Part One. Synthesis of iso-polydiacetylenic precursor. Part Two. The study of asymmetric addition of dimethyl thiophosphite to imines

Troy Mason Voelker
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PART ONE
SYNTHESIS OF ISO-POLYDIACETYLENIC PRECURSOR

PART TWO
THE STUDY OF ASYMMETRIC ADDITION OF DIMETHYL
THIOPHOSPHITE TO IMINES

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presented in partial fulfillment of the requirements
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This research has been divided into two subjects. The first was comprised of the study of vinyl triflates and terminal acetylenes used as precursors for iso-polydiacetylenic precursors. The second study was to assess the asymmetric addition of dimethyl thiophosphite to imines with chiral auxiliaries.

In the first study, palladium catalyzed cross-coupling of a terminal acetylene and vinyl triflate, which produced a dimer that could be converted into either a terminal acetylene or vinyl triflate was accomplished. The dimer was then converted into a terminal acetylene and coupled with the vinyl triflate monomer to produce a trimer that allowed linear chain growth. The sequence was repeated until the tetramer was produced establishing the possibility for iso-polydiacetylenic precursors to be made by the established method.

In the second study dimethyl thiophosphite was added across imines made from chiral amines and aldehydes. The diastereomer ratio ranged from poor (10:9) to good (11:2) and was independent of hydrogen bonding auxiliaries. Separation of diastereomers was accomplished with some of the compounds using silica chromatography. The asymmetric dealkylation using potassium ethyl xanthate was studied and found to be successful on the compounds that did not possess a hydroxy functional group on the chiral amine.
Dedication

It is with my utmost respect and pleasure to dedicate this thesis to my parents Don and Mary Voelker, my brothers Todd and Tyler Voelker, and my wife Sarah Voelker and all of their loved ones. Without their love and support my life would never be complete.
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I must also express my admiration of the Thompson group whom all have become more than just people with whom I work. Professor Sean Esslinger has been a constant friend and associate that I could approach with any problem whether it was about science or life. Dr. Joseph Degraw for all of his insight, patience, and the countless reminder of what a true chemist lives for; golf and beer. Travis Denton and James Cox for their camaraderie in the lab and conversations at the watering holes. Todd Talley, whom always had the answer on how to feel better and Katie George for her constant support and insight on what really matters. Jason Mullins for reminding me to not go back to graduate school along with Nate Tamblyn, Lisa Sandoval, Holly and, of course, Karma.
There is no way to list all who helped me get here today but I know that I would not be a chemist if Dr. Eric D. Edstrom had not spent so many days mentoring me through undergraduate school.

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List of Abbreviations

ACE = angiotensin converting enzyme
anal. calc = elemental composition; calculated value
anhyd = anhydrous
ANP = atrial natriuretic peptide
BNP = brain natriuretic peptide
br = broad
BTSE = bis-trimethylsilylethylene glycol
calcd = calculated
CHF = congestive heart failure
CNP = C-type natriuretic peptide
CP = conjugated polymers
d = doublet
DBQ = 2,6-dibromo-N-chloro-p-benzoquinoneimine
dd = doublet of doublets
DMF = dimethylformamide
dt = doublet of triplets
ESI = electron spray ionization
Et = ethyl
Et2O = ether
EtOAc = ethyl acetate
EtOH = ethyl alcohol
\( g = \text{gram(s)} \)

GC = gas chromatography

h = hour(s)

hex = hexanes

HRMS = high resolution mass spectrum

Hz = hertz

IR = infra red

Iso-PDA = \( \text{iso-polydiacetylene} \)

\( J \) = coupling constant (NMR) in hertz

\( K_2\text{CO}_3 \) = potassium carbonate

KOH = potassium hydroxide

LCS = linear \( \pi \)-conjugated systems

LED = light-emitting diodes

M = moles per liter

m = multiplet (spectral) milli

m/z = mass to charge ratio

Me = methyl

MeOH = methyl alcohol

Mg = milligram

MHz = megahertz

min = minute(s)

mL = milliliter

mmol = millimole
mol = mole(s)
NaNH = sodium hydroxide
NEP = neutral endopeptidase
NMR = nuclear magnetic resonance
PAM = phenylacetylene macrocycles
PAS = phenylacetylene sequences
PEX = potassium ethyl xanthate
ppm = parts per million
q = quartet
RAS = renin-angiotensin system
Rt = retention time
rt = room temperature
s = singlet
TEA = triethylamine
TES = triethyl silyl
THF = tetrahydrofuran
TIPS = triisopropyl silyl
TLC = thin layer chromatography
TMEDA = N,N,N',N'-Tetramethylethylenediamine
TMS = trimethyl silyl
Ts = tosyl, p-toluenesulfonyl
TS = transition state
UV = ultra violet
\[ \delta = \text{chemical shifts in parts per million} \]

\[ \mu = \text{micro} \]
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Chapter 1

Introduction

A. Molecular materials

For more than a century, synthetic chemists have demonstrated their ability to construct molecules and materials capable of fulfilling a huge variety of requirements in terms of mechanical, physical, and chemical properties. The electronic properties of linear $\pi$-conjugated systems (LCSs) have been the subject of growing importance in many areas of modern chemistry and also in the physics of condensed matter.\(^{[2]}\) At the molecular level, LCSs represent the simplest models of molecular wires, which together with their complementary functions such as molecular switches, or logical gates, have contributed to the emergence of the concept of molecular electronics.\(^{[3]}\) LCSs constitute the elemental bricks of molecular materials that have given rise to considerable research effort in basic and applied material science with the view to develop new materials and technological advancements.\(^{[2]}\)

Until recently, conjugated polymers (CP's) represented the best known prototypes of LCSs. The unique properties of CP's originate from their extended $\pi$-conjugated system delocalized over a large number of recurrent monomer units. The basic model of this class of materials consists of alternate single and double carbon-carbon bonds. A compound is in conjugation when two or more double bonds are separated by alternating single bonds as in 1,3-butadiene. The $\pi$ systems of the two conjugated double bonds are delocalized over the four carbons and electron flow may be possible.
In plastics, such as polyacetylene, the conjugation extends along the complete backbone of the polymer and has been used to install electronic properties in materials that would normally be considered insulators. In becoming electrically conductive, a polymer has to imitate a metal, that is, the electrons needs to be free to move and not localized to individual atoms. The first condition for this is that the polymer consists of conjugated double bonds. However, it is not enough to have conjugated double bonds. To become electrically conductive, the polymer has to be disturbed - either by removing electrons from (oxidation), or inserting them into (reduction) the material. The process is known as doping.

When describing polymer molecules, \( \sigma \) bonds and \( \pi \) bonds are distinguished by their individual properties. The \( \pi \) bonds are fixed, immobile, and form the covalent bonds between the carbon atoms. The \( \pi \) electrons in a conjugated double bond system are also relatively localized, though not as strongly bound as the \( \sigma \) electrons. Before an electrical current can flow along the molecule, one or more electrons have to be removed or inserted. If an electrical field is then applied, the electrons constituting the \( \pi \) bonds can move rapidly along the molecule chain. The conductivity of the polymer chains will be limited by the fact that the electrons have to "jump" from one molecule to the next. Hence, the chains have to be well-packed in ordered rows for conducting to occur.
As mentioned earlier, there are two types of doping, oxidative or reductive. In the case of polyacetylene, the oxidation or reduction reaction equations are written as follows:

Oxidation with halogen (p-doping): \[ \text{[CH]}_n + \frac{3x}{2} \text{I}_2 \longrightarrow \text{[CH]}_n^{x^+} + x \text{I}_3^- \]

Reduction with alkali metal (n-doping): \[ \text{[CH]}_n + x \text{Na} \longrightarrow \text{[CH]}_n^{x^-} + x \text{Na}^+ \]

where \[ \text{[CH}_n \] = \begin{array}{c}
\text{H} \\
\text{H} \\
\text{H} \\
\text{H} \\
\text{H} \\
\end{array} \]

The doped polymer is a salt. However, it is not the iodide or sodium ions that move to create the current, but the electrons from the conjugated double bonds. Furthermore, if a strong enough electrical field is applied, the iodide and sodium ions can move either towards or away from the polymer. This means that the direction of the doping reaction can be controlled and the conductive polymer can easily be switched on or off. Applications of such materials have been used in the production of photodiodes, flat television screens based on light-emitting diodes (LED), and luminous traffic signs.

The game illustrated in Fig. 1.1 offers a simple model of a doped polymer. The pieces cannot move unless there is at least one empty "hole". In the polymer each piece is an electron that jumps to a hole vacated by another one. This creates a movement along the molecule or an electric current.
Figure 1.1. A game that illustrates polymer doping. In order to align the numbers of the puzzle a piece must be moved into the hole creating a new hole which then allows another piece to be moved.

B. Aromaticity

Another principle in chemistry that is a direct result of a conjugated bond system is aromaticity. The most common aromatic compound is benzene. Benzene has the molecular formula C₆H₆ and is cyclic compound with three double bonds that are in conjugation, and therefore, the \( \pi \) electrons are delocalized around the entire ring. Unlike other alkenes, when benzene undergoes electrophilic addition it is quickly followed by elimination to restore the stable aromaticity, suggesting that this class of compounds should be treated different then other alkenes. In 1926, a British chemist named Sir Robert Robinson, recognized that the compounds that had been classified as aromatic always had six electrons, in either \( \pi \) or nonbonding orbitals, in a planar ring. Robinson suggested that there was a special stability associated with what he called an aromatic
Figure 1.2. Molecular orbital diagram of benzene.

sextet. As the theory of molecular bonding developed, it became clear that a sextet was indeed a significant number, corresponding to the number of electrons that fill the three bonding molecular orbitals of benzene (Fig. 1.2). Fewer electrons would leave unpaired electrons in the bonding orbitals and create a species with radical characteristics. Additional electrons would have to go in antibonding molecular orbitals and would create an unstable, high-energy species. Benzene has a filled shell of molecular orbitals, just as an element such as argon has a filled shell of atomic orbitals.

In 1931, a German chemist named Erich Hückel demonstrated that there were other numbers of electrons that corresponded to filled shells for smaller or larger rings. He proposed what is now known as Hückel's rule: "any conjugated monocyclic polyene that is planar and has $4n + 2$ π electrons ($n = 0, 1, 2$, etc.) will exhibit the same stability that is associated with benzene."

Benzene is the ideal aromatic compound. The six carbon atoms in the ring are $sp^2$-hybridized, so their regular bond angles of 120° create a perfect hexagon. The
molecule is planar and totally symmetrical. Because all the ring atoms are carbon, no differences in polarity are introduced by the presence of other elements in the ring. The electrons are delocalized evenly over the entire ring. Larger rings with conjugated cis double bonds cannot be planar because the bond angles required for such geometrical figures cannot be achieved. However, trans double bonds are possible for large carbon rings. Such a configuration puts hydrogen atoms on the inside of the ring where they occlude each other and prevent the molecule from being planar. Only in rings consisting of 18 or more carbon atoms is this steric hindrance removed from the molecule (Fig. 1.3). Although these larger rings, called annulenes, do not have the stability associated with benzene they do have some of the properties associated with aromaticity. Annulenes share properties with aromatic compounds and LCSs. These carbon allotropes possess complete π-conjugated systems that have a limitless boundary of carbon skeletons.\(^6\)

![Figure 1.3. Annulene](image-url)
C. Annulenes

A continuing question in the concept of aromaticity is the effect of ring size. Various theoretical predictions have suggested that in the upper range of ring size, roughly estimated to be anywhere from 18-30 carbons, the special properties associated with aromatic rings will disappear and these allotropes will begin to behave like simple polyenes.\(^7\) Although past synthetic work has provided rings of this size,\(^8\) the conformational mobility of these simple annulenes made it difficult to evaluate their properties in terms of polyene vs. aromatic character.\(^9\) The dehydroannulenes, containing acetylene-cumulene bonds, are much more structurally rigid and provide a more ideal platform for the study of aromatic properties in large rings. However, assessing the question of polyyne vs. aromatic character in this series is again difficult because of the unknown alkyne-cumulene anisotropy effects along with the unknown effect of having short fixed bonds in the annulene perimeter.\(^10\)

The ideal molecules for studying the aromatic conjugation are those that possess rigid, planar annulene perimeters in which all of the annulene bonds are equivalent. Sondheimer and coworkers accomplished the first synthesis of \(^{[1]}\)annulene 1, which contains a continuous molecular orbital around the ring. Cyclic compound 1 possesses 18 \(\pi\)-electrons in conjugation and was the first fully conjugated monocyclic system known with more than the classical sextet.\(^{[12]}\) This milestone in both synthetic and physical organic chemistry provided experimental support.
for the Hückel rule defining aromaticity and provided a spark for the synthesis of a
diverse array of macrocyclic π-electron perimeters.

D. Oxidative Acetylene Coupling

The synthesis of fully conjugated macrocyclic systems is primarily based on
acetylenic coupling (Fig. 1.4). First discovered by Glaser\textsuperscript{[13]} in 1869 and later applied by
Baeyer\textsuperscript{[14]} to the formation of near infinite, all carbon chains known as carbyne, the
reaction never achieved broad application because the isolation of explosive copper
acetylide was required for the coupling reaction. It was not until modifications to the
Glaser coupling by Englinton and Galbraith\textsuperscript{[15]} whom made use of cupric salts and donor
solvents such as pyridine, or Hay\textsuperscript{[16]} whom discovered the conditions for homocoupling,
and Cadiot-Chodkiewicz\textsuperscript{[17]} that developed the heterocoupling reaction that acetylene-
based molecular architecture took hold in synthetic organic chemistry.\textsuperscript{[18]} Considerable
synthetic efforts are currently targeting uncommon molecular architectures and
functionally advanced materials using acetylene coupling methodologies.
Glaser reaction

\[
\begin{align*}
2 \text{C}_6\text{H}_5\text{H} & \xrightarrow{2 \text{CuCl}} \text{C}_6\text{H}_5\text{Cu}, \\
\text{NH}_4\text{OH, EtOH} & \quad \text{NH}_4\text{OH, EtOH}
\end{align*}
\]

Eglinton and Galbraith

\[
2 \text{R} = = \text{H} \xrightarrow{\text{CuOAc}} \text{R} = = \text{R}
\]

Hay coupling

\[
2 \text{R} = = \text{H} + 0.5 \text{O}_2 \xrightarrow{\text{CuCl\cdotTMEDA (cat.)}} \text{solvent} \xrightarrow{\text{R} = = \text{R}} \text{R} = = \text{R}
\]

Cadiot-Chodkiewicz heterocoupling

1) \text{CuCl, NH}_2\text{OH\cdotHCl, EtNH}_2\text{MeOH, N}_2

\[
\begin{align*}
\text{R} = = \text{H} & \xrightarrow{\text{EtNH}_2\text{MeOH, N}_2} \text{R} = = \text{R} \\
2) \text{R} = = \text{Br, MeOH} & \xrightarrow{\text{R} = = \text{R}} \text{R} = = \text{R}
\end{align*}
\]

Figure 1.4. Acetylenic coupling reaction conditions.

E. Radialene

Within the family of annulene chemistry is a subset called radialenes. Radialenes are a series of all-methylidene-substituted cyloalkanes of molecular formula C\(_n\)H\(_n\) (Fig. 1.5) and has been an active field of chemistry for the past twenty years.\(^[19]\) By inserting ethynediyl or buta-1,3-diynediyl moieties into the cyclic radialene framework, a carbon rich homologous series of expanded radialenes is obtained with the molecular
Radialene and expanded radialenes’s defined by the formula $C_{x}H_{n} (x = 1, 2, 3)$.

Figure 1.5. Radialene and expanded radialenes’s defined by the formula $C_{x}H_{n} (x = 0, 1, 2, ...etc.)$.

formula $C_{n}H_{2n}$ and $C_{n}H_{3n}$.\(^{[20]}\) In contrast to their constitutional isomers with endocyclic, linearly conjugated double bonds such as benzene and cyclooctatetraene, radialenes possess an uninterrupted cyclic arrangement of cross-conjugated $\pi$ systems. The first radialene synthesis reported was hexaethylidenecyclohexane 2 (Fig 1.6) in 1961.\(^{[21]}\)

Radialenes have always been overshadowed by the linearly conjugated polyenes and the arenes in preparative and industrial organic chemistry. Yet recent interest in this radialenes has increased significantly because new methods of synthesis make them more accessible, and they are potential candidates for the construction of organic conductors and ferromagnets.\(^{[22]}\)

The highly symmetrical structure of radialenes, suggested by their constitutional formulas is not only aesthetically appealing but also stimulates the fantasy of the chemist.
It is thus not surprising that the search for a fitting trivial name for these regular structures with the double bonds “radiating” from the central ring led to the name “radialene”.\textsuperscript{[22]} Although this term was coined originally for the hexamethylenecyclohexane \textsuperscript{2}, it was quickly adopted for the whole series. To differentiate between its various members, a nomenclature was adopted in which the number of the exocyclic double bonds was put in parentheses in front of the word radialene, e.g., \textsuperscript{[23]}radialene \((n = 3, 4, ..).

![Figure 1.6. Two thermalytic routes to radialene (2).](image)

Methods of preparing radialenes fall into two general synthetic strategies: (1) olefin-forming reactions at an already existing cycloalkane skeleton, and (2) thermal, photolytic, or metal-induced cylooligomerization reactions of suitable\textsuperscript{[23]} cumulenes (Fig. 1.6). The latter method has turned out to be the more versatile and successful one so far, although it still cannot compete in product selectivity with the former. Diederich and Boldi\textsuperscript{[24]} were the first to synthesize a series of expanded radialenes \textsuperscript{3-5} (Fig. 1.7). The most direct synthetic route to these compounds would be by means of an Eglinton-Glaser cyclization of tetraethynylethene 6. Unfortunately, this method did not afford any isolable quantities of the macrocycles but instead led to oligomeric material.\textsuperscript{[24]} To favour cyclization and provide \textsuperscript{3-5} over competing polymerization
R = TIPS (triisopropylsilyl; iPr$_3$Si-)

Figure 1.7. Perethynylated expanded radialenes synthesized by Diederich and Boldi.\textsuperscript{19}

reactions, the authors had to access the radialenes by first making the dimeric compound 9 (Scheme 1.1). The dimer was prepared by sequential, kinetically controlled deprotection of trimethylsilyl (TMS) and triethylsilyl (TES) in the presence of the analogous triisopropylsilyl (TIPS) protected groups of compound 7. After removal of the
Scheme 1.1. Synthesis of perenthynylated radialenes.
TMS group, 8 was homocoupled under Eglinton-Galbraith conditions to afford dimer 9. Removal of the TES protecting group to afford 10 and a second homocoupling, produced 3 and 5 in 15% and 20% yield.

In order to prepare the pentagon radialene 4, the trimer 11 had to first be produced via a heterocoupling between 6 and 8. After removal of the TES protecting group an oxidative cyclization between 12 and 10 produced 4 (15%) as the major product with a small amount of 3 and 5 from the respective homocoupling reaction. All three radialenes are readily soluble, highly stable compounds with melting points above 260EC. The stability of the radialenes indicates the absence of free terminal alkyne groups which generally destabilize polyacetylenic π systems.

The particularly high stability of 3-5 may well be due to inefficient cross-conjugation, which cannot compete with linear π-electron conjugation in these systems. Inefficient cross-conjugation could be a general stabilizing principle of unsaturated carbon-based matter: fullerene C_{60} is best described as a cross-conjugated molecule with radialene substructures and graphite may also be viewed as cross-conjugated. The high solubility of 3-5 probably is more due to the presence of the TIPS groups rather than cross conjugation but this phenomenon still leaves hope for the construction of larger molecules with similar properties.

Although the production of expanded radialenes is remarkable in itself there are some drawbacks to the syntheses. It is four steps to prepare the tetraethynylethene 6 and although the protecting groups are a product of the coupling reaction to make 6 the subsequent selective, kinetically controlled deprotection to afford the dimer 9 and trimer 10 resulted in overall low yields and significant losses of material from over deprotection. An important facet to radialene synthesis that arose from these experiments is the
methodology of a stepwise synthesis of polymeric molecular rods. Although the direct synthesis from a single monomer unit is possible the by-products from such a reaction obviates any type of purification.

F. Iterative Radialene Synthesis

The challenge exists to develop supramolecular chemistry into a more systematic science, and therefore, methods need to be available for encoding molecular building blocks in a way that is simple, yet offers total specificity.\cite{26} In order to mimic the best-known natural supramolecular systems such as the nucleic acids and proteins, synthetic methods that allow efficient, but versatile construction of complex molecular objects from simple building blocks are desirable. Dissections of structures 3-5 indicate that the major drawback to the syntheses is not the low yielding oxidative coupling but rather the inefficient choice of tetraethynylethene as the monomer unit. The numerous steps to produce tetraethynylethene is simply too extensive to make production of the macrocycles on a large scale impossible. So there is an obvious need for further development of acetylenic building blocks that are easy to prepare and functionalize.

The stepwise construction of networks, in other words the transformation of appropriate monomers into successively larger oligomers, is the most promising approach. In this approach the solubility of the larger system can be estimated from the properties of the smaller molecules. If necessary the monomers can be modified to improve the desired properties of the anticipated network. Any repetitive methodology for assembling covalently linked sequences of monomeric units must be able to control chain length, sequence order of monomers, and end-group functionality. In addition, the
method must be efficient and versatile. The solid-phase methods pioneered by
Merrifield\textsuperscript{[27]} remain the methods of choice of complex polypeptides, especially when
each successive unit is different from the previous. However, it is clear that this
approach is not easily adapted to other complex molecular systems.

![Diagram of iterative cycle to synthesis of radialene precursors]

\textbf{Figure 1.8.} Iterative cycle to synthesis of radialene precursors.\textsuperscript{22}

For sequences that are periodic or sequences that have extensive periodic
segments, the repetitive method established by Moore\textsuperscript{[28]} (Fig 1.8) is considerably more
efficient as long as you can avoid solubility and purification problems. The cycle begins
by selective deprotection at each of the two ends of the diprotected monomer \(A_pB_p\),
yielding the monoprotected intermediates \(A_pB\) and \(AB_p\). These are often coupled to give
the dimer \(A_pBAB_p\) protected in the same manner as the monomer unit. The process can
be repeated \(n\) times to give a sequence of length \(2^n\). Sequences of length other than \(2^n\), as
well as sequences having particular arrangement of co-monomer units, can be realized by
merging parallel repetitive cycles. For example, a hexamer sequence is achieved by
combining tetramer and dimer sequences.

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A common feature in the preparation of carbon-rich systems is the use of the C-C triple bond as a functional group and linking unit. The alkyne group is the only easily preparable all-carbon building block; terminal alkyne functions can be linked to \( \text{sp}^2 \)-\( C-X \) (\( X = \text{halogen} \)) molecules readily and in high yields with certain oxidative coupling procedures. Thus, the acetylene function is indispensable not only in the construction of carbon-rich structures but also in the synthesis of larger rings. The relative rigidity and linearity of the acetylene fragment is a further advantage in the synthesis of rigid and uniformly shaped structures. So if the acetylene functional group can not be removed from the methodology of the synthesis then the vinyl coupling unit is the only functional group left to be explored.

A typical synthesis is illustrated by the palladium-catalyzed cross coupling\(^{[29]}\) of monomers TMS!A!Br and H!B!N=N!NEt\(_2\) which has trimethylsilylacetylene and 1-aryl-3,3-diethyltriazene end group functionalities (Scheme. 1.2).\(^{[26]}\) Reaction of TMS!AB!N=N!NEt\(_2\) with methanol in the presence of a catalytic amount of potassium carbonate gives terminal acetylene H!AB!N=N!NEt\(_2\). In contrast, reaction of TMS!AB!N=N!NEt\(_2\) with methyl iodide gives aryl iodide TMS!AB!I. Intermediates H!AB!N=N!NEt\(_2\) and TMS!AB!I are then cross-coupled to give tetramer H! (AB)\(_2\)N=N!NEt\(_2\) which has the exact same end group functionalities as dimer.
Scheme 1.2. Controlled synthesis of phenylacetylene sequences.
H!AB!N=N!NEt₂. This process can be repeated \( n \) times to give a sequence of length \( 2^n \).

A key to the success of this approach is that trimethylsilylacetylene and 1-aryl-3,3-diethyltriazene function very effectively as complementary protecting groups for terminal acetylene and aryl iodide. Each of the two protecting groups can be selectively removed in the presence of the other, and both protecting groups are stable to the cross-coupling reaction conditions. After a few iterations of the sequence well-defined phenylacetylene sequences (PAS) can be used to prepare phenylacetylene macrocycles (PAM). Deprotection of both masking groups from the sequence’s termini give difunctional intermediates such as H-(AB)₆-I. Upon intramolecular cyclization of PAS’s rigid macrocycles are obtained with larger internal diameters in high yield under optimized cyclization conditions. Although the synthesis does not provide a route to radialene compounds it does define the methodology employed by most in the radialene research community.

While studying triflate derivatives, Stang
\(^{[30]}\) prepared compound 13, which was singled out as a class of molecules ideally suited to probe the electronic characteristics of cross-conjugation in organic molecules and provide a monomer unit suitable for radialene synthesis. Tykwinski
\(^{[31]}\) reported the iterative synthesis and characterization of this class of oligomers. Palladium-catalyzed coupling of terminal alkynes with vinyl triflates
\(^{[32]}\) provided an alternative to coupling terminal alkynes to vinyl halides and an efficient route to the desired oligomers. Tykwinski assembled the \( \text{iso-polydiacetylene (PDA)} \) beginning with a palladium catalyzed cross-coupling of trimethylsilylacetylene and vinyl triflate monomer 13 to afford 14 (Scheme 1.3). Protodesilylation of 14 gave the deprotected diyne that could then be coupled with 13 to afford trimer 16. Using 16, iteration of the protodesilylation and coupling sequence gave pentamer 18. The assembly

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of radialene 21 required protodesilylation of 18 followed by cross coupling with
dibromoolefin 20. Although dilute reaction conditions was necessary to delineate the
formation of linear oligomers the cyclization reaction did require seven days for
completion. Work up and column chromatography gave the pure macrocycle in modest
yields due to decomposition during purification.

Scheme 1.3. Iterative synthesis of iso-PDA’s.
The work done by Tykwinski proved that 13 is an effective and efficient building block for the production of iso-PDA’s and radialenes. The synthesis does have a limit in terms of radialene synthesis due to the fact that chain elongation takes place on both ends of the iso-PDA. Plus, this type of approach does not allow for chain growth to proceed in a geometric fashion due to only the monomer unit being used for chain elongation rather then being able to couple a dimer to a dimer, or a trimer to a dimer, etc.

G. Statement of the Problem

Expanded radialenes, molecular wires, and polymers with polyacetylenic, conjugated backbones are compounds of extended C-cores and are materials with unusual structural and electronic properties. Macrocyclic ring systems containing multiple acetylenic units may have possible ligand binding properties or provide a core for the construction of expanded dendrimers. Rigid acetylenic molecular rods of defined length can serve as precursors to molecular wires for potential electronic devices. The preparation of acetylenic precursors for the construction of such molecules has been an interest in synthetic chemistry in the past decade. The goal of this project is to develop a novel synthetic route to the construction of polyacetylenic backbone precursors based on palladium cross-coupling reactions between vinyl triflates and terminal acetylenes.

It was in the interest of this investigator to study the possibility of using 13 in an iterative synthesis that would adapt to Moore’s methodology. By using 22, the precursor to 13 and readily accessed from bis-trimethylsilyl acetylene and the acid chloride, an iso-PDA with geometric growth may be realized (Scheme 1.4). By using the ketone and the trimethylsilyl group of 22, a set of complimentary protecting groups for the coupling
reaction is realized. Specifically, if 22 were converted to triflate from the ketone, and then cross-couple the triflate with the terminal acetylene acquired from desilylation of 22, a dimer would be produced that has the same protection scheme as the monomer. Through the iterative process established by Moore, an iso-PDA could be produced with the same 2ⁿ chain growth. Once the iso-PDA has reached the length desired for the macrocycle both ends of the chain are then deprotected and fused to make the radialene.
Scheme 1.4. Proposed synthetic design for iso-PDA’s.
Chapter 2

Synthesis

A. Iso-PDA framework

An iterative method of chain elongation was used to assemble the cross-conjugated precursors to the radialenes by utilizing palladium-catalyzed cross-coupling of terminal acetylenes and vinyl triflates. The coupling sequence is sufficiently mild enough to tolerate the unsaturated iso-PDA framework of the precursors, including the protecting groups masking the active coupling units, and allows modular incorporation of the different substituents required to maintain solubility or vary electronic effects.

The monomer unit 22 is made from the reaction of bis-(trimethylsilyl)acetylene with isopropyl acyl halide under Friedel-Crafts conditions (Scheme 2.1). Isopropyl acyl chloride was chosen as the acid chloride because once it is converted to the isopropylidene it could be distinguished by its singlet in the $^1$H NMR, making spectral analysis less complex on the radialene product. The reaction is conceived as an electrophilic displacement of one trimethylsilyl moiety by an acyl group which proceeds cleanly because of the electron-withdrawing power of the acyl substituent deactivates the ethynyl-silicon bond of the product toward further attack.[33] The acyl group which inhibits the breaking of the ethynyl-silicon bond under Friedel-Crafts conditions enhances the reactivity of the trimethylsilyl group toward nucleophilic displacement and can be cleaved by weak base. The TMS group of 22 was selectively cleaved with a 1:8 solution of 0.01M borax($aq$) in methanol in 36 h at rt but was found to be too volatile for the synthetic scheme. So to add some bulk to the monomer unit and some diversity to the
Scheme 2.1. Synthesis of monomer coupling compounds (vinyl triflate and terminal acetylene).
\(^1\)H NMR spectrum, cyclohexyl acid chloride was used as a co-monomer in the production of the radialene precursors. Gas chromatography (GC) was used to track the progress of the desilation reaction by monitoring the decrease in area of the 1-cyclohexyl-3-trimethylsilyl-propynone peak (R\(_t\) = 8.40 min) and the rise of the 1-cyclohexyl propynone, \(31\), peak (R\(_t\) = 6.44 min).

Standard conditions for making the triflate\(^{[34]}\) were attempted, but all afforded, along with left over starting material, tarring and very low yields, or no desired product. The triflates \(32\) and \(33\) were eventually prepared by the reaction of the corresponding ketone with the bulky base 2,6-di-t-butyl-4-methylpyridine and triflic anhydride in CH\(_2\)Cl\(_2\) in 78\% and 74\% yield.\(^{[12]}\) Use of the non-nucleophilic, sterically hindered 2,6-di-t-butyl-4-methylpyridine rather than standard bases was essential in this step. Triethyl amine, pyridine, and collidine result in heterogenous reaction conditions as the anhydride reacts with these nucleophiles affording amine salts that precipitate out of solution. The sterically hindered base 2,6-di-t-butyl-4-methylpyridine acts as a true Lewis base and is incapable of reacting with the anhydride which allows the carbonyl oxygen to attack the anhydride and increases the rate of the reaction.\(^{[30]}\) After nucleophilic attack by the carbonyl oxygen 2,6-di-t-butyl-4-methylpyridine scavenges the proton from the \(\alpha\) carbon and forms a salt. The amine salt formed in the triflate reaction can be collected and the 2,6-di-t-butyl-4-methylpyridine base can be recycled if desired for future reactions. A GC was used to monitor both of the triflate reactions and the structures of \(32\) and \(33\) were confirmed by the trifluoromethyl quartet in the \(^{13}\)C spectrum (Fig. 2.1).
Cross-coupling of 32 and 34 under standard Heck conditions did not afford the desired product 35 (Scheme 2.1), but rather an insoluble thick black tar and recovered vinyl triflate. The initial thought was that the enol tautomer of the ketone was forming under the basic conditions of the cross-coupling reaction and thus consuming the terminal acetylene starting material. Since the carbonyl group is essential to make the vinyl triflate a protection scheme was devised in order to stop the enol formation (Scheme 2.2).

Figure 2.1. $^{13}$C NMR spectrum of the isopropyl vinyl triflate showing the quartet of $-\text{CF}_3$
Scheme 2.2. Protection of the α,β-unsaturated ketone and palladium cross-coupling.

Since the protecting group would have to be stable to the basic reaction conditions used in the desilation and cross-coupling reactions the 1,3-dioxolane moiety was the most attractive choice. Several attempts at ketalization of 22 with typical methods\(^{[35]}\) using ethylene glycol in the presence of various catalysts, such as pyridinium \(p\)-toluenesulfonate, \(p\)-toluenesulfonic acid, or Amberlyst 15 ion exchange resin, resulted in
either no product or a complex mixture of products as indicated by NMR, GC, and TLC analysis. The most successful standard procedure was the use of 2,2-dimethylpropanediol and p-TsOH refluxed in benzene for 48 hrs which still only produced a 45% yield of the ketal.

To avoid such forcing conditions, a more facile approach was needed. Noyori reported an efficient ketalization procedure involving bis-trimethylsilylethylene glycol (BTSE) and Me₃SiOTf as reagents on α,β-unsaturated ketals. The idea is to shift the equilibrium of the reaction toward the products by forming the very stable hexamethyldisiloxane. The isopropyl monomer 22 was chosen to make the ketal because the ¹H peaks of the methyl groups could be easily distinguished from the ketal protons. The Noyori reaction conditions were conducted on 22, the progress monitored by GC, and the isolated product had the expected ¹H spectrum for the ketal group (Fig. 2.2). The yield was vastly improved (87%) and 36 became available for further study.

Once the ketal was installed desilation could be done under more basic conditions without the loss of starting material or possible Michael additions. The use of K₂CO₃ decreased the time of this step from 36 to 3 h. Palladium catalyzed cross-coupling of 33 and 38 under standard Heck conditions led to the formation of 39 (Scheme 2.2). But all attempts to remove the ketal protecting group from 39 led to little or no product 40. It became obvious that the direct coupling of 32 and 34 should be explored under a variety of conditions in order to avoid a large loss of material to protection and deprotection.
In order to explore different conditions for the Heck reaction it was prudent to examine the mechanism of the cross-coupling reaction (Fig. 2.3). The first step of the catalytic cycle is the oxidative addition aryl halide to the putative 14-electron complex Pd⁰(PPh₃)₂ to afford a σ-aryl palladium (II) complex. The second step is a nucleophilic attack on trans-ArPdX(PPh₃)₂ with a syn-addition of the olefin. The final product is generated by a β-hydride elimination via a base. Cross-coupling reactions are catalyzed by Pd⁰(PPh₃)₄, but it has been established that Pd⁰(PPh₃)₂, which forms after two
decomplexations of PPh₃ is the transient reactive species in the oxidative addition of ArX. However, its concentration is extremely low because it is involved in an endergonic reaction with the major but unreactive species Pd⁰(PPh₃)₃. The result of the equilibrium is a low turnover rate in the cross-coupling cycle which in turn means longer reactions times and loss of the terminal acetylene due to base instability. Reducible palladium (II) complexes such as Pd⁴Cl₂(PPh₃)₂ was considered a possible better precursor since their reduction in situ was expected to yield quantitatively the reactive Pd⁰(PPh₃)₂ complex, but the need for a strong base again negated this route. Since the
problem of the reaction seemed to be with the base, a study was done testing the stability of 34 with a variety of bases.

By simply placing a small aliquot of 34 in some test tubes with benzene followed by the addition of an amine base and monitoring by GC and color the decomposition of 34 could be followed by the reduction in peak 6.44 min and the rise of several peaks that were never analyzed. The three bases that had little or no reaction with 34 were the bulky base 2,6-di-t-butyl-4-methylpyridine, 2,6-di-t-butylpiperidine, and Hunig’s base. Although all three bases still resulted in decomposition of 34 it was a slow degradation. All the bases were tried in the cross-coupling reaction and only Hunig’s base gave satisfactory results. 2,6-Di-t-butyl-4-methylpyridine and 2,6-di-t-butylpiperidine reaction times were long requiring several days and up to 10 eq. of 34 in order for full consumption of vinyl triflate 32. The reaction with Hunig’s base was complete in 48 h and only used 5 eq. of 34 to complete the reaction. With the cross-coupling reaction conditions established, 35 was made successfully via coupling of 32 to 34 (Scheme 2.3). The reaction was monitored by GC with the decrease of the starting material peaks (32, Rₜ = 7.1 min; 32, Rₜ = 6.4 min) and the growth of a new peak (Rₜ = 11.2 min). ¹H NMR analysis confirmed product 35 with the ¹H NMR spectra showing both the methyl singlets from 32 and the TMS singlet from 34 (Fig. 2.4, 2.5).

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Figure 2.4. $^1$H NMR spectra of 1-Cyclohexyl-4-isopropylidene-6-trimethylsilyl-2,5-hexyne-1-one 35.
Figure 2.5. A; $^{13}$C NMR full spectra of 1-Cyclohexyl-4-isopropylidene-6-trimethylsilyl-2,5-hexyne-1-one 35. B; 220-90 ppm. C; 60 - (-)10 ppm.
Scheme 2.3. Heck coupling with Hunig's base, attempt at the production of the dimer vinyl triflate, and route to the dimer terminal acetylene.

With dimer 35 made successfully, the next iteration step required the preparation vinyl triflate 41 and terminal acetylene 42 (Fig. 2.6). The terminal acetylene was prepared successfully using the same conditions (0.1 M borax(aq)) used to make the monomer unit. The vinyl triflate dimer 36 was detected by $^{13}$C NMR but was unstable to silica gel and proved to be difficult to purify. Other methods of purification were tried; alumina gel led to the same decomposition as silica, vacuum distillation never resulted in any product, the oil was simply to unsuitable for recrystallization, and even freezing the product in a benzene matrix still resulted in a black tar. Using crude 35 in the Heck coupling reaction

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Figure 2.6. A, $^1$H NMR and B, $^{13}$C NMR of the dimer terminal acetylene.
was tried but either the vinyl triflate decomposed during the reaction or byproducts poisoned the palladium catalyst. In an effort to save product and time a new scheme was put in place that would make the *iso*-PDA radialene precursor via coupling a monomer unit to the chain at each step (Scheme 2.4). The new approach would still allow many different monomer units to be incorporated into the *iso*-PDA but would require extra steps to achieve the desired chain length.

**Scheme 2.4.** Chain elongation via monomer coupling.
The heterocoupling of 42 and 33 led to the successful production of the trimer 43. The compounds had too high of a boiling point to be monitored by GC so all of the remaining reactions were analyzed by TLC. A silica column with EtOAc/hex (1:99) eluted pure 43 as light yellow oil and was verified by $^1$H and $^{13}$C NMR (Fig. 2.7, 2.8). Desilation of 43 was accomplished with 0.1 M borax to afford 44, but its purification was never fully elucidated due to the similar Rf values of both 43 and 44 creating an overlap with the two compounds in the NMR spectra. Since, 43 would be fairly inert to the conditions of coupling 44 with 32, it was used impure and did successfully produce 40, although in modest yield. A silica column with EtOAc/hex (0.5:99.5) eluted pure 45 as dark yellow oil, that quickly turned dark red if left at rt and was verified by $^1$H and $^{13}$C NMR (Fig. 2.9, 2.10).
Figure 2.7. $^1$H spectra of 1-Cyclohexyl-7-cyclohexyldene-4-isopropylidene-9-trimethylsilanyl-nona-2,5,8-triyn-1-one (43).
Figure 2.8. $^{13}$C spectra of 1-Cyclohexyl-7-cyclohexylidene-4-isopropylidene-9-trimethylsilyl-nona-2,5,8-triyn-1-one (43).
Figure 2.9. $^1$H spectra of 1-cyclohexyl-7-cyclohexylidene-4,10-isopropylidene-12-trimethylsilanyl-2,5,8,11-dodecyn-1-one (45).
Figure 2.10. $^{13}$C spectra of 1-Cyclohexyl-7-cyclohexylidene-4,10-isopropylidene-12-trimethylsilyl-2,5,8,11-dodecyn-1-one (45).
Chapter 3

Conclusions and Future Work

A. New Radialene Scheme

The use of acyl chlorides and bis-(trimethylsilyl)acetylene as precursors for iso-PDA framework has been a success. Unfortunately, making the vinyl triflate with the dimer was unsuccessful and thus chain elongation could only be achieved with the monomer units. In order to realize the radialenes from the iso-PDA precursors, a new scheme for the production of a vinyl halide will have to be implemented (Scheme 3.1). Pross and Sternhell\textsuperscript{[37]} established a route to vinyl iodides via a hydrazone intermediate. The hydrazone is prepared by condensing hydrazine hydrate and the ketone in EtOH. Then a solution of the hydrazone and TEA is cooled to 0 EC and a saturated solution of iodine and THF is added to yield the vinyl iodide. An intramolecular cross-coupling reaction with the terminal acetylene and vinyl iodide would then yield the expanded radialene. Once the radialene has been made, a UV-vis spectroscopic analysis will be conducted to establish the contribution of the cross-conjugation to the overall π-electron delocalization in the ring.
Scheme 3.1. Future radialene scheme.
Chapter 4

Introduction

A. Endogenous Vasoactive Peptides

Since their discovery, endogenous vasoactive peptides have attracted considerable interest because of their concerted actions on the heart, vascular smooth muscle and kidney, as well as their ability to alter the release of hormones and neurotransmitters. Although much information has been obtained regarding their vasoconstrictor and mitogenic actions, their involvement in modulating the activity of the cardiovascular system under normal conditions has not been fully elucidated. A better understanding of their role in pathogenesis of a variety of diseases such as hypertension, arteriosclerosis or active renal failure is required. The endogenous vasoactive peptides presumably act through a variety of mechanisms to control vascular tone and peripheral blood flow. Some of these peptides (angiotensin II, vasopressin, and neuropeptide Y) are potent vasoconstrictors while others (atrial natriuretic peptide and bradykinin) are vasodilators acting in parallel to maintain homeostasis. These peptides are known to be released mostly by endothelial cells to mediate vasoconstriction in response to various chemical and physical stimuli. Since the initial description of angiotensin II-mediated hypertension some 40 years ago, basic and clinical investigations of the renin-angiotensin system (RAS) have resulted in broader understanding of cardiovascular pathophysiology and significant advances in therapy. Angiotensin-converting enzyme inhibitors
and angiotensin receptor antagonists are now widely prescribed for the treatment of hypertension and left ventricular dysfunction.\(^{43}\)

Renin is released by juxluloglomerular cells in the kidney in response to renal hypoperfusion, decreased sodium delivery, and sympathetic activation (Fig. 4.1). Angiotensinogen produced by the liver is cleaved by renin, through the hydrolysis of a Leu-Val bond, to yield the inactive N-terminal decapeptide angiotensin I. Circulating angiotensin I is in turn, converted to angiotensin II in the lungs by the action of ACE, which cleaves a two amino acid fragment from the C-terminus end of angiotensin I.\(^{44}\) ACE, or kininase II, also plays a key role in the kallikrein-kinin system by cleaving bradykinin to inactive peptides. In addition to the hormonal effects of circulating
angiotensin II, all of the necessary components of the RAS exist in several organs and tissues, including the heart, kidneys, and vasculature.\textsuperscript{[45]}

Angiotensin II exerts its actions in target organs and tissues by binding to both angiotensin II type 1 and 2 (AT\textsubscript{1} and AT\textsubscript{2}) receptors, although adverse effects in humans seem to be mediated primarily by the AT\textsubscript{1} receptor (Fig. 4.2).\textsuperscript{[46]} In the kidney, angiotensin II causes sodium and water retention and efferent arteriolar vasoconstriction. Constriction of the systemic vasculature by angiotensin II causes hypertension, whereas

\begin{figure}
\centering
\includegraphics[width=\textwidth]{diagram.png}
\caption{Synergistic effects resulting from combined ACE and NEP inhibition are due to similar mechanisms, leading to blockade of angiotensin (AT) synthesis and concomitant potentiation of effects of natriuretic peptides and bradykinin, leading to vasodilatation, natriuresis, and improvement in myocardial function. ET-1 indicates endothelin; ECE, endothelin-converting enzyme; AT, angiotensin; Thr, thrombin; Bk, bradykinin; pGC, particulate guanylate cyclase; and sGC, soluble guanylate cyclase (Scheme from Zhang \textit{et al.} 1998).}
\end{figure}

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coronary vasoconstriction may cause myocardial ischemia and arrhythmias\[47\]. Angiotensin II-stimulated secretion of aldosterone by the adrenal cortex and arginine vasopressin by the posterior pituitary contributes to extra cellular volume expansion and sympathetic activation\[48\]. Of particular relevance to the progression of cardiovascular disease is the recognition that angiotensin II and aldosterone exert pleiotropic effects in the heart and systemic vasculature that result in myocardial and vascular remodeling\[45\]. Important biological events include cellular hypertrophy, interstitial fibrosis, apoptosis, inflammation, and thrombosis. Increasing evidence points to the RAS as a major contributor to the progression of atherosclerosis\[49\].

The key factor in the production of angiotensin II is the enzyme ACE, and thus is a major target for therapy in cardiovascular disease. ACE, a glycoprotein widely distributed in mammals, is a membrane-bound enzyme localized mainly in endothelial cells with an expression level higher in atria than in ventricles\[50\]. ACE is also a well known zinc-containing carboxypeptidase's that has a central role in blood pressure homeostasis\[51\]. ACE transforms angiotensin I into angiotensin II and degrades bradykinin, a potent stimulator of the L-arginine and cyclooxygenase pathways\[52\]. Therefore, inhibitors of ACE not only prevent the formation of a potent vasoconstrictor with proliferate properties but also increase local concentrations of bradykinin and, in turn, the production of NO and prostacyclin\[53\]. The latter may participate in the vascular protective effects of ACE inhibitors by improving blood flow and preventing platelet activation.

In clinical studies, ACE inhibitors decreased systemic vascular resistance without an increased heart rate and promoted natriuresis\[54\]. The favorable effect of ACE inhibition has been documented in many large, randomized trials in hypertension, after
myocardial infarction, and in congestive heart failure (CHF). More recently, ACE inhibitors have been shown to decrease clinical events in high-risk patients with atherosclerosis with and without ventricular dysfunction and before and after myocardial infarction. Moreover, studies have confirmed that ACE inhibitors are vascular-protective independent of their effects on blood pressure and ventricular remodeling.

The clinical effects of ACE inhibitors in the treatment of hypertension and CHF underline the importance of neurohumoral blockade. Since the introduction of captopril in 1975, many long-acting molecules have been developed. Despite their clinical efficacy, however, a substantial number of hypertensive are not adequately controlled with ACE inhibitors and need combination therapy with diuretics, β-blockers, and/or calcium antagonists. Furthermore, clinical studies in early stages of CHF demonstrated that ACE inhibitors are less effective in patients with high levels of ANP, epinephrine, and renin activity. Thus, the development of new drugs that act on neurohumoral systems other than the RAS may be advantageous.

B. Natriuretic Peptides

The family of natriuretic peptides consist of three isoforms: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). Both ANP and BNP are synthesized in the atrium of the heart under physiological conditions and in the ventricles in the presence of ventricular hypertrophy, and CNP in endothelial cells. ANP infusion reduces blood pressure while increasing urine volume, urinary excretion of sodium, and inhibits renin and aldosterone secretion, and
increases the hypotensive effect of BNP.\textsuperscript{[64]} ANP also inhibits endothelin production and proliferation of vascular smooth muscle cells and myocardial hypertrophy, and ANP has been shown to have significant sympatholytic effects as well.\textsuperscript{[65]} Because of its biological effect as an antagonist to angiotensin II, ANP is an endogenous inhibitor of the RAS (Table 4.1).\textsuperscript{[66]} ANP and BNP production in myocardium is induced by increased atrial pressure, as may occur with increased sodium intake and by decreased left ventricular function.\textsuperscript{[66]}

\textbf{Table 4.1.} Natriuretic Peptides Have Contrasting Biological Effects to Angiotensin II.

<table>
<thead>
<tr>
<th>Angiotensin II</th>
<th>Natriuretic Peptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ Blood pressure</td>
<td>↓</td>
</tr>
<tr>
<td>↓ Renal sodium secretion</td>
<td>↑</td>
</tr>
<tr>
<td>↑ Aldosterone</td>
<td>↓</td>
</tr>
<tr>
<td>↓ Renin secretion</td>
<td>↓</td>
</tr>
<tr>
<td>↑ Sympathetic nerve activity</td>
<td>↓</td>
</tr>
<tr>
<td>↑ Cell proliferation</td>
<td>↓</td>
</tr>
<tr>
<td>↑ Hypertrophy</td>
<td>↓</td>
</tr>
</tbody>
</table>

Circulating ANP, BNP, and CNP are quickly metabolized and inactivated by the specific enzyme neutral endopeptidase (NEP).\textsuperscript{[67]} The short half-life of the natriuretic peptides, as well as the fact that a peptide is difficult to administer and expensive to produce, limits the option of an exogenous application of the peptide as a possible therapeutic strategy. It should be noted that BNP has emerged as an efficacious
intravenous agent for the treatment of CHF. Therefore, pharmacological inhibition of the metabolism of natriuretic peptides is an attractive alternative therapeutic target.

C. Neutral Endopeptidase (NEP)

NEP is an endothelial, membrane-bound Zn-metallopeptidase similar to ACE that cleaves peptides at the amino side of hydrophilic residues. The membrane-bound metalloproteinase has a catalytic unit similar to ACE. NEP is widely distributed in endothelial cells, smooth muscle cells, cardiac myocytes, renal epithelial cells, and fibroblasts. NEP is also found in the lung, gut adrenal glands, brain, and heart. It catalyzes the degradation of vasodilator peptides, including ANP, BNP, CNP, and bradykinin, as well as vasoconstrictor peptides like angiotensin II.

Selective NEP inhibitors prevent, in vitro and in vivo, the degradation of natriuretic peptides and increase their biological activity. In addition to degrading vasoactive peptides to inactive products, NEP is also involved in the enzymatic conversion of big endothelin to its active form, the vasoconstrictor peptide endothelin-1. Certain NEP inhibitors cause vasoconstriction rather than vasodilation and infers that vasoconstriction peptides, such as angiotensin II and endothelin-1, can be substrates for NEP. This explains why NEP inhibitors, such as candoxatril, thiorphan, and phosphoramidon increase blood pressure in normotensives.

NEP is also involved in the metabolism of kinins. In most tissues, NEP accounts for only a small portion of the metabolism of kinins, but in human cardiac tissue, NEP accounts for nearly half of the metabolism of bradykinin. When ACE is inhibited, however, NEP becomes a major pathway for bradykinin metabolism. In experimental
studies, the reduction of ischemia and reperfusion damage after NEP inhibition is kinin-mediated.\textsuperscript{[73]} Interestingly in hypertension, the selective NEP inhibitor candoxatril (Fig. 4.3) led to only minimal blood pressure reduction, whereas its combination with an ACE inhibitor caused a marked decrease in blood pressure.\textsuperscript{[74]}

![Figure 4.3 Structure of Candoxatril.](image)

**D. Combined ACE and NEP Inhibition**

In many cardiovascular diseases, an array of regulatory mechanisms is involved, making drugs with multiple modes of action promising. In other words, using a single drug for a single target is not as beneficial when the system has more than one target to hit. Because ACE inhibition leads to normalization of the physiological effect of ANP and NEP inhibitors lower blood pressure more effectively in salt-and-volume-dependent than in renin-dependent forms of hypertension, the combination of ACE and NEP inhibition may be especially useful in hypertension and CHF.\textsuperscript{[75]} Indeed, hypertension and CHF, the hemodynamic and renal effects (i.e., urine volume and sodium excretion) achieved after simultaneous inhibition of ACE and NEP are more pronounced than after selective inhibition of both enzymes.\textsuperscript{[75],[76]}
The synergistic effect of combined NEP and ACE inhibition is based on similar modes of action, including blockade of angiotensin synthesis and simultaneous unmasking and potentiation of the effects of peptides such as ANP, BNP, and bradykinin (by preventing their degradation), in turn inducing vasodilation and diuresis and improving myocardial function (Fig. 4.2).\(^{[77]}\)

Unfortunately, ACE and NEP structure elucidation has been a very difficult task due to size and heavy glycosylation.\(^{[45]}\) At present, ACE structural information is not useful for inhibitor design, and the most relevant factors in inhibitor preparation remain in knowledge of the enzymes substrate specificity, the use of computer programs to flexibly superimpose potent conformationally restricted inhibitors (pharmacophore development), coupled with an understanding of the catalytic mechanism.\(^{[78]}\) Our understanding of the hydrolytic mechanism of these physiologically important mammalian peptidases, and their interaction with inhibitors, has been aided greatly by study of the related bacterial endopeptidase thermolysin (TLN). Although the amino acid sequence of thermolysin is not necessarily related to the sequence of other zinc-requiring peptidases such as ACE and NEP,\(^{[79]}\) there is increasing evidence that the active sites of these zinc enzymes have common features.\(^{[80],[81],[82]}\) As such, recent X-ray structures of thermolysin-inhibitor complexes have proven useful in elucidating the mode of binding of related zinc peptidases including ACE and NEP.\(^{[83]}\) Moreover, commercial availability and cost makes thermolysin easier to obtain and study. Therefore, understanding thermolysin’s mode of action and interaction with potential inhibitors can aid better understanding of ACE and NEP.
E. Thermolysin

Thermolysin (TLN) is a thermostable zinc-containing endopeptidase of molecular weight 34,600 isolated from the thermophilic bacterium *Bacillus thermoproteolyticus*.[84] In order to understand the catalytic mechanism of thermolysin, its crystal structure has been determined, both in its native state and in complexes with inhibitors.[85] TLN is related to other peptidases such as ACE, NEP, collagenase, and enkephalinase, which have been recognized to play important roles in cellular and hormonal metabolism.[84] In addition, thermolysin has proven to be an advantageous system for the detailed evaluation of protein-ligand binding interactions.[86]

TLN is particularly suited for studying the mechanism of zinc-promoted peptide hydrolysis because a variety of crystallographic, inhibitor, substrate, and chemical modification data are available. Importantly, the crystal and solution conformations appear to be very similar on the basis of available experimental comparisons,[87] and moreover, the crystalline enzyme still hydrolyzes peptides. The relevance of the crystal structure is further enhanced by the fact that thermolysin changes very little upon binding numerous inhibitors; this feature makes thermolysin a good model to study the hydrolysis mechanism by zinc peptidases.[87]

The crystal structure of thermolysin together with computer graphics analysis of the active site, and the x-ray structure of a N-carboxymethyl dipeptide inhibitor[88] have led to a proposal of its mechanism (Fig. 4.4).[89] The protein consists of two spherical domains with a deep left cleft in the middle, which constitutes the active site. In its native state, thermolysin has a single water molecule bound to the zinc ion. The inhibitor, N-(1-carboxy-3-phenylpropyl)-l-leucyl-l-tryptophan, has been observed to
bind to thermolysin with the carboxyl group close to leucine interacting with zinc. In addition, the leucyl side chain was found to be required by the active site due to its nestled location in the hydrophobic specificity pocket.

Together with three ligands from the protein (Glu-166, His-146, and His-142), the zinc coordination is essentially tetrahedral. An incoming peptide is presumed to displace zinc bound water molecule toward Glu-143, forming a pentacoordinate complex at Zn. The water molecule, activated by the combined influence of the metal ion and Glu-143, attacks the carbonyl carbon to form tetrahedral intermediate. The tetrahedral intermediate is presumed to form a bidentate complex with the zinc and is stabilized, in part, by hydrogen bonds from His-213 and Tyr-157. Glu-143 accepts the proton from the activated water molecule and is presumed to subsequently donate the proton to the scissile nitrogen, although proton donation by solvent water is not excluded as an alternative. Glu-143 is presumed to also shuttle the remaining proton from the hydroxyl derived from the nucleophilic water molecule to the generated primary amine to yield the two peptide products. [87]
Figure 4.4. Proposed mechanism of thermolysin-catalyzed hydrolysis. The reaction proceeds by the attack of the zinc-bound water molecule on the scissile bond. The resulting tetrahedral intermediate, with enzyme residues involved in its stabilization, is shown. This subsequently decomposes to yield the products. Adapted from Matthews, the nomenclature used for the individual amino acids (P₁, P₁', etc.) of a substrate and the subsites (S₁, S₁', etc.) of the enzyme is that of Schechter and Berger. Amino acid residues of substrates numbered P₁, P₂, etc. are toward the N-terminal direction, and P₁', P₂', etc. are toward the C-terminal direction from the scissile bond (figure from Fernandez et al. 2001).

**F. Design of Dual ACE/NEP Inhibitors**

As previously stated, both ACE and NEP are zinc MMP’s with similar mechanism of actions and, hence, some structural similarities. The region of the enzyme where catalysis occurs are almost identical: the zinc, the zinc binding atoms, and nearby catalytically important glutamic acid of each enzyme could readily be superimposed on
the corresponding atoms of the other protease.[78] Most relevant to inhibitor design, the regions of the structure which mimic the scissile bond of the substrate also adopt a common conformation. However, beyond this region, the conformation that the inhibitor backbone and side chains adopt is significantly different. These differences reflect the variation in the shapes of the binding site of the different enzymes. These variations in binding site topology are responsible, at least in part, for the differences in substrate specificity.[78]

ACE is a dipeptidyl carboxypeptidase usually cleaving the C-terminal dipeptide residue of its substrates. Potent ACE inhibitors have been designed to mimic both di- and tripeptides. Extensive structure activity studies of ACE inhibitors revealed three important structural features required for tight binding to ACE: a functional group acting as a zinc binder, an amido carbonyl group, and a C-terminal carboxylic acid.

NEP has a broader substrate specificity than ACE and can act both as an expopeptidase and as an endopeptidase. This behavior is reflected in the relatively large structural variations observed with NEP inhibitors. Some potent NEP inhibitors resemble tri- and tetrapeptides, while other smaller inhibitors are dipeptide mimics. The features critical for an NEP inhibitor include the following: a zinc binding group, a $\text{P}_1$ hydrophobic group, and an amide group (or amide surrogate) available for forming two hydrogen bonds. A terminal carboxylic acid group seems advantageous, although it is not essential nor is its precise position as critical at it is for ACE inhibitors.

Therefore, in order for a single molecule to inhibit both ACE and NEP, it must possess a zinc binding group. The atoms connecting this group to the rest of the inhibitor must be able to adopt the common conformation found in the zinc MMP x-ray data. In addition, this molecule must include the functional groups required for tight binding to ACE. 

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each enzyme, and these groups must be able to adopt the conformation appropriate for the different binding sites.

Conformational restriction is an attractive strategy to maximize the binding of an inhibitor to a specific active site by reducing the loss of conformational entropy experienced when a flexible molecule is removed from solvent and forced to fit into a binding site.[90] However, at first glance, this approach does not appear suited for the design of dual inhibitors. Indeed, an inhibitor which is to efficiently occupy the binding sites of two structurally different enzymes cannot be totally rigid. To overcome this problem, a molecule can be designed to include conformational constrained sections designed to interact with certain areas in the binding site of each enzyme. These structural units can then be connected by a flexible hinge, allowing the entire molecule to adopt conformations complementary to the active site of each enzyme.

The active site of an enzyme performs the twofold function of binding the substrate and catalyzing the reaction. The efficiency of these actions determines the overall activity of the enzyme towards the particular substrate, which in turn determines the specificity of the enzyme. In order for an enzyme to be selective its active site is composed of "sub sites," each accommodating one amino acid residue of the substrate. The substrate is visualized as fitting into the groove, binding to several sub sites of specific geometry (Fig. 4.5).[91]

Although NEP and ACE have significant analogies in their active sites, as shown by their capacity to cleave in vitro identical peptides at the same bond.[92] The selectivity of molecules inhibiting these enzymes results from subtle differences in the active site of both peptidases. Efficient NEP inhibitors preferentially contain aromatic residues in the $P_1$ position,[64] and most ACE inhibitors are characterized by a small residue (Ala) in the...
P$_1$ position and a cyclic amino acid (Pro) in the P$_2$ position.$^{[93]}$ However, it has been possible to design dual inhibitors of these peptides by introducing more hydrophobic residues in the selective inhibitor of NEP and have shown relatively good affinities for both enzymes.$^{[94]}$

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**Figure 4.5.** Schematic representation of an enzyme-substrate complex. The active site of the enzyme is composed of 6 “subsites”, (S$_3$-S$_3$), located within the catalytic site of the enzyme. The peptide positions, (P$_3$-P$_3$), on the peptide substrate are counted from the point of cleavage and thus have the same numbering as the subsites they occupy.
Recently, more potent dual inhibitors containing hindered substituents have been designed giving new information about the structures of the binding domains of each enzyme. Thus, the development of RB 105 [(2S,3R)-HSCH₂CH(CH₃CHCH₃)Phe]CO-Ala] has shown that the S₁⁻ of both peptidases is able to accommodate constrained moieties such as a β-methylbenzyl group. However, conformational analysis of RB 105 (Fig. 4.6) and its derivatives, to determine thermodynamically acceptable overlaps between stable conformers of these inhibitors and the ACE pharmacophore, designed from rigid selective inhibitors, showed that the presence of the β-methylbenzyl group in the P₁⁻ position, precludes the presence of bulky residues in the P₂⁻ position. Recently, a great number of molecules containing polycyclic residues at the P₂⁻ position have been described, showing that NEP seems to possess, like ACE, a large S₂⁻ domain in agreement with earlier observations. Taken together, these data support the idea that the S₁⁻ and S₂⁻ sub sites of NEP and ACE constitute hydrophobic domains which could be efficiently occupied by cyclic or linear structures.

Since the specificity of ACE and NEP is essentially due to van der Walls and ionic interactions between the S₁⁻, S₂⁻, and S₃⁻ sub sites of NEP and the S₁, S₁⁻, and S₂⁻ sub
sites of ACE a working template for \( P_1, P_2 \), and \( P_3 \) should be established: (1) the substrate has to have a zinc binding group, (2) the \( S_1 \) pocket in both enzymes can accommodate a large hydrophobic residue, (3) the \( S_1 \) sub site of ACE and \( S_2 \) sub site of NEP are also hydrophobic but do not seem to be as open as the \( S_1 \) sub site in both enzymes, (4) although not as important as the previous three, the \( S_2 \) sub site of ACE and the \( S_3 \) sub site of NEP favor a cyclic peptide, and (5) a terminal carboxylic acid.

G. Transition State and Enzyme Catalysis

The term transition state (TS) refers to the configuration along the reaction coordinate that a species must pass through on its way to product formation. The transition state corresponds to the highest energy state in the course of a reaction; it is a dynamic, reactive and unstable state in which bonds are only partially formed or broken. According to transition state theory, the physical entities under consideration during a reaction are the reagents, or ground states, and the most unstable species in the reaction pathway, the transition state(s). The importance of transition state theory is that it relates the rate of a reaction to the difference in Gibbs Free Energy (\( G \)) between the transition state and the ground state. This is an important consideration when comparing the relative reactivity of pairs of substances or the rates of a given reaction under different sets of conditions.

The difference in height on the energy surface between the starting materials and the transition state is the reaction’s activation energy (or \( \Delta G^\ddagger \)), and is the energy barrier
that must be overcome before the reactants can convert to product. The higher a reaction’s activation energy is, the more difficult the path to products. An enzyme, however, can speed up a reaction by lowering the activation energy thus providing a smaller energy “hill” in the reaction pathway (Fig 4.6). Thus, enzymes function as catalysts in reactions, meaning that they alter the rate of a reaction without themselves undergoing change. The enzyme is able to function as a catalyst by converting the substrate into a specific product through the formation of a reactive TS. One of the enzymes primary functions in its role as a catalyst, however, is not its ability to strongly

Figure 4.7. Effect of catalyst on reaction pathway.
bind the substrate or the product but rather its ability to discriminate between substrate, product and transition state. In 1946, Linus Pauling suggested that an enzyme binds more strongly to the transition state than to the reactants or products.\textsuperscript{99} When enzymes capture the transition state, it is stabilized and as a result, less energy is needed to access this structure. The energy barrier is subsequently lowered and the reaction is generally accelerated – often by factors of several orders of magnitude (Fig. 4.8). The enzyme’s effect is catalytic because the molecules immediately diffuse away from the active site following product formation, enabling the enzyme to bind and transform new substrates repeatedly.\textsuperscript{100} To provide for the catalytic affinity, interactions between the enzyme and the substrate are extensive with a specificity reflected in the transition state.\textsuperscript{101} The binding interactions are quite numerous and can include hydrophobic, ionic, \(\pi\)-stacking and hydrogen bonding.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{diagram.png}
\caption{Energy levels in catalysis vs. normal reaction}
\end{figure}
H. Transition State Analogues

The concept of transition state analogues has been very useful in designing potent enzyme inhibitors.\textsuperscript{101} Such transition state analogues are designed on the assumption that structures which mimic the normal transition state have optimal interactions with the enzyme in that site. During catalysis, favorable binding interactions, not available to the ground-state enzyme-substrate complex, develop as the enzyme-substrate transition-state complex forms. These enhanced binding interactions assist in lowering the activation energy of the reaction, and hence in accelerating the enzymatic rate.\textsuperscript{102}

TS analogue inhibitors take advantage of these additional interactions by possessing, in their stable structures, key structural elements of the unstable enzyme-substrate transition-state complex.\textsuperscript{103} Pauling predicted that it would be possible to utilize transition state analogues as enzyme inhibitors.\textsuperscript{99} He predicted that, given a reaction for which an enzyme exists, a stable substance might mimic the native, reactive transition state in shape and charge. Such a transition state analogue should bind very tightly to the enzyme and inhibit its catalytic action by filling the binding site at its most complementary state, and thereby preventing the active site from binding to its true substrate.\textsuperscript{100} This theory has been validated over the last twenty years, as a significant number of compounds have been synthesized that behave as TS analogues in just the same manner Pauling predicted.\textsuperscript{101} An example of the depiction of enzyme action is supported by studies that show that stable mimics of transition states (such as phosphonates) are held tightly by enzymes that employ the putative mechanism (Fig 4.9).\textsuperscript{101} In this mechanism, a phosphorus atom has been substituted for a reaction...
carbonyl in the transition state’s tetrahedral ensemble, yielding a stable compound known as a phosphonate ester. The distribution of charge on the phosphonate oxygen atoms of the molecule resembles that of the TS. In addition, the phosphorus-oxygen bonds are about twenty percent longer than ordinary carbon-oxygen bonds, which enables the analogue to mimic the elongated bonds of the TS.\textsuperscript{100} Importantly, phosphorous esters and amides by virtue of their $sp^3$ hybridized center, closely resemble the TS achieved during the hydrolysis of certain carbonyl compounds.\textsuperscript{104} As a result, many of these phosphorus-based analogues have been used as enzyme inhibitors\textsuperscript{105,106} and as haptens for the production of catalytic antibodies.\textsuperscript{100}

The arrival of stable TS analogues, conceived to mimic the structure of an intermediate in the path of a substrate’s transformation to product, have made it possible
to examine the degree of enzyme binding/distortion energy, and to probe the specificity
and selectivity of this interaction. Most TS analogues behave as potent inhibitors
because, unlike the native substrate, they are not transformed into product and remain
tightly held in the catalytic domain, thereby inactivating the enzyme. Some enzyme
inhibitors have been used to study the role of individual enzymes, to understand enzyme
mechanisms, and to aid in the development of pharmacological and agricultural
agents.\textsuperscript{107} Among them, peptides containing a transition state-analogue in place of a
hydrolyzing amide bond have received considerable attention. Typically, peptide TS
analogues contain a phosphonoamidate or a sulfonamide moiety,\textsuperscript{108} which show the best
resemblance to the TS during the hydrolysis of the amide bond from both a steric and
electronic point of view (Fig. 4.10). These TS analogues have been used in the
development of protease inhibitors such as thermolysin, renin and pepsin.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4_10.png}
\caption{Amide bond hydrolysis intermediate and two TS analogues.}
\end{figure}
I. Phosphorus-Containing TS Analogues as Thermolysin Inhibitors

Some of the most potent inhibitors of thermolysin are transition state analogues that incorporate phosphorus-containing tetrahedral groups capable of binding to the active site zinc in a bidentate fashion.\textsuperscript{[109],[110]}

Phosphonate, phosphinate, phosphonamidate and phosphoramidate analogues (Fig. 4.11) designed to the same TS show different binding propensity to thermolysin.\textsuperscript{[84]} The enhanced binding and inhibitory potency of the phosphonamidate analog has been attributed to specific hydrogen-bonding by the amidate.\textsuperscript{[107]} Grobelny and co-workers noted that the binding energy difference was due to increased basicity of the phosphonamidate anion (better ligates the active zinc atom), whereas solvation effects were negligible.\textsuperscript{[111]}

A well known phosphorus-containing inhibitor used to probe the mechanism of thermolysin is phosphoramidon [N-(\(\alpha\)-L-rhamnopyranosyloxyhydroxyphosphinyl)-L-}
leucyl-L-tryptophan] (Fig. 4.12). This naturally occurring potent inhibitor
($K_3 = 2.8 \times 10^{-8}$), consists of a rhamnose sugar group attached to a leu-trp moiety through
a phosphate. The phosphoramidon-thermolysin complex was crystallized, and showed
monodentate Zn-ligation to one of the phosphate oxygen’s, 2.0 angstroms from the zinc
atom, resulting in a tetrahedral complex which closely resembles the transition state
during the catalytic process. Additionally, the crystal reaffirmed that the N-phosphoryl
group mimics a hydrated peptide since it was seen that the P-O bonds were only slightly
longer than the corresponding C-O bonds, and both the phosphoryl group and a hydrated
peptide have tetrahedral geometries. Interestingly, removal of the rhamose moiety
from phosphoramidon resulted in a slightly tighter binding inhibition indicating the sugar
is not essential for thermolysin recognition.

Based on the effectiveness of phosphoramidon as an inhibitor of thermolysin, a
series of related phosphoramidates and phosphonamidates have been synthesized and
Figure 4.13. Bertenshaw’s truncated version of phosphoramidon.

\[ X = \text{CH}_2, \text{O} \]

proved to be potent inhibitors not only of thermolysin, but also of other zinc peptidases including carboxypeptidase A, endothelin converting enzyme (ECE), and ACE. Bertenshaw truncated the phosphoramidon structure in an attempt to inhibit ECE (Fig. 4.13). The study found that the sugar moiety was not essential for anti-ECE activity. Previous studies also reaffirmed that the rhamnose moiety was of very little importance for the inhibition of ACE or ECE, whereas the phosphoryl group of phosphoramidon was an absolute requirement. Furthermore, the tryptophan residue of phosphoramidon appeared to be important for the ECE inhibition while thermolysin inhibition seemed to depend greatly on the leucine residue. It was concluded that in vivo ECE and thermolysin differ only in the way they recognize phosphoramidon.

J. Phosphorus Nomenclature

Phosphorus compounds can exist as di, tri, tetra, and pentavalent species. The nomenclature of various substituted pentavalent organophosphorus compounds are as
follows. A phosphate has four oxygen’s directly linked, a phosphonate has three oxygen’s and one carbon, an H-phosphonate or phosphinate has two oxygen’s, one carbon, and one hydrogen bonded to phosphorus. A phosphoryl is a general term referring to solely the phosphorus-oxygen double bond (P=O) with the term “thio” added as a prefix when oxygen is replaced by sulfur.

It is important to recognize that when the four substituents around the central phosphorus atom are different, the phosphorus becomes a chiral center. This characteristic is important since it will add a chiral center in the compound where it will most likely ligate with the zinc atom in the metalloproteases. With enantioenriched compounds tested with thermolysin new information can be divulged about the relationship of the inhibitor and the 3D structure of the enzymes active site.

K. α-Aminophosphonic Acid and the Pudovik Addition

The phosphorus analogs of α-amino carboxylic acids, α-aminophosphonic have attracted significant attention owing to their synthetic and biological value as both agrochemical (herbicides, pesticides, growth regulator in plants) and medicinal (antibiotics, antivirals, enzyme inhibitors) products with broad applications. The bioactivity of these compounds is known to be strongly dependent on their absolute configurations, and some methods, including diastereomeric and chemoenzymatic resolutions and asymmetric synthesis, are available for the synthesis of optically pure α-aminophosphonic acids. Of the various methods for the preparation of α-aminophosphonic acids, the most general route is the Pudovik addition of
dialkylphosphonate to a Schiff base, followed by hydrolytic or reductive cleavage of
diester precursors to either the mono or diphosphonic acid.

The Pudovik addition is the addition of esters of phosphorous (III) acids with
unsaturated systems like carbonyl’s, Michael adducts, or imines producing dialkyl N-
substituted, α-substituted, and α-aminophosphonates. The reaction is attributed to the
slow tautomerism of the pentavalent phosphonate to the more reactive tricoordinate
phosphite (Fig. 4.13). The more nucleophilic tricoordinate phosphorus atom attacks the
electrophilic carbon of the unsaturated system, forming a P-C bond, and generating a
positive charge on the phosphorus atom. A nonbonding electron pair of the hydroxyl
group forms a pi bond with phosphorus, neutralizing the phosphorus atom. Subsequent
proton transfer from the protonated phosphonyl to the amide anion yields the desired
products. The addition of dimethyl phosphite to asymmetrical imines, carbonyl’s, and
Michael adducts results in a chiral carbon bonded to phosphorus (Fig. 4.13).
Unfortunately, the addition usually produces a racemic solution due to the slow reaction
time and a reaction center that can be attacked equally on either side of the eletrophilic
carbon (Fig. 4.13). The resulting racemic mixture has to then be separated by either
diasteromeric or chemoenzymatic resolution.
Figure 4.14. Dimethyl phosphite tautomerism between pentavalent and trivalent phosphorus. The reactive trivalent phosphorus attacks the electrophilic carbon of imine [1] leads the subsequent generation of the phosphonium ion [4]. The nonbonding electrons of the hydroxyl group form a double bond with phosphorus to give intermediate [5] which then looses a proton to afford α-aminophosphonate.
L. Asymmetric Synthesis

The asymmetric synthesis of organophosphorus compounds is a relatively new field, which has developed mostly during the past two decades.\textsuperscript{[119]} For the preparation of enantiomerically homogenous molecules, chemists have basically two options. The molecules can either be synthesized in racemic form and resolved, or the synthesis can be performed in an enantioselective fashion so as to produce enantiomerically enriched products. Steroselectivity in the synthesis of chiral molecules can be realized by asymmetric induction of chirality (asymmetric synthesis)\textsuperscript{[121]} or via kinetic resolution.\textsuperscript{[122]}

The term ‘asymmetric synthesis’ was first used in 1894 by Emil Fisher and defined in 1904 by W. Markwald as a reaction which produces optically active substances from symmetrically constituted compounds with the intermediate use of optically active materials but with exclusion of analytical processes. Morrison and Mosher\textsuperscript{[121]} consider the asymmetric synthesis as a reaction in which an achiral unit in an ensemble of substrate molecules is converted by a reactant into a chiral unit in such a manner that the steroisomeric products are formed in unequal amounts. Kagan and Fiaud\textsuperscript{[122]} suggested that, “kinetic resolution can be defined as a process in which one of the enantiomer constituents of a racemic mixture is more readily transformed into a product than the other (enantioselective reaction).” If the Pudovik reaction is stopped before completion, for example unequal amounts of the diasteromerically substituted organophosphorus compounds should thus be obtained. The remaining unreacted starting material will also exhibit optical activity.\textsuperscript{[123]}

Pentavalent tetracoordinate organophosphorus compounds can exist in optically active states and are configurationally stable.\textsuperscript{[124]} On the basis of optically active
organophosphorus compounds, syntheses of different chiral organic compounds have been developed in which asymmetric induction involves; the transfer of chirality from phosphorus to another center, an asymmetric addition to an achiral molecule due to steric or kinetics, and reactions in the course of which the formation of a new stereogenic center is accompanied by elimination of the phosphorus moiety.

Silyl phosphite esters \((\text{RO})_3\text{POSiR'}\) have been shown to be remarkably versatile phosphorylating reagents.\(^{[125]}\) Chiral silylated organophosphorus reagents can be used in the asymmetric phosphorylation of prochiral unsaturated organic substrates such as aldehydes. One is the asymmetric variant of the Abramov reaction. This was applied to compounds possessing \(\text{C}_2\)-symmetry where only a single stereoisomeric form exists for the silylated organophosphorus (II) reagent and hence only two possible isomers can be produced in the reaction with benzaldehyde.\(^{[126]}\),\(^{[127]}\)

The silylphosphite compounds \(46\) have been synthesized by the reaction of binaphthalatochlorophosphites \(\text{wR}_3\text{SiOH}\).\(^{[128]}\) These chiral compounds are active asymmetric phosphorylating reagents. They undergo the Abramov reaction with benzaldehyde at rt to afford new silyloxy esters \(47\) in high yield and with good stereoselectivity. Silylphosphites derived from chiral (+)-dimethyl L-tartaric esters \(48\) as auxiliaries have been obtained, however, they are not sufficiently useful because they do not react very cleanly with benzaldehyde (Scheme. 4.1).\(^{[129]}\)

The 2-triorganosiloxy-1,3,2-oxazaphospholidines \(49\) undergo the Abramov reaction with benzaldehyde at rt to afford new esters in high yield and with good stereoselectivity. Recrystallization of diastereomeric mixtures from pentane affords \(\alpha\)-siloxyphosphonate esters \(50\) as white crystalline solids in up to 88% isolated yield and 95% isomeric purity.\(^{[130]}\),\(^{[131]}\) The reaction is kinetically controlled and the transfer of the
silyl group to the oxygen is intramolecular, with results in retention of relative configuration at the phosphorus atom (Scheme 4.2).[^132]

![Scheme 4.1. The Abramov reaction with binaphthalatochlorophosphites and benzaldehyde.](image)

(Scheme 4.1. The Abramov reaction with binaphthalatochlorophosphites and benzaldehyde.

![Scheme 4.2. The Abramov reaction with 2-triorganosiloxy-1,3,2-oxazaphospholidines and benzaldehyde.](image)

(Scheme 4.2. The Abramov reaction with 2-triorganosiloxy-1,3,2-oxazaphospholidines and benzaldehyde.

The reaction of dialkylphosphite anions with aldehydes is a method for the synthesis of α-hydroxy phosphonamides. As stated previously, the absolute

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configuration at the α-position in substituted phosphonic acids is very important for biological activity.

Spilling and co-workers investigated the use of chiral phosphorus compounds as reagents for symmetric synthesis, and have developed a method for the asymmetric synthesis of α-hydroxyacid derivatives formed by addition of chiral phosphoric acid diamide to aldehydes. Addition of aldehydes to chiral phosphoric acid anions in THF solution proceeds stereoselectively to α-hydroxyphosphonamides 51 in good yield and good stereoselectivity (54-93% de).\textsuperscript{[133]} The diastereoselectivity was strongly dependent upon the diamide used and ranged from poor to good.\textsuperscript{[134]} It was found that the reaction proceeds irreversibly and under kinetic control. The phosphonamides were hydrolyzed with aqueous HCl in dioxane to provide α-hydroxyphosphonic acids 52 (Scheme 4.3).\textsuperscript{[134],[135]}

Gordon and Evans described the condensation of diastereoisomeric 2-methoxy-1,3,2-oxazaphosphorinanes 53 (75:25 diastereomeric ratio) (Scheme 4.4).\textsuperscript{[132]}

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Scheme 4.3. Asymmetric Pudovik addition with chiral phosphoric acid diamide to aldehydes.

Optically pure samples of both enantiomers of 55a and 55b were obtained by the asymmetric induction method, described initially by Gilmore and McBride. The synthesis involved preparation of Schiff’s bases 54 from R-(+)- and S(-)-1-methybenzylamine and corresponding aldehydes, addition of diethyl phosphite, hydrolysis, and hydrogenolysis (Scheme. 4.5). By $^{31}$P NMR analysis of crude esters 55 revealed that diethyl phosphite addition, when carried out at rt without solvent yielded...
Scheme 4.5. Asymmetric induction method.

55a and 55b as mixtures of diastereoisomers formed in ratios of 6:1. At higher temperatures the induction was less pronounced, e.g. heating 54 with diethyl phosphite at 140°C without catalyst, as specified by Gilmore and McBride, produced a mixture of diastereoisomeric 55a and 55b in a ratio of 2:1. Mechanistic rationale for asymmetric induction stems from the formation of the Schiff base and steric hindering the addition of diethyl phosphite on the bulky phenyl side of the compound (Fig. 4.15). Thus, heating the reaction would provide the Schiff base with the energy needed to be in a higher energy conformation and leaving both sides open for attack with diethyl phosphite.
Figure 4.15. Addition of diethyl phosphite to the Schiff base formed from benzaldehyde and R-(+)-phenethylamine. Sterics from the phenyl ring hinder attack of diethyl phosphate vs. the attack on the methyl side.

M. α-Aminophosphonothioic Acids

Despite all of the advances in the preparation of enantioenriched APA’s, the syntheses of the corresponding phosphonothionate analogs of APA’s, α-
aminophosphonothioic acids, has not been as thoroughly researched. Phosphorothioic acids are sulfur-containing analogs of phosphate groups in nucleoside chemistry and because the thiol group imparts chemical characteristics that differ from the

\[ \text{thiono-tautomer} \quad \text{thiolo-tautomer} \]

1: \( \alpha \)-aminophosphonothioic acid (\( R = H \))
2: \( \alpha \)-aminophosphonothioate ester (\( R = \text{alkyl} \))

**Figure 4.16.** General structures of \( \alpha \)-aminophosphonothioic acids (1) and phosphonothioate esters (2) and their possible tautomers.

corresponding phosphate group (i.e. nucleophilicity, ligand characteristics, etc.) show value as new substrates. Such novel properties could also be beneficial additions to TS analogs of enzymes. Furthermore, the tautomeric non-equivalent form of the \( \alpha \)-aminophosphonothioic acids (thiono-thiolo equilibrium: Fig 4.16) and the capability to generate a new asymmetric center at phosphorus when in the monoester form, provides further benefits for this class of compounds.

The use of Lawesson’s reagent (2,4-bis(4-methoxyphenyl)-1,3-diathia-2,4-diphosphetane-2,4-disulfide) to convert the \( P=O \) for \( P=S \) would be the most direct route to form an APTA. Unfortunately, the preferred reactivity of the thionating agent at the amino or amine protecting group makes this route extremely difficult. Forming the
asymmetric APTA monoester by displacement of the phosphorus ester group by \( \text{SH}^- \) fails due to competing dealkylation reactions at the phosphorus ester groups. As previously stated, dialkyl phosphites add to imines (prepared from chiral amines) with stereocontrol to afford \( \alpha \)-amino phosphonates. From previous work in this lab, it was found that dimethyl thiophosphite (DMTP) adds rapidly and in high yield to a variety of electrophiles including imines. By adapting the DMTP addition to imines with chiral auxiliaries to provide the \( \alpha \)-amino phosphonothionates bearing an enantioenriched \( \alpha \)-carbon center (Scheme 4.6) would allow direct access to the desired APTA's via hydrolysis of the ester.

\[
\begin{align*}
\text{R} & = \text{alkyl, aryl} \\
\alpha\text{-aminophosphonothionate}
\end{align*}
\]

**Scheme 4.6.** Addition of dimethyl thiophosphite to chiral imines.

**N. Statement of the Problem**

Phosphorus-containing analogues have made significant contributions to our understanding of TS structure, however, several drawbacks to their application (resulting in deficits in current understanding) do remain. Some of these drawbacks include; (1) a lack of an essential hydrogen-bonding or solvation interaction in some phosphonates and phosphinates leaves then ineffectual, (2) instability to acid for many phosphonamidates,
and, (3) when used as haptens, failure to result in antibodies with substantial catalytic activity suggesting that key elements in binding amidases may be absent in phosphonamidates.\textsuperscript{[106]}

Despite these drawbacks, phosphorus-containing analogues do offer useful advantages. Certain phosphorus esters and amides, by virtue of their sp\textsuperscript{3} hybridized center, closely resemble the transition state achieved during the hydrolysis of certain carbonyl compounds. Another important advantage is the use of \textsuperscript{31}P NMR, a sensitive nucleus, capable of revealing highly diagnostic chemical and biochemical information. In addition, x-ray techniques as in the case of the crystal structure of a thermolysin-phosphoramidate TS analogue, has given tremendous insight into the TS-protein interaction. Doubtless, the systematic design and use of phosphorus-containing analogues will remain a promising path toward more specific and potent inhibitors (or probes) of protein structure and function.

It is well established that \(\alpha\)-aminophosphonic acids, the phosphorus analogs of \(\alpha\)-amino acids, exhibit novel biological properties. Substitution of a sulfur atom in place of a phosphoryl oxygen forms \(\alpha\)-aminophosphonothioic acids (APTA's), a new class of compounds in which the carboxyl group of an \(\alpha\)-amino acid has been replaced by a phosphonothioic acid moiety. This alteration introduces chirality at the phosphorus center, as observed by the tautomERICally non-equivalent \(\text{P[137]}\text{SH} = \text{P(S)OH}\) equilibrium. As a result of this unique asymmetric center, APTA's are predicted to exhibit different chemical properties from both \(\alpha\)-aminophosphonic acids and the corresponding \(\alpha\)-amino acids. Since \(\alpha\)-amino acids serve as building blocks in several
structures important to biological activity, APTA’s could have broad applications as amino acid mimics.

The proposed research will devise new methods for the preparation of APTA’s, use the new methodology to prepare peptide mimics of the TS of zinc MMP’s, and preliminary testing of the substrates as inhibitors of NEP and ACE.
A. Dimethyl Thiophosphite

The transformation of dimethyl phosphite 56 to dimethyl thiophosphite 57 was brought about with Lawesson’s reagent. Lawesson’s reagent acts as a thionating agent, converting carbonyl oxygen into sulfurs. The mechanism of the reaction of Lawesson’s reagent with carbonyl compounds is believed to occur through Wittig type intermediates, with the formation of 2,4,6-tris(p-methoxyphenyl)-1,3,5-trioxatriphosphorinane 2,4,6-trisulfide (Fig. 5.1). Lawesson’s investigation of the stoichiometry of the reaction revealed that in facile reactions proceeding as low temperatures, resulting in little loss of Lawesson’s reagent to decomposition, the ratio of 0.5:1.0 reagent to reactant yields near quantitative thionation with attendant formation of the intermediate trimer 2,4,6-tris(p-methoxyphenyl)-1,3,5-trioxatriphosphorinane 2,4,6-

\[ \text{MeO} - \text{P} - \text{S} - \text{S} - \text{P} - \text{S} - \text{S} - \text{O} - \text{Me} \]

Lawesson’s reagent

\[ \text{S} - \text{Ar} - \text{O} - \text{P} - \text{O} - \text{S} - \text{Ar} - \text{Ar} \]

Substituted 2,4,6-trisulfide

**Figure 5.1.** Lawesson’s reagent and the known trimer 2,4,6-tris(p-methoxyphenyl)-1,3,5-trioxatriphosphorinane 2,4,6-trisulfide formed during thination of carbonyls using Lawesson’s reagent.
Scheme 5.1. Synthesis of dimethyl thiophosphite (57) from dimethyl phosphite.

trisulfide, lending credibility to the proposed mechanism. The use of Lawesson’s reagent for the thionation of dimethyl phosphite was reported by N. Zabirov and R. Shabana, with benzene and acetonitrile as the chosen solvents. Toluene was also believed to be a suitable solvent since it is closely related to benzene but much less carcinogenic. It was found that warming of the reaction in toluene was necessary for complete solubility of Lawesson’s reagent, or the reaction would occur in low yields. Once completely dissolved, thionation of dimethyl phosphite was nearly quantitative, however, failure to quench the reaction immediately resulted in overall yield reduction (Scheme 5.1). DBQ staining was useful in monitoring the thionation reaction by TLC, as neither dimethyl phosphite nor dimethyl thiophosphite contain a strong enough chromophore to be UV active. 2,6-Dibromo-N-chloro-p-benzoquinoneimine (DBQ) reveals the presence of a phosphorus-sulfur pi bond by producing a maroon colored spot after heating on TLC.
B. Addition of DMTP to Imines Bearing Chiral Auxiliary Group

![Figure 5.2. Diastereofacial addition of dimethyl thiophosphite to an aldimine.](image)

The strategy to the stereoselective addition of dimethyl thiophosphite and dimethyl phosphate to the aldimines is to distinguish between the re and si faces of the Schiff base (Fig 5.2). The synthesis of the aldimines was conducted via the condensation of the aldehyde (benzaldehyde, isobutyraldehyde, n-butyraldehyde, phenylacetylaldehyde, and 2-methylbutyraldehyde) with the amine containing a chiral auxiliary (phenyl glycinol, α-methyl benzyl amine, serine, and threonine methyl esters, Scheme 5.2). The removal of water the reaction was necessary in order to drive the reaction to completion. TLC of the product indicated that the starting material was consumed, however, the product Rf was inconclusive, as imines are known to hydrolyze upon contact with silica. It was found that the best way to monitor the reactions were by...
either LC MS+ or ¹H NMR. Evaporation of the solvent followed by Kugelror distillation afforded the desired aldimines in acceptable purity as confirmed by the aldimine proton near δ 8.5 ppm.

The synthesis of dimethyl α-amino thiophosphonate's from dimethyl thiophosphite 57 was brought about via Pudovik methodology. The aldimine was dissolved in toluene and dimethyl phosphite was added via syringe and stirred at rt. The reaction was monitored by unlocked ³¹P NMR and by TLC with the aid of DBQ resulting in the production of a maroon colored spot. When the reaction was complete the solvent was evaporated and the residual oil was then analyzed by ³¹P NMR to calculate the diastereomer ratio of the reaction (Table 5.1, Fig.5.3). Silica chromatography of the oil under refined conditions led to enantioenriched dimethyl α-amino thiophosphonate diasteromers (Fig 5.4). All compounds were analyzed with ¹H (Fig 5.5) and ¹³C NMR.

Figure 5.3. Example of ³¹P NMR spectra used to determine diastereomeric ratio of dimethyl thiophosphite or dimethyl phosphite addition.
Figure 5.4. $^{31}$P analysis of the separation of diastereomers with silica chromatography; [1], mixture of two diastereomers [4] and [5], separated diastereomers after a silica column using 1% MeOH/CHCl$_3$ as eluent.
Figure 5.5. A) $^1$H spectrum of [1-(2-hydroxy-1-phenyl-ethylamino)-butyl]-phosphonothioic acid O,O-dimethyl ester. B) Example of the phosphorus methyl ester coupling.
Table 5.1. Addition of dimethyl thiophosphite and dimethyl phosphite to imines bearing a chiral auxiliary. Ratio's were determined by $^{31}$P NMR.

<table>
<thead>
<tr>
<th>Imine</th>
<th>Product</th>
<th>%Yield</th>
<th>$^{31}$P</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="structure-image" alt="Structure of 56, X = S" /></td>
<td><img src="structure-image" alt="Structure of 57, X = O" /></td>
<td>56, $X = S$ 57, $X = O$</td>
<td>$X = S$, 68%</td>
<td>$108.2/104.9$</td>
</tr>
<tr>
<td><img src="structure-image" alt="Structure of 58" /></td>
<td></td>
<td>58</td>
<td>$X = O$, 48%</td>
<td>$33.0/31.3$</td>
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<td><img src="structure-image" alt="Structure of 59, X = S" /></td>
<td><img src="structure-image" alt="Structure of 60, X = O" /></td>
<td>59, $X = S$ 60, $X = O$</td>
<td>$X = S$, 75%</td>
<td>$109.4/106.4$</td>
</tr>
<tr>
<td><img src="structure-image" alt="Structure of 61, X = S" /></td>
<td><img src="structure-image" alt="Structure of 62, X = O" /></td>
<td>61, $X = S$ 62, $X = O$</td>
<td>$X = S$, 76%</td>
<td>$107.3/105.3$</td>
</tr>
<tr>
<td><img src="structure-image" alt="Structure of 63" /></td>
<td></td>
<td>63</td>
<td>$X = S$, 62%</td>
<td>$98.1/97.3$</td>
</tr>
</tbody>
</table>

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Table 5.1. Addition of dimethyl thiophosphite and dimethyl phosphite to imines bearing a chiral auxiliary. Ratio’s were determined by $^{31}$P NMR (continued).

<table>
<thead>
<tr>
<th>Imine</th>
<th>Product</th>
<th>% Yield</th>
<th>$^{31}$P ppm</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="64.png" alt="Image" /></td>
<td>$^{31}$P ppm</td>
<td>X = S, 85%</td>
<td>108.2/104.9</td>
<td>1 : 0.47</td>
</tr>
<tr>
<td><img src="65.png" alt="Image" /></td>
<td>X = O, 75%</td>
<td>32.7/31.6</td>
<td>0.9 : 1</td>
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</tr>
<tr>
<td><img src="66.png" alt="Image" /></td>
<td>X = S, 73%</td>
<td>106.4/104.4</td>
<td>1 : 0.6</td>
<td></td>
</tr>
<tr>
<td><img src="67.png" alt="Image" /></td>
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<td>100.5/98.1</td>
<td>1 : 0.32</td>
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</tr>
<tr>
<td><img src="68.png" alt="Image" /></td>
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<td>1 : 0.25</td>
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<tr>
<td><img src="69.png" alt="Image" /></td>
<td>X = S, 18%</td>
<td>98.1/97.2</td>
<td>1 : 0.38</td>
<td></td>
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</table>

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C. Asymmetric Dealkylation of Phosphorus Methyl Esters with PEX

The methodology for the synthesis of enantioenriched dimethyl \( \alpha \)-amino thiophosphonic monoacids was based on the dealkylation of less sterically hindered side of the phosphonate diester with potassium ethyl xanthate (PEX). The hypothesis was that PEX would attack the less sterically confined ester carbon resulting in an asymmetric dealkylation to afford enantioenriched dimethyl \( \alpha \)-amino thiophosphonic monoacids (Fig. 5.6, Table. 5.2). The dimethyl \( \alpha \)-amino thiophosphonate's were dissolved in acetone and then PEX was added and stirred at rt until the reaction had completed. The progress was monitored by unlocked \( ^{31} \text{P} \) NMR by following the movement of peaks from \( \delta 110-95 \) to around \( \delta 80 \) ppm for P=S and from \( \delta 30-20 \) to \( \delta 20-10 \) for P=O (Fig. 5.7). Once the reaction had come to completion the monoacids were purified and analyzed by NMR (Fig. 5.8), then biologically tested as inhibitors of thermolysin.

![Figure 5.6. PEX attacking the less sterically hindered methyl ester.](image)
Figure 5.7. \(^{31}\text{P}\) NMR monitoring th reaction progress of the dealkylation of P=\(S\) and P=\(O\) with PEX.
Table 5.2. Dealkylation of the phosphorus methyl ester with PEX.

<table>
<thead>
<tr>
<th>Product</th>
<th>% Yield</th>
<th>$^{31}$P</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Compound 70" /></td>
<td>21%</td>
<td>74.6/72.3/71.6/68.2</td>
<td>10:8:7:5</td>
</tr>
<tr>
<td><img src="image2.png" alt="Compound 72" /></td>
<td>18%</td>
<td>73.4/72.9</td>
<td>3:2</td>
</tr>
</tbody>
</table>

The compound 72, which was made from a single diasteromer, shows that asymmetric dealkylation is possible with PEX. Since only one diasteromer was used in the reaction you would expect a 1:1 ratio if both methyl esters were sterically equivalent. Since the ratio is 2:3 there must be some steric bulk that is hindering PEX from attacking one of the methyl esters. Although the asymmetric dealkylation of the phosphorus methyl ester was successful, there were some problems with the isolation of compounds with a hydroxy functional group. The phosphonic acids with an auxiliary hydroxy group were all followed by $^{31}$P NMR and appeared to have a successful reaction, but when worked up the isolated products always had numerous $^{31}$P peaks in the spectrum, and
none of them correlated with the expected ppm shift. It is possible that upon purification the hydroxy group could act as a nucleophile and form a ring with phosphorus. Unfortunately the phosphorus acids proved to be extremely hard to purify due to its polar characteristics. Reverse phase chromatography was tried but was never very successful, the most efficient method was recrystallization with either ether or acetone, but this was still never successful with the hydroxy compounds.

D. Conclusions

The asymmetric addition of DMTP and DMP to imines with a chiral auxiliary was successful. The diastereomer ratios ranged from poor (65) to good (63) and expected considering the high reactivity of DMTP (as compared to DMP), distance from reaction center to asymmetric center, and weakly stereodifferentiating system topology. Somewhat interesting was the finding that the hydroxy group present in the chiral auxiliary phenylglycinol had no additional effect relative to α-methylbenzylamine, whereas the presence/absence of a hydroxy group on the amino acids did alter the diastereoselectivity. For example, changing the auxiliary from alanine to serine increased the ratio from 61:39 to 73:27. Conversely, the use of threonine (branching at the hydroxyl group) as an auxiliary led to lowered diastereoselectivity possibly as the result of steric and conformational limitations. Attempts to use cysteine methyl ester as auxiliary to examine the role of a sulfhydryl was conducted however the reaction with benzaldehyde forms the tetrahydrothiazole prior to imine formation.

It is worthy to note that the addition of DMTP was about 1000 fold faster than the addition of DMP. The faster rate of DMTP made it possible to make the APTA
precursors with imines that were not stable for a long period of time plus enhancing asymmetric addition. In the case of DMP only imines that were stable for days could be used in the reaction due to amount of time required for the reaction to come to completion. All of the DMTP and DMP addition products were stable for days at room temperature but the DMTP adducts had to be kept under Ar\(_{(g)}\) to prevent oxidation. The separation of diastereomers was achieved on some the compounds, but a more thorough study could be with separation with chiral HPLC columns. The asymmetric dealkylation was accomplished with PEX on all of the precursors but isolation was difficult with the auxiliary hydroxy compounds due to side reactions during the work up resulting in numerous products. It is worthy to note that the use of a chiral dealkylating agent may enhance the asymmetric dealkylation of the precursors.
Chapter 6

Preliminary Assay of α-Aminophosphonothioic Acid as Thermolysin Inhibitors

A. Kinetics

Kinetics are concerned with the rates of reactions. This is a very important matter for the body which maintains its steady state by adjusting reaction rates in response to the environment and to hormonal controls. Chemical reactions are classified according to the number of reactants. In the simplest case, the first order reaction, there is just one reactant and the rate of the reaction is proportional to the concentration of that reactant. Hence, a graph of reaction rate against reactant concentration is a straight line for a first order chemical reaction (Fig 6.1).

![Figure 6.1. First Order Chemical Reaction, V vs. [A].](image)

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In the case of enzyme-catalyzed reactions the kinetics are not so simple except under carefully chosen conditions. The course of an enzyme-catalyzed reaction is generally complex and enzyme reactions are usually characterized by measuring the initial rate of the reaction, \(V_0\), which is the reaction rate at time equal to zero. In a closed system, such as a test-tube, an enzyme reaction can be started by adding the enzyme to a solution of the substrate and other compounds that are required for the reaction. The reaction will start at a high rate and slow down over time for several reasons: (1) the substrate will be used up and so the reaction rate will slow down as each enzyme molecule spends more time diffusing through the solution before it collides with a new substrate molecule, (2) as the reaction proceeds, product will accumulate and this may tend to inhibit the enzyme, (3) the enzyme molecules may gradually lose activity due to random hydrolysis or denaturation.\(^{140}\)

To simplify the kinetics, we will start by considering only the reaction rate right at the beginning of the reaction, so that we do not have to worry about the three points previously noted are not of concern. The reaction rate at time 0, \(V_0\) is the initial slope of the graph of product concentration against time (Fig. 6.2). \(V_0\) can be measured for a variety of initial substrate concentrations, in order to understand how the reaction rate will change in response to changes in substrate concentration. At very low substrate concentrations, a graph of reaction velocity \((v)\) against substrate concentration will be a straight line, like the first order chemical reaction. However, for an enzyme-catalyzed reaction, even with only one substrate and one product, this graph will curve at higher substrate concentrations and will level off at very high substrate concentration.\(^{140, 141}\)
In other words: when the substrate is at a low concentration, it is the limiting factor and so an increase in substrate concentration produces a proportional increase in reaction rate. However, as the substrate concentration increases, the enzyme concentration becomes the limiting factor and then a further increase in substrate produces a less-than-proportional increase in reaction rate. Eventually, the enzyme becomes “saturated” and the reaction rate reaches a constant value that does not increase significantly as yet more substrate is added. The maximum reaction rate that is achieved is called the $V_{\text{max}}$.

If the enzyme concentration is then increased, the $V_{\text{max}}$ will increase because there are more enzyme molecules available to carry out the reaction. The $V_{\text{max}}$, therefore, is only proportional to the enzyme concentration (i.e. $V_{\text{max}} = k_{\text{cat}} \times [E]$ where $K_{\text{cat}}$ is a constant).
The equation used to describe the time course of an enzyme reaction is called the Michaelis-Menten equation:

$$v = \frac{V_{\text{max}} [S]}{K_m + [S]}$$

where $v$ = rate of the enzyme  
$V_{\text{max}} = k_{\text{cat}} \times [E]$ and $[E]$ = enzyme concentration  
$K_m = \frac{1}{2} V_{\text{max}}$  
$[S]$ = substrate concentration

The Michaelis-Menten equation can be derived from the chemical kinetics of a very simplified enzyme reaction with the assumption that there is only one intermediate state, the enzyme-substrate complex (ES). Since we are dealing with initial velocities it is not necessary to consider the back reaction. Thus, the chemical equation for an enzyme catalyzed reaction is:

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{\text{cat}}}{\rightarrow} E + P$$

$K_{\text{cat}}$ is the turnover number of the enzyme. The turnover number is the number of substrate molecules converted into product per active site at very high substrate concentration. The velocity of an enzyme reaction increases as $[S]$ increases. When $[S]$ becomes much larger than $K_m$ the velocity approaches a constant, i.e., as $[S] \rightarrow \infty$, $V_0 \rightarrow V_{\text{max}}$. Hence, $V_{\text{max}}$ is the maximum velocity that can be achieved with a given total enzyme concentration and it can be measured as the limiting velocity obtained as $[S]$ is increased. Since the theory shows that $V_{\text{max}} = k_{\text{cat}} [E]$ where $[E]$ is the total enzyme
concentration, the $V_{\text{max}}$ is proportional to enzyme concentration. Thus $k_{\text{cat}}$ is a property of the enzyme and its substrate and, unlike $V_{\text{max}}$, is independent of enzyme concentration. The $V_{\text{max}}$ of an enzyme is a measure of how fast the reaction it catalyzes can proceed once the enzyme-substrate complex is formed.

$K_m$ is the Michaelis-Menten constant and represents the substrate concentration that produces half the maximum velocity (Fig. 6.3). Therefore if $K_m$ is large, the whole curve is towards the right and the reaction rate at low substrate concentration is very low. Alternatively, if the $K_m$ is small, the curve is towards the left and the reaction rate at low substrate concentration is relatively high. If $K_m$ and $k_{\text{cat}}$ are known for a given enzyme, we can use the Michaelis-Menten equation to calculate the reaction rate, $V_0$.

![Figure 6.3. $K_m$ defined on a graph.](image)
The Michaelis-Menten equation can be converted to a linear form by plotting $1/V_0$ against $1/[S]$ and the result is called a double reciprocal plot or a Lineweaver-Burk plot (Fig 6.4). The plot can be used to evaluate $K_m/V_{max}$ and $1/V_{max}$ from the slope and y-intercept. The equation is:

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \frac{1}{[S]}$$

The fact that the “curve” is a straight line is a useful indicator that the Michaelis-Menten equation does actually describe the kinetics of the enzyme under study.

**Figure 6.4.** A Lineweaver-Burk plot of $1/v$ vs. $1/[S]$. The intercept on the y-axis is equal to $1/V_{max}$ and the slope is equal to $K_m/V_{max}$.
An alternative of the Michaelis-Menten equation occurs when $v/[S]$ vs. $v$ is plotted, which is called the Eadie-Hofstee plot (Fig. 6.5). It is fitted by the equation:

$$\frac{v}{[S]} = \frac{V_{\text{max}}}{K_m} - v \frac{1}{K_m}$$

The slope of the graph is $-1/K_m$, the intercept on the $v/[S]$ axis is $V_{\text{max}}/K_m$, and on the $v$ axis it is $V_{\text{max}}$.

**Figure 6.5.** The Eadie-Hofstee plot of $v/[S]$ vs. $v$. The slope is $-1/K_m$, the intercept on the $y$-axis is $V_{\text{max}}/K_m$, and the intercept on the $x$-axis is $V_{\text{max}}$. 

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The Lineweaver-Burk plot is useful for distinguishing between types of inhibition. The Eadie-Hofstee plot is better than the Lineweaver-Burk plot at picking up deviations from the Michaelis-Menten equation.

### B. Competitive Inhibition

If a reversible inhibitor can bind to the enzyme active site in place of the substrate, it is described as a "competitive inhibitor." In pure competitive inhibition, the inhibitor is assumed to bind to the free enzyme but not to the enzyme-substrate (ES) complex. The binding is described as shown below:

![Diagram of competitive inhibition](image)

Here $K_i$ is the dissociation constant for the enzyme inhibitor-complex (EI). Using the steady state theory and writing a mass balance equation that includes EI along with the enzyme and ES, an equation relating rate to substrate concentration that is analogous to the Michaelis-Menten is obtained. However $K_m$ is replaced by an apparent $K_m'$ defined as:

$$K_m' = K_m \left(1 + \frac{[I]}{K_i}\right)$$
EI does not react to form $E + P$, and the enzyme is unable to bind both $S$ and $I$ at the same time. The Michaelis-Menten, Lineweaver-Burk, and Eadie-Hofstee equations can all be modified to include a term that describes the inhibition by $I$ when substituting $K_m'$ for $K_m$.

A common test for competitive inhibition is to use the refined Lineweaver-Burk and Eadie-Hofstee equations in the absence of inhibitor and in the presence of one or more fixed concentration of $I$. The result is a series of lines that converge on one of the axes at the value of $1/V_{max}$. Since the inhibitor only the effects the formation of the ES complex and the not the conversion of ES to $E+P$, the maximum velocity is unchanged by the presence of the inhibitor.\[141\]

C. Thermolysin Photometric Assay

A number of furylacryloyl dipeptide substrates (Fig. 6.6) for the neutral proteases have been prepared which permit the dipeptide hydrolysis to be monitored spectrophotometrically.\[142\] The study of thermolysin-catalyzed hydrolysis of these substrates has yielded kinetic information of this enzyme($k_{cat}/K_m = 2.11$). The substrate 3-(2-furylacryloyl)-glycyl-L-leucine amide (FAGLA) is cleaved by thermolysin by hydrolyzing the glycine-leucine bond (Fig. 6.6). FAGLA’s advantage is that thermolysin can cleave it in only one place (as shown in Figure 1), making the reaction specific enough that the decrease of substrate could be easily quantified by measuring the
Thermolysin catalyzed proteolytic cleavage of N-(3-[2-furyl]acryloyl)-glycyl-L-leucine-amide (FAGLA).

Figure 6.6. Thermolysin catalyzed proteolytic cleavage of N-(3-[2-furyl]acryloyl)-glycyl-L-leucine-amide (FAGLA).

decrease in optical density at $\lambda = 345$nm. The activity (both inhibited and uninhibited) of thermolysin can thus be determined using UV-Visible spectrophotometric analysis.$^{[143]}$

In other words, when the enzyme is not inhibited FAGLA is cleaved by the active site and a decrease in absorbance at 345nm is observed, but when the enzyme is inhibited, FAGLA can not be cleaved by the active site and the absorbance at 345nm stays constant (Fig. 6.7).

Since the original assay was designed to be carried out in 1 cm cuvettes the procedure was modified for a micro plate reader in order to gain a higher throughput. When the experiment was changed from a 1 cm cuvette to the microwell plate reader a drop in sensitivity of the assay became apparent. The assay still worked for strong inhibitors of thermolysin, like phosphoramidon, but not for weak inhibitors, so the assay was used as a screen for the panel of compounds made in the Thompson lab. The data was collected and analyzed using the software Soft Max Pro and interpreted in Excel (Fig 6.8, Table 6.1)
Figure 6.7. UV monitoring of thermolysin substrates using FAGLA by observing absorbance at 345nm using a micro plate reader and Soft max pro. A) thermolysin uninhibited (decrease in 345 nm). B) thermolysin inhibited by 70 (345nm stays constant).
Trial 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>[S]</th>
<th>[S] corr</th>
<th>1/[S]</th>
<th>v</th>
<th>v corr</th>
<th>1/v</th>
<th>[E]</th>
<th>kcat/Km</th>
<th>v/[S]</th>
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Average: -8.54E+06
Std Dev: 8.06E+05

Slope: -3.26409 r^2
Y int: -6129.32
Vmax: -0.00016
Ave Km: 3.35E-04
[I]/Kl: 0.588
Ave kcat/Km: -5.33E+06

Figure 6.8. Data imported into Excel from Soft Max Pro and put into the Eadie-Hofstee equation to help calculate average K_i of 70.

Trial 2

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<td>3.07E-08</td>
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</tr>
</tbody>
</table>

Average: -7.45E+06
Std Dev: 6.20E+05

Slope: -3.33171 r^2
Y int: -8034.08
Vmax: -1.24E-04
Ave Km: 3.35E-04
[I]/Kl: 0.236
Kl: 6.20E-06
kcat: -2.50E+03

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Table 6.1. $K_i$ values of substrates tested on thermolysin using FAGLA and a micorwell plate reader

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<th>Substrate</th>
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Although the results did not show substantial inhibition of thermolysin they did show partial inhibition. This was not to much of a surprise since the compounds tested did not have carboxylic acid functional group which has been proven to help guide the peptide into the active site. But the preliminary results show that there is some coordination to the active site suggesting that the $P=S$ and $P=O$ can coordinate with the
zinc atom within the site. To help bind the inhibitor an additional peptides may be added in order to fill more S pockets of the enzyme. Plus, resolving the diastereomers and testing them separate would most likely lead to a higher inhibition since the substrates would not be competing with its diastereomer for the active site.
Chapter 7

Conclusions

A. Synthesis and Testing of APTA Compounds

In conclusion, DMTP adds with good diastereoselectivity to imines containing N-chiral auxiliaries. Using readily available chiral amines, α-aminophosphonothionates may be prepared in good yield and high stereoselectivity. Using these findings, the synthesis of APTA’s containing a P-chiral group will be possible. The asymmetric dealkylation of the phosphorus methyl esters was accomplished with PEX, but the use of a chiral nucleophile may emphasize asymmetric attack by capitalizing on the steric of the methyl ester and should be explored.

Adapting the thermolysin assay for the micro plate reader was accomplished, but it only showed strong inhibition and can only be used for preliminary results. The main problem with the assay was the background noise was often too high and would make the subtle drop in absorbance difficult to quantify. Work is being done on developing a MS assay using single ion monitoring and should prove to be a better assay.
Chapter 8

Future Work

A. Building a Tripeptide Mimic

Since the S and S' pockets of ACE have been elucidated it is known that there are three primary sites that guide and hold the peptide in the active site. It would then seem prudent to match a peptide for each pocket of the enzyme. In other words, if there are three subsites in the enzyme than the inhibitor should have three peptides to fully exploit its inhibitive properties. Thus, the dipeptides that have been made in this study could be improved with the addition of another peptide.

Initial attempts to functionalize the hydroxyl group of the \( \alpha \)-aminophosphonothioates with phenyl glycinol, serine methyl ester, and threonine methyl ester (63, 67, and 69) were unsuccessful but had interesting results. The Mitsunobu reaction with triphenylphosphine and diethyl azodicarboxylate resulted in the oxidation of the hydroxy unit to an aldehyde. Although it was not the expected outcome, the aldehyde could provide some interesting synthetic routes. Following dealkylation of the phosphorus ester an imine could be made which could be attacked by sulfur to form a mechanistic transition state adduct (Fig. 7.1).

A third amino acid could also be coupled with the phosphonic monoacid via a DCC coupling reaction. Once the tripeptide is made by the coupling of an amine with the phosphonic acid a second dealkylation would provide a TS analog that has the zinc binding group incorporated into the peptide framework which would mimic the
angiotensin enzyme more closely than the dipeptide with the zinc binding group in the terminal position (Fig 7.2).

![Mitsunobu reaction and possible route to mechanistic transition state inhibitor.](image)

**Figure 7.1.** Mitsunobu reaction and possible route to mechanistic transition state inhibitor.
Figure 7.2. APTA coupling with DCC and an amine followed by dealkylation with PEX to afford a tripeptide transition state inhibitor.
Chapter 9

Experimental

A. iso-Polydiacetylene Compounds

Unless otherwise noted, reactions were run in flame-dried apparatus under argon atmosphere. Anhydrous reagent grade solvents (CH$_2$Cl$_2$; THF; toluene; CH$_3$OH; benzene) were purchased from Aldrich Chemical Co. in sure sealed bottles and used without further purification. Protected amino acids, phenyl glycinol, $\alpha$-methylbenzylamine, isobutyryl chloride, isobutyryl aldehyde, cyclohexanecarbonyl chloride, trimethyl phosphite, 3-methyl-butyraldehyde, phenyl-acetaldehyde, Pd(PPh$_3$)$_2$, Cul, TEA, diisopropyl ethyl amine, $t$-butyl alcohol, and lawesson’s reagent were purchased from Aldrich Chemical Co. and used without further purification. bis-Trimethylsilyl acetylene was purchased from Petrarch and used without further purification. Analytical thin-layer chromatography (TLC) was performed on silica gel (Whatman) aluminum-backed plates. Flash chromatography was performed with silica gel (Merck, 230-240 mesh). Compounds were detected using a UV lamp at 254 nm and/or stained with ninhydrin or 2,6-dibromoquinone-chlorimide. Yields are optimized. $^1$H NMR (400 MHz), $^{13}$C NMR (100 MHz), $^{31}$P NMR (171 MHz) spectra were determined on a Varian 400 MHz Unity Plus spectrometer. Chemical shifts are reported in parts per million (ppm, $\delta$) using CHCl$_3$ (7.26 ppm for $^1$H), CDCl$_3$ (77 ppm for $^{13}$C) or 85% H$_3$PO$_4$ (0 ppm for $^{31}$P) as references. High resolution mass spectra (HRMS) were obtained using electrospray ionization (ESI) Micromass LCT connected with water 2790
HPLC with C-18 reversed phase column (2.1 mm i.d., 5 cm long). Elemental analyses for C, H, and N were performed by Midwest Microlab, Indianapolis, IN. All the HRMS and elemental analyses of the novel compounds are within acceptable error range.
1-Trimethylsilyl-4-methyl-pent-1-yn-3-one (22). In a flask was placed CH₂Cl₂ (100 mL), bis(trimethylsilyl)acetylene (10.24 g; 60.0 mmol), and isobutyryl chloride (6.36 g; 60.0 mmol) and reduced temperature to 0 °C with an acetone/ice bath. To this was added AlCl₃ (8.00 g; 60.0 mmol) and let contents stir at ambient temperature for 6.0 h. The reaction changed from a cloudy opaque color to a cloudy dark amber. The reaction was then concentrated in vacuo resulting in a dark red oil. The oil was diluted with EtOAc (50 mL) and then poured into a beaker with ice-cold 10% HCl (150 mL) transmuting the dark amber color to bright yellow. The reaction was extracted with EtOAc (3x100 mL) followed by extraction of the organic phase with H₂O, sat. NaHCO₃, and brine. The organic phase was then dried over anhydrous Na₂SO₄ for 0.5 h and concentrated in vacuo resulting in dark red oil. Compound 22 (8.98 g; 53.4 mmol; 89 %) was isolated as a colorless oil after distillation at 43 °C at 0.25 mmHg.

¹H NMR (CDCl₃, 400 MHz): δ 2.50-2.57 (m, J = 7.1 Hz, 1H), 1.09 (d, J = 7.1 Hz, 6 H), 0.15 (s, 9H);

¹³C NMR (CDCl₃, 100 MHz): δ 192.6, 102.0, 99.5, 43.7, 18.8, 0.2.
1-cyclohexyl-3-trimethylsilyl-propyn-1-one (31). To a flask was added CH₂Cl₂ (100 mL), bis(trimethylsilyl)acetylene (5.12 g; 30.0 mmol), and cyclohexanecarboxylic acid chloride (4.39 g; 30.0 mmol). The temperature was brought to 0 °C with an acetone/ice bath, and AlCl₃ (4.00 g; 30.0 mmol) was added and let stir at ambient temperature for 6.0 h. The reaction mixture changed from a cloudy opaque color to a cloudy dark amber during this period. The reaction mixture was then concentrated in vacuo resulting in a dark red oil. The oil was diluted with EtOAc (50 mL) and poured into a beaker that contained iced 10% HCl (150 mL) transmuting the dark amber color to bright yellow. The reaction mixture was separated, extracted with EtOAc (3x100 mL) followed by extraction of the organic phase with H₂O, sat. NaHCO₃, and brine. The organic phase was then dried over anhydrous Na₂SO₄ for 0.5 h and then concentrated in vacuo resulting in dark red oil. Compound 31 (3.88 g; 18.62 mmol; 62 %) was isolated as a colorless oil after distillation at 76 °C at 0.25 mmHg. Anal. Calcd for: C₁₂H₁₄O₃Si: C, 71.24; H, 6.97; Found: C, 71.04; H, 7.01.

$^1$H NMR (CDCl₃, 400 MHz): δ 2.36-2.48 (m, $J = 3.8$ Hz, 1 H), 2.00-1.96 (m, $J = 3.8$ Hz, 2 H), 1.81-1.77 (m, $J = 3.8$ Hz, 2 H), 1.68-1.65 (m, $J = 3.8$ Hz, 1 H), 1.46-1.19 (m, $J = 3.8$ Hz, 5 H), 0.18 (s, 9 H);

$^{13}$C NMR (CDCl₃, 100 MHz): δ 192.4, 102.5, 99.5, 53.1, 29.2, 26.8, 26.4, 0.4.
2-methyl-1-trimethylsilyl-ethyl-propenyl trifluoromethanesulfonate (32). To a 100 ml RB flask was added CH$_2$Cl$_2$ (50 mL), 2,6-di-tert-butyl-4-methylpyridine (1.33 g; 6.5 mmol), and 22 (1.00 g; 6.0 mmol). It was then cooled to -78 °C under Ar (g).

Trifluoromethane sulfonic acid (2.01 g; 7.20 mmol) was added via syringe and then the reaction was warmed to rt while stirring. After 8.0 h the reaction had turned to a dark brown color and was concentrated in vacuo resulting in a chunky brown salt. Pentane (100 mL) was added to the brown precipitate and the insoluble triflate/2,6-di-tert-butyl-4-methylpyridine salt was gravity filtered. The brown organic layer was then extracted with iced 10% HCl, H$_2$O, sat. NaHCO$_3$, brine, and dried over Na$_2$SO$_4$. The solution was then concentrated in vacuo to afford dark brown oil. The oil was distilled at 0.25 mmHg and 65 °C resulting in a clear colorless oil (1.41 g; 4.69 mmol; 78%).

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 1.98 (s, 3 H), 1.91 (s, 3 H), 0.21 (s, 9 H);

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 138.6, 127.7, 119.0(q, $J = 320$ Hz), 101.2, 95.5, 22.1, 19.6, 0.48.
l-Cyclohexylidene-3-trimethylsilyl-prop-2-ynyl trifluoromethanesulfonate (33).

l-cyclohexyl-3-trimethylsilyl-propyn-1-one (5.41 g; 26.0 mmol) and 2,6-di-tert-butyl-4-methylpyridine (6.42 g; 31.3 mmol) in CH₂Cl₂ (225 mL) was chilled to -78 °C and trifluoromethane sulfonic acid (8.62 g; 5.14 ml; 30.5 mmol) was added dropwise via syringe. The reaction was warmed to rt after 0.5 h and at 8.0 h the reaction had turned to a dark brown color. The reaction concentrated in vacuo resulting in a chunky brown solid. Pentane (100 mL) was added to the brown precipitate and the insoluble triflate/2,6-di-tert-butyl-4-methylpyridine salt was gravity filtered. The brown organic layer was then extracted with iced 10% HCl, H₂O, sat. NaHCO₃, brine, and dried over Na₂SO₄. Evaporation in vacuo followed by flash chromatography with hexanes gave 33 as clear yellow oil (6.55 g; 19.2 mmol; 74%).

¹H NMR (CDCl₃, 400 MHz): δ 2.42-2.34 (m, J = 6.3 Hz, 4 H), 1.68-1.58 (m, J = 6.3 Hz, 6 H), 0.21 (s, 9 H);

¹³C NMR (CDCl₃, 100 MHz): δ 146.1, 127.7, 119.0 (q, J = 320 Hz), 101.2, 95.5, 31.2, 28.6, 27.0, 26.6, 25.7, 0.48.
1-cyclohexyl-2-propyn-1-one (34). To a 100 mL RB flask was added MeOH (35 mL), 0.01 M Borax (3.9 mL), and 1-cyclohexyl-3-trimethylsilyl-propyn-1-one (500 mg, 2.40 mmol). The reaction was stirred at rt for 12 h and the mixture changed from colorless to pale yellow. The reaction was then quenched at 0 EC with iced 10% HCl (50 mL). The solution was extracted with hexanes (3 × 30 mL). The organic layers were combined and extracted with sat. NaHCO₃ and dried over Na₂SO₄ for 15 min. The organic layer was then filtered and concentrated in vacuo to afford a crude yellow oil. The yellow oil was then distilled at 0.25 mmHg and 49 EC to afford 34 (0.286 g, 2.10 mmol; 87%). Anal. Calcd for: C₉H₁₂O: C, 79.37; H, 8.88; Found: C, 79.45; H, 8.64.

¹H NMR (CDCl₃, 400 MHz): δ 3.23 (s, 1 H), 2.36-2.48 (m, J = 3.8 Hz, 1 H), 1.99-1.95 (m, J = 3.8 Hz, 2 H), 1.78-1.75 (m, J = 3.8 Hz, 2 H), 1.65-1.63 (m, J = 3.8 Hz, 1 H), 1.45-1.14 (m, J = 3.8 Hz, 5 H);

¹³C NMR (CDCl₃, 100 MHz): δ 191.7, 80.7, 78.9, 52.1, 27.8, 25.6, 25.2.
1-Cyclohexyl-4-isopropylidene-6-trimethylsilyl-2,5-hexyne-1-one (35). To a 100 mL RB 3 neck flask containing anhydrous benzene (15 mL), 32 (2.00 g; 6.64 mmol), Hunig's base (2.52 g; 2.4 mL; 9.92 mmol), Pd(PPh$_3$)$_4$ (168 mg; 0.14 mmol, 2.2 mol%), and Cul (6 mg; 0.0026 mmol) was degassed with Ar (g) for 0.75 h. To this solution was added, via syringe pump at 0.01 mL/min, 34 (1.32 g; 9.71 mmol) dissolved in anhydrous benzene (3 mL). When the addition was complete the reaction was diluted with Et$_2$O:Hex (1:1) (100 mL) and then extracted with 10% aqueous HCl. The combined aqueous layers were back extracted with Et$_2$O:Hex (1:1) (2 x 30 mL) followed by extraction of the organic layer with H$_2$O, aqueous NaHCCL, brine, and dried over MgSO$_4$ for 0.5 h. The solution was gravity filtered and concentrated in vacuo to afford dark brown oil. 35 (1.084 g; 3.86 mmol, 58%) was isolated after flash chromatography with silica and 0.3% EtOAc/hex (5 L) as yellow oil. Anal. Calcd for: C$_{18}$H$_{26}$OSi: C, 75.46; H, 9.15; Found: C, 75.51; H, 9.03.

$^1$H NMR (CDCl$_3$, 400 MHz): δ 2.39–2.46 (m, $J = 3.9$ Hz, 1 H), 2.08 (s, 3 H), 2.06 (s, 3 H), 2.00–1.96 (m, $J = 3.2$ Hz, 2 H), 1.79–1.74 (m, $J = 3.9$ Hz, 2 H), 1.65–1.61 (m, $J = 3.9$ Hz, 1 H), 1.49–1.14 (m, $J = 3.2$ Hz, 5 H) 0.19 (s, 9 H);
\(^{13}\text{C NMR (CDCl}_3, 100 \text{ MHz): } \delta \text{ 192.4, 163.5, 101.4, 100.8, 98.8, 90.6, 89.7, 53.4, 29.4, 26.9, 26.5, 24.4, 24.3, 0.9.} \)
2-methyl-3-[1,3]dixolane-1-trimethylsilyl-1-pentyne (36). To a 100 mL RB flask was added CH$_2$Cl$_2$ (25 mL) and trimethylsilyl trifluoromethanesulfonate (11.3 mg; 9.0 µL; 0.05 mmol) at -78 °C. To this solution was added 1,2-bis(trimethylsilyloxy)ethane (1.05 g; 1.25 mL; 5.10 mmol) and 4-methyl-1-trimethylsilyl-pent-1-yn-3-one (0.840 g; 5.00 mmol) and stirred at -78 °C for 1.0 h. The reaction was warmed to rt and after 13 h the color had changed from colorless to a dark amber. Anhydrous pyridine (5.0 µL) was added via syringe and then stirred for 1.0 h. The reaction was then transferred to a separatory funnel and extracted with aqueous Na$_2$SO$_4$ and CH$_2$Cl$_2$ (3 x 40 mL). The organic layers were combined and dried over Na$_2$SO$_4$ followed by evaporation in vacuo to afford dark amber oil. The oil was distilled to yield 36 (0.917 g; 4.32 mmol, 87%).

Anal. Calcd for: C$_{11}$H$_{20}$O$_2$Si: C, 62.21; H, 9.49; Found: C, 62.03; H, 9.33.

$^1$H NMR (CDCl$_3$, 400 MHz): δ 4.12-4.09 (m, J = 3.8 Hz, 2 H), 3.99-3.96 (m, J = 3.8 Hz, 2 H), 1.99 (m, J = 6.4 Hz, 1 H), 1.06 (d, J = 6.4 Hz, 6 H), 0.18 (s, 9 H);

$^{13}$C NMR (CDCl$_3$, 100 MHz): δ 107.4, 102.7, 90.2, 65.9, 37.6, 18.2, 0.9.
1-cyclohexyl-1-[1,3]dioxolane-3-trimethylsilyl-2propyn-1-one (37). A 100 ml RB flask, purged with Ar(g), containing CH$_2$Cl$_2$ (25 mL) and trimethylsilyl trifluoromethanesulfonate (11.3 mg; 9.0 µL; 0.05 mmol) was cooled down to -78 °C. To this solution was added 1,2-bis(trimethylsilyloxy)ethane (1.05 g; 1.25 mL; 5.10 mmol) and 4-methyl-1-trimethylsilyl-pent-1-yn-3-one (1.04 g; 5.00 mmol) and stirred at -78 °C for 1 h. The reaction was warmed to rt and after 13 h the color had changed from colorless to a dark amber. Anhydrous pyridine (5.0 µL) was added via syringe and then stirred for 1 h. The reaction was then transferred to a separatory funnel and extracted with aqueous Na$_2$SO$_4$ and CH$_2$Cl$_2$ (3 x 40 mL). The organic layers were combined and dried over Na$_2$SO$_4$ followed by evaporation in vacuo to afford dark amber oil. The oil was distilled to yield 37 (866 mg; 3.43 mmol, 68%). Anal. Calcd for: C$_{14}$H$_{24}$O$_2$Si: C, 66.61; H, 9.58 C, 66.61; H, 9.58; Found: C, 66.05; H, 9.52.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 4.12-4.08 (m, $J = 3.8$ Hz, 2 H), 3.96-3.92 (m, $J = 3.8$ Hz, 2 H), 2.10-1.21 (m, 11 H), 0.18 (s, 9 H);

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 106.6, 103.1, 90.3, 65.7, 47.1, 28.2, 27.3, 26.9, 0.9.

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2-methyl-3-[1,3]dixolane-1-pentyne (38). To a 100 mL RB flask was added 35 ml MeOH (35 mL), K₂CO₃ (1.0 g, 7.23 mmol), and 37 (500 mg, 2.35 mmol). The reaction was stirred at rt for 2.0 h where the solution changed from colorless to pale yellow. The reaction was then quenched at 0 EC with iced 10% HCl (50 mL). The solution was extracted with hexanes (3 x 30 mL). The organic layers were combined and extracted with sat. NaHCO₃ and dried over Na₂SO₄ for 15 min. The organic layer was then filtered and concentrated in vacuo to afford a crude yellow oil that had the smell of pine. The yellow oil was then distilled at 0.25 mmHg at rt to afford 38 (0.275 g, 1.95 mmol; 83%).

¹H NMR (CDCl₃, 400 MHz): δ 4.12-4.09 (m, J = 3.8 Hz, 2 H), 3.99-3.96 (m, J = 3.8 Hz, 2 H), 2.51 (s, 1 H), 1.95 (m, J = 6.4 Hz, 1 H), 1.06 (d, J = 6.4 Hz, 6 H);

¹³C NMR (CDCl₃, 100 MHz): δ 106.6, 102.1, 82.2, 65.0, 36.6, 18.4.
2-Methyl-6-cyclohexylidene-3-[1,3]dioxane-2-Methyl-6-trimethylsilyl-4,7-heptyne

To a 100 mL RB 3 neck flask containing anhydrous benzene (15 mL), 33 (2.00 g; 5.87 mmol), TEA (0.89 g; 1.23 mL; 8.77 mmol), Pd(PPh₃)₄ (148 mg; 0.12 mmol, 2.2 mol%), and CuI (6 mg; 0.0026 mmol) was degassed with Ar (g) for 0.75 h. To this solution was added, via syringe pump at 0.01 mL/min, 38 (1.20 g; 8.58 mmol) dissolved in anhydrous benzene (3 mL). When the addition was complete the reaction was diluted with Et₂O:Hex (1:1) (100 mL) and then extracted with 10% aqueous HCl. The combined aqueous layers were back extracted with Et₂O:Hex (1:1) (2 x 30 mL) followed by extraction of the organic layer with H₂O, aqueous NaHCO₃, brine, and dried over MgSO₄ for 0.5 h. The solution was gravity filtered and concentrated in vacuo to afford dark brown oil. 39 (0.87 g; 2.64 mmol, 45%) was isolated after flash chromatography with silica and 0.3% EtOAc/hex (3 L) as yellow oil.

¹H NMR (CDCl₃, 400 MHz): δ 4.11-4.09 (m, J = 3.8 Hz, 2 H), 3.98-3.97 (m, J = 3.8 Hz, 2 H), 2.02-1.98 (m, J = 3.8 Hz, 4 H), 1.96-1.93 (m, J = 6.4 Hz, 1 H), 1.83-1.46 (m, J = 3.8 Hz, 6 H) 1.06 (d, J = 6.4 Hz, 6 H);

¹³C NMR (CDCl₃, 100 MHz): δ 157.1, 107.3, 101.9, 97.4, 89.3, 82.4, 65.6, 47.3, 28.3, 27.3, 26.9, 23.8, 1.0.
1-Cyclohexyl-4-isopropylidene-6-trimethylsilyl-2,5-bexyne-1-one (42). 35 (1.00 g; 3.57 mmol) and KF (230 mg; 3.96 mmol) was put in a 250 mL RB flask containing CH$_3$CN (100 mL) and stirred at rt for 48 h. The reaction was then concentrated in followed by dilution with EtOAc (100 mL). The solution was then extracted with sat. NaHCO$_3$ (aq), H$_2$O, and brine. The organic layer was dried over Na$_2$SO$_4$ for 0.5 h, gravity filtered, and then concentrated in vacuo leaving yellow oil. The oil was chromatographed with silica and hex:EtOAc (99:1) to afford 42 (680 mg; 3.17 mmol; 91%). Anal. Calcd for: C$_{15}$H$_{18}$O: C, 84.07; H, 8.47; Found: C83.92; H, 8.81.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 3.12 (s, 1 H), 2.42-2.34 (m, $J = 3.9$ Hz, 1 H), 2.10 (s, 3 H), 2.05 (s, 3 H) 1.96-1.93 (m, $J = 2.6$ Hz, 2 H), 1.76-1.72 (m, $J = 3.9$ Hz, 2 H), 1.63-1.60 (m, $J = 3.9$ Hz, 1 H) 1.44-1.151 (m, $J = 2.6$ Hz, 5 H);

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 192.2,164.3, 99.1, 89.5, 88.0, 81.5, 80.4, 78.7, 53.3, 29.3, 26.8, 26.4, 24.3.
1-Cyclohexyl-7-cyclohexylidene-4-isopropylidene-9-trimethylsilyl-nona-2,5,8-
triyn-1-one (43). A 100 mL 3 neck flask containing benzene (40 mL), trifluoro-
methanesulfonic acid 1-cyclohexylidene-3-trimethylsilyl-prop-2-ynyl ester (5.416 g;
15.9 mmol), Hunig’s base (6.03 g; 5.7 mL; 46.6 mmol), Pd(PPh₃)₄ (349 mg; 0.30 mmol;
2.2 mol%), and CuI (15 mg; 0.0079 mmol) was degassed with Ar (g) for 45 min. To this
solution was added, via syringe pump at 0.01 mL/min, 1-Cyclohexyl-4-ethynyl-5-methyl-
hex-4-en-2-yn-1-one (5.02 g; 17.13 mmol) dissolved in benzene (5 mL). When the
addition was complete the reaction was diluted with Et₂O:Hex (1:1) (100 mL) and gravity
filtered. The filtrate was extracted with 10% aqueous HCl (3 x 100 mL) and combined
aqueous layers were back extracted with Et₂O:hex (1:1) (2 x 30 mL) followed by
extraction of the organic layer with H₂O, aqueous NaHCO₃, and brine. The organic layer
was then dried over MgSO₄ for 0.5 h and then concentrated in vacuo to afford dark
brown oil. 43 was isolated after flash chromatography with silica and 5 L of 0.6%
EtOAc/hex as yellow oil (3.73 g; 9.31 mmol; 59%).

¹H NMR (CDCl₃, 400 MHz): δ 2.52-2.40 (m, 5 H), 2.10 (s, 3 H), 2.09 (s, 3 H), 2.00-1.18
(m, 16 H), 0.18 (s, 9 H);
$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 192.4, 163.3, 161.8, 102.1, 101.4, 99.1, 97.2, 90.3, 90.2, 87.8, 53.4, 33.9, 33.8, 29.4, 28.6, 27.2, 26.9, 26.5, 24.3, 1.0.
1-Cyclohexyl-7-cyclohexylidene-4-isopropylidene-nona-2,5,8-triyn-1-one (44). 43 (3.73 g; 9.37 mmol) and KF (600 mg; 10.3 mmol) were put in a 500 mL RB flask containing CH$_3$CN (250 mL) and stirred at rt for 168 h. The reaction was then concentrated in vacuo followed by dilution with EtOAc (100 mL). The solution was extracted with sat. NaHCO$_3$ (aq), H$_2$O, and brine. The organic layer was dried over Na$_2$SO$_4$ for 0.5 h, gravity filtered, and then concentrated in vacuo to afford yellow oil. The oil was chromatographed with silica and hex:EtOAc (99:1) to yield 1-Cyclohexyl-7-cyclohexylidene-4-isopropylidene-nona-2,5,8-triyn-1-one as yellow oil (2.62 g; 7.88 mmol; 84%).
1-Cyclohexyl-7-cyclohexylidene-4,10-isopropyldene-12-trimethylsilyl-2,5,8,11-dodecyn-1-one (45). A 100 mL 3 neck flask containing benzene (40 mL), trifluoromethanesulfonic acid 2-methyl-1-trimethylsilanylethynyl-propenyl ester (4.12 g; 13.7 mmol), Hunig’s base (6.81 g; 6.5 mL; 53.9 mmol), Pd(PPh₃)₄ (454 mg; 0.394 mmol; 2.2 mol%), and Cul (18 mg; 0.095 mmol) was degassed with Ar (g) for 0.75 h. To this solution was added, via syringe pump at 0.01 mL/min, 1-Cyclohexyl-7-cyclohexylidene-4-isopropyldene-nona-2,5,8-triyn-1-one (3.57 g; 15.1 mmol) dissolved in benzene (5 mL). When the addition was complete the reaction was diluted with Et₂O:Hex (1:1) (100 mL) and gravity filtered. The filtrate was extracted with 10% aqueous HCl (3 x 100 mL) and the combined aqueous layers were extracted with Et₂O:Hex (1:1) (2 x 30 mL) followed by extraction of the organic layer with H₂O, aqueous NaHCO₃, and brine. The organic layer was dried over MgSO₄ for 0.5 h and then concentrated in vacuo to afford dark brown oil. 45 was isolated after flash chromatography with silica and 5 L of 0.6% EtOAc/hex as orange oil (2.38 g; 4.93 mmol; 36%). HRMS calcd for M+H⁺ of C₃₃H₄₂O₂Si 483.3083, found 483.3067.
$^1$H NMR (CDCl$_3$, 400 MHz): δ 2.52-2.37 (m, 5H), 2.10 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.78-1.17 (m, 16H), 0.17 (s, 9H);

$^{13}$C NMR (CDCl$_3$, 100 MHz): δ 192.5, 161.7, 155.8, 102.7, 101.3, 99.0, 96.9, 90.4, 90.2, 89.1, 89.0, 87.5, 53.4, 34.0, 33.9, 30.8, 29.4, 28.7, 27.2, 26.9, 26.5, 24.3, 23.8, 23.7, 1.1.
B. Compounds 56-69

**General synthesis for α-amino phosphonates and α-amino thiophosphonates:** The amine (1.0 eq) was weighed into a 100 mL RB flask with 20 mL of toluene and 0.5 g of 3 Å molecular sieves then heated to dissolve the amine (if necessary). When the amine was fully dissolved, the aldehyde (0.9 eq) was added via syringe while stirring and the solution would turn cloudy. After 20 min, the reaction was clear and checked by $^1$H NMR or LC MS for the formation of the imine. Once reaction was complete, the toluene was evaporated under reduced pressure and a thick oil was left behind. To the oil was added toluene (2-5 mL) and a stir bar, and the flask was then cooled to 0° C with an ice bath and purged with Ar(g). Either dimethyl phosphite (5-10 eq) or dimethyl thiophosphite (0.85 eq) was added via syringe and the reaction was stirred at ambient temperature for either 10 h for dimethyl thiophosphite or up to 4 d for dimethyl phosphite. All reactions were monitored by unlocked $^{31}$P NMR and TLC (DBQ stain). When the reaction was complete, the solvent was removed under reduced pressure and excess phosphite was removed with a Kugelrohr to afford a thick oil. All reactions were purified with silica chromatography.
**O,O-Dimethyl ester [2-Methyl-1-(1-phenyl-ethylamino)-propyl]-phosphonothionate (56).** [144]-α-Methylbenzylamine (1.88 g, 15.5 mmol) was dissolved in 10 mL of toluene followed by the addition of isobutyryl aldehyde (1.01 g, 1.27 mL, 13.96 mmol) and stirred at rt for 1 h. The reaction was concentrated to oil followed by the addition of 2 mL toluene, dimethyl thiophosphite (1.5 g, 11.87 mmol), and stirred at rt for 8 h. After concentrating to oil a silica column eluted with 5% EtOAc/hex afforded colorless oil 47 as a mixture of diastereomers (10:7), (2.42 g, 8.02 mmol, 68%). HRMS calcd for M+H⁺ of C₁₄H₂₄NO₂PS 302.1343, found 302.1246.

**1H NMR (CDCl₃, 400 MHz):** δ 7.39-7.21 (m, 5 H), 4.11-4.08 (m, 1H), 3.76 (d, JPOCH₃ = 12.9 Hz, 1.5 H), 3.73 (d, JPOCH₃ = 12.9 Hz, 1.5 H), 3.70 (d, JPOCH₃ = 12.9 Hz, 1.5 H), 3.68 (d, JPOCH₃ = 12.9 Hz, 1.5 H), 2.98 (dd, JPOCH = 14.7, 2.6 Hz, 0.5 H), 2.71 (dd, JPOCH = 11.0, 2.6 Hz 0.5 H), 2.36-2.25 (m, J = 2.6 Hz, 0.5 H), 2.17-2.06 (m, J = 2.6 Hz, 0.5 H) 1.77 (bs, 1 H), 1.34 (d, J = 6.4 Hz, 1.5 H), 1.30 (d, 6.4 Hz, 1.5 H), 1.10 (dd, J = 7.1, 2.0 Hz, 1.5 H), 0.98 (d, J = 7.1 Hz, 1.5 H), 0.93 (d, J = 7.1 Hz, 1.5 H), 0.82 (dd, J = 6.4, 1.3 Hz, 1.5 H);

**13C NMR (CDCl₃, 100 MHz):** δ 146.8,145.8, 129.5, 129.2,128.7, 128.2, 128.1, 63.2 (d, JPOCH = 94.6 Hz), 63.0 (d, JPOCH = 106.8 Hz), 57.2, 54.5 (d, JPOCH₃ = 9.2 Hz), 54.1 (d,
$J_{POCH} = 9.2$ Hz), 53.9 (d, $J_{POCH} = 6.1$ Hz), 53.1 (d, $J_{POCH} = 9.2$ Hz), 30.1 (d, $J = 9.1$ Hz), 30.0 (d, $J = 9.1$ Hz), 26.3, 24.1, 22.8 (d, $J = 15.3$ Hz), 22.4 (d, $J = 15.3$ Hz), 18.3, 17.8.

$^{31}$P NMR (CDCl$_3$, 171 MHz): $\delta$ 108.2, 104.9.
O,O-Dimethyl ester [2-Methyl-1-(1-phenyl-ethylamino)-propyl]-phosphonate (57).

[144]-α-Methylbenzylamine (1.88 g, 15.5 mmol) was dissolved in 10 mL of toluene followed by the addition of isobutyryl aldehyde (1.01 g, 1.27 mL, 13.96 mmol) and stirred at rt for 1 h. The reaction was then concentrated and followed by the addition of 2 mL toluene, dimethyl phosphite (3.24 g, 29.46 mmol), and stirred at rt for 72 h. After concentrating to oil a silica column eluted with 1% MeOH/CHCl₃ afforded colorless oil 57 as a mixture of diastereomers (12:9), (2.23 g, 7.42 mmol, 48%). HRMS calcd for M+H⁺ of C₁₄H₂₄N₂O₃P 286.1572, found 286.1549.

¹H NMR (CDCl₃, 400 MHz): δ 7.26-7.21 (m 5 H), 4.10-4.07 (m, 1 H), 3.71 (d, JPOCH₃ = 10.4 Hz, 3 H), 3.70 (d, JPOCH₃ = 10.4 Hz, 3 H), 2.60 (m, 1 H), 2.12-2.05 (m, 1 H), 1.36 (d, J = 6.4 Hz, 3 H), 1.09 (d, 6.4 Hz, 3 H), 1.01 (d, J = 6.4 Hz, 3 H);

¹³C NMR (CDCl₃, 100 MHz): δ 146.4,145.5, 129.8, 129.2,128.9, 128.3, 128.1, 53.8 (d, JPOCH₃ = 9.2 Hz), 53.1 (d, JPOCH₃ = 6.1 Hz), 53.0 (d, JPOCH₃ = 6.1 Hz), 52.7 (d, JPOCH₃ = 6.1 Hz), 51.5 (d, JPOCH₃ = 138.8 Hz), 51.1 (d, JPOCH₃ = 120.8 Hz), 29.9 (d, J = 9.1 Hz), 29.6 (d, J = 9.1 Hz), 26.2, 24.2, 22.4 (d, J = 12.2 Hz), 21.9 (d, J = 12.2 Hz).
$^{31}$P NMR (CDCl$_3$, 171 MHz): δ 33.0, 31.3.
O,O-Dimethyl ester [1-(2-Hydroxy-1-phenyl-ethylamino)-2-methyl-propyl]-
phosphonothionate (58). [144]-Phenylglycinol (1.13 g, 18.3 mmol) dissolved in 10 mL
of toluene followed by the addition of isobutyryl aldehyde (0.59 g, 8.20 mmol) and
stirred at rt for 1 h. The reaction was then concentrated, followed by the addition of 2 mL
of toluene, dimethyl thiophosphite (0.93 g, 7.38 mmol), and stirred at rt for 8 h. The
reaction was then concentrated to oil followed by a silica column eluted with 1%
MeOH/CHCl₃ to afford colorless oil 58 as a mixture of diastereomers (11:8), (2.23 g,
7.42 mmol, 48%). HRMS calc'd for M+H⁺ of C₁₄H₂₄N₀₃PS 318.1292, found 318.1201.

¹H NMR (CDCl₃, 400 MHz): δ 7.33-7.23 (m 5 H), 4.14-3.98 (m, 1H), 3.72 (d, JPOCH₃ =
12.9 Hz, 1.5 H), 3.71 (d, JPOCH₃ = 12.9 Hz, 1.5 H), 3.69-3.66 (m, J = 3.9 Hz, 2 H) 3.62-
3.55 (m, J = 3.9 Hz, 1 H) 3.59 (d, JPOCH₃ = 12.9 Hz, 1.5 H), 3.55 (d, JPOCH₃ = 12.9 Hz,
1.5 H), 2.95 (dd, J₈₅ CH = 16.2), 2.6 Hz, 0.5 H), 2.81 (dd, J₈₅ CH = 9.7, 2.6 Hz, 0.5 H), 2.33-
2.14 (m, J = 7.2 Hz, 1 H), 1.09 (d, J = 6.4 Hz, 1.5 H), 1.01 (d, 6.4 Hz, 1.5 H), 0.92 (d, J =
6.4 Hz, 1.5 H), 0.85 (d, J = 6.4 Hz, 1.5 H);

¹³C NMR (CDCl₃, 100 MHz): δ 141.6, 141.4, 129.4, 129.3, 129.1, 129.0, 128.7, 68.8,
68.2, 64.9, 64.5, 55.9 (d, JPOCH₃ = 134.3 Hz), 57.7 (d, JPOCH₃ = 152.6 Hz), 54.0 (d, JPOCH₃ =
6.1 Hz), 53.9 (d, JPOCH₃ = 9.2 Hz), 53.2 (d, JPOCH₃ = 6.1 Hz), 53.1 (d, JPOCH₃ = 9.2 Hz),

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30.4 (d, $J = 6.1$ Hz), 29.5 (d, $J = 6.1$ Hz), 21.8 (d, $J = 12.2$ Hz), 21.2 (d, $J = 12.2$ Hz), 19.6, 18.5.

$^{31}$P NMR (CDCl$_3$, 171 MHz): $\delta$ 106.3, 103.6.
O,O-Dimethyl ester [3-Methyl-1-(1-phenyl-ethylamino)-butyl]-phosphonothionate (59). [144]-α-Methylbenzylamine (1.13 g, 9.3 mmol) dissolved in 10 mL of toluene followed by the addition of 3-methyl-butyraldehyde (0.81 g, 9.3 mmol) and stirred at rt for 1 h. The reaction was then concentrated, followed by the addition of 2 mL of toluene, dimethyl thiophosphite (1.06 g, 8.37 mmol), and stirred at rt for 10 h. After concentrating to oil a silica column eluted with 0.5% EtOAc/hex afforded colorless oil 59 with partial separation of diastereomers (10:8), (2.19 g, 6.94 mmol, 75%). HRMS calcd for M+H+ of C_{18}H_{26}NO_{2}PS 316.1500, found 316.1555.

Major isomer:

\(^1^H\) NMR (CDCl\(_3\), 400 MHz): δ 7.35-7.18 (m 5 H), 4.07 (dq, J = 6.4, 1.9 Hz, 1H), 3.74 (d, \(J_{PCH3} = 12.9\) Hz, 3 H), 3.68 (d, \(J_{PCH3} = 12.9\) Hz, 3 H), 3.02 (dt, \(J_{PCH} = 8.4, 5.2\) Hz, 1 H), 1.92-1.86 (m, \(J = 6.4\) Hz, 1 H), 1.66-1.54 (m, \(J = 5.2\) Hz, 1 H), 1.42 (d, \(J = 8.4\) Hz, 2 H), 1.31 (d, 6.4 Hz, 3 H), 0.89 (d, \(J = 6.4\) Hz, 3 H), 0.84 (d, \(J = 6.4\) Hz, 3 H);

\(^{13}\)C NMR (CDCl\(_3\), 100 MHz): δ 145.9, 129.3, 128.5, 128.2, 57.0, 56.3 (d, \(J_{PCH} = 103.8\) Hz), 54.8 (d, \(J_{PCH3} = 6.1\) Hz), 53.7 (d, \(J_{PCH3} = 9.2\) Hz), 41.3 (d, \(J = 9.2\) Hz), 26.0, 24.8, 24.6, 22.0.
$^3$P NMR (CDCl$_3$, 171 MHz): $\delta$ 109.4.

Minor isomer:

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.35-7.21 (m 5 H), 4.07 (dq, $J = 6.4$, 1.9 Hz, 1H), 3.75 (d, $J_{POCH_3} = 12.9$ Hz, 3 H), 3.02 (dt, $J_{PCH} = 8.4$, 5.2 Hz, 1 H), 1.92-1.86 (m, $J = 6.4$ Hz, 1 H), 1.64-1.56 (m, $J = 5.2$ Hz, 1 H), 1.41 (d, $J = 8.4$ Hz, 2 H), 1.31 (d, 6.4 Hz, 3 H), 0.88 (d, $J = 6.4$ Hz, 3 H), 0.83 (d, $J = 6.4$ Hz, 3 H);

$^{13}$C NMR (CDCl$_3$, 400 MHz): $\delta$ 146.6, 129.4, 128.1, 128.0, 56.9, 56.5 (d, $J_{PCH} = 116.0$ Hz), 54.8 (d, $J_{POCH_3} = 9.2$ Hz), 53.8 (d, $J_{POCH_3} = 6.1$ Hz), 41.3 (d, $J = 9.1$ Hz), 25.9, 24.8, 24.2, 23.1.

$^3$P NMR (CDCl$_3$, 400 MHz): $\delta$ 106.1.
O,O-Dimethyl ester [3-Methyl-1-(1-phenyl-ethylamino)-butyl]-phosphonate (60).

[144]-α-Methylbenzylamine (1.88 g, 15.5 mmol) dissolved in 10 mL of toluene followed by the addition of 3-methyl-butyraldehyde (1.30 g, 15.45 mmol) and stirred at rt for 1 h. The reaction was then concentrated followed by the addition of 2 mL of toluene, dimethyl phosphite (3.41 g, 31.02 mmol) and stirred at rt for 96 h. After concentrating to oil a silica column eluted with 70% CHCl₃/hex afforded colorless oil 60 as a mixture of diastereomers (10:4), (2.93 g, 9.81 mmol, 63%). HRMS calcd for M+H⁺ of C₁₃H₂₆NO₃P 300.1728, found 300.1697.

¹H NMR (CDCl₃, 400 MHz): δ 7.27-7.14 (m 5 H), 4.10 (dq, J = 6.4, 3.2 Hz, 1 H), 3.71 (d, JPOCH₃ = 10.4 Hz, 3 H), 3.70 (d, JPOCH₃ = 10.4 Hz, 3 H), 2.65-2.59 (m, JPOCH = 8.4, 3.2 Hz, 1 H), 1.77-1.67 (m, J = 6.4 Hz, 1 H), 1.62-1.52 (m, J = 6.4 Hz, 2 H), 1.31 (d, 6.4 Hz, 3 H), 0.76 (d, J = 7.1 Hz, 3 H), 0.35 (d, J = 7.1 Hz, 3 H);

¹³C NMR (CDCl₃, 100 MHz): δ 146.2, 145.8, 129.4, 129.3, 128.3, 128.2, 128.1, 128.0, 57.2, 56.7 (d, JPOCH₃ = 9.2 Hz), 54.1 (d, JPOCH₃ = 6.1 Hz), 53.7 (d, JPOCH₃ = 6.1 Hz), 53.5 (d, JPOCH₃ = 6.1 Hz), 50.8 (d, JPOCH = 152.6 Hz), 50.6 (d, JPOCH = 146.5 Hz), 41.2, 40.8, 25.9, 25.6, 25.2, 24.8, 24.6, 23.9, 21.8.
$^{31}\text{P NMR (CDCl}_3, 171 \text{ MHz): } \delta \ 33.1, 32.0.$
O,O-Dimethyl ester [1-(2-Hydroxy-1-phenyl-ethylamino)-3-methyl-butyl]-phosphonothionate (61). [144]-Phenylglycinol (1.20 g, 8.73 mmol) dissolved in 10 mL of toluene followed by the addition of 3-methyl-butyraldehyde (0.745 g, 8.69 mmol) and stirred at rt for 1 h. The reaction was then concentrated followed by the addition of 2 mL of toluene, dimethyl thiophosphite (0.99 g, 7.87 mmol) and stirred at rt for 8 h. After concentrating to oil a silica column eluted with CHCl₃ afforded colorless oil 61 as a mixture of diastereomers (2.08 g, 6.61 mmol, 76%). HRMS calcd for M+H⁺ of C₁₅H₂₆NO₃PS 331.1371, found 331.1333.

¹H NMR (CDCl₃, 400 MHz): δ 7.33-7.23 (m 5 H), 4.16-4.12 (m, 1H), 4.02 (dd, J = 7.1, 2.6 Hz 2 H), 3.76 (d, J_PCH₃ = 12.9 Hz, 1.5 H), 3.73 (d, J_PCH₃ = 12.9 Hz, 1.5 H), 3.66 (d, J_PCH₃ = 12.9 Hz, 1.5 H), 3.64 (d, J_PCH₃ = 12.9 Hz, 1.5 H), 3.04-2.97 (m, J_PCH = 8.4, 2.6 Hz, 1 H), 1.88-1.74 (m, J = 7.1 Hz, 1 H), 1.62-1.34 (m, J = 7.1 Hz, 2 H), 0.86 (d, J = 7.1 Hz, 1.5 H), 0.83 (d, J = 7.1 Hz, 1.5 H), 0.81 (d, J = 6.4 Hz, 1.5 H), 0.48 (d, J = 6.4 Hz, 1.5 H);

¹³C NMR (CDCl₃, 100 MHz): δ 141.5, 141.3, 129.6, 129.0, 128.9, 128.8, 128.7, 68.5, 67.8, 64.2, 63.4 (d, J_PCH₃ = 9.2 Hz), 56.9 (d, J_PCH = 119.0 Hz), 56.7 (d, J_PCH = 112.9 Hz).
Hz), 54.7 (d, \( J_{POCH3} = 9.2 \) Hz), 54.4 (d, \( J_{POCH3} = 6.1 \) Hz), 53.9 (d, \( J_{POCH3} = 9.2 \) Hz), 41.2 (d, \( J = 6.1 \) Hz), 41.0 (d, \( J = 6.1 \) Hz), 26.2, 26.0, 24.9, 24.8, 24.1, 23.1.

\(^{31}\text{P} \text{NMR (CDCl}_3, 171 \text{ MHz)}: \delta 107.3, 105.3.\)
O,O-Dimethyl ester [1-(2-Hydroxy-1-phenyl-ethylamino)-3-methyl-butyl]-phosphonate (62). [144]-Phenylglycinol (1.74 g, 12.7 mmol) dissolved in 10 mL of toluene was put into a 50 mL RB, followed by the addition of 3-methyl-butyraldehyde (0.98 g, 11.43 mmol) and stirred at rt for 1 h. The reaction was then concentrated followed by the addition of 4 mL of toluene, dimethyl phosphite (2.52 g, 22.86 mmol), and stirred at rt for 72 h. After concentrating to oil a silica column eluted with 1% MeOH/CHCl₃ afforded colorless oil 62 as a mixture of diastereomers (4:1), (1.65 g, 5.24 mmol, 46%). HRMS calcd for M+H⁺ of C₁₅H₂₆N₀₄P 315.1599, found 315.1632.

¹H NMR (CDCl₃, 400 MHz): δ 7.34-7.23 (m 5 H), 4.11-4.00 (m, 1H), 4.02 (dd, J = 7.1, 2.6 Hz 2 H), 3.81 (d, JPOCH₃ = 10.4 Hz, 1.5 H), 3.75 (d, JPOCH₃ = 10.4 Hz, 1.5 H), 3.71 (d, JPOCH₃ = 10.4 Hz, 1.5 H), 3.70 (m, 2 H), 3.66 (d, JPOCH₃ = 10.4 Hz, 1.5 H), 2.93-2.84 (m, JPOCH = 8.4 Hz, 1 H), 1.83-1.71 (m, J = 6.4 Hz, 1 H), 1.64-1.45 (m, J = 6.4 Hz, 2 H), 0.86 (d, J = 6.4 Hz, 1.5 H), 0.84 (d, 6.4 Hz, 1.5 H), 0.78 (d, J = 6.4 Hz, 1.5 H), 0.55 (d, J = 6.4 Hz, 1.5 H);

¹³C NMR (CDCl₃, 100 MHz): δ 141.8, 141.5, 129.6, 129.4, 129.3, 128.8, 128.7, 68.5, 67.8, 65.2, 63.5, 63.4, 54.5 (d, JPOCH₃ = 6.1 Hz), 54.2 (d, JPOCH₃ = 6.1 Hz), 53.6 (d, JPOCH₃
= 6.1 Hz), 53.5 (d, $J_{POCH} = 9.2$ Hz), 51.9, (d, $J_{PCH} = 149.5$ Hz), 50.9 (d, $J_{PCH} = 152.6$
Hz), 41.4, 40.8, 26.0, 24.7, 23.8, 23.4, 21.9.

$^{31}$P NMR (CDCl$_3$, 171 MHz): $\delta$ 32.7, 32.0.
O,O-Dimethyl ester [1-(2-Hydroxy-1-phenyl-propylamino)-3-phenyl-methyl]-phosphonate (63). L-threonine methyl ester (1.039 g, 7.8 mmol) dissolved in 10 mL of CH₂Cl₂ was put into a 50 mL RB, followed by the addition of benzaldehyde (0.79 mL, 0.828 g, 7.8 mmol), K₂CO₃ (1.62 g, 11.7 mmol), and stirred at rt for 3 h. The reaction was then concentrated followed by the addition of 2 ml of toluene, dimethyl thiophosphite (0.84 g, 6.66 mmol), and stirred at rt for 10 h. After concentrating to oil a silica column eluted with 70% CH₂Cl₂-hex afforded colorless oil 63 as a mixture of diastereomers (8:3), (1.65 g, 5.24 mmol, 46%). HRMS calcd for M+H⁺ of C₁₄H₂₃NO₅PS 348.1034, found 348.1046.

Major isomer:

¹H NMR (CDCl₃, 400 MHz): δ 7.40-7.20 (m, 5H), 4.07 (d, 1 H, J_PCH = 14.8 Hz), 3.86-3.79 (m, 1H), 3.75 (d, 3 H, J_POC₃ = 13.6 Hz), 3.37 (s, 3 H), 3.33 (d, 3 H, J_POC₃ =12.8 Hz), 2.95 (d, 1 H, J = 6.4 Hz), 1.15 (d, 3 H, J = 6.4 Hz);

¹³C NMR (CDCl₃, 100 MHz): δ 173.20, 134.9, 133.8, 129.2, 128.7, 128.6, 128.4, 68.2, 66.7, 65.9 (d, J_CP = 127.50 Hz), 54.2 (d, J_POC₃ = 9.1 Hz), 53.7 (d, J_POC₃ = 6.1 Hz) 51.8, 19.1.
\[ ^{31}\text{P NMR (CDCl}_3, 171 \text{ MHz): } \delta 97.2. \]

Minor isomer:

\[ ^{1}\text{H NMR (CDCl}_3, 400 \text{ MHz): } \delta 7.40-7.20 \text{ (m, 5H)}, 4.11 \text{ (d, } J_{PCH} = 14 \text{ Hz, 1 H), 3.75-3.70} \text{ (m, 1 H), 3.67 (s, 3 H), 3.64 (d, } J_{PCH3} = 13.6 \text{ Hz, 3 H), 3.52 (d, } J_{POCH3} = 13.6 \text{ Hz, 3 H), 2.99 (d, } J = 6.4 \text{ Hz) 1.13 (d, } J = 6.4 \text{ Hz, 3 H);} \]

\[ ^{13}\text{C NMR (CDCl}_3, 100 \text{ MHz): } \delta 173.0, 134.9, 133.8, 129.2, 129.1, 128.7, 128.4, 67.9, 66.6, 64.5 (d, } J_{CP} = 118.4 \text{ Hz), 54.3 (d, } J_{POCH3} = 6.1 \text{ Hz), 53.6 (d, } J_{POCH3} = 6.1 \text{ Hz) 51.9, 19.3.} \]

\[ ^{31}\text{P NMR (CDCl}_3, 171 \text{ MHz): } \delta 96.8. \]
**O,O-Dimethyl ester [1-(1-Phenyl-ethylamino)-butyl]-phosphonothionate (64).** [144]-

α-Methylbenzylamine (1.88 g, 15.51 mmol) dissolved in 10 mL of toluene was put into a
50 mL RB, followed by the addition of n-butyraldehyde (1.062 g, 14.73 mmol) and
stirred at rt for 1 h. The reaction was then concentrated followed by the addition of
dimethyl thiophosphite (1.67 g, 13.26 mmol), and stirred at rt for 8 h. After
concentrating to oil a silica column eluted with 1% EtOAc/hex afforded colorless oil 64
as partial separation of diastereomers (9:19), (3.38 g, 11.21 mmol, 85%). HRMS calcd
for M+H⁺ of C₁₄H₂₄N₂O₂PS 302.1343, found 302.1301.

Major isomer:

^1^H NMR (CDCl₃, 400 MHz): δ 7.35-7.21 (m, 5 H), 4.03 (q, J = 6.4, 1 H), 3.74 (d, _J_PoCH₃_
= 12.9 Hz, 3 H), 3.67 (d, _J_PoCH₃ = 12.9 Hz, 3 H), 2.92 (dt, _J_PCH = 8.4, 2.6 Hz, 1 H), 1.84-
1.37 (m, _J = 7.1 Hz, 5 H), 1.32 (d, _J = 6.4 Hz, 3 H), 0.90 (t, _J = 7.1 Hz, 3 H);

^1^C NMR (CDCl₃, 100 MHz): δ 146.2, 129.5, 128.2, 128.0, 58.0 (d, _J_PCH = 116.0 Hz),
56.6, 54.8 (d, _J_PoCH₃ = 6.1 Hz), 54.1 (d, _J_PoCH₃ = 6.1 Hz), 33.4 (d, _J = 6.1 Hz), 25.0, 20.5,
15.2.

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$^3$P NMR (CDCl$_3$, 171 MHz): $\delta$ 105.0.

Minor isomer:

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.30-7.20 (m, 5 H), 4.10 (q, $J = 6.4$, 1 H), 3.77 (d, $J_{POCH3} = 12.9$ Hz, 3 H), 3.72 (d, $J_{POCH3} = 12.9$ Hz, 3 H), 2.77 (dt, $J_{PCH} = 8.4$, 3.2 Hz, 1 H), 1.63 (bs, 1 H), 1.58-1.49 (m, $J = 7.1$ Hz, 4 H), 1.31 (d, $J = 6.4$ Hz, 3 H), 0.71 (t, $J = 7.1$ Hz, 3 H);

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 146.0, 129.3, 128.4, 128.1, 57.8 (d, $J_{PCH} = 131.2$ Hz), 57.4, 54.7 (d, $J_{POCH3} = 9.2$ Hz), 53.8 (d, $J_{POCH3} = 9.2$ Hz), 34.3 (d, $J = 6.1$ Hz), 26.1, 20.1, 14.8.

$^3$P NMR (CDCl$_3$, 171 MHz): $\delta$ 108.6.
O,O-Dimethyl ester [1-(1-Phenyl-ethylamino)-butyl]-phosphonate (65). [144]-α-Methylbenzylamine (1.88 g, 15.51 mmol) dissolved in 10 mL of toluene was put into a 50 mL RB, followed by the addition of n-butyraldehyde (1.062 g, 14.73 mmol) and stirred at rt for 1 h. The reaction was then concentrated followed by the addition of 2 mL of toluene, dimethyl phosphite (3.24 g, 29.45 mmol), and stirred for 96 h. After a concentrating to oil a silica column eluted with 1% MeOH/CHCl₃ afforded colorless oil 65 as a mixture of diastereomers (9:10), (3.30 g, 11.56 mmol, 75%). HRMS calcd for M+H⁺ of C₁₄H₂₄N₀₃P 286.1572, found 286.1602.

¹H NMR (CDCl₃, 400 MHz): δ 7.28-7.15 (m 5 H), 4.08 (dq, J = 6.4, 2.6 Hz, 1 H), 3.71 (d, JPOCH₃ = 10.4 Hz, 3 H), 3.70 (d, JPOCH₃ = 10.4 Hz, 3 H), 2.62 (dt, J_PCH = 9.1, 3.8 Hz, 1 H), 1.58-1.32 (m, J = 7.1 Hz, 4 H), 1.26 (d, J = 6.4 Hz, 3 H), 0.67 (t, J = 7.1 Hz, 3 H);

¹³C NMR (CDCl₃, 100 MHz): δ 145.9, 129.4, 128.2, 128.1, 57.4, 53.7 (d, JPOCH₃ = 9.2 Hz), 53.6 (d, JPOCH₃ = 6.1 Hz), 52.3 (d, J_PCH = 143.4), 34.0 (d, J = 6.1 Hz), 25.7, 20.1, 14.7.

O,O-Dimethyl ester [1-(2-Hydroxy-1-phenyl-ethylamino)-butyl]-phosphonothionate (66). [144]-Phenylglycinol (3.52 g, 25.69 mmol) dissolved in 10 mL of toluene was put into a 50 mL RB, followed by the addition of n-butyraldehyde (1.80 g, 24.9 mmol) and stirred at rt for 1 h. The reaction was then concentrated followed by the addition of 2 mL toluene, dimethyl thiophosphite (2.92 g, 23.12 mmol), and stirred at rt for 7 h. After concentrating to oil a silica column eluted with 5% EtOAc/hex afforded colorless oil 66 as a mixture of diastereomers (10:6), (3.38 g, 24.9 mmol, 85%). HRMS calcd for M+H⁺ of C₁₄H₂₄NO₃PS 318.1292, found 318.1331.

¹H NMR (CDCl₃, 400 MHz): δ 7.34-7.26 (m 5 H), 4.03-4.00 (m, J = 7.1 Hz, 1 H), 3.78-3.70 (m, J = 7.1 Hz, 2 H), 3.68 (d, Jₚₒｃḥ = 12.9 Hz, 1.5 H), 3.66 (d, Jₚₒｃḥ = 12.9 Hz, 3 H), 3.60 (d, Jₚₒｃḥ = 12.9 Hz, 1.5 H), 3.58 (d, Jₚₒｃḥ = 12.9 Hz, 1.5 H), 2.95 (m, Jₚᶜḥ = 7.7 Hz, 1 H), 1.84-1.71 (m, J = 7.1 Hz, 2 H), 1.65-1.37 (m, J = 7.1 Hz, 2 H), 0.87 (t, J = 7.1 Hz, 3 H), 0.74 (t, J = 7.1 Hz, 3 H);

¹³C NMR (CDCl₃, 100 MHz): δ 141.4, 141.3, 129.6, 129.5, 128.9, 128.8, 128.7, 68.6, 67.9, 64.4, 63.3, 58.4 (d, Jₚᶜᴴ = 109.9 Hz), 58.2 (d, Jₚᶜᴴ = 119.0 Hz), 54.8 (d, Jₚₒᶜḥ = 9.2 Hz), 54.6 (d, Jₚₒᶜḥ = 6.1 Hz), 54.3 (d, Jₚₒᶜḥ = 6.1 Hz), 53.9 (d, Jₚₒᶜḥ = 9.2 Hz), 34.2 (d, J = 6.1 Hz), 33.2 (d, J = 6.1 Hz), 20.6, 20.5, 20.2, 20.1, 15.2, 14.8.
$^{31}$P NMR (CDCl$_3$, 171 MHz): $\delta$ 106.5, 104.3.
3RS, 1R- O,O-Dimethyl ester [(2-Hydroxy-1-phenyl-ethylamino)-phenyl-methyl]-phosphonothionate (67). [144]-Phenylglycinol (0.51 g, 3.72 mmol) dissolved in 10 mL of toluene was put into a 50 mL RB, followed by the addition of benzaldehyde (0.40 g, 3.74 mmol) and stirred at rt for 1 h. The reaction was then concentrated followed by the addition of 3 mL of toluene, dimethyl thiophosphite (0.46 g, 3.79 mmol), and stirred at rt for 7 h. After concentration to oil a silica column eluted with 50% hex/Et₂O afforded colorless oil 67 as a mixture of diastereomers (0.99 g, 2.77mmol, 56%). Anal. Calcd for C₁₇H₂₂N₃O₃PS: C, 58.11; H, 6.31; N, 3.99. Found: C, 57.90; H, 6.42; N, 3.89.

Major isomer:

¹H NMR (CDCl₃, 400 MHz): δ 7.33-7.21 (m, 10 H), 4.20 (d, J_PCH = 17.2 Hz, 1H), 4.01-3.95 (m, 1 H), 3.78 (d, J_POCH₃ = 15.6 Hz, 3 H), 3.79-3.73 (m, 1H), 3.32 (d, J_POCH₃ =12.8 Hz, 3 H), 2.64 (br s, 1 H);

¹³C NMR (CDCl₃, 100 MHz): δ 140.0, 135.7, 128.5, 128.4, 128.3, 128.1, 127.7, 127.4, 66.0, 62.9 (d, J_PC = 116.0 Hz), 62.5, 62.4, 54.5 (d, J_POCH₃ = 6.1 Hz), 53.8 (d, J_POCH₃ = 9.1 Hz);

³¹P NMR (CDCl₃, 171 MHz): δ 100.5.
Minor isomer:

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.36-7.21 (m, 10H), 3.97 (d, $J_{PCH} = 18.8$ Hz, 1 H), 3.74 (d, $J_{POCH_3} = 12.8$ Hz, 3 H) 3.68-3.58 (m, 3H), 3.45 (d, $J_{POCH_3} = 12.8$ Hz, 3 H), 2.28 (br s, 1H);

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 138.7, 134.4, 129.0, 128.9, 128.7, 128.4, 128.3, 127.9, 127.6, 67.0, 62.3 (d, $J_{PC} = 122.0$ Hz), 61.5, 61.3, 54.1 (d, $J_{POCH_3} = 6.1$ Hz) 53.3 (d, $J_{POCH_3} = 6.1$ Hz);

$^{31}$P NMR (CDCl$_3$, 171 MHz): $\delta$ 98.1.
O,O-Dimethyl ester [[144]-Phenyl-(1-phenyl-ethylamino)-methyl]-phosphonothionate (68). [144]-Phenylglycinol (0.75 g, 6.2 mmol) dissolved in 10 mL of toluene was put into a 50 mL RB, followed by the addition of benzaldehyde (0.66 g, 6.2 mmol), and stirred at rt for 1 h. The reaction was then concentrated followed by the addition of 2 mL of toluene, dimethyl thiophosphite (0.78 g, 6.2 mmol), and stirred at rt for 8 h. After concentrating to oil a silica column eluted with 2% Et₂O/hex to yield colorless oil 68 as a mixture of diastereomers (8:2), (1.32 g, 3.95 mmol, 64%). HRMS calcd for M+H⁺ of C₁₇H₂₂NO₂PS calcd for 336.1187, found 336.1194.

¹H NMR (CDCl₃, 400 MHz): δ 7.37-7.21 (m, 10 H), 4.24 (d, J_PCH = 19.2 Hz, 1 H), 3.93 (q, J = 6.4 Hz, 1 H), 3.84 (d, J_POC₃ = 12.8 Hz, 3 H), 3.36 (d, J_POC₃ = 12.8 Hz, 3 H), 2.45 (br s, 1H), 1.36 (d, J = 6.4 Hz, 3 H);

¹³C NMR (CDCl₃, 100 MHz): δ 145.0, 135.9, 128.4, 128.4, 128.3, 128.1, 127.0, 126.7, 62.6 (d, J_CP = 115.3 Hz), 55.2 (d, J_POC₃ = 9.1 Hz), 54.1 (d, J_POC₃ = 6.1 Hz), 53.7, 23.0;

³¹P NMR (CDCl₃, 171 MHz): δ 101.6.
O,O-Dimethyl ester 2-\{[\text{(Dimethoxy-thiophosphoryl)-phenyl-methyl} \text{amino}]\}-3-hydroxy-propionate (69). To a suspension of L-serine methyl ester hydrochloride (0.54 g, 3.47 mmol) in THF (15 mL) was added TEA (0.48 mL, 0.35 g, 3.44 mmol). The reaction was stirred at 0 °C for 30 min and filtered rapidly under vacuum. The solvent was evaporated to dryness to give L-serine methyl ester as an off-yellow oil (0.34 g, 2.82 mmol). To a solution of L-serine, methyl ester in CH$_2$Cl$_2$ (5 mL) was added benzaldehyde (0.27 mL, 0.28 g, 2.66 mmol) and molecular sieves (3 Å, 0.5 g). The reaction mixture was stirred at rt for 2 h and filtered through Celite. Dimethyl thiophosphite 5 (0.24 mL, 0.36 g, 2.85 mmol) was added and the reaction mixture was stirred at 0 °C for 12 h. The solvent was evaporated to dryness to give crude product (0.688 g) as yellowish oil that was purified by column chromatography (30% hex/Et$_2$O) to yield 69 as partially separated diastereomers. Anal. Calcd for C$_{13}$H$_{20}$NO$_5$PS: C, 46.84; H, 6.05; N, 4.20; Found: C, 46.49; H, 5.96; N, 4.21.

Major isomer:

$^1$H NMR (CDCl$_3$, 400 MHz): δ 7.39-7.26 (m, 5H), 4.16 (d, $J_{CPh} = 20.4$ Hz, 1 H), 3.81-3.55 (m, 2H), 3.75 (d, $J_{POCH_3} = 13.2$ Hz, 3 H), 3.60 (s, 3H), 3.45-3.32 (m, 1H), 3.38 (d, $J_{POCH_3} = 13.6$ Hz, 3 H), 2.91 (br s, 1H);
\[ {^{13}}\text{C NMR (CDCl}_3, 100 \text{ MHz): } \delta 172.4, 134.7, 128.5, 128.5, 128.3, 63.7 (d, J_{PC} = 128.1 \text{ Hz}), 61.6, 60.2, 54.3 (d, J_{POCH3} = 9.1 \text{ Hz}), 53.7 (d, J_{POCH3} = 6.1 \text{ Hz}), 52.2; \]

\[ {^{31}}\text{P NMR (CDCl}_3, 171 \text{ MHz): } \delta 97.8. \]

Minor isomer:

\[ {^1}\text{H NMR (CDCl}_3, 400 \text{ MHz): } \delta 7.42-7.26 (m, 5\text{H}), 4.29 (d, J_{PCH} = 15.6 \text{ Hz}, 1 \text{H}), 3.74-3.64 (m, 2\text{H}), 3.69 (d, J_{POCH} = 13.2 \text{ Hz}, 3 \text{H}), 3.70 (s, 3\text{H}), 3.53 (d, J_{POCH} = 13.2 \text{ Hz}, 3 \text{H}), 3.35-3.30 (m, 1\text{H}); \]

\[ {^{13}}\text{C NMR (CDCl}_3, 100 \text{ MHz): } \delta 172.6, 134.2, 129.1, 129.1, 128.4, 128.3, 64.2 (d, J_{PC} = 119.1 \text{ Hz}), 63.1, 60.0, 54.3 (d, J_{POCH} = 6.1 \text{ Hz}), 53.7 (d, J_{POCH} = 6.1 \text{ Hz}), 52.1; \]

\[ {^{31}}\text{P NMR (CDCl}_3, 171 \text{ MHz): } \delta 97.2. \]
D. Compounds 70-72

General synthesis for α-amino phosphonic and α-aminothiophosphonic acids:

α-Aminophosphonate or α-aminothiophosphonate (0.7-1.7 mmol) was placed into a 50 mL RB flask with 10 mL of acetone and a water condenser. To this solution was added potassium ethyl xanthate (0.9 eq) and then the reaction was refluxed for 8-20 h while monitoring by reaction with unlocked $^{31}$P NMR until the reaction was complete. The work up for each reaction was specific for the compound and is listed with the spectral data.
[2-Methyl-1-(1-phenyl-ethylamino)-propyl]-phosphonothioic acid O-methyl ester (70). Compound 56 (0.44 g, 1.48 mmol) was reacted with PEX (0.23 mg, 1.4 mmol) at reflux for 8 h and then concentrated under reduced pressure to afford light yellow oil. The oil was dissolved in Et₂O and a white powder crashed out of solution. The precipitate was isolated and then analyzed by \(^1\)H, \(^{13}\)C, and \(^{31}\)P NMR showing a mixture of 4 diasteromers (10:8:7:5), (0.086 g, 0.30 mmol, 21%). HRMS calcd for M+H⁺ of C₁₃H₂₂NO₂PS 288.1187, found 288.1225.

\(^1\)H NMR (CDCl₃, 400 MHz): δ 7.70-7.35 (m, 5H), 4.68 (q, J = 6.4 Hz, 0.5 H), 4.38 (q, J = 6.4 Hz, 0.5 H), 3.72 (d, J = 12.9 Hz, 0.75 H), 3.70 (d, J = 12.9 Hz, 0.75 H), 3.47 (d, J = 12.9 Hz, 0.75 H), 3.34 (d, J = 12.9 Hz, 0.75 H), 2.85-2.74 (m, 1 H), 2.53-2.38 (m, J = 7.1 Hz, 1 H), 1.97-1.90 (m, J = 7.1 Hz, 3 H), 1.88 (d, J = 7.1 Hz, 0.75 H), 1.29 (d, J = 7.1 Hz, 0.75 H), 1.27 (d, J = 7.1 Hz, 0.75 H), 1.17 (d, J = 7.1 Hz, 0.75 H), 1.09 (d, J = 7.1 Hz, 0.75 H), 1.06 (d, J = 7.1 Hz, 0.75 H), 0.81 (d, J = 7.1 Hz, 0.75 H), 0.76 (d, J = 7.1 Hz, 0.75 H).

\(^{13}\)C NMR (CDCl₃, 100 MHz): δ 137.6, 137.3, 137.0, 136.6, 130.7, 130.6, 130.5, 130.3, 130.2, 129.9, 129.8, 129.0, 128.9, 66.2, 65.3, 65.1, 64.3, 62.5 (d, J\(_{PCCH}\) = 103.8 Hz), 62.4
(d, $J_{PCH} = 119.0$ Hz), 61.5 (d, $J_{PCH} = 109.9$ Hz), 61.1 (d, $J_{PCH} = 128.2$ Hz), 53.3 (d, $J_{POCH}$ = 6.1 Hz), 53.1 (d, $J_{POCH} = 6.1$ Hz), 52.8 (d, $J_{POCH} = 6.1$ Hz), 51.9 (d, $J_{POCH} = 6.1$ Hz), 30.0, 29.4, 28.1, 23.7, 23.5, 23.0, 22.2, 21.5, 21.4, 21.1, 21.0, 18.7, 18.5.

$^{31}$P NMR (CDCl$_3$, 171 MHz): δ 74.6, 72.3, 71.6, 68.2
[1-(1-Phenyl-ethylamino)-butyl]-phosphonic acid monomethyl ester (71). Compound 65 (0.48 g, 1.69 mmol) and PEX (0.26 g, 1.6 mmol) were brought to reflux for 12 h and then concentrated under reduced pressure to afford light yellow oil. The oil was dissolved in Et₂O and a white powder crashed out of solution. The precipitate was isolated and then analyzed by \(^1\)H, \(^{13}\)C, and \(^{31}\)P NMR showing a mixture of 2 diasteromers (0.057 g, 0.02 mmol, 13%). HRMS calcd for M+H\(^+\) of C\(_{13}\)H\(_{22}\)N\(_2\)O\(_3\)P 272.1415, found 272.1456.

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta\) 7.58-7.34 (m, 5H), 4.69 (q, \(J = 6.4\) Hz, 0.6 H), 4.32 (q, \(J = 6.4\) Hz, 0.4 H), 3.60 (d, \(J = 11.0\) Hz, 2.1 H), 3.41 (d, \(J = 11.9\) Hz, 0.9 H), 2.76-2.51 (m, 1 H), 2.09-1.11 (m, \(J = 7.1\) Hz, 4 H), 1.82-1.79 (m, \(J = 6.4\) Hz, 3 H), 0.83 (t, \(J = 7.1\) Hz, 0.9 H), 0.64 (t, \(J = 7.1\) Hz, 2.1 H).

\(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \(\delta\) 138.3, 138.2, 130.3, 130.2, 130.0, 129.0, 128.8, 61.2, 59.7, 57.8 (d, \(J_{POCH3} = 9.2\) Hz), 55.3 (d, \(J_{PCH} = 149.5\) Hz), 53.9 (d, \(J_{PCH} = 147.3\) Hz), 52.9 (d, \(J_{POCH3} = 6.1\) Hz), 32.0, 29.9, 22.2 (d, \(J = 9.2\) Hz), 20.8 (d, \(J = 9.2\) Hz), 15.2, 14.3.

\(^{31}\)P NMR (CDCl\(_3\), 171 MHz): \(\delta\) 14.8, 14.6.

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[3-Methyl-1-(1-phenyl-ethylamino)-butyl]-phosphonothioic acid O-methyl ester (72). Compound 59 (0.25 g, 0.78 mmol) and PEX (0.12 g, 0.74 mmol) were refluxed for 10 h and then transferred to a separatory funnel. Then 50 mL of 0.2 M NaOH was added to the funnel followed by an extraction with CHCl₃ (3 x 40 mL). After the CHCl₃ extraction, solid citric acid was added until the pH was about 5 and then CHCl₃:isopropyl alcohol (4:1) was used to extract the product from the water layer. All organic layers from the CHCl₃:isopropyl alcohol extractions were combined and dried over Na₂SO₄ followed by concentration by reduced pressure to afford a light tan foam. The foam was isolated and then analyzed by ¹H, ¹³C, and ³¹P NMR showing a mixture of 2 diasteromers (3:2), (0.041 g, 0.01 mmol, 18%). HRMS calcd for M+H⁺ of C₁₄H₂₄NO₂PS 302.1343, found 302.1389.

¹H NMR (CDCl₃, 400 MHz): δ 7.57-7.32 (m, 5H), 4.71-4.51 (m, J = 7.1 Hz, 1 H), 3.72 (d, J = 12.9 Hz, 1.2 H), 3.62 (d, J = 12.9 Hz, 1.8 H), 2.93-2.81 (m, 1 H), 1.88-1.83 (m, J = 6.4 Hz, 3 H), 1.80-1.35 (m, J = 6.4 Hz, 3 H), 0.64 (d, J = 6.4 Hz, 1.2 H), 0.55 (d, J = 6.4 Hz, 1.8 H), 0.37 (d, J = 6.4 Hz, 1.2 H), 0.21 (d, J = 6.4 Hz, 1.8 H).

¹³C NMR (CDCl₃, 100 MHz): δ 137.7, 137.3, 130.6, 130.5, 130.4, 130.3, 129.6, 129.4, 61.5, 61.0, 58.6 (d, J_PCH = 106.8 Hz), 58.0 (d, J_PCH = 103.8 Hz), 52.5 (d, J_POC_3 = 6.1
Hz), 52.1 (d, $J_{POCH_3} = 6.1$ Hz), 39.3, 38.0, 32.8, 30.3, 25.9 (d, $J = 9.2$ Hz), 25.7 (d, $J = 6.1$ Hz), 23.5, 22.9, 22.4, 22.2.

$^{31}$P NMR (CDCl$_3$, 171 MHz): $\delta$ 73.4, 72.9.
ACE Assay

Phosphoramidon

1mL of HEPES shall be added to the bottle of Phosphoramidon.

50μL shall be transferred into each of 20 Eppendorf tubes.

950μL of 1e-4M HEPES shall be added to each tube when needed.

\[ [\text{Phosphoramidon}] = 9.19963 \times 10^{-5} \text{M} \]

FAGLA

277.8μL of 95%EtOH shall be used to dissolve 25mg of dipeptide.

40μL shall be transferred into each of 5 Eppendorf tubes.

960μL of 1e-4M HEPES shall be added to each tube when needed.

\[ [\text{FAGLA}] = 1.1747 \times 10^{-2} \text{M} \]

Thermolysin

13.3067mL of HEPES shall be used to dissolve 25mg of protein in approximately

2mL aliquots at a time.

The aliquots were then transferred to a small centrifuge tube.

1mL of suspension shall be transferred into each of 13 Eppendorf tubes.

100μL of concentrate shall be diluted with 900μL of 1e-4M HEPES when needed.

\[ [\text{Thermolysin}] = 5.01 \times 10^{-6} \text{M} \]

APTA compounds
Weigh approximately 1-2mg of compound and dissolve in 500μL of EtOH.

Calculate concentration and amount of concentrate needed to produce 1mL of 9.19963e-5M compound.

\[ [\text{APTA's}] = 9.19963e-5 \text{M} \]

500μL of 2.8279x10^{-3}M FAGLA were diluted in 500μL of 5.01x10^{-5} M enzyme and 500μL of pH 7.2, 1x10^{-3}M HEPES in a 96 well plate. The well plate was then incubated at 30°C for 5 min in the well plate reader. The decrease in the absorbance of FAGLA at λ=345 was measured over a time of 800 (later 180) seconds. A negative control of 500μL of FAGLA solution diluted in 1000μL of HEPES was also performed to make sure that the decreasing of A_{345} was due to the hydrolysis of FAGLA instead of its natural decomposition. A second control of 500μL of thermolysin in 1000μL of HEPES was also performed to guarantee that the enzyme did not absorb at 345nm. Finally 500μL of FAGLA were mixed with 500μL of thermolysin and 500μL of inhibitor, either the known inhibitor phosphoramidon, or an APTA compound.

These three solutions were then also analyzed on the Beckman UV-Visible spectrophotometer. The data was collected by Softmax pro and analyzed in Excel.


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