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Syntheses of aza analogs of kainoids

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

SYNTHESES OF AZA ANALOGS OF KAINOIDS

A dissertation submitted in partial fulfillment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

by

Mingping Di

2006

To: Interim Dean Mark Szuchman
College of Arts and Sciences

This dissertation, written by Mingping Di, and entitled Syntheses of Aza Analogs of Kainoids, 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.

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Florida International University, 2006

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DEDICATION

I dedicate this dissertation to my parents and my brothers, who have supported me in all my crazy endeavors; and most importantly, my wife, who, since I married her, has always supported me with unconditional love.

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ABSTRACT OF THE DISSERTATION
SYNTHESES OF AZA ANALOGS OF KAINOIDS

by

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Florida International University, 2006

Miami, Florida

Professor Kathleen S. Rein, Major Professor

The kainoids are a class of non-proteinogenic pyrrolidine dicarboxylates that exhibit both excitatory and excitotoxic activities. These activities are a result of the ability of the kainoids to act as glutamate receptor agonists by activating ionotropic glutamate receptors. The parent of this group of compounds is α -kainic acid. Kainic acid is isolated from the seaweed *Digenea simplex* and has been used in Asian countries as a treatment for intestinal worms in children. In addition it is used extensively by neuropharmacologists for the study of glutamate receptors. Several years ago, the world's sole supplier of kainic acid discontinued this product. Since that time, other sources have appeared, however, the price of kainic acid remains significantly higher than it once was. We have thus been working on synthesizing aza analogs of kainoids which would be less costly but potentially potent alternatives to kainic acid via the dipolar cycloadditions of diazoalkanes with *trans* diethyl glutaconate. These 1, 3-dipolar cycloadditions yielded 2-pyrazolines or pyrazoles. The 2-pyrazolines may be precursors to aza analogs of kainoids. The regioselectivity of these 1, 3-dipolar cycloadditions and isomerization of the 1-pyrazolines to 2-pyrazolines was evaluated. Reductions of the 2-pyrazolines yielded aza analogs of kainoids.

TMS diazomethane, due to the commercial availability, has been frequently used as a synthetic reagent in 1, 3-dipolar cycloadditions, particularly in the preparation of novel amino acid analogs. A survey of the recent literature indicates that the regioselectivity of the double bond isomerization of TMS substituted 1-pyrazolines is variable and at first glance, unpredictable. In an effort to develop a mechanistic rationale for the isomerization which could account for the products obtained, a systematic survey of dipolar cycloadditions between TMS diazomethane and α , β -unsaturated dipolarophiles was undertaken. It was suggested that the steric demand of the dipolarophiles had a profound effect on both the relative stereochemistry of dipolar cycloaddition reactions of TMSCHN_2 and the preferred direction of isomerization of the resulting 1-pyrazoline.

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1. Introduction

1.1 Ionotropic Glutamate Receptors (iGluRs)

The acidic amino acid (*S*)-glutamic acid (Glu) **1** (Figure 1) is a major excitatory neurotransmitter in the mammalian central nervous system (CNS), where it plays an important physiological role in mediating synaptic plasticity and processes such as learning and memory. Glu exerts strong excitatory action on most neurons in the CNS. It was shown to have powerful excitatory effects on neurons more than forty years ago.¹ Besides having a key role in excitatory transmission, Glu has also been shown to be implicated in neurodegenerative processes.² This was originally based on the observations developed by Lucas and Newhouse³ that administering glutamate systemically to mice induced degeneration of retinal neurons and has been well documented over the last forty years through numerous *in vitro* and *in vivo* experiments.⁴ Strong support has been provided for the involvement of at least partly a cytotoxic action of Glu in acute neurologic diseases such as ischemia, stroke, trauma and hypoglycemia. Glu may also contribute to certain chronic neurodegenerative diseases such as Huntington's chorea and Alzheimer's disease,⁵ although the actual mechanisms remain to be characterized.²

Glu acts by binding to glutamate receptors, which can be divided into two types: metabotropic glutamate receptors (mGluRs) and ionotropic glutamate receptors (iGluRs). The mGluRs belong to the superfamily of G-protein coupled receptors and comprise mGluR1-8.^{6,7} The iGluRs are cation-selective ligand-gated ion channels, regulating the flux of cations (Na^+ , K^+ , and Ca^{2+}) across the synaptic membrane. They have been classified into three classes: NMDA, AMPA and KA receptors based on their selective

interaction with the agonists *N*-methyl-D-aspartic acid **2** (Figure 1), 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid **3** (Figure 1) and kainic acid **4** (Figure 1), respectively.^{7, 8} NMDA receptors are distinct from AMPA and KA receptors due to the presence of potent and selective NMDA receptor antagonists. The difference between AMPA receptors and KA receptors is less distinct than those distinguishing NMDA receptors from AMPA and KA receptors, thus the latter two subtypes were often collectively referred to as non-NMDA receptors.⁹ While NMDA shows highly selective affinity for NMDA receptors, KA also has a moderate affinity for AMPA receptors in addition to its high affinity for KA receptors.¹⁰⁻¹² Therefore, the descriptive names for Glu receptor subtypes (AMPA and KA receptors) may be rather misleading due to a nonexisting specificity, and alternative names have been proposed.¹³ However, none of them have been widely accepted, and the aforementioned names are still predominantly used.⁹

Based on our knowledge of the iGluRs,¹⁴⁻¹⁸ the NMDA receptors gate an ion channel that is highly permeable to Ca^{2+} but also allows passage of Na^{+} and K^{+} . They show a voltage dependent block by Mg^{2+} and Zn^{2+} and are modulated by glycine, a coagonist of these receptors. NMDA receptors are widely distributed in the mammalian CNS and enriched in the cerebral cortex and hippocampus. They have been shown to be implicated in long-term potentiation (LTP), a form of synaptic plasticity in which NMDA receptors activation triggers a long-lasting increase in synaptic efficacy. This long-term potentiation can last for weeks and plays an important role in some forms of memory and learning processes mediated by the hippocampus which is believed to control the consolidation of short-term memory into long-term memory. Both the AMPA receptors

and kainate receptors gate an ion channel that is selective for monovalent cations Na^+ and K^+ , and also for some receptors Ca^{2+} . They mediate fast excitatory synaptic transmission in the CNS and are rapidly activated and desensitized by glutamate. Kainate receptors have been demonstrated to be involved in the induction of LTP at synapses where NMDA receptors do not have a role, in particular at mossy fiber synapses in the hippocampus.¹⁹

Figure 1. Multiplicity of iGluRs and structure of selected agonists.

The cloning of a number of iGluR subunits supported the aforementioned classification of iGluRs which is originally derived primarily from agonist pharmacology, but in the meantime brought about a subdivision of the three classes of iGluRs.²⁰ The iGluR receptor subunits can be divided into six groups (Figure 1). One group, containing the iGluR1-iGluR4 subunits, forms the AMPA receptors. The low affinity KA receptor subunits, iGluR5-iGluR7, and the high affinity KA receptor subunits, KA1 and KA2, comprise the KA receptors, whereas the NMDA receptor subunits are divided into three groups-the NR1, NR2A-NR2D, and NR3A, B.²¹ Each of these receptor subunits is membrane-anchored and is believed to contain a large extracellular N-terminal domain,

three membrane-spanning domains, and an ion channel-forming reentrant loop.⁷ It is believed that these subunits combine with other subunits from the same class to form pentameric or more likely tetrameric receptor ion channel complexes through a homomeric and/or heteromeric assembly.²²⁻²⁴

Glutamate receptors are thought to convey most of the fast excitatory transmission in the mammalian CNS and to be crucial for induction and maintenance of various forms of synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD).^{25, 26} In addition, glutamate receptors are thought to be crucially involved in neuronal degeneration associated with acute brain insults such as cerebral ischemia /hypoxia, hypoglycemia, cerebral trauma and epilepsy^{27, 28} and certain slowly progressing neurodegenerative diseases such as Huntington's chorea, Parkinson's disease, Alzheimer's disease.^{5, 27, 29} It is now generally agreed that all subtypes of the GluRs are potential targets for therapeutic intervention in a number of diseases. Neuronal damage, a phenomenon referred to as excitotoxicity^{29, 30} can be caused by excessive activation of glutamate receptors. Depending on the type of brain pathology, a pathophysiological overstimulation of glutamate receptors can be induced by an enhanced release of excitatory amino acid neurotransmitters such as Glu, increased levels of excitotoxins in the cerebral parenchyma, an impaired neurotransmitter uptake or metabolism, an imbalance between excitatory and inhibitory stimuli or an enhanced efficacy of the glutamatergic neurotransmission.^{27, 31} Prolonged glutamate receptor activation can result in a serious disturbance in the cellular signaling system which is mainly caused by uncontrolled transmembrane ion fluxes through glutamate receptor-mediated ionophores. In particular, it has been shown that the entry of Ca^{2+} is crucially implicated in glutamate-

induced neuronal injury and death.^{32,33} Among the iGluRs, NMDA receptors have received extensive attention as they can directly mediate abnormal rises in intracellular Ca^{2+} that may induce degenerative process. However, an involvement of non-NMDA receptors in pathological processes has also been demonstrated.³⁴⁻³⁷ For example, historically, kainate receptors have long been associated with epilepsy.

Kainate receptor subunit proteins are widely expressed in excitatory and inhibitory neurons throughout the brain.³⁸⁻⁴⁰ Kainate receptors are targeted to a variety of presynaptic and postsynaptic locations often within the same neuron. At these locations, they perform specific tasks associated with the regulation of presynaptic function of glutamate transmission and transmission mediated by other transmitters, most notably γ -aminobutyrate (GABA), postsynaptic neurotransmission, or regulation of membrane excitability.⁴¹⁻⁴⁴ Kainate receptors have been clearly established as major players in excitatory transmission. It has been demonstrated that kainate receptors at mossy fiber-CA3 synapses play an important role in frequency-dependent synaptic facilitation, a form of short-term plasticity in which the strength of transmission increases with repetitive stimulation.^{45,46} Kainate receptors have also been shown to be required for long-term potentiation (LTP) between mossy fibers and CA3 pyramidal cell.^{19,45} The pharmacological characterization of kainate receptors has been hampered by a lack of selective ligands. Typically, natural products such as kainic acid (Figure 1, **4**) and domoic acid (Figure 2, **13**) were used as reference standard agonists, although they also show interaction with AMPA receptors. KA (Figure 1, **4**) and domoic acid (Figure 2, **13**) are two naturally occurring pyrrolidine dicarboxylates in which the amino groups are incorporated into pyrrolidine rings, making them the conformationally restricted

glutamate analogs. They are not only potent kainate receptor agonists but are also known to have highly neurotoxic effects and have therefore been extensively used as pharmacological tools.⁴⁷

1.2 Kainoids

(-)- α -Kainic acid (Figure 1, **4**) is the parent of a well-known group of naturally occurring and synthetic nonproteinogenic pyrrolidine dicarboxylic acids known as the kainoids which have general structure **5** (Figure 2). They all have three asymmetric centers at carbons 2, 3, 4 of the pyrrolidine ring. They vary in the nature of the C-4 substituent generally containing π -unsaturation, and the absolute configuration at this position. This variation gives rise to various members of the kainoid family. The parent member, (-)- α -Kainic acid (Figure 1, **4**, originally known as digenic acid) was first isolated in 1953 from the Japanese marine algae *Digenea simplex*⁴⁸ along with its C-4 epimer (+)-allokainic acid (Figure 2, **6**). Since then it has been found to occur in the related algae *Centrocerus clavulatum*⁴⁹ and in the Corsican moss, *Alsidium helminthocorton*.^{50, 51}

(-)-Domoic acid (Figure 2, **13**) was originally isolated in 1958 from another Japanese marine algae – the warm water algae *Chondria armata*.⁵² Since then it has been found in the phytoplankton *Nitzschia pungens*^{53, 54} and the algae *Alsidium corallinum*⁵⁰. A number of kainoids related to domoic acid have also been isolated from the same algae, which include the isodomoic acids A-F (Figure 2, **14-19**),^{55, 56} the C-5' domoic acid diastereomer (Figure 2, **20**)⁵⁷ and the domoilactones A and B (Figure 2, **21a** and **21b**).⁵⁸ The acromelic acids A and B (Figure 2, **8** and **9**), containing a functionalized 2-pyridone

as the C-4 substituent, were found in a quite different organism to the previously

described kainoids, namely the poisonous Japanese mushroom *Clitocybe acromelalga*.^{59,}

⁶⁰ They were first isolated in sub-milligram quantities in 1983. Since their isolation, a

Figure 2. Representative synthetic and naturally occurring kainoids

number of other kainoids have been isolated including the acromelic acids C, D and E (Figure 2, **10-12**) which have been shown to be minor constituents of *Clitocybe acromelalga*,^{61, 62}

Interest in the kainoids arose from their pronounced insecticidal, anthelmintic and principally neuroexcitatory properties. The ability of the kainoids to be insecticides has long been used in Japan.⁵⁵ An extract of red algae, from which kainic acid and domoic acid have been isolated, was used for its fly-killing properties. The algae *Digenea simplex* has been used for its anthelmintic properties for more than a thousand years in Japan.⁶³ The active component, (-)- α -Kainic acid (Figure 1, **4**) has been shown to have a strong anthelmintic effect, about 10 times that of santonin without side effects.⁶³ Similar properties have been reported for (-)-domoic acid (Figure 2, **13**).

The pronounced neuroexcitatory properties of the kainoids have been well documented and this class of molecule is now inextricably associated with neuroscience research.^{18, 64-66} The kainoids have been shown to selectively block neuronal processes and thus are valuable tools in the study of neurofunctioning. The neuronal degeneration caused by the kainoids have been shown to closely resemble that observed in patients suffering from neuronal diseases such as epilepsy⁶⁷ and Huntington's chorea⁶⁸. In addition, it is possible that neuronal death caused by kainoids is a good experimental model for neuronal cell loss in senile dementia.⁶⁴ The potent neuroexcitatory activity of the kainoids can be attributed to their action as conformationally "locked" analogues of the neurotransmitter L-glutamic acid (Figure 3), causing neuronal death in glutaminergic system found in the brain. Numerous structure-activity investigations of the kainoids and their analogues have been carried out.^{9, 69-74} Based on these results, it has been shown that

the nature of C-4 substituent, its conformation and stereochemistry play a critical role in binding and functional activation at the recognition site. The activity of the kainoids is effectively lost if the absolute configuration of any one the three stereocenters is inverted.¹⁴ Further, a single study suggests that the activity of the unsubstituted kainoid, 2-carboxy-3-pyrrolidine-3-acetic acid (CPAA) (Figure 2, 7) is significantly less than that of kainic acid.⁷⁵ (-)-Domoic acid (Figure 2, 13) has been shown to be more neuroexcitatory than (-)- α -kainic acid (Figure 1, 4). It has been identified as the toxin in paralytic shellfish poisons (PSPs) and was determined to be responsible for an outbreak

Figure 3. Structural similarity of kainoids and (S)-glutamic acid

of mussel poisoning in Canada in 1987.^{76, 77} During the incident, over a hundred people were hospitalized as a result of the consumption of mussels contaminated with domoic acid (Figure 2, 13). Among them, three people died and many of the survivors of this incident continue to suffer severe dementia and memory loss. The contamination of the mussel was thought to occur by ingestion of domoic acid by the mussels through its main food source, a diatom, *Nitzschia pungens*, a primary producer of the toxin, which had formed a dense bloom near the mussel bed. In the U. S., a domoic acid incident occurred in Monterey Bay in 1991, and spread as far north as Oregon and Washington.^{78, 79}

Fortunately, due to extensive monitoring of shellfish, no human exposures were reported. However, with increased international commerce, contaminated seafood products have the potential for rapid widescale distribution.

Of the naturally occurring kainoids, the acromelic acids have been shown to have the most potent neuroexcitatory activity. The acromelic acid B (Figure 2, **9**) was shown to be slightly less potent than acromelic acid A (Figure 2, **8**)^{80, 81} and both were shown to be more potent than domoic acid. Acromelic acid A and B (Figure 2, **8** and **9**) were reported to be lethally toxic to mice at a dose of 7 and 8mg/kg respectively. Acromelic acid C (Figure 2, **10**) was shown to have similar lethal dose of 10mg/kg.⁶¹ Acromelic acid D (Figure 2, **11**) was shown to be a neuroexcitant as potent as kainic acid (Figure 1, **4**).⁸² Synthetic acromelic analogues such as **22-24** (Figure 4) have also been tested for neuroexcitatory activity and the phenyl derivative **22** (Figure 4) showed activity comparable to kainic acid (Figure 1, **4**).⁸³⁻⁸⁶ The phenol derivative **23** (Figure 4) was shown to be more potent than acromelic acid B (Figure 2, **9**) or domoic acid (Figure 2, **13**). The methoxyphenyl derivative **24** (Figure 4) was 3- to 5- fold more potent than acromelic acid A (Figure 2, **8**).

1.3 Crystal structures of iGluRs ligand binding cores: Molecular mechanisms underlying ligand-binding affinity and specificity and iGluR gating properties

iGluRs are modular proteins which are formed by distinct ligand-binding and channel-forming domains.^{87, 88} They have a similar overall structure in which four homologous subunits surround a central cation-selective pore. A clam shell-like ligand-binding core contains the agonist and antagonist binding sites^{89, 90} and is connected to the

three transmembrane domains (M1, M3, M4) and a reentrant loop (M2) that form the ion channel (Figure 5). Eukaryotic iGluRs contain an amino-terminal domain (ATD) that does not participate in ligand binding,⁹¹ but plays an important role in subtype-specific assembly⁹² and mediation of intersubunit interactions.⁹³ The ≈ 120 -aa between the end of the ATD and M1 (termed S1) form one half of the ligand binding core, and the ≈ 140 -aa

22 R = H

23 R = OH

24 R = OMe

Figure 4. Synthetic acromelic acid analogues

between M3 and M4 (S2) is the second half.^{89, 91} The structural elements required by the wild-type iGluR receptors to respond to agonists and antagonists are contained in the S1S2 domains.⁹¹ In the cases of AMPA, kainate, and NMDA receptors, S1S2 domain can be expressed as a water-soluble construct that is independent of the transmembrane domains and retains wild-type ligand binding affinities.^{89, 94, 95} The S1S2 constructs that have been expressed include the ligand-binding core of the AMPA receptor GluR2 (GluR2-S1S2J)⁹⁶, of the NMDA receptor NR1⁹⁷ and of the kainate receptor GluR5 and GluR6.⁹⁸⁻¹⁰⁰ The successful X-ray crystallographic resolution of structures of these constructs in complex with different agonists and antagonists have provided a wealth of information about binding modes and mechanism of action of iGluRs.^{24, 96, 98, 101, 102}

Figure 5. A. iGluR topology, showing the S1 and S2 segments in turquoise and pink, respectively. 'cut' and 'linker' represent the edges of the S1S2 construct. The ligand binding domain comprising S1 and S2 can be expressed as a soluble polypeptide, excluding the amino terminal domain and transmembrane domains M1-M4. SP, signal peptide. B. Ribbon representation of the structure of GluR2-S1S2 in complex with kainate (black ball-and-stick representation) (pdb code 1GR2). Arg 485 from helix D, Ser 654 and Thr 655 from helix F, and Thr 686 and Glu 705 from helices H and I either interact with kainate or are involved in interdomain contacts. TM, transmembrane domain. These models are modified from Nanao et al.¹⁰⁰ and Armstrong et al.⁹⁰.

1.3.1 AMPA receptor

High resolution structures of GluR2 S1S2 in the *apo* state and in the presence of glutamate (Figure 1, **1**) AMPA (Figure 1, **3**), kainate (Figure 1, **4**) and the antagonist 6,7-dinitro-2,3-quinoxalinedione (DNQX) (Figure 6, **25**) have been determined.⁹⁶

Comparison of these five structures has revealed reasonable mechanisms underlying receptor activation, antagonism and the molecular basis for ligand specificity.^{90, 96}

In the *apo* state or antagonist-bound state, the ion channel is closed. This closed channel state has a ligand binding core containing separated domains 1 and 2 and an open receptor cleft. The open-cleft conformation in the *apo* state is stabilized via intradomain interactions. The antagonist DNQX bound state possesses the open-cleft conformation stabilized via antagonist-receptor interactions.

Figure 6. Agonist (**26**), antagonist (**25**) and allosteric modulator (**27**)

Interestingly, the receptor binding sites for the α -substituents of glutamate (Figure 1, **1**), AMPA (Figure 1, **3**), and kainate (Figure 1, **4**) are essentially the same. α -Carboxyl and α -amino group directly make contact with the ligand binding core through seven ion pair and hydrogen bonding interactions to domains 1 and 2. α -Carboxyl group forms essential interaction with the guanidinium group of Arg-485(domain1), the main-chain NH group of Thr 480 (domain 1) and Ser 654 (domain2). Arg 485 is conserved in all glutamate receptors. The α -amino group is bound to domain 1 and 2 by interactions with a carboxylate oxygen of Glu 705, the carbonyl oxygen of Pro 478 and the sidechain hydroxyl of Thr 480.

In contrast, the binding sites for the anionic position attached to γ carbons of these three agonists are distinct. The ' γ ' substituent of AMPA (Figure 1, **3**), isoxazole ring, binds to different sites from the γ -carbonyl of kainate and glutamate. It interacts with a water molecule (#4) which forms interactions with an α -carbonyl oxygen, and residues at the base of helix F. The inclusion of this water molecule by AMPA converts AMPA into a good bioisosteric mimic of glutamate. The isoxazole nitrogen forms a hydrogen bond with the backbone NH of Glu-705.

The glutamate and kainate binding clefts mainly differ in the position of Leu-650, which moves substantially further into the cleft in the glutamate-bound state and thus would sterically clash with the kainate isopropenyl group when kainate structure is superimposed with the glutamate-bound structure. When this leucine is replaced by a valine or a threonine in GluR1, the affinity of the resulting mutants for kainate is significantly increased, with the EC_{50} decreased by 6- and 20-fold respectively.¹⁰³ This can be attributed to the adoption of a glutamate-like conformation by the ligand binding core in complex with kainate (Figure 1, **4**) after Leu 650 is replaced by valine or threonine.

The extent of the domain closure the ligand can induce appears to have a close correlation with ligand efficacy. The full agonists of AMPA receptors, such as glutamate (Figure 1, **1**) and AMPA (Figure 1, **3**) induce the same greatest degree of domain closure ($\sim 20^\circ$) relative to the unligated (*apo*) form. In contrast, kainate (Figure 1, **4**), a partial agonist at AMPA receptors,^{104, 105} induces only $\sim 12^\circ$ of domain closure, whereas in the antagonist DNQX bound state, a small degree of domain closure ($\sim 2.5^\circ$) is induced. It has thus been proposed that substantial closure of the ligand binding cleft results in

activation (opening) of the ion channel gate. The intermediate domain closure induced by the partial agonist results in an intermediate conformational change of the receptor at the transmembrane gate, and thus partially activates the ion channel. Antagonists, stabilizing the ligand binding core in an open-cleft conformation, induce no or very small conformational change of the receptor, precluding the opening of the ion channel gate.

1.3.2 Kainate receptor

Among the ionotropic glutamate receptors, the kainate receptor is the last one for which the crystal structures of the receptor ligand binding cores have become available. Prior to this, the GluR2-S1S2 structure had been used as a model in the study of kainate receptors, since the primary sequences of the agonist-binding domains of AMPA and kainate receptor subunit are similar (48-55%).^{106, 107} However, only limited inferences can be obtained for the kainate receptor from a comparison with the GluR2 structure since the properties of AMPA and kainate receptors are different. This situation changed when crystal structures of the GluR5 and GluR6 ligand binding cores were acquired in complex with glutamate (Figure 1, **1**), kainate (Figure 1, **4**), domoic acid (Figure 2, **13**) and (2*S*, 4*R*)-4-methylglutamate (Figure 6, **26**),⁹⁸⁻¹⁰⁰ a ligand that has a 1000-fold higher affinity for kainate versus AMPA receptors.^{108, 109} This has enabled the uncovering of the modes of agonist binding of the kainate receptor, the identification of the differences in the mode of agonist binding and the intersubunit contacts between the GluR2, GluR5 and GluR6 subunits, and thus the exploration of the relevance of these structural differences to the function of AMPA and kainate receptors. The molecular mechanisms underlying receptor selectivity have to some extent been revealed.

The S1S2 domains of GluR5, GluR6, GluR2 and NR1 have essentially the same overall folding pattern, with all of them sharing a bilobate structure. However, the ligand binding pocket in GluR6 (volume $255 \pm 15 \text{ \AA}^3$) is smaller than that in GluR5 (volume $305 \pm 6 \text{ \AA}^3$), but larger than that for GluR2 (volume $218 \pm 4 \text{ \AA}^3$), due to amino acid side chain substitutions unique to the binding site of each subunit. The α -carboxyl and α -amino groups of glutamate bind to the ligand binding cores of GluR5 and GluR6 through ion pair and hydrogen bond contacts with conserved Arg and Glu side chains, and with main chain peptide bonds in both domains 1 and 2, in a way nearly identical to that observed for AMPA receptor GluR2 subunit.⁹⁶ The glutamate γ -carboxyl group binds only to domain 2 and interacts through hydrogen bonding with residues near the N termini of helices F, H, and I that are same in GluR5 and GluR6, whereas, in the GluR2 glutamate complex, all of these are solvent mediated except a pair of hydrogen bonds formed with the hydroxyl group and main chain NH group of T655 at N terminus of helix F.

Some water molecules are trapped in the kainate GluR5 and GluR6 ligand binding pocket, similar to that observed in the GluR2 glutamate complex.⁹⁶ Consistent with the larger volume of the ligand binding pocket in the kainate receptor structures, five and six water molecules are contained in the GluR6 and GluR5 glutamate complex, compared to four trapped water molecules in the GluR2 complex. One of the additional water molecules in the kainate receptors interacts via hydrogen bonding with the ligand α -amino group and together with another water molecule forming a network linking domain 2 with domain 1. It would be expected that this interdomain contact can contribute to the stability of the glutamate-bound kainate receptor (GluR5 and GluR6) complex, which is better than that of AMPA GluR2 glutamate complex as indicated by the domain closure

of ca. 26° of the kainate (GluR5 and GluR6) glutamate bound complex relative to the *apo* structure of GluR2-S1S2, being ca 6° larger than that of the GluR2-S1S2 glutamate complex.

Kainic acid (Figure 1, **4**) has a much higher affinity for Kainate receptors (K_d 56 nM for GluR6 S1S2) than that for AMPA receptors (K_d 14.5 μ M for GluR2 S1S2).^{96, 98} The underlying mechanisms for this observation has been put forth.⁹⁸ One feature that contributes to high-affinity binding of Kainate to GluR6 is that, in GluR6 kainate complex, the hydrophobic 4-isopropenyl group is completely shielded from water, which is energetically favorable compared to its environment in aqueous solution. The second mechanism involves the high degree of domain closure in GluR6 kainate complex (23.3°), which is only slightly less than that for GluR6 glutamate complex (26°) and much greater than that observed for the GluR2 kainate complex (12.3°). This permits the formation of multiple interdomain contacts between domain 1 and helix F in domain 2 in GluR6 kainate complex, which are not observed in the GluR2 kainate complex due to its smaller degree of domain closure. The higher degree of domain closure in the GluR6 kainate complex possibly results from the reduced steric hindrance between the ligand isopropenyl group and the amino acid residues within the ligand binding core. The isopropenyl group is placed between L650 and M708 in GluR2 kainate complex whereas it exists between the smaller side chains V654 and T710 in the GluR6 kainate complex, resulting in a higher degree of domain closure.

Compared to glutamate (Figure 1, **1**), (2*S*, 4*R*)-4-methylglutamate (Figure 6, **26**) derived from the introduction of a single methyl group on the C γ atom of glutamate (Figure 1, **1**) has a 170- and 100-fold higher affinity for GluR5 and GluR6 respectively,

but 14- and 28-fold lower affinity for the AMPA receptor GluR1 and GluR3.¹⁰⁹ Probable mechanisms for the high affinity of (2*S*, 4*R*)-4-methylglutamate (Figure 6, **26**) for kainate receptors have been suggested. First, (2*S*, 4*R*)-4-methylglutamate (Figure 6, **26**) binds to GluR6 in a way identical to that for glutamate (Figure 1, **1**) except that the 4-methyl group makes additional contacts with Y457 and V654 via Van der Waals contacts, providing extra binding energy. Second, the hydrophobic methyl group is shielded in the ligand binding cavity, which is energetically favorable compared to its environment in aqueous solution. Third, there are more extensive contacts between domains 1 and 2 in kainate receptor (2*S*, 4*R*)-4-methylglutamate complex than AMPA receptor (2*S*, 4*R*)-4-methylglutamate complex, which contribute to the stability of agonist complexes. Superimposition of the GluR2 glutamate and GluR6 (2*S*, 4*R*)-4-methylglutamate structures reveals that both structures are almost superimposable except that 4-methyl group has an unfavorable contact with the GluR2 L650 chain, which is substituted by the smaller valine side chain in GluR6.

1.3.3 Gating mechanism

Ligand-gated ion channels convert chemical signals into electrical impulses through opening of a transmembrane pore after binding one or more neurotransmitter molecules. The typical action of an agonist at a receptor includes activating the receptor followed by either deactivation of the receptor (agonist unbinding) or desensitization of receptor to protect the cell from overactivation. Receptor desensitization, an inactive state with agonist remaining tightly bound, is a functionally important phenomenon occurring in most ligand-gated ion channels such as iGluRs.^{8, 110}

It has been indicated that iGluR ligand binding cores are assembled as dimeric units in a tetrameric form which has local rotational 2 fold axes of symmetry and the channel-forming region of iGluRs forms a tetramer with an approximate 4-fold axis of rotational symmetry.^{24, 88, 96, 99, 100, 111} It has been shown that stabilization of the dimer interface by either mutations or allosteric modulators such as cyclothiazide (Figure 6, 27) results in the decrease of desensitization and destabilization of the dimer interface increase the desensitization.¹⁰⁰

A mechanism for the activation and desensitization of the iGluRs has been proposed (Figure 7).^{24, 96, 100, 112} When agonists bind to the receptor, it probably first interacts with domain 1, which promotes the rotation of domain 2 toward domain 1 to close the ligand binding domain and trap the agonist in the ligand-binding cleft. Upon activation, the dimer interface is still intact and the conformational stress resulted from domain closure is translated to the ion channel gate, and thus the gate is opened. It has been suggested that the interaction between adjacent domain 1 keeps the domains fixed relative to each other in the moving of the channel during gating caused by rotation of domain 2 toward domain 1. When the agonist is present for a longer time, the dimer interface rapidly destabilizes and a rearrangement of the dimer interface takes place. The consequence of this action is that no conformational changes caused by the domain closure are translated to the ion channel gate and the decoupling of domain closure from the channel gate occurs, which promotes entry to the desensitized state to protect the cell from overactivation. For the native receptor, the activation of the receptor is faster than the desensitization of the receptor because the energy barrier for receptor desensitization is higher than for receptor activation. However, the receptor is more stable in the

Figure 7. Glutamate receptor activation and desensitization model. This model is modified from Sun et al.²⁴ D1 and D2 represent domain 1 and domain 2 of the ligand-binding core, respectively. Each subunit binds a single agonist (red circle) and has three different conformations: closed, open and desensitized. Transmembrane regions of each subunit are represented by a single green cylinder and the N-terminal domain (ATD) is not shown in the model.

desensitized state than in the activated state, and thus most of the receptors enter the desensitized state after the receptor is incubated with the agonist for a prolonged duration.

The binding of agonist to iGluRs induces a complex allosteric transition which involves rearrangements of both the ligand binding core and the ion channel. It should be noted that the information about iGluR binding and gating mechanisms that has been obtained so far, some of which are described above, has stemmed from the analysis of the crystal structures of the iGluR S1S2 constructs other than the crystal structures of the

intact iGluRs which have not been successfully obtained. Clearly, crystal structures for the intact iGluRs in different states such as *apo*, agonist-bound (active or desensitized), or antagonist-bound states are needed to completely understand the iGluR binding and gating mechanisms.

1.4 Synthesis of kainoids

A considerable synthetic challenge is involved in the synthesis of kainoids. The formation of a pyrrolidine with two carboxylic acid groups with specific stereochemistry at the three contiguous chiral centers around the ring needs to be addressed. Special consideration needs to be given to the *cis* disposition of the C3 and C4 substituents contained in most of the naturally occurring kainoids. Meanwhile, an ideal synthesis should enable the introduction of various side chains at the C4 position in a convergent way to afford all the known kainoids and various kainoid analogues in the relatively high yield.

The many synthetic approaches to the kainoids can be conveniently divided into two categories: those that involve pyrrolidine ring synthesis from an acyclic precursor and those that involve the modification of an existing pyrrolidine ring. The published syntheses of kainoids are too many for a comprehensive review. A few representative examples of various approaches are presented.

1.4.1 Pyrrolidine ring synthesis from an acyclic precursor

The majority of the approaches to the construction of the kainoid ring system involve the stereoselective formation of the 3, 4-bond. Typically, the configuration at C2 of the kainate ring is derived from a readily available chiral starting material (often an

Figure 8. Intramolecular ene reaction as a key step in the synthesis of kainoids

amino acid) and it is this stereocenter that directs the formation of two new stereocenters at C3 and C4. Selected examples are described below.

A stereocontrolled intramolecular ene reaction has been used as the key step in the enantioselective synthesis of kainic acid.¹¹³⁻¹¹⁶ As shown in Figure 8, the ene cyclization reaction occurred to provide the desired trisubstituted pyrrolidine **29** under the steric control of the C2 stereocenter when the key intermediate diene **28** synthesized from L-glutamic acid was subjected to thermolysis in hot toluene.¹¹³ The pyrrolidine **29** was converted to α -kainic acid (Figure 1, **4**) in six straightforward steps.

An intramolecular hetero-Diels-Alder cyclization has been employed to synthesize α -kainic acid (Figure 9).¹¹⁷ The key intermediate **30**, originally prepared from diethyl L-tartrate and not isolated, spontaneously underwent the [4+2] cyclization to furnish the tricyclic adduct **31** with the desired stereoselectivity, which was converted to α -kainic acid (Figure 1, **4**) after several steps including ketal hydrolysis, hydrogenation, amine protection and manipulation of the 3,4-substituents.

A cobalt-mediated cyclization reaction has also been used as the key step in kainoid synthesis (Figure 10).^{118, 119} By treatment with cobaloxime (I), cyclization of the iodide **32** provided a mixture of the separable *syn*- and *anti*- pyrrolidines **33** and **34** in the ratio of 5:3 with 80% yield. Significantly, the isopropenyl group was successfully

Figure 9. Intramolecular hetero-Diels-Alder cyclisation as a key step in the synthesis of kainoids

established at the C4 position along with the formation of the pyrrolidine ring. Compound **33** was converted to α -kainic acid (Figure 1, **4**) in six additional steps while the *anti*-isomer **34** was elaborated to *allo*-kainic acid (Figure 2, **6**) in a similar way. The same methodology has also been utilized in the preparation of the domoic acid analogue and acromelic acid A (Figure 2, **8**).^{120, 121}

Figure 10. Cobalt-mediated cyclisation reaction as a key step in the synthesis of kainoids

Figure 11. Pauson-Khand reaction as a key step in the synthesis of kainoids

The Pauson-Khand reaction has been employed in the kainoid synthesis.¹²²⁻¹²⁴ By treatment with dicobalt octacarbonyl followed by trimethylamine N-oxide, the ene-yne **35** derived from glutamic acid underwent the Pauson-Khand reaction to furnish a 1.7:1 mixture of inseparable enone diastereomers **36** and **37** in a yield of 95% (Figure 11).¹²² The enone **36** was elaborated to α -kainic acid (Figure 1, **4**) after several steps.

Figure 12. Tandem Michael addition as a key step in the synthesis of kainoids

A tandem Michael addition has been employed in the kainoid synthesis.^{125, 126} As shown in Figure 12, the amine **38** derived from D-serine was reacted with the alkene, 2-nitro-3-methyl-buta-1,3-diene, generated *in situ* from the nitro compound **39** to provide the desired pyrrolidine **40** in 88% yield. The nitro group of **40** was then removed regio- and stereoselectively by a hydride transfer reaction in order to synthesize α -kainic acid (Figure 1, **4**). The same synthetic methodology has also been employed in the synthesis of acromelic acid A (Figure 2, **8**).¹²⁷

Takano and coworkers have employed a 1,3-dipolar cycloaddition reaction of an azomethine ylide to construct the pyrrolidine ring (Figure 13).¹²⁸ On heating to 310 °C in xylene, aziridine **41** was converted to the all *syn*-adduct **42** with a yield of 70%. In this case, 2, 3 and 4, 5 bonds of the pyrrolidine ring were established in the same reaction. The adduct **42** was transformed to α -kainic acid in a number steps which include

Figure 13. 1,3-Dipolar cycloaddition reaction as a key step in the synthesis of kainoids inversion of C2 stereochemistry. The same group has used a similar methodology to synthesize acromelic acid A and B (Figure 2, **8** and **9**).^{129, 130}

Yoo et al. have utilized an intramolecular Michael reaction to construct the pyrrolidine ring in the synthesis of kainoid analog CPAA (Figure 2, **7**) including seven steps in total.¹³¹ Thus the key intermediate **43**, prepared from 3-amino-1-propanol, was treated with NaOEt to give a mixture of **44** (*trans*:*cis*=95:5) in 94% yield. The *trans* isomer of **44** was converted to **7** after acid hydrolysis.

Figure 14. Intramolecular Michael reaction as a key step in the synthesis of kainoids

1.4.2 Modification of an existing pyrrolidine ring

Modification of an existing pyrrolidine ring has also been employed in kainoid synthesis. Substituents have been introduced to pre-existing rings through a variety of methods. Selected examples are described in the following.

Ohfuné and Tomita have employed a Diels-Alder reaction to introduce the C3 and C4 substituents to the pyrrolidine ring.¹³² The key intermediate **45** (Figure 15), prepared from pyroglutamic acid, was reacted with 2-(trimethylsilyl)oxy-1,3-pentadiene to give a single adduct, lactam **46** with the desired facial selectivity in a stereospecific process via [4+2] cycloaddition. The lactam **46** was elaborated to (-)-domoic acid (Figure 2, **13**) in a number of steps.

Figure 15. Diels-Alder reaction as a key step in the synthesis of kainoids

Figure 16. Intramolecular radical cyclization as a key step in the synthesis of kainoids

An intramolecular radical cyclization has been employed to introduce the C3 substituent to the pyrrolidine ring in the synthesis of acromelic acid analogs **52-55**,⁸⁴ as illustrated in Figure 16. Radical cyclization of bromoacetal **48**, prepared from *trans*-4-hydroxyl-L-proline **47**, followed by epimerization of the C2 substituent using DBU in hot toluene and lactol oxidation, afforded the bicycle **49**, which was elaborated to tosylate **50**. A displacement of tosylate **50** by various lithium diaryl cuprates with retention of configuration at C4 position followed by esterification of the C-2 carboxylic acid furnished pyrrolidine **51**, which was then elaborated to the kainoid analogs **52-55**.

A Michael addition has been used to introduce C4 substituent in the synthesis of kainoid analog CPPA (Figure 2, 7).¹³³ Shown in Figure 17, the key step is a stereocontrolled Michael addition of appropriate nucleophiles such as dimethyl sodiomalonate to the pyrrolidone **56**, affording the Michael adduct **57** in 85% yield, which was transformed to the kainoid analog CPAA (Figure 2, 7) in several steps including decarboxylation, reduction, deprotection, Jones oxidation and basic hydrolysis.

1.5 Objective of research

Kainic acid (Figure 1, 4) is a research tool mostly used by neuroscientists for the study of glutamate receptors such as kainate receptors. The study of kainate receptors helps researchers to understand Alzheimer's disease, epilepsy, and other brain disorders. Kainic acid (Figure 1, 4) is extracted from a red seaweed of the genus *Digenea*, which can be found in many tropical and subtropical waters. The alga containing the highest concentrations of kainic acid is *Digenea simplex*, known to grow only in disputed waters in the South China Sea. It is claimed that in some *Digenea*, the concentration can be as

high as 276mg per 100g of dry weight. The use of *Digenea simplex* is practically essential to achieve the commercially viable extraction of pure kainic acid. All efforts to synthesize this compound in a commercially viable way have failed. For a while, the only source was a Taiwanese company that extracted kainic acid from *Digenea simplex*.

Figure 17. Michael addition as a key step in the synthesis of kainoids

However, several years ago, the world's sole supplier of kainic acid discontinued this product, hampering the neuroscience research.¹³⁴ Although some alternative sources have been available, the prices of kainic acid are still significantly higher than once it was.¹³⁵ Numerous syntheses of kainoids have been published,¹³⁶ however none have proven to be simple and practical. This is largely due to the synthetic challenge arising from the typical kainoid skeleton containing three contiguous stereocenters in a *trans* (C2-C3), *cis* (C3-C4) arrangements around the pyrrolidine ring.^{136, 137} The above observation prompted us to search for a less costly but still potent alternative to kainic acid. We envisaged that the aza analog of kainic acid **58** (Figure 18) would be a good substitute for kainic acid as the probe in the study of glutamate receptors due to its structural similarity to kainic acid and theoretically much simpler synthetic accessibility compared with kainic acid (see Section 1-7). Molecular modeling studies showed that **58** fits well in the kainate receptor. Similarly, the aza analogs of kainoids **59** and **60** would also be good substitutes for the kainoids **23** (Figure 4) and **7** (Figure 2) respectively.

The purpose of this dissertation is thus to design and to synthesize aza analogs of kainic acid, which would be good substitutes for the corresponding kainoids in the study of glutamate receptors and which could be synthesized more easily compared to the corresponding kainoids. The targets are aza analogs of kainoids **58-60** (Figure 18), which have the same structure as their corresponding kainoids except that the methylene group at the 5 position in the kainoids is replaced by an NH group. Such compounds should still be potent agonists at kainate receptors as supported by modeling studies described in Section 1.6 and they should be more synthetically accessible than their kainoid counterparts as discussed in Section 1.7. I attempted to synthesize these aza analogs of kainoids via a 1, 3-dipolar cycloaddition followed by reduction of the resulting cycloadducts and functional group transformations.

1.6 Modeling study of aza analog of kainoids 58 in kainate receptor GluR6

The design of selective agonists has, until recently, been hampered by the lack of a three-dimensional model of iGluRs. Early computer models, which were ultimately found to predict the structure of the ligand binding core with remarkable accuracy,¹³⁸ were based on homologies between iGluRs and bacterial glutamine binding proteins (QBP), and lysine/arginine/ornithine binding proteins (LAOBP).^{91, 139, 140} Later, these models were supported by site-directed mutagenesis experiments¹⁴¹⁻¹⁴⁴ and, more recently, by the acquisition of crystal structures (Section 1.3).

The resolution of a three-dimensional model for the ligand binding core of iGluR2, iGluR5 and iGluR6 co-crystallized with several ligands and in various stages of closure not only provided a tremendous advance in our understanding of the mechanism of ligand

binding and gating of ionotropic glutamate receptors (See Section 1-3), but will no doubt prove to be a tremendous advantage in the structure-based design of ligands of pharmacological interest in the study of many neurological disorders. Molecular modeling study of the aza analog of kainoids **58** in the ligand binding core of iGluR6, when co-crystallized with domoic acid (PDB Id: 1YAE) suggests that it would be a good substitute for α -kainic acid as an agonist at kainate receptors.

Figure 18. Some kainoids and aza analogs of kainoids

Low-mode conformational search (LMCS) addresses the complex problem of docking a flexible ligand into a flexible receptor.^{145, 146} The LMCS method consists of a Monte Carlo (MC) step in which the atoms in the freely flexible parts of the structure are moved along a trajectory that is consistent with the low frequency normal modes of vibration for that part of the structure. In low-mode docking searches, ligands are additionally subject to explicit translations and rotations. Thus conformational, orientational and positional space is sampled. Each Monte Carlo step is followed by energy minimization (EM) of the geometrically perturbed structure. A comparison of the

ability of commonly used computational methods (Catalyst, Confort, Flo99, Macromodel and Omega) to reproduce protein–ligand assemblies has determined that the low-mode conformational search algorithm (using the AMBER force field with GB/SA solvation) as implemented by Macromodel was superior to all other methods examined.¹⁴⁷

Carcache et al. reported the low-mode docking calculations of the kainic acid analogs, domoic acid and isodomoic acids, in the iGluR2 receptor.¹⁴⁸ Remarkably, this method was able to accurately predict the relative stabilities of a series of structurally related ligands with only slight variations in relative binding affinities. The low-mode docking search in iGluR homology model¹⁴⁹ identified three critical residues of ionotropic glutamate receptors that strongly influence ligand selectivity, Leu650, Thr649, and Leu704. The latter two residues have not previously been implicated in ligand selectivity.

To show that aza analog of kainoids **58** would be a good agonist at kainate receptors, low-mode docking searches of **58** in the ligand binding core of iGluR6 when cocrystallized with domoic acid (Figure 2, **13**) (PDB Id: 1YAE) were undertaken. As one would anticipate, these calculations indicate that aza analog **58** is able to adopt a geometry that resembles the conformation of kainic acid identified in the crystal structure (PDB Id: 1TT1) and allows it to bridge the two domains when docked in the iGluR6 via interactions between the receptor and compound **58** that are similar to those between kainic acid and the receptor identified in the crystal structure. The results of the calculations are summarized in the following.

Five-membered rings may occupy one of two conformations (Figure 19), the envelope (E) and the twist (T). Each atom may assume any position in the various conformations. Thus ten envelopes and ten twists, along with intermediate geometries

along the pseudorotational path, are available to kainoids. The conformation of kainic acid within the ligand binding core of iGluR6 (PDB Id: 1TT1) corresponds to E₃.⁹⁸ This structure served as the starting geometry for low-mode docking searches of aza analog of kainoids **58** in iGluR6. Since compound **58** may have two different ionic structures **58a** and **58b** which differ in the nitrogen on the ring that is protonated (Figure 19) (to avoid confusion we use the same numbering system for pyrazolidines throughout this dissertation as is used for kainoids, even though it is not the correct system for pyrazolidines), the low-mode docking searches of **58a** and **58b** in iGluR6 were both carried out.

Figure 19. Envelop (E) and half-chair (T) conformations of cyclopentane. Two ionic structures of aza-KA **58**. View of the E₄ conformation of aza-KA **58**. The subscript refers to the out of plane atom.

A LMCS/MOLS search of aza analog of kainoids **58** in ionic form **58a** in the ligand binding core of iGluR6 S1S2 was performed. The receptor was represented as a fixed shell within which various orientations and conformations of **58a** were sampled. Eight structures within a 25 kJ/mol energetic window (Table 1) were identified for **58a**. These eight structures represented three ring conformations: E₄, E₅, and E₁, represented four, two and two times. The RMS for superposition of the five ring atoms on the kainic acid crystal structure, as well as the comparison of the side chain orientation, reveal that the global minimum structure 1 (Table 1, entry 1) corresponds closely to that of kainic

acid bound to iGluR6. Important hydrogen bonding interactions similar to those identified in the kainic acid crystal structure are observed in this structure. The hydrogen bonding interactions between the ligands and receptor are summarized in Table 2 and shown in Figure 20a-20c. Although no hydrogen bond from the γ -carboxylate of **58a** to Thr690 (domain 2), compared to the kainic acid crystal structure (Figure 20a), is present in the global minimum structure of **58a** identified in the docking calculation (Figure 20b), it is compensated for by a hydrogen bond to Lys 762 (domain 2). Thus **58a** is able to maintain important domain bridging interactions in iGluR6.

Table 1. Conformations of aza-KA **58a** in the iGluR6 receptor

Entry	Conf.	RMS ^a (Å)	Relative energy (kcal / mol)	C3-4-1'-2' dihedral angle (crystal = 124.8°)
1	E ₄	0.178	0	120.9
2	E ₁	0.243	3.29	103.2
3	E ₄	0.169	3.49	117.8
4	E ₁	0.236	4.69	-103.6
5	E ₄	0.172	4.89	-33.7
6	E ₄	0.178	5.14	-98.7
7	E ₅	0.245	5.53	104.7
8	E ₅	0.239	5.89	115.3

^aFor superposition of the five ring atoms on the kainic acid crystal structure conformation.

Table 2. Hydrogen bonding interactions between ligands and iGluR6 receptor

Amino acid residues in iGluR6 receptor	KA in iGluR6 (PDB Id: 1TT1)	Aza-KA 58a docked in iGluR6	Aza-KA 58b docked in iGluR6
Arg523 (domain 1)	guanidinium NH to the α -carboxylate of KA	guanidinium NH to the α -carboxylate of 58a	guanidinium NH to the α -carboxylate of 58b
Arg523 (domain 1)	guanidinium NH ₂ to the α -carboxylate of KA		guanidinium NH ₂ to the α -carboxylate of 58b
Ala518 (domain 1)	NH to the α -carboxylate of KA	NH to the α -carboxylate of 58a	
Pro516 (domain 1)	carbonyl oxygen to NH of KA	carbonyl oxygen to N(1)H of 58a	carbonyl oxygen to N(5)H of 58b
Glu738 (domain 2)	γ -carboxylate to NH of KA	γ -carboxylate to N(1)H of 58a	
Glu738 (domain 2)		γ -carboxylate to N(5)H of 58a	γ -carboxylate to N(5)H of 58b
Ala689 (domain 2)	NH to the α -carboxylate of KA	NH to the α -carboxylate of 58a	NH to the α -carboxylate of 58b
Thr690 (domain 2)	NH to the γ -carboxylate of KA		
Lys762 (domain 2)		ϵ -NH ₂ to the γ -carboxylate of 58a	ϵ -NH ₂ to the γ -carboxylate of 58b

Figure 20a. Kainic acid in the iGluR6 S1S2 receptor (part of crystal structure with water molecules excluded for simplicity, PDB Id: 1TT1). Dashed purple lines represent hydrogen bonds within the receptor. Green lines represent hydrogen bonds between the ligand and receptor.

Figure 20b. Global minimum conformation of aza analog **58** in ionic form **58a** in the iGluR6 S1S2 receptor. Dashed purple lines represent hydrogen bonds within the receptor. Green lines represent hydrogen bonds between the ligand and receptor.

Figure 20c. Global minimum conformations of aza analog **58** in ionic form **58b** in the iGluR6 S1S2 receptor. Dashed purple lines represent hydrogen bonds within the receptor. Green lines represent hydrogen bonds between the ligand and receptor.

Within a 25 kJ/mol energetic window, fourteen geometries are available to compound **58b** with N5 protonated (Table 3). These fourteen structures represented three ring conformations: E₄, E₅, and E₁, represented six, five, and three times. The RMS for superposition of the five ring atoms on the crystal structure, the comparison of the side chain orientation, as well as the consideration of the relative energy, suggest that the global minimum structure 1 corresponds closely to that of kainic acid bound to iGluR6. Similar hydrogen bonding interactions to those identified in the kainic acid crystal structure are also observed in this structure (Table 2, Figure 20c). This structure forms hydrogen bonds between the α -carboxylate and Arg523 (guanidium NH), between the α -carboxylate and Arg523 (guanidium NH₂), between N5H and Pro516 (carbonyl oxygen) on domain 1, and between N5H and Glu 738 (γ -carboxylate), between the α -carboxylate

and Ala689 (NH), between the γ -carboxylate and Lys762 (ϵ -NH₂) on domain 2. These hydrogen bonding interactions are maintained from structure 1 to structure 3. In structure 3, additional hydrogen bond from N1H to Glu738 (γ -carboxylate) is present. Thus, **58b** is also able to maintain the important domain spanning interactions which are critical to the domain closure of iGluR6.

Table 3. Conformations of aza-KA **58b** in the iGluR 6 receptor

Entry	Conf.	RMS ^a (Å)	Relative energy (kcal / mol)	C3-4-1'-2' dihedral angle (crystal = 124.8°)
1	E ₄	0.186	0	116.9
2	E ₄	0.184	1.50	-24.7
3	E ₄	0.168	1.89	117.5
4	E ₄	0.164	2.16	-121.5
5	E ₁	0.236	3.25	106.0
6	E ₄	0.167	3.28	-25.9
7	E ₄	0.174	4.21	-97.2
8	E ₅	0.254	4.44	115.1
9	E ₅	0.265	4.76	105.3
10	E ₅	0.222	4.78	-101.8
11	E ₁	0.233	4.87	-101.3
12	E ₅	0.239	5.36	107.3
13	E ₅	0.231	5.37	114.7
14	E ₁	0.258	5.39	103.3

^aFor superposition of the five ring atoms on the kainic acid crystal structure conformation.

Docking energies are compared for the global minima of **58a** and **58b** in Table 4, which suggests that **58b** has slightly higher binding affinity than **58a** at iGluR6. Docking energies were estimated as the energy difference between the energy (E_{inside}) calculated for the receptor–ligand complex when the ligand is bound to the receptor in its global

minimum energy conformation and orientation, and the sum of the energies (E_{outside}) calculated for the separated ligand (in its bound global minimum energy conformation) and the receptor, to be specific, docking energy = ($E_{\text{outside}} - E_{\text{inside}}$).

Table 4. Estimated relative docking energies for **58a** and **58b**

Agonist	Relative docking energy (kcal/mol)
58a	0
58b	0.52

In conclusion, the aza analog of kainoids **58** can have interactions with the iGluR6 receptors similar to those between kainic acid and iGluR6 to achieve the domain closure and thus may be as potent as kainic acid at kainate receptors.

1.7 Research design

As shown in Figure 21, the skeleton of aza analogs of kainoids (pyrazolidines) can be constructed via the 1, 3-dipolar cycloaddition of the appropriate diazoalkane (diazoacetone,¹⁵⁰ phenyldiazomethane,¹⁵¹ and diazomethane respectively) and the dipolarophile diethyl glutaconate followed by the reduction of the resultant cycloadducts (1-pyrazolines). The relative configuration in the aza analogs of kainoids is highly predictable in the cycloaddition based on the consideration of the stereochemistry in the 1, 3-dipolar cycloaddition of diazoalkanes and alkenes. When an α , β -unsaturated ester such as *trans* diethyl glutaconate is used as the dipolarophile, the regioselectivity will be such that the terminal nitrogen of the diazoalkane will be connected with the α -carbon of the ester due to diazoalkane HOMO- dipolarophile LUMO interactions (Figure 22).¹⁵²

R = CH₃CO, **61**

R = Ph, **62**

R = H, **63**

Figure 21. Retrosynthetic analysis of aza analogs of kainoids

a. Reduction of 2-pyrazolines

b. Reduction of 1-pyrazolines

Figure 22. Proposed synthesis of aza analogs of kainoids

However, some exceptions have also been observed.^{153, 154} The relative configuration of the dipolarophile will be retained in the cycloadduct since the cycloaddition is stereospecific and *syn* with respect to the dipolarophile. Depending on the nature of the substituents, the cycloadduct containing either *trans* or *cis* relative configuration between

the substituents at the 4 and 5 positions of the 1-pyrazoline ring may be obtained (to avoid confusion we use the numbering system for pyrazolines shown in Figure 21 throughout this dissertation). The *cis* relative configuration will be favored if there may be π interactions between those two substituents in the transition state.¹⁵⁵ Therefore the 1, 3-dipolar cycloaddition of *trans* diethyl glutaconate with diazoalkanes will produce the appropriate 3, 4-*trans*, 4, 5-*cis* relative configurations in the cycloadduct if the cycloaddition follows the expected course. The reduction of the 1-pyrazolines will yield **61**, **62** and **63**, respectively (route b, Figure 22). Hydrolysis of **62** and **63** will give the desired targets **59** and **60** (Figure 18), respectively. Conversion of **61** to the target **58** will be achieved after functional group transformations.

Figure 23. Isomarization of 1-pyrazoline

However, prior studies on the cycloadditions of diazoalkanes with α , β -unsaturated esters have shown that the 1-pyrazolines initially formed in such cycloadditions readily isomerized to the corresponding conjugated 2-pyrazolines (Figure 23).^{153, 156} Therefore, it is possible that 1-pyrazolines formed in the cycloadditions of diazoalkanes with *trans* diethyl glutaconate will isomerize to the 2-pyrazolines with the direction of double bond isomerization dependent on the substituents (Figure 22), resulting in the loss of one of the newly formed stereocenters. If this is the case, aza analogs **61**, **62** and **63** (Figure 22) will be obtained after the selective reduction of C=N

double bond in the 2-pyrazolines, respectively (route a, Figure 22). Thus this research would provide the easy access to the aza analogs of kainoids which are potentially potent alternative to the corresponding kainoids.

2. Results and Discussion

2.1 1,3-Dipolar cycloadditions between diazoalkanes and *trans*-diethyl glutaconate

The dipolar cycloadditions of diazoalkanes with *trans*-diethyl glutaconate have been evaluated and the results are summarized in Table 5 and Figure 24. Some 2-pyrazolines **64b**, **65a**, **65b**, **66a** (Figure 24) have been obtained in good yields and were converted to Cbz derivatives to prevent oxidation.

Table 5. 1, 3-Dipolar Cycloaddition of Diazoalkanes and *trans* Diethyl glutaconate

R	#eq. RCHN ₂	Conditions	Yield ^a	Product ratio a:b
H	2.2	Et ₂ O, 23°C, 15 hr.	90%	0:100 ^c
Ph	0.9	THF, -78°C-23°C, 15 hr.	73%	80:20 ^c
CO ₂ Et	1.2	C ₆ H ₆ , reflux, 72hr.	39%	13.5:0:1 ^d (66a:66b:68)
“ “	1.2	PhCH ₃ , reflux, 72hr.	37%	4:0:1 ^d (66a:66b:68)
“ “	2	C ₆ H ₆ , reflux, 72hr.	64%	3:0:1 ^d (66a:66b:68)
“ “	2	PhCH ₃ , reflux, 72hr.	89%	3:0:1 ^d (66a:66b:68)
TMS	6	PhCH ₃ /hexane, 23°C, 3 d.	58% (96% ^b)	1.7:1 ^{d,c}
TMS	2	C ₆ H ₆ /hexane, reflux, 8.5h; 1.1eq. CbzCl, 1.1eq. NaHCO ₃ , 1:1 CH ₂ Cl ₂ / H ₂ O	51% ^f	1:1.4 ^d (69c:69d)
TMS	3	C ₆ H ₆ /hexane, reflux, 8.5h; 10eq. EtO ₂ COCl, 2eq. AgOTf, CH ₂ Cl ₂	24% ^f	1:5 ^d (69e:69f)

^aPurified.

^bBased on recovered diethylglutaconate

^cDetermined by NMR

^dBased on isolated yield

^eRegioisomers were not specifically assigned.

^fTwo-step overall yield

a) See Table 5 for conditions and yields. b) Cbz-chloride, CH₂Cl₂, Na₂CO₃(aq.) c) N₂CHCO₂Et/ Δ

Figure 24. 1,3-Dipolar cycloadditions between diazoalkanes and *trans*-diethyl glutaconate

The 1-pyrazolines either isomerized to the 2-pyrazolines or became oxidized to the pyrazoles. Because of the tendency of the 2-pyrazolines to become oxidized (phenyl substituted pyrazolines in particular), they were converted to the Cbz derivatives, which tends to inhibit oxidation, for full characterization.

The cycloaddition of diazomethane and *trans*-diethyl glutaconate yielded, as expected, a single 2-pyrazoline product (**64b**) in 90% yield at ambient temperature under nitrogen after 15h. **64b** was obtained in relatively high purity from the cycloaddition and can be used for further transformation without purification. It was found that this cycloaddition was completed in less than 2 hours. Although it is not stable on standing, the ¹H NMR, ¹³C NMR, COSY, APCI mass spectrum of **64b** were able to be acquired to help assign its structure. After the cycloaddition immediately followed by treatment with CbzCl (1.1eq.) in the presence of NaHCO₃, **64b** was converted to its Cbz derivative **64d** in 93% yield and no formation of oxidation product of **64b** or its Cbz derivative **70** was observed based on the crude ¹H NMR spectra of **64b** and **64d**. In this case, the crude **64d** was of sufficient purity to allow its use in subsequent reactions without further purification. However, when **64b** was treated with CbzCl after it stood at ambient temperature for 3 days, its Cbz derivative **64d** was obtained in 48% yield along with the Cbz derivative of its oxidation product, **70**, in 13% yield.

The reduction of the C=N double bond of **64b** or **64d** is required to furnish the target aza analog of kainoids **60** (Figure 18). Since the Cbz group can not survive some reducing conditions such as catalytic hydrogenation and reduction with lithium aluminum hydride, **64b** was also protected as other derivatives shown in Figure 25. **64b** was converted to the carbamate **71** in 74% yield by treatment with ClCO₂Et in CH₃CN mixed with aqueous saturated K₂CO₃ solution.¹⁵⁷ By treatment of **64b** with PhCOCl in the presence of NaHCO₃ in 1:1 ratio of H₂O and THF, the amide **72** was obtained in 98% yield.¹⁵⁸ By treatment of **64b** with CH₃COCl in the presence of NaHCO₃ in 1:1 ratio of H₂O and THF, the amide **73** was obtained in 92% yield.¹⁵⁸ Attempt to prepare the *N*-

sulfonyl derivative of **64b** was not successful by treatment of **64b** with 1.5eq. p-toluenesulfonyl chloride¹⁵⁹ in methylene chloride in the presence of 1.5eq. imidazole and 1.5eq. triethylamine at room temperature for 3 days.

Conditions: (a) 2eq. ClCO₂Et, Aqueous Sat. K₂CO₃, CH₃CN.
 (b) 1.1eq. CH₃COCl, 2eq. NaHCO₃, 1:1 H₂O/THF
 (c) 1.1eq. PhCOCl, 2eq. NaHCO₃, 1:1 H₂O/THF

Figure 25. Protection of 2-pyrazoline **64b**

The cycloaddition of phenyldiazomethane¹⁵¹ and *trans* diethyl glutaconate conducted in dry THF overnight under nitrogen yielded two 2-pyrazoline adducts (**65a** and **65b**) in a ratio of 4:1 as indicated by crude ¹H NMR spectrum and an overall isolated yield of 73%. On standing, this mixture readily oxidizes to a single pyrazole (**67**) confirming that **65a** and **65b** are not regioisomers with respect to the cycloaddition. Upon treatment of the mixture of **65a** and **65b** with CbzCl, their Cbz derivatives **65c** and **65d** were obtained in 68% and 56% yield, respectively. The double bond regioisomers (**65a** and **65b** and their Cbz derivatives) are readily distinguished on the basis of ¹³C and ¹H NMR. The relative configuration of the 3, 4-substituents of **65a** is expected to be *trans*

and is confirmed by the similarity of the ^1H NMR to similarly substituted 2-pyrazolines¹⁵³ and [4, 5]-dehydropyrrolidines^{160, 161} in terms of the vicinal coupling constant. The relative configuration of the 4, 5- substituents of **65b** is assigned as *cis* on the basis of NOESY spectrum of its Cbz derivative **65d**, the vicinal coupling constant (12 Hz)¹⁶⁰ and the large upfield shift of one of the diastereotopic C4 side-chain methylene protons (δ 2.12 ppm and δ 2.09 ppm) for **65b** and **65d**, respectively compared to a range from δ 2.51 ppm to δ 2.95 ppm for **64d**, **65a**, **65c**, **66a** and **66c**. This large upfield shift, due to shielding of the C4 side-chain methylene, is consistent with 3, 4-*cis* substituted kainoids with an aromatic ring at the 4-position^{160, 161} and is also consistent with a recent empirical analysis of NMR data of various natural and synthetic kainoids.¹⁶² The *trans* isomer of **65b** was not observed, suggesting that the relative stereoselectivity of the cycloaddition is *cis*.

Ethyldiazoacetate was unreactive at room temperature due to the electron withdrawing effect of the carbonyl. Thus the cycloaddition was carried out in refluxing benzene or toluene providing the *trans* isomer **66a**, along with a by-product **68**, which arose from carbene insertion into the N-H bond of the 2-pyrazoline **66a**. Assignment of relative configurations of **66a** was based on coupling constants of the ring protons¹⁶⁰ and NOESY spectrum of its Cbz derivative **66c**. Assignment of relative configurations of **68** was confirmed by conversion of **66a** to **68** by treatment with ethyldiazoacetate. When **66a** was reacted with 2eq. ethyldiazoacetate in refluxing toluene for 72h, **68** was obtained in 16% yield. The use of two equivalents of ethyldiazoacetate in refluxing toluene or benzene improved the overall cycloaddition yield, but also increased the proportion of the by-product **68**. Failure to identify the *cis* product **66b** suggests either that the preferred

stereoselectivity of the cycloaddition is *trans* or that the double bond isomerization is regioselective toward a *cis* substituted carbonyl group, by virtue of the relief of torsional strain which would be present in a 3,4-*cis* substituted 1-pyrazoline intermediate. Attempts to improve the yield by extending the reaction time (up to 10 days) or increasing the amount of ethyldiazoacetate (up to 3eq.) were not successful.

Figure 26. Preparation of some pyrazolidine compounds starting from $TMSCHN_2$

It was reported that the cycloaddition of trimethylsilyldiazomethane and chiral acrylamides produced 2-pyrazoline **74** (Figure 26) via proteodesilylation¹⁶³⁻¹⁶⁵ which was reduced to the pyrazolidine compound **75** (Figure 26) upon treatment with sodium cyanoborohydride in acetic acid¹⁶³ or were converted to the pyrazolidine compounds **76** (Figure 26) with a new substituent being introduced at C-4 position around the pyrazolidine ring via Lewis acid promoted addition of some nucleophiles to the $C=N$

bond of the 2-pyrazolines¹⁶⁶ (Figure 26). It was envisaged that this strategy can be applied towards the synthesis of aza analogs of kainoids such as the target compound **60** (Figure 18). Thus, the reaction between *trans*-diethyl glutaconate and trimethylsilyldiazomethane was carried out in the hope of obtaining the 2-pyrazoline **64a** (Figure 24) which contains no substituent at C-4 position around the pyrazoline ring. However, the cycloaddition conducted in the mixture of toluene and hexane at room temperature for 3 days, failed to provide 2-pyrazolines. Instead, a mixture of pyrazoles (**69a** and **69b**) (Figure 24) in a ratio of 1.7:1 (Table 5) was obtained. Apparently, the regioselectivity of the cycloaddition is partially reversed as the size of R increases and steric effects overcome electronic effects. The desired protected 2-pyrazoline **69d** (Figure 24) was obtained in 30% yield along with the TMS retained 2-pyrazoline **69c** (Figure 24) containing an isolated C=N double bond in 21% yield when *trans*-diethyl glutaconate was treated with 2eq. trimethylsilyldiazomethane in refluxing benzene and hexane mixture for 8.5h followed by the treatment of the crude cycloadduct mixture with CbzCl. Treatment of the crude cycloadduct mixture with 10eq. ethyl chloroformate and 2eq. AgOTf in methylene chloride at room temperature did not exclusively afford the protected desilylated product **69f** (Figure 24) as expected^{164, 165} although it significantly increased the proportion of the desilylated product. In this case, the protected desilylated product **69f** (Figure 24) was obtained in 20% yield along with the silylated product **69e** (Figure 24) in 4% yield, in which the C=N double bond is conjugated to the ester group. The relative configuration of the 4, 5-substituents of **69e** was tentatively assigned as *trans* based on the vicinal coupling constant (5.6Hz). The above observations that the same cycloaddition gave rise to different products under different conditions aroused our

interest in the reactivity of trimethylsilyldiazomethane in the 1, 3-dipolar cycloaddition with different dipolarophiles. We then carried out a systematic survey of dipolar cycloadditions between TMS diazomethane and α, β -unsaturated dipolarophiles. This work will be described in Section 2.3. The reduction of the protected 2-pyrazoline **69f** to yield the corresponding pyrazolidine compound will be described in Section 2.2.2.

Figure 27. Cycloaddition of hydrazones with olefins

Kobayashi et al reported that the cycloaddition of hydrazone **77** with olefin **78** was achieved to afford the pyrazolidine **79** in the presence of $\text{BF}_3 \cdot \text{OEt}_2$ or a catalytic amount of $\text{Zr}(\text{OTf})_4$, $\text{Hf}(\text{OTf})_4$, or $\text{Sc}(\text{OTf})_3$ under mild conditions (Figure 27).¹⁶⁷ The easy construction of the pyrazolidine ring in these cycloadditions drew our attention and prompted us to explore the possibility of applying this strategy in the synthesis of our target compounds. Unfortunately, no reaction of tosylhydrazone with diethyl glutaconate (3eq.) occurred in the presence of 1.4eq. $\text{BF}_3 \cdot \text{OEt}_2$ at room temperature for 24h. The

same thing happened when tosylhydrazone was treated with 3eq. diethyl glutaconate in the presence of 1.4eq. $\text{BF}_3 \cdot \text{OEt}_2$ in refluxing methylene chloride for 5 days. When the reaction was conducted in refluxing benzene for 42h, no formation of the cycloadduct was observed and diethyl glutaconate was recovered with tosylhydrazone being converted to the intractable materials possibly due to its decomposition at the reaction temperature based on the crude ^1H NMR.

2.2 Reductions of 2-pyrazolines

The dipolar cycloaddition of diazoalkanes and *trans*-diethylglutaconate provides the appropriate pyrazoline ring which may be converted into aza analogs of kainoids via reduction. Reductions of the 2-pyrazolines and their Cbz derivatives have been carried out and the results are summarized in the following.

2.2.1 Reductions of unprotected 2-pyrazolines **64b**, **65a** and **65b**

When **64b** (Figure 24) was treated by NaBH_4 ¹⁶⁸⁻¹⁷⁰ in ethanol overnight, no formation of the corresponding pyrazolidine compound was observed based on the crude ^1H NMR and mass spectrum. Low mass balance was obtained from this reaction. The reduction of the imine $\text{C}=\text{N}$ double bond of **64b** (Figure 24) was not effected when **64b** (Figure 24) was treated by H_2 (1atm or ~50psi) in the presence of Pd ^{160, 171} on activated carbon in Et_2O or EtOH or in the presence of Raney Ni ¹⁷² in EtOH respectively. Most of **64b** (Figure 24) was recovered along with some intractable materials in these reactions.

When the mixture of **65a** and **65b** (Figure 24) was treated with NaBH_4 in EtOH , NaBCNH_3 in EtOH ¹⁷³, H_2 (55 psi)/ Pd on 10% charcoal in EtOH , H_2 (55 psi) / Pd on activated carbon in EtOH , at room temperature, respectively, no reduction of the $\text{C}=\text{N}$

bond in **65a** and **65b** (Figure 24) was observed and the oxidation product **67** (Figure 24) was obtained in the yield of 8%, 8%, 8%, 6%, respectively along with intractable materials. The reduction of the unprotected 2-pyrazoline was thus not pursued further due to their potential oxidation to the corresponding pyrazoles.

2.2.2 Reductions of protected 2-pyrazolines

When **65c** and **65d** (Figure 24) were treated with H₂ (1atm) in the presence of palladium on activated carbon in EtOH at room temperature, the removal of Cbz group was effected to yield the unprotected 2-pyrazolines **65a** and **65b** (Figure 24), but no reduction of the C=N bond was observed. The application of higher pressure of H₂ (55psi) to the reaction yielded only intractable materials. Treatment of **65c** and **65d** with 0.5eq. lithium borohydride^{169, 174} in dry THF at ambient temperature for 30h yielded the intractable materials with 12% of the starting material being recovered. When 1.8eq. superhydride¹⁵⁷ was used to treat **65c** and **65d** (Figure 24) in dry THF at room temperature for 65h, intractable materials were obtained along with some recovered starting material.

Due to the aforementioned failure of the reduction of the C=N double bond of **65c** and **65d** (Figure 24), our attention was turned to the reduction of the C=N double bond of **64d** (Figure 24) as a model system for the reduction as it is more easily synthesized compared to **65c** and **65d** (Figure 24). Many reducing agents that have been reported to be capable of reducing an imine C=N double bond have been used to treat **64d** (Figure 24). Unfortunately, no such reduction has been achieved. The results of these reductions are summarized in Table 6.

Figure 28. Some products obtained from the reduction of **64d**

Table 6. Reduction of **64d**

Entry	Reducing agent	Conditions	Results
1	NaCNBH ₃ (2.5eq.)	CH ₃ COOH, R.T. (1) 1.5hrs or (2) 17h	Most of the starting material was recovered.
2	NaCNBH ₃ (10eq.)	<i>p</i> -toluenesulfonic acid, 1:1 DMF-dioxane, reflux, 22h	No reaction
3	NaBH ₄ (2.5 eq.)	EtOH, R.T., 15h	Probably 80 (Figure 28) was formed
4	NaBH ₄ (2.5 eq.)	EtOH, reflux, 15h	intractable materials
5	NaBH ₄ (2.5 eq.)	AcOH, R.T., 18h	No reaction
6	NaBH ₄ (10eq.)	AcOH, R.T., 1.5h; Reflux, 3h; R.T., 15.5h	No reaction
7	NaBH ₄ (1eq.), CeCl ₃ (1eq.)	MeOH, 0 °C, 5h, then R.T. , 12h	81 (Figure 28) (55% yield)
8	NaBCNH ₃ (2.5eq.), CeCl ₃ (1eq.)	EtOH, R.T., 17h	No reaction
9	LiBH ₄ (0.75 eq.)	THF, R.T., 24h	Complicated mixture
10	LiAlH ₄ (3eq.)	THF, R.T., 22h; then reflux, 6h	Complicated mixture
11	BH ₃ (1.1 eq.), phthalic acid (1.1 eq.)	(1) THF, -78°C, 2h; or (2) THF, R.T., 19h.	Most of the starting material was recovered.
12	BH ₃ (0.4 eq.)	THF, -78°C, 5h; then -78°C to R.T., overnight	Complicated mixture
13	SmI ₂ (2.5eq.), Et ₃ N(5eq.) H ₂ O(6.25eq.)	THF, R.T., <10s	Intractable materials and Low mass balance

14	SmI ₂ (2.5eq.), Et ₃ N(5eq.) H ₂ O(6.25eq.)	THF, -78°C, 2h; then another 2.5eq SmI ₂ , -78°C to R.T., overnight.	Nothing changed after 2h at -78°C. Intractable materials were obtained. The mass balance is low (11%).
15	SmI ₂ (2.5eq.)	THF, R.T., 22h	The starting material was not completely consumed. A complicated mixture was obtained.
16	H ₂ (50psi), Pd on activated carbon	EtOH, R.T., 18h	Cbz group was removed. A complicated mixture was obtained.
17	Mg (10 eq.)	MeOH, R.T., 5h	A complicated mixture
18	Et ₃ SiH(2eq.)	Refluxing CF ₃ COOH 5h	Removal of Cbz group and intractable materials
19	Me ₂ NH.BH ₃ (1eq.)	Gaseous HCl, PhCH ₃ R.T., 20h	No reaction and the starting material was recovered.
20	Me ₂ NH.BH ₃ (1.6eq.)	<i>p</i> -toluenesulfonic acid (6eq.), CH ₂ Cl ₂ +MeOH, 0°C 2h; R.T., 15h	No reduction product was obtained and some starting material was recovered.
21	Me ₂ NH.BH ₃ (10eq.)	<i>p</i> -toluenesulfonic acid (6eq.), PhCH ₃ +MeOH, reflux, 21h	No reaction occurred and the starting material was recovered.
22	Me ₃ N.BH ₃ (1eq.)	Gaseous HCl, PhCH ₃ R.T., 17h	No reaction occurred and the starting material was recovered.
23	Bu ₃ SnH(5eq.)	BF ₃ .OEt ₂ (4eq.), CH ₂ Cl ₂ , -78°C to R.T., 45h	No reaction occurred and the starting material was recovered.
24	Bu ₃ SnH(2.5eq.)	BF ₃ .OEt ₂ (6eq.), CH ₂ Cl ₂ , R.T., 26h	No reaction occurred and the starting material was recovered.
25	LiB(C ₂ H ₅) ₃ H (1.16eq)	CH ₂ Cl ₂ , -78°C, 4.5h	84 (Figure 29), 42% yield; 85 (Figure 29) was probably produced.

26	LiB(C ₂ H ₅) ₃ H (1.1eq.)	CH ₂ Cl ₂ , -78°C, 20min; then another 0.6eq of LiB(C ₂ H ₅) ₃ H, -78°C to R.T., overnight. Followed by treatment with ClCO ₂ Et.	86 (Figure 29), 16% yield. 87 was observed.
27	LiB(C ₂ H ₅) ₃ H (1.16eq.)	CH ₂ Cl ₂ , -78°C, 4h; -78°C to R.T., overnight.	80 (Figure 28), 84 and 85 (Figure 29) were probably formed.
28	LiB(C ₂ H ₅) ₃ H (1.16eq.)	CH ₂ Cl ₂ , R.T., 20h.	80 (Figure 28) and 85 (Figure 29) were probably formed. Low mass balance (36%)
29	LiB(C ₂ H ₅) ₃ H (2.16eq.)	CH ₂ Cl ₂ , -78°C, 5h	88 (Figure 29) was probably formed.
30	LiB(C ₂ H ₅) ₃ H (3.16eq.)	CH ₂ Cl ₂ , -78°C, 5h	Complicated mixture
31	LiB(C ₂ H ₅) ₃ H (1.1eq.)	CH ₂ Cl ₂ , -78°C, 3h; then another 1.1eq of LiB(C ₂ H ₅) ₃ H, -78°C, 3h.	Complicated mixture
32	LiB(C ₂ H ₅) ₃ H (1.1eq.)	CH ₂ Cl ₂ , -21°C, 3.5h; then another 1.1eq of LiB(C ₂ H ₅) ₃ H, overnight.	80 (Figure 28) and 84 (Figure 29) were probably formed.

Compared to the normal imine C=N double bond, the C=N double bond of **64d** (Figure 24) is more robust as indicated by the fact that no reduction of **64d** (Figure 24) occurred and most of the starting material was recovered in the reduction attempts with sodium cyanoborohydride in acetic acid^{163, 173, 175, 176} (Table 6, Entry 1), sodium cyanoborohydride in 1:1 ratio of dimethylformamide and dioxane in the presence of 0.6eq. p-toluenesulfonic acid monohydrate at reflux¹⁷⁷ (Table 6, Entry 2), sodium borohydride in acetic acid at room temperature for 18h or 10eq. sodium borohydride in acetic acid at reflux for 3h^{176, 178, 179} (Table 6, Entry 5-6).

Instead of the reduction of C=N bond of **64d** (Figure 24), **64d** was reduced to the alcohol **80** (Figure 28) by sodium borohydride in EtOH at room temperature based on the crude ^1H NMR and mass spectrum in less than 50% crude yield (Table 6, Entry 3). When the reaction was conducted in EtOH at reflux for 15h, **64d** (Figure 24) was converted into intractable materials (Table 6, Entry 4). When **64d** (Figure 24) was treated with 1eq. sodium borohydride in methanol in the presence of 1eq. CeCl_3 ¹⁸⁰ at 0°C for 5h and then at room temperature for 12h, the transesterification occurred to yield compound **81** (Figure 28) in 55% yield (Table 6, Entry 7) instead of the reduction of C=N bond of **64d**, indicating that the polarization of the ester group by CeCl_3 occurred and CeCl_3 was unable to polarize C=N bond of **64d** to increase its reactivity towards the hydride attack. However, **64d** was recovered when sodium cyanoborohydride was used as the reducing agent in ethyl alcohol in the presence of CeCl_3 at room temperature for 17h (Table 6, Entry 8). The same thing happened when **64d** was treated with 1.1eq. BH_3 in dry THF in the presence of 1.1eq. phthalic acid (Table 6, Entry 11).¹⁸¹ A complicated mixture was obtained when **64d** was treated with 0.4eq. BH_3 in dry THF (Table 6, Entry 12)¹⁸²⁻¹⁸⁴ or with 0.75eq. lithium borohydride in dry THF at room temperature for 24h (Table 6, Entry 9) or with 3eq. lithium aluminum hydride in dry THF at room temperature for 22h and then at reflux for 6h (Table 6, Entry 10).

SmI_2 has been shown to successfully reduce various nitrogen functionality (nitro group¹⁸⁵⁻¹⁸⁸, imines^{185, 189}, oximes¹⁸⁵, hydrazones^{185, 190}, isoxazoles¹⁹¹ and azides¹⁹². The SmI_2 -mediated reduction of imines has been shown to be instantaneous in the presence of H_2O and an amine in THF.¹⁹³ However, SmI_2 -mediated reduction of the C=N double bond of **64d** was unsuccessful, resulting only in intractable materials (Table 6, Entry 13-

14) or a complicated mixture (Table 6, Entry 15). A complicated mixture was obtained when **64d** was treated with 10eq. Mg¹⁹⁴ in methanol at room temperature for 5h (Table 6, Entry 17). Treatment of **64d** with H₂ (55psi) in the presence of palladium on activated carbon in EtOH at room temperature for 18h resulted in the removal of Cbz group and yielded only a complicated mixture (Table 6, Entry 16). However, when the same reducing system was applied to compound **71** (Figure 25) at room temperature for 6 days, no reaction occurred and **71** (Figure 25) was recovered. No reduction of C=N bond of compound **72** (Figure 25) was observed and some starting material was not consumed based on TLC analysis, ¹H NMR and mass spectrum when **72** (Figure 25) was treated with H₂ (55psi) in the presence of PtO₂ (up to 30% of the weight of the starting material)^{171, 195} in EtOH at room temperature for 71h.

Reaction of **64d** with 2eq. Et₃SiH in refluxing trifluoroacetic acid^{160, 196} for 5h resulted in the removal of the Cbz group and formation of intractable materials (Table 6, Entry 18). When compound **71** (Figure 25) was treated with 2eq. Et₃SiH in refluxing trifluoroacetic acid for 23h, most of **71** (Figure 25) was recovered and no reduction of C=N bond was observed based on TLC analysis, crude ¹H NMR and mass spectrum. In contract to the reports that the BF₃-promoted hydrostannation of N-heteroatom-substituted imines¹⁹⁷⁻¹⁹⁹ such as hydrazones, oximes, nitrones, and N-sulfonyl imines successfully resulted in the reduction of the C=N bond, **64d** was recovered from treatment with Bu₃SnH in the presence of freshly distilled BF₃.OEt₂ in dry dichloromethane (Table 6, Entry 23-24). Amino-borane complexes have drawn the attention of many synthetic organic chemists. Their stability, tolerance to acids, solubility in many solvents and good reducing power have led to a number of laboratory and

industrial applications.²⁰⁰⁻²⁰² One of the most important applications of amino-borane complexes is the reduction of the C=N double bond to form C-N.^{200, 203-205} Two particularly interesting amino-borane complexes are dimethylamine-boron complex (DMAB) and trimethylamine-boron complex (TMAB). These solid complexes are safe, economical, and soluble in protic and aprotic solvents and have been shown to effectively reduce hydrazones to hydrazines.^{206, 207} These two amino-boron complexes were thus applied to the reduction of the C=N double bond of **64d**, a fully substituted hydrazone. Unfortunately, no expected reduction of the C=N double bond of **64d** has been realized (Table 6, Entry 19-22).

Since superhydride (LiBEt₃H) has been used to successfully reduce the C=N double bond of 2-pyrazoline compound **82** to afford the pyrazolidine compound **83** (Figure 29),¹⁵⁷ it was used to treat the protected 2-pyrazoline **64d** and its related compounds in the hope of realizing the reduction of C=N double bond of these compounds. Treatment of **64d** with 1.16eq. superhydride in dry methylene chloride at -78°C for 4.5h (Figure 29) effected the selective reduction of the ester group conjugated to the C=N bond of **64d** to furnish the aldehyde **84** in 42% yield (Table 6, Entry 25). In this reaction, another impure compound was also isolated in small amount in less than 6% yield and assigned to the structure **85** based on its ¹H NMR and mass spectrum and the observation that, as monitored by the ¹H NMR and mass spectrum, it turned into the starting 2-pyrazoline **64d** after ca. 50 days possibly by undergoing slow oxidation in the air.¹⁵⁷ This indicated that the reduction of C=N bond of **64d** occurred to a modest extent and superhydride prefers to reduce the ester group conjugated to the C=N bond rather than the C=N double bond. Interestingly, when **64d** was treated with 1.1eq. superhydride

in dry methylene chloride at -78°C for 20min and then another 0.6eq. superhydride from -78°C to room temperature overnight, the ester group conjugated to the $\text{C}=\text{N}$ bond of **64d** was reduced to the hydroxyl group. This was suggested by the isolation of compound **86** (Figure 29) in 16% yield starting from **64d** after treatment of the crude reduction product

Conditions:

- a: $\text{LiB}(\text{C}_2\text{H}_5)_3\text{H}$ (1.2eq), CH_2Cl_2 , -78°C , 4.5h
- b: (1) $\text{LiB}(\text{C}_2\text{H}_5)_3\text{H}$ (1.1eq), CH_2Cl_2 , -78°C , 20min;
 $\text{LiB}(\text{C}_2\text{H}_5)_3\text{H}$ (0.6eq), CH_2Cl_2 , -78°C -R.T., overnight.
 (2) ClCOOEt
- c: $\text{LiB}(\text{C}_2\text{H}_5)_3\text{H}$ (2.16eq), CH_2Cl_2 , -78°C , 5h

Figure 29. Reduction of **64d** and **82** with superhydride

with ethyl chloroformate in saturated K_2CO_3 in acetonitrile, which was initially expected to protect the possible reduction product **85** but did not succeed since **87** was not isolated. Attempts to increase the yield of **84** (Figure 29) by increasing the number of the

equivalents of superhydride, the reaction temperature, and/or the reaction time were not successful (Table 6, Entry 27-32). Increasing the number of the equivalents of superhydride and/or the reaction temperature results in the lower chemoselectivity in the reduction. For example, treatment of **64d** with 2.16eq. superhydride in dry methylene chloride at -78°C for 5h (Table 6, Entry 29) probably afforded **88** (Figure 29) based on its ¹H NMR and mass spectrum, indicating the reduction of both ester groups.

Similar to **64d**, the Cbz protected 2-pyrazoline **66c** was reduced to the aldehyde **89** in 34% yield when it was treated with 1.1eq. superhydride in methylene chloride at -78 °C for 5h (Figure 30).

Figure 30. Reduction of 2-pyrazoline **66c** with superhydride

It was felt that the conjugation between the electron-withdrawing carbonyl group and the C=N double bond of **64d** and its analogs such as **66c** is likely to contribute to the resistance of the C=N double bond towards the common C=N reducing agents. Efforts were thus directed to interrupt such conjugation by converting the aldehyde **84** to its acetals. The aldehyde **84** was first converted to the dimethyl acetal **90** in 28% yield with **84** being recovered in 26% by treatment with 2.2eq. dimethoxypropane in the presence of a catalytic amount of p-toluenesulfonic acid monohydrate at room temperature for 19h

(Figure 31).²⁰⁸⁻²¹⁰ Similar results were obtained when **84** was treated with methanol in the presence of a catalytic amount of bismuth nitrate at room temperature for 21h.²¹¹ The low yield of the acetal **90** was probably due to its great tendency to go back to the starting 2-pyrazoline **64d** to reestablish the favored conjugation of the carbonyl group and the C=N

Figure 31. Protection of the aldehyde **84**

double bond under the reaction conditions. Increasing the yield of the acetal **90** was not pursued further since it was felt that the acetal **90** would not survive the mild acetic condition such as acetic acid used as the reaction solvent for reduction with NaBCNH₃. Efforts were then directed to convert **64d** to the cyclic acetal **91** by treatment with 1, 2-ethanediol which is likely to be more stable under the mild acidic condition than the dimethyl acetal **90**. Thus, the cyclic acetal **91** was obtained in 48% yield when **64d** was treated with 2eq. 1, 2-ethanediol in refluxing benzene for 7h in the presence of a catalytic amount of p-toluenesulfonic acid monohydrate with the water being removed from the

reaction mixture by using a Dean-Stark trap.²¹² The cyclic acetal **91** was treated with 5eq. sodium cyanoborohydride^{173, 175, 176} in acetic acid at room temperature overnight in the hope of reducing the isolated C=N bond of **91**. Unfortunately, no reduction occurred and most of the starting cyclic acetal **91** was recovered based on the crude ¹H NMR, mass spectrum and TLC analysis.

Efforts were subsequently directed to selectively reduce the ester group conjugated to the C=N double bond of **64d** to the hydroxyl group so that the corresponding conjugation will be interrupted. The use of NaBH₄ as the reducing agent to achieve such reduction was investigated since **64d** was reduced to the alcohol **80** (Figure 28) with sodium borohydride in EtOH at room temperature for 23h based on the crude ¹H NMR and mass spectrum although the crude yield was less than 50%. It was noticed that NaBH₄ was used to reduce the ester group (isolated or conjugated to C=N bond) to the hydroxyl group in the mixture of CH₃OH and THF (1:1 ratio) in good to excellent yield.^{157, 166, 213} Thus the reaction of **64d** with NaBH₄ in the mixture of CH₃OH and THF (1:1 ratio) under different conditions was studied. The results are summarized in Figure 32 and Table 7.

When **64d** was treated with 1.1eq. NaBH₄ at room temperature for 7h, three products **80**, **81** and **92** were obtained (Table 7, Entry 1), indicating that the transesterification competed with the reduction of the ester groups conjugated to the C=N bond. Increasing the equivalents of NaBH₄ suppressed the transesterification but resulted in the reduction of both ester groups of **64d**. The use of 3.8eq. NaBH₄ (Table 7, Entry 2) furnished two products **80** and **93** in 39% and 27%, respectively. Further increase of the equivalents of NaBH₄ reduced the yield of **80** and increased the yield of **93** as evidenced

by the result that the use of 8eq. NaBH₄ (Table 7, Entry 3) provided the two products **80** and **93** in 30% and 35% yield respectively and the use of 22eq. NaBH₄ (Table 7, Entry 4) provided mainly **93** based on TLC and the crude ¹H NMR. Thus the reaction outcome is mainly affected by the amount of NaBH₄ used in the reduction. Either **80** or **93** can be the major product depending on the amount of NaBH₄ used in the reduction. The use of fewer equivalents of NaBH₄ favors the formation of **80**. The opposite is true for **93**.

Figure 32. Reduction of 2-pyrazoline **64d** with NaBH₄ and protection of the alcohol **80** as a silyl ether

Table 7. Reduction of **64d** with NaBH₄ in MeOH / THF

Entry	No.# of NaBH ₄	Temperature	Reaction time (h)	Results
1	1.1	R.T.	7	80 , 31% yield; 81 , 32% yield; 92 , 4% yield.
2	3.8	0 °C	5	80 , 39% yield; 93 , 27% yield.
3	8	0 °C	5	80 , 30% yield; 93 , 35% yield.
4	22	R.T.	21h	The product mostly is 93 based on crude ¹ H-NMR & TLC analysis.

Similar to **64d**, the aldehyde **84** (Figure 29) was converted to **80** and **93** in about 1:1 ratio based on the crude ^1H NMR spectrum when it was treated with 2.5eq. NaBH_4 in ethanol at room temperature for 18h, indicating that the aldehyde group conjugated to the $\text{C}=\text{N}$ bond can not be easily distinguished from the ester group by NaBH_4 .

Since the $\text{C}=\text{N}$ bond of **80** and **93** is isolated, the reduction of **80** and **93** was then investigated. Compound **93** did not change when it was treated with 5eq. sodium borohydride in methanol at room temperature for 5h and was converted to the intractable materials when the reaction mixture was refluxed for 3h based on TLC analysis and the crude ^1H NMR. The reaction of **80** with superhydride was then conducted.¹⁵⁷ To prevent the likely participation of the OH group in the reduction process, **80** was converted to the silyl ether **94** (Figure 32) in 90% yield by treatment with tributyltrimethylsilyl chloride in the presence of imidazole in methylene chloride.²¹⁴⁻²¹⁶ The silyl ether **94** was subsequently treated with 1.1eq. superhydride in methylene chloride at $-78\text{ }^\circ\text{C}$ for 5.5h. However, in contrast to the reaction of **82** with superhydride to afford the pyrazolidine **83** (Figure 29),¹⁵⁷ no reduction occurred and some of **94** was converted into **80** via the removal of the silyl group along with some recovered starting silyl ether **94** based on TLC analysis, the crude ^1H NMR and mass spectrum.

To determine if the isolated $\text{C}=\text{N}$ double bond can be reduced with superhydride, my attention was turned to the model compound **95** which contains only one ester group. Thus cycloaddition of ethyl acrylate with diazomethane in ethyl ether at room temperature overnight followed by treatment of the crude cycloadduct with CbzCl furnished **95** in 93% yield in two steps (Figure 33), which was converted to the alcohol **96** in 76% yield by treatment with 3eq. NaBH_4 in the mixture of methanol and THF (1:1

ratio) at room temperature for 5h. The alcohol **96** was then converted into the silyl ether **97** in 72% yield by treatment with tributyldimethylsilyl chloride in the presence of imidazole in methylene chloride overnight. Treatment of the silyl ether **97** with 2.5eq. superhydride in methylene chloride from -78 °C to room temperature for 22h followed by exposure of the crude reduction product to CbzCl achieved the reduction of the isolated C=N bond of **97** to afford the pyrazolidine **98** in 74% yield in two steps. I noted that the same reduction reaction followed by treatment of the crude reduction product with 2eq. (Boc)₂O in the presence of NEt₃ and 4-dimethylaminopyridine in CH₂Cl₂ did not afford the expected BOC-protected pyrazolidine compound, most likely due to the steric hindrance exerted by the neighboring bulky silyl group since this condition successfully resulted in the formation of the BOC derivative of the 2-pyrazoline **64b**.

Reagents: (a) i. Et₂O, R.T., overnight; ii. CbzCl. (b) NaBH₄, MeOH/THF. (c) TBDMSCl (3.5eq.), Imidazole (3.5eq.), CH₂Cl₂, overnight. (d) i. LiBEt₃H, -78 °C - R.T., CH₂Cl₂; ii. CbzCl

Figure 33. Conversion of 2-pyrazoline **95** to the pyrazolidine compound **98**

The failure to reduce the silyl ether **94** with superhydride, structurally similar to the silyl ether **97**, is not fully understood and further experiments need to be conducted to determine if **94** can be reduced with superhydride. This was not done since the 2-

pyrazoline **80** was successfully reduced to the corresponding pyrazolidine compound with NaBCNH₃/HOAc reducing system (Figure 34). when 2-pyrazoline **80** was treated with 6eq. NaBCNH₃^{163, 173, 175, 176} in acetic acid at room temperature for 6h followed by treatment of the crude reduction product with CbzCl, three Cbz protected pyrazolidine products **99**, **100** and **101** were obtained in 10%, 39% and 11% yield in two steps, respectively. The relative configuration of the 3, 4-substituents of **99** and **100** is assigned as *trans* and that of **101** as *cis* on the basis of their NOESY spectrum. Thus 2-pyrazoline **80** was successfully reduced to the corresponding pyrazolidine compound in good yield (~61% for two steps) in 82% diastereoselectivity. It was noted that nothing was obtained when the reduction mixture was quenched with 1M NaOH solution¹⁷⁵ instead of saturated potassium carbonate solution.¹⁶³

Encouraged by the successful reduction of the C=N bond of **80**, the model compound **95** (Figure 33) was attempted to be treated with NaBCNH₃ in acetic acid to reduce its C=N bond. Unfortunately, no reduction occurred and most of the starting **95** stayed intact based on the TLC analysis, the crude ¹H NMR and mass spectrum after the model compound **95** was treated with 26eq. NaBCNH₃ in acetic acid at room temperature for 29h or 26eq. NaBCNH₃ in acetic acid at reflux for 17h. When the mixture of **65c** and **65d** was treated with 26eq. NaBCNH₃ in acetic acid at room temperature for 48h, no change of the starting mixture was observed based on TLC analysis. When additional 10eq. NaBCNH₃ was added to this reaction mixture and the resulting mixture was refluxed for 6h, no reduction occurred and most likely the Cbz groups of **65c** and **65d** (Figure 24) were replaced by the acetyl group based on the crude ¹H NMR and mass spectra. Based on the above observations, it was indicated that the C=N double bonds of

Conditions: i. 6eq. NaBCNH₃, AcOH; ii. 2eq. CbzCl, 2eq. NaHCO₃, 1:1 CH₂Cl₂/H₂O

Figure 34. Reduction of 2-pyrazolines with NaBCNH₃/HOAc

64d and its related compounds are resistant to the common C=N double bond reducing agents due to its conjugation with the carbonyl group or phenyl group.

As expected, the protected 2-pyrazoline **69f** was reduced to the corresponding pyrazolidine compound **102** (Figure 34) in 41% yield in two steps after it was treated with 6eq. NaBCNH₃ in acetic acid for 5h followed by the treatment of the crude cycloadduct mixture with 1.1eq. CbzCl in the presence of NaHCO₃ in the mixture of CH₂Cl₂/H₂O (1:1). I noted that the treatment of the crude cycloadduct mixture with 2eq. (Boc)₂O in the presence of NEt₃ and 4-dimethylaminopyridine in CH₂Cl₂ for 18h¹⁵⁷ failed to afford the expected protected pyrazolidine compound and resulted in the formation of intractable materials. One of the synthetic targets, compound **60** (Figure 18), will be obtained upon deprotection of the pyrazolidine compound **102**. Obviously, compound **100** can also be converted to the target compound **60** (Figure 18) after functional group transformations.

Figure 35. Possible routes to access the target compounds **58** and **59**

2.2.3 Possible routes to access the target compounds **58** and **59**

The strategy used for the conversion of the 2-pyrazoline **64d** to the pyrazolidine **100** can be applied in the synthesis of the target compounds **58** and **59**. Shown in Figure 35, selective reduction of the ester group conjugated to the C=N bond of the 2-pyrazoline

66c will lead to formation of the alcohol **103**, which can be converted to the pyrazolidine compound **104** after protection of the hydroxyl group of **103** followed by reduction of the C=N bond with NaBCNH₃/HOAc reducing system. The target compound **58** will be obtained after functional group transformations of the pyrazolidine compound **104**. Similar route can be used for the synthesis of the target compound **59** (Figure 35).

2.3 1,3-dipolar cycloadditions of trimethylsilyldiazomethane revisited

Trimethylsilyldiazomethane has been most frequently used as a source of carbene in the synthesis of Me₃Si-substituted cyclopropanes²¹⁷ and has enjoyed only limited synthetic utility in 1, 3-dipolar cycloadditions. In fact, Seyferth reported that among a series of dipolarophiles examined, only acrylonitrile reacted with TMS diazomethane to produce a cycloadduct in a synthetically useful yield.^{218, 219} Recently, with the commercial availability of TMS diazomethane, it has become somewhat more popular as a synthetic reagent, particularly in the preparation of novel amino acid analogs.^{163, 220}

As stated in Section 1.7 and shown in Figure 22, 1, 3-dipolar cycloadditions of diazoalkanes and α,β -unsaturated esters yield 1-pyrazolines, with the regioselectivity being such that the terminal nitrogen of the diazoalkane attacks the α -carbon of the ester, which tend to be unstable and isomerize to typically yield the conjugated 2-pyrazoline.

A survey of the recent literature indicates that the regioselectivity of the double bond isomerization of TMS substituted 1-pyrazolines is variable and at first glance, unpredictable. Carreira and Kanemasa have reported the dipolar cycloaddition of TMSCHN₂ with camphorsultam and oxazolidinone derivatives, respectively.^{163, 220, 221} These 1-pyrazoline products undergo proteodesilylation to yield 2-pyrazolines (**107a** and

107b) or loss of the proton α to TMS (**108**) (Figure 36). On the other hand, Barluenga et al.¹⁵⁷ recently described the cycloaddition of the menthol ester of *trans*-cinnamic acid to produce the conjugated 2-pyrazoline **109**, with retention of the TMS group. Moreover, it was found that the cycloaddition of trimethylsilyldiazomethane to diethyl glutaconate yielded different products under different conditions (See Section 2.1 and Ref.²²²).

Figure 36. Some 2-pyrazolines from the 1,3-dipolar cycloaddition of TMSCHN₂ and α , β -unsaturated esters

In an effort to develop a mechanistic rationale for the isomerization which could account for the products obtained, a systematic survey of dipolar cycloadditions between TMS diazomethane and α , β -unsaturated dipolarophiles was undertaken by me and another doctoral student, Dragan Simovic. The dipolarophiles vary in terms of the size of the electron withdrawing group (EWG) and the substituents at the β -carbon. The results derived from my work are summarized in Table 8. Because 1 and 2-pyrazolines oxidize readily to pyrazoles, the cycloaddition products were immediately converted to the Cbz

derivatives of the 2-pyrazolines. As shown in Table 8, three products may be obtained from the isomerization of the intermediate 1-pyrazoline; the conjugated 2-pyrazoline (**a**), the desilylation product (**c**), and in some instances, we also observed a third type of 2-pyrazoline (**b**) that results from a loss of the proton α to the TMS group.

In contrast to earlier reports, it was found that the acrylates react with trimethylsilyldiazomethane at ambient temperature to provide cycloadducts in good to excellent yield (Entries 1-4). Products **b** and **c** were formed exclusively with little selectivity.

Methyl acrylate, derived from the treatment of acryloyl chloride with methanol in toluene in the presence of pyridine, was reacted with trimethylsilyldiazomethane in a mixture of toluene and hexane at room temperature for 8.5h followed by exposure of the crude cycloadduct mixture to CbzCl to afford the two protected products **110b** and **110c** in 12% and 8% yield starting from acryloyl chloride respectively (Table 8, Entry 1). In this reaction, the crude methyl acrylate was not purified due to the formation of the azeotrope of methanol and methyl acrylate and directly subjected to cycloaddition. Some of it was probably lost during workup, possibly responsible for the low overall yield.

The results from the reactions of *t*-butyl acrylate with trimethylsilyldiazomethane are not consistent. When it was reacted with 3eq. trimethylsilyldiazomethane in the mixture of toluene and hexane at room temperature for 22h followed by the treatment of the crude cycloadduct mixture with CbzCl, two products **111a** and **111c** were obtained in the yield of 1.5% and 72.5% in two steps, respectively (Table 8, Entry 3) after careful purification of the crude reaction product. Product **111a** was not stable and oxidized on standing as indicated by the high resolution

mass spectrum. When the same reaction was done the second time, two products **111b** and **111c** were obtained in the yield of 3% and 73% in two steps, respectively (Table 8, Entry 4). **111b** and **111c** were afforded in 31% and 40% yield, respectively (Table 8,

Table 8. Dipolar cycloadditions of TMS diazomethane and various dipolarophiles

Entry	Cmpd.	R	R'	Conditions	%Yield ^a	Product Ratio a:b:c
1	110	H	OMe	2eq. TMSCHN ₂ , PhCH ₃ /hexane (1:1), RT, 8.5 hr	20 ^b	0:60:40
2	111	H	O-tBu	3eq. TMSCHN ₂ , PhCH ₃ /hexane (1:1), RT, 22 hr	71	0:44:56
3	111	H	O-tBu	3eq. TMSCHN ₂ , PhCH ₃ /hexane (1:1), RT, 22 hr	74	2:0:98
4	111	H	O-tBu	3eq. TMSCHN ₂ , PhCH ₃ /hexane (1:1), RT, 22 hr	76	0:4:96
5	112	Me	OEt	3eq. TMSCHN ₂ , Toluene, reflux, 8.5 hr	49	16:16:68
6	112	Me	OEt	3eq. TMSCHN ₂ , PhCH ₃ /hexane (1:1), reflux, 8.5 hr	41	78:0:22
7	113	Ph	OEt	2eq. TMSCHN ₂ , PhCH ₃ /hexane (1:1), reflux, 8.5 hr	31	15:0:85

^aIsolated yield. ^bYield based on acryloyl chloride.

Entry 2) when the same reaction was done the third time. The above discrepancy is not understood. Probably the cycloaddition reaction and/or the isomerization of the 1-pyrazoline are so sensitive to the reaction conditions and/or the reaction workup conditions that subtle change of these conditions results in different product distributions. The cycloaddition of *t*-butyl acrylate with trimethylsilyldiazomethane followed by exposure of the crude cycloadduct mixture to acid ($\text{CF}_3\text{COOH}/\text{CH}_2\text{Cl}_2$)¹⁶³ afforded the expected 2-pyrazoline **111d** (Figure 37) in 47% yield via the proteodesilylation. The

Figure 37. Some other products from the dipolar cycloaddition of TMSCHN_2 and α,β -unsaturated esters

same process followed by treatment of the crude cycloadducts with CbzCl furnished the protected 2-pyrazoline **111c** in 38% overall yield.

The β -substituted dipolarophiles (Entries 5-7) require elevated temperatures or extended reaction times to provide cycloadducts in moderate yields. Ethyl crotonate was not sufficiently reactive in the cycloaddition with trimethylsilyldiazomethane at room temperature. When Ethyl crotonate was reacted with 3eq. trimethylsilyldiazomethane in the mixture of toluene and hexane (1:1 ratio) at room temperature for 23h followed by the

acidic workup ($\text{CF}_3\text{COOH} / \text{CH}_2\text{Cl}_2$),¹⁶³ the formation of the two compounds **112d** and **112f** (Figure 37) was indicated before acidic workup and the formation of the two pyrazoles **112d** and **112e** (Figure 37) after acidic workup by the crude mass spectrum. However, the crude yield was very low (less than 15%) before acidic workup and after acidic workup. Although the formation of the two compounds **112d** and **112f** (Figure 37) was indicated when the same cycloaddition was done in the presence of 2.1eq. $\text{BF}_3 \cdot \text{OEt}_2$ ¹⁶⁷ without acidic workup, low crude yield (30%) was obtained. A complicated mixture was obtained in low crude yield (45%) when the same reaction was conducted for 19 days without acidic work up. When ethyl crotonate was reacted with 3eq. trimethylsilyldiazomethane in the mixture of toluene and hexane at reflux for 22h followed by acidic workup ($\text{CF}_3\text{COOH}/\text{CH}_2\text{Cl}_2$),¹⁶³ the two pyrazoles **112d** and **112e** (Figure 37) were obtained in 6% and 33%, respectively. When the reaction was carried out at reflux for 8.5h followed by treatment with CbzCl , the conjugated 2-pyrazoline **112a** and the pyrazole **112g** (Figure 37), derived from the oxidation of the desilylated 2-pyrazoline **112f** (Figure 37), were obtained in 32% and 9% yield, respectively (Table 8, Entry 6). The relative configuration of the 4, 5 substituents of **112a** is assigned as *trans* on the basis of its NOESY spectrum. At the same time, two pyrazole compounds containing TMS group were also obtained in 5% and 4% yield, respectively. They turned out to be the regioisomers **112h** and **112i** (Figure 37) in terms of the position of the Cbz group, which is supported by the observation that they were converted to the same unprotected 2-pyrazole **112d** (Figure 37) upon the removal of the Cbz group by treatment with H_2 (1atm) in the presence of palladium on activated carbon. The specific regioisomers were not determined. However, when ethyl crotonate was reacted with 3eq.

trimethylsilyldiazomethane in toluene at reflux for 8.5h followed by treatment with CbzCl, the conjugated 2-pyrazoline **112a** and the 2-pyrazoline **112b** and the desilylated 2-pyrazoline **112c** were obtained in the ratio of 16:16:68 in 49% overall yield (Table 8, Entry 5).

Reaction of ethylcinnamate with 2eq. trimethylsilyldiazomethane in the mixture of toluene and hexane at reflux for 8.5h followed by treatment with CbzCl furnished the conjugated 2-pyrazoline **113a** in 5% yield, the desilylated 2-pyrazoline **113c** in 15% yield, the pyrazole **113d** (Figure 37) in 11% yield and the unprotected **113e** (Figure 37) in 2% yield (Table 8, Entry 7). Formation of **113b** was not observed.

The cycloaddition of ethyl 3, 3-dimethylacrylate with trimethylsilyldiazomethane was not achieved under different conditions. No reaction occurred when ethyl 3,3-dimethylacrylate was treated with 3eq. trimethylsilyldiazomethane in the mixture of toluene and hexane at room temperature for 22h based on the crude ^1H NMR and mass spectrum. Intractable material was obtained in low mass balance when the same reaction was carried out for 19 days, or when ethyl 3,3-dimethylacrylate was treated with 3eq. trimethylsilyldiazomethane in the mixture of toluene and hexane at reflux for 22h or at room temperature for 22h in the presence of 2.1eq. $\text{BF}_3\cdot\text{OEt}_2$ based on the crude ^1H NMR and mass spectra.

Based on the above observations, we speculate that product distributions of dipolar cycloadditions between TMS diazomethane and α , β -unsaturated dipolarophiles may be affected by the cycloaddition conditions, the reaction workup conditions, and/or the nature of the dipolarophiles. Probably the cycloaddition reaction and/or the isomerization of the 1-pyrazoline are so sensitive to the reaction conditions and/or the

reaction workup conditions that subtle change of these conditions results in different product distributions. The complete picture of the cycloaddition of trimethylsilyldiazomethane is under investigation.

3. Experimental Section

3.1 General procedure

All reactions involving air-sensitive compounds were carried out under a N₂ atmosphere. All common reagents and solvents were obtained from commercial suppliers and used without any further purification unless otherwise indicated. Solvents were dried by standard methods. Melting points were obtained using a MEL-TEMP capillary apparatus and are uncorrected. Thin layer chromatography was performed using ANALTECH 0.25mm silica gel GHLF plates. The chromatograms were visualized under ultraviolet light and/or by staining with a Ce/Mo reagent (prepared by dissolving phosphomolybdic acid (2g), cerium(iv) sulfate (1g), and conc. sulfuric acid (10 mL) in H₂O (90 mL)) or Iodine. Flash column chromatography was carried out on silica gel 60, 230 ± 240 mesh. Routine NMR measurements were recorded on Bruker AVANCE-400 or AVANCE-600 spectrometers. ¹H NMR: splitting pattern abbreviations are: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. ¹³C NMR: multiplicities were determined by DEPT; abbreviations are: q, CH₃ ; t, CH₂ ; d, CH; s, quaternary carbons. NOESY, COSY, HETCOR experiments were carried out on Bruker AVANCE-400 or AVANCE-600 spectrometers. High-resolution mass spectra (HRMS) were obtained with a VG 7070 mass spectrometer at University of California Riverside Mass Spectrometry Facility.

3.2 Materials and methods for molecular modeling study

The crystal structures (Protein Data Bank Id: 1YAE, 1TT1 and 1GR2) were downloaded as PDB files from the Protein Data Bank. Residue numbers referred to in this work correspond to those of iGluR6 downloaded from The National Center for

Biotechnology Information (accession number NP_062182 for rat iGluR6). A fixed shell of residues within 10 Å of the ligand was used as the receptor. To adapt the crystal structure to the AMBER* force field explicit hydrogen atoms were added, then deleted. All crystallographically determined water molecules were removed to simplify the calculations. Amino acid side chains and ligands were subjected to energy minimizations using the AMBER* united atom force field with the programs MacroModel (v. 6.5) and Batchmin (v. 6.5)²²³ running on a Silicon Graphics O₂ workstation. No solvation was employed; however, a distance dependent dielectric constant of 4r was used. Calculations for low-mode docking searches were performed as described above. In addition ligands were subjected to explicit translation/rotations, using the Batchmin MOLS command, randomly varied from 0.1–0.5 Å and 0–90° respectively, to ensure efficient orientational sampling of all allowed binding modes (LMCS/MOLS). Backbone movements of the receptor were not allowed. A global search approach was undertaken in which the starting geometry for each Monte Carlo/Energy Minimization (MC/EM) cycle is taken randomly from the pool of structures found thus far, and the one selected is the one that has been used the least number of times previously as a starting structure. A minimum of one thousand, but not more than five thousand, MC/EM cycles were performed for each ligand–receptor pair. Gradient convergence to 0.05 kJ/Ång-mol RMS and an energy window for saved structures of 25 kJ/mol was employed.

3.3 *Synthesis*

General Procedure for Cycloaddition Reactions: Reactions were performed under the conditions described in Table 1 and Table 8. Solvent was evaporated under reduced pressure and the residue was purified by either gravity or flash column chromatography

(silica gel) using ethyl acetate and hexane as the eluant. **General Procedure for**

Preparation of Cbz derivatives: CbzCl (1.1mmol) and a solution of NaHCO₃ (1.5mmol) in H₂O (5ml) were sequentially added to a solution of the corresponding 2-pyrazoline (1mmol) in CH₂Cl₂ (5ml) at room temperature. The resulting mixture was stirred at room temperature overnight. The organic layer was extracted with CH₂Cl₂ (3 x 15mL), dried over Na₂SO₄, and filtered. Solvents were evaporated under reduced pressure and the residue was purified by flash chromatography over silica gel with hexane/ethyl acetate as eluents. Protected 2-pyrazolines were obtained in good yields, and they proved to be air-stable.

4-Ethoxycarbonylmethyl-4, 5-dihydro-1*H*-pyrazole-3,5-dicarboxylic acid ethyl ester [64b]

Yellow oil; 90% yield. ¹H NMR (CDCl₃, 400MHz) δ 6.74 (bs, 1H), 4.26 (q, 2H, J= 7.1 Hz), 4.11 (q, 2H, J=7.2Hz), 3.81 (t, 1H, J= 10.2Hz), 3.64-3.56 (m, 1H), 3.45 (dd, 1H, J=6.9, 10.1Hz), 2.86 (dd, 1H, J=3.5, 16.4Hz), 2.46 (dd, 1H, J=10.4, 16.4Hz), 1.31 (t, 3H, J=7.1Hz), 1.22 (t, 3H, J=7.1Hz). ¹³C NMR (CDCl₃, 100.6MHz) δ 172.10 (s), 162.76 (s), 144.07(s), 61.40, 61.11, 55.09, 40.34, 35.35, 14.62, 14.52. LC MS (MH⁺) 229.

4-Ethoxycarbonylmethyl-4, 5-dihydro-pyrazole-1, 3-dicarboxylic acid 1-benzyl ester 3-ethyl ester [64d]

Light yellow solid; m.p. 91-94°C; 93% yield. ¹H NMR (CDCl₃, 400MHz) δ 7.45-7.28 (m, 5H), 5.29 (d, 1H, J=12.3Hz), 5.25 (d, 1H, J=12.3Hz), 4.32 (q, 2H, J=7.1Hz), 4.21 (s, 1H), 4.13 (q, 2H, J=7.1Hz), 3.75-3.82 (m, 2H), 2.83 (dd, 1H, J=2.9, 16.7Hz), 2.53 (dd, 1H,

$J=9.9, 16.8\text{Hz}$), 1.33 (t, 3H, $J=7.1\text{Hz}$), 1.23 (t, 3H, $J=7.1\text{Hz}$). ^{13}C NMR (CDCl_3 , 100.6MHz) δ 171.23 (s), 161.87 (s), 152.78 (s), 149.00 (s), 135.98 (s), 128.95, 128.94, 128.84, 68.68, 62.31, 61.47, 53.37, 41.36, 36.40, 14.58, 14.53. HRMS (DCI) calculated for $\text{C}_{18}\text{H}_{23}\text{N}_2\text{O}_6$ (MH^+) 363.1556; found 363.1567.

***trans*-4-Ethoxycarbonylmethyl-5-phenyl-3,4-dihydro-2H-pyrazole -3-carboxylic acid ethyl ester [65a, major isomer]**

Yellow oil; 73% yield. ^1H NMR (CDCl_3 , 400MHz) δ 7.35-7.26 (m, 5H), 4.77 (d, 1H, $J=6.4\text{Hz}$), 4.32-4.23 (m, 2H), 4.20-4.07 (m, 2H), 3.54 (ddd, 1H, $J=3.7, 6.3, 9.9\text{Hz}$), 2.85 (dd, 1H, $J=3.7, 16.2\text{Hz}$), 2.67 (dd, 1H, $J=9.8, 16.2\text{Hz}$). 1.34 (m, 3H), 1.21 (m, 3H). ^{13}C NMR (CDCl_3 , 100.6MHz) mixture of isomers, δ 171.80, 162.73, 141.41, 141.12, 129.30, 128.89, 128.57, 128.55, 126.42, 70.95, 61.48, 61.20, 49.90, 35.37, 14.64, 14.52. LC MS (MH^+) 305.

***trans* -4-Ethoxycarbonylmethyl-3-phenyl-4, 5-dihydro-pyrazole-1, 5-dicarboxylic acid 1-benzyl ester 5-ethyl ester [65c]**

Colorless oil; 68% yield. ^1H NMR (CDCl_3 , 400MHz) δ 7.40-7.20 (m, 10H), 5.25 (d, 2H, $J=4.1\text{Hz}$), 5.16 (d, 1H, $J=4.0\text{Hz}$), 4.36 (q, 2H, $J=7.1\text{Hz}$), 4.18 (q, 2H, $J=7.1\text{Hz}$), 3.65 (ddd, 1H, $J=3.7, 4.0, 10.0\text{Hz}$), 2.95 (dd, 1H, $J=3.4, 16.6\text{Hz}$), 2.67 (dd, 1H, $J=10.2, 16.6\text{Hz}$), 1.38 (t, 3H, $J=7.1\text{Hz}$), 1.25 (t, 3H, $J=7.1\text{Hz}$). ^{13}C NMR (CDCl_3 , 100.6MHz) δ 170.95 (s), 161.79 (s), 152.52 (s), 147.71 (s), 140.28 (s), 135.77 (s), 129.43, 128.81, 128.67, 128.59, 125.78, 69.15, 68.68, 62.48, 61.58, 51.70, 36.24, 14.60, 14.55. HRMS (DCI) calculated for $\text{C}_{24}\text{H}_{27}\text{N}_2\text{O}_6$ (MH^+) 439.1869; found 439.1855.

***cis* -4-Ethoxycarbonylmethyl-5-phenyl-4, 5-dihydro-pyrazole-1, 3-dicarboxylic acid
1-benzyl ester 3-ethyl ester [65d]**

Yellow oil; 55% yield. ^1H NMR (CDCl_3 , 400MHz) δ 7.32-7.27 (m, 7H), 7.07-7.05 (m, 3H), 5.65 (d, 1H, $J=12.2\text{Hz}$), 5.21 (d, 1H, $J=12.3$), 5.11 (d, 1H, $J=12.3\text{Hz}$), 4.36 (q, 2H, $J=7.1\text{ Hz}$), 4.27 (ddd, 1H, $J=3.5, 11.1, 12\text{Hz}$), 3.93 (q, 2H, $J=7.1\text{Hz}$), 3.03 (dd, 1H, $J=3.5, 17.5\text{Hz}$), 2.09 (dd, 1H, $J=11.0, 17.5\text{Hz}$), 1.39 (t, 3H, $J=7.1\text{Hz}$), 1.11 (t, 3H, $J=7.1\text{Hz}$). ^{13}C NMR (CDCl_3 , 100.6MHz) δ 171.66, 162.12, 152.36, 147.53, 135.90, 135.81, 129.10, 128.80, 128.76, 128.55, 128.45, 68.51, 66.63, 62.41, 61.14, 46.52, 32.19, 14.60, 14.39. HRMS (DCI) calculated for $\text{C}_{24}\text{H}_{27}\text{N}_2\text{O}_6$ (MH^+) 439.1869; found 439.1865.

***trans*-4-Ethoxycarbonylmethyl-4, 5-dihydro-1 *H*-pyrazole-3, 5-dicarboxylic acid
diethyl ester [66a]**

Yellow oil; yield: 49% (refluxing benzene) or 67% (refluxing toluene). ^1H NMR (CDCl_3 , 400MHz) δ 6.58 (bs, 1H), 4.28 (d, 1H, $J=4.5\text{Hz}$), 4.22 (q, 2H, $J=7.1\text{Hz}$), 4.15 (q, 2H, $J=7.1\text{Hz}$), 4.09 (q, 2H, $J=7.1\text{ Hz}$), 3.84 (ddd, 1H, $J=3.9, 4.4, 10.0\text{Hz}$), 2.77 (dd, 1H, $J=3.8, 15.8\text{Hz}$), 2.51 (dd, 1H, $J=10.0, 15.8\text{Hz}$), 1.27 (t, 3H, $J=7.1\text{Hz}$), 1.22 (t, 3H, $J=7.1\text{Hz}$), 1.19 (t, 3H, $J=7.1\text{Hz}$). ^{13}C NMR (CDCl_3 , 100.6MHz) δ 171.24(s), 171.21(s), 161.99(s), 143.41(s), 66.97, 62.42, 61.62, 61.25, 44.81, 35.01, 14.53, 14.46, 14.40. HRMS (DCI) calculated for $\text{C}_{13}\text{H}_{21}\text{N}_2\text{O}_6$ (MH^+) 301.1400, found 303.1404.

***trans* -4-Ethoxycarbonylmethyl-4, 5-dihydro-pyrazole-1, 3, 5-tricarboxylic acid 1-benzyl ester 3, 5-diethyl ester [66c]**

Yellow oil; 87% yield. ^1H NMR (CDCl_3 , 400MHz) δ 7.42-7.32 (m, 5H), 5.37-5.28 (m, 2H), 4.72 (d, 1H, $J=5.5\text{Hz}$), 4.35 (q, 2H, $J=7.1\text{Hz}$), 4.16 (q, 4H, $J=7.1\text{Hz}$), 3.84-3.79 (m, 1H), 2.94 (dd, 1H, $J=3.7, 16.3\text{Hz}$), 2.71 (dd, 1H, $J=8.8, 16.3\text{Hz}$), 1.37 (t, 3H, $J=7.1\text{Hz}$), 1.25 (t, 6H, $J=7.1\text{Hz}$). ^{13}C NMR (CDCl_3 , 100.6MHz) δ 170.24 (s), 169.13(s), 161.38 (s), 152.37(s), 147.22 (s), 135.68 (s), 128.94, 69.08, 66.02, 62.61, 61.71, 47.30, 36.09, 14.56, 14.49, 14.38. HRMS (DCI) calculated for $\text{C}_{21}\text{H}_{27}\text{N}_2\text{O}_8$ (MH^+) 435.1767; found 435.1758.

Ethyl 3-(4-ethyl carboxymethylene-5-phenyl) pyrazole carboxylate [67]

Yellow oil; ^1H NMR (CDCl_3 , 400MHz) δ 9.37 (bs, 1H), 7.51-7.38 (m, 5H), 4.32 (q, 2H, $J=7.1\text{Hz}$), 4.17 (q, 2H, $J=7.1\text{Hz}$), 3.86 (s, 2H), 1.32 (t, 3H, $J=7.1\text{Hz}$), 1.24 (t, 3H, $J=7.1\text{Hz}$). ^{13}C NMR (CDCl_3 , 100.6MHz) δ 171.64, 161.44, 130.08, 129.32, 128.56, 61.55, 61.39, 30.44, 14.57, 14.49. HRMS (DCI) calculated for $\text{C}_{16}\text{H}_{19}\text{N}_2\text{O}_4$ (MH^+) 303.1345, found 303.1351.

3, 4-cis-4, 5-trans-3-Ethoxycarbonylmethyl-1, 5-diaza-bicyclo[3.1.0]hexane-2, 4, 6-tricarboxylic acid triethyl ester [68]

Yellow oil; 15% yield (refluxing benzene) or 22% (refluxing toluene). ^1H NMR (CDCl_3 , 400MHz) δ 4.49 (d, 1H, $J=18.2\text{Hz}$), 4.41 (d, 1H, $J=8.8\text{Hz}$), 4.36-4.07 (m, 10H), 3.17 (dd, 1H, $J=3.6, 16.4\text{Hz}$), 2.71 (dd, 1H, $J=10.2, 16.4\text{Hz}$), 1.34 (t, 3H, $J=7.1\text{Hz}$), 1.31 (t, 3H, $J=7.1\text{Hz}$), 1.27 (t, 3H, $J=7.1\text{Hz}$), 1.25 (t, 3H, $J=7.1\text{Hz}$). ^{13}C NMR (CDCl_3 , 100.6MHz) δ

171.54 (s), 169.72 (s), 169.68 (s), 162.15 (s), 140.20 (s), 71.13, 62.30, 61.65, 61.63, 61.25, 52.71, 46.38, 36.59, 14.74, 14.57, 14.53, 14.51. HRMS (DCI) calculated for $C_{17}H_{26}N_2O_8$ (MH^+) 387.1767; found 387.1758.

Ethyl 3-(4-ethyl carboxymethylene-5-trimethylsilyl) pyrazole carboxylate [69a] and Ethyl 4-(3-ethyl carboxymethylene-5-trimethylsilyl) pyrazole carboxylate [69b]

Yellow oil; 58 % overall yield. **Major isomer;** 1H NMR ($CDCl_3$, 400MHz) δ 7.32 (bs, 1H), 4.36 (q, 2H, $J=7.1$ Hz), 4.17 (q, 2H, $J=7.1$ Hz), 3.90 (s, 2H), 1.36 (t, 3H, $J=7.1$ Hz), 1.26 (t, 3H, $J=7.1$ Hz), 0.40 (s, 9H). ^{13}C NMR ($CDCl_3$, 100.6MHz) δ 171.19, 161.97, 145.15, 140.65, 124.21, 61.58, 61.39, 31.04, 14.61, 14.57, 1.43. HRMS (DCI) calculated for $C_{13}H_{23}N_2O_4Si$ (MH^+) 299.1427, found 299.1416. **Minor isomer;** 1H NMR ($CDCl_3$, 400MHz) δ 9.08 (bs, 1H), 4.38 (q, $J=7.2$ Hz, 2H), 4.18 (q, $J=7.1$ Hz, 2H), 3.84 (s, 2H), 1.38 (t, $J=7.1$ Hz, 3H), 1.27 (t, $J=7.2$ Hz, 3H), 0.36 (s, 9H). ^{13}C NMR ($CDCl_3$, 100.6MHz) δ 171.53, 161.83, 144.24, 141.61, 124.95, 61.50, 61.35, 30.58, 14.61, -0.94. HRMS (DCI) calculated for $C_{13}H_{23}N_2O_4Si$ (MH^+) 299.1427, found 299.1437.

4-Ethoxycarbonylmethyl-3-trimethylsilanyl-4,5-dihydro-pyrazole-1,5-dicarboxylic acid 1-benzyl ester 5-ethyl ester [69c]

Yellow oil; 1H NMR ($CDCl_3$, 400MHz) δ 7.39-7.21 (m, 5H), 5.25-5.10 (m, 2H), 4.37 (d, 1H, $J=4.8$ Hz), 4.09 (q, 2H, $J=7.2$ Hz), 4.08 (q, 2H, $J=7.2$ Hz), 3.53-3.49 (m, 1H), 2.60 (dd, 1H, $J=3.6, 15.9$ Hz), 2.32 (dd, 1H, $J=9.6, 15.9$ Hz), 1.18 (t, 6H, $J=7.0$ Hz), 0.20 (s, 9H);

^{13}C NMR (CDCl_3 , 100.6MHz) δ 171.63(s), 171.52 (s), 164.53(s), 153.81(s), 129.99, 129.74, 69.30, 64.59, 63.20, 62.82, 54.81, 37.94, 15.63, 15.56, 0.00. HRMS (DCI / NH_3) calcd for $\text{C}_{21}\text{H}_{31}\text{N}_2\text{O}_6\text{Si}$ (MH^+) 435.1951; found 435.1951.

**4-Ethoxycarbonylmethyl-4,5-dihydro-pyrazole-1,5-dicarboxylic acid 1-benzyl ester
5-ethyl ester [69d]**

Yellow oil; ^1H NMR (CDCl_3 , 400MHz) δ 7.36-7.27 (m, 5H), 6.87 (s, 1H), 5.30-5.19 (m, 2H), 4.42 (d, 1H, $J=5.6\text{Hz}$), 4.16 (q, 4H, $J=7.2\text{Hz}$), 3.60-3.55 (m, 1H), 2.60 (d, 2H, $J=7.6\text{Hz}$), 1.24 (t, 6H, $J=7.2\text{Hz}$); ^{13}C NMR (CDCl_3 , 100.6MHz) δ 170.23(s), 169.67(s), 152.76(s), 147.04, 135.98(s), 128.81, 128.64, 68.38, 63.12, 62.27, 61.60, 48.72, 36.61, 14.42, 14.28. HRMS (DCI / NH_3) calcd for $\text{C}_{18}\text{H}_{23}\text{N}_2\text{O}_6$ (MH^+) 363.1556; found 363.1562.

**4-Ethoxycarbonylmethyl-5-trimethylsilanyl-4,5-dihydro-pyrazole-1,3-dicarboxylic
acid diethyl ester [69e]**

Yellow oil; ^1H NMR (CDCl_3 , 400MHz) δ 4.22 (q, 2H, $J=6.9\text{Hz}$), 4.20 (q, 2H, $J=6.9\text{Hz}$), 4.02 (q, 2H, $J=7.2\text{Hz}$), 3.69 (d, 1H, $J=5.6\text{Hz}$), 3.55-3.50 (m, 1H), 2.64 (dd, 1H, $J=3.4$, 16.2Hz), 2.44 (dd, 1H, $J=9.4$, 16.2Hz), 1.25 (t, 3H, $J=7.0\text{Hz}$), 1.24 (t, 3H, $J=7.2\text{Hz}$), 1.14 (t, 3H, $J=7.0\text{Hz}$), 0.00 (s, 9H); ^{13}C NMR (CDCl_3 , 100.6MHz) δ 170.63(s), 161.76(s), 152.74(s), 148.19(s), 62.92, 61.87, 60.94, 51.73, 43.64, 37.56, 14.55, 14.17, - 2.84. HRMS (DCI / NH_3) calcd for $\text{C}_{16}\text{H}_{29}\text{N}_2\text{O}_6\text{Si}$ (MH^+) 373.1795; found 373.1792.

4-Ethoxycarbonylmethyl-4,5-dihydro-pyrazole-1,5-dicarboxylic acid diethyl ester

[69f] Yellow oil; ^1H NMR (CDCl_3 , 400MHz) δ 6.80 (s, 1H), 4.36 (d, 1H, $J=5.6\text{Hz}$), 4.16 (q, 2H, $J=7.2\text{Hz}$), 4.11 (q, 2H, $J=7.1\text{Hz}$), 3.55-3.49 (m, 1H), 2.56 (d, 2H, $J=7.2\text{Hz}$), 1.21 (t, 3H, $J=7.0\text{Hz}$), 1.20 (t, 3H, $J=7.0\text{Hz}$); ^{13}C NMR (CDCl_3 , 100.6MHz) δ 169.93(s), 169.42(s), 152.67(s), 146.40, 62.75, 62.47, 61.94, 61.30, 48.38, 36.36, 14.49, 14.10, 14.06. HRMS (DCI / NH_3) calcd for $\text{C}_{13}\text{H}_{21}\text{N}_2\text{O}_6$ (MH^+) 301.1400; found 301.1401.

4-Ethoxycarbonylmethyl-pyrazole-1,3-dicarboxylic acid 1-benzyl ester 3-ethyl ester **[70]**

Yellow oil; ^1H NMR (CDCl_3 , 400MHz) δ 8.19 (s, 1H), 7.49-7.46 (m, 2H), 7.41-7.37 (m, 3H), 5.48 (s, 2H), 4.40 (q, 2H, $J=7.1\text{Hz}$), 4.17 (q, 2H, $J=7.1\text{Hz}$), 3.80 (s, 2H), 1.38 (t, 3H, $J=7.1\text{Hz}$), 1.25 (t, 3H, $J=7.1\text{Hz}$). ^{13}C NMR (CDCl_3 , 100.6MHz) δ 170.78(s), 162.17(s), 149.08(s), 146.33(s), 134.34(s), 132.34, 129.55, 129.47, 129.16, 119.62 (s), 70.96, 61.97, 61.52, 30.44, 14.62, 14.56. LCMS (MH^+) 361.

4-Ethoxycarbonylmethyl-4,5-dihydro-pyrazole-1,3-dicarboxylic acid diethyl ester **[71]**

Aqueous saturated K_2CO_3 solution (3ml) and ClCOOEt (0.16mL, 1.69mmol) were sequentially added to a solution of the pyrazoline **64b** (193mg, 0.85mmol) in CH_3CN (3mL) at room temperature. The resulting mixture was stirred overnight. The organic layer was extracted with ethyl acetate, dried over Na_2SO_4 and filtered. Solvents were evaporated and the residue was purified by column chromatography using silica gel with hexane/ethyl acetate 80/20 and 65/35 as eluents to furnish the 2-pyrazoline **71** (188mg,

74%) as yellow solid. ^1H NMR (CDCl_3 , 400MHz) δ 4.33 (q, 2H, $J=7.0\text{Hz}$), 4.31 (q, 2H, $J=7.0\text{Hz}$), 4.24-4.21 (m, 1H), 4.15 (q, 2H, $J=7.1\text{Hz}$), 3.83-3.74 (m, 2H), 3.00 (dd, 1H, $J=3.1, 16.8\text{Hz}$), 2.54 (dd, 1H, $J=9.6, 16.8\text{Hz}$), 1.35 (t, 3H, $J=7.2\text{Hz}$), 1.34 (t, 3H, $J=7.2\text{Hz}$), 1.25 (t, 3H, $J=7.2\text{Hz}$). ^{13}C NMR (CDCl_3 , 100.6MHz) δ 171.22(s), 161.89(s), 152.90(s), 148.65(s), 63.33, 62.26, 61.43, 53.28, 41.24, 36.41, 14.91, 14.53, 14.50. LRMS (MH^+) 301.

1-Benzoyl-4-ethoxycarbonylmethyl-4,5-dihydro-1H-pyrazole-3-carboxylic acid ethyl ester [72]

To a stirred solution of 2-pyrazoline **64b** (1.90g, 8.33mmol) in THF- H_2O (1:1, 20mL) at 0°C was added NaHCO_3 (1.34 g, 16.0mmol). The mixture was stirred and cooled to 0°C . To this mixture was slowly added benzoyl chloride (1.29g, 9.17mmol). The resulting mixture was stirred at R.T. overnight and extracted with CH_2Cl_2 (3x20 mL). The combined organic layers were dried over Na_2SO_4 and solvents removed under vacuum. The oily residue was purified by column chromatography on silica gel, with hexane/ethyl acetate 85/15 and 65/35 as eluents, yielding the amide **72** (2.71g, 98% yield) as yellow oil. ^1H NMR (CDCl_3 , 400MHz) δ 7.92-7.90 (m, 2H), 7.52-7.40 (m, 3H), 4.43 (dd, 1H, $J=12.0, 12.6\text{Hz}$), 4.32 (q, 2H, $J=7.1\text{Hz}$), 4.17 (q, 2H, $J=7.2\text{Hz}$), 4.03 (dd, 1H, $J=7.2, 12.6\text{Hz}$), 3.84-3.76 (m, 1H), 3.00 (dd, 1H, $J=3.6, 16.8\text{Hz}$), 2.66 (dd, 1H, $J=9.6, 16.8\text{Hz}$), 1.33 (t, 3H, $J=7.2\text{Hz}$), 1.26 (t, 3H, $J=7.2\text{Hz}$). ^{13}C NMR (CDCl_3 , 100.6MHz) δ 170.79(s), 167.84(s), 161.47(s), 149.79(s), 132.74(s), 131.69, 130.13, 127.91, 61.95, 61.17, 52.69, 40.00, 36.14, 14.16, 14.11. LRMS (MH^+) 333.

1-Acetyl-4-ethoxycarbonylmethyl-4,5-dihydro-1H-pyrazole-3-carboxylic acid ethyl ester [73] 2-Pyrazoline **64b** (1.8 g, 7.9mmol) was treated with acetyl chloride (0.7g, 8.9mmol) in THF-H₂O (1:1, 20mL) as described for **72** to furnish the amide **73** (1.96g, 92% yield) as yellow oil. ¹H NMR (CDCl₃, 400MHz) δ 4.29 (q, 2H, J=7.1Hz), 4.17-4.12 (m, 1H), 4.08 (q, 2H, J=7.1Hz), 3.77-3.66 (m, 2H), 2.89 (dd, 1H, J=3.2, 16.8Hz), 2.52 (dd, 1H, J=9.2, 16.8Hz), 2.13 (s, 3H), 1.31 (t, 3H, J=7.0Hz), 1.19 (t, 3H, J=7.0Hz). ¹³C NMR (CDCl₃, 100.6MHz) δ 170.71(s), 170.48(s), 161.29(s), 148.94(s), 61.97, 61.11, 51.31, 40.74, 36.14, 21.28, 14.13, 14.11. LRMS (MH⁺) 271.

4-Ethoxycarbonylmethyl-3-hydroxymethyl-4,5-dihydro-pyrazole-1-carboxylic acid benzyl ester [80]

To a stirred solution of the 2-pyrazoline **64d** (1.01g, 2.79mmol) in 20ml of 1:1 solution of THF/CH₃OH at room temperature was added sodium borohydride (116mg, 3.07mmol). The reaction mixture was stirred for 7h, till TLC analysis showed complete disappearance of the starting material. The reaction was then carefully hydrolyzed with saturated aqueous ammonium chloride solution (15ml). The resulting mixture was extracted with ethyl acetate. The combined organic extracts were washed with saturated aqueous sodium bicarbonate solution and brine, dried over Na₂SO₄, filtered and concentrated by rotary evaporation. The residue was purified by column chromatography on silica gel, with hexane/ethyl acetate 80/20, 65/35, 50/50 and 20/80 as eluents, to give the alcohol **80** (277mg, 31% yield) as yellow oil. ¹H NMR (CDCl₃, 400MHz) δ 7.31-7.19 (m, 5H), 5.13 (s, 2H), 4.32 (d, 1H, J= 14.6Hz), 4.25 (d, 1H, J= 14.8Hz), 4.09-3.98 (m, 3H), 3.60-3.50 (m, 2H), 2.72 (dd, 1H, J=4.4, 16.6Hz), 2.40 (dd, 1H, J=8.8, 16.6Hz), 1.25

(t, 3H, J=7.1Hz) ^{13}C NMR (CDCl_3 , 100.6MHz) δ 171.24(s), 160.06 (s), 152.92(s), 136.03(s), 128.50, 128.28, 128.26, 67.63, 61.13, 58.60, 51.46, 41.83, 35.69, 14.09. HRMS (DCI) calculated for $\text{C}_{16}\text{H}_{21}\text{N}_2\text{O}_5$ (MH^+) 321.1450; found 321.1461.

4-Ethoxycarbonylmethyl-4,5-dihydro-pyrazole-1,3-dicarboxylic acid 1-benzyl ester 3-methyl ester [81] The 2-pyrazoline **64d** was treated with NaBH_4 as described for **80** to give **81** (311mg, 32% yield) as light yellow powder, m.p. 98-100°C. ^1H NMR (CDCl_3 , 400MHz) δ 7.31-7.27(m, 5H), 5.30-5.23 (m, 2H), 4.25-4.17 (m, 1H), 4.11 (q, 2H, J=7.1Hz), 3.83 (s, 3H), 3.81-3.72 (m, 2H), 2.96 (dd, 1H, J=3.2, 16.8Hz), 2.53 (dd, 1H, J=9.6, 16.8Hz), 1.21(t, 3H, J=7.1Hz) ^{13}C NMR (CDCl_3 , 100.6MHz) δ 171.21(s), 161.90 (s), 152.32(s), 148.16(s), 135.63(s), 128.50, 128.46, 128.39, 68.26, 61.01, 52.95, 52.49, 40.92, 35.93, 14.09. LRMS (MH^+) 349.

4-Ethoxycarbonylmethyl-3-formyl-4,5-dihydro-pyrazole-1-carboxylic acid benzyl ester [84]

A solution of superhydride (1.0M in THF, 1.0mL, 1.0 mmol) was added dropwise to a solution of the 2-pyrazoline **64d** (329mg, 0.91mmol) in the dry CH_2Cl_2 (10mL) at -78°C. The resulting mixture was stirred for 4.5h at -78°C, till TLC analysis showed complete disappearance of the starting material. The reaction was then hydrolyzed with 1M NaOH (5 mL) and the organic residue extracted with CH_2Cl_2 (3x15 mL). The combined organic layers were dried over Na_2SO_4 and solvents removed under vacuum. The oily residue was purified by column chromatography on silica gel, with hexane/ethyl acetate 85/15, 65/35 and 50/50 as eluents, yielding the aldehyde **84** (121mg, 42% yield) as yellow oil; ^1H

NMR (CDCl₃, 400MHz) δ 9.81 (s, 1H), 7.43-7.32 (m, 5H), 5.33 (d, 1H, J= 12.4Hz), 5.30 (d, 1H, J= 12.8Hz), 4.28 (t, 1H, J= 11.3Hz), 4.14(q, 2H, J=7.2Hz), 3.85-3.72 (m, 2H), 2.99 (dd, 1H, J=3.3, 17.0Hz), 2.53 (dd, 1H, J=9.4, 16.9Hz), 1.24 (t, 3H, J=7.1Hz) ¹³C NMR (CDCl₃, 100.6MHz) δ 187.41, 171.06(s), 155.77(s), 152.49(s), 135.65 (s), 129.04, 128.92, 69.07, 61.47, 54.16, 45.37, 35.74, 14.49. HRMS (DCI) calculated for C₁₆H₁₉N₂O₅ (MH⁺) 319.1294; found 319.1303.

4-Ethoxycarbonylmethyl-3-ethoxycarbonyloxymethyl-4,5-dihydro-pyrazole-1-carboxylic acid benzyl ester [86]

A solution of superhydride (1.0M in THF, 0.36mL, 0.36mmol) was added dropwise to a solution of the 2-pyrazoline **64d** (118.6mg, 0.33mmol) in the dry CH₂Cl₂ (10mL) at -78°C. The reaction mixture was stirred for 20min at -78°C and a solution of superhydride (1.0M in THF, 0.2mL, 0.2mmol) was added dropwise. The resulting mixture was allowed to warm to room temperature overnight, till TLC analysis showed complete disappearance of the starting material. The reaction was then hydrolyzed with 1M NaOH (5 mL) and the organic residue extracted with CH₂Cl₂ (3x10mL). The combined organic layers were dried over Na₂SO₄ and solvents removed under vacuum. The oily residue was dissolved in acetonitrile (5ml). To this solution was added aqueous saturated K₂CO₃ (3mL) and ClCOOEt (2equiv.). The reaction was stirred overnight. The resulting reaction mixture was extracted with methylene chloride. The combined organic extracts were washed with brine, dried over Na₂SO₄, filtered and concentrated by rotary evaporation. The resulting residue was purified by column chromatography on silica gel, with hexane/ethyl acetate 85/15 and 70/30 as eluents, affording the pyrazoline **86** (21mg,

16% yield starting from the pyrazoline **64d**) as yellow oil; ^1H NMR (CDCl_3 , 400MHz) δ 7.44-7.33 (m, 5H), 5.28 (s, 2H), 4.98-4.90 (m, 2H), 4.24 (q, 2H, $J=7.1\text{Hz}$), 4.19-4.14 (m, 3H), 3.75-3.61 (m, 2H), 2.80 (dd, 1H, $J=4.2, 16.6\text{Hz}$), 2.52 (dd, 1H, $J=9.3, 16.6\text{Hz}$), 1.33 (t, 3H, $J=7.1\text{Hz}$), 1.27 (t, 3H, $J=7.1\text{Hz}$) ^{13}C NMR (CDCl_3 , 100.6MHz) δ 171.03(s), 155.02 (s), 154.50(s), 153.12(s), 136.37(s), 128.92, 128.77, 128.70, 68.24, 65.12, 63.24, 61.58, 52.14, 42.19, 35.95, 14.59, 14.50. HRMS (DCI) calculated for $\text{C}_{19}\text{H}_{25}\text{N}_2\text{O}_7$ (MH^+) 393.1662; found 393.1657.

4-Ethoxycarbonylmethyl-3-formyl-4,5-dihydro-pyrazole-1,5-dicarboxylic acid 1-

benzyl ester 5-ethyl ester [89] A solution of superhydride (1.0M in THF, 0.44mL,

0.44mmol) was added dropwise to a solution of the 2-pyrazoline **66c** (174mg, 0.4mmol)

in the dry CH_2Cl_2 (10mL) at -78°C . The resulting mixture was stirred for 5h at -78°C , till

TLC analysis showed complete disappearance of the starting material. The reaction was

then hydrolyzed with 1M NaOH (5 mL) and the organic residue extracted with CH_2Cl_2

(3x15mL). The combined organic layers were dried over Na_2SO_4 and solvents removed

under vacuum. The oily residue was purified by column chromatography on silica gel,

with hexane/ethyl acetate 85/15, 65/35 and 50/50 as eluents, yielding the aldehyde **89**

(53mg, 34% yield) as yellow oil; ^1H NMR (CDCl_3 , 400MHz) δ 9.82 (s, 1H), 7.40-7.33

(m, 5H), 5.40-5.28 (m, 2H), 4.73 (d, 1H, $J=5.9\text{Hz}$), 4.15 (q, 4H, $J=7.1\text{Hz}$), 3.82-3.76 (m,

1H), 2.90 (dd, 1H, $J=3.9, 16.6\text{Hz}$), 2.73 (dd, 1H, $J=8.5, 16.7\text{Hz}$), 1.24 (t, 6H, $J=7.1\text{Hz}$)

^{13}C NMR (CDCl_3 , 100.6MHz) δ 186.86, 170.09, 168.93(s), 153.78 (s), 135.33 (s), 129.07,

129.03, 128.90, 69.46, 66.64, 62.70, 61.69, 45.37, 35.37, 14.46, 14.34. HRMS (DCI)

calculated for $\text{C}_{19}\text{H}_{23}\text{N}_2\text{O}_7$ (MH^+) 391.1505; found 391.1511.

3-Dimethoxymethyl-4-ethoxycarbonylmethyl-4,5-dihydro-pyrazole-1-carboxylic acid benzyl ester [90]

Method A: The aldehyde **84** (126mg, 0.396mmol) was dissolved in methanol (5ml) and 2, 2-dimethoxypropane (90.8mg, 0.872mmol) was added while stirring. The stirred solution is cooled to 0°C, then 15mg of p-toluenesulfonic acid monohydrate is added and the mixture is stirred at room temperature for 19h. After the volatile material is removed on a rotary evaporator, the remaining yellow oil is neutralized with 10 mL of aqueous saturated sodium bicarbonate solution and diluted with 15 mL of methylene chloride. The aqueous layer is separated and extracted with methylene chloride. The combined organic layers are washed with brine, dried over Na₂SO₄ and concentrated by rotary evaporation. The residual yellow oil is purified by column chromatography on silica gel, with hexane/ethyl acetate 70/30 as the eluent, yielding the dimethyl acetal **90** (40.4mg, 28% yield) as a yellow oil. **Method B:** The carbonyl compound **84** (92.4mg, 0.29mmol) was dissolved in methanol (4 mL), and bismuth nitrate (14.1mg, 0.029mmol) was added while stirring. After the starting material was consumed as indicated by TLC, the methanol was evaporated. The crude product was extracted with methylene chloride, washed with aqueous saturated sodium bicarbonate solution, and dried over Na₂SO₄, and the solvent was evaporated. The residual yellow oil is purified by column chromatography on silica gel, with hexane/ethyl acetate 70/30 as the eluent, yielding the dimethyl acetal **90** (30.7mg, 29% yield) as a yellow oil. ¹H NMR (CDCl₃, 400MHz) δ 7.42-7.28 (m, 5H), 5.30-5.26 (m, 2H), 4.98 (s, 1H), 4.18-4.10 (m, 3H), 3.70-3.62 (m, 2H), 3.42 (s, 3H), 3.41 (s, 3H), 3.02 (dd, 1H, J=2.8, 17.2Hz), 2.46 (dd, 1H, J=9.9, 17.2Hz), 1.24 (t, 3H, J=7.2Hz)

^{13}C NMR (CDCl_3 , 100.6MHz) δ 171.70(s), 156.43(s), 153.27(s), 136.52(s), 128.88, 128.74, 128.63, 68.06, 61.22, 55.87, 55.84, 52.37, 40.97, 36.39, 14.54. HRMS (DCI) calculated for $\text{C}_{18}\text{H}_{25}\text{N}_2\text{O}_6$ (MH^+) 365.1713; found 365.1707.

3-[1, 3] Dioxolan-2-yl-4-ethoxycarbonylmethyl-4,5-dihydro-pyrazole-1-carboxylic acid benzyl ester [91]

A 25mL round-bottomed flask was charged with the aldehyde **84** (235.7mg, 0.74mmol), benzene (10mL), 1, 2-ethanediol (92mg, 1.48mmol) and 15mg of p-toluenesulfonic acid monohydrate. The flask is attached to a water separator (Dean-Stark trap) and a reflux condenser fitted with a drying tube. The reaction mixture is refluxed for 7h until the starting material was consumed as indicated by TLC. The reaction mixture is cooled to room temperature, extracted successively with 1M sodium hydroxide solution and water, dried over Na_2SO_4 , and the solvent was evaporated. The residual yellow oil is purified by column chromatography on silica gel, with hexane/ethyl acetate 65/35 as the eluent, yielding the cyclic acetal **91** (129mg, 48% yield) as yellow oil. ^1H NMR (CDCl_3 , 400MHz) δ 7.43-7.33 (m, 5H), 5.59 (s, 1H), 5.32-5.25(m, 2H), 4.19-3.92 (m, 7H), 3.76-3.66 (m, 2H), 2.89 (dd, 1H, $J=3.5$, 17.2Hz), 2.49 (dd, 1H, $J=10.0$, 17.1Hz), 1.27 (t, 3H, $J=7.2\text{Hz}$) ^{13}C NMR (CDCl_3 , 100.6MHz) δ 171.57(s), 155.76(s), 153.18(s), 136.42(s), 128.91, 128.76, 128.67, 100.26, 68.20, 65.99, 65.71, 61.35, 52.89, 40.43, 36.79, 14.54. HRMS (DCI) calculated for $\text{C}_{18}\text{H}_{23}\text{N}_2\text{O}_6$ (MH^+) 363.1556; found 363.1545.

3-Hydroxymethyl-4-methoxycarbonylmethyl-4,5-dihydro-pyrazole-1-carboxylic acid benzyl ester [92]

The 2-pyrazoline **64d** was treated with NaBH₄ as described for **80** to give **92** (35mg, 4% yield) as colorless oil. ¹H NMR (CDCl₃, 400MHz) δ 7.43-7.28 (m, 5H), 5.17 (s, 2H), 4.44 (d, 1H, J= 14.6Hz), 4.38 (d, 1H, J= 14.6Hz), 4.19-4.12 (m, 1H), 3.72 (s, 3H), 3.70-3.63 (m, 2H), 2.82 (dd, 1H, J=5.0, 16.8Hz), 2.55 (dd, 1H, J=8.2, 16.8Hz). ¹³C NMR (CDCl₃, 100.6MHz) δ 171.71(s), 159.33 (s), 152.88(s), 136.05(s), 128.52, 128.35, 128.29, 67.72, 58.94, 52.19, 51.69, 41.71, 35.67. LRMS (MH⁺) 307.

4-(2-Hydroxy-ethyl)-3-hydroxymethyl-4,5-dihydro-pyrazole-1-carboxylic acid

benzyl ester [93] To a stirred solution of the 2-pyrazoline **64d** (2.08g, 5.71mmol) in 40ml of 1:1 solution of THF/CH₃OH at room temperature was added sodium borohydride (1.74g, 45.9mmol) The reaction mixture was stirred at 0 °C for 5h, until TLC analysis showed complete disappearance of the starting material. The reaction was then carefully hydrolyzed with saturated aqueous ammonium chloride solution (30ml). The resulting mixture was extracted with ethyl acetate. The combined organic extracts were washed with saturated aqueous sodium bicarbonate solution and brine, dried over Na₂SO₄, filtered and concentrated by rotary evaporation. The residue was purified by column chromatography on silica gel, with hexane/ethyl acetate 20/80, ethyl acetate/methanol 90/10, ethyl acetate/methanol 70/30 as eluents, to give the diol **93** (0.559g, 35% yield) as yellow oil. ¹H NMR (CDCl₃, 400MHz) δ 7.39-7.32 (m, 5H), 5.24 (s, 2H), 4.45-4.35 (m, 2H), 4.02 (t, 1H, J=11.0Hz), 3.76-3.71 (m, 1H), 3.66-3.61 (m, 2H), 3.48-3.40 (m, 1H), 2.00-1.96 (m, 1H), 1.71-1.68 (m, 1H). ¹³C NMR (CDCl₃, 100.6MHz) δ 162.51(s), 153.21(s), 136.00(s), 128.54, 128.30, 128.20, 67.66, 59.72, 58.35, 51.07, 42.68, 33.55. HRMS (DCI) calculated for C₁₄H₁₉N₂O₄ (MH⁺) 279.1345; found 279.1347.

3-(tert-Butyl-dimethyl-silanyloxymethyl)-4-ethoxycarbonylmethyl-4,5-dihydro-pyrazole-1-carboxylic acid benzyl ester [94]

TBDMSCl (167.0mg, 1.11mmol) and imidazole (75.3mg, 1.11mmol) were successively added at R.T. to a solution of the corresponding alcohol **80** (102.5mg, 0.32 mmol) in CH₂Cl₂ (5 mL) and the resulting mixture stirred at R.T. for 3h, until TLC analysis showed complete disappearance of the starting material. The reaction was hydrolyzed with H₂O (5 mL) and the organic residue extracted with CH₂Cl₂ (3x5 mL). The combined organic layers were dried over Na₂SO₄ and solvents removed under vacuum. The oily residue was purified by column chromatography on silica gel, using as eluent hexane/ethyl acetate 90/10 and 70/30, yielding the corresponding silyl ethers **94** (123.5mg, 90%) as yellow oil. ¹H NMR (CDCl₃, 400MHz) δ 7.33-7.19 (m, 5H), 5.17 (s, 2H), 4.39 (d, 1H, J= 24.0Hz), 4.36 (d, 1H, J= 24.0Hz), 4.08-3.99 (m, 3H), 3.62-3.53 (m, 2H), 2.80 (dd, 1H, J=3.4, 16.6Hz), 2.37 (dd, 1H, J=9.8, 16.6Hz), 1.15 (t, 3H, J=7.0Hz), 0.80 (s, 9H), 0.00 (s, 3H), 0.01 (s, 3H) ¹³C NMR (CDCl₃, 100.6MHz) δ 171.00(s), 159.32 (s), 152.91(s), 136.25(s), 128.48, 128.28, 128.15, 67.55, 60.95, 59.70, 51.35, 41.93, 35.56, 25.73, 18.14(s), 14.11, -5.48, -5.54. HRMS (DCI) calculated for C₂₂H₃₅N₂O₅Si (MH⁺) 435.2315; found 435.2330.

4, 5-Dihydro-pyrazole-1,3-dicarboxylic acid 1-benzyl ester 3-ethyl ester [95]

Yellow solid; m.p. 88-91 °C, 93% yield. ¹H NMR (CDCl₃, 400MHz) δ 7.45-7.33 (m, 5H), 5.31 (s, 2H), 4.35 (q, 2H, J=7.1Hz), 4.02 (t, 2H, J=10.8Hz), 3.11 (t, 2H, J=10.8Hz), 1.36 (t, 3H, J=7.1Hz). ¹³C NMR (CDCl₃, 100.6MHz) δ 161.77 (s), 152.60 (s), 148.26 (s), 135.80 (s), 128.51, 128.44, 128.36, 68.15, 61.84, 46.72, 31.59, 14.17. LRMS (MH⁺) 277.

3-Hydroxymethyl-4,5-dihydro-pyrazole-1-carboxylic acid benzyl ester [96]

To a stirred solution of the 2-pyrazoline **95** (2.03g, 7.35mmol) in 20ml of 1:1 solution of THF/CH₃OH at room temperature was added sodium borohydride (835mg, 22.1mmol). The reaction mixture was stirred for 5h, until TLC analysis showed complete disappearance of the starting material. The reaction was then carefully hydrolyzed with saturated aqueous ammonium chloride solution (15ml). The resulting mixture was extracted with ethyl acetate. The combined organic extracts were washed with saturated aqueous sodium bicarbonate solution and brine, dried over Na₂SO₄, filtered and concentrated by rotary evaporation. The residue was purified by column chromatography on silica gel, with hexane/ethyl acetate 70/30 and ethyl acetate/methanol 95/5 as eluents, to give the alcohol **96** (1.31g, 76% yield) as yellow oil. ¹H NMR (CDCl₃, 400MHz) δ 7.42-7.27 (m, 5H), 5.26 (s, 2H), 4.38(s, 2H), 3.89 (t, 2H, J=10.2Hz), 2.91 (t, 2H, J=10.2Hz); ¹³C NMR (CDCl₃, 100.6MHz) δ 159.98 (s), 153.06(s), 136.16(s), 128.54, 128.47, 128.28, 67.57, 59.76, 45.17, 31.93. HRMS (DCI) calculated for C₁₂H₁₅N₂O₃ (MH⁺) 235.1083; found 235.1077.

3-(tert-Butyl-dimethyl-silyloxymethyl)-4,5-dihydro-pyrazole-1-carboxylic acid benzyl ester [97]

TBDMSCl (1.01g, 6.70mmol) and imidazole (0.458g, 6.73mmol) were successively added at R.T. to a solution of the corresponding alcohol **96** (0.450g, 1.92mmol) in CH₂Cl₂ (15 mL) and the resulting mixture stirred at R.T. overnight, until TLC analysis showed complete disappearance of the starting material. The reaction was hydrolyzed with H₂O (15 mL) and the organic residue extracted with CH₂Cl₂ (3x25 mL). The

combined organic layers were dried over Na_2SO_4 and solvents removed under vacuum. The oily residue was purified by column chromatography on silica gel, using as eluents hexane/ethyl acetate 90/10 and 70/30, yielding the corresponding silyl ethers **97** (0.482g, 72%) as yellow oil. ^1H NMR (CDCl_3 , 400MHz) δ 7.34-7.19 (m, 5H), 5.18 (s, 2H), 4.35 (s, 2H), 3.79 (t, 2H, $J=10.2\text{Hz}$), 2.85 (t, 2H, $J=10.2\text{Hz}$), 0.81 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (CDCl_3 , 100.6MHz) δ 159.50 (s), 153.03(s), 136.37(s), 128.46, 128.25, 128.13, 67.45, 60.59, 44.97, 32.46, 25.76, 18.21(s), -5.41. HRMS (DCI) calculated for $\text{C}_{18}\text{H}_{29}\text{N}_2\text{O}_3\text{Si}$ (MH^+) 349.1947; found 349.1942.

3-(tert-Butyl-dimethyl-silanyloxymethyl)-pyrazolidine-1,2-dicarboxylic acid dibenzyl ester [98]

A solution of superhydride (1.0M in THF, 4.1mL, 4.1mmol) was added dropwise to a stirred solution of the silyl ether **97** (573.8mg, 1.65mmol) in the dry CH_2Cl_2 (15mL) at -78°C . The resulting mixture was allowed to warm to room temperature overnight, till TLC analysis showed complete disappearance of the starting material. The reaction was then hydrolyzed with 1M NaOH (15mL) and the organic residue extracted with CH_2Cl_2 (3x20mL). The combined organic layers were dried over Na_2SO_4 and solvents removed under vacuum. The oily residue was dissolved in methylene chloride (15ml). To this solution was added H_2O (15mL), NaHCO_3 (2equiv.) and CbzCl (2equiv.). The reaction was stirred overnight. The resulting reaction mixture was extracted with methylene chloride. The combined organic extracts were washed with brine, dried over Na_2SO_4 , filtered and concentrated by rotary evaporation. The resulting residue was purified by column chromatography on silica gel, with hexane/ethyl acetate 85/15 and 70/30 as

eluents, affording the pyrazolidine **98** (590mg, 74% yield starting from the silyl ether **97**) as light yellow liquid; ^1H NMR (CDCl_3 , 400MHz) δ 7.37-7.26 (m, 10H), 5.20-5.12 (m, 4H), 4.29 (m, 1H), 4.15-4.08 (m, 1H); 3.79 (dd, 1H, $J = 4.4, 10.2\text{Hz}$), 3.47 (dd, 1H, $J = 7.2, 10.2\text{Hz}$), 3.24 (dd, 1H, $J = 8.6, 19.4\text{Hz}$), 2.17-2.13 (m, 1H), 2.10-2.01 (m, 1H), 0.86 (s, 9H), 0.01 (s, 3H), 0.00 (s, 3H). ^{13}C NMR (CDCl_3 , 100.6MHz) δ 157.01 (s), 156.91(s), 136.11(s), 136.00 (s), 128.49, 128.46, 128.09, 128.05, 127.80, 127.76, 67.95, 67.90, 64.56, 60.16, 47.00, 28.62, 25.84, 18.25, -5.47, -5.53. HRMS (DCI) calculated for $\text{C}_{26}\text{H}_{37}\text{N}_2\text{O}_5\text{Si}$ (MH^+) 485.2472; found 485.2477.

3-Benzoyloxycarbonyloxymethyl-4-ethoxycarbonylmethyl-pyrazolidine-1,2-dicarboxylic acid dibenzyl ester [99]

To a solution of the 2-pyrazoline **80** (102.3mg, 0.32mmol) in glacial acetic acid (5ml) was added NaCNBH_3 (121mg, 1.93mmol). The reaction was stirred at R.T. for 6h, at which time the reaction mixture was diluted with ethyl acetate and quenched by addition of a saturated aqueous solution of potassium carbonate. The organic layer was washed with brine and dried over Na_2SO_4 . Solvent was removed by rotary evaporation and the resulting residue dissolved in methylene chloride (5ml). To this solution was added H_2O (5mL), NaHCO_3 (2equiv.) and CbzCl (2equiv.). The reaction was stirred overnight. The resulting reaction mixture was extracted with methylene chloride. The combined organic extracts were washed with brine, dried over Na_2SO_4 , filtered and concentrated by rotary evaporation. The resulting residue was purified by column chromatography on silica gel, with hexane/ethyl acetate 85/15, 70/30 and 50/50 as eluents, affording the pyrazolidine

99 (19mg, 10% yield from the 2-pyrazoline **80**) as yellow oil. ^1H NMR (CDCl_3 , 400MHz) δ 7.40-7.25(m, 15H), 5.24-5.06(m, 6H), 4.40(dd, 1H, $J=8.0$, 11.2Hz), 4.34-4.29(m, 1H), 4.26-4.21(m, 2H), 4.14(q, 2H, $J=7.2\text{Hz}$), 2.97(dd, 1H, $J=8.6$, 11.4Hz), 2.81-2.71(m, 1H), 2.54(dd, 1H, $J=6.8$, 16.6Hz), 2.42(dd, 1H, $J=7.6$, 16.6Hz), 1.26(t, 3H, $J=7.0\text{Hz}$). ^{13}C NMR (CDCl_3 , 100.6MHz) δ 170.88(s), 156.88(s), 156.46(s), 154.72(s), 135.86(s), 135.73(s), 135.02(s), 128.60, 128.52, 128.47, 128.42, 128.19, 128.03, 127.82, 127.60, 69.85, 68.25, 68.12, 67.81, 62.57, 61.02, 52.29, 38.77, 36.91, 14.14. HRMS (DCI/ NH_3) calculated for $\text{C}_{32}\text{H}_{35}\text{N}_2\text{O}_9$ (MH^+) 591.2343; found 591.2319.

4-Ethoxycarbonylmethyl-3-hydroxymethyl-pyrazolidine-1, 2-dicarboxylic acid dibenzyl ester [100]

The 2-pyrazoline **80** (102.3mg, 0.32mmol) was treated with NaCNBH_3 (121mg, 1.93mmol) in glacial acetic acid (5ml) followed by the treatment of the crude product with CbzCl as described for **99** to afford the pyrazolidine **100** (57mg, 39% starting from the 2-pyrazoline **80**) as light yellow oil. ^1H NMR (CDCl_3 , 400MHz) δ 7.39-7.34 (m, 10H), 5.26-5.12 (m, 4H), 4.36 (dd, 1H, $J=7.6$, 11.6Hz), 4.15 (q, 2H, $J=7.2\text{Hz}$), 4.02-3.99 (m, 1H), 3.85-3.82 (m, 1H), 3.52-3.48 (m, 1H), 2.97 (dd, 1H, $J=9.6$, 11.6Hz), 2.79-2.70 (m, 1H), 2.53 (dd, 1H, $J=6.2$, 16.2Hz), 2.41 (dd, 1H, $J=8.2$, 16.2Hz), 1.26 (t, 3H, $J=7.2\text{Hz}$). ^{13}C NMR (CDCl_3 , 100.6MHz) δ 171.04(s), 157.62(s), 156.79(s), 135.72(s), 135.51(s), 128.64, 128.57, 128.44, 128.27, 128.07, 127.87, 68.59, 68.25, 66.16, 62.33, 60.99, 53.12, 37.90, 36.17, 14.15. HRMS (DCI/ NH_3) calculated for $\text{C}_{24}\text{H}_{29}\text{N}_2\text{O}_7$ (MH^+) 457.1975; found 457.1956.

5-Oxo-hexahydro-pyrano[3,4-c]pyrazole-1,2-dicarboxylic acid dibenzyl ester [101]

The 2-pyrazoline **80** (102.3mg, 0.32mmol) was treated with NaCNBH₃ (121mg, 1.93mmol) in glacial acetic acid (5ml) followed by the treatment of the crude product with CbzCl as described for **99** to afford the pyrazolidine **101** (14mg, 11% starting from the 2-pyrazoline **80**) as light yellow oil. ¹H NMR (CDCl₃, 400MHz) δ 7.32-7.22 (m, 10H), 5.14-5.03 (m, 4H), 4.57 (dd, 1H, J=7.6, 13.6Hz), 4.38 (dd, 1H, J=5.2, 12.0Hz), 4.00-3.94 (m, 1H), 3.34 (dd, 1H, J=7.6, 12.0Hz), 3.00-2.92 (m, 1H), 2.52 (dd, 1H, J=6.4, 15.5Hz), 2.25 (dd, 1H, J=8.6, 15.5Hz); ¹³C NMR (CDCl₃, 100.6MHz) δ 170.28(s), 156.85(s), 155.83(s), 135.45(s), 135.42(s), 128.62, 128.56, 128.42, 128.33, 127.96, 68.79, 68.52, 67.48, 55.50, 52.79, 36.89, 32.20. HRMS (DCI/NH₃) calculated for C₂₂H₂₃N₂O₆ (MH⁺) 411.1556; found 411.1554.

4-Ethoxycarbonylmethyl-pyrazolidine-1,2,3-tricarboxylic acid 1-benzyl ester 2,3-diethyl ester [102]

To a solution of the 2-pyrazoline **69f** (110mg, 0.37mmol) in glacial acetic acid (5ml) was added NaCNBH₃ (140mg, 2.23mmol). The reaction was stirred at R.T. for 5h, at which time the reaction mixture was diluted with ethyl acetate and quenched by addition of a saturated aqueous solution of potassium carbonate. The organic layer was washed with brine and dried over Na₂SO₄. Solvent was removed by rotary evaporation and the resulting residue dissolved in methylene chloride (5ml). To this solution was added H₂O (5mL), NaHCO₃ (2equiv.) and CbzCl (2equiv.). The reaction was stirred overnight. The resulting reaction mixture was extracted with methylene chloride. The combined organic extracts were washed with brine, dried over Na₂SO₄, filtered and concentrated by rotary

evaporation. The resulting residue was purified by column chromatography on silica gel, with hexane/ethyl acetate 85/15, 70/30 and 50/50 as eluents, affording the pyrazolidine **102** (66mg, 41% yield from the 2-pyrazoline **69f**) as yellow liquid; ^1H NMR (CDCl_3 , 400MHz) δ 7.35-7.23 (m, 5H), 5.22 (d, 1H, $J=12.8\text{Hz}$), 5.11 (d, 1H, $J=12.8\text{Hz}$), 4.43 (br.s, 1H), 4.30 (br.s, 1H), 4.22-4.07 (m, 6H), 2.97 (m, 2H), 2.59 (dd, 1H, $J=3.6, 16.2\text{Hz}$), 2.42 (dd, 1H, $J=6.0, 16.2\text{Hz}$), 1.23-1.15 (m, 9H); ^{13}C NMR (CDCl_3 , 100.6MHz) δ 170.59(s), 169.94(s), 156.93(s), 156.21(s), 135.99(s), 128.42, 128.06, 127.72, 68.09, 64.65, 63.04, 61.73, 61.06, 51.91, 40.95, 36.52, 14.34, 14.13, 14.02. HRMS (DCI/ NH_3) calculated for $\text{C}_{21}\text{H}_{29}\text{N}_2\text{O}_8$ (MH^+) 437.1924; found 437.1921.

3-Trimethylsilanyl-4,5-dihydro-pyrazole-1,5-dicarboxylic acid 1-benzyl ester 5-methyl ester [110b]

Light yellow oil; 12% yield starting from acryloyl choride. ^1H NMR (CDCl_3 , 400MHz) δ 7.40-7.25 (m, 5H), 5.35 (d, 1H, $J=12.6\text{Hz}$), 5.22 (d, 1H, $J=12.6\text{Hz}$), 4.64 (dd, 1H, $J=6.4, 12.4\text{Hz}$), 3.66 (br. s, 3H), 3.23 (dd, 1H, $J=12.4, 18.0\text{Hz}$), 2.96 (dd, 1H, $J=6.0, 18.0\text{Hz}$), 0.26 (s, 9H); ^{13}C NMR (CDCl_3 , 100.6MHz) δ 173.55 (s), 164.14 (s), 154.66 (s), 138.47 (s), 130.73, 130.48, 130.42, 69.95, 59.17, 54.77, 45.73, 0.00. HRMS (DCI / NH_3) calcd for $\text{C}_{16}\text{H}_{23}\text{N}_2\text{O}_4\text{Si}$ (MH^+) 335.1427; found 335.1422.

1-Benzyl 5-methyl 1H-pyrazole-1, 5(4H,5H)-dicarboxylate [110c]

Yellow oil; 8% yield starting from acryloyl choride. ^1H NMR (CDCl_3 , 400MHz) δ 7.31-7.20 (m, 5H), 6.77 (s, 1H), 5.24 (d, 1H, $J=12.4\text{Hz}$), 5.13 (d, 1H, $J=11.6\text{Hz}$), 4.66 (dd, 1H, $J=5.8, 12.4\text{Hz}$), 3.61 (bs, 3H), 3.17 (ddd, 1H, $J=1.3, 12.4, 18.4\text{Hz}$), 2.89 (ddd, 1H,

$J=1.6, 6.0, 18.4\text{Hz}$) ^{13}C NMR (CDCl_3 , 100.6MHz) δ 169.24 (s), 151.23 (s), 143.22, 134.53 (s), 127.18, 126.97, 126.95, 66.65, 55.56, 51.33, 37.75. HRMS (DCI / NH_3) calcd for $\text{C}_{13}\text{H}_{15}\text{N}_2\text{O}_4$ (MH^+) 263.1032; found 263.1027.

5-Trimethylsilanyl-4,5-dihydro-pyrazole-1,3-dicarboxylic acid 1-benzyl ester 3-tert-butyl ester [111a] Yellow oil; ^1H NMR (CDCl_3 , 400MHz) δ 7.45-7.30 (m, 5H), 5.34 (d, 1H, $J=12.1\text{Hz}$), 5.25 (d, 1H, $J=12.2\text{Hz}$), 3.76 (dd, 1H, $J=11.9, 13.5\text{Hz}$), 3.28 (dd, 1H, $J=13.6, 18.3\text{Hz}$), 2.90 (dd, 1H, $J=11.8, 18.3\text{Hz}$), 1.56 (s, 9H), 0.12 (s, 9H); ^{13}C NMR (CDCl_3 , 100.6MHz) δ 161.57(s), 153.72(s), 149.46 (s), 136.31(s), 128.86, 128.80, 128.66, 83.39(s), 68.37, 50.83, 35.82, 28.42, -2.24. LRMS (MH^+) 377. HRMS (DCI / NH_3) found 375. Product has oxidized.

3-Trimethylsilanyl-4, 5-dihydro-pyrazole-1, 5-dicarboxylic acid 1-benzyl ester 5-tert-butyl ester [111b]

Yellow oil; ^1H NMR (CDCl_3 , 400MHz), δ 7.16-7.02 (m, 5H), 5.01 (br. s, 2H), 4.25 (dd, 1H, $J=5.7, 12.3\text{Hz}$), 2.95 (dd, 1H, $J=12.3, 17.9\text{Hz}$), 2.65 (dd, 1H, $J=5.6, 18.0\text{Hz}$), 1.12 (s, 9H), 0.00 (s, 9H); ^{13}C NMR (CDCl_3 , 100.6MHz) δ 172.10, 164.06, 154.83, 138.51, 130.73, 130.46, 130.39, 84.36, 69.82, 60.05, 45.89, 30.10, 0.00. HRMS (M^+) calculated for $\text{C}_{19}\text{H}_{28}\text{O}_4\text{N}_2\text{Si}$: 376.181836, found: 376.183081.

4,5-Dihydro-pyrazole-1,5-dicarboxylic acid 1-benzyl ester 5-tert-butyl ester [111c]

Yellow oil; ^1H NMR (CDCl_3 , 400MHz) δ 7.38-7.24 (m, 5H), 6.80 (s, 1H), 5.27-5.20 (m, 2H), 4.57 (dd, 1H, $J=5.6, 12.5\text{Hz}$), 3.19 (dd, 1H, $J=12.6, 18.4\text{Hz}$), 2.88 (dd, 1H, $J=5.6,$

18.6Hz), 1.37 (s, 9H); ^{13}C NMR (CDCl_3 , 100.6MHz) δ 169.52 (s), 152.93 (s), 144.95, 136.32 (s), 128.84, 128.63, 128.57, 82.80 (s), 68.13, 58.02, 39.54, 28.15. HRMS (DCI / NH_3) calcd for $\text{C}_{16}\text{H}_{21}\text{N}_2\text{O}_4$ (MH^+) 305.1501; found 305.1492.

3, 4-Dihydro-2H-pyrazole-3-carboxylic acid tert-butyl ester [111d]

Yellow oil; ^1H NMR (CDCl_3 , 400MHz) 7.0 (s, 1H), 4.02 (dd, 1H, $J=4.9$, 11.0Hz), 2.93 (dd, 1H, $J=1.7$, 5.0Hz), 2.90 (dd, 1H, $J=1.4$, 11.2Hz), 1.39 (s, 9H); ^{13}C NMR (CDCl_3 , 100.6MHz) δ 172.41(s), 143.10, 82.59 (s), 59.45, 37.83, 28.25. HRMS (DCI / NH_3) calcd for $\text{C}_8\text{H}_{15}\text{N}_2\text{O}_2$ (MH^+) 171.1134; found 171.1130.

4-Methyl-5-trimethylsilanyl-4,5-dihydro-pyrazole-1,3-dicarboxylic acid 1-benzyl ester 3-ethyl ester [112a]

Yellow oil; ^1H NMR (CDCl_3 , 400MHz) δ 7.41-7.26 (m, 5H), 5.31 (d, 1H, $J=12.2\text{Hz}$), 5.23 (d, 1H, $J=12.2\text{Hz}$), 4.30 (q, 2H, $J=7.1\text{Hz}$), 3.45 (d, 1H, $J=7.6\text{Hz}$), 3.32 (quintet, 1H, $J=7.0\text{Hz}$), 1.33 (t, 3H, $J=7.1\text{Hz}$), 1.28 (d, 3H, $J=6.9\text{Hz}$), 0.06 (s, 9H); ^{13}C NMR (CDCl_3 , 100.6MHz) δ 162.15(s), 153.27 (s), 151.36 (s), 136.24 (s), 128.90, 128.85, 128.68, 68.46, 62.02, 59.30, 43.00, 20.44, 14.55, -2.61. HRMS (DCI / NH_3) calcd for $\text{C}_{18}\text{H}_{27}\text{N}_2\text{O}_4\text{Si}$ (MH^+) 363.1740; found 363.1748.

4-Methyl-3-trimethylsilanyl-4,5-dihydro-pyrazole-1,5-dicarboxylic acid 1-benzyl ester 5-ethyl ester [112b] Yellow oil; ^1H NMR (CDCl_3 , 400MHz) δ 7.10-7.00 (m, 5H), 5.03-4.96 (m, 2H), 3.92 (d, 1H, $J=5.3\text{Hz}$), 3.84 (broad signal, 2H), 3.02-2.95 (m, 1H), 0.99 (d, 3H, $J=7.3\text{Hz}$), 0.89(broad signal, 3H), 0.00(s, 9H); ^{13}C NMR (CDCl_3 ,

100.6MHz) δ 171.88, 167.29, 154.02, 137.68, 130.24, 129.96, 129.62, 69.10, 66.60, 62.98, 53.62, 20.01, 15.58, 0.00. HRMS (M^+) calculated for $C_{18}H_{26}N_2O_4Si$: 362.1662, found: 362.1656.

4-Methyl-4,5-dihydro-pyrazole-1,5-dicarboxylic acid 1-benzyl ester 5-ethyl ester [112c]

Yellow oil; 1H NMR ($CDCl_3$, 400MHz) δ 7.37-7.27 (m, 5H), 6.76 (s, 1H), 5.30-5.17 (m, 2H), 4.29 (d, 1H, $J=5.6$ Hz), 4.14 (broad signal, 2H), 3.24 (m, 1H), 1.27 (t, 3H, $J=7.2$ Hz), 1.19 (broad signal, 3H); ^{13}C NMR ($CDCl_3$, 100.6MHz) δ 169.82(s), 152.65 (s), 149.19, 135.78(s), 128.59, 128.49, 128.27, 67.96, 64.72, 61.77, 47.20, 17.49, 14.00. HRMS (EI) calcd for $C_{15}H_{18}N_2O_4 (M^+)$ 290.1267; found 290.1268.

4-Methyl-5-trimethylsilanyl-2H-pyrazole-3-carboxylic acid ethyl ester [112d]

Yellow solid; m.p. $>120^\circ C$; 6% yield; 1H NMR ($CDCl_3$, 400MHz) 4.30 (q, 2H, $J=7.1$ Hz), 2.36 (s, 3H), 1.24 (t, 3H, $J=7.1$ Hz), 0.29 (s, 9H). ^{13}C NMR ($CDCl_3$, 100.6MHz) δ 163.6 (s), 142.3 (s), 141.1 (s), 127.3(s), 60.6, 14.6, 10.7, -1.2. HRMS (DCI) calculated for $C_{10}H_{19}N_2O_2 (MH^+)$ 227.1216; found 227.1212.

4-Methyl-2H-pyrazole-3-carboxylic acid ethyl ester [112e]

Yellow solid; m.p. $152-155^\circ C$; 33% yield; 1H NMR ($MeOH-d_4$, 400MHz) δ 7.47 (s, 1H), 4.34 (q, 2H, $J=7.1$ Hz), 2.26 (s, 3H), 1.36 (t, 3H, $J=7.1$ Hz) ^{13}C NMR ($MeOH-d_4$, 100.6MHz) δ 161.2 (s), 141.1 (s), 129.7 (s), 120.3 (s), 60.6, 13.6, 8.7. HRMS (DCI / NH_3) calcd for $C_7H_{11}N_2O_2 (MH^+)$ 155.0821; found 155.0816.

4-Methyl-pyrazole-1,5-dicarboxylic acid 1-benzyl ester 5-ethyl ester [112g]

Yellow oil; ^1H NMR (CDCl_3 , 400MHz) δ 7.95 (s, 1H), 7.42-7.35 (m, 5H), 5.47 (s, 2H), 4.39 (q, 2H, $J = 7.1\text{Hz}$), 2.29 (s, 3H), 1.41 (t, 3H, $J = 7.1\text{Hz}$) ^{13}C NMR (CDCl_3 , 100.6MHz) δ 162.51 (s), 149.26 (s), 146.79 (s), 141.42 (s), 134.50 (s), 131.15, 129.39, 129.15, 128.89, 70.76, 61.76, 14.65, 10.05. HRMS (DCI / NH_3) calcd for $\text{C}_{15}\text{H}_{17}\text{N}_2\text{O}_4$ (MH^+) 289.1188; found 289.1189.

4-Methyl-3-trimethylsilanyl-pyrazole-1,5-dicarboxylic acid 1-benzyl ester 5-ethyl ester [112h] and 4-Methyl-5-trimethylsilanyl-pyrazole-1,3-dicarboxylic acid 1-benzyl ester 3-ethyl ester [112i]

Isomer 1: Yellow oil; ^1H NMR (CDCl_3 , 400MHz) δ 7.48-7.35 (m, 5H), 5.44 (s, 2H), 4.24 (q, 2H, $J = 7.1\text{Hz}$), 2.20 (s, 3H), 1.26 (t, 3H, $J = 7.1\text{Hz}$) 0.37 (s, 9H). ^{13}C NMR (CDCl_3 , 100.6MHz) δ 161.98(s), 158.64(s), 155.50(s), 149.52 (s), 134.83(s), 134.71(s), 129.27, 129.02, 128.98, 70.29, 62.24, 14.35, 10.15, -0.96. LRMS (MH^+) 361 Isomer 2: Yellow oil; ^1H NMR (CDCl_3 , 400MHz) δ 7.52-7.33 (m, 5H), 5.50 (s, 2H), 4.42 (q, 2H, $J = 7.1\text{Hz}$), 2.39 (s, 3H), 1.41 (t, 3H, $J = 7.1\text{Hz}$) 0.36 (s, 9H). ^{13}C NMR (CDCl_3 , 100.6MHz) δ 163.01(s), 151.09(s), 146.65(s), 145.40(s), 134.85(s), 131.23(s), 129.35, 129.26, 129.02, 70.55, 61.66, 14.66, 11.10, 1.27. LRMS (MH^+) 361.

4-Phenyl-5-trimethylsilanyl-4,5-dihydro-pyrazole-1,3-dicarboxylic acid 1-benzyl ester 3-ethyl ester [113a] Yellow oil; ^1H NMR (CDCl_3 , 400MHz) δ 7.34-7.02 (m, 10H), 5.26 (d, 1H, $J = 12.4\text{Hz}$), 5.17 (d, 1H, $J = 12.4\text{Hz}$), 4.24 (d, 1H, $J = 6.8\text{Hz}$), 4.12-4.03 (m, 2H), 3.83 (d, 1H, $J = 6.8\text{Hz}$), 1.10 (t, 3H, $J = 7.2\text{Hz}$), 0.00 (s, 9H); ^{13}C NMR (CDCl_3 ,

100.6MHz) δ 163.00(s), 154.30 (s), 142.90 (s), 137.43(s), 130.77, 130.19, 130.05, 129.36, 128.81, 69.96, 63.40, 62.76, 54.75, 15.65, -1.25. HRMS (DCI / NH_3) calcd for $\text{C}_{23}\text{H}_{29}\text{N}_2\text{O}_4\text{Si}$ (MH^+) 425.1897; found 425.1884.

4-Phenyl-4,5-dihydro-pyrazole-1,5-dicarboxylic acid 1-benzyl ester 5-ethyl ester [113c]

Yellow oil; ^1H NMR (CDCl_3 , 400MHz) δ 7.28-7.17 (m, 8H), 7.04-7.01 (m, 2H), 6.79 (s, 1H), 5.21-5.07 (m, 2H), 4.53 (d, 1H, $J=5.2\text{Hz}$), 4.22 (dd, 1H, $J=1.6, 5.6\text{Hz}$), 4.15-4.01 (broad signal, 2H), 1.10 (broad signal, 3H); ^{13}C NMR (CDCl_3 , 100.6MHz) δ 167.69(s), 150.63 (s), 144.56, 135.23(s), 133.84(s), 127.57, 126.68, 126.61, 126.48, 125.48, 66.28, 64.12, 60.15, 55.86, 12.21. HRMS (EI) calcd for $\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}_4$ (M^+) 352.1423; found 352.1430.

4-Phenyl-pyrazole-1,5-dicarboxylic acid 1-benzyl ester 5-ethyl ester [113d]

Yellow oil; ^1H NMR (CDCl_3 , 400MHz) δ 8.14 (s, 1H), 7.46-7.29 (m, 10H), 5.46 (s, 2H), 4.31 (q, 2H, $J=7.1\text{Hz}$), 1.26 (t, 3H, $J=7.2\text{Hz}$); ^{13}C NMR (CDCl_3 , 100.6MHz) δ 161.74(s), 148.78(s), 145.08(s), 141.03(s), 133.95(s), 130.67, 130.11(s), 129.18, 129.13, 128.83, 128.52, 128.22, 128.18, 70.72, 61.70, 14.10. LRMS (MH^+) 351.

4-Phenyl-5-trimethylsilanyl-2H-pyrazole-3-carboxylic acid ethyl ester [113e]

Yellow oil; ^1H NMR (CDCl_3 , 400MHz) δ 7.31-7.12 (m, 5H), 4.09 (q, 2H, $J=7.1\text{Hz}$), 1.05 (t, 3H, $J=7.0\text{Hz}$), 0.00 (s, 9H); ^{13}C NMR (CDCl_3 , 100.6MHz) δ 163.35, 144.66, 142.02, 134.67, 131.47, 129.70, 128.72, 128.58, 61.81, 15.18, 0.00. LRMS (MH^+) 289.

4. Conclusion

The kainoids are a class of non-proteinogenic pyrrolidine dicarboxylates that exhibit both excitatory and excitotoxic activities. These activities are a result of the ability of the kainoids to act as glutamate receptor agonists by activating ionotropic glutamate receptors. The parent of this group of compounds is α -kainic acid. Kainic acid is isolated from the seaweed *Digenea simplex* and has been used in Asian countries as a treatment for intestinal worms in children. In addition it is used extensively by neuropharmacologists for the study of glutamate receptors. Several years ago, the world's sole supplier of kainic acid discontinued this product. Since that time, other sources have appeared, however, the price of kainic acid remains significantly higher than it once was. Numerous syntheses of kainoids have been published¹³⁶ however none have proven to be practical. This is largely due to its synthetic challenge arising from the typical kainoid skeleton containing three contiguous stereocenters in a *trans* (C2-C3), *cis* (C3-C4) arrangement around the pyrrolidine ring. A less costly but still potent alternative to kainic acid needs to be identified and available. It was envisaged that aza analogs of kainoids would be less costly but potentially potent alternatives to kainic acid as supported by molecular modeling evidence and theoretically their much easier synthetic accessibility. I attempted to synthesize these aza analogs of kainoids via 1, 3-dipolar cycloadditions of diazoalkanes with *trans* diethyl glutaconate followed by reduction of the resulting cycloadducts and functional group transformations.

The 1, 3-dipolar cycloadditions of diazoalkanes (except TMS diazomethane) with *trans* diethyl glutaconate yield 1-pyrazolines which have the expected regioselectivity, being such that the terminal nitrogen of the diazoalkane is connected with the α -carbon

of the ester. The 1-pyrazolines either isomerized to the 2-pyrazolines which may be precursors to aza analogs of kainoids or became oxidized to the pyrazoles. The isomerization of the 1-pyrazolines to the 2-pyrazolines with the direction of double bond isomerization dependent on the substituents results in the loss of one of the newly formed stereocenters.

The reduction of C=N double bond of the 2-pyrazolines would furnish the pyrazolidine compounds which can be converted to aza analogs of kainoids after functional group transformations. Such reduction has been successfully achieved for the 2-pyrazoline **69f** which contains no substituents at C-5 position around the pyrazoline ring to afford the pyrazolidine compound **102** (Figure 34). Upon deprotection, the pyrazolidine compound **102** can be converted to one of the synthetic targets **60** (Figure 18).

However, the reduction of the C=N double bond has not been achieved for the 2-pyrazolines (protected or unprotected) which have C=N double bond conjugated to either an ester group or a phenyl group. It was shown that the C=N double bond of these 2-pyrazolines is resistant to the common C=N double bond reducing agents possibly due to its conjugation with the carbonyl group or phenyl group. When the conjugation of the C=N double bond with the ester group in the 2-pyrazoline **64d** was interrupted by reduction of that ester group to the hydroxyl group (Figure 32), the C=N double bond of the resulting 2-pyrazoline **80** was easily reduced to afford the pyrazolidine compounds **99-101** (Figure 34) which may be converted to aza analog of kainoids **60** (Figure 18) after functional group transformation. However, in contrast to the successful reduction of the C=N double bond of the 2-pyrazoline **97** (Figure 33) with the superhydride, the

reduction of the C=N double bond of 2-pyrazoline **94** (Figure 32) with the superhydride was not achieved. This observation suggests that, besides the conjugation of the C=N double bond to the ester group or the phenyl group, some other factors, such as the nature of the C4 substituent, may also play a role in the resistance of the C=N double bond of the 2-pyrazolines towards the common C=N double bond reducing agents.

Although the original strategy to access the synthetic targets has not been realized, its simpleness is so attractive that further efforts to directly reduce the cycloaddition products (either 1-pyrazolines or 2-pyrazolines) to the pyrazolidine compounds are still worthwhile. Meanwhile, the successful conversion of the 2-pyrazoline **64d** to the pyrazolidine compounds **99-101** via the selective reduction of the ester group conjugated to the C=N double bond followed by the reduction of the resulting isolated C=N double bond suggests that the synthetic targets **58** and **59** may be accessed in a similar way. The suggested route illustrated in Figure 35 may be used as an alternative to the original strategy to access these two synthetic targets.

TMS diazomethane, due to the commercial availability, has been frequently used as a synthetic reagent in 1, 3-dipolar cycloadditions, particularly in the preparation of novel amino acid analogs. A survey of the recent literature indicates that the regioselectivity of the double bond isomerization of TMS substituted 1-pyrazolines is variable and at first glance, unpredictable. In an effort to develop a mechanistic rationale for the isomerization which could account for the products obtained, a systematic survey of dipolar cycloadditions between TMS diazomethane and α , β -unsaturated dipolarophiles was undertaken. Based on what I have obtained, I speculate that product distributions of dipolar cycloadditions between TMS diazomethane and α , β -unsaturated

dipolarophiles may be affected by the cycloaddition conditions, the reaction workup conditions, and/or the nature of the dipolarophiles. Probably the cycloaddition reaction and/or the isomerization of the 1-pyrazoline are so sensitive to the reaction conditions and/or the reaction workup conditions that subtle change of these conditions results in different product distributions. The complete picture of the cycloaddition of trimethylsilyldiazomethane is under investigation.

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