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Asymmetric Syntheses of Analogs of Kainic Acid

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ASYMMETRIC SYNTHESES OF ANALOGS OF KAINIC ACID

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the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

by

Wentian Wang

2012

To: Dean Kenneth G. Furton
College of Arts and Sciences

This dissertation, written by Wentian Wang, and entitled Asymmetric Syntheses of Analogs of Kainic Acid, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

_____
David Becker

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Watson Lees

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Kevin O'Shea

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Philip Stoddard

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Kathleen S. Rein, Major Professor

Date of Defense: November 02, 2012

The dissertation of Wentian Wang is approved.

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Dean Kenneth G. Furton
College of Arts and Sciences

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Dean Lakshmi N. Reddi
University Graduate School

Florida International University, 2012

DEDICATION

This dissertation is dedicated to my parents

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ABSTRACT OF THE DISSERTATION

ASYMMETRIC SYNTHESES OF ANALOGS OF KAINIC ACID

by

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Florida International University, 2012

Miami, Florida

Professor Kathleen Rein Major Professor

Kainic acid has been used for nearly 50 years as a tool in neuroscience due to its pronounced neuroexcitatory properties. However, the significant price increase of kainic acid resulting from the disruption in the supply from its natural source, the alga *Digenea Simplex*, as well as inefficient synthesis of kainic acid, call for the exploration of functional mimics of kainic acid that can be synthesized in a simpler way.

Aza kainoids analog could be one of them. The unsubstituted aza analog of kainoids has demonstrates its ability as an ionotropic glutamate receptor agonist and showed affinity in the chloride dependent glutamate (GluCl) binding site. This opened a question of the importance of the presence of one nitrogen or both nitrogens in the aza kainoid analogs for binding to glutamate receptors. Therefore, two different pyrrolidine analogs of kainic acid, *trans*-4-(carboxymethyl)pyrrolidine-3-carboxylic acid and *trans*-2-carboxy-3-pyrrolidineacetic acid, were synthesized through multi-step sequences. The lack of the affinity of both pyrrolidine analogs in GluCl binding site indicated that both nitrogens in aza kainoid analogs are involved in hydrogen bonding with receptors, significantly enhancing their affinity in GluCl binding site.

Another potential functional mimic of kainic acid is isoxazolidine analogs of kainoids whose skeleton can be constituted directly via a 1, 3 dipolar cycloaddition as the key step. The difficulty in synthesizing N-unsubstituted isoxazolidines when applying such common protecting groups as alkyl, phenyl and benzyl groups, and the requirement of a desired enantioselectivity due to the three chiral ceneters in kainic acid, pose great challenges. Hence, several different protected nitrones were studied to establish that diphenylmethine nitrone may be a good candidate as the dipole in that the generated isoxazolidines can be deprotected in mild conditions with high yields. Our investigations also indicated that the exo/endo selectivity of the 1, 3 dipolar cycloaddition can be controlled by Lewis acids, and that the application of a directing group in dipolarophiles can accomplish a satisfied enantioselectivity. Those results demonstrated the synthesis of isoxazoldines analogs of kainic acid is very promising.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

LIST OF SCHEMES

SCHEME PAGE

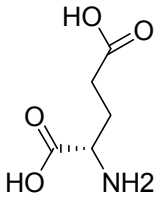
1. Introduction

1.1 Neurotransmitter receptors

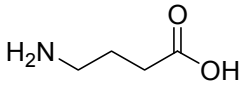
Neurotransmitter receptors function as integral components that allow communication between adjacent cells of the nervous system. They span the width of the postsynaptic membrane and protrude into the synaptic cleft and into the cell cytoplasm. The effects of neurotransmitters are mediated through a noncovalent, reversible interaction, causing a conformational change in the receptor protein. Consequently, this change results in either an alteration in the permeability of certain ions through the membrane (transduction mechanism 1) or the activation of intracellular enzymes (transduction mechanism 2). Ion channel receptors are multisubunit proteins and mediate rapid synaptic transmission. The majority of neurotransmitter receptors that activate intracellular enzymes are metabotropic or G protein-coupled receptors and are single-subunit proteins that mediate slower responses at the synapse [Stephenson, F. A. and Hawkins, L. M. 2001].

Activation of the neurotransmitter receptors is a result of noncovalent and reversible interaction with neurotransmitters. To be considered a neurotransmitter, a compound must meet four criteria: (1) it is presynaptically localized in specific neurons and released from synaptic vesicles; (2) it is released by physiological stimuli in concentrations high enough to generate postsynaptic responses; (3) there are mechanisms that will rapidly terminate its action; and (4) it exhibits pharmacological identity with the transmitter, including response to antagonists [Cooper, J. R. et al.1992][Fonnum, F. 1984].

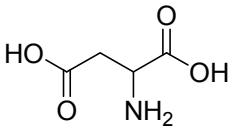
The early concept that each neurotransmitter only activates a single type of receptor was revolutionized with the advent of molecular cloning. It is currently believed that a family of neurotransmitter receptors exists for each neurotransmitter. More than 50 neurotransmitters have been discovered, and each one binds to its own family of neurotransmitter receptors [Stephenson, F. A. and Hawkins, L. M. 2001]. Neurotransmitters fall into three main categories: amino acids (mostly glutamic acid (Glu) **1**, γ-amino butyric acid **2**, aspartic acid **3** and glycine **4**), peptides (e.g., vasopressin, somatostatin, neurotensin), and amines (norepinephrine **5**, dopamine **6,** serotonin **7** and acetylcholine **8)** [Rousseaux. C. G. 2008]. The specificity of selectivity makes the neurotransmitter receptors a primary target for pharmacological intervention. Drugs that can trigger responses by binding to the same receptors as neurotransmitters are receptor agonists, whereas receptor antagonists are drugs that block or dampen agonist-mediated responses and do not provoke any biological response upon binding to the same site on the neurotransmitter receptor [Stephenson, F. A. and Hawkins, L. M. 2001] [Cooper, J.R. et al. 1992].



glutamic acid
1



gamma-amino butyric acid
2



aspartic acid
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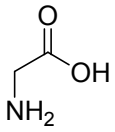
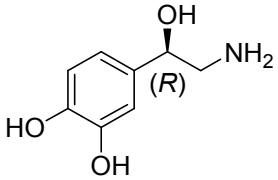
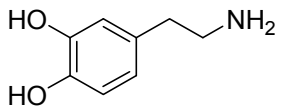


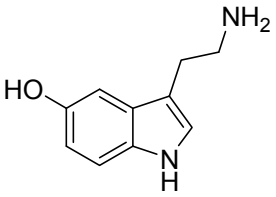
glycine
4



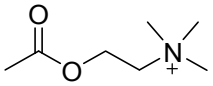
norepinephrine
5



dopamine
6



serotonin
7



acetylcholine
8

1.2 Glutamic acid and its receptors

L-glutamic acid (Glu), one of 20 proteinogenic amino acids, possesses numerous functions as listed in Table 1 [Rousseaus, C. G. 2008].

Table 1. Biological functions of glutamate

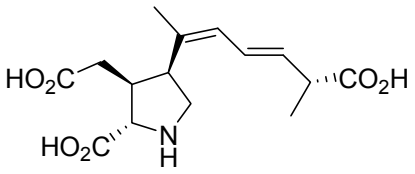
1	Substrate for protein synthesis
2	Precursor of glutamine
3	N transport (muscle-glutamine; brain)
4	Neurotransmitter (and γ-aminobutyrate)
5	Polyglutamate and cell signaling
6	D-Carboxylation of glutamate
7	Substrate for glutathione production
8	Precursor of *N*-acetylglutamate
9	Active sites of enzymes
10	Inhibitor of glutaminase reaction
11	Citric acid cycle intermediate
12	Energy source for some tissues (mucosa)

Glu and its metabolism and function in the detoxification of ammonia in the brain, as well as its role as a building block in the synthesis of proteins and peptides, have been studied for decades. However, the notion that Glu functions as a neurotransmitter was controversial when first proposed in the early 1950s [Hayashi, T. 1956][Curtis, D. R. and Watkins, J. C. 1960][Cotman, C. W. and Iversen, L. L. 1987][Fonnum, F. 1984]. Starting in the early 1980s, strong evidence accumulated from several studies to support the role of glutamic acid as an excitatory neurotransmitter in the mammalian central nervous system (CNS) [Fonnum, F. 1984]. Glu is synthesized, stored, and released from the presynaptic terminal. It binds to specific neurotransmitter receptors localized on the postsynaptic cells and is then removed from the synaptic cleft. Therefore, it fulfills the four requirements of a neurotransmitter. Through both ligand-gated ion channels at

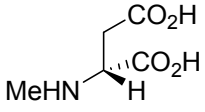
ionotropic receptors (iGluRs) and at G protein-coupled metabotropic receptors (mGluRs), L-Glu activates its corresponding receptors, contributing to basal excitatory synaptic transmission. Therefore, it is an excitatory neurotransmitter [Rousseaus, C. G. 2008].

In the mammalian CNS, Glu and aspartate (Asp) act as major excitatory neurotransmitters to stimulate or excite postsynaptic neurons. Glu is estimated to mediate approximately 50% of all synaptic transmissions in the CNS and is involved in almost all aspects of normal brain function including learning, memory, movement, cognition, and development [Rousseaus, C. G. 2008].

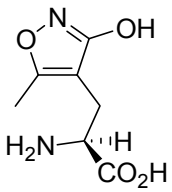
However, Glu, Asp, and structurally similar compounds, such as domoic acid **9**, can be neurotoxic when they excessively stimulate the same excitatory receptors, and this action is postulated to be involved in the pathogenesis of some types of acute and chronic insults to the CNS [Rousseaus, C. G. 2008][Choi, D. W. 1995]. This excitotoxic effect can be significant during acute diseases such as ischemic stroke and trauma, or moderate but prolonged in such chronic neurodegenerative diseases as Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis [Beal, M. F. 1995][Starr, M. S. 1995][Plaitakis, A. et al. 1996][Shaw, P. J. and Ince, P. G. 1997].



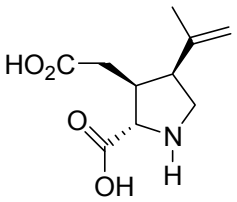
domoic acid
9



NMDA
10



AMPA
11



Kainate
12

Early studies classified glutamate receptors as either *N*-methyl-D-aspartate (NMDA) **10** receptors or non-NMDA receptors based on the NMDA-selective activation of these receptors. In the early 1980s, under conditions in which a prolonged excitatory postsynaptic potential was observed in response to intense stimulation of presynaptic fibers, NMDA receptors were demonstrated to be involved in several central synaptic pathways, acting together with non-NMDA receptors. Such activation results in the phenomenon of long-term potentiation, which is associated with lasting changes in synaptic efficacy (synaptic plasticity) and is considered an important process in memory and learning [Rousseaux. C. G. 2008] [Bliss, T. V. P. and Collingridge, G. L. 1993][Chapman. P. F. et al. 1990]. Almost simultaneously, a new class of glutamate receptors in the brain that are distinct from NMDA and non-NMDA receptors were discovered that mediate biochemical changes. This dichotomy was later addressed in the early 1990s with the identification of a second family of glutamate receptors, the mGluRs [Rousseaux. C. G. 2008]. Later, non-NMDA receptors were further divided into α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) **11** and kainate (KA) **12** receptors in terms of the agonist's preferential activation of these receptors. Presently, as shown in Figure 1, glutamate receptors are broadly characterized into two classes: the

mGluRs and iGluRs. Both mGluRs and iGluRs participate in a range of central synaptic processes, including synaptic plasticity. The former are coupled to intracellular signal transduction through G proteins and mediate slow excitatory responses. By contrast, the latter are responsible for fast excitatory responses by functioning as ion channels [Bunch, L. and Larsen, P. K. 2009][Lujan, R. et al. 1996]. iGluRs are cation channels and are further classified into three subtypes: NMDA, AMPA, and KA receptors. However, not all iGluRs are exclusively cation channels. A small family of iGluRs with glutamate-gated chloride channels has been discovered, although these receptors are not well studied and are still under investigation [Brockie, P. J. and Maricq, A. V. 2006]. The classification of glutamate receptors is shown in Figure 1.

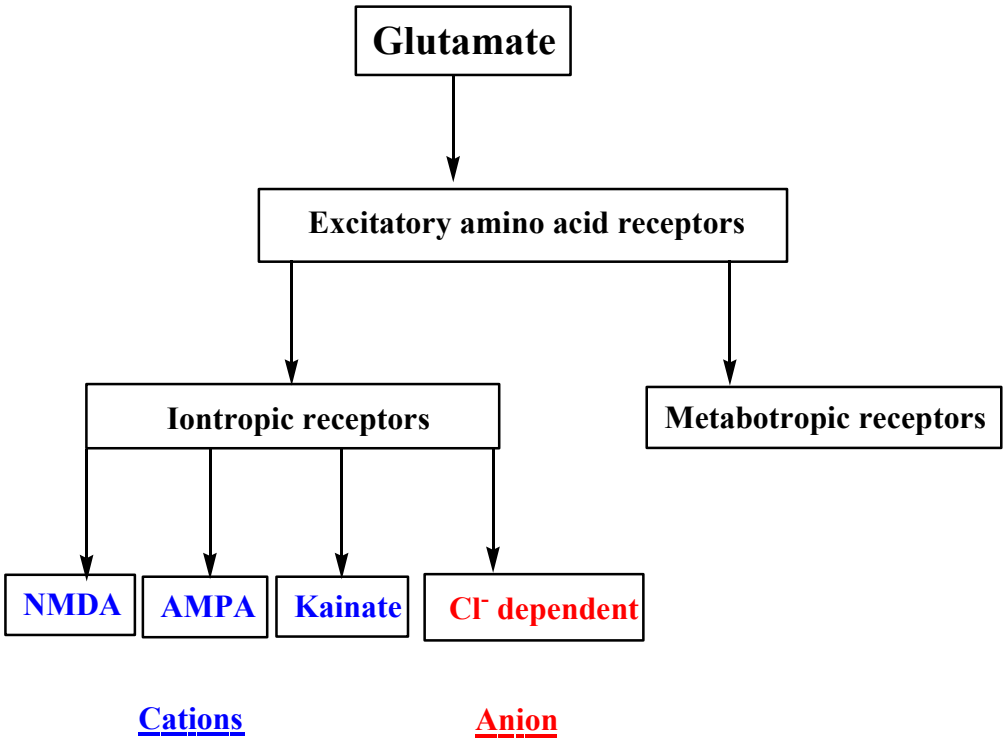


Figure 1. Classification of glutamate receptors

Because iGluRs with anion channels account for a very small portion of iGluRs, the term "iGluRs" refers only to ionotropic glutamate receptors with cation channels, unless otherwise specified.

iGluRs are ligand-gated ion channels and mediate the vast majority of excitatory neurotransmission in the brain. iGluRs have different physiological and pharmacological properties and are differentially localized throughout the CNS, where they form heteromeric subunit assemblies and are composed of different subunits [Rousseaux. C. G. 2008]. Most iGluRs incorporate ion channels that allow cations to permeate. However, the membrane channels associated with these receptors exhibit different selectivity to sodium (Na^+), potassium (K^+), and calcium (Ca^{++}). The non-NMDA receptors, including AMPA and KA receptors, control a nonselective cationic channel permeable to the monovalent cations K^+ and Na^+, whereas NMDA gates an ion channel that is more permeable to Ca^{++} ions than the AMPA or KA receptors [Rousseaux. C. G. 2008][Meldrum, B. S. 2000].

Several gene families encoding iGluRs have been identified by recombinant DNA technology. The AMPA family consists of iGluR 1-4 (iGluRA-D); the KA family is composed of iGluR 5-7 and KA 1-2; and the NMDA family includes NMDAR 1, NMDAR 2A-D, and NMDAR 3 (Figure 2).

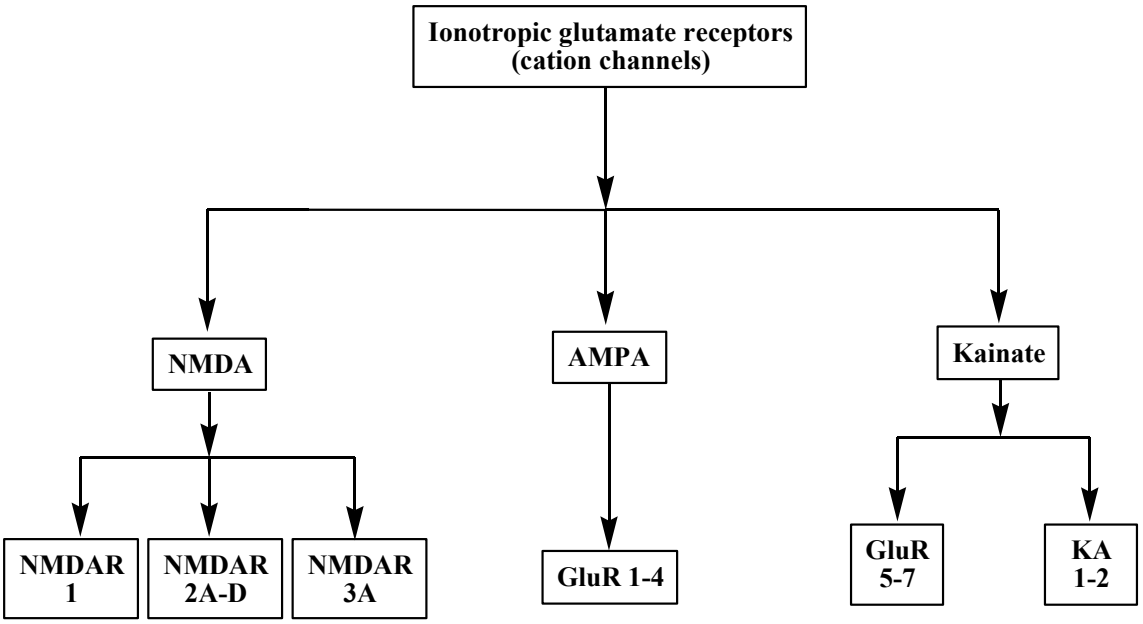


Figure 2. Families of ionotropic glutamate receptors

Learning and memory rely on persistent changes in synaptic plasticity that require neuronal gene expression. AMPA and NMDA GluRs play different roles in controlling synaptic plasticity. AMPA receptors influence short-term changes in synaptic plasticity, whereas NMDA receptors mediate gene expression that participates in the long-term maintenance of these changes [Rao, V. R. and Finkbeiner, S. 2007].

The subunits that assemble to form iGluRs may or may not be homologous, and different combinations of subunits are found. However, through gene transcription, different regions of the resultant mRNA molecule can be spliced together, leading to multiple mRNAs that are translated into different proteins. This phenomenon is known as splice variation, a very common finding among neuroreceptors. The C-terminus of iGluRs is the site of extensive splice variation, leading to different important functions, because the C-

terminus is also the site of multiple protein-protein interactions. RNA editing is a further modification leading to diversification, and some nucleotides in the mRNA sequence transcribed from the gene sequence are selected and enzymatically modified, changing the amino acid coded by the mRNA sequence. RNA editing can lead to fundamental consequences for the Ca^{++} permeability of subunits, e.g., the AMPA-type subunit GluR2 and the KA receptor subunit GluR5 [Clarke V. R. J. et al. 1997][Rousseaux. C. G. 2008].

Diverse NMDA receptors [Katsuwada, T. et al. 1992], which are perhaps the best characterized of the iGluRs, are composed of assemblies of NMDAR 1 subunits and one or more NMDAR 2A-D subunits. The Glu-binding domain of the receptor is present at the junction of the NMDAR 1 and NMDAR 2 subunits. Hence, expression of both subunits is necessary to form functional channels. Among those subunits, NMDAR 1 is more highly distributed in the cortex, caudate, and hippocampus and forms the channel; other subunits (NMDAR 2A-D) are involved in receptor modulation. NMDAR 1 is linked to a Na^{+} and Ca^{++} ion channel that has five different binding sites, including two distinct agonist recognition sites, one for Glu and the other for Glycine (Gly). The NMDA receptor requires both Glu and a co-agonist, Gly, to bind for receptor function. Of the NMDAR 2 groups, NMDAR 2A is expressed ubiquitously with the greatest densities in hippocampal regions. The NMDAR 2B subunit has a binding site for polyamines, regulatory molecules that mediate the function of the NMDA receptor [Chenard B. L. and Menniti, F. S. 1999][Rousseaux. C. G. 2008]. NMDAR 2C and NMDAR 2D may form heteroligomeric receptors with both NMDAR 1 and NMDAR

2A, providing a basis for the development of drugs selectively aimed at spinal cord disorders [Sundstrom E. et al. 1997].

NMDA receptors are inactive at resting membrane potentials because of a voltage-dependent block of ion flow through the channel pore by Mg^{++} ions. Sustained activation of AMPA receptors depolarizes the postsynaptic cell, releasing the channel inhibition and allowing NMDA receptor activation [Rousseaux. C. G. 2008]. NMDA receptor activation results in Ca^{++} influx into the postsynaptic cells. Thus, NMDA receptors are highly permanent for Ca^{++} ions. They demonstrate slower gating kinetics when the channel is blocked in a voltage- and use-dependent manner by physiological concentrations of Mg^{++} ions [Nowak, L. et al. 1984]. These properties provide NMDA receptors with their role as a coincidence detector underlying synaptic plasticity in learning, chronic pain, drug tolerance, and dependence [Rousseaux. C. G. 2008].

AMPA receptors, localized in the hippocampus and striatum, play an important role in mediating most forms of fast glutamatergic neurotransmission in response to a Ca^{++} influx [Chavez, A. E. et al. 2006]. AMPA receptors have four known subunits, GluR1 to GluR4, which are widely but differentially distributed throughout the CNS [Hollmann, M. and Heinemann, S. 1994]. The types of subunits forming a receptor usually determine the receptor's biophysical properties and pharmacological sensitivity [Rousseaux. C. G. 2008].

"Flip" and "flop," two alternative splice variants of GluR1 to GluR4 subunits, exhibit varying levels of expression throughout the brain and during development and impart distinct pharmacological properties [Partin K. M. et al. 1996][Parsons C. G. et al. 1993]. The GluR2 subunit controls the permeability of Ca^{++} into AMPA receptor channels. Posttranscriptional editing of GluR2 mRNA, which changes a single amino acid in the transmembrane II (TMII) region from glutamine (Q) to arginine (R), determines the Ca^{++} permeability of the GluR2 subunit. This phenomenon is called the Q/R editing site. When it is in the GluR2(Q) form, it is Ca^{++} permeable, whereas GluR2(R) is not. Almost all GluR2 protein expressed in the CNS is in the GluR2(R) form, leading to Ca^{++}-impermeable native AMPA receptors. Based on this and the interactions with other intracellular proteins, GluR2 has been described by some as the most important AMPA receptor subunit [Rousseaux. C. G. 2008].

Like AMPA, KA receptors are also localized in the hippocampus and striatum. They may play a role in the generation of seizures if overstimulated [Vignes M. et al. 1997]. Multimeric assemblies of GluR5-7 and KA-1-/2 subunits form KA receptors. KA receptors have an extracellular N-terminus that produces the ligand-binding domain and a re-entrant loop (TMII), combined with a loop between TMIII and TMIV, to form the lining of the pore region in the ion channel. Both splice variation and RNA editing also provide the KA receptor family with numerous possible receptors with different pharmacological and functional properties [Chittajallu, R. et al. 1999]. KA receptors can not only act as presynaptic autoreceptors to facilitate synaptic transmission [Contractor

A. et al. 2001], but are also involved postsynaptically in neurotransmission in some pathways [Rousseaux. C. G. 2008].

In addition to cation-permeable iGluRs, there is a small family of glutamate-gated chloride channels (GluCl) that has been identified in *Caenorhabditis elegans* [Brockie, P. J. and Maricq, A. V. 2006]. Based on the investigation of Cully and associates [Cully, D. F. et al. 1994], those channels are permeant to chloride ions and are sensitive to avermectin, an anthelmintic drug. Thus far, the GluCl channels seem to be specific to invertebrates. Six genes that encode GluCl subunits in *C. elegans* have been discovered: *glc-1*, *glc-2*, *glc-3*, *glc-4*, *avr-14/gbr-2*, and *avr-15* [Cully, D. F. et al. 1994][Dent, J. A.; 1997][Vassilatis, D. K. et al. 1997][Laughton, D. L. 1997][Dent, J. A. et al. 2000**]**Horoszok, L. et al. 2001]. GluCls function in pharyngeal pumping, sensory perception, and locomotion [Brockie, P. J. and Maricq, A. V. 2006].

mGluRs were discovered and established as a new type of excitatory amino acid receptor, with a family of eight single-polypeptide chain receptors that function via coupling to G proteins. mGluRs are classified into four groups (Group I-IV) according to amino acid sequence similarities, agonist pharmacology, and the signal transduction pathways to which the mGluRs are coupled [Narahashi, T. et al. 1992]. The co-assembly of several subunits forms each different group of receptor. All eight subunits (mGluRs 1-8) have been cloned [Rousseaux. C. G. 2008]. mGluRs 1, 5, and 6 form Group I, which stimulates inositol phosphate metabolism and mobilization of intracellular Ca^{++}. Both

Group II (mGluRs 2 and 3) and Group III (mGluRs 4, 6-8) are coupled to adenyl cyclase, whereas Group IV is coupled to the activation of phospholipase D (Figure 3) [Rousseaux. C. G. 2008]. However, Group IV is activated more efficiently by L-cysteinesulfonic acid than Glu, suggesting that L-cysteinesulfonic acid probably functions as an endogenous agonist of this receptor [Conn, P. J. and Pin, J. P. 1997].

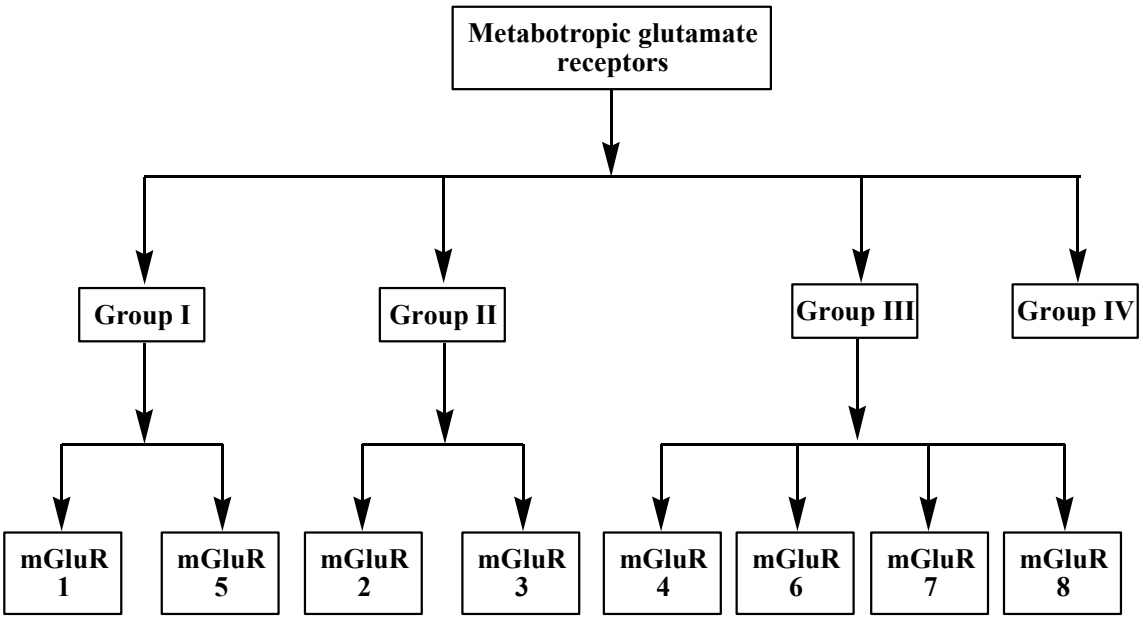


Figure 3. Classification of mGluRs

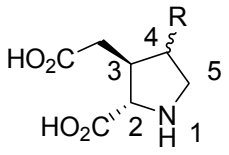
mGluRs are similar to iGluRs in that they both bind Glu and Glu binding can affect the permeability of ion channels. However, mGluRs have certain features that are different from iGluRs. First, mGluRs modulate the activity of neurons instead of mediating fast synaptic neurotransmission. Second, mGluRs are localized differently and heterogeneously. Different subclasses are uniquely distributed at both the anatomical and cellular levels; for example, mGluRs 2 and 3 are more concentrated in the cerebral

cortex, whereas mGluR 4 is expressed at high density in the thalamus and mGluR 6 is exclusively found in the retina [Rousseaux. C. G. 2008].

Activation of mGluRs changes local transmitter release and behaviors of experimental animals. In particular, mGluRs mediate phosphorylation of several key signaling proteins, such as protein kinases and transcription factors, leading to significant changes in immediate early gene and neuropeptide gene expression in striatal neurons. mGluRs are considered to play a pivotal role in the development of synaptic and neuronal plasticity underlying long-term adaptive changes in cellular physiology, which is related to various neurological disorders [Rousseaux. C. G. 2008]. mGluRs also mediate dopamine transmission, which is substantially involved in many neurological disorders [Hu, G. et al. 1999].

1.3 Kainic acid and its synthesis

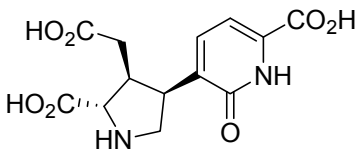
Kainic acid **12** is the parent of a class of non-proteinogenic amino acids known as the kainoids **13**.



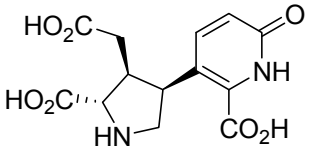
Kainoids
13

For the past 50 years, the neuroexcitatory properties of kainoid amino acids have provoked numerous investigations. Kainoid amino acids have a structure similar to

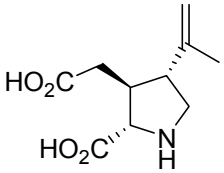
glutamic acid, a mammalian CNS neurotransmitter [Parsons, A. F. 2005]. The kainoid amino acids are a special group of non-proteinogenic pyrrolidine dicarboxylic acids. They have a general structure **13** with three asymmetric centers at positions 2, 3, and 4 of the pyrrolidine ring. The substituent at the C-4 position of the ring, generally containing π-unsaturation, can vary, giving rise to various members of the kainoid family [Parsons, A. F. 1996]. For instance, when the substituent at C-4 is an octadienoic side chain, the kainoid is domoic acid **9,** which was originally isolated from a Japanese marine algae, the warm water algae *Chondria armata* [Takemoto, T. and Daigo, K. 1958]. The acromelic acids A **14** and B **15** are kainoids with a functional 2-pyridone substituent at C-4. They were first isolated from the mushroom *Clitocybe acromelalga* [Konno, K. et al. 1983]. Both (−)-kainic acid and (+)-allokainic acid **16** bear an isopropenyl group at the C-4 position but with opposite configurations. Kainic acid and its C-4 epimer, allokainic acid, were first isolated from a Japanese marine algae *Digenea simplex* in 1953 [Murakami, S. et al. 1953].



Acromelic acid A
14



Acromelic acid B
15

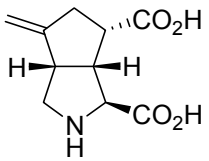


(+)-allokainic acid
16

Kainoids have been extensively investigated for their pronounced neuroexcitatory properties. The kainoids selectively block neuronal processes and are consequently valuable tools in the study of neurofunctioning. Their excitotoxicity in both the vertebrate

and invertebrate glutamergic system causes specific neuronal death in the brain [Parsons, A. F. 1996]. Researchers have discovered that after injection of kainoids, the pharmacological effects and patterns of neuronal degeneration are very similar to the symptoms observed in patients who suffer from neurological diseases such as epilepsy and Huntington's disease [Sperk, G. 1994][Coyle, J. T. and Schwarcz, R. 1976][Mcgeer, E. G. and Mcgeer, P. L. 1976].

Numerous structure-activity investigations of the naturally occurring and synthetic kainoids have established that the C-4 stereochemistry, the nature of the C-4 substituent, and its conformation play an essential role in binding and functional activation at the recognition site. i.e., kainic acid is more neuroexcitatory than its C-4 epimer, and allokainic has the opposite configuration at the C-4 position [Hansen, J. J. and Kroqsqaard-larsen, P. 1990]. An investigation by Ishida, M. et al.. [Ishida, M. and Shinozaki, H. 1991] indicated that an unsaturated substituent at C-4 is significantly related to the excitatory activity. Hashimoto, K. et al. synthesized and studied a series of conformationally constrained analogs **17-20** of kainic acid and found that all those compounds showed very weak activities in newborn rat spinal motoneurons compared with kainic acid [Hashimoto, K. et al.1995].



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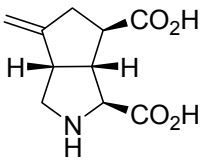

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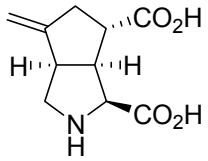

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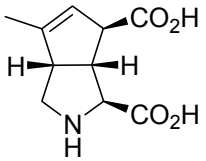
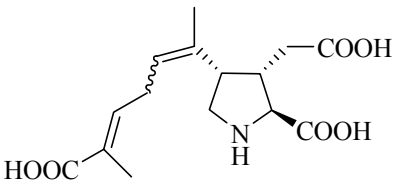
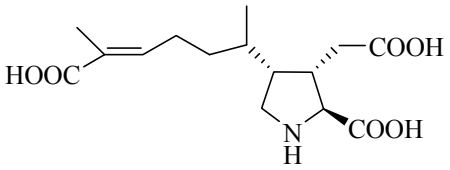


20

In addition to the pronounced neuroexcitatory properties, kainoids also draw considerable research interest due to their insecticidal and anthelmintic activities. Inhabitants of Yakushima Island in Japan have long used an extract of red algae, from which kainic acid and domoic acid are isolated, to kill flies [Parsons, A. F. 1996]. In addition, Meda, M. et al.. found that subcutaneous injection of domoic acid and the isodomoic acids A-C **21-23** into the abdomen of the American cockroach (*Periplaneta americana*) can lead to this insect's death [Meda, M. et al. 1986], demonstrating their potent insecticidal properties. However, research has also revealed that the insecticidal properties greatly rely on the nature of the side chain at the C-4 position of the pyrrolidine ring. Therefore, the potency of domoic acid in killing the American cockroach is 23 times that of isodomoic acid C [Parsons, A. F. 1996].



Isodomic acid A (Z, E) **21**
Isodomic acid B (E, E) **22**



Isodomic acid C **23**

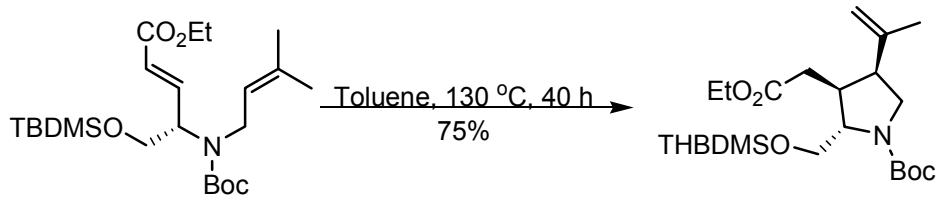
For over 1000 years, the Japanese have taken advantage of the anthelmintic (anti-intestinal worm) activities of *Digenea simplex*, the algae from which kainic acid was first

isolated [Parsons, A. F. 1996]. The active component, kainic acid, was extensively investigated and found to have powerful anthelmintic activity without side effects [Watase, H. et al. 1958]. Interestingly, the *cis* relative configuration between C-3 and C-4 is also essential to the anthelmintic function. This is why kainic acid has a much stronger anthelmintic effect than its C-4 epimer, allokainic acid [Parsons, A. F. 1996].

Due to these crucial biological properties of kainoids, they not only arouse numerous functional investigations but also attract enormous attention for synthesizing these compounds. However, synthesis of kainoid amino acids is a considerable challenge. Any synthesis must address the formation of a pyrrolidine-2-carboxylic acid with defined stereochemistry at the three continuous chiral centers of the pyrrolidine ring. Usually, the *cis* relative configuration between C-3 and C-4 is preferred. The other consideration for ideal synthesis of kainoids is having the option to easily introduce different side chains at C-4 to produce various known kainoids and their analogs [Parsons, A. F. 1996].

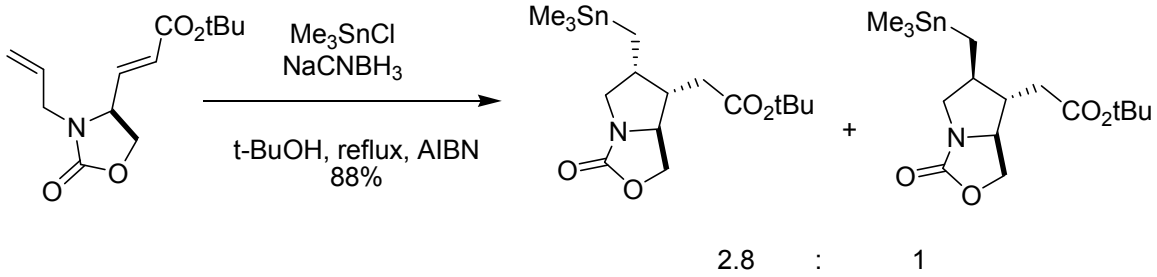
Among kainoid amino acids, kainic acid, the prototypical kainoid, has attracted the most interest in syntheses. A number of syntheses have been explored. As of 2012, 66 syntheses of kainic acid are present in the Chemical Abstracts CAS file. Early syntheses were relatively inefficient and nonstereoselective [Parsons, A. F. 1996]. Later, a variety of stereoselective syntheses were developed with the common feature that the stereocontrolled formation of the C-3 to C-4 bond is achieved under the steric effect of the C-2 substituent via cyclization, Claisen rearrangement reaction, Pauson-Khand reaction, tandem Michael addition, etc. [Parsons, A. F. 1996].

In 1982, a stereocontrolled intramolecular ene reaction (Scheme 1) reported by Oppolzer and Thirring [Oppolzer, W. and Thirring, K. 1982] stands as the first enantioselective synthesis of kainic acid. The key step, the ene cyclization reaction, was conducted with 75% yield, and the overall yield of the 10-step sequence was 4.8%. Their work also resulted in the establishment of absolute configuration of (−)-kainic acid.



Scheme 1. Intramolecular ene cyclization

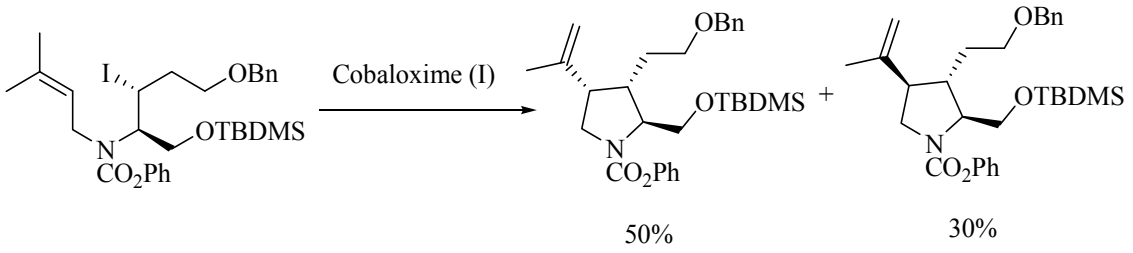
Hanessian, S. and Ninkovic, S. [Hanessian, S. and Ninkovic, S. 1996] employed a diastereoselective radical cyclization to generate a pyrrolidine intermediate that was converted into (−)-kainic acid after eight additional steps. This cyclization resulted in an excellent 88% yield with a 2.8:1 *cis*/*trans* selectivity (Scheme 2).



Scheme 2. Diastereoselective radical cyclization

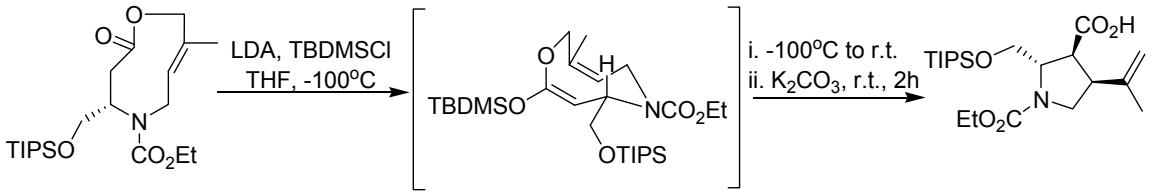
Baldwin and co-workers [Baldwin, J. E. and Li, C-S. 1987] used a cobalt-mediated cyclization reaction to prepare kainic acid (Scheme 3). In the presence of cobaloxime (I),

the cyclization gave rise to a separable 5:3 *syn*- to *anti*-pyrrolidine mixture in an 80% total yield.



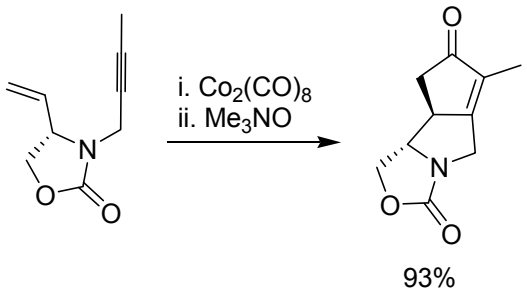
Scheme 3. Cobalt-mediated cyclization

Cooper and co-workers used a stereocontrolled enolate Claisen rearrangement (Scheme 4) to obtain the desired relative stereochemistry at the 3, 4-position. A deprotonation step by Lithium diisopropylamide in the presence of a silyl trapping agent formed an enolate, which produced the Claisen rearrangement via a boat-like transition state to produce the pyrrolidine [Cooper, J. et al. 1987].



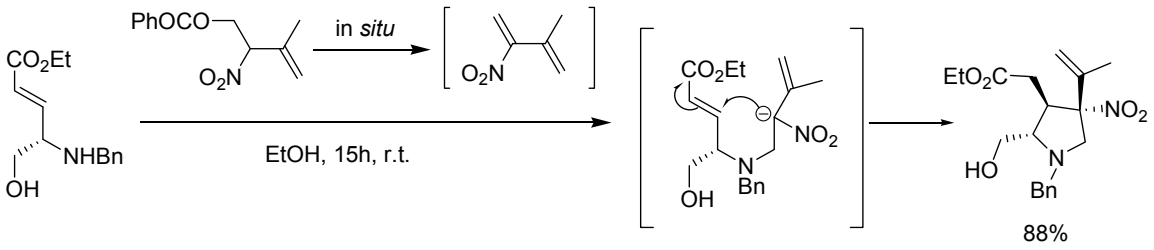
Scheme 4. Stereocontrolled enolate Claisen rearrangement

The Pauson-Khand reaction (Scheme 5) was used by Yoo, S-E and Lee, S. H. in the synthesis of (−)-kainic acid. An oxazolidinone ene-yne reacted with dicobalt octacarbonyl, followed by trimethylamine *N*-oxide, to form an enone, a desired kainic acid precursor in 93% yield [Yoo, S-E. and Lee, S. H. 1994].



Scheme 5. Pauson-Khand reaction to prepare a kainic acid precursor

A tandem Michael addition reaction (Scheme 6) was developed by Benetti and co-workers as the key step to prepare (−)-kainic acid. A secondary amine reacted with 2-methyl-3-nitrobuta-1,3-diene formed *in situ* from 3-methyl-2-nitrobut-3-enyl benzoate to yield the desired pyrrolidine in 88% yield. Later, for the synthesis of (−)-kainic acid, the nitro group was removed through a hydride transfer reaction, with retention of configuration, in the presence of a palladium catalyst [Barco, A. et al. 1992].



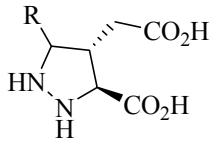
Scheme 6. Tandem Michael addition

All examples used a stereoselective ring closure under the steric effect of the C-2 substituent to constitute the highly functionalized pyrrolidine ring of kainic acid. Although these methods successfully realized the three contiguous asymmetric centers of (−)-kainic acid, their yields were not satisfactory [Parsons, P. 2011].

Kainic acid has been used for nearly 50 years as a tool in neuroscience due to its pronounced neuroexcitatory properties. For decades, kainic acid was commercially available from its most abundant natural source, the alga *Digenea simplex* [Murakami, S. et al. 1953]. In 1999, however, a disruption in the supply of kainic acid [Tremblay, J.-F. 2000] resulted in a significant price increase, making development of an efficient synthetic route for kainic acid more desirable. Unfortunately, the challenges in kainic acid synthesis prohibit large-scale preparation, even though numerous total syntheses and synthetic approaches have been reported. This greatly limits kainic acid's application in research.

1.4 Objective of research

To address this problem, analogs of kainic acid that exhibit the same biological activity as kainic acid but are easier to prepare are of considerable interest. Aza kainoid analogs **24** could be one type of analog.

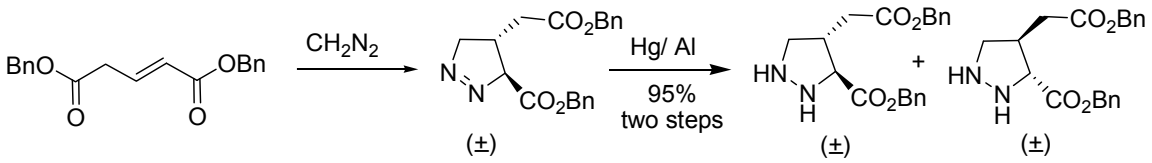


24

Molecular modeling/docking studies fitting the aza analog of KA into the binding site of the iGluR6 suggest that the aza-kainoid analogs can fit well and fully reproduce all critical ligand receptor interactions [Di, M. 2006].

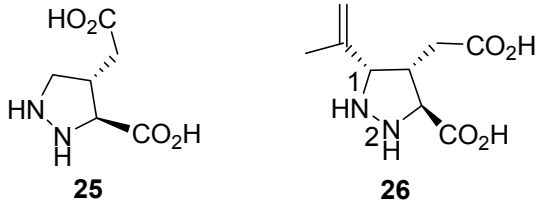
A simple unsubstituted aza kainoid **25** was prepared in racemic form (Scheme 7). During an Hg/Al reduction step, the all-*trans* adduct was converted to a mixture of *cis* and *trans*.

However, the two diastereomers were separable, which allowed for testing of the *trans* pyrazolidine after hydrogenolysis of the benzyl protecting groups [Simovic, D. 2008].



Scheme 7. Reduction of 1-pyrazoline prior to isomerization

Preliminary activity studies using *Aplysia californicus* indicated that application of this unsubstituted aza-kainoid **25** induced an inward current in *Aplysia* neuronal cells, demonstrating the ability of aza kainoids to function as iGluR agonists [Simovic, D. 2008].



The affinity of compound **25** was tested in AMPA-, KA-, NMDA-, and chloride-dependant glutamate binding sites (Figure 4). The affinity of **25** for the chloride-dependent glutamate binding site was clear [Simovic, D. 2008].

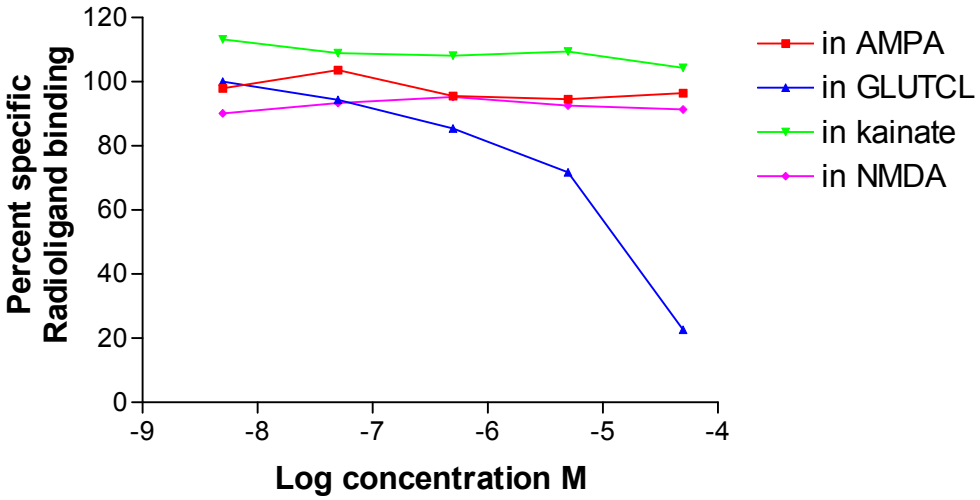


Figure 4. The affinity of compound **25** at AMPA- (■), Kainate- (▼), NMDA- (♦), and chloride-dependent (▲) glutamate binding sites.

In the crystal structures of iGluRs bound to kainic acid, the NH of kainic acid hydrogen bonds with the side chain of a Glu residue and a proline carbonyl [Bunch, L. and Krogsgaard-Larsen, P. 2009]. Molecular modeling/docking studies using the program Macromodel docking show (3*S*,4*S*,5*S*)-4-(carboxymethyl)-5-(prop-1-en-2-yl)pyrazolidine-3-carboxylic acid **26** in the ionotropic glutamate receptor binding site of iGluR6 (Figure 5), suggesting that the nitrogen at position 1 of this aza analog may reproduce some the critical interactions between the kainic acid -NH and the receptor [Di, M. 2006]. This led us to ask if both nitrogens or a single nitrogen in compound **25** are required for binding.

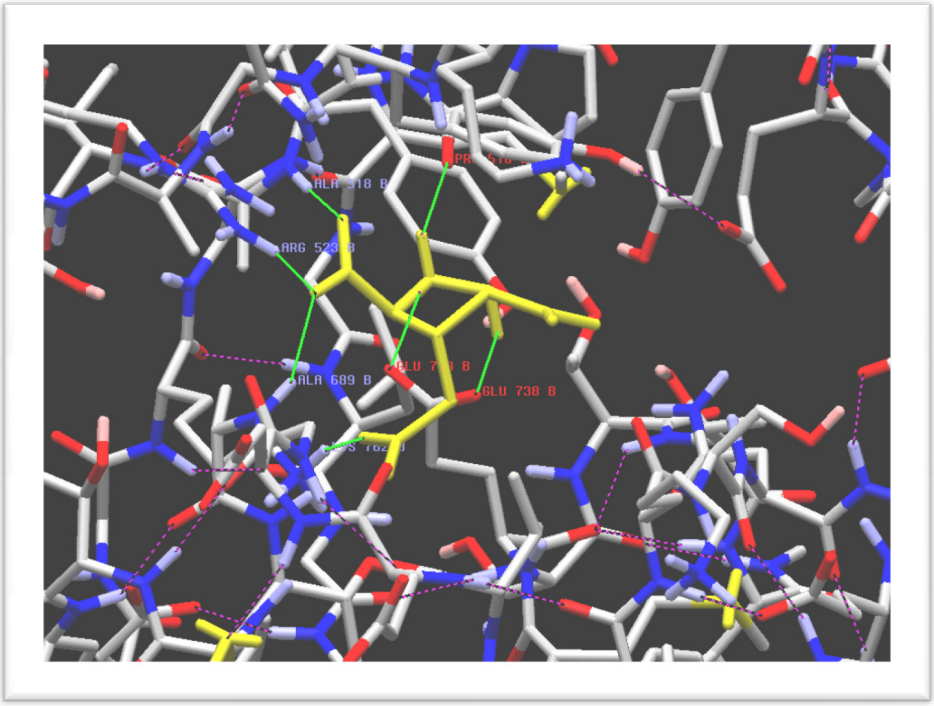
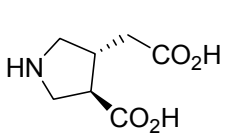


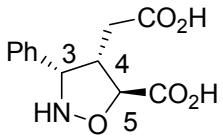
Figure 5. Compound **26** inserted into the iGluR6 crystal structure and minimized with Macromodel using the AMBER force field. Purple lines are hydrogen bonds within the receptor. Green lines are hydrogen bonds between the receptor and ligand.

Therefore, our first objective was to identify the amine group(s) involved in hydrogen bonding with iGluR in compound **25**. We proposed the synthesis of *trans*-4-(carboxymethyl)pyrrolidine-3-carboxylic acid **27**. To our surprise, this compound has never been synthesized or tested as a glutamate antagonist. A single citation appears in the literature for this compound [Miyamoto, M. et al. 1957]. The abstract of this paper describes this as an active component isolated from *Diginea simplex*, the same seaweed that produces kainic acid. If **27** can activate iGluR, not only will the -NH at position 5 of compound **25** be confirmed to be the one bonding with iGluR, but this result will also open the opportunity to explore another new class of kainoid analog, isoxazolidines, which may also be prepared by a dipolar cycloaddition. The rationale behind this

assumption is that isoxazolidines bear a nitrogen located at the same position in the five-membered ring as compound **27** does. Thus, it is highly possible for isoxazolidine kainoid analogs to possess the ability to activate iGluR. As such, compound **28** was designed to test its ability to activate iGluR.



27



28

The objectives of this proposal are summarized briefly below

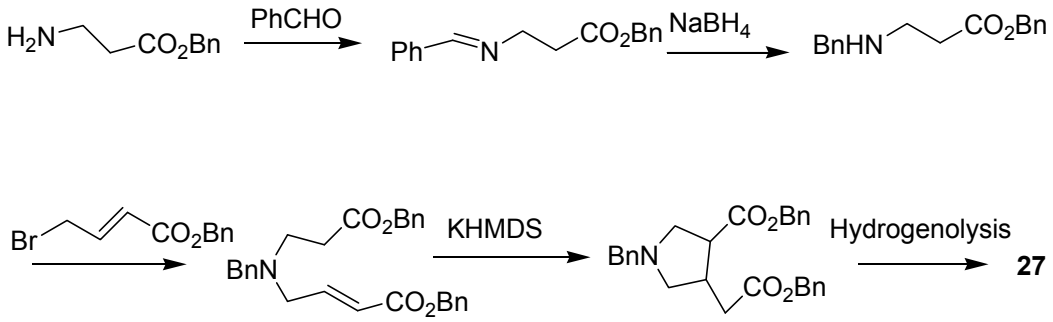
1. Synthesize compound **27** in order to determine if the location of the nitrogen is critical to binding and activity of kainoids at iGluR receptors.
2. Explore the synthesis of isoxazolidine **28** via 1, 3-dipolar cycloaditions using nitrones as the dipole.
3. Evaluate affinity of kainic acid analogs for glutamate receptors by competitive assays against [^{3}H] kainic acid.

1.5 Experimental Design

I. Synthesis of *trans*-4-(carboxymethyl)pyrrolidine-3-carboxylic acid **27**

Scheme 8 illustrates the planned synthesis of **27**. Reductive alkylation of benzyl-β-alanine with benzaldehyde will produce N-benzyl benzyl-β-alanine. After alkylation of the amine with benzylbromocrotonate, an intramolecular Michael addition will provide a

pyrrolidine intermediate. We anticipate the more stable *trans* product will be more abundant. However, we also expect the *cis/trans* diastereomers to be readily separable. Hydrogenolysis of the benzyl prtotecting group will yield **27**.



Scheme 8. Synthetic design of targeted compound **27**

II. Synthesis of isoxazolidine analog of kainoids **28**

Kainoid analog **28** will be prepared by 1, 3 dipolar cycloaddition. It is a very powerful synthetic reaction for the construction of variously functionalized five-membered heterocycles [Pellissier, H. 2007] with control over relative stereochemistry. This reaction involves 2 π-electrons of the dipolarophile and 4 π-electrons of the dipole which take part in a concerted, pericyclic shift. This reaction is a [2s+4s] suprafacial cycloaddtion, so it is stereoconservative and the relative configuration of the dipolarophile will be retained [Carey, F. A. and Sundberg, R. J. 1991]. The relative orbital energies of both the dipolarophile and the dipole HOMOs and LUMOs govern regioselectivity. Generally, electron-withdrawing groups on the dipolarophile favor an interaction of the LUMO of

the dipolarophile with the HOMO of the dipole while electron donating groups on the dipolarophile normally favor the inverse of this interaction [Houk, K. N. 1972].

Therefore, when acting as a dipolarophile, an α, β-unsaturated ester will favor the interaction of the LUMO of the dipolarophile with the HOMO of the dipole, resulting in the regioselectivity such that the terminal heteroatom of the dipole will attack the α-carbon of the ester [Carey, F. A.; Sundberg, R. J. 1991][Houk, K. N. 1972]. For instance, in the 1, 3 dipolar cycloaddition of nitrones with α, β-unsaturated esters, the oxygen atom of nitrones will form a new bond with the α-carbon of the esters(Figure 6).

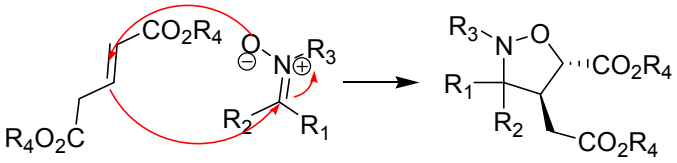
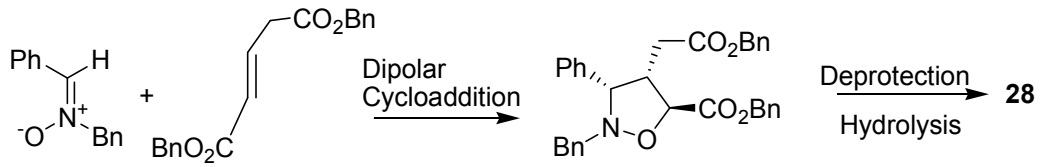


Figure 6. Interaction of the LUMO of α, β-unsaturated esters with the HOMO of nitrones

Isoxazolidine analog of kainoids **28** will be prepared via 1, 3 dipolar cycloaddition from a specific nitrone. The benzyl protecting group of the nitrone is used for optimizing the cycloaddtion (scheme 9), but we are aware of the need to develop an alternative protection/deprotection strategy.

N-benzylidene-benzylamine N-oxide is synthesized according to the method described by Sharma, V. B *et al.* [Sharma, V. B. et al. 2003]. The immediate product of the cycloaddition is an isoxazolidine which will not require reduction. This will avoid the isomerization issue of the 1, 3 dipolar cycloaddition between diazoalkanes and unsaturated esters, thus preventing the loss of one chiral center.

In scheme 9, because of the π-interaction in the transition state [Huisgen, R. and Eberhard, P. 1971], the 4, 5-*cis*-relative configurations in kainoid analog **28** after the 1,3 dipolar cycloaddition will be expected to predominate. In order to control the absolute configuration, chiral catalysts may be used. Kanemasa, S. *et al.* [Kanemasa, S. and Kanai. T. 2000] and Gothelf, K. V. *et al.* [Gothelf, K. V. et al. 1994] have applied some chiral catalysts to 1, 3 dipolar cycloaddition reactions of nitrones.



Scheme 9. Synthetic design of isoxa analog **28**

II. Evaluation of **27** and **28** for kainite receptor affinity

The binding affinity of compounds **27** and **28** will be evaluated in a competitive displacement assay against [^{3}H] kainic acid. This test will be done by a commercial vendor (Caliper).

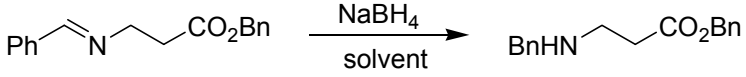
2. Synthesis of pyrrolidine analogs of kainic acid

2.1 Synthesis of trans-4-(carboxymethyl)pyrrolidine-3-carboxylic acid

2.1.1 Experimental results and discussion

In our original synthetic plan (Scheme 8), two intermediates **29** and **30** were designed to be prepared with an intention of removing all benzyl groups by hydrogenolysis after Michael addition. The preparation of **29** was attempted in different solvents (Table 2). However, transesterification always occurred when alcohols were employed as solvents while the imine was not stable in a mixture of an ether and water.

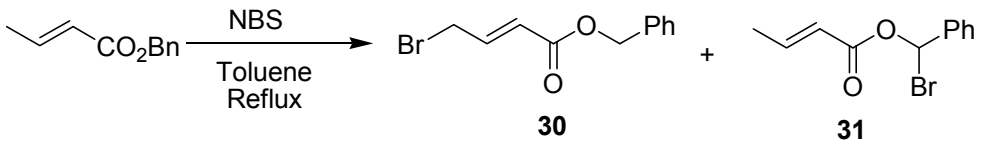
Table 2. imine reduction in different solvent systems



29

Solvent	Result
Methanol	Transesterification
Ethanol	Transesterification
t-butanol	Transesterification
Isopropanol	Transesterification
THF/water	Imine hydrolysis
Dioxane/water	Imine hydrolysis

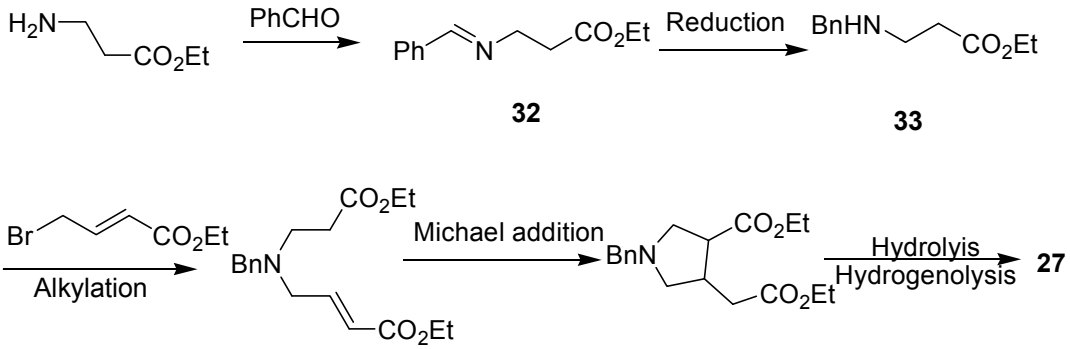
Additionally, the bromination of benzyl crotonate occurred not only on the desired *gamma* methyl group but also on the benylic methylene to form **31**, when N-Bromosuccinimide (NBS) was applied (Scheme 10).



30 **31**

Scheme 10. Bromination of benzyl crotonate

Based on these results, our synthetic route was modified slightly as shown in Scheme 11 to utilize **32** and **33**, the ethyl esters, rather than benzyl esters **29** and **30.** Accordingly, after Michael addition, a hydrolysis of both esters of **35** will be performed.



32 **33**

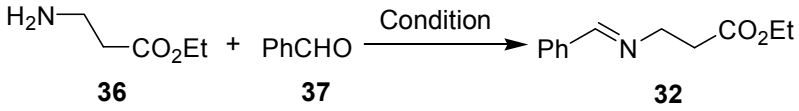
34 **35**

Scheme 11. Modified synthetic route to prepare **27**

In the synthesis of imine **32**, two different methods were examined in the reaction of β-alanine ethyl ester hydrochloride **36** with benzaldehyde **37** (Table 3). Interestingly, both

methods provided **32** with excellent yields. The use of *tert*-butylmethyl ether (TBME) as the solvent in the presence of triethylamine (Et_3N) for 24 hours gave a quantitative yield. By contrast, the formation of the imine **32** in CH_2Cl_2 had a lower yield (92%) and required a longer reaction time (86 h).

Table 3. Synthesis of imine **32**

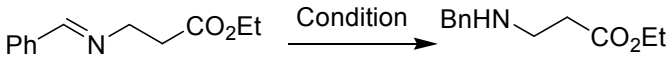


36 **37** **32**

Reaction condition	Yield
Et_3N, TBME, Na_2SO_4, rt, 24h	100%
Et_3N, CH_2Cl_2, $NaSO_4$, rt, 86h	92%

Several reaction conditions for the reduction of imine **32** to amine **33** were evaluated in order to optimize the preparation of N-benzyl-β-alanine ethyl ester **33**. These conditions are summarized in Table 4.

Table 4. Reduction of imine **32**



32 **33**

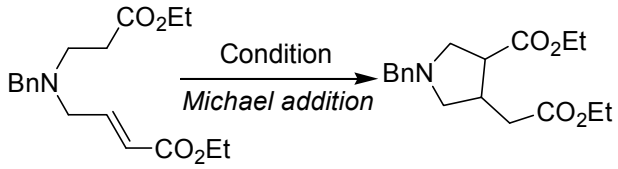
Conditions/reagents	Reaction time	Result
$NaCNBH_3$, glacial acetic acid, rt	3 h 35 min	6.7% yield
$NaCNBH_3$, glacial acetic acid, rt	overnight	Unknown product
H_2, Pd/C, Methanol, rt	24 h	Unknown product
$NaBH_4$, Methanol, rt	Overnight	Transesterification
$NaBH_4$, Ethanol, rt	Overnight	29%
$NaBH_4$, Ethanol, rt	40 min	90%

Reaction of **32** with $NaBH_4$ in ethanol at room temperature for 40 mintutes afforded a 90% yield of **33**.

The alkylation of amine **33** was carried out in acetonitrile with 4-bromoethyl crotonate in the presence of potassium carbonate overnight, giving rise to a Michael addition precursor **34** in a 73% yield after chromatographic purification.

The pyrrolidine ring in compound **35** was generated by a cyclization of **34**. This cyclization was realized by an intramolecular Michael addition in which a formed enolate attacked the α, β-unsaturated carbonyl moiety of precursor **34** to produce a new carbon-carbon bond, thus generating the pyrrolidine ring. The formation of the enolate ion was achieved by deprotonation of **34**. Aimed at discovering the optimal reaction condition, several bases and reaction times were investigated (Table 5).

Table 5. Synthesis of pyrrolidine **35**



34 **35**

Conditions/reagents	Reaction time	Result
TMG, ethanol, rt	Overnight	No reaction
NaH, ethanol, rt	Overnight	No reaction
LiHMDS, dry THF, -78^{o}C	3 h	No reaction
LiHMDS, dry THF, -78^{o}C-rt	3 h	39%
LiHMDS, dry THF, -78^{o}C-rt	25 min	79%

This investigation showed both 1, 1, 3, 3-tetramethylguanidine(TMG) and NaH were not strong enough as bases. Conversely, lithium hexamethyldisilazide (LiHMDS) in dry THF was capable of deprotonating compound **34**, generating a lithium enolate which ultimately cyclized to the desired pyrrolidine ring. This investigation also discovered that the reaction time affected the yield. It was necessary to quench the reaction as soon as **34** was consumed as monitored by TLC. Otherwise, numerous by productes are produced, resulting in a lower yield. An optimal 79% yield was obtained using LiHMDS in dry THF with a 25 min reaction time.

The hydrolysis of the Michael adduct **35** was carried out refluxing 1N HCl solution overnight, followed by the hydrogenolysis under H_2 (48psi) in the presence of Pd/C, affording the final product **27** in a 75% two steps overall yield.

Through analysis of the 700 MHz 2D NOESY NMR spectra, the relative configuration of compound **27** was determined to be *trans*. NOESY correlations which confirm this assignment are shown in Figure 7. The proton Ha is correlated with proton Hc_2 while the proton Hb has a cross peak with protons Hd_1. Those correlations reveal that Ha and Hc_2 are on the same face, and that Hb and Hd_1 are located on the same face. Also, the protons Hc_2 and Hd_2 were found to be correlated. Both Hc_1 and Hc_2 attach to a same carbon, indicating that they should lie on the opposite faces. Same rule can be applied to Hd_1 and Hd_2 which share a same carbon. Accordingly, Hc_2 and Hd_1 are on the different faces since Hc_2 and Hd_2 have a cross peak. Therefore, Ha and Hb reside on the opposite faces. As such, the relative configuration of compound **27** was assigned as *trans*.

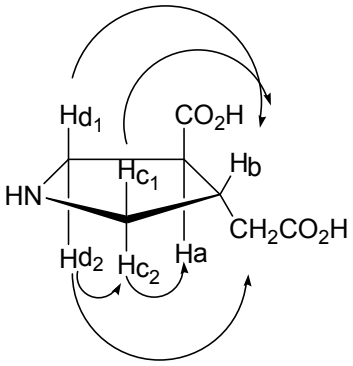
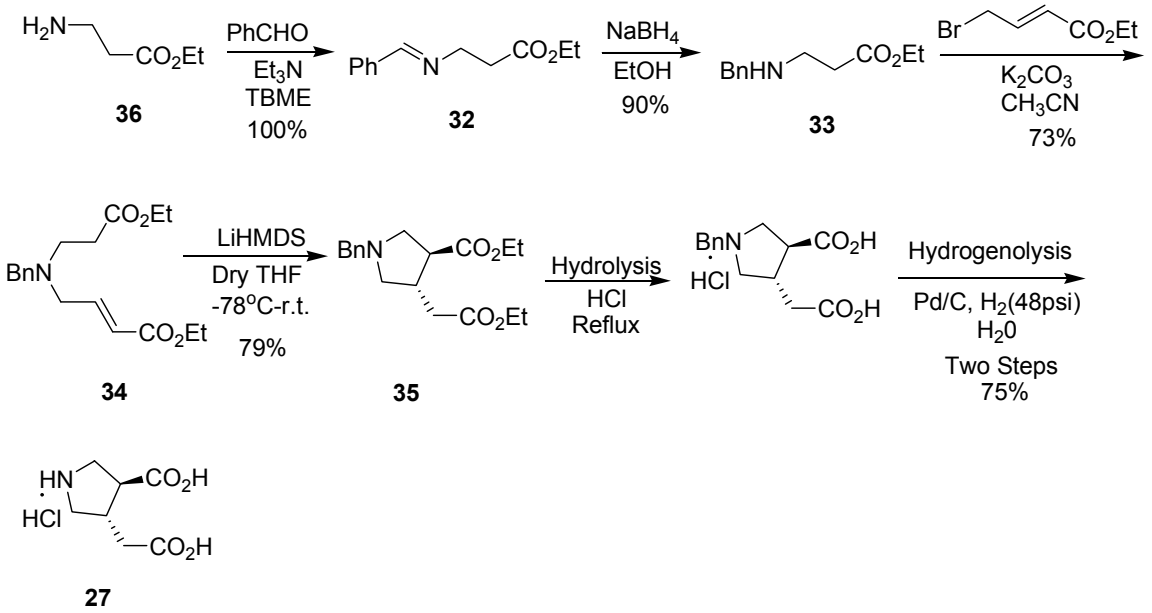


Figure 7. Relative configuration of 4-(carboxymethyl)pyrrolidine-3-carboxylic acid **27**. Important NOE correlations are indicated with arrows.

Based on this NOE spectrum, we may also conclude that the Michael adduct **35** has a *trans* relative configuration as **27** does. As a consequence, on the basis of the discussion above, an optimal synthetic route for **27** can be summarized in Scheme 12.



Scheme 12. Synthesis of *trans*-4-(carboxymethyl)pyrrolidine-3-carboxylic acid **27**

The binding of compound **27** was tested in AMPA, Kainate, NMDA and chloride dependant glutamate binding sites (Figure 8). However, in light of the affinity of **25** for the chloride dependant glutamate binding site, the absence of binding for **27** was surprising. This suggests that the nitrogen at position 2 of aza kainoid **25** may be responsible for its affinity in the chloride dependant glutamate binding site. This would also suggest that the known unsubstituted *trans*-2-carboxy-3-pyrrolidineacetic acid (CPAA) **38**, may have the affinity in the chloride dependant glutamate binding site.

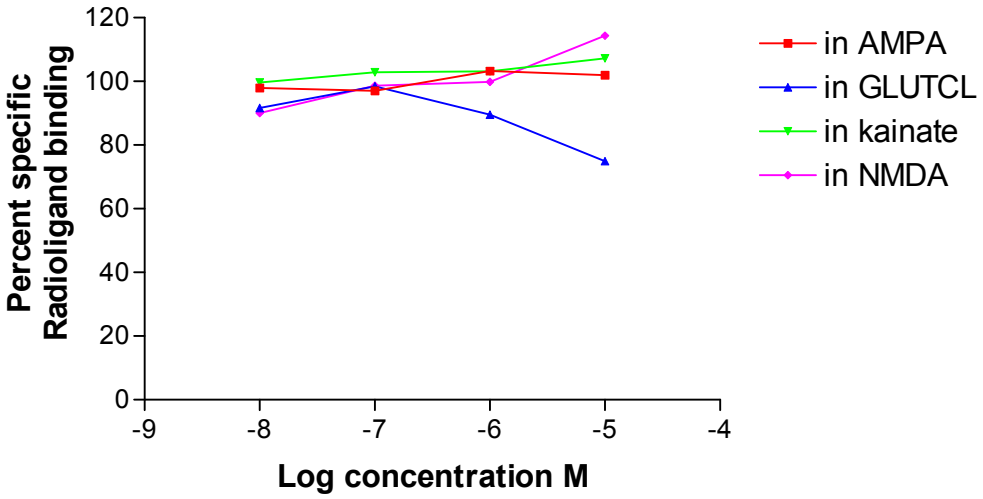
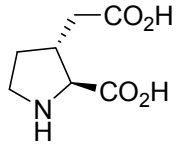


Figure 8. The affinity of compound **27** evaluated in AMPA(■), Kainate(▼) , NMDA(♦) and chloride dependant glutamate(▲) binding sites.

2.2 Synthesis of trans-2-carboxy-3-pyrrolidineacetic acid (CPAA)

CPAA **38** is a known compound for which numerous syntheses have been published [Yoo, S.; Lee, S. and Kim, N. 1988] [Karoyan, P. and Chassaing, G. 2002][Oba, M. et al. 2009]. A single study [Tsai, C. et al. 1988] reported that **38** has no affinity for the kainite

receptor. Therefore, we proposed to synthesize **38** in racemic form and determine its affinity for the chloride dependant glutamate receptor.

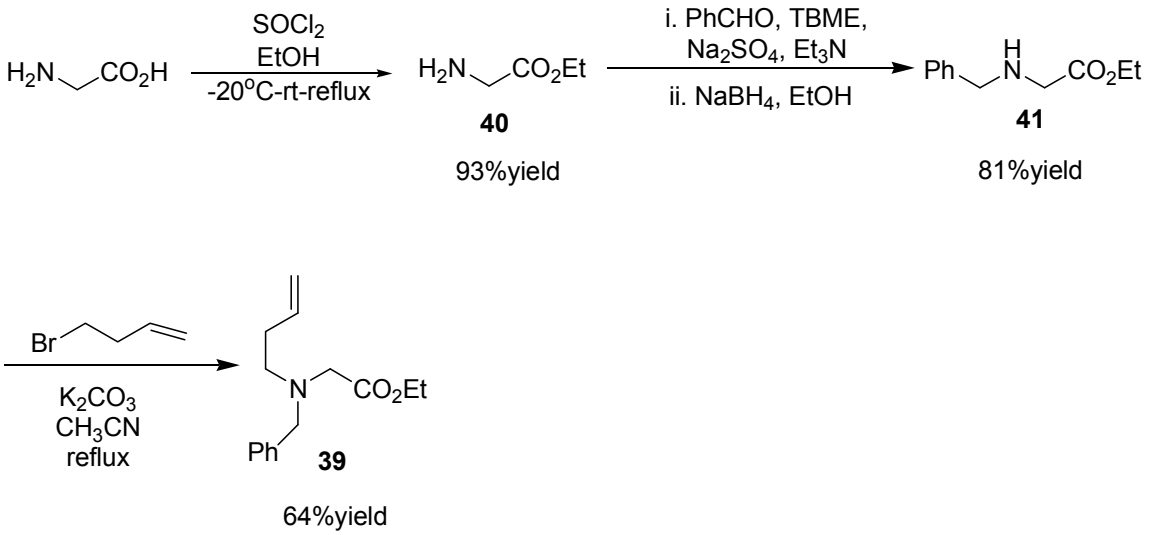


38

Karoyan P. and his associates [Karoyan, P. and Chassaing, G. 2002] utilized a zinc enolate cyclizaiton to stereoselectively synthesize (2*S*, 3*R*)-3-(carboxymethyl)pyrrolidine-2-carboxylic acid, a method that we chose to employ with some modifications.

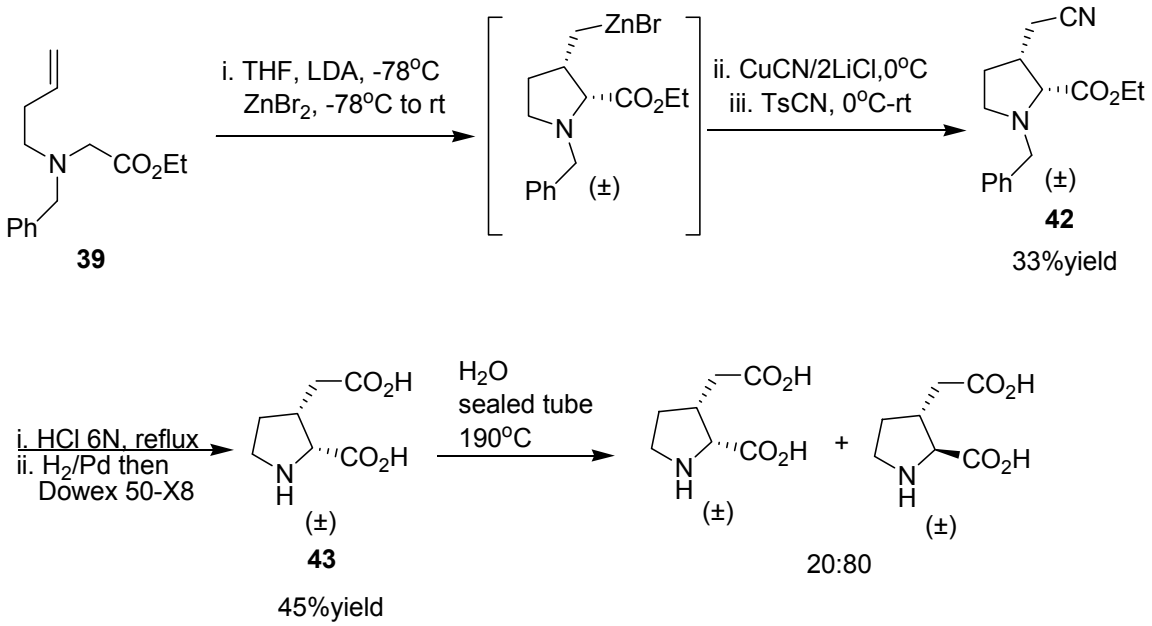
2.2.1 Experimental results and discustion

In the effort to synthesize the *trans* CPAA in racemic form, a cyclization precursor **39** (Scheme 13) was prepared. The preparation of **39** began with an esterification, giving rise to ethyl glycine **40** in 93% yield. The ethyl glycine **40** reacted with benzaldehyde **37** to give an imine intermediate which was reduced with $NaBH_4$ to form an amine **41**. The overall yield for the two steps was 81%. The alkylation of this amine **41** with 4-bromobutene provided the cyclization precursor **39** in a 64% yield.



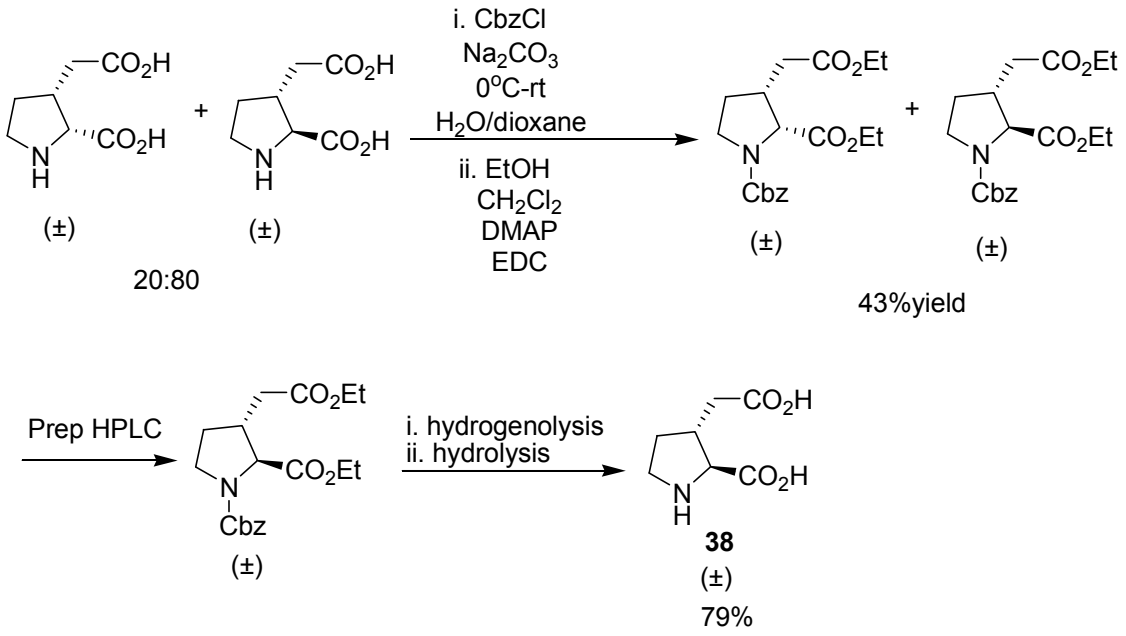
Scheme 13. Synthesis of cyclization precursor **39**

Following the procedure of Karoyan P. et al. [Karoyan, P. and Chassaing, G. 2002], compound **39** was deprotonated by Lithium diisopropylamide (LDA) to form a lithim enolate. Transmetallation with ZnBr provided the zinc enolate which went through a cyclization to produce the *cis* pyrrolidine intermediate in the form of a new Zinc enolate. After a second transmetalation with the addition of THF soluble CuCN/2LiCl salts, an organozinccopper species was produced and reacted with tosylcyanide, leading to compound **42**. This process is extremely sensitive to water, requiring the use of dry reagents and solvents in anhydrous conditions. The hydrolysis and hydrogenolysis of compound **42**, followed by purification using ion-exchange chromatography, gave the *cis* CPAA **43**. The epimerization was realized by heating *cis* CPAA **43** in water at 190°C for 12 hours, producing a mixture of *trans/cis* CPAA (80/20), as determined by ^{1}H NMR (Scheme 14).



Scheme 14. Synthesis of a mixture of *tran*/*cis* CPAA

However, the unsuccessful attempts to separate *trans* CPAA from its *cis* isomer by recrystallization, prompted us to prepare their Cbz protected derivatives (Scheme 15) with an intention of obtaining the pure *trans* derivative by chromatography. The mixture of *trans/cis* CPAA (80/20) was treated with benzyl chloroformate, to form the Cbz protected *cis/trans* CPAA mixture, followed by an esterification with ethanol in the presence of 4-dimethylaminopyridine (DMAP) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), leading to the mixture of Cbz protected *cis/trans* CPAA derivatives in an overall 43% yield. Preparative HPLC was employed to afford only the *trans* derivative. The hydrogenolysis and hydrolysis of this *trans* derivative gave the desired *trans* CPAA **38** in racemic form.



Scheme 15. Preparation of *tran* CPAA **38** from a mixture of *trans*/*cis* CPAA (80/20)

The binding of the *trans* CPAA **38** was tested in AMPA and chloride dependant glutamate binding sites (Figure 9). But, surprisingly, no affinity for any of those receptors was observed.

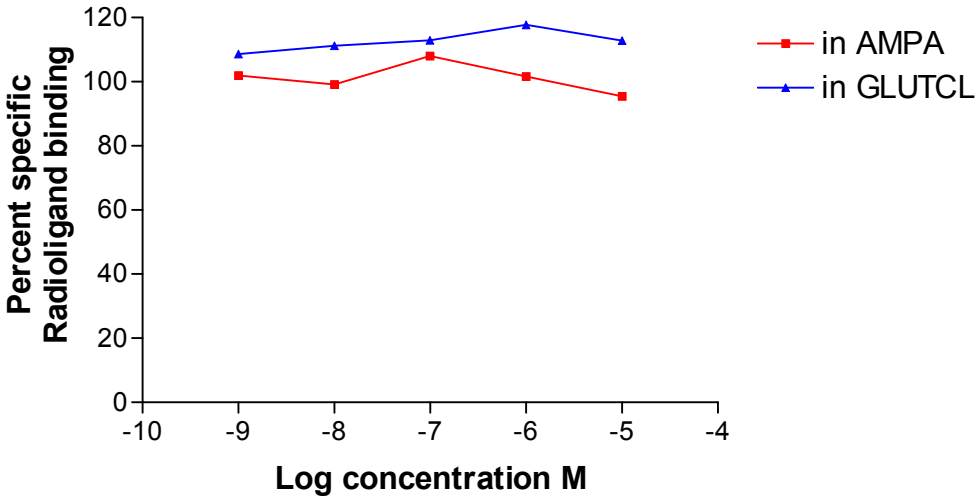


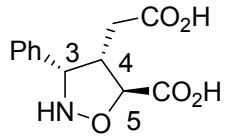
Figure 9. The affinity of *trans* CPAA **38** evaluated in AMPA(■) and chloride dependant glutamate(▲) binding sites.

2.3 Conclusion

The lack of the affinity of both compound **27** and the *trans* CPAA **38** for chloride dependant glutamate binding site suggested that both nitrogens in aza kainoid **25** are involved in hydrogen bonding, thus significantly enhancing the affinity of **25** in the chloride glutamate binding site.

3. Synthesis of isoxazolidine analog of kainic acid

3.1 1, 3 dipolar cycloaddtion as a key step realizing desired absolute configurations



28

Isoxazolidine analog **28** of kainic acid has the *S*, *S* and *S* absolute configurations at its three chiral centers. Those configurations will be generated by a key reaction: 1, 3 Dipolar cycloaddition of an unsaturated α, β ester with a nitrone. 1, 3 dipolar cycloaddition requires consideration of regioselectivity as well as relative stereoselectivity (*exo*/*endo*) and absolute stereoselectivity (diastereofacial). The regioselectivity has been discussed above in the introduction section and on the basis of the assumption that the α, β-unsaturated ester (the dipolarophile) will favor the interaction of the HOMO of the dipolarophile with the LUMO of the nitrone (the dipole), so the terminal oxygen atom of the nitrone is predicted to attack the α-carbon of the ester.

Assuming this regioselectivity, the *exo*/*endo* selectivity is outlined in Figure 10. According to the two transition states, the *endo* pathway will yield a 3, 4-*cis* relative configuration, as preferred in our synthetic plan for **28**. On the other hand, the *exo* route will form a 3, 4-*trans* configuration.

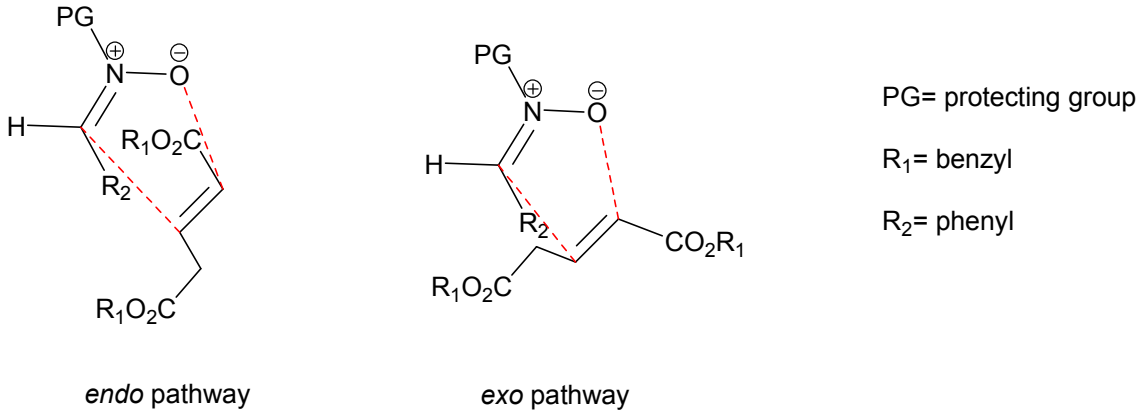


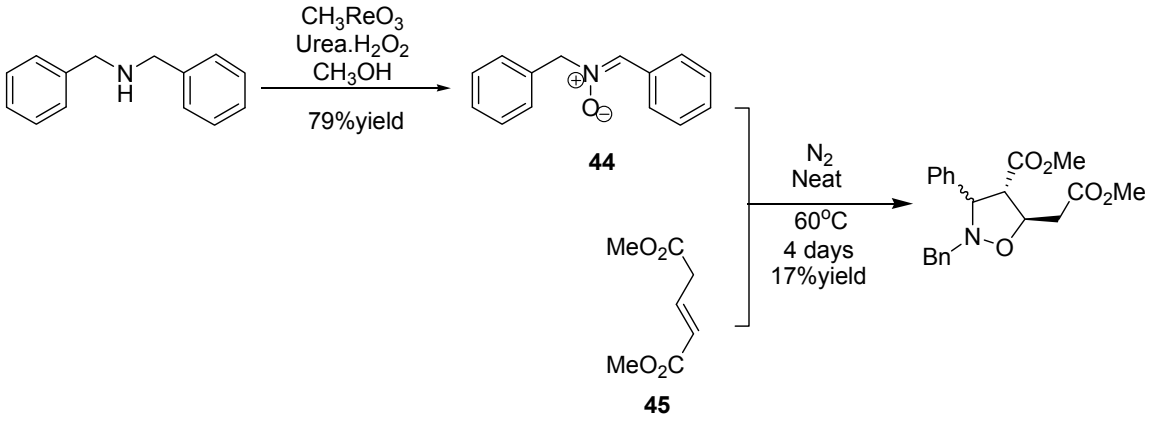
Figure 10. *Exo/Endo* selectivity in synthesis of **29** given a proposed regioselectivity

Given the selected *endo* pathway, the diastereofacial selectivity may offer two different enatiomers, determined by the attack of the dipole on the *Re*/*Si* face of the unsaturated ester at α position. *Si* facial approach will give the *S*, *S* and *S* absolute configurations at position 3, 4 and 5, whereas the *Re* face attack will afford a *R*, *R* and *R* absolute configurations at position 3, 4 and 5. Chiral catalysts may be applied to achieve the desired diastereofacial selectivity as mentioned in the introduction.

3.2 Model study of 1, 3 dipolar cycloaddtion between trans-dibenzyl glutaconate and benzyl protected nitrone

With the aim of confirming those hypotheses regarding to the regioselectivity and stereoselectivities, a model study, 1, 3 dipolar cycloaddition of benzyl protected nitrone **44** with dimethyl glutaconate **45** (Scheme 16) was launched. However, the undesired regioselectivity along with the production of multiple diastereomers suggested the need to revise the synthetic plans for **28**. Additionally, as mentioned in the experimental design, our concerns regarding the protecting groups of nitrones also came to mind. Most

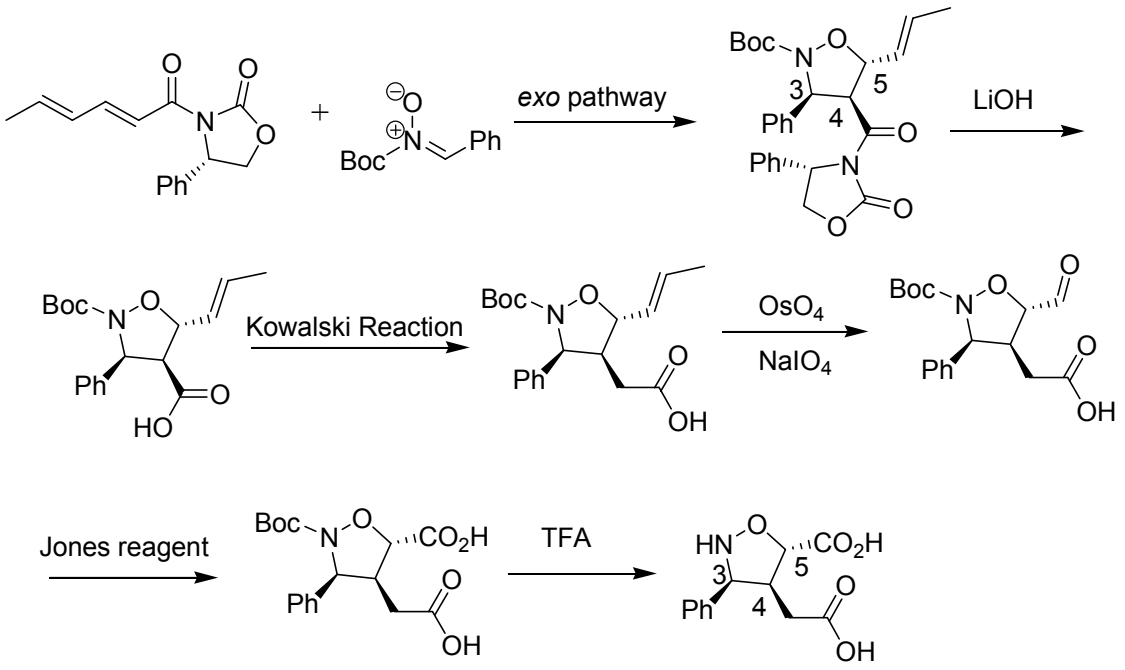
nitrones are protected by alkyl, phenyl and benzyl groups. Unfortunately, no method for the removal of the former two groups from the generated isoxazolidines has been devised. Hydrogenolysis can cleave the benzyl group, but it will also result in the reductive cleavage of the N-O bond of the isoxazolidine ring.



Scheme 16. 1, 3 Dipolar cycloaddition between benzyl protected nitrone **44** and dimethyl glutaconate **45** yields undesired regioselectivity and multiple diastereomers.

3.3 An alternative synthetic plan

Thus, a new synthetic plan (Scheme 17) for **28** was proposed. A chiral oxazolidinone diene derivative could react with a Boc protected nitrone using Lewis acids as catalysts to provide an isoxazolidine cycloadduct. Upon the removal of the oxazolidinone moiety, a *Kowalski* reaction was planned to insert a methylene group between the isoxazolidine ring and the carboxylate group. A new carboxylic acid group could be formed through the oxidation of the vinyl group. The deprotection of the Boc protected amine by TFA will lead to the target compound **28**.



Scheme 17. Revised synthetic plan of isoxazolidine analog **28**

3.3.1 Influences of Lewis acids on 1, 3 dipolar cycloaddition

In this synthetic plan, the 1, 3 dipolar cylcoaddition is still a key reaction whose transition state is controlled by the frontier molecular orbitals (FMO) of dipoles and dipolarophiles, i.e. The HOMO dipolarophile can react with the LUMO dipole while the LUMO dipolarophile with the HOMO dipole [Gothelf, K. V. and Jorgensen, K. A. 1998]. On the basis of Sustmann's classification, 1, 3-dipolar cycloadditions were divided into three types, according to the relative FMO energies between the dipole and the dipolarophile (Figure 11) [Sustmann, R. 1971] [Houk, K. N. et al. 1973].

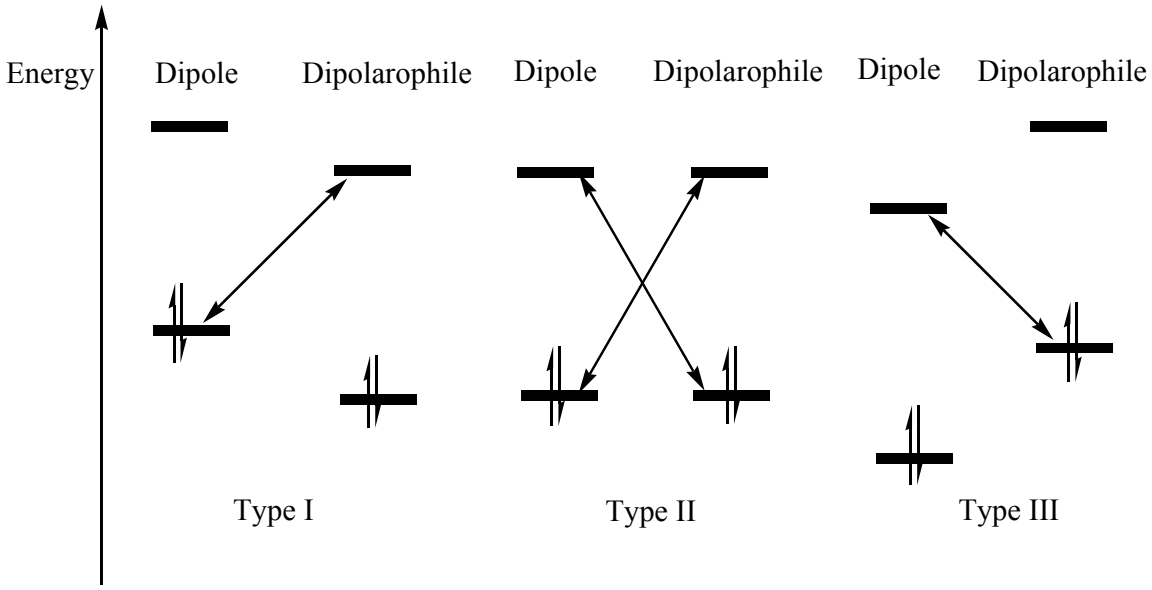
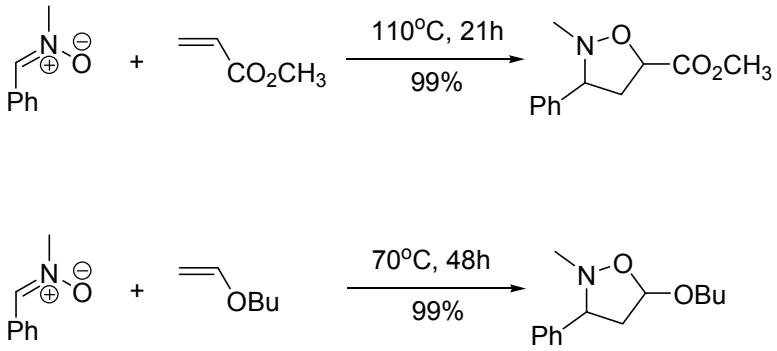


Figure 11. Sustmann's classification of 1, 3-dipolar cycloaddtions: type I, a HOMOdipole-LUMOdipolarophile interaction; type II, interaction of both HOMOdipole-LUMOdipolarophile and LUMOdipole-HOMOdipolarophile; type III, a LUMOdipole-HOMOdipolarophile interaction.

In this classification, the type I involves HOMO dipole-LUMO dipolarohile interactions in the transition state.; in type II, the similarity of the dipole and dipolarophile FMO energies suggests that the 1, 3-dipolar cycloadditon can undergo either the HOMO dipole-LUMO dipolarohile interaction or the HOMO dipolarophile-LUMO dipole interaction; in type III, the dominant interaction is that between the LUMO dipole and the HOMO dipolarophile..

The 1, 3 dipolar cycloaddition of nitrones are normally classified as type II, therefore, its transition state can be controlled by either HOMO dipole-LUMO dipolarophile interaction or HOMO dipolarophile-LUMO dipole interaction. For instance, in Scheme

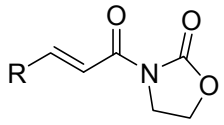
18, when N-methyl-C-phenylnitrone reacts with methyl acrylate, this 1, 3 dipolar cycloaddition is controlled by the HOMO dipole-LUMO dipolarophile, whereas the 1, 3 dipolar cycloaddition between the same nitrone and vinyl ether is controlled by the LUMO dipole-HOMO dipolarophile [Gothelf, K. V.; Jorgensen, K. A. 1998][Perez, P. et al. 2003].



Scheme 18. 1, 3 dipolar cycloaddition of N-methyl-C-phenylnitrone with methyl acrylate and vinyl ether, performed by Perez, P. et al.. [2003]

The presence of a Lewis acid influences the FMO-energy of nitrones or dipolarophiles. For instance, the coordination of a Lewis acid, such as $BF_3{\cdot}OEt_2$ or $AlCl_3$ to a nitrone, lowers both HOMO and LUMO energies of the nitrone. Thus, if the nitrone acts as electrophile in the cycloaddition, one would predict that this coordination would accelerate the reaction. But, according to Gothelf, K. V. et al., on a model 1, 3-dipolar cycloaddition of benzylidenemethylamine N-oxide with styrene, the transition state energy calculations indicated that the coordination between a Lewis acid like $BF_3{\cdot}OEt_2$ or $AlCl_3$ and a nitrone increased the transition state energy of 1, 3 dipolar cycloaddition, as compared to a reaction without the involvement of Lewis acid [Gothelf, K. V. and

Jorgensen, K. A. 1994]. Therefore, the catalytic effect of these Lewis acids is difficult to predict. On the other hand, Gothelf and his associate found that dipolarophile 3-acyl-1, 3-oxazolidin-2-one **46** can chelate titanium (IV) complexes. This chelation between the conjugated carbonyls of **46** and the Lewis acid (titanium complexeses), considerably accelerated the cycloaddition reaction [Gothelf, K. V. and Jorgensen, K. A. 1994].



46

The proposed application of Lewis acids will also have a significant influence on the stereoselectivities. The *exo/endo* selectivity dictating the relative configuration of the newly formed carbon-carbon bond is affected by the coordination of dipolarophile to a Lewis acid catalyst [Hein, J. E.; Julttin, P. G. 2005][Gothelf, K. V. et al. 1996]. Hence, the desired *cis* relative configuration between position 3 and 4 of our target compound **28** can be accomplished by the correct choice of Lewis acids.

The regioselectivity of the oxazolidinone substituted ene-ones towards nitrones is the same as that of dimethyl glutaconate. The *exo*/*endo* selectivity using oxazolidinone substituted ene-ones as dipolarophiles can be depicted in Figure 12. From those transition states, the *exo* selectivity will give a 3, 4-*cis* relative configuration in the generated isoxazolidnes, as preferred for our target compound **28**. By contrast, the *endo* selectivity will form a 3, 4-*trans* relative configuration.

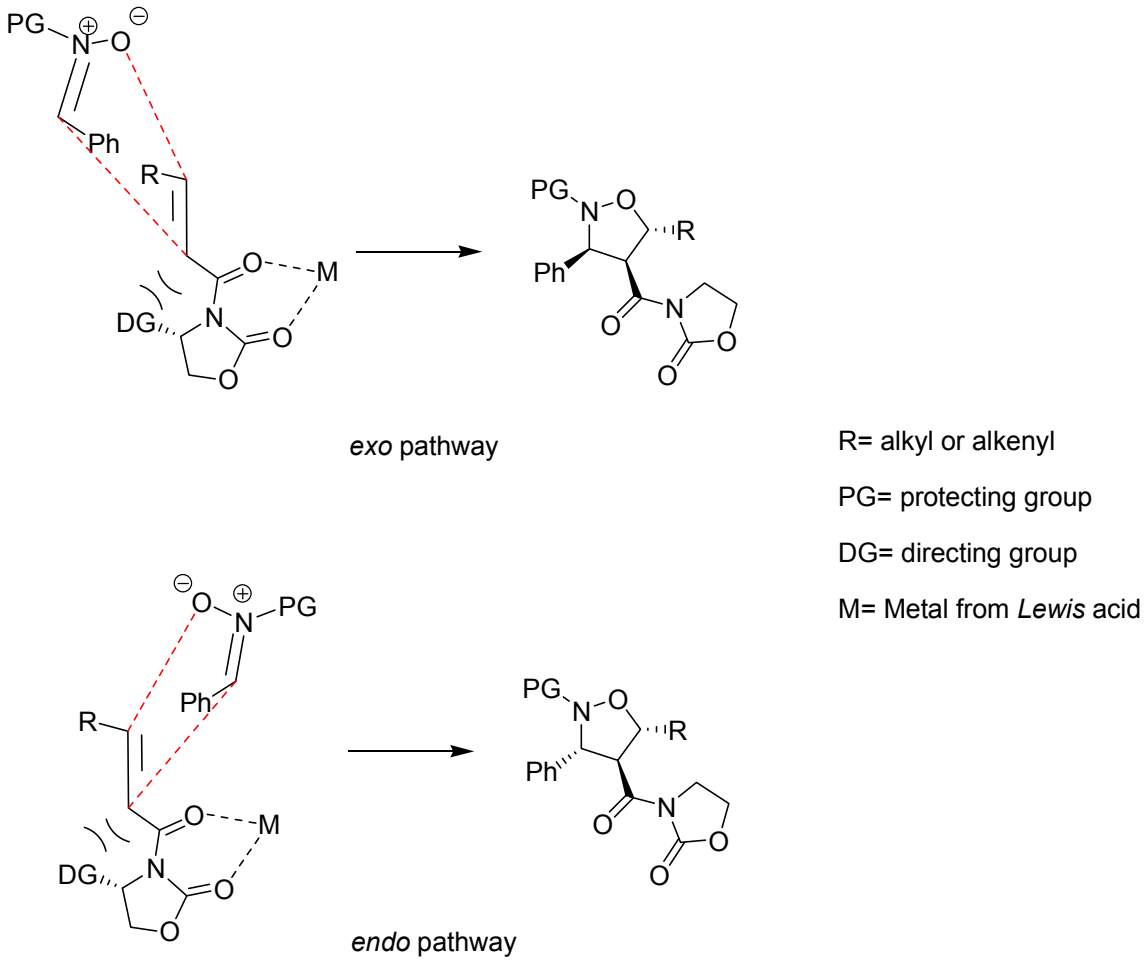


Figure 12. *Exo*/*Endo* selectivity using oxazolidinone alkene dipolarophiles

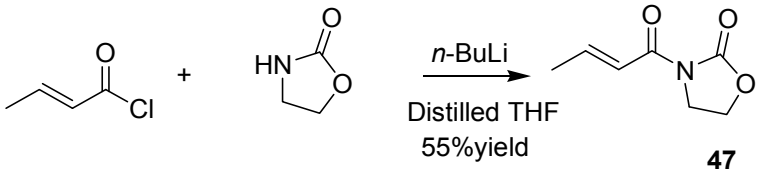
A study by Gothelf, K. V. et al. [Gothelf, K. V. et al. 1996] established that the octahedral $Ti(O\text{-}iPr)_2Cl_2$ Lewis acid catalyst favored the *exo* pathway and tetrahedral metal complex catalysts favored the *endo* pathway.

The diastereofacial preference of the dipolarophile dictates the formation of the absolute configurations of the major product. Selectivity is governed by both chelation of the carbonyls of the dipolarophile to the metal catalyst [Hein, J. E.; Julttin, P. G. 2005][Faita,

G. et al. 2001], and the steric effect of a substituent (the directing group) in the oxazolidinone ring (Figure 12). In the synthetic route (Scheme 17), the phenyl directing group controls the direction of approach of the dipole (opposite the phenyl group) towards the Cα-*Si* face, favoring the formation of the *S* configuration at position 4 of the isoxazolidine ring.

3.3.2 1, 3 dipolar cycloaddtion between 3-crotonyloxazolidinone and benzyl protected nitrone using different Lewis acids

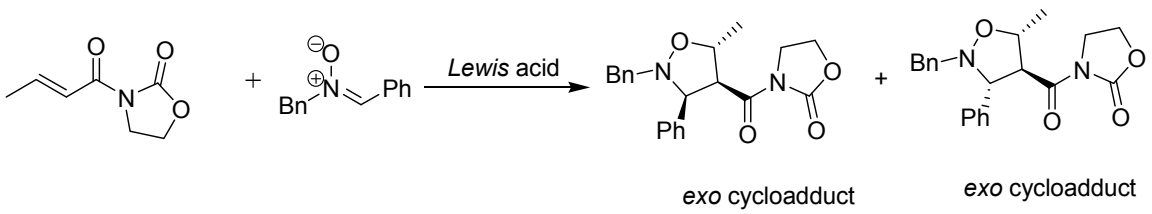
3-Crotonyloxazolidinone, an achiral dipolarophile **47** was prepared through a nucleophilic acyl substitution reaction of deprotonated 2-oxazolidone and crotonyl chloride in dry THF in a 55% yield (Scheme 19).



Scheme 19. Preparation of achiral dipolarophile **47**

Intending to find optimal reaction conditions for this 1, 3 dipolar cycloaddition, a model study (Table 6), using this simplified and achiral dipolarophile **47**, was examined with several different Lewis acids.

Table 6. 1, 3 Dipolar cycloaddition between oxazolidinone derivative **47** and benzyl protected nitrone using different Lewis acids

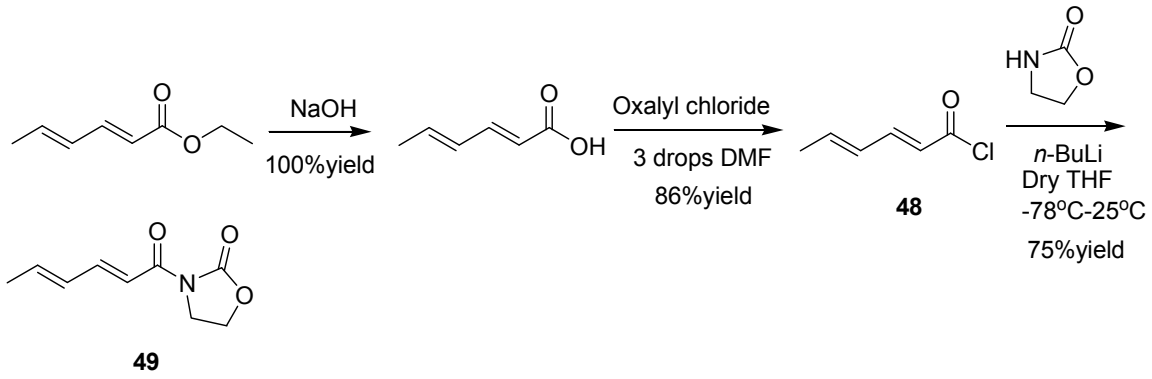


Lewis acid	Solvent/temperature	Time	Result
$BF_3{\cdot}(OEt)_2$ (1.2eq)	Dry CH_2Cl_2/r.t.	7 days	No reaction (NR)
$AlCl_3$(1.2eq)	Dry CH_2Cl_2/r.t.	5 days	NR
$ZnCl_2$(1.2eq)	Dry CH_2Cl_2/r.t.	5 days	NR
$Sc(TfO)_3$ (1.2eq)	Dry CH_2Cl_2/r.t.	2 days	NR
$CeCl_3$(1.2eq)	Dry CH_2Cl_2/r.t.	2 days	NR
$CuBr_2$ (1.2eq)	Dry CH_2Cl_2/r.t.	2 days	NR
$SnCl_2{\cdot}2H_2O$(1.2eq)	Toluene/55^{o}C	5 days	NR
$ZnCl_2$(1.2eq)	Toluene/55^{o}C	1 day	Small amount of product but most of starting materials unreacted based on NMR
$Sc(TfO)_3$ (0.1eq)	Dry CH_2Cl_2/r.t. with 4A Molecular Sieve (M.S.)	3 days	Small amount of product but most of starting materials unreacted based on NMR
$Sc(TfO)_3$ (0.1eq)	Dry CH_2Cl_2/r.t. with 6eq water	3 days	NR
$TiCl_4$(0.2eq)	Dry CH_2Cl_2/r.t. with 4A M.S.	7 days	About 1/3 starting material remained based on the NMR
$Ti(i\text{-}OPr)_2Cl_2$ (0.2eq)	Dry CH_2Cl_2/r.t. with 4A M.S.	4 days	Crude product (*exo/endo*: 77/23) based on NMR and after the purification, *exo/endo*:9:1 37%yield

This examination of Lewis acids provided several potential catalysts for the proposed 1, 3 dipolar cycloaddition of the chiral oxazolidinone diene dipolarophile. Among them, however, $Ti(i\text{-}OPr)_2Cl_2$ gave predominantly the *exo* cycloadduct with a better conversion, as determined by comparison with existing NMR data of the *exo* and *endo* cycloadducts [Gothelf, K. V. and Jorgensen, K. A. 1994]. The result that $Ti(i\text{-}OPr)_2Cl_2$ preferred the *exo* pathway is consistent with Gothelf's finding [Gothelf, K. V. et al. 1996][Gothelf, K. V. and Jorgensen, K. A. 1994].

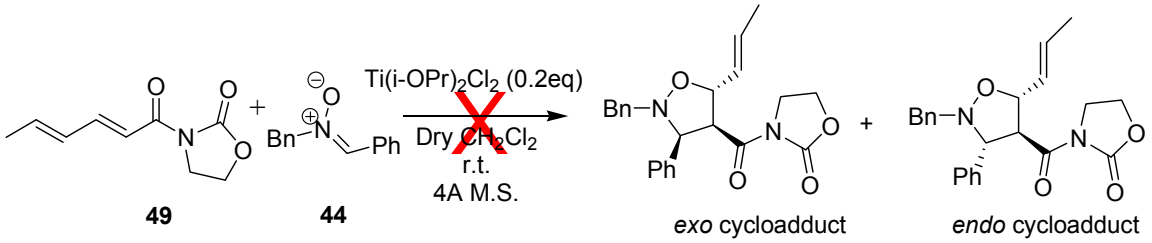
3.3.3 1, 3 dipolar cycloaddition of oxazolidine diene dipolarophile and synthesis of Boc protected nitrone

Encouraged by this result, an achiral oxazolidinone diene derivative **49** was prepared (Scheme 20) in a manner similar to the preparation of **47**, with a 75% yield in the substitution reaction between sorbyl chloride **48** and deprotonated 2-oxazolidone.



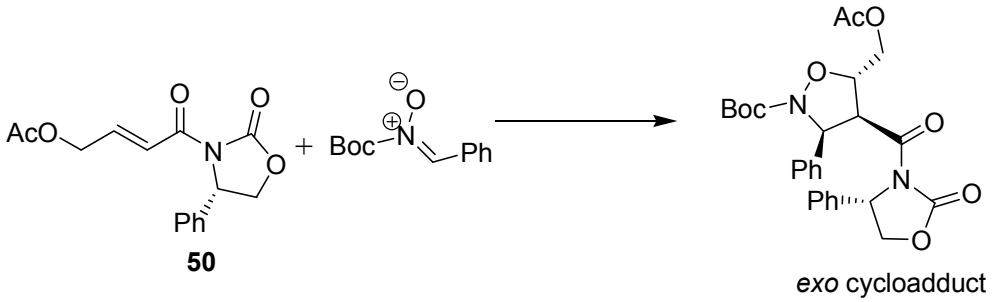
Scheme 20. Preparation of oxazolidinone diene derivative **49**

Compound **49** was used in additional model studies (Scheme 21) to examine its utility in a 1, 3 dipolar cycloaddition with benzyl protected nitrone **44**.



Scheme 21. Model study between diene **49** and benzyl protected nitrone **44**

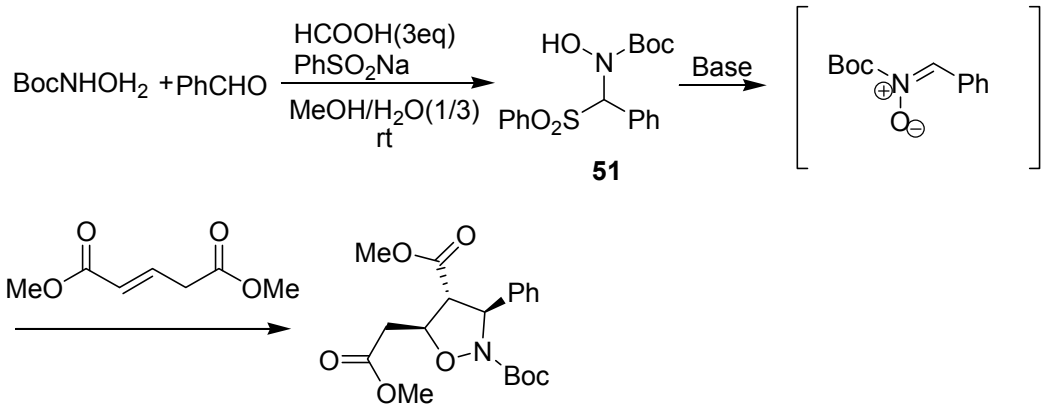
Discouragingly, this reaction did not proceed as expected. The product was not purified for characterization. However, based on the ^{1}H NMR of crude product, the vinyl methyl doublet in the dipolarophile shifted from 2 ppm to 1.1 ppm, suggesting that a cycloaddition or Diels Alder reaction occurred involving the γ, δ double bond. To address this problem, a new dipolarophile **50** with acetoxymethyl moiety was designed for the cycloaddition (Scheme 22) with the expectation that the acetyl group could be hydrolyzed and the resulting alcohol oxidized to the carboxylic acid..



Scheme 22. Proposed 1, 3 dipolar cycloaddition between Boc protected nitrone and dipolarophile **50**

Being aware of that the N-benzyl was not the optimal protecting group, concurrent to the experiment above, a different nitrogen protecting group was sought. The Boc protected

hydroxylamine **51** has been reportedly prepared and converted in *situ* to the corresponding Boc protected nitrone with the aid of a base, followed by a reaction with a dipolarophile in a one pot process (Scheme 23) [Gioia, G. 2009]. It should be noted that the reaction of this N-Boc protected nitrone proceeds through a two step polar process instead of a 1, 3-dipolar cycloaddition, possibly providing the undesired all *trans* trisubstituted isoxazolidines as Gioia, G.; et al. obtained. With this consideration as well as numerous unsuccessful attempts to obtain this hydroxylamine **51**, an alternate approach was taken.



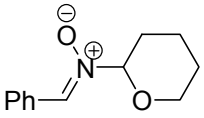
Scheme 23. Preparation of Boc protected hydroxylamine **51** and its conversion for 1, 3 dipolar cycloadditioin, performed by Gioia, G. *et al.*

3.3.4 1, 3 dipolar cycloaddition of aminal protected nitrones

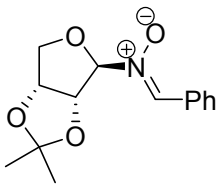
3.3.4.1 Aminal protected nitrones and their corresponding synthetic plans

A search of the literature revealed two aminal protected nitrones, oxane protected ntirone **52** [Abiko, A. 1995] and glycosylnitrone **53** [Cicchi, S. et al. 2003], which have been used in 1, 3-dipolar cycloadditions. Each of these nitrones incorporates an amino

protecting group that can be removed under mild acidic conditions after the cycloaddition. This addresses the issue of reductive cleavage of the isoxazolidine N-O bond caused by hydrogenolysis when utilizing benzyl protecting group.

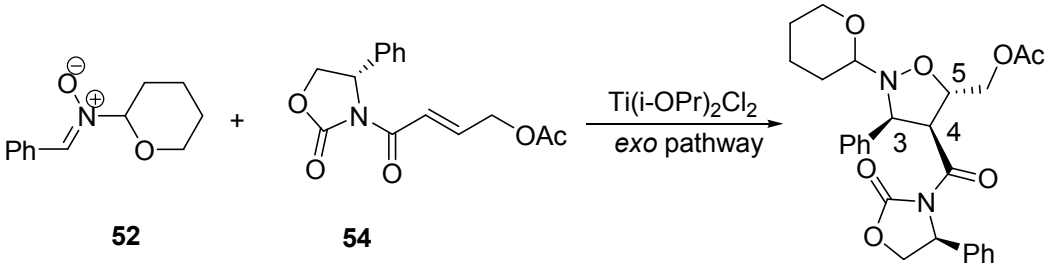


52



53

Nitrone **52** was previously prepared by Abiko, A. [Abiko, A. 1995] without a detailed preparation procedure. As this nitrone **52** will be formed in racemic form, a chiral oxazolidinone derivative **54** was designed to as the dipolarophile, with the expectation that the stereoselectivity will be controlled predominantly by this dipolarophile. Therefore, nitrone **52** could be used as shown in Scheme 24. In this approach, the oxane protected ntirone **52** will react with a chiral oxazolidinone derivative **54** in the presence of $Ti(i\text{-}OPr)_2Cl_2$ to form a desired *exo* cycloadduct with the 3*S*, 4*S* and 5*S* absolute configurations.

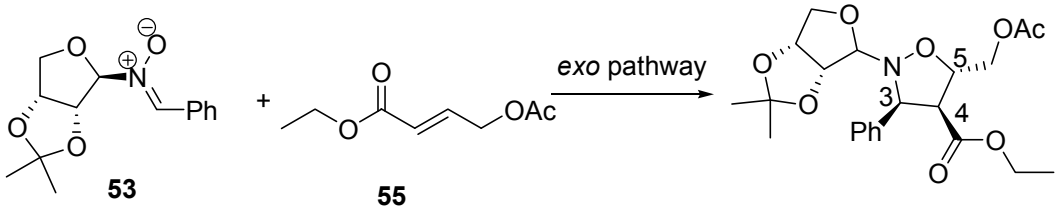


52 **54**

Scheme 24. Proposed cycloaddition between nitrone **52** and oxazolidinone derivative **54**

Nitrone **53** could be used as shown in Scheme 25. In this approach, the dipole **53,** instead of the achiral dipolarophile **55,** possesses a chiral auxiliary to influence both

diastereoselectivity and enatioselectivity. Consequently, this cycloaddition may produce a predominant diastereomer, hopefully, with the desired configurations at positions 3, 4 and 5.

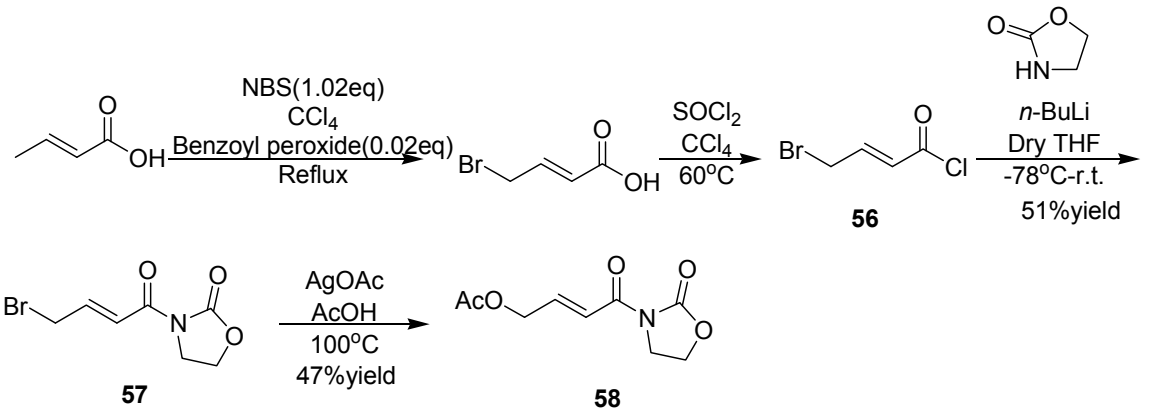


Scheme 25. Proposed cycloaddition of nitrone **53** and crotonate derivative **55**

3.3.4.2 Synthesis of oxane protected nitrone and achiral dipolarophile

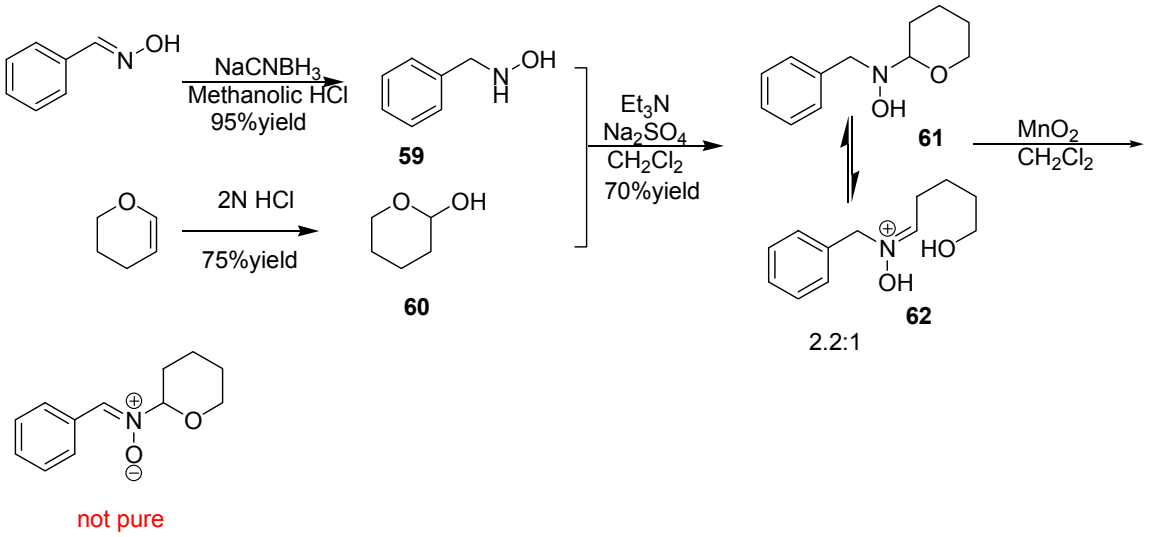
The planned application of $Ti(i\text{-}OPr)_2Cl_2$ and the oxazolidinone moiety in chiral dipolarophile **54**, took a priority in attempting the synthetic route of Scheme 24 because the $Ti(i\text{-}OPr)_2Cl_2$ has proven to be an *exo* selectivity catalyst in chelating to the carbonyl of oxazolidinone moiety.

An achiral dipolarophile **58** was prepared using the multi-step sequence shown below (Scheme 26). It is worth noting that both allylic bromide and acyl chloride moieties of intermediate **56** are suseptible to attack by the activated oxazolidone. Therefore, the deprotonated oxazolidone was transferred very slowly into a flask of excess **56** via a cannula at -78^{o}C. The formed product **57** was treated with silver acetate in glacial acetic acid, converting to the achiral dioplarophile **58** with precipitation of AgBr.



Scheme 26. Preparation of achiral dipolarophile **58**

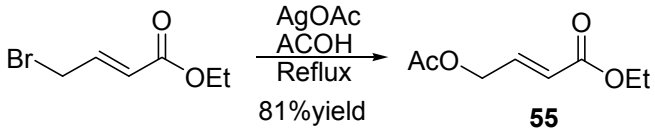
Scheme 27 illustrates the synthetic route for the preparation of nitrone **52**. Benzaldehyde oxime was reduced with $NaCNBH_3$ in a 95% yield to form N-benzylhydroxylamine **59**. Reacting with hemiactal **60**, compound **59** was converted to an equilibrating mixture of oxane protected hydroxylamine **61** and an open-chain nitrone **62** with a ratio of 2.2:1, determined by NMR. The nitrone **52** was formed by oxidizing the mixture of **61** and **62** using MnO_2**.** Due to the decomposition of nitrone **52** on silica gel and no feasible solvents discovered for recrystallization, pure nitrone **52** was not obtained. As a result, the focus was shifted to study nitrone **53** and its cycloaddition.



Scheme 27. Synthesis of nitrone **52**

3.3.4.3 1, 3 dipolar cycloaddition between glycosyl protected nitrone and its corresponding dipolarophile

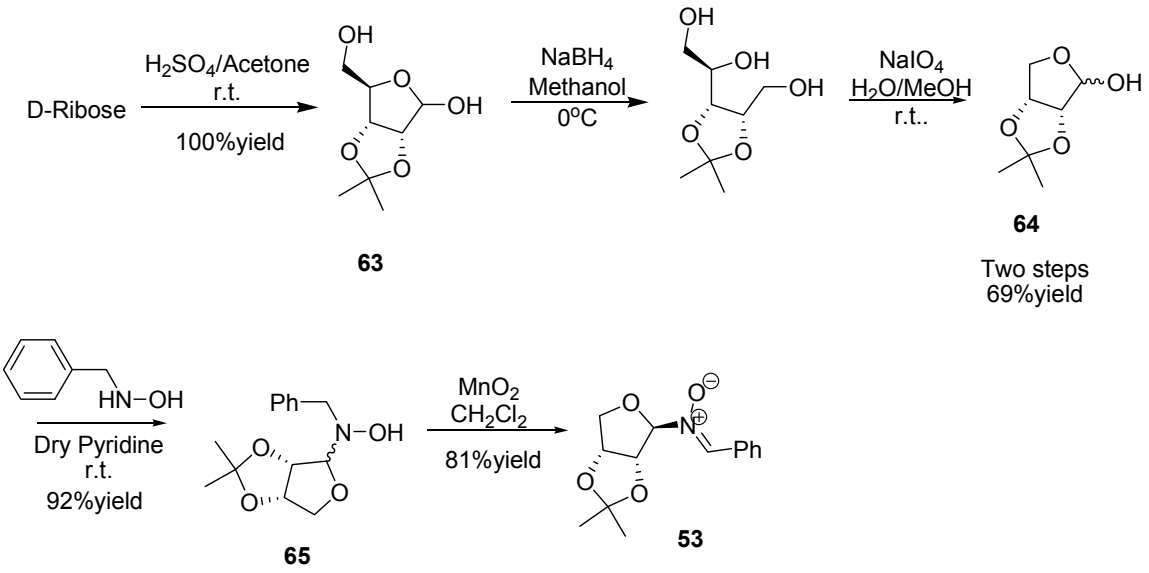
A simple synthesis (Scheme 28) offered dipolarophile **55** in an 81% yield from ethyl 4-bromocrotonate.



Scheme 28. Preparation of dipolarophile **55**

The synthesis of nitrone **53** resulted from a mult-step sequence (Scheme 29). In the presence of concentrated H_2SO_4, the diol of D-ribose reacts with acetone to form the acetonide, compound **63**. $NaBH_4$ was used to reductively open the hemiacetal of **63** to form a triol. This newly formed vicinal diol is subjected to oxidative cleavage by $NaIO_4$,

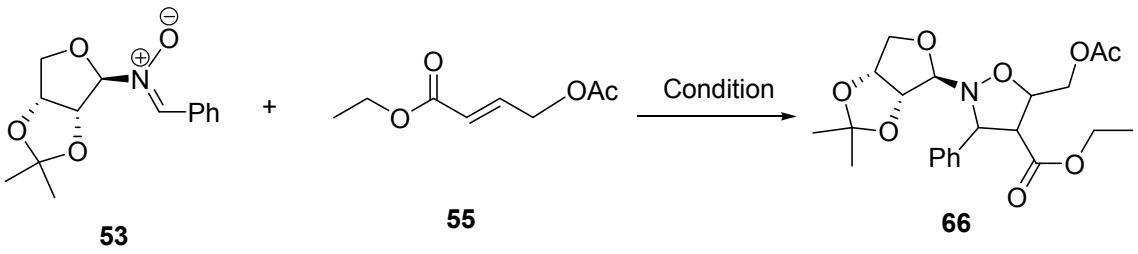
to give an aldehyde which immediately reacts with the *gamma* hydroxyl group to generate the hemiacetal **64**. Benzylhydroxylamine **59** reacts with **64** in the presence of pyridine to give glycosyl protected hydroxylamine **65** in a 92% yield. Glycosylnitrone **53** was obtained by the oxidation of **65** using MnO_2.



Scheme 29. Preparation of nitrone **53**

The 1, 3 dipolar cycloaddition between the glycosylnitrone **53** and the crotonate derivative **55** was examined in two different conditions (Table 7). The use of Ti(i-OPr)$_2$Cl$_2$ did not facilitate the cycloaddition, probably because dipolarophile **55** lacks the ability to chelate the titanium as the oxazolidinone derivatives. However, the reaction of chiral nitrone **53** with **55** refluxing refluxing toluene under N_2 afforded only one diastereomer **66** in a 44% yield.

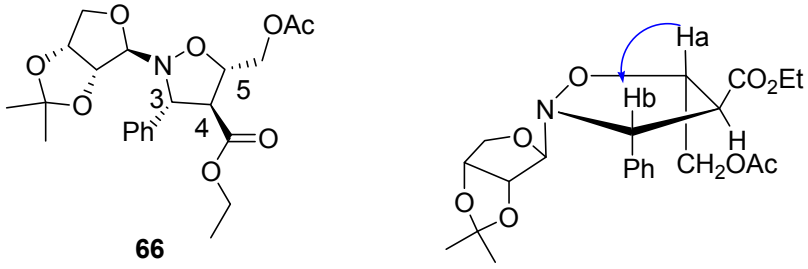
Table 7. 1, 3 Dipolar cycloaddition between nitrone **53** and a crotonate derivative **55**



53 **55** **66**

Reaction condition	Result
$TiCl_2(i\text{-}OPr)_2$/Dry CH_2Cl_2/r.t. 5days	No reaction based on NMR
Toluene/Reflux 6 days	44%yield

Unfornately, the correlation between H_a and H_b in the NOESY spectra (Figure 13) indicated that this cycloaddition proceeded through an *endo* pathway, giving rise to a cycloadduct with *trans* relative configuration between positions 3 and 4, not the preferred *cis* configuration. This result also demonstrates the importance of the chelation between the metal catalyst and oxazolidinone substituted dipolarophiles, in affecting the *exo/endo* selectivity of 1, 3-dipolar cycloaddition

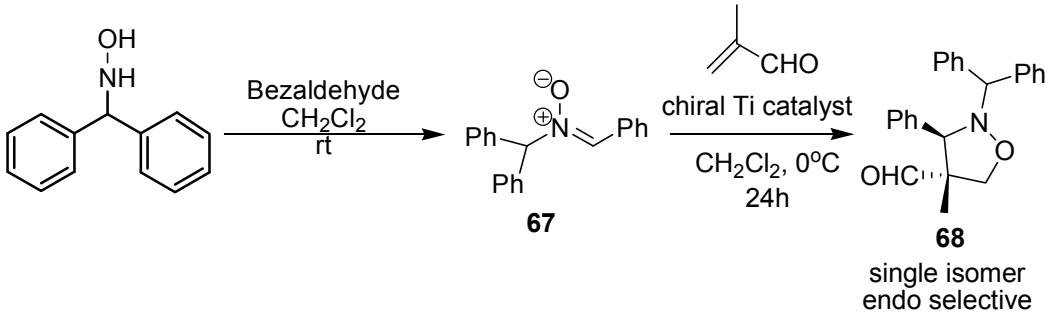


66

Figure 13. Structure and relative configurations of compound **66**. Important NOE correlations are shown with arrows.

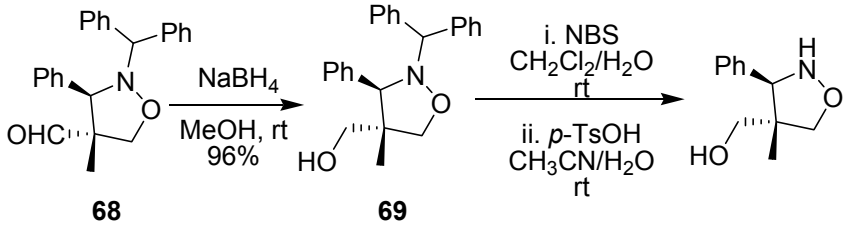
3.3.5 Diphenylmethine protected nitrone and a series of investigations

Nitrone **67,** which is protected by diphenylmethine group, was synthesized by Hashimoto, T. and his coworkers and applied to 1, 3 dipolar cycloaddition (Scheme 30) [Hashimoto, T. et al. 2007] .



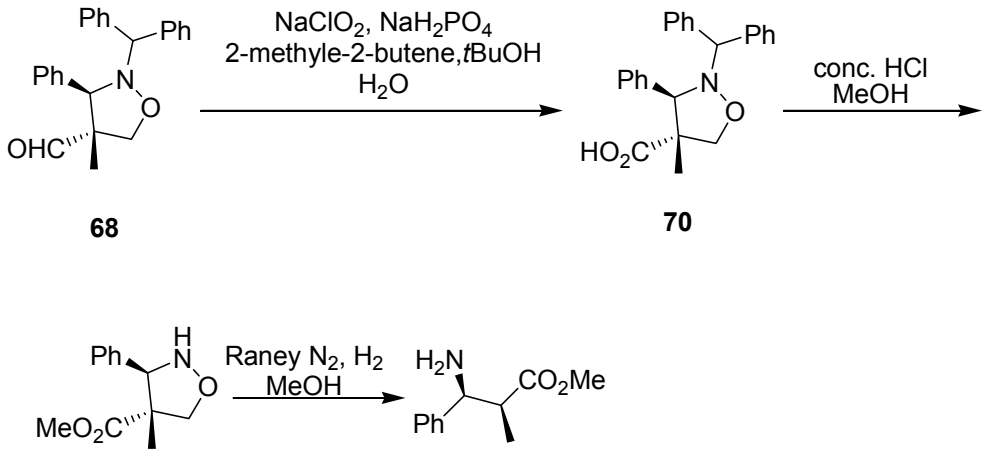
Scheme 30. Preparation of nitrone **67** and its application in 1, 3 dipolar cycloaddition to form isoxazolidine **68**, performed by Hashimoto, T. *et al.*

Hashimoto reported the removal of the diphenylmethine protecting group from isoxazolidines **68** and **70** (Shemes 32 and 33) using two different approaches. The first method involved the treatment of diphenylmethine protected isoxazolidine **69**, with NBS in CH_2Cl_2, followed by acidic hydrolysis using *p*-TsOH (Scheme 31) [Hashimoto, T. et al. 2007].



Scheme 31. Removal of diphenylmethine protecting group using NBS and *p*-TsOH, performed by Hashimoto, T. *et al.*

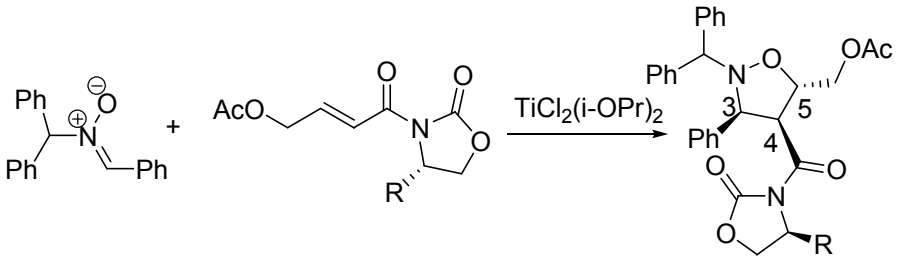
The second methods involved the hydrolysis of diphenylmethine protected isoxazolidine **70**, with concentrated HCl in methanol. The deproteceted isoxazolidine was then hydrogenolyzed to a 1, 3 amino alcohol (Scheme 32) [Hashimoto, T. et al. 2008].



68 **70**

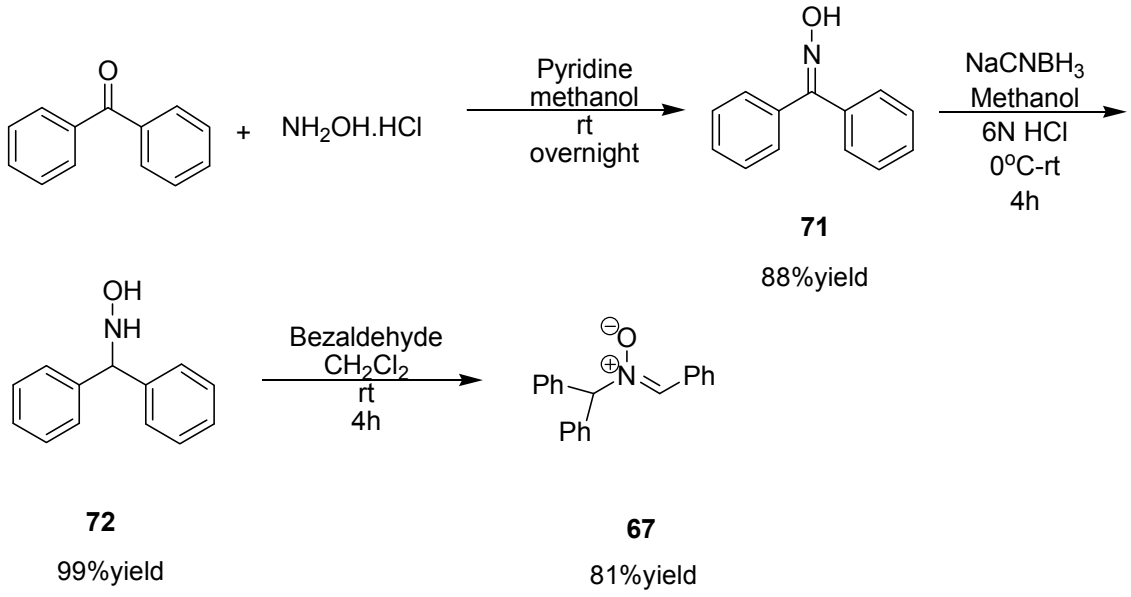
Scheme 32. Removal of diphenylmethine protecting group using conc. HCl in methanol, performed by Hashimoto, T. *et al.*

We concluded that the isoxazolidine ring could be deprotected while maintaining the N-O bond by using a diphenylmethine protecting group, prompting the design of a 1, 3 dipolar cycloadditon using nitrone **67** and a chiral dipolarophile having a directing group R, with the intention of obtaining a deprotectable *exo* cycloadduct which has the desired absolute configuration through the use of the $TiCl_2(O\text{-}i\text{-}Pr)_2$ catalyst (Scheme 33).



Scheme 33. Proposed 1, 3 dipolar cycloaddition of nitrone **67** to form an isoxazolidine with a specific configuration

Based on the literatures [Liu, S.; Yu, Y. and Liebeskind, L. S. 2007][Sivappa, R. et al. 2007], preparation of nitrone **67** (Scheme 34) began with an oxime formation **71** (88% yield) from benzophenone and hydroxylamine, followed by a reduction to give a hydroxylamine **72** in an excellent 99% yield. In terms of the method described by Hashimoto, T. [Hashimoto, T. et al. 2007] with some modifications, compound **72** was converted to the diphenylmethine protected nitrone **67** (81%).

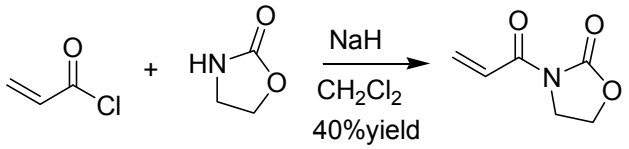


Scheme 34. Synthesis of nitrone **67**

Prior to launching the designed cycloaddition, several model reactions were investigated.

i. *Exo/endo* selectivity

The targeted compound **28** has the same relative configurations as *exo* cycloadducts, therefore *exo* selectivity is preferred. The main goal of this model study is to determine the *exo/endo* selectivity of nitrone **67** in 1, 3 dipolar cycloaddition by using different Lewis acids. Two dipolarophiles **47** and **73** were employed in this study. The synthesis of crotonate dipolarophile **47** has been mentioned above. As shown in Scheme 35, the acrylate dipolarophile **73** was prepared as previously described [Hird, A. W. and Hoveyda, A. H. 2003].

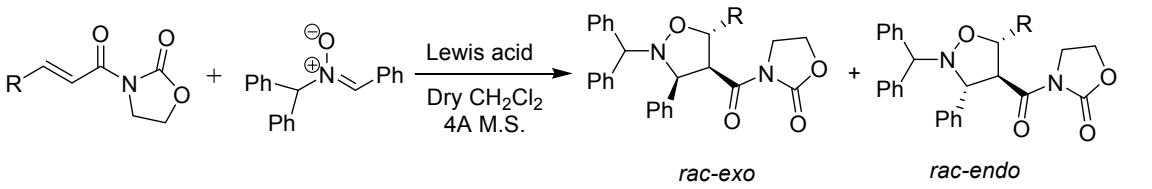


Scheme 35. Preparation of acrylate dipolarophile **73**

As for the *exo* selectivity investigation, $Ti(i\text{-}OPr)_2Cl_2$, a catalyst which has been studied in the 1,3 dipolar cycloaddition between benzyl protected nitrone **44** and crotonate dipolarophile **47** in table 6, was examined. As anticipated, this catalyst provided the *exo* products as the major diastereomers. The reaction (entry 3 in Table 8) between nitrone **67** and dipolarophiles **47** produced an *exo* predoninant mixture with a 2.6/1 ratio of *exo* adduct **74** to *endo* adduct **75** as determined by NMR. After chromatographic purification, the ratio increased to 8.6:1 with a 61% purified yield. The pure *exo* cycloadduct **74** can be recrystallized from anisole/henxane (1:4). The acrylate derivative **73** as the dipolarophile

provided a lower selectivity (entry 2 in Table 8) with a mixture of *exo* adduct **76** and *endo* adduct **77** (1.7/1).

Table 8. Various dipolarophiles and Lewis acids in 1, 3 dipolar cycloaddition of nitrone **67**



73 R = H
47 R = CH_3
58 R=CH_2OCOCH_3
78 R=Phenyl

76&77 R = H
74&75 R = CH_3
79&80 R=CH_2OCOCH_3

Entry	R	Lewis acid	Condition	*Exo/endo*[a]	yield (*exo*/*endo*)
1	CH_3	No catalyst	Room temperature/9days	No reaction	No reaction
2	H	$TiCl_2(i\text{-}OPr)_2$	Room temperature/9days	1.7:1(**76**/**77**)	43% (3:1)[b]
3	CH_3	$TiCl_2(i\text{-}OPr)_2$	Room temperature/9days	2.6:1(**74**/**75**)	61% (9:1)[b]
4	$CH_3CO_2CH_2$	$TiCl_2(i\text{-}OPr)_2$	Room temperature/9days	3.4:1(**79**/**80**)	66%[c]
5	$CH_3CO_2CH_2$	$TiCl_2(i\text{-}OPr)_2$	Reflux/9days	2.9:1(**79**/**80**)	48% (3.1:1)[b]
6	Ph	$TiCl_2(i\text{-}OPr)_2$	Room temperature/14days	No reaction	No reaction
7	Ph	$TiCl_2(i\text{-}OPr)_2$	Reflux/5days	No reaction	No reaction
8	H	$Sn(OTf)_3$	Room temperature/9days	1:1.1(**76**/**77**)	60%[c]

9	H	$Sn(OTf)_3$	Reflux/9days	1:1.0(**76**/**77**)	72% (1:1) [b]
10	CH_3	$Sn(OTf)_3$	4°C/9days/no stirring	1:2.6(**74**/**75**)	62%[c]
12	CH_3	$Sn(OTf)_3$	Room temperature/9days	1:5.2(**74**/**75**)	92%[c]
13	CH_3	$Sn(OTf)_3$	Room temperature/about 5 months	1:10.5(**74**/**75**)	98%[c]
14	CH_3	$Sn(OTf)_3$	Reflux/9dadys	1:3.8(**74**/**75**)	70%(0:1) [b]
15	$CH_3CO_2CH_2$	$Sn(OTf)_3$	Room temperature/9days	only found *endo* adduct	15%[c]
16	$CH_3CO_2CH_2$	$Sn(OTf)_3$	Reflux/9dadys	only found *endo* adduct	14%[c]
17	CH_3	MgI_2-phenanthroline	Room temperature/9days	1:1.7(**74**/**75**)	6%[c]
18	CH_3	MgI_2-phenanthroline	Reflux/9days	1:1.1(**74**/**75**)	19%[c]

a, determined by ^{1}H NMR of crude products; b, after purification; c, determined by ^{1}H NMR of the crude product

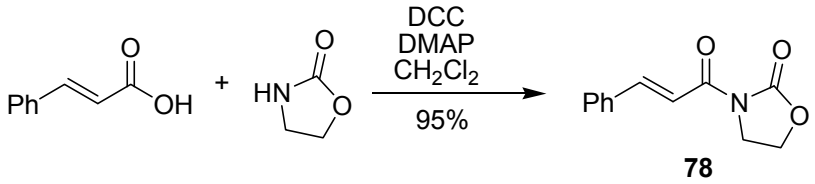
The examination of the *endo* selectivity was also performed in the precence of MgI_2-Phenantroline or $Sc(OTf)_3$. Surprisingly, MgI_2-Phenantroline (entry 17 and 18 in Table 8) whose chelation with crotonate dipolarophile **47** provided a major *endo* adduct in 1, 3 diopar cycloaddition with phenyl protected nitrone [Desimoni,G. et al. 1999], gave a poor yield in the reaction with **67** either at room temperature or in refluxing CH_2Cl_2. This probably resulted from the steric constraints of both bulky MgI_2-Phenantroline and bulky protecting group. On the other hand, the use of $Sc(OTf)_3$, an *endo* selective catalyst, provided the *endo* cycloadduct **75** in refluxing CH_2Cl_2 in a 70% yield in the cycloaddition reaction of **47** and **67** (entry 14 in Table 8). Interestingly, the reaction of acrylate

dipolarophile **73** in refluxing CH_2Cl_2 yielded cycloadducts with a poor selectivity as 1:1 *exo* to *endo* adducts were obtained (entry 9 in Table 8).

It should be noted that the assignment of *exo* and *endo* isomers were based on the coupling constants between H3 and H4 in the isoxazolidine rings [Huisgen, R. et al. 1969][Gothelf, K. et al. 1996]. The *exo* adduct with a *cis* relative configuration between H3 and H4, has a larger H3-H4 coupling constant than the *endo* isomer which has a *trans* relative configuration between H3 and H4. As *exo* adduct **74** and *endo* adduct **75** as an example, according to our NMR experiments, the cycloadduct **74** with 10.4 Hz coupling constant between H3 and H4 was therefore identified as *exo* adduct while isomer **75** with 6.0 Hz coupling constant was then assigned as *endo* adduct.

ii. Various dipolarophiles in 1, 3 dipolar cycloaddition with nitrone **67**

To further understand the influence of substituents in 3-acyl-1, 3-oxazolidin-2-one **46** toward the 1, 3 dipolar cycloaddition with nitrone **67**, dipolarophiles **78** bearing a phenyl substituent prepared (Scheme 36) based on the literature [Andrade, C. K. et al. 2003], together with dipolarophiles **47**, **73** and **58**, was studied.



Scheme 36. Preparation of cinnamate dipolarophile **78**

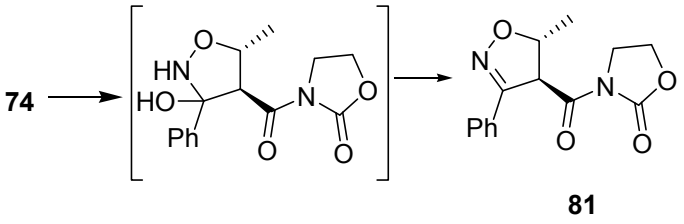
As shown in Table 8, electronic effects of the dipolarophile influence the *exo/endo* selectivity: the higher electronegativity a substitent has, the higher the diastereoselectivity will be. For instance, in the presence of $TiCl_2(i\text{-}OPr)_2$, an *exo* catalyst, the *exo* selectivity increased from 1.7:1 to 2.6:1 to 3.4:1 as the substituent changed from a hydrogen to methyl group to acetoxymethyl group (entry 2, 3 and 4 in Table 8), respectively. A similar result was observed in the *endo* selective cycloaddition using $Sn(OTf)_3$ as the catalyst in refluxing CH_2Cl_2. As such, the *endo* selectivity jumped from 1:1 to 3.8:1 with the change of substituent from a proton to methyl group (entry 8 and 14 in Table 8). Those results are in a good agreement with the finding of Badoiu, A. and Kundig, E. P. [Badoiu, A. and Kundig, E. P. 2012] who determined that the formed *exo* diastereomer increased with the increasing electron-withdrawing character of substituent in dipolarophiles in non-catalyzed 1, 3 dipolar cycloaddition reaction between 4-(trifluoromethyl)benzylidene methylamine N-oxide and 3, 5-substituted isoxazolidines.

On the basis of Table 8, the size of substituents at the β carbon of 3-acyl-1, 3-oxazolidin-2-one affects the 1, 3 dipolar cycloadditon reaction, that is to say that the larger substituent made this cycloaddition more difficult to proceed. For example, in the presence of $TiCl_2(i\text{-}OPr)_2$, when the substituent was a small group such as a proton and methyl group, the cycloaddition can be achieved at room temperature. But when the substituent is a larger group like an acetoxymethyl group, the reaction had to be performed under refluxing CH_2Cl_2 to complete the reaction. When the size of the substituent continued to increase as it was a phenyl group, this cycloaddition cannot be carried on even under refluxing CH_2Cl_2.

The 1, 3 dipolar cycloaddditions between achiral dipolarophile **58** and nitrone **67** without the involvement of any chiral auxiliary (entry 4 and 5 in Table 8) also serves as a model study to investigate the feasibility of proposed 1, 3 dipolar cycloaddition (Scheme 33). The evaluation of the reaction conditions in this model study showed that at room temperature, even after 9 days, significant amount of starting materials are present. This reaction, therefore, was carried out on refluxing CH_2Cl_2 for 9 days, resulting in a 3.1/1 ratio of *exo* to *endo* selectivity with the complete consumption of starting reagents. After chromatographic purification, a 48% yield was obtained.

iii. Deprotection

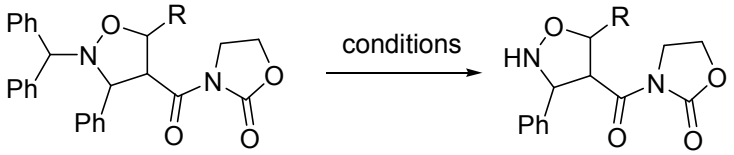
The removal of the protecting group without reductive cleavage of the isoxazolidine ring is an essential step in the synthesis of isoxazolidine analogs of kainic acid. Our first attempt involved treatment of the *exo* adduct **74** with N-bromosuccinimide in CH_2Cl_2, followed by acidic hydrolysis, according to the method described by Hashimoto, T. et al.. [2007]. Unfortunately, in our hands, the method gave a dihydroisoxazole final product **81**, likely through the aminal intermediate (Scheme 37)



Scheme 37. Attempted deprotection of **74** with NBS

Aimed at preparing the unprotected isoxazolidines, several different conditions were studied. Attempts at deprotection using methanolic HCl (4:1) or tifluoromethylsulfonic acid in TFA also failed, returning only starting material or an intractable mixture. On the other hand, refluxing the diphenylmethine protected isoxazolidine **74** in CH_2Cl_2 with 2.5 equivalents of Et_3SiH in the presence of TFA provided deprotected isoxazolidine **82** (Table 9, entry 3).

Table 9. Investigation of the deprotection of isoxazolidines



76&77 R = H
74&75 R = CH_3

84&85 R = H
82&83 R = CH_3

Entry	Substrate	Conditions	Outcome
1	**76**	Et_3SiH (2.5 eq), TFA, CH_2Cl_2,reflux 2.5 h	100% **84**
2	**77**	Et_3SiH (2.5 eq), TFA, CH_2Cl_2,reflux 2.5 h	87% **85**
3	**74**	Et_3SiH (2.5 eq), TFA, CH_2Cl_2,reflux 2.5 h	93% **82**
4	**75**	Et_3SiH (2.5 eq), TFA, CH_2Cl_2,reflux 2.5 h	83% **83**
5	**74**	i. NBS (1.1 eq) ,CH_2Cl_2/H_2O,rt. ii. *p*-TsOH,CH_3CN/H_2O,rt	47% yield of **81**
6	**74**	TfOH, anisole,0^oC-rt	No reaction
7	**74**	TfOH, TFA, 0^o	No reaction
8	**74**	TfOH, TFA, 0^oC-rt	Intractable mixture
9	**74**	MeOH:HCl (4:1), room temp	No reaction
10	**74**	MeOH:HCl (4:1), reflux 30 min	No reaction

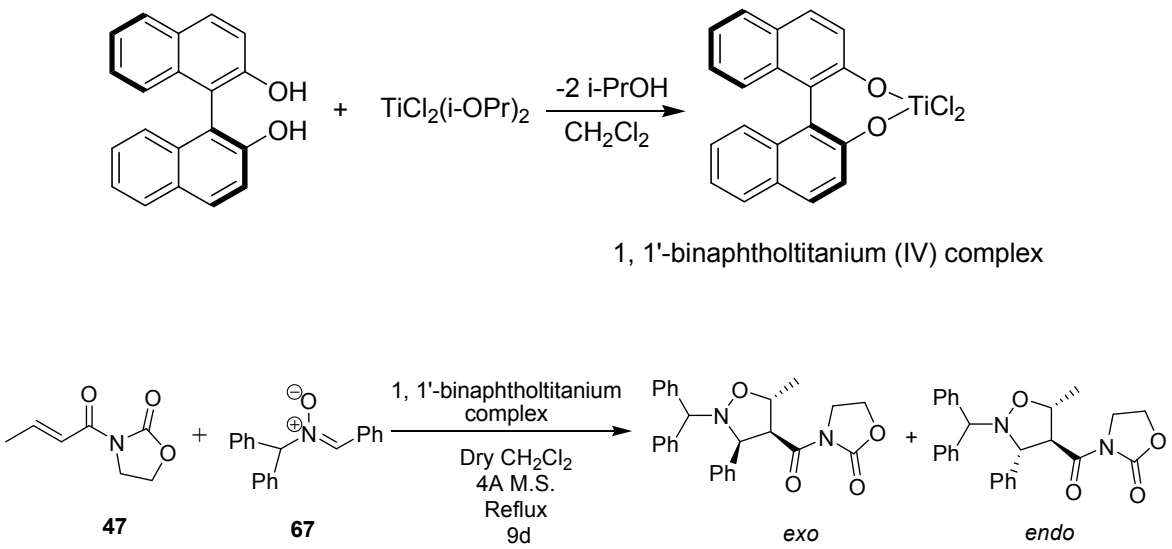
Encouraged by this result, we extended this method to several different diphenylmethine protected isoxazolidines **75**, **76** and **77**. This method provided the corresponding N-unsubstituted isoxazolidines **83, 84** and **85** in yields range from 83 to 100%.

The benzyhryl protecting group is not particularly common, but has found applications in the protection of aziridines [Patwardhan, A. P. et al. 2005], azetidines [Laurent, M. et al. 2003], in particular β-lactams, and in the selective protection of uracil nitrogens [Wu, F. et al. 2004]. Deprotection is typically achieved through catalytic hydrogenation or vigorous acid treatment. More novel methods involve oxidation to the benzhydrol either with NBS followed by hydrolysis [Laurent, M. et al. 2003] or ozone treatment followed by $NaBH_4$ reduction [Patwardhan, A. P. et al. 2005], although yields are poor (~50%) in the later method. A single report describes the reductive cleavage of the N-diphenylmethine group with Et_3SiH [Neumann, W. L. et al. 1991]. Although another report noted the instability of the N-diphenylmethine group during reductive cleavage of t-butyl esters using Et_3SiH [Kirincich, S. J. et al. 2009]. We have found that the diphenylmethine protecting group may be readily cleaved from isoxazolidines by reduction with Et_3SiH while maintaining the sensitive N-O bond.

iv. Aymmetric synthesis

The use of a chiral Lewis acid catalyst is one approach to the induction of enantioselectivity. Chiral 1, 1'-binaphthol complexes of titanium have been successfully applied in the enantioselecitve addition of nucleophiles to carbonyl groups [Duthaler, R. O. and Hafner, A. 1992]. However, when using this catalyst in the 1, 3 dipolar cycloaddion of phenyl protected nitrone with dipolarophile **47**, Gothelf K. V. et al. [Gothelf. K. V. 1994] found only an 8% ee of the *exo* adduct. Later, Hoshimoto, T. et al. [Hoshimoto, T. et al. 2007] reported that cycloadducts of the diphenylmethine protected nitrone **67** were obtained with a higher enantioselectivity in the asymmetric 1, 3 dipolar

cycloaddition reaction. This made us wonder if chiral 1, 1'-binaphthol complexes of titanium could generate high enantioselectivity in the 1, 3 dipolar cycloaddition of diphenylmethine protected nitrone **67** with dipolarophile **47**. Thus, chiral 1, 1'-binaphtholtitanium complex was prepared as previously described [Gothelf. K. V. 1994] and used as a catalyst in the 1, 3 dipolar cycloaddition of **67** and **47** (Scheme 38). The crude product was obtained in 67% yield with 1.1:1 *exo/endo* selectivity. However, it only induced about 6% ee of *exo* adduct purified through radial chromatography and recrystallization, as determined by ^{1}H NMR using Eu(hfc)$_3$ as chiral shift reagent.

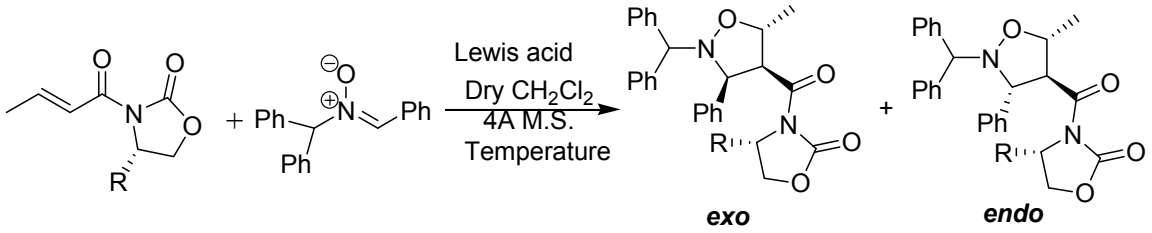


Scheme 38. Application of chiral 1, 1'-binaphtholtitanium complex

Another method to induce a high enantioselectivity/diastereoselectivity is the employment of chiral auxiliary. A model study was undetaken to identify a chiral auxiliary which can significantly enhance the enantioselectivity of the 1, 3 dipolar cycloaddition to predominantly form the diastereomer with the desired 3*S*, 4*S* and 5*S*

absolute configuration. We investigated two chiral auxiliaries, the oxazolidone derivatives **86** & **87** with either isopropyl directing group or benzyl directing group, both prepared using the procedure developed for the preparation of **47**. The dipolar cycloaddition between dipolarophile **86** and nitrone **67** gave a 3.1/1 mixture of *exo* to *endo* cycloadducts after 9 days of reaction, with a 58% yield of *exo* cycloadduct after purification isomers. Meanwhile, the dipolar cycloaddition between dipolarophile **87** and nitrone **67** afforded a 3.3/1 ratio of *exo* to *endo* isomers after 11 of days reaction, with a 41% yield of *exo* cycloadduct after purification (Table 10). Thus it was determined that dipolarophiles **86** and **87** gave similar *exo/endo* selectivity. However, dipolarophile **86** was judged to be superior as its use resulted in a higher yield in a shorter reaction time as compared to **87**. We therefore chose the isopropyl directing group over the benzyl group.

Table 10. Investigation of chiral auxiliaries using dipolarophiles **86** and **87**



86 R = $CH(CH_3)_2$
87 R = Bn

88 (*exo*) R = $CH(CH_3)_2$
89 (*exo*) R = Bn

Entry	R	Lewis acid	Condition	*Exo/endo*[a]	Yield
1	Isopropyl	$TiCl_2(i\text{-}OPr)_2$	Room temperature/9days	3.1/1	58% (pure *exo* **88**)
2	Ph	$TiCl_2(i\text{-}OPr)_2$	Room temperature/11days	3.3/1	41%(pure *exo* **89**)
3	Isopropyl	$Sn(OTf)_3$	Room temperature/9days	No reaction	No reaction
4	Isopropyl	$Sn(OTf)_3$	Reflux/9days	No reaction	No reaction

a, determined by ^1H NMR of crude products

v. Determination of the absolute configuration using Mosher's method

Radial chromatography afforded the major *exo* isomer **88** but the absolute configuration of the three new stereocenters remained unknown. Several examples of the asymmetric syntheses of isoxazolines can be found in the chemical literature. However, the methods used to determine absolute configuration involved either X-ray crystallography [Cicchi, S. et al. 2003][Blanarikova-Hlobilova, I. et al. 2003] or conversion of the cycloadduct to a known compound for which the sign of optical rotation is known. [Gothelf K. V. et al. 1996]. However, the former may not be helpful since not all of the molecules of interest will form a uniform crystal and the cost can be very high [Allen, D. A. et al. 2008]. The latter has more limitations because it requires a known compound with the assigned absolute configurations for comparsion, which is not always available. We therefore sought a simple and straightforward method to determine the absolute configuration of isoxazolidines. Mosher demonstrated that the determination of chiral amines and alcohols can be realized by the analysis of ^{1}H or ^{19}F NMR spectra of methoxy(trifluoromethyl)phenylacetyl (MTPA) derivatives of those compounds [Dale, J. A. and Dull, D. L. 1969][Dale, J. A. and Mosher, H. S. 1973]. Since then, the application of Mosher derivatives for the purpose of determining absolute configurations of chiral amines and alcohols has become common place. For the determination of chiral primary amines, the experiments of Kusumi and Kakisawa [Kusumi, T. et al. 1991][Allen, D. A. et al. 2008] indicated the dominant conformation of the resulting amides (Figure 14) in which the α-proton of the amine, the carbonyl oxygen and the trifluoromethyl gourps are coplanar and *syn*. In Figure 14, because the R_1in *S*-MTPA amide is on the same side of the phenyl of the MTPA moiety, the protons of R_1 group are shielded by the phenyl.

Therefore in ^{1}H NMR spectra, the chemical shifts of those protons are upfield as compared to the protons of R_1 in *R*-MTPA amide which is on the different side of the phenyl group. Same result can be observed between the protons (upfield) of R_2 group in *R*-MTPA amide and the protons (downfield) of R_2 group in *S*-MTPA amide. Through the assessment of the chemical shift differences $\Delta\delta$ (δ_S-δ_R) of the protons of R_1 group between *S*-MTPA and *R*-MTPA amides as well as the $\Delta\delta$ of the protons of R_2 group between *S*-MTPA and *R*-MTPA amides, the assignment of the absolute configuration of the chiral amine moiety can be achieved [Kusumi, T. et al. 1991][Allen, D. A. et al. 2008]. In the favored conformation shown in Figure 14, the α-CF_3 group experiences anisotropic deshielding by the amide carbonyl. However, if R_1 or R_2 are bulky, the anisotropic deshielding may be affected. For example, if R_1 is bulky and is larger than R_2, the interation of R_1 and the bulky phenyl in *S*-MTPA amide, which can be either steric, electronic, or both, will force the α-CF_3 group out of the coplanarity with the carbonyl group. Consequently, α-CF_3 will no longer experience the deshielding effect of the carbonyl and the chemical shift for the ^{19}F signal of the *S*-MTPA amide will be lower as compared with *R*-MTPA amide whose bulky R_1 is on the opposite side of the phenyl substituent [Sullivan, G. R. et al. 1973].

Figure 14. *S*-MTPA amide and *R*-MTPA amide

The use of Mosher's amide derivatives for the determination of absolute configuration has been principally applied to primary amines. Application of this approach to cyclic amines has been limited in the past and the use of Mosher derivatives for determination of absolute configuration of chiral isoxazolidines has not been reported. This is in part due to the difficulty of preparing N-unsubstituted isoxazolidines. As we have been able to deprotect our isoxazolidines cycloadducts, we wished to develop a model for the determinination of the absolute configuration of isoxazolidines using the Mosher derivatives. Travers and Rauk [Khan, M. A. et al. 1982][Rauk, A. et al. 1983] studied a number of MTPA amides of piperidines. The MTPA amide of *cis*-2, 6-dimethylpiperidine exists predominantly as two slowly interconverting diastereomeric rotamers (Figure 15), which appear as two distinct species in the ^{1}H NMR spectra. This observation suggests the potential for the assignment of absolute configuration of cyclic amines by way of the Mosher derivatives. Likewise, the Mosher derivatives of 2-substituted pyrrolidines reportedly also esist as two slowly interconverting rotamers as observed by ^{1}H NMR [Hoye, T. R. and Renner, M. K. 1996][Vidal, P. et al. 2007].

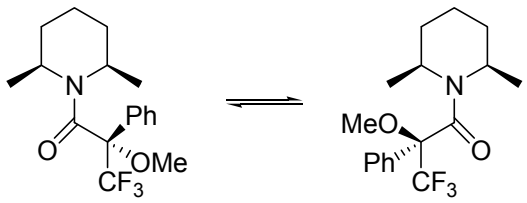
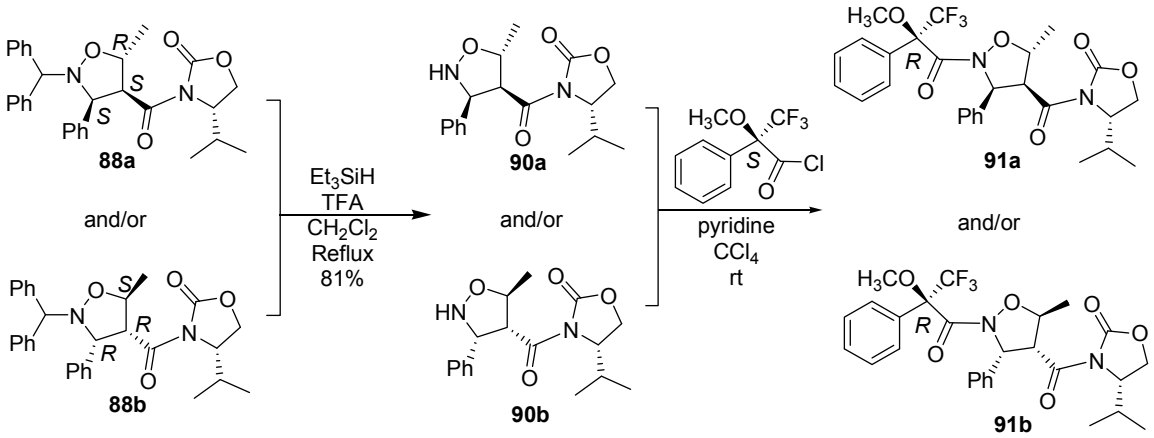


Figure 15. Two rotamers of *cis*-2, 6-dimethylpiperidine MTPA amide

With these earlier studies in mind, we undertook an analysis of the Mosher derivative of cycloadduct **88**. Two *exo* adducts of **88** may potentially be produced (diastereomers **88a**

and **88b**), resulting from attack on diastereotopic faces of the dipolarophile **86**. Although the ^{1}H NMR spectra of the purified *exo* adduct appeared to be a single diastereomer, we cannot make this assumption. Therefore, after deprotection, the *exo* adduct **88** was converted to the *R*-MTPA amide derivative by reacting with *S*-MTPA-Cl (Scheme 39). The ratio of isomers was determined by ^{19}F NMR.



Scheme 39. Preparation of *R*-MTPA amide; the *S* configuration of *S*-MTPA-Cl was changed to the *R* configuration of the generated MTPA amide

In $CDCl_3$, two peaks were observed in the ^{19}F NMR spectra with a ratio of 1 to 2 (Figure 17a). There are two possible explanations for the observation of two peaks in the ^{19}F NMR. The first possibility is that there are two different diasteromers **91a** and **91b**. The other explanation is that there is a single isomer which exists as two slowly interconverting rotamers (Figure 16), consistent with the coexistence of *syn* and *anti* rotamers of cyclic amine MTPA derivatives such as piperidine and pyrrolidines [Khan, M. A. et al. 1982][Rauk, A. et al. 1983][Vidal, P. et al. 2007].

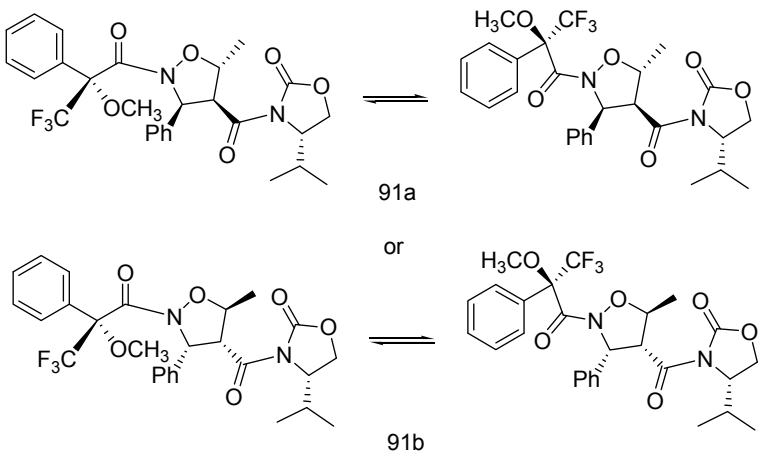
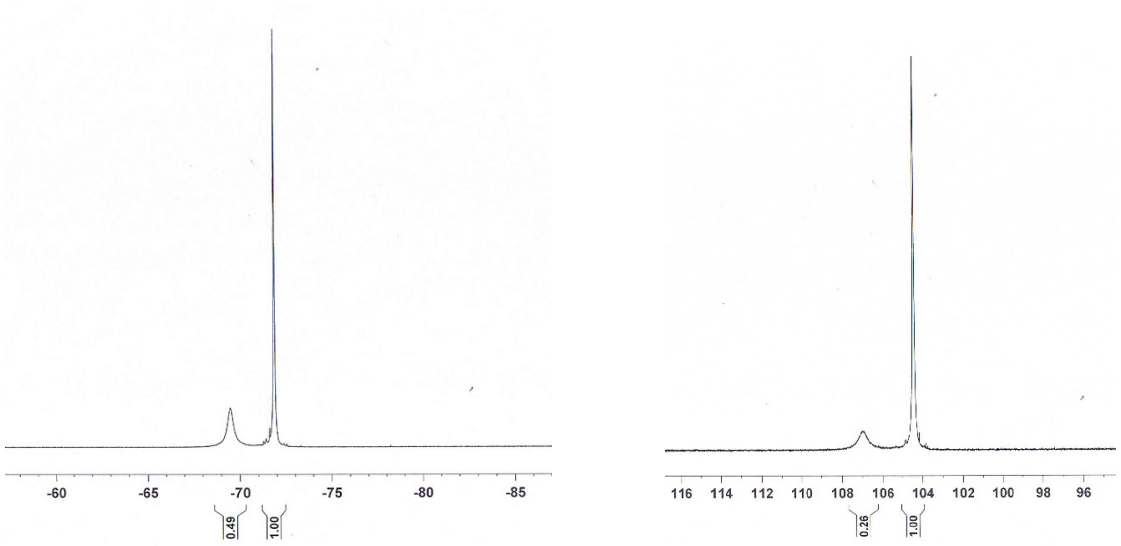


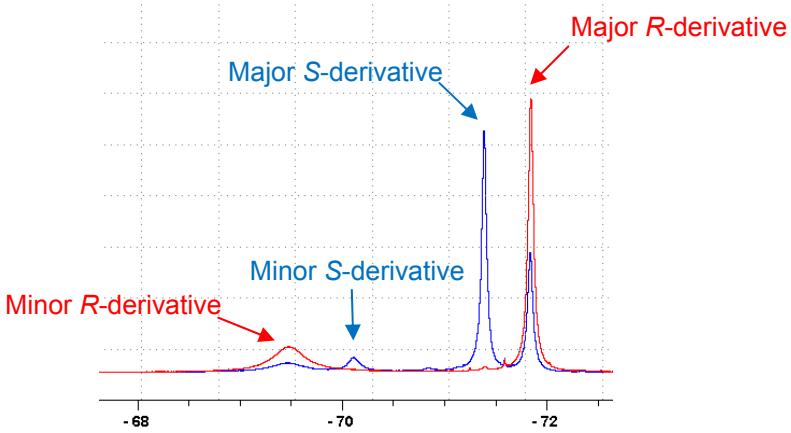
Figure 16. Two possible romators of **91**a or **91**b

When the ^{19}F NMR was acquired in d_6-acetone, the peak ratio changed to 1 to 4 (Figure 17b). Because the diastereomer ratio would not be solvent dependent, we concluded that the two peaks represent slowly equilibrating amide rotamers. This observation, that there are only two equilibrating rotamers, also suggests the presence of a single diastereomer, either *exo* **88**a or *exo* **88**b. Those two *exo* adducts have the opposite absolute configurations for the isoxazolidine ring substituents.

Intending to determine the absolute configuration of the *exo*, the deprotected *exo* adduct **90** was also derivatized with racmic Mosher's acid chloride for comparison of their ^{19}F and ^{1}H NMR spectra. The ^{19}F NMR spectra of the Mosher derivative prepared from racemic MTPA showed four peaks, two of them coincident with those observed in the *R*-MTPA amide obtained from the reaction of **90** with *S*-MTPA-Cl (Figure 17C). We conclude that the two new peaks belong to the *S*-MTPA amide produced by the reaction of **90** with *R*-MTPA-Cl.



a. ^{19}F NMR spectrum in $CDCl_3$ b. ^{19}F NMR spectrum in d_6-acetone

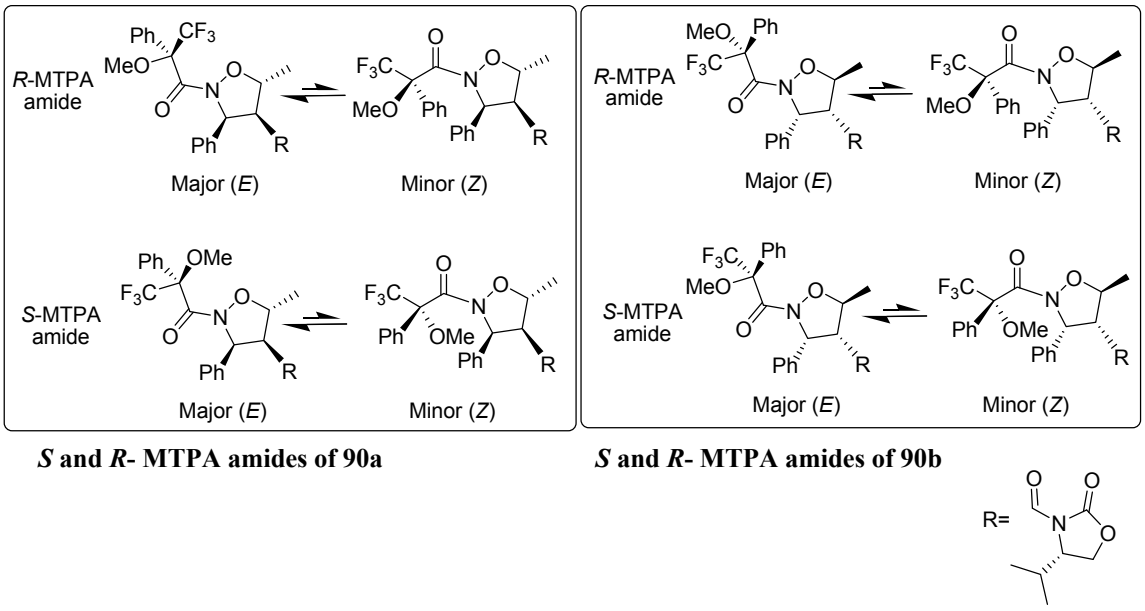


c. ^{19}F NMR spectrum in $CDCl_3$

Figure 17. ^{19}F NMR spectra of MTPA amide; a, ^{19}F NMR spectra of *R*-MTPA amide, determined in $CDCl_3$; b, ^{19}F NMR spectra of *R*-MTPA amide, determined in d_6-acetone; c, ^{19}F NMR spectra of *R*-MTPA amide and racmic MTPA amides, determined in $CDCl_3$, red peaks from *R*-MTPA amide and blue peaks from racmic MTPA amide

To investigate the conformational properties of the isoxazolidine ring in the diastereomeric Mosher's amides, we conducted a conformational search using the MMX force field within program Batchmin. As observed with the piperidine and pyrrolidine MTPA amides, and supported by our ^{19}F NMR spectra, these calculations predict that the

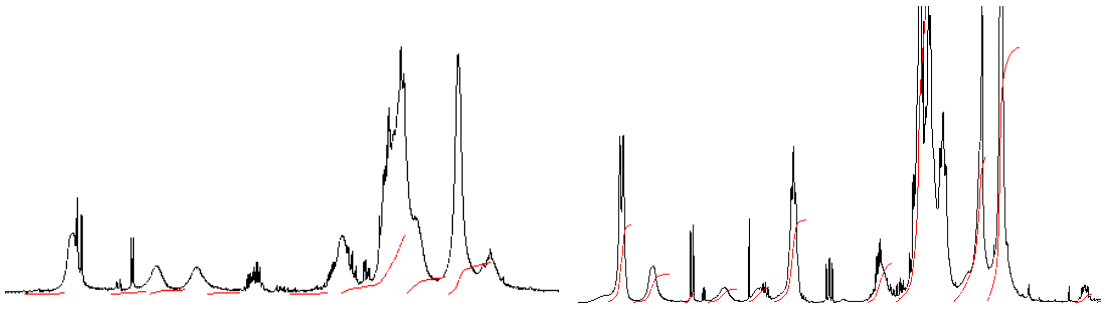
isoxazolidine MTPA derivative may adopt two conformations. The *E* and *Z* forms are assigned, in our case, on the basis of the relationship between the Cα-CO and the N-O bonds. For both the *R* and *S* derivatives, the *E* rotamer is lower in energy than the *Z* form as shown in Figure 18.



***S* and *R*- MTPA amides of 90a** ***S* and *R*- MTPA amides of 90b**

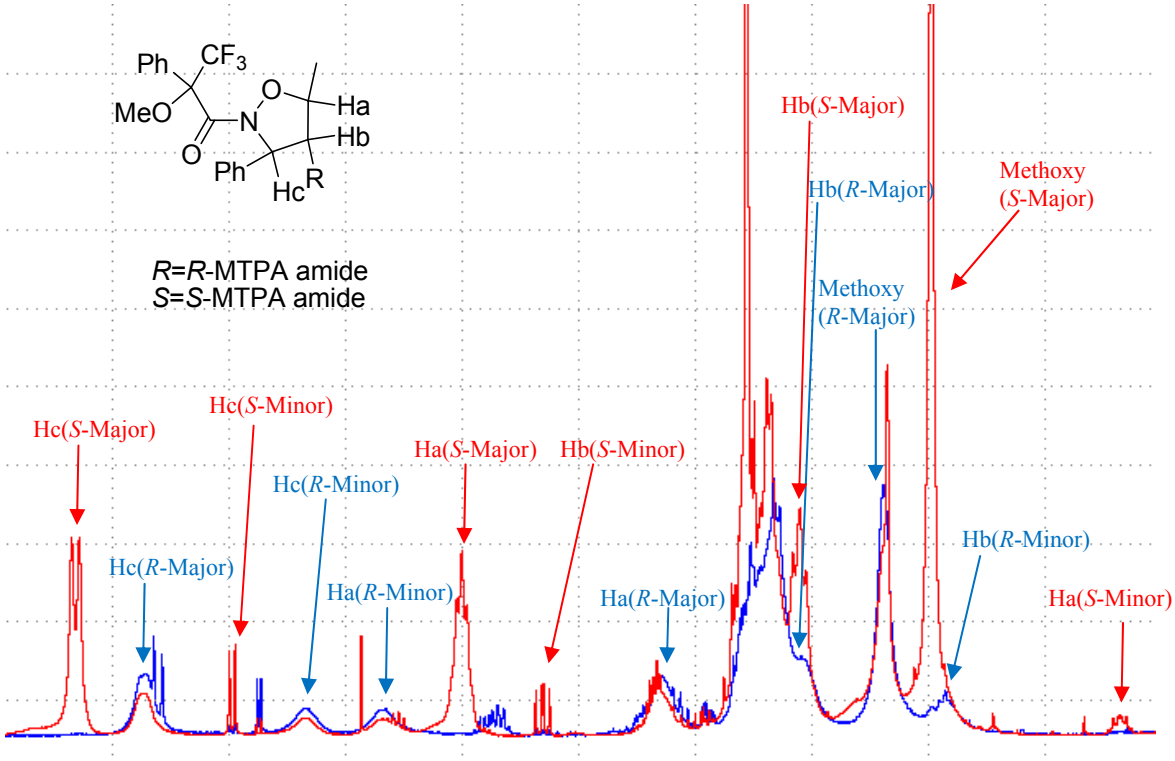
Figure 18. Conformational preferences of *S* and *R* MTPA amides of **90**a (left) and **90**b (right), according to MMX force field

On the basis of these computational results, the relative chemical shifts of diagnostic signals in the ^{19}F, ^{1}H NMR of *R* and *S*-MTPA amides may be predicted, resulting in the assignment of the absolute configuration. The diagnostic peaks (Figure 19C) in ^{1}H NMR were assigned with the assistance of COSY experiments.



a, ^{1}H NMR spectrum of R-MTPA amide

b, ^{1}H NMR spectrum of racemic-MTPA amide



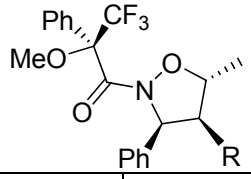
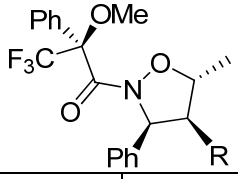
c, overlap of ^{1}H NMR spectra of *R* and racemic-MTPA amide

Figure 19. ^{1}H NMR spectra of the product of MTPA amide; a, ^{1}H NMR spectrum of *R*-MTPA amide; b, ^{1}H NMR spectra of the product of racemic-Mosher MTPA amides; c, ^{1}H NMR spectra of *R*-MTPA amide and racmic MTPA amides, blue peaks from *R*-MTPA amide and red peaks from racemic MTPA amides

According to MMX force field, in the *E* rotamers, the MTPA-phenyl groups of *R* and *S* MTPA amides are on the same side of the isoxazolidine ring. This results from the preference for the α-OCH_3 of the major *E* rotamer of the *R*-MTPA amide to adopt an eclipsed arrangement with the C=O double bond, whereas the major *E* rotamer of the *S*-MTPA amide adopts the more typical conformation in which the α-CF_3 is eclipsed with the C=O double bond. Beacause of the preference for different conformations, the protons on the isoxazolidine ring in both the *R* and *S* derivatives experience similar anisotropic shielding effects of the phenyl group. It is therefore, not helpful to examine relative chemical shifts of the ring protons of the major rotamers to determine absolute configuration. However, the chemical shifts of methoxy and trifluoromethyl groups may be affected by the phenyl of isoxazolidine moiety. If the single diastereomer is **88**a, in the *E* rotamer of the *R*-MTPA amide, the α-CF_3 is shielded by the phenyl of the isoxazolidine while in the *E* rotamer of the *S*-MTPA amide, the α-CF_3 group experiences anisotropic deshielding by the amide carbonyl since the MTPA-phenyl and the isoxazolidine-phenyl are on the different side. Additionally, in the *E* rotamer of the *R*-MTPA amide, the methoxyl group experiences anisotropic deshielding by the amide carbonyl while in the *E* rotamer of the *S*-MTPA amide, the methoxyl group is shielded by the phenyl of the isoxazolidine. These analyses suggest that the chemical shift for the ^{19}F α-CF_3 signal of the *R*-MTPA amide must be upfield as compared with *S*-MTPA amide and the chemical shift of the protons of methoxyl in the *R*-MTPA amide must be higher than in the *S*-MTPA. We indeed observed these results in ^{19}F NMR spectra (Figure 17c) and ^{1}H NMR spectra (Figure 19c and summarized in Table 11). If the single diastereomer is **88**b, the chemical shift differences of α-CF_3 and methoxyl groups between the *E* rotamers of the

R-MTPA amide and the *S*-MTPA amide will conflict with the experimental results. This demonstrated that the single diastereomer is **88**a which has 3*S*, 4*S* and 5*R* absolute configurations in the isoxazolidine ring.

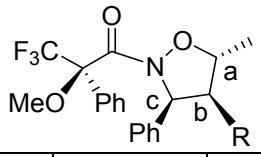
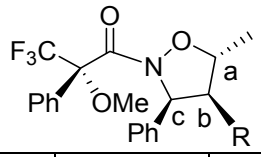
Table 11. Analysis of the major rotamers of MTPA amides of **90**a

	R-MTPA amide of 90a		*S*-MTPA amide of 90a	
Major rotamer (*E*)				
	CF_3	OCH_3	CF_3	OCH_3
C=O	Not affected	deshielded	deshielded	Not affected
Isoxazolidine-Ph	shielded	Not affected	Not affected	Shielded
δ ppm(expected)	lower	higher	Higher	Lower
δ ppm(experimental)	-71.8	3.60	-71.4	3.35

The inspection of the minor (*Z*) rotamers of the *R*- and *S*-MTPA isoxazolidine amides is perhaps more informative and more generally applicable. The conformation of the Z rotamers of both the *R*- and *S*-MTPA derivatives is more similar to that of acyclic amides and a similar analysis may be applied. In the *Z* rotamer of *R*-MTPA of **90**a (Figure 18, left), the Hb and Hc are shielded by the MTPA-phenyl, whereas in the *Z* rotamer of *S*-MTPA of **90**a, only Ha is shielded by the MTPA-phenyl (Table 12). In addition, because the bulky MTPA-phenyl and isoxazolidine-phenyl are on the same side of the isoxazolidine ring in the *Z* rotamer of the *S*-MTPA amide, the α-CF_3 will be shifted away from co-planarity with the C=O, thus it will no longer be subjected to the anisotropic deshielding of the C=O group. The chemical shift for the ^{19}F α-CF_3 signal of the *S*-MTPA amide must be lower as compared with *R*-MTPA amide. In other words, the relative

chemical shifts of the α-CF_3 groups of the *Z* rotamers should be opposite that of the *E* rotamers. These results were all observed in ^{1}H NMR spectra (19c) and ^{19}F NMR spectra (17c)

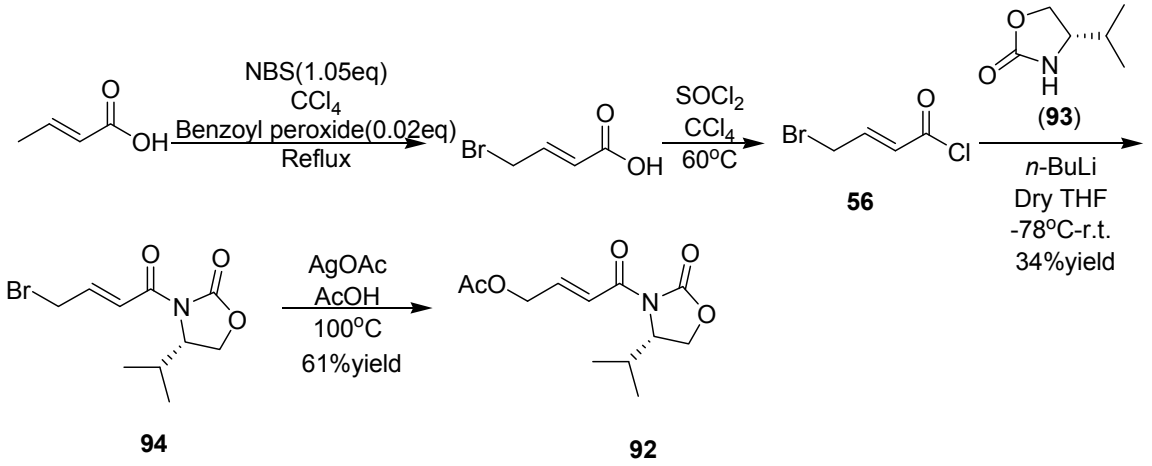
Table 12. Analysis of minor rotamers of MTPA amides of **90**a

	R-MTPA amide of 90a			*S*-MTPA amide of 90a		
Minor rotamer (*Z*)						
	H_a	**H_b**	**H_c**	**H_a**	**H_b**	**H_c**
α-Ph	Not effected	Shielded	Shielded	Shielded	Not effected	Not effected
δ ppm	5.0	3.4	5.25	2.8	4.5	5.45

Oxazolidinone chiral auxiliaries provide high diastereofacial discrimination in a wide variety of reactions including 1, 3-dipolar cycloadditions. On the basis of the discussion regarding the *Si*/*Re* facial selectivity in cycloadditions, it can be reliably predicted that the cycloadduct resulting from *Si* face approach is preferred since it arises from the attack of the nitrone on the dipolarophile from the face opposite the directing group. Therefore, this diastereomer can be predicted to have the 3*S*, 4*S* and 5*R* absolute configurations in the isoxazolidine ring, which is consistent with the absolute configuration of **88**a.

Encouraged by the results of model studies, a chiral dipolarophile **92** was synthesized (Scheme 40), in a route similar to the preparation of achiral dipolarophile **58.** (*R*)-4-isopropyloxazolidin-2-one **93** reacted with excess intermediate **56** to γ-bromocrotonyl

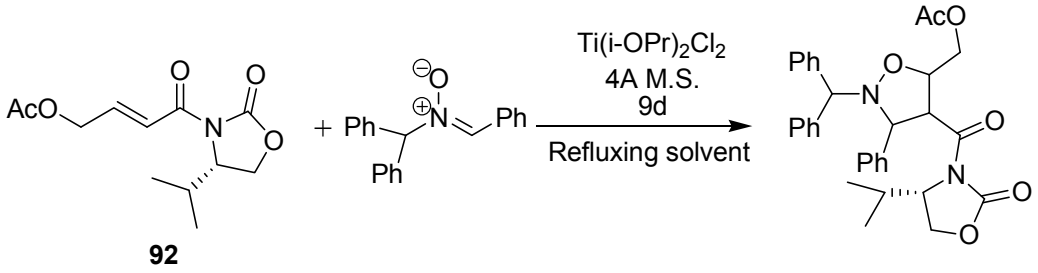
oxazolidinone derivative **94** (34%), which was converted to the dipolarophile **92** in a 61% yield, by treatment with silver acetate.



Scheme 40. Synthesis of chiral dipolarophiles **92**

The 1,3-dipolar cycloaddition between nitrone **67** and a chiral dipolarohile **92** was launched for evaluation (Table 13).

Table 13. 1, 3 dipolar cycloaddition of nitrone **67** with chiral dipolarophile **92** at refluxing CH_2Cl_2 and $CHCl_3$



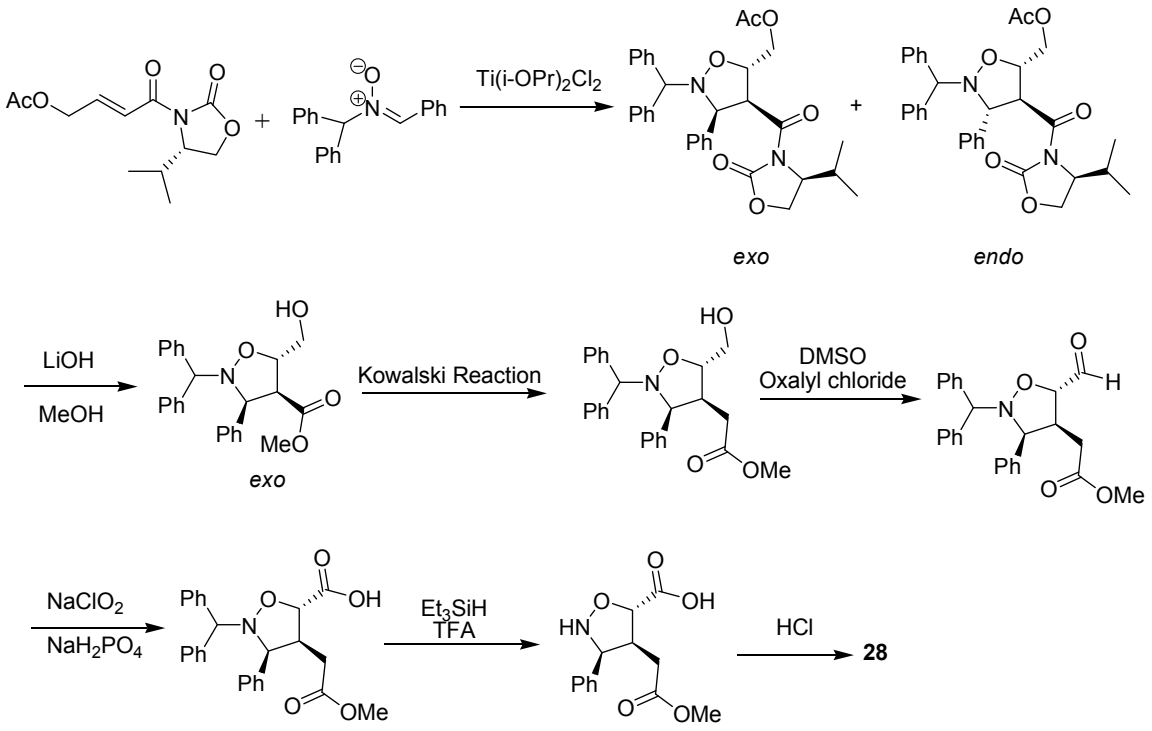
Reflux solvent	Ratio of *exo* to *endo*	Yield[a]
CH_2Cl_2	3.2:1	32%
$CHCl_3$	2.0:1	26%

a, determined by ^1H NMR of crude products

However, even after 9 days this reaction is not complete in either refluxing CH_2Cl_2 or refluxing $CHCl_3$. Yields, as determined by ^1H NMR of the crude product, were 32% in refluxing CH_2Cl_2 and 26% in refluxing $CHCl_3$. In refluxing CH_2Cl_2, the ratio of *exo* to *endo* was 3.2:1 while in refluxing $CHCl_3$, the ratio dropped to 2.0:1 due to the increased reaction temperature. Attempts to separate *exo* and *endo* adducts by radial chromatography failed. The *exo* and *endo* adducts were separable by preparative HPLC but according to the ^1H NMR spectra, 10% of *exo* adduct seemed to go through isomerization during the purification.

*3.4 Future synthetic plan for preparing isoxazolidine analogs of kainic acid **28***

Although the 1, 3 dipolar cycloaddition between nitrone **67** and dipolarohile **92** in refluxing CH_2Cl_2 proceeded in poor yield, it may yet be a promosing approach for preparing the target compound **28**. The determination of enantiomeric excess of *exo* **88** using the Mosher derivatives suggested the *exo* adduct formed in the reaction of **67** and **92** may be a single diastereomer of the desired absolute configuration. The inseparable mixture of *exo* and *endo* adducts could be used directly in the next reaction and the goal of separating them may be realized after the removal of oxazolidinone moiety. If not, the removal of diphenylmethine, followed by the protection with Boc group, could be conducted to attempt the separation. A different reaction solvent like dry toluene may improve the percent yield of the cycloaddition reaction, but perhaps at the expense of stereoselectivity.



Scheme 41. Future plan for the Synthesis of 2**8**

Therefore, our future plan for synthesizing **28** (Sheme 41) is similar to the previous plan depicted in Scheme 17. After the 1, 3 dipolar cycloaddition of **67** with **92**, the oxazolidinone moiety will be removed with a hope of separating *exo* and *endo* adducts. A Kowalski reaction will then be employed to insert a methylene group between the isoxazolidine ring and the carboxylate group. A new carboxylic acid group could be formed through the oxidation of the formed hydroxyl group. The deprotection of the diphenylmethine protected amine, followed by hydrolysis, will lead to the target compound **28**.

4. Conclusion

Kanic acid, an analog of glutamic acid, posseeses pronounced biological properties due to its silimar structure to glutamic acid. During the past three decades or so, numerous investigations on its biological activities and its synthesis have been conducted. However, selective formation of the three tetrahedral stereocenters of the pyrrolidine ring poses a great challenge to the synthetic efforts. As a result, attention was shifted to the functional mimics of kainic acid that may be readily synthesized with control over absolute and relative stereoselectivity. Aza kainoids analog is one such example. The unsubstituted aza analog of kainoids has demonstrated its ability to function as an ionotropic glutamate receptor agonist by inducing an inward current in *Aplysia californica* neuronal cells and showed its affinity to the chloride dependent glutamate binding site. This observation promted the question of the importance of the presence of one nitrogen or both nitrogens in the pyrazolidine skeleton of aza kainoid analog for binding to glutamate receptors. Thus, two different pyrrolidine analogs of kainic acid, *trans*-4-(carboxymethyl)pyrrolidine-3-carboxylic acid **27** and *trans*-2-carboxy-3-pyrrolidineacetic acid **38**, were synthesized. The demonstrated lack of affinity of both pyrrolidine analogs towards the chloride dependant glutamate binding site indicated that both nitrogens of the aza kainoid analog **25** are involved in hydrogen bonding to the receptor, significantly enhancing the affinity for the chloride dependant glutamate binding site.

Isoxazolidine analogs of kainoids are another family of potential functional mimics of kainic acid. Thereotically, the isoxazolidine skeleton can be constituted directely via a

simple 1, 3 dipolar cycloaddition without a need of a further reduction. This avoided the trouble of the isomerization caused by the reduction in the synthesis of aza kainoid analogs. However, due to the sensitive N-O bond of isoxazolidines, the preparation of N-unsubstituted isoxazolidines is problematic when common protecting groups such as alkyl, phenyl and benzyl groups are employed. Additionally control over absolute configuration in the isoxazolidine analogs must be achieved. Therefore, the asymmetric synthesis of 1, 3 dipolar cycloaddition for the purpose of obtaining the desired absolute configurations of isoxazolidine analogs was pursued. Unfortunately, glycosyl protected nitrone whose protecting group can be removed under mild acidic condition, gave rise to an undesired *endo* selective isoxazolidine. However, a series of investigations domenstrated that diphenylmethine protected nitrone could be a good substrate for the synthesis of isoxazolidine analogs of kainoid. Model studies showed that the *exo/endo* selectivity in the 1, 3 dipolar cycloaddition of the diphenylmethine protected nitrone with 3-acyl-1, 3-oxazolidin-2-one can be controlled by using different Lewis acid catalysts, and that the deprotection of diphenylmethine group with the intact isoxazolidine ring left can be realized by the treatment with Et_3Si and TFA in refluxing CH_2Cl_2. Model studies revealed that with the involvement of a directing group in dipolarophiles, high enantioselectivity can be achieved. Although the target isoxazolidine analog **28** was not obtained, these model studies solved key problems in the synthesis of **28**, including a new deprotection method for preparing N-unsubstituted isoxazolidines without the cleavage of the weak N-O bond, as well as obtaining the desired diastereomers via the 1, 3 dipolar cycloaddition reaction. The resolution of these problems makes the synthesis of isoxazoldines analogs of kainic acid very promosing.

5. Experimental procedure

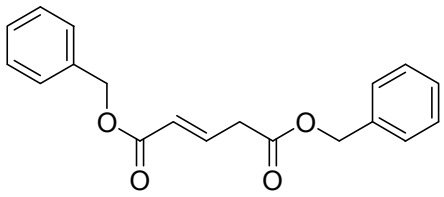
^{1}H and ^{13}C NMR spectra were obtained using Bruker AVANCE 400 spectrometer. Abbrevations for signal couplings are as follows: s, singlet; b, broad; d, doublet; t, triplet, q, quartet and m, multiplet. Melting point of solid samples were performed using MEL-TEMP melting point apparatus uncorrected. Analytical thin layer chromatography (TLC) was performed on 0.25 mm Analtech silica gel GHLF plates. The chromatograms were visualized under UV light or by staining with iodide. Radial chromatography was performed on a chromatotron using plates coated with silica gel 60 PF_{254} containing gypsum. All air sensitive reactions were carried out under nitrogen environment. Unless otherwise noted, all common reagents and solvents purchased from commercial sources were used without further purification. All glassware used for moisture-sensitive reactions were either oven dried overnight or flame-dried.

Preparation of dry solvents and reagents

(1) dry THF was prepared by distilling over calcium hydride; (2) dry ethyl ether was distilled from sodium metal/benzophenone when the solution becomes blue; (3) dry methylene chloride was distilled out into a flask with 4A molecular sieve on the bottom after being refluxed with calcium hydride for 1 hour; (4) dry $ZnBr_2$ (in dry ethyl ether): $ZnBr_2$ was heated under flame until it melted and then cooled to room temperature under vacuum and placed under nitrogen atmosphere. A magnetic stirrer and dry ethyl ether were added and the solution is stirred until complete dissolution of the zinc salts; (5) dry CuCN/2LiCl (in dry THF): CuCN and 2 equivalents LiCl was heated by flame under

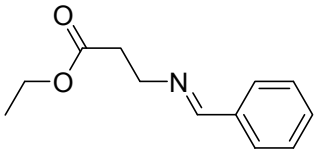
vacuum while being stirred by a magnetic stirring bar. The salts mixture was then cooled to room temperature under vacuum and placed under nitrogen atmosphere before adding dry THF. The suspension was stirred until it became a dark green solution.

Preparation of *trans*-dibenzyl glutaconate



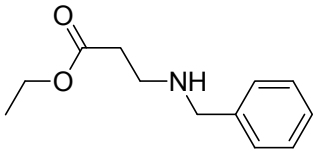
Dimethyl glutaconate (mixture of 83% *trans* and 17% *cis*, Fluka) was purified two times by flash chromatography on a silica column using solvent gradient 5%-20% ethyl acetate in hexane to obtain *trans* dimethyl glutaconate (>97%). *Trans* dimethyl glutaconate (10. 0243 g, 0.063 mol) was dissolved in 100 ml H_2O and NaOH (5.8974 g, 0.147 mol) was added. The reaction mixture was stirred under room temperature for 2.5 hours and 10% sulfuric acid was added until pH <2.0. The resulting solution was extracted by ethyl acetate (3x150 ml). The combined organic extracts were dried by Na_2SO_4, filtered and concentrated in *vacuo*, to give glutaconic acid (7.1013 g, 0.055 mol, 86% yield) as white crystals. A 250ml round bottom flask equipped with a Dean-Stark trap was charged with glutaconic acid (5.9156 g, 0.046 mol), benzyl alcohol (9.6 ml, 0.093 mol), toluene (60 ml) and $TsOH{\cdot}H_2O$ (187.7 mg, 1 mmol). The stirred mixture was refluxed for 4 hours and then cooled to room temperature. Upon the removal of the solvent in *vacuo*, the residue was purified by chromatography on silica gel (hexane/EtOAc 9:1) to give *trans*-dibenzyl glutaconate (11.1582 g, 0.036 mol, 79% yeild) as colorless oil. ^{1}H NMR was consistent with literature [Gioia, C. et al. 2009]. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.41-7.35 (m, 10H), 7.15 (dt, 1H, J=15.6, 6.8 Hz), 6.05 (d, 1H, J=15.6 Hz), 5.22 (s, 2H), 5.19 (s, 2H), 3.33 (dd, 2H, J = 7.2, 1.2 Hz).

Preparation of ethyl 3-(benzylideneamino)propanoate (32)



To a 250 ml round bottom flask were added β-alanine ethyl ester hydrochloride (7.6390 g, 0.05 mol), *tert*-butylmethyl ether (TBME, 42 ml), anhydrous sodium sulfate (4.2795 g, 0.03 mol), and distilled benzaldehyde (5.5947 g, 0.05 mol) sequentially. The stirred suspension was cooled to 0^{o}C in an ice bath and triethylamine (10 ml, 0.07 mol) was added dropwise over 10 min. The reaction suspension was then allowed to warm to room temperature and stirred for 24 hours. The mixture was filtered over a pad of Celite and evaporated under reduced pressure to give the desired imine (10.1913 g, 100% yield) as yellow oil which can be used directly in the next step. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.34 (s, 1H*),* 7.73 (m, 2H), 7.41(m, 3H), 4.14(q, 2H, *J*=7.2 Hz), 3.90(dt, 2H, *J*=6.8, 1.6 Hz), 2.74(t, 2H, *J*=6.8 Hz), 1.24(t, 3H, *J*=7.2 Hz,); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 172.0, 162.3, 136.1, 130.7, 128.6, 128.2, 60.4, 56.7, 35.5, 14.2

Preparation of ethyl 3-(benzylamino)propanoate (33)

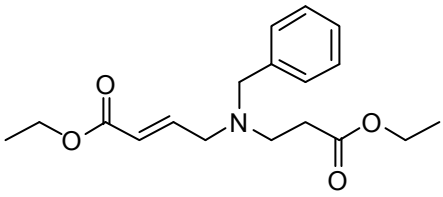


A 500ml round bottom flask was charged with ethyl 3-(benzylideneamino)propanoate (11.5685 g, 0.056 mol) and 100 ml EtOH. The mixture was cooled to 0^{o}C in an ice bath and sodium borohydride (4.3016 g, 0.113 mol) was introduced in three portions over 10 min. The reaction mixture was stirred at room temperature for further 40 min and was quenched by slowly adding saturated $NaHCO_3$ solution (140 ml). After filtration, the resulting solution was extracted by ethyl acetate (3x200 ml). The combined organic

extracts were dried by Na_2SO_4, filtered and concentrated in *vacuo* to give the desired product (10.5090 g, 90% yield) as yellow oil. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.42-7.24(m, 5H), 4.16(q, 2H, *J*=7.2 Hz), 3.83(s, 2H), 2.92(t, 2H, *J*=6.4 Hz), 2.55(t, 2H, *J*= 6.4 Hz), 1.27(t, 3H, *J*=7.2 Hz); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 172.7, 140.2, 128.4, 128.1, 126.9, 60.4, 53.7, 44.5, 34.8, 14.2. **HR-MS(ESI/APCI)** calcd for: $C_{12}H_{18}NO_2$ (MH^+): 208.1332, found: 208.1333

If the reaction time is too long, which will lead to the formation of impurities, chromatography (petroleum ether/EtOAc 1:1) can be employed to afford the pure amine.

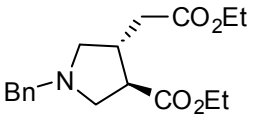
Preparation of (*E*)-ethyl 4-(benzyl(3-ethoxy-3-oxopropyl)amino)but-2-enoate (34)



To a 100ml round bottom flask were added 3-(benzylamino)propanoate (2.0672 g, 0.010 mol), acetonitrile (35 ml) and potassium carbonate (1.4004 g, 0.010 mol) sequentially. Ethyl 4-bromocrotonate (75%, 2.5921 g, 0.010 mol) was then added dropwise over 25 min. The resulting suspension was stirred overnight, filtered and concentrated under reduced pressure. The residue was purified by chromatography (petroleum ether/acetone 9.5:0.5) to give the yellow oil product (2.3252 g, 73% yield). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.30 (m, 5H), 6.96 (dt, 1H, *J*=15.6, 6.0 Hz,), 6.02 (d, 1H, *J*=15.6 Hz), 4.18 (m, 4H), 3.65 (s, 2H), 3.25 (d, 2H, *J*=5.6 Hz), 2.87 (t, 2H, *J*=7.2 Hz), 2.58 (t, 2H, *J*=7.2 Hz), 1.29 (m, 6H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 172.3, 166.2,

145.8, 138.7, 128.8, 128.4, 127.3, 123.3, 60.5, 60.4, 58.3, 54.4, 49.5, 32.9, 14.2, 14.2. **HR-MS(ESI/APCI)** calcd for: $C_{18}H_{26}NO_4$ (MH^+): 320.1856, found: 320.1857

Preparation of *trans*-ethyl 1-benzyl-4-(2-ethoxy-2-oxoethyl)pyrrolidine-3-carboxylate (35)



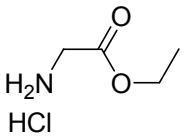
To a 100 ml flame-dried round bottom flask with a magnetic stir bar were added anhydrous (*E*)-ethyl 4-(benzyl(3-ethoxy-3-oxopropyl)amino)but-2-enoate (1.5294 g, 4.8 mmol) and dry THF (15 ml). The flask was then flushed with N_2 gas and sealed by a rubber septum which was fitted with a nitrogen balloon. The stirred mixture was cooled to -78^{o}C in a dry ice/acetone bath and lithium hexamethyldisilazide (1M in THF, 7.2 ml) was introduced via syringe. The reaction mixture was allowed to warm to room temperature and stirred for 25 min. The reaction was then quenched by H_2O (50 ml) and THF was evaporated under reduced pressure. The resulting aqueous solution was extracted by EtOAc (3x100 ml). The combined organic extracts were dried by Na_2SO_4, filtered and concentrated in *vacuo* to afford the brown oil product (1.2026 g, 79% yield) without a further purification. **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 7.38(m, 5H), 4.21(m, 4H), 3.76 (s, 2H), 3.26 (q, 1H, *J*=7.8 Hz), 3.14 (m, 2H), 3.02 (m, 1H), 2.83 (t, 1H, *J*=8.6 Hz), 2.57 (dd, 1H, *J* = 6.7, 16.3 Hz), 2.46(dd, 1H, *J* =16.3, 8.7 Hz), 2.32(t, 1H, *J* =8.9 Hz), 1.33(m, 6H). **^{13}C NMR** ($CDCl_3$, 100 MHz), δ173.4, 172.0,132.4, 128.9, 128.4, 127.3, 60.6, 60.5, 60.0, 59.0,55.9,45.2, 36.8, 34.6, 14.2, 14.1. **HR-MS(ESI/APCI)** calcd for: $C_{18}H_{26}NO_4$ (MH^+): 320.1856, found: 320.1854

If the reaction time is too long, which will lead to the formation of impurities, chromatography (Hexane/EtOAc 3:1 and then pure EtOAc) can be employed to afford the desired product.

Preparation of *trans*-4-(carboxymethyl)pyrrolidine-3-carboxylic acid (27)

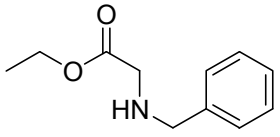
A 25ml round bottom flask was charged with *trans*-ethyl 1-benzyl-4-(2-ethoxy-2-oxoethyl)pyrrolidine-3-carboxylate (0.2061 g, 0.65 mmol) and 10ml 1N HCl solution. The mixture was stirred under reflux for overnight. The resulting solution was washed by Et_2O (2x20 ml) and was evaporated under reduced pressure to give 0.1811 g crystals. Those crystals was dissolved in 5 ml H_2O and transferred into a Pyrex 9800 test tube, followed by the addition of 2 spatulas of 10% palladium on activated carbon. The test tube was placed into a PARR reaction bottle (500 ml) which was shaken under H_2 (48 psi) in a pressure reaction apparatus for 26 h at room temperature. The reaction suspension was filtered over a pad of celite and showed a red spot on TLC by ninhydrin. The filtrate was concentrated in *vacuo* to give yellow crystals (0.1019 g, 75% overall yield). **^{1}H NMR** (D_2O, 400 MHz) δ 3.55-3.65(m, 2H), 3.40(dd, 1H, J =12.4, 6.8 Hz), 3.30(dt, 1H, J =6.9, 2.9 Hz), 3.06(t, 1H, J =10.8 Hz), 2.95(m, 1H), 2.54(d, 2H, J =7.5 Hz). **^{13}C NMR** (D_2O, 100 MHz), δ175.4, 175.2, 48.4, 47.6, 45.5,36.6, 33.1. **HR-MS(ESI/APCI)** calcd for: $C_7H_{12}NO_4$ (MH^+): 174.0761, found: 174.0760

Preparation of glycine ethyl ester hydrochloride (40)



Glycine (2.1103 g, 28.1 mmol), ethanol (60 ml) and thionyl chloride (2.5 ml, 34.4 mmol) were mixed and stirred at -20°C for 20 min. The reaction mixture was then warmed to room temperature and a second portion of glycine (1.9969 g, 26.6 mmol) was added. After stirring for 1 hour, the reaction mixture was refluxed for 2 more hours before being cooled to room temperature. Ethyl ether was added to precipitate the product out and after the vacuum filtration, the final product was obtained as white solid (4.4675 g, 93%). **^{1}H NMR** (D_2O, 400 MHz) δ 4.24(q, 2H, J=6.8 Hz), 3.84(s, 2H), 1.22(t, 3H, J=6.8 Hz); **^{13}C NMR** (D_2O, 100 MHz) δ 168.3, 63.5, 40.3, 13.3 [Cheng, C. et al. 2009].

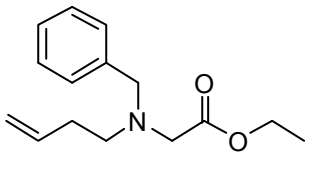
Preparation of ethyl 2-(benzylamino)acetate (41)



To a 100 ml round bottom flask were added glycine ethyl ester hydrochloride (2.0980 g, 0.015 mol), *tert*-butylmethyl ether (TBME, 30 ml), anhydrous sodium sulfate (2.0469 g, 0.014 mol), and distilled benzaldehyde (1.5604 g, 0.015 mol) sequentially. The stirred suspension was cooled to 0°C in an ice bath and triethylamine (2.928 g, 0.023 mol) was added dropwise over 10 min. The reaction suspension was then allowed to warm to room temperature and stirred for 24 hours. After the filtration and evaporation, the yellow oil was dissolved in 50 ml EtOH. The mixture was cooled to 0°C in an ice bath and sodium borohydride (1.1583 g, 0.03 mol) was introduced in three portions over 10 min. The reaction mixture was stirred at room temperature for further 35 min and was quenched by slowly adding saturated $NaHCO_3$ solution (50 ml). After filtration, the resulting solution

was extracted by ethyl acetate (3x50 ml). The combined organic extracts were dried by Na_2SO_4, filtered and concentrated in *vacuo*. The residue was purified by chromatography (petroleum ether/EtOAc 3:2) to give the yellow oil product (2.3112 g, 81% yield). ^{1}H NMR was consistent with literature [Yoon, U. C. et al. 1992] **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.30(m, 5H), 4.20 (q, 2H, J =7.2 Hz), 3.80(s, 2H), 3.40(s, 2H), 1.28 (t, 3H, J =7.2 Hz)

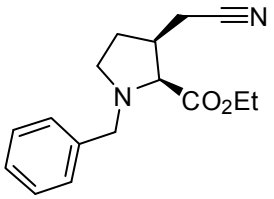
Preparation of ethyl 2-(benzyl(but-3-enyl)amino)acetate (39)



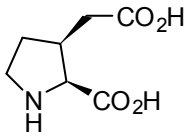
A 100 ml round bottom flask was charged with ethyl 2-(benzylamino)acetate (1.0842 g, 5.62 mmol), acetonitrile (25 ml) and K_2CO_3 (2.4304 g, 17.61 mmol). 4-bromobut-1-ene (2.2929 g, 17.0 mmol) was added dropwise at room temperature. The mixture was stirred under reflux for 24h and was then filtered, concentrated under reduced pressure. After purification by chromatography (Hexane/EtOAc 4:1), the yellow oil alkene product (0.8916 g, 64%) was given. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.35(m, 5H), 5.83(m, 1H), 5.05(m, 2H), 4.18(q, 2H, J=7.2 Hz), 3.83(s, 2H), 3.35(s, 2H), 2.76(t, 2H, J=7.2 Hz), 2.30(m, 2H), 1.29(t, 3H, J=7.2 Hz). **^{13}C NMR** ($CDCl_3$, 100 MHz), δ171.5, 139.1, 136.6, 128.9, 128.2, 127.0, 115.6, 60.2, 58.1, 54.2, 53.4, 32.2, 14.3. **HR-MS(ESI/APCI)** calcd for: $C_{15}H_{22}NO_2$ (MH^+): 248.1645, found: 248.1650

Preparation of *cis*-ethyl 1-benzyl-3-(cyanomethyl)pyrrolidine-2-carboxylate (42)

To a 25 ml flame-dried round bottom flask were added anhydrous ethyl 2-(benzyl(but-3-enyl)amino)acetate (0.3406 g, 1.38mmol) and 2ml dry THF. The flask was then flushed with N_2 gas and sealed by a rubber septum which was fitted with a nitrogen balloon. The stirred mixture was cooled to -78^{o}C and LDA (1.5 M in cyclohexane, 1.1 ml) was added dropwise. The reaction temperature was increased to -20^{o}C and then cooled to -100^{o}C at which Zinc Bromide (1 M in Et_2O, 3.4 ml) was introduced dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 2 hours. CuCN/2LiCl (1.35 M in THF, 1.4 ml) was added at 0^{o}C, followed by the addition of tosylcyanide (0.3001 g, 1.66 mmol) after 10 min. The reaction suspension was allowed to warm to room temperature and stirred for overnight. This reaction was then quenched by saturated NH_4Cl (30 ml). The resulting solution was extracted by Et_2O (30 ml) and CH_2Cl_2 (2x30 ml). The combined organic extracts were dried by Na_2SO_4, filtered and concentrated in *vacuo*. After chromatography (Hexane/EtOAc 9:1), the yellow oil product (0.1220g, 33%) was obtained. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.20 (m, 5H), 4.11(q, 2H, *J*=7.2 Hz), 3.49-3.82(AB, 2H), 3.40(d, 1H, *J*=7.6 Hz), 3.05(m, 1H), 2.69(m, 1H), 2.51(m, 1H), 2.40-2.29(m, 2H), 2.11(m, 1H), 1.68(m, 1H), 1.21(t, 3H, *J*=7.2 Hz). **^{13}C NMR** ($CDCl_3$, 100 MHz), δ171.3, 138.0, 129.0, 128.3, 127.3, 118.4, 67.4, 60.9, 57.5, 51.3, 37.9, 29.7, 19.6, 14.3. **HR-MS(ESI/APCI)** calcd for: $C_{16}H_{21}N_2O_2$ (MH^+): 273.1598, found: 273.1593

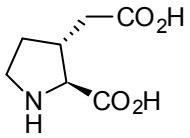


Preparation of *cis*-3-carboxymethyl-pyrrolidine-2-carboxylic acid (*cis*-CPAA) (43)



A 25 ml round bottom flask was charged with *cis*-ethyl 1-benzyl-3-(cyanomethyl)pyrrolidine-2-carboxylate (0.1220 g, 0.45 mmol) and 7 ml 6N HCl solution. The mixture was stirred under reflux for overnight. The resulting solution was concentrated under reduced pressure to give yellow crystals. Those crystals was dissolved in 5 ml H_2O and transferred into a Pyrex 9800 test tube, followed by the addition of 1 spatulas of 10% palladium on activated carbon. The test tube was placed into a PARR reaction bottle (500 ml) which was shaken under H_2 (50 psi) in a pressure reaction apparatus for 24h at room temperature. The reaction suspension was filtered over a pad of Celite and the filtrate was concentrated in *vacuo* to give yellow crystals (0.1252 g). After purification by ion-exchange chromatography (Dowex 50-X8, washed by water and then 1M NH_4Cl), the desired compound was obtained as a colorless crystals (0.0414 g, 45%). The structure was confirmed by comparison of their spectral data with those reported in the literature. [Karoyan, P. and Chassaing, G. 2002] **^{1}H NMR** (D_2O, 400 MHz) δ 4.09 (d, 1H, *J*=8.0 Hz), 3.47(m, 1H), 3.24(m, 1H), 2.83(m, 1H), 2.37(dd, 1H, *J*= 15.6, 4.8 Hz), 2.13(m, 2H), 1.74(m, 1H).

Preparation of *trans*-3-carboxymethyl-pyrrolidine-2-carboxylic acid (*trans*-CPAA) (38)



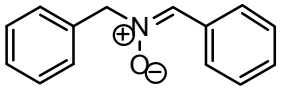
Cis-3-carboxymethyl-pyrrolidine-2-carboxylic acid (0.1142 g, 0.55 mmol) was acidified by 1N HCl, dissolved into 3 ml H_2O and transferred into a pressure tube. The mixture was heated at 190^{o}C for 12h to give a mixture of *cis/trans* 20:80 as seen when comparing the intensities of the doublet peak at

4.32 ppm (the α hydrogen of the *cis* isomer) to the doublet peak at 3.99 ppm (the α hydrogen of the *trans* isomer). Upon evaporation, the crystals were dissolved in 8 ml H_2O, cooled to 0^oC at which Na_2CO_3 (0.1998 g, 1.88 mmol) and benzyl chloromate (0.1116 g, 0.66 mmol) in 2 ml 1, 3-dioxane were added. The reaction solution was then allowed to warm to room temperature and stirred for 21 hours. 15 ml H_2O was added and the resulting solution was washed by Et_2O (2x20 ml). The aqueous layer was acidified by 1N HCl solution until its pH<2. The resulting aqueous solution was extracted by EtOAc (4x30 ml). The combined organic extracts were dried by Na_2SO_4, filtered and concentrated in *vacuo* to give an intermediate product. This intermediate was dissolved in 3 ml CH_2Cl_2, cooled to 0^oC at which 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (0.2524 g, 1.31 mmol), 4-Dimethylaminopyridine (0.0532 g, 0.44 mmol) and 1 ml EtOH were added. The reaction solution was then allowed to warm to room temperature and stirred for 20 hours. After the removal of solvents, chromatography (hexane/EtOAc 3:1) was used to obtain a mixture of *trans/cis* Cbz protected CPAA derivative (0.0855 g, 43%).

Preparative HPLC (methanol/H_2O 65:35, flow rate: 8 ml/min) was employed to afford the pure *trans* Cbz protected CPAA derivative (retention time: 52 min, 0.028 g, 0.077 mmol). This *trans* derivative was dissolved in 3 ml Methanol, treated with a spatula of 10% palladium on activated carbon and went through hydrogenolysis under H_2 (50 psi) for 24 h at room temperature. After filtration and evaporation, the hydrolysis of the residue was carried on in refluxing 4N HCl overnight to give the desired product (0.0128 g, 79%). The structure was confirmed by comparison of their spectral data with those

reported in the literature. [Karoyan, P. and Chassaing, G. 2002][Oba, M. et al. 2009] **^{1}H NMR** (D_2O, 400 MHz) δ 3.99(d, 1H, J=8.0 Hz), 3.41(m, 2H), 2.70-2.85(m, 2H), 2.55(m, 1H), 2.29(m, 1H), 1.78(m, 1H). **^{13}C NMR** (D_2O, 100 MHz), δ175.5, 171.4, 63.3, 45.2, 38.6, 36.3, 29.8.

Preparation of N-benzylidene-benzylamine N-oxide (44)



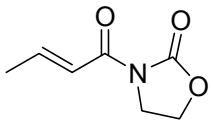
To a 250 ml two-necked flask equipped with a magnetic stir bar, thermometer and a reflux condenser fitted with a rubber septum and a balloon of nitrogen were added a solution of methyltrioxorhenium (MTO) (10 mg, 0.04 mmol) in 15 ml methanol and urea hydrogen peroxide (UHP) (1.4650 g, 15.6 mmol). The flask was cooled in an ice bath and dibenzylamine (1 ml, 5.21 mmol) was added dropwise via syringe over 10 min. The ice bath was then removed and the reaction mixture was stirred at room temperature. After 20 min, another four portions of MTO (14 mg each in 5 ml methanol) were added at 30 min intervals. 30 min after the last addition, the flask was cooled in an ice bath and sodium thiosulfate pentahydrate (1.3318 g, 5.37 mmol) was added in portions over 20 min. One hour later, the solution was decanted into a 250 ml flask and the solvent was evaporated under reduced pressure. CH_2Cl_2 (50 ml) was added and the urea was filtered through a pad of celite. The filtrate was concentrated to afford the nitrone (0.8732 g, 79% yield) as a yellow solid. If desired, a recrystallization can be performed in diethyl ether to provide the nitrone as a white solid. M.P. 77-78^{o}C. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ8.17-8.20 (m, 2H), 7.38-7.49 (m, 9H), 5.09 (s, 2H); **^{13}C NMR** ($CDCl_3$, 100 MHz), δ134.1, 133.4, 130.5, 130.4, 129.2, 128.9, 128.6, 128.4, 71.3 [Goti, A. et al. 2005].

Preparation of titanium dichlorodiisoproxide (2M in CH_2Cl_2) To a 25 ml flame-dried round bottom flask fitted with a rubber septum and a balloon of nitrogen were added dry CH_2Cl_2 (10 ml), Ti(*i*-OPr)$_2$ (6.1 ml) and $TiCl_2$ (2.2 ml) via syringes under an ice bath. The stirred mixture was then warm to room temperature and stirred for one hour. The solution was stored under nitrogen in a freezer (-20^{o}C).

Preparation of MgI_2-Phenanthroline (0.1M in CH_2Cl_2) [Gothelf, K. V. et al. 1996] Magnesium (103.5 g, 4.6 mmol), I_2(448.6 mg,1.8 mmol) and diethyl ether (10 ml) were placed in a 25 ml flask with a magnetic stirring bar under N_2. The mixture was stirred at room temperature 3h in which the iodine color disappeared. A syringe was employed to transfer the clear solution into a 100ml flame-dried flask. The ethyl ether was evaporated to form white MgI_2. Immediately, dry CH_2Cl_2 (40 ml) and phenanthroline (450 mg in 10 ml dry CH_2Cl_2, 2.5 mmol) was added. After the milky suspension was stirred for 2 hours, I_2(430.5 mg,1.7 mmol) was added. The deep red suspension was stirred for another 2 hours before use.

Preparation of (*E*)-3-but-2-enoyloxazolidin-2-one (47)

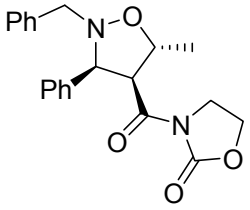
To a 100 ml flame-dried round bottom flask with a magnetic stir bar were added anhydrous 2-oxazolidinone (1.7461 g, 0.02 mol) and dry



THF (20 ml). The flask was then flushed with N_2 gas and sealed by a rubber septum which was fitted with a nitrogen balloon. The stirred mixture was cooled to -78^{o}C in a dry ice/acetone bath and n-butyl lithium (1.6M in hexane, 12.5 ml) was introduced via syringe. After stirring for 1 hour, crotonoyl chloride (90%, 2.2 ml, 0.021 mol) was added

through syringe and the reaction solution was slowly warmed to room temperature. After stirring for further 3 hours, the reaction was quenched by adding saturated NH_4Cl solution (60 ml). The mixture was extracted by EtOAc (3x50 ml). The combined organic extracts were dried by Na_2SO_4, filtered and concentrated in *vacuo*. The residue was purified by chromatography (1:1 hexane/EtOAc) to give the white solid product (1.7194 g, 55% yield). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.26-7.18(m, 2H), 4.42(t, 2H, *J*=8.0 Hz), 4.03(t, 2H, *J*=8.0 Hz), 1.92(d, 3H, *J*=6.4 Hz); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 195.9, 165.2, 146.8, 121.4, 62.0, 42.6, 18.5 [Nakamura, T. et al. 2007].

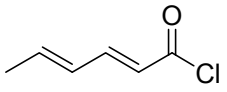
Preparation of 3-(2-benzyl-5-methyl-3-phenylisoxazolidine-4-carbonyl)oxazolidin-2-one



A 25 ml flame-dried flask were charged with *N*-benzylidene-benzylamine *N*-oxide (107.2 mg, 0.51 mmol), (*E*)-3-but-2-enoyloxazolidin-2-one (62.8 mg, 0.41 mmol), powered 4A molecular sieve (54.1 mg) and dry CH_2Cl_2 (3 ml). The flask was then flushed with N_2 gas and sealed by a rubber septum which was fitted with a nitrogen balloon. $TiCl_2(i\text{-}OPr)_2$ (2M in CH_2Cl_2, 0.05 ml) was added via syringe. The resulting suspension was stirred at room temperature for 4 days. A mixture of 5% methanol and 95% CH_2Cl_2 (10 ml) was added and the cloudy solution was then filtered. After the evaporation in *vacuo*, the crude product was purified by chromatography (3:1 EtO_2/petroleum ether) gave a mixture of *exo* adduct and *endo* adduct (9:1) (0.0555 g, 37% yield). The *exo* adduct (major product) was confirmed by comparison of their spectral data with those reported in the literature. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.43-

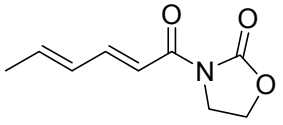
7.23(m, 10H), 4.94(m, 1H), 4.30-4.42(m, 2H), 4.12(m, 1H), 3.93(d, 1H, *J*=14.4 Hz), 3.80(d, 1H, *J*=14.4 Hz), 3.66(m, 2H), 3.08(m, 1H), 1.35(d, 3H, *J*=6.0 Hz); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ170.3, 152.9, 137.4, 136.9, 129.0, 128.7, 128.4, 128.2, 127.0, 74.6, 73.4, 61.9, 59.7, 59.6, 42.4, 18.0 [Gothelf, K. V. and Jorgensen, K. A. 1994].

Preparation of (*E*, *E*)-Hexa-2, 4-dienoyl chloride (sorbyl chloride) (48)



A 25 ml flask equipped with a $CaCl_2$ dring tube was charged with (*E*, *E*)-hexa-2, 4-dienoic acid (sorbic acid) (2.9445 g, 26.4 mmol), oxalyl chloride (4 ml, 45.5 mmol), dry CH_2Cl_2 (40 ml) and 3 drops DMF. The reaction solution was stirred for 4.5 hours and the solvent was evaporated under reduced pressure. The crude product was purified via Kugelrohr distillation to afford the yellow oil product (2.9163 g, 86%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.44 (dd, 1H, *J*=14.8, 10.8 Hz), 6.46-6.22(m, 2H), 6.00(d, 1H, *J*=14.8 Hz), 1.94(d, 3H, *J*=6.0 Hz) [Davies, S. G. et al. 2004].

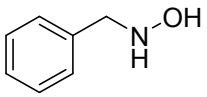
Preparation of 3-(2*E*, 4*E*)-hexa-2,4-dienoyloxazolidin-2-one (49)



A 100 ml flame-dried round bottom flask with a magnetic stir bar was charged with anhydrous 2-oxazolidinone (1.0953 g, 12.6 mmol) and dry THF (10 ml). The flask was then flushed with N_2 gas and sealed by a rubber septum which was fitted with a nitrogen balloon. The stirred mixture was cooled to -78^{o}C in a dry ice/acetone bath and n-butyl lithium (1.6M in hexane, 8 ml) was introduced via syringe. After stirring for 3 hour, (*E*, *E*)-Hexa-2, 4-dienoyl chloride (sorbyl chloride) (2.1713 g, 16.8 mmol) was added through syringe and the reaction solution was slowly warmed to room temperature. After stirring for further

2.5 hours, the reaction was quenched by adding saturated NH_4Cl solution (30 ml), followed by adding brine (20 ml). The mixture was extracted by EtOAc (3x50 ml). The combined organic extracts were washed by water (3x50 ml) and brine (50 ml). The resulting organic layer was dried by Na_2SO_4, filtered and concentrated in *vacuo* to give the white solid product (1.6995 g, 75%yield). **M.P.** 123-126^oC; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.47(dd, 1H, *J*=15.2, 10.8 Hz), 7.22(d, 1H, *J*=15.2 Hz), 6.38-6.21(m, 2H), 4.43(t, 2H, *J*=8.4 Hz), 4.10(t, 2H, *J*=8.4 Hz), 1.90(d, 3H, *J*=6.4 Hz); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 165.7, 153.5, 146.8, 141.1, 130.4, 117.5, 62.0, 42.8, 18.8.

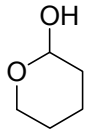
Preparation of *N*-benzylhydroxylamine hydrochloride (59)



To a 25 ml flask were added benzaldehyde oxime (0.1850 g, 1.53 mmol), methanol (1 ml) and a little methyl orange. The solution was cooled to 0^{o}C in an ice bath and methanol-HCl solution (carefully mixed 3:1 methanol/acetyl chloride under an ice bath) was dropwise added to make the solution turn to pink. $NaCNBH_3$ (0.0747 mg, 1.19 mmol) was added in three portions and methanol-HCl solution was used to maintain the pink color of the reaction solution. Upon the addition of $NaCNBH_3$, the reaction solution was warmed to room temperature and was stirred for 2 and half hours while staying pink by adding methanol-HCl solution occasionally. Methanol was evaporated under reduced pressure and 1ml water was added to the residue. The formed suspension was basified until the pH was higher than 9 using 25% NaOH solution. Brine (20 ml) was added and the solution was extracted by CH_2Cl_2 (3x25 ml) and the combined organic layers were dried by Na_2SO_4, filtered and concentrated in *vacuo* to offer the yellow solid product (0.1780 g, 95%). **^{1}H NMR**

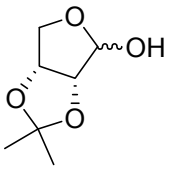
($CDCl_3$, 400 MHz) δ7.44-7.28(m, 5H), 6.13(s, 2H, exch D_2O), 3.99 (s, 2H) [Maskill, H. and Jencks, W. P. 1987].

Preparation of tetrahydro-2*H*-pyran-2-ol (60)



2, 3-dihydropyran (4.3376 g, 51.6 mmol) was mixed with 2N HCl solution (10 ml) at 0 °C, stirred for 40 min and then stirred at room temperature for 1 h. The reaction solution was neutralized by saturated $NaHCO_3$ solution. The resultant mixture was extracted with CH_2Cl_2 (2x60 ml). The combined organic layer was dried by $NaSO_4$, filtered and concentrated in *vacuo* to afford a crude prodeuct. After a vacuum distillation, the desired product was collected as colorless oil (3.9508 g, 75%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ4.92(m, 1H), 4.02(m, 1H), 3.52 (m, 1H), 1.83(m, 2H), 1.51(m, 4H) [Murphy, P. J. et al. 1996].

Preparation of (3a*R*, 6a*R*)-2,2-dimethyl-tetrahydrofuro[3,4-d][1,3]dioxol-4-ol (64)

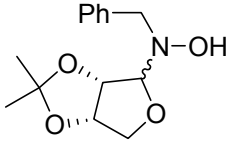


To a 100ml flask were added D-Ribose (1.9499 g, 13.0 mmol), anhydrous acetone (10 ml) and concentrated sulfuric acid (0.1 ml). The suspension was stirred at room temperature and after 30min the cloudy suspension graduately became clear. After stirring for further 1 hour, 10 ml acetone was added and $Ca(OH)_2$ was employed to adjust the pH to 7. The resulting suspension was filtered through a pad of celite and evaporated to give the yellow oil product (2.4769 g, 100%) as (3a*R*, 6*R*, 6a*R*)-6-(hydroxymethyl)-2, 2-dimethyl-tetrahydrofuro[3,4-*d*][1,3]dioxol-4-ol which can be used directly in next step without the need of purification.

(3a*R*, 6*R*, 6a*R*)-6-(hydroxymethyl)-2, 2-dimethyl-tetrahydrofuro[3,4-*d*][1,3]dioxol-4-ol (3.2071 g, 16.9 mmol) was dissolved in methanol (75 ml). The solution was cooled to 0^oC in an ice bath and $NaBH_4$ (2.7 g, 71.1 mmol) was added in 3 portions over 30 min. The solution was stirred for 2 more hours at 0^oC and was then neutralized by glacial acetic acid. After the ice bath was removed, water (50 ml) and $NaIO_4$ (6.7374 g, 31.5 mmol) was added sequentially. The mixture was stirred for 3 hours and was filtered and concentrated to remove the methanol under reduced pressure. The residue was extracted by EtOAc (3x40 ml). The combined extracts were washed by brine (100 ml) and dried by Na_2SO_4, filtered and concentrated in *vacuo*. The residue was purified by a short pad of silica gel, eluated by 1:1 hexane/EtOAc mixture to give the colorless oil product (1.7503 g, 69%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ5.45(s, 1H), 4.86(m, 1H), 4.61(d, 1H, *J*=5.6 Hz), 4.08(m, 2H), 1.50(s, 3H), 1.35(s, 3H) [Hudlicky, T. et al. 1990].

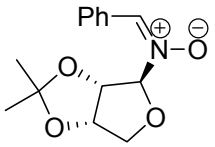
Preparation of (3a*R*, 4*R*, 6a*R*)-N-Benzyl-tetrahydro-N-hydroxy-2,2-dimethylfuro-[3,4-*d*]-1,3-dioxol-4-amine (65)

N-benzylhydroxylamine hydrochloride (0.1236 g, 1.0 mmol), (3a*R*, 6a*R*)-2,2-dimethyl-tetrahydrofuro[3,4-d][1,3]dioxol-4-ol (0.1407 g, 0.88 mmol), 3A molecular sieve (33.6 mg) and dry pyridine (2 ml) were mixed together in a 10 ml flask fitted with a $CaCl_2$ drying tube and stirred overnight. The mixture was then diluted by adding 2 ml CH_2Cl_2, filtered through a pad of celite and concentrated under reduced pressure. The residue was purified by a short pad of silica gel, eluated by 3:1 Petroleum ether/EtOAc solvent mixture to give a light yellow solid product (0.2154 g, 92%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.34(m, 5H), 4.93(d, 1H,



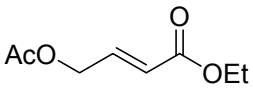
J= 6.0Hz), 4.89(dd, 1H, *J*=6.0, 4.4 Hz), 4.74(s, 1H), 4.28(dd, 1H, *J*=9.6, 4.4 Hz), 4.10(m, 2H), 3.89(d, 1H, *J*= 13.2Hz), 1.52(s, 3H), 1.36(s, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ137.0, 129.5, 128.5, 127.5, 112.1, 99.7, 83.9, 81.1, 76.4, 59.4, 26.5, 24.8 [Cicchi, S. et al. 2003].

Preparation of (3a*R*, 4*R*, 6a*R*)-Tetrahydro-2,2-dimethyl-N-(phenylmethylene)furo-[3,4-*d*][1,3]dioxol-4-amine N-Oxide (53)



(3a*R*, 4*R*, 6a*R*)-N-Benzyl-tetrahydro-N-hydroxy-2,2-dimethylfuro-[3,4-*d*]-1,3-dioxol-4-amine (0.1907 g, 0.71 mmol) was dissolved in CH_2Cl_2 (3 ml). The solution was cooled to 0^{o}C under an ice bath and MnO2 (85%, 0.1109 g, 1.08 mmol) was added. The suspension was warmed to room temperature and stirred overnight. The suspension was then filtered through one layer of celite (top) and one layer of Na_2SO_4 (bottom), followed by CH_2Cl_2 washing those two layers. The solvent was evaporated in *vacuo* to give the solid product (0.1533 g, 81%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.26(m, 2H), 7.63(s, 1H), 7.45(m, 3H), 5.53(s, 1H), 5.35(d, 1H, *J*=6.0 Hz), 5.03(dd, 1H, *J*=6.0, 4.0 Hz), 4.53(dd, 1H, *J*=10.0 ,4.0 Hz), 4.32(d, 1H, *J*=10.0 Hz), 1.55(s, 3H), 1.39(s, 3H); **^{13}C NMR**($CDCl_3$, 100 MHz) δ 132.8, 131.0, 129.6, 128.9, 128.5, 112.9, 104.0, 84.4, 80.5, 77.2, 26.3, 24.7 [Cicchi, S. et al. 2003].

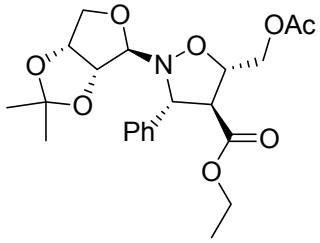
Preparation of (*E*)-ethyl 4-acetoxybut-2-enoate (55)



Ethyl 4-bromocrotonate (75%, 0.3889 g, 1.51 mmol), acetic acid (5 ml) and silver acetate (0.2848 g, 1.71 mmol) were mixed together in a 25 ml flask fitted with a condenser and drying tube ($CaCl_2$). The formed suspension

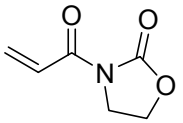
was stirred at reflux at for 23h. The solution was filtered and washed by EtOAc. The solvents were evaporated in *vacuo* and the residue was purified by chromatography (4:1 hexane/EtOAc) to give an oil product (0.2095 g, 81% yield). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 6.96(dt, 1H, *J*=15.6, 4.4 Hz), 6.04(d, 1H, *J*=15.6 Hz), 4.75(dd, 2H, *J*=4.4, 1.6 Hz), 4.22(q, 2H, *J*=7.2 Hz), 2.14(s, 3H), 1.31(t, 3H, *J*=7.2 Hz).

Preparation of (3*R*, 4*S*, 5*S*)-ethyl 5-(acetoxymethyl)-2-((3a*R*, 4*R*, 6a*R*)-2,2-dimethyl-tetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-3-phenylisoxazolidine-4-carboxylate (66)



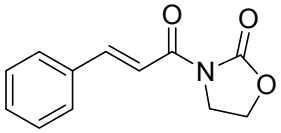
To a 50 ml flask equipped with a magnetic stir bar and a reflux condenser were added (3a*R*, 4*R*, 6a*R*)-Tetrahydro-2,2-dimethyl-N-(phenylmethylene)furo-[3,4-*d*][1,3]dioxol-4-amine N-Oxide (532.6 mg, 2.03 mmol), (*E*)-ethyl 4-acetoxybut-2-enoate (175.8 g, 1.02 mmol), powered 4A molecular sieve (100.2 mg) and Toluene (10 ml). The reaction mixture was refluxed for 6 days and was filtered through a pad of celite. The solution was concentrated under reduced pressure and the residue was purified by chromatography (3:1 hexane/EtOAc) to afford the final product (0.1965 g, 44%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.44-7.24(m, 5H), 4.99(d, 1H, *J*=6.0 Hz), 4.83-4.76(m, 3H), 4.73(m, 1H), 4.35(dd, 1H, *J*=12.0, 3.6 Hz), 4.29-4.20(m, 3H), 3.88(t, 1H, *J*=10.4 Hz), 3.77(dd, 1H, *J*=10.4, 4.0 Hz), 3.30(dd, 1H, *J*=7.2, 6.4 Hz), 1.98(s, 3H), 1.49(s, 3H), 1.35(s, 3H), 1.29(t, 3H, *J*=6.8 Hz); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 170.9, 170.6, 140.5, 128.6, 127.6, 126.8, 112.2, 97.8, 83.2, 80.6, 79.1, 73.9, 68.7, 63.2, 61.7, 59.2, 26.3, 24.9, 20.6, 14.1. **LC MS** calcd for: $C_{22}H_{30}NO_3$ (MH^+): 436.2, found: 436.4

Preparation of 3-(2-Propenoyl)-l,3-oxazolidin-2-one (73)



To a 100 ml flask were charged with 20 ml CH_2Cl_2 and NaH (60%, 1.6823 g, 42.1 mmol). The suspension was cooled to 0°C at which 2-oxazolidinone (1.7824 g, 20.7 mmol) was added, resulting in bubbling. After the bubbling stops, the suspension was cooled to -78°C and propenoyl chloride (1.46 ml, 18.1 mmol) was introduced. The reaction solution was then warmed to room temperature and stirred for 23 hours. The reaction solution was filtered through a short pad of silica gel, eluted by CH_2Cl_2. The resulting solution was evaporated under reduced pressure at 30°C to form 3-(2-Propenoyl)-l,3-oxazolidin-2-one as white solid (1.0163 g, 40% yield). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.45(m, 1H), 6.50(dd, 1H, *J*=16.8, 1.6 Hz), 5.84(dd, 1H, *J*=10.4, 1.6 Hz), 4.38(t, 2H, *J*=8.4 Hz), 4.02(t, 2H, *J*=8.4 Hz) [Narasaka, K. et al. 1989]

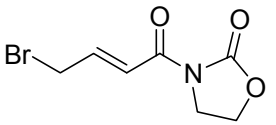
Preparation of 3-cinnamoyloxazolidin-2-one (78)



Oxazolidinone (0.8704 g, 10.0mmol), DMAP (0.2524 g, 2.1 mmol) and cinamic acid (1.9293 g, 13.0 mmol) was mixed in 20 ml CH_2Cl_2 in a 100 ml flask. The suspension was then cooled to 0°C and DCC (2.6701 g, 10.0 mmol) was added. After 10 min, the temperature was raised to room temperature and the reaction solution was stirred for 4 hours. The precipitate was fitered out and washed by CH_2Cl_2. The filtrate was washed by saturated $NaHCO_3$ (50 ml), dried by Na_2SO_4 and concentrated in *vacuo*. The residue was purified by radial chromatography (3:1 hexane/EtOAc) to afford the desired product (2.0449 g, 95%). **^{1}H NMR** ($CDCl_3$, 400

MHz) δ7.82(m, 2H), 7.55(m, 2H), 7.35(m, 3H), 4.40(t, 2H, *J*=8.0 Hz), 4.10(t, 2H, *J*=8.0 Hz) [Sibi, M. P. and Manyem, S. 2002].

Preparation of (*E*)-3-(4-bromobut-2-enoyl)oxazolidin-2-one (57)



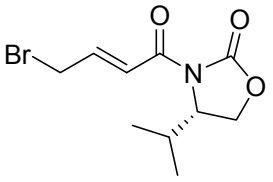
Crotonic acid (0.2752 g, 3.2 mmol) was dissolved in CCl_4 (8 ml), followed by adding NBS (0.5981 g, 3.4 mmol) and benzoyl peroxide (75%, 0.0211 g, 0.1 mmol). The resulting suspension was refluxed under N_2 atmosphere overnight and cooled to room temperature. After filtration, thionyl chloride (0.4 ml, 4.5 mmol) was added to the solution and the mixture was stirred at 60^{o}C in a round bottom flask equipped with a condenser and drying tube ($CaCl_2$) overnight. The solution was concentrated in *vacuo* to give crude (*E*)-4-bromobut-2-enoyl chloride which was dissolved in dry THF (3 ml). The flask of this solution was then flushed by N_2 flow and sealed by a rubber septum.

To a 100 ml flame-dried round bottom flask with a magnetic stir bar were added anhydrous 2-oxazolidone (0.2500 g, 2.9 mmol) and dry THF (4 ml). The flask was then flushed with N_2 gas and sealed by a rubber septum which was fitted with a nitrogen balloon. The stirred mixture was cooled to -78^{o}C in a dry ice/acetone bath and n-butyllithium (1.6M in hexane, 1.8 ml, 2.9 mmol) was introduced via syringe. After one hour, more dry ice was added to the dry ice/acetone bath and the activated 2-oxazolidone was added dropwise via a cannula to the flask containing (*E*)-4-bromobut-2-enoyl chloride which was also cooled to -78^{o}C. After the addition, the reaction mixture was allowed to warm to room temperature and stirred for 3 hours. The reaction was then

quenched by H_2O (10 ml) and brine (20 ml). The mixture was extracted by EtOAc (3x35 ml). The combined organic extracts were dried by Na_2SO_4, filtered and concentrated in *vacuo*. The residue was purified by chromatography (9:1 hexane/EtOAc then 7:3 hexane/EtOAc) to give the light yellow oil product (0.3404 g, 51% yield). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.48(d, 1H, *J*=15.2 Hz), 7.18(dt, 1H, *J*=15.2, 7.6 Hz), 4.45(t, 2H, *J*=7.6 Hz), 4.09(m, 4H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ164.1, 153.3, 143.3, 122.8, 62.2, 42.6, 29.3. **HR-MS(ESI/APCI)** calcd for: $C_7H_9NO_3Br(MH^+)$: 233.9760, found: 233.9768

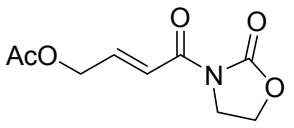
Preparation of (*S*, *E*)-3-(4-bromobut-2-enoyl)-4-isopropyloxazolidin-2-one (94)

This compound was prepared in the similar way of preparing (*E*)-3-(4-bromobut-2-enoyl)oxazolidin-2-one. Crotonic acid was treated with NBS and thionyl chloride sequentially to give (*E*)-4-bromobut-2-enoyl chloride which reacted with (*S*)-4-isopropyloxazolidin-2-one in the presence of n-butyllithium. 34% yield. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.50(dt, 1H, *J*=14.8, 1.2 Hz), 7.17(dt, 1H, *J*=14.8, 7.6 Hz), 4.52(m, 1H), 4.27(m, 2H), 4.10(dt, 2H, *J*=7.6, 1.2 Hz), 2.45(m, 1H), 0.96(d, 3H, *J*=7.2 Hz), 0.91(d, 3H, *J*=6.8 Hz). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ164.0, 153.9, 143.1, 123.3, 63.5, 58.6, 29.4, 28.4, 18.0, 15.0. **HR-MS(ESI/APCI)** calcd for: $C_{10}H_{15}NO_3Br$ (MH^+): 276.0230, found: 276.0222



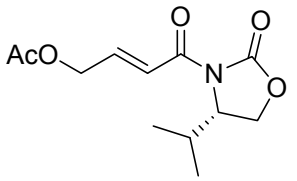
Preparation of (*E*)-4-oxo-4-(2-oxooxazolidin-3-yl)but-2-enyl acetate (58)

(*E*)-3-(4-bromobut-2-enoyl)oxazolidin-2-one (0.1121 g, 0.48 mmol) was dissolved in acetic acid (5 ml), followed by adding silver acetate (0.0888 g,



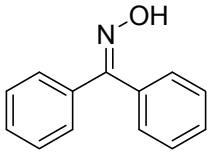
0.53 mmol). The formed suspension was stirred at 100°C under a drying tube (CaCl2) for 22h. The solution was filtered and water (25 ml) was added to the filtrate, followed by extraction by EtOAc (3x25 ml). The combined organic layers were washed by saturated $NaHCO_3$ solution until all of the acetic acid was removed. The solution was then dried by Na_2SO_4, filtered and concentrated in *vacuo*. The residue was purified by chromatography (3:1:1 hexane/EtOAc/CH_2Cl_2) to give a yellow oil product (0.0476 g, 47% yield). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.45(d, 1H, *J*=15.6 Hz), 7.12(dt, 1H, *J*=15.6, 4.4 Hz), 4.81(d, 2H, *J*=4.4 Hz), 4.45(t, 2H, *J*=8.0 Hz), 4.09(t, 2H, *J*=8.0 Hz), 2.15(s, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz), δ170.4, 164.4, 153.4, 143.0, 120.6, 62.9, 62.1, 42.6, 20.7. **HR-MS(ESI/APCI)** calcd for: $C_9H_{12}NO_5$ (MH^+): 214.0710, found: 214.0714

Preparation of (*S*, *E*)-4-(4-isopropyl-2-oxooxazolidin-3-yl)-4-oxobut-2-enyl acetate (92)



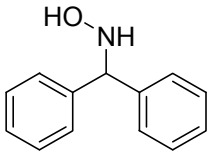
This compound was prepared in the similar way of preparing (*E*)-4-oxo-4-(2-oxooxazolidin-3-yl)but-2-enyl acetate. (*S*, *E*)-3-(4-bromobut-2-enoyl)-4-isopropyloxazolidin-2-one was stirred with silver acetate in reflux acetic acid for 24h. 61% yield. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.41(dt, 1H, *J*=15.6, 2.0 Hz), 7.02(dt, 1H, *J*=15.6, 4.8 Hz), 4.75(dd, 2H, *J*=4.8, 2.0 Hz), 4.43(m, 1H), 4.23(dd, 1H, *J*=9.2, 8.4 Hz), 4.16(dd, 1H, *J*=9.2, 3.2 Hz), 2.34(m, 1H), 2.08(s, 3H), 0.87(d, 3H, *J*=6.8 Hz), 0.82(d, 3H, *J*=7.2 Hz); **^{13}C NMR** ($CDCl_3$, 100 MHz), δ170.3, 164.2, 153.9, 142.8, 121.1, 63.5, 62.9, 58.6, 28.5, 20.7, 18.0, 14.7. **HR-MS(ESI/APCI)** calcd for: $C_{12}H_{18}NO_5$ (MH^+): 256.1179, found: 256.1176

Preparation of benzophenone oxime (71)



To a 250 ml flask with a magnetic stir bar were added benzophenone (5.5012 g, 30.2 mmol), hydroxylamine hydrochloride (7.6079 g, 109.4 mmol), methanol (100 ml) and pyridine (12 ml). The mixture was stirred at room temperature overnight. The solvents were evaporated in *vacuo* and a mixture of hexane (35 ml) and EtOAc (35 ml) was added to the residue. The liquid part was decanted into a separatory funnel, washed by 1N HCl solution (50 ml), water (50 ml) and brine (50 ml) sequentially. The solution was dried by Na_2SO_4, filtered and concentrated in *vacuo* to give the desired product as white solid (5.2307 g, 88%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ7.87(s, br, 1H), 7.54-7.33(m, 10H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ157.8, 136.1, 132.6, 129.5, 129.2, 129.1, 128.3, 128.2, 127.8 [Liu, S. et al. 2007].

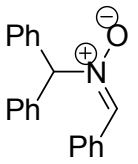
Preparation of *N*-diphenylmethylhydroxylamine (72)



Benzophenone oxime (5.2307 g, 26.4 mmol) was dissolved in methanol (50 ml) in a flask. After a little methyl orange was added, the solution was cooled to 0^{o}C in an ice bath and 6N HCl solution was dropwise added to make the solution turn to pink. $NaCNBH_3$(4.5228 g, 71.8 mmol) in methanol (10 ml) was then dropwise added into the solution and 6N HCl was used to maintain the pink color of the reaction solution. Upon the addition of $NaCNBH_3$, the reaction solution was warmed to room temperature and was stirred for 4 hours while staying pink by adding 6N HCl occasionally. Saturated $NaHCO_3$ solution was employed

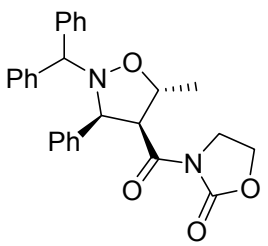
to basify the solution, after which methanol was evaporated under reduced pressure. The residue was extracted by CH_2Cl_2 (3x100 ml) and the combined organic layers were dried by Na_2SO_4, filtered and concentrated in *vacuo* to offer the yellow oil product (5.2471 g, 99%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ7.45-7.26(m, 10H), 5.26(s, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ140.5, 128.8, 127.9, 127.8, 70.7 [Sivappa, R. et al. 2007].

Preparation of (*Z*)-*N*-Benzylidenediphenylmethylamine *N*-Oxide (67)



The method described by Hashimoto et al. was followed with some modification. To an 100ml flask were added *N*-diphenylmethylhydroxylamine(4.4160 g, 22.01 mmol), benzaldehyde(3.5205 g, 33.21 mmol) and CH_2Cl_2(15 ml). The mixture was stirred at room temperature for 4h. The solvent was evaporated in *vacuo* and cool ethyl ether was added to the residue. The vacuum filtration afforded the nitrone as white solid (5.1370 g, 81%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ8.29-8.24 (m, 2H), 7.51 (s, 1H), 7.46-7.30 (m, 13H), 6.40 (s, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ137.1, 134.9, 130.5, 130.4, 128.8, 128.76, 128.75, 128.6, 128.5, 83.8 [Hashimoto, T. et al. 2007].

Preparation of 3-(2-benzhydryl-5-methyl-3-phenylisoxazolidine-4-carbonyl)oxazolidin-2-one (*exo* adduct) (74)

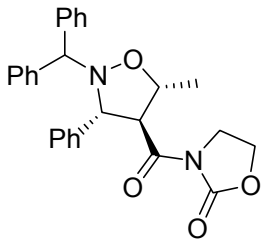


To a 100 ml flame-dried flask were added *N*-benzylidene-benzhydrylamine *N*-oxide (1.0627 g, 3.7 mmol), (*E*)-3-but-2-enoyloxazolidin-2-one (0.6780 g, 4.4 mmol), powered 4A

molecular sieve (1.0121 g) and dry CH_2Cl_2 (12 ml). The flask was then flushed with N_2 gas and sealed by a rubber septum which was fitted with a nitrogen balloon. $TiCl_2(i\text{-}OPr)_2$ (2M in CH_2Cl_2, 0.5 ml) was added via syringe. The resulting suspension was stirred at room temperature for 9 days. A mixture of 20% methanol and 80% CH_2Cl_2 (25 ml) was added and the cloudy solution was then filtered through one layer of celite (top) and one layer of silica gel (bottom), followed by CH_2Cl_2 (20 ml) washing those two layers. The solvent was evaporated in *vacuo*. The chromatography (4:1:1 hexane/EtOAc/CH_2Cl_2) gave a mixture of *exo* adduct and *endo* adduct (8.6:1) (1.0055 g, 61% yield). The pure *exo* adduct was recrystallized out as white solid from a mixture of anisole/hexane at -20°C overnight. M.P. 189-192°C; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.52-7.14(m, 15H), 5.11(m, 1H), 5.04(s, 1H), 4.52(d, 1H, *J*=10.4 Hz), 4.35(dd, 1H, *J*=10.4, 9.0 Hz), 4.21(m, 1H), 3.75(m, 2H), 3.10(m, 1H), 1.42(d, 3H, *J*=6.1 Hz); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ170.1, 152.8, 140.8, 140.0, 137.9, 129.2, 128.8, 128.6, 128.0, 127.7, 127.6, 127.4, 127.2, 126.9, 74.2, 72.5, 69.8, 61.9, 59.9, 42.3, 17.9. **HR-MS(ESI/APCI)** calcd for: $C_{27}H_{27}N_2O_4$ (MH^+): 443.1965, found: 443.1958

Preparation of 3-(2-benzhydryl-5-methyl-3-phenylisoxazolidine-4-carbonyl)oxazolidin-2-one (*endo* adduct) (75)

To a 50 ml flame-dried flask equipped with a magnetic stir bar and a reflux condenser fitted with a rubber septum and a balloon of nitrogen were added *N*-benzylidene-benzhydrylamine *N*-oxide (0.3182 g, 1.1 mmol), (*E*)-3-but-2-enoyloxazolidin-2-one (0.1586

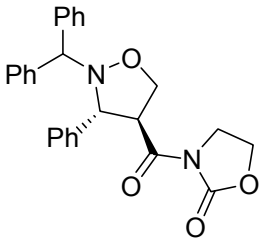


g, 1.0 mmol), powered 4A molecular sieve (193 mg), $Sc(OTf)_3$ (0.0531 g, 0.11 mmol) and dry CH_2Cl_2 (8 ml). The reaction mixture was refluxed for 9 days. A mixture of 10% methanol and 90% CH_2Cl_2 (10 ml) was added and the cloudy solution was then filtered through one layer of celite (top) and one layer of silica gel (bottom), followed by CH_2Cl_2 (15 ml) washing those two layers. The solvent was evaporated in *vacuo*. The chromatography (3:1:1 hexane/EtOAc/CH_2Cl_2) afforded the pure *endo* adduct as white solid (0.2309 g, 70% yield). M.P. 87-90^oC; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.50-7.08(m, 15H), 5.36(s, 1H), 4.68(dd, 1H, *J*=6.7, 6.0 Hz), 4.59(d, 1H, *J*=6.0 Hz), 4.50(m, 1H), 4.28(m, 2H), 3.98(m, 2H), 1.49(d, 3H, *J*=6.1 Hz); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ172.5, 152.8, 141.5, 141.3, 141.2, 128.5, 128.3, 128.2, 128.1, 127.4, 127.2, 127.1, 127.0, 78.8, 72.4, 71.9, 61.9, 61.8, 43.0, 19.0. **HR-MS(ESI/APCI)** calcd for: $C_{27}H_{27}N_2O_4$ (MH^+): 443.1965, found: 443.1952

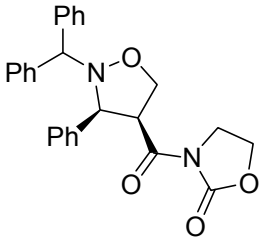
Preparation of 3-(2-benzhydryl-3-phenylisoxazolidine-4-carbonyl)oxazolidin-2-one

Following the procedure descrided above, a mixture of *exo* and *endo* cycloadducts were obtained from the 1, 3-dipolar cycloaddition between 3-acryloyloxazolidin-2-one and N-benzylidene-benzhydrylamine N-oxide in the presence of either $TiCl_2(i\text{-}OPr)_2$ or $Sc(OTf)_3$. When $TiCl_2(i\text{-}OPr)_2$ was the catalyst, radial chromatography (5:1:1 hexane/EtOAc/CH_2Cl_2) gave a mixture of *exo* and *endo* cycloadducts in a ratio of 74 to 26 (43% yield). When $Sc(OTf)_3$ was utilized, radial chromatography (5:1:1 hexane/EtOAc/CH_2Cl_2) afforded a mixture of *exo* and *endo* cycloadducts in a ratio of 50 to 50 (72% yield).

Endo adduct (**77**): although radial chromatography (1:1 Et_2O/petroleum ether) cannot separate *exo* and *endo* adducts completely, pure *endo* adduct (eluated after *exo* adduct) was able to obtain as white solid for characterization. M.P. 96-98^oC; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.48-7.10(m, 15H), 5.13(s, 1H), 4.68(d, 1H, *J*=5.6 Hz), 4.61(t, 1H, *J*=8.8 Hz), 4.50-4.36(m, 3H), 4.06-4.01(m, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ171.6, 153.1, 141.2, 140.8, 128.6, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.3, 127.2, 127.1, 72.1, 69.4, 68.3, 62.2, 61.9, 57.9, 42.8. **HR-MS(ESI/APCI)** calcd for: $C_{26}H_{25}N_2O_4$ (MH^+): 429.1809, found: 429.1803

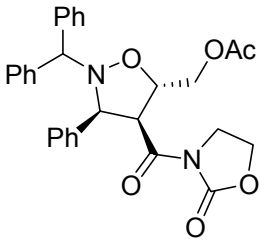


Exo adduct (**76**): *Exo* adduct (92%) with minor *endo* adduct (8%) was recrystallized from a solvent mixture of anisole/Hexane for characterization. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.41-7.11(m, 15H), 4.99(s, 1H), 4.89(m, 1H), 4.74(t, 1H, *J*=8.4 Hz), 4.49(d, 1H, *J*=10.0 Hz), 4.21(dd, 1H, *J*=8.4, 8.4 Hz), 4.12(m, 1H), 3.70(m, 2H), 3.01(m, 1H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ170.0, 152.9, 141.1, 140.3, 128.7, 128.6, 128.2, 128.0, 127.9, 127.6, 127.3, 127.1, 72.2, 68.9, 66.5, 62.0, 53.0, 42.4. **HR-MS(ESI/APCI)** calcd for: $C_{26}H_{25}N_2O_4$ (MH^+): 429.1809, found: 429.1811

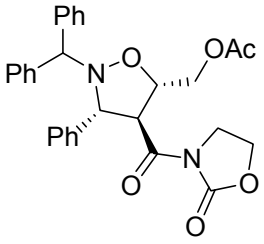


Preparation of (2-benzhydryl-4-(2-oxooxazolidine-3-carbonyl)-3-phenylisoxazolidin-5-yl)methyl acetate

This 1, 3 dipolar cycloaddition was conducted between *N*-benzylidene-benzhydrylamine *N*-oxide (0.1301 g, 0.45 mmol) and **(***E*)-4-oxo-4-(2-oxooxazolidin-3-yl)but-2-enyl acetate (0.0880 g, 0.41 mmol) in refluxing dry CH_2Cl_2 (8 ml) in the presence of $TiCl_2(i\text{-}OPr)_2$ (2M in CH_2Cl_2, 0.04 ml) and powered 4A molecular sieve (120.1 mg) under N_2 atmosphere for 9 days. The purification was realized from two times chromatography (3:1:1 hexane/EtOAc/CH_2Cl_2 and 1:1 Petroleum ether/ethyl ether) to afford the pure *exo* and *endo* adducts.



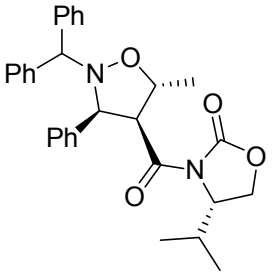
Exo adduct (**79**): white solid; 26% yield (0.0536 g); M. P. 83-86^oC ; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.21-7.02(m, 15H), 5.03(m, 1H), 4.83(s, 1H), 4.53(dd, 1H, *J*=10.0, 8.0 Hz), 4.34(broad, 1H), 4.23-4.14(m, 2H), 4.02(m, 1H), 3.61(m, 2H), 2.99(m, 1H), 1.93(s, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ171.6, 170.8, 152.8, 141.1, 139.2, 136.7, 129.5, 128.9, 128.2, 128.0, 127.9, 127.8, 127.4, 126.9, 75.7, 71.8, 68.9, 63.9, 62.2, 54.4, 43.0, 20.8. **HR-MS(ESI/APCI)** calcd for: $C_{29}H_{29}N_2O_6$ (MH^+): 501.2020, found: 501.2026



Endo adduct (**80**): white solid; 12% yield (0.0254 g); M. P. 68-71^oC ; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.37-7.00(m,15H), 5.16(s, 1H), 4.64-4.56(m, 2H), 4.46(m, 2H), 4.34-4.22(m, 3H), 3.95-3.89(m, 2H), 1.94(s, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ171.6, 170.8, 152.9, 140.9, 140.3, 139.5, 128.9, 128.5, 128.4, 128.1, 128.0, 127.7, 127.6, 127.2,

127.1, 79.5, 71.8, 71.1, 63.9, 61.9, 57.6, 43.0, 20.7. **HR-MS(ESI/APCI)** calcd for: $C_{29}H_{29}N_2O_6$ (MH^+): 501.2020, found: 501.2024

Preparation of (4*S*)-3-(2-benzhydryl-5-methyl-3-phenylisoxazolidine-4-carbonyl)-4-isopropyloxazolidin-2-one (*exo* adduct) (88)

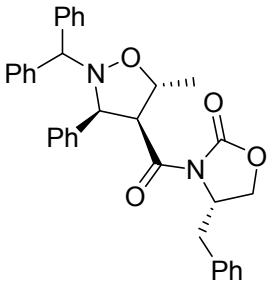


This 1, 3 dipolar cycloaddition was conducted between *N*-benzylidene-benzhydrylamine *N*-oxide (0.2834 g, 0.99 mmol) and **(***S*,*E*)-3-but-2-enoyl-4-isopropyloxazolidin-2-one (0.2356 g, 1.20 mmol) in refluxing dry CH_2Cl_2 (5 ml) in the presence of $TiCl_2$(i-OPr)$_2$ (2M in CH_2Cl_2, 0.12 ml) and powered 4A molecular sieve (215.6 mg) under N_2 atmosphere for 9 days. The chromatography (5:1 hexane/EtOAc) afforded the pure *exo* adduct as white solid (0.2770 g, 58% yield). M.P. 82-85^{o}C; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.37-7.01(m, 15H), 4.97(m, 1H), 4.86(s, 1H), 4.56(d, 1H, *J*=10.8 Hz), 4.16-3.90(m, 4H), 1.53(m, 1H), 1.27(d, 3H, *J*=6.0 Hz), 0.61(d, 3H, *J*=6.8 Hz), -0.01(d, 3H, *J*=6.8 Hz); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ169.3, 153.4, 141.0, 140.3, 130.0, 129.1, 128.4, 128.0, 127.9, 127.7, 127.6, 127.1, 126.8, 75.2, 72.7, 70.3, 63.3, 59.8, 58.6, 28.3, 18.1, 13.9. **HR-MS(ESI/APCI)** calcd for: $C_{30}H_{33}N_2O_4$ (MH^+): 485.2435, found: 485.2444

Preparation of (4*S*)-3-(2-benzhydryl-5-methyl-3-phenylisoxazolidine-4-carbonyl)-4-benzyloxazolidin-2-one (*exo* adduct) (89)

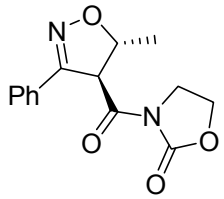
This 1, 3 dipolar cycloaddition was conducted between *N*-benzylidene-benzhydrylamine *N*-oxide (0.2543 g, 0.89 mmol) and (*S*,*E*)-4-benzyl-3-but-2-enoyloxazolidin-2-one (0.2636 g, 1.08 mmol) in refluxing dry CH_2Cl_2 (5 ml) in the presence of $TiCl_2$(i-OPr)$_2$

(2M in CH_2Cl_2, 0.11 ml) and powered 4A molecular sieve (216.3 mg) under N_2 atmosphere for 11 days. The chromatography (5:1 hexane/EtOAc) afforded the pure *exo* adduct as white solid (0.1922 g, 41% yield). M.P. 183-186^oC; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.35-6.90(m, 20H), 4.95(m, 1H), 4.90(s, 1H), 4.51(d, 1H, *J*=10.8 Hz), 4.22(m, 1H), 4.04(dd, 1H, *J*=10.8, 9.2 Hz), 3.87(t, 1H, *J*=8.8 Hz), 3.72(dd, 1H, *J*=8.8 ,2.8 Hz), 2.24(dd, 1H, *J*=13.6, 3.2 Hz), 1.24(d, 3H, *J*=6.0 Hz), 1.10(dd, 1H, J=13.6, 11.6 Hz); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ169.4, 152.8, 140.7, 140.6, 135.8, 129.4, 129.0, 128.9, 128.8, 128.7, 128.0, 127.9, 127.7, 127.4, 127.1, 127.0, 74.7, 73.3, 70.1, 66.2, 60.2, 54.8, 36.1, 17.8. **HR-MS(ESI/APCI)** calcd for: $C_{34}H_{33}N_2O_4$ (MH^+): 533.2435, found: 533.2453



***trans*-5-methyl-3-phenyl-4,5-dihydroisoxazole-4-carbonyl)oxazolidin-2-one (81)**

3-(2-benzhydryl-5-methyl-3-phenylisoxazolidine-4-carbonyl)oxazolidin-2-one (*exo* adduct) (0.0542 g, 0.12 mmol) was stirred with *N*-bromosuccinimide (0.0272 g, 0.15 mmol) in a mixuture of CH_2Cl_2/H_2O (2 ml/0.2 ml) at room temperature for 24 hours. The reaction solution was quenched by aqueous $NaHSO_3$ and extracted with CH_2Cl_2 (3x10 ml). The combined organic layers were dried by $NaSO_4$ and concentrated in *vacuo*. The resulting residue was stirred with *p*-$TsOH{\cdot}H_2O$ (0.0272 g, 0.14 mmol) in CH_3CN/H_2O (1.5 ml/1.5 ml) overnight. The reaction solution was quenched by with Et_3N. After the evaporation of CH_3CN in vacuo, followed by the addition of H_2O (10 ml), the solution was extracted by



CH_2Cl_2 (3x10 ml). The combined organic layers were dried by $NaSO_4$ and concentrated under reduced pressure. The residue was purified by radial chromatography on silica gel (hexane/EtOAc 1:1) to provide the product (0.0159 g, 47% yield) along with a small amount of the *cis* isomer as a result of epimerization at position 4 of the isoxazole. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.50(m, 2H), 7.31(m, 3H), 5.33(d, 1H, *J*=4.0 Hz), 4.84(m, 1H), 4.42(m, 2H), 3.96(m, 2H), 1.44(d, 3H, *J*=6.0 Hz); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 169.1, 154.4, 153.7, 130.2, 128.9, 126.8, 83.0, 62.4, 53.1, 42.7, 20.8. **HR-MS (ESI/APCI)** calcd for: $C_{14}H_{15}N_2O_4$ (MH^+): 275.1026, found: 275.1025

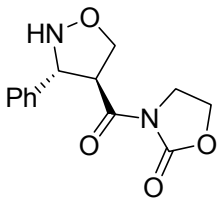
General procedure for deprotection

A 100 ml flask was charged with diphenylmethine protected isoxazolidine, dry CH_2Cl_2/TFA (4:1) and tiethylsilane (2.5 eq). The flask was flushed by N_2 flow and equipped with a reflux condenser fitted with a rubber septum and a balloon. The reaction mixture was refluxed for 2.5 hours and was cooled to room temperature. The flask was then placed into an ice bath and was dropwise added saturated $NaHCO_3$ solution until the pH became basic. The solution was extracted by CH_2Cl_2 (3x30 ml) and the combined organic layers were dried by Na_2SO_4, filtered and concentrated under reduced pressure. The chromatography purification (2:1 Hexane/EtOAc then 1:1 Hexane/EtOAc) provided the *N*-unsubstituted isoxazolidine.

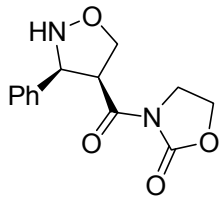
3-(5-methyl-3-phenylisoxazolidine-4-carbonyl)oxazolidin-2-one (deprotected *exo* adduct) (82): 3-(2-benzhydryl-5-methyl-3-phenylisoxazolidine-4-carbonyl)oxazolidin-2-one (*exo* adduct) (0.0807 g, 0.18 mmol) was stirred with triethylsilane(0.0610 g, 0.53 mmol) and TFA (2 ml) in reflux CH_2Cl_2 (8 ml) for 2.5 hours; White solid; 93% yield (0.0467 g); M. P. 106-109°C; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.28-7.23(m, 2H), 7.21-7.17(m, 3H), 4.91(d, 1H, *J*=9.6 Hz), 4.74(m, 1H), 4.43(dd, 1H, *J*=9.6, 7.2 Hz), 4.09(m, 1H), 3.67(m, 2H), 3.09(m, 1H), 1.37(d, 3H, *J*=6.0 Hz); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ171.2, 152.9, 134.9(small), 128.5, 128.4, 127.6, 81.6(small), 68.2, 61.9, 59.5, 42.3, 18.5. **HR-MS(ESI/APCI)** calcd for: $C_{14}H_{17}N_2O_4$ (MH^+): 277.1183, found: 277.1174

3-(5-methyl-3-phenylisoxazolidine-4-carbonyl)oxazolidin-2-one (deprotected *endo* adduct) (83): 3-(2-benzhydryl-5-methyl-3-phenylisoxazolidine-4-carbonyl)oxazolidin-2-one (*endo* adduct) (0.1146 g, 0.26 mmol) was stirred with triethylsilane(0.0810 g, 0.70 mmol) and TFA (2 ml) in reflux CH_2Cl_2 (8 ml) for 2.5 hours; White solid; 83% yield (0.0593 g); M. P. 131-134°C ; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.39(d, 1H, *J*=7.2 Hz), 7.27(m, 2H), 7.20(m, 1H), 4.76(d, 1H, *J*=3.6 Hz), 4.68(dd, 1H, *J*=5.2, 3.6 Hz), 4.37(m, 2H), 4.27(m, 1H), 4.02(m, 2H), 1.29(d, 3H, *J*=6.0 Hz); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ173.7, 153.1, 139.3, 128.7, 127.7, 127.0, 84.4, 69.1, 62.0, 59.7, 43.0, 19.2. **HR-MS(ESI/APCI)** calcd for: $C_{14}H_{17}N_2O_4$ (MH^+): 277.1183, found: 277.1181

3-(3-phenylisoxazolidine-4-carbonyl)oxazolidin-2-one (deprotected *endo* adduct) (85): 3-(2-benzhydryl-3-phenylisoxazolidine-4-carbonyl)oxazolidin-2-one (*endo* adduct) (0.0556 g, 0.13 mmol) was stirred with triethylsilane(0.0456 g, 0.39 mmol) and TFA (1.5 ml) in reflux CH_2Cl_2 (6 ml) for 2.5 hours; White solid; 87% yield (0.0295 g); M. P. 144-146^oC ; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.39-7.18(m, 5H), 4.87(d, 1H, *J*=4.0 Hz), 4.60(m, 1H), 4.40-4.28(m, 3H), 4.02-3.93(m, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ172.8(small), 153.3, 128.8, 127.9, 127.5, 127.0, 74.0, 66.4, 62.3, 55.3, 42.7. **HR-MS(ESI/APCI)** calcd for: $C_{13}H_{15}N_2O_4$ (MH^+): 263.1026, found: 263.1034



3-(3-phenylisoxazolidine-4-carbonyl)oxazolidin-2-one (deprotected *exo* adduct) (84): 3-(2-benzhydryl-3-phenylisoxazolidine-4-carbonyl)oxazolidin-2-one (92% *exo* adduct and 8% *endo* adduct) (0.0603 g, 0.14 mmol) was stirred with triethylsilane(0.0467 g, 0.40 mmol) and TFA (1.5 ml) in reflux CH_2Cl_2 (6 ml) for 2.5 hours to give predominant deprotected *exo* adduct with small amount of deprotected *endo* adduct (0.0372 g, quantative yield). Deprotected *exo* adduct: **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.25-7.16(m, 5H), 5.00(m, 1H), 4.79(d, 1H, *J*=8.8 Hz), 4.50(m, 1H), 4.13-4.06(m, 2H), 3.76-3.61(m, 2H), 3.05(m, 1H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ171.1(small), 153.0, 128.7, 128.4, 128.2, 127.5, 72.9, 67.6, 62.0, 55.2, 42.4. **HR-MS(ESI/APCI)** calcd for: $C_{13}H_{15}N_2O_4$ (MH^+): 263.1026, found: 263.1036



(4*S*)-4-isopropyl-3-(5-methyl-3-phenylisoxazolidine-4-carbonyl)oxazolidin-2-one (deprotected *exo* adduct) (91): (4*S*)-3-(2-benzhydryl-5-methyl-3-phenylisoxazolidine-4-carbonyl)-4-isopropyloxazolidin-2-one (*exo* adduct) (0.0896 g, 0.19 mmol) was stirred with triethylsilane(0.0596 g, 0.51 mmol) and TFA (2 ml) in reflux CH_2Cl_2 (8 ml) for 3 hours; White solid; 81% yield (0.0479 g); M. P. 120-123^oC; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ7.25(m, 5H), 5.00(d, 1H, *J*=9.6 Hz), 4.70(m, 1H), 4.32(dd, 1H, *J*=9.6, 8.0 Hz), 4.19(m, 1H), 4.12(dd, 1H, *J*=8.8, 8.4 Hz), 4.01(dd, 1H, *J*=8.8, 2.4 Hz), 1.59(m, 1H), 1.39(d, 3H, *J*=6.0 Hz), 0.64(d, 3H, *J*=6.8 Hz), 0.00(d, 3H, *J*=6.8 Hz); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ171.0, 153.5, 135.0, 128.7, 128.6, 128.5 128.3, 83.0, 68.5, 63.2, 59.0, 58.7, 28.3, 18.6, 18.0, 13.7. **HR-MS(ESI/APCI)** calcd for: $C_{17}H_{23}N_2O_4$ (MH^+): 319.1652, found: 319.1662

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