Altering the Configuration of the Secondary Alcohol and Quaternary Carbon in Pantothenate Derivatives: Synthesis of Novel *N*-Pentylpantothenamides

Hamed Darabi

Master of Science

Department of Chemistry

McGill University

Montreal, Quebec, Canada

July 8, 2013

©Copyright 2013 All rights reserved.

DEDICATION

This document is dedicated to the graduate students of the McGill University.

ACKNOWLEDGMENTS

I thank my parents for supporting me throughout my studies over the years, and I hope to return the favour one day. It has been a great pleasure to work under the supervision of Prof. Karine Auclair. She has helped me to troubleshoot throughout my research, especially when I was trying to extract my product from slurry of yeast and water. Earlier in my Masters, I received my organic synthesis training from Dr. Kayode Akinnusi, thus I would like to thank him for leading me in the initial stage of my research. In the end it has been six years now that I have been studying at McGill, and I will miss all of the great memories from this lively and eventful campus in the heart of downtown Montreal.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF SCHEMES

LIST OF FIGURES

ABBREVIATION

EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ACP	acyl carrier protein
ADP	adenosine di-phosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
Ala	alanine
cat.	catalytic
δ	chemical shift
CoA	coenzyme A
J	coupling constant
DCM	dichloromethane
d	doublet
ESI	electrospray ionization
E. coli	Escherichia coli
EtOH	ethanol
Et	ethyl
EtOAc	ethyl acetate
Hz	hertz
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
HCl	hydrochloric acid
H	hydrogen
HOBt	hydroxybenzotriazole
LC-MS	liquid chromatography - mass spectrometry
MIC	mean inhibitory concentration
MeOH	methanol
MRSA	methicillin-resistant *Staphyloccocus aureus*
OMe	methoxy
Me	methyl
µl	microliter
µM	micromolar
µM	micromolar
mg	milligram
m	multiplet
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	*N*,*N*-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
nM	nanomolar
pH	negative logarithm of hydrogen ion concentration

NADH	Nicotinamide adenine dinucleotide
N5-pan	*N*-pentylpantothenamide
NMR	nuclear magentic resonance
HATU	*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate
HBTU	O-(benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate
$(COCl)_2$	oxalyl chloride
PanK	pantothenate kinase
ppm	parts per million
PPAT	phosphopantetheine adenylyltransferase
PPCDC	phosphopantothenoylcysteine decarboxylase
PPCS	phosphopantothenoylcysteine synthetase
PP_i	pyrophosphate
R_f	retention time
sat. aq.	saturated aqueous
s	singlet
$NaHCO_3$	sodium bicarbonate
SAR	structure activity relationships
THF	tetrahydrofuran
TLC	thin layer chromatography
NEt_3	triethylamine
TEA	triethylamine
t	triplet
H_2O	water

ABSTRACT

The synthesis of pantothenamides with various but defined stereoconfigurations is promising for medicinal chemistry studies. *N*-pentylpantothenamides are antibacterial agents. Previous SAR studies reported an MIC of 3.6 μM against *S. aureus* for the allyl derivative with *anti* stereoconfiguration with respect to the secondary alcohol and the quaternary carbon. With the goal of improving the MIC, it was envisaged that compounds with the *syn* stereoconfiguration might have different activity. Thus a synthetic approach was developed which set the *anti* stereochemistry at the di alkyl germinal carbon via Frater's alkylation. Next the alcohol was oxidized to the ketone, and selectively reduced to generate the *syn* stereoconfiguration.

Over the course of the synthesis of the *syn* stereoconfiguration, several different chemical and biocatlytic reducing agents were used. The chemical reducing agents tested were $NaBH_4$, DIBAL-H, CBS and $Zn_2(BH_4)_2$. None of these chemical reducing agents are able to overcome the energetic barriers of forming the *syn* product, instead favouring the formation of the *anti* product which is thermodynamically favoured. Interestingly with Baker's yeast the *syn* product is the major product. Next the scope of this reaction was studied by varying the alkyl chain at the quaternary carbon, ranging from small to large groups. Remarkably the Baker's yeast accepted a wide range of substrates.

The compounds were finally extended to the full pantothenamide derivatives via the previously reported methodology. The final pantothenamide compounds will be tested for antibacterial activity, hopefully improving on the MIC

of the parent compound. Furthermore these compounds have shown to be active antimalarial agents.

ABRÉGÉ

La synthèse de pantothenamides avec des stéréoconfigurations variées mais définies est prometteur pour les études en chimie médicinale. Le *N*-pentylpantothenamide est un agent anti-bactérien. Des études SAR précédantes ont raporté un CMI de 3.6 μM contre *S. aureus* pour le derivative allyl avec stereoconfiguration *anti* de l'alcool secondaire et du carbone quartenaire. Avec le but d'améliorer le CMI, nous attendions que les composés avec la stereoconfiguration *syn* aurait une activité différemment. Ainsi, nous avons développé une nouvelle approche synthétique qui a mis la stéréochimie *anti* au carbone di aklkyl via alkylation de Frater avant d'avoir oxidé l'alcool du cétone suivi par une réduction sélective afin de générer la stéréoconfiguration *syn*.

Au cours de cette stratégie de synthèse pour développer la stéréoconfiguration *syn*, différentes agents réducteurs et réductions biocatalytiques, tel que la levure de Baker, ont été utilisées. Les agents réducteurs utilisé étaient $NaBH_4$, DIBAL-H, CBS and $Zn_2(BH_4)_2$. Aucun de ces agents ont été capables de surmonter les barrières énergétiques nécessaire pour former le produit *syn*. À la place, la formation du produit *anti* est thermodynamiquement favorisée. Intéressamment, le levure de Baker donne le produit *syn* comme produit dominant. Ensuite, le potentiel de cette réaction a été exploré en variant la chaîne d'alkyl au carbone quartenaire, allant d'un petit à de larges groupes. La levure de Baker a accepté une grande variété de substrats.

Au final, les composés ont été agrandi en dérivatifs de pantothenamide via la méthode précédente. Les panthothenamides finals seront testés pour activité antibactérienne dans l'espoir, d'améliorer la CMI du composé parent. De plus, ces composés ont démontré de l'activité antimalariale.

1 INTRODUCTION

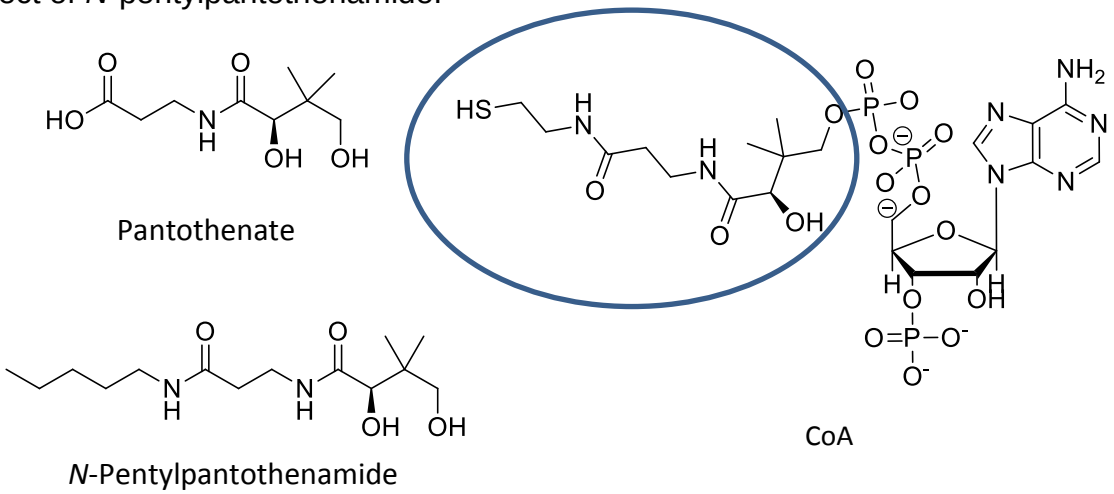
Bacteria are increasingly countering antibiotics through various resistance mechanisms. Intense usage of antibiotics has created a pressure to select for resistant strains. Moreover bacteria can transfer resistance genes through bioconjugation, transformation or transduction which aggravates the problem.[1] The rapid evolution of bacteria to various antibiotics is a global concern because the phenomenon of antibiotic resistance is widespread in hospitals across all continents, including North America. It is therefore necessary to develop new agents to treat resistant infections.

A large variety of resistance mechanisms have been identified.[1] They encompass: 1) mutations on the target protein in bacteria; 2) expression of efflux pumps that excrete the drug out of the cell; 3) expression of bypass pathways; and 4) and cell impermeability. In addition, bacteria can express enzymes that cleave or modify the drug to render them inactive. For example aminoglycoside antibiotics can undergo acetylation, adenylylation or phosphorylation by enzymes expressed in bacteria.[2] Another example includes β-lactam antibiotics such as penicillin which are inactivated by β-lactamases.[3] The latter cleave the β-lactam ring of these antibiotics. Of special concern is the fact that bacteria can gain multiple resistance mechanisms simultaneously.

1.1 Pantothenate Analogs as Potential Antibacterial Agents

Coenzyme A (CoA, Figure 1-1) is an essential cofactor involved in a large number of processes, including fatty acid and amino acid biosynthesis.[4]

Pantothenate is a key precursor in the biosynthesis of CoA in bacteria.[5] Lactic acid bacteria rely on exogenous sources of pantothenic acid, whereas most other bacterial strain can synthesize pantothenate.[6,7] Pantothenamide derivatives such as *N*-pentylpantothenamide (**1.1**, Figure 1-1) often show antibacterial activity due to their ability to mimic pantothenate, and thus affect the CoA biosynthetic and utilization pathways. For example **1.1** shows an MIC of 2 µM against *E. coli*.[8] This MIC was not affected by the presence of added pantothenate. In lactic acid bacteria, however, a high concentration of pantothenate was found to reverse the effect of *N*-pentylpantothenamide.[8]



1.1

Figure 1-1: Structure of pantothenate, *N*-pentylpantothenamide and CoA

Bacteria convert pantothenate into CoA through the CoA biosynthetic pathway. In this process, the pantothenate undergoes five biotransformations which are catalyzed by the enzymes CoaA to CoaE (Figure 1-2).[5] This pathway is highly conserved in prokaryotes. First, pantothenate is phosphorylated at the primary hydroxyl position by pantothenate kinase (PanK or CoaA) producing 4'-phosphopantothenate.[9] This intermediate

undergoes a condensation reaction with L-cysteine catalyzed by the enzyme phosphopantothenoylcysteine synthetase (PPCS or CoaB), yielding 4'-phosphopantothenoyl cysteine.[10] Next phosphopantothenoylcysteine decarboxylase (PPCDC or CoaC) catalyzes decarboxylation of the cysteine moiety to produce 4'-phosphopantetheine.[10] This is followed by adenylyl transfer onto the primary alcohol by phosphopantetheine adenylyl transferase (PPAT or CoaD) to yield dephospho-CoA.[11] Finally the enzyme dephosphocoenzyme A kinase (DPCK or CoaE) catalyzes phosphorylation at the 3'-hydroxyl position of the ribose group, producing CoA.[12]

The non-natural analog *N*-pentylpantothenamide (**1.1**) was demonstrated to be similarly converted to ethyldethia-coenzyme A, when incubated with the CoA biosynthetic enzymes.[5]

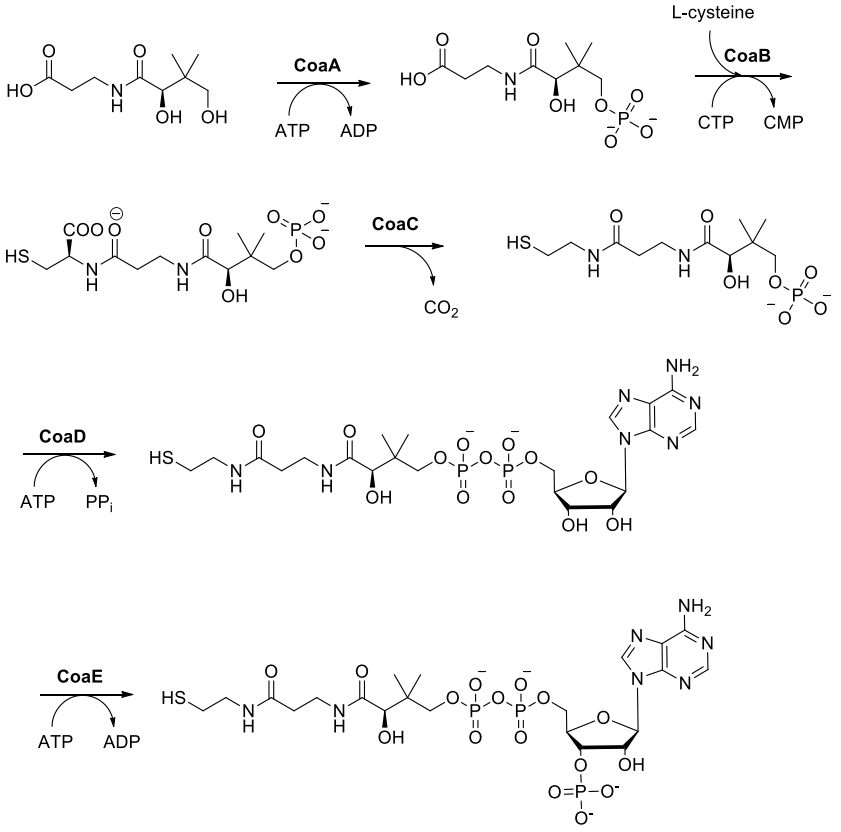


Figure 1-2: CoA biosynthetic pathway from pantothenate

Three enzymes of the CoA biosynthetic pathway are involved in converting *N*-pentylpantothenamide (**1.1**), namely PanK, PPAT and DPCK (Figure 1-3).[5] The turnover rate for the formation of ethyldethia-coenzyme A from *N*-pentylpantothenamide is 10.5 times faster than that of the natural analog pantothenate in *E. coli*.[5]

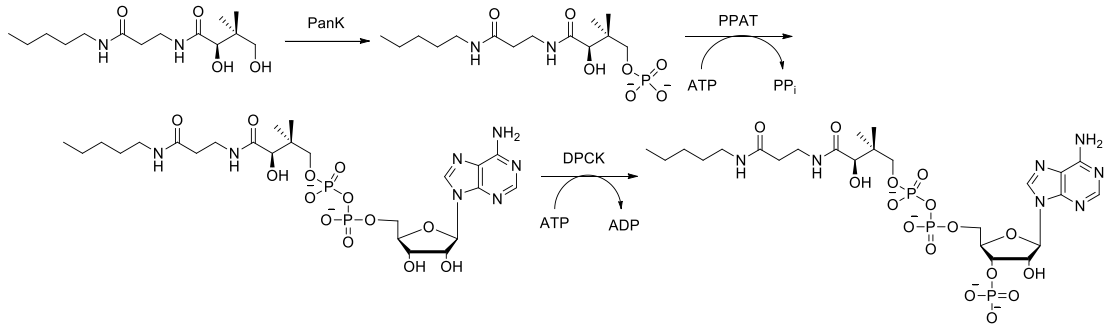


Figure 1-3: Conversion of *N*-pentyl pantothenamide to ethyldethia CoA by PanK, PPAT and DPCK

The ethyldethia-CoA thus produced has been shown to negatively affect the CoA-utilizating pathway, for example by incorporating into the acyl carrier protein (ACP). ACP is involved in the transfer of acyl moeities in the biosynthesis of fatty acids. ACP is normally activated by ACP synthase (AcpS) via formation of a phosphoester bond with the pantetheine fragment of CoA.[9] When ethyldethia-CoA is used instead of CoA, an ACP variant lacking the thiol group is generated (Figure 1-4).[4] This thiol is required for the transfer of acyl moieties during fatty acid biosynthesis.[4] Thus the antibacterial activity of compound **1.1** results both from diminishing the levels of CoA in cell, and inhibiting the CoA-utilizing enzymes.

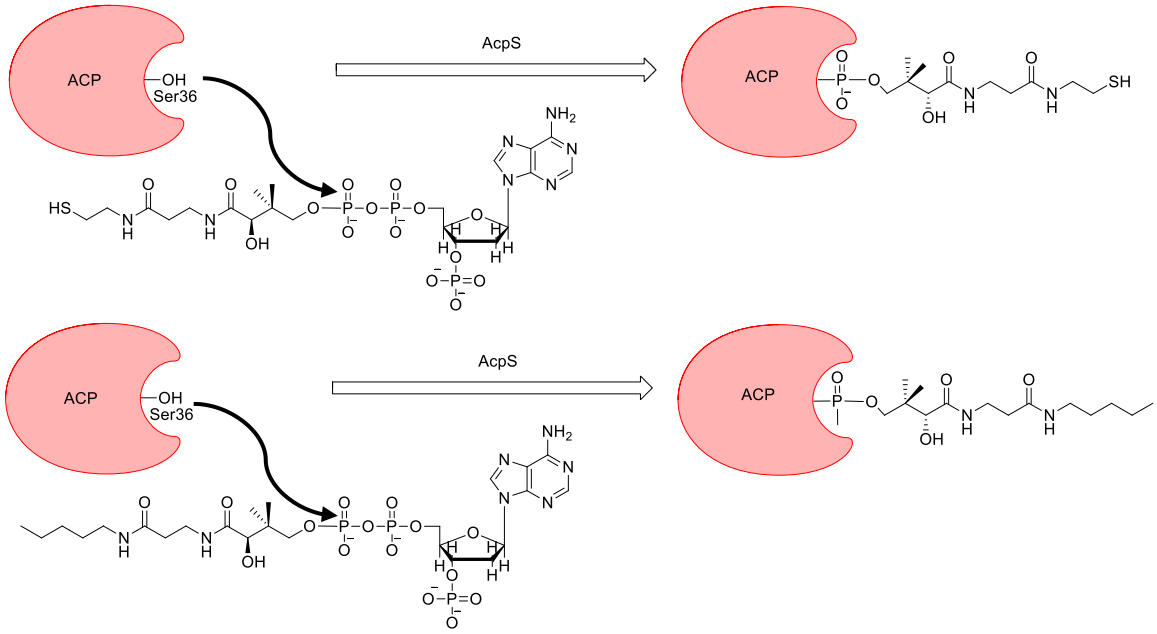


Figure 1-4: CoA and ethyldethia CoA coupling to ACP by Acp Synthase

The crystal structure of pantothenate bound to the *E. coli* PanK reveals multiple key interactions between the substrate and the enzyme. These interactions give useful information for developing new analogs. The C-1 (Figure 1-5) hydroxyl group interacts with the Asp-127 residue via a hydrogen bond; this is essential for the phosphorylation activity.[13] Hydrogen bonds are also formed between the C-3 hydroxyl and the imidazole ring of His177, the C-4 amide group and both Tyr175 and Asn282, and the C-8 acid moiety with Asn282 and Tyr240.[13] Ho-pantothenate (**1.2**) is an analog of pantothenate with an extra carbon in the C-6 and C-7 spacer region (Figure 1-5). In an *in vitro* assay, the inhibitory activity of both *R* and *S* enantiomers at C-3 of Ho-pantothenate were measured in different bacteria, and the results from this assay show the *R* isomer to be active while the *S* isomer to have lost most activity.[14]

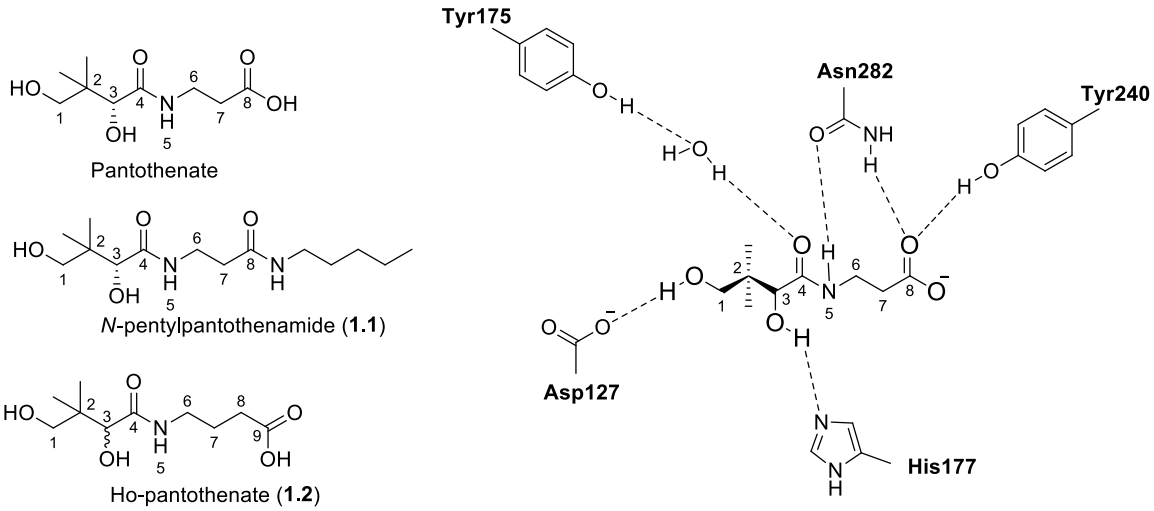


Figure 1-5: Binding interactions of pantothenate and PanK or CoaA, including structures of pantothenate, pantothenamide and Ho-pantothenate

SAR studies have shown that removal of the C-8 carbonyl is detrimental for binding, and the optimal spacer for C-6 and C-7 is two carbons in length.[14] In pantothenamide analogs the acid group is replaced with an amide bond at the C-8 position, which is tolerated. The pantothenamide amide extension projects into a hydrophobic pocket, containing several aromatic residues, such as Phe-244, Tyr-258, Phe-259 and Tyr-262.[14,15] With MIC of 78 nM against *S. aureus*, N7-pan was the most active derivatives among those with linear alkyl chain on the amide.[14]

The Auclair group has developed a methodology to synthesize pantothenamide derivatives with one or both of the methyl substituents at the C-2 position replaced with larger groups. There are two possible relative stereoconfigurations for the alcohol and C-2, specifically they can be *anti* and *syn*. The *anti* derivatives were synthesized by replacing the Pro*R* methyl

substituent with various alkyl groups starting from any given alcohol configuration.[16] Antibacterial activity studies on these variants (Table 1-1) revealed interesting SARs. Replacing the ProR methyl with an ethyl group (**1.3**) had no significant effect on the MIC.[16] Other derivatives with longer alkyl chains were synthesized by replacement of the same methyl with either a propyl (**1.4**) or hexyl (**1.5**). These modifications further decreased the activity, with MIC values 13 μM and 715 μM respectively (Table 1-1).[16] Bulkier alkyl chains like isobutyl (**1.6**) and cyclobutyl (**1.7**) were all detrimental to the activity of pantothenamides (Table 1-1).[16] In contrast, the allyl chain (**1.8**) had an antibacterial activity of 3.6 μM (Table 1-1).[16] A double substitution of the methyl groups with allyl groups (**1.9**) however reduced the activity, suggesting that large groups are not tolerated at the Pro*S* methyl pocket (Table 1-1).[16]

Based on these results, it was envisaged that accessibility to the other *anti* derivative (*S* alcohol) and to the *syn* derivatives might provide extra SARs. In order to achieve this goal, one synthetic strategy involves oxidation of the secondary alcohol at C-2, followed by stereoselective reduction of the carbonyl.

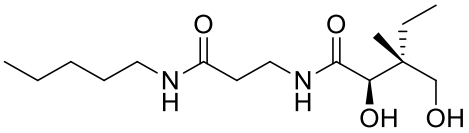
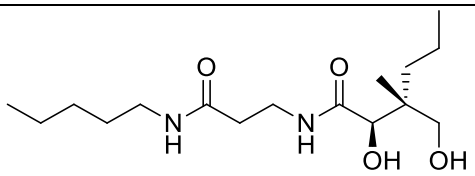
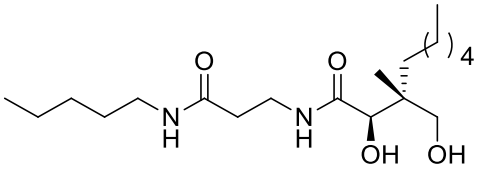
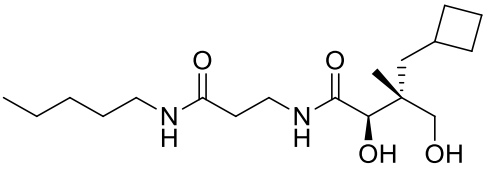
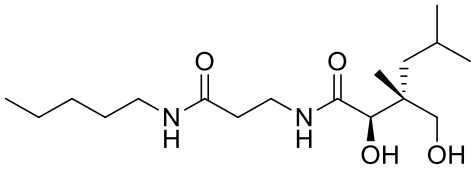
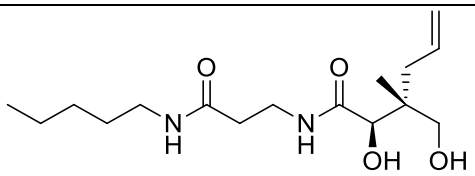
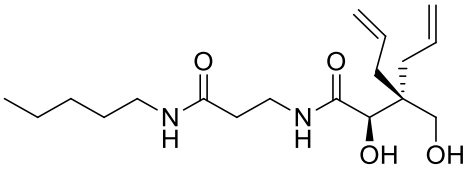
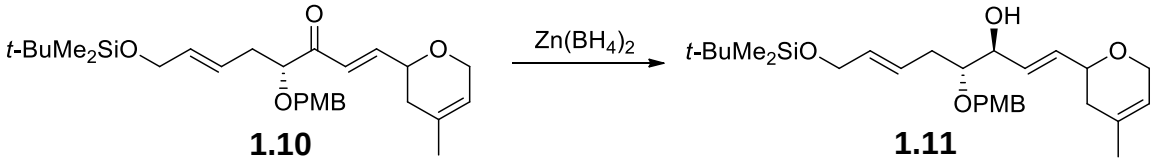
Compound	Structure	MIC (μM) in *S. aureus*	MIC (μM) in MRSA
1.3		7±6	7±3
1.4		13±7	13±2
1.5		>715	>715
1.6		374±187	374±153
1.7		101±45	51±15
1.8		3.2±0.8	3.2±0.9
1.9		326±217	376±109

Table 1-1: SAR of pantothenamide derivatives prepared in the Auclair group.[16]

1.2 Chemical Reductions

Lithium aluminum hydride is commonly used to reduce carbonyl compounds, specifically aldehydes, ketones, carboxylic acid, anhydrides, acid chlorides, esters, lactones and amides.[17] In general the hydride gets transferred to the less sterically hindered face of the prochiral ketone.[18] For a given subset of compounds it is possible to predict the outcome of a reaction through modeling based on Cram's model, Cram chelation model and Felkin-Anh model.[19-21] Borohydrides are also useful reagents for the reduction of carbonyls into alcohols. Sodium borohydride for example is selective for reducing aldehydes and ketones over esters, amides, and lactones.[22]

Zinc borohydride is another reducing agent used in the diastereoselective reduction of carbonyls that are adjacent to chiral alcohols. The zinc borohydride coordinates to the chiral alcohol, and selectively transfers the hydride from the same plane as the chiral alcohol.[23] In the total synthesis of (-)-laulimalide for example, zinc borohydride was used to reduce the allylic ketone **1.10** to the allylic alcohol **1.11**, yielding 77% of product with a dr ratio of >98:2 (Figure 1-6).[24]



1.10 **1.11**

Figure 1-6: Diastereoselective reduction with zinc borohydride

Bulkier borohydride reducing agents, such as L-selectride and K-selectride, are complimentary agents which proceed to reduce ketones from the less hindered face. For example K-selectride was used in the synthesis of (+)-

phorboxazole A, by reducing ketone **1.12** to alcohol **1.13** in 95% yield, and a dr ratio of 9:1 (Figure 1-7).[25]

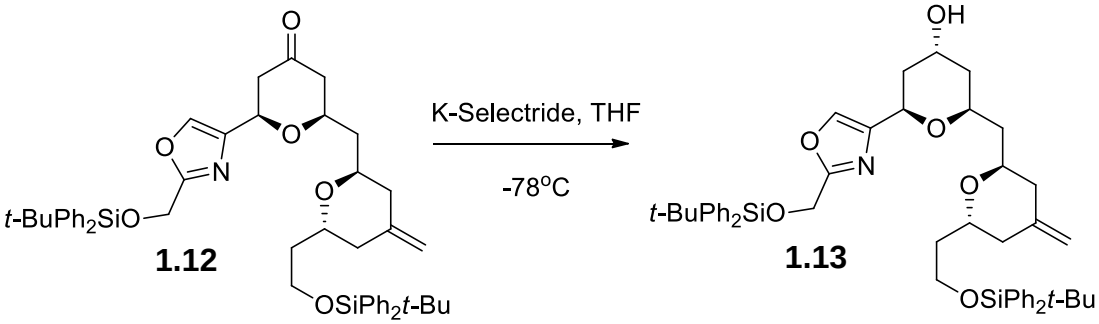


Figure 1-7: K-Selectride reduction used on an intermediate in the synthesis of (+)-phorboxazole A

An alternative reagent, DIBAL-H, reduces ketones to yield the alcohol anti to the large methoxy group. For example, reduction of chiral ketone **1.14** with DIBAL-H yields 99% of *anti*-alcohol **1.15** (Figure 1-8).[26]

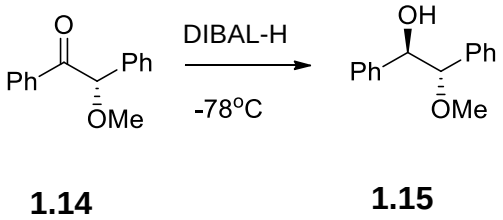
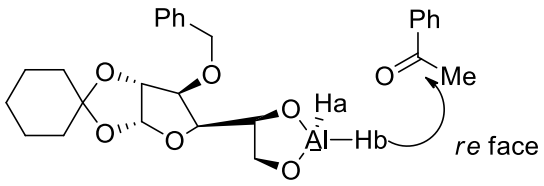


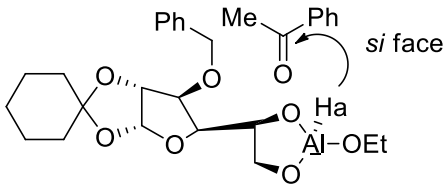
Figure 1-8: Diastereoselective reduction with DIBAL-H

Chiral reducing agents on the other hand, can enantioselectively reduce ketones to chiral alcohols. Chiral derivatives of lithium aluminum hydride are synthesized by substituting the hyrides with chiral alkyl chains.[23] The chiral groups favour one specific chiral complex, thus resulting in higher e.e. Landor compared the enantioselectivity of two chiral lithium aluminum hydride derivatives. The first reagent (**1.17**, Figure 1-9) had two hydride remaining while

the other reagent (**1.18**, Figure 1-9) only had a single hydride.[27] Reducing acetophenone with reagent **1.17** was found to yield a mixture of products, whereas reduction with **1.18** yielded only the *R* alcohol.[27] According to computer modelling the acetophenone can undergo attack by either hydride H_a or H_b hence substitution of H_b with an ethoxy group results in better selectivity.[27] The H_b hydride reacts at the *re* face to yield the (*S*)-alcohol, while the H_a hydride attacks the *si* face of the ketone yielding the (*R*)-alcohol.[27]



1.17



1.18

Figure 1-9: Enantioselectivity of chiral lithium aluminum hydride derivatives

Chiral borohydride derivatives have also been used in asymmetric reduction.[28] The reducing agent Alpine borane for example, contains α-pinene as shown on Figure 1-10. This reagent was reported to reduce butanal to butanol with high enantioselectivity, yielding 1-deuterobutanol with 83% e.e.[28] First the carbonyl is coordinated with borane, second the small group and large group of the ketone interact with the α-pinene. Both *re* and *si* faces are accessible, however the *re* face attack is favoured because steric interactions are minimized.[28] On the *re* face, the R group is away from the pinene ring and the tertiary β hydrogen is near the ketone.[28]

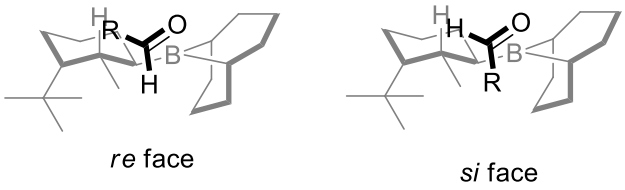


Figure 1-10: Comparing the configuration of (*S*)-Alpine Borane reduction

In the synthesis of (-)-archazolid, a propargylic ketone (**1.19**) was reduced with (*S*)-Alpine borane yielding 89% of alcohol **1.20** with a dr ratio of 20:1 (Figure 1-11).[29]

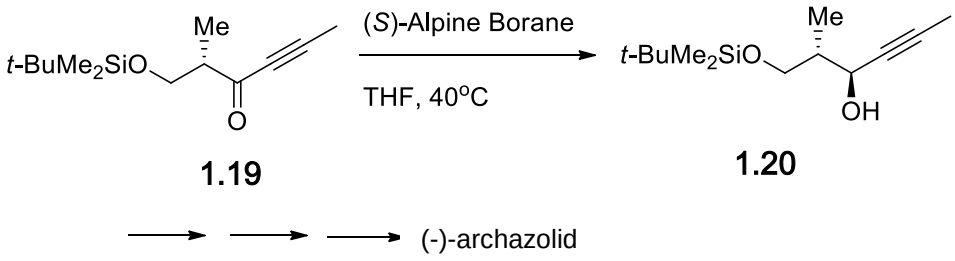


Figure 1-11: Alpine Borane reduction as used in the synthesis of (-)-archazolid

For the purpose of this thesis, a reducing agent is needed in the diastereoselective transformation of a β keto-ester. Oxazaborilidines are important chiral borohydrides used in the enantioselective reduction of keto esters into chiral alcohols.[30] This is exemplified with the Corey-Bakshi-Shibata reagent used in the synthesis of (+)-cryptophycin (Figure 1-12). It yielded 84% of the desired chiral alcohol in 97% optical purity.[31]

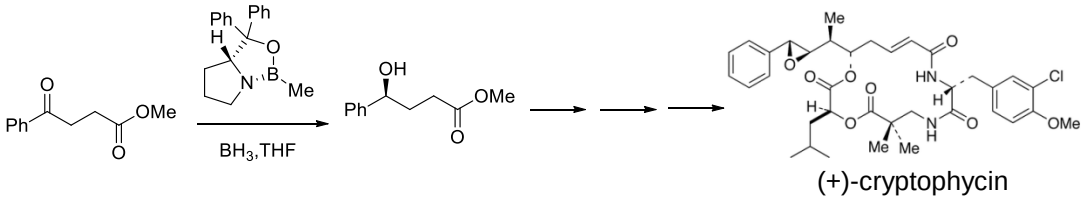


Figure 1-12: Enantioselective reduction with an oxazaborolidine chiral catalyst

1.3 Baker's Yeast Reduction

Biocatalysts are widely used in research labs and the industry to complement chemical reagents. The pros cover chemoselectivity, safety, environmental impact, and scaling up.[32] A main advantage is that reactions catalyzed by enzymes tend to be stereoselective and regioselective.[32] Biocatalytic reactions are also often safer as they are generally performed in aqueous environments and under ambient temperatures.[32] Moreover, biocatalysts are typically non-toxic.[32] Biocatalytic reactions can be scaled up but the recovery of product from the mixture is challenging.[32] Regardless, they remain a useful alternative to chemical catalysts for transforming a wide range of substrates.

The selectivity of biocatalytic reductions typically lies in control of the geometry in the complex during hydride transfer. Nicotinamide adenine dinucleotide phosphate NAD(P)H is the typical hydride donor used by alcohol dehydrogenases and reductases. Depending on the enzyme, the NAD(P)H is bound differently with respect to the prochiral ketone double bond to be reduced. Its hydride group can either sit on the *re* face or the *si* face of the carbonyl.[33] In addition some enzymes transfer the pro*S* or pro*R* hydride of the NAD(P)H cofactor, leading to four possible known mechanisms of hydride transfer, labelled as E1 to E4 in Figure 1-13.[33] For example one alcohol dehydrogenase from *Pseudomnas sp.* transfers the pro*R* hydride of NADH to the *si* face of the carbonyl, resulting in the (*S*)-alcohol.[33] On the other hand, at least one of the alcohol dehydrogenases from yeast transfers the pro*R* hydride of NADPH on the *re* face to yield the (*R*)-alcohol.[33]

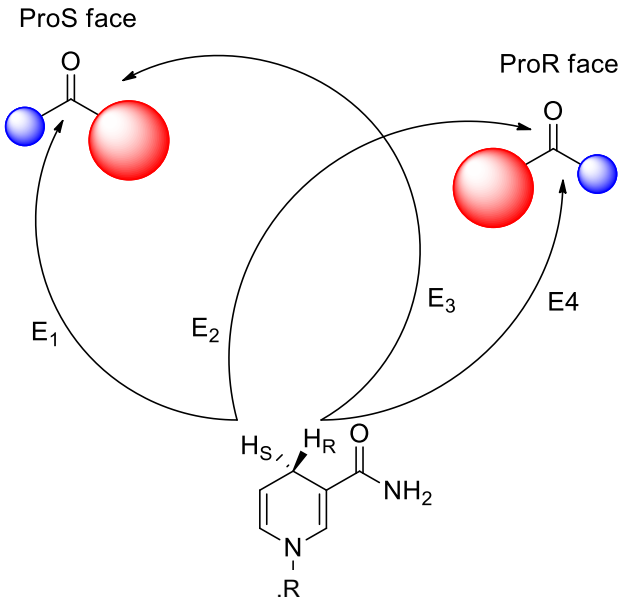


Figure 1-13: Hydride transfer mechanism in dehydrogenases and reductases

Biocatalytic reductions are typically highly enantioselective. According to Brook *et al.* biocatalysts often even distinguish between enantiotopic homomorphic substrates.[34] Enantiotopic homomorphic molecules, such as **1.25**, are *meso* compounds with a prochiral center imbedded between two prochiral ketones. Some enzymes can differentiate the chirality of the center, and selectively reduce one prochiral ketone over the other.[35] For example the reduction of **1.25** by Baker's yeast whole cells yields 9:1 (syn/anti) of the (2*S*,3*S*)-2-allyl-2-methyl-3-hydroxycyclopentanone (**1.26**) and (2*R*,3*S*)-2-allyl-2-methyl-3-hydroxycyclopentanone (**1.27**) (Figure 1-14).[34] In comparison, the chemical reducing agent $NaBH_4$ only yields 3:1 syn:anti for the same reaction.[34]

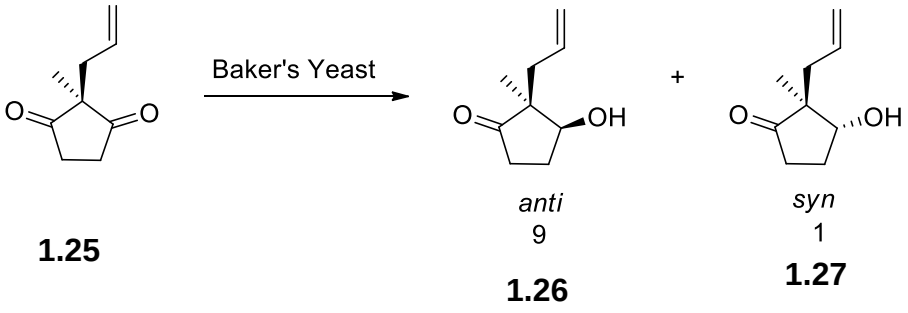


Figure 1-14: Baker's yeast reduction of an enantiotopic homomorphic molecule

The efficiency and selectivity of a given biocatalyst can be enhanced or modified by varying the reaction conditions. In microorganisms for example, there are several dehydrogenases and reductases with different chemoselectivity. It is possible to inactivate some of the undesired enzymes by changing the reaction conditions, such as the temperature and solvent.[33] The reduction of ketone **1.28** by *Debaryomyces hansenjii* gave 95% ee when the cells were lyophilized and rehydrated before reaction, and 80% ee when cells were untreated (Figure 1-15).[36]

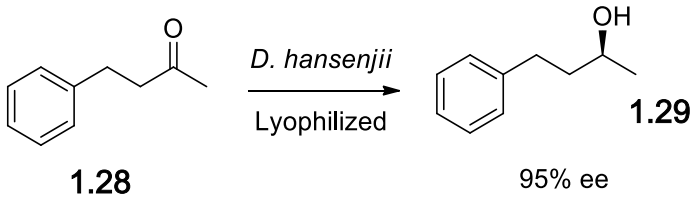
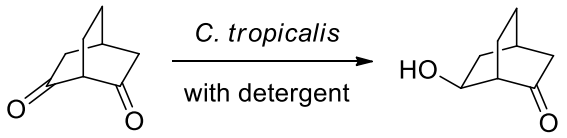


Figure 1-15: A lyophilized yeast strain to improve enantioselectivity

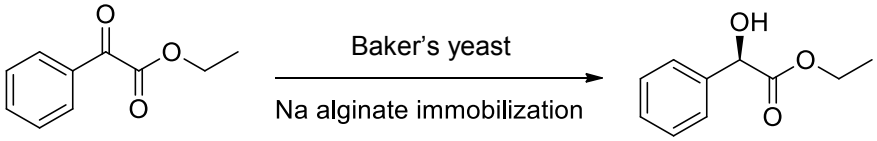
As another example, *Candida tropicalis* was treated with detergent to separate the *endo* alcohol reductase in the cytosol from the *exo*-alcohol reductase bound to membranes, and yielded the *exo* bicyclic product with high enantioselectivity when the detergent fraction was used (Figure 1-16).[37]



1.30 1.31

Figure 1-16: Yeast reduction with detergent favours *exo* product in the asymmetrization of the diketone bicylic

Immobilizing enzymes or Baker's yeast onto a solid matrix has in some cases improved the efficiency of biotransformation. Immobilization is believed to affect the effective concentration of the biocatalysts, and facilitate recycling of the biocatalyst.[33] For example Baker's yeast in alginate particles catalyzed the transformation of ethyl benzoylformate (**1.32**) to (*R*)-ethylmandelate (**1.33**) in a yield of 82%, with 92% ee (Figure 1-17).[38] The use of organic solvents for biocatalytic reduction has also in some cases proven beneficial to improve the effective concentration of substrate and enhancing the biotransformation.[33]



ethyl benzoylformate (*R*)-ethylmandelate

1.32 1.33

Figure 1-17: Immobilized Baker's yeast for the reduction of ethyl benzoylformate

Baker's yeast can reduce chiral compounds to yield diastereomers with high enantiospecificity. The reduction of racemic α-alkyl-β-ketoesters can produce as many as four different diastereoisomers (Figure 1-18).[33]

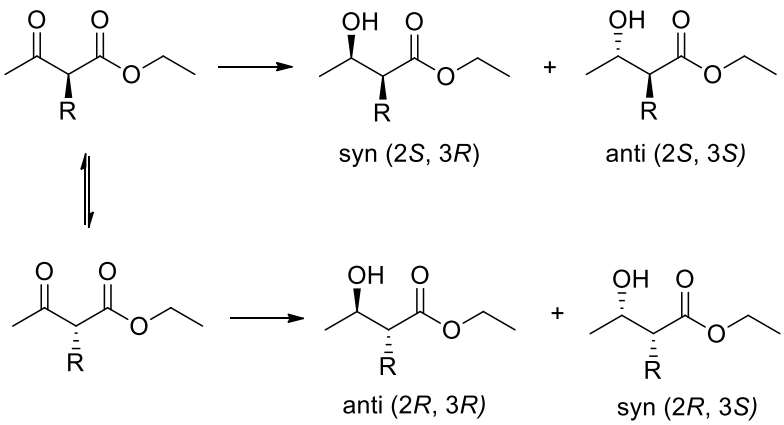
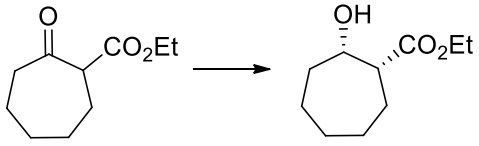


Figure 1-18: Reduction of α-alkyl-β-ketoesters yields four possible diastereoisomers

This is of interest here because one of the goals of this thesis is to identify a reducing agent for synthesizing the *syn* diastereomer of such a system. Baker's yeast can differentiate the enantiomers and reduce only one of them enantioselectively while leaving the other unreacted. The ability to react with a single enantiomer out of a mixture of equilibrating racemates to yield a single diastereomer is known as dynamic kinetic resolution. For example, the reduction of racemic ethyl-2-oxo-cycloheptanecarboxylate (**1.34**) by Baker's yeast yields only the *cis*-(1*R*, 2*S*) product **1.35** (Figure 1-19).[33]



cis-(1*R*, 2*S*)

1.34 **1.35**

Figure 1-19: Reduction of ethyl-2-oxo-cycloheptanecarboxylate with Baker's yeast

The reaction of racemic 2-allyl-2-methyl-3-oxo-ethylbutanoate (**1.36**) with Baker's yeast is another example of dynamic kinetic resolution (Figure 1-20).[39] Frater synthesized the racemic mixture by α-alkylation, and then reduced it with Baker's yeast to yield a single diastereomer, the anti-(2*S*,3*S*) of **1.37**, in 20% yield.[39] The reaction conditions weren't optimized since the yeast wasn't preincubated with any crosslinking agent at high temperatures, as will be discussed below.

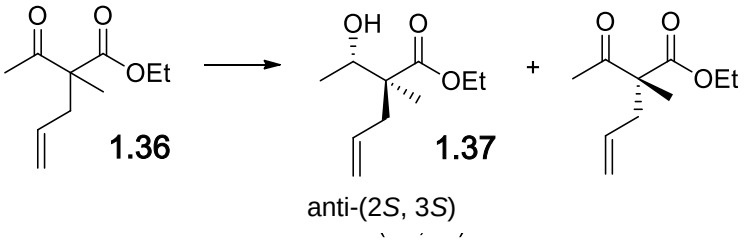


Figure 1-20: Reduction of substituted β-keto esters with Baker's yeast

Baker's yeast finds widespread use in the reduction of ketones to chiral alcohols. Seebach and Frater have reduced several β-keto esters and α-keto esters with Baker's yeast to yield a variety of chiral building blocks for the synthesis of natural products.[39,40] From these extensive studies, they propose rules, to predict stereoselectivity of the Baker's yeast reduction known as the Prelog rules (Figure 1-21).[40] According to the Prelog rules the prochiral ketone having a large and small substituent is oriented by the enzyme, and undergoes hydride transfer through the pro*S* face to yield the *S*-alcohol.

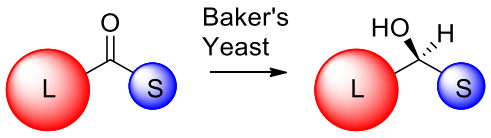


Figure 1-21: Baker's yeast reduction follows the Prelog rule

In the case of α and β keto esters, the main product is the (*S*)-alcohol, with enantiomeric excesses ranging from 40 to 97%.[40] A number of examples are reproduced in Table 1-2. The standard protocol involves incubating at 30°C for 24 hours, and dissolving 250 mg of yeast in 60 ml water, yet the enantiomeric excesses of the product when using this protocol is often poor.

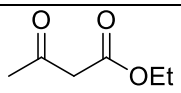
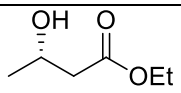
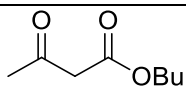
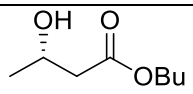
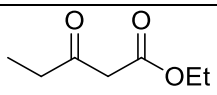
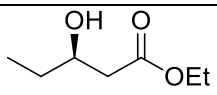
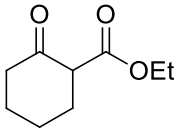
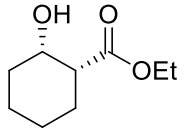
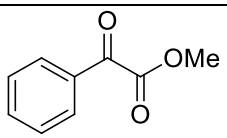
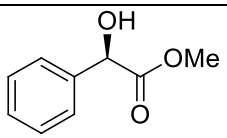
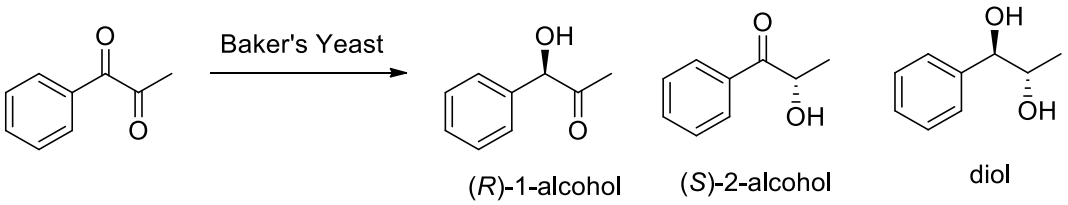
Substrate	Product	% yield	% ee
		57-67	84-87
		58	90
		67	40
		65	86
		59	>97

Table 1-2: Reduction of β-keto esters and α-keto esters with Baker's yeast

The reduction of α-diketones with Baker's yeast typically yields a mixture of α-hydroxy ketone and diol. Specifically the Baker's yeast reduction of 1-phenyl-1,2-propanedione (**1.39**) yields the alcohol products (*R*)-1-phenyl-1-hydroxy-2-propanone (**1.40**) and (*S*)-1-phenyl-2-hydroxy-1-propanone (**1.41**), and the diol product (1*R*,2*S*)-1-phenyl-1,2-propanediol **1.42** (Figure 1-22).[41] To block formation of the diol, the yeast is treated with a crosslinking agent (methyl vinyl ketone or MVK). Other common crosslinking agents include 2-cyclopenten-1-one

and ethyl chloroacetate.[41] Besides slowing down diol formation, MVK crosslinking also improves the regioselectivity of the α-hydroxy product from 32:68 to 22:78.[41] The regioselectivity was further increased to 4:96 when the yeast was preincubated at 50^{o}C to inactivate some selected dehydrogenases.[41]

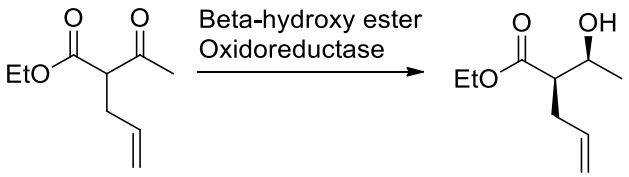


1.39 1.40 1.41 1.42

Figure 1-22: Baker's yeast reduction of diketone

The yield of enantiopure product varies with the substrate being reduced. Baker's yeast produces several dehydrogenases, each with a different stereoselectivity. They are classified as the L-(-) or D-(+) enzymes.[42] The enantioselectivity of the Baker's yeast depends on the number of reducing enzymes which can transform a given substrate. The product's enantiomeric excess can be enhanced by selectively blocking the activity of the undersired dehydrogenases.

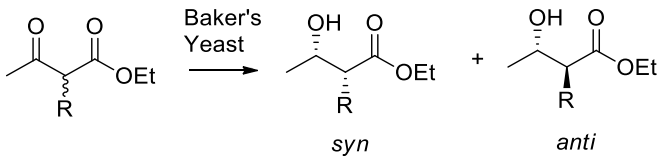
One of the yeast enzymes is the β-hydroxy ester oxidoreductase. When isolated, this enzyme can reduce ethyl 2-allyl-3-oxobutanoate **1.43** to yield 70% ethyl (2*R*,3*S*)-2-allyl-3-hydroxybutanoate **1.44** with greater than 97% ee (Figure 1-23).[43]



1.43 **1.44**

Figure 1-23: Reduction of β-keto ester with isolated enzyme

As mentioned above cross linking agents have been used to improve the enantioselectivity of Baker's yeast reductions. MVK is an α,β-unsaturated ketone that can react with various dehydrogenases and form intra-enzyme covalent bridges, mainly at the lysine residues, this results in enzyme inactivation. By treating the yeast with MVK, ethyl (2*R*,3*S*)-syn-2-allyl-3-hydroxybutanoate **1.44** was obtained with 84% ee in comparison to untreated yeast that only yields mostly the ethyl (2R,3S)-anti-2-allyl-3-hydroxybutanoate.[44] The authors concluded that MVK inhibited the dehydrogenases that favour the anti-products. In addition, preincubation of yeast at 50^{o}C has also been shown to increase enantioselectivity for some substrates. The increase in temperature inactivates the dehydrogenases favouring the anti-product.[45] Furthermore combining the crosslinking and temperature effect synergistically improved the enantioselectivity of the reaction. Various substrates with different alkyl groups were reduced with Baker's yeast following this protocol (Table 1-3).[45]



R group	Syn/Anti ratio (% conversion rate)		
	No heat treatment	Heat (50^{o}C for 30 min)	Heat and MVK
Methyl	87/13 (99)	93/7 (92)	97/3 (94)
Ethyl	66/34 (99)	80/20 (91)	92/8 (79)
Propyl	74/26 (99)	92/8 (71)	93/7 (70)
Allyl	30/70 (99)	65/35 (92)	96/4 (94)
Propargyl	66/34 (97)	86/14 (99)	94/6 (99)

Table 1-3: Baker's yeast reduction with or without MVK and/or temperature treatment[45]

An important goal of this thesis is to establish a route to synthesize the allyl derivative of *N*-pentylpantothenamide with *syn* and *anti* stereoconfiguration about the C-3 alcohol and C-2 carbon. Since Frater's alkylation leads to the *anti* configuration, it was thus envisaged to set the absolute stereochemistry at the C-2 using this alkylation methodology, followed by oxidation of the C-2 alcohol and stereoselective reduction. Hereby we wish to access the three diastereomers not reported in the literature (Figure 1-24).

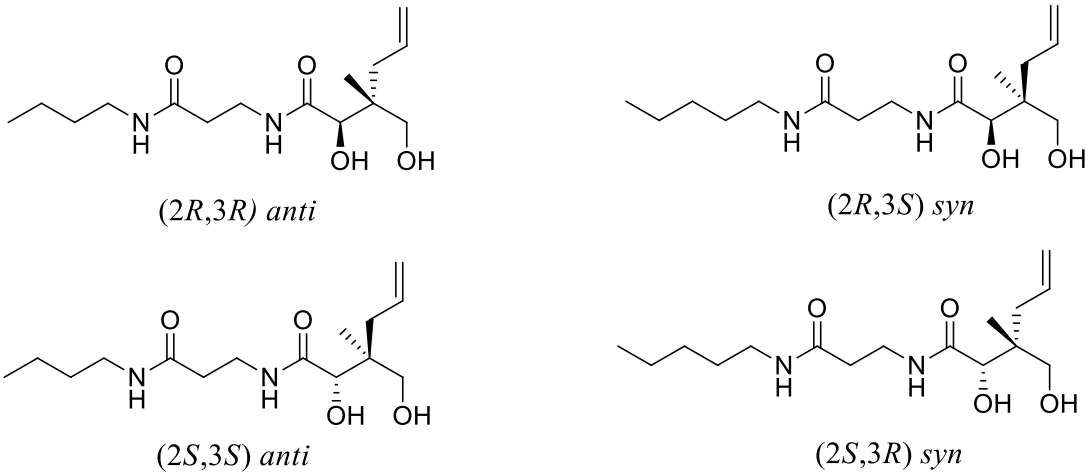


Figure 1-24: Four possible diastereomers of allyl derivative of *N*-pentylpantothenamide

2 RESULTS AND DISCUSSION

2.1 Preface

Previously in the Auclair group a library of pantothenamide derivatives with substitutions at the geminal dimethyl group were reported.[16] This methodology was instrumental for the synthesis of the stereoisomers discussed in this chapter. Indeed, one of the goals of this thesis work was to vary the stereochemistry at the secondary alcohol and at the modified quaternary carbon to yield other diastereomers. A library of dialkylated malic acid esters with the secondary alcohol in the syn stereoconfiguration was synthesized. One of these, the methyl allyl derivative, was extended into the full pantothenamide for biological studies. All synthetic work reported in this thesis was carried out by the author, while the antibacterial studies were performed by Mr. Eric Habib in the Auclair group, and the antimalarial data were from the group of Prof. Kevin Saliba at Australian National University.

2.2 Introduction

In bacteria, pantothenamide derivatives are extended by the coenzyme A (CoA) biosynthetic pathway, and the resulting modified CoA derivatives proceed to affect CoA utilizing enzymes. Structure-activity relationships reported for antibacterial activity of pantothenamide derivatives suggest that substitution of one methyl at the geminal dimethyl group can improve antibacterial activity and stability in blood (unpublished data from a collaborator). Interestingly the allyl methyl derivative **1.8** showed improved antibacterial activity against *S. aureus*

compared to the standard geminal dimethyl compound **1.1**.[16] CoA naturally has the alcohol in the *R* configuration, and the pantothenamides previously reported by the group[16] have a new substituent at the quaternary carbon that is *anti* to the alcohol, as in the *anti* (2*R*, 3*R*)-diastereoisomer **1.8** shown (Figure 2-1). The goal of this thesis is to vary the stereoconfiguration to yield the other diastereomers (boxed in Figure 2-1). However, the methodology developed here did not give access to the *syn*-(2*S*, 2*R*)-product.

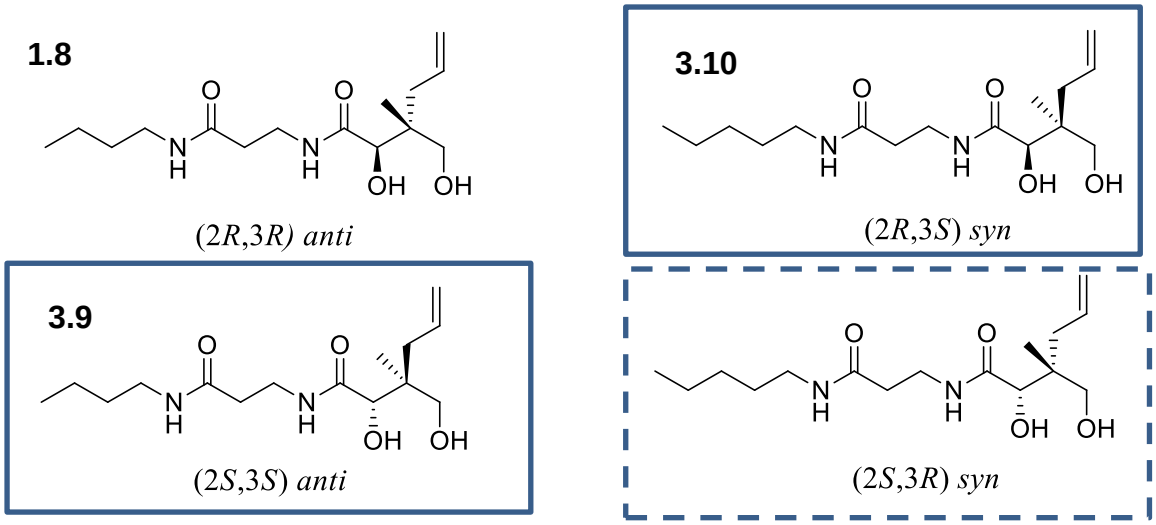


Figure 2-1: Four possible pantothenamide diastereoisomers with *syn* and *anti* configurations. The boxed molecules were the target for this thesis.

From a retrosynthetic perspective, two amide bonds link three fragments. These fragments are pentyl amine, β-alanine and a pantoyl group. The pantoyl fragment contains the two stereogenic centers, one at the secondary alcohol and one at the quaternary carbon. For the reported synthesis of the *anti*-(2*R*, 3*R*)-isomer[16], D-malic acid was esterified, followed by two Frater alkylations hereby setting the stereochemistry at the quaternary carbon (Figure 2-2). The di-substituted malic ester was next reduced and selectively protected with an acetal,

before oxidation of the free alcohol to the acid needed in the pantoyl fragment. Similarly, for the synthesis of the *syn*-(2*R*, 3*S*)-isomer it was envisaged that after setting the stereochemistry at the quaternary carbon with the Frater alkylations as for the *anti* compound, the alcohol would be oxidized before stereoselective reduction to yield the compound with reverse configuration at the quaternary carbon (Figure 2-2).

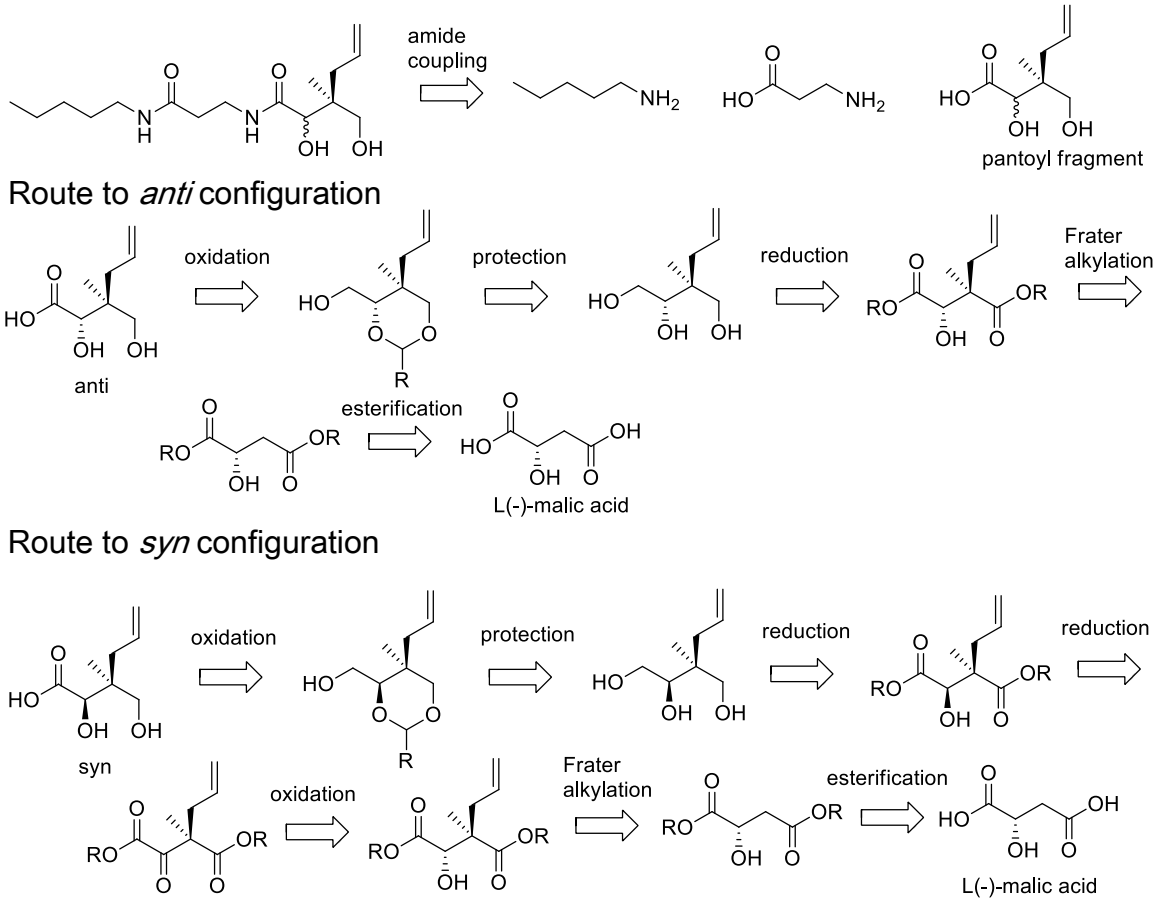


Figure 2-2: Retrosynthesis of dialkyl substituted pantothenamides

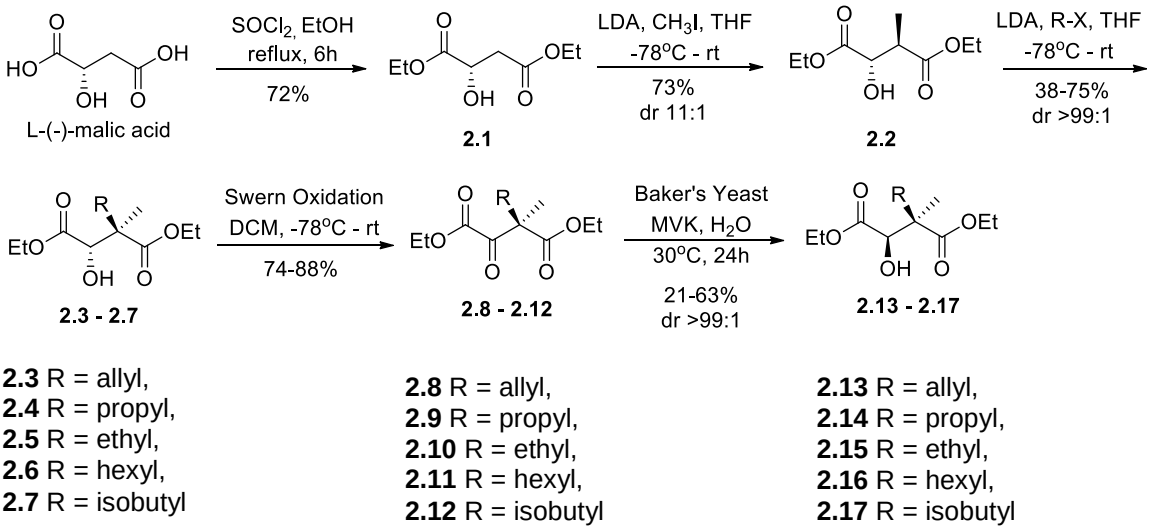
The oxidation reaction did not pose a special challenge, but the following reduction was expected to require optimization. Several reducing agents exist for

reducing prochiral carbonyls into secondary alcohols. These include for example: $LiAlH_4$, DIBAL-H, $NaBH_4$, $Zn_2(BH_4)_2$. Most carbonyls undergo nucleophilic attack by the hydride on the sterically less hindered face, and products can be predicted using the Felkin Ahn's model.[18] There are exceptions; for example $Zn_2(BH_4)_2$ forms a chelate and the product can be predicted via Cram's chelate model.[19]

Chiral reducing agents can reduce one face of the prochiral ketone by orienting the compound into a thermodynamically stable conformation. These include (*R*)-CBS, (*S*)-CBS, and ruthenium BINAP complexes. In particular the Corey-Bakshi-Shibata (CBS) reagents are based on chiral oxazaborolidine rings.[28] Biocatalysts such as Baker's yeast have also been reported to reduce β-ketoesters with high enantioselectivity as was detailed in the introduction.[44]

2.3 Results and Discussion

Stereoselective synthesis of the di-alkyl substituted malic acid ester was the first milestone. In order to synthesize the *syn*-(2*R*, 3*S*)-isomer **3.10**, one must start from L-(-)-malic acid (2*S*-alcohol), whereas D-(+)- malic acid (2*R*-alcohol) is used to access the anti-(2*R*, 3*S*)-isomer **3.9**. Thus enriched L-(-)-malic acid was esterified by reaction with thionyl chloride and absolute ethanol (Scheme 2-1). For this reaction to proceed to completion freshly added reagents must be added twice, with product concentration in between. This reaction yields malic acid ester **2.1** in very good yield.



2.3 R = allyl,
2.4 R = propyl,
2.5 R = ethyl,
2.6 R = hexyl,
2.7 R = isobutyl

2.8 R = allyl,
2.9 R = propyl,
2.10 R = ethyl,
2.11 R = hexyl,
2.12 R = isobutyl

2.13 R = allyl,
2.14 R = propyl,
2.15 R = ethyl,
2.16 R = hexyl,
2.17 R = isobutyl

Scheme 2-1: Synthesis of the *anti* and *syn* di-substituted malic acid ester

Compound **2.1** is next treated with two successive alkylations using the method developed by Seebach and Frater.[39] First lithium diisopropylamine is generated *in situ* from butyllithium and diisopropylamine at 0°C, before reaction with **2.1** in the presence of methyl iodide to yield **2.2** in 73% yield. According to Seebach and Frater[39], the enolate forms a six-membered ring chelate with the secondary alcohol and the alkoxide of the enolate (Figure 2-3).[39] Moreover the stereoconfiguration of the secondary alcohol determines the face of the ring that attacks the electrophile. This yields the alkylated product with the *anti* stereoconfiguration. Here the diastereomeric ratio (dr) of **2.2** was 11:1 anti:syn (as measured by NMR from the crude sample). Similar conditions were used for the second alkylation with various alkyl halides. The yield of this step was highly dependent on the alkyl group, and ranged from 38-75% for **2.3**-**2.7**. The dr ratio was consistently excellent (>99:1), as predicted by the results of Seebach and Frater's. Again the *anti* stereoconfiguration was favoured.

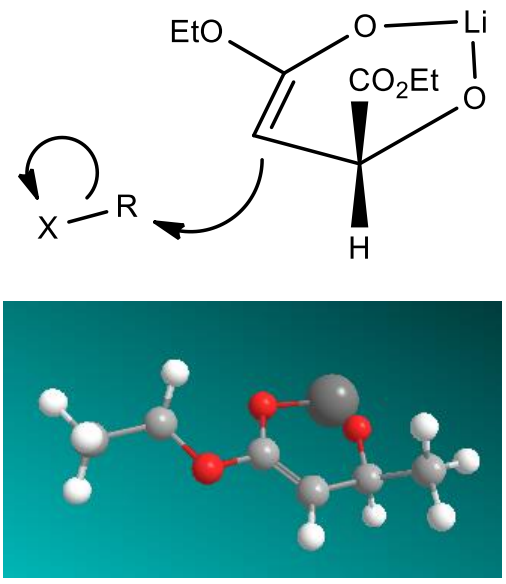
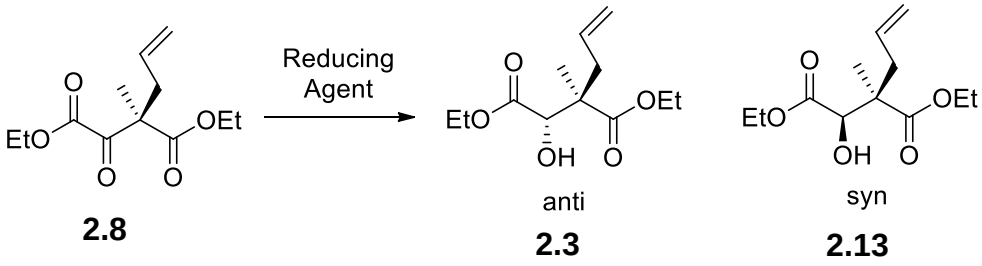


Figure 2-3: Model for Frater's alkylation

The initial configuration of the alcohol carbon is determined by the malic acid isomer used, both of which are commercially available. This initial configuration determines the outcome of the alkylation product (*anti*). In order to access the *syn* isomers, it was envisaged to reverse the configuration at the alcohol using Swern oxidation followed by enantioselective reduction. Hereby Swern oxidation of **2.3**-**2.7** proceeded with yields of 74-88% for **2.8**-**2.12**, depending on the substrate, with larger R groups giving lower yields.

A library of commercial reducing agents was next tested to evaluate their ability to yield the *syn* alcohol product from **2.8**-**2.12**. As shown in Table 2-1, DIBAL-H at -78^{o}C yielded predominantly the *anti*-product **2.3** with a dr ratio of 1.5:1. The dr ratio was determined by integrating the characteristic NMR peaks for the methyl group at the quaternary carbon, specifically the signal at 1.16 ppm from the *anti* product[16], and the signal at 1.05 ppm from the *syn* product.

Table 2-1: Reduction of ketones adjacent to di-alkyl germinal centers



2.8 **2.3** **2.13**

Reducing Agent	Diastereomeric Ratio
DIBAL-H	1.5:1
$NaBH_4$	2.9:1
$ZnBH_4$	2.3:1
(*R*)-CBS	3.6:1
(*S*)-CBS	1.9:1
Baker's yeast	>99:1 (syn)

$NaBH_4$ is a milder reducing agent, which was also tested, yet the major product of this reaction was again the *anti*-isomer of **2.3** with a dr ratio of 2.9:1. $Zn_2(BH_4)_2$ was explored because it is expected to lock the β-ketoester into a 6-member ring-like chelate by coordinating to the ketone and the ester. This positions the borohydride on the less bulky face of the ring, which is the face opposing the large allyl group, thus the hydride is expected to be transfered on the less hindered face, affording the *syn*-isomer. Nonetheless the reaction didn't react according to predictions, and yielded predominantly the *anti*-isomer of **2.3** with a dr ratio of 2.3:1.

Since none of the typical achiral reducing agents favoured the *syn*-stereoconfiguration, efforts were next turned towards achiral reducing agents.

The Corey-Bakshi-Shibata reagent is an oxazaborolidine catalyst that is used to reduce prochiral ketones to chiral alcohols. This chiral catalyst is a boron based catalyst that can coordinate to the prochiral ketone. Its substituents orient the ketone's large and small group to minimize steric clashes, before hydride transfer from the boron species occurs. Here the (*R*)-CBS yielded the *anti* product **2.3** from the ketone **2.8** with a dr ratio of 3.6:1. Similarly, the (*S*)-CBS yielded the *anti* product **2.3** with a dr ratio of 1.9:1. Neither enantiomer of the CBS reagent afforded the desired *syn* product.

Alternatively ketones can be reduced by reductases from the Baker's yeast.[45] Whole cell Baker's yeast has been reported to enantioselectively reduce β-ketoesters to enantiopure alcohols.[44] To our pleasure, reduction of **2.8** with Baker's yeast yielded the desired *syn* product, (2*R*,3*R*)-diethyl 2-allyl-3-hydroxy-2-methylsuccinate **2.13** as the major product (dr ratio of >99:1), and in 63% yield after purification. The yeast was pre-incubated with MVK at 50^{o}C for 30 min before addition of the ketone, and the reaction was allowed to proceed for 24 hours at 30^{o}C.

The absolute stereochemistry of the product was confirmed by coupling the alcohol with both the (*R*) and (*S*) Mosher's acids (MPA) to form the corresponding esters. Notably the model implies that the signal corresponding to the substituents labeled as a blue circle (Figure 2-4) shift to higher field.[46] On the other hand, the group labeled with the red circle (Figure 2-4) is expected to shift towards the lower field (larger ppm). Measuring the Δppm^{RS} between the NMR spectra of the (*R*)-MPA derivative **2.18** and (*S*)-MPA derivative **2.19** confirmed

the *R* configuration about the secondary alcohol. In fact the methyl substituent was found to shift by 0.1 ppm and the ethyl ester was found to shift by -0.15 ppm. Overall, compound **2.13** was prepared from L-malic acid (*S* configuration at the alcohol carbon) via oxidation of the alcohol followed by Baker's yeast reduction to give the *R*-alcohol.

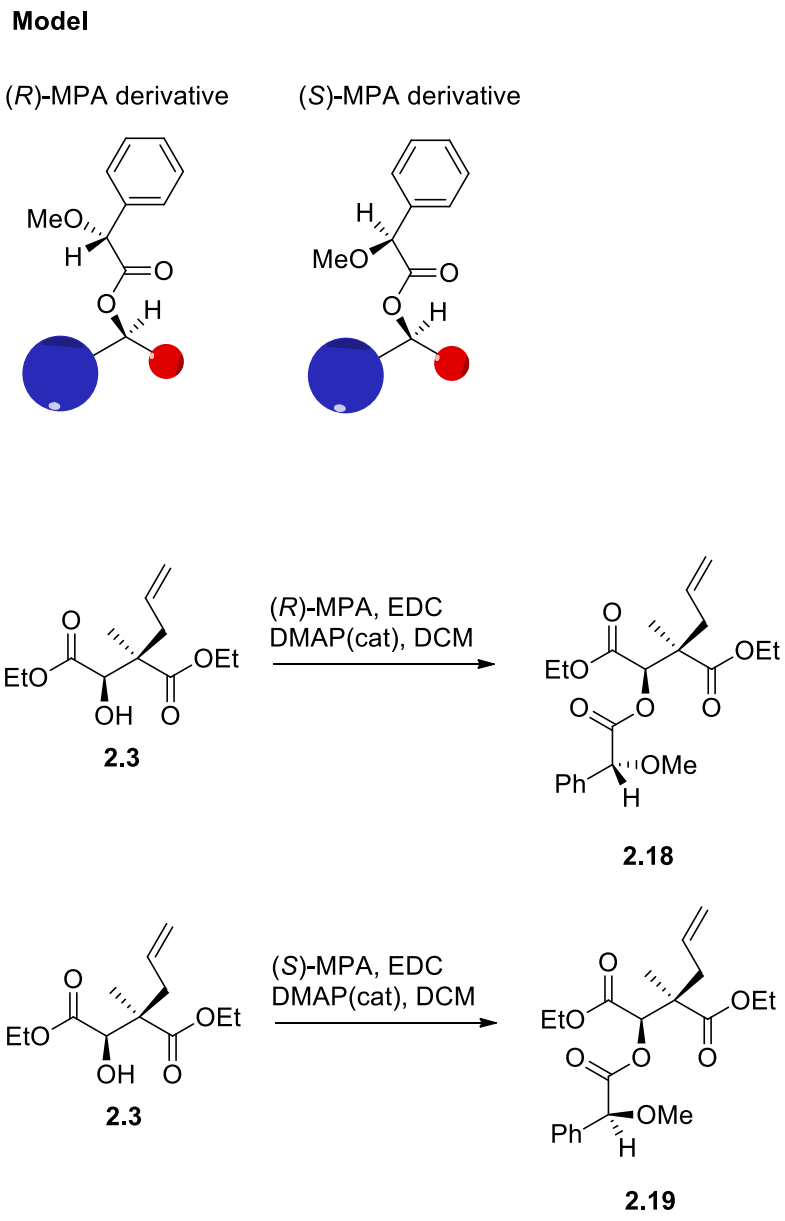
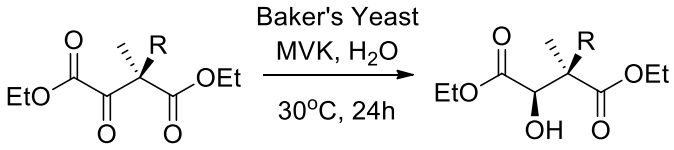


Figure 2-4: Determination of absolute stereochemistry via MPA derivatization

The scope of the Baker's yeast reduction was evaluated with other β-ketoesters (**2.8**-**2.12**), the diastereomeric ratio (dr) was measured by NMR on the crude product and the yield was calculated after purification (Table 2-2). As mentioned before reduction of allyl derivative **2.8** afforded the syn-product **2.13** in 63% yield and a dr ratio of >99:1 (Table 2-2). The propyl derivative **2.9**, when reduced by Baker's yeast, yielded 31% of syn-product **2.14** with a high dr ratio (Table 2-2). When reduced in the same way, the ethyl derivative **2.10** afforded 37% of syn-product **2.15** with a high dr ratio. The ethyl substituent is smaller than the propyl or allyl subtituents hence might fit better in the reductase, resulting in a higher yield. A bulkier substituent, specifically the hexyl found in derivative **2.11** affected the Baker's yeast reduction; the *syn*-product **2.16** was generated in only 21% yet with a high dr ratio (Table 2-2). Finally the isobutyl derivative **2.12** was reduced with Baker's yeast in 26% yield, again favouring the *syn*-product **2.17** with a high dr ratio. There too, the low yield is attributed to the large size of the alkyl group, likely it well not fit in the reductase substrate binding pocket. Overall, Baker's yeast is an excellent biocatalyst for chiral reductions of the systems.

Table 2-2: Baker's yeast reduction di-alkyl substituted malic acid esters



Compound	R group	Diastereomeric Ratio	% yield
2.13	allyl	>99:1	63%
2.14	ethyl	98:1	37%
2.15	propyl	>99:1	31%
2.16	hexyl	>99:1	21%
2.17	isobutyl	98:1	26%

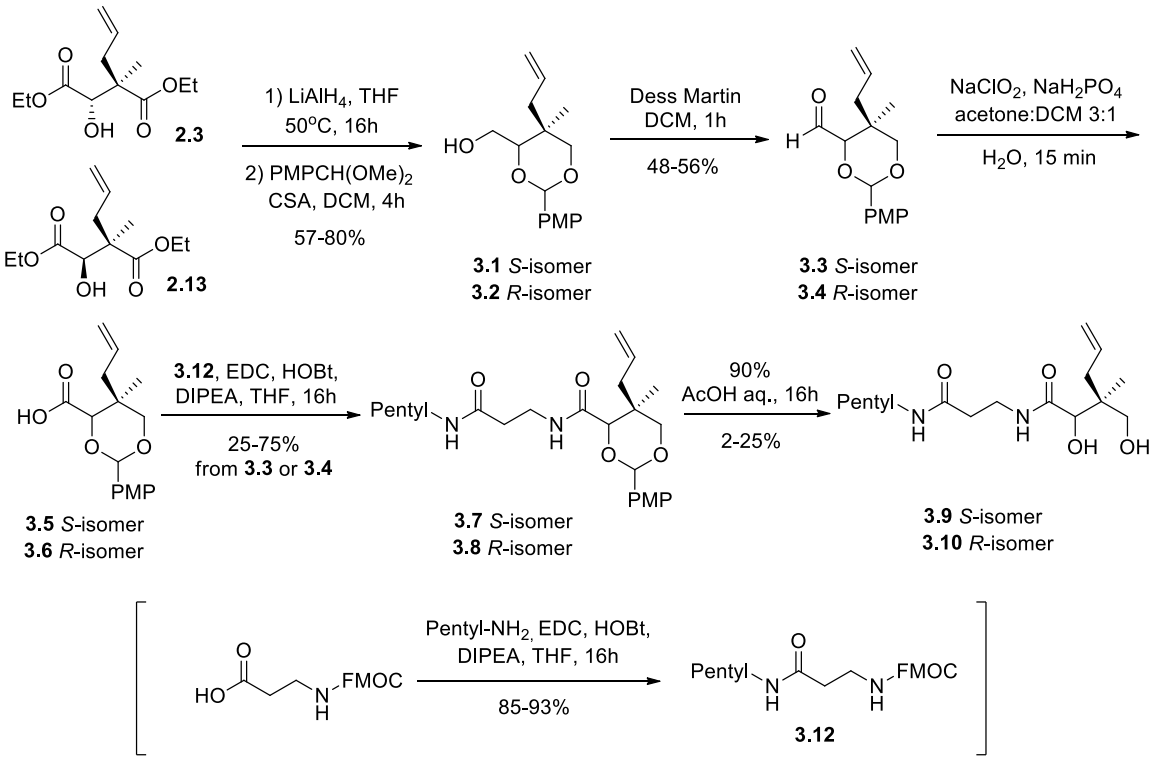
Synthesis of pantothenamide derivatives with *syn* and *anti* configuration:

With the stereochemistry established at these 2 centers, it was possible to extend the methyl allyl derivatives **2.3** and **2.13** to the corresponding pantothenamides using the established route (Scheme 2-2). Derivatives **2.3** and **2.13** were selected because the corresponding (2R, 3R) diastereoisomer was the most active amongst those tested by the group. Thus 2.3 and 2.13 were separately reduced with LiAlH4 at reflux temperature for 16 hours to generate the corresponding triols. Next the crude triols were separately protected yielding the 1,3-protected triols **3.1** and **3.2**. Anisaldehyde acetal favours the six membered ring.

The terminal alcohol of the 1,3-protected triols were next oxidized to the aldehydes **3.3** and **3.4** separately with the oxidant Dess Martin Periodinane, in

yields ranging from 57-80%. Unlike the reported protocol[16], here the aldehyde is purified to minimize impurities. The purified aldehydes were further oxidized separately to generate acids **3.5** and **3.6** via the Pinnick Oxidation. Here the crude acid was used directly in the amide coupling because of its low stability.

The amine needed for the coupling, 3.12, was synthesized from Fmoc protected β–alanine and pentylamine (Scheme 2-2). With the coupling agent EDC the reaction proceeded in excellent yield (85-93%). Acids **3.5** and **3.6** were also separately coupled to **3.12** using EDC. Prior to the amide coupling however the amine of **3.12** is deprotected. Thus the **3.12** was reacted with piperidine to form the free amine, which was reacted with crude acids **3.5** and **3.6** to yield amides **3.7** and **3.8**. Finally, the acetal protecting group was removed using 90% acetic acid, to produce pantothenamides **3.9** and **3.10** in 2-25% yield.



Scheme 2-2: Synthesis of pantothenamide with *syn* and *anti* stereoconfiguration

With the compounds in hand they were tested for antibacterial activity. They were also sent to the Saliba group in Australia for antimalarial tests. The antimalarial test have shown some promising results (Figure 2-5), as can be seen both *anti* and *syn* derivatives showed MICs below 10 µM. Therefore these compounds will be further studied by the group.

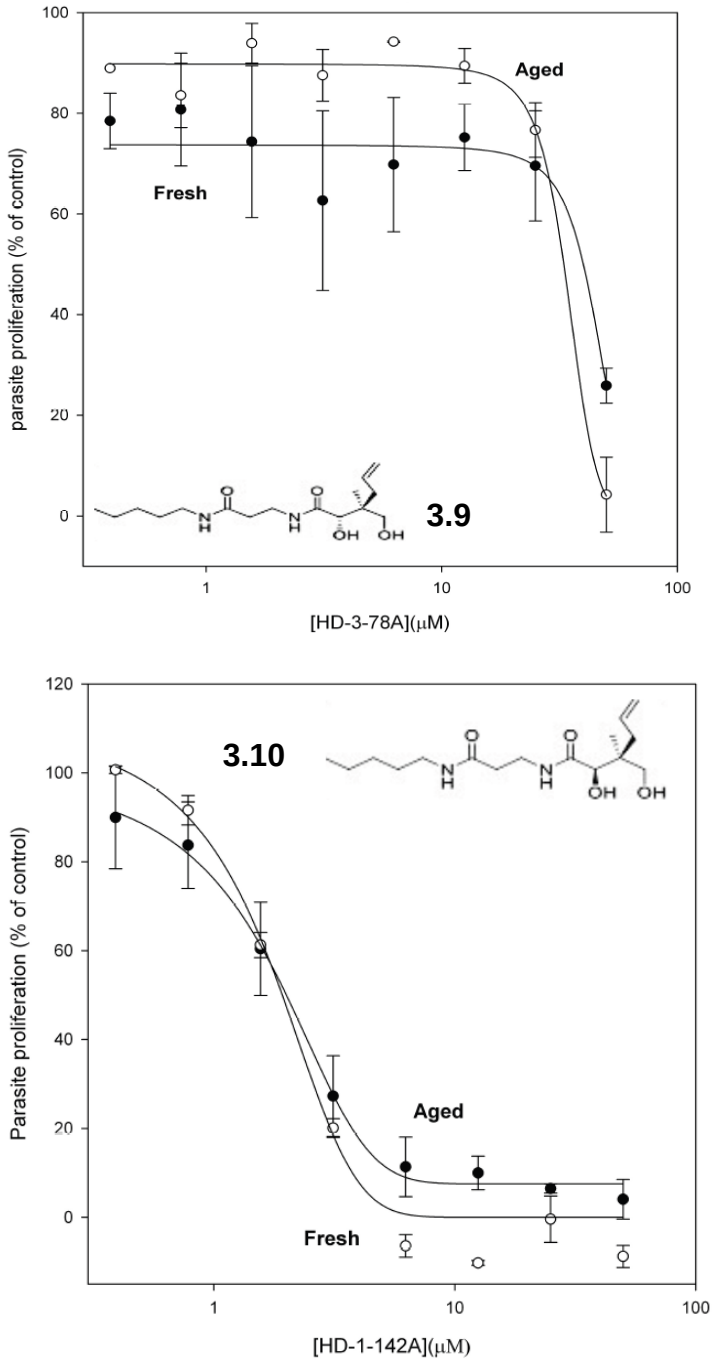
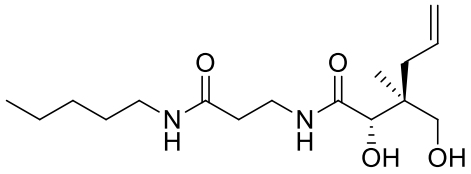


Figure 2-5: MIC results for pantothenamide (**3.9** and **3.10**) activity against malaria. “Fresh” media was incubated for 48 hrs at 4^{o}C, while “aged” media was incubated for 1 week at 4^{o}C, before use. These results are from the Saliba research group at Australia National University.

Here a successful methodology has been established for the synthesis of both *syn* and *anti* diastereomers of pantothenamides. A key step in this process involved the use of Baker's yeast for the *syn* selective reduction of α-ketomalonates. Overall the Baker's yeast is a wide scope reaction. Finally, although the target compounds did not show significant antibacterial activity, they have demonstrated potential as antimalarial agents.

3 CONTRIBUTIONS AND FUTURE DIRECTIONS

While efforts to discover new antibiotics have slowed dramatically in recent decades, bacteria are becoming resistant to the current antibiotics. Resistance mechanisms are diverse and span target modification, drug modification, impermeability, and efflux pumps. There are several ways to tackle the problem of antibiotic resistance, including the development of new drugs, diagnostic, awareness and prevention. In this thesis the approach was to develop new antibacterial agents in the class of pantothenamides. In the Auclair group various pantothenamide derivatives have shown activity against MRSA, with the most active compound being the *anti N*-pentyl allyl-derivative (**3.9**).



3.9

Figure 3-1: Structure of *anti N*-pentyl allyl-derivative

Based on the positive results for compound **3.9** we embarked on discovering a synthetic route to access the three other possible diastereomers of **3.9**. Using the previously reported synthetic route, two additional steps were incorporated to reverse the stereochemistry. Specifically the secondary alcohol was oxidized and enantioselectively reduced to yield the *syn* configuration. Various chemical reducing agents were tried but they all failed. Baker's yeast however gave the *syn* product with high diastereoselectivity. This emphasizes how different the reactivity of chemical and biological catalysts can be. By synthesizing the *syn* pantothenamide we have validated the use of Baker's yeast

methodology for modifying the stereochemistry of α-hydroxy ester functionalities. Chiral alcohols are common in biologically active molecules, and this methodology may find use in the preparation of other such molecules.

The products of the Baker's yeast reaction were extended to full length pantothenamides. These compounds are currently being tested for antibacterial activity. In collaboration with the Saliba group one of the compounds was found to show antimalarial activity. If the pantothenamide derivatives were to show promising antibacterial activity, this result could potentially lead to the development of a new antibiotic against resistant bacteria. Furthermore the pantothenamides have proven useful molecular probes for studies of the CoA biosynthetic pathway, and even the biosynthesis of fatty acids. The methodology reported here can be applied to prepare other derivatives for further SAR studies of these systems.

Another project in the group focuses on inhibiting a resistance-causing enzyme, AAC(6′)-Ii. The methodology reported here will be used by my colleagues to generate the *syn* stereoconfiguration of previous inhibitors. According to computer modeling the *syn* isomer of some of the groups' best inhibitors look promising. By modifying the methyl into an allyl derivative, cross metathesis will also be used to access a larger variety of compounds. These are some of the potential directions for this project in the future.

4 EXPERIMENTAL

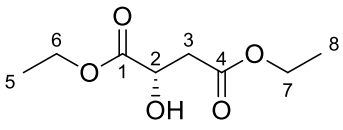
4.1 General Method

All reagents were purchased from Sigma-Aldrich Canada, Ltd. (Oakville, Ontario, Canada) unless otherwise noted. They were used from the bottle without any titration or purification. Dry solvents however were obtained from the solvent distillation system. All anhydrous chemical reactions were run under an atmosphere of nitrogen and the dry techniques were applied throughout the experimental setup. Compounds were purified using the CombiFlash Rf system from Teledyne Isco, depending on the scale of the reaction the compounds were purified using Gold Silica columns ranging from 12 g to 80 g in size. Some compounds were also purified using the Biotage Isolera Spektra One using Biozip silica columns ranging from 5 g to 45 g in size. The preparative TLC and TLC analysis were performed on 60 F_{254} silica gel plates obtained from EMD (Gibbstown, NJ).

The ^{1}H spectra were determined using one of the Varian Mercury 300, 400 or 500 MHz NMR spectrometers. The chemical shifts are reported in parts per million (ppm) relative to residual proton in chloroform-d (7.26 ppm). The different splitting patterns are reported as follows: s, singlet; d, doublet; dd, doublet of doublet; ddd, doublet of doublet of doublet; t, triplet; q, quartet; m, multiplet; br s, broad singlet. The coupling constants (*J*) are reported in Hertz (Hz). Similarly the ^{13}C NMR spectra were acquired using the Varian Mercury 300 MHz NMR spectrometer. The chemical shift are reported in parts per million, relative to the position of the deuteriochloroform triplet peak at 77.0 ppm. The exact mass of

compounds were measured using a Thermo Fischer Scientific Inc. Exactive Orbitrap system.

4.2 Baker's Yeast Reduction



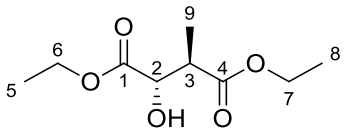
(*S*)-Diethyl-2-hydroxysuccinate (2.1):

Compound **2.1** was synthesized from starting material (-)-L-malic acid by esterification in the presence of thionyl chloride and ethanol.[16] Thus, (-)-L-malic acid (10.1 g, 75.3 mmol) was dissolved in ethanol (50 mL), and a solution of thionyl chloride (2.5 mL, 2.0 M in DCM, 34.5 mmol, 0.5 eq) was added to the mixture. The reaction was refluxed for 3 hours at 100°C, before evaporation of the solvent under reduced pressure to yield a clear liquid. To fully bring the reaction to completion, the esterification reaction was repeated once more with freshly added ethanol (50 mL) and thionyl chloride (2.5 mL, 2.0 M in DCM, 34.5 mmol, 0.5 eq). The final residue was diluted in diethyl ether (200 mL) then washed with a saturated solution of sodium bicarbonate (2 × 80 mL), followed by a brine wash (1 × 120 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated to yield the crude product. Finally the crude compound **2.1** was purified via silica gel chromatography. Recovered yield: 10.3 g of oily residue, 72%. R_f 0.58 (ethyl acetate/hexanes, 4:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.47 (dd, *J* = 4.7, 5.7, 1H, H-2), 4.27 (q, *J* = 7.2, 2H, H-6 or H-7), 4.17 (q, *J* = 7.2, 2H, H-6 or H-7), 3.21 (s, 1H, OH), 2.82 (dd, *J* = 4.7, 16.3, 1H, H-3a), 2.80 (dd, *J* = 5.7, 16.3, 1H, H-3b), 1.30 (t, *J* = 7.2, 3H, H-5 or H-8), 1.26 (t, *J* = 7.2, 3H, H-5 or H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4 (C-1 or C-4), 172.8 (C-1

or C-4), 77.0 (C-2), 62.0 (C-6 or C-7), 61.0 (C-6 or C-7), 38.7 (C-3), 14.1 (C-5, C-8).

General protocol for alkylation (used to generate compounds 2.2-2.7)[16]:

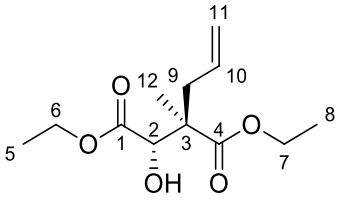
Before setting up the reaction, all flasks, syringes and needles were oven-dried and purged with nitrogen. Dry THF was first added (40 mL per 5 mmol of sample) and cooled to 0°C. To make the lithium diisopropylamide (LDA) reagent, diisopropylamine (DIPA, 2.4 eq) and butyllithium (BuLi, 2.2 eq) were added to the reaction flask. The mixture was allowed to react for 15 min in an ice bath. After having formed the LDA species, the reaction mixture was cooled to -78°C, before addition of compound **2.1** (1 eq), or its monoalkylated derivative compound **2.2** (1 eq). The mixture was allowed to warm up to -40^{o}C over 1 hour. Next, the alkyl halide (2.7 eq) of interest was added and the reaction was allowed to react for 16 hours. Finally, the reaction was cooled to -45°C before quenching with saturated ammonium chloride (30 mL). The product was extracted in ethyl acetate (3 × 50 mL). Finally the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Purification was achieved via silica gel chromatography.



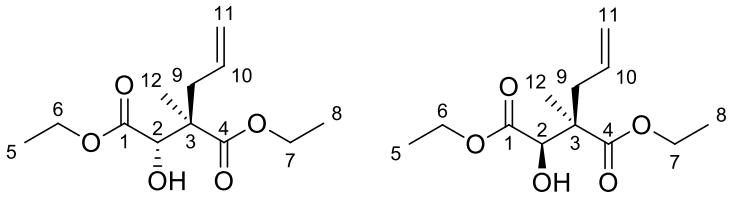
(2*S*,3*R*)-Diethyl 3-methyl-2-hydroxysuccinate (2.2):

The known general alkylation protocol was followed for the synthesis of the new diastereomer **2.2**.[16] From compound **2.1** (3.06 g, 16 mmol), the compound **2.2** was obtained as a yellow oil in 73% yield (2.4 g). R_f 0.37 (diethyl ether/hexanes, 1:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.59 (d, J = 3.6, 1H, H-2), 4.26 (m, 2H, H-6

or H-7), 4.14 (q, *J* = 7.1, 2H, H-6 or H-7), 3.01 (dq, *J* = 3.6, 7.4, 1H, H-3), 1.29 (t, *J* = 7.1, 3H, H-5 or H-8), 1.29 (d, *J* = 7.4, 3H, H-9), 1.24 (t, *J* = 7.1, 3H, H-5 or H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3 (C-1 or C-4), 172.8 (C-1 or C-4), 77.0 (C-2), 62.0 (C-6 or C-7), 60.9 (C-6 or C-7), 43.1 (C-3), 14.1 (C-5, C-8 or C-9), 14.1 (C-5, C-8 or C-9), 13.0 (C-5, C-8 or C-9).



(2*R*,3*S*)-Diethyl 2-allyl-3-hydroxy-2-methylsuccinate (2.3): The known general alkylation protocol was used for the synthesis of the new diastereoisomer **2.3**.[16] Compound **2.3** was synthesized by reacting compound **2.2** (1.11 g, 5.4 mmol) with allyl bromide following the general alkylation procedure described above. Compound **2.2** was isolated as an orange oily liquid with a final yield of 0.77 g, 58%. R_f 0.37 (ethyl acetate/hexanes, 1:4, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.75 (m, 1H, H-10), 5.1 (m, 2H, H-11), 4.25 (m, 2H, H-6 or H-7), 4.17 (q, *J* = 7.2, 2H, H-6 or H-7), 3.37 (s, 1H, OH), 2.53 (dd, *J* = 7.0, 13.7, 1H, H-9a), 2.33 (dd, *J* = 7.8, 13.7, 1H, H-9b), 1.30 (t, *J* = 7.2, 3H, H-5 or H-8), 1.27 (t, *J* = 7.2, 3H, H-5 or H-8), 1.18 (s, 3H, H-12). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3 (C-1 or C-4), 172.8 (C-1 or C-4), 133.1 (C-10), 118.9 (C-11), 77.5 (C-2), 61.8 (C-6 or C-7), 60.9 (C-6 or C-7), 49.8 (C-3), 39.5 (C-9), 17.9 (C-5 or C-8), 14.1 (C-5 or C-8), 14.1 (C-12).



(2*R*,3*S*)-Diethyl 2-allyl-3-hydroxy-2-methylsuccinate (2.3) and (2*S*,3*S*)-Diethyl 2-allyl-3-hydroxy-2-methylsuccinate (2.13):

Compound 2.3 from reduction of compound 2.8 with DIBAL-H:

Compound **2.3** was synthesized from compound **2.8** using DIBAL-H as the reducing agent. Dry THF (4.1 mL) was cooled to -78^{o}C and stirred. The DIBAL-H solution (0.28 mL, 0.73 M in THF, 1 eq) was added followed by compound **2.8** (50 mg, 0.21 mmol). The mixture was allowed to react overnight while warming up to room temperature. Then the reaction was quenched with a solution of saturated ammonium chloride (4 mL), and the product was extracted in ethyl acetate (3 × 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The diastereomeric ratio was determined to be 1.5:1 of *anti*/*syn* products, by measuring the integration of the H-12 peak of the *anti* and *syn* at 1.16 ppm and 1.05 ppm respectively. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.80-5.62 (m, 1H, H-10), 5.13-5.03 (m, 2H, H-11), 4.27-4.09 (m, 5H, H-6, H-7, H-2), 2.56-2.26 (m, 2H, H-9), 1.29-1.22 (m, 6H, H-5, H-8), 1.16 (s, 3H, H-12), 1.05 (s, 3H, H-12).

Compound 2.3 from reduction of compound 2.8 with $NaBH_4$:

Compound **2.3** was also synthesized from compound **2.8** by $NaBH_4$ reduction. $NaBH_4$ (15 mg, 0.08 mmol, 0.4 eq) was dissolved in dry methanol (20 mL) before

cooling to 0^{o}C. Compound **2.8** (50 mg, 0.21 mmol) was added and the reaction was left to stir overnight, while warming up to room temperature. Then the reaction was quenched with aqueous HCl (5 mL, 2 N). The product was extracted in ethyl acetate (3 × 15 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The diastereomeric ratio was determined to be 2.9:1 of *anti*/*syn* products based on the integration of the H-12 peak for the *anti* and *syn* products at 1.16 ppm and 1.05 ppm respectively. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.80-5.55 (m, 1H, H-10), 5.14-5.03 (m, 2H, H-11), 4.34-4.06 (m, 5H, H-6, H-7, H-2), 2.57-2.27 (m, 2H, H-9), 1.32-1.22 (m, 6H, H-5, H-8), 1.16 (s, 3H, H-12), 1.05 (s, 3H, H-12).

Compound 2.3 from reduction of compound 2.8 with $ZnBH_4$:

Compound **2.3** was also synthesized from compound **2.8** by reduction with $ZnBH_4$. Dry THF (4.1 mL) was cooled to -78^{o}C before the addition of $ZnBH_4$ solution (1.7 mL, 0.21 mmol, 0.12 M in THF, 1 eq). Compound **2.8** (50 mg, 0.21 mmol, 1 eq) was added and the mixture was left to stir overnight while warming up to room temperature. To quench the reaction, saturated ammonium chloride (4 mL) was added and the mixture was allowed to stir for 10 min. The product was extracted in ethyl acetate (3 × 10 mL), and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The diastereomeric ratio for the mixture obtained was 2.3:1 of *anti*/*syn* products, as determined by measuring the integration of the H-12 peak for the *anti* and *syn* at 1.16 ppm and 1.05 ppm respectively. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.82-5.61 (m,

1H, H-10), 5.14-5.03 (m, 2H, H-11), 4.29-4.08 (m, 5H, H-6, H-7, H-2), 2.57-2.26 (m, 2H, H-9), 1.32-1.21 (m, 6H, H-5, H-8), 1.16 (s, 3H, H-12), 1.05 (s, 3H, H-12).

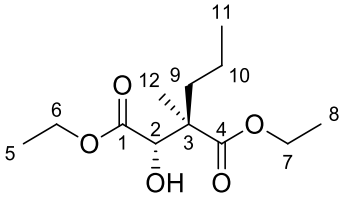
Compound 2.3 from borohydride reduction of compound 2.8 with the (*R*)-CBS catalyst:

Compound **2.8** was also reduced with the (*R*)-(+)-2-methyl-CBS-oxazaborolidine catalyst in combination with boron dimethyl sulfide to give **2.3**. A catalytic amount of (*R*)-CBS (25 mg, 0.08 mmol, 0.1 eq) was dissolved in THF (25 mL) and cooled to -20^{o}C. Next the boron dimethyl sulfide (170 µL, 1.7 mmol, 2.1 eq) was added, followed by dropwise addition of compound **2.8** dissolved in THF (200 mg, 0.83 mmol, in 5 mL). The reaction was left to stir overnight at -20^{o}C. Finally the reaction was quenched with aqueous HCl (5 mL, 2 N) and the product was extracted in diethyl ether (1 × 40 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduce pressure. The diastereomeric ratio was determined to be 3.6:1 of *anti*/*syn* product based on the integration of the H-12 peak for the *anti* and *syn* at 1.18 ppm and 1.05 ppm respectively. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.90-5.63 (m, 1H, H-10), 5.15-5.04 (m, 2H, H-11), 4.34-4.09 (m, 5H, H-6, H-7, H-2), 2.59-2.28 (m, 2H, H-9), 1.34-1.23 (m, 6H, H-5, H-8), 1.18 (s, 3H, H-12), 1.05 (s, 3H, H-12).

Compound 2.3 from borohydride reduction of compound 2.8 with the (*S*)-CBS catalyst:

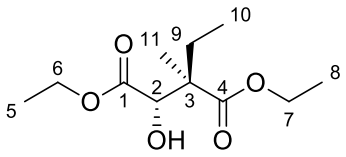
Compound **2.8** was also reduced with the boron dimethyl sulfide in the presence of (*S*)-(-)-2-methyl-CBS-oxazaborolidine catalyst to yield compound **2.3**. A catalytic amount of (*S*)-CBS (25 mg, 0.09 mmol, 0.1 eq) was dissolved in dry THF

(25 mL) and cooled to -20°C. The boron dimethyl sulfide (170 µL, 2.0 eq) was added, followed by dropwise addition of compound **2.8** (210 mg, 0.87 mmol, dissolved in 5 mL of THF). The reaction was allowed to stir overnight at -20°C. Then the reaction was quenched with aqueous HCl (10 mL, 2 N) and the product was extracted in diethyl ether (1 × 40 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The diastereomeric ratio was determined to be 1.9:1 *anti*/*syn* products based on the integration of the H-12 peak for the *anti* and *syn* products at 1.18 ppm and 1.05 ppm respectively. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.83-5.62 (m, 1H, H-10), 5.15-5.04 (m, 2H, H-11), 4.32-4.10 (m, 5H, H-6, H-7, H-2), 2.59-2.27 (m, 2H, H-9), 1.33-1.24 (m, 6H, H-5, H-8), 1.18 (s, 3H, H-12), 1.05 (s, 3H, H-12).

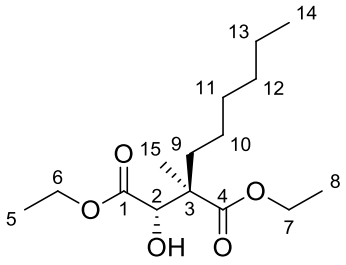


(2*R*,3*S*)-Diethyl 2-propyl-3-hydroxy-2-methyl succinate (2.4): The general alkylation protocol was used for the synthesis of compound **2.4**.[16] Compound **2.4** was synthesized by reacting compound **2.2** (1.42 g, 7.0 mmol) with propyl iodide following the general alkylation procedure described above. The final yield was 1.03 g of yellow oily liquid, 60%. R_f 0.30 (ethyl acetate/hexanes, 1:4, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.28-4.20 (m, 3H, H-6 or H-7, H-2), 4.17 (q, *J* = 7.2, 2H, H-6 or H-7), 3.38 (s, 1H, OH), 1.75 (dt, *J* = 4.4, 12.9, 1H, H-9a), 1.50 (t, *J* = 4.4, 12.9, 1H, H-9b), 1.29 (t, *J* = 7.2, 3H, H-5 or H-8), 1.27 (t, *J* = 7.2, 3H, H-5 or H-8), 1.24-1.17 (m, 2H, H-10), 1.14 (s, 3H, H-12), 0.90 (t, *J* = 7.1, 3H, H-11). ^{13}C NMR (75 MHz, $CDCl_3$) 175.0 (C-1 or C-4),

172.9 (C-1 or C-4), 75.8 (C-2), 61.7 (C-6 or C-7), 60.9 (C-6 or C-7), 50.1 (C-3), 37.7 (C-10), 17.7 (C-11, C-5 or C-8), 17.3 (C-11, C-5 or C-8), 14.5 (C-12, C-5 or C-8), 14.1 (C-12, C-5 or C-8).

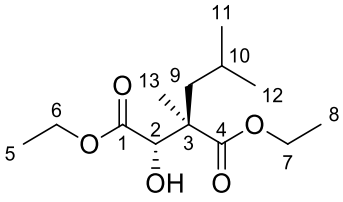


(2*R*,3*S*)-Diethyl 2-ethyl-3-hydroxy-2-methylsuccinate (2.5): The general alkylation protocol was used for the synthesis of compound **2.5**.[16] Compound **2.5** was synthesized by reacting compound **2.2** (1.04 g, 5.1 mmol) with ethyl iodide, using the general alkylation protocol described above. The final yield was 0.89 g of yellow oily liquid, 75%. R_f 0.28 (diethyl ether/hexanes, 1:1, v/v).^{1}H NMR (500 MHz, $CDCl_3$) δ 4.28-4.18 (m, 5H, H-2, H-6, H-7), 3.36 (s, 1H, OH), 1.88-1.81 (m, 1H, H-9a), 1.63-1.56 (m, 1H, H-9b), 1.30 (t, *J* = 7.2, 3H, H-5 or H-8), 1.28 (t, *J* = 7.1, 3H, H-5 or H-8), 1.15 (s, 3H, H-11), 0.88 (t, *J* = 7.5, 3H, H-10).



(2*R*,3*S*)-Diethyl 2-hexyl-3-hydroxy-2-methylsuccinate (2.6): The general alkylation protocol was used for the synthesis of compound **2.6**.[16] Compound **2.6** (2.05 g, 10 mmol), was synthesized by reacting compound **2.2** with iodohexane, following the general alkylation procedure described above. The overall yield was 1.10 g of yellow oil, 38%. R_f 0.40 (diethyl ether/hexanes, 1:1, v/v).^{1}H NMR (300 MHz, $CDCl_3$) δ 4.28-4.20 (m, 3H, H-6 or H-7, H-2), 4.18 (q, *J* = 7.1, 2H, H-6 or H-7), 3.30 (br s, 1H, OH), 1.82-1.46 (m, 2H, H-9), 1.32-1.23

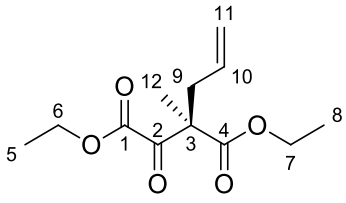
(m, 14H, H-5, H-8, H-10 to H-13), 1.15 (s, 3H, H-15), 0.87 (t, *J* = 6.7, 3H, H-14). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.0 (C-1 or C-4), 172.9 (C-1 or C-4), 75.8 (C-2), 61.7 (C-6 or C-7), 60.9 (C-6 or C-7), 50.1 (C-3), 35.4 (C-9), 31.6 (C-10), 29.7 (C-11), 24.2 (C-12), 22.6 (C-13), 17.3 (C-15), 14.1 (C-14, C-5 or C-8), 14.1 (C-14, C-5 or C-8), 14.0 (C-14, C-5 or C-8).



(2*R*,3*S*)-Diethyl 2-isobutyl-3-hydroxy-2-methylsuccinate (2.7): The general alkylation protocol was used for the synthesis of compound **2.7**.[16] Compound **2.7** (0.71 g, 2.8 mmol), was synthesized by reacting compound **2.2** with 2-methyl-1-iodopropane, following the general alkylation procedure described above. The overall yield was 0.73 g (81%) of yellow oil. R_f 0.34 (diethyl ether/hexanes, 1:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.28-4.19 (m, 3H, H-6 or H-7, H-2), 4.17 (q, *J* = 7.2, 2H, H-6 or H-7), 1.82 (dd, *J* = 7.0, 13.7, 1H, H-9a), 1.75-1.61 (m, 1H, H-10), 1.50 (dd, *J* = 5.2, 13.7, 1H, H-9b), 1.30 (t, *J* = 7.2, 3H, H-5 or H-8), 1.28 (t, *J* = 7.2, 3H, H-5 or H-8), 1.16 (s, 3H, H-13), 0.92 (d, *J* = 6.5, 3H, H-11 or H-12), 0.84 (d, *J* = 6.5, 3H, H-11 or H-12). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2 (C-1 or C-4), 172.7 (C-1 or C-4), 61.7 (C-6 or C-7), 60.9 (C-6 or C-7), 49.7 (C-3), 43.9 (C-9), 24.7 (C-10), 24.6 (C-11 or C-12), 23.2 (C-11 or C-12), 16.9 (C-5, C-8 or C-13), 14.2 (C-5, C-8 or C-13), 14.0 (C-5, C-8 or C-13).

General protocol for the Swern oxidation (used to generate compounds 2.8-2.12):

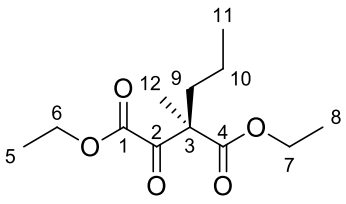
DCM (40 mL) was cooled to -78^{o}C under nitrogen. The oxalyl chloride solution (2.0 M in DCM, 2 eq) was added, followed by dimethylsulfoxide (DMSO, 4 eq). After addition, a gas was generated and it vented out through the small needle opening. The reaction mixture was stirred for 30 min, after which a DCM solution of the alcohol (1 eq in 5 mL) was added. The reaction was left to react for 1 hour at -78^{o}C. Triethylamine (6 eq) was added and the reaction was left to stir overnight while warming up to room temperature. Finally the organic layer was washed with hydrochloric acid (20 mL, 10% aqueous solution) followed by a saturated solution of sodium bicarbonate (30 mL). The organic layer was dried over Na_2SO_4 and concentrated to yield the crude ketone. Finally the product was purified by silica gel chromatography.



(*R*)-Diethyl 2-allyl-2-methyl-3-oxosuccinate (2.8):

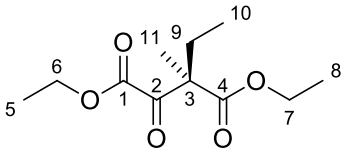
Compound **2.8** was synthesized from compound **2.3** (2.14 g, 8.8 mmol), following the Swern oxidation protocol described above. An orange oil was obtained in a final yield of 1.59 g, 75%. R_f 0.47 (ethyl acetate/hexanes, 1:4, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.71-5.56 (m, 1H, H-10), 5.08 (m, 2H, H-11), 4.31 (q, *J* = 7.2, 2H, H-6 or H-7), 4.17 (q, *J* = 7.2, 2H, H-6 or H-7), 2.67 (m, 2H, H-9), 1.42 (s, 3H, H-12), 1.35 (t, *J* = 7.2, 3H, H-5 or H-8), 1.21 (t, *J* = 7.2, 3H, H-5 or H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.3 (C-2), 171.5 (C-1 or C-4), 160.1 (C-1 or C-4), 131.7 (C-

10), 119.5 (C-11), 62.6 (C-6 or C-7), 61.5 (C-6 or C-7), 56.2 (C-3), 39.2 (C-9), 19.3 (C-12), 14.0 (C-5 and C-8). HRMS (ESI) calculated for $C_{12}H_{18}O_5$ (M+23): 265.10464; found: 265.10437.



(*R*)-Diethyl 2-propyl-2-methyl-3-oxosuccinate (2.9):

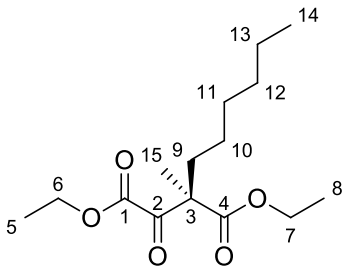
Compound **2.9** was synthesized from compound **2.4** (1.51 g, 6.1 mmol), using the general Swern oxidation protocol described above. After purification an oily orange liquid was obtained with an overall yield of 1.32 g, 88%. R_f 0.51 (ethyl acetate/hexanes, 1:4, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.30 (q, *J* = 7.2, 2H, H-6 or H-7), 4.17 (q, *J* = 7.2, 2H, H-6 or H-7), 1.88 (t, *J* = 8.6, 2H, H-9), 1.43 (s, 3H, H-12), 1.35 (t, *J* = 7.2, 3H, H-5 or H-8), 1.22 (t, *J* = 7.2, 3H, H-5 or H-8), 0.91 (t, *J* = 7.2, 3H, H-11). ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.0 (C-2), 172.1 (C-1), 160.4 (C-4), 62.5 (C-6 or C-7), 61.4 (C-6 or C-7), 56.4 (C-3), 37.0 (C-9), 19.5 (C-12), 17.4 (C-10), 14.4 (C-11), 14.0 (C-5 or C-8), 13.9 (C-5 or C-8). HRMS (ESI) calculated for $C_{12}H_{20}O_5$ (M+23): 267.12029; found: 267.12044.



(*R*)-Diethyl 2-ethyl-2-methyl-3-oxosuccinate (2.10):

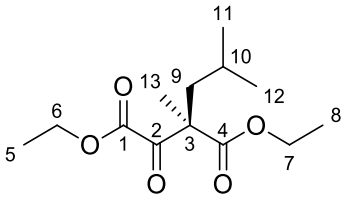
To synthesize compound **2.10** from compound **2.5** (1.90 g, 8.2 mmol), the general Swern oxidation protocol described above was used. This compound was purified as an orange oil with a yield of 1.58 g, 84%. R_f 0.54 (ethyl acetate/hexanes, 1:4, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.30 (q, *J* = 7.2, 2H, H-6

or H-7), 4.18 (q, *J* = 7.2, 2H, H-6 or H-7), 1.96 (q, *J* = 7.7, 2H, H-9), 1.42 (s, 3H, H-11), 1.35 (t, *J* = 7.2, 3H, H-5 or H-8), 1.22 (t, *J* = 7.2, 3H, H-5 or H-8), 0.86 (t, *J* = 7.7, 3H, H-10).



(*R*)-Diethyl 2-hexyl-2-methyl-3-oxosuccinate (2.11):

Compound **2.11** was synthesized from compound **2.6** (0.56 g, 1.9 mmol), using the general Swern oxidation protocol described above. The overall yield was 0.41g of yellow oil, 74%. R_f 0.47 (ethyl acetate/hexanes, 1:4, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.30 (q, *J* = 7.2, 2H, H-6 or H-7), 4.17 (q, *J* = 7.2, 2H, H-6 or H-7), 1.90 (m, 2H, H-9), 1.42 (s, 3H, H-15), 1.35 (t, *J* = 7.2, 3H, H-5 or H-8), 1.32-1.10 (m, 11H, H-5 or H-8, H-10 to H-13), 0.86 (t, *J* = 6.8, 3H, H-14). ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.2 (C-2), 172.1 (C-1), 172.1 (C-4), 62.4, (C-6 or C-7), 61.3 (C-6 or C-7), 56.3 (C-3), 34.8 (C-9), 31.4 (C-10), 29.5 (C-11), 23.8 (C-12), 22.5 (C-13), 19.5 (C-15), 14.0 (C-5, C-8 or C-14), 14.0 (C-5, C-8 or C-14), 13.9 (C-5, C-8 or C-14). HRMS (ESI) calculated for $C_{15}H_{26}O_5$ (M+1): 287.18530; found: 287.18507.

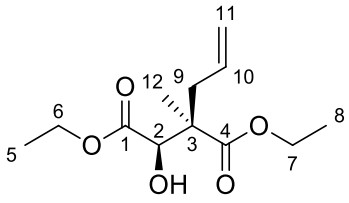


(*R*)-Diethyl 2-isobutyl-2-methyl-3-oxosuccinate (2.12): Compound **2.12** was synthesized from compound **2.7** (0.56 g, 2.2 mmol), using the general Swern oxidation protocol described above. The final yield was 0.45 g of yellow oil, 81%. R_f 0.48 (ethyl acetate/hexanes, 1:4, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.31 (q, *J* = 7.2, 2H, H-6 or H-7), 4.16 (q, *J* = 7.2, 2H, H-6 or H-7), 1.90 (m, 2H, H-9) 1.66-1.54 (m, 1H, H-10), 1.46 (s, 3H, H-13), 1.35 (t, *J* = 7.2, 3H, H-5 or H-8), 1.21 (t, *J* = 7.2, 3H, H-5 or H-8), 0.88 (d, *J* = 6.6, 3H, H-11 or H-12), 0.83 (d, *J* = 6.6, 3H, H-11 or H-12). ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.5 (C-2), 172.3 (C-1 or C-4), 160.6 (C-1 or C-4), 62.5 (C-6 or C-7), 61.4 (C-6 or C-7), 56.3 (C-3), 43.6 (C-10), 24.2 (C-9), 20.3 (C-11 or C-12), 20.3 (C-11 or C-12), 14.0 (C-5 or C-8), 13.9 (C-5 or C-8). HRMS (ESI) calculated for $C_{13}H_{22}O_5$ (M+1): 259.15400; found: 259.15390.

General protocol for enantioselective reduction with Baker's Yeast (used to generate compounds 2.13-2.17:

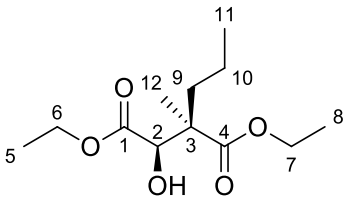
The compounds **2.13**-**2.17** were synthesized from compounds **2.8**-**2.12** by reducing the ketone with Baker's yeast. Thus distilled water (60 mL per 250 mg of ketone) was added to a 1 L Erlenmeyer flask and stirred. Baker's yeast (20 g per 250 mg of ketone) was added followed by methyl vinyl ketone (292 µL per 250 mg of ketone for a final concentration of 0.06 M). The mixture was stirred for 30 min at 50^{o}C. Then the ketone (**2.8**-**2.12**, 250 mg) was added and allowed to react for 24 hours at 30^{o}C with low stirring.

To work up the Baker's yeast reaction, first the mixture was poured into a 1 L sized beaker containing a magnetic stir bar, before addition of ethyl acetate (400 mL) and HyFlo celite (50-100 g). The mixture was stirred for 30 min at room temperature. Then the mixture was filtered through Hyflo celite (1cm thickness) using a Buchner filter. During this process, the surface of the celite layer was gently scratched with a spatula to prevent clogging. The filtrate containing the product was collected and the layers were separated. The aqueous layer was washed with ethyl acetate (2 × 50 mL) and the combined organic layers were washed with a solution of saturated NaCl (150 mL). Finally the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified using silica gel chromatography twice (ethyl acetate/hexanes, 1:9, v/v followed by ethyl acetate/dichloromethane, 1:9, v/v).

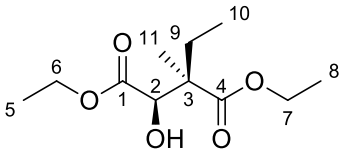


(2*R*,3*R*)-Diethyl 2-allyl-3-hydroxy-2-methylsuccinate (2.13): Compound **2.13** was synthesized from compound **2.8** (0.94 g, 3.9 mmol) using the Baker's yeast reduction protocol described above. The overall yield was 0.60 g of yellow oil, 63%. 1st purification Rf 0.11 (ethyl acetate/hexanes, 1:9, v/v), and Rf 0.56 (ethyl acetate/dichloromethane, 1:9, v/v). 1H NMR (300 MHz, CDCl3) δ 5.80-5.63 (m, 1H, H-10), 5.08 (m, 2H, H-11), 4.45 (s, 1H, H-2), 4.29-4.18 (m, 2H, H-6 or H-7), 4.15 (q, J = 7.1, 2H, H-6 or H-7), 2.56 (dd, J = 7.2, 13.9, 1H, H-9a), 2.40 (dd, J = 7.7, 13.9, 1H, H-9b), 1.27 (t, J = 7.1, 3H, H-5 or H-8), 1.27 (t, J = 7.1, 3H, H-5 or H-8), 1.06 (s, 3H, H-12). 13C NMR (75 MHz, CDCl3) δ 173.8

(C-1 or C-4), 173.3 (C-1 or C-4), 133.1 (C-10), 118.7 (C-11), 76.6 (C-2), 62. 1 (C-6 or C-7), 60.7 (C-6 or C-7), 50.3 (C-3), 40.8 (C-9), 15.2 (C-12), 14.2 (C-5 or C-8), 14.0 (C-5 or C-8). HRMS (ESI) calculated for $C_{12}H_{20}O_5$ (M+23): 267.12029; found: 267.12024.

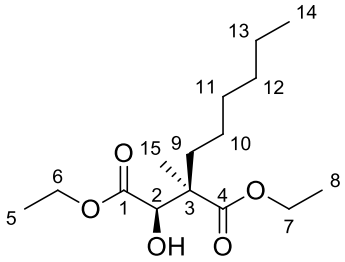


(2*R*,3*R*)-Diethyl 2-propyl-3-hydroxy-2-methyl succinate (2.14): Compound **2.9** (1.1 g, 4.6 mmol) was reduced with Baker's yeast to yield compound **2.14**, using the Baker's yeast methodology described above. The overall yield was 0.35 g, 31% of yellow oil. R_f 0.12 (ethyl acetate/hexanes, 1:9, v/v), and 2nd purification R_f 0.35 (ethyl acetate/dichloromethane, 1:19, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.45 (s, 1H, H-2), 4.29-4.11 (m, 4H, H-6, H-7), 3.05 (s, 1H, OH), 1.81-1.56 (m, 2H, H-9), 1.43-1.08 (m, 2H, H-10), 1.27 (t, *J* = 7.1, 3H, H-5 or H-8), 1.26 (t, *J* = 7.1, 3H, H-5 or H-8), 1.04 (s, 3H, H-12), 0.90 (t, *J* = 7.3, 3H, H-11). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6 (C-1 or C-4), 170.7 (C-1 or C-4), 74.5 (C-2), 62.1 (C-6 or C-7), 60.7 (C-6 or C-7), 50.3 (C-3), 17.6 (C-9), 15.1 (C-10), 14.5 (C-5 or C-8), 14.1 (C-5 or C-8), 14.0 (C-11).



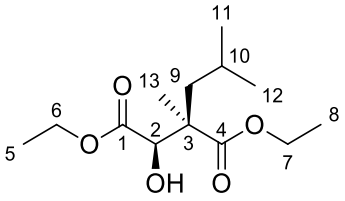
(2*R*,3*R*)-Diethyl 2-ethyl-3-hydroxy-2-methylsuccinate (2.15): Compound **2.15** was synthesized by Baker's yeast reduction of compound **2.10** (1.03 g, 4.5 mmol) using the protocol described above. The overall yield was

0.39 g of a yellow oil, 37%. R_f 0.10 (ethyl acetate/hexanes, 1:9, v/v), and R_f 0.44 (ethyl acetate/dichloromethane, 1:9, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.46 (s, 1H, H-2), 4.29-4.12 (m, 4H, H-6, H-7), 3.04 (s, 1H, OH), 1.84 (dq, *J* = 7.4, 14.0, 1H, H-9a), 1.68 (dq, *J* = 7.7, 14.0, 1H, H-9b), 1.28 (t, *J* = 7.1, 3H, H-5 or H-8), 1.26 (t, *J* = 7.1, 3H, H-5 or H-8) 1.03 (s, 3H, H-11), 0.86 (t, *J* = 7.6, 3H, H-10). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3 (C-1 or C-4), 173.7 (C-1 or C-4), 74.7 (C-2), 62.1 (C-6 or C-7), 60.6 (C-6 or C-7), 50.7 (C-3), 29.5 (C-10), 14.6 (C-5, C-8 or C-12), 14.2 (C-5, C-8 or C-12), 14.0 (C-5, C-8 or C-12), 8.7 (C-11). HRMS (ESI) calculated for $C_{11}H_{20}O_5$ (M+1): 233.13835; found: 233.13836.



(2*R*,3*R*)-Diethyl-2-hexyl-3-hydroxy-2-methyl succinate (2.16): To synthesize compound **2.16**, compound **2.11** (0.25 g, 0.9 mmol) was reduced with Baker's yeast using the general reduction protocol described above. The overall yield was 0.052 g of a yellow oil, 21%. R_f 0.11 (ethyl acetate/hexanes, 1:9, v/v), and R_f 0.26 (ethyl acetate/dichloromethane, 1:9, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.45 (s, 1H, H-2), 4.31-4.08 (m, 4H, H-6, H-7), 1.81-1.58 (m, 2H, H-9), 1.37-1.22 (m, 14H, H-5, H-8, H-10 to H-13), 1.04 (s, 3H, H-15), 0.87 (m, 3H, H-14). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.4 (C-1 or C-4), 173.6 (C-1 or C-4), 75.0 (C-2), 62.1 (C-6 or C-7), 60.3 (C-6 or C-7), 50.3 (C-3), 36.5 (C-9), 31.9 (C-10), 29.4 (C-11), 24.7 (C-12), 22.7 (C-13), 15.1 (C-15), 14.1 (C-5, C-8 or

C-14), 14.1 (C-5, C-8 or C-14), 14.0 (C-5, C-8 or C-14). HRMS (ESI) calculated for $C_{15}H_{28}O_5$ (M+1): 289.20048; found: 289.200095.

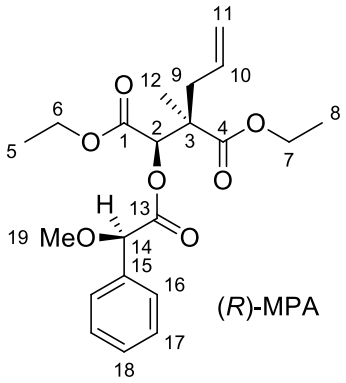


(2*R*,3*R*)-Diethyl 2-isobutyl-3-hydroxy-2-methyl succinate (2.17): Compound **2.17** was synthesized from compound **2.12** (0.25 g, 1 mmol) using the Baker's Yeast protocol described above. Thus 0.066 g of a yellow oil was isolated, 26%. R_f 0.10 (ethyl acetate/hexanes, 1:9, v/v), and R_f 0.29 (ethyl acetate/dichloromethane, 1:9, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.45 (s, 1H, H-2), 4.28-4.10 (m, 4H, H-6, H-7), 1.70-1.57 (m, 2H, H-9), 1.32-1.22 (m, 6H, H-5, H-8), 1.06 (s, 3H, H-13), 0.92 (d, *J* = 6.5, 3H, H-11 or H-12), 0.83 (d, *J* = 6.5, 3H, H-11 or H-12). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.9 (C-1 or C-4), 174.9 (C-1 or C-4), 75.6 (C-2), 62.2 (C-6 or C-7), 60.5 (C-6 or C-7), 50.2 (C-3), 44.6 (C-10), 30.9 (C-9), 25.0 (C-11, 12 or C-13), 24.5 (C-11, 12 or C-13), 23.0 (C-11, 12 or C-13), 14.9 (C-5 or C-8), 14.0 (C-5 or C-8). HRMS (ESI) calculated for $C_{13}H_{24}O_5$ (M+1): 261.16965; found: 261.16939.

Determination of absolute stereochemistry of the secondary alcohol in compound 2.13:

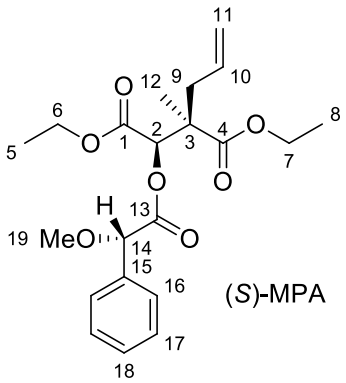
Compound **2.18** and **2.19** were synthesized by coupling with (*R* or *S*)-methoxy-phenylacetic acid to yield a chiral derivative that was used for NMR analysis of the absolute stereochemistry of the secondary chiral alcohols. Thus compound **2.13** (25 mg, 0.1 mmol, 1eq) was esterfied by adding (*R* or *S*)-MPA (16.6 mg, 0.1 mmol, 1eq), and the coupling agent EDC (23.0 mg, 0.12 mmol, 1.2

eq) with DMAP (1 mg, 0.01mmol, 0.1 eq). The reaction was allowed to stir in DCM (2 mL) for 16 hours at room temperature. The reaction was quenched with NH_4Cl (2 mL), and extracted with ethyl acetate (3 × 5 mL). The solution was concentrated and the product purified by preparative TLC. Finally the ^1H NMR of the purified (*R*)-MPA derivative and (*S*)-MPA were measured.



(*R*)-MPA

(*R*)-MPA derivative (2.18): Compound **2.18** was synthesized by coupling with (*R*)-MPA. R_f 0.72 (hexane/ethyl acetate, 1:1, v/v). ^1H NMR (300 MHz, $CDCl_3$) δ 7.51-7.44 (m, 2H, H-16), 7.42-7.32 (m, 3H, H-17, H-18), 5.72-5.54 (m, 1H, H-10), 5.46 (s, 1H, H-14), 5.00-4.87 (m, 3H, H-2, H-11), 4.13 (q, *J* = 7.0, 2H, H-7), 4.01 (q, *J* = 7.0, 2H, H-6), 3.51 (s, 3H, H-19), 2.38 (dd, *J* = 7.3, 13.7, 1H, H-9a), 2.17 (dd, *J* = 7.5, 13.7, 1H, H-9b), 1.23 (t, *J* = 7.0, 3H, H-8), 1.14 (s, 3H, H-12), 1.06 (t, *J* = 7.0, 3H, H-5).



(*S*)-MPA

(*S*)-MPA derivative (2.19): Compound **2.19** was synthesized by coupling with (*S*)-MPA. R_f 0.72 (hexane/ethyl acetate, 1:1, v/v). ^1H

NMR (300 MHz, $CDCl_3$) δ 7.51-7.44 (m, 2H, H-16), 7.42-7.32 (m, 3H, H-17, H-18), 5.57-5.37 (m, 2H, H-10, H-14), 4.94-4.87 (m, 2H, H-2, H-11a), 4.75-4.64 (d, *J* = 17.4, 1H, H-11b), 4.16 (m, 2H, H-6), 4.07 (q, *J* = 7.3, 2H, H-7), 2.09 (dd, *J* = 6.7, 13.9, 1H, H-9a), 1.70 (dd, *J* = 7.7, 13.9, 1H, H-9b), 1.21 (t, *J* = 7.3, 6H, H-5, H-8), 1.04 (s, 3H, H-12).

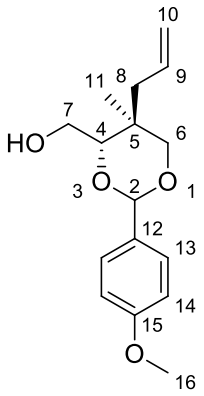
4.3 Pantothenamide Synthesis

General procedure for LiAlH4 reduction and selective triol protection (used to generate compounds 3.1 and 3.2):

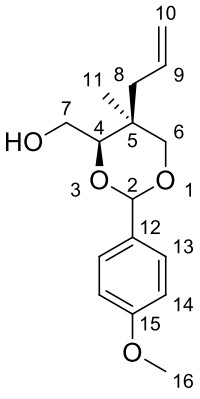
$LiAlH_4$ was used to reduce compounds **2.3** and **2.13**. $LiAlH_4$ (0.23 g, 6.1 mmol, 3 eq) was dissolved in dry THF (30 mL) and cooled down to 0^oC while stirring. The diester (**2.3** or **2.13**, 2.1 mmol, 1 eq) was added dropwise as a solution of THF (3 mL). Small amounts of H_2 gas were produced. The reaction was left to reflux over an oil bath at 85^oC for 16 hours. The remaining $LiAlH_4$ was quenched with an aqueous saturated solution of ammonium chloride (20 mL) and the product was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure.

The resulting 1,3-diol was selectively protected as a para-methoxy phenyl (PMP) acetal. Thus DCM (15 mL) was added to the reaction flask with anisaldehyde dimethyl acetal (0.51 mL, 3.0 mmol, 1.5 eq) and a catalytic amount of camphor sulfonic acid (46 mg, 0.2 mmol, 0.1 eq). Then the intermediate triol, prepared by $LiAlH_4$ reduction, (2 mmol, 1 eq), was dissolved in DCM (3 mL)

before addition to the reaction mixture. The reaction was left to stir for 4 hours at room temperature before quenching with triethylamine (NEt_3, 3 mL). Finally the mixture was concentrated under reduced pressure to afford the crude product. Purification was achieved with silica gel chromatography.



(4*S*,5*S*)-5-Allyl-2-(4-methoxyphenyl)-5-methyl-1,3-dioxan-4-yl)methanol (3.1): This known compound was synthesized using the general protection procedure.[16] Compound **3.1** was synthesized from crude **2.3** (0.49 g, 2 mmol), by $LiAlH_4$ reduction and selective protection of the 1,3-diol as the anisaldehyde dimethyl acetal, using the general protocol described above.[16] The overall yield of the yellow oily residue was 0.32 g, 57%. R_f 0.14 (ethyl acetate/hexanes, 1:4, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, *J* = 8.6, 2H, H-13), 6.90 (d, *J* = 8.6, 2H, H-14), 5.86-5.67 (m, 1H, H-9), 5.44 (s, 1H, H-2), 5.14-5.05 (m, 2H, H-10), 3.82-3.62 (m, 5H, H-4, H-6, H-7), 3.80 (s, 3H, H-16), 2.05-1.98 (m, 2H, H-8), 1.16 (s, 3H, H-11). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.1 (C-15), 132.3 (C-9 or C-12), 130.8 (C-9 or C-12), 127.6 (C-13), 118.8 (C-10), 113.7 (C-14), 101.9 (C-2), 84.7 (C-4), 77.0 (C-7), 61.3 (C-6), 55.4 (C-16), 40.4 (C-8), 34.3 (C-5), 17.5 (C-11).

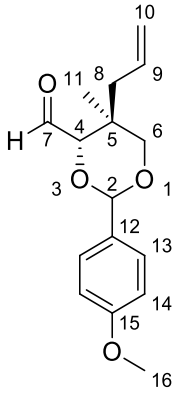


(4*R*,5*S*)-5-Allyl-2-(4-methoxyphenyl)-5-methyl-1,3-dioxan-4-yl)methanol (3.2): This known compound was synthesized using the general protection procedure.[16] Compound **3.2** was synthesized from compound **2.13** (0.50 g, 2.1 mmol) by $LiAlH_4$ reduction and selective protection of the 1,3-diol as the anisaldehyde dimethyl acetal, using the general protocol described above.[16] The overall yield was 0.46 g (80%) of an oily yellow residue. R_f 0.26 (ethyl acetate/hexanes, 3:7, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, *J* = 8.7, 2H, H-13), 6.91 (d, *J* = 8.7, 2H, H-14), 5.92-5.76 (m, 1H, H-9), 5.50 (s, 1H, H-2), 5.19-5.06 (m, 2H, H-10), 3.82-3.68 (m, 5H, H-4, H-6, H-7), 3.80 (s, 3H, H-16), 2.22-1.87 (m, 2H, H-8), 0.80 (s, 3H, H-11). ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.0 (C-9), 133.0 (C-12), 127.6 (C-13), 118.6 (C-10), 113.7 (C-14), 102.2 (C-2), 86.8 (C-4), 74.4 (C-7), 61.1 (C-6), 55.4 (C-16), 35.2 (C-8), 34.5 (C-5), 18.3 (C-11).

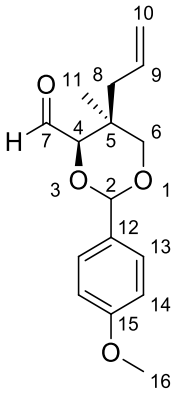
General procedure for oxidation of alcohols 3.1 and 3.2 to the corresponding aldehyde 3.3 and 3.4:

The alcohol **3.1** or **3.2** (2.2 mmol, 1 eq) was dissolved in DCM (15 mL) before the addition of the Dess-Martin periodinane (3.3 mmol, 1.5 eq). The reaction was allowed to stir for 2 hours at room temperature. The mixture was washed with a solution of sodium thiosulfate $Na_2S_2O_3$ (prepared by mixing 1.5 g

$Na_2S_2O_3$ in 5 mL of aqueous saturated sodium bicarbonate). Finally the organic layer was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The intermediate was purified silica gel chromatography to afford **3.3** and **3.4**.



(4*S*,5*S*)-5-Allyl-2-(4-methoxyphenyl)-5-methyl-1,3-dioxan-4-yl)formaldehyde (3.3): This known compound was synthesized using the general oxidation procedure.[16] Following the general protocol described above, the intermediate compound **3.3** was synthesized from compound **3.1** (0.32 g, 1.2 mmol). The yield for compound **3.3** was 0.18 g, 56% of yellow oily residue. R_f 0.55 (ethyl acetate/hexanes, 1:4, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.66 (s, 1H, H-7), 7.47 (d, *J* = 8.7, 2H, H-13), 6.92 (d, *J* = 8.7, 2H, H-14), 5.86-5.68 (m, 1H, H-9), 5.48 (s, 1H, H-2), 5.18-5.09 (m, 2H, H-10), 4.06 (s, 1H, H-4), 3.83-3.78 (m, 5H, H-6, H-16), 2.23 (d, *J* = 7.4, 2H, H-8), 1.24 (s, 3H, H-11). ^{13}C NMR (75 MHz, $CDCl_3$) 202.0 (C-7), 160.4 (C-15), 131.9 (C-12), 130.1 (C-9), 127.5 (C-13), 119.4 (C-10), 113.8 (C-14), 101.3 (C-2), 85.7 (C-4), 60.4 (C-6), 55.4 (C-16), 39.4 (C-5 or C-8), 36.1 (C-5 or C-8), 17.7 (C-11).



(4*R*,5*S*)-5-Allyl-2-(4-methoxyphenyl)-5-methyl-1,3-dioxan-4-yl)formaldehyde (3.4): This known compound[16] was synthesized using the general protection procedure. Following the general protocol described above, compound **3.4** was synthesized from compound **3.2** (0.46 g, 1.7 mmol) by oxidizing the alcohol to the aldehyde with Dess Martin periodinane. The yield was 0.22 g (48%) of yellow oily residue. R_f 0.45 (ethyl acetate/hexanes, 1:4, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.69 (s, 1H, H-7), 7.49 (d, *J* = 8.7, 2H, H-13), 6.93 (d, *J* = 8.7, 2H, H-14), 5.88-5.74 (m, 1H, H-9), 5.53 (s, 1H, H-2), 5.21-5.11 (m, 2H, H-10), 3.99 (s, 1H, H-4), 3.96 (d, *J* = 11.8, 1H, H-6a), 3.81 (s, 3H, H-16), 3.49 (d, *J* = 11.8, 1H, H-6b), 2.81 (dd, *J*= 7.2, 13.7, 1H, H-8a), 2.17 (dd, J = 7.7, 13.7, 1H, H-8b), 1.20 (s, 3H, H-11). ^{13}C NMR (75 MHz, $CDCl_3$) 190.0 (C-7), 164.5 (C-15), 132.0 (C-12), 132.0 (C-9), 129.9 (C-13), 114.2 (C-10), 114.2 (C-14), 76.5 (C-4), 55.7 (C-16), 31.0 (C-5), 31.0 (C-8), 14.2 (C-11).

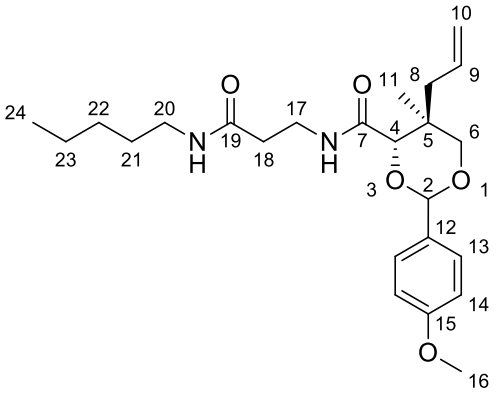
General protocol for the synthesis of carboxylic acid 3.5 & 3.6 from the aldehyde 3.3 & 3.4 using Pinnick oxidation:

For the Pinnick oxidation, a mixture of sodium chlorite ($NaClO_2$, 3.2 mmol, 5 eq) and sodium phosphate monobasic (NaH_2PO_4, 6.4 mmol, 10 eq) was dissolved in water (1.25 mL). The aldehyde **3.3** or **3.4** (0.18 g, 0.64 mmol, 1 eq) was dissolved in a mixture of acetone/DCM (8 mL, 3:1, v/v) before addition of the

$NaClO_2/Na_2PO_4$ aqueous solution. The reaction mixture was allowed to stir for 30 min at room temperature. The reaction was quenched with a solution of saturated aqueous sodium sulfite (5 mL) and the product was extracted with ethyl acetate (3 × 15 mL). Finally the combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude acid was used directly into the next step without any further purification.

General procedure for amide coupling (used to generate compounds 3.7 and 3.8):

Compounds **3.7** and **3.8** were synthesized by coupling the acid (**3.5** or **3.6**) and amine **3.12** with the coupling agent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). The FMOC protected amine (**3.12**, 103 mg, 0.3 mmol, 1.5 eq) was dissolved in DMF (10 mL) before piperidine (1.5 mL) was added, for *in situ* Fmoc deprotection. The reaction was allowed to stir for 10 min before concentration of the free amine under reduced pressure. Next EDC (110 mg, 0.6 mmol, 3 eq) and HOBT (87 mg, 0.6 mmol, 3 eq) were added to the reaction mixture and the reaction flask was purged with nitrogen gas, before addition of dry THF (5 mL). A solution of the acid (0.2 mmol, 1 eq) in THF (1 mL) was added, followed by addition of DIPEA (0.33 ml, 1.8 mmol, 10 eq). The reaction was allowed to react for 16 hours at room temperature, before quenching with a saturated aqueous solution of ammonium chloride NH_4Cl (5 mL) and extraction in ethyl acetate (3 × 10 mL). Finally the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel chromatography.



(4*S*,5*S*)-5-Allyl-2-(4-methoxyphenyl)-5-methyl-*N*-(3-oxo-3-(pentylamino)propyl)-1,3-dioxane-4-carboxamide (3.7): The crude acid **3.5** (≤0.2 mmol) was coupled with amine **3.12** using the EDC amide coupling procedure above. This is a known compound.[16] Overall the amount of yellow oily residue obtained was 25 mg, 33%. R_f 0.23 (ethyl acetate/hexanes, 4:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.7, 2H, H-13), 6.91 (d, *J* = 8.7, 2H, H-14), 5.93-5.77 (m, 1H, H-9), 5.41 (s, 1H, H-2), 5.19-5.05 (m, 2H, H-10), 4.19 (s, 1H, H-4), 3.87-3.74 (m, 2H, H-20), 3.82 (s, 3H, H-16), 3.63-3.47 (m, 2H, H-17), 2.48-2.36 (m, 4H, H-8, H-18), 1.37-1.22 (m, 6H, H-21, H-22, H-23), 1.10 (s, 3H, H-11), 0.88 (t, *J* = 6.6, H-24). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 169.5, 162.0, 160.2, 132.8, 130.1, 127.4, 118.8, 113.7, 101.1, 82.0, 76.5, 76.1, 55.4, 40.5, 39.6, 36.2, 35.5, 35.0, 29.2, 29.0, 22.3, 17.7, 14.0.

Alternative amide coupling reactions:

Compound **3.7** was also synthesized by coupling the acid (**3.5**) and amine (**3.12**) with the coupling agent 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HBTU). The Fmoc protected amine (**3.12**, 103 mg, 0.3 mmol, 1.5 eq) was dissolved in THF (10 mL) and piperidine (1.5 mL) was added, for *in situ* Fmoc deprotection. The reaction was allowed to stir for 10 min before concentration of the free amine under

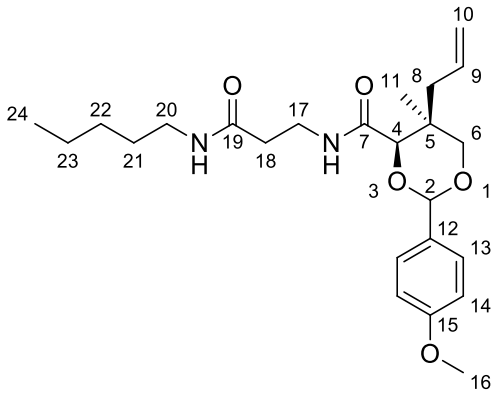
reduced pressure. Next HBTU (22 mg, 0.6 mmol, 3 eq) and HOBT (87 mg, 0.6 mmol, 3 eq) were added to the reaction mixture and the reaction flask was purged with nitrogen gas before addition of dry THF (5 mL). A solution of the acid (0.2 mmol, 1 eq) in THF (1 mL) was added, followed by addition of DIPEA (0.33 mL, 2.0 mmol, 10 eq). The reaction was allowed to react for 16 hours at room temperature, before quenching with a saturated aqueous solution of ammonium chloride NH_4Cl (5 mL) and extraction in ethyl acetate (3 × 10 mL). Finally the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel chromatography.

The crude acid **3.5** (≤0.2 mmol) was coupled with amine **3.10** using the HBTU amide coupling procedure. The overall amount of yellow oily residue **3.7** obtained was 15 mg, 19%. *Rf* 0.23 (ethyl acetate/hexanes, 4:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (d, J = 8.7, 2H, H-13), 6.90 (d, J = 8.7, 2H, H-14), 5.97-5.72 (m, 1H, H-9), 5.41 (s, 1H, H-2), 5.18-5.05 (m, 2H, H-10), 4.19 (s, 1H, H-4), 3.86-3.64 (m, 2H, H-20), 3.81 (s, 3H, H-16), 3.60-3.39 (m, 2H, H-17), 2.45-2.29 (m, 4H, H-8, H-18), 1.35-1.19 (m, 6H, H-21, H-22, H-23), 1.10 (s, 3H, H-11), 0.88 (t, J = 6.6, H-24).

Compound **3.7** was also synthesized by coupling the acid (**3.5**) and amine **3.12** using the coupling agent O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N′*,*N′*-tetramethyluronium hexafluorophosphate (HATU). The Fmoc protected amine (**3.12**, 102.9 mg, 0.3 mmol, 1.5 eq) was dissolved in THF (10 mL) before piperidine (1.5 mL) was added, for *in situ* Fmoc deprotection. The reaction was allowed to stir for 10 min before concentration of the free amine under reduced

pressure. Next HATU (22 mg, 0.6 mmol, 3 eq) and HOBT (87 mg, 0.6 mmol, 3 eq) were added to the reaction mixture and the reaction flask was purged with nitrogen gas before addition of dry THF (5 mL). A solution of the acid (0.2 mmol, 1 eq) in THF (1 mL) was added, followed by addition of DIPEA (0.33 mL, 2.0 mmol, 10 eq). The reaction was allowed to react for 16 hours at room temperature, before quenching with a saturated aqueous solution of ammonium chloride NH_4Cl (5 mL), and extraction in ethyl acetate (3 × 10 mL). Finally the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel chromatography.

The crude acid **3.5** (≤0.2 mmol) was coupled to the amine **3.10** using the HBTU amide coupling procedure. The overall amount of yellow oily compound **3.7** obtained was 18 mg, 23%. *Rf* 0.23 (ethyl acetate/hexanes, 4:1, v/v). 1H NMR (300 MHz, CDCl3) δ 7.42 (d, *J* = 8.7, 2H, H-13), 6.91 (d, *J* = 8.7, 2H, H-14), 6.00-5.69 (m, 1H, H-9), 5.41 (s, 1H, H-2), 5.18-5.06 (m, 2H, H-10), 4.18 (s, 1H, H-4), 3.93-3.69 (m, 2H, H-20), 3.81 (s, 3H, H-16), 3.60-3.45 (m, 2H, H-17), 2.48-2.32 (m, 4H, H-8, H-18), 1.37-1.20 (m, 6H, H-21, H-22, H-23), 1.10 (s, 3H, H-11), 0.88 (t, *J* = 6.6, H-24).

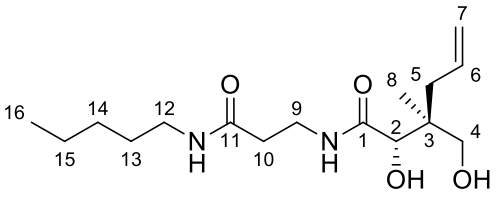


(4*R*,5*S*)-5-Allyl-2-(4-methoxyphenyl)-5-methyl-*N*-(3-oxo-3-(pentylamino)propyl)-1,3-dioxane-4-carboxamide (3.8):

The acid **3.6** (221 mg, 0.8 mmol) was coupled with the amine **3.10** using the EDC amide coupling procedure above.[16] The overall amount of yellow oily residue obtained was 257 mg, 75 %. R_f 0.24 (ethyl acetate/hexanes, 4:1, v/v).

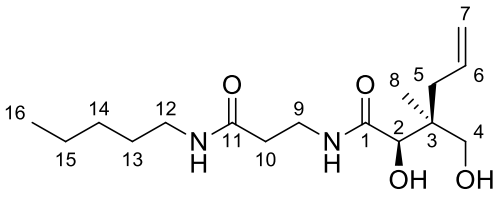
General procedure for the deprotection of the alcohols (used to generate compounds 3.9 and 3.10):

To cleave the PMP group, compound **3.9** or **3.10** (0.08 mmol) were separately mixed with a solution of acetic acid (5 mL, 90% aqueous) and stirred for 16 hours at room temperature. The acetic acid was evaporated under reduced pressure and the product was lyophilized. Finally the final product was purified by silica gel chromatography.

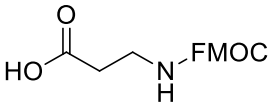


(2*S*,3*S*)-2-Hydroxy-3-(hydroxymethyl)-3-methyl-*N*-(3-oxo-(pentylamino)propyl)hex-5-enamide (3.9): Compound **3.9** was synthesized by deprotection of compound **3.7** (33 mg, 0.08 mmol), with acetic acid as described above.[16] The overall yield was 5.6 mg, 24% of yellow oily residue. R_f 0.45 (MeOH/DCM, 1:9, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (br s, 1H, NH), 6.13 (br s, NH), 5.91-5.73 (m, 1H, H-6), 5.15-5.02 (m, 2H, H-7), 4.00 (s, 1H, H-2), 3.63-3.46 (m, 5H, H-2, H-9, H-16), 3.20 (d, *J* = 12.4, 1H, H-4a), 3.19 (d, *J* = 12.4, 1H, H-4b), 2.43 (t, *J* = 6.2, 2H, H-10), 2.27 (dd, *J* = 7.5, 13.2, 1H, H-5a), 2.09 (dd, *J* = 7.5, 13.2, 1H, H-5b), 1.52-1.40 (m, 2H, H-13), 1.34-1.21 (m, 4H, H-14, H-15), 0.94 (s, 3H, H-8), 0.88 (t, *J* = 6.9, 3H, H-16). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9 (C-1 or C-11), 171.5 (C-1 or C-11), 133.9 (C-6), 118.3 (C-7), 76.8

(C-2), 55.5 (C-4), 41.9 (C-9 or C-12), 39.8 (C-9, or C-12), 35.7 (C-10), 29.1 (C-13), 22.3 (C-14), 18.7 (C-8), 14.0 (C-16).

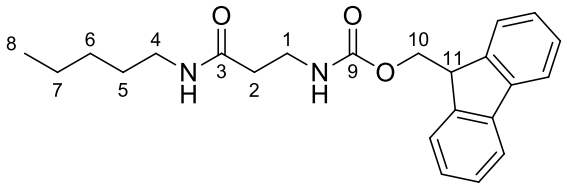


(2*R*,3*S*)-2-Hydroxy-3-(hydroxymethyl)-3-methyl-N-(3-oxo-(pentylamino)propyl)hex-5-enamide (3.10): Compound **3.10** was synthesized by deprotection of compound **3.8** (257 mg, 0.6 mmol), in the presence of 90% acetic acid.[16] The overall yield was 4 mg, 2% of yellow oily residue. R_f 0.45 (MeOH/DCM, 1:9, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (br s, 1H, NH), 5.99-5.82 (m, 1H, H-6), 5.71 (br s, 1H, NH), 5.19-5.07 (m, 2H, H-7), 4.09 (s, 1H, H-2), 3.63-3.53 (m, 4H, H-9, H-12), 3.48 (s, 2H, OH), 3.22 (d, *J* = 13.1, 1H, H-4a), 3.20 (d, *J* = 13.1, 1H, H-4b), 2.42 (t, *J* = 5.8, 2H, H-10), 2.34 (dd, *J* = 7.9, 13.6, 1H, H-5a), 2.18 (dd, *J* = 7.9, 13.6, 1H, H-5b), 1.52-1.45 (m, 2H, H-13), 1.34-1.23 (m, 4H, H-14, H-15), 0.89 (t, *J* = 6.8, 3H, H-16), 0.85 (s, 3H, H-8).



(9H-Fluoren-9-yl)methyl-3-oxo-3-hydroxypropyl-carbamate (3.11): Fmoc protected β-alanine (**3.11**) was synthesized from β-alanine by amide coupling.[47] Initially, β-alanine (688 mg, 7.7 mmol, 1 eq) was mixed with a solution of sodium bicarbonate (20 mL, 10% aqueous), followed by addition of *p*-dioxane (10 mL). The mixture was cooled down to 0^{o}C in an ice bath. In a separate flask, Fmoc-Cl (2.00 g, 7.7 mmol, 1 eq) was dissolved in *p*-dioxane (20 mL), before being added dropwise into the reaction mixture containing β-alanine. First the reaction mixture was stirred for 1 hour at 0^{o}C and

then for 3 hours at room temperature. Finally the reaction was worked up by dilution in diethyl ether (100 mL) and acidification to pH 1 with concentrated HCl. The product was extracted in ethyl acetate (3 × 50 mL). The organic layers were combined and dried over sodium sulfate, before removal of the solvent under reduced pressure. The crystallized product was collected and washed with hexane (20 mL). The product yield was 2.19 g, 91% of white solid.

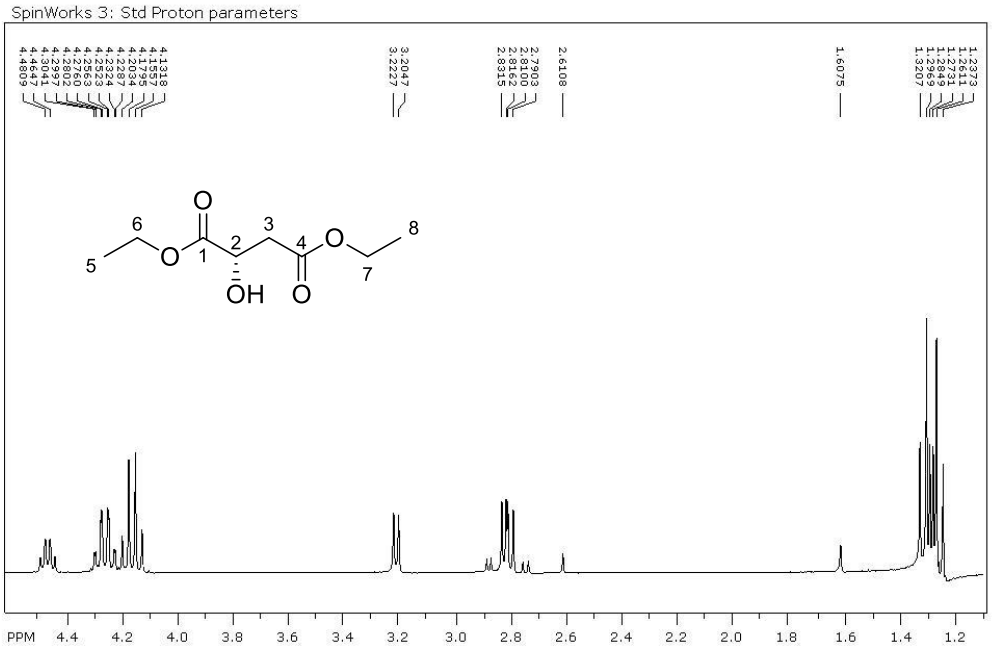


(9H-Fluoren-9-yl)methyl-3-oxo-3-(pentylamino)propyl-carbamate (3.12): Compound **3.12** was synthesized from the Fmoc protected β-alanine (**3.11**), by coupling to pentylamine using EDC as a coupling agent.[16] Thus compound **3.11** (2.19 g, 7.0 mmol, 1 eq) was mixed with EDC (1.60 g, 8.3 mmol, 1.2 eq) and HOBT (1.59 g, 12 mmol, 1.7 eq) in dry THF (30 mL). The reaction mixture was stirred under nitrogen for 16 hrs. Next pentylamine (0.88 mL, 7.6 mmol, 1.1 eq) and DIPEA (6.04 mL, 35 mmol, 5 eq) were added. The reaction mixture was stirred for 16 hours before quenching it with aqueous saturated ammonium chloride (NH_4Cl, 30 mL). The product was extracted in ethyl acetate (3 × 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The overall yield was 2.30 g, 85% of yellow solid. R_f 0.49 (MeOH/DCM, 1:19, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78-7.67 (m, 2H, H-Ar), 7.59 (d, *J* = 7.4, 2H, H-Ar). 7.43-7.27 (m, 4H, H-Ar), 5.74 (br s, 1H, NH), 5.60 (br s, 1H, NH), 4.35 (d, *J* = 7.4, 2H, H-10), 4.20 (t,

J = 6.9, 1H, H-11), 3.50 (m, 2H, H-1), 3.27-3.23 (m, 2H, H-4), 2.46-2.32 (m, 2H, H-2), 1.54-1.42 (m, 2H, H-5), 1.36-1.21 (m, 4H, H-6, H-7), 0.88 (t, *J* = 6.7, 3H).

APPENDIX

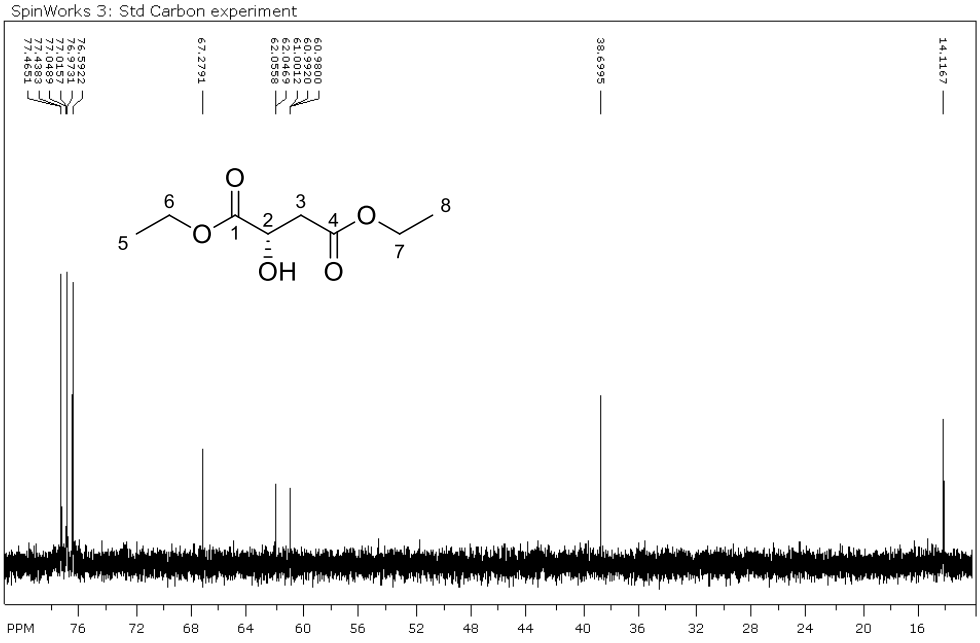
Compound 2.1 ^{1}H:



file: ...\NMR\EarlystageNMR\HD-1-5A.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 300.056790 MHz
time domain size: 28786 points
width: 4800.77 Hz = 15.9995 ppm = 0.166774 Hz/pt
number of scans: 8

freq. of 0 ppm: 300.054990 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 42.428 ppm/cm: 0.14140

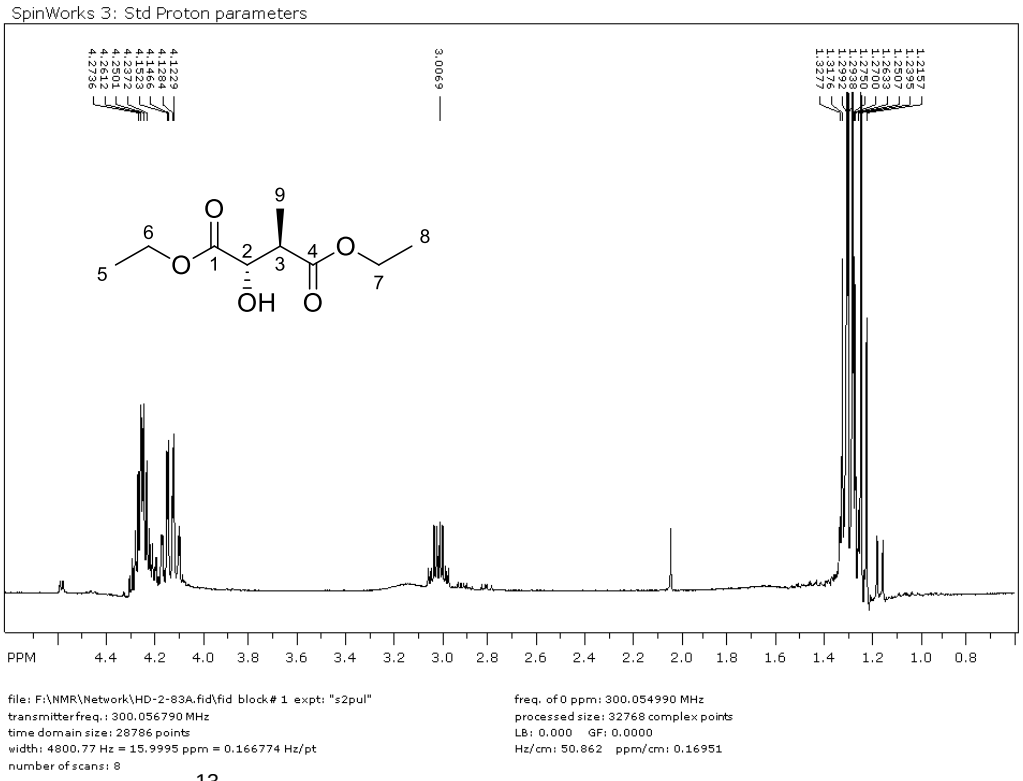
Compound 2.1 ^{13}C:



file: ...NMR\EarlystageNMR\HD-1-5Ac.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 75.456809 MHz
time domain size: 47120 points
width: 18115.94 Hz = 240.0836 ppm = 0.384464 Hz/pt
number of scans: 128

freq. of 0 ppm: 75.448887 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 209.557 ppm/cm: 2.77718

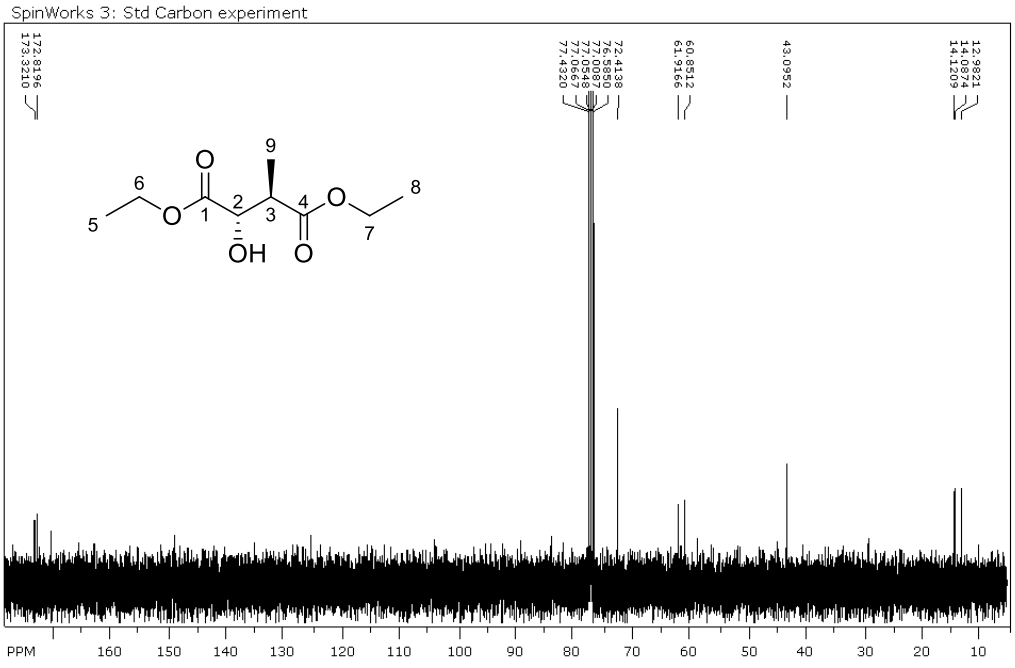
Compound 2.2 ^{1}H:



file: F:\NMR\Network\HD-2-83A.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 300.056790 MHz
time domain size: 28786 points
width: 4800.77 Hz = 15.9995 ppm = 0.166774 Hz/pt
number of scans: 8

freq. of 0 ppm: 300.054990 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 50.862 ppm/cm: 0.16951

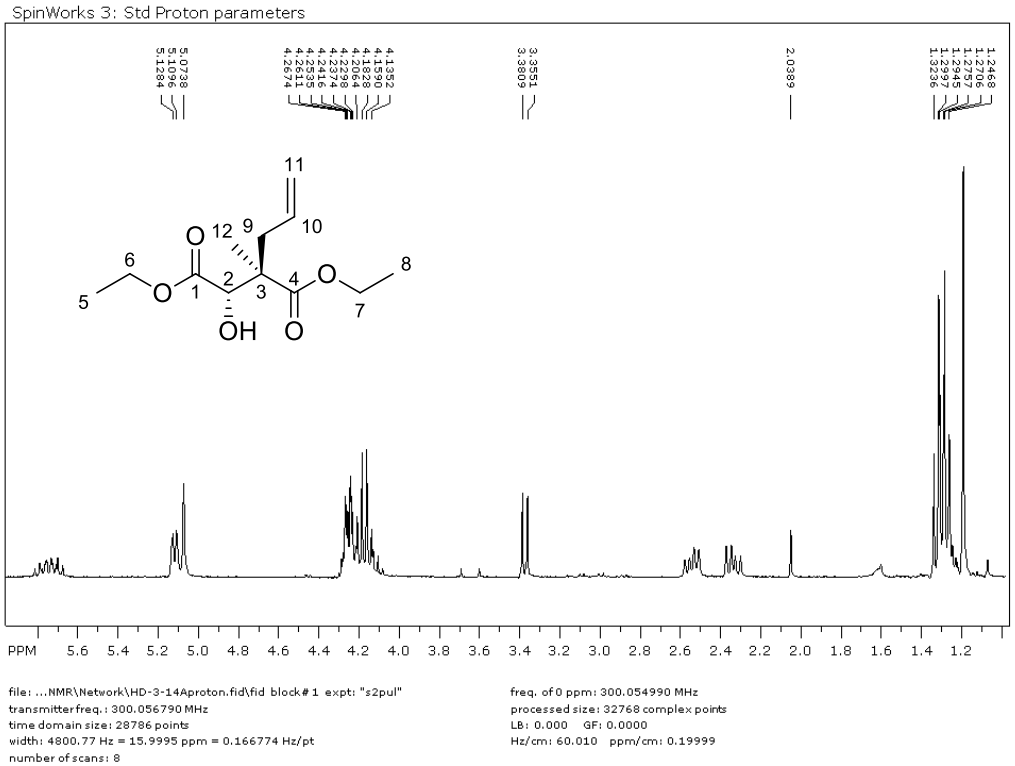
Compound 2.2 ^{13}C:



file: ...R\EarlystageNMR\HD-1-9AC13.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 75.456809 MHz
time domain size: 47120 points
width: 18115.94 Hz = 240.0836 ppm = 0.384464 Hz/pt
number of scans: 256

freq. of 0 ppm: 75.448887 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 523.404 ppm/cm: 6.93647

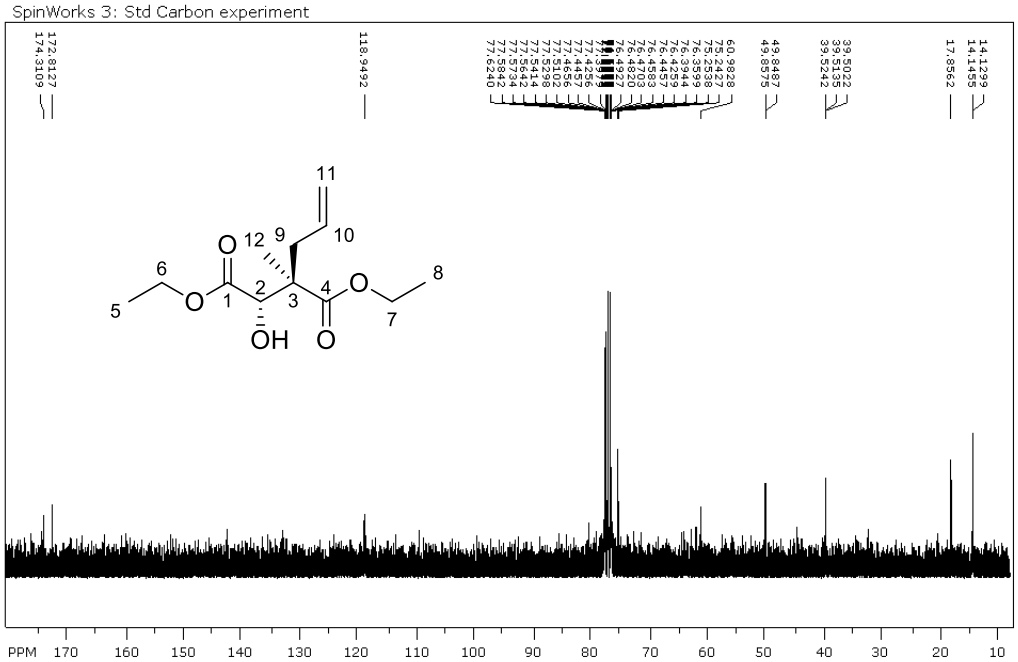
Compound 2.3 ^{1}H:



file: ...NMR\Network\HD-3-14Aproton.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 300.056790 MHz
time domain size: 28786 points
width: 4800.77 Hz = 15.9995 ppm = 0.166774 Hz/pt
number of scans: 8

freq. of 0 ppm: 300.054990 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 60.010 ppm/cm: 0.19999

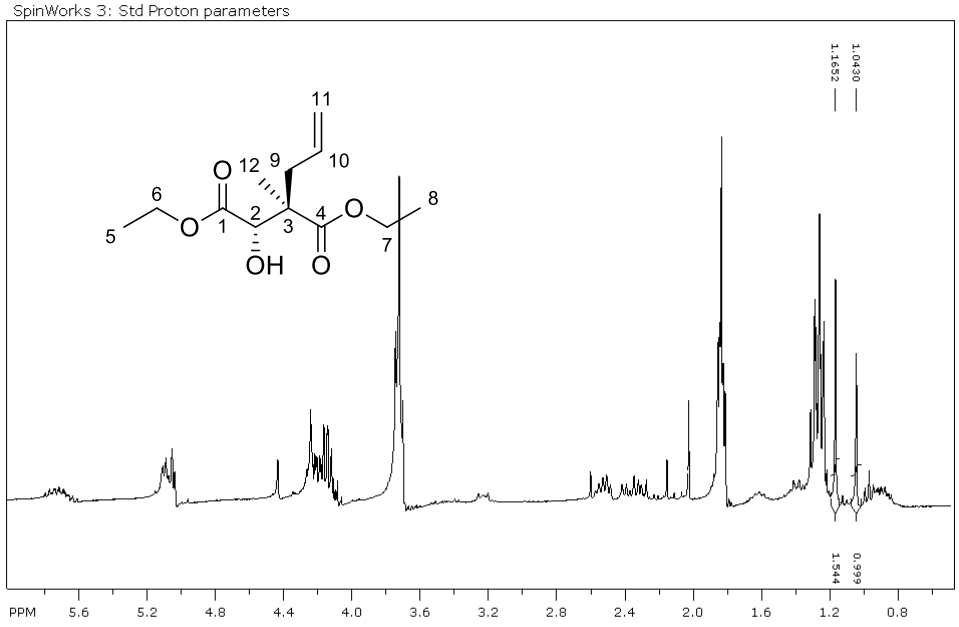
Compound 2.3 ^{13}C:



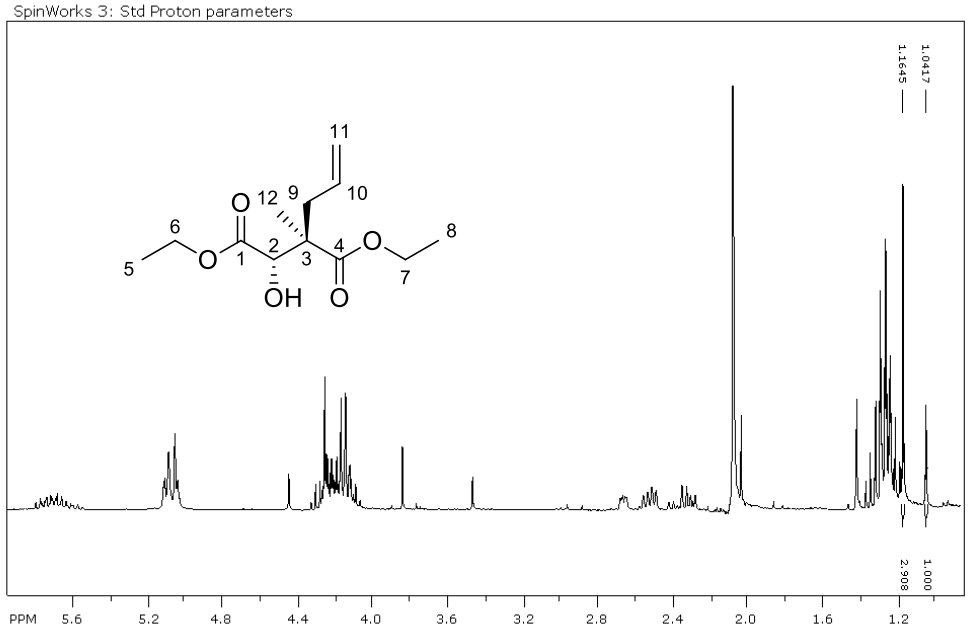
file: ...NMR\Network\HD-3-14Acarbon.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 75.456809 MHz
time domain size: 47120 points
width: 18115.94 Hz = 240.0836 ppm = 0.384464 Hz/pt
number of scans: 192

freq. of 0 ppm: 75.448887 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 522.451 ppm/cm: 6.92384

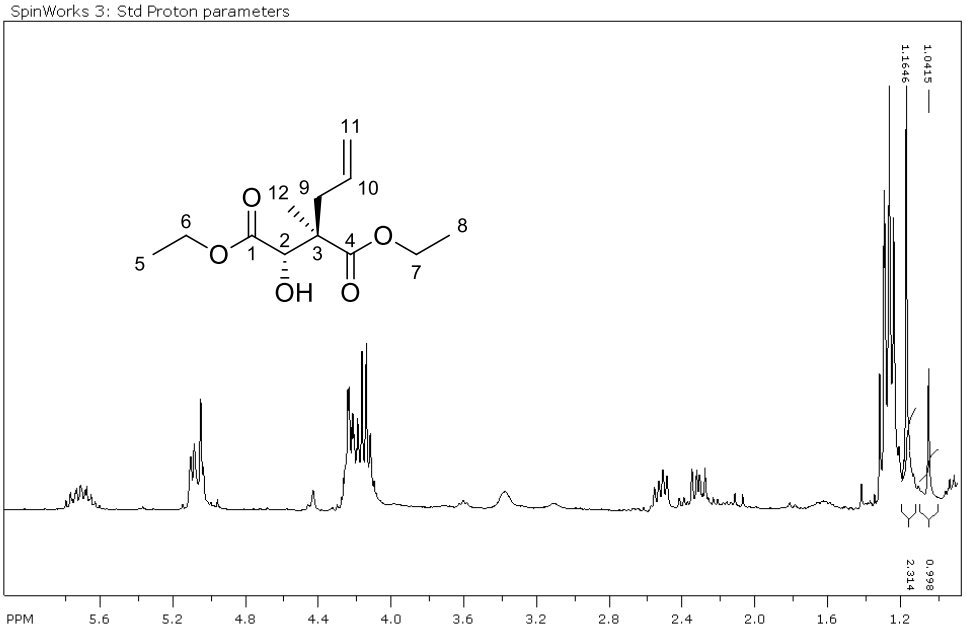
Compound 2.3 ^{1}H from reduction with DIBAL-H (crude sample used to determine *syn*/*anti* ratio):



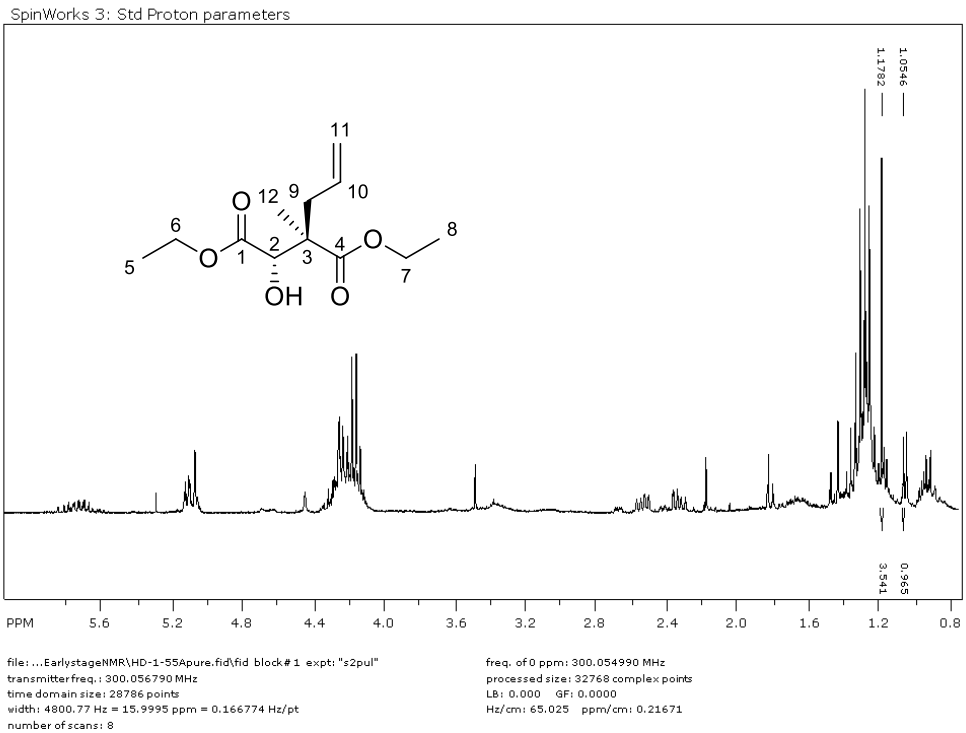
Compound 2.3 ^{1}H from reduction with $NaBH_4$ (crude sample used to determine *syn*/*anti* ratio):



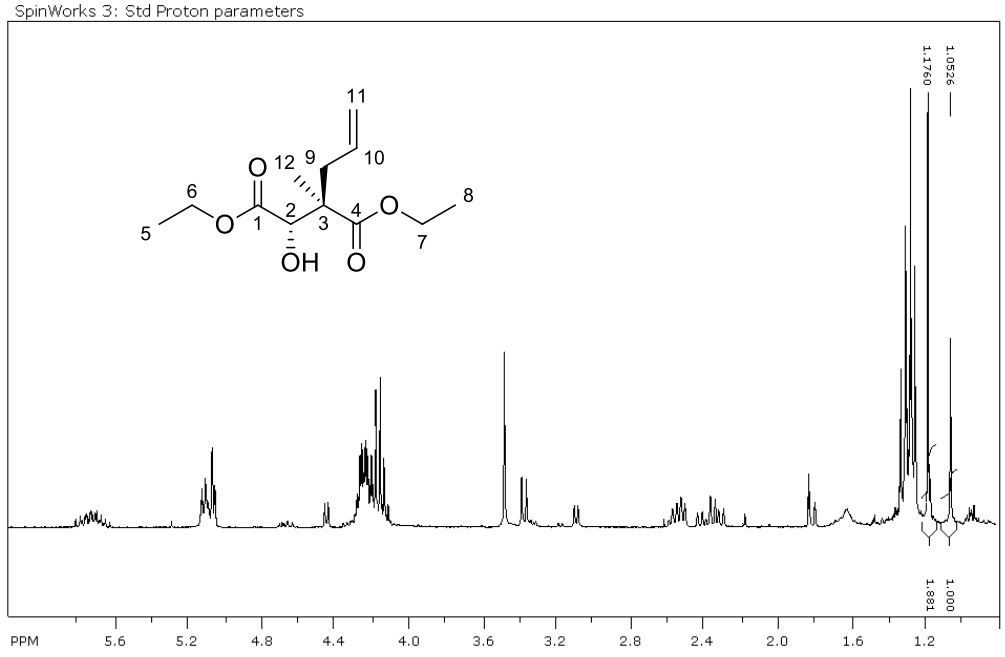
Compound 2.3 ^{1}H from reduction with $ZnBH_4$ (crude sample used to determine *syn*/*anti* ratio):



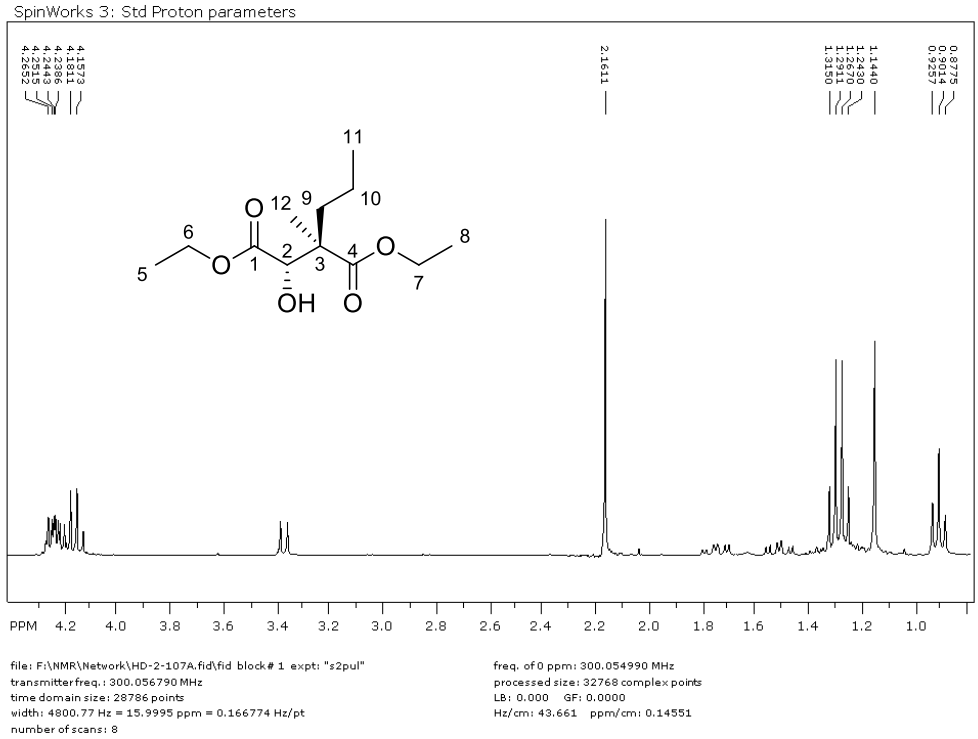
Compound 2.3 ^{1}H from borohydride reduction with (*R*)-CBS catalyst (crude sample used to determine *syn*/*anti* ratio):



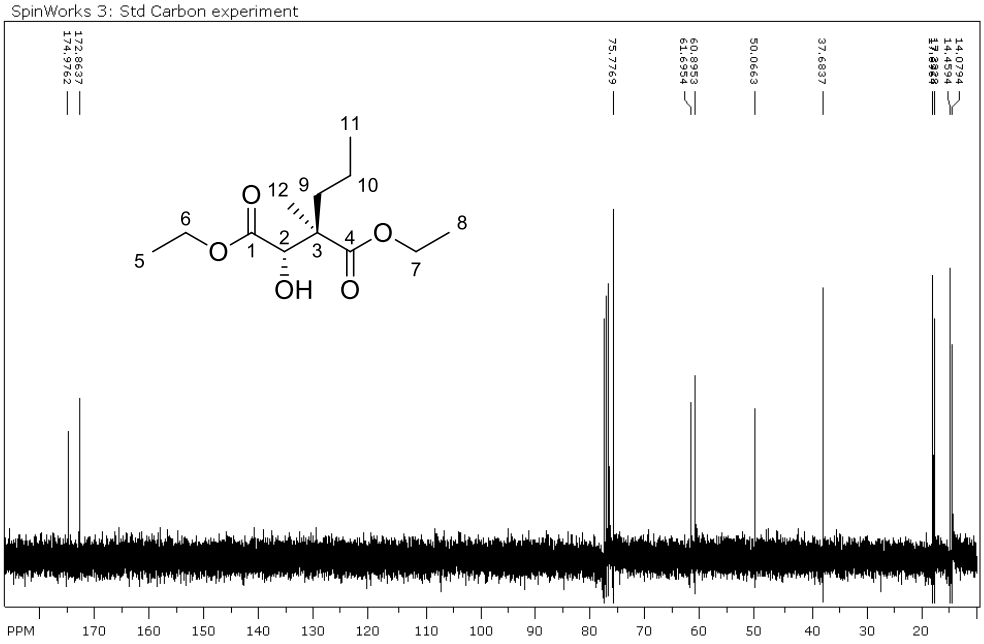
Compound 2.3 ^{1}H from borohydride reduction with (*S*)-CBS catalyst (crude sample used to determine *syn*/*anti* ratio):



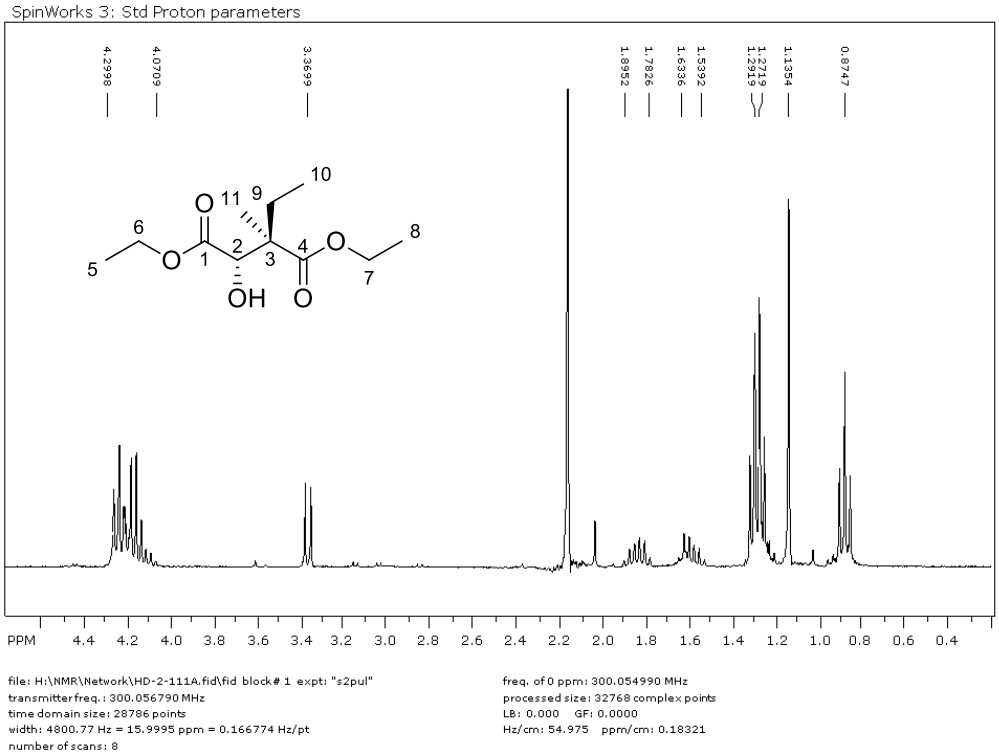
Compound 2.4 ^{1}H:



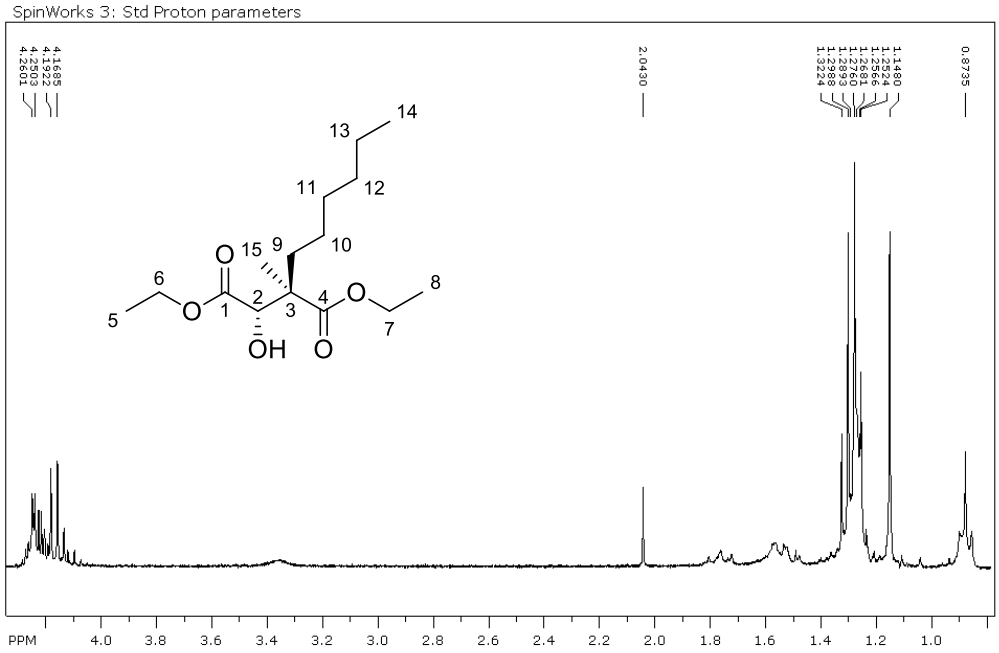
Compound 2.4 ^{13}C:



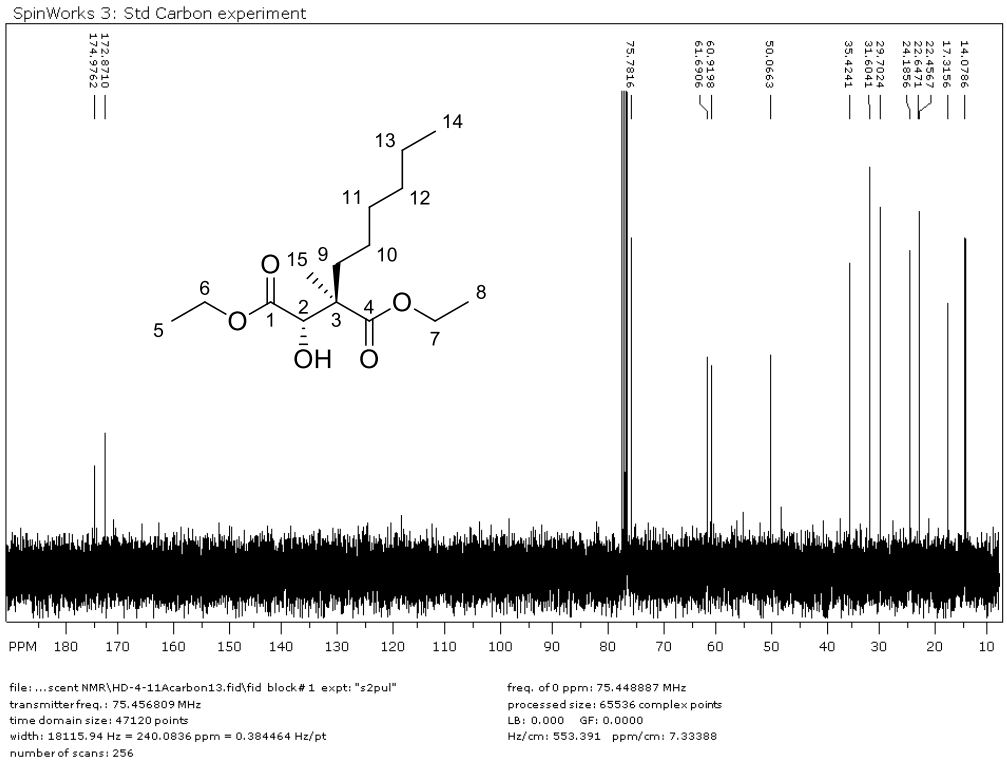
Compound 2.5 ^{1}H:



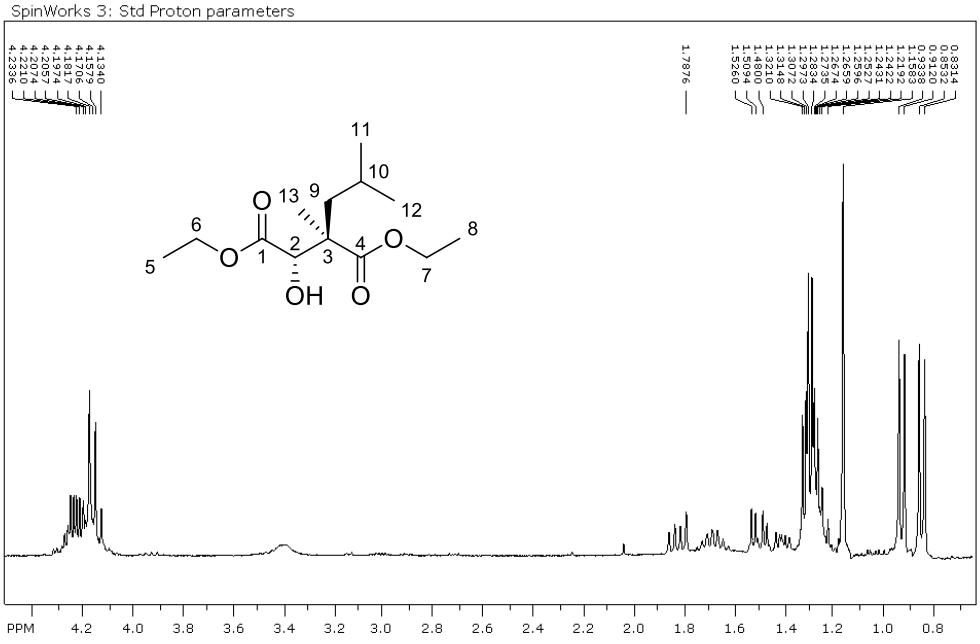
Compound 2.6 ^{1}H:



Compound 2.6 ^{13}C:



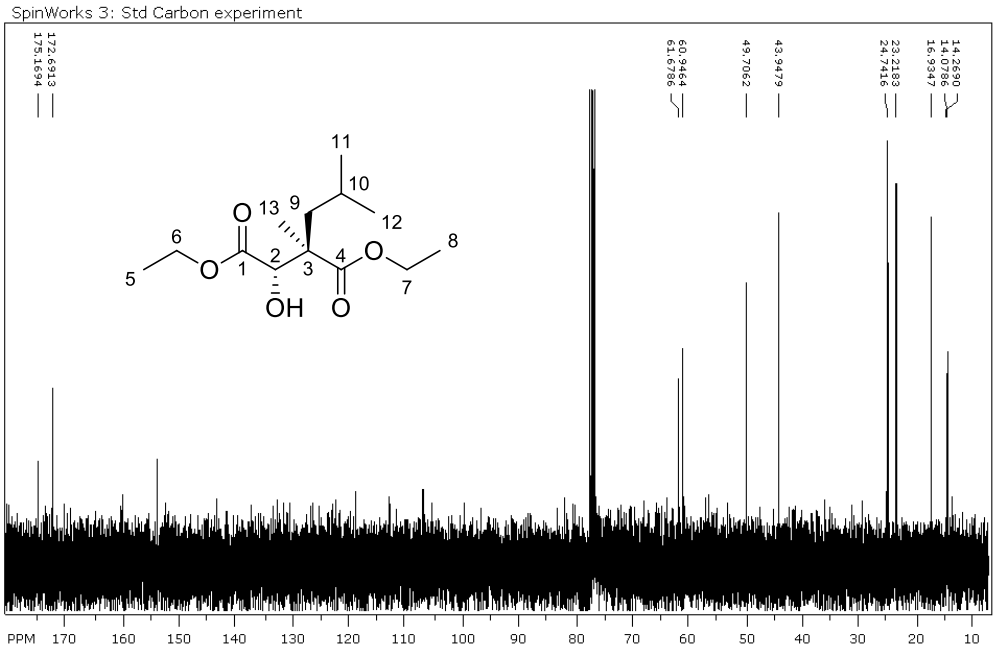
Compound 2.7 ^{1}H:



file: I:\NMR\Network\HD-1-131B.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 300.056790 MHz
time domain size: 28786 points
width: 4800.77 Hz = 15.9995 ppm = 0.166774 Hz/pt
number of scans: 8

freq. of 0 ppm: 300.054990 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 46.599 ppm/cm: 0.15530

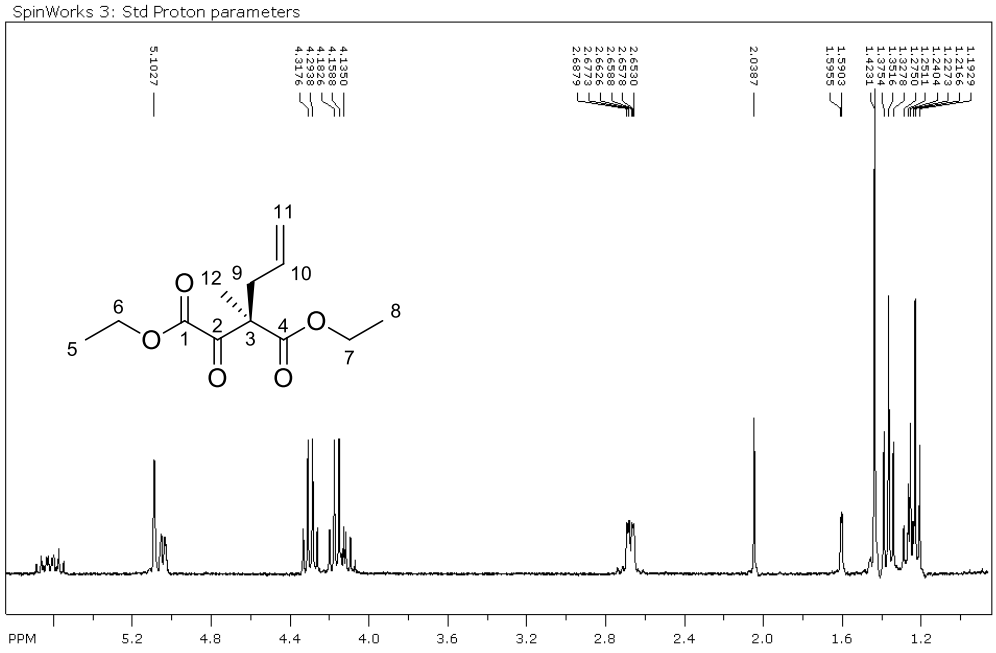
Compound 2.7 ^{13}C:



file: ...scent NMR\HD-4-12Acarbon13.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 75.456809 MHz
time domain size: 47120 points
width: 18115.94 Hz = 240.0836 ppm = 0.384464 Hz/pt
number of scans: 256

freq. of 0 ppm: 75.448887 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 525.808 ppm/cm: 6.96833

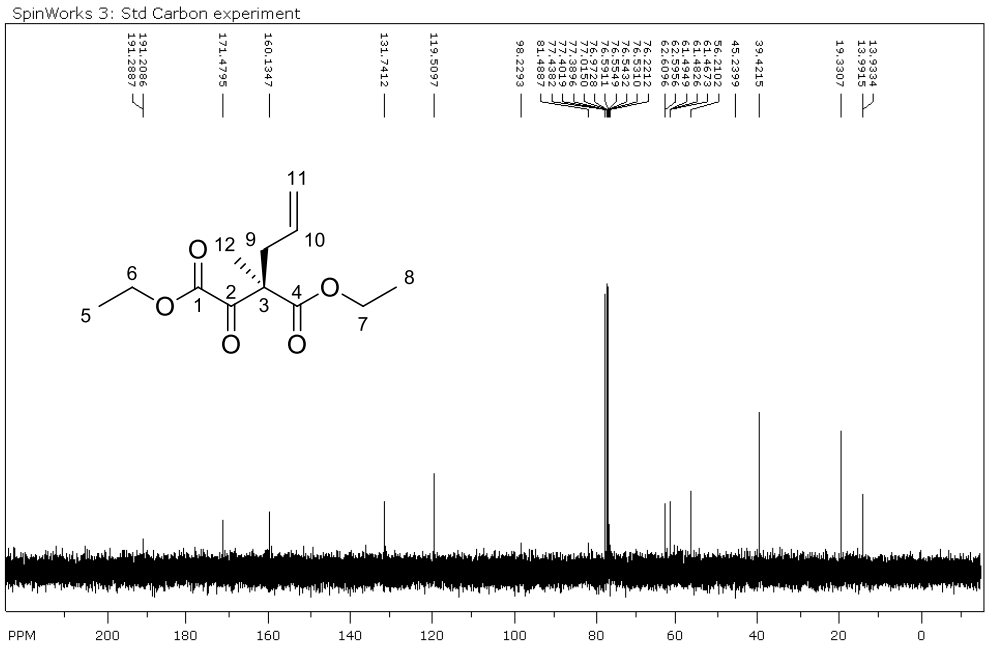
Compound 2.8 ^{1}H:



file: ...NMR\Network\HD-3-67Aproton.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 300.056790 MHz
time domain size: 28786 points
width: 4800.77 Hz = 15.9995 ppm = 0.166774 Hz/pt
number of scans: 8

freq. of 0 ppm: 300.054990 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 60.204 ppm/cm: 0.20064

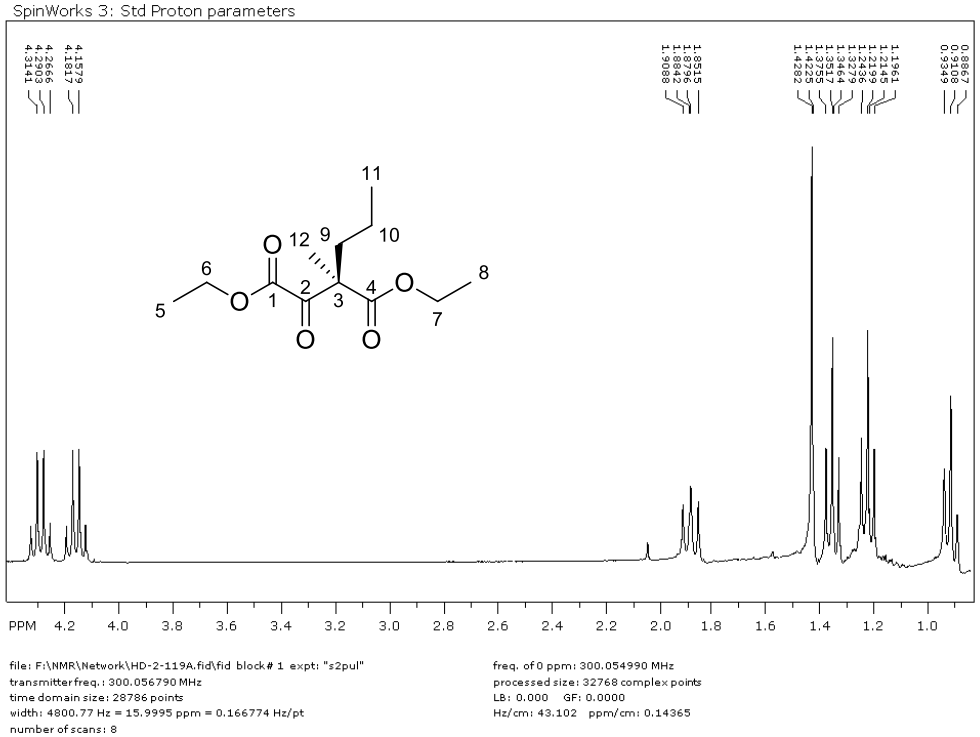
Compound 2.8 ^{13}C:



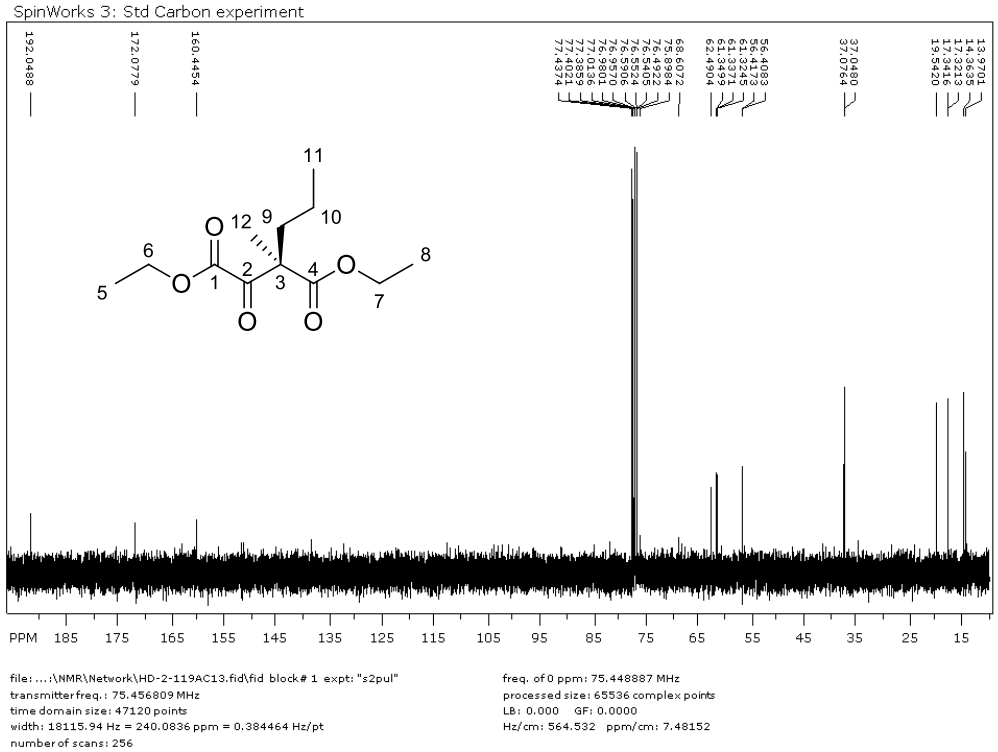
file: F:\NMR\Network\HD-3-67AC13.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 75.456809 MHz
time domain size: 47120 points
width: 18115.94 Hz = 240.0836 ppm = 0.384464 Hz/pt
number of scans: 256

freq. of 0 ppm: 75.448887 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 724.638 ppm/cm: 9.60334

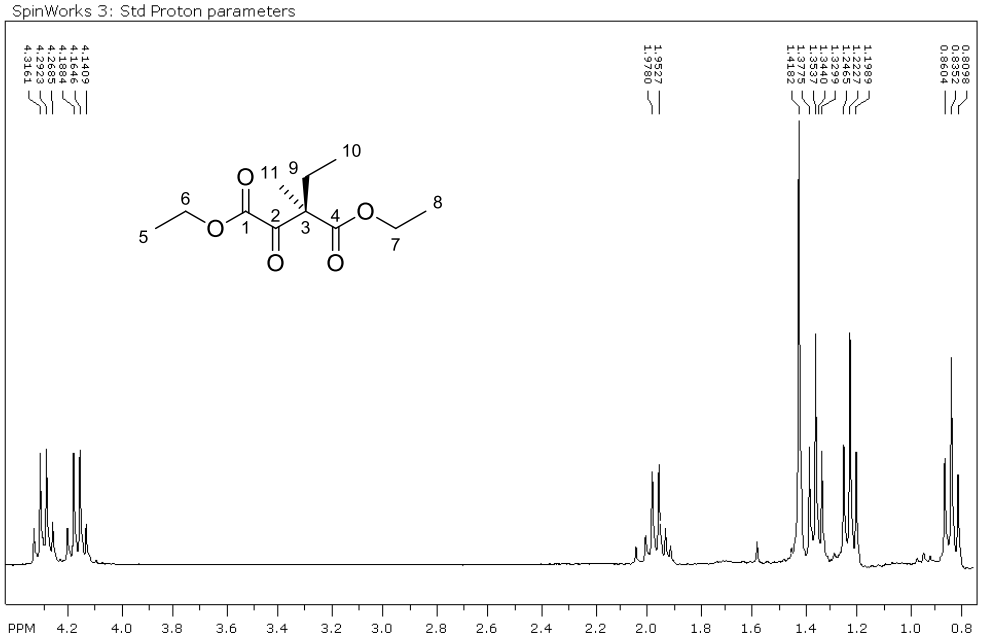
Compound 2.9 ^{1}H:



Compound 2.9 ^{13}C:



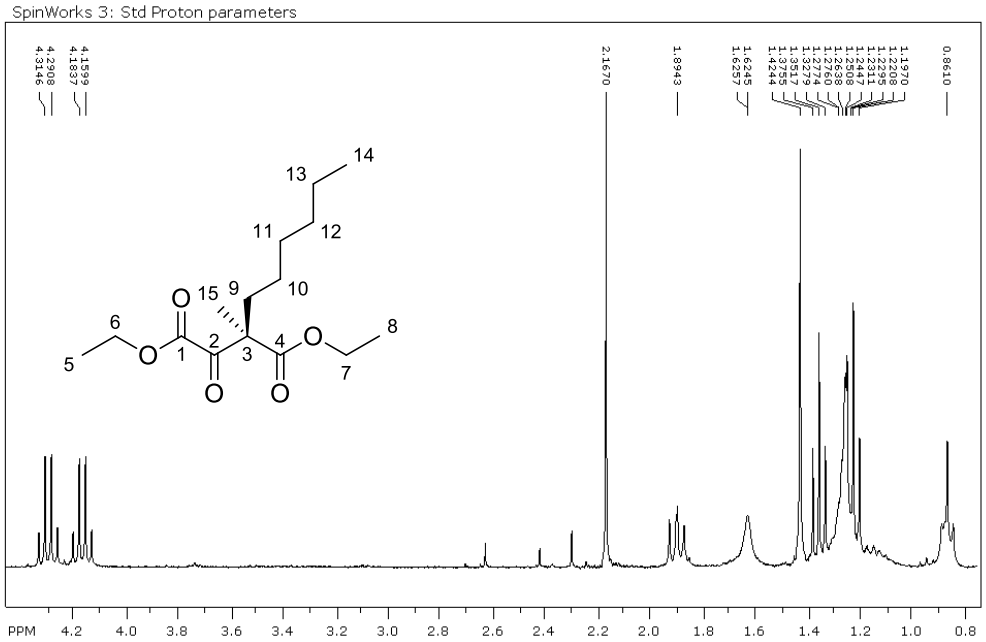
Compound 2.10 ^{1}H:



file: F:\NMR\Network\HD-2-121A.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 300.056790 MHz
time domain size: 28786 points
width: 4800.77 Hz = 15.9995 ppm = 0.166774 Hz/pt
number of scans: 8

freq. of 0 ppm: 300.054990 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 44.378 ppm/cm: 0.14790

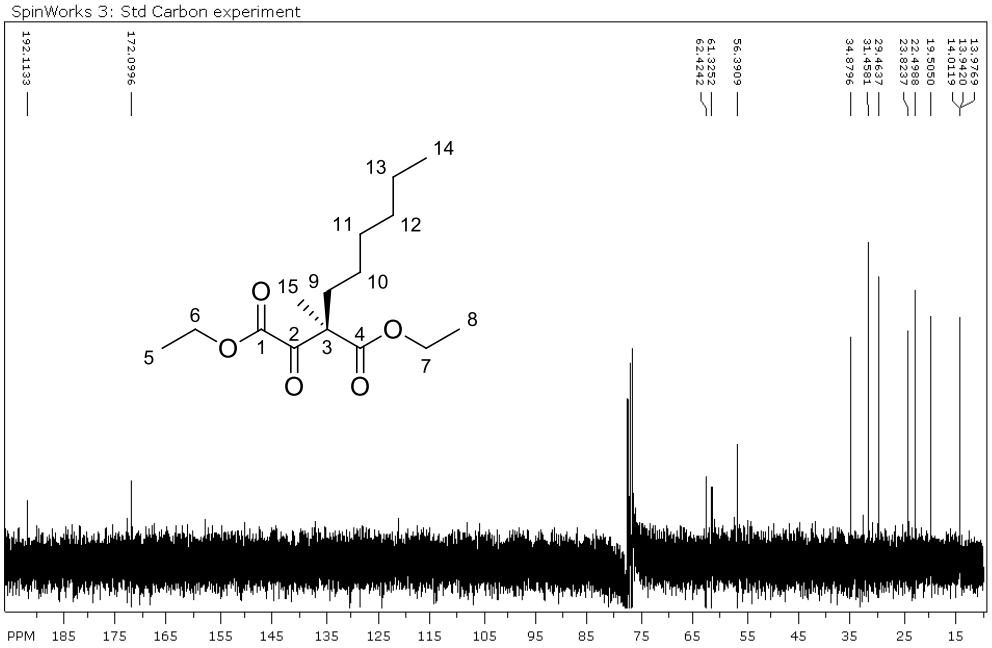
Compound 2.11 ^{1}H:



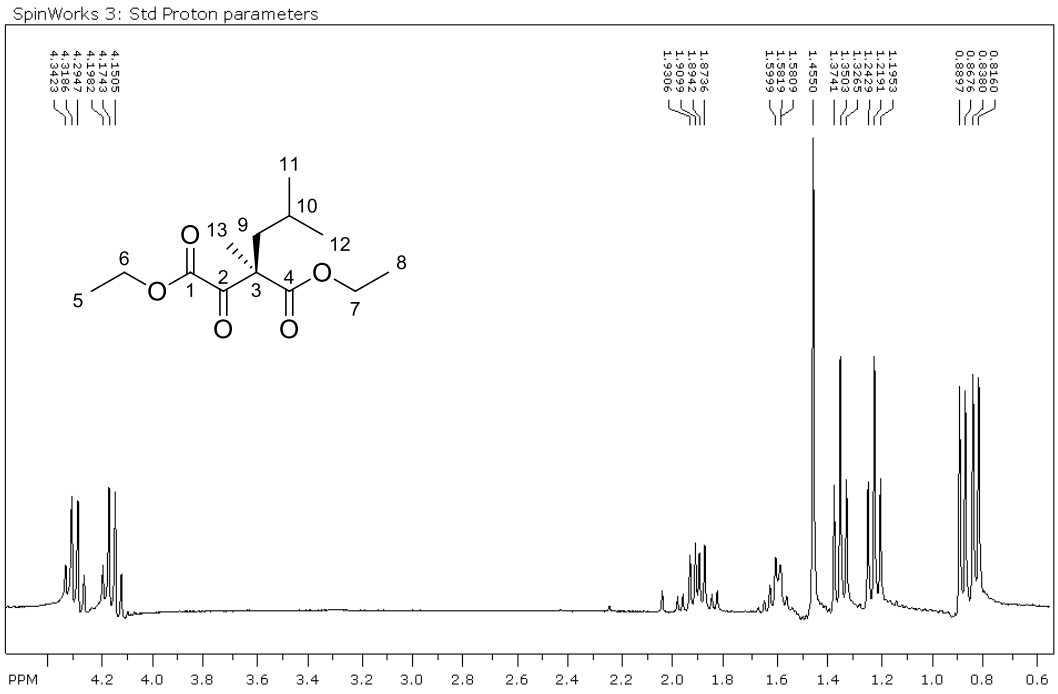
file: F:\NMR\Network\HD-1-133B.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 300.056790 MHz
time domain size: 28786 points
width: 4800.77 Hz = 15.9995 ppm = 0.166774 Hz/pt
number of scans: 8

freq. of 0 ppm: 300.054990 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 44.634 ppm/cm: 0.14875

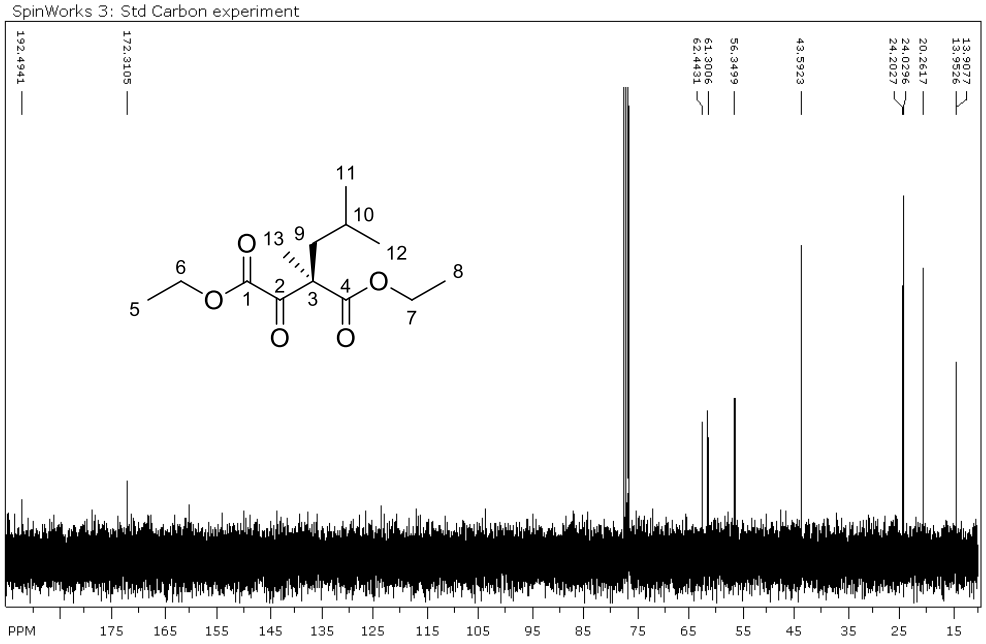
Compound 2.11 ^{13}C:



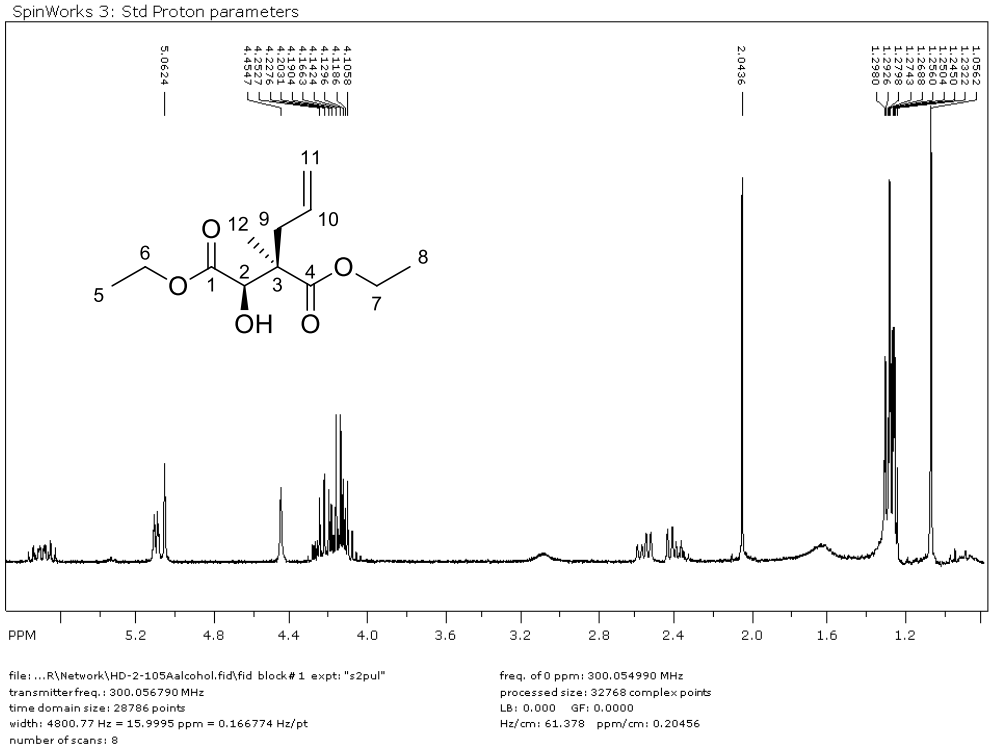
Compound 2.12 ^{1}H:



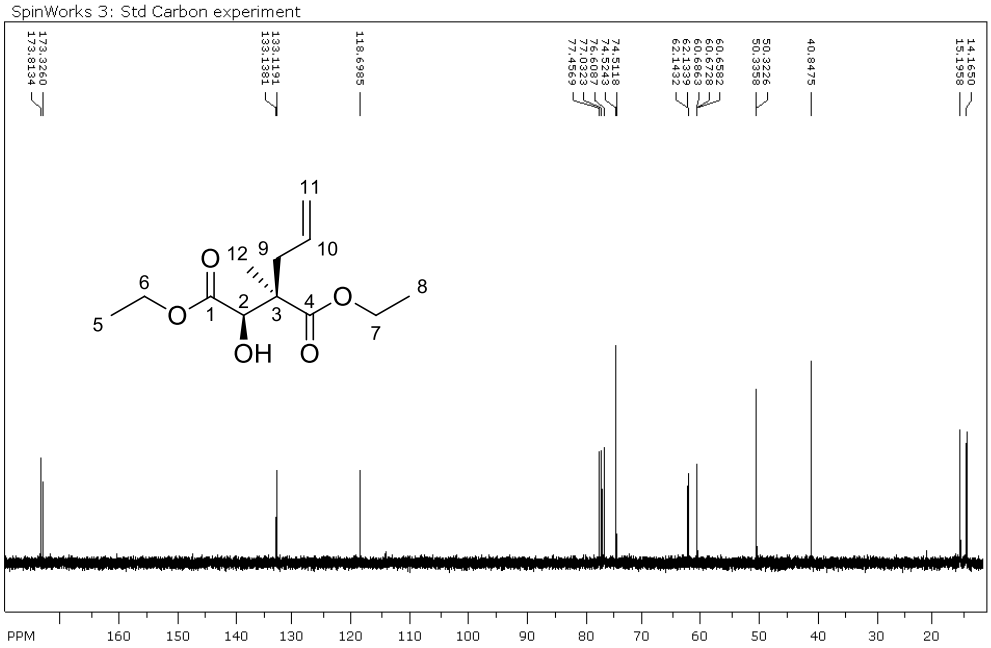
Compound 2.12 ^{13}C:



Compound 2.13 ^{1}H:



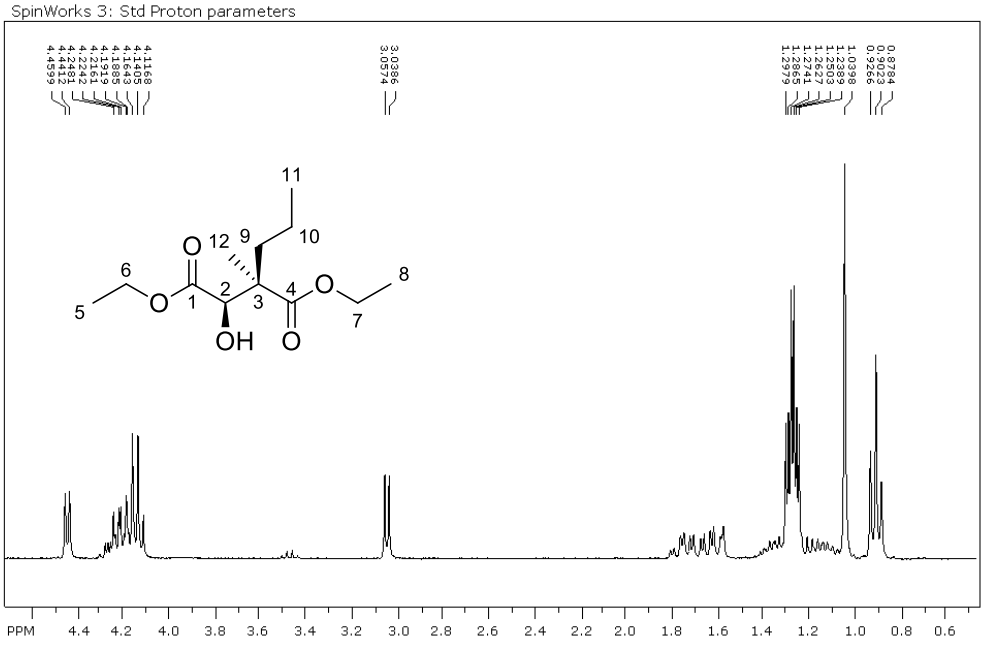
Compound 2.13 ^{13}C:



file: ...:\NMR\Network\HD-2-105Ac13.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 75.456809 MHz
time domain size: 47120 points
width: 18115.94 Hz = 240.0836 ppm = 0.384464 Hz/pt
number of scans: 256

freq. of 0 ppm: 75.448887 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 509.143 ppm/cm: 6.74747

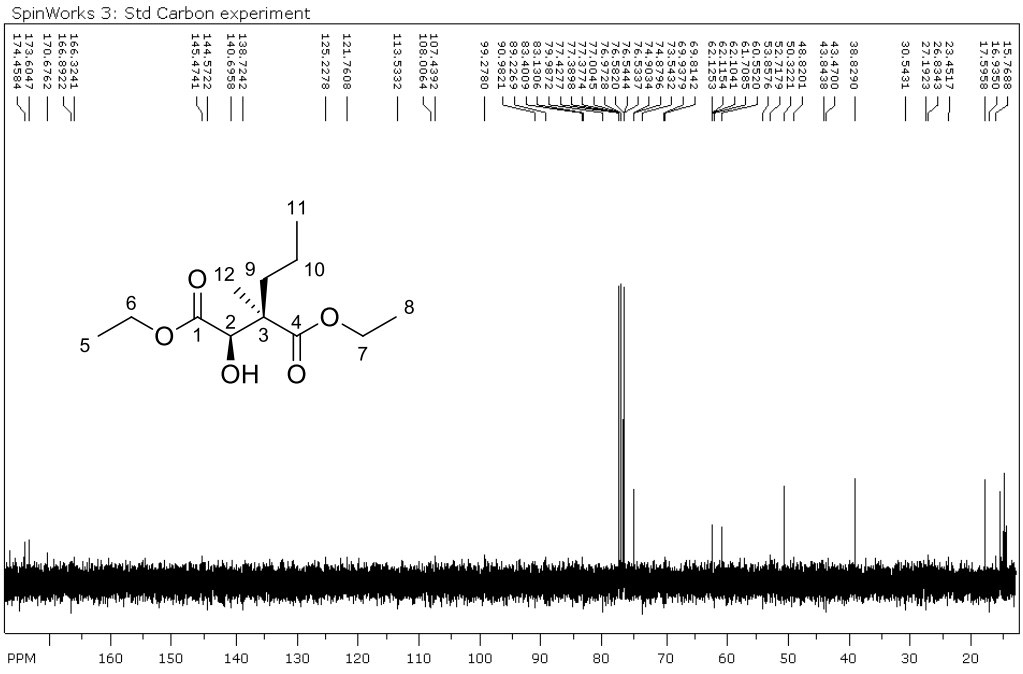
Compound 2.14 ^{1}H:



file: ...etwork\HD-2-127Aprotonpure.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 300.056790 MHz
time domain size: 28786 points
width: 4800.77 Hz = 15.9995 ppm = 0.166774 Hz/pt
number of scans: 8

freq. of 0 ppm: 300.054990 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 51.168 ppm/cm: 0.17053

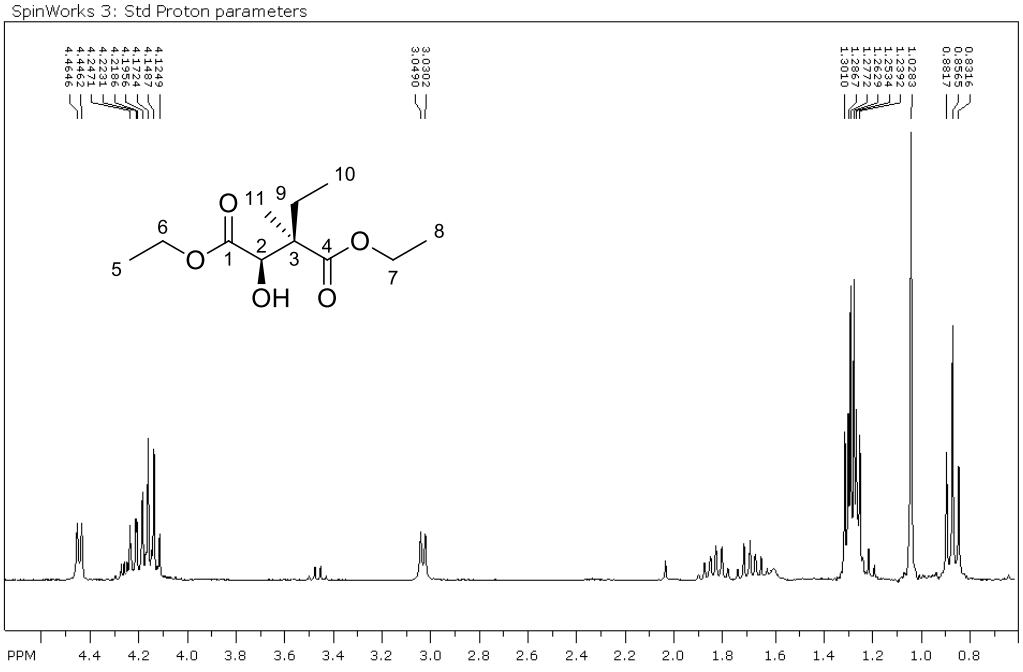
Compound 2.14 ^{13}C:



file: ...:\NMR\Network\HD-2-127AC13.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 75.456809 MHz
time domain size: 47120 points
width: 18115.94 Hz = 240.0836 ppm = 0.384464 Hz/pt
number of scans: 192

freq. of 0 ppm: 75.448887 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 498.224 ppm/cm: 6.60277

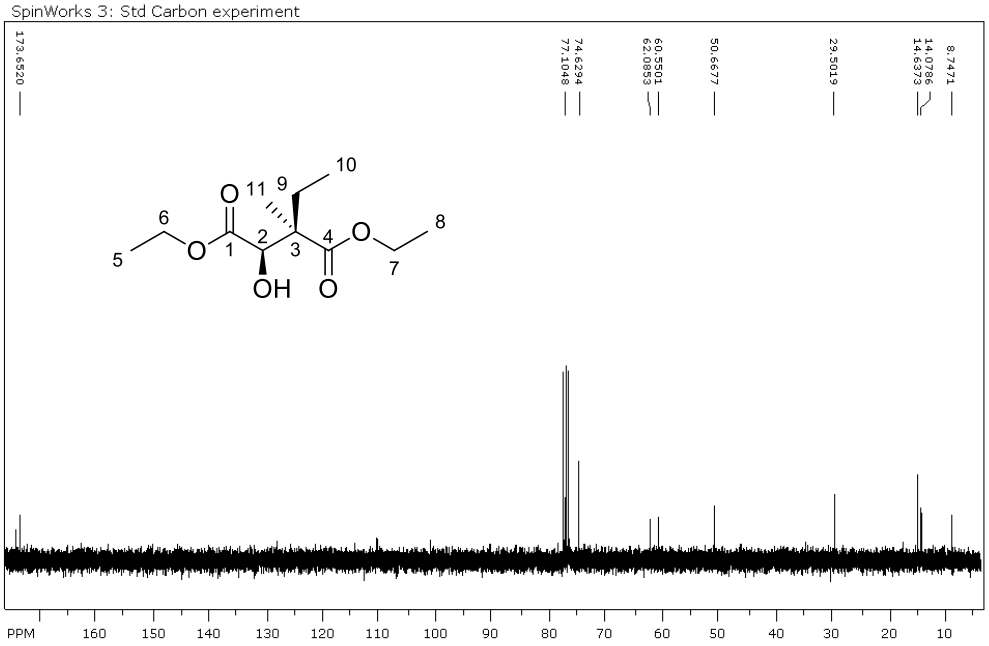
Compound 2.15 ^{1}H:



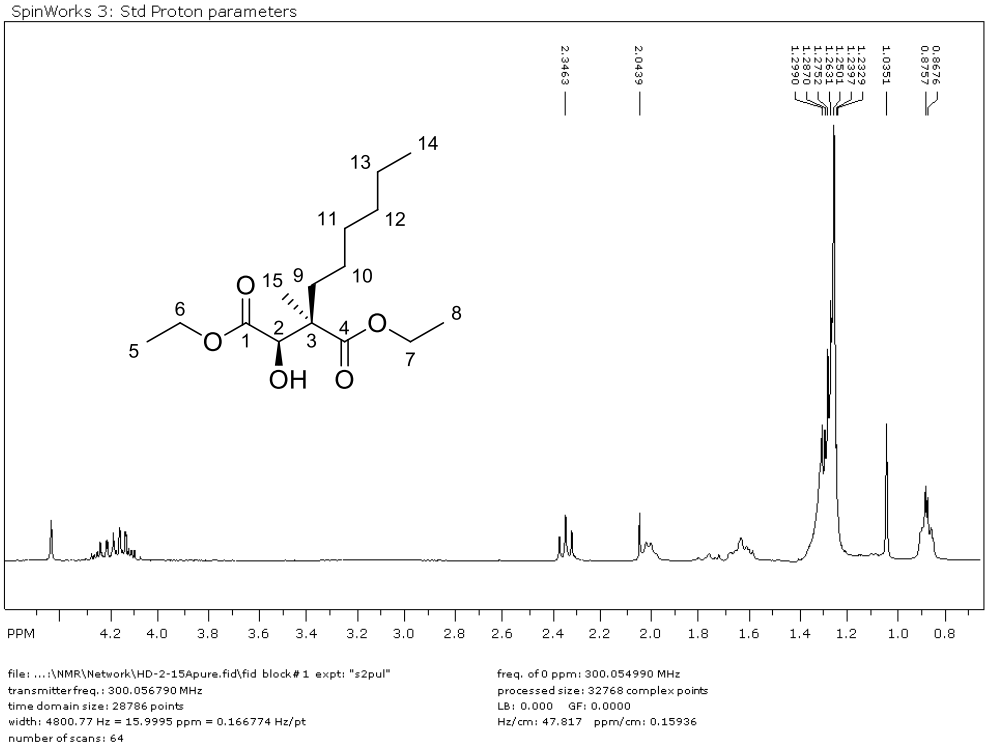
file: ...NMR\Network\HD-2-129AHpure.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 300.056790 MHz
time domain size: 28786 points
width: 4800.77 Hz = 15.9995 ppm = 0.166774 Hz/pt
number of scans: 8

freq. of 0 ppm: 300.054990 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 49.949 ppm/cm: 0.16647

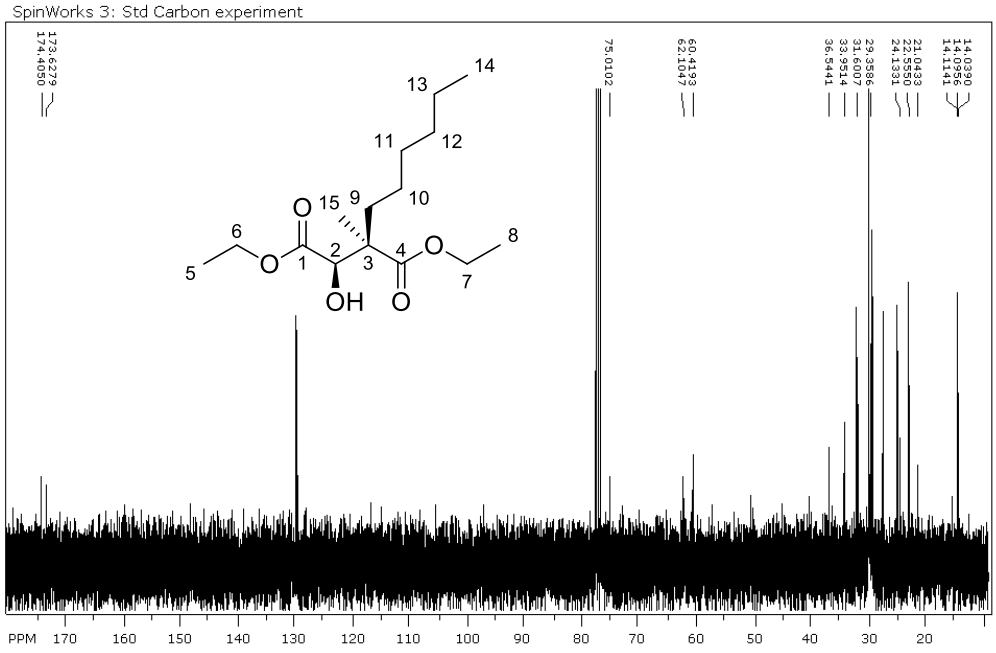
Compound 2.15 ^{13}C:



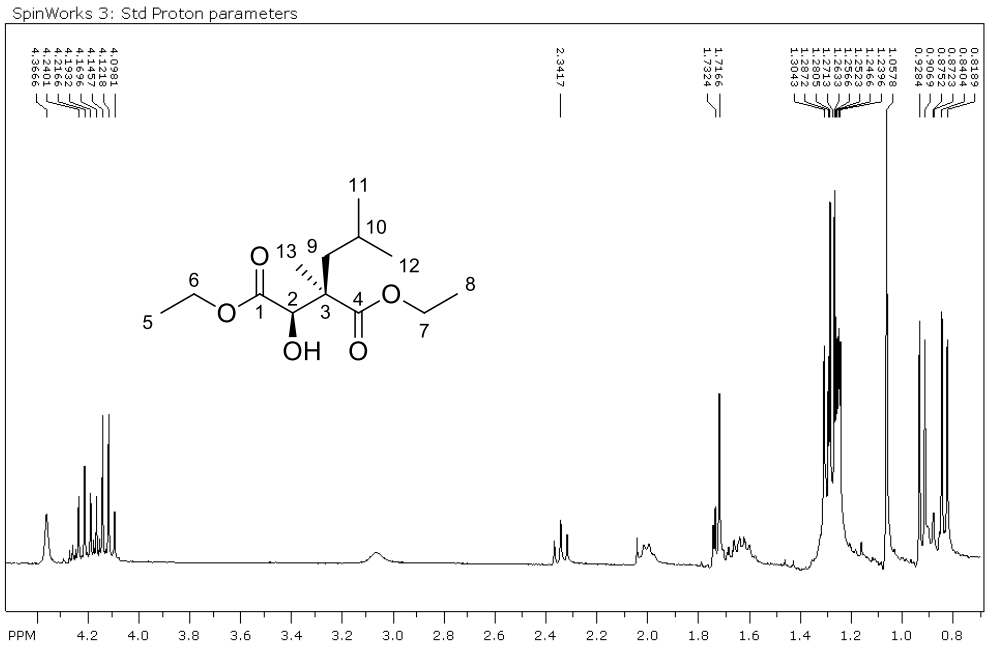
Compound 2.16 ^{1}H:



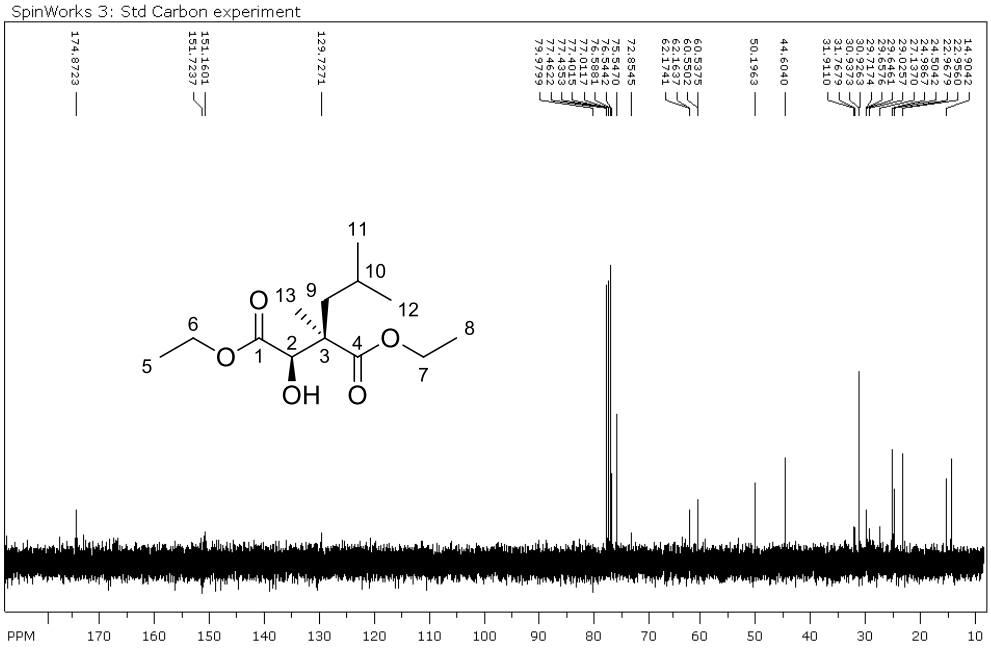
Compound 2.16 ^{13}C:



Compound 2.17 ^{1}H:



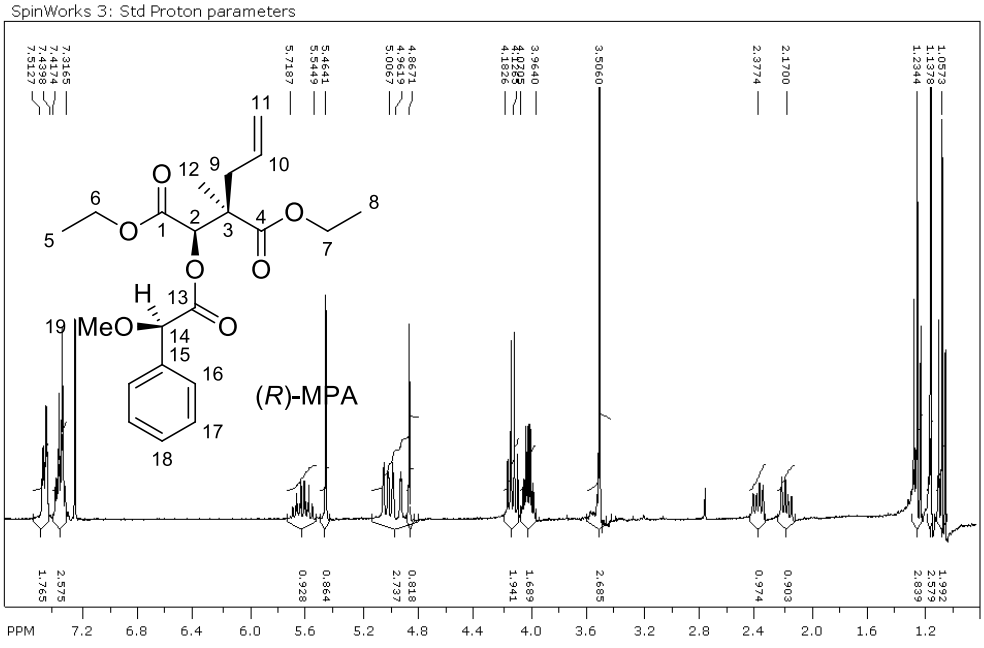
Compound 2.17 ^{13}C:



file: ...NMR\Network\HD-2-98pureC13.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 75.456809 MHz
time domain size: 47120 points
width: 18115.94 Hz = 240.0836 ppm = 0.384464 Hz/pt
number of scans: 256

freq. of 0 ppm: 75.448887 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 542.473 ppm/cm: 7.18918

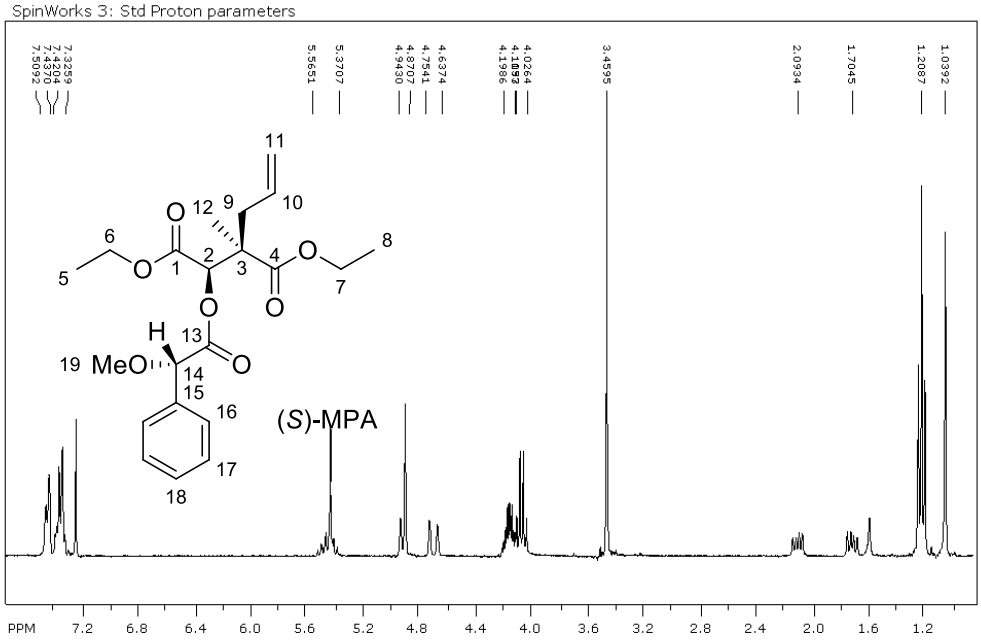
Compound 2.18 ^{1}H:



file: ...pbox\NMR\network\HD-2-117A.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 300.056790 MHz
time domain size: 28786 points
width: 4800.77 Hz = 15.9995 ppm = 0.166774 Hz/pt
number of scans: 8

freq. of 0 ppm: 300.054990 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 83.378 ppm/cm: 0.27787

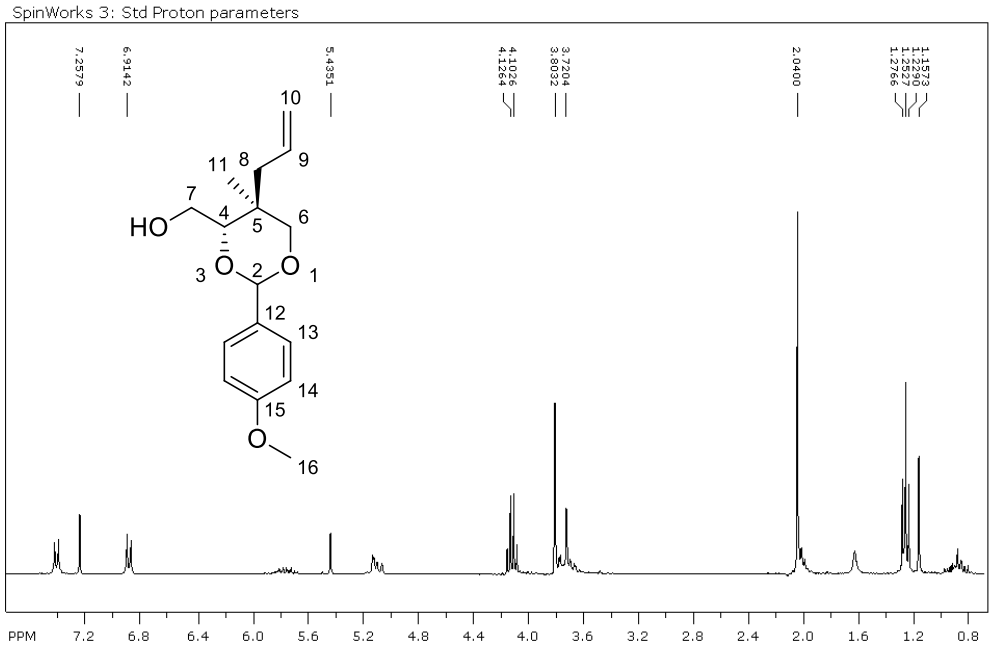
Compound 2.19 ^{1}H:



file: ...pbox\NMR\network\HD-2-117B.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 300.056790 MHz
time domain size: 28786 points
width: 4800.77 Hz = 15.9995 ppm = 0.166774 Hz/pt
number of scans: 8

freq. of 0 ppm: 300.054990 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 83.069 ppm/cm: 0.27685

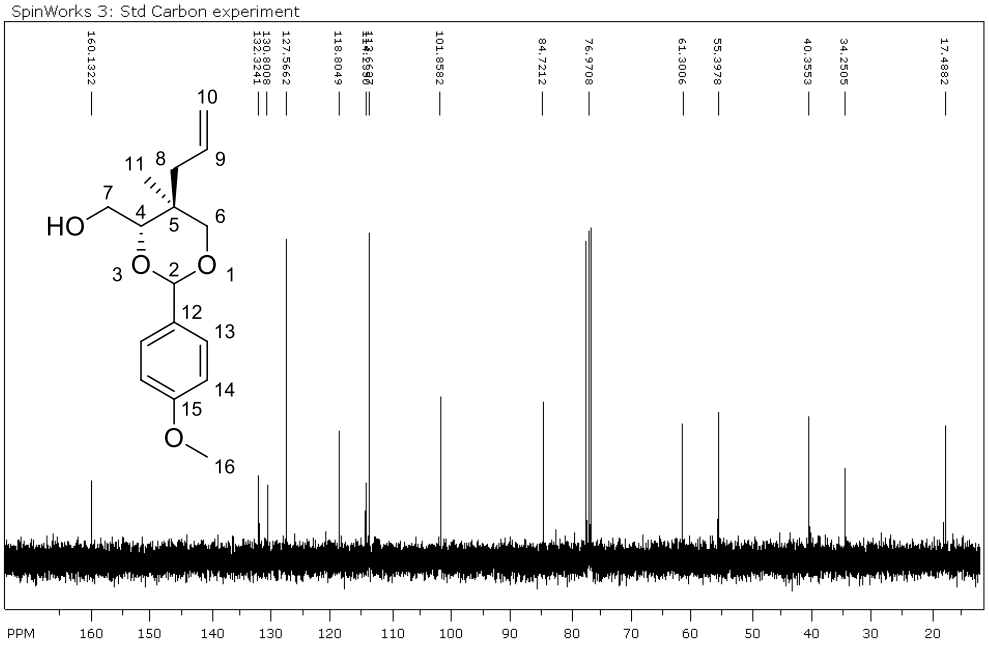
Compound 3.1 ^{1}H:



file: F:\NMR\Network\HD-3-73A.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 300.056790 MHz
time domain size: 28786 points
width: 4800.77 Hz = 15.9995 ppm = 0.166774 Hz/pt
number of scans: 8

freq. of 0 ppm: 300.054990 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 85.376 ppm/cm: 0.28453

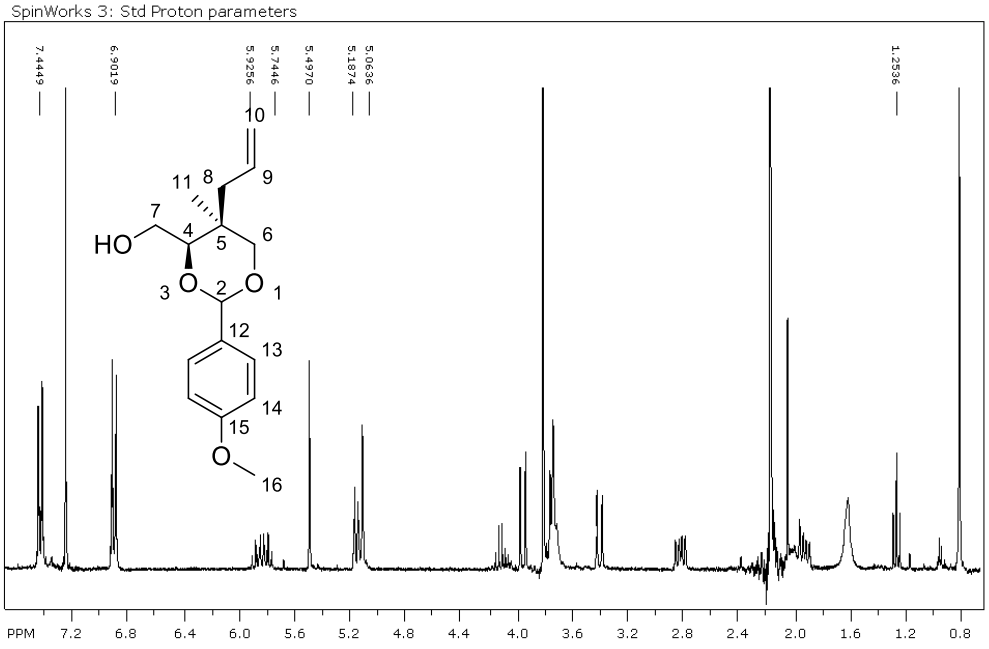
Compound 3.1 ^{13}C:



file: ...st rescent NMR\HD-4-25AC13.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 75.456809 MHz
time domain size: 47120 points
width: 18115.94 Hz = 240.0836 ppm = 0.384464 Hz/pt
number of scans: 128

freq. of 0 ppm: 75.448887 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 491.903 ppm/cm: 6.51900

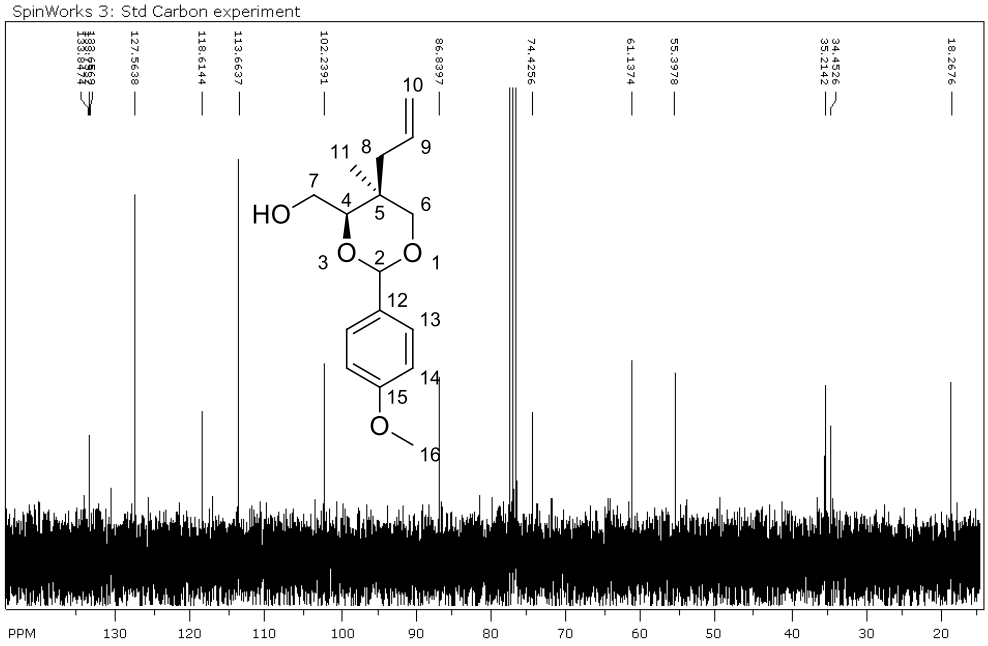
Compound 3.2 ^{1}H:



file: ...:\NMR\rescentNMR\HD-1-146A.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 300.056790 MHz
time domain size: 28786 points
width: 4800.77 Hz = 15.9995 ppm = 0.166774 Hz/pt
number of scans: 8

freq. of 0 ppm: 300.054990 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 84.597 ppm/cm: 0.28194

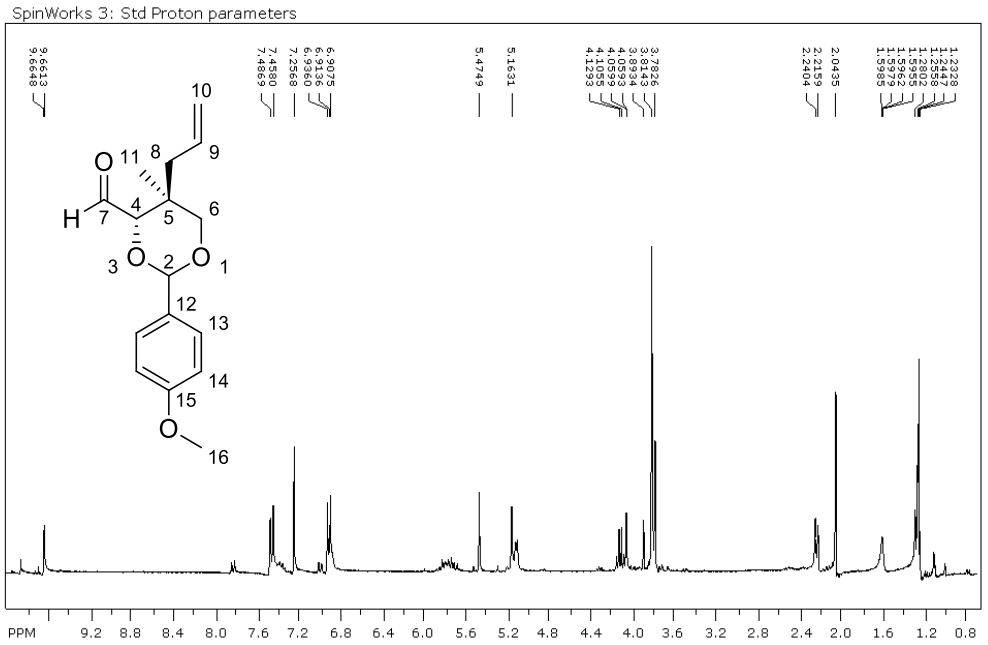
Compound 3.2 ^{13}C:



file: ...escent NMR\HD-4-29ApureC13.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 75.456809 MHz
time domain size: 47120 points
width: 18115.94 Hz = 240.0836 ppm = 0.384464 Hz/pt
number of scans: 192

freq. of 0 ppm: 75.448887 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 393.637 ppm/cm: 5.21673

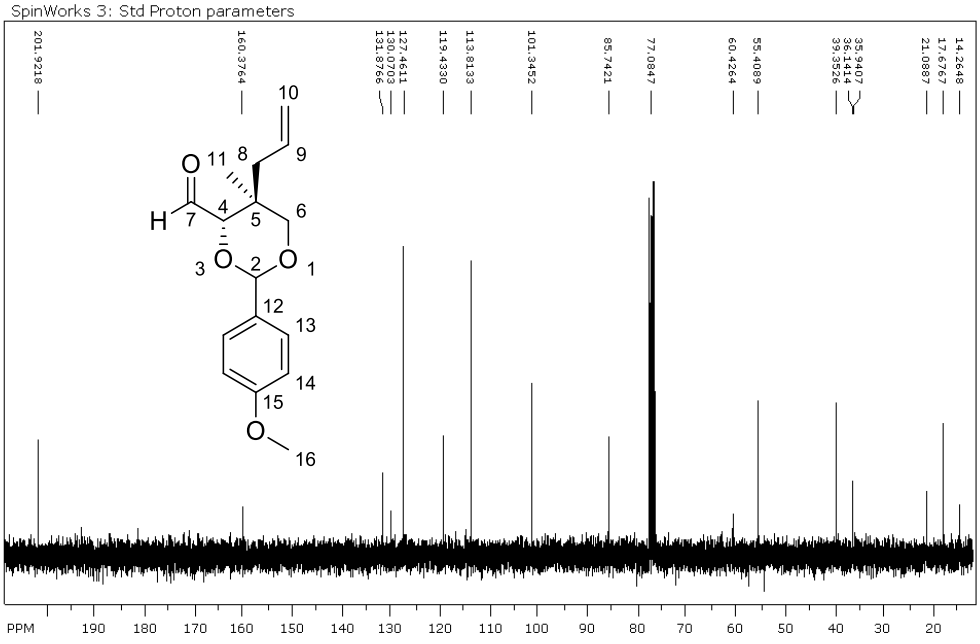
Compound 3.3 ^{1}H:



file: F:\NMR\Network\HD-3-74A.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 300.056790 MHz
time domain size: 28786 points
width: 4800.77 Hz = 15.9995 ppm = 0.166774 Hz/pt
number of scans: 8

freq. of 0 ppm: 300.054990 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 112.234 ppm/cm: 0.37404

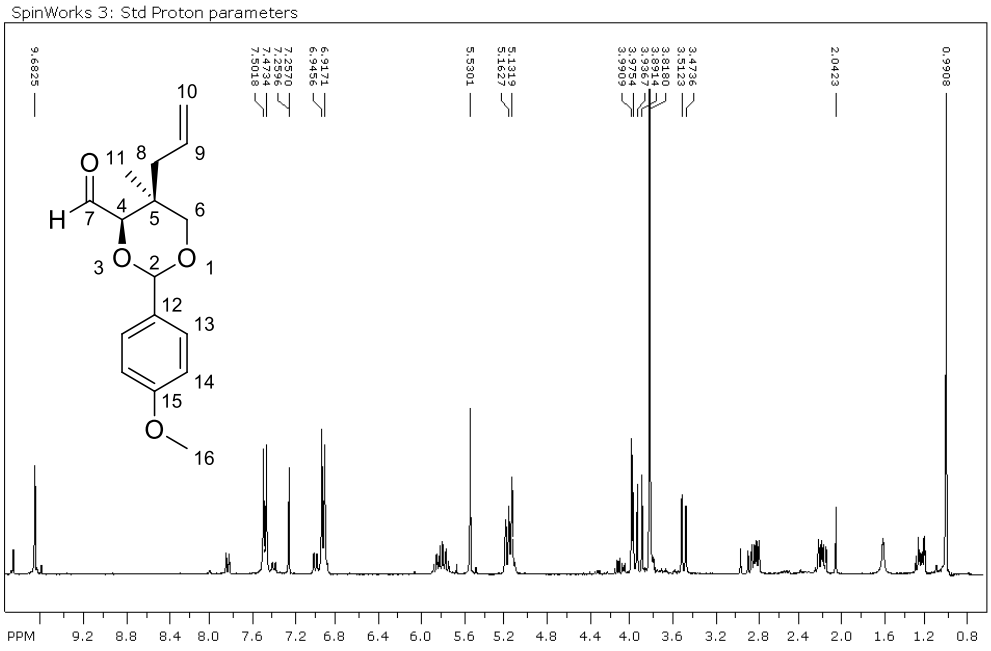
Compound 3.3 ^{13}C:



file: ...ers\Hamed\Dropbox\NMR\HD-4-72A\fid block# 1 expt: "s2pul"
transmitter freq.: 75.457187 MHz
time domain size: 32768 points
width: 18867.92 Hz = 250.0481 ppm = 0.575803 Hz/pt
number of scans: 256

freq. of 0 ppm: 75.448887 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 594.809 ppm/cm: 7.88274

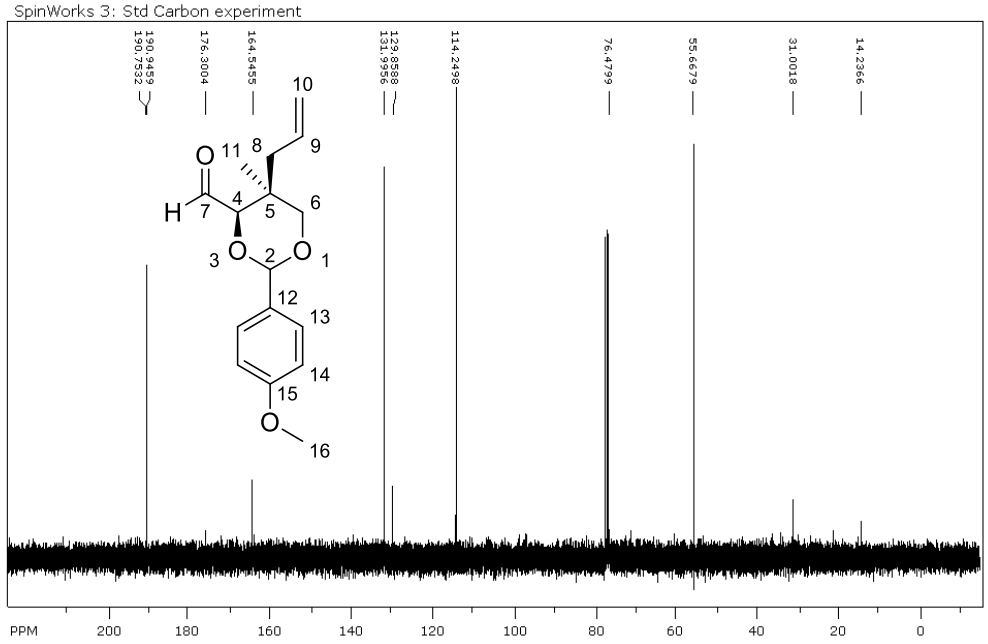
Compound 3.4 ^{1}H:



file: F:\NMR\Network\HD-1-140A.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 300.056790 MHz
time domain size: 28786 points
width: 4800.77 Hz = 15.9995 ppm = 0.166774 Hz/pt
number of scans: 8

freq. of 0 ppm: 300.054990 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 111.975 ppm/cm: 0.37318

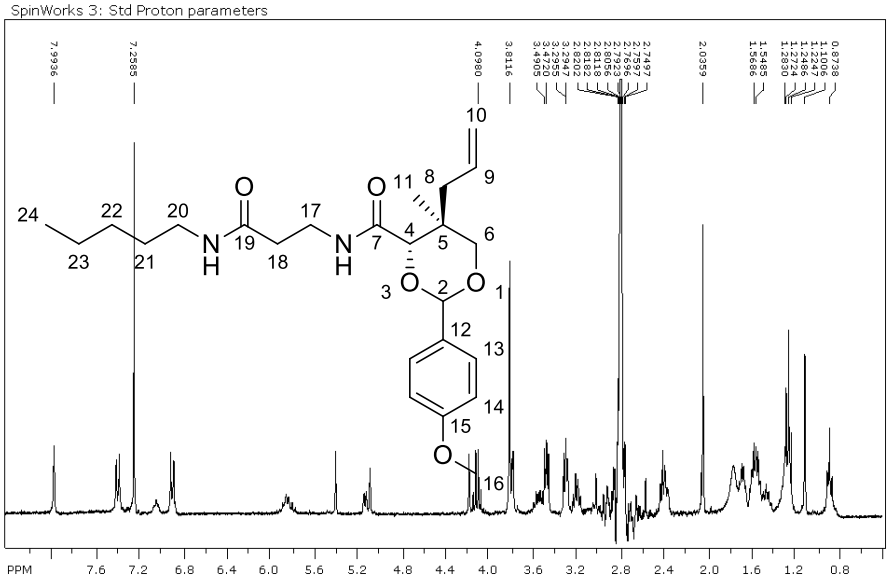
Compound 3.4 ^{13}C:



file: ...\Hamed\Dropbox\NMR\HD-4-18Ac13\fid block# 1 expt: "s2pul"
transmitter freq.: 75.456809 MHz
time domain size: 47120 points
width: 18115.94 Hz = 240.0836 ppm = 0.384464 Hz/pt
number of scans: 256

freq. of 0 ppm: 75.448887 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 724.638 ppm/cm: 9.60334

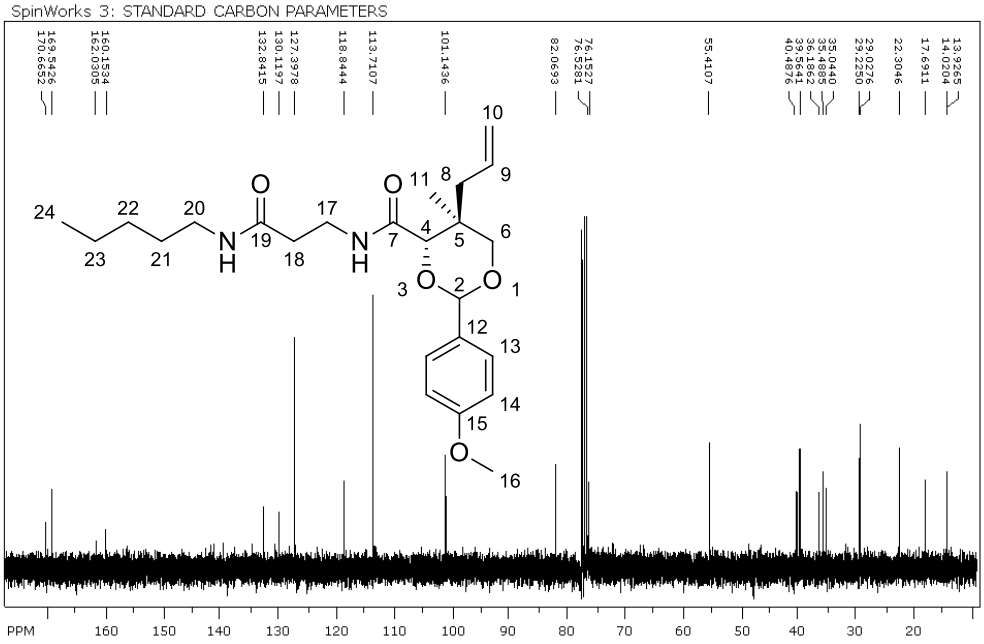
Compound 3.7 ^{1}H:



file: F:\NMR\Network\HD-3-75A.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 300.056790 MHz
time domain size: 28786 points
width: 4800.77 Hz = 15.9995 ppm = 0.166774 Hz/pt
number of scans: 8

freq. of 0 ppm: 300.054990 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 96.664 ppm/cm: 0.32215

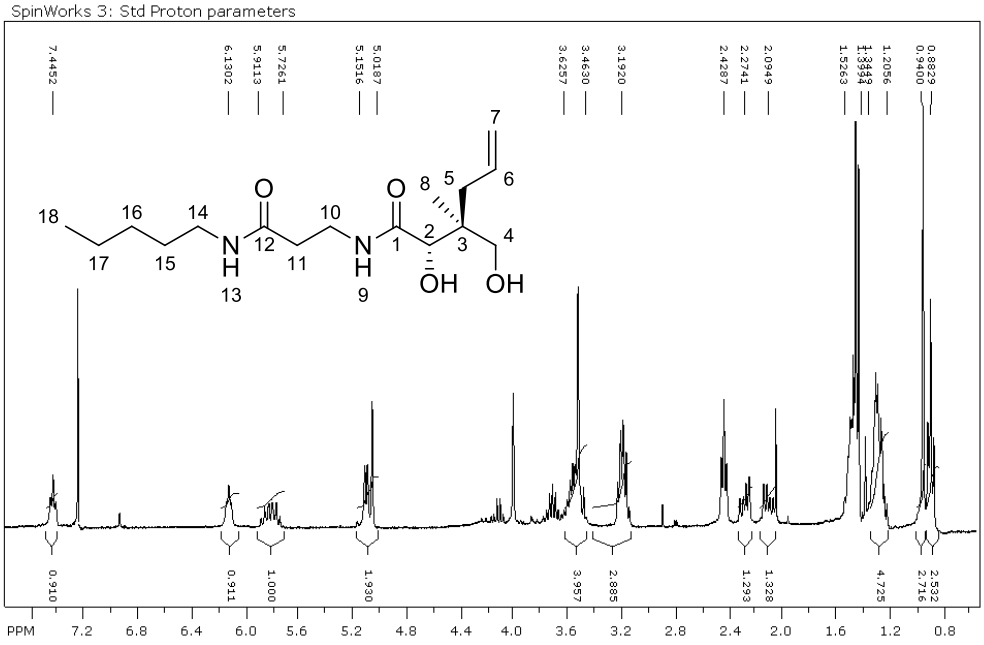
Compound 3.7 ^{13}C:



file: ...s\Hamed\Dropbox\NMR\HD-5-5Ac13\fid block# 1 expt: "s2pul"
transmitter freq.: 75.457187 MHz
time domain size: 39322 points
width: 18867.92 Hz = 250.0481 ppm = 0.479831 Hz/pt
number of scans: 256

freq. of 0 ppm: 75.448887 MHz
processed size: 65536 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 509.853 ppm/cm: 6.75685

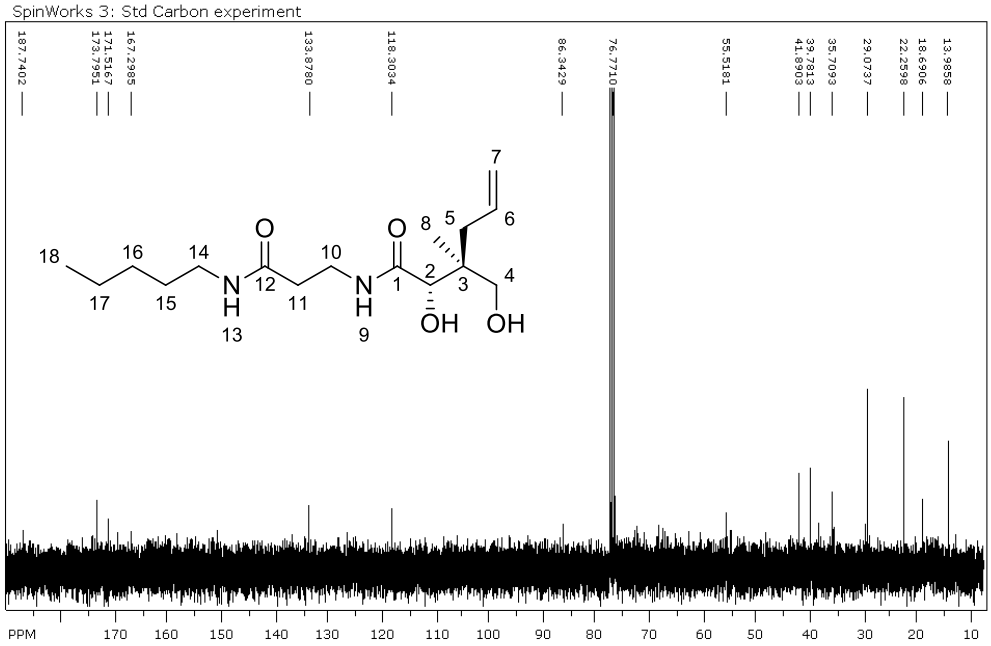
Compound 3.9 ^{1}H:



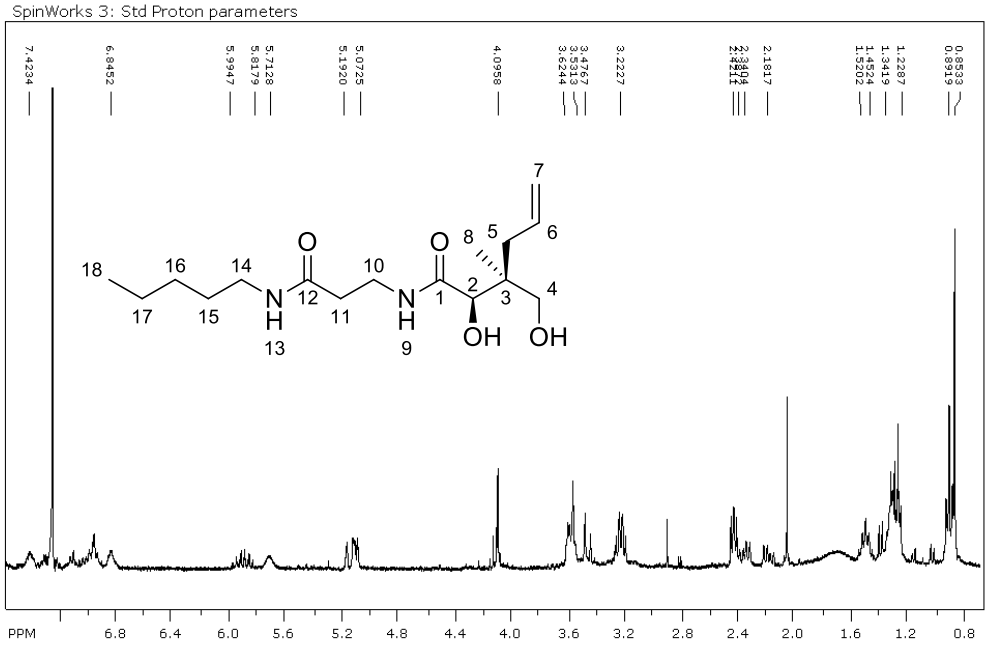
file: ...centNMR\HD-3-78Apureproton.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 300.056790 MHz
time domain size: 28786 points
width: 4800.77 Hz = 15.9995 ppm = 0.166774 Hz/pt
number of scans: 8

freq. of 0 ppm: 300.054990 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 87.192 ppm/cm: 0.29059

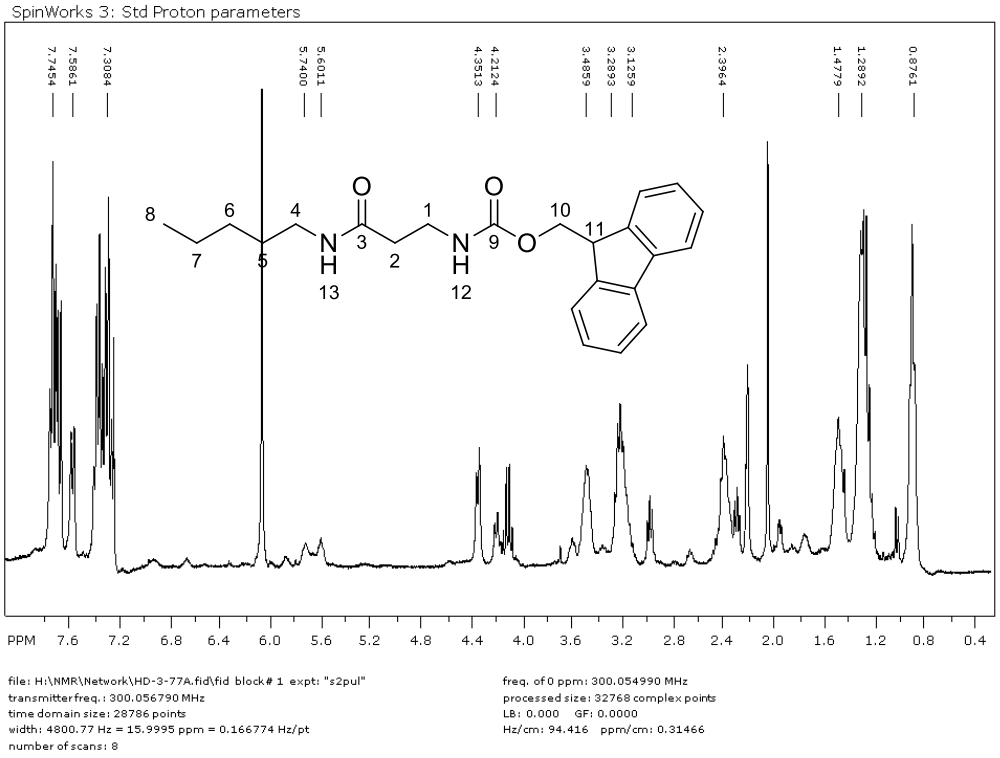
Compound 3.9 ^{13}C:



Compound 3.10 ^{1}H:



Compound 3.12 ^{1}H:



file: H:\NMR\Network\HD-3-77A.fid\fid block# 1 expt: "s2pul"
transmitter freq.: 300.056790 MHz
time domain size: 28786 points
width: 4800.77 Hz = 15.9995 ppm = 0.166774 Hz/pt
number of scans: 8

freq. of 0 ppm: 300.054990 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 94.416 ppm/cm: 0.31466

LIST OF REFERENCES

1 Davies, J., Davies, D. Origins and Evolution of Antibiotic Resistance. *Microbiology and Molecular Biology Reviews* **74**, 417-433 (2010).

2 Gao, F. *et al.* Synthesis and Structure−Activity Relationships of Truncated Bisubstrate Inhibitors of Aminoglycoside 6‘-N-Acetyltransferases. *Journal of Medicinal Chemistry* **49**, 5273-5281, doi:10.1021/jm060732n (2006).

3 House, H. O. *Modern Synthetic Reactions*. 2nd edn, (Benjamin, 1972).

4 Zhang, Y.-M. *et al.* Acyl Carrier Protein Is a Cellular Target for the Antibacterial Action of the Pantothenamide Class of Pantothenate Antimetabolites. *Journal of Biological Chemistry* **279**, 50969-50975 (2004).

5 Strauss, E. & Begley, T. P. The Antibiotic Activity of N-Pentylpantothenamide Results from Its Conversion to Ethyldethia-Coenzyme A, a Coenzyme A Antimetabolite. *Journal of Biological Chemistry* **277**, 48205-48209 (2002).

6 Snell, E. E., Strong, F. M. & Peterson, W. H. Pantothenic and Nicotonic Acids as Growth Factors for Lactic Acid Bacteria. *Journal of the American Chemical Society* **60**, 2825-2825 (1938).

7 Cronan, J. E. Beta-Alanine Synthesis in Escherichia coli. *Journal of Bacteriology* **141**, 1291-1297 (1980).

8 Clifton, G., Bryant, S. R. & Skinner, C. G. N1-(Substituted) Pantothenamides, Antimetabolites of Pantothenic Acid. *Archives of Biochemistry and Biophysics* **137**, 523-528 (1970).

9 Magnuson, K., Jackowski, S., Rock, C. O. & Cronan, J. E. Regulation of Fatty Acid Biosynthesis in Escherichia coli. *Microbiological Reviews* **57**, 522-542 (1993).

10 Strauss, E., Kinsland, C., Ge, Y., McLafferty, F. W. & Begley, T. P. Phosphopantothenoylcysteine Synthetase from Escherichia coli. *Journal of Biological Chemistry* **276**, 13513-13516 (2001).

11 Geerlof, A., Lewendon, A. & Shaw, W. V. Purification and Characterization of Phosphopantetheine Adenylyltransferase from Escherichia coli. *Journal of Biological Chemistry* **274**, 27105-27111 (1999).

12 Mishra, P. k., Park, P. K. & Drueckhammer, D. G. Identification of YacE (CoaE) as the Structural Gene for Dephosphocoenzyme A Kinase inEscherichia coli K-12. *Journal of Bacteriology* **183**, 2774-2778 (2001).

13 Ivey, R. A. *et al.* The Structure of the Pantothenate Kinase·ADP·Pantothenate Ternary Complex Reveals the Relationship between the Binding Sites for Substrate, Allosteric Regulator, and Antimetabolites. *Journal of Biological Chemistry* **279**, 35622-35629 (2004).

14 Virga, K. G. *et al.* Structure–activity Relationships and Enzyme Inhibition of Pantothenamide-Type Pantothenate Kinase Inhibitors. *Bioorganic & Medicinal Chemistry* **14**, 1007-1020 (2006).

15 Spry, C., Kirk, K. & Saliba, K. J. Coenzyme A Biosynthesis: an Antimicrobial Drug Target. *FEMS Microbiology Reviews* **32**, 56-106 (2008).

16 Akinnusi, T. O., Vong, K. & Auclair, K. Geminal Dialkyl Derivatives of N-Substituted Pantothenamides: Synthesis and Antibacterial Activity. *Bioorganic and Medicinal Chemistry* **19**, 2696-2706 (2011).

17 Larock, R. C. *Comprehensive Organic Transformations*. 2nd edn, (Wiley-VCH, 1999).

18 Fieser, L. F. F., M. *Reagents for Organic Synthesis*. Vol. 1 (Wiley, 1967).

19 Cram, D. J. & Elhafez, F. A. A. Studies in Stereochemistry. X. The Rule of “Steric Control of Asymmetric Induction” in the Syntheses of Acyclic Systems. *Journal of the American Chemical Society* **74**, 5828-5835 (1952).

20 Eliel, E. L., Wilsen, S. H. & Mander, L. N. *Stereochemistry of Organic Compounds*. (Wiley, 1994).

21 Chérest, M., Felkin, H. & Prudent, N. Torsional Strain Involving Partial Bonds. The Stereochemistry of the Lithium Aluminium Hydride Reduction of Some Simple Open-Chain Ketones. *Tetrahedron Letters* **9**, 2199-2204 (1968).

22 Chaikin, S. W. & Brown, W. G. Reduction of Aldehydes, Ketones and Acid Chlorides by Sodium Borohydride. *Journal of the American Chemical Society* **71**, 122-125 (1949).

23 Smith, M. B. *Organic Synthesis*. 347-490 (Academic Press, 2010).

24 Crimmins, M. T., Stanton, M. G. & Allwein, S. P. Asymmetric Total Synthesis of (−)-Laulimalide: Exploiting the Asymmetric Glycolate Alkylation Reaction. *Journal of the American Chemical Society* **124**, 5958-5959 (2002).

25 Smith, A. B., Razler, T. M., Ciavarri, J. P., Hirose, T. & Ishikawa, T. (+)-Phorboxazole A Synthetic Studies. A Highly Convergent, Second Generation Total Synthesis of (+)-Phorboxazole A. *Organic Letters* **7**, 4399-4402 (2005).

26 Davis, F. A., Haque, M. S. & Przeslawski, R. M. Asymmetric Synthesis of the Methyl and Benzyl Ethers of Erythro-.Alpha.,.Beta.-Diphenyl-.Beta.-Hydroxyethanol and Erythro-.Alpha.,.Beta.-Diphenyl-.Beta.-Hydroxyethylamine from (+)-(S)-Benzoin. *The Journal of Organic Chemistry* **54**, 2021-2024 (1989).

27 Landor, S. R., Miller, B. J. & Tatchell, A. R. Asymmetric synthesis. Part II. The Reduction of Ketones with the Lithium Aluminium Hydride-3-O-Benzyl-1,2-O-Cyclohexylidene-[Small Alpha]-D-Glucofuranose Complex. *Journal of the Chemical Society C: Organic* **0**, 2280-2282 (1966).

28 Midland, M. M., Greer, S., Tramontano, A. & Zderic, S. A. Chiral Trialkylborane Reducing Agents. Preparation of 1-Deuterio Primary Alcohols of High Enantiomeric Purity. *Journal of the American Chemical Society* **101**, 2352-2355 (1979).

29 Roethle, P. A., Chen, I. T. & Trauner, D. Total Synthesis of (−)-Archazolid B. *Journal of the American Chemical Society* **129**, 8960-8961 (2007).

30 Corey, E. J., Bakshi, R. K., Shibata, S., Chen, C. P. & Singh, V. K. A Stable and Easily Prepared Catalyst for the Enantioselective Reduction of

Ketones. Applications to Multistep Syntheses. *Journal of the American Chemical Society* **109**, 7925-7926 (1987).

31 Ghosh, A. K. & Swanson, L. Enantioselective Synthesis of (+)-Cryptophycin 52 (LY355703), a Potent Antimitotic Antitumor Agent. *The Journal of Organic Chemistry* **68**, 9823-9826 (2003).

32 Nakamura, K., Yamanaka, R., Matsuda, T. & Harada, T. Recent Developments in Asymmetric Reduction of Ketones with Biocatalysts. *Tetrahedron: Asymmetry* **14**, 2659-2681 (2003).

33 Matsuda, T., Yamanaka, R. & Nakamura, K. Recent Progress in Biocatalysis for Asymmetric Oxidation and Reduction. *Tetrahedron: Asymmetry* **20**, 513-557 (2009).

34 Brooks, D. W., Mazdiyasni, H. & Grothaus, P. G. Asymmetric Microbial Reduction of Prochiral 2,2,-Disubstituted Cycloalkanediones. *The Journal of Organic Chemistry* **52**, 3223-3232 (1987).

35 Brooks, D. W., Grothaus, P. G. & Irwin, W. L. Chiral Cyclopentanoid Synthetic Intermediates via Asymmetric Microbial Reduction of Prochiral 2,2-Disubstituted Cyclopentanediones. *The Journal of Organic Chemistry* **47**, 2820-2821 (1982).

36 Erdélyi, B. *et al.* Stereoselective Production of (S)-1-Aralkyl- and 1-Arylethanols by Freshly Harvested and Lyophilized Yeast Cells. *Tetrahedron: Asymmetry* **17**, 268-274 (2006).

37 Katz, M., Johanson, T. & Gorwa-Grauslund, M.-F. Mild Detergent Treatment of Candida Tropicalis Reveals a NADPH-Dependent Reductase in the Crude Membrane Fraction, Which Enables the Production of Pure Bicyclic Exo-Alcohol. *Yeast* **21**, 1253-1267 (2004).

38 Milagre, H. M. S., Milagre, C. D. F., Moran, P. J. S., Santana, M. H. A. & Rodrigues, J. A. R. Reduction of Ethyl Benzoylformate Mediated by Saccharomyces cerevisiae Entrapped in Alginate Fibers with Double Gel Layers in a Continuously Operated Reactor. *Enzyme and Microbial Technology* **37**, 121-125 (2005).

39 Fráter, G., Müller, U. & Günther, W. The Stereoselective α-Alkylation of Chiral β-Hydroxy Esters and Some Applications Thereof. *Tetrahedron* **40**, 1269-1277 (1984).

40 Seebach, D. S., A., M., Weber, R. H. & Züger, M. F. Yeast Reduction of Ethyl Acetoacetate: (S)-(+)-Ethyl 3-Hydroxybutanoate. *Organic Syntheses* **63** (1985).

41 Nakamura, K. *et al.* Enantio- and Regioselective Reduction of α-Diketones by Baker's yeast. *Tetrahedron: Asymmetry* **7**, 409-412 (1996).

42 Shieh, W., Gopalan, A. S. & Sih, C. J. Stereochemical Control of Yeast Reductions. 5. Characterization of the Oxidoreductases Involved in the Reduction of Beta.-Keto Esters. *Journal of the American Chemical Society* **107**, 2993-2994 (1985).

43 Nakamura, K., Miyai, T., Kawai, Y., Nakajima, N. & Ohno, A. Stereoselective Synthesis of (2R, 3S)-Syn-2-Allyl-3-Hydroxy Butanoate Mediated by an Enzymatic System from Baker's Yeast. *Tetrahedron Letters* **31**, 1159-1160 (1990).

44 Nakamura, K., Kawai, Y., Miyai, T. & Ohno, A. Stereochemical Control in Diastereoselective Reduction with Baker's yeast. *Tetrahedron Letters* **31**, 3631-3632 (1990).

45 Nakamura, K., Kawai, Y. & Ohno, A. Stereochemical Control in Microbial Reduction. 19. Effect of Heat-treatment on the Diastereoselectivity in the Reduction with Baker's yeast. *Tetrahedron Letters* **32**, 2927-2928 (1991).

46 Seco, J. M., Quiñoá, E. & Riguera, R. A Practical Guide for the Assignment of the Absolute Configuration of Alcohols, Amines and Carboxylic Acids by NMR. *Tetrahedron: Asymmetry* **12**, 2915-2925, doi:10.1016/S0957-4166(01)00508-0 (2001).

47 Bain, J. D., Wacker, D. A., Kuo, E. E. & Chamberlin, A. R. Site-Specific Incorporation of Non-Natural Residues into Peptides: Effect of Residue Structure on Suppression and Translation Efficiencies. *Tetrahedron* **47**, 2389-2400, doi:10.1016/S0040-4020(01)81776-2 (1991).