Chemically synthesized substrates for bio-polymerization

Maria Francisca Jofre

Iowa State University

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Chemically synthesized substrates for bio-polymerization

by

Maria Francisca Jofre

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Organic Chemistry

Program of Study Committee:
Nicola Pohl, Major Professor
Thomas Greenbowe
Gordon Miller

Iowa State University
Ames, Iowa
2003
Graduate College
Iowa State University

This is to certify that the master's thesis of

Maria Francisca Jofre

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$C NMR</td>
<td>carbon nuclear magnetic resonance</td>
</tr>
<tr>
<td>$^1$H NMR</td>
<td>hydrogen nuclear magnetic resonance</td>
</tr>
<tr>
<td>3HA</td>
<td>3-hydroxyalkanoate</td>
</tr>
<tr>
<td>3HBCoA</td>
<td>3-hydroxybutyrate coenzyme A</td>
</tr>
<tr>
<td>AcCl</td>
<td>acetyl chloride</td>
</tr>
<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-butoxycarbonyl</td>
</tr>
<tr>
<td>BTCA</td>
<td>benzyltrichloroacetimidate</td>
</tr>
<tr>
<td>CoA</td>
<td>coenzyme A</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-Diazabicyclo[5,4,0]undec-7-ene</td>
</tr>
<tr>
<td>DCC</td>
<td>N,N'-Dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DMAP</td>
<td>N,N-Dimethyl-p-hydroxyaniline</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DPPA</td>
<td>diphenylphosphonic azide</td>
</tr>
<tr>
<td>FAS</td>
<td>fatty acid synthase</td>
</tr>
<tr>
<td>HA</td>
<td>hydroxyalkanoates</td>
</tr>
<tr>
<td>HACoA</td>
<td>coenzyme A hydroxyalkanoates</td>
</tr>
<tr>
<td>HF</td>
<td>hydrogen fluoride</td>
</tr>
<tr>
<td>KOH</td>
<td>potassium hydroxide</td>
</tr>
<tr>
<td>LDA</td>
<td>Lithium diisopropylamide</td>
</tr>
<tr>
<td>MCL</td>
<td>medium-chain-length</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Chemical Name</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>NAD⁺</td>
<td>nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NADH</td>
<td>nicotinamide adenine dinucleotide, reduced form</td>
</tr>
<tr>
<td>NADPH</td>
<td>nicotinamide adenine dinucleotide phosphate, reduced form</td>
</tr>
<tr>
<td>PHAs</td>
<td>poly(hydroxyalkanoates)</td>
</tr>
<tr>
<td>PHAS</td>
<td>poly(hydroxyalkanoates) synthase</td>
</tr>
<tr>
<td>PHB</td>
<td>poly(3-hydroxybutyrate)</td>
</tr>
<tr>
<td>PKS</td>
<td>polyketide synthase</td>
</tr>
<tr>
<td>psi</td>
<td>pound per square inch</td>
</tr>
<tr>
<td>Py</td>
<td>pyridine</td>
</tr>
<tr>
<td>SCL</td>
<td>short-chain-length</td>
</tr>
<tr>
<td>TBDMS</td>
<td>tert-butyldimethylsilyl</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>TFAA</td>
<td>trifluoroacetic anhydride</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>trimethylsilyl</td>
</tr>
<tr>
<td>TMSCl</td>
<td>trimethylsilyl chloride</td>
</tr>
</tbody>
</table>
INTRODUCTION

In the last century petrochemical polymers have become one of the most used materials in our society given their versatility and low cost; however, it is known that these polymers are not environmentally friendly (Zinn, 2001). Thus, given the growing concern over the harmful effect of olefin-derived plastics on the environment, poly(hydroxyalkanoates) (PHAs) have been identified as environmentally friendly biological plastics due to their thermoplastic properties and high biodegradability in soil (see table 1) (Merrick, 2002; Zinn, 2001; Jendrossek, 1996). In addition PHAs can be used as a base for the synthesis of pharmacological and medical products (Zinn, 2001; Lee, 1999). However, the biodiversity these polymers exhibit and the potential of these materials as alternatives for chemically synthesized polymers have only begun to be explored.

Table 1. Potential applications of PHAs

<table>
<thead>
<tr>
<th>Type of application</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replacement of petrochemical</td>
<td>Bottles, films, fibers, packaging materials,</td>
</tr>
<tr>
<td>plastics</td>
<td></td>
</tr>
<tr>
<td>Wound management</td>
<td>Sutures, skin substitutes, nerve cuffs, surgical meshes, staples</td>
</tr>
<tr>
<td>Vascular system</td>
<td>Heart valves, cardiovascular fabrics, vascular grafts</td>
</tr>
<tr>
<td>Drug delivery</td>
<td>Micro- and nanospheres for anticancer therapy</td>
</tr>
<tr>
<td>Substitutes</td>
<td>Dairy cream substitutes (Yalpani, 1993)</td>
</tr>
<tr>
<td>Delivery agent</td>
<td>Flavor delivery agent in different types of food (Yalpani 1993)</td>
</tr>
<tr>
<td>Dental</td>
<td>Barrier material for guided tissue regeneration in periodontitis</td>
</tr>
</tbody>
</table>
A wide array of bacterial species produce biodegradable PHAs as carbon and energy reserves from a variety of carbon sources and they are usually produced under conditions of limiting nutritional elements such as N, P, S, or O in the presence of excess carbon (Zhang, 2002; Amara, 2002; Kamachi, 2001; Steinbüchel, 2001; Lee, 1999; Kraak, 1997; Jendrosessek, 1996). PHAs are deposited intracellularly in the form of granules and can amount to 90% of the dry cell mass under controlled fermentation conditions (Zhang, 2002; Jendrosessek, 1996). PHAs can have a variety of different monomers but it is essential for the biodegradability and biocompatibility of these polymers to have complete stereospecificity; all chiral carbon atoms in the backbone of the polymer have R configuration (see Figure 1) (Zinn, 2001).

![Chemical structure of poly(hydroxyalkanoates)](image)

**Figure 1.** Chemical structure of poly(hydroxyalkanoates). All monomers have a chiral center (*) in the R configuration and R varies according to the alkyl chain of the monomer.

Chiral hydroxycarboxylic acids, the monomer unit of PHAs, can be used as the base for a variety of chemicals such as antibiotics, vitamins, and pheromones (Lee, 1999). Unfortunately, these acids contain a chiral center and two functional groups (OH and COOH) that make their chemical synthesis cumbersome and expensive. Consequently, it can be thought that enantiomerically pure aldol acetates, 2-alkenoic acids, β-hydroxyalkanols, β-acyllactones, and β-hydroxyacid esters can be prepared by depolymerizing biosynthesized PHAs (Qi, 2000; Madison, 1999; Kraak, 1997). In addition, if the polymerase only utilizes R
monomers for polymer biosynthesis it could be possible to separate a racemic mixture, because the \((R)-(\cdot)-\)hydroxycarboxylic acids would be polymerized into PHAs and the \((S)-(\cdot)-\)hydroxycarboxylic acids would remain in solution as monomers.

![Chemical structure](image)

Where Coenzyme A is:

![Coenzyme A structure](image)

**Figure 2.** Biosynthesis of poly(hydroxyalkanoates) with the concomitant release of coenzyme A

In most cases the PHAs are polymerized in the cell from coenzyme A (CoA) thioester derivatives of hydroxyalkanoates (HACoA) by a PHA synthase (PHAS) to make PHAs with the release of CoA as shown in Figure 2 (Zhang, 2002; Amara, 2002; Steinbüchel, 2001; Ballard, 1987). The most common and abundant PHA is poly(3-hydroxybutyrate), (PHB) (Figure 1; \(R=\text{Me}\)), and its biosynthesis has been the focus of most studies. However, this class of polymers demonstrates great biodiversity. In the last three
decades nearly 125 monomer units have been identified in bacterially produced PHAs (Zhang, 2001; Jendrosessek, 1996).

**Classification of PHA Synthases**

On the basis of their substrate specificity and protein products PHAs can be arranged into three different types (Zhang, 2002; Amara, 2002; Kamachi, 2001; Steinbüchel, 2001; Rhem, 2001; Müh, 1999). Type I and type II PHA synthases consist of only one type of subunit (polypeptide chain) and recent studies indicate that the active site consists of aggregates of more than one subunit (Steinbüchel, 2001). Type III PHA synthases consist of two different types of subunits (polypeptide chains) that aggregate.

**Table 2. Properties of PHA synthases.**

<table>
<thead>
<tr>
<th></th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species that represents each type</td>
<td><em>Ralstonia eutropha</em></td>
<td><em>Pseudomonas oleovorans</em></td>
<td><em>Allochromatium vinosum</em></td>
</tr>
<tr>
<td>Substrate</td>
<td>C₃-C₅ HACoAs</td>
<td>C₆-C₁₄ HACoAs</td>
<td>C₃-C₅ HACoAs</td>
</tr>
<tr>
<td>Subunit</td>
<td>One type</td>
<td>One type</td>
<td>Two types</td>
</tr>
<tr>
<td>Molecular mass of subunit</td>
<td>~64 kDa</td>
<td>~62 kDa</td>
<td>~40 kDa</td>
</tr>
</tbody>
</table>

Type I synthases, represented by PHA synthase from *Ralstonia eutropha*, prefer short-chain-length (SCL) monomers that contain from 3 to 5 carbon atoms (Zhang, 2002, Steinbüchel, 2001; Müh, 1999). Type II synthases, represented by *Pseudomonas oleovorans*,
incorporate preferentially 3-hydroxyfatty acid monomers of medium-chain-length (MCL) that contain 6 to 14 carbon atoms. Finally, the type III synthases, represented by the PHA synthase from *Allochromatium vinosum*, have a preference for SCL monomers, therefore, having similar substrate specificity as type I synthases. The properties of these polymerases are summarized in Table 2.

**Historical Outline**

The interest of PHAs dates to 1926 when the French scientist Lemoigne, at the Institute Pasteur, first observed the production of poly(3-hydroxybutyrate) (PHB) (Zinn, 2001; Jendrossek, 1996; Merrick, 1961; Lemoigne, 1926). All of his studies were carried out on a bacterium called *Bacillus megaterium* and he was able to identify PHB as an inclusion body in the bacteria. In addition, Lemoigne realized that PHB was a homopolymer that consisted of 3-hydroxybutyric acid linked through ester bonds between the 3-hydroxyl group and the carboxylic group of the next monomer.

In 1961, Merrick and Doudoroff studied the bacterial production of PHB from its CoA-thioester monomers in both *Bacillus megaterium* and *Rhodospirillum rubrum* (Merrick, 1961). Merrick and coworkers carried out *in vitro* kinetic studies on the polymerization of 3HBCoA, showing for the first time the role of PHB synthase as a catalyst in the polymerization reaction of 3HBCoA (Griebel, 1968). The authors also proposed that the active site of the enzyme contained a thiol group which would be covalently bound to the polymer. In the same study they were also able to determine the effect of incubation time,
protein concentration, and pH on the rate of polymerization with the bound PHA synthase. In a later study, Griebel and Merrick unsuccessfully tried to obtain an active PHA synthase in soluble form (Griebel, 1971). Tomita and coworkers were the first group to isolate a soluble PHA in 1976. In their studies the authors were able to obtain an active enzyme and studied both the granule-bound and the soluble forms of PHA synthase of Zoogloea ramigera (Fukui, 1976).

A more detailed mechanism for the polymerization of 3HBCoA was not proposed until 1987 (Ballard, 1987). They proposed that two thiolates in the active site of the synthase participate in the covalent catalysis of the polymer synthesis as shown in Figure 2. In this mechanism one thiol group (S₁H) covalently binds to a hydroxyalkanoic acid received from the CoA-thioester. The growing polymer is covalently bound to the second thiol group as shown in Figure 3.

According to this mechanism the condensation step occurs via a four component transition state having a thiol group free at all times for a new monomer to be incorporated into the polymer chain. Ballard and coworkers also suggested that the polymer chain goes back and forth between the two thiol groups in the active site. Stubbe and coworkers, in order to understand the differences between type I and type III synthases, researched and characterized the mechanism of polymerization by the PHB synthase of Chromatium vinosum, a type III synthase (Müh, 1999). This study further demonstrated that two thiol groups and covalent catalysis are required for the polymerization process. Later, Stubbe and coworkers illustrated the similarities in the catalytic mechanism and architecture of the active

![Chemical structure](image)

**Figure 3.** Proposed model for the reaction mechanism of PHAS.

The activities of different PHA synthases were studied almost simultaneously. Dawes and coworkers conducted *in vitro* polymerization studies on R. eutropha using CoA derived monomers containing longer alkyl chains, from 6 to 10 carbon atoms (Haywood, 1989). Their results showed that the enzyme was only active towards monomers containing 4 to 5 carbon atoms as previous studies had shown. In addition, they found that the enzyme was only active with (R) monomers and not with the (S) isomer. Similar results were obtained by Fukui and coworkers when studying the PHA synthase of Z. ramigera (Fukui, 1976). In 1997, Witholt and coworkers studied the first MCL polymerase. They assayed the *in vitro* activity of P. oleovorans and discovered that the PHA synthase had its highest activity towards (R)-3-hydroxyoctanoylCoA and no activity towards either isomer of 3HBCoA (Kraak, 1997).
Importance of Medium-Chain-length PHAs

Witholt and coworkers were the first to discover medium-chain-length PHAs in 1983 (De Smet, 1983). The elemental analysis of the granules obtained showed that *P. oleovorans* formed poly(β-hydroxyoctanoate) instead of poly(β-hydroxybutanoate) which was the most common polymer obtained in previous studies. MCL-poly(3HA) is not a single polymer but a family of biopolymers that are formed by different CoA-thioester monomers. In the last two decades different functional groups, such as epoxy-, cyano-, carboxyl-, nitrophenoxy-, have been introduced into MCL-poly(3HA) (Kamachi, 2001). MCL polymers have elastomeric properties that had not been observed in their short chain counterpart (Lee, 2002). New studies of MCL-poly(3HA) have been hindered in part because their chiral monomers are not commercially available. In addition, the coenzyme A leaving group used by the MCL polymerase is exceedingly expensive. Other enzymes, such as the polyketide (PKSs) and fatty acid synthases (FASs), that accept CoA-thioester substrates, have been shown to tolerate and turnover truncated versions of the substrates that are less costly and easier to chemically synthesize (Khosla, 1999). It is unclear whether PHA synthases, which are proposed to be related to lipases rather than FASs (Jia, 2000), would exhibit a similar substrate tolerance.

The high cost of the CoA leaving group and the fact that MCL chiral monomers are not commercially available are delaying the studies of MCL polymerase. Consequently, if the polymerase is able to accept and turnover truncated versions of the thioesters, without the CoA-derivative, the low cost and ease of a chiral synthesis would promote further studies on this type of polymerase. To probe the specificity requirements of the *Pseudomonas*
aeruginosa, a MCL polymerase, the synthesis of chiral β-hydroxy-thioesters is reported herein.

**Previous Syntheses of Chiral β-hydroxy Acids**

Most studies on PHAS have been completed on type I and III synthases given the commercial availability of the chiral hydroxybutyric acids, which makes the synthesis of CoA-thioesters straightforward. Unfortunately, the chiral β-hydroxycarboxylic acids required to make the monomers needed to biosynthesize MCL- poly(3HA) are not commercially available, which makes the production of the CoA-thioesters a particular challenge.

![Chemical synthesis diagram](image)

**Figure 4.** Frequently used route to CoA-thioester substrates (Yuan, 2001; Jia, 2000).

The most commonly used chemical synthesis to obtain SCL CoA-thioesters substrates (see Figure 4) starts with 3-(R)-hydroxybutyric acid (R=H), which is commercially available. In the first step, the hydroxyl group is protected, followed by coupling with benzenethiol and
deprotection of the alcohol. The resulting thioester is coupled to CoA at pH 8 (Yuan, 2001; Jia, 2000).

Another pathway, to this class of structures, is to use biosynthesis to obtain SCL and MCL CoA-thioesters substrates (Zinn, 2001). Steinbüchel and coworkers synthesize MCL substrates using a racemic mixture of (R,S)-3-hydroxydecanoyl, CoA, and acyl-CoA synthetase as the catalyst for the reaction. Unfortunately, this method yields a racemic mixture and requires the expensive CoA derivative for polymerase testing (Rehm, 1998). Clearly, acyl-CoA synthetase demonstrates no preference for the β-stereocenter. Another frequent way to obtain PHB biosynthetically is through a two-step reaction starting with acetyl-CoA (see Figure 5). In this reaction the enzyme β-thiolase makes the acetoacetyl-CoA which is reduced by the NADH-dependent enzyme acetoacetyl-CoA reductase, giving the desired product. This route still requires a relatively expensive CoA-derivative, however.

![Figure 5. PHB monomer biosynthesis.](image)
RESULTS AND DISCUSSION

The original goal of this project was to synthesize enantiomerically pure β-hydroxythioesters with different carbon chain lengths as well as different functionalities (R₃ and R₄ in Figure 6). The synthesis would start with a chiral compound and the chirality would be kept throughout the synthetic route. A β-hydroxyacid would be synthesized and then coupled to a wide variety of thiols in order to test the tolerance and turnover of the truncated versions of the substrates by the polymerase. This method would provide an inexpensive and simple way to chemically synthesize a wide variety of thioesters that can be used to study the specificity requirements of different kinds of type II PHA synthase. A good starting point for the synthesis was thought to be commercially available (S)-3-hydroxy-γ-butyrolactone.

Retrosynthetic Analysis

The principal disconnections for the retrosynthetic analysis used are illustrated in Figure 6. In the retrosynthesis of compound I, convenient disconnections could be made at C1 and C3 (Figure 6). As a result, a strategy for the synthesis of chiral β-hydroxy-thioesters analogs could arise from coupling different thiols to β-hydroxycarboxylic acids. The different functionalities and length of the alkane chain of R₄, in figure 6, would vary according to the thiol of choice. The array of β-hydroxycarboxylic acids could be obtained by modifying the carbon chain length of intermediate II (R₃ in Figure 6) via the Wittig reaction, and, by hydrolysing the protected carboxylate terminus, at C1, of intermediate III. Finally, compound III could be attained by opening the protected (S)-3-hydroxy-γ-butyrolactone.
Therefore, the main points of the synthesis plan call for protecting the alcohol at C3, until late in the synthesis, and the proper protection of the carboxylate terminus at C1.

\[
\begin{align*}
R^4S & \quad \text{OH} \quad R^1 \quad \rightarrow \quad \text{O} \quad \text{OTBDMS} \quad \rightarrow \quad \text{R}^1 \quad \text{N} \quad \text{R}^2 \quad \text{H} \quad \rightarrow \quad \text{O} \quad \text{O} \quad \text{Bn} \\
\text{I} & \quad \text{II} & \quad \text{III} & \quad \text{IV}
\end{align*}
\]

**Figure 6.** Retrosynthetic analysis for thioester analogs.

**First Synthesis and Discussion of Problems**

The first route (Figure 7) to (R)-β-hydroxyoctanoic acid involved the protection of the hydroxyl group of the lactone using benzyltrichloroacetimidate (Larsen, 1999). This protecting group was chosen for its stability under different conditions, such as basic and mildly acidic media. The lactone ring was then opened using benzyl amine as the nucleophile with an 80% yield as previously reported (Kanno, 2000).

\[
\begin{align*}
\text{BTCA, TfOH} & \quad \text{CH}_2\text{Cl}_2 \quad \text{Cyclohexane} \quad \rightarrow \quad \text{O} \quad \text{OBn} \quad \rightarrow \quad \text{Benzylamine} \quad \text{Benzene} \quad \rightarrow \quad \text{73%} \\
\text{Bn} & \quad \text{N} \quad \text{OBn} \quad \text{Oxidation} \quad \rightarrow \quad \text{Bn} \quad \text{N} \quad \text{OBn} \quad \text{H}
\end{align*}
\]

**Figure 7.** First proposed synthetic route to thiol analogs.
In order to obtain the aldehyde needed for the Wittig reaction two different types of oxidations, Parikh-Doering (Smith, 1996) and Swern (Omura, 1978), were tried unsuccessfully. Instead of yielding the aldehyde only the cyclized N-acyl hemiaminal could be isolated as the major product (Figure 8). Cyclizations of this type have been observed previously (Smith, 1996). Clearly, the amide hydrogen would need to be protected to prevent this cyclization pathway.

\[
\begin{align*}
\text{OBn} & \quad \text{Py} \cdot \text{SO}_3 \\
\text{OH} & \quad \text{Bn} \quad \text{N} \\
\text{TEA, DMSO} & \quad \text{Bn} \quad \text{N} \\
\text{H} & \quad \text{BnO} \\
\text{O} & \quad \text{O} \\
\end{align*}
\]

\text{Figure 8.} Formation of N-acyl hemiaminal upon oxidation of the primary alcohol of the benzyl amide.

\text{Second Synthesis and Discussion of Problems.}

One method to avoid an amide hydrogen would involve making a tertiary amide from a secondary amine. There are a vast number of disubstituted amines that could have been used. 2-Oxazolidinone was the first one tried to open the lactone ring via the Weinreb protocol (Figure 9) (Basha, 1977; Lipton, 1980). According to the literature, similar reactions have proven to yield the desired product (Romo, 1998; Smith, 1996). Despite the fact that a variety of conditions were tried, such as reflux for 13 and 48 hours, and reflux at 90 °C (using dichloroethane) for 12 and 24 hours, no product was observed by thin layer chromatography (TLC) nor by hydrogen nuclear magnetic resonance spectroscopy (\(^1\text{H NMR}\)).
The next attempt involved dibenzyl amine as the nucleophile. This reagent was coupled with the protected (S)-3-hydroxy-γ-butyrolactone via the Weinreb protocol (Basha, 1977; Lipton, 1980), providing the desired alcohol in 30% yield (Figure 10). The low yield obtained in this reaction was in part due to the difficulty of removing the coordinated aluminum complex in the acidic workup.

**Figure 9.** Initial attempt to open the protected (S)-3-hydroxy-γ-butyrolactone using 2-oxazolidinone

**Figure 10.** Synthetic route to thiol analogs using benzyl amine as nucleophile.
The lactone opening was followed by Dess-Martin oxidation to afford the desired aldehyde (Dess, 1983). This oxidation was chosen due to the mild conditions of the reaction which would not recognize the stereochemistry at the chiral center of the starting material, and the simplicity of the workup procedure. The resulting aldehyde was immediately reacted, in order to avoid decomposition, with butyltriphenylphosphonium bromide in a Wittig reaction to yield the desired product (White, 2000). Unfortunately, the simultaneous hydrogenation of the double bond, deprotection of the hydroxyl group, and the removal of the benzyl from the amide could not be completed.

**Table 3.** Reaction and conditions for concomitant hydrogenation of olefin, O-benzyl group, and amide benzyl protecting group.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Solvent</th>
<th>Pressure</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palladium on Carbon (Pd/C)</td>
<td>Methanol</td>
<td>14.7 psi</td>
<td>16 hours</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Pd/C</td>
<td>Ethanol</td>
<td>14.7 psi</td>
<td>16 hours</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Pd/C</td>
<td>Ethanol</td>
<td>50 psi</td>
<td>1.5 hours</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Pd/C and ammonium formate</td>
<td>Ethanol</td>
<td>50 psi</td>
<td>2.0 hours</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Palladium Hydroxide [Pd(OH)$_2$]</td>
<td>Ethanol</td>
<td>50 psi</td>
<td>16 hours</td>
<td>60 °C</td>
</tr>
<tr>
<td>Pd(OH)$_2$ and ammonium formate</td>
<td>Ethanol</td>
<td>50 psi</td>
<td>16 hours</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Pd(OH)$_2$ and ammonium formate</td>
<td>Ethanol</td>
<td>50 psi</td>
<td>16 hours</td>
<td>60 °C</td>
</tr>
<tr>
<td>Pd(OH)$_2$</td>
<td>Ethanol</td>
<td>500 psi</td>
<td>16 hours</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Pd(OH)$_2$</td>
<td>Ethanol</td>
<td>1000 psi</td>
<td>16 hours</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Pd(OH)$_2$</td>
<td>Ethanol</td>
<td>1200 psi</td>
<td>16 hours</td>
<td>Room temperature</td>
</tr>
</tbody>
</table>
Different conditions were tried for the concomitant hydrogenation (Table 3) but debenzylation of the benzylacetamide by hydrogenolysis was not achieved. The difficulty of removing a benzyl group of an amide by hydrogenolysis is not unprecedented (Greene, 1999). The use of a strong base, such as sodium, lithium, potassium tert-butoxide, t-butyl lithium, is known to debenzylate the benzylacetamide when hydrogenolysis has not worked. Unfortunately, the use of a strong base would cause elimination of the benzyl protected hydroxyl group, in the compound, given the high acidity of the neighboring hydrogens.

In view of the fact that removal of the benzyl group of the amide by hydrogenolysis was not possible under relatively mild or basic conditions and that the use of a mono-substituted amine ended in cyclization of the starting material after the oxidation of the primary alcohol, some modifications to the first synthetic route were made.

**A Variant of the First Synthesis.**

To prevent closure of the ring after the oxidation (Figure 8), the amide and the hydroxyl group would have to be selectively protected. Therefore, a temporary protecting group is required in order to mask nitrogen and not oxygen for protection of the alcohol until late in the synthesis. A one pot reaction for the protection of both functional groups would be the best synthetic approach. The tert-butoxycarbonyl (Boc) substituent is one of the most widely used in synthetic organic chemistry due to the ease of introducing and removing Boc and due to the fact that it can easily be employed with other protecting groups (Agami, 2002). This synthesis required a hydroxyl protecting group that could be easily inserted and removed.
under neutral or acidic conditions, in order to avoid elimination. In addition, the reaction setting had to be similar to the conditions required to protect the nitrogen with a Boc substituent. As a result, trifluoroacetic anhydride (TFAA) in pyridine (Lansbury, 1996) was chosen (Figure 11); however, the nitrogen protection did not take place.

Another hydroxyl protecting group that met all the required characteristics was trimethylsilyl chloride. The alcohol protection was done successfully but protection of the nitrogen, with a Boc substituent, did not proceed. Partial purification to remove most of the salts and excess reagents, which could interfere with the introduction of the Boc substituent, was tried unsuccessfully; the trimethylsilyl (TMS) would invariably be cleaved to produce the starting material again. Transient protection schemes were abandoned at this point in favor of routes with more stable protecting groups.

At this point an achiral model compound was synthesized to try different protecting groups for the alcohol and the nitrogen (Figure 13). Protection of the alcohol as a tert-butyldimethylsilyl (TBDMS) ether proved to be stable and partial purification was carried out followed by the addition of the Boc substituent. Removal of the TBDMS group with hydrogen fluoride in pyridine afforded the desired product (Figure 13). Next, the same
synthetic procedure was tried on the chiral substrate. The protection steps were successful, though the yields for the protected amide were lower than in the model compound (75% yield for chiral material over two steps). Unfortunately, the deprotection of the alcohol with hydrogen fluoride in pyridine afforded starting material. Other deprotection methods were tried (such as boron trifluoride, diethyl etherarate, acetic acid, and tetra-n-butylammonium fluoride) yielding only starting material.

![Chemical diagram]

**Figure 12.** Synthesis of model compound.

The choice of amide protecting group was a p-methoxybenzyl moiety. Unfortunately, the reaction of the amide with base and p-methoxybenzyl chloride (Akiyama, 1990) afforded only low yields of the protected amide under a variety of reaction conditions (Figure 13). As a result, after several futile attempts to protect the carboxylate terminus it was apparent that this protection proved to be more difficult than expected. A new approach to synthesize the thioesters analogs was developed.
Figure 13. Amide protection with $p$-methoxybenzyl chloride. Conditions used: DBU, acetonitrile, 50 °C; NaH, THF, 0 °C; KH, THF, 0 °C

Final Synthesis Using Braun’s Chiral Auxiliary

Despite recent progress made in stereoselective aldol reactions (Alcaide, 2002; Palomo, 2002), the problem of addition of an $\alpha$-unsubstituted acetate enolate to aldehydes in order to obtain enantiomerically pure $\beta$-hydroxycarboxylic acids has not been resolved (Lalic, 2003; Palomo, 2002; Braun, 1984). Therefore, the final approach to obtain the thioester analogs was based on the work done by Braun and coworkers on chiral acetate enolates, although the final product would not be as enantiomerically pure as with a route relying on a biologically-derived chiral starting material (Braun, 1984; Devant, 1988). Indeed, the synthesis of $\beta$-hydroxyacids using Braun’s chiral auxiliary, synthesized as described in Figure 14 (Braun, 1984; Devant, 1988), gave a mixture, of 75% ($R$)-$\beta$-hydroxycarboxylic acid and 25% ($S$)-$\beta$-hydroxycarboxylic acid.
**Figure 14.** Final synthetic route to thiol analogs
The next step was protection of the hydroxyl group to avoid complications in the thioester formation step. In order to achieve this goal the alcohol and the carboxylic acid were protected using TBDMSI; selective removal of the TBDMS group, attached to the acid portion of the molecule, was performed from the resulting di-TBDMS-protected compound (see Figure 14). The coupling of the thiol to the acid was a low yielding reaction (55-37% yield) as has been observed previously (Jacobsen, 1998), but the three desired thioesters were all obtained in quantities necessary for the enzymatic studies. In conclusion, after deprotection of the silyl protecting groups and purification, an inexpensive and straightforward route to chemically synthesize an ample array of thioesters that can be used to study specificity requirements of different kinds of type II PHA synthases has been achieved.
CONCLUSIONS

The initial goal of this project was to synthesize enantiomerically pure β-hydroxythioesters with different carbon chain length as well as different functionalities in an inexpensive way. The main points of the synthesis plan called for protection of the alcohol at C3 and the proper protection of the carboxylate terminus at C1 (Figure 6). The first two synthetic routes that were attempted failed at protecting the carboxylate terminus. The first path (Figure 7) used a mono-substituted amine which ended in cyclization of the starting material after oxidation of the primary alcohol. In the second synthesis (Figure 10) the main problem was removal of the benzyl group of the amide by hydrogenolysis. The failure of these syntheses lead to the idea of using selective and temporary protecting groups for the amide nitrogen and the primary hydroxyl group (Figures 11 – 12). Unfortunately, after several futile attempts to protect the carboxylate terminus, it was clear that this protection proved to be more difficult than expected and development of a new route was needed. Therefore, the final approach to obtain thioester analogs was based on the work done by Braun and coworkers on chiral acetate enolates, even though the final product was not enantiomerically pure (Braun, 1984). Nevertheless, we succeeded in the synthesis of a natural product obtaining good yields since Braun’s chiral auxiliary can be easily recovered. Consequently, an in vitro system using purified synthases and chemically synthesized thioesters, as proposed in this thesis, can be used to study the specificity requirements of different kinds of type II synthases, beyond that obtainable from living systems.
MATERIALS AND METHODS

Except as otherwise indicated, reactions were carried out under nitrogen atmosphere in flame- or oven-dried glassware, and solvents were freshly distilled. Reactions were monitored and Rf values were determined by thin layer chromatography (TLC) on EM Science 250 µm precoated silica gel plates (60 F254). TLC plates were visualized with UV light (254 nm). Purification of products was performed by column chromatography on silica gel (32-63) and HPLC grade solvents. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise indicated. 'H spectra were recorded on a Varian VXR-300, 13C spectra were recorded on a Varian VXR-400, and HETCOR spectra were recorded on a Bruker DRX-400.

Benzyl trichloroacetimidate (BTCA) (2).

To a solution of benzyl alcohol (2.4 mL, 23 mmol) in dichloromethane (CH2Cl2) (30mL) was added aqueous potassium hydroxide (25 mL, 50% w/w, 0.23 mol) and tetrabutylammonium hydrogen sulfate (33 mg, 11 mmol). The resulting solution was stirred at -15 °C for 5 minutes. Trichloroacetonitrile was added dropwise and the reaction was stirred for 30 minutes at -15 °C. Then, the solution was allowed to warm up to room temperature to stir for an additional 30 minutes. The aqueous solution was separated and then extracted with CH2Cl2 (3 X 10 mL). These extracts were combined with the organic layer and dried over MgSO4. The excess solvent was removed under reduced pressure and the product was filtered through a celite pad (1 cm) to yield the crude product as a yellow oil. 1H NMR: (300 MHz, CDCl3) 5.34 (s, 2H), 7.43 (m, 5H), 8.42 (s, 1H).
Benzyloxydihydrofuran-2-one (3).

A solution of (S)-3-hydroxy-γ-butyrolactone (0.9 g, 9 mmol) in CH₂Cl₂ (493 mL) and cyclohexane (250 mL) under nitrogen was cooled to 0 °C. BTCA (5.0 g, 20 mmol) was added followed by the dropwise addition of trifluoromethanesulfonic acid (triflic acid, 0.05 mL, 0.3 mmol). The resulting reaction was stir for 6 hours allowing the solution to warm up to room temperature. Hexanes (250 mL) were added to the reaction to precipitate the out the trichloroacetimidate, which was filtered off. The organic layer was washed with saturated sodium bicarbonate (250 mL) and dried (MgSO₄). The product was chromatographed (30% ethyl acetate in petroleum ether) to yield a clear colorless oil (418 mg, 73%, yield). Rf 0.70 (30% ethyl acetate in hexanes); H NMR: (300 MHz, CDCl₃) 2.67 (m, 1H), 4.38 (m, 3H), 4.53 (m, 2H), 7.33 (m, 1H).

(S)-N-Benzyl-3-benzyloxy-4-hydroxybutyramide (4).

4-Benzylkoxydihydrofuran-2-one (57 mg, 0.30 mmol) was dissolved in benzene (1.0 mL) under nitrogen. Benzylamine (0.065 mL, 0.60 mmol) was added to the reaction and the resulting solution was stirred for 2.0 hours at 50 °C. The mixture was diluted with ethyl acetate (5 mL), quenched with aqueous hydrochloric acid (HCl) (10 ml, 1N). The organic layer was washed with saturated aqueous sodium chloride (NaCl) and dried (MgSO₄). The product was chromatographed (50% ethyl acetate in petroleum ether) to yield a clear colorless oil (62 mg, 80%, yield). Rf 0.17 (50% ethyl acetate in hexanes); H NMR: (300 MHz, CDCl₃) 2.60 (m, 2H), 3.81 (m, 2H), 4.13 (m, 2H), 4.53 (m, 2H), 4.60 (m, 2H), 6.23 (s, 1H), 7.39 (m, 10H).
(S)-N,N-Dibenzyl-3-benzyloxy-4-hydroxybutyramide (5).

Dry dibenzylamine (0.10 mL, 0.52 mmol) was dissolved in dry CH$_2$Cl$_2$ (3 mL) under nitrogen and cooled to 0 °C. Trimethylaluminum (0.31 mL, 2.0 M in toluene, 0.63 mmol) was added dropwise, under nitrogen, and the resulting mixture was stirred for 2.0 hours while warming up to room temperature. The mixture was then cooled to 0 °C and a solution of 4-Benzyloxydihydrofuran-2-one (0.122 g, 0.65 mmol) in dry CH$_2$Cl$_2$ (10 mL) was added slowly. The reaction was allowed to warm up to room temperature. After 20 hours, a solution of Dry dibenzylamine (0.10 mL, 0.52 mmol) was dissolved in dry CH$_2$Cl$_2$ (3 mL) under nitrogen and cooled to 0 °C. Then trimethylaluminum (0.31 mL, 2.0 M in toluene, 0.63 mmol) was added dropwise, under nitrogen, and the resulting mixture was stirred for 2.0 hours while warming up to room temperature. This solution was added to the original reaction while both were at 0 °C and the resulting mixture was stirred at room temperature for 5 hours. The reaction was cooled to 0 °C and slowly quenched with aqueous HCL (10 ml, 1N). The aqueous solution was then extracted with ethyl acetate (5 X10 mL) and CH$_2$Cl$_2$ (5 X10 mL). The combined organic layers were dried over MgSO$_4$ and excess solvent was removed under reduced pressure. The product was chromatographed (50% ethyl acetate in petroleum ether) to yield a clear yellow oil (0.072 mg, 30%, yield). R$_f$ 0.44 (50% ethyl acetate in hexanes); $^1$H NMR: (300 MHz, CDCl$_3$) 1.26 (t, 1H), 2.10 (s, 1H), 2.81 (m, 2H), 3.75 (m, 2H), 4.23 (m, 2H), 4.54 (s, 2H), 4.60, (m, 4H), 7.39 (m, 15H).

(S)-N,N-Dibenzyl-3-benzyloxy-4-oxobutyramide (6).

To a solution of Dess-Martin reagent (0.5g, solution of periodinane 15% by w/w in CH$_2$Cl$_2$, 0.175 mmol) under nitrogen was added (S)-N,N-Dibenzyl-3-benzyloxy-4-
hydroxybutyramide (63 mg, 0.162 mmol dissolved in 2 mL of CH₂Cl₂). The resulting solution was stirred at room temperature for 20 minutes, diluted with diethyl ether (3 mL), and washed with 3 mL of a 1:1 (v/v) solution of sodium bicarbonate and sodium sulfate (10% w/w in water). The aqueous layer was separated and extracted with diethyl ether (2X10 mL). The combined organic layers were washed with water, saturated sodium chloride, and dried over MgSO₄ and excess solvent was removed under reduced pressure. The product was run through a 1 cm celite pad followed by a silica plug (2.0 cm of silica gel and eluted with 10% ethyl acetate in hexanes) The excess solvent was removed under reduced pressure and the resulting yellow oil was co evaporated with chloroform. The crude product was immediately carried on to the next step (0.52 mg). Rf 0.28 (30% ethyl acetate in hexanes); \(^1\)H NMR: (300 MHz, CDCl₃) 2.96 (m, 2H), 4.54 (m, 4H), 4.81 (m, 3H), 7.29 (m, 15H), 9.91 (s, 1H).

\((S)-\text{N,}N\text{-Dibenzyl-3-benzyloxy-4-enebutyramide} (7).\)

To a solution of n-butyltriphenylphosphonium bromide (82 mg, 1.9 mmol), in toluene (3 mL), at 0 °C, was added potassium hexamethyldisilazane (KHMDS) (0.7 mL, 0.5 M in toluene, 0.35 mmol). The orange solution was stirred for 30 minutes and then it was cooled down to -78 °C. \((S)-\text{N,}N\text{-Dibenzyl-3-benzyloxy-4-oxobutyramide} (52 mg crude) was dissolved in toluene (1 mL) and added to the original reaction while both were at -78 °C and the resulting mixture was stirred at room temperature for 12 hours. The reaction was slowly quenched with aqueous ammonium chloride (3 mL, 1N). The aqueous solution was then extracted with ethyl acetate (3X3 mL) and CH₂Cl₂ (5X3 mL). The combined organic layers were washed with saturated aqueous sodium chloride and dried over MgSO₄. The excess solvent was removed under reduced pressure and the product was chromatographed (10%
ethyl acetate in petroleum ether) giving a two step yield of 50% (34 mg,). \( R_f \) 0.42 (10% ethyl acetate in hexanes); \(^1\)H NMR: (300 MHz, CDCl\(_3\)) 0.99 (m, 3H), 1.45 (m, 2 H), 2.21 (m, 2H), 2.48 (m, 1H), 2.95 (m, 1H), 4.41 (m, 2H), 4.60, (m, 2H), 4.96 (m, 2H), 5.39, (m, 1H), 5.72 (m, 1H), 7.26, (m, 15H).

**(S)-N,N-Dibenzy1-3benzyloxy-4-oxobutyramide (8).**

To a solution of Dess-Martin reagent (1 g, solution periodinane 15% w/w in CH\(_2\)Cl\(_2\), 0.35 mmol) was added a solution of (S)-N,N-Dibenzy1-3-benzyloxy-4-enebutyramide (125 mg, 32 mmol) in CH\(_2\)Cl\(_2\) (3 mL). The mixture was stirred for 20 minutes, diluted with diethyl ether (3 mL) and washed with 5 mL of a 1:1 (v/v) solution of saturated aqueous NaHCO\(_3\) and Na\(_2\)S\(_2\)O\(_3\) (10% w/w in water). The aqueous layer was extracted with diethyl ether (2 X 20 mL). The combined organic layers were washed with water and saturated aqueous NaCl and dried over MgSO\(_4\). The excess solvent was removed under reduced pressure and the product was partially purified through a silica plug (eluted with 5 % ethyl acetate in hexanes). The solvent was removed in vacuo giving a yellow oil (104 mg) that was carried immediately to the following step.

**N-Benzyl-4-hydroxybutyramide (9)**

4-Benzyloxydihydrofuran-2-one (300 mg, 3.5 mmol) was dissolved in benzene (3.0 mL) under nitrogen. Benzylamine (0.76 mL, 7.0 mmol) was added to the reaction and the resulting solution was stirred for 2.0 hours at 50 °C. The mixture was diluted with ethyl acetate (15 mL), quenched with aqueous hydrochloric acid (HCL) (30 ml, 1N). The organic layer was washed with saturated aqueous sodium chloride (NaCl) and dried (MgSO\(_4\)). The
product was chromatographed (50% ethyl acetate in petroleum ether) to yield a clear
colorless oil (490 mg, 80%, yield). \( R_f \) 0.14 (50% ethyl acetate in hexanes); \( ^1H \) NMR: (300 MHz, CDCl₃) 1.22 (t, 1H), 1.88 (m, 2H), 2.28 (m, 2H), 3.68 (m, 2H), 4.32 (s, 1H), 6.28 (s, 1H), 7.26 – 7.35, (m, 5H).

N-Benzyl-4-\([\{(1,1\text{-dimethylethyl)}\text{dimethylsilyl}oxy\}\]-butyramide (10).

A solution of N-Benzyl-4-hydroxybutyramide (150 mg, 0.85 mmol) in dichloromethane (5 mL) was treated with imidazole (115 mg, 1.7 mmol) and chloro-\( \text{tert} \)-butyldimethylsilane (TBDMS) (190 mg, 1.3 mmol) at room temperature, and stirred for one hour. The organic layer was washed with saturated aqueous NaCl and dried over MgSO₄. The product was partially purified using column chromatography (70% ethyl acetate in petroleum ether) to yield a clear colorless oil (243 mg). \( R_f \) 0.84 (100% ethyl acetate). \( ^1H \) NMR: (300 MHz, CDCl₃) 0.018 (s, 6H), 0.88 (s, 9H), 1.93 (m, 2H), 2.94 (t, 2H), 3.63 (t, 2H), 4.15 (s, 1), 4.88 (s, 2H), 7.28 (s, 5H).

(1-oxo-4-\([\{(1,1\text{-dimethylethyl)}\text{dimethylsilyl}oxy\}\]-butyl)-(phenylmethyl)-, 1,1-
dimethylethyl ester (11).

A solution of N-Benzyl-4-\([\{(1,1\text{-dimethylethyl)}\text{dimethylsilyl}oxy\}\]-butyramide (230 mg, 0.79 mmol) in THF (6mL) was allowed to stir with di-\( \text{tert} \)-butyl dicarbonate (377 mg, 1.73 mmol) for 24 hours at room temperature. The mixture was washed with an aqueous solution of HCL (1N), water, a saturated aqueous solution of sodium bicarbonate, and a saturated aqueous solution of NaCl. The organic layer was dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (20% ethyl acetate in
hexanes) to give the desired product (230 mg, 87 % yield). \( R_f \) 0.75 (20% ethyl acetate in hexanes). \(^1\)H NMR: (300 MHz, CDCl \(_3\)) 0.27 (s, 6H), 0.88 (s, 9H), 1.41 (s, 9H), 1.87 (q, 2H), 2.97 (t, 2H), 3.66 (t, 2H), 4.89, (s, 2H), 7.26, (m, 5H).

\((1\text{-oxo-4-butanol})(\text{phenylmethyl}), 1,1\text{-dimethylethyl ester (12).}\)

The protected alcohol (11) (60 mg, 0.18 mmol) was dissolved in THF (2.0 mL), cooled to 0 °C, and hydrofluoric acid (0.5 mL, 10 mmol, 48% aqueous) was added. The reaction was allowed to warm up to room temperature and stirred for 45 minutes. The mixture was quenched with saturated aqueous sodium bicarbonate until pH 8.0 was reached. The THF was removed in vacuo, and the aqueous residue was extracted with dichloromethane (5X10 mL). The organic layers were combined and dried (MgSO\(_4\)). The product was chromatographed (10% ethyl acetate in petroleum ether) to give the desired product (48 mg, 98% yield) \( R_f \) 0.70 (10% ethyl acetate in hexanes). \(^1\)H NMR: (300 MHz, CDCl \(_3\)) 0.90 (t, 1H), 1.42 (s, 9H), 1.94 (q, 2H), 3.04 (t, 2H), 3.70 (t, 2H), 4.89 (s, 2H), 7.26, (m, 5H).

\((S)\text{-1,1,2-Triphenyl-1,2-ethanediol (13)}\)

\((S)\)-mandelic acid methyl ester (1.00 g, 6 mmol) was dissolved in 8.00 mL of dry tetrahydrofuran (THF). This mixture was added drop wise, at 0°C, to a solution of phenylmagnesium bromide in THF, prepared in the usual way from magnesium (0.690 g, 28 mmol) and bromobenzene (2.6 ml, 16 mmol). The mixture was refluxed for three hours. The reaction was quenched by the slow addition of 10 mL saturated aqueous ammonium chloride, and the resulting mixture was extracted with chloroform (2x30mL). The combined organics were dried (MgSO\(_4\)) and concentrated under reduced pressure. The residue was
purified by chromatography (30% ethyl acetate in hexanes) to give the desired product (1.04 g, 69% yield in one step). Rf 0.35 (20% ethyl acetate in hexanes). 1H NMR: (300 MHz, CDCl₃) 2.04 (s, 1H), 3.12 (s, 1H), 5.65 (d, 1H), 7.07-7.72 (m, 15H).

1,1,2-Triphenyl-1,2-ethanediol-2-acetate (14).

To a solution of (S')-1,1,2-Triphenyl-1,2-ethanediol (507 mg, 1.75 mmol) in dichloromethane, (8 mL), at 0 °C, was added pyridine (280 mg, 3.54 mmol) and the mixture was allowed to stir for 5 minutes. A solution of acetyl chloride (0.160 mL, 2.25 mmol) in dichloromethane (2 mL) was added dropwise, under nitrogen, and the resulting mixture was stirred for 2.0 hours while warming up to room temperature. The reaction was quenched by the addition of 5 mL of water; the solvent was evaporated under reduced pressure and the crystals were collected from the water layer. The product was washed with an aqueous solution of HCL (1N) and water. 1H NMR: (300 MHz, CDCl₃) 1.99 (s, 3H), 2.82 (s, 1H), 6.68 (s, 1H), 7.03-7.57 (m, 15H).

3-hydroxy-, 2-hydroxy-1,2,2-triphenylethyl ester (15).

A solution of the (S)-1,1,2-Triphenyl-1,2-ethanediol-2-acetate (14). (100 mg, .33 mmol) in dry THF (3 mL) was slowly added to a cooled (-78 °C) solution of LDA (0.81 mmol) in dry THF (5 mL). The mixture was stirred at this temperature for 1 hour and allowed to reach 0 °C. The mixture was cooled again to -78 °C and a solution of butyraldehyde (0.032 mL, 0.33 mmol) in dry THF (1 mL) was dropwise added within 5 minutes. The mixture was stirred at -78 °C for 1 hour and quenched with saturated NH₄Cl solution (10 mL). The mixture was extracted with chloroform (5X15) and the combined organic fractions were collected, washed...
with water and dried over MgSO₄. The solvent was removed in vacuo to yield a yellowish oil that was flash column chromatographed (90% chloroform in ethyl acetate) affording the desired product in 75% yield. \( R_f 0.48 \) (90% chloroform in ethyl acetate). \(^1\)H NMR: (300 MHz, CDCl₃) 0.87 (t, 3H), 1.31 (m, 4H), 2.38 (m, 2H), 2.80 (s, 1H), 3.84 (m, 1H), 7.04-7.52 (m, 15H).

**Hexanoic acid (16)**

Potassium hydroxide (400 mg, mmol) was added to a solution of 3-hydroxy-, 2-hydroxy-1,2,2-triphenylethyl ester (240 mg, 60 mmol) in methanol (30 mL) and water (10 mL). The mixture was refluxed for 2 hours under nitrogen. The resulting solution was cooled to room temperature and the methanol was removed under reduced pressure. The aqueous layer was extracted with chloroform (3×40 mL) to extract the chiral adduct. After the extraction 30 g of ice were placed in the aqueous solution, the mixture was acidified with HCl (1 N) was until pH 3 was reached. The resulting solution was saturated with NaCl and extracted with diethyl ether (5×60 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure. The product is a colorless oil (70 mg, %): \(^1\)H NMR (300 MHz, CDCl₃) 0.91 (t, 3H), 1.51 (m, 4H), 2.5 (m, 2H), 4.06 (m, 1H).

**Hexanoic acid, 3-[(1,1-dimethylethyl)dimethylsilyl]oxy] (17)**

Imidazole (0.36 g, 5.3 mmol) was added to a solution of tert-butyldimethylsilyl chloride (TBDMSCl) (0.28 g, 1.8 mmol) in dry DMF (3.0 mL). The solution was stirred in an ice bath under nitrogen for 15 min, followed by the addition of β-hydroxy acid (0.070 g, 0.53 mmol) in DMF (1.0 mL). The reaction was stirred overnight at room temperature. The mixture was
poured in a saturated solution of NaCl (10 mL) and extracted with a 1:3 mixture of diethyl ether and petroleum ether (5 X 10 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated to give the crude bis-silylated material. This material was dissolved in a mixture of methanol (7.0 mL), and tetrahydrofuran (THF, 3.0 mL). Potassium carbonate (0.15 g) dissolved in water (2.0 mL) was added, and the mixture was stirred overnight at room temperature. The solution was diluted with a saturated solution of NaCl (5.0 mL) and acidified to a pH 3.0 with 2N HCl. The solution was then extracted with a 1:3 mixture of diethyl ether and petroleum ether (5 X 10 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated. The TBDMS alcohol was removed in vacuo (2 Torr, overnight). The product is a colorless oil (0.11 g, 83 %): ¹H NMR (300 MHz, CDCl₃) 0.12 (d, 6H), 0.89 (s, 9H), 0.94 (t, 3H), 1.32 (m, 2H), 1.48 (m, 2H), 2.50 (m, 2H), 4.11 (m, 1H). ¹³C NMR (100 Mhz, CDCl₃) -4.6, -4.3, -4.2, -3.9, 14.31, 14.73, 18.6, 19.1, 26.0, 26.4, 39.6, 40.1, 42.0, 42.4, 69.5, 69.9, 178.3.

Hexanethioic acid, 3-hydroxy-, S-phenyl ester (18)

In 3 mL of dry DMF was dissolved 0.040 g (0.16 mmol) of Hexanoic acid, 3-[[[(1,1-dimethylthyl)dimethylsilyl]oxy]. The solution was cooled to 0 °C, and diphenylphosphoryl azide (DPPA) (0.11 mL, 0.48 mmol) and triethylamine (TEA) (0.090 mL, 0.65 mmol) were added. After two hours, benzene thiol (0.050 mL, 0.48 mmol) was added dropwise. The reaction was allowed to warm slowly to room temperature. After 24 hours, 4.0 mL of water were added, and the resulting mixture was extracted with diethyl ether (3X5 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated in vacuo. The residue was partially purified by chromatography (5% ethyl acetate in petroleum ether) to
give the intermediate thioester. The protected thioester was dissolved in acetonitrile (3.0 mL) and water (1.0 mL), and hydrofluoric acid (0.5 mL, 10 mmol, 48% aqueous) was added. After 2h, saturated aqueous sodium bicarbonate was added until pH 8.0 was reached. The acetonitrile was removed in vacuo, and the aqueous residue was extracted with dichloromethane (5X10 mL). The organic layers were combined and dried (MgSO₄). The product was chromatographed (10% ethyl acetate in petroleum ether) to give the desired product (17 mg, 44%, two steps). \( R_f 0.125 \) (10% ethyl acetate in hexanes); \(^1\)H NMR (300 MHz, CDCl₃) 0.94 (t, 3H), 1.54 (m, 4H), 2.84 (m, 2H), 4.11 (m, 1H), 7.42 (s, 5H). \(^{13}\)C NMR (100 Mhz, CDCl₃) 14.2, 19.5, 39.0, 51.8, 70.15, 122.2, 129.5, 130.0, 133.8, 193.7

Hexanethioic acid, 3-hydroxy-, S-[2-(acetylamino) ethyl] ester (19).

In 3 mL of dry DMF was dissolved 0.040 g (0.16 mmol) of Hexanoic acid, 3-[[[1,1-dimethylethyl]dimethylsilyl]oxy]. The solution was cooled to 0 °C, and diphenylphosphoryl azide (DPPA) (0.11 mL, 0.48 mmol) and triethylamine (TEA) (0.090 ml, 0.65 mmol) were added. After two hours, N-acetylcysteamine (0.052 mL, 0.48 mmol) was added dropwise. The reaction was allowed to warm slowly to room temperature. After 24 hours, 4.0 mL of water were added, and the resulting mixture was extracted with diethyl ether (3X5 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated in vacuo. The residue was partially purified by chromatography (50% ethyl acetate in petroleum ether) to give the intermediate thioester. The protected thioester was dissolved in acetonitrile (3.0 mL) and water (1.0 mL), and hydrofluoric acid (0.5 mL, 10 mmol, 48% aqueous) was added. After 2h, saturated aqueous sodium bicarbonate was added until pH 8.0 was reached. The acetonitrile was removed in vacuo, and the aqueous residue was extracted with
dichloromethane (5X10 mL). The organic layers were combined and dried (MgSO₄). The product was chromatographed (70% ethyl acetate in petroleum ether) to give the desired product (14 mg, 37%, two steps). Rf 0.21 (70% ethyl acetate in hexanes); ¹H NMR (300 MHz, CDCl₃) 0.93 (t, 3H), 1.54 (m, 4H), 1.96 (s, 3H), 2.74 (m, 2H), 3.04 (t, 2H) 3.46 (m, 2H), 4.11 (m, 1H). ¹³C NMR (100 Mhz, CDCl₃) 14.2, 19.5, 22.4, 30.2, 39.11, 39.2, 50.1, 71.0, 170.5, 194.8

Hexanethioic acid, 3-hydroxy-, S-butyl ester (20)

In 3 mL of anhydrous DMF was dissolved 0.040 g (0.16 mmol) of hexanoic acid, 3-[(1,1-dimethylethyl)dimethylsilyl]oxy]. The solution was cooled to 0 °C, and diphenylphosphoryl azide (DPPA) (0.11 mL, 0.48 mmol) and triethylamine (TEA) (0.090 ml, 0.65 mmol) were added. After two hours, n-butanethiol (0.051 mL, 0.48 mmol) was added dropwise. The reaction was allowed to warm slowly to room temperature. After 24 hours, water (4.0 mL) was added, and the resulting mixture was extracted with diethyl ether (3 x 5 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated in vacuo. The residue was partially purified by flash chromatography (5% ethyl acetate in petroleum ether) to give the intermediate thioester. The protected thioester was dissolved in acetonitrile (3.0 mL) and water (1.0 mL), and hydrofluoric acid (0.5 mL, 10 mmol, 48% aqueous) was added. After 2h, saturated aqueous sodium bicarbonate was added until pH 8.0 was reached. The acetonitrile was removed in vacuo, and the aqueous residue was extracted with dichloromethane (5 x 10 mL). The organic layers were combined and dried (MgSO₄). The residue was purified by flash chromatography (10% ethyl acetate in petroleum ether) to give
the desired product (18 mg, 52%, two steps). $R_f$ 0.30 (10% ethyl acetate in hexanes). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0.90 (m, 6H), 1.48 (m, 8H), 2.65 (m, 2H), 2.90 (t, 2H), 4.10 (m, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 13.4, 14.8, 19.5, 21.6, 28.7, 30.9, 39.6, 50.5, 70.9, 197.8

Coenzyme A, 3-Hydroxyhexanoic acid (21)

CoA (5 mg, 0.0063 mmol) was dissolved in 125 µL of 50mM potassium phosphate (KPi), pH 7.8 (saturated with nitrogen) in a 5 mL flask. The pH of the solution was adjusted to 7.8 by addition of 1 N NaOH. To this solution was added 3-hydroxy-hexanethioic acid, S-butyl ester (7.5 mg, 0.035 mmol) in 19 µL of acetonitrile. The flask was sealed, and the reaction mixture was stirred at 22 °C for 20h. The reaction mixture was extracted with diethyl ether (5x1 mL) to remove benzenethiol and excess hexanethioic acid, 3-hydroxy-, S-butyl ester.
Figure 15. $^1$H NMR of benzyl trichloroacetimidate (2)
Figure 16. $^1$H NMR of Benzyloxydihydrofuran-2-one (3).
Figure 17. $^1$H NMR of (S)-N-Benzyl-3-benzyloxy-4-hydroxybutyramide (4).
Figure 18. $^1$H NMR of (S)-N,N-Dibenzyl-3-benzyloxy-4-hydroxybutyramide (5).
Figure 19. Crude $^1$H NMR of (S)-N,N-Dibenzyl-3-benzyloxy-4-oxobutyramide (6).
Figure 20. $^1$H NMR of (R)-N,N-Dibenzyl-3-benzyloxy-4-enebutyramide (7)
Figure 21. $^1$H NMR of (R)-N,N-Dibenzyl-3-benzyloxybutyramide after first hydrogenation.
Figure 22. $^1$H NMR (S)-N,N-Dibenzyl-3-benzyloxy-4-oxobutyramide (8).
Figure 23. $^1$H NMR N-Benzyl-4-hydroxybutyramide (9)
Figure 24. $^1$H NMR of (1-oxo-4-[[1,1-dimethylethyl]dimethylsilyl]oxy]-butyl)(phenylmethyl)-, 1,1-dimethylethyl ester (11).
Figure 25. 1H NMR of (1-oxo-4-butanol)(phenylmethyl)-, 1,1-dimethylethyl ester (12).
Figure 26. $^1$H NMR of 1,1,2-Triphenyl-1,2-ethanediol (13).
Figure 27. $^1$H NMR of 1,1,2-Triphenyl-1,2-ethanediol-2-acetate (14).
Figure 28. $^1$H NMR of 3-hydroxy-, 2-hydroxy-1,2,2-triphenylethyl ester (15).
Figure 29. $^1$H NMR of hexanoic acid (16).
Figure 30. $^1$H NMR of Hexanoic acid, 3-[[1,1-dimethylethyl]dimethylsilyl]oxy] (17).
Figure 31. $^{13}$C NMR of Hexanoic acid, 3-[[((1,1-dimethylethyl)dimethylsilyl]oxy]] (17).
Figure 32. HETCOR of Hexanoic acid, $3-	ext{[(1,1-dimethylethyl)dimethylsilyl]oxy]}$ (17).
Figure 33. $^1$H NMR of Hexanethioic acid, 3-hydroxy-, S-phenyl ester (18)
Figure 34. $^{13}$C NMR of Hexanethioic acid, 3-hydroxy-, S-phenyl ester (18)
Figure 35. HETCOR of Hexanethioic acid, 3-hydroxy-, S-phenyl ester (18)
Figure 36. $^1$H NMR of Hexanethioic acid, 3-hydroxy-, S-[2-(acetylamino) ethyl] ester (19)
Figure 37. $^{13}$C NMR of Hexanethioic acid, 3-hydroxy-, S-[2-(acetylamino) ethyl] ester (19)
Figure 38. HETCOR of Hexanethioic acid, 3-hydroxy-, S-[2-(acetylamino) ethyl] ester (19)
Figure 39. $^1$H NMR of Hexanethioic acid, 3-hydroxy-, S-Butyl ester (20)
Figure 40. $^{13}$C NMR of Hexanethioic acid, 3-hydroxy-, S-Butyl ester (20)
Figure 41. HETCOR of Hexanethioic acid, 3-hydroxy-, S-butyl ester (20)
Figure 42. $^1$H NMR of Coenzyme A, 3-hydroxyhexanoic acid (21).
Figure 43. HETCOR of Coenzyme A, 3-hydroxyhexanoic acid (21).
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