Nonactin biosynthesis: studies toward the synthesis of stable isotope labeled potential precursors

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NONACTIN BIOSYNTHESIS:
STUDIES TOWARD THE SYNTHESIS OF STABLE ISOTOPE LABELED POTENTIAL
PRECURSORS

by
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NONACTIN BIOSYNTHESIS: STUDIES TOWARD THE SYNTHESIS OF STABLE ISOTOPE LABELED POTENTIAL PRECURSORS

Nonactin is a polyether antibiotic macrotetrolide produced by *Streptomyces griseus* that is a potent inhibitor of the P170-glycoprotein efflux pump responsible for drug resistance in cancer cell lines. Nonactin is an atypical polyketide that consists of four monomer units of nonactate. Previous studies have confirmed that the primary metabolic precursors of nonactate are acetate, propionate and succinate. The late intermediate in the nonactate biosynthesis has been confirmed by feeding studies. The biosynthesis pathway from the primary metabolites to the late intermediate have so far not been confirmed.

A synthesis for an unconfirmed intermediate, a [2,3-\(^{13}\)C]-6,8-dihydroxy-2-methylnonanethioic ester, has been proposed with unlabeled material and nearly completed. The synthesis is based on the Mg\(^{2+}\)-pyridine dependent coupling of \(\beta\)-ketoesters and acyl halides and the introduction of stereochemistry by stereoselective reduction using chiral ruthenium complexes to afford anti-1,3-diols. The most promising synthesis was completed through (3R,5R)-3,5-diacetoxyhexan-1-ol, which at this point requires conversion into (4R,6R)-4,6-diacetoxyheptan-1-al. In order to couple propionate stereospecifically to (4R,6R)-4,6-diacetoxyheptan-1-al, the chiral auxiliary (4S)-4-(phenylmethyl)-propanoyl-1,3-oxazolidinone was prepared. An efficient synthesis for [2-\(^{13}\)C]-propionic acid was developed with unlabeled material.
For Gisela and Bengt
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CHAPTER 1

INTRODUCTION

1.1 Natural Products and Antibiotics

Natural products have long been a source of compounds of medicinal value. A wide range of biologically active secondary metabolites has been identified by screening for antibacterial and antifungal properties, but with the advent of modern screening methods, many natural products with antiviral, antitumor, antimetabolic, and immunological activities have been identified. Among the natural products of clinical or agricultural importance, most are produced by Actinomycetes, in particular by the genus *Streptomyces*.

With modern assays, natural products can not only be screened for antiproliferative properties but also tested for inhibition of enzymes, gene expression, and signal transduction. Traditionally, antibacterial compounds were the most studied category within the natural products. The emergence of new infectious diseases, many of which can not be treated effectively, has led to renewed research for new active compounds. Aside from emerging diseases, many bacterial strains are exhibiting antibiotic resistance, which has become a major public health issue, as the number of infective diseases that can be treated effectively decreases. Even for antibiotics such as vancomycin, that have been used only as a last defense when all else fails, the number of resistant strains has drastically increased. The ultimate goal of antibiotic research is to discover and characterize fundamentally different and new compounds.
and to study in detail all the applications of the natural products that are commercially available using new technology and assays.\(^5\)

Natural products are intrinsically complex and thus difficult to synthesize. For example the first total synthesis of penicillin (1) by Sheehan et al. took nearly ten years to develop.\(^6\)

\[
\text{penicillin V (1)}
\]

Synthesis can, however, be used to study the natural biosynthesis pathways and the mechanisms by which the organism assembles the final natural product. Research of the genetics involved in the process complements the chemistry.\(^7\) Knowledge of the genes involved in the biosynthesis permits the manipulation of the biosynthesis pathways.\(^8\) The microbial organism can, for example, be used for the production of the final product, or a late intermediate can be isolated by modifying the genes. The intermediate can be chemically converted into a range of analogues. Libraries of potential active compounds can be produced in this manner, which is much more effective than synthesizing them individually. Once the chemistry is known and the genetics have been elucidated, the pathway can be manipulated for development and finally the production of new drugs.
Polyketides constitute a broad class of natural products. They are synthesized by large protein complexes, referred to as polyketide synthase (PKS) complexes. Polyketide biosynthesis is related to fatty acid biosynthesis, so it is important to understand fatty acid biosynthesis. All reactions in fatty acid biosynthesis are catalyzed by a complex of enzymes called the fatty acid synthase (FAS). The FAS complex from *Escherichia coli* for example consists of seven proteins. The structure of the FAS complex differs from organism to organism, but the reactions it catalyzes are universal. Fatty acid biosynthesis is a four-step elongation process (Figure 1). Initially, an acyl carrier protein ketosynthase (ACP-KS) of the enzyme complex accepts an acetyl group with its free thiol, a process catalyzed by acetyl-CoA-ACP transacylase, and the acetyl group is transferred to a neighboring ketosynthase (KS). The acyl carrier protein ketosynthase (ACP-KS) now has a free cysteine residue again which is charged with a malonyl group, catalyzed by malonyl-CoA-ACP transferase. The enzyme complex is now charged for the first step, a decarboxylative Claisen condensation. A β-ketoacyl-ACP synthase (KS) catalyzes the reaction, which generates a molecule of carbon dioxide. In the second step, the β-keto carbonyl of the acetoacetyl group is reduced by β-ketoacyl-ACP reductase (KR) with the reduced form of nicotinamide adenine dinucleotide (NADPH) serving as the electron donor, resulting in a β-hydroxyacyl-ACP complex. In the following step, β-hydroxyacyl-ACP dehydratase (DH) catalyzes a dehydration reaction to give the corresponding trans-Δ^2^-acyl-ACP complex. The double bond is reduced in the final step by an enoyl-ACP reductase (ER) to yield the acyl-ACP complex. This is an NADPH-dependent step as well. Finally, the chain is transferred back to the KS by an acyl transferase (AT) to free up the ACP for a new
Figure 1: Fatty Acid Biosynthesis
molecule of malonate. This process is repeated in an iterative fashion to elongate the chain to the desired length.

Fatty acid synthase complexes are divided into two main categories based on structural differences. Type I FASs are usually found in fungi and animals. They consist of a large modular protein complex. Each individual protein has catalytic domains for multiple functions, so each protein can carry out different reactions. Type II FASs are usually found in plants and bacteria. They consist of multiple proteins that are encoded by individual genes. The individual enzymes each catalyze a unique reaction. Since the reactions that are carried out are the same for either type, the catalytic domains of type I FASs are in function analogous to the individual enzymes of a type II FAS.

Like FAS complexes, systems for the synthesis of polyketides fall into two main structural categories, type I or type II. Type I PKS complexes synthesize the final natural product in a processive fashion. Modifications are carried out after each elongation step before the next extender unit is added. Type II PKS complexes on the other hand assemble the entire backbone of the molecule before modifying it. Although type I and type II PKS utilize the same reactions, they result in distinctly different compounds.

1.2.1 Type I Polyketides

Two major classes of type I polyketides have been studied extensively, macrolides (Figure 2) and polyethers (Figure 3). The term macrolide was introduced by Woodward to define a number of natural antibiotics that possessed a lactone macrocycle. The first macrolide antibiotic reported in the literature was pikromycin (2). Methymycin (4) was the first macrolide whose structure was determined. The most extensively studied type I polyketide is erythromycin (6). Erythromycin is a 14-

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membered macrolactone originally isolated from *Saccharopolyspora erythraea*. The erythromycin PKS consists of three large proteins that in turn each have two modules. The putative product of the PKS is 6-deoxyerythronolide B (10, 6-DEB), which illustrates the 'processive' assembly (Figure 4). The final antibiotic is obtained after post-PKS modifications (Figure 5). The modules contain the sites for catalytic activity of modifications, such as reductions or eliminations. After addition of a malonyl extender unit, the chain is modified before addition of the following extender unit. This means that each catalytic domain has a specific activity that is only utilized once per molecule synthesized.

![Chemical structures](image)

**Figure 2: Type I Polyketides – Macrolides**

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Polyethers are the second major class of type I polyketides. Monensin A (7), an antibiotic used in veterinary medicine, is a polyether ionophore isolated from *Streptomyces cinnamonensis*. The biosynthesis of polyethers has not been studied as extensively as that of macrolides. In 1979, Kishi et al. disclosed the first total synthesis for monensin. Soon after, Still et al. proposed a different route. Initially, monensin was proposed to be biologically assembled from an all-(E) triene intermediate, following the Cane-Celmer-Westley hypothesis. The triene was synthesized, but in a feeding study, it failed to incorporate. This lead to the development of an alternative hypothesis for the biosynthesis, the Townsend-Basak-McDonald proposal. The suggestion was that monensin is obtained from an all-(Z) triene intermediate.
intermediate is proposed to undergo a series of metal-mediated syn-oxidative cyclization reactions to give the sequence of cyclic ethers. The difference between the Cane-Celmer-Westley hypothesis and the Townsend-Basak-McDonald proposal is the stereochemistry of the product (Figure 6). There is biomimetic experimental evidence that supports the Townsend-Basak-McDonald proposal.
6-deoxyerythronolide B

Erythronolide B (12)

TDP-myrcarose glycosyltransferase

TDP-desosamine glycosyltransferase

Erythromycin D (14)

C-12 Hydroxylase

3-O-Mycarosyl erythronolide B (13)

Erythromycin C (16)

Erythromycin B (15)

Erythromycin A (6)

Figure 5: Post-PKS Modifications of 6-DEB
1.2.2 Type II Polyketides

Type II polyketide synthase complexes resemble type II FAS complexes. The synthesis is performed by individual proteins, rather than domains of a large complex. Each protein, with the exception of ketoreductase (KR) enzymes, has a specific function that can be utilized more than once in the synthesis of a single molecule. The modifications, such as reductions and eliminations, are likely carried out after the backbone has been assembled. The chain elongation is exclusively carried out with malonyl-CoA as an extender unit. The intermediates have been proposed to be poly-β-ketones. The diversity in type II polyketides stems from the variation in chain length, the number of possibilities in folding and cyclization patterns, as well as the modifications after the intermediate is assembled. The typical products of type II PKS are polycyclic, aromatic compounds, such as daunorubicin (17), actinorhodin (19) and oxytetracycline (20) (Figure 7).
Figure 7: Type II Polyketides

The best studied type II polyketide biosynthesis is the actinorhodin system. Actinorhodin (19) is an antibiotic isolated from *Streptomyces coelicolor A3(2)*. The actinorhodin gene cluster was the first PKS gene cluster to be characterized, so it has served as a model system since then. The biosynthesis cluster contains a minimal PKS, a ketoreductase (KR), a cyclase (CYC) and an aromatase (ARO). The minimal PKS is a three-protein complex, with a ketoacyl synthase/acyl transferase (KS/AT), later renamed as KSα, a chain length factor (CLF), later renamed as KSβ, and an acyl carrier protein (ACP) (Figure 8).
The biosynthesis of actinorhodin is typical for a type II polyketide (Figure 9). The nonactin biosynthesis cluster on the other hand encodes an atypical PKS. The genes are homologues of type II PKS genes, but they differ noticeably from other type II PKS genes and they encode the synthesis of a non-aromatic, non-polycyclic compound, nonactin.
Figure 9: Biosynthesis of Actinorhodin
1.3. Nonactin

Nonactin (27) is the parent compound of a series of homologous macrocyclic ionophore macrotetrolide antibiotics (Figure 10). Macrotetrolides were initially isolated by Prelog et al. from *Streptomyces griseus* ETH A7796, and subsequently from *S. longisori* and *S. chrysomallus*. The structures were elucidated by spectroscopy and degradation. The crystal structure confirmed both structure and stereochemistry later on. The macrotetrolides are 32-membered macrocycles assembled from four monomer units (Figure 11). Nonactin consists of alternating (+)-nonactate (32) and (-)-nonactate units, so each nonactin molecule contains two (+)-nonactate and two (-)-nonactate molecules. The resulting macrotetrolide has $S_4$ symmetry; therefore it is achiral. The nonactate units can be replaced by homononactate (33) or bishomononactate (34). The replacement of a nonactate unit with a homononactate unit results in monactin (28). Further substitution can lead to even higher homologues, the other naturally occurring macrotetrolides.

\[
\begin{align*}
\text{Nonactin (27)} & \quad \text{Me} & \text{Me} & \text{Me} & \text{Me} \\
\text{Monactin (28)} & \quad \text{Me} & \text{Me} & \text{Et} & \text{Me} \\
\text{Dinactin (29)} & \quad \text{Et} & \text{Me} & \text{Et} & \text{Me} \\
\text{Trinactin (30)} & \quad \text{Et} & \text{Et} & \text{Et} & \text{Me} \\
\text{Tetranactin (31)} & \quad \text{Et} & \text{Et} & \text{Et} & \text{Et}
\end{align*}
\]

Figure 10: Naturally occurring macrotetrolides
R = Me  (+)-nonactic acid (32)  
R = Et  (+)-homononactlc acid (33)  
R = 'Pr  (+)-bishomononactic acid (34)

Figure 11: Naturally occurring monomer homologues

Macrotetrolides are ionophores which can bind mono- and divalent ions. As such, nonactin and its homologues can transport ions across membranes. The structures of nonactin-ion complexes have been studied and it was found that the carbonyl oxygen atoms and oxygen atoms in the tetrahydrofuran rings coordinate to the ion while the molecule is wrapped around the ion.\(^{30}\) The ability for ion transport is the basis for its antimicrobial activity and cytotoxicity.\(^{31}\)

The macrotetrolides exhibit a range of activity. Nonactin is an antibiotic effective against Gram positive bacteria, fungi and mycobacteria.\(^{26}\) All homologues are active \textit{in vitro} against tumor cell lines.\(^{32}\) They also have shown anti-tumor activity \textit{in vivo} in mice.\(^{33}\) Nonactin is an efficient inhibitor of the P170 glycoprotein-mediated efflux of 4'-O-tetrahydropyranyladriamycin in multi-drug resistant erythroleukemia K562 cells.\(^{34}\) Nonactin shows cytotoxic effects in mammalian cells, which unfortunately precludes its use in clinical trials. Nonactin is a useful lead compound, and when its mechanism for antimicrobial activity is fully understood, the information can be used for the development of new compounds.
Figure 12: Hypothesis for nonactin biosynthesis
1.4. Nonactin Biosynthesis

According to early studies with radioisotope labeled compounds, nonactin is synthesized from acetate, propionate and succinate.\textsuperscript{35} The monomer nonactate has been shown to be present in the culture. Since the initial study, nonactin biosynthesis has been studied by extensive feeding studies of compounds labeled with radioisotopes and stable isotopes.\textsuperscript{36-41} Robinson et al.\textsuperscript{39} have postulated a biosynthesis pathway for nonactin based on polyketide biosynthesis, which has since been expanded by Priestley et al.,\textsuperscript{42} and now branches out into two enantiocomplementary pathways to yield an enantiomeric pair of acyclic diols (45 and 46) (Figure 12). Each enantiomer is cyclized by enzymes to result in (-)-nonactate and (+)-nonactate, respectively. The monomeric units are linked stereospecifically by enzymes to form the macrotetrolides.

The earliest sequencing work was done by Plater and Robinson.\textsuperscript{43} They isolated a 3.3 kbp DNA fragment from \textit{S. griseus} that conferred tetranactin resistance (\textit{nonR}) on \textit{S. lividans}. The \textit{nonR} gene encodes a deduced protein with an active site motif found in serine proteases and esterases. This early work has been continued and so far approximately 40 kbp have been sequenced.\textsuperscript{44-46} The cluster appears to encompass a total of 39 kbp comprising 30 open reading frames (ORF). Out of these ORFs, ten appear to be part of the nonactate PKS (Figure 13). These genes encode ketoacyl synthase units (\textit{orf7}, \textit{nonN}, \textit{nonQ}, \textit{nonK} and \textit{nonJ}), ketoacyl reductase units (\textit{nonO}, \textit{nonP}, \textit{nonM} and \textit{nonE}) and a 3-ketoacid:succinyl-CoA CoA-transferase (\textit{orf8}). The genes \textit{nonS} and \textit{nonS'} encode a pair of nonactate synthases, one gene potentially for each enantiomer. The enzyme \textit{nonR} is a serine esterase, as mentioned above. Another resistance mechanism is encoded on genes \textit{orf5/6}. The deduced protein of \textit{orf5/6} has homology to an ABC transporter. The deduced protein \textit{NonD} shows a strong
homology with cocaine esterase. By homology, nonL encodes a putative ligase. The gene nonG has been postulated to encode a transcriptional repressor. The function of nonB, nonC and nonF has not been deciphered yet.

![Figure 13: Current map of the nonactate biosynthesis cluster](image)

The synthesis of one enantiomer of nonactate requires three condensation and three reduction reactions. If each enzyme was only used once in the synthesis, and each enantiocomplementary pathway requires its own protein, the synthesis would require six synthases and six reductases. If each enzyme could catalyze the synthesis of either enantiomer, the cluster should have three synthases and three reductases. The cluster has five synthase and four reductase genes, so some of the enzymes are used in both pathways and some are only used in one of the enantiocomplementary pathways. The challenge lies in determining how the genes are shared in the two pathways and in understanding how stereochemical control in the synthesis is attained.
1.5. Current Chemical Work

There are two generally accepted methods for studying biosynthesis pathways. Advances in biotechnological tools opened up the possibility of studying natural product biosynthesis by isolation and characterization of biosynthesis genes and the proteins they encode. Genetic modification of the original organism, followed by studies of how the mutation affects the organism's ability to make the natural product under investigation, have allowed the assignment of conversions in the biosynthesis to specific enzymes.

The classic technique for elucidating biosynthesis pathways is the feeding of isotopically labeled compounds into fermentative cultures. The natural products are subsequently isolated from the culture and analyzed. Both radioactive and stable isotopes have been used, but radioactive isotopes require that the substrate is chemically degraded, so the position of the radioactive label may be determined. Stable isotopes are most widely used since advances in nuclear magnetic resonance (NMR) spectroscopy make it possible to assign the position of the label from the spectrum of an entire molecule and determine the level of isotope incorporation. The most commonly encountered stable isotopes are $^{13}$C, $^2$H, $^{15}$N, and $^{18}$O which is used in mass spectroscopy (MS) studies. Single $^{13}$C labeled compounds only provide information about the final location of an individual atom, much like radioactive isotope studies. They do not provide information about what bonds are broken in the synthesis or the fate of the hydrogen atoms. This has led to the use of dual labels.

Dual labels may consist of $^2$H used adjacent to $^{13}$C or of two adjacent $^{13}$C atoms. Because of deuterium's low natural abundance of 0.012%, minimal incorporation is...
required for detection even with a single label. Deuterium labels alone have the
drawback of resulting in broad peaks in the $^2\text{H}$ NMR spectrum and exhibiting low
sensitivity. When $^2\text{H}$ is adjacent to $^{13}\text{C}$, an $\alpha$-isotope shift in the $^{13}\text{C}$ NMR spectrum is
observed, so dual $^2\text{H}$-$^{13}\text{C}$ labeled compounds have been used to track the fate of
deuterium atoms.

An alternative tool is the incorporation of a dual $^{13}\text{C}$-$^{13}\text{C}$ label. Adjacent $^{13}\text{C}$
labels are easily identified by a characteristic coupled doublet in the $^{13}\text{C}$ NMR spectrum.
With a natural abundance of 1%, the natural occurrence of adjacent $^{13}\text{C}$ atoms is about
0.01%. The detection limit for incorporation is very low. The coupling giving the
observed doublet indicates that the bond is still intact. If the bond was broken and the
$^{13}\text{C}$ isotopes detected individually, the doublet would not be observed, so the
observation of this labeling pattern has also been referred to as ‘bond labeling’. Bond
labels are very useful because they supply the information about whether the labeled
bond was broken in vivo.

Dual $^{13}\text{C}$-labeled potential intermediates will be used to probe the nonactin
biosynthesis pathway. Nonactin is an achiral tetramer of nonactate units. In the
assembly of the macrocycle, both enantiomers of nonactate are required. In order to
target a minimal number of likely intermediates to synthesize, initial target compounds
were chosen that are close to confirmed intermediates.

In the nonactin biosynthesis pathway recently proposed by Priestley et al. (Figure
12) $^{42}$, a single ketoreductase (KR) reduces two of the carbonyls in 36, but each
enantiomer requires its unique KR, so two KRs are required to obtain 37 and 38. The
following transthioesterification is most likely performed by the 3-ketoacid:succinyl CoA
CoA-transferase (TRA) encoded by orf8. The diols 39 and 40 thus obtained require two
more ketoacyl synthases (KS) for the condensation with a methylmalonyl extender unit,
one for each enantiomer. The β-ketone of each enantiomer is then reduced by a unique reductase enzyme to give 43 and 44. The final steps of elimination and cyclization have been assigned to nonS and nonS'. This pathway would explain why there are five ketoacyl synthase genes and four ketoacyl reductase genes.

The last confirmed intermediate 6,8-dihydroxy-2-methylnon-2E-enethioate ester (54) is preceded by the putative intermediate 6,8-dihydroxy-2-methylnonanethioate ester (53).
In order to establish the mechanism for the step previous to the elimination
catalyzed by nonS/S', the synthesis of all eight stereoisomers of thioester activated [2,3-
\cite{56}-13C]-3,6,8-trihydroxy-2-methylnonanethioate was attempted (56).

The placement of dual \(^{13}\text{C}\) labels on carbon-2 and carbon-3 will serve to
establish that the only modification taking place to get to the intermediate 54 is the
elimination of the hydroxyl on carbon-3 and that the labeled bond is not broken in the
process.

\textbf{Figure 15: Target Compounds (56)}
CHAPTER 2

RESULTS AND DISCUSSION

2.1. Retrosynthetic Analysis

In our current hypothesis for the nonactin biosynthesis, the earliest and latest intermediates have been confirmed by feeding studies with isotopically labeled molecules. In order to confirm more of the biosynthesis pathway, the latest not yet confirmed intermediate was chosen as a synthetic target. The objective of this research project was to first develop a synthetic route to [2,3-^13C]-S-[2-(acetylamino)ethyl] (2S,3S,6R,8R)-3,6,8-trihydroxy-2-methylnonanethioate (57) that could then be readily adapted to synthesize the other seven stereoisomers.

Retrosynthetic analysis began with a disconnection at the thioester of 57 (Figure 16). The fragment (58) carrying the two labels was disconnected at the labeled bond between C2 and C3 to yield [1-^13C]-4,6-dihydroxyheptanal (60) and [2-^13C]-propionate (61). Since the propionate had to be added stereospecifically, a chiral auxiliary was necessary. Oxazolidinones have been well-studied and show excellent stereoselectivity when they are used in boron-enolate coupling. [2-^13C]-Propionate was not commercially available, so it became necessary to synthesize it. In order to synthesize 60, the anti-1,3-diol backbone had to be set up, which was the most complex feature. Coupling reactions of acetoacetate esters with halides in the presence of magnesium chloride and an amine base, such as pyridine, have been developed as a way to set up asymmetric 1,3-diketones. It seemed to be a promising route to utilize
the stereospecific hydrogenation developed by Noyori\textsuperscript{56,57} to reduce the asymmetric 1,3-diketone to the corresponding \textit{anti}-1,3-diol. As an alternative, the 1,3-dioxan-4-one chemistry studied by Rychnovsky\textsuperscript{58-61} was an option. Here the stereochemistry of the 1,3-diol is embedded in the dioxane, which is prepared from the corresponding 2-hydroxycarboxylic acid.
2.2. Synthesis of [2-\textsuperscript{13}C]-Propionate

The synthesis of [2-\textsuperscript{13}C]-propionate was studied by two routes. In the first case (Figure 17), the starting material, anisaldehyde, was converted into the corresponding 1,3-dithiane and then substituted with ethyl bromide. The label would have been incorporated by using [2-\textsuperscript{13}C]-ethyl bromide. Oxidation with \textit{m}-chloroperoxybenzoic acid (\textit{mCPBA}) and base hydrolysis yielded propionate. As an alternative, carbon dioxide was added to ethyl magnesium bromide in a simple Grignard reaction. In the corresponding labeled synthesis, [2-\textsuperscript{13}C]-ethyl bromide would have been converted into the Grignard reagent [2-\textsuperscript{13}C]-ethyl magnesium bromide.

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

Figure 17: Synthesis of [2-\textsuperscript{13}C]-Propionic Acid via Dithiane
As a classic example of an Umpolung synthon, 1,3-dithianes have been used to convert aldehydes into ketones. The carbonyl in the aldehyde is electrophilic, but by converting it into a 1,3-dithiane, the carbon now carries a fairly acidic hydrogen. Treatment with a strong base, such as n-butyl lithium (nBuLi), yields the dithiane anion, a very nucleophilic species. The dithiane anion undergoes $S_{N}2$ substitution with halides. Stütz and Stadler used the anion of 2-phenyl-1,3-dithiane as a nucleophile in addition reactions to prepare 2-alkylsulfanyl-2-phenyl-1,3-dithiane. The 1,3-dithiane was easily hydrolyzed to yield the corresponding ketone. Two prevalent methods are currently used for the regeneration of the carbonyl. Hirano et al. pioneered a heterogeneous cleavage reaction using $Fe(NO_3)_3$-silica gel in hexanes. Stütz and Stadler used anhydrous $CuCl_2$ and $CuO$ in acetone. The latter method is known to be a very reliable and versatile reaction.

In this synthesis, anisaldehyde was chosen as the starting material because the aromatic ring chromophore made it much easier to track the progress of the synthesis by TLC. The formation of 63 was adopted from the standard procedure for the protection of diols as dioxanes. The yield was 65.9% which did not reflect accurately the actual efficiency of the reaction since a substantial amount of product was lost during the purification. The subsequent step of generating the anion with nBuLi and then adding the nucleophile was adopted from the procedure of Stütz and Stadler. The anion was generated at $-22^\circ C$ and trapped with ethyl bromide to give the desired product 64 in 95.9% yield. Compound 64 was cleaved to yield 65 using $CuO$ and $CuCl_2$. Problems arose with the separation and purification of the product. During the aqueous base wash of the reaction mixture, $CuCl_2$ dissolved in the aqueous phase, but $CuO$ formed a fine suspension of deep orange color in the organic phase and could not be removed except by chromatography on silica gel. The final yield of 59.1% probably reflected that some
of the product was lost with the CuO. This chemistry was very elegant, but the added steps decreased the overall yield dramatically. The overall yield for the sequence from anisaldehyde to 65 was 37.4%. This was particularly poor considering that the $^{13}$C-label would be introduced before the reaction with the lowest yield, so most of the labeled compound would be lost. The sequence was not particularly amenable to scale-up. A larger scale preparation of 63 was attempted from which no product was isolated.

![Chemical Reaction]

Figure 18: Synthesis of [2-$^{13}$C]-Propionate via Grignard

In parallel to the above route, propionate was generated via a Grignard reaction. The nucleophilic addition of carbon dioxide to a Grignard reagent yields a magnesium salt that is hydrolyzed to give the corresponding acid during the work-up with aqueous dilute acid. Propionate could therefore be prepared by the addition of carbon dioxide to ethyl magnesium bromide. For the synthesis with the labeled starting material, the Grignard reagent would have to be prepared from [2-$^{13}$C]-ethyl bromide.

Dry magnesium powder in a tetrahydrofuran (THF) suspension was activated with a small amount of 1,2-dibromoethane. The by-product, ethene, was observed to bubble out of solution. Ethyl bromide was added to the activated magnesium to form ethyl magnesium bromide. Barium carbonate was treated with concentrated hydrochloric acid as a source of dry carbon dioxide. The carbon dioxide produced was directly channeled through a drying tube into the solution with the Grignard reagent and frozen out in a liquid nitrogen bath. Once the flask was sealed, it was allowed to thaw,
thus placing the flask under a positive carbon dioxide pressure. Propionate was isolated by lyophilization in 86% yield. This was an efficient synthesis for $[2-^{13}\text{C}]$-propionate.

The major advantages of this route were that it was quick, since it involved a single step, and that excess carbon dioxide could be used, since it is economical and readily available. Lyophilization was not a quick way to remove excess solvent, but the procedure can be performed in a reasonable timeframe if the solvent volume is kept to a minimum and a good vacuum is applied. Overall, propionate was obtained in sufficient yield in one step.

2.3. Synthesis of Oxazolidinones

Substituted oxazolidinones were first prepared by Newman and Kutner. They developed a method to convert ketones $\text{RCOR'}$ into aldehydes $\text{RR'CHCHO}$ by substituting 3-nitroso-2-oxazolidinones and then decomposing the oxazolidinones under mild conditions. In an example, methyl bromoacetate was coupled with acetone in the presence of activated zinc (Reformatsky reaction) to yield 68 (Figure 19). The corresponding hydrazide 69 was prepared by addition of hydrazine. The hydrazide was rearranged in the presence of nitrite to yield oxazolidinone 70. The nitroso-derivative 71 was obtained after treatment of 70 with nitrosyl chloride. Decomposition of 71 under basic conditions yielded 72. In an alternative route, oxazolidinones were prepared from the corresponding aminoalcohol with phosgene to obtain authentic material.

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Mukaiyama studied dialkylboron triflates to regioselectively generate Z-enolates (Figure 20). A ketone, such as 1-phenylbutan-1-one (73), was enolized with a dialkylboron triflate and a nitrogenous base. The borane-adduct was allowed to condense with an aldehyde. High stereoselectivity and regioselectivity were observed under these relatively mild conditions.®^ Evans continued the study of Mukaiyama-type boron-enolate coupling reactions and discovered that oxazolidinones were useful chiral auxiliaries in these reactions (Figure 21). Their major advantages were high enantioselectivity and their economical nature because they were easily recycled.®^ Initially, Evans employed the oxazolidinones derived from valinol (77) and norephedrine, but a variety of amino alcohols have been shown to be suitable precursors.®^ The oxazolidinones were enolized with either lithium diisopropylamine (LDA) or dibutylboron...
triflate to give only the Z-enolate. These enolates underwent Aldol condensations with various aldehydes to yield only the erythro-adduct (79) (Figure 21).

Since Evans began studying oxazolidinones, alternate methods for their preparation have been developed. Originally, Newman and Kutner employed either phosgene or diethyl carbonate and methoxide. Davies and Doisneau discovered that anhydrous potassium carbonate was more reliable than methoxide and since then, their method has become generally used.
Oxazolidinones (80) readily undergo substitution at the amide nitrogen when they are treated with a strong base, such as nBuLi, followed by addition of an acyl halide (Figure 22). Alternatively, a mild nitrogenous base like triethylamine was used in the presence of catalytic 4-(dimethylamino)pyridine (DMAP) to add an anhydride. In this particular synthesis, the preparation of the labeled propionate is the limiting factor. The anhydride would have to be prepared with a label on both acetate groups and half of the labeled compound would be wasted. Therefore, the method of Ager et al. was not used.

Two different routes were studied to introduce the $^{13}\text{C}$-label. In the direct route the oxazolidinone was substituted with propionyl chloride. The disadvantage of this particular synthesis is the expense of [2-$^{13}\text{C}$]-ethyl bromide. Alternatively, acetylation of
the oxazolidinone with acetyl chloride was explored, followed by addition of a methyl group. Kelly et al. used this approach to introduce $^{13}$C-labels with $[^{13}$C]-methyl iodide in various positions of the propyl substituent. This route had two advantages over the direct addition of labeled propionate. Excess methyl iodide could be used to ensure a complete reaction because it would be unlabeled. $[^{2-13}$C]-Acetyl chloride is significantly less expensive than $[^{2-13}$C]-ethyl bromide.

Methyl ester 83 was prepared from L-valine to activate the acid towards reduction (Figure 23). The methyl ester was reliably prepared in 85% yield. Poindexter and Meyers had observed that both sodium borohydride and lithium aluminum hydride reduced various amino acid esters to the corresponding amino alcohol under retention of configuration. In this sequence, 83 was readily reduced by lithium aluminum hydride to give 84 in over 60% yield. For the cyclization of 84 to give oxazolidinone 85, the
method of Davies and Doisneau was employed and resulted in a yield of 81%. In a modification of various literature preparations, 95% sodium hydride was used to generate the anion of 85, which was then trapped with either acetyl or propionyl chloride. The use of nBuLi was problematic because the anion had to be generated cold, but the deprotonation proceeded very slowly at low temperatures, so the reaction required careful modulation of time and temperature to ensure complete anion formation while leaving the oxazolidinone intact. With the use of 95% sodium hydride, the anion was reliably generated in 1 hour at 0 °C. The reaction with acetyl chloride to give 86 proceeded rapidly and resulted in a 99.6% yield. With freshly distilled acetyl chloride the
reaction proceeded rapidly and the product was pure as evidenced by NMR. The reaction of the anion of 85 with propionyl chloride to give 87 was not quite as reliable. The yield of 48.5% might be improved, but the hydrochloric acid content of the propionyl chloride would always interfere with a clean reaction. Sodium hydride was not titrated to accurately determine its concentration, so loss of product could be due to incomplete deprotonation. Overall, the reaction with propionyl chloride was less reliable than that with acetyl chloride.

Oxazolidinone 86 was prepared in 42.7% overall yield, which was acceptable because all the reactions with lesser yields occurred before the $^{13}$C-label would be added. The losses would occur with unlabeled material based. Oxazolidinone 87 on the other hand was prepared in 20.8% overall yield. This was particularly poor because a 50% loss occurred in the reaction in which the label was to be added, and this was the most expensive starting material because of the high cost of labeled ethyl bromide. Without major improvements in the last step, this was not a viable route.

These reactions were difficult to track since progress of all of the reactions was monitored by TLC. The valine-derived oxazolidinone 85 had no useful chromophore and was therefore hard to detect. As an alternative, norephedrine and phenylalanine were chosen as the starting materials since both have an aromatic ring that introduced a chromophore. Norephedrine is an amino alcohol, so it was cyclized directly to give the corresponding oxazolidinone 88 (Figure 24), as discussed in the literature.\textsuperscript{54}

The preparation of 88 was analogous to the preparation of 85. The reaction was monitored by TLC to ensure completion, yet after recrystallization the yield was only 56.5%. The crystals were very fine, so it is possible that some product was lost in the filtration. Compound 88 was treated with 95% sodium hydride, as previously 85 was in
Figure 24: Synthesis of (4R, 5S)-3-Acetyl-4-methyl-5-phenyl-1,3-oxazolidin-2-one

the synthesis of 86, to prepare 89 in 72.0% yield. This corresponds to an overall yield of 40.7% for two steps. Norephedrine is a relatively expensive starting material compared to the amino acids L-valine and L-phenylalanine, so this route was not considered viable.

L-Phenylalanine has a chromophore like norephedrine. Compared to L-valine, L-phenylalanine is more active towards reduction, allowing direct reduction of the acid to the alcohol.\(^3\)

L-Phenylalanine was reduced with lithium aluminum hydride to give 90 in a single step in 69.9% yield, following the literature preparation (Figure 25).\(^7\) While this was not spectacular, 84 was prepared in two steps in 52.9% overall yield from the starting material L-valine, so the direct reduction of L-phenylalanine was more efficient.

Compound 90 was converted into the oxazolidinone 91 in 72.2% yield using the same procedure as described previously for the preparation of 85 and 88. This was an acceptable yield considering that this is still unlabeled material and that it was
Figure 25: Synthesis of (4S)-4-phenylmethyl-1,3-oxazolidinone derivatives

prepared in two steps from the abundant precursor L-phenylalanine. As previously with 85, both the acetyl and propionyl chloride substituted oxazolidinones were prepared. Compound 92 was crystalline and obtained in 91.1% yield. This was an excellent route, since 92 was prepared in 46.0% overall yield, and in an analogous labeled synthesis, the label would have been introduced in the highest yielding step. The methylation proved difficult and proceeded in 23.1% yield. Methyl iodide was filtered through basic alumina to remove traces of acid that were decomposing the oxazolidinone. A large excess of methyl iodide was required to force the reaction. The problematic step was most likely the anion formation with sodium hexamethyldisilane (NaHMDS). The terminal
methyl group had to be deprotonated without cleavage of the acetyl group from the
oxazolidinone. This reaction might be optimized further, but the literature suggested that
there were inherent problems with this particular reaction.\textsuperscript{71} Compound 93 was
prepared in 15.2\% overall yield from L-phenylalanine, with a 77\% loss in the last step, in
which the starting material would be labeled. In comparison, 93 was prepared in 86.3\%
yield from 90. By directly adding the propionyl group, 93 was prepared in 43.6\% overall
yield, with minor losses in the step in which the label would be added.

The synthesis with L-phenylalanine as a precursor for the chiral auxiliary was
preferable to the routes based on L-valine or norephedrine as starting materials.
Oxazolidinone 16 was prepared in 43.6\% overall yield in three steps. The $^{13}\text{C}$-label
would be introduced in the last step and only minor losses of label would result from the
last step. This synthesis was the most efficient, economic and reliable for the
preparation of substituted oxazolidinones. For the labeled synthesis, this would be the
best method.

2.4. Synthesis of 1,3-anti-Diol “Backbone”

The anti-1,3-diol in target molecule 57 could be synthesized by multiple different
routes. Katsuki and Sharpless used kinetic resolution of allylic alcohols, such as 94,
which underwent asymmetric epoxidation with a peroxide in the presence of diethyl
tartrate and Ti(O'Pr)$_4$ to give only one enantiomer 95 (Figure 26).\textsuperscript{74} The stereochemistry
was determined by the choice of enantiomer of diethyl tartrate. The epoxide was
opened using Red-Al\textsuperscript{®} to give the anti-1,3-diol, such as 96. Noyori pioneered a series of
ruthenium catalysts (Figure 27) that allow stereoselective reduction of 1,3-diketones to
give specifically the anti-1,3-diol.\textsuperscript{57,75} Both monomeric (97) and
dimeric (98) catalysts have been used. The active intermediate species is still under study in various laboratories.

Rychnovsky developed an elegant method in which a single stereoisomer of a 3-hydroxycarboxylic acid, such as 99, was protected with pivalaldehyde as the corresponding 1,3-dioxane 100 (Figure 28). The stereochemistry at the second stereocenter was induced during the 1,3-dioxane formation. \[58,59\]
We initially tried a Katsuki-Sharpless approach and attempted to synthesize hepta-1,5-dien-4-ol via a Grignard reaction of crotonaldehyde with allyl bromide. The analog octa-2,7-dien-4-ol has been prepared in this laboratory. Spontaneous elimination of the alcohol gave hepta-1,3,5-triene. The elimination proved difficult to suppress, since a conjugated system of three double bonds was formed. The route via the Katsuki-Sharpless epoxidation was abandoned.
A series of 1,3-diketones have been synthesized based on the method by Rathke and Cowan.\textsuperscript{55} Originally, ethyl acetoacetate (103) was coupled with acid chlorides. The coupling has since been performed with imidazolides formed \textit{in situ} as well, but it was observed that the reaction proceeded cleaner and more completely with acid chlorides.\textsuperscript{9} The chlorides required in the syntheses discussed here, if they were not commercially available, were synthesized. The resulting esters (104) were easily deprotected to give the corresponding 1,3-diketone (105).

![Acetoacetate coupling](image)

\textbf{Figure 29: Acetoacetate coupling} \textsuperscript{76}

Because the ruthenium catalysts were developed recently, their full range of applications has yet to be explored. Kawano et al. reduced simple aliphatic 1,3-diketones.\textsuperscript{77} Kitamura extended the application of the catalysts to the reduction of functionalized ketones.\textsuperscript{56} Noyori initially developed them for efficient stereoselective reduction of $\beta$-keto esters.\textsuperscript{57} Since then, other research groups have experimented with a variety of ligands coordinated to the ruthenium center.\textsuperscript{78} In this synthesis, (+)-Ru(binap)(PPh$_3$)$_2$Cl$_2$ (97) was used. Both enantiomers of this catalyst are commercially available.
Figure 30: Synthesis of Benzyl 2-Acetyl-5-bromo-3-oxopentanoate

First, the synthesis of 106 was attempted (Figure 30). The coupling was performed, but 106 was never isolated. The major product of the reaction appeared to be benzyl 2-methyl-4-oxo-5,6-dihydro-4H-pyran-3-carboxylate (107), so the bromide had cyclized to form the dihydropyran ring. A strategy to avoid having such a good leaving group that can add intramolecularly was to have a terminal alcohol and protect it as an ether. The alcohol could later be converted into a good leaving group, such as a tosylate. Nucleophilic addition of the organocuprate formed from [2-\textsuperscript{13}C]-\{2-iodovinyl\}-benzene and subsequent ozonolysis of the double bond would yield the labeled aldehyde.

Compound 109 was not commercially available, so it was prepared from methyl 3-methoxypropionate (Figure 31). Hydrolysis of methyl 3-methoxypropionate with aqueous lithium hydroxide in methanol and THF gave 108 in 72% yield. The reaction was very good, but the disadvantage was the large volumes of solvent. Methyl 3-methoxypropionate is insoluble in water, so the mixture of methanol and THF was
required to dissolve the starting material. Compound 108 was converted into the chloride 109 in 92.9% yield. Freshly distilled 109 was used in the coupling reaction with benzyl acetoacetate to yield 110 in 100% yield. The progress of the reaction depended greatly on the purity of the starting materials. Commercially available benzyl acetoacetate was purified by Kugelrohr distillation prior to use. Even if an excess of the chloride was used, the high yield could not be attained when the chloride contained impurities. The benzyl group was removed by hydrogenation to give 111 in 79.2% yield. At the time, the reaction could not be scaled up easily because the volume of hydrogen needed was large.

Figure 31: Synthesis of (3R,5R)-3,5-Bis-[(2,2-dimethyl)-propyl]-dimethylsilanyloxy]-1-methoxyhexane

acetoacetate was purified by Kugelrohr distillation prior to use. Even if an excess of the chloride was used, the high yield could not be attained when the chloride contained impurities. The benzyl group was removed by hydrogenation to give 111 in 79.2% yield. At the time, the reaction could not be scaled up easily because the volume of hydrogen needed was large.
required increased to the point that the balloon needed to be refilled frequently, and therefore the reaction proceeded slowly. The only hydrogenation apparatus available held a small reactor (20 mL).

The following reduction of 111 to 112 was a very sensitive process based on the procedure by Kitamura at al.\textsuperscript{56} The yield of 51.8% was not completely optimized. The reaction greatly depended on the purity and dryness of the substrate and solvent as well as the quality of the catalyst. At this point the catalyst was purchased from Aldrich. Better results were obtained later on with catalysts obtained from Strem Chemicals. The pressure was crucial as well. Too high a pressure resulted in loss of selectivity, too low a pressure resulted in incomplete reaction. The temperature was estimated from the external heating bath. For later reactions, an internal temperature probe was wired in to allow monitoring of the actual internal temperature. All in all, this was a useful reaction, but it required careful control of all the variables since it was intolerant to minor changes. Compound 112 was protected by the procedure in Greene and Wuts\textsuperscript{65} to give 113 in 100% yield.

The overall yield for this route through compound 113 was 27.4%, which was satisfactory for six steps. Removal of the methyl ether proved impossible without losing the protection of the 1,3-diol. Among the mildest conditions to remove a methyl ether are the methods developed by Jung and Lyster.\textsuperscript{79} They used trimethylsilyl iodide (TMS-I) in chloroform at 25°C. Olah et al.\textsuperscript{80} pointed to shortfalls in Jung's method and offered slightly modified conditions. In 6-10 hours, methyl ethers were removed by TMS-I in acetonitrile. Olah et al. prepared their TMS-I in situ from trichloromethylsilane and sodium iodide. In this synthesis, the methyl group could not be removed without removing the tert-butyldimethylsilyl (TBS) ethers on the diol. The most common method
for the cleavage of methyl ethers employs boron tribromide in dichloromethane \(^{65}\), but TBS-groups will be cleaved under these conditions.

To solve this problem a more labile ether was used instead of the methyl ether. Benzyl ether was chosen because it can be removed under neutral conditions, allowing selective removal of a benzyl ether while retaining the TBS ethers. Previous preparation of the methyl ether has proven that it was possible to stereoselectively reduce the diketone to the desired *anti*-1,3-diol.

\[ 
\text{PhOH} + \text{NaOH} \xrightarrow{\text{H}_2\text{O}} \text{BnO} \xrightarrow{\text{KOH}} \text{HO} \xrightarrow{\text{H}_2\text{O}, \text{H}_2\text{O}_2} \text{OBn} 
\]

\[ 
\text{114} \rightarrow \text{115} \rightarrow \text{116} \rightarrow \text{117} \rightarrow \text{118} 
\]

**Figure 32: Synthesis of 1-Phenylmethoxyhexa-3,5-dione**

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Since 3-(phenylmethoxy)-propionyl chloride was not commercially available, it was synthesized (Figure 32). Acrylonitrile and benzyl alcohol were reacted by the method of Gutman et al.\textsuperscript{81,82} to give 114 in 99.0% yield. The reaction was very robust and clean. Hydrolysis of the nitrile in 114 to give 115 was achieved with potassium hydroxide in 30% hydrogen peroxide. This reaction gave the product in 29.8% yield, but milder alternative methods failed to hydrolyze 114. A major problem with this reaction was that it was quite exothermic at first, so when the substrate 114 was dissolved in hydrogen peroxide and aqueous potassium hydroxide was added, the reaction mixture heated up. The temperature usually stabilized around 65 °C, but with larger scale reactions it proved difficult to keep the vessel cool enough to prevent the reaction from getting out of control and yet let it warm up enough to allow the reaction to proceed.

The acid 115 was converted into the chloride using thionyl chloride according to the procedure by Li et al.\textsuperscript{73} An alternative route to prepare 3-(phenylmethoxy)-propanoyl chloride with oxalyl chloride and catalytic DMF was reported by Eberlein, West and Tester.\textsuperscript{83} Crude 3-(phenylmethoxy)-propanoyl chloride was directly used to prepare 116 in 71.6% yield. The tert-butyl ester was cleaved with trifluoroacetic acid (TFA) in dichloromethane using the method of Bryan et al.\textsuperscript{84} Only 13.2% of the product 118 was obtained. This was likely due to decomposition, so a different ester that could be deprotected under milder conditions should work better. As the immediate analogue, the corresponding benzyl ester 117 was prepared from 115. As reported previously, 115 was converted \textit{in situ} into 3-(phenylmethoxy)-propanoyl chloride and coupled with benzyl acetoacetate to give 117 in 67.7% yield.

To remove the benzyl ester in the presence of a benzyl ether required a more specific catalyst than previous hydrogenations. Sajiki et al. reported an ethylenediamine-doped palladium catalyst that could selectively remove benzyl esters in
the presence of other functionalities sensitive to hydrogenation.\textsuperscript{85} The reaction was run under the same conditions as previous hydrogenations, at room temperature under a balloon with hydrogen. Compound 118 was prepared in 95.7\% yield using this method.

The benzyl ester was preferred over the \textit{tert}-butyl ester since it was removed under very mild conditions. The preparation of 118 via the \textit{tert}-butyl ester resulted in an overall yield of 2.7\%, so this route was abandoned. Compound 118 was prepared in 19.1\% overall yield from acrylonitrile and benzyl alcohol, which is satisfactory. Previous to the nitrile hydrolysis, which was the only low yield step, only readily available reagents had been used in the reaction making it amenable to scale-up.

Problems arose with the hydrogenation of 118 to give (3\textit{R},5\textit{R})-1-phenylmethoxyhexa-3,5-diol. The substrate 118 was either not reduced completely or the terminal benzyl group was removed as well. The catalyst (97) was reported to be selective enough to not hydrogenate benzyl ethers, yet the only systems it had been used with were aliphatic 1,3-diketones with a phenyl group\textsuperscript{77} or simple esters such as \textit{tert}-butyl or methyl.\textsuperscript{86} The common method for the removal of benzyl ethers is hydrogenation in the presence of palladium on carbon in ethanol.\textsuperscript{65} Therefore, the hydrogenation was unlikely to proceed without removal of the benzyl group even if optimized. At this point an alternate strategy appeared to be a better solution to the problem.

Instead of looking at other ethers to protect the alcohol, the terminal bromide could be obtained by functional group interconversion from the aldehyde. Aldehydes are easily formed by ozonolysis of an alkene. Subsequent reduction and introduction of the bromide with triphenylphosphonium bromide would yield the terminal bromide. Vinylacetic acid was commercially available, so the chloride could be prepared and used in the coupling as previously.
3-Butenoyl chloride was prepared by adding oxalyl chloride to neat vinylacetic acid. During the first few reactions, 3-butenoyl chloride was prepared according to the method of Marson, Grabowska and Fallah using thionyl chloride. The product was impure, so it was isolated and purified, but this is a highly corrosive compound that is difficult to handle and does not store well even at low temperatures. Therefore, it was prepared \textit{in situ} using the much cleaner reaction conditions of oxalyl chloride, and directly used in the coupling reaction to give 119 in 48.7\% yield (Figure 33). Although this yield was not as good as yields previously attained in this reaction, it was sufficient to make substantial amounts of 119.

Problems with this reaction appeared in the hydrogenation of 119 when hept-5-ene-2,4-dione (120) was obtained instead of hept-6-ene-2,4-dione (121). The double bond always rearranged into conjugation with the 1,3-diketone. This type of compound had been the focus of an investigation by Linstead and Williams.\textsuperscript{88} They found these...
systems prone to rearrangement and as predicted, the rearrangement was observed to occur in a reaction very similar to the one attempted here. Linstead and Williams solved the problem by using styrylacetic acid instead of vinylacetic acid. The phenyl group in conjugation with the double bond prevented the rearrangement.

Since 4-phenyl-3-butenoyl chloride was not commercially available, it was prepared by the method of Hoye and Richardson (Figure 34). An alternate method by Kumar et al. used silica gel as a catalytic surface and microwave radiation. Although this approach was tried, the method of Hoye and Richardson proved more reliable and consistently produced 122 in 65.6% yield.
The chloride was prepared from 123 with oxalyl chloride in ether. This was the first chloride prepared that was a solid at room temperature, so it was recrystallized before using it in the coupling reaction. After recrystallization, the chloride was only produced in 40.6% yield, but since both malonic acid and phenylacetaldehyde are readily available, the method was not optimized. The coupling reaction produced 123 in 89.1% yield. The tert-butyl ester was cleaved with trifluoroacetic acid in dichloromethane. This system proved good enough to yield 88.0% of 124 after treatment with acid.

Overall, this was a solid route with 20.9% overall yield in 4 steps. All the steps with low yield involved only inexpensive reagents in the early part of the synthesis. The problematic step was the reduction of 124 to give 1-phenylheptene-4,6-diol. The product was lacking the double bond, so the alkene was saturated in the hydrogenation. Ohta et al. had used ruthenium-binap catalysts in the asymmetric reduction of alkenes. The conditions to reduce the 1,3-diketone were forced, so the alkene was probably saturated at the same rate as the carbonyls were reduced. Selective reduction of the 1,3-diketone over the alkene seemed impossible.

Phenyl rings have been used as latent carboxylic acids because they are very unreactive, so most common functional group interconversions do not affect phenyl rings. Since ruthenium tetraoxide was introduced as an oxidant for a variety of oxidation reactions in 1953 by Djerassi and Engle, it has been used in catalytic systems involving periodate or hypochlorite as oxidants. Traditionally, about 1-5% of ruthenium catalyst was added to the substrate in carbon tetrachloride and water, but these reactions were frequently slow or failed if the substrate included a carboxylic acid or if an acid was formed by the oxidation, since the ruthenium catalyst forms carboxylate complexes and therefore loses activity. Carlsen et al. modified the procedure to a
biphasic solvent system of carbon tetrachloride, water and acetonitrile to make it more amenable to the oxidation of phenyl rings. Additionally, a large excess of metaperiodate was used with ruthenium trichloride hydrate to be able to oxidize phenyl rings. This has now become the most commonly used method.\textsuperscript{95} Núñez and Martin\textsuperscript{96} modified the procedure by using periodic acid instead of metaperiodate, since the metaperiodate tends to form a precipitate that has been shown to prevent the reaction from going to completion.

Although not used as frequently, ozonolysis can be used to decompose the aromatic ring. The work-up determines the final product. Oxidative work-up with hydrogen peroxide yields the acid. Reductive work-up with sodium borohydride should yield the alcohol, but in the literature only the oxidative work-up has been used, followed by borane reduction to give the alcohol.\textsuperscript{97}

Since phenylacetyl chloride was commercially available, it was coupled with benzyl acetoacetate to give 125 in 74.0\% yield (Figure 35). The benzyl ester was removed by hydrogenation to give 126 in 74.4\% yield. The 1,3-diketone was reduced to 127 under 52 atm of hydrogen pressure. The reaction proceeded slowly, taking 3 days, but it was determined that with a shorter reaction time, significant amounts of monoreduced substrate were recovered, which were difficult to separate from 127. After the full reaction time of 3 days, the product was obtained in 92.2\% yield.
The TBS-protection used so far was unsuitable for the oxidation reaction, so a more robust protective group was needed. Dioxanes are very stable, so the diol was protected as the 1,3-dioxane with 2,2-dimethoxypropane. The reaction required lengthy reflux to go to completion and a substantial amount of 127 degraded. The product 128 was only obtained in 45.7% yield.
As an alternative, 127 was protected as the diacetate. Initially, the procedure of Robinson et al. was followed, but long reaction times and moderate yields called for improvement. Höfle et al. reported that acetylation reactions proceeded up to $10^4$ times faster in the presence of catalytic 4-(dimethylamino)pyridine (DMAP) compared to running the reaction only with pyridine. The modified procedure with DMAP worked well and 129 was prepared in 66.1% yield in 2 hours.

The ozonolysis of 129 to give 130 followed the procedure of Bednarski and Danishefsky. It was run at room temperature which is an unusual feature. Ordinarily, ozonolyses are done at $-78 \, ^\circ\text{C}$. In order to keep the solvent from evaporating the flask was equipped with a dry ice trap. Acid 130 was purified by column chromatography and obtained in 23.2% yield. This yield did not reflect the efficiency of the actual reaction because some of the product was lost on the column. Sometimes 1% acetic acid was used as an additive to the eluent, but the acetic acid was difficult to remove completely. Most of the time the crude extract was directly used in the borane reduction, as Bednarski and Danishefsky had done. The borane reduction was problematic. Product 131 was only obtained in 13.5% yield, which was very poor. Borane was purchased as a solution and not titrated, so there was no guarantee for its actual concentration and purity. This reaction has only been run on a fairly small scale due to limited amounts of 130, so any discrepancies in the concentration of borane and any impurities in the substrate 130 had a major impact on the final yield.

Compound 129 was prepared in 33.6% overall yield in four steps. This was very promising, especially since the following two steps are proven to work. They only require some further optimization, which has not been done due to time constraints. In parallel to the above synthesis, the analogue based on 3,4-dimethoxyphenylacetyl chloride was prepared. The ozonolysis proceeded very slowly, so activation in the form
of substituents on phenyl ring was assumed to improve this reaction. As a commercially available precursor, 3,4-dimethoxyphenylacetyl chloride was chosen.

This route proceeded much like its analogous preparation of 129 (Figure 36). 3,4-Dimethoxy-phenylacetyl chloride was coupled with benzyl acetoacetate to give 132 in 76.3% yield. Hydrogenation of the benzyl ester and decarboxylation resulted in 133 in 91.1% yield. The 1,3-diketone 133 was reduced to 134 under 82 atm of hydrogen. This reaction went slower than previous reductions with the ruthenium catalyst. A reaction time of 5 days was required to force the reaction to go to completion, compared to a reaction time of 3 days to prepare 127. Product 134 was isolated in only 48.6% yield. A
problem with this reaction was the high pressure and long reaction time required since the high pressure reduced selectivity. The catalyst was of good quality and was used for both 127 and 134, resulting consistently in high yields of 127 and mediocre yields of 134. The diacetate was prepared by Spavold's route\textsuperscript{41}, so 134 was simply stirred in pyridine and acetic anhydride added cold. The product 135 was isolated in 21.3\% yield.

Compound 135 was prepared in 7.2\% overall yield from 3,4-dimethoxy-phenylacetyl chloride and benzyl acetoacetate. The ozonolysis of 135 proceeded slightly faster than that of 129. Still, the slight gain was not worth the loss of material and the longer reaction time for the reduction experienced with this particular route. The substitution of the phenyl ring seemed to make the 1,3-diketone 133 less active towards reduction to 134. It was interesting to test an alternative substrate to make 131, but this proved to be an inferior route.

At this point, it was worthwhile considering whether the introduction of the labeled carbon as cyanide would make the process easier (Figure 37). The nitrile was then to be hydrolyzed to give the acid that could be converted into the aldehyde.

![Figure 37: Retrosynthesis - introduction of labeled cyanide](image-url)
3-Cyanopropionic acid 143 has been prepared in one step from \( \beta \)-propiolactone by ring-opening with sodium cyanide, as reported by Gresham et al. (Figure 38).\textsuperscript{99} The acid was easily converted into 3-cyanopropionyl chloride with thionyl chloride in ether by Dolby et al.\textsuperscript{100}

![Figure 38: Literature preparation of 3-cyanopropionic acid](image)

\( \beta \)-Propiolactone is an undesirable starting material because it tends to polymerize upon storage and cannot be purchased in large quantities. The alternative of using 3-bromopropionic acid as the starting material was explored.

![Figure 39: Synthesis of 3-cyanopropionic acid](image)

Cyanide was added in a simple nucleophilic displacement to 144 (Figure 39). The most common method is to use sodium or potassium cyanide in aqueous ethanol. Alternatively, lithium cyanide has been used in THF. Lithium cyanide has the advantage
that it is soluble in THF and more reactive than potassium cyanide.\textsuperscript{101} Lithium cyanide has been prepared from acetone cyanohydrin.\textsuperscript{102} In this synthesis, the standard method of treatment with potassium cyanide in aqueous ethanol was chosen.

3-Bromopropionic acid was esterified with methanol and catalytic sulfuric acid by the same method as L-valine to yield 144 in 85.2\% yield. Just as before, this was a good reaction. After trying different solvent mixtures for the nitrile synthesis, it appeared that ethanol:water (1:1) works best. For safety, the potassium cyanide was weighed out into a preweighed flask in the hood and the amount of substrate was adjusted accordingly. Only 28.4\% of 145 was obtained. Part of the problem appeared to be that 145 was somewhat soluble in water, so some of the material was never recovered in the extraction. Hydrolysis under basic conditions, similar to the hydrolysis of methyl 3-methoxypropionate previously, yielded 43.5\% of the product 143. The problem of solubility increased in this step. Compound 143 was very soluble in water, so the mixture was concentrated to minimal solvent and extracted with copious volumes of ether to assure that as much of the product as possible was recovered.

This route has not been pursued in enough depth to make predictions about its viability. The displacement with cyanide to give 145 needs drastic improvements before this should be considered an alternative to the route via 131.

As a radical departure from the acetoacetate ester coupling, a dioxane was synthesized to set up the backbone of target molecule 57 (Figure 40). Rychnovsky\textsuperscript{59} began studying dioxanes as a general way to construct the framework of anti-1,3- diols based on prior work by Seebach et al.\textsuperscript{60,61,103} The dioxanes readily underwent coupling with dialkylzinc reagents to elongate the chain. The dioxane was stable enough to allow other functional group modifications to take place.
The backbone of target molecule 57 dictated the use of 3-hydroxybutyric acid as starting material which was prepared from benzyl acetoacetate (Figure 40). Racemic 146 was obtained by reducing benzyl acetoacetate with sodium borohydride (NaBH₄). The procedure was slightly modified from the one by Nelson⁹ and gave 146 in 99.9% yield. Alternatively, hydrogenation of benzyl acetoacetate in the presence of (+)-Ru(binap)(PPh₂)₂Cl₂ (97) according to Noyori et al.⁵⁷ gave the pure R-enantiomer of 146 in 100.0% yield. The hydrogenation was forced with 82 atm hydrogen over a reaction time of 36 hours. Since there was no other reaction possible, the product was obtained quite pure out of the reaction mixture. The ester had to be hydrolyzed in a nonaqueous...
system since 147 was very water-soluble and difficult to isolate from an aqueous extract. Benzyl acetoacetate is relatively expensive compared to, for example, methyl acetoacetate, but the reduction and hydrolysis of methyl acetoacetate yielded only insignificant amounts of 147. The benzyl ester was easily removed by hydrogenation in the presence of palladium on carbon because it was done under non-aqueous conditions. The hydrogenation resulted in a yield of 97.4% of 147.

Compound 147 was efficiently prepared from benzyl acetoacetate in 97.3% overall yield. The problems with this route started with the protection of 147. Initially, the procedure of Rychnovsky et al.® was adopted, where the hydroxyl-groups were first protected as the corresponding trimethylsilyl ethers with trimethylsilyl chloride and triethylamine. The resulting product was distilled at 70 °C (0.2 torr) and treated with pivalaldehyde, 2,6-lutidine and trimethylsilyl triflate. This reaction showed no trace of product. Seebach et al.® protected 3-hydroxybutyric acid with pivalaldehyde in the presence of pyridinium p-toluenesulfonate (PPTS). In a different communication, Powell and Rychnovsky® utilized scandium triflate and molecular sieves to achieve the same reaction. Following Powell and Rychnovsky's procedure, only 26.1% of the product 148 was obtained. The reaction was very sensitive, so the solvent was freshly distilled and dry, the molecular sieves were dried in an Abderhalden apparatus, and both 147 and the pivalaldehyde were freshly distilled. The quality of the scandium triflate was never ascertained, but when the alternative procedure by Seebach et al. was followed, freshly recrystallized and dried pyridinium p-toluenesulfonate (PPTS) was used and the reaction nevertheless did not go any cleaner or better. In the subsequent reaction 148 was reduced by dibutylaluminum hydride (DIBALH) and treated with acetic anhydride to give 149 in 16.9% yield. At this point, this route was abandoned.
A major problem arose with the use of pivalaldehyde, which was synthesized. Pivalaldehyde is very volatile and unstable, so it should only be stored short-term and frozen. The synthesis proved difficult not in producing pivalaldehyde but rather in isolating it. The continuous supply of pivalaldehyde began to be a problem because it has a low molecular weight and is used in excess in the protection reaction.

Pivalaldehyde was most successfully produced by oxidation of neopentyl alcohol with dichromate according to Vogel’s procedure. The aldehyde was continuously distilled out of the reaction mixture for a yield of 31%. The only other method with any promise was published by Nazarki et al. The Grignard reagent of tert-butyl chloride was formed and dimethylformamide (DMF) added as a formate donor. The resulting reaction mixture was stabilized with hydroquinone. Pivalaldehyde was distilled out to give a yield of 13.1%. In light of the promise shown by the acetoacetate coupling reactions and the problems encountered early on in this particular route, this synthesis was abandoned.

2.5. Conclusions

The synthetic route via 131 showed the most promise in the preparation of the target molecule 57. The alternate syntheses have been shown to fail. In order to complete the synthesis and prepare 57, the alcohol 131 will have to be converted into either the iodide 150 or the tosylate to provide a good leaving group. This leaving group will be substituted by $^{13}$C-labeled potassium cyanide to give nitrile 151. Before hydrolyzing 151 to the corresponding acid 152, it will be necessary to exchange the acetate protecting groups on the 1,3-diol for tert butyldimethylsilyl (TBS) ethers. The acid will then be reduced to the aldehyde 153 by DIBALH. Alternatively, the acid could be reduced to the alcohol and then oxidized to 153 via a Swern oxidation. The aldehyde 153 will be
coupled with the oxazolidinone in an *Evans*-aldol reaction to give the acid 154. The acid is easily converted into the *N*-caprylcysteamine thioester 57, which is fed into the fermentative culture.

There is still much work to be done, but hopefully the synthetic route described in this thesis will proved useful in directing future studies.

![Chemical structures and reactions](image-url)
CHAPTER 3

EXPERIMENTAL SECTION

General Procedures

Solvents were purchased from Fisher Scientific. All other chemicals, unless noted otherwise, were obtained from Aldrich Chemical Co (Milwaukee, WI). The Ru(binap)(PPh₂)₂Cl₂ catalyst was purchased from Strem Chemicals (Newburyport, MA). The solvents CH₂Cl₂ (over CaH₂), Et₂O (over Na/K-benzophenone), THF (over Na/K-benzophenone), and MeOH (over Me(OMe)₂) were dried and distilled prior to use. Solutions were dried over anhydrous MgSO₄ and concentrated by evaporation in vacuo. All synthesis procedures were carried out under a slight positive pressure of dry argon gas. Column chromatography was performed with Silicycle Ultra Pure Silica Gel 230-400 Mesh (Quebec, QC, Canada). IR spectra were acquired on a Nicolet Nexus 870 FT-IR. NMR spectra were acquired on a Varian Unity 400 MHz, were referenced to residual solvent, and are reported as chemical shift (δ/ppm), intensity, splitting pattern, and coupling constant (J/Hz).

Synthesis

2-(4-Methoxyphenyl)-1,3-dithiane: Anisaldehyde (3.0 mL, 25.0 mmol), p-tosic acid (0.24 g, 1.25 mmol), and 1,3-propanedithiol (7.5 mL, 75.0 mmol) were dissolved in benzene (61 mL) and heated at reflux overnight in a flask equipped with a Dean-Stark trap. The mixture was concentrated and gave a yellow solid,
which was crystallized from benzene-Et₂O to yield white crystals (3.73 g, 65.9%).
Mp 114-116 °C (benzene-Et₂O); IR (ATR): 1506 (s), 1247 (s), 1029 (s), 757 (m),
674 (m) cm⁻¹.

4-(2-Ethyl-1,3-dithlan-2-yl)-1-methoxybenzene: A solution of n-BuLi (8.0 mmol)
in hexanes (3.2 mL) was carefully added by syringe into a stirred solution of 63
(1.81 g, 8.0 mmol) in dry THF (100 mL) at -22 °C. After 15 min EtBr (0.37 mL,
5.0 mmol) was added by syringe and the reaction left stirring overnight, allowing it
to warm to 15 °C. The reaction was quenched with aq. sat. NH₄Cl (15 mL) and
extracted with Et₂O (3×50 mL). The extracts were combined, dried, filtered, and
concentrated. The crude yellow oil obtained was purified by chromatography on
silica gel, eluting with EtOAc-hexanes (1:3), to give a clear liquid (1.22 g, 95.9%).
¹H NMR (400 MHz, CDCl₃) δ 7.80 (2H, d, J₁H-₁H = 7.9 Hz), 6.65 (2H, d, J₁H-₁H = 7.9
Hz), 3.83 (3H, s), 2.68 (4H, m), 2.04 (2H, q, J₁H-₁H = 7.1 Hz), 1.93 (2H, m), 0.81
(3H, t, J₁H-₁H = 6.7 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 181.9, 158.4, 133.8, 130.1,
113.8, 55.7, 38.0, 17.9, 16.8, 8.5.

1-(4-Methoxyphenyl)propan-1-one: A solution of 64 (1.78 g, 7.0 mmol) in dry
acetone (14 mL) was slowly added to a suspension of CuO (0.69 g, 8.4 mmol)
and anhyd. CuCl₂ (2.25 g, 16.8 mmol) in dry acetone (56 mL) and DMF (1.4 mL)
at reflux. After 2 h the orange precipitate was removed by filtration and washed
with CH₂Cl₂ (70 mL). The organic fractions were combined and washed with 2 M
Na₂CO₃ (70 mL), followed by aq. sat. NaCl (50 mL). The combined extracts were
dried, filtered, and concentrated to give a dark-red liquid that was purified by
chromatography on silica gel, eluting with EtOAc-hexanes (1:3), to give the
product as a pale orange liquid (0.68 g, 59.1%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$
7.82 (2H, d, $J_{1H-1H} = 7.8$ Hz), 6.86 (2H, d, $J_{1H-1H} = 7.8$ Hz), 3.81 (3H, s), 2.88 (2H, q, $J_{1H-1H} = 6.9$ Hz), 1.19 (3H, t, $J_{1H-1H} = 6.5$ Hz); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$
198.7, 163.0, 130.1, 129.9, 113.8, 54.4, 31.2, 8.3.

**Sodium propanoate:** 1,2-Dibromoethane (5 $\mu$L) was added to a stirred suspension of Mg (0.5 g, 20.0 mmol) in dry THF (20 mL). After gas evolution stopped, distilled EtBr (0.75 mL, 10.0 mmol) was added via syringe. Dry CO$_2$ was generated from BaCO$_3$ (9.9 g, 50.0 mmol) and HCl (5 M, 10 mL). The Ar atmosphere was replaced with CO$_2$, which was passed through a drying tube. The reaction was left stirring overnight at rt. The mixture was quenched with 1 M NaOH (20 mL) and extracted with Et$_2$O (2×20 mL). The pH of the aqueous layer was adjusted to 1.5 with 1 M HCl. Propionic acid and H$_2$O were isolated by lyophilization. The pH of the collected lyophilizate was adjusted to 6.5 and H$_2$O was removed by lyophilization, leaving sodium propionate (0.69 g, 86.0 %). IR (ATR): 1553 (s), 1368 (s) cm$^{-1}$; $^1$H NMR (400 MHz, D$_2$O) $\delta$ 2.2 (2 H, q, $J_{1H-1H} = 7.8$ Hz), 0.9 (3H, t, $J_{1H-1H} = 7.8$ Hz); $^{13}$C NMR (100 MHz, D$_2$O) $\delta$ 186.6, 31.7, 11.2.

**83 (S)-Methyl 2-amino-3-methylbutanoate:** Distilled SOCl$_2$ (14.6 mL, 0.2 mol) was added dropwise to a stirred suspension of l-Valine (11.7 g, 0.1 mol) in dry MeOH (250 mL) at 0 °C. The reaction mixture was heated to reflux. After 19 h the mixture was allowed to cool to rt and quenched by pouring onto ice (300 mL). The pH was adjusted to 10 with aq. c. NH$_4$OH. The mixture was extracted with CH$_2$Cl$_2$ (3×100 mL). The extracts were combined, dried, filtered, and concentrated. The crude product was distilled to give a colorless oil (11.05 g,
85.5%). Bp 47-49 °C (35 torr); IR (ATR): 1729 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 3.57 (3H, s), 3.14 (1H, d, J₁H-₁H = 6.0 Hz), 1.86 (1H, m), 1.28 (2H, s), 0.82 (3H, d, J₁H-₁H = 4.0 Hz), 0.76 (3H, d, J₁H-₁H = 3.9 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 176.0, 60.6, 52.6, 33.3, 20.4, 18.1.

84 (S)-2-Amino-3-methylbutan-1-ol: A solution of 83 (19.0 g, 147 mmol) in dry THF (50 mL) was added dropwise into a stirred suspension of LiAlH₄ (11.2 g, 294 mmol) in dry THF (500 mL) at 0 °C. After 30 min, H₂O (11.2 mL) was added very carefully, followed by 15% (w/v) aq. NaOH (11.2 mL) and then H₂O (33.6 mL). The mixture was stirred vigorously for 30 min and the white solid that was formed removed by filtration. The filtrate was concentrated and the crude product distilled to give a colorless oil (9.4 g, 61.9%). Bp 114-116 °C (36 torr); ¹H NMR (400 MHz, CDCl₃) δ 3.19 (1H, m), 2.90 (1H, t, J₁H-₁H = 9.1 Hz), 2.65 (1H, br s), 2.16 (1H, m), 1.21 (1H, m), 0.51 (6 H, q, J₁H-₁H = 5.3 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 63.3, 57.6, 29.9, 18.5, 17.6.

85 (4S)-4-(1-Methylethyl)-1,3-oxazolidin-2-one: Anhyd. K₂CO₃ (0.14 g, 1.0 mmol) was added to a solution of 84 (2.36 mL, 20 mmol) in (EtO)₂CO (4.8 mL, 40 mmol). The reaction mixture was fractionally distilled through a Vigreux column to leave a yellowish solid. The solid was purified by crystallization from toluene and recrystallization from hexanes as white crystals (2.09 g, 81.0%). Mp 67-69 °C (hexanes); ¹H NMR (400 MHz, CDCl₃) δ 6.68 (1H, s), 4.43 (1H, t, J₁H-₁H = 9.0 Hz), 4.08 (1H, m), 3.59 (1H, m), 1.74 (1H, m), 0.94 (3H, d, J₁H-₁H = 7.0 Hz), 0.85 (3H, d, J₁H-₁H = 6.4 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 154.0, 69.9, 59.5, 33.8, 19.3, 18.8.
(4S)-4-(Methylethyl)-3-acetyl-1,3-oxazolidinone: Dry 85 (2.58 g, 20.0 mmol) was added into a stirred suspension of 95% NaH (0.58 g, 24.0 mmol) in dry THF (40 mL) at 0 °C. After 1 h AcCl (1.4 mL, 20.0 mmol) was added via syringe. After 1 h the mixture was allowed to warm to rt and stirred overnight. The reaction was quenched by the addition of aq. sat. NH₄Cl (10 mL) and the organic phase extracted with Et₂O (3x50 mL). The extracts were combined, dried, filtered, and concentrated to give a clear liquid (3.41 g, 99.6%) that was used without further purification. IR (ATR): 1779 (s), 1701 (s), 1375 (s), 1303 (s), 1206 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) 4.36 (1H, m), 4.20 (2H, m), 2.46 (3H, s), 2.32 (1H, m), 0.67 (3H, d, J₁H₁-₁H = 6.7 Hz), 0.60 (3H, d, J₁H₁-₁H = 6.7 Hz); ¹³C NMR (100 MHz, CDCl₃) 170.2, 154.1, 63.1, 58.2, 28.5, 24.0, 18.1, 14.9.

(4S)-4-(1-Methylethyl)-3-propanoyl-1,3-oxazolidin-2-one: A solution of dry 85 (0.52 g, 4.0 mmol) in dry THF (2.0 mL) was added carefully to a stirred suspension of 95% NaH (0.12 g, 4.8 mmol) in dry THF (2.0 mL). After 20 min, propionyl chloride (0.26 mL, 4.0 mmol) was added by syringe and the reaction stirred overnight. The mixture was quenched with aq. sat. NH₄Cl (5 mL) and extracted with CH₂Cl₂ (3x10 mL). The extracts were combined, dried, filtered, and concentrated. The crude yellow oil obtained was purified by chromatography on silica gel, eluting with EtOAc-hexanes (1:1), to give a pale liquid (0.36 g, 48.5%). ¹H NMR (400 MHz, CDCl₃) 4.39 (1H, m), 4.19 (2H, m), 2.89 (2H, m), 2.32 (1H, m), 1.12 (3 H, t, J₁H₁-₁H = 8.0 Hz), 0.87 (3H, d, J₁H₁-₁H = 4.0 Hz), 0.83 (3H, d, J₁H₁-₁H = 6.4 Hz); ¹³C NMR (100 MHz, CDCl₃) 174.0, 154.0, 63.2, 58.5, 26.7, 26.1, 18.0, 14.4, 8.5.
(4R, 5S)-4-Methyl-5-phenyloxazolidin-2-one: Anhyd. K₂CO₃ (0.14 g, 1.0 mmol) was added into a solution of norephedrine (1.51 g, 10.0 mmol) in dry (EtO)₂CO (3.0 mL, 25.0 mmol). The reaction mixture was heated to 150 °C for 3 h and MeOH removed by distillation. The mixture was allowed to cool and then concentrated. The residue was dissolved in CH₂Cl₂ (10 mL) and washed with H₂O (2×10 mL). The organic layer was dried, filtered, and concentrated to give a white solid. After crystallization from EtOAc-hexanes, white crystals were obtained (1.00 g, 56.5 %). Mp 113-115 °C (EtOAc-hexanes); IR (ATR): 3264 (b), 1713 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.21 (5H, m), 5.79 (1H, br s), 5.70 (1H, d, J₁H-₁H = 6.6 Hz), 4.18 (1H, m), 0.79 (3H, d, J₁H-₁H = 4.0 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 153.2, 134.6, 128.2, 126.0, 80.7, 52.3, 17.6.

(4R, 5S)-3-Acetyl-4-methyl-5-phenyl-1,3-oxazolidin-2-one: Compound 88 (0.89 g, 5.0 mmol) was added into a stirred suspension of 95% NaH (0.14 g, 6.0 mmol) in dry THF (10 mL) at 0 °C. After 3 h, AcCl (0.4 mL, 5.0 mmol) was added via syringe. The reaction mixture was stirred overnight at rt. The reaction mixture was quenched by the addition of aq. sat. NH₄Cl (5 mL) and extracted with Et₂O (3×5mL). The extracts were combined, dried, filtered, concentrated, and purified by chromatography on silica gel, eluting with EtOAc-hexanes (1:3), to give a clear liquid (0.78 g, 72.0 %). ¹H NMR (400 MHz, CDCl₃) δ 7.35 (5H, m), 5.63 (1H, d, J₁H-₁H = 7.7 Hz), 4.72 (1H, m), 2.49 (3H, s), 0.83 (3H, d, J₁H-₁H = 5.2 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 176.1, 170.0, 152.6, 132.8, 128.3, 125.9, 78.4, 54.2, 24.0, 14.1.
(2S)-2-Amino-3-phenylpropan-1-ol: A slurry of L-phenylalanine (8.26 g, 50.0 mmol) in dry THF (50 mL) was dropped slowly into a slurry of LiAlH₄ (3.80 g, 100.0 mmol) in dry THF (150 mL) at 0 °C. The mixture was stirred for 2 h at 0 °C, then 2 h at rt. Finally, it was heated at reflux for 20 h. The reaction mixture was cooled to 0 °C and quenched by the addition of H₂O (3.8 mL), 15% (w/v) NaOH (3.8 mL) and then H₂O (11.4 mL). The resulting precipitate was stirred for 30 min before being collected by filtration and washed with Et₂O (100 mL). The combined filtrate and washes were concentrated to give a yellow solid, which was crystallized from benzene to give white crystals (5.28 g, 69.9 %). Mp 84-88 °C (benzene); IR (ATR): 3356 (s), 3298 (b), 1576 (s), 1064 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.21 (5H, m), 3.61 (1H, dd, J₁H-₁H = 3.2 Hz, 7.9 Hz), 3.37 (1H, q, J₁₁H-₁₁H = 5.8 Hz), 3.10 (1H, m), 2.79 (1H, dd, J₁₁H-₁₁H = 2.7 Hz, 13.3 Hz), 2.53 (1H, q, J₁₁H-₁₁H = 8.0 Hz), 2.17 (3H, br s); ¹³C NMR (100 MHz, CDCl₃) δ 158.2, 128.6, 128.3, 126.2, 66.0, 54.1, 40.9.

(4S)-4-Phenylmethyl-1,3-oxazolidin-2-one: Anhyd. K₂CO₃ (0.28 g, 2.0 mmol) was added to a solution of dry 90 (3.02 g, 20.0 mmol) in dry (EtO)₂CO (6.1 mL, 50.0 mmol). The reaction mixture was heated to 150 °C for 3 h and MeOH removed by distillation. The mixture was allowed to cool and then concentrated to give a white solid. After recrystallization from EtOAc-hexanes, white crystals were obtained (2.56g, 72.2%). Mp 85-86 °C (EtOAc-hexanes); IR (ATR): 3264 (b), 1751 (s), 1697 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.28 (5H, m), 5.33 (1H, br s), 4.45 (1H, t, J₁₁H-₁₁H = 8.0 Hz), 4.14 (1H, dd, J₁₁H-₁₁H = 4.8 Hz, 8.0 Hz), 4.07 (1H, m), 2.84 (2H, d, J₁₁H-₁₁H = 5.3 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 169.9, 136.1.
(4S)-3-Acetyl-4-(phenylmethyl)-1,3-oxazolidin-2-one: Compound 91 (1.77 g, 10.0 mmol) was added into a stirred suspension of 95% NaH (0.29 g, 12.0 mmol) in dry THF (10 mL) at 0 °C. After 3 h, AcCl (0.7 mL, 10.0 mmol) was added via syringe and the reaction stirred overnight at rt. The mixture was quenched by the addition of aq. sat. NH₄Cl (5 mL) and extracted with Et₂O (3×10 mL). The extracts were combined, dried, filtered, and concentrated. The crude solid obtained was crystallized from EtOAc-hexanes to give white crystals (2.00 g, 91.1%). Mp 87-89 °C (EtOAc-hexanes); ¹H NMR (400 MHz, CDCl₃) δ 7.27 (5H, m), 4.69 (1H, m), 4.17 (2H, m), 3.28 (1H, dd, J₁H-₁H = 2.7 Hz, 13.3 Hz), 2.77 (1H, q, J₁H-₁H = 7.1 Hz), 2.56 (3H, s); ¹³C NMR (100 MHz, CDCl₃) δ 172.4, 170.1, 154.9, 129.8, 129.1, 127.4, 66.1, 55.0, 47.8, 23.7.

(4S)-4-(Phenylmethyl)-3-propanoyl-1,3-oxazolidin-2-one: Procedure 1:

Compound 91 (0.89 g, 5.0 mmol) was added into a stirred solution of 95 % NaH (0.14 g, 6.0 mmol) in dry THF (10 mL) at 0 °C. After 3 h, propionyl chloride (0.4 mL, 5.0 mmol) was added via syringe and the reaction mixture stirred overnight at rt. The reaction was quenched by the addition of aq. sat. NH₄Cl (5 mL) and extracted with Et₂O (3×10 mL). The extracts were combined, dried, filtered, and concentrated. The residue obtained was purified by chromatography on silica gel, eluting with EtOAc-hexanes (1:1), to give a faint yellow oil (1.01 g, 86.3%).

Procedure 2: A solution of NaHMDS (6.0 mmol) in THF (6.0 mL) was added slowly via syringe to a stirred solution of 92 (1.10 g, 5.0 mmol) in dry THF (20 mL) at 0 °C. The solution turned bright yellow, an indicator for complete anion
formation. Freshly distilled CH$_3$I was filtered through basic alumina and added via syringe into the reaction mixture. After 15 min the solution was allowed to warm to rt and then stirred for 3 h. The reaction was quenched with aq. sat. NH$_4$Cl (10 mL). The mixture was diluted with 1 M Na$_2$S$_2$O$_3$ (10 mL) and extracted with Et$_2$O (3×20 mL). The combined organic extracts were washed with H$_2$O (30 mL), dried over anhyd. Na$_2$SO$_4$, filtered, and concentrated. The product was purified by chromatography on silica gel, eluting with EtOAc-hexanes (1:1), as a faint yellowish oil (269 mg, 23.1%). IR (ATR): 1778 (s), 1703 (s), 1212 (s), 703 (s) cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.28 (5H, m), 4.65 (1H, m), 4.15 (2H, m), 3.25 (1H, dd, $J_{1H-1H} = 3.2$ Hz, 13.6 Hz), 2.92 (2H, m), 2.75 (1H, q, $J_{1H-1H} = 7.8$ Hz), 1.17 (3H, t, $J_{1H-1H} = 7.1$ Hz); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 174.0, 153.7, 133.2, 129.3, 128.8, 127.2, 66.1, 55.0, 37.8, 29.0, 8.2.

**Benzyl 2-Acetyl-5-bromo-3-oxopentanoate:** Dry pyridine (3.22 mL, 40 mmol) was added via syringe into a stirred suspension of benzyl acetoacetate (3.46 mL, 20 mmol) and anhyd. MgCl$_2$ (1.90 g, 20 mmol) in dry CH$_2$Cl$_2$ (20 mL) at 0 °C. After 15 min 3-bromopropionyl chloride (2.02 mL, 20 mmol) was added via syringe. The mixture was allowed to warm to rt after 1 h. The reaction was quenched with c. HCl (5 mL) and the mixture extracted with Et$_2$O (3×50 mL). The extract was dried, filtered, and concentrated to give a yellow oil that was fractionated by chromatography on silica gel eluting with 20% EtOAc/hexanes. Only benzyl acetoacetate and benzyl 2-methyl-4-oxo-5,6-dihydro-4H-pyran-3-carboxylate were isolated. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$; 7.36 (5H, m), 5.24 (2H, s), 4.39 (2H, t, $J_{1H-1H} = 5.7$ Hz), 2.27 (2H, t, $J_{1H-1H} = 5.2$ Hz), 2.05 (3H, s); $^{13}$C NMR
3-Methoxypropanoic acid: Methyl 3-methoxypropionate (11.7 mL, 100 mmol) was added into a stirred solution of LiOH·2H₂O (8.39 g, 200 mmol) in H₂O (80 mL), MeOH (160 mL), and THF (240 mL) and then stirred overnight. The pH was adjusted to 2.5 with 5 M HCl and the mixture extracted with CH₂Cl₂ (3×100 mL). The extracts were combined, dried, filtered, and concentrated. The crude product was purified by distillation to yield a faint yellow liquid (7.49 g, 72%). Bp 65-66 °C (0.4 torr); ¹H NMR (400 MHz, CDCl₃) δ 11.73 (1H, br s), 3.63 (2H, t, J₁H-₁H = 6.1 Hz), 3.33 (3H, s), 2.58 (2H, t, J₁H-₁H = 6.1 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 177.2, 67.5, 58.6, 34.7.

3-Methoxypropanoyl chloride: Oxalyl chloride (1.4 g, 16.5 mmol) was added into a stirred solution of 108 (1.56 g, 15.0 mmol) in dry Et₂O (30 mL) at 0 °C. A catalytic amount of DMF (100 µL) was added. The solution was stirred overnight at rt. The reaction mixture was concentrated. The liquid obtained was fractionally distilled to give the product as a clear liquid (1.70 g, 92.9%). ¹H NMR (400 MHz, CDCl₃) δ 3.67 (2H, t, J₁H-₁H = 6.1 Hz), 3.35 (3H, s), 3.09 (2H, t, J₁H-₁H = 6.0 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 172.0, 67.1, 59.0, 46.9.

Phenylmethyl 2-acetyl-5-methoxy-3-oxopentanoate: Pyridine (11.0 mL, 137.0 mmol) was added via syringe into a stirred suspension of anhyd. MgCl₂ (6.5 g, 68.5 mmol) and benzyl acetoacetate (11.8 mL, 68.5 mmol) in dry CH₂Cl₂ (70 mL) at 0 °C. After 15 min, 109 (8.4 g, 68.5 mmol) was added via cannula. The mixture...
was allowed to warm to rt and stirred overnight. The reaction mixture was
acidified with 0.1 M HCl and extracted with Et₂O (3×100 mL). The extracts were
combined, dried, filtered, and concentrated. The crude product was purified by
chromatography on silica gel, eluting with EtOAc-hexanes (1:3), to give a pale
yellow liquid (17.13 g, 100%). IR (ATR): 1709 (s), 1072 (s), 752 (s); ¹H NMR (400
MHz, CDCl₃) δ 17.85 (1H, s), 7.36 (5H, m), 5.24 (2H, s), 3.63 (2H, t, J₁H₁H = 7.4
Hz), 3.27 (3H, s), 2.93 (2H, t, J₁H₁H = 4.4 Hz), 2.24 (3H, s); ¹³C NMR (100 MHz,
CDCl₃) δ 197.7, 196.2, 166.5, 135.6, 128.6, 128.5, 128.4, 128.2, 108.8, 68.4,
67.3, 59.0, 25.9.

6-Methoxyhexane-2,4-dione: Compound 110 (17.13 g, 68.5 mmol) and Pd on
activated carbon (1.7 g, 10% Pd by weight) were suspended in dry THF (75.0 mL)
and stirred vigorously under a H₂ atmosphere (balloon) overnight. Celite was
added to the reaction mixture, which was then filtered, and the residue washed
with CHCl₃ (3×100 mL). The filtrates were combined, concentrated, and purified
by chromatography on silica gel, eluting with EtOAc-hexanes (1:3), to give a pale
liquid (7.82 g, 79.2%). ¹H NMR (400 MHz, CDCl₃) δ 15.39 (1H, s), 5.50 (1H, s),
3.60 (2H, m), 3.28 (3H, s), 2.47 (2H, m), 2.01 (3H, s); ¹³C NMR (100 MHz, CDCl₃)
δ 191.6, 100.2, 68.3, 58.5, 38.7, 24.6.

(3R,5R)-1-Methoxyhexa-3,5-diol: Compound 111 (1.44 g, 10.0 mmol) and (+)-
Ru(binap)(PPh₂)₂Cl₂ (33.0 mg, 20.0 μmol) were dissolved in dry MeOH (2.0 mL) in
a reactor which was placed in a Parr hydrogenation apparatus. The reaction was
stirred at ca. 50 °C under 50 atm (740 psi) H₂ pressure for 3 days. Celite was
added to the resulting orange solution and removed by filtration. The dark-red

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filtrate was concentrated and purified by chromatography on silica gel, eluting with EtOH-hexanes (1:3), to give a clear oil (0.76 g, 51.8%). IR (ATR): 3390 (b), 1454 (m), 1379 (m), 1169 (s), 1084 (s), 737 (s), 697 (s); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 4.21 (2H, m), 3.60 (1H, m), 3.56 (1H, m), 3.39 (2H, s), 3.32 (3H, s), 1.83 (2H, m), 1.58 (2H, m), 1.19 (3H, d, \(J_{1H-1H} = 7.2\) Hz); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 71.8, 69.0, 64.7, 59.1, 44.3, 36.2, 23.6.

113 (3R,4R)-3,5-Bis-[(2,2-dimethyl)-propyl]-dimethylsilanyloxy]-1-methoxyhexane: Compound 112 (2.93 g, 20.0 mmol), TBSCI (9.04 g, 60.0 mmol), and imidazole (8.17 g, 120.0 mmol) were dissolved in dry DMF (20.0 mL) and stirred for 28 hrs. The reaction was quenched by the addition of H\(_2\)O (80 mL) and extracted with CH\(_2\)Cl\(_2\) (3x60 mL). The organic extracts were dried, filtered, and concentrated. The crude oil obtained was fractionated by chromatography on silica gel, eluting with EtOH-hexanes (1:3), to give the product as an oil (5.53 g, 100%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 4.04 (1H, q, \(J_{1H-1H} = 10.7\) Hz), 3.81 (1H, m), 3.39 (2H, m), 3.24 (3H, s), 1.66 (2H, m), 1.61 (2H, m), 1.05 (3H, d, \(J_{1H-1H} = 5.3\) Hz), 0.82 (18H, s), 0.01 (12H, s); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 69.4, 67.8, 66.3, 60.4, 58.5, 48.3, 37.9, 36.0, 34.2, 18.0, -0.42.

114 Phenylmethoxy 2-cyanoethane: A solution of NaOH (1.40 g, 35.0 mmol) in H\(_2\)O (4.7 mL) was added dropwise to BnOH (51.7 mL, 0.5 mol) at 0 °C. Acrylonitrile (131.7 mL, 2.0 mol) was slowly added into the stirred mixture. After 30 min the reaction was allowed to warm to rt and stirred overnight. The reaction mixture was quenched with 1 M HCl (50 mL) and extracted with CH\(_2\)Cl\(_2\) (5x200 mL). The extracts were combined, dried, filtered, and concentrated to yield the product, a
clear liquid (79.8 g, 99.0%). IR (ATR): 2251 (m), 1101 (s), 739 (s), 698 (s) cm⁻¹;
₁H NMR (400 MHz, CDCl₃) δ 7.35 (5H, m), 4.55 (2H, s), 3.62 (2H, t, J₁H₋₁H = 6.4 Hz), 2.56 (2H, t, J₁H₋₁H = 6.1 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 137.0, 128.2, 127.6, 127.4, 117.8, 72.8, 64.2, 18.5.

115 3-(Phenylmethoxy)-propanoic acid: Compound 114 (58.0 g, 360.0 mmol) was slowly added into a stirred solution of KOH (30.3 g, 540.0 mmol) in H₂O (1080 mL) and 30% H₂O₂ (140 mL). The suspension warmed up to 65 °C. After the temperature stabilized, the reaction was heated at reflux overnight. An aliquot (250 µL) was tested with aq. sat. FeSO₄ (1 mL) for H₂O₂ content. The solution remained green, as a qualitative test that no peroxide was present. The reaction mixture was quenched with 5 M HCl (150 mL) and saturated with NaCl. The mixture was extracted with EtOAc (4×500 mL). The extracts were combined, dried, filtered, and concentrated. The residue obtained was purified by chromatography on silica gel, eluting with EtOAc-hexanes (1:4), to give a clear liquid (10.73 g, 29.8%). ₁H NMR (400 MHz, CDCl₃) δ 7.36 (5H, m), 4.58 (2H, s), 3.76 (2H, t, J₁H₋₁H = 7.2 Hz), 2.69 (2H, J₁H₋₁H = 6.9 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 177.5, 137.5, 128.2, 127.6, 127.5, 72.9, 65.0, 34.7.

116 (2,2-Dimethyl)-propyl 2-acetyl-5-benzyloxy-3-oxopentanoate: Thionyl chloride (2.9 mL, 40 mmol) was added dropwise into neat 115 (3.60 g, 20.0 mmol) at 0 °C. The reaction mixture was allowed to warm to rt and then heated at reflux. The reaction mixture was concentrated after 2 h to give 3-(phenylmethoxy)-propanoyl chloride. Dry pyridine (2.4 mL, 30.0 mmol) was added via syringe into a stirred suspension of tert-butyl acetoacetate (2.5 mL, 15.0 mmol) and anhyd. MgCl₂ (1.43
g, 15.0 mmol) in dry CH₂Cl₂ (15.0 mL) at 0 °C. After 15 min, 3-(phenylmethoxy)-propanoyl chloride was added. The mixture was allowed to warm to rt and stirred overnight. The reaction mixture was acidified with 0.1 M HCl and extracted with Et₂O (3×75 mL). The combined extracts were dried, filtered, and concentrated. The residue obtained was purified by chromatography on silica gel, eluting with EtOAc-hexanes (1:3), to give a reddish oil (3.44 g, 71.6%). 

1H NMR (400 MHz, CDCl₃) δ 17.60 (1H, s), 7.32 (5H, m), 4.50 (2H, s), 3.74 (2H, t, J₁H-₁H = 6.6 Hz), 2.97 (2H, t, J₁H-₁H = 6.5 Hz), 3.30 (3H, s), 1.53 (9H, s); 13C NMR (100 MHz, CDCl₃) δ 195.7, 194.3, 166.0, 138.0, 128.2, 237.5, 127.4, 110.3, 81.4, 72.9, 65.8, 38.1, 28.0, 25.1.

**Phenylmethyl 2-acetyl-5-benzyloxy-3-oxopentanoate:** Thionyl chloride (14.5 mL, 200.0 mmol) was added dropwise into neat (18.0 g, 100.0 mmol) at 0 °C. The reaction mixture was allowed to warm to rt and then heated at reflux. The reaction mixture was concentrated after 2 h to give 3-(phenylmethoxy)-propanoyl chloride. Dry pyridine (14.5 mL, 180.0 mmol) was added via syringe into a stirred suspension of benzyl acetoacetate (15.6 mL, 90.0 mmol) and anhyd. MgCl₂ (8.57 g, 90.0 mmol) in dry CH₂Cl₂ (90 mL) at 0 °C. After 15 min, 3-(phenylmethoxy)-propanoyl chloride (19.87 g, 100.0 mmol) was added directly. The mixture was allowed to warm to rt after 1 h. The reaction was acidified with 0.1 M HCl and extracted with Et₂O (3×150 mL). The extracts were combined, dried, filtered, and concentrated. The product was obtained by chromatography on silica gel, eluting with EtOAc-hexanes (1:4), to give a faint yellow oil (21.59 g, 67.7%). 

1H NMR (400 MHz, CDCl₃) δ 17.97 (1H, s), 7.21 (10H, m), 5.21 (2H, s), 4.44 (2H, s), 3.73 (2H, t, J₁H-₁H = 6.7 Hz), 3.02 (2H, t, J₁H-₁H = 4.6 Hz), 2.11 (3H, s); 13C NMR (100 MHz, CDCl₃)
118 1-Phenylmethoxyhexa-3,5-dione:  

Procedure 1: TFA (12.0 mL, 156.0 mmol) was added by syringe to a solution of 116 (3.20 g, 10.0 mmol) in CH₂Cl₂ (36.0 mL). The color changed to red and within 15 min reverted back to a faint yellow, indicating completion of the reaction. The reaction mixture was diluted with CH₂Cl₂ (50 mL) and washed with aq. sat. NaHCO₃ (4×50 mL). The organic phase was dried, filtered, and concentrated. The product obtained was purified by chromatography on silica gel eluting with EtOAc-hexanes (1:3), as a clear liquid (290 mg, 13.2%).

Procedure 2: Compound 117 (3.54 g, 10.0 mmol) and palladium doped with ethylene diamine on activated carbon (0.35 g, 10% Pd(en) by weight) were suspended in dry THF (10.0 mL) and stirred vigorously under a H₂ atmosphere (balloon) for 24 h. Celite was added to the reaction mixture, which was then filtered, and the residue washed with CHCl₃ (80 mL). The combined organic fractions were concentrated, and the product obtained by chromatography on silica gel, eluting with EtOAc-hexanes (1:3), as a pale yellowish liquid (2.11 g, 95.7%). ¹H NMR (400 MHz, CDCl₃) δ 7.29 (5H, m), 5.41 (2H, s), 4.52 (2H, s), 3.69 (2H, t, J₁H–₇H = 6.5 Hz), 3.49 (2H, t, J₁H–₇H = 5.8 Hz), 1.99 (3H, s); ¹³C NMR (100 MHz, CDCl₃) δ 192.7, 139.1, 129.5, 128.8, 128.7, 101.6, 74.1, 67.0, 39.9.

119 (2,2-Dimethyl)-propyl 2-acetyl-3-oxohex-5-enoate: Oxalyl chloride (10.0 mL, 120.0 mL) was added via syringe to stirred neat vinylacetic acid (9.3 mL, 110.0 mmol) at 0 °C, followed by the addition of 2 drops of DMF. The solution was
stirred for 2.5 h to generate 3-butenoyl chloride. Dry pyridine (16.1 mL, 200.0 mmol) was added via syringe into a stirred suspension of tert-butyl acetoacetate (16.6 mL, 100.0 mmol) and anhyd. MgCl₂ (9.52 g, 100.0 mmol) in dry CH₂Cl₂ (100 mL) at 0 °C. After 15 min the crude 3-butenoyl chloride was added directly. The mixture was allowed to warm to rt and stirred overnight. The reaction mixture was acidified and extracted with Et₂O (3×50 mL). The extracts were combined, dried, filtered, and concentrated. The crude product obtained was purified by chromatography on silica gel, eluting with EtOAc-hexanes (1:3), to give a clear oil (11.0 g, 48.7%). ¹H NMR (400 MHz, CDCl₃) δ 17.41 (1H, s), 5.91 (1H, m), 5.15 (2H, m), 3.42 (2H, d, J_H-H = 3.9 Hz), 1.52 (9H, s), 1.44 (3H, s); ¹³C NMR (100 MHz, CDCl₃) δ 196.0, 166.4, 133.0, 118.7, 51.3, 42.1, 31.8, 28.0, 14.1.

122 4-Phenyl-3-butenolic acid: A stirred suspension of malonic acid (4.94 g, 47.5 mmol) in phenylacetaldehyde (5.8 mL, 50.0 mmol) and dry pyridine (4.0 mL, 50.0 mmol) was heated at reflux for 24 hrs. The reaction mixture was allowed to cool and then quenched with H₂O (100 mL). The aqueous phase was acidified with 1 M HCl and extracted with EtOAc (2×100 mL). The organic extracts were combined and washed with 1M NaOH (100 mL). The aqueous phase was acidified with c. H₂SO₄ and extracted with EtOAc (100 mL). The combined extracts were dried, filtered, and concentrated. The crude solid obtained was recrystallized to give fine white crystals (5.05 g, 65.6%). Mp 69-71 °C (Et₂O-hexanes); IR (ATR): 1698 (s), 1221 (m), 1209 (m), 743 (s), 692 (s); ¹H NMR (400 MHz, CDCl₃) δ 7.37 (5H, m), 6.50 (1H, d, J_H-H = 15.7 Hz), 6.30 (1H, m), 3.31 (2H, d, J_H-H = 6.8 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 178.4, 136.6, 134.0, 128.5,
(2,2-Dimethyl)-propyl 2-acetyl-3-oxo-6-phenylhex-5-enoate: Oxalyl chloride (2.6 mL, 30.0 mmol) was added via syringe to a stirred solution of 122 (2.43 g, 15.0 mmol) in dry Et₂O (4.0 mL), followed by addition of 2 drops of DMF. The reaction mixture was stirred at room temperature for 4 h. The excess oxalyl chloride was evaporated and the crude solid recrystallized to yield 4-phenyl-3-butenoyl chloride as orange crystals (1.10 g, 40.6%). Mp 40-42 °C (petroleum ether). Dry pyridine (3.2 mL, 40.0 mmol) was added via syringe to a stirred solution of tert-butyl acetoacetate (3.3 mL, 20.0 mmol) and anhyd. MgCl₂ (1.90 g, 20.0 mmol) in dry CH₂Cl₂ (20 mL) at 0 °C. 4-Phenyl-3-butenoyl chloride was added, the mixture was allowed to warm to rt and stirred overnight. The reaction mixture was acidified with 0.1 M HCl and extracted with Et₂O (3×100 mL). The extracts were combined, dried, filtered, and concentrated. The crude product was purified by chromatography on silica gel, eluting with EtOAc-hexanes (1:9), as a colorless oil (5.39 g, 89.1%). IR (ATR): 2977 (b), 1703 (s), 1082 (s), 754 (m), 691 (s); ¹H NMR (400 MHz, CDCl₃) δ 17.58 (1H, s), 7.25 (5H, m), 6.40 (1H, d, J₁H₁H = 16.0 Hz), 6.34 (1H, m), 3.60 (2H, d, J₁H₁H = 5.7 Hz), 2.31 (3H, s), 0.54 (9H, s); ¹³C NMR (100 MHz, CDCl₃) δ 196.0, 194.4 (enol resonance), 166.1, 136.8, 133.5, 128.4, 127.3, 126.1, 122.4, 109.9, 81.6, 41.5, 28.1, 25.1.

7-Phenylhept-6-ene-2,4-dione: Neat TFA (12 mL, 156 mmol) was added into a stirred solution of 123 (3.21 g, 10.6 mmol) in CH₂Cl₂ (36 mL). The color changed to Bordeaux-red. Within 5 min, it reverted back to light yellow, indicating complete reaction. The reaction mixture was diluted with CH₂Cl₂ (50 mL) and washed with
aq. sat. NaHCO₃ (3×75 mL). The organic phase was aqed, filtered, and concentrated. The yellow oil obtained was purified by Kugelrohr distillation to yield 124 as a faint yellow oil (1.89 g, 88.0%). ¹H NMR (400 MHz, CDCl₃) δ 15.43 (1H, br s), 7.31 (5H, m), 6.47 (1H, d, J_H-H = 15.4 Hz), 6.28 (1H, m), 5.55 (1H, s), 3.20 (2H, d, J_H-H = 5.1 Hz), 2.04 (3H, s); ¹³C NMR (100 MHz, CDCl₃) δ 192.8, 190.9 (enol resonance), 136.8, 133.8, 128.6, 127.6, 126.3, 122.5, 121.3, 99.5, 42.5, 24.6.

125 Phenylmethyl 2-acetyl-3-oxobutanoate: Dry pyridine (16.2 mL, 200.0 mmol) was added via syringe to a stirred solution of benzyl acetoacetate (17.3 mL, 100.0 mmol) and anhyd. MgCl₂ (9.52 g, 100.0 mmol) in dry CH₂Cl₂ (90 mL) at 0 °C. After 15 min phenylacetyl chloride (14.5 g, 110.0 mmol) in dry CH₂Cl₂ (10 mL) was added. The mixture was allowed to warm to rt and then stirred overnight. The reaction mixture was acidified with 0.1 M HCl and extracted with Et₂O (3×100 mL). The combined extracts were dried, filtered, and concentrated. The crude product was purified by chromatography on silica gel, eluting with EtOAc-hexanes (1:3), as a colorless oil (23.0 g, 74.0%). ¹H NMR (400 MHz, CDCl₃) δ 17.80 (1H, s), 7.35 (10H, m), 5.25 (2H, s), 4.03 (2H, s), 2.28 (3H, s); ¹³C NMR (100 MHz, CDCl₃) δ 196.7, 196.1 (enol resonance), 167.0, 135.4, 134.9, 129.5, 129.4, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 108.5, 67.0, 43.9, 25.8.

126 1-Phenyl-2,4-pentadione: Compound 125 (69.7 g, 225.0 mmol) and Pd on activated carbon (3.48 g, 5% Pd by weight) were suspended in dry THF (200.0 mL) and stirred vigorously under a H₂ atmosphere (balloon) overnight. Celite was added to the reaction mixture, which was then filtered, and the residue washed.
with CHCl₃ (4×100mL). The combined filtrates were concentrated to give an orange liquid that was purified by Kugelrohr distillation (29.5 g, 74.4%). IR (ATR): 1712 (s), 1599 (s), 1236 (s), 697 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.30 (5H, m), 5.42 (1H, s), 3.63 (1H, s), 3.55 (2H, s), 1.96 (3H, s); ¹³C NMR (100 MHz, CDCl₃) δ 191.5, 129.7, 129.4, 128.8, 128.6, 127.1, 99.9, 45.1, 41.0, 24.8.

127 (2R, 4R)-1-Phenyl-2,4-pentadiol: Compound 126 (8.81 g, 50.0 mmol) and (+)-Ru(binap)(PPh₂)₂Cl₂ (80.0 mg, 100.0 μmol) were dissolved in dry MeOH (7.5 mL) in a reactor which was placed in a Parr hydrogenation apparatus. The reaction was stirred at 50 °C under 52 atm (760 psi) H₂ pressure for 3 days. Celite was added to the solution, removed by filtration, and washed with MeOH (100 mL). The filtrates were concentrated, and purified by Kugelrohr distillation under vacuum to yield a faint yellow liquid (8.31 g, 92.2%). Bp 100 °C (0.08 torr); IR (ATR): 3358 (b), 1454 (s), 1436 (s), 699 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.23 (5H, m), 4.13 (2H, br s), 2.73 (2H, m), 2.24 (2H, br s), 1.61 (2H, m), 1.20 (3H, d, J₁H-₁H = 2.7 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 138.2, 129.4, 128.6, 126.5, 70.1, 65.3, 44.0, 43.5, 23.5.

128 (4R, 6R)-4-Phenylmethyl-2,2,6-trimethyl-1,3-dioxane: Dry 2,2-dimethoxypropane (2.5 mL, 20.0 mmol) was added to a stirred solution of 127 (1.80 g, 10.0 mmol) in dry benzene (10.0 mL). Recrystallized p-TsOH (100 mg, 0.5 mmol) was added and the mixture heated at reflux for 8 h. The reaction mixture was diluted with H₂O (10 mL) and extracted with Et₂O (3×20 mL). The extracts were combined, dried, filtered, and concentrated. The residue obtained was purified by chromatography on silica gel, eluting with Et₃N-EtOAc-hexanes...
(2.5%:1%:96.5%), to give a clear liquid (1.02 g, 45.7%). \(^1\)H NMR (400 MHz, CD\(_2\)D\(_3\)) \(\delta\) 7.28 (5H, m), 4.10 (1H, m), 3.98 (1H, m), 2.79 (2H, dq, \(J_{1H-1H} = 6.2\) Hz, \(J_{1H-1H} = 93.3\) Hz), 1.70 (H, m), 1.37 (3H, s), 1.33 (3H, s), 1.15 (3H, d, \(J_{1H-1H} = 6.7\) Hz); \(^1^3\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 129.2, 128.4, 128.2, 127.4, 126.2, 100.2, 67.4, 62.8, 42.1, 39.5, 25.0, 24.9, 21.7.

129 (2\(R\), 4\(R\))-1-Phenyl-2,4-pentadiol acetate: Distilled Ac\(_2\)O (2.8 mL, 30.0 mmol) was added to a solution of 127 (1.80 g, 10.0 mmol), pyridine (2.4 mL, 30.0 mmol), and DMAP (98 mg, 0.8 mmol) in dry CH\(_2\)Cl\(_2\) (40 mL) at 0 °C. The mixture was allowed to warm to rt after 1 h, and then stirred for 1 h. The reaction mixture was diluted with H\(_2\)O (40 mL) and extracted with CH\(_2\)Cl\(_2\) (3\(\times\)20 mL). The extracts were combined, washed with aq. sat. NaCl, dried, filtered, and concentrated. The residue obtained was purified by chromatography on silica gel, eluting with EtOAc-hexanes (1:3), to give a clear liquid that solidified upon standing (1.75 g, 66.1%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.19 (5H, m), 5.16 (1H, m), 4.95 (1H, m), 2.85 (2H, dq, \(J_{1H-1H} = 6.7\) Hz, \(J_{1H-1H} = 28.1\) Hz), 2.00 (6H, d, \(J_{1H-1H} = 5.5\) Hz), 1.72 (2H, m), 1.19 (3H, d, \(J_{1H-1H} = 6.1\) Hz); \(^1^3\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 170.6, 170.3, 136.9, 129.5, 128.4, 126.6, 70.6, 66.8, 40.9, 39.6, 21.2, 21.0, 20.4.

130 (3\(R\), 5\(R\))-3,5-Diacetyloxyhexanoic acid: Ozone was bubbled through a solution of 129 (3.96 g, 15.0 mmol) in dry CH\(_2\)Cl\(_2\) (150 mL) at rt for 8 h. The characteristic blue color of excess ozone was observed. The solution was purged with oxygen for 5 min. The reaction mixture was diluted with H\(_2\)O (150 mL), quenched with 30% H\(_2\)O\(_2\) (90 mL), and left stirring at rt for 12 h. The mixture was extracted with CH\(_2\)Cl\(_2\) (3\(\times\)150 mL). The extracts were combined, dried, filtered, and
concentrated. The residue obtained was purified by chromatography on silica gel, eluting with a gradient of EtOAc/hexanes (1:3 to 1:1), to give 130 as a clear oil (808 mg, 23.2%). IR (ATR): 2984 (b), 1736 (s), 1242 (s), 1177 (m), 907 (s), 728 (s) cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 10.86 (1H, b), 5.24 (1H, m), 4.94 (1H, m), 2.59 (2H, m), 2.07 (3H, s), 2.01 (3H, s), 1.84 (2H, m), 1.21 (3H, d, \(J\)\(_{IH-HH}\) = 6.1 Hz); \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 175.2, 170.8, 170.3, 66.7, 66.4, 39.9, 38.9, 21.0, 20.8, 20.2.

131 (3R, 5R)-3,5-Diacet oxyhexanol: A solution of BH\(_3\)-THF (14.4 mmol) in THF (14.4 mL) was added via syringe into a stirred solution of 130 (2.79 g, 12.0 mmol) in dry THF (48 mL) at 0 °C. The reaction mixture was allowed to warm to rt and then stirred for 5 h. The reaction was quenched with aq. sat. NH\(_4\)Cl (20 mL). The mixture was extracted with CH\(_2\)Cl\(_2\) (3×75 mL). The combined extracts were washed with aq. sat. NaCl (75 mL), dried, filtered, and concentrated. The residue obtained was purified by chromatography on silica gel, eluting with EtOAc-hexanes (1:1), to give a faint yellow oil (353 mg, 13.5%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 4.99 (1H, m), 4.76 (1H, m), 3.47 (2H, m), 2.98 (1H, b), 1.86 (6H, d, \(J\)\(_{IH-HH}\) = 14.9 Hz), 1.68 (2H, m), 1.59 (2H, m), 1.13 (3H, d, \(J\)\(_{IH-HH}\) = 1.99 Hz); \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 171.6, 170.5, 67.4, 66.5, 57.9, 40.6, 37.4, 20.9, 20.7, 20.2.

132 Phenylmethyl 2-acetyl-4-(3,4-dimethoxyphenyl)-3-oxobutanoate: Dry pyridine (3.2 mL, 40.0 mmol) was added via syringe to a stirred solution of benzyl acetoacetate (3.5 mL, 20.0 mmol) and anhyd. MgCl\(_2\) (1.90 g, 20.0 mmol) in dry CH\(_2\)Cl\(_2\) (15 mL) at 0 °C. After 15 min 3,4-dimethoxyphenylacetyl chloride (4.94 g, 23.0 mmol) in dry CH\(_2\)Cl\(_2\) (5 mL) was added. The mixture was allowed to warm to
rt and then stirred overnight. The reaction mixture was acidified with 0.1 M HCl and extracted with Et2O (3×50 mL). The extracts were combined, dried, filtered, and concentrated. The crude product was purified by chromatography on silica gel, eluting with EtOAc-hexanes (1:3), to give a colorless oil (5.65 g, 76.3%). IR (ATR): 1710 (s), 1514 (s), 1262 (s), 1238 (s), 1070 (s), 1030 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.36 (5H, m), 6.74 (3H, m), 5.42 (2H, s), 3.94 (2H, s), 3.83 (3H, s), 3.79 (3H, s), 2.30 (3H, s); ¹³C NMR (100 MHz, CDCl₃) δ 196.5, 195.6 (enol resonance), 166.7, 148.6, 147.9, 135.1, 128.5, 128.4, 128.3, 121.4, 112.4, 110.9, 66.7, 55.6, 55.5, 43.1, 25.4.

133 1-(3,4-Dimethoxyphenyl)-penta-2,4-dione: Compound 132 (5.37 g, 14.5 mmol) and Pd on activated carbon (0.5 g, 10% Pd by weight) were suspended in dry THF (15.0 mL) and stirred vigorously under a H₂ atmosphere (balloon) overnight. Celite was added to the reaction mixture, which was then filtered, and the residue washed with CHCl₃ (3×100 mL). The combined organic phases were concentrated to give an orange liquid that was purified by chromatography on silica gel, eluting with EtOAc-hexanes (1:1), to give a pale yellowish oil (3.12 g, 91.1%). ¹H NMR (400 MHz, CDCl₃) δ 15.39 (0.9 H, br s, enol), 6.79 (1H, d, J₁H-1H = 7.9 Hz), 6.76 (1H, d, J₁H-1H = 1.8 Hz), 6.73 (1H, s), 5.41 (0.9H, s, enol), 3.85 (3H, s), 3.84 (3H, s), 3.49 (2H, s), 1.99 (3H, s); ¹³C NMR (100 MHz, CDCl₃) δ 192.7, 190.9 (keto), 148.9 (keto), 148.0 (enol), 127.4, 121.7, 121.4, 112.3, 111.3, 111.2, 99.6 (enol), 55.7, 44.6, 24.6.

134 (2R, 4R)-1-(3,4-Dimethoxyphenyl)-penta-2,4-diol: Compound 133 (7.33 g, 31.0 mmol) and (+)-Ru(binap)(PPh₂)₂Cl₂ (50.0 mg, 62.0 µmol) were dissolved in dry
MeOH (6.0 mL) in a reactor which was placed in a Parr hydrogenation apparatus. The reaction was stirred at 45 °C under 82 atm (1200 psi) H\textsubscript{2} pressure for 5 days. Celite was added to the solution, which was then filtered. The filtrate was concentrated and purified by Kugelrohr distillation under vacuum (0.15 torr) to yield a very viscous oil (4.89 g, 48.6%). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 6.77 (3H, m), 4.12 (2H, m), 3.87 (6H, s), 2.56 (1H, \(J_{1H-1H} = 6.5\) Hz), 2.43 (1H, \(J_{1H-1H} = 6.5\) Hz), 2.04 (3H, s), 1.88 (2H, m), 1.25 (3H, s).

135 (2\textsubscript{R,4\textsubscript{R}})-1-(3,4-Dimethoxyphenyl)-penta-2,4-diol acetate: Distilled Ac\textsubscript{2}O (14.1 mL, 150.0 mmol) was added to a solution of 134 (4.86 g, 15.0 mmol) in dry pyridine (24.2 mL, 300.0 mmol) at 0 °C. The mixture was allowed to warm to rt after 1 h and then stirred overnight. The reaction mixture was diluted with CH\textsubscript{2}Cl\textsubscript{2} (100 mL) and washed twice with aq. CuSO\textsubscript{4} (100 mL), followed by a wash with H\textsubscript{2}O (100 mL) and then aq. sat. NaHCO\textsubscript{3} (100 mL). The organic phase was dried, filtered, and concentrated. The residue obtained was purified by chromatography on silica gel, eluting with EtOAc-hexanes (1:3), to give a clear liquid (1.03 g, 21.3%). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 6.74 (3H, m), 6.68 (5H, m), 5.01 (1H, m), 4.92 (1H, m), 3.84 (7H, d, \(J_{1H-1H} = 1.2\) Hz), 3.83 (7H, d, \(J_{1H-1H} = 2.4\) Hz), 2.53 (2H, t, \(J_{1H-1H} = 7.6\) Hz), 2.40 (2H, t, \(J_{1H-1H} = 7.3\) Hz), 2.09 (2H, s), 2.05 (2H, s), 1.97 (6H, s); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \(\delta\) 170.6, 170.3, 148.6, 147.3, 121.4, 120.1, 112.5, 70.6, 66.8, 55.8, 55.7, 40.3, 39.5, 34.5, 25.2, 21.1, 20.3.

144 Methyl 3-bromopropanoate: A solution of 3-bromopropionic acid (38.25 g, 250.0 mmol) in CH\textsubscript{3}OH (100 mL) was cooled to 0 °C and c. H\textsubscript{2}SO\textsubscript{4} (1.3 mL, 25.0 mmol) was added. The solution was heated at reflux for 12 h. The reaction mixture was
cooled, diluted with CH$_2$Cl$_2$ (200 mL), and washed with aq. sat. NaHCO$_3$ (2×150 mL). The organic extracts were combined, dried, filtered, and concentrated to yield a colorless liquid (35.6 g, 85.2%). IR (ATR): 1735 (s), 1237 (m), 1214 (s), 616 (m) cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 3.68 (3H, s), 3.53 (2H, t, $J_{1H-1H} = 9.9$ Hz, 2.49 (2H, t, $J_{1H-1H} = 9.3$ Hz); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.8, 51.8, 37.3, 25.7.

145 Methyl 3-cyanopropanoate: A solution of 144 (5.83 g, 35.0 mmol) in EtOH (35 mL) was added to a solution of KCN (2.27 g, 35.0 mmol) in H$_2$O (35.0 mL) and then stirred for 2 days at rt. Carefully, 4 M HCl was added to adjust the pH to 6. The reaction mixture was extracted with Et$_2$O (3×75 mL). The combined organic extracts were washed with H$_2$O (2×100 mL), dried, filtered, and concentrated. The residue was purified by chromatography on silica gel, eluting with an EtOAc-hexanes (1:3 to 1:1) gradient, to give an oil (1.13 g, 28.4%). IR (ATR): 2253 (w), 1736 (s), 1244 (m), 1206 (m), 1177 (m), 1046 (w), 752 (s) cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 3.70 (3H, s), 2.63 (4H, m); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.4, 118.4, 52.1, 29.4, 12.7.

143 3-Cyanopropanoic acid: A solution of LiOH (361 mg, 8.6 mmol) in H$_2$O (3.5 mL) was added to a solution of 145 (487 mg, 4.3 mmol) in THF (3.5 mL). The mixture was stirred for 6 h at rt. The reaction mixture was concentrated to ca. 2 mL and dissolved in H$_2$O (3 mL). The solution was extracted with Et$_2$O (3×50 mL). The combined extracts were dried, filtered, and concentrated. The resulting viscous liquid crystallized upon cooling on ice. After recrystallization from CHCl$_3$, the product was collected as white crystals (185 mg, 43.5%). $^1$H NMR (400 MHz,
D$_2$O $\delta$ 2.60 (4H, m); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 175.9, 95.5, 30.3, 13.4.

146 Benzyl 3-hydroxybutanoate: Powdered NaBH$_4$ (0.11 g, 3.0 mmol) was added to a solution of benzyl acetoacetate (1.7 mL, 10.0 mmol) in H$_2$O (15.0 mL) in dry THF (85.0 mL) at 0 °C. The reaction was stirred for 40 min before dilution with H$_2$O (70 mL) and extraction with Et$_2$O (3×50 mL). The extracts were combined, dried, filtered, and concentrated to give a yellow oil, which was purified by chromatography on silica gel, eluting with EtOAc-hexanes (1:3), to give a colorless oil (1.94 g, 99.9%).

(3R)-Benzyl 3-hydroxybutanoate: Benzyl acetoacetate (8.6 mL, 50.0 mmol) and (+)-Ru(binap)(PPh$_2$)$_2$Cl$_2$ (25 mg, 20 µmol) were dissolved in dry MeOH (10.0 mL) in a reactor which was placed in a Parr hydrogenation apparatus. The reaction was stirred at ca. 30 °C under 82 atm (1200 psi) H$_2$ pressure for 36 h. Celite was added to the reaction mixture, which was then filtered. The filtrate was concentrated and purified by Kugelrohr distillation to give 146 (9.71 g, 99.96%).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.26 (5H, m), 5.03 (2H, s), 4.13 (1H, m), 2.42 (2H, d, $J_{1H-1H} = 9.8$ Hz), 1.12 (3H, d, $J_{1H-1H} = 7.9$ Hz); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 172.2, 135.4, 128.1, 128.0, 126.6, 66.1, 64.0, 42.8, 22.3.

147 3-Hydroxybuturic acid: Compound 146 (11.37 g, 58.5 mmol) and Pd on activated carbon (1.14 g, 10% Pd by weight) were suspended in dry THF (100.0 mL) and stirred vigorously overnight under a H$_2$ atmosphere (balloon). Celite was added to the reaction mixture, which was then filtered, and the residue washed with CHCl$_3$ (3×75 mL). The combined organic fractions were concentrated, and the product purified by vacuum distillation to give a clear liquid (5.93 g, 97.4%).
Bp 63 °C (0.15 torr); IR (ATR): 2975 (b), 1709 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.54 (2H, br s), 4.19 (1H, m), 2.42 (2H, m), 1.16 (3H, d, J₃₋₁H = 6.7 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 176.5, 64.3, 42.4, 22.2.

148 2-(1,1-Dimethylethyl)-6-methyl-1,3-dioxan-4-one: Compound 147 (1.85 mL, 20.0 mmol), Sc(OTf)₃ (0.39 g, 0.8 mmol), and pivalaldehyde (3.26 mL, 30.0 mmol) were dissolved in dry CH₂Cl₂ (200 mL) and heated at reflux. The condensed vapors were dripped through a Soxhlett extractor with 4Å molecular sieves. After 6 h the reaction mixture was allowed to cool and washed with H₂O (3×75 mL). The organic layer was dried, filtered, and concentrated. The residue obtained was purified by chromatography on silica gel, eluting with EtOAc-hexanes (1:9), to give 148 as a clear oil (0.9 g, 26.1%). ¹H NMR (400 MHz, CDCl₃) δ 4.85 (1H, s), 3.96 (1H, m), 2.58 (1H, dd, J₁H₋₁H = 4.8 Hz, J₁H₋₁H = 16.0 Hz), 2.34 (1H, dd, J₁H₋₁H = 8.0 Hz, J₁H₋₁H = 16.0 Hz), 1.25 (3H, d, J₁H₋₁H = 7.8 Hz), 0.90 (9H, s); ¹³C NMR (100 MHz, CDCl₃) δ 168.3, 108.5, 70.4, 37.4, 35.1, 23.9, 21.1.

149 2-(1,1-Dimethylethyl)-6-methyl-1,3-dioxan-4-yl acetate: Compound 148 (1.41 g, 8.2 mmol) was dissolved in dry CH₂Cl₂ (32 mL) and cooled to -78 °C. DIBALH (9.0 mmol) in CH₂Cl₂ (9 mL) was slowly added via syringe, with the temperature remaining below -70 °C. After 3 h, pyridine (2.0 mL, 24.6 mmol) was added, followed by DMAP (1.10 g, 9.0 mmol) dissolved in dry CH₂Cl₂ (16 mL), and then distilled Ac₂O (3.1 mL, 32.8 mmol). The mixture was allowed to warm to rt and stirred for 2 h. The reaction was quenched with aq. sat. NH₄Cl (25 mL) and then stirred for 30 min. The mixture was extracted with CH₂Cl₂ (3×20 mL), and the organic phase washed twice with 1 M aq. Oxone® (20 mL), twice with aq. sat.
NaHCO₃ (20 mL), and once with aq. sat. NaCl (20 mL). The organic fraction was dried, filtered, and concentrated. The product was obtained by chromatography on silica gel, eluting with Et₃N-EtOAc-hexanes (1:10:89), as a clear liquid (0.3 g, 16.9%). ¹H NMR (400 MHz, CDCl₃) δ 5.81 (1H, dd, Jₐₐ-₁H = 1.3 Hz, Jᵢᵢ-₁H = 10.7 Hz), 4.22 (1H, s), 3.75 (1H, m), 2.10 (3H, s), 1.73 (2H, m), 1.26 (3H, s), 0.92 (9H, s); ¹³C NMR (100 MHz, CDCl₃) δ 169.2, 105.1, 93.4, 70.9, 37.4, 34.6, 24.6, 24.3, 21.2.
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