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Synthesis and Biological Evaluation of Rigid Analogues of Methamphetamines

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Synthesis and Biological Evaluation of Rigid Analogues of Methamphetamines

A Dissertation

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To Ronald L. Norris III and Satchiko, for being the best two things that have ever happened to me. I am extremely thankful for Ron's constant love and support as well as his atomic model-painting skills. Satchiko and her love of strange cheeses and exotic head rubs have brightened many of my days.

To my family for their love and support.

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ABSTRACT

A series of rigid azetidenyl-based methamphetamine analogs were synthesized from commercially available *N*-Boc-azetidinone. The benzylideneazetidine analogs were prepared via a Wittig olefination via the ylides generated from the corresponding triphenylphosphonium benzylhalide salts. The substituted benzylazetidine analogs were synthesized from the corresponding benzylideneazetidienes via hydrogention over palladium and platinum catalysts. The benzylideneazetidine and benzyliazetidine analogs were evaluated at monoamine transporters as a part of preliminary structure-activity study for the development of novel monoamine transporter ligands. The binding affinities of the azetidine analogs were determined at dopamine (DAT) and serotonin (SERT) transporters in rat brain tissue preparations. The preliminary in vitro binding studies revealed that the rigid scaffold of the azetidine ring system was an effective substitution for the 2-aminopropyl group of methamphetamine and led to compounds with nanomolar binding affinity at dopamine and serotonin. In general, the benzylideneazetidine analogs were more potent than the corresponding benzylazetidine analogs. In addition, the azetidine analogs were more selective for the serotonin transporter than the dopamine transporter. The 3-(3,4-dichlorobenzylidene)azetidine (**24m**) was the most potent analog of the series with K_i values of 139 nM for SERT and 531 nM for DAT (DAT/SERT = 3.8).

Key words: dopamine, serotonin, norepinephrine, methamphetamine, amphetamine, azetidine, psychostimulants

INTRODUCTION

Psychoactive substances are substances that when taken, alter perception, mood and/or consciousness. Humanity has a lengthy history of using psychoactive substances for reasons ranging from spiritual to recreational. In today's world, most of these substances are prohibited without proper medical oversight. Despite the legal prohibition, illicit drugs are a global phenomenon and addiction appears to be pandemic.

One group of particular psychoactive substances are amphetamines. The most recent SAMHSA reports show that while the use of amphetamine type stimulants (ATS) appears to be declining in the US, the UN has recently reported that globally, ATS use is increasing.[1,2] These stimulants are easily synthesized, cheap, readily available and highly addictive. Unlike some other psychostimulants, methamphetamine is known possess neuro-toxicity.[3] Problems stemming from amphetamine addiction range from poor individual health, criminal activity and environmental hazards stemming from meth labs. As of this writing, there are no good pharmacotherapies for those seeking treatment.

Addiction

Drug addiction is a cyclical condition typified by two major characteristics: the compulsion to take the drug and the user's loss of intake control.[4] The DSM-IV breaks the addiction cycle down further into three stages: preoccupation/anticipation, binge-intoxication and withdrawal-negative effect.[5] Between these stages the user may take

larger than intended amounts of the substance and develop tolerance which leads to further health complications.

Statistically, many more people try psychostimulants than actually become addicted to them. Research has shown that there are both genetic and environmental factors which influence our ability to become addicted and subsequently, our likelihood for relapse. Studies focusing on families and twins have shown genetic influence estimates ranging from 30-60%.[6] Differences in the dopaminergic network have also been linked to impulsivity and drug craving.[6,7] The underlying neurochemical bases for certain psychiatric conditions (e.g. schizophrenia, bipolar, depression, etc) is often temporarily ameliorated by the effects of psychostimulants, however the user also runs a significant risk of developing dependence due to habitual self-medication.

The chronic exposure to drugs of abuse induces molecular neuroadaptations that occur throughout the nervous system as well as in the dopamine receptors in the nucleus accumbens.[4] These gradual changes in the neural substrate have given rise to the theory of addiction as a disease of gradual neural changes that are driven by the body's need to maintain homeostasis. As exposure to the drug persists, other physiological systems are recruited along with the neuroadaptations causing the organism to set new 'normal' parameters termed allostasis.[4]

The prolonged exposure to psychoactive substances alters neuropathways and substrates to create a new allostatic physiological state which can have many adverse effects to mental and physical health. Since any given physiological parameter (e.g. blood pressure) can be controlled by numerous but mutually interacting signals; altering

any of these can lead to a multitude of changes in various feedback loops, further complicating the system's ability to regulate. Given that many psychostimulants act on one or more neuroreceptors, this increasingly complicates the homeostatic picture.

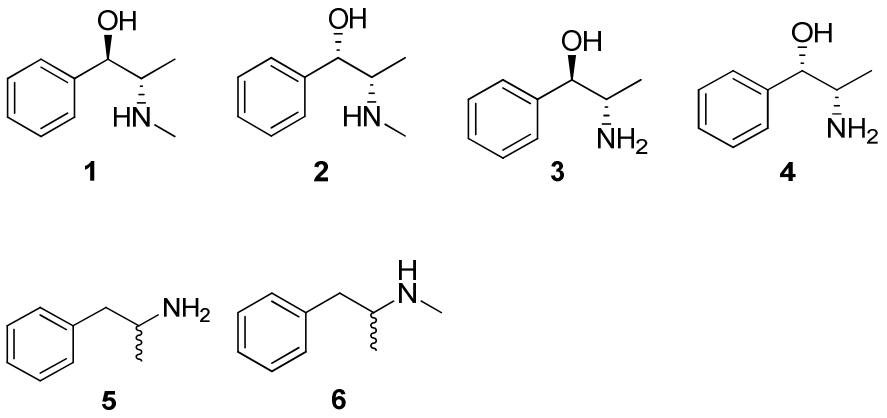
Since levels of neurotransmitters and other variables have been significantly altered, the system goes into what has been termed an allostatic state. While an individual is in this state, their ability to cope with further stress is impaired as the margins for coping have decreased due to their altered physiological state.[4] The constant stress of the system and its impaired ability to relax increase the odds of breakdown and illness. The term "allostatic load" is used to describe this physiological tab the body runs while performing under such stress. This running tab leads to the quickened deterioration of tissues of the brain, skin and/or vital organs. Other side effects are due to the impaired judgment of the user, such as needle-sharing, which can easily lead to the spread of disease. Users of psychoactive substances often show heightened emotional states and compulsive urges to use or get drugs, making them potential hazards to their communities.

Amphetamine History

The story of amphetamines begins with over 5,000 years ago in China, where herbalists and healers used Ephedra as a circulatory stimulant, diaphoretic, antipyretic and respiratory aid. Other species of *Ephedra* were utilized for medicinal value in Greece, Russia, India and the Americas.[10] Its value as an appetite suppressant, respiratory aid and (in the Americas) venereal disease treatment, made it an extremely popular medicine. Its dominance as a respiratory aid went virtually without competition for many years. Japanese chemists began to study the medicinal quality of the plant in

the 19th century. In 1885, Nagayoshi Nagai isolated the alkaloids responsible for activity, l-ephedrine (1, 40-90% of the total alkaloids), d-pseudoephedrine (**2**). Two other notable alkaloids were isolated, norephedrine (**3**) and norpseudoephedrine (**4**), which have limited therapeutic use. On the other side of the world in 1887, amphetamine (**5**) was synthesized in Germany. In 1893, Nagai went on to synthesize a derivative of ephedrine, methamphetamine (**6**).

Figure 1: Ephedrine alkaloids and derivatives



In 1887 a colleague of Nagai named Miura performed various animal studies on l-ephedrine to demonstrate any pharmaceutical qualities. His findings suggested that the drug was "too toxic" for use in the whole animal, but it could be safely used to safely (reversibly) dilate the pupils - a stark contrast from atropine (a drug that was commonly used at the time, whose effects may last upwards of a week).[11] The epinephrine-like qualities of the ephedra alkaloids were described by several other chemists, but it was not until the 1924 paper by Chen and Schmidt that Western science began to examine ephedra.

Their subsequent discoveries of ephedra's side effects - specifically the capillary-constriction effect offered a unique therapeutic for a common problem - nasal decongestion.[12] It was introduced into the market in 1926 by Lily and became very popular, especially among sufferers of asthma.[11,12] Since the active ingredients are readily available from nature, many sought to control the import of the plant. The import control coupled with the fact that extracts of ephedra contained a significant portion of d-pseudoephedrine and the 1937 invasion of China by the Japanese (which led to ephedra shortages) spurred a surge of research into alternatives.[11] It is this search for alternatives that ultimately led to the mass production and use of amphetamines.

During the 1920s, Gordon Alles was one of those researchers seeking to capitalize on ephedra's anti-allergy abilities. His research focused in on the sulfate salt of phenylisopropylamine (amphetamine) which he tested on animals and observed it had similar effects and was not "terribly toxic."[12] In 1929, he tested the compound on himself and observed the following: "a feeling of well-being, palpitation, and eventually a sleepless night ... in which his mind seemed to race from one subject to another."[12] However, it was amphetamine's usefulness as a bronchodilator and decongestant that eventually led to it overtaking ephedra and found Alles with a patent on amphetamine.[13]

Smith, Kline and French filed a separate patent on an amphetamine inhaler called "Benzedrine sulfate" in 1933 which was little more than amphetamine in a small tube.[12] A year later, Alles would transfer his patent to Smith, Kline and French who further developed the amphetamine salts as drugs. In 1937, Smith, Kline and French received the approval from the American Medical Association to advertize benzedrine sulfate for conditions ranging from narcolepsy, post-encephalitic Parkinsonism (a condition caused

virally that leads to the deterioration of the cells of the substantia nigra and Parkinson's-like symptoms) and minor depression.[13] The popularity of benzedrine (amphetamine) eventually led to the marketing and over use of specific stereoisomers and methamphetamine (methedrine).

Global Usage and Addiction of Amphetamines

The would-be "golden age" of American meth- amphetamine use could be thought of as a period from the late 1920s to around 1970.[12,13,14] Even before the onset of World War II, the drugs had gained widespread attraction for their ability to increase the activity and enhance the mood of the user as well as the drugs' ability to enhance weight loss.[13] In 1939 benzedrine sulfate (amphetamine sulfate) received FDA approval for the treatment of obesity.[13] Compared to other methods of treating depression (such as the popular frontal lobotomy), amphetamines allowed for a much less intrusive treatment of the condition.[13]

The ability of meth- amphetamine to keep people alert for days on end was exploited during World War II.[12-15] The Allied and Axis sides both rationed out benzedrine (amphetamine) to soldiers to help alleviate fatigue and improve mood and endurance.[14,15] After the war, a paper by Monroe and Drell suggested a link in military service and "agitated hallucinating patients" whom were addicted to "eating the contents of benzedrine inhalers" (~250 mg of amphetamine).[13,16] Both physicians were stationed at a military prison and had noted that only 11% of the addicted patients had used a form of amphetamine before their military career.[13,16] A significant amount (27%) had been exposed to amphetamine while in the military, usually by an officer in the form of a

pill compared with 5% of non-addicted inmates.[13,16] It is estimated that around 16 million Americans had been exposed to amphetamine (in the form of benzedrine sulfate) during their service in the military.[13] Since the benzedrine inhaler was readily available without a prescription, this had the effect of a massive "word of mouth" campaign amphetamine exposure and potential addiction.

By the end of the war in 1945, over 500,000 civilians in the US were using amphetamine for everything from weight loss to depression.[12-17] Stockpiles of amphetamine (and methamphetamine) became more readily available in the years following the war, and misuse was becoming more common.[12-17] As legal usage increased, so did the illicit trade and addiction of amphetamines, though it would take years before the addictive properties of the drugs were recognized. The black market of amphetamines provided a product for anyone looking to improve his performance either in studying or athletically, or looking to stay awake during long-hauls.

During the 1950s, the first references to intravenous amphetamine usage appear, but was not a widespread practice until the 1960s.[14] In the early '60s the government began to crackdown on pharmacies which sold injectable amphetamines. This crackdown led to illicit production going underground and giving rise to the now infamous home laboratories. By the 1970s, the usage of amphetamines declined considerably thanks to the DEA crackdowns and FDA scheduling of the drug.[14,18] In the 1990s, the DEA began a crackdown of cocaine which began a resurgence in amphetamines thanks to traffickers bringing the drugs from Mexico.

Outside of the US, amphetamine usage has been prevalent since the 1950s and 1960s.[2,12-15,17] Japan has had two amphetamine addiction epidemics. During World War II, the Japanese also rationed out methamphetamine to their troops as well as their factory workers to increase out-put. During and after the war, supplies of methamphetamine became freely available to the public.[2,17,19] At one point, 5% of the population of Japan was addicted to meth- amphetamines, around one-tenth of which were estimated to have experienced methamphetamine-induced psychotic symptoms.[19] This led to intervention by the government in 1951 when the stimulant control act was passed. A resurgence of amphetamine addiction occurred again in the 1980s and is ongoing. This second wave of amphetamines are either made in clandestine labs or are trafficked in from abroad. Given the problematic history of amphetamines in Japan, in 2008 a decision was made to ban *all* amphetamines - including pharmaceutical preparations (Adderall).[2,20] Travelers now bringing such medications into the country are arrested on the spot.[20]

In eastern Asia, methamphetamine abuse has taken over as the most commonly abused drug, surpassing heroin, cocaine, opium and cannabis. While many of these drugs are manufactured in places such as China, Myanmar or the Philippines, methamphetamine seized in places such as Japan and South Korea often can be traced to west Africa.[2] Western and southern Asia are experiencing a similar situation as seizures of amphetamines are increasing and countries which one imported the stimulants are now exporters (Iran).[2,22] This may change as countries around the world are drafting much harsher legislation and are receiving advanced training in the detection and prevention of drug production and distribution.[21,23]

Europe's usage levels of amphetamines appears to be steady.[2] Seizures in countries such as Germany are increasing, but the overall usage throughout the continent remains consistent. Unlike the global trend, amphetamine is much more popular than methamphetamine. Amphetamine is usually produced in Poland or the Netherlands, whereas the Czech Republic is the main manufacturer of methamphetamine.[2,23]

Detailed knowledge of the production and use of amphetamine-type stimulants in Africa is lacking.[2] There are three major known sites of production within the continent, Egypt, Nigeria and South Africa. Data about lifetime use is limited given the perennially stretched resources of most governments in the region. It is known that amphetamine production has occurred throughout Egypt since before 2000 but its role as a regional supplier is not well understood. A tanker of methamphetamine that had its origins in Egypt was recently seized in Saudi Arabia.[2] Recent reports from Nigeria present a potential epicenter both as a regional manufacturer and a global distributer. Methamphetamine from Nigeria has been seized by Japanese authorities in increasing amounts since 2009.[2] Similarly, South Africa has been implicated as a significant supplier of methamphetamine to Australia. While the country continues to strengthen legislation controlling the import of important precursors, increasing amounts of amphetamines are being seized.[23]

In central and South America, there appears to be an on-going exchange with Europe of cocaine for amphetamines. While few labs have been raided, production is known to be on-going in places such as Argentina and Brazil. Use of amphetamines is highest in countries such as El Salvador (3.3%), Belize (1.4%) and Costa Rica (1.3%).[2]

The current concern is now focused on the increase of production given that countries in North America have tightened controls on precursors.

As of this writing, amphetamine type stimulants are the second most commonly abused drug in the world (behind cannabis) - far outstripping heroin and cocaine.[2] Smuggling is a major issue in many countries, and given that the origins of many seized dosages are from unstable or authoritarian regimes, there are legitimate security questions (fighting wars, sponsoring terrorism, etc). The physiological and psychological toll amphetamines take on the user and his community, these drugs present a global sociological hazard. In 1971, the DEA scheduled methamphetamine and amphetamine as Schedule II substances.[24]

Amphetamine Mode of Action Overview

Amphetamines can be ingested orally, intravenously or by smoking. Users describe different experiences depending on how the drug is ingested; smoking and injection leads to an intense rush that lasts only a few minutes, while snorting and oral routes lead to a less intense high. In many countries, amphetamines have become the number one illicit injecting drug. The half-life of the drug depends on the method of ingestion, where oral administration has an elimination half-life of 10.1 hours and intravenous administration has a half-life of 12.2 hours.[19,25]

Peak plasma concentrations also vary by method of ingestion; injection leads very quickly to peak concentrations, smoking meth- amphetamines requires a few minutes and oral intake gives a peak plasma concentration after around 3 hours.[19,25] The high attributed to amphetamine usage can dissipate before the blood concentrations of the

drug fall substantially, leading many users to ingest very high dosages of the substance and further fueling tolerance.[26] Typical of stimulant users, amphetamine type stimulant users tend to follow a "binge and crash"-type pattern of use where users will be awake for days taking the drug before finally 'crashing' (often sleeping for several days).[26]

Once ingested, amphetamines readily cross the blood brain barrier. Once in the central nervous system, the drugs are extremely active with the monoaminergic (especially dopaminergic) transporters. Generally, the extracellular amphetamines bind to the uptake carriers where they are then transported into the cell. Once in the cytosol, amphetamines are able to enter the neurotransmitter storage vesicles by bonding to the vesicular monoamine transporter (VMAT-2) which begins to exchange the neurotransmitter (e.g. dopamine) for the amphetamine.[27] The loss of the reductive influence of the vesicles results in cytosolic oxidative stress which may also play a role in the neurotoxicity typical of methamphetamine.[17] In the case of dopamine, as the concentration of it increases in the cytosol, the dopamine transporter bonds to the cytosolic dopamine and transports it from the terminal into the synaptic space.

There has also been some consideration of a 'weak base model' which states that amphetamines can redistribute catecholamines from synaptic vesicles by collapsing the vesicle proton gradient.[17] The proton gradient usually provides the free energy required for catechol accumulation, with this gradient disestablished they simply enter the synapse. This theory also states that even non-stimulant weak bases should also work in this manner.[17]

There is a great deal of structural similarity between amphetamine (**5**) and the endogenous ligand β-phenylethylamine (**7**) (Figure 2). Both molecules act as releasers of various monoamines (Table 1). The fact that the monoaminergic neurotransmitters may serve as releasers may indicate a feedback mechanism as of yet not understood. Intoxication from the substance is a result of the 'overflow' of these monoamines. Symptoms due to amphetamine intoxication results in tachycardia, hypertension, pupillary dilation, profuse sweating, rapid breathing, peripheral hyperthermia, hyperpyrexia, light sensitivity, anxiety, headaches, paranoia, delusions, and hallucinations.[19,25] Users also experience dry mouth, bruxism (teeth grinding - a contributing factor to "meth mouth"), the sensation of "bugs" crawling on the skin (delusional parasitosis) and sores; which can be attributed to the overabundance of norepinephrine.[28,29] Long-term effects of meth- amphetamine usage also include changes in neuronal structures as well as transporter levels which have been shown to persist even after extended periods of abstinence.[30,31,32]

Figure 2: SAR Comparison of Amphetamine to β-Phenethylamine

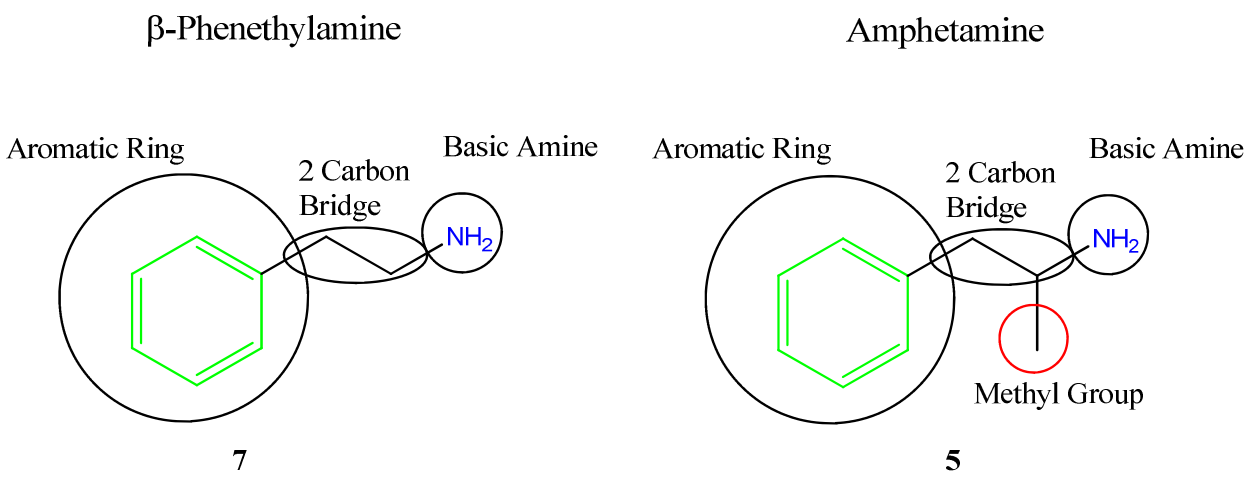
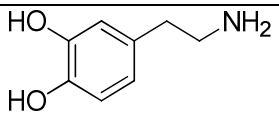
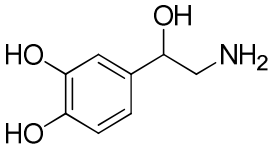
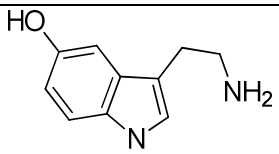
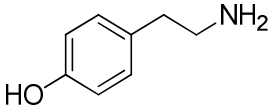
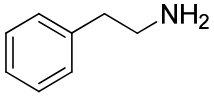
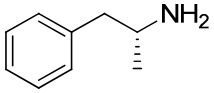
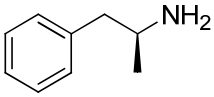
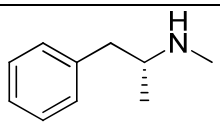
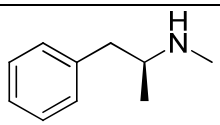


Figure modeled after Palmer.[33]

Table 1: Comparison of DAT, SERT and NET Releasing Activity of Neurotransmitters and Amphetamines (EC_{50})

Compound	Name	Structure	DAT (nM)	SERT (nM)	NET (nM)
8	Dopamine		86.9	Inhibitor	66.2
9	Norepinephrine		869	Inactive	164
10	Serotonin		1960	44.4	Inhibitor
11	Tyramine		119	2775	40.6
7	β-Phenethylamine		39.5	Inactive	10.9
12	R-(-)-amphetamine		27.7	-	9.5
13	S-(+)-amphetamine		8.7	-	10.2
14	R-(-)-methamphetamine		416	-	28.5
15	S-(+)-methamphetamine		24.5	-	12.3

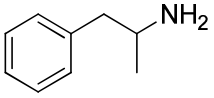
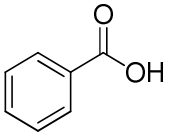
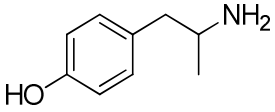
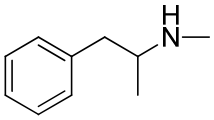
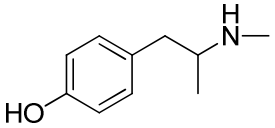
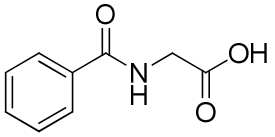
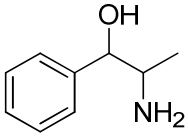
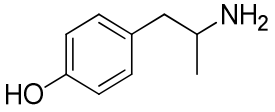
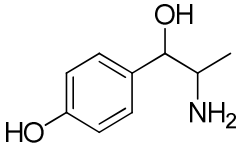
Adapted from Blough.[34]

The alpha methyl group on meth- amphetamine prevents ready metabolism of these compounds and in effect allows them to inhibit monoamine oxidase (MAO).[35] Inhibiting MAO prevents the metabolism of neurotransmitters, increasing the number of neurotransmitter present in the cytosol and allowing them to interact with other molecules within the cell. In the dopaminergic cells, this leads to an amphetamine-induced but dopamine-dependent neurotoxicity where the nerve terminals "burn out" and leave the body of the neuron intact.[36] The neuronal toxicity of the dopaminergic pathways exhibited by amphetamines (especially methamphetamine) is usually observed after long term, high dose use.[36,37] Sustained high dosages of amphetamines in experimental animals produces a persistent depletion of dopamine associated with terminal degeneration as well as neuronal chromatolysis in several areas of the brain (brain stem, cortex and striatum).[17] Compared to continuous high dosage of another psychostimulant (cocaine), no terminal degeneration in the frontal cortex or striatum was observed.[17] The toxicity of methamphetamine has been shown to be inhibited by dopamine synthesis inhibitor alpha-methyl-p-tyrosine; dopamine/NDA-receptor antagonists an dopamine or serotonin re-uptake inhibitors (which protect their specific neurons). There is also a link between the hyperthermia induced by amphetamines, the social interactions of its users and potential neurotoxicity.[38]

Amphetamines are neither quickly removed nor metabolized by the body. Both drugs and their metabolites are cleared primarily through the urine (Table 2).[39,40] In the case of orally ingested amphetamine, a study found that over a four day period, 90% of the initial dose was excreted.[39] Of this, 60-65% was retrieved on the first day, with 30% of the mixture being the unchanged drug, 21% benzoic acid (**16**) and 3%

hydroxyamphetamine (**17**).[39] In the case of methamphetamine, the clearance pattern is similar, with 90% being recovered over a 4 day period. Unlike amphetamine, approximately 50% of the dose recovered was the unchanged drug (22% of which was excreted in the urine on day one).[40] The other metabolites of methamphetamine are 4-hydroxymethamphetamine (**18**) (15% recovered) and several minor metabolites: hippuric acid (**19**), norephedrine, 4-hydroxyamphetamine (**17**) and 4-hydroxynorephedrine (**20**).[40] The slow clearance and low-metabolism of these drugs allows them to remain active physiologically much longer than the initial high. As a comparison, the half-life of cocaine is about 40 minutes and 80-90% of injected cocaine (most bioavailable method of ingestion) is rapidly metabolized.[41] Only 1-5% of cocaine is excreted as the unchanged drug within 6 hours of use.

Table 2: Metabolites of Amphetamines[39,40]

Compound	Name	Structure	Amount (%)
5	Amphetamine*		>30%
16	Benzoic acid*		21%
17	Hydroxyamphetamine*		3%
6	Methamphetamine**		50%
18	4-hydroxymethamphetamine**		15%
19	Hippuric acid**		Minor
3	Norephedrine**		Minor
17	4-hydroxyamphetamine**		Minor
20	4-hydroxynorephedrine**		Minor

* - Metabolites of amphetamine. ** - Metabolites of methamphetamine

It should be noted that while the above lists the physiological effects of long term meth/amphetamine use, low dosages under a physician's care can be safe and effective for some neurological conditions. One of the most common treatments for ADD/ADHD and narcolepsy is Adderall or dexedrine which are amphetamine salts. These drugs can both increase attention as well as aid in wakefulness that is desired by both conditions, improving quality of life significantly for patients. Blood concentrations of these drugs usually are in the range of 0.02-0.05 mg/L, compared to recreational users whose concentrations range from 0.01-2.5 mg/L (concentrations above which may be toxic).[25]

Monoamines, Their Receptors and Transporters

While the roles of the monoamines and their receptors are discussed at length in the following, we would like to take the opportunity to discuss some of the shared characteristics of their transporters. Monoamine transporters belong to a super-family of facilitated transporters, specifically the 12 transmembrane domain neurotransmitter: sodium symporter family (NSS).[42-44] These transporters are able to translocate their substrates across the membrane bilayer by exploiting an ion gradient, which is driven by the naturally occurring sodium ion gradient.[44] This allows them to operate in two directions, depending on concentration gradients/net driving force.[42,43] Cotransport of chloride ion is also required for the following transporters, with additional (and particular) needs for the dopamine and serotonin transporters.

These transporters also act similarly by releasing their neurotransmitter (serotonin, norepinephrine and dopamine) when binding amphetamines.[43] This ability of amphetamines to cause efflux *while* they are being transported into the cell is not

adequately explained by the alternate access model. The facilitated exchange diffusion model is similarly inadequate as it fails to explain why the transporter would release neurotransmitter instead of meth- amphetamine. It has since been suggested that monoaminergic transporters operate as counter-transporters in the presence of amphetamines.[43] Release of neurotransmitter and uptake of amphetamines occurs through separate by coupled moieties which are also coupled in changes of the ionic gradients.[43] A second stimulus by a protein kinase C isoform primes the inward facing conformation for outward transport, which accounts for the influx/efflux behavior.[43]

Norepinephrine

As a neurotransmitter, norepinephrine is principally a excitatory. It is ultimately derived from vesicular dopamine through the action of dopamine β-hydroxylase. Almost every neural region receives input from these neurons. The cell bodies are located in seven regions of the pons, the medulla and one region of the thalamus.[45] The locus coeruleus-noradrenergic system supplies norepinephrine throughout the central nervous system through a far-reaching series of efferent projections.[46] Unlike dopamine, or serotonin; the release of norepinephrine does not occur at synaptic buttons, but rather along axonal varicosities (beadlike swellings along the branches of the neuron).[45]

Norepinephrine Receptors

Receptors are sensitive to both epinephrine (secreted by the adrenal medulla) and norepinephrine. Two major types of receptors have been described for norepinephrine;

the α- and β- types of which there are a total of five subtypes (α_1, α_2; β_1, β_2, and β_3).[45] All of these subtypes are found outside of the CNS, and so far the β_3 receptor appears to be exclusively located outside of the CNS (predominantly in adipose tissue). All of these subtypes are able to produce excitatory and inhibitory neural effects, although behavioral effects are almost exclusively excitatory. In the brain, almost all autoreceptors appear to be of the α_2 subtype. In the brain, α_1 receptors are able to produce slow, depolarizing effects on the post-synaptic cell membrane (excitatory). Alternatively, the α_2 receptor produces a slow hyperpolarization (inhibitory). Both β_1 and β_2 neuro-receptors increase the response of post-synaptic neurons to excitatory inputs which likely leads to the increased alertness experienced by subjects.[45]

Norepinephrine is known to modulate serotonin and dopamine release, especially in the thalamocortical regions.[47] The entire system is extremely important in the control of concentration, alertness, arousal, mood, sleep/wake cycle, emotions, blood pressure and pain regulation.[47,48,49] Depression is thought, in part to be caused by dysfunction of any of these pathways. It is thought that post-synaptic α_1, α_2 receptors in the frontal cortex play a significant role in mood, attention and comprehension.[47]

The Norepinephrine Transporter

The norepinephrine transporter was isolated in 1991 by expression cloning; the gene is located on human chromosome 16q 12.2.[49] The amino acid sequence of the transporter predicted a protein of 617 amino acid which contain 12 transmembrane domains. The structure of the transporter is homologous to other transporters of monoamine neurotransmitters (e.g. dopamine, serotonin, etc). This group of

transporters are members of a larger group of sodium and chloride dependent transport proteins located in neuronal and glial plasma membranes.[42,49] The reuptake of norepinephrine by the norepinephrine transporter is primarily how the synapse is cleared and how the biological effects of the neurotransmitter are ceased. Of the synaptic norepinephrine, 70-90% of it is cleared by the transporter while the remaining 10-30% goes into circulation or spills into extraneuronal tissue.[49] In particular, cardiac noradrenergic synapses tend to be three times narrower than distances of synapses in the vasculature and the extraneuronal uptake (uptake-2) plays only a small role in cardiac norepinephrine clearance.[49] These observations suggest that the heart is more dependent on the norepinephrine transporter for norepinephrine clearance of synapses in the vascular beds. It follows that any impairment of the norepinephrine transporter should lead to an increase in norepinephrine levels in the synaptic clefts of the heart.[49]

Serotonin, Receptors and Transporter

The behavioral effects of serotonin are complex and its roles range from mood regulation, appetite, sleep, arousal, sex, regurgitation, perception, motor function, gastrointestinal function, neurotrophism, vascular function, dreaming and regulation of pain.[45,50] Serotonergic neurons are found in nine clusters in the brain, many of which are located within the raphe nuclei of the midbrain, pons and medulla. Two principal clusters are the dorsal (D-system) and medial raphe nucleus (M-system). The axons of both these clusters project into the cerebral cortex. The dorsal raphe nuclei innervates a part of the hippocampal formation called the dentate gyrus. The two systems have other distinguishing features as well.

The D-system axonal fibers are thin with spindle-shaped varicosities that appear not to form synapses with other neurons. Instead, the varicosities release serotonin that then diffuses throughout the system. The M-system axonal fibers are thick and its varicosities are rounded - appearing much like beads along a string. Unlike the D-system varicosities, these appear to be adjacent to post-synaptic membranes forming conventional synapses.[45]

Serotonin Receptors

Seven families of serotonin receptors have been described, and along with them many subtypes have also been reported. The families are referred to as 5-HT_{1-7}, with subtypes being referenced as a letter after the number (e.g. 5-HT_{1a}). All of these receptors are metabotropic except for 5-HT_3 which is ionotropic.[45] It has also been observed that these subtypes have further variants due to alternative splicing, leading to isoforms. Alternative splicing appears to be fairly common with several of the subtypes (5-HT_{2C}, 5-HT_4 and 5-HT_7).[50] These variants may range from being severely truncated and having no obvious function (5-HT_{2C}), to differences in length and composition in the carboxyl terminus (5-$HT_{4(a)}$ - 5-$HT_{4(f),}$ 5-$HT_{7(a)}$ - 5-$HT_{7(d)}$).[50] Further variations of the 5-HT_{2C} receptor involve RNA editing which was the first G-coupled protein found to be edited. These variations of subtypes could present more of a challenge to the development drugs targeting these receptors (which isoform to test), but also present the possibility of further specification of the drug target for optimum binding.

The 5-HT_1 has high concentrations of 5-HT1a binding sites and mRNA expression in many areas throughout the brain (dorsal raphe nucleus, hippocampal pyramidal cell

layer, cerebral cortex).[50] All members of the 5-HT_1 family tend to have inhibitory effects, whether they are pre- or post-synaptic.[45] This appears to be accomplished by the opening of inwardly rectifying K^+ channels or a closing of voltage-gated Ca^{2+} channels, which induces hyperpolarization.[50] The 5-HT_{1b} and 5-HT_{1d} serve as presynaptic autoreceptors. In both the D- and M-systems, the 5-HT_{1a} receptors serve as autoreceptors in the membranes of both the dendrites and somas.

All 5-HT_2 receptors tend to have slow excitatory effects through a decrease in K^+ or an increase in non-selective cation conductance.[45,50] These effects have been observed in various regions throughout the brain from motor neurons to the nucleus accumbens, cerebral cortex and the substantia nigra pars reticulata. Studies have shown that these receptors have high concentrations of binding sites and mRNA in some regions of the forebrain (neocortex, piriform cortex, claustrum and olfactory tubercle).[50] Outside of the motor nuclei and the nucleus tractus solitarius, these receptors and their mRNA are found at low concentration within the spinal cord and brain stem.[50] These receptors are also highly expressed in the motorneurons of the face. These receptors are also known to play an important role in cortical information processing.

The 5-HT_3 subtype can produce either excitatory or inhibitory effects. 5-HT_3 can produce an inhibitory effect on postsynaptic potentials through a chloride ion channel. The excitatory responses attributed to this receptor are typically rapid onset and rapid sensitization (typical of ligand-gated ion channels).[50] This subtype can cause inhibition in a round-about way by exciting inhibitory interneurons (GABAergic).[50]

The 5-HT_4 receptors are found in several regions of the mammalian brain (striatum, substantia nigra, olfactory tubercle and hippocampus). Since these regions also show

significant levels of 5-HT_4 mRNA suggesting that these receptors probably function post-synaptically, in order to mediate some actions of serotonin. In the hippocampal pyramidal cells of the CA1 region, these receptors have been shown to mediate an inhibition of calcium activated potassium current which is ultimately responsible for the cells ability to respond to excitatory inputs with "a robust spike activity."[50]

The 5-HT_5 receptor appear to be relegated to the central nervous system, though its role therein is not well characterized, although some studies suggest that it may play a role in neuroendocrine secretion.[51] While two subtypes (5-HT_{5a}, 5-HT_{5b}) have been found in humans, only one (5-HT_{5a}) appears functional.[52] 5-HT_6 receptors are almost exclusively located in the limbic and cortical regions.[53] These receptors regulate cholinergic and glutamatergic activity and may play some role in cognition, mood, seizures and feeding.[53] 5-HT_7 receptors are found in both the central nervous system and the peripheral tissues and mediate the relaxation of smooth muscle (cardiovascular system and gastrointestinal tract).[54] In the central nervous system, these receptors appear to play a role in the pacemaker of the circadian rhythm which is located in the suprachiasmatic nucleus of the hypothalamus.[50]

Serotonin Transporter

The serotonin transporter is homologous to the norepinephrine and dopamine transporters. The description in 1994 by Lesch et al noted that serotonin transporter from the human midbrain raphe complex and that of platelets could be attributed to a single copy of a gene located on chromosome 17 (17q11.1-q12).[55] The human serotonin transporter is comprised of ~630 amino acid residues and contains 12

putative transmembrane domains. The human serotonin transporter is 92% homologous to that of the rat. Like the other monoamine transporters, its activity is regulated by extracellular concentrations of sodium and chloride, and is an anti-porter of potassium ions.[42,55] Unlike the norepinephrine or dopamine transporters, the activity of the serotonin transporter is sensitive to extracellular concentrations of potassium ion. If extracellular concentrations of potassium ion increases, or if extracellular concentrations of sodium or chloride ions should plummet, the transporter has been noted to reverse direction, effluxing substrates and ions.[42]

Genetic polymorphism of the serotonin transporter typically is seen in the promoter region of the gene where a person has two copies of the so-called short (s) allele. This polymorphism has been associated with decreased serotonin transporter activity in the brain as well as decreased serotonin transporter mRNA expression and serotonin uptake in lymphoblasts compared to a serotonin transporter with two copies of the long (l) allele.[55] The debate of whether or not this polymorphism is significant in neuroticism, anxiety and other mood disorders is on-going. Early when this polymorphism was reported, Lesch et al. had suggested that individuals with this polymorphism displayed increased bias towards higher rates of neuroticism and harm avoidance than those with two copies of the l-type allele.[55] However, further studies and meta-analyses have found conflicting results, making it seems likely that these conditions are polygenetic.

Dopamine, Receptors and Transporter

The neurotransmitter dopamine serves multiple roles dependent on its location in the brain although it appears to always cause a slow postsynaptic effect.[56] While there are

several systems of dopaminergic neurons throughout the brain, there are three systems of major importance that both originate in the midbrain.[45] The nigrostriatal system originates in the substantia nigra of the midbrain. The nigrostriatal system's axons project into the neostriatum (the caudate nucleus and the putamen) which is an important part of the basal ganglia. These neurons play an important role in movement and Parkinson's disease. The second major dopaminergic system is that of the mesolimbic system which originates from the ventral tegmental area (VTA) of the midbrain. These neurons project their axons into several parts of the limbic system (nucleus accumbens and its shell region, amygdala and hippocampus) which is known to play a role in learning, motivation and reward. The third major system is the mesocortical system which also originates from the ventral tegmental area. These axons are projected to and have an excitatory effect on the prefrontal cortex. They play a role in the function of short-term memory, strategizing and problem solving.

Dopamine Receptors

There are 5 identified subtypes of the dopamine receptor protein. These can be broken into two homologous groups: D_1-type, comprised of subtypes D_1 and D_5; and D_2-type, comprised of subtypes D_2, D_3, and D_4.[57] These two groups are similar in their homology as well as the brain regions in which they are found, but have several key differences. D_1-like receptors are G-protein coupled receptors which are coupled to G_s and mediate excitatory neurotransmission. Activation of these receptors activates adenylyl cyclase which increases the intracellular concentration of cyclic adenosine monophosphate (cAMP). These receptors mostly regulate neuronal growth and

development, modulate D_2-mediated events and mediate some behavioral responses. Of the two D_1-like receptors, D_1 is the most commonly expressed throughout the brain whereas D_5 is primarily found in the limbic system.

The D_2-like receptors are coupled to a different G-protein ($G_{\alpha i}$) which directly inhibits adenylate cyclase (thereby inhibiting cAMP production). The D_2-type transporters are found primarily in the nucleus accumbens, the ventral tegmental area and the basal ganglia. D_2-like receptors mediate inhibitory neurotransmission. Both types of receptors are found throughout the body; notably in the cardio-pulmonary system as well as the renal system.

The Dopamine Transporter

The role of the dopamine transporter is to transport synaptic dopamine into the cell body. It is the primary mechanism by which synaptic dopamine is cleared. The transporter is a plasma-membrane bound transporter protein with 12 transmembrane domains with both the amino- and carboxyl termini within the cytoplasm.[5,58] The transporter is a symporter, which transports two Na^+ ions and one Cl_2^- are co-transported with each molecule of dopamine.[59] For each molecule of dopamine transported into the cell, two net positive charges will also accompany it (because dopamine is positively charged at physiological pH) and it is this movement which generates an inward current.[59] Once inside of the cell dopamine is then sequestered into vesicles by vesicular monoamine transport protein (VMAT-2). Any dopamine that is not sequestered by VMAT-2 is metabolized by monoamine oxidase (MAO), an outer-membrane bound mitochondrial enzyme. The genetic sequence encoding the dopamine

transporter appears to be highly conserved across species (human : monkey, 98%; human : rat, 92%, human : bovine, 88%).[60] Additionally, the DNA encoding the dopamine transporter is very similar to the sequences of other cloned monoamine receptors in the rat (norepinephrine transporter similarity 67%; serotonin transporter similarity 49%, etc).[60]

Amphetamines Versus Cocaine

Since cocaine and amphetamines are the most popular stimulants globally, it is important to compare and contrast their effects on their users. Cocaine shares a past similar to amphetamines' parent compound (ephedra), as it was used medicinally for thousands of years in the New World. Cocaine is utilized in both the free base ("crack" cocaine) and salt forms (cocaine hydrochloride). According to the 2010 UN drug report, there are approximately 15.9 million users of cocaine worldwide compared to 13.7 - 52.9 million users of amphetamine type stimulants.[62] Both cocaine and amphetamines share similar intoxicating effects on the user due to both acting as dopamine agonists.[45,63] However, there are some notable differences between the two.

Differences Between Cocaine and Amphetamine Binding and Neuronal Effects

Although both drugs act as dopamine agonists, they share several important differences in their binding and neuronal effects. Cocaine and amphetamines directly interact with monoaminergic neurons.[31,63,64] Cocaine tends to bind at the cell surface by inhibiting the dopamine transporter (DAT). This inhibition leads to an increased concentration of dopamine in the synaptic cleft by preventing the re-uptake of

neurotransmitter.[31,63,64] Cocaine is not limited to interacting with the DAT, it also binds to and inhibits other monoaminergic transporters (norepinephrine and serotonin).

Like meth- amphetamines, cocaine also acts on the vesicular monoamine transporters (VMAT-2) by enhancing the storage of monoamines inside the synaptic vesicles.[27] Given more neurotransmitter in each vesicle, this leads to significantly greater synaptic dopamine (or other monoamine) upon depolarization of the neuron.[31]

The binding and activity of amphetamines is somewhat dependent on concentration. At low concentration, amphetamine is transported by the dopamine transporter into the cytosol; whereas at higher concentrations, amphetamines may diffuse into the cell due to their lipophilicity.[31] Once in the cytosol, amphetamines also bind to VMAT-2; however, unlike cocaine, amphetamines alter its function by trafficking the neurotransmitters out of the vesicle and into the cytosol.[27] At higher concentrations, amphetamines may also diffuse into the vesicles. The ability of amphetamines to interfere with the synthesis of dopamine is also dependent on concentration. At lower concentrations, the drugs boost dopamine synthesis.[31] At higher concentrations or prolonged exposure, the drugs inhibit tyrosine hydroxylase or decrease the protein's levels.[31]

As a weak base, amphetamines are able to accept protons from the acidic environment of the vesicles (pH ~5.5) which leads to the alkalinization/collapse of the proton gradient of the vesicles. This in turn nixes the active transport of amphetamines into the vesicles.[31] The weak base effect also explains mitochondrial dysfunction induced by amphetamines in the absence of dopamine (collapse of proton gradient

which disrupts the mitochondrial potential which may cause a decrease in intracellular ATP).[31]

A study by Jones et al in 1999 showed that as the concentration of amphetamine inside of the cell increases, this also leads to an increase in the number of inward-facing dopamine transporters.[65] These transporters then reverse their normal behavior by transporting dopamine from the cytosol into the synaptic cleft.[31,65] This leads to higher concentrations of extracellular dopamine which can lead to neurotoxic consequences.[31] The reversal of transporter direction and subsequent efflux of neurotransmitter is also evident in the norepinephrine and serotonin transporters. Interestingly, while studies have found methamphetamine-related neurotoxicity in regards to the dopamine and serotonin transporters; it appears that the norepinephrine transporter is merely down-regulated.[66,67]

Whereas amphetamines show a distinct preference for both the norepinephrine and dopamine transporters, cocaine binds roughly equally to all three (SERT, DAT and NET).[68] Cocaine is also readily metabolized by the body and has a much shorter half-life than amphetamines; about 0.8 ± 0.2 hours for cocaine compared to around 10.1 hours for amphetamines.[25] Peak plasma concentrations vary by method of administration and range from 0.22 mg/L at 30 minutes for nasal administration; 0.21 mg/L at 1 h for oral administration; 0.23 mg/L at 45 minutes for smoking (50 mg dose used).[25] This is significantly faster than the 2.6-3.6 hours it takes for peak plasma concentrations of amphetamines.

Long Term Effects of Cocaine versus Amphetamine Use

The long-term effects of both drugs are similar with users sharing symptoms such as irritability, aggressiveness, stereotyped behavior and a paranoid-like psychosis.[63] Like meth- amphetamine users, chronic cocaine has been linked to neurological deficits. These deficits include: seizures, optic neuropathy, cerebral infarction, subarachnoid hemorrhage, intracerebral hemorrhage, multifocal cerebral ischemia, cerebral atrophy of the frontal cortex and basal ganglia, myocardial infarction leading to global brain ischemia, movement disorders and edema.[31,69,] Many of cocaine's side effects have been reported to be caused by vasoconstriction and subsequent hypoxia, instead of the neurotoxic mechanisms seen in amphetamines.[31]

Brain glucose metabolism is positively correlated to neuronal activity and cognitive function.[69] The dopamine transporter is known to play a role in glucose metabolism of the brain, although the precise mechanism has not been discovered.[69,70] Cocaine is also known to alter brain glucose metabolism, possibly through its interactions with the dopamine transporter. Cocaine depresses the level of glucose metabolism in the brain both during use and during 'late-phase' abstinence.[69] During the first week or so after the start of abstinence, the user's brain has a higher than normal (read: non-user) glucose metabolism in the medial orbitofrontal cortex and basal ganglia.[71] Following this period, glucose metabolism will fall below that of normal and persist at this level for about three months.[71] Levels of glucose metabolism in the dorsolateral prefrontal cortex can serve as a predictor for visual and verbal memory scores.[72] Glucose metabolism levels in the anterior cingulate gyrus were also found to be predictive for

attention/executive function (note that these detriments are still considered mild compared to other neuropsychiatric conditions, e.g. schizophrenia).[72]

Similarly to meth- amphetamine use, chronic cocaine users also see a marked decrease in dopamine receptors. These receptors (D_2) are often most severely effected in the orbitofrontal cortex and the cingulate gyrus - areas which receive the dopamine afferent neurons. This decrease has been noted to persist despite years of abstinence. The depletion of these receptors in combination with the decrease in brain glucose metabolism is also seen in Parkinson's disease, a condition which has also been associated with chronic cocaine use.

Methamphetamine use has been shown to decrease striatal glucose metabolism that may be reflective of its selective toxicity to dopaminergic neurons.[73] Users who underwent extended abstinence showed metabolic recovery in the thalamus which may indicate neuro-adaptations for the loss of dopamine.[73]

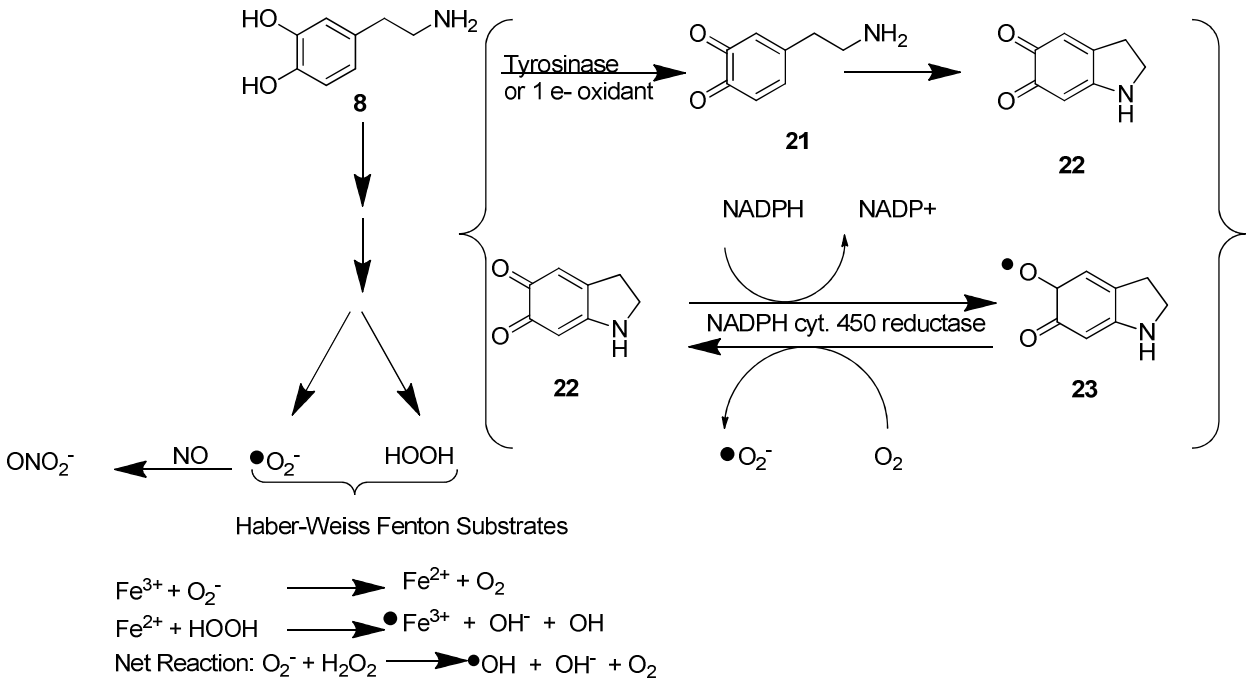
Mechanism of Amphetamine-Induced Neurotoxicity

The chronic use of amphetamines shows profound damage and cell death in areas of the brain containing high concentrations of dopaminergic neurons. Dopamine is known to be neurotoxic at high concentrations both *in vivo* and *in vitro*.[31] The combination of the abilities of amphetamines to efflux dopamine and inhibit its clearance from the synaptic terminal, and the fact that these molecules have a long half-life creates an extremely problematic scenario. Dopamine is easily oxidized by both enzymatic and non-enzymatic mechanisms which may lead to oxidative stress in the dopaminergic neurons as well as surrounding brain tissue (Figure 3 A).[31] Auto-oxidation of dopamine leads to the production of superoxide ion ($O_{2\cdot}^{-}$) and hydrogen peroxide.[31] Both hydrogen

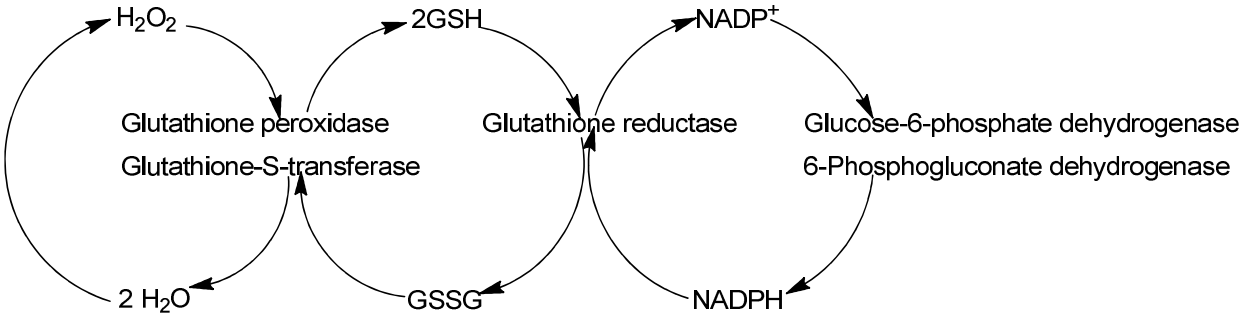
peroxide and superoxide may then interact with transition metals in a Haber-Weiss/Fenton reaction to give the hydroxyl radical (·OH). Superoxide can also interact with nitric oxide to produce peroxynitrite (ONO_2^-), another powerful oxidant. There are some neuro-protective enzymes that mediate the toxic effects of these chemicals. Hydrogen peroxide can be dulled by glutathione peroxidase which catalyzes one molecule of hydrogen peroxide to two molecules of water (Figure 3 B). Superoxide dismutase (SOD) catalyzes a reaction which produces diatomic oxygen and hydrogen peroxide which may contribute to the high levels of hydrogen peroxide.

Figure 3 - Dopamine Neurotoxicity(A) and Inactivation of Hydrogen Peroxide via Glutathione Peroxidase (B)

A:



B:



GSH = Glutathione monome; GSSG = Glutathione disulfide.

(B) Adapted from a figure by Tandoğan and Ulusu.[74]

Cell death due to reactive oxidizing species is often due to the oxidation of macromolecules (amino acids, phospholipids, nucleic acids, etc). In a normal system,

acute exposure to hydrogen peroxide leads to apoptopic cell death. In the system of a chronic amphetamine user, there is evidence of cell resistance to acute toxicity due to higher levels of antioxidant enzymes in several areas of the brain.[75]

Developmental Approaches for Pharmacotherapies of Psychostimulant Dependence

Two major drug classes exist for drugs targeting neuro-transporters, releasers and re-uptake inhibitors.[76] Releasers work by promoting efflux of a neurotransmitter by way of a transporter mediated exchange (e.g. extracellular methamphetamine for cytoplasmic dopamine), and also disrupt the storage of neurotransmitters in vesicles, increasing their concentration in the cytoplasm.[76] Re-uptake inhibitors work by binding to the transporter and preventing the reuptake of the neurotransmitter from the synapse in order to increase synaptic concentration.

There are several approaches in the development of pharmacotherapies for psychostimulant addiction. Two major approaches exist; that of an agonist based pharmacotherapy and the dual deficit approach.[76] The agonist approach involves increasing the release and/or concentration of a particular (or several) neurotransmitters, which is thought to help with cravings and withdrawal symptoms. Two well known antagonists are bupropion (Wellbutrin or Zycam) and modafinil (Provigil, Alertec).[77,78] Bupropion acts by inhibiting the reuptake of norepinephrine and dopamine, of which it is twice as selective in preventing reuptake of dopamine than norepinephrine. It has also been implicated as being a dopamine and norepinephrine releaser.[79] For these reasons, it was recently tested for its possible effectiveness as a pharmacotherapy for methamphetamine abuse.[77] While the drug was found to be

effective in 'light' users, chronic users who would benefit the most, showed no effectiveness. Similarly, the narcolepsy drug modafinil also works as a dopamine and norepinephrine releaser/reuptake inhibitor. A recent study found no clear evidence of any decrease in methamphetamine use when users were taking this drug. Other agonists showed a similar trend of failure.[76,77,78]

The major issue with treating psychostimulant addiction with dopaminergic agonists is that their mode of action creates abuse liability.[76] Activation of the mesolimbic dopaminergic while mediating some of the effects of withdrawal, can create similar circumstances in the neurocircuitry that may prompt addiction. Research has suggested that interplay between dopaminergic and serotonergic systems may hold the key to reducing the liability of psychostimulant pharmacotherapies.[76]

The dual deficit approach is an alternative that relies on the action of two neurotransmitter systems (serotonin and dopamine), rather than focusing on just one. It is centered on the observation of disruption in the serotonergic and dopaminergic systems in substance abusers.[76] The withdrawal experienced by psychostimulant abusers includes symptoms that have underlying causes that are typical of low serotonergic and dopaminergic activity (e.g. anhedonia and craving; obsessive thoughts and impulsivity, respectively; Figure 4). This observation has been shown to be accurate in human brain imaging studies which have shown a marked reduction in evoked dopamine release and D2 binding potential in the striatum, as well as diminished response in serotonin releasers in abstinent cocaine users.[76] By focusing on both the dopamine and serotonergic pathways, the increased activation of the serotonergic neurons should provide an inhibitory effect on mesolimbic dopamine. This

should reduce the abuse liability for these pharmacotherapies, as well as alleviating the symptoms of depressed serotonergic activity.[76]

Figure 4 - Schematic of Dual Deficit

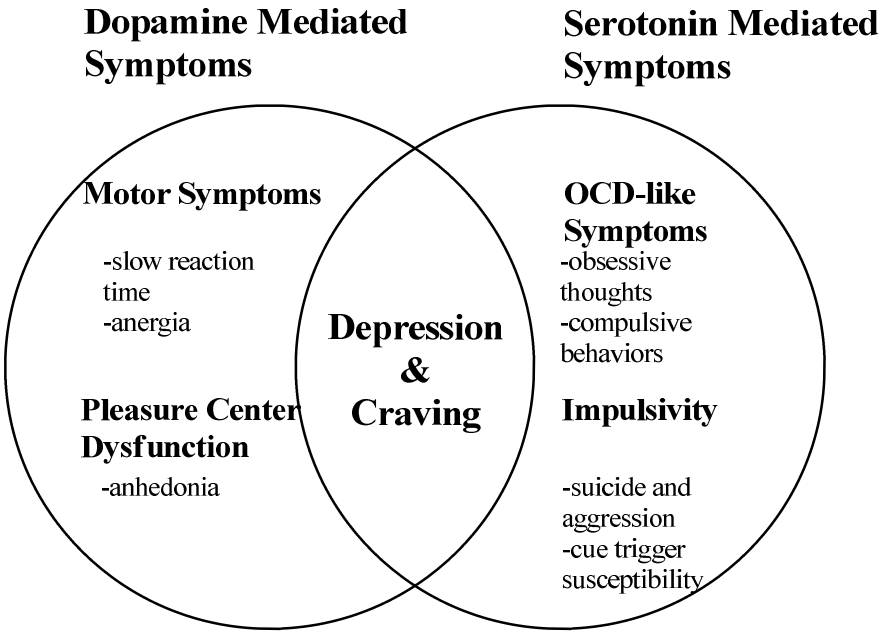


Figure based on Rothman, Blough and Baumann.[76]

Compound Classification, Binding Affinities and Calculation of Inhibition Constant

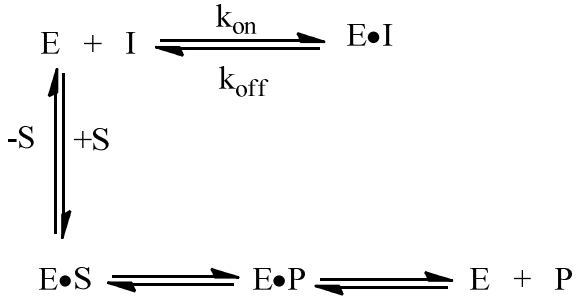
Compounds may be classified by their effects once bound to a receptor. Structures which bind to the receptor and cause a cellular response are termed agonists. A full agonist has maximal effect on the desired receptor. Partial agonists are structures which can dampen the effects of the original substrate and can act as antagonists in the case of overexposure to the substrate (these compounds have less than a full agonist

effect on the receptor). Inverse agonists are compounds which bind to a receptor and initiate the opposite response of the agonist. Antagonists are compounds which bind to the receptor but cause no cellular response. These compounds have affinity for the receptor, but lack efficacy (their only effect is to prevent another substrate from acting).

In the case of drugs of abuse, an antagonist is sought to block the receptors from binding the ingested drug and therefore preventing the high. In order to evaluate the effectiveness of these analogs, either a K_i or IC_{50} value is used. The K_i value is defined as the dissociation constant for the enzyme-inhibitor complex (Figure 5). The smaller the value of the K_i; the more potent the inhibitor. Since the K_i value is a rate constant, one finds its value by determining enzyme-catalyzed reactions while varying the concentrations of substrate and inhibitor.[80] In general, the method of establishing the K_i value is much more intensive than generating the IC_{50}, although the IC_{50} may be used to calculate a K_i.

The IC_{50} value is a quantitative measurement of the amount of substance needed to inhibit a specific enzyme concentration by half. Like the K_i value, a low IC_{50} number means less compound is required to inhibit 50% of the normal activity of an enzyme. The IC_{50} value is determined by using one concentration of enzyme over a range of inhibitor concentrations.[80] This allows for a much quicker assessment of compounds, especially when a series must be tested.

Figure 5: Competitive Inhibition



Adopted from Silvermann.[81]

Calculating the K_i of a receptor or enzyme can be achieved by multiplying the concentration of inhibitor and enzyme and then dividing the product by the concentration of enzyme/inhibitor complex (Figure 6a). In order to calculate the IC_{50} from the K_i value, the Cheng-Prusoff equation (Figure 6b) can be used. While using this formula (Figure 6b) provides an only approximate value of K_i, the fact that it is quick to calculate allows for a fast screening of many compounds for evaluation.[81]

Figure 6: K_i Formulas

a) $K_i = ([E][I])/ [EI])$

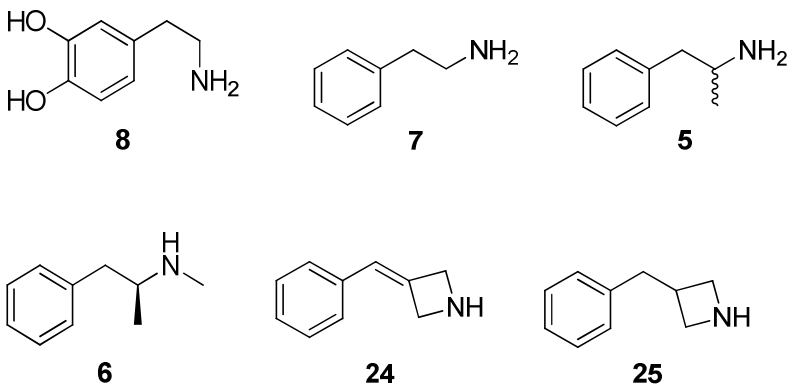
b) $K_i = \dfrac{IC_{50}}{([S]/K_m)}$

* K_m is the equilibrium constant for the enzyme/substrate complex.

Synthetic Targets

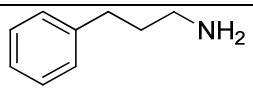
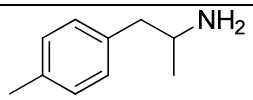
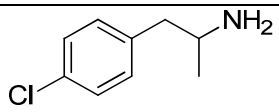
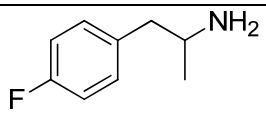
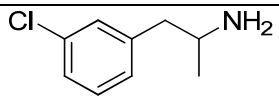
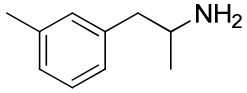
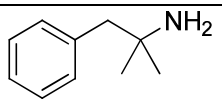
Given the structural similarity between amphetamines and other endogenous neurotransmitters (Figure 7), we decided to examine the binding affinities for azetidine-ring based analogs (**24** and **25**). This template gives a more rigid placement of the nitrogen as well as provides a chance to examine the effects of olefination on the carbon chain. This also gives a slight difference in the length of the chain as well as a slight difference in angle of the nitrogen. The goal was to examine how these changes impact binding at the desired transporter.

Figure 7:



Previous studies have focused on the substituents on the phenyl ring and various modifications to the two carbon linkage and the alpha methyl group (Table 3).

Table 3: Comparison of Releasing Activity of Amphetamine Analogs

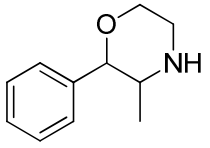
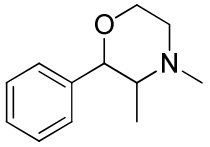
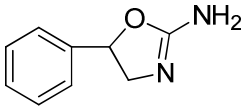
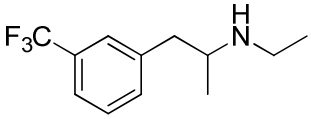
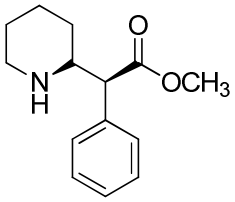
			EC_{50} (nm)		
Compound	**Name**	**Structure**	**DAT**	**NET**	**SERT**
26	Phenylpropylamine		1491	222	-
27	p-methylamphetamine		44.1	22.2	-
28	p-chloroamphetamine		68.5	23.5	-
29	p-fluoroamphetamine		51.5	28	-
30	m-chloroamphetamine		11.8	9.4	-
31	m-methylamphetamine		33.3	18.3	-
36	Phentermine		262	39.4	3511

Data from Blough[34].

The modification of the two carbon linkage to that of a three carbon linkage greatly increases the amount required for activity at the dopamine and norepinephrine transporters. Similarly, an additional methyl group attached at the alpha carbon also increases concentrations required for all three transporters' activity. Substitutions on the aromatic ring are well tolerated and seem to follow a meta > para preference.

The releasing activity of other, amphetamine-like compounds were also recently examined for their releasing activity (Table 4). These compounds have more hindered amines, either heterocyclic (**33-36**, **38**), or additional substituents (**34**, **37**) while retaining the two carbon linkage of meth- amphetamine. Some of these compounds (**34** and **37**) show inhibition activity at the dopamine transporter. This inhibition is desirable as the compound shows no releasing activity as well as providing a longer presence of dopamine within the synaptic cleft (aiding in withdrawal). It is hoped that the structure of the azetidine analogs are similar enough to share this inhibition as well as inhibition at the serotonergic transporter. The evaluation of the azetidine system represents a novel structure for this pursuit.

Table 4: Comparison of releasing activity of other compounds

			EC_{50} (nm)		
Compound	**Name**	**Structure**	**DAT**	**NET**	**SERT**
33	Phenmetrazine		131	50.4	-
34	Phendimetrazine		Inhibitor	Inhibitor	-
35	Aminorex		49.4	26.4	-
37	Fenfluramine		Inhibitor	739	79.3
38	dl-Threo-methylphenidate (Ritalin)**		17 +/- 2.0	-	>1000

Data from Blough[34].

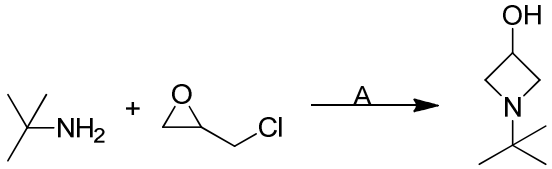
** - Data from Meltzer et al J Med Chem 2003.[83]

Synthetic Routes for the Azetidine Ring System

Syntheses for the azetidine ring system have been reported since the 1888.[84] The early syntheses involved extremely low yields (<1%) and were relatively infeasible for library development. Due to the constrained nature of the ring, high yielding procedures for azetidine synthesis are a relatively new development in chemistry. The first high

yielding azetidine synthesis did not occur until 1967 (Scheme 1).[85] Gaertner's synthesis focused on primary hindered amines (t-butylamine, **37**). While this method offered good yields, it suffered from very slow reaction time (2.5 months), as well as being untested for secondary amines or for less hindered *N*-substituted azetidines (e.g. *N*-benzyl).

Scheme 1

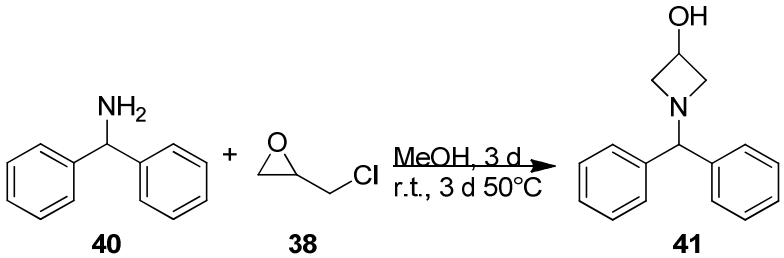


37 **38** **39**

A. Stand at r.t. 3 d and then divided into half. One half was stirred at 60°C for 3 d and then the product was recrystallized. The other half was condensed under reduced pressure at 40°C and the residue allowed to stand for 2.5 m at r.t. before being worked up as usual.

In 1968, Chatterjee and Triggle reported a modified version of this synthesis, but failed to present any experimental data along with this publication.[86] A few years later, Anderson and Lok (Scheme 2) in 1972 published a new modification that offered much higher yields with a bulky substituent and a significantly faster reaction time (~6 days).[87]

Scheme 2



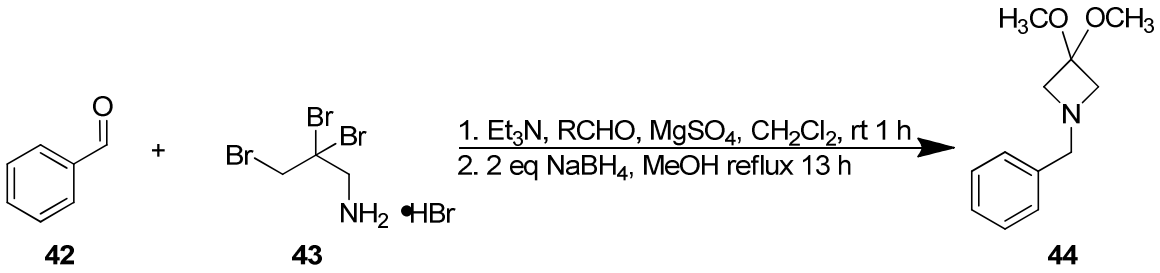
40 **38** **41**

This procedure is also one pot, but also has the added benefit of a removable nitrogen protecting group. This allows not only for easier manipulation at the 3-position,

but prevents side product formation at the nitrogen. This also opened the door for future modifications at the amine. The down side to this synthesis is the long reaction time as well as the fickleness of the starting material to react. Many labs have published slight modifications of this reaction claiming faster reaction times and superior results.[88-90]

The generation of the azetidine ring can be accomplished via other methods as well. A recent paper by de Kimpe et al published a two step procedure involving the formation of the imine and subsequent reduction into the azetidine ring (Scheme 3).[91] A modification of this procedure was also developed in our labs using benzaldehyde starting material.[92] These procedures have their strength in the ease in which one can generate less-hindered azetidine systems. Both procedures were attempted during the course of this work.

Scheme 3



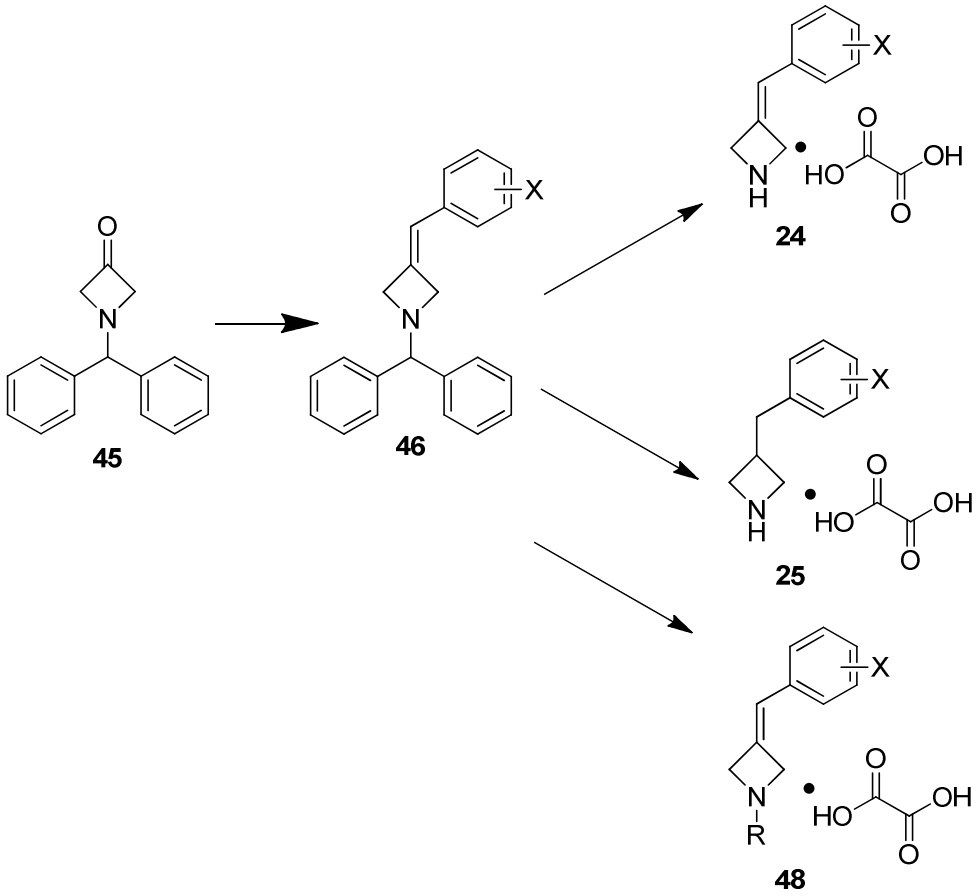
42 **43** **44**

Synthetic Strategy

Our strategy illustrated in Scheme 4, centered around the formation of a key intermediate, the N-benzhydrylazetidin-3-one (**45**). From there, we could perform a Wittig olefination to create the benzylidene **46** which would allow us to generate several classes of compounds for evaluation and SAR development. From the benzylidene **46**, a deprotection could allow us to test the 3-benzylidene oxalate series **24** or we could

perform a series of N-alkylations to generate N-substituted azetidines **48**. The benzylidene could also undergo hydrogenation to introduce a bit more conformational freedom to afford 3-benzylazetidine derivatives **25**.

Scheme 4

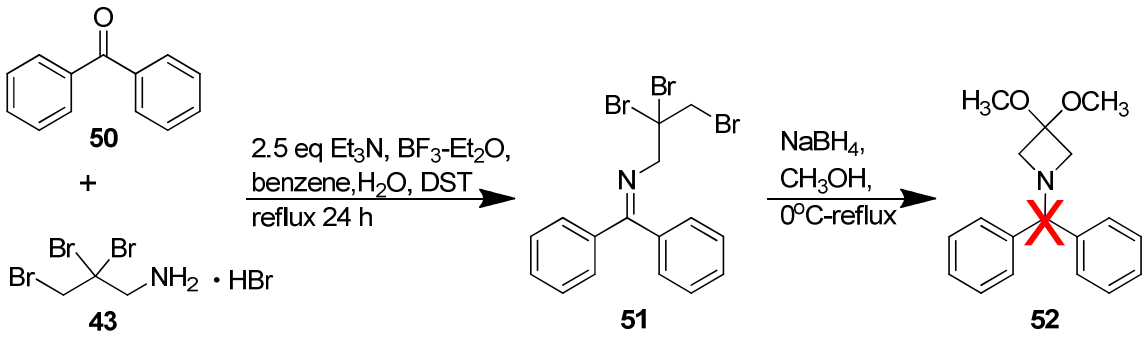


RESULTS AND DISCUSSION

Attempted Synthesis of Azetidine Ring

When we first approached this project, we searched for a commercially available source for N-benzhydryl-azetidin-3-one (**45**). Unfortunately, the prices for what was available at the time made this unreasonable. So we examined several routes to form the azetidine ring. The first of which was an attempt to replicate a method which was developed by a previous group member.[92] The reaction that was developed in our lab used benzaldehyde instead of benzophenone. It had been noted that the N-benzyl-azetidin-3-one was not as stable as we would have liked, and had a half-life of about eight hours at room temperature. It had been shown that the N-benzhydrylazetidin-3-one was stable for months at a time at room temperature, which made it ideal for library development.[93] The N-benzhydrylazetidin-3-ol (**43**) thus became our target.

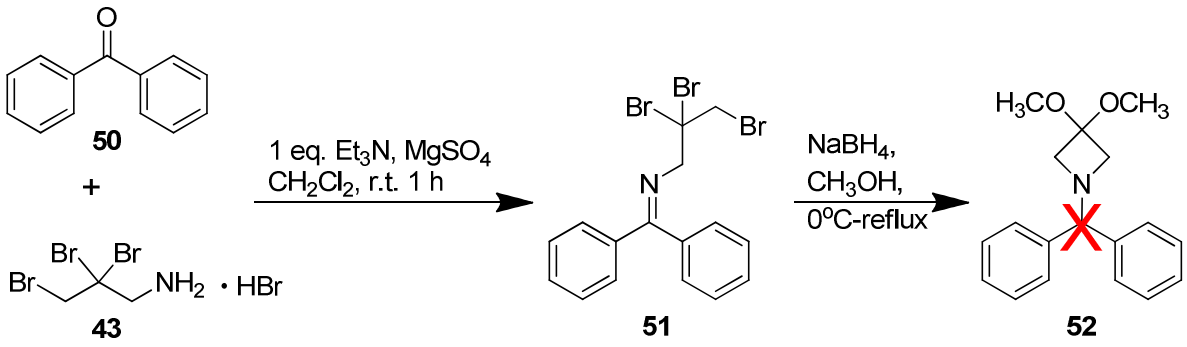
Scheme 5:



As illustrated in Scheme 5, we attempted to prepare the N-benzhydryl-3,3-dimethoxyazetidine **52**. After numerous attempts, we were unable to achieve the synthesis of acetal **52**. It had been noted in previous studies that excess of the boron trifluoride diethyl etherate was deleterious to the reaction yield.[92] It had noted that

anything more than a few drops negatively impacted yields. So the reaction was repeated paying careful consideration to the amount of the catalyst, but after multiple attempts there was no improvement in yield. Therefore we abandoned this route and examined a similar method put forth by the de Kimpe group (Scheme 6).[91]

Scheme 6:



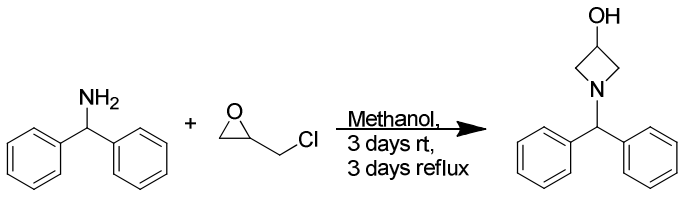
In the De Kimpe procedure (Scheme 6) magnesium sulfate was used in place of boron triflouride diethyl etherate as a Lewis acid catalyst. However, this mild modification did not furnish the desired acetal **52**. After this reaction similarly failed, we set about to see if perhaps there was some unidentified problem with the method. The reaction sequence was repeated (Scheme 5) with benzaldehyde. The procedure afforded the 3,3'-dimethoxyazetidine **44** in good yield.

Based upon these results we more closely examined why the benzophenone reactions were failing. An NMR study of both reactions was performed. It was clear from the NMR that after formation of the imine **51**, the reductive alkylation did not take place and only benzhydrol was observed resulting for the reduction and hydrolysis of the imine **51**. At this point, it became clear that the benzhydryl- group was much more easily

hydrolyzed than we had originally anticipated; so we searched for new route to achieve the desired cyclization.

A search of the literature turned up another method, specifically designed for the synthesis of benzhydryl azetidinols. Unlike the procedures above, this reaction started with benzhydrylamine and epichlorohydrin. The reaction involved attack of the amine at the chloride bearing carbon which takes place over the course of the first three days, and subsequent epoxide opening and then azetidine formation. Ultimately, the scheme put forth by Anderson and Lok (Scheme 7) proved to be successful in synthesizing the N-benzhydrylazetidin-3-ol (**41**) in good yield.[87]

Scheme 7:



40 **38** **41**

Unfortunately, this reaction suffered from two major draw backs; variability and long reaction times (6-7 days). At times the reaction would give good yields (72%) of a an easily purified product. Other times, the reaction would generate only viscous yellow residue that despite additional reaction time, yielded none of the desired product. Attempts were made to gauge the source of the variability of the reaction; after repeated syntheses we determined that impurities in the benzhydrylamine (**40**) were a key factor. The amine that was used in this work has a 97% purity from the vendor. The best results had been noted to occur from the use of a newly arrived and freshly opened

bottle of the benzhydrylamine. At times, bottles from our vendor would arrive with small precipitates formed. We reasoned that these precipitates were normal to the compound, and perhaps were un-reacted starting material. We noted that unopened containers of the amine in our labs would also form precipitates the longer they sat in our labs. These precipitates would grow over time to the point where they would comprise most of the sample. Later, we would find that exposure of benzyhdrylamine to carbon dioxide forms impurities that are known but not fully characterized by the vendor.

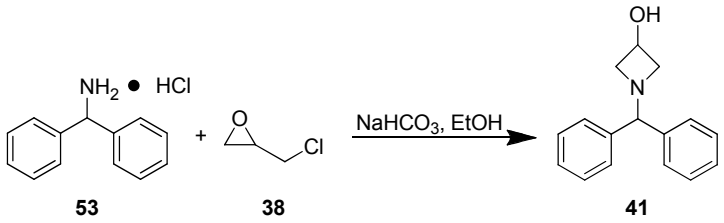
These impurities in the starting material further complicated the issue of long reaction time. Normally, the long reaction time could be overcome by scaling up the reaction so that enough material would be generated for numerous subsequent reactions. Due to the variability of this reaction, if a scaled up reaction were to fail, it would take another week to run another reaction and yet another if more of the amine had to be ordered. This could put work potentially one to two weeks behind, making scale up an improbable and cost ineffective solution to our problem.

We looked again at the literature and found that there were some variations of our previous method. It was thought that at least one of these methods would dampen the interference of impurities in the reaction and perhaps cut down on the reaction time needed. These variations altered both solvents and reaction time (Schemes 8-10), but these were also ultimately abandoned given their much lower (if any) yields.[88-90]

The first of these modifications was put forth by a patent by Aventis (Scheme 8).[90] The procedure in the patent promised a much shorter reaction time in a one pot reaction. The patent starts with freebasing the amine hydrochloride before the addition

of epichlorohydrin and subsequent azetidine formation that takes place over the period of several hours. The use of the more stable benzhydrylamine salt should have cut down on the variability of this reaction. Unfortunately, the only thing we were able to produce with this procedure was a brown sludge. The time frame suggested by the patent seemed at odds with what we were seeing occur in our flasks (and yield wise), so after several failed attempts we pursued other schemes.

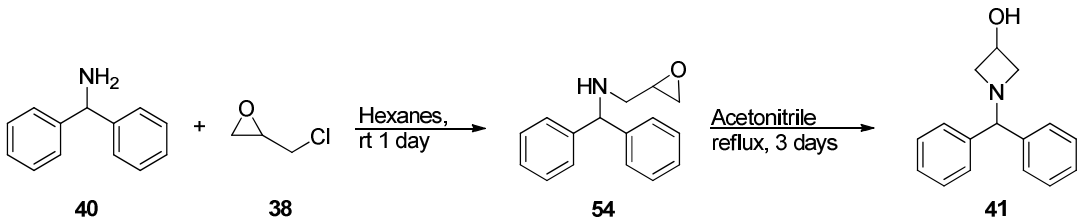
Scheme 8:



53 **38** **41**

The next method was published by Okutani et al (Scheme 9).[88] This procedure breaks the one-pot synthesis of Anderson and Lok into two separate steps with different solvents. The first involves the formation of the epoxypropylamine **54** that takes place in hexanes over the period of 24 h. After this step in the reaction is completed, the solution is evaporated under reduced pressure and then dissolved in acetonitrile and refluxed. This part of the synthesis takes 3 days, similar to the Anderson method but takes off two days of the initial reaction. We repeated this synthesis several times with yields ranging from around 10-59%. Unfortunately, this method also suffered from similar issues of variability due to its use of benzhydrylamine and a lengthy reaction time.

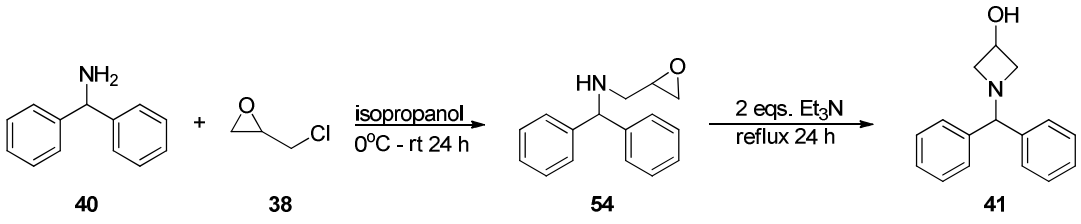
Scheme 9:



40 38 54 41

The last modification we tried was put forth by Oh et al, and it is very similar to the Okutani method (Scheme 10).[89] Unlike the other methods, this suggested a purification of the 2,3-epoxypropylamine (**54**) before the cyclization with triethylamine. It was thought that the inclusion of the purification step would allow for a more easily isolatable azetidinol 41.However, we found that at least two columns were required in order to successfully purify the 2,3-epoxypropylamine **54**. The multiple purifications required to isolate 54 resulted in an overall increase in time required for this procedure. This resulted in nullifying our goal of a shorter (overall) reaction time. The cyclization of both purified and crude materials were attempted and similarly failed. The combination of failed azetidine synthesis and a similar lab time (~5 days) led to this route being abandoned.

Scheme 10:



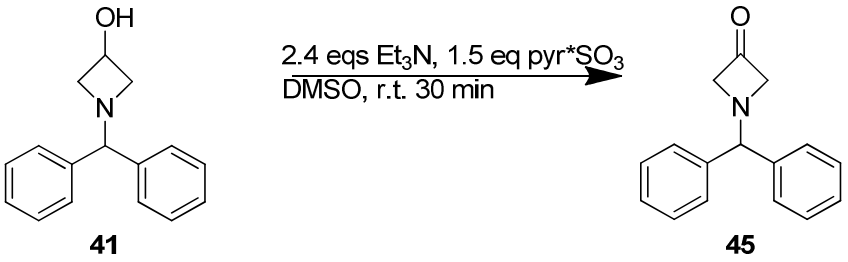
40 38 54 41

The reactions that performed the best for us were Anderson and Lok (Scheme 7) and Okutani et al (Scheme 9). While the two procedures suffered notable problems, their

ability to give good yields and ease of product isolation were desirable. At this point, enough the benzhydrylazetidin-3-ol had been synthesized through various methods that we could proceed with the multiple oxidations to the azetidin-3-one (**45**). The first procedure we used was one by Morimoto for the oxidation of 1-diphenylmethylazetidin-3-ol (Scheme 11).[93] They used a variation of the Swern oxidation, the Parikh-Doering, to generate the desired *N*-benzhydrylazetidin-3-one (**45**). Morimoto and other groups claimed fast reaction time (30 min-1.5 h), mild conditions (room temperature), and good yield.[93,94]

Unlike Swern oxidation, the Parikh-Doering uses pyridine sulphur trioxide complex and DMSO to generate the oxidizing species (alkoxysulfonium ion). The reaction involved first adding anhydrous DMSO to a flame dried round bottom flask which contained the azetidinol. Triethylamine was then added and allowed to stir at room temperature before the addition of pyridine-SO_3 complex in DMSO. The appearance of a yellow color had been noted as an indicator of the success of the oxidation. While we did sometimes observe the appearance of a yellow when the pyridine sulfurtrioxide complex was added, subsequent Wittig olefination of the crude generated very little product. TLCs of the crude ketone showed multiple spots. Since the ketone **45** has been noted to be stable at room temperature, it appeared that that the problem was not due to the degradation of **45**, but low conversion of the starting material. The scheme was repeated with longer reaction times, but yielded similar results.

Scheme 11:



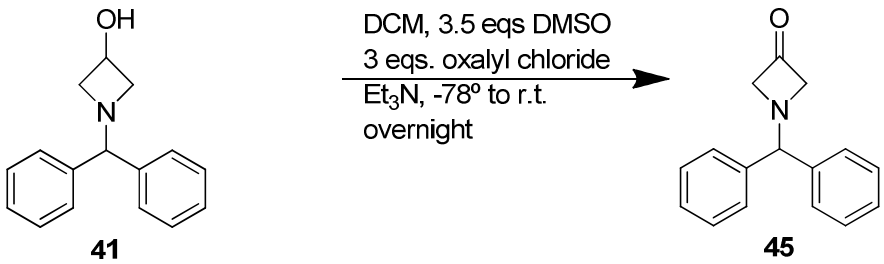
41 **45**

Since the Parikh-Doering procedure failed to yield much of our desired product, we decided to try a traditional Swern oxidation (Scheme 12).[95,96] The oxidizing species can be generated either by the use of trifluoroacetic anhydride or oxalyl chloride. Care must be taken to keep the reaction at low temperature (at least -30°C when using TFAA and -60°C with oxalyl chloride), as intermediates are known to be unstable. Both reagents are extremely reactive to DMSO and without the use of solvent, can be explosive when mixed.

Keeping this in mind, we fitted a dried reaction flask with anhydrous dichloromethane before placing it on a dry ice/isopropanol bath at -78°C. A nitrogen atmosphere was established in the flask before the addition of oxalyl chloride. We next added anhydrous DMSO drop-wise into the solution. The temperature of the reaction was carefully monitored throughout the experiment, especially during the addition of DMSO. After allowing the mixture to stir for 20 minutes or so, the alcohol in anhydrous dichloromethane was added. This is allowed to stir at -78°C for an hour before triethylamine is slowly added. The reaction is then allowed to come up to room temperature over night. Using this methodology, the azetidinone was able to be generated but was not isolated. The crude was dried under vacuum for several hours before being submitted to Wittig olefination. This resulted in a complex mixture of

product and side-products and after several columns, we decided to re-evaluate our overall synthetic approach.

Scheme 12:



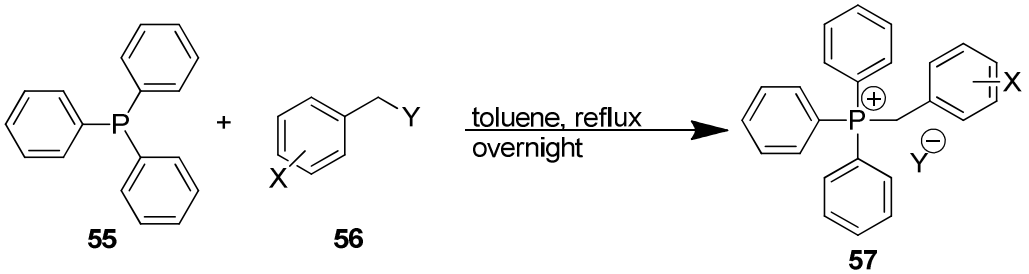
41 **45**

Up until this point in time, we had focused on a benzhydrylazetidin-3-one as our key intermediate. Our initial searches for a commercial supplier yielded very few actual vendors. Of those vendors, their prices for the intermediate were well above what we considered reasonable for library development. Now that some time had passed, a new search revealed that a new supplier had the N-boc-azetidin-3-one available for a good price with high purity (5 g, $125) as opposed to previous suppliers. The decision to use commercially available starting material saved substantial lab time and money via the elimination of several synthetic steps.

With the N-boc-azetidin-3-one in hand, the reaction scheme was considerably shortened. Our next target was the synthesis of phosphonium salts (Scheme 13).[97] The preparation of the Wittig reagent was easily set up and purification was very simple. Triphenylphosphorane and the appropriate benzyl bromide were heated to reflux in toluene. The reaction was allowed to reflux over night and in the morning, was allowed to cool and the precipitate was filtered, washed and dried under reduced pressure. This reaction provided excellent yields (72-98%) part of the desired phosphonium salts.

Yields were considerably better when the benzyl bromide was used instead of the chloride (15% for 3-fluorobenzyltriphenylphosphonium chloride versus 71% for the corresponding bromide), although some of the benzyl halides were only available as the chloride (4-methoxybenzyl chloride). The yields of the phosphonium salts are summarized in Table 5.

Scheme 13:



55 **56** **57**

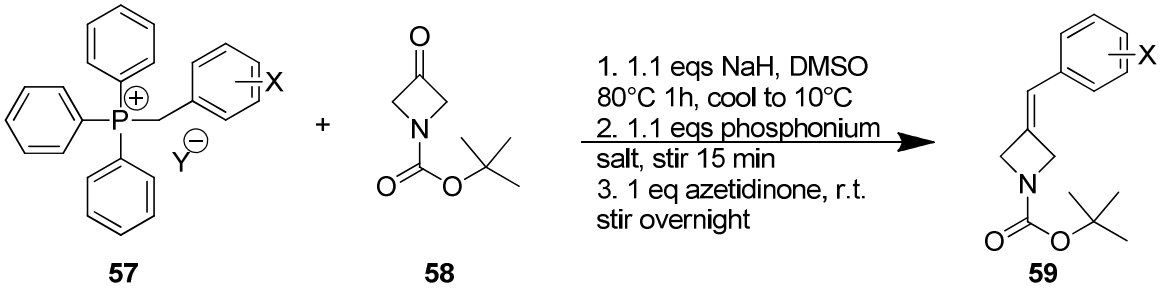
X = H, CH_3, OCH_3, CF_3, Cl, F, Br, I
Y = Br, Cl

Table 5: Summary of triphenylphosphonium salts 57

Compound	X	Y	Yield (%)
57a	H	Br	98
57b	4-CH_3	Br	86
57c	4-OCH_3	Cl	72
57d	4-CF_3	Br	98
57e	2-F	Br	77
57f	3-F	Br	71
57g	4-F	Br	99
57h	3,4-Cl_2	Br	91
57i	3,5-F_2	Br	99
57j	2-Cl	Br	94
57k	3-Cl	Br	94
57l	4-Cl	Br	95
57m	3,4-Cl_2	Br	65
57n	4-Br	Br	97
57o	4-I	Br	77

With both the phosphonium salts **57** and the azetidinone **58** in hand, the olefination could proceed (Scheme 14).[97] The procedure we used involved the production of the dimsyl sodium by heating sodium hydride in DMSO for 1 h. The original publication called for the solution to be chilled to 5℃, but we found that at that temperature, the entire flask of solution would end up freezing. We found that 10℃ was a good compromise, as the reaction was cool enough to handle the excess heat produced by deprotonation of the phosphonium salt **57**, yet the flask was not so cold that the DMSO would freeze entirely. After the addition of the phosphonium salt, the solution would generally change color to a bright orange. The flask was allowed to stir for approximately 15 minutes so that it was uniform in color and any "clumps" would have time to break apart. The color would generally change again once the N-boc-azetidin-3-one **58** was added, usually changing to a clear yellow. After the addition, the mixture was allowed to warm back up to room temperature over night. After an aqueous work-up, the benzylidenes **59** were obtained in good yields (Table 6). The only problems that we experienced running this reaction generally came in the form of high humidity, which negatively impacted yields. Fortunately, unlike some of the previous syntheses the olefination did not experience much variability at all. So the poor yield of some of these reactions could be ameliorated by simply increasing the scale of the reaction. While this would not improve the yield appreciably, it would allow for the generation of the desired amount of benzylidene for testing or hydrogenation.

Scheme 14:



57 **58** **59**

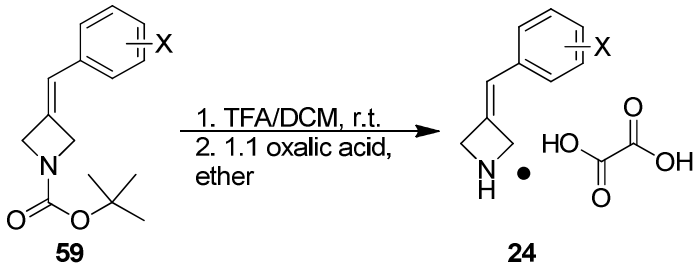
Y = Br, Cl

Table 6: Summary of 3-arylmethylene azetidine carboxylates 59

Compound	X	Yield (%)
59a	H	13
59b	4-CH_3	31
59c	4-OCH_3	16
59d	4-CF_3	59
59e	2-F	63
59f	3-F	60
59g	4-F	61
59h	3,4-F_2	18
59i	3,5-F_2	20
59j	2-Cl	50
59k	3-Cl	59
59l	4-Cl	26
59m	3,4-Cl_2	53
59n	4-Br	43
59o	4-I	51

Deprotection of the benzylidene (Scheme 15) allowed us to probe a more rigid conformation of the azetidine analogs and how our structure would compare with the activity of known amphetamine analogs.

Scheme 15:



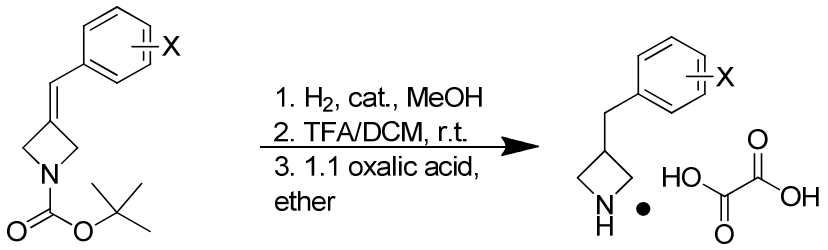
59 **24**

While the deprotection was fairly straightforward on paper, care had to be taken when performing the reaction. It is important for the carboxylate to be dissolved in dichloromethane before the addition of the trifluoroacetic acid. It also was beneficial for an excess amount of dichloromethane compared to the acid as well. We found that for a deprotection of solution concentration of 0.06 M of the carboxylate **59**, in dichloromethane (5 mL) to 1.5-2 mL of trifluoroacetic acid worked best. Amounts less than 1.5 mL of acid deprotected too slowly and allowed the formation of side products. Amounts greater than 2 mL reacted quickly, but suffered from increased side product formation. Upon the addition of the acid, the solution changed to a brownish color and from then on had to be carefully monitored via TLC (1:4 ethyl acetate : hexanes). Most

deprotections carried out in this way took about twenty to forty minutes. It is imperative that once the deprotection is finished, that the reaction be worked up quickly before decomposition products begin to form. After work up, the azetidine is smoothly freebased and then the oxalate salt formed.

The hydrogenated product **25** introduced a somewhat more flexible substrate for us to evaluate (Scheme 16). Like the benzylidene **24**, the position of the nitrogen and the alpha carbon are more rigidly fixed than that of methamphetamine, but the overall geometry is a bit less constrained than that of the benzylidene product.

Scheme 16:

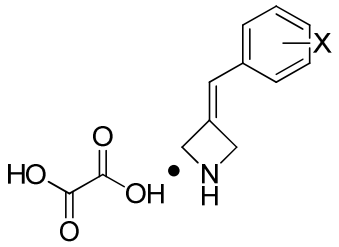


59 **25**

Most substituents (F, CF_3, CH_3) were able to undergo hydrogenation by using palladium on charcoal (10%). The chloro- and iodo- substituents required the use of platinum oxide to prevent dehalogenation. We had attempted several hydrogenations at room temperature using hydrogen balloons, but all of these attempts were unsuccessful in any appreciable addition to the double bond. Therefore, we used a Parr hydrogenator that allowed us to perform the reaction at 45 psi. Even with the hydrogenator, all of the substituents often required at least eight hours for completion. Many benzylidenes were very sluggish, often running 8 hours or more.

With a series of benzylidene derivatives **24** and 3-benzylazetidine derivatives **25** in hand, the compounds were evaluated for binding affinity at the dopamine and serotonin receptors (Table 7). Binding affinities for the dopamine and serotonin transporters were determined by the ability of the drug to displace the radiolabeled ligands [^{3}H]WIN 35,428, and [^{3}H]citalopram, respectively, from the monoamine transporters obtained from rat brain tissue using previously reported assays.[98,99]

Table 7: Binding affinities for benzylideneazetidine analogs at serotonin (SERT) and dopamine (DAT) transporters.



24

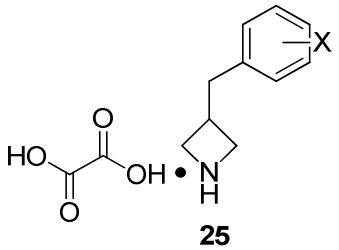
Compound	Code[a]	X	ClogP[b]	SERT[c] K_i (nM)	DAT[c] K_i (nM)	DAT/SERT
24a	AF IV 123	H	2.06	TBD	TBD	TBD
24b	AF IV 109	4-CH_3	2.37	TBD	TBD	TBD
24c	AF IV 133	4-OCH_3	1.95	TBD	TBD	TBD
24d	AF IV 49	4-CF_3	2.82	1,220 ± 50	28,000 ± 8,800	23
24e	AF IV 51	2-F	2.12	1,070 ± 342	3190 ± 322	3
24f	AF IV 119	3-F	2.12	TBD	TBD	TBD
24g	AF IV 169	4-F	2.12	1,840 ± 335	3,190 ± 320	1.7
24h	AF IV 55	3,4-F	2.18	TBD	TBD	TBD
24i	AF IV 77	3,5-F	2.18	TBD	TBD	TBD
24j	AF IV 93	2-Cl	2.67	TBD	TBD	TBD
24k	AF IV 57	3-Cl	2.67	1,330 ± 140	2,820 ± 1,080	2.1
24l	AF IV 105	4-Cl	2.67	TBD	TBD	TBD
24m	AF IV 175	3,4-diCl	3.28	139 ± 32	531 ± 162	3.8
24n	AF IV 171	4-Br	2.76	664 ± 39	1,520 ± 260	2.3
24o	AF IV 121	4-I	2.99	TBD	TBD	TBD

[a]All compounds were tested as the oxalate salts. TBD To be determined
[b]ClogP: see reference.[100]
[c] All values are the mean ± SEM of three experiments preformed in triplicate.

Data as to how benzyl substituent position effects the binding affinities is still forthcoming as more compounds are being tested. Comparing the binding affinities of the two fluoro-substituted analogs; 3-(2-fluorobenzylidene)azetidine oxalate **24e** to the 3-(4-fluorobenzylidene)azetidine oxalate **24g,** hints that placement in the ortho position may favor binding. Of the compounds tested, lipophilicity appears to play the larger role as the most potent compounds tend to be more lipophilic than their cohorts. The best compound in the series was the 3,4-dichloro analog **24m** which had the best binding affinities displayed thus far, but also showed about a 3-fold preference for the serotonin transporter versus the dopamine transporter. A similar trend was seen with the 4-bromo analog **24n**, with an almost 50% better selectivity for the serotonin transporter over the dopamine transporter.

The hydrogenated compounds **25** followed a similar pattern, having better affinities for serotonin transporter versus the dopamine transporter (Table 8). These compounds have an overall lower lipophilicity (1.93-3.09 as compared to 1.95-3.28 in the benzylidene series) than their corresponding benzylidene counterparts which may account for the overall lower affinities. Similar to the benzylidene series, the hydrogenated products which have higher lipophilicity display better affinity. The best compound in the series so far has been the 4-iodo compound **25j** which displays also shows a higher affinity for the serotonin transporter than the dopamine transporter.

Table 8: Binding affinities of 3-benzylazetidines at DAT and SERT.



25

Compound	Code[a]	X	ClogP[b]	SERT K_i (nM)[c]	DAT K_i (nm)[c]	DAT/SERT
25a	AF IV 257	4-CH_3	2.18	TBD	TBD	TBD
25b	AF IV 125	4-CF_3	2.63	TBD	TBD	TBD
25c	AF IV 251	2-F	1.93	TBD	TBD	TBD
25d	AF IV 207	3-F	1.93	3,200 ± 300	5,200 ± 1400	1.6
25e	AF IV 187	4-F	1.93	2,730 ± 460	7,670 ± 338	2.8
25f	AF IV 81	3,4-diF	1.98	TBD	TBD	TBD
25g	AF IV 179	3,5-diF	1.98	2,800 ± 50	12,600 ± 1,400	4.5
25h	AF IV 231	4-Cl	2.48	2,250 ± 460	2,240 ± 310	0.99
25i	AF IV 259	3,4-diCl	3.09	TBD	TBD	TBD
25j	AF IV 227	4-I	2.8	455 ± 55	1,290 ± 400	2.8

[a]All compounds were tested as the oxalate salts.
[b]ClogP see reference.[100]
[c] All values are the mean ± SEM of three experiments preformed in triplicate.

It is interesting to note that the 3,5-difluoro analog **25g**, displays such poor binding affinities for both transporters. Comparing the series of products; the benzylidenes provide better selectivity and higher affinities for the dopamine and the serotonin transporters. Of the currently tested compounds, the data suggests that lipophilicity

plays a significant role in the binding affinities of these analogs as the more lipophilic they are the better their K_i value.

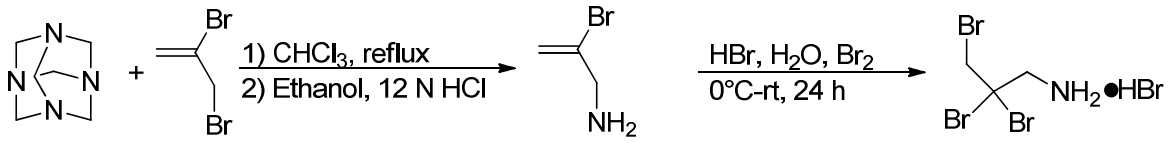
CONCLUSION

A series of 3-benzylideneazetidines (**24**) and 3-benzylazetidines (**25**) were synthesized from N-Boc-3-azetidinone. N-Boc-azetidin-3-one was converted into the 3-benzylideneazetidine carboxylates **59** via Wittig olefination in good yields (13-63%). Subsequent deprotection and conversion to the oxalate salt gave the 3-benzylideneazetidines (**24**). The 3-benzylazetidines (**25**) were also prepared from **59** via hydrogenation followed by deprotection and conversion into the oxalate salt.

Based upon preliminary binding studies at the dopamine and serotonin transporters, the 3-benzylideneazetidines (**24**) and 3-benzylazetidines (**25**) meet the pharmacophore requirements for binding. Although the affinity of some analogues was low, the lipophilic analogues (**24m, 24n** and **25j**) indicate that with the proper substituent it may be possible to develop more potent ligand. Clearly the results of these studies indicate that the azetidine ring system could be a useful scaffold for development of novel compounds to explore the binding motifs at monoamine transporters.

EXPERIMENTAL SECTION

All chemicals were purchased from Sigma Aldrich Chemical Company and used as received unless otherwise noted. Proton and carbon NMR were recorded on a Varian 400 MHz nuclear magnetic resonance spectrometer at ambient temperature in deuterated chloroform ($CDCl_3$) from Cambridge Isotope Laboratories Inc, unless otherwise noted. ^1H NMR chemical shifts are reported as delta values (ppm) relative to chloroform-d (7.26 ppm). Melting points (mp) were measured with an Electrothermal R Mel-Temp apparatus and are uncorrected.



102 **103** **104** **43**

2,2,3-tribromopropylamine hydrobromide (43)[92]

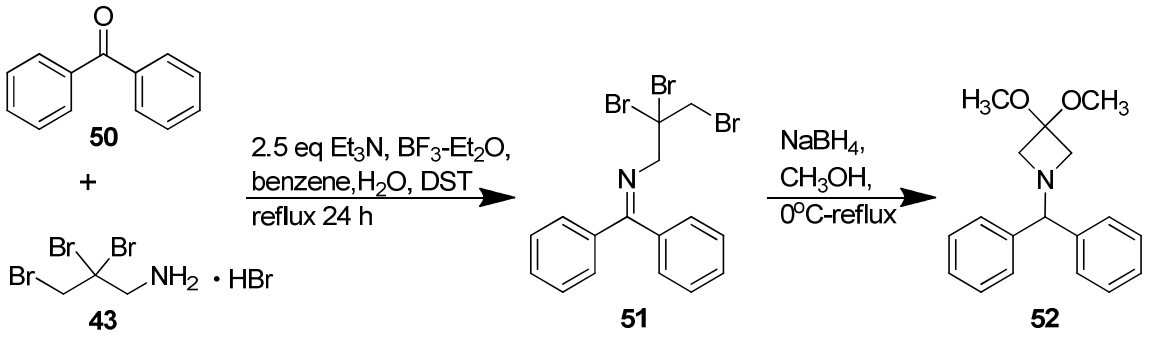
Hexamethylenetetramine (100 grams, 0.71 mol) was added to a 1 L three-neck round bottom flask where it was dissolved in chloroform (800mL). The flask was fitted with a condenser and an addition funnel. The dibromopropene (126 g, 0.898 mol) was added drop-wise over a period of 40 min while the mixture was heated to reflux. The solution went from clear to a milky yellow color and refluxed for an additional 3 h. The mixture was then allowed to cool and stand overnight. The flask was then placed on an ice bath to precipitate the salt. The precipitate was then vacuum filtered and the salt was allowed to air dry overnight to yield an off-white solid.

In a 3 L round bottom flask, 2 liters of ethanol were added along with 480 mL of 12N HCl, 400 mL of H_2O and the salts stirred for 1 h at room temperature and then allowed

to stand for 24 h. This led to a precipitate which was vacuum filtered. The filtrate was then concentrated to near 600 mL to precipitate more of the orange salt. The filtrate was filtered again before being concentrated to dryness. This residue was then dissolved in 300 mL of water and pH adjusted with 6N NaOH to pH 13. This mixture was then extracted with ether (3 x 100mL) and the combined ether solutions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was then distilled under reduced pressure to afford the amine as an off-white solid which was immediately used in the next step.

The amine (41.8 g, 307 mM) was dissolved in 70 mL of water and cooled to 0 C via an ice bath. HBr (48% solution) (40.5 mL, 0.36 mol) was added slowly with stirring, followed by slow addition of Br_2 (25 mL, 0.49 mol). The mixture was stirred at room temperature for 24 h. The mixture was then concentrated under reduced pressure to afford the amine salt (43) as an orange-white solid (74 g, 49% yield). ^{1}H NMR (400 MHz, D_2O) δ 4.35 (s, 2 H), 3.82 (s, 3 H).

Attempted Synthesis of Azetidine 52[92]

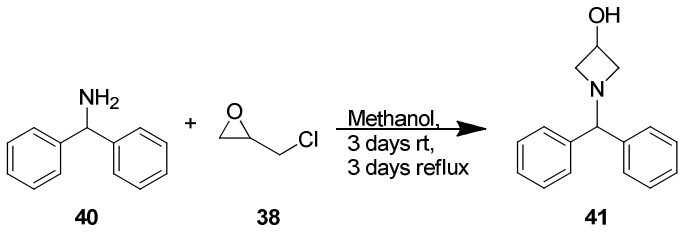


To a 250 mL round bottom flask, the 2,2,3-tribromopropylamine hydrobromide (4.02 g, 11 mmol) was added along with a stir bar and 100 mL benzene and 10 mL of water.

Next, 4,4’-dichlorobenzophenone (2.78 g, 11 mmol) was added along with 2.5 equivalents of triethylamine (3 mL, 21.5 mmol). Five drops of boron trifluoride diethyl etherate were added to promote the reaction. A Dean Stark trap was fitted to the reaction apparatus to siphon off any of the water produced by the reaction, to help drive the equilibrium. This reaction was heated to reflux and run overnight and monitored by TLC. The reaction was then cooled to room temperature and diethyl ether was added to precipitate the ammonium chloride salt which was then filtered. The filtrate was concentrated under reduced pressure to afford the crude imine which was then immediately utilized in the next step.

The crude imine was dissolved in dry methanol and placed in an ice bath under Nitrogen. Three equivalents of sodium borohydride(1.13 g, 29.8 mmol) were added, portion-wise over a period of forty-five minutes. The mixture was allowed to stir and come back up to room temperature before being refluxed over night. Despite numerous procedures and manipulations, the reduction failed to yield the acetal and instead generated the corresponding benzhydrol.

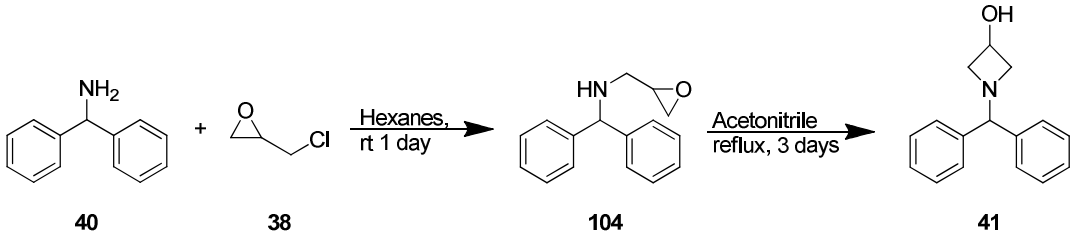
Anderson and Lok Procedure for the preparation of N-benzhydryl azetidin-3-ol (41)[87]



40 38 41

A stir bar was added to a 250 mL round bottom flask that was then hooked up to a condenser and placed on a heating mantle over a stir plate. This was then flushed with dry Nitrogen gas for 5 min and wrapped in foil to prevent light from entering the sides of the vessel. Benzhydrylamine (3.1 mL, 17 mmol) was added first and then 25-30 mL of methanol. Epichlorohydrin (1.4 mL, 17 mmol) was then added to the stirring mixture along with an additional 20-25 mL of methanol. Once the epichlorohydrin and methanol were added, the solution was allowed to stir at room temperature for 3 d, protected from light. After three days, the reaction was heated to reflux for 3 d. After the three days, the mixture was evaporated under reduced pressure, revealing a thick, amber residue. The residue was washed with acetone (3 x 30 mL) and the solid reserved. The wash can be evaporated under reduced pressure and then refluxed in methanol for an increased yield, but this was found to give only a fractional percentage increase. The solid was then partitioned between ether and a 6 N NaOH solution. The ether was evaporated to give N-benzhydryl azetidin-3-ol (41) in 48-60% yield. m.p 85-90℃ (sublimation). (lit. m.p. 113℃).[87] ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.86-2.89 (m, 2 H), 3.51-3.55 (m, 2 H), 4.33 (s, 1 H), 4.46 (s, 1 H), 7.16-7.28 (m, 8 H), 7.37-7.51 (m, 3 H). ^{13}C (400 MHz, $CDCl_3$) 62.4, 63.6, 78.6, 127.3, 128.6, 142.2.

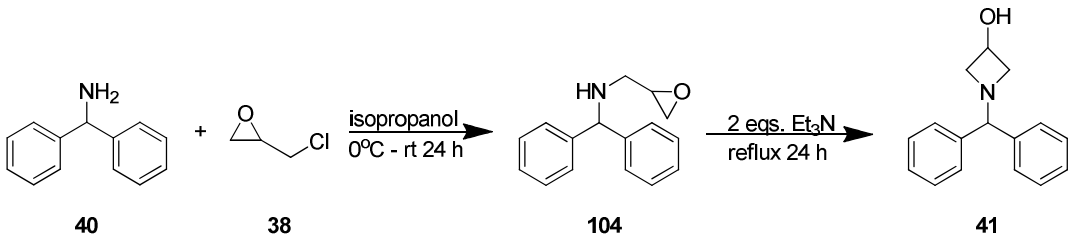
Alternative Method for N-Benzhydryl Azetidin-3-ol (41)[88]



40 **38** **104** **41**

To a 250 mL round bottom flask, epichlorohydrin (2 ml, 2.5 mmol) was added slowly to benzhydrylamine (3 mL, 17 mmol) in hexanes (10 mL). This was allowed to stand at room temperature for 1 day. After the time was completed, the solvent was removed under reduced pressure and the residue is dissolved in acetonitrile (30mL) and refluxed for 3 days. The solution was then evaporated under reduced pressure and partitioned between ether and 12 N NaOH. The ether layer was reserved, dried and condensed under reduced pressure to reveal the N-benzhydrylazetidin-3-ol (2.4 g, 59%).

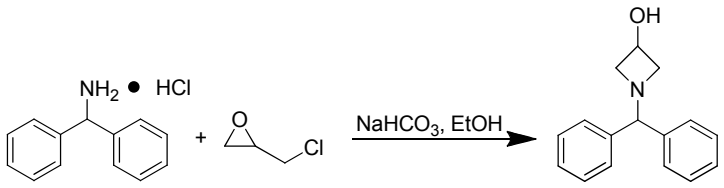
Alternative Method for the synthesis of N-Benzhydryl Azetidin-3-ol (41)[89]



40 **38** **104** **41**

A 250 mL round bottom flask containing benzhydrylamine (1 g, 5.5 mmol) in isopropanol (10 mL) under a Nitrogen atmosphere was placed on an ice bath (0° C). Epichlorohydrin (0.5 mL, 5.5 mmol) was added drop-wise with stirring. The mixture was allowed to warm up to room temperature over night with stirring. After 24 h, the solution was concentrated under reduced pressure and the residue purified by flash chromatography (SiO_2, 10:1 hexanes:ethyl acetate). The epoxyamine 106 is dissolved in acetonitrile (36 mL) and then added to a 250 mL round bottom flask. Three equivalents of triethyl amine (8 mL, 57 mmol) is added and the solution allowed to reflux for 24 h. The reaction was then cooled to room temperature and concentrated under reduced pressure. The residue was then purified by column chromatography (2:1 hexanes: ethyl acetate), but yielded none of the desired product.

Alternative Method for N-Benzhydryl Azetidin-3-ol (41)[90]

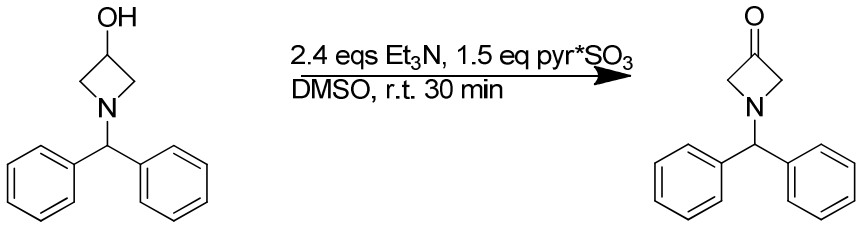


53 **38** **41**

The benzhydrylamine hydrochloride (0.2 g, 0.9 mmol) was added to a 50 mL round bottom flask with stir bar. Ethanol (13 mL) was also added, along with sodium bicarbonate (0.14 g, 1.7 mmol) was added and the flask was fitted to a condenser and heated to 80℃. After 1 h, the reaction was allowed to cool to 55℃ and epichlorohydrin (0.2 mL, 2.47 mmol) was added drop-wise. The reaction was allowed to continue stirring at 55℃ for the next 4 hours. Next, the reaction was allowed to cool to 30℃ and toluene (15 mL) and water (15 mL) were added. The reaction was allowed to spin for 30 min. The water layer was discarded and the organics were washed with unionized water (25 mL). Hydrobromic acid (48% solution; 4 mL) was added and the flask placed on an ice-bath to try to aid crystallization, but only a brown sludge was observed.

Oxidation of N-Benzhydrylazetidin-3-ol (41) to N-Benzhydrylazetidin-3-one (45)

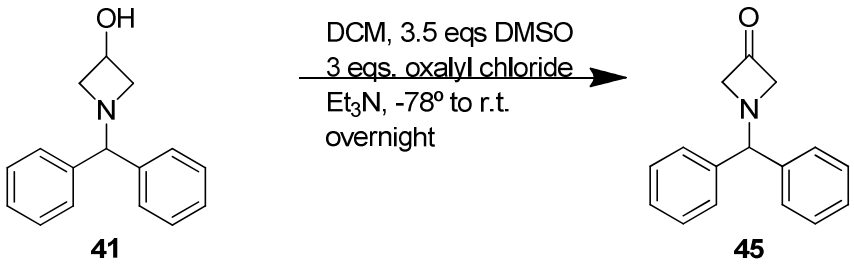
Parikh-Doering Oxidation[94]



41 **45**

To a 100 mL, dry round bottom flask, a solution of dry DMSO (10 mL) and azetidinol (0.4 g, 1.7 mmol) was added. Triethylamine (0.6 mL, 4.3 mmol) and the pyridine sulfur trioxide complex (0.602 g, 3.8 mmol) in DMSO (10 mL) were added at room temperature and the mixture was allowed to stir for 30 min. This was then poured into 50 mL of ice water and then extracted with ethyl acetate (3 x 30 mL). The organics were combined and then washed with water (50 mL) and dried over $MgSO_4$ and concentrated under reduced pressure. This reaction failed to yield an appreciable amount of the desired azetidinone.

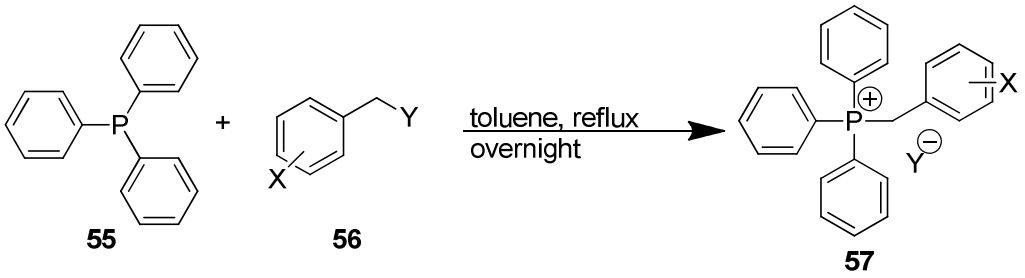
Swern Oxidation[95,96]



41 **45**

To a flame dried, 250 mL round bottom flask, a stir bar and 10 mL of dry dichloromethane were added. This was placed on a dry ice/isopropanol bath (-78°C) and allowed to stir while being flushed with dry nitrogen. Oxalyl chloride (0.6 mL, 7 mmol) was added and allowed to stir before the slow addition of DMSO (0.6 mL, 8.44 mmol). The mixture was allowed to stir 20 min. Benzhydryl azetidinol (0.66 g, 2.75 mmol) was weighed out and dissolved in dry dichloromethane before being added drop-wise to the mixture. After one hour of stirring at -78°C, triethylamine (2 mL, 14 mmol) was added slowly. The mixture was allowed to stir and warm up over night. The reaction was worked up by diluting it with 25 mL of ethyl acetate and concentrated under reduced pressure on low heat. The residue was dried under high vacuum and used as the crude in subsequent Wittig olefination.

General Procedure A. Synthesis of Benzyl Phosphonium Salts (57)[97]

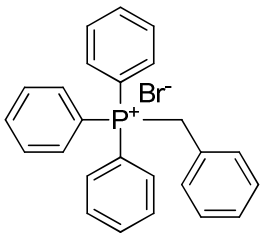


55 **56** **57**

X = H, CH_3, OCH_3, CF_3, Cl, F, Br, I
Y = Br, Cl

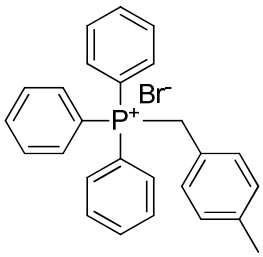
The benzyl halide (3 mL, 21.3 mmol) was added to a dry, 250 mL round-bottom flask containing 1.1 equivalents of triphenylphosphine (5.5 g, 23.4 mmol). The mixture was then dissolved in toluene (70 mL) and refluxed over night. The reaction was cooled to room temperature and filtered. The precipitate (usually a white solid) was washed with diethyl ether (3x50 mL) and dried thoroughly under vacuum before use.

(benzyl)triphenylphosphine bromide (57a)



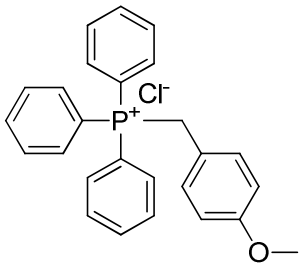
General Procedure A; white solid (5.5 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.42 (d, 2 H, J = 12), 7.08-7.14 (m, 4 H), 7.60-7.66 (m, 6 H), 7.71-7.78 (m, 9 H). m.p. 280-292°C. (lit. m.p.296-297°C).[101]

(4-methylbenzyl)triphenylphosphonium bromide (57b)



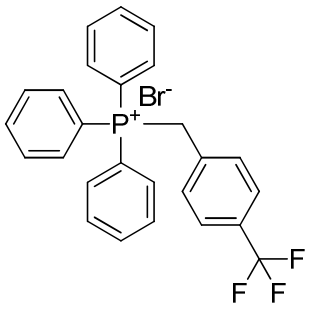
General Procedure A; white solid (2.0 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.25 (d, 3 H, J = 4), 5.34 (d, 2 H, J = 14), 6.92-6.97 (m, 4 H), 7.61-7.64 (m, 5 H), 7.71-7.79 (m, 9H). m.p. 254-260°C. (lit. m.p.256-258°C).[102]

(4-methoxybenzyl)triphenylphosphonium chloride (57c)



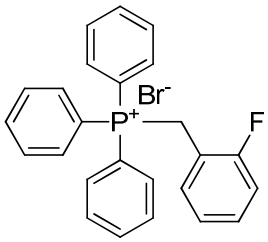
General Procedure A; white solid (5.3 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78-7.73 (m, 9 H), 7.63-7.60 (m, 6 H), 7.03-7.00 (m, 2 H), 6.66-6.64 (d, 2 H), 5.43 (d, 2 H), 3.72 (s, 3 H). m.p. 222-226°C. (lit. m.p. 228-230°C).[103]

(4-trifluoromethylbenzyl)triphenylphosphine bromide (57d)



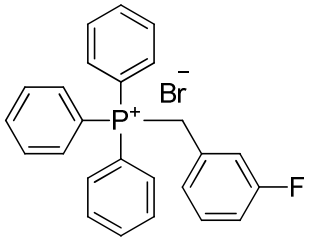
General Procedure A; white solid (4.1 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.64 (d, 2 H, J = 16), 7.29-7.36 (m, 2 H), 7.37 (d, 2 H, J = 8), 7.62-7.66 (m, 6 H), 7.75-7.80 (m, 9 H). m.p. 225-230℃.

(2-fluorobenzyl)triphenylphosphonium bromide (57e)



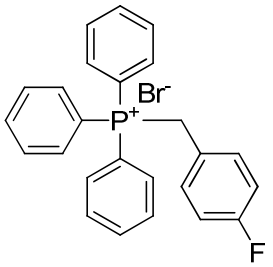
General Procedure A; white solid (5.6 g, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34 (d, 2 H, J = 14.4 Hz), 6.81-6.86 (t, 1 H, J = 8.8 Hz), 6.99-7.04 (m, 1 H), 7.42-7.46 (m, 2 H), 7.64-7.81 (m, 13 H). m.p. 230-242℃.

(3-fluorobenzyl)triphenylphosphonium bromide (57f)



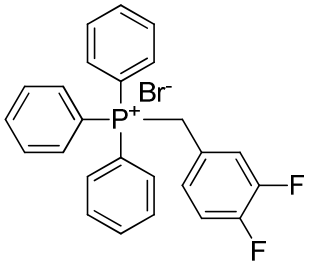
General Procedure A; white solid (5.1 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.53 (d, 2 H, J = 16 Hz) 6.70 (d, 1 H J = 12), 6.91-6.95 (t, 1 H J = 8), 7.05-7.14 (m, 2 H), 7.63-7.68 (m, 6 H), 7.75-7.81 (m, 9 H). m.p. 298-308℃. (lit. m.p. 290-292℃).[102]

(4-fluorobenzyl)triphenylphosphonium bromide (57g)



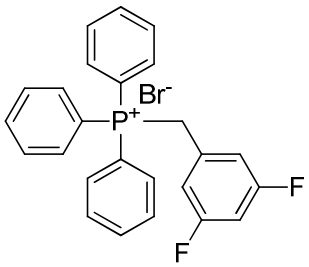
General Procedure A.; white solid (8.7 g, 99% yield). ^{1}H NMR (400 MHz, CDCl3) δ 5.51 (d, 2 H, J =14 Hz), 6.78-6.8 (m, 2 H), 7.12-7.16 (m, 2 H), 7.61-7.65 (m, 6 H), 7.79-7.74 (m, 9H). m.p. 297-303℃. (lit. m.p. 280-282℃).[102]

(3,4-difluorobenzyl)triphenylphosphonium bromide (57h)



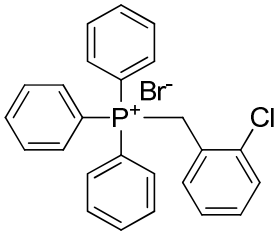
General Procedure A; off-white solid (4.9 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.46 (d, 2 H, J = 14.4 Hz), 6.86 (t, 1 H, J = 18.4 Hz), 6.91-6.99 (m, 1 H), 7.65-7.82 (m, 15 H). m.p. 312-318℃.

(3,5-difluorobenzyl)triphenylphosphonium bromide (57i)



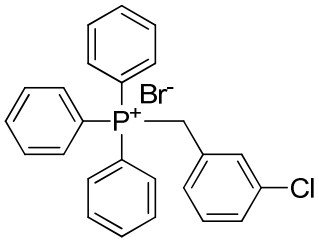
General Procedure A; white solid (7.0 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.71 (d, 2 H, J = 15.2 Hz), 6.58-6.68 (m, 1 H), 6.73 (d, 2 H, J = 8 Hz) 7.62-7.67 (m, 6 H), 7.76-7.85 (m, 9 H). m.p. 335-344℃. (lit. m.p.> 250℃).[104]

(2-chlorobenzyl)triphenylphosphonium bromide (57j)



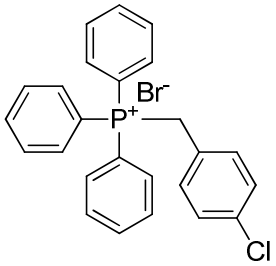
General Procedure A; white solid (4.6 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.69 (d, 2 H, J = 12 Hz), 7.13-7.22 (m, 3 H), 7.59-7.62 (m, 7H), 7.71-7.81 (m, 8 H). m.p. 197-201℃.

(3-chlorobenzyl)triphenylphosphonium bromide (57k)



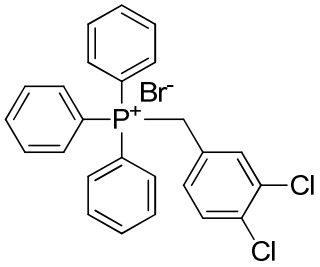
General Procedure A; white solid (5.8 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.55 (d, 2 H, J = 14.8 Hz), 6.80 (s, 1 H), 7.09 (t, 1 H, J = 8 Hz), 7.18 (s, 1 H), 7.63-7.66 (m, 6 H), 7.77-7.88 (m, 9 H). m.p. 296-300℃. (lit. m.p. 310℃).[105]

(4-chlorobenzyl)triphenylphosphonium bromide (57l)



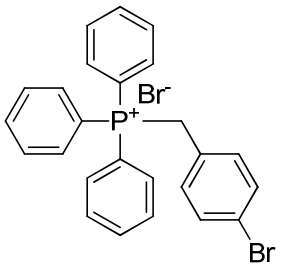
General Procedure A; white solid (4.8 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.71 (d, 2 H, J = 12 Hz),6.98 (t, 1 H, J = 8 Hz), 7.52-7.66 (m, 6 H), 7.75-7.86 (m, 10 H). m.p. 270-275℃.

(3,4-dichlorobenzyl)triphenylphosphonium bromide (57m)



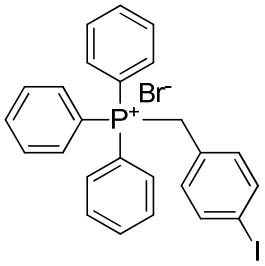
General Procedure A; white solid (3.6 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.57 (d, 2 H, J = 14.8 Hz), 6.93 (t, 1 H, J = 2 Hz), 7.194-7.256(m, 2 H), 7.63-7.68 (m, 6 H), 7.77-7.83 (m, 9 H) m.p. 296-305℃.

(4-bromobenzyl)triphenylphosphonium bromide (57n)



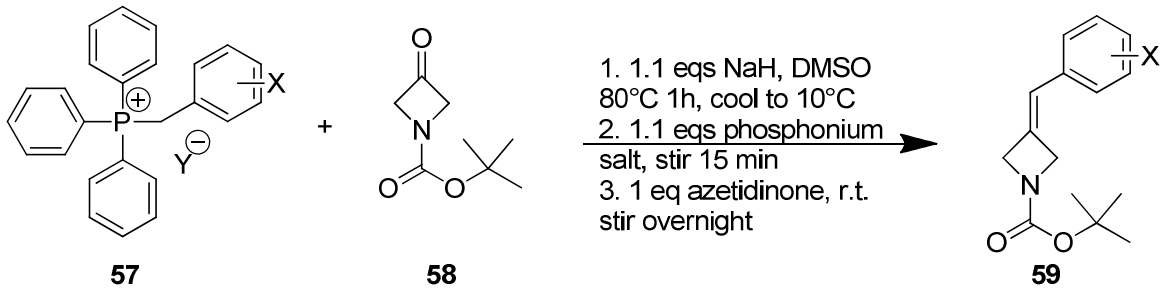
General Procedure A; white solid (5.5749 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.55 (d, 2 H, J = 16), 7.00-7.06 (m, 2 H), 7.22 (d, 2 H, J = Hz), 7.60-7.65 (m, 5 H), 7.75-7.90 (m, 10 H). m.p. 268-272°C. (lit. m.p. 276-277°C).[106]

(4-iodobenzyl)triphenylphosphonium bromide (57o)



General Procedure A; brown solid (2.2 g, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.81-6.84 (m, 2 H), 7.5 (d, 2 H, J = 16), 7.64-7.69 (m, 12 H), 7.78-7.81 (m, 3 H). m.p. 231-240°C. (lit. m.p. 255-256°C).[107]

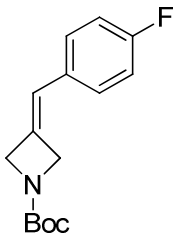
General Procedure B. Synthesis of 3-(arylmethylene)-azetidines (59)



57 **58** **59**

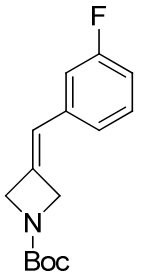
To a flame dried, 3-neck 250 mL round bottom flask, 1.1 equivalents of sodium hydride (60% dispersion in mineral oil) was added. Nitrogen was continuously blown through the flask as 25 mL of anhydrous DMSO was added. The mixture was heated to 80°C for about one hour. The mixture was then cooled to 5°C on an ice-bath and allowed to stir. The atmosphere was maintained as 1.1 equivalents of the appropriate phosphonium salt was added. The mixture was allowed to stir for ten minutes before adding 1 equivalent of N-Boc-azetidin-3-one. The mixture was allowed to warm to room temperature and stir overnight. The reaction was worked up by being poured into an icy, saturated sodium bisulfate solution. This was extracted with dichloromethane (3 x 50 mL) and dried over sodium sulfate before being concentrated. The residue was purified via column chromatography (SiO_2, 1:4 EtOAc:Hexanes).

***t*-butyl 3-(4-fluorobenzylidene)azetidine-1-carboxylate (59a)**



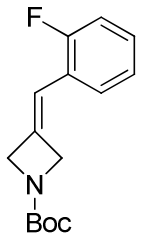
General Procedure B; white solid (0.48 g, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57 (s, 9 H), 4.63 (s, 2 H), 4.80(s, 2 H), 6.22 (s, 2 H), 7.00-7.09 (m, 4 H). ^{13}C NMR (300 MHz, $CDCl_3$) 28.4, 35.8, 115.6, 115.8, 128.2, 128.6, 128.7. m.p. 60-69℃. Anal. Calc. for $C_{15}H_{18}FNO_3$: C 68.41; H 6.89; N 5.31. Found: C 77.68, H 10.67, N 2.47.

***t*-butyl 3-(3-fluorobenzylidene)azetidine-1-carboxylate (59b)**



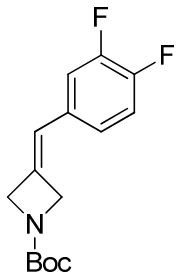
General Procedure B; white solid (0.46 g, 60%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (s, 9 H), 4.82 (s, 2 H), 4.64 (s, 2 H), 6.24 (s, 1 H), 6.77-6.79 (m, 1 H), 6.87-6.94 (m, 2 H), 7.30-7.32 (m, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 28.9, 55.9, 114.0, 114.2, 121.6, 130.4, 135.5. m.p. 75-80℃. Anal. Calc. for $C_{15}H_{18}FNO_3$: C 68.41; H 6.89; N 5.31. Found: C 67.79, H 6.99, N 5.14.

***t*-butyl 3-(2-fluorobenzylidene)azetidine-1-carboxylate (59c)**



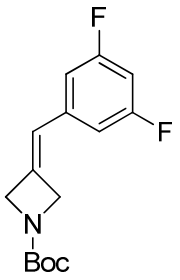
General Procedure B; off-white solid (0.7 g, 63%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 9 H), 4.20 (s, 2 H), 4.78 (s, 2 H), 6.5 (s, 1 H), 6.99-7.19 (m, 3 H), 7.18-7.21 (m, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$) 28.4, 79.9, 114.6, 115.4, 115.8, 124.2, 127.6, 128.6, 128.7. m.p 49-52℃. Anal. Calc. for $C_{16}H_{18}FNO_2$: C 68.41, H 6.89, N 5.31. Found: C 68.64, H 7.06, N 5.14.

***t*-butyl 3-(3,4-difluorobenzylidene)azetidine-1-carboxylate (59d)**



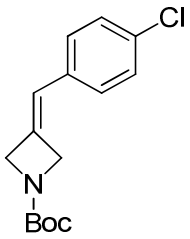
General Procedure B; white solid, (0.18 g, 18%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (s, 9 H), 4.63 (s, 2 H), 4.78 (s, 2 H), 6.18 (s, 1 H), 6.83-6.99 (m, 2 H), 7.10-7.15 (m, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 28.6, 58.8, 115.7, 115.9, 117.7, 177.9, 120.7, 123.4. m.p. 116-120℃. Anal. Calc. for $C_{16}H_{17}F_2NO_2$: C 64.04; H 6.09; N 4.98. Found: C 63.87, H 6.21, N 4.94.

***t*-butyl 3-(3,5-difluorobenzylidene)azetidine-1-carboxylate (59e)**



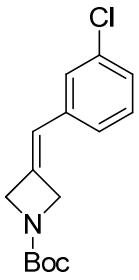
General Procedure B; white solid (0.2 g, 20%).^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (s, 9 H), 4.64 (s, 2 H), 4.81 (s, 2 H), 6.2 (s, 1 H), 6.59-6.68 (m, 3 H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 31.2, 57.0, 101.6, 101.9, 102.3, 109.7, 109.9, 119.6, 161.6, 164.9. m.p. 118-125℃. Anal. Calc. for $C_{16}H_{17}F_2NO_2$: C 64.04; H 6.09; N 4.98. Found: C 63.65, H 6.32, N 4.83.

***t*-butyl 3-(4-chlorobenzylidene)azetidine-1-carboxylate (59f)**



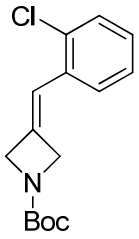
General Procedure B; white solid (0.42 g, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3 (d, 2 H), 7.0 (d, 2 H), 6.22 (s, 1 H), 4.8 (s, 2 H), 4.6 (s, 2 H), 1.4 (s, 9 H). ^{13}C NMR (300 MHz, $CDCl_3$) 134.8, 131.7, 129.1, 128.5, 121.4, 80.1, 58.9, 28.6. m.p. 130-135℃. Anal. Calc. for $C_{15}H_{18}NO_2Cl$: C 64.4; H 6.48; N 5.00. Found: C 64.22, H 6.64, N 4.92.

***t*-butyl 3-(3-chlorobenzylidene)azetidine-1-carboxylate (59g)**



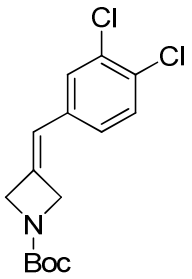
General Procedure B; thick, yellow oil (0.58 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 9 H), 4.64 (s, 2 H), 4.81 (s, 2 H), 6.21 (s, 1 H), 6.98 (d, 1H, J = 4), 7.18-7.28 (m, 2 H). ^{13}C NMR (300 MHz, $CDCl_3$) 28.6, 58.9, 121.4, 125.4, 130.1. Anal. Calc. for $C_{15}H_{18}NO_2Cl$: C 64.4; H 6.48; N 5.00. Found: C 61.56; H 6.47; N 4.57.

***t*-butyl 3-(2-chlorobenzylidene)azetidine-1-carboxylate (59h)**



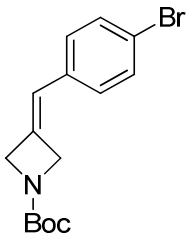
General Procedure B; yellow oil (0.42 g, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (s, 9 H), 4.67 (s, 2 H), 4.78 (s, 2 H), 6.67 (s, 1 H), 7.10-7.18 (m, 3 H), 7.38 (d, 1 H, J= 8 Hz). ^{13}C NMR (300 MHz, $CDCl_3$) δ 28.6, 58.8, 80.1, 118.9, 127.1, 127.6, 128.5, 130.1, 131.8, 132.4, 132.5, 133.2, 133.4.

***t*-butyl 3-(3,4-dichlorobenzylidene)azetidine-1-carboxylate (59i)**



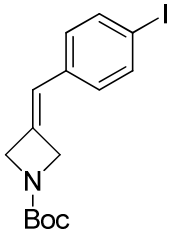
General Procedure B; white solid, (0.62 g, 53%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55 (s, 9 H), 4.64 (s, 2 H), 4.8 (s, 2 H), 6.18 (s, 1 H), 6.92 (d, 2 H, J= 7.5 Hz), 7.18 (s, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 28.4, 58.7, 120.5, 126.4, 129.1, 130.9. m.p. 90-105°C. Anal. Calc. for $C_{16}H_{17}Cl_2NO_2$: C 57.33; H 5.45; N 4.46. Found: C 54.65, H 5.21, N 4.17.

***t*-butyl 3-(4-bromobenzylidene)azetidine-1-carboxylate (59j)**



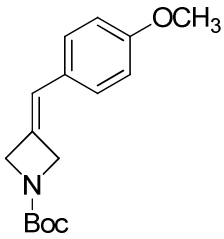
General Procedure B; white solid, (0.38 g, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 9 H), 4.62 (s, 2 H), 4.78 (s, 2 H), 6.20 (s,1 H), 6.97 (t, 2 H, J = 8 Hz), 7.45 (d, 2 H, J = 8 Hz). ^{13}C NMR (300 MHz, $CDCl_3$) δ 28.6, 100.2, 121.5, 128.8, 132.1. m.p. 110-115°C. Anal. Calc. for $C_{15}H_{18}NO_2Br$: C 55.57; H 5.60; N 4.32. Found: C 55.28, H 5.45, N 4.35.

***t*-butyl 3-(4-iodobenzylidene)azetidine-1-carboxylate (59k)**



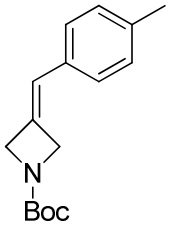
General Procedure B; yellowish solid (0.4 g, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 9 H), 4.61 (s, 2 H), 4.77 (s, 2 H), 6.18 (s, 1 H), 6.83 (d, 2 H, J = 8 Hz), 7.65 (d, 2 H, J = 8 Hz). ^{13}C NMR (300 MHz, $CDCl_3$) δ 28.4, 58.7, 115.6, 115.8, 128.2, 128.6, 128.7, 135.2, 162.9. m.p. 78-85°C. Anal. Calc. for $C_{15}H_{18}NO_2I$: C 48.53; H 4.89; N 3.77. Found: C 50.71, H 5.11, N 3.32.

_t_-butyl 3-(4-methoxybenzylidene)azetidine-1-carboxylate (59l)



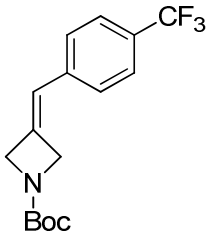
General Procedure B; waxy solid (0.18 g, 16%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 9 H), 3.80 (s, 3 H), 4.61 (s, 2 H), 4.79 (s, 2 H), 6.19 (s, 1 H), 6.86 (d, 2 H, J = 4 Hz), 7.03 (d, 2 H, J = 8 Hz). ^{13}C NMR (300 MHz, $CDCl_3$) δ 28.6, 29.9, 55.5, 59.1, 114.4, 122.5, 127.3 (2), 127.6, 128.5, 129.9. m.p. 40-45°C. Anal. Calc. for $C_{16}H_{21}NO_3$: C 69.79; H 7.68; N 5.09. Found: C 72.46, H 8.63, N 3.92.

_t_-butyl 3-(4-methylbenzylidene)azetidine-1-carboxylate (59m)



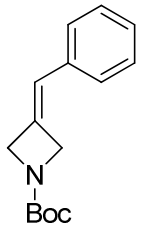
General Procedure B; white solid (0.21 g, 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 9 H), 2.33 (s, 3 H), 4.62 (s, 2 H), 4.81 (s, 2 H), 6.22 (s, 1 H), 7.00 (d, 2 H,J = 8 Hz) 7.14 (d, 2 H, J = 8 Hz) ^{13}C NMR (300 MHz, $CDCl_3$) δ 21.4, 28.6, 29.9, 122.3, 127.3, 129.6, 133.6, 137.1, 156.5. m.p. 63-65°C. Anal. Calc. for $C_{16}H_{21}NO_2$: C 74.10; H 8.16; N 5.40. Found: C 73.61, H 8.22, N 5.38.

***t*-butyl 3-(4-trifluoromethylbenzylidene)azetidine-1-carboxylate (59n)**



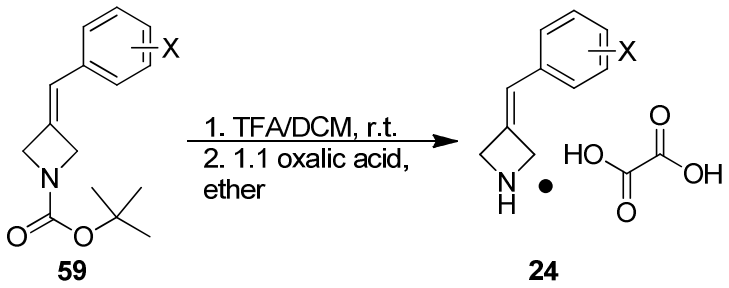
General Procedure B; light yellow solid (0.64 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 9 H), 4.66 (s, 2 H), 4.84 (s, 2 H), 6.31 (s, 1 H), 7.20 (d, 2 H, J = 8 Hz), 7.58 (d, 2 H, J = 8 Hz). ^{13}C NMR (300 MHz, $CDCl_3$) δ 28.6, 59.0, 121.5, 125.9 (2), 127.4, 134.2. m.p. 110-115°C. Anal. Calc. for $C_{16}H_{18}F_3NO_2$: C 56.79; H 5.36; N 4.14. Found: C 61.15, H 5.81, N 4.39.

***t*-butyl 3-(benzylidene)azetidine-1-carboxylate (59o)**



General Procedure B; white solid (0.09g, 13%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 9 H), 4.64 (s, 2 H), 4.83 (s, 2 H), 6.26 (s, 1 H), 7.10 (d, 2 H, J = 4 Hz), 7.20 (t, 1 H, J = 8 Hz), 7.33 (t, 2 H, J = 8 Hz). ^{13}C NMR (300 MHz, $CDCl_3$) δ 28.6, 58.9, 122.5, 127.3, 128.9, 136.3, 156.5. m.p. 53-58°C. Anal. Calc. for $C_{15}H_{19}NO_2$: C 73.43; H 7.80; N 5.70. Found: C 73.61, H 8.23, N 5.17.

General Procedure C. Syntheis of 3-Benzylideneazetidies 24



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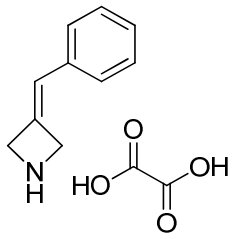
The boc-protected azetidine (0.08 g, 0.32 mmol) was added to a 50 mL round bottom flask. It was then dissolved in 5 mL of dichloromethane and allowed to stir. Trifluoroacetic acid (1.5 mL, 19.6 mmol) was added to the mixture and allowed to stir at room temperature. The reaction was monitored by TLC (1:4 EtOAc:Hexanes) for the duration of the deprotection. Once the trifluoroacetic salt had formed, the mixture was then condensed under reduced pressure without heat.

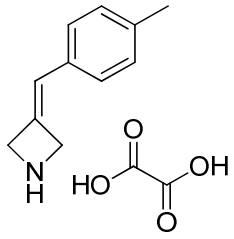
The trifluoroacetate salt was dissolved in water (10 mL) and sodium bicarbonate was added slowly. After reaching pH ~9, ammonium hydroxide was added in drop-wise until pH 12. The solution was then extracted with dichloromethane (3 x 30 mL) and dried over sodium bicarbonate. This was filtered and the organics were condensed under reduced pressure at room temperature. The residue was then dissolved in a minimal amount of anhydrous diethyl ether. Oxalic acid (0.032 g, 0.352 mmol) was then dissolved in ether (5 mL) and added to the solution drop-wise. The salt was allowed to precipitate overnight before being triturated and dried under reduced pressure.

3-(benzylidene)azetidine oxalate (24a)



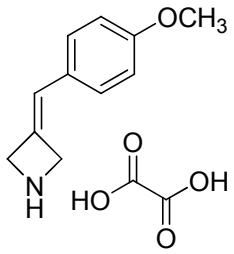
General Procedure C; white solid (0.56 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.07 (s, 1 H), 4.43 (s, 2 H), 4.61 (s, 2 H), 6.13 (s, 1 H), 7.07-7.09 (m, 2 H), 7.16-7.20 (m, 1 H), 7.29-7.22 (m, 2 H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 57.2, 57.4, 121.1, 126.6, 128.3, 137.1, 140.3. m.p.155-165℃. Anal. Calc. for $C_{10}H_{10}N\bullet C_2H_2O_4$: C 61.27; H 5.57; N 5.95. Found: C 59.94; H 5.51; N 5.80.

3-(4-methylbenzylidene)azetidine oxalate (24b)



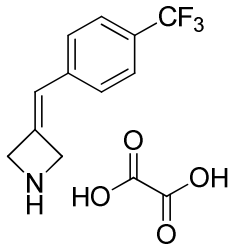
General Procedure C; white solid (0.19 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16-7.11 (m, 2 H), 2.17 (s, 1 H), 2.54 (s, 3 H), 4.42 (s, 2 H), 4.60 (s, 2 H), 6.10 (s, 1 H), 6.98 (d, 2 H, J = 9 Hz), 7.12 (d, 2 H, J = 6 Hz). ^{13}C NMR (300 MHz, $CDCl_3$) δ 57.4, 100.2, 120.9, 127.2, 129.5. m.p. 133-140℃. Anal. Calc. for $C_{11}H_{13}N\bullet C_2H_2O_4$: C 62.63; H 6.06; N 5.62. Found: 61.30, H 6.21, N 5.17.

3-(4-methoxybenzylidene)azetidine oxalate (24c)



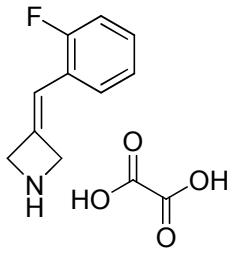
General Procedure C; white solid (0.045 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.18 (s, 1 H), 3.8 (s, 3 H) 4.42 (s, 2 H), 4.59 (s, 2 H), 6.07 (s, 1 H), 6.85 (d, 2 H, J = 9 Hz), 7.06 (d, 2 H, J = 12). ^{13}C NMR (300 MHz, $CDCl_3$) δ 57.2, 114.2, 120.4, 128.5. m.p.175-180℃. Anal. Calc. for $C_{11}H_{13}NO\bullet C_2H_2O_4$: C 58.86; H 5.7; N 5.27. Found: C 57.83; H 5.65; N 5.09.

3-(4-trifluoromethylbenzylidene)azetidine oxalate (24d)



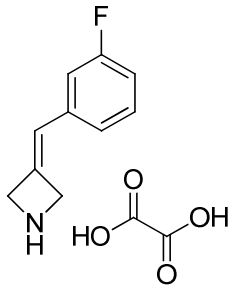
General Procedure C; white solid (0.046 g, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.41 (s, 1 H), 4.46 (s, 2 H), 4.63 (s, 2H), 6.18 (s, 1 H), 7.16 (d, 2 H, J = 9 Hz), 7.54-7.63 (m, 2 H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 57.2, 57.3, 120.2, 125.7 (3), 125.8, 127.4. m.p. 121-130℃. Anal. Calc. for $C_{11}H_{10}F_3N\bullet C_2H_2O_4$: C 51.49; H 3.99; N 4.62. Found: C 49.65; H 4.00; N 4.59.

3-(2-fluorobenzylidene)azetidine oxalate (24e)



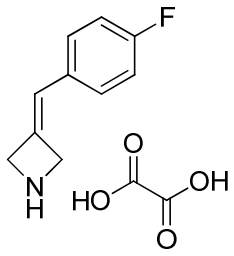
General Procedure C; white solid (0.05 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.17 (bs, 1 H), 4.42 (s, 2 H), 4.60 (s, 2 H), 6.07 (s, 1 H), 6.94-6.99 (m, 1 H), 7.05 (s, 1 H), 7.45-7.53 (m, 1 H), 7.65-7.75 (m, 1 H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 57.2 (2), 119.9, 125.4, 128.7, 131.8, 138.8, 142.3. m.p. 140-152°C. Anal. Calc. for $C_{10}H_{10}FN\bullet C_2H_2O_4$: C 56.92; H 4.78; N 5.53. Found: C 53.56; H 4.65; N 5.04.

3-(3-fluorobenzylidene)azetidine oxalate (24f)



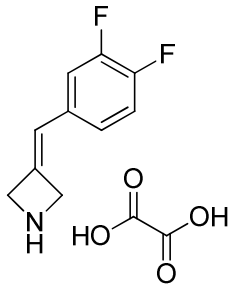
General Procedure C; white solid (0.0508 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.17 (s, 1 H), 4.58 (s, 2 H), 4.75 (s, 2 H), 6.14 (s, 1 H), 6.72-6.89 (m, 4 H).^{13}C NMR (400 MHz, $CDCl_3$) δ 29.9, 115.6, 128.7, 128.8, 131.8 (2)m 132.4, 132.6. m.p. 62-75°C. Anal. Calc. for $C_{10}H_{10}FN\bullet C_2H_2O_4$: C 56.92; H 4.78; N 5.53. Found: C 56.15; H 4.71; N 5.28.

3-(4-fluorobenzylidene)azetidine oxalate (24g)



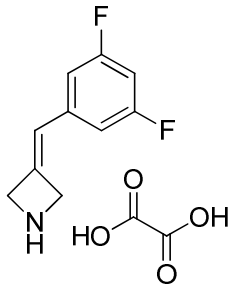
General Procedure C; white solid (0.054 g, 97%) . ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.16 (s, 1 H), 4.40 (s, 2 H), 4.58 (s, 2 H), 6.09 (s, 1 H) 6.97-7.05 (m, 4 H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 29.9 (3), 32.1, 57.1, 115.6, 115.8, 120.0, 128.7, 128.8, 162.8. m.p. 305-312℃. Anal. Calc. for $C_{10}H_{10}FN\bullet C_2H_2O_4$: C 56.91; H 4.77; N 5.52. Found: C 51.84; H 4.82; N 4.49.

3-(3,4-difluorobenzylidene)azetidine oxalate (24h)



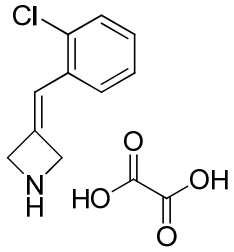
General Procedure C; white solid (0.051 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.16 (bs, 1 H), 4.42 (s, 2 H), 4.57 (s, 2 H), 6.05 (s, 1 H), 6.76-6.88 (m, 2 H), 7.04-7.13 (m, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 57.0 (2), 60.6, 115.5, 117.4, 119.3, 123.3 (2) ,123.4. m.p. 150-158℃. Anal. Calc. for $C_{10}H_9F_2N\bullet C_2H_2O_4$: C 53.41; H 4.09; N 5.16. Found: C 52.30; H 4.00; N 5.35.

3-(3,5-difluorobenzylidene)azetidine oxalate (24i)



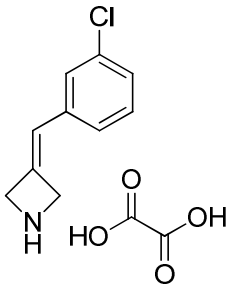
General Procedure C; white solid (0.066 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.17 (s, 1 H), 4.17 (s, 2 H), 4.59 (s, 2 H), 6.07 (s, 1 H), 6.56-6.65 (m, 3 H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 57.1, 101.6, 101.9, 102.3, 109.7, 109.8, 109.9, 110, 119.6. 170-185°C. Anal. Calc. for $C_{10}H_9F_2N\bullet C_2H_2O_4$: C 53.14; H 4.08; N 5.16. Found: C 43.94; H 3.91; N 6.05.

3-(2-chlorobenzylidene)azetidine oxalate (24j)



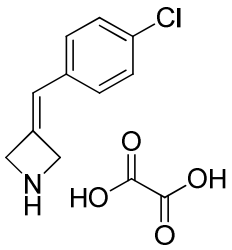
General Procedure C; pale yellow solid (0.62 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.94 (bs, 1 H), 4.47 (s, 2 H), 4.58 (s, 2 H), 6.55 (s, 1 H), 7.10-7.37. ^{13}C NMR (300 MHz, $CDCl_3$) δ 29.9, 57.0, 57.3, 100.2, 117.4, 126.9, 127.7, 127.8, 130.0. m.p. 107-110°C. Anal Calc. for $C_{10}H_{10}ClN\bullet C_2H_2O_4$: C 53.44; H 4.49; N 5.19. Found: C 50.64; H 4.78; N 3.70.

3-(3-chlorobenzylidene)azetidine oxalate (24k)



General Procedure C; white solid (0.05 g, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.17 (s, 1 H), 4.41 (s, 2 H), 4.58 (s, 2 H), 6.07 (s, 1 H), 6.93-7.17 (m, 4 H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 52.8, 55.1, 57.1, 100.2, 125.4, 126.7, 127.7, 128.6, 129.9. m.p. 155-162°C. Anal. Calc. for $C_{10}H_{10}ClN\bullet C_2H_2O_4$: C 53.44; H 4.49; N 5.19. Found: C 53.61; H 4.85; N 4.60.

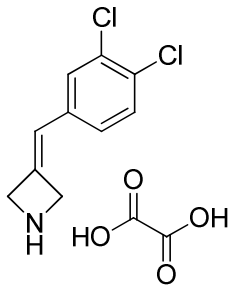
3-(4-chlorobenzylidene)azetidine oxalate (24l)



General Procedure C; cream colored solid (0.06 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.17 (s, 1 H), 4.42 (s, 2 H), 4.58 (s, 2 H), 6.09 (s, 1 H), 6.99-7.01 (m, 2 H), 7.26-7.28 (m, 2 H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 57.2, 119.9, 128.5 (2), 128.9 (2), 132.3, 135.5,

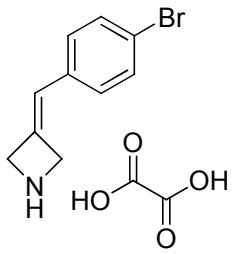
141.2. m.p. 163-169°C. Anal. Calc. for $C_{10}H_{10}ClN\bullet C_2H_2O_4$: C 53.44; H 4.49; N 5.19. Found: C 52.2, H 4.81, N 4.67.

3-(3,4-dichlorobenzylidene)azetidine oxalate (24m)



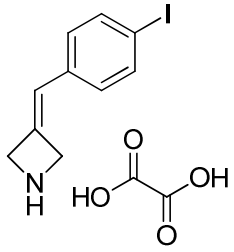
General Procedure C; white solid (0.54 g, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.17 (s, 1 H), 4.41 (s, 2 H), 4.57 (s, 2 H), 6.03 (s, 1 H), 6.88 (d, 1 H, J = 9 Hz), 7.13-7.18 (m, 1 H), 7.18 (s, 1 H), 7.34-7.48 (m, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 57.1 (2), 119.0, 126.4, 128.9, 130.6, 137.0, 142.9. m.p. 150-165°C. Anal. Calc. for $C_{10}H_9Cl_2N\bullet C_2H_2O_4$: C 47.39; H 3.64; N 4.60. Found: C 44.42; H 3.95; N 3.86.

3-(4-bromobenzylidene)azetidine oxalate (24n)



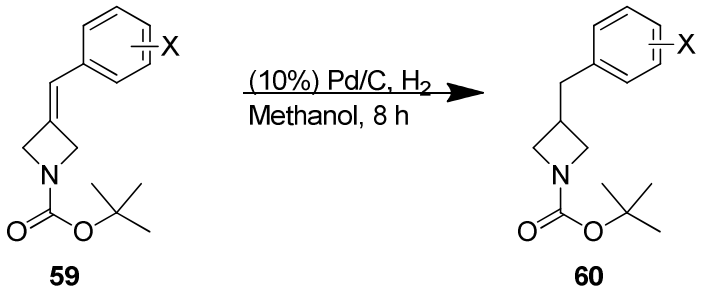
General Procedure C; white solid (0.053 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.17 (s, 1 H), 4.40 (s, 2 H), 4.57 (s, 2 H), 6.07 (s, 1 H), 6.89-6.93 (m, 2 H), 7.37-7.56 (m, 2 H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 57.2, 120.1, 120.4, 128.8, 131.9, 135.9, 141.3. m.p. 178-184°C. Anal. Calc. for $C_{10}H_{10}BrN\bullet C_2H_2O_4$: C 45.88; H 3.85; N 4.45. Found: C 46.40; H 4.18; N 3.90.

3-(4-iodobenzylidene)azetidine oxalate (24o)



General Procedure C; soft yellow solid (0.52 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.78 (s, 1 H), 4.43 (s, 2 H), 4.59 (s, 2 H), 6.07 (s, 1 H), 6.82 (d, 2 H, J = 6 Hz), 7.61-7.73 (m, 2 H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 57.1, 91.9, 120.6, 129.0, 136.3, 137.8 (2), 137.9, 138,3, 140.4. m.p. 182-188°C. Anal. Calc. for $C_{10}H_{10}IN\bullet C_2H_2O_4$: C 39.91; H 3.35; N 3.88. Found: C 40.05; H 3.31; N 3.81.

General Procedure D. Synthesis of 3-benzyl-azetidines (25)



59 **60**

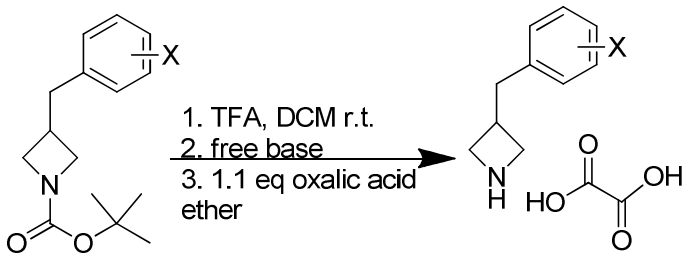
To a hydrogenation flask, 10% wt. of palladium on carbon (0.11 g) (or other catalyst in cases where noted) was added. The flask was then flushed with dry nitrogen for 10 to 15 minutes. A nitrogen balloon was placed on the flask and anhydrous methanol (40 mL) was added. The alkylidene 59 (0.10 g, 0.36 mmol) was then dissolved in dry methanol (10 mL) and added to the mixture. The flask was then placed under reduced pressure for approximately five minutes and flushed with hydrogen. The hydrogen was then released and the flask was evacuated again before being filled with hydrogen (~45 psi) and the mixture was allowed to stir for 8 h. The catalyst was then filtered on Celite and the filter-cake was washed with methanol (3 x 30 mL). The filtrate was then evaporated under reduced pressure and dried under vacuum to yield the desired compound.

In cases where hydrogenation was sluggish or there was concern for dehalogenation of the aromatic ring, platinum oxide was used as a catalyst. To a dry, 100 mL round bottom flask, platinum oxide (0.015g) and a stir bar were added. The flask was covered with a septa and flushed with nitrogen for approximately five min. Dry methanol (20 mL) was then added to the flask. The azetidine (0.08g) was dissolved in methanol and

added to the flask. The flask was then flushed with hydrogen before being placed under a hydrogen atmosphere and heated to 50℃ and allowed to stir overnight.

In both procedures, hydrogenations were allowed to proceed until a full conversion of starting material had occurred.

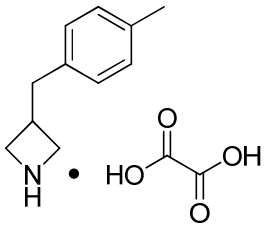
General Procedure E. Deprotection of Boc Group and formation of oxalate salt



59 **25**

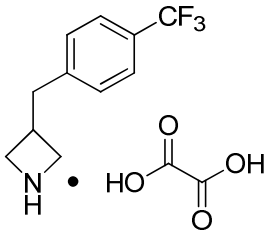
To a dry 50 mL flask, the azetidine (0.036 g, 0.13 mmol) and stir bar were added. The azetidine was dissolved in dichloromethane (5 mL) and then trifluoroacetic acid (2 mL) was added with stirring. The reaction was monitored by TLC until completion. The flask was then condensed to dryness. Water (10 mL) was added to the residue and sodium bicarbonate was added until pH ~9. Ammonium hydroxide (~10 mL) was then added until pH 12. This was then extracted with dichloromethane (3 x 30 mL). The organics were dried with sodium sulfate and condensed at room temperature under reduced pressure. The azetidine was then dissolved in a minimal amount of anhydrous diethyl ether. Oxalic acid (0.013 g, 0.14 mmol) in diethyl ether was added drop-wise into the solution. The salt was allowed to precipitate over night before being triturated and dried under vacuum.

3-(4-methylbenzyl)azetidine oxalate (25a)



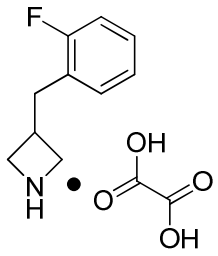
General Procedures D and E; orange, oily wax-like solid (0.03 g, 97%). Boc compound: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (d, 2 H), 7.03 (d, 2 H), 3.97 (t, 2 H), 3.63 (t, 2 H), 2.85 (d, 2 H), 2.79-2.77 (m, 1 H), 2.31 (s, 3 H), 1.43 (s, 9 H). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69-7.48 (m, 3 H) Anal. Calc. for $C_{11}H_{15}N\bullet C_2H_2O_4$: C 62.13; H 6.82; N 5.58. Found: C 52.85, H 8.69, N 0.89.

3-(4-trifluoromethyl)azetidine oxalate (25b)



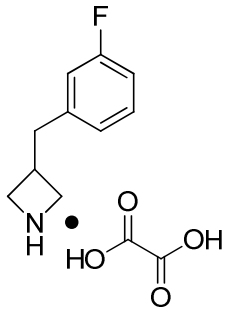
General Procedures D and E; white solid (0.39 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75-7.73 (m, 1 H), 7.55-7.33 (m, 3 H), 4.02-3.98 (m, 2 H), 3.65-3.48 (m, 2 H), 2.96 (s, 1 H), 2.87-2.76 (m, 2 H), 1.48 (s, 9 H). m.p. for $C_{10}H_{12}ClN\bullet C_2H_2O_4$: 115-130℃. Anal. Calc. for $C_{11}H_{12}F_3N\bullet C_2H_2O_4$: C 51.15; H 4.62; N 4.59. Found: C 48.98; H 4.80; N 4.36.

3-(2-fluorobenzyl)azetidine oxalate (25c)



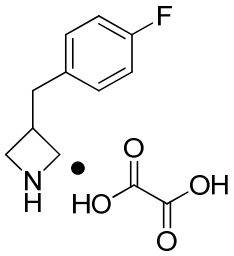
General Procedures D and E; brownish solid (0.031 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.17 (s, 2 H), 2.91-3.00 (m, 3 H), 3.44 (s, 2 H), 3.66 (s, 2 H), 6.98-7.18(m, 4 H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 33.6, 52.9, 115.4, 115.6, 124.3, 130.8. Anal. Calc. for $C_{10}H_{12}FNO\bullet C_2H_2O_4$: C 56.46; H 5.52; N 5.48. Found: C 21.58, H 4.54, N 0.19.

3-(3-fluorobenzyl)azetidine oxalate (25d)



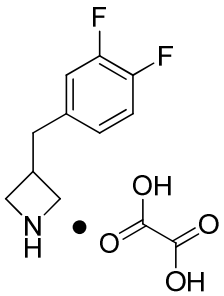
General Procedures D and E, white solid (0.15 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.09 (bs, 1 H), 2.81-2.96 (m, 3 H), 3.41 (s, 2 H),3.67 (s, 2 H), 6.82-6.92 (m, 3 H), 7.22-7.24 (m, 1 H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 53.1, 100.2, 113.0, 113.3, 124.3, 130.1. m.p. 50-60℃. Anal. Calc. for $C_{10}H_{12}FNO\bullet C_2H_2O_4$: C 56.46; H 5.52; N 5.48. Found: C 55.04, H 5.97, N 4.30.

3-(4-fluorobenzyl)azetidine oxalate (25e)



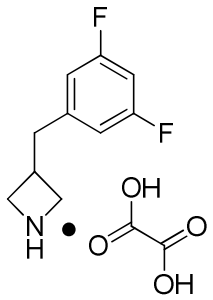
General Procedures D and E, white solid (0.044 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.91 (s, 2 H), 2.86-2.88 (m, 2 H), 2.92-3.02 (m, 1 H), 3.40 (t, 2 H), 3.66 (t, 2 H), 6.93-6.98 (m, 2 H), 7.07-7.10 (m, 2 H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 29.9, 36.5, 38.8, 53, 115.3, 115.5, 129.9 (2). m.p. 68-80℃. Anal. Calc. for $C_{10}H_{12}FNO\bullet C_2H_2O_4$: C 56.46; H 5.52; N 5.48. Found: C 57.96, H 6.56, N 4.36.

3-(3,4-difluorobenzyl)azetidine oxalate (25f)



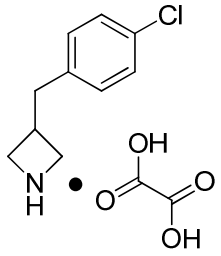
Beige solid (0.54 g, 99%). Boc compound: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 (s, 9H), 2.86 (d, 2 H, J = 6 Hz), 3.98 (t, 2 H, J = 6 Hz), 6.82-6.85 (m, 1 H), 6.90-6.95 (m, 1 H), 7.02-7.09 (m, 1 H).^{13}C NMR (300 MHz $CDCl_3$) δ 29.9, 53.0, 117.1 (2), 117.4 (2), 124.4. m.p. for $C_{10}H_{11}F_2N\bullet C_2H_2O_4$ 75-85 ℃. Anal. Calc. for $C_{10}H_{11}F_2N\bullet C_2H_2O_4$: C 52.74; H 4.79; N 5.12. Found: C 58.09, H 7.04, N 3.46.

3-(3,5-difluorobenzyl)azetidine oxalate (25g)



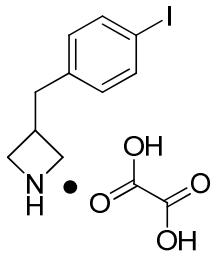
General Procedures D and E; yellow solid (0.044 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (s, 9H), 2.77-2.80 (m, 1 H), 2.89 (s, 2 H), 3.62 (s, 2 H), 4.01 (s, 2 H), 6.65-6.66 (m, 3 H). ^{13}C NMR (300 MHz, DMSO) δ 51.0, 54.2, 112.5. m.p. for $C_{15}H_{19}F_2NO_2$: 50-58℃. m.p. for $C_{10}H_{11}F_2N\bullet C_2H_2O_4$: 100-110℃. Anal. Calc. for $C_{10}H_{11}F_2N\bullet C_2H_2O_4$: C 52.94; H 4.44; N 5.14. Found: C 47.06; H 5.44; N 3.52.

3-(4-chlorobenzyl)azetidine oxalate (25h)



General Procedures D and E; white solid (0.1 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.05 (s, 1 H), 2.86-2.95 (m, 2 H), 3.28 (m, 1 H), 3.40 (s, 2 H), 3.65 (s, 2 H), 6.99-7.15 (m, 2 H), 7.19-7.23 (m, 2 H) ^{13}C NMR (300 MHz, $CDCl_3$) 14.3, 22.9, 29.9, 53.1, 128.7, 129.9. m.p. for $C_{10}H_{12}ClN\bullet C_2H_2O_4$: 110-120 ℃. Anal. Calc. for $C_{10}H_{12}ClN\bullet C_2H_2O_4$: C 53.04; H 5.19; N 5.15. Found: C 54.2, H 6.00, N 4.06.

3-(4-iodobenzyl)azetidine oxalate (25j)



General Procedures D and E; white solid (0.1 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 9 H), δ 1.42 (s, 9 H), 2.75-2.79 (m, 1H), 2.86 (d, 1 H, J = 16 Hz), 3.59-3.64 (m, 2 H), 3.95-4.00 (m, 2 H), 6.97 (d, 1 H, J=6 Hz), 7.05 (d, 1 H, J = 12 Hz), 7.19 (d, 1 H, J = 8 Hz), 7.60 (d, 1 H, J = 8 Hz). Anal. Calc. for $C_{10}H_{12}IN\bullet C_2H_2O_4$: C 39.68; H 3.88; N 3.85. Found: C 49.68, H 5.68, N 3.57.

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