Molecular Recognition in Water: Design, Synthesis, and Characterization of Rigid Molecular Receptors and Enzymatic Mechanistic Probes

Robert William Hoppe
University of Wisconsin-Milwaukee

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MOLECULAR RECOGNITION IN WATER: DESIGN, SYNTHESIS, AND CHARACTERIZATION OF RIGID MOLECULAR RECEPTORS AND ENZYMATIC MECHANISTIC PROBES

by

Robert William Hoppe

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Chemistry at The University of Wisconsin-Milwaukee

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ABSTRACT

MOLECULAR RECOGNITION IN WATER: DESIGN, SYNTHESIS, AND CHARACTERIZATION OF RIGID MOLECULAR RECEPTORS AND ENZYMATIC MECHANISTIC PROBES

by

Robert William Hoppe

The University of Wisconsin-Milwaukee, 2016
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Molecular recognition can be defined as a selective and reversible binding between two or more molecules through non-covalent interactions. Multiple weakly attractive intermolecular forces work in concert to achieve selectivity in association. Such discrimination is critical to the physiological processes of catalysis, transport, antigen recognition, and storage. To better understand this phenomenon, acyclic synthetic molecular receptors also known as “molecular tweezers” were made to study the inclusion of small molecule guests from water. Three anionic tweezers derivatives with syn-cofacial orientation were constructed from a 1,2:4,5-bis-Tröger’s Base skeleton that differed in the amount and distribution of negative charge about the receptors. These binding isomers were found to undergo self association to form dimers in solution by inclusion of the naphthalene wall of one tweezers into the cleft of another. Small molecule binding was evaluated at different ionic strengths and under concentration regimes in which either the desired monomeric tweezers or the dimeric complex predominated.

Small molecules were also created in order to probe the mechanisms by which enzymes facilitate their transformations. Cinnamylidene pyruvate and fluorinated pyruvate derivatives were synthesized to better understand why SbADC, an enzyme classified as an acetoacetate decarboxylase, demonstrates aldolase activity.

Enduracidinidine is an unsaturated amino acid found in the potent antibiotics mannopeptimycin, teixobactin, and enduracidin. To investigate the biosynthesis of this amino acid, a new preparation of L-vinylglycine was devised. L-vinylglycine is a potential general precursor to the formation β,γ-unsaturated amino acids. Conditions were identified in which such unsaturated amino acids are formed by olefin metathesis of protected L-vinylglycine and alkenes including allylamine derivatives.
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I. INTRODUCTION

A. Molecular Recognition

Molecular recognition can be defined as a selective and reversible binding between two or more molecules through non-covalent interactions. Multiple weakly attractive intermolecular forces work in concert to achieve selectivity and thus makes it substantially more difficult to predict than the formation of covalent chemical bonds. Intercalaction of a smaller molecule into the cavity of a larger molecule often describes the mode of action in which two molecules bind together. While net forces like van der Waals attractions or the hydrophobic effect\textsuperscript{1}, which is a property of the solvent, are primer movers in this phenomenon other electrostatic interactions like dipole-dipole, aromatic stacking\textsuperscript{2}, the cation-π effect\textsuperscript{3}, ion pairing\textsuperscript{4}, and hydrogen bonding\textsuperscript{5} in arrays\textsuperscript{6}, and ditopic coordination\textsuperscript{7} provide the means for exceptionally strong affinities and substrate discrimination in binding.

Molecular recognition also known as Host-Guest chemistry\textsuperscript{8} is critical to the physiological processes of catalysis, transport, antigen recognition, and storage. In studying what can lead to selective and strong binding these powerful capabilities demonstrated by nature could be replicated. For theses reasons the concepts of molecular recognition have been applied to molecular acuators\textsuperscript{9}, drug discovery\textsuperscript{10}, and the self assembly of molecular capsules\textsuperscript{11}.
Figure 1: Equilibrium for association between a molecular receptor (host, H) and an associating molecule (guest, G) in solution to form a complex (C)

The strength of the binding interactions is described in terms of an equilibrium constants as either the forward direction of the association of components in complex formation ($K_a$ in units L/mol) or the backwards direction of the dissociation of the complex to the individual compounds ($K_d$ in units mol/L). However as these associations occur in a solvent there exists another equilibrium for the solvation of the host and guest molecules individually to come together to form a solvated complex. The strength of the association to form this solvated complex depends upon solvation energies, temperature, and ionic strength among many other factors lending to a small change in the energies between the two states. For these reasons, predicting what molecules will bind together strongly and selectively is difficult and thus requires the use of experimentation to elucidate fundamental principles to understand this interaction.

B. Precidented Natural and Synthetic Molecular Hosts

1. Cyclic Molecular Receptors

Cyclodextrin, a macrocyclic polysaccharide comprised of glucose units, was the first molecule reported to bind another molecule by way of inclusion of elemental iodine into its
cavity.\textsuperscript{12} In the decades following many prominent researchers took up investigating this bucket shaped structure and its propensity to intercalate small molecules.

Figure 2: Example of a cyclodextrin: $n=6$ \(\alpha\)-cyclodextrin, $n=7$ \(\beta\)-cyclodextrin, $n=8$ \(\gamma\)-cyclodextrin

Cramer and Hettler\textsuperscript{13} were among the first to profile complex formation with cyclodextrins while researchers like Saenger\textsuperscript{14} and Breslow\textsuperscript{15} studied the inclusion complexes of derivatized cyclodextrins. Cyclodextrins have also been exploited for pharmaceutical purposes to deliver drugs\textsuperscript{16} or release over time to create a continuous dosage.

While naturally occurring substances like cyclodextrins and enzymes hold enormous potential for understand what entails the formation of a complex the creation of synthetic molecular receptors could hold even greater potential. Synthetic receptors would allow for the modification of the binding cavity identify of the specific non-covalent interactions responsible for binding. The first artificial host molecule were macrocyclic polyethers synthesized by Pedersen in 1967.\textsuperscript{17} This class of compounds dubbed crown ethers were called so for their ability to “crown” metal cations and thioureas\textsuperscript{18} by inclusion into their cavity by ion-dipole interactions.
These crown ethers, especially 18-crown-6, bind cationic substrates with high affinities as all of the oxygens are orientated towards the interior of the macrocycle whereas the alkyl linkers are positioned on the exterior. This demonstrates the concept of “preorganization”. Preorganization is the concept that the host and guest molecules bind with the strongest affinities if the functional groups responsible for complexation are orientated in a cooperative fashion prior to the components coming into proximity. An example of this would be in the theoretical comparison of 18-crown-6 to its acyclic analogue with the assumption that the acyclic version has the capability to bind potassium ion.

The acyclic version would not bind the the alkali ion as strongly as 18-crown-6 as it contains many more rotational degrees of freedom and thus contains many conformations unsuitable for complex formation. This would result in a lower $\Delta G$ term in the Gibbs Free Energy equation compared to the crown ether as the acyclic version $\Delta S$ term would be more positive upon complex formation. This loss of entropy results from the acyclic version having to orient...
itself to allow for all six oxygen atoms to coordinate to the cation like the crown ether and overall weaker affinity.

From these concepts, Koga reported the first unambiguous example of a water soluble, macrocyclic synthetic molecular receptor.\textsuperscript{20} Comprised of two diamino diphenylmethanes with two n-butyl alkyl chains linking connecting the amino groups the macrocycle was found to dissolve in aqueous media below pH2.

![Figure 5: Koga's macrocycle](image)

This host molecule was able to fully include hydrocarbons like durene, naphthalene, and \( p \)-xylene. A dissociation constant for 8-anilino-1-naphthalene sulfonic acid (\( K_d = 1.6 \times 10^{-4} \) M) was determined by fluorescent emission and the orientation of 2,7-dihydroxynaphthalene bound into the cavity was determined using \(^1\text{H} \) NMR. In using these spectroscopic techniques, Koga was able to demonstrate the utility of these techniques and were widely adopted afterwards. This work went on to inspire others in the study of small molecule binding with significant contributions made by Diedertich and Dougherty. Diederich focused on making macrocyclic receptors that were soluble in water at pH7\textsuperscript{21} that were capable of binding hydrophobic moieties like pyrene and facilitating their transport through aqueous media.\textsuperscript{22} He was also able to correlate the binding affinities in electron donor-acceptor interactions with electron poor naphthalene derivatives binding stronger to the electron rich macrocycle.\textsuperscript{23} Dougherty too
focused on synthetic macrocycles soluble in neutral water. He reported\textsuperscript{24} that his receptor was able to form inclusion complexes with quaternary ammonium ions. He later went on to show that binding can not only be driven by the hydrophobic effect but by the attraction of the cation to the $\pi$ electrons in the aromatic walls of the macrocycle.\textsuperscript{25} This interaction, coined the cation-$\pi$ effect, was found to be so favorable that it orientated the quaternary ammonium to the interior of the cavity while the hydrophobic part of the ammonium salt was left exposed to the aqueous environment.\textsuperscript{26}

2. Acyclic (non-macrocyclic) Molecular Receptors

Acyclic, synthetic molecular receptors were dubbed “molecular tweezers” by Whitlock in the first known publication of this architecture in 1978.\textsuperscript{27} Molecular tweezers are usually characterized by two aromatic walls that are approximately parallel to one another in a syncofacial configuration and held apart by a rigid linker. This initial design was intended to act as a bis-intercalater for DNA that was restricted to a single strand of DNA. Whitlock constructed his tweezers by alkylating caffeine molecules with a rigid diyne linker and prove that it was able to associate to 2,6-dihydroxybenzoate by binding between the aromatic walls to form a sandwich complex by $\pi$-$\pi$ stacking. From this elegant design the next iteration of molecular recognition studies was birthed. Early designs utilized diphenyl glycourils\textsuperscript{28} or a derivatized Kemp’s triacid\textsuperscript{29} the work provided by Zimmerman provided foundational examples in constructing molecular tweezers. His motif displays a high degree of preorganization with an aromatic linker and a syncofacial arrangement of acridine walls connected in the 9- position.\textsuperscript{30}
With the anthracenes twisted out of conjugation due to steric hinderance they can rotate until to walls achieve a conformation until the walls are parallel and approximately 7Å apart so that they can bind a flat, aromatic guest. The import of the rigid spacer was demonstrated in a later publication where Zimmer substituted the rigid linker for a butyl linker to connect the anathcene sized walls and found that the binding affinity towards 2,4,7-trinitrofluorene decreased by a factor at least 40. He also demonstrated that binding affinities can be greatly enhanced through the use of cooperative non-covalent interactions. When a carboxylic acid group was incorporated into the spacer the combination of π-π interactions and hydrogen bonding the tweezer was able to bind 9-propyladenine with high affinity ($K_d = 4.0 \times 10^{-5}$ M) in CDCl$_3$. When the carboxylic acid was converted to a methyl ester the binding affinity diminished precipitously with no detectable binding was observed by $^1$H NMR when 9-propyladenine was added to the tweezers in up top four fold stoichiometric excess.

The next contribution in the development of molecular tweezers come from Harmata. His methodology progressed from the synthesis of asymmetric Kagan’s ethers to symmetrical bis-Kagan’s ethers. Kagan’s ether is a diaryl, [6.6.1] bicyclic ether with the oxygen atom bridging the two rings while a bis-Kagan’s ether is two of these structures connected by a common benzene ring. This structure is more preorganized than Zimmerman’s tweezers as the
aromatic walls are enforced into being parallel and are precluded from rotating by being incorporated into the cyclic ether.

![Figure 7: bis-Kagan's ether (left) and Harmata's molecular tweezers based on a bis-Kagan's ether architecture](image)

However, this structure was not able to bind electron poor, aromatic molecules due to the small aromatic surface area in the cavity. When the benzene walls were replaced with dibenzofurans an X-ray crystal structure was obtained with 1,3,5-trinitrobenzene bound into the 7Å cavity forming the classic sandwich complex to corroborating the function of the desired shape. The use of bicyclic polyethers and dibenzofuran sized derivatives as walls have seen continued use since Harmata’s publication to bind quaternary ammonium ions and aromatic guests respectively.

The molecular tweezers developed by Klärner utilized a scheme which employed Diels-Alder reactions to attach a dimethyldihydroquinone norbornen derivative to an aromatic norbornadiene. If one Diels-Alder reaction occurs then a U-shaped molecular tweezers is afforded, if done twice then a C-shaped molecular clip is produced. In each of these cases it was found that these structures were able to intercalate electron poor, armomatic small molecules into their cavities.
While initially confined to organic solvents, these structures were given water solubility by converting the dimethylhydroquinone ethers to bis-phosphates and bis-methyl phosphonates which allowed for the binding of thiazolium salts in aqueous media. These receptors were studied further and found to inhibit enzyme activity in a novel fashion: alcohol dehydrogenase by binding its cofactor NAD$^+$ and G6PD by binding to NADP$^+$. In binding to flavium salts Klärner observed a spectroscopic response in the UV-Vis region which demonstrates the potential for molecular tweezes to be useful as molecular sensors. To date, this class of water soluble molecular tweezers has shown the most robust utility in small molecule complex formation.

C. Tröger’s Base and bis-Tröger’s Base

While the previously detailed works into molecular tweezers were being published, a parallel strategy was being developed using an easier to synthesize moiety that also generated a well defined cleft for molecular binding. Tröger’s Base, composed of two aromatic amines and three formaldehyde equivalents, was chosen by Wilcox to suite these criteria. First synthesized by Julius Tröger in 1887 Tröger’s Base is a chiral compound formed from $p$-toluidine and formalin in the presence of concentrated hydrochloric acid. The chirality in this
structure comes not from chiral carbons but from the sterogenic nitrogens in the bicyclic ring system which cannot undergo inversion. If inversion were to occur the bridging methylene unit would have to pass through to the other face of the ring system, ring would place the atoms in the same plane in the process, but the energetic barrier provided by steric hindrance and bond angle strain prevent this.

Figure 9: Formation of the two Tröger’s Base isomers from p-toluidine and formaldehyde

Since its discovery, Tröger’s Base was subject of intensive investigation into its structure, mechanism of formation, and origin of its chiral nature. Wganer probed the mechanism of formation by generating Tröger’s Base from intermediates isolated along the synthetic pathway whereas, Spielman was able to confirm the structure of Tröger’s Base through its degradation products. Prelog and Wieland were the first to report the separation of the isomers the assignment of the absolute configuration of these chiral partners was not conducted until 1967 where circular dichroism was used to analyze the (+)- and (-)- isomers. From these experiments it was concluded that the (+)- isomer was of the 1R, 3R configuration while the (-)- isomer was 1S, 3S. Later studies however contradicted these assignments with the acquisition of an X-ray crystal structure and isomeric resolution utilizing a chiral Brønsted acid. It wasn’t until 2000 that the initial assignment were unambiguously refuted and the true
configuration of the chiral Tröger’s Base isomers were determined as the (+)- being 1S, 3S and the (-)- isomer being 1R, 3R.\textsuperscript{52}

Among the first to take advantage of the shape of Tröger’s Base to generate molecular tweezers was Wilcox.\textsuperscript{53} In taking advantage of the fact the the aromatic faces in Tröger’s Base are in the range of 92 to 104° apart, 9,10-dihydro-9,10-ethenoanthracen-2-amine was condensed with formaldehyde to generate a molecular receptor which was found to have two ethanol molecules in its cavity via X-Ray crystallographic analysis.\textsuperscript{54}

![Figure 10: A Wilcox molecular tweezers based on Tröger’s Base that binds pyrimidin-2-amine](image)

He later developed a molecular tweezers utilizing a \textit{bis}-carboxylic acid motif to bind to 2-aminopyrimidine through hydrogen bonding\textsuperscript{55} which later elucidated the influence of water included in the active site on binding affinities.\textsuperscript{56} His work demonstrated that the rigid, well defined structure of Tröger’s Base was of utility in constructing rigid molecular frameworks that could be incorporated in architectures ranging from metallo-macrocycles\textsuperscript{57} to colorometric sensors for anion recognition.\textsuperscript{58}

Analogous to Harmata’s \textit{bis}-Kagan’s ether tweezers and the recognition of the perpendicular nature of the aromatic elements in Tröger’s Base, 2001 saw the introduction of a \textit{bis}-Tröger’s Base into the catalogue of molecular tweezers.\textsuperscript{59} Starting with a Tröger’s Base
derived from p-toluidine and 4-nitroaniline Pardo et. al. was able to synthesize a diastereomeric mixture of bis-Tröger’s Bases via Friedel-Crafts alkylation. This process was only able to proceed under the fairly harsh conditions of anhydrous ethanol and concentrated hydrochloric acid at 90°C for 24h and resulted in regio-selective cyclization with a 29% overall yield affording the 1,2; 4,5 isomer in a 4:1 ratio of the syn-coficial isomer to the anti- isomer.

![Figure 11: Precidented 1,2:4,3-bis-Tröger’s Base isomer and desired 1,2:4,5- bis-Tröger’s Base](image)

This nomenclature is derived from the substitution pattern around the central benzene spacer. The 1 and 4 numbers denote the position of the amino groups in the benzene while the 2 and 3 number describe the positions in that benzene ring where Friedel-Crafts alkylation occurred. The observed ratios invites intriguing questions. What was the reason for the favorablility of the syn- isomer, the one with the aromatic walls on the same side of the spacer unit, over the anti- isomer, the one with the walls on the opposite sides of the linker? What was the origin of the regio-selectivity in cyclization to form the bis-Tröger’s Base? To address the first question, the Pardo group subjected the anti- isomer to the previously described cyclization conditions and recovered the same 4:1 ratio of syn- : anti- isomers. They attributed this ratio to the syn- isomer being more thermodynamically favorable due to the π-stacking interactions of the parallel aromatic walls. While this may be true, I propose it to be a
more complicated mechanism. Under the acidic conditions and isomerization concentrations, this bis-Tröger’s Base is able to isomerize freely between the two isomers with the syn-isomer able to associate with itself, albeit weakly, to form a dimer by π-π stacking interactions. This would remove the self associating syn-isomer from the equilibrium and by the Le Châtelier’s Principle would drive its continued formation.

With regards to the regioselectivity of Friedel-Crafts alkylation, the Pardo group expected a statistical formation of the 1,2:4,5 isomer alongside the 1,2:4,3 isomer but as stated before observed the latter exclusively. They postulated that even though the 1,2:4,3 isomer is more sterically hindered it was formed preferentially due to the π-stacking of the aromatic walls. This however seems unlikely as the cavity, like the bis-Kagan’s ether, is approximately 7Å apart in either configuration.

In a later synthesis of bis-Tröger’s Base starting from p-phenlenediamine, the 1,2:4,3 isomer was also afforded exclusively. To overcome this regioselectivity, the cyclization to the 1,2:4,5 isomer was enforced by using 1,4-diamino-2,5-dimethylbenzene as the spacer unit. The isolated yield of the cavity shaped syn-isomer was 7% en route to generating linear oligo-Tröger’s Bases. Dolenský would later go on to incorporate naphthalene sized walls into a bis-Tröger’s Base tweezers synthesis of the 1,2:4,3 isomer in order to provide enough aromatic surface area to potentially bind small molecules. The synthetic procedure was improved to utilize a one-pot procedure but only afforded bis-Tröger’s Bases in low yield (8-13%). To date, there are no known examples of a 1,2:4,5 bis-Tröger’s Base tweezers without substitution on the core benzene linker to compel the Friedel-Crafts alkylation into this pattern.
D. Rationale and Design of Target \textit{bis}-Tröger’s Base Molecular Receptor

Inspired by the design of Harmata’s tweezers, the desired 1,2;4,5 \textit{bis}-Tröger’s base molecular receptor has the same connectivity to afford parallel naphthalene sized walls held approximately 7Å apart by a rigid spacer. In reviewing the previous syntheses of \textit{bis}-Tröger’s Bases a common feature held in common was the occurrence of the Friedel-Crafts on the central benzene spacer generating the 1,2;4,3- isomer preferentially. The designed spacer unit for desired \textit{bis}-Tröger, the tweezers core, depicted below will circumvent. By already possessing the bonding required for the 1,2;4,5 isomer on the spacer units Trögerization should occur unambiguously to said isomer. This tweezers core not only allows for modular synthesis by condensation with a wide range of aromatic amines but could also facilitate others in their studies of \textit{bis}-Tröger’s Base molecular receptors as convenient starting compounds are not readily available.\textsuperscript{63}

![Molecular "tweezers"

Figure 12: Retro-synthetic scheme for modular construction of the desired water soluble \textit{bis}-Tröger’s Base molecular tweezers

Water solubility is installed into the molecular tweezers by the transformation of the imine into an \textit{α}-amino phosphonate which will then be dealkylated subsequently. Although this functionality provides significant water solubilization it also introduces a not insignificant amount of steric bulk. The consequence of this is that upon cyclization to form the \textit{bis}-Tröger’s
Base, the stereochemistry of the bridging methylene is determined by the stereochemistry of the α-aminophosphate to generate the syn-cofacial or anti-isomers in an expected 1:1 ratio. These orientations cannot be interconverted under acidic conditions as seen in previous bis-Tröger’s bases as its formation is sensitive to steric interactions. The alternative to generating a statistical mixture of syn-cofacial and anti-isomers and isolate more of the desired binding syn-isomer would be to either build in water solubilizing functional groups into the walls of the molecule or employ a stereoselective method of forming the α-amino phosphonate.

![Figure 13: 1,2;4,5-bis-Tröger’s Base molecular tweezer with naphthalene walls illustrating their offset nature](image)

In contrast to the 1,2;4,3-isomer, inclusion of naphthalene sized hydrocarbons for the walls in the 1,2;4,5- leads to them existing in an offset orientation mimicking the pattern observed in the crystal structure of benzene. This feature should facilitate easier inclusion of aromatic guest molecules in the cavity compared to the 1,2;4,3-isomer which has its walls directly over one another.
II. RESULTS AND DISCUSSION

A. Formation of the key intermediate N,N’-di-t-Boc-2,5-diaminoterephthaldehyde

![Chemical reaction diagram]

Figure 14: Overall synthetic sequence to produce key intermediate dialdehyde 8

1. Synthesis of Dimethyl 2,5-diaminocyclohexa-1,4-diene-1,4-dicarboxylate

![Chemical reaction diagram]

Figure 15: Synthesis of bis-enamine 1 from dimethyl succinyl succinate

Succinyl succinate diester derivatives have been condensed with amines for over 100 years utilizing molten NH₄OAc and NH₃ in ethanol. However, neat alkyl amines and NH₄OAc in solution provide for more facile methods to the desired bis-enamines. In a method initially developed by Kanchan Tiwari, she chose to go the route of NH₄OAc in ethanol as we needed the primary enamine. Baeyer describes uses ten equivalents of NH₄OAc, but she found that reducing it to five equivalents was sufficient with 95% yield on a one gram scale of
dimethyl succinyl succinate (DMSS). Use of ethanol as the solvent allowed 1 to crystallize from the reaction medium eliminating the need for subsequent purification. In scaling the reaction up to 55g scale, the equivalents of NH₄OAc were reduced to three and four equivalents with each producing yields of 87% and 88% respectively and identical melting points of 212-214°C. On this scale addition of DMSS needs to be added in portions to the refluxing ethanol to ensure it dissolves.

2. Synthesis of Dimethyl 2,5-bis((tert-butoxycarbonyl)amino)terephthalate

![Diagram of the synthesis](image)

Figure 16: Scheme for aromatization and di-Boc protection of bis-enamine 1

Use of Pd-C has been demonstrated to be able to oxidize heterocycles⁷¹ and cyclic enamines⁷² to the corresponding anilines, but required the use of a sacrificial oxidant to prevent disproportionation of the enamine. Fortunately, refluxing in toluene for 13hr with 30% by weight 10% Pd-C afforded the aromatized 1 in 98% yield with no reduced 1 observed. Boc protection of both amines was accomplished in 66% yield by Kanchan Tiwari in boiling toluene with 5% Pd-C with 4eq Boc₂O followed by 2eq Boc₂O after 4hr. Boc₂O was added in several portions because it is unstable under the reactions conditions and 1 does not acylate until it has been aromatized. This was taken into consideration when increasing the scale of 1 production.

Under my direction, John Lukesh III increased the initial concentration of 1 by a factor of two on a 1g scale. This was considered feasible as 2 is more soluble in boiling toluene than is 1.
A greater concentrations of Boc₂O (7eq, added in two portions) was utilized with complete conversion to 2 was observed after 22hr. He was able to acquire a 92% yield of the desired product in four crops from recrystallization from CH₂Cl₂/hexanes. In further testing conditions for scale up, the concentration of 1 was increased further in efforts to decrease the amount of the most expensive reagent, Boc₂O, required for conversion to 2. Coupled with the aromatization of 1 before the addition of Boc₂O, this alteration to the synthetic methodology was considered to be beneficial. However, this was not the case with side product formation observed. This complication is described subsequently.

**a. Diarylamine formation**

In the effort to make 8 on a large scale so attempted was 2 but with isolated yields of only ~50%. In the small scale preparations of 2, Pd-C and Boc₂O were added concurrently to 1 but as Boc₂O thermally decomposes it was thought prudent to aromatize 1 to 3 beforehand. On testing these changes desirable for large scale reaction, it was observed that TLC did not indicate complete reaction over the same time scale. Aromatization initially occurred in distilling toluene to azeotropically remove residual water or ethanol from 1 to prevent it from later reacting with Boc₂O. This resulted in a yellow-green distillate and a pungent smelling vapor being produced that was basic to pH paper. Due to the composition of the mixture and the odor of the gas, it was concluded that ammonia was being produced and was taking as the first evidence that another transformation was occurring.

A potential source of ammonia was residual NH₄OAc in the 1. This was ruled out by washing with H₂O and EtOH, and use of pure 1 with a melting point of 212-214°C. With
exogenous ammonia ruled out, hydrolysis of the bis-enamine was considered the next most likely mechanism. After isolating 2 from the mixture by crystallization, its mother liquor was analyzed by $^1$H NMR and observed to be a mixture of DE, Boc$_2$O, and an unidentified side product that was not mono Boc protected 2. The excess Boc$_2$O present suggested that the amine functionality was lost and replaced with something that would not reaction with the Boc$_2$O, possibly a hydroxyl group. To test the phenol hypothesis, 6g of the concentrated mother liquor was dissolved in CH$_2$Cl$_2$ and extracted with sat. Na$_2$CO$_3$. The aqueous phase was removed, acidified to pH6, and extracted with CH$_2$Cl$_2$. After drying the organic phase and concentrating to dryness 1.8mg of material was afforded that identified as not the side product by $^1$H NMR. This did not fully rule out the phenol as the side product as the phenoxide may not have been able to be partitioned into water.

To rule out hydrolysis, a portion of 1 was split into two portions for a side by side reaction. The first portion was not azeotropically dried by distillation of toluene and heated at reflux without 5% Pd-C for 3hr with no change in pH paper observed above the condenser. 5% Pd-C was then added and after 12min pH Hydridron paper indicated pH11 vapor above the condenser. After heating at reflux for 18hr, no pH change was observed above the condenser as testing with pH paper. The second portion of 1 was dried azeotropically by distillation of toluene and heated at reflux for 3hr with no observed change in pH paper before the addition of Pd-C. After 5min the pH paper changed to pH9-10. The products from each of the portions were isolated and analyzed by $^1$H NMR to afford identical spectra containing 3.
Figure 17: Deprotection of 2 to afford aromatic diamine 3

To corroborate the signals, a very pure sample of authentic aromatic diamine 3 was synthesized in 77% overall yield from the TFA deprotection 2 and subsequent deprotonation in 75% conc. NH$_3$(aq). No side product was produced with this method. The $^1$H NMR spectrum matched that of the aromatic 3 described above. 3 was also exposed to 5% Pd-C in refluxing toluene with no change in composition as observed by TLC. This also ruled out the possibility of 3 reacting with itself to evolve ammonia. It was shown that if the previous protocol of concurrent addition of Pd-C and Boc$_2$O was used 2 was generated in 88% yield after recrystallization form toluene. 2 was also made from 3 with a 92% yield with no observation of 4 in the isolated product.

Figure 18: Compound 4, the diaryl amine side product formed from condensation of 2
Mass spectrometric analysis of the mother liquor from a synthesis of 2 in which side product formed proved that the side product was not a phenol. The signals produced had a m/z of 442 (2+Na\(^+\)) and 632 which is a consistent with 4. This data ruled out hydrolysis as a mechanism of side product formation. This mixture was purified by flash chromatography in which diarylamine 4 was isolated and characterized by \(^1\)H NMR and ESI-MS confirmed to be the side product generated by Pd-C oxidation. With this diarylamine in hand, it was hypothesized that en route to being aromatized by Pd-C, condenses with itself to form the diaryl amine in the absence of Boc\(_2\)O which resulted in the evolution of NH\(_3\). This surprising results raised the question of when the coupling occurred. The most plausible pathway for condensation would be the transamination of the aromatic amine 3 with the imine form of 2. As this process is expected to be acid catalyzed it was checked to see if this process could be inhibited by the use of base washed catalyst and glassware. What was determined was that it had no effect in decreasing diarylamine formation.

b. Exploration on mechanism of diaryl amine formation

To further elucidate this process, 1 and \(p\)-toluidine were heated in refluxing toluene together without Pd-C. No change was observed by TLC over 17h. Upon addition of 5% Pd-C however, two new spots were observed and after 16-41hr of reflux 5 and 6 were isolated by
flash chromatography in a 1:1 molar ratio with 71% conversion of 1 into the mono- and bis-variants. To ensure exchange of the amine was not due to acid catalysis, the 5% Pd-C was washed with 15% NaOH and rinsed with nanopure water until the filtrate was neutral. Following above procedure, 5 and 6 were observed to be in a 2.3:1 molar ratio respectively with 63% mol conversion of 1 into the desired products. In obtaining compounds 5 and 6, the hypothesis of having an aniline condense with 1 en route to aromatization was confirmed.

3. Synthesis of Di-tert-butyl (2,5-\textit{bis}(hydroxymethyl)-1,4-phenylene)dicarbamate

LiBH$_4$ in CH$_3$OH or IpOH was utilized initially by Kanchan Tiwari reduced diester 2 to afford only trace amounts of diol 3. When the solvent was switched to THF, a 63% yield by NMR with complete reaction after 4h even though 2 was still only moderately soluble. She observed that if pH7 buffer was not used in the workup of the LiBH$_4$ the crude product was contaminated with a \textit{bis}-cyclic urethane from the addition of the benzyl alchol in the the carbonyl of the Boc group. The solubility issues of 2 in the reduction medium were ultimately overcome by a two solvent method employing LiAlH$_4$.

LiAlH$_4$ has been used to reduce a vast array of organic compounds\textsuperscript{73} but we were leery of its use with its ability to reduce the Boc group. This concern did not come to fruition however as deprotonation of the Boc protected NH preserved the protecting group. As LiAlH$_4$ is soluble in Et$_2$O but not in CH$_2$Cl$_2$ and 2 is readily soluble in CH$_2$Cl$_2$ but not in Et$_2$O a solution was made of
each in the appropriate solvent. 2 in solution was then added to refluxing LiAlH₄ to produce the desired diol in ~2hr over the scale of 0.5g to 27g scale with 71-88% yield. (22g scale, 79% yield, mp 162-166; 27g scale, 82% yield mp 154-157. Most pure product 6g scale, 86% yield, mp 177-180 extracted quenched rxn mix/celite cake with CH₂Cl₂ only)

4. Synthesis of N,N'-di-t-Boc-2,5-diaminoterephthaldehyde

MnO₂ has been shown to be a mild oxidant for selective oxidation of benzylic of vinylic alcohols after systematic testing and although documented to have difficulty in oxidizing ortho- amino benzyl alcohols, it was not the case for our particular substrate. Although its chemioselectivity is desired, MnO₂ has many complicating factors in its use. First is that it must be freshly synthesized, usually by the Attenburrow method, and generates a fine solid that requires a large investment in time to collect by filtration and washed. Its activity also varies based upon its method of production and to what extent it is dried. If applicable, Goldman has found that wet MnO₂ can be stored for extended periods of time without precipitous loss in activity which greatly reduces the time in preparing the oxidant. Another issue in MnO₂ oxidation is that it requires a large stoichiometric excess which translates to a large volume of solid and can make efficient stirring tricky although recent work has found that
substoichiometric “active” MnO₂ from Sigma-Aldrich has been used under an atmosphere of O₂ to circumvent some of these concerns.

Oxidation of diol 7 to dialdehyde 8 was reliable when carried out using MnO₂ prepared by the Attenburrow procedure and dried in an oven at 110°C with periodic mixing until a free flowing powder resulted. When the oxidation was conducted in refluxing CH₂Cl₂ yields averaged 40%, which was attributed to the inability of the diol and mono-aldehyde to desorb from the MnO₂ surface. Yields improved to 85-95% when the oxidation was carried out in 20% CH₂Cl₂ in acetone. However care needs to be taken in the order of addition of the solvent. If 7 and MnO₂ were mixed together as solid, the addition of a flammable solvent first resulted in ignition of the materials. Combination of the mixed solids with CH₂Cl₂, followed by acetone avoided combustion.

**a. Over oxidation from MnO₂ and alternate Cu(I) catalyzed oxidation**

![Figure 22: Selective oxidation to dialdehyde with MnO₂](image)

![Figure 23: Over oxidation of DA to mono-aldehyde mono-carboxylic acid with MnO₂](image)
Scale up of the oxidation commenced with MnO₂ synthesized via the Attenburrow procedure using newly purchased reagents. Yields of the dialdehyde unexpectedly dipped to a 38% and 53%. Extraction of the MnO₂ from the reaction with 50% CH₃OH/CH₂Cl₂ produced a new solid. Analysis by ¹H NMR in DMSO-d₆ and ESI in negative ion mode confirmed that the reaction with MnO₂ somehow produced the mono carboxylic acid, mono aldehyde 9 by generating m/z= 370 (M-H⁺, 100%) and singlet integrating to 2H at 13ppm. Even though the MnO₂ is described in the literature needing to be alkaline for the reaction to proceed, it was postulated that excess hydroxide or other water soluble contaminants like KMnO₄ were present.

A new batch of MnO₂ was made and washed with water until the filtrate was colorless and neutral to pH Hydrion paper. Reaction with 25eq MnO₂ at 17hr reflux and 35eq MnO₂ at reflux for 24hr produced 58% 8 with 21% mono acid mono aldehyde and 43% 8 with 34% mono acid mono aldehyde 9 respectively. To rule out potential oxidation by Fe³⁺ or other dissolved metal ions from the metal instruments used in the synthesis of MnO₂ ceramic tools with analogous results as described above. Yield improved to 74-87% when the MnO₂ synthesized was washed with hot 0.6M pH7 phosphate buffer. CH₃OH was added to the system to decrease adsorption of 7 to MnO₂ in hopes of limiting the chance of over oxidation. However with a combination of 10% CH₃OH/ 20% CH₂Cl₂/70% acetone the reaction was incomplete over 2d at reflux and 75eq. MnO₂. The carboxylic acid side product began to form after 3d and incomplete transformation of 7 observed.

An alternative method to MnO₂ attracted our attention which would be to make an “active” MnO₂ layer deposited on the surface of activated carbon. The solid was easily
filtered. Its washing with water until the filtrate was neutral took a fraction of the time compared to the Attenburrow method. Use of a $12 \text{MnO}_2$ : $1 \text{C}$ mass ratio was used in 80% THF/CH$_2$Cl$_2$ resulted in trace dialdehyde formation after 24h at refluxing temperature. This method was no longer explored proving impractical for our purposes and should not be taken as an indictment of this clever technique. As Carpino noted in his work that the activated carbon employed came from the J.T. Baker company and other sources of activated carbon produced minimally active or non-reactive MnO$_2$ on C.

As the MnO$_2$ made did not have consistent chemical reactivity over its many preparations coupled with the days required for 100+g scale preparation it was determined to not be worth the effort or the sacrifice of diol. Although the Swern oxidation is known to selectively oxidize an alcohol to an aldehyde and has been optimized$^{82}$, it was not attempted due to concerns of solubility of the diol at the low temperature required. In collaboration with Surajudeen Omolabake we found an aerobic oxidation utilizing Cu(I)$^{83}$ the ideal for $7$. Complete conversion occurred overnight when exposed to the atmosphere at room temperature with rapid stirring. It was accidentally discovered that the oxidation was able to occur in the absence of 2,2′-bipyridyl with 50% mol ratio NMI as opposed to the 20% prescribed by the literature. Yields in the 80% range were average with crystallization of the product from the reaction medium occurring if CH$_3$CN was used as the solvent.

Figure 24: Selective oxidation to DA with Cu(I) catalyst
B. Synthesis of acyclic bis-Tröger’s Base Molecular Receptor

1. Preparation of bis-imines

With the key intermediate dialdehyde in hand, construction of the molecular tweezers began by condensation of a naphthyl amine with it to form a bis-imine was first conducted in collaboration with Nzagi Nyakirangani\textsuperscript{84}. The naphthyl amine initially chosen was methyl 6-amino-2-naphthoate which was prepared in 94\% yield (mp 154-157˚C) by refluxing 6-amino-2-naphthoic acid in dry CH\textsubscript{3}OH for 2h using 18M H\textsubscript{2}SO\textsubscript{4} as the acid catalyst. The dialdehyde was combined with excess 10 in boiling toluene using a Dean-Stark trap to drive the reaction to completion by azeotropic removal of water. Over the course of 17h the dialdehyde was no longer detectable by TLC and an orange solid formed. This orange solid was also observed by Nzagi Nyakirangani who had also had difficulties in identifying this material. This solid was insoluble in ethanol, DMSO, and sparingly soluble in CDCl\textsubscript{3} in which a $^1$H NMR spectrum was acquired. From the spectrum the material was speculated to be composed of 10, the mono imine, and the bis-imine but was sufficiently complicated to prohibit confident assignment. The synthesis was conducted again with an acid catalyst, the hydrochloride salt of 10 as well as a chemical dehydrating agent, bis-(trimethylsilyl)acetamide (BSA), were employed in an effort to completely form the desired bis-imine. After mixing at room temperature overnight a greater
amount of the orange solid was produced, which was sufficiently insoluble to prevent NMR analysis. In order to rule out the possibility that the orange solid was some kind of polymer derived from 8, a similar reaction was carried out in the absence of naphthylamine 10. No orange solid was formed, as no new spots were observed by TLC.

The insoluble orange solid was treated in glacial acetic acid with trimethyl phosphite for if the orange solid was indeed the desired bis-imine these reagents would be able to convert it into the corresponding bis-α-aminophosphonates. After 10d of vigorous stirring at room temperature, the desired aminophosphonates were formed, confirming that the organ solid was indeed the bis-imine. The crystallinity of the bis-imine was was exploited subsequently to drive its formation and eliminate the need for removal of water. Use of CH₂Cl₂ as a solvent and glacial acetic used the acid catalyst gave 11 in 88% yield.

![Figure 26: Optimized condition for synthesis of bis-imine 13](image)

Ethyl 6-amino-2-naphthoate 12 was made by Fischer esterification in anhydrous ethanol using concentrated sulfuric acid as the catalyst. After complete esterification and partitioning between CH₂Cl₂ and saturated aqueous NaHCO₃, yields ranged from 62-97. The purity of the material typically afforded after isolation is usually good enough to carry on without purification. 13 was first made by the condensation of 8 with a slight excess of the two equivalents of 12 needed in CH₂Cl₂ in the presence of Yb(OTf)₃. The lanthanide catalyst was
used to develop conditions later used in a one pot synthesis of the *bis*-aminophosphonates.

After 4hr at room temperature 8 was no longer seen by TLC, and a large amount of orange solid was seen in the reaction volume. After collecting by vacuum using ethyl acetate or methanol to transfer, 13 was afforded in 63% and 82% yield respectively. Synthesis was optimized using CH$_2$Cl$_2$ and glacial acetic acid on a 1.6g scale of the 8 to give the *bis*-imine in 91% yield in two crops with a melting point of 282-286°C of the first crop when allowed to stir at room temperature overnight and washed with EtOAc. After many preparations it was correlated that a purer crude product was produced after prolonged time stirring at room temperature. Due to its highly crystalline nature and the small amount of the *bis*-imine that can go into solution it is able to selectively complex with itself and enriching the solid in the desired material. *Bis*-imine 13, with ethyl esters instead of methyl esters, was soluble enough in CDCl$_3$ to be characterized by $^1$H, a $^{13}$C NMR and MS. In the $^1$H NMR spectrum, a singlet is observed at δ11.8 ppm which was attributed to the N-H of the Boc protected amine which is intramolecularly hydrogen bonded to the imine nitrogen. The consequence of this would be to lock all atoms participating in this six membered ring into a planar conformation that enhances crystallinity.

Solvents screened to determine not only a good solvent for recrystallization but a good solvent to put 13 into solution for reaction. The best solvents for dissolving 13 were as follows: THF ≈ CH$_2$Cl$_2$ >> EtOAc, toluene, Et$_2$O >> CH$_3$CN, DMSO, hexane.
2. Synthesis *bis*-α-aminophosphonates

![Diagram of synthesis process]

**Figure 27: Formation *bis*-α-aminophosphonates 14 and 15 from *bis*-imine 13**

The first attempt to improve *bis*-α-aminophosphonates, elevated temperature to shorten reaction times and to increase the solubility of *bis*-imine 13 were investigated. However, at temperatures above 80°C, the Boc protecting groups were removed. With degradation at higher temperatures and room temperature consigning reaction times to prohibitively long periods, investigation into altering the phosphite and catalyst species was conducted. It has been demonstrated in the literature that metal catalysts based on indium, scandium, and ytterbium facilitate α-aminophosphonate formation from imines with dialkylphosphites. Of these, ytterbium was chosen by Nzagi Nyakirangani and used by myself due to its ability to catalyze condensation of amines and ketones or aldehydes in β-enamino synthesis, its use in one pot preparations as it poorly catalyzes the dialkyl phosphite addition to the aldehyde, and its future potential for asymmetric aminophosphonation using chiral ligands. To confirm that a dialkyl phosphite would not add to 8 in the presence of an ytterbium catalyst a mixture of 8, Yb(OTf)₃, and dimethyl phosphite in CDCl₃. This was monitored by NMR spectroscopy over the course of a day with no changes observed in the ¹H or ³¹P resonances for the components.
Introduction of $\text{P(OMe)}_3$ however readily added to aldehyde at room temperature, so should only be used if the bis-imine is preformed.

When 8, 10, Yb(OTf)$_3$, and HPO(OMe)$_2$ were combined in either CH$_2$Cl$_2$ or CH$_3$CN, only trace amounts of the bis-aminophosphonates were formed after 4d at room temperature. This lack of activity was attributed to the poor solubility of 11 in either solvent which reduces concentrations below those that rapidly react. When done in neat HPO(OMe)$_2$ at 50°C for 18h 47% of the meso- diastereomer 14 and 31% of the rac- diastereomer 15 were isolated after silica gel flash chromatography with Nzagi Nyakirangani initially isolating only the meso- isomer. The diastereomers were readily separated on silica as they have marked different $R_f$ values. We tentatively assigned the meso- isomer 14 as the faster moving isomer with higher $R_f$.

Presumably, phosphonate group association with silica will dominate chromatographic retention, being most strongly retained when both phosphonates contact the surface While free rotation about the single bond allows placement of phosphonate on either face of the central benzene ring, there must be a lowest energy conformation. In the rac- diastereomer 15, with the same chirality at each of the aminophosphonate centers, both phosphonates will be on the same face in the lowest energy conformation. If formation of 14 and 15 was conducted in neat HPO(OMe)$_2$ at 90°C the desired materials were not produced. The isolated material resembled neither the starting material nor the products but with apparent loss of the Boc protecting groups. This preliminary data reinforced the idea that the insolubility of the bis-imine is the major impediment to reactivity that needs to be overcome.
Bisaminophosphonate synthesis from dialdehyde

Investigation into forming 16 and 17 efficiently starting by formation of bis-imine 13 in situ first using CH₂Cl₂ and glacial AcOH, followed by the addition of P(OMe)₃. The rationale behind this was to determine if the lanthanide catalyst could be omitted and then later used in developing asymmetric phosphonation. What was found though was that even with circa 10x stoichiometric excess of P(OMe)₃ in the presence of acid and at room temperature did not facilitate quantitative transformation. When Yb(OTf)₃ was introduced and the mixture heated to 60°C for 3h the reaction was complete. Analysis by ¹H NMR showed that the Boc groups remained intact but ³¹P NMR showed signatures for the remaining trimethylphosphite, the bis-aminophosphonate diastereomers, and a new signal at δ34 ppm which is attributed to dimethyl methylphosphonate, believed to be formed by the following mechanism:

When the solvent was changed to THF using 60°C temperature, the reaction time decreased to 14h but unfortunately P(OMe)₃ was still too reactive and not only formed dimethyl methylphosphonate but reacted with this solvent in the presence of ytterbium (III)
triflate. A side product was tentatively assigned as dimethyl (4-hydroxybutyl)phosphite which was formed, presumably by ring opening of lantanide corrodinated to THF.

![Proposed mechanism for reaction of P(OMe)₃ with THF in the presence of Yb(OTf)₃](image)

After silica gel flash chromatography the bis-aminophosphonates were afforded in an estimated 50% total yield that were not separated from the methyl dimethylphosphate or the dimethyl (4-hydroxybutyl)phosphite. From these results continued use of trialkyl phosphites were put aside due to its demonstrated propensity to form side products at the expense of the desired compounds and focus shifted to conditions in which dialkyl phosphites could be employed.

**Bisaminophosphonate Formation using HPO(OMe)₂**

![“One pot” bis-aminophosphonate synthesis with HPO(OMe)₂](image)

Its been shown in the literature that transformation of an imine to an aminophosphonate with a dialkylyphosphite and a catalyst as a homogeneous mixture occurs over the range of 0.5 to 4h with a wide range of compatible aldehydes and amines. Building off of the previous work done by Nzagi Nyakirangani, prospecting began using the “one pot”
method for bis-aminophosphonate formation. Bis-imine 13 was formed first in the presence of Yb(OTf)₃ with 4.5eq dimethyl phosphite in CH₂Cl₂ for 4d to give 34% yield (28% meso- 16, 6% rac- 17) after silica gel chromatography. This contrasted greatly from the 1:1 diastereomeric ratio observed in the NMR of the crude product before purification. Resolution suffered as the remaining dimethyl phosphite interfered with chromatography. Analysis of the diastereomers by ¹H and ³¹P NMR showed clean isolation of products from the dimethyl phosphite, but the rac- portion contained more than one signal in the ³¹P spectrum and lower integration ratio of the phosphoesters in ¹H spectrum. From this it was surmised that degradation of the aminophosphonates was occurring on silica gel by the lack of mass balance after this purification technique and the retention of color on the silica. As the ratio of aminophosphonate diasteromers formed should be 1:1 as the conditions for aminophosphonation are unselective, the rac- isomer was being degraded to a greater degree as it was isolated in a significantly lower amount. Put forward here is a mechanism by which degradation occurs which seems feasible as it is similar to the removal of a methyl phosphonate ester by excess trimethyl phosphite and provides and explanation as to why the rac- isomer is affected disproportionately. The longer contact time with the slower migrating racemic isomer 17 may explain the greater degree of degradation.

If the silica gel acidity was the problem, neutralization may avoid it. The first protocol employed was to expose silica gel to gaseous ammonia. To do this a sample of silica gel was exposed to the vapor over a container holding concentrated aqueous ammonia for one week with periodic mixing over that time. When this silica gel was used for chromatographic separation it indeed significantly decreased the polarity of the eluent needed but caused the
diastereomers to collute. However, if the silica gel that was treated in this manner was left exposed to the atmosphere for a day with periodic mixing the separation was accomplished with the meso- isomer being eluted with 40% CH₃CN/CH₂Cl₂ instead of 80% CH₃CN/CH₂Cl₂ and the rac- isomer was eluted with 80-100% CH₃CN/CH₂Cl₂ instead of 10% CH₃OH/CH₂Cl₂. Use of the silica gel also produced a lesser degree of degradation upon separation of the diasteromers. Although encouraging we chose not to follow up on this due to questions of reproducibility.

With quantitation in mind though, deactivated silica gel was prepared by exposure of silica gel to well defined amounts of Et₃N in CH₂Cl₂ as a slurry. Mixtures of 2.5% and 5% allowed for sufficient deactivation while still affording separation. The 2.5% mixture behaved analogously to the gaseous ammonia exposed silica while the 5% mixture produced the largest disparity in eluents needed to elute the products with the meso- isomer being collected with 10% CH₃CN/CH₂Cl₂ and the rac- isomer being collected with 100% CH₃CN. Deactivation of the silica indeed afforded a greater amount of the rac- isomer but still failed to completely prevent degradation.

<table>
<thead>
<tr>
<th>% by mass NEt₃/silica</th>
<th>Yield 16</th>
<th>Yield 17</th>
<th>Total Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>28%</td>
<td>6%</td>
<td>34%</td>
</tr>
<tr>
<td>2.5%</td>
<td>39%</td>
<td>19%</td>
<td>58%</td>
</tr>
<tr>
<td>5%</td>
<td>32%</td>
<td>21%</td>
<td>53%</td>
</tr>
</tbody>
</table>

Table 1: bis-aminophosphonate diastereomers afforded from deactivated silica gel chromatography

With the anemic activity of the dimethyl phosphite and the use of trimethyl phosphite producing side products a species of intermediate activity was desired. Dimethyl phosphite is in
equilibrium with its trivalent tautomer but the equilibrium heavily favors that of the pentavalent form. This can be shifted though by the introduction of a silylating agent. BSA was chosen not only for its two equivalents of silylating group per molecule but also acts as a base to facilitate proton transfer in isomerization.

![Chemical structure](image)

**Problem**

**Solution**

Figure 32: Use of BSA to shift HPO(OMe)\(_2\) equilibrium and prevent methyl transfer

Dimethylphosphite was combined with BSA and monitored by 31P NMR over the course of a day over which time the signal at \(\delta 15\) ppm from HPO(OMe)\(_2\) decreased and a new signal grew in at \(\delta 129\) ppm. As this new resonance was similar in chemical shift to P(OMe)\(_3\) (\(\delta 141\) ppm) this was taken as evidence of the desired conversion to dimethyl trimethylsilylphosphite\(^9^4\). Along with this change another modification to the synthetic procedure was introduced in order to still utilize the large excesses of dimethyl phosphite required but allow it to be removed as this cannot be easily done by simple evaporation. It well known that use of I\(_2\) in pyridine and water can oxidize dimethyl phosphite to a phosphinic acid\(^9^5\) which could then be removed by washing with aqueous base. When the dialkyl phosphite was injected with an equimolar amount of BSA reaction times averaged 18-30h at room
temperatures with yield increasing to a consistent 50% of the diastereomeric mixture after silica gel chromatography. It was discovered that quenching of the silylating agent was paramount by addition alcohol prior to being exposed to the I$_2$/aqueous pyridine mixture for if this was not done then only trace amounts of the desired products were isolated. This could possibly be attributed to I$^-$ demethylating the methyl phosphonates too quickly under aprotic conditions.

It wasn’t until much later that the oxidative workup was suspected to be in participating in the dealkylation of the phosphonates in 16 and 17 even after quenching the BSA. This was tested by taking a crude mixture of the diastereomers, splitting it into two portions, and treating one of the halves. Analysis of the untreated half when viewed with $^{31}$P NMR showed signals for the excess dimethyl phosphite and the two desired bis-aminophosphonates wereas the treated half had the resonances of the products along wth an array of signals upfield confirming the removal of the phosphomethyl esters. The mechanism of dealkylation is suspected to be by S$_{N}$2 reaction with I$^-$. This avenue of excess phosphite removal was abandoned as subsequently developed methodologies do not utilize chromatographic separation.

With the susceptibility of the dimethyl phosphonates to degradation on silica gel established the phosphonating agent was switched to diethyl phosphite in order to slow the rate of ethyl phosphonate ester S$_{N}$2 cleavage. This approach was validated by isolation of 73% total yield of the bis-aminophosphonates after chromatographic separation. From this result it was deemed worthy to carry forward with diethyl phosphite as the phosphonating agent as
well as pursuing tweezers synthesis without isolation of the bis-aminophosphonates which will be discussed in a later section.

3. Trögerization Affording Molecular Receptors Isomers

![Chemical Structures]

**Figure 33**: Trogerization to form desired rac-(18) and control meso- (19) molecular receptors isomers

With isolated 17 and 16 on hand cyclization to form the bis-Tröger’s base tweezers commenced. The acid chosen, trifluoroacetic acid (TFA), and the formaldehyde equivalent, dimethoxymethane (DMM), were chosen for Trögerization as both of these required reagents are volatile so that when used in excess remaining amounts of them can be removed under reduced pressure. In using these two as reactive solvents two things were observed: that a reflux temperature at 60°C or above was required to drive Tröger’s base formation to completion and that polyformaldehyde was generated as a side product. The polyformaldehyde was not separated from the desired product through silica gel flash chromatography but was able to be removed only after extensive washing with a saturated NaHCO₃ solution.

Fortunately it was found that the addition of either methanol or ethanol as a cosolvent
prevented polyformaldehyde formation by condensing with the formaldehyde equivalent as well as elevating the reflux temperature of the mixture. Additionally crude 17 was cyclized in the presence of dimethyl phosphite, dimethyl methylphosphonate, and dimethyl (4-hydroxybutyl)phosphonate with no observable interference in Tröger’s base formation. 18 and 19 molecular tweezers isomers displayed similar behavior on silica gel as its bis-aminophosphonate counterparts with regards to degradation occurring over the course of flash chromatography, albeit to a lesser degree. Although these tweezers isomers were not still not separated from the phosphite derivatives they were still able to be identified by $^1$H NMR and provided samples for monitoring their formation by TLC.

Figure 34: "One pot" molecular tweezers synthetic scheme using HPO(OMe)$_2$

Synthesis to form the desired molecular tweezers built upon the previous procedure in which the bisimine 13 was formed in situ followed by the introduction of the aminophosphininating reagents followed by exposure to condition to form the bis-Tröger’s base skeleton. Synthesis proceeded in one flask starting with 8 and 12. The formation of the intermediates 13, 16, and 17 were followed by TLC until the desired products were completely formed. When the desired compound was formed the mixture was concentrated by rotary evaporation and exposed to the next set of reaction conditions without removal of Yb(OTf)$_3$. This proved to be detrimental as the more stable meso-isomer 19 was afforded in only 17% yield but a 17% yield the rac-isomer 18 was also obtained. Even though this sequence did not
produce an overall increase in yield of the 18 after chromatographic isolation, it did provide an amount of material on par with the protocol utilizing chromatographic isolation in each synthetic step. When the lanthanide was removed prior to cyclization conditions with an EDTA/NaHCO₃ solution, yields did not substantially increase.

![Figure 35: "One pot" molecular tweezers synthetic scheme using diethyl phosphite](image)

Figure 35: "One pot" molecular tweezers synthetic scheme using diethyl phosphite

With the increased stability of the ethyl phosphonate esters previously demonstrated exploration of the “one pot” synthetic method was conducted using diethyl phosphite with removal of the lanthanide before Trögerization. The resulting tweezers isomers were were separated by flash chromatography to afford a substantial increase in yield compared to the dimethyl phosphonate analogues with an average yield of 76% for the meso- isomer 21 and 58% average yield for the desired rac- isomer 20. The meso- and rac- isomers exhibited dramatically different physical properties. For example, the meso- isomer was isolated as a crystalline white solid with a fairly high melting point range of 299-303°C while the binding rac- isomer is a semi-solid with a low melting point range of 50-60°C.

From these observations and with an appreciable amount of the two isomers now in hand we set about a separation by crystallization. The solubilites of the two were then screened against a panel of solvents. While the rac- isomer, 20, was found to be soluble in all the solvents tested save for hexanes, 21 on the other hand proved to be insoluble or sparingly soluble in
CH$_3$CN, toluene, ethanol, and EtOAc and could be recrystallized from any of these solvents. When a mixture of 20 and 21 was triturated with EtOAc, toluene, or CH$_3$CN the meso- isomer remained solid and was isolated by vacuum filtration while the desired rac- isomer remained in the supernatents. Most attempts to recrystallize the rac- isomer failed: addition of hexanes to a solution of 20 in any of the solubilizing solvents caused oiling out. What was found to be an effective method to solidify 20 was to take a solution in toluene (56mL/g) and add it dropwise to ten volumes of stirring hexanes.

Scale up of the molecular tweezers synthesis was now conducted with an optimized “one pot” procedure and a method of non-chromatographic separation. The synthetic protocol began with the easily isolated and recrystallized bis-imine 13 as opposed to forming it in situ. This avoids formation of Tröger’s base derivative formed from 12, which required silica gel chromatography for removal. After aminophosphonation, extractive removal of the lanthanide catalyst, and cyclization to form the bis-Tröger’s bases, the volatiles were removed by rotary evaporation before neutralization. The resulting crude solid was then triturated with hexanes to remove any remaining diethyl phosphite before being triturated with EtOAc to leave behind 21 as a white solid. The EtOAc supernatents were concentrated to dryness, dissolved in toluene, and added dropwise to stirring hexanes to generate the solidified 20. In the end, an 84% yield
of non-binding meso-isomer 21 and a 72% yield of the desired binding rac-isomer 20 were afforded. This process was repeated several times to give yields of 21 averaging 86% and 20 averaging 75%.

When this synthesis was done on a 1g scale or above of the bis-imine 13 using the developed methodology the transformation to the corresponding bis-aminophosphonates was not complete after the usual 18hr time period. Instead, it required three days and additional portions of Yb(OTf)₃, diethyl phosphite, and BSA reaching these final concentrations and ratios: Yb(OTf)₃ = 0.39eq, 7x10⁻³M; HPO(OEt)₂ = 99eq, 1.8M; BSA = 49eq, 0.9M. This change in behavior was attributed to the increased purity and thus the increased crystallinity of the 13 used as well as a diminishing in the activity of the lanthanide catalyst over time. As mentioned previously, the chemical literature thoroughly demonstrates rapid aminophosphonation on time scales up to hours in length. This implies that the catalyst if active in this time frame. Undergraduate Angel Corona added various ligands and attempted to study varying catalyst compositions and stoichiometries to maximize reaction rate. He found a dependence on order of ligand addition and irreproducible rates. Sometimes bis-imine 13 was solubilized in minutes, with apparent completion of reaction by TLC in that time. Unfortunately, identical conditions required days for completion if the starting imine did not quickly dissolve. We interpret these results to say that a transient lanthanide complex acts as a much more active catalyst before diminishing over time.
In an attempt to circumvent the insolubility of the bis-imine 13 formation of the tetraBoc derivative was prepared. The reason for this is that the bis-imine is a flat, aromatic molecule with a large amount of surface area that allows for a multitude of π-π interactions. If this could be inhibited by reducing its planarity, it would make a poor crystal and more easily dissolve. Addition of another Boc group on the Boc protected nitrogen would eliminate the intramolecular hydrogen bond that enforces the shape. Use of DMAP and Boc₂O in CH₃CN has been shown to accomplish this.³⁶ The described conditions did not facilitate additional Boc protection which was attributed again to the poor solubility of 13 in CH₃CN. When the solvent was switched to THF, and triethylamine was added, the desired tetraBoc compound was completely formed after 17hr and isolated in 89% yield of 22 by silica gel chromatography. This material was then exposed to the aminophosphonation conditions as a homogeneous solution with the derivative bis-imine being consumed after 4hr. Analysis of the mixture by ³¹P NMR however did not show just the two signals anticipated for the aminophosphonate disastereomers but instead contained ten major peaks in the chemical shift range of the desired materials (δ25-30ppm). This multitude of signals could have arisen from the transfer of a Boc groups from the phenylamine to the naphthylamine. If this were the case it should not prohibit cyclization as the trifluoroacetic acid in the next reaction step would remove the Boc groups. This technique seems promising for efficient conversion of the 13 on a large scale and should be investigated by future researchers. However, due to the number of the signals and ambiguity in interpretation this avenue was not followed up.
With a developed “one pot” protocol and the ability to separate the isomers non-chromatographically, it was decided to go back to the dimethylphosphonate version of the molecular tweezers. The reason for this would be a simpler $^1$H NMR spectrum which would allow for unambiguous interpretation when evaluating conditions for phosphonate ester removal. The $^1$H NMR spectrum for the dimethyl phosphonates exist as two doublets as each of the diastereotopic methyl groups is coupled to the phosphorus nucleus. On the other hand, the splitting pattern of the diethyl phosphonates is substantially more complex. Each of the two diastereotopic ethyls has a pair of diastereotopic hydrogens that couple each other, are doubled by the $^{31}$P, and coupled into a quartet by the neighboring methyl, yielding 4 sets of ddq for 64 peaks in a 0.2ppm spectral range. The carbethoxy group also has a nearby quartet.

Transformation of 13 on a 1.6g scale to 16 and 17 required the same prolonged reaction time; 11d and four additions of Yb(OTf)$_3$. The tweezers isomers were separated non-chromatographically using toluene to afford an average yield of 86% 19 and 83% 18. The desired rac-isomer was purified by dissolving in minimal 1,4-dioxane and precipitating by addition of eight volumes hexanes. It was observed that the rac-isomer 18 needs to be kept in cold storage as the material appears to degrade if kept at room temperature. This was concluded when old samples of 18, which were kept at room temperature from four months to
one year were combined with toluene which produced a heterogeneous mixture. The degraded material was able to be separated from intact 18 by vacuum filtration as the degraded material was not soluble in toluene. Investigation into the composition of this degraded material was not pursued as proper storage and separation techniques were indentified, but $S_N2$ attack at the phosphonate methyl is plausible.

4. Reactions to Generate Water Soluble Molecular Receptors

To turn the molecular tweezers into a water soluble form, the esters need to be removed. Detailed below are the formations of three different water soluble derivatives that vary in the amount and distribution of negative charge on the molecular tweezers. Known synthetic methods were employed to take advantage of the selectivity in ester cleavage based on the atom the ester is connected to. These techniques were also used to make water soluble version of the meso- tweezers isomers to serve as a negative control in small molecule titration to evaluate complex formation with the desired rac- binding isomer.

![Diagram of the reactions](image)

**Figure 39: TMSBr deprotection of tweezers isomers to afford carboxethoxy tetra acids derivatives**

Bromotrimethylsilane (TMSBr) was first described in 1977\(^97\) to selectively cleave alkyl phosphonate esters in the presence of carboxylate esters swiftly at room temperature in high yield. For this reason this method has been used subsequently\(^98\) with demonstrated tolerance
for a multitude of functional groups. The conversion of 18 to the desired carbethoxy tetraacid compound was done with large excesses (10x stoichiometric) of TMSBr in CH$_2$Cl$_2$ at reflux for 1.5 to 16h or at room temperature over 22hr without apparent degradation or side reactions. However, this is dependent upon the purity of the TMSBr. If the TMSBr is colorless or a faint yellow color removal of the phosphoesters occurred without complication. If the TMSBr was orange in color or darker, which is indicative of Br$_2$ from HBr formed, the afforded material could not be identified and was concluded to have been degraded. Complete removal of the P-Me esters was determined by NMR spectroscopy when doublets in $^1$H at δ4.04 and 3.98ppm disappeared and signals in $^{31}$P converged to δ17ppm. After this the volatiles were removed by vacuum in an anhydrous environment to prevent HBr formation until a solid remained after which it was exposed to methanol to remove trimethylsilyl esters. Dealkylation of the meso-isomer 19 required refluxing temperature to go to completion in a reasonable amount of time but could also be accomplished in 7d at room temperature if time is not a factor. Both of the isomers were purified by dissolving in boiling EtOH, cooling to room temperature, and precipitation with hexanes to yield 77% 23 and 86% 24 respectively.

Figure 40: Saponification of tweezers isomers to form the methyl phosphonate tetra anion derivatives

rac- isomer (25): 83%
meso- isomer (26): 87%
Another water soluble derivative was made when the tweezers isomers were exposed to aqueous hydroxide to saponify the carboxylic esters and selectively remove a single phosphonate methyl ester on each of the phosphonates. Rabinowitz demonstrated this selectivity\(^{100}\) in dealkylation utilizing aqueous hydroxide at refluxing temperatures over the course of 16h resulting in high yield. The origin of this selectivity is attributed to coulombic repulsion of subsequent hydroxide after the first equivalent breaks the first P-O bond through an addition elimination mechanism.

The saponification procedure was developed using 19 as it was in abundance. Lithium hydroxide was chosen as even though it is the weakest of the alkali hydroxides it can be used in excess as it is readily removed after neutralization with HBr: LiBr is soluble in THF. 19 was mixed with a four fold excess of LiOH in a combination of 1,4-dioxane and D\(_2\)O so progress could be monitored by \(^1\)H NMR spectroscopy. TLC analysis would not be able to distinguish the degree of ester removal over time. After 15h all of the desired esters were removed and the absence of the benzyl hydrogen in the aminophosphonate was noted. When submitted to mass spectrometry analysis a m/z of 743 in the negative ion mode and a m/z of 745 were observed suggesting a molar mass of the resulting compound as 744 g/mol. This molar mass is two mass units higher than that of the tetracid form of the saponified meso- tweezers which is what seen in MS analysis due to the use of formic acid as the modifier. From this mass increase and lack of signal in the NMR it was determined that H/D exchange occurred on that benzyl position under the aqueous basic conditions before cleavage of the phosphonate ester. This was proved when saponification was performed in H\(_2\)O and no loss of benzyl signal was seen and no increase in mass in spectrometric analysis.
The desired saponified tweezers isomers are formed and stable in the refluxing hydroxide solution after 15h after which it is concentrated to dryness to remove the dioxane as to not react with the hydrobromic acid. After redissolving in water, neutralizing to pH7 with 4.8% HBr, and trituration with THF 25 was afforded in 83% yield and 26 was afforded in 87% yield.

**Figure 41:** Hexa-anionic tweezers isomers formed by saponification of the TMSBr dealkylated derivatives

The fully deprotected hexa-anionic tweezers were produced by saponification of the bromotrimethylsilane dealkylated water soluble tweezers 18 and 19. The LiOH saponification protocol described previously was used with saponification complete after 6hr at reflux in the aquoues hydroxide. Neutralization with HBr and trituration with THF afforded 27 in 61% yield and 28 in 66% yield.
C. Characterization of Molecular Receptor Isomers

Isomers 18 and 19 displayed markedly different behaviors when analyzed by $^1$H NMR spectroscopy. The fully esterified rac- isomer 18 was initially difficult to identify as the resonances seen were very broad but at least were in chemical shifts regions consistent for the chemical bonds for the hydrogen atoms in the desired material. In contrast, the meso- isomer 19 gave sharp signals in the same region. To test whether the broadening is due to binding, dimerization only of 18, we acquired $^1$H NMR spectra at various concentrations from $10^{-2}$M down to $1.4\times10^{-4}$M, roughly the detection limit for the 300MHz Bruker NMR for 30min acquisition time. Over the course of the dilution, the aromatic hydrogens in the naphthalene walls shifted downfield while the aromatic signals in the benzene core shifted upfield with no more apparent movement at concentrations of $3.4\times10^{-4}$M and below. This behavior is consistent with the binding, rac- form of the molecular receptors self association by the formation of a dimer the inclusion of the naphthalene wall of one molecule of the tweezers into the cavity of another molecule of the tweezers. In dimer formation by this mechanism the
delocalized electrons in the aromatic naphthalene walls from the tweezers would shield the hydrogens on the other copy of the tweezers while the benzene hydrogens would be deshielded by the edge on interaction with the naphthalene quadrupole.

![Figure 43: ¹H NMR dilution of the fully esterified rac- tweezers 18 in CDCl₃](Image)

From the NMR dilution study the chemical shifts of the naphthalene hydrogens versus concentration were plotted and analyzed via non-linear least squares fit via the derived equation below to see whether the changes are consistent with dimerization and to determine the dimerization constant of 18 in CDCl₃. The value calculated from this data was $1.5 \times 10^{-1} \text{M}$ but it should be stressed that this value is just an estimate based on the number of data points (4) used in the analysis and at least ten data points are required to garner an accurate estimate.
Figure 44: Equation used for the non-linear least squares fit for the dimerization of the fully esterified rac-tweezers utilizing NMR chemical shifts (top) and fit using chemical shift proton A (bottom)

\[ [C] = \frac{(4[H_o] + K_{dimer}) - \sqrt{(8[H_o] * K_{dimer} + K_{dimer}^2))}}{8} \]

\[ \delta_{obs} = \delta_{Ho} \left( \frac{H}{H_o} \right) + \delta_c \left( \frac{2C}{H_o} \right) \]

Figure 45: Chemical shifts, coupling constants, and NOESY correlations for the full esterified binding rac-isomer 18 in CDCl$_3$ at 3x10$^{-3}$ M concentration

When at a concentrations that were monomeric the hydrogen signals of rac-isomer 18 in the bis-Tröger’s base skeleton were able to be distinguished by their distinct coupling
constants. The hydrogens on the methylene bridging the two nitrogens had a coupling constant of 13Hz, the hydrogens on the methylene connecting the naphthalene wall exhibited a coupling constant of 17Hz, and the benzyl hydrogen in the aminophosphonate displayed a signal with 23Hz due to its coupling with the phosphorous.

![Figure 46: $^1$H NMR spectrum of the fully esterified non-binding meso- tweezers isomer 19](image)

When the $^1$H NMR spectrum was acquired for the control meso- isomer 19 no broadening of the hydrogen signals were observed at concentrations where that phenomenon was seen in the rac- isomer. From this it was concluded that meso- isomer was not associating with itself while in solution, consistent with our design.

**D. Dimerization of Water Soluble Receptor Isomers in Water**

**1. Analysis by $^1$H NMR Spectroscopy**

All of the water soluble derivatives of the binding rac- isomer also showed the propensity for self assembly in aqueous enviornments. This made initial characterization of all of these compounds difficult as in the case of carbethoxy tetra anion 23, which produced a $^1$H NMR spectrum containing ill defined singals over broad chemical shift ranges.
Figure 47: Comparison of the $^1$H NMR spectra of rac- carboxethoxy tetra anionic tweezers 23 in D$_2$O and 50% TFA/CDCl$_3$ versus the non-binding meso- isomer 24 in D$_2$O

The sample in D$_2$O was exposed to temperatures ranging from 274K to 313K in hopes of increasing the resolution of the peaks by either increasing or decreasing the rate of exchange of the tweezer molecules in dimer formation but this was not observed. This suggests that rapid exchange on an NMR scale is not accessible at a reasonable temperature. Pyridine-$d_5$ was used as a solvent in effort to produce monomeric species by insertion into the cavity and did seem to work when the concentration of the rac- tweezers 23 was around 5x10$^{-4}$M but the signals from the residual solvent and their $^{13}$C satellite peaks obscured the majority of the aromatic region. DMSO-$d_6$ was successful in producing a spectrum with resolved aromatic signals but a broad singlet was seen in the spectrum in the 3 to 4ppm range which is in the area needed to evaluate if the P-Me esters are absent. What did produce the best results for characterization was dissolving the initially isolated tetra acid 23 in a mixture of 50% TFA in CDCl$_3$. Although not as well resolved as the meso- isomer 24, it produced data sufficient for accurate integration and assignment of the hydrogens. The lack of doublets at $\delta$4ppm with $J$=10Hz confirms the absence of methyl phosphonates and the efficiency of the TMSBr dealkylation method. The control
meso- carbethoxy tetra anion isomer 24, like the full esterified form, gave sharp peaks which were interpreted as no self association in D₂O at concentrations ranges equal to or greater than those where the binding rac- isomer 23 appears to do so.

\[ \text{Figure 48: Comparison of the } ^1\text{H NMR spectra of rac- methyl phosphonate tetra anionic isomer 25 in D}_2\text{O and 50% TFA/CDCl}_3 \text{ versus the non-binding meso- isomer 26 in D}_2\text{O} \]

As opposed to 23, the rac- methyl phosphonate tetra anionic tweezers 25 when in D₂O did not produce broad signals but an array of signals presumed to be a mixture of species that are exchanging with each other in solution. This presumably is able to occur as this saponified form of the molecular tweezers has an energetic barrier to dimerization from the negatively charged carboxylates on the edges of the naphthalene. The \(^1\text{H NMR is consistent with the desired cleavage by hydroxide having taken place. The } ^1\text{H NMR simplified slightly upon dilution in D}_2\text{O but not to the point of producing a single set of signals. Use of 50% TFA/CDCl}_3 \text{ in NMR analysis was able to produce a well resolved set of signals that allowed for characterization of 25. It appears that the molecule is not stable to strongly acidic conditions: an impurity forms}

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showing a doublet at δ3.9ppm with J=11Hz in proximity to the methyl phosphonate signal. This may be due to partial transformation of the Tröger’s Base skeleton.

This phenomenon also occurs for the meso- isomer 26 in 50% TFA/CDCl₃ but it is not required as like the other control isomers affords well resolved NMR spectra at mM concentrations. The residual solvent signal in D₂O however does reside in the same chemical shift range of the Tröger’s base signals and 50% TFA/CDCl₃ is needed to see these resonances as the residual solvent signals in this mixture do not mask them. After replicating the saponification procedure for both isomers multiple times the integrity of the Tröger’s base skeleton remained intact under the alkaline conditions with the TFA/CDCl₃ needed only to absolutely confirm the structure.

Figure 49: Comparison of the ¹H NMR spectra of rac- hexa anionic tweezers 27 in D₂O and 50% TFA/CDCl₃ versus the non-binding meso- isomer 28 in D₂O.
The binding rac- (27) and control meso- (28) isomers for the fully deprotected hexa anionic derivative exhibited the same behavior in D$_2$O and 50% TFA/CDCl$_3$ as their other water soluble counterparts. The set of signals in D$_2$O were less broad than that of the methyl phosphonate tetra anion and did seem to coalesce to a single set between 6.3x10$^{-4}$ and 6.3x10$^{-5}$ M but a sufficient signal to noise ratio required to assign the peaks could not be achieved.

2. Analysis by Fluorescent Spectroscopy

BACKGROUND AND GENERAL PROCEDURES

As $^1$H NMR spectroscopy could not be used at sufficiently low concentrations to determine a dimerization constant for the water soluble tweezers varieties, fluorescent spectroscopy was used. This technique would allow for the dilution of the tweezers to concentrations below the level of detection of NMR by monitoring its fluorescent emission. It is expected that the dimerized complex and the free monomeric species would emit differently to allow to for a way to identify the concentration in which dimerization occurs. A deviation from linearity in fluorescent emission upon dilution of the tweezers would provide evidence of association. If a single species exists in solution its fluorescence will decrease proportionally with concentration with a constant excitation wavelength as described by the Beer-Lambert Law.
Figure 50: Derivation of the equation used to determine $K_{\text{dim}}$ for the dimerization of the binding rac-tweezers by fluorescence spectroscopy

Correction of fluorescence for screening of exciting light was used. In cases where the absorbance of the tweezers isomers were above a value of 0.1 the intensity of the fluorescent emission was corrected using the equation of $I = I^* 10^{(A/2)}$. The term of $A/2$ was used to compensate for the diminution of emission produced at a point halfway through the sample where fluorescence is monitored. This correction factor was used only for absorbance values between 0.1 and 0.2 as values corrections exceeding 0.2 will be inconsequential.

**Carbethoxy tetra acid tweezers 23**

The Ocean Optics SD2000 was initially used in trying to determine monomeric concentrations of the *rac*-carbethoxy tetra anion tweezers 23 which was irradiated with broadband light centered at 365nm. A $7.2 \times 10^{-2}$M solution in 10mM pH7 phosphate buffer was serially diluted by a factor of 2 until a final concentration of $7.0 \times 10^{-5}$M was reached. Linear decrease in fluorescent emission was observed from $2.25 \times 10^{-3}$M and lower by monitoring the emission maximum (495nm) of the tweezers. Fluorescence was non-linear in range of $7.2 \times 10^{-2}$ to $2.25 \times 10^{-3}$M with fluorescence increasing upon dilution. This linearized using our screening
correction of the exciting light and not from dimer to monomer transition. The Fluorolog-3 fluorimeter was used subsequently to test concentration ranges below the level of detection of the Ocean Optics, with more control of excitation wavelength.

23 was dissolved in an aqueous solution containing 150mM NaCl and 10mM pH7 phosphate buffer and serially diluted by factors of 1/3 from an initial concentration of 2.50x10^{-5} to a final concentration of 2.89x10^{-7} M. Spectra were acquired using an excitation wavelength of 300nm. A broad emission centered at 445nm was seen down to 10^{-7} M. A plot of tweezers concentration versus fluorescent emission at 445nm showed a linear correlation in the decrease in emission.

![Figure 51: Plot of fluorescent emission (445nm) versus concentration for rac-carbethoxy tetra anion 23 tweezers using 300nm excitation](image)

This is consistent with a single species present over this concentration range, either monomer or a dimeric. 23 is expected to dimerize most strongly and if it is dimeric, the K_{dim} < 3x10^{-7} M the limit of detection for this compound with the Fluorolog fluorimeter.
Methyl phosphonate tetra anion isomers 25 and 26

It is expected that the methyl phosphonate tweezers, bearing carboxylate anions on their walls, should not dimerize as readily. To keep ionic strength roughly constant an aqueous solution of 150mM NaCl and 10mM pH7 phosphate buffer was used in the dilution studies. Dimerization behavior was again evaluated using deviation from linearity in fluorescent emission with dilution. Using 300nm excitation the emission spectrum of the binding isomer displayed a fine structure with emission maxima at 405 and 430nm. The rac- methyl phosphonate tetra anion isomer 25 was diluted from $2.5 \times 10^{-5}$ M to $4.3 \times 10^{-7}$ M. A plot of fluorescence at 405nm versus tweezers concentration made and analyzed using the non-linear least squares fit to determine $K_{\text{dim}}$. Although it converged it produced error of equal value to the dimerization constant ($K_{\text{dim}} = 1.9 \times 10^{-4}$ M ± $2.1 \times 10^{-4}$ M) which makes sense as the plot of fluorescence vs. concentration appeared to have only a slight curvature to it, and dimer would be mostly dissociated even at the highest concentration.

Figure 52: Fluorescence of rac- methyl phosphonate tweezer displaying fine structure at 300nm excitation
This result from the concentration range tested again is suggestive of a single species in solution while being diluted. Using 300nm excitation, concentrations above $2.5 \times 10^{-5}$M could not be studied because of screening of light. Dilution was repeated using the ionic solution over the range of $2.5 \times 10^{-4}$ to $1.5 \times 10^{-5}$M which was anticipated to be a regime in which dilution would facilitate the change from dimer to monomer. The plot of fluorescent emission at 430nm versus rac- isomer concentration produced a line with slightly more curvature resulting in a $K_{\text{dim}}$ of $4.5 \times 10^{-4}M \pm 9.8 \times 10^{-5}M$ with the standard error being satisfactory for the value for $K_{\text{dim}}$. This suggests a significant association of the tweezers to form a dimer, overcoming a substantial amount of coulombic repulsion in the process.

![Figure 53: Fluorescent emission and plot of fluorescence (430nm) versus concentration for rac- methyl phosphonate tetra anion tweezers 25](image)

From these data it appears that fluorescence spectroscopy is limited in its ability to determine a dimerization constant with low standard error using 300 and 365nm light. 345nm excitation light however may be just right if this dilution is to be repeated as it might allow for solutions of high enough concentration to contain predominantly dimer but not have the rapidly diminishing emission upon dilution so that concentrations lower than $1.5 \times 10^{-5}$M can be
investigated. The ideal concentration range to study dimerization would span from 80% dimer to 80% monomer, but this method is limited to 50% dimer. Dimerization was subsequent investigated by UV-Vis spectroscopy and will be discussed in a later section.

The control isomer, meso- methyl phosphonate tetra anion 26, was diluted from $2.5 \times 10^{-4}$ M to $4.3 \times 10^{-6}$ M but only data from $6.25 \times 10^{-5}$ M and below were used in the plot of emission at 405nm vs. concentration as absorbance values for concentrations above that were above 0.2 using 300nm excitation. As anticipated the resulting plot was linear and was able to converge when fit to a non-linear least squares model producing a $K_{\text{dim}}$ of $1.9 \times 10^{-7}$ M ± $6.8 \times 10^{-6}$ M. The enormous error range indicates that this behavior is not appropriately modeled by dimerization. The standard amount of error was an order of magnitude larger than the derived value and linear decrease of fluorescence are consistent with the behavior.

To confirm that the meso- isomer was not self associating, the fluorescent emission with variable excitation of a $1.8 \times 10^{-4}$ M solution of the control meso- isomer was monitored. As seen previously with the rac- isomer 25, different excitation wavelengths produced different shapes in the fluorescent emission spectrum due to the differences in the absorbance of either the
dimer or monomer. If only a single species is present then a change in fluorescent intensity would be expected with no change in the shape of the emission spectrum.

![Emission meso- methyl phosphonate tetra anion (1.76E-4M) at various Excitation Wavelengths](image)

**Figure 55:** Fluorescent emission of control meso- methyl phosphonate tetra anion 26 at various excitation wavelengths plotted to include excitation wavelengths

This is exactly what was observed. Fluorescence increase from 260 to 315nm as screening of the light went away and fluorescent intensity diminished with the longer wavelengths supporting what was observed with NMR that the control meso- isomer is not dimerizing when in solution.

**Hexa-anions 27 and 28**

The final water soluble derivative, the fully de-esterified hexa-anion 27 is expected to dimerize the weakest. It was diluted in the same manner to determine the dimerization equilibrium constant. Using the solution of 150mM NaCl and 10mM pH7 phosphate buffer to dilute to mitigate the change in ionic strength the rac- hexa anion was diluted from 2.5x10^{-4}M to 1.7x10^{-7}M using 345nm excitation. The emission at 405nm versus tweezers concentration produced the greatest deviation from linearity in the plot and when fit to the dimerization equation it produced a \( K_{\text{dim}} = 1.2x10^{-4} \text{M} \pm 9.0x10^{-6} \text{M}. \)
Figure 56: Fluorescent emission and plot of emission (405nm) vs. concentration for rac-hexa anionic tweezers using 345nm excitation

From the small error in the $K_{\text{dim}}$ value it shows that the model is appropriate in describing the observed phenomenon. The binding rac-hexa anion 27 also has the weakest $K_{\text{dim}}$ of the three due to the not only the carboxylates on the edge of the tweezers providing the coulombic barrier but also from having the greatest amount of negative charge per tweezers molecule. The average amount of negative charge per hexa-anion in solution at pH7 is presumably that of a penta-anion when comparing it to other bis-aminophosphonic acids.$^{101}$ Overcoming coulombic repulsion to dimerize a penta-anion at $10^{-4}$M implies a significant association.
The control meso-isomer 28 was evaluated using the same protocol for dilution monitored by fluorescent emission as the rac-isomer. When the plot of emission at 405 nm versus meso-isomer concentration was fit it was able to converge but produced a value or error greater in magnitude for the calculated \( K_{\text{dim}} \). This again demonstrated that dimerization is a poor model for characterizing the decrease in fluorescence and that the control meso-isomer does not complex with itself in aqueous media.

![Meso Hexa Tweezer Dilution Studies, Corrected where \( l = 1 \times 10^{-4} (A/2) \)](image)

**Figure 57:** Plot of fluorescent emission (405 nm) versus concentration for the control meso-hexa anionic isomer using 345 nm excitation

### 3. Analysis by UV-VIS Spectroscopy

**Methyl phosphonate tetra anion 25 and 26**

In the effort to find a more convenient alternative to fluorescence for determining dimerization UV-Vis absorbance was investigated. Absorbance spectra for the binding rac-(25) and control meso-(26) isomers were monitored over a concentration range of \( 4.4 \times 10^{-4} \) M to \( 3.4 \times 10^{-6} \) M using water containing 150 mM NaCl and 10 mM pH 7 phosphate buffer as the diluent.
Excitation coefficients were calculated for each wavelength by dividing the absorbance spectrum by the concentration of the sample. Plots of wavelength versus these epsilon values at each concentration were constructed. What was seen from this was that at 297 nm there were deviation in epsilon for the rac- isomer whereas no deviation was seen in the meso-isomer at the same wavelength providing another spectroscopic example a difference in behavior for the binding rac- isomer 25 and control meso- isomer 26.

![Diagram of rac- Methyl phosphonate Tetra Anion and Meso- Control isomer]

**Figure 58:** Plot of wavelength vs. epsilon for the binding 25 (left) and the control 26 (right) methyl phosphonate tetra anion isomers

The concentration 25 versus calculated epsilon was plotted and fit using non-linear least squares. The equation used was the same as that for determining $K_{\text{dim}}$ using NMR except epsilon values were used instead of chemical shifts. Unfortunately, when fit it produced errors larger than the $K_{\text{dim}}$ value but this is not due to dimerization being a poor model. When analyzing the fit, it showed that the majority of the data did not fall on the actual curvature which is required for an accurate calculation. If this dilution study were to be repeated,
absorbance data at concentrations below $7 \times 10^{-6}$M need to be acquired to collect more data points and to get closer to the predicted $K_{\text{dim}}$ value.

![Rac Sap Tweezer Dilution Studies (Abs at 297nm)](image)

Figure 59: Plot of concentration vs. epsilon for 25 fit by non-linear least squares

**E. Binding Studies of Molecular Receptor by Small Molecule Titration**

Titration of the water soluble rac- binding molecular tweezers and the meso-nonbinding control isomer were carried out by fluorescence spectroscopy, monitoring the decrease in fluorescent emission of the tweezers isomer upon titration of the small molecule guest. Guest solutions contained the tweezers isomer at the same concentration as the solution of tweezers isomer being evaluated as not to dilute the tweezers isomer upon addition of the titrant. Fluorescent emission of the host was monitored at a particular wavelength with increasing guest concentration and fit to the equation below which models a 1:1 binding interaction and fit using non-linear least squares to determine the dissociation constant ($K_d$) of the complex. Adherence to this model would be consistent with decrease in fluorescence by static (intramolecular) quenching. This could be confirmed with fluorescence lifetime...
measurements of the host as the fluorescence lifetime of the complex will not change with increasing concentration of the small molecule.

\[
H + G \xrightleftharpoons{K_a}{K_d} C
\]

\[
K_d = \frac{[H][G]}{[C]}
\]

\[
H_o = [H] + [C]
\]

\[
G_o = [G] + [C]
\]

\[
I = I_o[H] + I_c[C]
\]

\[
K_d = \frac{[H_o - C][G_o - C]}{[C]} \to [H_o][G_o] - ([H_o] + [G_o])[C] + [C]^2 = K_d[C]
\]

\[
[C] = \frac{([H_o] + [G_o] + K_d) \cdot \sqrt{([H_o] + [G_o] + K_d)^2 - 4[H_o][G_o]}}{2}
\]

\[
I = I_o([H_o] - [C]) + I_c[C] = I_o[H_o] - I_o[C] + I_c[C] = I_o[H_o] + (I_c - I_o)[C]
\]

\[
\to I[H_o] + \frac{I_c - I_o}{2} \left([H_o] + [G_o] + K_d\right) \cdot \sqrt{\left([H_o] + [G_o] + K_d\right)^2 - 4[H_o][G_o]}
\]

Figure 60: Derivation of equation used to determine \(K_d\) in small molecule binding titrations where \(H=host\), \(G=guest\), and \(C=complex\)

The fluorescent data were also fit to the Stern-Volmer equation which models dynamic (intermolecular) quenching of fluorescence with adherence to this model is taken as evidence of intermolecular quenching. Use of fluorescent lifetime measurements could be used if required to confirm dynamic quenching as the fluorescent lifetime of the host molecule will decrease with increasing concentration of the small molecule.

\[
\text{Stern-Volmer Equation} \quad \frac{I_0}{I} = 1 + kt[Q]
\]

Figure 61: Stern-Volmer equation where \(I_o=\text{initial fluorescence}\), \(I=\text{fluorescence with quencher present}\), \(k=\text{quencher rate coefficient}\), and \(t=\text{lifetime of the excited state of the emitting molecule}\)
1. Carbethoxy tetra anion isomers 23 and 24

The titrations of 23 with the small molecules described below were conducted using an Ocean Optics SD2000 fiber optic spectrometer. The titrations of the water soluble tweezers were conducted at concentrations (5x10^{-5} and 1x10^{-5}M where the self associated dimer predominates. As a consequence, the titrants are presumably in competition with the binding pocket of the dimer.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Apparent K_d (M) (Titration Medium)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HO-</td>
<td>2.3x10^{-2} ± 1.6x10^{-3} (10mM pH7 phosphate buffer)</td>
</tr>
<tr>
<td><img src="image1" alt="Image" /></td>
<td>3.7x10^{-4} ± 1.6x10^{-5} (10mM pH7 phosphate buffer)</td>
</tr>
<tr>
<td><img src="image2" alt="Image" /></td>
<td>&gt;5.5x10^{-2} (10mM pH7 phosphate buffer)</td>
</tr>
<tr>
<td><img src="image3" alt="Image" /></td>
<td>3.2 x 10^{-2} ± 1.2x10^{-1} (10mM pH7 phosphate buffer)</td>
</tr>
<tr>
<td><img src="image4" alt="Image" /></td>
<td>1.2 x 10^{-1} ± 3.5x10^{-4} (10mM pH7 phosphate buffer)</td>
</tr>
<tr>
<td><img src="image5" alt="Image" /></td>
<td>1.4x10^{-1} ± 4.8x10^{-4} (10mM pH7 phosphate buffer)</td>
</tr>
<tr>
<td><img src="image6" alt="Image" /></td>
<td>8.3 x 10^{-4} ± 3.3x10^{-4} (10mM pH7 phosphate buffer)</td>
</tr>
<tr>
<td><img src="image7" alt="Image" /></td>
<td>2.6x10^{-4} ± 7.0x10^{-4} (10mM pH7 phosphate buffer)</td>
</tr>
<tr>
<td><img src="image8" alt="Image" /></td>
<td>3.6x10^{-5} ± 3.0x10^{-5} (10mM pH7 phosphate buffer)</td>
</tr>
<tr>
<td><img src="image9" alt="Image" /></td>
<td>2.4x10^{-5} ± 2.7x10^{-5} (10mM pH7 phosphate buffer)</td>
</tr>
<tr>
<td><img src="image10" alt="Image" /></td>
<td>3.0x10^{-4} ± 7.0x10^{-4} (100mM pH7 phosphate buffer)</td>
</tr>
</tbody>
</table>

Table 2: Observed dissociation constants from the titration of the proposed rac- carbethoxy tetra anionic tweezers 23 dimer

Hydroquinone and sodium benzenesulfonate were determined to not bind with the dimer. It was concluded that hydroquinone was not able to bind strongly enough into the molecular tweezers to compete with the dimer as evidenced by its adherence to Stern-Volmer behavior. The sodium benzensulfonate was speculated to not bind because it has too much

68
steric bulk in proximity to the benzene group. Results from sodium benzenesulfinate titration are suggestive as being able to intercalate into the binding cavity as it did not display a Stern-Volmer relationship but the calculated dissociation constant had fifty percent error. The naphthylphosphates and methyl viologen both appear to bind as they both fit to the binding equation and both deviate from Stern-Volmer behavior. Interestingly enough, both of these substrates have dissociation constant on par with one another yet they are oppositely charged supporting the hypothesis that the carbethoxy tetra anion binding isomer is dimeric.

The preliminary study was continued by selecting the cationic guests, methyl viologen and L-tyrosine ethyl ester hydrochloride, and performing titrations with the meso- nonbinding control isomer to determine if the differences in fluorescent quenching would demonstrate binding behavior in the rac- binding isomer.

![Figure 62: Titration of rac- and meso- carbethoxy tetraanion isomers with L-Tyr Et ester HCl](image)

When the concentration of L-Tyr ethyl ester hydrochloride versus fluorescent intensity of the tweezers isomers were plotted on the same graph seemed to exhibit binding behavior by the curvature in decrease in fluorescence and its deviation from linearity in the Stern-Volmer plot. From the results of later titrations with cationic guests and the methyl
phosphonate form of the molecular tweezers it was concluded that the guest molecule was likely to be associated with the dimer coulombically not being inserted into the binding isomer’s cavity. The fluorescence of 24 stayed fairly constant but increased with increasing concentration of the titrant. It also did not exhibit Stern-Volmer behavior as the value of I₀/I decreased, not increased, with increasing guest concentration. This behavior is counterintuitive as this data suggests that addition of L-Tyr ethyl ester HCl is able increase the fluorescence of the meso- isomer by some mechanism as the guest does not emit at the excitation wavelength used to irradiate the non-binding isomer. It is speculated that the meso- isomer 24 is not complexing with the guest molecule but the results unfortunately do not provide the evidence for it.

Figure 63: Titration of rac- and meso- carbethoxy tetra anion isomers with methyl viologen

Titration of the rac- and meso- carbethoxy tetra-anionic isomers displayed similar fluorescent behavior when titrated with methyl viologen. Rac- tweezers 23 appears to be binding methyl viologen by fitting to the 1:1 binding equation and not following Stern-Volmer behavior. However, the quenching of fluorescence for the binding isomer appears to be extrapolating to zero with increasing quest concentration. This is rationalized by having not only
the intramolecular form of quenching occurring but also the quenching of the fluorescence of the complex by intermolecular collision and can be seen in the later half of the titration by an apparent linear decrease in emission. What was expected to be seen on binding however would be a shift in fluorescent emission by formation of a charge transfer complex between the two but this phenomenon was not observed. This suggests that the methyl viologen is associating tightly to the exterior of the tweezers dimer in the low ionic strength of the titration medium to form a complex that is an ion pair. The control isomer 24’s behavior was consistent with its structure by an overall linear decrease in fluorescence, non-convergance to the binding isotherm, and adherence to the Stern-Volmer relationship. Interestingly when compared to the meso- control of the methyl phosphonate tetra-anion, whose decrease in fluorescent emission when titrated with cationic guests mimicked binding, the meso- carbothoxy analogue did not. There is no explaination for the difference in the behavior at this time.

As the binding affinities afforded from the titrations were of the same approximate value, it was concluded that the guests were not binding into the cavity of the tweezers.

2. Methyl phosphonate tetra anionic isomers 25 and 26

Fluorescent titrations where conducted on a Fluorolog-3 Model FL3-22 by Horiba Jobin-Yvon using nanopure water as the titration medium. Concentrations used for titration for the rac- binding and meso-control isomers were either 5.0x10^-6 or 5.0x10^-7 M which were assumed to be predominated by monomeric species. This assumption was supported by observing the fluorescent emission of 25 at various excitation wavelengths. If a single species is present,
change in excitation wavelength should change the intensity of the emission but not change the shape of the emission profile which is what was observed.

![Fluorescence Emission Spectra of rac-tetra anion 25 (5.20E-6M) at Varying Excitation Wavelengths](image)

**Figure 64:** Fluorescent emission of tetra anion tweezers 25 with varying excitation wavelength plotted to include excitation wavelengths

All of the small molecules screened were fit by non-linear least squares to a 1:1 binding equation and did not adhere to intermolecular quenching as described by the Stern-Volmer equation. Titration of neutral and anionic small molecule appeared to bind weakly with $K_d$'s on the order of $10^{-2}$M which was attributed to the small amount of aromatic surface in the benzene sized substrates. For this reason it was expected that 1-naphthylacetic acid would bind stronger than sodium phenylacetate but appeared to bind with the same affinity. This was attributed to the sparing solubility of the 1-naphthylacetic acid in nanopure water which prohibited a solution of sufficient concentration to follow probe the guest. If this material were to be titrated in the future it is suggested to form a salt of the 1-naphthylacetic acid to circumvent the limitation in solubility. The cationic guests afforded dissociation constants on average of an order of magnitude stronger when compared to the other titrants for 1-Me nicotininium chloride and 100x stronger for methyl viologen.
<table>
<thead>
<tr>
<th>Substrate</th>
<th>Apparent $K_d$ (M) (Titration Medium)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Molecule1" /></td>
<td>$5.9 \times 10^{-2} \pm 3.8 \times 10^{-3}$ (NP Water)</td>
</tr>
<tr>
<td><img src="image2.png" alt="Molecule2" /></td>
<td>$2.1 \times 10^{-4} \pm 1.6 \times 10^{-5}$ (NP Water)</td>
</tr>
<tr>
<td><img src="image3.png" alt="Molecule3" /></td>
<td>$3.6 \times 10^{-3} \pm 1.2 \times 10^{-4}$ (NP Water) $2.5 \times 10^{-3} \pm 1.4 \times 10^{-4}$ (NP Water) $6.0 \times 10^{-3} \pm 5.0 \times 10^{-4}$ (150mM NaCl, 10mM pH7)</td>
</tr>
<tr>
<td><img src="image4.png" alt="Molecule4" /></td>
<td>$3.8 \times 10^{-2} \pm 2.2 \times 10^{-2}$ (NP Water)</td>
</tr>
<tr>
<td><img src="image5.png" alt="Molecule5" /></td>
<td>$1.8 \times 10^{-2} \pm 3.1 \times 10^{-3}$ (NP Water)</td>
</tr>
<tr>
<td><img src="image6.png" alt="Molecule6" /></td>
<td>$4.3 \times 10^{-2} \pm 4.5 \times 10^{-2}$ (NP Water)</td>
</tr>
</tbody>
</table>

Table 3: Apparent $K_d$ values for small molecules titrated with binding rac- methyl phosphonate tetra anion tweezers 25

These results were less encouraging when the small molecules were evaluated against the control isomer 26. For the non-cationic guests, the shape of the plot of decreasing fluorescence versus titrant concentration and the total decrease in fluorescence were eerily similar save for the last few data points where they began to diverge at the highest concentrations. The plots of 26 had the same slight curvature as the plots for the binding isomer 25 did and for this reason were able to fit to the 1:1 binding equation. These control isomer plots were analyzed for a Stern-Volmer relationship and showed inconsistent behavior. Sodium phenylacetate and nictotinamide showed Stern-Volmer correlation where pyridine did not. With conflicting data for the behavior of 26 in acting as a negative control and noisy data from the minimal response in quenching of fluorescence in the concentration ranges tested for
the small molecules the behaviors between the two isomers could not be distinguished from one another with any confidence. This could possibly be accomplished by future reseaches with increasing the concentrations of titrant solutions as well as increasing the ionic strength of the titration medium.

![Figure 65: Titration of binding rac- and meso- methyl phosphonate isomers with 1-Me nicotinamide chloride in nanopure water](image)

In tritrations using the cationic guests methyl viologen and 1-Me nicotinamide chloride the binding rac- isomer 25 and the control meso- isomer 26 looked to be behaving in the same manner as if binding was occurring in both cases. For titrations with methyl viologen both isomers deviated from linearity when modeled by the Stern-Volmer and both converged to the binding equation with $2.1 \times 10^{-4} \text{M} \pm 1.6 \times 10^{-5} \text{M}$ and $6.2 \times 10^{-4} \text{M} \pm 8.7 \times 10^{-5} \text{M}$ for the binding rac- isomer and the control meso- isomer respectively. Shown below is the data from the titration of the binding rac- methyl phosphonate tetra anion tweezers with 1-Me nicotinamide chloride in nanopure water.
Figure 66: 1:1 binding and Stern-Volmer plot for titration of rac- methyl phosphonate tweezers 25 with 1-Me nicotinamide chloride in nanopure water

The small amount of error and the even distribution of errors as plotted by the residuals in the 1:1 binding fit show that this is a good description for binding interaction of the guest molecule. The curvature of the \(I_0/I\) values with increasing guest concentration when compared to the linear regression for the Stern-Volmer plot demonstrate that the decrease in fluorescence observed is not described well by an intermolecular quenching model.

When the negative control meso- isomer 26 was titrated with the same protocol it quenched fluorescence in an apparent non-linear fashion but produced errors equal to the calculated \(K_d\) when fit to the binding isotherm demonstrating that intramolecular quenching is not a good model as expected for the isomer that lacks the cavity to allow intercalation of a small molecule. However, when the data was evaluated by the Stern-Volmer relationship it demonstrated the same behavior was the binding isomer. It was from the culmination of these
results in titrating cationic guest that the ionic strength of the titration medium was thought to be of import when trying to demonstrate the differences in the isomers of the molecular receptors. In the very low ionic strength condition of nanopure water it was postulated that the cationic guest would be able to mimic the spectroscopic profile of static (intramolecular) quenching by forming a tight ionic pair with the tetra anionic host which would be interesting as the coulombic attraction between the charged species would be more stable than the solvated ion in water.

Figure 67: 1:1 binding and Stern-Volmer plot for titration of control meso- methyl phosphonate tetra anion isomer with 1-Me nicotinamide chloride in nanopure water

This association was explored using $^1$H NMR spectroscopy to compare the chemical shifts of free 26, free 1-Me nicotinamide chloride, and a 1:1 mixture of the two at a concentration similar to the end points of the titration condition in pure D$_2$O and D$_2$O of increased ionic strength. The ionic strength of the D$_2$O was modified by addition NaCl to
160mM concentration which was approximation of the 150mM NaCl, 10mM pH7 phosphate buffer used in subsequent titration.

Figure 68: \(^1\)H NMR comparison of the aromatic region of free meso- methyl phosphonate tetra anion 26, free 1-Me nicotinamide chloride, and a 1:1 mixture of the two at 1.8x10^{-2} M concentration

When the components were combined in a 1:1 ratio in pure D\(_2\)O large upfield shifts were observed in the 1-Me nicotinamide Cl and all but one of the signals in the aromatic region of the meso- control. Upon mixing it was also observed that while the meso- isomer’s signals remained sharp while those of the nicotinamide became broadened, consistent with its associating. From the upfield shifts of the hydrogens in the naphthyl wall of the meso-compound along with the concurrent down field shift of hydrogen F that resides on the central benzene ring it is proposed that the nicotinamide salt is associated on the face of the
naphthylene on the opposite side of the phosphonate moiety and perpendicular to the benzene ring. The 1-Me nicotinamide Cl would need to be on the opposite face was when the complex was made using molecular models the 1-Me nicotinamide Cl would not be in proximity to the hydrogens on the benzene core. The ionic strength of the mixture was then adjusted to 160mM strength by the addition of NaCl with only minor downfield shifts for the 1-Me nicotinamide chloride in the $^1$H spectrum which shows that even with increased ionic strength the cationic guest and the tetra anionic isomer are still closely associated.

![Figure 69: Comparison of $K_d$ for binding 25 and control 26 isomer to 1-Me nicotinamide chloride in nanopure water and 150mM NaCl, 10mM pH7 phosphate buffer](image)

The titration of the methyl phosphonate tetra anion isomer with 1-Me nicotinamide chloride was conducted again but now in aqueous 150mM NaCl, 10mM pH7 phosphate buffer so that the ionic strength of the medium would not appreciable change over the course of the
experiment. What was found was in this solution the dissociation constant for the binding rac-isomer decreased by a factor of 1.7 and for the control meso-isomer decreased by a factor of 5 which is evidence for the binding isomer forming an inclusion complex with the guest. If the association of the binding isomer was strictly coulombic it would be expected to have its apparent $K_d$ value diminished by the same amount as the control meso-isomer. When the $K_d$ values of the binding and control isomer where compared to one another under the new ionic conditions the binding isomer was able to associate with the 1-Me nicotinamide Cl 3.5x stronger whereas in nanopure water the two isomers associated with the guest molecule with the same affinity. This too was taken as evidence as a demonstration of the difference in behavior of the binding isomer 25 and the control isomer 26.

3. Hexa-anionic isomers 27 and 28

The best chance of binding a small molecule into the cavity of the bis-Tröger’s Base tweezers would be with the hexa-anionic derivative. As this form has a dimer that is held together the weakest, it can be titrated at concentrations where ne is confident that the tweezers exists as a monomer. In pH9 borate buffer, 1-Me nicotinamide chloride appeared to associate with approximately the same affinity to the hexa anion 27 ($K_d = 8.2 \times 10^{-3}$ M) as it did to the rac- methyl phosphonate tetra anion tweezers under the same ionic strength regime. Borate buffer was used to ensure that the hexa-anionic tweezers were fully deprotonated so there was no ambiguity to the charge on the host molecule.
Table 4: Apparent $K_d$ values substrate association to binding rac- hexa anionic tweezers at $5.0\times10^{-6}$ M concentrations. $K_d$ generated by fitting the curvature in the data to the 1:1 binding equation

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Apparent $K_d$ (M) (Titration Medium)</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Substrate Structure]</td>
<td>$8.2\times10^{-3} \pm 1.0\times10^{-3}$ (150mM NaCl, 10mM pH9 borate buffer)</td>
</tr>
<tr>
<td>![Substrate Structure]</td>
<td>$7.2\times10^{-3} \pm 4.4\times10^{-3}$ (150mM NaCl, 10mM pH7 phosphate buffer)</td>
</tr>
<tr>
<td>![Substrate Structure]</td>
<td>ambiguous</td>
</tr>
<tr>
<td>![Substrate Structure]</td>
<td>$4.2\times10^{-4} \pm 4.2\times10^{-5}$ (150mM NaCl, 10mM pH9 borate buffer)</td>
</tr>
</tbody>
</table>

Titrations of the rac- tweezers 27 and the meso- non-binding control 28 with caffeine provided the best evidence for the discrimination between the two. Upon addition, the fluorescence of the binding isomer decreased sharply initially and appeared to reach and asymptotic value. This data did not exhibit Stern-Volmer behavior and when fit this data was fit to the non-linear least squares fit it converged to generate a $K_d$ of $4.2\times10^{-4}$ M which is similar to binding affity reported by other synthetic acyclic receptors. In contrast, when the meso- hexa anionic 28 was exposed to increasing concentrations of caffeine its fluorescent emission vacillated around a constant value before decreasing, increased at the end of the addition, and did converge when fit to the 1:1 binding equation. This increase in fluorescence is not understood at the present time as the absorbance of caffeine at 365nm is 0.002 and thus should not lead to emission form the guest. However, as their behaviors are so different one can be confident that 27 is actually binding to caffeine presumably via intercalation between its naphthalene walls.
Figure 70: Comparison of fluorescent emissions of the binding rac-hexa anionic tweezers (top) and the non-binding meso-control isomer (bottom). Stern-Volmer plot also included for binding isomer 27.

When titrations of 27 were carried out in water containing 10mM pH7 phosphate buffer, 150mM NaCl with 1- or 2-naphthylphosphate disodium salt the data acquired by fluorescent titrations were ambiguous to stay the least. When the 1-naphthylphosphate analogue was titrated with the binding isomer its fluorescent emission increased upon the first addition of guest then fluctuated up and down until 0.06M concentration of the guest was reached. Upon increasing the concentration, the fluorescence of the tweezers decreased to 66% of its initial value before increasing in value after 0.016M of the guest. Then the data points corresponding to the curvature was analyzed with the 1:1 binding model a $K_d$ of $7.2 \times 10^{-3}$ M was produced after convergence. With regards to the 2-naphthylphosphate, it too...
increased in fluorescent emission with the first addition of guest before leveling off until 0.004M of the guest was achieved. After that, fluorescence decreased in a curve before reaching an apparent minimum before increasing with the last addition. When the data in the curvature were analyzed with the 1:1 binding model it did not converge presumably due to the lack data points. These fluorescent data from the naphthylphosphate guest molecules suggests the the mechanism of binding may be either more complexed than initially estimated or that the phenomenon responsible for the increase in emission signature cannot be fully understood at the present time. Due these observations, titrations of the control isomer 28 were not conducted with the phosphate salts.

Figure 71: Titration of hexa anionic tweezers 27 with disodium 2-naphthylphosphate
F. Quinolone Synthesis for Functionalized Walls for *bis*-Tröger’s Base Molecular Receptors

Quinolones have been reported as therapeutic agents ranging in use from the treatment of Herpes Simplex Type I (HSV-1), as an antitumor agent for Burkitt’s lymphoma, as iron coordinating compounds to inhibit Factor Inhibiting Hypoxia-inducible factor, an antifilarial chemotherapy agent, and an inhibitor to the binding of Nerve Growth Factor p75 to diminish neural apoptosis. Although these properties may prove beneficial for specific recognition in the future, the rational in synthesizing the quinolones described below would be to exploit the differences in reactivity of its functional groups to allow for selective instillation of a wide range function groups. The consequence is this would be multiple non-covalent interactions leading to selectivity in substrate binding and could potentially produced catalytic activity. However, the dipole moments of the cavity induced by the quinolones in the *bis*-Tröger’s base tweezers and its inherent fluorescent properties to monitor binding by fluorescence or absorbance are of immediate interest.

7-Amino-4-oxo-1,4-dihydro-quinoline-2-carboxylic acid ethyl ester when exposed to cyclization conditions should cyclize in the 8 position of the quinolone exclusively yielding a single orientation of tweezers.

![Chemical Structure Image]

Figure 72: 7-Amino-4-oxo-1,4-dihydro-quinoline-2-carboxylic acid ethyl ester and its resulting *bis*-Tröger’s Base tweezer
However when incorporating 6-amino-derivative into the tweezer’s architecture there is a question of regioselectivity and could undergo Friedel-Crafts alkylation on the 5 or 7 position of the quinolone to produce a mixture of differently bonded molecular receptors in which some will not have the correct shape to intercalate substrates.

![Figure 73: 6-Amino-4-oxo-1,4-dihydro-quinoline-2-carboxylic acid ethyl ester and the possible combinations of bis-Tröger's Base tweezers](image)

Below is a general scheme envisioned for the transformations needed to produce the quinolones:

![Figure 74: General scheme for aminoquinolone synthesis](image)
Diethyl oxaloacetate was first used\textsuperscript{107} and is commonly used to generate the 2-carboxy-4-oxo-quinolone variety along with diethyl acetylenedicarboxylate which can form enamines even with less reactive or sterically hindered anilines\textsuperscript{108}. Although the reactivity of diethyl acetylenedicarboxylate is greater than that of diethyl oxaloacetate, so is its price leading to the use of diethyl oxaloacetate. Quinolone synthesis has been reported in the literature as early as 1887\textsuperscript{109} by Conrad and Limpach reporting the formation of 4-oxoquinolone formation under high temperature conditions which have been modified overtime to induced thermal cyclization\textsuperscript{110,111}. Before 1946 it was contended that cyclizing an enamine made from an aniline and diethyl oxaloacetate generated either a 2-oxo quinolone isomer or the 7- substituted 4-oxo isomer cleanly before Surrey and Hammer demonstrated that the resulting material generated upon thermolysis was that of a mixture of 7- and 5- substituted 4-oxo-quinlones\textsuperscript{112} and occurs even in the gas phase\textsuperscript{113}

1. Preparation of 7-Amino-4-oxo-1,4-dihydro-quinoline-2-carboxylic acid ethyl ester

a. From (3-Amino-phenyl)-carbamic acid tert-butyl ester Route

\[
\begin{align*}
\text{H}_2\text{N} & \text{NH}_2 \quad \text{Boc}_2\text{O}, \text{EtOAc} \quad \text{RT} \quad 17\text{hr} \quad 79\% \quad \text{BOcHN} \quad \text{NH}_2 \\
& \quad \Downarrow \quad \text{29} \\
& \quad \text{EtO} \quad \text{COOEt} \quad \text{Na} \quad \text{AcOH, CH}_2\text{Cl}_2 \quad \text{ms4A, RT} \quad 96\% \quad \text{BOcHN} \quad \text{O} \quad \text{Et} \\
& \quad \Downarrow \quad \text{30} \\
& \quad \text{Ph}_2\text{O}, \text{reflux} \quad 20\text{min} \quad 78\% \quad \text{H}_2\text{N} \quad \text{NH}_2 \quad \text{OEt} \quad \text{H} \quad \text{NH}_2 \quad \text{OEt} \\
& \quad \Downarrow \quad \text{31} \quad \text{2:3 ratio} \quad \text{32}
\end{align*}
\]

\textit{Figure 75: Synthetic route to produce 7-amino-2-carboxy from 1,3-diaminobenzene}
Mono-Boc protection of 1,3-diaminobenzene to generate 29 was afforded in 79% yield by using a 100% excess of the diamine over Boc₂O. Utilizing the ability to partition the diamine into water to remove its excess and recrystallization from hexane to remove the diBoc protected side product gave a pure product. Formation of the enamine 30 from the sodium salt of diethyl oxaloacetate in glacial acetic acid cleanly generated the enamine in 4h if 4Å molecular sieves were used to drive the equilibrium by physical removal of water. The excess diethyl oxaloacete was removed by partitioning the reaction mixture between hexane and sat. Na₂CO₃ to produce the enamine in 96% yield as an orange oil. Diphenyl ether (258°C) was used due to its chemical stability at high temperatures. Refluxing the enamine oil in diphenyl ether for 20min facilitated quinolone formation as well as Boc protecting group removal. Analysis of the quinolone mixture by ¹H NMR spectroscopy revealed a 2:3 ratio of 31 to 32. This was determined by the ratio of integrations of the doublet of doublets signal at δ6.69ppm for the 7-NH₂ isomer and the triplet at δ7.24ppm indicative of the 5-NH₂ compound. From this data it was hypothesized that when the Boc group was removed and the resulting amino group was able to direct the Fridel-Crafts acylation ortho- to it. The installation of a thermally stable protecting was deemed necessary and thus cyclization with a trifluoroacetyl protecting group was investigated.
b. From N-(3-aminophenyl)-2,2,2-trifluoroacetamide Route

The mono-trifluoroacetyl 1,3-diaminobenzene (33) used was synthesized, isolated, and purified by John Lukesh III which was then condensed using the glacial acetic acid, 4Å molecular sieves and workup method to generate the enamine 34 cleanly in 89% yield as a lime green oil. After exposuring the enamine to refluxing diphenyl ether, analysis of the crude product with $^1$H NMR spectroscopy revealed a mol ratio of 1:5 for the 7-trifluoroacetyl quinolone 35 to 5-trifluoroacetyl quinolone 36 and a 1:3.5 mol ratio of 35:36 when the experiment was replicated. As an alternative to thermal cyclization, strongly acidic conditions were also known to facilitate cyclization. Published by Eaton in 1973$^{114}$ demonstrated that Eaton’s reagent (1:10 solution by weight of P$_4$O$_{10}$:CH$_3$SO$_3$H) not only induced ring closure but sidestepped the difficulties with the physical properties of polyphosphoric acid and thus has been used since then as an alternative.$^{115}$ Unfortunately when Eaton’s reagent was used the ratio increased even more in favor of the 36 to a 6.7:1 mol ratio compared to the desired 35. Ring formation using POCl$_3$ was also used but appeared to produce 36 as the major product. Quinolone formation was attempted with the enamine adsorbed to either silica gel or basic alumina which was placed...
into a thermal well in refluxing diphenyl ether which afforded incomplete conversion of the starting material and low mass recovery off of the solid support. From these results it was postulated that this seeming regioselectivity could be due to the formation on an intramolecular hydrogen bond upon the formation of the ketene intermediate.

If this mechanism were true, then eliminating the ability to hydrogen bond should prevent ortho- direction. This informed the change to a nitro group which would be subsequently reduced to the amine functionality to generate the 7-Amino-4-oxo-1,4-dihydroquinoline-2-carboxylic acid ethyl ester.

**c. From 3-nitroaniline Route**

Enamine formation was first attempt with the glacial acetic acid, 4Å molecular sieves method but only afforded the desired enamine 37 in 54% yield. This was presumably due to the
inductive effect of the nitro reducing the nucleophilicity of the amine and thus requiring harsher conditions to increase yield. Condensation of the 3-nitroaniline and sodium diethyl oxaloacetate was driven then by elevated temperature and physical removal of water via azeotrope with a distilling solvent returned via Dean-Stark trap. It was discovered that choice of solvent was not trivial as use of toluene (b.p. 110°C) or 1,2-dichloroethane (b.p. 84°C) lead to substantial formation of a crystalline, orange side product. This material was not fully characterized but evidence $^1$H NMR gave speculation that it was likely produced by condensation of two 3-nitroanilines by reaction of the amine functionality with a protonated nitro group. Dichloromethane (b.p. 40°C) was employed next due to its lower boiling point and ability to azeotropically remove water albeit poor. It was due to this poor azeotrope (99 CH$_2$Cl$_2$·1H$_2$O) that reaction times were extended considerably to ranges of overnight to several days. It was later discovered that on the synthetic scale that this transformation was attempted that the water removed by the dichloromethane was not able to quantitatively separate and allowed for return of water back into the reaction mixture. If however the heavier than water Dean Stark trap was packed with 4Å molecular sieves atop a loosely fit plug of cotton the reaction could be reliably completed overnight. On larger scales though replacing of the sieves may be required to thoroughly remove all of the water generated from the reaction.

37 was then subjected to the thermolytic cyclization protocol of 20min exposure in refluxing diphenyl ether and unfortunately still produced the mixture of 7-NO$_2$ isomer 38 and 5-NO$_2$ isomer 39. In replicating this transformation many times it was observed that although the mol ratio of the quinolone mixture improved with regards to 7-NO$_2$ to 5-NO$_2$ isomers it was inconsistent, ranging from 7:1 to 1:1, with no correlation able to be determined for this
observed behavior. Jackie Koch developed a procedure the exploited the greater crystallinity of the quinolone 38 to isolate it exclusively via recrystallization using a mixture of ethanol and toluene and is described in detail in the experimental section. She also found that if only ethanol was used, 38 could still be separated but the amount of material recovered decreased significantly. Interestingly enough when the enamine 37 was cyclized using Eaton’s reagent at 110°C the mol ratio of the resulting quinolone mixture was observed to be 2.3:1 in preference of the 5-NO₂ isomer. From these results it appears that although neither the 39 or 38 isomer can be made exclusively there is at least some ability in directing ring closure by either substituent effects or cyclization conditions.

Reduction of the nitro group was first attempted on a mixture of the 38 and 39 using ammonium formate (\(\text{NH}_4\text{O}_2\text{CH}\)), 5% Pd-C, and methanol as a solvent. Ammonium formate is attractive as the reducing agent because in the presence of Pd-C it decomposes to produce a concentration of hydrogen gas \textit{in situ} which corresponds to a high pressure of \(\text{H}_2\) over the reaction volume. This eliminates the necessity for a specialized high pressure apparatus and the

\[
\begin{array}{|c|c|c|}
\hline
R & \text{Conditions} & \text{Mol Ratio} \\
\hline
\text{BocHN}^- & A & 2 & 3 \\
\text{CF}_3(\text{CO})\text{HN}^- & A & 1 & 3.5-5 \\
\text{CF}_3(\text{CO})\text{HN}^- & B & 1 & 6.7 \\
\text{O}_2\text{N}^- & A & 7-1 & 1 \\
\text{O}_2\text{N}^- & B & 1 & 2.3 \\
\hline
\end{array}
\]

\textit{Table 5: Quinolone mol ratios with varying substituents and reaction conditions}
safety concerns that come with it. Transformation to the amino group occurred as expected but under these conditions trans-esterification with the alcohol solvent occurred resulting in the methyl ester of the corresponding aminoquinolones. Although unexpected in retrospect should not have been wholly unanticipated due to the weakly acidic nature of the ammonium formate. When repeated with ethanol as the solvent the desired aminoquinolone 31 was afforded in 66% yield after isolation by flash chromatography and an average 47% overall yield from the starting 3-nitroaniline.

Attempts to isolate 31 non-chromatographically proved to be problematic. When the starting quinolone 38 was observed to be absent by TLC analysis the mixture was filtered to which CH$_2$Cl$_2$ was added to the filtrate to decrease the solvent polarity to cause an unreacted ammonium formate to precipitate. Although precipitation of the salt did occur the removal of the ammonium formate was not quantitative. Reducing the volume of the filtrate or concentrating to dryness resulted in the formation of solid aminoquinolone which was not separated from the excess salt. It was observed that if left to stir for excessive time in the presence of Pd-C the ammonium formate is able to be converted to into insoluble ammonium carbonate however with no way to conveniently monitor this conversion it was not considered as a method of separation. It was later found that use of H$_2$(g) is not detrimental to the rate of reaction and will be discussed subsequently.
2. Preparation of 6-Amino-4-oxo-1,4-dihydro-quinoline-2-carboxylic acid ethyl ester

![Synthetic scheme for production of 6-amino-2-carboxy-4-oxoquinolone ethyl ester](image)

Figure 79: Synthetic scheme for production of 6-amino-2-carboxy-4-oxoquinolone ethyl ester

With a developed methodology in hand, formation of 42 proceeded smoothly especially without question of regioselectivity in ring closure. A major contribution in the reduction step was provided by Tyler Fenske who observed that use of H$_2$(g) reduced the nitro group at the same rate as that of the ammonium formate and in 90% yield with effortless removal of the excess, gaseous reducing agent.

III. CONCLUSION

The dialdehyde 8 was afforded in a 50% overall yield starting from the dimethyl ester of succinyl succinate without chromatography and the most of the intermediates being able to crystallize from the reaction mixture, eliminating the need for subsequent purification steps. The diarylamino side products were demonstrated to be formed from the bisenamine 2 in the absence of Boc$_2$O with bis-aryl amino derivatives able to be produced from the corresponding anilines with the mono-substituted version predominating. This opens up a different route by which acridones can be synthesized as well as the possibility for asymmetric quinacridones.

The desired hexa-methyl ester of 1,2:4,5-bis-Tröger’s Base stereoisomer was afforded as a diastereomeric mixture of the syn-cofacially oriented rac- isomer and the control meso-isomer in 69% overall yield from the key intermediate dialdehyde 8. This proceeded through
the bis-imine formation from the core dialdehyde, transformation into α-aminophosphonates, then cyclization to the final bis-Tröger’s Base. Each of these isomers was then converted into water soluble analogues via selective dealkylation protocols employing combinations of bromotrimethylsilane and aqueous lithium hydroxide. Two tetra anionic molecular tweezers derivatives were afforded each with a different distribution of charge across its surface along with a fully deprotected hexa anionic receptor.

Each of the organic and water soluble forms of the rac- and meso- 1,2;4,5-bis-Tröger’s Bases were characterized using spectroscopic techniques and spectrometric techniques to confirm their identity. It was shown that the desired binding rac- isomers underwent self-association to presumably form a dimeric complex in solution whereas the non-binding control meso- isomers did not. $^1$H NMR analysis of the fully esterified rac- isomer in CDCl$_3$ produced broad resonances what sharpened and shifted downfield upon dilution, which is behavior consistant with the interpretation dimer formation. The water soluble derivatives were not able to produce unambiguous spectra upon dilution in D$_2$O but were able to generate well resolved spectra in a mixture of 50% TFA/CDCl$_3$ to allow for their identification. In contrast, the non-binding meso- isomers produced well resolved spectra in all cases even at sample concentrations greater than those were dimerization was observed with the rac- isomers. This demonstrated the desired lack of self association for the non-binding meso- isomers so that it may serve as a negative control for dimeration and titration studies.

Equilibrium constants for dimer formation were determined for the rac- isomers using a combination of $^1$H NMR and fluorescence spectroscopy and analyzed using a dimeration equation fit using a non-liner least squares method. $K_{\text{dim}}$ for the fully esterified tweezers was
calculated to be $1.5 \times 10^{-1}$ M in CDCl$_3$ but this is just an estimate based upon the low number of data points acquired in the NMR dilution experiment. Deviation from Beer’s Law in fluorescent emission upon dilution was used to determine the strength of self association for the water soluble molecular tweezers. The rac- hexa anionic tweezers formed the weakest dimer with a $K_{\text{dim}}$ value of $1.2 \times 10^{-4}$ M $\pm$ $9.0 \times 10^{-6}$ M. The tetra anions formed progressively stronger dimers with the rac- methyl phosphonate analogue producing $K_{\text{dim}}= 4.5 \times 10^{-4}$ M $\pm$ $9.8 \times 10^{-5}$ M and the TMSBr deallylated rac- carbethoxy $K_{\text{dim}} < 4.4 \times 10^{-7}$ M. In the case of the rac- carbethoxy tweezers a linear decrease in fluorescence was observed upon dilution down the $4.4 \times 10^{-7}$ M, which was the limit of detection for this compound and prevented fitting to the dimerization model.

Each of the water soluble binding rac- and control meso- bis-Trögers Base derivatives were titrated with small molecules monitoring the decrease in fluorescent emission to acquire the data to be fit to the 1:1 binding model which describes intra-molecular (static) quenching. Data was also fit the Stern-Volmer equation, which models inter-molecular (dynamic) quenching, as a way to discriminate between the binding and non-binding isomers. The rac- carbethoxy tetra anion similar dissociation constants ($K_d$) across a range of guest molecules reinforcing the notion that it exists as a dimer at $10^{-5}$ M concentration regime the titrations were conducted at. The ionic strength of the aqueous media was also observed to be of import with regards to distinguishing between the binding and non-binding isomers during small molecule titrations. In a low ionic strength environment, like nanopure water, it appeared that both the rac- methyl phosphonate and control meso- methyl phosphonate tetra anions exhibited binding behavior in the presence of cationic guest molecules like 1-Me nicotinamide chloride. When ionic strength was increased so that the water contained 150 mM NaCl and
10mM pH7 phosphate buffer, both isomers were still able to converge to the 1:1 binding model when titrated with 1-Me nicotinamide chloride. However, the calculated $K_d$ diminished by a factor of 5 for the control meso-isomer between the low and high ionic strength conditions whereas the $K_d$ for binding rac-isomer diminished only by a factor of 1.7.

From this it was concluded that the cationic guest molecule was associating to the control isomer through coulombic attraction which in retrospect is not surprising. To confirm that the nicotinamide salt was indeed forming a pair, $^1$H NMR was used to analyze a mixture of the 1-Me nicotinamide chloride with the control meso-isomer. What was observed was a significant upfield shift and broadening of the guest’s resonances along with minor upfield shift in the naphthyl signature of the meso-isomer. When the ionic strength was increased to 160mM NaCl the peaks for the 1-Me nicotinamide chloride shifted only slightly back downfield and still appeared to be significantly associated to the control meso-isomer. Titration with charge neutral guest like pyridine or nicotinamide, or anionic guests like sodium phenylacetate did not sufficiently quench either isomer upon addition. This prohibited confidently distinguishing between the modes of intra- or inter-molecular quenching between the two isomers.

The rac-hexa anionic tweezers provided the best data with regards to small molecule titrations using cationic or neutral guest. Ionic strength of the aqueous media was accounted for by containing 150mM NaCl and 10mM pH9 borate buffer which ensures that the hexa anionic tweezers fully deprotonated. Titration of the binding rac-isomer with 1-Me nicotinamide chloride resulted convergence of the 1:1 binding model, negative Stern-Volmer correlation, and a $K_d$ value of $8.2 \times 10^{-3} \text{M} \pm 1.0 \times 10^{-3} \text{M}$. This value is similar to the dissociation
constant afforded by the rac- methyl phosphonate tetra anion tweezers. Titration with caffeine resulted in the same spectroscopic behavior and yielded a $K_d$ value of $4.2 \times 10^{-4} M \pm 4.2 \times 10^{-5} M$.

The future direction of this work should continue small molecule titration with the binding rac- hexa anionic tweezers to determine the scope and trends in its ability to intercalate guest molecules. As it appears that quenching of fluorescence it not adequate in order to determine dissociation constants in some cases it is recommended that the walls of the molecular tweezers be modified to a potentially more responsive moiety.

![Molecular tweezers diagram]

**Figure 80: 1,2:4,5-bis-Troger’s Base tweezer scheme displaying desired quinolones for future inclusion**

This can be accomplished by incorporating the synthesized amino-quinolone derivatives, 7-Amino-4-oxo-1,4-dihydro-quinoline-2-carboxylic acid ethyl ester (47% overall yield) and 6-Amino-4-oxo-1,4-dihydro-quinoline-2-carboxylic acid ethyl ester (60% overall yield), into the modular tweezers synthetic scheme. These structures would provide a different fluorescent signature to monitor binding as well a potentially upon up the range of small molecules the tweezers may bind by the presence of dipole-dipole interactions in the cavity.
PART TWO: SMALL MOLECULE SYNTHESIS FOR ENZYMATIC MECHANISTIC PROBES

IV. SMALL MOLECULE PROBES FOR EVALUATING SbADC ALDOLASE ACTIVITY

A. SbADC Background Information

*S. Bingchenggensis* Acetoacetate Decarboxylase (SbADC) is a tetrameric enzyme first isolated from *Streptomyces Bingchenggensis* from Harbin, China and having sequence homology to that of Group V acetoacetate decarboxylases. However, SbADC does not display the same properties of its brethren and instead was discovered to catalyze Aldol Condensation and Aldol Dehydration as well as the retro variants of these reactions with α-keto acids. It was discovered in the Silvaggi lab that it reacted preferentially with pyruvate and various aldehydes. The purpose of the enzyme for its native species is as of yet unknown but characterization of its aldolase functionality was conducted.

The mode of active site catalysis for SbADC was studied by stop flow, steady state, and inhibition kinetics as well as X-ray crystallography conducted by Lisa Mueller to achieve kinetic parameters in intermolecular interactions in the active site to determine which residues are involved in the transformations. When allowed to react with benzaldehyde; pyruvate and acetoacetate were the only nucleophiles to react with catalytic SbADC action with rates of 2.112µM s⁻¹ and 0.012 µM s⁻¹ rate where as glyoxylate had non-enzymatic reaction and 2-oxobutyrate did not react leading to the conclusion that pyruvate was the preferred α-keto substrate. Aldehyde substrates were then screened with *trans*-cinnamaldehyde observed to the bind the stongest (42-48µM) with $k_{cat}/K_m = 3.9\times10^5$ M⁻¹s⁻¹. Soaking with hexanal produced no reaction and imidazole-2-carboxaldehyde reacted but did not turn over. Inbetween these
results with increasing reactivity: benzaldehyde $< trans$-2-hexenal, $trans$-2,4-heptadienal $< trans$-2-octenal leading to the conclusion that an $\alpha,\beta$-unsaturated aldehyde with required and if steric bulk is in an aldehyde (like a benzyl group) it has to be at least two carbons away from the aldehyde. $Trans$-cinnamaldehyde analogues were screened. Ortho substitution proved detrimental to binding ($o$-methoxy $K_d=185\mu M$) while hydrogen bonding substituents in the para position enhanced binding ($o$-methoxy $K_d= 21.5\mu M$, $o$-nitro $K_d=7.6\mu M$).

When SbADC was soaked with sodium pyruvate and the X-ray crystal structure was acquired it appeared as a hemiaminal in three of the four active sites and a Schiff base in the last. However when SbADC had the active site mutation of Y252F and soaked with cinnamylidene pyruvate it was observed to be the Schiff base in all four active sites leading to the speculation that Y252 is needed for the retro Aldol activity as it hydrogen bonds to a water molecule and orients to close to the $\alpha$-keto moiety. $\alpha$-Keto acid enones and dieneones have been synthesized from pyruvate for almost one hundred years\textsuperscript{116} utilizing silyl enol ethers and Lewis acids\textsuperscript{117}, two carbon ethylene homologation\textsuperscript{118}, and base catalyzed Aldol Condensation and Dehydration\textsuperscript{119,120}. For evaluating the X-ray crystal structures of benzylidene pyruvate, cinnamylidene pyruvate, 4-nitrocinnamylidene pyruvate with SbADC the dieneones used were mixtures with pyruvate. For the kinetic studies these substrates were purified to be 98-99% pyruvate free to obtain accurate enzymatic parameters.

**B. Rational for SbADC Molecular Probes**

In the attempt to elucidate the reason why SbADC does not facilitate the decarboxylation of pyruvate to acetaldehyde, SbADC was to be soaked with various
combinations of substrates to study X-ray crystal structures. This series of X-ray crystal structures when viewed in sequence would produce a “molecular” movie which would show the position of the enzyme’s active site residues, their proximity to substrate functionalities, and thus their role in the catalyzing Aldol reactions. Shown below is the schematic for the desired “molecular movie” showing the steps in the reaction that would be of interest.

![Figure 81: Proposed use of trifluoropyruvate and difluoropyruvate for mechanistic determination of SbADC aldol pathway](image)

The structures in square brackets are the combinations that are already observed by X-ray crystallography while the ones in curly brackets are the ones desired to be produced. For these trifluoropyruvate and difluoropyruvate were synthesized. The purpose of the trifluoropyruvate would be to soak the enzyme with a substrate that cannot undergo Aldol condensation. Trifluoropyruvate would be concurrently soaked with cinnamaldehyde to see the proximity of the substrates to one another and the positions of the active site residues that would be getting into bonding distance of the substrates. Difluoropyruvate would be employed as it is able to undergo aldol condensation but not aldol dehydration. This attenuated reactivity
would also allow for the identification of which side chains participate in Aldol dehydration reaction by their proximity to the Aldol product.

Ethyl trifluoropyruvate is commercially available so the sodium salt of the hydrate of trifluoropyruvate can be made by acidic hydrolysis and neutralization. This method minimizes the presence of excess hydroxide which would denature the enzyme being studied. Ethyl difluoropyruvate is not commercially available so it or an analogue is needed to be made so its salt can be used.

Of the literature examples for synthesizing difluorocompounds, only a few seemed attractive for difluoropyruvate. One of these was as follows:

![Figure 82: An example synthetic scheme in formation of difluoropyruvate analogues](image)

This method\textsuperscript{121} used (diethylamino)sulfur trifluoride (DAST) to make \(\beta,\beta\)-difluoro compounds by reaction with the \(\alpha\)-keto carbonyl and subsequently regenerating it by reduction of the ester to to the aldehyde level followed by substitution with cyanide, oxidation, then acid hydrolysis. This method worked well for cases when \(R\) was either alkyl or aryl substituents. No examples of \(R=H\) were reported, leading one to believe that it may be unsuccessful with the method. The other route was the Mg\textsuperscript{0} promoted mono-defluorination\textsuperscript{122} of trifluromethyl ketones. This simple preparation was attempted with ethyl trifluoropyruvate even though there was no precedent for an \(\alpha\)-keto trifluoromethyl moiety to get to difluoropyruvate directly. The most reliable route and the one ultimately used was trifluoroacetylation of 2-methylfuran by
Friedel-Crafts acylation then Mg$^0$ promoted monodefluorination$^{123}$ followed by ozonolysis of the resulting 5-difluoroacetyl-2-methylfuran$^{124}$.

V. RESULTS AND DISCUSSION

C. Formation of CinnamylidenePyruvate and 4-NO$_2$ CinnamylidenePyruvate

The sodium benzylidene pyruvate, sodium and potassium cinnamylidene pyruvate salts, and sodium and postassium 4-nitrocinnamylidene pyruvate salts were synthesized from their corresponding aldehydes using the base catalyzed aldol chemistry. Preliminary studies began with the synthesis of sodium benzylidene pyruvate to determine an appropriate method for cinnamylidene preparation. Benzylidene pyruvate was prepared from benzaldehyde and sodium pyruvate with sodium hydroxide as described$^5$ and isolated in 34% purified yield. The initial precipitate from the reaction mixture was isolated and determined to be a mixture of the condensation and dehydration product in ~1:2 molar ratio and free of pyruvate. The mixture was purified from aqueous media and acidified to pH1 with the desired dieneone precipitating from solution. Acidification shifts the α-hydrogen from δ6.9ppm to δ7.6ppm.

![Figure 83: Formation of cinnamylidene pyruvate sodium salt](image)

43 was synthesized using the same method, but at 55°C with a yellow solid precipitating from the reaction mixture in 68% crude yield as mixture of the desired dieneone and pyruvate (27% by mass of the mixture). The mixture was made pyruvate free by the same precipitation
method from acidic aqueous media with a 42% yield and exhibited the same large change in chemical shift of the hydrogen α to the ketone in either the salt or acid form. However the dieneone was reactive to aqueous acid. Extraction of the supernatant of EtOAc afforded a yellow oil in 36% crude mass that was a mix of the desired acid (74% by mass), ethyl acetate, and what was hypothesized to be the Aldol condensation product formed by hydration of the dienone. Total estimated yield from the precipitate and extract is ~60%.

![Figure 84: Formation of 4-nitrocinnamylidene pyruvate sodium salt](image)

The 4-NO₂ analogue 44 was synthesized with the previous methods and placed through the same workup protocol but to pH2. This dienone however proved to be very sensitive to aqueous acid and afforded none of the desired material. Synthesis without acidic workup but with precipitation of the salts with isopropyl alcohol afforded a 74% yield of the product as a mixture that was 84% by mass 44 and 14% by mass sodium pyruvate.

![Figure 85: General scheme for isolating sodium pyruvate free cinnamylidene pyruvate analogues](image)

The acid sensitivity of 45 and 46 prevented straightforward extraction to remove contaminating pyruvate. However compounds 45 and 46 could be extracted from their aqueous solutions in triethylammonium bicarbonate into CH₂Cl₂. Evaporation yielded the oily
triethylammonium salts. The crystallize potassium salts were obtained by dissolution in ethyl acetate and precipitated upon addition of a solution of potassium 2-ethylhexanoate\textsuperscript{125} generating 15\% yield \textbf{46} and 5\% yield \textbf{46} with 1-2\% by mass pyruvate as determined by \textsuperscript{1}H NMR spectroscopy. The desired products were able to be isolated pure as when the triethyl ammonium salt acquired from extraction when exposed to ethyl acetate the large majority of it did not dissolve. The resulting supernatant was decanted from the undissolved oil and mixed with a solution of potassium 2-ethylhexanoate in ethyl acetate precipitating the dieneone salt. When the insoluble oil, which was dissolved in CH\textsubscript{2}Cl\textsubscript{2}, was mixed with potassium 2-ethylhexanoate no precipitate was formed even when the salt was just in large excess. Analysis of the EtOAc insoluble oil by \textsuperscript{1}H NMR showed that the doublet characteristic of the hydrogen α to the ketone of the dieneone were reduced to 75\% signal intensity relative to the dieneone and instead had a new set of signals from δ3.5 to δ3.2 that appeared to be a triplet and a doublet.

![Figure 86: \textsuperscript{1}H NMR spectra of 4-nitrocinnamylidene pyruvate over the course of sodium pyruvate removal](image)

A \textsuperscript{1}H NMR spectrum was acquired of the triethyl ammonium bicarbonate extract and looked the same as the EtOAc insoluble oil. From this data it was concluded that the dieneones still undergo hydration to form the Aldol product even under these mild conditions. Attempts
to minimize hydration by raising the pH of the reaction mixture to 12 from 8 by bubbling in of CO₂ before exposure to triethylammonium bicarbonate did not mitigate the amount of product degradation. The cinnamylidene pyruvate derivatives containing 1-2% pyruvate were then used by Lisa Mueller to obtain kinetic parameters for SbADC by monitoring the retro Aldol reactions in stop flow experiments.

D. Difluoropyruvate Synthesis

![Diagram of difluoropyruvate synthesis](image)

Figure 87: Scheme for sodium trifluoropyruvate hydrate

Ethyl trifluoropyruvate hydrolysis was monitored by ¹H NMR to ensure complete hydrolysis by watching the ester quartet turn to that of the quartet of ethanol. ¹H NMR was deemed necessary for evaluating the reaction mixture as ethyl trifluoropyruvate readily hydrates, analogous to that of hexafluoroacetone, and was unclear if it could migrate upon silica gel for TLC analysis. After overnight reflux with a pyruvate concentration of 0.37M gave a 93% yield of 47 after neutralization with NaOH.

![Diagram of simplified difluoropyruvate formation](image)

Figure 88: Proposed synthetic route for simplified difluoropyruvate formation

If Mg⁰ reduction and mono defluorination proceeds as for CF₃COAr,¹²³ this has the potential to provide a succinct route to difluoropyruvates. When ethyl trifluoropyruvate was
exposed to the mono-defluorination conditions reduction occurred but afforded a mixture of the E and Z isomers of bis-silyl enol ether 48 without elimination of fluoride. This material exposed to a range of conditions in efforts to produce ethyl difluoropyruvate (49) by the most succinct route known. The first was exposure to aqueous hydrochloric acid. The purpose of this admittedly was not to yield difluoropyruvate but confirm the identity of the product afforded from the monodefluorination reaction. Upon addition of acid the quartet indicative of the ethyl ester of trifluoropyruvate was observed as well as the quartet seen of the alpha hydrogen in ethyl trifluorolactate. The second condition was addition of (Bu)$_4$NF in portions over time. The goal in using this organic soluble fluoride was to react in an E$_2$ fashion kicking out fluoride by making trimethylsilyl fluoride in the following fashion:

![Figure 89: Desired E2 elimination of O,O-(bis-trimethylsilyl) ethyl trifluoropyruvate](image)

After 20hr with 6eq of (Bu)$_4$NF added over that time, trifluoroacetic acid was added was to remove the trimethylsilyl groups and to see if a triplet at 6ppm would be produced. This singal is indicative of the desired difluorocompound but no change in the $^1$H NMR spectrum observed. If the removal of the silyl groups by fluoride did not occur, then the combination of ethyl trifluoropyruvate and ethyl trifluorolactate would be observed after protonation. The resonances for the compounds were also not seen in the NMR. From this data, it was postulated that the E$_2$ elimination occurred but the resulting difluoroalkene also acted as a
Michael acceptor which condensed with the bis-silylenol ether precursor. The proposed mechanism is diagramed below:

![Proposed E2 elimination of O,O-(bis-trimethylsilyl) ethyl trifluoropyruvate that occurred](image)

With the limited success using ethyl trifluoropyruvate the ozonolysis scheme was pursued by way of an ozone generator on loan with many thanks to Dr. Tysoe.

![Literature based synthesis for ozonolysis precursor](image)

Trifluoroacetylation of 2-methylfuran with trifluoroacetic anhydride was conducted by the literature procedure to afford 50 in 73% yield followed by the magnesium facilitated monodeflourination in 44% purified yield after vacuum distillation of 51.

![Ozonolysis scheme for synthesis of methyl difluoropyruvate methyl hemiacetal](image)

In the end, ozonolysis of the difluorofuran does produce the methyl ester 52. Katagiri, Ozaki, and Tanaka describe ozonolysis “until a green color is formed” which proved to be
problematic in reproducing what they accomplished. We found bubbling of gas into the reaction mixture until a yellow-green color was apparent to produce a mixture of methyl difluoropyruvate hydrate, 52, and unreacted difluoroacetyl methyl furan. We found if ozone was bubbled into the reaction mixture when the greenish color formed, and then disappeared, the conversion of 51 to 52 was complete.

Separation of 52 from contaminating CF₂HCO₂H was described by passage through silica gel. Repeated attempts at chromatographic separation on silica failed, using various CH₃OH/CH₂Cl₂ or CH₃CN/CH₂Cl₂ eluents. Acidic byproducts were successfully removed by passage through a column of Amberlyst A-21, a polystyrene supported tertiary amine ion exchange resin. This treatment successfully removed the acidic components but elution with ethanol caused exchange with the methyl hemiacetal of the desired product to produce a mixture of the ethyl and methyl hemiacetals of methyl difluoropyruvate. Using the same methodology for the formation of 47, compound 53 was afforded in 69% yield after acidic hydrolysis and neutralization.

Soaking SbADC crystals with 50mM 53 did not produce sufficient electron density in the active site to conclude that it was complexed in the cavity. This is more than likely due to the propensity of the difluoropyruvate to hydrate and not have enough of the keto form present to form an imine with the active site lysine. As 50 to 150mM pyruvate was used to generate 1.5Å resolution images, a concentration of 150mM or greater would probably be needed to the 53 to form a Schiff Base with the enzyme. When 47 and cinnamaldehyde were soaked with SbADC concurrently no electron density was observed in the active site. This result was not expected for if the pair would not occupy the active site at least the Schiff base of 47 was expected to be
seen. The reason for this remains unknown but conducting the soaks at various concentrations of the two components might generate the desired crystallographic structure.

VI. SMALL MOLECULE PROBES FOR MppR AND MppP

A. Enduracidididine and Enduracidin Background Information

Second to vaccines, antibiotics seem to be the most effective means to treat disease and illness. Unfortunately, this boon is being combated by the organisms we eliminate by their evolution of antibiotic resistance. Infectious agents now exist that are either resistant or immune to our most potent substances like vancomycin or methicillin. The mode of action of these last resort antibiotics, like vancomycin, impede the growth of these bacteria by inhibiting the expansion of their cell walls upon growth causing the cells to lyse in the process due to internal osmotic pressure. Antibiotics such as ramoplanin, enduracidin, and mannopeptimycin\textsuperscript{126} prohibit growth in much the same way but seem to exhibit a peculiar ability to not be adapted against.

All three of these structures are similar in their construction and anitibiotic potency. In 2002, Cudic \textit{et. al.} investigated the mechanism of ramoplanin\textsuperscript{127} to inhibit the late stage glycosyltransferase reactions necessary for bacterial growth. Different from the recognition of the N-acyl-D-Ala-D-Ala moiety like vancomycin, Somner and Reynold postulated that it was inhibition of Lipid I to Lipid II conversion that stopped the glycosyltransference.\textsuperscript{128,129} Testing of this hypothesis was problematic for some time as acquisition of pure murG, the enzyme that facilitates transglycosylation, and assay soluble version of Lipid I and II were not available until 1998 when the Walker labs synthesized a biomimetic analogue that was water soluble to test
the activity of the murG enzyme.\textsuperscript{130} It was also stated in this paper that murG has no human equivalent and that the muramyl-pentapeptide moiety is unique to bacterial organisms. Their work continued\textsuperscript{131} by following the accumulation of radiolabelled Lipid II in increasing amounts of ramoplanin present to suggest a new mechanism of action for the antibiotic: binding to Lipid II by ramoplanin to prevent its polymerization and thus prevent cell wall biosynthesis. These two modes were tested later in 2006 to confirm which one of these pathways was taken.\textsuperscript{132} Using inhibition kinetics and binding assays it was demonstrated that Lipid II binds approximately ten times stronger to enduracidin and ramoplanin than Lipid I but was only favored in competition experiments by a factor of five. Although capture of Lipid II is slightly favorable both of these pathways appear to happen equally. It is this that makes these antibiotics so resistant to adaptive mutation in that they target and disrupt a process so fundamental to the bacteria’s survival that in order to evolve against it would require an alternate method to construct its cellular membranes.

Enduracidin was first observed from \textit{Streptomyces fungicidicus} from the soil of Nishinomia, Japan and found to have potent antibiotic activity against gram-positive bacteria and moderate activity against acid-fast and phytopathogenic organisms while having no activity against gram-negative bacteria, fungi, or yeasts tested.\textsuperscript{133} Later in the year enduracidin was isolated and characterized\textsuperscript{134} demonstrating its most potent activity against antibiotic resistant strains of \textit{S. aureus} with minimum inhibition concentrations (MIC) ranging from 0.1-0.2µg/mL.

The antibiotic’s efficacy was demonstrated\textsuperscript{135} \textit{in vitro} and \textit{in vivo} when administered intravenously, intraperitoneally, and subcutaneously but not orally against \textit{S. aureus}, \textit{S. pyogenes}, and \textit{D. pneumoniae}. Digestion of enduracidin in 6M HCl yielded nine types of known
amino acids along with two as of then unidentified residues. In a follow up publication\textsuperscript{136} the structures of the known amino acids were determined and called enduracididine and allo-enduracididine, the enantiomer of enduracididine.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figures/enduracididine.png}
\caption{Enduracididine (left) and allo-enduracididine (right)}
\end{figure}

The origin of enduracididine was investigated by feed experiments of \textit{S. fungicidicus} with radiolabelled amino acids.\textsuperscript{137} For these it was concluded that aspartic acid, threonine, serine, glycine, alanine, ornithine, and citrullene were directly incorporated into the cyclic polypeptide whereas the 4-hydroxyphenyl and 3,5-dichloro-4-hydroxyphenyl glycine residues were derived from tyrosine. For enduracididine it was presumed that either arginine or histidine was the precursor to enduracididine but with this study it shows that the radiolabelled arginine was the amino acid used to make enduracididine providing solid evidence that arginine was the precursor.
B. Roles of MppP and MppR

Using *Streptomyces eurocidius* Eguchi\(^ {138} \) uncovered more detail as to the specific transformations arginine undergoes along the metabolic pathway to enduracididine when looking at the role of arginine in the biosynthesis of 2-nitroimidazole. When the bacteria was heated at 50°C the production of 2-aminoimidazole, the precursor to 2-nitroimidazole, ended. When arginine was administered to the bacteria followed by 50°C heat treatment a guanidine positive material was seen to accumulate. Exposure of the guanidine positive fraction to H\(_2\)O\(_2\) to decarboxylate the amino acids present afforded 3-hydroxy-4-guanidino-butyric acid implying that γ-OH-arginine was the material that was building up. Pyridoxal phosphate (PLP) and oxygen were also confirmed as necessary to the reaction for if the cofactor was omitted and the reaction was conducted under an inert atmosphere 2-aminoimidazoxole production was not seen.
The proposed biosynthetic pathway was the oxidation of arginine followed by loss of pyruvate from the oxidized arginine’s α-keto analogue to produce 2-aminoimidazole for conversion to the 2-nitroimidazole antibiotic. When given arginine and its cofactors, pyridoxal phosphate and molecular oxygen, 2-aminoimidazole production proceeded. However if deprived of these or if the enzyme was heated to 50°C 2-aminoimidazole was not formed but instead a guanidine positive material accumulated. When this accumulated fraction was treated with H₂O₂ to induce decarboxylation 3-hydroxy-4-guanidinobutyric acid was found as the product implying that the guanidine positive substance was γ-hydroxy-arginine which underwent transamination to the α-keto acid then decarboxylated. Interestingly enough, when γ-OH-arginine was given to the enzyme in the absence of pyridoxal phosphate and/or molecular oxygen 2-aminoimidazole was formed along with an equal amount of pyruvate. Although not identified, it was postulated that an aldolase type enzyme would be required to affect the transformation described above.

Genetic sequencing and experimentation with cloned variants lead to the identification of the 17 amino acid gene cluster for the production of enduracididine. It was found that both *S. enduricidicus* and *S. hygroscopius* contained the three gene operon for antibiotic EndP, EndQ, EndR and MppP, MppQ, MppR for the biosynthesis of enduracidin and mannopeptimycin respectively. They also held in common a non-heme based oxygenase that utilized 2-oxo-
glutarate that does not hydroxylate arginine but instead hydroxylates enduracididine in the β position.\textsuperscript{140} MppP and MppQ were both shown to be pyridoxal based aminotransferases with the role of MppR still undetermined.

The structural and functional capability of it was later reported\textsuperscript{141} demonstrating its activity as an aldolase and confirming the previous hypothesis that an aldolase is needed to be present as was postulated by the feed experiments of \textit{S. eurocidius}. Bioinformatic analysis shows that MppR has a low but significant sequence homology to that of a previously uncharacterized group of acetoacetate decarboxylase family with the similarity notably occurring in the active site. X-ray crystallography of MppR crystallized from HEPES buffer showed a HEPES molecule bound in the active site with the sulfonyl group hydrogen bonded to the active site lysine L156. Treatment of MppR with sodium pyruvate either as a soak or being present during crystallization covalently bonded to L156 as a Schiff base with no coordinated water proximal to the former α-keto functionality probably due to the hydrophobic nature of the active site itself.

![Aldol condensation and dehydration product of imidazole carboxaldehyde and sodium pyruvate formed by MppR](image)

Encouraged by this observation, imidazole-2-carboxaldehyde was soaked with MppR and sodium pyruvate with the resulting crystal structure showing the Aldol product between the two, however, with no enzymatic turnover. HPLC analysis with UV/VIS detection showed none of the enone in the supernatant. To test the role of MppR in the biosynthesis of enduracididine it was treated with 2-oxo-γ-OH-arginine which resulted from the exposure of 4(R/S)-hydroxy-
2(S)-arginine with an L-amino acid oxidase (LAAO) from *C. atrox*. Over time this produced the 2-oxo derivative of enduracididine with the same stereochemistry at C4 as seen in mannopeptimycins and making the same intermolecular interactions with the active site residues as pyruvate did.

(2S,4R)-2-amino-5-guanidino-4-hydroxypentanoic acid, colloquially known as γ-OH-arginine, was first isolated from the sea cucumber *Polycheira rufescens*. Identification of the amino acid was derived from material isolated after reaction of treated extract with an L-argininase and comparing the compounds to synthetically produced γ-OH ornithine and γ-OH-arginine.

![Chemical structure](image)

**Figure 97: Literature route to γ-OH arginine**

γ-OH-arginine was made in 4.6% overall yield by first making γ-OH ornithine which was then treated with S-methylthioisourea to install the guanidine group. The stereochemistry of the γ-OH-arginine isolated from *P. rufescens* was published by Fujita one year later as the S chirality on the hydroxyl containing carbon and R as the chirality of the amino bearing carbon. A later report altered the route used by Fujita by first reacting epichlorohydrin with sodium diethyl malonate instead of phthalimide.
After treatment with ammonia the guanidine was formed from OMe isourea. Reaction with bromine, then ammonia, then trypsin give the desired product in 1.3% yield as a mixture of the R and S hydroxyl diastereomers with no isolation of the intermediates after formation of the epichlorohydrin malonate adduct. The low yield was attributed to the multitude of side reactions undoubtedly produced by reaction with bromine as well as trypsin hydrolyzing only two of the four total diastereomers present. They also replicated Fujita’s method using OMe isourea but did not report a yield however it was stated that use of an acidic eluent for ion exchange chromatography drove the γ-OH-arginine to lactone formation.

More recent methodologies focus on stereoselectivity to increase yield of the (2S,4R) isomer to study the biosynthesis of enduracidin, synthesis of a fully protected, organic soluble enduracididine in 3-8% overall yield using a single enantiomer of a key aziradine intermediate, and synthesis of β-OH enduracididine derivatives to generate modified mannopeptimycins via peptide coupling. Synthesis of γ-OH-arginine and its dehydro analogue were undertaken to more thoroughly explore MppR as well as to open a pathway to make
modified enduracididines which would hopefully be incorporated into the biosynthesis resulting in modified enduracids to fight antibiotic resistant bacteria in the future.

C. Attempts to Make Guanidiniumacetaldehydye for MppR Evaluation

\[
\begin{align*}
\text{H}_2\text{N} & \text{NH}_2^+ \quad \text{COO}^- \quad \text{NH}_3^+ \\
\text{BocHN} & \text{N} \text{-O} + \text{O} \text{ONa}
\end{align*}
\]

Figure 99: Retro-synthetic analysis for dehydro-L-Arginine by Aldol reactions route

The above retrosynthetic analysis shows that the most expedient route to γ-hydroxy-arginine or dehydro-arginine would be through Aldol Condensation of sodium pyruvate with guanidinoacetaldehyde. Good reagents have been developed for installing guanidino functionality on imines but Drake, Petel, and Lebl have made an extremely convenient and efficient guanidynylating agent (used exclusively from here on in) and if reacted with the dimethyl acetal of amino acetaldehyde acidic hydrolysis would provide the desired material.

\[
\begin{align*}
\text{BocHN} & \text{N} \text{-Boc} \quad \text{OMe} \quad \text{H}_2\text{N} \quad \text{OMe} \\
\text{BocHN} & \text{N} \text{-Boc} \quad \text{OMe} \quad \text{OMe} \\
\text{CH}_3\text{CN, RT overnight} \quad \text{BocHN} & \text{N} \text{H-Boc} \quad \text{OMe} \quad \text{OMe}
\end{align*}
\]

Figure 100: Guanidinylation of aminoacetaldehyde dimethyl acetal 54

When the dimethyl acetal was exposed to the pyrazolyl diBoc-carboxamidine the resulting guanidine was afforded in an average of 98% yield with two degree melting point range due to the easy removal of pyrazole side product due to its volatility.
Hydrolysis of dimethyl acetal 54 in 11M trifluoroacetic acid in deuterium oxide was monitored by $^1$H NMR with complete removal of the Boc groups after 20min when data was first acquired. When the spectrum of the sample was obtained a second time 18hr later, only singlets corresponding to methanol and 2-aminoimidazole were present. As acid hydrolysis resulted in cyclization and aromatization, removal of the Boc groups to generate the guanidinium salt was conducted in hopes to prevent cyclization. When the acetal was combined with two equivalents of methanesulfonic acid at -78°C and warmed to 0°C no removal of the Boc groups was observed. It was only after warming to room temperature that removal of only one Boc group occurred as determined by $^1$H NMR. Even after 46hr at room temperature no change was observed from the now monoBoc guanidine. Hydrolysis was undertaken again but with 12M HCl at 0°C and followed by no D NMR. Complete conversion of the substrate to 2-aminoimidazole occurred in ≤ 48min. Under these conditions it was deemed an unsuitable method for formation of the guanidinoacetaldehyde as cyclization and aromatization could not be prevented. In all of these cases the characteristic resonance of an aldehyde in $^1$H NMR was not observed, even transiently.
D. Towards $\beta,\gamma$-dehydroarginine for MppP Evaluation

With acidic hydrolysis of the dimethyl acetal demonstrated to not be a viable route to make guanidinoacetaldehyde an alternative method was adopted. Oxidative cleavage of the appropriate alkene precursor to produce the aldehyde first while keeping the Boc groups intact to prevent cyclization was the rationale for the new approach. Guanidinylation of allyl amine to afford 55 averaged yields of 97% with two degree melting point range. Its been shown that generation of OsO$_4$ *in situ* with NaIO$_4$ can be used to convert an alkene to a diol which then is cleaved by NaIO$_4$ to the corresponding carbonyl compounds can be performed conveniently and in decent yield.$^{151}$ When di-Boc allylguanidine was exposed to these conditions only unreacted starting material was recovered in 83%, even after 35hr time and was not observed anymore on TLC analysis. The reaction was performed again with none of the desired aldehyde 56 observed even after 3days. However, after three days a new signal was observed by $^1$H NMR at $\delta$ 6.7ppm and interpreted to be the diBoc derivative of 2-aminomidazole and present in only as minor amount.

![Figure 102: Desired oxidative cleavage of guanidinylated allylamine](image)

Perplexed by this lack of activity on what appeared to be a suitable substrate the quality of the reagents were tested by way of reaction with a different allyl compound.
Figure 103: Model reactions to test conditions for oxidative cleavage of an alkene with K$_2$OsO$_4$

N-Benzoyl allylamine was synthesized in 96% yield and exposed to the oxidation regime to produce the corresponding aldehyde in 62% yield. In comparing the structure of the di-Boc allylguanidine and the benzoyl allylamine it was hypothesized that the benzoyl analogue was able to react was that the lone pair of electrons on the nitrogen were involved in resonance were as the lone pair on the allyl moiety were sitting as a free lone pair which can coordinate to the osmium when the osmate ester is formed preventing diol formation by either occupying a required coordination site by chelation preventing the ligand transfer needed for the oxidative regeneration of the osmium.

The hypothesis was tested by deprotection of 55 in refluxing 50% CF$_3$COOH/CH$_2$Cl$_2$ for 1hr. Elevated temperature was needed as exposure to the acid at room temperature only removed one of the Boc groups which is agreement with previous observations as a di-cation would need to be made to remove the second Boc group.

The resulting trifluoroacetate salt 57 was treated with the oxidation reagents in a mixture of D$_2$O and CD$_3$OD and monitored by $^1$H NMR.
Figure 105: Cyclized hemiaminal generated upon treatment of allylguanidinium trifluoroacetate with osmium tetroxide

After 3.5 hr the alkene resonances were no longer observed but neither was that of the desired aldehyde. Instead signals of $\delta 5.6$, $\delta 3.9$, $\delta 3.5$ were seen, each as a doublet of doublets. From this it was concluded that the aldehyde did form but cyclized to hemiaminal 58 even with the protonated guanidine at neutral pH due to the germinal coupling constants. Also observed in the reaction mixture was formalin, corroborating that the oxidative cleavage took place. 2-aminoimidazole was also produced but in trace amounts which implies that 58 is the kinetic product, and aromatization to the imidazole occurs over time.

The reaction mixture was split into two portions to which was added hydroxylamine to one and sodium bisulfite added to the other. Each was added to react with the aldehyde in a manner that could be reversed and to keep it acyclic so it could not aromatize. Exposure to hydroxylamine hydrochloride did not produce the desired oxime but instead catalyzed the transformation to 2-aminoimidazole completely. Addition of 20 eq of sodium bisulfite on the other hand did not aromatize the hemiaminal and made the sulfonate of the hemiaminal proving that elimination to the aromatic species is avoidable. After accounting for these two compounds another doublet of doublets was seen but wasn’t enough to confirm the presence of the acyclic bisulfite adduct. The splitting pattern, chemical shift, and coupling constants of the cyclic sulfonate shifted upfield to $\delta 5.3$ for methyne hydrogen and downfield shifts of $\delta 5.0$
and δ4.9 for the methylene hydrogens. Since the identity of the corresponding anion from the reaction may oxidize MppR when exposed to it the reaction was repeated and the hemianimal was purified to the chloride salt via ion exchange chromatography with Dowex 2X-8 resin.

As hemiaminal 58 had to be produced by cyclization of the guanidine group into an aldehyde, there presumably some mechanism by which the equilibrium can be perturbed so that the aldehyde can be present. Even if it could not open back up, it could lose a hydroxyl group to form an iminium ion which could go on to react with pyruvate in an aldol manner. Based on these possibilities, the Silvaggi group combined 58, sodium pyruvate, and MppR. Unfortunately, they found that no reaction between the components was observed.

Figure 106: Synthethis diBoc protected cyclic hemiaminal from diBoc protected allylguanidine

With the lone pair hypothesis validated, the di-Boc allylguanidine was treated with K₂OsO₄ and NaIO₄ again but this time in the presence of 1eq p-toluenesulfonic acid to determine if protonation would be a viable way to oxidize the allyl group while keeping the guanidine Bocs intact. By ¹H NMR it was unable to be determined if the cyclized hemiaminal was a salt or became deprotonated to become neutral but the alkene signals went away and resonances similar to that of 58 were seen. As the mixture was taken in CDCl₃ it was presumed to be the neutral species. The mixture was partitioned against water to remove the inorganic salts and concentrated before being allowed to react with sodium pyruvate as per the cinnamylidine method to produce 59.
After 17hr at 55°C 59 was longer seen by TLC. Analysis by $^1$H NMR in D$_2$O showed no Boc signals present nor any other the alkene signals anticipated for the Aldol product. The crude product was extracted from triethylammonium bicarbonate with CH$_2$Cl$_2$ to isolate the desired product if present. What was observed by $^1$H NMR did not have signals correlating to the alkene 60 and the starting material 59 was not isolated. One thought would be that the Boc protected nitrogen in the guanidine could Michael add into the enone product to cyclize to 61. This was considered a possibility as demonstrated by the guanidine’s propensity for cyclization but based on the ambiguity of the $^1$H NMR spectrum, and the low amount of material recovered, it was deemed an unattractive method to make the dehydroarginine. However, more investigation should be undertaken to conclude if the material generated was indeed α-keto enduracidididine as the methodology used would provide a succinct path for its synthesis.

1. L-Vinylglycine Synthesis

Vinylglycine$^{152}$ is a simple chiral substance allowing transformation of each of the three groups bonded to the chiral center.$^{153}$ It has become even more valuable since the development of efficient olefin metathesis catalysts that can convert it to other unsaturated amino acids. The unsaturation diverts pyridoxal-mediated transformations, leading to suicide substrate behavior of substituted vinylglycines with several pyridoxal based enzymes.$^{154,155,156}$
GABA transaminase irreversible inhibitor has also been prepared by a similar means.\textsuperscript{157} Even in the absence of pyridoxal, the α-proton of vinylglycine ester is considerably more acidic than that of saturated amino acid esters: in mild base β-γ unsaturation of vinylglycine esters is rapidly converted to α-β unsaturation. Even in neutral D\textsubscript{2}O, vinylglycine esters α-deuterate and gradually isomerize.\textsuperscript{158} This obviously limits the conditions and procedures appropriate for preparation and transformation of such unsaturated amino acids.

Vinylglycine has been prepared by a variety of methods\textsuperscript{159,160} including sulfoxide or selenoxide elimination from methionine derivatives,\textsuperscript{161,162,163,164,165,166} oxidative decarboxylation of glutamic acid,\textsuperscript{167} asymmetric allylic substitution and subsequent transformation,\textsuperscript{168} vinyl Grignard reaction with an α-bromoglycine,\textsuperscript{169} and an interesting route involving Neber rearrangement.\textsuperscript{170} All these procedures yield vinylglycine in a protected or racemic form, from which L-vinylglycine itself can be isolated by deprotection and/or enzymatic resolution.

Perhaps the most common route is that reported by the Rapoport group in 1980 involving vapor-phase thermolysis of the Cbz protected methyl ester of methionine sulfoxide.\textsuperscript{92,171} Contamination with isomerization products and variable yields encouraged modifications of conditions over the years, leading ultimately to a 79% yield of 95% pure protected vinylglycine.\textsuperscript{96} Deprotection of chromatographically-isolated Cbz vinylglycine ester in refluxing hydrochloric acid allows subsequent transformation.\textsuperscript{161} However, the gas phase reaction limits reaction scale, and requires careful control of conditions inconvenient with standard laboratory glassware. Attempts to increase throughput by solution phase approaches met with difficulties.\textsuperscript{93} Despite this procedure, and the significant number of papers and patents describing vinylglycine and its use,\textsuperscript{172,173} vinylglycine is not readily available commercially.
We report here a modification to the Rappoport approach to unprotected vinylglycine that allows convenient reaction at larger scale using standard apparatus and short reaction times. We have replaced two protection steps, thermolysis, chromatographic purification of protected vinylglycine, and deprotection, with a single simpler process using in situ protection, more convenient thermolysis, and a simple workup that effects deprotection and isolation of crystalline vinylglycine without chromatography. We have optimized for convenience rather than yield: conversion of methionine into pure crystalline vinylglycine requires two simple operations.

a. L-Selenomethionine selenoxide Route

The mildest procedures leading to vinylglycines appear to involve phenylselenoxide elimination. A succinct method would be the by β,γ-syn elimination of the selenoxide analogue of L-Methionine with the chemical literature demonstrating that the α,β- elimination to form enones occurs rapidly, usually with no ability to isolate the corresponding selenoxide.

![Chemical Reaction Diagram]

Figure 108: α,β -unsaturated ketone formation by arylselenoxide elimination and desired elimation of a methyl selenoxide to form vinylglycine

While not as facile as the α,β- elimination, the β,γ-elimination was deemed feasible. L-selenomethionine was converted to the methyl ester 62, N-Boc protected to 63, and oxidized
the selenoxide 64 using standard protocols. While the α,β-elimination can occur between 78°C and 0°C, elevated temperature were employed in investigation of the β,γ-elimination to generated the vinylglycine derivative 65.

![Chemical Reaction Diagram]

**Figure 109: Synthetic scheme of N-Boc-L-vinyglycine methyl ester synthesis from L-selenomethionine**

It has been previously reported that a derivative of L-Selenomethionine oxide reduces back to its L-Selenomethionine derivative in acetone over 7d at room temperature in the absence of light. What was surprising was not the extent of this conversion in our experimental observations but the rapidity at which this transformation took place. Tabulated below are the experimental parameters and results from the β,γ-elimination of 64:

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conc SeO</th>
<th>Conc P(OMe)_3</th>
<th>Temp(°C)</th>
<th>Time</th>
<th>Solvent</th>
<th>χ(vinyl:Se)</th>
<th>%yield(NMR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0×10^{-2} M</td>
<td>-</td>
<td>138</td>
<td>2h</td>
<td>p-xylene</td>
<td>1 : 4</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>1.0×10^{-2} M</td>
<td>-</td>
<td>81</td>
<td>2h</td>
<td>CH_{2}CN</td>
<td>0 : 1</td>
<td>39 L-MetSe</td>
</tr>
<tr>
<td>3</td>
<td>9.0×10^{-3} M</td>
<td>0.09 M</td>
<td>110</td>
<td>20min</td>
<td>Toluene</td>
<td>1 : 2.5</td>
<td>50 → 0</td>
</tr>
<tr>
<td>4</td>
<td>8.0×10^{-3} M</td>
<td>0.10 M</td>
<td>110</td>
<td>20min</td>
<td>Toluene</td>
<td>1 : 1.8</td>
<td>41</td>
</tr>
<tr>
<td>5</td>
<td>4.0×10^{-3} M</td>
<td>-</td>
<td>111</td>
<td>25min</td>
<td>P(OMe)_3</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>1.0×10^{-2} M</td>
<td>3.60 M</td>
<td>reflux</td>
<td>25min</td>
<td>Hexane</td>
<td>1 : 10</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>2.0×10^{-2} M</td>
<td>0.27 M</td>
<td>110</td>
<td>40min</td>
<td>Toluene</td>
<td>0 : 1</td>
<td>97 L-MetSe</td>
</tr>
<tr>
<td>8</td>
<td>1.5×10^{-2} M</td>
<td>-</td>
<td>110</td>
<td>20min</td>
<td>Toluene</td>
<td>1 : 2.5</td>
<td>36</td>
</tr>
</tbody>
</table>

**Table 6: Parameters and results in thermolysis of NBoc-L-selenomethionine oxide methyl ester**
In our hands the methyl selenoxide 64 eliminated less well: only low yields (10-41%) of 65 were obtained on thermolysis. The major product was the precededented reduction to selenide. Poor results were found at various temperatures, even in the presence of excess periodate, though it helped in a related phenylselenoxide case.

b. L-Methinonine sulfoxide Route

i. Gas Phase Pyrolysis

With the failure to produce vinyl glycine by β,γ-elimination from 64 in appreciable amounts, we turned to the harsh sulfoxide elimination procedure using the N-Boc methyl ester derivative of methionine sulfoxide, as reported by Rich which provided the most detail for pyrolysis as well as an attractive yield before Rappoport’s report and thus this method was chosen. L- methionine was converted to 68 using the same protocols in the derviatization of L-selenomethione to 64. Vapor or aerosol introduction of 68 in xylene to a hot tube at 266°C led in our hands to variable yields (2-18%) of vinylglycine methyl ester with loss of Boc protection and on average 50% recovery of 68. Pyrolysis was successful on one account affording a 59% yield of 69 as estimated by NMR a mixture with DEHP. The capricious behavior was attributed
to the low volatility of starting material and to lability of product, as isomerization of desired vinylglycine to α-β unsaturated side product was also observed.

Figure 111: Illustration of apparatus constructed for gas phase pyrolysis

The thermal Boc removal is not entirely unwelcome, as its removal would be the next step in our sequence, but consideration of other protecting groups led to a useful modification. Protection of methionine sulfoxide in an organic soluble and more volatile form is needed, as we found pyrolysis of L-methionine sulfoxide (70) itself does not lead to L-vinylglycine (71). As stocks of 68 were depleted, pyrolysis of a different protected form of L-Methionine sulfoxide was performed we chose trimethylsilyl groups to protect both the acid and the amine functionality, partly to increase volatility, and partly because both groups could be added before and removed after elimination as one procedure. Vapor phase thermolysis proceeded in a similar efficiency as before, though exposure of product to water caused complete
deprotection to free vinylglycine. Previous solution phase thermolyses led to problems because of decomposition of products on longer high temperature exposure times. Pyrolysis proved successful and reproducible with the silylated methionine sulfoxide. The parameter that determined reproducibility was not only the temperature of pyrolysis, but the position of the stainless steel packing material in the pyrolysis tube.

ii. *in situ* Thermolysis

As the silylated methionine sulfoxide solution was able to be transformed into vinyl glycine by pyrolysis and vinyl glycine was recovered from the packing material at temperatures up to 310°C, it was hypothesized that the reaction could be performed in solution with the high boiling solvent diphenyl ether. The thermolysis was performed with variable amount of time with the solution at reflux. The table below details the distribution of materials was identified by $^1$H NMR.

<table>
<thead>
<tr>
<th>t (reflux)</th>
<th>Vinyl glycine</th>
<th>Side product</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 min</td>
<td>1.0</td>
<td>0.17</td>
</tr>
<tr>
<td>5 min</td>
<td>1.0</td>
<td>0.36</td>
</tr>
<tr>
<td>8 min</td>
<td>1.0</td>
<td>0.39</td>
</tr>
<tr>
<td>16 min</td>
<td>1.0</td>
<td>1.66</td>
</tr>
</tbody>
</table>

![Figure 112: NMR spectra of isolated materials from *in situ* thermolysis of silylated L-methionine](image)
Aqueous workup of the 5min trial afforded a 34% NMR yield of vinyl glycine.

Encouraged, thermolysis of non-silylated 70 was done for 3.5min in refluxing diphenyl ether.

Undetermined degradation products were produced as determined by NMR with signals observed that did not correspond to L-Methionine sulfoxide, vinyl glycine, or the side product, concluding that vinyl glycine cannot be produced without the presence of N,O-(bistrimethylsilyl)acetamide. Thermolysis of the silylated L-Methionine sulfoxide was scaled up and replicated with experimental parameters and results tabulated below:

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conc. L-MetSO</th>
<th>Conc. BSA</th>
<th>Mol Ratio L-Met SO:BSA</th>
<th>BSA:Ph₂O (v:v)</th>
<th>Time to Reflux</th>
<th>Time at Reflux</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.09 M</td>
<td>0.39 M</td>
<td>1 : 4.3</td>
<td>1 : 11</td>
<td>4 min</td>
<td>1 min</td>
</tr>
<tr>
<td>2</td>
<td>0.10 M</td>
<td>0.37 M</td>
<td>1 : 3.6</td>
<td>1 : 10</td>
<td>3 min</td>
<td>2 min</td>
</tr>
<tr>
<td>3</td>
<td>0.15 M</td>
<td>0.59 M</td>
<td>1 : 4.0</td>
<td>1 : 6</td>
<td>3 min</td>
<td>3 min</td>
</tr>
<tr>
<td>4</td>
<td>0.28 M</td>
<td>0.91 M</td>
<td>1 : 3.3</td>
<td>1 : 3.5</td>
<td>2 min</td>
<td>3 min</td>
</tr>
</tbody>
</table>

*Table 7: Experimental parameters in screening *in situ* thermolysis for L-vinylglycine formation*

<table>
<thead>
<tr>
<th>Entry</th>
<th>Vinyl Gly</th>
<th>Acetamide</th>
<th>Side Product</th>
<th>L-Met SO</th>
<th>Estimated NMR yield L-Vgy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49</td>
<td>37</td>
<td>7</td>
<td>7</td>
<td>68%</td>
</tr>
<tr>
<td>2</td>
<td>52</td>
<td>37</td>
<td>11</td>
<td></td>
<td>54%</td>
</tr>
<tr>
<td>3</td>
<td>43</td>
<td>39</td>
<td>7</td>
<td>11</td>
<td>68%</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>65</td>
<td>20</td>
<td></td>
<td>30%</td>
</tr>
</tbody>
</table>

*Table 8: Results from tested conditions *in situ* thermolysis parameters in L-vinylglycine synthesis*

**iii. Addition and Distillation Method**

Because of the expected substantially greater volatility of trimethylsilyl derivatives, we tried solution-phase heating of the silylated methionine sulfoxide. This provides a dramatic operational simplification.
Simply stirring L-methionine sulfoxide\textsuperscript{177} in N\textsubscript{2}O\textsubscript{-bis}(trimethylsilyl)acetamide (BSA) caused gradual dissolution as the zwitterionic starting material was converted to the trimethylsilyl ester and likely the N-trimethylsilyl derivative as well.\textsuperscript{178}

Surprisingly the material in the still pot after aqueous workup was determined by \textsuperscript{1}H NMR to contain trace amounts of vinyl glycine with the majority being the unidentified side product and other degradation products accounting for 15\% of the theoretical mass. Analysis of the distillate showed the presence of the silylated vinyl glycine. After aqueous workup the crude product was determined to be 3\% by mass side product, 50\% by mass acetaminde, and 47\% by mass vinyl glycine corresponding to a 48\% yield by NMR and the cleanest crude product seen to date. The experiment was replicated with similar success. The tables below are the parameters and results for the subsequent trails:

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conc SO (M)</th>
<th>Conc. BSA (M)</th>
<th>Ratio BSA:Ph\textsubscript{2}O(mols)</th>
<th>Time to Reflux</th>
<th>Distill Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>136</td>
<td>0.16</td>
<td>0.56</td>
<td>1:3.5</td>
<td>5.5 min</td>
<td>4 min</td>
</tr>
<tr>
<td>138</td>
<td>0.12</td>
<td>0.43</td>
<td>1:3.6</td>
<td>8 min</td>
<td>6 min</td>
</tr>
<tr>
<td>130</td>
<td>0.30</td>
<td>0.67</td>
<td>1:2.2</td>
<td>10 min</td>
<td>9 min</td>
</tr>
<tr>
<td>140</td>
<td>0.27</td>
<td>1.92</td>
<td>1:7.1</td>
<td>7 min</td>
<td>5 min</td>
</tr>
<tr>
<td>142</td>
<td>0.18</td>
<td>0.72</td>
<td>1:4</td>
<td>15 min</td>
<td>4 min</td>
</tr>
</tbody>
</table>

\textbf{Table 9: Experimental parameters from \textit{in situ} thermolysis with distillation}
Thermolysis with distillation has consistently lead to the best molar ratios of vinyl glycerine to unknown side product (on average 1:0.04 as determined by ratio of vinyl integrals to the triplet at 3.86ppm). Fractions can be collected during the course of distillation to obtain a purer crude product with the head temperature range of 175-246°C containing all of the vinyl product. However, the head temperature range of 175-200°C does contain vinyl glycerine it only averages ~1% by mass and ~1% by mass side product with the remainder of the mass being acetamide. Interestingly, a large excess of BSA did not lead to an increased yield of vinyl glycerine and corresponded to an increased amount of acetamide. The current hypothesis is that not only does amount of BSA correspond to the best yield but the ratio of volumes of BSA and diphenyl ether. This is presumably due to the time at which the reaction volume reaches the boiling temperature of diphenyl ether.

Table 10: Experimental results from in situ thermolysis with distillation

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amount Distilled</th>
<th>L-vinylglycine</th>
<th>Acetamide</th>
<th>Side Product</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>136</td>
<td>2/3 rxn V</td>
<td>47</td>
<td>50</td>
<td>3</td>
<td>48</td>
</tr>
<tr>
<td>138</td>
<td>4/5 rxn V</td>
<td>45</td>
<td>52</td>
<td>3</td>
<td>51</td>
</tr>
<tr>
<td>130</td>
<td>To 246°C head temp</td>
<td>16</td>
<td>80</td>
<td>4</td>
<td>22</td>
</tr>
<tr>
<td>140</td>
<td>To 246°C head temp</td>
<td>22</td>
<td>73</td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td>142</td>
<td>175-253°C head temp</td>
<td>34</td>
<td>62</td>
<td>4</td>
<td>44</td>
</tr>
</tbody>
</table>
Figure 113: Synthetic scheme for L-vinylglycine formation by in situ silylation followed by thermolysis with distillation

Though promising the in situ silylation and distillation method has the drawback of scale. A volume ratio of diphenyl ether to N,O-(bis-trimethylsilyl)acetamide (BSA) of 6:1 was determined to be optimal and therefore the reaction volume increases rapidly with scale. To circumvent this limitation the silylated methionine sulfoxide was added to distilling diphenyl ether. This method proved to be successful in maintaining decent yield along with bringing the silylated mixture to boiling Ph₂O temperature quickly, something not obtainable with lower than 6:1 Ph₂O:BSA. The in situ thermolyses where the ratio of Ph₂O to BSA was less than 6:1(v/v) a greater proportion of side product were observed in the isolated material.

Heating caused direct distillation of silylated vinylglycine from the reaction mixture. The addition of the silylated L-methionine sulfoxide mixture via Hirschberg constant addition funnel allowed for the production of vinyl glycine with low amounts of side product (estimated 1%-4% by mass across scale up) while going down to a ratio of Ph₂O:BSA of 2.3:1 (v/v). This protocol was used for reaction scales of 1.4g, 10.6g, 20.1g, 36.0g, and 50.4g with an average yield of vinylglycine of 38%. Of these, the 1.4g scale reaction proved to be anomalous in the fact that no side product was observed in the isolated crude by NMR and would obviously be the ideal method of preparation. The workup procedure was identical across the reactions with regards to hydrolysis and precipitation protocol. In this case, the silylation vessel was flame dried, N₂.
flushed, silylated mixture diluted by about half with Ph₂O prior to addition, the pot temperature minimum was 254°C and 40% of the combined volumes used was collected as distillate. This result may be anomalous, as the only factors not carried on throughout the scale were flame dried glassware which was N₂ flushed and only distilling 40% of the combined volumes.

The other concern with scale up, especially with the 50g reaction, is the length of time needed to do the reaction. In this case the addition occurred over 54min at which time almost all Ph₂O was distilled with 92% of the combined volumes being collected as the distillate. Although dilution with Ph₂O does not seem to be needed with smaller scales (1-10g), it is recommended to dilute it with Ph₂O to ensure the distillation pot does not go dry. This also opens up the opportunity to use smaller glassware or work with larger scale as the Ph₂O being distilled is being replaced. Overall, the addition is robust enough with increasing scale while still use standard glassware. The problem of consistently not producing the side product during synthesis remains as well as a way to separate it from the desired product.

The initial isolation of 71 by precipitation of the aqueous phase with CH₃CN was still used, but done twice with scales greater than 20g. At the 20g scale, the crude product was determined to be 28% by mass acetamide which produced a sticky semisolid, whereas if the first precipitate was redissolved in water and precipitated again with acetonitrile, the average mass contribution was 3%. This protocol, although convenient and used for expediency, probably used more water than required to dissolve and thus increased the required amount of acetonitrile to precipitate. If time is not a factor, it is recommended to precipitate once and dry under vacuum overnight followed by tritiation of the crude product in boiling ethanol to limit the use of acetonitrile.
Since this method of vinylglycine synthesis appears to be the most succinct when compared to the literature and employs H₂O while still using standard synthetic glassware, exploration of the silylation conditions were carried out to make the overall procedure more amiable to industrial scale up. First, the typical heat treatment was monitored over time with ¹H NMR. The spectra showed from room temperature to 81°C over the course of 100min the α-hydrogen shifted upfield by 0.3ppm and the CH₃ signal transform from a doublet to a singlet. From 125min to 18hr of heating the spectrum did not change from the 100min spectrum and was determined that silylation was complete at 100min. Second, temperatures above 80°C were investigated. Heating at reflux for 2hr and 100°C produced identical spectra which were different from the 80°C and afforded 12% and 6% yield of vinylglycine with an estimated 5:2 and 2:1 molar ratio of vinylglycine to sideproduct. Lastly, cooler temperatures were employed. L-Methionine sulfoxide was silylated at 80°C until the mixture was homogeneous followed by room temperature stirring overnight or stirring at room temperature overnight. These two methods produced NMR spectrum like that of the 80°C overnight silylated product.

However, as slow heating caused starting material decomposition to form side products, and prolonged heating also destroyed the product, we settled upon dropwise addition to distilling diphenyl ether (258°C), which caused rapid heating, elimination, and carryover of product. Hydrolysis, partitioning between water and hexane, and addition of acetonitrile to the aqueous layer caused precipitation of free vinylglycine in 95% purity. This protocol was used for reaction scales of 1-50g, with a 38% average yield of purified vinylglycine with reaction times ranging from 11-54min. No epimerization was apparent as determined by
HPLC separation of the dipeptide diastereomers prepared by reaction of vinylglycine with Fmoc-L-Ala-OSu and Fmoc-D-Ala-OSu.

Using these much less careful conditions, we have found effective elimination to vinylglycine without isomerization or epimerization. When N-Cbz vinylglycine methyl ester is used in thermolysis there had been substantial difficulties which are avoided by very short contact times with high temperatures. We wondered which features in our case allowed the simpler reaction conditions. The free acid is, as expected, quite resistant to isomerization compared to the ester: in contrast to the Boc protection of the free acid, bicarbonate in methanol causes complete isomerization during Boc protection of vinylglycine methyl ester in under 4h. In contrast, dissolution of vinylglycine methyl ester in BSA and triethylamine led to no detectable isomerization, even after 50 h. In order to separate effects of ester, and N-substituent on isomerization rates, we carried out the following studies.\textsuperscript{179}
Comparison of N-Boc with N-TMS groups was made by dissolution of Boc-vinylglycine or free vinylglycine 1 in BSA, followed by addition of Et$_3$N. Boc vinylglycine TMS ester isomerized with a half time of ca. 1.6h, while the N-TMS derivative had an isomerization half-time of ca. $10^3$h, demonstrating that the N protecting group is a substantial part of the difference. We compared TMS with H: vinylglycine methyl ester hydrochloride, on exposure to Et$_3$N, isomerized with a half time of ca 5.4h while vinylglycine exposed to TMSCl and Et$_3$N isomerized considerably more slowly, with a half time of ca. $1.7 \times 10^2$h. While N-Boc facilitates isomerization, it appears the N-TMS group slows it down compared to the N-H material, though the ester also changed. In comparing methyl ester with TMS ester, each in BSA solution, we found that in comparison to the N-TMS TMS carboxy ester half time of ca. $10^3$h, the N-TMS methyl ester showed no detectable isomerization in that sample after 50h exposure to Et$_3$N, corresponding to a half time $>2 \times 10^3$h. Consequently, the faster isomerization of NH, methyl ester vs NTMS, TMS ester substrate is not due to the ester.

While the low isomerization tendency of silylated vinylglycine may not be critical to the success of the preparation, it is gratifying that this medium is less conducive to isomerization.
<table>
<thead>
<tr>
<th>Substrate</th>
<th>Conditions</th>
<th>Solvent</th>
<th>[vinyl] (M)</th>
<th>[S. agent] (M)</th>
<th>[Net₃] (M)</th>
<th>Time (hr)</th>
<th>Mol Ratio (vinyl:enamine)</th>
<th>t ½ (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CO₂TMS</strong> NHTMS</td>
<td>BSA/NEt₃</td>
<td>CDCl₃</td>
<td>0.092</td>
<td>0.31</td>
<td>1.05</td>
<td>64.5</td>
<td>1 : 0.045</td>
<td>1.0x10³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>92.5</td>
<td>1 : 0.06</td>
<td>1.1x10³</td>
</tr>
<tr>
<td></td>
<td>TMSCl/NEt₃</td>
<td>CDCl₃</td>
<td>0.141</td>
<td>0.91</td>
<td>0.99</td>
<td>18</td>
<td>1 : 0.09</td>
<td>1.4x10³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>108</td>
<td>1 : 0.73</td>
<td>1.4x10³</td>
</tr>
<tr>
<td><strong>CO₂Me</strong> NHTMS</td>
<td>BSA/NEt₃</td>
<td>CDCl₃</td>
<td>0.004</td>
<td>0.25</td>
<td>1.50</td>
<td>50</td>
<td>1 : 0.02</td>
<td>&gt;2.3x10³</td>
</tr>
<tr>
<td></td>
<td>TMSCl/NEt₃</td>
<td>CDCl₃</td>
<td>0.136</td>
<td>0.88</td>
<td>0.96</td>
<td>69</td>
<td>1 : 0.33</td>
<td>1.7x10³</td>
</tr>
<tr>
<td><strong>CO₂Me</strong> NH₃Cl</td>
<td>NEt₃</td>
<td>CDCl₃</td>
<td>0.094</td>
<td>0</td>
<td>0.99</td>
<td>19</td>
<td>1 : 6.7</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42.5</td>
<td>&lt;1 : 28</td>
<td>&lt;8.7</td>
</tr>
<tr>
<td><strong>CO₂TMS</strong> NHBoc</td>
<td>BSA/NEt₃</td>
<td>CDCl₃</td>
<td>0.03</td>
<td>0.95</td>
<td>0.90</td>
<td>1</td>
<td>1 : 2</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>&lt;1 : 22</td>
<td>&lt;1.2</td>
</tr>
<tr>
<td></td>
<td>TMSCl/NEt₃</td>
<td>CDCl₃</td>
<td>0.075</td>
<td>0.91</td>
<td>0.99</td>
<td>1.5</td>
<td>1 : 7</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18.5</td>
<td>&lt;1 : 33</td>
<td>&lt;3.6</td>
</tr>
</tbody>
</table>

Table 11: Pseudo-first order kinetic data for the isomerization of L-vinylglycine derivatives. All reactions conducted at 22°C and in CDCl₃.

Boc group installation attempted on the C-Me L-Vgy HCl with Boc₂O in CH₃OH in the presence of NaHCO₃ to yield a mixture of the enamine isomers exclusively after 4h as observed by TLC and confirmed by ¹H NMR. Properties of derivatives of vinylglycine appropriate to peptide coupling have not been described in the literature, despite the report that incorporation from the Fmoc derivative was superior to incorporation of a phenylselenide followed by elimination: an uncharacterized Fmoc derivative was used.¹⁸⁰ Fmoc vinylglycine (73) prepared and characterized. The Boc derivative 74 is an oil at room temperature, so it was converted into its crystalline dicyclohexylammonium salt 75.
Olefin methathesis (OM) can convert vinylglycine into other unsaturated amino acids, though steric hindrance prevents its homodimerization. Olefin metathesis in the presence of a more reactive alkene can lead to crossmetathesis.\textsuperscript{181} Most examples of efficient vinylglycine and vinyl amine olefin metathesis involve intramolecular reaction.\textsuperscript{182,183} Intermolecular reactions of vinyl glycine derivatives\textsuperscript{100,103,184,185} and related substances\textsuperscript{186} are reported, but some attempts have led to other products.\textsuperscript{187} As we hoped to prepare unsaturated substances related to arginine for mechanistic studies\textsuperscript{188} we screened several allyl derivatives for OM cross coupling to vinylglycine.

While simple terminal alkenes react under olefin metathesis conditions to cross couple with less reactive alkenes such as vinylglycine, the pairing of less reactive alkenes is problematic. Early attempts to cross couple protected allyamine with vinylglycine failed, even in an intramolecular case.\textsuperscript{118}
2. Olefin Cross Metathesis of L-Vinylglycine Derivatives

a. Background on Olefin Metathesis

The net transformation of olefin metathesis is to cleave the strongest (double) bond, and reconnect the fragments. It proceeds by reversible cycloaddition of metalloccarbenes to olefins forming a metalloccyclobutane. It has become a powerful and versatile tool in the pursuit of sp² C-C bond formation. These metal catalysts are either molybdenum or ruthenium based, with each containing a metal carbene bonded to their metal center which serves as the driving force for olefin metathesis.

![Figure 117: Examples of intramolecular olefin metathesis with amino acid derivatives](image)

Some of the earlier examples of metathesis on amino acid or peptide scaffolds were attempts to perform ring closing metathesis on side chains containing allyl functionalites to make cyclic peptides\(^\text{189}\) which remain a topic of interest as cyclic peptides provide a physiologically novel structures.\(^\text{190}\) However when one of the allyl groups was for a vinyl group, intramolecular ring closure was prevented.\(^\text{191}\)
Once the lactone was saponified, ring closing metathesis occurred in 95% yield. Whether this was due to steric hinderence or the pre-Grubbs 1st generation catalyst not being active enough was unknown at the time. Another attempt at ring closing metathesis was an N-allyl derivative of N-Boc-vinylglycine methyl ester. While this did not have the previous constraint of being cyclic, instead of cyclizing with 5mol% catalyst at 25°C, the vinyl side chain isomerized to the α,β-unsaturated side chain and cross metathesized with itself to form a homodimer.

The Schrock metathesis catalyst which can have difficulties performing cross metathesis if the substrates are not reactive enough was employed a year later to perform cross metathesis on N-Cbz-vinylglycine methyl ester without isomerization to the α,β-unsaturated sidechain. The cross metathesis product between the vinyl moiety and allyl trimethylsilane
was accomplished by use of a more reactive alkene, but it is limited in application due to the Schrock catalyst’s need anhydrous and anerobic reaction conditions.

![Schrock's, Grubb's 1st gen, and Grubb's 2nd gen metathesis catalysts](image)

**Figure 120:** From left to right Schrock's, Grubb's 1st gen, and Grubb's 2nd gen metathesis catalysts

The Grubbs 1st generation catalyst, made by substitution to a styrenyl carbene, was used to perform cross metathesis on unsaturated α-amino acids to systematically test the parameters of C-ester steric hindrance as well as proximity of the olefin to the amino acid backbone with regard to the ability to perform cross metathesis. N-Boc homoallylglycine was used as a model compound to evaluate ester steric when metathesized with styrene or hexene. The esters were methyl, benzyl, t-butyl, and one instance of the free acid ranged from 52-66% yield showing no appreciable effect from increasing steric bulk at this carbon chain length. The contributing factor that affected the propensity towards cross metathesis was the proximity of the double bond to the amino acid's chiral center: the yield of the desired product decreased as it approached the α-amino carbon. When cross metathesis was conducted using hexene with homoallylglycine, allylglycine, and vinylglycine yields dropped as bulk increased from 66% to 45% to 7% respectively.

![Yield of cross metathesis product](image)

**Figure 121:** Afforded yields of cross metathesis product as steric hindrance around olefin increases
The sensitivity to sterics for the 1st generation Grubbs catalyst was quantified by the rate of metathesis with increasing encumberance around the α-carbon to the olefin.\textsuperscript{196}

![Figure 122: Effects of increasing steric hindrance on rate of metathesis](image)

If hydrogens were substituted for methyl groups β- to the olefins (or presumably even further away) the rate decreased slightly from 1.48 to 1.02x10\(^{-3}\) L/mol·s. When this same change in substituents took place α- to the alkene, resulting in a 3° carbon, the rate dropped by an order of magnitude to 2.5x10\(^{-4}\) L/mol·s had only trace amounts of metathesis product observed after 4 days if 4° carbon was in proximity. The electronic characteristics were also correlated to a rate which increased by an overall factor of four when changed from p-NO\(_2\) styrene to p-Me styrene to p-OMe styrene, suggesting electron rich species react faster.

Over the course of these studies it was observed that the E isomers were isolated or formed preferentially over the Z isomer. A paper on a new methodology for cross metathesis with Grubbs 1st generation catalyst for greater E selectivity demonstrated homodimerization of terminal olefins before cross metathesis occurred but at the cost of an overall lower yield of the cross metathesis product.\textsuperscript{197} The 2nd generation Grubbs catalyst also shares this tendency\textsuperscript{198} towards formation of the E isomer. Compatibility with aliphatic alcohol, ester, or ether containing functionalities was no problem affording yields on average of 70% and E:Z ratios ranging from 3-10:1. The only detriment to homodimerization in these cases was reaction temperature. If the temperature were to exceed that of the boiling point of dichloromethane
the yield would decrease, which was attributed to the decomposition of the metathesis catalyst.

The tolerance of alkenes with nitrogen substituents towards metathesis were also studied. Though not as widely explored, the examples of Boc protected allyl amine and amides in peptides did not interfere with cross coupling. What did prevent facile metathesis were amides alpha to the terminal olefin. When metathesis was attempted on N-methoxy-N-methylacrylamide, a Weinreb amide, it gave a low yield of 17% and the low E:Z ratio of 1.9:1. This lack of reactivity was speculated to be due to intramolecular coordination of the amide to the ruthenium center when it became attached as a carbene to stabilize it as an intermediate and interrupt the catalytic cycle.

In the year 2000 Hoveyda advanced metathesis technology with his modification to the Grubbs catalyst by replacing the initial styrenyl carbene with an intramolecular coordinating isoproxystyrene\textsuperscript{199} increasing the reactivity of the catalyst, as well as stabilizing it, allowing for a more robust set of experimental conditions in which metathesis may be conducted. This ruthenium carbene complex is known as the Hoveyda-Grubbs 2\textsuperscript{nd} generation catalyst. Substrates previously unresponsive to cross metathesis with previous catalysts were now able to be reevaluated for these purposes. N-Boc-2-vinyl-pyrrolidine was able to do metathesis with methyl 3-butenoate\textsuperscript{200} in 31% yield in a 10:1 E:Z ratio under conditions where the 1\textsuperscript{st} generation Grubbs catalyst could not perform and N-benzyl-N-Boc-ethenamine with methyl 3-butenoate in 60% yield. The 1\textsuperscript{st} generation Hoveyda-Grubbs catalyst, in which the tricyclohexylphosphine ligand was replaced with an N,N'-\textit{bis}-mesitylimidazole carbene, is also able to effectuate
vinylglycine metathesis due to the enhanced reactivity of the metal afforded by the additional carbene moiety.

![Hoveyda-Grubbs's 2nd generation metathesis catalyst](image)

**Figure 123: Hoveyda-Grubbs's 2nd generation metathesis catalyst**

In the synthesis of D-glycosyl-asparagines, α- or β- allyl glycosides were coupled with vinylglycine derivatives containing different combinations of N-protecting groups and carboxy esters. What was found from the Orlando group was that metathesis of α- or β- positions displayed slight preference towards the α- position due to the lesser degree of steric interaction. Neither N-Cbz, N-Boc, N-Fmoc nor C-Me, C-Bn- or C-tBu esters altered the yields of the desired glycoside metathesis product substantially. However, when the alkene on the glycoside was change to vinyl from allyl no cross metathesis was observed. Isomerization of the vinyl group in the vinylglycine derivative to the α,β-unsaturated alkene was observed when exposed to refluxing CCl₄ (76°C) and 1,2-dichloroethane (84°C).

Cross metathesis of protected vinylglycines was also used to make other biologically important molecules like diaminopimelic acid, a component in peptidoglycan biosynthesis in gram-negative bacteria as well as in the synthetic route to make the natural products Syringolin A and B. Syringolin A and B have been identified as a proteasome inhibitor which act as Michael receptors for conjugate addition to N-terminal threonine residues, acting as virulence factors for plant pathogens as well as a potential therapeutic for human neuroblastoma.
Early in the sequence 4-bromobutene was coupled with a vinylglycine analogue in 69% yield using 10mol% catalyst over two additions and five hours of reaction time.

As these catalysts are non-polar and conducting olefin metathesis in physiological media has become of interest, water soluble varieties of the Hoveyda-Grubbs catalyst have been synthesized and determining their limitations in metathesis has been undertaken. One of the first attempts involved attaching a polyethylene glycol chain to the imidazole ligand in the Hoveyda-Grubbs 2\textsuperscript{nd} generation catalyst which resulted in an average molar mass of 5000g/mol.\textsuperscript{204} This was able to perform ring closing metathesis on a range of substrates and cross metathesis on allyl alcohol but failed to produce homodimers of acrylic acid or allyl ammonium chloride. Altering the pH of the aqueous media did not perturb the reactivity of the catalyst to either induce metathesis on the unreactive substrates or prohibit the metathesis of the successful species.

The other water solubilizing changes made to the Hoveyda-Grubbs 2\textsuperscript{nd} gen. catalyst were quaternary ammoniums on the iospropoxy styrene ligand\textsuperscript{205} or use of a solvent mixture consisting of water and an organic cosolvent\textsuperscript{206} in which the main drawback was the low maximum concentration of the Hoveyda-Grubbs 2\textsuperscript{nd} gen catalyst. Ring closing was able to be performed on secondary ammonium chlorides to form five and six member rings and tertiary amines to close to a five member ring in good yield if the amine was a trifluoroamide or tosylamide. The cases were ring closure did not occur were if quaternary ammoniums were present or if the nitrogen was replaced with a diphenyl silane suggesting that the steric bulk around these moieties hindered metathesis.
b. Synthesis and Screening of Allylamine Derivatives for Cross Metathesis

Our metathesis experimentation began with evaluating the ability of desired alkenes to homodimerize in the presence of Hoveyda-Grubbs 2nd generation catalyst to screen for substrates suitable to perform cross-metathesis on an analogue of vinylglycine. Allyl amine was tried first and when combined with 11mol% of the catalyst in CH$_2$Cl$_2$. Upon addition of the amine the color of the solution changed from an emerald green to a brown color and after refluxing for 17hr no metathesis was observed. This was attributed to the coordination of the amine to the ruthenium, inactivating the metal towards metathesis. This observation prompted the creation of a series of derivatives.

<table>
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<th>Reaction</th>
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</thead>
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<tr>
<td><strong>NH$_2$</strong></td>
<td>17M AcOH</td>
<td>94%</td>
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<tr>
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<td>75%</td>
</tr>
<tr>
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</tr>
<tr>
<td><strong>NH$_2$</strong></td>
<td>CF$_3$C(O)OEt</td>
<td>76%</td>
</tr>
<tr>
<td><strong>NH$_2$</strong></td>
<td>NaN$_3$, NaHCO$_3$</td>
<td>68%</td>
</tr>
</tbody>
</table>

Table 12: Synthesis of allyl amine derivatives for homodimerization screening
The duo of protonated allyl amines, allyl ammonium chloride and allyl ammonium acetate, were made by combining with 12M HCl and glacial acetic acid respectively. After concentration with rotary evaporation, azeotropic drying by evaporation of toluene, and exposure to vacuum to remove residual solvent, 71% yield of 76 and 94% yield of 77 were obtained. Synthesis of acyl analogues proceeded next. Boc protection of allyl amine occurred quite rapidly using Boc anhydride and excess of allyl amine in methanol at room temperature. Mixing of the two produced a large exotherm and effervescence upon mixing and complete conversion after 20min as seen by TLC (silica, KMnO₄, 5% CH₃OH/CH₂Cl₂, Rf amine= 0.0, Rf Boc amine= 0.78). The white solid afforded after workup corresponded to 75% yield 78 and had a melting point range of 28-34˚C. 78 was also observed to be volatile which was discovered after leaving a previous preparation under vacuum overnight to remove residual solvent to find it had evaporated away. This implies that sublimation should be employed in future preparations to purify this substance.

Fmoc protection used the N-hydroxy succinimide ester of fluorenyl methyloxycarbonyl with an excess of allyl amine in CH₂Cl₂ to produce 79 in 95% yield with 121-122˚C melting point in one hour. 80 was created by treating allyl amine with two equivalents ethyl trifluoroacetate, a volatile trifluoroacetylationating agent that does not require base to be present. Mixing at room temperature in ethanol produced a large exotherm and complete reaction after 15min (silica gel, 40% EtOAc/hexane, KMnO₄ visualized, Rf product= 0.55). Isolation of the desired product became troublesome as its boiling point was lower than that of either ethanol or ethyl trifluoroacetate meaning that removal of the solvent or excess reagent came at the expense of the yield as the small scale prohibited convenient distillation. From this procedure, the
trifluoroacetate 80 was afforded in 76% yield. Allylamine is the lowest in boiling point of the combination so subsequent syntheses should employ it in excess coupled with its slow addition to ethyl trifluoroacetate in an ice bath using neat conditions.

The final derivative made was 81. NaHCO₃, as an extra precaution to prevent the formation of hydrazoic acid, was dissolved in water along with NaN₃ before the addition of allyl bromide as per the literature.²⁰⁷ A biphasic mixture resulted with the organic layer on the bottom. After 30min of stirring at room temperature, the organic phase switched positions with the aqueous phase and is an indication that the reaction is proceeding, but not a sign of complete reaction. After 4hr though TLC showed complete conversion to the allyl azide (silica, 10% EtOAc/hexane, KMnO₄, Rf amine= 0.57, Rf azide= 0.64). As with 80 and 78, isolation was problematic due to its volatility. Previous preparations utilized ethyl ether to partition the allyl azide away from the aqueous layer, but use of rotary evaporation or vacuum to remove the solvent also evaporated allyl azide resulting in negligible mass. It was determined then to separate 81 neat and pass it over a column of MgSO₄ in pasteur pipet to give the desired material in 68% yield.

_Homodimer Formation_

\[
\begin{array}{c}
\text{2 R} - \text{cis} + \text{2 R} - \text{cis} \rightarrow \text{R} - \text{cis} + \text{R} - \text{cis} + 2 \parallel
\end{array}
\]

Figure 125: General scheme for homodimer formation via olefin metathesis

Cross metathesis with the vinylglycine moiety is predicated upon the formation of a metathesis homodimer with the allylamine derivatives. This is due to the fact that the allyl
functionalities have a lesser amount of steric hinderence about the olefin compared to the vinyl and thus will undergo metathesis first. As a result, selectivity in cross metathesis can be achieved by having the allyl homodimer then react with the vinyl group. Cross metathesis of L-vinylglycine is desirable as it would provide the most direct route to dehydroarginine. Allylammonium salts and aqueous cosolvent conditions were used as described by the Raines publication\textsuperscript{206} to investigate metathesis. A solution of 76 in D\textsubscript{2}O as added to a solution of the Hoveyda-Grubbs 2\textsuperscript{nd} generation catalyst (5mol\%) in 1,2-dimethoxyethane (DME) which caused some of the catalyst to precipitate. The mixture was stirred at room temperature over the course of five days while being periodically monitored by \textsuperscript{1}H NMR with no change observed in the spectra over that time. The same results were seen with 77 in CH\textsubscript{2}Cl\textsubscript{2} and H\textsubscript{2}O/acetone solvent systems with 10mol\% catalyst at reflux and 55°C respectively over the course of 24hr. The mixtures were allowed to sit for an additional 12d at room temperature in the hopes of seeing metathesis proceed, but to no avail. Lack of metathesis was determined to be due to the proximity of the positive charge to the allyl functionality and not the chloride ion.
Allyl guanidinium chloride was investigated next with the thought that maybe a more delocalized cation would not be an impediment. The reaction appeared to proceed with 12mol% catalyst in 20% H$_2$O/acetone at reflux for 17hr at which time the $^1$H spectrum was taken and showed a lack of the starting alkene signals. The mixture was washed with CH$_2$Cl$_2$ and the aqueous phase concentrated to give a 68% crude yield. The crude product was analyzed by ESI-MS to give a peak at m/z=307.5 which would correspond to a vapor phase complex between the desired homodimer and allyl guanidinium chloride with a bridging chloride between them and further evidence that metathesis had occurred. However, an M+2 peak at 25% intensity indicative of the $^{37}$Cl isotope was absent. Comparison its NMR spectrum to that of the homodimer formed from 78 showed a similar set of signals. The resonances of this homodimer were broad singlets at $\delta$5.62, $\delta$4.57, and $\delta$3.72 and those of the allylguanidinium homodimer were broad singlets at $\delta$5.74, $\delta$5.30, $\delta$4.20, $\delta$4.12 but these signals were only a
fraction of the signals present or their intensities. From these data, metathesis of this substrate appears to occur but is sufficiently ambiguous to prevent a definitive conclusion.

As the cationic allyl amines proved stagnant to homodimerization, 55 was tried next with 11mol% metathesis catalyst in CDCl₃ at room temperature. This mixture was monitored by ¹H NMR over 22.5hr with no appreciable change in the spectral signatures of the catalyst or the starting olefin. Although initially perplexing, recalling the coordination of the imine lone pair to the osmium tetroxide to attempt oxidative cleavage of the alkene and how it prevented reaction could explain this lack of metathesis reactivity. If the lone pair on the guanidine could be occupied by being protonated it could facilitate olefin metathesis much like the case of oxidative cleavage to proceed to form 59. The experiment was replicated with 13mol% catalyst and 10 eq glacial acetic acid and heated at 40°C in CD₂Cl₂. After heating overnight this mixture only starting materials were observed to be present. The reaction volume was vented with a slow stream of N₂ to the atmosphere and set back to reflux for another 24hr. ¹H NMR analysis showed no ethylene present or starting materials and TLC did show new spots which were isolated with flash chromatography. These spots were then submitted for ESI-MS analysis but did not give any peaks corresponding to the molecular ion of the desired homodimer product. The conclusion from this experiment that as the neutral compound the imine lone pair prevented metathesis by coordination to the ruthenium and trying to circumvent this by protonation also prohibited homodimerization for the same reason as the allyl ammonium compounds, the proximity of the formal positive charge.
Being cationic and sterically bulky has been demonstrated to be detrimental to olefin metathesis allyl azide was exposed to metathesis conditions next. The metathesis catalyst was added at reflux in CDCl$_3$ with allyl azide in two portions up to a total of 13mol% over the course of two days which resulted in a 1:3 molar ratio of the homodimer to the monomer. The solution changed from a green to a dark brown color upon reaching reflux similar to the attempted metathesis of allyl amine which was assumed to correlate to catalyst decomposition. However when conducted at room temperature with 20mol% catalyst added in two portions over two days a 1:6 molar ratio of homodimer to monomer was produced. Allyl azide also seems to be a poor substrate for olefin metathesis under these conditions.

![Figure 126: Homodimer formation via olefin metathesis of Boc protected allyl amine](image)

Thankfully not all of the allyl amine derivatives were unreactive. The acyl derivatives were able to homodimerize but with reactivity dependent on solvent observed. In the case of Boc protected 78 use of 30% H$_2$O/DME resulted in no metathesis while reflux in CH$_2$Cl$_2$ afforded complete metathesis in 16hr in a 75% yield in a 1:1.6 ratio of Z:E isomers 82 and 83. Fmoc derivative 79 underwent complete conversion in refluxing dichloromethane with 10mol% catalyst in 1.5hr. 58% yield of this homodimer 84 was afforded but mass was only acquired from the crystalline material from the reaction mixture, the E isomer. A more quantitative yield can be generated by utilizing flash chromatography on the crude product but was deemed unnecessary, as looking for metathesis activity was the primary goal.
N-allyl-trifluoroacetamide also underwent complete homodimer formation overnight with 12mol% catalyst in refluxing CH$_2$Cl$_2$ even though the color of the mixture changed to a dark brown upon reaching reflux temperature. A 43% yield of homodimer 85 was analyzed by ESI-MS (-ion mode) and produced ions corresponding to M-H$^+$, M+HCOO$^-$, and M+CF$_3$COO$^-$ confirming that metathesis occurred. As expected the less hindered alkene 4-pentenoic acid, underwent olefin metathesis smoothly to form homodimer 86 in 88% isolated yield.

c. Cross Metathesis of L-Vinylglycine Derivatives and Selected Allylamine Derivatives

Even with the lack of precedence of cross metathesis between two ammonium bearing olefins, the metathesis of vinylglycine hydrochloride with allyl guanidinium chloride was attempted to make the dehydroarginine directly. Using conditions described by Raines$^{206}$, no change was observed in the composition of the mixture by $^1$H NMR over the course of 89hr. The next attempt at cross metathesis was done with allyl ammonium acetate (77) and N-Boc-L-vinylglycine (74) in 28% D$_2$O/DME with 6mol% catalyst. Even though it seems unlikely as the allyl ammonium acetate did not form a homodimer, it may be possible with a cationic and neutral olefin as opposed to bringing two cationic species together to metathesize into a dicationic species. After heating at 55°C for 24hr the $^1$H spectrum remain unchanged. The mixture was brought to room temperature and monitored sporatically over the course of 13d with still no change seen in the resonances.

![Figure 127: Example olefin cross metathesis using Hoveyda-Grubbs 2nd generation catalyst](image-url)
Effort was then shifted from the charged olefins to neutral, organic soluble derivatives with the first combination being 78 and 74. Utilizing 11mol% catalyst and refluxing CH2Cl2 only partial reaction was observed over 18hr. The mixture was none the less concentrated, redissolved in EtOAc, and extacted with 29% NH3(aq) to give a crude product that was partially separated by flash chromatography. The fraction containing the largest mol fraction of the cross metathesis product 87 was subjected to ESI-MS (- ion) analysis and produced peaks of m/z= 329 (M-H+) and 530 (M+ 74 vapor complex). Accounting for the unreacted 74 a 17% yield based on conversion was isolated. This low yield could be attributed to the low partitioning of the desired product into aqueous ammonia as well as the incomplete reaction. Recommendations in improving the yield of the cross metathesis product can be done by increasing the ratio of 4:1 78: 74, increasing the amount of catalyst to 20mol% in two portions, and chromatography of the reaction mixture should be done in future preparations.

The Fmoc derivatives of vinylglycine coupled to the Boc allyl amine (78) is desirable as cross metathesis would generate a product containing orthogonal protecting groups. This will allow for installation of the guanidine functionality unambiguously on the resulting side chain amine. The dicyclohexylammonium salt of Fmoc-L-vinylglycine was prepared and mixed with 6eq Boc allyl amine with 20mol% catalyst in refluxing CH2Cl2 for 24hr with no change as seem by TLC. An additional 20mol% calatyst was added and left at reflux for 4d before analysis of the mixture by TLC was done again and still looked like the starting materials. The proton spectrum confirmed that it was still reactants along with the NBoc allyl amine homodimers 82 and 83. The steric hindrance around the vinylglycine olefin was too bulky to effectuate metathesis.
73 was employed next with Boc allyl amine under similar metathesis conditions but with only 20mol% catalyst. After 4d at reflux no new spots by TLC or a molecular ion from ESI-MS analysis of the reaction mixture was observed.

This continued reticence Fmoc vinylglycine towards metathesis prompted the swapping of the protecting groups on the coupling partners to Fmoc allyl amine and N-Boc-L-vinylglycine (74). A minimum of two equivalents Fmoc allyl amine (79) and 25mol% metathesis catalyst were left in refluxing CH₂Cl₂ for 4d before TLC analysis which showed the absence of 74. The reaction, though complete, has yet to have the cross metathesis product 88 isolated. Extraction with ammonia did not partition the product into the aqueous phase, using silica gel flash chromatography with 10% AcOH/CH₂Cl₂, or ion exchange chromatography with Dowex 2X-8 (quaternary ammonium, strong anion exchanger) failed to generate materials that produced the desired molecular ion with ESI-MS. Use of chromatography before any separation should be used subsequently in attempts to isolate the product and should not be abandoned as a possibility.

Metathesis between N-allyl trifluoroacetamide (80) and 74 was able to be done with 6eq of 80 and 18mol% catalyst added in two portions in refluxing CH₂Cl₂ over two days. The product was attempted to be isolated by extraction with pH9 water but was not able to partition it into the water and confirmed by lack of molecular ion when analyzed by ESI-MS. This product should be isolated by chromatography.

Success was also found in the coupling of 4-pentenoic acid and 74 with 6eq of the acid and 15mol% metathesis catalyst added in two portions over two days in refluxing CH₂Cl₂ to
generate cross metathesis product. Isolation of the cross metathesis product was accomplished by trifluoroacetic acid removal of the Boc group, dissolving the resulting salt in water, followed by washing with EtOAc to afford 89 in 58% yield.

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<th>Entry</th>
<th>Entry</th>
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<th>mol% catalyst</th>
<th>Temp</th>
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</tbody>
</table>

Table 14: Summary of results for combinations of metathesis substrates

VII. CONCLUSION

Cinnamylidene pyruvate and (4-nitro)cinnamylidene pyruvate salts were synthesized in a facile manner that allowed subsequent researchers to generate more material and additional pyruvate adducts to further probe the reactivity of SbADC. Triethylammonium bicarbonate extraction of the cinnamylidene pyruvates allowed of the removal of excess sodium pyruvate for kinetic stop flow experiments but at the cost of a large amount of the desired pyruvate.
adducts with most of the desired diene reverting back to the Aldol condensation product by hydration of the diene.

The method for sodium difluoropyruvate synthesis was improved on from the described literature methods by detailing ozonolysis conditions that does not generate a mixture of furan starting material and desired difluoropyruvate ester as well as employing an anion exchange resin to simply the removal of acidic byproducts. Unfortunately the limited amount of material that could be made under the time constraints did not yield solutions of a sufficient concentration to occupy the active site of SbADC to afford unambiguous x-ray crystallography data.

A synthetic route that affords L-vinylglycine in two steps that can be conducted on a 50g scale utilizing conventional laboratory glassware in an average 39% yield was also developed. This method proved to be a dramatic simplification from the literature in the amount of material that could be generated with regard to time invested. Utilizing relatively inexpensive reagents, easily obtainable temperature, and standard laboratory techniques researchers in need of this material are stronger encouraged to use this methodology.

The L-vinylglycine made was derivatized with Boc and Fmoc protecting and made into a crystalline dicyclohexylammonium salt so that it could be used in subsequent screening for olefin metathesis reaction conditions en route to forming β,γ-dehydroarginine. It was found that Boc-L-vinylglycine was able to undergo cross metathesis with Boc protected allyl amine, Fmoc protected allyl amine, trifluoroacetyl allylamine, and 4-pentenoic acid opening up the potential to for other non-canonical dehydro amino acids.
Future researchers who pick up this almost completed project are suggested to follow the route shown above as it is known that cross metathesis between Fmoc allyl amine and Boc-L-vinylglycine is able to go to completion with the presence of the desired product confirmed by ESI-MS. This cross metathesis product is the most desirable as its protecting groups are orthogonal allowing for the Fmoc group to be removed while retaining the Boc group allowing for unambiguous guanidinylation. However, the desired material is yet to be isolated from the reaction mixture with silica gel and reverse phase flash chromatography proving to be ineffective in this task. It is suggested to use preparative HPLC for initial isolation of the desired cross metathesis product followed by development of a method to separate it from the Fmoc allylamine homodimer. The remaining steps in the sequence are well known and described in detail for analogous compounds in the experimental.
VIII. EXPERIMENTAL SECTION

General Procedures:

Reagents and solvents purchased from suppliers were used as received unless otherwise noted in experimental procedures. $^1$H NMR spectra were collected on a 300MHz Brucker DPX 300 NMR unless otherwise noted. Chemical shifts (δ) are reported as downfield in parts per million of tetramethylsilane. Coupling constants (J) are reported in Hertz and splitting patterns denoted as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), multiplet (m), and broad (b). $^{13}$C NMR spectra were $^1$H decoupled and collected on a 300 MHz Brucker DPX 300 NMR with chemical shifts (δ) reported as downfield of tetramethylsilane. The $^{31}$P NMR spectra were $^1$H decoupled, and collected on a 300MHz Brucker DPX 300 spectrometer with chemical shifts (δ) reported downfield of an 85% H$_3$PO$_4$ external standard. Low resolution mass spectrometry data was collected on a Hewlett-Packard 5985 B GC-mass spectrometer and a Shimadzu LCMS-2020 Single Quadrupole. High resolution mass spectrometry data was acquired with a Shimadzu LCMS-IT-TOF. Infrared spectroscopy was performed on a Shimadzu IRAffinity-1S FTIR. Elemental analysis was conducted on a Perkin-Elmer 240C carbon, hydrogen, and nitrogen analyzer. Melting points were determined using a MEL-TEMP II apparatus without correction. HPLC analysis was performed on Agilent Technologies 1220 Infinity LC. UV-Vis absorption data was collected on a Perkin Elmer Lambda 650 using quartz absorbance cells. Fluorescent measurements were collected using right angle scattering on a Fluorolog-3 Model FL3-22 by Horiba Jobin-Yvon using quartz fluorescent cells or an Ocean Optics SD2000 dual channel fiber optic spectrometer. Analytical TLC was conducted on Merck silica gel 60 F$_{254}$
plates and visualized using shortwave ultraviolet light unless noted otherwise. Flash chromatography employed Sorbent Technologies silica gel 60Å, 32-63µm Standard Grade.

**Dimethyl 2,5-diaminocyclohexa-1,4-diene-1,4-dicarboxylate (1)**

70.82g (0.9969mol) NH₄OAc was combined with 2.0L anhyd. EtOH, placed under an atmosphere of N₂, and heated to reflux. 54.93g (0.2407mol) dimethyl 2,5-dioxocyclohexane-1,4-dicarboxylate was added to the boiling solution in three equal portions, waiting for the previous portion to dissolve before the next addition with 400mL anhyd. EtOH added with the last portion. The mixture continued to heat at reflux for 2.5h before TLC showed the reaction to be complete (10% CH₃CN/CH₂Cl₂, silica, R<sub>fSM</sub> = 0.76, R<sub>fBE</sub> = 0.38) over which time orange crystals formed on the side of the reaction flask. The mixture was allowed to cool to room temperature slowly before being placed into an ice bath for 1h. The resulting orange solid was collected by vacuum filtration and washed with 500mL cold anhyd. EtOH. After drying under vacuum to remove residual solvent overnight 47.72g (88% yield, mp = 212-214°C) of a cream orange colored solid.

<sup>1</sup>H NMR (300MHz, CDCl₃): δ3.71 (s, 6H); 3.13 (s, 4H)

<sup>13</sup>C NMR (300MHz, CDCl₃): δ169.3; 155.6; 86.8; 50.8; 30.4

ESI-MS (DUIS, + ion): 268 (M + CH₃CNH⁺, 100%); 227 (M + H⁺, 98%)

160
*Dimethyl 2,5-bis((tert-butoxycarbonyl)amino)terephthalate (2)*

3.708g (16.39mmol) 1 was suspended in 180mL toluene, heated to boiling, and had 80mL toluene removed via distillation. 22.11g (101.3mmol) Boc₂O dissolved in 40mL toluene was added to the reaction mixture followed immediately by 0.409g 5% Pd-C and heated at reflux for 15h before the addition of 3.917g (17.95mmol) Boc₂O dissolved in 8mL toluene. The mixture was allowed to reflux for an additional 8h before the addition of 8.229g (37.71mmol) Boc₂O dissolved in 12mL toluene. With an additional 20h reflux time, TLC showed the reaction to be complete (10% CH₃CN/CH₂Cl₂, silica, R_f product = 0.72). The boiling mixture was filtered over a pad of Celite and rinsed with boiling toluene until the filtrate appeared colorless (90mL). The filtrate was boiled down to 75mL total volume, allowed to cool to room temperature slowly, and placed in an ice bath for 30min. The resulting solid was collected by vacuum filtration, rinsed with three 10mL portions cold toluene, and placed under vacuum overnight to remove residual solvent to yield 6.146g (88% yield, mp = 248.5-250°C) of a lemon yellow solid.

^1H NMR (300MHz, CDCl₃): δ 9.96 (s, 2H); δ 9.03 (s, 2H); δ 3.94 (s, 6H); δ 1.52 (s, 18H)

^13C NMR (300MHz, CDCl₃): δ 167.8; 152.8; 135.2; 120.9; 119.2; 80.6; 52.7; 28.2

ESI-MS (DUIS, + ion): 448 (M + CH₃CN + Na⁺, 31%); 442 (M + NH₄⁺, 100%); 310 (56%); 266 (M - 2Boc + CH₃CNH⁺, 30%)
Dimethyl 2,5-diaminoterephthalate (3)

0.4165g (0.9813mmol) 2 was dissolved in 15mL CH₂Cl₂ to which was added 10mL CF₃COOH. The mixture was placed under an atmosphere of nitrogen before it was heated at reflux for 5min at which time TLC showed the deprotection was complete (Rᶠ: 0.62, silica, shortwave UV, 25% CH₃CN/CH₂Cl₂). The reaction mixture was concentrated with rotary evaporation to dryness before being placed under vacuum overnight to remove residual solvent to afford 0.3746g of a yellow solid. The resulting solid was combined with 75% conc. NH₃(aq) upon which created a suspension and a color change of yellow to orange in the visible solid. After stirring rapidly for 5min the solid was collected by vacuum filtration, washed with two 10mL potions RT DI H₂O followed by 10mL RT EtOH. (Note: Upon EtOH rinse, filtrate turned orange, don’t use EtOH to wash away water) The orange solid was placed under vacuum for 1h to remove residual solvent to yield 0.1708g (78% overall yield) of the desired diamine as an orange tinged yellow solid.

¹H NMR (300MHz, CDCl₃): δ7.28 (s, 2H); 4.97 (s, 4H); 3.88 (s, 6H)

ESI-MS (DUIS, + ion): 266 (M + CH₃CNH⁺, 100%); 225 (M + H⁺, 93%)
**Tetramethyl 5,5’-azanediylbis(2-((tert-butoxycarbonyl)amino)terephthalate)** (4)

4.541g of the concentrated mother liquor from recrystallization of 2 was combined with 1.085g (14.46mmol) L-Glycine, 4.027g (47.94mmol) NaHCO₃, and 95mL CH₃OH. The mixture was left to stir at room temperature for 17h before the remaining solid was removed by vacuum filtration and rinsed with CH₂Cl₂ until the filtrate ran colorless (150mL). The filtrate was concentrated to dryness with rotary evaporation and placed under vacuum overnight to remove residual solvent to afford 5.199g of a pale orange solid. 5.153g of the resulting solid was boiled in 200mL toluene for 5min before filtering remaining solid with vacuum filtration. The filtrate was washed with three 100mL portion sat. NaHCO₃, dried over MgSO₄, concentrated to dryness with rotary evaporation, and placed under vacuum overnight to remove residual solvent to yield 2.727g of a light orange solid which was determined to be 33% by mass 2 and 67% by mass 4 by ¹H NMR. 0.4081g of the new crude product was purified by flash chromatography (8” silica, 2”/min flow rate, 10mL fraction, eluted with 200mL CH₂Cl₂ then 200mL 5% CH₃CN/CH₂Cl₂) to afford 0.1755g.

¹H NMR (300MHz, CDCl₃): δ10.64 (s, 1H); 9.78 (s, 2H); 8.97 (s, 2H); 8.10 (s, 2H); 3.97 (s, 6H); 3.90 (s, 6H); 1.54 (s, 18H)

ESI-MS (DUIS, + ion): 654 (M + Na⁺, 54%); 632 (M + H⁺, 100%)

HRMS (IT-TOF-ESI, + ion): Calc’d for C₃₀H₃₇N₃O₁₂: 632.2450. Found: 632.2451
Dimethyl 2-amino-5-(p-tolylamino)terephthalate (5)

0.2764g (1.222mmol) 1 and 1.920g (17.92mmol) p-toluidine were combined with 100mL toluene. 30mL toluene was distilled off before the addition of 0.0568g 5% Pd-C. The mixture was then allowed to heat at reflux for 41h (Note: pH paper neutral when placed above the condenser periodically over the course of the reaction) after which the boiling reaction mixture was filtered over a pad of Celite and rinsed with boiling toluene until the filtrate was observed to be colorless (75mL). The filtrate was then washed with two 100mL portion pH5 AcOH\(_{aq}\) followed by two 100mL pH3 AcOH\(_{aq}\). (Note: use 4x100mL pH3 portions instead) The organic phase was then removed, dried over MgSO\(_4\), filtered, rinsed with 30mL toluene, concentrated with rotary evaporation, and placed under vacuum overnight to afford 0.335g or a reddish-orange solid (87% crude yield). 0.321g of the crude product from purified via flash chromatography (20mm column, 2in/min flow rate, 10mL fraction, 7.75” silica, 50mL CH\(_2\)Cl\(_2\), then 100mL 5% CH\(_3\)CN/CH\(_2\)Cl\(_2\), 50mL 10% CH\(_3\)CN/CH\(_2\)Cl\(_2\), then 50mL 100% CH\(_3\)CN) Fracs 7-10 5% + Frac 1-3 10% afforded 0.140g 5.

\(^1\)H NMR (300MHz, CDCl\(_3\)): δ7.86 (s, 1H); 7.35 (s, 1H); 7.11 (d, J=8.1, 2H); 7.05 (d, J=8.4, 2H); 3.89 (s, 3H); 3.83 (s, 3H); 2.32 (s, 3H)

ESI-MS (DUIS, + ion): 356 (M + CH\(_3\)CN\(^+\), 100%); 315 (M + H\(^+\), 46%)

HRMS (IT-TOF-ESI, + ion): Calc’d for C\(_{17}\)H\(_{18}\)N\(_2\)O\(_4\): 315.1339. Found: 315.1340
Dimethyl 2,5-bis(p-tolylamino)terephthalate \((6)\)

0.2764g (1.222mmol) \(1\) and 1.920g (17.92mmol) \(p\)-toluidine were combined with 100mL toluene. 30mL toluene was distilled off before the addition of 0.0568g 5% Pd-C. The mixture was then allowed to heat at reflux for 41h (Note: pH paper neutral when placed above the condenser periodically over the course of the reaction) after which the boiling reaction mixture was filtered over a pad of Celite and rinsed with boiling toluene until the filtrate was observed to be colorless (75mL). The filtrate was then washed with two 100mL portion pH5 AcOH\(_{aq}\) followed by two 100mL pH3 AcOH\(_{aq}\). (Note: use 4x100mL pH3 portions instead) The organic phase was then removed, dried over MgSO\(_4\), filtered, rinsed with 30mL toluene, concentrated with rotary evaporation, and placed under vacuum overnight to afford 0.335g or a reddish-orange solid (87% crude yield). 0.321g of the crude product from purified via flash chromatography (20mm column, 2in/min flow rate, 10mL fraction, 7.75” silica, 50mL CH\(_2\)Cl\(_2\), then 100mL 5% CH\(_3\)CN/CH\(_2\)Cl\(_2\), 50mL 10% CH\(_3\)CN/CH\(_2\)Cl\(_2\), then 50mL 100% CH\(_3\)CN) Fracs 5-8 100% CH\(_2\)Cl\(_2\) + f1 5% afforded 0.170g \(6\).

\(^1\)H NMR (300MHz, CDCl\(_3\)): 8.66 (s, 2H); 7.92 (s, 2H); 7.14 (d, J=8.1Hz, 4H); 7.08 (d, J=8.4Hz, 4H); 3.84 (s, 6H); 2.33 (s, 6H)

ESI-MS (DUIS, + ion): 405 (M + H\(^+\), 100%); 446 (M + CH\(_3\)CNH\(^+\), 19%)

HRMS (IT-TOF-ESI, + ion): Calc’d for C\(_{24}\)H\(_{24}\)N\(_2\)O\(_4\): 405.1809. Found: 405.1810
13.99g (368.6mmol) LiAlH₄ was combined with 750mL anhydrous Et₂O and heated at reflux for 30min before the addition of 21.95g (51.72mmol) of 2 dissolved in 730mL CH₂Cl₂. The diester solution was added as quickly as possible while still having the color of the solution disappear upon addition. **Note: Evolution of H₂ gas at this scale can produce increases of pressure that need to be taken into consideration** The reaction was heated at reflux for 1.5h before TLC showed the reaction was complete (Rᵋ = 0.79 diester, Rᵋ = 0.14 diol 10% CH₃CN/CH₂Cl₂). The mixture was removed from the heat and quenched by sequential, dropwise, addition of 14mL DI H₂O, 14mL 15% NaOH (aq), then 42mL DI H₂O. The mixture was filtered over Celite with the filter pad being removed, boiled in 400mL 50% Et₂O/CH₂Cl₂, for 2min before being filtered over Celite again. The filtrates were combined, dried over MgSO₄, filtered, concentrated to dryness with rotary evaporation, and placed under vacuum overnight to yield 15.00g (79% yield) of 7 as a white solid (mp = 162-166°C)

**¹H NMR (300MHz, CDCl₃):** δ 7.78 (s, 2H); 7.59 (s, 2H); 4.67 (s, 4H); 1.92 (s, 2H); 1.51 (s, 18H)

**¹³C NMR (300MHz, CDCl₃):** δ 153.7; 133.2; 129.7; 121.7; 80.6; 64.2; 28.5

**MS (EI, 70eV):** 368 (M⁺, 43%); 312 (M⁺ - C₄H₈, 30%); 256 (312 - C₄H₈, 100%); 221 (M⁺ - C₅H₁₀O₂, 30%); 212 (256 - CO₂, 48%); 194 (212 - H₂O, 76%); 150 (194 - CO₂, 88%); 132 (150 - H₂O, 32%)

**ESI-MS (DUIS, - ion):** 367(M - H⁺, 90%); 293(M - 2Boc + CF₃COO⁻, 100%)
HRMS (IT-TOF-ESI, + ion): Calc’d for C₁₈H₂₈N₂O₆ + NH₄⁺; 386.2291. Found: 368.2288

IR (KBr pellet): IR (KBr pellet): 3384 cm⁻¹ (N-H); 3195 cm⁻¹ (O-H); 3011 cm⁻¹, 2979 cm⁻¹, 2931 cm⁻¹, 2856 cm⁻¹ (C-Hs); 1686 cm⁻¹, 1677 cm⁻¹ (C=O); 1537 cm⁻¹ (aromatic C-H); 1167 cm⁻¹ (C-O)

Elem. Anal.: Calc’d for C₁₈H₂₈N₂O₆: C, 58.68; H, 7.66; N, 7.60. Found C, 58.54; H, 7.73; N, 7.52

\[ \text{N,N'-di-t-Boc-2,5-diaminoterephthaldehyde (8)} \]

**MnO₂ method**

3.018g 7 (8.194mmol) was placed into a 500mL round bottom flask along with 42.47g (488.5mmol) freshly prepared, dried in a 135° C oven until powdry with periodic grinding, MnO₂. 90mL CH₂Cl₂ was added to the reaction vessel before the addition of 210mL acetone. The reflux apparatus was placed under an N₂ atmosphere and heated to reflux while stirring rapidly. After 15h, the reaction was incomplete by TLC (\( R_f = 0.85 \), \( R_f \) monoaldehyde intermediate = 0.60 10% CH₃CN/CH₂Cl₂) and an additional 4.519g (51.98mmol) 1 day old MnO₂ was added to the reaction and set back to reflux. After an additional 5h TLC showed the reaction was complete. The reaction was filtered hot over a pad of Celite and rinsed with CH₂Cl₂ until the filtrate ran clear (200mL). The MnO₂/Celite pad was placed into a 500mL beaker along with 100mL CH₂Cl₂, 100mL THF, and 100mL MeOH, stirred rapidly, and boiled for ~1-2min before filtering over a pad of Celite. This process was repeated once more. The filtrates were
combined, dried over MgSO₄, filtered, concentrated with rotary vacuum, and dried over high to remove residual solvent overnight to yield 2.831g 8 (95% yield). 1.015g crude 8 recrystallized from 45mL CH₃CN (44.3mL CH₃CN / g 8) (58% recovery, mp = 219-221˚C) this recovery is not typical though after multiple recrystallizations.

[Cu(MeCN)₄]OTf method

0.3036g (0.8240mmol) 7 was combined with 0.0325g (0.0883mmol) [Cu(I)(MeCN)₄]OTf, 0.0013g (0.0083mmol) bpy, 13.4µL (0.168mmol) NMI, 0.0013g (0.0083mmol) TEMPO, and dissolved in 30mL CH₃CN. The mixture was set to stir rapidly open to the air at room temperature for 23h at which time TLC showed incomplete reaction. 0.0130g (0.0832mmol) TEMPO dissolved in 2mL CH₃CN and 20µL (0.251mmol) NMI were added along with 8mL CH₂Cl₂ to rinse the walls of the vessel. The mixture was set to stir rapidly open to the air for 18h at which time TLC showed complete reaction then filtered over a plug of silica (4.5cm dia., 0.75cm tall) and rinsed with CH₂Cl₂ until the filtrate ran clear (30mL). The filtrate was diluted with 20mL CH₃OH (to prevent bumping), concentrated to dryness with rotary evaporation, and placed under vacuum for 2h to afford 0.286g 8 (95% yield, mp 212-217˚C). 0.2641g of crude 8 was recrystallized from 10mL CH₃CN, cooled, and placed in a -20˚C freezer overnight to yield 0.2191g (83% recovery, mp = 218-220˚C) as orange needles. The mother liquor was concentrated, dissolved in 1.5mL CH₂Cl₂, and diluted with 12mL hexane. The mixture was boiled down to ½ volume, cooled, and placed in a -20˚C freezer overnight to afford 0.0101g DA as yellow plates. 8 afforded in 83% purified yield in two crops from recrystallization.

¹H NMR (300MHz, CDCl₃): δ 10.11 (s, 2H); 10.01 (s, 2H); 8.88 (s, 2H); 1.55 (s, 18H)
$^{13}$C NMR (300MHz, CDCl$_3$): δ 195.6; 153.2; 135.3; 126.0; 125.2; 81.5; 28.4

MS (EI, 70eV): 364 ($M^+$, 35%); 308 ($M^+ - C_4H_8$, 39); 252 (308 - C$_4$H$_8$, 84%); 208 (252 - CO$_2$, 48%);
191 (252 - CH$_3$NO$_2$, 33%); 164 (208 - CO$_2$, 100%); 136 (164 - CO, 27%); 108 (136 - CO, 25%)

ESI-MS (DLIS, - ion): 458 (54%); 431 ($M^- + Na^+ + HCOO^-$, 78%); 363 ($M^- + H^+$, 100%)

HRMS (IT-TOF-ESI, + ion): Calc’d for C$_{18}$H$_{24}$N$_2$O$_6$: 382.1978. Found: 382.1973

IR (KBr pellet): 3314cm$^{-1}$ (N-H); 3003cm$^{-1}$, 2976 cm$^{-1}$, 2933cm$^{-1}$ (methyl C-H); 2886cm$^{-1}$ (aldehyde C-H);
1724cm$^{-1}$ (amide C=O); 1674cm$^{-1}$ (aldehyde C=O); 1550cm$^{-1}$ (aromatic C-H); 1148cm$^{-1}$ (C-O)

Elem. Anal. Calc’d for C$_{18}$H$_{24}$N$_2$O$_6$: C, 59.33; H, 6.64; N, 7.69. Found: C, 56.68; H, 6.42; N, 7.22

**Ethyl 6-amino-2-naphthoate** (12) H$_2$N

0.9425g (5.035mmol) 6-amino-2-naphthalene-2-carboxylic acid was combined with 24mL anhyd. ethanol to which was added 1.3mL conc. H$_2$SO$_4$, causing a purplish brown solid to precipitate from the mixture. This mixture was placed under and atmosphere of N$_2$ and heated to reflux, which causing the mixture to become homogeneous. After 2h TLC showed the reaction to be complete. The mixture was then allowed to cool slightly before it was poured into a separator funnel containing 50mL CH$_2$Cl$_2$ and 50mL sat. NaHCO$_3$. The organic layer was removed and washed with 50mL sat. NaHCO$_3$. Another portion of 50mL CH$_2$Cl$_2$ was used to countercurrently extract the two aqueous phases. The organic portions were combined, dried over MgSO$_4$, filtered, concentrated with rotary evaporation, and placed under vacuum
overnight to remove residual solvent to afford 1.054g (97%, m.p = 68-71°C) of the desired product as a purple hued brown solid.

\[ ^1H \text{ NMR (300MHz, CDCl}_3\] : \( \delta 8.45 (s, 1H) \), 7.94 (dd, \( J = 8.6 \text{ Hz}; 1.5\text{ Hz}, 1H) \), 7.75 (d, \( J = 9.3\text{ Hz}, 1H) \), 7.58 (d, \( J = 8.6\text{ Hz}, 1H) \), 6.96 (dd, \( J = \) overlapped, 1H), 6.95 (d, \( J = \) overlapped, 1H), 4.41 (q, \( J = 7.1\text{ Hz}, 2H) \), 3.94 (bs, 2H), 1.42 (t, \( J = 7.1\text{ Hz}, 3H) \)

\[ ^{13}C \text{ NMR (300MHz, CDCl}_3\] : \( \delta 167.2, 146.6, 137.6, 131.1, 131.0, 126.8, 126.1, 125.8, 124.3, 118.8, 108.0, 60.9, 14.9 \)

MS (EI, 70eV) 215 (M\(^+\), 100%), 187 (M\(^+\) - C\(_2\)H\(_4\), 38%), 170 (M\(^+\) - C\(_2\)H\(_5\)O, 79%), 142 (M\(^+\) - C\(_3\)H\(_5\)O\(_3\)), 115 (142 - CHN, 31%)

IR (KBr pellet): Peaks showed the following functional groups: 3431 cm\(^{-1}\), 3349 cm\(^{-1}\) (NH\(_2\)), 1690 cm\(^{-1}\) (C=O); 1294 cm\(^{-1}\) (C-O)

Elem. Anal. Calc’d for C\(_{13}\)H\(_{13}\)NO\(_2\): C, 72.54; H, 6.09; N, 6.51. Found C, 71.82; H, 6.13; N, 6.36

**Diethyl 6,6′-(((1E,1′E)-((2,5-bis((tert-butoxycarbonyl)amino)-1,4-phenylene)bis(methanylylidene))bis(azanylylidene))bis(2-naphthoate)** (13)

1.003g (2.752mmol) 8 and 1.192g (5.536mmol) 7 were dissolved in 30mL distilled CH\(_2\)Cl\(_2\) before the addition of 1.35mL (23.58mmol) glacial acetic acid. The mixture was set to stir at room temperature under an atmosphere of N\(_2\) 21h at which time TLC was used to confirm the reaction was complete. Over the course of the reaction it became heterogeneous while orange
solid. The reaction mixture was concentrated to ca. 1/3 volume before diluting with 20mL EtOAc and collecting the orange solid by centrifugation. The supernatant was decanted and the orange solid was combined thoroughly with 20mL EtOAc before centrifuging, decanting and drying the solid under vacuum overnight. 1.792g (86%, mp = 280-282°C) **13** was afforded as a bright orange solid. The supernatants were combined, concentrated with rotary evaporation, diluted with 10mL EtOAc, and centrifuged to collect another portion of orange solid. The supernatant was decanted, washed with 7mL EtOAc, centrifuged, supernatant decanted, and dried under vacuum for 5h to afford 0.1086g (5%) **13** as an orange solid for a total of 1.900g (91% yield)

**1H NMR (300MHz, CDCl₃):** δ11.84 (s, 2H); 8.85 (s, 2H); 8.77 (s, 2H); 8.63 (s, 2H); 8.12 (d, J=8.7Hz, 2H); 8.04 (d, J=9Hz, 2H); 7.92 (d, J=8.7, 2H); 7.72 (s, 2H); 7.56 (d, J=8.7Hz, 2H); 4.47 (q, J=7.2Hz, 4H); 1.59 (s, 18H); 1.47 (t, J=7.2Hz, 6H)

**13C NMR (300MHz, CDCl₃):** δ166.8; 163.2; 153.8; 149.3; 136.4; 135.0; 131.7; 131.0; 130.9; 128.4; 128.0; 126.3; 124.2; 123.2; 121.7; 118.7; 80.6; 61.3; 28.6; 14.6

**IR (KBr pellet) 3410cm⁻¹; 2975 cm⁻¹; 2359 cm⁻¹; 2341 cm⁻¹; 1713 cm⁻¹; 1273 cm⁻¹, 1241 cm⁻¹**

**ESI-MS (DUIS, + ion):** 759 (M + H⁺, 100%)

**HRMS (IT-TOF-ESI):** Calc’d for C₄₄H₄₆N₄O₈ + H⁺: 759.3388 Found: 759.3381

Diethyl 6,6'-(((1R,1'S)-(2,5-bis((tert-butoxycarbonyl)amino)-1,4-phenylene)bis((dimethoxyphosphoryl)methylene))bis(azanediyl))bis(2-naphthoate) (16)

0.1072 g (0.4981 mmol) 12, 0.0865 g (0.2374 mmol) 8, and 0.0543 g (0.0875 mmol) Yb(OTf)₃ were combined and dissolved in 5 mL CH₂Cl₂ under an atmosphere of N₂. After 2.75 h TLC showed that NEDA fully formed. The mixture was made homogeneous with the addition of 7 mL CH₂Cl₂ before the addition of 0.25 mL (4.240 mmol) P(OMe)₃. After 21 h, the mixture was observed to be homogenous but with the monoamine, mono-aminophosphonate still present so 0.20 mL (1.696 mmol) P(OMe)₃ was added. After stirring for 21 h the reaction appeared complete by TLC. The mixture was poured into a sep. funnel containing 50 mL 80% sat. Na₂CO₃ and 25 mL CH₂Cl₂. The organic layer was countercurrently washed with 50 mL 80% sat. Na₂CO₃. A 40 mL portion CH₂Cl₂ run through the countercurrent extraction apparatus before the organic layers were combined, dried over MgSO₄, filtered, concentrated with rotary evaporation, and placed under vacuum to remove residual solvents to afford 16 and 17 (0.2337 g, 100%) as a brown solid. Purified by flash chromatography (100 mL 10% CH₃CN/CH₂Cl₂, 200 mL 40% CH₃CN/CH₂Cl₂, 200 mL CH₃CN) to afford 16 (0.0377 g, 32%) as an off white solid.

¹H NMR (300 MHz, CDCl₃): δ 8.39 (s, 2H); 7.86 (dd, J=8.6;1.6, 2H); 7.77 (bs, 2H); 7.72 (d, J=8.7, 2H); 7.59 (bs, 2H); 7.38 (d, J=8.7, 2H); 6.99 (dd, J=8.8;2.3, 2H); 6.54 (nd, J=1.9, 2H); 5.13 (dd, J=22.1; 5.4, 2H); 4.99 (dd, J=10.2;5.7, 2H); 4.39 (q, J=7.1, 4H); 3.67 (d, J=10.5, 6H); 3.61 (d, J=10.3, 6H); 1.51 (s, 18H); 1.41 (t, J=7.1, 6H)
\(^{13}\text{C} \text{NMR (300MHz, CDCl}_3\): } \delta 167.1, 154.0, 137.4, 134.3, 130.9, 130.8, 128.6, 127.1, 126.1, 126.0, 125.8, 124.6, 118.7, 106.2, 80.5, 60.9, 55.5, 53.8, 53.0, 51.1, 28.6, 14.1

\(^{31}\text{P} \text{NMR (300MHz, CDCl}_3\): } \delta 24.94

ESI-MS (DUIS, + ion): 997 (M + H\(^+\), 100%)

\textit{Diethyl 6,6'-\{((1S,1'S)-(2,5-bis((tert-butoxycarbonyl)amino)-1,4-phenylene)bis([dimethoxyphosphoryl]methylene))bis(azanediyl)]bis(2-naphthoate) (17)}

0.1072g (0.4981mmol) 12, 0.0865g (0.2374mmol) 8, and 0.0543g (0.0875mmol) Yb(OTf)\(_3\) were combined and dissolved in 5mL CH\(_2\)Cl\(_2\) under an atmosphere of N\(_2\). After 2.75h 13 fully formed. The mixture was made homogeneous with the addition of 7mL CH\(_2\)Cl\(_2\) before the addition of 0.25mL (4.2402mmol) P(OMe)_3. After 21h, the mixture was observed to be homogenous but with the monoamine, mono-aminophosphonate still present so 0.20mL (1.696mmol) P(OMe)_3 was added. After stirring for 21h the reaction appeared complete by TLC. The mixture was poured into a sep. funnel containing 50mL 80% sat.Na\(_2\)CO\(_3\) and 25mL CH\(_2\)Cl\(_2\). The organic layer was countercurrently washed with 50mL 80% sat.Na\(_2\)CO\(_3\). A 40mL portion CH\(_2\)Cl\(_2\) run through the countercurrent extraction apparatus before the organic layers were combined, dried over MgSO\(_4\), filtered, concentrated with rotary evaporation, and placed under vacuum to remove residual solvents to afford 16 and 17 (0.2337g, 99.7%) as a brown solid.

Purified by flash chromatography (100mL 10%CH\(_3\)CN/CH\(_2\)Cl\(_2\), 200mL 40% CH\(_3\)CN/CH\(_2\)Cl\(_2\), 200mL CH\(_3\)CN) to afford EDPB (0.0344g, 29%) as an tan solid.
$^1$H NMR (300MHz, CDCl$_3$): δ 8.38 (s, 2H), 8.00 (bs, 2H), 7.87 (dd, J = 8.6;1.6, 2H), 7.77 (s, 2H), 7.69 (d, J = 8.8, 2H), 7.44 (d, J = 8.6, 2H), 6.96 (dd, J = 8.8;2.2, 2H), 6.65 (s, 2H), 5.08 (dd, J = 2.94;6.7), 4.99 (d, J = 7.0, 2H), 4.39 (q, J = 7.1, 4H), 3.58 (d, J = 10.5, 2H), 1.46 (s, 18H), 1.41 (t, J = 7.1, 6H)

$^{13}$C NMR (300MHz, CDCl$_3$): δ 167.1, 153.8, 145.7, 145.5, 137.3, 134.1, 130.8, 130.8, 127.1, 126.5, 126.1, 124.6, 118.7, 106.5, 80.5, 60.9, 54.7, 54.2, 52.5, 28.5, 14.5

$^{31}$P NMR (300MHz, CDCl$_3$): δ 25.65

ESI-MS (DUIS, - ion): 977 (M - H$^+$, 100%)

$C$-Me, $P$-Me rac- tweezers (18)

0.1107g (0.1122mmol) EDPB was combined with 3mL (74.064mmol) MeOH and 1.5mL (16.932mol) methylal and placed under an atmosphere of N$_2$ before the addition of 7mL (90.861mmol) CF$_3$COOH. The mixture was heated at reflux for 22hr before concentration over rotary evaporation. The mixture was poured into a sep. funnel containing 55mL 80% sat. Na$_2$CO$_3$ and 30mL CH$_2$Cl$_2$ and 15mL sat. NaCl. The organic layer was removed and countercurrently washed with 55mL 80% sat. Na$_2$CO$_3$ and 15mL sat. NaCl 20mL CH$_2$Cl$_2$ run through the extraction apparatus before the organic portions were combined, dried over MgSO$_4$, filtered, concentrated with rotary evaporation, and placed under vacuum to remove residual solvents to afford 0.0835g 18 (90% yield) as a tan solid. Purified by flash chromatography (silica, 75% CH$_3$CN/CH$_2$Cl$_2$, 10% MeOH/CH$_2$Cl$_2$).
Largest Scale:

1.886g (1.927mmol) of the 1:1 mixture of 16 and 17 was combined with 14.0mL anhyd. ethanol, 14.0mL methylal (90mmol), and 34.0mL CF₃COOH. The mixture was placed under an atmosphere of N₂ and heated at reflux for 20hr after which it was cooled to room temperature, diluted with 30mL toluene and concentrated to dryness with rotary evaporation. The concentrate was dissolved in 100mL CH₂Cl₂ and washed with two 50mL portions sat. NaHCO₃. The aqueous washes were combined and back extracted with two 30mL portions CH₂Cl₂. The organic extracts were combined, dried over MgSO₄, filtered, concentrated to dryness with rotary evaporation and placed under vacuum overnight to remove residual solvent to afford 1.6862g of a beige solid. 0.577g of the crude product was combined with 30mL boiling toluene, concentrated to 25mL total volume, cooled to room temperature, and placed on ice for 15min. The resulting mixture was filtered and rinsed with 15mL toluene. The filtrate was concentrate to dryness with rotary evaporation, dissolved in 10mL 1,4-dioxane and precipitate using 100mL hexane. The solid was collected by centrifugation, washed with 40mL hexane, and placed under vacuum overnight to remove residual solvent to afford 0.2253g (81% yield, mp = 308°C (s), 320°C (d)) of the light tan solid 18.

1H NMR (300MHz, CDCl₃, at 3x10⁻³M): 6.828 (s, 2H); 7.88 (d, J=7.8Hz, 2H); 7.62 (d, J=8.7Hz, 2H); 7.48 (d, J=8.7, 2H); 7.20 (s, 2H); 7.18 (d, J=8.4Hz, 2H); 5.01 (d, J=12.0Hz, 2H); 4.89 (d, J=17.1Hz, 2H); 4.61 (d, J=23.7Hz, 2H); 4.36 (d, overlapped, 2H); 4.34 (q, J=7.2Hz, 4H); 4.22 (d, J=13.0Hz, 2H); 4.04 (d, J=10.2Hz, 6H); 3.98 (d, J=10.5Hz, 6H); 1.34 (t, J=7.2Hz, 6H)
$^{13}$C NMR (300MHz, CDCl$_3$): δ166.7; 166.5; 147.9; 133.6; 133.2; 131.5; 131.1; 130.2; 129.9; 129.4; 126.8; 126.5; 126.1; 125.6; 124.9; 124.7; 123.3; 122.3; 121.3; 64.4; 63.6; 62.3; 61.25; 61.20; 56.4; 55.3; 53.2; 28.2; 14.5

$^{31}$P NMR: δ23.8

ESI-MS (DUIS, + ion): 868(M + CH$_3$CNH$^+$, 100%); 827(M + H$^+$, 42%)

HRMS (IT-TOF-ESI, + ion): Calc’d for [C$_{42}$H$_{44}$N$_4$O$_{10}$P$_2$ + H$^+$] 827.2605    Found: 827.2610

**C-Me, P-Me meso- isomer (19)**

0.1501g (0.1521mmol) **16**, 1.2mL (13.545mmol) methylal, and 2.5mL MeOH (61.720mmol) were combined under an atmosphere of N$_2$ before 4mL (341.36mmol) CF$_3$COOH was added. The mixture was heated at reflux for 21hr concentrating the mixture with rotary evaporation. 10mL CH$_2$Cl$_2$ was to the concentrate and countercurrently washed with 50mL 80% sat. Na$_2$CO$_3$ in the first sep. funnel and 50mL 80% sat. Na$_2$CO$_3$ and 30mL sat. NaCl in the second sep.funnel. 60mL CH$_2$Cl$_2$ run through the extraction apparatus before the organic portions were combined, dried over MgSO$_4$, filtered, concentrated with rotary evaporation, and placed under high vacuum to afford **19** (0.1062g, 84%) as a solid. Crude product is purified by flash chromatography (40% CH$_3$CN/CH$_2$Cl$_2$, 5% MeOH/CH$_2$Cl$_2$, and 10% MeOH/CH$_2$Cl$_2$ if not eluted out in the 5% portion).
**Largest Scale:**

1.886g (1.927mmol) of the 1:1 mixture of 16 and 17 was combined with 14.0mL anhyd. ethanol, 14.0mL methylal (90mmol), and 34.0mL CF₃COOH. The mixture was placed under an atmosphere of N₂ and heated at reflux for 20hr after which it was cooled to room temperature, diluted with 30mL toluene and concentrated to dryness with rotary evaporation. The concentrate was dissolved in 100mL CH₂Cl₂ and washed with two 50mL portions sat. NaHCO₃. The aqueous washes were combined and back extracted with two 30mL portions CH₂Cl₂. The organic extracts were combined, dried over MgSO₄, filtered, concentrated to dryness with rotary evaporation and placed under vacuum overnight to remove residual solvent to afford 1.6862g of a beige solid. 0.577g of the crude product was combined with 30mL boiling toluene, concentrated to 25mL total volume, cooled to room temperature, and placed on ice for 15min. The resulting mixture was filtered with 15mL toluene used to rinse the collected solid which was then placed under vacuum overnight to afford 0.241g (84% yield, mp = 322˚C (s), 338˚C (d)) of the meso-isomer 19.

**¹H NMR (300MHz, CDCl₃):** δ8.52 (s, 2H); 8.05 (d, J=8.7Hz, 2H); 7.84 (d, J=9.0Hz, 2H); 7.61 (d, J=9.0Hz, 2H); 7.31 (s, 2H); 7.28 (d, overlapped, 2H); 5.07 (d, J=16.8Hz, 2H); 5.00 (d, J=13.8Hz, 2H); 4.75 (d, J=24.3Hz, 2H); 4.45 (q, overlapped, 2H); 4.43 (q, overlapped, 4H); 4.25 (d, J=12.9Hz, 2H); 3.94 (d, J=10.5Hz, 6H); 3.85 (d, J=10.8Hz, 6H); 1.43 (t, J=7.2Hz, 6H)

**¹³C NMR (300MHz, CDCl₃):** δ6166.8; 133.7; 131.6; 130.3; 129.8; 127.1; 126.5; 125.8; 124.9; 123.5; 122.2; 121.4; 64.4; 63.6; 62.3; 61.2; 57.1; 55.2; 53.3; 14.5

**³¹P NMR (300MHz, CDCl₃):** δ23.7
ESI-MS (DUIS, + ion): 827 (M + H⁺, 100%); 424 ((M + 2H⁺)/2, 40%); 270 (26%)

HRMS (IT-TOF-ESI, + ion): Calc’d for [C_{42}H_{44}N_{4}O_{10}P_{2} + H⁺] 827.2605  Found: 827.2600

rac-carbethoxy tetra acid tweezers (23)

0.121g (0.146mmol) rac- naphthyl tweezers was dissolved in 6mL CH₂Cl₂ (distilled from CaH₂) and placed under an atmosphere of N₂ before the addition of 0.80mL (6.0mmol) bromotrimethylsilane. The mixture was stirred at room temperature for 16hr before the mixture was concentrated to dryness with vacuum. The crude product was dissolved in 10mL boiling ethanol and cooled to room temperature before the addition of 40mL hexane causing a solid to precipitate and stored overnight at -20°C. The solid was collected by centrifugation, washed with 15mL hexane, and placed under vacuum overnight to remove residual solvent to afford 0.087g (77% yield, mp = 350°C (d)) 23.

¹H NMR (1:1 dTFA/CDCl₃): δ8.58 (s, 2H); 8.49 (s, 2H); 8.07 (d, J=9.3Hz, 2H); 8.04 (d, J=9.6Hz, 2H); 7.58 (d, J=9.0Hz, 2H); 7.53 (d, J=9.0, 2H); 5.89 (d, J=11.4Hz, 2H); 5.62 (d, J=15.0Hz, 2H); 5.19 (d, J=25.5Hz, 2H); 4.94 (overlapped, 4H); 4.47 (q, J=6.9Hz, 4H); 1.45 (t, J=7.2Hz, 6H)

³¹P NMR (300MHz, DMSO-d₆): δ17.2

ESI-MS (DUIS, - ion): 769 (M + H⁺, 100%)
HRMS (IT-TOF-ESI, + ion): Calc’d for C_{38}H_{34}N_{10}O_{10}P_{2} + H^+: 771.1979  Found: 771.1975

*meso-* carbethoxy tetra acid isomer (24)

0.212g (0.1256mmol) 19 was suspended in 10mL CH₂Cl₂ (distilled from CaH₂) and placed under an atmosphere of N₂ before the addition of 0.90mL (6.8mmol) bromotrimethylsilane. The mixture was heated at reflux for 18hr after which time none of the starting material was seen by TLC (R_f =0.35, 10% CH₃OH/CH₂Cl₂, silica). The reaction mixture was concentrated to dryness under vacuum. The 16mL boiling ethanol was added to the crude product, allowed to cool to room temperature, diluted with 25mL hexane, and stored overnight at -20°C. The solid was collected by centrifugation, washed with 15mL hexane, and placed under vacuum overnight to remove residual solvent to yield 0.170g (86% yield, mp = 340°C (darkens), 380°C (shrinks), >400°C) of 24.

¹H NMR (300MHz, D₂O): δ8.50 (s, 2H); 7.99 (d, J=8.4Hz, 2H); 7.88 (d, J=8.7Hz, 2H); 7.82 (d, J=8.7Hz, 2H); 7.54 (d, J=9.0Hz, 2H); 7.29 (s, 2H); 4.98 (d, J=12.3Hz, 2H); 4.9-4.7 (obscured by HOD, 4H); 4.55 (d, J=21.3Hz, 2H); 4.40 (q, J=6.9Hz, 4H); 4.07 (d, J=11.7Hz, 2H); 1.40 (t, J=7.2Hz, 6H)

³¹P NMR (300MHz, D₂O): δ18.2

ESI-MS (DUIS, - ion): 770 (M - H^+, 100%); 384 (M - 2H^+, 40%)

179
HRMS (IT-TOF-ESI, + ion): [Calc’d for C_{38}H_{37}N_{4}O_{10}P_{2} + H^{+}] 771.1979  Found: 771.1970

*rac*-methyl phosphonate tetra anionic tweezers (25)

0.076g (0.0.092mmol) 18 was dissolved in 2mL 1,4-dioxane to which was added 0.018g (0.752mmol) LiOH in 5.5mL 18Ω H₂O and heated at reflux for 22hr. The mixture was allowed to cool to room temperature, concentrated to dryness with rotary evaporation, dried azeotropically by the evaporation of 50mL toluene, and placed under vacuum overnight to yield 0.0734g of a mixture that was 87% by mass 25. 0.0491g of the crude solid was dissolved in 10mL 18Ω H₂O, titrated to pH7 with 4.8% HBr (0.89M), concentrated to dryness with rotary evaporation, dissolved in 12mL 18Ω H₂O, and concentrated to dryness again. 10mL THF was added to the solid and swirled periodically over the course of 20min after which the THF was removed. This process was repeated with a 20mL portion THF then followed by a 6mL portion THF before being placed under vacuum for 3d to remove residual solvent to afford 0.0401g of 25 (96% by mass, 90% recovery) as a mixture with THF.

^1^H NMR (1:1 TFA:CDCl₃): δ8.67 (s, 2H); 8.52 (s, 2H); 8.16 (d, J=8.7Hz, 2H); 8.06 (d, J=9.0Hz, 2H); 7.60 (d, J=9.0Hz, 2H); 7.54 (d, J=8.7Hz, 2H); 5.94 (d, J=11.7Hz, 2H); 5.63 (d, J=17.1Hz, 2H); 5.19 (d, J=25.8Hz, 2H); 5.02 (overlapped, 4H); 4.14 (d, J=10.8Hz, 6H)

^3^1^P NMR (1:1 TFA:CDCl₃): δ17.2
ESI-MS (- ion mode): 748 (M - 2H⁺ +Li⁺, 50%); 741 (M - H⁺, 100%)

HRMS (IT-TOF-ESI, + ion): Calc’d for C₃₆H₃₂N₄O₁₀P₂ + H⁺: 743.1666  Found: 743.1671

*meso*-methylphosphonate tetra anionic isomer (26)

0.095g (0.115mmol) 19 was suspended in 15mL 1,4-dioxane to which was added 0.025g (1.03mmol) LiOH in 4.5mL 18Ω H₂O. The mixture was placed under an atmosphere of N₂ and heated at reflux for 29hr. The mixture was concentrated to dryness with rotary evaporation, combined with 4mL 18Ω H₂O, and filtered through a plug of Celite. The filtrate was concentrated to dryness with rotary evaporation, dried azeotropically by the evaporation of 25mL toluene, and placed under vacuum for 3d for yield 0.0889g of 26 in 86% yield as an 85% by mass mixture with LiOH. 0.0125g of the crude product was dissolved in 10 mL 18Ω H₂O and titrated to pH7 with 4.8% HBr (0.89M). The mixture was concentrated to dryness with rotary evaporation and triturated with 10mL THF by period swirling over 20min. The THF was decanted and the process was repeated one more time before the solid was placed under vacuum overnight to remove residual solvent to afford 0.011g 26 (100% recovery)

¹H NMR (300MHz, D₂O): δ8.46 (s, 2H); 7.94 (d, overlapped, 2H); 7.94 (d, overlapped, 2H); 7.82 (nd, J=7.2Hz); 7.42 (d, J=8.7Hz, 2H); 7.27 (s, 2H); 4.97 (d, J=16.5Hz, 2H); 4.95-4.55 (obsured by HOD, 6H); 4.18 (d, J=12.0Hz, 2H); 3.56 (d, J=9.0Hz, 6H)
$^{13}$C NMR (300MHz, D$_2$O): δ176.1; 146.8 (d); 143.5; 133.6; 132.8; 130.7; 130.4; 130.1; 127.2; 126.2; 125.6; 125.2; 122.5; 121.9; 65.1; 63.0 (d); 56.8; 53.1

$^{31}$P NMR (300MHz, D$_2$O): δ16.9

ESI-MS (DUIS, - ion): 747(M - H$^+$ - Li$^+$, 100%); 741(M - H$^+$, 58%)

HRMS (IT-TOF-ESI): Calc'd for C$_{36}$H$_{32}$N$_4$O$_4$P$_2$ + H$^+$: 743.1666 Found: 743.1666

rac-hexa anionic tweezers (27)

0.041g (0.053mmol) 23 was combined with 0.024g (0.994mmol) LiOH and 8mL 18Ω H$_2$O. The mixture was heated at reflux for 24hr after which time the mixture was allowed to cool to room temperature and brought to pH7 by dropwise addition of 4.8% HBr (0.89M). The mixture was concentrated to dryness with rotary evaporation, dissolved in 10mL 18Ω H$_2$O, and concentrated to dryness again. The crude product was combined with 11mL THF, mixed thoroughly, centrifuged, and THF decanted. This process was repeated twice more before the solid was placed under vacuum for 2d to remove residual solvent to afford 0.0276g 27 as a 90% by mass mixture (62% yield) with THF.
$^1$H NMR (300MHz, 50% TFA/CDCl$_3$): δ8.69 (s, 2H); δ8.54 (s, 2H); δ8.16 (d, J=8.1Hz, 2H); δ8.08 (d, J=7.8Hz, 2H); δ7.62 (d, J=8.1Hz, 2H); δ7.58 (d, J=9.3Hz, 2H); δ5.93 (d, J=12.9Hz, 2H); δ5.62 (d, J=14.1Hz, 2H); δ5.22 (d, J=25.2Hz, 2H); δ5.02 (d, overlapped, 4H)

$^{31}$P NMR (50% TFA/CDCl$_3$): δ17.6

ESI-MS (DUIS, - ion): 713 (M - H$^+$, 85%); 473 (100%)

HRMS (IT-TOF-ESI, + ion): Calc’d for C$_{34}$H$_{28}$N$_4$O$_{10}$P$_2$ + H$^+$: 715.1353  Found: 715.1357

*meso-hexa anionic isomer* (28)

0.079g (0.103mmol) 24 was combined with 0.050g (0.2.07mmol) LiOH in 10mL 18Ω H$_2$O and heated at reflux for 25hr after it was allowed to cool to room temperature and neutralized to pH7 with 4.8% HBr (0.89M). The mixture was concentrated to dryness with rotary evaporation, redissolved in 10mL 18Ω H$_2$O, and concentrated to dryness again. The crude solid was mixed thoroughly with 10mL THF, centrifuged, and THF was decanted. This process was repeated twice more before being placed under vacuum overnight to afford 0.0517g (66% yield) of the *meso-hexa anion tweezer* as a pale yellow solid.
**H NMR (50% TFA/CDCl₃):** δ8.80 (s, 2H); 8.28 (d, obscured, 2H); 8.27 (s, 2H); 8.17 (d, J=8.7Hz, 2H); 7.80 (d, J=8.4Hz, 2H); 7.58 (d, J=8.7Hz, 2H); 5.87 (d, J=12.6Hz, 2H); 5.61 (d, J=15.9Hz, 2H); 5.20 (d, J=22.5Hz, 2H); 5.11 (d, J=16.2Hz, 2H); 4.97 (d, J=12.3Hz, 2H)

**3¹P NMR (50% TFA:CDCl₃):** δ16.0

ESI-MS (DUIS, - ion): 713 (M - H⁺, 100%); 473 (89%)

HRMS (IT-TOF-ESI, + ion): Calc’d for C₃₄H₂₈N₄O₁₀P₂ + H⁺: 715.1353 Found: 715.1398

*tert-butyl (3-aminophenyl)carbamate (29)*

2.064g 1,3-diaminobenzene (19.09mmol) was dissolved in 15mL of EtOAc at room temperature to which as added 2.033g Boc₂O (9.315mmol) dropwise under an atmosphere of N₂. After 90min, the starting diamine and mono Boc material observed by TLC. At 120min the diacylated product started to show by TLC (50% EtOAc/CH₂Cl₂, silica, short UV, Rₜ diamine= 0.34 Rₜ mono Boc = 0.72, Rₜ DiBoc= 0.92). The reaction mixture was diluted with 50mL of CH₂Cl₂ and washed with eight 250mL portion DI H₂O. The organic layer was removed, dried over MgSO₄, concentrated over rotary vacuum, and dried over vacuum overnight to yield 1.528g 29 (79% yield) as a smokey white solid. Recrystallized from hexanes using hot filtration to afford white crystals (mp = 108-109°C).

**H NMR (300MHz, CDCl₃):** δ7.32 (t, J=8.0Hz, 1H); 6.92 (1H, s); 6.56 (ddd, J=7.3; 1.8; 0.6, 1H); 6.56 (s, 1H); 6.33 (ddd, J=7.9; 2.1; 0.7, 1H); 3.23 (bs, 2H); 1.51 (s, 9H)
$^{13}$C NMR (300MHz, CDCl$_3$): δ152.6; 147.2; 139.3; 129.6; 109.8; 108.5; 105.0; 80.2; 28.3

MS (EI, 70eV): 208 (M$^+$, 25%); 152 (M$^+$ - C$_4$H$_8$, 100%); 108 (152 - CO$_2$, 78%); 57 (C$_4$H$_9^+$, 84%)

2-(3-tert-Butoxycarbonylamino-phenylamino)-but-2-enedioic acid diethyl ester (30)

![Chemical structure of 2-(3-tert-Butoxycarbonylamino-phenylamino)-but-2-enedioic acid diethyl ester (30)](image)

0.9982g (4.794mmol) tert-butyl (3-aminophenyl)carbamate 29 and 2.192g (10.43mmol) sodium diethyl oxaloacetate were added to 11mL CH$_2$Cl$_2$ and 8.9mL glacial AcOH. The mixture was stirred until homogeneous before the addition of 4Åms (25% reaction volume). The mixture was swirled periodically over 4h at which time TLC showed the reaction was done (25% EtOAc/CH$_2$Cl$_2$, silica, short UV, R$_f$ amine=0.5, R$_f$ enamine=0.9). The mixture was poured into 125mL sat. Na$_2$CO$_3$ and 100mL hexanes. The organic phase was removed and the aqueous player was extracted with an addition two 100mL portions hexanes. The organic extracts were combined, washed with four 100mL portions DI H$_2$O then 60mL pH2 phosphate buffer before drying over MgSO$_4$. After drying the mixture was filtered, concentrated with rotary evaporation, and placed under vacuum overnight to yield the 1.558g (96% yield) desired enamine 30 as an orange oil.

$^1$H NMR (300MHz, CDCl$_3$): δ9.61 (s, 1H); 7.15 (unresolved, 2H); 6.95 (dd, J=6.7 Hz; 1.4 Hz, 1H); 6.57 (dd J=6.9 Hz; 2.0 Hz, 1H); 6.42 (s, 1H); 5.37 (s, 1H); 4.20 (q, J=7.1 Hz, 4H); 1.51 (s, 9H); 1.32 (t, J=7.1Hz, 3H); δ1.15 (t, J=7.1Hz, 3H)

$^{13}$C NMR (300MHz, CDCl$_3$): δ169.8; 164.7; 152.7; 148.6; 141.4; 139.6; 129.7; 115.6; 114.2; 111.2; 94.5; δ81.0; 62.5; 60.3; 28.6; 14.7; 14.0

185
MS (EI, 70eV): 378 (M⁺, 18%); 322 (M⁺ - C₄H₆, 22%); 249 (322 - C₃H₅O₂, 39%); 203 (249 - C₂H₂O, 68%); 185 (203 - H₂O, 36%); 159 (203 - CO₂, 100%); 132 (159 - CO, 32%)

7-Amino-4-oxo-1,4-dihydro-quinoline-2-carboxylic acid ethyl ester (31)

0.0326g (0.1243mmol) 38 and 0.0109g 5% Pd-C were suspended in 10mL anhyd. EtOH to which was added 0.1930g (3.061mmol) ammonium formate. The mixture was stirred rapidly for 3h before TLC showed the absence of starting material (50% EtOAc/CH₂Cl₂, silica, short UV Rf nitro= 0.39, Rf amino= 0.08). The mixture was filtered through Celite, rinsed with 40mL anhyd. EtOH, and concentrated to dryness with rotary evaporation. The crude was dissolved in 5.5mL EtOH and diluted with 30mL CH₂Cl₂ causing NH₄O₂CH to precipitate which was removed by filtration. The filtrate was concentrated to dryness with rotary evaporation and purified by flash chromatography (5% IpOH/CH₃CN) to yield 0.019g (66% yield) of 31.

¹H NMR (300MHz, DMSO-d₆): δ11.40 (s,1H); 8.03 (d, J=8.7Hz, 1H); 6.81 (d, J=1.8Hz, 1H); 6.64 (dd, J=8.8; 2.1Hz, 1H); 6.42 (d, J=2.1Hz, 1H); 6.03 (s, 2H); 4.41 (q, J=7.1Hz, 2H); 1.42 (t, J=7.1Hz, 3H)

¹³C NMR (300MHz, DMSO-d₆): δ162.8; 153.1; 142.8; 137.1; 126.4; 117.7; 114.4; 110.6; 110.0; 98.1; 62.6; 14.3

MS (EI, 70eV): 232 (M⁺, 49%); 158 (M⁺ - C₃H₅O₂, 10%); 130 (158 - CO); 104 (130 - C₂H₂, 27%); 57 (C₃H₅O⁺, 56%)

ESI-MS (DUIS, + ion): 465 (2M + H⁺, 28%); 291 (M + 59, 38%); 233 (M + H⁺, 100%)
Ethyl 5-amino-4-oxo-1,4-dihydroquinoline-2-carboxylate (32)

\[
\text{H NMR (300MHz, DMSO-d}_6\text{: }\delta 11.52 \text{ (s, 1H); } 7.51 \text{ (bs, 2H); } 7.23 \text{ (t, } J=8.8\text{Hz, 1H); } 6.86 \text{ (dd, } J=7.3; 0.8\text{Hz, 1H); } 6.40 \text{ (d, } J=1.9\text{Hz, 1H); } 6.20 \text{ (dd, } J=7.4; 0.7\text{Hz, 1H); } 4.40 \text{ (q, } J=7.1\text{Hz, 2H); } 1.35 \text{ (t, } J=7.1\text{Hz, 3H)}
\]

\[
\text{C NMR (300MHz, DMSO-d}_6\text{: }\delta 181.9; 162.4; 151.1; 142.7; 137.2; 133.6; 112.7; 110.7; 107.0; 103.7; 62.8; 14.3
\]

Diethyl 2-((3-(2,2,2-trifluoroacetamido)phenyl)amino)fumarate (34)

0.154g (0.753mmol) N-(3-aminophenyl)-2,2,2-trifluoroacetamide (33) and 0.404g (1.92mmol) sodium diethyl oxaloacetate were dissolved in 5mL CH\textsubscript{2}Cl\textsubscript{2} and 0.3mL glacial AcOH. After homogeneous 0.709g 4Åms were added to the mixture and swirled periodically over the course of 27h at which time the reaction was observed to be complete by TLC (1:1:1 EtOAc/CH\textsubscript{2}Cl\textsubscript{2}/NE\textsubscript{t}, silica, short UV R\textsubscript{f} amine=0.54, R\textsub_{f enamine}=0.82). The reaction volume was poured into 20mL sat. Na\textsubscript{2}CO\textsubscript{3} and 100mL hexanes. The organic phase was removed and the aqueous layer was extracted with an addition two 50mL portions hexanes. The organic extracts were combined, washed with 20mL sat. Na\textsubscript{2}CO\textsubscript{3}, dried over MgSO\textsubscript{4}, filtered, concentrated with rotary evaporation, and placed under vacuum overnight to remove residual solvent to yield 0.252g (89% yield) of 34 as a yellow green oil.
\(^1\)H NMR (300MHz, CDCl\(_3\)): δ 9.61 (s, 1H); 8.15 (s, 1H); 7.26 (obscured, 1H); 7.24 (t, J=8.0Hz, 1H); 7.14 (d, J=8.2Hz, 1H); 6.73 (d, J=7.8Hz, 1H); 5.47 (s, 1H); 4.24 (q, J=7.2Hz, 4H); 1.30 (t, J=7.1Hz, 3H); 1.18 (t, J=7.1Hz, 3H)

\(^13\)C NMR (300MHz, CDCl\(_3\)): δ 169.3; 164.2; 154.8; 147.3; 141.0; 136.2; 129.5; 118.0; 115.7; 115.5; 112.7; 95.4; 62.4; 60.8; 14.1; 13.5

MS (El, 70eV): 374 (M\(^+\), 12%); 301 (M\(^+\) - C\(_3\)H\(_5\)O\(_2\), 13%); 255 (301 - C\(_2\)H\(_6\)O, 100%); 228 (256 - CO, 18%)

**Ethyl 4-oxo-7-(2,2,2-trifluoroacetamido)-1,4-dihydroquinoline-2-carboxylate (35)**

![Chemical structure](image)

**Thermolysis method**

0.0526g (0.1405mmol) diethyl 2-((3-(2,2,2-trifluoroacetamido)phenyl)amino)fumarate (34) was combined with 25mL Ph\(_2\)O and heated at reflux under an atmosphere of N\(_2\) for 20min after which it was cooled to room temperature below the addition of 170mL hexane. The mixture was stored in a 0°C refrigerator with crystals being collected by vacuum filtration the following day. After rinsing with hexanes and residual solvent removed 0.0249g (54% yield) quinolones afforded in a 1:3.5 mol ratio of the 7- : 5-trifluoroacetyl isomers.

**Eaton’s Reagent method**

0.149g (0.394mol) 34 was dissolved in 10mL Eaton’s Reagent and heated at 116°C for 50min after which the reaction volume was poured into 20mL CH\(_2\)Cl\(_2\) to which was added sat.
Na$_2$CO$_3$ until no more effervescence was observed. The organic layer was removed and the aqueous phase was extracted with two 60mL portions CH$_2$Cl$_2$. The organic layers were combined, dried over MgSO$_4$, filtered, concentrated with rotary evaporation, and placed under vacuum to remove residual solvent yield 0.105g (81% yield) of the quinolones in a 1:7 mol ratio of the 7- : 5-trifluoroacetyl isomers.

$^1$H NMR (300MHz, DMSO-$d_6$): δ12.26 (s, 1H); 11.64 (s, 1H); 8.13 (d, J=8.9Hz, 1H); 7.96 (d, J=2.0Hz, 1H); 7.52 (dd, J=9.0; 2.1Hz, 1H); 6.89 (s, 1H); 4.47 (q, J=7.1Hz, 2H); 1.39 (t, J=7.1Hz, 3H)

$^{13}$C NMR (300MHz, DMSO-$d_6$): δ168.7; 165.3; 161.4; 155.1; 140.5; 138.9; 131.9; 127.1; 123.5; 115.8; 114.2; 107.3; 62.3; 14.2

MS (EI, 70eV): 328 (M$^+$, 100%); 299 (M$^+$ - C$_2$H$_5$, 25%); 256 (299 +H - CO$_2$, 34%); 203 (256 + H - C$_3$H$_2$O, 16%); 157 (256 - H - C$_2$HF$_3$O, 22%); 132 (157 + H - C$_2$H$_2$, 40%); 69 (CF$_3$$^+$, 45%)

**Ethyl 4-oxo-5-(2,2,2-trifluoroacetamido)-1,4-dihydroquinoline-2-carboxylate (36)**

$^1$H NMR (300MHz, CDCl$_3$): δ14.79 (s, 1H); 9.26 (s, 1H); 8.57 (d, J=8.1Hz, 1H); 7.69 (t, J=8.3Hz, 1H); 7.23 (d, J=8.4; 0.9Hz, 1H); 6.96 (s, 1H); 4.52 (q, J=7.1Hz, 2H); 1.45 (t, J=7.1Hz, 3H)
Diethyl 2-((3-nitrophenyl)amino)fumarate (37)

1.024 (7.417mmol) m-nitroaniline, 3.118g (14.84mmol) sodium diethyl oxaloacetate, 2.982g TsOH·H₂O (15.68mmol) were combined in 60mL of CH₂Cl₂, fitted with a heavier than water Dean-Stark Trap and heated to reflux overnight. After the starting aniline had been seen to be consumed (silica, 1:1:1 EtOAc: CH₂Cl₂: NEt₃, short UV Rᵣ aniline=0.65, Rᵣ enamine=0.83) the reaction mixture was poured into 50mL of hexane and washed with 200mL of a 0.1M K₃PO₄ solution. The organic phase was then washed with 200mL of DI H₂O, dried over MgSO₄, filter, concentrated over rotary vacuum, and placed on high vacuum to removal residual solvent to yield 2.085g (92% yield) of the desired enamine as a yellow oil.

¹H NMR (300MHz, CDCl₃): δ9.77 (s, 1H); 7.92 (dd, J=8.1; 1.5Hz, 1H); 7.72 (t, J=2.1Hz, 1H); 7.44 (t, J=8.1Hz, 1H); 7.20 (dd, J=8.0; 2.1Hz, 1H); 5.62 (s, 1H); 4.24 (q, J=7.2Hz, 4H); 1.32 (t, J=7.1Hz, 3H); 1.21 (t, J=7.1Hz, 3H)

¹³C NMR (300MHz, CDCl₃): δ169.2; 163.5; 148.7; 146.3; 141.8; 129.8; 126.2; 118.3; 115.0; 97.6; 62.5; 60.5; 14.3; 13.8

MS (EI, 70eV): 308 (M⁺, 18%); 235 (M⁺ - C₃H₅O₂, 28%); 189 (235 - C₂H₆O, 100%); 162 (189 - CO, 23%); 143 (189 - NO₂, 24%); 89 (C₃H₇NO₂⁺, 23%); 76 (C₆H₄⁺, 49%)
**Ethyl 7-nitro-4-oxo-1,4-dihydroquinoline-2-carboxylate (38)**

**Thermolysis**

0.282g (0.916mmol) 37 was dissolved in 80°C 40mL Ph$_2$O. The reaction mixture was degassed and backfilled with N$_2$ three times, placed under an atmosphere of N$_2$, and heated at reflux for 21min before the mixture was allowed to cool to room temperature over which time a yellow solid precipitated. 50mL hexanes were added to ensure complete precipitation before the solid was collected by vacuum filtration, washed with an additional 100mL hexane, and dried under vacuum overnight to remove residual solvent to generate 0.194g (81%) of the quinolones in a mol ratio of 4:1 of the 7-NO$_2$: 5-NO$_2$ isomers.

**Eaton’s Reagent**

0.273g (0.886mmol) 37 to which was added 5mL Eaton’s Reagent. The mixture was placed under a N$_2$ atmosphere and heated at 110°C 3h over which time it changed color to a dark red color and was removed from the heat. After 20h at room temperature the mixture was poured into 50mL CH$_2$Cl$_2$ and 60mL sat. Na$_2$CO$_3$. The organic layer was removed and the aqueous phase was extracted with an additional two 40mL portions CH$_2$Cl$_2$. The organic extracts were combined, dried over MgSO$_4$, filtered, concentrated with rotary evaporation, and placed under vacuum overnight to remove residual solvent to yield 0.254g (109% yield) of the quinolones in a mol ratio of 1:2.3 of the 7-NO$_2$: 5-NO$_2$ isomers.
Recrystallization procedure developed by Jackie Koch

0.1006 g of a 2.6:1 mol ratio 7-NO₂ : 5-NO₂ quinolone isomers mixture was suspended in 8mL boiling EtOH for 3 min to which was added 10mL boiling toluene and continued to heat at boiling for 9 min. The mixture was placed on ice for 15 min with the resulting solid collected by centrifugation. It was then washed with 20mL hexanes and placed under vacuum overnight to afford 0.0639 g of the 38 in 88% recovery (mp = 308-310°C)

¹H NMR (300MHz, DMSO-d₆): δ12.47 (s, 1H); 8.87 (d, J=2.2Hz, 1H); 8.29 (d, J=8.9, 1H); 8.09 (dd, J=8.9; 2.2Hz, 1H); 6.79 (s, 1H); 4.44 (q, J=7.1Hz, 2H); 1.38 (t, J=7.1, 3H)

¹³C NMR (300MHz, DMSO-d₆): saturated solution too dilute to acquire all signals

Signals observed: δ161.8; 149.6; 128.5; 127.0; 117.5; 111.1; 62.8; 13.9

MS (EI, 70eV): 262 (M⁺, 67%); 188 (M⁺ - C₃H₆O₂, 98%); 142 (188⁺ - NO₂); 114 (142⁺ - CO, 75%); 88 (114⁺ - C₂H₂, 61%); 75 (C₃H₇O₂⁺, 100%); 62 (88⁺ - C₂H₂, 58%)

Ethyl 5-nitro-4-oxo-1,4-dihydroquinoline-2-carboxylate (39)

¹H NMR (300MHz, DMSO-d₆): δ14.86 (1H, s); 8.19 (d, J=8.5, 1H); 7.86 (1H, t, J=7.9Hz); 7.62 (1H, d, J=7.4Hz); 6.69 (1H, s); 4.47 (2H, q, J=6.8Hz); 1.40 (3H, t, J=7.4Hz)
Diethyl 2-((4-nitrophenyl)amino)fumarate (40)

0.6405g (4.637mmol) p-nitroaniline, 1.951g (9.282mmol) sodium diethyl oxaloacetate, 1.855g (9.752mmol) TsOH·H₂O, and 50mL CH₂Cl₂ were combined in heated at reflux with a heavier than water Dean-Stark trap with 0.596g loosely packed cotton contained within. After 3d the aniline was no longer seen by TLC (1:1:1 EtOAc:CH₂Cl₂:NEt₃, silica, shortUV, R_f aniline=0.37, R_f enamine=0.86) and the mixture was allowed to cool to room temperature before being poured into 120mL hexanes. The mixture was washed with three 200mL portions sat. Na₂CO₃. The aqueous phase was back extracted with 200mL hexane. The organic extracts were combined, dried over MgSO₄, filtered, concentrated with rotary evaporation, and placed under vacuum overnight to yield 1.216g of the desired product as a mixture with the starting aniline. The desired enamine 40 was present in a 92:8 mol ratio to provide an NMR yield of 78%

¹HNMR (300MHz, CDCl₃): δ9.82 (s,1H); 8.15 (d, J=9.0Hz, 2H); 6.90 (d, J=9.0Hz, 2H); 5.69 (1H, s); 4.27 (q, J=7.1Hz, 2H); 4.23 (q, J=7.1Hz, 2H); 1.31 (q, J=7.1Hz, 3H); 1.23 (q, J=7.1Hz, 3H)

Ethyl 6-nitro-4-oxo-1,4-dihydroquinoline-2-carboxylate (41)

1.112g (3.607mmol) 40 was combined with 25mL Ph₂O, placed under an atmosphere of N₂, and heated at reflux for 20min. The mixture was cooled to room temperature, diluted with 250mL hexanes, and left to sit overnight. The resulting precipitate was collected by vacuum
filtration and placed under vacuum overnight to afford 0.807g (85% yield) of the desired quinolone 41.

$^1$H NMR (300MHz, DMSO-$d_6$): δ12.54 (s, 1H); 8.80 (d, J=2.6Hz, 1H); 8.48 (dd, J=9.2; 2.6Hz, 1H); 8.11 (d, J=9.2Hz, 1H); 6.76 (s, 1H); 4.44 (q, J=7.1Hz, 2H); 1.38 (q, J=7.1Hz, 3H)

$^{13}$C NMR: δ177.1; 161.7; 143.8; 143.1; 139.2; 126.5; 124.7; 121.4; 121.2; 111.3; 62.9; 13.8

MS (EI, 70eV): 262 (M$^+$, 96%); 216 (M$^+$ - C$_2$H$_6$O, 25%); 188 (216 - CO, 100%); 142 (188 - NO$_2$, 40%)

**Ethyl 6-amino-4-oxo-1,4-dihydroquinoline-2-carboxylate (42)**

0.1075g (0.4100mmol) 41, 0.6093g (9.577mmol) NH$_4$O$_2$CH, 0.0282g 5% Pd-C, and 5mL anhyd. EtOH were combined under an atmosphere of N$_2$. The mixture was mixed at a rapid pace for 6d at which time the mixture was filtered over Celite and rinsed with 60mL anhyd. EtOH. The filtrate was concentrated to dryness with rotary evaporation and placed under vacuum for 10d to generate 0.096g (100% yield) of the desired product.

$^1$H NMR (300MHz, CDCl$_3$): δ9.01 (s, 1H); 7.55 (d, J=2.6, 1H); 7.29 (d, J=8.8Hz, 1H); 7.09 (dd, J=8.7; 2.6Hz, 1H); 6.91 (s, 1H); 4.47 (q, J=7.1Hz, 2H); 3.91 (s, 2H); 1.43 (t, J=7.1Hz, 3H)

MS (EI, 70eV): 232 (M$^+$, 73%); 186 (M$^+$ - C$_2$H$_6$O, 9%); 158 (186 - CO, 100%); 130 (158 - CO, 17%); 104 (130 - C$_2$H$_2$, 16%)
Potassium (3E,5E)-2-oxo-6-phenylhexa-3,5-dienoate (45)

3.292g (24.91mmol) cinnamaldehyde, 2.804g (25.48mmol) sodium pyruvate, and 0.1123g (2.808mmol) NaOH were combined with 25mL CH\textsubscript{3}OH before being placed under an atmosphere of N\textsubscript{2} and heated to reflux for 17h. The volume was observed to be heterogeneous with yellow solid but TLC showed that the starting aldehyde was still present (25\% CH\textsubscript{3}CN/CH\textsubscript{2}Cl\textsubscript{2} R\textsubscript{f}: 0.85) and concentrated to dryness with rotary evaporation. 0.4030g (10.08mmol) NaOH and 46mL CH\textsubscript{3}OH were placed into the reaction vessel and heated at reflux for 3h before the starting aldehyde was no longer visible by TLC. The yellow solid was collected by vacuum filtration, rinsed with two 100mL portions CH\textsubscript{3}OH, and placed under vacuum overnight to afford 2.083g (37\% crude yield). CO\textsubscript{2} was bubbled into the filtrate until it reached pH 8 before being concentrated to dryness with rotary evaporation before being combined with 150mL 18Ω H\textsubscript{2}O and 75mL triethylamine (TEA). CO\textsubscript{2} was bubbled into the mixture while on ice until it became homogeneous before being extracted with five 50mL CH\textsubscript{2}Cl\textsubscript{2}. The organic portions were combined, dried over MgSO\textsubscript{4}, filtered, concentrated with rotary evaporation, and placed under vacuum overnight to yield 2.050g of an auburn oil. The oil was combined with 100mL EtOAc, heated to boiling, decanted from the remaining undissolved semisolid, and cooled to room temperature. The supernatant was combined with 1.643g (9.013mmol) potassium 2-ethylhexanoate (K-2EHA) dissolved in 30mL EtOAc which produced a precipitate. The solid was collected by centrifugation, washed with two 30mL portions EtOAc, and placed under vacuum overnight to remove residual solvent to afford 0.433g of crude 45 as a yellow solid. 2.045g of the initially isolated yellow solid was dissolved in 185mL 2.4M
triethylammonium bicarbonate (TEABC) and extracted with four 50mL portions CH₂Cl₂. The organic extracts were combined, dried over MgSO₄, filtered, concentrated with rotary evaporation, and placed under vacuum overnight to yield 1.0963g of an auburn oil. The oil was combined with 50mL EtOAc and stirred at room temperature before the supernatant was decanted and mixed with 0.8073g (4.429mmol) K-2EHA producing a solid upon mixing. The solid was collected by centrifugation, washed with three 30mL portions EtOAc, and dried under vacuum to afford 0.7836g of crude 45 as a yellow solid. 1.118g of crude 45 was recrystallized from 55mL CH₃OH and 100mL EtOAc and boiled down to 1/3 of the total volume and stored in a -10°C freezer overnight. The resulting solid was collected by centrifugation and dried under vacuum to yield 0.914g 45 as a yellow solid (15% yield, 99.4% 45, 0.6% by mass pyruvate, mp = 204°C (darkens to tan), 228°C (shrinks, darkens to brown), 260°C (d)

1H NMR (300MHz, D₂O): 67.63 (d, J=8.1Hz, 2H); 7.51 (q<sub>AB</sub>, J=15.6Hz, 9.6Hz, 1H); 7.48-7.41 (m, 3H); 7.20-7.07 (m, J=15.6Hz; 8.7Hz, 2H); 6.40 (d, J=15.6Hz, 1H)

13C NMR (300MHz, D₂O): δ198.3; 173.0; 151.7; 144.7; 136.4; 130.6; 129.7; 128.4; 127.4; 126.0

ESI-MS (DUIS, + ion): 519 (2M + K⁺, 100%); 279 (M + K⁺, 96%)

ESI-MS (DUIS, - ion): 441 (2M⁻ + K⁺, 89%); 201 (M⁻, 100%)

UV-VIS: λ<sub>max</sub>=335nm; ε=38868 L mol⁻¹ cm⁻¹
1.456g (8.222mmol) 4-nitrocinnamaldehyde and 1.351g (12.28mmol) sodium pyruvate were suspended in 25mL. 0.1133g (2.832mmol) NaOH was dissolved in 25mL CH$_3$OH, added to the reaction mixture, and quantitatively transferred with 10mL CH$_3$OH. The mixture was placed under an atmosphere of N$_2$ and heated at reflux for 3.5h before the starting aldehyde was no longer visible by TLC (25% CH$_3$CN/CH$_2$Cl$_2$ R$_f$: 0.87). The mixture was allowed to cool to room temperature, concentrated with rotary evaporation, and placed under vacuum overnight to afford 2.133g of a brown solid. 2.132g of the crude product was added to 120mL 18Ω H$_2$O and 100mL TEA. CO$_2$ was bubbled into the mixture until it became homogeneous, extracted with six 100mL portions CH$_2$Cl$_2$. The organic extracts were combined, dried over MgSO$_4$, filtered, concentrated with rotary evaporation, and placed under vacuum to yield 2.372g of a dark brown solid. 1.539g of the dark brown solid was mixed with 140mL EtOAc and heated to boiling before the supernatant was decanted, cooled to room temperature. 1.357g potassium 2-ethylhexanoate dissolved in 50mL EtOAc was added to the supernatant, producing a precipitate. The solid was collected by centrifugation, washed with two 40mL portions EtOAc, and dried under vacuum to produce 0.2123g of the crude product which was recrystallized from 12mL CH$_3$OH and 15mL EtOAc. After concentrating to 10mL total volume the mixture brought to room temperature and placed on ice for several minutes before collecting the solid by centrifugation and drying under vacuum to yield 0.1257g (5% yield) 46 (98% pure, 2% pyruvate mp = 198˚C (darkens from brown to dark brown), > 400˚C
1H NMR (300MHz, D₂O): δ8.21 (d, J=8.4Hz, 2H); 7.72 (d, J=8.7Hz, 2H); 7.46 (q<sub>AB</sub>, J=15.3Hz, 8.4Hz, 1H); 7.20 (m, 2H); 6.46 (d, J=15.6Hz, 2H)

13C NMR (300MHz, D₂O): δ198.9; 173.6; 150.9; 149.0; 144.1; 142.4; 132.3; 129.9; 129.0; 125.8

MS (DUIS, + ion): 324 (M + K⁺, 89%), 150 (C₈H₇NO₂ + H⁺, 100%)

MS (DUIS, - ion): 531 (2M⁻+K⁺, 65%); 493 (2M⁻+H⁺, 69%); 246 (M⁻, 38%); 202 (M⁻ - CO₂, 100%)

UV-VIS: λ<sub>max</sub>=356nm; ε=16922 L mol⁻¹ cm⁻¹

**Sodium trifluoropyruvate (47)**

3.760g ethyl trifluoropruvate was combined with 50mL 1.2M HCl and heated at reflux for 21h at which time hydrolysis appeared complete by TLC (R<sub>f</sub> ester= 0.22, R<sub>f</sub> prod=0.0, 10% CH₃CN/CH₂Cl₂, DNP stain). The reaction mixture was concentrated to dryness with rotary evaporation, redissolved in 50mL 18Ω H₂O, and concentrated to dryness with rotary evaporation again. The crude product was dissolved in 30mL 18Ω H₂O neutralized to pH7 with NaOH(aq), concentrated to near dryness with rotary evaporation, and azeotropically dried by evaporation of toluene. The 47 was placed under vacuum overnight to generate 3.542g (91% yield, mp = 206°C(d)) as a white solid.

1H NMR (300MHz, D₂O): no signals observed

13C NMR (300MHz, D₂O): δ 171.1; 122.4 (q); 92.2 (q)
ESI-MS (DUIS, - ion): 227 (M + HCO$_2^-$, 41%); 159 (M$^-$ (hydrate), 100%); 141 (M$^-$ (keto form), 85%)

5-methyl-2-(2,2,2-trifluoroacetyl)furan (50)

8.0mL 2-methylfuran (88.67mol) and 60mL hexane were combined in a 200mL round bottom flask. The reaction mixture was placed under an atmosphere of N$_2$ and submerged into an ice bath and stirred for 20min to allow for thermo-equilibration. 8.25mL (59.35mmol) trifluoroacetic anhydride was then injected into the reaction mixture dropwise and stirred rapidly at 0°C for 2.5h. The reaction mixture was concentrated with rotary evaporation to 1/6 volume before being poured into a separatory funnel containing 150mL 0°C sat.NaHCO$_3$ and 30mL Et$_2$O. The organic layer was removed with the aqueous phase being countercurrently extracted with three 30mL portions of Et$_2$O. The organic phases were combined, dried over MgSO$_4$, filtered, rinsed with 10mL Et$_2$O, and concentrated with rotary evaporation to afford 12.439g (118% crude yield) of a free flowing orange liquid. The crude product was purified by vacuum distillation with the following parameters:

Pressure: 20torr Fraction 1 collected with 80°C head temp and oil bath temp 119-126°C

Pressure: 20torr Fraction 2 collected with 80°C head temp and oil bath temp 128-139°C

Fraction 1: 6.7228g Fraction 2: 1.034g were both 50 to generate a 74% purified yield

$^1$H NMR (300MHz, CDCl$_3$): δ7.45 (d, bs, 1H); 6.33 (d, J=3.6Hz, 1H); 2.48 (s, 3H)
1.354g Mg\(^0\) (55.71mmol) was crushed with a mortar and pestle, placed into a 250mL round bottom flask, and placed under an atmosphere of N\(_2\). 110mL THF (distilled, Na\(^0\)/benzophenone) and 14mL (110.3mmol) TMSCl were placed into the reaction vessel before being submerged in an ice bath while stirring rapidly. A solution of 5.004g (28.09mmol) \(\text{50}\) in 28mL THF was added dropwise at the rate of 1drop/5sec. After 1h the reaction mixture darkened to a blackish blue. 30min after all of the \(\text{50}\) solution was added the reaction mixture was filtered. The crude yellow liquid was combined with 20mL THF and added to rapidly stirring 4.4mL conc. HCl on an ice bath. The mixture was stirred at 0°C for 30min before being poured into a separatory funnel containing 30mL Et\(_2\)O and 35mL sat. NaHCO\(_3\). The organic layer was removed and the aqueous phase was extracted with two 30mL portions of Et\(_2\)O. The organic portions were combined, dried over MgSO\(_4\), filtered, concentrated with rotary evaporation, and placed under vacuum to remove residual solvent for 30s to afforded 3.838g (85% crude yield) of a yellow liquid. \(^1\)H NMR analysis determined the crude product was 88% by mass for afford a corrected mass to be 3.3889g (75% NMR yield). The reaction mixture was concentrated to 1/8 total volume with rotary evaporation before diluting with 300mL hexane, causing a white solid to precipitate. The white solid was removed via filtration over Celite. The filtrate was concentrated with rotary evaporation and placed under vacuum to remove residual solvent for 30s to afford 5.200g (80% crude yield) of a yellow liquid. Crude \(\text{51}\) purified by vacuum distillation with the following parameters: Pressure: 20torr Fraction 1: 96-105°C head temp,
130-140°C oil temp, Pressure: 20torr Fraction 2: 105-103°C head temp, 140-160°C oil temp.

64% yield of pure 51

\(^1\)H NMR (300MHz, CDCl\(_3\)): \(\delta 7.39\) (bs, 1H); 6.24 (d, \(J=3.6\)Hz, 1H); 6.12 (t, \(J=53.7\)Hz, 1H); 2.40 (s, 3H)

_Methyl difluoropyruvate methyl hemiacetal (52)_

0.518g (3.237mmol) 51 was combined with 16mL CH\(_3\)OH in a 50mL round bottom flask was placed under a nitrogen atmosphere and submerged in a -78°C bath. After stirring for 3min a mixture of O\(_3\)/O\(_2\) was bubbled into the reaction mixture with the following parameters: A2Z O\(_3\) generator Model#A2Z-5GLAB, 0.56Amp on meter(100% production), 13PSI O\(_2\) from the regulator, 0.5L/min bubble flow rate. After 25min of bubbling the reaction mixture was observed to be faint greenish yellow in color and after 35min of bubbling, the reaction mixture became colorless. After 1h, the O\(_3\)/O\(_2\) bubbling was ceased and N\(_2\) then bubbled through the reaction mixture for 18min. The reaction vessel was then moved to a -47°C bath (m-xylene/N\(_2\)(l)) for 2min before the dropwise addition of 0.07mL conc. H\(_2\)SO\(_4\) and 0.2126g 3Å molecular sieves. After warming to room temperature the mixture was heated at reflux for 16h. The reaction mixture was concentrated to ~4mL total volume with rotary evaporation. The H\(_2\)SO\(_4\) was removed by passing over a column (49mmx24mm) silica gel and eluted with 10% CH\(_3\)OH/CH\(_2\)Cl\(_2\) collecting 2.5mL fractions. Desired product collected in fractions 5-16 with \(^1\)H NMR yield of the 35% of the desired product as a mixture with CF\(_2\)HCOOH. (Note: Use ionic exchange technique only)
Removal of the acidic side product via ion exchange chromatography by the following method: 10.2231g Amberlyst A-21 (swollen with 18.1MΩ H₂O) was used to make a 10.25” tall by 10mm wide column. 300mL 5M NH₃ was passed through the column followed by 18.1Ω H₂O until the effluent was neutral to pH Hydron paper then 150mL MeOH. The reaction concentrate was loaded on top of the column and eluted with MeOH. Seventeen 3mL fraction were collected at a rate of ~1drop/s. Eight 5mL fraction were then collected at a rate of ~2drops/s. Fractions 3-19 contained the desired product was visualized by KMnO₄ stain on silica.

^1^H NMR (300MHz, CDCl₃): δ5.81 (t, J=54.9Hz, 1H); 3.94 (s, 3H); 3.34 (s, 3H)

Sodium difluoropyruvate hydrate (53)

0.0652g (0.4178mmol) mixture of 52 and the ethyl hemiacetal derivative were dissolved in 20mL 3M HCl, placed under an atmosphere of N₂, and heated to reflux for 17h being cooled to room temperature. The reaction mixture was filtered (as specks of brown solid formed in the mixture overnight), concentrated with rotary evaporation, and dried azeotropically with CH₃CN and toluene, and placed under vacuum to remove residual solvent for 2h to afford 0.0464g (78% crude yield) of a yellow solid (difluoropyruvic acid). The crude product was dissolved in 15mL 18.1MΩ H₂O and titrated to neutrality with a solution of NaOH. The mixture was concentrated with rotary evaporation, dried azeotropically with CH₃CN and toluene, and placed under vacuum overnight to remove residual solvent to afford 0.0470g (69%crude yield) of a tannish solid.
0.253g (0.816mmol) N,N’-Di-Boc-1H-pyrazole-1-carboxamidine was placed under an atmosphere of \( \text{N}_2 \) before being dissolved in 4.1mL CH\(_3\)CN. The mixture was set to stir before the addition of 0.27mL (2.48mmol) aminoacetaldehyde dimethyl acetal. The mixture was allowed to stir for 1.5h before TLC showed only the presence of the desired product (0.43 25% EtOAc/CH\(_2\)Cl\(_2\), silica). The mixture was concentrated with rotary evaporation and placed under vacuum overnight to remove residual solvent to afford 0.218g \( \text{54} \) (99% yield, mp = 101-104°C) as a white solid.

\( ^1\text{H NMR (300MHz, CDCl}_3\): } \delta 11.46 (s, 1H); \delta 8.48 (s, 1H); \delta 4.48 (t, J=5.4Hz, 1H); \delta 3.59 (q\(_{AB}\), J=5.4Hz, 2H); \delta 3.40 (s, 6H)

\( ^{13}\text{C NMR (300MHz, CDCl}_3\): } \delta 163.6; 156.4; 153.1; 102.0; 83.2; 79.4; 54.1; 42.3; 28.4; 28.2

MS (EI, 70eV): 347 (M\(^+\), 100%); 290 (M\(^+\) - C\(_4\)H\(_8\), 30%); 236 (35%)

0.159g (0.512mmol) N,N’-Di-Boc-1H-pyrazole-1-carboxamidine was dissolved in 3mL CH\(_3\)CN before 0.12mL (1.600mmol) allyl amine was added to the reaction mixture and subsequently
placed under an inert atmosphere. The mixture was allowed to stir at room temperature for 3h before the reaction mixture was concentrated with rotary evaporation and placed under vacuum overnight to remove residual solvent to afford 0.147g (96% yield) of off-white solid 55.

\[ \text{1}^H \text{ NMR (300MHz, CDCl}_3\text{): } \delta 11.52 \text{ (s, 1H); 8.41 (s, 1H); 5.89 (m, J= 15.9; 10.2; 5.1Hz, 1H); 5.24 (d, J= 17.1; 1.2Hz, 1H); 5.17 (d, J= 10.2Hz, 1H); 4.08 (dd, J= 5.4; 1.2Hz, 2H); 1.50 (s, 18H) } \]

\[ \text{13}^C \text{ NMR (300MHz, CDCl}_3\text{): } \delta 163.7; 156.2; 153.4; 133.4; 116.9; 83.7; 79.5; 43.4; 28.4; 28.2 \]

\[ \text{MS (Cl): } 299 (\text{M}^+ \text{, 10%}); 243 (\text{M-C}_3\text{H}_7\text{N+H}^+ \text{, 17%}); 188 (\text{M}^+-111, 100%) \]

**N-allyl guanidinium trifluoracetate (57)**

0.106g (0.355mmol) 55 was dissolved in 3.0mL CH\(_2\)Cl\(_2\) before the addition of 1.0mL CF\(_3\)COOH. The reaction mixture was set to stir and heated at reflux for 3h. The reaction mixture was allowed to cool to room temperature, concentrated by rotary evaporation, and dried azeotropically via evaporation of toluene. After being placed under vacuum overnight to remove residual solvent afforded 0.0819g (108%) 57.

\[ \text{1}^H \text{ NMR (300MHz, D}_2\text{O): } \delta 5.86 \text{ (m, 1H); 5.28 (dd, J= 12.3; 0.9Hz, 1H); 5.23 (dd, overlapped, 2H); 3.83 (d, J= 4.8Hz, 2H) } \]

\[ \text{13}^C \text{ NMR (300MHz, CD}_3\text{CN): } \delta 158.8; 133.6; 117.3; 44.2 \]

\[ \text{ESI-MS (DUIS, + ion): } 100 (\text{M-CF}_3\text{COO}^+, 100%) \]
0.0331g (0.1459mmol) 1-allylguanidinium trifluoroacetate and 0.0856g (0.4002mmol) NaIO₄ were combined in a 25mL pear bottom flask along with 0.08mL CD₃OD. 0.0037g (0.0100mmol) K₂OsO₄·2H₂O was dissolved in 0.50mL D₂O, injected into the reaction mixture, and quantitatively transferred with 0.50mL D₂O. The mixture was allowed to stir at room temperature. Shortly after mixing, a white solid was observed to precipitate from the reaction mixture. The reaction was allowed to mix for 2.5 h before being analyzed by ¹H NMR at which point the reaction was determined to be complete. The reaction mixture was filtered, rinsed with 6mL CH₃OH, and combined with NMR sample. Dowex 2-X8 ion exchange resin was swelled with 3M HCl. A column 28cm tall and 2cm wide was constructed with the resin and rinsed with 300mL 3M HCl. 18.1Ω H₂O was run through the column until the effluent was neutral to pH Hydron paper (180mL) followed by 150mL CH₃OH. The methanolic solution was loaded on the column and eluted with CH₃OH. Twenty 10mL fractions were collected at the rate of 10mL/min. All fractions were negative to starch iodide paper with TLC showing the majority of the material collected in fractions 3-8. All fractions were combined, concentrated with rotary evaporation, dried azeotropically by evaporation of toluene, and placed under vacuum overnight to remove residual solvent to afford 0.0254g (128% crude yield). ¹H NMR analysis in D₂O showed the product to be 92% by mass 58, 5% by mass 2-aminoimidizole, and 3% by mass toluene.

¹H NMR (300MHz, D₂O): δ5.57 (dd, J= 6.9; 1.5Hz, 1H); 3.89 (dd, J= 11.7; 6.9Hz, 1H); 3.52 (dd, J= 11.7; 1.5Hz, 1H)
12.0mL anhyd. CH$_3$OH was placed into a flame dried 25mL round bottom flask placed into an ice/salt bath, allowed to thermoequilibrare before the dropwise addition of 1.0mL (13.71mmol) SOCl$_2$. 0.5242g (2.673mmol) L-Selenomethionine added to the mixture, placed under an atmosphere of N$_2$, sceptum wired on, and allowed to warm to room temperature in the air while stirring for 22h. TLC was take and showed the reaction to be complete (4:1:1 nBuOH:AcOH:H$_2$O, silica, KMnO$_4$, R$_f$ a.a: 0.65, R$_f$ ester: 0.38) Note: Mixture should be concentrated with rotary evaporation to dryness and dissolved in CH$_3$OH before precipitation. The reaction mixture was quantitatively transferred to a beaker with 5.0mL distilled CH$_3$OH before the addition of 150mL cold, anhyd. Et$_2$O. The beaker was placed into an ice bath for 5 min before the collection of the white solid that formed by vacuum filtration and dried under vacuum overnight 0.4598g (70% yield, mp = 158-161°C) 62 as a white solid.

$^1$H NMR (300MHz, D$_2$O): δ4.29 (t, J=6.3, 1H); 3.86 (s, 3H); 2.67 (t, J=6.9Hz, 2H); 2.23 (m, 2H); 2.02 (s, 3H)

$^{13}$C NMR (300MHz, D$_2$O): δ176.3; 59.5; 58.4; 24.7; 9.1

ESI-MS (DUIS, + ion): 253 (M$^+$ + CH$_3$CN, 57%); 212 (M$^+$, 100%); 210 (M$^+$ ($^{78}$Se), 56%)
0.4078g (1.6538mmol) 62 and 0.4644g (5.529mmol) NaHCO₃ were combined in 5.0mL distilled CH₃OH. 0.6652g (3.048mmol) Boc₂O was dissolved in 3.0mL distilled CH₃OH, injected into the reaction mixture, and quantitatively transferred using 2.0mL distilled CH₃OH. The mixture was placed under an atmosphere of N₂ and stirred at room temperature for 15h at which time TLC was taken and showed the reaction to be complete (4:1:1 nBuOH:AcOH:H₂O, silica, KMnO₄ visualized Rᶠ SM: 0.44, Rᶠ prod: 0.94). 0.1131g (1.507mmol) glycine was added to the reaction mixture and stirred for 1h before the reaction mixture was filtered and concentrated with rotary evaporation before being diluted with 20mL CH₂Cl₂ and poured into a separatory funnel containing 50mL sat. NaHCO₃ and 20mL CH₂Cl₂. The organic layer was removed and the aqueous phase was extracted with two 25mL portions CH₂Cl₂. The organic portions were combined, dried over MgSO₄, filtered, concentrated with rotary evaporation, and placed under vacuum overnight to remove residual solvent to afford 0.4046g (79% yield) of the desired product 63 as a pale yellow oil.

¹H NMR (300MHz, CDCl₃): δ5.09 (s, 1H); 4.41 (s, 1H); 3.76 (s, 3H); 2.55 (t, J=8.1Hz, 2H); 2.20 (m, 1H); 2.00 (m, 1H); 2.00 (s, 3H); 1.45 (s, 9H)

¹³C NMR (300MHz, CDCl₃): δ172.7; 155.2; 79.7; 53.4; 52.2; 33.1; 28.2; 20.3; 4.0

ESI-MS (DUIS, + ion): 312 (M + H⁺, 33%); 253 (M + H⁺- COOME, 54%); 212 (M + H⁺-Boc, 100%)
N-Boc-L-Selenomethionine oxide methyl ester (64)

0.3192g (1.029mmol) 63 was dissolved in 13mL distilled CH$_3$OH, diluted with 7.0mL 18Ω H$_2$O, and submerged in an ice bath. The mixture was allowed to thermoequilibrate before the addition of 0.2347g (1.097mmol) NaIO$_4$. The mixture was set to stir rapidly in the ice bath for 2h before it was confirmed to be complete by TLC (4:1:1 nBuOH/AcOH/H$_2$O, silica, KMnO$_4$ R$_f$ Se: 0.94, R$_f$ SeO: 0.60). The reaction mixture was filtered, rinsed with 10mL distilled CH$_3$OH, and concentrated with rotary evaporation to remove the methanolic portion before it was diluted with 13mL 80% sat. NaCl. The aqueous phase was then countercurrently extracted with three 40mL portions EtOAc. The organic portions were combined, dried over MgSO$_4$, filtered, concentrated with rotary evaporation, and placed under vacuum overnight to afford 0.2335g of a pale yellow oil that was 4.6% by mass EtOAc to yield 0.2247g (67% yield) 64.

$^1$H NMR (300MHz, CDCl$_3$): δ5.57 (d, J=6.6Hz, 1H); 4.41 (m, 1H); 3.78 (s, 3H); 2.95 (m, 1H); 2.79 (m, 1H); 2.54 (s, 3H); 2.27 (m, 1H); 2.13 (m, 1H); 1.45 (s, 9H)

L-Methionine methyl ester hydrochloride (66)

12.0mL CH$_3$OH (distilled from Mg$^0$/I$_2$) was placed into a 25mL flame dried round bottom flask. The vessel was submerged in an ice/salt bath (-12°C) and allowed to thermoequilibrate before the dropwise addition of 2.5mL (34.27mmol) SOCl$_2$. 1.003g (6.721mmol) L-Methionine was then added to the methanolic mixture, placed under an atmosphere of N$_2$, sceptum wired
on, and allowed to warm to room temperature in air for 17h. TLC was then taken, showing the reaction to be complete. (4:1:1 nBuOH/AcOH/H₂O, silica, KMnO₄, L-Met Rₖ a.a.: 0.35, Rₖ ester:0.45) Note: Reaction mixture should be concentrated with rotary evaporation to dryness and dissolved in MeOH before continuing. The reaction mixture was cooled to 0°C before the addition of 150mL cold Et₂O, causing a white solid to precipitate. The white solid was collected by vacuum filtration and dried under vacuum overnight (1ˢᵗ crop). White solid was observed to have formed in the filtrate which was collected by vacuum filtration and dried under vacuum overnight (2ⁿᵈ crop). The two crops were combined to afford 1.215g 66 (90% yield) as a white solid.

¹H NMR (300MHz, D₂O); δ4.29 (t, J=6.3Hz, 1H); 3.86 (s, 3H); 2.69 (t, J=7.2Hz, 2H); 2.26 (m, 2H); 2.12 (s, 3H)

_N-Boc-L-Methionine Methyl Ester (67)_

0.7251g (3.631mmol) 66 and 0.7234g (8.613mmol) NaHCO₃ were combined with 5.0mL distilled CH₃OH. 1.083g (4.963mmol) Boc₂O was dissolved in 3.0mL distilled CH₃OH, injected into the reaction mixture, and quantitatively transferred using 2.0mL distilled CH₃OH. The mixture was placed under an atmosphere of N₂, and stirred rapidly at room temperature for 18h. TLC was taken, showing only the presence of 67 (4:1:1 nBuOH:AcOH:H₂O, silica, KMnO₄ visualized Rₖ SM: 0.49, Rₖ prod: 0.93). 0.1828g (2.435mmol) glycine was added to the reaction mixture and stirred at room temperature for 3h before the mixture was filtered, concentrated with rotary evaporation to near dryness, diluted with 20mL CH₂Cl₂ and poured into a separatory funnel containing 20mL CH₂Cl₂ and 50mL sat. NaHCO₃. The organic layer was removed and the
aqueous phase was extracted with two 25mL portions CH\(_2\)Cl\(_2\). The organic portions were combined, dried over MgSO\(_4\), filtered, concentrated with rotary evaporation, and placed under vacuum overnight to remove residual solvent to afford 0.8652g (90\% yield) of 67 as a pale yellow oil.

\(^1\)H NMR (300MHz, CDCl\(_3\)): δ5.11 (s, 1H); 4.41 (s, 1H); 3.76 (s, 3H); 2.54 (t, J=7.8Hz, 2H); 2.10 (s, 3H); 2.10 (m, 1H); 1.94 (m, 1H); 1.45 (s, 9H)

\(N\)-Boc-\(L\)-Methionine sulfoxide Methyl Ester (68)

0.5496g (2.087mmol) 67 was dissolved in 7.0mL distilled CH\(_3\)OH to which was added 4.0mL 18Ω H\(_2\)O. The mixture was submerged in an icebath and stirred rapidly for 3min to thermoequilinate before the addition of 0.4712g (2.203mmol) NaIO\(_4\). The mixture was stirred rapidly for 1.5h during which a white solid formed in the reaction volume. After 1.5h TLC showed the reaction to be complete (4:1:1 nBuOH:AcOH:H\(_2\)O, silica, KMnO\(_4\) visualized S R\(_f\): 0.93, SO R\(_f\): 0.58). The reaction mixture was filtered and concentrated with rotary evaporation to near dryness before being diluted with 15mL 80\% sat. NaCl. The aqueous phase was countercurrently extracted with four 40mL portions of EtOAc. The organic phases were combined, dried over MgSO\(_4\), filtered, concentrated with rotary evaporation, and placed under vacuum overnight to remove residual solvent to afford 0.6495g of a pale yellow oil which was determined to be 16.9\% by mass EtOAc to yield 0.5397g (93\% yield) 68.
$^1$H NMR (300MHz, CDCl$_3$): δ5.27 (s, 1H); 4.43 (m, 1H); 3.78 (s, 3H); 2.79 (t, J=7.8, 2H); 2.57 (s, 3H); 2.35 (m, 1H); 2.14 (m, 1H); 1.45 (s, 9H)

$L$-Methionine sulfoxide (70)

3.005g (20.14mmol) L-Methionine was suspended in 10mL DI H$_2$O in a 25mL round bottom flask. 2.2mL 30% H$_2$O$_2$ was added to the suspension in the following manner: 0.36mL over the course of 2min, dropwise with 5min of stirring until 1.44mL total volume of 30% H$_2$O$_2$ was added. At this point the mixture became homogeneous. The mixture was allowed to stir for 1.5h (Note: Does not appear to be necessary). After this time 0.36mL 30% H$_2$O$_2$ followed by a 0.40mL 30% H$_2$O$_2$ portion added to the reaction in the same manner as described above. After 1.2h of stirring at RT, TLC showed all of the starting material consumed. 150mL 200proof EtOH was then added to the aqueous solution causing a white solid to precipitate. The mixture sat at room temperature for 30min before the white solid was collected by vacuum filtration, rinsed with 20mL 200proof EtOH, and dried under vacuum overnight to remove residual solvent to afford 3.096g 70 (93% yield, mp = 254˚C(d)) as a white solid.

$^1$H NMR (300MHz, D$_2$O): δ3.87 (q, J=6.3Hz, 1H); 3.02 (m, 2H); 2.74 (s, 3H); 2.33 (q, J=7.2Hz, 2H)

$^{13}$C NMR (300MHz, D$_2$O): δ173.8; 54.0; 48.9; 37.1; 24.4

ESI-MS (DUIS, + ion): 188 (M + Na$^+$, 100%); 166 (M + H$^+$, 14%)
20.09g (0.1216mol) L-Methionine sulfoxide was combined with 120mL (0.4872mol) bis-(trimethylsilyl)acetamide and set to stir in a 25-80°C oil bath for 19h after which it was diluted with 80mL Ph₂O. The mixture was added to 300mL boiling Ph₂O over the course of 33.5min. Distillate was collected with pot temperature not dropping below 250°C and a head temperature range of 238-248°C. The 275mL EtOH and 30mL H₂O was poured into the distillate (417mL). The resulting mixture thickened and required being shaken by hand periodically over 20min. Volatiles were removed by rotary evaporation before the addition of 20mL H₂O and 150mL hexane. The mixture was shaken well and allowed to separate before the removal of the organic phase. The aqueous phase was washed with another 150mL hexane before the addition of 400mL CH₃CN which caused a tan solid to precipitate. The solid was collected by vacuum filtration, rinsed with 40mL CH₃CN, and placed under vacuum overnight to remove residual solvent to afford 7.672g. 7.267g of the crude product was triturated with 155mL boiling anhyd. EtOH for 5min before being collected by vacuum filtration, rinsed with 20mL cold EtOH, and dried under vacuum overnight to yield 4.568g 71 (37% yield, 95% purity as estimated by NMR, mp = 216°C(d)) (35% yield overall).

^1^H NMR (300MHz, D₂O); 85.97 (ddd, J=10.2; 6; 4.5Hz, 1H); 5.48 (d, J= 10.2Hz, 1H); 5.48 (d, J=6.3Hz, 1H); 4.27 (d, J=4.5Hz, 1H)

^1^3^C NMR (300MHz, D₂O); 6173.5; 130.8; 122.0, 57.7

ESI-MS (DUIS, + ion): 102 (M + H⁺, 22%)

HRMS (IT-TOF-ESI, + ion): Calc’d C₄H₇NO₂ (M + H⁺): 102.0550. Found: 102.0553
(S)-2-((R)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)but-3-enoic acid (72)

0.3371g (0.8090mmol) 98% Fmoc-L-Ala-OSu was dissolved in 10mL acetone to which was added 0.1317g (1.568mmol) NaHCO₃ dissolved in 5mL H₂O (using 2mL H₂O to quant. trans.) which caused the bicarbonate to precipitate. 0.1144g (1.075mmol) 71 dissolved in 5mL H₂O was then added to the mixture, using 2mL H₂O to quantitatively transfer. Upon addition the mixture became homogeneous and was set to stir at room temperature for 3h at which time TLC showed the reaction to be complete (R_f OSu=0.54, silica, 10% CH₃OH/CH₂Cl₂). The mixture was acidified to pH1 with 1mL 12M HCl and extracted with three 35mL CH₂Cl₂. The organic portions were combined, washed with three 35mL portions 3M HCl, dried over MgSO₄, filtered, concentrated with rotary evaporation, and placed under vacuum overnight to remove residual solvent to afford 0.3131g of the desired dipeptide 72 as a white solid (98% yield, mp = 176˚C (shrinks), 184-188˚C)

¹H NMR (300MHz, CD₃OD): δ7.79 (d, J=7.5Hz, 2H); 7.67 (dd, J=7.2Hz, 2H); 7.39 (t J=7.2Hz, 2H); 7.31 (t, J=7.2Hz, 2H); 6.00 (m, J=16.8Hz, 2H); 5.38 (d, J=16.8Hz, 1H); 5.25 (d, J=10.2Hz, 1H); 4.98 (d, J=5.1Hz, 1H); 4.36 (t, J=6.9Hz, 2H); 4.22 (d, J=6.6Hz, 1H); 4.22 (d, J=6.6Hz, 1H); 1.37 (d, J=7.2Hz, 3H)

¹³C NMR (300MHz, CD₃OD): δ175.3; 173.1; 158.3; 145.4; 145.2; 142.6; 133.5; 128.8; 128.2; 126.3; 120.9; 117.9; 68.0; 56.0; 51.8; 18.2

HRMS (IT-TOF-ESI, -ion): Calc’d (C₂₂H₂₂N₂O₅): 393.1456. Found: 393.1458
1.024 g (2.974 mmol) Fmoc-OSu was dissolved in 13 mL acetone to which was added 0.3344 g (3.143 mmol) 71 dissolved in 11 mL H₂O and 0.5330 g (6.344 mmol) NaHCO₃ dissolved in 9 mL H₂O. Upon addition, the vinylglycine precipitated out. The mixture was heated at 65°C for 1 h before the mixture was observed to be homogeneous. The mixture was removed from the heat and stirred at room temperature for 26 h at room temperature at which time TLC did not show Fmoc-OSu (Rₛ=0.73, silica, 10% CH₃OH/CH₂Cl₂). The mixture was then acidified to pH 1 with 1 mL 12 M HCl and extracted with three 35 mL portions CH₂Cl₂. The organic phases were combined, washed with three 35 mL portions 4 M HCl, dried over MgSO₄, filtered, rinsed with 20 mL CH₂Cl₂, concentrated with rotary evaporation, and placed under vacuum overnight to remove residual solvent to afford 0.876 g of the desired product as an off-white solid (91% yield, mp = 146-149°C). 0.0484 g of the crude product was dissolved in 0.6 mL EtOAc and diluted with 14 mL hexane producing a cloudy white mixture which was allowed to sit at room temperature for 1 h to produce crystals. The solid was collected by centrifugation, washed with 10 mL hexane, and dried under vacuum to remove residual solvent to afford 0.0448 g 73 (93% recovery, mp = 160-161°C).

¹H NMR (300 MHz, CD₃OD): δ 7.80 (d, J=7.5 Hz, 2H); 7.68 (d, J=6.9 Hz, 2H); 7.39 (t, J=7.5 Hz, 2H); 7.31 (t, J=7.5 Hz, 2H); 6.01 (ddd, J=16.8, 10.2, 5.7 Hz, 1H); 5.36 (d, J=17.4 Hz, 1H); 5.26 (d, J=10.2 Hz, 1H); 4.78 (d, J=5.4 Hz, 1H); 4.36 (m, 2H); 4.24 (t, J=7.2 Hz, 1H)
$^{13}$C NMR (300MHz, CD$_3$OD): δ145.3; 145.2; 133.9; 128.8; 128.2; 128.2; 126.5; 120.9; 117.8; 68.1; 57.8; 48.4

MS (DUIS, - ion): 322 (M - H$^+$, 100%)

HRMS (IT-TOF-ESI, - ion): Calc’d C$_{19}$H$_{17}$NO$_4$ (M - H$^+$): 322.1085. Found: 322.1091

$$\text{(S)-2-((tert-butoxycarbonyl)amino)but-3-enoic acid (74)}$$

0.1624g L-Vinylglycine (1.542mmol) was combined with 0.5228g (2.428mmol) Boc$_2$O, 0.6813g (8.112mmol) NaHCO$_3$, and suspended in 12mL CH$_3$OH. The mixture was set to stir at room temperature for 24h at which time TLC show complete reaction (10% AcOH/CH$_2$Cl$_2$ $R_f$: 0.42). The mixture was filtered and the filtrate was concentrated to dryness with rotary evaporation, and dissolved in 20mL CH$_2$Cl$_2$ and 20mL sat. NaHCO$_3$. The organic layer was removed and the aqueous phase was washed with two 20mL CH$_2$Cl$_2$ before acidifying to pH3 with conc. H$_3$PO$_4$. After extracting with four 20mL portion EtOAc the organic phases were combined, dried over MgSO$_4$, filtered, concentrated with rotary evaporation, diluted with 30mL toluene, concentrated to dryness, and placed under vacuum overnight to remove residual solvent to yield 0.2601g (84%) of a pale yellow oil.

$^1$H NMR (300MHz, CD$_3$OD): δ5.97 (ddd, J=16.8; 10.8; 4.5Hz, 1H); 5.34 (d, J=17.1Hz, 1H); 5.23 (d, J=10.5Hz, 1H); 4.69 (d, J=4.5Hz, 1H); 1.45 (s, 9H)

$^{13}$C NMR (300MHz, CD$_3$OD): δ174.0; 157.7; 134.3; 117.4; 80.7; 57.5; 28.7

ESI-MS (DUIS, - ion): 423 (2M$^- + $ Na$^+$, 52%); 401 (2M$^- + $ H$^+$, 100%); 200 (M - H$^+$, 72%)

HRMS (IT-TOF-ESI, + ion) Calc’d C$_9$H$_{15}$NO$_4$Na (M+Na$^+$): 224.0893. Found 224.0890
Dicyclohexyl ammonium 2-((tert-butoxycarbonyl)amino)but-3-enoate (75)

0.1682g (0.8360mmol) 74 was dissolved in 1.5mL CH₂Cl₂ to which was added 0.20mL (1.006mmol) dicyclohexylamine. The mixture was brought to a boil, diluted with 12mL hexanes added at a rate that kept the mixture boiling, and concentrated to one third total volume. The mixture was allowed to cool to room temperature before being placed into a -20°C freezer for 1.5h. The solid was collected by centrifugation, washed with 10mL hexane, and placed under vacuum overnight to remove residual solvent to afford 0.2760g (86% yield, mp = 152°C (d)) of the desired salt 75 as a yellow solid.

¹H NMR (CDCl₃): δ6.00 (m, J=16.2; 11.1; 6.3Hz, 1H); 5.62 (d, J=17.1Hz, 1H); 5.25 (d, J= 4.8Hz, 1H); 5.09 (d, J= 10.2Hz, 1H); 4.48 (s, 1H); 2.95 (m, cyclohexyl, 2H); 1.99-1.67 (cyclohexyl, 10H); 1.43 (s, 9H); 1.40-1.20 (cyclohexyl, 10H)

¹³C NMR (CDCl₃): δ176.6; 157.2; 137.7; 114.2; 80.2; 59.9; 54.4; 30.6; 28.8; 26.2; 25.5

ESI-MS (DUIS, + ion): 223 (H₂NCy₂ + CH₃CN, 76%); 182 (HNCy₂ + H⁺, 100%)

ESI-MS (DUIS, - ion): 401 (2M⁻ + H⁺, 44%); 246 (M + HCOO⁻, 72%); 200 (M - H⁺, 100%)

HRMS (IT-TOF-ESI, + ion): calc’d C₁₂H₂₃N (HNCy₂ + H⁺): 182.9000. Found: 182.9003

HRMS (IT-TOF-ESI, - ion): calc’d (C₉H₁₃NO₄ - H⁺): 200.0928. Found: 200.0925
**Allylammonium chloride (76)**

3mL (40.02mmol) allyl amine was added to 6mL 12M HCl. The mixture was concentrated to dryness with rotary evaporation, dissolved in 15mL DI H$_2$O, and concentrated again using 5mL CH$_3$OH and 15mL toluene to dry by azeotropic evaporation. The resulting white solid was placed under vacuum overnight to yield 2.668g 76 (71% yield).

$^1$H NMR (300MHz, D$_2$O): δ5.94 (m, 1H); 5.43 (d, J=18.3Hz, 1H); 5.41 (d, J=9.9Hz, 1H); 3.62 (d, J=5.7Hz, 2H)

**tert-butyl allylcarbamate (78)**

2.096g (9.125mmol) Boc$_2$O was dissolved in 10mL CH$_3$OH. The mixture was set to stir rapidly before the slow addition of 0.85mL (11.330mmol) allyl amine. Upon addition the reaction volume became exothermic and evolved gas. After 30min TLC showed the consumption of Boc$_2$O (5% CH$_3$OH/CH$_2$Cl$_2$ Rf prod= 0.78, R$_f$ Boc$_2$O= 0.96). The reaction mixture was concentrated to near dryness with rotary evaporation, diluted with 20mL toluene, concentrated to dryness with rotary evaporation, and placed under vacuum for 1.5h to afford 1.075g (75% yield ,mp = 28-34°C) of 78 as a yellow solid.

Note: Product is volatile and will evaporate away if left under vacuum overnight. 1.5h appears to the the amount of time needed at this scale to give the driest product with the best yield.

$^1$H NMR (300MHz, CDCl$_3$): δ5.84 (m, J=17.1; 10.2; 1.5Hz, 1H); 5.18 (dd, J=17.1; 1.5Hz, 1H); 5.11 (dd, J=10.2; 1.2Hz, 1H); 4.60 (s, 1H); 3.75 (nt, unresolved, 2H); 1.45 (s, 9H)

$^{13}$C NMR (300MHz, CDCl$_3$): δ115.9; 135.0; 115.8; 79.4; 43.2; 28.5
2.000g (5.810mmol) Fmoc-OSu was dissolved in 20mL CH$_2$Cl$_2$ (distilled from CaH$_2$). 2.0mL (26.12mmol) allyl amine was injected into the mixture was set to stir at room temperature 1h after which time the Fmoc-OSu was not seen by TLC ($R_f$ Fmoc-OSu= 0.16, $R_f$ prod= 0.33). The mixture was filtered with vacuum over a pad of Celite and rinsed with 20mL CH$_2$Cl$_2$. The filtrate was washed with four 40mL portions 0.1M HCl$_{aq}$. The organic phase was dried over MgSO$_4$, filtered, concentrated with rotary evaporation, and placed under vacuum overnight to remove residual solvent to yield 1.5476g 79 (95% yield, mp = 121-122°C.) as a white solid.

$^1$H NMR (300MHz, CDCl$_3$): δ7.77 (d, J=7.2Hz, 2H); 7.60 (d, J=7.5Hz, 2H); 7.40 (t, J=7.2Hz, 2H); 7.31 (t, J=7.5Hz, 2H); 5.84 (m, 1H); 5.18 (d, J=17.6Hz, 1H); 5.14 (d, J=10.2Hz, 1H); 4.82 (t, J=7.5Hz, 2H); 4.43 (d, J=6.9Hz, 2H); 4.23 (t, J=6.9Hz, 1H); 3.83 (t, unresolved, 2H)

$^{13}$C NMR (300MHz, CDCl$_3$): δ156.4; 144.1; 141.4; 134.6; 127.8; 125.2; 120.1; 116.2; 66.8; 47.4; 43.6;

ESI-MS (DUIS, + ion): 581 (2M + Na$^+$, 95%); 334 (M + Na$^+$+CH$_3$OH, 54%); 302 (M + Na$^+$, 100%); 297 (M + NH$_4^+$, 94%); 280 (M + H$^+$, 65%); 179 (9-methylene-9H-fluorene+H$^+$, 26%)

(9H-fluoren-9-y)l methyl allylcarbamate (79) $\xrightarrow{\text{NHFmoc}}$
2.0mL (26.66mmol) allyl amine with combined with 2.0mL (54.62mmol) ethyl trifluoroacetamide producing a large exotherm upon addition. (Note: Use no solvent and an excess of allyl amine instead as it is more volatile and add it in portions to stirring ethyl trifluoroacetate stirring in an ice bath) After 15min TLC confirmed that the reaction was complete (silica gel, KMnO₄ visualized, R_f product= 0.55 40% EtOAc/hexane). The mixture was concentrated with rotary evaporation and placed under high vacuum for 25min to yield 3.117g (76% yield) of 80 as a light yellow liquid.

1H NMR (300MHz, CDCl₃): δ 7.52 (s, 1H); 5.77 (m, J=16.2; 10.2; 5.7; 0.9Hz, 1H); 5.18 (dd, J=16.2; 0.9Hz, 1H); 5.15 (dd, J= 10.2; 1.2Hz, 1H); 3.89 (t, J=5.7Hz, 2H)

13C NMR (300MHz, CDCl₃): δ 157.7 (q, J= 147Hz); 131.9; 117.7; 116.0 (q, J=1143Hz); 42.2

ESI-MS (DUIS, + ion): 329 (2M + Na⁺, 32%); 231 (69%); 211 (100%)

3-azidoprop-1-ene (81) \[\text{N}_3\] 

0.0872g (1.038mmol) NaHCO₃ and 3.078g (47.34mmol) NaN₃ were combined and dissolved in 11mL DI H₂O. 2.5mL (28.89mmol) allyl bromide was then added to the mixture resulting in a biphasic solution. The mixture was set to stir rapidly at room temperature for 30min at which time the aqueous phase was observed to be the bottom layer. TLC of the reaction mixture after 4h showed that it was complete (silica,10% EtOAc/hexane, KMnO₄ visualized, R_f allyl bromide= 0.56, R_f product= 0.61). The aqueous phase was removed and the organic phase was passed over a 2cm tall column of MgSO₄ in a glass Pasteur pipet to afford
1.6258g (68% yield) of **81** as a yellow liquid. (Note: Do not place product under vacuum for a prolonged period of time as it is volatile and cannot be separated from diethyl ether by means of rotary evaporation even)

$^1$H NMR (300MHz, CDCl$_3$): δ5.87 (m, 1H); 5.33 (d, J=17.1Hz, 1H); 5.30 (d, J=11.1Hz, 1H); 3.78 (d, J=6Hz, 2H)

(Z)-di-tert.-butyl but-2-ene-1,4-diyldicarbamate (**82**)  

0.0507g (0.3225mmol) **78** was placed into a flame dried, N$_2$ flushed 25mL round bottom flask to which was added 0.0235g (0.0375mmol) Hoveyda-Grubbs 2$^{nd}$ generation catalyst. The mixture was dissolved in 10mL CH$_2$Cl$_2$ (distilled from CaH$_2$), placed under an atmosphere of N$_2$, and heated at reflux for 18.5h at which time TLC showed the reaction to be complete (10% CH$_3$CN/CH$_2$Cl$_2$ $R_f$ SM = 0.73, $R_f$ prod = 0.41). The mixture was concentrated with rotary evaporation to dryness and placed under vacuum to remove residual solvent for 30min to yield 0.0897g (194% yield). The crude solid was purified by flash chromatography (silica gel 60, 9” silica, 10mm column, 2’/min flow rate, 5mL fraction, 100mL 10% CH$_3$CN/CH$_2$Cl$_2$, 50mL 20% CH$_3$CN/CH$_2$Cl$_2$) fraction 7-12 10% were combined, concentrated with rotary evaporation, and placed under vacuum overnight to afford 0.0134g **82** as a brown oil (58% yield of (Z)-TY)

$^1$H NMR (300MHz, CDCl$_3$): δ5.39 (s, 2H); 4.72 (s, 2H); 3.44 (m, 2H); 3.25 (m, 2H); 1.46 (s, 9H); 1.44 (s, 9H)

ESI-MS (DUIS, + ion): 309 (M + Na$^+$, 54%); 304 (M + NH$_4^+$, 100%); 287 (M + H$^+$, 42%)
\((E)-\text{di-tert.-butyl but-2-ene-1,4-diyldicarbamate} \) (83)

0.0507g (0.3225mmol) 78 was placed into a flame dried, N\(_2\) flushed 25mL round bottom flask to which was added 0.0235g (0.0375mmol) Hoveyda-Grubbs 2\(^{nd}\) generation catalyst. The mixture was dissolved in 10mL CH\(_2\)Cl\(_2\) (distilled from CaH\(_2\)), placed under an atmosphere of N\(_2\), and heated at reflux for 18.5h at which time TLC showed the reaction to be complete (silica, ninhydrin, 10% CH\(_3\)CN/CH\(_2\)Cl\(_2\) \(R_f\) \(_{SM}\) = 0.73, \(R_f\) \(_{prod}\) = 0.36). The mixture was concentrated with rotary evaporation to dryness and placed under vacuum to remove residual solvent for 30min to yield 0.0897g (194% yield). The crude solid was purified by flash chromatography (silica gel 60, 9” silica, 10mm column, 2’/min flow rate, 5mL fraction, 100mL 10% CH\(_3\)CN/CH\(_2\)Cl\(_2\), 50mL 20% CH\(_3\)CN/CH\(_2\)Cl\(_2\) fraction 13-21 10% were combined, concentrated with rotary evaporation, and placed under vacuum overnight to afford 0.0213g 83 as a tan solid (92% yield of \((E)-\text{TY}\)

\(^1\)H NMR (300MHz, CDCl\(_3\)): \(\delta\) 5.62 (s, 2H); 4.57 (s, 2H); 3.72 (s, 4H); 1.45 (s, 18H)

ESI-MS (DUIS, + ion): 309 (M + Na\(^+\), 100%); 304 (M + NH\(_4\)^\(+\), 50%)

\((E)-\text{bis([9H-fluoren-9-yl)methyl] but-2-ene-1,4-diyldicarbamate} \) (84)

0.0510g (0.1826mmol) 79 was combined with 0.0115g (0.0184mmol) Hoveyda-Grubbs 2\(^{nd}\) generation catalyst and dissolved in 5mL CH\(_2\)Cl\(_2\) (distilled from CaH\(_2\)). The mixture was placed under an atmosphere of N\(_2\) and reflux for 4h at which time \(^1\)H NMR showed consumption of the allyl amine. The mixture was diluted with 6mL hexanes and cooled to -78°C before the solid present was collected by centrifugation. The supernatant was removed, the white solid was washed with 12mL 50% CH\(_2\)Cl\(_2\)/hexane, and placed under vacuum overnight to remove residual solvent to afford 0.0229g (47% yield, mp = 191-192°C) 84 as a white solid.
$^1$H NMR (300MHz, CDCl$_3$): δ 7.76 (d, J=7.2Hz, 4H); 7.60 (d, J=7.2Hz, 4H); 7.40 (t, J=7.2Hz, 4H); 7.31 (t, J=7.2Hz, 4H); 5.60 (s, 2H); 4.79 (s, 2H); 4.44 (d, J=6.6Hz, 4H); 4.22 (t, J= 6.0Hz, 2H); 3.80 (s, 2H)

ESI-MS (DIUS, + ion): 553 (M + Na$^+$, 100%); 548 (M + NH$_4^+$, 39%)

(E/Z)-N,N’-(but-2-ene-1,4-diyl)bis(2,2,2-trifluoroacetamide) (85)

0.0539g (0.3521mmol) 80 was injected into a solution of 0.0266g (0.0424mmol) Hoveyda-Grubbs 2$^{\text{nd}}$ generation catalyst dissolved in 2mL CH$_2$Cl$_2$ and quantitatively transferred using two 2mL portions CH$_2$Cl$_2$. The mixture was placed under an atmosphere of N$_2$ and heated at reflux for 18h. 4min after mixing, the color of the mixture darkened to a brownish green color and turned completely brown after 13min when reflux was achieved. After 18h, TLC indicated that the reaction was complete by absence of the starting alkene (silica gel, KMnO$_4$, 40% EtOAc/hexane, $R_f$ SM= 0.55, $R_f$ Z and E products= 0.63 and 0.42). The mixture was concentrated to dryness with rotary evaporation and placed under vacuum overnight to remove residual solvent to yield 0.0478g of 85 (43% yield after subtraction of catalyst mass).

ESI-MS of crude product acquired.

ESI-MS (DIUS, - ion): 391 (M + CF$_3$COO$^-$, 47%); 323 (M + HCOO$^-$, 26%); 277 (M - H$^+$, 100%)
(E)-oct-4-enedioic acid (86)

0.05mL (0.4899mmol) 4-pentenoic acid was combined with 0.0273g (0.0436mmol) Hoveyda-Grubbs 2nd generation catalyst dissolved in 10mL CH2Cl2 (distilled from CaH2). The mixture was placed under an atmosphere of N2 and heated at reflux for 16.5h after which the solution was observed to still be green in color. Analysis of a concentrated aliquot by 1H NMR in CD3CN showed no more of the starting alkene. The reaction mixture and NMR sample were combined, dissolved in a combination of 5mL CH3CN and 15mL CH2Cl2, and extracted with 20mL sat. NaHCO3. The aqueous layer was removed and the organic phase was extracted with an additional 20mL sat. NaHCO3. The aqueous phases were combined, washed with two 20mL portions CH2Cl2, and acidified to pH1 were 12M HCl. The aqueous phase was extracted with two 35mL EtOAc. The organic extracts were combined, dried over MgSO4, filtered, concentrated with rotary evaporation to dryness, and placed under vacuum overnight to remove residual solvent to yield 0.0185g (44% yield) 86 as an off white solid.

1H NMR (300MHz, DMSO-d6): δ12.05 (s, 2H); 5.43 (m, 2H); 2.27-2.18 (m, 8H)

ESI-MS (DUIS, - ion): 387 (35%); 365 (2M^- + Na^+, 100%); 343 (M^- + M^-, 86%); 171 (M - H^+, 72%)

(S,E)-2,5-bis((tert-butoxycarbonyl)amino)hept-3-enoic acid (87)

0.0392g (0.2494mmol) 78 dissolved in 2.5mL CH2Cl2 was added to 0.0188g (0.0934mmol) 74 and 0.0067g (0.0107mmol) Hoveyda-Grubbs 2nd generation catalyst dissolved in 5mL CH2Cl2. The mixture was set to stir heated at reflux under an atmosphere of N2 for 18h at
which time the mixture was analyzed by $^1$H NMR revealing that the vinylglycine was almost
gone. The reaction mixture was concentrated to dryness with rotary evaporation, dissolved in
15mL EtOAc, and extracted with four 7mL portion conc. NH$_3$. The aqueous extracts were
combined, concentrated by rotary evaporation and dried azeotropically by evaporation of 5mL
CH$_3$OH and 15mL toluene. The concentrate was placed under vacuum overnight to yield
0.0333g (102% yield). 0.0144g of the crude product was separation was attempted by flash
chromatography (10mm column, 7.5” silica, 2”/min flow rate, 5mL fractions, slurry packed and
eluted with 100mL (2.5% AcOH/7.5%H$_2$O/90%CH$_2$Cl$_2$) to afford the desired product 87 as a 3:1
mixture with 74 (0.0043g, 32% yield, 39% based on conversion)

$^1$H NMR (300MHz, CDCl$_3$): $\delta$ 5.81-5.66 (m, 2H); $\delta$ 4.65 (d, J=17.7Hz, 1H); $\delta$ 3.65 (d, J=10.2Hz, 2H);
1.44 (s, 9H); 1.43 (s, 9H)

MS (DUIS, -ion): 530 (M$^+$+NBoc-L-Vgy, 31%); 329 (M-H$^+$, 100%)

(S,E)-5-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-((tert-butoxycarbonyl)amino)pent-3-
enoic acid (88)

0.0613g (0.9387mmol) 73 and 0.2622g (0.3047mmol) 79 were combined to which was
added 0.0315g (0.0503mmol) Hoveyda-Grubbs 2$^{nd}$ generation catalyst dissolved in 3mL CH$_2$Cl$_2$,
using two 2mL portions CH$_2$Cl$_2$ to quantitatively transfer. The mixture placed under an
atmosphere of N$_2$ and heated at reflux for 21h over which time the initial emerald green color
of the solution changed to an orange-green to brown in the first 1h of heating. After 21h TLC
showed that the vinylglycine was still present. The mixture was removed from the heat for 24h
before the addition of 0.0406g (0.1453mmol) 79, 0.0150g (0.0239mmol) Hoveyda-Grubbs 2$^{nd}$
generation catalyst and 3mL CH$_2$Cl$_2$ and heated at reflux for another 13h at reflux at which time the NBoc-L-Vgy was no longer seen by TLC (silica, ninhydrin, 10% AcOH/CH$_2$Cl$_2$, R$_f$ NBoc-L-Vgy= 0.38, R$_f$ homodimer= 0.56, R$_f$ CM product= 0.20). The mixture was concentrated to dryness with rotary evaporation and placed under vacuum overnight to remove residual solvent to yield 0.4060g crude product (294% yield). 0.1173g of the crude product was dissolved in 20mL CH$_2$Cl$_2$ and 10mL toluene and extracted with three 10mL portions sat. NaHCO$_3$. The aqueous portions were combined and washed with 10mL CH$_2$Cl$_2$. The aqueous phase was acidified to pH3 and extracted with three 30mL portions EtOAc. The organic extracts were combined, dried over MgSO$_4$, filtered, concentrated with rotary evaporation, and placed under vacuum overnight to remove residual solvent to afford 0.1200g which was submitted to MS analysis to afford M+NH$_4^+$ of the desired product.

ESI-MS (DUIS, - ion): 475 (M + Na$^+$, 98%); 470 (M + NH$_4^+$, 100%)

(S,E)-2-((tert-butoxycarbonyl)amino)hept-3-enoic acid (89)

0.1292g (0.6421mmol) 74 was dissolved in 6mL CH$_2$Cl$_2$ to which was added 0.20mL (1.956mmol) 4-pentenoic acid followed by 0.0377g (0.0602mmol) Hoveyda-Grubbs 2$^{nd}$ generation catalyst dissolved in 6mL CH$_2$Cl$_2$. The mixture was placed under an atmosphere of N$_2$ and heated to reflux for 24h after which time TLC showed the reaction to be incomplete.

0.20mL 4-pentenoic acid and 0.0259g (0.0413mmol) Hoveyda-Grubbs 2$^{nd}$ generation catalyst were added before resuming reflux for another 21h with a white precipitate forming over the course of the reaction (pentenoic acid homodimer). TLC analysis after this time showed that
starting vinylglycine was no longer present (silica, ninhydrin, 10% AcOH/CH₂Cl₂, Rₐ NBoc-L-Vgy = 0.35, Rₐ homodimer = 0.57, Rₐ product = 0.22). The mixture was filtered to remove the white precipitate, concentrated to dryness with rotary evaporation, and dissolved in 30mL CH₂Cl₂. The solution was extracted with three 10mL portions sat. NaHCO₃. The aqueous portions were combined, washed with two 25mL portions CH₂Cl₂, and acidified to pH2 with 85% H₃PO₄, and extracted with three 25mL portions EtOAc. The organic layers were combined, dried over MgSO₄, filtered, concentrated with rotary evaporation and placed under vacuum overnight to remove residual solvent to afford 0.1725g (98% crude yield). 0.0465g of the crude product was dissolved in 10mL CH₂Cl₂ and 10mL CF₃COOH and heated at reflux for 1.5h before concentrating with rotary evaporation to dryness. The concentrated crude was dissolved in 30mL EtOAc to which was added 15mL DI H₂O. The aqueous phase was removed and the organic phase was extracted with an addition two 10mL portions H₂O. The aqueous portions were combined, washed with three 20mL portions EtOAc, then concentrated with rotary evaporation to near dryness before being dried azeotropically by evaporation CH₃OH and toluene to afford 0.0290g of 89 as a light tan solid (16% yield, 58% based on amount used)

**Boc protected CM product**

ESI-MS (DUIS, - ion): 567 (2M⁻ + Na⁺, 30%); 545 (M + M⁻, 75%); 272 (M - H⁺, 100%)

**Deprotected TFA salt of CM product**

¹H NMR (300MHz, CDCl₃): δ6.01 (dt, J=15.6; 6.9Hz, 1H); 5.64 (dd, J= 15.6; 7.5Hz 1H); 4.29 (d, J= 8.1Hz, 1H); 2.52 (t, J= 6.3Hz, 2H); 2.41 (m, J= 14.1Hz; 6.9Hz, 2H)

ESI-MS (DUIS, - ion): 249 (M-2H⁺+NH₄⁺+AcO⁻, 92%); 172 (M-H⁺, 100%)

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IX. REFERENCES


69. Baeyer Ber., 1886, 19, 430.


178. NMR indicates changes subsequent to dissolution that correlate with higher yields.

Possibly this corresponds to a greater degree of N-silylation.

179. In each, base was held constant at 1M triethylamine, and isomerization monitored at 25˚C by 1H NMR. As we did not collect full time course data, and analysis times varied, we have chosen to express half times calculated assuming pseudo first order kinetics and using only 1-2 data points.


X. APPENDIX

A. Characterization Data for Selected Compounds

$^1$H NMR *Dimethyl 2,5-diaminocyclohexa-1,4-diene-1,4-dicarboxylate* (1)
240

C NMR (CDCl₃) bisename, mp: 212–214°C

Current Data Parameters
NAME 648-3-14713C
EXPN 1
PROCNO 3

F2 - Acquisition Params
Data 2014001f
Time 16.5s
INSTRUM spect
PROBDD 5 mm Multinuclear
PULPROG dupp3c
TD 653M
SOLVENT CDCl3
HS 67c
DS (c
GWM 17925.613
FNRES 0.274431
AQ 1.821950E
RG 456.3
SN 27.80k
SN 6.00
SN 306.0
D1 0.50000000
D1i 0.03000000
DELTAP 0.40000000
TD 3

------ CHANNEL #1 -----
NUT1 13C
P1 6.40
P1 6.00
SPOL 75.477223PM

------ CHANNEL #2 -----
CFP2R2 13C
NUC2 3
CPD2 100.00
P2 0.00
P1 16.40
P1 120.00
SPOL 300.111000

F2 - Processing params
ST 32761
RF 75.467777
SWW 9
SSB 1.00
GB (c
PC 1.40
MS Dimethyl 2,5-diaminocyclohexa-1,4-diene-1,4-dicarboxylate (1)
H NMR (CDCl3) recrystallized DE mp 248–250°C

Current Data Parameters
NAME  dimethyl 2,5-bis(tert-butoxycarbonyl)aminoterephthalate
KONTR  1
PROCNO  1
F2 - Acquisition Parameter
decay  2048101
Time  12.04
INSTRUM  specter
PROTOCOL  6 mm Multinuclear
SOLVENT  CDCl3
NS  16
DS  0
DM  5172.830
FID  0.181190
RSW  2.654270
DM  0
DE  0
TE  0
DS  0
DI  0
TDO  1

= CHANNEL 1 =
H  1H
P1  9.80
P2  -4.90
SPOL  300.133534

F2 - Processing parameters
S  304709
DP  300.125036
NEW  RM
RIN  C
LS  0.30
DS  0.5
PC  1.00
13C NMR (CDCl3) recrystallized DE mp 248–250°C
MS *Dimethyl 2,5-bis((tert-butoxycarbonyl)amino)terephthalate* (2)
1H NMR (CDCl₃) yellow solid from NH₃(aq) workup of TFA deprotected DE
4.8 mg sample
MS Dimethyl 2,5-diaminoterephthalate (3)
$^1$H NMR Tetramethyl 5,5’-azanediylbis[2-((tert-butoxycarbonyl)amino)terephthalate] (4)
MS *Tetramethyl 5,5'-azanediylbis(2-((tert-butoxycarbonyl)amino)terephthalate)* (4)
HRMS Tetramethyl 5,5'-azanediylbis(2-((tert-butoxycarbonyl)amino)terephthalate) (4)
$^1$H NMR *Dimethyl 2-amino-5-(p-tolylamino)terephthalate* (5)
ESI-MS *Dimethyl 2-amino-5-(p-tolylamino)terephthalate* (5)
HRMS *Dimethyl 2-amino-5-(p-tolylamino)terephthalate* (5)
$^1$H NMR\textit{ Dimethyl 2,5-bis(p-tolylamino)terephthalate (6)}
ESI-MS *Dimethyl 2,5-bis(p-tolylamino)terephthalate* (6)
HRMS *Dimethyl 2,5-bis(p-tolylamino)terephthalate* (6)
$^1$H NMR  *Di-tert-butyl (2,5-bis(hydroxymethyl)-1,4-phenylene)dicarbamate* (7)
$^{13}$C NMR (CDCl$_3$) saturated diol solution

Current Data Parameters:
NAME  RMI-2-991M
EXNO   1
PROCNO 1

P2 - Acquisition Parameters:
Date: 20130622:
Time: 14:41
INSTRUM  spect
PROBID  5 mm Multinuclear
PULPROG  299P3M
SOLVENT  CDCl$_3$
NS  3M
DS  (x
SNR  19856.11
SIFRES  0.27443
AQ  1.821955
MS  1149.0
FM  27.800
DE  50.0
TE  398.0
DD  0.0000000
DI  0.0000000
DDLTA  0.0000000
TD0  1

-------- CHANNEL 1 ---
NUC1  1M
PH  6.8
PLL  6.0
SF01  75.475295

-------- CHANNEL 2 ---
CPDPR02  wait3
NUC2  11
PD102  20.00
PL1  16.40
PL12  16.40
PL13  120.00
SF02  300.19200

P2 - Processing parameters:
SI  32768
SF  75.46775
WDM  3
MS  (x
LB  1.00
GB  1.40
MS (EI, 70eV): \textit{Di-tert-butyl (2,5-bis(hydroxymethyl)-1,4-phenylene)dicarbamate} (7)
ESI-MS (DUIS, -ion) *Di-tert-butyl (2,5-bis(hydroxymethyl)-1,4-phenylene)dicarbamate* (7)
HRMS (IT-TOF) *Di-tert-butyl (2,5-bis(hydroxymethyl)-1,4-phenylene)dicarbamate* (7)
IR Di-tert-butyl (2,5-bis(hydroxymethyl)-1,4-phenylene)dicarbamate (7)
Elem. Anal. *Di-tert-butyl (2,5-bis(hydroxymethyl)-1,4-phenylene)dicarbamate* (7)
$^1$H NMR \textit{N,N'-di-t-Boc-2,5-diaminoterephthaldehyde (8)}
C NMR N,N'-di-Boc-2,5-diaminoterephthaldehyde (8)
MS (EI, 70eV) \textit{N,N'\text{-di-t-Boc-2,5-diaminoterephthaldehyde}} (8)
ESI-MS $N,N'$-di-t-Boc-2,5-diaminoterephthaldehyde (8)
HRMS (IT-TOF) $N,N'$-di-t-Boc-2,5-diaminoterephthaldehyde (8)
IR (KBr pellet) \( N,N'-\text{di-t-Boc-2,5-diaminoterephthaldehyde} \) (8)
Elem. Anal. *N,N*-di-t-Boc-2,5-diaminoterephthaldehyde (8)
$^1$H NMR Ethyl 6-amino-2-naphthoate (12)
13C NMR (CDCl3) rextyl NE 76-78C mp range 18.7mg sample

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**Current Data Parameters**
- **NAME**: Ethyl 6-amino-2-naphthoate
- **EXPND**: 1
- **PROCND**: 1

**F2 - Acquisition Parameters**
- **DATE**: 20100215
- **TIME**: 15:18
- **INSTRUM**: dpx300
- **PROSHD**: 5 mm multinu
- **PULPROG**: updf
- **TD**: 65536
- **SOLVENT**: CDCl3
- **NS**: 1852
- **DS**:
  - **DRR**: 1015.941 Hz
  - **FIDRES**: 0.276427 Hz
  - **AQ**: 1.808848 sec
  - **HQ**: 2.642 .6
  - **DW**: 21.600 ussec
  - **DS**: 6.00 ussec
  - **TE**: 300.0 K
  - **DL**: 0.1000000 sec
  - **D11**: 0.0300000 sec

**CHANNEL F1**
- **NUC1**: 13C
- **P1**: 6.40 ussec
- **P1A**: -0.00 gH
- **SP01**: 75.4796659 MHz

**CHANNEL F2**
- **CPW1**: wex116
- **NUC2**: 1H
- **PUC2**: 1.600 ussec
- **PL2**: -0.00 gH
- **PL22**: 16.60 gH
- **SP02**: 300.1332005 MHz

**F2 - Processing parameters**
- **SI**: 32768
- **SF**: 75.4677399 MHz
- **WM**: 0 Hz
- **SSB**: 0 Hz
- **LB**: 3.00 Hz
- **GB**: 0 Hz
- **PC**: 1.40

**1D NMR plot parameters**
- **CP**: 3.00 cm
- **F1P**: 200.000 ppm
- **F1**: 1093.95 Hz
- **F2**: -10.000 ppm
- **F2**: -754.68 Hz
- **PNRM**: 9.5845 ppm/cm
- **EXCM**: 92.3738 Hz/cm
MS Ethyl 6-amino-2-naphthoate (12)
IR Ethyl 6-amino-2-naphthoate (12)

![Graphical representation of elemental analysis](image)

**C.H.N Elemental Analysis**

- **Version**: 1.06
- **Operator ID**: Mark Wang
- **Method Name**: Minute8
- **Sample ID**: RWM_3_43 (# 23)
- **Analysis Type**: Unknown (Area)
- **Chromatogram**: C:\EAW\F141120.DAT
- **Calib. method**: Using 'K Factors'
- **Sample weight**: 1.436
- **Company Name**: UWM Chemistry
- **Method File**: MINUTE8.MTH
- **Printed**: 2/15/2011 03:23
- **Channel**: E.A. Channel A

**Element Name** | **Element %** | **Ret.Time** | **Area** | **BC** | **Area ratio** | **K factor**
--- | --- | --- | --- | --- | --- | ---
Nitrogen | 6.3557 | 1.07 | 278897 | FU | 19.840360 | 285272E+07
Carbon | 71.8226 | 1.40 | 5533412 | FU | 1.000000 | 535241E+07
Hydrogen | 6.1295 | 3.41 | 1300089 | RS | 4.256179 | 147704E+08
$^1$H NMR: Diethyl 6,6’-((1E,1'E)-((2,5-bis((tert-butoxycarbonyl)amino)-1,4-phenylene)bis(methanylylidene))bis(azanylylidene))bis(2-naphthoate) (13)
$^{13}$C NMR Diethyl 6,6'-(1E,1'E)-(2,5-bis((tert-butoxycarbonyl)amino)-1,4-phenylene)bis(methanylylidene)bis(azanylylidene)bis(2-naphthoate) (13)
ESI-MS Diethyl 6,6’-((1E,1’E)-((2,5-bis((tert-butoxycarbonyl)amino)-1,4-phenylene)bis(methanylylidene))bis(azanylylidene))bis(2-naphthoate) (13)
IR $\text{Diethyl 6,6'}-((1E,1'E)-((2,5\text{-bis((tert-butoxycarbonyl)amino)-1,4-phenylene})\text{bis(methanylylidene)})\text{bis(azanylylidene)})\text{bis(2-naphthoate)}}$ (13)
Elem. Anal. *Diethyl 6,6'-(1E,1'E)-((2,5-bis((tert-butoxycarbonyl)amino)-1,4-phenylene)bis(methanylylidene))bis(azanylylidene))bis(2-naphthoate)* (13)
$^1$H NMR Diethyl 6,6'-(((1R,1'S)-(2,5-bis((tert-butoxycarbonyl)amino)-1,4-phenylene)bis((dimethoxyphosphoryl)methylene))bis(azanediyl))bis(2-naphthoate) (16)
$^{13}$C NMR Diethyl 6,6'-(((1R,1'S)-(2,5-bis((tert-butoxycarbonyl)amino)-1,4-phenylene)bis((dimethoxyphosphoryl)methylene))bis(azanediyl))bis(2-naphthoate) (16)
$^{31}$P NMR Diethyl 6,6'-(((1R,1'S)-(2,5-bis((tert-butoxycarbonyl)amino)-1,4-phenylene)bis((dimethoxyphosphoryl)methylene)bis(azanediyl))bis(2-naphthoate) (16)
ESI-MS *Diethyl 6,6’-(((1R,1’S)-2,5-bis((tert-butoxycarbonyl)amino)-1,4-phenylene)bis((dimethoxyphosphoryl)methylene))bis(azanediyl))bis(2-naphthoate) (16)*
$^1$H NMR Diethyl 6,6'-(((1S,1'S)-(2,5-bis((tert-butoxycarbonyl)amino)-1,4-phenylene)bis((dimethoxyphosphoryl)methylene))bis(azanediyl))bis(2-naphthoate) (17)
$\text{^{13}C NMR } \text{Diethyl 6,6'}-(((1S,1'S)-(2,5-bis((\text{tert-butoxycarbonyl})amino)-1,4-phenylene)bis((\text{dimethoxyphosphoryl})methylene))bis(\text{azanediyl})bis(2-naphthoate}) (17)$
$^{31}$P NMR Diethyl 6,6'-(((1S,1'S)-(2,5-bis((tert-butoxycarbonyl)amino)-1,4-phenylene)bis((dimethoxyphosphoryl)methylene)bis(azanediyl))bis(2-naphthoate) (17)
ESI-MS *Diethyl 6,6’-(((15,1’S)-2,5-bis((tert-butoxycarbonyl)amino)-1,4-phenylene)bis((dimethoxyphosphoryl)methylene))bis(azanediyl))bis(2-naphthoate)* (17)
$^1$H NMR C-Me, P-Me rac-tweezers (18)
13C NMR (CDCl3) rac-P-Me, C-Et naphthyl tweezers 21.3 mg sample

Current Data Parameters:
NAME  R50r-4-61raccl3C
EXPNO  1
PROCNO  1
F2 - Acquisition Params
Date_ 20140414
Time  17:41
INSTRUM spect
PROCWD  5 mm Multinuclear
PULPROP  zgpp31
TD  6533
SOLVENT CDCl3
NS  2400
DS  1
SNR  17985.613
FIDRES  0.274331
AQ  1.8219506
BG  64.5
DM  27.80
DE  6.0
FT  300.0
DI  0.500000N
dd1  0.0300000
DELT A  0.0000000
TDQ  1

---------- CHANNEL F1 ---
NOC1  13C
P1  6.8
PL1  -6.0
SFO1  75.479295

---------- CHANNEL F2 ---
CPDPRG2  valtet16
NOC2  13C
PCD2  100.00
P12  -6.0
PL12  16.41
PL13  120.01
SPO2  300.012200
F2 - Processing param
SI  22784
SF  75.479295
WDW  EB
SUB  1.0
GN  1.4
31P NMR (CDCl₃) conc. toluene mother liquor from rextl of tweezers mix
COSY C-Me, P-Me rac-tweezers (18)
Shimadzu LCMS-2020 Data Report

Mass Spectrum for Sample
RWH-4-108rac.lcd

Operator: Robert Hoppe

Data Filename: C:\LabSolutions\Data\Schwaber Alan\RWH-4-108rac.lcd
Spectrum Mode: Averaged
Retention Time: ---
Interface Type (ESI, APCI, DUIS): DUIS
Acquisition Mode: (Scan, SIM, Profile): Scan
Polarity: +
H2O/0.1% HCOOH, CH3CN/0.1% HCOOH

Intensity

Operator: Robert Hoppe

Data Filename: C:\LabSolutions\Data\Schwaber Alan\RWH-4-108rac.lcd
Spectrum Mode: Averaged
Retention Time: ---
Interface Type (ESI, APCI, DUIS): DUIS
Acquisition Mode: (Scan, SIM, Profile): Scan
Polarity: -
H2O/0.1% HCOOH, CH3CN/0.1% HCOOH

Intensity

ESI-MS C-Me, P-Me rac-tweezers (18)
HRMS C-Me, P-Me rac-tweezers (18)
$^1$H NMR C-Me, P-Me meso-isomer (19)
$^{13}$C NMR (CDCl$_3$) sat, meso-naphthyl tweezers in CDCl$_3$

Current Data Parameters:

NAME  RBM-4-inmeos31
EXPHO  1
PROCWO  1

P2 - Acquisition Parameters:
Date_  20140811
Time  19:24
INSTRUM  spect
PROJ1  5 mm Multinucle
PULPROG  pulse
TD  6583
SOLVENT  CDCl$_3$
NS  2071
DS  17935.451
SMR  0.274431
AQ  1.8219501
RG  1290.0
DW  27.801
DE  6.01
TE  100.4
D1  0.5000000
d0  0.0300000
DELTAD  0.4000000
TD0  1

---------- CHANNEL F1 ----------
NUPC  1
P1  6.86
PL1  -6.04
SFO1  75.4752951

---------- CHANNEL F2 ----------
CPPGNC  val:A
NUPC  11
PCPD2  100.0
PL2  -6.04
PL12  14.41
PL13  120.04
SFO2  300.131200

P2 - Processing parameters:
ST  32761
SP  75.4677371
MDW  82
SSE  1
LB  1.0
GB  1
PC  1.4

The diagram shows a 13C NMR spectrum of meso-naphthyl tweezers in CDCl$_3$. The parameters listed are for the acquisition and processing of the spectrum, including names, acquisition time, instrument details, solvent, and various phase and delay settings.
$^{31}$P NMR C-Me, P-Me meso-isomer (19)
COSY C-Me, P-Me meso-isomer (19)
ESI-MS C-Me, P-Me meso-isomer (19)
HRMS C-Me, P-Me meso-isomer (19)
H NMR (CDCl3/TFA) prod after ppt from crd prod of TMSBR depro of En, 4.0mg sampl
31P NMR (DMSO-d6) TMSBr depro rac naphthyl tweezers crude product 3.3mg

Current Data Parameters:
NAME: RMR-1116crd31
EXPRO: 1
PROCNO: 1

F2 - Acquisition Parameters
Date: 20160121
Time: 11:41
INSTRUM: agon
PROC: 5 mm Multinuclear
PULPROG: ngk
TD: 6553
SOLVENT: Acetone
NE: 61
DG: (6)
SNH: 17985.613
FDRES: 0.274421
AQ: 1.8241509
BG: 224.3
DN: 27.80
DE: 5.0
TE: 598.0
D1: 0.20000000
D11: 0.03000000
T200: 1

------- CHANNEL F1 -------
NUC1: 81
F1: 4.0
F1.1: -4.0
SP01: 121.497281

------- CHANNEL F2 -------
CPDPRG: walszt1
NUC1: 1
CPD0: 100.0
PL2: 6.4
PL12: 16.4
SP02: 306.131200

F2 - Processing parameters
SZ: 12761
SF: 121.494718
MDW: 28
SIGB: 1.0
SL: 1
UG: 1
PC: 1.41
ESI-MS rac-carboxy tetra acid tweezers (23)
HRMS *rac*-carbethoxy *tetra acid tweezers* (23)
\(^1\)H NMR \textit{meso- carbethoxy tetra acid isomer} (24)
31P NMR (D2O, tBuOH int std) crude TMSBR depro meso naphthyl tweezers

Current Data Parameters:
NAME 503-4-115crd31
EXPO 1
PC 1

F2 - Acquisition Params
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Time 12:11
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PRBIRD 5 mm Multinuclear
PULPROG AgN
TD 650H
SOLVENT Acetone
NS 61
DS
SNH 17885.611
PDRES 0.274431
AQ 1.8219501
RG 9195
DM 27.804
DE 0.0
TR 294.0
D1 0.2000000
D11 0.000000
T0 1

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MDC1 311
P1 8.00
P2 0.00
SF01 121.497201

****** CHANNEL f2:******
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MDC2 13
PCD2 100.00
F2 0.00
GL2 17.81
SF02 300.131200
F2 - Processing parameter
SI 3276
SF 121.494663
WDM 0
SSB 0
LH 1.00
GB 0
PC 1.41
ESI-MS *meso- carbethoxy tetra acid isomer* (24)
HRMS *meso*-carbethoxy tetra acid isomer (24)
$^{1}$H NMR rac-methylphosphonate tetra anionic tweezers (25)
31P NMR (TFA, TMS int std) LiOH sapon. rac tweezers 1.9mg sample

Current Data Parameters
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EXPS1  1
PROCNO  1

F2 - Acquisition Parameters
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PROBID  5 mm Multinuclear
PULPROG  spgd
TD2  65536
SOLVENT  Acetone
RS  11.1
DS  1.0
SNH  17999.513
FIDRES  0.274331
A2  1.821950
EG  9195.2
DM  27.800
DE  6.0
TE  100.0
DI  0.0000000
DD1  0.0300000
DD2  1

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NUC1  31P
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PC1  -6.0
SPO1  121.497281

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NUC2  31P
PCVD2  100.0
PL2  -6.0
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SPO2  300.131200

F2 - Processing parameters
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SP  121.494760
MDW  1.0
SSB  1.0
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FC  1.0
ESI-MS *rac-methylphosphonate tetra anionic tweezers* (25)
HRMS *rac-methylphosphonate tetra anionic tweezers* (25)
\(^1\)H NMR meso-methylphosphonate tetra anionic isomer (26)
\( ^{13}\text{C} \) NMR \textit{meso-methylphosphonate tetra anionic isomer} (26)
$^{31}$P meso-methylphosphonate tetra anionic isomer (26)
ESI-MS *meso-methylphosphonate tetra anionic isomer* (26)
HRMS *meso-methylphosphonate tetra anionic isomer* (26)
1H NMR (CDCl₃/TFA) crude prod from rac-hexa anion synthesis
318

IP NMR (1:1 TFA/CDCl3) sapon of rac anptyl tweezers after 2nd add of LiOH and refit
3.2mg sample
ESI-MS *rac-hexa anionic tweezers* (27)
HRMS NMR *rac-hexa anionic tweezers* (27)
1H NMR (1:1 TFA:CDCl3) crude prod from sapon of EN depro, 3.1mg sample, filtered
31P NMR (1:1 TFA/CDCI3) crude prod of sapon of EN depro, 3.1mg sample, filtered
ESI-MS *meso-hexa anionic isomer* (28)
HRMS *meso-hexa anionic isomer* (28)
$^1$H NMR tert-butyl (3-aminophenyl)carbamate (29)
$^{13}$C NMR tert-butyl (3-aminophenyl)carbamate (29)
MS (EI, 70eV) tert-butyl (3-aminophenyl)carbamate (29)
$^1$H NMR $2-(3\text{-}\text{tert-Butoxycarbonylamino-phenylamino})\text{-}\text{but-2-enedioic acid diethyl ester}$ (30)
$^{13}$C NMR 2-(3-tert-Butoxycarbonylamino-phenylamino)-but-2-enedioic acid diethyl ester (30)
MS (70eV) 2-(3-tert-Butoxycarbonylamino-phenylamino)-but-2-enedioic acid diethyl ester (30)
$^1$H NMR 7-Amino-4-oxo-1,4-dihydro-quinoline-2-carboxylic acid ethyl ester (31)
$^{13}$C NMR 7-Amino-4-oxo-1,4-dihydro-quinoline-2-carboxylic acid ethyl ester (31)
MS (EI, 70eV) 7-Amino-4-oxo-1,4-dihydro-quinoline-2-carboxylic acid ethyl ester (31)
MS (ESI, + ion) 7-Amino-4-oxo-1,4-dihydro-quinoline-2-carboxylic acid ethyl ester (31)
$^1$H NMR Diethyl 2-((3-(2,2,2-trifluoroacetamido)phenyl)amino)fumarate (34)
$^{13}$C NMR Diethyl 2-((3-(2,2,2-trifluoracetamido)phenyl)amino)fumarate (34)
MS (EI, 70eV) *Diethyl 2-((3-(2,2,2-trifluoroacetamido)phenyl)amino)fumarate* (34)
H NMR
Ethyl 4-oxo-7-(2,2,2-trifluoroacetamido)-1,4-dihydroquinoline-2-carboxylate (35)
$^{13}$C NMR Ethyl 4-oxo-7-(2,2,2-trifluoroacetamido)-1,4-dihydroquinoline-2-carboxylate (35)
MS (EI, 70eV) Ethyl 4-oxo-7-(2,2,2-trifluoroacetamido)-1,4-dihydroquinoline-2-carboxylate (35)
$^1$H NMR Ethyl 4-oxo-5-(2,2,2-trifluoroacetamido)-1,4-dihydroquinoline-2-carboxylate (36)
$^1$H NMR Diethyl 2-((3-nitrophenyl)amino)fumarate (37)
$^{13}$C NMR Diethyl 2-((3-nitrophenyl)amino)fumarate (37)
MS (EI, 70eV) *Diethyl 2-((3-nitrophenyl)amino)fumarate* (37)
$^1$H NMR Ethyl 7-nitro-4-oxo-1,4-dihydroquinoline-2-carboxylate (38)
$^{13}$C NMR Ethyl 7-nitro-4-oxo-1,4-dihydroquinoline-2-carboxylate (38)
MS (EI, 70eV) *Ethyl 7-nitro-4-oxo-1,4-dihydroquinoline-2-carboxylate* (38)
$^1$H NMR Ethyl 5-nitro-4-oxo-1,4-dihydroquinoline-2-carboxylate (39)
MS (El, 70eV) *Ethyl 5-nitro-4-oxo-1,4-dihydroquinoline-2-carboxylate* (39)
$^1$H NMR Diethyl 2-((4-nitrophenyl)amino)fumarate (40)
$^{1}$H NMR (d-DMSO) thermocyclization of p-nitroenamine

Ethyl 6-nitro-4-oxo-1,4-dihydroquinoline-2-carboxylate (41)
$^{13}$C NMR Ethyl 6-nitro-4-oxo-1,4-dihydroquinoline-2-carboxylate (41)
MS (EI, 70eV) Ethyl 6-nitro-4-oxo-1,4-dihydroquinoline-2-carboxylate (41)
$^1$H NMR Ethyl 6-amino-4-oxo-1,4-dihydroquinoline-2-carboxylate (42)
MS (EI, 70eV) *Ethyl 6-amino-4-oxo-1,4-dihydroquinoline-2-carboxylate* (42)
$^1$H NMR Potassium (3E,5E)-2-oxo-6-phenylhexa-3,5-dienoate (45)
**31P NMR (D2O, 1uL tBuOH) K salt after trit w/ EtOAc and vacuum to dry**

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Current Data Parameters
- NAME: RMB-B-46tritK
- EXPRO: 3
- PROCHO: 3

**F2 - Acquisition Parameters**
- Data: 20140111
- Time: 15.35
- INSTRUM: spc630
- PROBHD: 5 mm Multinucl
- PULPROG: sppg3
- TD: 6553
- SOLVENT: D2O
- NS: 85
- DS: 1
- SWH: 17985.613
- FIDRES: 0.2744536
- AQ: 1.82195E6
- GR: -1149.4
- DW: 27.800
- DM: 6.000
- TE: 298.1
- D1: 0.5000000
- G11: 0.0000000
- DELTA: 0.4000000
- DSG: 3

#### CHANNEL f1
- NUC1: 13K
- NUC2: 6.8K
- NUC3: 6.8K
- SFO1: 75.4752955

#### CHANNEL f2
- CPOPG2: waltz1f
- NUC1: 13K
- TCO1: 100.0K
- N11: -6.0K
- PL1: 16.4K
- PL2: 120.0K
- SFO2: 300.1312000
- F2 - Processing parameters
  - ST: 12761
  - SP: 75.4676953
  - WDM: 18
  - LSB: 1.0
  - LB: 1.0
  - GC: 1.0
  - PC: 1.0
ESI-MS Potassium (3E,5E)-2-oxo-6-phenylhexa-3,5-dienoate (45)
2D COSY S(D2O, 1uL tBuOH int std_ K salt diene of cinn + pyr
$^1$H NMR **Potassium (3E,5E)-6-(4-nitrophenyl)-2-oxohexa-3,5-dienoate (46)**
$^{13}$C NMR Potassium (3E,5E)-6-(4-nitrophenyl)-2-oxohexa-3,5-dienoate (46)
ESI-MS Potassium [3E,5E]-6-(4-nitrophenyl)-2-oxohexa-3,5-dienoate (46)
$^1$H NMR Sodium trifluoropyruvate (47)
13C NMR(D2O) sodium trifluoropyruvate 0.03g sample CH3CN int std
ESI-MS Sodium trifluoropyruvate (47)
\( ^1 \text{H} \text{NMR} \) \( 5\)-methyl-2-(2,2,2-trifluoroacetyl)furan (50)
$^1$H NMR 5-methyl-2-(2,2-difluoroacetyl)furan (51)
$^1$H NMR *Methyl difluoropyruvate methyl hemiacetal* (52)
$^1$H NMR Sodium difluoropyruvate hydrate (53)
MS Sodium difluoropyruvate hydrate (53)
$^1$H NMR $N,N'$-Di-Boc-$N''$-(2,2-dimethoxy-ethyl)-guanidine (54)
$^{13}$C NMR \textit{N,N'-Di-Boc-N"(2,2-dimethoxy-ethyl)-guanidine} (54)
MS $N,N'$-Di-Boc-$N''$-(2,2-dimethoxy-ethyl)-guanidine (54)
$^1$H NMR $N$-Allyl-$N',N''$-Di-Boc-guanidine (55)
$^{13}$C NMR of *N-Allyl-N',N''-Di-Boc-guanidine* (55)
MS *N- Allyl-N',N''-Di-Boc guanidine* (55)
$^1$H NMR $N$-allyl guanidinium trifluoracetate (57)
$^{13}$C NMR $N$-allyl guanidinium trifluoracetate (57)
MS *N-allyl guanidinium trifluoracetate* (57)
$^1$H NMR 2-Amino-5-hydroxy-4,5-dihydro-3H-imidazolium chloride (58)
1H NMR(D2O) C-methyl ester L-selenomethionine HCl 1uL CH3CN int std

Current Data Parameters
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EXPNO  1
PROCNO  1

F2 - Acquisition Parameters
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F2 - Processing parameters
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$^{13}$C NMR L-Selenomethionine methyl ester hydrochloride (62)
ESI-MS *L-Selenomethionine methyl ester hydrochloride* (62)
$^1$H NMR $N$-Boc-$L$-Selenomethionine methyl ester (63)
$^{13}$C NMR $N$-Boc-$L$-Selenomethionine methyl ester (63)
MS N-Boc-L-Selenomethionine methyl ester (63)
\(^1\)H NMR \textit{N-Boc-L-Selenomethionine oxide methyl ester} (64)
$^1$H NMR $L$-Methionine methyl ester hydrochloride
$^1$H NMR \textit{N-Boc-L-Methionine methyl ester} (67)
$^1$H NMR *N-Boc-L-Methionine sulfoxide methyl ester* (68)
$^1$H NMR (D2O) White ppt after treatment of L-Met with 30% H2O2 and dilution with EtOH 8.6mg s
4µL ACN internal std.
$^{13}$C NMR \textit{L-Methionine sulfoxide} (70)
MS L-Methionine sulfoxide (70)
$^1$H NMR $L$-Vinylglycine (71)
$^{13}$C NMR $L$-Vinyglycine (71)
ESI-MS *L-Vinylglycine* (71)
HRMS (IT-TOF) *L-Vinylglycine* (71)

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<th>Ioni</th>
<th>Meas. m/z</th>
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2D COSY NMR (D2O) Ppt crude of vinyl glycine from H2O w/ tBuOH

0.5uL tBuOH int std

Current Data Parameters
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EXPS
PROC0

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Time: 10:21:09
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POLPOD 5 mm Multiple
POLENG: coupling
TO: 31
SOLVENT: 1
DS
SNR 1798.1
FIDRES 0.5706
DE 21
SN 279.4
DS 6
TE 291
dd 0.000000
DI 1.235200
dl 0.000000
DI6 0.000000
IND 0.000000

****** CHANNEL F1:
NCP 9
F1 9
F1 4
FGP1 300.1311

****** CHANNEL F2:
GPAK1 SUN: 1.0
F16 1000.0
F2 300.1
FIDRES 14.088
SNK 0.1
FMODE

F3 - Processing para:
SI 3
SF 300.1300
GWO 0
SHD 1
LB 0
GQ 1
PC 1

F1 - Processing para:
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MC2 300.1300
GWO 1
SHD 0
LB 0
GQ 0
$^1$H NMR

(S)-2-((R)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)but-3-enolic acid (72)
$^{13}$C NMR

(S)-2-((R)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)but-3-enoic acid (72)
HRMS (IT-TOF)

(S)-2-((R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)but-3-enoic acid (72)
$^1$H NMR (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)but-3-enoic acid (73)
$^{13}$C NMR (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)but-3-enoic acid (73)
ESI-MS (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)but-3-enoic acid (73)
HRMS (IT-TOF) \((S)-2-(((9H\text{-fluoren}-9-\text{-yl})\text{methoxy})\text{carbonyl})\text{amino})\text{but-3-enoic acid}\ (73)
$^1$H NMR (S)-2-((tert-butoxycarbonyl)amino)but-3-enoic acid (74)
$^{13}$C NMR (5)-2-((tert-butoxycarbonyl)amino)but-3-enoic acid (74)
ESI-MS (S)-2-((tert-butoxycarbonyl)amino)but-3-enoic acid (74)
HRMS (IT-TOF) (S)-2-((tert-butoxycarbonyl)amino)but-3-enoic acid (74)
$^1$H NMR: Dicyclohexyl ammonium 2-((tert-butoxycarbonyl)amino)but-3-enoate (75)
$^{13}$C NMR *Dicyclohexyl ammonium 2-((tert-butoxycarbonyl)amino)but-3-enoate* (75)
ESI-MS Dicyclohexyl ammonium 2-((tert-butoxycarbonyl)amino)but-3-enoate (75)
HRMS (IT-TOF, + ion) *Dicyclohexyl ammonium 2-((tert-butoxycarbonyl)amino)but-3-enoate* (75)
HRMS (IT-TOF, -ion) *Dicyclohexyl ammonium 2-((tert-butoxycarbonyl)amino)but-3-enoate* (75)
$^1$H NMR *Allylammonium chloride* (76)
ESI-MS Allylammnonium chloride (76)
$^1$H NMR tert-butyl allylcarbamate (78)
$^{13}$C NMR (CDCl$_3$) N-Boc allyl amine 69.6mg sample

Current Data Parameters:
NAME  BMB-1-101
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PROCNO  1
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Time_  15.15
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PROBNO_ 5 mm Multinuclear
PULPROG_ zgpp1
TD_  6538
SOLVENT_ CDCl$_3$
NS_  191
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F2 - Processing parameters
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ESI-MS tert-butyl allylcarbamate (78)
\( ^1\text{H NMR} \) \((9\text{H-fluoren-9-yl)methyl allylcarbamate}\) (79)
$^{13}$C NMR (9H-fluoren-9-yl)methyl allylcarbamate (79)
ESI-MS (9H-fluoren-9-yl)methyl allylcarbamate (79)
$^1$H NMR $N$-allyl-2,2,2-trifluoroacetamide (80)
$^{13}$C NMR $N$-allyl-2,2,2-trifluoroacetamide (80)
ESI-MS *N*-allyl-2,2,2-trifluoroacetamide (80)
$^1$H NMR 3-azidoprop-1-ene (81)
$^1$H NMR (Z)-di-tert.-butyl but-2-ene-1,4-diyl dicarbamate (82)
ESI-MS (Z)-di-tert.-butyl but-2-ene-1,4-diyl dicarbamate (82)
$^1$H NMR (E)-di-tert.-butyl but-2-ene-1,4-diyl dicarbamate (83)
ESI-MS (E)-di-tert.-butyl but-2-ene-1,4-diyl dicarbamate (83)
$^1$H NMR (E)-bis((9H-fluoren-9-yl)methyl) but-2-ene-1,4-diylidicarbamate (84)
ESI-MS (E)-bis((9H-fluoren-9-yl)methyl) but-2-ene-1,4-diyldicarbamate (84)
ESI-MS (E/Z)-N,N’-(but-2-ene-1,4-diyl)bis(2,2,2-trifluoroacetamide) (85)
1H NMR(dDMSO) white solid filtered from initial rxn mix

H NMR (E)-oct-4-enedioic acid (86)
ESI-MS (E)-oct-4-enedioic acid (86)
$^1$H NMR (S,E)-2,5-bis((tert-butoxycarbonyl)amino)hept-3-enoic acid (87)
ESI-MS (S,E)-2,5-bis((tert-butoxycarbonyl)amino)hept-3-enoic acid (87)
ESI-MS

(S,E)-5-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-((tert-butoxycarbonyl)amino)pent-3-enoic acid (88)

Shimadzu LCMS-2020 Data Report

Mass Spectrum for Sample
RWH-8-72 cld.kcd

Operator: Mark Wang

Data Filename: C:\LabSolutions\Data\Schwabacher Alan\RWH-8-72 cld.kcd

Spectrum Mode: Single

Retention Time: 0.150

Interface Type (ESI, APCl, DUlS): DUlS

Acquisition Mode: (Scan, SIM, Profile): Scan

Polarity: -
1H NMR (D2O) deprotected NBoc-L-Vgly + 4PA cross metathesis crude product
ESI-MS (S,E)-2-((tert-butoxycarbonyl)amino)hept-3-enoic acid (89)
XI. CURRICULUM VITAE

Robert W. Hoppe

Highlights

• Experience in developing, optimizing and scaling up organic synthetic procedures
• Extensive experience in characterization of molecular structure and behavior using NMR, MS, HPLC, and fluorescence spectroscopy
• Directed research of multiple undergraduates emphasizing fundamental synthetic technique, prudent record keeping, and spectroscopic analysis
• Managing daily laboratory operations including chemical purchasing, organization and storage, and maintenance and repair of equipment

Education

• Dissertation Title: “Molecular Recognition in Water: Rigid Synthetic Receptors and Enzymatic Mechanistic Probes”
  Adviser: Professor Alan W. Schwabacher

• BS, Chemistry, University of Wisconsin-Oshkosh, Oshkosh WI, 2006
  Adviser: Professor Brant L. Kedrowski

Research Experience

2007-Present  Graduate Assistant, University of Wisconsin-Milwaukee
  Adviser: Professor Alan W. Schwabacher

• Synthesis and characterization of a water soluble artificial molecular receptor
• Dramatic simplification of thermal reactions: L-Vinylglycine synthesis
• Small molecule synthesis for catalytic and kinetic characterization of enzymes
• Identification of a novel synthesis of diaryl amines in optimization of a large scale synthesis
• Quinolone heterocycles synthesis and correlating factors in regioselectivity in formation

2004-2006  Undergraduate Research Assistant, University of Wisconsin Oshkosh
  Adviser: Brant L. Kedrowski

• Studied natural product synthesis
Teaching Experience

General Chemistry (5 terms), Survey of Biochemistry (4 terms), Organic Chemistry (4 terms), Advanced Organic Laboratory (5 terms)

Affiliations

- American Chemical Society

Publications


References

Professor Alan W. Schwabacher, Dept. of Chemistry & Biochemistry, Univ. of Wisconsin-Milwaukee, Milwaukee WI 414-229-4410 awschwab@uwm.edu

Professor Nicholas Silvaggi, Dept. of Chemistry & Biochemistry, Univ. of Wisconsin-Milwaukee, Milwaukee WI 414-229-2647 silvaggi@uwm.edu

Professor Anja Blecking, Dept. of Chemistry & Biochemistry, Univ. of Wisconsin-Milwaukee, Milwaukee WI 414-229-2974 blecking@uwm.edu