MOLECULAR PROBES FOR THE MECHANISM OF ACTION OF THE ENZYME
BIOTIN SYNTHASE

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To the most important person in my life. You know who you are.
My predecessor Dr. Corey Fugate raised the bar for dissertation acknowledgements and I have been at this so long that I have become indebted to so many people it is impossible to recall, let alone thank, all of them properly. But I will try to make the best of it.

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Abstract

The enzyme biotin synthase uses two equivalents of the cofactor S-adenosylmethionine to generate in its active site a 5'-deoxyadenosyl carbon radical that can abstract a hydrogen atom from the substrate dethiobiotin. The resulting dethiobiotinyl carbon radicals can form carbon-sulfur bonds to close a tetrahydrothiophene ring that converts dethiobiotin into biotin. This transformation may be better understood by investigating the effect of exposing the enzyme to a series of rationally designed substrate analogs. These analogs could include either substrate homologs with selected modifications made at or near the reactive positions, or by isotopic substitutions to monitor a change in reaction kinetics. The initial efforts to refine a goal-oriented synthetic methodology to produce a library of dethiobiotin analogs was unsuccessful due to a series of reactions with low yields, which made it impractical for the generation of a library of compounds. A second approach involving defunctionalization of biotin itself was hampered by the high cost of the starting material and a lack of selectivity in the reaction pathway. A third approach involving the synthesis of a natural product precursor in the biotin biosynthetic pathway was limited by an unexpected incompatibility of substrate and catalysts. No effective route to produce the library of interest has yet been demonstrated.
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<table>
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<td>Ac₂O</td>
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</tr>
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<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
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Chapter 1

Introduction

1.1 Biotin precursors in synthesis

Biotin 1 (Figure 1.1.1) is a natural product that is essential to all plant and animal life.\(^1\) Nature has evolved a sequence of enzymatic reactions that produces biotin from L-alanine and pimelic acid, with the last step a radical reaction that incorporates a sulfur atom into the molecule.\(^2,3\) Despite biotin’s biological significance and the unusual chemical mechanism by which it is produced the precursors in the biosynthetic pathway – which could be useful in elucidating the workings of this peculiar enzyme – have received scant attention from the community of synthetic organic chemists. This gap in the literature is problematic for study of the enzyme because of the limited ability of the biological pathway to produce substrate analogs. A synthetic route to produce analogs of biotin precursors would facilitate the studies of the mechanisms of enzymes in the pathway, including biotin synthase (BioB).

1.2 Chemical Structure of Biotin

The natural product biotin (Vitamin H) contains two fused five-membered rings with a carboxylic acid side chain. It plays a critical role in fatty acid biosynthesis, in branched chain amino acid catabolism, and in the first step of
gluconeogenesis. Structurally, biotin is composed of a tetrahydrothiophene ring fused to a five-membered imidazolidone, and an alkyl chain terminated by a carboxylic acid. The structure contains three chiral centers at sequential carbon atoms, which produce an overall concave structure. While the biological role of biotin as a carboxylation catalyst is carried out by the imidazolidone ring, the tetrahydrothiophene contributes important steric bulk that stabilizes carboxylated biotin intermediate against water hydrolysis.

Biotin contains a linear nine carbon chain beginning with the carboxylic acid carbon atom and terminating in the methylene carbon atom embedded within the tetrahydrothiophene. These carbon atoms are referenced according to their position in this chain with the carboxylic acid carbon is known as “C1” and the methylene carbon in the tetrahydrothiophene as “C9”. The same numbering scheme is also used in biotin precursors where the nonane subunit is present. Biotin synthase abstracts a hydrogen atom from the C6 methylene and C9 methyl groups in dethiobiotin to allow formation of two new carbon-sulfur bonds, oxidizing these atoms to a methine and methylene, respectively.

1.3 Biotin Biosynthesis

1.3.1 Conserved Steps from Pimelic Acid to Biotin

Biotin is produced in nature by means of a conserved five-step biosynthetic pathway starting from pimelic acid 2 (Figure 1.3.1). The initial transformation involves the conversion of diacid 2 to the corresponding Coenzyme A-derived
thioester 3 pimelyl CoA. The steps used to accomplish this vary between different organisms but thereon the synthetic routes converge for the final four steps. Pimelyl CoA is first condensed with alanine by 8-amino-7-oxononanoate synthase (BioF) to afford 7-keto-8-amino pelargonic acid (KAPA) 4. KAPA undergoes enantioselective reductive amination to produce 7,8-diaminopelargonic acid (DAPA) 5, which reacts spontaneously with CO₂ to generate an unstable carbamate. This species is then ring-closed by dethiobiotin synthase (BioD), which uses activation by ATP to drive the dehydration reaction, resulting in the addition of a carbonyl group bridging the diamine and affording the cyclic imidazolidone functionality in dethiobiotin 6.¹,⁶

![Chemical structure diagram](image.png)

**Figure 1.3.1** Biotin biosynthesis from pimelic acid

### 1.3.2 Mechanism of tetrahydrothiophene formation

Biotin synthase is a radical S-adenosylmethionine enzyme that acts by abstracting hydrogen atoms from dethiobiotin, facilitating formation of new C-S bonds between the substrate and a [2Fe-2S]²⁺ cluster in the enzyme. S-Adenosylmethionine undergoes a single-electron reduction and is homolytically cleaved to generate an equivalent of methionine and a 5'-deoxyadenosyl radical;
this radical abstracts a hydrogen atom from the terminal methyl group (C9) of dethiobiotin. The process is then repeated with a second equivalent of S-adenosylmethionine to form a second C-S bond with the C6 methylene group, closing the tetrahydrothiophene ring and completing the biosynthesis of 1.\textsuperscript{7}

1.4 History of the total synthesis of biotin

Unlike its biological precursors, biotin itself has drawn the attention of synthetic chemists and been the target of numerous successful total syntheses. While known routes to biotin may be elegant in their own right, the unique chemistry of biotin synthase could preclude this prior art from being a useful guide in designing a synthesis of earlier substrates in the biotin pathway.

1.4.1 Wolf and Harris

Biotin was first synthesized as a racemate by Wolf and Harris in 1943 (Figure 1.4.1).\textsuperscript{8,9}
**Figure 1.4.1** The first chemical synthesis of biotin and its stereoisomers

Beginning with glutaric anhydride the final structure was obtained in seven steps as a mixture of both enantiomers of biotin as well as other possible biotin stereoisomers. While the planar structure of the final product is correct the lack of any control of stereochemistry, as well as early incorporation of the tetrahydrothiophene ring, makes this approach not useful for the purposes of designing a synthetic method to reach our goal of the production of a single enantiomer of dethiobiotin.
1.4.2 Goldberg and Sternbach

The first report of a synthesis of a single enantiomer of biotin came in a patent application by Goldberg and Sternbach in 1949 (Figure 1.4.2).\textsuperscript{10}

\begin{center}
\includegraphics[width=\textwidth]{Goldberg-Sternbach_synthesis.png}
\end{center}

**Figure 1.4.2** Goldberg-Sternbach synthesis of (+)-biotin

Starting with fumaric acid biotin was produced in nine steps. Clearly the starting material is achiral, as are all reagents used for the first seven steps, so to obtain synthetic biotin as a single enantiomer it was necessary to derivatize the product with a chiral compound to form a diastereomeric salt mixture and then separate. This method, while inelegant by modern standards, did allow for the first
production of synthetic biotin. This work is also notable because of its use of the concavity of the fused ring system to block approach on one face of the alkene, thus allowing control of relative stereochemistry at C6 (indicated in Figure 1.4.2 with an asterisk) in the hydrogenation reaction. The method, however, is not a good guide for the synthesis of biotin precursors due to the presence of and reliance on the sulfur atom early in the scheme.

1.4.3 Hoffman-La Roche

The Hoffman-La Roche method is now the standard modern method to access synthetic biotin. Biotin is produced as a single enantiomer starting from dicysteine methyl ester in eight steps (Figure 1.4.3).11

Figure 1.4.3 Hoffman-La Roche total synthesis of biotin.

The choice of a starting material from the chiral pool facilitates production of a single enantiomer of the product with the correct absolute stereochemistry.
While this synthesis is the gold standard for biotin production, it also falls short in the production of biotin precursors as it relies on the sulfur atom – already present in the starting material and carried to the end – the enforce stereoselectivity of later steps. The sulfur dependence of the Hoffman-La Roche synthesis makes it not suitable to the production of sulfur-free biotin precursors.

1.5 Substrate Analogs
Rationally designed analogs of the substrate of a particular enzyme can be useful in the elucidating the chemical mechanism of the enzyme itself. As an example, selective isotopic labeling of a substrate enables tracking the fate of specific atoms over the course of a reaction. Alternatively, if the breaking of a particular bond is suspected to be the rate-determining step of a reaction, and therefore corresponds to the highest energy transition state for the reaction, then labeling an atom in that bond with a heavy atom isotope may cause a noticeable change in reaction kinetics.\(^\text{12}\)

1.5.1 Possible Effects of Substrate Analogs on the Enzyme
Structural analogues may also be useful in elucidating mechanism. Carefully selected chemical modifications of a substrate at or near the active positions can either enhance or preclude reactivity of the substrate with either the enzyme or its cofactors.
**Inhibitors**

Isotopic substitution is a well-precedented method for not only tracking the fate of an atom through the course of a reaction, but also of altering the velocity of a reaction. Chemical bonds involving atoms with heavier isotopes have lower zero-point energies, which consequently makes them marginally harder to break. If a given bond-breaking step in a transformation is the overall rate-determining step for a reaction then making an isotopic substitution of one of the atoms in that bond may depress reaction kinetics compared to the analogous non-isotopically labeled version of that same reaction.\textsuperscript{13} In biotin synthase this effect would manifest itself by slowing the relative production of biotin from a deuterium-labelled substrate as compared to the normal nondeuterated analog. If another step in the mechanism – for example, the formation of the SAM radical – were the rate-determining step then an isotopic substitution at the active positions would have an insignificant effect on the reaction rate.

**Kinetic Isotope Effects**

The issue of measuring isotope effects can be complicated because the extent and level of isotopic substitution can further alter reaction kinetics beyond the simple rate retardation described previously. Heavier uncommon isotopes present near a reaction center but not participating in the reaction itself can contribute to a change in reaction rate, known as a secondary isotope effect.
Hyperconjugation of the non-reactive carbon-deuterium bonds at the reactive carbon can lower the energy of either a carbocation or a carbon radical transition state and thus affect overall reaction kinetics. In biotin synthase it is expected that isotopically substituting deuterium for hydrogen at either of the reactive carbon atoms would produce a secondary isotope effect if the enzyme does not select the deuterium atom for reaction, a primary isotope effect if deuterium is selected and a combination of the two if multiple deuterium atoms are present at the active positions. It is thus critical that we are able to control the degree of deuteration at the active positions within the substrate, and also that we can locate and quantitate any deuterium present within the final products.

1.6 Known Isotope Effects in SAM radical enzymes

Observable isotope effects have been observed for other enzymes within the SAM radical superfamily. Roach and coworkers showed the enzyme lipoil synthase (LipA) exhibits a large isotope effect in a substrate analog in which the hydrogen atoms at the active positions have been substituted with deuterium (Figure 1.6.1).

![Figure 1.6.1 Aliphatic Csp3-H bond replacement by sulfur via LipA enzyme.](image-url)
Although they did not quantitate this isotope effect, they did describe it as “large”. The chemical transformation achieved by BioB is remarkably similar to that of LipA so the observation of an isotope effect in the LipA examples suggests BioB may experience similar effects. Marquet and co-workers reported no observable isotope effect in BioB in vivo; however, they were not able to study the enzyme in vitro so were not able to determine whether extraneous factors in the biological milieu were influencing or masking the reaction kinetics.\(^\text{17}\)

### 1.7 Known Effects of Structural Analogs on Biotin Synthase

#### 1.7.1 Dethiobiotin Racemates

Structural analogs of dethiobiotin have been shown to have inhibitory effects on biotin synthase. Total synthesis has been employed by previous investigators to afford a family of dethiobiotin structural homologues, though no prior art exists that generates a single dethiobiotin enantiomer produced by synthesis to study biotin synthase in vivo. Parry and coworkers synthesized dethiobiotin synthetically from racemic starting materials and produced a single enantiomer of biotin by relying on the enzyme to select only the correct enantiomer of dethiobiotin to produce biotin after carrying a racemate through the entire pathway (Figure 1.7.1).\(^\text{18, 19}\)
While this method is effective to produce a substrate for biotin synthase, the possibility of competitive binding by the undesired enantiomer – and the inherent potential for affecting reaction rates – makes use of this method problematic in the quest to accurately quantitate rate constants and kinetic isotope effects. Interestingly, Parry noted there was little to no isotope effect observed when tritium in substituted into the active methyl groups using unpurified biotin synthase in crude cell extracts. The result stands in sharp contrast the large isotope effect observed by Roach in other radical SAM enzymes with chemistry similar to biotin synthase.
1.7.2 Regioisomeric Sulfur-containing Analogs

Alternatively, biotin precursors have been accessed synthetically by defunctionalizing biotin itself. In an effort to determine the regioselectivity of the biotin synthase reaction Marquet opened the tetrahydrothiophane ring of biotin to afford 6-mercapto and 9-mercaptodethiobiotin, and then individually fed both compounds to the enzyme in vivo to determine whether they could be transformed back into the natural product. Biotin precursors were produced according to the method described in Figure 1.7.2.

![Thiophene ring opening with C6 & C9 functionalization.](image)

**Figure 1.7.2** Thiophene ring opening with C6 & C9 functionalization.

The free carboxylic acid was esterified, then the thioether converted to a hemithioacetal and finally reductively cleaved via Raney nickel reduction, or alternatively the methyl ester can be treated with cyanogen bromide to open the ring in one step with differing functional groups at both active positions. While no isotopically labeled compounds were produced in this study, the chemistry is amenable to the production of a large number of synthetic variants by
substituting the orthogonal functional groups present on both active carbon atoms.

1.7.3 Partially labeled mixtures

A notable attempt was made by Marquet and coworkers to produce deuterium-labeled dethiobiotin analogs. Starting with biotin itself, Marquet replaced the hydrogen atoms by deuterium at the active positions by converting the thioether to a sulfoxide, followed by exchanging the adjacent protons for deuterons by soaking in sodium deuteroxide and D₂O for long periods of time then reducing the sulfur-carbon bonds to afford isotopically-labeled dethiobiotin (Figure 1.7.3).²¹

![Figure 1.7.3 Synthesis of d,d,d-dethiobiotin from biotin](image)

While the prospect of an 11 day reaction to exchange protons for deuterons does have a certain appeal to a graduate student living in Hawai‘i, the process proved to be inefficient for producing cleanly labeled substrates; all exchange reactions reported by Marquet produced mixtures of dethiobition analogs possessing
different degrees of deuteration at the active positions, ranging between completely perdeuterated down to a significant (7%) amount of substrate which still contained a single hydrogen at both active positions. The presence of these inseparable partially-labeled contaminants complicates the use of this method to produce labeled substrates to quantitate potential kinetic isotope effects.

1.8 Synthesis of earlier biotin precursors

While no enantioselective synthesis of dethiobiotin is known to us, there are examples of enantioselective syntheses of other biotin precursors. 7-Keto-8-aminopelargonic acid (KAPA), two steps back in the biotin biosynthesis from dethiobiotin, was recently produced in a four step synthesis from Boc-protected alanine (Figure 1.8.1).

![Figure 1.8.1 Synthesis of Boc-KAPA from alanine.](image)

The Weinreb amide of Boc-alanine was alkylated to form a β-ketophosphonate. A Horner-Wadsworth-Emmons reaction added the aliphatic side-chain, and subsequent hydrogenation to reduce the alkene and remove the protecting groups afforded KAPA. While this method involves relatively few steps and
proceeds from a readily available starting material, the end product still requires additional chemical manipulation to be useful for our purposes. Additionally, the use of the Horner-Wadsworth-Emmons reaction dictates that the carbon, which will become C6 (denoted in Figure 1.8.1 with an asterisk) in dethiobiotin, will be $sp^2$ hybridized thus limiting our ability to install a single deuterium with control of absolute stereochemistry at this position.

1.9 Thesis Objectives

While evidence exists that supports the presence of a kinetic isotope effect when the enzyme biotin synthase is exposed to dethiobiotin analogs with deuterium substituted at the reactive positions, no one has been able to quantitate these effects because there is no method in the literature to cleanly produce deuterated dethiobiotin analogs. This thesis describes our efforts to produce and refine a goal-oriented synthetic method, which will be useful for the production of a family of dethiobiotin analogs.
References


Chapter 2
Efforts Towards a Goal-Oriented Enantioselective Total Synthesis of Dethiobiotin

2.1 Introduction

Our initial efforts centered on a total synthesis of the natural product dethiobiotin that would allow for maximum versatility in the production of substrate analogs. As this was a goal-oriented synthesis the normal considerations that go into planning a synthetic route - such as employing the fewest number of steps or achieving the highest possible yields - were secondary considerations to a establishing a route which would allow for the facile production of a library of substrate analogs. These analogs would include isotopic labels at the active positions or unsaturation $\alpha$ to the active carbons.

Parry and coworkers had previously shown that biotin synthase selects for the pro-\textit{S} hydrogen at C6.\textsuperscript{1} As our goal was to accurately quantitate isotope effects at the reactive C6 and C9 positions, the ability to install a single deuterium at C6 with control of absolute stereochemistry was important. Parry had previously prepared (±)-dethiobiotin in which the C6 position was selectively singly tritiated (Figure 2.1.1), and we believed this method could serve as an effective starting point for our dethiobiotin synthesis.\textsuperscript{2} Our aim was to build on this work by labeling with deuterium stereospecifically in an enantioselective total synthesis.
2.2 Carbon chain synthesis

2.2.1 C6 Isotope Control

We began with the commercially available and relatively inexpensive ethyl-6-hydroxyhexanoate 1 as our starting material. The alcohol was oxidized to aldehyde 2 quantitatively via a Swern oxidation and purified by extraction with no chromatography necessary. The aldehyde was then protected as dioxolane 3 by refluxing with ethylene glycol and an acid catalyst in benzene (Figure 2.2.1). Despite numerous attempts to optimize this protection reaction the typical yields remained frustratingly low, (50-60%). This problem was exacerbated by a competing side reaction that consumed starting material irreversibly. Based on crude \(^1\text{H}\) NMR evidence it appears a competitive transesterification reaction involving the ethyl ester and ethylene glycol was taking place, and no conditions were found to suppress this side reaction.
Figure 2.2.1 Synthesis of biotin aliphatic chain precursor

At this point in the reaction scheme the degree and position of deuteration that will be at C6 in the final compound can be controlled. The atom that is currently the carbonyl carbon in the ester moiety in 3 will become C6 in dethiobiotin (Figure 2.2.2).

Figure 2.2.2 Tracking of ester carbon in 3 through the scheme.

To produce a non-deuterated or perdeuterated C6, either lithium aluminum hydride in THF or lithium aluminum deuteride in THF, respectively, can be used to reduce the ester to the corresponding alcohol in quantitative yield; to produce a monodeuterated C6 an additional two steps are needed. To obtain a substrate that will have a single deuterium in the pro-S position for which biotin synthase selects, the substrate will be oxidized to the aldehyde and then reduced enzymatically using horse liver alcohol dehydrogenase and NADH in D₂O to
afford a chiral primary alcohol (Figure 2.2.3). The deuteron in this substrate is correctly oriented to end the synthesis at the position with which biotin synthase reacts and thus possibly experience a primary kinetic isotope effect. For the opposite alcohol stereochemistry, the di-deuterated substrate will be oxidized to the aldehyde then reduced enzymatically in an analogous fashion using NADH in H_{2}O to afford the opposite alcohol stereoisomer which may be useful to study secondary kinetic isotope effects.

Figure 2.2.3 Selective deuterium incorporation at C6 precursor carbon.
2.2.2 C9 functionality

Alkylation

With the C6 deuterium now installed the oxidation state of this carbon atom cannot be changed, and the effect of any inversion of this atom must be accounted for when preparing singly deuterated analogs. With this in mind the alcohol was converted to the bromide using CBr$_4$ and PPh$_3$ in CH$_2$Cl$_2$ to afford 10 as a yellow oil in 88% yield after column chromatography. The bromide was displaced by lithio-ethylN,N,N-trimethylsilane - thus reinverting the C6 stereocenter - and the crude product was stirred in methanol with a suspension of excess potassium carbonate to afford, after column chromatography, terminal alkyne 11 as a colorless oil in 79% yield (Figure 2.2.4).

\[
\begin{align*}
\text{HO} & \quad \xrightarrow{\text{CBr}_4, \text{Ph}_3\text{P}} \quad \text{Et}_3\text{N} \quad \xrightarrow{\text{CH}_2\text{Cl}_2} \\
\text{Br} & \quad \xrightarrow{\text{TMS}, \text{Li}} \quad \xrightarrow{\text{K}_2\text{CO}_3, \text{MeOH}} \\
\text{H} & \quad \xrightarrow{} 
\end{align*}
\]

Figure 2.2.4 Synthesis of terminal octyne dethiobiotin precursor

Sharpless Asymmetric Dihydroxylation

Multiple routes were envisioned from octyne 11 to dethiobiotin, each with their own potential advantages and drawbacks. The conversion of a vicinal syn-diol to a vicinal syn-diazide with net inversion of stereochemistry has been reported in the literature, and this was viewed this as the shortest possible route from 11 to
dethiobiotin, installing two vicinal heteroatoms in a single step with control of absolute stereochemistry at C7 and C8. Octyne 11 was homologated by first deprotonating with nBuLi then alkylating with methyl iodide to afford alkyne 12 as a colorless oil after purification via flash chromatography. The alkyne was reduced to the cis-alkene 13 by treatment with hydrogen gas and Lindlar’s Catalyst in 93% total yield over two steps (Figure 2.2.5).  

![Figure 2.2.5](image_url)

**Figure 2.2.5** Asymmetric dihydroxylation precursor synthesis

cis-Disubstituted alkenes are notoriously poor substrates for Sharpless asymmetric dihydroxylation reactions, but the titillating possibility of a short synthesis required a thorough investigation. Alkene 13 was treated with AD-mix α to afford the diol 14 (65% yield, 28% ee by Mosher’s analysis) (Figure 2.2.6).

![Figure 2.2.6](image_url)

**Figure 2.2.6** Diol addition at C7 and C8.

Enantioselectivity for this reaction was lower than desired, although in-line with the expectation for the substrate. The low enantioselectivity of the
dihydroxylation strategy was unsuitable for our goals and therefore this route was discarded.

**Sharpless Asymmetric Epoxidation**

As described above, we had envisioned multiple routes from octyne 11 to dethiobiotin. After the failure of the dihydroxylation strategy we returned to octyne 11 to pursue a route that, while longer, we believed would circumvent the previous problems we had confronted. After deprotonation with nBuLi, the alkyne was homologated with paraformaldehyde in THF to afford the propargylic alcohol 15, which was reduced to the trans-allylic alcohol 16 with excess LiAlH₄ with an overall yield of 66% for two steps.⁸,⁹ A trans-allylic alcohol was selected as a synthetic intermediate so as to control absolute stereochemistry in a Sharpless asymmetric epoxidation reaction. We envisioned the epoxide functionality serving as the precursor to the dethiobiotin cyclic urea, making the success of this reaction key to the production of our target compounds as a single enantiomer. Asymmetric epoxidation of the substrate under standard conditions was successful, though yields were low with most reactions affording large quantities of returned starting material after chromatography (Figure 2.2.7).¹⁰
Figure 2.2.7 Introduction of epoxide at C7-C8 precursor atoms.

A screen of reaction conditions using varying reaction times, reagent bottles and excess equivalents of peroxide was not successful in improving yield of product, though in all cases unreacted starting material could be recovered and reused. Chiral HPLC indicated a 90:10 enantiomer ratio of the epoxide product (Figure 2.2.8).

Figure 2.2.8 Chiral HPLC trace of compound 17

2.3 Cyclic Urea

The epoxide was regioselectively cleaved with Ti(OiPr)$_4$ and TMS-N$_3$ in benzene in 65% yield after chromatography to introduce the first urea nitrogen and set the
stereochemistry at C7. 11 While we were able to produce the target azido diol 18 no unreacted starting material was recovered from this reaction. The yield, while modest, was in line with what is known for this type of reaction, so despite the shortcomings we viewed this reaction as serviceable. The presence of an alcohol at what will become C9 in dethiobiotin is advantageous as it allows the possibility of the introduction of multiple deuterons at an active position late in the synthesis. In light of these considerations it becomes necessary to preserve this alcohol functional group as long as possible. Exposure of the azido diol to a slight stoichiometric deficiency of TBS-Cl in DMF with catalytic DMAP afforded a singly protected species with no observed protection of the secondary alcohol (Figure 2.3.1). 12

![Chemical Structure](image)

**Figure 2.3.1** Epoxide opening with azide and alcohol protection.

The remaining alcohol at C8 has the incorrect absolute stereochemistry to allow direct inversion of the carbon by a nitrogen nucleophile, so the center must be inverted before displacement of the oxygen. The alcohol was esterified with monochloroacetic acid under Mitsunobu conditions in 38% overall yield after chromatography - typical for this substrate under the selected conditions. 13 No competing Staudinger product was observed. The choice of monochloroacetic acid was deliberate to avoid a potential problematic side reaction in the
The inverted alcohol was produced in 29% yield over two steps. With the center now inverted, treatment of the secondary alcohol with MsCl and Et$_3$N in CH$_2$Cl$_2$ at low temperature afforded the corresponding C8 tosylate (Figure 2.3.2).

Displacement of the C8 mesylate with sodium azide in either DMF or DMSO proved problematic due to low yields (35-40%) and the consumption of the tosylate starting material by a competing elimination reaction that became more prevalent as the temperature increased.$^{15}$ Attempts to prevent elimination by using a more active azide source at a lower temperature were similarly plagued by very low yields and consumption of the starting material due to a side reaction with the silicon to afford the unprotected diol, an epimer of precursor 18. The second azide installation concludes a series of five sequential steps in which four of five reactions had overall yields were no greater than 65%, or where reaction
conditions consumed unreacted starting material such that recycling of precursors was impossible, or both.

### 2.4 Discussion

The synthetic scheme suffered from many low yields at late steps. While it may have been possible with further study to address some of these problems, the chances of solving all of them by raising yields to a level sufficient to justify carrying though a large number of costly isotopically-labelled substrates seemed remote. In addition to the aforementioned issues some potentially problematic questions remained on the horizon with future steps, particularly the ability to maintain stereochemical integrity at C8 if the oxidation state of C9 is raised to allow deuterium introduction at that position. The ultimate goal of the synthesis of dethiobiotin is to function as a tool to produce numerous dethiobiotin analogs. Despite the large investment of time and resources in the route described above, we came to realize the problems could not be overcome, and that the production of a library of dethiobiotin analogs would not be practical using this strategy. All work on this route was abandoned at this point and the synthesis remains unfinished.
References:

Chapter 3

The Production of Dethiobiotin Analogs Derived from Biotin.

3.1 Introduction

In light of the difficulties associated with preparing a library of dethiobiotin analogs by employing total synthesis from a simple precursor, it might alternatively be possible to use biotin itself as a starting material where selective defunctionalization affords substrates of interest. Biotin is a costly starting material, but if the desired compounds can be obtained in an economical number of steps then this route may prove effective in affording many of the target molecules the total synthesis could not provide.

Andrée Marquet reported using biotin methyl ester as the starting material to produce a ring-opened analog by reaction with cyanogen bromide (Figure 3.1.1).¹ This afforded a biotin derivative with orthogonal functionality at the two active positions used by biotin synthase. Marquet would go on to produce 6-mercaptopdethiobiotin using a strategy that is potentially amenable to the production of both isotopically labelled dethiobiotin analogs as well as other structural analogs with varying functionality around the active positions.

Figure 3.1.1 Tetrahydrothiophene opening with cyanogen bromide.
In the same paper Marquet reported the production of a hemithioacetal analog of biotin produced via a Pummerer rearrangement of biotin sulfoxide methyl ester (Figure 3.1.2). The potential utility of this compound is obvious for the production of useful dethiobiotin analogs, most notably 9-hydroxydethiobiotin 1, a planned late-stage intermediate in our previously discarded total synthesis discussed in Chapter 2. Marquet was able to produce 9-hydroxybiotin from biotin in four steps.

![Reaction Scheme]

**Figure 3.1.2** Formation of 9-hydroxybiotin from biotin

Although this report appears to be a promising source, it cannot escape note that experimental detail is limited in this reference. Clearly this makes replication of the results an additional challenge.

Following the publication of Marquet’s seminal work a more detailed and thorough reexamination of the synthesis by Alice Ting and co-workers\(^2\) confirmed the synthesis of 9-hydroxybiotin methyl ester using Marquet’s listed reagents, though the yield was a modest 21% with no report of recovered starting material.
Despite the first literature report being less complete than might be desired, and the more clearly described subsequent report promising only moderate yields, these reports do suggest a shorter route to a class dethiobiotin analogs. The cost of biotin as a starting material is a concern, but the possibility of a short scheme with late stage deuterium introduction warranted thorough investigation.

3.2 Ring opening with cyanogen bromide

As noted above, it was reported that the tetrahydrothiophene functionality in biotin methyl ester can be opened regioselectively by reaction with cyanogen bromide; however, the report of this reaction contained no experimental details beyond the identity of the reagent. The principal investigator of this work has since retired from academic research and so we were not able to contact her for further details about the work described in this report. Given the lack of supporting information it was necessary to screen conditions to attempt to optimize this reported reaction. Biotin was converted to biotin methyl ester by reaction with anhydrous methanol and catalytic HCl, which afforded the product in 97% yield after extraction with saturated aqueous NaHCO$_3$ and removal of the solvent.$^2$ The ester was then exposed to cyanogen bromide under a variety of conditions to test for formation of the reported bromide and thiocyanate (Figure 3.2.1).
Figure 3.2.1 Attempted ring opening with cyanogen bromide

Despite repeated attempts to open the ring using a variety of solvents, temperatures and reaction times none of the expected product was produced, though \(N\)-formylation was observed in the case of refluxing formic acid (Figure 3.2.2). In all other cases TLC showed no other species present in the reaction media except and biotin methyl ester, which was recovered quantitatively.

Figure 3.2.2 Formylation of biotin methyl ester by solvent.

Attempts to functionalize the sulfur with acetyl chloride and acetyl bromide led to either no reaction or of decomposition of the starting material, respectively.

This methodology was plagued by the availability of neither experimental detail nor product characterization data in the original reference it was drawn from, a lack of other known examples of this reaction in the published literature and an inability to contact the authors for further information. In light of these difficulties
and the failure of other nucleophiles to open the tetrahydrothiophene ring this strategy was abandoned.

### 3.3 9-Hydroxybiotin methyl ester

A second compound of interest described in the same paper by Marquet as the cyanogen bromide reaction dismissed above was 9-hydroxybiotin methyl ester 2, produced by a Pummerer rearrangement and hydrolysis of biotin sulfoxide methyl ester. As was the case with the cyanogen bromide example, this reaction was also not supported by experimental detail or spectroscopic data so the results were treated with a slight degree of skepticism. These concerns were alleviated when another group led by Alice Ting reported an identical reaction along with full characterization data.²

Biotin methyl ester 2 was oxidized to the corresponding sulfoxide 5 with sodium periodate in quantitative yield. Pummerer rearrangement was induced by exposure to TFAA at low temperature in dichloromethane, with hydrolysis of the trifluoroacetate ester taking place on workup (Figure 3.3.1).
This reaction completely consumed the sulfoxide starting material and afforded two products derived from biotin: the expected 9-hydroxybiotin methyl ester 6 as a minor product (21% yield) as well as a major product that is a biotin-derived substrate 7 which has undergone elimination (59% yield). The major product appears to result from a Pummerer rearrangement taking place with oxidation at C6 in biotin, which affords a tertiary alcohol after ester hydrolysis (Figure 3.3.2). This tertiary alcohol 8 is adjacent to a thioether, which facilitates an elimination reaction to form 7.

**Figure 3.3.1** Pummerer rearrangement to from 9-hydroxy derivative.

**Figure 3.3.2** Elimination of tertiary alcohol to form major product.
The formation of multiple products appeared fortuitous as it opened several possibilities for access to some of the polyfunctional biotin derivatives originally envisioned from the failed cyanogen bromide experiments. Attempts to isolate alcohol 8, or regenerate it from the eliminated product proved unsuccessful. Less active Pummerer reagents such as acetic anhydride\textsuperscript{3} were ineffective in forming either rearrangement product, and all attempts at hydration of the alkene afforded only returned starting material (Figure 3.3.3).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.3.3}
\caption{Unsuccessful attempts to trap or regenerate 8.}
\end{figure}
The desulfurized analog of 7 (Figure 3.3.4) was also a compound of interest to us and so efforts were made to reduce the thioether while leaving the rest of the molecule intact. Raney nickel proved too active, performing both the desulfurization and hydrogenation of the alkene to afford dethiobiotin methyl ester 9 at room temperature with no isolable intermediate products. Efforts to modulate the reactivity of the catalyst by poisoning it with varying ratios of triethylamine did differentiate the speed of the competing reactions; however, it was observed that the hydrogenation reaction occurred faster than the desulfurization. Careful monitoring of the reduction reactions as they proceeded indicates the presence of unreacted starting material 7, biotin methyl ester 2 and dethiobiotin methyl ester 9 with no desulfurized alkene-containing product present (Figure 3.3.5). In light of the observed difference in reaction rates we abandoned attempts to isolate our target molecule.
Figure 3.3.5 Attempted desulfurization of 7

Also unsuccessful were attempts to ring open the hemithioacetal ring in 9-hydroxybiotin methyl ester 6 by trapping the aldehyde as the tosylhydrazone (Figure 3.3.6).

Figure 3.3.6 Attempted trapping of aldehyde from hemithioacetal.
While these failed attempts to preserve functionality in the substrate were undoubtedly troublesome, our original goal of attempting isotopic substitution at C9 was still feasible.

3.4 Deuterium incorporation at C9

9-Hydroxybiotin methyl ester 6 was desulfurized by Raney nickel reduction in ethanol/water to afford 9-hydroxydethiobiotin methyl ester 10 in yields ranging from 15-25%. A consistent yield was not achieved, nor was recovery of unreacted starting material, possibly due to the affinity of the substrate for the catalyst. Alcohol 10 was derivatized with tosyl chloride in dichloromethane to afford the corresponding tosylate 11 (Figure 3.4.1).

Figure 3.4.1 Tosylation of 9-hydroxydethiobiotin methyl ester.

Deuterium incorporation into this substrate proved problematic. LiAlD₄ is a candidate for displacement of the tosylate by a deuteron, but this reagent also presents compatibility problems with other functionalities in the molecule.⁴ The methyl ester group will undoubtedly be reduced by this reagent, though reoxidation to the carboxylic acid is possible. The cyclic urea group is also known to undergo reduction to the alkane, a significantly more problematic side
reaction. In light of these facts we chose to displace the tosylate using NaBD$_4$, a milder agent that was expected to leave all other functional groups untouched. Exposure of tosylate 11 to excess NaBD$_4$ in THF afforded returned starting material, even after refluxing for up to 14 h (Figure 3.4.2).

![Figure 3.4.2 Attempted deuterium substitution on primary tosylate.](image)

This observation buttresses an earlier result when a similar deuterium introduction failed on a tosylated derivative of Garner’s aldehyde (see: Appendix A). It is speculated that in both cases the steric bulk of the neighboring groups blocks backside attack by the approaching deuteron nucleophile, thus preventing insertion of deuterium.

### 3.5 Discussion

This approach to dethiobiotin analogs was hampered by the high cost of using biotin as a starting material, low yields which consume large quantities of this expensive starting material, questionable literature precedent, and unanticipated difficulties controlling side reactions which would have afforded many of the useful compounds that made this route attractive in the first place. These issues
combined to afford a methodology through which few useful compounds could potentially be produced, and in those that were produced, an inability to further chemically modify the functional groups persisted. In light of all these issues the efforts to pursue dethiobiotin derivatives from biotin itself was abandoned. It should not escape notice that this method was able to afford a substrate very similar to one of the targeted late stage molecules in the total synthesis discussed in Chapter 2: 9-hydroxydethiobiotin 1. The failure to introduce deuterium into this substrate indicates that had the total synthesis been seen through to completion, despite its shortcomings it would not have been a useful means to access some of the key dethiobiotin analogs that inspired the work initially, thus validating the decision to abandon that methodology *in toto*. 
References:


Chapter 4

Efforts towards the synthesis of KAPA, a dethiobiotin precursor

4.1 Introduction

The goal of this thesis was to employ organic synthesis to obviate the limitations of the biological system for the production of dethiobiotin analogs; however, to the extent that the biological system is a viable tool, it can be used in conjunction with total synthesis in a hybrid methodology for the production of the target compounds. The principal difficulties associated with using the biotin biosynthetic pathway to produce analogs result from the limitations of the enzymes BioW and BioF, which together transform pimelic acid and L-alanine to 7-keto-8-aminopelargonic acid (KAPA) (Figure 4.1.1).

Figure 4.1.1 Biotin biosynthetic pathway

BioW exerts its enzymatic action under buffer conditions that differ from the remainder of the pathway and so the product, pimeloyl CoA, must be purified by HPLC. BioF is highly selective for L-alanine as a substrate, limiting the scope of any enzymatic synthesis. The methyl side chain of alanine becomes C9 in dethiobiotin, and as a result this high selectivity precludes the use of BioF for the production of functionalized dethiobiotin derivatives. Moreover, BioF is not
amenable to prolonged storage and therefore must be produced, purified and used expeditiously each time it is needed.

The two remaining enzymes in the dethiobiotin pathway, BioA and BioD, are stable proteins that function under identical reaction conditions and appear to have enough space in their respective active sites to allow the uptake of slightly larger substrate analogs. These features make this series of enzymes a candidate for service as a catalytic system for producing dethiobiotin analogs of interest. In light of these facts the biosynthetic intermediate KAPA was viewed as a possible synthetic target for dethiobiotin analog production. In this strategy, chemistry may overcome the limits of biology, and biology may afford an advantageous method to obtain the final target molecules.

With KAPA as our new target our synthesis was redesigned drawing from the work of Aldrich and co-workers. The Aldrich method is attractive because it is relatively short, and it involves an inexpensive chiral starting material (L-alanine) from which the absolute stereochemistry of the final product is derived. Alanine is also readily available in various isotopically labeled pure forms. In addition the side chain of the amino acid does not play a role in proposed pathway, and so a variety of functionalized analogs can be accessed by employing alternate amino acids as a starting material.
4.2 Aldrich synthesis of KAPA

In order to verify that this method would be useful to produce a library of compounds from amino acid derivatives we chose to first confirm that the report could be successfully duplicated in our hands. Alanine 1 was protected at nitrogen with a $t$-butyl carbonate group by stirring the free amino acid with di-$t$-tert-butyl carbonate in aqueous THF (1:1 THF:water) to afford protected amine 2. The carboxylic acid group was then converted to the corresponding Weinreb amide 3 in 96% overall yield in a two step sequence (Figure 4.2.1) without the need for purification.  

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{O} \\
\text{CH}_3 & \quad \text{OH} \\
1 & \quad (\text{Boc})_2\text{O} \\
\text{THF, Et}_3\text{N} & \quad \text{BocHN} \quad \text{O} \\
\text{CH}_3 & \quad \text{OH} \\
2 & \quad \text{CDI, CH}_3\text{NHOCH}_3 \\
\text{CH}_2\text{Cl}_2 & \quad \text{BocHN} \quad \text{O} \\
\text{N} & \quad \text{OCH}_3 \\
3 & \quad \text{CH}_3 \\
\end{align*}
\]

**Figure 4.2.1** Synthesis of protected Weinreb amide from alanine.

The Weinreb amide was treated with isopropylmagnesium chloride and lithium methyl dimethylphosphonate to afford $\beta$-ketophosphonate 4, which owing to instability was carried on to the next step without purification. Phosphonate 4 was treated with excess NaH, then exposed to aldehyde 5 to afford enone 6 in 22% percent overall yield for two steps (Figure 4.2.2).
The aldehyde 5 proved to be problematic, presumably due to its poor stability on the bench. Aldehyde 5 was prepared in three steps from δ-valerolactone (Figure 4.2.3). Although prior reports suggest the esterification of the sodium carboxylate to the benzyl ester 8 proceeds in high yields, we found this reaction to be inefficient and proceeded with disappointingly low yield. Oxidation of the alcohol was achieved conclusively but once isolated the aldehyde 5 cyclizes at room temperature in a matter of hours to give a 1,3,5-trioxirane, necessitating storage of the aldehyde as a solution in benzene at −20°C.
In our hands, the Aldrich procedure as reported gave an overall yield of aldehyde 5 of 5% over three steps. While the saponification reaction to form carboxylate 7 was quantitative, yields for ester 8 were not greater than 25% under any set of conditions. The oxidation of 8 to 5 was similarly problematic owing to the aforementioned low bench stability of the aldehyde product regardless of oxidation procedure. The alkylation reaction to produce 6 calls for the aldehyde 5 to be used in excess, and the excess material could not be recovered and recycled at the end of the reaction. In addition, due to the strong base required to execute this alkylation reaction, epimerization of our material was observed by polarimetry. The combination of unserviceable yields and substrate instability observed in duplicating this work called into question its efficacy to produce our compounds of interest.

**4.3 Cross-metathesis based KAPA synthesis**

**4.3.1 Aldrich protection strategy**

In light of the difficulties encountered during this synthesis the route to KAPA was reimagined with an alternate method of joining the two separate moieties. The Weinreb amide 3 was prepared as described above starting from Boc-alanine and alkylated with excess allylmagnesium bromide to afford enone 9 as a colorless oil in 61% yield after column chromatography.4 4-Pentenoic acid was esterified with benzyl alcohol using dicyclohexylcarbodiimide and catalytic 4-
dimethylaminopyridine to afford ester 10 as a colorless oil in 95% yield (Figure 4.3.1).§

Figure 4.3.1 Synthesis of cross-metathesis precursors

Attempts to join enone 9 and ester 10 under the action of ruthenium-based catalysts proved unsuccessful. Homocoupling of 10 to form 11 was observed but neither 9 nor any related compounds, nor our product of interest were recovered from the crude reaction mixture. \(^1\)H NMR analysis of the crude reaction mixture afforded no evidence of the presence of 9 or any other tert-butyl containing species in significant quantity, suggesting decomposition of the substrate during the reaction. (Figure 4.3.2) The use of Lewis acid co-catalysts, either titanium (IV) isopropoxide or chlorocatecholborane,\(^6\) or of rigorously anhydrous conditions had no discernable effect on the reaction.

Figure 4.3.2 Attempted metathesis to form KAPA carbon chain.
4.3.2 Alternate protection strategies

A second-generation strategy was developed employing a more robust nitrogen protecting group, and shorter conjugated enone group to obviate the possibility of isomerization of the alkene into conjugation with the ketone. The carboxylic acid group in N-benzyloxycarbonyl alanine was converted to the corresponding Weinreb amide 12 in 96% yield and used without purification.\(^2\) The Weinreb amide 12 was alkylated with excess vinylmagnesium bromide to afford enone 13 as a colorless oil in 54% yield after chromatography.\(^7\) It was observed that excess nucleophile was necessary to afford 13 in useful quantities. 5-Hexenoic acid was esterified with benzyl alcohol using dicyclohexylcarbodiimide and catalytic 4-dimethylaminopyridine to afford ester 14 as a colorless oil in 93% yield (Figure 4.3.3.).\(^8\)

![Chemical Reaction](attachment:reaction_image)

**Figure 4.3.3** Synthesis of cross metathesis substrates.

Enone 13 and ester 14 were joined under the action of the 2\(^{nd}\) generation Hoveyda-Grubbs catalyst (15 mol %) in 14 h (Figure 4.3.4). Mass spectrometry
indicated formation of 15 but yields were too low to be serviceable for later synthesis, as 15 could not be isolated by column chromatography.

![Chemical structure](image)

**Figure 4.3.4** Enone formation by cross metathesis

In an effort to drive the reaction further towards completion the reaction was repeated under neat conditions, at reduced pressure (50 torr) and at 40°C. Homocoupling of ester 14 was again observed, though no traces of the starting enone 13 or coupled enone 15 were visible in the crude ¹H NMR spectrum. The observations are consistent with the results of the attempted coupling of 9 and 10 as described in Section 4.3.1 for the more labile Boc nitrogen protecting group under milder reaction conditions.

In light of the observed instability of the Cbz- group to metathesis conditions a new substrate was prepared where alanine was protected at nitrogen with a 9-fluorenylmethyloxycarbonyl group. N-Fmoc alanine was converted to the Weinreb amide 16 under analogous conditions as the Weinreb amides described above affording a highly crystalline solid in 94% yield (Figure 4.3.5).² Attempts to
alkylate amide 16 under standard conditions for this system\textsuperscript{7} were unsuccessful owing the insolubility of 16 in THF.

\[
\begin{align*}
\text{FmocHN} & \quad \text{CDI, CH\textsubscript{3}NHOCH\textsubscript{3}} \\
\hat{\text{C}}\text{H}_3 & \quad \text{THF, 0°C to rt} \\
\text{FmocHN} & \quad \hat{\text{C}}\text{H}_3 \\
\hat{\text{C}}\text{H}_3 & \quad \hat{\text{C}}\text{H}_3 \\
\end{align*}
\]

Figure 4.3.5 Synthesis of N-Fmoc protected enone.

4.4 Discussion

Duplication of the Aldrich method for the production of KAPA appears unsatisfactory owing to yields, bench instability of substrates and the lack of stereochemical integrity of the products. An alternative cross metathesis-based strategy has not yet been proven effective, though preliminary evidence indicates this method does afford some amount of the compounds of interest, albeit in unserviceable yields. Optimization of the system remains a possibility. Work in this area remains ongoing.
References:

1) Unpublished results


Conclusions

Despite numerous attempts involving a variety of approaches a clear method for the production of a target library of dethiobiotin analogs remains out of reach. Total synthesis affords the most control over the substrate, including the quantity and positions of desirable isotope labels but yields for the method described in Chapter 2 and the number of steps involved combine to make this strategy unsuitable for the production of a large number of dethiobiotin analogs. Rather than suffering due to the failure of a single step, a series of steps combined to make this approach untenable. While the substrate control afforded by this approach was appealing, the shortfalls in the chemistry cannot be overcome.

Biotin defunctionalization ultimately suffered from the limited scope of materials that were accessible from this starting material. Difficulties duplicating literature procedures almost immediately prevented access to many of the potentially useful biotin derivatives that initially drew us to this method. Those procedures which could be duplicated afforded derivatives that were of limited utility in the production of a library of compounds. As an aside, the reported biological activities of the dethiobiotin derivatives generated in the prior report by Marquet may be viewed as suspect.

An attempted synthesis of KAPA remains incomplete but future refinement may be possible to make this a viable route to access compounds of interest. Despite
apparent decomposition under some conditions, preliminary evidence suggests it may be possible to form selected compounds, which could serve as the basis for a library of molecular probes. The synthetic route requires relatively few steps with serviceable yields until the non-optimized cross metathesis reaction. Additional efforts at optimization may resolve this problem, affording a substrate that requires only alkene reduction and deprotection to generate synthetic KAPA.
Appendices
Appendix

Monodeuteroalanine from a Garner’s aldehyde

L-alanine-3-$d$ 1 was a molecule of interest, but its synthesis has not been described and it is not commercially available. To access this compound we envisioned a synthetic method employing the known route to Garner’s aldehyde, which would afford us a deuterated alanine precursor. The commercially available D-serine methyl ester was protected at nitrogen with a tert-butyloxycarbonyl group 2. The alcohol and amino groups were then bridged with 2,2-dimethoxypropane to form the dimethyloxazolidine 3, and the ester functionality was reduced to the alcohol by excess lithium aluminum hydride to afford 4. (Figure A.2)

Tosyl chloride converted the alcohol 4 to the corresponding tosylate 5. Displacement of this tosylate by action of a deuteride nucleophile proved difficult.

Figure A.2 Synthesis of Garner’s aldehyde precursor from serine.
Initial attempts to reduce the tosylate with lithium aluminum deuteride at both 0°C and at room temperature afforded only returned starting material 5, though model system studies indicated the lithium aluminium deuteride used in the reaction was active and could displace a primary tosylate at 0°C (Figure A.3). Heating the sample to reflux for 3 h resulted in loss of the tert-butyloxy carbonyl group but the tosylate remained undisturbed.

**Figure A.3** Attempted deuterium substitution of primary alcohol.

We hypothesized the inability to displace the tosylate might result from decreased reactivity of deuteride relative to hydride and therefore altered our scheme to allow deuteride introduction to a more active functional group and to use of a more active nucleophile to displace the tosylate. Returning to alcohol 4 we completed the classic Garner’s aldehyde scheme by oxidizing the alcohol to the aldehyde 7. Aldehyde 7 was reduced by sodium borodeuteride to afford deuterium-containing alcohol 8 (Figure A.4).
Figure A.4 Deuteride reduction of Garner's aldehyde.

Tosyl chloride converted alcohol 8 to the corresponding tosylate 9. As was observed in our earlier efforts to displace tosylate 5 with a deuteride nucleophile, all attempts to substitute tosylate 9 with hydride were ineffective. At 0°C and room temperature the tosylate group persisted, and the nitrogen protecting group was lost without the tosylate being disturbed at reflux (Figure A.5). In light of these difficulties the use of a Garner’s aldehyde-based strategy to access monodeuteroalanine was abandoned.

Figure A.5 Attempted hydride substitution of primary alcohol
References

Experimental

Chapter 2

\[
\text{EtO} \quad \text{EtO} \quad \text{EtO} \quad \text{CH} \quad \text{Cl} \quad \text{2}
\]

To a solution of oxalyl chloride (0.64 mL, 7.49 mmol) under an atmosphere of dry nitrogen gas in 20 mL of anhydrous dichloromethane at -78°C was slowly added dimethylsulfoxide (0.62 mL, 8.74 mmol). After 15 minutes a solution of ethyl-6-hydroxyhexanoate 2.1 (1.0 g, 6.24 mmol) in anhydrous dichloromethane (10 mL) was added slowly in order to maintain the temperature. The reaction mixture was stirred for an additional 30 minutes, then triethylamine (2.6 mL, 18.7 mmol) was added and the flask was allowed to warm to room temperature over 30 minutes then quenched with H₂O. Dichloromethane was removed under reduced pressure, then the residue was diluted with a 2:1 mixture of hexane and ethyl acetate. The resulting biphasic material was extracted sequentially with 1M HCl, a saturated aqueous NaHCO₃ solution and brine then dried over MgSO₄. Removal of the solvent under reduced pressure afforded the ethyl-6-oxyhexanoate 2.2 (0.98 g, 6.20 mmol, 99% yield) as a colorless oil which was used without further purification.
To aldehyde 2.2 (0.98 g, 6.20 mmol) in benzene (30 mL) was added ethylene glycol (1.1 mL, 15.9 mmol) and p-toluenesulfonic acid monohydrate (0.06 g, 0.03 mmol). The flask was fitted with a Dean-Stark apparatus and heated to reflux for 12 h. The solution was extracted with aqueous NaHCO₃, dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified via flash chromatography using a gradient elution of 10%, 20% and 40% ethyl acetate in hexane affording the protected dioxolane 2.3 (0.83 g, 3.8 mmol) as a colorless oil in 63% yield.

Dioxolane 2.3 (0.50 g, 2.27 mmol) was diluted in anhydrous tetrahydrofuran (20 mL) and cooled to 0°C under an atmosphere of dry nitrogen gas. LiAlH₄ (0.09 g, 2.40 mmol) was added portionwise and the reaction was stirred for an additional 1 hr. The reaction was quenched with sequential addition of 2.0 mL water, 2.0 mL 3M NaOH and 6.0 mL water. The mixture was then warmed to room temperature, dried over anhydrous MgSO₄ for 15 minutes and then filtered to
remove the solids. The solvent was removed under reduced pressure to afford alcohol 2.4 (0.36 g, 2.27 mmol) as a colorless oil in quantitative yield that was used without further purification.

Alcohol 2.4 (0.43 g, 2.68 mmol) was diluted in 20 mL of anhydrous dichloromethane and cooled to 0°C. Anhydrous triethylamine (1.1 mL, 7.90 mL), carbon tetrabromide (1.34 g, 4.04 mmol) and triphenylphosphine (1.04 g, 3.97 mmol) were added sequentially and the reaction was stirred for 12 h. The reaction was quenched with 5 mL H₂O. Dichloromethane was removed under reduced pressure, the residue was redissolved in a 2:1 mixture of hexanes and ethyl acetate, and extracted sequentially with 1M HCl and brine, dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified via flash chromatography using a gradient elution of 0%, 5%, 10% and 20% ethyl acetate in hexanes to afford the purified bromide 2.10 (0.53 g, 2.36 mmol) as a pale yellow oil in 88% yield.
Ethynyltrimethylsilane (0.42 mL, 2.97 mmol) was dissolved in 20 mL of a 10:1 mixture of tetrahydrofuran and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone under an atmosphere of dry nitrogen gas and cooled to -78°C. A 1.9 M solution of nBuLi (1.4 mL, 2.66 mmol) was slowly added and the mixture was allowed to stir for 1 h. A solution of bromide 2.10 (0.53 g, 2.36 mmol) in 10 mL tetrahydrofuran was added dropwise and the reaction was allowed to slowly warm to room temperature and stirred for 12 h. The solvent was removed under reduced pressure to afford a brown residue, which was washed into an Erlenmeyer flask with 40 mL methyl alcohol. Potassium carbonate (5.0 g, 36.2 mmol) was added and the resulting suspension was stirred for 24 h. The solvent was removed under reduced pressure and the crude product was purified via flash chromatography using a gradient elution of 5%, 10% and 20% ethyl acetate in hexanes to afford alkyne 2.11 as a colorless oil in 79% yield (0.31 g).

Alkyne 2.11 (0.31 g, 1.86 mmol) was dissolved in anhydrous tetrahydrofuran (10 mL) under an atmosphere of dry nitrogen gas and cooled to -78°C. A 1.9 M
solution of \( n\)-BuLi (1.08 mL, 2.05 mmol) was added and the mixture was stirred for 30 min. Methyl iodide (0.15 mL, 2.41 mmol) was added and the solution was allowed to warm to 0°C over 1 h. The reaction was quenched with sat. aqueous \( \text{NH}_4\text{Cl} \), dissolved in 60 mL 2:1 hexane : ethyl acetate, washed with brine, dried over anhydrous MgSO\(_4\), and concentrated en vacuo. The resulting orange residue was purified via flash chromatography using a gradient elution of 10% to 20% ethyl acetate in hexane to afford alkyne \textbf{2.12} as a colorless oil in 95% yield (0.33 g, 1.75 mmol).

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{O} \\
\text{H}_3\text{C} & \quad \text{O} \\
\text{H}_3\text{C} & \quad \text{O} \\
\text{H}_3\text{C} & \quad \text{O} \\
\text{H}_3\text{C} & \quad \text{O} \\
\end{align*}
\]

Alkyne \textbf{2.12} (0.2 g, 1.10 mmol) was dissolved in 15 mL hexanes in a round bottomed flask equipped with a magnetic stir bar. Lindlar’s Catalyst (0.08 g) and quinoline (0.013 g, 0.10 mmol) were added, the flask was fitted with a rubber septum and quickly evacuated en vacuo. Following removal of the atmosphere, the flask was fitted with a double balloon containing hydrogen gas and stirred for 12 h. The resulting liquid was filtered through Celite, concentrated en vacuo and purified via flash chromatography using a gradient elution of 10% to 20% ethyl acetate in hexane to afford \textit{cis}-alkene \textbf{2.13} as a colorless oil in 98% yield (0.198 g, 1.07 mmol).
Alkene 2.13 (0.1 g, 0.54 mmol) was dissolved in 10 mL of a 1:1 mixture of tert-butanol and water. Methylsulfonamide (0.058 g, 0.61 mmol) and AD-mix α (0.142 g) were added and the mixture was stirred at 0°C for 6 hours. The reaction was quenched with aqueous sodium sulfite, diluted with ethyl acetate and the layers were separated. The organic layer was washed with brine, concentrated en vacuo and purified via flash column chromatography using a gradient elution of 20%, 40% and 60% ethyl acetate in hexanes to afford diol 2.14 in 65% yield (0.077 g, 0.35 mmol). Mosher’s acid analysis indicated the produce e.r. of approximately 2:1

Alkyne 2.11 (0.24 g, 1.43 mmol) was dissolved in 15 mL of anhydrous tetrahydrofuran under an atmosphere of dry nitrogen and cooled to -78°C. A 1.9 M solution of nBuLi (0.90 mL, 1.71 mmol) was added dropwise and the reaction was stirred for 1 h. A suspension of paraformaldehyde (0.2 g, 4.5 equivalents) in anhydrous tetrahydrofuran was canulated into the flask and the resulting suspension was stirred for 24 h. The reaction was quenched an aqueous
ammonium chloride, dried over anhydrous MgSO$_4$ and the solvent was removed under reduced pressure. The residue was purified via flash chromatography using a gradient elution of 10%, 20% and 40% ethyl acetate in hexanes to afford propargylic alcohol 2.15 as a colorless oil in 74% yield (0.21 g, 1.06 mmol).

Propargylic alcohol 2.15 (0.10 g, 1.0 mmol) was dissolved in 10 mL of anhydrous tetrahydrofuran under an atmosphere of dry nitrogen and cooled to 0°C. Lithium aluminum hydride (0.078 g, 2.1 mmol) was added portionwise over 30 minutes, then the reaction was slowly warmed to room temperature. The reaction was then heated to reflux for 12 hours, cooled back to 0°C and then quenched with sequential addition of 0.5 mL water, 0.5 mL 3M NaOH and 1.5 mL water. The resulting suspension was filtered through Celite, dried over anhydrous MgSO$_4$, filtered and concentrated under reduced pressure to afford allylic alcohol 2.16 as a colorless oil in 89% yield (0.090 g, 0.89 mmol).
A dry three-neck flask was charged with 500 mg of freshly activated 4Å molecular sieves and 20 mL anhydrous dichloromethane, and cooled to -20°C. L-(+)-tartrate (0.75 mL, 4.38 mmol) and Ti(OiPr)$_4$ (0.73 mL, 2.46 mmol) were added sequentially. tert-butylhydroperoxide (3.1 mL, 5 M in decane, 15.5 mmol) was added dropwise and the reaction was stirred for 30 minutes. A solution of allylic alcohol 2.16 (0.52 g, 2.60 mmol) in anhydrous dichloromethane was then added dropwise over 30 minutes to maintain temperature. The reaction was stirred for 2 h, quenched with 3M NaOH, allowed to warm to room temperature over 1 h then filtered through a Celite pad. The filter was washed with ethyl acetate (50 mL) and the combined filtrate was dried over anhydrous MgSO$_4$. The solvents were removed under reduced pressure and the crude product was purified via flash chromatography using a gradient elution of 20%, 40% and 60% ethyl acetate in hexanes to afford epoxide 2.17 as a colorless oil in 19% yield (0.085 g, 0.39 mmol, 69% yield BRSM).
A round bottom flask was charged with 5.0 mL dry benzene, Ti(OiPr)$_4$ (0.41 mL, 1.39 mmol) and Me$_3$SiN$_3$ (0.37 mL, 2.77 mmol) under an atmosphere of dry nitrogen. The mixture was heated to reflux for 6 h until the solution became clear. A solution of epoxide 2.17 (0.25 g, 1.16 mmol) in dry benzene (2.0 mL) was added to the hot mixture and was stirred for an additional 15 min. The flask was then cooled to room temperature and the solvent was removed under reduced pressure. The yellow residue was resuspended in diethyl ether and quenched with 1M H$_2$SO$_4$. The layers were separated, the aqueous layer was back-extracted with dichloromethane. The organic phases were combined, dried over anhydrous MgSO$_4$, concentrated under reduced pressure and purified via flash chromatography using a gradient elution of 30%, 60% and 80% ethyl acetate in hexanes to afford azido diol 2.18 (0.186 g, 0.72 mmol) in 62% yield.

Azido diol 2.18 (0.20 g, 0.77 mmol) and 4-Dimethylaminopyridine (0.010 g, 0.08 mmol) were dissolved in 4.0 mL anhydrous dichloromethane at 0°C. Triethylamine (0.22 mL, 1.6 mmol) and tert-butyldimethylsilyl chloride (0.11 g,
0.73 mmol) were added sequentially and the solution was allowed to warm to room temperature and stirred for 12 h. The solution was diluted with 20 mL of a 3:1 mixture of hexanes and ethyl acetate, extracted with aqueous NaHCO₃, dried over anhydrous MgSO₄, concentrated en vacuo and purified via flash chromatography using a gradient elution of 5%, 10% and 20% ethyl acetate in hexanes to afford the monoprotected silyl ether 2.19 in 98% yield (0.267 g, 0.715 mmol).

A mixture of alcohol 2.19 (0.25 g, 0.67 mmol), monochloroacetic acid (0.079, 0.84 mmol) and PPh₃ (0.279 g, 1.07 mmol) in 2.0 mL anhydrous tetrahydrofuran was cooled to 0°C. DIAD (0.20 mL ,1.0 mmol) was slowly added dropwise and the reaction was slowly allowed to warm to room temperature over 14 h. The solvent was removed under reduced pressure and the residual crude product was purified via flash chromatography to afford the monochloroacetate ester in 38% yield (0.115 g, 0.255 mmol 81% BRSM).
Monochloroacetate (0.10 g, 0.222 mmol) was dissolved in 2.0 mL aqueous ethanol. Thiourea (0.21 g, 2.76 mmol) and sodium bicarbonate (0.17 g, 2.02 mmol) were added and the resulting suspension was stirred at room temperature for 16 h. The reaction mixture was diluted with a 3:1 mixture of hexanes and ethyl acetate (10 mL) and extracted with water and brine. The organic layer was dried over anhydrous MgSO₄, concentrated and purified via flash chromatography to afford alcohol 2.20 76% in yield (0.063 g, 0.169 mmol).

\[ ^{1}H \text{ NMR (CDCl}_3, 300 \text{ MHz): } \delta 4.84 \text{ (t, 1H), } 3.8-4.0 \text{ (m, 4H), } 3.29 \text{ (m, 1H), } 2.32 \text{ (m, 1H), } 1.3-1.7 \text{ (m, 10H), } 0.89 \text{ (s, 9H), } 0.08 \text{ (s, 6H)} \]

Alcohol 2.20 (0.11 g, 0.294 mmol) was dissolved in anhydrous dichloromethane (4.0 mL) and cooled to 0°C. Triethylamine (0.13 mL, 0.93 mmol) was added, followed by a dropwise addition of a 0.4 M solution of mesyl chloride (0.84 mL, 0.336 mmol) in anhydrous dichloromethane, then was slowly warmed to room temperature over 14 h, after which the reaction was quenched with 3 mL
deionized water. The solvent was removed under reduced pressure, the residue was resuspended in a 3:1 mixture of hexanes and ethyl acetate and washed sequentially with water and brine. The organic phase was then separated, dried over anhydrous MgSO₄ and purified via flash chromatography to afford the mesylate in 90% yield (0.12 g, 0.265 mmol).

¹H NMR (CDCl₃, 300 MHz): δ 4.93 (t, 1H), 4.60 (q, 1H), 3.92 (m, 2H) 3.84 (m, 4H), 3.55 (m, 1H), 3.04 (s, 3H), 1.32-1.72 (m, 10H), 0.94 (s, 9H), 0.10 (s, 6H)

The mesylate (0.05 g, 0.11 mmol) was dissolved in anhydrous DMSO (4.0 mL). A suspension of sodium azide (0.062 g, 0.95 mmol) in DMSO was added via syringe transfer and the solution was heated at 80°C with constant stirring for 16 h. The solution was cooled, diluted with Et₂O (10 mL) and transferred to a separatory funnel with 5 mL water. The layers were separated, the organic layer was extracted twice with 5 mL water, then the combined aqueous washes were back extracted with 10 mL Et₂O. The combined ethereal phases were dried over MgSO₄, filtered, concentrated under reduced pressure and loaded onto a silica column packed with 10% EtOAc in hexanes. Gradient elution with 20% EtOAc afforded the diazide 2.21 in 40% yield (0.017 g, 0.044 mmol).
The mesylate (0.05 g, 0.11 mmol) was dissolved in anhydrous tetrahydrofuran (4.0 mL). Azidotrimethylsilane (25 µL, 0.19 mmol) was added at room temperature, followed by a suspension of cesium fluoride (0.02 g, 0.13 mmol) in anhydrous tetrahydrofuran (1.5 mL). The solution was heated to 40°C and stirred for 4 h. After cooling the reaction mixture was diluted with 15 mL EtOAc and washed with water. The organic layer was separated, dried over MgSO₄, filtered and concentrated under reduced pressure. ¹H NMR indicated only a small amount of 2.21 relative to undesired side products so purification was no attempted.
Anhydrous methanol (10 mL) was cooled to 0°C under an atmosphere of dry nitrogen gas and acetyl chloride (1.1 mL, 12.7 mmol) was added dropwise over 5 minutes. This solution was cannulated into a suspension of biotin (0.75 g, 3.1 mmol) in anhydrous methanol (5 mL). The suspension was stirred at room temperature for three hours, resulting in a colorless solution. This solution was concentrated in vacuo, diluted in 25 mL 95:5 dichloromethane : methanol and extracted with a saturated aqueous solution of sodium bicarbonate. The layers were separated, the aqueous phase was washed with 10 mL 95:5 dichloromethane : methanol, the organic layers were combined, dried over MgSO₄ and concentrated under reduced pressure to afford biotin methyl ester \textbf{3.2} in 98% yield (0.775 g, 3.0 mmol)

\(^1\)H NMR (CDCl₃, 300 MHz): \(\delta\) 5.58 (bs, 1H), 5.18 (bs, 1H), 4.49 (m, 1H), 4.28 (m, 1H), 3.63 (s, 3H), 3.13 (m, 1H), 2.90 (dd, 1H), 2.70 (dd, 1H), 2.32 (t, 2H) 1.60-1.74 (m, 4H), 1.37-1.48 (m, 2H)
Biotin methyl ester 3.2 (0.2 g, 0.77 mmol) was diluted in anhydrous ethanol (8 mL). A solution of cyanogen bromide (0.12 g, 1.1 mmol) in anhydrous ethanol (2 mL) was syringe transferred into the flask and the reaction was heated to reflux for 24 h. TLC showed no change in reaction composition and on cooling and concentration of the crude mixture, $^1$H NMR showed only 3.2 to be the only species present. The starting material was recovered quantitatively.

Biotin methyl ester 3.2 (0.2 g, 0.77 mmol) was dissolved in 90% formic acid (8 mL). A solution of cyanogen bromide (0.12 g, 1.1 mmol) in formic acid (2 mL) was added to the flask and the reaction was heated to reflux for 16 h. The reaction mixture was diluted with 30 mL dichloromethane and the layers were separated. The acid was washed with dichloromethane (2 x 15 mL), then the combined organic layers were washed twice with saturated aqueous sodium bicarbonate (2 x 30 mL). The organic layer was dried over anhydrous MgSO$_4$, 

75
filtered and concentrated under reduced pressure to afford \(N\)-formyl biotin methyl ester \(3.4\) in quantitative yield (0.22 g, 0.77 mmol).

To a suspension of biotin methyl ester \(3.2\) (0.25 g, 0.97 mmol) in methanol (15 mL) at room temperature was a solution of sodium periodate (0.22 g, 1.02 mmol) in water (2 mL), and the resulting solution was vigorously stirred for 16 h. The resulting suspension was filtered and the liquid was concentrated under reduced pressure. The residue was redissolved in 30 mL of a 95:5 dichloromethane/methanol solution. This solution was dried over anhydrous MgSO\(_4\), filtered and concentrated \textit{en vacuo} to afford biotin sulfoxide methyl ester \(3.5\) in 93% yield (0.247 g, 0.90 mmol). No additional purification was necessary. 

\(^1\)H NMR (CDCl\(_3\), 300 MHz): \(\delta\) 5.58 (bs, 1H), 5.21 (bs, 1H), 4.72 (m, 1H), 4.67 (m, 1H), 3.65 (s, 3H), 3.30 (dd, 1H), 3.06 (m, 2H), 2.36 (t, 2H), 1.52-1.76 (m, 6H)
A suspension of biotin sulfoxide methyl ester 3.5 (0.3 g, 1.1 mmol) in chloroform (10 mL) was cooled to -60°C. Trifluoroacetic anhydride (0.5 mL, 3.6 mmol) was added dropwise and the resulting solution was allowed to slowly warm to room temperature over 30 minutes. The solvent was evaporated and the residue was redissolved in a 1:1 mixture of tetrahydrofuran and water (15 mL) for 14 h. The organic solvent was removed en vacuo and the resulting water solution was diluted with saturated aqueous sodium bicarbonate (10 mL) and a 95:5 mixture of dichloromethane/methanol (20 mL). The layers were separated, the aqueous phase was washed twice with 10 mL 95:5 dichloromethane/methanol, the combined organic layers were dried over anhydrous MgSO₄, filtered, concentrated en vacuo and purified by column chromatography on silica gel using a gradient elution of 4%-10% methanol in dichloromethane to afford 3.6 in 19% yield (0.058 g, 0.21 mmol) and 3.7 in 65% yield (0.182 g, 0.71 mmol).

3.6

^1^H NMR (CDCl₃, 300 MHz): δ 5.92 (bs, 1H), 5.68 (bs, 1H), 5.11 (s, 1H), 4.36-4.48 (m, 2H), 3.74 (m, 1H), 3.64 (s, 3H), 3.55 (bs, 1H), 3.45 (d, 1H), 2.32 (t, 2H), 1.61-1.73 (m, 4H), 1.41 (p, 2H)

^1^3^C NMR (CDCl₃, 75 MHz): δ 174.4, 163.5, 86.9, 68.6, 61.9, 52.9, 51.7, 33.7, 28.3, 28.2, 24.7

3.7
$^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 5.53 (t, 1H), 5.44 (bs, 1H), 5.23 (bs, 1H), 4.64 (d, 1H), 4.47 (m, 1H), 3.64 (s, 3H), 3.21 (dd, 1H), 3.00 (dd, 1H), 2.31 (t, 2H), 2.12 (m, 2H), 1.62-1.78 (m, 4H)

Alkene 3.7 (0.05 g, 0.2 mmol) was dissolved in 95% ethanol in water (4 mL). A 50% slurry of Raney nickel in water (0.25 mL) was added and the system was heated to reflux for 1 h. After cooling the heterogeneous mixture was filtered through a pad of silica gel and flushed with absolute ethanol (50 mL). The filtrate was evaporated to dryness en vacuo, redissolved in dichloromethane, dried over anhydrous MgSO$_4$, filtered, concentrated and purified on silica gel via flash column chromatography using a gradient elution from 5%-15% methanol in dichloromethane to afford dethiobiotin methyl ester 3.9 in 18% yield (0.008 g, 0.035 mmol).
To a 50% slurry of Raney nickel in water (0.25 mL) was added 4 mL 95% ethanol and triethylamine (0.1 mL, 0.7 mmol). The mixture was stirred for 30 minutes and a solution of alkene \(3.7\) (0.05 g, 0.2 mmol) was added with constant stirring. The mixture was heated to 50°C for 2 h, cooled to room temperature and filtered through a pad of silica gel. The pad was washed with absolute ethanol and the filtrate was concentrated \textit{en vacuo}. The residue was subjected to ESI mass spectrometry revealing the presence of unreacted starting material in addition to dethiobiotin methyl ester \(3.9\) and biotin methyl ester \(3.2\). No further isolation or characterization was performed.

HR ESI+ Mass Spec. calculated for C\(_{11}\)H\(_{19}\)N\(_2\)O\(_3\)S: 259.1116, found 259.1197 (31.3 ppm error)

9-Hydroxybiotin methyl ester \(3.6\) (0.1 g, 0.36 mmol) was dissolved in 95% ethanol in water (5 mL). A 50% slurry of Raney nickel in water (0.25 mL) was added and the system was heated to reflux for 1 h. After cooling the heterogeneous mixture was filtered through a pad of silica gel and flushed with absolute ethanol (50 mL). The filtrate was evaporated to dryness \textit{en vacuo}, redissolved in dichloromethane, dried over anhydrous MgSO\(_4\), filtered,
concentrated and purified on silica gel via flash column chromatography using a gradient elution from 5%-15% methanol in dichloromethane to afford 9-hydroxydethiobiotin methyl ester 3.10 in 24% yield (0.021 g, 0.086 mmol).

Alcohol 3.10 (0.05 g, 0.2 mmol) was dissolved in anhydrous dichloromethane (8 mL) under an atmosphere of dry nitrogen gas and triethylamine (0.1 mL, 0.7 mmol) was added via syringe transfer. The solution was cooled to 0°C and a solution of tosyl chloride (0.046 g, 0.24 mmol) in anhydrous dichloromethane (2 mL) was added via cannulation. The solution was stirred for 3 h, quenched with 2 mL water and then concentrated en vacuo. The residue was redissolved in a 3:1 mixture of hexane/ethyl acetate (15 mL) and washed sequentially with saturated aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous MgSO₄, filtered, concentrated and purified on silica gel using a gradient elution from 10% to 30% ethyl acetate in hexanes to afford tosylate 3.11 in 93% yield (0.076 g, 0.19 mmol).
Tosylate 3.11 (0.1 g, 0.25 mmol) was dissolved in anhydrous dimethyl sulfoxide (4 mL) under an atmosphere of dry nitrogen gas. A solution of sodium borodeuteride (0.035 g, 0.84 mmol) in anhydrous dimethyl sulfoxide (2 mL) was added via cannulation and the system was heated to reflux for 14 h. The flask was cooled to room temperature, excess borodeuteride was quenched with 2 mL water and the solution was diluted with diethyl ether (12 mL). The organic layer was washed twice with water, dried over anhydrous MgSO₄, filtered and concentrated en vacuo. ¹H NMR of the residue indicated 3.11 was the only species present with no evidence of the target. Further purification was not attempted.
A suspension of (S)-alanine hydrochloride (3.0 g, 23.9 mmol) in tetrahydrofuran (75 mL) and triethylamine (6.8 mL, 48.8 mmol) was cooled to 0°C. A solution of di-tert-butyl dicarbonate (5.48 g, 25.1 mmol) in tetrahydrofuran (40 mL) was added slowly over 10 minutes and the flask was allowed to warm to room temperature while continued for 16 h. The resulting homogeneous solution was concentrated en vacuo and partitioned between diethyl ether (75 mL) and saturated aqueous sodium bicarbonate (75 mL). The layers were separated, the aqueous layer was extracted with diethyl ether (3 x 40 mL) and the combined organic layers were dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure to afford carbonate 4.2 as a colorless oil in 98% yield (4.43 g, 23.4 mmol). No further purification was necessary.
Boc-Ala-OH \textbf{4.2} (1.0 g, 5.3 mmol) was dissolved in CH$_2$Cl$_2$ (30 mL) in a round bottom flask. Carbonyl diimidazole (0.94 g, 5.8 mmol) was slowly added with constant stirring at room temperature. After 1 h N,O-dimethylhydroxylamine hydrochloride (0.57 g, 5.8 mmol) was added and the mixture was stirred for an additional 16 h. The reaction mixture was then diluted with ethyl acetate (40 mL), washed successively with 1 M HCl (20 mL), saturated aqueous NaHCO$_3$ (20 mL) and brine (20 mL), then dried over MgSO$_4$, filtered and concentrated \textit{en vacuo} to afford Weinreb amide \textbf{4.3} as a white solid in 97% yield (1.19 g, 5.1 mmol). No further purification was necessary.

Weinreb amide \textbf{4.3} (0.5 g, 2.2 mmol) was dissolved in anhydrous tetrahydrofuran (10 mL) and cooled to -30°C. A solution of isopropyl magnesium chloride (4.5 mL, 1.0 M in THF, 4.5 mmol) was slowly added over 5 minutes and the flask was slowly warmed to 0°C over 1 h. While the amide solution as warming a separate
solution of dimethymethylphosphonate (0.35 mL, 3.2 mmol) in anhydrous tetrahydrofuran (12 mL) was prepared, cooled to -78°C and a solution of n-butyllithium (1.9 mL, 1.8 M in hexanes, 3.4 mmol) was added to it dropwise over 15 minutes. This solution was then stirred for 1 h at -78°C. The Weinreb amide solution was then cannulated into the phosphonate solution at -78°C and stirred for 2 h. The reaction was quenched with saturated aqueous ammonium chloride (5 mL). The layers were separated and the organic layer was washed with ethyl acetate (2 x 10 mL). The combined organic layers were dried over anhydrous MgSO₄, filtered and concentrated en vacuo to afford β-ketophosphonate 4.4, which was immediately carried on to the next step without purification.

Crude β-ketophosphonate 4.4 was dissolved in anhydrous tetrahydrofuran (10 mL) and cooled to -30°C. This solution was cannulated into a flask containing a suspension of sodium hydride (0.07 g, 2.9 mmol) in tetrahydrofuran at -30°C over 15 minutes. The reaction was stirred for 30 minutes, warmed to -20°C, and a solution of aldehyde 4.5 (0.5 g, 2.4 mmol) in tetrahydrofuran (3 mL) was added dropwise over 10 minutes. The reaction was stirred for 3 h while maintaining temperature. The reaction was quenched with saturated aqueous ammonium...
chloride (5 mL), the layers were separated and the aqueous phase was washed with ethyl acetate (3 x 15 mL). The combined organic layers were washed with brine, dried over anhydrous MgSO₄, filtered, concentrated en vacuo, then purified on silica gel using a gradient elution of 10%-30% ethyl acetate in hexanes to afford enone 4.6 in 22% yield (0.18 g, 0.48 mmol) from the Weinreb amide 4.3.

A 1 M aqueous solution of sodium hydroxide (50 mL, 50 mmol) was heated to 70°C and δ-valerolactone (4.64 mL, 50 mmol) was quickly added to the warm flask. The solution was stirred for 16 h while maintaining temperature. The flask was then cooled and the solvent evaporated to afford sodium δ-hydroxybutyrate 4.7 as a white solid in quantitative yield (7.0 g, 50 mmol).

To a flask containing a suspension of sodium δ-hydroxybutyrate 4.7 (7.0 g, 50 mmol) in acetone (50 mL) was added tetrabutylammonium bromide (0.80 g, 2.5
mmol). Benzyl bromide (7.1 mL, 60 mmol) was added and the suspension was stirred at room temperature for 16 h. The solvent was evaporated and the residue was partitioned between dichloromethane (50 mL) and saturated aqueous ammonium chloride (50 mL). The layers were separated, the aqueous layer was extracted with dichloromethane (3 x 25 mL), the combined organic layers were washed with brine, dried over anhydrous MgSO$_4$, filtered, concentrated 	extit{en vacuo} and purified on silica gel using a gradient elution of 0-30% ethyl acetate in hexanes to afford ester 4.8 in 11% yield (1.14 g, 5.5 mmol).

Ester 4.8 (0.21 g, 1 mmol) was dissolved in dry acetonitrile (3 mL). Copper (I) bromide (7.2 mg, 0.05 mmol), 2,2'-bipyridine (7.8 mg, 0.05 mmol), TEMPO (7.8 mg, 0.05 mmol), and N-methyl imidazole (8.0 µL, 0.1 mmol) were added sequentially and the reaction was stirred for 3 h until TLC showed consumption of 4.8. The crude reaction mixture was concentrated 	extit{en vacuo} and purified on silica gel using a gradient elution from 0% to 40% ethyl acetate in hexanes to afford aldehyde 4.5 in 42% yield (0.087 g, 0.42 mmol).

$^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 9.78 (s, 1H), 7.38 (m, 5H), 5.14 (s, 2H), 2.53 (t, 2H), 2.42 (t, 2H), 1.96 (m, 2H)
Weinreb amide 4.3 (0.5 g, 2.2 mmol) was dissolved in anhydrous tetrahydrofuran (30 mL) under an atmosphere of dry nitrogen and cooled to 0°C. A solution of allyl magnesiumbromide (7.5 mL, 1.0 M in THF, 7.5 mmol) was added dropwise and the reaction was allowed to warm to room temperature for 12 h with constant stirring. The reaction was quenched with 5 mL sat. aq. NH₄Cl, the crude mixture was diluted and extracted with 8 mL DI H₂O, the separated organic layer was dried over MgSO₄, filtered, concentrated and purified via flash chromatography (0-15% EtOAc : hexanes) to afford the target alkene 4.9 in 46% yield (0.21 g, 1 mmol).

4-Pentenoic acid (1.0 mL, 9.8 mmol), 4-dimethylaminopyridine (0.12 g, 0.98 mmol) and N,N-Dicyclohexylcarbodiimide (2.2 g, 10.7 mmol) were dissolved in anhydrous CH₂Cl₂ (20 mL) under an atmosphere of dry nitrogen and cooled to 0°C. A solution of dry benzyl alcohol (1.5 mL, 14.5 mmol) in anhydrous CH₂Cl₂
(10 mL) was added via syringe transfer, the ice-water bath was removed and the reaction was allowed to stir at room temperature for 4 h. The crude reaction mixture was then filtered, concentrated *en vacuo*, and purified via flash chromatography (0 to 15% ethyl acetate in hexanes) to afford the target ester 4.10 in 95% yield (1.77 g, 9.3 mmol).

Terminal alkene 4.9 (0.05 g, 0.23 mmol) and ester 4.10 (0.09 g, 0.47 mmol) were dissolved in 3 mL anhydrous dichloromethane under and atmosphere of dry nitrogen gas. A solution of Hoveyda-Grubbs’ second generation catalyst (0.02 g, 0.03 mmol) in anhydrous dichloromethane was added and the solution was stirred under dry nitrogen at room temperature for 16 hours. The crude mixture was filtered through a plug of silica gel, washed with 30 mL dichloromethane and concentrated under reduced pressure. Homocoupling of ester 4.10 to produce the dimeric species 4.11 was the only isolvable organic product from this reaction.
Cbz-Ala-OH (0.5 g, 2.2 mmol) was dissolved in 20 mL CH₂Cl₂ in a round bottom flask at room temperature. Carbonyl diimidazole (0.4 g, 2.5 mmol) was slowly added with constant stirring. After 1 h N,O-dimethylhydroxylamine hydrochloride (0.24 g, 2.5 mmol) was added and the mixture was stirred for an additional 16 h. The reaction mixture was then diluted with ethyl acetate (30 mL), washed successively with 1 M HCl (10 mL), sat. NaHCO₃ (10 mL) and brine (10 mL), then dried over MgSO₄, filtered and concentrated en vacuo to afford 4.12 in 97% yield (0.58 g, 2.18 mmol) as a white solid. No further purification was necessary.

![Diagram](insert diagram here)

Weinreb amide 4.12 (0.5 g, 1.9 mmol) was dissolved in anhydrous tetrahydrofuran (20 mL) under an atmosphere of dry nitrogen and cooled to 0°C. A solution of vinyl magnesiumbromide (7.5 mL, 1.0 M in THF, 7.5 mmol) was added dropwise and the reaction was allowed to warm to room temperature for 12 h with constant stirring. The reaction was poured into 10 mL 1 M HCl and diluted with 25 mL ethyl acetate. The organic layer was separated, the aqueous layer was washed with additional ethyl acetate (2x20 mL), the combined organic
layers were dried over MgSO$_4$, filtered, concentrated and purified via flash chromatography (0-20% ethyl acetate : hexanes) to afford the target enone in 52% yield (0.23 g, 0.99 mmol).

$^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.38 (m, 5H), 6.45 (m, 2H), 5.94 (dd, 1 H), 5.72 (d, 1H), 5.13 (s, 2H), 4.73 (p, 1H), 1.40 (d, 3H)

5-Hexenoic acid (1.0 g, 8.8 mmol), 4-dimethylaminopyridine (0.11 g, 0.9 mmol) and N,N-Dicyclohexylcarbodiimide (2.17 g, 10.5 mmol) were dissolved in anhydrous CH$_2$Cl$_2$ (20 mL) under an atmosphere of dry nitrogen and cooled to 0°C. A solution of dry benzyl alcohol (1.4 mL, 13.5 mmol) in anhydrous CH$_2$Cl$_2$ (5 mL) was added via syringe transfer, the ice-water bath was removed and the reaction was allowed to stir at room temperature for 4 h. The crude reaction mixture was then filtered, concentrated en vacuo, and purified via flash chromatography (0 to 15% ethyl acetate in hexanes) to afford the target ester 4.14 in 96% yield (1.72 g, 8.4 mmol).

$^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.39 (m, 5H), 5.91 (m, 1H), 5.15 (s, 2H), 5.01 (m, 2H), 2.42 (t, 2H), 2.12 (q, 2H), 1.79 (m, 2H)
A flask was charged with enone 4.13 (0.05 g, 0.21 mmol) and ester 4.14 (0.088 g, 0.43 mmol), and the oils were dissolved in 3 mL dichloromethane. Hoveyda-Grubbs’ second generation catalyst (0.02 g, 0.03 mmol) was added and the solution was stirred for 16 h. The product was filtered through a plug of silica gel, washed with 30 mL dichloromethane and the filtrate was concentrated en vacuo. ESI mass spectrometry analysis of the residue indicated the presence of enone 4.15, though none of this target material could be isolated. Repeating the reaction under reduced pressure in the absence of solvent at 40°C afforded none of the target material and 1H NMR analysis of the crude material indicates loss of 4.13 with no product derived from this substrate visible.

HR ESI+ Mass Spec. calculated for C_{24}H_{27}NO_5Na: 432.1787, found 432.1781 (1.4 ppm error)

Fmoc-Ala-OH (0.2 g, 0.64 mmol) was dissolved in 20 mL CH_2Cl_2 in a round bottom flask at room temperature. Carbonyl diimidazole (0.12 g, 0.74 mmol) was
slowly added with constant stirring. After 1 h \( N,O \)-dimethylhydroxylamine hydrochloride (0.07 g, 0.72 mmol) was added and the mixture was stirred for an additional 16 h. The reaction mixture was then diluted with ethyl acetate (30 mL), washed successively with 1 M HCl (10 mL), sat. NaHCO\(_3\) (10 mL) and brine (10 mL), then dried over MgSO\(_4\), filtered and concentrated \textit{en vacuo} to afford 4.12 in 93% yield (0.21 g, 0.59 mmol) as a white solid. No further purification was necessary.
Appendix A

A suspension of (R)-serine methyl ester (1.0 g, 8.4 mmol) in tetrahydrofuran (10 mL) and triethylamine (2.4 mL, 17 mmol) was cooled to 0°C. A solution of di-tert-butyl dicarbonate (2.0 g, 9.2 mmol) in tetrahydrofuran (8 mL) was added slowly over 10 minutes and the flask was allowed to warm to room temperature while continued for 16 h. The resulting homogeneous solution was concentrated en vacuo and partitioned between diethyl ether (15 mL) and saturated aqueous sodium bicarbonate (15 mL). The layers were separated, the aqueous layer was extracted with diethyl ether (3 x 10 mL) and the combined organic layers were dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure to afford carbonate A.2 as a colorless oil in 99% yield (1.82 g, 8.3 mmol). No further purification was necessary.

To a solution of N-Boc-R-serine methyl ester (1.82 g, 8.3 mmol) in acetone (25 mL) is added 2,2-dimethoxypropane (10 mL, 82 mmol) and boron trifluoride
etherate (0.1 mL, 0.8 mmol). The solution was stirred for 2.5 h. The reaction was quenched with 1 mL triethylamine and the solvent was removed under reduced pressure. The residue was partitioned between diethyl ether (30 mL) and saturated aqueous sodium bicarbonate (50 mL). The layers were separated and the aqueous phase was extracted with diethyl ether (2 x 30 mL). The combined organic phases were dried over anhydrous MgSO$_4$, filtered and concentrated under reduced pressure to afford oxazolidine A.3 as a yellow oil in 90% yield (1.94 g, 7.5 mmol). The product was used without further purification.

A flask was charged with lithium aluminum hydride (0.14 g, 3.69 mmol) and anhydrous tetrahydrofuran (10 mL) and cooled to 0°C. A solution of oxazolidine A.3 (0.5 g, 1.93 mmol) in anhydrous tetrahydrofuran (10 mL) was added dropwise over 10 minutes and the reaction was stirred for 1 h. The reaction was then quenched with sequential addition of 0.5 mL water, 0.5 mL 3M NaOH and 1.5 mL water. The resulting suspension was filtered through a pad of Celite, dried over anhydrous MgSO$_4$, filtered and concentrated under reduced pressure to afford alcohol A.4 as a colorless oil in 92% yield (0.41 g, 1.77 mmol).
A solution of alcohol A.4 (0.1 g, 0.43 mmol) and triethylamine (0.2 mL, 1.43 mmol) were dissolved in anhydrous dichloromethane (6 mL). The flask was cooled to 0°C and a solution of tosyl chloride (0.1 g, 0.52 mmol) in anhydrous dichloromethane (2 mL) was added via syringe transfer. The reaction was stirred for 2 h then quenched with 2 mL saturated aqueous sodium bicarbonate. The solution was concentrated under reduced pressure, redissolved in a 30% solution of ethyl acetate in hexanes (12 mL), then washed sequentially with water (4 mL) and brine (4 mL). The organic phase was dried over anhydrous MgSO₄, filtered, concentrated under reduced pressure and purified on silica gel using a gradient elution from 10% to 20% ethyl acetate in hexanes to afford tosylate A.5 as a colorless oil in 88% yield (0.146 g, 0.38 mmol).

¹H NMR (CDCl₃, 300 MHz): δ 7.74 (d, 2H), 7.32 (d, 2H), 3.70-4.11 (m, 5H), 2.39 (s, 3H), 1.41 (s, 3H), 1.39 (s, 3H), 1.36 (s, 9H)

¹³C NMR (CDCl₃, 75 MHz): δ 151.1, 144.9, 132.5, 129.8, 127.7, 94.0, 80.4, 67.5, 64.5, 60.2, 55.4, 28.1, 26.5, 21.5
A flask was charged with lithium aluminum deuteride (0.022 g, 0.52 mmol) and anhydrous tetrahydrofuran (6 mL) and cooled to 0°C. A solution of tosylate A.5 (0.1 g, 0.26 mmol) in anhydrous tetrahydrofuran (6 mL) was added dropwise over 10 minutes and the reaction warmed to room temperature stirred for 16 h. The reaction was then quenched with sequential addition of 0.5 mL water, 0.5 mL 3M NaOH and 1.5 mL water. The resulting suspension was filtered through a pad of Celite, dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. Tosylate A.5 was recovered quantitatively with no A.6 visible by ¹H NMR.

A solution of dimethyl sulfoxide (0.1 mL, 1.4 mmol) in anhydrous dichloromethane (10 mL) was cooled to -78°C. Oxalyl chloride (0.1 mL, 1.2 mmol) was slowly added via syringe transfer and the solution was stirred for 5 min. A solution of alcohol A.4 (0.25 g, 1.08 mmol) in anhydrous dichloromethane (4 mL) was added dropwise over 10 min while maintaining temperature. The reaction was stirred
for 1 h, followed by the addition of triethylamine (0.5 mL, 3.6 mmol). The reaction was warmed to room temperature and quenched with 2 mL water. The solution was concentrated under reduced pressure, the residue was redissolved in 30% ethyl acetate in hexanes (20 mL) and extracted sequentially with 1 M HCl (10 mL), saturated aqueous sodium bicarbonate (10 mL) and brine (10 mL). The organic phase was dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure to afford aldehyde A.7 in 93% yield (0.23 g, 1.0 mmol).

Aldehyde A.7 (0.1 g, 0.43 mmol) was dissolved in methanol (5 mL) and cooled to 0°C. Sodium borodeuteride (0.035 g, 0.84 mmol) was added portionwise over 5 minutes and the reaction was stirred at 0°C for an additional 2 h. The reaction was quenched with slow addition of 3 M NaOH (2 mL) and warmed to room temperature. The crude mixture was diluted with ethyl acetate (20 mL) and washed with brine (6 mL). The organic phase was dried over anhydrous MgSO₄, filtered, concentrated under reduced pressure and purified on silica gel using a gradient elution from 10% to 30% ethyl acetate in hexanes to afford alcohol A.8 in 86% yield (0.087 g, 0.37 mmol).
A solution of alcohol A.8 (0.1 g, 0.43 mmol) and triethylamine (0.12 mL, 0.86 mmol) were dissolved in anhydrous dichloromethane (5 mL). The flask was cooled to 0°C and a solution of tosyl chloride (0.1 g, 0.52 mmol) in anhydrous dichloromethane (3 mL) was added via syringe transfer. The reaction was stirred for 2 h then quenched with 2 mL saturated aqueous sodium bicarbonate (2 mL). The solution was concentrated under reduced pressure, redissolved in a 30% solution of ethyl acetate in hexanes (10 mL), then washed sequentially with water and brine. The organic phase was dried over anhydrous MgSO₄, filtered, concentrated under reduced pressure and purified on silica gel using a gradient elution from 10% to 20% ethyl acetate in hexanes to afford tosylate A.9 as a colorless oil in 88% yield (0.146 g, 0.38 mmol).

¹H NMR (CDCl₃, 300 MHz): δ 7.74 (d, 2H), 7.32 (d, 2H), 3.70-4.11 (m, 4H), 2.39 (s, 3H), 1.41 (s, 3H), 1.39 (s, 3H), 1.36 (s, 9H)

¹³C NMR (CDCl₃, 75 MHz): δ 151.2, 145.0, 132.6, 129.9, 127.8, 94.1, 80.6, 67.5, 64.8, 60.2, 55.4, 28.2, 26.6, 21.6
A flask was charged with lithium aluminum hydride (0.02 g, 0.53 mmol) and anhydrous tetrahydrofuran (6 mL) and cooled to 0°C. A solution of tosylate A.9 (0.1 g, 0.26 mmol) in anhydrous tetrahydrofuran (4 mL) was added dropwise over 10 minutes and the reaction warmed to room temperature stirred for 16 h. The reaction was then quenched with sequential addition of 0.5 mL water, 0.5 mL 3M NaOH and 1.5 mL water. The resulting suspension was filtered through a pad of Celite, dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. Tosylate A.9 was recovered quantitatively with no A.6 visible by ¹H NMR.