SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF PRIMARY AMINO ACID DERIVATIVES (PAADs): NOVEL NEUROLOGICAL AGENTS FOR THE TREATMENT OF EPILEPSY AND NEUROPATHIC PAIN

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ABSTRACT

AMBER MARIE KING: Synthesis and Pharmacological Evaluation of
Primary Amino Acid Derivatives (PAADs):
Novel Neurological Agents for the Treatment of Epilepsy and Neuropathic Pain
(Under the direction of Dr. Harold Kohn)

Epilepsy and neuropathic pain (NP) are chronic neurological disorders that result from dysregulations in neuronal function. Currently, there is a lack of adequate therapeutic agents available to treat these disorders and the need remains to develop compounds that possess a novel mechanism of action to address the shortcomings of current medications. Recently, the role of voltage-gated sodium channels (VGSCs) has been implicated in the pathophysiological mechanisms of NP, while their role in epilepsy has been known for some time. The functionalized amino acid (FAA) (*R*)-lacosamide is an emerging antiepileptic drug (AED) that has been shown to selectively promote VGSCs into the slow inactivated state and has recently been approved by the EMEA and the US FDA under the trademark Vimpat® for the adjuvant treatment of partial-onset seizures in adults. (*R*)-Lacosamide has also demonstrated clinical efficacy in treating painful diabetic neuropathy, but has yet to gain regulatory approval for this indication.

The pharmaceutical industry has made advances in developing peripheral nervous system (PNS)-specific agents that target specific isoforms of VGSCs for the treatment of NP. We combined the concept of PNS-selectivity with our knowledge of FAAs and proposed that primary amino acid derivatives (PAADs) may selectively target PNS receptor sites, thereby avoiding potential CNS side effects that makes adherence to pain therapy difficult. Additionally, we examined the effect of PAADs on CNS function due to the excellent

anticonvulsant activity of FAAs. We synthesized and evaluated over 50 PAADs in whole animal models of epilepsy and NP, and developed a structure-activity relationship (SAR) that defined the structural requirements for PAAD activity. The SAR revealed excellent anticonvulsant activity and pain attenuation for a novel class of compounds, the C(2)-hydrocarbon PAADs. Then, we synthesized over 40 additional PAADs to optimize anticonvulsant activity and pain attenuation. From our optimization studies, we discovered two PAADs that displayed superior anticonvulsant activity and may rival the therapeutic capabilities of (*R*)-lacosamide. Finally, we evaluated the most active PAADs in a series of binding and enzymatic assays but we did not reveal any new binding targets of therapeutic relevance.

To my youngest sisters, Hailey and Zoe Smith:

You can achieve any goal with confidence and perseverance.

PREFACE

As a young child, I possessed the gift of gab and a sense of confidence that often resulted in borderline inappropriate conservations with strangers when my parents dared to venture into public with me. As I grew older, I retained those qualities but learned the value of modesty, which can be misinterpreted as shyness. My family and friends know better.

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LIST OF ABBREVIATIONS

Å	Ångström
AAA	α-Aminoamide
Ac	Acetyl
ACN	Acetonitrile
ADD	Antiepileptic drug development
Ag_2O	Silver (I) oxide
Alloc	Allyloxycarbonyl
AP	Action potential
app. t	Apparent triplet
Ar	Aryl (spectral)
Ar	Argon
ASP	Anticonvulsant screening project
atm	Atmosphere
ATP	Adenosine triphosphate
Azoc	Azidomethyl carbonyl
BBB	Blood-brain barrier
BF_3	Boron trifluoride
Bn	Benzyl
Boc_2O	Di-*tert*-butyl dicarbonate
B:P	Brain-to-plasma ratio
br	Broad (spectral)
Br_2	Bromine
BZP	Benzodiazepine

°C	Degrees Celsius
$C(CH_3)_3$	*tert*-Butyl
C_6H_5	Phenyl
Ca^{2+}	Calcium
Calcd	Calculated
cat	Catalytic
Cbz	Benzyloxycarbonyl
CD_3OD	Deuterated methanol
$CDCl_3$	Deuterated chloroform
CDMT	2-Chloro-4,6-dimethyl-1,3,5-triazine
CF_3	Trifluoromethyl
$CH(CH_3)_2$	Isopropyl
CH_2Cl_2	Dichloromethane
CH_3	Methyl
CH_3CN	Acetonitrile
CH_3I	Methyl iodide
$CHCl_3$	Chloroform
Cl^-	Chloride
Cl	Chloro
cm^{-1}	Wavenumbers
CNS	Central nervous system
$(COCl)_2$	Oxalyl chloride
COSY	Correlation spectroscopy
CRMP	Collaspin response mediator protein
CVD	Cardiovascular disease

d	Doublet
DAT	Dopamine transporter
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DEAD	Diethyl azodicarboxylate
DHP	Dihydropyran
DIEA	*N*,*N*-Diisopropylethylamine
DMAP	4-(*N*,*N*-Dimethylamino)pyridine
DMEM	Dulbecco's modified eagle medium
DMF	*N*,*N*-Dimethylformamide
DML	Designed multiple ligand
DMSO	Dimethyl sulfoxide
DMSO-d_6	Deuterated dimethyl sulfoxide
DMTMM	4-(4,6-Dimethoxy-1,3,5-traizin-2-yl)-4-methylmorpholine
DOR	Delta opioid receptor
DRG	Dorsal root ganglion
DTPP	Diethoxytriphenylphosphorane
ED_{50}	Dose effective in 50% of test subjects
E_{ion}	Ionic potential
EMEA	European Medicines Agency
ESI	Electrospray ionization
Et_2O	Diethyl ether
Et_3N	Triethylamine
EtOAc	Ethyl acetate
EtOH	Ethanol
F	Fluoro

FAA	Functionalized amino acid
FAK	Functionalized amino ketone
FDA	Food and Drug Administration
Fmoc	Fluorenylmethyloxycarbonyl
Form	Formalin
FT	Fourier transform
g	Gram
GABA	γ-Aminobutyric acid
GI	Gastrointestinal
GPCR	G-protein coupled receptor
h	Hour
H^+	Hydrogen ion
H_2O	Water
HCl	Hydrogen chloride
hERG	Human ether-a-go-go related gene
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum correlation
HPLC	High pressure liquid chromatography
HRMS	High resolution mass spectrometry
Hz	Hertz
I	Current
IBCF	Isobutyl chloroformate
IC_{50}	Inhibitory concentration in 50% of receptors
ip	Intraperitoneally
IR	Infrared

J	Coupling constant
K^+	Potassium ion
K_2CO_3	Potassium carbonate
K_d	Dissociation constant
kg	Kilogram
$KHSO_4$	Potassium bisulfate
K_i	Binding affinity
KOR	Kappa opioid receptor
LC	Liquid chromatography
Li_2CuCl_4	Dilithium tetrachlorocuprate
$LiAlH_4$	Lithium aluminum hydride
LiOH	Lithium hydroxide
lit.	Literature value
LRMS	Low resolution mass spectrometry
M	Moles per liter
M^+	Parent molecular ion
mA	Milliamp
MAC	Mixed anhydride coupling
MAD	Minimal active dose
MAO	Monoamine oxidase
MAO_A	Monoamine oxidase A
MAO_B	Monoamine oxidase B
mbar	millibar
MeOH	Methanol
MES	Maximal electroshock seizure

mg	Milligram
Mg^{2+}	Magnesium ion
$MgSO_4$	Magnesium sulfate
MHz	Megahertz
min	Minute
mL	Milliliter
mM	Millimolar
mmol	Millimole
μM	Micromolar
MOA	Mechanism of action
mol	Mole
MOR	Mu opioid receptor
mp	Melting point
MPLC	Medium pressure liquid chromatography
MS	Mass spectrometry
MsCl	Methanesulfonyl chloride
MTD	Maximal tolerated dose
N	Normal
N_2	Nitrogen
Na^+	Sodium ion
Na_2CO_3	Sodium carbonate
Na_2SO_4	Sodium sulfate
NADPH	Nicotinamide adenine dinucleotide phosphate
$NaHCO_3$	Sodium bicarbonate
NaOEt	Sodium ethoxide

NaOH	Sodium hydroxide
n-BuLi	*n*-Butyl lithium
NCE	New chemical entity
ND	Not determined
NET	Norepinephrine transporter
NIMH	National Institute of Mental Health
NINDS	National Institute of Neurological Disorders and Stroke
nm	Nanometers
NMM	*N*-Methylmorpholine
NMR	Nuclear magnetic resonance
NP	Neuropathic pain
OCF_3	Trifluoromethoxy
OCH_3	Methoxy
PAAD	Primary amino acid derivative
PAE	Primary amino ester
PAK	Primary amino ketone
Pd-C	Palladium on carbon
PDSP	Psychoactive drug screening program
PEG	Poly(ethylene)glycol
PEPD	Paroxysmal extreme pain disorder
PET	Positron emission tomography
Ph	Phenyl
PI	Protective index
PK	Pharmacokinetics
pM	picomolar

PNS	Peripheral nervous system
po	Orally
ppm	Parts per million
p-TSA	*para*-Toluenesulfonic acid
q	Quartet
R_f	Retention factor
rpm	Revolutions per minute
rt	Room temperature
s	Singlet
SAAD	Secondary amino acid derivative
SAR	Structure-activity relationship
scMET	Subcutaneous metrazol®
SERT	Serotonin transporter
SiO_2	Silicon dioxide
SVP2	Synaptic vesicle protein 2
t	Triplet
TAAD	Tertiary amino acid derivative
t-Boc	*tert*-Butoxycarbonyl
t-BuOH	*tert*-Butanol
TcBoc	2,2,2-Trichloro-*tert*-butyloxycarbonyl
TD_{50}	Dose toxic in 50% of test subjects
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TMS	Tetramethylsilane

Tox	Toxicity
TPP	Triphenylphosphine
TPPO	Triphenylphosphine oxide
TrCl	Trityl chloride
Troc	2,2,2-Trichloroethoxycarbonyl
TTX	Tetrodotoxin
*μ*M	Micromolar
UV	Ultraviolet
V	Voltage
VGIC	Voltage-gated ion channels
VGPC	Voltage-gated potassium channels
VGSC	Voltage-gated sodium channels
w/w	Weight per unit wieght

Chapter 1. Introduction

1. Central nervous system disorders

Central nervous system (CNS) disorders, including but not limited to epilepsy and neuropathic pain (NP), are some of the most poorly treated illnesses in modern medicine. The lack of new chemical entities (NCEs) for CNS disorders is a multifaceted problem involving the complexity of the brain, blood-brain barrier (BBB) penetration, CNS side effects, and a lack of validated biomarkers.[1] Not only are CNS therapies less likely to reach the market compared to other therapeutic areas, such as cardiovascular disease (CVD) (clinical success rates: CNS, 8%; CVD, 20%), their time in development can be significantly longer (years in development: CNS, 12.6; CVD, 6.3).[1,2] A shift in the drug discovery paradigm over the last several decades to design high-specificity compounds could be one of the largest contributing factors to the current unmet medical need of CNS disorders, and perhaps another shift is necessary to adequately address these issues.

The birth of synthetic organic chemistry in the 19th century altered the identification of therapeutic agents from empirical methods, which are blind to mechanism, to a more "rational" hypothesis-driven approach. Post-19th century drug discovery has become increasing more sophisticated, first incorporating cellular analysis (biochemistry), followed by target analysis (molecular biology), with the overarching goal of creating a therapeutic agent, or "magic bullet," that can selectively target and treat a single disease state.[3,4] While the magic bullet approach has experienced limited success in areas such as oncology (monoclonal antibodies, kinase inhibitors),[5,6] it presents a challenge when addressing

disorders that are multifactorial in origin, such as CNS disorders. Treatment of these complex processes may benefit from a multitargeted or "magic shotgun" approach, rather than a high-specificity, magic bullet approach.[7,8] Analysis of currently marketed anticonvulsants[7] and antipsychotics[8] revealed that interaction with multiple molecular targets are responsible for their pharmacological effects. We agree that the magic shotgun approach may be the more appropriate choice for the treatment of CNS disorders and, accordingly, we have developed several possible lead compounds based on structure-activity relationships (SAR) in whole animal models of epilepsy and neuropathic pain, independent of mechanism.

1.1. Epilepsy

Epilepsy is a serious neurological disorder affecting up to 1% of the world's population, including more than two million Americans.[9-12] Currently, the estimated annual medical cost of epilepsy exceeds $15 billion and it is estimated that 140,000 new cases of epilepsy will be diagnosed in the US in 2010.[12] It is a common misconception to define epilepsy as a singular disease, but it is more appropriate to refer to epilepsy as a heterogeneous mixture of disorders with the commonality of neuronal dysregulations as a result of varying external, brain developmental, or genetic causes.[10,11,13-16] Broadly defined, epilepsy is characterized by reoccurring, unprovoked seizures, which results from neuronal hyperexcitability and hypersynchronous neuronal firing, and is classified as either partial-onset (localized within one hemisphere of the brain) or generalized-onset (involving both brain hemispheres).[11,17] Treatment of epilepsy is limited to antiepileptic drugs (AEDs), dietary regulations (ketogenic diet), or surgery. Although newer generation AEDs have made improvements (reviewed in Section 1.3), it is estimated that 30% of epilepsy patients fail at least two first-line AED treatments, deeming these patients pharmacoresistant, while ~40% of the patients that respond to AEDs experience adverse side effects.[9-11,18,19]

Pharmacoresistant patients represent a substantial market with an important unmet medical need. Therefore, the need remains for highly potent AEDs with improved efficacy, decreased toxicity, and favorable pharmacokinetic (PK) properties with the hope of not only suppressing seizures, but also reducing the patient's susceptibility to future seizures.

1.2. Neuropathic pain

Neuropathic pain (NP) is caused by damage, disease, or dysfunction in the nervous system.[20] However, this is an oversimplification due to the sheer complexity of pathophysiological events that occur during the development of NP, and this complexity often results in poorly diagnosed and improperly managed therapy.[21,22] The source of nervous system damage can range from genetic factors to traumatic factors, but symptoms commonly include spontaneous, unpleasant burning sensations (dysesthesia), exaggerated pain sensations (hyperalgesia), and/or hypersensitivity to normally non-painful stimuli (allodynia).[20,22,23] Currently, treatment options rely on pharmacological management and/or surgical management, but both options fail to adequately relieve pain in the majority of patients.[24] First line medications include nonsteroid anti-inflammatory drugs (NSAIDs), opioids, antidepressants, and anticonvulsants, but nonspecific mechanisms of CNS depression cause a range of inconsistent and unwanted side effects, which often limits their use.[23-26] Recently, the role of voltage-gated ion channels (VGICs) and downstream effectors of neurotrophins in NP have been investigated.[20,26,27] Ultimately, inadequate pain control can be attributed to the combination of ill-defined pathophysiological mechanisms and limited treatment options, but large efforts are underway to clearly define and specifically disrupt the development of neuropathies with therapeutic agents that possess a novel mechanism of action.[28-33]

1.3. Antiepileptic drugs

Presently, AEDs are classified into three groups based on the timeframe in which they were discovered: established (Figure 1), recent (Figure 2), or emerging (Figure 3). Established or traditional AEDs span until ~1978, after which there was a period of inactivity (~15 years) before a resurgence of AED research in the early 1990s. The pathophysiological processes of most AEDs fall into one of four categories: increase of inhibitory neurotransmission, decrease of excitatory neurotransmission, blocking VGICs, and interference with intracellular signaling pathways.[13,16,18,34-38]

1.3.1. Established AEDs: 1800s–1978

Antiepileptic drugs have a relative short history in the grand scheme of medicinally relevant agents. Bromide salts were the principle AED throughout the 19th century until the anticonvulsant properties of phenobarbital (**5**) were discovered serendipitously at the turn of the 20th century.[39] Then, the development of electrically-induced[40] and chemically-induced[41] animal seizure models aided the discovery of phenytoin (**6**), primidone (**7**), and ethosuximide (**4**) in the 1930s–1950s. In 1962, the anticonvulsant activity of valproic acid (**8**) was also discovered serendipitously when it was used as a solvent for compounds that were screened for seizure protection. The 1970s saw a re-purposing of known CNS agents, such as the antipsychotic carbamazepine (**1**), and the anxiolytics clobazam (**2**) and clonazepam (**3**), as they began to be recognized for their anti-seizure activities.[39]

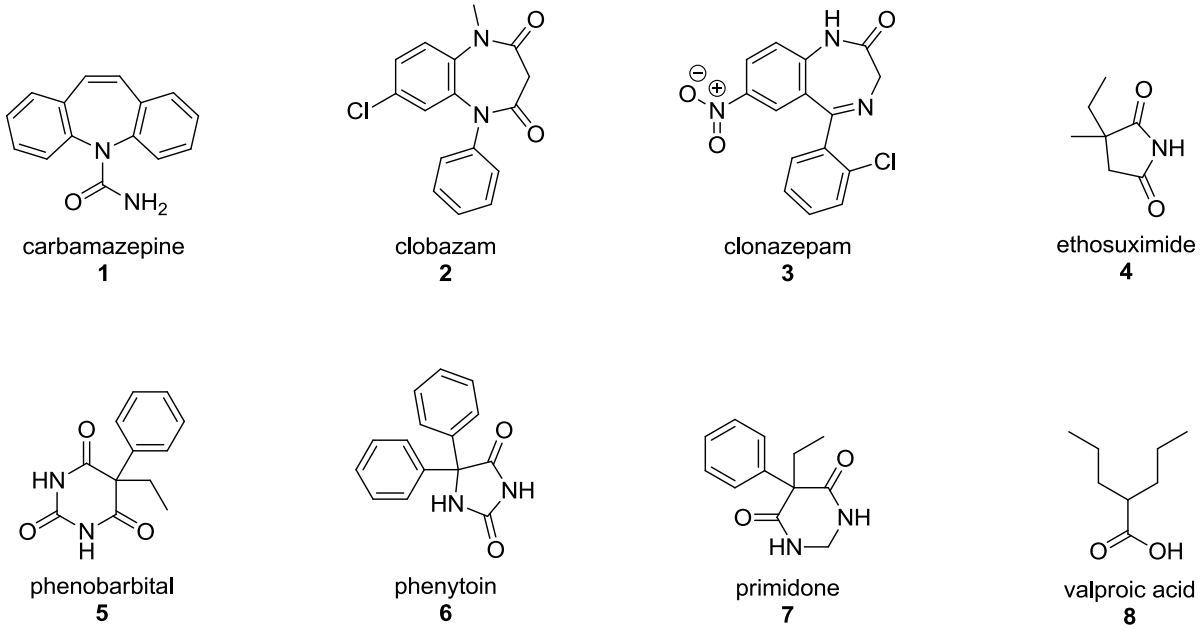


Figure 1. Established AEDs

1.3.2. Recent AEDs: 1993–Early 2000s

The Epilepsy Branch of the National Institute of Neurological Disorders and Stroke (NINDS) started the Antiepileptic Drug Development (ADD) program in 1975 to facilitate the development of new therapeutics for the treatment of seizure disorders as a response to the decline in novel anticonvulsant agents. Within the ADD program, the Anticonvulsant Screening Project (ASP) served as the preclinical component to identify lead compounds.[42] Several novel compounds, including felbamate (**10**) and topiramate (**18**), were identified through the collaborative efforts of pharmaceutical companies and the ADD. Felbamate (**10**) was the first anticonvulsant to be approved by the United States Food and Drug Administration (US FDA) since the 1978, and topiramate (**18**) followed suit a few years later.[42]

Among the recent AEDs, several are second generation anticonvulsants that have tried to improve efficacy/safety or circumvent unfavorable side effects/PK properties compared with the established parent AEDs, while others are novel chemical structures.

Some second generation AEDs include fosphenytoin (**9**) and oxacarbazepine (**14**). Fosphenytoin (**9**) was developed as a phosphate ester prodrug of phenytoin (**6**) and, unlike phenytoin (**6**), it is freely soluble in aqueous solutions,[18,43,44] while oxacarbazepine (**14**) avoids the inducible cytochrome CYP3A4-mediated oxidative metabolism of carbamazepine (**1**).[45] Many new chemical structures that possess anticonvulsant activity are gabapentin (**11**), lamotrigine (**12**), levetiracetam (**13**), rufinamide (**16**), tiagabine (**17**), vigabatrin (**19**), and zonisamide (**20**). Pregabalin (**15**) is a follow up to gabapentin (**11**) and has demonstrated higher efficacy than gabapentin (**11**) in preclinical models of epilepsy, neuropathic pain, and anxiety.[45]

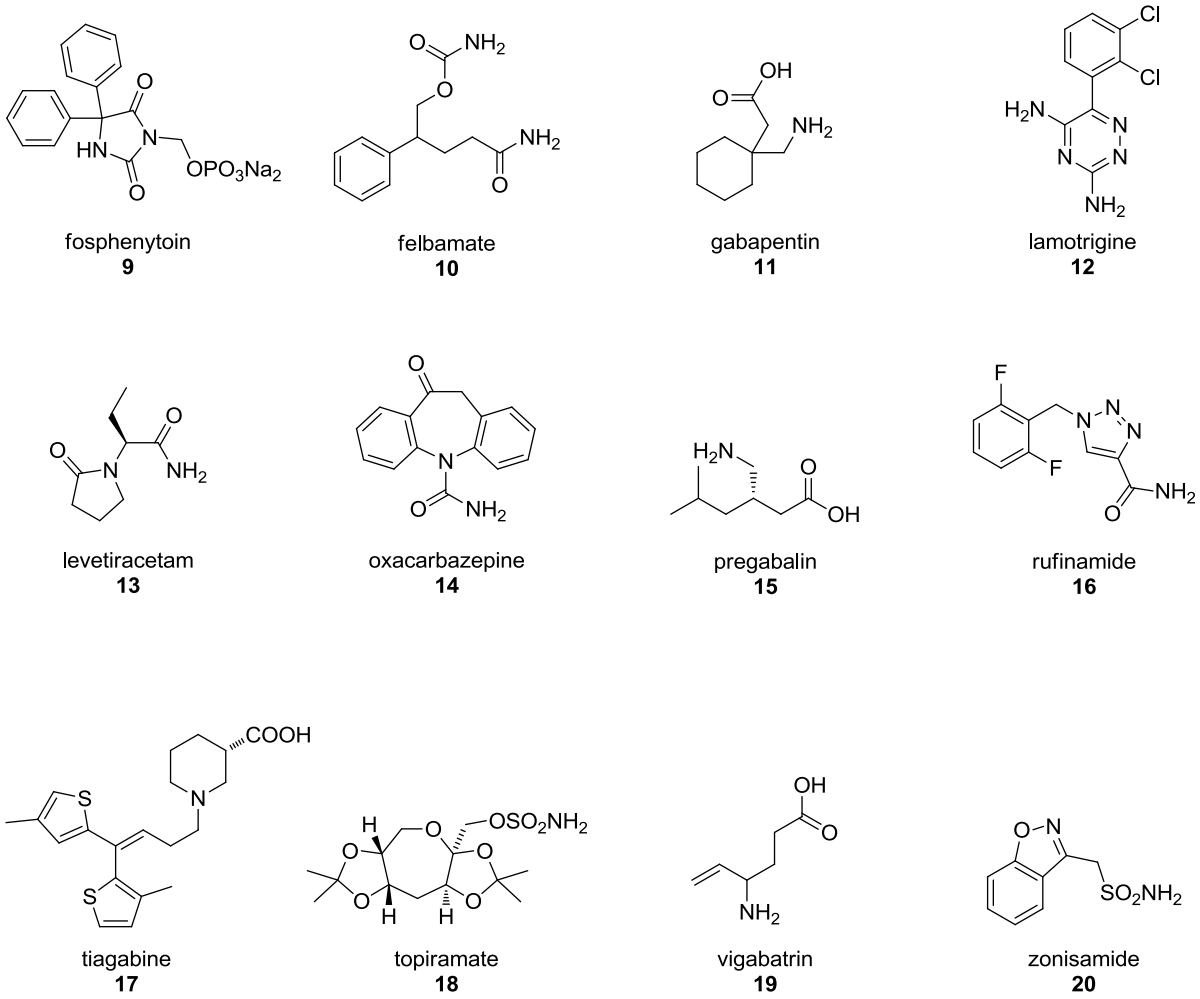


Figure 2. Recent AEDs

1.3.3. Emerging AEDs: Early 2000s–2010

Several emerging AED candidates are second generation to recent AEDs, but many are novel in structure. Second generation emerging AED candidates include brivaracetam (**21**), carisbamate (**22**), eslicarbazepine (**24**), JZP-4 (**27**), propylisopropyl acetamide (**29**), and valrocemide (**31**). Brivaracetam (**21**) has ~10-fold higher affinity than levetiracetam (**13**) for synaptic vesicle protein 2 (SVP2) and it expands upon the mechanism of action (MOA) to include neuronal voltage-gated sodium channels (VGSCs).[37,45-47] Carisbamate (**22**) was developed to avoid the toxic metabolites of felbamate (**10**), and also displays a broader

spectrum of activity.[37,47] Eslicarbazepine (**24**), second generation to oxacarbazepine (**14**) and third generation carbamazepine (**1**), was designed as a prodrug to obtain patent rights. The active, non-patentable metabolite of eslicarbazepine (licarbazepine, **25**) displayed higher bioavailability than oxacarbazepine (**14**) and still avoids the inducible cytochrome CYP3A4-mediated oxidative metabolism associated with carbamazepine (**1**).[45-47] JZP-4 (**27**) has better efficacy in the 6 Hz seizure model than lamotrigine (**12**), and has demonstrated a greater selectivity for neuronal VGSCs over peripheral tetrodotoxin-resistant (TTX) VGSCs.[46] Propylisopropyl acetamide (**29**) and valrocemide (**31**) are just two of the many valproic acid (**8**) derivatives in development, and these compounds decrease the teratogenicity and hepatotoxicity associated with valproic acid (**8**).[45-47] New scaffolds with seizure protection are neuroactive sugars (2-deoxy-D-glucose, **23**), neuroactive steroids (ganaxolone, **26**), functionalized amino acids (lacosamide, **28**), and α-aminoamides (safinamide, **30**).[47,48]

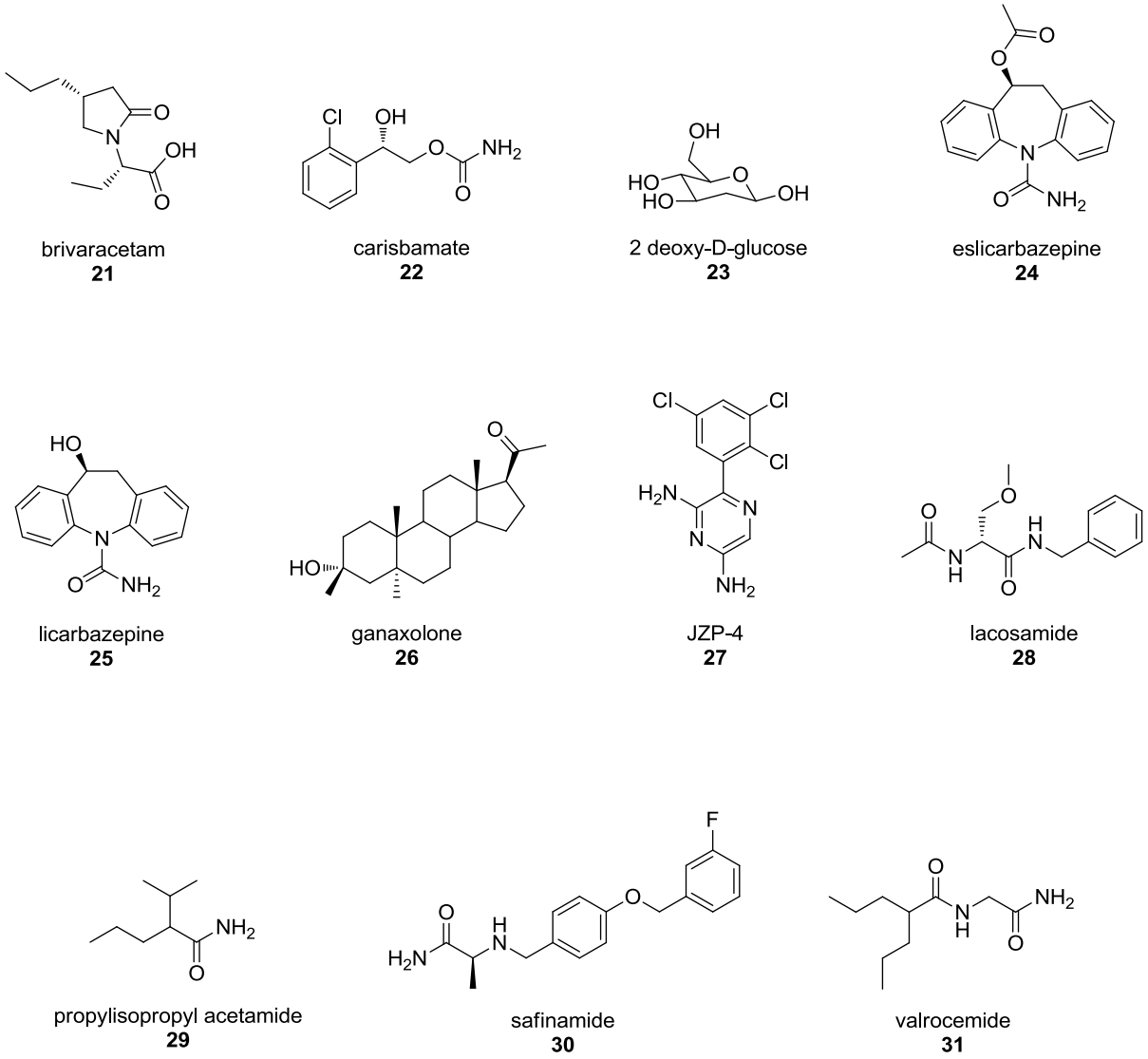


Figure 3. Emerging AEDs

1.3.3.1. Lacosamide

Of the emerging AEDs with anticonvulsant activity, we have a particular connection with lacosamide (**28**) because the inception of functionalized amino acids (FAAs), and the subsequent discovery of lacosamide (**28**), occurred in the Kohn laboratory (Figure 4). Before FAAs, the Kohn laboratory examined the pharmacological activity of substituted imidazolidinethiones and thioimidazolines,[49] followed by the effect of structural modification

of the hydantoin ring on anticonvulsant activity.[50] Concurrent analysis of the literature of emerging anticonvulsants[51] led the Kohn laboratory to examine the pharmacological activities of protected amino acid derivatives (**33**), which were conceptually open-chain analogs of hydantoins (i.e., **32**). These compounds were termed Functionalized Amino Acids (FAAs), and racemic FAA **33** (R = CH_3) displayed significant anticonvulsant activity in mice (<100 mg/kg).[50,52] Next, the Kohn laboratory demonstrated that the seizure protection of FAAs were associated with the D-amino acid configuration (typically (*R*)-stereoisomer) of FAAs ((*R*)-**33**).[53] Then, a series of studies were published probing the 2-position of FAAs,[54-58] and the 2-furanyl FAA (*R*)-**34** was identified as a lead compound (ED_{50} = 3.3 mg/kg in mice).[56] However, toxicity in preclinical assessment at the Eli Lilly Laboratories (Indianapolis, IN) halted further advancement into phase I clinical trial.[59] Further studies of heteroatom-containing 2-substituents in FAAs revealed the potent anticonvulsant properties of (*R*)-lacosamide ((*R*)-**28**) (ED_{50} = 4.5 mg/kg in mice),[60] which attracted the interest of Harris FRC Corporation (Holmdel, NJ), and subsequently Schwarz Pharma (Monheim, Germany; acquired by UCB Pharma (Brussels, Belgium) in 2006).

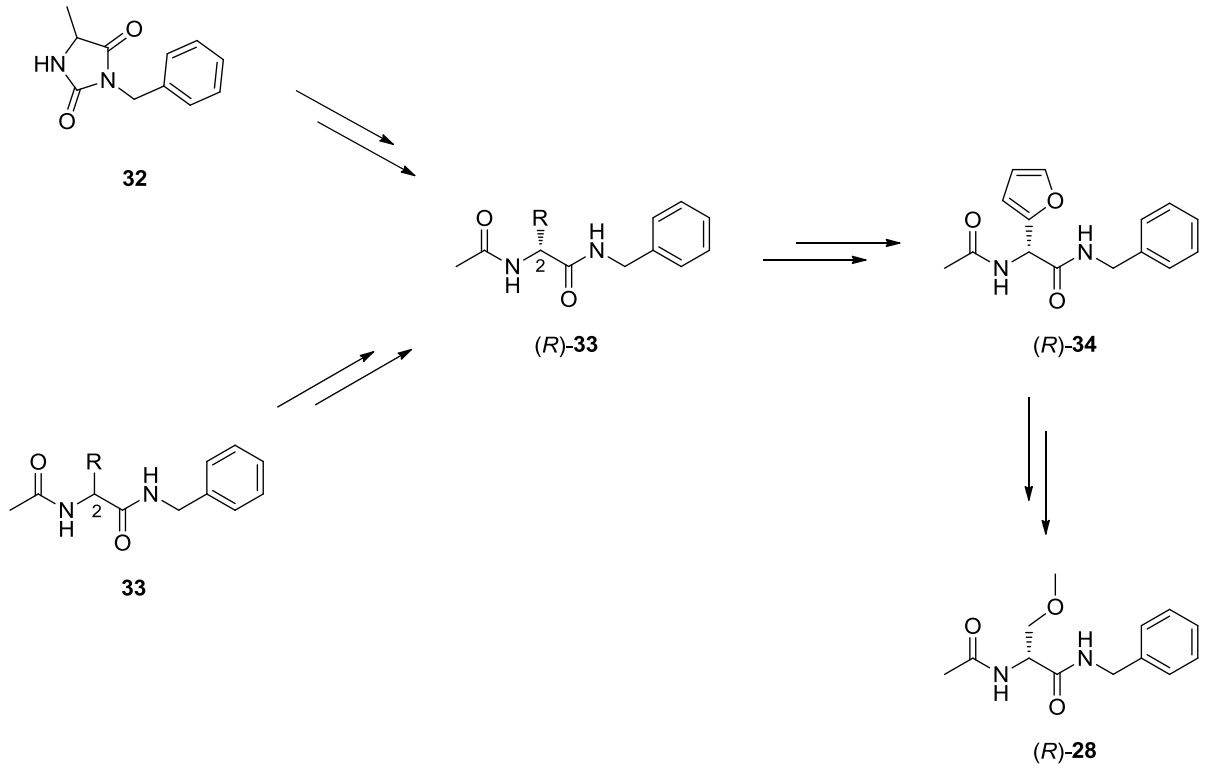


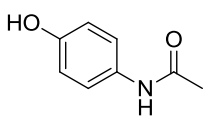
Figure 4. Highlights on the path to discovering (*R*)-lacosamide ((*R*)-28)

(*R*)-Lacosamide ((*R*)-**28**) has recently been approved by the European Medicines Agency (EMEA) and the US FDA under the trademark Vimpat® for the adjuvant treatment of partial-onset seizures in adult patients with epilepsy.[61] (*R*)-**28** is a new generation AED due to its unique structure and *in vivo* pharmacological profile. The molecular mode of action is currently unknown, but recent research indicates that (*R*)-**28** promotes selective enhancement of sodium channel slow inactivation (reviewed in Section 2.1.2). Recent studies have also shown that (*R*)-**28** binds to the collaspin-response mediator protein 2 (CRMP-2)[62-64] and carbonic anhydrase,[65] but the functional significance of these interactions have not been determined. While (*R*)-**28** has demonstrated clinical efficacy in treating painful diabetic neuropathy in two out of three phase III clinical trials and was determined suitable for long-term use with a desirable safety profile, additional testing is needed for approval for this indication.[66]

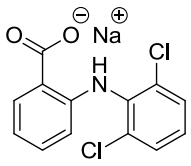
1.4. Pain attenuating drugs

First line treatment of NP relies heavily on therapeutic agents that were originally developed for other medical conditions, such as inflammation, acute pain, depression, and seizures.[23] These include, but are not limited to, the nonsteroid anti-inflammatory drugs (NSAIDs) acetaminophen (**35**), diclofenac sodium (**36**), ibuprofen (**37**), and indomethacin (**38**); the opioids codeine (**39**), morphine (**40**), and oxycodone (**41**); the antidepressants amitriptyline (**42**), despiramine (**43**), duloxetine (**44**), and nortryptyline (**45**); and the anticonvulsants carbamazepine (**1**), clonazepam (**3**), gabapentin (**11**), and pregabalin (**15**) (Figure 5).[27] There are limited US FDA approved drugs specifically for NP, but those that have received approval include carbamazepine (**1**, facial pain),[67] duloxetine (**44**, painful diabetic peripheral neuropathy and fibromyalgia),[68] gabapentin (**11**, post-herpetic neuralgia),[69] and pregabalin (**15**, post-herpetic neuralgia, painful diabetic peripheral neuropathy, and fibromyalgia).[70] Current pharmacological management of NP usually consists of a combination of agents from several of the listed drug classes, but it provides only 30–50% pain reduction in ~50% of patients.[23] Therefore, the need remains to identify and validate new targets that are involved in NP and design novel therapeutic agents to address those targets.

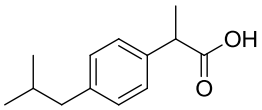
NSAIDS



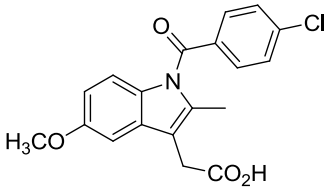
acetaminophen
35



diclofenac sodium
36

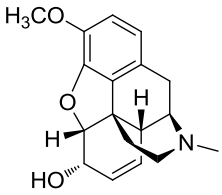


ibuprofen
37

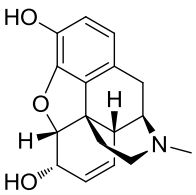


indomethacin
38

Opioids



codeine
39



morphine
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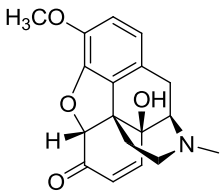

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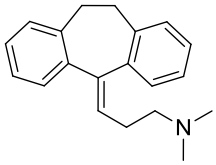
Antidepressants



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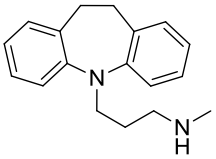

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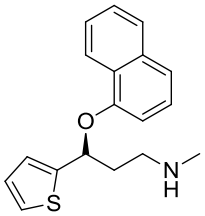

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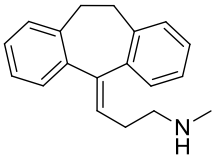

nortryptyline
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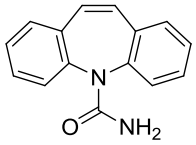
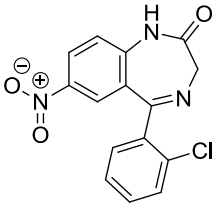
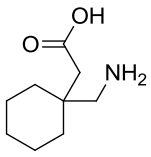
AEDs



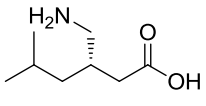
carbamazepine
1



clonazepam
3



gabapentin
11



pregabalin
15

Figure 5. Drugs used for the management of neuropathic pain

2. Communication methods of the nervous system

Communication between the central and peripheral nervous systems (CNS and PNS, respectively) and the brain is a highly regulated process involving the sending, receiving, and integration of electrical and chemical impulses. Neurons are responsible for sending these signals over considerable distances by the propagation of action potentials (electrical transmission) or through the release of endogenous chemicals (chemical transmission).

2.1. Electrical transmission

2.1.1. The action potential

The intracellular and extracellular sides of the neuronal cell plasma membrane are surrounded by ions (K^+, Na^+, Ca^{2+}, Cl^-). The differential distribution in ionic concentration (intracellular relative to extracellular) defines an equilibrium potential (E_{ion}) for each ion (Table 1) and the differential distribution in ionic concentrations and the relative permeabilities of each ion under normal physiological conditions create the resting membrane potential.[71] When neurons are stimulated, voltage-, ligand-, or stretch-activated ion channels open or close, causing a change in ionic permeabilities and, consequently, a change in the membrane potential. The constant flux of ions across the plasma membrane requires active transport mechanisms that use the hydrolysis of ATP (i.e., Na^+/K^+ ATPase, H^+/K^+ ATPase, Ca^{2+} ATPase, Mg^{2+} ATPase) and co-transport mechanisms (symport and antiport transporters) to maintain ionic homeostasis. An action potential (AP) is generated if the change in membrane potential reaches a particular value (threshold) before ionic homeostasis can be restored by ionic pumps and transporters.

Table 1. Physiological mammalian ion gradients and equilibrium potentials

Ion	[Outside][a] (mM)	[Inside][b] (mM)	E_{ion} (mV)
K^+	3	135	-102
Na^+	150	18	+56
Ca^{2+}	1.2	0.0001	+125
Cl^-	120	7	-76

[a] Concentration of extracellular ions. *Fundamental Neuroscience*. Academic Press: Boston, **2003**, p. 140. [b] Concentration of intracellular ions. *Fundamental Neuroscience*. Academic Press: Boston, **2003**, p. 140.

In the generation of an AP as depicted in Figure 6, the membrane potential slowly becomes less negative compared with the resting potential (1) due to extrinsic stimulation or intrinsic pacemaker depolarization until the membrane potential reaches its threshold (2). At this point, the neuron is committed to the generation of the AP, and the membrane potential rapidly becomes less negative than the resting potential (depolarization, 3) due to the activity of VGSCs. As the membrane potential depolarizes, inactivation of VGSCs and activation of voltage-gated potassium channels (VGPCs) causes repolarization (4), and often becomes more negative than the original resting potential (afterhyperpolarization, 5), before equilibrating back to the resting potential (1). A hypothetical voltage-clamp analysis of the ionic current that underlies an AP (Figure 7) shows that the total membrane current (3) is a combination of the outward conductance of K^+ (1) through VGPCs and the inward conductance of Na^+ (2) through VGSCs.[71]

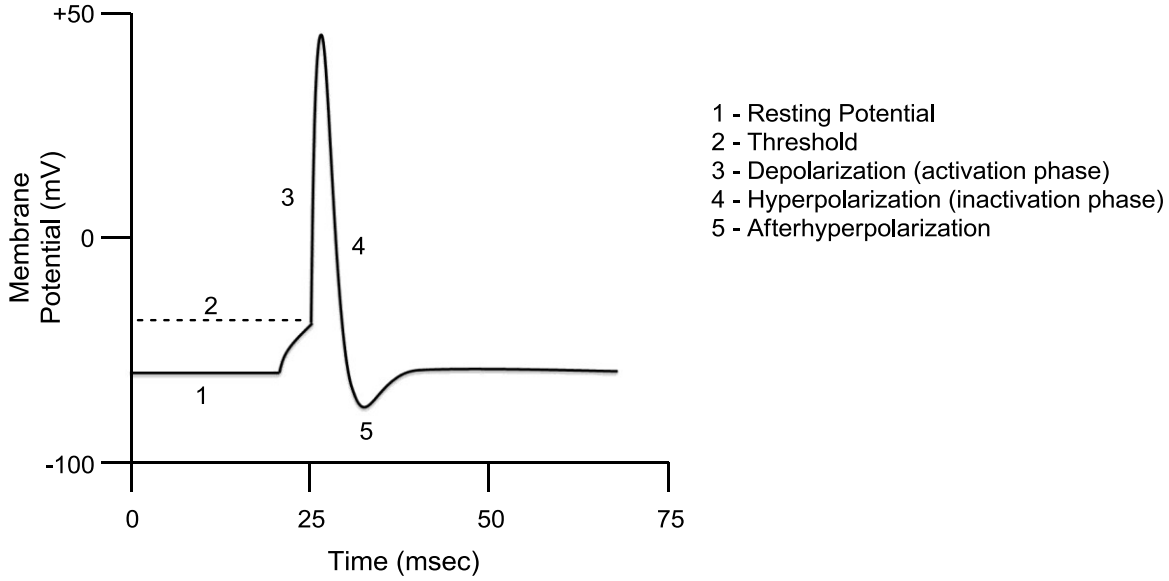


Figure 6. Hypothetical action potential

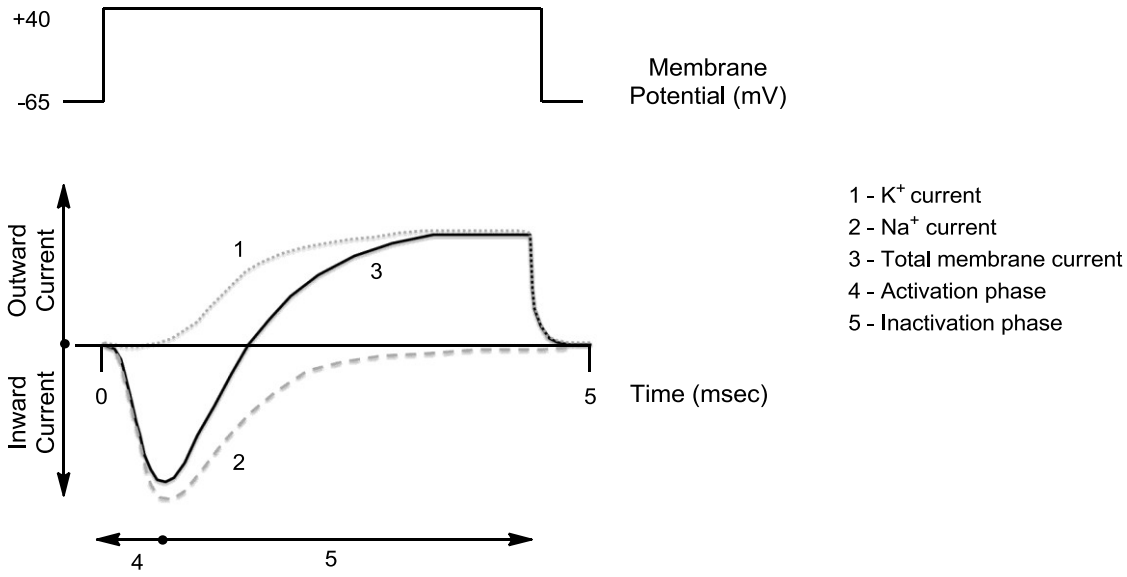


Figure 7. Hypothetical voltage-clamp analysis of ionic currents underlying an action potential[71]

2.1.2. VGSCs

Many mutated genes that encode for VGICs have been identified in idiopathic epilepsies[72-74] and pain disorders[75,76] (Table 2). Therefore, it is not surprising that VGICs have become potential targets for AEDs.[77] Specifically, the Kohn laboratory is focused on the role of VGSCs in epilepsy and neuropathic pain since reports indicate that lacosamide ((*R*)-**28**) promotes selective enhancement of sodium channel slow inactivation.[63,64]

Table 2. Mutated genes that encode for VGICs that are associated with idiopathic epilepsies and pain disorders

Gene	Ion Channel	Syndrome
KCNQ2	K^+	Benign familial neonatal seizures
KCNQ3	K^+	Benign familial neonatal seizures
SCN1A	Na^+	Generalized epilepsy with febrile seizures
SCN1B	Na^+	Generalized epilepsy with febrile seizures
SCN2A	Na^+	Benign familial neonatal seizures
SCN9A	Na^+	Paroxysmal extreme pain disorder
SCN9A	Na^+	Erythromelalgia
CACNB4	Ca^{2+}	Juvenile myoclonic epilepsy
CLCN2	Cl^-	Idiopathic generalized epilepsy

2.1.2.1. Structural properties of VGSCs

VGSCs are transmembrane proteins, comprised of an α-subunit (~260 kDa) (Figure 8A) and an accessory β-subunit, that allow the influx of sodium during cell depolarization and have been implicated in nociception, hyperexcitability, and increased pain sensitivity.[75] The α-subunit consists of four internally homologous domains (DI–DIV) (Figure 8C) that are

further divided into six transmembrane segments (S1–S6) (Figure 8B). The S4 segment contains a positively charged residue at every third position and creates the voltage-sensing mechanism.[78-85] Nine isoforms ($Na_v1.1$–1.9) are involved in physiological processes such as cognition, locomotion, and nociception. Generally, $Na_v1.1$–$Na_v1.3$, and $Na_v1.6$ are extensively found in the CNS, $Na_v1.4$ is present in muscle tissue, $Na_v1.5$ is present is cardiac tissue, and $Na_v1.7$–$Na_v1.9$ are distributed in the PNS.[78,83-87] However, expression can change significantly under pathological conditions such as NP and multiple sclerosis.[75,83] In conjunction with tissue distribution, sodium channel classification is also based on sensitivity to TTX, a powerful neurotoxin that selectivity binds to sodium channels with nanomolar affinity. $Na_v1.1$–1.4, 1.6, and 1.7 are TTX-sensitive while $Na_v1.5$, 1.8, and 1.9 are TTX-resistant.[85,86] Each subtype exhibits different voltage and kinetic profiles and is responsible for the rapid upstroke of the AP in neurons, and consequently leads to the initiation and propagation of electrical signals in the CNS and PNS.[83]

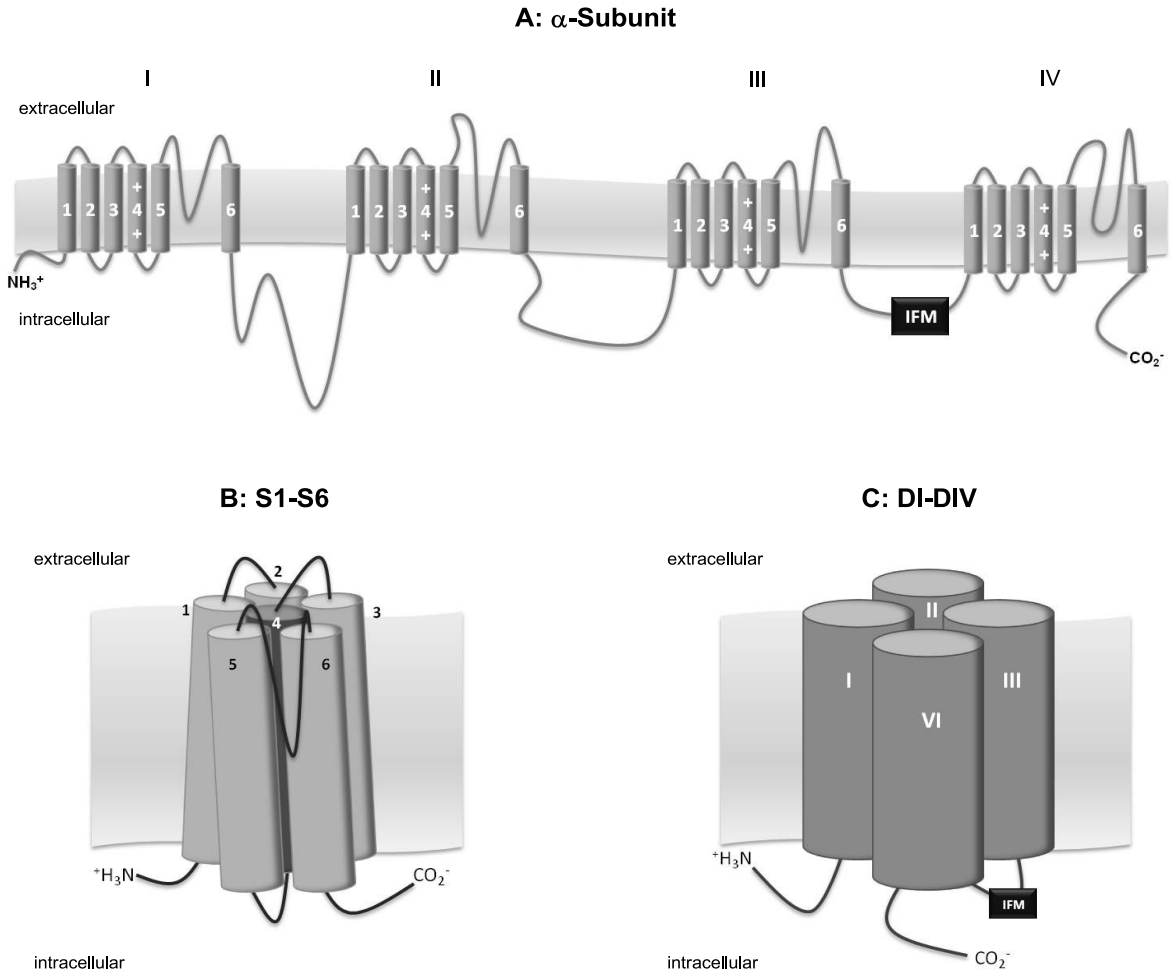


Figure 8. Topology of the VGSC

2.1.2.2. Functional states of the VGSC

The voltage-dependence of sodium channels mediates the opening, closing, and inactivation of the channel in response to cell depolarization (Figure 9). Upon depolarization, the sodium channel transitions from the closed (resting) state to the opened state, and allows the influx of sodium into the cell. Local anesthetics (e.g., lidocaine) have been shown block the open state by interacting with the cytoplasmic region of the inner pore.[64,88] Next, the channel transitions into the inactivated state to terminate the influx of sodium before returning back to the closed state. Inactivation serves as a biophysical mechanism that essentially makes the channel unavailable, thereby regulating the firing frequency of the AP

and controlling the excitability of tissues.[62,80,85] Two distinct kinetic classes of inactivation have been characterized: fast inactivation and slow inactivation, appropriately described by the time scale on which they occur (5–20 milliseconds and 500–1000 milliseconds, respectively).[62,80,85] Fast inactivation is well characterized and arises when the channel pore is physically blocked by a cytoplasmic region located between DIII and DIV of the sodium channel (Figure 8A and Figure 9D). This cytoplasmic region is distinguished by a specific amino acid sequence (IFM motif) and obstructs the pore in a 'ball-and-chain' or 'hinged lid' mechanism.[78,80,84,85] Several established anticonvulsants (e.g., carbamazepine) have been shown to selectively bind and enhance fast inactivation.[62,63] Slow inactivation is functionally and structurally distinct and much less understood. It is thought to occur via a conformational rearrangement experienced during repeated neuronal firing or sustained depolarization that impedes ionic flow through the channel.[62,80,85] In Figure 9C, slow inactivation is depicted as obstructing sodium flow on the extracellular side of the channel but the precise structural mechanism for sodium impedance remains elusive. (*R*)-**28** has been shown to promote the entry of sodium channels into the slow inactivated state.[63,64]

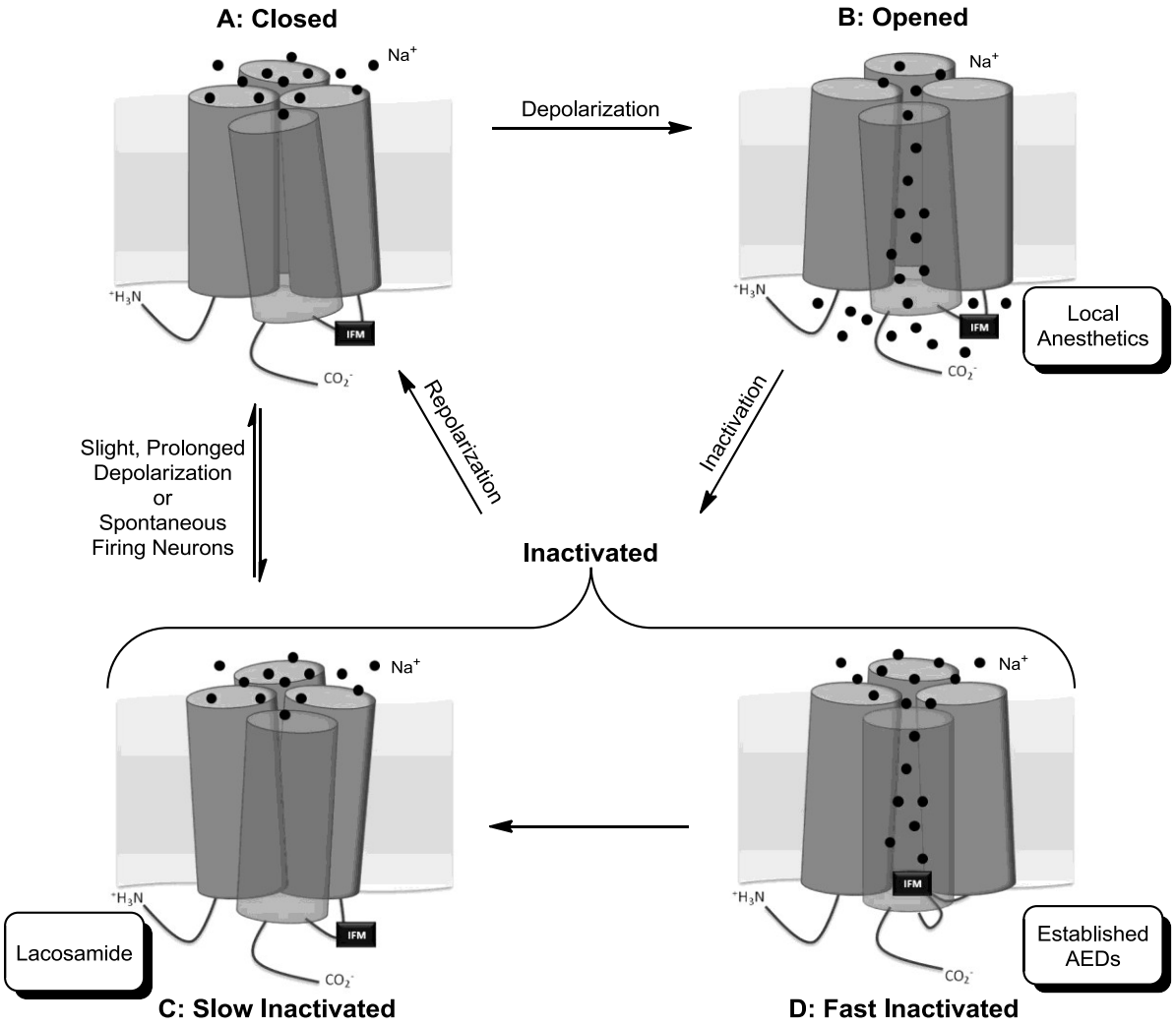


Figure 9. Functional states of the VGSC

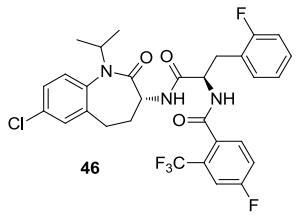
Recently, Lees *et al.* demonstrated that (*R*)-**28** inhibits sodium channel activation by shifting the slow inactivation voltage-dependence in the hyperpolarizing direction in primary neuronal cultures. Significantly, use of the (*S*)-stereoisomer of **28** did not show any modulation of the slow inactivation process.[63] Cummins *et al.* demonstrated that (*R*)-**28** enhances inactivation of rat $Na_v1.3$ and human $Na_v1.7$ in stably expressed HEK293 cells, as well as $Na_v1.8$-type TTX-R currents from dorsal root ganglion (DRG) neurons, with IC_{50} values of 415, 182, and 16 μM, respectively, without altering steady-state fast inactivation. It was also demonstrated that carbamazepine affects the fast inactivated state, while lidocaine

shows evidence of affecting both the fast and slow inactivated states. When comparing the resting state IC_{50} values of cells expressing $Na_v1.3$, $Na_v1.7$, and $Na_v1.8$ channels to the IC_{50} values of chronically depolarized cells expressing $Na_v1.3$, $Na_v1.7$, and $Na_v1.8$ channels, (*R*)-**28** was 123-250 times more selective for the chronically depolarized state, while carbamazepine and lidocaine were only 3-16 times more selective for the chronically depolarized state. This indicates that (*R*)-**28** can selectively block the activity of nociceptive neurons ($Na_v1.3$, $Na_v1.7$, and $Na_v1.8$) that are chronically depolarized, a pathological phenomenon that may characterize NP.[64]

2.1.2.3. The role of VGSCs in nociception

Recently, several sodium channel isoforms have been demonstrated to play a role in peripheral nociception and NP.[64,75,76,83,86] Gain-of-function mutations of the peripheral $Na_v1.7$ channel results in extremely painful disorders, such as erythromelalgia and paroxysmal extreme pain disorder (PEPD). Conversely, deletion of the $Na_v1.7$ channel results in an inability to experience pain without affecting non-nociceptive sensory functions.[33,75] Along with $Na_v1.7$, $Na_v1.3$ and $Na_v1.8$ are thought to be involved in NP and recent studies reported promising PNS-specific agents that target these sodium channel isoforms.[28-33,75] Among these, researchers at Merck Research Laboratories (Whitehouse Station, NJ) have characterized a novel class of benzazepinones as potent inhibitors of $hNa_v1.7$ channels for the potential treatment of NP.[29,30,33] Extensive SAR studies, with a focus on PK-enhancing properties, led to the discovery of **46** (IC_{50} = 30 nM).[29,30,33] Merck also developed CDA54 (**47**) to selectively inhibit injury-induced nerve signaling by blocking the inactivated state of $hNa_v1.7$ and $hNa_v1.8$ channels (IC_{50} = 0.25 μM and 0.18 μM, respectively).[28] Similarly, investigators at Abbott Laboratories (Abbott Park, IL) have developed and evaluated a series of 5-aryl-2-furfuramides, which led to the discovery of A-803467 (**48**) as a selective $hNa_v1.8$ channel blocker (IC_{50} = 140 nM) that reduced mechanical allodynia.[31,32] As

demonstrated by Merck Research Laboratories and Abbott Laboratories, preferential binding of small molecules to specific sodium channel isoforms could lead to therapeutically relevant molecules for NP with improved efficacy and safety. We will revisit the importance of **48** as it relates to this project in Chapter 3.



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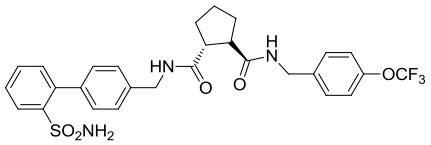

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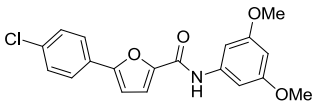
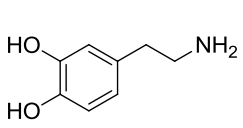


48

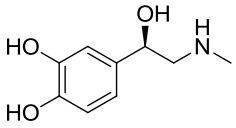
2.2. Chemical transmission

Neuronal communication consists not only of electrically-mediated transmission, but also chemically-mediated transmission. The chemical messengers can be broadly defined as classic neurotransmitters (small molecules) and non-classical neurotransmitters (peptides). Classic neurotransmitters include the biogenic monoamines dopamine (**49**), epinephrine (**50**), norepinephrine (**51**), and serotonin (**52**); the amino acids aspartate (**53**), GABA (**54**), glutamate (**55**), glycine (**56**), and serine (**57**); as well as acetylcholine (**58**) (Figure 10).

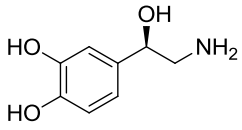
Monoamine Neurotransmitters



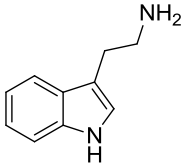
dopamine
49



epinephrine
50

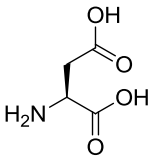


norepinephrine
51

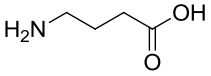


serotonin
52

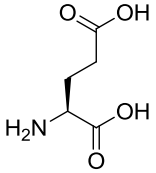
Amino Acid Neurotransmitters



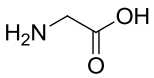
aspartate
53



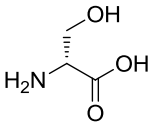
GABA
54



glutamate
55

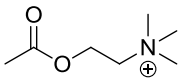


glycine
56



serine
57

Other Neurotransmitters



acetylcholine
58

Figure 10. Classic neurotransmitters

As the AP propagates along the axon of a neuron, the abrupt changes in the plasma membrane potential signals the release of neurotransmitters from the presynaptic nerve terminal into the synaptic cleft (Figure 11). Once released into the synapse, the neurotransmitters interact with receptors embedded within the plasma membrane of the postsynaptic nerve terminal. This interaction can be excitatory (i.e., causes a depolarization of the membrane potential) or inhibitory (i.e., causes a hyperpolarization of the membrane potential) in nature, but both result in changes of the postsynaptic membrane potential, which affects the AP. Equally important as the release of neurotransmitters is the termination of the neurotransmission by active mechanisms (i.e., reuptake through specific

transporter proteins, enzymatic degradation) or passive mechanisms (diffusion). Failure to terminate neurotransmission results in continual stimulation of the postsynaptic nerve terminal and can lead to neuronal disorders, such as epilepsy and neuropathic pain.[71]

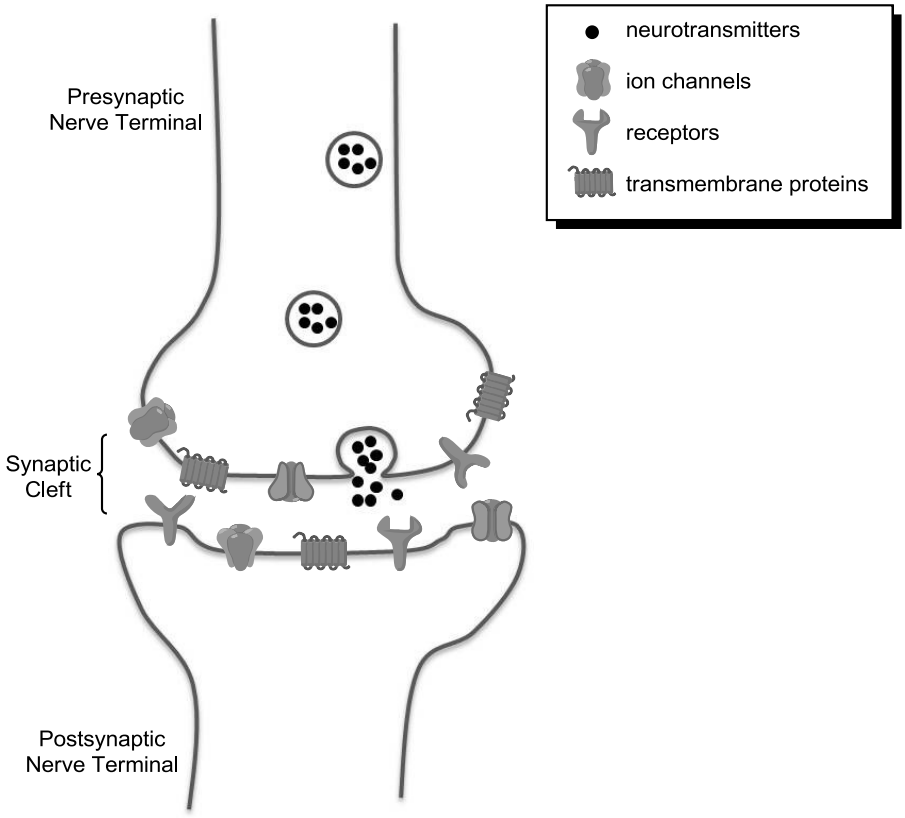


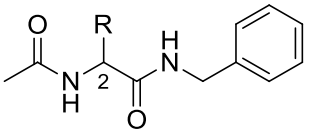
Figure 11. Neurotransmission

3. Primary amino acid derivatives as a treatment for CNS disorders

The extensive work on FAAs (**33**) by the Kohn laboratory,[49-58,60,89-105] and the approval of (*R*)-**28** for the adjuvant treatment of partial-onset seizures by the US FDA and EMEA, has led us to hypothesize that primary amino acid derivatives (PAADs, **59**) may be useful agents for the treatment of CNS disorders due to their close structural similarities with

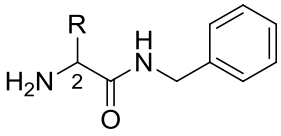
FAAs (**33**). To provide proof-of-concept, a literature search revealed that PAADs **60**–**63** have shown moderate-to-excellent anticonvulsant activity in maximal electroshock seizure (MES) tests (Table 3).[55,56,89,92] With few known literature examples, much of the PAAD chemical space is largely unexplored.[1]

Functionalized Amino Acids



33

Primary Amino Acid Derivatives



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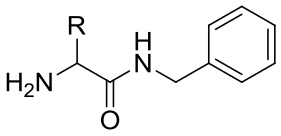
Lacosamide ((*R*)-**28**) has shown significant protection in NP models for inflammatory pain, osteoarthritis pain, and diabetic neuropathy, but it is unclear if the site of action occurs in the CNS, PNS, or both.[66,106-111] With the reported efficacy of FAA (*R*)-**28** in several NP models and the recent emergence of PNS-selective agents,[28-33] we proposed that the amino structural counterpart of FAAs, the PAADs, after adsorption through the gastrointestinal tract (GI) would be less likely to cross the blood-brain barrier (BBB) due to their expected increased water solubility compared with the corresponding FAA (Figure 12). Significantly, at physiological pH, the primary amine (**59**) is protonated to give the corresponding ammonium salt (**64**), thus possibly hindering its ability to cross the anionic surface of the BBB. We recognize from the onset that targeting the PNS was a challenge and selectivity would depend on the hydrophilicity of the PAAD and the possible role of drug transporters. Indeed, the fact that PAADs **60**–**63** showed pronounced seizure protection in the MES test documents that these PAADs can access the CNS. Finally, the presence of the protonated primary amine also allows greater diversity of substitution at the C(2) and *N*-benzylamide

[1] In Chapter 3, we briefly review earlier reports on the pharmacological activity of secondary amino acid derivatives (SAADs) and tertiary amino acid derivatives (TAADs).

sites, while still maintaining a high degree of water solubility. This permits the synthesis and evaluation of hydrophilic PAADs where the FAA counterpart may not have been extensively transported into systemic circulation because of poor water solubility. Thus, PAADs may represent an ideal class of compounds that can be tailored at the C(2) and *N*-benzylamide sites to selectively target PNS sites and reduce neurological toxicities typically associated with CNS active drugs.

Table 3. Previously reported MES activity of PAADs in mice and rats



		Mice (ip)[a]	Rat (po)[b]
Cmpd No.	**R**	**MES,[c] ED_{50}**	**MES,[c] ED_{50}**
(*R*)-**60**[d]	CH_3	>10, <30	19 [2.0] (13-25)
(*R*)-**61**[d,e]	CH_2OCH_3	48 [0.25] (40-61)	18 [4.0]
(*R,S*)-**62**[d]	Ph	>100, <300	>30, <100
(*R,S*)-**63**[f]	$N(H)C(O)CH_3$	65 [0.5] (56-75)	ND[g]
phenytoin[h]		9.5 [2.0] (8.1–10)	30 [4.0] (22-39)
phenobarbital[h]		22 [1.0] (15–23)	9.1 [5.0] (7.6-12)
valproate[h]		270 [0.25] (250–340)	490 [0.5] (350-730)

[a] The compounds were administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} values are in mg/kg. [b] The compounds were administered orally to adult male albino Sprague Dawley rats. ED_{50} values are in mg/kg. A dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets). Numbers in parentheses are 95% confidence intervals. [c] MES = maximal electroshock seizure test. [d] Béguin, C. *et al. Bioorg. Med. Chem*. **2004**, *12*, 3079–3096. [e] Andurkar, S. *et al. Tetrahedron: Asymmetry,* **1998**, *9*, 3841–3854. [f] Kohn, H. *et al. J. Med. Chem*. **1991**, *34*, 2444–2452. [g] ND = not determined. [h] Porter, R.J. *et al. Cleveland Clin. Q.* **1984**, *51*, 293–305.

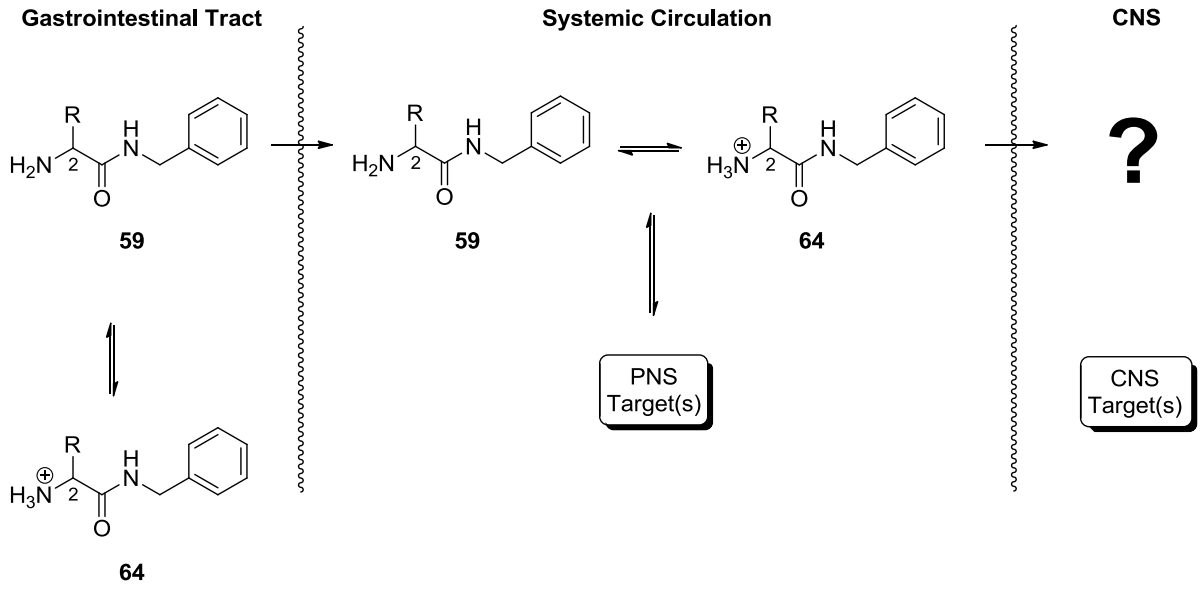


Figure 12. Proposed distribution of PAADs

Although our primary objective was to synthesize and then evaluate PAADs to determine if their site of action was restricted to the PNS, thereby avoiding potential CNS side effects that makes adherence to pain therapy difficult, we also examined the effect of PAADs on CNS function. Specifically, we aimed to (1) use the pharmacological data generated from whole animal models of epilepsy and NP to develop a SAR that defined the C(2)-structural requirements for PAAD activity; (2) optimize PAADs that displayed anticonvulsant activity and/or pain attenuation by functionalization of either the *N*-terminal amine or *N*-benzylamide sites; and (3) subject the most active PAADs to *in vitro* binding and enzymatic assays to explore their mechanism of action. Two[2] whole animal models of epilepsy (MES test and 6 Hz test) were used to identify PAADs that could prevent seizures in mice, and one whole animal model of NP (formalin test) was used to identify PAADs that could attenuate pain in mice. Many PAADs were also evaluated using the MES test in rats.

[2] Subcutaneous pentylenetetrazol (Metrazol®) (scMET) screening was also performed at the NINDS ASP as a method to identify seizure protection, but PAADs were generally inactive in this test (Chapter 2 and Chapter 3).

Neurotoxicity was determined by the rotorod test in mice, while behavioral toxicity was monitored in rats.

We synthesized over 50 C(2)-substituted PAADs that were evaluated in whole animal models of epilepsy and NP (Chapter 2). A subset of PAADs displayed excellent anticonvulsant activity and pain attenuation and surpassed the activity of several established AEDs. Next, we synthesized over 40 PAADs to determine the affect of functionalization of both the *N*-terminal amine and the *N*-benzylamide moiety of PAADs on anticonvulsant activity and pain attenuation (Chapter 3). Anticonvulsant data suggests that several of the optimized PAADs can arguably rival the therapeutic value of lacosamide ((*R*)-**28**). Then, we initiated studies on the MOA of the most active PAADs through a series of binding and enzymatic assays (Chapter 4). Finally, we comment on the therapeutic significance of PAADs for the treatment of epilepsy and NP and suggest further studies that could prove beneficial for the development of PAADs (Chapter 5).

CHAPTER 2. Defining the Structural Requirements for PAADs

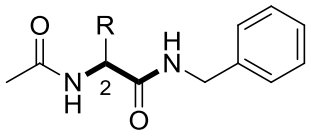
1. Introduction

Over the past 25 years, the Kohn laboratory has synthesized more than 300 FAAs that have been evaluated for anticonvulsant activity.[50,52-58,60,89-93,96-105,112,113] With the clinical success of lacosamide ((*R*)-**28**), we have proposed that PAADs may serve as useful neurological agents for the treatment of epilepsy and NP. Our choice of compounds in this study was driven by the *in vivo* behavioral tests that were conducted by UCB Pharma and the NINDS ASP for anticonvulsant activity, pain attenuation, and neurotoxicity.

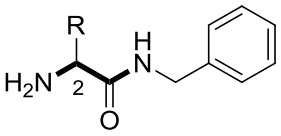
2. Results and discussion

The SAR of the comprehensively studied FAAs (**33**) revealed excellent anticonvulsant activities in the MES test and, accordingly, the FAA structural blueprint was the foundation for the development of PAADs (**59**) due to their similar pharmacophores. Hallmarks of the FAA SAR include: (1) the *N*-acetyl; (2) the diamine backbone; (3) the carbonyl group within the diamine backbone; (4) the *N'*-benzylamide; (5) the heteroatom one atom removed from the chiral C(2) center; (6) the substitution of the heteroatom; and (7) the stereochemistry corresponding to the D-amino acid at the chiral C(2) center.[50,52-58,60] Building the SAR of *primary* amino acid derivatives inherently precludes hallmark one. Hallmarks two, three, and four are retained in the structural blueprint of PAADs, thereby directing the

majority of the initial SAR exploration at hallmarks five, six, and seven. Therefore, our studies began by simultaneously examining the requirement for a heteroatom one carbon removed from the C(2) center, the heteroatom substitution, and the stereochemistry at the C(2) position. After examining the FAA hallmarks in the context of PAADs, we investigated C(2)-hydrocarbon substituents that deviated from the traditional FAA SAR.



FAAs
33



PAADs
59

2.1. Choice of compounds: PAADs

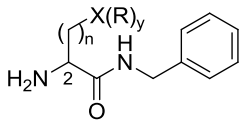
Using lacosamide ((*R*)-**28**) as the prototypical FAA structural template, we systematically modified the C(2) position of the PAADs according to three categories: (1) C(2)-$(CH_2)_n$XR PAADs (**61**, **65**–**76**), where n equals the number of methylene carbons, X is an oxygen, nitrogen, or sulfur, and R is an alkyl group; (2) C(2)-6-membered and 5-membered aromatic and heteroaromatic PAADs (**62**, **77**–**94**); and (3) C(2)-hydrocarbon PAADs (**60**, **95**–**102**).

2.1.1. C(2)-$(CH_2)_n$X$(R)_y$ PAADs

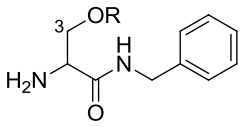
The C(2)-$(CH_2)_n$X$(R)_y$ PAADs simultaneously examined the heteroatom one-to-two carbons removed from the C(2) center, the heteroatom substitution, and in select cases, the stereochemistry at the C(2) position. The $(CH_2)_n$X$(R)_y$ PAADs are further classified according to three sub-categories: (1) C(3)-alkoxy PAADs (**61**, **65**–**69**); (2) C(3)-amino PAADs (**70**–**73**); and (3) C(4)-substituted PAADs (**74**–**76**). In the C(3)-alkoxy series, n equals one, X is oxygen, and R (y = 1) was successively replaced with a methyl, ethyl,

propyl, allyl, and propargyl group. All of the C(3)-alkoxy PAADs were synthesized in the (*R,S*)-configuration with the exception of the hydroxy PAAD (**65**) and the methoxy PAAD (**61**), which were synthesized in the (*R*)-, (*S*)-, and (*R,S*)-configurations. In the C(3)-amino series, n equals one, X is nitrogen, and R was successively replaced with either one methyl group (y = 1) or two methyl groups (y = 2), or the C(3)-amino group was embedded within a morpholino moiety. All C(3)-amino PAADs were synthesized in the (*R,S*)-configuration. In the C(4)-substituted series, n equals two, X is oxygen or sulfur, and R (y = 1) is either a hydrogen or methyl group. All C(4)-substituted PAADs were synthesized in the (*R*)-configuration. Inspiration for the C(3)-alkoxy and C(4)-substituted PAADs was directly obtained from the structure of lacosamide ((*R*)-**28**), the prototypical FAA where n equals one, X is oxygen, and R (y = 1) is a methyl group. Like lacosamide ((*R*)-**28**), excellent anticonvulsant activity was also observed for other oxygen-substituted FAA analogs, provided the oxygen was one atom removed from the C(2) center. Our interest in C(3)-amino PAADs was increased by the notion that these compounds might exhibit decreased penetration into the CNS. We expected that under physiological condition that the C(3)-amino groups would be protonated, thus hindering passive diffusion across the blood-brain barrier.

C(2)-$(CH_2)_nX(R)_y$ PAADs

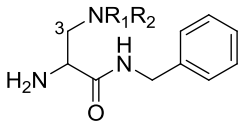


C(3)-Alkoxy PAADs



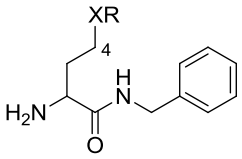
(*R*)-, (*S*)-, (*R,S*)-**65** R = H
(*R*)-, (*S*)-, (*R,S*)-**61** R = CH_3
(*R,S*)-**66** R = CH_2CH_3
(*R,S*)-**67** R = $CH_2CH_2CH_3$
(*R,S*)-**68** R = $CH_2CH{=}CH_2$
(*R,S*)-**69** R = $CH_2C{\equiv}CH$

C(3)-Amino PAADs



(*R,S*)-**70** R_1 = H, R_2 = H
(*R,S*)-**71** R_1 = CH_3, R_2 = H
(*R,S*)-**72** R_1 = CH_3, R_2 = CH_3
(*R,S*)-**73** R_1R_2 = $(CH_2CH_2)_2O$ (morpholino)

C(4)-Substituted PAADs

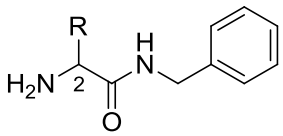


(*R*)-**74** X = O, R = H
(*R*)-**75** X = O, R = CH_3
(*R*)-**76** X = S, R = CH_3

2.1.2. 6-Membered and 5-membered aromatic and heteroaromatic PAADs

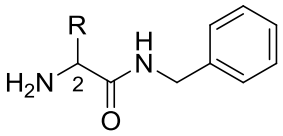
Excellent anticonvulsant activity in the MES test has been observed for 6-membered and 5-membered aromatic and heteroaromatic compounds in the FAA series, and activity typically increased when a heteroatom was located one atom removed from the C(2) chiral carbon (e.g., 2-furanyl, 2-pyrrolyl, 1-pyrazolyl, 2-oxazolyl, 2-thiazolyl, 2-pyridyl, 2-pyrimidyl, 2-pyrazinyl).[53-57,60] Utilizing the knowledge of the heteroaromatic FAAs, we synthesized 6-membered aromatic and heteroaromatic PAADs **62**, **77**–**88** and 5-membered heteroaromatic PAADs **89**–**94**. Except where R equals phenyl (**62**), all 6-membered and 5-membered aromatic and heteroaromatic PAADs (**77**–**94**) were synthesized by Drs. Pranjal Baruah, Jason Dinsmore, and Christophe Salomé. PAAD **62** served as a reference compound and was synthesized in the (*R*)-, (*S*)-, and (*R,S*)- configurations, while compounds **77**–**94** were synthesized as the (*R,S*)-configuration only.

C(2)-6-Membered Aromatic and Heteroaromatic PAADs



(*R*)-, (*S*)-, (*R,S*)-**62** R = phenyl
(*R,S*)-**77** R = 2-pyridyl
(*R,S*)-**78** R = 3-pyridyl
(*R,S*)-**79** R = 4-pyridyl
(*R,S*)-**80** R = 2-pyrazinyl
(*R,S*)-**81** R = 1-naphthyl
(*R,S*)-**82** R = 1-isoquinolinyl
(*R,S*)-**83** R = 2-naphthyl
(*R,S*)-**84** R = 2-quinolinyl
(*R,S*)-**85** R = 2-quinoxalinyl
(*R,S*)-**86** R = 4-flurorphenyl
(*R,S*)-**87** R = 4-methylphenyl
(*R,S*)-**88** R = 4-methoxyphenyl

C(2)-5-Membered Heteroaromatic PAADs



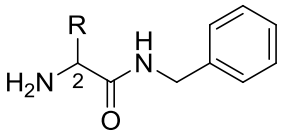
(*R,S*)-**89** R = 2-furanyl
(*R,S*)-**90** R = 5-methyl-2-furanyl
(*R,S*)-**91** R = 2-benzofuranyl
(*R,S*)-**92** R = thiophen-2-yl
(*R,S*)-**93** R = 2-thiazolyl
(*R,S*)-**94** R = benzo[*b*]thiophen-2-yl

2.1.3. C(2)-Hydrocarbon PAADs

Initially, we synthesized the hydrocarbon equivalent of PAAD **61** (O→CH_2, **96**) in the (*R*)-, (*S*)-, and (*R,S*)-configurations to further examine the requirement for a heteroatom one atom removed from the C(2) center in conjunction with the need for stereochemical specificity at the C(2) position. Modest anticonvulsant activity and NP protection was observed for (*R*)- and (*R,S*)-**96** (reviewed in Section 2.3.3), which prompted the synthesis of an expanded series of C(2)-hydrocarbon PAADs (**60**, **95**, **97**–**102**). Therefore, we prepared C(2)-hydrocarbon PAADs where R is a linear alkyl group (methyl, ethyl, and *n*-butyl), a branched alkyl group (isopropyl, *t*-butyl, and 2-methyl-propyl), or a cyclic group (cyclohexyl and benzyl). PAADs **97** and **101** were synthesized by Drs. Jason Dinsmore and Christophe Salomé, respectively. Since the C(2)-$(CH_2)_nXR$ PAADs revealed a preference for stereochemistry in the D-amino acid configuration, all of the hydrocarbon PAADs were synthesized in the (*R*)-configuration, except PAAD **100**, which contained two chiral centers and was a mixture of stereoisomers. The excellent anticonvulsant activity of PAADs (*R*)-**98**

and (*R*)-**99** prompted the synthesis of the corresponding (*S*)-stereoisomers to determine if pharmacological activity paralleled the C(2)-$(CH_2)_nX(R)_y$ PAADs, where activity preferentially resided in the C(2)-D-amino acid configuration.

C(2)-Hydrocarbon PAADs



(*R*)-**60** R = CH_3
(*R*)-**95** R = CH_2CH_3
(*R*)-, (*S*)-, (*R,S*)-**96** R = $(CH_2)_2CH_3$
(*R*)-**97** R = $(CH_2)_3CH_3$
(*R*)-, (*S*)-**98** R = $CH(CH_3)_2$
(*R*)-, (*S*)-**99** R = $C(CH_3)_3$
(*R,S*)-**100** R = $CH(CH_3)CH_2CH_3$
(*R*)-**101** R = C_6H_{11}
(*R*)-**102** R = $CH_2C_6H_5$

2.2. Synthesis

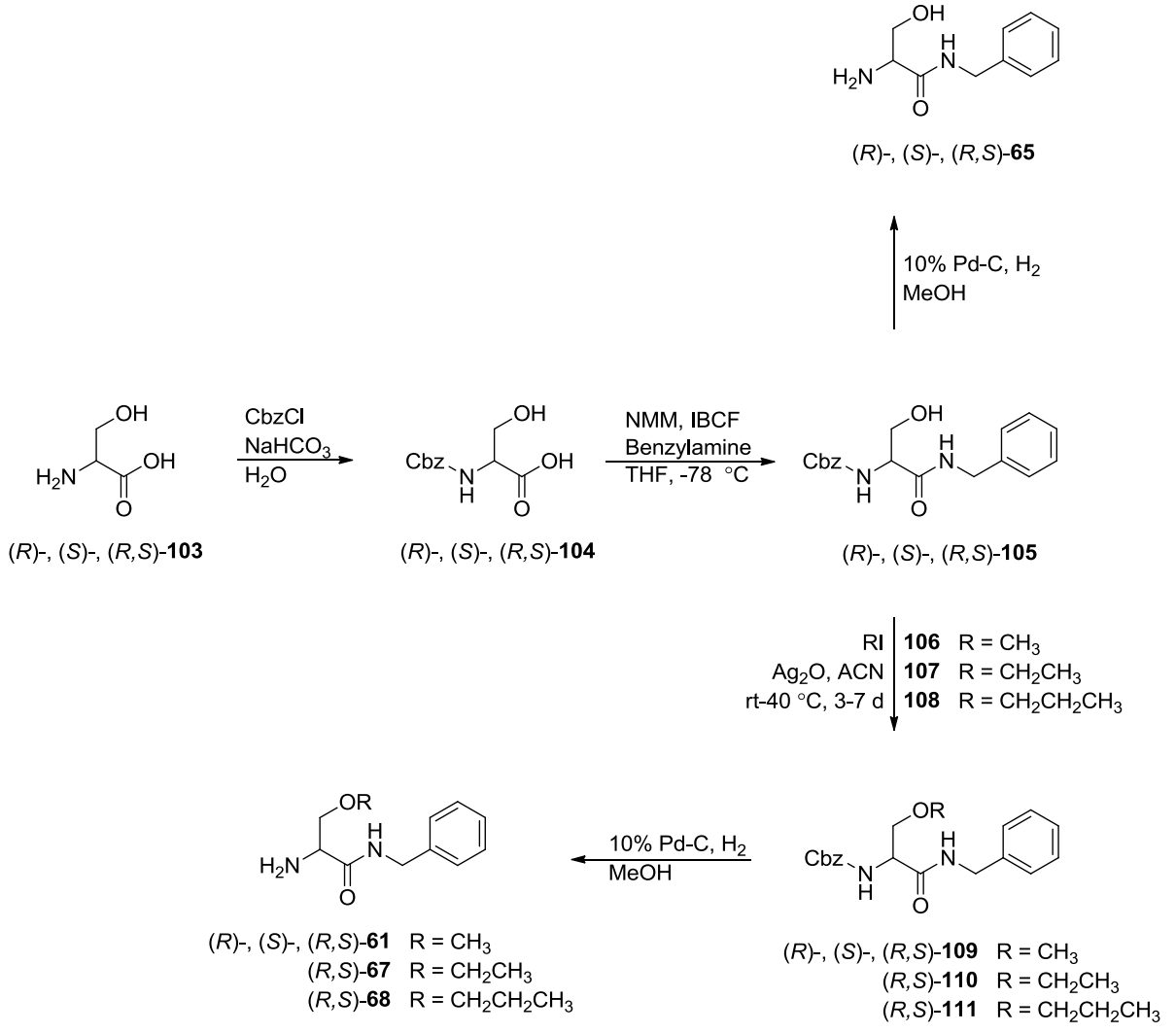
2.2.1. C(2)-$(CH_2)_nX(R)_y$ PAADs

2.2.1.1. C(3)-Hydroxy and C(3)-alkoxy PAADs

PAADs **61**, **65**–**69** were synthesized in three to four steps using commercially available reagents and established synthetic procedures (Scheme 1). Treatment of (*R*)-, (*S*)-, and (*R,S*)-serine (**103**) with benzyl chloroformate under basic conditions gave (*R*)-, (*S*)-, and (*R,S*)-**104**,[89,98] which was then converted to the amides (*R*)-, (*S*)-, and (*R,S*)-**105** using the mixed anhydride coupling (MAC) procedure,[89,98] followed by subsequent hydrogenolysis to obtain the corresponding PAADs (*R*)-, (*S*)-, and (*R,S*)-**65**.[89,98] Alkylation of (*R*)-, (*S*)-, and (*R,S*)-**105** using methyl iodide (**106**) and Ag_2O gave (*R*)-, (*S*)-, and (*R,S*)-**109** before hydrogenolysis to the corresponding PAADs (*R*)-, (*S*)-, and (*R,S*)-**61**.[92] Similarly, treatment of (*R,S*)-**105** with either ethyl iodide (**107**) or propyl iodide (**108**) and Ag_2O gave (*R,S*)-**110**

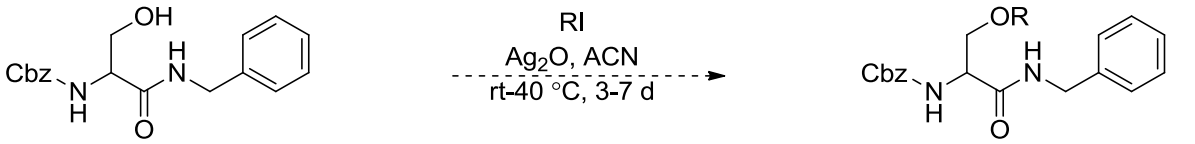
and (*R,S*)-**111**, respectively, followed by hydrogenolysis to their respective PAADs (*R,S*)-**67** and (*R,S*)-**68**. We observed an increase in reaction time and temperature, as well as a decrease in yield as the alkyl iodide went from methyl to ethyl to propyl. However, this was not unexpected as work on the corresponding FAAs by Choi *et al.* resulted in similar trends.[60]

Scheme 1. Synthesis of C(3)-hydroxy PAAD 65 and C(3)-alkoxy PAADs 61, 67, and 68



We attempted to synthesize (*R,S*)-**114** and (*R,S*)-**115** using the alkylation conditions from Scheme 1, but no reaction was observed (Scheme 2).

Scheme 2. Attempted alkylation of (*R,S*)-105 using allyl iodide (112) and propargyl iodide (113)



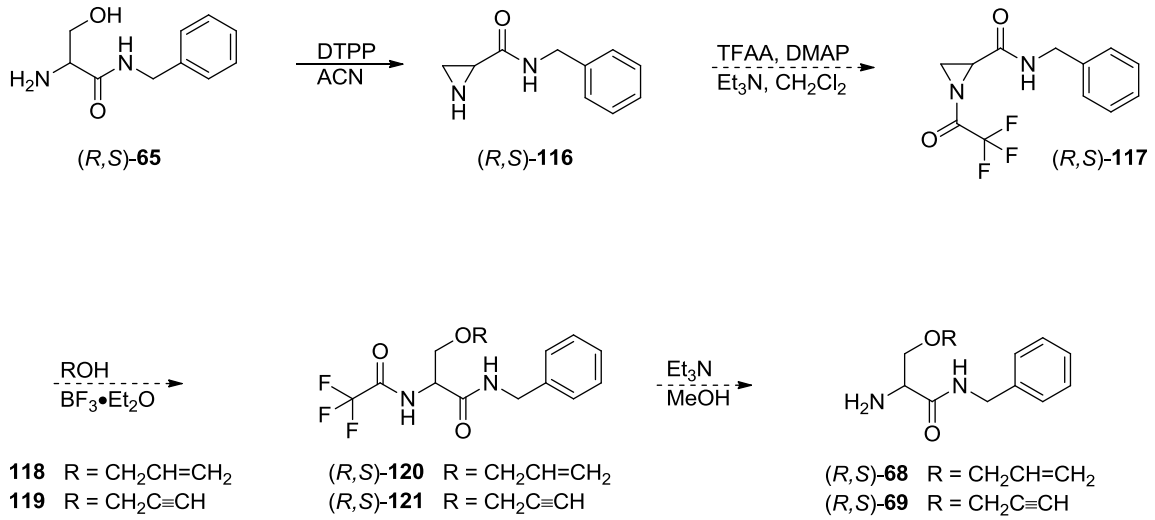
(*R,S*)-**105**

112 R = $CH_2CH{=}CH_2$
113 R = $CH_2C{\equiv}CH$

(*R,S*)-**114** R = $CH_2CH{=}CH_2$
(*R,S*)-**115** R = $CH_2C{\equiv}CH$

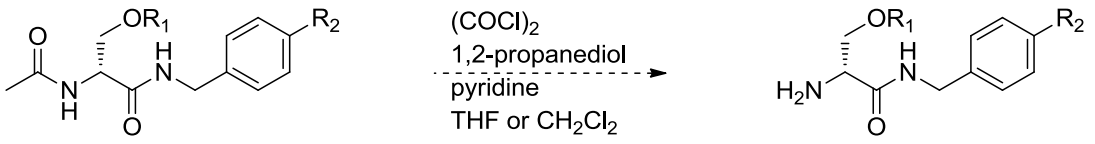
Therefore, we attempted to synthesize PAADs (*R,S*)-**68** and (*R,S*)-**69** via an aziridine intermediate (Scheme 3). Aziridines are important intermediates in the derivatization of amino acids due to their highly strained three-membered rings that are readily opened by a nucleophile under Lewis-acid catalyzed conditions.[114-118] A highly desirable trait of aziridine chemistry is the retention of stereochemistry of the aziridine precursor in the ring-opened product. The Kohn group utilizes two methods for aziridine formation: a one-step cyclodehydration using 2-amino alcohols and diethoxytriphenylphosphorane (DTPP),[100,119,120] and a three-step *N*-trityl protection of methyl ester followed by cyclodehydration.[94-97] We successfully converted (*R,S*)-**65** to the corresponding aziridine (*R,S*)-**116** using DTPP on gram scale, but we were unable to *N*-protect the aziridine using trifluoroacetic anhydride. In each attempt, the ^{1}H NMR displayed signs of ring opening and the trifluoroacetyl-protection route was abandoned.

Scheme 3. Attempted synthesis of PAADs (*R,S*)-68 and (*R,S*)-69



An alternative route to synthesize PAADs (*R,S*)-**68** and (*R,S*)-**69** was based on a study by Koenig and coworkers (Scheme 4).[121] In this report, the researchers illustrate how imidoyl chlorides, generated from secondary acetamides and oxalyl chloride in the presence of base, can be selectively deprotected to give the primary amine, without racemization of the chiral center. Encouraged by the scope of the results, we attempted three model reactions to convert FAAs (*R*)-**122**, (*R*)-**123**, and (*R*)-**124**, generously provided by Drs. Ki Duk Park and Christophe Salomé, to PAADs (*R*)-**125**, (*R*)-**126**, and (*R*)-**127**, respectively. Ideally, the secondary acetyl is converted to the imidoyl chloride in the presence of base and releases a mixture of carbon monoxide and carbon dioxide. The reaction is quenched at 0 ºC with 1,2-propanediol, converting the imidoyl chloride to an imidoyl ester. Finally, warming the reaction to room temperature releases the carboxylic ester and amine salt. However, we were unable to generate PAADs using this method.

Scheme 4. Attempted synthesis of C(3)-alkoxy PAADs by acetyl deprotection



(*R*)-**122** $R_1 = CH_3$ $R_2 = CH_3$
(*R*)-**123** $R_1 = CH_3$ $R_2 = OCF_3$
(*R*)-**124** $R_1 = CH_2C{\equiv}CH$ $R_2 = CH_3$

(*R*)-**125** $R_1 = CH_3$ $R_2 = CH_3$
(*R*)-**126** $R_1 = CH_3$ $R_2 = OCF_3$
(*R*)-**127** $R_1 = CH_2C{\equiv}CH$ $R_2 = CH_3$

Reverting back to the aziridine chemistry, we decided to modify our approach using a different protecting group based on chemistry that was familiar to the Kohn laboratory (Scheme 5).[101] We successfully executed a three-step procedure beginning with the *N*-tritylation protection of commercially available (*R,S*)-**128**, followed by cyclodehydration to give (*R,S*)-**130**.[122-127] Optimization of the remaining steps to obtain PAADs (*R,S*)-**68** and (*R,S*)-**69** required consideration of (1) the sensitivity of the *N*-protecting group to $BF_3{\bullet}Et_2O$; and (2) the sensitivity of the unsaturated alkenyl or alkynyl group to the final deprotection conditions.

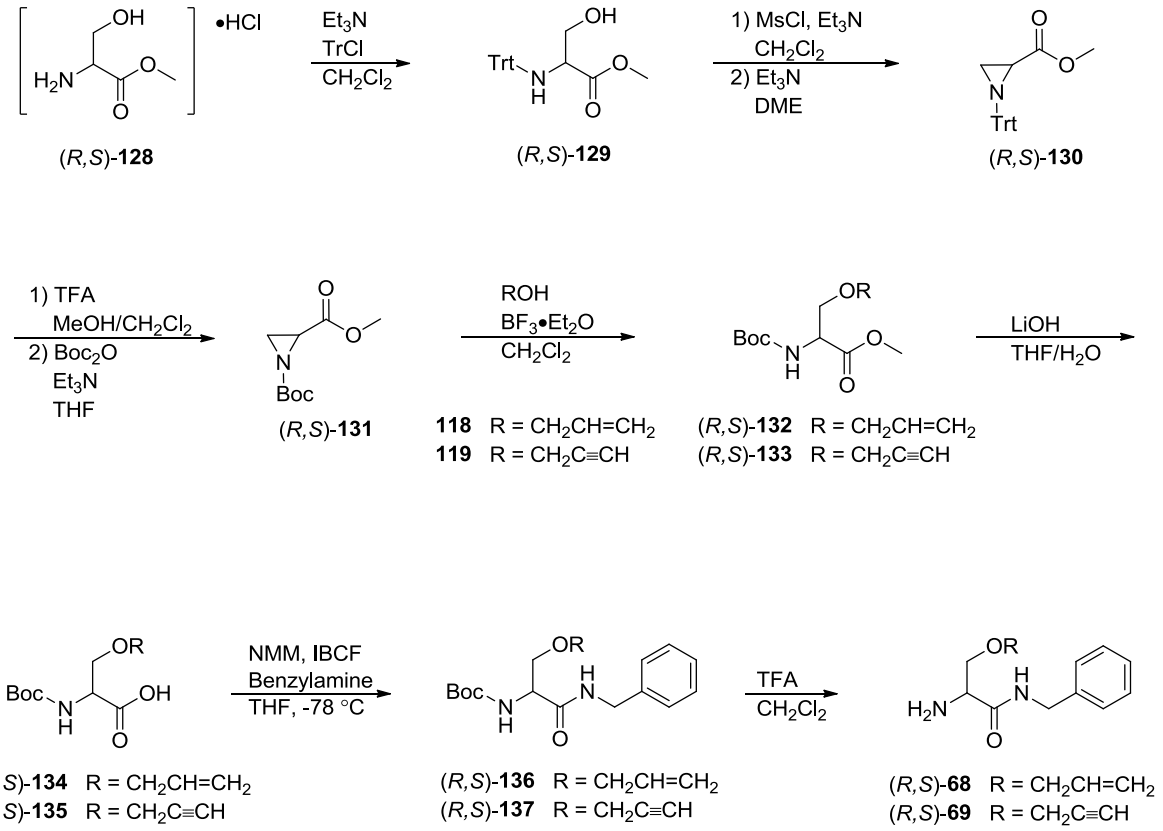
Generally, aziridines exhibit only modest reactivity toward ring opening by oxygen nucleophiles.[128] Therefore, we considered replacing the bulky electron-donating trityl group with an electron-withdrawing group that could coordinate with a Lewis acid to enhance ring opening. Protecting groups considered were *tert*-butyloxycarbonyl (*t*Boc), 2,2,2-trichloro-*tert*-butyloxycarbonyl (TcBoc), 2,2,2-trichloroethoxycarbonyl (Troc), and an azidomethyl carbonyl (Azoc). *t*Boc is a classic protecting group that undergoes cleavage in acidic media and has been widely used in aziridine chemistry.[125,129-134] TcBoc and Troc are stable against acids and bases, making them orthogonal to classic protecting groups (e.g., Cbz, *t*Boc, Fmoc, and Alloc) and are removed by freshly activated zinc dust in acetic acid at room temperature.[135-141] Cases of TcBoc removal in the presence of an allyl functionality have been reported,[138,139] however we found literature evidence that zinc could possibly reduce the

terminal alkynyl group.[142-144] Azoc is a more recent alternative that is stable against acids and bases, providing control over neighboring functional groups, and undergoes fast deprotection (<2 min) using phosphines.[145,146]

Immediate accessibility and familiarity with Boc_2O led us to choose *t*Boc as the protecting group. In a model reaction, propargyl ether was exposed to the acidic *t*Boc deprotection conditions (TFA/CH_2Cl_2) and did not show signs of decomposition after several hours (TLC and ^1H NMR analysis). Further support for pursuing the *t*Boc aziridine came from the reported preparation of (*S*)-**131** by Wei and coworkers.[133] Hence, we felt confident that PAADs (*R,S*)-**68** and (*R,S*)-**69** could be synthesized under our optimized conditions using *t*Boc as the protecting group.

Deprotection of (*R,S*)-**130** under acidic conditions was followed by reprotection with Boc_2O under basic conditions to give aziridine (*R,S*)-**131**.[129-131] Ring-opening with allyl alcohol (**118**) or propargyl alcohol (**119**) in the presence of BF_3•Et_2O gave (*R,S*)-**132** and (*R,S*)-**133**, respectively, which were hydrolyzed to give (*R,S*)-**134** and (*R,S*)-**135**, respectively, and then directly used for the MAC coupling to give amides (*R,S*)-**136** and (*R,S*)-**137**, respectively. Finally, amides (*R,S*)-**136** and (*R,S*)-**137** were deprotected under acidic conditions (TFA) to the corresponding PAADs (*R,S*)-**68** and (*R,S*)-**69**, respectively.

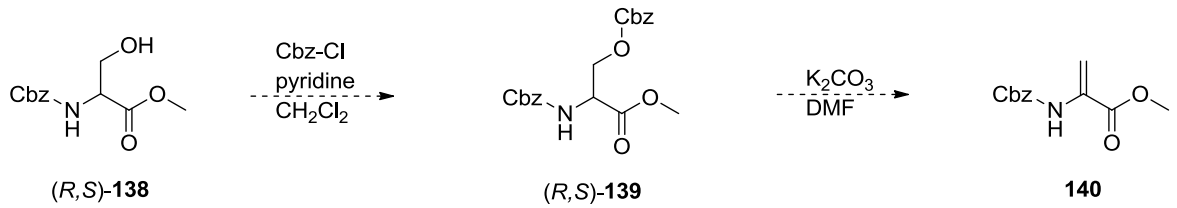
Scheme 5. Synthesis of unsaturated C(3)-alkoxy PAADs (*R,S*)-68 and (*R,S*)-69



2.2.1.2. C(3)-Amino PAADs

Previously, the Kohn laboratory has reported the expedient synthesis of racemic 2,3-diaminopropionic acid derivatives by the addition of amines to dehydroalanine derivatives.[147] Similarly, we attempted a two-step, one-pot reaction to form the dehydroalanine, followed by the Michael addition of various amines to obtain the desired C(3)-amino intermediates. First, we attempted to *O*-Cbz protect commercially available (*R,S*)-**138** using benzyl chloroformate in the presence of base at low temperature to give the carbonate (*R,S*)-**139**, but reaction completion and purification from the starting material posed some difficulties (Scheme 6).[148]

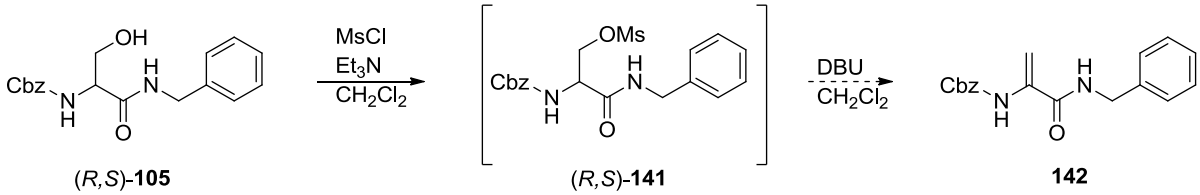
Scheme 6. Attempted synthesis of Cbz-protected dehydroalanine methyl ester intermediate 140



(*R,S*)-**138** (*R,S*)-**139** **140**

Next, alcohol (*R,S*)-**105** was converted to a mesylate that was expected to quickly undergo elimination upon the addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (Scheme 7). We were unable to isolate (*R,S*)-**142** and instead isolated a mixture of products with unique ^{1}H NMR spectra (Scheme 8).

Scheme 7. Attempted synthesis of Cbz-protected dehydroalanine *N*-benzylamide intermediate 142

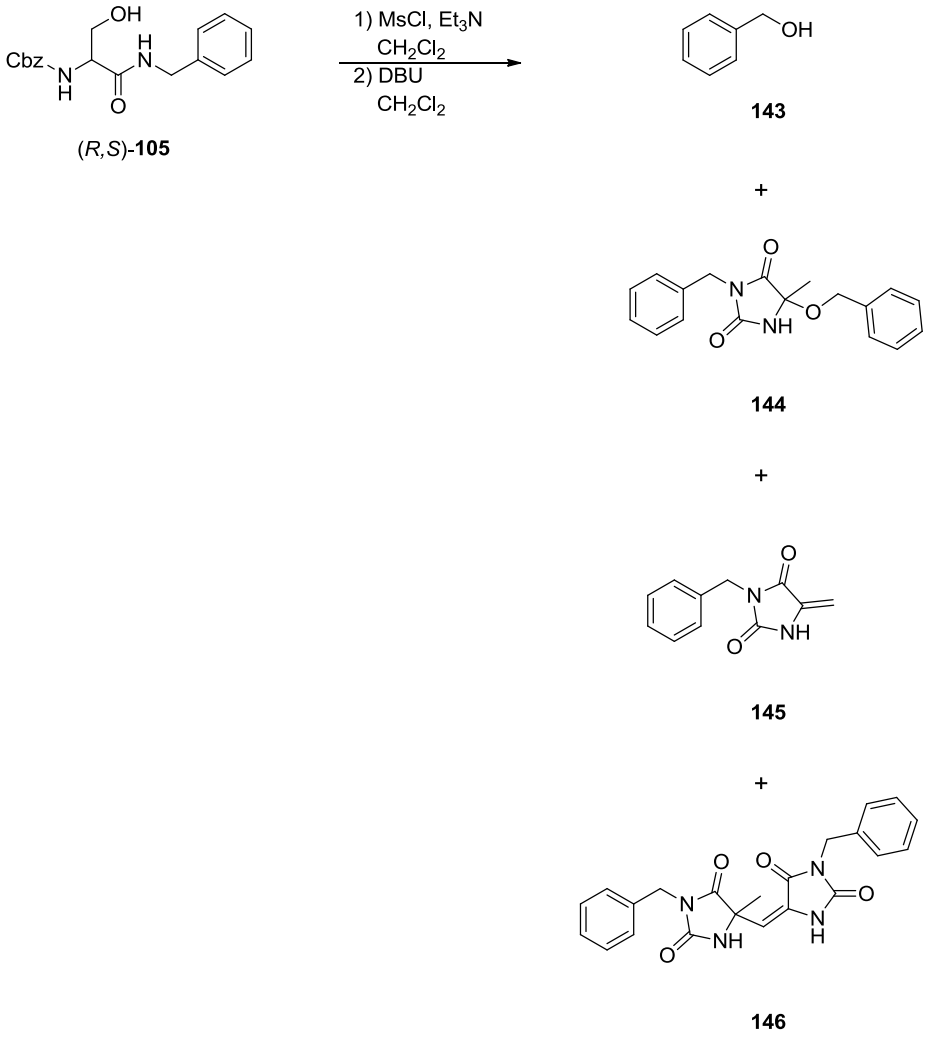


(*R,S*)-**105** (*R,S*)-**141** **142**

A total of four compounds (**143**–**146**) were isolated and characterized from the reaction conditions indicated in Scheme 8. In order of elution from a flash column using an increasing gradient of EtOAc in hexanes, benzyl alcohol (**143**) was isolated in the highest yield (37%), followed by hydantoins **144**–**146** (3%, 27%, and 11%, respectively). The first indication of an unexpected reaction was the absence of the α-proton in all four of the ^{1}H NMR spectra. The second indication was the dramatic change in the coupling constant for the *N*-benzyl methylene protons. Typically, we observe a doublet of doublets for these

protons with a coupling constant of ~6 Hz, but under the current reaction conditions we generated compounds that showed a doublet of doublets with a coupling constant of ~11 Hz. Unable to unequivocally identify the compounds by NMR techniques, we submitted compound **144** for crystallographic x-ray analysis, which was identified as the functionalized hydantoin **144**. From there, we were able to suggest a structure for the remaining two compounds **145** and **146**, and proposed a mechanism to account for the formation of each compound (Scheme 9).

Scheme 8. Isolated products from the attempted synthesis of 142



(*R,S*)-**105**

143

+

144

+

145

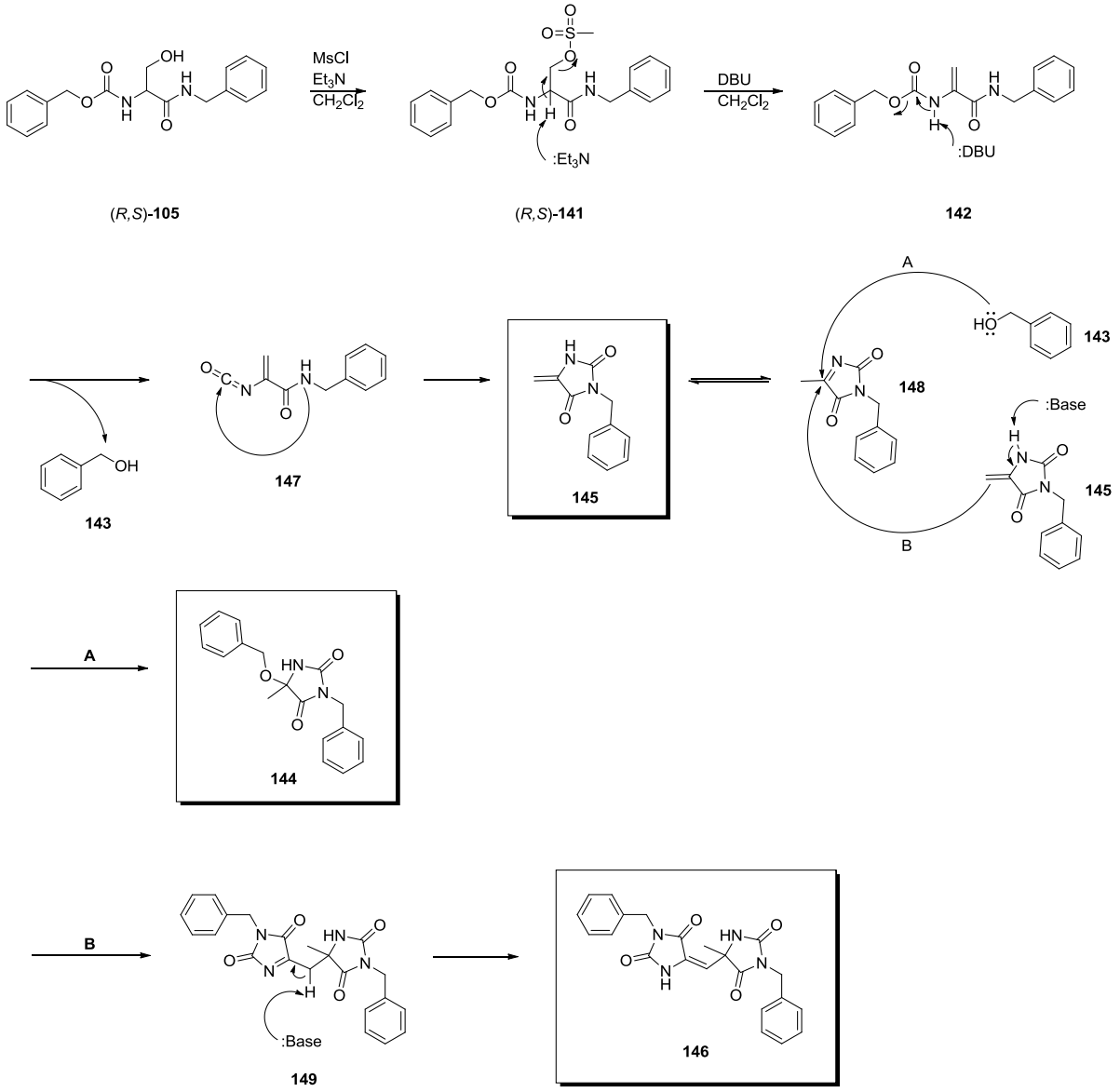
+

146

Based on literature precedent,[149] we expect that formation of mesylate (*R,S*)-**141** from (*R,S*)-**105** allowed the subsequent DBU-mediated elimination to give dehydroalanine **142** (Scheme 9). Deprotonation of **142** generated isocyanate **147** and benzyl alcohol (**143**). Cyclization of **147** by a 5-exo-dig ring closure process gave hydantoin **145**. Subsequent tautomerization of **145** to **148** permitted the Michael addition of **143** (route A) or **145** (route B) to give **144** or **149**, respectively, and **149**'s tautomer **146**.

A more in depth search of the literature revealed the same mesylation-elimination-cyclization sequence was reported by Cernak and coworkers.[149] These researchers showed that the desired *N*-protected dehydroalanine intermediate was isolated with DBU when the protecting group was *t*Boc but when the protecting group was Cbz (**105**), elimination was accompanied by cyclization to the hydantoin **145**. They prevented unwanted cyclization by transforming mesylate **141** to the corresponding iodide followed by elimination under milder conditions (Et_3N in acetone).

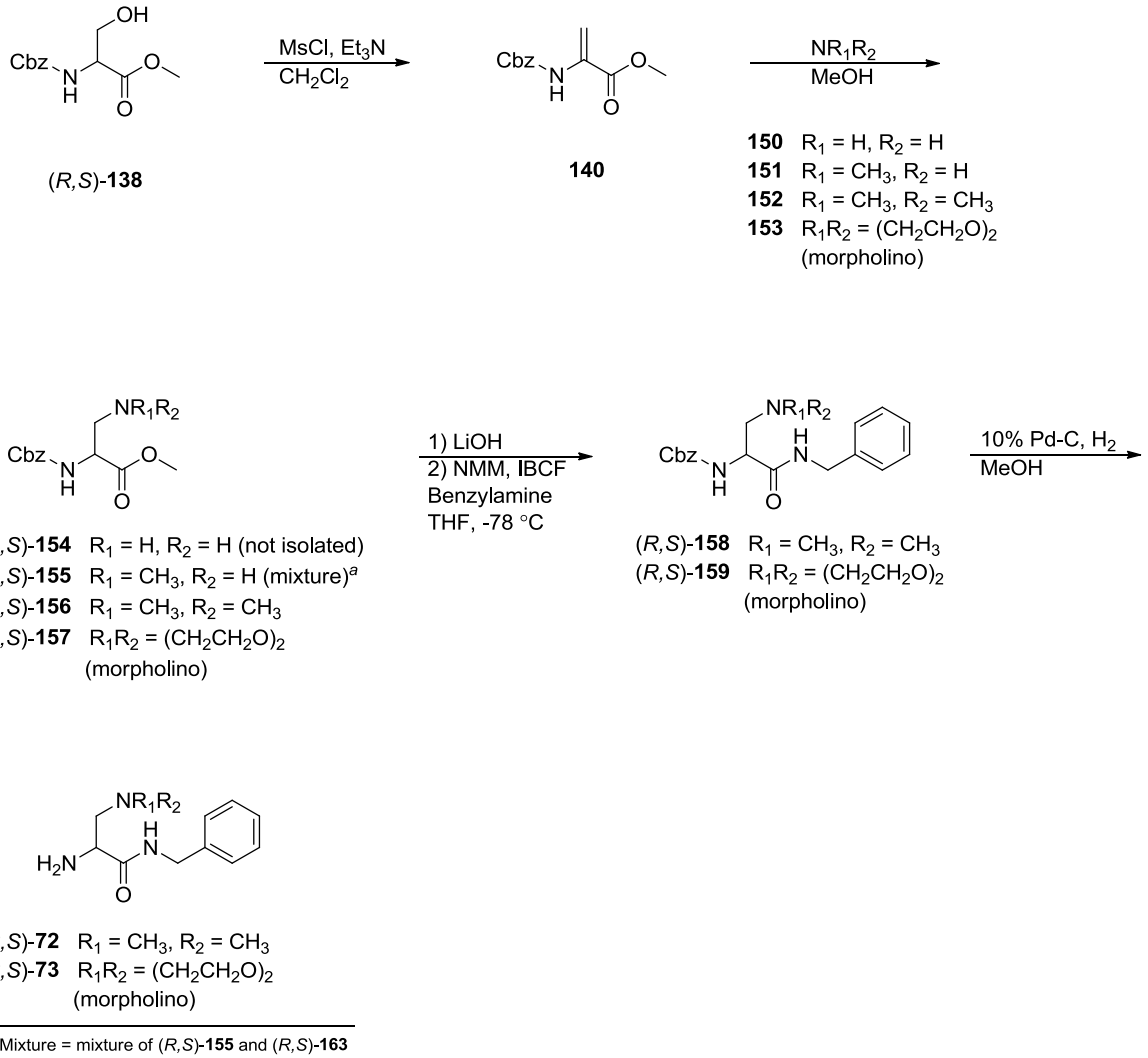
Scheme 9. Proposed mechanism for the formation of hydantoin products 144–146



We continued in our effort to develop a general synthetic method that could utilize a two-step, one-pot reaction that entailed Michael addition of amines to a suitable dehydroalanine intermediate to obtain the desired C(3)-amino intermediates. We successfully generated dehydroalanine **140**[149] via the mesylation and elimination of (*R,S*)-**138** using Et_3N (Scheme 10). Michael addition of dimethylamine (**152**) or morpholine (**153**) to **140** gave (*R,S*)-**156** and (*R,S*)-**157**, respectively, which were subsequently hydrolyzed

and directly used for the MAC coupling to give the corresponding amides (*R,S*)-**158** and (*R,S*)-**159**, respectively. Deprotection of amides (*R,S*)-**158** and (*R,S*)-**159** by hydrogenolysis gave the corresponding PAADs (*R,S*)-**72** and (*R,S*)-**73**, respectively. Use of ammonia (**150**) and methylamine (**151**) in this protocol were unsuccessful and in the case of methylamine, we observed a competing side reaction.

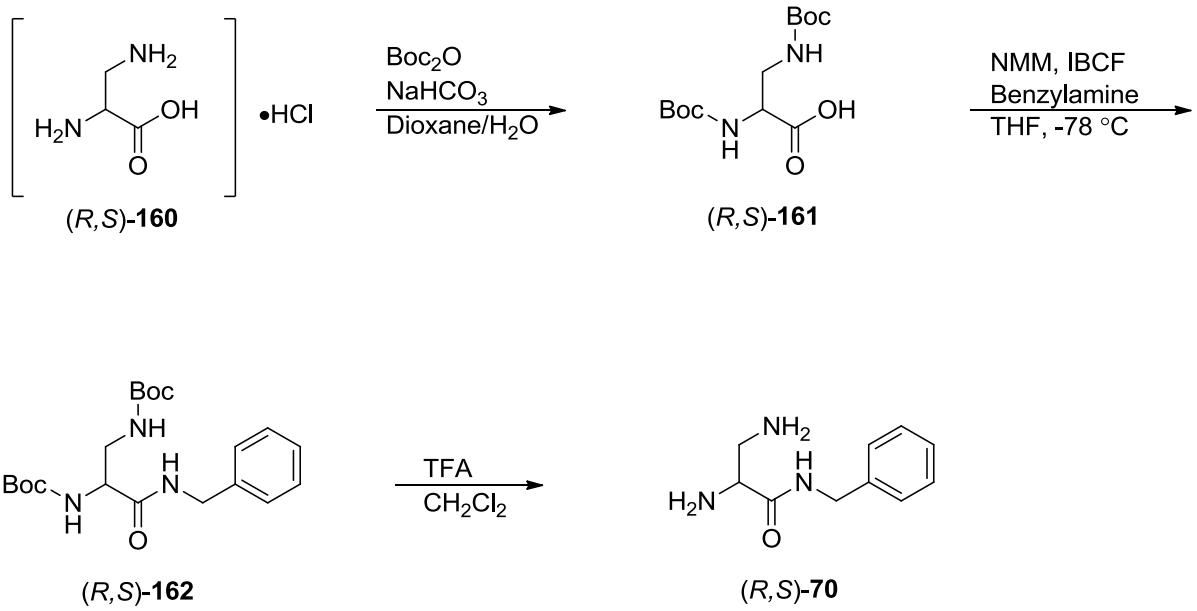
Scheme 10. General pathway for the synthesis of C(3)-amino PAADs utilizing Cbz-protected dehydroalanine methyl ester 140



[a] Mixture = mixture of (*R,S*)-**155** and (*R,S*)-**163**

Therefore, we modified our approach and treated commercially available diamine (*R,S*)-**160** with Boc_2O under basic conditions to give (*R,S*)-**161**.[150-153] (*R,S*)-**161** was coupled with benzylamine using the MAC method to give amide (*R,S*)-**162**, followed by acid deprotection to give PAAD (*R,S*)-**70** (Scheme 11).

Scheme 11. Synthesis of (*R,S*)-*N*-benzyl 2,3-diaminopropionamide (*R,S*)-70

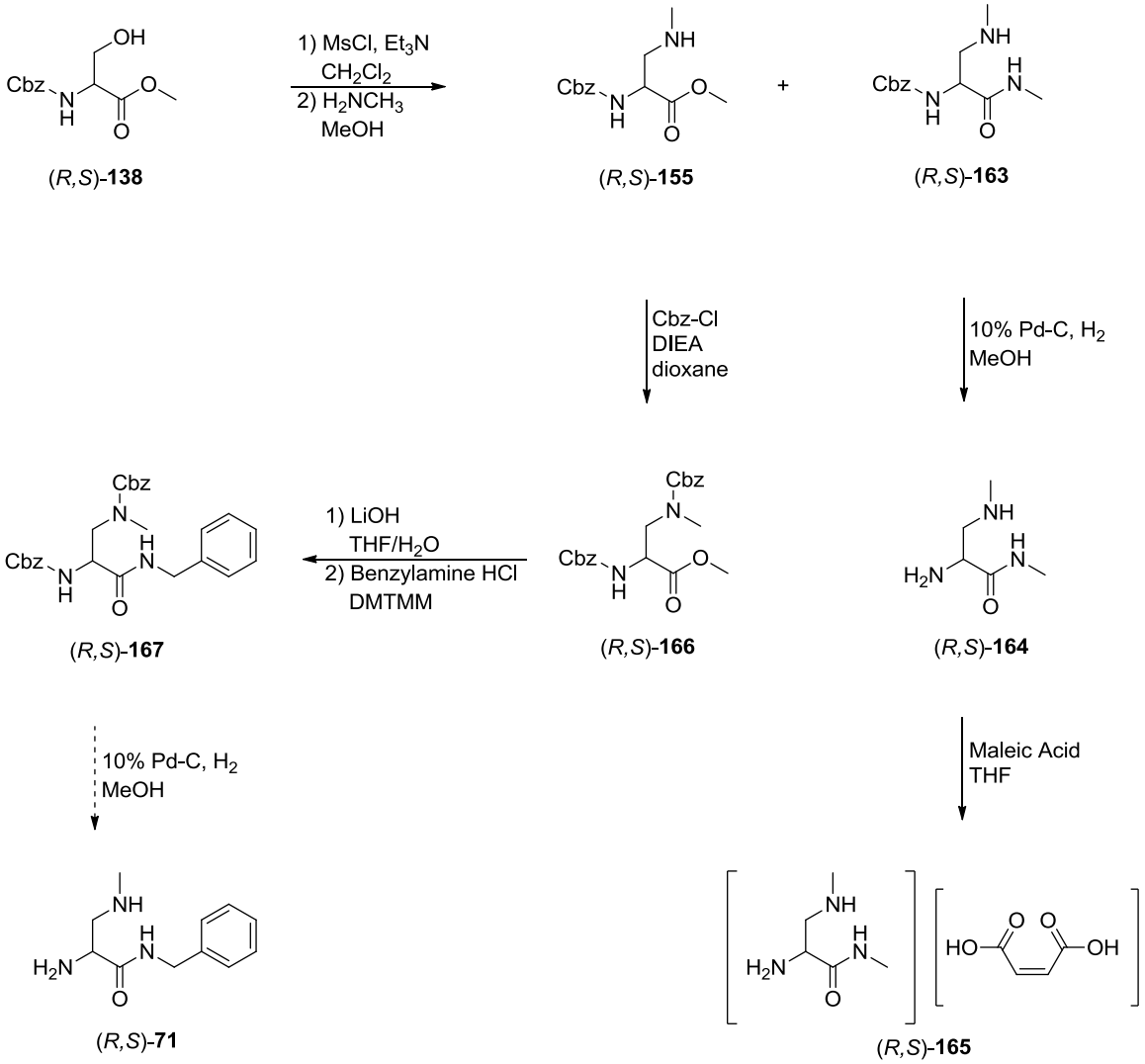


(*R,S*)-**160** (*R,S*)-**161** (*R,S*)-**162** (*R,S*)-**70**

Attempted Michael addition of methylamine (**151**) to **140** (generated from (*R,S*)-**138**) gave a mixture of products: the desired methyl ester (*R,S*)-**155** as the minor product and the *N*-methyl amide (*R,S*)-**163** as the major product (Scheme 12). We scaled up the reaction in an attempt to accumulate enough (*R,S*)-**155** to continue with the remaining synthetic procedure, but the percentage of (*R,S*)-**163** generated increased as we scaled up. However, we viewed the competing side reaction as a possible opportunity to evaluate the importance of the *N*-benzylamide unit to the PAAD pharmacological activity. Therefore, hydrogenolysis of (*R,S*)-**163** gave PAAD (*R,S*)-**164**, but stability and purification issues prompted us to convert the free amine ((*R,S*)-**164**) to the maleic salt ((*R,S*)-**165**). An attempt was made to

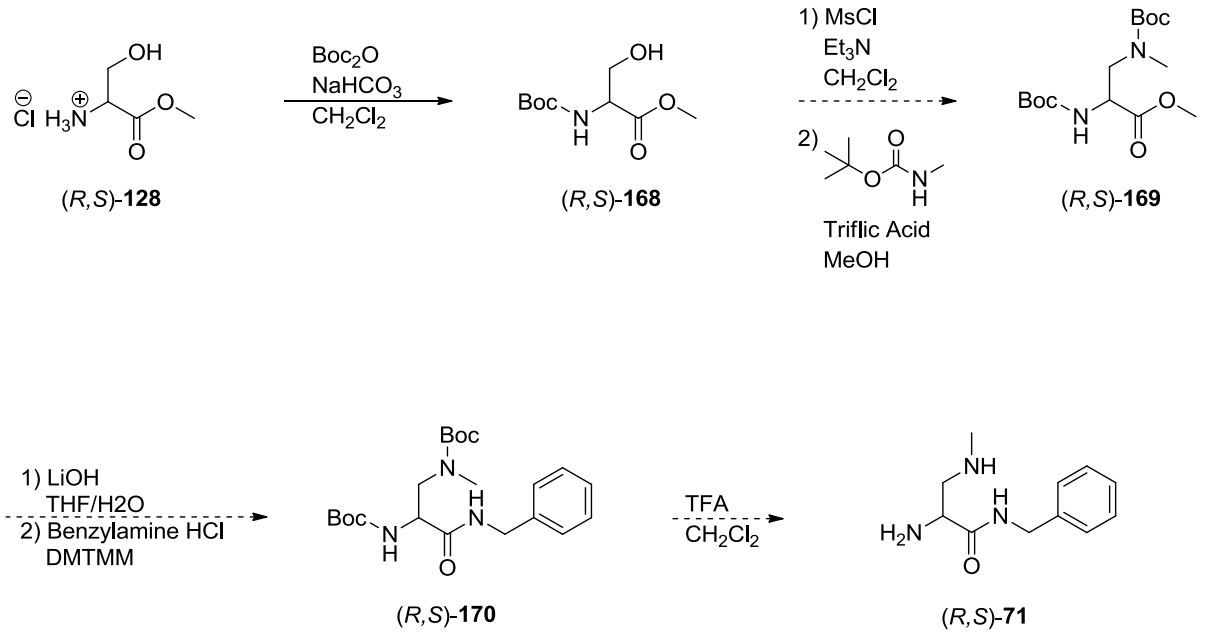
complete the proposed synthesis of PAAD (*R,S*)-**71** using the limited amount of (*R,S*)-**155**. We successfully generated the double *N*-Cbz-protected methyl ester (*R,S*)-**166** from (*R,S*)-**155** using benzyl chloroformate under basic conditions,[154] followed by hydrolysis and MAC coupling with benzylamine to give the amide (*R,S*)-**167**. However, at this point we did not have enough material to continue with the deprotection.

Scheme 12. Attempted synthesis of (*R,S*)-*N*-benzyl 3-*N'*-(methyl)aminopropionamide (*R,S*)-71 (Method A)



In Scheme 13, we outline a similar Michael addition route that used *t*Boc as the amine protecting group and 1,1-dimethylethyl methyl carbamate as the nucleophile.[155,156] Accordingly, (*R,S*)-**168** was readily synthesized from commercially available (*R,S*)-**128** using Boc_2O under basic conditions. Subsequent treatment of (*R,S*)-**168** with MsCl and Et_3N followed by 1,1-dimethylethyl methylcarbamate gave a complex mixture (TLC, ^1H NMR analysis). The ^1H NMR spectrum supported the formation of (*R,S*)-**169**, but purification and low yield limited the utility of this method.

Scheme 13. Attempted synthesis of (*R,S*)-*N*-benzyl 3-*N'*-(methyl)aminopropionamide (*R,S*)-71 (Method B)

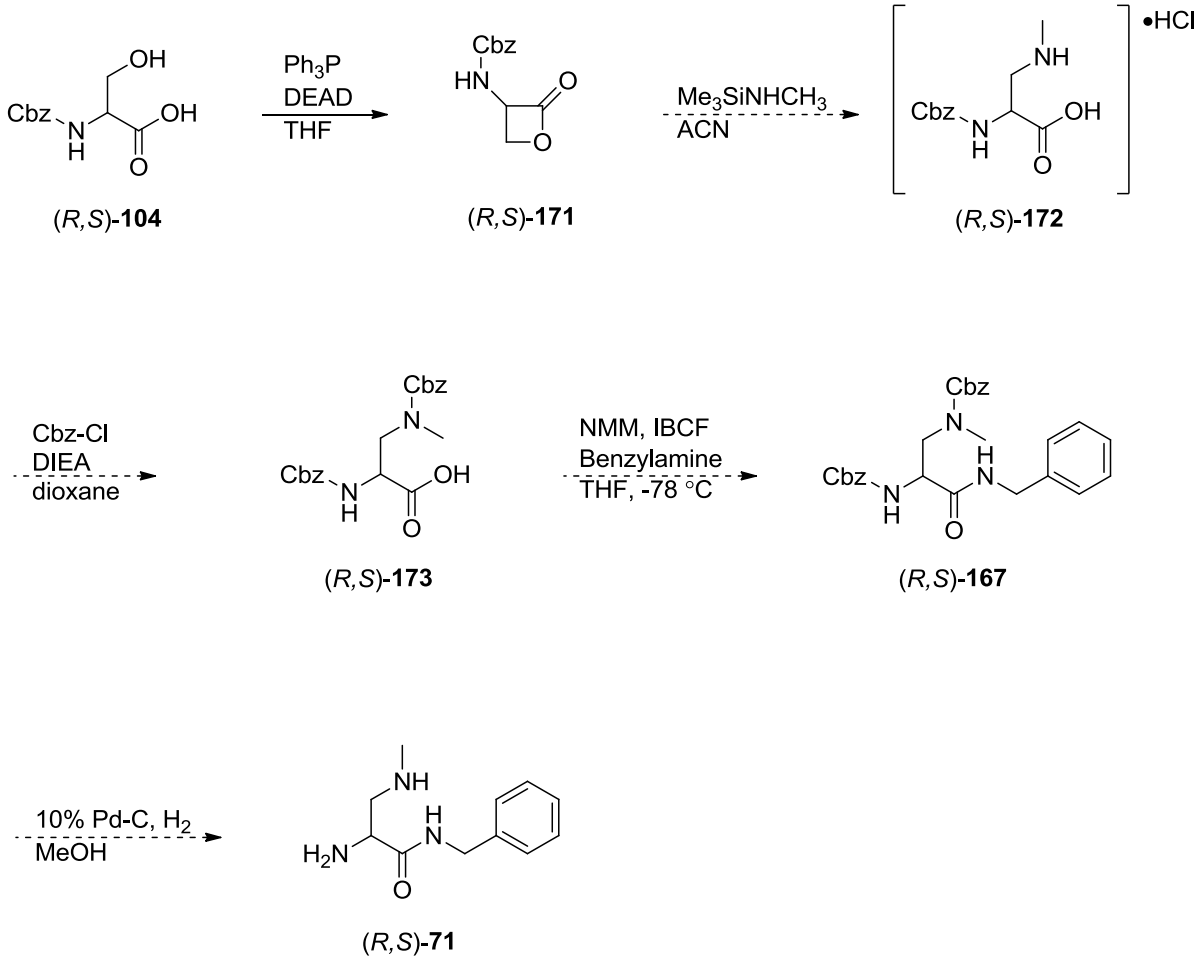


In a final attempt to synthesize PAAD (*R,S*)-**71**, we adapted a protocol reported by Ratemi and Vederas (Scheme 14).[157] They reported the stereoselective and regioselective synthesis of several *N*-alkyl derivatives upon ring opening of *N*-Cbz-L-serine β-lactone. Like dehydroalanine derivatives, β-lactones can serve as a powerful intermediate for the functionalization of amino acids that are not easily accessible by other methods.[157-160] However, the inherent ring strain of β-lactones results in a highly reactive species that can

generate a mixture of two products upon the addition of a nitrogen nucleophile: (1) the amide arising from acyl-oxygen cleavage; and (2) the amino acid arising from alkyl-oxygen cleavage. Ratemi and Vederas demonstrated that alkyl regioselectivity can be achieved by using a weaker nucleophile (*N*-trimethylsilylamine)[161] and a more polar solvent (ACN) to afford the highest acid:amide ratio.[157]

We successfully synthesized lactone (*R,S*)-**171** from (*R,S*)-**104**,[158,162] however several attempts to ring open (*R,S*)-**171** with *N*-methyl-*N*-(trimethylsilyl)amine gave the undesired amide (acyl-oxygen cleavage) (data not shown).[163,164] At this point in time, initial pharmacological data from the other C(3)-amino PAADs did not warrant further efforts to prepare PAAD (*R,S*)-**71**.

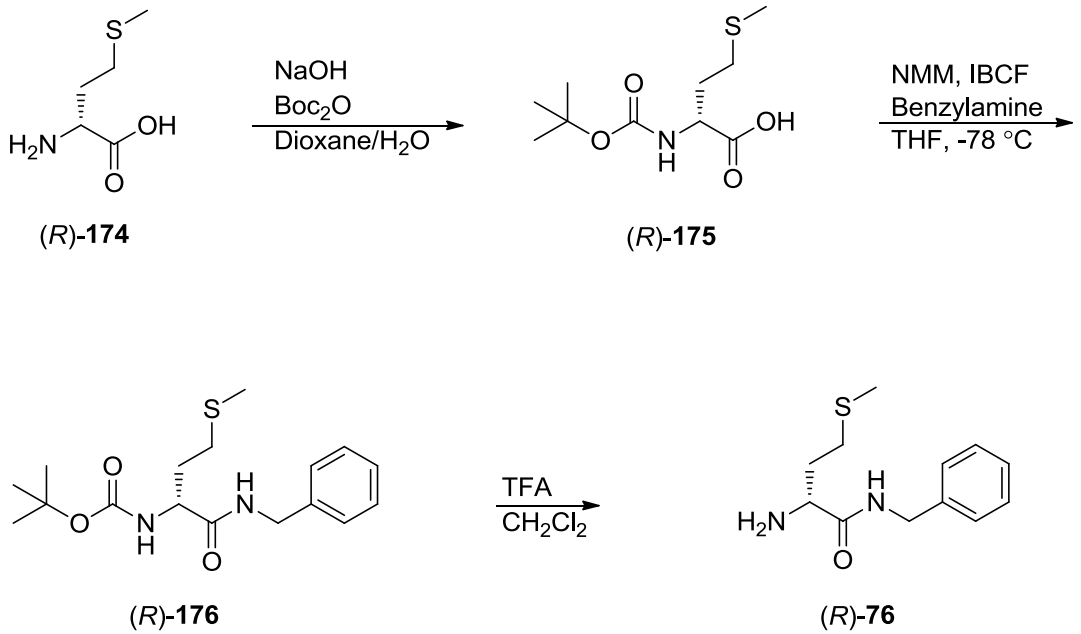
Scheme 14. Attempted synthesis of (*R,S*)-*N*-benzyl 3-*N*'-(methyl)aminopropionamide (*R,S*)-71 (Method C)



2.2.1.3. C(4)-Substituted PAADs

To conclude the C(2)-$(CH)_nX(R)_y$ series, we explored the effect of the heteroatom *two* atoms removed from the chiral C(2) center. Evaluation of (*R*)-, (*S*)-, and (*R,S*)-**61** indicated that the pharmacological activity resided largely in the D-amino acid configuration (See Section 2.3.1.1). Therefore, all C(4)-substituted PAADs were synthesized in the (*R*)-configuration. Synthesis of (*R*)-**76** was achieved without complications starting from commercially available D-methionine (**174**). (*R*)-**174** was *N*-*t*Boc protected following standard procedures, coupled with benzylamine using the MAC method, and deprotected to give (*R*)-**76** (Scheme 15).[97,165-167]

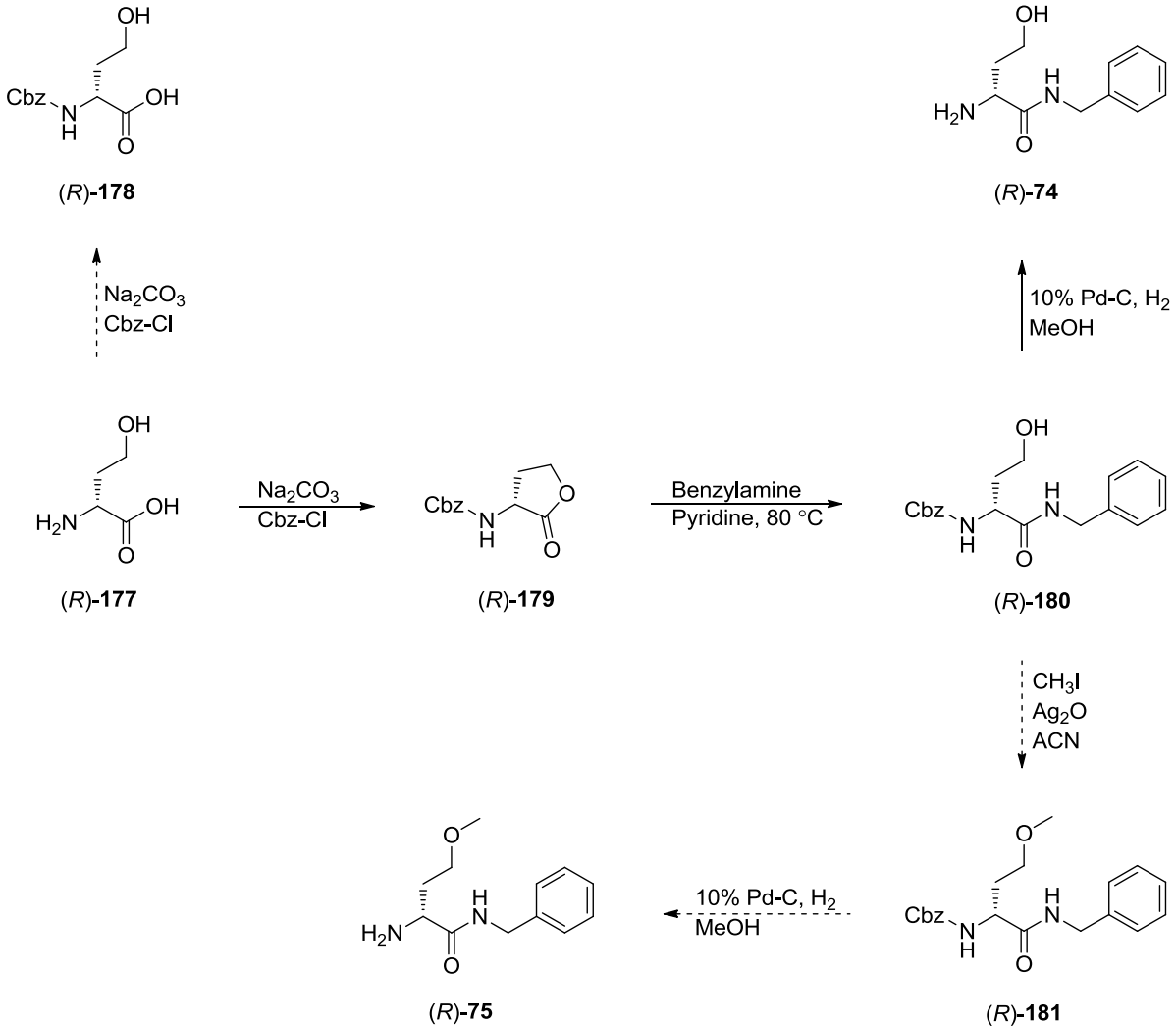
Scheme 15. Synthesis of (*R*)-*N*-benzyl 2-amino-4-(methylthio)butanamide ((*R*)-76)



(*R*)-**174** (*R*)-**175** (*R*)-**176** (*R*)-**76**

To synthesize the oxygen equivalent of (*R*)-**76** (*S* → *O*, (*R*)-**75**), we initially planned to follow the synthetic route depicted in Scheme 1, using commercially available D-homoserine ((*R*)-**177**) to obtain the C(4)-hydroxy PAAD ((*R*)-**74**) and the C(4)-methoxy PAAD ((*R*)-**75**). However, lactonization during *N*-Cbz protection of (*R*)-**177** gave (*R*)-**179**, and accordingly, we modified our route to take advantage of (*R*)-**179** (Scheme 16).[168,169] Treatment of (*R*)-**179** under basic conditions with benzylamine gave amide (*R*)-**180**,[170] followed by hydrogenolysis to obtain the corresponding PAAD (*R*)-**74** (Scheme 16). Several attempts were made to obtain (*R*)-**75**, but efforts to methylate (*R*)-**180** were unsuccessful and prompted us to switch the *N*-protecting group.

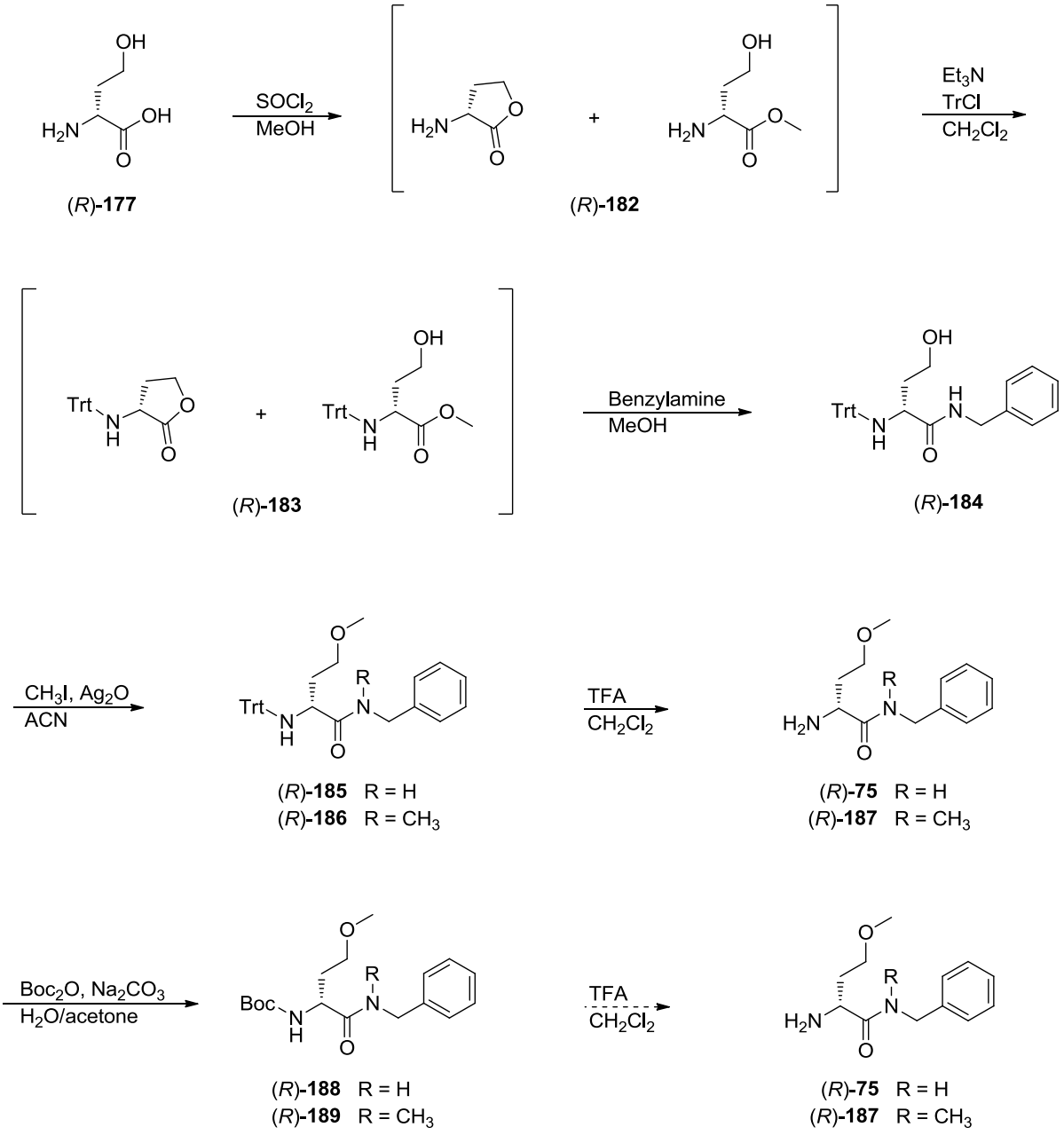
Scheme 16. Synthesis of (*R*)-*N*-benzyl 2-amino-4-hydroxybutanamide ((*R*)-74) and attempted synthesis of (*R*)-*N*-benzyl 2-amino-4-methoxybutanamide ((*R*)-75)



Accordingly, we proposed to use a *N*-trityl protecting group (Scheme 17). Following literature procedure, treatment of commercially available (*R*)-**177** with thionyl chloride in MeOH, followed by subsequent treatment with trityl chloride in the presence of base, gave a 5:1 mixture of *N*-trityl protected γ-lactone (major) and methyl ester (minor) ((*R*)-**183**).[171,172] The mixture was treated with benzylamine in MeOH to give amide (*R*)-**184**. We successfully alkylated (*R*)-**184** on small scale using CH_3I and Ag_2O to give (*R*)-**185**, which was deprotected under acidic conditions to the corresponding PAAD ((*R*)-**75**). However, scale up

attempts of the methylation resulted in a mixture of (*R*)-**185** and the dimethylated analog (*R*)-**186**, where amide methylation had occurred. 1D and 2D (COSY) NMR experiments supported the assignment of methylation at the amide nitrogen, however a degree of ambiguity leaves the possibility of methylation at the tritylated nitrogen. We attempted to purify (*R*)-**185** from (*R*)-**186** by flash column chromatography, but their similar chromatographic properties prevented their separation. In a final effort to recover the desired PAAD, we deprotected the mixture of (*R*)-**185** and (*R*)-**186** under acidic conditions, and then reprotected with Boc_2O under basic conditions, to give (*R*)-**188** and (*R*)-**189**. Once again, attempts to purify sufficient amounts of (*R*)-**188** by flash column chromatography were unsuccessful.

Scheme 17. Attempted synthesis of (*R*)-*N*-benzyl 2-amino-4-methoxybutanamide ((*R*)-75)

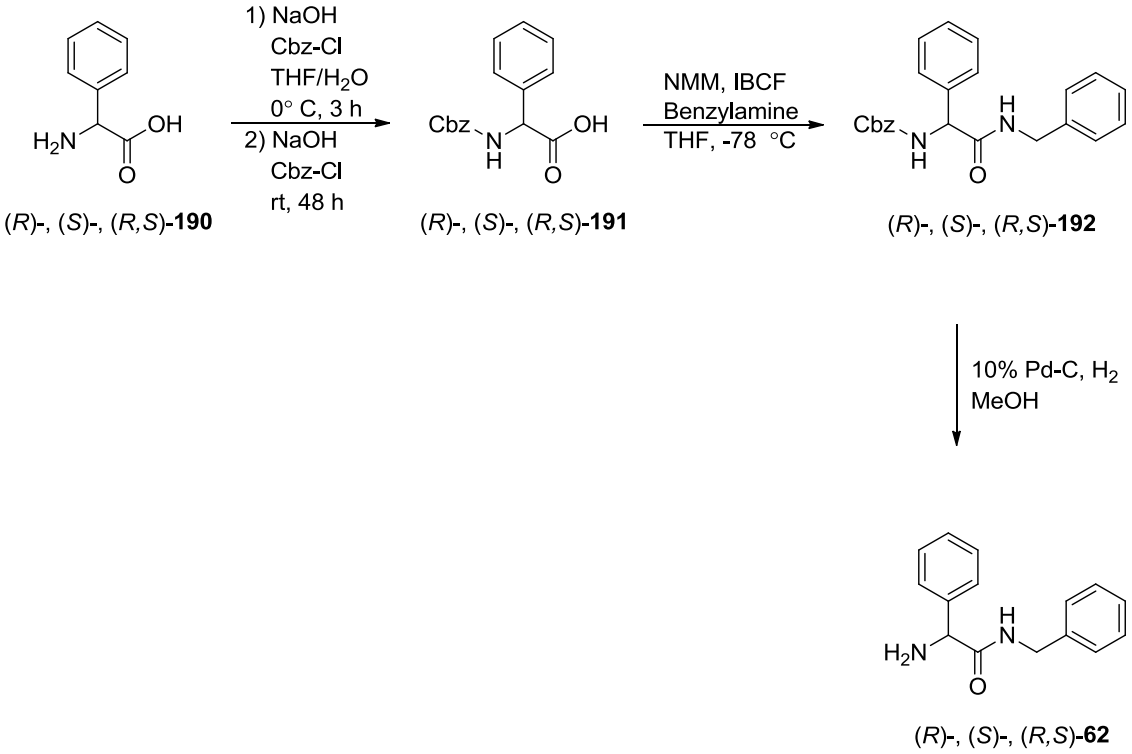


2.2.2. C(2)-6-Membered and 5-membered aromatic and heteroaromatic PAADs

C(2)-6-Membered and 5-membered aromatic and heteroaromatic PAADs **77**–**94** were synthesized by Drs. Jason Dinsmore, Pranjal Baruah, and Christophe Salomé. (*R*)-,

(*S*)-, and (*R,S*)-**62** were synthesized according to Scheme 18, and in the case of the (*R*)- and (*S*)-**62**, we were mindful of the potential for C(2) racemization during the amide coupling and the *N*-Cbz protection steps. Therefore, commercially available (*R*)-, (*S*)-, and (*R,S*)-**190** was treated at low temperature with benzyl chloroformate under basic conditions in two portions to give (*R*)-, (*S*)-, and (*R,S*)-**191**.[173-175] Optical rotations of (*R*)- and (*S*)-**191** were consistent with previously reported values,[173] and (*R*)-, (*S*)-, and (*R,S*)-**191** were converted to the amides (*R*)-, (*S*)-, and (*R,S*)-**192**[176] using the MAC procedure. Finally, deprotection using 10% Pd-C and H_2 gave the corresponding PAADs (*R*)-, (*S*)-, and (*R,S*)-**62**.[52]

Scheme 18. Synthesis of *N*-benzyl 2-amino-2-phenylacetamide (*R*)-, (*S*)-, and (*R,S*)-62

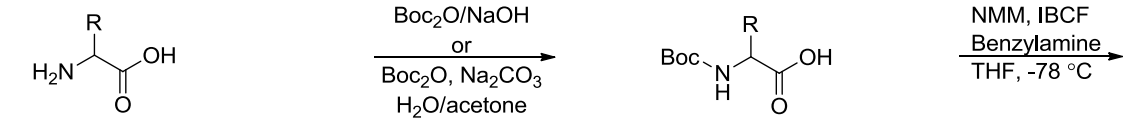


2.2.3. C(2)-Hydrocarbon PAADs

As previously stated, we synthesized the hydrocarbon equivalent of **61** (O→CH_2, **96**) in the (*R*)-, (*S*)-, and (*R,S*)- configurations to examine the requirement for a heteroatom one

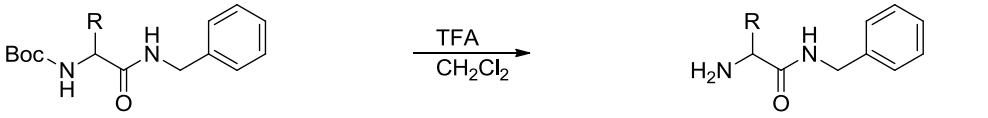
atom removed from the C(2) center, as well as the need for stereochemical specificity at the C(2) position. Modest anticonvulsant activity and NP protection was observed for (*R*)- and (*R,S*)-**96**, and prompted the synthesis of an expanded series of C(2)-hydrocarbon PAADs (**95**, **97–102**) (Scheme 19). Commercially available amino acids (*R*)-**193**, -**195,** -**196,** -**199**, (*S*)-**195**, -**196** and (*R,S*)-**197** were *N*-*t*Boc protected following standard procedures, coupled with benzylamine using the MAC method, and then deprotected to give PAADs (*R*)-**95**, -**98**, -**99**, -**102**, (*S*)-**98**, -**99**, and (*R,S*)-**100**, respectively. (*R*)-**97** and (*R*)-**101** were synthesized by Drs. Jason Dinsmore and Christophe Salomé, respectively, in the same manner as depicted in Scheme 19.

Scheme 19. Synthesis of C(2)-hydrocarbon PAADs 95–102



(*R*)-**193** R = CH_2CH_3
(*R*)-**194** R = $(CH_2)_3CH_3$
(*R*)-, (*S*)-**195** R = $CH(CH_3)_2$
(*R*)-, (*S*)-**196** R = $C(CH_3)_3$
(*R,S*)-**197** R = $CH(CH_3)CH_2CH_3$
(*R*)-**198** R = C_6H_{11}
(*R*)-**199** R = $CH_2C_6H_5$

(*R*)-**200** R = CH_2CH_3
(*R*)-**201** R = $(CH_2)_3CH_3$
(*R*)-, (*S*)-**202** R = $CH(CH_3)_2$
(*R*)-, (*S*)-**203** R = $C(CH_3)_3$
(*R,S*)-**204** R = $CH(CH_3)CH_2CH_3$
(*R*)-**205** R = C_6H_{11}
(*R*)-**206** R = $CH_2C_6H_5$



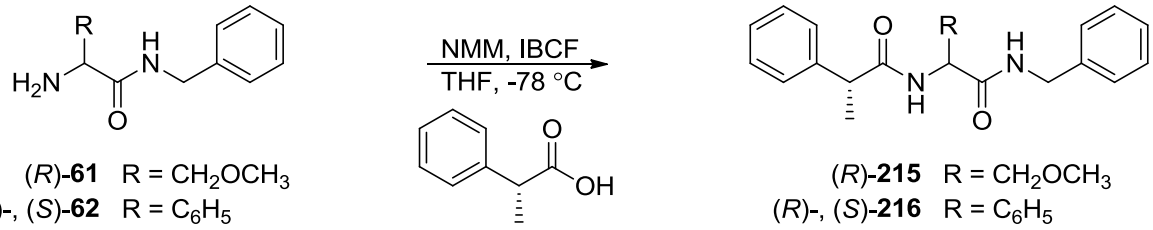
(*R*)-**207** R = CH_2CH_3
(*R*)-**208** R = $(CH_2)_3CH_3$
(*R*)-, (*S*)-**209** R = $CH(CH_3)_2$
(*R*)-, (*S*)-**210** R = $C(CH_3)_3$
(*R,S*)-**211** R = $CH(CH_3)CH_2CH_3$
(*R*)-**212** R = C_6H_{11}
(*R*)-**213** R = $CH_2C_6H_5$

(*R*)-**95** R = CH_2CH_3
(*R*)-**97** R = $(CH_2)_3CH_3$
(*R*)-, (S)-**98** R = $CH(CH_3)_2$
(*R*)-, (S)-**99** R = $C(CH_3)_3$
(*R,S*)-**100** R = $CH(CH_3)CH_2CH_3$
(*R*)-**101** R = C_6H_{11}
(*R*)-**102** R = $CH_2C_6H_5$

2.2.4. Assessment of PAAD optical purity

We determined the enantiomeric purity of select PAADs to assess the pharmacological data. Accordingly, we confirmed that (*R*)-**61**, (*R*)-**62**, and (*S*)-**62** were prepared without racemization. (*R*)-**61** was selected based on the central role of lacosamide ((*R*)-**28**) and its PAAD equivalent ((*R*)-**61**) in this study. (*R*)- and (*S*)-**62** were selected based on their presumed higher tendency to racemize during synthesis due to the acidity of the α-proton. Unfortunately, comparing the optical rotation or melting point of enantiomeric PAADs is not sufficient due to the possibility of equal racemization. The selected PAADs also have poor solubility in $CDCl_3$, making it impossible to verify enantiomeric purity by the addition of the chiral resolving agent mandelic acid.[177] Therefore, each isomer was coupled with (*R*)-2-phenylpropionic acid (**214**) using the MAC procedure to give (*R*)-**215**, (*R*)-**216**, and (*S*)-**216**. Each derivative contained two stereocenters that were analyzed for racemization by ^1H NMR prior to extensive purification and shown to be enantiomerically pure (>95%).

Scheme 20. Synthesis of (*R*)-*N*-benzyl 2-*N'*-((*R*)-2-phenylpropionyl)amino-3-methoxypropionamide ((*R,R*)-215), (*R*)-*N*-benzyl 2-*N'*-((*R*)-2-phenylpropionyl)amino-2-phenylacetamide ((*R,R*)-216), and (*S*)-*N*-benzyl 2-*N'*-((*R*)-2-phenylpropionyl)amino-2-phenylacetamide ((*R,S*)-216)



(*R*)-**61** R = CH_2OCH_3
(*R*)-, (*S*)-**62** R = C_6H_5

214

(*R*)-**215** R = CH_2OCH_3
(*R*)-, (*S*)-**216** R = C_6H_5

2.3. Pharmacological evaluation

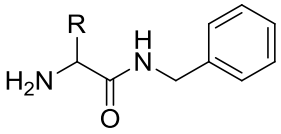
PAADs **61**, **62**, and **65**–**94** were evaluated for anticonvulsant activity using the MES test at either UCB Pharma, following the procedures described by Klitgaard,[178] or at the

NINDS ASP, following the procedures described by Stables and Kupferberg,[42] or both. Anticonvulsant activity using the 6 Hz test was performed either at UCB Pharma following the procedures described by Kaminski and coworkers (44 mA),[179] or at the NINDS ASP, following the procedures described by Stables and Kupferberg (32 mA),[42] or both. PAADs evaluated at UCB Pharma were also tested for NP protection (formalin test).[180] Recently, Visser and coworkers demonstrated a good correlation between findings in the second phase of the formalin test and results for cold allodynia in the chronic constriction injury (CCI) model for both rats (r = 0.72) and gerbils (r = 0.68) using drugs with proven pain attenuating effects in humans.[181] Therefore, the formalin model of NP is advantageous compared to other well-characterized models of NP (CCI) due to the ease of administration and standardization, and is an effective tool to prescreen compounds for NP protection. All compounds were administered intraperitoneally (ip) to mice at UCB Pharma or ip to mice and orally (po) to rats at the NINDS ASP. The pharmacological data from the MES, 6 Hz, and formalin tests are summarized in Tables 5, 7, 9–11, 13, and 14. The MES activities of PAADs are compared with the MES activities of their corresponding FAAs in Tables 6, 8, 12, 15, and 16. The pharmacological data from PAADs synthesized as individual isomers ((*R*), (*S*), and (*R,S*)) are summarized in Table 17. Several compounds were evaluated at both UCB Pharma and the NINDS ASP and displayed comparable activities. Therefore, we conclude the initial PAAD SAR by comparing the MES activities of PAADs that were obtained at UCB Pharma and the NINDS ASP (Table 18). The tables list the results obtained from qualitative (dose range) or quantitative (ED_{50}) testing in mice (ip) and rats (po). We also include qualitative (dose range) or median neurological impairing dose (TD_{50}) values in mice (ip) using the rotorod test in mice, and the behavioral toxicity effects observed in rats (po). The protective indices ($PI = TD_{50}/ED_{50}$) are provided, when applicable. PAADs tested at the NINDS ASP were evaluated in the subcutaneous Metrazol® (scMET) seizure model but protection was not observed at the doses (30, 100, 300 mg/kg) and times

(0.5 and 4 h) tested (data not shown), with two exceptions. (*R*)-**98** displayed protection in the scMET seizure model for one out of eight mice treated with 62 mg/kg of compound and the remaining seven mice displayed continuous seizure activity. Similarly, (*R*)-**99** protected one out of eight mice treat with 63 mg/kg and the remaining seven mice displayed continuous seizure activity, while three out of eight mice treated with 75 mg/kg were protected and the remaining five mice displayed continuous seizure activity.

Early evaluation of (*R*)-**61**, (*R*)-**65**, and (*R,S*)-**77** surprisingly revealed good brain-to-plasma (B:P) ratios in mice (Table 4). (*R*)-**61** and (*R*)-**65** were detected in a B:P ratio of 1.2:1 and (*R,S*)-**77** was detected in a B:P ratio of 2.7:1. We had hypothesized (Chapter 1, Figure 12) that protonation of the amino group in PAADs at physiological pH, to give the corresponding ammonium ion, would hinder penetration of the predominately negatively charged phospholipid head groups of the BBB. Good penetration of the BBB indicated that these hydrophilic PAADs do not *selectively* target the PNS, but rather possibly exert their mechanism of action through a combination of interactions within the CNS and PNS.

Table 4. Plasma and brain levels of PAADs in mice



Cmpd No.[a]	R	Plasma level (μM)[b]	Brain level (μM)[b]	Brain:Plasma Ratio
(*R*)-**65**	CH_2OH	5.5	6.6	1.2:1
(*R*)-**61**	CH_2OCH_3	8.2	10	1.2:1
(*R,S*)-**77**	2-pyridyl	12	32	2.7:1

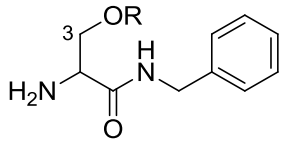
[a] The compounds were administered intraperitoneally (10 mg/kg) to adult male NMRI mice (n = 3) under the auspices of UCB. [b] Plasma and brain levels are in μM and were determined 30 min after ip administration.

2.3.1. C(2)-$(CH_2)_nX(R)_y$ PAADs

2.3.1.1. C(3)-Hydroxy and C(3)-alkoxy PAADs

Table 5 lists the neurological activities for C(3)-oxy *N*-benzylamide PAADs (*R*)- and (*S*)-**61**, (*R*)-, (*S*)-, and (*R,S*)-**65** and (*R,S*)-**66**–**69** in mice, where we systematically evaluated the effect of a hydrogen, methyl, ethyl, propyl, allyl, and propargyl group placed at the C(3)-oxy terminus (R) on anticonvulsant activity and pain attenuation. Throughout this study, we used ((*R*)-**28**) and the clinical AEDs, phenytoin, phenobarbital, and valproate, as reference compounds. The MES values for (*R*)-, (*S*)-, and (*R,S*)-**65** were not determined due to (1) the MES test was implemented after the initial testing; (2) the absence of activity in the 6 Hz and formalin tests; and (3) the FAA counterpart ((*R*)-**217**) was inactive in the MES test (Table 6). The MES activities of PAADs (*R*)-, (*S*)-, and (*R,S*)-**61**, and (*R,S*)-**66–69** remained relatively constant throughout the series, but we observed a slight increase in activity as we went from a saturated alkyl group ((*R,S*)-**67**, ED_{50} = 69 mg/kg) to unsaturated hydrocarbon groups (ED_{50} (mg/kg): (*R,S*)-**68**; 45; (*R,S*)-**69**, 46). (*R*)-**61** displayed the highest anticonvulsant activity in this series (ED_{50} = 34 mg/kg) but it was a ~10-fold drop in activity from the corresponding FAA (*R*)-**28** (ED_{50} = 3.3 mg/kg). PAAD **61** also showed a ~2-fold preference in regard to MES activity for the (*R*)-isomer. This selectivity was considerably lower than that exerted for (*R*)-**28**, where there was ~22-fold difference in activity between the (*R*)- and (*S*)-isomers in mice. In all instances, the MES activity was greater than the 6 Hz activity (44 mA and 32 mA) and no significant pain attenuation was observed. Comparison of the MES activities of PAADs **61**, **65**, **66**, **68**, and **69** with their corresponding FAAs (Table 6) revealed a consistent drop in activity (3–10-fold) as we went from the FAA to the PAAD. Therefore, we conclude that the C(3)-hydroxy and C(3)-alkoxy PAADs do not provide any significant advantage for the prevention of seizures or NP.

Table 5. Pharmacological activities of C(3)-oxy *N*-benzylamide PAADs in mice (mg/kg) at UCB and the NINDS ASP

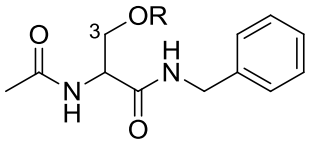


			Mice (ip)[a]						
Cmpd No.	**Test Site**	**R**	**MES,[b] ED_{50}**	**6 Hz,[c] ED_{50}**	**Formalin, ED_{50}**	**Tox,[d] TD_{50}**	**Com-ments[e]**	**PI,[f] MES**	**PI,[f] Form**
(*R*)-**28**	UCB	LCM	3.3	10	15	19	Ref	5.8	1.3
(*R*)-**28**[g]	NINDS	LCM	4.5 [0.5] (3.7–5.5)	10	ND[h]	27 [0.25] (26–28)	Ref	6.0	
(*R*)-**65**	UCB	H	ND[h]	>62	>62	>110			
(*S*)-**65**	UCB	H	ND[h]	>62	>62	>110			
(*R,S*)-**65**	UCB	H	ND[h]	>62	>62	>110			>1.8
(*R*)-**61**	UCB	CH_3	34	>67	>67	>120		>3.5	>1.8
(*R*)-**61**	NINDS	CH_3	48 [0.25] (40–61)	ND[h]	ND[h]	>30, <100 [0.25]			
(*S*)-**61**	UCB	CH_3	64	>70	120	63		1.0	0.5
(*R,S*)-**66**	UCB	CH_2CH_3	73	120	71[i] (19%)	ND[h]			
(*R,S*)-**67**	UCB	$CH_2CH_2CH_3$	69	>130	>130	ND[h]	230 (C)		

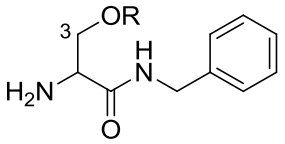
(*R,S*)-**68**	UCB	$CH_2CH{=}CH_2$	45	130 (MAD)[j]	75[i] (12%)	ND[h]		
(*R,S*)-**69**	UCB	$CH_2C{\equiv}CH$	46	130 (MAD)[j]	74[i] (27%)	47		1.0
phenytoin[k]			9.5 [2.0] (8.1–10)			66 [0.5] (53–72)	Ref	
phenobarbital[k]			22 [1.0] (15–23)			69 [0.5] (63–73)	Ref	
valproate[k]			270 [0.25] (250–340)			430 [0.25] (370–450)	Ref	

[a] The compounds were administered either intraperitoneally to adult male NMRI mice under the auspices of UCB or administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration (UCB) or a dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets) (NINDS ASP). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] 6 Hz test = psychomotor seizure model (44 mA, UCB; 32 mA, NINDS ASP). [d] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [e] Dose in mg/kg is followed by whole animal pharmacological observation (Ref = reference, C = convulsions). [f] PI = protective index (TD_{50}/ED_{50}). [g] Choi, D. *et al. J. Med. Chem.* **1996**, *39*, 1907–1916. [h] ND = not determined. [i] Single dose experiments where the mg/kg used is followed by the percentage protected in parenthesis. [j] MAD = minimal active dose. [k] Porter, R.J. *et al. Cleveland Clin. Q.* **1984**, *51*, 293–305.

Table 6. Comparison of the pharmacological activities of C(3)-oxy *N*-benzylamide FAAs and their PAAD counterparts in mice (mg/kg)



FAA



PAAD

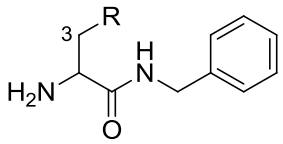
			Mice (ip)[a]				Mice (ip)[a]	
R	**FAA Cpd No.**	**FAA Test Site**	**FAA MES,[b] ED_{50}**	**FAA Tox,[c] TD_{50}**	**PAAD Cpd No.**	**PAAD Test Site**	**PAAD MES,[b] ED_{50}**	**PAAD Tox,[c] TD_{50}**
H	(*R*)-**217**[d]	NINDS	53 [2.0] (38–67)	>500 [2.0]	(*R*)-**65**	UCB	>62	>110
H	(*R,S*)-**217**[d]	NINDS	>100, <300	<300	(*R,S*)-**65**	UCB	>62	>110
CH_3	(*R*)-**28**[d]	NINDS	4.5 [0.5] (3.7–5.5)	27 [0.25] (26–28)	(*R*)-**61**	NINDS	48 [0.25] (40–61)	>30, <100 [0.25]
CH_3	(*S*)-**28**[d]	NINDS	>100, <300	> 300	(*S*)-**61**	UCB	64	63
CH_3	(*R,S*)-**28**[d]	NINDS	8.3 [0.5] (7.9–9.8)	43 [0.25] (38–47)	(*R,S*)-**61**[e]	NINDS	84 [0.25] (65–97)	290 [0.25] (240–320)
CH_2CH_3	(*R,S*)-**218**[d]	NINDS	17 [0.25] (15–19)	78 [0.25] (64–90)	(*R,S*)-**66**	UCB	73	ND[g]
$CH_2CH{=}CH_2$	(*R,S*)-**219**[d]	NINDS	>30, <100	>30, <100	(*R,S*)-**68**	UCB	45	ND[g]
$CH_2C{\equiv}CH$	(*R,S*)-**220**[f]	NINDS	16 [0.25] (13–19)	59 [0.25] (55–66)	(*R,S*)-**69**	UCB	46	47

[a] The compounds were administered either intraperitoneally to adult male NMRI mice under the auspices of UCB or administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration (UCB) or a dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets) (NINDS ASP). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [d] Choi, D. *et al. J. Med. Chem.* **1996**, *39*, 1907–1916. [e] Béguin, C. *et al. Bioorg. Med Chem*. **2004**, *12*, 3079–3096. [f] Park, K. *et al. J. Med. Chem*. **2009**, *52*, 6897–6911. [g] ND = not determined.

2.3.1.2. C(3)-Amino PAADs

Table 7 lists the neurological activities for C(3)-amino *N*-benzylamide PAADs (*R,S*)-**70**, -**72**, and -**73**. In this series, we substituted the C(3)-amino with two methyl groups or embedded the C(3)-amino within a morpholino moiety. We proposed that the increased polarity and the possibility of C(3)-amino protonation could lead to an increase in PNS versus CNS biodistribution (Chapter 1, Figure 12). However, no significant neurological activity was observed in either the anticonvulsant models or the pain model, and toxicity was not evaluated due to the lack of activity. Comparison of the C(3)-amino PAADs with their corresponding FAAs (Table 8) showed that there is neither an advantage nor disadvantage of the C(3)-amino group on anticonvulsant activity.

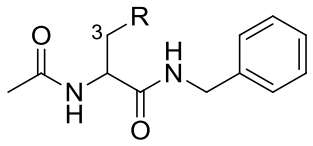
Table 7. Pharmacological activities of C(3)-amino *N*-benzylamide PAADs in mice (mg/kg) at UCB



		Mice (ip)[a]					
Cmpd No.	**R**	**MES,[b] ED_{50}**	**6 Hz,[c] ED_{50}**	**Formalin, ED_{50}**	**Tox,[d] TD_{50}**	**PI,[e] MES**	**PI,[e] Form**
(*R*)-**28**	LCM	3.3	10	15	19	5.8	1.3
(*R,S*)-**70**	NH_2	>68	ND[f]	ND[f]	ND[f]		
(*R,S*)-**72**	$N(CH_3)_2$	94	>110	83 (14%)[g]	ND[f]		
(*R,S*)-**73**	morpholino	89	>84	69	ND[f]		

[a] The compounds were administered intraperitoneally to adult male NMRI mice under the auspices of UCB. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration. [b] MES = maximal electroshock seizure test. [c] 6 Hz test = psychomotor seizure model (44 mA). [d] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [e] PI = protective index (TD_{50}/ED_{50}). [f] ND = not determined. [g] Single dose experiments where the mg/kg used is followed by the percentage protected in parenthesis.

Table 8. Comparison of the pharmacological activities of C(3)-amino *N*-benzylamide FAAs and their PAAD counterparts in mice (mg/kg)



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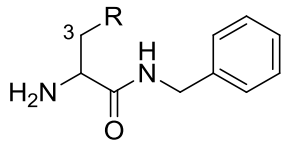


PAAD

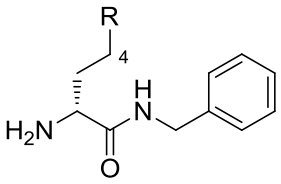
			Mice (ip)[a]				Mice (ip)[a]	
R	**FAA Cpd No.**	**FAA Test Site**	**FAA MES,[b] ED_{50}**	**FAA Tox,[c] TD_{50}**	**PAAD Cpd No.**	**PAAD Test Site**	**PAAD MES,[b] ED_{50}**	**PAAD Tox,[c] TD_{50}**
NH_2	(*R,S*)-**221**[d]	NINDS	>100	>100	(*R,S*)-**70**	UCB	>68	ND[e]
$N(CH_3)_2$	(*R,S*)-**222**[d]	NINDS	>30, <100	>100, <300	(*R,S*)-**72**	UCB	94	ND[e]
morpholino	(*R,S*)-**223**[d]	NINDS	>100, <300	300	(*R,S*)-**73**	UCB	89	ND[e]

[a] The compounds were administered either intraperitoneally to adult male NMRI mice under the auspices of UCB or administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration. [b] MES = maximal electroshock seizure test. [c] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [d] Choi, D. *et al.* unpublished results. [e] ND = not determined.

2.3.1.3. C(4)-Substituted PAADs

Next, we examined the C(4)-substituted *N*-benzylamide PAADs (*R*)-**74** and (*R*)-**76** (Table 9). PAADs containing an oxygen ((*R*)-**74**) or a sulfur ((*R*)-**76**) two atoms from the C(2) carbon were inactive in the MES test (ED_{50} (mg/kg): (*R*)-**74**, >160; (*R*)-**76**, 75). However, substitution of a carbon atom for the heteroatom that is two atoms removed from the C(2) carbon, giving (*R*)-**96** and (*R*)-**97**, provided PAADs with considerable anticonvulsant activity (ED_{50} (mg/kg): (*R*)-**96**, 21; (*R*)-**97**, 23). The activities of (*R*)-**96** and (*R*)-**97** will be discussed in detail in Table 13 and are presented in Table 9 for comparative purposes. (*R*)-**74** and (*R*)-**76** were also inactive in the 6 Hz and formalin tests (ED_{50} >60 mg/kg), therefore the inactivity did not justify toxicity studies. It is difficult to make generalizations based on the limited set of compounds but in conjunction with the data given in Table 5 and Table 7, we conclude that C(2)-acyclic PAADs containing a heteroatom two atoms removed from the C(2)-center did not show appreciable seizure protection, and provide a distinctive departure of the PAAD SAR from the corresponding FAAs.

Table 9. Pharmacological activities of C(4)-substituted *N*-benzylamide PAADs in mice (mg/kg) at UCB



		Mice (ip)[a]					
Cmpd No.	**R**	**MES,[b] ED_{50}**	**6 Hz,[c] ED_{50}**	**Formalin, ED_{50}**	**Tox,[d] TD_{50}**	**PI,[e] MES**	**PI,[e] Form**
(*R*)-**28**	LCM	3.3	10	15	19	5.8	1.3
(*R*)-**74**	OH	>160	>120	>67	ND[g]		
(*R*)-**76**	SCH_3	75	130 (MAD)[f]	>76	ND[g]		
(*R*)-**96**	CH_3	21	66 (MAD)[f]	35	57	2.8	1.6
(*R*)-**97**	CH_2CH_3	23	93	>71	ND[g]		

[a] The compounds were administered intraperitoneally to adult male NMRI mice under the auspice of UCB. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration. [b] MES = maximal electroshock seizure test. [c] 6 Hz test = psychomotor seizure model (44 mA). [d] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [e] PI = protective index (TD_{50}/ED_{50}). [f] MAD = minimal active dose. [g] ND = not determined.

2.3.2. 6-Membered and 5-membered aromatic and heteroaromatic PAADs

The neurological activities for C(2)-6-membered aromatic and heteroaromatic *N*-benzylamide PAADs (*R,S*)-**62** and (*R,S*)-**77**–**88** in mice are listed in Table 10. Using (*R,S*)-**62** as a non-heteroatom-containing aromatic reference, we evaluated the effect of positioning either a 6-membered aromatic group [i.e., 2-, 3-, and 4-pyridyl ((*R,S*)-**77**–**79**), 2-pyrazinyl ((*R,S*)-**80**), 4-fluorophenyl ((*R,S*)-**86**), 4-methylphenyl ((*R,S*)-**87**), 4-methoxyphenyl ((*R,S*)-**88**)], or a benzoannulated 6-membered aromatic group [i.e., 1- and 2-naphthyl ((*R,S*)-**81** and -**83**), 1-isoquinolinyl ((*R,S*)-**82**), 2-quinolinyl ((*R,S*)-**84**), 2-quinoxalinyl ((*R,S*)-**85**)] at the C(2)-site on anticonvulsant activity and pain attenuation. The aromatic and heteroaromatic compounds showed a general lack of protection in the MES (>30 mg/kg) and

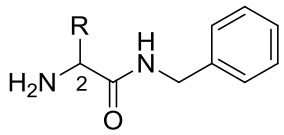
6 Hz (>30 mg/kg) tests in mice. Interestingly, we observed moderate activity in the formalin test for the 6-membered heteroaromatic PAADs (*R,S*)-**77**–**79**, -**82**, -**84**, and -**85** (ED_{50} (mg/kg): (*R,S*)-**77**, 33; (*R,S*)-**78**, 74; (*R,S*)-**79**, 67; (*R,S*)-**82**, 29; (*R,S*)-**84**, 54; (*R,S*)-**85**, 42) indicating that these compounds were more effective in the pain model compared with the seizure models. This finding differed from the PAADs containing a C(2)-acyclic moiety (i.e. -CH_2OR, -CH_2NR, -CH_2CH_2R) where we consistently observed lower activity in the formalin test compared with the MES seizure model. There was lack of toxicity data gathered due to the inactivity in the MES and 6 Hz tests. In the pyridyl series, (*R,S*)-**77** showed the greatest protection in the formalin test and protection was slightly improved upon benzoannulation (ED_{50} (mg/kg): (*R,S*)-**77**, 33; (*R,S*)-**82**, 29), while little or no protection was observed for the same compounds in the MES and 6 Hz tests (MES ED_{50} (mg/kg): (*R,S*)-**77**, 100 (MAD); (*R,S*)-**82**, >52); (6 Hz ED_{50} (mg/kg): (*R,S*)-**77**, >100; (*R,S*)-**82**, >93). The formalin activity of (*R,S*)-**82** (ED_{50} = 29 mg/kg) is within 2-fold of the formalin activity of our benchmark (*R*)-**28** (ED_{50} = 15 mg/kg). Benzoannulation of (*R,S*)-**80** to give (*R,S*)-**85** also resulted in an increase in activity in the formalin test (ED_{50} (mg/kg): (*R*)-**80**, >160 mg/kg; (*R*)-**85**, 42 mg/kg). This result differed from patterns observed for the anticonvulsant activities of FAAs, where benzoannulation led to a sharp loss in seizure protection (see Table 12).[54] Therefore, the 6-membered heteroaromatic substituents and their benzoannulated analogs, where the heteroatom is one atom removed from the C(2)-center, represent the first examples of appreciable neuropathic pain protection in PAADs. Furthermore, these heteroaromatic PAADs ((*R,S*)-**77**, (*R,S*)-**79**, and (*R,S*)-**85**) are ~3-fold more selective for pain attenuating effects over anticonvulsant activity.

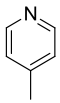
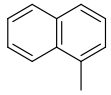
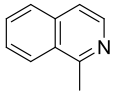
We expanded the heteroaromatic PAADs to include C(2)-5-membered heteroaromatic *N*-benzylamide PAADs and select benzoannulated analogs (Table 11). We evaluated the effect of oxygen-containing (2-furanyl, (*R,S*)-**89**; 5-methyl-2-furanyl, (*R,S*)-**90**) and sulfur-containing (thiophen-2-yl, (*R,S*)-**92**; 2-thiazolyl, (*R,S*)-**93**) 5-membered

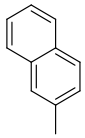
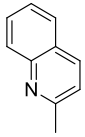
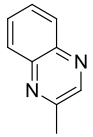
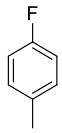
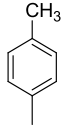
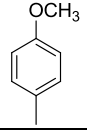
heteroaromatic PAADs and also tested the benzo-ring fused systems (2-benzofuranyl, (*R,S*)-**91**); benzo[*b*]thiophen-2-yl, (*R,S*)-**94**) on anticonvulsant and pain activity in mice. In contrast with the 6-membered heteroaromatic PAADs, the 5-membered heteroaromatic PAADs ((*R,S*)-**89**–(*R,S*)-**94**) displayed modest anticonvulsant activity (ED_{50} >67, <120 mg/kg) in the MES test, but were insensitive in the 6 Hz test (ED_{50} >90 mg/kg). (*R,S*)-**93** was the most active in this series (ED_{50} = 67 mg/kg) regarding the MES test, but (*R,S*)-**93** is still ~20-fold less active than our benchmark (*R*)-**28** (ED_{50} = 3.3 mg/kg). (*R,S*)-**89** displayed considerable activity in the formalin test (ED_{50} = 29 mg/kg) and is within 2-fold of the formalin activity of (*R*)-**28** (ED_{50} = 15 mg/kg). Unlike the 6-membered heteroaromatic series, benzoannulation of both (*R,S*)-**89** and (*R,S*)-**92** to give (*R,S*)-**91** and (*R,S*)-**94**, respectively, decreased pain attenuation ((ED_{50} (mg/kg): (*R,S*)-**89**, 29; (*R,S*)-**91**, 90 (31% reduction); (*R,S*)-**92**, 48; (*R,S*)-**94**, 66). Collectively, heteroaromatic systems containing a heteroatom one atom removed from the C(2) center in PAADs attenuate pain in the formalin test and are 1.5 to 3-fold selective for neuropathic pain protection over anticonvulsant activity.

Comparison of the MES activities of active heteroaromatic PAADs (*R*)-, (*S*)-, and (*R,S*)-**62**, (*R,S*)-**77**, (*R,S*)-**80**, (*R,S*)-**83**, and (*R,S*)-**88**–**94** with their corresponding FAAs (Table 12) revealed a drop in activity up to 10-fold going from FAA to PAAD. Therefore, the C(2)-6-membered and 5-membered aromatic and heteroaromatic PAADs do not provide any significant advantage for the prevention of seizures. It would be informative to conduct a similar comparison of the formalin activity in PAADs and their corresponding FAAs but extensive formalin data is not available in the FAA series.

Table 10. Pharmacological activities of C(2)-6-membered aromatic and heteroaromatic *N*-benzylamide PAADs in mice (mg/kg) at UCB

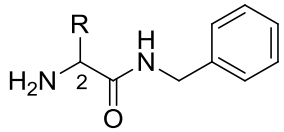


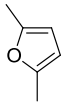
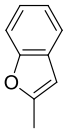
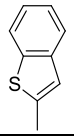
		Mice (ip)[a]						
Cmpd No.	**R**	**MES,[b] ED_{50}**	**6 Hz,[c] ED_{50}**	**Formalin, ED_{50}**	**Tox,[d] TD_{50}**	**Comments[e]**	**PI,[f] MES**	**PI,[f] Form**
(*R*)-**28**	LCM	3.3	10	15	19	Ref	5.8	1.3
(*R,S*)-**62**		>30	>43	24[g] (37%)	51	77 (C), 140 (MT)	>1.7	>1.2
(*R,S*)-**77**		100 (MAD)[h]	>100	33	180 (MTD)[i]			
(*R,S*)-**78**		120 (MAD)[h]	>210	74	ND[j]			
(*R,S*)-**79**		210 (MAD)[h]	>120	67	ND[j]			
(*R,S*)-**80**		85	28 (MAD)[h]	>160	>160		>1.9	>1.0
(*R,S*)-**81**		>69	>59	>33	ND[j]	105 (C, MT)		
(*R,S*)-**82**		>52	>93	29	ND[j]			

(*R,S*)-**83**		45	>110	<110 (inactive) 110[g] (100%)	110 (MTD)[i]	59 (T, W) 110 (C, M)	<2.4
(*R,S*)-**84**		>41	>130	54	ND[j]	130 (W, C)	
(*R,S*)-**85**		>120	>120	42	ND[j]		
(*R,S*)-**86**		>83	>83	26 (MAD)[h]	ND[j]	83 (C)	
(*R,S*)-**87**		140 (MAD)[h]	>140	<25 (inactive)	ND[j]	81 (C)	
(*R,S*)-**88**		150 (MAD)[h]	>86	<27 (inactive) 86[g] (48%)	ND[j]	270 (C, MT)	

[a] The compounds were administered intraperitoneally to adult male NMRI mice under the auspice of UCB. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration. [b] MES = maximal electroshock seizure test. [c] 6 Hz test = psychomotor seizure model (44 mA). [d] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [e] Dose in mg/kg is followed by whole animal pharmacological observation (Ref = reference, T = tremors, C = convulsions, MT = mortality, W = writhing). [f] PI = protective index (TD_{50}/ED_{50}). [g] Single dose experiments where the mg/kg used is followed by the percentage protected in parenthesis. [h] MAD = minimal active dose. [i] MTD = maximal tolerated dose. [j] ND = not determined.

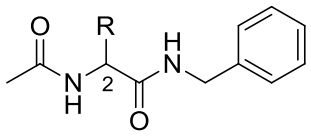
Table 11. Pharmacological activities of C(2)-5-membered heteroaromatic *N*-benzylamide PAADs in mice (mg/kg) at UCB



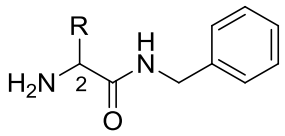
		Mice (ip)[a]						
Cmpd No.	**R**	**MES,[b] ED_{50}**	**6 Hz,[c] ED_{50}**	**Formalin, ED_{50}**	**Tox,[d] TD_{50}**	**Comments[e]**	**PI,[f] MES**	**PI,[f] Form**
(*R*)-**28**	LCM	3.3	10	15	19	Ref	5.8	1.3
(*R,S*)-**89**		85	>150	29	>150		>1.8	>5.1
(*R,S*)-**90**		100 (MAD)[g] 140[h] (100%)	>140	>78	ND[i]	100 (IG, T)		
(*R,S*)-**91**		90	>120	90[h] (31%)	ND[i]	90 (T)		
(*R,S*)-**92**		68 (MAD)[g]	>91	48	51 (MTD)[j]	160 (C)	<0.8	<1.1
(*R,S*)-**93**		67	>140	45	140 (MTD)[j]		<2.1	<3.1
(*R,S*)-**94**		120	>170	66	ND[i]			

[a] The compounds were administered intraperitoneally to adult male NMRI mice under the auspices of UCB. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration. [b] MES = maximal electroshock seizure test. [c] 6 Hz test = psychomotor seizure model (44 mA). [d] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [e] Dose in mg/kg is followed by whole animal pharmacological observation (Ref = reference, T = tremors, C = convulsions, IG = impaired gait). [f] PI = protective index (TD_{50}/ED_{50}). [g] MAD = minimal active dose. [h] Single dose experiments where the mg/kg used is followed by the percentage protected in parenthesis. [i] ND = not determined. [j] MTD = maximal tolerated dose.

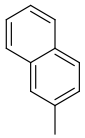
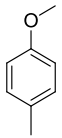
Table 12. Comparison of the pharmacological activities of C(2)-6-membered and 5-membered aromatic and heteroaromatic FAAs and their PAAD counterparts in mice (mg/kg)

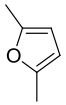
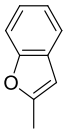
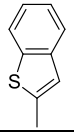


FAA



PAAD

			Mice (ip)[a]				Mice (ip)[a]	
R	**FAA Cpd No.**	**FAA Test Site**	**FAA MES,[b] ED_{50}**	**FAA Tox,[c] TD_{50}**	**PAAD Cpd No.**	**PAAD Test Site**	**PAAD MES,[b] ED_{50}**	**PAAD Tox,[c] TD_{50}**
	(*R*)-**224**[d]	Lilly	26 [0.5] (21–32)	>80	(*R*)-**62**	UCB	43 (MAD)[e]	ND[f]
	(*S*)-**224**[d]	Lilly	>300	>100, <300	(*S*)-**62**	UCB	ND[f]	140 (MTD)[g]
	(*R,S*)-**224**[d]	Lilly	32 [0.5] (28–40)	>40	(*R,S*)-**62**	UCB	>30	51
	(*R,S*)-**225**[h]	NINDS	11 [0.5] (9–12)	>25, <100	(*R,S*)-**77**[i]	UCB	100 (MAD)[e]	180 (MTD)[g]
	(*R,S*)-**226**[h]	NINDS	15 [0.5] (13–17)	58 [0.5] (46–73)	(*R,S*)-**80**[i]	UCB	85	>160
	(*R,S*)-**227**[i]	NINDS	>300	>300	(*R,S*)-**83**[i]	UCB	45	110 (MTD)[g]
	(*R,S*)-**228**[j]	NINDS	>300	ND	(*R,S*)-**88**[i]	UCB	150 (MAD)[e]	ND[f]

	(*R,S*)-**34**[j]	NINDS	10 [0.5] (9–12)	~ 40 [0.5]	(*R,S*)-**89**[k]	UCB	85	>150
	(*R,S*)-**229**[j]	NINDS	19 [0.5] (16–24)	75 [0.5]	(*R,S*)-**90**[k]	UCB	100 (MAD)[e]	ND[f]
	(*R,S*)-**230**[j]	NINDS	>100, <300	>100, <300	(*R,S*)-**91**[k]	UCB	90	ND[f]
	(*R,S*)-**231**[l]	NINDS	45 [0.5] (39–51)	>30, <100	(*R,S*)-**92**[k]	UCB	68 (MAD)[e]	51 (MTD)[g]
	(*R,S*)-**232**[h]	NINDS	12 [0.5] (10–15)	69 [0.5] (62–79)	(*R,S*)-**93**[k]	UCB	67	140 (MTD)[g]
	(*R,S*)-**233**[j]	NINDS	>100, <300	>100, <300	(*R,S*)-**94**[k]	UCB	120	ND[f]

[a] The compounds were administered either intraperitoneally to adult male NMRI mice under the auspices of UCB or administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP or Lilly Research Laboratories. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration (UCB) or a dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets) (NINDS ASP and Lilly Research Laboratories). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] Tox = neurological toxicity. TD_{50} value determined from the rotorod test (UCB and NINDS) or the horizontal screen test (Lilly Research Laboratories). [d] Kohn, H. *et al. Brain Res*. **1988**, *457*, 371–375. [e] MAD = minimal active dose. [f] ND = not determined. [g] MTD = maximal tolerated dose. [h] Bardel, P. *et al. J. Med. Chem*. **1994**, *37*, 4567–4571. [i] Baruah, P. *et al.* unpublished results. [j] Kohn, H. *et al. J. Med. Chem.* **1990**, *33*, 919–926. [k] Dinsmore, J. *et al.* unpublished results. [l] Kohn, H. *et al. J. Med. Chem*. **1993**, *36*, 3350–3360.

2.3.3. C(2)-Hydrocarbon PAADs

Table 13 lists the neurological activities in mice for C(2)-hydrocarbon *N*-benzylamide PAADs (*R*)-, (*S*)-, and (*R,S*)-**60**, (*R*)-**95**, (*R*)-, (*S*)-, and (*R,S*)-**96**, (*R*)-**97**, (*R*)- and (*S*)-**98**, (*R*)- and (*S*)-**99**, (*R,S*)-**100**, (*R*)-**101**, and (*R*)-**102**. Initially, we synthesized (*R*)-**96** to serve as the hydrocarbon counterpart to (*R*)-**61** (Table 5) and (*R*)-**97** to serve as the hydrocarbon counterpart for (*R*)-**74** and (*R*)-**76** (Table 9). To our surprise, both hydrocarbon references ((*R*)-**96** and (*R*)-**97**) resulted in an increase in MES activity. However, the increase in MES activity was correlated with an increase in neurotoxicity. C(3)-Substitution of oxygen ((*R*)-**61**) with a methylene group ((*R*)-**96**) increased the anticonvulsant activity 1.6-fold (ED_{50} (mg/kg): (*R*)-**61**, 34; (*R*)-**96**, 21). C(4)-Substitution of oxygen ((*R*)-**74**) and sulfur ((*R*)-**76**) with a methylene group ((*R*)-**97**) increased the anticonvulsant activity 3–7-fold (ED_{50} (mg/kg): (*R*)-**74**, >160; (*R*)-**76**, 75 mg/kg; (*R*)-**97**, 23). (*R*)-**96** and (*R*)-**97** also displayed significant activity in the formalin test (ED_{50} (mg/kg): (*R*)-**96**, 35; (*R*)-**97**, 20). The considerable anticonvulsant activity and pain attenuation of the propyl and *n*-butyl PAADs prompted the pharmacological evaluation of the corresponding C(2)-methyl, -ethyl, -isopropyl, -*tert*-butyl, -2-methylpropyl, -cyclohexyl, and -benzyl PAADs. For PAADs **60** and **96**, we determined the anticonvulsant activity and NP protection for the (*R*)-, (*S*)-, and (*R,S*)-stereoisomers, and for **98** and **99**, the individual (*R*)- and (*S*)-enantiomers. During the C(2)-hydrocarbon investigation, we transitioned from pharmacological evaluation at UCB Pharma to pharmacological evaluation at the NINDS ASP. Several C(2)-hydrocarbons were evaluated at both facilities and the testing site is indicated in column two of Table 13. All of the PAADs evaluated at both UCB Pharma and the NINDS ASP are later presented in Table 18.

The C(2)-hydrocarbon PAADs displayed significant anticonvulsant activity in the MES test, and to a lesser degree in the 6 Hz test. MES activity slightly decreased as the length of the alkyl chain increased (ED_{50} (mg/kg): ethyl ((*R*)-**95**, 16) > propyl ((*R*)-**96**, 21) > *n*-butyl ((*R*)-**97**, 23)) but the MES activity slightly improved with an increase in branching (ED_{50}

(mg/kg): ethyl ((*R*)-**95**, 16) < isopropyl ((*R*)-**98**, 15) < *tert*-butyl ((*R*)-**99**, 13)). However, the anticonvulsant activity of (*R,S*)-**100** (ED_{50} = 46 mg/kg (100% reduction)) was lower than its unbranched constitutional isomer (*R*)-**97** (ED_{50} = 23 mg/kg). Substitution of the terminal methyl group of (*R*)-**95** (ED_{50} = 16 mg/kg) with a phenyl group ((*R*)-**102**, ED_{50} = 40 mg/kg) resulted in a 2.5-fold decrease in MES activity. The same trends were observed for anticonvulsant activity in the 6 Hz test, although 3–5-fold less sensitive. (*R*)-**95**, (*R*)-**96**, and (*R*)-**98** displayed excellent activity in the formalin test (ED_{50} (mg/kg): (*R*)-**95**, 22; (*R*)-**96**, 35; (*R*)-**98**, 20) and approached the formalin activity of (*R*)-**28** (ED_{50} = 15 mg/kg). We did not observe a correlation of C(2)-alkyl length and activity regarding formalin activity, as seen in the MES test. There was an initial 3-fold increase in activity from methyl to ethyl (ED_{50} (mg/kg): (*R*)-**60**, 69; (*R*)-**95**, 22) but then a steady decrease in activity was observed as the C(2)-alkyl length increased (ED_{50} (mg/kg): ethyl ((*R*)-**95**, 22) > propyl ((*R*)-**96**, 35) > *n*-butyl ((*R*)-**97**, >71). We observed an increase in activity in the formalin test upon branching. A three carbon C(2)-substituent displayed greater activity when in the isopropyl configuration ((*R*)-**98**, ED_{50} = 20 mg/kg) compared with the *n*-propyl configuration ((*R*)-**96**, ED_{50} = 35 mg/kg). Similarly, a four carbon C(2)-substituent displayed greater activity when in the *tert*-butyl configuration ((*R*)-**99**, ED_{50} >22 mg/kg) compared with the *n*-butyl configuration ((*R*)-**97**, ED_{50} >71 mg/kg). All C(2)-hydrocarbon PAADs that were tested as individual enantiomers (**60**, **96**, **98**, and **99**) revealed a 2–20-fold preference for anticonvulsant activity in the (*R*)-isomer. The isopropyl group at the C(2)-carbon displayed the largest (*R*)- versus (*S*)- selectivity (ED_{50} (mg/kg): (*R*)-**98**, 15; (*S*)-**98**, >300), but the stereoselectivity was diminished to 3-fold using the *tert*-butyl group (ED_{50} (mg/kg): (*R*)-**99**, 14; (*S*)-**99**, 42). The stereoselectivity of (*R*)-**98** resembles that of (*R*)-**28**, where (*R*)-**28** is ~22-fold more potent than its (*S*)-stereoisomer. The large variation in (*R*)- versus (*S*)-selectivity in activity for the C(2)-hydrocarbon series was unexpected. In the FAAs, the eudismic ratio[182] (the ratio of the affinity between the more tightly bound isomer [eutomer] and the less tightly bound isomer

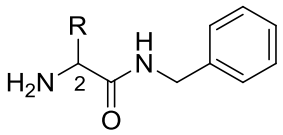
[distomer]) ranged between 10–22. We are uncertain why this ratio varied within the PAAD series, but suspect that multiple factors (e.g., site selectivity, metabolism, transport, efflux) affected the observed anticonvulsant activities for the individual stereoisomers. The protective indices in the MES test in mice (PI: TD_{50}/ED_{50}) of (*R*)-**98** (PI = 4.7) and (*R*)-**99** (PI = 4.7) are similar to (*R*)-**28** (5.8–6.0).

In summary, considering the MES activity, formalin activity, and protective indices in mice, (*R*)-**98** and (*R*)-**99** both approached the therapeutic potential of (*R*)-**28**, the FDA-approved FAA and benchmark for this study. The anticonvulsant activity of (*R*)-**98** was slightly improved in rats upon oral administration (ED_{50} = 11 mg/kg) and with no apparent behavioral toxicity (TD_{50} >500 mg/kg) (Table 14).

Comparison of the MES activities of PAADs (*R*)-, (*S*)-, and (*R,S*)-**60**, (*R*)-, and (*R,S*)-**95**, (*R,S*)-**96**, (*R*)-**98**, and (*R*)-**99** with their corresponding FAAs (*R*)-**234**, (*R*)-**235**, (*R*)-**236**, (*R*)-**237**, and (*R*)-**238**, respectively, in mice (ip) (Table 15) revealed similar activities of linear alkyl chains (i.e., (*R,S*)-**96**, ED_{50} = 39 mg/kg versus (*R,S*)-**236**, ED_{50} = 38 mg/kg) but there was a significant increase in PAAD activity of branched alkyl chains (i.e., (*R*)-**98**, 15 mg/kg versus (*R*)-**237**, ED_{50} >100, <300 mg/kg; (*R*)-**99**, 14 mg/kg versus (*R*)-**238**, ED_{50} >300 mg/kg). FAAs (*R*)-**237** and (*R*)-**238** were inactive (ED_{50} (mg/kg): (*R*)-**237**, >100, <300; (*R*)-**238**, >300) but the corresponding PAADs (*R*)-**98** and (*R*)-**99** displayed excellent activity (ED_{50} (mg/kg): (*R*)-**98**, 15; (*R*)-**99**, 14). Table 16 compared the MES activities of PAADs (*R,S*)-**60**, (*R,S*)-**96**, (*R*)-**98**, and (*R*)-**99** with their corresponding FAAs (*R*)-**234**, (*R*)-**236**, (*R*)-**237**, and (*R*)-**238**, respectively, in rats (po). It is difficult to make generalizations based on the limited PAAD rat data, but PAADs (*R*)-**60** and (*R*)-**98** displayed an increase in anticonvulsant activity compared with their FAA counterparts (ED_{50} (mg/kg): (*R*)-**60**, 14; (*R*)-**234**, 48; (*R*)-**98**, 11; (*R*)-**237**, >30). Unlike the increase in PAAD toxicity observed in mice, (*R*)-**60** and (*R*)-**98** did not display any behavioral toxicity in rats (TD_{50} >500 mg/kg).

The data from Tables 13–16 suggest that a C(2)-heteroatom one atom removed from the C(2) center is not necessary for PAAD anticonvulsant activity or NP protection, and that the activities of C(2)-hydrocarbon PAADs reside predominantly in the (*R*)-stereoisomer, but the effect of stereochemistry on activity was less pronounced than reported for FAAs except for (*R*)-**98**. Furthermore, the dramatic improvement in MES seizure protection for PAADs (*R*)-**98** and (*R*)-**99** compared with their FAA counterparts suggests that C(2)-hydrocarbon PAADs may function, in part, by different pharmacological pathways than other PAADs and the FAA class. If this is the case, the basic structural premise of the PAAD backbone (**59**) may be invalid. In Chapter 3, we test these assumptions. PAADs (*R*)-**98** and (*R*)-**99** from the C(2)-hydrocarbon series emerged as leading compounds. These PAADs surpassed the MES activity of the traditional antiepileptic phenobarbital (22 mg/kg) and are approaching the activity of the antiepileptic phenytoin (9.5 mg/kg). Therefore, we chose (*R*)-**98** and (*R*)-**99** for SAR optimization (Chapter 3).

Table 13. Pharmacological activities of C(2)-hydrocarbon *N*-benzylamide PAADs in mice (mg/kg) at UCB and the NINDS ASP

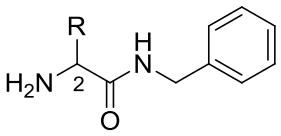


			Mice (ip)[a]						
Cmpd No.	**Test Site**	**R**	**MES,[b] ED_{50}**	**6 Hz,[c] ED_{50}**	**Formalin, ED_{50}**	**Tox,[d] TD_{50}**	**Com-ments[e]**	**PI,[f] MES**	**PI,[f] Form**
(*R*)-**28**	UCB	LCM	3.3	10	15	19	Ref	5.8	1.3
(*R*)-**28**[g]	NINDS	LCM	4.5 [0.5] (3.7–5.5)	10	ND[h]	27 [0.25] (26–28)	Ref	6.0	
(*R*)-**60**[i]	NINDS	CH_3	>10, <30	ND[h]	69	>100, <300			
(*S*)-**60**[i]	NINDS	CH_3	>300	ND[h]	ND[h]	>300			
(*R,S*)-**60**[i]	NINDS	CH_3	>100, <300	ND[h]	ND[h]	>300			
(*R*)-**95**	UCB	CH_2CH_3	16	62 (MAD)[j]	22	ND[h]			
(*R*)-**95**	NINDS	CH_2CH_3	18 [0.25] (10–25)	ND[h]	ND[h]	80 [0.25] (65–95)		4.4	
(*R*)-**96**	UCB	$(CH_2)_2CH_3$	21	66 (MAD)[j]	35	57	66 (LR)	2.8	1.6
(*S*)-**96**	UCB	$(CH_2)_2CH_3$	>37	>210	100	ND[h]			
(*R,S*)-**96**	UCB	$(CH_2)_2CH_3$	39	120 (MAD)[j]	68[k] (17%)	ND[h]	68 (T), 160 (IG, LR, C)		
(*R*)-**97**	UCB	$(CH_2)_3CH_3$	23	93	>71	ND[h]			

(*R*)-**98**	UCB	$CH(CH_3)_2$	16 (MAD)[j]	74	20	47			2.4
(*R*)-**98**	NINDS	$CH(CH_3)_2$	15 [0.25] (13–18)	<100 [0.25–1.0]	ND[h]	70 [0.25] (63–80)		4.7	
(S)-**98**	NINDS	$CH(CH_3)_2$	>300 [0.5]	ND[h]	ND[h]	>300 [0.5]			
(*R*)-**99**	UCB	$C(CH_3)_3$	13	>71	>22	ND[h]	70 (C)		
(*R*)-**99**	NINDS	$C(CH_3)_3$	14 [0.25] (11–17)	ND[h]	ND[h]	66 [0.25] (58–73)		4.7	
(*S*)-**99**	NINDS	$C(CH_3)_3$	42 [0.25] (37–46)	ND[h]	ND[h]	100 [0.25] (100–110)			
(*R,S*)-**100**	UCB	$CH(CH_3)CH_2CH_3$	46 (MAD)[j] 46[k] (100%)	>120	>22	ND[h]	70 (C,T,IG)		
(*R*)-**101**	UCB	C_6H_{11}	28	140 (MAD)[j]	25 (inactive) 79[k] (95%)	ND[h]	140 (C, MT)		
(*R*)-**102**	UCB	$CH_2C_6H_5$	40	81 (MAD)[j]	>37	ND[h]	120 (C)		

[a] The compounds were administered intraperitoneally to adult male NMRI mice under the auspices of UCB or administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration (UCB) or a dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets) (NINDS ASP). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] 6 Hz test = psychomotor seizure model (44 mA, UCB; 32 mA, NINDS ASP). [d] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [e] Dose in mg/kg is followed by whole animal pharmacological observation (Ref = reference, LR = loss of righting reflex, T = tremors, C = convulsions, IG = impaired gait, MT = mortality). [f] PI = protective index (TD_{50}/ED_{50}). [g] Choi, D. *et al. J. Med. Chem.* **1996**, *39*, 1907–1916. [h] ND = not determined. [i] Bégiun, C. *et al. Bioorg. Med. Chem*. **2004**, *12*, 3079–3096. [j] MAD = minimal active dose. [k] Single dose experiments where the mg/kg used is followed by the percentage protected in parenthesis.

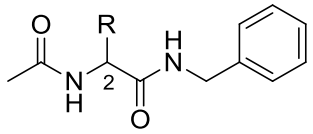
Table 14. Pharmacological activities of C(2)-hydrocarbon *N*-benzylamide PAADs in rats (mg/kg) at the NINDS ASP



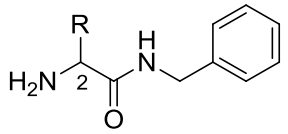
		Rat (po)[a]		
Cmpd No.	**R**	**MES,[b] ED_{50}**	**Tox,[c] TD_{50}**	**PI[d]**
(*R*)-**28**[f]	LCM	3.9 [0.5] (2.6–6.2)	>500 [0.5]	>130
(*R*)-**60**[g]	CH_3	19 [2.0] (13–25)	>30	>1.5
(*S*)-**60**[g]	CH_3	>80	>80	
(*R,S*)-**60**[g]	CH_3	14 [1.0] (7–22)	>500	>36
(*R*)-**98**	$CH(CH_3)_2$	11 [0.25] (9.1–13)	>500	>45
(*S*)-**99**	$C(CH_3)_3$	>30 [0.25–4.0]	>30 [0.25–4.0]	
phenytoin		30 [4.0] (22–39)	>3000	>100
phenobarbital		9.1 [5.0] (7.6–12)	61 [0.5] (44–96)	6.7
valproate		490 [0.5] (350–730)	280 [0.5] (190–350)	0.6

[a] The compounds were administered orally to adult male albino Sprague Dawley rats. ED_{50} and TD_{50} values are in mg/kg. A dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] Tox = behavioral toxicity. [d] PI = protective index (TD_{50}/ED_{50}). [f] Choi, D. *et al. J. Med. Chem.* **1996**, *39*, 1907–1916. [g] Béguin, C. *et al. Bioorg. Med. Chem*. **2004**, *12*, 3079–3096. [h] Porter, R.J. *et al. Cleveland Clin. Q.* **1984**, *51*, 293–305.

Table 15. Comparison of the pharmacological activities of C(2)-hydrocarbon FAAs and their PAAD counterparts in mice (mg/kg)



FAA

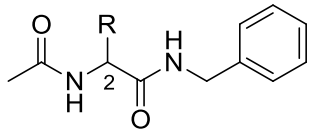


PAAD

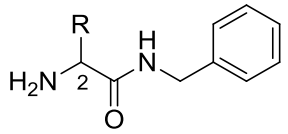
			Mice (ip)[a]				Mice (ip)[a]	
R	**FAA Cpd No.**	**FAA Test Site**	**FAA MES,[b] ED_{50}**	**FAA Tox,[c] TD_{50}**	**PAAD Cpd No.**	**PAAD Test Site**	**PAAD MES,[b] ED_{50}**	**PAAD Tox,[c] TD_{50}**
CH_3	(*R*)-**234**[d]	NINDS	55 [0.5] (50–60)	210 [0.5] (150–260)	(*R*)-**60**[e]	NINDS	>10, <30	>100, <300
CH_3	(*S*)-**234**[d]	NINDS	550 [0.5] (460–740)	840 [0.5] (690–950)	(*S*)-**60**[e]	NINDS	>300	>300
CH_3	(*R,S*)-**234**[d]	NINDS	76 [0.5] (67–89)	450 [0.5] (420–500)	(*R,S*)-**60**[e]	NINDS	>100, <300	>300
CH_2CH_3	(*R*)-**235**	ND[f]	ND[f]	ND[f]	(*R*)-**95**	NINDS	18 [0.25] (10–25)	80 [0.25] (65–95)
CH_2CH_3	(*R,S*)-**235**[g]	NINDS	>100, <300	>300	(*R,S*)-**95**	ND[f]	ND[f]	ND[f]
$CH_2CH_2CH_3$	(*R,S*)-**236**[h]	NINDS	38 [0.25] (35–45)	160 [0.25] (150–170)	(*R,S*)-**96**	UCB	39	ND[f]
$CH(CH_3)_2$	(*R*)-**237**[i]	NINDS	>100, <300	>300	(*R*)-**98**	NINDS	15 [0.25] (13–18)	70 [0.25] (63–80)
$C(CH_3)_3$	(*R*)-**238**[i]	NINDS	>300	>300	(*R*)-**99**	NINDS	14 [0.25] (11–17)	66 [0.25] (58–73)

[a] The compounds were administered intraperitoneally to adult male NMRI mice under the auspices of UCB or administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration (UCB) or a dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets) (NINDS ASP). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [d] Kohn, H. *et al. Brain Res*. **1988**, *457*, 371–375. [e] Béguin, C. *et al. Bioorg. Med Chem*. **2004**, *12*, 3079–3096. [f] ND = not determined. [g] Shen, M. *et al. J. Med. Chem*. **2002**, *45*, 2811–2823. [h] LeTiran, A. *et al. J. Med. Chem*. **2002**, *45*, 4762–4773. [i] Salomé, E. *et al. J. Med. Chem*. **2010**, *53*, 1288–1305.

Table 16. Comparison of the pharmacological activities of C(2)-hydrocarbon FAAs and their PAAD counterparts in rats (mg/kg)



FAA



PAAD

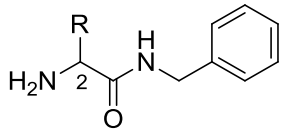
			Rat (po)[a]				Rat (po)[a]	
R	**FAA Cpd No.**	**FAA Test Site**	**FAA MES,[b] ED_{50}**	**FAA Tox,[c] TD_{50}**	**PAAD Cpd No.**	**PAAD Test Site**	**PAAD MES,[b] ED_{50}**	**PAAD Tox,[c] TD_{50}**
CH_3	(*R,S*)-**234**[d]	NINDS	48 [1.0] (32–72)	>1000	(*R,S*)-**60**[e]	NINDS	14 [1.0] (7–22)	>500
$CH_2CH_2CH_3$	(*R,S*)-**236**[e]	NINDS	~30	>30	(*R,S*)-**96**	UCB	ND[f]	ND[f]
$CH(CH_3)_2$	(*R*)-**237**	NINDS	>30 [0.25–4.0]	>30 [0.25–4.0]	(*R*)-**98**	NINDS	11 [0.25] (9.1–13)	>500
$C(CH_3)_3$	(*R*)-**238**	NINDS	>30 [0.25–4.0]	>30 [0.25–4.0]	(*R*)-**99**	NINDS	ND[f]	ND[f]

[a] The compounds were administered orally to adult male albino Sprague Dawley rats. ED_{50} and TD_{50} values are in mg/kg. A dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] Tox = behavioral toxicity. [d] Béguin, C. *et al. Bioorg. Med Chem*. **2004**, *12*, 3079–3096. [e] LeTiran, A. *et al. J. Med. Chem*. **2002**, *45*, 4762–4773. [f] ND = not determined.

2.3.4. C(2) Stereochemistry and pharmacological activity

PAADs evaluated as individual stereoisomers from Tables 5, 12, and 13 are presented in Table 17 to assess the importance of PAAD stereochemistry on neurological activity in mice. A global assessment revealed that MES activity resided predominantly in the (*R*)-isomer. The general insensitivity of PAADs to the 6 Hz test prevents the determination of stereochemical preference in this model. Similarly, although there are several examples of C(2)-hydrocarbon PAADs that displayed significant activity in the formalin test, a lack of data from the corresponding (*S*)-isomers prevents any generalized conclusions of (*R*) versus (*S*) selectivity. Not surprisingly, the PAADs that were active in the MES test showed higher neurological toxicity associated with the (*R*)-configuration compared with the (*S*)-configuration. Collectively, PAADs follow the FAA anticonvulsant activity trend with respect to stereochemistry in the MES test. However, the degree of separation between (*R*) versus (*S*) specificity is typically 3–4-fold in PAADs compared with the 10–20-fold difference in the FAAs.

Table 17. Pharmacological activities of C(2)-*N*-benzylamide PAAD chiral sets in mice (mg/kg) at UCB and the NINDS ASP



			Mice (ip)[a]						
Cmpd No.	**Test Site**	**R**	**MES[b], ED_{50}**	**6 Hz,[c] ED_{50}**	**Formalin, ED_{50}**	**Tox[d], TD_{50}**	**Comments[e]**	**PI[f], MES**	**PI[f], Form**
(*R*)-**28**	UCB	LCM	3.3	10	15	19	Ref	5.8	1.3
(*R*)-**28**[g]	NINDS	LCM	4.5 [0.5] (3.7–5.5)	10	ND[h]	27 [0.25] (26-28)	Ref	6.0	
(*S*)-**28**[g]	NINDS	LCM	>100, <300	ND[h]	ND[h]	>300	Ref		
(*R*)-**65**	UCB	CH_2OH	ND[h]	>62	>62	>110			
(*S*)-**65**	UCB	CH_2OH	ND[h]	>62	>62	>110			
(*R,S*)-**65**	UCB	CH_2OH	ND[h]	>62	>62	>110			
(*R*)-**61**	UCB	CH_2OCH_3	34	>67	>67	>120		>3.5	
(*R*)-**61**	NINDS	CH_2OCH_3	48 [0.25] (40–61)	ND[h]	ND[h]	>30, <100 [0.25]			
(*R*)-**61**[i]	NINDS	CH_2OCH_3	>30, <100	ND[h]	ND[h]	>100, <300			
(*S*)-**61**	UCB	CH_2OCH_3	64	>70	120	63		1.0	0.5
(*R*)-**60**[j]	NINDS	CH_3	>10, <30	ND[h]	ND[h]	>100, <300			
(*S*)-**60**[j]	NINDS	CH_3	>300	ND[h]	ND[h]	>300			
(*R,S*)-**60**[j]	NINDS	CH_3	>100, <300	ND[h]	ND[h]	>300			

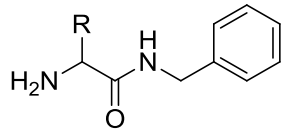
(*R*)-**96**	UCB	$CH_2CH_2CH_3$	21	66 (MAD)[k]	35	57	66 (LR)	2.8	1.6
(*S*)-**96**	UCB	$CH_2CH_2CH_3$	>37	>210	100	ND[h]			
(*R,S*)-**96**	UCB	$CH_2CH_2CH_3$	39	120 (MAD)[k]	68[l] (17%)	ND[h]	68 (T), 160 (IG, LR, C)		
(*R*)-**98**	UCB	$CH(CH_3)_2$	16 (MAD)[k] 16[l] (100%)	74	20	47	320 (S, H)	2.9	2.4
(*R*)-**98**	NINDS	$CH(CH_3)_2$	15 [0.25] (13–18)	<100 [0.25–1.0]	ND[h]	70 [0.25] (63–80)		4.7	
(*S*)-**98**	NINDS	$CH(CH_3)_2$	>300 [0.5]	ND[h]	ND[h]	>300 [0.5]			
(*R*)-**99**	UCB	$C(CH_3)_3$	13	>71	>22	ND[h]	70 (C)		
(*R*)-**99**	NINDS	$C(CH_3)_3$	14 [0.25] (11–17)	ND[h]	ND[h]	66 [0.25] (58–73)		4.7	
(*S*)-**99**	NINDS	$C(CH_3)_3$	42 [0.25] (37–46)	ND[h]	ND[h]	100 [0.25] (100–110)			
(*R*)-**62**	UCB	C_6H_5	43 (MAD)[k]	>24	24[l] (61%)	ND[h]	77 (MT)		
(*S*)-**62**	UCB	C_6H_5	ND[h]	>77	110	140 (MTD)[m]	>140 (C)		
(*R,S*)-**62**	UCB	C_6H_5	>30	>43	24[l] (37%)	51	77 (C), 140 (MT)		

[a] The compounds were administered intraperitoneally to adult male NMRI mice under the auspices of UCB or administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration (UCB) or a dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets) (NINDS ASP). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] 6 Hz test = psychomotor seizure model (44 mA, UCB; 32 mA, NINDS ASP). [d] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [e] Dose in mg/kg is followed by whole animal pharmacological observation (Ref = reference, LR = loss of righting reflex, T = tremors, C = convulsions, S = sedation, H = hypothermia, MT = mortality). [f] PI = protective index (TD_{50}/ED_{50}). [g] Choi, D. *et al. J. Med. Chem.* **1996**, *39*, 1907–1916. [h] ND = not determined. [i] Andurkar, S.V. *et al. Tetrahedron: Asymmetry* **1998**, *9*, 3841–3854. [j] Béguin, C. *et al. Bioorg. Med. Chem*. **2004**, *12*, 3079–3096. [k] MAD = minimal active dose. [l] Single dose experiments where the mg/kg used is followed by the percentage protected in parenthesis. [m] MTD = maximal tolerated dose.

2.3.5. Comparison of the data acquired at UCB and the NINDS ASP

The expiration of contractual obligations with UCB Pharma did not coincide with the conclusion of this project and we shifted the pharmacological evaluation of PAADs from UCB Pharma to the NINDS ASP. However, to allow our comparison of the data from different testing facilities, we determined the pharmacological activities of several active PAADs at both sites. Accordingly, (*R*)-**61**, (*R*)-**95**, (*R*)-**98**, and (*R*)-**99** were evaluated at UCB Pharma and the NINDS ASP (Table 18). We chose (*R*)-**61** due its direct connection to (*R*)-**28**, and (*R*)-**95**, (*R*)-**98**, and (*R*)-**99** because they displayed the highest activities in the MES test. Table 18 shows that the MES activities obtained from UCB Pharma and the NINDS ASP were in excellent agreement. However, there is a variance on the neurological toxicities reported, leading to different protective indices (PI). The PI values obtained from the NINDS ASP are consistently higher than those reported from UCB Pharma but differ by less than a factor of two. Overall, the data from UCB Pharma, and the NINDS ASP are consistent and comparisons between the two testing facilities are made in confidence. We build upon this table in Chapter 3 to include optimized PAADs.

Table 18. Comparison of the pharmacological activities of PAADs evaluated in mice (mg/kg) at UCB and the NINDS-ASP



		UCB[a]			NINDS-ASP[b]		
Cmpd No.	**R**	**MES,[c] ED_{50}**	**Tox,[d] TD_{50}**	**PI[e]**	**MES,[c] ED_{50}**	**Tox,[d] TD_{50}**	**PI[e]**
(*R*)-**28**	LCM	3.3	19	5.8	4.5 [0.5] (3.7–5.5)	27 [0.25] (26–28)	6.0
(*R*)-**61**	CH_2OCH_3	34	>117	>3.4	48 [0.25] (40–61)	>30, <100 [0.25]	
(*R*)-**95**	CH_2CH_3	16	46	2.9	18 [0.25] (10–25)	80 [0.25] (65–95)	4.4
(*R*)-**98**	$CH(CH_3)_2$	16 (MAD)[f]	47	2.9	15 [0.25] (13–18)	70 [0.25] (63–80)	4.8
(*R*)-**99**	$C(CH_3)_3$	13	ND[g]		14 [0.25] (11–17)	66 [0.25] (58–73)	4.7

[a] The compounds were administered intraperitoneally to adult male NMRI mice under the auspices of UCB. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration. [b] The compounds were administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg. A dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets). Numbers in parentheses are 95% confidence intervals. [c] MES = maximal electroshock seizure test. [d] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [e] PI = protective index (TD_{50}/ED_{50}). [f] MAD = minimal active dose. [g] ND = not determined.

3. Conclusions

We have developed the PAAD SAR from the pharmacological evaluation of more than 50 compounds in whole animal models of epilepsy and NP. We examined select substituents at the C(2) carbon (CH_2OR, $CH_2N(R)R'$, CH_2CH_2XR, 6-membered aromatic and heteroaromatic, 5-membered heteroaromatic, and hydrocarbon) that tested the requirement for a heteroatom one atom removed from the C(2) center, the need for heteroatom substitution, and the stereochemical preference at the C(2) position. The SAR suggested that PAAD activity, unlike FAAs, does not improve with the inclusion of a substituted heteroatom one atom removed from the C(2) center. Although the PAADs that displayed the highest anticonvulsant activity in the MES test are 4–10-fold less active than (*R*)-**28**, four hydrocarbon PAADs (ED_{50} (mg/kg): (*R*)-**95**, 16; (*R*)-**96**, 21; (*R*)-**98**, 15; and (*R*)-**99**, 13) surpassed the MES activity observed for the traditional antiepileptic agent phenobarbital (ED_{50} = 22 mg/kg), and the majority of PAADs tested surpassed the MES activity observed for the traditional antiepileptic agent valproate (270 mg/kg).

The original testing paradigm at UCB Pharma consisted of only the 6 Hz assay for anticonvulsant activity, but the MES test was included to serve as a complementary approach because each assay has shown different sensitivities to distinct classes of antiepileptic agents. A prime example is the efficacy of levetiracetam in the 6 Hz test but not in the MES test.[178] Conversely, traditional antiepileptic agents are active in the MES test but are largely inactive in the 6 Hz test.[183] The inclusion of the MES test proved beneficial in determining the anticonvulsant activity of PAADs since many were insensitive to the 6 Hz test.

The finding that the C(2)-hydrocarbon PAADs (*R*)-**98** (ED_{50} = 15 mg/kg) and (*R*)-**99** (ED_{50} = 14 mg/kg) exhibited superb protection in the MES test in mice was surprising. Their corresponding FAAs were inactive in the MES test (ED_{50} (mg/kg): (*R*)-**98**, >100, <300; (*R*)-

99, >300). These findings raised the intriguing thought that these PAADs may function by different pathways from FAAs, and perhaps other PAADs. (*R*)-**95**, (*R*)-**96**, (*R*)-**98**, and (*R*)-**99** also displayed significant activity in the formalin test (ED_{50} <35 mg/kg), and were only 1.3–2.3-fold less active than (*R*)-**28** in this test. Evaluation of the individual stereoisomers of PAADs **61**, **62**, **96**, **98**, and **99** demonstrated that the (*R*)-stereoisomer exhibited greater anticonvulsant activity and pain attenuation than the (*S*)-isomer for these compounds. Nonetheless, we observed a general decrease in (*R*)- versus (*S*)- stereospecificity in the PAADs (3–4-fold) compared with the FAAs (10–20-fold), except for (*R*)-**98**, where the eudismic ratio was greater than 20. The modest (*R*)- versus (*S*)- difference in activities for the PAADs raises the possibility that factors (e.g. metabolism, transport, efflux) other than interaction with the site of function(s) may be responsible for this difference. The potency of small branched hydrocarbon PAADs (*R*)-**98** and (*R*)-**99** in both the MES and formalin tests, and the importance of the lacosamide PAAD (*R*)-**61** to both the PAAD and FAA projects, prompted their selection as candidates for optimization studies. We will explore structural modifications of the *N*-terminal amine and *N'*-benzylamide moiety to determine their effects on anticonvulsant activity and pain attenuation.

In addition to the C(2)-hydrocarbon PAADs that displayed significant activity in the formalin test (ED_{50} (mg/kg): (*R*)-**95**, 22; (*R*)-**96**, 35; (*R*)-**98**, 20; (*R*)-**99**, 22), several C(2)-6-membered and 5-membered heteroaromatic PAADs also displayed significant activity in the same animal model (ED_{50} (mg/kg): (*R,S*)-**77**, 33; (*R,S*)-**82**, 29; (*R,S*)-**85**, 42, (*R,S*)-**89**, 29). PAADs (*R,S*)-**77**, (*R,S*)-**82**, and (*R,S*)-**89** were only 2-fold less active than (*R*)-**28** in the formalin test (ED_{50} = 15 mg/kg). However, it was unexpected that these PAADs were 1.5–3-fold selective for pain attenuation over anticonvulsant activity (MES ED_{50} (mg/kg), formalin ED_{50} (mg/kg): (*R,S*)-**77**, 100 (MAD), 33; (*R,S*)-**82**, >52, 29; (*R,S*)-**85**, >120, 42; (*R,S*)-**89**, 85, 29). The C(2)-6-membered and 5-membered heteroaromatic PAADs do not provide any significant advantage for the prevention of seizures compared with FAAs (~10-fold drop in

activity going from FAA to PAAD) but optimized C(2)-6-membered and 5-membered heteroaromatic PAADs could possess therapeutic value as pain modulating agents. Unfortunately, we cannot determine if this pattern is unique to PAADs due to the lack of formalin data in the FAA series.

Comparison of the PAAD pharmacological data with their corresponding FAAs uncovered complicated SAR trends. First, it was clear that the inclusion of a heteroatom one atom removed from the C(2)-center in PAADs did not afford the same seizure protection as their FAA equivalent. On the contrary, C(2)-hydrocarbon PAADs displayed the greatest anticonvulsant activity in the PAAD series. However, there was a similar trend between the MES activities of C(3)-alkoxy PAADs and C(3)-alkoxy FAAs, but the activity was decreased ~10-fold in magnitude and there was a sharp drop in C(2) (*R*)-stereospecificity for seizure protection. Therefore, C(3)-alkoxy PAADs could be interacting with the same receptor binding sites as C(3)-alkoxy FAAs, but with either a lower affinity or the relative brain concentration of the PAADs may be lower than that for FAAs at a given dose. We were unable to correlate the activities of C(3)-amino PAADs and C(3)-amino FAAs due to the limited data set. It is unclear whether C(2)-6-membered and 5-membered heteroaromatic PAADs are functioning in a similar manner to their FAA counterparts. The C(2)-heteroaromatic PAAD series is 5–10-fold less sensitive in the MES test compared with FAAs (similar to that seen for C(3)-alkoxy PAADs), but ambiguous data points (minimal active dose) prevents reliable comparison with the FAAs. Moreover, when comparing the C(2)-heteroaromatic PAADs and FAAs, we observed that benzoannulation in the former series resulted in anticonvulsant activities that either remained the same or improved, while in the latter set of compounds benzoannulation led to a precipitous drop in anticonvulsant activity. Therefore, while these PAADs may function at many of the same receptor sites used by FAAs, we cannot exclude the possibility of interaction with other receptor sites that affect NP

and possibly seizures. Lastly, the unexpected excellent activity for C(2)-hydrocarbon PAADs suggests binding interactions could be occurring at receptors not involved with FAA function.

4. Experimental

4.1. General methods

Melting points were determined in open capillary tubes using a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on an ATI Mattson Genesis FT-IR spectrometer. Absorption values are expressed in wavenumbers (cm^{-1}). Optical rotations were obtained on a Jasco P-1030 polarimeter at the sodium D line (589 nm) using a 1 dm path length cell. NMR spectra were recorded at 300 or 400 MHz (^{1}H) and 75 or 100 MHz (^{13}C) using tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are reported in parts per million (ppm) from TMS. Low-resolution mass spectra (LRMS) were recorded with a BioToF-II-Bruker Daltonics spectrometer by Drs. M. Crowe and S. Habibi at the University of North Carolina Department of Chemistry. The high-resolution mass spectra (HRMS) were recorded on a Bruker Apex-Q 12 Telsa FTICR spectrometer by Drs. M. Crowe and S. Habibi. Microanalyses were performed by Atlantic Microlab, Inc. (Norcross, GA). Reactions were monitored by analytical thin-layer chromatography (TLC) plates (Aldrich, catalog no. Z12272-6, or Dynamic Adsorbents Inc., catalog no. 84111) and analyzed with 254 nm light. The reaction mixtures were purified by medium pressure liquid chromatography (MPLC, CombiFlash *Rf*) with self-packed columns (silica gel from Dynamic Adsorbents Inc., catalog no. 02826-25) or by flash column chromatography using silica gel (Dynamic Adsorbents Inc., catalog no. 02826-25). All chemicals and solvents were reagent grade and used directly from commercial sources without further purification. THF was distilled from blue sodium benzophenone ketyl. Yields reported are for purified products and were not optimized. All compounds were checked by

TLC, ^{1}H and ^{13}C NMR, MS, and elemental analyses. The analytical results are within 0.40% of the theoretical value. The TLC, NMR, and analytical data confirmed the purity of the products was ≥95%.

General Procedure for the Preparation of *N*-Benzyloxycarbonyl Amino Acids and *N*-Benzyloxycarbonyl Amino Methyl Esters (Method A). $NaHCO_3$ (2.5–3.3 equiv) was added to an aqueous solution of amino acid (0.4–0.5 M) and the solution was cooled to 0 ºC in an ice bath under an inert atmosphere (Ar or N_2) before benzylchloroformate (1.1–1.5 equiv) was added dropwise. The reaction proceeded for 45 min at 0 ºC and then allowed to warm to room temperature (24 h). Amino acid derivatives: The aqueous layer was washed with EtOAc (3x), acidified to pH ~1 with aqueous concentrated HCl, and extracted with EtOAc (3x). The second set of organic layers were combined, dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was used for the next step without further purification or purified by recrystallization from hot EtOAc/hexanes. Amino methyl ester derivatives: The aqueous layer was extracted with CH_2Cl_2 (3x), dried (Na_2SO_4), concentrated *in vacuo*, and purified by column chromatography (SiO_2).

General Procedure for the Preparation of *N*-Benzylamide Amino Acid Derivatives Using the Mixed Anhydride Coupling (MAC) Method (Method B). An anhydrous THF solution of carboxylic acid (0.5–2.0 M) was cooled to -78 ºC in a dry ice/acetone bath under an inert atmosphere (Ar or N_2), and 4-methylmorpholine (NMM) (1.3–1.5 equiv) was added. After the mixture was stirred (2–10 min), isobutyl chloroformate (IBCF) (1.1–1.5 equiv) was added leading to the precipitation of a white solid. The reaction was allowed to proceed for an additional 15–25 min, and then benzylamine (1.05–1.36 equiv) was added at -78 ºC. The reaction mixture was allowed to stir at room temperature (1.5 h), and then the insoluble salts

were filtered. The organic layer was concentrated *in vacuo*, and the product was purified by column chromatography (SiO_2).

General Procedure for the Conversion of Cbz-Protected PAADs to PAADs Using Pd-Catalyzed Hydrogenation (Method C). A MeOH solution of Cbz-protected PAAD (0.05–0.1 M) was hydrogenated (1 atm) in the presence of 10% Pd-C at room temperature (3 h–7 d). The mixture was filtered through a bed of Celite®, the filtrate was evaporated *in vacuo*, and the product was purified by column chromatography (SiO_2).

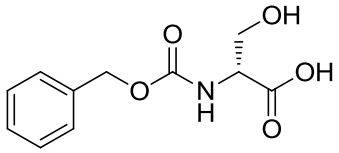
General Procedure for the Preparation of Alkoxy-Substituted Cbz-PAADs Using Silver (I) Oxide-Mediated Alkylation (Method D). To a CH_3CN solution of Cbz-protected PAAD (0.01–0.1 M) were successively added Ag_2O (5 equiv) and the alkyl iodide (10 equiv) at room temperature. The reaction mixture was stirred (room temperature-50 ºC, 5–7 d) and then the insoluble salts were filtered and the filtrate evaporated *in vacuo*. The product was purified by column chromatography (SiO_2) followed by recrystallization.

General Procedure for PAAD Preparation Using TFA Deprotection (Method E). TFA (15 equiv) was added to an anhydrous CH_2Cl_2 solution of the *N*-*t*-butoxycarbonyl *N*'-benzylamide (0.3 M) at room temperature. The solution was stirred (1 h) and then the solvent was evaporated *in vacuo*. The crude product was diluted with CH_2Cl_2 and extracted with aqueous 1 M HCl (3x). The combined aqueous layers were washed with CH_2Cl_2 (2x), basified (pH 10–12) with aqueous 4 M NaOH, and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with brine (2x), dried (Na_2SO_4), evaporated *in vacuo*, and purified by column chromatography (SiO_2).

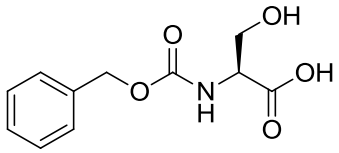
General Procedure for the Preparation of *N*-(Benzyloxycarbonyl)-3-*N*-Aminopropanoic Methyl Ester Derivatives Using the Michael Addition of Amines to Dehydroalanine Derivatives (Method F). An anhydrous CH_2Cl_2 solution of serine methyl ester (0.2 M) was cooled to 0 ºC under an inert atmosphere (Ar or N_2), and triethylamine (Et_3N) (1.2 equiv) was added. After the mixture was stirred (10 min), methanesulfonyl chloride (MsCl) (1.2 equiv) was added. The reaction was allowed to proceed for an additional 1 h, and then Et_3N (1.2 equiv) was added at 0 ºC. The reaction was allowed to stir at room temperature (3 h), and then the solvent was evaporated *in vacuo* to give the crude Cbz-dehydroalanine-methyl ester. An anhydrous primary, secondary, or tertiary amine solution in THF (5–20 equiv) and MeOH (1:1) was added directly to the crude material and stirred overnight. The organic layer was concentrated *in vacuo* to give the crude product, which was diluted with CH_2Cl_2, successively washed with saturated aqueous Na_2CO_3 (3x) and brine (2x), dried (Na_2SO_4), concentrated *in vacuo*, and purified by column chromatography (SiO_2).

General Procedure for the Preparation of *N*-Benzylamide Amino Acid Derivatives Using the LiOH Hydrolysis/DMTMM Amide Coupling Method (Method G). Methyl ester (0.1 M) was dissolved in THF and H_2O (1:2) and LiOH (1 equiv) was added. The reaction was stirred at room temperature (1.5 h) and then benzylamine hydrochloride (1.2 equiv) and DMTMM (1.2 equiv) were added and the solution stirred overnight (18 h). The organic layer was evaporated *in vacuo*, and CH_2Cl_2 was added to the remaining aqueous layer. The binary mixture was basified (pH 9–10) with aqueous 1 M NaOH, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3x). The organic layers were combined, washed with brine (3x), dried (Na_2SO_4), and purified by column chromatography.

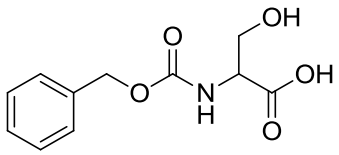
4.2. Synthesis



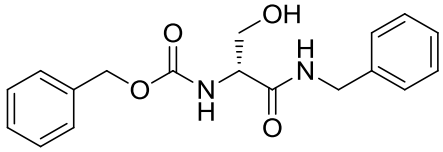
(*R*)-*N*-(Benzyloxycarbonyl)-2-amino-3-hydroxypropionic Acid ((*R*)-104).[162] Utilizing Method A and using D-serine (5.00 g, 47.6 mmol), $NaHCO_3$ (10.0 g, 118.9 mmol), H_2O (100 mL) and benzyl chloroformate (10.0 mL, 71.4 mmol) gave the crude product after workup that was further purified by recrystallization from hot EtOAc/hexanes to give the desired product (7.38 g, 65%) as a white solid: mp 115–116 °C (lit.[162] mp 117–119 ºC); $[\alpha]^{25}_D$ –5.12° (*c* 6.0, acetic acid) (lit.[184] (*S*): $[\alpha]^{12}_D$ +5.8º (*c* 6, acetic acid)); R_f = 0.43 (1:3 MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.67 (d, J = 4.2 Hz, C**H**$_2$OH), 4.03–4.09 (m, C**H**), 4.83–4.96 (br s, N**H**), 5.04 (s, C**H**$_2$Ph), 7.32–7.38 (m, Ph**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 56.7 (**C**H), 61.4 (**C**H$_2$OH), 65.5 (**C**H$_2$Ph), 127.7, 127.8, 128.4, 137.0 (**C**$_6$H$_5$), 156.1 (O**C**(O)NH), 172.2 (**C**(O)OH).



(*S*)-*N*-(Benzyloxycarbonyl)-2-amino-3-hydroxypropionic Acid ((*S*)-104).[184] The previous procedure was repeated using L-serine (5.00 g, 47.6 mmol), $NaHCO_3$ (10.0 g, 118.9 mmol), H_2O (100 mL), and benzyl chloroformate (10.0 mL, 71.4 mmol) to give the desired product (7.94 g, 70%) as a white solid: mp 116–117 °C (lit.[184] mp 117–119 ºC); $[\alpha]^{25}_D$ +5.51° (*c* 6.0, acetic acid) (lit.[184] $[\alpha]^{12}_D$ +5.8º (*c* 6, acetic acid)); R_f = 0.41 (1:3 MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.66 (d, J = 4.5 Hz, C**H**$_2$OH), 4.02–4.09 (m, C**H**), 4.83–4.94 (br s, N**H**), 5.04 (s, C**H**$_2$Ph), 7.29–7.38 (m, Ph**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 56.6 (**C**H), 61.3 (**C**H$_2$OH), 65.4 (**C**H$_2$Ph), 127.7, 127.8, 128.3, 137.0 (**C**$_6$H$_5$), 156.0 (O**C**(O)NH), 172.1 (**C**(O)OH).

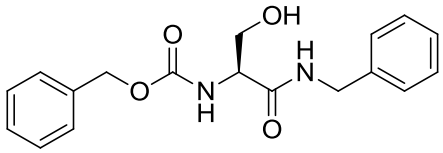


(*R,S*)-*N*-(Benzyloxycarbonyl)-2-amino-3-hydroxypropionic Acid ((*R,S*)-104).[185] The previous procedure was repeated using DL-serine (20.00 g, 0.19 mol), $NaHCO_3$ (40.00 g, 0.48 mol), H_2O (400 mL), and benzyl chloroformate (40.0 mL, 0.29 mol) to give the desired product (38.04 g, 84%) as a white solid: mp 124–125 °C (lit.[185] mp 120–122 ºC); R_f = 0.39 (1:3 MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.66 (d, J = 4.8 Hz, C**H**$_2$OH), 4.03–4.09 (m, C**H**), 4.72–4.97 (br s, N**H**), 5.04 (s, C**H**$_2$Ph), 7.28–7.40 (m, Ph**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 56.6 (**C**H), 61.3 (**C**H$_2$OH), 65.4 (**C**H$_2$Ph), 127.7, 127.8, 128.3, 137.0 (**C**$_6$H$_5$), 156.0 (O**C**(O)NH), 172.1 (**C**(O)OH).

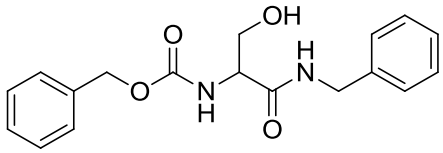


(*R*)-*N*-Benzyl 2-*N*-(Benzyloxycarbonyl)amino-3-hydroxypropionamide ((*R*)-105).[89] Utilizing Method B, (*R*)-*N*-(benzyloxycarbonyl)-2-amino-3-hydroxypropionic acid (5.00 g, 20.9 mmol), NMM (2.98 mL, 27.2 mmol), IBCF (2.98 mL, 23.0 mmol), and benzylamine (2.40 mL, 21.9 mmol) gave the crude product that was purified by flash column chromatography (SiO_2; 1:20 MeOH/$CHCl_3$) to give the desired product (5.16 g, 75%) as a white solid: mp 146–147 °C (lit.[89] mp 147–149 ºC); $[\alpha]^{25}_D$ +5.1° (*c* 2.1, MeOH) (lit.[89] $[\alpha]^{23}_D$ +4.6° (*c* 2.0, MeOH); R_f = 0.44 (5% MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.50–3.67 (m, C**H**$_2$OH), 4.06–4.13 (m, C**H**), 4.30 (d, J = 6.3 Hz, NHC**H**$_2$), 4.90 (t, J = 5.7 Hz, O**H**), 5.04 (s, C**H**$_2$OC(O)), 7.20–7.38 (m, Ph**H** and OC(O)N**H**), 8.42 (t, J = 6.0 Hz, C(O)N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 42.0 (**C**H$_2$NH), 57.4 (**C**H), 61.8 (**C**H$_2$OH), 65.5 (O**C**H$_2$Ph),

126.6, 127.0, 127.7, 127.8, 128.2, 128.3, 137.0, 139.3 (2 $\mathbf{C}_6H_5$), 155.9 (**C**(O)O), 170.2 (**C**(O)NH).

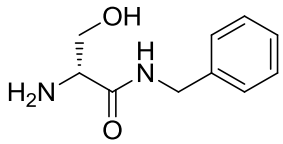


(*S*)-*N*-Benzyl 2-*N*-(Benzyloxycarbonyl)amino-3-hydroxypropionamide ((*S*)-105).[98] The previous procedure was repeated using (*S*)-*N*-(benzyloxycarbonyl)-2-amino-3-hydroxypropionic acid (5.00 g, 20.9 mmol), NMM (2.98 mL, 27.2 mmol), IBCF (2.98 mL, 23.0 mmol), and benzylamine (2.40 mL, 21.9 mmol) to give the desired product (4.84 g, 71%) as a white solid: mp 148–149 °C (lit.[98] mp 148–149.5 ºC); $[\alpha]^{25}_D$ –5.8° (*c* 2.1, MeOH) (lit.[98] $[\alpha]^{23}_D$ –5.4° (*c* 1.04, MeOH)); R_f = 0.45 (1:20 MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.54–3.68 (m, C$\mathbf{H}_2$OH), 4.06–4.13 (m, C**H**), 4.30 (d, *J* = 6.0 Hz, NHC$\mathbf{H}_2$), 4.90 (t, *J* = 5.4 Hz, O**H**), 5.04 (s, C$\mathbf{H}_2$OC(O)), 7.20–7.38 (m, Ph**H** and OC(O)N**H**), 8.42 (t, *J* = 6.3 Hz, C(O)N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 42.0 (**C**H_2NH), 57.3 (**C**H), 61.8 (**C**H_2OH), 65.5 (O**C**H_2Ph), 126.6, 127.0, 127.7, 127.8, 128.2, 128.3, 137.0, 139.3 (2 $\mathbf{C}_6H_5$), 155.9 (**C**(O)O), 170.1 (**C**(O)NH).

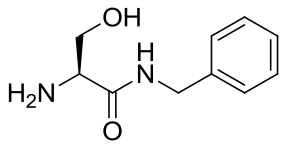


(*R,S*)-*N*-Benzyl 2-*N*-(Benzyloxycarbonyl)amino-3-hydroxypropionamide ((*R,S*)-105).[186] The previous procedure was repeated using (*R,S*)-*N*-(benzyloxycarbonyl)-2-amino-3-hydroxypropionic acid (10.00 g, 41.8 mmol), NMM (5.97 mL, 54.4 mmol), IBCF (5.96 mL, 46.0 mmol), and benzylamine (4.79 mL, 43.9 mmol) to give the desired product (12.25 g, 89%) as a white solid: mp 140–141 °C (lit.[186] mp 142 ºC); R_f = 0.33 (1:20 MeOH/$CHCl_3$); ^{1}H

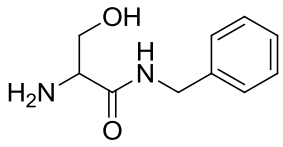
NMR (300 MHz, DMSO-d_6) δ 3.54–3.67 (m, C**H**$_2$OH), 4.06–4.13 (m, C**H**), 4.30 (d, J = 6.0 Hz, NHC**H**$_2$), 4.90 (t, J = 5.7 Hz, O**H**), 5.02 (s, C**H**$_2$OC(O)), 7.20–7.38 (m, Ph**H** and OC(O)N**H**), 8.42 (t, J = 6.0 Hz, C(O)N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 42.0 (**C**H$_2$NH), 57.3 (**C**H), 61.8 (**C**H$_2$OH), 65.5 (O**C**H$_2$Ph), 126.6, 127.0, 127.7, 128.2, 128.3, 137.0, 139.3 (2 **C**$_6$H$_5$), 155.9 (**C**(O)O), 170.1 (**C**(O)NH), one aromatic peak was not detected and is believed to overlap with nearby signals.



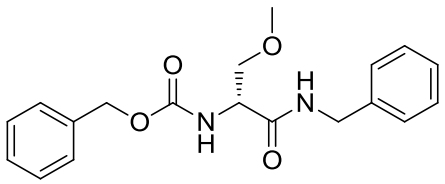
(*R*)-*N*-Benzyl 2-Amino-3-hydroxypropionamide ((*R*)-65). Utilizing Method C and using (*R*)-*N*-benzyl 2-*N*-(benzyloxycarbonyl)amino-3-hydroxypropionamide (1.82 g, 5.53 mmol), 10% Pd-C (180 mg), and MeOH (200 mL) (8 h) gave the crude product that was purified by medium pressure liquid chromatography to give the desired product (0.56 g, 53%) as a white solid: mp 95–96 °C; $[\alpha]^{25}_{D}$ –0.48° (*c* 1.5, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.84 (br s, N**H**$_2$), 3.25 (dd, J = 4.8, 4.8 Hz, C**H**), 3.42–3.56 (m, C**H**$_2$OH), 4.29 (d, J = 6.0 Hz, C**H**$_2$NH), 4.72–4.86 (br s, O**H**), 7.20–7.33 (m, Ph**H**), 8.35 (t, J = 6.0 Hz, N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 41.8 (NH**C**H$_2$), 57.0 (**C**H), 64.4 (**C**H$_2$OH), 126.6, 127.1, 128.2, 139.6 (**C**$_6$H$_5$), 173.4 (**C**(O)NH); HRMS (ESI) 217.0953 [M + Na$^+$] (calcd for $C_{10}H_{14}N_2O_2Na^+$ 217.0953); Anal. Calcd for $C_{10}H_{14}N_2O_2$: C, 61.84; H, 7.27; N, 14.42. Found C, 61.94; H, 7.27; N, 14.31.



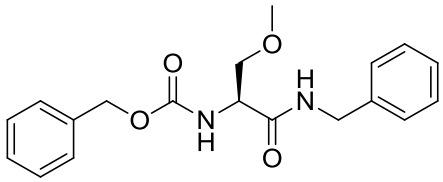
(*S*)-*N*-Benzyl 2-Amino-3-hydroxypropionamide ((*S*)-65). The previous procedure was repeated using (*S*)-*N*-benzyl 2-*N*-(benzyloxycarbonyl)amino-3-hydroxypropionamide (1.46 g, 4.44 mmol), 10% Pd-C (140 mg), and MeOH (200 mL) (8 h) to give the desired product (0.54 g, 63%) as a white solid: mp 91–92 °C; $[\alpha]^{25}_{D}$ +4.88° (*c* 1.5, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.27 (dd, *J* = 5.1, 5.1 Hz, C**H**), 3.42–3.57 (m, C**H**$_2$OH), 4.30 (d, *J* = 6.2 Hz, C**H**$_2$NH), 7.19–7.34 (m, Ph**H**), 8.37 (t, *J* = 6.2 Hz, N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 41.8 (NH**C**H$_2$), 57.0 (**C**H), 64.3 (**C**H$_2$OH), 126.6, 127.1, 128.2, 139.6 (**C**$_6$H$_5$), 173.2 (**C**(O)NH); HRMS (ESI) 217.0953 [M + Na$^+$] (calcd for $C_{10}H_{14}N_2O_2Na^+$ 217.0953); Anal. Calcd for $C_{10}H_{14}N_2O_2$: C, 61.84; H, 7.27; N, 14.42. Found C, 61.91; H, 7.31; N, 14.21.



(*R,S*)-*N*-Benzyl 2-Amino-3-hydroxypropionamide ((*R,S*)-65). The previous procedure was repeated using (*R,S*)-*N*-benzyl 2-*N*-(benzyloxycarbonyl)amino-3-hydroxypropionamide (1.33 g, 4.07 mmol), 10% Pd-C (130 mg), and MeOH (200 mL) (8 h) to give the desired product (0.52 g, 66%) as a white solid: mp 90–91 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.14 (br s, N**H**$_2$), 3.27 (dd, *J* = 4.5, 4.8 Hz, C**H**), 3.42–3.57 (m, C**H**$_2$OH), 4.30 (d, *J* = 6.5 Hz, C**H**$_2$NH), 4.62–4.88 (br s, O**H**), 7.19–7.34 (m, Ph**H**), 8.37 (t, *J* = 6.5 Hz, N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 41.8 (NH**C**H$_2$), 57.0 (**C**H), 64.2 (**C**H$_2$OH), 126.6, 127.1, 128.2, 139.5 (**C**$_6$H$_5$), 173.2 (**C**(O)NH); HRMS (ESI) 217.0953 [M + Na$^+$] (calcd for $C_{10}H_{14}N_2O_2Na^+$ 217.0953); Anal. Calcd for $C_{10}H_{14}N_2O_2$: C, 61.84; H, 7.27; N, 14.42. Found C, 61.69; H, 7.33; N, 14.41.

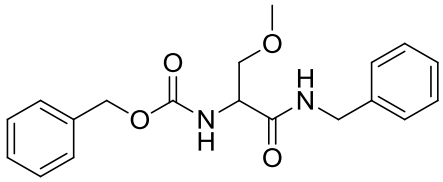


(*R*)-*N*-Benzyl 2-*N*-(Benzylcarboxycarbonyl)amino-3-methoxypropionamide ((*R*)-109).[89] Utilizing Method D and using (*R*)-*N*-benzyl 2-*N*-(benzyloxycarbonyl)amino-3-hydroxypropionamide (4.00 g, 12.2 mmol), Ag_2O (14.12 g, 60.9 mmol), MeI (7.59 mL, 121.9 mmol), and CH_3CN (400 mL) at room temperature (5 d) gave the crude product that was purified by flash column chromatography (SiO_2, 1:10 MeOH/CH_2Cl_2) followed by recrystallization from hot EtOAc to give the desired product (2.86 g, 69%) as a white crystalline solid: mp 129–130 ºC (lit.[89] mp 128–130 ºC); $[\alpha]^{25}_D$ +2.8° (*c* 1.0, MeOH) (lit.[89] $[\alpha]^{23}_D$ +2.8° (*c* 1.1, MeOH)); R_f = 0.39 (1:1 EtOAc/hexanes); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.25 (s, OC**H**$_3$), 3.46–3.55 (m, C**H**$_2$OCH$_3$), 4.24–4.30 (m, C**H**, NHC**H**$_2$Ph), 5.04 (s, OC**H**$_2$Ph), 7.22–7.38 (m, 2 Ph**H**), 7.47 (d, *J* = 8.1 Hz, N**H**C(O)O), 8.53 (t, *J* = 5.7 Hz, N**H**C(O)); ^{13}C NMR (75 MHz, DMSO-d_6) δ 41.7 (NH**C**H$_2$), 54.3 (**C**H), 57.7 (O**C**H$_3$), 65.1 (O**C**H$_2$Ph), 71.6 (**C**H$_2$OCH$_3$), 126.3, 126.6, 127.3, 127.4, 127.8, 127.9, 136.6, 138.8 (2 **C**$_6$H$_5$), 155.6 (O**C**(O)), 169.3 (**C**(O)NH).

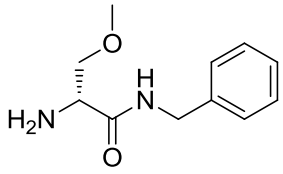


(*S*)-*N*-Benzyl 2-*N*-(Benzylcarboxycarbonyl)amino-3-methoxypropionamide ((*S*)-109).[98] The previous procedure was repeated using (*S*)-*N*-benzyl 2-*N*-(benzyloxycarbonyl)amino-3-hydroxypropionamide (4.00 g, 12.2 mmol), Ag_2O (14.12 g, 60.9 mmol), MeI (7.59 mL, 121.9 mmol), and CH_3CN (400 mL) to give the desired product (3.13 g, 75%) as a white crystalline solid: mp 131–132 ºC (lit.[98] mp 130–132 ºC); $[\alpha]^{25}_D$ –2.9° (*c* 1.1, MeOH) (lit.[98] $[\alpha]^{24}_D$ –3.3° (*c* 1.1, MeOH)); R_f = 0.36 (1:1 EtOAc/hexanes); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.25 (s, OC**H**$_3$), 3.47–3.57 (m, C**H**$_2$OCH$_3$), 4.25–4.30 (m, C**H**, NHC**H**$_2$Ph), 5.04 (s, OC**H**$_2$Ph), 7.20–7.37 (m, 2 Ph**H**), 7.48 (d, *J* = 8.1 Hz, N**H**C(O)O), 8.53 (t, *J* = 5.4 Hz, N**H**C(O)); ^{13}C NMR (75

MHz, DMSO-d_6) δ 41.7 (NH**C**H_2), 54.3 (**C**H), 57.8 (O**C**H_3), 65.1 (O**C**H_2Ph), 71.6 (**C**H_2OCH_3), 126.3, 126.6, 127.3, 127.4, 127.8, 127.9, 136.6, 138.9 (2 **C**$_6H_5$), 155.6 (O**C**(O)), 169.3 (**C**(O)NH).

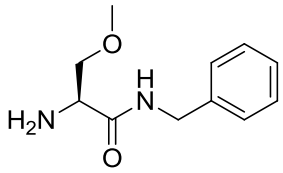


(*R,S*)-*N*-Benzyl 2-*N*-(Benzylcarboxycarbonyl)amino-3-methoxypropionamide ((*R,S*)-109). The previous procedure was repeated using (*R,S*)-*N*-benzyl 2-*N*-(benzyloxycarbonyl)amino-3-hydroxypropionamide (4.00 g, 12.2 mmol), Ag_2O (14.12 g, 60.9 mmol), MeI (7.59 mL, 121.9 mmol), and CH_3CN (400 mL) to give the desired product (2.53 g, 61%) as a white crystalline solid: mp 126–127 ºC; R_f = 0.39 (1:1 EtOAc/hexanes); IR (nujol mull) 3220, 2910, 1692, 1639, 1542, 1459, 1376, 1310, 1265, 1125, 1051, 967, 752, 698 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.25 (s, OC**H**$_3$), 3.50–3.52 (m, C**H**$_2$OCH_3), 4.22–4.34 (m, C**H**, NHC**H**$_2$Ph), 5.04 (s, OC**H**$_2$Ph), 7.23–7.36 (m, 2 Ph**H**), 7.48 (d, J = 8.1 Hz, N**H**C(O)O), 8.53 (t, J = 5.4 Hz, N**H**C(O)); ^{13}C NMR (75 MHz, DMSO-d_6) δ 41.7 (NH**C**H_2), 54.3 (**C**H), 57.7 (O**C**H_3), 65.1 (O**C**H_2Ph), 71.6 (**C**H_2OCH_3), 126.3, 126.6, 127.3, 127.4, 127.8, 127.9, 136.7, 138.9 (2 **C**$_6H_5$), 155.6 (O**C**(O)), 169.3 (**C**(O)NH).



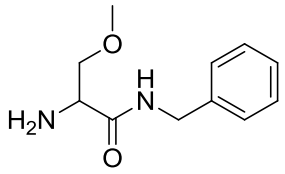
(*R*)-*N*-Benzyl 2-Amino-3-methoxypropionamide ((*R*)-61).[89] Utilizing Method C and using (*R*)-*N*-benzyl 2-*N*-(benzylcarboxycarbonyl)amino-3-methoxypropionamide (2.00 g, 5.85 mmol), 10% Pd-C (0.2 g), and MeOH (100 mL) (5 h) gave the crude product that was

purified by flash column chromatography (SiO_2; 1:10 MeOH/$CHCl_3$). The resulting oil was dissolved in CH_2Cl_2 (20 mL) and was extracted with aqueous 0.1 N HCl (3 x 20 mL). The aqueous layers were combined and washed with CH_2Cl_2 (2 x 60 mL). The aqueous layer was basified to pH 10–12 with aqueous 0.1 N NaOH, and then extracted with CH_2Cl_2 (3 x 100 mL). The CH_2Cl_2 layers were combined, dried ($MgSO_4$), and concentrated *in vacuo* to give the desired product (0.89 g, 73%) as a waxy solid: mp 39–40 °C; $[\alpha]^{25}_D$ –1.5° (*c* 1.6, MeOH) (lit.[89] $[\alpha]^{23}_D$ –2.0° (*c* 1.5, MeOH)); R_f = 0.26 (1:20 MeOH/$CHCl_3$); IR (nujol mull) 3366, 3302, 3140, 2904 (br), 1955, 1886, 1815, 1667, 1457, 1373, 1152, 966, 726 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.82 (s, N**H**$_2$), 3.25 (s, OC**H**$_3$), 3.35–3.44 (m, C**H**C**H**$_2$), 4.23–4.36 (m, C**H**$_2$Ph), 7.20–7.34 (m, Ph**H**), 8.36–8.44 (br t, N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 41.5 (**C**H_2PH), 54.3 (**C**H), 57.8 (**C**H_3), 74.8 (CH**C**H_2), 126.3, 126.7, 127.8, 139.1 (**C**$_6H_5$), 172.6 (**C**(O)); Anal. Calcd for $C_{11}H_{16}N_2O_2 \cdot 0.18H_2O$: C, 62.49; H, 7.80; N, 13.25. Found C, 62.13; H, 7.82; N, 13.10.

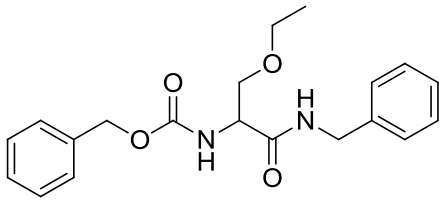


(*S*)-*N*-Benzyl 2-Amino-3-methoxypropionamide ((*S*)-61).[98] The previous procedure was repeated using (*S*)-*N*-benzyl 2-*N*-(benzylcarboxycarbonyl)amino-3-methoxypropionamide (2.00 g, 5.85 mmol), 10% Pd-C (0.2 g), and MeOH (100 mL) to give the desired product (1.12 g, 92%) as a waxy solid: mp 39–40 °C; $[\alpha]^{25}_D$ +1.7° (*c* 1.5, MeOH) (lit.[98] $[\alpha]^{23}_D$ +1.8° (*c* 0.8, MeOH)); R_f = 0.39 (1:20 MeOH/$CHCl_3$); IR (nujol mull) 3474, 3365, 3125 (br), 1949, 1885, 1817, 1662, 1520, 1457, 1369, 1240, 1189, 1106, 970, 727 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.84 (s, N**H**$_2$), 3.25 (s, OC**H**$_3$), 3.35–3.44 (m, C**H**C**H**$_2$), 4.23–4.36 (m, C**H**$_2$Ph), 7.20–7.34 (m, Ph**H**), 8.36–8.45 (br t, N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 41.5 (**C**H_2PH), 54.3 (**C**H), 57.8 (**C**H_3), 74.8 (CH**C**H_2), 126.3, 126.7, 127.8, 139.1 (**C**$_6H_5$), 172.6 (**C**(O)); Anal.

Calcd for $C_{11}H_{16}N_2O_2 \cdot 0.15H_2O$: C, 62.56; H, 7.79; N, 13.28. Found C, 62.25; H, 7.91; N, 13.16.

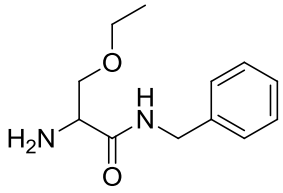


(*R,S*)-*N*-Benzyl 2-Amino-3-methoxypropionamide ((*R,S*)-61). The previous procedure was repeated using (*R,S*)-*N*-benzyl 2-*N*-(benzylcarboxycarbonyl)amino-3-methoxypropionamide (2.00 g, 5.85 mmol), 10% Pd-C (0.2 g), and MeOH (100 mL) to give the desired product (0.42 g, 32%) as a pale yellow oil: R_f = 0.37 (1:20 MeOH/$CHCl_3$); IR (neat) 3227, 3177, 3063, 2900 (br), 1959, 1884, 1814, 1540, 1456, 1361, 1253, 1187, 1110, 927, 739, 701 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.82 (br s, N**H**$_2$), 3.25 (s, OC**H**$_3$), 3.37–3.44 (m, C**H**C**H**$_2$), 4.23–4.36 (m, C**H**$_2$Ph), 7.19–7.34 (m, Ph**H**), 8.36–8.44 (br t, N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 41.5 (**C**H_2PH), 54.3 (**C**H), 57.8 (**C**H_3), 74.8 (CH**C**H_2), 126.3, 126.7, 127.8, 139.1 (**C**$_6H_5$), 172.6 (**C**(O)); Anal. Calcd for $C_{11}H_{16}N_2O_2$: C, 63.44; H, 7.74; N, 13.45. Found C, 63.18; H, 7.66; N, 13.41.



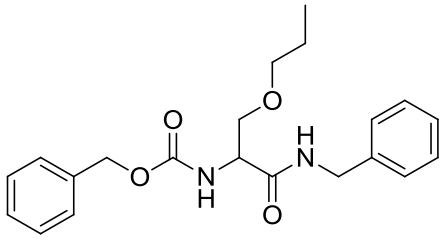
(*R,S*)-*N*-Benzyl 2-*N*-(Benzylcarboxycarbonyl)amino-3-ethoxypropionamide ((*R,S*)-110). Utilizing Method D and using (*R,S*)-*N*-benzyl 2-*N*-(benzyloxycarbonyl)amino-3-hydroxypropionamide (1.88 g, 5.73 mmol), Ag_2O (6.64 g, 8.59 mmol), EtI (6.94 mL, 85.9 mmol), and CH_3CN (400 mL) (40–50 °C, 7 d) gave the crude product that was further purified by flash column chromatography (SiO_2, 1:10 MeOH/CH_2Cl_2) followed by

recrystallization from hot toluene to give the desired product (1.04 g, 51%) as a white crystalline solid: mp 100–101 ºC; R_f = 0.48 (1:1 EtOAc/hexanes); IR (nujol mull) 3297, 2941 (br), 2729, 2680, 1688, 1645, 1538, 1457, 1374, 1242, 727 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.08 (t, J = 6.9 Hz, C**H**$_3$), 3.44 (q, J = 6.9 Hz, OC**H**$_2$CH$_3$), 3.50–3.60 (m, CHC**H**$_2$), 4.22–4.37 (m, C**H**, C**H**$_2$Ph), 5.04 (s, OC**H**$_2$Ph), 7.22–7.46 (m, 2 Ph**H**), 8.53 (t, J = 5.7 Hz, N**H**C(O)); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.6 (OCH$_2$**C**H$_3$), 41.6 (NH**C**H$_2$), 54.5 (**C**H), 65.1 (O**C**H$_2$CH$_3$ or O**C**H$_2$Ph), 65.3 (O**C**H$_2$Ph or O**C**H$_2$CH$_3$), 70.0 (**C**H$_2$OCH$_2$CH$_3$), 126.3, 126.6, 127.3, 127.4, 127.8, 127.9, 136.6, 138.9 (2 **C**$_6$H$_5$), 155.5 (**C**(O)O), 169.4 (**C**(O)NH); HRMS (ESI) 357.1814 [M + H^+] (calcd for $C_{20}H_{24}N_2O_4H^+$ 357.1814); Anal. Calcd for $C_{20}H_{24}N_2O_4$; C, 67.40; H, 6.79; N, 7.86. Found C, 67.12; H, 6.67; N, 7.89.



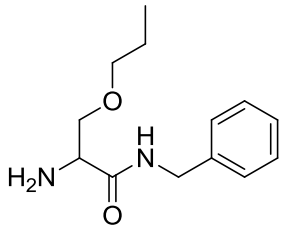
(*R,S*)-*N*-Benzyl 2-Amino-3-ethoxypropionamide ((*R,S*)-67). Utilizing Method C and (*R,S*)-*N*-benzyl 2-*N*-(benzylcarboxycarbonyl)amino-3-ethoxypropionamide (1.00 g, 2.81 mmol), 10% Pd-C (0.1 g), and MeOH (30 mL) (18 h) gave the crude product that was purified by flash column chromatography (SiO_2; 1:10 MeOH/CH_2Cl_2). The resulting oil was dissolved in CH_2Cl_2 (10 mL) and was extracted with aqueous 0.1 N HCl (3 x 10 mL). The aqueous layers were combined and washed with CH_2Cl_2 (2 x 30 mL). The aqueous layer was basified to pH 10–12 with aqueous 0.1 N NaOH, and then extracted with CH_2Cl_2 (3 x 60 mL). The CH_2Cl_2 layers were combined, dried ($MgSO_4$), and concentrated *in vacuo* to give the desired product (0.42 g, 68%) as a pale yellow oil: R_f = 0.33 (1:1 EtOAc/hexanes); IR (neat) 3319, 3177, 3062, 2865, 1957, 1883, 1812, 1662, 1535, 1455, 1361, 1254, 1107, 1022, 870, 738 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.10 (t, J = 7.2 Hz, C**H**$_3$), 1.82 (s, N**H**$_2$), 3.29–3.49 (m,

C**H**C**H**$_2$OC**H**$_2$CH$_3$), 4.22–4.37 (m, C**H**$_2$Ph), 7.20–7.33 (m, Ph**H**), 8.36–8.45 (br t, N**H**C(O)); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.7 (OCH$_2$**C**H$_3$), 41.5 (NH**C**H$_2$), 54.5 (**C**H), 65.3 (O**C**H$_2$CH$_3$), 72.7 (**C**H$_2$OCH$_2$CH$_3$), 126.3, 126.7, 127.8, 139.2 (**C**$_6$H$_5$), 172.8 (**C**(O)NH); HRMS (ESI) 223.1450 [M + H$^+$] (calcd for $C_{12}H_{18}N_2O_2H^+$ 223.1447); Anal. Calcd for $C_{12}H_{18}N_2O_2 \cdot 0.06CH_2Cl_2$; C, 63.63; H, 8.02; N, 12.30. Found C, 63.67; H, 8.21; N, 12.32.

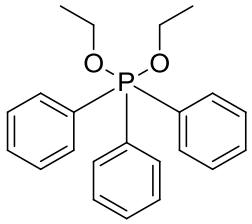


(*R,S*)-*N*-Benzyl 2-*N*-(Benzylcarboxycarbonyl)amino-3-propoxypropionamide ((*R,S*)-111). Utilizing Method D and using (*R,S*)-*N*-benzyl 2-*N*-(benzyloxycarbonyl)amino-3-hydroxypropionamide (3.29 g, 10.0 mmol), Ag_2O (11.59 g, 50.1 mmol), propyl iodide (14.7 mL, 150.4 mmol), and CH_3CN (100 mL) (40–50 °C, 7 d) gave the crude product that was further purified by flash column chromatography (SiO_2, 1:10 MeOH/CH_2Cl_2) followed by recrystallization from hot EtOAc/hexanes gave the desired product (1.71 g, 46%) as a pale orange crystalline solid: mp 101–102 ºC; R_f 0.58 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3279, 2927 (br), 2728, 1682, 1644, 1544, 1459, 1377, 1237, 696 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.83 (t, J = 6.9 Hz, C**H**$_3$), 1.42–1.53 (m, C**H**$_2$CH$_3$), 3.33 (1/2 ABq, J = 6.0 Hz, OC**H**H'CH$_2$CH$_3$), 3.35 (1/2 ABq, J = 6.0 Hz, OCH**H'**CH$_2$CH$_3$), 3.50–3.60 (m, CHC**H**$_2$), 4.23–4.36 (m, C**H**, C**H**$_2$Ph), 5.01 (1/2 ABq, J = 12.6 Hz, OC**H**H'Ph), 5.07 (1/2 ABq, J = 12.6 Hz, OCH**H**'Ph), 7.23–7.37 (m, 2 C$_6$**H$_5$**), 7.43 (d, J = 8.1 Hz, N**H**C(O)), 8.52 (t, J = 6.3 Hz, N**H**CH$_2$Ph); ^{13}C NMR (75 MHz, DMSO-d_6) δ 10.0 (**C**H$_3$), 21.9 (**C**H$_2$CH$_3$), 41.7 (NH**C**H$_2$Ph), 54.5 (**C**H), 65.1 (O**C**H$_2$Ph), 69.8 (**C**H$_2$OCH$_2$ or CH$_2$O**C**H$_2$CH$_2$), 71.5 (**C**H$_2$OCH$_2$ or CH$_2$O**C**H$_2$CH$_2$), 126.3, 126.6, 127.3, 127.4, 127.8, 127.9, 136.6, 138.9 (2 **C$_6$**H$_5$), 155.6 (**C**(O)O), 169.4 (**C**(O)NH); HRMS (ESI) 393.1790 [M + Na$^+$] (calcd for $C_{21}H_{26}N_2O_4Na^+$

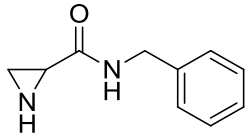
393.1790); Anal. Calcd for $C_{21}H_{26}N_2O_4$; C, 68.09; H, 7.07; N, 7.56. Found C, 68.20; H, 7.19; N, 7.64.



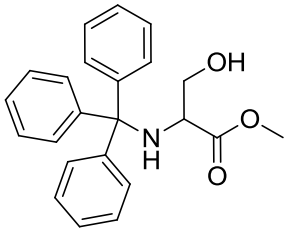
(*R,S*)-*N*-Benzyl 2-Amino-3-propoxypropionamide ((*R,S*)-68). Utilizing Method C and (*R,S*)-*N*-benzyl 2-*N*-(benzylcarboxycarbonyl)amino-3-propoxypropionamide (1.42 g, 3.85 mmol), 10% Pd-C (0.15 g), and MeOH (50 mL) (6 h) gave the crude product that was purified by flash column chromatography (SiO_2; 1:100 MeOH/CH_2Cl_2). The resulting oil was dissolved in CH_2Cl_2 (10 mL) and extracted with aqueous 0.1 N HCl (3 x 10 mL). The aqueous layers were combined and washed with CH_2Cl_2 (2 x 30 mL). The aqueous layer was basified to pH 10–12 with aqueous 0.1 N NaOH, and then extracted with CH_2Cl_2 (3 x 60 mL). The second set of CH_2Cl_2 layers were combined, dried ($NaSO_4$), and concentrated *in vacuo* to give the desired product (0.66 g, 72%) as a pale orange oil: R_f 0.52 (1:100 MeOH/CH_2Cl_2); IR (neat) 3338, 3123, 2935, 2868, 1661, 1528, 1457, 1362, 1256, 1108, 699 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.85 (t, J = 7.8 Hz, C**H**$_3$), 1.44–1.56 (m, C**H**$_2$CH$_3$), 1.82 (br s, N**H**$_2$), 3.31–3.46 (m, C**H**C**H**$_2$OC**H**$_2$CH$_2$), 4.23–4.37 (m, C**H**$_2$Ph), 7.19–7.32 (m, C$_6$**H**$_5$), 8.36–8.45 (br t, N**H**C(O)); ^{13}C NMR (75 MHz, DMSO-d_6) δ 10.1 (**C**H$_3$), 22.0 (**C**H$_2$CH$_3$), 41.5 (NH**C**H$_2$), 54.4 (**C**H), 71.6 (**C**H$_2$OCH$_2$ or CH$_2$O**C**H$_2$CH$_2$), 72.8 (**C**H$_2$OCH$_2$ or CH$_2$O**C**H$_2$CH$_2$), 126.2, 126.6, 127.7, 139.2 (**C**$_6$H$_5$), 172.7 (**C**(O)NH); Anal. Calcd for $C_{13}H_{20}N_2O_2$; C, 66.07; H, 8.53; N, 11.85. Found C, 65.80; H, 8.29; N, 11.58.



Diethoxytriphenylphosphorane (377).[100] Triphenylphosphine (75.00 g, 0.29 mol) was dissolved in anhydrous CH_2Cl_2 (600 mL) and cooled to -78 ºC. While stirring, Br_2 (14.7 mL, 0.29 mol) was added all at once with a syringe and the reaction was stirred at -78 ºC (30 min). NaOEt in denatured EtOH (21% w/w, 213 mL 0.57 mol) was added dropwise over 4 h. The reaction was stirred at -78 ºC (2 h), and allowed to warm to room temperature (12 h). The supernatant was decanted and filtered over a Celite® bed. The remaining suspension was centrifuged at 4500 rpm for 15 min. The supernatant layers obtained from both fractions were combined and evaporated at 30 ºC. Hexanes (500 mL) were added to the oily residue and the mixture was shaken for 5 min. The triphenylphosphine oxide (TPPO) was filtered, and the solvent removed in vacuo. Additional hexanes (500 mL) were added to the solid and the flask was let stand on ice for 30 min. Additional TPPO was filtered and the solvent evaporated to yield DTPP as a white to pale yellow solid (36.40 g, 36% yield, 85% pure by wt): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.75 (t, J = 9.0 Hz, $P(OCH_2C\mathbf{H_3})_2$), 2.48–2.58 (m, $P(OC\mathbf{H_2}CH_3)_2$), 7.37–7.54 (m, 9 Ar**H**), 8.04–8.12 (m, 6 Ar**H**); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.4 (d, J = 5.3 Hz, $P(OCH_2\mathbf{C}H_3)_2$), 57.6 (d, J = 7.5 Hz, $P(O\mathbf{C}H_2CH_3)_2$), 127.7, 129.4, 132.8 (15 Ar**C**), 139.2 (d, J = 173.3 Hz, 3 Ar**C**). The DTPP obtained under these conditions was ~85% pure by weight and was contaminated with TPPO (^{1}H NMR analysis).

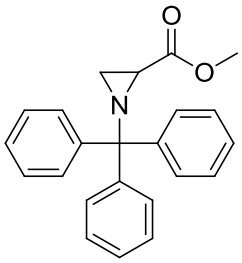


(*R,S*)-*N*-Benzyl 1-Aziridine-2-carboxamide ((*R,S*)-116).[187] Utilizing Method C and using (*R,S*)-*N*-benzyl 2-*N*-(benzyloxycarbonyl)amino-3-hydroxypropionamide (4.27 g, 13.01 mmol), 10% Pd-C (0.45 g), and MeOH (200 mL) (3 h) gave crude (*R,S*)-*N*-benzyl 2-amino-3-hydroxypropionamide that was then directly dissolved in CH_3CN (130 mL). DTPP (8.39 g, 14.31 mmol, 60% w/w) was added and the reaction was stirred overnight (24 h), filtered, and the filtrate evaporated *in vacuo*. The crude product was purified by flash column chromatography (SiO_2; 1:100 MeOH/CH_2Cl_2) to give the desired product (0.92 g, 40%) as an orange oil: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.03–1.11 (N**H**CH), 1.47–1.90 (m, NHC**H$_2$**CH), 2.36–2.40 (br t, NHC**H**C(O)), 4.22–4.35 (m, NHC**H$_2$**), 7.23–7.36 (m, C$_6$**H$_5$**), 8.89–8.90 (br t, N**H**CH$_2$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 29.0 (NH**C**H$_2$), 30.3 NH**C**H), 42.2 (NH**C**H$_2$Ph), 126.6, 127.0, 128.0, 139.9 (**C$_6$**H$_5$), 170.4 (**C**(O)).



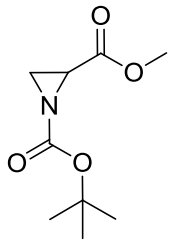
(*R,S*)-Methyl 3-Hydroxy-2-(*N*-tritylamino)propionate ((*R,S*)-129).[127] To a solution of DL-serine methyl ester hydrochloride (20.00 g, 0.13 mol) and Et_3N (35.82 mL, 0.26 mol) in CH_2Cl_2 (80 mL) at 0 ºC, was added in one portion a solution of TrCl (36.53 g, 0.13 mol) in CH_2Cl_2 (80 mL). The mixture was allowed to stir at 0 ºC (18 h) under N_2 and then successively washed with aqueous 10% citric acid (120 mL) and saturated aqueous brine (120 mL). The organic layer was dried (Na_2SO_4) and evaporated *in vacuo* to give the crude product (44.58 g, 96%) as a pale yellow crystalline solid. The product was used in the next step without further purification: R_f 0.72 (1:10 EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.38–2.54 (br s, 1 H), 2.89–3.02 (br s, 1 H), 3.26 (s, OC**H$_3$**), 3.50–3.59 (m, C**H**, C**H**H'OH), 3.69–3.73 (m, CH**H'**OH), 7.15–7.29 (m, 9 Ph**H**), 7.44–7.50 (6 Ph**H**); ^{13}C NMR (75 MHz,

$CDCl_3$) δ 52.1 (O**C**H_3), 58.0 (**C**H), 65.1 (**C**H_2OH), 126.8, 128.1, 128.9, 145.8 (3 $\mathbf{C_6}H_5$), 174.1 (**C**(O)).

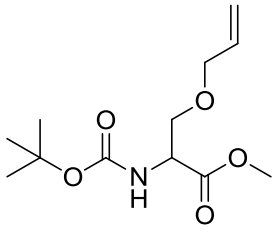


(*R,S*)-Methyl *N*-Tritylaziridine-2-carboxylate ((*R,S*)-130).[125] Crude (*R,S*)-methyl 3-hydroxy-2-(*N*-tritylamino)propionate (44.00 g, 0.12 mol) was dissolved in CH_2Cl_2 (250 mL) and cooled to 0 ºC under N_2. Methanesulfonyl chloride (10.37 mL, 0.13 mol) was added to the cooled solution, followed by the dropwise addition of Et_3N (25.47 mL, 0.18 mol). The resulting solution was allowed to stir at 0 ºC (30 min) and then successively washed with aqueous 10% citric acid (250 mL) and saturated aqueous brine (250 mL), dried (Na_2SO_4), and evaporated *in vacuo* to give the crude mesylate. The crude mesylate was dissolved in DME (250 mL) and Et_3N (33.97 mL, 0.24 mol) was added. The mixture was stirred at 80 ºC (72 h) and then concentrated to dryness *in vacuo*. The crude product was dissolved in EtOAc (250 mL), successively washed with aqueous 10% citric acid (250 mL) and saturated aqueous brine (250 mL), dried (Na_2SO_4), evaporated *in vacuo*, and purified by recrystallization from hot EtOH to give the desired product (26.43 g, 63%) as an off white solid: mp 131–132 ºC (lit.[125] mp 133 ºC); R_f 0.16 (1:10 EtOAc/hexanes); IR (nujol mull) 2927, 2859, 1719, 1596, 1454, 1375, 1291, 1216, 1077, 1027, 923, 869, 754, 707, 637 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (dd, J = 1.6, 6.2 Hz, NC**H**H'CH), 1.89 (dd, J = 2.7, 6.2 Hz, CHH'C**H**N), 2.26 (dd, J = 1.6, 2.7 Hz, NCH**H'**CH), 3.75 (s, OC$\mathbf{H_3}$), 7.18–7.30 (9 Ph**H**), 7.48–7.52 (6 Ph**H**); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.8 (N**C**H_2CH), 31.9 (CH_2**C**HN), 52.3 (O**C**H_3),

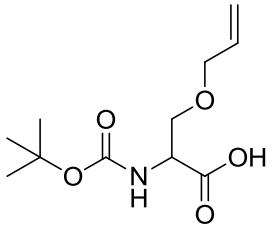
74.6 (N**C**Ph_3), 127.1, 127.9, 129.5, 143.8 (3 $\mathbf{C_6}H_5$), 172.1 (**C**(O)); HRMS (ESI) 344.1663 [M + H^+] (calcd for $C_{23}H_{21}NO_2H^+$ 344.1651).



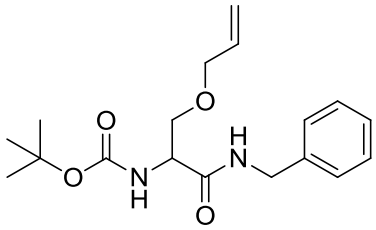
(*R,S*)-Methyl *N*-(*t*-Butoxycarbonyl)aziridine-2-carboxylate ((*R,S*)-131).[132] (*R,S*)-Methyl *N*-(trityl)aziridine-2-carboxylate (16.48 g, 48.02 mmol) was dissolved in CH_2Cl_2 (240 mL) and MeOH (1.53 mL, 48.02 mmol) was added and the solution was cooled to 0 ºC in an ice bath under N_2 before TFA (7.13 mL, 96.05 mmol) was added dropwise. Et_3N (33.47 mL, 0.24 mol) was added dropwise at 0 ºC and the reaction stirred at 0 ºC (10 min) before Boc_2O (11.53 g, 52.83 mmol) in CH_2Cl_2 (60 mL) was added dropwise. The reaction was allowed to warm to room temperature (18 h) and then successively washed with aqueous 10% citric acid (3 x 300 mL), H_2O (3 x 300 mL), and saturated aqueous brine (2 x 300 mL), dried (Na_2SO_4), evaporated *in vacuo*, and purified by flash column chromatography (SiO_2; 1:100–1:10 MeOH/CH_2Cl_2) to give the desired product (6.79 g, 70%) as a pale yellow oil and as a 1:1 mixture of isomers A and B: R_f 0.47 (1:10 EtOAc/hexanes); IR (neat) 3483, 3427, 2980, 1737, 1630, 1449, 1378, 1328, 1156, 1029, 965, 853, 801, 751, 686, 597, 530 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.45, 1.46 (2 s, (C(C$\mathbf{H_3})_3$), 2.52–2.58 (m, NHC$\mathbf{H_2}$CH), 3.03–3.06 (m, NHCH_2C**H**), 3.78, 3.79 (2 s, OC$\mathbf{H_3}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.6 (C(**C**$H_3)_3$), 31.0 (N**C**H_2CH), 34.6 (CH_2**C**HN), 52.3 (O**C**H_3), 81.9 (**C**$(CH_3)_3$), 159.3 (N**C**(O)O), 168.6 (**C**(O)OCH_3); HRMS (ESI) 224.0909 [M + Na^+] (calcd for $C_9H_{15}NO_4Na^+$ 224.0899).



(*R,S*)-Methyl 2-*N'*-(*t*-Butoxycarbonyl)amino-3-allyloxypropionate ((*R,S*)-132). (*R,S*)-Methyl *N*-(*t*-butoxycarbonyl)aziridine-2-carboxylate (6.22 g, 30.93 mmol) was dissolved in anhydrous CH_2Cl_2 (30 mL) and cooled to 0 ºC in an ice bath. Then, allyl alcohol (6.31 mL, 92.79 mmol) followed by BF_3•Et_2O (3.82 mL, 30.93 mmol) were successively added. The reaction was continued at 0 ºC (45 min) and then saturated aqueous $NaHCO_3$ (30 mL) was added and stirred (30 min). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 30 mL). Both sets of organic layers were combined, dried (Na_2SO_4), evaporated *in vacuo*, and purified by flash column chromatography (SiO_2; 1:10–1:1 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired product (1.52 g, 19%) as a pale yellow oil: R_f 0.55 (1:10 EtOAc/hexanes); IR (neat) 3379, 3301, 2930, 1716, 1507, 1453, 1362, 1167, 929, 866, 781, 658 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, C(C**H$_3$**)$_3$), 3.65 (dd, J = 3.6, 9.2 Hz, C**H**H'OCH_2), 3.76 (s, OC**H$_3$**), 3.85 (dd, J = 3.2, 9.2 Hz, CH**H'**OCH_2), 3.93–4.03 (m, CH_2OC**H$_2$**), 4.42–4.44 (m, C**H**), 5.17–5.27 (m, OCH_2CHC**H$_2$**), 5.42 (d, J = 9.2 Hz, N**H**), 5.79–5.95 (m, OCH_2C**H**CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.4 (C(**C**H_3)$_3$), 52.5 (O**C**H_3), 54.1 (**C**H), 70.0 (**C**H_2OCH_2), 72.3 (CH_2O**C**H_2), 80.0 (**C**$(CH_3)_3$), 117.4 (OCH_2CH**C**H_2), 134.2 (OCH_2**C**HCH_2), 155.6 (**C**(O)N), 171.3 (**C**(O)OCH_3).

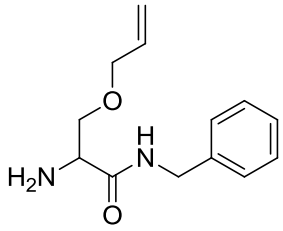


(*R,S*)-Methyl 2-*N'*-(*t*-Butoxycarbonyl)amino-3-allyloxypropionic Acid ((*R,S*)-134). (*R,S*)-Methyl 2-*N'*-(*t*-butoxycarbonyl)amino-3-allyloxypropionate (1.13 g, 4.36 mmol) was dissolved in anhydrous THF (44 mL) and a solution of LiOH (0.6 M, 7.27 mL, 4.36 mmol) was added at room temperature (18 h) and then the solvent was evaporated *in vacuo*. The crude product was diluted with H_2O (25 mL), acidified to pH 3 with aqueous 1 M $KHSO_4$, and extracted with EtOAc (6 x 25 mL). The combined organic layers were dried (Na_2SO_4) and evaporated *in vacuo* to give the crude product (1.07 g, 99%) as a pale yellow oil. The product was used for the next step without further purification: R_f 0.44 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, CD_3OD) δ 1.45 (s, C(C**H$_3$**)$_3$), 3.68 (dd, J = 3.4, 9.8 Hz, C**H**H'OCH_2), 3.79 (dd, J = 5.0, 9.8 Hz, CH**H'**OCH_2), 3.95–4.04 (m, CH_2OC**H$_2$**), 4.30 (t, J = 4.0 Hz, C**H**), 5.14–5.30 (m, OCH_2CHC**H$_2$**), 5.83–5.93 (m, OCH_2C**H**CH_2); ^{13}C NMR (100 MHz, CD_3OD) δ 27.3 (C(**C**H_3)$_3$), 53.8 (**C**H), 69.4 (**C**H_2OCH_2), 71.7 (CH_2O**C**H_2), 79.3 (**C**$(CH_3)_3$), 116.0 (OCH_2CH**C**H_2), 134.3 (OCH_2**C**HCH_2), 156.4 (**C**(O)N), 172.4 (**C**(O)OH).

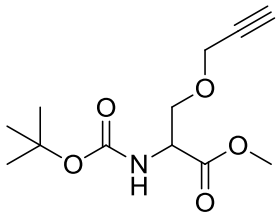


(*R,S*)-*N*-Benzyl 2-*N'*-(*t*-Butoxycarbonyl)amino-3-allyloxypropionamide ((*R,S*)-136). Employing Method B and using (*R,S*)-methyl 2-*N'*-(*t*-butoxycarbonyl)amino-3-allyloxypropionic acid (1.05 g, 4.28 mmol), NMM (0.62 mL, 5.57 mmol), IBCF (0.61 mL, 4.71 mmol), and benzylamine (0.49 mL, 4.50 mmol) gave the crude product that was purified by flash column chromatography (SiO_2; 10–50% EtOAc/hexanes followed by 10% MeOH/CH_2Cl_2) to give the desired product (1.25 g, 87%) as a pale yellow solid: R_f 0.19 (10% EtOAc/hexanes); mp 70–71 C; IR (neat) 3305, 3037, 2968, 1707, 1532, 1365, 1250, 1167, 1041, 948, 869, 744, 700 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, C(C**H$_3$**)$_3$), 3.56

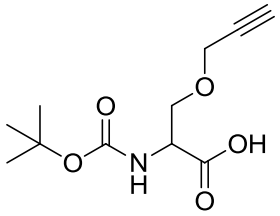
(dd, J = 6.4, 9.4 Hz, C**H**H'OCH_2), 3.87 (dd, J = 4.0, 9.4 Hz, CH**H'**OCH_2), 3.95–4.04 (m, $CH_2OC\mathbf{H_2}$), 4.26–4.34 (m, C**H**), 4.47 (d, J = 5.6 Hz, NHC$\mathbf{H_2}$Ph), 5.15–5.25 (m, $OCH_2CHC\mathbf{H_2}$), 5.37–5.52 (br d, C(O)N**H**), 5.78–5.88 (m, $OCH_2C\mathbf{H}CH_2$), 6.81–6.87 (br t, N**H**CH_2Ph), 7.23–7.33 ($C_6\mathbf{H_5}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.4 (C(**C**$H_3)_3$), 43.6 (NH**C**H_2Ph), 54.3 (**C**H), 69.9 (**C**H_2OCH_2), 72.4 ($CH_2O\mathbf{C}H_2$), 80.4 (**C**$(CH_3)_3$), 117.7 ($OCH_2CH\mathbf{C}H_2$), 127.5, 127.6, 128.8 (3 Ar**C**), 134.1 ($OCH_2\mathbf{C}HCH_2$), 138.1 (1 Ar**C**), 155.6 (O**C**(O)N), 171.3 (C**C**(O)N).



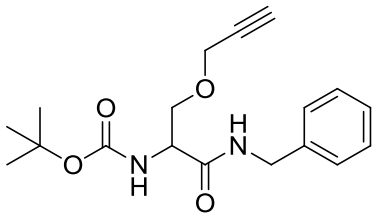
(*R,S*)-*N*-Benzyl 2-Amino-3-allyloxypropionamide ((*R,S*)-68). Utilizing Method E and using (*R,S*)-*N*-benzyl 2-*N'*-(*t*-butoxycarbonyl)amino-3-allyloxypropionamide (1.17 g, 3.50 mmol), TFA (3.90 mL, 52.52 mol), and CH_2Cl_2 (12 mL) gave the crude product after workup that was further purified by flash column chromatography (SiO_2; 1:100–1:10 MeOH/CH_2Cl_2) to give the desired product (285 mg, 35%) as a pale yellow oil: R_f 0.44 (1:20 MeOH/CH_2Cl_2); IR (neat) 3446, 3299, 2920, 2863, 1658, 1530, 1454, 1355, 1256, 1090, 1001, 930, 737, 700 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.61–1.73 (br s, N$\mathbf{H_2}$), 3.62 (t, J = 5.6 Hz, C**H**), 3.66–3.74 (m, C$\mathbf{H_2}OCH_2$), 4.00 (app. t, J = 1.2 Hz, $CH_2OC\mathbf{H}$H'), 4.02 (app. t, J = 1.6 Hz, $CH_2OCH\mathbf{H'}$), 4.41–4.51 (m, NHC$\mathbf{H_2}$Ph), 5.17–5.29 (m, $OCH_2CHC\mathbf{H_2}$), 5.84–5.93 (m, $OCH_2C\mathbf{H}CH_2$), 7.24–7.35 ($C_6\mathbf{H_5}$), 7.73–7.81 (br t, N**H**CH_2Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ 43.3 (NH**C**H_2Ph), 55.2 (**C**H), 72.3 (**C**H_2OCH_2), 72.4 ($CH_2O\mathbf{C}H_2$), 117.5 ($OCH_2CH\mathbf{C}H_2$), 127.5, 127.8, 128.8 (3 Ar**C**), 134.5 ($OCH_2\mathbf{C}HCH_2$), 138.6 (1 Ar**C**), 172.8 (**C**(O)N); HRMS (ESI) 235.1452 [M + H$^+$] (calcd for $C_{13}H_{18}N_2O_2H^+$ 235.1447); Anal. Calcd for $C_{13}H_{18}N_2O_2$•0.20H_2O: C, 65.61; H, 7.80; N, 11.77. Found: C, 65.21; H, 7.92; N, 11.60.



(*R,S*)-Methyl 2-*N*-(*t*-Butoxycarbonyl)amino-3-(prop-2-ynyloxy)propionate ((*R,S*)-133). (*R,S*)-Methyl *N*-(*t*-butoxycarbonyl)aziridine-2-carboxylate (6.85 g, 34.06 mmol) was dissolved in anhydrous CH_2Cl_2 (48 mL) and cooled to 0 ºC in an ice bath. Then, propargyl alcohol (6.03 mL, 102.18 mmol) followed by BF_3•Et_2O (4.20 mL, 34.06 mmol) were successively added. The reaction was continued at 0 ºC (15 min) and then saturated aqueous $NaHCO_3$ (48 mL) was added and stirred (30 min). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL). Both sets of organic layers were combined, dried (Na_2SO_4), evaporated *in vacuo*, and purified by flash column chromatography (SiO_2; 1% MeOH/CH_2Cl_2) to give the desired product (1.06 g, 12%) as a pale yellow oil: R_f 0.35 (1:10 EtOAc/hexanes); IR (neat) 3396, 3265, 2976, 2880, 2118, 1713, 1507, 1451, 1358, 1213, 1169, 1108, 1065, 925, 869, 778, 663 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, C(C**H**$_3$)$_3$), 2.45 (t, J = 2.4 Hz, CH_2CC**H**), 3.77 (dd, J = 3.6, 9.2 Hz, C**H**H'OCH_2), 3.78 (s, OC**H**$_3$), 3.96 (dd, J = 2.8, 9.2 Hz, CH**H'**OCH_2), 4.15 (d, J = 2.4 Hz, CH_2OC**H**$_2$), 4.43–4.44 (m, C**H**), 5.38 (d, J = 8.4 Hz, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.5 (C(**C**H_3)$_3$), 52.7 (O**C**H_3), 54.0 (**C**H), 58.7 (**C**H_2CCH), 69.9 (**C**H_2OCH_2), 75.2 (CH_2C**C**H), 79.0 (CH_2**C**CH), 80.2 (**C**$(CH_3)_3$), 155.6 (**C**(O)N), 171.1 (**C**(O)OCH_3); HRMS (ESI) 280.1167 [M + Na^+] (calcd for $C_{12}H_{19}NO_5Na^+$ 280.1161).

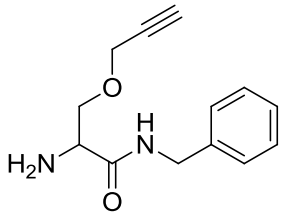


(*R,S*)-2-*N*-(*t*-Butoxycarbonyl)amino-3-(prop-2-ynyloxy)propionic Acid ((*R,S*)-135). (*R,S*)-Methyl 2-*N*-(*t*-butoxycarbonyl)amino-3-(prop-2-ynyloxy)propionate (959 mg, 37.30 mmol) was dissolved in anhydrous THF (37 mL) and a solution of LiOH (0.6 M, 6.22 mL, 37.30 mmol) was added at room temperature (18 h) and then the solvent was evaporated *in vacuo*. The crude product was diluted with H_2O (25 mL), acidified to pH 3 with aqueous 1 M $KHSO_4$, and extracted with EtOAc (6 x 25 mL), dried (Na_2SO_4), and evaporated *in vacuo* to give the crude product (916 mg, 99%) as a pale yellow, waxy solid. The product was used for the next step without further purification: R_f 0.46 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (300 MHz, CD_3OD) δ 1.45 (s, C(C**H$_3$)$_3$**), 2.87 (t, J = 2.3 Hz, CH_2CC**H**), 3.76 (dd, J = 3.9, 9.6 Hz, C**H**H'OCH_2), 3.89 (dd, J = 4.6, 9.6 Hz, CH**H'**OCH_2), 4.17 (d, J = 2.3 Hz, CH_2OC**H$_2$**), 4.32 (t, J = 4.2 Hz, C**H**); ^{13}C NMR (75 MHz, CD_3OD) δ 28.8 (C(**C**$H_3)_3$), 55.2 (**C**H), 59.3 (**C**H_2CCH), 70.6 (**C**H_2OCH_2), 76.4 (CH_2**C**CH), 80.2 (CH_2**C**CH), 80.9 (**C**$(CH_3)_3$), 158.1 (**C**(O)N), 173.7 (**C**(O)OCH_3).



(*R,S*)-*N*-Benzyl 2-*N'*-(*t*-Butoxycarbonyl)amino-3-(prop-2-ynyloxy)propionamide ((*R,S*)-137). Employing Method B and using (*R,S*)-methyl 2-*N*-(*t*-butoxycarbonyl)amino-3-(prop-2-ynyloxy)propionic acid (894 mg, 3.68 mmol), NMM (0.53 mL, 4.78 mmol), IBCF (0.52 mL, 4.05 mmol), and benzylamine (0.42 mL, 3.86 mmol) gave the crude product that was purified by flash column chromatography (SiO_2; 10–50% EtOAc/hexanes) to give the desired product (1.10 g, 90%) as a pale yellow solid: mp 100–101 ºC; R_f 0.77 (1:1 EtOAc/hexanes); IR (nujol mull) 3332, 3263, 2878, 2114, 1710, 1660, 1539, 1457, 1369, 1300, 1249, 1166, 1092, 1020, 943, 871, 663 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, C(C**H$_3$)$_3$**), 2.45 (t, J =

2.4 Hz, $CH_2CC\mathbf{H}$), 3.70 (dd, *J* = 6.2, 9.2 Hz, C**H**H'OCH_2), 3.97 (dd, *J* = 3.8, 9.2 Hz, CH**H'**OCH_2), 4.11–4.23 (m, $CH_2OC\mathbf{H_2}$), 4.25–4.39 (m, C**H**), 4.49 (d, *J* = 5.2 Hz, NHC$\mathbf{H_2}$Ph), 5.31–5.42 (br d, C(O)N**H**), 6.66–6.72 (br t, N**H**CH_2Ph), 7.25–7.34 (m, $C_6\mathbf{H_5}$); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 28.5 (C($\mathbf{C}H_3)_3$), 43.7 (NH$\mathbf{C}H_2$Ph), 54.3 (**C**H), 58.8 ($\mathbf{C}H_2CCH$), 69.7 ($\mathbf{C}H_2OCH_2$), 75.4 ($CH_2C\mathbf{C}H$), 79.1 ($CH_2\mathbf{C}CH$), 80.6 ($\mathbf{C}(CH_3)_3$), 127.7, 127.8, 128.9, 138.1 ($\mathbf{C_6}H_5$), 155.2 (O**C**(O)N), 170.2 (C**C**(O)N); HRMS (ESI) 355.1645 [M + Na^+] (calcd for $C_{18}H_{24}N_2O_4Na^+$ 355.1634); Anal. Calcd for $C_{18}H_{24}N_2O_4$: C, 65.04; H, 7.28; N, 8.43. Found: C, 65.26; H, 7.44; N, 8.42.



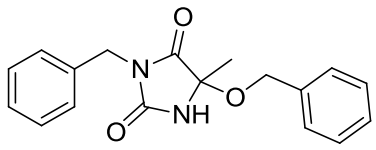
(*R,S*)-*N*-Benzyl 2-Amino-3-(prop-2-ynyloxy)propionamide ((*R,S*)-69). Utilizing Method E and using (*R,S*)-*N*-benzyl 2-*N'*-(*t*-butoxycarbonyl)amino-3-(prop-2-ynyloxy)propionamide (973 mg, 2.93 mmol), TFA (3.26 mL, 43.94 mol), and CH_2Cl_2 (10 mL) gave the crude product after workup that was further purified by flash column chromatography (SiO_2; 1:100–1:10 MeOH/CH_2Cl_2) to give the desired product (512 mg, 75%) as a pale yellow oil: R_f 0.29 (1:100 MeOH/CH_2Cl_2); IR (neat) 3344, 3295, 3142, 3066, 2909, 2114, 1659, 1527, 1454, 1357, 1258, 1095, 1023, 916, 737, 697 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) *δ* 1.64–1.72 (br s, N$\mathbf{H_2}$), 2.45 (t, *J* = 2.4 Hz, $CH_2CC\mathbf{H}$), 3.62 (dd, *J* = 4.0, 6.2 Hz, C**H**), 3.76 (dd, *J* = 6.2, 9.2 Hz, C**H**H'OCH_2), 3.84 (dd, *J* = 4.0, 9.2 Hz, CH**H'**OCH_2), 4.13–4.24 (m, $CH_2OC\mathbf{H_2}$), 4.41–4.51 (m, NHC$\mathbf{H_2}$Ph), 7.24–7.35 (m, $C_6\mathbf{H_5}$), 7.73–7.81 (br t, N**H**CH_2Ph); ^{13}C NMR (400 MHz, $CDCl_3$) *δ* 43.4 (NH$\mathbf{C}H_2$Ph), 55.1 (**C**H), 58.7 ($\mathbf{C}H_2CCH$), 72.3 ($\mathbf{C}H_2OCH_2$), 75.1 ($CH_2C\mathbf{C}H$), 79.4 ($CH_2\mathbf{C}CH$), 127.6, 127.8, 128.8, 138.5 ($\mathbf{C_6}H_5$), 172.5 (C**C**(O)N); HRMS (ESI) 233.1294 [M +

H^+] (calcd for $C_{13}H_{16}N_2O_2H^+$ 233.1290); Anal. Calcd for $C_{13}H_{16}N_2O_2 \bullet 0.09CH_2Cl_2$: C, 65.59; H, 6.80; N, 11.69. Found: C, 65.54; H, 6.97; N, 11.68.

Synthesis of hydantoin derivatives

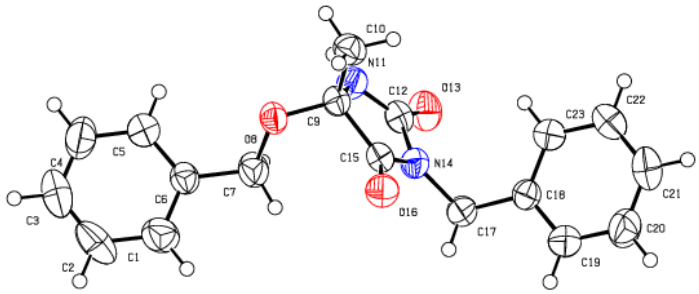
A suspension of (*R,S*)-*N*-benzyl-2-*N*-(benzyloxycarbonyl)amino-3-hydroxypropionamide (2.25 g, 6.86 mmol) in anhydrous CH_2Cl_2 (70 mL) was cooled to 0 ºC and triethylamine (2.1 mL, 15.09 mmol) and methanesulfonyl chloride (0.9 mL, 11.66 mmol) were successively added. After 1 h at 0 ºC, DBU (2.1 mL, 13.71 mmol) was added and the reaction was maintained at 0 ºC for 30 min before warming to room temperature (4 h). Then, DBU (2.1 mL, 13.71 mmol) was added and the reaction continued overnight. The reaction was diluted with Et_2O (30 mL), and successively washed with aqueous 10% citric acid (3 x 100 mL), saturated aqueous $NaHCO_3$ (3 x 100 mL), and saturated aqueous brine (2 x 100 mL), dried (Na_2SO_4), concentrated *in vacuo*, and further purified by flash column chromatography (SiO_2; 1:10–1:0 EtOAc/hexanes) to give benzyl alcohol (0.27 g, 37%) as a pale yellow oil, 3-benzyl-5-(benzyloxy)-5-methylimidazolidine-2,4-dione (0.06 g, 3%) as a pale yellow solid, 3-benzyl-5-methyleneimidazolidine-2,4-dione (0.37 g, 27%) as a colorless oil, and compound C (0.31 g, 11%) as a white solid.

Benzyl alcohol (143): R_f 0.38 (1:5 EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.42 (s, O**H**), 4.60 (s, C**H$_2$**Ph), 7.22–7.36 (m, C$_6$**H$_5$**); ^{13}C NMR ($CDCl_3$) δ 65.3 (**C**H$_2$Ph), 127.1, 127.7, 128.7, 141.0 (**C$_6$**H$_5$).



3-Benzyl-5-(benzyloxy)-5-methylimidazolidine-2,4-dione was further purified by recrystallization from EtOAc (144): mp 130–131 ºC; R_f 0.34 (1:5 EtOAc/hexanes); ^{1}H

NMR (300 MHz, $CDCl_3$) δ 1.64 (s, C**H**$_3$), 4.18 (d, J = 10.5 Hz, NC**H**H'Ph), 4.37 (d, J = 10.5 Hz, NCH**H'**Ph), 4.66 (1/2 ABq, J = 14.6 Hz, OC**H**H'Ph), 4.72 (1/2 ABq, J = 14.6 Hz, OCH**H'**Ph), 5.92 (s, N**H**), 7.22–7.43 (m, 2 C$_6$**H**$_5$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.7 (**C**H$_3$), 42.5 (N**C**H$_2$Ph), 66.4 (O**C**H$_2$Ph), 87.0 (**C**CH$_3$), 128.1, 128.2, 128.3, 128.6, 128.7, 129.0, 136.0, 136.7 (2 **C**$_6$H$_5$), 155.5 (N**C**(O)N), 171.9 (C**C**(O)N); HRMS (ESI) 333.1216 [M + Na^+] (calcd for $C_{18}H_{18}N_2O_3Na^+$ 333.1215); Anal. Calcd for $C_{18}H_{18}N_2O_3$; C, 69.66; H, 5.85; N, 9.03. Found C, 69.93; H, 5.67; N, 9.04.

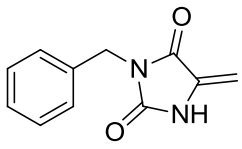


Crystallographic data collection and processing parameters for 3-benzyl-5-(benzyloxy)-5-methylimidazolidine-2,4-dione (Table 19).

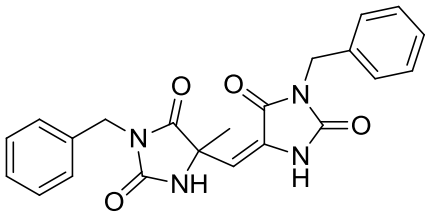
Table 19. Crystal data and structure refinement for 3-benzyl-5-(benzyloxy)-5-methylimidazolidine-2,4-dione

Empirical formula	$C_{18}H_{18}N_2O_3$
Formula weight	310.34
Temperature	296(2) K
Wavelength	1.54178 Å
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	a = 18.6606(15) Å
	b = 8.1395(7) Å
	c = 11.1590(10) Å
	α= 90°.

	β= 106.943(5)°.
	γ = 90°.
Volume	1621.3(2) $Å^3$
Z	4
Density (calculated)	1.271 Mg/m^3
Absorption coefficient	0.712 mm^{-1}
F(000)	656
Crystal size	0.30 x 0.25 x 0.25 mm^3
Theta range for data collection	2.48 to 66.58°.
Index ranges	-22<=h<=21, -9<=k<=9, -11<=l<=13
Reflections collected	16778
Independent reflections	2811 [R(int) = 0.0393]
Completeness to theta = 66.58°	98.1%
Absorption correction	Numerical
Max. and min. transmission	0.8420 and 0.8147
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2811 / 0 / 210
Goodness-of-fit on F2	1.049
Final R indices [I>2sigma(I)]	R1 = 0.0389, wR2 = 0.1014
R indices (all data)	R1 = 0.0473, wR2 = 0.1061
Extinction coefficient	0.0040(5)
Largest diff. peak and hole	0.224 and -0.264 e.$Å^{-3}$



3-Benzyl-5-methyleneimidazolidine-2,4-dione[149] (145): R_f 0.61 (1:1 EtOAc/Hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.72 (s, NC**H$_2$**Ph), 4.92 (d, J = 2.0 Hz, CC**H**H'), 5.44 (d, J = 2.0 Hz, CCH**H'**), 7.21–7.41 (m, C_6**H$_5$**), 8.28–8.36 (br s, N**H**).



Compound C was further purified by recrystallization from hot EtOAc (**146**): mp 228–229 ºC; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.56 (C**H$_3$**), 4.55 (s, NC**H$_2$**Ph), 4.62 (m, NC**H$_2$**Ph), 5.62 (s, C**H**), 7.22–7.37 (m, 2 C$_6$**H$_5$**), 8.56 (s, N**H**), 10.28 (s, N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 23.7 (**C**H$_3$), 41.0 (N**C**H$_2$Ph), 41.1 (N**C**H$_2$Ph), 59.8 (**C**CH$_3$), 108.4 (**C**H), 126.8, 127.0, 127.1, 127.2, 128.2, 128.6, 135.9, 136.0 (2 **C$_6$**H$_5$), 153.9 (CH$_3$C**C**(O)N), 154.8 (CHCN**C**(O)N), 162.7 (CHC**C**(O)N), 173.9 (CH$_3$C**C**(O)N); HRMS (ESI) 405.1566 [M + H$^+$] (calcd for $C_{22}H_{20}N_4O_4H^+$ 405.1556); Anal. Calcd for $C_{22}H_{20}N_4O_4$; C, 65.34; H, 4.98; N, 13.85. Found C, 65.39; H, 4.83; N, 13.50. HSQC (Figure 13) and HMBC (Figure 14) experiments were performed to corroborate the assigned structure.

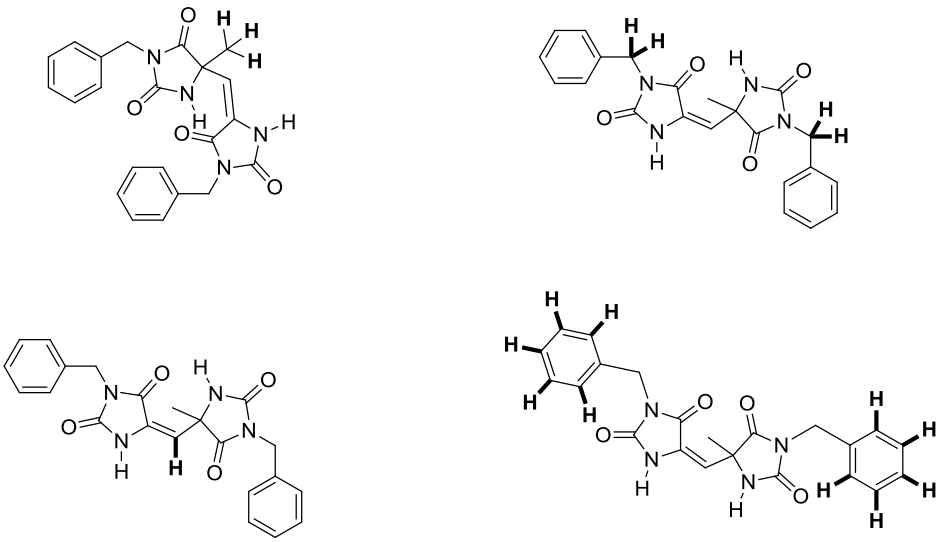


Figure 13. Bond correlations obtained from HSQC experiment

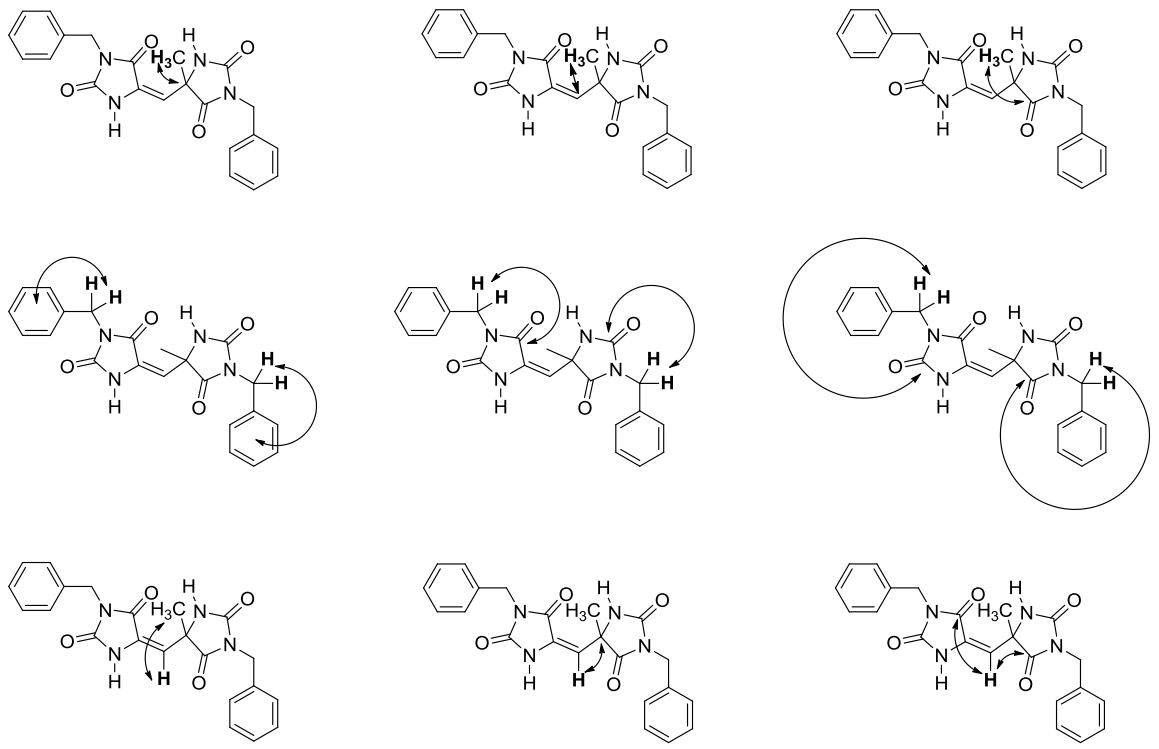
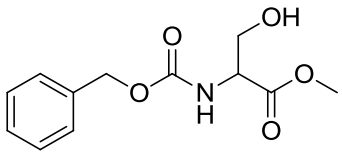
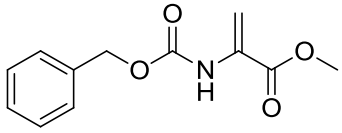


Figure 14. Two and three bond correlations obtained from HMBC experiment

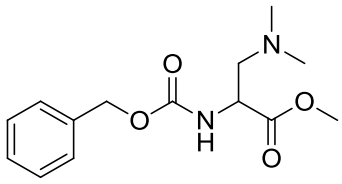


(*R,S*)-*N*-(Benzyloxycarbonyl)-serine Methyl Ester ((*R,S*)-138).[188] Utilizing Method A and using DL-serine methyl ester hydrochloride (10.0 g, 64.3 mmol), $NaHCO_3$ (17.8 g, 212.1 mmol), and H_2O (130 mL) gave the crude product after workup that was further purified by flash column chromatography (SiO_2; 1:100 MeOH/CH_2Cl_2) to give the desired product (12.17 g, 75%) as a pale yellow oil: R_f 0.29 (1:100 MeOH/CH_2Cl_2); IR (neat) 3413, 3362, 3033, 2954, 2893, 1708, 1522, 1219, 1065, 912, 746, 700 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 3.59–3.69 (m, C**H**$_2$OH, C**H**$_3$), 4.14–4.20 (m, C**H**), 4.98 (t, J = 6.0 Hz, O**H**), 5.05 (s, OC**H**$_2$Ph), 7.29–7.38 (m, C_6**H**$_5$), 7.55 (d, J = 7.8 Hz, N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 51.5 (**C**H$_3$), 56.3 (**C**H), 60.9 (**C**H$_2$OH), 65.3 (O**C**H$_2$Ph), 127.4, 127.5, 128.0, 136.6 (**C**$_6$H$_5$), 155.7

(N**C**(O)O), 170.9 (CH**C**(O)); HRMS (ESI) 276.0844 [M + Na^+] (calcd for $C_{12}H_{15}NO_5Na^+$ 276.0848).

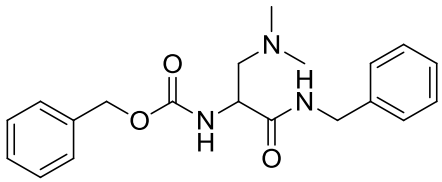


(*R,S*)-*N*-(Benzyloxycarbonyl)amino-dehydroalanine Methyl Ester ((*R,S*)-140). (*R,S*)-*N*-(Benzyloxycarbonyl)-serine methyl ester (1.00 g, 3.95 mmol) was dissolved in anhydrous THF (20 mL) and cooled to 0 ºC before the dropwise addition of Et_3N (0.66 mL, 4.74 mmol). After 10 min, MsCl (0.37 mL, 4.74 mmol) was added dropwise at 0 ºC, which led to the formation of a precipitate. After 1 h at 0 ºC, Et_3N (0.66 mL, 4.74 mmol) was added and the reaction was warmed to room temperature (3 h). The precipitate was filtered, washed with anhydrous THF, and the filtrate was evaporated *in vacuo* to give a crude oil (0.58 g, 62%) that was used without further purification: R_f 0.54 (1:10 EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.30–3.75 (br s, N**H**), 3.83 (s, OC**H$_3$**), 5.16 (s, OC**H$_2$**Ph), 5.79 (s, C**H**H'), 6.25 (s, CH**H'**), 7.26–7.39 (m, C_6**H$_5$**).



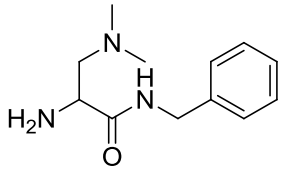
(*R,S*)-2-*N*-(Benzyloxycarbonyl)amino-3-(*N',N'*-dimethyl)aminopropionic Methyl Ester ((*R,S*)-156). Utilizing Method F and using (*R,S*)-*N*-(benzyloxycarbonyl)-serine methyl ester (6.11 g, 24.14 mmol), Et_3N (4.0 mL, 28.97 mmol), MsCl (2.2 mL, 28.97 mmol), dimethylamine (in 2 M THF, 100 mL, 193.1 mmol), and MeOH (100 mL) gave the crude product after workup that was further purified by flash column chromatography (SiO_2, 1:100–1:10 MeOH/CH_2Cl_2) to give the desired product (4.51 g, 67%) as an orange oil: R_f 0.47 (1:20

MeOH/CH_2Cl_2); IR (neat) 3494, 3248, 3145, 1718, 1524, 1458, 1213, 1056, 908, 850, 745, 700 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.21 (s, N(C**H**$_3$)$_2$), 2.57–2.69 (m, C**H**$_2$N(CH$_3$)$_2$), 3.73 (s, OC**H**$_3$), 4.30–4.36 (m, C**H**), 5.10 (s, OC**H**$_2$Ph), 5.89 (d, J = 6.3 Hz, N**H**), 7.27–7.36 (m, C$_6$**H**$_5$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 45.6 (N(**C**H$_3$)$_2$), 52.4 (**C**H$_3$ or **C**H), 52.8 (**C**H$_3$ or **C**H), 59.9 (**C**H$_2$N(CH$_3$)$_2$), 67.0 (O**C**H$_2$Ph), 128.2, 128.5, 136.4 (**C**$_6$H$_5$), 156.2 (N**C**(O)O), 172.4 (CH**C**(O)), one aromatic peak was not detected and is believed to overlap with nearby signals; HRMS (ESI) 303.1322 [M + Na^+] (calcd for $C_{14}H_{20}N_2O_4Na^+$ 303.1321); Anal. Calcd for $C_{14}H_{20}N_2O_2$; C, 59.99; H, 7.19; N, 9.99. Found C, 60.17; H, 7.29; N, 9.85.

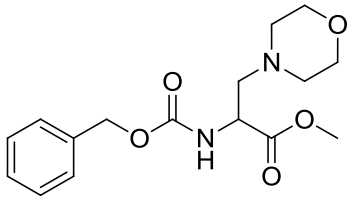


(*R,S*)-*N*-Benzyl 2-*N'*-(Benzyloxycarbonyl)amino-3-(*N'',N''*-dimethyl)aminopropionamide ((*R,S*)-158). Utilizing Method G and using (*R,S*)-*N*-(benzyloxycarbonyl)amino-3-(*N',N'*-dimethyl)aminopropionic methyl ester (3.88 g, 13.85 mmol), LiOH (0.33 g, 13.85 mmol), benzylamine hydrochloride (2.39 g, 16.62 mmol), DMTMM (4.60 g, 16.62 mmol), and THF/H_2O (140 mL/70 mL) gave the crude product after workup that was further purified by flash column chromatography (SiO_2, 1:3 CH_2Cl_2/EtOAc followed by 1:10 MeOH/CH_2Cl_2) to give the desired product (1.67 g, 34%) as an orange solid: mp 82-83 ºC; R_f 0.44 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3403, 3149, 2856 (br), 1706, 1655, 1550, 1457, 1375, 1255, 1058, 904, 850, 732, 694 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.26 (s, N(C**H**$_3$)$_2$), 2.43 (dd, J = 8.7, 12.4 Hz, C**H**H'N(CH$_3$)$_2$), 2.60 (dd, J = 6.2, 12.4 Hz, C**H**H'N(CH$_3$)$_2$), 4.09–4.19 (m, C**H**), 4.38 (dd, J = 5.3, 14.9 Hz, NHC**H**H'Ph), 4.53 (dd, J = 6.2, 14.9 Hz, NHCH**H'**Ph), 5.08 (1/2 ABq, J = 12.2 Hz, OC**H**H'Ph), 5.13 (1/2 ABq, J = 12.2 Hz, OCH**H'**Ph), 5.86–6.01 (br d, N**H**C(O)), 7.23–7.36 (m, 2 C$_6$**H**$_5$), 8.36–8.55 (br t, N**H**CH$_2$Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ

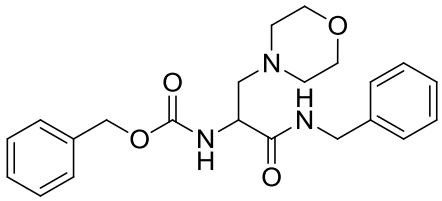
43.5 (NH**C**H_2Ph), 45.2 (N(**C**H_3)$_2$), 51.2 (**C**H), 61.2 (**C**H_2N(CH_3)$_2$), 67.1 (O**C**H_2Ph), 127.5, 127.6, 128.2, 128.3, 128.7, 128.8, 136.4, 138.5 (**C$_6$**H_5), 156.4 (N**C**(O)O), 171.1 (CH**C**(O)); HRMS (ESI) 378.1798 [M + Na^+] (calcd for $C_{20}H_{25}N_3O_3Na^+$ 378.1794); Anal. Calcd for $C_{20}H_{25}N_3O_3$; C, 67.58; H, 7.09; N, 11.82. Found C, 67.58; H, 7.15; N, 12.02.



(*R,S*)-*N*-Benzyl 2-Amino-3-(*N'*,*N'*-dimethyl)aminopropionamide ((*R,S*)-72). Utilizing Method C and using (*R,S*)-*N*-benzyl 2-*N'*-(benzyloxycarbonyl)amino-3-(*N''*,*N''*-dimethyl)aminopropionamide (1.08 g, 3.03 mmol), 10% Pd-C (0.1 g), and MeOH (30 mL) gave a crude oil that was purified by flash column chromatography (SiO_2; 1:100–1:5% MeOH/CH_2Cl_2) to give the desired product (0.40 g, 60%) as a pale orange oil: R_f 0.26 (1:10 MeOH/CH_2Cl_2); IR (neat) 3447, 3116, 3006, 2848, 2731, 1661, 1526, 1458, 1362, 1259, 1113, 1037, 935, 870, 739, 701, 602 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.86 (s, N**H$_2$**), 2.26 (s, N(C**H$_3$**)$_2$), 2.42 (dd, *J* = 8.8, 12.3 Hz, C**H**H'N(CH_3)$_2$), 2.60 (dd, *J* = 6.0, 12.3 Hz, CH**H'**N(CH_3)$_2$), 3.50 (dd, *J* = 6.0, 8.8 Hz, C**H**), 4.38–4.52 (m, NHC**H$_2$**Ph), 7.24–7.36 (m, C_6**H$_5$**), 8.20–8.34 (br t, N**H**CH_2Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 43.2 (NH**C**H_2Ph), 45.4 (N(**C**H_3)$_2$), 52.5 (**C**H), 63.0 (**C**H_2N(CH_3)$_2$), 127.5, 127.8, 128.8, 138.8 (**C$_6$**H_5), 174.4 (CH**C**(O)); HRMS (ESI) 244.1434 [M + Na^+] (calcd for $C_{12}H_{19}N_3ONa^+$ 244.1426); Anal. Calcd for $C_{12}H_{19}N_3O_2$•0.33H_2O; C, 63.41; H, 8.72; N, 18.49. Found C, 63.38; H, 8.52; N, 18.21.

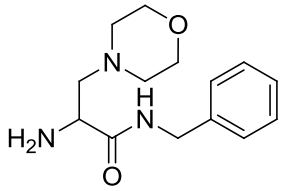


(*R,S*)-2-*N*-(Benzyloxycarbonyl)amino-3-morpholinopropionic Methyl Ester ((*R,S*)-157). Utilizing Method F and using (*R,S*)-*N*-(benzyloxycarbonyl)-serine methyl ester (7.00 g, 27.66 mmol), Et_3N (4.6 mL, 33.19 mmol), MsCl (2.6 mL, 33.19 mmol), and morpholine (24.2 mL, 276.6 mmol) gave the crude product after workup that was further purified by flash column chromatography (SiO_2, 1:100–1:5% MeOH/CH_2Cl_2) to give the desired product (6.58 g, 74%) as a white solid: mp 69–70 ºC; R_f 0.53 (1:10 MeOH/CH_2Cl_2); IR (nujol mull) 3284, 2904 (br), 1749, 1679, 1528, 1457, 1373, 1302, 1269, 1207, 1159, 1109, 1054, 1007, 904, 862, 737 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.37–2.50 (m, N(C**H**$_2$$CH_2$)$_2$O), 2.65–2.76 (m, C**H**$_2$N($CH_2CH_2$)$_2$O), 3.64 (t, *J* = 4.5 Hz, N(CH_2C**H**$_2$)$_2$O), 3.75 (s, OC**H**$_3$), 4.35–4.41 (m, C**H**), 5.09 (1/2 ABq, *J* = 12.2 Hz, OC**H**H'Ph), 5.14 (1/2 ABq, *J* = 12.2 Hz, OCH**H'**Ph), 5.67–5.69 (d, *J* = 6.6 Hz, N**H**), 7.31–7.40 (m, C_6**H**$_5$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 52.2 (O**C**H_3), 52.5 (**C**H), 53.8 (N(**C**H_2CH_2)O), 59.2 (**C**H_2N(CH_2CH_2)O), 67.0 (N(CH_2**C**H_2)O), 67.2 (O**C**H_2Ph), 128.4, 128.7, 136.4 (**C**$_6$$H_5$), 156.1 (N**C**(O)O), 172.4 (CH**C**(O)), one aromatic peak was not detected and is believed to overlap with nearby signals; HRMS (ESI) 345.1431 [M + Na^+] (calcd for $C_{16}H_{22}N_2O_5Na^+$ 345.1427); Anal. Calcd for $C_{16}H_{22}N_2O_5$; C, 59.61; H, 6.88; N, 8.69. Found C, 59.53; H, 6.79; N, 8.59.



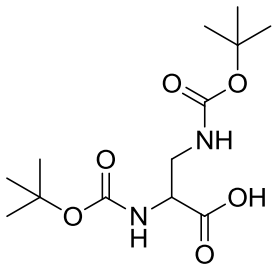
(*R,S*)-*N*-Benzyl 2-*N'*-(Benzyloxycarbonyl)amino-3-morpholinopropionamide ((*R,S*)-159). Utilizing Method G and using (*R,S*)-2-*N*-(benzyloxycarbonyl)amino-3-morpholinopropionic methyl ester (5.00 g, 15.52 mmol), LiOH (0.37 g, 15.52 mmol), benzylamine hydrochloride (2.67 g, 18.62 mmol), DMTMM (5.15 g, 18.62 mmol), and THF/H_2O (150 mL/75 mL) gave the crude product after workup that was further purified by

column chromatography (SiO_2, 1:100–1:10 MeOH/CH_2Cl_2) followed by recrystallization from hot EtOAc/hexanes to give the desired product (3.49 g, 57%) as a white solid: mp 103–104 ºC; R_f 0.50 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3418, 3290, 3140, 2912 (br), 1658, 1554, 1457, 1376, 1260, 1113, 1037, 869, 732, 697 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.26–2.82 (m, C**H**$_2$N(C**H**$_2$CH$_2$)$_2$O), 3.41–3.56 (m, N(CH$_2$C**H**$_2$)$_2$O), 4.10–4.21 (m, C**H**), 4.33 (dd, *J* = 4.8, 14.6 Hz, NC**H**H'Ph), 4.54 (dd, *J* = 6.3, 14.6 Hz, NCH**H'**Ph), 5.08 (1/2 ABq, *J* = 12.3 Hz, OC**H**H'Ph), 5.13 (1/2 ABq, *J* = 12.3 Hz, OCH**H'**Ph), 5.87–5.94 (br d, N**H**C(O)), 7.25–7.39 (m, C$_6$**H**$_5$) 8.10–8.23 (m, N**H**CH$_2$Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 43.9 (NH**C**H$_2$Ph), 50.6 (**C**H), 53.5 (N(**C**H$_2$CH$_2$)O), 60.3 (**C**H$_2$N(CH$_2$CH$_2$)O), 67.0 (N(CH$_2$**C**H$_2$)O), 67.2 (O**C**H$_2$Ph), 127.9, 128.0, 128.3, 128.4, 128.7, 129.0, 136.4, 138.2 (2 **C**$_6$H$_5$), 156.3 (N**C**(O)O), 170.7 (CH**C**(O)); HRMS (ESI) 420.1884 [M + Na^+] (calcd for $C_{22}H_{27}N_3O_4Na^+$ 420.1899); Anal. Calcd for $C_{22}H_{27}N_3O_4$; C, 66.48; H, 6.85; N, 10.57. Found C, 66.52; H, 6.82; N, 10.64.

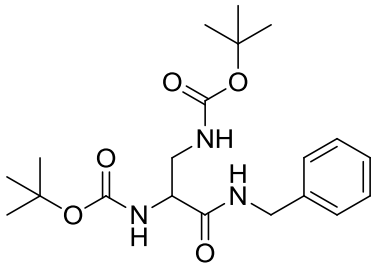


(*R,S*)-*N*-Benzyl 2-Amino-3-morpholinopropionamide ((*R,S*)-73). Utilizing Method C and using (*R,S*)-*N*-benzyl 2-*N*'-(benzyloxycarbonyl)amino-3-morpholinopropionamide (2.50 g, 6.29 mmol), 10% Pd-C (0.25 g), and MeOH (60 mL) gave the crude product that was further purified by flash column chromatography (SiO_2; 1:100 MeOH/CH_2Cl_2) to give the desired compound (0.94 g, 57%) as a white solid: mp 84–85 ºC; R_f 0.24 (5% MeOH/CH_2Cl_2); IR (nujol mull) 3401, 3237, 3168, 3096, 2920 (br), 1650, 1598, 1458, 1375, 1115, 946, 876, 720 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.83 (s, N**H**$_2$), 2.37–2.55 (m, C**H**H'N(C**H**$_2$CH$_2$)$_2$O), 2.67 (dd, *J* = 5.7, 12.3 Hz, CH**H'**N(CH$_2$CH$_2$)$_2$O), 3.53–3.69 (m, C**H**, N(CH$_2$C**H**$_2$)$_2$O), 4.43 (d, *J* = 5.7 Hz, NHC**H**$_2$Ph), 7.24–7.36 (m, C$_6$**H**$_5$), 8.06–8.18 (br t, N**H**CH$_2$Ph); ^{13}C NMR (75 MHz,

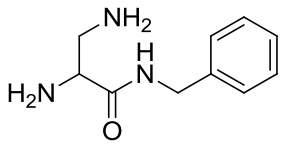
$CDCl_3$) δ 43.2 (NH**C**H_2Ph), 51.6 (**C**H), 53.5 (N(**C**H_2CH_2)O), 61.9 (**C**H_2N(CH_2CH_2)O), 67.0 (N(CH_2**C**H_2)O), 127.5, 127.8, 128.8, 138.5 (**C**$_6$$H_5$), 174.1 (CH**C**(O)); HRMS (ESI) 286.1533 [M + Na^+] (calcd for $C_{14}H_{21}N_3O_2Na^+$ 286.1532); Anal. Calcd for $C_{14}H_{21}N_3O_2$; C, 63.85; H, 8.04; N, 15.96. Found C, 63.71; H, 7.99; N, 15.77.



(*R,S*)-*N,N*'-2,3-Bis(*t*-butoxycarbonyl)aminopropionic Acid ((*R,S*)-161).[150,152] 2,3-Diaminopropionic acid hydrochloride (5.00 g, 35.57 mmol) and $NaHCO_3$ (29.88 g, 0.36 mol) were dissolved in H_2O (150 mL) and dioxane (150 mL). Di-*tert*-butyl-dicarbonate (31.05 g, 142.3 mmol) was added and the reaction was stirred at room temperature (18 h). The mixture was diluted with H_2O (100 mL), washed with CH_2Cl_2 (2 x 150 mL), acidified to pH 2 with aqueous concentrated HCl, and extracted with CH_2Cl_2 (3 x 200 mL). The organic layers were combined, washed with H_2O (3 x 200 mL), dried (Na_2SO_4), and evaporated *in vacuo*. The residue was recrystallized from hot toluene to give the desired product (6.72 g, 62%) as a white solid: mp 126–127 ºC (lit.[152] mp 118–124 ºC); IR (nujol mull) 3311, 2920 (br), 1741, 1689, 1530, 1469, 1372, 1284, 1161, 1107, 1044, 868, 773, 719, 671 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41 (s, C(C**H$_3$**)$_3$), 1.42 (s, (C(C**H$_3$**)$_3$)'), 3.24 (t, J = 5.9 Hz, C**H$_2$**NH), 3.96–4.03 (m, C**H**), 6.80 (t, J = 5.9 Hz, $(CH_3)_3$COC(O)N**H**), 6.88–6.91 (d, J = 8.1 Hz, $(CH_3)_3$COC(O)N**H'**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.8 (2 **C**$(CH_3)_3$), 40.8 (**C**H_2NH), 53.4 (**C**H), 77.6, 77.8 (2 **C**$(CH_3)_3$), 155.0, 155.3 (2 **C**(O)OC$(CH_3)_3$), 171.9 (**C**(O)OH).

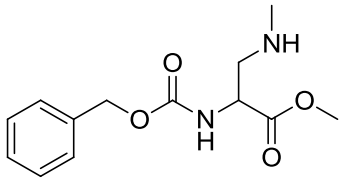


(*R,S*)-*N*-Benzyl *N',N"*-2,3-Bis(*t*-butoxycarbonyl)aminopropionamide ((*R,S*)-162). Utilizing Method B and using (*R,S*)-*N,N'*-2,3-bis(*t*-butoxycarbonyl)amino-2,3-propionic acid (1.77 g, 5.82 mmol), NMM (0.83 mL, 7.57 mmol), IBCF (0.75 mL, 6.40 mmol), and benzylamine (0.64 mL, 6.11 mmol) gave the crude product that was recrystallized twice from hot EtOAc to give the desired product (1.54 g, 67%) as a white solid: mp 141–142 ºC; R_f 0.52 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3319, 2919, 2860, 1687, 1531, 1456, 1371, 1304, 1247, 1169, 1044, 952, 873, 746, 698, 639 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.42 (s, C(C**H**$_3$)$_3$), 1.43 (s, C(C**H**$_3$)$_3$**'**), 3.45–3.60 (m, C**H**$_2$NH), 4.18–4.29 (m, C**H**), 4.39 (dd, *J* = 5.0, 15.4 Hz, NHC**H**H'Ph), 4.51 (dd, *J* = 5.6, 15.4 Hz, NHCH**H'**Ph), 5.11–5.24 (br t, $(CH_3)_3COC(O)N$**H**), 5.89 (d, *J* = 6.0 Hz, $(CH_3)_3COC(O)N$**H'**), 6.96–7.05 (br t, N**H**CH_2Ph), 7.24–7.35 (C_6**H**$_5$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.3, 28.4 (2 **C**$(CH_3)_3$), 42.5 (NH**C**H_2Ph), 43.5 (**C**H_2NH), 56.0 (**C**H), 80.0, 80.4 (2 **C**$(CH_3)_3$), 127.5, 127.6, 128.8, 138.1 (**C**$_6$$H_5$), 156.4, 157.3 (2 **C**(O)$OC(CH_3)_3$), 170.8 (**C**(O)$NHCH_2Ph$); HRMS (ESI) 416.2151 [M + Na^+] (calcd for $C_{20}H_{31}N_3O_5Na^+$ 416.2162); Anal. Calcd for $C_{20}H_{31}N_3O_5$: C, 61.05; H, 7.94; N, 10.68. Found: C, 61.16; H, 7.98; N, 10.49.

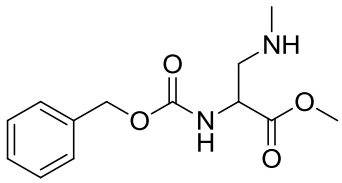


(*R,S*)-*N*-Benzyl 2,3-Diaminopropionamide ((*R,S*)-70). Utilizing Method E and using (*R,S*)-*N*-benzyl-*N',N"*-2,3-bis(*t*-butoxycarbonyl)aminopropionamide (1.71 g, 4.35 mmol), TFA (4.86 mL, 65.23 mmol), and CH_2Cl_2 (15 mL) gave the crude product after workup that was further

purified by recrystallization from hot EtOAc/hexanes to give the desired product (427 mg, 51%) as a white solid: mp 119–120 ºC; R_f 0.17 (1–10 MeOH/CH_2Cl_2); IR (nujol mull) 3316, 2924, 2858, 1645, 1523, 1459, 1376, 731 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 2.70–2.95 (m, C**H**$_2$$NH_2$), 3.35–3.42 (m, C**H**), 4.40 (s, NHC**H**$_2$Ph), 7.21–7.35 (m, C_6**H**$_5$); ^{13}C NMR (75 MHz, CD_3OD) δ 44.2 (NH**C**H_2Ph), 46.7 (**C**H_2NH_2), 57.5 (**C**H), 128.5, 128.8, 129.8, 140.0 (**C**$_6$$H_5$), 175.9 (**C**(O)NH); HRMS (ESI) 216.1107 [M + Na^+] (calcd for $C_{10}H_{15}N_3ONa^+$ 216.1113); Anal. Calcd for $C_{10}H_{15}N_3O$•H_2O: C, 61.91; H, 7.84; N, 21.66. Found: C, 61.54; H, 7.90; N, 21.38.

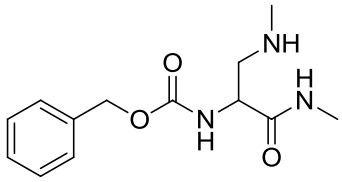


(*R,S*)-2-*N*-(Benzyloxycarbonyl)amino-3-(*N'*-methylamino)propionic Methyl Ester ((*R,S*)-155).[189,153] Utilizing Method F and using (*R,S*)-*N*-(benzyloxycarbonyl)-serine methyl ester (4.67 g, 18.45 mmol), Et_3N (3.1 mL, 22.14 mmol), MsCl (1.7 mL, 22.14 mmol), methylamine (in 2 M THF, 92.3 mL, 184.51 mmol), and MeOH (92.3 mL) gave the crude product after workup that was further purified by flash column chromatography (SiO_2, 1–20% MeOH/CH_2Cl_2) to give the desired product (1.38 g, 28%) as an orange oil and (*R,S*)-*N*-methyl-2-*N*'-(benzyloxycarbonyl)amino-3-(*N''*-methylamino)propionamide (2.06 g, 42%) as an orange solid.

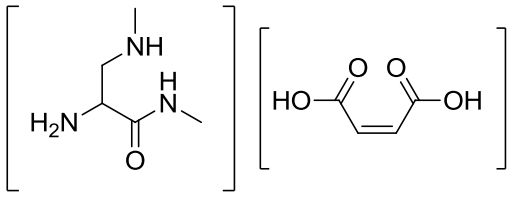


(*R,S*)-2-*N*-(Benzyloxycarbonyl)amino-3-(*N'*-methylamino)propionic Methyl Ester ((*R,S*)-155): R_f 0.26 (1:20 MeOH/CH_2Cl_2); IR (neat) 3326, 3032, 2952, 2853, 2800, 2360,

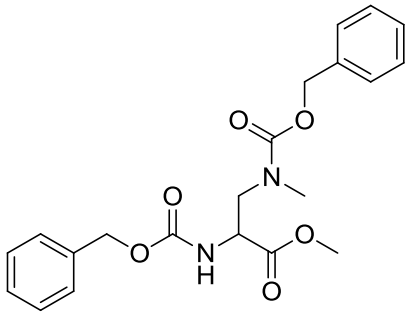
1960, 1716, 1530, 1451, 1218, 1054, 912, 746, 700 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.16–1.26 (br s, N**H**CH_3), 2.40 (s, NHC**H$_3$**), 2.89–3.02 (m, C**H$_2$**NHCH_3), 3.75 (s, OC**H$_3$**), 4.42–4.48 (m, C**H**), 5.12 (s, OC**H$_2$**Ph), 5.81 (d, J = 7.5 Hz, N**H**C(O)O), 7.27–7.37 (m, C_6**H$_5$**); ^{13}C NMR (75 MHz, $CDCl_3$) δ 36.4 (NH**C**H_3), 52.7 (O**C**H_3 or **C**H_2NHCH_3), 52.8 (O**C**H_3 or **C**H_2NHCH_3), 53.9 (**C**H), 67.2 (O**C**H_2Ph), 128.2, 128.3, 128.7, 136.4 (**C$_6$**H_5), 156.3 (N**C**(O)O), 172.3 (CH**C**(O)); HRMS (ESI) 267.1352 [M + H^+] (calcd for $C_{13}H_{18}N_2O_4H^+$ 267.1345); Anal. Calcd for $C_{13}H_{18}N_2O_4$; C, 58.63; H, 6.81; N, 10.52. Found C, 58.36; H, 6.82; N, 10.67.



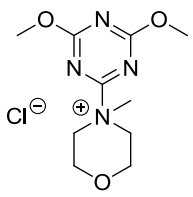
(*R,S*)-*N*-Methyl 2-*N*'-(Benzyloxycarbonyl)amino-3-(*N''*-methylamino)propionamide ((*R,S*)-163): mp 122–123 ºC; R_f 0.32 (1:10 MeOH/CH_2Cl_2); IR (nujol mull) 3319, 3217, 3170, 3041 (br), 1719, 1660, 1593, 1547, 1457, 1377, 1250, 1153, 1052, 850, 735 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.72 (s, N**H**CH_3), 2.42 (s, NHC**H$_3$**), 2.64 (dd, J = 8.0, 11.7 Hz, C**H**H'NHCH_3), 2.80 (d, J = 4.8 Hz, C(O)NHC**H$_3$**), 3.11 (dd, J = 3.6, 11.7 Hz, CH**H'**NHCH_3), 4.08–4.14 (m, C**H**), 5.11 (s, OC**H$_2$**Ph), 6.11 (d, J = 5.7 Hz, N**H**C(O)), 7.28–7.36 (m, C_6**H$_5$**) 7.49–7.60 (br s, OC(O)N**H**CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.3 (C(O)NH**C**H_3), 36.3 (NH**C**H_3), 53.2 (**C**H, CH**C**H_2), 67.2 (O**C**H_2Ph), 128.2, 128.4, 128.7, 136.4 (**C$_6$**H_5), 156.6 (N**C**(O)O), 171.8 (CH**C**(O)); HRMS (ESI) 288.1327 [M + Na^+] (calcd for $C_{13}H_{19}N_3O_3Na^+$ 288.1324); Anal. Calcd for $C_{13}H_{19}N_3O_3$; C, 58.85; H, 7.22; N, 15.84. Found C, 58.82; H, 7.22; N, 15.66.



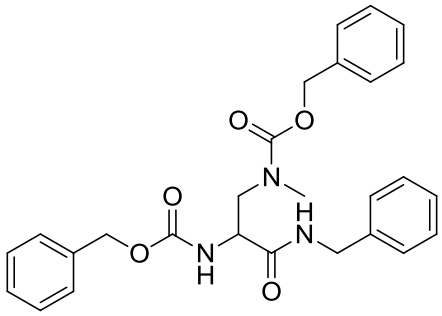
(*R,S*)-*N*-Methyl 2-Amino-3-(*N*'-methylamino)propionamide Maleate Salt ((*R,S*)-165). Utilizing Method C and using (*R,S*)-*N*-methyl 2-*N*'-(benzyloxycarbonyl)amino-3-(*N"*-methylamino)propionamide (4.00 g, 15.09 mmol), 10% Pd-C (0.40 g), and MeOH (150 mL) gave a crude oil that was then dissolved in THF (13.7 mL). A THF solution (13.7 mL) of maleic acid (6.36 g, 54.89 mmol) was added dropwise to the crude product and allowed to stand at room temperature (18 h) before the precipitate was collected as a yellow crystalline solid. The precipitate was purified by recrystallization from MeOH/Et_2O (2x) to give the desired product (2.53 g, 67%) as a white solid and as a 1:2 ratio of PAAD:maleic acid: mp 95–96 ºC; R_f 0.17 (1:4 MeOH/CH_2Cl_2); IR (nujol mull) 3156, 2906 (br), 1694, 1574, 1458, 1373, 1208, 866, 721, 650, 574 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 2.64 (s, $CH_2NHC\mathbf{H_3}$), 2.69 (d, J = 4.7 Hz, $C(O)NC\mathbf{H_3}$), 3.20 (dd, J = 6.5, 13.7 Hz, C**H**H'$NHCH_3$), 3.30 (dd, J = 5.3, 13.7 Hz, CH**H'**$NHCH_3$), 3.96–4.02 (br t, C**H**), 6.11 (s, $HCO_2C\mathbf{H}C\mathbf{H}CO_2H$), 8.43–8.48 (m, N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 26.0 ($CH_2NH\mathbf{C}H_3$), 33.5 ($C(O)NH\mathbf{C}H_3$), 49.0, 49.8 ($\mathbf{C}H_2NH$, $\mathbf{C}H$), 135.4 ($HCO_2\mathbf{C}H\mathbf{C}HCO_2H$), 166.9 ($\mathbf{C}(O)NHCH_3$), 167.5 ($H\mathbf{C}O_2CHCH\mathbf{C}O_2H$); LRMS (ESI) 132.10 [M + H^+] (calcd for $C_5H_{13}N_3OH^+$ 132.10); Anal. Calcd for $C_{13}H_{21}N_3O_9$•0.47CH_3OH: C, 42.36; H, 6.20; N, 10.92. Found: C, 42.76; H, 6.09; N, 11.10.



(*R,S*)-Methyl 2-*N*-(Benzyloxycarbonyl)amino-3-(*N*'-benzyloxycarbonyl-*N*'-methyl)aminopropionate ((*R,S*)-166). Benzyl chloroformate (0.65 mL, 4.64 mmol) was added dropwise to a solution of (*R,S*)-methyl 2-*N*-(benzyloxycarbonylamino)-3-(*N'*-methylamino)propanoate. (1.18 g, 4.42 mmol) and DIEA (0.81 mL, 4.64 mmol) in dioxane (23 mL) at room temperature while stirring under N_2. The reaction was maintained at room temperature (18 h) before the solvent was evaporated *in vacuo*. The resulting crude oil was dissolved in EtOAc (25 mL) and was successively washed with aqueous 10% citric acid (3 x 25 mL), saturated aqueous $NaHCO_3$ (3 x 25 mL), and saturated aqueous brine (2 x 25 mL), dried (Na_2SO_4), evaporated *in vacuo*. The crude product was purified by flash column chromatography (SiO_2, 1:100–1:5 MeOH/CH_2Cl_2) followed by a second flash column (SiO_2, 1:10–1:1% EtOAc/hexanes) to give the desired compound (0.33 g, 18%) as a pale yellow oil: IR (neat) 3343, 3033, 2953, 1712, 1518, 1454, 1403, 1341, 1218, 1059, 746, 700, 605 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.95 (s, NC**H$_3$**), 3.57–3.79 (m, C**H$_2$**NCH$_3$, OC**H$_3$**), 4.48–4.55 (m, C**H**), 5.10 (s, 2 OC**H$_2$**Ph), 5.90 (d, J = 7.2 Hz, N**H**C(O)O), 7.26–7.38 (m, 2 C$_6$**H$_5$**); ^{13}C NMR (75 MHz, $CDCl_3$) δ 35.8 (N**C**H$_3$), 50.8 (O**C**H$_3$ or **C**H$_2$NCH$_3$), 51.2 (O**C**H$_3$ or **C**H$_2$NCH$_3$), 54.0 (**C**H), 67.6, 68.1 (2 O**C**H$_2$Ph), 128.4, 128.5, 128.6, 128.7, 129.1, 136.9, 137.1 (2 **C$_6$**H$_5$), 156.6, 157.9 (2 N**C**(O)O), 171.6 (CH**C**(O)), one aromatic peak was not detected and is believed to overlap with nearby signals; HRMS (ESI) 423.1539 [M + Na^+] (calcd for $C_{21}H_{24}N_2O_6Na$ 423.1532); Anal. Calcd for $C_{21}H_{24}N_2O_6$: C, 62.99; H, 6.04; N, 7.00. Found: C, 62.71; H, 6.11; N, 6.98.

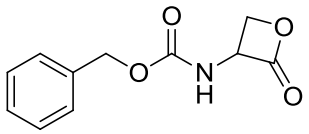


4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium Chloride (378).[5] 2-Chloro-4,6-dimethoxy-1,3,5-triazine (5.00 g, 28.5 mmol) was dissolved in reagent grade THF (570 mL) and NMM (3.44 mL, 31.4 mmol) was added at room temperature. After 1 h, the white precipitate was filtered, washed with THF, and dried *in vacuo* to give the desired product (5.32 g, 68%) as a white powder: mp 126–127 ºC (lit.[5] mp 120–122 ºC); ^{1}H NMR (300 MHz, CD_3OD) δ 3.55 (s, NC**H**$_3$), 3.80–3.96 (m, 2 C**H**$_2$), 4.04–4.11 (m, C**H**$_2$), 4.18 (s, 2 OC**H**$_3$), 4.52–4.57 (m, C**H**$_2$); ^{13}C NMR (75 MHz, CD_3OD) δ 56.6 (N**C**H$_3$), 57.7 (2 N**C**H$_2$C), 61.5 (2 O**C**H$_3$), 63.3 (2 O**C**H$_2$C), 171.9 (N**C**N), 175.5 (2 N**C**O).

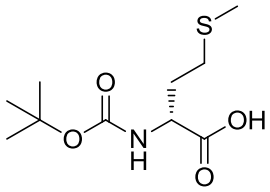


(*R,S*)-*N*-Benzyl 2-*N*'-(Benzyloxycarbonyl)amino-3-(*N*''-benzyloxycarbonyl-*N*''-methyl)aminopropionamide ((*R,S*)-167). Utilizing Method G and using (*R,S*)-methyl 2-*N*-(benzyloxycarbonyl)amino-3-(*N*'-benzyloxycarbonyl-*N*'-methyl)aminopropionate (259 mg, 0.65 mmol), LiOH (15 mg, 0.65 mmol), benzylamine hydrochloride (110 mg, 0.78 mmol), DMTMM (215 mg, 0.78 mmol), and THF/H_2O (6 mL/3 mL) gave the crude product after workup that was further purified by recrystallization from hot EtOAc/hexanes to give the desired product (205 mg, 69%) as a white solid: mp 119–120 ºC; R_f 0.44 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3298, 2932 (br), 1699, 1645, 1547, 1458, 1375, 1235, 1137, 1065, 728 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.93 (s, NC**H$_3$**), 3.57–3.81 (m, C**H$_2$**NCH$_3$), 4.28–4.50 (m, C**H**, NHC**H$_2$**Ph), 5.09 (s, 2 OC**H$_2$**Ph), 6.32–6.44 (br d, N**H**C(O)O), 6.81–6.89 (br t, N**H**CH$_2$Ph), 7.16–7.35 (m, 3 C$_6$**H$_5$**); ^{13}C NMR (75 MHz, $CDCl_3$) δ 36.4 (N**C**H$_3$), 44.1

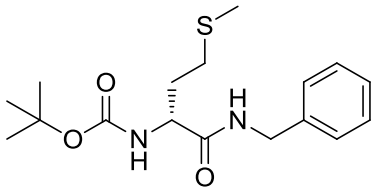
(NH**C**H_2Ph), 52.2 (**C**H_2NCH_3), 56.6 (**C**H), 67.8, 68.3 (2 O**C**H_2Ph), 128.1, 128.2, 128.4, 128.7, 128.9, 129.2, 129.3, 136.7, 136.8, 138.3 (3 $\mathbf{C_6}H_5$), 156.6, 157.9 (2 N**C**(O)O), 171.6 (CH**C**(O)), two aromatic peaks were not detected and are believed to overlap with nearby signals; HRMS (ESI) 476.2184 [M + H^+] (calcd for $C_{27}H_{29}N_3O_5H^+$ 476.2185); Anal. Calcd for $C_{27}H_{29}N_3O_5$: C, 68.19; H, 6.15; N, 8.84. Found: C, 68.22; H, 6.10; N, 8.85.



(*R,S*)-3-*N*-(Benzylcarboxycarbonyl)amino β-Propiolactone ((*R,S*)-171).[158] PPh_3 (5.49 g, 20.91 mmol) was dissolved in anhydrous THF (140 mL) and cooled to -78 ºC. DEAD (40 wt. in toluene, 9.52 mL, 20.91 mL) was added dropwise, followed by the dropwise addition of an anhydrous THF solution (30 mL) of (*R,S*)-2-*N*-(benzyloxycarbonyl)amino-3-hydroxypropanoic acid (5.00 g, 90.91 mmol). The solution stirred at -78 ºC (30 min) and then warmed to room temperature (3 h). The solvent was evaporated in vacuo to give the crude product that was further purified by recrystallization from hot $CHCl_3$/hexanes to give the desired product (1.00 g, 22%) as a white solid: mp 107-109 ºC (lit.[158] mp 114-116 ºC); R_f 0.84 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2981 (br), 1839, 1685, 1532, 1459, 1376, 1270, 1105, 1019, 885, 752 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.42–4.44 (br d, OC$\mathbf{H_2}$), 5.07–5.17 (m, C**H**, OC$\mathbf{H_2}$Ph), 5.67 (d, J = 6.9 Hz, N**H**), 7.32–7.36 (m, $C_6\mathbf{H_5}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 59.6 (NH**C**H), 66.3 (O**C**H_2Ph), 67.8 (O**C**H_2CH), 128.3, 125.5, 128.6, 135.5 ($\mathbf{C_6}H_5$), 155.2 (N**C**(O)), 168.8 (C**C**(O)); HRMS (ESI) 353.9730 [M + Cs^+] (calcd for $C_{11}H_{11}NO_4Cs^+$ 353.9742).

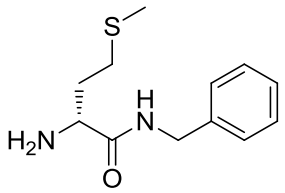


(*R*)-2-*N*-(*t*-Butoxycarbonyl)amino-4-(methylthio)butanoic Acid ((*R*)-175).[166] D-Methionine (5.00 g, 33.55 mmol) was dissolved in dioxane (20 mL) and aqueous 1.25 M NaOH (25 mL) and then cooled to 0 ºC in an ice bath under N_2. Boc_2O (7.69 g, 35.22 mmol) in dioxane (5 mL) was added dropwise and the reaction was allowed to warm to room temperature (18 h) before the organic layer was evaporated *in vacuo*. The remaining aqueous layer was diluted with aqueous 1 M $KHSO_4$ (50 mL) and extracted with EtOAc (3 x 75 mL). The organic layers were combined and washed with brine (2 x 200 mL), dried (Na_2SO_4), and *evaporated in* vacuo to give the crude product (8.19 g, 98%) as a pale yellow oil. The product was used for the next step without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (s, C(C**H**$_3$)$_3$), 1.95–2.06 (m, C**H**H'CH_2SCH_3), 2.11 (s, SC**H**$_3$), 2.12–2.26 (m, CH**H'**CH_2SCH_3), 2.58 (t, J = 8.0 Hz, C**H**$_2$$SCH_3$), 4.18–4.64 (m, C**H**), 5.24–5.27 (d, J = 8.0 Hz, N**H**C(O)).

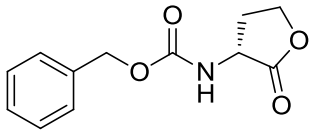


(*R*)-*N*-Benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-4-(methylthio)butanamide ((*R*)-176). Utilizing Method B and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-4-(methylthio)butanoic acid (7.10 g, 28.50 mmol), NMM (4.07 mL, 37.05 mmol), IBCF (4.04 mL, 31.35 mmol), and benzylamine (3.27 mL, 29.92 mmol) gave the crude product that was purified by recrystallization (2x) from hot EtOAc/hexanes to give the desired compound (4.21 g, 44%) as a white solid: mp 104–105 ºC; R_f 0.59 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3369, 3305, 2924, 1655, 1527, 1456, 13.73, 1328, 1293, 1247, 1170, 1055, 856, 739, 692 cm^{-1}; ^{1}H NMR

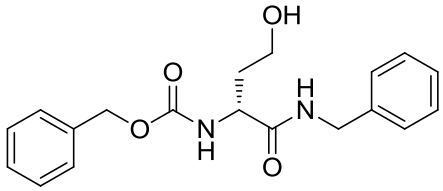
(300 MHz, $CDCl_3$) δ 1.40 (s, C(C**H**$_3$)$_3$), 1.87–1.99 (m, C**H**H'CH_2SCH_3), 2.06 (s, SC**H**$_3$), 2.07–2.16 (m, CH**H'**CH_2SCH_3), 2.50–2.60 (m, C**H**$_2$$SCH_3$), 4.29–4.48 (m, C**H**, NHC**H**$_2$Ph), 5.40–5.42 (d, J = 7.5 Hz, N**H**C(O)), 6.88–6.96 (br t, N**H**CH_2Ph), 7.23–7.34 (C_6**H**$_5$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.0 (S**C**H_3), 28.0 (C(**C**H_3)$_3$), 30.0 (**C**H_2SCH_3), 31.4 (**C**$H_2CH_2SCH_3$), 43.1 (NH**C**H_2Ph), 53.2 (**C**H), 79.8 (**C**$(CH_3)_3$), 127.1, 127.3, 128.4, 137.7 (m, **C**$_6$$H_5$), 155.4 (N**C**(O)O), 171.3 (N**C**(O)C); HRMS (ESI) 339.1751 [M + H^+] (calcd for $C_{17}H_{26}N_2O_3SH^+$ 339.1742); Anal. Calcd for $C_{17}H_{26}N_2O_3S$: C, 60.33; H, 7.74; N, 8.28; S, 9.47. Found: C, 60.18; H, 7.80; N, 8.25; S, 9.18.



(*R*)-*N*-Benzyl 2-Amino-4-(methylthio)butanamide ((*R*)-76). Utilizing Method E and using (*R*)-*N*-benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-4-(methylthio)butanamide (1.64 g, 4.85 mmol), TFA (5.40 mL, 72.74 mol), and CH_2Cl_2 (15 mL) gave the crude product that was further purified by flash column chromatography (SiO_2; 1:100–1:10 MeOH/CH_2Cl_2) to give the desired product (0.87 g, 76%) as a pale yellow oil: R_f 0.58 (1:20 MeOH/CH_2Cl_2); IR (neat) 3315, 3032, 2918, 1655, 1524, 1441, 1356, 1249, 1082, 1027, 956, 737, 700 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, N**H**$_2$), 1.73–1.87 (m, C**H**H'CH_2SCH_3), 2.10 (s, SC**H**$_3$), 2.16–2.27 (m, CH**H'**CH_2SCH_3), 2.62 (t, J = 7.4 Hz, C**H**$_2$$SCH_3$), 3.55 (dd, J = 4.4, 8.3 Hz, C**H**), 4.45 (d, J = 6.0 Hz, NHC**H**$_2$Ph), 7.25–7.36 (m, C_6**H**$_5$), 7.57–7.64 (br t, N**H**CH_2Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.5 (S**C**H_3), 30.9 (**C**H_2SCH_3), 34.3 (**C**$H_2CH_2SCH_3$), 43.4 (NH**C**H_2Ph), 54.5 (**C**H), 127.6, 127.9, 128.9, 138.6 (**C**$_6$$H_5$), 174.6 (N**C**(O)); HRMS (ESI) 239.1224 [M + H^+] (calcd for $C_{12}H_{18}N_2OSH^+$ 239.1218); Anal. Calcd for $C_{12}H_{18}N_2OS$: C, 60.47; H, 7.61; N, 11.75; S, 13.45. Found: C, 60.17; H, 7.62; N, 11.52; S, 13.69.

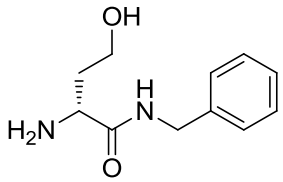


(*R*)-2-(Benzyloxycarbonyl)amino-γ-lactone ((*R*)-179).[169] D-Homoserine (5.00 g, 42.00 mmol) was dissolved in aqueous 1 M Na_2CO_3 (50 mL) and cooled to 0 ºC in an ice bath under N_2. Benzyl chloroformate (6.48 mL, 46.20 mmol) was added dropwise and the reaction was maintained at 0 ºC (30 min) before warming to room temperature (18 h). The solution was acidified to pH 2 with aqueous concentrated HCl and extracted with EtOAc (3 x 75 mL). The combined organic layers were dried (Na_2SO_4), evaporated *in vacuo*, and purified by recrystallization from hot EtOAc/hexanes to give the desired product (7.32 g, 74%) as a white solid: mp 130–131 ºC (lit.[169] mp 118–120 ºC); R_f 0.67 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3428, 3062, 2856, 1777, 1694, 1550, 1458, 1380, 1295, 1176, 1076, 1011, 946, 844, 739, 694, 629 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.14–2.29 (m, CHC**H**H'), 2.74–2.82 (m, CHCH**H'**), 4.21–4.29 (m, **C**H), 4.34–4.48 (m, $CHCH_2C\mathbf{H_2}O$), 5.13 (s, $OC\mathbf{H_2}Ph$), 5.34–5.42 (br d, N**H**), 7.32–7.38 (m, $C_6\mathbf{H_5}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.2 ($CH\mathbf{C}H_2CH_2O$), 50.6 ($\mathbf{C}HCH_2CH_2O$), 65.9 ($CHCH_2\mathbf{C}H_2O$), 67.4 ($O\mathbf{C}H_2Ph$), 128.3, 128.5, 128.7, 136.0 ($\mathbf{C_6}H_5$), 156.3 (N**C**(O)O), 175.3 (**C**(O)O); HRMS (ESI) 258.0753 [M + Na^+] (calcd for $C_{12}H_{13}NO_4Na^+$ 258.0742); Anal. Calcd for $C_{12}H_{13}NO_4$: C, 61.27; H, 5.57; N, 5.59. Found: C, 61.26; H, 5.65; N, 6.00.



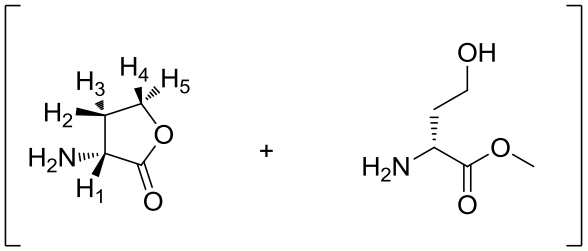
(*R*)-*N*-Benzyl 2-*N'*-(Benzyloxycarbonyl)amino-4-hydroxybutanamide ((*R*)-180). A mixture of (*R*)-2-(benzyloxycarbonyl)amino-γ-lactone (4.20 mg, 17.87 mmol) and benzylamine (3.90 mL, 35.73 mmol) was stirred in anhydrous pyridine (70 mL) at 80 ºC (18

h). The mixture was allowed to cool to room temperature and was diluted with CH_2Cl_2 (50 mL). The organic layer was successively washed with aqueous 1 M HCl (3 x 50 mL), H_2O (3 x 50 mL), and brine (2 x 50 mL), dried (Na_2SO_4), and evaporated *in vacuo*. The crude product was purified by recrystallization from hot EtOAc to give the desired product (3.57 g, 58%) as a white solid: mp 126–127 ºC; R_f 0.56 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3087, 2916 (br), 1694, 1656, 1552, 1457, 1343, 1255, 1159, 1085, 1019, 727 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.65–1.89 (m, CHC**H$_2$**), 3.40-3.46 (m, C**H$_2$**OH), 4.10-4.17 (m, C**H**), 4.21-4.35 (m, NHC**H$_2$**), 4.59 (t, O**H**), 5.03 (s, OC**H$_2$**Ph), 7.23–7.37 (m, 2 C_6**H$_5$**), 7.46 (d, J = 8.1 Hz, OC(O)N**H**), 8.46 (t, J = 6.0 Hz N**H**CH_2Ph); ^{13}C NMR (75 MHz, DMSO-d_6) δ 34.9 (CH**C**H_2), 42.0 (NH**C**H_2), 52.3 (**C**H_2OH), 57.5 (**C**H), 65.4 (O**C**H_2Ph), 126.6, 127.0, 127.7, 127.7, 128.2, 128.3, 137.0, 139.5 (2 **C$_6$**H_5), 155.9 (O**C**(O)), 172.1 (C**C**(O)); HRMS (ESI) 365.1462 [M + Na^+] (calcd for $C_{19}H_{22}N_2O_4Na^+$ 365.1477).

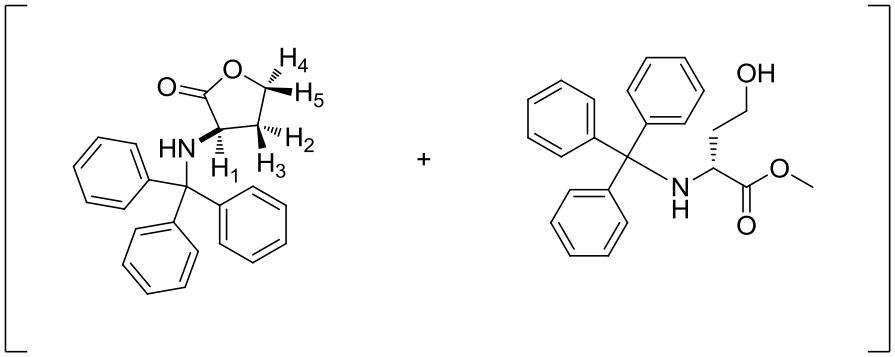


(*R*)-*N*-Benzyl 2-Amino-4-hydroxybutanamide ((*R*)-74). Utilizing Method C and using (*R*)-*N*-benzyl 2-*N*'-(benzyloxycarbonyl)amino-4-hydroxybutanamide (1.50 g, 4.38 mmol), 10% Pd-C (0.15 g), and MeOH (45 mL) gave the crude product that was further purified by flash column chromatography (SiO_2; 1:100–1:10 MeOH/CH_2Cl_2) to give the desired product (0.53 g, 57%) as a white solid: mp 88–89 ºC; R_f 0.40 (1:10 MeOH/CH_2Cl_2); $[\alpha]^{25}_D$ + 4.8° (*c* 1.1, CH_2Cl_2); IR (nujol mull) 3265, 2965 (br), 1649, 1540, 1457, 1365, 1251, 1142, 1080, 1024, 952, 801, 724, 602, 549 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.79–1.90 (m, C**H**H'CH_2OH), 1.93–2.19 (m, CH**H'**CH_2OH, N**H$_2$**), 3.61 (t, J = 6.6 Hz, C**H**), 3.76–3.88 (CH_2C**H$_2$**OH), 4.46 (d, J = 6.3 Hz, NHC**H$_2$**Ph), 7.25–7.38 (m, C_6**H$_5$**), 7.56–7.64 (br t, N**H**CH_2Ph); ^{13}C NMR (75 MHz,

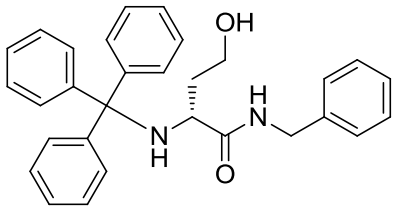
$CDCl_3$) δ 37.6 (**C**H_2CH_2OH), 43.0 (NH**C**H_2Ph), 54.1 (**C**H), 60.6 (CH_2**C**H_2OH), 127.3, 127.5, 128.5, 137.9 (**C**$_6H_5$), 175.0 (**C**(O)N); HRMS (ESI) 209.1288 [M + H^+] (calcd for $C_{11}H_{16}N_2O_2H^+$ 209.1290); Anal. Calcd for $C_{11}H_{16}N_2O_2$: C, 63.44; H, 7.74; N, 13.45. Found: C, 63.31; H, 7.86; N, 13.31.



(*R*)-2-Amino-γ-lactone and (*R*)-Methyl 4-Hydroxy-2-aminobutanoate ((*R*)-182).[171,172] An anhydrous MeOH solution (35 mL) was cooled to 0 ºC in an ice bath under N_2 and $SOCl_2$ (2.44 mL, 33.60 mmol) was added dropwise. After 30 min at 0 ºC, D-homoserine (4.00 g, 33.60 mmol) was added and the reaction warmed to room temperature (18 h). The solvent was evaporated *in vacuo* and the crude product was triturated with Et_2O (3 x 50 mL) to give a 5:1 mixture of lactone:ester. The crude product was used for the next step without further purification: R_f 0.00 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, D_2O) Lactone: δ 2.45–2.56 (m, **H$_2$**), 2.83–2.90 (m, **H$_3$**), 4.47–4.56 (m, **H$_1$**, **H$_5$**), 4.67 (t, J = 9.2 Hz, **H$_4$**); Ester: δ 2.17–2.40 (m, C**H$_2$**CH_2OH), 3.84 (t, J = 5.6 Hz, C**H$_2$**OH), 3.90 (s, OC**H$_3$**), 4.33–4.39 (m, C**H**).

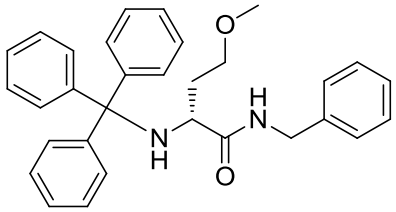


(*R*)-2-*N*-(Trityl)amino-γ-lactone and (*R*)-Methyl 4-Hydroxy-2-(*N*-trityl)aminobutanoate ((*R*)-183).[171,172] A mixture of (*R*)-2-amino-γ-lactone and (*R*)-methyl 4-hydroxy-2-aminobutanoate (4.47 g, 33.60 mmol) was dissolved in CH_2Cl_2 (25 mL) and Et_3N (9.37 mL, 67.20 mmol) was added at room temperature. The mixture was cooled to 0 ºC in an ice bath and TrtCl (9.37 g, 33.60 mmol) in CH_2Cl_2 (25 mL) was added in one portion. The mixture was stirred overnight (18 h) at 0 ºC and then the solvent was evaporated *in vacuo*. The crude product was diluted with CH_2Cl_2 (50 mL) and washed with aqueous 10% citric acid (3 x 50 mL). The aqueous layers were combined and washed with CH_2Cl_2 (2 x 50 mL). Both sets of organic layers were combined, washed with brine (2 x 200 mL), dried (Na_2SO_4), and evaporated *in vacuo* to give the crude product (11.83 g, 94%) as a pale orange solid and as a 5:1 mixture of modified lactone:ester. The product was used for the next step without further purification: R_f 0.38, 0.63 (1:10 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) Lactone: δ 1.15–1.21 (m, $\mathbf{H_3}$), 1.53–1.64 (m, $\mathbf{H_2}$), 3.38–3.43 (m, $\mathbf{H_1}$), 3.74–3.80 (m, $\mathbf{H_5}$), 4.09 (t, J = 8.8 Hz, $\mathbf{H_4}$), 7.15–7.31 (m, 9 Ph**H**), 7.39–7.55 (m, 6 Ph**H**); Ester: δ 1.83–2.07 (m, C$\mathbf{H_2}$CH$_2$OH), 3.20 (s, OC$\mathbf{H_3}$), 3.56 (t, J = 6.4 Hz, C$\mathbf{H_2}$OH), 7.15–7.31 (m, 9 Ph**H**), 7.39–7.55 (m, 6 Ph**H**).



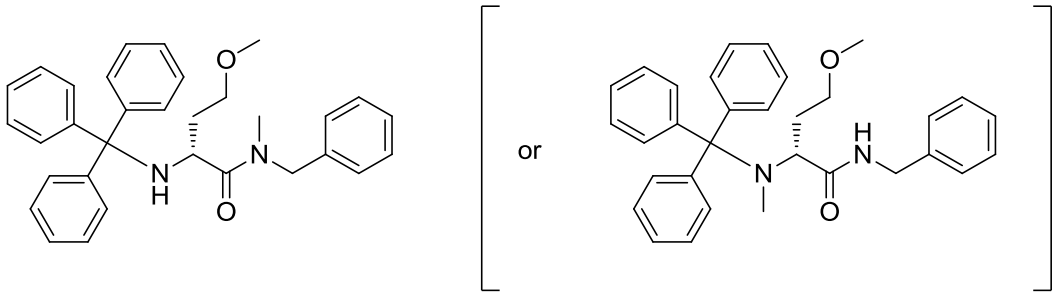
(*R*)-*N*-Benzyl 2-*N'*-(Trityl)amino-4-hydroxybutanamide ((*R*)-184). Benzylamine (8.96 mL, 82.04 mmol) was added to a suspension of (*R*)-2-*N*-(trityl)amino-γ-lactone and (*R*)-methyl 4-hydroxy-2-(*N*-trityl)aminobutanoate (10.26 g, 27.35 mmol) in anhydrous MeOH (35 mL). The mixture was heated to 50 ºC (48 h) and cooled to room temperature before evaporating the solvent *in vacuo*. The crude product was purified by flash column chromatography (SiO_2; 5–50% EtOAc/hexanes followed by 10% MeOH/CH_2Cl_2) to give the desired product (5.03 g,

41%) as a white solid: mp 64–65 ºC; R_f 0.65 (1:1 EtOAc/hexanes); $[\alpha]^{28}_D$ + 59.8° (*c* 1.0, $CHCl_3$); IR (nujol mull) 3319, 3062, 2931 (br), 2863, 1958, 1890, 1815, 1651, 1456, 1377, 1240, 1122, 1071, 906, 704 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.44–1.52 (m, C**H**H'CH_2OH), 1.63–1.71 (m, CH**H'**CH_2OH), 2.88–3.02 (br s, O**H**), 3.45 (dd, *J* = 4.2, 7.8 Hz, C**H**), 3.54–3.64 (m, C$\mathbf{H_2}$OH), 4.05 (dd, *J* = 6.0, 14.8 Hz, NHC**H**H'), 4.20 (dd, *J* = 6.0 Hz, 14.8 Hz, NHCH**H'**), 7.18–7.38 (m, 4 $C_6\mathbf{H_5}$, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 37.0 (**C**H_2CH_2OH), 43.4 (NH**C**H_2), 56.9 (**C**H), 60.2 (**C**H_2OH), 71.9 (**C**$(C_6H_5)_3$), 126.9, 127.5, 127.7, 128.0, 128.7, 128.8, 138.1, 145.5 (4 ($\mathbf{C_6}H_5$)), 175.3 (**C**(O)); HRMS (ESI) 583.1352 [M + Cs^+] (calcd for $C_{30}H_{30}N_2O_2Cs^+$ 583.1362); Anal. Calcd for $C_{30}H_{30}N_2O_2$•0.18EtOAc: C, 79.12; H, 6.79; N, 6.01. Found: C, 78.74; H, 6.76; N, 6.08.



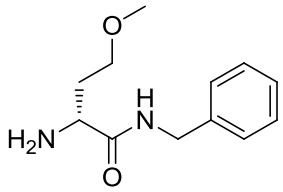
(*R*)-*N*-Benzyl 2-*N'*-(Trityl)amino-4-methoxybutanamide ((*R*)-185). Utilizing Method D and using (*R*)-*N*-benzyl 2-*N'*-(trityl)amino-4-hydroxybutanamide (90 mg, 0.20 mmol), Ag_2O (232 mg, 1.01 mmol), CH_3I (0.13 mL, 2.02 mmol), and CH_3CN (3 d) gave the crude product that was purified by flash column chromatography (SiO_2; 1:100–1:10 MeOH/CH_2Cl_2) to give the desired product (64 mg, 68%) as a white solid: mp 120–121 ºC; R_f 0.29 (1:5 EtOAc/hexanes); $[\alpha]^{28}_D$ + 40.5° (*c* 1.0, $CHCl_3$); IR (nujol mull) 2962, 2866, 1635, 1558, 1456, 1377, 1306, 1206, 1117, 1031, 899, 744, 704 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.41–1.49 (m, C**H**H'CH_2OCH_3), 1.89–1.96 (m, CH**H'**CH_2OCH_3), 3.18 (s, OC$\mathbf{H_3}$), 3.27–3.32 (m, C**H**), 3.38–3.43 (m, C$\mathbf{H_2}OCH_3$), 4.04 (dd, *J* = 5.6, 14.8 Hz, NHC**H**H'), 4.17 (dd, *J* = 6.2, 14.8 Hz, NHCH**H'**), 7.07 (t, *J* = 5.6 Hz, N**H**CH_2), 7.18–7.40 (m, 4 $C_6\mathbf{H_5}$, N**H**CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 33.4 (**C**$H_2CH_2OCH_3$), 43.3 (NH**C**H_2), 57.1 (**C**H), 58.7 (O**C**H_3), 70.2 (**C**H_2OCH_3),

71.7 (**C**(C_6H_5)$_3$), 126.7, 127.3, 127.8, 127.9, 128.6, 128.9, 138.5, 145.9 (4 (**C$_6$**H$_5$)), 174.5 (**C**(O)); HRMS (ESI) 597.1518 [M + Cs^+] (calcd for $C_{31}H_{32}N_2O_2Cs^+$ 597.1552); Anal. Calcd for $C_{31}H_{32}N_2O_2$•0.04H_2O: C, 80.01; H, 6.95; N, 6.02. Found: C, 79.64; H, 6.94; N, 6.02.

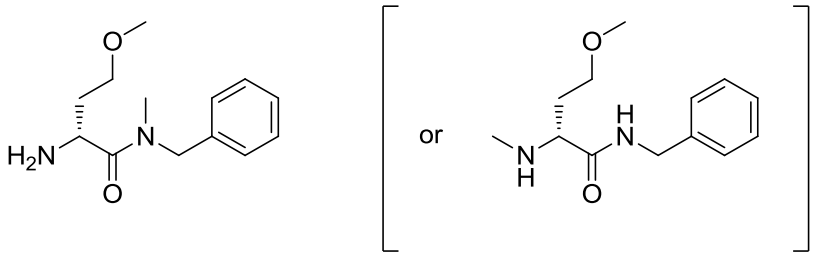


(*R*)-*N*-Benzyl *N*-Methyl 2-*N'*-(Trityl)amino-4-methoxybutanamide ((*R*)-186). The previous procedure was repeated using (*R*)-*N*-benzyl 2-*N'*-(trityl)amino-4-hydroxybutanamide (3.00 g, 6.66 mmol), Ag_2O (7.71 g, 33.31 mmol), CH_3I (4.14 mL, 66.63 mmol), and CH_3CN (66 mL) (3 d) to isolate three fractions: (1) (*R*)-*N*-benzyl 2-*N'*-(trityl)amino-4-methoxybutanamide (~300 mg) as a pale yellow solid; (2) a 2:1 mixture of (*R*)-*N*-benzyl 2-*N'*-(trityl)amino-4-methoxybutanamide and (*R*)-*N*-benzyl *N*-methyl 2-*N'*-(trityl)amino-4-methoxybutanamide (2.70 g, 85%) as a pale yellow solid; (3) (*R*)-*N*-benzyl *N*-methyl 2-*N'*-(trityl)amino-4-methoxybutanamide (~200 mg) as a pale yellow solid.

(*R*)-*N*-Benzyl *N*-methyl 2-*N'*-(trityl)amino-4-methoxybutanamide ((*R*)-186): mp 126–127 ºC; R_f 0.75 (1:1 EtOAc/hexanes); IR (nujol mull) 2858 (br), 1634, 1557, 1458, 1376, 1300, 1206, 1118, 1029, 897, 704 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.91–1.99 (CHC**H$_2$**), 2.39 (s, NC**H$_3$**), 3.16 (d, J = 14.8 Hz, N(CH_3)C**H**H'), 3.23 (s, OC**H$_3$**), 3.28–3.30 (m, C**H**), 3.65–3.73 (m, C**H$_2$**OCH_3), 4.75 (d, J = 14.8 Hz, N(CH_3)CH**H'**), 7.04–7.52 (m, 4 C_6**H$_5$**, N**H**), a COSY spectrum supported the proton-proton connectivity pattern; ^{13}C NMR (100 MHz, $CDCl_3$) δ 33.7 (CH**C**H_2), 36.0 (N**C**H_3), 50.0, 50.9 (**C**H, NH**C**H_2), 58.4 (O**C**H_3), 68.9 (**C**H_2OCH_3), 71.1 (**C**(C_6H_5)$_3$), 126.3. 127.1, 127.5, 128.2, 128.4, 129.0, 137.4, 146.4 (4 **C$_6$**H$_5$), 174.3 (**C**(O)); HRMS (ESI) 611.1644 [M + Cs^+] (calcd for $C_{32}H_{34}N_2O_2Cs^+$ 611.1675).

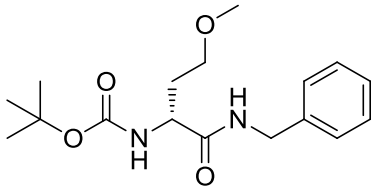


(*R*)-*N*-Benzyl 2-Amino-4-methoxybutanamide ((*R*)-75). (*R*)-*N*-Benzyl 2-*N'*-(trityl)amino-4-methoxybutanamide (30 mg, 0.07 mmol) was dissolved in 1% TFA/CH_2Cl_2 solution (0.5 mL). The reaction was stirred at room temperature (5 h) before the solvent was evaporated *in vacuo* to give the crude product (12 mg, 83%) as a pale yellow solid: R_f 0.33 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.76 (s, N**H**$_2$), 1.77–1.88 (m, C**H**H'CH_2OCH_3), 2.09–2.17 (m, CH**H'**CH_2OCH_3), 3.30 (s, OC**H**$_3$), 3.50–3.59 (m, C**H**, C**H**$_2OCH_3$), 4.40–4.50 (m, NHC**H**$_2$), 7.24–7.35 (m, C_6**H**$_5$), 7.68–7.74 (br t, N**H**).

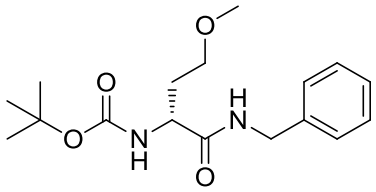


(*R*)-*N*-Benzyl *N*-Methyl 2-*N'*-Amino-4-methoxybutanamide. ((*R*)-187) The previous procedure was repeated using a 2:1 mixture of (*R*)-*N*-benzyl 2-*N'*-(trityl)amino-4-methoxybutanamide and (*R*)-*N*-benzyl *N*-methyl 2-*N'*-(trityl)amino-4-methoxybutanamide (2.45 g, 5.12 mmol) and 1% TFA/CH_2Cl_2 solution (35 mL) (5 h) to isolate three fractions: (1) (*R*)-*N*-benzyl 2-amino-4-methoxybutanamide (~100 mg) as a pale yellow solid; (2) a 2:1 mixture of (*R*)-*N*-benzyl 2-amino-4-methoxybutanamide and (*R*)-*N*-benzyl *N*-methyl 2-*N'*-amino-4-methoxybutanamide (0.97 g, 80%) as a pale yellow solid; (3) (*R*)-*N*-benzyl *N*-methyl 2-*N'*-amino-4-methoxybutanamide (~50 mg) as a pale yellow solid and as a 2:1 mixture of conformers A (major) and B (minor).

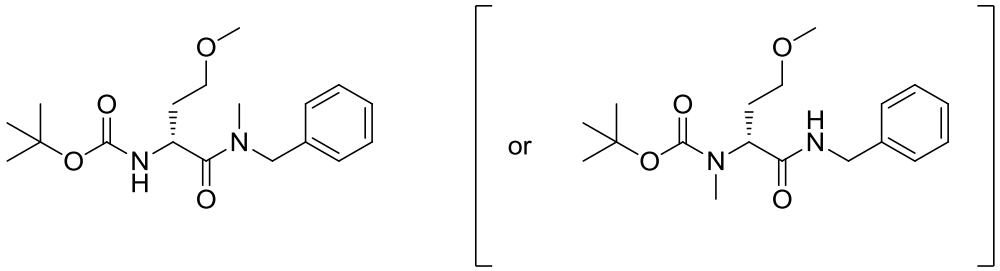
(*R*)-*N*-Benzyl *N*-Methyl 2-*N'*-Amino-4-methoxybutanamide ((*R*)-187): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60–1.74 (m, N**H$_2$**, CHC**H**H'), 1.86–1.96 (m, CHCH**H'**), 2.96 (s, NC**H$_3$**), 3.30 (s, (OC**H$_3$**)$_B$), 3.32 (s, (OC**H$_3$**)$_A$), 3.34–3.48 (m, C**H**H'OCH$_3$), 3.53–3.65 (m, CH**H'**OCH$_3$), 3.88 (dd, J = 4.2, 8.8 Hz, (C**H**)$_B$), 3.92 (dd, J = 4.2, 8.8 Hz, (C**H**)$_A$), 4.40 (d, J = 16.8 Hz, (N(CH$_3$)C**H**H')$_B$), 4.46 (d, J = 14.7 Hz, (N(CH$_3$)C**H**H')$_A$), 4.76 (d, J = 14.7 Hz, (N(CH$_3$)CH**H'**)$_A$), 4.80 (d, J = 16.8 Hz, (N(CH$_3$)CH**H'**)$_B$), 7.17–7.39 (m, C$_6$**H$_5$**).



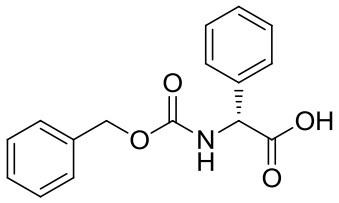
(*R*)-*N*-Benzyl 2-*N'*-(*t*-Butoxycarbonyl)amino-4-methoxybutanamide ((*R*)-188). A 2:1 mixture of (*R*)-*N*-benzyl 2-*N'*-amino-4-methoxybutanamide and (*R*)-*N*-benzyl *N*-methyl 2-*N'*-amino-4-methoxybutanamide (0.91 g, 3.85 mmol) and Na_2CO_3 (1.02 g, 9.63 mmol) was dissolved in a mixture of H_2O/acetone (10 mL/10 mL). Boc_2O (0.93 g, 4.24 mmol) was added in one portion at room temperature and the mixture stirred overnight (18 h). The reaction was partially evaporated *in vacuo* and the remaining aqueous layer was washed with Et_2O (3 x 20 mL). The organic layers were combined, dried (Na_2SO_4), evaporated *in vacuo*, and purified by flash column chromatography (SiO_2; 1:100 MeOH/CH_2Cl_2). Three fractions were isolated: (1) (*R*)-*N*-benzyl *N*-methyl 2-*N'*-(*t*-butoxycarbonyl)amino-4-methoxybutanamide (0.19 g, 15%) as a colorless oil; (2) a 2:1 mixture of (*R*)-*N*-benzyl 2-*N'*-(*t*-butoxycarbonyl)amino-4-methoxybutanamide and (*R*)-*N*-benzyl *N*-methyl 2-*N'*-(*t*-butoxycarbonyl)amino-4-methoxybutanamide (1.06, 81%) as a colorless oil; (3) (*R*)-*N*-benzyl 2-*N'*-(*t*-butoxycarbonyl)amino-4-methoxybutanamide (0.03 g, 2%) as a colorless oil.



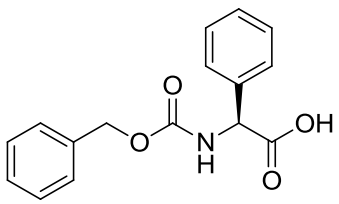
(*R*)-*N*-Benzyl 2-*N'*-(*t*-Butoxycarbonyl)amino-4-methoxybutanamide ((*R*)-188): R_f 0.25 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, C(C**H$_3$**)$_3$), 2.03–2.07 (m, CHC**H$_2$**), 3.27 (s, OC**H$_3$**), 3.41–3.54 (m, C**H$_2$**OCH$_3$), 4.24–4.32 (m, C**H**), 4.41–4.51 (m, NHC**H$_2$**), 5.62 (d, J = 7.2 Hz, OC(O)N**H**), 6.76–6.82 (br t, N**H**CH$_2$), 7.20–7.34 (m, C$_6$**H$_5$**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.3 (C(**C**H$_3$)$_3$), 32.1 (CH**C**H$_2$), 43.4 (NH**C**H$_2$), 53.3 (**C**H), 58.8 (O**C**H$_3$), 70.0 (**C**H$_2$OCH$_3$), 80.1 (**C**(CH$_3$)$_3$), 127.4, 127.6, 128.6, 138.1 (**C$_6$**H$_5$), 155.7 (O**C**(O)), 171.6 (C**C**(O)).



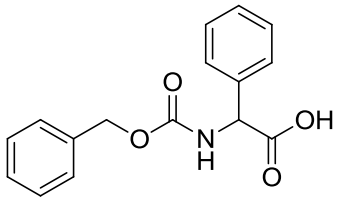
(*R*)-*N*-Benzyl *N*-Methyl 2-*N'*-(*t*-Butoxycarbonyl)amino-4-methoxybutanamide ((*R*)-189). R_f 0.29 (1:20 MeOH/CH_2Cl_2); IR (neat) 3067 (br), 1669, 1533, 1453, 1365, 1254, 1169, 1116, 910, 862, 739, 701 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, C(C**H$_3$**)$_3$), 1.77–1.99 (m, CHC**H$_2$**), 3.01 (s, NC**H$_3$**), 3.31 (s, OC**H$_3$**), 3.38–3.49 (C**H**, C**H$_2$**OCH$_3$), 4.48 (d, J = 14.4 Hz, NHC**H**H'), 4.72 (d, J = 14.4 Hz, NHCH**H'**), 5.45 (d, J = 8.0 Hz, N**H**), 7.21–7.37 (m, C$_6$**H$_5$**); HRMS (ESI) 469.1102 [M + Cs$^+$] (calcd for $C_{18}H_{28}N_2O_4Cs^+$ 469.1103).



(*R*)-2-*N*-(Benzyloxycarbonyl)amino-2-phenylacetic Acid ((*R*)-191).[173] NaOH (1.32 g, 33.1 mmol) was added to a suspension of D-phenylglycine (5.00 g, 33.1 mmol) in THF/H_2O (100:100 mL) at 0 ºC. After the suspension dissolved, benzylchloroformate (4.64 mL, 33.1 mmol) in THF (15 mL) was added drop wise. The reaction was maintained at 0 ºC (3 h), and then a second equivalent of NaOH (1.32 g, 33.1 mmol) followed by benzylchloroformate (4.64 mL, 33.1 mmol) in THF (15 mL) were added and then the reaction was allowed to warm to room temperature (48 h). The mixture was filtered and the filtrate was concentrated to half of its volume *in vacuo* and the remaining aqueous layer was basified to pH ~12 with aqueous 10 M NaOH, and then extracted with CH_2Cl_2 (3 x 100 mL). The aqueous layer was acidified (pH 1) with aqueous concentrated HCl and extracted with EtOAc (3 x 100 mL). The EtOAc layers were combined, dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was recrystallized from hot EtOAc/hexanes to give the desired product (7.53 g, 80%) as a light yellow solid: mp 123–124 °C (lit.[173] mp 125–128 ºC); $[\alpha]^{25}_D$ –108.5° (*c* 1.3, MeOH) (lit.[173] $[\alpha]_D$ –108.5º (*c* 1.0, MeOH)); R_f = 0.29 (1:10 MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.04 (s, C**H**$_2$Ph), 5.13 (d, *J* = 8.1 Hz, C**H**), 7.26–7.42 (m, 2 Ph**H**), 8.02 (d, *J* = 8.1 Hz, N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 58.2 (**C**H), 65.5 (O**C**H_2Ph), 127.6, 127.7, 127.8, 128.3, 136.9, 137.6 (2 **C**$_6$H$_5$), 155.8 (N**C**(O)O), 172.0 (CH**C**(O)), two aromatic peaks were not detected and are believed to overlap with nearby signals.

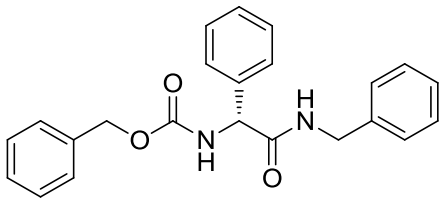


(*S*)-2-*N*-(Benzyloxycarbonyl)amino-2-phenylacetic Acid ((*S*)-191). The previous procedure was repeated using L-phenylglycine (5.00 g, 33.1 mmol) in THF/H_2O (100:100 mL), NaOH (1.32 g, 33.1 mmol), benzylchloroformate (4.64 mL, 33.1 mmol) in THF (15 mL), followed by a second equivalent of NaOH (1.32 g, 33.1 mmol) and benzylchloroformate (4.64 mL, 33.1 mmol) in THF (15 mL) to give the desired product (8.06 g, 85%) as a light yellow solid: mp 125–126 °C (lit.[173] mp 125–128 ºC); $[\alpha]^{25}_D$ +108.8° (*c* 1.1, MeOH) (lit.[173] (*R*): $[\alpha]_D$ –108.5º (*c* 1.0, MeOH)); R_f = 0.26 (1:10 MeOH/$CHCl_3$); IR (nujol mull) 3441, 3363, 2935 (br), 1737, 1666, 1458, 1375, 1303, 1245, 1160, 1051, 724 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 5.05 (s, C**H**$_2$Ph), 5.18 (d, *J* = 8.1 Hz, C**H**), 7.29–7.43 (m, 2 Ph**H**), 8.14 (d, *J* = 8.1 Hz, N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 58.0 (**C**H), 65.6 (O**C**H_2Ph), 127.7, 127.8, 127.9, 128.3, 128.4, 136.9, 137.1 (2 **C**$_6H_5$), 155.8 (N**C**(O)O), 172.0 (CH**C**(O)), one aromatic peak was not detected and is believed to overlap with nearby signals.

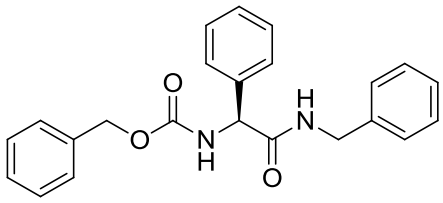


(*R,S*)-2-*N*-(Benzyloxycarbonyl)amino-2-phenylacetic Acid ((*R,S*)-191).[175] The previous procedure was repeated using DL-phenylglycine (5.00 g, 33.1 mmol) in THF/H_2O (100:100 mL), NaOH (1.32 g, 33.1 mmol), benzylchloroformate (4.64 mL, 33.1 mmol) in THF (15 mL), followed by a second equivalent of NaOH (1.32 g, 33.1 mmol) and benzylchloroformate (2.32 mL, 16.6 mmol) in THF (15 mL) to give the desired product (5.73 g, 61%) as a light yellow solid: mp 130–131 °C (lit.[175] mp 128–130 ºC); R_f = 0.31 (1:10 MeOH/$CHCl_3$); ^1H NMR (300 MHz, DMSO-d_6) δ 5.06 (s, C**H**$_2$Ph), 5.18 (d, *J* = 8.1 Hz, C**H**), 7.30–7.43 (m, 2 Ph**H**), 8.14 (d, *J* = 8.1 Hz, N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 58.0 (**C**H), 65.6 (O**C**H_2Ph), 127.7,

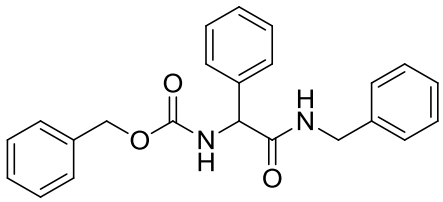
127.8, 127.9, 128.3, 128.4, 136.9, 137.1 (2 **C**$_6$H$_5$), 155.8 (N**C**(O)O), 172.0 (CH**C**(O)), one aromatic peak was not detected and is believed to overlap with nearby signals.



(*R*)-*N*-Benzyl 2-*N*-(Benzylcarboxycarbonyl)amino-2-phenylacetamide ((*R*)-192).[190] Utilizing Method B and using (*R*)-2-*N*-(benzyloxycarbonyl)amino-2-phenylacetic acid (3.50 g, 12.3 mmol), NMM (1.75 mL, 16.0 mmol), IBCF (1.58 mL, 13.5 mmol), and benzylamine (1.41 mL, 12.9 mmol) gave the crude product that was recrystallized from hot EtOAc/hexanes to give the desired compound (3.51 g, 77%) as a white solid: mp 186–187 °C (lit.[190] mp 186–190 °C); $[\alpha]^{25}_{D}$ –105.5° (*c* 0.6, CH_2Cl_2) (lit.[190] $[\alpha]^{23}_{D}$ –105° (*c* 0.5, CH_2Cl_2)); R_f = 0.67 (1:1 EtOAc/hexanes); ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.27 (d, J = 5.7 Hz, C**H**$_2$Ph), 5.05 (s, C**H**$_2$OC(O)), 5.29 (d, J = 8.3 Hz, C**H**), 7.13–7.47 (m, 3 C$_6$**H$_5$**), 7.97 (d, J = 8.3 Hz, N**H**C(O)O), 8.72 (t, J = 5.7 Hz, N**H**C(O)); ^{13}C NMR (75 MHz, DMSO-d_6) δ 41.7 (NH**C**H$_2$), 58.0 (**C**H), 65.2 (O**C**H$_2$Ph), 126.4, 126.7, 126.9, 127.3, 127.4, 127.8, 127.9, 128.0, 136.6, 138.2, 138.7 (3 **C**$_6$H$_5$), 155.3 (O**C**(O)), 169.6 (**C**(O)NH), one aromatic peak was not detected and is believed to overlap with nearby signals; HRMS (ESI) 397.1522 [M + Na$^+$] (calcd for $C_{23}H_{22}N_2O_3Na^+$ 397.1528); Anal. Calcd for $C_{23}H_{22}N_2O_3$; C, 73.78; H, 5.92; N, 7.48. Found C, 73.90; H, 5.91; N, 7.47.

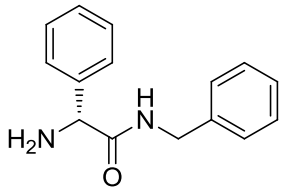


(*S*)-*N*-Benzyl 2-*N*-(Benzylcarboxycarbonyl)aminophenylacetamide ((*S*)-192). The previous procedure was repeated using (*S*)-2-*N*-(benzyloxycarbonyl)amino-2-phenylacetic acid (5.00 g, 17.5 mmol), NMM (2.51 mL, 22.8 mmol), IBCF (2.49 mL, 19.3 mmol), and benzylamine (2.01 mL, 18.4 mmol) to give the crude product that was recrystallized from hot EtOAc/hexanes to give the desired compound (4.56 g, 70%) as a white solid: mp 186–187 °C (lit.[9] mp 186–190 °C); $[\alpha]^{25}_{D}$ +107.5° (*c* 0.6, CH_2Cl_2) (lit.[9] (*R*): $[\alpha]^{23}_{D}$ –105° (*c* 0.5, CH_2Cl_2)); R_f = 0.74 (1:1 EtOAc/hexanes); IR (nujol mull) 3416, 3165, 2865 (br), 1645, 1527, 1458, 1373, 1248, 1156, 1065, 707 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 4.27 (d, *J* = 5.7 Hz, C**H**$_2$Ph), 5.05 (s, C**H**$_2$OC(O)), 5.30 (d, *J* = 8.6 Hz, C**H**), 7.13–7.47 (m, 3 C_6**H**$_5$), 7.98 (d, *J* = 8.6 Hz, N**H**C(O)O), 8.72 (t, *J* = 5.7 Hz, N**H**C(O)); ^{13}C NMR (75 MHz, DMSO-d_6) δ 41.8 (NH**C**H$_2$), 58.0 (**C**H), 65.2 (O**C**H$_2$Ph), 126.4, 126.7, 127.0, 127.3, 127.4, 127.8, 127.9, 128.0, 136.6, 138.2, 138.7 (3 **C**$_6$H$_5$), 155.3 (O**C**(O)), 169.6 (**C**(O)NH), one aromatic peak was not detected and is believed to overlap with nearby signals; HRMS (ESI) 397.1522 [M + Na^+] (calcd for $C_{23}H_{22}N_2O_3Na^+$ 397.1528); Anal. Calcd for $C_{23}H_{22}N_2O_3$; C, 73.78; H, 5.92; N, 7.48. Found C, 73.74; H, 5.94; N, 7.54.



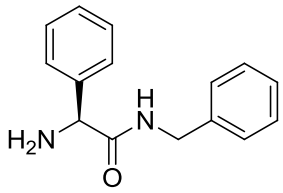
(*R,S*)-*N*-Benzyl 2-*N*-(Benzylcarboxycarbonyl)aminophenylacetamide ((*R,S*)-192). The previous procedure was repeated using (*R,S*)-2-*N*-(benzyloxycarbonyl)amino-2-phenylacetic acid (5.00 g, 17.5 mmol), NMM (2.51 mL, 22.8 mmol), IBCF (2.49 mL, 19.3 mmol), and benzylamine (2.01 mL, 18.4 mmol) to give the crude product that was recrystallized from hot EtOAc/hexanes to give the desired compound (2.84 g, 44%) as a white solid: mp 178–179 °C (lit.[9] mp 186–190 °C); R_f = 0.70 (1:1 EtOAc/hexanes); IR (nujol mull) 3423, 2889 (br),

2358, 1649, 1457, 1374, 724 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 4.27 (d, J = 5.9 Hz, C**H**$_2$Ph), 5.05 (s, C**H**$_2$OC(O)), 5.30 (d, J = 8.4 Hz, C**H**), 7.13–7.47 (m, 3 C_6**H**$_5$), 7.97 (d, J = 8.4 Hz, N**H**C(O)O), 8.72 (t, J = 5.9 Hz, N**H**C(O)); ^{13}C NMR (75 MHz, DMSO-d_6) δ 41.7 (NH**C**H$_2$), 58.0 (**C**H), 65.2 (O**C**H$_2$Ph), 126.4, 126.7, 127.0, 127.3, 127.4, 127.8, 127.9, 128.0, 136.6, 138.1, 138.7 (3 **C**$_6$H$_5$), 155.4 (O**C**(O)), 169.6 (**C**(O)NH), one aromatic peak was not detected and is believed to overlap with nearby signals; HRMS (ESI) 397.1522 [M + Na^+] (calcd for $C_{23}H_{22}N_2O_3Na^+$ 397.1528); Anal. Calcd for $C_{23}H_{22}N_2O_3$; C, 73.78; H, 5.92; N, 7.48. Found C, 73.50; H, 5.94; N, 7.53.

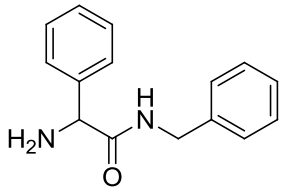


(*R*)-*N*-Benzyl 2-Amino-2-phenylacetamide ((*R*)-62). Utilizing Method C and using (*R*)-*N*-benzyl 2-*N*-(benzyloxycarbonyl)amino-2-phenylacetamide (2.50 g, 6.68 mmol), 10% Pd-C (250 mg), and MeOH (100 mL) (18 h) gave the crude product that was purified by flash column chromatography (SiO_2; 1:10 MeOH/$CHCl_3$). The oil was dissolved in CH_2Cl_2 (20 mL) and was extracted with aqueous 0.1 N HCl (3 x 20 mL). The aqueous layers were combined and extracted with CH_2Cl_2 (2 x 60 mL). The aqueous layer was basified to pH 10–12 with aqueous 0.1 N NaOH, and then extracted with CH_2Cl_2 (3 x 100 mL). The CH_2Cl_2 layers were combined, dried ($MgSO_4$), and concentrated *in vacuo* to give the desired product (1.31 g, 82%) as a waxy solid: mp 87–88 °C; $[\alpha]^{25}_D$ –75.1° (*c* 0.7, CH_2Cl_2); R_f = 0.39 (1:20 MeOH/$CHCl_3$); IR (nujol mull) 3374, 2873 (br), 1647, 1458, 700 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 2.26 (s, N**H**$_2$), 4.27 (d, J = 6.0 Hz, C**H**$_2$), 4.40 (s, C**H**), 7.17–7.42 (m, 2 Ph**H**), 8.57 (t, J = 6.0 Hz, N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 41.6 (**C**H$_2$PH), 58.7 (**C**H), 126.3, 126.5, 126.7, 127.6, 127.8, 139.1, 142.5 (2 **C**$_6$H$_5$), 172.9 (**C**(O)), one aromatic peak was not

detected and is believed to overlap with nearby signals; HRMS (ESI) 241.1342 [M + H^+] (calcd for $C_{15}H_{16}N_2OH^+$ 241.1341); Anal. Calcd for $C_{15}H_{16}N_2O$; C, 74.97; H, 6.71; N, 11.66. Found C, 74.72; H, 6.72; N, 11.57.

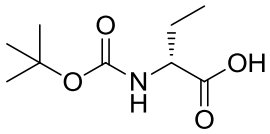


(*S*)-*N*-Benzyl 2-Amino-2-phenylacetamide ((*S*)-62). The previous procedure was repeated using (*S*)-*N*-benzyl 2-*N*-(benzyloxycarbonyl)amino-2-phenylacetamide (3.00 g, 8.02 mmol), 10% Pd-C (300 mg), and MeOH (100 mL) to give the desired product (1.60 g, 83%) as a waxy solid: mp 85–86 °C; $[\alpha]^{25}_D$ +74.1° (*c* 0.7, CH_2Cl_2); R_f = 0.43 (1:20 MeOH/$CHCl_3$); IR (nujol mull) 3152, 2957 (br), 1647, 1458, 700 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.25 (s, N**H**$_2$), 4.27 (d, *J* = 6.2 Hz, C**H**$_2$), 4.40 (s, C**H**), 7.17–7.43 (m, 2 Ph**H**), 8.57 (t, *J* = 6.2 Hz, N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 41.6 (**C**H_2PH), 58.7 (**C**H), 126.3, 126.5, 126.7, 127.6, 127.8, 139.1, 142.5 (2 **C**$_6H_5$), 172.9 (**C**(O)), one aromatic peak was not detected and is believed to overlap with nearby signals; HRMS (ESI) 241.1342 [M + H^+] (calcd for $C_{15}H_{16}N_2OH^+$ 241.1341); Anal. Calcd for $C_{15}H_{16}N_2O$; C, 74.97; H, 6.71; N, 11.66. Found C, 74.69; H, 6.73; N, 11.53.

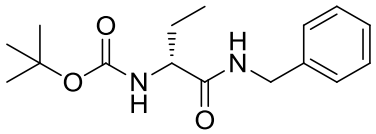


(*R,S*)-*N*-Benzyl 2-Amino-2-phenylacetamide ((*R,S*)-62).[52] The previous procedure was repeated using (*R,S*)-*N*-benzyl 2-*N*-(benzyloxycarbonyl)amino-2-phenylacetamide (2.00 g, 5.35 mmol), 10% Pd-C (250 mg), and MeOH (100 mL) to give the desired product (1.07 g,

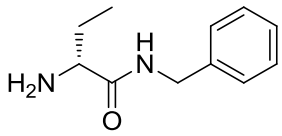
83%) as a waxy solid: mp 67–68 °C; R_f = 0.52 (1:20 MeOH/$CHCl_3$); IR (nujol mull) 3361, 2856 (br), 1646, 1459, 699 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 2.25 (s, N**H**$_2$), 4.27 (d, J = 5.9 Hz, C**H**$_2$), 4.40 (s, C**H**), 7.17–7.43 (m, Ph**H**), 8.57 (t, J = 5.9 Hz, N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 41.6 (**C**H_2PH), 58.7 (**C**H), 126.3, 126.5, 126.7, 127.6, 127.8, 139.1, 142.5 (2 **C**$_6H_5$), 172.9 (**C**(O)), one aromatic peak was not detected and is believed to overlap with nearby signals; HRMS (ESI) 241.1342 [M + H^+] (calcd for $C_{15}H_{16}N_2OH^+$ 241.1341); Anal. Calcd for $C_{15}H_{16}N_2O$; C, 74.97; H, 6.71; N, 11.66. Found C, 74.74; H, 6.76; N, 11.61.



(*R*)-2-*N*-(*t*-Butoxycarbonyl)aminobutanoic Acid ((*R*)-200).[191] D-2-Aminobutanoic acid (3.50 g, 33.94 mmol) and Na_2CO_3 (8.99 g, 84.85 mmol) was dissolved in a mixture of H_2O/acetone (70 mL/70 mL). Boc_2O (8.15 g, 37.34 mmol) was added in one portion at room temperature and the mixture stirred overnight (18 h). The organic layer was evaporated *in vacuo* and the remaining aqueous layer was washed with Et_2O (50 mL), acidified to pH ~2 with aqueous 1 M $KHSO_4$, and extracted with EtOAc (3 x 50 mL). The second set of organic layers was combined, dried (Na_2SO_4), and evaporated *in vacuo* to give the crude product (6.83 g, 99%) as a colorless oil and as a 2:1 mixture of conformers A (major) and B (minor). The product was used for the next step without further purification: R_f 0.55 (1:20 MeOH/CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 0.98 (t, J = 7.6 Hz, CH_2C**H$_3$**), 1.45 (s, C(C**H$_3$**)$_3$), 1.68–1.79 (m, C**H**H'CH_3), 1.86–1.95 (m, CH**H'**CH_3), 4.04–4.13 (m, C**H$_B$**), 4.24–4.35 (m, C**H$_A$**), 5.11 (d, J = 7.6 Hz, N**H$_A$**), 6.41 (d, J = 7.6 Hz, N**H$_B$**), 9.20–9.65 (br s, O**H**); ^{13}C NMR (400 MHz, $CDCl_3$) δ 9.8 ((CH_2**C**H_3)$_A$), 9.9 ((CH_2**C**H_3)$_B$), 25.8 (**C**H_2CH_3), 28.5 (C(**C**H_3)$_3$), 54.6 ((**C**H)$_A$), 56.0 ((**C**H)$_B$), 80.3 ((**C**$(CH_3)_3$)$_A$), 81.8 ((**C**$(CH_3)_3$)$_B$), 155.8 ((O**C**(O)N)$_A$), 157.1 ((O**C**(O)N)$_A$), 177.2 ((**C**(O)OH)$_A$), 177.5 ((**C**(O)OH)$_B$).

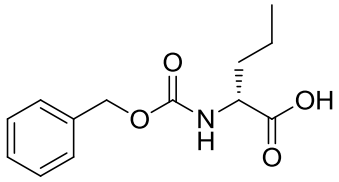


(*R*)-*N*-Benzyl 2-*N'*-(*t*-Butoxycarbonyl)aminobutanamide ((*R*)-207). Utilizing Method B and using (*R*)-2-*N*-(*t*-butoxycarbonyl)aminobutanoic acid (6.16 g, 30.33 mmol), NMM (4.33 mL, 39.43 mmol), IBCF (4.30 mL, 33.36 mmol), and benzylamine (3.48 mL, 31.84 mmol) gave the crude product that was recrystallized from hot EtOAc/hexanes to give the desired product (5.94 g, 67%) as a white solid: mp 70–71 ºC; R_f 0.33 (1:10 EtOAc/hexanes); $[\alpha]^{25}_D$ + 18.6° (*c* 1.0, CH_2Cl_2); IR (nujol mull) 3317, 2869 (br), 1688, 1648, 1525, 1457, 1376, 1245, 1166, 1058, 1012, 908, 865, 757, 656 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.93 (t, *J* = 7.6 Hz, $CH_2C\mathbf{H_3}$), 1.39 (s, $C(C\mathbf{H_3})_3$), 1.58–1.69 (m, C**H**H'CH_3), 1.80–1.91 (m, CH**H'**CH_3), 4.04–4.16 (m, C**H**), 4.36 (dd, *J* = 6.0, 14.8 Hz, NHC**H**H'Ph), 4.45 (dd, *J* = 6.0, 14.8 Hz, NHCH**H'**Ph), 5.26 (d, *J* = 8.4 Hz, N**H**), 6.82–6.89 (br t, N**H**CH_2Ph), 7.22–7.31 (m, 2 $C_6\mathbf{H_5}$); ^{13}C NMR (400 MHz, $CDCl_3$) δ 10.2 ($CH_2\mathbf{C}H_3$), 26.0 ($\mathbf{C}H_2CH_3$), 28.4 ($C(\mathbf{C}H_3)_3$), 43.5 (NH$\mathbf{C}H_2$Ph), 56.0 (**C**H), 80.0 ($\mathbf{C}(CH_3)_3$), 127.5, 127.7, 128.8, 138.3 ($\mathbf{C_6}H_5$), 156.0 (O**C**(O)N), 172.3 (**C**(O)NH); HRMS (ESI) 315.1694 [M + Na^+] (calcd for $C_{16}H_{24}N_2O_3Na^+$ 315.1685); Anal. Calcd for $C_{16}H_{24}N_2O_3$: C, 65.73; H, 8.27; N, 9.58. Found: C, 66.00; H, 8.33; N, 9.56.

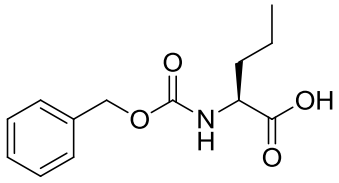


(*R*)-*N*-Benzyl 2-Aminobutanamide ((*R*)-95). Utilizing Method E and using (*R*)-*N*-benzyl 2-*N'*-(*t*-butoxycarbonyl)aminobutanamide (4.87 g, 16.67 mmol), TFA (18.57 mL, 0.25 mol), and CH_2Cl_2 (55 mL) gave the crude product after workup that was further purified by flash column chromatography (SiO_2; 1:100–1:10 MeOH/CH_2Cl_2) to give the desired compound (1.86 g, 58%) as a pale yellow oil: R_f 0.53 (1:20 MeOH/CH_2Cl_2); IR 2966, 1659, 1529, 1456, 1357, 1292, 1082, 1025, 928, 736, 700 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, *J* = 7.2

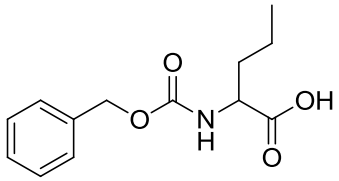
Hz, $CH_2C\mathbf{H_3}$), 1.38–1.46 (br s, $N\mathbf{H_2}$), 1.51–1.66 (m, C**H**H'CH_3), 1.83–1.97 (m, CH**H'**CH_3), 3.35 (dd, *J* = 4.5, 7.8 Hz, C**H**), 4.44 (d, *J* = 5.7 Hz, NHC$\mathbf{H_2}$Ph), 7.26–7.35 (m, $C_6\mathbf{H_5}$), 7.61–7.73 (br t, N**H**CH_2Ph); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 10.2 ($CH_2\mathbf{C}H_3$), 28.2 (**C**H_2CH_3), 43.2 (NH**C**H_2Ph), 56.5 (**C**H), 127.5, 127.8, 128.8, 138.7 ($\mathbf{C_6}H_5$), 175.0 (**C**(O)N); HRMS (ESI) 193.1348 [M + H^+] (calcd for $C_{11}H_{16}N_2OH^+$ 193.1341); Anal. Calcd for $C_{11}H_{16}N_2O$•0.06CH_2Cl_2: C, 67.22; H, 8.22; N, 14.17. Found: C, 67.23; H, 8.31; N, 14.36.



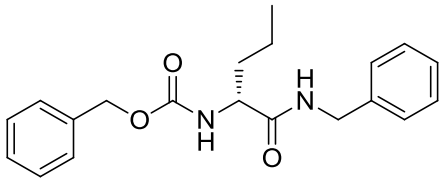
(*R*)-2-*N*-(Benzyloxycarbonyl)aminopentanoic Acid ((*R*)-379).[192] Utilizing Method A and using D-norvaline (5.00 g, 42.7 mmol), $NaHCO_3$ (8.97 g, 106.8 mmol), benzyl chloroformate (9.00 mL, 64.1 mmol) and H_2O (100 mL) gave the desired product (9.15 g, 85%) as a white solid after workup and the compound was used for the next step without further purification: mp 88–89 °C (lit.[192] mp 84–85 ºC); $[\alpha]^{25}_D$ + 4.2° (*c* 2.0, acetone) (lit.[193] (*S*): $[\alpha]^{12}_D$ –4.2º (*c* 2, acetone)); R_f 0.61 (1:1 EtOAc/hexanes); ^{1}H NMR (300 MHz, DMSO-d_6) *δ* 0.86 (t, *J* = 7.8 Hz, $CH_2C\mathbf{H_3}$), 1.24–1.41 (m, $C\mathbf{H_2}CH_3$), 1.51–1.67 (m, $CHC\mathbf{H_2}$), 3.91–3.99 (m, C**H**), 5.03 (s, $OC\mathbf{H_2}$Ph), 7.27–7.41 (m, $C_6\mathbf{H_5}$), 7.58 (d, *J* = 8.1 Hz, N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) *δ* 13.1 (**C**H_3), 18.4 (**C**H_2CH_3), 32.5 (CH**C**H_2), 53.2 (**C**H), 65.0 (O**C**H_2Ph), 127.4, 127.5, 128.0, 136.7 ($\mathbf{C_6}H_5$), 155.9 (N**C**(O)O), 173.7 (CH**C**(O)).



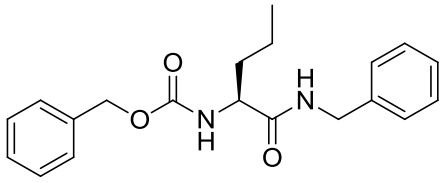
(*S*)-2-*N*-(Benzyloxycarbonyl)aminopentanoic Acid ((*S*)-379).[193] The previous procedure was repeated using L-norvaline (5.00 g, 42.7 mmol), $NaHCO_3$ (8.97 g, 106.8 mmol), and benzyl chloroformate (9.00 mL, 64.1 mmol), and H_2O (100 mL) to give the desired product (7.78 g, 73%) as a white solid: mp 85–86 °C (lit.[193] mp 86 ºC); $[\alpha]^{25}_D$ – 4.3° (*c* 2.1, acetone) (lit.[193] $[\alpha]^{12}_D$ –4.2º (*c* 2, acetone)); R_f = 0.43 (1:10 MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (t, J = 7.5 Hz, $CH_2C\mathbf{H}_3$), 1.25–1.41 (m, $C\mathbf{H}_2CH_3$), 1.50–1.71 (m, $CHC\mathbf{H}_2$), 3.90–3.98 (m, C**H**), 5.03 (s, $OC\mathbf{H}_2Ph$), 7.29–7.37 (m, Ph**H**), 7.58 (d, J = 8.1 Hz, N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.4 ($\mathbf{C}H_3$), 18.7 ($\mathbf{C}H_2CH_3$), 32.8 ($CH\mathbf{C}H_2$), 53.5 (**C**H), 65.3 ($O\mathbf{C}H_2Ph$), 127.7, 127.8, 128.3, 137.0 ($\mathbf{C}_6H_5$), 156.2 (N**C**(O)O), 174.0 (CH**C**(O)).



(*R,S*)-2-*N*-(Benzyloxycarbonyl)aminopentanoic Acid ((*R,S*)-379).[194] The previous procedure was repeated using DL-norvaline (5.00 g, 42.7 mmol), $NaHCO_3$ (8.97 g, 106.8 mmol), benzyl chloroformate (9.00 mL, 64.1 mmol), and H_2O (100 mL) to give the desired product (8.55 g, 80%) as a white solid: mp 90–91 °C (lit.[193] mp 86 ºC); R_f 0.57 (1:1 EtOAc/hexanes); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (t, J = 7.2 Hz, $CH_2C\mathbf{H}_3$), 1.26–1.42 (m, $C\mathbf{H}_2CH_3$), 1.51–1.69 (m, $CHC\mathbf{H}_2$), 3.91–3.99 (m, C**H**), 5.04 (s, $OC\mathbf{H}_2Ph$), 7.27–7.39 (m, $C_6\mathbf{H_5}$), 7.58 (d, J = 8.1 Hz, N**H**); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.1 ($\mathbf{C}H_3$), 18.4 ($\mathbf{C}H_2CH_3$), 32.5 ($CH\mathbf{C}H_2$), 53.2 (**C**H), 65.0 ($O\mathbf{C}H_2Ph$), 127.4, 127.5, 128.0, 136.7 ($\mathbf{C_6}H_5$), 155.8 (N**C**(O)O), 173.7 (CH**C**(O)).

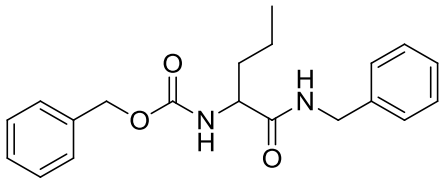


(*R*)-*N*-Benzyl 2-*N*-(Benzyloxycarbonyl)aminopentanamide ((*R*)-380). Utilizing Method B and using (*R*)-2-*N*-(benzyloxycarbonyl)aminopentanoic acid (5.00 g, 19.9 mmol), NMM (2.85 mL, 25.9 mmol), IBCF (2.82 mL, 21.9 mmol), and benzylamine (2.28 mL, 20.9 mmol) gave the crude product that was purified by flash column chromatography (SiO_2; 10% MeOH/CH_2Cl_2) followed by recrystallization from hot toluene to give the desired compound (3.49 g, 52%) as a white solid: mp 139–141 °C; $[\alpha]^{25}_D$ + 11.5° (*c* 1.1, MeOH); R_f 0.27 (1:100 MeOH/CH_2Cl_2); IR (nujol mull) 3290, 2934 (br), 2358, 1692, 1643, 1540, 1458, 1375, 1258, 1058, 737, 694 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.85 (t, J = 7.5 Hz, $CH_2C\mathbf{H}_3$), 1.20–1.37 (m, $C\mathbf{H}_2CH_3$), 1.46–1.67 (m, $C\mathbf{H_2}CH_2CH_3$), 3.98–4.06 (m, C**H**), 4.28 (d, J = 5.9 Hz, $C\mathbf{H}_2Ph$), 5.03 (s, $OC\mathbf{H}_2Ph$), 7.23–7.37 (m, 2 $C_6\mathbf{H_5}$), 7.43 (d, J = 8.1 Hz, N**H**C(O)), 8.43 (t, J = 5.9 Hz, $N\mathbf{H}CH_2Ph$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.2 ($\mathbf{C}H_3$), 18.3 ($\mathbf{C}H_2CH_3$), 33.6 ($CH\mathbf{C}H_2$), 41.6 ($\mathbf{C}H_2Ph$), 54.1 (**C**H), 65.0 ($O\mathbf{C}H_2Ph$), 126.3, 126.7, 127.3, 127.4, 127.8, 127.9, 136.7, 139.0 (2 $\mathbf{C_6}H_5$), 155.6 (N**C**(O)O), 171.7 (CH**C**(O)); HRMS (ESI) 363.1686 [M + Na^+] (calcd for $C_{20}H_{24}N_2O_3Na^+$ 363.1685); Anal. Calcd for $C_{20}H_{24}N_2O_3 \cdot 0.12H_2O$; C, 70.11; H, 7.13; N, 8.18. Found C, 69.75; H, 7.20; N, 8.35.



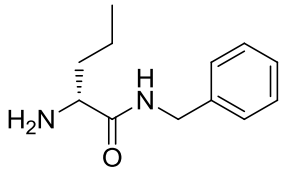
(*S*)-*N*-Benzyl 2-*N*-(Benzyloxycarbonyl)aminopentanamide ((*S*)-380). The previous procedure was repeated using (*S*)-2-*N*-(benzyloxycarbonyl)aminopentanoic acid (5.00 g, 19.9 mmol), NMM (2.85 mL, 25.9 mmol), IBCF (2.82 mL, 21.9 mmol), and benzylamine

(2.28 mL, 20.9 mmol) to give the crude product that was further purified by flash column chromatography (SiO_2; 1:10 MeOH/$CHCl_3$) to give the desired compound (4.02 g, 60%) as a white solid: mp 134–135 °C; $[\alpha]^{25}_D$ – 13.2° (*c* 1.1, MeOH); R_f 0.28 (1:100 MeOH/CH_2Cl_2); IR (nujol mull) 3285, 2938 (br), 1693, 1644, 1542, 1458, 1377, 1260, 1112, 1059, 739, 695 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.85 (t, J = 7.5 Hz, CH_2CH_3), 1.20–1.38 (m, CH_2CH_3), 1.46–1.65 (m, $CH_2CH_2CH_3$), 3.98–4.06 (m, CH), 4.28 (d, J = 6.0 Hz, CH_2Ph), 5.03 (s, OCH_2Ph), 7.20–7.44 (m, 2 PhH, NHC(O)), 8.42 (t, J = 6.0 Hz, $NHCH_2Ph$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.2 (CH_3), 18.3 (CH_2CH_3), 33.6 ($CHCH_2$), 41.6 (CH_2Ph), 54.1 (CH), 65.0 (OCH_2Ph), 126.7, 126.9, 127.3, 127.4, 127.8, 127.9, 136.7, 139.0 (2 C_6H_5), 155.6 (NC(O)O), 171.7 (CHC(O)); HRMS (ESI) 363.1687 [M + Na^+] (calcd for $C_{20}H_{24}N_2O_3Na^+$ 363.1685); Anal. Calcd for $C_{20}H_{24}N_2O_3$; C, 70.56; H, 7.11; N, 8.23. Found C, 70.28; H, 7.19; N, 8.31.



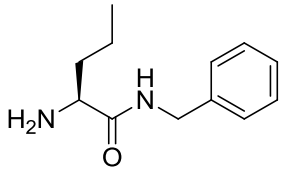
(*R,S*)-*N*-Benzyl 2-*N*-(Benzyloxycarbonyl)aminopentanamide ((*R,S*)-380).[105] The previous procedure was repeated using (*R,S*)-2-*N*-(benzyloxycarbonyl)aminopentanoic acid (5.00 g, 19.9 mmol), NMM (2.85 mL, 25.9 mmol), IBCF (2.82 mL, 21.9 mmol), and benzylamine (2.28 mL, 20.9 mmol) to give the crude product that was further purified by flash column chromatography (SiO_2; 1:10 MeOH/CH_2Cl_2) followed by recrystallization from hot toluene to give the desired compound (4.72 g, 70%) as a white solid: mp 136–137 °C (lit.[105] mp 138–139 ºC); R_f 0.27 (1:100 MeOH/CH_2Cl_2); IR (nujol mull) 3290, 2930 (br), 1689, 1641, 1538, 1459, 1375, 1257, 1057, 753, 702 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.85 (t, J = 6.9 Hz, CH_2CH_3), 1.17–1.40 (m, CH_2CH_3), 1.47–1.68 (m, $CH_2CH_2CH_3$), 3.99–4.06 (m, CH), 4.28 (d, J = 5.9 Hz, CH_2Ph), 5.03 (s, OCH_2Ph), 7.23–7.37 (m, 2 C_6H_5), 7.43 (d, J = 8.4 Hz, NHC(O)),

8.43 (t, J = 5.9 Hz, N**H**CH_2Ph); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.2 (**C**H_3), 18.3 (**C**H_2CH_3), 33.6 (CH**C**H_2), 41.6 (**C**H_2Ph), 54.1 (**C**H), 65.0 (O**C**H_2Ph), 126.3, 126.7, 127.3, 127.4, 127.8, 127.9, 136.7, 139.1 (2 **C$_6$**H$_5$), 155.6 (N**C**(O)O), 171.7 (CH**C**(O)); HRMS (ESI) 363.1685 [M + Na^+] (calcd for $C_{20}H_{24}N_2O_3Na^+$ 363.1685); Anal. Calcd for $C_{20}H_{24}N_2O_3$; C, 70.56; H, 7.11; N, 8.23. Found C, 70.36; H, 7.17; N, 8.19.

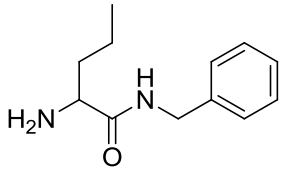


(*R*)-*N*-Benzyl 2-Aminopentanamide ((*R*)-96). Utilizing Method C and using (*R*)-*N*-benzyl 2-*N*-(benzyloxycarbonyl)aminopentanamide (2.00 g, 5.88 mmol), 10% Pd-C (0.2 g), and MeOH (60 mL) gave the crude product further purified by flash column chromatography (SiO_2; 1:100 MeOH/CH_2Cl_2). The resulting oil was dissolved in CH_2Cl_2 (10 mL) and was extracted with aqueous 1 M HCl (3 x 10 mL). The aqueous layers were combined and extracted with CH_2Cl_2 (2 x 30 mL). The aqueous layer was basified to pH 10–12 with aqueous 1 M NaOH, and then extracted with CH_2Cl_2 (3 x 60 mL). The CH_2Cl_2 layers were combined, dried ($NaSO_4$), and concentrated *in vacuo* to give the desired product (0.95 g, 79%) as a pale yellow oil: $[\alpha]^{25}_D$ –9.4° (*c* 1.0, MeOH); R_f 0.64 (1:10 MeOH/CH_2Cl_2); IR (neat) 3307, 3033, 2956, 2870, 1658, 1525, 1456, 1358, 1250, 700 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (t, J = 7.4 Hz, CH_2C**H**$_3$), 1.19–1.42 (m, C**H**$_2CH_3$, C**H**H'CH_2CH_3), 1.47–1.61 (m, CH**H**'CH_2CH_3), 1.78 (br s, N**H**$_2$), 3.15–3.20 (app. t, C**H**), 4.28 (d, J = 5.7 Hz, C**H**$_2$Ph), 7.20–7.34 (m, C_6**H$_5$**), 8.34 (t, J = 5.7 Hz, N**H**CH_2Ph); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J = 7.2 Hz, CH_2C**H**$_3$), 1.32–1.58 (m, C**H$_2$**CH_3, C**H**H'CH_2CH_3, N**H**$_2$), 1.81–1.93 (m, CH**H'**CH_2CH_3), 3.41 (dd, J = 4.1, 8.0 Hz, C**H**), 4.45 (d, J = 6.0 Hz, C**H**$_2$Ph), 7.24–7.37 (m, C_6**H$_5$**), 7.60–7.72 (br s, N**H**CH_2Ph); ^{1}H NMR (300 MHz, CD_3OD) δ 0.92 (t, J = 7.2 Hz, CH_2C**H**$_3$), 1.28–1.42 (m, C**H$_2$**CH_3), 1.44–1.56 (m, C**H**H'CH_2CH_3), 1.58–1.71 (m,

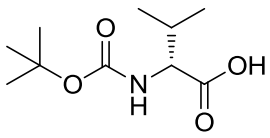
CH**H**'CH_2CH_3), 3.28–3.33 (m, C**H**), 4.35 (1/2 ABq, *J* = 15.0 Hz, C**H**H'Ph), 4.41 (1/2 ABq, *J* = 15.0 Hz, CH**H**'Ph), 4.86 (s, N$\mathbf{H}_2$), 7.20–7.34 (m, $C_6\mathbf{H_5}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.5 (**C**H_3), 18.2 (**C**H_2CH_3), 37.1 (CH**C**H_2), 41.5 (**C**H_2Ph), 54.2 (**C**H), 126.3, 126.8, 127.8, 139.3 ($\mathbf{C_6}H_5$), 175.0 (CH**C**(O)); HRMS (ESI) 229.1317 [M + Na^+] (calcd for $C_{12}H_{18}N_2ONa^+$ 229.1317); Anal. Calcd for $C_{12}H_{18}N_2O$; C, 69.87; H, 8.80; N, 13.58. Found C, 69.98; H, 8.73; N, 13.37.



(*S*)-*N*-Benzyl 2-Aminopentanamide ((*S*)-96). The previous procedure was repeated using (*R*)-*N*-benzyl 2-*N*-(benzyloxycarbonyl)aminopentanamide (3.00 g, 8.82 mmol), 10% Pd-C (0.3 g), and MeOH (100 mL) to give the desired product (1.76 g, 97%) as a pale yellow oil: $[\alpha]^{25}_D$ +9.4° (*c* 1.5, MeOH); R_f 0.65 (1:10 MeOH/CH_2Cl_2); IR (neat) 3351, 3265, 3032, 2928, 2873, 1655, 1534, 1456, 1358, 1250, 701 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (t, *J* = 6.6 Hz, $CH_2C\mathbf{H}_3$), 1.22–1.42 (m, C$\mathbf{H}_2CH_3$, C**H**H'CH_2CH_3), 1.48–1.61 (m, CH**H**'CH_2CH_3), 1.78 (br s, N$\mathbf{H}_2$), 3.17 (t, *J* = 6.9 Hz, C**H**), 4.28 (d, *J* = 6.0 Hz, C$\mathbf{H}_2$Ph), 7.20–7.34 (m, Ph**H**), 8.34 (t, *J* = 4.8 Hz, N**H**CH_2Ph); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.5 (**C**H_3), 18.2 (**C**H_2CH_3), 37.1 (CH**C**H_2), 41.5 (**C**H_2Ph), 54.2 (**C**H), 126.3, 126.8, 127.8, 139.3 ($\mathbf{C}_6H_5$), 175.0 (CH**C**(O)); LRMS (ESI) 207.12 [M + H^+] (calcd for $C_{12}H_{18}N_2OH^+$ 207.12); Anal. Calcd for $C_{12}H_{18}N_2O$•0.32H_2O; C, 67.99; H, 8.86; N, 13.22. Found C, 67.93; H, 8.76; N, 13.21.

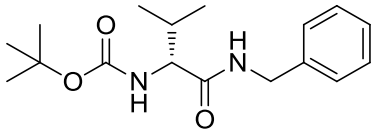


(*R,S*)-*N*-Benzyl 2-Aminopentanamide ((*R,S*)-96). The previous procedure was repeated using (*R,S*)-*N*-benzyl 2-*N*-(benzyloxycarbonyl)aminopentanamide (2.00 g, 11.76 mmol), 10% Pd-C (0.2 g), and MeOH (60 mL) to give the desired product (0.88 g, 72%) as a pale yellow oil: R_f 0.65 (1:10 MeOH/CH_2Cl_2); IR (neat) 3292, 3064, 2957, 1655, 1528, 1457, 1358, 1250, 700 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.86 (t, J = 6.6 Hz, CH_2CH_3), 1.24–1.42 (m, CH_2CH_3, $CHH'CH_2CH_3$), 1.48–1.61 (m, $CHH'CH_2CH_3$), 1.74 (br s, NH_2), 3.15-3.18 (app. t, CH), 4.28 (d, J = 5.9 Hz, CH_2Ph), 7.20–7.34 (m, C_6H_5), 8.36 (t, J = 5.9 Hz, $NHCH_2Ph$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J = 7.2 Hz, CH_2CH_3), 1.32–1.58 (m, CH_2CH_3, $CHH'CH_2CH_3$, NH_2), 1.81–1.93 (m, $CHH'CH_2CH_3$), 3.41 (dd, J = 4.2, 8.1 Hz, CH), 4.45 (d, J = 6.0 Hz, CH_2Ph), 7.24–7.37 (m, C_6H_5), 7.58–7.70 (br s, $NHCH_2Ph$); ^1H NMR (300 MHz, CD_3OD) δ 0.92 (t, J = 7.2 Hz, CH_2CH_3), 1.28–1.43 (m, CH_2CH_3), 1.44–1.56 (m, $CHH'CH_2CH_3$), 1.58–1.71 (m, $CHH'CH_2CH_3$), 3.28–3.32 (m, CH), 4.35 (1/2 AB_q, J = 14.9 Hz, $CHH'Ph$), 4.41 (1/2 AB_q, J = 14.9 Hz, $CHH'Ph$), 4.88 (s, NH_2), 7.21–7.34 (m, C_6H_5); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.5 (CH_3), 18.2 (CH_2CH_3), 37.1 (CH_2), 41.5 (CH_2Ph), 54.2 (CH), 126.3, 126.8, 127.9, 139.3 (C_6H_5), 175.1 (C(O)); HRMS (ESI) 229.1320 [M + Na^+] (calcd for $C_{12}H_{18}N_2ONa^+$ 229.1317); Anal. Calcd for $C_{12}H_{18}N_2O \cdot 0.18H_2O$; C, 68.81; H, 8.83; N, 13.37. Found C, 68.48; H, 8.81; N, 13.27.

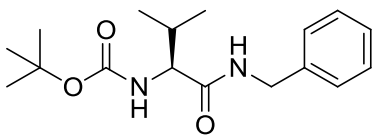


(*R*)-2-*N*-(*t*-Butoxycarbonyl)amino-3-methylbutanoic Acid ((*R*)-202).[195] D-Valine (5.00 g, 42.71 mmol) was dissolved in aqueous 2 M NaOH (43 mL) and cooled to 0 ºC in an ice water bath. Boc_2O (11.18 g, 51.25 mmol) was slowly added and the reaction was allowed to warm to room temperature (18 h). The mixture was acidified to pH 2 using aqueous concentrated HCl and then extracted with EtOAc (3 x 50 mL). The combined organic layers

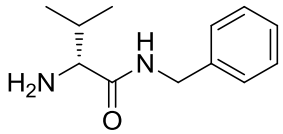
were dried (Na_2SO_4) and evaporated *in vacuo* to give the crude product as a pale yellow oil (8.50 g, 92%). The product was used for the next step without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83–1.02 (m, CH(C**H$_3$)$_2$), 1.45 (s, C(C**H$_3$)$_3$), 2.15–2.28 (m, C**H**(CH$_3$)$_2$), 4.27 (dd, *J* = 4.7, 9.1 Hz, C**H**CH(CH$_3$)$_2$), 5.06 (d, *J* = 9.1 Hz, N**H**).



(*R*)-*N*-Benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-209).[196,197] Utilizing Method B and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (8.50 g, 39.14 mmol), NMM (5.60 mL, 50.89 mmol), IBCF (5.55 mL, 43.06 mmol), and benzylamine (4.50 mL, 41.10 mmol) gave the crude product that was recrystallized from hot EtOAc to give the desired compound (6.64 g, 55%) as a white solid: mp 122–123 ºC (lit.[197] mp 112–115 ºC); R_f 0.67 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3297, 2900 (br), 1690, 1645, 1530, 1458, 1378, 1299, 1247, 1166, 1020, 930, 875, 744, 693, 585, 508 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (d, *J* = 6.9 Hz, CH(C**H$_3$**)CH$_3$), 0.95 (d, *J* = 6.6 CH(CH$_3$)C**H$_3$**), 1.40 (s, C(C**H$_3$**)$_3$), 2.18–2.26 (m, C**H**(CH$_3$)$_2$), 3.94–4.00 (br t, C**H**CH(CH$_3$)$_2$), 4.36 (dd, *J* = 6.0, 14.7 Hz, NHC**H**H'Ph), 4.46 (dd, *J* = 6.0, 14.7 Hz, NHCH**H'**Ph), 5.25 (d, *J* = 9.3 Hz, N**H**C(O)), 6.71–6.79 (br t, N**H**CH$_2$Ph), 7.21–7.33 (C$_6$**H$_5$**); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.5 (CH(**C**H$_3$)(CH$_3$)'), 19.9 (CH(CH$_3$)(**C**H$_3$)'), 28.9 (C(**C**H$_3$)$_3$), 31.4 (**C**H(CH$_3$)(CH$_3$)'), 43.9 (NH**C**H$_2$Ph), 60.7 (**C**H), 80.4 (**C**(CH$_3$)$_3$), 128.0, 128.2, 129.2, 138.7 (**C$_6$**H$_5$), 156.6 (N**C**(O)O), 172.3 (C**C**(O)N).

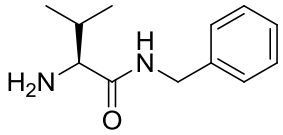


(*S*)-*N*-Benzyl 2-*N'*-(*tert*-Butoxycarbonyl)amino-3-methylbutanamide ((*S*)-209).[198,199] The previous procedure was repeated using commercially available (*S*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (4.00 g, 18.42 mmol), NMM (2.63 mL, 23.95 mmol), IBCF (2.61 mL, 20.26 mmol), and benzylamine (2.11 mL, 19.34 mmol) to give the crude product that was recrystallized from hot EtOAc to give the desired compound (2.89 g, 51%) as a white solid: mp 122–123 ºC (lit.[199] mp 123–124 ºC); R_f 0.24 (1:10 EtOAc/hexanes); IR (nujol mull) 3116, 2910 (br), 1689, 1646, 1527, 1458, 1375, 1301, 1249, 1163, 1018, 725 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.92 (d, J = 7.2 Hz, CH(C**H$_3$**)CH_3), 0.97 (d, J = 7.2 Hz, $CH(CH_3)$C**H$_3$**), 1.42 (s, (C**H$_3$**)$_3$), 2.12–2.23 (m, C**H**$(CH_3)_2$), 3.92 (dd, J = 6.2, 8.6 Hz, C**H**), 4.39–4.50 (m, NHC**H$_2$**), 5.06–5.14 (br d, C(O)N**H**), 6.36–6.44 (br t, N**H**CH_2Ph), 7.25–7.34 (C_6**H$_5$**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.9 (CH(**C**H_3)(CH_3)'), 19.4 ($CH(CH_3)$(**C**H_3)'), 28.3 ((**C**$H_3)_3$), 30.7 (**C**H$(CH_3)_2$), 43.5 (NH**C**H_2), 60.2 (**C**H), 80.0 (**C**$(CH_3)_3$), 127.5, 127.7, 128.7, 138.0 (**C$_6$**H_5), 156.0 (O**C**(O)), 171.6 (C**C**(O)).

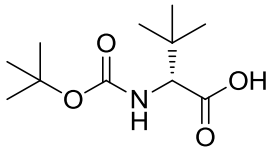


(*R*)-*N*-Benzyl 2-Amino-3-methylbutanamide ((*R*)-98).[196] Utilizing Method E and using (*R*)-*N*-benzyl 2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanamide (4.00 g, 13.06 mmol), TFA (14.56 mL, 0.20 mol), and CH_2Cl_2 (45 mL) gave the crude product after workup that was further purified by flash column chromatography (SiO_2; 1:100–1:10 MeOH/CH_2Cl_2) to give the desired compound (2.25 g, 83%) as a pale yellow oil: R_f 0.70 (5% MeOH/CH_2Cl_2); IR (neat) 3316, 3064, 2961, 1952, 1881, 1812, 1655, 1525, 1459, 1364, 1240, 1081, 1028, 882, 700, 608, 488 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.82 (d, J = 6.8 Hz, CH(C**H$_3$**)CH_3), 0.97 (d, J = 6.8 Hz, $CH(CH_3)$C**H$_3$**), 1.31 (s, N**H$_2$**), 2.24–2.35 (m, C**H**$(CH_3)_2$), 3.23 (d, J = 3.9 Hz, C**H**$CH(CH_3)_2$), 4.35–4.49 (m, NHC**H$_2$**Ph), 7.21–7.33 (m, C_6**H$_5$**), 7.72–7.81 (br t,

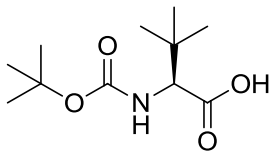
N**H**CH_2Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.1 (CH(**C**H_3)CH_3), 19.7 (CH(CH_3)**C**H_3), 30.1 (**C**H(CH_3)$_2$), 43.0 (NH**C**H_2Ph), 60.2 (**C**H), 127.2, 127.7, 128.6, 138.7 (**C$_6$**H_5), 174.4 (N**C**(O)); HRMS (ESI) 207.1501 [M + H^+] (calcd for $C_{12}H_{18}N_2OH^+$ 207.1497); Anal. Calcd for $C_{12}H_{18}N_2O$•0.04CH_2Cl_2: C, 68.93; H, 8.69; N, 13.35. Found: C, 68.97; H, 8.82; N, 13.37.



(*S*)-*N*-Benzyl 2-Amino-3-methylbutanamide ((*S*)-98).[198] The previous procedure was repeated using (*S*)-*N*-benzyl 2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanamide (2.57 g, 8.39 mmol), TFA (9.35 mL, 0.13 mol), and CH_2Cl_2 (28 mL) to give the crude product after workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 10% MeOH/CH_2Cl_2) to give the desired compound (1.65 g, 96%) as a white solid: mp 52–53 ºC (lit.[198] mp 53–54 ºC); $[\alpha]^{28.5}_D$ – 27.1° (*c* 0.6, CH_2Cl_2) (lit.[196] $[\alpha]^{20}_D$ – 27.2° (*c* 0.5, CH_2Cl_2)); R_f 0.47 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3012, 2904 (br), 1645, 1550, 1458, 1375, 1165, 1078, 1028, 965, 727 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (d, *J* = 6.8 Hz, CH(C**H$_3$**)CH_3), 0.99 (d, *J* = 6.8 Hz, CH(CH_3)C**H$_3$**), 1.43 (s, N**H$_2$**), 2.28–2.40 (m, C**H**(CH_3)$_2$), 3.27 (d, *J* = 4.0 Hz, C**H**), 4.42 (dd, *J* = 6.2, 14.8 Hz, NHC**H**H'), 4.48 (dd, *J* = 6.0, 14.8 Hz, NHCH**H'**), 7.24–7.34 (C$_6$**H$_5$**), 7.62–7.70 (br t, N**H**CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.1 (CH(**C**H_3)CH_3), 19.8 (CH(CH_3)**C**H_3), 30.9 (**C**H(CH_3)$_2$), 43.1 (NH**C**H_2), 60.2 (**C**H), 127.3, 127.7, 128.6, 138.6 (m, **C$_6$**H_5), 174.3 (**C**(O)); HRMS (ESI) 207.1502 [M + H^+] (calcd for $C_{12}H_{18}N_2OH^+$ 207.1497); Anal. Calcd for $C_{12}H_{18}N_2O$•0.30H_2O: C, 68.09; H, 8.86; N, 13.23. Found: C, 67.71; H, 8.89; N, 13.19.

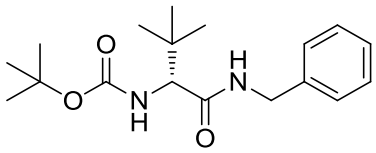


(*R*)-2-*N*-(*t*-Butoxycarbonyl)amino-3,3-dimethylbutanoic Acid ((*R*)-203).[200] Boc_2O (9.16 g, 41.96 mmol) was added to an acetone/H_2O (75/75 mL) solution of D-*tert*-leucine (5.00 g, 38.14 mmol) and Na_2CO_3 (10.11 g, 95.35 mmol). The reaction was stirred at room temperature (18 h) and then Et_2O was added. The layers were separated and the aqueous layer was acidified with an aqueous 1 M solution of $KHSO_4$ to pH 1–2. The acid aqueous layer was extracted with EtOAc (3 x 100 mL) and then the EtOAc layers were combined, dried (Na_2SO_4), and evaporated *in vacuo* to give the crude product (8.12 g, 92%) as a pale yellow solid and as a 2:1 mixture of conformers A (major) and B (minor). The product was used for the next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (s, CC(C$\mathbf{H_3}$)$_3$), 1.45 (s, OC(C$\mathbf{H_3}$)$_3$), 3.85–3.92 (br d, C$\mathbf{H_B}$), 4.13 (d, J = 8.0 Hz, C$\mathbf{H_A}$), 5.12 (d, J = 8.0 Hz, N$\mathbf{H_A}$), 6.16–6.22 (br d, N$\mathbf{H_B}$), 10.02–10.45 (br s, O**H**). ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.5 (CHC(**C**H_3)$_3$), 28.3 (OC(**C**H_3)$_3$), 34.0 (CH**C**$_\mathbf{B}$(CH_3)$_3$), 34.5 (CH**C**$_\mathbf{A}$(CH_3)$_3$), 61.6 (**C**H_A), 63.6 (**C**H_B), 80.0 (O**C**$_\mathbf{A}$(CH_3)$_3$), 81.6 (O**C**$_\mathbf{B}$(CH_3)$_3$), 155.6 (O**C**$_\mathbf{A}$(O)), 156.6 (O**C**$_\mathbf{B}$(O)), 176.8 (C**C**(O)).

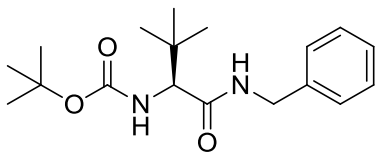


(*S*)-2-*N*-(*t*-Butoxycarbonyl)amino-3,3-dimethylbutanoic Acid ((*S*)-204).[194,198,201] Boc_2O (8.24 g, 37.76 mmol) was added to a *t*-butyl alcohol/H_2O (45 mL/45 mL) solution of L-*tert*-leucine (4.50 g, 34.33 mmol) and NaOH (1.51 g, 37.76 mmol). The reaction was stirred at room temperature (18 h) and then the organic layer was evaporated *in vacuo*. The aqueous layer was acidified with an aqueous 1 M $KHSO_4$ to pH 2–3. The aqueous layer was

extracted with EtOAc (3 x 50 mL) and the organic layers were combined, dried (Na_2SO_4), and evaporated *in vacuo* to give the crude product (7.55 g, 95%) as a white solid and as a 2:1 mixture of conformers A (major) and B (minor): mp 105–106 ºC (lit.[201] mp 122 ºC); $[\alpha]^{25}_D$ – 2.8° (*c* 1.1, CH_2Cl_2); R_f 0.77 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2910 (br), 1660, 1531, 1458, 1373, 1223, 1161, 1061, 1011, 900, 850, 724 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.02 (s, CC(C$\mathbf{H_3}$)$_3$), 1.45 (s, OC(C$\mathbf{H_3}$)$_3$), 3.88 (d, *J* = 7.4 Hz, C$\mathbf{H_B}$), 4.14 (d, *J* = 9.5 Hz, C$\mathbf{H_A}$), 5.16 (d, *J* = 9.5 Hz, N$\mathbf{H_A}$), 6.38 (d, *J* = 7.4 Hz, N$\mathbf{H_B}$), 10.91–11.16 (br s, CO_2**H**). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.7 (CHC(**C**H_3)$_3$), 28.5 (OC(**C**H_3)$_3$), 34.7 (CH**C**(CH_3)$_3$), 61.8 ($\mathbf{C_A}$HC(CH_3)$_3$), 63.8 ($\mathbf{C_B}$HC(CH_3)$_3$), 80.2 (O$\mathbf{C_A}$(CH_3)$_3$), 81.8 (O$\mathbf{C_B}$(CH_3)$_3$), 155.9 (O$\mathbf{C_A}$(O)NH), 157.7 (O$\mathbf{C_B}$(O)NH), 176.9 (**C**(O)OH).

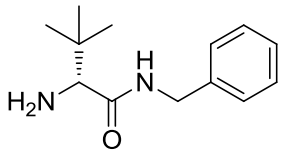


(*R*)-*N*-Benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3,3-dimethylbutanamide ((*R*)-210). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3,3-dimethylbutanoic acid (3.50 g, 15.14 mmol), 4-methylmorpholine (2.16 mL, 19.68 mmol), isobutyl chloroformate (2.15 mL, 16.66 mmol), and benzylamine (1.74 mL, 15.90 mmol) in anhydrous THF (150 mL) gave the crude product (3.97 g, 82%) as a pale solid. The product was used immediately for the next step without further purification.



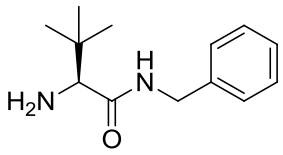
(*S*)-*N*-Benzyl 2-*N'*-(*tert*-Butoxycarbonyl)amino-3,3-dimethylbutanamide ((*S*)-210).[198,196] The previous procedure was repeated using (*S*)-2-*N*-(*t*-butoxycarbonyl)amino-3,3-

dimethylbutanoic acid (4.53 g, 19.60 mmol), NMM (2.80 mL, 25.48 mmol), IBCF (2.78 mL, 21.56 mmol), and benzylamine (2.25 mL, 20.57 mmol) in anhydrous THF (200 mL) to give the crude product that was purified by flash column chromatography (SiO_2; 1:10–1:1 EtOAc/hexanes) to give the desired product (4.58 g, 73%) as a white solid: mp 150–151 ºC; $[\alpha]^{25}_D$ + 3.1° (*c* 1.0, CH_2Cl_2); R_f 0.36 (1:10 EtOAc/hexanes); IR (nujol mull) 2911 (br), 1714, 1644, 1543, 1459, 1374, 1228, 1170, 1066, 732 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.00 (s, CHC(C**H$_3$**)$_3$), 1.39 (s, OC(C**H$_3$**)$_3$), 3.91 (d, *J* = 9.5 Hz, C**H**), 4.35 (dd, *J* = 5.4, 15.0 Hz, NHC**H**H'Ph), 4.48 (dd, *J* = 5.9, 15.0 Hz, NHCH**H'**Ph), 5.35 (d, *J* = 9.5 Hz, OC(O)N**H**), 6.44–6.51 (br m, CC(O)N**H**), 7.20–7.33 (m, C$_6$**H$_5$**); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.6 (CC(**C**H$_3$)$_3$), 28.3 (OC(**C**H$_3$)$_3$), 34.5 (C**C**(CH$_3$)$_3$), 43.4 (NH**C**H$_2$Ph), 62.3 (**C**H), 79.6 (O**C**(CH$_3$)$_3$), 127.3, 127.7, 128.6, 138.1 (**C$_6$**H$_5$), 156.0 (O**C**(O)N), 171.1 (**C**(O)N).

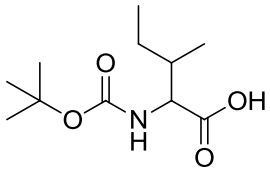


(*R*)-*N*-Benzyl 2-*N'*-Amino-3,3-dimethylbutanamide ((*R*)-99).[202] Utilizing Method E and using (*R*)-*N*-benzyl 2-*N'*-(*tert*-butoxycarbonyl)amino-3,3-dimethylbutanamide (3.80 g, 11.87 mmol), TFA (13.22 mL, 0.18 mol), and CH_2Cl_2 (40 mL) to give the crude product after workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (2.17 g, 83%) as a pale yellow solid: mp 65–66 ºC (lit.[202] mp 53–54 ºC); $[\alpha]^{28.5}_D$ + 21.8° (*c* 0.5, CH_2Cl_2); R_f 0.48 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3291, 2957, 1643, 1555, 1452, 1361, 1259, 1188, 1018, 831 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.00 (s, (C**H$_3$**)$_3$), 1.45 (s, N**H$_2$**), 3.12 (s, C**H**), 4.42 (dd, *J* = 4.0, 12.8 Hz, NHC**H**H'), 4.46 (dd, *J* = 4.0, 12.8 Hz, NHCH**H'**), 7.05–7.13 (br m, N**H**), 7.24–7.34 (m, C$_6$**H$_5$**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.8 ((**C**H$_3$)$_3$), 34.2 (**C**(CH$_3$)$_3$), 43.1 (N**C**H$_2$), 64.4 (**C**H), 127.4, 127.9, 128.6, 138.6 (**C$_6$**H$_5$), 173.5 (**C**(O));

HRMS (+ESI) 221.1654 $[M+H]^+$ (calcd. for $C_{13}H_{20}N_2OH^+$ 221.1653). Anal. Calcd for $C_{13}H_{20}N_2O$: C, 70.87; H, 9.25; N, 12.72. Found: C, 70.83; H, 9.20; N, 12.75.

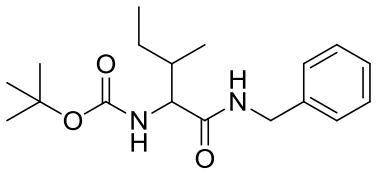


(*S*)-*N*-Benzyl 2-Amino-3,3-dimethylbutanamide ((*S*)-99).[198,196] The previous procedure was repeated using (*S*)-*N*-benzyl 2-*N'*-(*tert*-butoxycarbonyl)amino-3,3-dimethylbutanamide (2.50 g, 7.81 mmol), TFA (8.70 mL, 0.12 mol), and CH_2Cl_2 (26 mL) to give the crude product after workup that was further purified by flash column chromatography (SiO_2; 1:100–1:10 MeOH/CH_2Cl_2) to give the desired compound (1.58 g, 92%) as a white solid: mp 65–66 ºC (lit.[1] mp 53–54 ºC); $[\alpha]^{25}_D$ – 15.2° (*c* 0.51, CH_2Cl_2) (lit.[3] $[\alpha]^{20}_D$ – 17.5° (*c* 0.56, CH_2Cl_2); R_f 0.19 (100% EtOAc); IR (nujol mull) 2912 (br), 1649, 1555, 1459, 1372, 1260, 1192, 1023, 941, 839, 707 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.00 (s, C(C**H**$_3$)$_3$), 1.47 (s, N**H**$_2$), 3.14 (s, C**H**), 4.44 (d, *J* = 5.7 Hz, NHC**H**$_2$Ph), 7.04–7.12 (br t, C(O)N**H**), 7.24–7.36 (m, C$_6$**H**$_5$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.0 (C(**C**H$_3$)$_3$), 34.4 (**C**(CH$_3$)$_3$), 43.4 (NH**C**H$_2$Ph), 64.7 (**C**H), 127.6, 128.1, 128.9, 138.8 (**C**$_6$H$_5$), 173.6 (**C**(O)N); HRMS (ESI) 221.1652 [M + H^+] (calcd for $C_{13}H_{20}N_2OH^+$ 221.1654); Anal. Calcd for $C_{13}H_{20}N_2O$: C, 70.87; H, 9.15; N, 12.72. Found: C, 70.90; H, 9.10; N, 12.54.



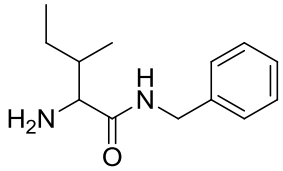
(*R,S*)-2-*N*-(*t*-Butoxycarbonyl)amino-3-methylpentanoic Acid ((*R,S*)-204).[203] A solution of DL-isoleucine (5.00 g, 38.14 mmol) in a mixture of dioxane (75 mL) and aqueous 1 M NaOH (37.5 mL) was cooled to 0 ºC in an ice water bath. Boc_2O (9.16 g, 41.97 mmol) was added

slowly and the reaction was allowed to warm to room temperature (18 h), and then the solvent was evaporated *in vacuo*. The resulting crude oil was dissolved in EtOAc (50 mL), the aqueous layer was acidified to pH 2 with aqueous 1 M $KHSO_4$ and extracted with EtOAc (3 x 50 mL). The combined organic layers were dried (Na_2SO_4) and evaporated *in vacuo* to give the crude product with minor impurities (9.10 g) as a pale yellow oil and as a 1:1 mixture of conformers A and B. The product was used for the next step without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82–0.98 (m, 2 C$\mathbf{H_3}$), 1.18–1.32 (m, C$\mathbf{H_2}$), 1.45 (s, C(C$\mathbf{H_3}$)$_3$), 1.82–2.03 (m, C**H**), 4.33 (dd, *J* = 4.4, 8.7 Hz, NHC$\mathbf{H_A}$), 4.43 (dd, *J* = 3.5, 9.5 Hz, NHC$\mathbf{H_B}$), 5.02 (d, *J* = 9.5 Hz, N$\mathbf{H_B}$), 5.08 (d, *J* = 8.7 Hz, N$\mathbf{H_A}$), 10.52–11.35 (br s, O**H**).

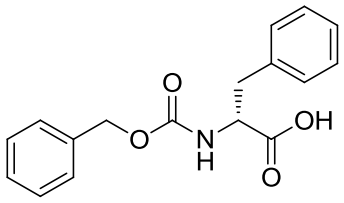


(*R,S*)-*N*-Benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylpentanamide ((*R,S*)-211). Utilizing Method B and using (*R,S*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylpentanoic acid (9.10 g, 39.37 mmol), NMM (5.63 mL, 51.18 mmol), IBCF (5.59 mL, 43.31 mmol), and benzylamine (4.52 mL, 41.34 mmol) gave the crude product that was purified by recrystallization from hot EtOAc/hexanes to give the desired compound (7.16 g, 57%) as a white solid and as a 1:1 mixture of conformers A and B: mp 104–105 ºC; R_f 0.59 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3393, 3282, 2912, 1658, 1554, 1458, 1375, 1308, 1253, 1168, 1041, 723 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84–0.96 (m, 2 C$\mathbf{H_3}$), 1.01–1.26 (m, C$\mathbf{H_2}$), 1.40, 1.41 (2 s, OC(C$\mathbf{H_3}$)$_3$), 1.74–2.04 (m, CHC**H**), 3.98 (dd, *J* = 6.7, 8.7 Hz, NHC$\mathbf{H_A}$ or NHC$\mathbf{H_B}$), 4.15 (dd, *J* = 3.5, 9.5 Hz, NHC$\mathbf{H_B}$ or NHC$\mathbf{H_A}$), 4.36–4.51 (m, NHC$\mathbf{H_2}$Ph), 4.96–5.18 (m, $(CH_3)_3COC(O)N\mathbf{H}$), 6.54–6.62 (br t, N**H**CH_2Ph), 7.17–7.35 (m, $C_6\mathbf{H_5}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.8, 12.1 ($CH_2\mathbf{C}H_3$), 14.9, 16.1 (CH**C**H_3), 19.6, 22.3 (**C**H_2CH_3), 26.8, 28.8 (C(**C**H_3)$_3$), 37.9, 38.0 (CH**C**H), 43.8, 43.9 (NH**C**H_2Ph), 58.9, 59.8 (C**H**), 80.2, 80.3 (**C**$(CH_3)_3$),

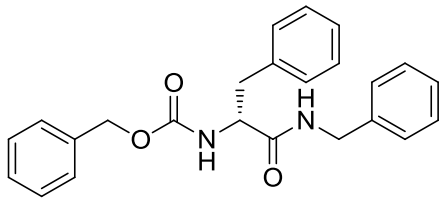
127.9, 128.2, 129.0, 129.1, 138.7, 138.8 (($\mathbf{C_6}H_5)_A$, ($\mathbf{C_6}H_5)_B$), 156.3, 156.6 (N**C**(O)O), 172.4, 172.6 (C**C**(O)N), two aromatic peaks were not detected and are believed to overlap with nearby signals; HRMS (ESI) 321.2187 [M + H^+] (calcd for $C_{18}H_{28}N_2O_3H^+$ 321.2178); Anal. Calcd for $C_{18}H_{28}N_2O_3$: C, 67.47; H, 8.81; N, 8.74. Found C, 67.22; H, 9.00; N, 8.65.



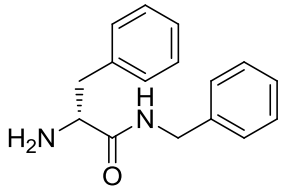
(*R,S*)-*N*-Benzyl 2-Amino-3-methylpentanamide ((*R,S*)-100). Utilizing Method E and using (*R,S*)-*N*-benzyl-2-N'-(*t*-butoxycarbonyl)amino-3-methylpentanamide (4.00 g, 12.49 mmol), TFA (13.92 mL, 0.19 mol), and CH_2Cl_2 (40 mL) gave the crude product after workup that was further purified by flash column chromatography (SiO_2; 1:100–1:10 MeOH/CH_2Cl_2) to give the desired compound (2.24 g, 81%) as a pale yellow oil and as a 1:1 mixture of diastereomers: R_f 0.73 (1:20 MeOH/CH_2Cl_2); IR (neat) 3308, 3068, 2962, 2926, 2876, 1656, 1521, 1457, 1365, 1247, 1081, 1026, 934, 852, 737, 700 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.77–0.96 (m, 2 C$\mathbf{H_3}$), 1.00–1.46 (m, (C$\mathbf{H_2}CH_3$), N$\mathbf{H_2}$), 1.92–2.17 (m, CHC**H**), 3.26 (d, J = 3.6 Hz, (C**H**)$_A$ or (C**H**)$_B$), 3.36 (d, J = 3.6 Hz, (C**H**)$_A$ or (C**H**)$_B$), 4.36–4.49 (m, NHC$\mathbf{H_2}$Ph), 7.21–7.33 (m, $C_6\mathbf{H_5}$), 7.74–7.82, 7.83–7.91 (2 br t, N**H**CH_2Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.9 ($CH_2\mathbf{C}H_3$), 13.1, 16.2 (CH**C**H_3), 23.7, 26.7 (**C**H_2CH_3), 37.1, 38.0 (CH**C**H), 42.9, 43.0 (NH**C**H_2Ph), 57.9, 59.9 (C**H**), 127.2, 127.6, 127.7, 128.5, 138.7, 138.7 (($\mathbf{C_6}H_5)_A$, ($\mathbf{C_6}H_5)_B$), 174.4, 174.7 (C**C**(O)N), two aromatic peaks were not detected and are believed to overlap with nearby signals; HRMS (ESI) 221.1663 [M + H^+] (calcd for $C_{13}H_{20}N_2OH^+$ 221.1654); Anal. Calcd for $C_{13}H_{20}N_2O$•0.03CH_2Cl_2: C, 70.20; H, 9.07; N, 12.57. Found: C, 70.19; H, 9.21; N, 12.54.



(*R*)-2-*N*-(Benzyloxycarbonyl)amino-3-phenylpropionic Acid ((*R*)-206).[204,205] Benzyl chloroformate (5.09 mL, 36.35 mmol) and aqueous 4 M NaOH (12 mL) were added simultaneously over a 30 min period to a vigorously stirred solution of D-phenylalanine (5.00 g, 30.29 mmol) dissolved in aqueous 4 M NaOH (10 mL)/aqueous 1 M $NaHCO_3$ (30 mL) at 0 ºC. The mixture was then warmed to room temperature and stirred overnight (18 h). The reaction was washed with Et_2O (2 x 100 mL) and then the aqueous mixture was added to a stirred mixture of aqueous 4 M HCl (66 mL) and EtOAc (100 mL). The aqueous layer was separated and then extracted with EtOAc (3 x 100 mL). All of the organic layers were combined, successively washed with H_2O (3 x 100 mL) and brine (2 x 100 mL), dried (Na_2SO_4), and then evaporated *in vacuo* to give the desired product (6.74 g, 74%) as an off-white solid and as a 2:1 mixture of conformers A (major) and B (minor): mp 88–89 ºC (lit.[204] mp 85–88 ºC); $[\alpha]^{25}_D$ + 4.3° (*c* 1.0, MeOH) (lit.[204] $[\alpha]^{25}_D$ +4.1º (*c* 1.0, MeOH)); R_f 0.52 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3461, 3319, 2919, 1695, 1531, 1457, 1260, 1053, 904, 735 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.87–2.95 (m, (CHC**H$_2$**Ph)$_B$), 3.07 (dd, *J* = 6.6, 14.1 Hz, (CHC**H**H'Ph)$_A$), 3.19 (dd, *J* = 5.6, 14.1 Hz, (CHCH**H'**Ph)$_A$), 4.60–4.53 (m, C**H$_B$**), 4.65–4.72 (m, C**H$_A$**), 5.03 (d, *J* = 4.5 Hz, (OC**H$_2$**Ph)$_B$), 5.07 (d, *J* = 3.3 Hz, (OC**H$_2$**Ph)$_A$), 5.36 (d, *J* = 8.1 Hz, N**H$_A$**), 6.45 (d, *J* = 8.1 Hz, N**H$_B$**), 7.12–7.32 (m, 2 C$_6$**H$_5$**), 10.32–10.58 (br s, O**H**); ^{13}C NMR (75 MHz, $CDCl_3$) δ 37.8 (CH**C**H$_2$Ph), 54.8 (**C**H), 67.3 (O**C**H$_2$Ph), 127.3, 128.2, 128.4, 128.7, 128.7, 129.5, 135.7, 136.2 (2 **C$_6$**H$_5$), 156.1 (O**C**(O)N), 176.2 (**C**(O)OH).

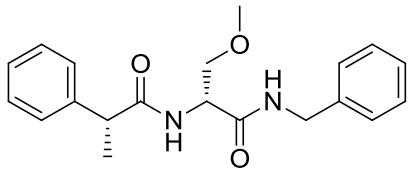


(*R*)-*N*-Benzyl 2-*N'*-(Benzyloxycarbonyl)amino-3-phenylpropionamide ((*R*)-213). Utilizing Method B and using (*R*)-2-*N*-(benzyloxycarbonyl)amino-3-phenylpropionic acid (5.00 g, 16.71 mmol), NMM (2.39 mL, 21.73 mmol), IBCF (2.37 mL, 18.38 mmol), and benzylamine (1.92 mL, 17.55 mmol) gave the crude product that was recrystallized from hot EtOAc to give the desired compound (4.67 g, 72%) as an off-white solid: mp 154–155 ºC; $[\alpha]^{25}_{D}$ + 5.1° (*c* 1.1, CH_2Cl_2); R_f 0.85 (1:100 MeOH/CH_2Cl_2); IR (nujol mull) 3412, 3292, 2923, 2860, 1688, 1645, 1537, 1457, 1380, 1288, 1239, 1045, 743, 696 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.99–3.15 (m, CHC**H$_2$**Ph), 4.24–4.44 (m, C**H**, NHC**H$_2$**Ph), 5.46 (d, *J* = 7.2 Hz, N**H**), 6.10–6.19 (br t, N**H**CH_2Ph), 7.03–7.36 (m, 3 C_6**H$_5$**); ^{13}C NMR (75 MHz, $CDCl_3$) δ 39.0 (CH**C**H_2Ph), 43.7 (NH**C**H_2Ph), 56.7 (**C**H), 67.3 (O**C**H_2Ph), 127.2, 127.7, 127.9, 128.2, 128.4, 128.7, 128.8, 128.9, 129.5, 136.2, 136.6, 137.7 (3 **C$_6$**H_5), 156.2 (O**C**(O)N), 170.9 (C**C**(O)N); HRMS (ESI) 411.1697 [M + Na^+] (calcd for $C_{24}H_{24}N_2O_3Na^+$ 411.1685); Anal. Calcd for $C_{24}H_{24}N_2O_3$: C, 74.21; H, 6.23; N, 7.21. Found: C, 74.18; H, 6.28; N, 7.28.



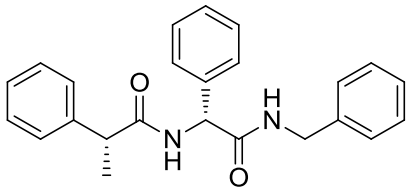
(*R*)-*N*-Benzyl 2-Amino-3-phenylpropionamide ((*R*)-102). Utilizing Method C and using (*R*)-*N*-benzyl 2-*N'*-(benzyloxycarbonyl)amino-3-phenylpropionamide (3.50 g, 9.02 mmol), 10% Pd-C (0.35 g), and MeOH (90 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 1:10 MeOH/CH_2Cl_2) to give the desired compound (2.22 g, 97%) as a pale yellow solid: mp 66–67 ºC; $[\alpha]^{25}_{D}$ + 63.3° (*c* 1.4, CH_2Cl_2); R_f 0.43 (1:20

MeOH/CH_2Cl_2); IR (nujol mull) 3293, 2924, 2859, 1641, 1539, 1457, 1373, 1261, 1105, 1031, 950, 845, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.98–1.62 (br s, N**H$_2$**), 2.75 (dd, *J* = 9.0, 13.7 Hz, CHC**H**H'Ph), 3.29 (dd, *J* = 4.2, 13.7 Hz, CHCH**H'**Ph), 3.65 (dd, *J* = 4.2, 9.0 Hz, C**H**), 4.37–4.50 (m, NHC**H$_2$**Ph), 7.20–7.35 (m, 2 C_6**H$_5$**), 7.56–7.64 (br t, N**H**CH_2Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 41.2 (CH**C**H_2Ph), 43.3 (NH**C**H_2Ph), 56.6 (**C**H), 127.0, 127.5, 127.9, 128.8, 128.9, 129.5, 138.0, 138.5 (2 **C$_6$**H_5), 171.3 (C**C**(O)N); HRMS (ESI) 277.1321 [M + Na^+] (calcd for $C_{16}H_{18}N_2ONa^+$ 277.1317); Anal. Calcd for $C_{16}H_{18}N_2O$: C, 75.56; H, 7.13; N, 11.01. Found: C, 75.53; H, 7.16; N, 10.85.



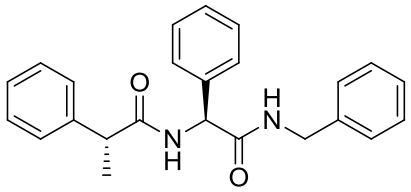
(*R*)-*N*-Benzyl 2-*N*-((*R*)-2-Phenylpropionyl)amino-3-methoxypropionamide ((*R,R*)-215). Utilizing Method B and using (*R*)-2-phenylpropionic acid (41 mg, 0.27 mmol), NMM (39 *μ*L, 0.36 mmol), IBCF (34 *μ*L, 0.26 mmol), and (*R*)-*N*-benzyl 2-amino-3-methoxypropionamide (60 mg, 0.29 mmol) gave the crude product that was purified by flash column chromatography (SiO_2; 1:100 MeOH/CH_2Cl_2). The major fraction gave the following spectrum: ^1H NMR (300 MHz, DMSO-d_6) δ 1.32 (d, *J* = 7.2 Hz, C**H$_3$**), 3.26 (s, OC**H$_3$**), 3.50 (d, *J* = 6.3 Hz, C**H$_2$**OCH_3), 3.81 (q, *J* = 7.2 Hz, C**H**CH_3), 4.23 (d, *J* = 6.3 Hz, C**H**$_2$Ph), 4.50–4.56 (m, C**H**CH_2), 7.14–7.34 (m, 2 C_6**H$_5$**), 8.23 (d, *J* = 7.8 Hz, N**H**C(O)), 8.37 (t, *J* = 6.3 Hz, N**H**CH_2Ph), minor signals attributed to impurities were detected. The major fraction was recrystallized from hot EtOAc to give the desired product (52 mg, 53%) as a white solid: mp 146–147 °C; R_f 0.63 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3287, 2859 (br), 1633, 1544, 1458, 1374, 1227, 1099, 906, 704 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.32 (d, *J* = 7.4 Hz, C**H$_3$**), 3.26 (s, OC**H$_3$**), 3.50 (d, *J* = 6.3 Hz, C**H$_2$**OCH_3), 3.79 (q, *J* = 7.4 Hz, C**H**CH_3), 4.23 (d,

J = 6.0 Hz, C**H**$_2$Ph), 4.49–4.52 (m, C**H**CH$_2$), 7.14–7.34 (m, 2 C$_6$**H$_5$**), 8.23 (d, *J* = 8.1 Hz, N**H**C(O)), 8.37 (t, *J* = 6.0 Hz, N**H**CH$_2$Ph); ^{13}C NMR (75 MHz, DMSO–*d*$_6$) *δ* 18.3 (CH**C**H$_3$), 41.6 (**C**H$_2$Ph), 44.0 (**C**HCH$_3$), 52.0 (**C**HCH$_2$), 57.9 (O**C**H$_3$), 71.7 (**C**H$_2$OCH$_3$), 126.0, 126.3, 126.5, 127.0, 127.7, 127.8, 138.6, 141.7 (2 **C$_6$**H$_5$), 169.1 (CHNH**C**(O)), 173.0 (CH$_2$NH**C**(O)); HRMS (ESI) 363.1686 [M + Na$^+$] (calcd for $C_{20}H_{24}N_2O_3Na^+$ 363.1685); Anal. Calcd for $C_{20}H_{24}N_2O_3 \cdot 0.05CH_2Cl_2$; C, 69.83; H, 7.04; N, 8.14. Found C, 69.87; H, 6.83; N, 8.01.



(*R*)-*N*-Benzyl 2-*N*-((*R*)-2-Phenylpropionyl)amino-2-phenylacetamide ((*R,R*)-216). Utilizing Method B and using (*R*)-2-phenylpropionic acid (60 mg, 0.40 mmol), NMM (57 *μ*L, 0.52 mmol), IBCF (49 *μ*L, 0.38 mmol), and (*R*)-*N*-benzyl 2-amino-2-phenylacetamide (100 mg, 0.42 mmol) gave the crude product that was purified by flash column chromatography (SiO_2; 1:100 MeOH/CH_2Cl_2). The major fraction gave the following spectra: ^{1}H NMR (300 MHz, DMSO-*d*$_6$) *δ* 1.29 (d, *J* = 7.1 Hz, C**H**$_3$), 3.95 (q, *J* = 7.1 Hz, C**H**CH$_3$), 4.22 (d, *J* = 5.7 Hz, C**H**$_2$Ph), 5.53 (d, *J* = 7.8 Hz, C**H**Ph), 7.06–7.46 (m, 3 C$_6$**H$_5$**), 8.70–8.78 (m, N**H**C(O), N**H**CH$_2$Ph), minor signals attributed to impurities were detected; ^{13}C NMR (75 MHz, DMSO-*d*$_6$) *δ* 18.3 (**C**H$_3$), 41.6 (**C**H$_2$Ph), 43.7 (**C**HCH$_3$), 55.8 (**C**HPh), 126.1, 126.4, 126.5, 126.6, 127.0, 127.1, 127.8, 127.9, 138.5, 138.8, 141.8 (3 **C$_6$**H$_5$), 169.4 (CHNH**C**(O)), 172.6 (CH$_2$NH**C**(O)), one aromatic peak was not detected and is believed to overlap with nearby signals. The major fraction was recrystallized from hot $CHCl_3$ to give the desired product (83 mg, 54%) as a white solid: mp 213-214 °C; $[\alpha]^{25}_D$ –48.8° (*c* 0.9, DMSO); R_f 0.48 (1:100 MeOH/CH_2Cl_2); IR (nujol mull) 3313, 2934 (br), 1634, 1531, 1458, 1375, 1216, 724 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-*d*$_6$) *δ* 1.28 (d, *J* = 6.9 Hz, C**H**$_3$), 3.94 (q, *J* = 6.9 Hz, C**H**CH$_3$), 4.22 (d,

J = 6.0 Hz, C**H**$_2$Ph), 5.52 (d, *J* = 8.4 Hz, C**H**Ph), 7.05-7.46 (m, 3 C_6**H$_5$**), 8.70-8.77 (m, N**H**C(O), N**H**CH_2Ph); ^{13}C NMR (75 MHz, DMSO-d_6) δ 18.3 (**C**H_3), 41.6 (**C**H_2Ph), 43.7 (**C**HCH_3), 55.8 (**C**HPh), 126.1, 126.4, 126.6, 127.0, 127.1, 127.7, 127.8, 127.9, 138.5, 138.8, 141.8 (3 **C$_6$**H_5), 169.4 (CHNH**C**(O)), 172.6 (CH_2NH**C**(O)), one aromatic peak was not detected and is believed to overlap with nearby signals; HRMS (ESI) 395.1735 [M + Na^+] (calcd for $C_{24}H_{24}N_2O_2Na^+$ 395.1736).



(*S*)-*N*-Benzyl 2-*N*-((*R*)-2-Phenylpropionyl)amino-2-phenylacetamide ((*R,S*)-216). The previous procedure was repeated using (*R*)-2-phenylpropionic acid (60 mg, 0.40 mmol), NMM (57 *μ*L, 0.52 mmol), IBCF (56 *μ*L, 0.44 mmol), and (*S*)-*N*-benzyl 2-amino-2-phenylacetamide (100 mg, 0.42 mmol) to give the crude product that was purified by flash column chromatography (SiO_2; 1:100 MeOH/CH_2Cl_2). The major fraction gave the following spectra: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.34 (d, *J* = 6.5 Hz, C**H**$_3$), 3.94 (q, *J* = 6.5 Hz, C**H**CH_3), 4.29 (d, *J* = 5.9 Hz, C**H**$_2$Ph), 5.47 (d, *J* = 8.1 Hz, C**H**Ph), 7.14–7.33 (m, 3 C_6**H$_5$**), 8.63 (d, *J* = 7.8 Hz, N**H**C(O)), 8.82 (t, *J* = 5.9 Hz, N**H**CH_2Ph), minor signals attributed to impurities were detected; ^{13}C NMR (75 MHz, DMSO-d_6) δ 17.9 (**C**H_3), 41.7 (**C**H_2Ph), 43.8 (**C**HCH_3), 56.0 (**C**HPh), 126.1, 126.4, 126.6, 126.7, 127.0, 127.1, 127.7, 127.8, 127.9, 138.3, 138.7, 141.7 (3 **C$_6$**H_5), 169.5 (CHNH**C**(O)), 172.7 (CH_2NH**C**(O)); The major fraction was recrystallized from hot $CHCl_3$ to give the desired product (82 mg, 53%) as a white solid: mp 224–225 °C; $[\alpha]^{25}_D$ +47.3° (*c* 1.0, DMSO); R_f 0.57 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3297, 2924 (br), 1636, 1537, 1457, 1375, 1218, 702 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.34 (d, *J* = 7.4 Hz, C**H**$_3$), 3.91 (q, *J* = 7.4 Hz, C**H**CH_3), 4.28 (d, *J* = 5.3 Hz, C**H**$_2$Ph), 5.46 (d, *J* =

7.4 Hz, C**H**Ph), 7.14–7.33 (m, 3 C_6**H**$_5$), 8.63 (d, *J* = 7.4 Hz, N**H**C(O)), 8.82 (t, *J* = 5.3 Hz, N**H**CH_2Ph); ^{13}C NMR (75 MHz, DMSO-d_6) δ 18.0 (**C**H_3), 41.7 (**C**H_2Ph), 43.8 (**C**HCH_3), 55.9 (**C**HPh), 126.1, 126.4, 126.5, 126.7, 127.0, 127.1, 127.7, 127.8, 127.9, 138.3, 138.7, 141.7 (3 **C**$_6$$H_5$), 169.5 (CHNH**C**(O)), 172.7 (CH_2NH**C**(O)); HRMS (ESI) 395.1734 [M + Na^+] (calcd for $C_{24}H_{24}N_2O_2Na^+$ 395.1736); Anal. Calcd for $C_{24}H_{24}N_2O_2$·0.64H_2O; C, 75.07; H, 6.64; N, 7.30. Found C, 74.69; H, 6.24; N, 7.17.

4.3. Pharmacology

Compounds were screened under the auspices of UCB Pharma (Braine L'Alleud, Belgium) and the NINDS ASP (Rockville, MD). Pharmacological evaluation by UCB Pharma consisted of four mice assays: the 6 Hz test and the MES test to assess anticonvulsant activity, the formalin test to assess NP protection, and the rotorod test to assess neurological toxicity. After the expiration of contractual obligations with UCB Pharma, compounds were screened under the auspices of the NINDS ASP. Initial pharmacological evaluation by the NINDS ASP consisted of the MES test (mice and rats) and the subcutaneous pentylenetetrazol (Metrazol®) (scMET) (mice) seizure threshold test to assess anticonvulsant activity, the rotorod test to assess neurological toxicity (mice), and the positional sense test or gait and stance test to assess behavioral toxicity (rats). The effective dose (50%) (ED_{50}) values were obtained in quantitative screenings for compounds that showed significant activity. Also provided were the median doses for neurological impairment (50%) (TD_{50}) in mice using the rotorod test, and the behavioral toxicity effects observed in rats. TD_{50} values were determined for those compounds that demonstrated significant activity in the MES test.

4.3.1. Pharmacological evaluation conducted at UCB Pharma

All experiments were performed with the use of male NMRI mice (Charles River, France) weighing 25–30 g. All mice were kept on a 12/12 h light/dark cycle with lights on at 6 am and were housed at a temperature maintained at 20–24 ºC and at a humidity of 40–70%. The mice were kept in groups of 10 in Makrolon cages (Type III, 425 x 266 x 155 mm) containing a bedding layer of sawdust. The mice were allowed *ad libitum* access to standard dry pellet food and tap water before random assignment to experimental groups. Each experiment consisted of several groups of 10 mice, one group receiving the vehicle control and the other groups receiving different doses of compounds. All compounds were dissolved in 0.5% methylcellulose and injected ip (10 mL/kg volume) 30 min before testing. An effective dose protecting 50% of the mice (ED_{50}) against the convulsive endpoint and its associated 95% confidence interval, and a toxic dose impairing the rotorod performance of 50% of the mice (TD_{50}), were calculated using a non-linear fitting of the dose-response curve with GraphPad Prism 4 (GraphPad Software, San Diego, CA). In cases where the dose-response could not be established, the minimal active dose (MAD) or maximal tolerated dose (MTD) was determined after statistical comparison (Fisher's exact test). This occurred when no further increase in protection was observed at a higher dose (plateau effect), a higher dose could not be tested because of CNS side effects, or there was loss of activity at higher doses.

In the 6 Hz test, partial-onset seizures were induced by a stimulator (ECT Unit 57800, Ugo Basile, Comerio, Italy) using a current intensity of 44 mA, delivered with 0.2 msec monopolar pulses at 6 Hz, for a duration of 3 sec through corneal electrodes as described by Kaminski and coworkers.[179] A drop of saline/Unicain 0.1% was placed on the eyes to ensure good conductivity and mild local anesthesia before electrical stimulation. After stimulation, each mouse was observed for 30 sec and the duration of immobility (stunned posture) was noted. Untreated mice reliably respond with seizures. After

compound treatment, the mice were considered as protected against seizures if the duration of immobility was shorter than 7 sec.

The maximal electroshock seizures (MES) test identified compounds that prevent seizure spread and was induced by a stimulator (WITT Industrie Elektronik, Berlin, Germany) using a current of 50 mA, delivered with a pulse frequency of 50 Hz for 0.2 sec, through corneal electrodes as described by Klitgaard and coworkers.[178] Stimulation with this current caused tonic hindlimb extension in 100% of the vehicle-treated mice. The mice were considered as protected by the compound if they did not exhibit the tonic hindlimb extension following stimulation.

In the formalin test, mice were pretreated ip with compound or vehicle 30 min before intrapaw injection of formalin and then returned to their cage. Twenty min after pretreatment (10 min before the injection of formalin), the mice were individually placed in 6 mm thick Plexiglas cages (26 cm x 17 cm x 28 cm) and observed for drug induced side effects over a 10 min period. Then, 25 µL of 1.5% formalin (1.5% aqueous solution of formaldehyde) was injected into the midplantar aspect of the right hindpaw (subplantar injection) and the mice were returned to the observation cage. The nociceptive response was defined as the duration of licking directed at the right hindpaw and was measured to the nearest sec for each consecutive 5 min time bin for 30 min after the injection of formalin. A typical triphasic pattern of nociceptive response occurs.[180] An immediate (0–5 min) bout of intense flinching, licking, or biting behavior is followed by nociceptive silence (5–10 min), followed by a gradual increase in nociceptive licking that peaks at 15–20 min. The nociceptive response gradually decays to zero by 25–30 min after injection.[181] The bin 0–5 min after formalin injection constituted the early phase, where pain is due to direct excessive stimulation of primary afferent neurons. The sum of the remain four time bins (10–15 min, 15–20 min, 20–25 min, and 25–30 min) constituted the late phase, where pain is due to peripheral and central sensitization. The compound's efficacy in the late phase is viewed as predictive of

efficacy in animal models of NP. Therefore, the compound's primary outcome measure is the late-phase value at which 50% of mice display a paw lick duration ≤72 sec (ED_{50}), which corresponds to a 50% reduction of the nociceptive response relative to the mean of vehicle-treated mice. ED_{50} values, with 95% confidence limits where applicable, were calculated using JMP Version 5 for Windows (SAS Institute Inc.).

The adverse effects on motor coordination were assessed in a rotorod test (Treadmill for Mice, Ugo Basile, Italy) as described by Klitgaard and coworkers.[178] The device consisted of a rod with a diameter of 3 cm rotating at a constant speed of 6 rpm. Mice were pretrained and only mice that were able to remain on the rotorod for at least 60 sec in three consecutive trials were retained for testing. Within 24 h, the compound was administered and the number of mice unable to remain on the rod for at least 60 sec was recorded.

4.3.2. Pharmacological evaluation conducted at the NINDS ASP

Experiments were performed with the use of male albino Carworth Farms No. 1 mice (18–25 g) or male albino Sprague-Dawley rats (100-150 g). Housing, handling, and feeding were all in accordance with the recommendations in the "Guide for the Care and Use of Laboratory Animals".[206] All compounds were dissolved in 0.5% methylcellulose and administered ip (10 mL/kg volume) in mice and po (20 mL/kg) in rats. An effective dose protecting 50% of the mice and rats (ED_{50}) against the convulsive endpoint and its associated 95% confidence interval, as well as the toxic dose impairing the rotorod performance of 50% of the mice, or the positional sense/gait and stance performance of 50% of the rats (TD_{50}), were calculated by a computer program based on the methods described by Finney.[207]

The MES test was induced by a stimulator using a current of 50 mA in mice, or 150 mA in rats, and delivered with a pulse frequency of 60 Hz for 0.2 sec through corneal electrodes.[42] A drop of 0.5% butacaine hemisulfate in 0.9% sodium chloride was placed on

the eyes to ensure good conductivity and mild local anesthesia before electrical stimulation. Qualitative assessment of activity in mice was determined at 30 min and 4 h following doses of 30, 100, and 300 mg/kg of compound. Rats were tested at 0.25–4 h in 30 min intervals at a dose of 30 mg/kg. The mice or rats were considered as protected by the compound if they did not exhibit the tonic hindlimb extension following stimulation.

The scMET model primarily identifies compounds that raise the seizure threshold and was induced by subcutaneous injection of 0.5% solution of pentylenetetrazol in the posterior midline (85 mg/kg) in mice. This produces clonic seizures that last at least 5 sec in 97% (CD_{97}) of animals tested.[42] Mice were tested at 30 min and 4 h following doses of 30, 100, and 300 mg/kg of compound. The animals were considered protected when the compound abolished the effect of pentylenetetrazol on seizure threshold.

The adverse effects on motor coordination in mice were assessed in a rotorod test, similar to that conducted at UCB Pharma. Mice were positioned on a rod with a diameter of 1 in rotating at a constant speed of 6 rpm, and the ability of the mice to remain on the rod for at least 60 sec was recorded. Mice were tested at 30 min and 4 h following doses of 30, 100, and 300 mg/kg of compound. The adverse effects on motor coordination in rats were assessed in a positional sense test or gait and stance test. In the positional sense test, one hind leg was lowered over the edge of the table and the ability to transition back to a stable stance was recorded. In the gait and stance test, neurotoxicity was indicated by circular or zig-zag gait, ataxia, abnormal stance or posture, tremor, somnolence, stupor, or catalepsy.[42] Rats were tested at 0.25–4 h in 30 min intervals at a dose of 30 mg/kg.

CHAPTER 3. PAAD Optimization

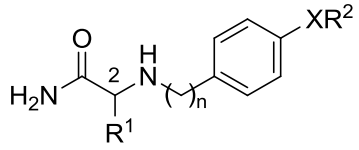
1. Introduction

In this study, we discovered a series of C(3)-*O*-methoxy and C(2)-hydrocarbon PAADs that possessed significant anticonvulsant activity and notable NP protection. To optimize the neurological activities of PAADs, we demonstrate that anticonvulsant activity can be improved upon substitution at the 4'-*N*-benzyl position in C(3)-*O*-methoxy, C(2)-isopropyl, and C(2)-*tert*-butyl PAADs. The 4'-substituents included simple electron-donating or electron-withdrawing groups, as well as more complex substituents using a rationally designed multiple ligand (DML) approach.[208]

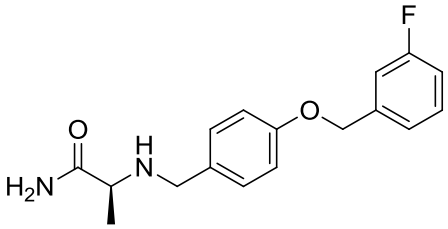
DMLs challenge the current "one-target, one-disease" paradigm and are developed to modulate multiple targets simultaneously with the goal of enhancing efficacy or improving safety. DMLs are compounds that contain multiple ligands that are responsible for the compound's pharmacological activity, and the single formulation is thought to provide a different risk-benefit profile compared to drug cocktails (two or more active ingredients in separate formulations) or multicomponent drugs (two or more active ingredients in one formulation). The risk involved with the increased complexity and optimization of DMLs is concentrated toward the earlier (and less expensive) stages of the drug discovery process. Also, DMLs possibly lower the risk of drug-drug interactions compared with cocktails or multicomponent drugs.[208]

Multiple ligand-containing compounds are rationally designed by connecting the ligands via a linker (conjugated DMLs), directly attaching the ligands (fused DMLs), or

overlapping common functional groups within the ligands (merged DMLs).[208] We took the latter approach and overlapped commonalities of PAADs (**59**) and α-aminoamides (AAAs, **239**), a class of amino acid-based anticonvulsants that display exhibit excellent activities in various animal seizure models,[209,210] to create 4'-chimeric PAADs. The archetypal AAA, safinamide (**30**), was initially discovered due to its anticonvulsant activity and has recently moved into phase III clinical trials for the treatment of Parkinson's disease.[209,211-213]



AAAs
239



Safinamide
30

2. Results and discussion

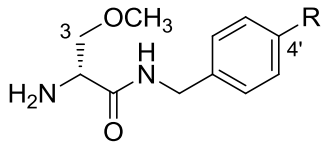
2.1. Choice of compounds: 4'-*N*-Benzyl substituted PAADs

We systematically modified the 4'-*N*-benzyl position according to three categories: (1) 4'-*N*-benzyl substituted C(3)-*O*-methoxy PAADs (**240**–**242**); (2) 4'-*N*-benzyl substituted C(2)-hydrocarbon PAADs (**243**–**253**); and (3) 4'-chimeric C(3)-*O*-methoxy PAADs and 4'-chimeric C(2)-hydrocarbon PAADs (**254**–**262**). The stereochemical preference determined from Chapter 2 prompted the synthesis of all 4'-*N*-benzyl substituted PAADs in the (*R*)-configuration, and select compounds were synthesized in the (*S*)-configuration to confirm that the stereochemical preference applied to optimized PAADs. Since most optimized PAADs were evaluated at the NINDS ASP rather than UCB, the majority of the whole animal pharmacological data was in seizure and neurotoxicity models rather than NP screens.

2.1.1. 4'-*N*-Benzyl substituted C(3)-*O*-methoxy PAADs

The 4'-*N*-benzyl position of (*R*)-**61** was systematically modified with a bromo, trifluoromethyl, trifluoromethoxy, and phenyl group ((*R*)-**240**, -**241**, -**126**, and -**242**, respectively) by Dr. Christophe Salomé and Elise Salomé-Grosjean. We wanted to determine the importance of size, electronics, and hydrophobic interactions of the 4'-substituents on anticonvulsant activity. Although several C(2)-hydrocarbon PAADs displayed higher anticonvulsant activity than (*R*)-**61**, the short series of optimized C(3)-*O*-methoxy PAADs provided a useful comparison for the C(2)-hydrocarbon optimizations, and also served as the PAAD counterpart to a recently published FAA SAR study of (*R*)-**28** by the Kohn laboratory.[102]

4'-*N*-Benzyl-substituted C(3)-*O*-methoxy PAADs



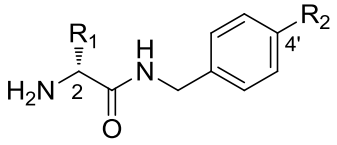
(*R*)-**61** R = H
(*R*)-**240** R = Br
(*R*)-**241** R = CF_3
(*R*)-**126** R = OCF_3
(*R*)-**242** R = C_6H_5

2.1.2. 4'-*N*-Benzyl substituted C(2)-hydrocarbon PAADs

The 4'-*N*-benzyl position of (*R*)-**98** was systematically modified with a fluoro, chloro, methyl, trifluoromethyl, methoxy, trifluoromethoxy, and phenyl group ((*R*)-**243**–**249**). Similarly, the 4'-*N*-benzyl position of (*R*)-**99** was modified with a chloro, trifluoromethyl, trifluoromethoxy, and phenyl group ((*R*)-**250**–**253**). Like the C(3)-*O*-methoxy derivatives, we wanted to determine the importance of size, electronics, and hydrophobic interactions of the 4'-substituents on anticonvulsant activity. Initially, we began with optimization of the C(2)-

isopropyl PAAD ((*R*)-**98**) because of its significant MES activity (MAD: 16 mg/kg) *and* formalin activity (20 mg/kg). Then, we paralleled the 4'-*N*-benzyl substitution of (*R*)-**98** using C(2)-*tert*-butyl PAAD ((*R*)-**99**) after the results of (*R*)-**99** showed a slight improvement in MES activity (13 mg/kg) compared with (*R*)-**98** (MAD 16 mg/kg). We omitted further *tert*-butyl derivatives due to the relatively high cost of D-*tert*-leucine.

4'-*N*-Benzyl-substituted C(2)-hydrocarbon PAADs

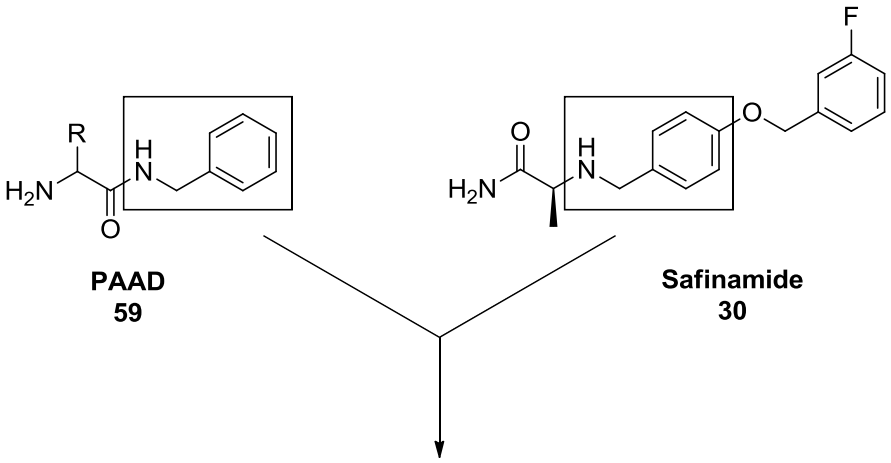


(*R*)-**98**	$R_1 = CH(CH_3)_2$	$R_2 = H$
(*R*)-**243**	$R_1 = CH(CH_3)_2$	$R_2 = F$
(*R*)-**244**	$R_1 = CH(CH_3)_2$	$R_2 = Cl$
(*R*)-**245**	$R_1 = CH(CH_3)_2$	$R_2 = CH_3$
(*R*)-**246**	$R_1 = CH(CH_3)_2$	$R_2 = CF_3$
(*R*)-**247**	$R_1 = CH(CH_3)_2$	$R_2 = OCH_3$
(*R*)-**248**	$R_1 = CH(CH_3)_2$	$R_2 = OCF_3$
(*R*)-**249**	$R_1 = CH(CH_3)_2$	$R_2 = C_6H_5$
(*R*)-**99**	$R_1 = C(CH_3)_3$	$R_2 = H$
(*R*)-**250**	$R_1 = C(CH_3)_3$	$R_2 = Cl$
(*R*)-**251**	$R_1 = C(CH_3)_3$	$R_2 = CF_3$
(*R*)-**252**	$R_1 = C(CH_3)_3$	$R_2 = OCF_3$
(*R*)-**253**	$R_1 = C(CH_3)_3$	$R_2 = C_6H_5$

2.1.3. 4'-Chimeric C(2)-hydrocarbon PAADs

Over the last decade, AAAs have emerged as powerful neurological agents that possess significant anticonvulsant activity.[211-213] Therefore, we overlaid the *N*-benzylamino unit in PAADs and AAAs (see structural unit in box in **59** and **30**) to create a series of chimeric PAADs in an effort to improve anticonvulsant activity and neuropathic pain protection, and reduce neurological toxicity (**254**–**258, 260, 261**). Initial pharmacological data suggested that small substituents at the 4'-*N*-benzyl position of C(3)-*O*-methoxy and C(2)-hydrocarbon PAADs improved anticonvulsant activity, and this chimeric series evaluated the effect of a substantially larger substituent on pharmacological activity. Each

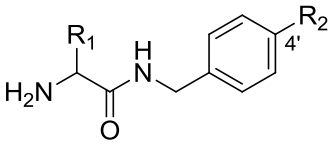
chimeric PAAD was defined by the R_1 and R_2 regions, where R_1 consists of the PAAD functionality and R_2 consists of the AAA functionality. R_1 is either CH_2OCH_3, CH_3, $CH(CH_3)_2$, or $C(CH_3)_3$, and R_2 either is a safinamide-like pharmacophore or a safinamide-like pharmacophore with a reversed ether linkage (-CH_2O- as opposed to -OCH_2-). All chimeric PAADs were synthesized in the (*R*)-configuration. The excellent activities of (*R*)-**255** and (*R*)-**258** prompted the synthesis of the (*S*)-stereoisomers to determine if the anticonvulsant activity of chimeric PAADs resided in the (*R*)-stereoisomer. An attempt was made to synthesize a chimeric agent with a sulfonamide linker (*R*)-**262** (-$NHSO_2$- as opposed to -OCH_2-). Sulfonamides possess diverse therapeutic application, including broad-spectrum anticonvulsant activity as exemplified by the anticonvulsant topiramate (**18**).[214,215] However, purification issues were experienced late in the synthesis of (*R*)-**262** and we were unable to complete the synthesis of the sulfonamide analog.



PAAD
59

Safinamide
30

4'-Chimeric PAADs



(*R*)-**254** $R_1 = CH_2OCH_3$ $R_2 = OCH_2C_6H_4(mF)$
(*R*)-, (*S*)-**255** $R_1 = CH_2OCH_3$ $R_2 = CH_2OC_6H_4(mF)$

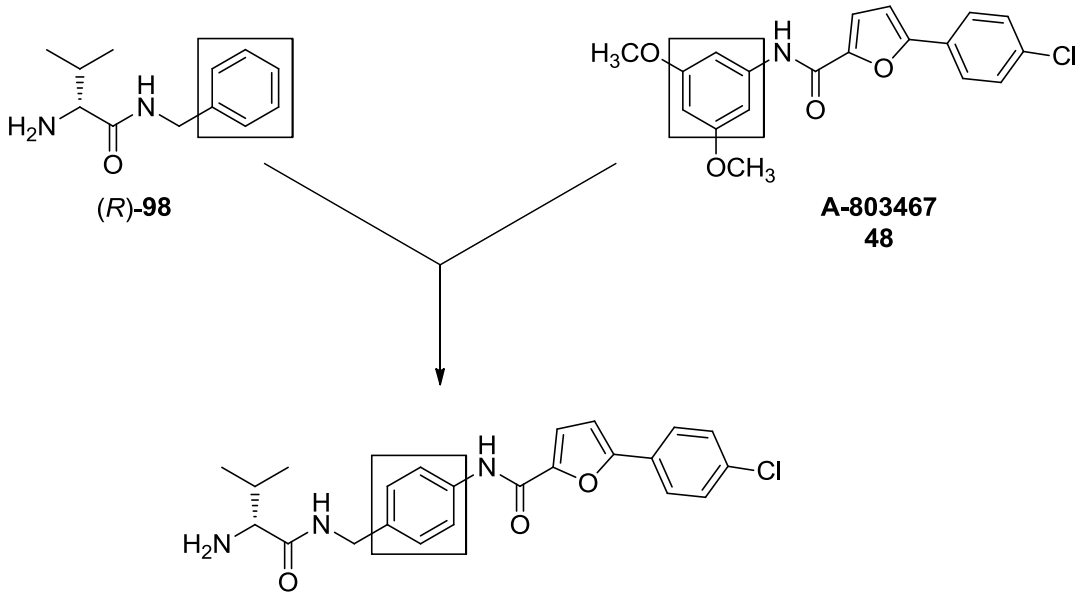
(*R*)-**256** $R_1 = CH_3$ $R_2 = OCH_2C_6H_4(mF)$

(*R*)-**257** $R_1 = CH(CH_3)_2$ $R_2 = OCH_2C_6H_4(mF)$
(*R*)-, (*S*)-**258** $R_1 = CH(CH_3)_2$ $R_2 = CH_2OC_6H_4(mF)$
(*R*)-**259** $R_1 = CH(CH_3)_2$ $R_2 = NHC(O)(C_4H_2O)C_6H_4(pCl)$

(*R*)-**260** $R_1 = C(CH_3)_3$ $R_2 = OCH_2C_6H_4(mF)$
(*R*)-**261** $R_1 = C(CH_3)_3$ $R_2 = CH_2OC_6H_4(mF)$
(*R*)-**262** $R_1 = C(CH_3)_3$ $R_2 = NHSO_2C_6H_4(mF)$

(*R*)-**259** is an exception in the chimeric series, where R_1 is $CH(CH_3)_2$ but R_2 is a 5-aryl-2-furfuramide. Interest in the 5-aryl-2-furfuramide substituent came after Abbott Laboratories reported the discovery and biological evaluation of a series of 5-aryl-2-furfuramides.[31,32] Within the series, A-803467 (**48**) was identified as a potent (IC_{50} = 8 nM) and selective peripheral sodium channel blocker ($hNa_v1.8$) in recombinant human cells (HEK-293).[31] We wanted to capitalize on the reported neuropathic pain attenuation of A-803467 (**48**) and proposed a 4'-chimeric PAAD that overlaid the amide ring of A-803467 (**48**)

with the benzyl ring of (*R*)-**98** (see structural unit in box in **48** and **98**). We chose to overlap the amide ring, instead of the 5-aryl ring, due to the commercial availability of the reagents needed to complete the synthesis of (*R*)-**259**.

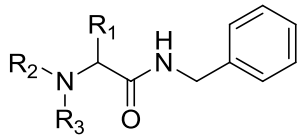


2.2. Choice of compounds: *N*-Substituted 3-methylbutanamides

Currently, our strategy has focused on *primary* amino acid derivatives (PAADs). We wanted to determine the effect of functionalization of the terminal amine on pharmacological activity. Literature support for this investigation was obtained from Paruszewski and coworkers, who demonstrated that *N*-methyl (*secondary* amino acid derivatives, SAADs) and *N*,*N*-dimethyl (*tertiary* amino acid derivatives, TAADs) *N*'-benzyl propionamides were potent anticonvulsants (ED_{50} <100 mg/kg).[216-218] Correspondingly, the Kohn laboratory previously altered the *N*-terminus of C(2)-methyl, C(2)-phenyl, and C(3)-*O*-methoxy *N*'-benzylamides (PAADs) by incorporating acyclic, cyclic alkylamine, and alkoxyamino units,

and found inconsistent anticonvulsant activity trends as the terminal amine went from unsubstituted, to monomethyl-substituted, to dimethyl-substituted (Table 20).[92]

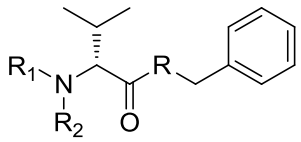
Table 20. Pharmacological activities of primary (PAAD), secondary (SAAD), and tertiary (TAAD) amino acid analogs in mice (mg/kg)



					Mice (ip)[a]		
Cmpd No.	**Class**	**R_1**	**R_2**	**R_3**	**MES[b], ED_{50}**	**Tox,[c] TD_{50}**	**PI,[d] MES**
(*R,S*)-**60**[e]	PAAD	CH_3	H	H	>100, <300	>300	
(*R,S*)-**263**[e,f]	SAAD	CH_3	CH_3	H	31 (21–41)	99 (75–121)	3.2
(*R,S*)-**264**[e,f]	TAAD	CH_3	CH_3	CH_3	75 [0.25] (61–90)	160 [0.25] (140–180)	2.1
(*R,S*)-**62**[e]	PAAD	C_6H_5	H	H	>100, <300	>100, <300	
(*R,S*)-**265**[e]	SAAD	C_6H_5	CH_3	H	46 [0.25] (34–59)	83 [0.25] (64–104)	1.8
(*R,S*)-**266**[e]	TAAD	C_6H_5	CH_3	CH_3	36 [0.25] (30–46)	72 [0.25] (57–86)	2.0
(*R,S*)-**61**[e]	PAAD	CH_2OCH_3	H	H	84 [0.25] (65–97)	290 [0.25] (240–320)	3.5
(*R,S*)-**267**[e]	SAAD	CH_2OCH_3	CH_3	H	68 [0.25] (55–96)	290 [0.25] (250–330)	4.3
(*R,S*)-**268**[e]	TAAD	CH_2OCH_3	CH_3	CH_3	>300	~300	

[a] The compounds were administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg. A dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] Tox = neurological toxicity. TD_{50} values determined from the rotorod test. [d] PI = protective index (TD_{50}/ED_{50}). [e] Bégiun, C. *et al. Bioorg. Med. Chem*. **2004**, *12*, 3079–3096. [f] Paruszewski, R. *et al. Pharmazie*, **1996**, *51*, 145–148.

Not knowing the effect of *N*-substitution on D-valine amino acid derivatives, we selected to synthesize the *secondary* amino acid derivative (SAAD) (*R*)-**269** and the *tertiary* amino acid derivative (TAAD) (*R*)-**270**. We chose to examine the functionalization of (*R*)-**98** due to the combination of (1) the excellent activity in the MES and formalin tests; (2) the availability and inexpensive cost of the D-amino acid starting material; and (3) the reported synthesis of the intermediate *N*,*N*-dimethyl-D-valine.[219,220]



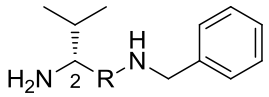
(*R*)-**98** R_1 = H, R_2 = H
(*R*)-**269** R_1 = CH_3, R_2 = H
(*R*)-**270** R_1 = CH_3, R_2 = CH_3

2.3. Choice of compounds: C(2)-isopropyl PAAD analogs

Several C(2)-hydrocarbon PAADs possess significant anticonvulsant activity and activity was improved upon functionalization of the 4'-*N*-benzyl position. Our studies indicated that many of the FAA hallmarks do not apply to PAADs. Therefore, it was necessary to interrogate the original proposed structural framework of PAADs. We investigated the importance of six properties that were common between the PAADs and the FAAs (**271**–**284**): (1) the carbonyl unit (Site A); (2) the amide bond (Site B); (3) the amide methylene linker length (Site C); (4) the *N*-benzylamide regiosubstitution (Site D); (5) the need for an arylamide (Site E); and (6) the C(2)-amino functionality (Site F). All C(2)-isopropyl PAAD analogs were synthesized in the (*R*)-configuration, except **283**, which was synthesized in the (*S*)- and (*R,S*)-configurations, and **284** was synthesized in the (*R*)-configuration.

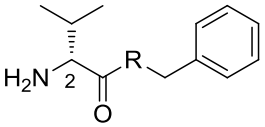
C(2)-Isopropyl PAAD Analogs

Site A



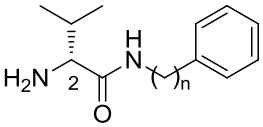
(*R*)-**98** R = C=O
(*R*)-**271** R = C=S
(*R*)-**272** R = CH_2

Site B



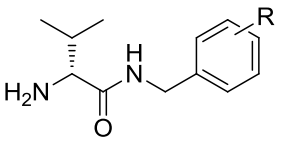
(*R*)-**98** R = NH
(*R*)-**273** R = CH_2
(*R*)-**274** R = O

Site C



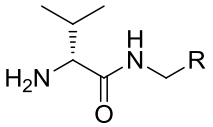
(*R*)-**275** n = 0
(*R*)-**98** n = 1
(*R*)-**276** n = 2
(*R*)-**277** n = 3
(*R*)-**278** n = 4

***N'*-Benzyl Substituted Regioisomers Site D**



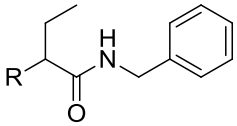
(*R*)-**279** R = 2'-OCF_3
(*R*)-**280** R = 3'-OCF_3
(*R*)-**248** R = 4'-OCF_3

***N'*-Substituted Isopropyl PAADs Site E**



(*R*)-**98** R = C_6H_5
(*R*)-**281** R = C_6H_{11}

α-Substituted *N*-Benzylbutanamides Site F



(*R*)-**95** R = NH_2
282 R = H
(*S*)-, (*R,S*)-**283** R = CH_3
(*R*)-**284** R = OH

2.3.1. C(2)-Isopropyl PAAD analogs (Sites A–C)

The importance of the carbonyl unit to FAAs has been demonstrated by an isoelectronic substitution of the amide carbonyl with a thiocarbonyl group,[56] and we conducted a similar investigation using (*R*)-**271**. We further examined the value of the carbonyl unit by substitution with a methylene group ((*R*)-**272**). The Kohn laboratory has previously reported that functionalized amido ketones (FAK) exhibited significant anticonvulsant activities that were comparable to FAAs however, an increase in neurological toxicity was also observed.[91] In a similar manner, we replaced of the nitrogen of the amide bond of (*R*)-**98** with a methylene group ((*R*)-**273**) or oxygen ((*R*)-**274**) to create either a primary amino ketone (PAK) or primary amino ester (PAE), respectively. Several

perturbations are analogous to the work completed in the FAA series, but the distance between the amide bond and the aromatic ring has not been extensively investigated. Therefore, we examined the anticonvulsant activities of PAADs containing 0–4 methylene units between the amide bond and the aromatic ring ((*R*)-**275–**(*R*)-**278**).

2.3.2. *N*-Benzyl substituted regioisomeric PAADs (Site D)

Recently, Salomé and coworkers systematically evaluated both a fluoro and trifluoromethoxy group at the 2'-, 3'-, and 4'-postions of the *N*-benzyl moiety of FAAs and determined that the 4'-modified derivatives displayed the highest degree of anticonvulsant activity,[102] which is in agreement with previous studies conducted by the Kohn laboratory.[60,98] Accordingly, we evaluated the anticonvulsant activities at the 2'-, 3'- and 4'-positions of (*R*)-**98** using a trifluoromethoxy group ((*R*)-**279**, (*R*)-**280**, and (*R*)-**248**).

2.3.3. *N*-Substituted C(2)-isopropyl PAADs (Site E)

The benzylamide unit is vital for the excellent anticonvulsant activity of FAAs. The Kohn laboratory evaluated several *N*-substituted FAAs, including *N*-methylamide, *N*-benzhydrylamide, and two derivatives where an electron-donating (methoxy) or electron-withdrawing (fluoro) group was placed at the meta position of the aromatic ring.[52] We altered our approach compared with previous studies and examined the importance of aromaticity for pharmacological activity. Accordingly, we substituted the aromatic ring in (*R*)-**98** for a cyclohexyl ring ((*R*)-**281**) to examine the influence of the planar ring system on anticonvulsant activity

2.3.4. α-Substituted *N*-benzylbutanamides (Site F)

The final analysis examined the choice of the amino group. The limited availability of 2-substituted 3-methylbutanoic acid reagents prompted the investigation of *N*-

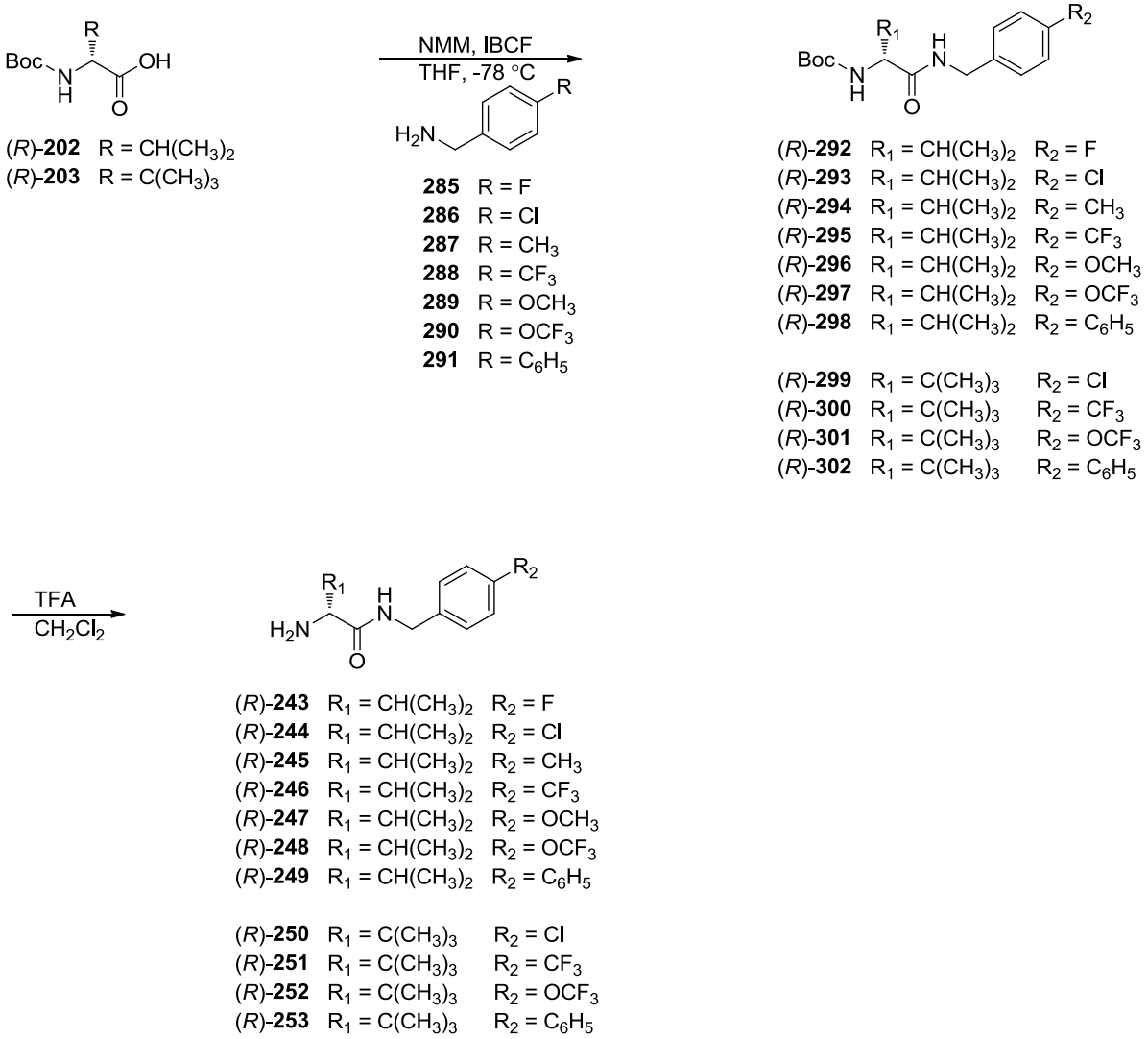
benzylbutanamide derivatives instead of *N*-benzyl 3-methylbutanamide derivatives. Previously, the 2-acetamido of (*R,S*)-*N*-benzyl 2-acetamido-2-phenylacetamide ((*R,S*)-**224**, ED_{50} = 20 mg/kg) was systematically replaced by a hydrogen, methyl, hydroxy, or halogen group, and the hydroxy and methoxy groups provided moderate MES activities (>30, <100 mg/kg).[93] In a similar study, the 2-acetamido group of (*R,S*)-*N*-benzyl 2-acetamido-3-methoxypropionamide ((*R,S*)-**28**, ED_{50} = 8.3 mg/kg) was replaced with a methyl, hydroxy, and amino group and the methyl and amino groups also resulted in moderate MES activities (>30, <100 mg/kg).[90] Both studies concluded that the 2-acetamido group resulted in superior anticonvulsant activity. We conducted a similar study that systematically replaced the amino group with a hydrogen (**282**), methyl ((*R,S*)-**283**), or hydroxy group ((*R*)-**284**). The moderate activity of (*R,S*)-**283** prompted our efforts to prepare (*R*)-**283** and (*S*)-**283** to determine if anticonvulsant activity resided in the (*R*)-stereoisomer. We successfully synthesized (*S*)-**283** but were unable to complete the synthesis of (*R*)-**283**.

2.4. Synthesis

2.4.1. 4'-*N*-Benzyl substituted C(2)-hydrocarbon PAADs

4'-*N*-Benzyl substituted C(2)-hydrocarbon PAADs (*R*)-**243**–**253** were synthesized from either (*R*)-**202** or (*R*)-**203** following standard MAC procedures and using commercially available 4'-modified benzylamines (**285**–**291**) (Scheme 21).

Scheme 21. Synthesis of 4'-*N*-benzyl substituted hydrocarbon PAADs (*R*)-243–253

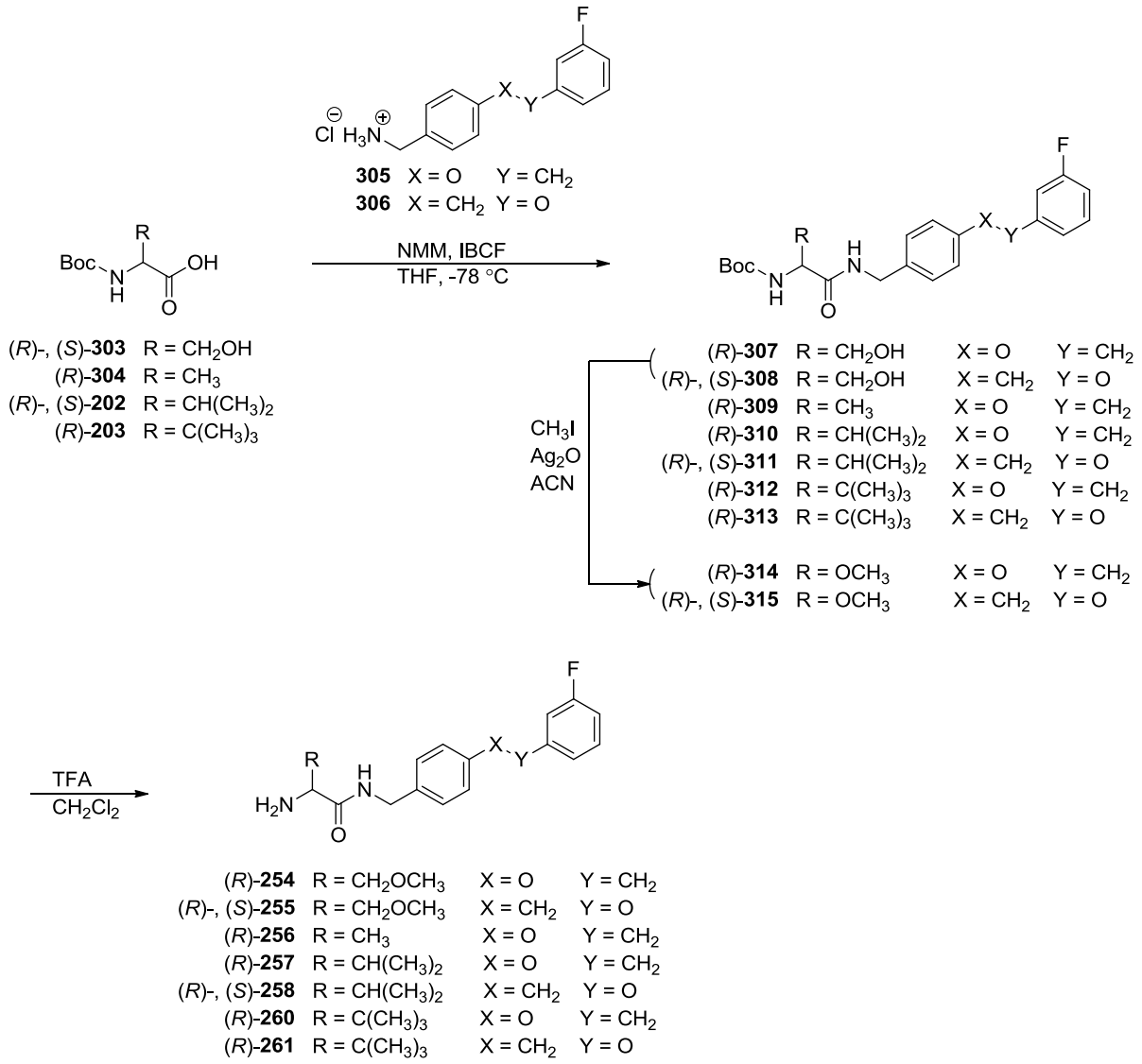


2.4.2. 4'-Chimeric C(2)-hydrocarbon PAADs

PAADs (*R*)-**256**, (*R*)-**257**, (*R*)-**258**, (*S*)-**258**, (*R*)-**260**, and (*R*)-**261** were synthesized as described in Scheme 22 from either (*R*)-**304**, (R)-**202**, (*S*)-**202**, or (*R*)-**203** following standard MAC procedures and using 4'-modified benzylamines **305** or **306** (Scheme 23). PAADs (*R*)-**254**, (*R*)-**255**, and (*S*)-**255** were synthesized in the same manner beginning with (*R*)- and (*S*)-**303**, except alkylation of (*R*)-**307**, (*R*)-**308**, and (*S*)-**308** using methyl iodide

(**106**) and Ag_2O gave (*R*)-**314**, (*R*)-**315**, and (*S*)-**315**, respectively, before acidic deprotection to the corresponding PAADs.

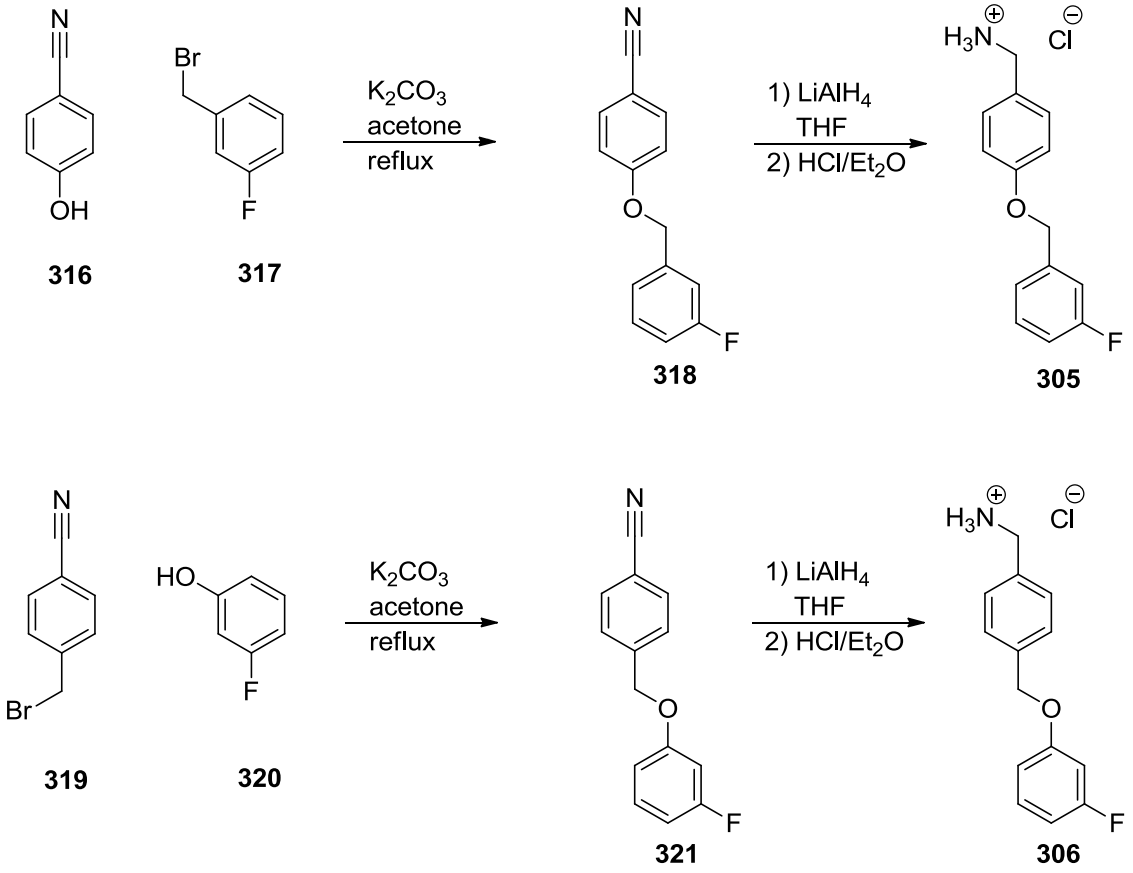
Scheme 22. Synthesis of 4'-chimeric PAADs 254–258, 260, and 261



Amines **305** and **306** were prepared by the Williamson reaction of phenols **316** and **320** with organohalides **317** and **319**, respectively, followed by $LiAlH_4$ reduction of the nitriles

318 and **321** to their corresponding amines, which were immediately converted to the hydrochlorides using HCl in Et_2O (Scheme 23).[105]

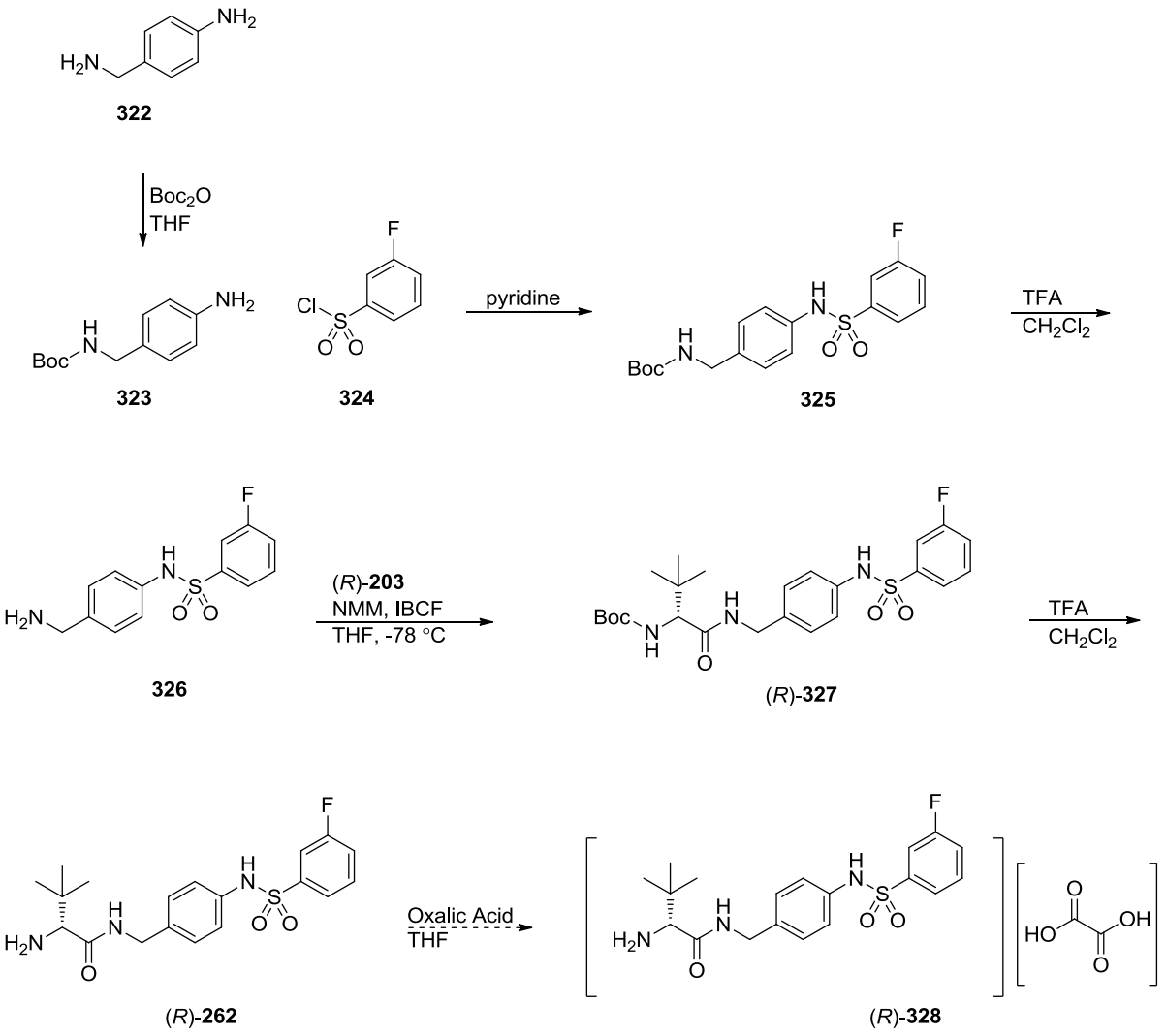
Scheme 23. Synthesis of 4'-modified benzylamines 305 and 306



We attempted to synthesize sulfonamide (*R*)-**262** (Scheme 24). 4-Aminobenzylamine (**322**) was *N*-protected with Boc_2O to give **323**, then converted to the sulfonamide **325**, and deprotected in acidic media to obtain the corresponding amine **326**.[221] Amine **326** was coupled with (*R*)-**203** following the standard MAC procedure to give sulfonamide (*R*)-**327**, which was then deprotected in acidic media to give the corresponding PAAD (*R*)-**262**.

Purification difficulties prompted the conversion to the oxalate salt (*R*)-**328** but we were unable to obtain a sufficient amount that met the purity standards for biological testing.

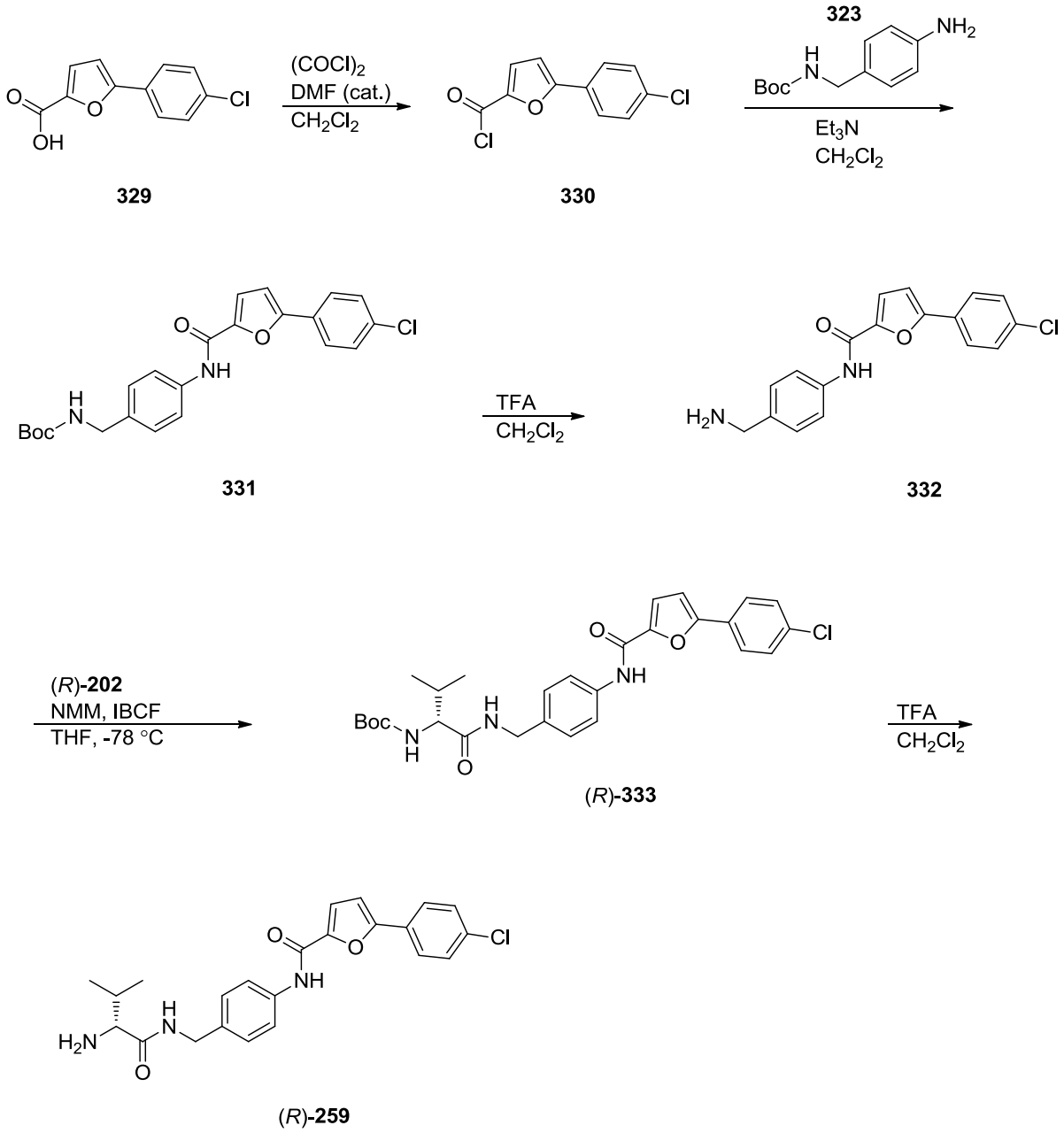
Scheme 24. Attempted synthesis of (*R*)-*N*-(4-((-3-fluorophenyl)sulfonamido)phenyl)benzyl 2-amino-3,3-dimethylbutanamide ((*R*)-262)



Chimeric (*R*)-**259** was prepared by a convergent synthesis, requiring (*R*)-**202** and amine **332** (Scheme 25). Amine **332** was prepared by reacting **323** with acyl chloride **330**, prepared from commercially available carboxylic acid **329** and oxalyl chloride in DMF, to

give **331** that was then deprotected (TFA).[32] MAC coupling of (*R*)-**202** with **332** provided (*R*)-**333** that was deprotected (TFA) in the final step to give the PAAD (*R*)-**259**.

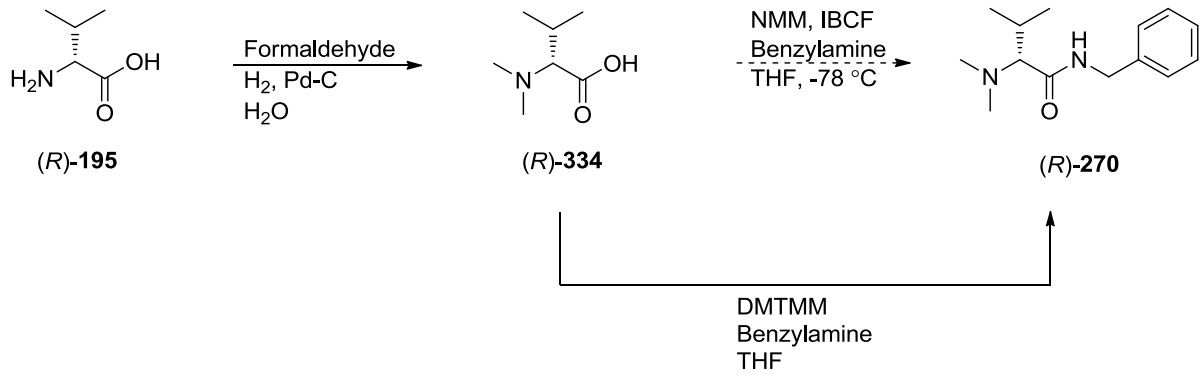
Scheme 25. Synthesis of (*R*)-4-((5-(4-chloro)phenyl)furan-2-carboxamido)benzyl 2-amino-3-methylbutanamide ((*R*)-259)



2.4.3. *N*-Benzyl *N',N'*-dimethylamino-3-methylbutanamide

Reductive condensation of (*R*)-**195** with formaldehyde using 10% Pd-C in the presence of H_2 gave (*R*)-**334**.[219,220,222] We attempted to couple (*R*)-**334** with benzylamine using the MAC protocol but the limited solubility of (*R*)-**334** in THF resulted in low yields (<5%) (Scheme 26). Accordingly, we used the condensing reagent DMTMM. Benzylamine was first added to a THF suspension of (*R*)-**334** and the resulting ammonium carboxylate dissolved after several minutes. Then, the reaction solution was treated with DMTMM to give (*R*)-**270** in 10% yield.

Scheme 26. Synthesis of (*R*)-*N*-benzyl *N,N*-dimethylamino-3-methylbutanamide ((*R*)-270)



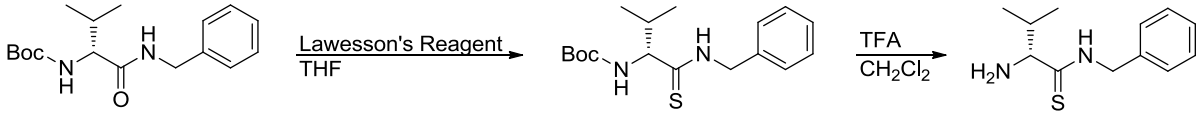
(*R*)-**195** (*R*)-**334** (*R*)-**270**

2.4.4. C(2)-Isopropyl PAAD analogs

Thioamide (*R*)-**355** was prepared directly from (*R*)-**209** upon the treatment with excess Lawesson's reagent (2.2 equiv) at reflux,[56] and then deprotected in acidic media to give PAAD (*R*)-**271** (Scheme 27). We were mindful of the potential for thiation of the carbamate carbonyl but the ^{13}C NMR spectrum contained a signal at ~155 ppm, the typical shift for carbamate carbon. If thiation of the carbamate carbonyl had occurred, we would have expected a significant downfield shift in the signal (~190 ppm).[223] In agreement with

the proposed structure of (*R*)-**271**, we observed the thioamide carbon signal in the ^{13}C NMR at 205 ppm.

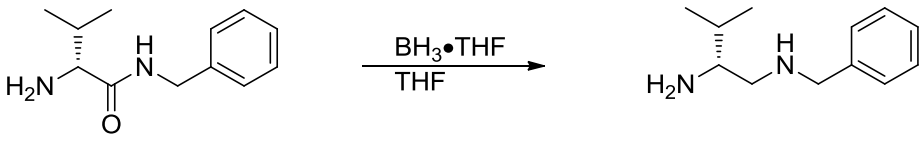
Scheme 27. Synthesis of (*R*)-*N*-benzyl 2-amino-3-methylthiobutanamide ((*R*)-271)



(*R*)-**209** (*R*)-**335** (*R*)-**271**

Diamine (*R*)-**272** was synthesized by the direct reduction of (*R*)-**98** with borane in THF (Scheme 28).[196] Previously, Ramalingam and coworkers purified (*R*)-**272** by Kugelrohr distillation (145 ºC/0.06 mbar),[196] but we used an acid/base extraction method followed by flash column chromatography. Using these conditions, we found that the purification of (*R*)-**272** was complicated by the ring opening of THF, to give trace amounts of 1,4-butanediol. Extraction of 2,3-butanediol from fermentation broths using an ethanol/phosphate system was reported by Jiang and coworkers[224] and we optimized their extraction system to fit our needs. We successfully removed 1,4-butanediol by washing a dichloromethane solution of (*R*)-**272** with a 1:1 mixture of EtOH/H_2O.

Scheme 28. Synthesis of (*R*)-1-*N*-benzylamino-2-amino-3-methylbutane ((*R*)-272)

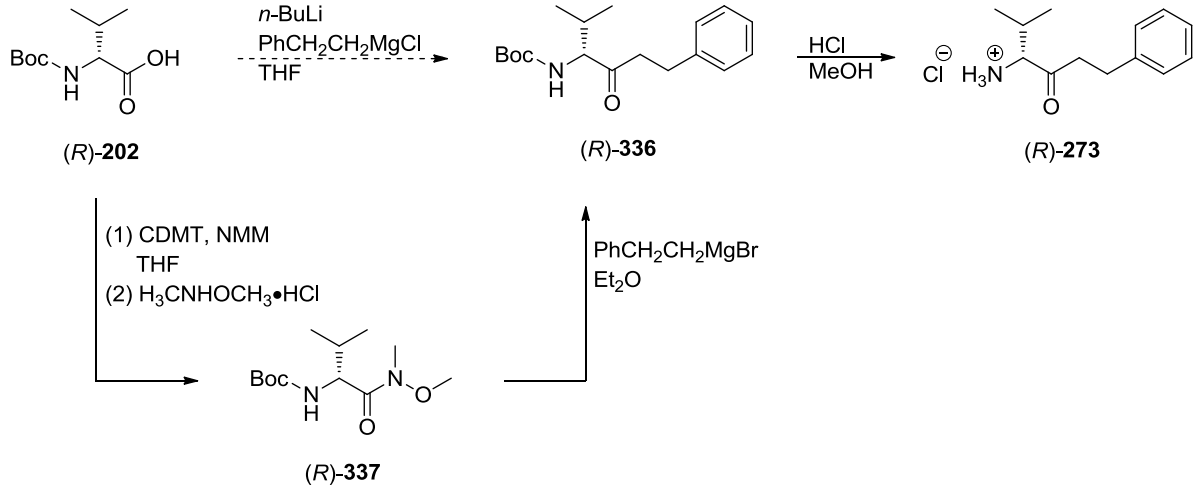


(*R*)-**98** (*R*)-**272**

We attempted to synthesize PAAD (*R*)-**273** via the intermediate (*R*)-**336** (Scheme 29). Previously, the Kohn laboratory introduced a ketone unit starting from either a *N*-acetyl

or *N*-Cbz amino acid.[91] Accordingly, we treated *N*-*t*Boc amino acid (*R*)-**202** with *n*-butyl lithium, followed by phenethylmagnesium chloride, in an effort to obtain (*R*)-**336**. However, the ^{1}H NMR spectrum for the crude reaction product showed a complex mixture. Therefore, we prepared Weinreb amide (*R*)-**337** by coupling (*R*)-**202** with *N*,*O*-dimethylhydroxylamine in the presence of CMDT and base.[225] Weinreb amides (*N*-methoxy-*N*-methylamides) readily react with Grignard reagents and are widely known as useful precursors to ketones.[225-227] Next, (*R*)-**337** was directly reacted with phenethylmagnesium bromide to give (*R*)-**336**. This method provided a relatively clean reaction mixture permitting isolation of (*R*)-**336**. Deprotection of this intermediate gave the corresponding PAAD (*R*)-**273**.

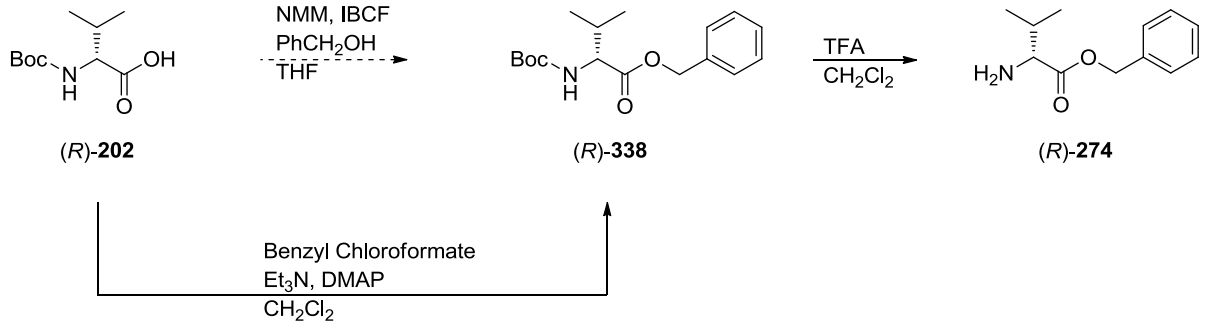
Scheme 29. Synthesis of (*R*)-4-amino-2-methyl-6-phenyl-4-hexanone hydrochloride ((*R*)-273)



We attempted to synthesize (*R*)-**338** from (*R*)-**202** and benzyl alcohol, following the standard MAC coupling procedure, in an effort to reach PAAD (*R*)-**274** (Scheme 30). However, we recovered starting material from the MAC reaction. Next, we used the mild esterification method reported by Kim and coworkers.[228] The desired ester (*R*)-**338** was

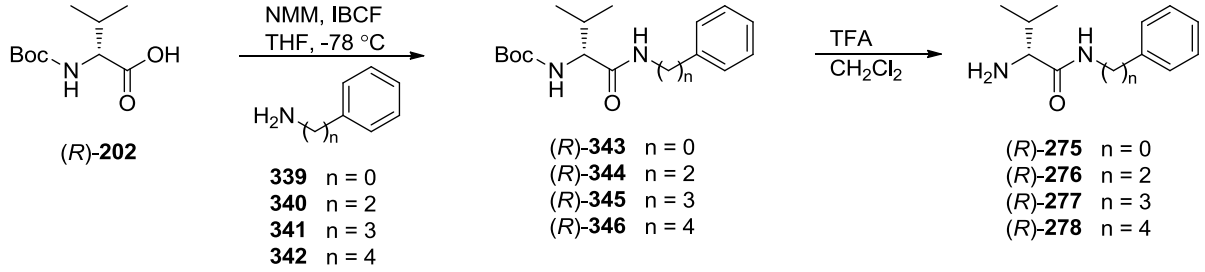
achieved by the addition of benzylchloroformate in the presence of base and catalytic amounts of DMAP. Subsequent deprotection of (*R*)-**338** in acidic media gave PAAD (*R*)-**274**.

Scheme 30. Synthesis of (*R*)-*O*-benzyl 2-amino-3-methylbutanoate ((*R*)-274)



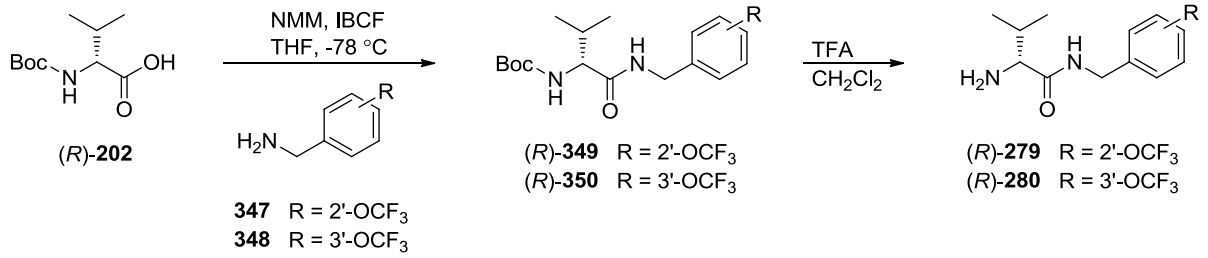
PAADs (*R*)-**275**–**278** were synthesized from (*R*)-**202** following standard MAC procedures and using commercially available amines (**339**–**342**) (Scheme 31).

Scheme 31. Synthesis of C(2)-isopropyl analog: the *N*-amide Site A ((*R*)-275–278)



Similarly, we prepared PAADs (*R*)-**279** and (*R*)-**280** from (*R*)-**202** and the commercially available amines **347** and **348** using the MAC procedure (Scheme 32).

Scheme 32. Synthesis of C(2)-isopropyl analogs: *N*-benzyl substituted regioisomers ((*R*)-279 and (*R*)-280)



(*R*)-**202**

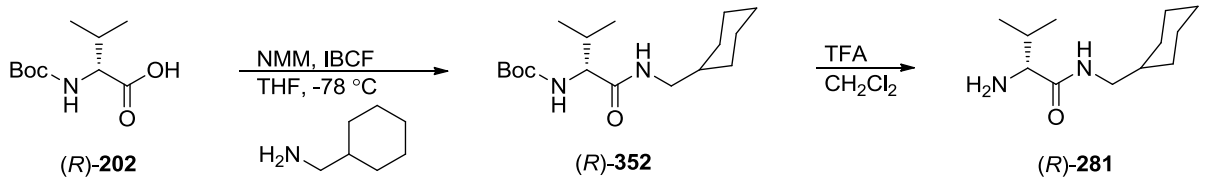
347 R = 2'-OCF_3
348 R = 3'-OCF_3

(*R*)-**349** R = 2'-OCF_3
(*R*)-**350** R = 3'-OCF_3

(*R*)-**279** R = 2'-OCF_3
(*R*)-**280** R = 3'-OCF_3

We found that the MAC protocol worked well for cyclohexylmethylamine (**351**). Thus, treatment of (*R*)-**202** with **351** yielded (*R*)-**352**. Removal of the *t*Boc protecting group in the final step gave (*R*)-**281** (Scheme 33).

Scheme 33. Synthesis of C(2)-isopropyl analogs: the *N*-amide Site B ((*R*)-281)



(*R*)-**202**

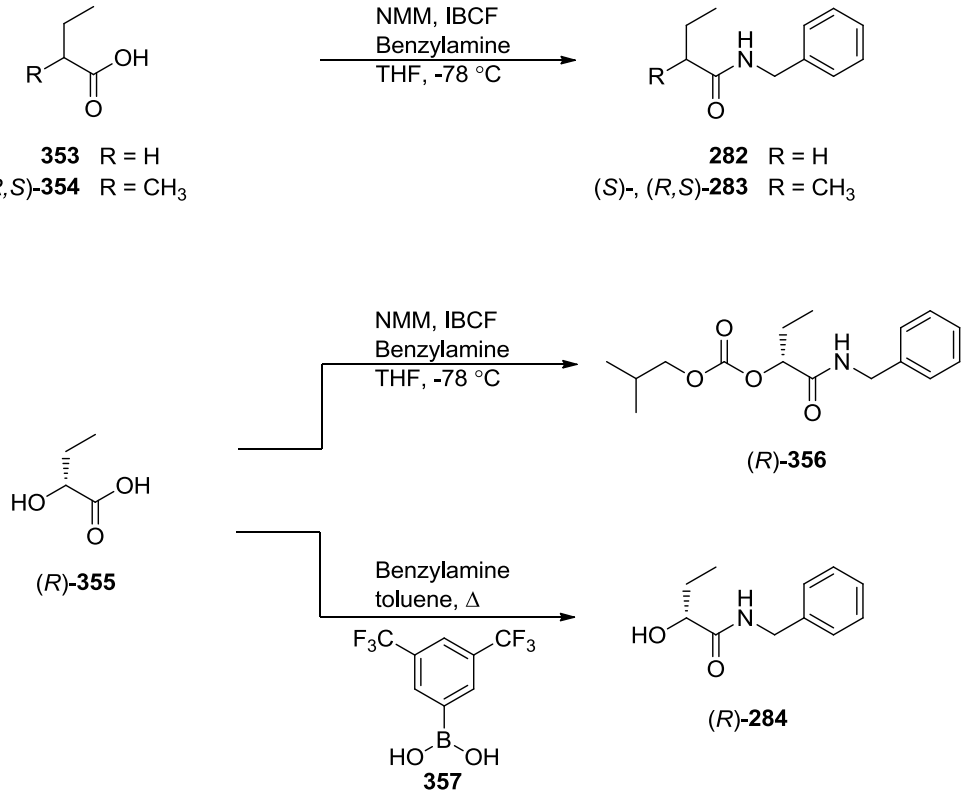
351

(*R*)-**352**

(*R*)-**281**

PAADs **282,** (*S*)-**283**, (*R,S*)-**283**, and (*R*)-**284** were synthesized from commercially available carboxylic acids **353**, (*S*)-**354**, (*R,S*)-**354**, and (*R*)-**355**, respectively (Scheme 34). An attempt was made to synthesize PAAD (*R*)-**284** from commercially available (*R*)-**355** using the MAC coupling procedure but we obtained carbonate (*R*)-**356** as the major product.

Scheme 34. Synthesis of C(2)-isopropyl analog: α-substituted *N*-benzylbutanamides (282, (*S*)-283, (*R,S*)-283, and (*R*)-284)

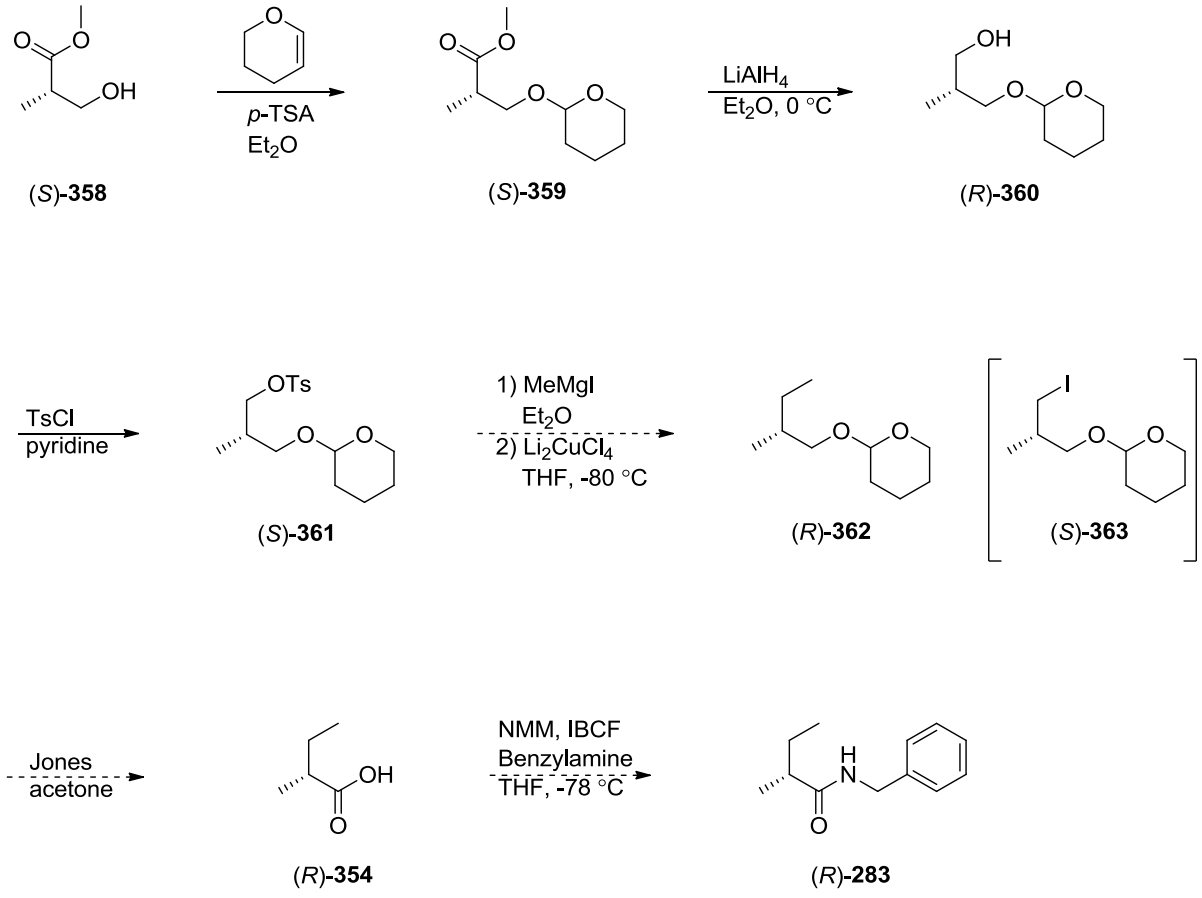


Therefore, we modified a procedure reported by Ishihara and coworkers that utilized an arylboronic acid bearing electron-withdrawing groups as an efficient catalyst for the amidation of (*R*)-**355**.[229] Accordingly, (*R*)-**355** was treated in the presence of benzylamine and commercially available 3,5-bis(trifluoromethyl)benzeneboronic acid (**357**) to give PAAD (*R*)-**284** (Scheme 34).

We wanted to prepare (*R*)-**283** to compare its pharmacological activity with (*S*)-**283** and (*R,S*)-**283**, but the lack of commercially available (*R*)-**354** required our use of a more extensive synthetic pathway (Scheme 35). Adapted from the synthesis of Santangelo and coworkers,[230] we protected the hydroxyl group of commercially available (*S*)-3-hydroxy-2-methylpropionate ((*S*)-**358**) using dihydropyran to give (*S*)-**359**, reduced the methylester to the corresponding alcohol ((*R*)-**360**) with $LiAlH_4$ and then protected the alcohol ((*R*)-**360**)

with tosyl chloride to give (*S*)-**361**. Next, we made several attempts to displace the tosylate group in (*S*)-**361** with MeMgI and Li_2CuCl_4 to give (*R*)-**362**, where we varied reagent concentrations and reaction temperatures. Copper reagents are widely used in organic synthesis for the formation of carbon-carbon bonds and the development of higher order cuprates has allowed the coupling of alkyl halides and alkyl sulfonates with several types of organometallic reagents.[231] We were mindful of possible by-products from competitive nucleophilic substitution and elimination reactions but recovered starting material after each attempt, with one exception. We isolated the iodo intermediate (*R*)-**363** upon lowering the reaction temperature to 0 °C but were unable to duplicate this result.

Scheme 35. Attempted synthesis of (*R*)-*N*-benzyl 2-methylbutanamide ((*R*)-283)



(*S*)-**358** (*S*)-**359** (*R*)-**360**

(*S*)-**361** (*R*)-**362** (*S*)-**363**

(*R*)-**354** (*R*)-**283**

2.5. Pharmacological evaluation

PAADs **240**–**284** were evaluated for anticonvulsant activity using the MES test at either UCB Pharma, following the procedures described by Klitgaard,[178] or at the NINDS ASP, following the procedures described by Stables and Kupferberg,[42] or both. Anticonvulsant activity using the 6 Hz test was performed either at UCB Pharma, following the procedures described by Kaminski and coworkers (44 mA),[179] or at the NINDS ASP, following the procedures described by Stables and Kupferberg (32 mA),[42] or both. PAADs evaluated at UCB Pharma were also tested for NP protection (formalin test).[180] All compounds were administered intraperitoneally (ip) to mice at UCB Pharma or ip to mice and orally (po) to rats at the NINDS ASP. The pharmacological data from the MES, 6 Hz, and formalin tests are summarized in Tables 21, 23, 24, 26, 27, and 29–35. The MES activities of PAADs are compared with the MES activities of their corresponding FAAs in Tables 22 and 28. (*R*)-**255** and (*R*)-**258** were evaluated at UCB Pharma and the NINDS ASP and these compounds were added to the list of four compounds (Chapter 2) that compared the MES activities of PAADs at both testing facilities (Table 36). The tables list the results obtained from either qualitative (dose range) or quantitative (ED_{50}) testing in mice (ip) and rats (po). We also included qualitative (dose range) or median neurological impairing dose (TD_{50}) values in mice (ip) using the rotorod test in mice and the behavioral toxicity effects observed in rats (po). The protective indices ($PI = TD_{50}/ED_{50}$) were provided, when applicable. PAADs tested at the NINDS ASP were evaluated in the subcutaneous Metrazol® (scMET) seizure model but protection was not observed at the doses (30, 100, 300 mg/kg) and times (0.5 and 4 h) tested (data not shown), with three exceptions. (*R*)-**248** displayed protection in the scMET seizure model for four out of eight mice treated with 62 mg/kg of compound and the remaining four mice displayed continuous seizure activity. Similarly, (*R*)-**251** protected one out of eight mice treated with 70 mg/kg and the remaining seven mice displayed continuous seizure activity, while two out of eight mice treated with 135 mg/kg of

(*R*)-**251** were protected and the remaining six mice displayed continuous seizure activity. Lastly, (*R,S*)-**283** was quantitatively determined to prevent seizures with an effective dose of 140 mg/kg (0.25 h) (95% confidence interval: 120–160 mg/kg).

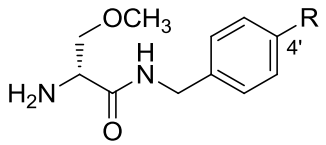
2.5.1. 4'-*N*-Benzyl substituted PAADs

2.5.1.1. 4'-*N*-Benzyl substituted C(3)-*O*-methoxy PAADs

The neurological activities for 4'-*N*-benzyl substituted C(3)-*O*-methoxy PAADs (*R*)-**126**, -**240**–**242** in mice are listed in Table 21. We systematically evaluated the effect of a bromo, trifluoromethyl, trifluoromethoxy, and phenyl group placed at the 4'-*N*-benzyl position on anticonvulsant and pain activity. The MES activities of all 4'-*N*-benzyl substituted C(3)-*O*-methoxy PAADs increased compared to the unsubstituted PAAD (*R*)-**61** (ED_{50} = 34 mg/kg). The 4'-phenyl derivative (*R*)-**242** (ED_{50} = 15 mg/kg) and the 4'-trifluoromethyl derivative (*R*)-**241** (ED_{50} = 19 mg/kg) displayed the highest MES activities, a ~2-fold improvement from (*R*)-**61**. However, the 4'-bromo derivative (*R*)-**240** (ED_{50} = 31 mg/kg) showed only marginal improvement in anticonvulsant activity. While PAADs (*R*)-**126**, -**240**–**242** displayed significant activity in the MES test, they are 2–6-fold less sensitive to the 6 Hz test. This result is not surprising because a similar decrease in sensitivity (~3-fold) was observed for (*R*)-**28** (MES ED_{50} = 3.3 mg/kg, 6 Hz ED_{50} = 10 mg/kg) and a >2-fold decrease in sensitivity was observed for (*R*)-**61** (MES ED_{50} = 34 mg/kg, 6 Hz ED_{50} >67 mg/kg). All 4'-*N*-benzyl substituted C(3)-*O*-methoxy PAADs displayed a lack of activity in the formalin test at the tested doses. Comparison of the MES activities of (*R*)-**126**, -**240**–**242** with their corresponding FAAs (Table 22) revealed a consistent drop in activity (2–10-fold) as we go from FAA to PAAD. This drop in activity is similar to the drop in activity observed for the unsubstituted C(3)-oxy PAADs (Table 6). Therefore, we conclude that substitution at the 4'-*N*-benzyl position of C(3)-*O*-methoxy PAADs moderately improves anticonvulsant activity in

the MES test, but the substitutions do not provide any advantage over the corresponding FAAs.

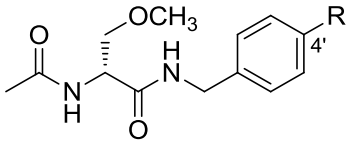
Table 21. Pharmacological activities of 4'-*N*-benzyl-substituted C(3)-*O*-methoxy PAADs in mice (mg/kg) at UCB



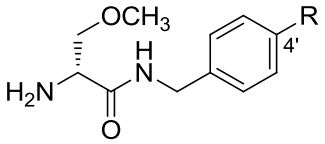
		Mice (ip)[a]						
Cmpd No.	**R**	**MES[b], ED_{50}**	**6 Hz,[c] ED_{50}**	**Formalin, ED_{50}**	**Tox[d], TD_{50}**	**Comments[e]**	**PI[f], MES**	**PI[f], Form**
(*R*)-**28**	LCM	3.3	10	15	19	Ref	5.8	1.3
(*R*)-**61**	H	34	>67	>67	>120		>3.5	
(*R*)-**240**	Br	31	>180	70	115		3.7	1.6
(*R*)-**241**	CF_3	19	55	>31	48	75 (LR)	2.5	
(*R*)-**126**	OCF_3	>10, <30	ND[g]	ND[g]	>30, <100			
(*R*)-**242**	C_6H_5	15	59 (MAD)[h]	>33	ND[g]	100 (C)		
phenytoin[i]		9.5 [2.0] (8.1–10)			66 [0.5] (53–72)	Ref		
phenobarbital[i]		22 [1.0] (15–23)			69 [0.5] (63–73)	Ref		
valproate[i]		270 [0.25] (250–340)			430 [0.25] (370–450)	Ref		

[a] The compounds were administered intraperitoneally to adult male NMRI mice under the auspices of UCB. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration. [b] MES = maximal electroshock seizure test. [c] 6 Hz test = psychomotor seizure model (44 mA). [d] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [e] Dose in mg/kg is followed by whole animal pharmacological observation (Ref = reference, LR = loss of righting reflex, C = convulsions). [f] PI = protective index (TD_{50}/ED_{50}). [g] ND = not determined. [h] MAD = minimal active dose.

Table 22. Comparison of the pharmacological activities of 4'-*N*-benzyl-substituted C(3)-*O*-methoxy FAAs and their PAAD counterparts in mice (mg/kg)



FAA



PAAD

			Mice (ip)[a]				Mice (ip)[a]	
R	**FAA Cpd No.**	**FAA Test Site**	**FAA MES,[b] ED_{50}**	**FAA Tox,[c] TD_{50}**	**PAAD Cpd No.**	**PAAD Test Site**	**PAAD MES,[b] ED_{50}**	**PAAD Tox,[c] TD_{50}**
H	(*R*)-**28**[d]	NINDS	4.5 [0.5] (3.7–5.5)	27 [0.25] (26–28)	(*R*)-**61**	UCB	34	>120
Br	(*R*)-**364**[e]	NINDS	8.7 [0.25] (7.2–10)	30 [0.25] (24–36)	(*R*)-**240**	UCB	31	115
CF_3	(*R*)-**365**[e]	NINDS	>10, <30 [0.5]	>100, <300 [0.5]	(*R*)-**241**	UCB	19	48
OCF_3	(*R*)-**123**[e]	NINDS	3.6 [0.25] (3.0–4.3)	13 [0.25] (9.2–19)	(*R*)-**126**	UCB	>10, <30	>30, <100
C_6H_5	(*R*)-**366**[e]	NINDS	8.0 [0.5] (5.3–12)	11 [0.5]	(*R*)-**242**	UCB	15	ND[f]

[a] The compounds were either administered intraperitoneally to adult male NMRI mice under the auspices of UCB or administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration (UCB) or a dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets) (NINDS ASP). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [d] Choi, D. *et al. J. Med. Chem.* **1996**, *39*, 1907–1916. [e] Salomé, C. *et al. J. Med. Chem.* **2010**, *53*, 1288–1305. [f] ND = not determined.

2.5.1.2. 4'-*N*-Benzyl substituted C(2)-hydrocarbon PAADs

The data from Chapter 2 suggested that a C(2)-heteroatom one atom removed from the C(2) center is not necessary for optimal PAAD anticonvulsant activity or NP protection. Hydrocarbon PAADs (*R*)-**98** and (*R*)-**99** displayed excellent activity in both the MES and formalin tests, and their MES activity surpassed the MES activity of the traditional antiepileptic phenobarbital (22 mg/kg) and approached the MES activity of the antiepileptic phenytoin (9.5 mg/kg). Therefore, we chose to expand the SAR of (*R*)-**98** and (*R*)-**99** to include functionalization of both the *N*-benzylamide moiety and the *N*-terminal amine in an attempt to optimize anticonvulsant activity. Unfortunately, we were unable to evaluate the optimized PAADs for NP modulation due to the shift of testing location from UCB Pharma to the NINDS ASP.

We gauged the importance of electronic effects, hydrophobic interactions, and size of the 4'-substitution in C(2)-hydrocarbon PAADs (*R*)-**243**–**253** on anticonvulsant activity in mice (Table 23). The MES activity for the 4'-chloro-substituted C(2)-isopropyl ((*R*)-**244**) and C(2)-*tert*-butyl ((*R*)-**250**) PAADs were similar (ED_{50} (mg/kg): (*R*)-**244**, 22; (*R*)-**250**, 25) but were slightly lower than their respective parent compounds (*R*)-**98** and (*R*)-**99** (ED_{50} (mg/kg): (*R*)-**98**, 15; (*R*)-**99**, 14 mg/kg). The 4'-flouro derivative (*R*)-**243** was evaluated in the isopropyl series and displayed similar activity to the 4'-chloro derivative (*R*)-**244** (ED_{50} (mg/kg): (*R*)-**243**, >10, <30; (*R*)-**244**, 22). Therefore, the electron-withdrawing effects of halogens (F and Cl) slightly decreased anticonvulsant activity compared with the unsubstituted parent compounds. A similar trend was observed in rats, where the 4'-fluoro-substituted isopropyl PAAD (*R*)-**243** resulted in a 2-fold decrease in seizure protection from the parent PAAD (*R*)-**98** (ED_{50} (mg/kg): (*R*)-**98**, 11; (*R*)-**243**, 21) (Table 24). Next, we compared the 4'-methyl-substitued C(2)-isopropyl PAAD (*R*)-**245** and the 4'-trifluoromethyl analog (*R*)-**246**. The 4'-methyl moiety resulted in the complete loss of anticonvulsant activity in the MES test (>300 mg/kg), while the 4'-trifluoromethyl moiety displayed excellent activity

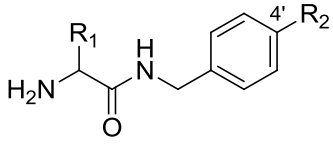
(ED_{50} = 14 mg/kg). These results were paralleled when we evaluated the 4'-methoxy ((*R*)-**247**) and 4'-trifluoromethoxy ((*R*)-**248**) derivatives in the C(2)-isopropyl series (ED_{50} (mg/kg): (*R*)-**247**, >300; (*R*)-**248**, 16). The 4'-trifluoromethyl ((*R*)-**251**) and 4'-trifluoromethoxy ((*R*)-**252**) compounds also displayed significant anticonvulsant activities in the C(2)-*tert*-butyl series (ED_{50} (mg/kg): (*R*)-**251**, 24; (*R*)-**252**, 28), although to a lesser degree than found in the isopropyl series, and were less active than the parent compound (*R*)-**99** (ED_{50} = 14 mg/kg). The 4'-methyl and 4'-methoxy analogs were not evaluated in the *tert*-butyl series due to the inactivity in the isopropyl series. (*R*)-**246**, -**248**, -**251**, and -**252** also showed substantial MES activity in the rat (ED_{50} (mg/kg): (*R*)-**246**, 13; (*R*)-**248**, ~20; (*R*)-**251**, <30; and (*R*)-**252**, 23). Finally, the 4'-substituted phenyl derivatives (*R*)-**249** and (*R*)-**253** displayed only moderate activity (ED_{50} >30, <100 mg/kg).

Several possible physiochemical properties may account for the observed 4'-*N*-benzyl SAR. Modification of the 4'-site in the C(2)-hydrocarbon PAADs altered the electronic and hydrophobic properties at this site in the drug candidates. Our data indicates that the electronic properties of 4'-*N*-benzyl substituents impacted pharmacological activity to a greater extent than the hydrophobic properties of 4'-*N*-benzyl substituents. We found that electron-withdrawing 4'-substituents (defined as a positive Hammett sigma value, Table 25) maintained or increased seizure protection while electron-donating groups (defined as a negative Hammett sigma value, Table 25) at this site dramatically decreased activity (Figure 15). We are at a loss to explain the abrupt loss of activity as the sigma value approached zero. Many, but not all, of the electron-withdrawing moieties contained fluorine-substituents. The greatest activity was observed in C(2)-isopropyl PAADs containing a CF_3 ((*R*)-**246**) or OCF_3 ((*R*)-**248**) group at the 4'-*N*-benzyl position. When the corresponding non-fluorinated moieties CH_3 ((*R*)-**245**) and OCH_3 ((*R*)-**247**) were incorporated at the 4'-*N*-benzylamide site, the compounds were inactive suggesting that hydrophobic interactions (defined by their π-value) may affect anticonvulsant activity (Table 25) (π: (*R*)-**246**, 0.88; and (*R*)-**248**, 1.04; (*R*)-

245, 0.56; and (*R*)-**247**, -0.02).[232,233] However, there is not a direct correlation between hydrophobicity and pharmacological activity (Figure 16). For example, the 4'-OCH_3 and 4'-F have similar π-values (-0.02 and 0.14) but strikingly different ED_{50} values (ED_{50} (mg/kg): (*R*)-**243**, >10, <30; (*R*)-**247**, >300). In addition, the 4'-phenyl derivative was the most hydrophobic substituent incorporated at the 4'-site, yet it exhibited only moderate anticonvulsant activity (ED_{50} >30, <100 mg/kg).

Our finding that the pharmacological activities of C(2)-hydrocarbon PAADs was highly dependent upon the electronic properties of the 4'-substitutent was unexpected. In a related study, Salomé and coworkers showed for 4'-substituted derivatives of the FAA (*R*)-**28**, anticonvulsant activity did not correlate with the electronic properties of the 4'-group, and that both electron-donating and electron-withdrawing substituents displayed excellent protection in the MES seizure model.[102] The sensitivity of the C(2)-hydrocarbon PAADs to the electronic properties of the 4'-*N*-benzyl substituent provides another difference in the SAR of this series of compounds compared with the findings reported for FAAs. We appreciate that these PAADs and FAAs differ not only by the absence or presence of the *N*-terminal acetyl group, but also in the nature of the C(2)-substituent (e.g., hydrocarbon, CH_2OCH_3).

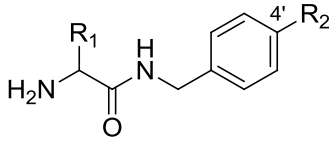
Table 23. Pharmacological activities of 4'-*N*-benzyl-substituted C(2)-hydrocarbon PAADs in mice (mg/kg) at the NINDS ASP



			Mice (ip)[a]				
Cmpd No.	**R_1**	**R_2**	**MES,[b] ED_{50}**	**6 Hz,[c] ED_{50}**	**Tox,[d] TD_{50}**	**PI,[e] MES**	**PI,[e] 6 Hz**
(*R*)-**28**	LCM	H	4.5 [0.5] (3.7–5.5)	10	27 [0.25] (26–28)	6.0	2.7
(*R*)-**98**	$CH(CH_3)_2$	H	15 [0.25] (13–18)	ND[f]	70 [0.25] (63–80)	4.8	
(*R*)-**243**	$CH(CH_3)_2$	F	>10, <30 [0.5]	ND[f]	>100, <300 [0.5]		
(*R*)-**244**	$CH(CH_3)_2$	Cl	22 [0.25] (20–25)	~50	74 [0.25] (72–78)	3.4	~1.5
(*R*)-**245**	$CH(CH_3)_2$	CH_3	>300 [0.5]	ND[f]	>300 [0.5]		
(*R*)-**246**	$CH(CH_3)_2$	CF_3	14 [0.5] (12–16)	>20, <60	57 [0.25] (54–54)	4.1	
(*R*)-**247**	$CH(CH_3)_2$	OCH_3	>300 [0.5]	ND[f]	>30, <100 [0.5]		
(*R*)-**248**	$CH(CH_3)_2$	OCF_3	16 [0.25] (14–20)	ND[f]	84 [0.25] (67–109)	5.3	
(*R*)-**249**	$CH(CH_3)_2$	C_6H_5	>30, <100 [0.5]	ND[f]	>300 [0.5]		
(*R*)-**99**	$C(CH_3)_3$	H	14 [0.25] (11–17)	ND[f]	66 [0.25] (58–73)	4.7	
(*R*)-**250**	$C(CH_3)_3$	Cl	25 [0.25] (21–29)	ND[f]	84 [0.5] (75–100)	3.4	
(*R*)-**251**	$C(CH_3)_3$	CF_3	24 [0.25] (21–28)	<100 [0.25-2.0]	133 [0.25] (93–197)	5.5	
(*R*)-**252**	$C(CH_3)_3$	OCF_3	28 [1.0] (22–34)	ND[f]	73 [0.25] (60–86)	2.6	
(*R*)-**253**	$C(CH_3)_3$	C_6H_5	>30, <100 [0.5]	ND[f]	>30, <100 [0.5]		

[a] The compounds were administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg. A dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] 6 Hz test = psychomotor seizure model (44 mA). [d] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [e] PI = protective index (TD_{50}/ED_{50}). [f] ND = not determined.

Table 24. Pharmacological activities of 4'-*N*-benzyl-substituted C(2)-hydrocarbon PAADs in rats (mg/kg) at the NINDS ASP



			Rat (po)[a]		
Cmpd No.	**R_1**	**R_2**	**MES,[b] ED_{50}**	**Tox,[c] TD_{50}**	**PI[d]**
(*R*)-**28**	LCM	H	3.9 [2.0] (2.9–6.2)	>500	>120
(*R*)-**98**	$CH(CH_3)_2$	H	11 [0.25] (9.1–13)	>500	>45
(*R*)-**243**	$CH(CH_3)_2$	F	21 [0.5] (13–31)	>500	>24
(*R*)-**244**	$CH(CH_3)_2$	Cl	ND[e]	ND[e]	
(*R*)-**245**	$CH(CH_3)_2$	CH_3	ND[e]	ND[e]	
(*R*)-**246**	$CH(CH_3)_2$	CF_3	13 [1.0] (9–18)	>500	>38
(*R*)-**247**	$CH(CH_3)_2$	OCH_3	ND[e]	ND[e]	
(*R*)-**248**	$CH(CH_3)_2$	OCF_3	~20 [0.25–2.0]	>30 [0.25–4.0]	
(*R*)-**249**	$CH(CH_3)_2$	C_6H_5	~30 [2.0]	>30 [0.25–4.0]	
(*R*)-**99**	$C(CH_3)_3$	H	ND[e]	ND[e]	
(*R*)-**250**	$C(CH_3)_3$	H	>30	>30	
(*R*)-**251**	$C(CH_3)_3$	Cl	~30 [0.25–0.5]	>30 [0.25–4.0]	
(*R*)-**252**	$C(CH_3)_3$	CF_3	<30 [0.5–4.0]	>30 [0.25–4.0]	
(*R*)-**253**	$C(CH_3)_3$	OCF_3	23 [2.0] (17–33)	>30 [0.25–4.0]	
(*R*)-**28**	$C(CH_3)_3$	C_6H_5	ND[e]	ND[e]	

[a] The compounds were administered orally to adult male albino Sprague Dawley rats. ED_{50} and TD_{50} values are in mg/kg. A dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] Tox = behavioral toxicity. [d] PI = protective index (TD_{50}/ED_{50}). [e] ND = not determined.

Table 25. Physical properties of aromatic substituents

R	σ_p[a]	π[a]	Pauling[b] electronegativity
H	0.00	0.00	2.28
Br	0.23	0.86	2.80
Cl	0.23	0.71	3.03
F	0.06	0.14	3.95
CH_3	-0.17	0.56	2.30
CF_3	0.54	0.88	3.35
OCH_3	-0.27	-0.02	NA[c]
OCF_3	0.35	1.04	NA[c]
C_6H_5	-0.01	1.96	3.0

[a] Hansch, C. *Substituent constants for correlation analysis in chemistry and biology*. Wiley: New York, **1979**, pp. 49–51. [b] Carey, F. A. *Advanced organic chemistry. Part A: Structure and mechanisms*. Springer: New York, **2006**, p. 17. [c] NA = not available.

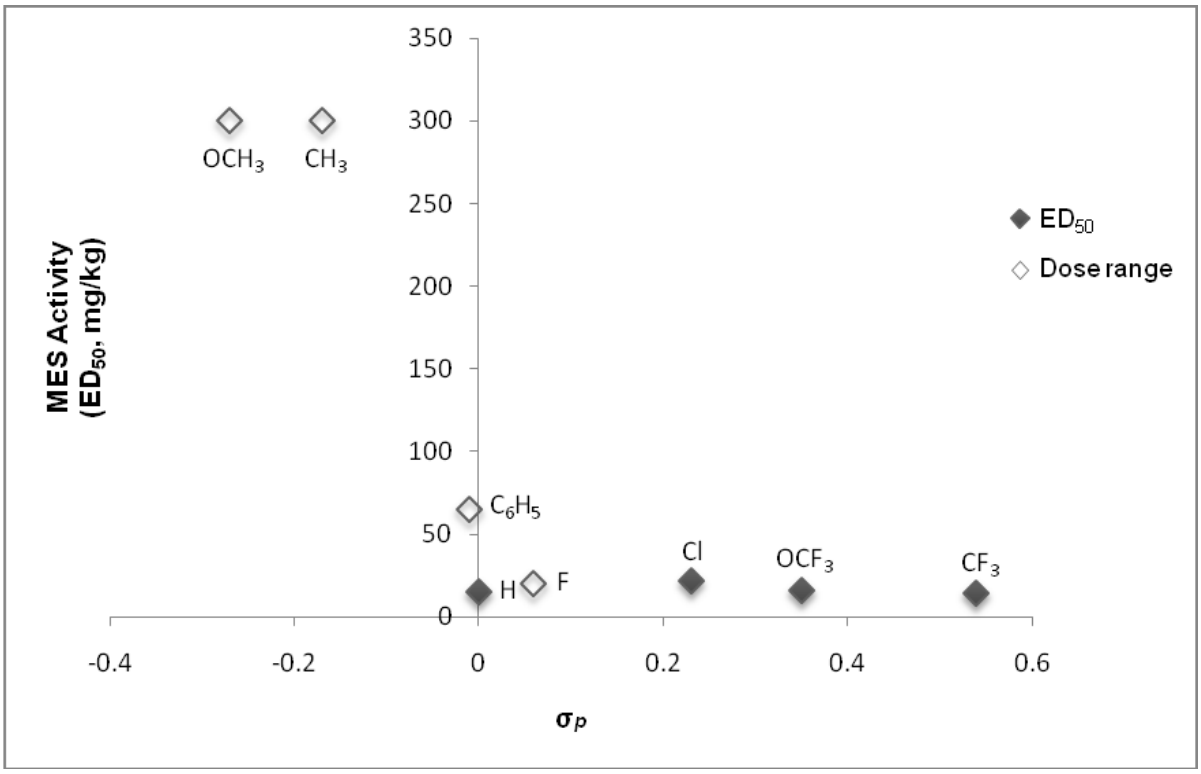


Figure 15. Effect of σ_p on MES activity

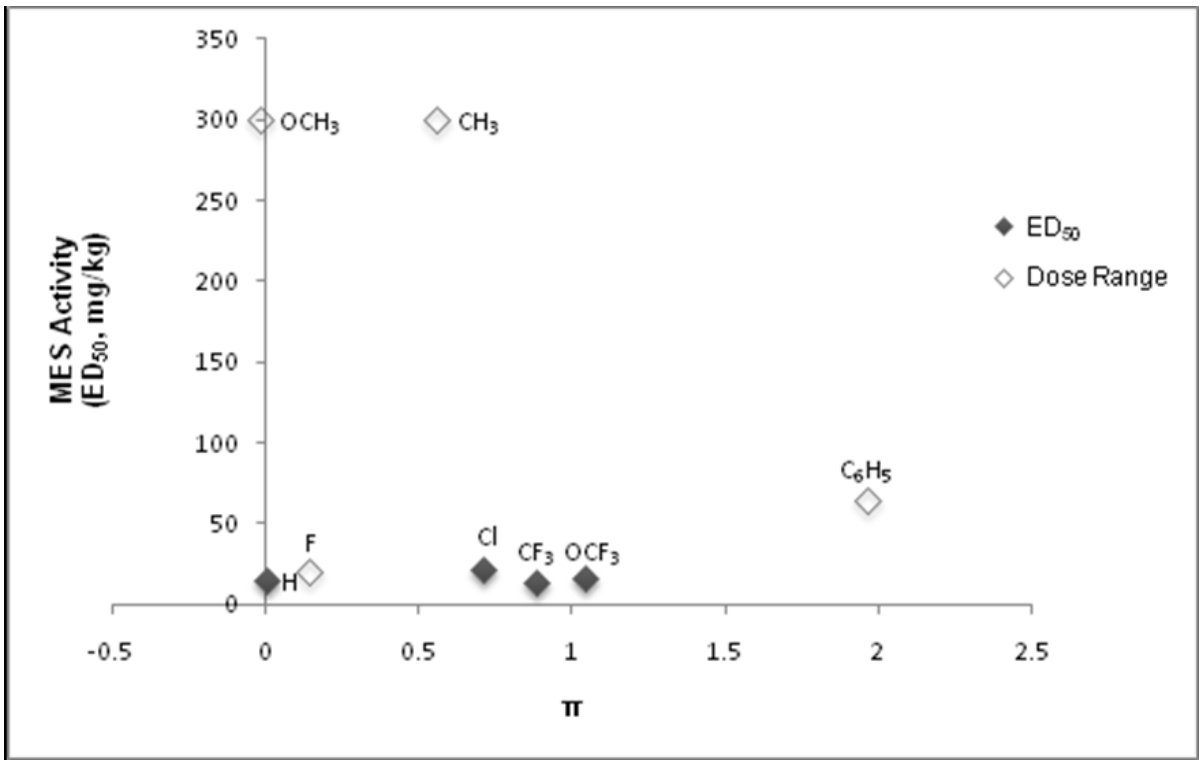


Figure 16. Effect of π on MES activity

2.5.1.3. 4'-Chimeric C(2)-hydrocarbon PAADs

Next, we evaluated the neurological activities of 4'-*N*-benzyl-chimeric PAADs (*R*)-**254**–**261** in mice (Table 26) and rats (Table 27). Evaluation occurred during the transition from UCB Pharma to the NINDS ASP and (*R*)-**255** and (*R*)-**258** were tested at both facilities (location indicated in column two of Table 26). First, we approached the data analysis from the standpoint of R_1, where R_2 was kept the same and we looked at the effect of R_1 on activity. Then, we examined the effect of R_2 on activity, where we kept R_1 the same and looked at the effect of R_2 on activity. The first method examined which R_1 unit was optimal for 4'-chimeric PAADs, irrespective of the R_2 unit, and the second method examined if there was a preference in the ether linkage orientation of R_2 (-OCH_2- versus –CH_2O-), irrespective of the R_1 unit. Finally, we evaluated the stereochemical preference for 4'-*N*-benzyl-chimeric PAADs **255** and **258**.

Using this approach, we determined how introduction of either a (3-fluoro)benzyloxy or a (3-fluoro)phenyloxymethyl moiety at the 4'-*N*-benzyl position (R_2) in the C(2)-CH_2OCH_3, C(2)-methyl, C(2)-isopropyl, or C(2)-*tert*-butyl PAADs affected pharmacological activity. When R_2 was (3-fluoro)benzyloxy, we did not observe any distinct trends in MES activity (mice) as we varied R_1 from C(2)-CH_2OCH_3 ((*R*)-**254**, ED_{50} = 15 mg/kg) to C(2)-methyl ((*R*)-**256**, ED_{50} = 17 mg/kg) to C(2)-isopropyl ((*R*)-**257**, ED_{50} = 12 mg/kg) to C(2)-*tert*-butyl ((*R*)-**260**, ED_{50} >30, <100 mg/kg). However, when R_2 was (3-fluoro)phenyloxymethyl, we observed a decrease in MES activity (mice) as we varied R_1 from C(2)-CH_2OCH_3 ((*R*)-**255**, ED_{50} = 8.9 mg/kg) to C(2)-isopropyl ((*R*)-**258**, ED_{50} = 12 mg/kg) to C(2)-*tert*-butyl ((*R*)-**261**, ED_{50} >30, <100 mg/kg). In both cases, the C(2)-*tert*-butyl derivatives (*R*)-**260** and (*R*)-**261** were considerably less active in the MES test than the corresponding C(2)-isopropyl derivatives (*R*)-**257** and (*R*)-**258**. Correspondingly, a C(2)-*tert*-butyl group at R_1 displayed only moderate MES activity in rats (ED_{50} (mg/kg): (*R*)-**260**, >30; (*R*)-**261**, ~30) while a C(2)-CH_2OCH_3 group displayed excellent activity, irrespective of the ether orientation in the R_2

unit (ED_{50} (mg/kg): (*R*)-**254**, <30; (*R*)-**255**, 12) (Table 27). The near equivalence in activity of the C(2)-CH_2OCH_3 and C(2)-isopropyl groups is unique to chimeric PAADs. In the unsubstituted PAADs (*R*)-**61** and (*R*)-**98**, we observed that C(2)-isopropyl (*R*)-**98** was 3.2-fold more active than C(2)-CH_2OCH_3 (*R*)-**61** (ED_{50} (mg/kg): (*R*)-**8**, 48; (*R*)-**44**, 15). Similarly, when the FAA counterpart of C(2)-CH_2OCH_3 PAAD ((*R*)-**367**), and C(2)-isopropyl ((*R*)-**370**) were compared, there is a remarkable difference in their anticonvulsant activities (ED_{50} (mg/kg): (*R*)-**367**, 13; (*R*)-**370**, >300) (Table 28).

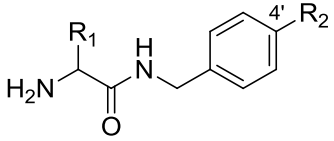
Next, we examined the C(2)-CH_2OCH_3, C(2)-isopropyl, and C(2)-*tert*-butyl PAADs containing either a (3-fluoro)benzyloxy or a (3-fluoro)phenyloxymethyl moiety (R_2) to determine if there was a preferred ether linker orientation between the two aromatic rings. Each R_1 set (C(2)-CH_2OCH_3, C(2)-isopropyl, C(2)-*tert*-butyl) displayed similar MES activities in mice for the two ether linkages (-OCH_2- ED_{50} (mg/kg): (*R*)-**254**, 15; (*R*)-**257**, 12; (*R*)-**260**, >30, <100; and -CH_2O- ED_{50} (mg/kg): (*R*)-**255**, 12; (*R*)-**258**, 12; and (*R*)-**261**, >30, <100). The only notable difference in regard to linker orientation was in the formalin test of the C(2)-isopropyl PAADs (*R*)-**257** (-OCH_2-, ED_{50} = 110 mg/kg (80% reduction)) and (*R*)-**258** (-CH_2O-, ED_{50} = 30 mg/kg). The formalin activity was evaluated for the C(2)-isopropyl PAAD (*R*)-**255** (ED_{50} = 37 mg/kg (94% reduction)) but the lack of data for (*R*)-**254**, (*R*)-**260**, and (*R*)-**261** prevents any generalized statements. Therefore, using the available data, both (3-fluoro)benzyloxy and (3-fluoro)phenyloxymethyl moieties at the 4'-*N*-benzyl position of C(2)-CH_2OCH_3 ((*R*)-**254** and (*R*)-**255**) and C(2)-isopropyl ((*R*)-**257** and (*R*)-**258**) PAADs resulted in an increase in MES activity from the unsubstituted parent compounds ((*R*)-**61** and (*R*)-**98**, respectively). Additionally, we observed no preference for the -CH_2O- linker orientation when R_1 was a C(2)-isopropyl group. Nonetheless, both 4'-*N*-benzyl substitutions provided PAADs with excellent anticonvulsant activities. The activity of (*R*)-**255** was noteworthy and is among the most active PAADs prepared. Using the conventional unit mg/kg, it was 2-fold less active than (*R*)-**28**, but it exhibited nearly the same PI value as (*R*)-**28** (PI: (*R*)-**255**, 5.4; (*R*)-**28**,

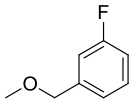
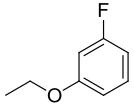
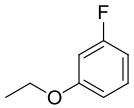
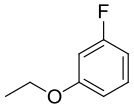
5.8). When the ED_{50} values are converted to μmol/kg, the difference in activities between (*R*)-**255** and (*R*)-**28** reduces to 1.4-fold (~30% increase).

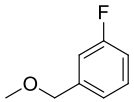
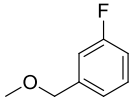
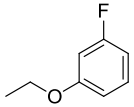
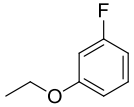
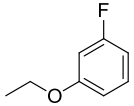
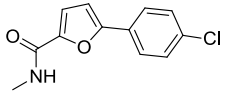
Comparison of the MES activities of (*R*)-**255** with (*S*)-**255**, and (*R*)-**258** with (*S*)-**258**, revealed that the higher activity was associated with the (*R*)-isomer (ED_{50} (mg/kg): (*R*)-**255**, 8.9; (*S*)-**255**, >30, <100; (*R*)-**258**, 12; (*S*)-**258**, >30, <100). This was not unexpected, as similar trends were observed in the unsubstituted PAADs (ED_{50} (mg/kg): (*R*)-**60**, >10, <30; (*S*)-**60**, >300; (*R*)-**61**, 34; (*S*)-**61**, 64; (*R*)-**96**, 21; (*S*)-**96**, >37; (*R*)-**98**, 15; (*S*)-**98**, >300; (*R*)-**99**, 14; (*S*)-**99**, 42) and in the FAA series (ED_{50} (mg/kg): (*R*)-**367**, 13; (*S*)-**367**, >300).

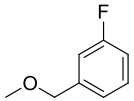
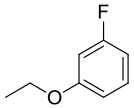
Lastly, we evaluated a 5-aryl-2-furfuramide at the 4'-*N*-benzyl position of (*R*)-*N*-benzyl 2-amino-3-methylbutanamide (a C(2)-isopropyl PAAD) ((*R*)-**259**) with the intention of observing pain attenuation. However, (*R*)-**259** displayed no MES activity (ED_{50} >300 mg/kg) compared with its parent compound ((*R*)-**98**, ED_{50} = 15 mg/kg) and as a result, the formalin test was not run.

Table 26. Pharmacological activity of 4'-*N*-benzyl-chimeric PAADs in mice (mg/kg) at UCB and the NINDS ASP



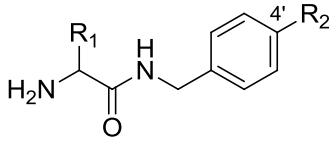
				Mice (ip)[a]				
Cmpd No.	**Test Site**	**R_1**	**R_2**	**MES,[b] ED_{50}**	**6 Hz,[c] ED_{50}**	**Formalin, ED_{50}**	**Tox,[d] TD_{50}**	**PI,[e]**
(*R*)-**28**	UCB	LCM	H	3.3	10	15	19	5.8
(*R*)-**28**[f]	NINDS	LCM	H	4.5 [0.5] (3.7–5.5)	10	ND[g]	27 [0.25] (26–28)	6.0
(*R*)-**61**	UCB	CH_2OCH_3	H	34	>67	>67	>120	>3.5
(*R*)-**61**	NINDS	CH_2OCH_3	H	48 [0.25] (40–61)	ND[g]	ND[g]	>30, <100 [0.25]	
(*S*)-**61**	UCB	CH_2OCH_3	H	64	>70	120	63	1.0
(*R*)-**254**	NINDS	CH_2OCH_3		15 [0.5] (13–17)	ND[g]	ND[g]	58 [0.25] (53–62)	3.9
(*R*)-**255**	UCB	CH_2OCH_3		8.9	58	12 (inactive) 37[h] (94%)	46	5.4
(*R*)-**255**	NINDS	CH_2OCH_3		~10 [0.5]	<30	ND[g]	>30, <100 [0.5]	
(*S*)-**255**	NINDS	CH_2OCH_3		>30, <100	ND[g]	ND[g]	>100, <300	

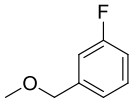
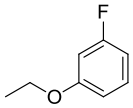
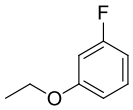
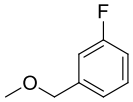
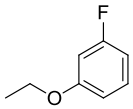
(*R*)-**60**	NINDS	CH_3	H	>10, <30	ND[g]	69	>100, <300	
(*S*)-**60**	NINDS	CH_3	H	>300	ND[g]	ND[g]	>300	
(*R*)-**256**	UCB	CH_3		17	>120	>37	ND[g]	
(*R*)-**98**	UCB	$CH(CH_3)_2$	H	16[i] (MAD)	74	20	47	2.9
(*R*)-**98**	NINDS	$CH(CH_3)_2$	H	15 [0.25] (13–18)	ND[g]	ND[g]	70 [0.25] (63–80)	4.7
(*S*)-**98**	NINDS	$CH(CH_3)_2$	H	>300	ND[g]	ND[g]	>300	
(*R*)-**257**	UCB	$CH(CH_3)_2$		12	>110	110[h] (80%)	ND[g]	
(*R*)-**258**	UCB	$CH(CH_3)_2$		12	>110	30	ND[g]	
(*R*)-**258**	NINDS	$CH(CH_3)_2$		>10, <30 [0.5]	ND[g]	ND[g]	>30, <100 [0.5]	
(*S*)-**258**	NINDS	$CH(CH_3)_2$		>30, <100			>100, <300	
(*R*)-**259**	NINDS	$CH(CH_3)_2$		>300 [0.5]	ND[g]	ND[g]	>300 [0.5]	
(*R*)-**99**	UCB	$C(CH_3)_3$	H	13	>71	>22	ND	
(*R*)-**99**	NINDS	$C(CH_3)_3$	H	14 [0.25] (11–17)	ND[g]	ND[g]	66 [0.25] (58–73)	4.7

(*R*)-**260**	NINDS	$C(CH_3)_3$		>30, <100 [0.5]	ND[g]	ND[g]	~300 [0.5]
(*R*)-**261**	NINDS	$C(CH_3)_3$		>30, <100 [0.5]	>75	ND[g]	>100, <300 [0.5]

[a] The compounds were administered intraperitoneally to adult male NMRI mice under the auspices of UCB or administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration (UCB) or a dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets) (NINDS ASP). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] 6 Hz test = psychomotor seizure model (44 mA, UCB; 32 mA, NINDS ASP). [d] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [e] PI = protective index (TD_{50}/ED_{50}). [f] Choi, D. *et al. J. Med. Chem.* **1996**, *39*, 1907–1916. [g] ND = not determined. [h] Single dose experiments where the mg/kg used is followed by the percentage protected in parenthesis. [i] MAD = minimal active dose.

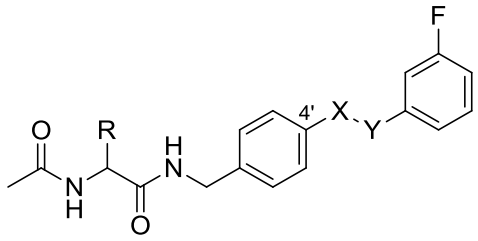
Table 27. Pharmacological activity of 4'-*N*-benzyl-chimeric PAADs in rats (mg/kg) at the NINDS ASP



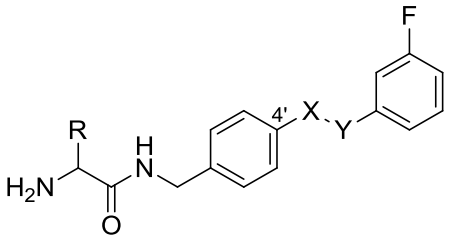
			Rat (po)[a]		
Cmpd No.	**R_1**	**R_2**	**MES,[b] ED_{50}**	**Tox,[c] TD_{50}**	**PI[d]**
(*R*)-**28**	LCM	H	3.9 [2.0] (2.9–6.2)	>500	>120
(*R*)-**61**	CH_2OCH_3	H	18 [4.0]	>500 [4.0]	>28
(*R*)-**254**	CH_2OCH_3		<30 [0.25–2.0]	>30 [0.25–4.0]	
(*R*)-**255**	CH_2OCH_3		12 [0.5] (8.2–18)	>500	>42
(*R*)-**98**	$CH(CH_3)_2$	H	11 [0.25] (9.1–13)	>500	>45
(*S*)-**258**	$CH(CH_3)_2$		>30 [0.25–4.0]	>30 [0.25–4.0]	
(*R*)-**260**	$C(CH_3)_3$		>30 [0.25–4.0]	>30 [0.25–4.0]	
(*R*)-**261**	$C(CH_3)_3$		~30 [2.0–4.0]	>30 [0.25–4.0]	

[a] The compounds were administered orally to adult male albino Sprague Dawley rats. ED_{50} and TD_{50} values are in mg/kg. A dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets) (NINDS ASP). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] Tox = behavioral toxicity. [d] PI = protective index (TD_{50}/ED_{50}).

Table 28. Comparison of the pharmacological activities of 4'-*N*-benzyl-chimeric FAAs and their PAAD counterparts in mice (mg/kg)



FAA



PAAD

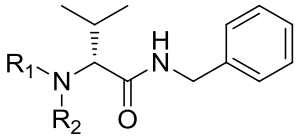
					Mice (ip)[a]					Mice (ip)[a]	
R	**X**	**Y**	**FAA Cpd No.**	**FAA Test Site**	**FAA MES,[b] ED_{50}**	**FAA Tox,[c] TD_{50}**	**PAAD Test Site**	**PAAD Cpd No.**	**PAAD MES,[b] ED_{50}**	**PAAD Tox,[c] TD_{50}**
CH_2OCH_3	O	CH_2	(*R*)-**367**[d]	NINDS	13 [0.25] (11–16)	26 [0.5] (21–34)	(*R*)-**254**	NINDS	>10, <30 [0.5]	>30, <100 [0.5]
CH_2OCH_3	O	CH_2	(*S*)-**367**[d]	NINDS	>300	>300	(*S*)-**254**	NINDS	ND[e]	ND[e]
CH_2OCH_3	CH_2	O	(*R*)-**368**[d]	NINDS	5.9 [0.25] (4.3–7.3)	10 [0.25] (9.1–13)	(*R*)-**255**	UCB	8.9	46
CH_2OCH_3	CH_2	O	(*S*)-**368**[d]	NINDS	ND[e]	ND[e]	(*S*)-**255**	NINDS	>30, <100	>100, <300
CH_3	O	CH_2	(*R*)-**369**[d]	NINDS	>30, <100	>300	(*R*)-**256**	UCB	17	ND[e]
$CH(CH_3)_2$	O	CH_2	(*R*)-**370**[d]	NINDS	>300	>300	(*R*)-**257**	UCB	12	ND[e]
$C(CH_3)_3$	O	CH_2	(*R*)-**371**[d]	NINDS	>300	>300	(*R*)-**260**	NINDS	>30, <100 [0.5]	~300 [0.5]

[a] The compounds were administered intraperitoneally to adult male NMRI mice under the auspices of UCB or administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration (UCB) or a dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets) (NINDS ASP). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [d] Salomé, C. *et al. J. Med. Chem*. **2010**, *53*, 3756–3771. [e] ND = not determined.

2.5.2. *N*-Substituted 3-methylbutanamides

The activities of the primary (PAAD), secondary (SAAD), and tertiary (TAAD) amino acid analogs of (*R*)-*N*-benzyl 2-amino-3-methylbutanamide are summarized in Table 29. Comparison of the SAAD (*R*)-**269** with the PAAD (*R*)-**98** showed a decrease in activity in the MES test (ED_{50} (mg/kg): (*R*)-**98**, 15; (*R*)-**269**, 25), as well as in the 6 Hz test (ED_{50} (mg/kg): (*R*)-**98**, 74; (*R*)-**269**, 82 (MAD)). The decrease in activity in the formalin test is much more pronounced (ED_{50} (mg/kg): (*R*)-**98**, 20; (*R*)-**269**, 82 (42% reduction)). Furthermore, comparison of the TAAD (*R*)-**270** with the SAAD (*R*)-**269** showed a decrease in activity in the MES test (ED_{50} (mg/kg): (*R*)-**269**, 25; (*R*)-**270**, >30, <100). Therefore, the MES data for PAAD (*R*)-**98**, SAAD (*R*)-**269**, and TAAD (*R*)-**270** revealed a linear decrease in anticonvulsant activity as we successively *N*-methylated the C(2)-amine. This trend differed from the reported pattern for racemic C(2)-methyl, C(2)-phenyl, and C(2)-CH_2OCH_3 PAADs, where successive *N*-methylation led to non-linear trends in MES activity in C(2)-methyl and C(2)-CH_2OCH_3 series, and resulted in a linear increase in anticonvulsant activity in the C(2)-phenyl series (Table 20).[92]

Table 29. Pharmacological activities of primary (PAAD), secondary (SAAD), and tertiary (TAAD) amino acid derivatives of (*R*)-*N*-benzyl 2-amino-3-methylbutanamide in mice (mg/kg) at UCB and the NINDS ASP



				Mice (ip)[a]				
Cmpd No.	**Test Site**	**R_1**	**R_2**	**MES[b], ED_{50}**	**6 Hz,[c] ED_{50}**	**Formalin, ED_{50}**	**Tox,[d] TD_{50}**	**PI,[e] MES**
(*R*)-**98**	UCB	H	H	16 (MAD)[h] 16[i] (100%)	74	20	47	
(*R*)-**98**	NINDS	H	H	15 [0.25] (13–18)	ND[g]	ND[g]	70 [0.25] (63–80)	4.7
(*R*)-**269**	UCB	CH_3	H	25	82 (MAD)[h]	82[i] (42%)	ND[g]	
(*R*)-**270**	NINDS	CH_3	CH_3	>30, <100 [0.5]	ND[g]	ND[g]	>100, <300 [0.5]	

[a] The compounds were administered intraperitoneally to adult male NMRI mice under the auspices of UCB or administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration (UCB) or a dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets) (NINDS ASP). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] 6 Hz test = psychomotor seizure model (44 mA). [d] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [e] PI = protective index (TD_{50}/ED_{50}). [f] Choi, D. *et al. J. Med. Chem.* **1996**, *39*, 1907–1916. [g] ND = not determined. [h] MAD = minimal active dose. [i] Single dose experiments where the mg/kg used is followed by the percentage protected in parenthesis.

2.5.3. C(2)-Isopropyl PAAD analogs

Compound (*R*)-**98** emerged as a potent anticonvulsant that possessed pain attenuating properties from the SAR investigation at the C(2)-carbon, and we improved the anticonvulsant activity in mice upon functionalization at the 4'-*N*-benzyl position ((*R*)-**246**, (*R*)-**248**, (*R*)-**257**, and (*R*)-**258**). The excellent activity of (*R*)-**98** came as a surprise since it did not follow the trends observed in the FAA series. Therefore, we questioned several aspects of the original PAAD structural framework to determine if the basic tenets of the FAA blueprint applied to this C(2)-hydrocarbon PAAD. We investigated the importance of six properties (Sites A–F) that were common with the FAAs and report their anticonvulsant activity and neurological toxicity in Tables 30–35.

First, we determined the effect of the carbonyl unit (Site A) on anticonvulsant activity (Table 30). Replacement of the carbonyl unit ((*R*)-**98**) with a thiocarbonyl unit ((*R*)-**271**) resulted in a decrease in MES activity in mice (ED_{50} (mg/kg): (*R*)-**98**, 15; (*R*)-**271**, >30, <100) but notable activity was observed in the rat (ED_{50} <30 mg/kg). Reduction of the carbonyl unit in (*R*)-**98** to a methylene group ((*R*)-**272**) led to decreased activity in both the mice (ED_{50} >30, <100 mg/kg) and rats (ED_{50} >30 mg/kg). Next, we determined the effect of the amide bond (Site B) on anticonvulsant activity (Table 31). Conversion of the amide (X = NH, (*R*)-**98**) to a ketone (X = CH_2, (*R*)-**273**) resulted in a decrease in MES activity (ED_{50} (mg/kg): (*R*)-**98**, 15; (*R*)-**273**, >30, <100). Conversion of the amide (X = NH, (*R*)-**98**) to an ester (X = O, (*R*)-**274**) abolished anticonvulsant activity (ED_{50} >300 mg/kg). Then, Site C looked at the optimal methylene linker length between the amide bond and the aromatic ring (Table 32). Direct linkage of the aromatic ring to the amide bond (n = 0, (*R*)-**275**) resulted in a significant drop in activity from the parent compound (n = 1, (*R*)-**98**) (ED_{50} (mg/kg): (*R*)-**98**, 15; (*R*)-**275**, >30, <100). However, extending the linkage of the parent compound (n = 1, (*R*)-**98**) by one methylene unit (n = 2, (*R*)-**276**) resulted in a ~30% increase in anticonvulsant activity (ED_{50} = 10 mg/kg). The increase in activity was also associated with an increase in toxicity

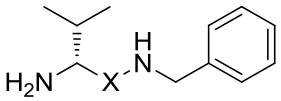
compared with the parent compound (TD_{50} (mg/kg): (*R*)-**98**, 70; (*R*)-**276**, 50), however, the protective indices of (*R*)-**98** (4.8) and (*R*)-**276** (5.0) are nearly equal. Extension of the linker by another carbon (n = 3, (*R*)-**277**) led to comparable activity with (*R*)-**98** (ED_{50} (mg/kg): (*R*)-**98**, 15; (*R*)-**277**, 16) but the increased toxicity for (*R*)-**277** (TD_{50} = 43 mg/kg) reduced the protective index to 2.7. A final extension to n = 4 ((*R*)-**278**) resulted in comparable activity with (*R*)-**98** and (*R*)-**277** (ED_{50} (mg/kg): (*R*)-**278**, >10, <30; (*R*)-**98**, 15; (*R*)-**277**, 16). Site D compared the regiosubstitution on the *N*-benzylamide, where we systematically placed a trifluoromethoxy group at the 2'-, 3'-, and 4'-positions on the *N*-benzylamide moiety (2': (*R*)-**279**, 3': (*R*)-**280**, and 4': (*R*)-**248**) (Table 33). All regiosubstitutions displayed excellent MES activities (ED_{50} (mg/kg): (*R*)-**279**, 9.2; (*R*)-**280**, >3, <10; (*R*)-**278**, 16). The 2'-substitution afforded the highest activity (ED_{50} = 9.2 mg/kg) and was 1.6-fold more active than the parent compound (*R*)-**98** (ED_{50} = 15 mg/kg). There was also an increase in the neurotoxicity observed for (*R*)-**279** compared with (*R*)-**98** (ED_{50} (mg/kg): (*R*)-**98**, 70; (*R*)-**279**, 51) but comparison of the protective indices revealed that (*R*)-**279** (PI = 5.5) may provide a slight advantage over (*R*)-**98** (PI = 4.8). However, comparison of the MES activity in rats (Table 33) showed ~3-fold drop in activity in the rat (ED_{50} (mg/kg): (*R*)-**98**, 11; (*R*)-**279**, 33). The MES activity of (*R*)-**280** in the rat (ED_{50} = 10 mg/kg) was comparable with (*R*)-**98** (ED_{50} = 11 mg/kg) but there was a sharp increase in behavioral toxicity (TD_{50} (mg/kg): (*R*)-**98**, >500; (*R*)-**280**, 43). Next, we examined the need for a benzyl moiety (Site E) (Table 34). We replaced the aromatic ring ((*R*)-**98**) with a cyclohexyl ring ((*R*)-**281**) and observed a decrease in anticonvulsant activity (ED_{50} (mg/kg): (*R*)-**98**, 15; (*R*)-**281**, >30, <100). When (*R*)-**281** was evaluated in the rat, we found this PAAD to have excellent activity (ED_{50} ~15 mg/kg). While our data indicates that the *N*-benzyl substituent is preferred over the saturated *N*-cyclohexylmethyl unit, the anticonvulsant activity of (*R*)-**281** was not anticipated. Finally, we investigated the importance of the C(2)-amino functionality (Site F) by comparing the unsubstituted (**282**, X = H), methyl-substituted ((*S*)-, and (*R,S*)-**283**, X = CH_3), and

hydroxy-substituted ((*R*)-**284**, X = OH) *N*-benzyl butanamides with the parent PAAD (*R*)-**95** (X = NH_2) (Table 35). Compounds **282** and (*R*)-**284** gave similar results, where modest activity was observed in mice (ED_{50} >30, <100 mg/kg) and there was a lack of activity in rats (ED_{50} >30 mg/kg). (*R,S*)-**283** also displayed modest protection in mice (ED_{50} = 56 mg/kg) with minimal neurotoxicity (TD_{50} = 165 mg/kg), and moderate activity was observed in the rat (ED_{50} = 51 mg/kg) without any detectable behavioral toxicity (TD_{50} >500 mg/kg). We were surprised by the activity of (*R,S*)-**283** since the methyl group lacks the hydrogen bonding capabilities afforded by an amino or a hydroxyl group. Evaluation of (*S*)-**283** resulted in a decrease in anticonvulsant activity (ED_{50} >100, <300 mg/kg), suggesting that activity predominantly resided in the (*R*)-isomer. Unfortunately, we were unable to complete the synthesis of (*R*)-**283** to confirm this suggestion.

Examination of Sites A–F of PAADs revealed that the amide bond (Sites A and B) were necessary for seizure protection. Alteration of this unit affects the hydrogen bonding properties of the compounds. Assessment of the methylene linker (Site C) showed that at least one carbon between the amide bond and the aromatic rings was necessary for anticonvulsant activity but incorporation of up to three additional methylene units still provided excellent activity. When considering activity, toxicity, and the protective index for these C(2)-isopropyl PAADs, the optimal linker length appeared to be when n = 2, instead of the n = 1 seen in FAAs. Similarly, we found that replacement of the benzylamide group by a cyclohexylmethylamide (Site E) led to lower anticonvulsant activity, but the drop was only modest. Thus, there seems to be structural latitude at Sites C and E, where modifications still retain excellent activities. Comparison of trifluoromethoxy regioisomers of (*R*)-*N*-benzyl 2-amino-3-methylbutanamide (Site D) revealed that superb seizure protection was associated with all positions but the increase in activity was associated with an increase in neurotoxicity. Lastly, we evaluated our choice of the C(2)-amino group (Site F) since the SAR project was centered around primary *amino* acid derivatives. Of the other C(2)-groups

investigated, the amino group was most active but further analysis of the C(2)-methyl group ((*R*)-isomer) and other analogs should be conducted to show that the C(2)-amino group is not mandatory for activity. Collectively, this data, along with the finding that activity for hydrocarbon PAADs does not improve with inclusion of a substituted heteroatom one atom removed from the C(2)-center, suggests that the C(2)-hydrocarbon PAADs have an unique SAR and possibly a pathway(s) of function that differ from FAA and other PAADs.

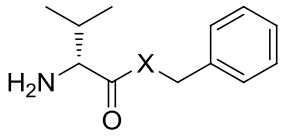
Table 30. Pharmacological activities of C(2)-isopropyl PAAD amide analogs (Site A) in mice (mg/kg) and rats (mg/kg) at the NINDS ASP



		Mice (ip)[a]			Rat (po)[b]		
Cmpd No	**X**	**MES,[c] ED_{50}**	**Tox,[d] TD_{50}**	**PI[e]**	**MES,[c] ED_{50}**	**Tox,[f] TD_{50}**	**PI[e]**
(*R*)-**98**	C=O	15 [0.25] (13–18)	70 [0.25] (63–80)	4.8	11 [0.25] (9.1–13)	>500	>45
(*R*)-**271**	C=S	>30, <100 [0.5]	>30, <100 [0.5]		<30 [0.5–2.0]	>30 [0.25–4.0]	
(*R*)-**272**	CH_2	>30, <100 [0.5]	>100, <300 [0.5]		>30 [0.25–4.0]	>30 [0.25–4.0]	

[a] The compounds were administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg. A dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets). Numbers in parentheses are 95% confidence intervals. [b] The compounds were administered orally to adult male albino Sprague Dawley rats under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg. [c] MES = maximal electroshock seizure test. [d] TD_{50} value determined from the rotorod test. [e] PI = protective index (TD_{50}/ED_{50}). [f] Tox = behavioral toxicity.

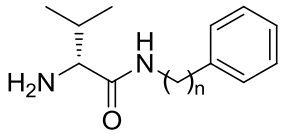
Table 31. Pharmacological activities of C(2)-isopropyl PAAD amide analogs (Site B) in mice (mg/kg) and rats (mg/kg) at the NINDS ASP



		Mice (ip)[a]			Rat (po)[b]		
Cmpd No	**X**	**MES,[c] ED_{50}**	**Tox,[d] TD_{50}**	**PI[e]**	**MES,[c] ED_{50}**	**Tox,[f] TD_{50}**	**PI[e]**
(*R*)-**98**	NH	15 [0.25] (13–18)	70 [0.25] (63–80)	4.8	11 [0.25] (9.1–13)	>500	>45
(*R*)-**273**	CH_2	>30, <100	>100, <300		ND[g]	ND[g]	
(*R*)-**274**	O	>300 [0.5]	>300 [0.5]		>30 [0.25]	>30 [0.25–4.0]	

[a] The compounds were administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg. A dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets). Numbers in parentheses are 95% confidence intervals. [b] The compounds were administered orally to adult male albino Sprague Dawley rats under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg. [c] MES = maximal electroshock seizure test. [d] TD_{50} value determined from the rotorod test. [e] PI = protective index (TD_{50}/ED_{50}). [f] Tox = behavioral toxicity. [g] ND = not determined.

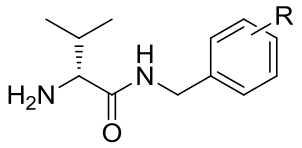
Table 32. Pharmacological activities of C(2)-isopropyl PAADs: *N*-Aryl and *N*-alkylaryl analogs (Site C) in mice (mg/kg) and rats (mg/kg) at the NINDS ASP



		Mice (ip)[a]			Rat (po)[b]		
Cmpd No.	**n**	**MES,[c] ED_{50}**	**Tox,[d] TD_{50}**	**PI[e]**	**MES,[c] ED_{50}**	**Tox,[f] TD_{50}**	**PI[e]**
(*R*)-**275**	0	>30, <100 [0.5]	>30, <100 [0.5]		ND[g]	ND[g]	
(*R*)-**98**	1	15 [0.25] (13–18)	70 [0.25] (63–80)	4.8	11 [0.25] (9.1–13)	>500	>45
(*R*)-**276**	2	10 [0.25] (8.3–14)	50 [0.25] (42–80)	5.0	ND[g]	ND[g]	
(*R*)-**277**	3	16 [0.25] 13–17	43 [0.25] (38–47)	2.7	<30 [0.25–0.5]	>30 [0.25–4.0]	
(*R*)-**278**	4	>10, <30 [0.5]	>30, <100 [0.5]		ND[g]	ND[g]	

[a] The compounds were administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg. A dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets). Numbers in parentheses are 95% confidence intervals. [b] The compounds were administered orally to adult male albino Sprague Dawley rats under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg. [c] MES = maximal electroshock seizure test. [d] TD_{50} value determined from the rotorod test. [e] PI = protective index (TD_{50}/ED_{50}). [f] Tox = behavioral toxicity. [g] ND = not determined.

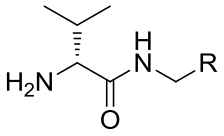
Table 33. Pharmacological activities of trifluoromethoxy regioisomers (Site D) of (*R*)-*N*-benzyl 2-amino-3-methylbutanamide in mice (mg/kg) and rats (mg/kg) at the NINDS ASP



		Mice (ip)[a]			Rat (po)[b]		
Cmpd No.	**R**	**MES,[c] ED_{50}**	**Tox,[d] TD_{50}**	**PI[e]**	**MES,[c] ED_{50}**	**Tox,[f] TD_{50}**	**PI[e]**
(*R*)-**98**	H	15 [0.25] (13–18)	70 [0.25] (63–80)	4.8	11 [0.25] (9.1–13)	>500	>45
(*R*)-**279**	2'-OCF_3	9.2 [0.25] (7.7–11)	51 [0.25] (38–65)	5.5	33 [0.5] (27–44)	>500	>15
(*R*)-**280**	3'-OCF_3	>3, <10 [0.5]	>30, <100 [0.5]		10 [1.0] (7.5–14)	43 [1.0] (35–57)	4.3
(*R*)-**248**	4'-OCF_3	16 [0.25] (14–20)	84 [0.25] (67–109)	5.3	~20 [0.25–4.0]	>20 [0.25–4.0]	

[a] The compounds were administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg. A dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets). Numbers in parentheses are 95% confidence intervals. [b] The compounds were administered orally to adult male albino Sprague Dawley rats under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg. [c] MES = maximal electroshock seizure test. [d] TD_{50} value determined from the rotorod test. [e] PI = protective index (TD_{50}/ED_{50}). [f] Tox = behavioral toxicity.

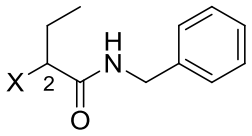
Table 34. Pharmacological activities of *N*-substituted C(2)-isopropyl PAADs (Site E) in mice (mg/kg) and rats (mg/kg) at the NINDS ASP



		Mice (ip)[a]			Rat (po)[b]		
Cmpd No.	**R**	**MES,[c] ED_{50}**	**Tox,[d] TD_{50}**	**PI[e]**	**MES,[c] ED_{50}**	**Tox,[f] TD_{50}**	**PI[e]**
(*R*)-**98**	C_6H_5	15 [0.25] (13–18)	70 [0.25] (63–80)	4.8	11 [0.25] (9.1–13)	>500	>45
(*R*)-**281**	C_6H_{11}	>30, <100 [0.5]	>100, <300 [0.5]		~15 [0.25-1.0]	>30 [0.25-4.0]	

[a] The compounds were administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg. A dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets). Numbers in parentheses are 95% confidence intervals. [b] The compounds were administered orally to adult male albino Sprague Dawley rats under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg. [c] MES = maximal electroshock seizure test. [d] TD_{50} value determined from the rotorod test. [e] PI = protective index (TD_{50}/ED_{50}). [f] Tox = behavioral toxicity.

Table 35. Pharmacological activities of C(2)-substituted *N*-benzyl butanamides (Site F) in mice (mg/kg) and rats (mg/kg) at the NINDS ASP



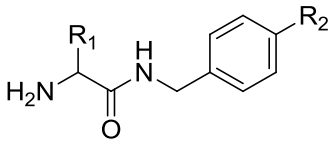
		Mice (ip)[a]			Rat (po)[b]		
Cmpd No.	**X**	**MES,[c] ED_{50}**	**Tox,[d] TD_{50}**	**PI[e]**	**MES,[c] ED_{50}**	**Tox,[f] TD_{50}**	**PI[e]**
(*R*)-**95**	NH_2	18 [0.25] (10–25)	80 [0.25] (65–95)	4.4	ND[g]	ND[g]	
282	H	>30, <100 [0.5]	>100, <300 [0.5]		>30 [0.25–4.0]	>30 [0.25-4.0]	
(*S*)-**283**	CH_3	>100, <300 [0.5]	~300 [0.5]		>30 [0.25–4.0]	>30 [0.25-4.0]	
(*R,S*)-**283**	CH_3	56 [0.25] (45–69)	165 [0.25] (148–180)	2.9	51 [0.5] (35–71)	>500	<9.8
(*R*)-**284**	OH	>30, <100 [0.5]	>100, <300 [0.5]		>30 [0.25–4.0]	>30 [0.25-4.0]	

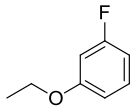
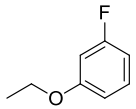
[a] The compounds were administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg. A dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets). Numbers in parentheses are 95% confidence intervals. [b] The compounds were administered orally to adult male albino Sprague Dawley rats under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg. [c] MES = maximal electroshock seizure test. [d] TD_{50} value determined from the rotorod test. [e] PI = protective index (TD_{50}/ED_{50}). [f] Tox = behavioral toxicity. [g] ND = not determined.

2.5.4. Comparison of data acquired at UCB and the NINDS ASP

Lastly, we expanded the list of PAADs evaluated at UCB Pharma and the NINDS ASP (Table 18) to include the 4'-*N*-benzyl-chimeric PAADs (*R*)-**255** and (*R*)-**258** (Table 36). In total, six PAADs that displayed excellent activity in the MES test ((*R*)-**61**, (*R*)-**95**, (*R*)-**98**, (*R*)-**99**, (*R*)-**255**, and (*R*)-**258**) were evaluated at both UCB Pharma and the NINDS ASP. The addition of (*R*)-**255** and (*R*)-**258** further supports the consistency of reported MES activities from the two testing facilities.

Table 36. Comparison of the pharmacological activities of PAADs evaluated in mice (mg/kg) at UCB and the NINDS ASP



			UCB[a]			NINDS-ASP[b]		
Cmpd No.	**R_1**	**R_2**	**MES,[c] ED_{50}**	**Tox,[d] TD_{50}**	**PI[e]**	**MES,[c] ED_{50}**	**Tox,[d] TD_{50}**	**PI[e]**
(*R*)-**28**	LCM	H	3.3	19	5.8	4.5 [0.5] (3.7–5.5)	27 [0.25] (26–28)	6.0
(*R*)-**61**	CH_2OCH_3	H	34	>117	>3.4	48 [0.25] (40–61)	>30, <100 [0.25]	
(*R*)-**255**[f,g]	CH_2OCH_3		8.9	46	5.2	~10 [0.5]	>30, <100 [0.5]	
(*R*)-**95**	CH_2CH_3	H	16	46	2.9	18 [0.25] (10–25)	80 [0.25] (65–95)	4.4
(*R*)-**98**	$CH(CH_3)_2$	H	16 (MAD)[h]	47	2.9	15 [0.25] (13–18)	70 [0.25] (63–80)	4.8
(*R*)-**258**	$CH(CH_3)_2$		12	ND[i]		>10, <30 [0.5]	>30, <100 [0.5]	
(*R*)-**99**	$C(CH_3)_3$	H	13	ND[i]		14 [0.25] (11–17)	66 [0.25] (58–73)	4.7

[a] The compounds were administered intraperitoneally to adult male NMRI mice under the auspices of UCB. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration. [b] The compounds were administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg. A dose-response curve was generated for all compounds that displayed sufficient activity and the dose-effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets). Numbers in parentheses are 95% confidence intervals. [c] MES = maximal electroshock seizure test. [d] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [e] PI = protective index (TD_{50}/ED_{50}). [f] 6 Hz (44 mA) ED_{50} = 58 mg/kg determined by UCB. [g] 6 Hz (32 mA) ED_{50} = <30 mg/kg determined by the NINDS ASP. [h] MAD = minimal active dose. [i] ND = not determined.

3. Conclusions

We evaluated more than 40 PAADs and analogs of PAADs in whole animal models of epilepsy and NP to optimize seizure protection and pain attenuation. We examined 4'-*N*-benzyl substituted C(3)-O-methoxy and C(2)-hydrocarbon PAADs using electron-withdrawing substituents, electron-donating substituents, and chimeric substituents, and also evaluated analogs of *N*-benzyl 2-amino-3-methylbutanamide (*R*)-**98** and *N*-benzyl 2-aminobutanamide (*R*)-**99**. The SAR suggested that 4'-*N*-benzyl substitution of C(3)-*O*-methoxy PAADs ((*R*)-**126**, -**240**–**242**) moderately improves anticonvulsant activity in the MES test compared with the parent PAAD (*R*)-**61**, but the substitutions do not provide any advantage over the corresponding FAAs ((*R*)-**123**, -**364**–**366**) (Table 22). Correspondingly, 4'-*N*-benzyl substitution of C(2)-hydrocarbon PAADs with electron-withdrawing groups provided compounds with excellent activity (<30 mg/kg), but most 4'-*N*-benzyl substituted C(2)-isopropyl and C(2)-*tert*-butyl PAADs did not provide an advantage over the unsubstituted parent compounds (*R*)-**98** and (*R*)-**99**. We discovered that the pharmacological activities of C(2)-hydrocarbon PAADs was highly dependent upon the electronic properties of the 4'-substitutent. This finding was unexpected since the anticonvulsant activity of 4'-*N*-benzyl substituted FAAs was independent of electronic factors.[102]

The 4'-chimeric C(3)-O-methoxy PAAD (*R*)-**255** and C(2)-isopropyl PAAD (*R*)-**258** displayed superb anticonvulsant activity (<15 mg/kg). This SAR pattern is unique to the PAADs because in the FAA series, C(2)-hydrocarbon 4'-chimeric FAAs ((*R*)-**370** and (*R*)-**371**) are devoid of anticonvulsant (>300 mg/kg), while the C(3)-O-methoxy 4'-chimeric FAAs ((*R*)-**367** and (*R*)-**368**) remain extremely active.[103] Finally, we observed that C(2)-*tert*-butyl 4'-chimeric PAADs displayed only moderate activity (>30, <100 mg/kg).

We determined the SAR for C(2)-isopropyl analogs with successive *N*-methylation (SAAD (*R*)-**269** and TAAD (*R*)-**270**), and found that the unmethylated derivative (PAAD (*R*)-**98**) provided the greatest seizure protection (ED_{50} = 15 mg/kg).

Next, we evaluated the importance of the PAAD structural backbone for bioactivity. Evaluation of *N*-benzyl 2-amino-3-methylbutanamide analogs at six sites (Sites A–F) concluded that the amide bond (Sites A and B) is necessary for anticonvulsant activity. However, changes in the length of the methylene linker between the amide bond and the aromatic ring (Site C), the position of substitution on the benzylamide ring (i.e., 2'-, 3'-, and 4'-substitution; Site D), reduction of the aromatic ring (Site E), and substitution for the C(2)-amino group (Site F) all provided compounds that showed anticonvulsant activity, indicating that these structural units are important, but perhaps dispensable, for drug function. Consideration of anticonvulsant activity, neurotoxicity, and the protective index of these C(2)-isopropyl PAAD structural analogs indicated that the optimal activity occurred when the linker was n =1 or n = 2, there was benzylamide group, and where the ring was substituted either at the 2'- or 3'-positions. Of the other C(2)-groups investigated, the amino group was most active, but further analysis of the C(2)-methyl group ((*R*)-isomer) and other analogs should be conducted to show that the C(2)-amino group is not necessary for activity.

When considering seizure protection, toxicity, the protective indices, and the amount of compound administered in *μ*mol/kg, optimized PAADs (*R*)-**255** and (*R*)-**279** displayed superior anticonvulsant activity that may rival the therapeutic capabilities of (*R*)-**28**. The activity of (*R*)-**255** (ED_{50} = 8.9 mg/kg) was noteworthy and is among the most active PAADs prepared. Using the conventional mg/kg dose it was 2-fold less active than (*R*)-**28**, but it exhibited nearly the same PI value as (*R*)-**28** (PI: (*R*)-**255**, 5.4; (*R*)-**28**, 5.8). When the ED_{50} values are converted to *μ*mol/kg, the difference in activities between (*R*)-**255** and (*R*)-**28** reduced to 1.4-fold (~30% increase). Similarly, the anticonvulsant activity of (*R*)-**279** (ED_{50} = 9.2 mg/kg) was 2-fold less potent than (*R*)-**28** (ED_{50} = 4.5 mg/kg). However, there was an

approximate 2-fold decrease in neurotoxicity in mice (ip) (TD_{50} (mg/kg): (*R*)-**279**; 51; (*R*)-**28**; 27), resulting in similar PI values ((*R*)-**279**, 5.5; (*R*)-**28**; 5.8).

Collectively, this data, along with the finding that activity for hydrocarbon PAADs does not improve upon inclusion of a substituted heteroatom one atom removed from the C(2)-center, suggests that the C(2)-hydrocarbon PAADs have an unique SAR and possibly a pathway(s) of function that differ from FAAs and other PAADs. The sensitivity of the C(2)-hydrocarbon PAADs to the electronic properties of the 4'-*N*-benzyl substituent provides another difference in the SAR of this series of compounds compared with the findings reported for FAAs. We recognize that the PAADs and FAAs discussed here not only differ by the absence or presence of the *N*-terminal acetyl group, but also in the nature of the C(2)-substituent (e.g., hydrocarbon, CH_2OCH_3).

4. Experimental

4.1. General methods

Melting points were determined in open capillary tubes using a Thomas-Hoover melting point apparatus and are uncorrected. IR were recorded on an ATI Mattson Genesis FT-IR spectrometer. Absorption values are expressed in wavenumbers (cm^{-1}). Optical rotations were obtained on a Jasco P-1030 polarimeter at the sodium D line (589 nm) using a 1 dm path length cell. NMR spectra were recorded at 300 or 400 MHz (^1H) and 75 or 100 MHz (^{13}C) using TMS as an internal standard. Chemical shifts (δ) are reported in ppm from TMS. LRMS were recorded with a BioToF-II-Bruker Daltonics spectrometer by Drs. M. Crowe and S. Habibi at the University of North Carolina Department of Chemistry. The HRMS were recorded on a Bruker Apex-Q 12 Telsa FTICR spectrometer by Drs. M. Crowe and S. Habibi. Microanalyses were performed by Atlantic Microlab, Inc. (Norcross, GA). Reactions were monitored by analytical TLC plates (Aldrich, catalog no. Z12272-6, or

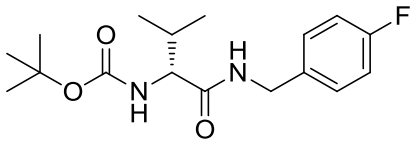
Dynamic Adsorbents Inc., catalog no. 84111) and analyzed with 254 nm light. The reaction mixtures were purified by MPLC (CombiFlash *Rf*) with self-packed columns (silica gel from Dynamic Adsorbents Inc., catalog no. 02826-25) or by flash column chromatography using silica gel (Dynamic Adsorbents Inc., catalog no. 02826-25). All chemicals and solvents were reagent grade and used directly from commercial sources without further purification. THF was distilled from blue sodium benzophenone ketyl. Yields reported are for purified products and were not optimized. All compounds were checked by TLC, ^{1}H and ^{13}C NMR, MS, and elemental analyses. The analytical results are within 0.40% of the theoretical value. The TLC, NMR, and analytical data confirmed the purity of the products was ≥95%.

General Procedure for the Preparation of *N*-Benzylamide Amino Acid Derivatives Using the Mixed Anhydride Coupling (MAC) Method (Method A). An anhydrous THF solution of carboxylic acid (0.5–2.0 M) was cooled to -78 ºC in a dry ice/acetone bath under an inert atmosphere (Ar or N_2), and NMM (1.3–1.5 equiv) was added. After the mixture was stirred (2–10 min), IBCF (1.1–1.5 equiv) was added leading to the precipitation of a white solid. The reaction was allowed to proceed for an additional 15–25 min, and then benzylamine (1.05–1.36 equiv) was added at -78 ºC. The reaction mixture was allowed to stir at room temperature (1.5 h), and then the insoluble salts were filtered. The organic layer was concentrated *in vacuo*, and the product was purified by column chromatography (SiO_2).

General Procedure for PAAD Preparation Using TFA Deprotection (Method B). TFA (15 equiv) was added to an anhydrous CH_2Cl_2 solution of the *N*-*t*-butoxycarbonyl protected *N*'-benzylamide PAAD (0.3 M) at room temperature. The solution was stirred (1 h) and then the solvent was evaporated *in vacuo*. The crude product was subjected either to an acidic workup or basic workup. Acidic: The crude product was diluted with CH_2Cl_2 and extracted with aqueous 1 M HCl (3x). The combined aqueous layers were washed with CH_2Cl_2 (2x),

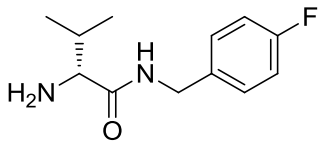
basified (pH 10–12) with aqueous 4 M NaOH, and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with brine (2x), dried (Na_2SO_4), evaporated *in vacuo*, and purified by column chromatography (SiO_2). Basic: The crude product was diluted with CH_2Cl_2 and washed with aqueous 1 M Na_2CO_3 (3x). The aqueous layers were combined and washed with CH_2Cl_2 (2x). All of the CH_2Cl_2 layers were combined and successively washed with H_2O (2x) and brine (2x), dried (Na_2SO_4), evaporated *in vacuo*, and purified by column chromatography (SiO_2).

4.2. Synthesis

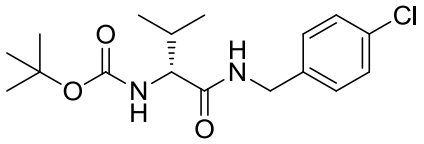


(*R*)-*N*-4'-(Fluoro)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-292). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (4.00 g, 18.42 mmol), NMM (2.63 mL, 23.95 mmol), IBCF (2.61 mL, 20.26 mmol), and 4-fluorobenzylamine (2.20 mL, 19.34 mmol) in anhydrous THF (185 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes) to give the desired compound (5.38 g, 90%) as a white solid: mp 129–130 ºC; $[\alpha]^{28}_D$ +10.1° (*c* 1.1, $CHCl_3$); R_f 0.85 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2856 (br), 1690, 1644, 1520, 1458, 1378, 1304, 1226, 1162, 1024, 928, 826, 693 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.92 (d, J = 7.0 Hz, CH(C**H$_3$**)CH_3), 0.95 (d, J = 7.0 Hz, CH(CH_3)C**H$_3$**), 1.41 (s, C(C**H$_3$**)$_3$), 2.10–2.20 (m, C**H**(CH_3)$_2$), 3.91 (dd, J = 6.6, 8.6 Hz, C**H**), 4.34–4.45 (m, NHC**H$_2$**), 5.06–5.14 (br d, OC(O)N**H**), 6.50–6.56 (br t, N**H**CH_2), 6.96–7.02 (m, 2 Ar**H**), 7.21–7.24 (m, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.9 (CH(**C**H_3)CH_3), 19.4 (CH(CH_3)**C**H_3), 28.3 (C(**C**H_3)$_3$), 30.6 (**C**H(CH_3)$_2$), 42.7 (NH**C**H_2), 60.3 (**C**H), 80.0 (**C**(CH_3)$_3$), 115.5 (d, J = 20.9 Hz, **C$_3$**), 129.3 (d, J

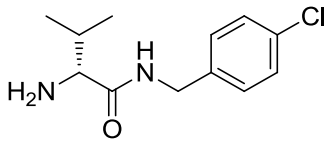
= 7.7 Hz, **C$_2$**), 133.9 (d, *J* = 3.1 Hz, **C$_1$**), 156.0 (O**C**(O)), 162.2 (d, *J* = 244.7 Hz, **C$_4$**), 171.7 (C**C**(O)); LRMS (ESI) 347.16 [M + Na^+] (calcd for $C_{17}H_{25}FN_2O_3Na^+$ 347.16); Anal. Calcd for $C_{17}H_{25}FN_2O_3$: C, 62.94; H, 7.77; F, 5.86; N, 8.64. Found: C, 63.17; H, 7.87; F, 5.76; N, 8.62.



(*R*)-*N*-4'-(Fluoro)benzyl 2-Amino-3-methylbutanamide ((*R*)-243). Utilizing Method B and using (*R*)-*N*-4'-(fluoro)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (4.50 g, 13.88 mmol), TFA (15.47 mL, 0.21 mol), and CH_2Cl_2 (46 mL) gave the crude product after acidic workup and further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (2.73 g, 87%) as a white solid: mp 86–87 ºC; $[\alpha]^{25}_D$ +32.9° (*c* 1.1, $CHCl_3$); R_f 0.47 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2724 (br), 1634, 1548, 1458, 1375, 1217, 1158, 1095, 1012, 827, 720 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (d, *J* = 6.8 Hz, CH(C**H$_3$**)CH_3), 0.99 (d, *J* = 6.8 Hz, CH(CH_3)C**H$_3$**), 1.35 (s, N**H$_2$**), 2.29–2.41 (m, C**H**(CH_3)$_2$), 3.27 (d, *J* = 3.2 Hz, C**H**), 4.39 (dd, *J* = 6.0, 15.0 Hz, NHC**H**H'), 4.45 (dd, *J* = 6.0, 14.8 Hz, NHCH**H'**), 6.97–7.03 (m, 2 Ar**H**), 7.23–7.27 (m, 2 Ar**H**), 7.64–7.72 (br t, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0 (CH(**C**H_3)CH_3), 19.7 (CH(CH_3)**C**H_3), 30.8 (**C**H(CH_3)$_2$), 42.4 (NH**C**H_2), 60.1 (**C**H), 115.4 (d, *J* = 20.9 Hz, **C$_3$**), 129.4 (d, *J* = 7.7 Hz, **C$_2$**), 134.5 (d, *J* = 3.9 Hz, **C$_1$**), 162.1 (d, *J* = 243.9 Hz, **C$_4$**), 174.3 (**C**(O)); LRMS (ESI) 225.13 [M + H^+] (calcd for $C_{12}H_{17}FN_2OH^+$ 225.13); Anal. Calcd for $C_{12}H_{17}FN_2O$: C, 64.26; H, 7.64; F, 8.47; N, 12.49. Found: C, 64.37; H, 7.73; F, 8.49; N, 12.47.

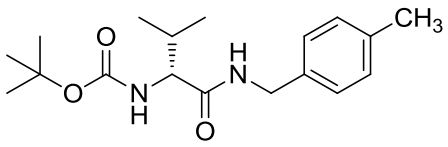


(*R*)-*N*-4'-(Chloro)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-293). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (3.00 g, 13.82 mmol), 4-methylmorpholine (1.97 mL, 17.96 mmol), isobutyl chloroformate (1.96 mL, 15.20 mmol), and 4-chlorobenzylamine (1.76 mL, 14.51 mmol) in anhydrous THF (140 mL) gave the crude product (3.43 g, 73%). The product was used immediately for the next step without further purification.

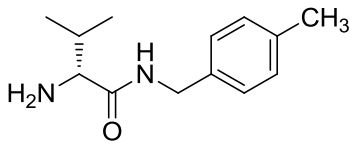


(*R*)-*N*-4'-(Chloro)benzyl 2-Amino-3-methylbutanamide ((*R*)-244). Utilizing Method B and using (*R*)-*N*-4'-(chloro)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (2.86 g, 8.41 mmol), TFA (6.09 mL, 82.02 mmol), and CH_2Cl_2 (18 mL) gave the crude product after acidic workup that was further purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (1.52 g, 76%) as a white solid: mp 72–73 ºC; $[\alpha]^{28.5}_D$ +26.7° (*c* 1.1, $CHCl_3$); R_f 0.26 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3116, 2732 (br), 1640, 1548, 1458, 1375, 1231, 1091, 1016, 801, 725 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.82 (d, *J* = 7.2 Hz, CH(C**H$_3$**)CH_3), 0.99 (d, *J* = 6.4 Hz, $CH(CH_3)$C**H$_3$**), 1.30 (s, N**H$_2$**), 2.28–2.37 (m, C**H**$(CH_3)_2$), 3.27 (d, *J* = 3.6 Hz, C**H**), 4.37 (dd, *J* = 6.0, 14.8 Hz, NHC**H**H'), 4.43 (dd, *J* = 6.0, 14.8 Hz, NHCH**H'**), 7.21 (d, *J* = 8.2 Hz, 2 Ar**H**), 7.28 (d, *J* = 8.2 Hz, 2 Ar**H**), 7.72–7.80 (br t, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0 (CH(**C**H_3)CH_3), 19.7 ($CH(CH_3)$**C**H_3), 30.8 (**C**H$(CH_3)_2$), 42.3 (NH**C**H_2), 60.1 (**C**H), 128.7, 129.1, 133.0, 137.3 (4 Ar**C**), 174.4 (**C**(O)); LRMS (ESI) 241.12 [M + H^+] (calcd for

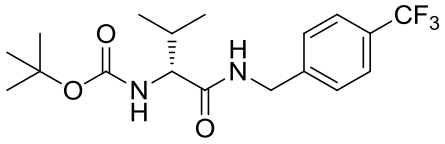
$C_{12}H_{17}ClN_2OH^+$ 241.12); Anal. Calcd for $C_{12}H_{17}ClN_2O$: C, 59.87; H, 7.12; Cl, 14.73; N, 11.64. Found: C, 59.97; H, 7.17; Cl, 14.60; N, 11.58.



(*R*)-*N*-4'-(Methyl)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-294). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (4.00 g, 18.42 mmol), NMM (2.63 mL, 23.95 mmol), IBCF (2.61 mL, 20.26 mmol), and 4-methylbenzylamine (2.46 mL, 19.34 mmol) in anhydrous THF (185 mL) gave the crude product that was purified by recrystallization from hot EtOAc to give the desired compound (3.95 g, 67%) as a white solid: mp 139–140 ºC; $[\alpha]^{28.5}{}_D$ +5.2° (*c* 1.1, $CHCl_3$); R_f 0.92 (1:1 EtOAc/hexanes); IR (nujol mull) 3372, 3265, 3188, 2934 (br), 1654, 1458, 1374, 1161, 726 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.92 (d, *J* = 7.0 Hz, CH(C**H$_3$**)CH_3), 0.96 (d, *J* = 7.0 Hz, $CH(CH_3)$C**H$_3$**), 1.42 (s, (C**H$_3$**)$_3$), 2.10–2.20 (m, C**H**$(CH_3)_2$), 2.32 (s, C**H$_3$**), 3.89–3.93 (m, C**H**), 4.34 (dd, *J* = 6.0, 14.8 Hz, NHC**H**H'), 4.42 (dd, *J* = 6.0, 14.8 Hz, NHCH**H'**), 5.07–5.14 (br d, OC(O)N**H**), 6.32–6.38 (br t, N**H**CH_2), 7.11 (d, *J* = 8.2 Hz, 2 Ar**H**), 7.15 (d, *J* = 8.2 Hz, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.8 (CH(**C**H_3)CH_3), 19.3 (CH(CH_3)**C**H_3), 21.1 (**C**H_3), 28.3 (C(**C**H_3)$_3$), 30.7 (**C**H$(CH_3)_2$), 43.2 (NH**C**H_2), 60.2 (**C**H), 79.9 (**C**$(CH_3)_3$), 127.7, 129.3, 135.0, 137.2 (**C$_6$**H_4), 155.9 (O**C**(O)), 171.5 (C**C**(O)); LRMS (ESI) 343.21 [M + Na^+] (calcd for $C_{18}H_{28}N_2O_3Na^+$ 343.21); Anal. Calcd for $C_{18}H_{28}N_2O_3$: C, 67.47; H, 8.81; N, 8.74. Found: C, 67.26; H, 8.81; N, 8.78.

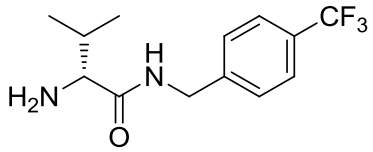


(*R*)-*N*-4'-(Methyl)benzyl 2-Amino-3-methylbutanamide ((*R*)-245). Utilizing Method B and using (*R*)-*N*-4'-methylbenzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (3.50 g, 10.93 mmol), TFA (12.18 mL, 0.16 mol), and CH_2Cl_2 (36 mL) gave the crude product after acidic workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (1.70 g, 71%) as a white solid: mp 67–68 ºC; $[\alpha]^{28.5}_D$ +26.7° (*c* 1.1, $CHCl_3$); R_f 0.53 (1:10 MeOH/CH_2Cl_2); IR (nujol mull) 3117, 3064, 2914, 2856, 1639, 1458, 1375, 1305, 1229, 1162, 801, 723 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, *J* = 7.0 Hz, CH(C**H$_3$**)CH_3), 0.99 (d, *J* = 7.0 Hz, CH(CH_3)C**H$_3$**), 1.33 (s, N**H$_2$**), 2.29–2.40 (m, C**H**(CH_3)$_2$), 2.33 (s, ArC**H$_3$**), 3.27 (d, *J* = 4.0 Hz, C**H**), 4.38 (dd, *J* = 6.0, 14.8 Hz, NHC**H**H'), 4.44 (dd, *J* = 6.0, 14.8 Hz, NHCH**H'**), 7.13 (d, *J* = 8.4 Hz, 2 Ar**H**), 7.17 (d, *J* = 8.4 Hz, 2 Ar**H**), 7.54–7.62 (br t, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0 (CH(**C**H_3)CH_3), 19.8 (CH(CH_3)**C**H_3), 21.7 (Ar**C**H_3), 30.8 (**C**H(CH_3)$_2$), 42.8 (NH**C**H_2), 60.2 (**C**H), 127.8, 129.3, 135.6, 137.0 (**C$_6$**H_4), 174.2 (**C**(O)); HRMS (ESI) 221.1664 [M + H^+] (calcd for $C_{13}H_{20}N_2OH^+$ 221.1654); Anal. Calcd for $C_{13}H_{20}N_2O \cdot 0.05H_2O$: C, 70.57; H, 9.16; N, 12.66. Found: C, 70.19; H, 9.17; N, 12.57.



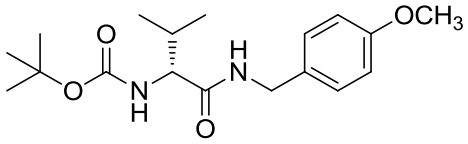
(*R*)-*N*-4'-(Trifluoromethyl)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-295). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (4.00 g, 18.42 mmol), NMM (2.63 mL, 23.95 mmol), IBCF (2.61 mL, 20.26 mmol), and 4-(trifluoromethyl)benzylamine (2.76 mL, 19.34 mmol) in anhydrous THF (185 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes) to give the desired product (4.40 g, 64%) as a white solid: mp 125–126 ºC;

$[\alpha]^{25}_{D}$ +8.5° (*c* 1.1, $CHCl_3$); R_f 0.90 (1:1 EtOAc/hexanes); IR (nujol mull) 2859 (br), 1659, 1528, 1458, 1375, 1332, 1247, 1166, 1120, 1070, 1021, 727 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.93 (d, *J* = 6.8 Hz, CH(C**H$_3$**)CH_3), 0.97 (d, *J* = 6.8 Hz, CH(CH_3)C**H$_3$**), 1.41 (s, CH(C**H$_3$**)$_3$), 2.15–2.20 (m, C**H**(CH_3)$_2$), 3.93 (dd, *J* = 6.6, 9.0 Hz, C**H**), 4.29–4.53 (m, NHC**H$_2$**), 5.12 (d, *J* = 7.6 Hz, OC(O)N**H**), 6.72–6.80 (br t, N**H**CH_2), 7.36 (d, *J* = 8.2 Hz, 2 Ar**H**), 7.65 (d, *J* = 8.2 Hz, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.9 (CH(**C**H_3)CH_3), 19.4 (CH(CH_3)**C**H_3), 28.2 (C(**C**H_3)$_3$), 30.5 (**C**H(CH_3)$_2$), 42.8 (NH**C**H_2), 60.3 (**C**H), 80.1 (**C**(CH_3)$_3$), 124.0 (q, *J* = 270.2 Hz, **C**F_3), 125.5 (q, *J* = 3.9 Hz, **C$_{3'}$**), 127.7 (**C$_{2'}$**), 129.7 (q, *J* = 31.8 Hz, **C$_{4'}$**), 142.3 (**C$_{1'}$**), 156.0 (O**C**(O)), 174.6 (C**C**(O)); LRMS (ESI) 397.13 [M + Na^+] (calcd for $C_{18}H_{25}F_3N_2O_3Na^+$ 397.13); Anal. Calcd for $C_{18}H_{25}F_3N_2O_3$: C, 57.74; H, 6.73; F, 15.22; N, 7.48. Found: C, 57.75; H, 6.78; F, 15.08; N, 7.62.

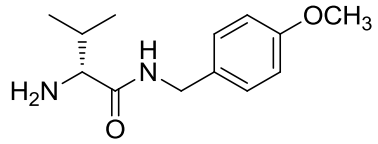


(*R*)-*N*-4'-(Trifluoromethyl)benzyl 2-Amino-3-methylbutanamide ((*R*)-246). Utilizing Method B and using (*R*)-*N*-4'-(trifluoromethyl)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (4.00 g, 10.69 mmol), TFA (11.91 mL, 0.16 mol), and CH_2Cl_2 (35 mL) gave the crude product after acidic workup that was further purified by flash column chromatography (SiO_2; 1:10–1:1 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (2.18 g, 75%) as a white solid: mp 86–87 ºC; $[\alpha]^{28.5}_{D}$ +26.0° (*c* 1.0, $CHCl_3$); R_f 0.37 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3335, 2961, 1641, 1512, 1327, 1161, 1107, 1067, 808 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, *J* = 7.2 Hz, CH(C**H$_3$**)CH_3), 1.00 (d, *J* = 7.2 Hz, CH(CH_3)C**H$_3$**), 1.32 (s, N**H$_2$**), 2.30–2.41 (m, C**H**(CH_3)$_2$), 3.30 (d, *J* = 4.0 Hz, C**H**), 4.47 (dd, *J* = 6.0, 15.4 Hz, NHC**H**H'), 4.53 (dd, *J* = 6.0, 15.4 Hz, NHCH**H'**), 7.39 (d, *J* = 7.6 Hz, 2 Ar**H**), 7.57 (d, *J* = 7.6 Hz, 2 Ar**H**), 7.84–7.92 (br t, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$)

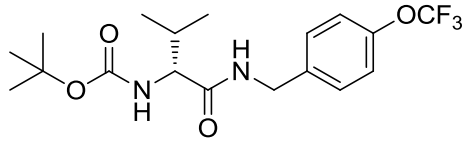
δ 16.0 (CH(**C**H_3)CH_3), 19.7 (CH(CH_3)**C**H_3), 30.8 (**C**H(CH_3)$_2$), 42.6 (NH**C**H_2), 60.2 (**C**H), 124.2 (q, J = 270.6 Hz, **C**F_3), 125.6 (q, J = 3.9 Hz, **C$_{3'}$**), 127.9 (**C$_{2'}$**), 129.6 (q, J = 31.8 Hz, **C$_{4'}$**), 142.9 (**C$_{1'}$**), 174.6 (**C**(O)); LRMS (ESI) 275.14 [M + H^+] (calcd for $C_{13}H_{17}F_3N_2OH^+$ 275.14); Anal. Calcd for $C_{13}H_{17}F_3N_2O$: C, 56.93; H, 6.25; F, 20.78; N, 10.21. Found: C, 57.06; H, 6.36; F, 20.61; N, 10.27.



(*R*)-*N*-4'-(Methoxy)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-296). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (4.00 g, 18.42 mmol), NMM (2.63 mL, 23.95 mmol), IBCF (2.61 mL, 20.26 mmol), and 4-methoxybenzylamine (2.51 mL, 19.34 mmol) in anhydrous THF (185 mL) gave the crude product that was purified by recrystallization from hot EtOAc/hexanes to give the desired compound (4.77 g, 77%) as pale yellow needles: mp 121–122 ºC; $[\alpha]^{28.5}_D$ +4.2° (*c* 1.0, $CHCl_3$); R_f 0.88 (1:1 EtOAc/hexanes); IR (nujol mull) 3163, 2858 (br), 1653, 1458, 1375, 1302, 1247, 1165, 1029, 726 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (d, J = 6.8 Hz, CH(C**H$_3$**)CH_3), 0.94 (d, J = 6.8 Hz, CH(CH_3)C**H$_3$**), 1.40 (s, (C**H$_3$**)$_3$), 2.04–2.22 (m, C**H**(CH_3)$_2$), 3.77 (OC**H$_3$**), 3.93–3.97 (br t, C**H**), 4.28 (dd, J = 5.6, 14.4 Hz, NHC**H**H'), 4.39 (dd, J = 5.6, 15.0 Hz, NHCH**H'**), 5.27 (d, J = 8.8 Hz, OC(O)N**H**), 6.68–6.76 (br t, N**H**CH_2), 6.82 (d, J = 8.6 Hz, 2 Ar**H**), 7.17 (d, J = 8.6 Hz, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0 (CH(**C**H_3)CH_3), 19.3 (CH(CH_3)**C**H_3), 28.3 (C(**C**H_3)$_3$), 30.9 (**C**H(CH_3)$_2$), 42.8 (NH**C**H_2), 55.2 (O**C**H_3), 60.1 (**C**H), 79.7 (**C**(CH_3)$_3$), 114.0, 129.0, 130.3 (3 Ar**C**), 155.9 (O**C**(O)), 158.9 (1 Ar**C**), 171.6 (C**C**(O)); HRMS (ESI) 469.1108 [M + Cs^+] (calcd for $C_{18}H_{28}N_2O_4Cs^+$ 469.1103); Anal. Calcd for $C_{18}H_{28}N_2O_4 \cdot 0.08H_2O$: C, 64.00; H, 8.40; N, 8.29. Found: C, 63.63; H, 8.47; N, 8.13.

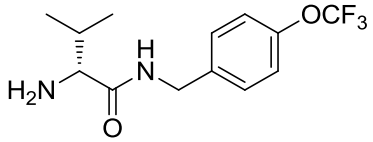


(*R*)-*N*-4'-(Methoxy)benzyl 2-Amino-3-methylbutanamide ((*R*)-247). Utilizing Method B and using (*R*)-*N*-4'-methoxybenzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (4.50 g, 13.38 mmol), TFA (14.91 mL, 0.20 mol), and CH_2Cl_2 (45 mL) gave the crude product after acidic workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (3.04 g, 96%) as a white solid: mp 81–82 ºC; $[\alpha]^{28.5}_D$ +25.5° (*c* 1.1, $CHCl_3$); R_f 0.42 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2728 (br), 1635, 1547, 1458, 1375, 1305, 1233, 1169, 1105, 1022, 840, 722 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, *J* = 7.0 Hz, CH(C**H$_3$**)CH_3), 0.99 (d, *J* = 7.0 Hz, CH(CH_3)C**H$_3$**), 1.33 (s, N**H$_2$**), 2.28–2.40 (m, C**H**(CH_3)$_2$), 3.26 (d, *J* = 3.6 Hz, C**H**), 3.79 (s, OC**H$_3$**), 4.36 (dd, *J* = 5.6, 14.6 Hz, NHC**H**H'), 4.41 (dd, *J* = 6.0, 14.6 Hz, NHCH**H'**), 6.86 (d, *J* = 8.6 Hz, 2 Ar**H**), 7.21 (d, *J* = 8.6 Hz, 2 Ar**H**), 7.52–7.58 (br t, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0 (CH(**C**H_3)CH_3), 19.7 (CH(CH_3)**C**H_3), 30.8 (**C**H(CH_3)$_2$), 42.5 (NH**C**H_2), 55.3 (O**C**H_3), 60.2 (**C**H), 114.0, 129.1, 130.8. 158.9 (4 Ar**C**), 174.1 (**C**(O)); LRMS (ESI) 237.17 [M + H^+] (calcd for $C_{13}H_{20}N_2O_2H^+$ 237.17); Anal. Calcd for $C_{13}H_{20}N_2O_2$: C, 66.07; H, 8.53; N, 11.85. Found: C, 65.94; H, 8.35; N, 11.60.



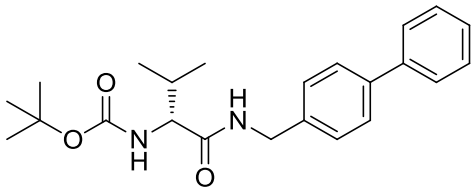
(*R*)-*N*-4'-(Trifluoromethoxy)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-297). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (4.30 g, 19.80 mmol), NMM (2.83 mL, 25.75 mmol), IBCF (2.81 mL, 21.78 mmol), and 4-(trifluoromethoxy)benzylamine (3.17 mL, 20.79 mmol) in anhydrous THF (200 mL) gave

the crude product that was purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes) to give the desired product (5.17 g, 67%) as a white solid: mp 101–102 ºC; $[\alpha]^{25}_D$ +7.6° (*c* 1.0, $CHCl_3$); R_f 0.94 (1:1 EtOAc/hexanes); IR (nujol mull) 2934 (br), 1652, 1524, 1458, 1375, 1276, 1222, 1159, 1021, 923, 843, 721 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.93 (d, *J* = 6.8 Hz, CH(C**H**$_3$)CH_3), 0.97 (d, *J* = 6.8 Hz, $CH(CH_3)$C**H**$_3$), 1.41 (C(C**H**$_3$)$_3$), 2.10–2.22 (m, C**H**$(CH_3)_2$), 3.91 (dd, *J* = 6.6, 8.6 Hz, C**H**), 4.43 (d, *J* = 5.6 Hz, NHC**H**$_2$), 5.12–5.22 (br d, N**H**CH), 6.56–6.62 (br t, N**H**CH_2), 7.15 (d, *J* = 8.2 Hz, 2 Ar**H**), 7.28 (d, *J* = 8.2 Hz, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.9 (CH(**C**H_3)CH_3), 19.4 ($CH(CH_3)$**C**H_3), 28.2 (C(**C**$H_3)_3$), 30.4 (**C**H$(CH_3)_2$), 42.6 (NH**C**H_2), 60.3 (**C**H), 80.1 (**C**$(CH_3)_3$), 120.4 (q, *J* = 255.6 Hz, **C**F_3), 121.1, 129.0, 136.9 (3 Ar**C**), 148.5 (**C**OCF_3), 156.0 (O**C**(O)); 171.8 (C**C**(O)); LRMS (ESI) 413.12 [M + Na^+] (calcd for $C_{18}H_{25}F_3N_2O_4Na^+$ 413.12); Anal. calcd. for $C_{18}H_{25}F_3N_2O_4$: C, 55.38; H, 6.45; F, 14.60; N, 7.18. Found: C, 55.45; H, 6.51; F, 14.65; N, 7.29.

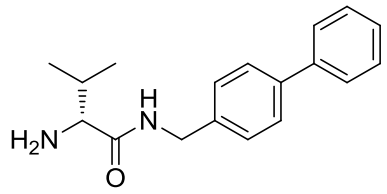


(*R*)-*N*-4'-(Trifluoromethoxy)benzyl 2-Amino-3-methylbutanamide ((*R*)-248). Utilizing Method B and using (*R*)-*N*-4'-(trifluoromethoxy)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (3.20 g, 8.20 mmol), TFA (9.14 mL, 1.23 mol), and CH_2Cl_2 (27 mL) gave the crude product after acidic workup and further purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (1.33 g, 56%) as a pale yellow solid: mp 63–64 ºC; $[\alpha]^{28.5}_D$ +26.6° (*c* 1.0, $CHCl_3$); R_f 0.47 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2906 (br), 1638, 1510, 1459, 1373, 1269, 1141, 1019, 889, 323, 728 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, *J* = 7.2 Hz, CH(C**H**$_3$)CH_3), 1.00 (d, *J* = 7.2 Hz, $CH(CH_3)$C**H**$_3$), 1.36 (s, N**H**$_2$), 2.33–2.41 (m, C**H**$(CH_3)_2$), 3.30 (d, *J* = 3.2 Hz, C**H**), 4.40–4.51 (m, NHC**H**$_2$), 7.17 (d, *J* = 8.4 Hz, 2 Ar**H**), 7.31 (d, *J* = 8.4 Hz, 2 Ar**H**),

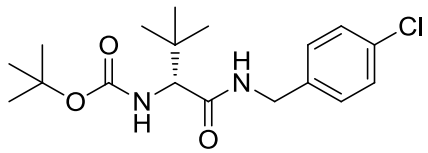
7.70–7.80 (br t, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.9 (CH(**C**H_3)CH_3), 19.7 (CH(CH_3)**C**H_3), 30.7 (**C**H(CH_3)$_2$), 42.3 (NH**C**H_2), 60.1 (**C**H), 120.4 (q, J = 255.6 Hz, **C**F_3), 121.1, 129.1, 137.5 (3 Ar**C**), 148.4 (**C**OCF_3), 174.4 (**C**(O)); LRMS (ESI) 291.15 [M + H^+] (calcd for $C_{13}H_{17}F_3N_2O_2H^+$ 291.15); Anal. calcd. for $C_{13}H_{17}F_3N_2O_2$: C, 53.79; H, 5.90; F, 19.63; N, 9.65. Found: C, 53.96; H, 5.92; F, 19.46; N, 9.66.



(*R*)-*N*-(Biphenyl-4'-yl)methyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-298). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (2.70 g, 12.44 mmol), NMM (1.78 mL, 16.17 mmol), IBCF (1.76 mL, 13.68 mmol), and 4-(phenyl)benzylamine (2.39 g, 13.06 mmol) in anhydrous THF (125 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 1:10-1:1 EtOAc/hexanes) to give the desired compound (3.25 g, 68%) as a white solid: mp 144–145 ºC; $[\alpha]^{25}_D$ –5.1° (*c* 1.2, CH_2Cl_2); R_f 0.81 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2911 (br), 1650, 1457, 1375, 1162, 725 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93–0.95 (d, J = 6.6 Hz, CH(C**H$_3$**)CH_3), 0.98–0.99 (d, J = 6.6 Hz, CH(CH_3)C**H$_3$**), 1.43 (s, C(C**H$_3$**)$_3$), 2.14–2.26 (m, C**H**(CH_3)$_2$), 3.91–3.94 (dd, J = 6.4, 8.8 Hz, C**H**), 4.49–4.50 (d, J = 5.6 Hz, NHC**H$_2$**), 5.02–5.09 (br d, OC(O)N**H**), 6.30–6.39 (br t, N**H**CH_2), 7.33–7.37 (m, 3 Ar**H**), 7.41–7.45 (m, 2 Ar**H**), 7.53–7.58 (m, 4 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0 (CH(**C**H_3)CH_3), 19.4 (CH(CH_3)**C**H_3), 28.3 (C(**C**H_3)$_3$), 30.8 (**C**H(CH_3)$_2$), 43.1 (NH**C**H_2), 60.2 (**C**H), 79.9 (**C**(CH_3)$_3$), 127.0, 127.3, 127.4, 128.1, 128.8, 137.2, 140.4, 140.7 (8 Ar**C**), 156.0 (O**C**(O)), 174.3 (C**C**(O)); HRMS (ESI) 515.1290 [M + Cs^+] (calcd for $C_{23}H_{30}N_2O_3Cs^+$ 515.1311); Anal. Calcd for $C_{23}H_{30}N_2O_3$: C, 72.22; H, 7.91; N, 7.32. Found: C, 72.08; H, 7.94; N, 7.34.

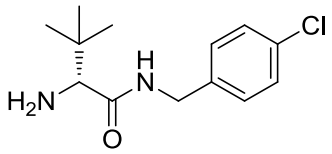


(*R*)-*N*-(Biphenyl-4'-yl)methyl 2-Amino-3-methylbutanamide ((*R*)-249).[234] Utilizing Method B and using (*R*)-*N*-(biphenyl-4'-yl)methyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (2.51 g, 6.57 mmol), TFA (7.32 mL, 98.50 mmol), and CH_2Cl_2 (22 mL) gave the crude product after basic workup that was further purified by flash column chromatography (SiO_2; 1:10–1:1 EtOAc/hexanes) to give the desired compound (1.81 g, 98%) as a white solid: mp 96–97 ºC; $[\alpha]^{25}_D$ +16.5° (*c* 1.0, CH_2Cl_2); R_f 0.28 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3139, 3089 (br), 2727, 1457, 1374, 1158, 1078, 963, 725 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.85–0.86 (d, *J* = 7.0 Hz, CH(C**H**$_3$)CH_3), 1.00–1.02 (d, *J* = 7.0 Hz, CH(CH_3)C**H**$_3$), 1.45 (s, N**H**$_2$), 2.33–2.42 (m, C**H**(CH_3)$_2$), 3.31–3.32 (d, *J* = 3.2 Hz, C**H**), 4.45–4.55 (m, NHC**H**$_2$), 7.32–7.37 (m, 3 Ar**H**), 7.43 (m, 2 Ar**H**), 7.56 (m, 4 Ar**H**), 7.62–7.68 (br t, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0 (CH(**C**H$_3$)CH_3), 19.8 (CH(CH_3)**C**H$_3$), 30.8 (**C**H(CH_3)$_2$), 42.8 (NH**C**H$_2$), 60.2 (**C**H), 127.0, 127.3, 127.4, 128.2, 128.8, 137.6, 140.3, 140.8 (8 Ar**C**), 174.3 (**C**(O)); HRMS (ESI) 283.1800 [M + H^+] (calcd for $C_{18}H_{22}N_2OH^+$ 283.1810); Anal. Calcd for $C_{18}H_{22}N_2O$•0.07CH_2Cl_2: C, 75.18; H, 7.73; N, 9.70. Found: C, 75.08; H, 7.68; N, 9.61.



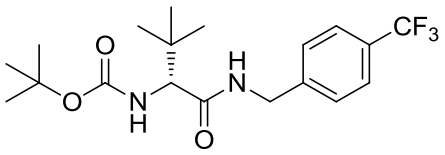
(*R*)-*N*-4'-(Chloro)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3,3-dimethylbutanamide ((*R*)-299). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3,3-dimethylbutanoic acid (2.22 g, 9.60 mmol), NMM (1.37 mL, 12.49 mmol), IBCF (1.36 mL, 10.56 mmol), and 4-chlorobenzylamine (1.23 mL, 10.08 mmol) in anhydrous THF (100 mL) gave the crude

product that was purified by flash column chromatography (SiO_2; 1:10–1:1 EtOAc/hexanes) to give the desired compound (2.76 g, 81%) as a white solid: mp 83–84 ºC; $[\alpha]^{25}_D$ –7.3° (*c* 1.2, CH_2Cl_2); R_f 0.21 (1:10 EtOAc/hexanes); IR (nujol mull) 2942 (br), 1658, 1458, 1373, 1243, 1170, 1082, 1011, 911, 805, 725 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.99 (s, CC(C**H**$_3$)$_3$), 1.40 (s, OC(C**H**$_3$)$_3$), 3.89 (d, *J* = 8.6 Hz, C**H**), 4.32 (dd, *J* = 6.0, 14.8 Hz, NHC**H**H'), 4.42 (dd, *J* = 6.0, 14.8 Hz, NHCH**H'**), 5.31 (d, *J* = 8.6 Hz, OC(O)N**H**), 6.48–6.56 (br t, N**H**CH_2), 7.19 (d, *J* = 8.4 Hz, 2 Ar**H**), 7.26 (d, *J* = 8.4 Hz, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.6 (CC(**C**H_3)$_3$), 28.3 (OC(**C**H_3)$_3$), 34.4 (C**C**$(CH_3)_3$), 42.7 (NH**C**H_2), 62.4 (**C**H), 79.8 (O**C**$(CH_3)_3$), 128.8, 129.1, 133.2, 136.6 (4 Ar**C**), 156.0 (O**C**(O)), 171.1 (C**C**(O)); LRMS (ESI) 377.10 [M + Na^+] (calcd for $C_{18}H_{27}ClN_2O_3Na^+$ 377.10); Anal. Calcd for $C_{18}H_{27}ClN_2O_3$: C, 60.92; H, 7.67; Cl, 9.99; N, 7.89. Found: C, 61.19; H, 7.70; Cl, 9.73; N, 7.78.

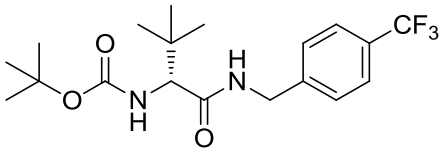


(*R*)-*N*-4'-(Chloro)benzyl 2-Amino-3,3-dimethylbutanamide ((*R*)-250). Utilizing Method B and using (*R*)-*N*-4'-(chloro)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3,3-dimethylbutanamide (2.50 g, 7.06 mmol), TFA (7.86 mL, 0.11 mol), and CH_2Cl_2 (23 mL) gave the crude product after acidic workup that was further purified by flash column chromatography (SiO_2; 1:100–1:10 MeOH/CH_2Cl_2) to give the desired compound (1.31 g, 73%) as a white solid: mp 78–79 ºC; $[\alpha]^{25}_D$ +14.2° (*c* 1.0, CH_2Cl_2); R_f 0.23 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2934 (br), 1457, 1374, 1160, 1082, 725 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.00 (s, C(C**H**$_3$)$_3$), 1.47 (s, N**H**$_2$), 3.14 (s, C**H**), 4.36 (1/2 ABq, *J* = 17.6 Hz, NHC**H**H'), 4.43 (1/2 ABq, *J* = 17.6 Hz, NHCH**H'**), 7.22 (d, *J* = 8.6 Hz, 2 Ar**H**), 7.29 (d, *J* = 8.6 Hz, 2 Ar**H**); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.0 (C(**C**H_3)$_3$), 34.4 (**C**$(CH_3)_3$), 42.6 (NH**C**H_2), 64.6 (**C**H), 128.9, 129.4, 133.3, 137.4 (4 Ar**C**), 173.7 (**C**(O)); HRMS (ESI) 255.1256 [M + H^+] (calcd for $C_{13}H_{19}ClN_2OH^+$ 255.1264);

Anal. Calcd for $C_{13}H_{19}ClN_2O$: C, 61.29; H, 7.52; Cl, 13.92; N, 11.00. Found: C, 61.35; H, 7.58; Cl, 13.76; N, 10.90.

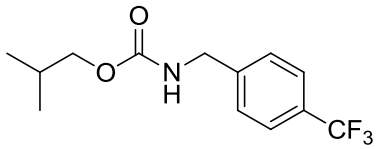


(*R*)-*N*-4'-(Trifluoromethyl)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3,3-dimethylbutanamide ((*R*)-300). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3,3-dimethylbutanoic acid (2.50 g, 10.82 mmol), NMM (1.55 mL, 14.06 mmol), IBCF (1.53 mL, 11.90 mmol), and 4-(trifluoromethyl)benzylamine (1.62 mL, 11.36 mmol) in anhydrous THF (110 mL) gave the crude product that was purified twice by flash column chromatography (SiO_2; 1:1 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give a ~4:1 mixture of (*R*)-*N*-4'-(trifluoromethyl)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3,3-dimethylbutanamide and *N*-4'-(trifluoromethyl)benzyl (isobutoxycarbonyl)carbamate (2.77 g, 66%) as a white solid.

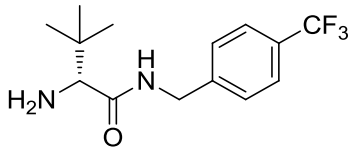


(*R*)-*N*-4'-(Trifluoromethyl)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3,3-dimethylbutanamide ((*R*)-300): R_f 0.81 (1:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 (s, CC(C**H$_3$**)$_3$), 1.37 (s, OC(C**H$_3$**)$_3$), 3.98 (d, J = 9.2 Hz, C**H**), 4.39 (dd, J = 5.8, 15.6 Hz, NHC**H**H'), 4.48 (dd, J = 5.8, 15.6 Hz, NHCH**H'**), 5.38 (d, J = 9.2 Hz, OC(O)N**H**), 7.00–7.08 (br t, N**H**CH$_2$), 7.35 (d, J = 7.8 Hz, 2 Ar**H**), 7.52 (d, J = 7.8 Hz, 2 Ar**H**). ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.6 (CC(**C**H$_3$)$_3$), 28.2 (OC(**C**H$_3$)$_3$), 34.3 (C**C**(CH$_3$)$_3$), 42.8 (NH**C**H$_2$), 62.3 (**C**H), 79.8 (O**C**(CH$_3$)$_3$), 124.0 (q, J = 270.3 Hz, **C**F$_3$), 125.4 (q, J = 3.1 Hz, **C$_{3'}$**), 127.7 (**C$_{2'}$**), 129.5 (q, J =

32.5 Hz, $\mathbf{C_{4'}}$), 142.3 ($\mathbf{C_{1'}}$), 156.1 (O**C**(O)), 171.5 (C**C**(O)); HRMS (ESI) 521.1028 [M + Cs^+] (calcd for $C_{19}H_{27}F_3N_2O_3Cs^+$ 521.1044).

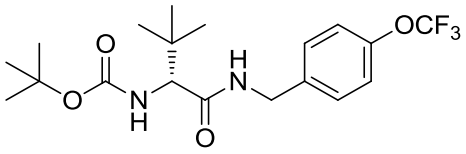


***N*-4'-(Trifluoromethyl)benzyl (isobutoxycarbonyl)carbamate:** R_f 0.85 (1:1 EtOAc/hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (d, J = 6.0 Hz, CH(C$\mathbf{H_3}$)$_2$), 1.80–1.98 (m, C**H**(CH_3)$_2$), 3.87 (d, J = 6.4 Hz, OC$\mathbf{H_2}$), 4.37–4.51 (m, NHC$\mathbf{H_2}$), 7.40 (d, J = 8.2 Hz, 2 Ar**H**), 7.58 (d, J = 8.2 Hz, 2 Ar**H**). ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.9 (CH(**C**H_3)$_2$), 28.0 (**C**H(CH_3)$_2$), 44.4 (NH**C**H_2), 71.3 (O**C**H_2), 124.1 (q, J = 270.2 Hz, **C**F_3), 125.5 (q, J = 3.9 Hz, $\mathbf{C_{3'}}$), 127.5 ($\mathbf{C_{2'}}$), 129.7 (q, J = 31.8 Hz, $\mathbf{C_{4'}}$), 142.9 ($\mathbf{C_{1'}}$), 156.9 (O**C**(O)).

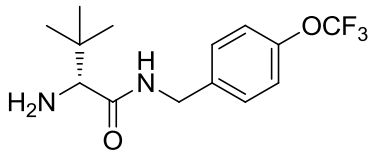


(*R*)-*N*-4'-(Trifluoromethyl)benzyl 2-Amino-3,3-dimethylbutanamide ((*R*)-251). Utilizing Method B and using (*R*)-*N*-4'-(trifluoromethyl)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3,3-dimethylbutanamide (2.50 g, 6.44 mmol), TFA (7.18 mL, 0.97 mol), and CH_2Cl_2 (21 mL) gave the crude product after acidic workup and further purified by flash column chromatography (SiO_2; 1:10–1:1 EtOAc/hexanes) to give the desired compound (0.68 g, 37%) as a white solid: mp 90–91 ºC; $[\alpha]^{25}_D$ +17.2° (*c* 1.0, CH_2Cl_2); R_f 0.14 (1:1 EtOAc/hexanes); IR (nujol mull) 3389, 3089, 2911 (br), 1648, 1557, 1459, 1373, 1335, 1264, 1165, 1112, 1021, 946, 820, 767 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (s, C(C$\mathbf{H_3}$)$_3$), 1.49 (s, N$\mathbf{H_2}$), 3.17 (s, C**H**), 4.44–4.54 (m, NHC$\mathbf{H_2}$), 7.29–7.37 (br t, N**H**), 7.40 (d, J = 7.6 Hz, 2 Ar**H**), 7.58 (d, J = 7.6 Hz, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.0 (C(**C**H_3)$_3$), 34.4

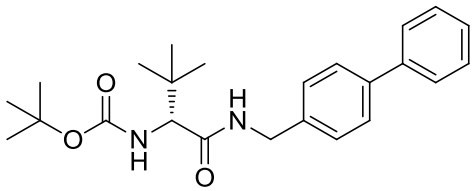
(**C**$(CH_3)_3$), 42.8 (NH**C**H_2), 64.5 (**C**H), 124.3 (q, *J* = 271.0 Hz, **C**F_3), 125.8 (q, *J* = 3.9 Hz, $\mathbf{C_{3'}}$), 128.2 ($\mathbf{C_{2'}}$), 129.8 (q, *J* = 31.8 Hz, $\mathbf{C_{4'}}$), 143.0 ($\mathbf{C_{1'}}$), 173.9 (**C**(O)); HRMS (+ESI) 289.1515 $[M+H]^+$ (calcd. for $C_{14}H_{19}F_3N_2OH^+$ 289.1528). Anal. Calcd for $C_{14}H_{19}F_3N_2O$: C, 58.32; H, 6.64; F, 19.77; N, 9.72. Found: C, 58.56; H, 6.57; F, 19.47; N, 9.73.



(*R*)-*N*-4'-(Trifluoromethoxy)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3,3-dimethylbutanamide ((*R*)-301). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3,3-dimethylbutanoic acid (2.00 g, 8.65 mmol), NMM (1.24 mL, 11.25 mmol), IBCF (1.23 mL, 9.52 mmol), and 4-(trifluoromethoxy)benzylamine (1.39 mL, 9.09 mmol) in anhydrous THF (90 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 1:10–1:1 EtOAc/hexanes) to give the desired compound (1.40 g, 40%) as a white solid: mp 62–63 ºC; $[\alpha]^{25}_D$ –4.9° (*c* 1.1, CH_2Cl_2); R_f 0.30 (1:10 EtOAc/hexanes); IR (nujol mull) 2725 (br), 1670, 1457, 1374, 1262, 1168, 1073, 1012, 924, 852, 725 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.00 (s, CC(C$\mathbf{H_3})_3$), 1.37 (s, OC(C$\mathbf{H_3})_3$), 3.96 (d, *J* = 9.2 Hz, C**H**), 4.34 (dd, *J* = 5.4, 14.8 Hz, NHC**H**H'), 4.45 (dd, *J* = 6.0, 14.8 Hz, NHCH**H'**), 5.36 (d, *J* = 9.2 Hz, OC(O)N**H**), 6.86–6.92 (br t, N**H**CH_2), 7.12 (d, *J* = 8.4 Hz, 2 Ar**H**), 7.27 (d, *J* = 8.4 Hz, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.6 (CC(**C**$H_3)_3$), 28.2 (OC(**C**$H_3)_3$), 34.3 (C**C**$(CH_3)_3$), 42.5 (NH**C**H_2), 62.3 (**C**H), 79.7 (O**C**$(CH_3)_3$), 120.4 (q, *J* = 255.6 Hz, **C**F_3), 121.0, 129.0, 137.0 (3 Ar**C**), 148.4 (**C**OCF_3), 156.0 (O**C**(O)), 171.3 (C**C**(O)); LRMS (ESI) 427.12 [M + Na^+] (calcd for $C_{19}H_{27}F_3N_2O_4Na^+$ 427.12); Anal. Calcd for $C_{19}H_{27}F_3N_2O_4$: C, 56.43; H, 6.73; F, 14.09; N, 6.93. Found: C, 56.54; H, 6.79; F, 14.06; N, 6.85.

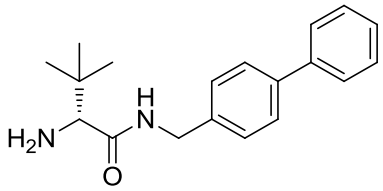


(*R*)-*N*-4'-(Trifluoromethoxy)benzyl 2-Amino-3,3-dimethylbutanamide ((*R*)-252). Utilizing Method B and using (*R*)-*N*-4'-(trifluoromethoxy)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3,3-dimethylbutanamide (2.00 g, 4.95 mmol), TFA (5.51 mL, 74.22 mmol), and CH_2Cl_2 (16 mL) gave the crude product after acidic workup that was further purified by flash column chromatography (SiO_2; 1:10–1:1 EtOAc/hexanes) to give the desired compound (0.93 g, 62%) as a white solid: mp 68–69 ºC; $[\alpha]^{25}_D$ +14.7° (*c* 1.1, CH_2Cl_2); R_f 0.29 (1:1 EtOAc/hexanes); IR (nujol mull) 2860 (br), 1649, 1457, 1374, 1267, 1166, 1018, 926, 838, 727 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.00 (s, C(C**H**$_3$)$_3$), 1.56 (s, N**H**$_2$), 3.14 (s, C**H**), 4.43 (d, *J* = 6.0 Hz, NHC**H**$_2$), 7.17 (d, *J* = 8.6 Hz, 2 Ar**H**), 7.23–7.29 (br s, N**H**), 7.31 (d, *J* = 8.6 Hz, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.8 (C(**C**H$_3$)$_3$), 34.3 (**C**(CH$_3$)$_3$), 42.4 (NH**C**H$_2$), 64.4 (**C**H), 120.5 (q, *J* = 255.6 Hz, **C**F$_3$), 121.2, 129.2, 137.5 (3 Ar**C**), 148.5 (**C**OCF$_3$), 173.6 (**C**(O)); HRMS (ESI) 305.1463 [M + H^+] (calcd for $C_{14}H_{19}F_3N_2O_2H^+$ 305.1477); Anal. Calcd for $C_{14}H_{19}F_3N_2O_2$: C, 55.26; H, 6.29; F, 18.73; N, 9.21. Found: C, 55.23; H, 6.14; F, 18.54; N, 9.18.



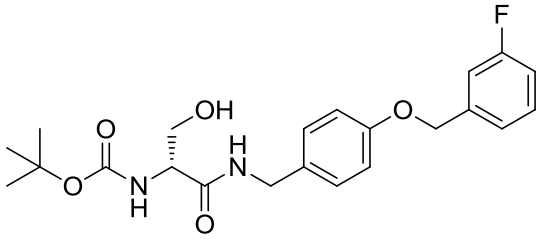
(*R*)-*N*-(Biphenyl-4'-yl)methyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3,3-dimethylbutanamide ((*R*)-302). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3,3-dimethylbutanoic acid (2.50 g, 10.82 mmol), 4-methylmorpholine (1.55 mL, 14.06 mmol), isobutyl chloroformate (1.53 mL, 11.90 mmol), and 4-phenylbenzylamine (2.08 g, 11.36

mmol) in anhydrous THF (110 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 5-50% EtOAc/hexanes) to give the desired compound (2.72 g, 63%) as a white solid: R_f 0.79 (1:1 EtOAc/hexanes); mp 125–126 ºC; IR (nujol mull) 2932 (br), 1650, 1527, 1459, 1374, 1249, 1173, 1078, 1009, 918, 824, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.02 (s, CC(C**H₃**)**₃**), 1.40 (s, OC(C**H₃**)**₃**), 3.93 (d, *J* = 8.5 Hz, C**H**), 4.40 (dd, *J* = 5.4, 14.7 Hz, NHC**H**H'), 4.50 (dd, *J* = 6.0, 14.7 Hz, NHCH**H'**), 5.37 (d, *J* = 8.5 Hz, OC(O)N**H**), 6.50–6.58 (br t, N**H**CH_2), 7.31–7.34 (m, 3 Ar**H**), 7.40–7.44 (m, 2 Ar**H**), 7.51–7.58 (m, 4 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 26.6 (CC(**C**H_3)$_3$), 28.3 (OC(**C**H_3)$_3$), 34.5 (C**C**$(CH_3)_3$), 43.2 (NH**C**H_2), 62.4 (**C**H), 79.7 (O**C**$(CH_3)_3$), 127.0, 127.2, 127.3, 128.2, 128.5, 137.0, 140.4, 140.7 (8 Ar**C**), 156.0 (O**C**(O)), 171.1 (C**C**(O)); LRMS (ESI) 419.18 [M + Na^+] (calcd for $C_{24}H_{32}N_2O_3Na^+$ 419.18); Anal. Calcd for $C_{24}H_{32}N_2O_3$: C, 72.70; H, 8.13; N, 7.06. Found: C, 72.97; H, 7.94; N, 6.94.

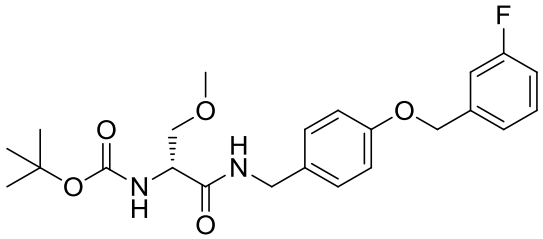


(*R*)-*N*-(Biphenyl-4'-yl)methyl 2-*N*'-Amino-3,3-dimethylbutanamide ((*R*)-253). Utilizing Method B and using (*R*)-*N*-(biphenyl-4'-yl)methyl 2-*N*'-(*t*-butoxycarbonyl)amino-3,3-dimethylbutanamide (1.53 g, 3.86 mmol), TFA (4.30 mL, 57.92 mmol), and CH_2Cl_2 (13 mL) gave the crude product after basic workup that was further purified by flash column chromatography (SiO_2; 1:10–1:1 EtOAc/hexanes) to give the desired compound (0.76 g, 67%) as a white solid: mp 75–76 ºC; $[\alpha]^{28.5}_D$ +14.4° (*c* 1.0, CH_2Cl_2); R_f 0.50 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2858 (br), 1635, 1458, 1375, 1158, 1093, 1010, 823, 728 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 1.03 (s, C(C**H₃**)**₃**), 1.50 (s, N**H₂**), 3.16 (s, C**H**), 4.44–4.54 (m, NHC**H₂**), 7.06–7.12 (br s, N**H**), 7.32–7.37 (m, 3 Ar**H**), 7.42–7.45 (m, 2 Ar**H**), 7.55–7.59 (m, 4

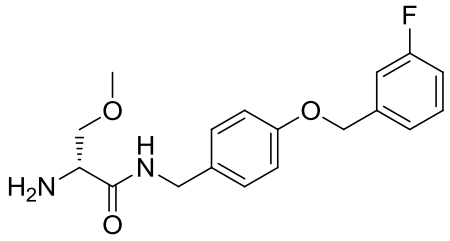
Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.8 (C(**C**H_3)$_3$), 34.3 (**C**(CH_3)$_3$), 42.9 (NH**C**H_2), 64.5 (**C**H), 127.1, 127.3, 127.4, 128.3, 128.8, 137.6, 140.3, 140.7 (8 Ar**C**), 173.5 (**C**(O)); LRMS (ESI) 297.20 [M + H^+] (calcd for $C_{19}H_{24}N_2OH^+$ 297.20); Anal. Calcd for $C_{19}H_{24}N_2O$: C, 76.99; H, 8.16; N, 9.45. Found: C, 77.14; H, 8.28; N, 9.49.



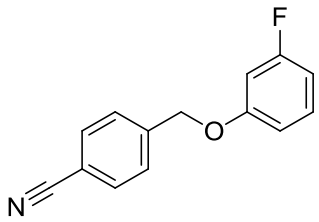
(*R*)-*N*-4'-((3''-Fluoro)benzyloxy)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-hydroxypropionamide ((*R*)-307).[103] Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-hydroxypropionic acid (6.00 g, 29.25 mmol), NMM (4.18 mL, 38.03 mmol), IBCF (4.15 mL, 32.18 mmol), and 4-((3-fluorobenzyloxy)benzylamine (7.09 g, 30.72 mmol) in anhydrous THF (300 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 5–50% EtOAc/hexanes followed by 10% MeOH/CH_2Cl_2) to give the desired compound (3.74 g, 30%) as a pale yellow solid: mp 92–93 ºC (lit.[103] mp 88–89 ºC); $[\alpha]^{25}_D$ +25.4° (*c* 0.7, $CHCl_3$) (lit.[103] $[\alpha]^{25.8}_D$ +25.8° (*c* 1.0, $CHCl_3$)); R_f 0.30 (1:1 EtOAc/hexanes); IR (nujol mull) 3221, 3175, 3106, 2946 (br), 1660, 1526, 1458, 1376, 1304, 1243, 1168, 1008, 866, 775, 726 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (s, (C**H$_3$**)$_3$), 3.36–3.48 (br m, C**H**H'OH), 3.61–3.72 (br m, CH**H'**OH), 4.00–4.10 (br m, C**H**), 4.11–4.20 (br m, O**H**), 4.30–4.52 (m, NHC**H$_2$**), 5.02 (s, OC**H$_2$**), 5.65 (d, *J* = 7.2 Hz, N**H**), 6.87–6.91 (m, 2 Ar**H**), 6.98–7.12 (m, 1 Ar**H**, N**H**), 7.16–7.18 (m, 4 Ar**H**), 7.30–7.36 (m, 1 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2 ((**C**H_3)$_3$), 42.8 (NH**C**H_2), 54.9 (**C**H), 62.8 (**C**H_2OH), 69.2 (**C**H_2O), 80.6 (**C**(CH_3)$_3$), 114.1 (d, *J* = 21.7 Hz, **C$_{4''}$** or **C$_{2''}$**), 114.8 (d, *J* = 20.9 Hz, **C$_{2''}$** or **C$_{4''}$**), 115.0 (**C$_1$**), 122.6 (d, *J* = 3.1 Hz, **C$_{6''}$**), 128.9 (Ar**C**), 130.1 (d, *J* = 7.8 Hz, **C$_{5''}$**), 130.4 (Ar**C**), 139.5 (d, *J* = 6.9 Hz, **C$_{1''}$**), 156.3 (O**C**(O)), 157.9 (**C$_4$**), 163.0 (d, *J* = 244.7 Hz, **C$_{3''}$**), 171.2 (C**C**(O)).



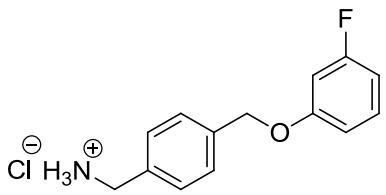
(*R*)-*N*-4'-((3''-Fluoro)benzyloxy)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methoxypropionamide ((*R*)-314).[103] Ag_2O (9.00 g, 38.86 mmol) was added to a CH_3CN solution (155 mL) of (*R*)-*N*-4'-((3''-fluoro)benzyloxy)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-hydroxypropionamide (3.25 g, 7.77 mmol), and CH_3I (4.84 mL, 77.72 mmol) at room temperature under Ar. The reaction mixture was stirred (4 d), filtered, and the filtrate concentrated *in vacuo*. The crude product was purified by flash column chromatography (SiO_2; 5–50% EtOAc/hexanes) to give the desired compound (3.22 g, 96%) as a white solid: mp 70–71 ºC (lit.[103] mp 68–70 ºC); $[\alpha]^{28.5}_D$ –15.9° (*c* 1.0, $CHCl_3$) (lit.[103] $[\alpha]^{24.3}_D$ –16.6° (*c* 1.0, $CHCl_3$)); : R_f 0.75 (1:1 EtOAc/hexanes); IR (nujol mull) 3307, 3255, 3157, 2929, 2858, 1647, 1521, 1458, 1375, 1247, 1167, 1048, 917, 776, 725 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, (CH_3)$_3$), 3.35 (s, OCH_3), 3.49 (dd, *J* = 6.2, 9.1 Hz, C**H**H'OCH_3), 3.82 (dd, *J* = 4.0, 9.1 Hz, CH**H'**OCH_3), 4.20–4.30 (br m, C**H**), 4.35–4.45 (m, $NHCH_2$), 5.04 (s, OCH_2), 5.38–5.42 (br d, OC(O)N**H**), 6.64–6.72 (br t, CC(O)N**H**), 6.91 (d, *J* = 8.8 Hz, 2 Ar**H**), 6.98–7.02 (m, 1 Ar**H**), 7.13–7.20 (m, 4 Ar**H**), 7.34 (q, *J* = 8.0 Hz, 1 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2 ((CH_3)$_3$), 42.9 ($NHCH_2$), 54.0 (**C**H), 59.1 (OCH_3), 69.2 (CH_2O), 72.1 (CH_2OCH_3), 80.3 ($C(CH_3)_3$), 114.1 (d, *J* = 21.7 Hz, $C_{4''}$ or $C_{2''}$), 114.8 (d, *J* = 20.9 Hz, $C_{2''}$ or $C_{4''}$), 115.0 (C_1), 122.6 (d, *J* = 3.1 Hz, $C_{6''}$), 128.8 (Ar**C**), 130.1 (d, *J* = 8.5 Hz, $C_{5''}$), 130.7 (Ar**C**), 139.6 (d, *J* = 7.7 Hz, $C_{1''}$), 155.5 (O**C**(O)), 157.9 (C_4), 163.0 (d, *J* = 244.7 Hz, $C_{3''}$), 170.2 (C**C**(O)).



(*R*)-*N*-4'-((3''-Fluoro)benzyloxy)benzyl 2-*N'*-Amino-3-methoxypropionamide ((*R*)-254). Utilizing Method B and using (*R*)-*N*-4'-((3''-fluoro)benzyloxy)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methoxypropionamide (3.00 g, 6.94 mmol), TFA (7.73 mL, 0.10 mol), and CH_2Cl_2 (23 mL) gave the crude product after basic workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired product (2.05 g, 89%) as a pale yellow solid: mp 76–77 ºC; $[\alpha]^{28.5}_D$ +7.9° (*c* 1.1, $CHCl_3$); R_f 0.74 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3120, 2911 (br), 1644, 1458, 1375, 1253, 1142, 1024, 781, 726 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.67 (s, N**H**$_2$), 3.36 (s, OC**H**$_3$), 3.56–3.64 (m, C**H**$_2$OCH$_3$, C**H**), 4.33–4.44 (m, NHC**H**$_2$), 5.04 (s, OC**H**$_2$), 6.91 (d, *J* = 8.4 Hz, 2 Ar**H**), 6.98–7.02 (m, 1 Ar**H**), 7.13–7.21 (m, 4 Ar**H**), 7.34 (q, *J* = 8.0 Hz, 1 Ar**H**), 7.68–7.72 (br t, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 42.6 (NH**C**H$_2$), 54.9 (**C**H), 58.9 (O**C**H$_3$), 69.2 (**C**H$_2$O), 74.5 (**C**H$_2$OCH$_3$), 114.2 (d, *J* = 21.7 Hz, **C**$_{4''}$ or **C**$_{2''}$), 114.8 (d, *J* = 21.7 Hz, **C**$_{2''}$ or **C**$_{4''}$), 115.0 (**C**$_1$), 122.7 (d, *J* = 2.3 Hz, **C**$_{6''}$), 129.0 (Ar**C**), 130.1 (d, *J* = 8.5 Hz, **C**$_{5''}$), 131.1 (Ar**C**), 139.6 (d, *J* = 7.7 Hz, **C**$_{1''}$), 157.8 (**C**$_4$), 163.0 (d, *J* = 244.7 Hz, **C**$_{3''}$), 172.5 (**C**(O)); LRMS (ESI) 333.17 [M + H^+] (calcd for $C_{18}H_{21}FN_2O_3H^+$ 333.17); Anal. Calcd for $C_{18}H_{21}FN_2O_3$: C, 65.05; H, 6.37; F, 5.72; N, 8.43. Found: C, 65.10; H, 6.39; F, 5.67; N, 8.34.

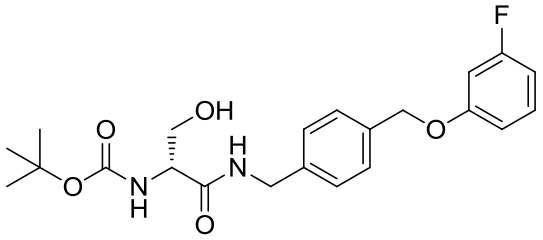


4'-((3''-Fluoro)phenoxy)methyl)benzonitrile (321).[103] A mixture of 4-cyanobenzyl bromide (10.00 g, 51.00 mmol), K_2CO_3 (29.66 g, 0.21 mol), and 3-fluorophenol (4.85 mL, 53.65 mmol) were heated at reflux in acetone (215 mL) (18 h). The volatiles were evaporated *in vacuo* and the residue was dissolved in CH_2Cl_2 (100 mL). The organic layer was washed with H_2O (2 x 100 mL), dried (Na_2SO_4), and evaporated *in vacuo* to give the desired product (11.61 g, 95%) as a white solid. The product was used for the next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.10 (s, C**H**$_2$O), 6.65–6.75 (m, 3 Ar**H**), 7.23 (q, J = 8.0 Hz, 1 Ar**H**), 7.53 (d, J = 7.6 Hz, 2 Ar**H**), 7.68 (d, J = 8.4 Hz, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 69.1 (**C**H$_2$O), 102.7 (d, J = 25.5 Hz, **C**$_{2'}$ or **C**$_{4'}$), 108.3 (d, J = 21.0 Hz, **C**$_{4'}$ or **C**$_{2'}$), 110.5 (d, J = 3.1 Hz, **C**$_{6'}$), 111.9 (Ar**C**), 118.6 (**C**N), 127.5 (Ar**C**), 130.4 (d, J = 10.1 Hz, **C**$_{5'}$), 132.4, 141.9 (2 Ar**C**), 159.5 (d, J = 10.8 Hz, **C**$_{1'}$), 163.6 (d, J = 244.7 Hz, **C**$_{3'}$).



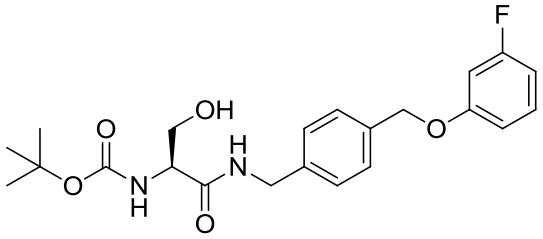
4'-((3''-Fluoro)phenoxy)methyl)benzylamine Hydrochloride (306).[103] To a $LiAlH_4$ (5.02 g, 132.12 mmol) suspension in THF (400 mL) was added dropwise at 0 ºC, a THF (30 mL) solution of 4'-((3''-fluoro)phenoxy)methyl)benzonitrile (10.00 g, 44.04 mmol). The mixture was stirred at room temperature (18 h). Then, H_2O (4 mL) was added dropwise at 0 ºC followed by an aqueous NaOH solution (2 mL, 15% w/w) and H_2O (4 mL). The mixture was stirred at room temperature (2 h), and the precipitate was filtered and the pad was washed with CH_2Cl_2, and the filtrate was concentrated *in vacuo*. The residue was solubilized in Et_2O (30 mL), and then HCl in Et_2O (1 M) was added dropwise at 0 ºC. The white precipitate was filtered to give the desired product (8.43 g, 72 %) as a white solid: mp 243–245 ºC (lit.[103] mp 240–245 ºC); R_f 0.00 (1:10 EtOAc/hexanes); IR (nujol mull) 3284, 2927, 2856, 1596, 1459,

1376, 1277, 1140, 1030, 964, 831, 771, 727 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 4.01 (s, C**H**$_2$NH$_3$), 5.14 (s, C**H**$_2$O), 6.74–6.79 (m, 1 Ar**H**), 6.85–6.92 (m, 2 Ar**H**), 7.31 (q, J = 7.6 Hz, 1 Ar**H**), 7.47 (d, J = 8.2 Hz, 2 Ar**H**), 7.55 (d, J = 8.2 Hz, 2 Ar**H**), 8.50–8.71 (br s, N**H**$_3$Cl); ^{13}C NMR (100 MHz, DMSO-d_6) δ 42.2 (**C**H$_2$NH$_3$), 69.6 (**C**H$_2$O), 102.8 (d, J = 24.8 Hz, **C**$_{2'}$ or **C**$_{4'}$), 107.8 (d, J = 20.9 Hz, **C**$_{4'}$ or **C**$_{2'}$), 111.7 (d, J = 2.3 Hz, **C**$_{6'}$), 128.3, 129.6 (2 Ar**C**), 131.2 (d, J = 10.1 Hz, **C**$_{5'}$), 134.3, 137.3 (2 Ar**C**), 160.1 (d, J = 11.6 Hz, **C**$_{1'}$), 163.4 (d, J = 241.6 Hz, **C**$_{3'}$).



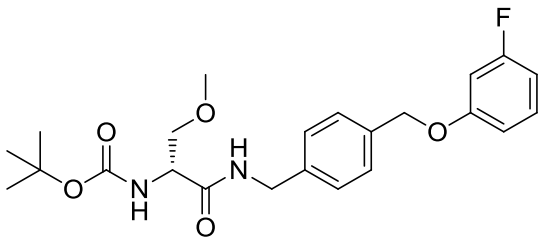
(*R*)-*N*-4'-((3''-Fluoro)phenoxy)methyl)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-hydroxypropionamide ((*R*)-308).[103] Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-hydroxypropionic acid (6.00 g, 29.25 mmol), NMM (8.04 mL, 73.14 mmol), IBCF (4.15 mL, 32.18 mmol), and 4-((3-fluorophenoxy)methyl)benzylamine hydrochloride (8.20 g, 30.72 mmol) in anhydrous THF (300 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (3.47 g, 28%) as a pale yellow solid: mp 85–86 ºC (lit.[103] mp 85–86 ºC); $[\alpha]^{28.5}_D$ +25.3° (*c* 0.8, $CHCl_3$) (lit.[103] $[\alpha]^{23.4}_D$ +27.9° (*c* 1.0, $CHCl_3$)); R_f 0.46 (1:1 EtOAc/hexanes); IR (nujol mull) 3434, 3377, 3158, 2934 (br), 1652, 1524, 1457, 1374, 1306, 1165, 1011, 961, 835, 727 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, (C**H**$_3$)$_3$), 3.30–3.40 (br m, C**H**H'OH), 3.62–3.76 (br m, CH**H'**OH), 4.07–4.13 (m, C**H**), 4.14–4.22 (br m, O**H**), 4.37–4.51 (m, NHC**H**$_2$), 5.01 (s, OC**H**$_2$), 5.65 (d, J = 6.8 Hz, N**H**), 6.64–6.74 (m, 3 Ar**H**), 7.10–7.22 (m, 1 Ar**H**, N**H**), 7.27 (d, J = 8.2 Hz, 2 Ar**H**), 7.36 (d, J = 8.2 Hz, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2 ((**C**H$_3$)$_3$), 43.1 (NH**C**H$_2$), 54.9 (**C**H), 62.8

(**C**H_2OH), 69.8 (**C**H_2O), 80.7 (**C**$(CH_3)_3$), 102.6 (d, *J* = 24.8 Hz, $\mathbf{C_{4''}}$ or $\mathbf{C_{2''}}$), 107.8 (d, *J* = 20.9 Hz, $\mathbf{C_{2''}}$ or $\mathbf{C_{4''}}$), 110.5 (d, *J* = 3.1 Hz, $\mathbf{C_{6''}}$), 127.7, 127.8 (2 Ar**C**), 130.2 (d, *J* = 10.1 Hz, $\mathbf{C_{5''}}$), 135.8, 137.8 (2 Ar**C**), 156.3 (O**C**(O)), 160.0 (d, *J* = 10.8 Hz, $\mathbf{C_{1''}}$), 163.6 (d, *J* = 243.9 Hz, $\mathbf{C_{3''}}$), 171.3 (C**C**(O)).

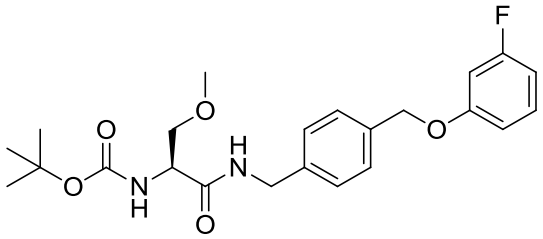


(*S*)-*N*-4'-((3''-Fluoro)phenoxy)methyl)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-hydroxypropionamide ((*S*)-308). The previous procedure was repeated using (*S*)-2-*N*-(*t*-butoxycarbonyl)amino-3-hydroxypropionic acid (2.50 g, 12.19 mmol), NMM (3.35 mL, 30.47 mmol), IBCF (1.73 mL, 13.41 mmol), and 4-((3-fluorophenoxy)methyl)benzylamine hydrochloride (3.42 g, 12.80 mmol) in anhydrous THF (120 mL) to give the crude product that was purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes) to give the desired compound (3.66 g, 72%) as a white solid: mp 89–90 ºC; $[\alpha]^{28}_D$ –24.0° (*c* 1.1, $CHCl_3$) (lit.[103] (*R*): $[\alpha]^{23.4}_D$ +27.9° (*c* 1.0, $CHCl_3$)); R_f 0.39 (1:1 EtOAc/hexanes); IR (nujol mull) 2927 (br), 1652, 1527, 1458, 1375, 1276, 1166, 1012, 835, 768 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, (C$\mathbf{H_3}$)$_3$), 3.42–3.52 (br m, C**H**H'OH), 3.62–3.74 (br m, CH**H'**OH), 4.06–4.10 (m, C**H**), 4.16–4.24 (br m, O**H**), 4.33–4.50 (m, NHC$\mathbf{H_2}$), 5.00 (s, OC$\mathbf{H_2}$), 5.68 (d, *J* = 7.6 Hz, N**H**), 6.63–6.68 (m, 2 Ar**H**), 6.72–6.74 (m, 1 Ar**H**), 7.09–7.27 (m, 3 Ar**H**, N**H**), 7.35 (d, *J* = 7.6 Hz, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2 ((**C**H_3)$_3$), 43.1 (NH**C**H_2), 55.0 (**C**H), 62.8 (**C**H_2OH), 69.8 (**C**H_2O), 80.6 (**C**$(CH_3)_3$), 102.6 (d, *J* = 24.8 Hz, $\mathbf{C_{4''}}$ or $\mathbf{C_{2''}}$), 107.7 (d, *J* = 20.9 Hz, $\mathbf{C_{2''}}$ or $\mathbf{C_{4''}}$), 110.5 (d, *J* = 3.1 Hz, $\mathbf{C_{6''}}$), 127.7, 127.8 (2 Ar**C**), 130.2 (d, *J* = 10.1 Hz, $\mathbf{C_{5''}}$), 135.7, 137.7 (2 Ar**C**), 156.3 (O**C**(O)), 160.0 (d, *J* = 10.9 Hz, $\mathbf{C_{1''}}$), 163.6 (d, *J* = 243.9 Hz, $\mathbf{C_{3''}}$), 171.3 (C**C**(O)); LRMS (ESI) 441.14 [M + Na^+] (calcd for $C_{22}H_{27}FN_2O_5Na^+$ 441.14); Anal.

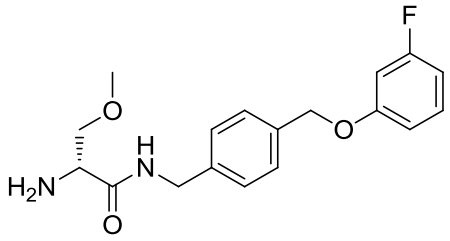
Calcd for $C_{22}H_{27}FN_2O_5$: C, 63.14; H, 6.50; F, 4.54; N, 6.69. Found: C, 63.22; H, 6.66; F, 4.29; N, 6.80.



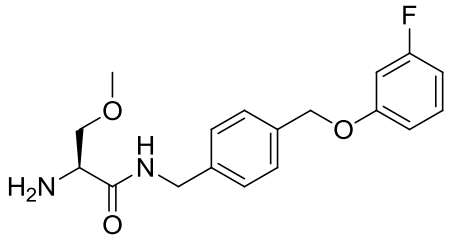
(*R*)-*N*-4'-((3''-Fluoro)phenoxy)methyl)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methoxypropionamide ((*R*)-315).[103] Ag_2O (8.70 g, 37.54 mmol) was added to a CH_3CN solution (150 mL) of (*R*)-*N*-4'-((3''-fluoro)phenoxy)methyl)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-hydroxypropionamide (3.14 g, 7.51 mmol), and CH_3I (4.67 mL, 75.09 mmol) at room temperature under Ar. The reaction mixture was stirred (4 d), filtered, and the filtrate concentrated *in vacuo*. The crude product was purified by flash column chromatography (SiO_2; 5–50% EtOAc/hexanes) to give the desired compound (2.80 g, 86%) as a white solid: R_f 0.72 (50% EtOAc/hexanes); mp 75–76 ºC (lit.[103] mp 77–79 ºC); $[\alpha]^{28.5}_D$ –16.7° (*c* 1.1, $CHCl_3$) (lit.[103] $[\alpha]^{26}_D$ –17.8° (*c* 1.0, $CHCl_3$)); IR (nujol mull) 3409, 2927, 1689, 1648, 1528, 1458, 1375, 1267, 1165, 1048, 961, 918, 825, 763, 726 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, (C**H**$_3$)$_3$), 3.37 (s, OC**H**$_3$), 3.50 (dd, *J* = 6.2, 9.2 Hz, C**H**H'OCH_3), 3.84 (dd, *J* = 4.0, 9.2 Hz, CH**H'**OCH_3), 4.24–4.30 (br s, C**H**), 4.44–4.48 (br d, NHC**H**$_2$), 5.02 (s, OC**H**$_2$), 5.36–5.44 (br m, OC(O)N**H**), 6.64–6.76 (m, 3 Ar**H**, CC(O)N**H**), 7.21 (q, *J* = 8.4 Hz, 1 Ar**H**), 7.29 (d, *J* = 8.2 Hz, 2 Ar**H**), 7.38 (d, *J* = 8.2 Hz, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2 ((**C**H_3)$_3$), 43.2 (NH**C**H_2), 54.0 (**C**H), 59.1 (O**C**H_3), 69.9 (**C**H_2O), 72.0 (**C**H_2OCH_3), 80.4 (**C**$(CH_3)_3$), 102.6 (d, *J* = 24.8 Hz, **C**$_4$'' or **C**$_2$''), 107.8 (d, *J* = 20.9 Hz, **C**$_2$'' or **C**$_4$''), 110.6 (d, *J* = 2.3 Hz, **C**$_6$''), 127.7, 127.8 (2 Ar**C**), 130.2 (d, *J* = 10.0 Hz, **C**$_5$''), 135.7, 138.0 (2 Ar**C**), 155.5 (O**C**(O)), 160.0 (d, *J* = 10.9 Hz, **C**$_1$''), 163.6 (d, *J* = 243.9 Hz, **C**$_3$''), 171.2 (C**C**(O)).



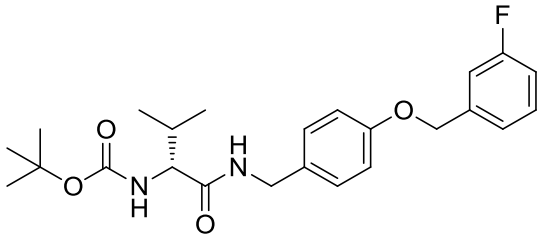
(*S*)-*N*-4'-((3''-Fluoro)phenoxy)methyl)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methoxypropionamide ((*S*)-315). The previous procedure was repeated using (*S*)-*N*-4'-((3''-fluoro)phenoxy)methyl)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-hydroxypropionamide (3.14 g, 7.51 mmol), Ag_2O (8.70 g, 37.54 mmol), CH_3I (4.67 mL, 75.09 mmol), and CH_3CN (140 mL) to give the crude product that was purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes) to give the desired compound (2.68 g, 83%) as a white solid: mp 83–84 ºC; $[\alpha]^{28}_D$ +16.0° (*c* 1.0, $CHCl_3$) (lit.[103] (*R*): $[\alpha]^{26}_D$ –17.8° (*c* 1.0, $CHCl_3$)); R_f 0.61 (1:1 EtOAc/hexanes); IR (nujol mull) 2867 (br), 1647, 1529, 1458, 1375, 1322, 1264, 1165, 1047, 960, 916, 823, 760 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, (C**H**$_3$)$_3$), 3.37 (s, OC**H**$_3$), 3.50 (dd, *J* = 6.4, 9.1 Hz, C**H**H'OCH_3), 3.84 (dd, *J* = 3.8, 9.1 Hz, CH**H'**OCH_3), 4.24–4.30 (br s, C**H**), 4.44–4.54 (br d, NHC**H**$_2$), 5.02 (s, OC**H**$_2$), 5.36–5.44 (br m, OC(O)N**H**), 6.64–6.69 (m, 2 Ar**H**), 6.73–6.78 (m, 1 Ar**H**, CC(O)N**H**), 7.19–7.24 (m, 1 Ar**H**), 7.29 (d, *J* = 8.0 Hz, 2 Ar**H**), 7.38 (d, *J* = 8.0 Hz, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2 ((**C**H_3)$_3$), 43.2 (NH**C**H_2), 54.0 (**C**H), 59.1 (O**C**H_3), 69.9 (**C**H_2O), 72.0 (**C**H_2OCH_3), 80.4 (**C**$(CH_3)_3$), 102.6 (d, *J* = 24.8 Hz, **C$_4$''** or **C$_2$''**), 107.8 (d, *J* = 21.6 Hz, **C$_2$''** or **C$_4$''**), 110.5 (d, *J* = 2.4 Hz, **C$_6$''**), 127.7, 127.8 (2 Ar**C**), 130.2 (d, *J* = 10.0 Hz, **C$_5$''**), 135.7, 138.0 (2 Ar**C**), 155.5 (O**C**(O)), 160.0 (d, *J* = 10.8 Hz, **C$_1$''**), 163.6 (d, *J* = 243.9 Hz, **C$_3$''**), 171.3 (C**C**(O)); LRMS (ESI) 455.18 [M + Na^+] (calcd for $C_{23}H_{29}FN_2O_5Na^+$ 455.18); Anal. Calcd for $C_{23}H_{29}FN_2O_5$: C, 63.87; H, 6.76; F, 4.39; N, 6.48. Found: C, 64.14; H, 6.73; F, 4.44; N, 6.43.



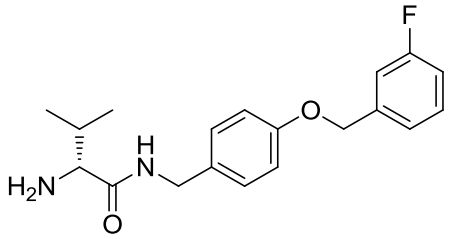
(*R*)-*N*-4'-((3''-Fluoro)phenoxy)methyl)benzyl 2-*N*'-Amino-3-methoxypropionamide ((*R*)-255). Utilizing Method B and using (*R*)-*N*-4'-((3''-fluoro)phenoxy)methyl)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methoxypropionamide (2.45 g, 5.67 mmol), TFA (6.32 mL, 85.03 mol), and CH_2Cl_2 (19 mL) gave the crude product after basic workup that was further purified twice by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) followed by recrystallization from hot EtOAc/hexanes to give the desired product (1.30 g, 69%) as a pale yellow solid: mp 60–61 ºC; $[\alpha]^{25}_D$ +6.1° (*c* 1.1, $CHCl_3$); R_f 0.76 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3132, 2877 (br), 1651, 1457, 1375, 1270, 1130, 1016, 774 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.63 (s, N**H$_2$**), 3.38 (s, OC**H$_3$**), 3.59–3.65 (m, C**H**, C**H$_2$**OCH_3), 4.42–4.52 (m, NHC**H$_2$**), 5.03 (s, OC**H$_2$**), 6.64–6.69 (m, 2 Ar**H**), 6.75 (d, *J* = 9.6 Hz, 1 Ar**H**), 7.22 (q, *J* = 8.4 Hz, 1 Ar**H**), 7.30 (d, *J* = 7.8 Hz, 2 Ar**H**), 7.38 (d, *J* = 7.8 Hz, 2 Ar**H**), 7.74–7.83 (br t, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 42.8 (NH**C**H_2), 54.9 (**C**H), 58.9 (O**C**H_3), 69.9 (**C**H_2O), 74.4 (**C**H_2OCH_3), 102.6 (d, *J* = 24.8 Hz, **C$_{4''}$** or **C$_{2''}$**), 107.8 (d, *J* = 21.7 Hz, **C$_{2''}$** or **C$_{4''}$**), 110.6 (d, *J* = 2.3 Hz, **C$_{6''}$**), 127.8, 127.9 (2 Ar**C**), 130.2 (d, *J* = 10.0 Hz, **C$_{5''}$**), 135.6, 138.4 (2 Ar**C**), 160.0 (d, *J* = 10.9 Hz, **C$_{1''}$**), 163.6 (d, *J* = 243.9 Hz, **C$_{3''}$**), 172.6 (**C**(O)); LRMS (ESI) 333.17 [M + H^+] (calcd for $C_{18}H_{21}FN_2O_3H^+$ 333.17); Anal. Calcd for $C_{18}H_{21}FN_2O_3$: C, 65.05; H, 6.37; F, 5.72; N, 8.43. Found: C, 64.83; H, 6.39; F, 5.58; N, 8.50.



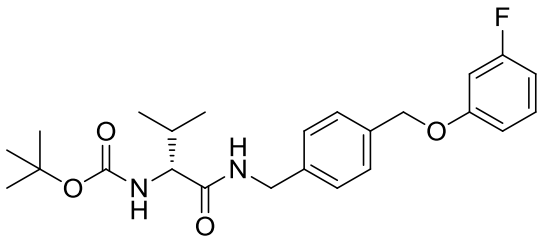
(*S*)-*N*-4'-((3''-Fluoro)phenoxy)methyl)benzyl 2-*N*'-Amino-3-methoxypropionamide ((*S*)-255). Utilizing Method B and using (*S*)-*N*-4'-((3''-fluoro)phenoxy)methyl)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methoxypropionamide (2.71 g, 6.27 mmol), TFA (6.99 mL, 94.05 mol), and CH_2Cl_2 (21 mL) gave the crude product after basic workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give a mixture of desired product and impurity as an orange oil. The crude oil obtained after the acid workup was then further purified by flash column chromatography (SiO_2; 1:100–1:10 MeOH/CH_2Cl_2) to give the desired compound (0.27 g, 19%) as a pale yellow solid: mp 52–53 ºC; $[\alpha]^{25}_D$ - 6.8° (*c* 1.1, $CHCl_3$); R_f 0.29 (1:20 MeOH/CH_2Cl_2); IR (nujol) 2938 (br), 1651, 1521, 1458, 1375, 1271, 1130, 1017, 964, 832, 774 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.68 (s, N**H$_2$**), 3.38 (s, OC**H$_3$**), 3.59-3.65 (m, C**H**, C**H$_2$**OCH$_3$), 4.42-4.52 (m, NHC**H$_2$**), 5.03 (s, OC**H$_2$**), 6.64-6.70 (m, 2 Ar**H**), 6.73-6.76 (m, 1 Ar**H**), 7.19-7.26 (m, 1 Ar**H**), 7.30 (d, *J* = 8.0 Hz, 2 Ar**H**), 7.38 (d, *J* = 8.0 Hz, 2 Ar**H**), 7.76-7.84 (br t, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 42.8 (NH**C**H$_2$), 54.8 (**C**H), 58.9 (O**C**H$_3$), 69.9 (**C**H$_2$O), 74.5 (**C**H$_2$OCH$_3$), 102.6 (d, *J* = 24.7 Hz, **C$_{4''}$** or **C$_{2''}$**), 107.7 (d, *J* = 20.9 Hz, **C$_{2''}$** or **C$_{4''}$**), 110.5 (d, *J* = 3.1 Hz, **C$_{6''}$**), 127.8, 127.9 (2 Ar**C**), 130.2 (d, *J* = 10.0 Hz, **C$_{5''}$**), 135.6, 138.4 (2 Ar**C**), 160.0 (d, *J* = 10.9 Hz, **C$_{1''}$**), 163.6 (d, *J* = 243.9 Hz, **C$_{3''}$**), 172.6 (**C**(O)); LRMS (ESI) 333.12 [M + H^+] (calcd for $C_{18}H_{21}FN_2O_3H^+$ 333.12); Anal. Calcd for $C_{18}H_{21}FN_2O_3$: C, 65.05; H, 6.37; F, 5.72; N, 8.43. Found: C, 64.89; H, 6.39; F, 5.45; N, 8.21.



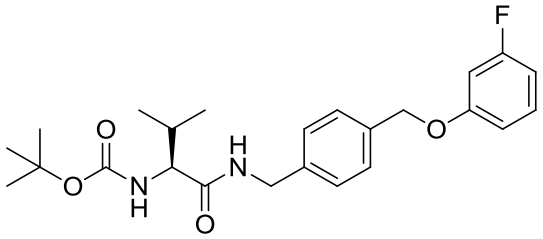
(*R*)-*N*-(4-(3-Fluoro)benzyloxy)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-310). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (3.07 g, 14.14 mmol), NMM (2.02 mL, 18.38 mmol), IBCF (1.84 mL, 15.55 mmol), and 4-(3-fluoro)benzyloxybenzylamine (3.43 g, 14.85 mmol) in anhydrous THF (15 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 1:100–1:10 MeOH/CH_2Cl_2) to give the desired compound (5.29 g, 87%) as a pale yellow solid: mp 109–110 ºC; $[\alpha]^{25}_D$ +4.3° (*c* 1.1, CH_2Cl_2); R_f 0.21 (1:10 EtOAc/hexanes); IR (nujol mull) 3298, 2947 (br), 1652, 1530, 1458, 1375, 1301, 1245, 1170, 1017, 879, 777 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.91 (d, J = 6.8 Hz, CH(C**H**$_3$)CH_3), 0.95 (d, J = 6.8 Hz, $CH(CH_3)$C**H**$_3$), 1.41 (s, C(C**H**$_3$)$_3$), 2.09–2.19 (m, C**H**$(CH_3)_2$), 3.86–3.92 (m, C**H**), 4.23–4.42 (m, NHC**H**$_2$Ph), 5.04 (s, OC**H**$_2$), 5.08–5.12 (br d, C(O)N**H**), 6.36 (t, J = 5.2 Hz, N**H**CH_2Ph), 6.88–6.93 (m, 2 Ar**H**), 6.98–7.03 (m, 1 Ar**H**), 7.12–7.22 (m, 4 Ar**H**), 7.31–7.36 (1 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.1 (CH(**C**H_3)CH_3), 19.6 ($CH(CH_3)$**C**H_3), 28.5 (C(**C**$H_3)_3$), 30.9 (**C**H$(CH_3)_2$), 43.1 (NH**C**H_2Ph), 60.4 (**C**H), 69.4 (O**C**H_2), 80.1 (**C**$(CH_3)_3$), 114.4 (d, J = 21.9 Hz, **C**$_{4'}$ or **C**$_{2'}$), 115.0 (d, J = 21.2 Hz, **C**$_{2'}$ or **C**$_{4'}$), 115.2 (**C**$_1$), 122.9 (d, J = 2.6 Hz, **C**$_{6'}$), 129.3 (Ar**C**), 130.3 (d, J = 8.3 Hz, **C**$_{5'}$), 130.9 (Ar**C**), 139.8 (d, J = 7.1 Hz, **C**$_{1'}$), 156.1 (O**C**(O)), 158.1 (**C**$_4$), 163.2 (d, J = 244.4 Hz, **C**$_{3'}$), 171.7 (C**C**(O)); HRMS (ESI) 453.2178 [M + Na^+] (calcd for $C_{24}H_{31}FN_2O_4Na^+$ 453.2166); Anal. Calcd for $C_{24}H_{31}FN_2O_4$: C, 66.96; H, 7.26; F, 4.41; N, 6.51. Found: C, 67.23; H, 7.22; F, 4.47; N, 6.28.



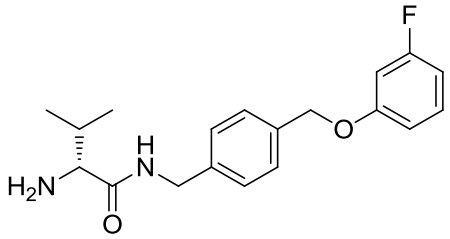
(*R*)-*N*-(4-(3-Fluoro)benzyloxy)benzyl 2-Amino-3-methylbutanamide ((*R*)-257). Utilizing Method B and using (*R*)-*N*-(4-(3-fluoro)benzyloxybenzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (2.00 g, 4.65 mmol), TFA (5.18 mL, 69.73 mmol), and CH_2Cl_2 (15 mL) gave the crude product after basic workup that was further purified by flash column chromatography (SiO_2; 1:100–1:10 MeOH/CH_2Cl_2) to give the desired compound (481 mg, 32%) as a white solid: mp 77–78 ºC; $[\alpha]^{25}_D$ +16.8° (*c* 1.0, CH_2Cl_2); R_f 0.57 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3458, 3408, 3350, 3265, 2915, 2859, 1630, 1458, 1375, 1251, 1137, 1032, 952, 831, 725 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, *J* = 6.8 Hz, CH(C**H$_3$**)CH_3), 0.99 (d, *J* = 7.2 Hz, CH(CH_3)C**H$_3$**), 1.31–1.58 (br s, N**H$_2$**), 2.31–2.38 (m, C**H**(CH_3)$_2$), 3.26 (d, *J* = 2.8 Hz, C**H**), 4.33–4.44 (m, NHC**H$_2$**Ph), 5.05 (s, OC**H$_2$**), 6.91 (d, *J* = 7.2 Hz, 2 Ar**H**), 7.00 (t, *J* = 8.4 Hz, 1 Ar**H**), 7.13–7.26 (m, 4 Ar**H**), 7.31–7.39 (m, 1 Ar**H**), 7.51–7.59 (br t, N**H**CH_2Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.2 (CH(**C**H_3)CH_3), 20.0 (CH(CH_3)**C**H_3), 31.0 (**C**H(CH_3)$_2$), 42.7 (NH**C**H_2Ph), 60.4 (**C**H), 69.4 (O**C**H_2), 114.4 (d, *J* = 21.9 Hz, **C$_{4'}$** or **C$_{2'}$**), 115.0 (d, *J* = 21.2 Hz, **C$_{2'}$** or **C$_{4'}$**), 115.2 (**C$_1$**), 122.9 (d, *J* = 3.2 Hz, **C$_{6'}$**), 129.4 (Ar**C**), 130.3 (d, *J* = 8.4 Hz, **C$_{5'}$**), 131.6 (Ar**C**), 139.8 (d, *J* = 7.0 Hz, **C$_{1'}$**), 158.0 (**C$_4$**), 163.2 (d, *J* = 245.0 Hz, **C$_{3'}$**), 174.4 (**C**(O)); HRMS (ESI) 331.1827 [M + H^+] (calcd for $C_{19}H_{23}FN_2O_2H^+$ 331.1822); Anal. Calcd for $C_{19}H_{23}FN_2O_2$: C, 69.07; H, 7.02; F, 5.75; N, 8.48. Found: C, 68.98; H, 6.90; F, 5.75; N, 8.32.



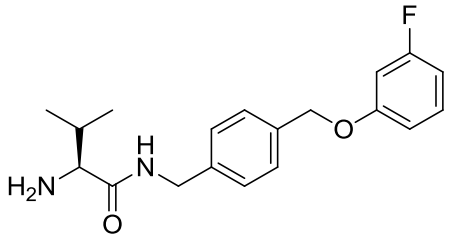
(*R*)-*N*-4-((3-Fluoro)phenoxymethyl)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-311). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (2.25 g, 10.36 mmol), NMM (1.48 mL, 13.47 mmol), IBCF (1.47 mL, 11.40 mmol), and 4-((3-fluorophenoxy)methyl)benzylamine (2.51 g, 10.88 mmol) in anhydrous THF (10 mL) gave the crude product. The compound was purified by flash column chromatography (SiO_2; 1:10–1:1 EtOAc/hexanes) to give the desired product (4.03 g, 90%) as a white solid: mp 115–116 ºC; $[\alpha]^{25}_D$ +2.6° (*c* 1.1, CH_2Cl_2); R_f 0.85 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3347, 2958, 1648, 1527, 1458, 1375, 1306, 1253, 1166, 1045, 961, 826, 769, 724 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (d, J = 6.8 Hz, CH(C**H**$_3$)CH_3), 0.95 (d, J = 6.8 Hz, $CH(CH_3)$C**H**$_3$), 1.40 (s, C(C**H**$_3$)$_3$), 2.05–2.19 (m, C**H**$(CH_3)_2$), 3.94 (dd, J = 5.6, 10.0 Hz, C**H**), 4.34–4.49 (m, NHC**H**$_2$Ph), 4.99 (s, OC**H**$_2$), 5.23 (d, J = 9.0 Hz, C(O)N**H**), 6.63–6.77 (m, 4 Ar**H**), 7.11–7.39 (m, 4 Ar**H**, N**H**CH_2Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.1 (CH(**C**H_3)CH_3), 19.2 ($CH(CH_3)$**C**H_3), 28.5 (C(**C**H_3)$_3$), 30.9 (**C**$H(CH_3)_2$), 43.3 (NH**C**H_2Ph), 60.4 (**C**H), 70.1 (**C**H_2O), 80.1 (**C**$(CH_3)_3$), 102.3 (d, J = 24.5 Hz, **C**$_{4'}$ or **C**$_{2'}$), 108.0 (d, J = 21.1 Hz, **C**$_{2'}$ or **C**$_{4'}$), 110.8 (d, J = 2.9 Hz, **C**$_{6'}$), 128.0 (d, J = 10.9 Hz, **C**$_{1'}$), 129.5 (Ar**C**), 130.4 (d, J = 10.3 Hz, **C**$_{5'}$), 135.9 (Ar**C**), 138.3 (Ar**C**), 156.2 (O**C**(O)), 160.2 (d, J = 10.8 Hz, **C**$_4$), 163.8 (d, J = 243.8 Hz, **C**$_{3'}$), 172.0 (C**C**(O)); HRMS (ESI) 453.2172 [M + Na^+] (calcd for $C_{24}H_{31}FN_2O_4Na^+$ 453.2166); Anal. Calcd for $C_{24}H_{31}FN_2O_4$: C, 66.96; H, 7.26; F, 4.41; N, 6.51. Found: C, 67.01; H, 7.41; F, 4.36; N, 6.38.



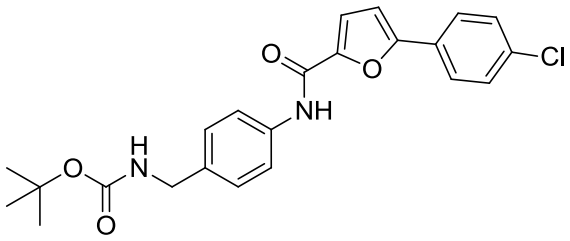
(*S*)-*N*-4-((3-Fluoro)phenoxymethyl)benzyl 2-*N'*-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*S*)-311). The previous procedure was repeating using (*S*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (2.50 g, 11.51 mmol), NMM (3.16 mL, 28.78 mmol), IBCF (1.63 mL, 12.67 mmol), and 4-((3-fluorophenoxy)methyl)benzylamine hydrochloride (3.23 g, 12.09 mmol) in anhydrous THF (115 mL) to give the crude product that was purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes) to give the desired compound (4.53 g, 91%) as a white solid: mp 115–116 ºC; $[\alpha]^{28}_D$ –2.6° (*c* 1.1, CH_2Cl_2); R_f 0.90 (1:1 EtOAc/hexanes); IR (nujol mull) 2911 (br), 1648, 1526, 1458, 1375, 1305, 1252, 1166, 1044, 962, 826, 724 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.92 (d, J = 7.0 Hz, CH(C**H**$_3$)CH$_3$), 0.96 (d, J = 7.0 Hz, CH(CH$_3$)C**H**$_3$), 1.41 (s, C(C**H**$_3$)$_3$), 2.10–2.20 (m, C**H**(CH$_3$)$_2$), 3.93 (dd, J = 6.4, 8.8 Hz, C**H**), 4.38–4.49 (m, NHC**H**$_2$Ph), 5.01 (s, OC**H**$_2$), 5.10–5.15 (br d, C(O)N**H**), 6.55 (t, J = 5.6 Hz, N**H**CH$_2$Ph), 6.64–6.75 (m, 3 Ar**H**), 7.10–7.24 (m, 1 Ar**H**), 7.27 (d, J = 8.0 Hz, 2 Ar**H**), 7.36 (d, J = 8.0 Hz, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.9 (CH(**C**H$_3$)CH$_3$), 19.4 (CH(CH$_3$)**C**H$_3$), 28.3 (C(**C**H$_3$)$_3$), 30.6 (**C**H(CH$_3$)$_2$), 43.1 (NH**C**H$_2$Ph), 60.2 (**C**H), 69.9 (**C**H$_2$O), 79.9 (**C**(CH$_3$)$_3$), 102.6 (d, J = 24.8 Hz, **C$_{4'}$** or **C$_{2'}$**), 107.7 (d, J = 20.9 Hz, **C$_{2'}$** or **C$_{4'}$**), 110.7 (d, J = 3.1 Hz, **C$_{6'}$**), 127.8 (d, J = 13.9 Hz, **C$_{1'}$**), 129.3 (Ar**C**), 130.2 (d, J = 10.0 Hz, **C$_{5'}$**), 135.7 (Ar**C**), 138.1 (Ar**C**), 155.9 (O**C**(O)), 160.0 (d, J = 10.8 Hz, **C$_4$**), 163.6 (d, J = 243.9 Hz, **C$_{3'}$**), 171.7 (C**C**(O)); LRMS (ESI) 563.10 [M + Cs^+] (calcd for $C_{24}H_{31}FN_2O_4Cs^+$ 563.10); Anal. Calcd for $C_{24}H_{31}FN_2O_4$: C, 66.96; H, 7.26; F, 4.41; N, 6.51. Found: C, 67.01; H, 7.33; F, 4.27; N, 6.43.



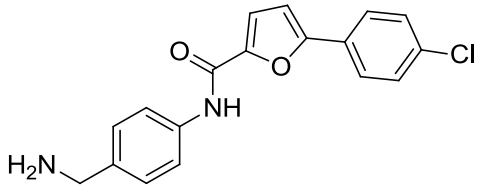
(*R*)-*N*-4-((3-Fluoro)phenoxymethyl)benzyl 2-Amino-3-methylbutanamide ((*R*)-258). Utilizing Method B and using (*R*)-*N*-4-((3-fluoro)phenoxymethyl)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (3.00 g, 6.97 mmol), TFA (7.77 mL, 0.10 mol), and CH_2Cl_2 (23 mL) gave the crude product after basic workup that was further purified by flash column chromatography (SiO_2; 1:100–1:10 MeOH/CH_2Cl_2) to give the desired compound (0.92 g, 40%) as a white solid: mp 78–79 ºC; $[\alpha]^{25}_D$ +17.5° (*c* 1.1, CH_2Cl_2); R_f 0.50 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3418, 3222, 2919, 2861, 1641, 1459, 1375, 1268, 1136, 1017, 960, 819, 776, 723 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.84 (d, *J* = 6.8 Hz, CH(C**H$_3$**)CH_3), 1.00 (d, *J* = 7.2 Hz, CH(CH_3)**C**H$_3$), 1.26–1.58 (br s, N**H$_2$**), 2.33–2.41 (m, C**H**(CH_3)$_2$), 3.29 (d, *J* = 3.6 Hz, C**H**), 4.41–4.52 (m, NHC**H$_2$**Ph), 5.03 (s, OC**H$_2$**), 6.64–6.69 (m, 2 Ar**H**), 6.73–6.76 (m, 1 Ar**H**), 7.12–7.39 (m, 5 Ar**H**), 7.62–7.84 (br t, N**H**CH_2Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.2 (CH(**C**H$_3$)CH_3), 20.0 (CH(CH_3)**C**H$_3$), 31.0 (**C**H(CH_3)$_2$), 43.0 (NH**C**H_2Ph), 60.4 (**C**H), 70.1 (**C**H_2O), 102.8 (d, *J* = 24.4 Hz, **C$_{4'}$** or **C$_{2'}$**), 108.0 (d, *J* = 21.2 Hz, **C$_{2'}$** or **C$_{4'}$**), 110.8 (d, *J* = 2.6 Hz, **C$_{6'}$**), 128.2 (d, *J* = 23.1 Hz, **C$_{1'}$**), 129.5 (Ar**C**), 130.4 (d, *J* = 9.7 Hz, **C$_{5'}$**), 135.8 (Ar**C**), 138.9 (Ar**C**), 160.2 (d, *J* = 11.0 Hz, **C$_4$**), 163.8 (d, *J* = 243.7 Hz, **C$_{3'}$**), 174.5 (**C**(O)); HRMS (ESI) 331.1828 [M + H^+] (calcd for $C_{19}H_{23}FN_2O_2H^+$ 331.1822); Anal. Calcd for $C_{19}H_{23}FN_2O_2$: C, 69.07; H, 7.02; F, 5.75; N, 8.48. Found: C, 68.77; H, 7.03; F, 5.48; N, 8.43.



(*S*)-*N*-4-((3-Fluoro)phenoxymethyl)benzyl 2-Amino-3-methylbutanamide ((*S*)-258). The previous procedure was repeated using (*S*)-*N*-4-((3-fluoro)phenoxymethyl)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (4.00 g, 9.30 mmol), TFA (10.36 mL, 0.14 mol), and CH_2Cl_2 (30 mL) to give the crude product after basic workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (2.67 g, 87%) as a white solid: mp 75–76 ºC; $[\alpha]^{28}_D$ –18.2° (*c* 1.2, CH_2Cl_2); R_f 0.45 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2902 (br), 1640, 1522, 1459, 1377, 1268, 1137, 1017, 958, 812, 778, 722 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.84 (d, J = 7.0 Hz, CH(C**H**$_3$)CH$_3$), 0.99 (d, J = 7.0 Hz, CH(CH$_3$)**C**H$_3$), 1.20–1.52 (br s, N**H**$_2$), 2.34–2.40 (m, C**H**(CH$_3$)$_2$), 3.24–3.34 (br d, C**H**), 4.41–4.52 (m, NHC**H**$_2$Ph), 5.02 (s, OC**H**$_2$), 6.64–6.70 (m, 2 Ar**H**), 6.73–6.76 (m, 1 Ar**H**), 7.12–7.25 (m, 1 Ar**H**), 7.30 (d, J = 8.2 Hz, 2 Ar**H**), 7.38 (d, J = 8.2 Hz, 2 Ar**H**), 7.64–7.70 (br t, N**H**CH$_2$Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0 (CH(**C**H$_3$)CH$_3$), 19.7 (CH(CH$_3$)**C**H$_3$), 30.8 (**C**H(CH$_3$)$_2$), 42.8 (NH**C**H$_2$Ph), 60.1 (**C**H), 69.9 (**C**H$_2$O), 102.6 (d, J = 24.7 Hz, **C$_4$**, or **C$_{2'}$**), 107.8 (d, J = 20.9 Hz, **C$_2$**, or **C$_{4'}$**), 110.5 (d, J = 2.4 Hz, **C$_{6'}$**), 128.2 (d, J = 23.2 Hz, **C$_{1'}$**), 129.3 (Ar**C**), 130.2 (d, J = 10.0 Hz, **C$_{5'}$**), 135.6 (Ar**C**), 138.6 (Ar**C**), 160.0 (d, J = 10.8 Hz, **C$_4$**), 163.6 (d, J = 243.9 Hz, **C$_{3'}$**), 174.3 (**C**(O)); LRMS (ESI) 331.14 [M + H^+] (calcd for $C_{19}H_{23}FN_2O_2H^+$ 331.14); Anal. Calcd for $C_{19}H_{23}FN_2O_2$: C, 69.07; H, 7.02; F, 5.75; N, 8.48. Found: C, 69.29; H, 7.06; F, 5.48; N, 8.60.

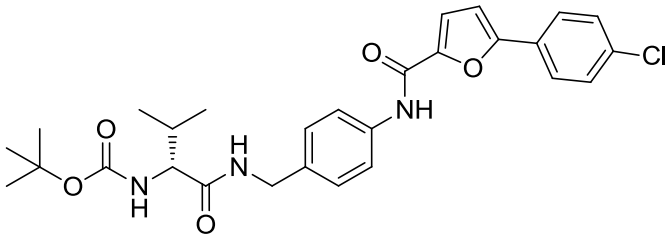


4-((5-(4-Chloro)phenyl)furan-2-carboxamido)benzyl *O*-(*t*-Butyl)carbamate (331). 5-(4-Chloro)phenyl-2-furoic acid (2.00 g, 8.98 mmol) was dissolved in anhydrous CH_2Cl_2 (90 mL) and treated with oxalyl chloride (1.16 mL, 13.48 mmol) and DMF (0.20 mL, cat.). The reaction was stirred at room temperature (2 h) and the solvent evaporated *in vacuo*. The crude acid chloride was dissolved in anhydrous CH_2Cl_2 (18 mL) and treated with *N*-(*t*-butoxycarbonyl) 4-(aminomethyl)aniline (2.00 g, 8.98 mmol) and Et_3N (2.75 mL, 19.76 mmol). The reaction was maintained at room temperature (2 h) before the solvent was evaporated *in vacuo*. The crude product was diluted with CH_2Cl_2 (30 mL) and successively washed with aqueous 1 M HCl (3 x 50 mL) and saturated aqueous brine (3 x 50 mL), dried (Na_2SO_4), and evaporated *in vacuo* to give the crude compound (3.66 mg, 96%) as an orange solid. The crude product was used for the next step without further purification: R_f 0.84 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, C(C**H**$_3$)$_3$), 4.31 (d, J = 5.6 Hz, NHC**H**$_2$), 4.79–4.91 (br t, N**H**CH_2), 6.79 (d, J = 3.2 Hz, 1 furan**H**), 7.29–7.32 (m, 1 furan**H**, 2 Ar**H**), 7.43 (d, J = 8.4 Hz, 2 Ar**H**), 7.64 (d, J = 8.4 Hz, 2 Ar**H**), 7.68 (d, J = 8.4 Hz, 2 Ar**H**), 8.05 (s, N**H**).



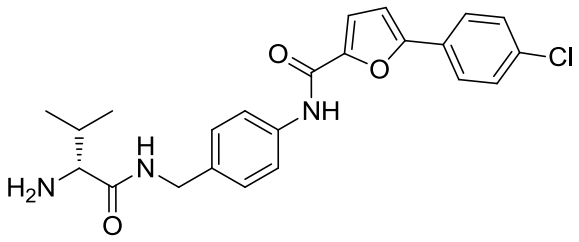
4-((5-(4-Chloro)phenyl)furan-2-carboxamido)benzylamine (332). Utilizing Method B and using 4-((5-(4-Chloro)phenyl)furan-2-carboxamido)benzyl *O*-(*t*-butyl)carbamate (1.60 g, 3.75

mmol), TFA (4.18 mL, 56.32 mmol), and CH_2Cl_2 (13 mL) gave the crude product (1.11 g, 91%) after basic workup as an orange solid. The crude product was used for the next step without further purification: R_f 0.34 (100% MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.48–3.67 (br s, N**H**$_2$), 3.79 (s, C**H**$_2$), 7.23 (d, J = 4.0 Hz, 1 furan**H**), 7.36 (d, J = 8.4 Hz, 2 Ar**H**), 7.42 (d, J = 4.0 Hz, 1 furan**H**), 7.57 (d, J = 8.4 Hz, 2 Ar**H**), 7.71 (d, J = 8.4 Hz, 2 Ar**H**), 8.02 (d, J = 8.4 Hz, 2 Ar**H**), 10.21 (s, N**H**); ^{13}C NMR (100 MHz, DMSO-d_6) δ 45.0 (**C**H$_2$), 109.0 (furan **C**$_3$ or **C**$_4$), 117.4 (furan **C**$_4$ or **C**$_3$), 121.0, 126.7, 128.0, 128.6, 129.4, 133.6, 137.3, 138.3 (2 **C**$_6$H$_4$), 147.4 (furan **C**$_2$), 154.4 (furan **C**$_5$ or **C**(O)N), 156.3 (**C**(O)N or furan **C**$_5$).



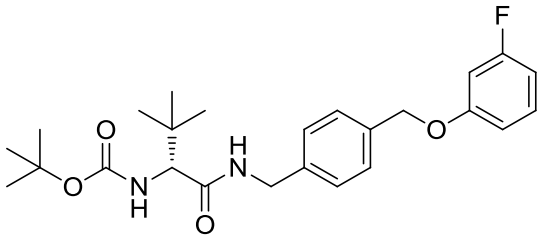
(*R*)-4-((5-(4-Chloro)phenyl)furan-2-carboxamido)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-333). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (0.70 g, 3.22 mmol), NMM (0.46 mL, 4.19 mmol), IBCF (0.45 mL, 3.55 mmol), and 4-((5-(4-chloro)phenyl)furan-2-carboxamido)benzylamine (1.10 g, 3.39 mmol) in anhydrous THF (40 mL) gave the crude product that was purified by recrystallization from hot EtOAc/hexanes to give the desired compound (0.87 g, 52%) as an orange solid: mp 204–206 ºC; $[\alpha]^{25}_D$ +10.7° (*c* 1.0, DMSO); R_f 0.56 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3194, 2897 (br), 1652, 1530, 1459, 1374, 1320, 1242, 1164, 1097, 1027, 966, 808, 747, 673 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83 (br s, CH(C**H**$_3$)CH$_3$), 0.85 (br s, CH(CH$_3$)C**H**$_3$), 1.40 (s, C(C**H**$_3$)$_3$), 1.92–1.97 (m, C**H**(CH$_3$)$_2$), 3.79 (t, J = 7.6 Hz, C**H**), 4.28 (d, J = 7.2 Hz, NHC**H**$_2$), 6.71 (d, J = 7.2 Hz, OC(O)N**H**), 7.23 (d, J = 3.6 Hz, 1 furan**H**), 7.26 (d, J = 8.8 Hz, 2 Ar**H**), 7.40 (d, J = 3.6 Hz, 1 furan**H**), 7.56–7.59 (m, 2 Ar**H**), 7.66 (d, J = 8.4 Hz, 2 Ar**H**), 7.99–8.03 (m, 2 Ar**H**), 8.33 (t, J = 6.0 Hz, N**H**CH$_2$), 10.18

(s, N**H**); ^{13}C NMR (100 MHz, DMSO-d_6) δ 18.8 (CH(**C**H$_3$)CH$_3$), 19.7 (CH(CH$_3$)**C**H$_3$), 28.7 (C(**C**H$_3$)$_3$), 30.6 (**C**H(CH$_3$)$_2$), 42.1 (NH**C**H$_2$), 60.5 (**C**H), 78.4 (**C**(CH$_3$)$_3$), 109.0 (furan **C$_3$** or **C$_4$**), 117.4 (furan **C$_4$** or **C$_3$**), 121.0, 126.7, 127.9, 128.6, 129.5, 133.7, 135.4, 137.4 (2 **C$_6$**H$_4$), 147.4 (furan **C$_2$**), 154.4 (furan **C$_5$** or **C**(O)N), 156.0 (O**C**(O)N), 156.3 (**C**(O)N or furan **C$_5$**), 171.9 (C**C**(O)); HRMS (ESI) 658.1076 [M + Cs$^+$] (calcd for $C_{28}H_{32}ClN_3O_5Cs^+$ 658.1085); Anal. Calcd for $C_{28}H_{32}ClN_3O_5$•0.09H_2O: C, 63.74; H, 6.13; Cl, 6.74; N, 7.99. Found: C, 63.35; H, 6.18; Cl, 7.01; N, 7.88.

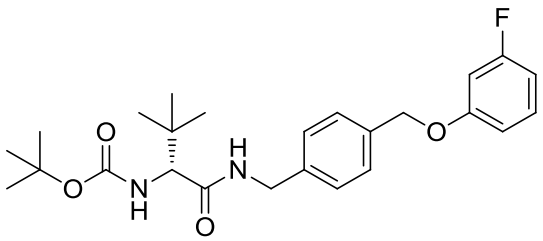


(*R*)-4-((5-(4-Chloro)phenyl)furan-2-carboxamido)benzyl 2-Amino-3-methylbutanamide ((*R*)-259). Utilizing Method B and using (*R*)-4-((5-(4-chloro)phenyl)furan-2-carboxamido)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (0.92 g, 1.75 mmol), TFA (1.95 mL, 26.28 mmol), and CH_2Cl_2 (6 mL) gave the crude product after basic workup that was further purified by flash column chromatography (SiO_2; 1:10–1:1 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired product (0.56 g, 75%) as an orange solid: mp 184–186 ºC; $[\alpha]^{25}_D$ –2.7° (*c* 1.0, DMSO); R_f 0.47 (100% MeOH); IR (nujol mull) 2904 (br), 1644, 1601, 1542, 1458, 1375, 1166, 1090, 1030, 963, 791, 724 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.80 (d, J = 6.8 Hz, CH(C**H$_3$**)CH$_3$), 0.88 (d, J = 6.8 Hz, CH(CH$_3$)C**H$_3$**), 1.85–2.01 (m, C**H**(CH$_3$)$_2$, N**H$_2$**), 2.99–3.00 (m, C**H**), 4.28 (d, J = 4.8 Hz, NHC**H$_2$**), 7.23 (d, J = 3.8 Hz, 1 furan**H**), 7.27 (d, J = 8.4 Hz, 2 Ar**H**), 7.40 (d, J = 3.8 Hz, 1 furan**H**), 7.57 (d, J = 8.4 Hz, 2 Ar**H**), 7.68 (d, J = 8.4 Hz, 2 Ar**H**), 8.01 (d, J = 8.4 Hz, 2 Ar**H**), 8.31 (t, J = 6.0 Hz, N**H**CH$_2$), 10.18 (s, N**H**); ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.6 (CH(**C**H$_3$)CH$_3$), 20.0 (CH(CH$_3$)**C**H$_3$), 32.1 (**C**H(CH$_3$)$_2$), 42.0 (NH**C**H$_2$), 60.6 (**C**H), 109.0 (furan

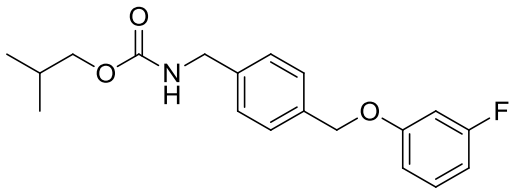
C_3 or **C_4**), 117.4 (furan **C_4** or **C_3**), 121.1, 126.7, 128.1, 128.6, 129.5, 133.6, 135.8, 137.4 (2 **C_6H_4**), 147.4 (furan **C_2**), 154.4 (furan **C_5** or **C**(O)N), 156.3 (**C**(O)N or furan **C_5**), 175.0 (C**C**(O)); HRMS (ESI) 558.0545 [M + Cs^+] (calcd for $C_{23}H_{24}ClN_3O_3Cs^+$ 558.0560); Anal. Calcd for $C_{23}H_{24}ClN_3O_3$•0.03 EtOAc: C, 64.79; H, 5.70; Cl, 8.27; N, 9.80. Found: C, 64.41; H, 5.80; Cl, 8.03; N, 9.50.



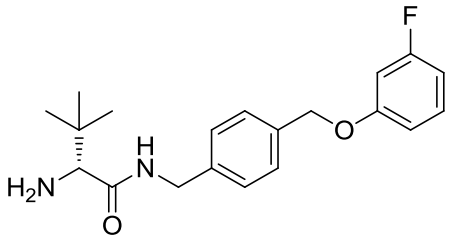
(*R*)-*N*-4'-((3''-Fluoro)phenoxymethyl)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3,3-dimethylbutanamide ((*R*)-313). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3,3-dimethylbutanoic acid (1.65 g, 7.14 mmol), NMM (1.02 mL, 9.28 mmol), IBCF (1.01 mL, 7.85 mmol), and 4-((3-fluoro)phenoxymethyl)benzylamine (1.73 g, 7.50 mmol) in anhydrous THF (65 mL) gave the crude product. The compound was purified by flash column chromatography (SiO_2; 1:10–1:1 EtOAc/hexanes) to give a ~2:1 mixture of (*R*)-*N*-4'-((3''-fluoro)phenoxymethyl)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3,3-dimethylbutanamide and *N*-4'-((3''-fluoro)phenoxymethyl)benzyl (isobutoxycarbonyl)carbamate (1.82 g, 57%) as a white solid.



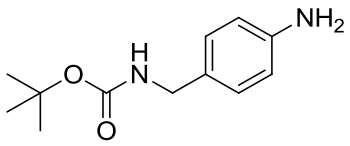
(*R*)-*N*-4'-((3''-Fluoro)phenoxymethyl)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3,3-dimethylbutanamide ((*R*)-313): R_f 0.88 (1:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 (s, CC(C**H**$_3$)$_3$), 1.40 (s, OC(C**H**$_3$)$_3$), 3.87 (d, J = 7.6 Hz, C**H**), 4.36–4.40 (m, NHC**H**H'), 4.44–4.50 (m, NHCH**H'**), 5.01 (s, CH_2O), 5.32 (d, J = 7.6 Hz, OC(O)N**H**), 6.42–6.48 (br t, N**H**CH_2), 6.64–6.74 (m, 3 Ar**H**), 7.18–7.49 (m, 5 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.6 (CC(**C**H$_3$)$_3$), 28.3 (OC(**C**H$_3$)$_3$), 34.4 (C**C**(CH$_3$)$_3$), 43.1 (NH**C**H$_2$), 62.3 (**C**H), 69.9 (**C**H$_2$O), 79.6 (O**C**(CH$_3$)$_3$), 102.6 (d, J = 24.7 Hz, **C$_4$**" or **C$_2$**"), 107.7 (d, J = 20.9 Hz, **C$_2$**" or **C$_4$**"), 110.6 (d, J = 2.3 Hz, **C$_6$**"), 127.7, 127.8 (2 Ar**C**), 130.2 (d, J = 9.3 Hz, **C$_5$**"), 135.4, 138.1 (2 Ar**C**), 156.0 (O**C**(O)), 160.0 (d, J = 10.8 Hz, **C$_1$**"), 163.6 (d, J = 243.9 Hz, **C$_3$**"), 171.2 (C**C**(O)); HRMS (ESI) 577.1479 [M + Cs$^+$] (calcd for $C_{25}H_{33}FN_2O_4Cs^+$ 577.1595).



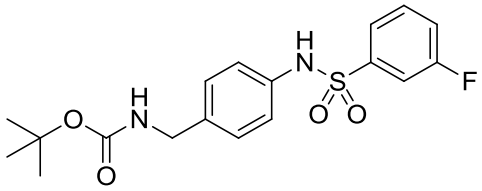
***N*-4'-((3''-Fluoro)phenoxymethyl)benzyl (Isobutoxycarbonyl)carbamate:** R_f 0.92 (1:1 EtOAc/hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (d, J = 6.4 Hz, CH(C**H**$_3$)$_2$), 1.86–1.95 (m, C**H**(CH$_3$)$_2$), 3.88–3.91 (br d, C(O)OC**H**$_2$), 4.36–4.40 (m, NHC**H**H'), 4.44–4.50 (m, NHCH**H'**), 5.02 (s, OC**H**$_2$), 6.64–6.74 (m, 3 Ar**H**), 7.18–7.49 (m, 5 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.0 (CH(**C**H$_3$)$_2$), 28.0 (**C**H(CH$_3$)$_2$), 44.7 (NH**C**H$_2$), 69.9, 71.2 (2 O**C**H$_2$), 102.5 (d, J = 24.8 Hz, **C$_4$**" or **C$_2$**"), 107.7 (d, J = 20.9 Hz, **C$_2$**" or **C$_4$**"), 110.6 (d, J = 2.3 Hz, **C$_6$**"), 127.8, 127.9 (2 Ar**C**), 130.2 (d, J = 9.3 Hz, **C$_5$**"), 135.6, 138.8 (2 Ar**C**), 156.9 (O**C**(O)), 160.0 (d, J = 10.8 Hz, **C$_1$**"), 163.6 (d, J = 243.9 Hz, **C$_3$**"); HRMS (ESI) 464.0638 [M + Cs$^+$] (calcd for $C_{19}H_{22}FNO_3Cs^+$ 464.0690).



(*R*)-*N*-4'-((3''-Fluoro)phenoxymethyl)benzyl 2-Amino-3,3-dimethylbutanamide ((*R*)-261). Utilizing Method B and using (*R*)-*N*-4'-((3''-fluoro)phenoxymethyl)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3,3-dimethylbutanamide (1.82 g, 4.10 mmol), TFA (4.56 mL, 61.45 mmol), and CH_2Cl_2 (14 mL) gave the crude product after basic workup that was further purified by flash column chromatography (SiO_2; 1:10–1:1 EtOAc/hexanes) to give the desired compound (1.03 g, 73%) as a white solid: mp 59–60 ºC; $[\alpha]^{25}_D$ +9.3° (*c* 1.1, CH_2Cl_2); R_f 0.30 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2732 (br), 1648, 1604, 1552, 1458, 1375, 1306, 1132, 1031, 961, 841, 726 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.01 (s, C(C**H**$_3$)$_3$), 1.50 (s, N**H**$_2$), 3.14 (s, C**H**), 4.45–4.46 (d, *J* = 4.2 Hz, NHC**H**$_2$Ph), 5.03 (s, OC**H**$_2$), 6.65–6.69 (m, 2 Ar**H**), 6.74–6.76 (d, *J* = 6.0 Hz, 1 Ar**H**), 7.02–7.14 (br t, N**H**CH_2), 7.19–7.25 (m, 1 Ar**H**), 7.31–7.32 (d, *J* = 5.3 Hz, 2 Ar**H**), 7.38–7.40 (d, *J* = 5.3 Hz, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.8 (C(**C**H_3)$_3$), 34.2 (**C**$(CH_3)_3$), 42.8 (NH**C**H_2), 64.4 (**C**H), 69.9 (**C**H_2O), 102.6 (d, *J* = 24.8 Hz, **C**$_{4''}$ or **C**$_{2''}$), 107.8 (d, *J* = 20.9 Hz, **C**$_{2''}$ or **C**$_{4''}$), 110.6 (d, *J* = 3.1 Hz, **C**$_{6''}$), 127.8, 128.2 (2 Ar**C**), 130.2 (d, *J* = 10.1 Hz, **C**$_{5''}$), 135.6, 138.6 (2 Ar**C**), 160.0 (d, *J* = 10.8 Hz, **C**$_{1''}$), 164.8 (d, *J* = 243.9 Hz, **C**$_{3''}$), 173.4 (**C**(O)); HRMS (ESI) 345.1977 [M + H^+] (calcd for $C_{20}H_{25}FN_2O_2H^+$ 345.1978); Anal. Calcd for $C_{20}H_{25}FN_2O_2$: C, 69.74; H, 7.32; F, 5.52; N, 8.13. Found: C, 69.76; H, 7.37; F, 5.39; N, 8.14.

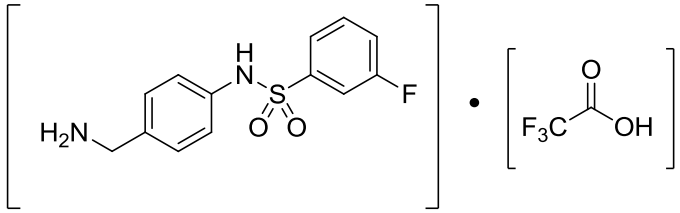


***tert*-Butyl *N*-(4-Aminobenzyl)carbamate (323).**[221] Boc_2O (10.59 g, 48.53 mmol) was added to a THF solution (55 mL) of 4-aminobenzylamine (5.00 mL, 44.12 mmol) at room temperature. The reaction was continued at room temperature (2 h) and then the solvent was evaporated *in vacuo* to give the crude product that was purified by column chromatography (SiO_2; 1:10–1:0 EtOAc/hexanes) to give the desired compound (6.60 g, 67%) as a yellow solid: mp 79–81 ºC (lit.[221] mp 74–75 ºC); R_f 0.39 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2667 (br), 1690, 1619, 1521, 1457, 1371, 1289, 1174, 1045, 1048, 946, 818, 726 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, C(C**H$_3$**)$_3$), 3.68 (s, N**H$_2$**), 4.16 (d, J = 5.4 Hz, NHC**H$_2$**), 4.80–4.92 (br t, N**H**), 6.59–6.63 (m, 2 Ar**H**), 7.04 (d, J = 8.1 Hz, 2 Ar**H**); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.5 (C(**C**H$_3$)$_3$), 44.4 (NH**C**H$_2$), 79.3 (**C**(CH$_3$)$_3$), 115.2, 128.8, 128.9, 145.9 (**C$_6$**H$_4$), 156.0 (**C**(O)); LRMS (ESI) 245.13 [M + Na^+] (calcd for $C_{12}H_{18}N_2O_2Na^+$ 245.13); HRMS (ESI) 355.0429 [M + Cs^+] (calcd for $C_{12}H_{18}N_2O_2Cs^+$ 355.0423).



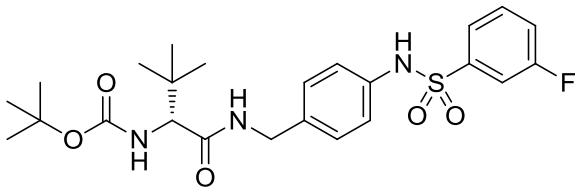
***tert*-Butyl *N*-(((4-(3-Fluorophenyl)sulfonamido)benzyl)carbamate (325).** *tert*-Butyl *N*-(4-aminobenzyl)carbamate (2.00 g, 9.00 mmol) was dissolved in anhydrous pyridine (18 mL) and cooled to 0 ºC before 3-fluorobenzenesulfonyl chloride (1.44 mL, 10.80 mmol) was added dropwise. The reaction was warmed to room temperature (18 h) and then cooled to 0 ºC before neutralizing with aqueous 1 M HCl. The solution was diluted with H_2O (10 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The organic layers were combined and successively washed with H_2O (2 x 50 mL) and brine (2 x 50 mL), dried (Na_2SO_4), evaporated in vacuo, and purified by column chromatography (SiO_2; 1:101:1 EtOAc/hexanes) to give the desired product (2.93 g, 86%) as a pale yellow solid. mp 147–150 ºC; R_f 0.64 (1:20 MeOH/CH_2Cl_2);

IR (nujol mull) 3328, 3116, 2988, 2927, 2839, 1689, 1457, 1374, 1158, 725 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, C(C**H**$_3$)$_3$), 4.18–4.28 (m, NHC**H**$_2$), 4.82–4.92 (br t, N**H**CH_2), 7.03 (d, J = 8.4 Hz, 2 Ar**H**), 7.14 (d, J = 8.4 Hz, 2 Ar**H**), 7.20–7.56 (m, 6 Ar**H**, N**H**). ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.3 (C(**C**H_3)$_3$), 43.9 (NH**C**H_2), 79.7 (**C**$(CH_3)_3$), 114.6 (d, J = 24.8 Hz, **C$_{2'}$** or **C$_{4'}$**), 120.2 (d, J = 21.0 Hz, **C$_{4'}$** or **C$_{2'}$**), 122.1 (Ar**C**), 122.9 (d, J = 3.1 Hz, **C$_{6'}$**), 128.3 (Ar**C**), 130.8 (d, J = 7.7 Hz, **C$_{5'}$**), 135.1, 136.5 (2 Ar**C**), 141.0 (d, J = 7.0 Hz, **C$_{1'}$**), 156.0 (**C**(O)), 162.3 (d, J = 250.9 Hz, **C$_{3'}$**), two aromatic peaks were not detected and are believed to overlap with nearby signals; HRMS (ESI) 513.0255 [M + Cs^+] (calcd for $C_{18}H_{21}FN_2O_4SCs^+$ 513.0260).

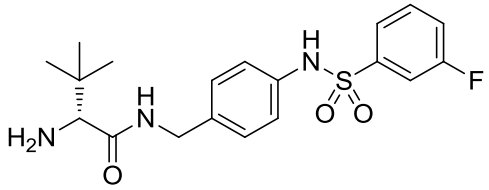


(4-((-3-Fluorophenyl)sulfonamido)phenyl)benzylamine Trifluoroacetate (326). *tert*-Butyl *N*-(((4-(3-fluorophenyl)sulfonamido)benzyl)carbamate (2.75 g, 7.23 mmol) was dissolved in anhydrous CH_2Cl_2 (70 mL) and cooled to 0 ºC before TFA (8.06 mL, 0.11 mol) was added dropwise. The reaction was maintained at 0 ºC (1.5 h) and then the solvent was evaporated *in vacuo* to give the desired product (2.73 g, 96%) as a pale yellow solid. The product was used in the next step without further purification: mp 209–211 ºC; R_f 0.00 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3416, 3308, 2858 (br), 1659, 1516, 1458, 1380, 1332, 1196, 1145, 924, 849, 794, 723 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.34–3.46 (br s, N**H**), 3.92 (s, NHC**H**$_2$), 7.14 (d, J = 8.8 Hz, 2 Ar**H**), 7.33 (d, J = 8.8, 2 Ar**H**), 7.47–7.64 (m, 4 Ar**H**), 8.06–8.21 (br s, N**H**$_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 41.6 (**C**H_2), 113.6 (d, J = 24.1 Hz, **C$_{2'}$** or **C$_{4'}$**), 119.9 (Ar**C**), 120.2 (d, J = 20.9 Hz, **C$_{4'}$** or **C$_{2'}$**), 122.9 (d, J = 3.1 Hz, **C$_{6'}$**), 129.7, 129.9 (2 Ar**C**), 131.7 (d, J = 7.8 Hz, **C$_{5'}$**), 137.4 (Ar**C**), 141.3 (d, J = 7.0 Hz, **C$_{1'}$**), 161.5 (d, J = 247.8

Hz, $\mathbf{C_{3'}}$), two aromatic peaks were not detected and are believed to overlap with nearby signals.

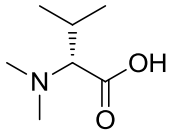


(*R*)-*N*-(4-((-3-Fluorophenyl)sulfonamido)phenyl)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3,3-dimethylbutanamide ((*R*)-327). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3,3-dimethylbutanoic acid (1.40 g, 6.06 mmol), NMM (1.41 mL, 15.48 mmol), IBCF (0.56 mL, 6.66 mmol), and (4-((-3-fluorophenyl)sulfonamido)phenyl)benzylamine trifluroracetate (2.50 g, 7.61 mmol) in anhydrous THF (75 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 1:10–1:1 EtOAc/hexanes) to give the desired compound (1.91 g, 64%) as a pale yellow solid with minor impurities: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 (s, CC(C$\mathbf{H_3}$)$_{\mathbf{3}}$), 1.39 (s, OC(C$\mathbf{H_3}$)$_{\mathbf{3}}$), 4.09–4.15 (m, C**H**), 4.27–4.44 (m, NHC$\mathbf{H_2}$), 5.18 (s, N**H**SO_2), 5.70 (d, J = 9.6 Hz OC(O)N**H**), 6.93–7.63 (m, 8 Ar**H**, N**H**CH_2).

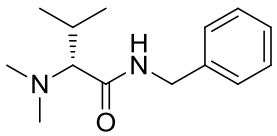


(*R*)-*N*-(4-((-3-Fluorophenyl)sulfonamido)phenyl)benzyl 2-Amino-3,3-dimethylbutanamide ((*R*)-262). Utilizing Method B and using (*R*)-*N*-(4-((-3-fluorophenyl)sulfonamido)phenyl)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3,3-dimethylbutanamide (1.80 g, 3.65 mmol), TFA (4.07 mL, 54.75 mol), and CH_2Cl_2 (12 mL) gave the crude product after basic workup as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84

(s, C(C**H**$_3$)$_3$), 3.71 (d, *J* = 6.8 Hz, C**H**), 4.04–4.17 (m, NHC**H**$_2$), 6.90–7.56 (m, 8 Ar**H**, N**H**SO$_2$), 8.09 (t, *J* = 5.6 Hz, N**H**CH$_2$); The ^{1}H NMR spectrum showed a small amount of EtOAc along with other minor impurities.

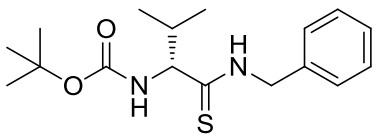


(*R*)-*N,N*-Dimethylamino-3-methylbutanoic acid ((*R*)-334).[220] D-Valine (10.00 g, 85.41 mmol) was dissolved in H_2O (450 mL) and formaldehyde (37% w/w, 13.86 mL, 0.17 mol) and 10% Pd-C (10.00 g) were added. The mixture was hydrogenated (1 atm) at room temperature (5 d), and then heated to reflux (10 min). The mixture was filtered through a bed of Celite® (3x) and the filtrate was evaporated *in vacuo*. The crude solid was dissolved in a 7:1 mixture of EtOH/H_2O and then evaporated *in vacuo* (5x) and then recrystallized from hot EtOH/acetone to give the desired product (11.89 g, 96%) as a white solid: mp 187–188 ºC (lit.[219] (*S*): mp 154 ºC); $[\alpha]^{28}_D$ –33.6° (*c* 2.1, EtOH); (lit.[219] (*S*): $[\alpha]^{16}_D$ +40.1° (*c* 2.02, EtOH)); IR (nujol mull) 2947 (br), 1726, 1459, 1373, 1196, 994, 884, 737 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.96 (d, *J* = 6.8 Hz, CH(C**H**$_3$)CH$_3$), 1.08 (d, *J* = 6.8 Hz, CH(CH$_3$)C**H**$_3$), 2.31–2.42 (m, C**H**(CH$_3$)$_2$), 2.80 (s, N(C**H**$_3$)$_2$), 3.91 (d, *J* = 4.8 Hz, C**H**); ^{13}C NMR (400 MHz, DMSO-d_6) δ 17.5 (CH(**C**H$_3$)CH$_3$), 20.7 (CH(CH$_3$)**C**H$_3$), 26.6 (**C**H(CH$_3$)$_2$), 41.7 (N(**C**H$_3$)$_2$), 71.9 (**C**H), 168.7 (**C**(O)); LRMS (ESI) 146.11 [M + H$^+$] (calcd for $C_7H_{15}NO_2H^+$ 146.11).



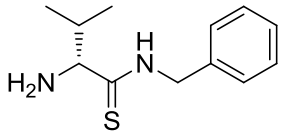
(*R*)-*N*-Benzyl *N,N*-Dimethylamino-3-methylbutanamide ((*R*)-270). (*R*)-*N,N*-Dimethylamino-3-methylbutanoic acid (2.28 g, 15.71 mmol) and benzylamine (2.06 mL,

18.86 mmol) were added to anhydrous THF (160 mL) at room temperature. The mixture stirred (15 min) and then DMTMM (5.22 g, 18.86 mmol) was added in one portion. The reaction continued at room temperature overnight (18 h) and then the insoluble salts were filtered, evaporated *in vacuo*, and purified by flash column chromatography (SiO_2; 2:1 EtOAc/CH_2Cl_2 followed by 10% MeOH/CH_2Cl_2) to give the desired product (0.38 g, 10%) as a white solid: mp 78–79 ºC; $[\alpha]^{28}_D$ –11.2° (*c* 0.5, $CHCl_3$); R_f 0.55 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2850 (br), 1635, 1555, 1458, 1375, 1234, 1034, 747, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (d, J = 6.6 Hz, CH(C**H**$_3$)CH_3), 1.03 (d, J = 6.6 Hz, CH(CH_3)C**H**$_3$), 2.04–2.17 (m, C**H**(CH_3)$_2$), 2.25 (s, N(C**H**$_3$)$_2$), 2.48 (d, J = 6.0 Hz, C**H**), 4.42–4.51 (m, NHC**H**$_2$Ph), 6.58–6.65 (br t, N**H**), 7.25–7.35 (m, C_6**H**$_5$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.5 (CH(**C**H_3)CH_3), 20.2 (CH(CH_3)**C**H_3), 27.7 (**C**H(CH_3)$_2$), 43.0, 43.1 (NH**C**H_2Ph, N(**C**H_3)$_2$), 76.3 (**C**H), 127.4, 128.0, 128.6, 138.5 (**C**$_6$$H_5$), 171.3 (**C**(O)); LRMS (ESI) 235.17 [M + H^+] (calcd for $C_{14}H_{22}N_2OH^+$ 235.17); Anal. Calcd for $C_{14}H_{22}N_2O$: C, 71.76; H, 9.46; N, 11.95. Found: C, 71.50; H, 9.29; N, 11.94.

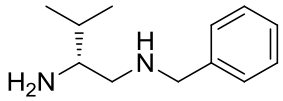


(*R*)-*N*-Benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylbutane-thioamide ((*R*)-335). (*R*)-*N*-Benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (4.00 g, 13.06 mmol) was dissolved in anhydrous THF (130 mL) and Lawesson's reagent (5.81 g, 14.37 mmol) was added at room temperature. The reaction was heated at reflux (4 h) and a second portion of Lawesson's reagent (5.81 g, 14.37 mmol) was added. The reaction was continued at reflux (18 h) before the solvent was evaproated *in vacuo*. The crude product was purified twice by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (3.18 g, 76%) as a pale yellow oil with minor

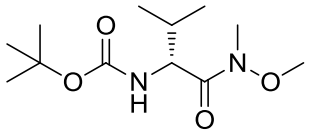
impurities: R_f 0.28 (1:10 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (d, J = 6.4 Hz, CH(C**H$_3$**)**$_2$**), 1.37 (s, C(C**H$_3$**)**$_3$**), 2.20–2.38 (br m, C**H**(CH_3)$_2$), 4.14 (t, J = 7.2 Hz, C**H**), 4.78 (dd, J = 5.2, 14.8 Hz, NHC**H**H'), 4.90 (dd, J = 5.2, 14.8 Hz, NHCH**H'**), 5.18–5.38 (br d, C(O)N**H**), 7.24–7.35 (m, C_6**H$_5$**), 8.38–8.48 (br t, C(S)N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.1 (CH(**C**H$_3$)CH$_3$), 19.7 (CH(CH$_3$)**C**H$_3$), 28.3 (C(**C**H$_3$)$_3$), 33.2 (**C**H(CH$_3$)$_2$), 49.7 (NH**C**H$_2$), 66.9 (**C**H), 80.2 (**C**(CH$_3$)$_3$), 128.0, 128.2, 128.9, 136.0 (**C$_6$**H$_5$), 155.9 (**C**(O)), 204.4 (**C**(S)); HRMS (ESI) 455.0786 [M + Cs^+] (calcd for $C_{17}H_{26}N_2O_2SCs^+$ 455.0769).



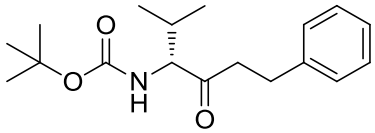
(*R*)-*N*-Benzyl 2-Amino-3-methylbutane-thioamide ((*R*)-271). Utilizing Method B and using (*R*)-*N*-benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutane-thioamide (1.72 g, 5.34 mmol), TFA (5.95 mL, 80.08 mmol), and CH_2Cl_2 (18 mL) gave the crude product after acidic workup and further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (0.89 g, 76%) as a yellow oil: $[\alpha]^{28.5}_D$ +43.7° (*c* 1.0, $CHCl_3$); R_f 0.50 (1:1 EtOAc/hexanes); IR (neat) 3253, 3201, 3035, 2959, 2873, 1601, 1520, 1456, 1382, 1327, 1362, 1181, 1068, 960, 740, 701 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.72 (d, J = 6.8 Hz, CH(C**H$_3$**)CH$_3$), 1.07 (d, J = 6.8 Hz, CH(CH$_3$)C**H$_3$**), 1.36 (s, N**H$_2$**), 2.75–2.87 (m, C**H**(CH_3)$_2$), 3.78 (d, J = 2.8 Hz, C**H**), 4.84 (dd, J = 4.6, 15.0 Hz, NHC**H**H'), 4.91 (dd, J = 5.0, 15.0 Hz, NHCH**H'**), 7.29–7.38 (m, C_6**H$_5$**), 9.75–9.95 (br s, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.4 (CH(**C**H$_3$)CH$_3$), 20.6 (CH(CH$_3$)**C**H$_3$), 32.9 (**C**H(CH$_3$)$_2$), 49.2 (NH**C**H$_2$), 66.6 (**C**H), 127.9, 128.2, 128.8, 136.5 (**C$_6$**H$_5$), 205.0 (**C**(S)); LRMS (ESI) 223.13 [M + H^+] (calcd for $C_{12}H_{18}N_2SH^+$ 223.14); Anal. Calcd for $C_{12}H_{18}N_2S$: C, 64.82; H, 8.16; N, 12.60. Found: C, 64.71; H, 8.24; N, 12.45.



(*R*)-1-*N*-Benzylamino-2-amino-3-methylbutane ((*R*)-272).[196] (*R*)-*N*-Benzyl 2-amino-3-methylbutanamide (3.38 g, 16.40 mmol) was dissolved in anhydrous THF (65 mL) and cooled to 0 ºC. BH_3•THF (1 M, 49.19 mL, 49.19 mmol) was added dropwise and the mixture was heated to reflux (18 h). The mixture was cooled to 0 ºC and aqueous 0.5 M HCl (100 mL) was slowly added. The THF was evaporated *in vacuo* and Et_2O (100 mL) was added to the acidic aqueous solution. The aqueous layer was separated and the organic layer was extracted with aqueous 0.5 M HCl (3 x 50 mL). All of the aqueous layers were combined and washed with Et_2O (2 x 100 mL). The aqueous layer was basified to pH 10–12 using aqueous 4 M NaOH and extracted with CH_2Cl_2 (3 x 100 mL). The CH_2Cl_2 layers were combined and were successively washed with a 1:1 mixture of EtOH/H_2O (2 x 100 mL) and saturated aqueous brine (2 x 100 mL), dried (Na_2SO_4), evaporated *in vacuo*, and purified three times by flash column chromatography (SiO_2; 1:100 MeOH/CH_2Cl_2) to give the desired product (0.53 g, 17%) as a pale yellow oil: $[\alpha]^{28}_D$ –31.4° (*c* 0.51, CH_2Cl_2) (lit.[196] $[\alpha]^{20}_D$ –30.4° (*c* 0.55, CH_2Cl_2)), $[\alpha]^{28}_D$ –31.4° (*c* 1.9, $CHCl_3$) (lit.[235] (*S*): $[\alpha]^{25.4}_D$ +33.4° (*c* 1.80, $CHCl_3$)); R_f 0.53 (1:20 MeOH/CH_2Cl_2); IR (neat) 3318, 3210, 3062, 2958, 2877, 1595, 1458, 1368, 1114, 1028, 851, 740, 701 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.90 (d, J = 6.8 Hz, CH(C**H$_3$**)**$_2$**), 1.58–1.69 (m, C**H**(CH_3)$_2$), 2.42–2.48 (m, C**H**H'N**H**, N**H$_2$**), 2.64–2.69 (m, C**H**), 2.74 (dd, J = 3.2, 12.0 Hz, CH**H'**NH), 3.78 (1/2 AB_q, J = 13.4 Hz, C**H**H'Ph), 3.84 (1/2 AB_q, J = 13.4 Hz, CH**H'**Ph), 7.22–7.35 (m, C_6**H$_5$**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0, 19.2 (CH(**C**H_3)$_2$), 32.0 (**C**H(CH_3)$_2$), 52.6, 53.8, 56.5 (**C**H, **C**H_2NH**C**H_2), 127.0, 128.2, 128.4, 140.0 (**C$_6$**H_5); HRMS (ESI) 193.1705 [M + H^+] (calcd for $C_{12}H_{20}N_2H^+$ 193.1695); Anal. Calcd for $C_{12}H_{20}N_2$•H_2O: C, 68.53; H, 10.54; N, 13.32. Found: C, 68.13; H, 10.97; N, 12.92.

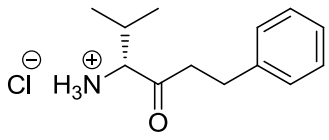


(*R*)-*N*-Methyl-*N*-methoxy 2-*N'*-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-337).[236] (*R*)-2-*N*-(*t*-Butoxycarbonyl)amino-3-methylbutanoic acid (5.00 g, 23.03 mmol) was dissolved in anhydrous THF (75 mL) and then 2-chloro-4,6-dimethoxy-1,3,5-triazine (4.85 g, 27.63 mmol) and NMM (7.60 mL, 69.08 mmol) were successively added leading to the precipitation of a white solid. The mixture stirred at room temperature (30 min) and then *N*,*O*-dimethylhydroxylamine hydrochloride (2.25 g, 23.03 mmol) was added. The mixture stirred at room temperature (18 h) and then the reaction was quenched with H_2O (75 mL) and extracted with Et_2O (3 x 75 mL). All of the organic layers were combined and were successively washed with saturated aqueous Na_2CO_3 (2 x 100 mL), aqueous 1 M HCl (2 x 100 mL), and saturated aqueous brine (2 x 100 mL). The organic layer was dried (Na_2SO_4) and evaporated *in vacuo* to give the desired product (5.89 g, 98%) as a white solid. The product was used for the next step without further purification: R_f 0.25 (1:10 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (d, J = 6.8 Hz, CH(C**H$_3$**)CH$_3$), 0.96 (d, J = 6.8 Hz, CH(CH$_3$)C**H$_3$**), 1.44 (s, C(C**H$_3$**)$_3$), 1.94–2.05 (m, C**H**(CH$_3$)$_2$), 3.22 (s, NC**H$_3$**), 3.78 (s, OC**H$_3$**), 4.52–4.64 (br m, C**H**), 5.16 (d, J = 8.8 Hz, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.4 (CH(**C**H$_3$)CH$_3$), 19.3 (CH(CH$_3$)**C**H$_3$), 28.3 (C(**C**H$_3$)$_3$), 31.3, 31.8 (**C**H(CH$_3$)$_2$, N**C**H$_3$), 54.9 (**C**H), 61.5 (O**C**H$_3$), 79.3 (**C**(CH$_3$)$_3$), 155.8 (O**C**(O)), 172.9 (C**C**(O)); LRMS (ESI) 283.15 [M + Na$^+$] (calcd for $C_{12}H_{24}N_2O_2Na^+$ 283.15).



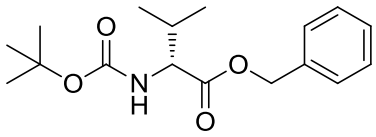
(*R*)-4-*N*-(*t*-Butoxycarbonyl)amino-5-methyl-1-phenyl-3-hexanone ((*R*)-336).[237,238] To anhydrous Et_2O (130 mL) was added magnesium turnings (1.69 g, 69.42 mmol) and 2-

(bromoethyl)benzene (8.97 mL, 65.45 mmol) in a 3 neck round bottom flask that was fitted with a thermometer and a condensor. An ice bath was applied when the reaction reached 40 ºC (5 min) and was removed when the reaction cooled to 20 ºC. The reaction was continued at room temperature (1.5 h) and then an anhydrous Et_2O solution (50 mL) of (*R*)-*N*-methyl-*N*-methoxy 2-*N'*-(*t*-butoxycarbonyl)amino-3-methylbutanamide (5.16 g, 19.83 mmol) was added to the reaction via a cannula. An ice bath was applied when the reaction reached 40 ºC (10 min) and was removed when the reaction cooled to 20 ºC. The reaction was continued at room temperature (1.5 h) and then was quenched with aqueous 1 M HCl (100 mL). The organic layer was separated and washed with saturated aqueous brine (2 x 100 mL), dried (Na_2SO_4), evaporated *in vacuo*, and purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes) to give the desired product (4.32 g, 71%) as a colorless oil: $[\alpha]^{28}_D$ –40.7° (*c* 2.2, $CHCl_3$); R_f 0.52 (1:10 EtOAc/hexanes); IR (neat) 3342, 2970, 1709, 1501, 1368, 1308, 1244, 1169, 1079, 1021, 872, 748, 701 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.70 (d, J = 6.8 Hz, CH(C**H**$_3$)CH_3), 0.96 (d, J = 6.8 Hz, $CH(CH_3)$C**H**$_3$), 1.44 (s, C(C**H**$_3$)$_3$), 2.07–2.15 (m, C**H**$(CH_3)_2$), 2.74–2.95 (m, C**H**$_2$C**H**$_2$Ph), 4.26 (dd, J = 4.2, 8.5 Hz, C**H**), 5.11 (d, J = 8.5 Hz, N**H**), 7.15–7.29 (m, C_6**H**$_5$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.6 (CH(**C**H_3)CH_3), 19.9 ($CH(CH_3)$**C**H_3), 28.3 (C(**C**$H_3)_3$), 29.4 (CH_2**C**H_2Ph), 30.1 (**C**$H(CH_3)_2$), 42.4 (**C**H_2CH_2Ph), 64.0 (**C**H), 79.6 (**C**$(CH_3)_3$), 126.2, 128.3, 128.5, 140.8 (**C**$_6H_5$), 155.9 (O**C**(O)), 208.7 (C**C**(O)); HRMS (ESI) 438.1025 [M + Cs^+] (calcd for $C_{18}H_{27}NO_3Cs^+$ 438.1045).



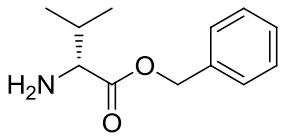
(*R*)-4-Amino-2-methyl-6-phenyl-4-hexanone Hydrochloride ((*R*)-273). (*R*)-4-*N*-(*t*-Butoxycarbonyl)amino-5-methyl-1-phenyl-3-hexanone (3.04 g, 9.96 mmol) was dissolved in MeOH (50 mL) and aqueous concentrated HCl (4.92 mL, 0.20 mol) was added. The reaction

was heated at reflux (1 h) and then cooled to room temperature before evaporating the solvent *in vacuo* to give a crude oil. The crude product was triturated with hexanes (3x) to give the desired product (0.60 g, 25%) as a white solid: mp 123–124 ºC; $[\alpha]^{28}_{D}$ –59.7° (*c* 1.1, $CHCl_3$); R_f 0.49 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2919 (br), 1716, 1590, 1458, 1375, 1284, 1078, 980, 926, 751, 703 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.97 (d, J = 7.0 Hz, CH(C**H**$_3$)CH_3), 1.17 (d, J = 7.0 Hz, CH(CH_3)C**H**$_3$), 2.34–2.44 (br m, C**H**(CH_3)$_2$), 2.56–2.98 (m, C**H**$_2$C**H**$_2$Ph), 4.18–4.26 (br d, C**H**), 7.16–7.24 (m, C_6**H**$_5$), 8.57 (br s, N**H**$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.1 (CH(**C**H_3)CH_3), 19.3 (CH(CH_3)**C**H_3), 29.0, 29.1 (**C**H(CH_3)$_2$, CH_2**C**H_2Ph), 42.4 (**C**H_2CH_2Ph), 64.2 (**C**H), 126.3, 128.4, 128.5, 140.3 (**C**$_6$$H_5$), 204.7 (**C**(O)); LRMS (ESI) 206.13 [M - Cl^-] (calcd for $C_{13}H_{20}NO$ 206.13); Anal. Calcd for $C_{13}H_{20}ClNO$: C, 64.59; H, 8.34; Cl, 14.66; N, 5.79. Found: C, 64.53; H, 8.47; Cl, 14.44; N, 5.78.



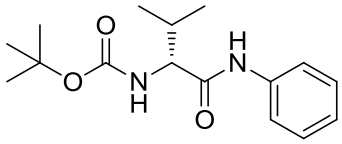
(*R*)-*O*-Benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylbutanoate ((*R*)-338).[228,239] Et_3N (2.38 mL, 17.13 mmol) was added to a solution of (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (3.72 g, 17.13 mmol) in anhydrous CH_2Cl_2 (60 mL) at 0 ºC. After the reaction was stirred at 0 ºC (10 min), benzyl chloroformate (2.41 mL, 17.13 mmol) was added dropwise. The reaction was continued at 0 ºC (10 min) and DMAP (0.21 g, 1.71 mmol) was added. The reaction was continued at 0 ºC (30 min) and then the solution was diluted with CH_2Cl_2 (40 mL), successively washed with saturated $NaHCO_3$ (2 x 100 mL), aqueous 0.1 M HCl (2 x 100 mL), and saturated aqueous brine (2 x 100 mL), dried (Na_2SO_4), and evaporated *in vacuo*. The crude product was purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes) to give the desired product (2.65 g, 50%) as a colorless oil: $[\alpha]^{28.5}_{D}$ +27.8° (*c* 1.1, MeOH) (lit.[239] $[\alpha]^{20}_{D}$ +32.1° (*c* 1.08, MeOH)); R_f 0.53 (1:10

EtOAc/hexanes); IR (neat) 2970, 1716, 1504, 1368, 1251, 1166, 1090, 1011, 747, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.85 (d, J = 6.4 Hz, CH(C**H$_3$**)CH_3), 0.94 (d, J = 6.4 Hz, $CH(CH_3)$C**H$_3$**), 1.44 (s, C(C**H$_3$**)$_3$), 2.10–2.19 (m, C**H**$(CH_3)_2$), 4.27 (dd, J = 4.6, 8.8 Hz, C**H**), 5.03 (d, J = 8.8 Hz, N**H**), 5.11–5.22 (m, OC**H$_2$**), 7.32–7.36 (m, C_6**H$_5$**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.4 (CH(**C**H_3)CH_3), 19.0 ($CH(CH_3)$**C**H_3), 28.3 (C(**C**$H_3)_3$), 31.3 (**C**H$(CH_3)_2$), 58.5 (**C**H), 66.8 (O**C**H_2), 79.7 (**C**$(CH_3)_3$), 128.3, 128.4, 128.5, 135.4 (**C$_6$**H_5), 155.6 (O**C**(O)), 172.2 (C**C**(O)).

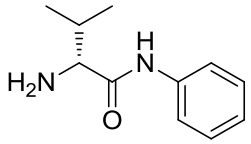


(*R*)-*O*-Benzyl 2-Amino-3-methylbutanoate ((*R*)-274).[240] 1 M HCl in Et_2O (165 mL) was added to a Et_2O solution (10 mL) of (*R*)-*O*-benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanoate (2.02 g, 6.58 mmol) at 0 ºC. The reaction was stirred at room temperature (18 h) to give a cloudy white solution. The solution was filtered but no substantial filtrate was collected. The solvent was evaporated *in vacuo* to give a crude oil that was then redissolved in CH_2Cl_2 (10 mL). The organic layer was extracted with aqueous 1 M HCl (3 x 10 mL). The aqueous layers were combined and washed with CH_2Cl_2 (2 x 30 mL). The aqueous layer was basified to pH 10–12 with aqueous 4 M NaOH and extracted with CH_2Cl_2 (3 x 50 mL). The second set of organic layers were combined and washed with saturated aqueous brine (2 x 150 mL), dried (Na_2SO_4), and evaporated *in vacuo.* The crude product was purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired product (0.99 g, 72%) as a pale yellow oil: $[\alpha]^{28.5}_D$ –10.2° (*c* 1.1, $CHCl_3$); R_f 0.39 (1:1 EtOAc/hexanes); IR (neat) 2962, 1602, 1460, 1378, 1163, 984, 912, 819, 747, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (d, J = 7.0 Hz, CH(C**H$_3$**)CH_3), 0.96 (d, J = 7.0 Hz, $CH(CH_3)$C**H$_3$**), 1.44 (s, N**H$_2$**), 1.99–2.10 (m, C**H**$(CH_3)_2$), 3.34 (d, J = Hz, C**H**), 5.14 (1/2

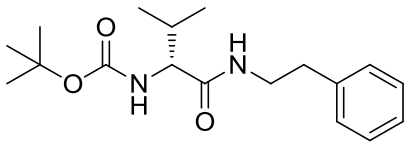
AB_q, J = 12.2 Hz, OC**H**H'), 5.18 (1/2 AB_q, J = 12.2 Hz, OCH**H'**), 7.30–7.39 (m, C_6**H**$_5$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.1 (CH(**C**H_3)CH_3), 19.3 (CH(CH_3)**C**H_3), 32.1 (**C**H(CH_3)$_2$), 59.9 (**C**H), 66.5 (O**C**H_2), 128.3, 128.4, 128.5, 135.8 (**C**$_6$$H_5$), 175.4 (**C**(O)); LRMS (ESI) 208.15 [M + H^+] (calcd for $C_{12}H_{17}NO_2H^+$ 208.15); Anal. Calcd for $C_{12}H_{17}NO_2$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.28; H, 8.44; N, 6.80.



(*R*)-*N*-Phenyl 2-*N'*-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-343).[241] Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (2.42 g, 11.15 mmol), NMM (1.59 mL, 14.49 mmol), IBCF (1.58 mL, 12.26 mmol), and aniline (1.07 mL, 11.70 mmol) in anhydrous THF (110 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 1:10–1:1 EtOAc/hexanes) to give the desired product (2.17 g, 67%) as a pale orange solid: mp 178–179 ºC; $[\alpha]^{25}_D$ +37.0° (*c* 1.1, CH_2Cl_2); R_f 0.24 (1:10 EtOAc/hexanes); IR (nujol mull) 3264, 3185, 2725 (br), 1677, 1606, 1457, 1375, 1297, 1167, 726 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (d, J = 6.8 Hz, CH(C**H$_3$**)CH_3), 1.04 (d, J = 6.8 Hz, CH(CH_3)C**H$_3$**), 1.44 (s, C(C**H$_3$**)$_3$), 2.18–2.22 (m, C**H**(CH_3)$_2$), 4.09 (t, J = 6.4 Hz, C**H**CH(CH_3)$_2$), 5.36–5.38 (br d, OC(O)N**H**), 7.06 (br t, 1 Ar**H**), 7.25 (d, J = 7.8 Hz, 2 Ar**H**), 7.48 (d, J = 7.8 Hz, 2 Ar**H**), 8.38–8.49 (br s, N**H**Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.4 (CH(**C**H_3)CH_3), 19.4 (CH(CH_3)**C**H_3), 28.3 (C(**C**H_3)$_3$), 30.9 (**C**H(CH_3)$_2$), 61.0 (**C**H), 80.1 (**C**(CH_3)$_3$), 120.0, 124.2, 128.8, 137.7 (**C**$_6$$H_5$), 156.5 (O**C**(O)), 170.7 (C**C**(O)); HRMS (ESI) 315.1685 [M + Na^+] (calcd for $C_{16}H_{24}N_2O_3Na^+$ 315.1685); Anal. Calcd for $C_{16}H_{24}N_2O_3$: C, 65.73; H, 8.27; N, 9.58. Found: C, 65.47; H, 8.32; N, 9.71.

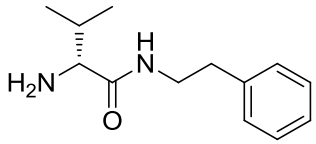


(*R*)-*N*-Phenyl 2-Amino-3-methylbutanamide ((*R*)-275).[242] Utilizing Method B and using (*R*)-*N*-phenyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (1.75 g, 5.99 mmol), TFA (6.67 mL, 89.84 mmol), and CH_2Cl_2 (20 mL) gave the crude product after acidic workup and was further purified by flash column chromatography (SiO_2; 1:10–1:1 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (0.94 g, 82%) as a pale orange oil: $[\alpha]^{25}_D$ +82.5° (*c* 1.6, CH_2Cl_2); R_f 0.34 (1:1 EtOAc/hexanes); IR (neat) 3059, 2878 (br), 1662, 1599, 1515, 1444, 1382, 1312, 1246, 1173, 1049, 1030, 982, 882, 753, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.87 (d, J = 7.2 Hz, CH(C**H**$_3$)CH_3), 1.04 (d, J = 7.2 Hz, $CH(CH_3)$C**H**$_3$), 1.46 (s, N**H**$_2$), 2.40–2.48 (m, C**H**$(CH_3)_2$), 3.37 (d, J = 3.6 Hz, C**H**), 7.09 (t, J = 7.2 Hz, 1 Ar**H**), 7.32 (t, J = 8.2 Hz, 2 Ar**H**), 7.60 (d, J = 8.2 Hz, 2 Ar**H**), 9.46–9.56 (br s, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0 (CH(**C**H_3)CH_3), 19.8 ($CH(CH_3)$**C**H_3), 30.8 (**C**H$(CH_3)_2$), 60.4 (**C**H), 119.4, 124.0, 128.9, 137.8 (**C**$_6$H$_5$), 172.6 (**C**(O)); HRMS (ESI) 193.1343 [M + H^+] (calcd for $C_{11}H_{16}N_2OH^+$ 193.1341); Anal. Calcd for $C_{11}H_{16}N_2O$•0.01H_2O: C, 68.65; H, 8.39; N, 14.56. Found: C, 68.27; H, 8.41; N, 14.32.



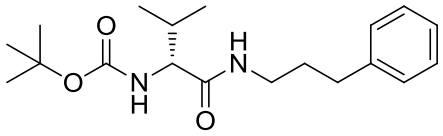
(*R*)-*N*-Phenethyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-344). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (2.40 g, 11.05 mmol), NMM (1.58 mL, 14.37 mmol), IBCF (1.57 mL, 12.16 mmol), and phenylethylamine (1.46 mL, 11.61 mmol) in anhydrous THF (110 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 1:10–1:1 EtOAc/hexanes) to give the desired

compound (1.87 g, 53%) as a white solid: mp 121–122 ºC; $[\alpha]^{25}_{D}$ +10.7° (*c* 1.0, CH_2Cl_2); R_f 0.77 (1:1 EtOAc/hexanes); IR (nujol mull) 2728 (br), 1653, 1457, 1375, 1162, 725 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.86 (d, *J* = 7.0 Hz, CH(C**H$_3$**)CH_3), 0.91 (d, *J* = 7.0 Hz, CH(CH_3)C**H$_3$**), 1.43 (s, C(C**H$_3$**)$_3$), 2.01–2.16 (m, C**H**(CH_3)$_2$), 2.81 (t, *J* = 6.8 Hz, C**H$_2$**Ph), 3.44–3.52 (m, NHC**H**H'), 3.54–3.63 (m, NHCH**H'**), 3.81 (dd, *J* = 6.4, 8.8 Hz, C**H**), 4.98–5.14 (br d, N**H**), 5.88–5.61 (br t, N**H**), 7.18–7.32 (m, C_6**H$_5$**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.8 (CH(**C**H_3)CH_3), 19.2 (CH(CH_3)**C**H_3), 28.3 (C(**C**H_3)$_3$), 30.8 (**C**H(CH_3)$_2$), 35.8 (**C**H_2Ph), 40.6 (NH**C**H_2), 60.1 (**C**H), 79.8 (**C**(CH_3)$_3$), 126.5, 128.6, 128.7, 138.7 (**C$_6$**H_5), 155.9 (O**C**(O)), 171.5 (C**C**(O)); HRMS (ESI) 453.1151 [M + Cs^+] (calcd for $C_{18}H_{28}N_2O_3Cs^+$ 453.1154); Anal. Calcd for $C_{18}H_{28}N_2O_3$•0.01H_2O: C, 67.43; H, 8.81; N, 8.74. Found: C, 67.04; H, 8.93; N, 8.97.

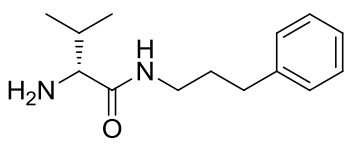


(*R*)-*N*-Phenethyl 2-Amino-3-methylbutanamide ((*R*)-276). Utilizing Method B and using (*R*)-*N*-phenethyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (1.37 g, 4.28 mmol), TFA (4.77 mL, 64.18 mmol), and CH_2Cl_2 (15 mL) gave the crude product after acidic workup and further purified by flash column chromatography (SiO_2; 1:10–1:1 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (873 mg, 93%) as a pale yellow solid: mp 36–37 ºC; $[\alpha]^{25}_{D}$ +32.9° (*c* 1.0, CH_2Cl_2); R_f 0.19 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3199 (br), 1656, 1524, 1458, 1371, 1243, 1085, 1035, 895, 745, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.77 (d, *J* = 7.2 Hz, CH(C**H$_3$**)CH_3), 0.96 (d, *J* = 7.2 Hz, CH(CH_3)C**H$_3$**), 1.20–1.26 (s, N**H$_2$**), 2.22–2.33 (m, C**H**(CH_3)$_2$), 2.77–2.88 (m, C**H$_2$**Ph), 3.19 (d, *J* = 4.0 Hz, C**H**), 3.46–3.62 (m, NHC**H$_2$**), 7.20–7.32 (m, C_6**H$_5$**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.9 (CH(**C**H_3)CH_3), 19.7 (CH(CH_3)**C**H_3), 30.7 (**C**H(CH_3)$_2$), 35.9 (**C**H_2Ph), 40.1 (NH**C**H_2),

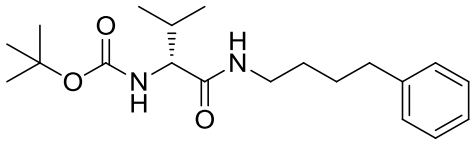
60.2 (**C**H), 126.4, 128.5, 128.7, 130.6 (**C$_6$**H$_5$), 174.2 (**C**(O)); HRMS (ESI) 221.1643 [M + H$^+$] (calcd for $C_{13}H_{20}N_2OH^+$ 221.1654); Anal. Calcd for $C_{13}H_{20}N_2O$: C, 70.87; H, 9.15; N, 12.72. Found: C, 70.65; H, 9.15; N, 12.72.



(*R*)-*N*-Phenylpropyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-345). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (4.00 g, 18.42 mmol), NMM (2.63 mL, 23.95 mmol), IBCF (2.61 mL, 20.26 mmol), and 3-phenyl-1-propylamine (2.76 mL, 19.34 mmol) in anhydrous THF (185 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes) to give the desired compound (6.08 g, 99%) as a white solid: mp 77–78 ºC; $[\alpha]^{28.5}_D$ +11.1° (*c* 1.1, $CHCl_3$); R_f 0.88 (1:1 EtOAc/hexanes); IR (nujol mull) 3137, 2726 (br), 1681, 1527, 1457, 1373, 1304, 1247, 1169, 1021, 929, 733 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (d, *J* = 7.2 Hz, CH(C**H$_3$**)CH$_3$), 0.95 (d, *J* = 6.4 Hz, CH(CH$_3$)C**H$_3$**), 1.43 (s, C(C**H$_3$**)$_3$), 1.79–1.86 (m, NHCH$_2$C**H$_2$**), 2.04–2.14 (m, C**H**(CH$_3$)$_2$), 2.63 (t, *J* = 8.0 Hz, C**H$_2$**Ph), 3.20–3.35 (m, NHC**H$_2$**), 3.86 (dd, *J* = 7.0, 8.5 Hz, C**H**), 5.16 (d, *J* = 8.5 Hz, OC(O)N**H**), 6.32 (br t, CC(O)N**H**), 7.15–7.20 (m, 3 Ar**H**), 7.25–7.29 (m, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0 (CH(**C**H$_3$)CH$_3$), 19.3 (CH(CH$_3$)**C**H$_3$), 28.3 (C(**C**H$_3$)$_3$), 30.8 (**C**H(CH$_3$)$_2$), 31.2 (NHCH$_2$**C**H$_2$), 33.2 (**C**H$_2$Ph), 39.0 (NH**C**H$_2$), 60.2 (**C**H), 79.8 (**C**(CH$_3$)$_3$), 126.0, 128.3, 128.4, 141.4 (**C$_6$**H$_5$), 156.0 (O**C**(O)), 171.6 (C**C**(O)); LRMS (ESI) 357.23 [M + Na$^+$] (calcd for $C_{19}H_{30}N_2O_3Na^+$ 357.23); Anal. Calcd for $C_{19}H_{30}N_2O_3$: C, 68.23; H, 9.04; N, 8.38. Found: C, 68.51; H, 9.14; N, 8.24.

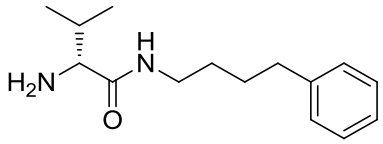


(*R*)-*N*-Phenylpropyl 2-Amino-3-methylbutanamide ((*R*)-277). Utilizing Method B and using (*R*)-*N*-phenylpropyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (5.00 g, 14.96 mmol), TFA (16.67 mL, 0.22 mol), and CH_2Cl_2 (50 mL) gave the crude product after acidic workup and further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (3.02 g, 86%) as a pale yellow oil: $[\alpha]^{28.5}_D$ +30.1° (*c* 1.2, $CHCl_3$); R_f 0.21 (1:1 EtOAc/hexanes); IR (neat) 3138, 2953 (br), 2870, 1953, 1878, 1806, 1651, 1533, 1457, 1369, 1303, 1240, 1188, 1092, 1036, 898, 746, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.81 (d, *J* = 7.2 Hz, CH(C**H$_3$**)CH_3), 0.98 (d, *J* = 7.2 Hz, CH(CH_3)C**H$_3$**), 1.30 (s, N**H$_2$**), 1.79–1.90 (m, NHCH_2C**H$_2$**), 2.23–2.34 (m, C**H**(CH_3)$_2$), 2.65 (t, *J* = 8.4 Hz, C**H$_2$**Ph), 3.19 (d, *J* = 4.0 Hz, C**H**), 3.22–3.37 (m, NHC**H$_2$**), 7.16–7.20 (m, 3 Ar**H**), 7.26–7.35 (m, 2 Ar**H**, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0 (CH(**C**H_3)CH_3), 19.7 (CH(CH_3)**C**H_3), 30.8 (**C**H(CH_3)$_2$), 31.3 (NHCH_2**C**H_2), 33.4 (**C**H_2Ph), 38.6 (NH**C**H_2), 60.2 (**C**H), 125.9, 128.4, 128.4, 141.6 (**C$_6$**H_5), 174.3 (**C**(O)); HRMS (ESI) 235.1818 [M + H^+] (calcd for $C_{14}H_{22}N_2OH^+$ 235.1810); Anal. Calcd for $C_{14}H_{22}N_2O$•0.16H_2O: C, 70.87; H, 9.48; N, 11.81. Found: C, 70.48; H, 9.60; N, 11.72.



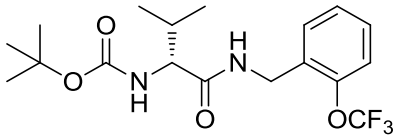
(*R*)-*N*-Phenylbutyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-346). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (4.24 g, 19.53 mmol), NMM (2.79 mL, 25.39 mmol), IBCF (2.77 mL, 21.48 mmol), and 4-phenylbutylamine (3.24 mL, 20.50 mmol) in anhydrous THF (195 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes) to give the desired compound (3.75 g, 55%) as a white solid: mp 73–74 ºC; $[\alpha]^{28.5}_D$ +12.8° (*c* 1.0, $CHCl_3$); R_f 0.89 (1:1 EtOAc/hexanes); IR (nujol mull) 3139, 2917 (br), 1650, 1530, 1458,

1374, 1303, 1250, 1171, 1023, 926, 744, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.91 (d, J = 7.0 Hz, CH(C**H**$_3$)CH$_3$), 0.94 (d, J = 7.0 Hz, CH(CH$_3$)C**H**$_3$), 1.42 (s, C(C**H**$_3$)$_3$), 1.48–1.58 (m, NHCH$_2$C**H**$_2$ or C**H**$_2$CH$_2$Ph), 1.59–1.68 (m, C**H**$_2$CH$_2$Ph or NHCH$_2$C**H**$_2$), 2.04–2.14 (C**H**(CH$_3$)$_2$), 2.62 (t, J = 7.6 Hz, C**H**$_2$Ph), 3.19–3.34 (m, NHC**H**$_2$), 3.84 (dd, J = 6.4, 8.8 Hz, C**H**), 5.10–5.12 (br d, OC(O)N**H**), 6.10–6.16 (br t, CC(O)N**H**), 7.14–7.19 (m, 3 Ar**H**), 7.25–7.28 (m, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0 (CH(**C**H$_3$)CH$_3$), 19.3 (CH(CH$_3$)**C**H$_3$), 28.3 (C(**C**H$_3$)$_3$), 28.6 (**C**H$_2$CH$_2$Ph or NHCH$_2$**C**H$_2$), 29.2 (NHCH$_2$**C**H$_2$ or **C**H$_2$CH$_2$Ph), 30.8 (**C**H(CH$_3$)$_2$), 35.4 (**C**H$_2$Ph), 39.2 (NH**C**H$_2$), 60.2 (**C**H), 79.8 (**C**(CH$_3$)$_3$), 125.8, 128.3, 128.4, 142.0 (**C**$_6$H$_5$), 155.9 (O**C**(O)), 171.6 (C**C**(O)); LRMS (ESI) 371.24 [M + Na^+] (calcd for $C_{20}H_{32}N_2O_3Na^+$ 371.24); Anal. Calcd for $C_{20}H_{32}N_2O_3$: C, 68.93; H, 9.26; N, 8.04. Found: C, 68.94; H, 9.44; N, 8.14.

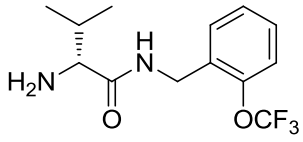


(*R*)-*N*-Phenylbutyl 2-Amino-3-methylbutanamide ((*R*)-278). Utilizing Method B and using (*R*)-*N*-phenylbutyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (3.18 g, 9.13 mmol), TFA (10.17 mL, 0.14 mol), and CH_2Cl_2 (30 mL) gave the crude product after basic workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (2.16 g, 96%) as a pale yellow oil: $[\alpha]^{28.5}_D$ +29.9° (*c* 1.0, $CHCl_3$); R_f 0.53 (1:20 MeOH/CH_2Cl_2); IR (neat) 3126, 3005, 2817 (br), 1652, 1530, 1458, 1371, 1302, 1240, 1180, 1091, 744, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.81 (d, J = 6.8 Hz, CH(C**H**$_3$)CH$_3$), 0.97 (d, J = 6.8 Hz, CH(CH$_3$)C**H**$_3$), 1.51–1.59 (m, NHCH$_2$C**H**$_2$ or C**H**$_2$CH$_2$Ph, N**H**$_2$), 1.62–1.70 (m, C**H**$_2$CH$_2$Ph or NHCH$_2$C**H**$_2$), 2.22–2.34 (m, C**H**(CH$_3$)$_2$), 2.63 (t, J = 8.0 Hz, CH$_2$C**H**$_2$Ph), 3.20 (d, J = 4.0 Hz, C**H**), 3.22–3.44 (m, NHC**H**$_2$), 7.15–7.19 (m, 3 Ar**H**), 7.25–7.36 (m, 2 Ar**H**, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ

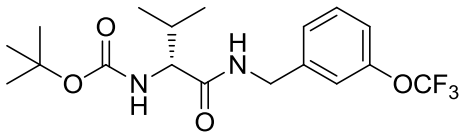
16.0 (CH(**C**H_3)CH_3), 19.7 (CH(CH_3)**C**H_3), 28.7 (**C**H_2CH_2Ph or NHCH$_2$**C**H_2), 29.3 (NHCH$_2$**C**H_2 or **C**H_2CH_2Ph), 30.8 (**C**H(CH_3)$_2$), 35.5 (**C**H_2Ph), 38.8 (NH**C**H_2), 60.2 (**C**H), 125.8, 128.3, 128.4, 142.2 (**C$_6$**H_5), 174.3 (**C**(O)); HRMS (ESI) 249.1954 [M + H^+] (calcd for $C_{15}H_{24}N_2OH^+$ 249.1967); Anal. Calcd for $C_{15}H_{24}N_2O$•0.0.6CH_2Cl_2: C, 71.29; H, 9.58; N, 11.04. Found: C, 71.01; H, 9.75; N, 10.88.



(*R*)-*N*-2'-(Trifluoromethoxy)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-349). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (4.00 g, 18.42 mmol), NMM (2.63 mL, 23.95 mmol), IBCF (2.61 mL, 20.26 mmol), and 2-(trifluoromethoxy)benzylamine (2.91 mL, 19.34 mmol) in anhydrous THF (185 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes) to give the desired compound (6.20 g, 86%) as a white solid: mp 133–134 ºC; $[\alpha]^{28.5}_D$ +11.1° (*c* 1.1, $CHCl_3$); R_f 0.91 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2918 (br), 1657, 1526, 1458, 1375, 1162, 725 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 (d, *J* = 6.6 Hz, CH(C**H$_3$**)CH_3), 0.95 (d, *J* = 6.6 Hz, CH(CH_3)C**H$_3$**), 1.42 (s, C(C**H$_3$**)$_3$), 2.12–2.22 (m, C**H**(CH_3)$_2$), 3.89 (dd, *J* = 6.4, 8.8 Hz, C**H**), 4.53 (d, J = 6.0 Hz, NHC**H$_2$**), 4.94–5.04 (br d, OC(O)N**H**), 6.24–6.36 (br t, CC(O)N**H**), 7.23–7.34 (m, 3 Ar**H**), 7.40–7.42 (m, 1 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.7 (C(**C**H_3)CH_3), 19.3 (C(CH_3)**C**H_3), 28.3 (C(**C**H_3)$_3$), 30.5 (**C**H(CH_3)$_2$), 38.1 (NH**C**H_2), 60.3 (**C**H), 80.1 (**C**(CH_3)$_3$), 120.5 (1 Ar**C**), 120.6 (q, *J* = 257.1 Hz, O**C**F_3), 127.1, 129.0, 130.2, 130.5 (4 Ar**C**), 147.3 (**C**OCF_3), 155.9 (O**C**(O)), 171.7 (C**C**(O)); LRMS (ESI) 413.15 [M + Na^+] (calcd for $C_{18}H_{25}F_3N_2O_4Na^+$ 413.15); Anal. Calcd for $C_{18}H_{25}F_3N_2O_4$: C, 55.38; H, 6.45; F, 14.60; N, 7.18. Found: C, 55.31; H, 6.52; F, 14.51; N, 7.15.

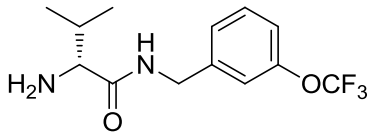


(*R*)-*N*-2'-(Trifluoromethoxy)benzyl 2-Amino-3-methylbutanamide ((*R*)-279). Utilizing Method B and using (*R*)-*N*-2'-(trifluoromethoxy)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (6.00 g, 15.38 mmol), TFA (17.13 mL, 0.23 mol), and CH_2Cl_2 (50 mL) gave the crude product after acidic workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (3.70 g, 83%) as a pale yellow solid: mp 54–55 ºC; $[\alpha]^{28.5}_D$ +28.0° (*c* 1.0, $CHCl_3$); R_f 0.59 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2954 (br), 1638, 1458, 1373, 1257, 1160, 897, 727 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.81 (d, J = 6.8 Hz, CH(C**H**$_3$)CH_3), 0.98 (d, J = 6.8 Hz, CH(CH_3)C**H**$_3$), 1.34 (s, N**H**$_2$), 2.28–2.39 (m, C**H**(CH_3)$_2$), 3.27 (d, J = 4.0 Hz, C**H**), 4.48–4.57 (m, NHC**H**$_2$), 7.22–7.33 (m, 3 Ar**H**), 7.41–7.43 (m, 1 Ar**H**), 7.71–7.79 (br t, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.9 (C(**C**H_3)CH_3), 19.7 (C(CH_3)**C**H_3), 30.8 (**C**H(CH_3)$_2$), 37.7 (NH**C**H_2), 60.2 (**C**H), 120.5 (Ar**C**), 120.6 (q, J = 256.4 Hz, O**C**F_3), 127.1, 128.8, 130.3, 131.2 (4 Ar**C**), 147.4 (**C**OCF_3), 174.5 (**C**(O)); LRMS (ESI) 291.12 [M + H^+] (calcd for $C_{13}H_{17}F_3N_2O_2H^+$ 291.12); Anal. Calcd for $C_{13}H_{17}F_3N_2O_2$: C, 53.79; H, 5.90; F, 19.63; N, 9.65. Found: C, 53.80; H, 5.83; F, 19.37; N, 9.45.



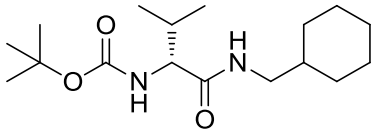
(*R*)-*N*-3'-(Trifluoromethoxy)benzyl 2-*N*'-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-350). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (2.20 g, 10.13 mmol), NMM (1.45 mL, 13.17 mmol), IBCF (1.43 mL, 11.15 mmol), and 3-(trifluoromethoxy)benzylamine (1.60 mL, 10.64 mmol) in anhydrous THF (100 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 1:20–1:1

EtOAc/hexanes) to give the desired compound (3.26 g, 82%) as a white solid: mp 103–104 ºC; $[\alpha]^{28.5}_{D}$ +12.9° (*c* 1.0, $CHCl_3$); R_f 0.88 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2917 (br), 1646, 1458, 1375, 1166, 725 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.93 (d, *J* = 7.0 Hz, CH(C**H$_3$**)CH_3), 0.96 (d, *J* = 7.0 Hz, CH(CH_3)C**H$_3$**), 1.41 (s, C(C**H$_3$**)$_3$), 2.09–2.22 (m, C**H**(CH_3)$_2$), 3.94 (dd, *J* = 6.9, 9.0 Hz, C**H**), 4.41 (dd, *J* = 6.0, 15.2 Hz, NHC**H**H'), 4.52 (dd, *J* = 6.0, 15.2 Hz, NHCH**H'**), 5.13 (d, *J* = 6.9 Hz, OC(O)N**H**), 6.72–6.78 (br t, CC(O)N**H**), 7.10–7.11 (m, 2 Ar**H**), 7.18–7.20 (m, 1 Ar**H**), 7.30–7.34 (m, 1 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.9 (CH(**C**H_3)CH_3), 19.4 (CH(CH_3)**C**H_3), 28.3 (C(**C**H_3)$_3$), 30.6 (**C**H(CH_3)$_2$), 42.7 (NH**C**H_2), 60.3 (**C**H), 80.1 (**C**(CH_3)$_3$), 119.8 (1 Ar**C**), 120.4 (q, *J* = 255.6 Hz, O**C**F_3), 120.0, 125.9, 130.0, 140.7 (4 Ar**C**), 149.5 (**C**OCF_3), 156.1 (O**C**(O)), 172.0 (C**C**(O)); LRMS (ESI) 413.14 [M + Na^+] (calcd for $C_{18}H_{25}F_3N_2O_4Na^+$ 413.14); Anal. Calcd for $C_{18}H_{25}F_3N_2O_4$: C, 55.38; H, 6.45; F, 14.60; N, 7.18. Found: C, 55.31; H, 6.46; F, 14.77; N, 7.08.

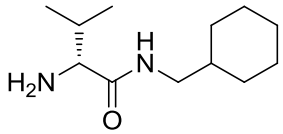


(*R*)-*N*-3'-(Trifluoromethoxy)benzyl 2-Amino-3-methylbutanamide ((*R*)-280). Utilizing Method B and using (*R*)-*N*-3'-(trifluoromethoxy)benzyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (3.00 g, 7.69 mmol), TFA (8.57 mL, 0.12 mol), and CH_2Cl_2 (25 mL) gave the crude product after acidic workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (1.81 g, 81%) as a pale yellow oil: $[\alpha]^{28.5}_{D}$ +20.6° (*c* 1.3, $CHCl_3$); R_f 0.47 (1:20 MeOH/CH_2Cl_2); IR (neat) 3325, 3077, 2963, 2471, 1657, 1253, 1454, 1358, 1261, 1166, 1088, 1025, 964, 874, 794, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, *J* = 7.0 Hz, CH(C**H$_3$**)CH_3), 1.00 (d, *J* = 7.0 Hz, CH(CH_3)C**H$_3$**), 1.38 (s, N**H$_2$**), 2.31–2.40 (m, C**H**(CH_3)$_2$), 3.30 (d, *J* = 4.0 Hz, C**H**), 4.43 (dd, *J* = 6.2, 15.0 Hz, NHC**H**H'), 4.51 (dd, *J* = 6.6, 15.0 Hz,

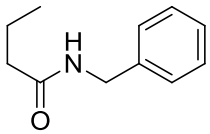
NHCH**H'**), 7.10–7.13 (m, 2 Ar**H**), 7.21–7.22 (m, 1 Ar**H**), 7.34 (t, *J* = 8.0 Hz, 1 Ar**H**), 7.78–7.86 (br t, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 16.0 (C(**C**H_3)CH_3), 19.7 (C(CH_3)**C**H_3), 30.8 (**C**H(CH_3)$_2$), 42.5 (NH**C**H_2), 60.2 (**C**H), 119.7, 120.0 (2 Ar**C**), 120.5 (q, *J* = 256.3 Hz, O**C**F_3), 126.0, 130.0, 141.3 (3 Ar**C**), 149.5 (**C**OCF_3), 174.6 (**C**(O)); LRMS (ESI) 291.11 [M + H^+] (calcd for $C_{13}H_{17}F_3N_2O_2H^+$ 291.11); Anal. Calcd for $C_{13}H_{17}F_3N_2O_4$: C, 53.79; H, 5.90; F, 19.63; N, 9.65. Found: C, 53.55; H, 6.11; F, 19.63; N, 9.66.



(*R*)-*N*-Cyclohexylmethyl 2-*N'*-(*t*-Butoxycarbonyl)amino-3-methylbutanamide ((*R*)-352). Utilizing Method A and using (*R*)-2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanoic acid (4.00 g, 18.42 mmol), NMM (2.63 mL, 23.95 mmol), IBCF (2.61 mL, 20.26 mmol), and cyclohexylmethylamine (2.52 mL, 19.34 mmol) in anhydrous THF (185 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes) to give the desired compound (5.01 g, 87%) as a white solid: mp 141–142 ºC; $[\alpha]^{28}_D$ +18.2° (*c* 1.1, $CHCl_3$); R_f 0.88 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2919 (br), 1651, 1529, 1458, 1375, 1307, 1249, 1170 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 0.87–0.97 (m, 2 cyclohexyl **H**), 0.92 (d, *J* = 7.0 Hz, CH(C**H$_3$**)CH_3), 0.96 (d, *J* = 7.0 Hz, CH(CH_3)C**H$_3$**), 1.09–1.28 (m, 3 cyclohexyl **H**), 1.44 (s, C(C**H$_3$**)$_3$), 1.64–1.74 (m, 6 cyclohexyl **H**), 2.06–2.20 (m, C**H**(CH_3)$_2$), 3.05–3.16 (m, NHC**H$_2$**), 3.83 (dd, *J* = 6.4, 8.8 Hz, NHC**H**), 5.04–5.10 (br d, OC(O)N**H**), 5.98–6.08 (br t, N**H**CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 17.9 (CH(**C**H_3)CH_3), 19.4 (CH(CH_3)**C**H_3), 25.8, 26.4 (2 cyclohexyl **C**), 28.3 (C(**C**H_3)$_3$), 30.6, 30.8 (**C**H(CH_3)$_2$, cyclohexyl **C**), 37.9 (cyclohexyl **C**), 45.7 (NH**C**H_2), 60.4 (**C**H), 79.9 (**C**(CH_3)$_3$), 155.9 (O**C**(O)), 171.6 (C**C**(O)); LRMS (ESI) 335.21 [M + Na^+] (calcd for $C_{17}H_{32}N_2O_3Na^+$ 335.21); Anal. Calcd for $C_{17}H_{32}N_2O_3$: C, 65.35; H, 10.32; N, 8.97. Found: C, 65.39; H, 10.35; N, 8.84.

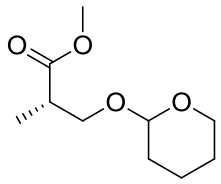


(*R*)-*N*-Cyclohexylmethyl 2-Amino-3-methylbutanamide ((*R*)-281). Utilizing Method B and using (*R*)-*N*-cyclohexylmethyl 2-*N*'-(*t*-butoxycarbonyl)amino-3-methylbutanamide (3.60 g, 11.53 mmol), TFA (12.84 mL, 0.17 mol), and CH_2Cl_2 (38 mL) gave the crude product after acidic workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (2.34 g, 96%) as a white solid: mp 85–86 ºC; $[\alpha]^{25}_D$ +38.1° (*c* 1.1, $CHCl_3$); R_f 0.59 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2934 (br), 1635, 1555, 1458, 1375, 1305, 1227, 1152, 1075, 982, 720 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.82 (d, *J* = 7.0 Hz, CH(C**H**$_3$)CH_3), 0.89–0.99 (m, 2 cyclohexyl **H**), 0.99 (d, *J* = 7.0 Hz, $CH(CH_3)$C**H**$_3$), 1.10–1.28 (m, 3 cyclohexyl **H**), 1.36 (s, N**H**$_2$), 1.41–1.52 (m, 1 cyclohexyl **H**), 1.65–1.74 (m, 5 cyclohexyl **H**), 2.26–2.37 (m, C**H**$(CH_3)_2$), 3.03–3.18 (m, NHC**H**$_2$), 3.23 (d, *J* = 3.6 Hz, NHC**H**), 7.30–7.40 (br t, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0 (CH(**C**H_3)CH_3), 19.8 ($CH(CH_3)$**C**H_3), 25.8, 26.4 (2 cyclohexyl **C**), 30.8, 30.9 (**C**$H(CH_3)_2$, cyclohexyl **C**), 38.0 (cyclohexyl **C**), 45.2 (NH**C**H_2), 60.3 (**C**H), 174.2 (**C**(O)); LRMS (ESI) 213.18 [M + H^+] (calcd for $C_{12}H_{24}N_2OH^+$ 213.18); Anal. Calcd for $C_{12}H_{24}N_2O$: C, 67.88; H, 11.39; N, 13.19. Found: C, 67.88; H, 11.50; N, 13.02.



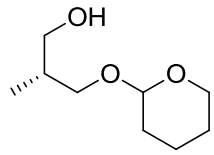
***N*-Benzyl Butanamide (282).**[194,243] Utilizing Method A and using n-butanoic acid (2.00 mL, 21.88 mmol), NMM (3.13 mL, 28.45 mmol), IBCF (3.10 mL, 24.07 mmol), and benzylamine (2.51 mL, 22.98 mmol) gave the crude product that was recrystallized from hot EtOAc/hexanes to give the desired compound (1.18 g, 30%) as a white solid: mp 54–55 °C (lit.[7] mp 36.9–38 ºC); R_f 0.67 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 3140, 2856 (br), 1457,

1375, 726 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.96 (t, J = 7.6 Hz, C**H$_3$**), 1.65–1.74 (m, CH_3C**H$_2$**), 2.20 (t, J = 8.0 Hz, C**H$_2$**CH_2CH_3), 4.45 (d, J = 5.6 Hz, NHC**H$_2$**), 5.66–5.78 (br s, N**H**), 7.26–7.35 (m, C_6**H$_5$**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8 (**C**H_3), 19.2 (**C**H_2CH_3), 38.7 (**C**$H_2CH_2CH_3$), 43.6 (NH**C**H_2), 127.5, 127.8, 128.7, 138.4 (**C**$_6H_5$), 172.7 (**C**(O)); HRMS (ESI) 178.1238 [M + H^+] (calcd for $C_{11}H_{15}NOH^+$ 178.1232); Anal. Calcd for $C_{11}H_{15}NO$•$0.06H_2O$: C, 74.06; H, 8.55; N, 7.85. Found: C, 73.72; H, 8.60; N, 7.93.

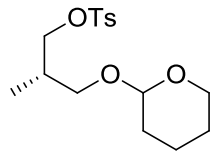


Methyl (*S*)- 3-(Tetrahydro-2-pyranyloxy)-2-methylpropionate ((*S*)-359).[230] (*S*)-3-Hydroxy-2-methylpropionate (4.50 mL, 40.80 mmol) was dissolved in anhydrous Et_2O (40 mL) and then dihydropyran (4.47 mL, 48.96 mmol) and *p*-toluenesulfonic acid (0.78 g, 4.08 mmol) were added at room temperature. The reaction was continued at room temperature (18 h) and then the solution was washed with saturated aqueous $NaHCO_3$ (2 x 40 mL), dried (Na_2SO_4), and evaporated *in vacuo* to give a crude oil that was purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes) to give the desired product (7.40 g, 90%) as an ~1:1 diastereomeric mixture (**A,B**) as a colorless oil: $[\alpha]^{28}_D$ +14.7° (*c* 3.9, Et_2O) (lit.[230] $[\alpha]^{25}_D$ +16.3° (*c* 3.9, Et_2O)); R_f 0.58 (1:10 EtOAc/hexanes); IR (neat) 2944, 2875, 1739, 1454, 1358, 1260, 1199, 1129, 1032, 974, 818 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.18 (d, J = 3.0 Hz) and 1.20 (d, J = 3.0 Hz) (CHC**H$_3$**; **A,B**), 1.49–1.89 (m, OCH_2C**H$_2$**C**H$_2$**C**H$_2$**; **A + B**), 2.73–2.81 (m, C**H**CH_3; **A + B**), 3.42–3.62 (m, C**H**H'OCHOC**H**H'; **A + B**), 3.69 (s) and 3.70 (s) (OC**H$_3$**; **A,B**), 3.74–3.93 (m, CH**H'**OCHOCH**H'**; **A + B**), 4.60 (app t, J = 3.4 Hz) and 4.62 (app. t, J = 3.4 Hz) (C**H**O; **A,B**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 13.9 (CH**C**H_3), 19.0, 19.2, 25.2, 30.3, 30.4 (OCH_2**C**H_2**C**H_2**C**H_2), 39.9, 40.1 (**C**HCH_3), 51.5 (O**C**H_3), 61.6, 61.9

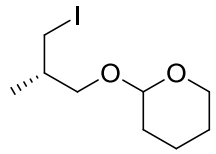
(O**C**H_2CH_2), 68.9, 69.2 (O**C**H_2CH), 98.3, 98.9 (O**C**HO), 175.1, 175.2 (**C**(O)), two peaks were not detected and are believed to overlap with nearby signals; LRMS (ESI) 214.11 [M + Na^+] (calcd for $C_{10}H_{18}O_4Na^+$ 214.11).



(*R*)-2-Methyl-3-(tetrahydro-2-pyranyloxy)-propan-1-ol ((*S*)-360).[230] Methyl (*S*)-3-(tetrahydro-2-pyranyloxy)-2-methylpropionate (6.39 g, 31.61 mmol) was dissolved in anhydrous Et_2O (30 mL) and added to a stirred solution of $LiAlH_4$ (0.96 g, 25.30 mmol) in anhydrous Et_2O (60 mL) at 0 ºC. The reaction was stirred at room temperature (18 h) and then cooled to 0 ºC. H_2O (6.4 mL) was added to the mixture, followed by aqueous 15% NaOH (6.4 mL) and H_2O (19.2 mL). The resulting mixture was filtered through Celite® and the filtrate was evaporated *in vacuo*. The crude product was purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes) to give the desired product (3.81 g, 98%) as an ~1:1 diastereomeric mixture (**A,B**) as a colorless oil: $[\alpha]^{28}_D$ +0.8° (*c* 1.5, Et_2O) (lit.[230] $[\alpha]^{25}_D$ +1.2° (*c* 1.5, Et_2O)); R_f 0.48 (1:10 EtOAc/hexanes); IR (neat) 3225, 2944, 1457, 1356, 1667, 1128, 1031, 902, 812 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.91 (d, *J* = 4.2 Hz) and 0.92 (d, *J* = 4.2 Hz) (CHC**H$_3$**; **A,B**), 1.48–2.08 (m, $OCH_2C\mathbf{H_2}C\mathbf{H_2}C\mathbf{H_2}$, O**H**; **A + B**), 2.93–3.00 (br s, C**H**CH_3; **A + B**), 3.34–3.47 (m, OC**H**H'CH; **A + B**), 3.49–3.55 (m, C**H**H'OCH; **A + B**), 3.58–3.64 (m, C**H$_2$**OH; **A + B**), 3.66–3.82 (m, OCH**H'**CH; **A + B**); 3.83–3.90 (m, CH**H'**OCH; **A + B**); 4.58 (app. t, *J* = 4.4 Hz, C**H**O; **A + B**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.5, 13.6 (CH**C**H_3), 19.5, 25.2, 25.3, 30.4, 30.5 ($OCH_2\mathbf{C}H_2\mathbf{C}H_2\mathbf{C}H_2$), 35.4, 35.6 (**C**$HCH_3$), 62.3, 62.4 (O**C**$H_2CH_2$), 66.7, 66.8 (**C**$H_2$OH), 71.6, 71.7 (O**C**$H_2$CH), 99.0, 99.2 (O**C**HO), one peak was not detected and is believed to overlap with nearby signals; LRMS (ESI) 197.10 [M + Na^+] (calcd for $C_9H_{18}O_3Na^+$ 197.10).

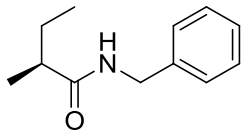


(*S*)-2-Methyl-3-(tetrahydro-2-pyranyloxy)-propyl-*p*-toluenesulfonate ((*S*)-361).[230] (*R*)-2-Methyl-3-(tetrahydro-2-pyranyloxy)-propan-1-ol (3.40 g, 19.53 mmol) was dissolved in anhydrous pyridine (25 mL) and cooled to 0 ºC and TsCl (4.84 g, 25.38 mmol) was added. The reaction was maintained at 5 ºC overnight (18 h) and then H_2O (10 mL) was added. The aqueous layer was extracted with Et_2O (3 x 10 mL). The Et_2O layers were combined and successively washed with aqueous 10% citric acid (3 x 30 mL), saturated $NaHCO_3$ (2 x 30 mL), and saturated aqueous brine (2 x 30 mL), dried (Na_2SO_4), and evaporated *in vacuo*. The crude product was purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes) to give the desired product (5.72 g, 89%) as an ~1:1 diastereomeric mixture (**A,B**) as a colorless oil: $[\alpha]^{28}_D$ –24.4° (*c* 7.1, Et_2O) (lit.[230] $[\alpha]^{25}_D$ +6.8° (*c* 7.01, Et_2O)); R_f 0.41 (1:10 EtOAc/hexanes); IR (neat) 2947, 2873, 1600, 1459, 1360, 1180, 1129, 1031, 970, 820 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.93 (d, J = 2.4 Hz) and 0.95 (d, J = 2.4 Hz) (CHC**H$_3$**; **A,B**), 1.42–1.78 (m, $OCH_2C\mathbf{H_2}C\mathbf{H_2}C\mathbf{H_2}$, **A + B**), 2.05–2.15 (m, C**H**CH_3; **A + B**), 2.44 (s, PhC**H$_3$**; **A + B**), 3.17–3.28 (m, OC**H**H'CH; **A + B**), 3.43–3.48 (m, C**H**H'OCH; **A + B**), 3.56–3.64 (m, OCH**H'**CH; **A + B**), 3.71–3.78 (m, CH**H'**OCH; **A + B**), 3.93–4.14 (m, $C\mathbf{H_2}OSO_2$), 4.44 (app. t, J = 3.8 Hz) and 4.47 (app. t, J = 3.8 Hz) (C**H**O; **A,B**), 7.34 (d, J = 8.2 Hz, 2 Ar**H**), 7.79 (d, J = 8.2 Hz, 2 Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.5, 13.6 ($CH\mathbf{C}H_3$), 19.2, 19.3, 25.3, 30.3, 30.4 ($OCH_2\mathbf{C}H_2\mathbf{C}H_2\mathbf{C}H_2$), 21.5 (Ph**C**$H_3$), 33.4, 33.6 (**C**$HCH_3$), 61.9, 62.1 (O**C**$H_2CH_2$), 67.8, 68.3 (**C**$H_2OSO_2$); 72.2, 72.3 (O**C**$H_2CH$), 98.5, 99.0 (O**C**HO), 132.9, 133.0, 127.8, 129.7, 132.9, 133.0, 144.6 (C_6H_4), three peaks were not detected and are believed to overlap with nearby signals; LRMS (ESI) 351.11 [M + Na^+] (calcd for $C_{16}H_{24}O_5SNa^+$ 351.11).

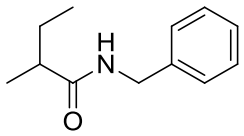


(*S*)-1-Iodo-2-methyl-3-(tetrahydro-2-pyranyloxy)propane ((*S*)-363).[244] A 3-neck round bottom flask was charged with magnesium turnings (0.17 g, 6.86 mmol) fitted with a thermometer and a condenser. Anhydrous Et_2O (7 mL) was added and the mixture was cooled to 0 ºC in an ice bath before MeI (0.43 mL, 6.86 mmol) was added. The mixture was allowed to warm to room temperature and was maintained between 20–25 ºC by intermittently applying an ice bath. After all of the magnesium turning had disappeared (1 h), the solution was transferred via cannula to a -78 ºC THF solution (14 mL) of (*S*)-2-methyl-3-(tetrahydro-2-pyranyloxy)-propyl-*p*-toluenesulfonate (0.45 g, 1.37 mmol). The mixture was stirred (5 min) and then $LiCuCl_4$ (0.1 M in THF, 0.27 mL) was added at -78 ºC. The mixture was allowed to warm to room temperature (18 h) and then washed with aqueous saturated NH_4Cl (2 x 25 mL). The aqueous layers were combined and washed with Et_2O (2 x 50 mL). All of the organic layers were combined and washed with H_2O (2 x 50 mL), dried (Na_2SO_4), and purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes) to give the starting material (0.02 g, 4%) and (*S*)-1-iodo-2-methyl-3-(tetrahydro-2-pyranyloxy)propane (0.23 g, 58%) as an ~1:1 diastereomeric mixture (**A,B**) as a pale yellow oil: R_f 0.61 (1:10 EtOAc/hexanes); IR (neat) 3330, 3059 (br), 1456, 1353, 1267, 1194, 1129, 1033, 973, 903, 813 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.99 (d, J = 6.8 Hz) and 1.02 (d, J = 6.8 Hz) ($CHC\mathbf{H_3}$; **A,B**), 1.51–1.86 (m, $OCH_2C\mathbf{H_2}C\mathbf{H_2}C\mathbf{H_2}$, $C\mathbf{H}CH_3$; **A + B**), 3.17–3.38 (m, $C\mathbf{H_2}I$, $OC\mathbf{H}H'CHCH_3$; **A + B**), 3.49–3.68 (m, $OCH\mathbf{H'}CHCH_3$, $OC\mathbf{H}H'CH_2$; **A + B**), 3.66–3.82 (m, $OCH\mathbf{H'}CH_2$; **A + B**); 4.60 (app. t, J = 4.0 Hz, $C\mathbf{H}O$; **A + B**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.5, 14.0 ($\mathbf{C}H_2I$), 17.6, 17.8 ($CH\mathbf{C}H_3$), 19.2, 19.5, 25.4, 30.5, 30.6 ($OCH_2\mathbf{C}H_2\mathbf{C}H_2\mathbf{C}H_2$), 35.1, 35.3 ($\mathbf{C}HCH_3$), 62.0, 62.3 ($O\mathbf{C}H_2CH_2$), 71.0, 71.4 ($O\mathbf{C}H_2CH$), 98.0, 99.2 ($O\mathbf{C}HO$), one peak was not

detected and is believed to overlap with nearby signals; HRMS (ESI) 307.0156 [M + Na^+] (calcd for $C_9H_{17}IO_2Na^+$ 307.0171).

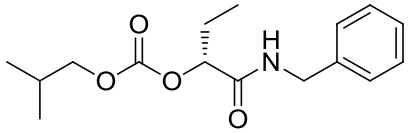


(*S*)-*N*-Benzyl 2-Methylbutanamide ((*S*)-283).[245] Utilizing Method A and using (*S*)-2-methylbutanoic acid (0.90 mL, 8.27 mmol), NMM (1.18 mL, 10.75 mmol), IBCF (1.17 mL, 9.10 mmol), and benzylamine (0.95 mL, 8.62 mmol) gave the crude product that was purified twice by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes) to give the desired compound (1.01 g, 64%) as a white solid: mp 55–56 ºC; $[\alpha]^{28}_D$ +15.5° (*c* 1.0, acetone) (lit.[245] $[\alpha]_D$ +16.96° (*c* 1.0, acetone)); R_f 0.80 (1:1 EtOAc/hexanes); IR (nujol mull) 2861 (br), 1644, 1550, 1458, 1374, 1245, 1107, 1024, 732 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.91 (t, *J* = 7.6 Hz, $CH_2C\mathbf{H_3}$), 1.16 (d, *J* = 7.6 Hz, $CHC\mathbf{H_3}$), 1.40–1.50 (m, C**H**H'CH_3), 1.65–1.75 (m, CH**H'**CH_3), 2.09–2.18 (m, C**H**), 4.39–4.49 (m, NHC$\mathbf{H_2}$Ph), 5.80–5.88 (br s, N**H**), 7.25–7.35 (m, $C_6\mathbf{H_5}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9 ($CH_2\mathbf{C}H_3$), 17.5 ($CH\mathbf{C}H_3$), 27.3 ($\mathbf{C}H_2CH_3$), 43.2, 43.4 (**C**H, NH**C**H_2), 127.4, 127.7, 128.6, 138.6 ($\mathbf{C}_6H_5$), 176.3 (**C**(O)); LRMS (ESI) 193.16 [M + H^+] (calcd for $C_{12}H_{17}NOH^+$ 193.16); Anal. Calcd for $C_{12}H_{17}NO$: C, 75.35; H, 8.96; N, 7.32. Found: C, 75.21; H, 8.96; N, 7.36.

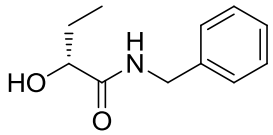


(*R,S*)-*N*-Benzyl 2-Methylbutanamide ((*R,S*)-283).[246] Utilizing Method A and using DL-2-methylbutanoic acid (2.00 mL, 18.33 mmol), NMM (2.62 mL, 23.83 mmol), IBCF (2.60 mL, 20.16 mmol), and benzylamine (2.10 mL, 19.25 mmol) gave the crude product that was purified twice by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes) to give the

desired compound (2.32 g, 66%) as a white solid: mp 54–55 ºC (lit.[8] mp 47.5–48.5 ºC); R_f 0.80 (1:1 EtOAc/hexanes); IR (nujol mull) 2967 (br), 1645. 1550. 1458. 1374, 1245, 1107, 1024, 732 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.91 (t, J = 7.2 Hz, $CH_2C\mathbf{H_3}$), 1.15 (d, J = 6.2 Hz, $CHC\mathbf{H_3}$), 1.39–1.50 (m, C**H**H'CH_3), 1.64–1.75 (m, CH**H'**CH_3), 2.09–2.17 (m, C**H**), 4.39–4.49 (m, NHC$\mathbf{H_2}$Ph), 5.79–5.89 (br s, N**H**), 7.26–7.35 (m, $C_6\mathbf{H_5}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1 ($CH_2\mathbf{C}H_3$), 17.7 ($CH\mathbf{C}H_3$), 27.5 ($\mathbf{C}H_2CH_3$), 43.4, 43.6 (**C**H, NH**C**H_2), 127.6, 127.9, 128.9, 138.8 ($\mathbf{C}_6H_5$), 176.5 (**C**O); HRMS (ESI) 214.1199 [M + Na^+] (calcd for $C_{12}H_{17}NOH^+$ 214.1208); Anal. Calcd for $C_{12}H_{17}NO$: C, 75.35; H, 8.96; N, 7.32. Found: C, 75.23; H, 8.91; N, 7.27.



(*R*)-*N*-Benzyl 2-(Isobutoxycarbonyl)oxybutanamide ((*R*)-356). Utilizing Method A and using (*R*)-2-hydroxybutanoic acid (2.00 g, 19.21 mmol), NMM (2.75 mL, 24.98 mmol), IBCF (2.73 mL, 21.13 mmol), and 4-(trifluoromethoxy)benzylamine (2.20 mL, 20.17 mmol) in anhydrous THF (190 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes) to give the desired compound (1.96 g, 35%) as a white solid: mp 67–68 ºC; R_f 0.89 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2849 (br), 1750, 1658, 1556, 1458, 1375, 1243, 1128, 1041, 963, 789, 740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.92 (s, CH(C$\mathbf{H_3}$)CH_3'), 0.94 (s, CH(CH_3)C$\mathbf{H_3}$'), 0.97 (t, J = 7.6 Hz, $CH_2C\mathbf{H_3}$), 1.90–2.04 (m, C**H**(CH_3)$_2$, C$\mathbf{H_2}CH_3$), 3.90–3.98 (m, OC$\mathbf{H_2}$), 4.23–4.53 (m, NHC$\mathbf{H_2}$), 5.09 (dd, J = 4.6, 6.6 Hz, C**H**), 6.49–6.56 (br t, N**H**), 7.26–7.36 (m, $C_6\mathbf{H_5}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.9 ($CH_2\mathbf{C}H_3$), 18.8 (CH($\mathbf{C}H_3$)$_2$), 25.3 ($\mathbf{C}H_2CH_3$), 27.7 (**C**H(CH_3)$_2$), 43.1 (NH**C**H_2), 74.7 (O**C**H_2), 78.4 (**C**H), 127.6, 127.7, 128.8, 137.8 ($\mathbf{C_6}H_5$), 154.2 (O**C**(O)), 169.3 (**C**(O)); HRMS (ESI) 426.0667 [M + Cs^+] (calcd for $C_{16}H_{23}NO_4Cs^+$ 426.0681).



(*R*)-*N*-Benzyl 2-Hydroxybutanamide ((*R*)-284). To anhydrous toluene (80 mL) was added (*R*)-2-hydroxybutanoic acid (1.70 g, 16.33 mmol), benzylamine (1.78 mL, 16.33 mmol), and 3,5-bis(trifluoromethyl)benzene boronic acid (0.42 g, 1.63 mmol). A pressure equalizing dropping funnel containing a cotton plug was filled 1/3 of the way with 3 Å molecular sieves that were oven dried (120 ºC) and a condenser was placed above the dropping funnel. The mixture was heated at reflux (18 h) before cooling to room temperature and then the solvent was evaporated *in vacuo*. The crude product was purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) followed by recrystallization from hot EtOAc/hexanes to give the desired compound (1.05 g, 33%) as a white solid: mp 63–64 ºC; $[\alpha]^{28.5}_D$ +28.3° (*c* 2.2, $CHCl_3$); R_f 0.56 (1:20 MeOH/CH_2Cl_2); IR (nujol mull) 2929 (br), 1625, 1530, 1458, 1375, 1302, 1241, 1092, 1038, 985, 724 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.96 (t, J = 7.6 Hz, C**H**$_3$), 1.63–1.74 (m, C**H**H'CH_3), 1.82–1.92 (m, CH**H'**CH_3), 3.32 (d, J = 4.8 Hz, O**H**), 4.07–4.11 (m, C**H**), 4.38–4.48 (m, NHC**H**$_2$), 6.95–7.20 (br t, N**H**), 7.24–7.34 (m, C_6**H**$_5$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.1 (**C**H_3), 27.9 (**C**H_2CH_3), 43.1 (NH**C**H_2), 73.1 (**C**H), 127.6, 127.7, 128.7, 138.0 (**C**$_6H_5$), 173.9 (**C**(O)); LRMS (ESI) 216.12 [M + Na^+] (calcd for $C_{11}H_{15}NO_2Na^+$ 216.12); Anal. Calcd for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.36; H, 7.95; N, 7.36.

4.3. Pharmacology

Compounds (*R*)-**126**, (*R*)-**240**–**242**, (*R*)-**255**–**258**, and (*R*)-**269** were screened under the auspices of UCB Pharma (Braine L'Alleud, Belgium) following the procedures described in Chapter 2, Section 4.3.1. Compounds (*R*)-**243**–**253**, (*R*)-**254**, (*R*)- and (*S*)-**255**, (*R*)- and (*S*)-**258**, (*R*)-**259**–**261**, (*R*)-**270**–**281**, **282**, (*S*)- and (*R,S*)-**283**, and (*R*)-**284** were screened

under the auspices of the NINDS ASP (Rockville, MD) following the procedures described in Chapter 2, Section 4.3.2. Pharmacological evaluation by UCB Pharma consisted of four assays: the 6 Hz test and the MES test to assess anticonvulsant activity, the formalin test to assess neuropathic pain protection, and the rotorod test to assess neurological toxicity. Initial pharmacological evaluation by the NINDS ASP consisted of the MES test and the subcutaneous pentylenetetrazol (Metrazol®) (scMET) (mice and rats) seizure threshold test to assess anticonvulsant activity, the rotorod test to assess neurological toxicity (mice), and the positional sense test or gait and stance test to assess behavioral toxicity (rats). The effective dose (50%) (ED_{50}) values were obtained in quantitative screenings for compounds that showed significant activity. The median doses for neurological impairment (50%) (TD_{50}) in mice using the rotorod test, and the behavioral toxicity effects observed in rats were reported, when applicable. TD_{50} values were determined for those compounds that demonstrated significant activity in the MES test.

CHAPTER 4. Exploring the Mechanism of Action of PAADs

1. Introduction

Our initial objective was to develop a class of compounds that could selectively target PNS sites, thereby reducing neurological toxicities typically associated with CNS active drugs. We proposed that PAADs represented an ideal class of compounds that may be able to achieve PNS selectivity because the presence of the protonated primary amine at physiological pH would allow both greater diversity of hydrophilic substituents at the C(2) and *N*-benzylamide sites, and prevent the transport of PAADs across the predominantly negatively charged BBB. We have demonstrated that several unsubstituted and substituted C(3)-*O*-methoxy and C(2)-hydrocarbon PAADs attenuate pain ((*R*)-**95**, (*R*)-**96**, (*R*)-**98**, (*R*)-**99**, (*R*)-**255**, (*R*)-**258**), possibly by interaction with PNS targets, but these PAADs also possess significant anticonvulsant activity. The observed anticonvulsant activities indicate that PAADs readily penetrate the BBB, demonstrating that PAADs do not *selectively* target the PNS, but rather possibly exert their mechanism of action through a combination of interactions within the CNS and PNS. The brain:plasma ratio quantification of (*R*)-**61**, (*R*)-**65**, and (*R*)-**77** (Table 4) confirmed that PAADs indeed penetrate the CNS. Therefore, we amend our original hypothesis and no longer propose that PAADs are PNS-selective agents.

Comparison of the PAADs with their corresponding FAAs suggests that the PAADs could be exerting their mechanism of action by one of three pathways: (1) interaction with receptor binding sites that are involved in FAA function; (2) interaction with receptor binding sites that are not involved with FAA function; or (3) a combination of both.

The C(3)-alkoxy PAADs possibly follow scenario one and interact with similar receptor binding sites as the C(3)-alkoxy FAAs. We found that these PAADs followed the C(3)-alkoxy SAR trends of the corresponding C(3)-alkoxy FAAs, but the PAADs were 10-fold less effective in seizure prevention (Table 6). Several factors may account for this decrease in anticonvulsant activity. These include the binding affinity of the drug candidate with their cognate receptors, the susceptibility of the PAAD to metabolism, and the CNS concentration levels. Without specific information concerning the target(s) of PAAD function, we are not able to speculate if the PAADs bind weaker to molecular targets responsible for anticonvulsant activity. It is difficult to attribute the reduced PAAD potency solely to reduced levels in the CNS, since (*R*)-**61** displayed a brain:plasma ratio of 1.2:1. Correspondingly, lacosamide ((*R*)-**28**) has ~100% bioavailability,[247] but we recognize that the reported bioavailability does not provide information or the blood-to-plasma distribution. A more likely cause for the 10-fold reduction in activity could be due to enhanced PAAD metabolism compared with its FAA counterpart. Lacosamide ((*R*)-**28**) undergoes negligible phase I metabolism.[248] In proceeding from FAA (*R*)-**28** to PAAD (*R*)-**61**, the terminal amide in the FAA has been converted to an amine. This structural change could permit phase I metabolism (e.g., deamination, hydroxylation) processes and/or phase II metabolism (e.g., methylation, sulphation, acetylation, glucuronidation).[249] It is also feasible that PAADs may bind to serum proteins to a greater extent than their corresponding FAAs, resulting in a lower amount of PAAD available to bind to its receptor sites. (*R*)-**28** has relatively low protein binding (<15%).[247] If the C(3)-alkoxy PAADs do interact with similar targets as their

corresponding C(3)-alkoxy FAAs, then left unexplained is the pronounced loss of stereospecificity for the (R)-stereoisomer observed for the PAAD in comparison to the FAA.

The C(2)-aromatic and C(2)-heteroaromatic PAADs pose a greater dilemma. Indeed, we do not consistently see evidence of key hallmarks of the FAA activity profile in the PAAD series. For example, inclusion of a heteroatom one atom removed from the C(2) site led, in some cases, to improved anticonvulsant activity ((*R,S*)-**62** versus (*R,S*)-**77**). Nonetheless, notable exceptions were observed ((*R,S*)-**62** versus (*R,S*)-**94**), and the improvement in anticonvulsant activity with heteroatom inclusion in the PAAD series was far less than in the FAA series.[55-57] Also, confounding our interpretation of the data was the enhanced pain protection for several C(2)-heteroaromatic PAADs compared with their anticonvulsant activity. Therefore, the C(2)-heteroaromatic PAADs may be functioning by scenario three, since there was a preferred selectivity for pain attenuation over anticonvulsant activity.

It is possible that the C(2)-hydrocarbon PAADs follow scenario two since they have a unique SAR. Most important, the FAA counterparts of the C(2)-hydrocarbon PAADs (e.g., FAA (*R*)-**237** versus PAAD (*R*)-**98**; FAA (*R*)-**238** versus PAAD (*R*)-**99**) displayed either minimal or no seizure protection in the MES test (Table 16), while the structurally similar PAADs showed excellent anticonvulsant activity. Further examination of Sites A–F of the C(2)-hydrocarbon PAADs revealed that a number of alterations to the PAAD structural backbone still produced pronounced pharmacological effects (Tables 30–35), while similar changes in the FAA structural backbone abolished activity. This structural flexibility with respect to pharmacology suggests that C(2)-hydrocarbon PAADs may interact with receptor binding sites not involved with FAA function. Furthermore, the observed SAR suggests that the receptor binding sites may not be size-limiting, but rather accommodates compounds of various sizes, and relies on both hydrogen bonding and hydrophobic interactions. Collectively, while PAADs may function at receptor sites not involved in FAA function, at this

time we cannot exclude the possibility of PAAD interaction with receptor sites involved in FAAs function.

Lacosamide ((*R*)-**28**) did not significantly bind to more than 100 receptors in a variety of radioligand binding assays (<20% inhibition at 10 μM) or inhibit neurotransmission (norepinephrine, dopamine, 5-HT, GABA) (<20% inhibition at 10 μM), and only weak binding was observed at the site 2 of VGSCs (~25% at 10 μM).[250] The molecular mode of action of (*R*)-**28** remains elusive but recent electrophysiology studies suggests that (*R*)-**28** selectively promotes the enhancement of sodium channels into the slow inactivated state (reviewed in Section 2.1.2).[63,64] However, it is difficult to determine which receptors are involved in FAA function due to the lack of binding in the lacosamide target identification experiments.

In this chapter, we continue our studies and focus on the C(3)-*O*-methoxy PAADs and the C(2)-hydrocarbon PAADs due to their excellent anticonvulsant activities in animal models. In an attempt to identify possible PAAD molecular target(s), we screened 17 PAADs in *in vitro* radioligand competition binding assays against 44 receptors, as well as in *in vitro* functional assays against 24 receptors, and one PAAD was subjected to an *in vitro* enzymatic assay.

2. Results and discussion

2.1. *In vitro* binding assays and functional receptor screens

Receptor binding profiles were provided by the National Institute of Mental Health's Psychoactive Drug Screening Program (NIMH PDSP), directed by Dr. Bryan L. Roth at UNC-Chapel Hill. The primary radioligand competition binding assay included receptors known to be involved in seizure and nociceptive processes, including $GABA_A$ ([^{3}H]-baclofen) and benzodiazepine ([^{3}H]-flunitrazepam).[251,252] The PSDP assessed the affinity of PAADs for 44 receptors (in quadruplicate at a concentration of 10 μM) to determine the percentage of

radioligand displacement (i.e., receptor binding). If displacement of the radioligand was greater than 50%, a secondary radioligand binding assay was performed to determine the binding affinity (K_i value) from a span of 11 dose points ranging from 10 pM to 10 μM. Functional activity using orphan 23 G-protein coupled receptors (GPCRs) and hERG was also screened (in triplicate at a concentration of 10 μM) by the PDSP. Another receptor of interest not currently available through the PDSP, the Na^+ channel site 2 ([^{3}H]-batrachotoxin), was carried out by Cerep (Poitiers, France), a company that offers a range of pre-clinical drug discovery services.

The 17 PAADs screened were categorized as either C(3)-*O*-methoxy-based ((*R*)-**61**, (*S*)-**61**), *N*-benzyl-safinamide-based ((*R*)-**254**, (*R*)-**255**, (*S*)-**255**, (*R*)-**257**, (*R*)-**258**, (*S*)-**258**), or C(2)-hydrocarbon-based ((*R*)-**98**, (*S*)-**98**, (*R*)-**246**, (*R*)-**248**, (*R*)-**276**, (*R*)-**277**, (*R*)-**280**, (*S*)-**283**, (*R,S*)-**283**). The radioligand binding assays looked at an array of receptor classes, including serotonin receptors, adrenergic receptors, dopamine receptors, GABA receptors, histamine receptors, muscarinic receptors, opioid receptors, amine transporters, sigma receptors, and site 2 of VGSCs. The functional assays looked at 22 orphan GPCRs and the K_v11.1 potassium ion channel encoded by the human ether-a-go-go related gene (hERG).

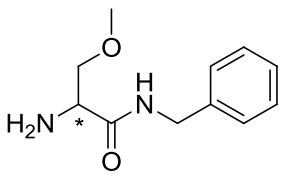
The GABA receptors, $GABA_A$ and benzodiazepine, as well as VGSCs, are known to play a role in epilepsy, and recently, VGSCs have been implicated in the pathology of NP (reviewed in Chapter 1).[28-33,75,253] Serotonin receptors, dopamine receptors, muscarinic receptors, and amine transporters play a central role in the regulation of neurotransmission and dysregulation can result in a range of neurological disorders, including, but not limited to, addiction, alcoholism, anxiety, attention deficit hyperactivity disorder, bipolar disorder, depression, and Parkinson's disease.[254-257] Adrenergic receptors generally cause a sympathetic response (i.e., fight-or-flight response)[258] and histamine receptors cause allergic and inflammatory responses.[259] Opioid receptors and sigma receptors, once thought to be a opioid receptor subtype due to their antitussive properties, are associated with CNS

disorders, such as pain, schizophrenia, and memory deficits.[260,261] Finally, hERG potassium channels are essential for normal electrical activity in the heart, and mutations of hERG channels can result in life-threatening arrhythmias. Therefore, hERG serves as an important anti-target in drug development.[262]

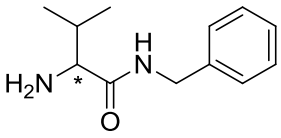
2.1.1. C(3)-*O*-Methoxy-based PAADs

Analysis of the whole animal pharmacological trends observed in the C(3)-alkoxy PAADs and the C(2)-hydrocarbon PAADs suggested that different molecular pathways may be responsible for their overall pharmacological function. The primary radioligand binding profile of C(3)-*O*-methoxy PAADs (*R*)-**61** and (*S*)-**61** did not reveal any substantial (>50%) binding partners at 10 μM (Table 37), including receptors known to be involved in seizures and nociceptive processes (e.g., $GABA_A$, BZP, Na^+ channel site 2).[263] This result was not unexpected, as lacosamide ((*R*)-**28**) did not significantly (>50% at 10 μM) bind to either $GABA_A$ or BZP, and only weakly interacted with the Na^+ channel site 2 (~25% at 10 μM). The binding profile of the C(2)-isopropyl PAAD (*R*)-**98** showed 76% inhibition of the dopamine transporter (DAT) at 10 μM (Table 37). Inhibition of DAT could also be stereospecific, as (*S*)-**98** displayed only 4.7% inhibition. The (*S*)-isomer also inhibited the histamine receptor H1 (51%) and sigma 2 (88%) at 10 μM, and the binding affinities were determined to be 3.5 μM and 4.9 μM, respectively (Table 45). The determination of the binding affinity of (*R*)-**98** for DAT is in progress. We would have expected that the binding affinity of (*S*)-**98** for sigma 2 would be greater (a lower numerical value) than H1 due to their primary inhibition values. However, the primary radioligand competition assay has been designed for preliminary screening purposes and quantitative comparisons should be made using the K_i values determined from the secondary radioligand competition assay. Therefore, we will also determine the binding affinity of (*S*)-**98** for DAT to definitively establish if the interaction of the C(2)-isopropyl PAAD is indeed stereospecific. Comparison

of the primary radioligand binding profile of C(3)-*O*-methoxy PAAD enantiomers ((*R*)-**61** and (*S*)-**61**) with the C(2)-isopropyl PAAD enantiomers ((*R*)-**98** and (*S*)-**98**) revealed one potential receptor, DAT, that may contribute to the unique SAR of the C(2)-hydrocarbon PAADs. DAT is important in maintaining neurotransmitter balance by removing dopamine from the synapse, and dysregulation in DAT expression can result in a range of neurological disorders, including Parkinson's disease, attention deficit hyperactivity disorder, bipolar disorder, depression, and alcoholism.[256] Recently, positron emission tomography (PET) scans revealed reduced DAT binding in patients with juvenile myoclonic epilepsy,[255,264] but further investigation needs to be conducted to determine the connection between DAT and seizure disorders. Lastly, neither (*R*)-**61**, (*S*)-**61**, (*R*)-**98**, nor (*S*)-**98** significantly diminished the functional activity of either the orphan GPCRs or hERG (Table 38).

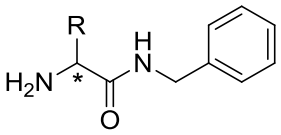


(*R*)-**61**
(*S*)-**61**



(*R*)-**98**
(*S*)-**98**

Table 37. Primary radioligand binding profile of PAADs (*R*)-61, (*S*)-61, (*R*)-98, and (*S*)-98: Percentage of inhibition at 10 *μ*M. Comparison of parent C(3)-*O*-methoxy PAADs versus C(2)-hydrocarbon PAADs

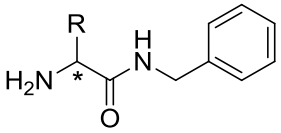


	R	(*R*)-CH_2OCH_3	(*S*)-CH_2OCH_3	(*R*)-$CH(CH_3)_2$	(*S*)-$CH(CH_3)_2$
Receptor Class	**Receptor**	**Compound**			
		(*R*)-**61**	(*S*)-**61**	(*R*)-**98**	(*S*)-**98**
Serotonin[a]	5ht1a	5.3	11	-13	-17
	5ht1b	9.6	17	-12	-7.6
	5ht1d	4.0	7.3	19	14
	5ht1e	8.9	0.7	-4.8	3.5
	5ht2a	-0.9	-4.9	-2.8	5.3
	5ht2b	-7.1	-11	17	9.9
	5ht2c	11	8.2	26	26
	5ht3	9.8	11	23	29
	5ht5a	1.7	-17	18	15
	5ht6	-2.1	-4.9	0.8	3.3
	5ht7	-15	-14	-10	0.1
Adrenergic[a]	Alpha 1A	0.9	10	8.3	-7.8
	Alpha 1B	7.4	-1.2	26	30
	Alpha 1D	-2.3	-0.2	13	-4.6
	Alpha 2A	34	25	-16	9.0
	Alpha 2B	-3.0	-8.8	-3.5	-4.4
	Alpha 2C	25	22	17	-1.1
	Beta 1	34	24	2.1	8.7
	Beta 2	-0.4	-0.5	-4.7	19
	Beta 3	2.2	3.5	24	1.7
Dopamine[a]	D1	14	-3.1	-1.0	-0.3
	D2	22	20	-2.4	-0.5
	D3	-0.2	6.1	-0.2	5.0
	D4	0.9	-0.6	-2.7	-0.1
	D5	-1.7	-14	-0.4	1.3
GABA[a]	$GABA_A$	31	-10	14	10
	BZP	30	32	23	23
Histamine[a]	H1	18	28	48	**51**[b]
	H2	19	25	21	20
	H3	15	12	31	28
	H4	29	12	23	27
Muscarinic[a]	M1	-16	-17	-23	-17

	M2	0.8	3.9	27	24
	M3	1.0	-5.8	-19	-15
	M4	38	24	-27	-16
	M5	3.9	26	-4.6	11
Opioid[a]	DOR	9.9	7.7	-17	-5.9
	KOR	32	21	-4.4	1.6
	MOR	-12	-15	6.4	0.4
Transporters[a]	SERT	21	26	0	-2.1
	NET	-5.7	13	-8.6	-0.7
	DAT	11	24	**76**[b]	4.7
Misc[a]	Sigma 1	-5.1	-3.4	30	48
	Sigma 2	-3.8	-0.1	48	**88**[b]
Ion Channel[c]	Na+ Channel[d]	-11	-6.0	5.0	-8.0

[a] The compounds were tested at 10 μM in quadruplicate under the auspices of the NIMH PDSP. % inhibition = 100% - % radioactivity bound. [b] Submitted for a secondary radioligand binding assay (Table 45). [c] The compounds were tested at 10 μM in duplicate by the auspices of Cerep, Inc. % inhibition = 100% - % radioactivity bound. [d] Site 2.

Table 38. Functional profile of PAADs (*R*)-61, (*S*)-61, (*R*)-98, and (*S*)-98: Percentage of inhibition at 10 *μ*M. Comparison of parent C(3)-*O*-methoxy PAADs versus C(2)-hydrocarbon PAADs



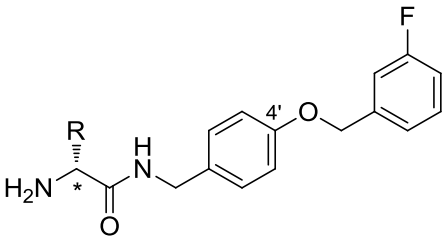
R	(*R*)-CH_2OCH_3	(*S*)-CH_2OCH_3	(*R*)-$CH(CH_3)_2$	(*S*)-$CH(CH_3)_2$
Receptor[a]	**Compound**			
	(*R*)-**61**	(*S*)-**61**	(*R*)-**98**	(*S*)-**98**
GPR1	-0.2	-2.2	-11	-11
GPR123	-0.6	1.8	3.6	6.0
GPR132	13	-5.0	16	20
GPR133	0.9	4.8	3	4.2
GPR15	-1.1	-1.2	8.9	7.2
GPR161	0.7	2.3	1.1	1.2
GPR31	7.9	5.3	4.2	-0.2
GPR39	4.5	-0.4	-0.2	4.5
GPR4	1.4	2.9	2.2	0.5
GPR41	-6.0	-4.7	-1.7	-1.8
GPR43	-0.5	-2.9	1.5	5.0
GPR45	2.3	1.9	4.0	10
GPR55	3.4	2.0	1.1	-1.0
GPR57	-2.1	0.1	-4.5	-5.5
GPR58	2.8	4.0	2.4	4.2
GPR62	-5.8	-2.5	-0.2	3.6
GPR63	2.3	3.2	2.5	-1.6
GPR68	0.2	-0.2	-1.6	0.9
GPR83	-4.5	0.7	0.4	6.7
GPR84	0.1	5.1	8.0	3.4
GPR87	-3.8	-1.6	12	11
GPR88	-10	0.8	-8.8	-3.5
hERG	1.4	5.6	-0.3	7.7

[a] The compounds were tested at 10 *μ*M in quadruplicate under the auspices of the NIMH PDSP.

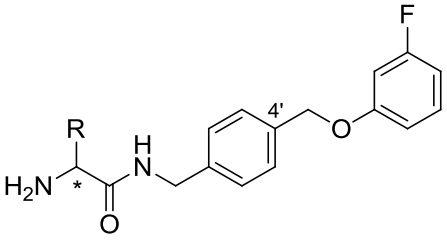
2.1.2. *N*-Benzyl safinamide-based PAADs

A global comparison of the safinamide-like chimeric PAADs ((*R*)-**254**, (*R*)-**255**, (*S*)-**255**, (*R*)-**257**, (*R*)-**258**, (*S*)-**258**) (Table 39) with the unsubstituted C(3)-*O*-methoxy PAADs ((*R*)-**61**, (*S*)-**61**) and C(2)-isopropyl PAADs ((*R*)-**98**, (*S*)-**98**) (Table 38) revealed a greater number of receptor classes experiencing significant inhibition at 10 μM upon inclusion of either a benzyloxybenzyl unit or a phenoxymethylbenzyl unit at the 4'-*N*-benzyl position. These include binding to serotonin receptors (5ht2a, 5ht2b, and 5ht2c), adrenergic receptors (alpha1A, alpha2A, alpha2B, and alpha2C), the dopamine receptor D5, histamine receptors (H1, H2, and H3), amine transporters (serotonin transporter (SERT), norepinephrine transporter (NET), and DAT), sigma 1 and 2, as well as the Na^+ channel site 2. The considerable inhibition of sigma 1 and the Na^+ channel site 2 was not unexpected, as safinamide (**30**) is known to interact with these receptors.[77,212] Within the safinamide-based PAADs, the compounds can be further classified as chimeric C(3)-*O*-methoxy PAADs ((*R*)-**254**, (*R*)-**255**, (*S*)-**255**) and chimeric C(2)-isopropyl PAADs ((*R*)-**257**, (*R*)-**258**, (*S*)-**258**). Using this subclassification, we observed that the (*R*)-stereoisomer of chimeric C(2)-isopropyl PAADs significantly inhibited (>50%) 4-fold more receptors than the (*R*)-stereoisomer of chimeric C(3)-*O*-methoxy PAADs at 10 μM (chimeric C(2)-isopropyl PAAD (*R*)-stereoisomers: 5ht2a, 5ht2b, 5ht2c, alpha1A, alpha2A, D5, H3, NET, DAT, sigma 1, sigma 2, Na^+ channel site 2; chimeric C(3)-*O*-methoxy PAAD (*R*)-stereoisomers: 5ht2b, DAT, sigma 1). We also observed significant binding interactions for the corresponding (S)-stereoisomers of these two different C(2)-substituted PAADs, where the C(3)-*O*-methoxy PAAD (*S*)-**255** significantly inhibited 5ht2a, 5ht2b, 5ht7, alpha1A, alpha2A, alpha2C, H1, SERT, NET, sigma 1, and the Na^+ channel site 2, and the C(2)-isopropyl PAAD (*S*)-**258** significantly inhibited 5ht2a, 5ht2b, 5ht2c, H1, H2, NET, sigma 1, sigma 2, and the Na^+ channel site 2. In instances where we observed significant binding (>50% at 10 μM) for both the (*R*)- and (*S*)-isomers of PAADs **255** and **258**, the binding affinities were in the low

micromolar range and were similar for both the (*R*)- and (*S*)-stereoisomers (PAAD **255** (*R*)-stereoisomer K_i (μM) versus (*S*)-stereoisomer K_i (μM): 5ht2b, 3.4 versus 2.8) (PAAD **258** (*R*)-stereoisomer K_i (μM) versus (*S*)-stereoisomer K_i (μM): 5ht2b, 1.2 versus 1.2; 5ht2c, 3.2 versus 1.7) (Table 45). This finding suggested that the enhanced receptor binding for the C(2)-hydrocarbon and C(3)-*O*-methoxy safinamide-based PAADs was independent of C(2)-stereochemistry, and binding was likely was attributed to the combined structural effects of the C(2)-substituent and the safinamide *N*-benzyl pharmacophore. If this notion is correct, then perhaps these binding interactions are not relevant for their seizure and/or NP activities, since seizure protection was greater in the (*R*)-stereoisomer versus the (*S*)-stereoisomer. Correspondingly, the binding to various receptor classes may signal that these compounds could exhibit other pharmacological effects, provided binding was appreciable and functionally significant. There was not any obvious difference in receptor inhibition with respect to the orientation of the ether linkage (-OCH_2- versus -CH_2O-) in either the chimeric C(3)-*O*-methoxy PAADs or the chimeric C(2)-hydrocarbon PAADs. Secondary radioligand binding assays of the safinamide-based PAADs revealed modest binding affinities (2–10 μM) for most receptors, although high nanomolar binding affinities for sigma 1 were observed (K_i (nM): (*R*)-**254**, 147; (*R*)-**255**, 92; (*S*)-**255**, 765; (*R*)-**257**, 896; (*R*)-**258**, 367; (*S*)-**258**; 1112) (Table 45). Again, this result was not unexpected because safinamide has been shown to bind to sigma 1 with an affinity of 19 nM.[212] However, the seizure protection afforded by safinamide is not thought to occur through the interaction with sigma 1, but rather by modulating VGICs, including VGSCs.[212] Alternatively, the affinity of the 4'-chimeric PAADs for sigma 1 may contribute to their toxicity profile (profile not determined) as sigma 1 receptors have been reported to be involved in behavioral psychosis.[210] Finally, neither (*R*)-**254**, (R)-**255**, (*S*)-**255**, (*R*)-**257**, (*R*)-**258**, nor (*S*)-**98** significantly diminished the functional activity of either the orphan GPCRs or hERG (Table 38).

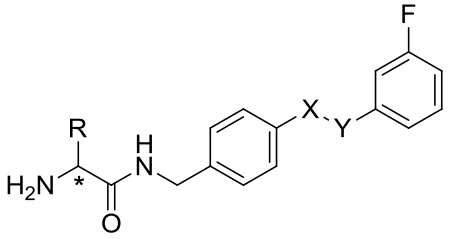


(*R*)-**254** R = CH_2OCH_3
(*R*)-**257** R = $CH(CH_3)_2$



(*R*)-, (*S*)-**255** R = CH_2OCH_3
(*R*)-, (*S*)-**258** R = $CH(CH_3)_2$

Table 39. Primary radioligand binding profile of (*R*)-254, (*R*)-255, (*S*)-255, (*R*)-257, (*R*)-258, and (*S*)-258: Percentage of inhibition at 10 *μ*M. The effect of the *N*-benzyl safinamide unit on C(3)-*O*-methoxy PAAD and C(2)-hydrocarbon PAAD activity



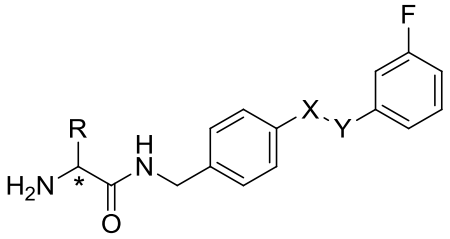
	R	(*R*)-CH_2OCH_3	(*R*)-CH_2OCH_3	(*S*)-CH_2OCH_3	(*R*)-$CH(CH_3)_2$	(*R*)-$CH(CH_3)_2$	(*S*)-$CH(CH_3)_2$
	X	O	CH_2	CH_2	O	CH_2	CH_2
	Y	CH_2	O	O	CH_2	O	O
Receptor Class	**Receptor**	**Compound**					
		(*R*)-**254**	(*R*)-**255**	(*S*)-**255**	(*R*)-**257**	(*R*)-**258**	(*S*)-**258**
Serotonin[a]	5ht1a	15	8.9	12	-12	14	8.4
	5ht1b	0.6	-12	-8.5	-11	-18	-20
	5ht1d	3.5	11	16	16	6.5	13
	5ht1e	2.4	-2.9	-6.8	-2.4	1.1	14
	5ht2a	34	43	**52**[b]	45	**55**[b]	**59**[b]
	5ht2b	8.2	**68**[b]	**57**[b]	**52**[b]	**81**[b]	**82**[b]
	5ht2c	48	37	43	**60**[b]	**59**[b]	**61**[b]
	5ht3	11	14	6.2	22	32	-14
	5ht5a	-20	6.3	11	7.9	1.6	14
	5ht6	2.7	3.8	-8.5	-7.7	-11	-20
	5ht7	-19	22	**56**[b]	3.4	2.7	6.2
Adrenergic[a]	Alpha 1A	46	-7.4	6.1	**89**[b]	-1.5	5.8
	Alpha 1B	3.0	29	19	28	20	36
	Alpha 1D	-0.3	-0.9	16	-1.5	-0.5	50

	Alpha 2A	24	-15	**>50**[b]	**57**[b]	**74**[b]	20
	Alpha 2B	-6.3	6.4	**>50**[b]	5.9	12	20
	Alpha 2C	36	20	**>50**[b]	34	37	44
	Beta 1	26	38	31	35	14	19
	Beta 2	-2.8	4.3	2.5	-16	-14	-1.3
	Beta 3	1.5	20	29	49	36	15
Dopamine[a]	D1	2.1	-8.9	45	3.8	-9.9	48
	D2	9.7	-4.1	-7.5	-5.9	-4.1	7.5
	D3	8.8	8.5	-0.9	11	6.4	7.3
	D4	27	30	-0.4	24	38	-19
	D5	-1.5	31	45	**59**[b]	**62**[b]	33
GABA[a]	$GABA_A$	-4.9	-1.3	10	-0.1	-0.9	5.7
	BZP	30	14	27	16	13	27
Histamine[a]	H1	37	17	**67**[b]	29	31	**56**[b]
	H2	20	22	37	33	35	**50**[b]
	H3	29	31	28	**57**[b]	**73**[b]	31
	H4	19	13	5	9.0	6.8	11
Muscarinic[a]	M1	-9.1	-17	-1.2	-17	-19	-7.1
	M2	14	13	20	8.9	16	6.7
	M3	5.6	-4.0	27	-15	-7.2	-7.7
	M4	14	4.6	16	-17	-7.2	12
	M5	35	6.4	17	8.4	-2.4	3.9
Opioid[a]	DOR	8.9	-7.3	1.0	-17	-18	10
	KOR	16	6.4	5.4	12	18	7.9
	MOR	-12	15	-13	19	17	-16
Transporters[a]	SERT	34	23	**50**[b]	25	41	25
	NET	25	42	**64**[b]	46	**60**[b]	**57**[b]
	DAT	**53**[b]	40	**50**[b]	**94**[b]	**73**[b]	49
Misc[a]	Sigma 1	**72**[b]	**94**[b]	**87**[b]	**96**[b]	**96**[b]	**69**[b]
	Sigma 2	46	31	41	**79**[b]	**90**[b]	**82**[b]

Ion Channel[c]	Na+ Channel[d]	48	**69**	**64**	**71**	**73**[f]	**86**

[a] The compounds were tested at 10 μM in quadruplicate under the auspices of the NIMH PDSP. % inhibition = 100% - % radioactivity bound. [b] Submitted for a secondary radioligand binding assay (Table 45). [c] The compounds were tested at 10 μM in duplicate under the auspices of Cerep, Inc. % inhibition = 100% - % radioactivity bound. [d] Site 2. [e] ND = not determined. [f] Submitted for a secondary assay to determine the IC_{50} from a span of 5 dose points ranging from 0.1 μM to 0.1 mM.

Table 40. Functional profile of (*R*)-254, (*R*)-255, (*S*)-255, (*R*)-257, (*R*)-258, and (*S*)-258: Percentage of inhibition at 10 *μ*M. The effect of the *N*-benzyl safinamide unit on C(3)-*O*-methoxy PAAD and C(2)-hydrocarbon PAAD activity



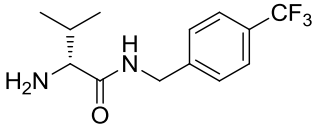
R	(*R*)-CH_2OCH_3	(*R*)-CH_2OCH_3	(*S*)-CH_2OCH_3	(*R*)-$CH(CH_3)_2$	(*R*)-$CH(CH_3)_2$	(*S*)-$CH(CH_3)_2$
X	O	CH_2	CH_2	O	CH_2	CH_2
Y	CH_2	O	O	CH_2	O	O
Receptor[a]	**Compound**					
	(*R*)-**254**	(*R*)-**255**	(*S*)-**255**	(*R*)-**257**	(*R*)-**258**	(*S*)-**258**
GPR1	4.5	12	13	-6.1	-3.5	18
GPR123	0.3	-7.6	4.6	-12	2.3	5.6
GPR132	7.5	4.6	-5.9	-0.3	26	8.9
GPR133	5.9	-4.2	2.2	-6.5	5.4	1.1
GPR15	-5.3	-12	-2.5	-33	4.1	4.3
GPR161	-0.6	-0.5	-0.1	-1.9	1.3	1.2
GPR31	1.1	-1.6	3.5	-3.7	4.8	2.5
GPR39	6.4	-7.3	-4.6	-0.7	7.1	-7.1
GPR4	-0.2	-4.2	4.0	-4.4	3.2	-1.4
GPR41	-5.6	-10	25	-15	0.8	17
GPR43	2.9	-6.3	7.6	-16	2.1	6.0
GPR45	-5.1	-14	0.6	-17	7.8	4.0
GPR55	3.0	1.3	1.5	-2.3	-1.2	1.4
GPR57	-2.3	-18	12	-21	5.7	21
GPR58	1.3	-6.9	0.8	-12	2.6	2.5
GPR62	0.3	-6.7	16	-6.7	-1.6	6.3

GPR63	0.1	-6.9	5.5	-2.8	3.0	1.6
GPR68	5.5	9.3	6.5	-1.8	4.1	13
GPR83	8.1	3.7	5.4	-10	-2.5	8.0
GPR84	-7.2	-12	-3.2	-24	0.8	16
GPR87	2.0	-7.4	-6.6	-2.3	8.0	11
GPR88	-4.4	-3.1	22	-12	-2.1	16
hERG	5.7	13	11	13	6.7	11

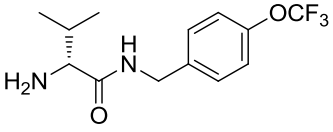
[a] The compounds were tested at 10 μM in quadruplicate under the auspices of the NIMH PDSP.

2.1.3. C(2)-Hydrocarbon-based PAADs

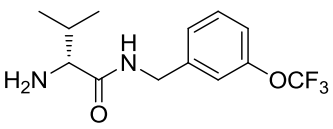
The primary radioligand receptor screening of the electron-withdrawing *N*-benzyl substituted C(2)-isopropyl PAADs ((*R*)-**246**, (*R*)-**248**, and (*R*)-**280**) showed minimal binding at 10 μM to the 44 receptors tested (Table 41). (*R*)-**246** inhibited radioligand binding in D1 (60%), DAT (83%), sigma 1 (58%), and sigma 2 (66%). (*R*)-**248** displayed a similar binding profile with inhibition of DAT (60%), sigma 1 (68%), and sigma 2 (84%). (*R*)-**280** did not significantly (>50% at 10 μM) inhibit radioligand binding in the receptors tested. (*R*)-**280** did not resemble the binding profiles of (*R*)-**246** and (*R*)-**248**, which was surprising since the anticonvulsant activities of (*R*)-**246**, (*R*)-**248**, and (*R*)-**280** were similar (ED_{50} (mg/kg): (*R*)-**246**, 14; (*R*)-**248**, 16; and (*R*)-**280**, >3, <10). This suggests that either the 4'-*N*-benzyl substituted PAADs function at different receptor site(s) compared with 3'-*N*-benzyl substituted PAADs or that the receptors profiled are not involved in PAAD function. We suspect the latter case, which was also supported by the 4'-chimeric primary radioligand binding profiles. More likely, the binding profiles may represent receptors involved in neurotoxicity. As seen in the 4'-chimeric PAADs, the 4'-*N*-benzyl substituted PAADs revealed high nanomolar binding affinities for sigma 1 (K_i (nM): (*R*)-**246**, 1858; (*R*)-**248**, 804) (Table 45). These collective findings suggest that there may be an inherent safety advantage in this series of PAADs for 3'-*N*-benzyl substituted compounds compared with the corresponding 4'-substituted isomers. It will be interesting to determine the receptor binding profile to the 2'-susbtituted isomer (*R*)-**279** (study in progress). Also in line with the previous PAADs profiled, (*R*)-**246**, (*R*)-**248**, and (*R*)-**280** did not significantly diminish the functional activity of either the orphan GPCRs or hERG (Table 42).



(*R*)-**246**

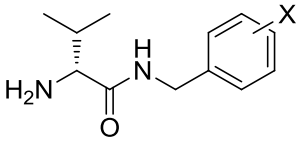


(*R*)-**248**



(*R*)-**280**

Table 41. Primary radioligand binding profile of (*R*)-98, (*R*)-246, (*R*)-248, and (*R*)-280: Percentage of inhibition at 10 *μ*M. Assessment of *N*-benzyl substitution in C(2)-isopropyl PAADs

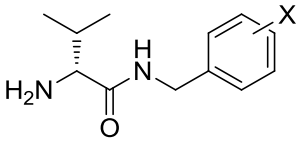


	X	H	4'-CF_3	4'-OCF_3	3'-OCF_3
Receptor Class	**Receptor**	**Compound**			
		(*R*)-**98**	(*R*)-**246**	(*R*)-**248**	(*R*)-**280**
Serotonin[a]	5ht1a	-13	2.7	-13	24
	5ht1b	-12	-18	-15	-18
	5ht1d	19	-6.4	11	-7.5
	5ht1e	-4.8	1.0	-3.8	-12
	5ht2a	-2.8	-4.6	-1.9	-7.5
	5ht2b	17	6.7	23	6.7
	5ht2c	26	7.8	15	5.1
	5ht3	23	-9.4	29	-3.7
	5ht5a	18	13	-5.9	6.2
	5ht6	0.8	-11	-3.8	-6.1
	5ht7	-10	-4.9	2.9	-4.7
Adrenergic[a]	Alpha 1A	8.3	-2.0	-0.3	-0.3
	Alpha 1B	26	-2.9	3.7	14
	Alpha 1D	13	18	14	6.6
	Alpha 2A	-16	-5.2	23	-1.1
	Alpha 2B	-3.5	24	-5.4	2.9
	Alpha 2C	17	30	-3.1	15
	Beta 1	2.1	-1.0	11	10
	Beta 2	-4.7	-0.3	17	19
	Beta 3	24	5.0	-1.6	8.6
Dopamine[a]	D1	-1.0	**60**[b]	1.9	29
	D2	-2.4	4.0	-3.2	-2.6
	D3	-0.2	-5.4	9.9	-3.9
	D4	-2.7	-18	2.9	-2.0
	D5	-0.4	40	-1.0	20
GABA[a]	$GABA_A$	14	28	13	17
	BZP	23	14	27	32
Histamine[a]	H1	48	15	49	21
	H2	21	31	18	25
	H3	31	12	23	2.1
	H4	23	-1.1	25	-0.6

Muscarinic[a]	M1	-23	-9.7	-13	-8.4
	M2	27	-4.2	-0.1	5.1
	M3	-19	-4.4	-5.7	-4.0
	M4	-27	2.8	-19	-2.5
	M5	-4.6	-2.9	-8.7	14
Opioid[a]	DOR	-17	-19	-6.0	-14
	KOR	-4.4	-6.4	7.8	0.2
	MOR	6.4	-14	23	-19
Transporters[a]	SERT	0	18	23	25
	NET	-8.6	-8.0	-2.6	3.7
	DAT	**76**[b]	**83**[b]	**60**[b]	-8.2
Misc[a]	Sigma 1	30	**58**[b]	**68**[b]	37
	Sigma 2	48	**66**[b]	**84**[b]	36
Ion Channel[c]	Na+ Channel[d]	5.0	39	41	47

[a] The compounds were tested at 10 μM in quadruplicate under the auspices of the NIMH PDSP. % inhibition = 100% - % radioactivity bound. [b] Submitted for a secondary radioligand binding assay (Table 45). [c] The compounds were tested at 10 μM in duplicate by the auspices of Cerep, Inc. % inhibition = 100% - % radioactivity bound. [d] Site 2.

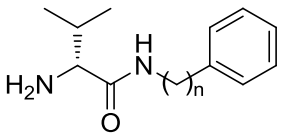
Table 42. Functional profile of (*R*)-98, (*R*)-246, (*R*)-248, and (*R*)-280: Percentage of inhibition at 10 *μ*M. Assessment of *N*-benzyl substitution in C(2)-isopropyl PAADs



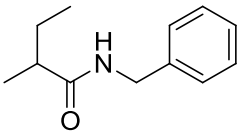
X	H	4'-CF_3	4'-OCF_3	3'-OCF_3
Receptor[a]	**Compound**			
	(*R*)-**98**	(*R*)-**246**	(*R*)-**248**	(*R*)-**280**
GPR1	-11	5.0	-11	15
GPR123	3.6	1.3	7.5	-0.8
GPR132	16	-14	-14	25
GPR133	3.0	-3.0	-0.4	6.7
GPR15	8.9	12	-0.1	9.1
GPR161	1.1	-1.7	-0.7	5.0
GPR31	4.2	-2.7	-3.4	2.4
GPR39	-0.2	-1.4	3.9	-0.7
GPR4	2.2	2.5	-4.5	0.5
GPR41	-1.7	5.1	-6.1	24
GPR43	1.5	-14	4.6	-0.2
GPR45	4.0	1.1	-6.9	-4.7
GPR55	1.1	-3.9	-0.6	5.3
GPR57	-4.5	6.0	-5.8	28
GPR58	2.4	-7.9	6.1	15
GPR62	-0.2	-3.3	2.9	13
GPR63	2.5	0.1	3.8	2.9
GPR68	-1.6	-1.1	-2.8	4.3
GPR83	0.4	1.9	4.4	3.5
GPR84	8.0	-2.5	0.3	12
GPR87	12	-5.0	7.9	8.2
GPR88	-8.8	0.8	5.7	26
hERG	-0.3	-3.4	0	5.3

[a] The compounds were tested at 10 *μ*M in quadruplicate under the auspices of the NIMH PDSP.

Finally, we looked at the binding profile of PAADs with extended *N*-terminal linkages ((*R*)-**276** and (*R*)-**277**), as well as the C(2)-hydrocarbon-like PAADs (*S*)-**283**, (*R,S*)-**283**. Of the PAADs with the extended *N*-terminal linkages, both (*R*)-**276** and (*R*)-**277** showed minimal binding at 10 μM to the chosen panel of receptors. For (*R*)-**276**, 54% radioligand displacement was observed for the dopamine receptor, D5 (Table 43). No significant binding (>50% at 10 μM) was observed with either (*S*)-**283** or (*R,S*)-**283**. Similarly, these PAADs did not affect the functional activity of either the orphan GPCRs or hERG (Table 44). We can still ascertain information from the lack of receptor binding. The C(2)-isopropyl PAADs with the extended *N*-terminal linkages afforded excellent seizure protection (ED_{50} (mg/kg): (*R*)-**276**, 10; (*R*)-**277**, 16) and the absence of receptor binding may indicate that these PAADs do not exert their pharmacological function through these receptors. Additionally, the absence of receptor binding indicated that potential adverse interaction did not occur with extension of the *N*-terminal linkage.

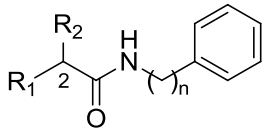


(*R*)-**276** n = 2
(*R*)-**277** n = 3



(*S*)-**283**
(*R,S*)-**283**

Table 43. Primary radioligand binding profile of (*R*)-98, (*R*)-276, (*R*)-277, (*S*)-283, and (*R,S*)-283: Percentage of inhibition at 10 *μ*M. Effect of C(2)-substituent and *N*-terminal linkage size in a series of C(2)-hydrocarbon PAAD-like compounds

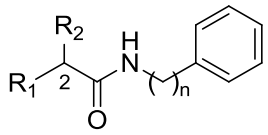


	R_1	NH_2	NH_2	NH_2	CH_3	CH_3
	R_2	(*R*)-$CH(CH_3)_2$	(*R*)-$CH(CH_3)_2$	(*R*)-$CH(CH_3)_2$	(*S*)-CH_2CH_3	(*R,S*)-CH_2CH_3
	n	1	2	3	1	1
Receptor Class	**Receptor**	**Compound**				
		(*R*)-**98**	(*R*)-**276**	(*R*)-**277**	(*S*)-**283**	(*R,S*)-**283**
Serotonin[a]	5ht1a	-13	4.2	6.9	-3.5	15
	5ht1b	-12	-13	-15	-14	-20
	5ht1d	19	2.1	-6.9	-13	-1.6
	5ht1e	-4.8	17	8.3	4.6	21
	5ht2a	-2.8	-8.0	-15	-19	4.0
	5ht2b	17	9.7	12	0.1	1.5
	5ht2c	26	-0.5	8.9	36	-1.1
	5ht3	23	1.0	0.4	-1.1	2.7
	5ht5a	18	13	14	7.2	8.6
	5ht6	0.8	-4.2	13	-4.3	-8.4
	5ht7	-10	-9.3	-3.9	-12	-6.6
Adrenergic[a]	Alpha 1A	8.3	12	-1.2	-2.5	2.3
	Alpha 1B	26	-0.1	14	22	2.5
	Alpha 1D	13	33	16	35	28
	Alpha 2A	-16	3.3	-1.1	0	-2.3
	Alpha 2B	-3.5	-6.7	3.7	8.2	-2.9
	Alpha 2C	17	19	28	**68**[b]	21

	Beta 1	2.1	8.8	9.2	28	4.3
	Beta 2	-4.7	-6.2	11	-0.6	17
	Beta 3	24	8.4	28	26	-1.2
Dopamine[a]	D1	-1.0	33	29	15	34
	D2	-2.4	8.1	0.4	-19	3.7
	D3	-0.2	-14	-6.2	-9.7	-12
	D4	-2.7	-20	-17	-20	-19
	D5	-0.4	**54**[b]	16	-5	38
GABA[a]	$GABA_A$	14	39	20	4.3	31
	BZP	23	14	24	24	13
Histamine[a]	H1	48	-1.9	13	35	-2.7
	H2	21	35	27	27	19
	H3	31	-0.4	-0.9	7.9	1.1
	H4	23	-18	-6.8	8.7	-14
Muscarinic[a]	M1	-23	-8.5	-11	-11	-12
	M2	27	-0.2	-2.5	-1.6	-4.1
	M3	-19	-9.0	-10	-2.4	-1.7
	M4	-27	-0.6	-2.2	10	-4.7
	M5	-4.6	8.0	-0.3	7.2	4.0
Opioid[a]	DOR	-17	-16	-18	-1.8	-16
	KOR	-4.4	3.9	-18	-0.2	-16
	MOR	6.4	-6.3	-9.5	-20	-16
Transporters[a]	SERT	0	12	21	18	16
	NET	-8.6	23	17	-10	-9.2
	DAT	**76**[b]	-1.5	6.4	11	0
Misc[a]	Sigma 1	30	21	43	7.9	14
	Sigma 2	48	-6.0	30	4.9	-11
Ion Channel[c]	Na+ Channel[d]	5.0	10	29	-7.0	-4.0

[a] The compounds were tested at 10 μM in quadruplicate under the auspices of the NIMH PDSP. % inhibition = 100% - % radioactivity bound. [b] Submitted for a secondary radioligand binding assay (Table 45). [c] The compounds were tested at 10 μM in duplicate by the auspices of Cerep, Inc. % inhibition = 100% - % radioactivity bound. [d] Site 2.

Table 44. Functional profile of (*R*)-98, (*R*)-276, (*R*)-277, (*S*)-283, and (*R,S*)-283: Percentage of inhibition at 10 *μ*M. Effect of C(2)-substituent and *N*-terminal linkage size in a series of C(2)-hydrocarbon PAAD-like compounds



R_1	NH_2	NH_2	NH_2	CH_3	CH_3
R_2	(*R*)-$CH(CH_3)_2$	(*R*)-$CH(CH_3)_2$	(*R*)-$CH(CH_3)_2$	(*S*)-CH_2CH_3	(*R,S*)-CH_2CH_3
n	1	2	3	1	1
Receptor	**Compound**				
	(*R*)-**98**	(*R*)-**276**	(*R*)-**277**	(*S*)-**283**	(*R,S*)-**283**
GPR1	-11	5.9	-5.6	11	0.6
GPR123	3.6	1.1	-5.5	6.7	0.3
GPR132	16	6.8	-1.1	21	-14
GPR133	3.0	-3.0	-7.5	4.6	0.6
GPR15	8.9	0.5	1.1	10	-1.6
GPR161	1.1	0.5	-2.1	0.1	-3.5
GPR31	4.2	-1.3	-5.0	-2.6	-6.0
GPR39	-0.2	2.5	-2.3	3.4	8.1
GPR4	2.2	-1.1	-3.0	2.7	-0.3
GPR41	-1.7	16	5.8	20	8.9
GPR43	1.5	6.4	-13	1.8	3.5
GPR45	4.0	8.5	-16	-4.6	3.1
GPR55	1.1	-0.8	-9.3	2.5	-2.4
GPR57	-4.5	2.6	-4.1	26	2.8
GPR58	2.4	0.2	-7.8	-2.9	-1.5
GPR62	-0.2	3.8	-8.0	10	5.6
GPR63	2.5	3.8	-0.6	-0.5	0.4
GPR68	-1.6	1.2	-9.8	1.1	-3.0
GPR83	0.4	4.1	-5.3	-3.4	1.0
GPR84	8.0	10	-24	12	-1.9
GPR87	12	-10	-16	1.1	-7.7
GPR88	-8.8	6.7	-9.1	29	12
hERG	-0.3	-3.8	-2.9	5.1	-9.6

[a] The compounds were tested at 10 *μ*M in quadruplicate under the auspices of the NIMH PDSP.

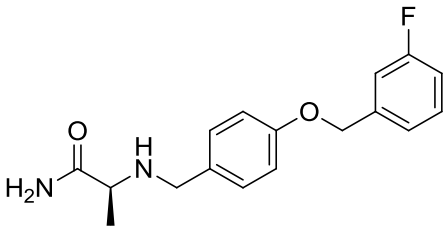
Table 45. Secondary radioligand binding profile: Binding affinity of PAADs

Compound[a]	Receptor	K_i (nM)	Compound[a]	Receptor	K_i (nM)
(*R*)-**98**[a]	DAT		(*R*)-**257**[a]	Alpha1A	>10,000
(*S*)-**98**[a]	H1	3516 ± 349	(*R*)-**257**[a]	Alpha2A	>10,000
(*S*)-**98**[a]	Sigma 2	4908 ± 521	(*R*)-**257**[a]	D5	>10,000
(*R*)-**246**[a]	DAT		(*R*)-**257**[a]	H3	2482 ± 538
(*R*)-**246**[a]	D1		(*R*)-**257**[a]	DAT	
(*R*)-**246**[a]	Sigma 1	1858 ± 118	(*R*)-**257**[a]	Sigma 1	147 ± 13
(*R*)-**246**[a]	Sigma 2	1267 ± 233	(*R*)-**257**[a]	Sigma 2	896 ± 107
(*R*)-**248**[a]	DAT		(*R*)-**258**[a]	5ht2a	2435 ± 200
(*R*)-**248**[a]	Sigma 1	804 ± 68	(*R*)-**258**[a]	5ht2b	2081 ± 105
(*R*)-**248**[a]	Sigma 2	2270 ± 220	(*R*)-**258**[a]	5ht2c	3245 ± 201
(*R*)-**254**[a]	DAT	9948 ± 778	(*R*)-**258**[a]	Alpha2A	>10,000
(*R*)-**254**[a]	Sigma 1	147 ± 13	(*R*)-**258**[a]	D5	>10,000
(*R*)-**255**[a]	5ht2b	3369 ± 145	(*R*)-**258**[a]	H3	7736 ± 612
(*R*)-**255**[a]	Sigma 1	92 ± 8	(*R*)-**258**[a]	DAT	
(*S*)-**255**[a]	5ht2a	6076 ± 650	(*R*)-**258**[a]	NET	4139 ± 463
(*S*)-**255**[a]	5ht2b	2800 ± 323	(*R*)-**258**[a]	Sigma 1	367 ± 32
(*S*)-**255**[a]	5ht7	3510 ± 474	(*R*)-**258**[a]	Sigma 2	407 ± 42
(*S*)-**255**[a]	Alpha2A	>10,000	(*R*)-**258**[b]	Na^+ (Site 2)	1600[c]
(*S*)-**255**[a]	Alpha2B	5403 ± 1058	(*S*)-**258**[a]	5ht2a	2422 ± 256
(*S*)-**255**[a]	Alpha2C	6025 ± 1173	(*S*)-**258**[a]	5ht2b	1153 ± 109
(*S*)-**255**[a]	H1	>10,000	(*S*)-**258**[a]	5ht2c	1676 ± 131
(*S*)-**255**[a]	DAT		(*S*)-**258**[a]	H1	>10,000
(*S*)-**255**[a]	NET		(*S*)-**258**[a]	H2	4247 ± 277
(*S*)-**255**[a]	SERT	>10,000	(*S*)-**258**[a]	NET	325 ± 49
(*S*)-**255**[a]	Sigma 1	765 ± 36	(*S*)-**258**[a]	Sigma 1	1112 ± 73
(*R*)-**257**[a]	5ht2b	5548 ± 254	(*S*)-**258**[a]	Sigma 2	83 ± 15
(*R*)-**257**[a]	5ht2c	4465 ± 215	(*S*)-**283**[a]	Alpha2C	>10,000

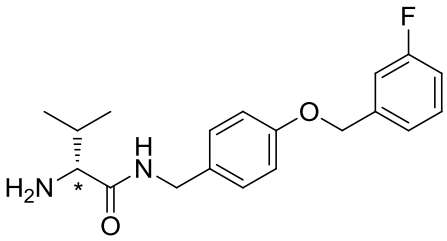
[a] The compounds were tested under the auspices of the NIMH PDSP. The IC_{50} was determined from a span of 11 dose points ranging from 10 pM to 10 μM, and used to obtain the K_i value by applying the Cheng-Prusoff approximation (K_i = IC_{50} / (1 + [ligand]/K_D), where [ligand] equals the assay radioligand concentration and K_D equals the affinity constant of radioligand for the target receptor). [b] The compound was tested under the auspices of the Cerep, Inc. The IC_{50} was determined from a span of 5 dose points ranging from 0.1 μM to 0.1 mM. [c] IC_{50} value.

2.2. *In vitro* MAO_B enzymatic assay

The isoenzymes, monoamine oxidase A and B (MAO_A and MAO_B), catalyze the oxidative deamination of monoamine neurotransmitters to form H_2O_2 and reactive oxygen species. Generally, MAO_A deaminates serotonin (**52**), epinephrine (**50**), and norepinephrine (**51**), whereas MAO_B deaminates phenylethylamine. Dopamine (**49**), epinephrine (**50**), and norepinephrine (**51**) are oxidized by both enzymes. Initially, inhibitors of MAO enzymes were developed as antidepressants, but their potential has been expanded to neurodegenerative disorders, such as Parkinson's disease and Alzheimer's disease.[265] Safinamide (**30**), originally developed as an anticonvulsant agent due to its voltage-sensitive channel blocking activity, has demonstrated *reversible* MAO_B inhibition.[212] This makes safinamide the only reversible MAO_B inhibitor in clinical development, and has indications for Parkinson's disease, epilepsy, and restless leg syndrome.[211,212] We have synthesized several safinamide-based PAADs that retained the benzyloxybenzyl pharmacophore ((*R*)-**254**, (*R*)-**256**, (*R*)-**257**, and (*R*)-**260**). We also investigated the reversed ether linkage (-CH_2O- versus -OCH_2-) between the aryl moieties ((*R*)-**255**, (*S*)-**255**, (*R*)-**258**, (*S*)-**258**, (*R*)-**261**). The primary structural feature distinguishing our compounds from **30** is the order in which the structural units appear. In **30**, the benzyloxybenzyl pharmacophore is directly attached to a substituted amine, while in our compounds this pharmacophore is attached to an amide. We chose to determine the MOA_B enzymatic activity of (*R*)-**257** due to its significant anticonvulsant activity and pain attenuation. The inhibition of MAO_B activity was determined by the spectrophotometric detection of benzyaldehyde, oxidized from benzylamine (0.5 mM), in human platelets at 10 μM (assessed in duplicate at Cerep, Inc). The lack of MOA_B enzymatic inhibition (-3%) suggested that the safinamide-based PAADs do not possess MOA_B enzymatic, as exemplified by (*R*)-**257**, a finding that likely resulted from the absence of the benzyloxybenzyl amine moiety.



safinamide
30



(*R*)-**257**

3. Conclusions

Many PAADs possess excellent anticonvulsant activities and can attenuate pain. These pharmacological events led us to conclude that PAADs are not PNS-selective agents, but rather exert function at both PNS and CNS receptor sites. We screened 17 PAADs in a series of primary radioligand binding assays, follow-up secondary radioligand binding assays to determine the binding affinity (K_i) when the primary assay indicated a radioligand displacement of >50%, functional receptor assays, and an MAO_B enzymatic assay. Overall assessment of the binding, functional, and enzymatic assays suggests that we have not identified potential receptor sites involved with anticonvulsant activity or NP function. Of the binding interactions that were identified, most were in the low micromolar range (2-10 μM) and could possibly indicate receptor sites involved in CNS toxicities, in particular the sigma 1 receptor. Nonetheless, in most cases these binding affinities were modest, suggesting that these interactions were not predictors of adverse toxicity profiles. Our results are not discouraging, as binding partners for lacosamide ((*R*)-**28**) have not been identified by similar methods. Therefore, it remains that PAADs could be exerting their mechanism of action by interaction with receptor binding sites that are involved in FAA function, interaction with receptor binding sites that are not involved with FAA function, or a combination of both.

4. Experimental

4.1. Primary radioligand receptor screen

The protocols for the PDSP primary binding assays, secondary binding assays, and functional assays are available online at http://pdsp.med.unc.edu/UNC-CH%20Protocol%20Book.pdf. The affinities of the PAADs for various receptors were assessed in quadruplicate at a concentration of 10 μM to determine the percentage of inhibition. Test compound (PAAD) and reference compound (positive control) were diluted to 5X final assay concentration (50 μM for a final assay concentration of 10 μM) in the appropriate radioligand binding buffer (Table 46). Then, 50 μL aliquots of buffer (negative control), test compound, and reference compound were added in quadruplicate to a 96-well plate, each of which contains 50 μL of 5X radioligand (see Table 46 for final assay concentration for each radioligand) and 100 μL of buffer. Finally, receptor-containing crude membrane fractions were resuspended in buffer and dispensed (50 μL per well) into a 96-well plate. Radioligand binding was allowed to equilibrate (typically for 1.5 h at room temperature), and then bound radioactivity was isolated by filtration onto 0.3% polyethyleneimine-treated, 96-well filter mats using a 96-well Filtermate harvester. The filter mats were dried, then scintillant was melted onto the filters and the radioactivity retained on the filters was counted in a Microbeta scintillation counter. Raw dpm data from the Microbeta counter was analyzed on the PDSP database. Total bound radioactivity was estimated from quadruplicate wells containing no test or reference compound and adjusted to 100%. Non-specifically bound radioactivity was assessed from quadruplicate wells containing 10 μM of a suitable reference compound (Table 46) and adjusted to 0%. The average bound radioactivity in the presence of the test compound (10 μM final assay concentration, quadruplicate determinations) was then expressed on the percent scale. The percent inhibition of radioligand binding was calculated as 100% minus the percentage of

radioactivity bound. The PDSP on-line data entry and analysis system calculated the variance of the quadruplicate determinations (for the total, non-specific, and test compound binding values) and variances greater than 20% were flagged for further inspection, and assays were repeated if necessary. Additionally, percent inhibition values that were greater than the total binding (i.e., 100%) by at least 20% were also flagged for inspection, as such results could indicate allosteric modulation of radioligand binding.

Table 46. Primary radioligand binding assay conditions

Receptor	Radioligand	[Assay] (nM)	Reference
5ht1a	[^{3}H]-8-OH-DPAT	0.5	Methysergide
5ht1b	[^{3}H]-GR127543	0.3	Ergotamine
5ht1d	[^{3}H]-GR127543	0.3	Ergotamine
5ht1e	[^{3}H]-5-HT	3.0	5-HT
5ht2a	[^{3}H]-Ketanserin	0.5	Chlorpromazine
5ht2b	[^{3}H]-LSD	1.0	Methylsergide
5ht2c	[^{3}H]-Mesulergine	0.5	Chlorpromazine
5ht3	[^{3}H]-LY278584	0.3	LY278584
5ht5a	[^{3}H]-LSD	1.0	Ergotamine
5ht6	[^{3}H]-LSD	1.0	Chlorpromazine
5ht7	[^{3}H]-LSD	1.0	Chlorpromazine
Alpha 1A	[^{3}H]-Prazosin	0.7	Urapidil
Alpha 1B	[^{3}H]-Prazosin	0.7	Corynanthine
Alpha 1D	[^{3}H]- Prazosin	0.7	Corynanthine
Alpha 2A	[^{3}H] –Clonidine	1.0	Oxymetazoline
Alpha 2B	[^{3}H] –Clonidine	1.0	Prazosin
Alpha 2C	[^{3}H] -Clonidine	1.0	Prazosin
Beta 1	[^{3}H]-Iodopindolol	0.1	Atenolol
Beta 2	[^{3}H] –Iodopindolol	0.1	ICI118551
Beta 3	[^{3}H] -Iodopindolol	0.1	IC118551
D1	[^{3}H]-SCH233930	0.2	SKF38393
D2	[^{3}H]-*N*-methylspiperone	0.2	Haloperidol
D3	[^{3}H] -*N*-methylspiperone	0.2	Chlorpromazine
D4	[^{3}H] -*N*-methylspiperone	0.3	Chlorpromazine
D5	[^{3}H]-SCH233930	0.2	SKF38393
$GABA_A$	[^{3}H]-Baclofen	20	GABA
BZP	[^{3}H]-Flunitrazepam	0.5	Diazepam
H1	[^{3}H]-Pyrilamine	0.9	Chlorpheniramine
H2	[^{3}H]-Tiotidine	3.0	Cimetidine

H3	[^{3}H]-α-Methylhistamine	0.4	Histamine
H4	[^{3}H]-Histamine	5.0	Clozapine
M1	[^{3}H]-QNB	0.5	Atropine
M2	[^{3}H] –QNB	0.5	Atropine
M3	[^{3}H] –QNB	0.5	Atropine
M4	[^{3}H] –QNB	0.5	Atropine
M5	[^{3}H] –QNB	0.5	Atropine
DOR	[^{3}H]-DADLE	0.3	Naltrindole
KOR	[^{3}H]-U69593	0.3	Salvinorin A
MOR	[^{3}H]-DAMGO	0.3	DAMGO
SERT	[^{3}H]-Citalopram	0.5	Amitriptyline
NET	[^{3}H]-Nisoxetine	0.5	Despiramine
DAT	[^{3}H]-Vasopressin	1.0	Vasopressin
Sigma 1	[^{3}H]-Pentazocine	3.0	Haloperidol
Sigma 2	[^{3}H]-DTG	3.0	Haloperidol
Na+ Channel[d]	[^{3}H]-Batrachotoxin	10	Veratridine

4.2. Secondary radioligand receptor screen

A solution of the test compound (PAAD) and reference compound (positive control) was prepared as a 1 mg/mL stock in standard binding buffer, or DMSO, according to its solubility. Eleven dilutions of the test and reference compounds (see Table 46) were prepared in standard binding buffer by serial dilution at concentrations of 0.05 nM, 0.5 nM, 1.5 nM, 5 nM, 15 nM, 50 nM, 150 nM, 500 nM, 1.5 μM, 5 μM, and 50 μM. The appropriate radioligand (see Table 46) was diluted to 5X the assay concentration in standard binding buffer. Aliquots (50 μL) of radioligand were dispensed in duplicate into the wells of a 96-well plate containing 100 μL of standard binding buffer. Finally, crude membrane fractions of cells expressing recombinant target were resuspended in 3 mL of chilled standard binding buffer, homogenized by several passages through a 26 gauge needle, then 50 μL was dispensed into each well. The 250 μL reactions were incubated at room temperature and shielded from light (to prevent photolysis of light-sensitive ligands) for 1.5 h, then harvested by rapid filtration onto Whatman GF/B glass fiber filters pre-soaked with 0.3%

polyethyleneimine using a 96-well Brandel harvester. Four rapid 500 μL washes were performed with chilled standard binding buffer to reduce non-specific binding. Filters were placed in 6 mL scintillation tubes and allowed to dry overnight. Then, 4 mL of EcoScint scintillation cocktail (National Diagnostics) were added to each tube. The tubes were capped, labeled, and counted by liquid scintillation counting. Raw data (dpm) representing total radioligand binding (i.e., specific + non-specific binding) was plotted as a function of the logarithm of the molar concentration of the competitor (i.e., test or reference compound). Non-linear regression of the normalized (i.e., percent radioligand binding compared to that observed in the absence of test or reference compound) raw data was performed in Prism 4.0 (GraphPad Software) using the built-in three parameter logistic model describing ligand competition binding to radioligand-labeled sites as "y = bottom + [(top-bottom)/(1 + 10x-logIC_{50})]," where bottom equals the residual radioligand binding measured in the presence of 10 μM reference compound (i.e., non-specific binding) and top equals the total radioligand binding observed in the absence of competitor. The log IC_{50} (i.e., the log of the ligand concentration that reduces radioligand binding by 50%) was then estimated from the data and used to obtain the K_i by applying the Cheng-Prusoff approximation (K_i = IC_{50}/(1 + [ligand]/K_D), where [ligand] equals the assay radioligand concentration and K_D equals the affinity constant of the radioligand for the target receptor).[266]

4.3. Functional receptor screen

For orphan GPCRs, receptor-expressing cell lines were seeded in glass-bottom 96- or 384-well, poly-L-lysine-coated plates 48 h prior to the assay (40,000 cells per well or 6,700 cells, respectively) in DMEM containing 5% dialyzed serum. Twenty hours prior to the assay, the medium was changed to serum-free DMEM. Then, the cells were preincubated in 30 μL (96-well plates) or 20 μL (384-well plates) of calcium dye-containing assay buffer (the lyophilized dye is reconstituted with 15 mL of assay buffer) at 37 ºC for 75 min in a

humidified incubator. During that time, serial dilutions of the test compounds were made at 2x assay concentration (10 μM final assay concentration). Just prior to the assay, the plates were allowed to cool to room temperature for 10 min, and then transferred to a FLIPR Tetra fluorescence image plate reader (Molecular Devices). Basal fluorescence (excitation 488 nm, emission 510–570 nm) was measured for 20 sec, then test compound (2x assay concentration) was added (30 μL for 96-well plates, 20 μL for 384-well plates, in triplicate) and fluorescence was measured for 60 sec. The maximum fluorescence values were measured during the baseline and test compound addition phases.

For hERG, a fluorescence-based membrane potential assay (Molecular Devices) was used. HEK293 cells stably expressing recombinant human HERG (provided by Dr. J. Overholt, Case Western Reserve University, Cleveland, OH; cells originally from Drs. A. Brown and E. Ficker, MetroHealth Medical Center, Cleveland, OH) were seeded in poly-L-lysine-coated 96-well plates (45,000 cells/100 μL DMEM supplemented with 10% fetal bovine serum/well) one day prior to the assay. The next day, the medium was removed and replaced with 30 μL/well of assay buffer containing the membrane potential dye (Molecular Devices) (the lyophilized dye was reconstituted with 15 mL of assay buffer). After a 15 min incubation at 37 ºC, 30 μL/well of 2X dilutions of terfenadine (a known HERG blocker used as a reference compound) or test compound (10 μM final assay concentration) was added to the cells (each concentration assayed in triplicate). Baseline fluorescence (excitation 530 nM, emission 565 nM) was measured over 15 min, then 140 μL of depolarization solution (143 mM KCl in distilled water) containing test or reference compound (1x) was added to the cells and fluorescence is recorded for 3 min. Raw fluorescence data was exported to GraphPad Prism 4.0 for further analysis. The last data value measured in each well (i.e., 3 min after addition of depolarization solution) was used for analysis, after subtraction of the mean background value obtained in the first 30 sec before depolarization of the cells. For negative controls, the value obtained using assay buffer alone was defined as 0% hERG

blockade, and for positive controls, the value obtained using 10 μM terfenadine was defined as 100% hERG blockade. Negative and positive terfenadine controls were done on each plate. Data from test compounds are scaled as follows: percent hERG blockade for test compound = 100 x (value for test compound - value for negative control)/(value for 10 μM terfenadine - value for negative control). "Hits" (or "blockers") were defined as those compounds with 20% or greater hERG blockade. hERG "openers" were defined as those compounds with values for hERG blockade less than -20%.

4.4. MAO_B enzymatic assay

Human platelets containing 0.5 mM benzylamine were incubated at 37 ºC for 45 min with 10 μM of (*R*)-**257**, or reference compound (deprenyl, IC_{50} = 0.35 nM), and the amount of benzylaldehyde generated was detected by spectrophotometry (in duplicate).[267] The IC_{50} value was determined by non-linear regression analysis of the inhibition curve generated with mean replicative values using Hill equation curve fitting ($y = d + [(a-d)/(1 + (c/c_{50})^{nH})]$, where y equals the specific activity, d equals the minimum specific activity, a equals the maximum specific activity, c equals the compound concentration, c_{50} equals the IC_{50}, and nH equals the slope factor. Analysis was performed using software developed at Cerep, Inc (Hill software) and validated by comparison with data generated by the commercial software Sigmaplot® 4.0 for Windows® (© 1997 by SPSS, Inc).

CHAPTER 5. General Conclusions and Future Directions

1. Conclusions

Epilepsy and NP are serious neurological disorders that result from dysregulations in neuronal function, and the lack of adequate therapeutic agents available to treat these disorders are compounded by issues including, but not limited to, pharmacoresistance, adverse CNS side effects, and a lack of efficacy. Therefore, there is a substantial market for the treatment of these neurological disorders and the need remains to develop compounds that possess a novel mechanism of action to address the shortcomings of current medications. Recently, the role of VGSCs has been implicated in the pathophysiological mechanisms of NP,[28,75,76] while their role in epilepsy has been known for some time.[72-74,77] The FAA (*R*)-lacosamide ((*R*)-**28**) is an emerging AED that has been shown to selectively promote VGSCs into the slow inactivated state[63,64] and has recently been approved by the EMEA and the US FDA under the trademark Vimpat® for the adjuvant treatment of partial-onset seizures in adult patients with epilepsy.[61] (*R*)-**28** has also demonstrated clinical efficacy in treating painful diabetic neuropathy, but has yet to gain regulatory approval for this indication.[66] Furthermore, the pharmaceutical industry has made advances in developing PNS-specific agents that target specific isoforms of VGSCs for the treatment of NP.[28-32]

Encouraged by the discovery of several PNS-selective agents, we combined the concept of PNS-selectivity with our knowledge of FAAs, and initially proposed that PAADs may be able to selectively target PNS sites for the treatment of NP, but we have since concluded that PAADs can access the CNS. Accordingly, we examined the effect of PAADs on CNS function due to the known excellent anticonvulsant activities of FAAs. We synthesized and evaluated over 50 PAADs in whole animal models of epilepsy and NP, and developed a SAR that defined the structural requirements for PAAD activity. The SAR revealed excellent anticonvulsant activity and pain attenuation for the C(2)-hydrocarbon PAADs. While these PAADs were slightly less active than (*R*)-**28**, many C(2)-hydrocarbon PAADs surpassed the MES activity observed for the traditional antiepileptic agent phenobarbital (ED_{50} = 22 mg/kg). Then, we synthesized 36 C(2)-hydrocarbon PAADs along with 7 C(3)-*O*-methoxy PAADs and determined that, in some cases, functionalization of the *N*-benzylamide moiety enhanced anticonvulsant activity and pain attenuation, while functionalization of the *N*-terminal amine reduced these effects. Additionally, a closer examination of the PAAD structural blueprint in the C(2)-hydrocarbon series revealed that at least four methylene units could be inserted between the amide bond and the aromatic ring without a loss of seizure protection. From our optimization studies, we discovered two PAADs ((*R*)-**255** and (*R*)-**279**) that displayed superior anticonvulsant activity that may rival the therapeutic capabilities of (*R*)-**28**. Finally, evaluation of our most active PAADs in a series of binding and enzymatic assays did not reveal any potential binding targets of therapeutic relevance.

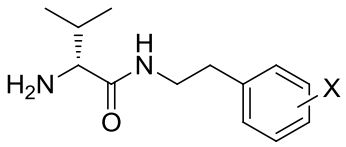
2. Future directions

The findings from this SAR project warrant further studies of the C(2)-hydrocarbon PAADs that could prove beneficial for the treatment of epilepsy and NP, including expanding

the SAR, attempting to prolong their duration of action, expanding target identification efforts (including, but not limited to, electrophysiology studies), metabolism studies, and toxicity studies.

2.1. Expanding the SAR of C(2)-hydrocarbon PAADs

The C(2)-hydrocarbon PAAD SAR revealed that at least four methylene units could be incorporated between the amide bond and the aromatic ring without a dramatic loss in anticonvulsant activity. Anticonvulsant activity for this series reached at maximum when the methylene linker was two (CH_2CH_2) (Table 32). Separately, we determined that substitution of the *N*-benzylamide moiety with an electron-withdrawing substituent improved seizure protection. Therefore, we intend to combine these two elements to synthesize a C(2)-isopropyl phenethylamide bearing an electron-withdrawing substituent (**372**) in an attempt to increase seizure protection, and possibly pain attenuation. Ideally, we would like to synthesize the 2'-, 3'-, and 4'-OCF_3 derivatives, but limited availability of the corresponding substituted phenethyl reagents would extend the synthetic route. A suitable alternative would be to synthesize the 2'-, 3'-, and 4'-fluoro derivatives because the corresponding substituted phenethyl reagents are readily available and the anticonvulsant activity of the parent 4'-OCF_3 ((*R*)-**248**) and 4'-fluoro ((*R*)-**243**) PAADs were similar (ED_{50} (mg/kg): (*R*)-**248**, 16; (*R*)-**243**, >10, <30).



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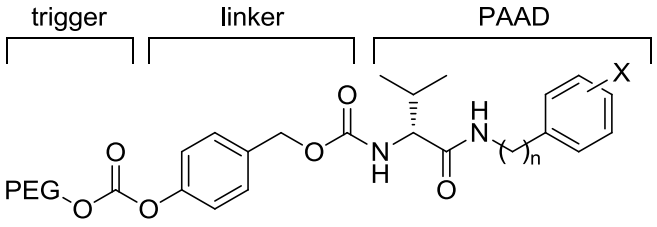
2.2. Prolonging the duration of action of C(2)-hydrocarbon PAADs

The C(2)-isopropyl PAAD (*R*)-**98** possessed significant anticonvulsant activity (ED_{50} = 15 mg/kg), but the time to peak effect occurred within the first 15 min of compound administration (4/4 mice protected). The number of mice protected reduced by half within 30 min of compound administration (2/4 mice protected), and then no protection was observed 1 h after administration (0/4 mice protected). By comparison, the time to peak effect for (*R*)-**28** in mice (ip) was 0.5 h.[60] Following a report published by Greenwarld and coworkers,[268] we propose that the synthesis of a poly(ethylene)glycol (PEG) prodrug employing a 1,4-elimination system (**373**) could prolong the duration of action of C(2)-hydrocarbon PAADs. Polymeric prodrugs are often designed to circumvent unfavorable pharmacokinetic properties, such as limited solubility, uncontrolled degradation, and a short plasma half-life.[269] This double-prodrug strategy first relies on enzymatic hydrolysis of a PEG "trigger", followed by a classic 1,4-elimination reaction of the linker to release the amine (PAAD).



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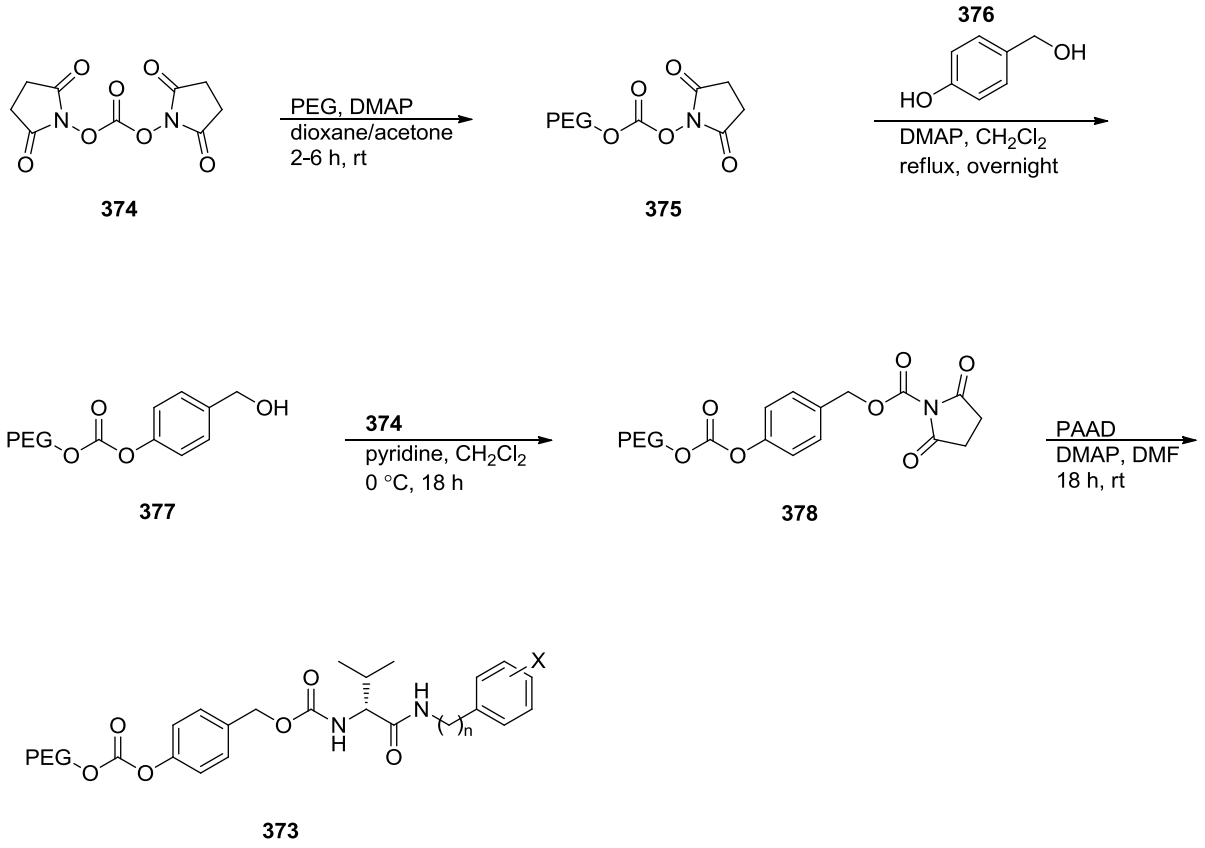
Synthesis of **373** can be achieved by convergent synthesis of the C(2)-isopropyl PAAD and the pegylated-linker **378** in the presence of DMAP (Scheme 36). The pegylated-linker **378** can be prepared in three steps by first activating PEG with *N*,*N*-disuccinimidyl carbonate (**374**) in the presence of DMAP[270] to give **375**, followed by treatment with 4-hydroxybenzyl alcohol (**376**) and in the presence of DMAP at reflux[268] to give **377**. Benzyl

alcohol **377** is then activated by *N*,*N*-disuccinimidyl carbonate[268] (**374**) to provide **378**, and then coupled with the PAAD of choice to generate prodrug **373**.

Scheme 36. Synthesis of C(2)-isopropyl PAAD prodrug



2.3. Electrophysiology studies

Recently, the Lees[63] and Cummins[64] laboratories have examined the mechanism of action of lacosamide ((*R*)-**28**) on VGSC function through a series of electrophysiological experiments (discussed in Section 2.1.2.2).[63,64] We have formed a collaboration with the Khanna laboratory at the Indiana University School of Medicine to determine the current-voltage (*I*-*V*) relationship of selected optimized PAADs on Na^+ channels at various concentrations on the resting state, fast inactivated state, and slow inactivated state of Na^+

channels using whole-cell patch clamp techniques. Recruiting a particular conformational state can be achieved by either manipulating the holding potential or varying the prepulse duration of the voltage protocol (Figure 17). The voltage protocols are applied in the absence of compound to determine the amount of current evoked, and the protocol is repeated after the application of PAAD to determine the amount of current inhibition. To examine the resting state, neurons are held at a potential of -100 mV and are stimulated by a 500 msec test pulse in 10 mV increments from -120 mV to +60 mV (Figure 17A). To examine the fast inactivated state, neurons are held at a potential of -100 mV and currents are evoked by 500 msec prepulses between -120 mV and -20 mV in 10 mV increments, followed by a 30 msec test pulse to +10 mV (Figure 17B). To examine the slow inactivated state, neurons are held at a potential of -100 mV and currents are evoked by 5 sec prepulses between -120 mV and -10 mV in 10 mV increments, allowed to return to the holding potential for 1 sec (to allow recovery from fast inactivation), followed by the test pulse to -10 mV to analyze the fraction of channels available (Figure 17C). The prepulses in the fast inactivated and slow inactivated voltage protocols allow PAAD binding to reach its steady state before the test pulses. A dose-response curve of current inhibition can be constructed by repeating the voltage protocols at various PAAD concentrations and provide insight into whether the PAAD mechanism of action occurs on resting, fast inactivated, or slow inactivated Na^+ channels.

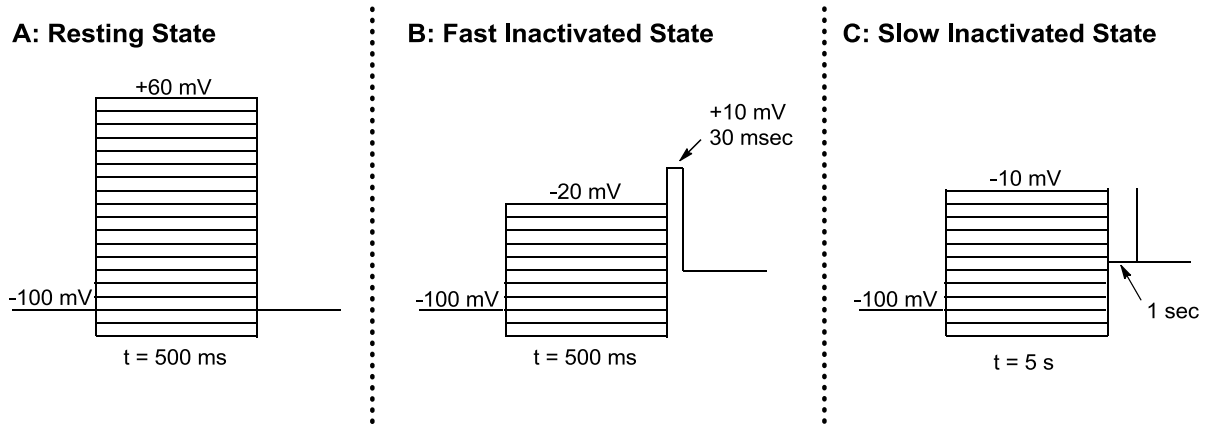


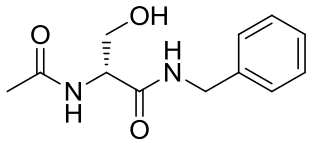
Figure 17. Proposed pulse protocol to evoke the resting state, fast inactivated state, or slow inactivated state of VGSCs

We are particularly anxious to compare the patch-clamp electrophysiological results for the C(3)-*O*-methoxy PAADs and the C(2)-hydrocarbon PAADs with those previously reported for (*R*)-**28**.[63,64] The whole animal pharmacological data for the C(3)-*O*-methoxy PAADs suggested that they mirrored those of C(3)-alkoxy FAAs (Table 6), and thus, the C(3)-*O*-methoxy may facilitate Na^+ channel entry into the slow inactivated state and show stereospecificity for function. Confirmation of this finding would strengthen our suspicion that these PAADs function similar to their FAA counterparts. Correspondingly, the SAR for the C(2)-hydrocarbon PAADs deviated from the blueprint established for the FAAs (Table 15). Moreover, the corresponding C(2)-hydrocarbon FAAs showed minimal or no anticonvulsant activity. These findings provide significant impetus for us to explore if the C(2)-hydrocarbon PAADs modulate sodium channel activity, and if so, how.

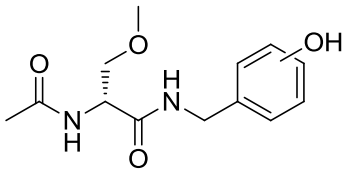
2.4. Metabolism studies

Metabolism is one of several components that influence drug pharmacokinetics (other parameters include adsorption, distribution, and excretion) and can have a dramatic effect on pharmacological activity. Minimal (4%) *in vitro* metabolism of (*R*)-**28** was detected

after 4 h of incubation with human hepatocytes, and there was no evidence of phase II metabolites. The major phase I metabolites identified were the *O*-desmethyl metabolite (*R*)-**374** and the phenolic metabolites (*R*)-**375**.[248] However, *in vivo* phase I metabolism of (*R*)-**28** was more notable in animal species. Plasma, urine, and feces samples were analyzed 1.5 h and 6 h following a single oral dose of [^{14}C]-(*R*)-**28** in mouse, rat, and dog, and the percentage of unchanged [^{14}C]-(*R*)-**28** ranged from 80–90% (1.5 h post dose) and 50–65% (6 h post dose). The major metabolites identified 6 h post dose were (*R*)-**374** (40%) and (*R*)-**375** (5%).[248]

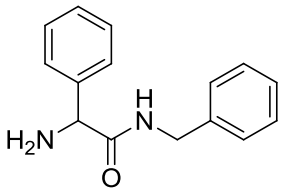


(*R*)-**374**

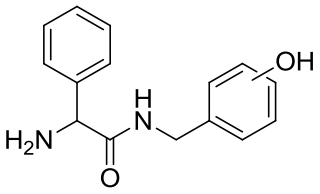


(*R*)-**375**

Although, metabolism of (*R*)-**28** is not thought to be responsible for its pharmacological activity, it is possible that PAAD metabolism could be responsible for the unique C(2)-hydrocarbon PAAD SAR. To rule out this option, it would be informative to determine the interaction(s) of PAADs with cytochrome P-450 using a hepatic microsomal assay. Previously, the Kohn laboratory has conducted similar work, which included metabolic studies of PAAD (*R,S*)-**62**, from which the phenolic PAAD (*R,S*)-**376** was identified as the major metabolite (25% after 1 h).[92] In a similar manner, C(2)-hydrocarbon PAADs are incubated with liver microsomes containing P-450 enzymes, in the absence and presence of NADPH (22 ºC, 0–60 min), and substrate disappearance is measured by high pressure liquid chromatography (HPLC). Metabolites can then be identified by LC-UV and MS/MS detection.



(*R,S*)-**62**



(*R*)-**376**

2.5. Toxicity studies

Preclinical and clinical toxicology studies are time and resource intensive, but it is a critical component to the success or failure of a drug candidate. Types of toxicity evaluated include, but are not limited to, single dose toxicity, repeat dose toxicity, genotoxicity, carcinogenicity, cardiotoxicity, and reproductive toxicity, and are performed to identify the possible human risk associated with the administration of the drug candidate. It is critical to identify the risks associated with AEDs and NP agents because these classes of drugs treat chronic medical disorders and are intended for long term use.

We have extensively studied the C(2)-hydrocarbon PAAD series and have optimized its anticonvulsant activity in the MES seizure model in rodents. The excellent anticonvulsant activity and pain attenuating properties of the C(2)-hydrocarbons PAADs, in conjunction with a possible novel mechanism of action, warrant acute toxicity studies. In the acute studies, we want to identify the dose at which toxic effects first appear and the highest dose where no adverse affects are observed. If the C(2)-hydrocarbon PAADs demonstrate an acceptable toxicological profile under acute conditions, that would provide preliminary assurance in the safety of this class of compounds and we could justify long term toxicity studies.

REFERENCES

1. Pangalos, M. N.; Schechter, L. E.; Hurko, O., Drug development for CNS disorders: Strategies for balancing risk and reducing attrition. *Nat. Rev. Drug Discov.* **2007,** *6* (7), 521-532.

2. Kola, I.; Landis, J., Can the pharmaceutical industry reduce attrition rates? *Nat. Rev. Drug Discovery* **2004,** *3* (8), 711-716.

3. Drews, J., Drug discovery: A historical perspective. *Science* **2000,** *287* (5460), 1960-1964.

4. Enna, S. J.; Williams, M., Challenges in the search for drugs to treat central nervous system disorders. *J. Pharmacol. Exp. Ther.* **2009,** *329* (2), 404-411.

5. Betz, U. A. K., How many genomics targets can a portfolio afford? *Drug Discov. Today* **2005,** *10* (15), 1057-1063.

6. Strebhardt, K.; Ullrich, A., Paul Ehrlich's magic bullet concept: 100 years of progress. *Nat. Rev. Cancer* **2008,** *8* (6), 473-480.

7. Bianchi, M. T.; Pathmanathan, J.; Cash, S. S., From ion channels to complex networks: Magic bullet versus magic shotgun approaches to anticonvulsant pharmacotherapy. *Med. Hypotheses* **2009,** *72* (3), 297-305.

8. Roth, B. L.; Sheffler, D. J.; Kroeze, W. K., Magic shotguns versus magic bullets: Selectively non-selective drugs for mood disorders and schizophrenia. *Nat. Rev. Drug Discov.* **2004,** *3* (4), 353-359.

9. Begley, C. E.; Lairson, D. R.; Reynolds, T. F.; Coan, S., Early treatment cost in epilepsy and how it varies with seizure type and frequency. *Epilepsy Res.* **2001,** *47* (3), 205-215.

10. McCorry, D.; Chadwick, D.; Marson, A., Current drug treatment of epilepsy in adults. *Lancet Neurol.* **2004,** *3* (12), 729-735.

11. Stafstrom, C. E., Epilepsy: A review of selected clinical syndromes and advances in basic science. *J. Cereb. Blood Flow Metab.* **2006,** *26* (8), 983-1004.

12. CDC - Epilepsy. http://www.cdc.gov/epilepsy/.

13. Duncan, J. S., The promise of new antiepileptic drugs. *Br. J. Clin. Pharmacol.* **2002,** *53* (2), 123-131.

14. Fisher, R. S.; Boas, W. v. E.; Blume, W.; Elger, C.; Genton, P.; Lee, P.; Jr., J. E., Epileptic seizures and epilepsy: Definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia* **2005,** *46* (4), 470-472.

15. Mohanraj, R.; Brodie, M. J., Measuring the efficacy of antiepileptic drugs. *Seizure* **2003,** *12* (7), 413-443.

16. Porter, R., New antiepileptic agents: Strategies for drug development. *Lancet* **1990,** *336* (8712), 423-424.

17. Curia, G.; Biagini, G.; Perucca, E.; Avoli, M., Lacosamide: A new approach to target voltage-gated sodium currents in epileptic disorders. *CNS Drugs* **2009,** *23* (7), 555-568.

18. Bauer, J.; Reuber, M., Medical treatment of epilepsy. *Expert Opin. Emerg. Drugs* **2003,** *8* (2), 457-467.

19. Picot, M. C.; Baldy-Moulinier, M.; Daurs, J. P.; Dujols, P.; Crespel, A., The prevalence of epilepsy and pharmacoresistant epilepsy in adults: A population-based study in a western European country. *Epilepsia* **2008,** *49* (7), 1230-1238.

20. Boucher, T. J.; McMahon, S. B., Neurotrophic factors and neuropathic pain. *Curr. Opin. Pharmacol.* **2001,** *1* (1), 66-72.

21. Dickinson, T.; Lee, K.; Spanswick, D.; Munro, F. E., Leading the charge - pioneering treatments in the fight against neuropathic pain. *Trends Pharmacol. Sci.* **2003,** *24* (11), 555-557.

22. Zimmermann, M., Pathobiology of neuropathic pain. *Eur. J. Pharmacol.* **2001,** *429* (1-3), 23-37.

23. Butera, J. A., Current and emerging targets to treat neuropathic pain. *J. Med. Chem.* **2007,** *50* (11), 2543-2546.

24. Florin, O.; Mark, V. B.; Samuel, K. R., Neuropathic pain: Review of mechanisms and pharmacologic management. *Neurorehabilitation* **2000,** *14* (1), 15-23.

25. Dworkin, R. H.; O'Connor, A. B.; Backonja, M.; Farrar, J. T.; Finnerup, N. B.; Jensen, T. S.; Kalso, E. A.; Loeser, J. D.; Miaskowski, C.; Nurmikko, T. J.; Portenoy, R. K.; Rice, A. S. C.; Stacey, B. R.; Treede, R.-D.; Turk, D. C.; Wallace, M. S., Pharmacologic management of neuropathic pain: Evidence-based recommendations. *Pain* **2007,** *132* (3), 237-251.

26. Pezet, S.; McMahon, S. B., Neurotrophins: Mediators and modulators of pain. *Annu. Rev. Neurosci.* **2006,** *29* (1), 507-538.

27. Williams, M.; Kowaluk, E. A.; Arneric, S. P., Emerging molecular approaches to pain therapy. *J. Med. Chem.* **1999,** *42* (9), 1481-1500.

28. Brochu, R. M.; Dick, I. E.; Tarpley, J. W.; McGowan, E.; Gunner, D.; Herrington, J.; Shao, P. P.; Ok, D.; Li, C.; Parsons, W. H.; Stump, G. L.; Regan, C. P.; Lynch, J. J.; Lyons, K. A.; McManus, O. B.; Clark, S.; Ali, Z.; Kaczorowski, G. J.; Martin, W. J.; Priest, B. T., Block of peripheral nerve sodium channels selectively inhibits features of neuropathic pain in rats. *Mol. Pharmacol.* **2006,** *69* (3), 823-832.

29. Hoyt, S. B.; London, C.; Gorin, D.; Wyvratt, M. J.; Fisher, M. H.; Abbadie, C.; Felix, J. P.; Garcia, M. L.; Li, X.; Lyons, K. A.; McGowan, E.; MacIntyre, D. E.; Martin, W. J.; Priest, B. T.; Ritter, A.; Smith, M. M.; Warren, V. A.; Williams, B. S.; Kaczorowski, G. J.; Parsons, W. H., Discovery of a novel class of benzazepinone Nav1.7 blockers: Potential treatments for neuropathic pain. *Bioorg. Med. Chem. Lett.* **2007,** *17* (16), 4630-4634.

30. Hoyt, S. B.; London, C.; Ok, H.; Gonzalez, E.; Duffy, J. L.; Abbadie, C.; Dean, B.; Felix, J. P.; Garcia, M. L.; Jochnowitz, N.; Karanam, B. V.; Li, X.; Lyons, K. A.; McGowan, E.; MacIntyre, D. E.; Martin, W. J.; Priest, B. T.; Smith, M. M.; Tschirret-Guth, R.; Warren, V. A.; Williams, B. S.; Kaczorowski, G. J.; Parsons, W. H., Benzazepinone Nav1.7 blockers: Potential treatments for neuropathic pain. *Bioorg. Med. Chem. Lett.* **2007,** *17* (22), 6172-6177.

31. Jarvis, M. F.; Honore, P.; Shieh, C.-C.; Chapman, M.; Joshi, S.; Zhang, X.-F.; Kort, M.; Carroll, W.; Marron, B.; Atkinson, R.; Thomas, J.; Liu, D.; Krambis, M.; Liu, Y.; McGaraughty, S.; Chu, K.; Roeloffs, R.; Zhong, C.; Mikusa, J. P.; Hernandez, G.; Gauvin, D.; Wade, C.; Zhu, C.; Pai, M.; Scanio, M.; Shi, L.; Drizin, I.; Gregg, R.; Matulenko, M.; Hakeem, A.; Gross, M.; Johnson, M.; Marsh, K.; Wagoner, P. K.; Sullivan, J. P.; Faltynek, C. R.; Krafte, D. S., A-803467, a potent and selective $Na_v1.8$ sodium channel blocker, attenuates neuropathic and inflammatory pain in the rat. *Proc. Natl. Acad. Sci. U. S. A.* **2007,** *104* (20), 8520-8525.

32. Kort, M. E.; Drizin, I.; Gregg, R. J.; Scanio, M. J. C.; Shi, L.; Gross, M. F.; Atkinson, R. N.; Johnson, M. S.; Pacofsky, G. J.; Thomas, J. B.; Carroll, W. A.; Krambis, M. J.; Liu, D.;

Shieh, C.-C.; Zhang, X.; Hernandez, G.; Mikusa, J. P.; Zhong, C.; Joshi, S.; Honore, P.; Roeloffs, R.; Marsh, K. C.; Murray, B. P.; Liu, J.; Werness, S.; Faltynek, C. R.; Krafte, D. S.; Jarvis, M. F.; Chapman, M. L.; Marron, B. E., Discovery and biological evaluation of 5-aryl-2-furfuramides, potent and selective blockers of the $Na_{1.8}$ sodium channel with efficacy in models of neuropathic and inflammatory pain. *J. Med. Chem.* **2008,** *51* (3), 407-416.

33. Williams, B. S.; Felix, J. P.; Priest, B. T.; Brochu, R. M.; Dai, K.; Hoyt, S. B.; London, C.; Tang, Y. S.; Duffy, J. L.; Parsons, W. H.; Kaczorowski, G. J.; Garcia, M. L., Characterization of a new class of potent inhibitors of the voltage-gated sodium channel $Na_v1.7$. *Biochemistry* **2007,** *46* (50), 14693-14703.

34. Dichter, M. A., Emerging insights into mechanisms of epilepsy: Implications for new antiepileptic drug development. *Epilepsia* **1994,** *35* (s4), S51-S57.

35. Gee, N. S.; Brown, J. P.; Dissanayake, V. U. K.; Offord, J.; Thurlow, R.; Woodruff, G. N., The novel anticonvulsant drug, gabapentin (neurontin), binds to the subunit of a calcium channel. *J. Biol. Chem.* **1996,** *271* (10), 5768-5776.

36. Landmark, C. J., Antiepileptic drugs in non-epilepsy disorders: Relations between mechanisms of action and clinical efficacy. *CNS Drugs* **2008,** *22* (1), 27-47.

37. Landmark, C. J.; Johannessen, S. I., Pharmacological management of epilepsy: Recent advances and future prospects. *Drugs* **2008,** *68* (14), 1925-1939.

38. Macdonald, R. L., Antiepileptic drug actions. *Epilepsia* **1989,** *30* (s1), S19-S28.

39. Brodie, M. J.; Dichter, M. A., Established antiepileptic drugs. *Seizure* **1997,** *6* (3), 159-174.

40. Merritt, H. H.; Putnam, T. J., A new series of anticonvulsant drugs tested by experiments on animals. *Arch Neurol Psychiatry* **1938,** *39* (5), 1003-1015.

41. Chen, G.; Portman, R.; Ensor, C. R.; Bratton, A. C., The anticonvulsant activity of α-phenyl succinimides. *J. Pharmacol. Exp. Ther.* **1951,** *103* (1), 54-61.

42. Stables, J. P.; Kupferberg, H. J., The NIH anticonvulsant drug development (ADD) program: Preclinical anticonvulsant screening project. In *Molecular and cellular targets for anti-epileptic drugs*, Avanzini, G.; Regesta, G.; Tanganelli, O.; Avoli, M., Eds. John Libby & Company Ltd: London, 1997; pp 191-198.

43. Yamaoka, Y.; Roberts, R. D.; Stella, V. J., Low-melting phenytoin prodrugs as alternative oral delivery modes for phenytoin: A model for other high-melting sparingly water-soluble drugs. *J. Pharm. Sci.* **1983,** *72* (4), 400-405.

44. Browne, T. R., Fosphenytoin (Cerebyx). *Clin. Neuropharmacol.* **1997,** *20*, 1-12.

45. Bialer, M., New antiepileptic drugs that are second generation to existing antiepileptic drugs. *Expert Opin. Invest. Drugs* **2006,** *15* (6), 637-647.

46. Bialer, M.; White, H. S., Key factors in the discovery and development of new antiepileptic drugs. *Nat. Rev. Drug Discov.* **2010,** *9* (1), 68-82.

47. Rogawski, M. A., Diverse mechanisms of antiepileptic drugs in the development pipeline. *Epilepsy Res.* **2006,** *69* (3), 273-294.

48. Bialer, M.; Johannessen, S. I.; Levy, R. H.; Perucca, E.; Tomson, T.; White, H. S., Progress report on new antiepileptic drugs: A summary of the Ninth Eilat Conference (EILAT IX). *Epilepsy Res.* **2009,** *83* (1), 1-43.

49. Kohn, H.; Kohn, B. A.; Steenberg, M. L.; Buckley, J. P., Syntheses and pharmacological activity of substituted imidazolidinethiones and thioimidazolines. *J. Med. Chem.* **1977,** *20* (1), 158-160.

50. Cortes, S.; Liao, Z. K.; Watson, D.; Kohn, H., Effect of structural modification of the hydantoin ring on anticonvulsant activity. *J. Med. Chem.* **1985,** *28* (5), 601-606.

51. Kohn, H.; Conley, J. D., New antiepileptic agents. *Chem. Br.* **1988,** *24*, 231-2, 234.

52. Conley, J. D.; Kohn, H., Functionalized DL-amino acid derivatives. Potent new agents for the treatment of epilepsy. *J. Med. Chem.* **1987,** *30* (3), 567-574.

53. Kohn, H.; Conley, J. D.; Leander, J. D., Marked stereospecificity in a new class of anticonvulsants. *Brain Res.* **1988,** *457* (2), 371-375.

54. Kohn, H.; Sawhney, K. N.; LeGall, P.; Conley, J. D.; Robertson, D. W.; Leander, J. D., Preparation and anticonvulsant activity of a series of functionalized α-aromatic and α-heteroaromatic amino acids. *J. Med. Chem.* **1990,** *33* (3), 919-926.

55. Kohn, H.; Sawhney, K. N.; LeGall, P.; Robertson, D. W.; Leander, J. D., Preparation and anticonvulsant activity of a series of functionalized α-heteroatom-substituted amino acids. *J. Med. Chem.* **1991,** *34* (8), 2444-2452.

56. Kohn, H.; Sawhney, K. N.; Bardel, P.; Robertson, D. W.; Leander, J. D., Synthesis and anticonvulsant activities of α-heterocyclic α-acetamido-*N*-benzylacetamide derivatives. *J. Med. Chem.* **1993,** *36* (22), 3350-3360.

57. Bardel, P.; Bolanos, A.; Kohn, H., Synthesis and anticonvulsant activities of α-acetamido-*N*-benzylacetamide derivatives containing an electron-deficient α-heteroaromatic substituent. *J. Med. Chem.* **1994,** *37* (26), 4567-4571.

58. Kohn, H.; Sawhney, K. N.; Robertson, D. W.; Leander, J. D., Anticonvulsant properties of *N*-substituted α,α-diamino acid derivatives. *J. Pharm. Sci.* **1994,** *83* (5), 689-691.

59. Personal communication between Eli Lilly and Harold Kohn.

60. Choi, D.; Stables, J. P.; Kohn, H., Synthesis and anticonvulsant activities of *N*-benzyl-2-acetamidopropionamide derivatives. *J. Med. Chem.* **1996,** *39* (9), 1907-1916.

61. UCB - Vimpat. http://www.ucb.com/products/product-list/cns/vimpat.

62. Beyreuther, B. K.; Freitag, J.; Heers, C.; Krebsfänger, N.; Scharfenecker, U.; Stöhr, T., Lacosamide: A review of preclinical properties. *CNS Drug Rev.* **2007,** *13* (1), 21-42.

63. Errington, A. C.; Stöhr, T.; Heers, C.; Lees, G., The investigational anticonvulsant lacosamide selectively enhances slow inactivation of voltage-gated sodium channels. *Mol. Pharmacol.* **2008,** *73* (1), 157-169.

64. Sheets, P. L.; Heers, C.; Stoehr, T.; Cummins, T. R., Differential block of sensory neuronal voltage-gated sodium channels by lacosamide [(2*R*)-2-(acetylamino)-*N*-benzyl-3-methoxypropanamide], lidocaine, and carbamazepine. *J. Pharmacol. Exp. Ther.* **2008,** *326* (1), 89-99.

65. Temperini, C.; Innocenti, A.; Scozzafava, A.; Parkkila, S.; Supuran, C. T., The coumarin-binding site in carbonic anhydrase accommodates structurally diverse inhibitors: The antiepileptic lacosamide as an example and lead molecule for novel classes of carbonic anhydrase inhibitors. *J. Med. Chem.* **2010,** *53* (2), 850-854.

66. Shaibani, A.; Biton, V.; Rauck, R.; Koch, B.; Simpson, J., Long-term oral lacosamide in painful diabetic neuropathy: A two-year open-label extension trial. *Eur. J. Pain* **2009,** *13* (5), 458-463.

67. Wiffen, P. J.; Derry, S.; Moore, R. A.; McQuar, H. J., Carbamazepine for acute and chronic pain. *Cochrane Database Syst. Rev.* **2005,** *20* (3), 1-40.

68. Lilly, E. FDA Approves Cymbalta for the Management of Fibromyalgia. http://newsroom.lilly.com/ReleaseDetail.cfm?ReleaseID=316740.

69. Pfizer *Neurontin*; 2002.

70. FDA FDA approves first drug for treating fibromyalgia. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2007/ucm108936.htm.

71. *Fundamental neuroscience*. Academic Press: Boston, 2003.

72. Graves, T. D., Ion channels and epilepsy. *QJM* **2006,** *99* (4), 201-217.

73. Lerche, H.; Weber, Y. G.; Jurkat-Rott, K.; Lehmann-Horn, F., Ion channel defects in idiopathic epilepsies. *Curr. Pharm. Des.* **2005,** *11* (21), 2737-2752.

74. Mulley, J. C.; Scheffer, I. E.; Petrou, S.; Berkovic, S. F., Channelopathies as a genetic cause of epilepsy. *Curr. Opin. Neurol.* **2003,** *16*, 171-176.

75. Cummins, T. R.; Sheets, P. L.; Waxman, S. G., The roles of sodium channels in nociception: Implications for mechanisms of pain. *Pain* **2007,** *131* (3), 243-257.

76. Dib-Hajj, S. D.; Binshtok, A. M.; Cummins, T. R.; Jarvis, M. F.; Samad, T.; Zimmermann, K., Voltage-gated sodium channels in pain states: Role in pathophysiology and targets for treatment. *Brain Res. Rev.* **2009,** *60* (1), 65-83.

77. Errington, A. C.; Stohr, T.; Lees, G., Voltage gated ion channels: Targets for anticonvulsant drugs. *Curr. Top. Med. Chem.* **2005,** *5* (1), 15-30.

78. Anger, T.; Madge, D. J.; Mulla, M.; Riddall, D., Medicinal chemistry of neuronal voltage-gated sodium channel blockers. *J. Med. Chem.* **2001,** *44* (2), 115-137.

79. Cestèle, S.; Catterall, W. A., Molecular mechanisms of neurotoxin action on voltage-gated sodium channels. *Biochimie* **2000,** *82*, 883-892.

80. Goldin, A., Mechanisms of sodium channel inactivation. *Curr. Opin. Neurobiol.* **2003,** *13* (3), 284-290.

81. Gordon, D., Sodium channels as targets for neurotoxins. In *Toxins and signal transduction*, Gutman, Y.; Lazarovici, P., Eds. Hardwood Academic Publishers: 1997; Vol. 43, p 520.

82. Kyle, D. J.; Ilyin, V. I., Sodium channel blockers. *J. Med. Chem.* **2007,** *50* (11), 2583-2588.

83. Termin, A.; Martinborough, E.; Wilson, D., Chapter 3. Recent advances in voltage-gated sodium channel blockers: Therapeutic potential as drug targets in the CNS. In *Annu. Rep. Med. Chem.*, John, E. M., Ed. Academic Press: 2008; Vol. Volume 43, pp 43-60.

84. Wang, S.-Y.; Wang, G. K., Voltage-gated sodium channels as primary targets of diverse lipid-soluble neurotoxins. *Cell. Signal.* **2003,** *15* (2), 151-159.

85. Yamaoka, K.; Vogel, S. M.; Seyama, I., Na^+ channel pharmacology and molecular mechanisms of gating. *Curr. Pharm. Des.* **2006,** *12*, 429-442.

86. Goldin, A. L., Resurgence of sodium channel research. *Annu. Rev. Physiol.* **2003,** *63* (1), 871-894.

87. Goldin, A. L.; Barchi, R. L.; Caldwell, J. H.; Hofmann, F.; Howe, J. R.; Hunter, J. C.; Kallen, R. G.; Mandel, G.; Meisler, M. H.; Netter, Y. B.; Noda, M.; Tamkun, M. M.; Waxman, S. G.; Wood, J. N.; Catterall, W. A., Nomenclature of voltage-gated sodium channels. *Neuron* **2000,** *28* (2), 365-368.

88. Lipkind, G. M.; Fozzard, H. A., Molecular model of anticonvulsant drug binding to the voltage-gated sodium channel inner pore. *Mol. Pharmacol.* **2010,** *78*, 631-638.

89. Andurkar, S. V.; Stables, J. P.; Kohn, H., Synthesis and anticonvulsant activities of (*R*)-(*O*)-methylserine derivatives. *Tetrahedron: Asymmetr* **1998,** *9* (21), 3841-3854.

90. Andurkar, S. V.; Stables, J. P.; Kohn, H., The anticonvulsant activities of *N*-benzyl 3-methoxypropionamides. *Bioorg. Med. Chem.* **1999,** *7* (11), 2381-2389.

91. Béguin, C.; Andurkar, S. V.; Jin, A. Y.; Stables, J. P.; Weaver, D. F.; Kohn, H., Functionalized amido ketones: New anticonvulsant agents. *Bioorg. Med. Chem.* **2003,** *11* (19), 4275-4285.

92. Béguin, C.; LeTiran, A.; Stables, J. P.; Voyksner, R. D.; Kohn, H., *N*-Substituted amino acid *N*'-benzylamides: Synthesis, anticonvulsant, and metabolic activities. *Bioorg. Med. Chem.* **2004,** *12* (11), 3079-3096.

93. Choi, D.; Stables, J. P.; Kohn, H., The anticonvulsant activities of functionalized *N*-benzyl 2-acetamidoacetamides. The importance of the 2-acetamido substituent. *Bioorg. Med. Chem.* **1996,** *4* (12), 2105-2114.

94. Kohn, H.; Liao, Z. K., *N*-Amidoyliminium ion cyclizations. Synthesis of annulated imidazolidinones. *J. Org. Chem.* **1982,** *47*, 2787-2789.

95. Kohn, H.; Salome, C.; Park, K. D. Preparation of 2-(acylamido)acetic acid and 2-(acylamido)propionic acid benzylamides as neurological agents. 2009.

96. Legall, P.; Sawhney, K. N.; Conley, J. D.; Kohn, H., Synthesis of functionalized non-natural amino acid derivatives via amidoalkylation transformation. *Int. J. Pept. Protein Res.* **1988,** *32*, 279-291.

97. LeTiran, A.; Stables, J. P.; Kohn, H., Functionalized amino acid anticonvulsants: Synthesis and pharmacological evaluation of conformationally restricted analogues. *Bioorg. Med. Chem.* **2001,** *9* (10), 2693-2708.

98. LeTiran, A.; Stables, J. P.; Kohn, H., Design and evaluation of affinity labels of functionalized amino acid anticonvulsants. *J. Med. Chem.* **2002,** *45* (21), 4762-4773.

99. , C.; Park, K. D.; Stables, J. P.; Kohn, H., The structure−activity relationship of the 3-oxy site in the anticonvulsant (*R*)-*N*-benzyl 2-acetamido-3-methoxypropionamide. *J. Med. Chem.* **2010,** *53* (15), 5716-5726.

100. Morieux, P.; Stables, J. P.; Kohn, H., Synthesis and anticonvulsant activities of *N*-benzyl (2*R*)-2-acetamido-3-oxysubstituted propionamide derivatives. *Bioorg. Med. Chem.* **2008,** *16* (19), 8968-8975.

101. Park, K. D.; Morieu , C.; Cotten, S. W.; Reamtong, O.; Eyers, C.; Gaskell, S. J.; Stables, J. P.; Liu, R.; Kohn, H., Lacosamide isothiocyanate-based agents: Novel agents to target and identify lacosamide receptors. *J. Med. Chem.* **2009,** *52* (21), 6897-6911.

102. Sa -Grosjean, E.; Park, K. D.; Morieux, P.; Swendiman, R.; DeMarco, E.; Stables, J. P.; Kohn, H., Synthesis and anticonvulsant activities of (*R*)-*N*-(4′-substituted)benzyl 2-acetamido-3-methoxypropionamides. *J. Med. Chem.* **2010,** *53* (3), 1288-1305.

103. -Grosjean, E.; Stables, J. P.; Kohn, H., Merging the structural motifs of functionalized amino acids and α-aminoamides: Compounds with significant anticonvulsant activities. *J. Med. Chem.* **2010,** *53* (9), 3756-3771.

104. Shen, M.; Béguin, C.; Golbraikh, A.; Stables, J. P.; Kohn, H.; Tropsha, A., Application of predictive QSAR models to database mining: Identification and experimental validation of novel anticonvulsant compounds. *J. Med. Chem.* **2004,** *47* (9), 2356-2364.

105. Shen, M.; LeTiran, A.; Xiao, Y.; Golbraikh, A.; Kohn, H.; Tropsha, A., Quantitative structure−activity relationship analysis of functionalized amino acid anticonvulsant agents Using k nearest neighbor and simulated annealing PLS methods. *J. Med. Chem.* **2002,** *45* (13), 2811-2823.

106. Bee, L. A.; Dickenson, A. H., Effects of lacosamide, a novel sodium channel modulator, on dorsal horn neuronal responses in a rat model of neuropathy. *Neuropharmacology* **2009,** *57* (4), 472-479.

107. Beyreuther, B.; Callizot, N.; Stohr, T., Antinociceptive efficacy of lacosamide in the monosodium iodoacetate rat model for osteoarthritis pain. *Arthritis Res. Ther.* **2007,** *9* (1), R14.

108. Beyreuther, B.; Callizot, N.; Stöhr, T., Antinociceptive efficacy of lacosamide in a rat model for painful diabetic neuropathy. *Eur. J. Pharmacol.* **2006,** *539* (1-2), 64-70.

109. Hao, J.-X.; Stöhr, T.; Selve, N.; Wiesenfeld-Hallin, Z.; Xu, X.-J., Lacosamide, a new anti-epileptic, alleviates neuropathic pain-like behaviors in rat models of spinal cord or trigeminal nerve injury. *Eur. J. Pharmacol.* **2006,** *553*, 135-140.

110. Rauck, R. L.; Shaibani, A.; Biton, V.; Simpson, J.; Koch, B., Lacosamide in painful diabetic peripheral neuropathy: A phase 2 double-blind placebo-controlled study. *Clin. J. Pain* **2007,** *23* (2), 150-158 10.1097/01.ajp.0000210957.39621.b2.

111. Stöhr, T.; Krause, E.; Selve, N., Lacosamide displays potent antinociceptive effects in animal models for inflammatory pain. *Eur. J. Pain* **2006,** *10* (3), 241-249.

112. Andurkar, S. V.; Beguin, C.; Stables, J. P.; Kohn, H., Synthesis and structural studies of aza analogues of functionalized amino acids: New anticonvulsant agents. *J. Med. Chem.* **2001,** *44* (9), 1475-1478.

113. Cortes, S.; Liao, Z.-K.; Watson, D.; Kohn, H., Additions and corrections - Effect of structural modification of the hydantoin ring on anticonvulsant activity. *J. Med. Chem.* **1985,** *28* (12), 1965-1965.

114. Nakajima, K.; Oda, H.; Okawa, K., Studies on 2-airidinecarboxylic acid. IX. Convenient synthesis of optically active S-alkylcysteine, threo-S-alkyl-β-methylcysteine, and lanthionine derivatives via the ring-opening reaction of aziridine by several thiols. *Bull. Chem. Soc. Jpn.* **1983,** *2*, 520-522.

115. Sekar, G.; Singh, V. K., Efficient method for cleavage of aziridines with aromatic amines. *J. Org. Chem.* **1999,** *64* (7), 2537-2539.

116. Tanner, D.; He, H. M.; Somfai, P., Regioselective nucleophilic ring opening of 2,3-aziridino alcohols. *Tetrahedron* **1992,** *48* (29), 6069-6078.

117. Nakajima, K.; Neya, M.; Yamada, S.; Okawa, K., Studies on 2-aziridinecarboxylic acid. VI. Synthesis of β-alkoxy-α-amino acids via ring-opening reaction of aziridine. *Bull. Chem. Soc. Jpn.* **1982,** *9*, 3049-3050.

118. Dahanukar, V.; Zavialov, L., Aziridines and aziridinium ions in the practical synthesis of pharmaceutical intermediates - A perspective. *Curr. Opin. Drug Discovery Dev.* **2002,** *5* (6), 918 - 927.

119. Kelly, J. W.; Anderson, N. L.; Evans, S. A., Cyclodehydration of N- and C-substituted β-amino alcohols to the corresponding aziridines with diethoxytriphenylphosphorane. *J. Org. Chem.* **1986,** *51* (1), 95-97.

120. Mathieu-Pelta, I.; Evans, S. A., A new and improved preparation of acyclic σ-dialkoxyphosphoranes. *J. Org. Chem.* **1994,** *59* (8), 2234-2237.

121. Koenig, S. G.; Vandenbossche, C. P.; Zhao, H.; Mousaw, P.; Singh, S. P.; Bakale, R. P., A facile deprotection of secondary acetamides. *Org. Lett.* **2008,** *11* (2), 433-436.

122. Kato, S.; Harada, H.; Morie, T., Efficient synthesis of (6*R*)-6-amino-1-methyl-4-(3-methylbenzyl)hexahydro-1H-1,4-diazepine from methyl (2*R*)- and (2*S*)-1-benzyloxycarbonylaziridine-2-carboxylates. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3219-3226.

123. Larsson, U.; Carlson, R., Synthesis of amino acids with modified principal properties. 2: Amino acids with polar side chains. *Acta Chem. Scand.* **1994,** *48*, 511-516.

124. Meyer, M.; Telford, J. R.; Cohen, S. M.; White, D. J.; Xu, J.; Raymond, K. N., High-yield synthesis of the enterobactin trilactone and evaluation of derivative siderophore analogs. *J. Am. Chem. Soc.* **1997,** *119* (42), 10093-10103.

125. Shtrumfs, B.; Hermane, J.; Kalvinsh, I.; Trapencieris, P., Unnatural amino acids. 3. Aziridinyl ketones from esters and amides of aziridine-2-carboxylic acids. *Chem. Heterocycl. Compd. (N. Y., NY, U. S.)* **2007,** *43* (2), 169-174.

126. Bhatia, S. K.; Hajdu, J., A new approach to the synthesis of thioether phospholipids. Preparation of 1-thiohexadecyl-2-N-acylaminodeoxyglycerophosphocholines. *Tetrahedron Lett.* **1988,** *29* (1), 31-34.

127. Webb, R. G.; Haskell, M. W.; Stammer, C. H., A nuclear magnetic resonance method for distinguishing α-amino acids from β and γ isomers. *J. Org. Chem.* **1969,** *34* (3), 576-580.

128. Hu, X. E., Nucleophilic ring opening of aziridines. *Tetrahedron* **2004,** *60* (12), 2701-2743.

129. Braga, A. L.; Schneider, P. H.; Paixão, M. W.; Deobald, A. M.; Peppe, C.; Bottega, D. P., Chiral seleno-amines from indium selenolates. A straightforward synthesis of selenocysteine derivatives. *J. Org. Chem.* **2006,** *71* (11), 4305-4307.

130. Michaelis, D. J.; Dineen, T. A., Ring opening of aziridines with ortho-bromophenyl metal reagents: synthesis of 2-substituted indolines. *Tetrahedron Lett.* **2009,** *50* (17), 1920-1923.

131. Sato, K.; Kozikowski, A. P., Construction of optically pure tryptophans from serine derived aziridine-2-carboxylates. *Tetrahedron Lett.* **1989,** *30* (31), 4073-4076.

132. Whitehouse, D.; Hu, S.; Zandt, M. C. V.; Parker, G. Preparation of substituted amino carboxylic acids. 2006.

133. Wei, Z.-L.; Petukhov, P. A.; Bizik, F.; Teixeira, J. C.; Mercola, M.; Volpe, E. A.; Glazer, R. I.; Willson, T. M.; Kozikowski, A. P., Isoxazolyl-serine-based agonists of peroxisome proliferator-activated receptor: Design, synthesis, and effects on cardiomyocyte differentiation. *J. Am. Chem. Soc.* **2004,** *126* (51), 16714-16715.

134. Kranz, M.; Kessler, H., Novel synthesis of a Phe-Gly E-alkene dipeptide isostere. *Tetrahedron Lett.* **1996,** *37* (30), 5359-5362.

135. Lapatsanis, L.; Milias, G.; Froussios, K.; Kolovos, M., Synthesis of *N*-2,2,2-(trichloroethoxycarbonyl)-L-amino acids and *N*-(9-fluorenylmethoxycarbonyl)-L-amino acids involving succinimidoxy anion as a leaving group in amino acid protection *Synthesis* **1983**, 671-673.

136. Eckert, H.; Breuer, W.; Geller, J.; Lagerlund, I.; Listl, M.; Marquarding, D.; Stuber, S.; Ugi, I.; Zahr, S.; Zychlinski, H., New methods in peptide synthesis, based in supernulceophiles. *Pure Appl. Chem.* **1979,** *51*, 1219-1233.

137. Eckert, H.; Listl, M.; Ugi, I., The 2,2,2-trichloro-*tert*-butyloxycarbonyl group (TcBoc) - An acid and base resistant protecting group removable under mild conditions. *Angew. Chem. Int. Ed. Engl.* **1978,** *17*, 361-362.

138. McKeever, B.; Pattenden, G., Total synthesis of the cytotoxic cyclopeptide mollamide, isolated from the sea squirt Didemnum molle. *Tetrahedron* **2003,** *59* (15), 2701-2712.

139. Pak, J. K.; Hesse, M., Synthesis of penta-*N*-protected homocaldopentamine and its selective acylation. *J. Org. Chem.* **1998,** *63* (23), 8200-8204.

140. Dong, Q.; Eric Anderson, C.; Ciufolini, M. A., Reductive cleavage of TROC groups under neutral conditions with cadmium-lead couple. *Tetrahedron Lett.* **1995,** *36* (32), 5681-5682.

141. - , M.; Albericio, F., Amino acid-protecting groups. *Chem. Rev.* **2009,** *109* (6), 2455-2504.

142. Alex, K.; Tillack, A.; Schwarz, N.; Beller, M., Zinc-promoted hydrohydrazination of terminal alkynes: An efficient domino synthesis of indoles. *Angew. Chem. Int. Ed. Engl.* **2008,** *47* (12), 2304-2307.

143. Kaufman, D.; Johnson, E.; Mosher, M. D., Zinc reduction of alkynes. *Tetrahedron Lett.* **2005,** *46* (34), 5613-5615.

144. Yavari, I.; Riazi-Kermani, F., Zinc-promoted Barbier-type reaction of propargyl bromide with aldehydes in aqueous media. *Synth. Commun.* **1995,** *25* (19), 2923 - 2928.

145. Loren, J. C.; Krasiński, A.; Fokin, V. V.; Sharpless, K. B., NH-1,2,3-Triazoles from azidomethyl pivalate and carbamates: Base-labile *N*-protecting groups. *Synlett* **2005,** *2005* (18), 2847,2850.

146. Pothukanuri, S.; Winssinger, N., A highly efficient azide-based protecting group for amines and alcohols. *Org. Lett.* **2007,** *9* (11), 2223-2225.

147. Choi, D.; Harold, K., Expedient syntheses of racemic 2,3-diaminopropanoic acid derivatives. *Tetrahedron Lett.* **1995,** *36* (41), 7371-7374.

148. Ramesh, R.; De, K.; Chandrasekaran, S., An efficient synthesis of dehydroamino acids and dehydropeptides from *O*-Cbz and *O*-Eoc derivatives of serine and threonine. *Tetrahedron* **2007,** *63* (42), 10534-10542.

149. Cernak, T. A.; Gleason, J. L., Synthesis of 5-chloromethylene hydantoins and thiohydantoins. *Heterocycles* **2007,** *71* (1), 117-134.

150. Chiu, F. C. K.; Brownlee, R. T. C.; Mitchell, K.; Phillips, D. R., 9-Aminoacridine-EDTA conjugates as hydroxy radical footprinting reagents with no intrinsic cutting specificity. *Bioorg. Med. Chem. Lett.* **1995,** *5* (15), 1689-1694.

151. Takashima, H.; Hirai, C.; Tsukahara, K., Selective and monofunctional guanosine 5′-monophosphate binding by chloro[3-(2,3-diaminopropionylamino)propionic acid](dimethyl sulfoxide)platinum(II) complex. *Bull. Chem. Soc. Jpn.* **2005,** *78*, 1629-1634.

152. Efange, S. M. N.; Kung, H. F.; Billings, J. J.; Blau, M., Synthesis and biodistribution of 99mTc-labeled piperidinyl bis(aminoethanethiol) complexes: potential brain perfusion imaging agents for single photon emission computed tomography. *J. Med. Chem.* **1988,** *31* (5), 1043-1047.

153. Webber, S. E.; Okano, K.; Little, T. L.; Reich, S. H.; Xin, Y.; Fuhrman, S. A.; Matthews, D. A.; Love, R. A.; Hendrickson, T. F.; Patick, A. K.; Meador, J. W.; Ferre, R. A.; Brown, E. L.; Ford, C. E.; Binford, S. L.; Worland, S. T., Tripeptide aldehyde inhibitors of human rhinovirus 3C protease: Design, synthesis, biological evaluation, and cocrystal structure solution of P1 glutamine isosteric replacements. *J. Med. Chem.* **1998,** *41* (15), 2786-2805.

154. Safavy, A.; Smith, D. C.; Bazooband, A.; Buchsbaum, D. J., De novo synthesis of a new diethylenetriaminepentaacetic acid (DTPA) bifunctional chelating agent. *Bioconjug. Chem.* **2002,** *13* (2), 317-326.

155. Nanchen, S.; Pfaltz, A., Chiral phosphino- and (phosphinooxy)-substituted *N*-heterocyclic carbene ligands and their application in iridium-catalyzed asymmetric hydrogenation. *Helv. Chim. Acta* **2006,** *89* (8), 1559-1573.

156. Qi, X.; Wang, X.; Wang, L.; Wang, Q.; Cheng, S.; Suo, J.; Chang, J., A solid-phase approach to DDB derivatives. *Eur. J. Med. Chem.* **2005,** *40* (8), 805-810.

157. Ratemi, E. S.; Vederas, J. C., Reaction of trimethylsilylamines with *N*-Cbz-L-serine-β-lactone: A convenient route to optically pure β-amino-L-alanine derivatives. *Tetrahedron Lett.* **1994,** *35* (41), 7605-7608.

158. Arnold, L. D.; Kalantar, T. H.; Vederas, J. C., Conversion of serine to stereochemically pure β-substituted α-amino acids via β-lactones. *J. Am. Chem. Soc.* **1985,** *107* (24), 7105-7109.

159. Moura, S.; Pinto, E., One-pot synthesis of *N*-Cbz-L-BMAA and derivatives from *N*-Cbz-L-serine. *Tetrahedron Lett.* **2007,** *48* (13), 2325-2327.

160. Wang, Y.; Tennyson, R. L.; Romo, D., β-Lactones: Intermediates for natural product total synthesis and new transformations. *Heterocycles* **2004,** *64*, 605-658.

161. Hall, H. K., Correlation of the base strengths of amines. *J. Am. Chem. Soc.* **1957,** *79* (20), 5441-5444.

162. Lall, M. S.; Ramtohul, Y. K.; James, M. N. G.; Vederas, J. C., Serine and threonine β-lactones: A new class of hepatitis A virus 3C cysteine proteinase inhibitors. *J. Org. Chem.* **2002,** *67* (5), 1536-1547.

163. Kretsinger, J. K.; Schneider, J. P., Design and application of basic amino acids displaying enhanced hydrophobicity. *J. Am. Chem. Soc.* **2003,** *125* (26), 7907-7913.

164. Kim, J.; Bott, S. G.; Hoffman, D. M., Synthesis of indium amide compounds. *Inorg. Chem.* **1998,** *37* (15), 3835-3841.

165. Colombo, R., Cleavage of peptide to sparrow-resin bond by catalytic transfer hydrogenolysis with 1,4-cyclohexadiene. *Chem. Lett.* **1980,** *9*, 1119-1122.

166. Macdonald, S. J. F.; Clarke, G. D. E.; Dowle, M. D.; Harrison, L. A.; Hodgson, S. T.; Inglis, G. G. A.; Johnson, M. R.; Shah, P.; Upton, R. J.; Walls, S. B., A flexible, practical, and stereoselective synthesis of enantiomerically pure trans-5-oxohexahydropyrrolo[3,2-

β]pyrroles (pyrrolidine-trans-lactams), a new class of serine protease inhibitors, using acyliminium methodology. *J. Org. Chem.* **1999,** *64* (14), 5166-5175.

167. Maity, P.; Zabel, M.; König, B., Tetrahydrofuran Cα-tetrasubstituted amino acids: Two consecutive β-turns in a crystalline linear tripeptide. *J. Org. Chem.* **2007,** *72* (21), 8046-8053.

168. Ozinskas, A. J.; Rosenthal, G. A., Synthesis of L-canaline and γ-functional 2-aminobutyric acid derivatives. *J. Org. Chem.* **1986,** *51* (26), 5047-5050.

169. Lall, M. S.; Karvellas, C.; Vederas, J. C., β-Lactones as a new class of cysteine proteinase inhibitors: Inhibition of hepatitis A virus 3C proteinase by *N*-Cbz-serine β-lactone. *Org. Lett.* **1999,** *1* (5), 803-806.

170. Sheradsky, T.; Knobler, Y.; Frankel, M. A. X., Synthesis of selectively protected homoserine and α,γ-diaminobutyric acid derivatives. *J. Org. Chem.* **1961,** *26* (5), 1482-1487.

171. Son, J.-K.; Kalvin, D.; Woodard, R. W., Assignment of the chemical shift values of *N*-trityl -homoserine lactone. *Tetrahedron Lett.* **1988,** *29* (33), 4045-4048.

172. Lee, K. M.; Ramalingam, K.; Son, J. K.; Woodard, R. W., A highly efficient and large-scale synthesis of (2S,3S)-[2,3-2H2]- and (2S,3R)-[3-2H]aspartic acids via an immobilized aspartase-containing microbial cell system. *J. Org. Chem.* **1989,** *54* (13), 3195-3198.

173. Janusz, J. M.; Gardlik, J. M.; Young, P. A.; Burkes, R. V.; Stoll, S. J.; Estelle, A. F.; Riley, C. M., High potency dipeptide sweeteners. 1. L-Aspartyl-D-phenylglycine esters. *J. Med. Chem.* **1990,** *33* (3), 1052-1061.

174. Kwon, C. H.; Iqbal, M. T.; Wurpel, J. N. D., Synthesis and anticonvulsant activity of 2-iminohydantoins. *J. Med. Chem.* **1991,** *34* (6), 1845-1849.

175. Zemlicka, J.; Bhuta, A.; Bhuta, P., Analogs of 2'(3')-*O*-L-phenylalanyladenosine as substrates and inhibitors of ribosomal peptidyltransferase. *J. Med. Chem.* **1983,** *26* (2), 167-174.

176. Kovacevic, M.; Herak, J.; Gaspert, B., Activation of the carboxylic acids by anhydride formation with *N*-acyl-*N*-alkyl carbamic acid. *Croat. Chem. Acta* **1983,** *56*, 141-155.

177. Parker, D.; Taylor, R. J., Direct ^{1}H NMR assay of the enantiomeric composition of amines and β-amino alcohols using *O*-acetyl mandelic acid as a chiral solvating agent. *Tetrahedron* **1987,** *43* (22), 5451-5456.

178. Klitgaard, H.; Matagne, A.; Gobert, J.; Wülfert, E., Evidence for a unique profile of levetiracetam in rodent models of seizures and epilepsy. *Eur. J. Pharmacol.* **1998,** *353* (2-3), 191-206.

179. Kaminski, R. M.; Livingood, M. R.; Rogawski, M. A., Allopregnanolone analogs that positively modulate $GABA_A$ receptors protect against partial seizures induced by 6-Hz electrical stimulation in mice. *Epilepsia* **2004,** *45* (7), 864-867.

180. Tjølsen, A.; Berge, O.-G.; Hunskaar, S.; Rosland, J. H.; Hole, K., The formalin test: An evaluation of the method. *Pain* **1992,** *51* (1), 5-17.

181. Vissers, K. C. P.; Geenen, F.; Biermans, R.; Meert, T. F., Pharmacological correlation between the formalin test and the neuropathic pain behavior in different species with chronic constriction injury. *Pharmacol. Biochem. Behav.* **2006,** *84* (3), 479-486.

182. Lehmann, P. A., Quantifying stereoselectivity or how to choose a pair of shoes when you have two left feet. *Trends Pharmacol. Sci.* **1982,** *3*, 103-106.

183. Barton, M. E.; Klein, B. D.; Wolf, H. H.; Steve White, H., Pharmacological characterization of the 6 Hz psychomotor seizure model of partial epilepsy. *Epilepsy Res.* **2001,** *47* (3), 217-227.

184. Moore, J. A.; Dice, J. R.; Nicolaides, E. D.; Westland, R. D.; Wittle, E. L., Azaserine, synthetic studies. I. *J. Am. Chem. Soc.* **1954,** *76* (11), 2884-2887.

185. Skinner, C. G.; McCord, T. J.; Ravel, J. M.; Shive, W., *O*-Carbamyl-L-serine, an inhibitory analog of L-glutamine. *J. Am. Chem. Soc.* **1956,** *78* (11), 2412-2414.

186. Morie, T.; Kato, S.; Harada, H.; Hufiwara, I.; Watanabe, K.; Matsumoto, J.-i., Ring expansion of nitrogen-containing chloromethylheteroalicycles via aziridium intermediates. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2565-2569.

187. Galonić, D. P.; van der Donk, W. A.; Gin, D. Y., Site-selective conjugation of thiols with aziridine-2-carboxylic acid-containing peptides. *J. Am. Chem. Soc.* **2004,** *126* (40), 12712-12713.

188. Scheurer, A.; Mosset, P.; Bauer, W.; Saalfrank, R., A practical route to regiospecifically substituted (*R*)- and (*S*)-oxazolylphenols. *Eur. J. Org. Chem.* **2001,** *2001* (16), 3067-3074.

189. Sakai, N.; Ohfune, Y., Total synthesis of galantin I. Acid-catalyzed cyclization of galantinic acid. *J. Am. Chem. Soc.* **1992,** *114* (3), 998-1010.

190. Kovacevic, M.; Herak, J. J.; Gaspert, B., Activation of the carboxylic acids by anhydride formation with *N*-acyl-*N*-alkyl carbamic acid. *Croat. Chem. Acta* **1983,** *56* (1), 141-155.

191. Ley, S.; Priour, A., Total synthesis of the cyclic peptide Argyrin B. *Eur. J. Org. Chem.* **2002,** *2002* (23), 3995-4004.

192. Oka, T.; Yasusa, T.; Ando, T.; Watanabe, M.; Yoneda, F.; Ishida, T.; Knoll, J., Enantioselective synthesis and absolute configuration of (-)-1-(benzofuran-2-yl)-2-propylaminopentane, ((-)-BPAP), a highly potent and selective catecholaminergic activity enhancer. *Bioorg. Med. Chem.* **2001,** *9* (5), 1213-1219.

193. Izumiya, N.; Yamamoto, T., Action of chymotrypsin. *J. Biochem. (Tokyo)* **1959,** *46*, 19.

194. *Commercially available: Sigma-Aldrich*.

195. Chen, J.; Corbin, S. P.; Holman, N. J., An improved large scale synthesis of the Schöllkopf chiral auxiliaries: (2R)- and (2S)-2,5-Dihydro-3,6-dimethoxy-2-isopropylpyrazine. *Org. Process Res. Dev.* **2005,** *9* (2), 185-187.

196. Ramalingam, B.; Neuburger, M.; Pfaltz, A., Synthesis of chiral C_2-symmetric methylene- and boron-bridged bis(imidazolines). *Synthesis* **2007,** *4*, 572-582.

197. Yu, Y.; Shao, X.; Cui, Y.; Liu, H. m.; Wang, C. l.; Fan, Y. z.; Liu, J.; Dong, S. l.; Cui, Y. x.; Wang, R., Structure–activity study on the spatial arrangement of the third aromatic ring of endomorphins 1 and 2 using an atypical constrained C-terminus. *ChemMedChem* **2007,** *2* (3), 309-317.

198. Vachal, P.; Jacobsen, E. N., Structure-based analysis and optimization of a highly enantioselective catalyst for the Strecker reaction. *J. Am. Chem. Soc.* **2002,** *124* (34), 10012-10014.

199. Wollweber, D.; Seitz, T.; Brandes, W. Fungicidal substituted amino acid amides. 5210084, 1993. DE.

200. Agosta, E.; Caligiuri, A.; D'Arrigo, P.; Servi, S.; Tessaro, D.; Canevotti, R., Enzymatic approach to both enantiomers of *N*-Boc hydrophobic amino acids. *Tetrahedron: Asymmetr* **2006,** *17* (13), 1995-1999.

201. Jenssen, K.; Sewald, K.; Sewald, N., Synthesis of marimastat and a marimastat conjugate for affinity chromatography and surface plasmon resonance studies. *Bioconjug. Chem.* **2004,** *15* (3), 594-600.

202. Vachal, P.; Jacobsen, E. N., Enantioselective catalytic addition of HCN to ketoimines. Catalytic synthesis of quaternary amino acids. *Org. Lett.* **2000,** *2* (6), 867-870.

203. Ray, S.; Drew, M. G. B.; Das, A. K.; Banerjee, A., The role of terminal tyrosine residues in the formation of tripeptide nanotubes: a crystallographic insight. *Tetrahedron* **2006,** *62* (31), 7274-7283.

204. Harding, C. I.; Dixon, D. J.; Ley, S. V., The preparation and alkylation of a butanedione-derived chiral glycine equivalent and its use for the synthesis of α-amino acids and α,α-disubstituted amino acids. *Tetrahedron* **2004,** *60* (35), 7679-7692.

205. Ramage, R.; Hopton, D.; Parrott, M. J.; Richardson, R. S.; Kenner, G. W.; Moore, G. A., Application of diphenylphosphinic carboxylic mixed anhydrides to peptide synthesis. *J. Chem. Soc., Perkin Trans. 1* **1985**, 461-470.

206. Institute of Laboratory Animal, R., *Guide for the care and use of laboratory animals*. National Academy Press: Washington, D.C., 1996.

207. Finney, D. J., *Probit analysis*. 3 ed.; Cambridge University Press: London, 1971.

208. Morphy, R.; Rankovic, Z., Designed multiple ligands. An emerging drug discovery paradigm. *J. Med. Chem.* **2005,** *48* (21), 6523-6543.

209. Pevarello, P.; Bonsignori, A.; Dostert, P.; Heidempergher, F.; Pinciroli, V.; Colombo, M.; McArthur, R. A.; Salvati, P.; Post, C.; Fariello, R. G.; Varasi, M., Synthesis and anticonvulsant activity of a new class of 2-[(arylalkyl)amino]alkanamide derivatives. *J. Med. Chem.* **1998,** *41* (4), 579-590.

210. Salvati, P.; Maj, R.; Caccia, C.; Cervini, M. A.; Fornaretto, M. G.; Lamberti, E.; Pevarello, P.; Skeen, G. A.; White, H. S.; Wolf, H. H.; Faravelli, L.; Mazzanti, M.; Mancinelli, E.; Varasi, M.; Fariello, R. G., Biochemical and electrophysiological studies on the mechanism of action of PNU-151774E, a novel antiepileptic compound. *J. Pharmacol. Exp. Ther.* **1999,** *288* (3), 1151-1159.

211. Chazot, P., Safinamide for the treatment of Parkinson's disease, epilepsy and restless legs syndrome. *Curr. Opin. Investig. Drugs* **2007,** *8* (7), 570 - 579.

212. Fariello, R. G., Safinamide. *Neurotherapeutics* **2007,** *4* (1), 110-116.

213. Leonetti, F.; Capaldi, C.; Pisani, L.; Nicolotti, O.; Muncipinto, G.; Stefanachi, A.; Cellamare, S.; Caccia, C.; Carotti, A., Solid-phase synthesis and insights into structure−activity relationships of safinamide analogues as potent and selective inhibitors of type B monoamine oxidase. *J. Med. Chem.* **2007,** *50* (20), 4909-4916.

214. Barrios, I. A.; Bruno-Blanch, L. E.; Estiú, G. L., Pharmacophoric pattern of anti-epileptic sulfonamides and sulfamates. Theoretical study of the associated requirements. *J. Mol. Struct.* **2002,** *580* (1-3), 243-250.

215. Parker, M. H.; Smith-Swintosky, V. L.; McComsey, D. F.; Huang, Y.; Brenneman, D.; Klein, B.; Malatynska, E.; White, H. S.; Milewski, M. E.; Herb, M.; Finley, M. F. A.; Liu, Y.; Lubin, M. L.; Qin, N.; Iannucci, R.; Leclercq, L.; Cuyckens, F.; Reitz, A. B.; Maryanoff, B. E., Novel, broad-spectrum anticonvulsants containing a sulfamide group: Advancement of *N*-((benzo[b]thien-3-yl)methyl)sulfamide (JNJ-26990990) into human clinical studies. *J. Med. Chem.* **2009,** *52* (23), 7528-7536.

216. Paruszewski, R.; Rostafinska-Suchar, G.; Strupinska, M.; Jaworski, P.; Stables, J. P., Synthesis and anticonvulsant activity of some amino acid derivatives. Part 1: Alanine derivatives. *Pharmazie* **1996,** *51*, 145-148.

217. Paruszewski, R.; Rostafinska-Suchar, G.; Strupinska, M.; Jaworski, P.; Winiecka, I.; Stables, J. P., Synthesis and anticonvulsant activity of some amino acid derivatives. Part 2: Derivatives of Gly, Ala, Leu, Pro, Trp, Phe(4 Cl), and Ala(α-Me). *Pharmazie* **1996,** *51*, 212-215.

218. Paruszewski, R.; Rostafinska-Suchar, G.; Strupinska, M.; Winiecka, I.; Stables, J. P., Synthesis and anticonvulsant activity of some amino acid derivatives. Part 3: Derivatives of Ala, Arg, Tzl, Gly, and χAbu. *Pharmazie* **2000,** *55*, 27-30.

219. Bowman, R. E.; Stroud, H. H., *N*-substituted amino-acids. Part I. A new method of preparation of dimethylamino-acids. *J. Chem. Soc.* **1950**, 1342-1345.

220. - , E.; Gerwick, W. H.; McPhail, K. L., Coibamide A, a potent antiproliferative cyclic depsipeptide from the Panamanian marine cyanobacterium *Leptolyngbya sp*. *J. Am. Chem. Soc.* **2008,** *130* (20), 6324-6325.

221. Lee, J.; Lee, J.; Kang, M.; Shin, M.; Kim, J.-M.; Kang, S.-U.; Lim, J.-O.; Choi, H.-K.; Suh, Y.-G.; Park, H.-G.; Oh, U.; Kim, H.-D.; Park, Y.-H.; Ha, H.-J.; Kim, Y.-H.; Toth, A.; Wang, Y.; Tran, R.; Pearce, L. V.; Lundberg, D. J.; Blumberg, P. M., *N*-(3-Acyloxy-2-benzylpropyl)-*N*'-[4-(methylsulfonylamino)benzyl]thiourea analogues:Novel potent and high affinity antagonists and partial antagonists of the vanilloid receptor. *J. Med. Chem.* **2003,** *46* (14), 3116-3126.

222. Pettit, G. R.; Singh, S. B. Synthesis of Dolastatin 10. 4,978,744, 1990. US.

223. Barton, D. H. R.; Fontana, G.; Yang, Y., The invention of radical reactions. Part XXXV. A novel radical fission reaction of *N*-sulfonylthioxocarbamates. *Tetrahedron* **1996,** *52* (8), 2705-2716.

224. Jiang, B.; Li, Z.-G.; Dai, J.-Y.; Zhang, D.-J.; Xiu, Z.-L., Aqueous two-phase extraction of 2,3-butanediol from fermentation broths using an ethanol/phosphate system. *Process Biochem.* **2009,** *44* (1), 112-117.

225. De Luca, L.; Giacomelli, G.; Taddei, M., An easy and convenient synthesis of Weinreb amides and hydroxamates. *J. Org. Chem.* **2001,** *66* (7), 2534-2537.

226. Katritzky, A. R.; Yang, H.; Zhang, S.; Want, M., An efficient conversion of carboxylic acids into Weinreb amides. *ARKIVOC* **2002,** *11*, 39-44.

227. Liu, J.; Ikemoto, N.; Petrillo, D.; Armstrong Iii, J. D., Improved syntheses of α-BOC-aminoketones from α-BOC-amino-Weinreb amides using a pre-deprotonation protocol. *Tetrahedron Lett.* **2002,** *43* (46), 8223-8226.

228. Kim, S.; Lee, J. I.; Kim, Y. C., A simple and mild esterification method for carboxylic acids using mixed carboxylic-carbonic anhydrides. *J. Org. Chem.* **1985,** *50* (5), 560-565.

229. Ishihara, K.; Ohara, S.; Yamamoto, H., 3,4,5-Trifluorobenzeneboronic acid as an extremely active amidation catalyst. *J. Org. Chem.* **1996,** *61* (13), 4196-4197.

230. Santangelo, E. M.; Zarbin, P. H. G.; Cass, Q. B.; Ferreira, J. T. B.; Corrêa, A. G., Synthesis of the four possible stereoisomers of *N*-2′-methylbutyl-2-methylbutylamide, the sex pheromone of the longhorn beetle *Migdolus fryanus* westwood. *Synth. Commun.* **2001,** *31* (23), 3685-3698.

231. Burns, D. H.; Miller, J. D.; Chan, H.-K.; Delaney, M. O., Scope and utility of a new soluble copper catalyst [CuBr−LiSPh−LiBr−THF]: A comparison with other copper catalysts in their ability to couple one equivalent of a Grignard reagent with an alkyl sulfonate. *J. Am. Chem. Soc.* **1997,** *119* (9), 2125-2133.

232. Leroux, F. R.; Manteau, B.; Vors, J.-P.; Pazenok, S., Trifluoromethyl ethers – synthesis and properties of an unusual substituent. *Beilstein J. Org. Chem.* **2008,** *4*, 13.

233. Hansch, C., *Substituent constants for correlation analysis in chemistry and biology*. Wiley: New York, 1979.

234. Napolitano, E. P., IT), Fiaschi, Rita (Pisa, IT), Bechini, Chiara (Pisa, IT), Brunetto, Gabriella (Livorno, IT) Process for resolving racemix mixtures and diasterisomeric complex of a resolving agent and an enantiomer of interest. 2009.

235. Ishihara, K.; Nakano, K., Design of an organocatalyst for the enantioselective diels−alder reaction with α-acyloxyacroleins. *J. Am. Chem. Soc.* **2005,** *127* (30), 10504-10505.

236. Katrizky, A. R.; Yang, H.; Zhang, S.; Want, M., An efficient conversion of carboxylic acids into Weinreb amides. *ARKIVOC* **2002,** *11*, 39-44.

237. McDonald, A.; Morgans, D.; Bergnes, G.; Dhanak, D.; Knight, S. Compounds, composition and methods. PCT/US/2003/022466, 2004.

238. Bergnes, G.; Dhanak, D. Compounds, compositions and methods. PCT/US2004/032569, 2005.

239. Shintou, T.; Kikuchi, W.; Mukaiyama, T., Efficient method for the preparation of carboxylic acid alkyl esters or alkyl phenyl ethers by a new-type of oxidation–reduction condensation using 2,6-dimethyl-1,4-benzoquinone and alkoxydiphenylphosphines. *Bull. Chem. Soc. Jpn.* **2003,** *76* (8), 1645-1667.

240. Pettit, G. R.; Knight, J. C.; Herald, D. L.; Pettit, R. K.; Hogan, F.; Mukku, V. J. R. V.; Hamblin, J. S.; Dodson, M. J.; Chapuis, J.-C., Antineoplastic agents. 570. Isolation and structure elucidation of bacillistatins 1 and 2 from a marine *Bacillus silvestris*. *J. Nat. Prod.* **2009,** *72* (3), 366-371.

241. Pirkle, W. H.; McCune, J. E., Separation of the enantiomers of *N*-protected α-amino acids as anilide and 3,5-dimethylanilide derivatives. *J. Chromatogr. A* **1989,** *479*, 419-423.

242. Li, X.; Yudin, A. K., Epimerization- and protecting-group-free synthesis of peptidomimetic conjugates from amphoteric amino aldehydes. *J. Am. Chem. Soc.* **2007,** *129* (46), 14152-14153.

243. Buehler, C. A.; Mackenzie, C. A., The action of benzylamine on aliphatic esters. *J. Am. Chem. Soc.* **1937,** *59* (2), 421-422.

244. Storer, R. I.; Takemoto, T.; Jackson, P. S.; Brown, D. S.; Baxendale, I. R.; Ley, S. V., Multi-step application of immobilized reagents and scavengers: A total synthesis of Epothilone C. *Chem.--Eur. J.* **2004,** *10* (10), 2529-2547.

245. Satoh, T.; Motohashi, S.; Kimura, S.; Tokutake, N.; Yamakawa, K., The asymmetric favorskii rearrangement: A synthesis of optically active α-alkyl amides from aldehydes and (-)-1-chloroalkyl p-tolyl sulfoxide. *Tetrahedron Lett.* **1993,** *34* (30), 4823-4826.

246. Dermer, O. C.; King, J., N-Benzylamides as derivatices for identifying the acyl group in esters 1,2. *J. Org. Chem.* **1943,** *08* (2), 168-173.

247. Doty, P.; Rudd, G. D.; Stoehr, T.; Thomas, D., Lacosamide. *Neurotherapeutics* **2007,** *4* (1), 145-148.

248. EMEA, Assessment report for vimpat. London, 2008.

249. Silverman, R. B., *The organic chemistry of drug design and drug action*. Elsevier Academic Press: Boston, 2004.

250. Errington, A. C.; Coyne, L.; Stöhr, T.; Selve, N.; Lees, G., Seeking a mechanism of action for the novel anticonvulsant lacosamide. *Neuropharmacology* **2006,** *50* (8), 1016-1029.

251. Tang, L.; Huger, F. P.; Klein, J. T.; Davis, L.; Martin, L. L.; Shimshock, S.; Effland, R. C.; Smith, C. P.; Kongsamut, S., 4-Aminopyridine derivatives: A family of novel modulators of voltage-dependent sodium channels. *Drug Dev. Res.* **1998,** *44* (1), 8-13.

252. Zilles, K.; Qü, M.; Schleicher, A.; Luhmann, H. J., Characterization of neuronal migration disorders in neocortical structures: quantitative receptor autoradiography of ionotropic glutamate, $GABA_A$ and $GABA_B$ receptors. *Eur. J. Neurosci.* **1998,** *10* (10), 3095-3106.

253. Shin, C.; Pedersen, H.; McNamara, J., γ-Aminobutyric acid and benzodiazepine receptors in the kindling model of epilepsy: A quantitative radiohistochemical study. *J. Neurosci.* **1985,** *5* (10), 2696-2701.

254. Langmead, C. J.; Watson, J.; Reavill, C., Muscarinic acetylcholine receptors as CNS drug targets. *Pharmacol. Ther.* **2008,** *117* (2), 232-243.

255. Ciumas, C.; Wahlin, T.-B. R.; Jucaite, A.; Lindstrom, P.; Halldin, C.; Savic, I., Reduced dopamine transporter binding in patients with juvenile myoclonic epilepsy. *Neurology* **2008,** *71* (11), 788-794.

256. Madras, B. K.; Fahey, M. A.; Goulet, M.; Lin, Z.; Bendor, J.; Goodrich, C.; Meltzer, P. C.; Elmaleh, D. R.; Livni, E.; Bonab, A. A.; Fischman, A. J., Dopamine transporter (DAT) inhibitors alleviate specific parkinsonian deficits in monkeys: Association with DAT occupancy *in vivo*. *J. Pharmacol. Exp. Ther.* **2006,** *319* (2), 570-585.

257. Bonaventure, P.; Kelly, L.; Aluisio, L.; Shelton, J.; Lord, B.; Galici, R.; Miller, K.; Atack, J.; Lovenberg, T. W.; Dugovic, C., Selective blockade of 5-hydroxytryptamine (5-HT)7 receptors enhances 5-HT transmission, antidepressant-like behavior, and rapid eye movement sleep suppression induced by citalopram in rodents. *J. Pharmacol. Exp. Ther.* **2007,** *321* (2), 690-698.

258. Elliott, J., α-Adrenoceptors in equine digital veins: Evidence for the presence of both alpha1 and alpha2-receptors mediating vasoconstriction. *J. Vet. Pharmacol. Ther.* **1997,** *20* (4), 308-317.

259. Stark, H., Histamine receptors. *BIOTREND Rev.* **2007,** *1*.

260. Guitart, X.; Codony, X.; Monroy, X., Sigma receptors: biology and therapeutic potential. *Psychopharmacology* **2004,** *174* (3), 301-319.

261. Corbett, A. D.; Henderson, G.; McKnight, A. T.; Paterson, S. J., 75 years of opioid research: the exciting but vain quest for the Holy Grail. *Br. J. Pharmacol.* **2006,** *147* (S1), S153-S162.

262. Sanguinetti, M. C.; Tristani-Firouzi, M., hERG potassium channels and cardiac arrhythmia. *Nature* **2006,** *440* (7083), 463-469.

263. Akada, Y.; Ogawa, S.; Amano, K.-i.; Fukudome, Y.; Yamasaki, F.; Itoh, M.; Yamamoto, I., Potent analgesic effects of a putative sodium channel blocker M58373 on formalin-induced and neuropathic pain in rats. *Eur. J. Pharmacol.* **2006,** *536* (3), 248-255.

264. Odano, I.; Varrone, A.; Ciumas, C.; Jucaite, A.; Halldin, C.; Farde, L.; Savic, I., Dopamine transporter binding in patients with juvenile myoclonic epilepsy using [11C]PE2I and PET. *J. Nucl. Med. Allied Sci.* **2010,** *51*, 493.

265. Youdim, M. B. H.; Edmondson, D.; Tipton, K. F., The therapeutic potential of monoamine oxidase inhibitors. *Nat. Rev. Neurosci.* **2006,** *7* (4), 295-309.

266. Cheng, Y.-C.; Prusoff, W. H., Relationship between the inhibition constant (K_I) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973,** *22* (23), 3099-3108.

267. Uebelhack, R.; Franke, L.; Schewe, H. J., Inhibition of platelet MAO_B by kava pyrone-enriched extract from Piper Methysticum Forster (kava-kava). *Pharmacopsychiatry* **1998,** *31* (05), 187,192.

268. Greenwald, R. B.; Pendri, A.; Conover, C. D.; Zhao, H.; Choe, Y. H.; Martinez, A.; Shum, K.; Guan, S., Drug delivery systems employing 1,4- or 1,6-elimination: Poly(ethylene glycol) prodrugs of amine-containing compounds. *J. Med. Chem.* **1999,** *42* (18), 3657-3667.

269. D'Souza, A. J. M.; Topp, E. M., Release from polymeric prodrugs: Linkages and their degradation. *J. Pharm. Sci.* **2004,** *93* (8), 1962-1979.

270. Miron, T.; Wilchek, M., A simplified method for the preparation of succinimidyl carbonate polyethylene glycol for coupling to proteins. *Bioconjug. Chem.* **1993,** *4* (6), 568-569.