Inhibition Studies of DapL Enzymes Orthologs and Synthesis of Proposed Biosynthetic Intermediates of Tabtoxin and Synthesis of a Hexaketide as a Standard for Lovastatin Studies

by

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Abstract

The enzyme LL-diaminopimelate aminotransferase (DapL) catalyzes the transformation of L-THDP to LL-DAP during the biosynthesis of lysine in plants, alga, and in some pathogenic bacteria like *Leptospira interrogans* and *Verrucomicrobium spinosum*. Because the biosynthesis of lysine is absent in mammals, specific inhibitors of DapL enzyme could potentially function as antibiotics with low toxicity to humans or as herbicides or algaecides. An inhibition study of DapL enzyme from three orthologs (*V. spinosum*, *L. interrogans,* and *C. reinhardtii*) was completed. Five potential pharmacophores were selected (derivatives of rhodanine, barbiturate, and thiobarbiturate). The results showed a different inhibition pattern between each ortholog. In order to help to understand the differences we used structural modeling and protein alignment of DapL orthologs.

Tabtoxin is a phytotoxic dipeptide from *P. syringae* that contains a non-proteinogenic amino acid, tabtoxinine-β-lactam (TβL). Once the peptide bond in tabtoxin is hydrolysed by aminopeptidases in the host or in the periplasm of the pathogen, TβL is released. TβL is the actual toxin because it is responsible for irreversible inactivation of glutamine synthetase. For example, this inhibition results in accumulation of high levels of ammonia causing chlorosis on the leaves, in tobacco wildfire disease. To date, the intermediates involved in the biosynthesis of tabtoxin, have not been elucidated. In this work we proposed a complete biosynthesis of tabtoxin. In order to demonstrate this pathway,

synthesis of three proposed intermediates in the biosynthesis of tabtoxin were pursued. Synthetic methodologies were developed towards the synthesis of labeled and/or unlabeled intermediates.

Lovastatin is a statin that reduces cholesterol levels through the inhibition of the enzyme HMG-CoA reductase. The biosynthesis of lovastatin is accomplished by a HR-PKS, LovB with involvement of LovC in *Aspergillus terreus*. LovB has a condensation (CON) domain of a non-ribosomal peptide synthetase (NRPS). CON domain is believed to be a natural Diels-Alderase that catalyzes the formation of the decalin ring. In this project the synthesis of a *N*-acetyl cysteamine (SNAC) ester of a hexaketide was pursued. This hexaketide may be used as a standard for GC-MS to investigate the substrate specificity of LovB. The enzymatic study would demonstrate if the CON domain is responsible for catalyzing the Diels-Alder reaction during the biosynthesis of lovastatin.

Preface

Most of chapter 1 has been published - McKinnie, S. M. K.; Rodriguez-Lopez, E. M.; Vederas, J. C.; Crowther, J. M.; Suzuki, H.; Dobson, R. C. J.; Leustek, T.; Triassi, A. J.; Wheatley, M. S.; Hudson, A. O., Differential response of orthologous L,L-diaminopimelate aminotransferases (DapL) to enzyme inhibitory antibiotic lead compounds. *Bioorganic & Medicinal Chemistry* **2014,** *22* (1), 523-530. DOI: 10.1016/j.bmc.2013.10.055.

Some of the work presented in section 2.2.2 has been published - Cochrane, S. A.; Findlay, B.; Bakhtiary, A.; Acedo, J. Z.; Rodriguez-Lopez, E. M.; Mercier, P.; Vederas, J. C., Antimicrobial lipopeptide tridecaptin A1 selectively binds to Gram-negative lipid II. *Proceedings of the National Academy of Sciences* **2016,** *113* (41), 11561-11566. DOI: 10.1073/pnas.1608623113.

The rest of the work presented in this thesis is unpublished at the time of writing.

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List of Abbreviations

$[\alpha]_D$	specific rotation
AAA	α-aminoadipic acid
Ac	acetyl
AcCl	acetyl chloride
Agl	allylglycine
Ala or A	alanine
AMP	adenosine monophosphate
AMR	antimicrobial resistance
aq.	aqueous
Arg or R	arginine
ASA	aspartate semialdehyde
Asn or N	asparagine
Asp or D	aspartic acid
AT	aminotransferase
ATP	adenosine triphosphate
BHT	butylated hydroxytoluene
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
br	broad
Bu	butyl
c	concentration
Cbz	carboxybenzyl

CDI	1,1'-carbonyldiimidazole
CM	cross metathesis
CoA	coenzyme A
C-terminal	carboxy terminal
δ	chemical shift
d	doublet
Da	dalton
DAP	diaminopimelic acid
DABCO	1,4-diazabicyclo[2.2.2]octane
DapA	dihydrodipicolinate synthase
DapB	dihydropicolinate reductase
DapC	*N*-acyl-2-amino-6-ketopimelate aminotransferase
DapD	tetrahydropicolinate acylase
DapE	*N*-acyl-L,L-2,6-diaminopimelate deacylase
DapF	diaminopimelate epimerase
DapL	LL-diaminopimelate aminotransferase
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N,N'*-dicyclohexylcarbodiimide
DCE	1,2-dichloroethane
DCU	dicyclohexylurea
Ddh	*meso*-diaminopimelate dehydrogenase
DHDP	dihydrodipicolinate
DIPEA	*N,N*-diisopropylethylamine

DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DML	dihydromonacolin L
DMP	Dess-Martin periodinane
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
EI	electron ionization
ESI, ES	electrospray ionization
Et	ethyl
Et_2O	diethylether
Et_3N	triethylamine
EtOAc	ethyl acetate
FAS	fatty acid synthase
FT	Fourier transform
GC	gas chromatography
GlcNAc	*N*-acetylglucosamine
Gln, Q	glutamine
Glu, E	glutamic acid
Gly, G	glycine
GMC	glucose-methanol-choline oxidoreductase
GNAT	*N*-acetyltransferase

GS	glutamine synthetase
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HWE	Horner-Wadsworth Emmons
IC_{50}	half maximal inhibitory concentration
IR	infrared
J	coupling constant
α-Kg	α-ketoglutarate
k_{cat}	turnover number
kDa	kilodalton
KR	ketoreductase domain
K_m	Michaelis constant
KS	ketosynthase domain
LNKS	Lovastatin nonaketide synthase
Lys or K	lysine
LysA	*meso*-diaminopimelate decarboxylase
m	multiplet
Me	methyl
MeOH	methanol
MeT	methyl transferase domain
MS	mass spectrometry
MSAS	6-methylsalicylic acid synthase

MSO	methionine sulfoximine
MT	Methyl transferase domain
MWCO	molecular weight cut off
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NCBI	National Center for Biotechnology Information
NMR	nuclear magnetic resonance
NPs	natural products
NR PKS	non-reducing polyketide synthase
NRPS	non-ribosomal peptide synthase
N-terminal	amino terminal
OAB	*o*-aminobenzaldehyde
PDB	protein data bank
PG	peptidoglycan
Ph	phenyl
PKS	polyketide synthase
PK-NRP	polyketide-non-ribosomal peptide
PKS-NRPS	polyketide synthase-non-ribosomal peptide synthase
PLP	pyridoxal-5'-phosphate
PMP	pyridoxamine-5'-phosphate
Ppant	phosphopantetheine
PP_i	pyrophosphate
ppm	part per million

Pptase	phosphopantetheinyl transferase
PR PKS	partially reducing polyketide synthase
PT	product template domain
pv	pathovars
q	quartet
R	reductase domain
rpm	revolutions per minute
s	singlet
Ser or S	serine
SAM	*S*-adenosyl methionine
SAR	structure activity relationship
SNAC	*N*-acetylcysteamine
sp.	species
spp.	multiple species
STI	sexually transmitted infections
t	triplet
TβL	tabtoxinine-β-lactam
*t*Bu	tertiary-butyl
TE	thioesterase domain
TFA	trifluoroacetic acid
THDP	tetrahydropicolinate
THF	tetrahydrofuran
Thr or T	threonine

TLC	thin layer chromatography
UV-Vis	ultra violet visible
v/v	volume per volume
V_{max}	enzyme maximum rate

Chapter 1. L,L-diaminopimelate aminotransferases

1.1 Introduction

Natural products (NPs) represent a large family of diverse chemical compounds with several applications in medicine and agriculture. NPs with industrial applications can be produced from primary or secondary metabolism of living organisms (plants, animals or microorganisms). Examples of primary metabolites are amino acids, vitamins and nucleotides, which are essential for growth, development, and reproduction of the organisms.[1] The NPs produced by an organism that are not required for survival are defined as secondary metabolites. In general, the main role of secondary metabolites are as mechanism of defense, regulation and communication.[2]

Since the discovery of penicillin more than 80 years ago, over 500,000 NPs have been characterized; approximately 20% show biological activity, and ~7% have been isolated from microbes. Moreover, from ~34,000 biologically active compounds that have been obtained from microorganisms, 35% are produced by actinomycetes, 44% by fungi and 21% by unicellular bacteria.[3]

NPs have long been an abundant source of drugs, but structural modification is necessary to optimize potency, pharmacokinetics and other important parameters. Actually, close to half of drugs approved between 1981 and 2014 are natural product derivatives, synthetic natural product mimics, or contain natural product pharmacophores.[4]

Lovastatin is a secondary metabolite produce by fungi (*Monascus ruber* and *Aspergillus terreus*). This compound belongs to the class of NPs known as statins, which are important for the treatment of hypercholesterolemia. Statins are among the world's best-selling anticholesterolemic. A remarkable fact is that this secondary metabolite is biosynthesized by polyketide synthases (PKS).[2] The study of the programming of this mega enzyme is currently under development and its reprogramming could lead to development of novel polyketides. The biosynthesis of lovastatin and the synthesis of a hexaketide substrate is discussed in Chapter 3.

Another example of a secondary metabolite is tabtoxin. This phytotoxin is produced by bacteria (*Pseudomonas syringae*) and contains a β-lactam ring that could have antibiotic activity. Tabtoxin biosynthesis branches off from the lysine biosynthetic pathway.[5] The biosynthesis of tabtoxin and the efforts to prepare proposed intermediates of its biosynthetic pathway are discussed in Chapter 2. The lysine biosynthesis is absent in mammals but is present in bacteria. A novel pathway of lysine biosynthesis in pathogenic bacteria is discussed in section 1.1.2. The study of specific inhibitors of enzymes involved in lysine biosynthesis is an attractive target for antibiotic development (section 1.1.3 and 1.2).

Nowadays, there is an urgent need for new antibiotics that are active against resistant bacteria. This resistance increasingly limits the effectiveness of current antimicrobial drugs. Today, NPs remain the main source for new therapeutic agents.[6]

In 2014, the UK government estimated that 700,000 people die every year from drug-resistant strains of bacteria, human immunodeficiency virus (HIV), tuberculosis (TB) and malaria.[7] By 2050, 10 million people a year would be at risk if the problem is not confronted. The loss of effective therapies to treat bacterial infections has caught the attention of government agencies to propose solutions to slow down the potential threat.

Antimicrobial drugs are medicines that are active against a range of infections, such as those caused by bacteria (antibiotics), viruses (antivirals), fungi (antifungals) and parasites (antiparasitics including antimalarials). Antibiotics are a special category of antimicrobial drugs that are medicines used to treat bacterial infections. Antibiotics are classified as bacteriostatic or bactericidal agents. A bacteriostatic agent stops bacteria from reproducing whereas a bactericidal agent kills the bacteria. Antibiotic resistance occurs when bacteria change in response to the use of these medicines. These bacteria may infect humans and animals, and the infections they cause are harder to treat than those caused by non-resistant bacteria. Antibiotic resistance leads to higher medical costs, prolonged hospital stays, and increased mortality.

Examples of bacterial resistance mechanisms include: antibiotic efflux, antibiotic inactivation, biofilm formation, and target modification.[8] Also, there are multidrug resistant (MDR) pathogens that are resistant to more than one class of antimicrobial agents. Some of the most problematic MDR organisms are *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli* and *Klebsiella pneumonia*, Vancomycin-resistant enterococci (VRE) and methicillin-

resistant *Staphylococcus aureus* (MRSA).[8] It is important to highlight that most of these examples are Gram-negative but *Enterococcus* and *Staphylococcus aureus* are Gram-positive.

Since 2000, just 30 new antibiotics have been approved worldwide. Of the 30 antibiotics, two are natural products (NP), twelve are natural product-derived (NP-derived), and 16 synthetic-derived.[9] Most of them are effective against both Gram-positive and Gram-negative bacteria; eight are selective for Gram-positive bacteria, and only two target Gram-negative bacteria.[9] This fact points to the urgent need of developing new classes of antibiotics with Gram-negative activity.

The increasing number of multidrug resistant bacteria has prompted the development of new antibiotics that are effective against bacterial diseases. Currently many drugs used to treat bacterial infections target pathways such as DNA replication, protein synthesis and cell wall biosynthesis.[10]

1.1.1 *meso*-Diaminopimelate in nature

meso-Diaminopimelate (*meso*-DAP, **28**) is a biosynthetic precursor of lysine in prokaryotes and plants.[11] Both *meso*-DAP (**28**) and lysine (**13**) are important for the synthesis of peptidoglycan (PG), a chemical component of the cell wall in bacteria. *meso*-DAP is found in specific eubacterial and archaeal lineages, in several groups of pathogenic bacteria, as well as many algal lineages.

Lysine (Lys), required for protein synthesis and growth, is one of the nine essential amino acids absent in mammals, and thus must be obtained from

dietary sources. In nature, prokaryotes and eukaryotes can synthesize lysine by one of the two known pathways: the diaminopimelate (DAP) or the α-aminoadipic acid (AAA) pathway. Most bacteria and plants use the diaminopimelate pathway to synthesize lysine from aspartate *via* diaminopimelate. Fungi and yeast employ the α-aminoadipate (AAA) pathway, which proceeds through α-aminoadipate (Figure 1.1).[12,13]

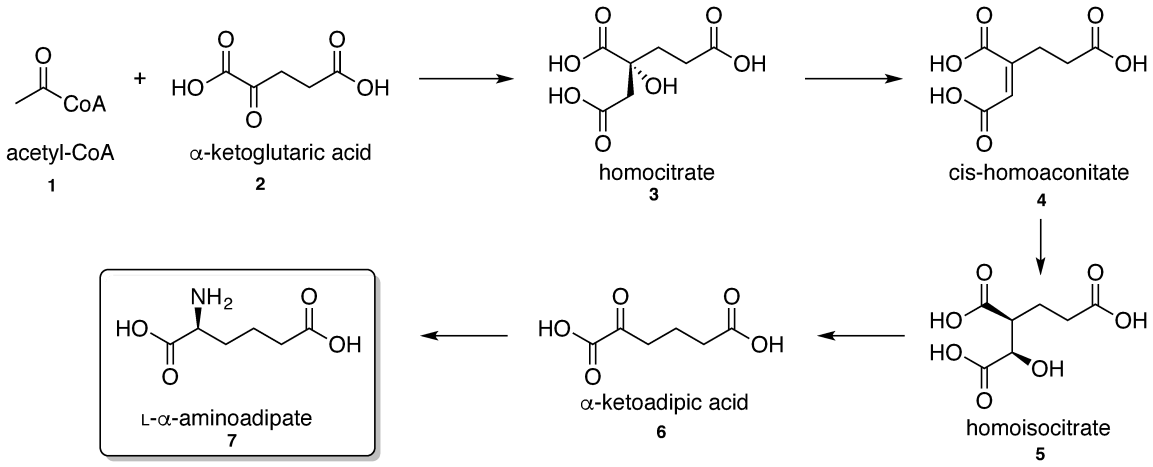


Figure 1.1. The first half of L-lysine biosynthesis from α-ketoglutaric acid in the α- aminoadipate (AAA) pathway.

The biosynthetic pathway starts with the synthesis of homocitrate from the condensation of acetyl-CoA with α-ketoglutarate by homocitrate synthase. Then homocitrate is oxidized to *cis*-homoaconitate followed by decarboxylation, which is catalyzed by homoisocitrate dehydrogenase to form α-ketoadipic acid. Finally, the 2-oxo group of α-ketoadipic acid undergoes transamination to yield L-α-aminoadipate.

The second half of the AAA pathway splits in two different pathways (Figure 1.2). In pathway A, which is followed by most fungi and yeast, the

synthesis of lysine proceeds through L-α-aminoadipic acid and saccharopine. Pathway B, is a modified AAA pathway similar to that of fungi and yeast, this pathway is followed by the extremely thermophilic bacterium, *Thermus thermophilus* HB27 (Figure 1.2).

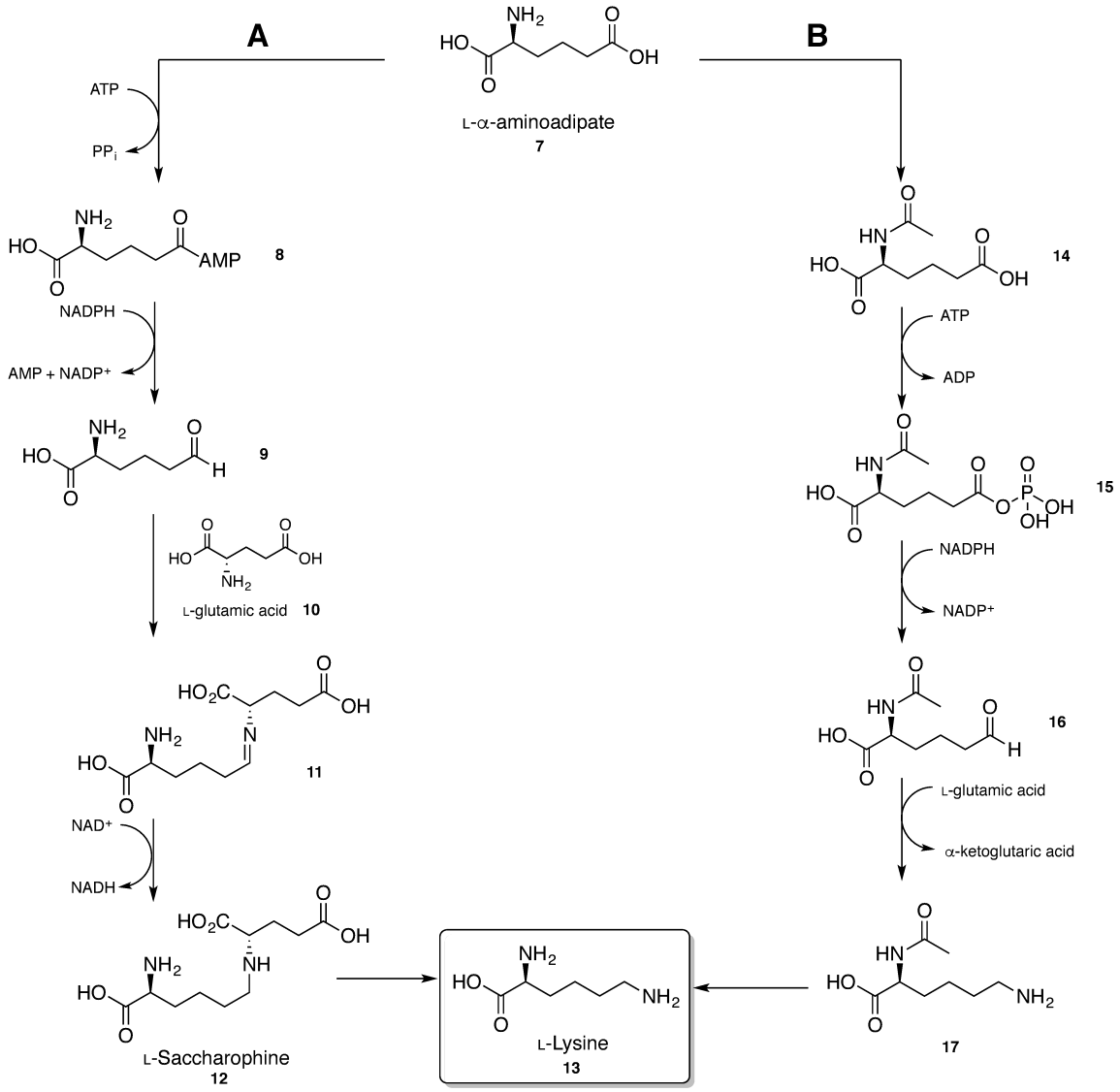


Figure 1.2. The second half of L-lysine biosynthesis from L-α-aminoadipic acid (AAA). Pathway A is followed by most of the fungi and yeast. Pathway B is a putative variant of *Thermus thermophiles*.

In contrast, most bacteria and photosynthetic organisms use the diaminopimelate (DAP) pathway to synthesize lysine. Since the DAP pathway is absent in mammals, specific inhibitors of enzymes involved in lysine biosynthesis could be a new class of antibiotics with low-toxicity to humans.

1.1.2 Lysine biosynthesis in bacteria and plants

In plants and bacteria, the DAP pathway of lysine biosynthesis is subdivided into four variants: the acylase pathway, which utilizes succinylated or acetylated intermediates; the *meso*-diaminopimelate dehydrogenase (Ddh) pathway; and the L,L-diaminopimelate aminotransferase (DapL) pathway.[14,15] The starting point of all four variants, is the synthesis of L-tetrahydrodipicolinate acid (L-THDP, **22**) from L-aspartate (asp, **18**). The reactions are accomplished by the enzymes aspartate kinase (LysC), aspartate semialdehyde dehydrogenase (asd), dihydrodipicolinate synthase (DapA) and dihydrodipicolinate reductase (DapB) (Figure 1.3).

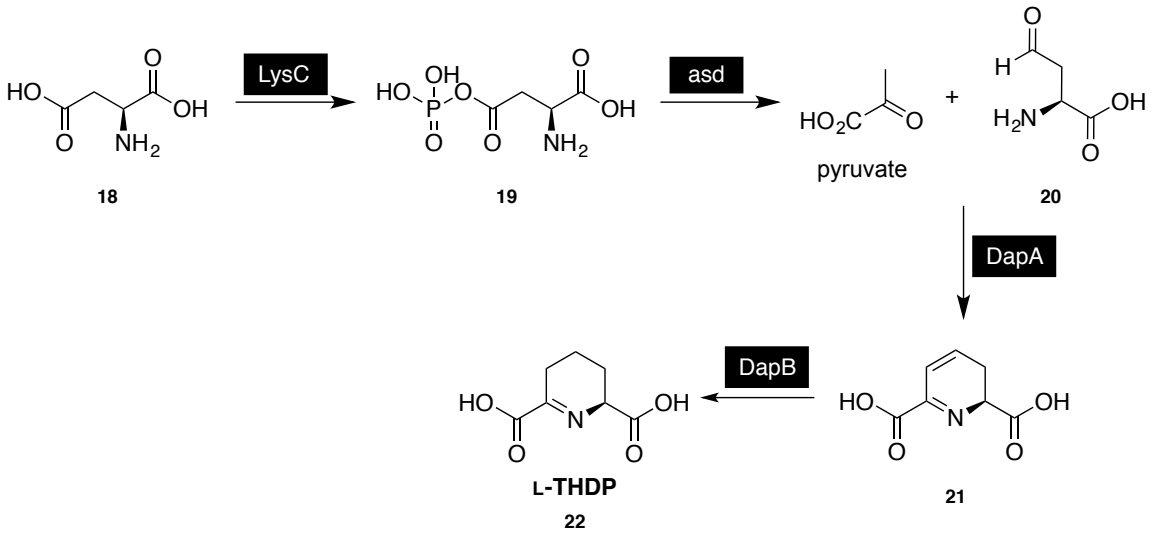


Figure 1.3. Synthesis of L-THDP from aspartate in DAP pathways.

The main difference between the DAP pathways is how they transform L-THDP to the common precursor *meso*-DAP. In the acyl pathways, this conversion is facilitated by four enzymes: tetrahydrodipicolinate acylase (DapD), *N*-acyl-2-amino-6-ketopimelate aminotransferase (DapC), *N*-acyl-L,L-2,6-diaminopimelate deacylase (DapE) and diaminopimelate epimerase (DapF) (Fig. 1.4).

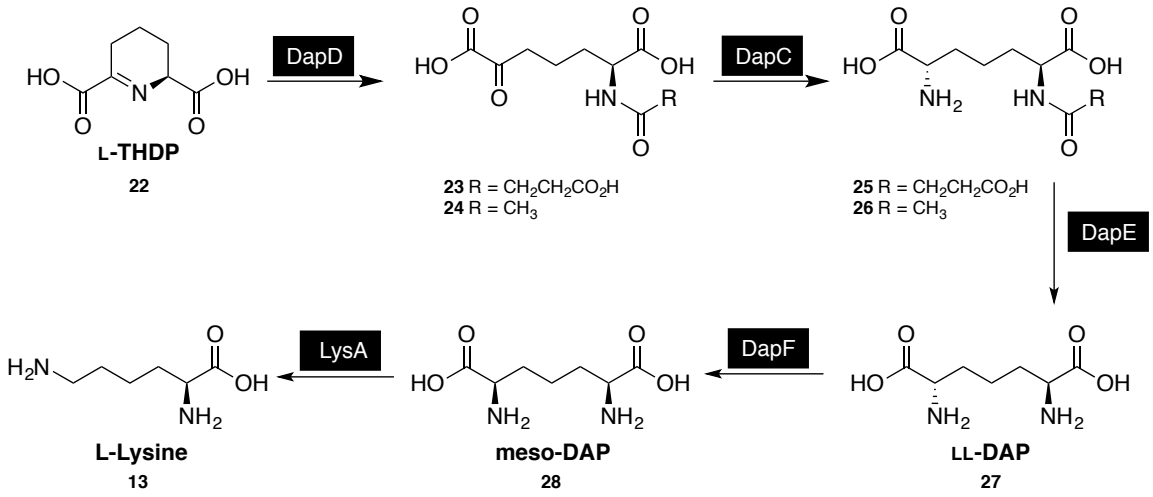


Figure 1.4. The acyl pathways towards the biosynthesis of L-Lysine from L-THDP.

In the dehydrogenase pathway, L-THDP is converted to *meso*-DAP in a single reaction by the enzyme *meso*-diaminopimelate dehydrogenase (Ddh). This single enzyme performs all four reactions of the enzymes in the acyl pathways: DapD, DapC, DapE and DapF (Figure1.5).

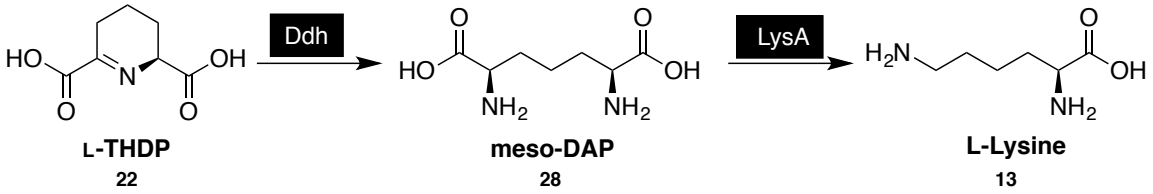


Figure 1.5. Dehydrogenase pathway

Lastly, in the DapL pathway, the enzymes DapD, DapC and DapE are bypassed by one single enzyme: the L,L-diaminopimelate aminotransferase (DapL). Another common feature to all four variants is the last step, the decarboxylation of *meso*-DAP to afford lysine, catalyzed by the enzyme *meso*-diaminopimelate decarboxylase (LysA) (Fig. 1.6).

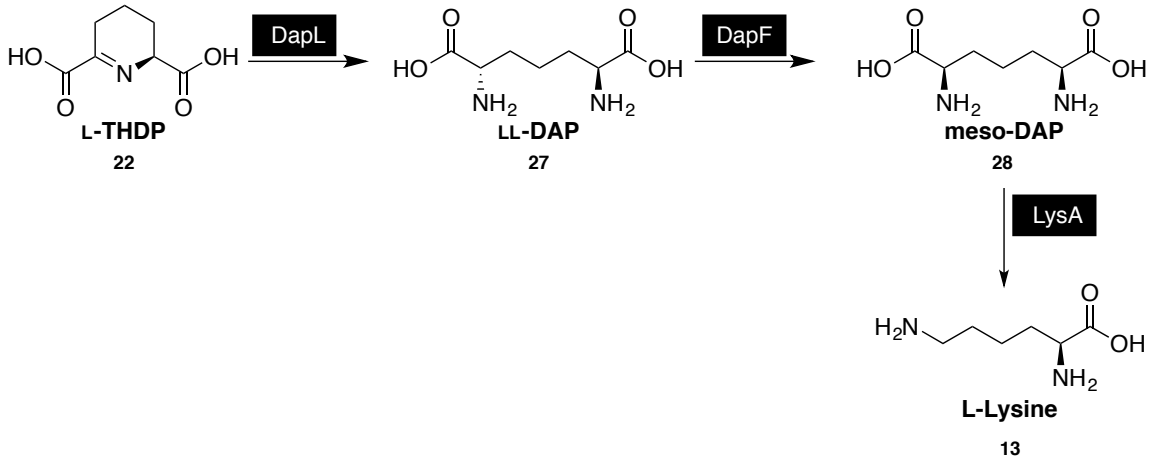


Figure 1.6. Biosynthesis of L-Lysine: the DapL pathway.

The direct precursor of lysine in this biosynthetic route is *meso*-DAP. The DAP pathway is a potential target for a new class of antibiotics or herbicides with low toxicity to mammals. This has prompted studies of not only the structures of the enzymes involved in DAP biosynthesis, but also the design of inhibitors for

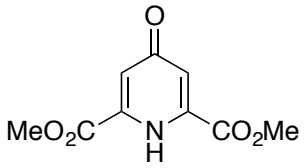
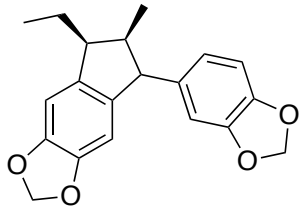
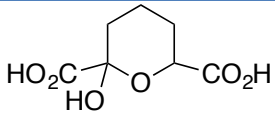
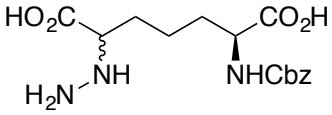
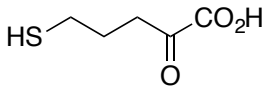
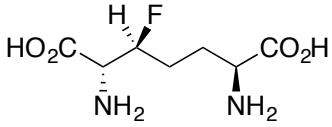
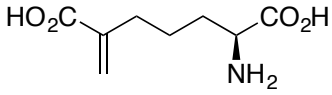
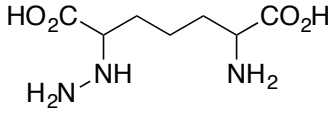
them. Another benefit of understanding the biogenesis of lysine is the potential to assist engineering plants with greater lysine content.

Peptidoglycan (PG), a component in the cell wall of most bacteria, has an essential role to protect the cytoplasmic membrane from osmotic stress. *meso*-DAP is the cross-linking amino acid in the cell wall of Gram-negative bacteria and lysine is the cross-linking amino acid in most Gram-positive bacteria.[16] Therefore, inhibition of the enzymes involved in DAP/Lys pathway will lead to cell death *via* lysis as a result of osmotic pressure disruption from the improperly constructed PG.[17] Also, the inhibition of these enzymes will interfere with the synthesis of lysine, which will cause bacterial cell death, as the bacteria will not be able to synthesize PG due to the scarcity of *meso*-DAP and lysine. Moreover, it has been demonstrated that compounds that are specific for enzymes involved in the synthesis of DAP/L-lysine pathway have antimicrobial activity,[18-20] and are likely to be non-toxic, since these enzymes are absent in humans.

1.1.3 Screening for DAP enzymes-inhibitors

The emerging antibiotic resistance has prompted the urgent need to find new classes of antibiotics. Several research groups including our group have studied different inhibitors to target specific enzymes of the DAP pathway. Some examples of compounds that have been tested to inhibit the activity of DAP enzymes are shown in Table 1.1.

Table 1.1. Selected inhibitors of different enzymes belonging to DAP pathways

Enzyme	Inhibitor	
DapA[16]	 **(29)**	IC_{50} = 14 mM
DapB[21]	 **(30)**	IC_{50} = 20 μM
DapD[22]	 **(31)**	K_i = 60 nM
DapC[23]	 **(32)**	K_i = 54 nM
DapE[20]	 **(33)**	IC_{50} = 43 μM
DapF[24]	 **(34)**	IC_{50} = 4 μM
Ddh[25]	 **(35)**	K_i = 5.3 μM
LysA[26]	 **(36)**	K_i = 0.1 mM

The plant *Arabidopsis thaliana* is an important model for identification of new proteins. In 2000, the publication of the complete sequence of *Arabidopsis*

thaliana, allowed the study of its genes for further identification of their functions.[27] Later, in 2005, Leustek and collaborators studied the genome of *Arabidopsis thaliana* to investigate the enzymes involved in the biosynthesis of lysine. Surprisingly, DapD, DapC, and DapE were not found in the *A. thaliana* genome.[28]

Following the findings of Leustek and collaborators, in 2006, Hudson and collaborators,[15] found a new variant of a DAP pathway for the synthesis of lysine in photosynthetic organisms. In this variant, a single enzyme, the L,L-diaminopimelate aminotransferase (DapL) from *Arabidopsis thaliana* directly converts L-THDP to LL-DAP, circumventing the DapD, DapC, and DapE steps of the acyl pathways found in prokaryotes.

The DapL pathway is also shared by *Protochlamydia amoebophila* and *Chlamydia trachomatis*, members of the Chlamydiae phylum. These bacteria are responsible for significant diseases worldwide in both humans and animals. For example, *C. trachomatis* causes sexually transmitted infections as well as neglected infectious blindness (trachoma). Moreover, coronary heart disease and atherosclerosis infections are caused by *C. pneumonia*. Poultry and livestock are also affected by a diverse number of *Chlamydophila* spp.[14] Recent genetic analysis has shown that DapL occurs in *Cyanobacteria, Desulfuromonadales, Firmicutes, Bacteroidetes, Chlamydiae, Spirochaeta*, and *Chloroflexi* and two archaeal groups, *Methanobacteriaceae* and *Archaeoglobaceae*.[11]

The fact that the DapL pathway for *meso*-DAP/lysine synthesis is exclusive to plants and some bacteria, makes DapL an attractive target for the

development of new antimicrobials or herbicides. This motivated another study conducted by our group to look for new possible antibiotics against Chlamydia genus. In this study a library of 29,201 drug-like compounds were tested against the DapL ortholog from the model plant *Arabidopsis thaliana* by robotic screening.[29] From this work, the top 46 compounds were manually retested, and IC_{50} values were measured, showing an inhibition of at least 13% of *At*DapL. These inhibitors contain one of four main structural moieties: hydrazide, rhodanine, barbiturate or thiobarbiturate, which were identified as potential pharmacophores. From the initial screening *o*-sulfonamido-arylhydrazide, a reversible inhibitor, showed the best inhibition with an IC_{50} ~ 5 μM. Further SAR studies of the hydrazide moiety led to the identification of an *o*-sulfonamido-*p*-fluorophenylhydrazide, with an IC_{50} value of 2.5 μM.[30]

1.1.4 Three dimensional studies of DapL

The DAP pathway is a target for a new class of antibiotics or herbicides with low toxicity to mammals. This has prompted the study of structures of the enzymes involved in DAP biosynthesis, as well as the design of inhibitors for them. To date, the crystal structures of three DapL orthologs have been solved: *A. thaliana* (*At*DapL),[31] *Chlamydia trachomatis* (*Ct*DapL),[32] and *Chlamydomonas reinhardtii* (*Cr*DapL).[33,34] The X-ray crystallography analysis of these orthologs showed that the holoenzymes are homodimeric, and PLP-dependent with a V-shaped active site. Although, *At*DapL and *Cr*DapL orthologs are highly specific

for LL-DAP, the *Ct*DapL ortholog is able to use both diastereomers, LL-DAP and *meso*-DAP, as substrates.[14] Watanabe *et al.* proposed that differences in substrate specificity could be due to flexibility of the loops in the active-site region.[32] This structural study provides insights into their primary and tertiary structures. A better understanding of the active site of DapL aminotransferase may guide the design of inhibitors.

It has been demonstrated, that DapL pathway is present in only 14.0 % of microbial genomes that have been sequenced.[11] Therefore, the possibility to explore inhibition of DapL enzyme in organisms that employ the DAP pathway could provide the opportunity to develop specific antimicrobials.

In the work present in this dissertation, a comparative study of DapL orthologs from a pathogenic bacterium *Leptospira interrogans*, *Verrucomicrobium spinosum* and from the alga *C. reinhardtii* is presented in order to help to understand the mode(s)/mechanism(s) of DapL inhibition and to facilitate the discovery or development of compounds that could be classified as antibiotics, algaecides or herbicides.

1.2 Results and discussion

1.2.1 DapL activity and kinetic studies

Our collaborator, Dr. André Hudson at Rochester Institute of Technology, (Rochester, NY, USA), sent to our research lab three DapL enzymes from different organisms: *Clamydomonas reinhardtii* (*Cr*DapL, green algae),

Leptospira interrogant (*Li*DapL, pathogenic bacterium) and *Verrucomicrobium spinosum* (*Vs*DapL, soil-dwelling bacterium). All DapL genes (LL-DAP aminotransferase enzymes) were previously cloned into plasmids (pET30A/B vectors) and transformed into BL21-CodonPlus (DE3)-RIPL strains of *Escherichia coli* by our collaborator Dr. A. Hudson. pET30A/B vectors carry kanamycin resistance genes; BL21-Codon Plus (DE3)-RIPL strains carry chloramphenicol resistance genes to doubly select for successfully transformed bacteria.

The expression of recombinant DapL enzymes with hexahistidine tags was induced using isopropylthiogalactoside (IPTG) and overexpressed in *E. coli.* Cells were lysed by sonication, enzymes were purified by nickel affinity chromatography, bound protein was eluted with increasing imidazole concentrations, and purity was assessed by SDS-PAGE.

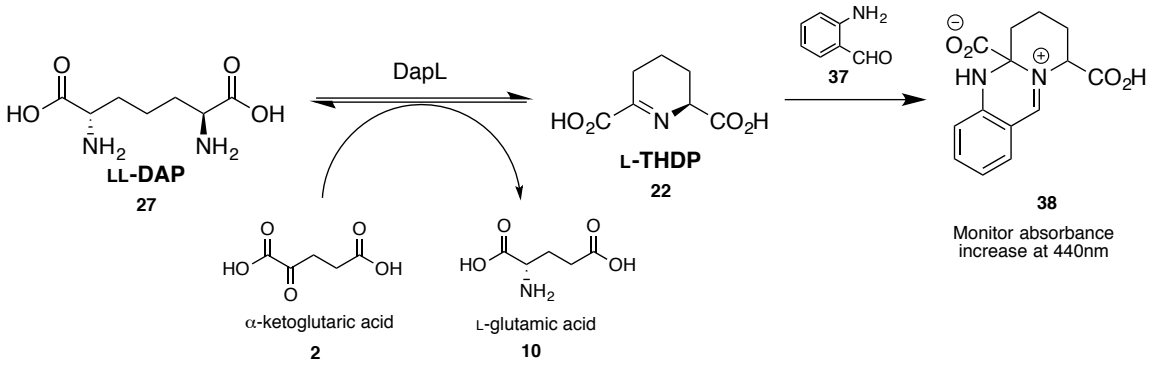


Figure 1.7. Coupled assay system used to determine activity of DapL enzymes.

To study DapL activity of the purified orthologs and their kinetic properties, a coupled assay system was used (Figure 1.7). The production of L-THDP was

measured using *ortho*-aminobenzaldehyde (OAB). This compound yields a dihydroquinazolium adduct that has an absorbance maximum at 440 nm.[15]

The concentration of LL-DAP was varied from 0.0625 to 8.0 mM. The increase in absorbance at 440 nm was monitored in triplicate over a period of 200 min. In order to obtain the data needed to calculate the kinetic parameters, absorptions at 440 nm (A_{440}) were collected every 60 seconds. The absolute concentrations were calculated using the Beer-Lambert Law $A_{440} = \varepsilon$ x [substrate]. The Michaelis constant (K_M) and the catalytic turnover (k_{cat}) for each enzyme were calculated by nonlinear regression analysis using GraphPad Prism version 4.0a (Table 1.2).

Table 1.2. K_M, k_{cat}, K_M/k_{cat} kinetic parameters for DapL orthologs

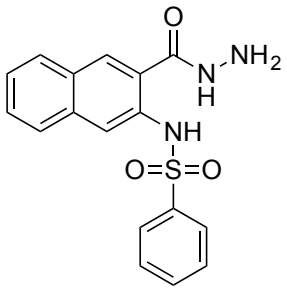
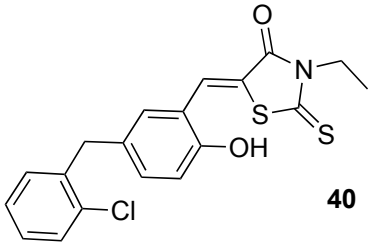
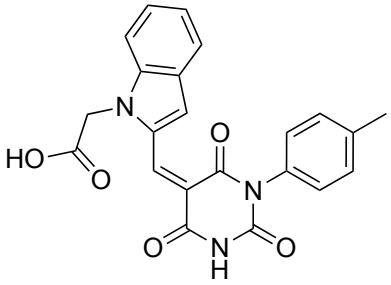
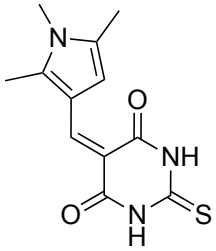
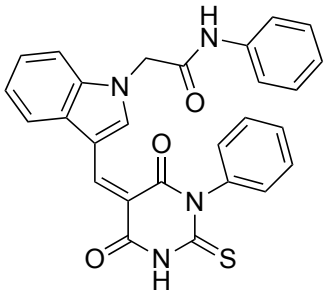
Enzyme	*Vs*DapL	*Li*DapL	*Cr*DapL
K_M for L,L-DAP (mM)	4 ± 1	1.1 ± 0.2	2.7 ± 0.7
k_{cat} (s^{-1})	1.6	5.7	16.4
K_M/k_{cat} (s^{-1} M^{-1})	0.4 x 10^3	5.2 x 10^3	6.1 x 10^3

The results shown in Table 1.2 indicate that *Li*DapL ortholog has the higher affinity for the substrate (L,L-Dap) and *Vs*DapL ortholog has the lowest affinity for the substrate. Moreover, *Cr*DapL and *Li*DapL have similar catalytic efficiency (K_M/k_{cat}), which represents the ability of the enzyme to convert the substrate into product.

1.2.2 Inhibition assay of DapL orthologs

IC_{50} is a measure of the inhibitory activity of a compound for a specific enzyme at the half maximal of inhibitory concentration. From the chemical library recently screened against DapL enzyme from *Arabidopsis thaliana* (*At*DapL),[29] five inhibitors were selected. The selection was based on IC_{50} values (half maximal inhibitory concentration). The inhibitors that showed the lower values of IC_{50} were more effective to inhibit *At*DapL ortholog. These compounds belong to one of the following categories: hydrazide, rhodanine, barbiturate, or thiobarbiturate (Table 1.3). Furthermore, these compounds were selected to evaluate if the inhibition activities of the three enzymes *Cr*DapL, *Li*DapL and *Vs*DapL were similar or comparable to those obtained for *At*DapL from *A. thaliana*.

Table 1.3. Inhibitors selected for DapL inhibition study.

Class of Inhibitor	ChemBridge ID number	Chemical Formula	Structure
Hydrazide	5925714	$C_{17}H_{15}N_3O_3S$	 **39**
Rhodanine	6523070	$C_{19}H_{16}NO_2S_2Cl$	 **40**
Barbiturate	6072466	$C_{22}H_{17}N_3O_5$	 **41**
Thiobarbiturate	6088649	$C_{12}H_{13}N_3O_2S$	 **42**
Thiobarbiturate (time-dependent)	6088649	$C_{27}H_{20}N_4O_3S$	 **43**

Enzymatic inhibition was measured against each inhibitor compound selected in a 1.0 mL cell containing 100 mM HEPES-KOH (pH=7.6), 8.5 mM 2-oxoglutarate, 7.0 mM *ortho*-aminobenzaldehyde, and 4.3 mg of enzyme for *Li*DapL and *Cr*DapL, or 43 mg for *Vs*DapL. The amount of enzyme was determined based on its level of activity. The orthologs *Vs*DapL and *Cr*DapL, showed a very different pattern compared with the model enzyme *At*DapL, while the ortholog *Li*DapL shows a similar inhibition pattern to *At*DapL. In contrast, *Cr*DapL was insensitive to hydrazide **39** (IC_{50} > 200 μM) and *Vs*DapL was insensitive to inhibitor **43** (no inhibition detected), but *Vs*DapL was sensitive to barbiturate **41** and thiobarbiturate **42**, IC_{50} ~5 μM and ~6 μM respectively. The results are summarized in Table 1.4.

Table 1.4. Calculated IC_{50} values in μM for inhibitor against DapL

Inhibitor	*Vs*DapL	*Cr*DapL	*Li*DapL	*At*DapL
Hydrazide (**39**)	47	[a]	6.8	5
Rhodanine (**40**)	250	119	48	46
Barbiturate (**41**)	4.7	56	48	37
Thiobarbiturate (**42**)	5.7	33	28	25
Thiobarbiturate (**43**)	No inhibition	62	25	33

a) <10% inhibition at 200 μM.

1.2.3 Structural insights into DapL

Structural modeling and protein alignment of DapL enzymes from *V. spinosum*, *L. interrogans* using the structural data from the *A. thaliana*, *C. trachomatis*, and *C. reinhardtii* orthologs showed that the amino acids that comprise the active site of DapL are conserved. Moreover, the comparison of the quaternary structure of each DapL enzyme showed that the five orthologs have a very similar active site (V-shape), but this cannot help to describe the differences in inhibition pattern obtained (Figure 1.8).

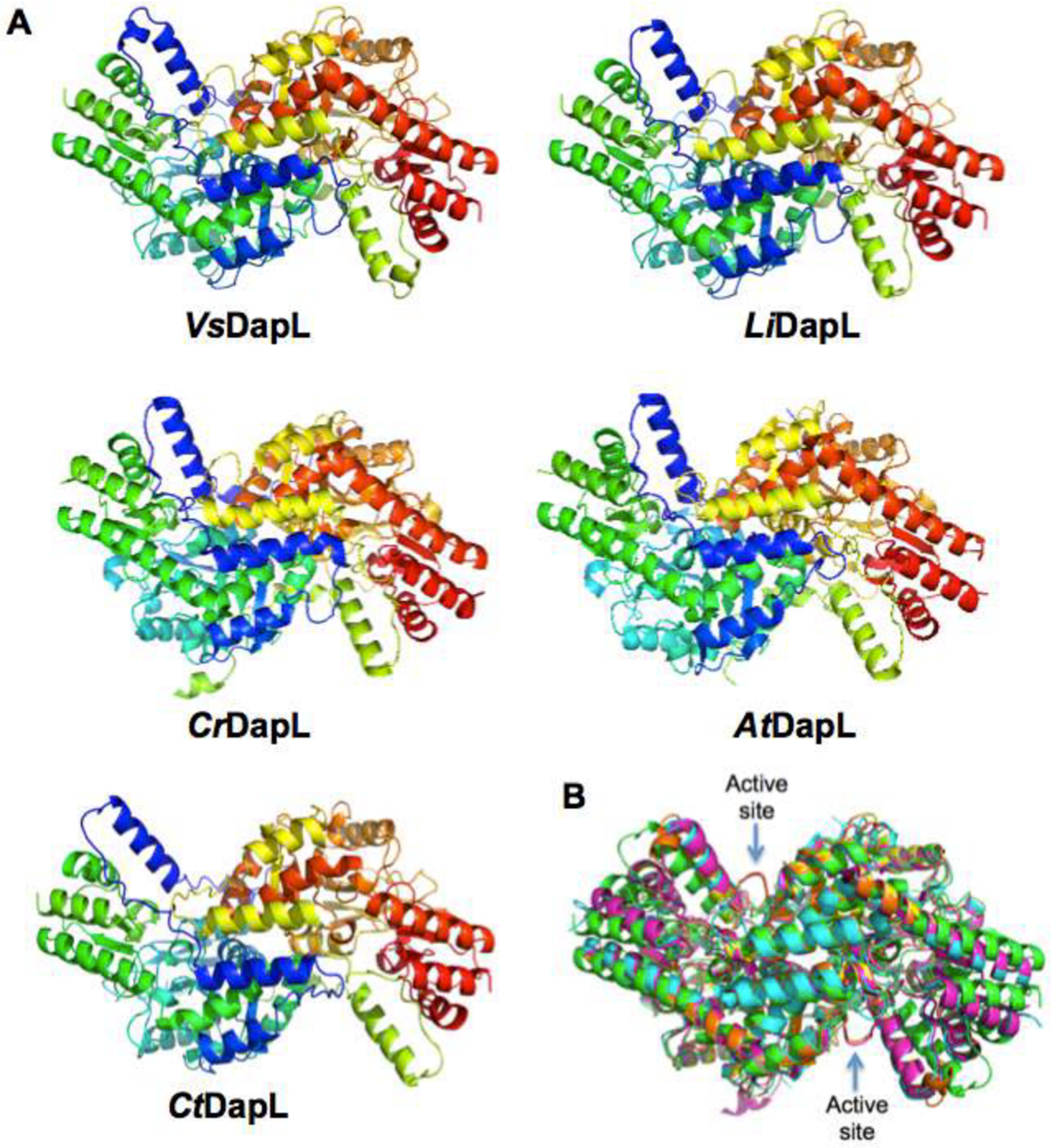


Figure 1.8. A. Cartoon representation of DapL orthologs: Structures of DapL from *V. spinosum*, *L. interrogans* (homology models generated using the Phyre2 server), *C. reinhardtii* (PDB id: 3QGU), *A. thaliana* (PDB id: 3E17) and *C. trachomatis* (PDB id: 3ASA). B. Structural comparison of DapL orthologs: *V. spinosum* in orange, *L. interrogans* in purple, *C. reinhardtii* in yellow, *A. thaliana* in blue and *C. trachomatis* in green.

1.3 Conclusions and Future work

From the three enzymes studied, *Vs*DapL has the lowest catalytic turnover compared with the other orthologs. While both *Cr*DapL and *Li*DapL have similar catalytic efficiency. The inhibition study showed that the selected compounds were effective inhibitors of the DAP orthologs and the IC_{50} values ranged from 4.7 to 250 μM. One of the orthologs (*Li*Dap) had a similar inhibition pattern with the model enzyme (*At*Dap), while the other orthologs showed a very different pattern. The inhibition studies of these key enzymes in the biosynthetic pathway of lysine and peptidoglycan could potentially be used for bactericides, algaecides or herbicides development.

Chapter 2. Tabtoxin

2.1 Introduction

2.1.1 Pseudomonas syringae

Pseudomonas are Gram-negative bacteria that are found in different environments like water, soil, plants and mammals.[35] To this genus belongs *Pseudomonas aeruginosa*, an opportunistic bacterium responsible for pneumonia in humans.[35] Another species of particular interest in this genus is *Pseudomonas syringae*, which is responsible for several diseases in crops. Different strains or pathovars (pv) of this pathogenic bacteria can affect a diversity of vegetables, fruits, legumes, cereals and plants. Some examples of crops affected by this bacterium are: tomato (*P. syringae* pv. tomato), bean (*P. syringae* pv. phaseolicola), rice (*P. syringae* pv. oryzae), kiwi fruit (*P. syringae* pv. actinidiae),[36] oat (*P. syringae* pv coronafaciens),[37] *A. thalina* (*P. syringae* pv. tomato), *Brassica* species (*P. syringae* pv. tomato), etc. These pathogenic bacteria can cause a wide variety of symptoms on the leaf or fruit such as blasts, cankers, galls, necrosis, and chlorosis depending on the strain of *Pseudomonas syringae*.[35]

The phytotoxins produced by *P. syringae* are diverse and some of them contain non-proteinogenic amino acids that are released after hydrolysis by peptidases in the infected host. For example, the hydrolysis of the tripeptide phaseolotoxin (**44**) releases diaminophosphinyl-sulfamoyl-L-ornithine (octicidine) that inhibits ornithine carbamoyltransferase.[38] Syringolin A (**45**) contains two non-proteinogenic amino acids (5-methyl-4-amino-2-hexenoic acid and 3,4-

dehydrolysine), and causes irreversible inhibition of the eukaryotic proteasome.[39] Coronatine (**46**) contains coronafacic acid linked to coronamic acid by a peptide bond, mimics the plant hormone jasmonic acid isoleucine (**47**) and promotes the opening of stomata for bacterial infection (Figure 2.1).[40]

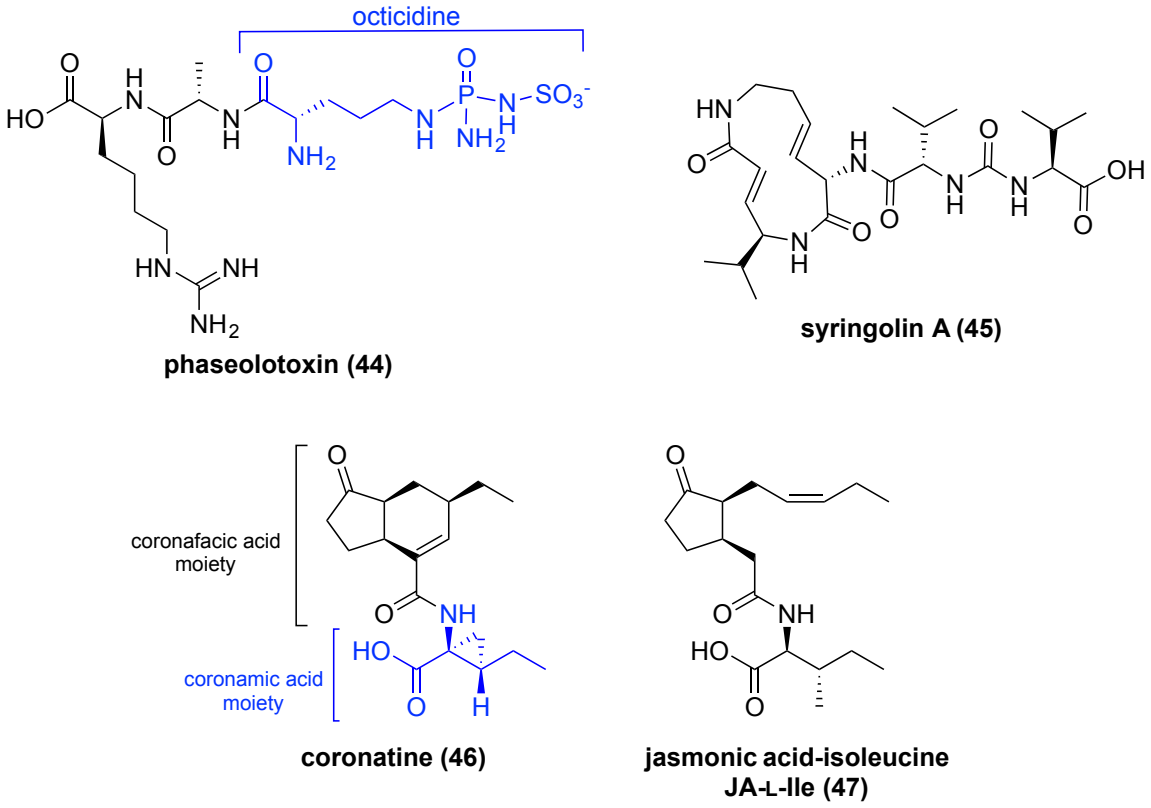
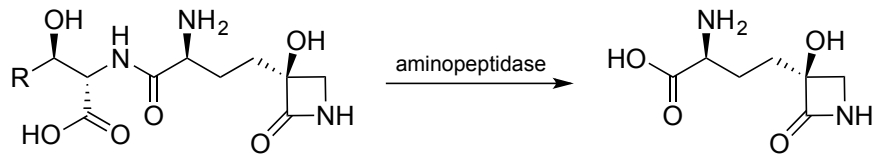


Figure 2.1. Examples of phytotoxins produced by *P. syringae*

2.1.2 Tabtoxin

Tabtoxin (**48**) is another example of a phytotoxin from *P. syringae* that contains a non-proteinogenic amino acid, tabtoxinine-β-lactam (TβL) (**49**). A minor variant of tabtoxin (TβL-Thr) contains L-serine (**50**)[41] instead of L-threonine (Figure 2.2). The Trojan Horse tabtoxin is produced by three different pathovars: *P. tabaci*, *P. coronafaciens* and *P. garcae*. Tobacco wildfire disease is caused by

P. syringae pv *tabaci*, and almost a century ago, it used to be a severe and devastating disease of tobacco causing whole fields to be eliminated.[42] This phytotoxin was named wildfire toxin due to its rapid and destructive spread.

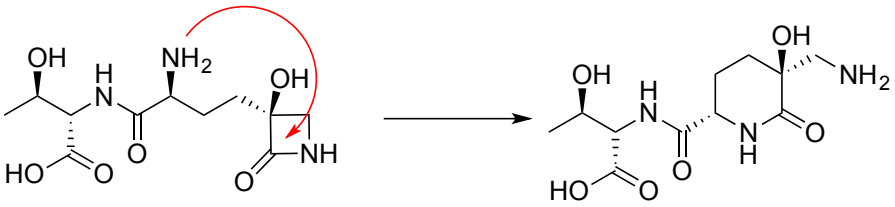


R = CH_3 β-Tabtoxin (TβL-Thr) (48)
R = H β-Tabtoxin (TβL-Ser) (50)

tabtoxinine-β-lactam (TβL) 49

Figure 2.2. Hydrolysis of amide bond in β-tabtoxin (**48**, **50**) by an aminopeptidase to afford the active moiety tabtoxinine-β-lactam (**49**)

Although tabtoxin was isolated by Wooley in 1952,[43] its structure was not elucidated until 1971 by Stewart.[44] This work also showed that tabtoxin can be isomerized as a result of translactamization from attack of the free amine on the β-lactam ring, yielding the six member ring δ-tabtoxin (**51**)[44,41] (Figure 2.3).



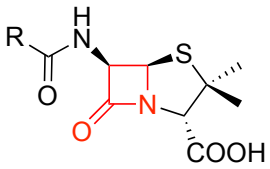
β-Tabtoxin (TβL-Thr) (48)

δ-Tabtoxin (TδL-Thr) (51)

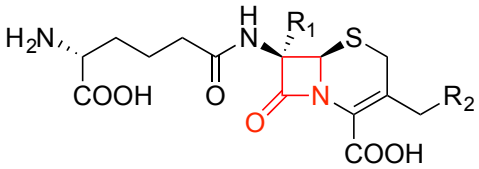
Figure 2.3. Isomerization of β-tabtoxin (TβL-Thr) to δ-Tabtoxin (TδL-Thr).

A remarkable feature of tabtoxin is the presence of the β-lactam ring, that is present in the family of penicillins (**52**), cephalosporins (**53**), clavams (**54**), and

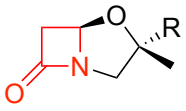
monocyclic β-lactams (**55**)[45] (Figure 2.4). This means, that tabtoxinine-β-lactam could have the potential of being a natural product with antibiotic properties.



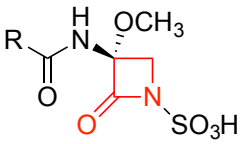
penicillins (52)



cephalosporins (53)



clavams (54)

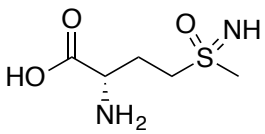


sulfazenic-type monobactams (55)

Figure 2.4. Examples of antibiotic compounds that contain the β-lactam ring.

2.1.3 Glutamine synthetase - Mode of action

Early studies on growth inhibition in plants showed that methionine sulfoximine (**56**, Figure 2.5) induce chlorosis in leaves of higher plants.[43,46] In mammals, the wildfire toxin produced by *P. syringae* pv *tabaci* was shown to cause seizures, similar to the symptoms that are produced by methionine sulfoximine (MSO).[47] This observation led to the hypothesis that tabtoxine could have a similar mode of action like MSO. Preliminary experiments conducted by Sinden and Durbin demonstrated that tabtoxin causes irreversible inactivation of glutamine synthetase (GS).[46]



Methionine sulfoximine (56)

Figure 2.5. Methionine sulfoximine (MSO)

In 1971, after demonstrating the structure of tabtoxin, Stewart suggested some possible mechanisms for inhibition of glutamine synthetase.[44] These were based on the mode of action of MSO, which is phosphorylated during its interaction with the enzyme.[48] He also suggested that the wildfire toxin could inhibit the enzyme through acylation in the active site analogous with the mechanism of action of penicillins (Figure 2.6).

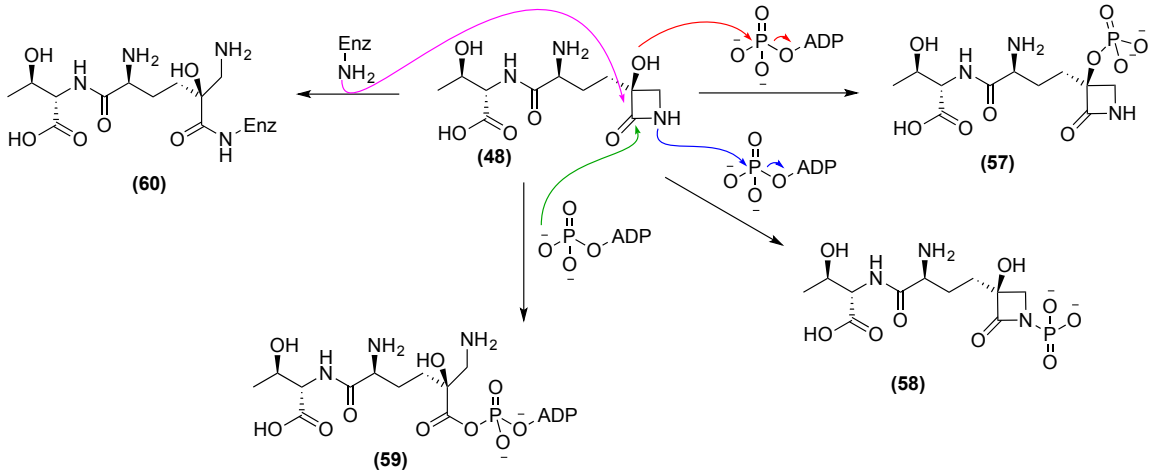


Figure 2.6. Proposed mechanisms of inactivation of GS by Steward (1971).[44]

Later in 1984, Durbin and collaborators,[49] demonstrated that tabtoxin is inactive. However, when the peptide bond in tabtoxin is hydrolysed by aminopeptidases in the host or in the periplasm of the pathogen, TβL is released.[50,51] TβL is toxic because it is responsible for irreversible inactivation of glutamine synthetase (GS) (Figure 2.7). TβL competes for the active site with the natural substrate, glutamate.[46,52]

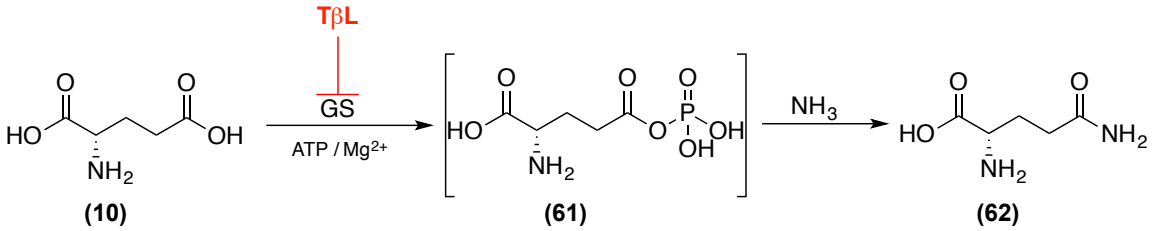


Figure 2.7. Synthesis of L-glutamine and enzyme targeted by TβL.

This inhibition results in accumulation of high levels of ammonia causing chlorosis on the leaves.[46] To date, the exact mechanism of inactivation of GS has not been demonstrated.

2.1.4 Biosynthesis of tabtoxin

Although the structure of tabtoxin was elucidated more than four decades ago, its biosynthetic intermediates have not been completely elucidated. In the late 80's, labeling experiments performed by Unkefer *et al*[53] and Tamm *et al*[54,55] demonstrated that the direct precursors of the moiety tabtoxinine-β-lactam (TβL) are L-aspartate and pyruvate, suggesting that the biosynthesis of tabtoxin branches off from the L-lysine pathway.

For the first time, in 1991, Kinscherf *et al*[5] reported the complete set of biosynthetic and resistance genes. This was the starting point that allowed the study and discovery of the enzymes involved in the biogenesis of tabtoxin. Immediately after this work appeared, Engst and Shaw[56] published the identification of the gene *tabA*, and its high homology to *lysA* which led to the proposal that the enzyme encoded by this gene could recognize substrates that are analogous to *meso*-DAP and L-THDP.

Subsequent genetic studies by Barta *et al.*,[57] led to the finding of the *tblA* gene and it was suggested that this gene may encode a biosynthetic enzyme or a regulatory factor.

In 1997, Liu and Shaw[58] reported the characterization of the *dapB* gene found in *P. syringae* pv tabaci (BR2.024), which encodes L-2,3-dihydrodipicolinate reductase (DapB), and they showed that this gene shares a high sequence homology (60-90%) with known DapB enzymes from Gram-negative bacteria. Also, their results demonstrated that the DapB enzyme is required for both lysine and tabtoxin biosynthesis. Based on their results, the authors suggested that L-THDP is the branch point of lysine and tabtoxin biosynthesis.

In another genetic study, Liu and Shaw[59] described the identification of the *tabB* gene and the enzyme encoded by this gene (TabB) was identified as an acetyltransferase that has a role similar to DapD in *E. coli*, suggesting that it can form an acetyl derivative that can be further transformed into TβL (Figure 2.8). Moreover, the authors mentioned that TabA has significant sequence homology with LysA, a decarboxylase from *E. coli* and *P. aeruginosa*, but it can't complement *E. coli* LysA mutant.[59] These findings, and the results of early studies led Liu and Shaw to suggest a preliminary biosynthetic pathway for TβL (Figure 2.8).

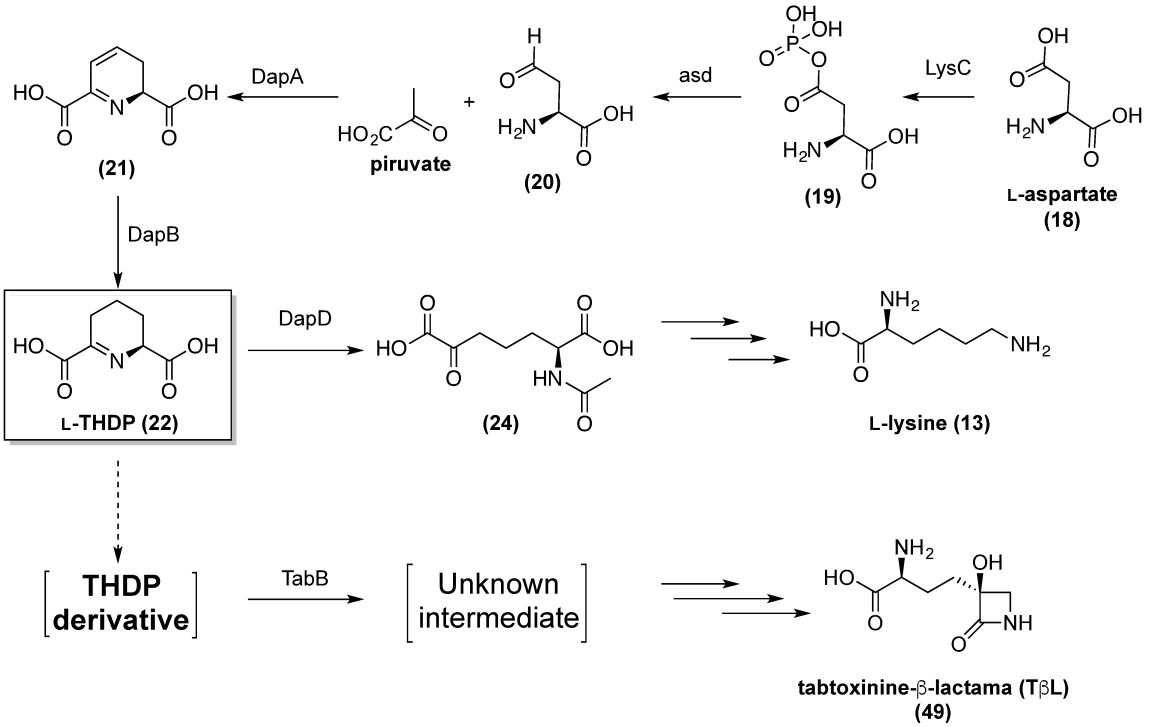


Figure 2.8. Biosynthesis of tabtoxinine-β-lactam (TβL) suggested by Liu and Shaw.

In this biosynthetic pathway, the first step is the phosphorylation of L-aspartate, followed by reduction of the L-4-aspartylphosphate to afford aspartic acid 4-semialdehyde (**20**). The condensation of the semialdehyde with pyruvate to form L-2,3-dihydrodipicolinate (**21**, L-DHDP) is catalyzed by DapA. Then, DapB catalyzes the reduction of L-DHDP to L-THDP (**22**). Based on their results, the authors suggested that L-THDP is the branching point in the biosynthesis of L-lysine and tabtoxin.[59]

In this model, the authors suggested that TabB is an acetyltransferase that converts an unknown structure of a L-THDP derivative into an acetyl derivative that is further transformed into TβL (**49**). Also, their results showed that TabB has

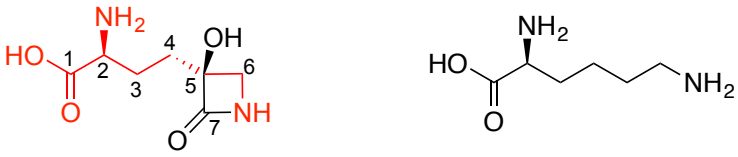
a high sequence homology with DapD from *E. coli,* and the *tabB* gene can replace a defective *dapD* in *E. coli* but is not required for DAP biosynthesis in *P. syringae* pv *tabaci* (BR2.024).[59]

In a more recent publication, Kinscherf and Willis,[60] reported a comparative analysis of the DNA sequence that contains the genes encoding the pathway for both tabtoxin production and host resistance using the GenBank database available on-line at the National Center for Biotechnology Information (NCBI).

Table 2.1. Comparison of Tab genes with similar protein sequences of GenBank[60]

Gene Name	Protein homology or similarity
tabP	Metallopeptidase
tabD	Aat-like aminotransferase
tabB	DapD, tetrahydrodipicolinate *N*-succinyltransferase
tabA	LysA, diaminopimelate decarboxylase
tblA	No matches in database
tabC	No matches in database
tblS	β-Lactam synthetase
tblC	Clavaminic acid synthetase (TauD, taurine dioxygenase)
tblD	Apparent fusion: GMC oxidoreductase-GNAT acetyltransferase
tblE	Membrane protein
tblF	D-ala D-ala ligase
tblR	Sugar transporter (MFS transporter)

According to this table and previous studies, *tabABD* genes are similar to genes in lysine biosynthesis; *tblSCDEF* genes encode enzymes that may participate in the synthesis of β-lactam ring.[60,61] Although some authors[59,61] have pointed to the structural analogy of TβL with lysine, the origin of both, the hydroxyl at C-5 and the carbonyl at C-7 is not clear yet (Figure 2.9).



tabtoxinine-β-lactama (TβL) (49) **L-Lysine (13)**

Figure 2.9. Structural analogy of TβL with L-lysine.

Based on the labeling and genetic studies already discussed, and after discussions with Dr. Christopher T. Walsh (Harvard Medical School) about what is known to date about the biosynthetic pathway of tabtoxin, a novel biosynthesis for the construction of the β-lactam ring can be proposed (Figure 2.10).

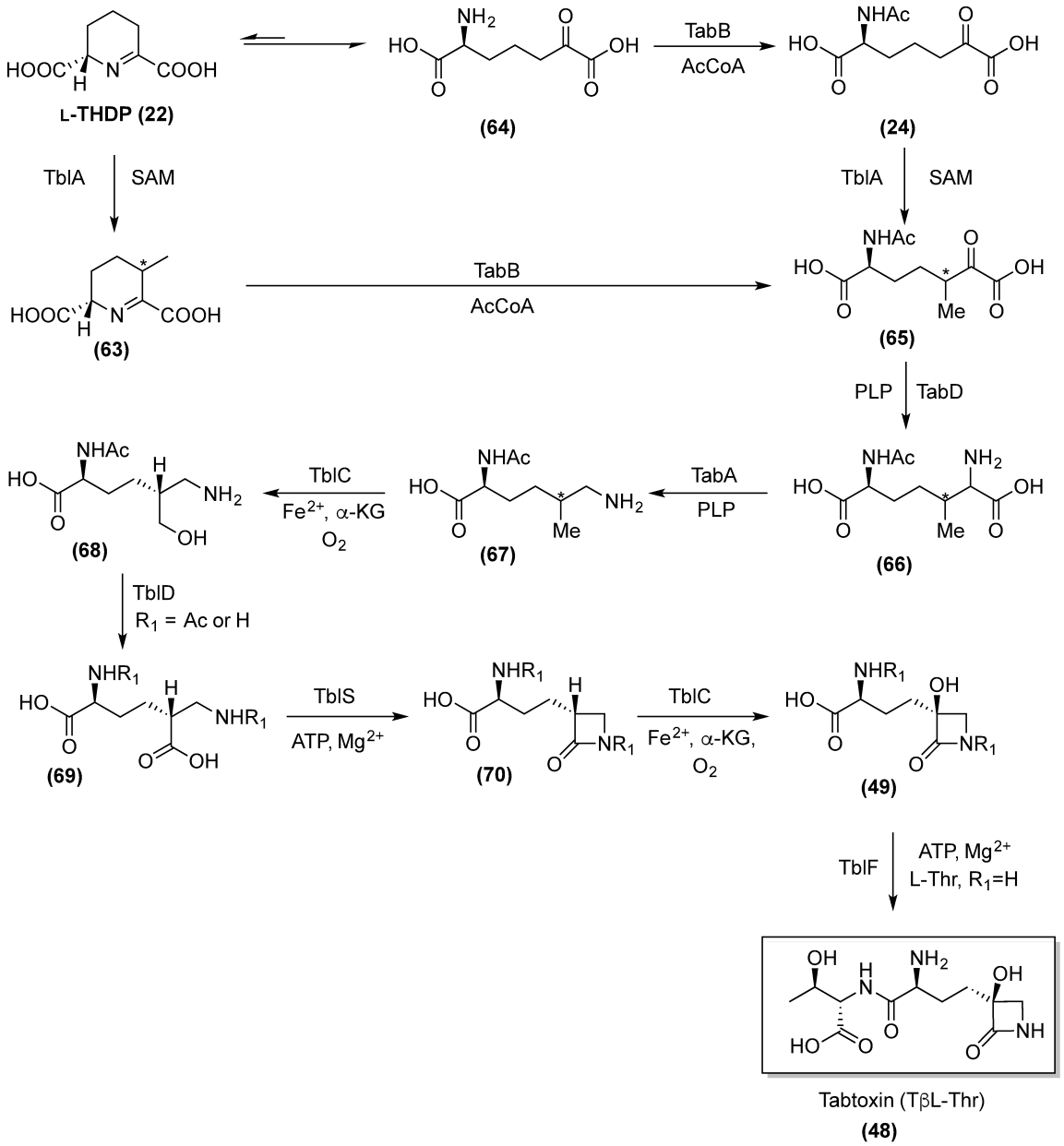


Figure 2.10. Proposed biosynthesis of tabtoxin (TβL-Thr)

This proposed biosynthetic pathway intends to complement what is already known about the biogenesis of tabtoxin. The first step can be branched in two ways. In the first path, L-THDP (**22**) can undergo acetylation on the free amine catalyzed by the enzyme TabB, after opening of the L-THDP ring (**64**), to

afford L-α-keto-ε(*N*-acetylamino)pimelate (**24**), similar to DAP acyl pathway. Then, intermediate (**24**) may undergo methylation catalyzed by TblA and SAM to yield (**65**). In the second option, L-THDP is first methylated by TblA and SAM to give THDP derivative (**63**). Then, the enzyme TabB would convert this methylated L-THDP derivative (**63**) into an acetyl derivative, L-α-keto-β-methyl-ε(*N*-acetylamino)pimelate (**65**) (Figure 2.10). These transformations are suggested based on the hypothesis proposed by Liu and Shaw (Figure 2.8).[59] The acetyl derivative (**65**) could undergo transamination catalyzed by TabD aminotransferase, to afford a methyl-diaminopimelate derivative (**66**). Then, TabA a pyridoxal phosphate (PLP) dependent enzyme, which is highly similar to diaminopimelate decarboxylase (LysA), could catalyze decarboxylation of (**66**) to yield a methyl-lysine derivative (**67**) in the presence of PLP. The inactivated methyl in the intermediate (**67**) would be oxidized to yield (**68**) by enzyme TblC, which has similarity to clavaminic acid synthase,[60] an iron / α-ketoglutarate dependent dioxygenase.[62,63] This oxidative reaction could be related to the reaction catalyzed by taurine dioxygenase (TauD),[60,63] which uses the oxidative decarboxylation of α-ketoglutarate (αKg) to activate dioxygen and catalyze the hydroxylation reaction (Figure 2.11).

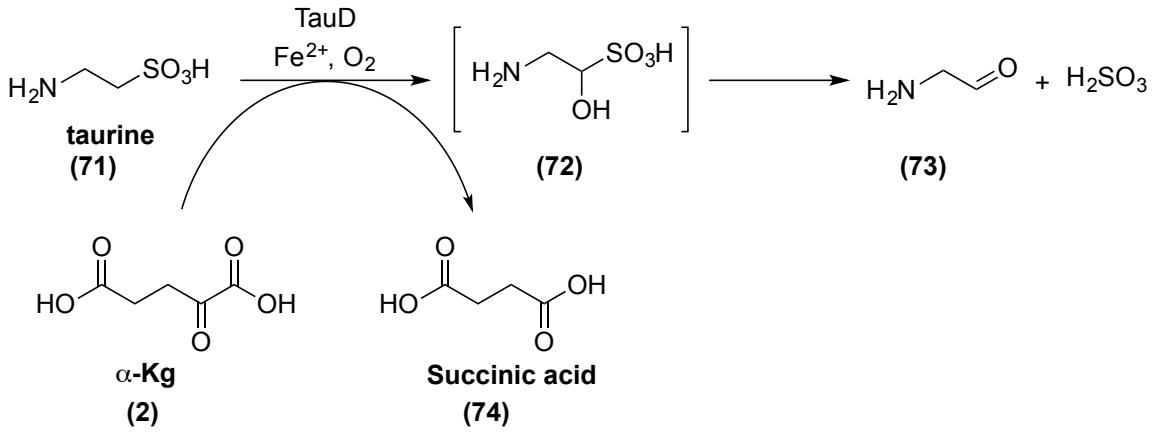


Figure 2.11. Catabolism of taurine by taurine dioxygenase (TauD).

Further oxidation of intermediate (**68**) could be catalyzed by TblD to afford 2-acetylamino-5-(aminomethyl)hexedioic acid (**69**). This enzyme seems to be a glucose-methanol-choline (GMC) oxidoreductase fashioned with a GNAT *N*-acetyltransferase domain on the carboxy-terminal end.[60] The β-lactam ring (**70**) would be constructed by cyclization of intermediate (**69**) catalyzed by TblS enzyme, a β-lactam synthetase homolog. The hydroxylation of the β-lactam intermediate (**70**) could be done by TblC, a non-heme iron-containing oxygenase, to afford the tabtoxinine-β-lactam (TβL, **49**). Finally, as a mechanism of self-protection in the bacteria, the non-proteinogenic amino acid tabtoxinine-β-lactam (TβL) would be linked to the amino acid L-threonine by TblF ligase[61] to yield tabtoxin (**48**).

Some limitations have delayed the complete elucidation of the biosynthesis of tabtoxin. Firstly, the scarcity of the pure toxin, and secondly, the instability of the β-lactam ring ($t_{½}$ = 24 h at 25 ºC and pH 7). TβL undergoes

intramolecular rearrangement to afford the stable six-membered ring, creating an inactive δ-lactam isotabtoxin (TδL).

The difficulty in obtaining intermediates could be due to rapid conversion of intermediates to the product, as well as the transfer of these intermediates to different domains within the enzyme before being released into the cytoplasm,[64] making the study of the biosynthetic pathway more challenging. Hence, the chemical synthesis of labeled intermediates could help to demonstrate the complete set of intermediates involved in the biosynthesis of tabtoxin, and at the same time, contribute to the understanding of the function of each enzyme involved.

The study of the biosynthetic pathway of tabtoxin may help to isolate useful intermediates and understand the biogenesis of the β-lactam ring, which belongs to the family of natural antibiotic monobactams. The significance of the biosynthesis and resistant mechanisms is essential for practical applications in agriculture.

2.1.5 Synthetic approach

The goal of this project is to synthesize three hypothesized tabtoxin intermediates labeled with ^{13}C or D to elucidate the biosynthetic pathway of tabtoxin with enzyme feeding experiments. In order to elaborate a synthetic methodology towards the labeled compounds, unlabeled intermediates were pursued (Figure 2.12).

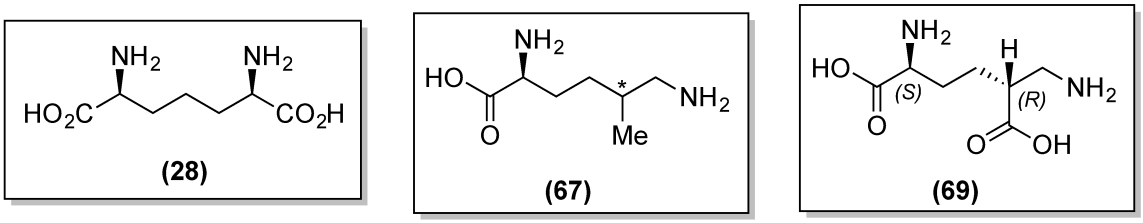


Figure 2.12. Selected hypothesized tabtoxin intermediates for synthesis.

meso-DAP amino acid (α,ε-diaminopimelate, **28**) was selected because it can be interconverted to L-THDP (**22**) by oxidative deamination using DAP dehydrogenase enzyme[65] (Figure 2.13). Therefore, a labeled L-THDP could be used in feeding experiments to test if TblA enzyme can produce the methylated L-THDP derivatived **63** (Figure 2.10). Moreover, with the three potential substrates in hand (**22, 67, 69**) the enzymes TblA, TabB, TabD, TblC, TblS and TblF can be tested to demonstrate the proposed biosynthesis of tabtoxin.

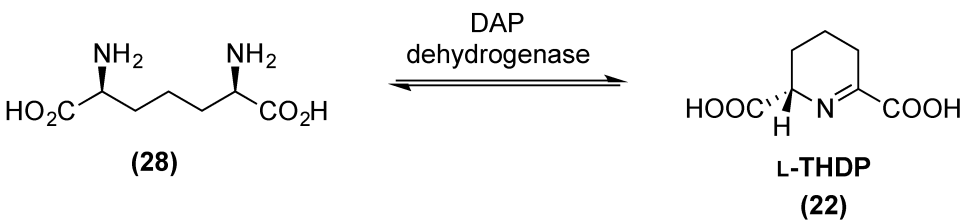
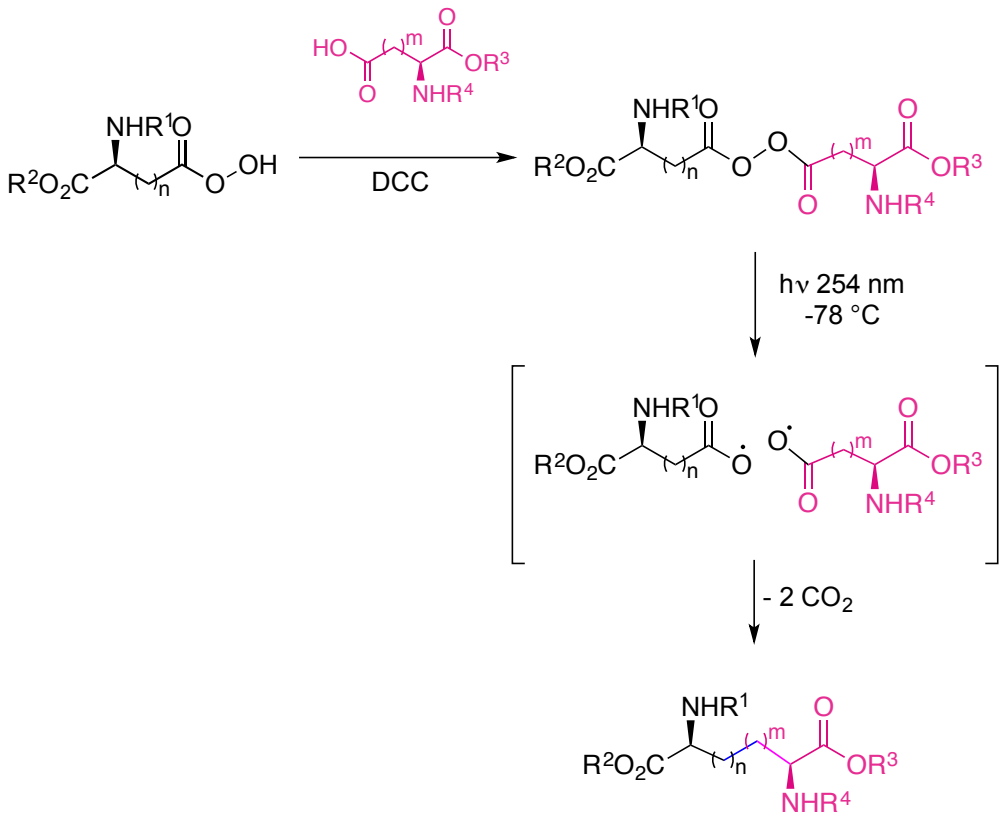


Figure 2.13. Enzymatic conversion of DAP (**28**) into L-THDP (**22**).

To the best of our knowledge, there is no precedent for the asymmetric synthesis of the novel functionalized amino acids **67** and **69**. A convenient methodology that has been successfully employed in our group for the synthesis of unusual amino acids is the photolysis of diacyl peroxides.[66,67] In these studies, L-aspartic acid or L-glutamic acid were used to generate diacyl peroxide derivatives (Scheme 2.1).

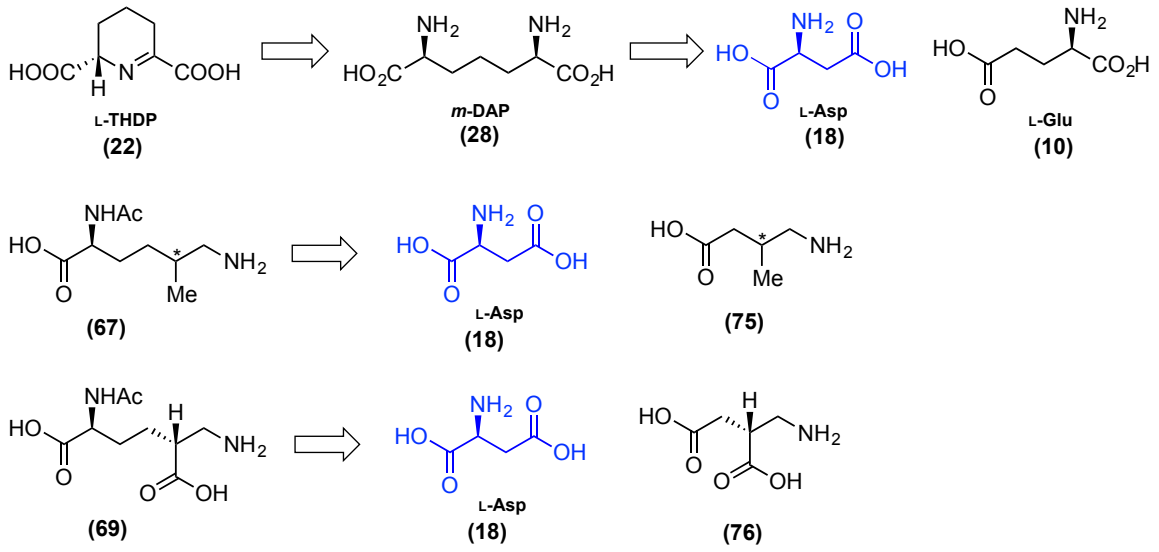


Scheme 2.1. Synthesis of functionalized amino acids by photolysis of diacyl peroxides.

The neat photochemical cleavage of the peroxide bond at low temperature resulted in decarboxylation, followed by coupling of the radical species formed *in situ*, yielding a functionalized α-amino acid which is orthogonally protected (Scheme 2.1). This method proved to be a good option to provide symmetrical

and unsymmetrical amino acids in good yields (52-58%) without affecting the chiral centers.[66,67]

The retrosynthetic analysis of the three proposed biosynthetic intermediates of tabtoxin (**22**, **67**, **69**) shows that L-aspartic acid (**18**) is a common precursor in the synthesis of the three proposed substrates using a diacyl peroxide approach (Scheme 2.2).

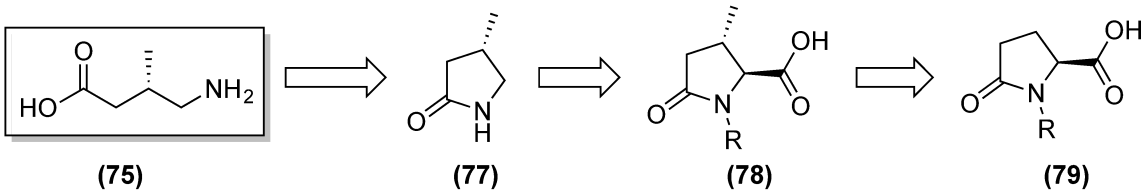


Scheme 2.2. Diacyl peroxide approach.

Therefore, the starting materials of *meso*-DAP (**28**) are the commercially available L-glutamic acid (**10**) and L-aspartic acid (**18**). However, in the case of the unusual amino acids, (2*S*)-2-acetamido-6-amino-5-methylhexanoic acid (**67**) and (2*S*,5*R*)-2-acetamido-5-(aminomethyl)hexanedioic acid (**69**), their respective starting material **75** and **76** need to be prepared using a different approach.

The retrosynthetic analysis of 4-amino-3-methylbutyric acid (**75**) shows that this compound could be made from hydrolysis of an oxopyrrolidine derivative

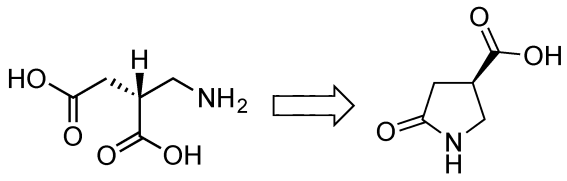
(**77**), which is the product of decarboxylation of a pyroglutamic acid derivative (**78**) that comes from the asymmetric methylation of pyroglutamic acid (**79**) (Scheme 2.3).



(75) **(77)** **(78)** **(79)**

Scheme 2.3. Retrosynthetic analysis of 4-amino-3-methylbutyric acid **75**.

Using a similar approach, (*R*)-2-(aminomethyl)butanedioic acid (**76**) can be envisioned as the product of hydrolysis of oxopyrrolidine derivative (**80**) as well (Scheme 2.4).

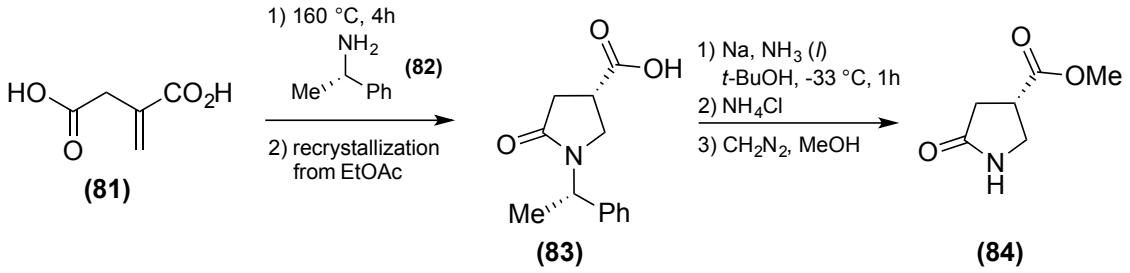


(76) **(80)**

Scheme 2.4. Retrosynthetic analysis of compound **76**.

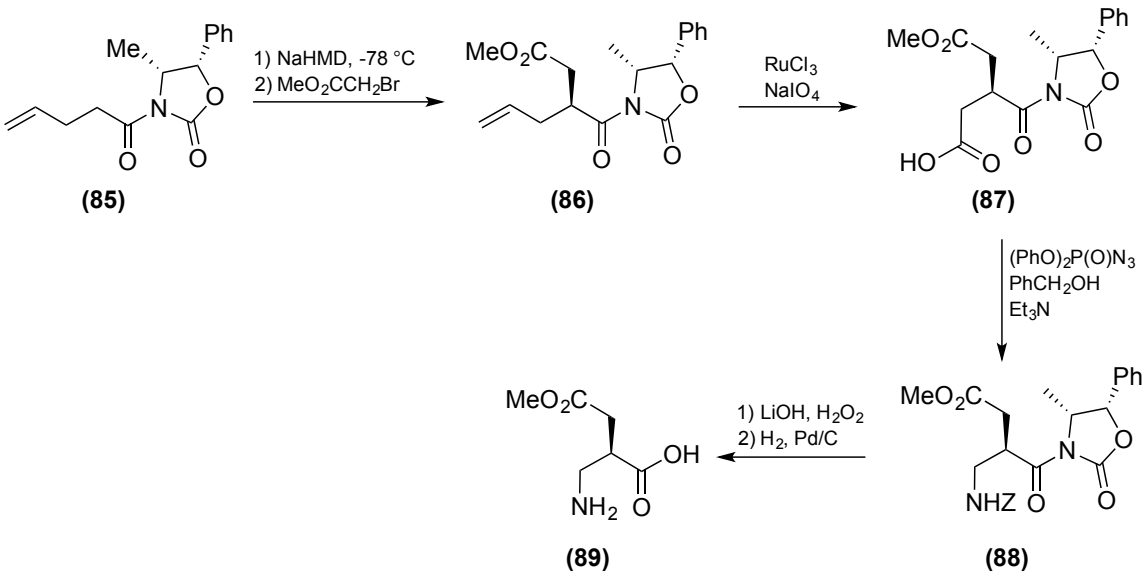
Wyatt *et al.* reported an asymmetric synthesis of the enantiomers of **76** and **80**[68] (Scheme 2.4). Heating 2-methylene succinic acid (**81**) with (*S*)-1-phenylethylamine (**82**) gave a 1:1 mixture of diastereomeric pyrrolidonecarboxylic acids. Recrystallization from ethyl acetate afforded the diastereomer with (*S*) configuration at C-4 (28%). The cleavage of the α-methylbenzyl moiety was effected by a Birch reduction, followed by esterification with diazomethane to yield compound **84** in 5%. Although this synthesis is short, the low yields and the

harsh conditions to remove the α-methylbenzyl moiety makes this approach not suitable (Scheme 2.5).



Scheme 2.5. Synthesis of **84** from 2-methylene succinic acid **81**.

Alternatively, Wyatt *et al.* prepared the enantiomer (*S*)-2-(aminomethyl)-butanedioic acid **89** by alkylation of a sodium enolate **85** bearing an Evans auxiliary with methyl bromoacetate.[68] Subsequent oxidation of the double bond in **86** afforded the carboxylic acid **87**, which undergoes Curtius rearrangement to yield **88**. Removal of the Evans auxiliary and protecting groups furnishes **89** (Scheme 2.6). Therefore, the adaptation of this methodology can be used to synthesize the desired enantiomer (*R*)-2-(aminomethyl)butanedioic acid (**76**).

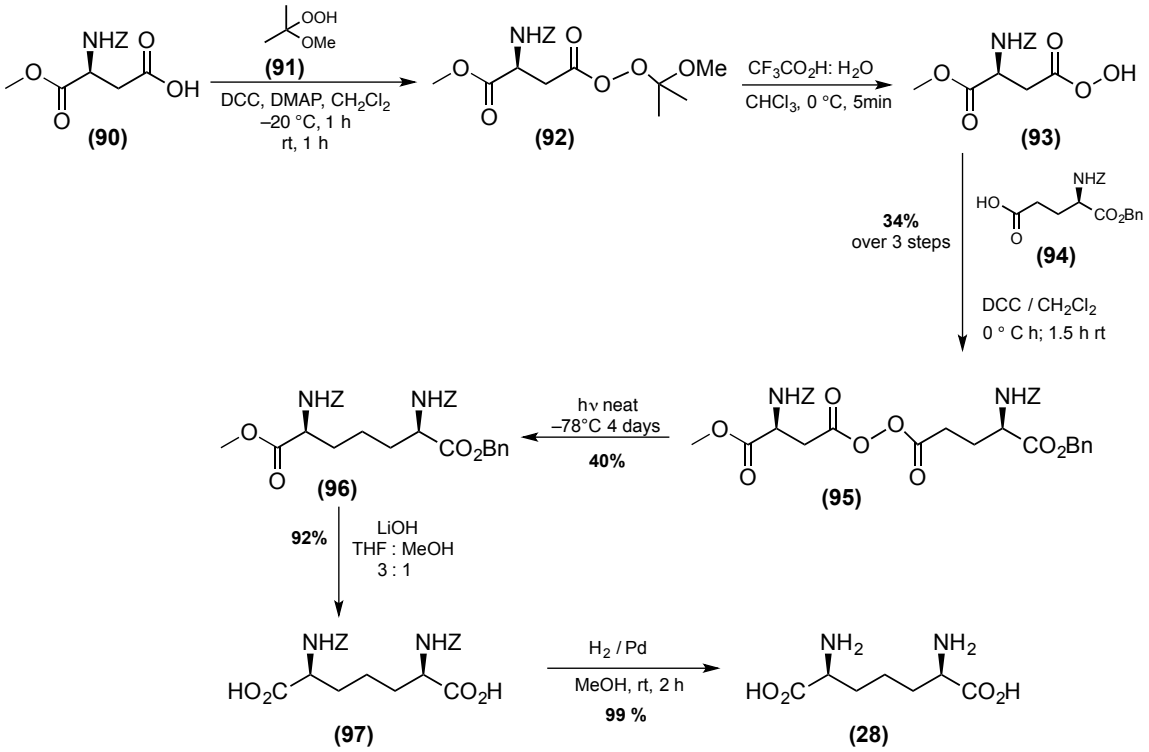


Scheme 2.6. Synthetic methodology of (*S*)-2-(aminomethyl)butanedioic acid **89** by Waytt.

2.2 Results and discussion

2.2.1 Asymmetric synthesis of *meso*-DAP by diacylperoxide approach

meso-DAP (**28**) was synthesized following a methodology previously reported in the literature[66] (Scheme 2.7). This method allowed the coupling of two different partners, which are orthogonally protected, Z-L-Asp-OMe (**90**) and Z-D-Glu-OBn (**94**) without affecting the chiral centers.



Scheme 2.7. Synthesis of *meso*-DAP by diacyl peroxide approach.

Firstly, Z-L-Asp-OMe (**90**) was activated with *N,N'*-dicyclohexylcarbodiimide (DCC) and coupled with 2-methoxyprop-2-yl hydroperoxide (**91**) to afford perester (**92**). After purification by column chromatography, acid deprotection with trifluoroacetic acid (TFA) yields the peracid (**93**), which was coupled with Z-D-Glu-OBn (**94**) to give the corresponding diacyl peroxide (**95**). The crude product was purified by column chromatography as well. Then, the pure product (**95**) was spread on the surface of a cylinder of 500 mL volume and left at neat conditions at –78 ºC under argon atmosphere. The photolysis was carried out with a UV-lamp for 4 days. The reaction mixture was collected and purified by flash chromatography to yield 40% of *meso*-DAP orthogonally protected (**96**). Basic hydrolysis of methyl ester **96** with LiOH gave

the free acid **97** in 92% yield. Finally, hydrogenolysis with palladium/carbon in methanol removes the amine protecting groups to afford *meso*-DAP (**28**). This approach consisted of 6 steps with an overall yield of 12.5% (unlabeled material).

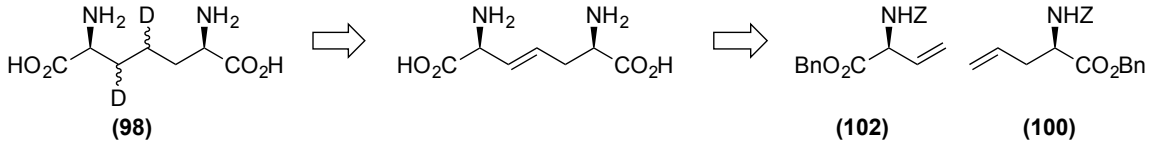
Although this methodology can provide *meso*-DAP, we could not envision a way to introduce deuterium or ^{13}C in a later stage of this synthesis. One alternative was to use labeled L-aspartic acid (2,3,3-3D), but this needs further orthogonal protection adding more steps at the beginning of this approach, which could be more time consuming and expensive. For that reason, I looked at the literature for an alternative that would allow me to prepare labeled material.

2.2.2 Asymmetric synthesis of *meso*-DAP by cross metathesis

The cross metathesis (CM) permits the coupling of two terminal alkenes with concomitant formation of a new alkene catalyzed by ruthenium carbene complexes (Scheme 2.8). The improvement in stability of the ruthenium catalysts has allowed a more extensive use of cross metathesis in organic synthesis as a key step to form C-C bonds in natural products,[69] amino acid derivatives,[70] as well as in the formation of heterocyclic rings and olefins with diverse functional groups that can be further transformed.[71]

Scheme 2.8. Cross metathesis of two terminal olefins.

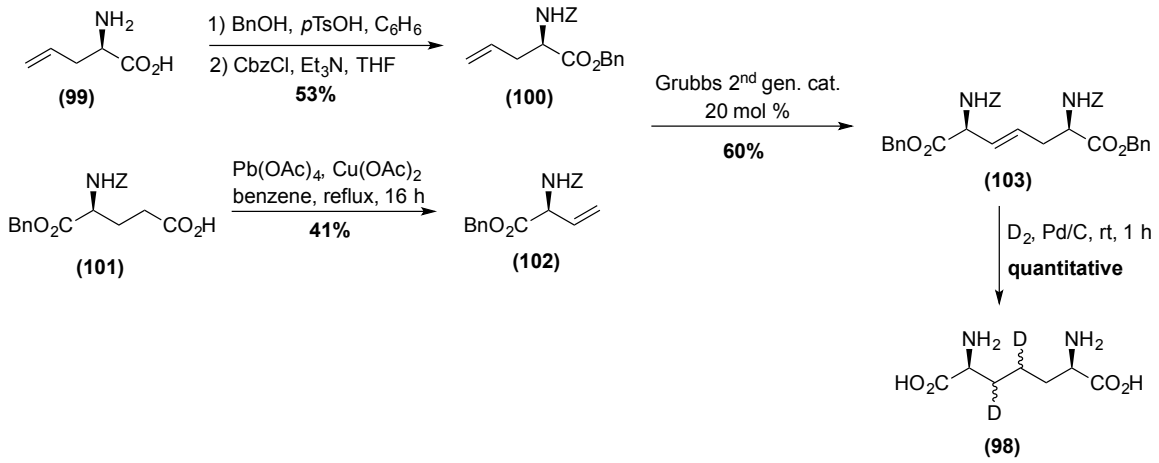
Recent publications have used this reaction to have access to diaminopimelic acid derivatives by preparation either in solution[72,73] or solid phase.[74] The advantage of this approach is that the starting materials allyl and vinyl glycine are easily accessible and in one-step, a diaminopimelic acid derivative containing a double bond is formed. Further reduction of the double bond would afford the diaminopimelic acid. Therefore, we considered the double bond as a potential route to introduce deuterium, leading to the preparation of labeled *meso*-diaminopimelic acid **98** (Scheme 2.9).



Scheme 2.9. Retrosynthetic analysis of labeled *meso*-DAP.

Thus, labeled *meso*-DAP was synthesized by modification of previously reported literature procedures.[73,75] This approach was based on a cross metathesis, which allowed for preparation of deuterium labeled material (Scheme 2.10). First, the commercially available D-allylglycine (**99**) was protected as benzyl ester, followed by protection of the amine as carbamate with the carboxybenzyl group (Z) to give Z-D-Agl-OBn (**100**). The vinylglycine derivative (**102**) was prepared using Hanessian's method,[75] which consists of decarboxylation of glutamic acid assisted by lead tetraacetate and cupric acetate. Coupling of Z-D-Agl-OBn (**100**) and Z-L-Vgl-OBn (**102**) was achieved using Grubbs second generation catalyst to afford *meso*-DAP derivative **103**.

Reduction of the double bond with deuterium gas with a catalytic amount of palladium on carbon allowed the introduction of deuterium into the alkene. Removal of the protecting groups in one step yielded the labeled *meso*-DAP (**98**) (Scheme 2.10).

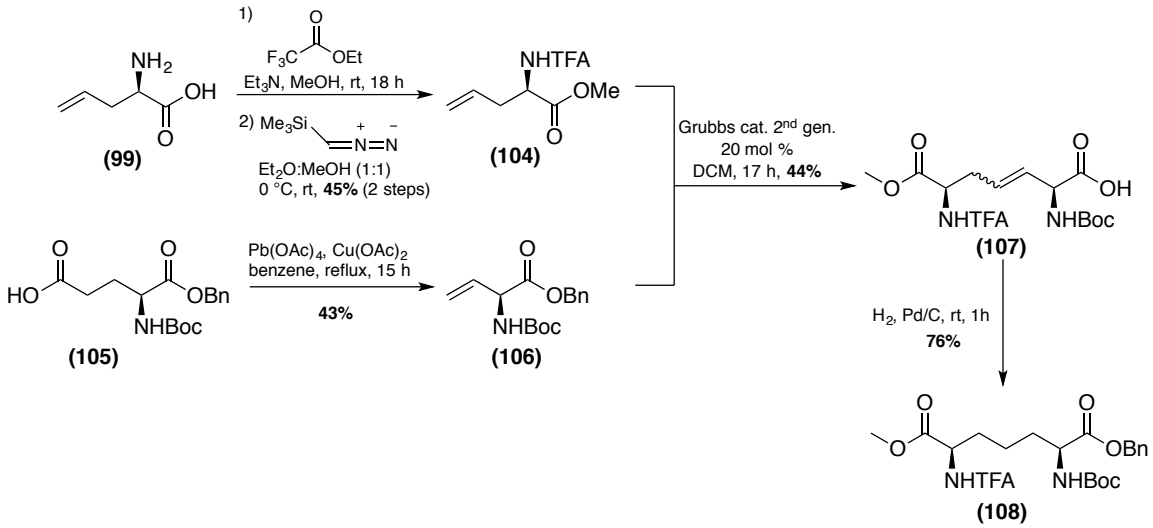


Scheme 2.10. Synthesis of labeled *meso*-DAP (**95**).

The overall yield is 13% for the CM approach, and it is shorter than the diacyl peroxide approach, although the overall yield is similar (12.5%). The advantage of the CM methodology is the introduction of deuterium through the alkene moiety to afford the labeled compound **98**.

Furthermore, after being successful in the synthesis of labeled *meso*-DAP with the ruthenium catalyzed CM reaction, we decided to use this methodology to prepare the orthogonally protected diaminopimelic acid **108** to investigate the mode and mechanism of action of Tridecaptin A_1 ($TriA_1$). This compound is a lipopeptide with selective antimicrobial activity against Gram-negative bacteria. In order to study its bactericidal effect, synthesis of an analog of lipid II was done in collaboration with several members of our research group: Stephen A. Cochrane,

Brandon Findlay, Alireza Bakhtiary and Jeella Z. Acedo. My contribution to this project was the syntheses of an orthogonally protected *meso*-DAP by using the cross metathesis methodology (Scheme 2.11).



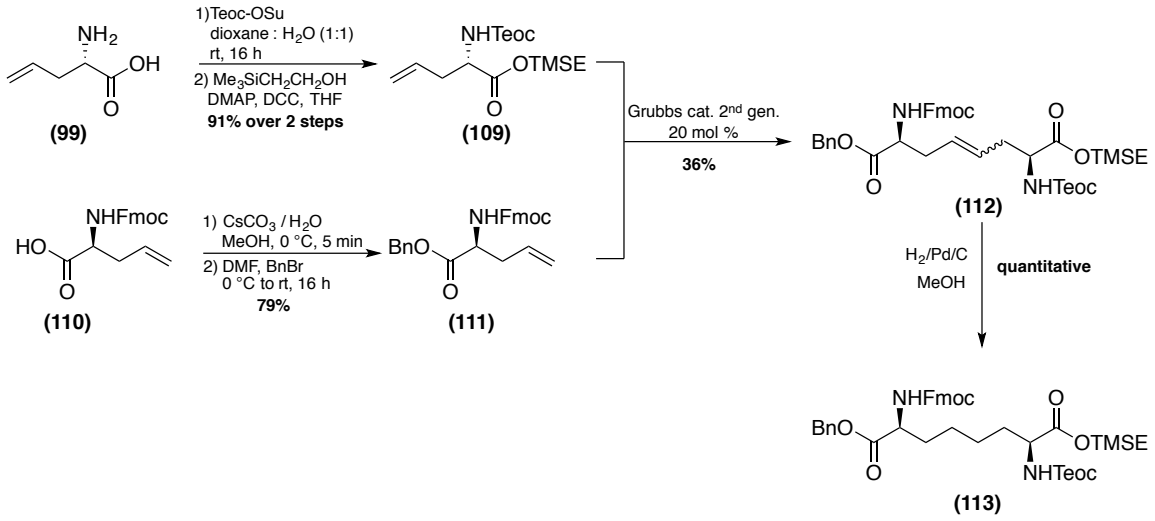
Scheme 2.11. Synthesis of orthogonally protected *meso*-DAP **108**.

This work has been already published as "The Antimicrobial Lipopeptide Tridecaptin A_1 Selectively Binds to Gram-Negative Lipid II" in Proceedings of the National Academy of Science of the United States of America (PNAS) in 2016.[76]

Additionally, the synthesis of an orthogonally protected (*S*,*S*)-2,7-diaminosuberic acid **113** was achieved by cross metathesis methodology as well. The purpose of the synthesis of compound **113** was to synthesize three carbon analogs of the natural neopetrosiamides A & B. In order to study if replacing one of the sulfur bridges with a carbon analog like (*S*,*S*)-2,7-diaminosuberic acid **113** would increase stability and/or activity of this potent inhibitors of cancer cells.

First, commercially available L-allylglycine was protected either with Teoc/TMSE or Fmoc/Bn protecting groups. The initial product of the cross metathesis was an unsaturated C4-bridge, that was an inseparable mixture of

both *E* and *Z* isomers (**112**). Later hydrogenation of compound **112** with palladium over carbon yields orthogonally protected (*S*,*S*)-2,7-diaminosuberic acid (**113**) (Scheme 2.12).

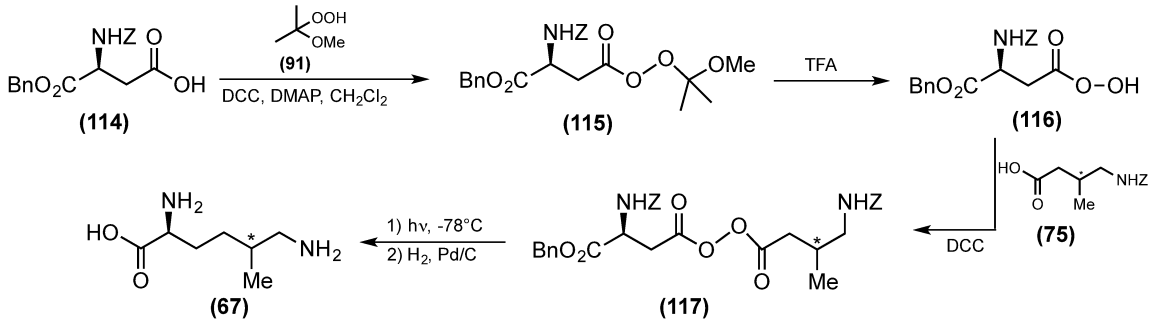


Scheme 2.12. Synthesis of orthogonally protected (*S*,*S*)-2,7-diaminosuberic acid.

The synthesis of the analogs of the natural neopetrosiamides A & B was conducted by Kaitlyn Towle.

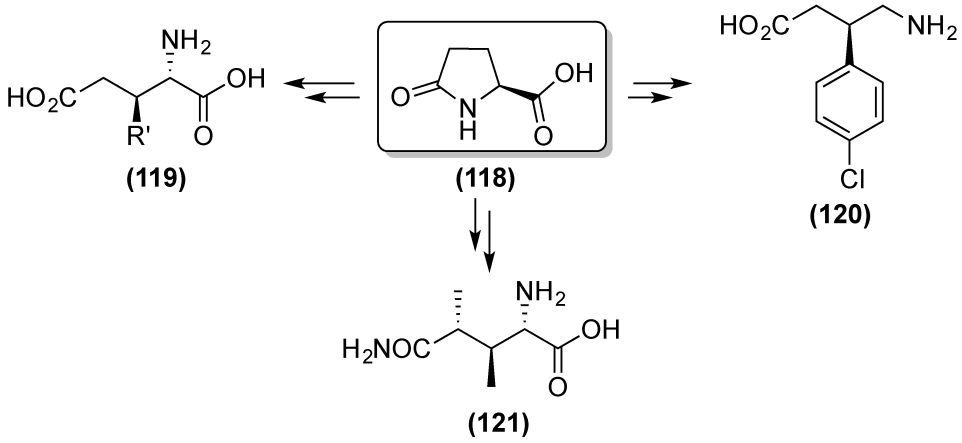
2.2.3 Pyroglutamic acid as synthetic scaffold

Our first approach for the synthesis of compound **67** was based on a diacyl peroxide approach (Scheme 2.2), where the γ-aminobutyric acid derivative **75** needs to be coupled with the aspartic acid derivative **116** by a similar methodology used for compound **28** (Scheme 2.7 and 2.13).



Scheme 2.13. Initial approach for the synthesis of compound **67**.

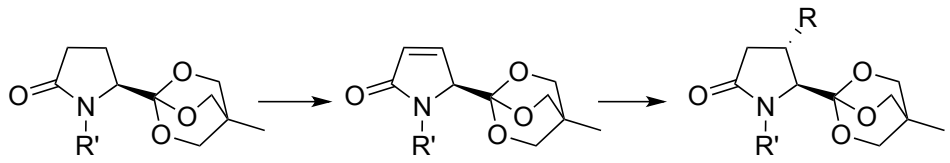
The synthesis of 4-amino-3-methylbutyric acid (**75**) could be accomplished from pyroglutamic acid (**118**). There are examples in the literature where pyroglutamic acid (**118**) has been used as a scaffold to prepare L-glutamic acid (**119**), γ-aminobutyric acid (**120**), and L-glutamine (**121**) derivatives (Scheme 2.14).[77,78]



Scheme 2.14. Pyroglutamic acid as scaffold to prepare amino acid derivatives.

Herdeis and Kelm reported a methodology[79] using Corey's protecting group 4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl (OBO-ester)[80] to protect the carboxylic acid moiety in the pyroglutamic acid (**118**) and prepare in a

diastereoselective manner 3-substituted (alkyl or aryl) pyroglutamic acids after the introduction of a double bond by established procedures (Scheme 2.15).

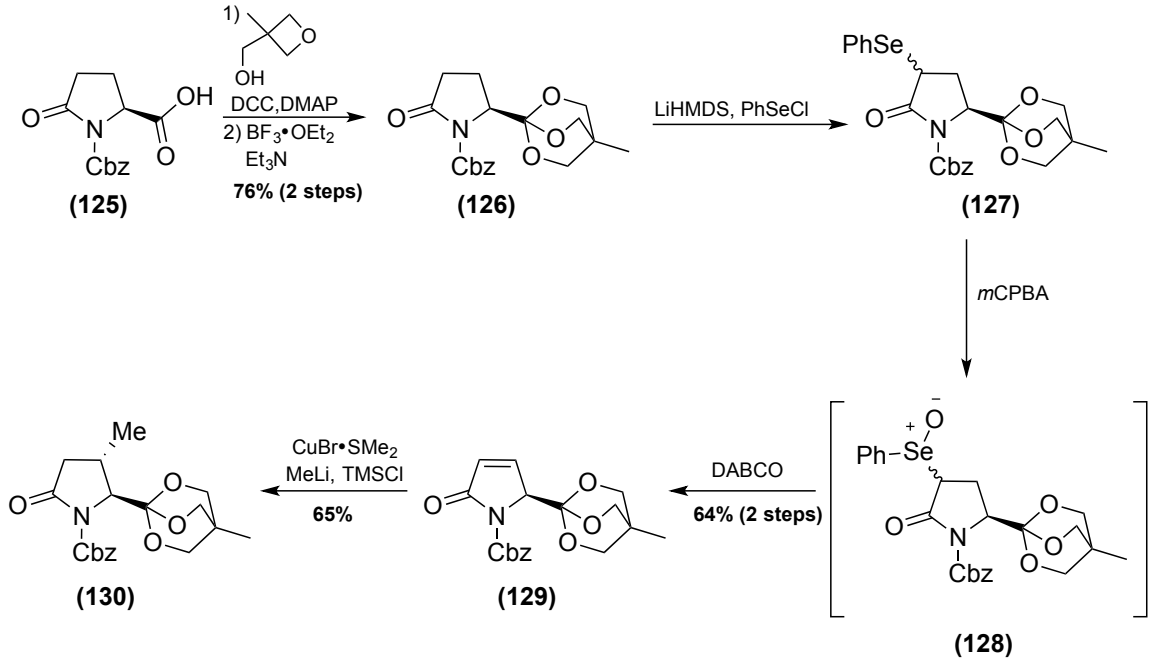


Scheme 2.15. Synthesis of 3-substituded pyroglutamic acid derivatives.

The purpose of using the OBO-ester protecting group has two advantages: first, to reduce the acidity of the α-proton, which allows further transformations without racemization of the stereochemistry at the α position; and secondly, to accomplish a 1,4-Michael addition of an organocuprate in a stereoselective fashion.

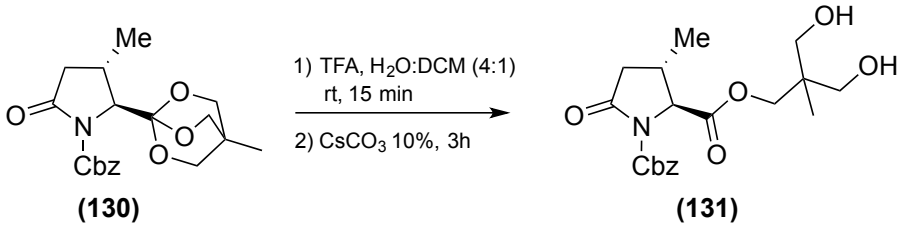
By modification of this methodology, I started the attempt to synthesize 4-amino-3-methylbutyric acid (**75**). Firstly, Z-L-pyroglutamic acid was coupled with 3-hydroxymethyl-3-methyloxetane, using DCC with catalytic amounts of DMAP in dichloromethane. After purification by flash chromatography, the resulting oxetane ester derivative undergoes cyclization, catalyzed by BF_3•OEt_2, to yield the OBO-ester **126** (76%, 2 steps). In order to introduce an unsaturation, a selenoxide was formed.[81-82] The enolate of compound **126** reacted with phenylselenyl chloride to form the α-phenylselenocarbonyl **127** followed by oxidation with *m*CPBA to yield the corresponding selenoxide **128**. The elimination is enhanced with base (DABCO), forming the α,β-unsaturated compound **129** (64%). The 1,4-addition of the methyl organocuprate formed *in situ* to **129** affords

the product **130** in a stereoselective manner with a moderate yield (65%) (Scheme 2.16).



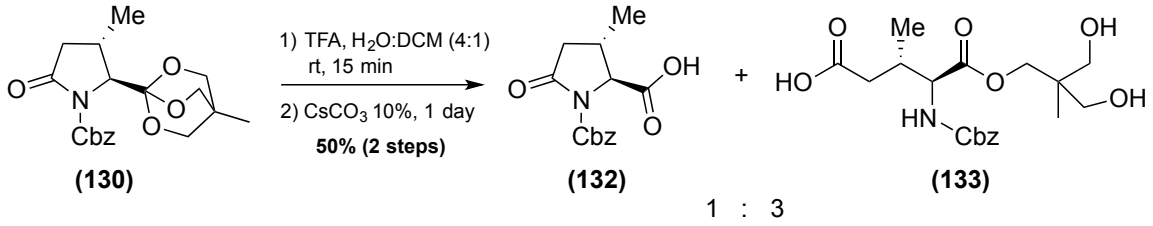
Scheme 2.16. Synthesis of 3-methyl-pyroglutamic acid derivative.

The next step was the attempt to regenerate the carboxylic acid moiety using a literature procedure.[79] The opening of the OBO ester **130** with aqueous trifluoroacetic acid in dichloromethane led to the corresponding dihydroxy ester **131**. Hydrolysis of the ester with cesium carbonate should give the carboxylic acid. However, the first attempt didn't yield the desired product **132**, instead dihydroxy ester **131** was recovered (Scheme 2.17).



Scheme 2.17. Removal of OBO ester in **130**.

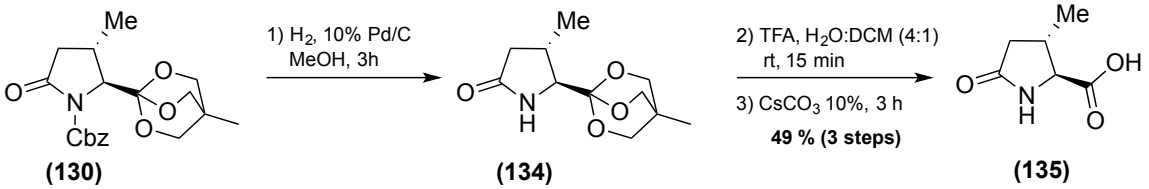
In order to afford the product, several conditions were tried. In the next attempt, we reduced the amount of TFA from 4 equivalents to 1.6 equivalents and reduced the amount of water to 11 equivalents, but this only extended the reaction time from 15 min to 60 min to afford dihydroxy ester **131**. The second attempt was the addition of a 10% aqueous solution of cesium carbonate and we decided to extend the reaction time until hydrolysis of dihydroxy ester **131** was observed. After one day of stirring the reaction mixture with the mild base, no starting material **130** was observed and a mixture of products was found (Scheme 2.18).



Scheme 2.18. Hydrolysis of OBO ester **130**.

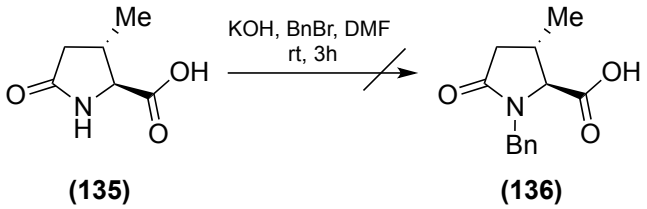
Although these reaction conditions afforded the desired product **132**, the main product was a (*S*)-3-methyl-L-glutamic acid derivative **133** (37%). Another drawback was the overall yield of the product **132** (13%). These results led us to conclude that the carboxybenzyl (Cbz) protecting group on nitrogen was making the carbonyl in the pyroglutamic ring more electron deficient and more susceptible to ring opening. For this reason, we decided first to remove the Cbz

group on nitrogen, followed by the deprotection of the carboxylic acid moiety (Scheme 2.19).



Scheme 2.19. Deprotection of nitrogen followed by removal of OBO ester.

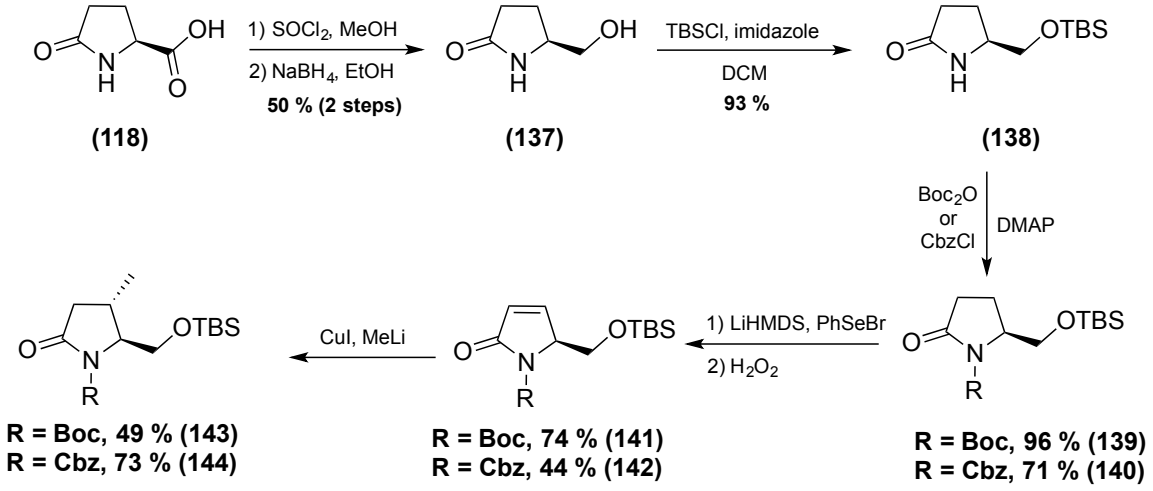
This modification afforded the product **135** in a better yield (49%) after three steps. However, the re-protection of the nitrogen with benzyl bromide under basic conditions following a literature procedure[83] for benzylation of proline did not yield the protected product **136** (Scheme 2.20).



Scheme 2.20. Attempt of benzylation of **135**.

Though there are protocols in the literature for benzylation of nitrogen on pyroglutamic acid, these procedures require a catalyst like copper-TMEDA[84] or they need to protect and deprotect the carboxylic acid moiety making the process longer.[85] Considering the difficulties we faced when trying to remove the OBO ester, we decided to follow another variant of this methodology, where the

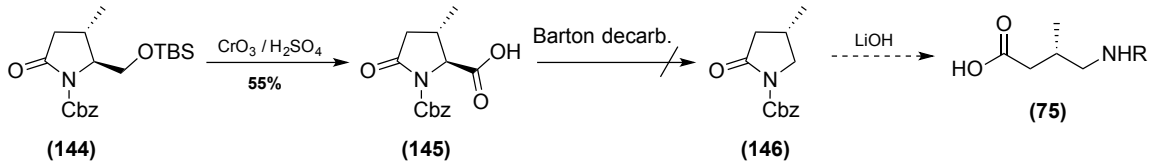
protecting group on the nitrogen does not need to be removed. Acevedo *et al.* reported a method to prepare (3*S*,4*R*)-3,4-dimethyl-L-pyroglutamic acid from L-pyroglutamic acid.[77] In order to prevent racemization of the α-proton, the authors reduced the L-pyroglutamic acid to (*S*)-5-hydroxymethyl-2-pyrrolidinone (**137**). Therefore, we decided to adapt this methodology to prepare a 3-methyl-pyrrolidinone derivative that has the nitrogen protected either with Boc (**143**) or Cbz protecting group (**144**) (Scheme 2.21).



Scheme 2.21. Synthesis of compounds **143** and **144**.

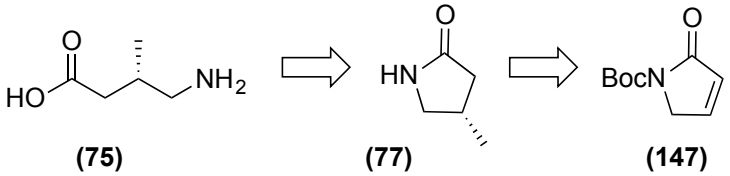
The pyroglutamic acid **118** was first converted to the corresponding methyl ester, followed by reduction with $NaBH_4$ to give (*S*)-5-hydroxymethyl-2-pyrrolidinone (**137**) in moderate yield (50%). Compound **137** was protected as a silyl ether in good yield (93%), with *tert*-butyldimethylsilyl chloride and imidazole in DMF. The nitrogen was protected with $(Boc)_2O$ and a catalytic amount of DMAP in acetonitrile to afford compound **139** in 96%, whereas the protection with

benzyl chloroformate yielded product **140** in 71%. Formation of the double bond by α-selenation of either **139** or **140** followed by oxidation-elimination gave a better yield when nitrogen was protected with Boc (74%) than with the Cbz group (44%). The Gilman reagent (Me_2CuLi) was formed *in situ* by treatment of copper iodide (1 eq) with methyllithium (2 eq) in THF at –78 ºC. A Michael addition of lithium dimethylcuprate to the α,β-unsaturated systems afforded the corresponding methyl derivatives **143** (49%) and **144** (73%), respectively (Scheme 2.21). The deprotection of the silyl ether moiety in **144** followed by oxidation was accomplished in one step using Jones reagent in 55%. Since the protecting group Boc is acid sensitive, it seemed that the yield would be lower for the deprotection/oxidation of compound **143** and this reaction was not attempted. After synthesizing the 3-methyl-pyroglutamic acid **145**, attempts to remove the carboxylic acid moiety by Barton decarboxylation were made but without success (Scheme 2.22).



Scheme 2.22. Efforts towards the synthesis of **75**.

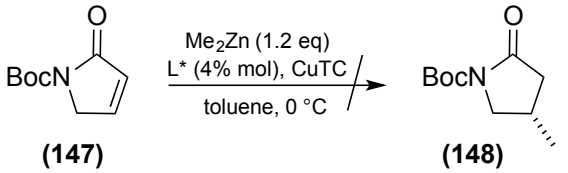
These results encouraged us to attempt a new approach to synthesize the desired 4-amino-3-methylbutyric acid **75** by a short pathway (Scheme 2.23). In this approach, an α,β-unsaturated pyrrolidone **147** could undergo a stereoselective addition to afford the methyl derivative **77**. Basic hydrolysis could yield compound **75** (Scheme 2.23).



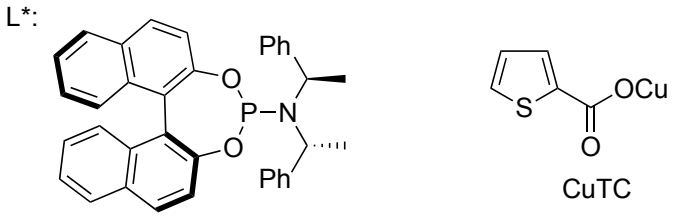
(75) **(77)** **(147)**

Scheme 2.23. Proposed retrosynthesis of 4-amino-3-methylbutyric acid **75**

There are examples in the literature of enantioselective conjugate additions of organozinc to cyclic enones catalyzed by copper complexed with chiral phosphorus amidite,[86] or aryl diphosphite[87] ligands derived from binol that proceeded with high enantiomeric excess (ee). Based on the results reported by Cook and coworkers,[88] we decided to apply their methodology to accomplish copper catalyzed conjugate addition of dimethylzinc to the commercially available α,β-unsaturated-γ-lactam **147** (Scheme 2.24).



(147) **(148)**

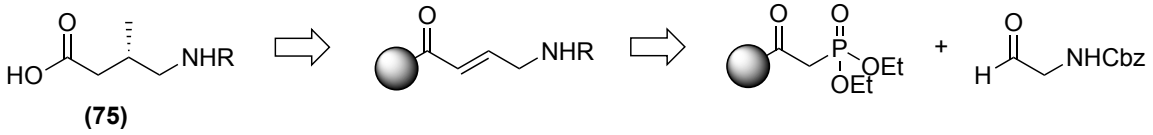


(149) **(150)**

copper(I)-thiophene-2carboxylate

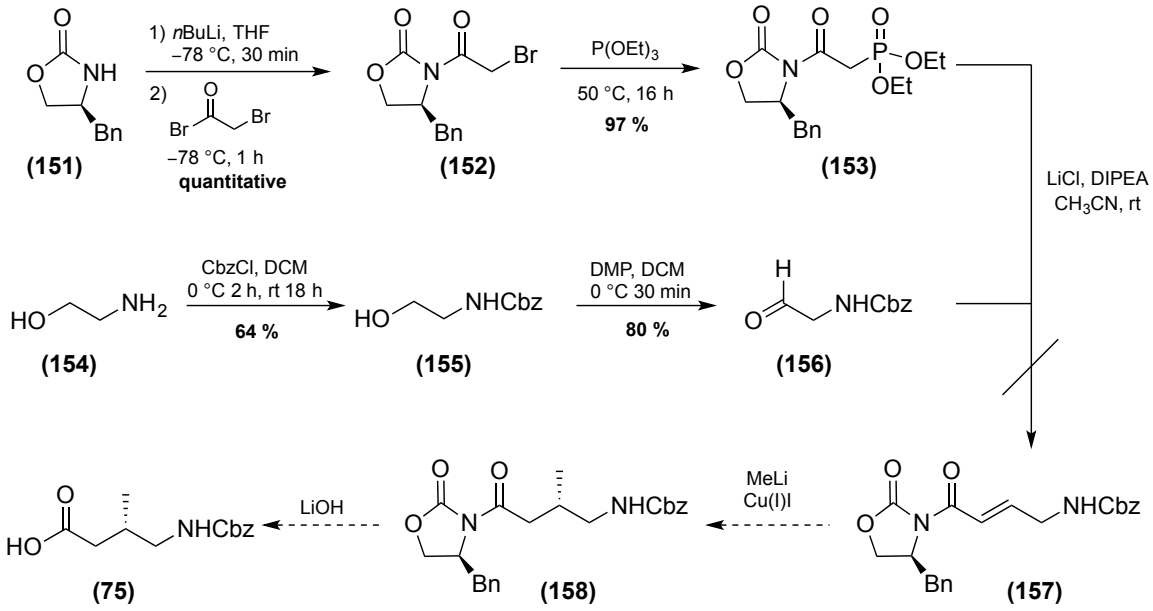
Scheme 2.24. Attempted of enantioselective conjugate addition to form the γ-lactam **148**.

Unfortunately, the reaction conditions were not able to produce the 1,4-adduct. Thus, this reaction requires further optimization. Due to the extent of variables that needed to be optimized, such as changing solvent, copper salt or ligands, we decided to switch to a different approach. Since we have been successful in adding organocuprates to α,β-unsaturated γ-lactams bearing a chiral moiety (**129**, **141**, **142**), we thought that an acyclic α,β-unsaturated carbonyl compound attached to a chiral auxiliary could undergo an enantioselective Michael addition. This intermediate can be constructed by a Horner-Wadsworth-Emmons reaction between a phosphonate and an amino aldehyde (Scheme 2.25).



Scheme 2.25. Alternative retrosynthetic analysis of **75**.

The commercially available oxazolidinone **151** was acylated with *n*-BuLi and bromoacetyl bromide to give compound **152**[89] in quantitative yield. Bromoacetyl oxazolidinone **152** was reacted with triethyl phosphite to form phosphonate derivative **153**[89] in very good yield (97%). The nitrogen on ethanolamine **154** was protected with carboxybenzyl group to generate compound **155**[90] (64%). Further oxidation of the hydroxyl moiety with Dess-Martin periodinane reagent (DMP)[91] yielded aldehyde **156** (80%) (Scheme 2.26).

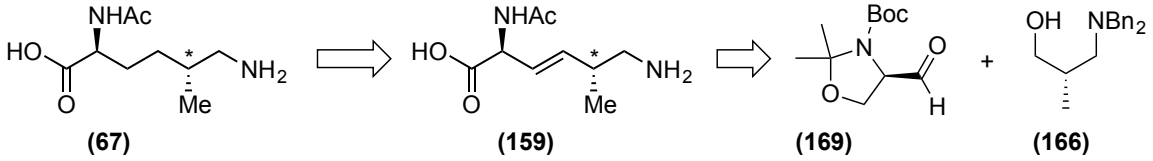


Scheme 2.26. Efforts towards the synthesis of **75**.

The Horner-Wadsworth-Emmons reaction between aldehyde **156** and the carbanion of phosphonate **153** did not lead to the desired α,β-unsaturated derivative **157** (Scheme 2.26). After several efforts to obtain **75** we decided to switch to a different approach because this compound still needs to be coupled with a peracid **108** derived from L-aspartic acid to form the diacyl product **109**. Afterwards this product could undergo photolytic cleavage to afford the orthogonally protected non-proteinogenic amino acid. Finally, removal of the protecting groups would yield the desired product **67** (Scheme 2.13). However, this strategy involves several steps, which would make the pathway longer and less suitable.

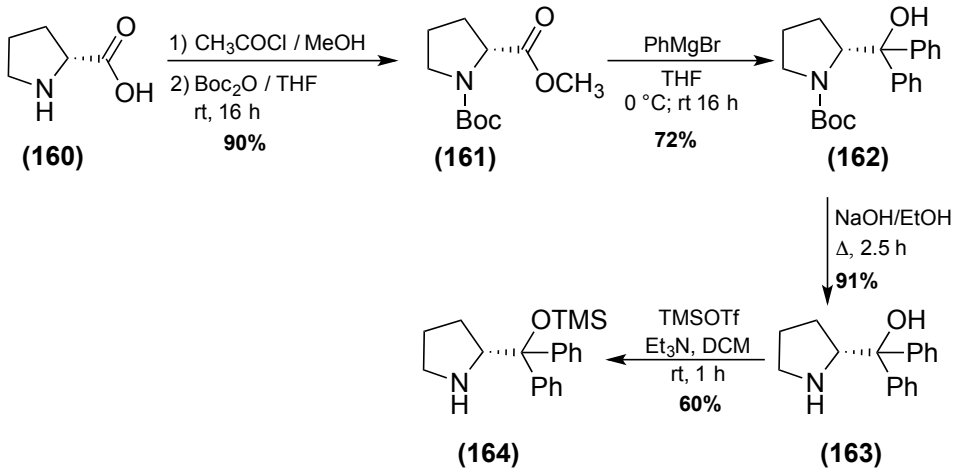
2.2.4 Wittig approach

In the new methodology, we envisioned product **67** could be formed from hydrogenolysis of an alkene derivative **159**, which could also allow for the introduction of deuterium needed for further labeling studies. Moreover, the unsaturated compound **159** could be the product of a Wittig reaction between the commercially available Garner aldehyde **169** with the ylide derived from the amino alcohol **166** (Scheme 2.27).



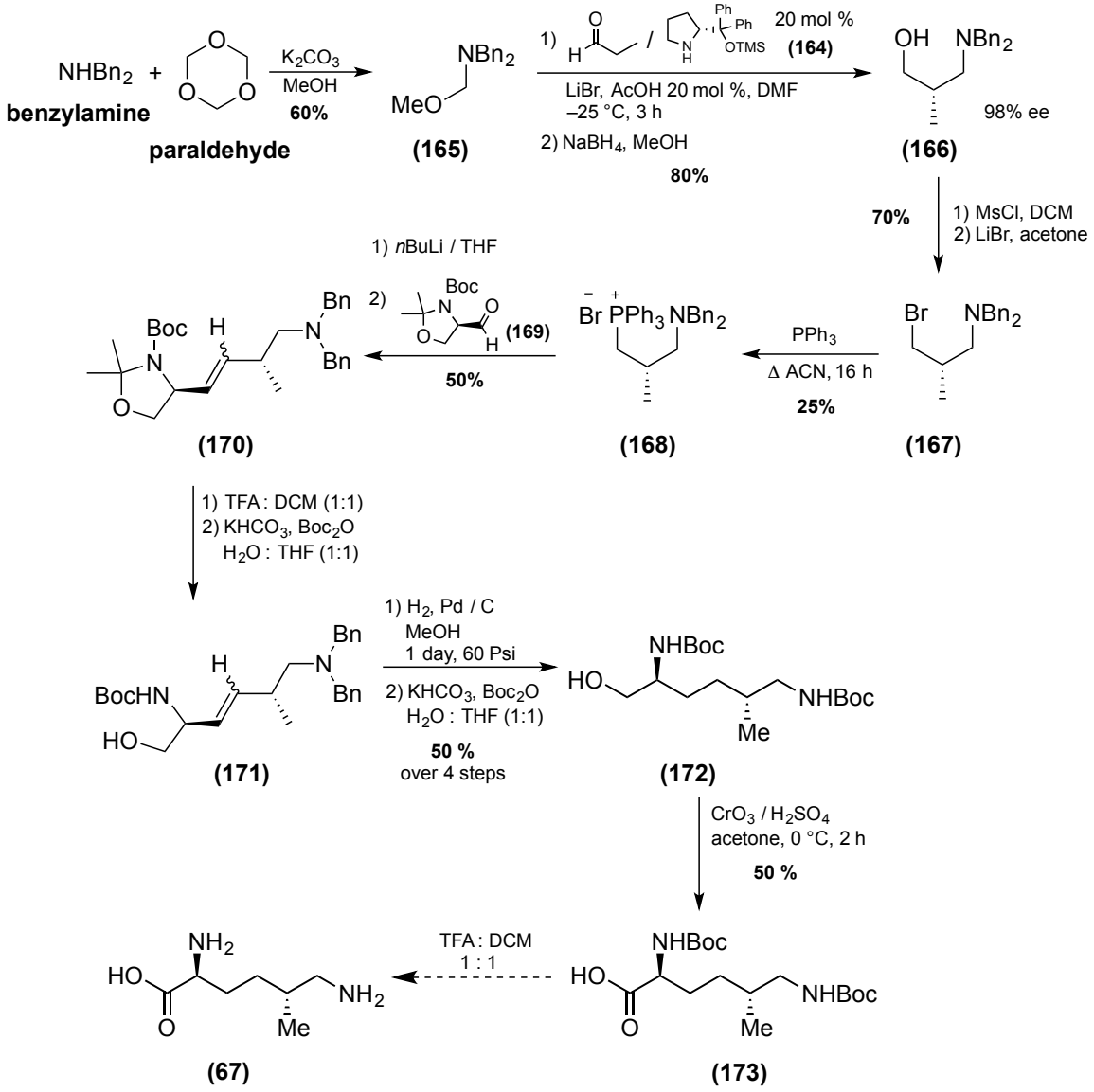
Scheme 2.27. Retrosynthetic analysis of compound **67**.

To prepare the enantiomerically pure amino alcohol **166** the catalyst **164** was prepared (Scheme 2.28). In a first step, D-proline **160** was converted into the methyl ester hydrochloride, and the crude salt was Boc-protected to give **161**[92,93] in good yield (90%). Addition of Grignard reagent to **161** afforded the alcohol derivative **162**[94] in moderate yield (72%). Deprotection of the amino group in compound **162** under basic conditions gave product **163**[95] (92%). Finally, protection of the hydroxy group in **163** as the trimethylsilyl ether (TMS) yielded the catalyst **164**[96] in moderate yield (60%).



Scheme 2.28. Synthesis of catalyst **164**.

Aminomethyl ether **165** was prepared in moderate yield (60%) by a reaction between dibenzylamine and paraldehyde in the presence of potassium carbonate and methanol.[97] With this compound in hand, the enantiopure amino alcohol **166** was prepared according to a literature procedure[98] through a Mannich condensation between aminomethyl ether **165** and propionaldehyde followed by *in situ* reduction (Scheme 2.29).



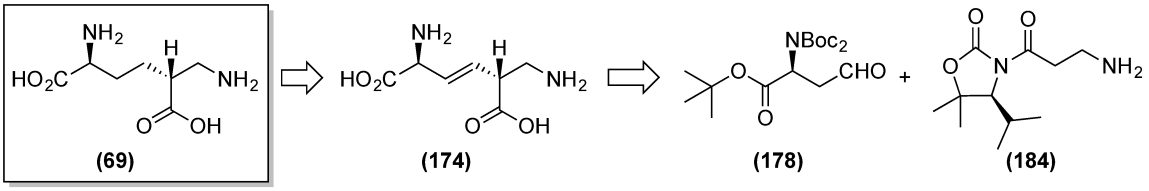
Scheme 2.29. Synthesis towards compound **67**.

The resulting amino alcohol **166** was converted to the corresponding halogenated compound **167** (70%) and then to the phosphonium salt **168** (25%). Subsequent reaction of **168** with *n*-BuLi produced the corresponding ylide, which reacted with Garner aldehyde **169** to form the alkene derivative **170** (50%). After deprotection of the acetonide moiety followed by Boc-reprotection of the amino

moiety in **170**, the double bond was reduced with hydrogen gas. Further protection of the amino moiety in **171** after deprotection with hydrogen yielded **172**. Oxidation of the alcohol group in **172** afforded the protected compound **173** (50%). Boc deprotection with TFA would give the final free amino acid **67** (Scheme 2.29).

2.2.5 Synthetic approach towards (2*S*,5*R*)-2-amino-5-(aminomethyl)hexanedioic acid (69)

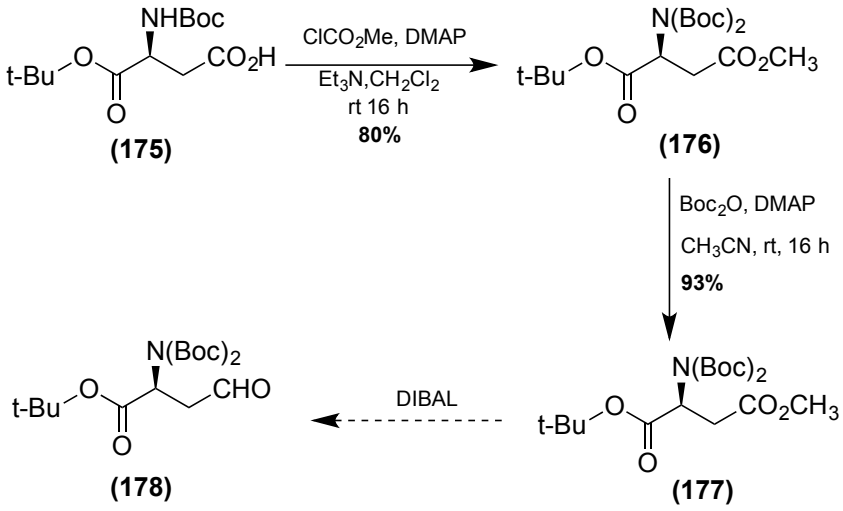
We envisioned the synthesis of compound (2*S*,5*R*)-2-amino-5-(aminomethyl)hexanedioic acid **69** (unlabeled and labeled) as the product of reduction of an alkene derivative **174**, which can be prepared from the aldol condensation between **178** and **184** (Scheme 2.30).



Scheme 2.30. Retrosynthetic analysis of compound **69**.

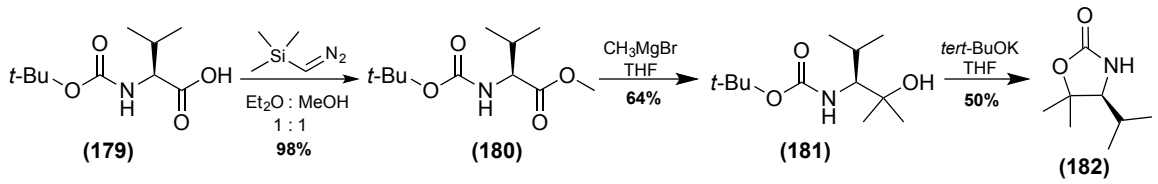
Hence, Boc-L-Asp-O*t*Bu **175** was esterified with methyl chloroformate in the presence of triethylamine and a catalytic amount of DMAP in dichloromethane to afford **176** in 80% yield. After purification by flash chromatography, a second protection on the amine group was introduced with Boc_2O, in the presence of DMAP in acetonitrile to give product **177** in good yield

(93%). Subsequent reduction of the methyl ester in **177** could be accomplished with DIBAL to afford aldehyde **178** (Scheme 2.31).



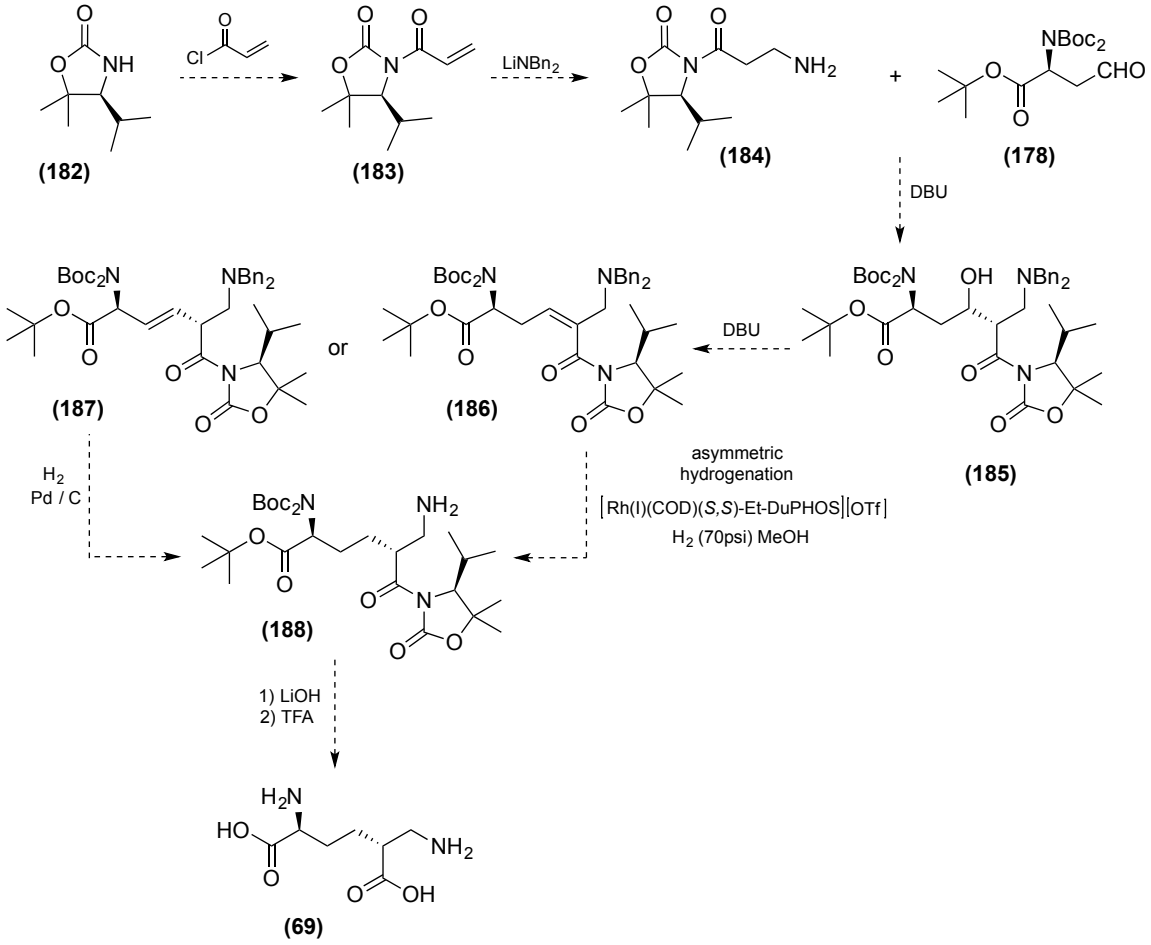
Scheme 2.31. Synthesis towards compound **175**.

The chiral auxiliary **182,** required for the synthesis of compound **184**, was prepared according to methodology reported by Bull *et al*.[99] Boc-L-valine **179** was esterified with trimethylsilyldiazomethane in a mixture of Et_2O:MeOH (1:1) to give the corresponding Boc-L-valine methyl ester **180** in excellent yield (98%). Boc-L-valine methyl ester **180** was reacted with four equivalents of methylmagnesium bromide in THF to afford alcohol derivative **181** in moderate yield (64%). Finally, cyclization of **181** in the presence of potassium *tert*-butoxide in THF furnished the desired oxazoline **182** in moderate yield (50%) (Scheme 2.32).



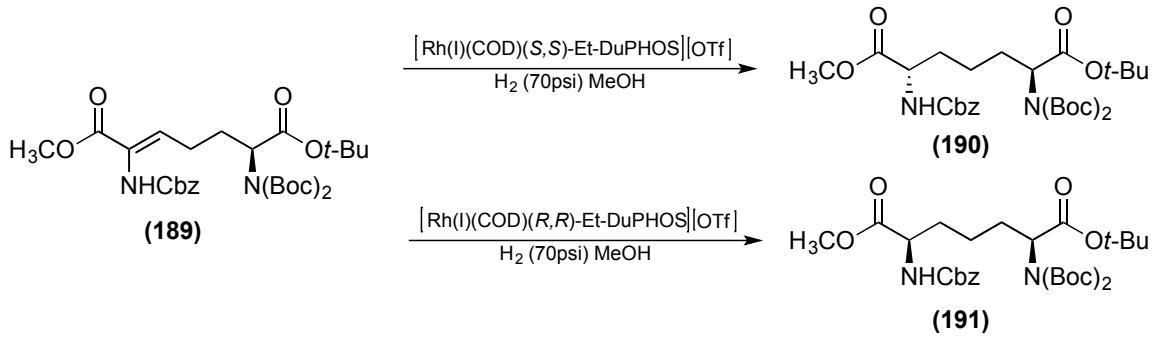
Scheme 2.32. Synthesis of compound **182**.

The chiral auxiliary **182** could be used to complete the synthesis of compound **184**. Firstly, acylation of oxazoline **182** with acryloyl chloride would afford the corresponding *N*-acryloyl oxazoline **183**, which could undergo conjugate addition of lithium dibenzylamide to yield compound **184** (Scheme 2.33).



Scheme 2.33. Synthetic approach towards compound **69**.

Compounds **178** and **184** could undergo an asymmetric aldol condensation, directed by the chiral auxiliary moiety in **184**, to form alcohol derivative **185**. Further elimination with DBU would afford either α,β-unsaturated compound **186** or unconjugated alkene derivative **187**. Compound **187** could undergo hydrogenolysis in the presence of palladium over charcoal to yield intermediate **188**. If product **186** were formed, then an asymmetric hydrogenation could be carried out with a proper catalyst like Burks' catalyst, [Rh(I)(COD)-(*S,S*) or -(*R,R*)-Et-DuPHOS)$^{+}$OTf^{-}. Wang *et al.*[100] have reported an efficient method to prepare orthogonally protected *meso*-DAP and LL-DAP derivatives from asymmetric hydrogenolysis of a dehydroamino acid (**189**) using Burks' catalyst in high enantiomeric excess (ee).



Scheme 2.34. Example of an asymmetric hydrogenolysis using Burks' catalyst.[100]

Finally, compound **188** could undergo removal of the chiral auxiliary in the presence of lithium hydroxide, follow by Boc deprotection with TFA to afford the desired compound **69** (Scheme 2.33).

2.3 Conclusions and future work

Synthesis of *meso*-DAP (**28**) was completed by a diacyl peroxide approach in six steps with an overall yield of 12.5%. The labeled *meso*-DAP (**98**) was prepared by a CM methodology with an overall yield of 13%. Protected **173** was successfully synthesized in unlabeled form through a Wittig approach. Synthesis of unlabeled **69** and labeled remains to be done. The synthesis of the proposed intermediates would allow studying the enzymes TblA, TabB, TabD, TblC, TblS and TblF in order to demonstrate the proposed biosynthesis of tabtoxin.

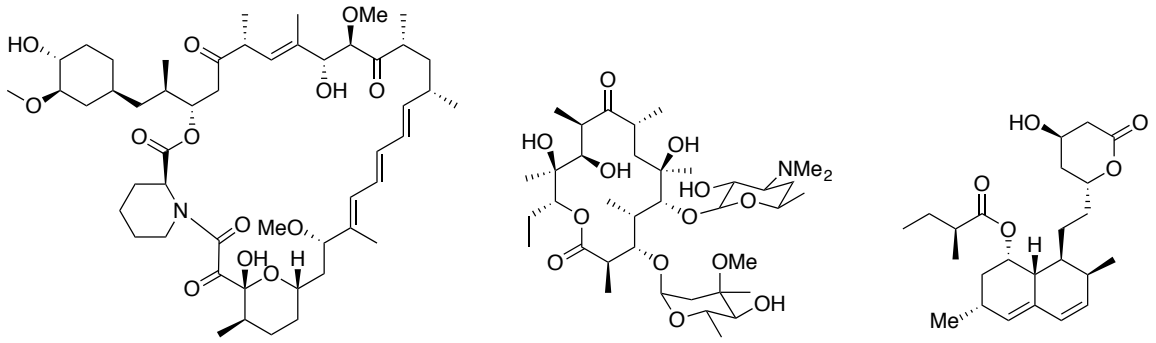
The synthesis of diamino acids derivatives using cross metathesis (CM) gives the opportunity to synthesize selectively protected *meso*-DAP **108** or (*S*,*S*)-2,7-diaminosuberic acid **113** for further incorporation into peptide chains to study natural products like tridecaptin A_1 ($TriA_1$)[76] or for the synthesis of carbon analogs to replace cysteine bridges in natural peptides like neopetrosiamides A&B.

Chapter 3: Chemical synthesis of a standard for LOV enzymes studies.

3.1 Introduction

3.1.1 Polyketides

Polyketides (PK) are secondary metabolites made by different organisms that have shown a wide spectrum of pharmaceutical activities with annual sales over $20 billion USD.[101] Some medical applications include antibiotics (erythromycin A), immunosuppressants (rapamycin), antiparasitics (plakortide P), cholesterol lowering (lovastatin), HIV inhibitor (equisetin) and antitumoral agents (mitomycin C) (Figure 3.1).



Rapamycin (192) **Erythromycin A (193)** **Lovastatin (194)**

Mitomycin C (195) **Plakortide P (196)** **Equisetin (197)**

Figure 3.1. Examples of pharmaceutically active polyketides.

These diverse structures are made by huge multidomain enzymes, which are known as polyketide synthases (PKS), and are able to build polyketide chains from acyl-CoA building blocks.[102] Several studies are in progress in order to understand how these enzymes work.[101,103] An interesting fact is that the biosynthesis of the polyketides highly resembles the biosynthesis of fatty acids (FA), and both use common precursors and enzymes for chain assembly.[102] The similarities in chemical steps and intermediates shared with fatty acids, has helped to understand the biosynthetic process of polyketides synthesis. They are alike in the chemical mechanism involved in chain extension and also in the common pool of precursors employed.

The central domain in fatty acid synthases (FASs) and polyketide synthases (PKSs) is the acyl carrier protein (ACP). The ACP is ~80-100 amino acids long that functions as a tether for the growing chain.[104] All PKSs and FASs require posttranslational modification of their ACP domain to become catalytically active. The inactive apo form is converted to the active holo form by posttranslational transfer of the 4'-phosphopantetheinyl (PPant) moiety from coenzyme A (CoA) to the side chain hydroxyl of a conserved serine residue in the ACP domain. This transformation is assisted by a serine-specific phosphopantetheinyl transferases (PPTases) (Figure 3.2).[104-106]

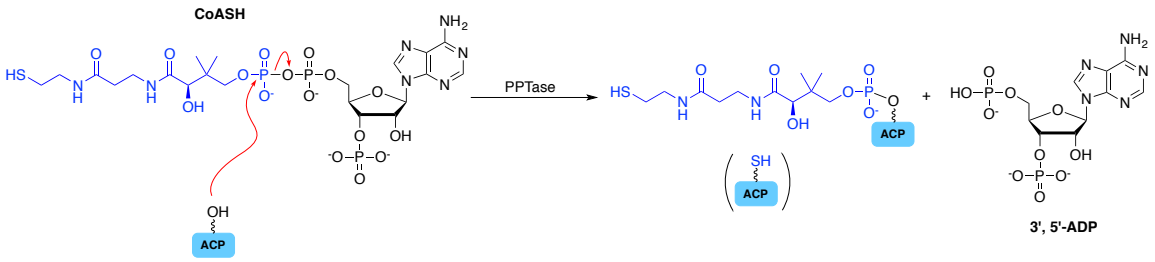


Figure 3.2. Formation of the active holo ACP from the inactive apo form.

The PPant moiety can be considered of as an “arm” of 20 Å that ACP uses to tether the growing acyl intermediates between the different domains in the megaenzymes. The –SH of the PPant moiety also acts as a nucleophile for acylation by a substrate, which may be acetyl-CoA or malonyl-CoA for FAS and PKS respectively.

Usually, polyketides are assembled from C_2 units by repeated head to tail linkage in the same fashion like fatty acids.[107] In the FA biosynthesis the starter substrate is an acetyl unit and the chain extender is a malonyl unit.[103] Analogously, the PK biosynthesis the starter unit is usually either acetyl or propionyl, and the extender unit could be malonyl or methylmalonyl.[103] First, a malonyl-CoA unit (extender) undergoes decarboxylation followed by a Claisen condensation with an acyl-CoA unit (starter) assisted by a β-ketoacylsynthase (KS). Then, the β-ketoacyl product is reduced by a ketoreductase (KR) to furnish a hydroxyl moiety, before elimination by a dehydratase (DH) to afford a double bond, which is reduced by an enoyl reductase (ER) to give a saturated carbon chain longer than the original by two methylene units, before the next round of condensation (Figure 3.3).

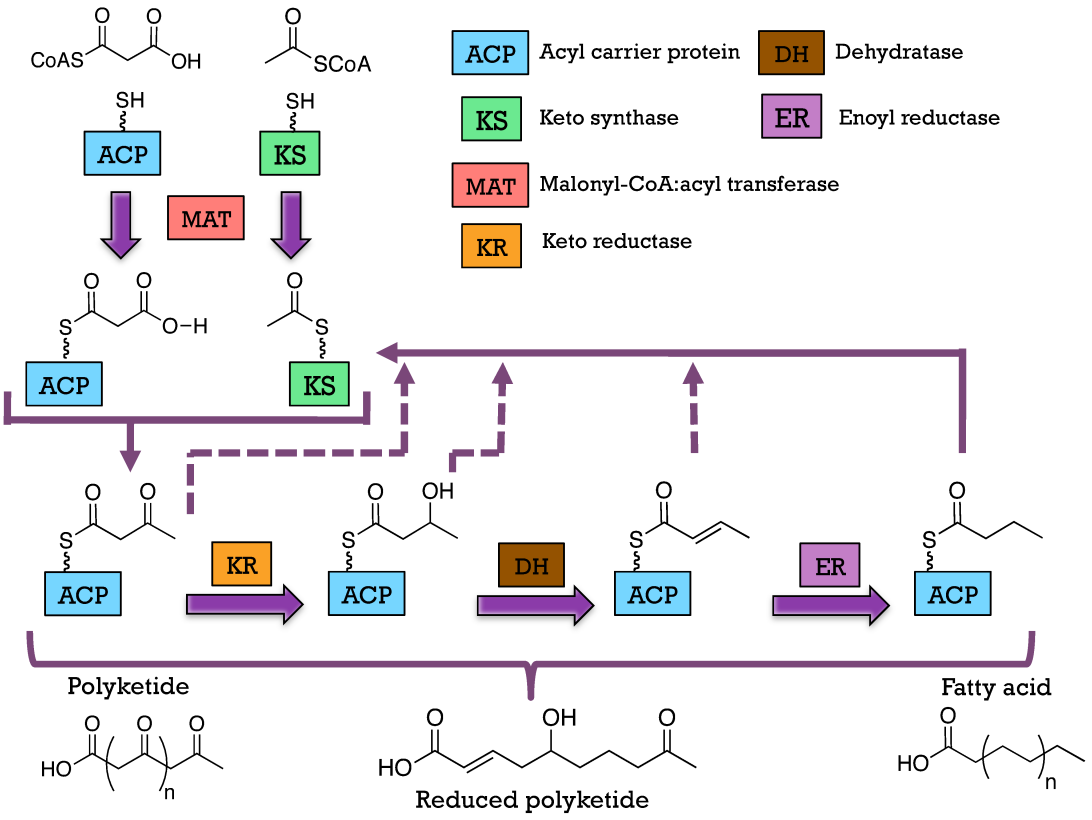


Figure 3.3. Biosynthesis of fatty acids and polyketides.

FAS can catalyze full reductive steps after each elongation, but PKS can skip some or all the reductive steps before the next cycle of chain elongation, which gives as a result diverse patterns along the chain. However, a particular PKS will usually produce only one discrete product with particular length and functionality.

3.1.2 Classification

The PKS enzymes have been classified into three main types, according to organism producing them and the mode of assembly of the chains.[102] This can be divided as modular or iterative in the case of type I. Furthermore, the degree of reduction of the chain can be subdivided as nonreducing (NR); partially reducing (PR) or highly reducing (HR).

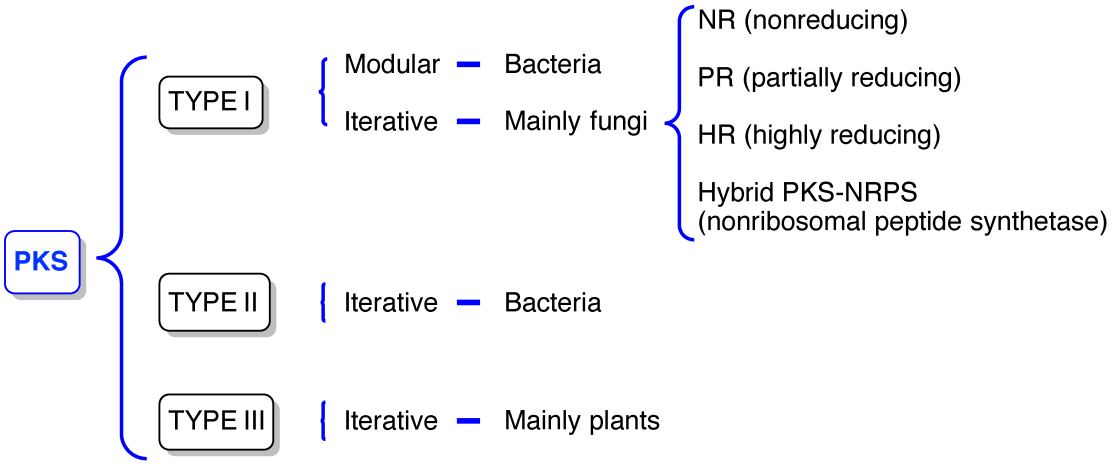


Figure 3.4. PKS classification.

3.1.2.1 Type I Modular

Modular PKSs are typically co-linear, *i.e.* they work like assembly lines, and each module contains a set of domains that are in charge of catalyzing one round of chain extension.

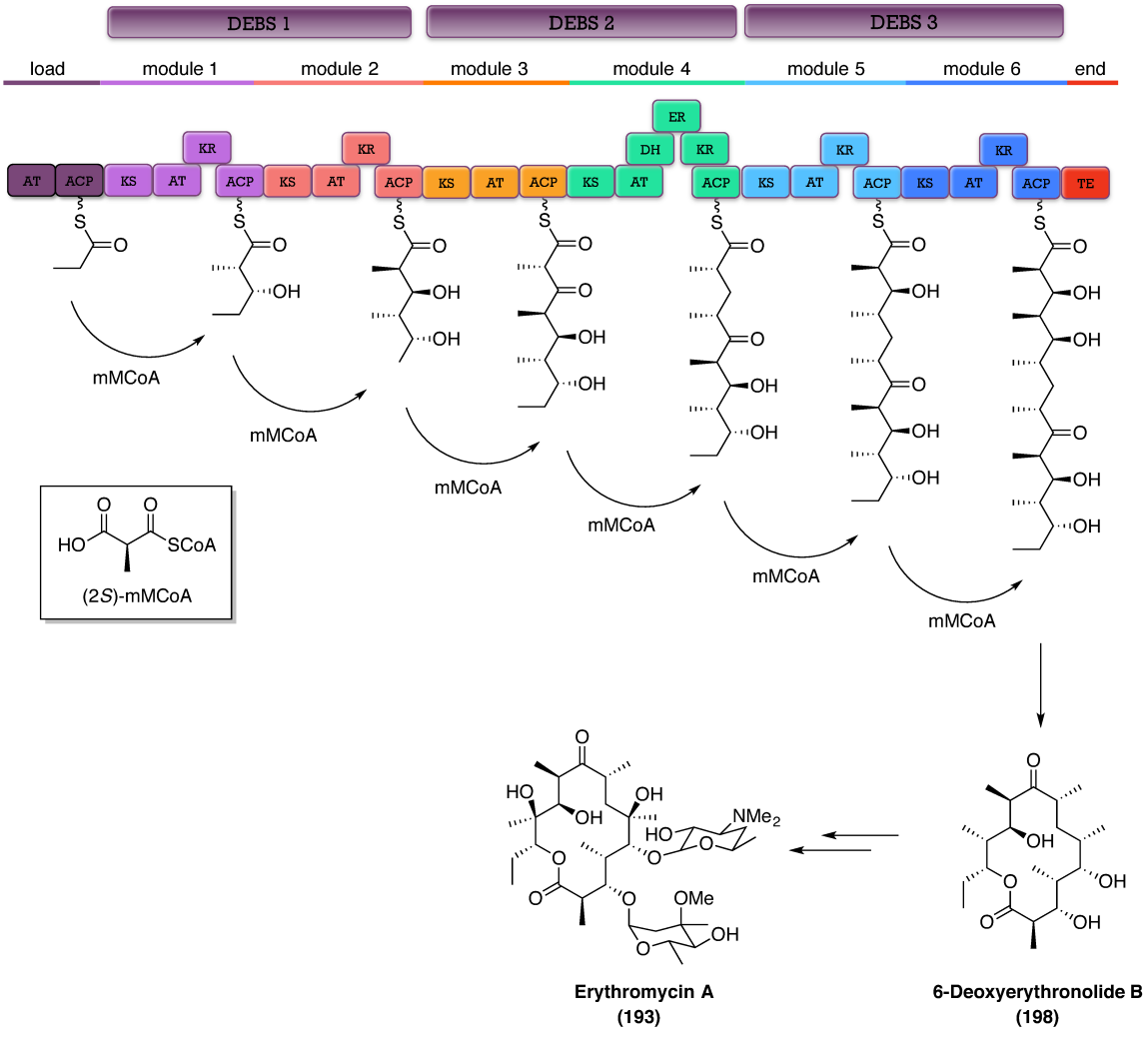


Figure 3.5. Example of a type I modular polyketide.

3.1.2.2. Type I iterative

Type I iterative polyketide synthases are typically found in fungi, and they are single enzymes that contain a set of catalytic domains, which are used iteratively to tailor the growing chain.[108-109] One example of this type of PKS that has been well studied is 6-MSAS (6-methylsalicylic acid synthase), which is responsible for the synthesis of 6-methylsalicylic acid.[107, 110] This compound is produced by *Penicillium patulum*, and is constructed from one molecule of acetyl-CoA and three molecules of malonyl-CoA. This 6-MSAS contains a set of domains: KS (ketosynthase), MAT (acyltransferase), DH (dehydratase), KR (ketoreductase) and ACP (acyl carrier protein), which are responsible for tailoring the growing chain. The first condensation is followed by a second condensation with malonyl-CoA. Next, the resulting tricarbonyl molecule undergoes reduction and dehydration of one carbonyl moiety. After the third condensation with a malonyl-CoA molecule the chain undergoes cyclization, dehydration and enolization to give the aromatic 6-MSA.

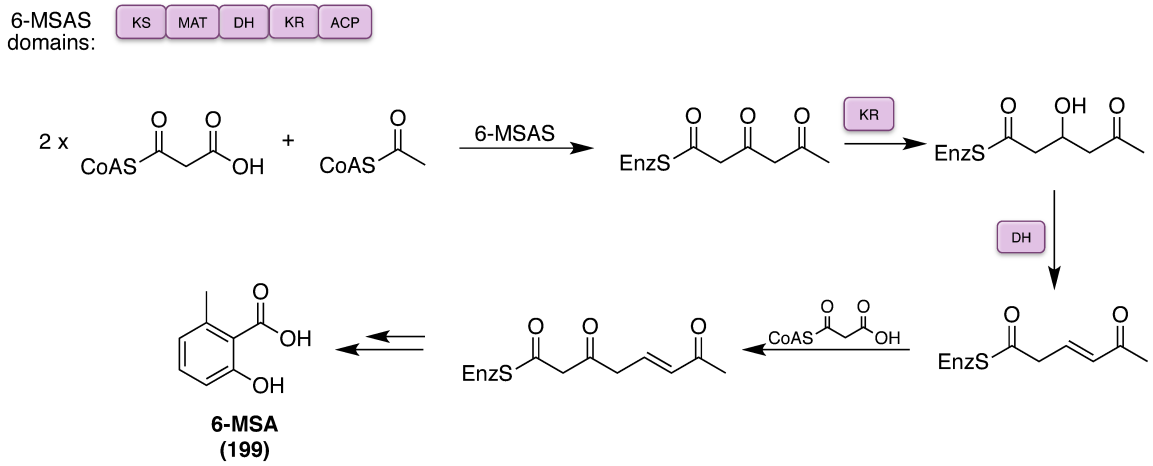
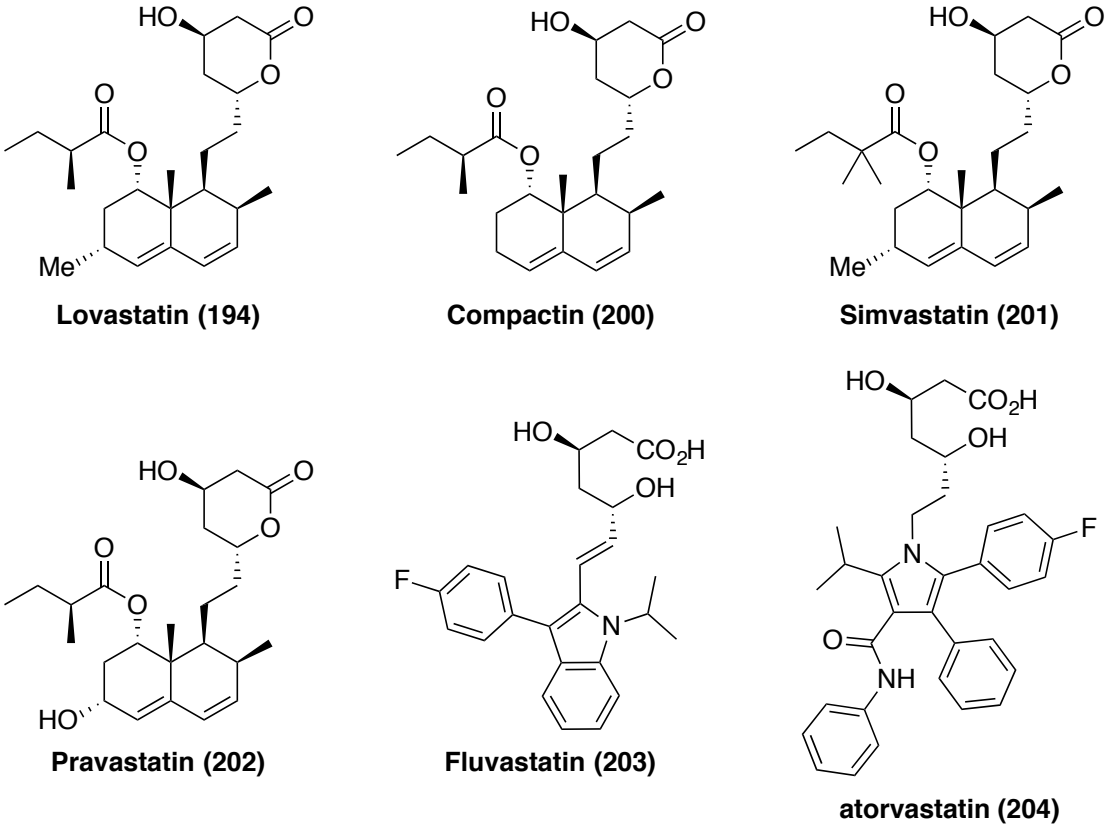


Figure 3.6. Example of a type I iterative polyketide.

Among the enzymes that make these compounds are the highly reducing iterative polyketide synthases (HR-IPKSs) found in fungi.[108] These enzymes use a set of different domains to produce diverse fungal metabolites. One example is lovastatin that is used as precursor to the semi-synthetic drug simvastatin: both are cholesterol-lowering drugs.[111] The key mode of action of these compounds is through the inhibition of (3*S*)-3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), which is the rate limiting enzyme in the biosynthesis of cholesterol.[112] The biosynthesis of lovastatin proceeds via the nonaketide dihydromonacolin L (DML). The proteins responsible for DML production are LovB and LovC.[111,113] The enzyme LovB is able to catalyze about 35 reactions to yield DML, and the most remarkable fact is that this enzyme catalyzes a Diels-Alder reaction to form the decalin ring system. Some efforts have been made to incorporate early-hypothesized polyketide intermediates into LovB as SNAC esters, but still the rest of the intermediates need to be confirmed.

3.1.3 Lovastatin

Lovastatin is a statin that is also known as monacolin K, mevinolin, or mevacor. Originally, it was isolated from *Aspergillus terreus* by a Merck Sharp & Dohne research group,[114] but it was also found in *Monascus ruber*.[115] An interesting feature of this compound is its ability to reduce cholesterol levels, which has led to several studies in order to understand its molecular and biosynthesis origin.[116],[117] Several unnatural analogs were also commercialized including simvastatin (Zocor), fluvastatin (Lescol), and atorvastatin (Lipitor) (Figure 3.7).



Lovastatin (194) **Compactin (200)** **Simvastatin (201)**

Pravastatin (202) **Fluvastatin (203)** **atorvastatin (204)**

Figure 3.7. Examples of statins.

The mode of action of the statins is through the inhibition of the enzyme (3*S*)-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase or HMGR), which controls the synthesis of cholesterol. The natural substrate of this enzyme is HMG-CoA, which is a key intermediate in the biosynthesis of cholesterol. Statins are attractive compounds because they mimic and block HMG-CoA but do not cause accumulation of toxic steroid intermediates.[118,119]

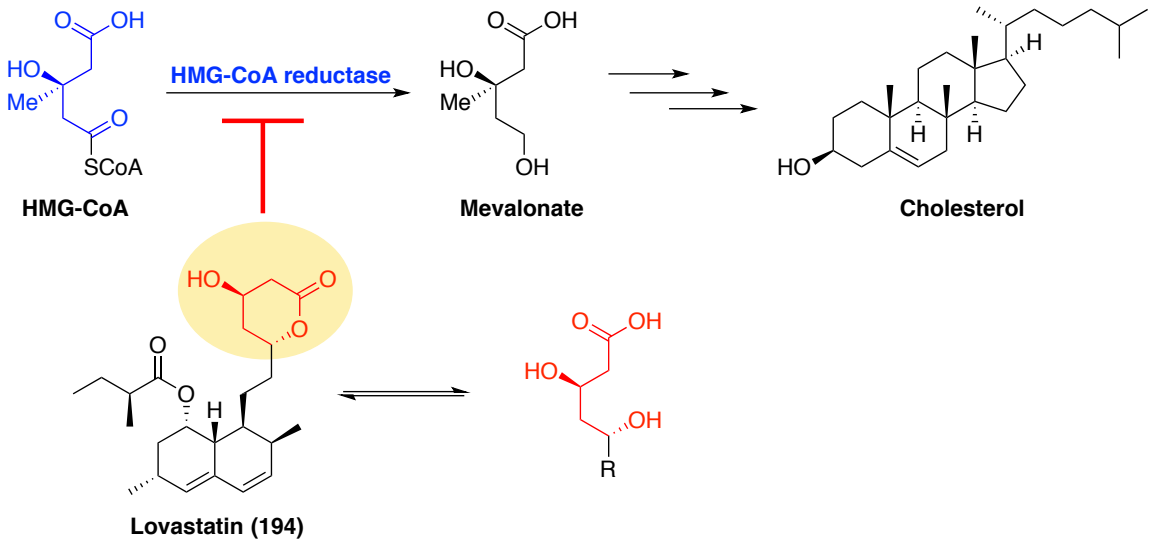


Figure 3.8. Mode of inhibition of the enzyme HMG-CoA reductase.

It has been demonstrated by labeling studies that lovastatin is generated via a polyketide biosynthetic pathway.[120-122]

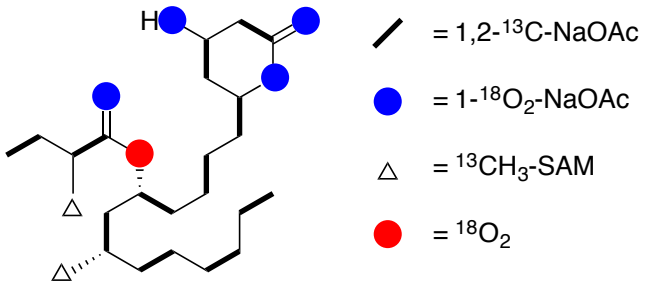


Figure 3.9. Labeling studies to demonstrate the polyketide nature of lovastatin.

3.1.3.1. Lovastatin biosynthetic studies

Lovastatin is a fungal secondary metabolite produced by a fungal megasynthase that represents a highly reduced polyketide synthase (HR-PKS).[109] In nature, these fungal megasynthases (PKS) are iterative, which means that each catalytic domain is used repetitively during the chain elongation.

The genes involved in the synthesis of lovastatin were studied in A. *terreus* initially by Reeves, McAda, and coworkers at MDS Panlabs Inc.[123] The first studies showed that the genes encoded a type I PKS. This protein was estimated to be ~335 kDa, and was later named lovastatin nonaketide synthase (LNKS) by our group. Further genetic studies by our group in collaboration with Hutchinson's group identified the complete gene cluster.[109] The cluster encodes 18 genes, but only nine are involved in lovastatin biosynthesis (Figure 3.10).

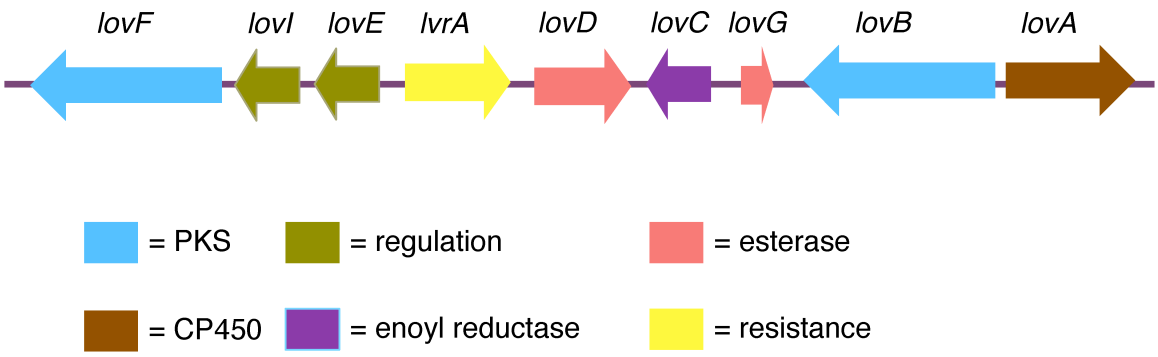


Figure 3.10. Genes involved in the biosynthesis of lovastatin.

Comparison of the catalytic domain arrangement between LNKS (LovB), LDKS (LovF), 6-MSA PKS and mammalian fatty acid synthase (rat FAS) shows that the organization of LovB and LovC highly resembles the mammalian FAS.

The LovB and LovC proteins have seven predicted active domains: KS, AT, DH, MeT, ER, KR, and ACP (Figure 3.11). Also, six out of seven domains are present in FAS, but not the methyl transferase (MeT) domain. The thioesterase (TE) domain that is commonly present in FASs and PKSs is replaced at the C-terminal with a 500 amino acid extension that resembles the condensation (CON) domain of a non-ribosomal peptide synthetase (NRPS), and was considered possibly responsible for the cyclization (Diels-Alder reaction). Another interesting feature is the inactivity of ER^0 domain in LovB.

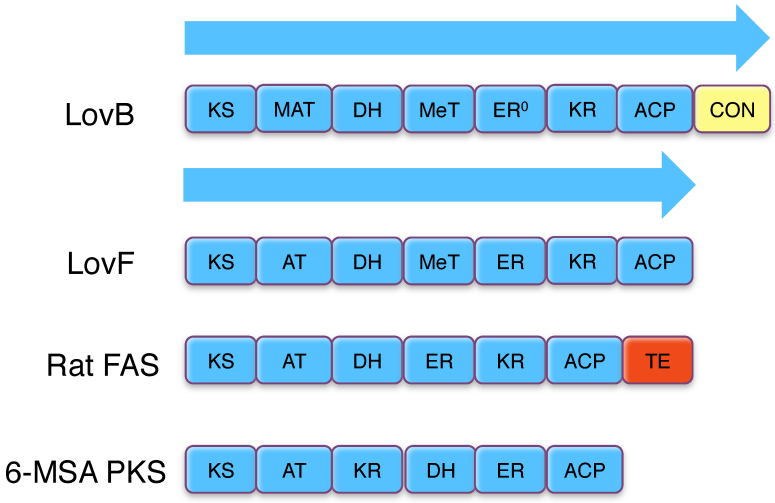


Figure 3.11. Comparison of LovB, LovF to 6-MSA PKS a mammalian FAS.

The intermediate DML is synthesized by the consecutive incorporation of nine acetate units to form a nonaketide, which undergoes further oxidations to install a hydroxyl group at C-8. The final product, lovastatin, is formed via esterification of the hydroxyl at position eight with a diketide (Figure 3.12).

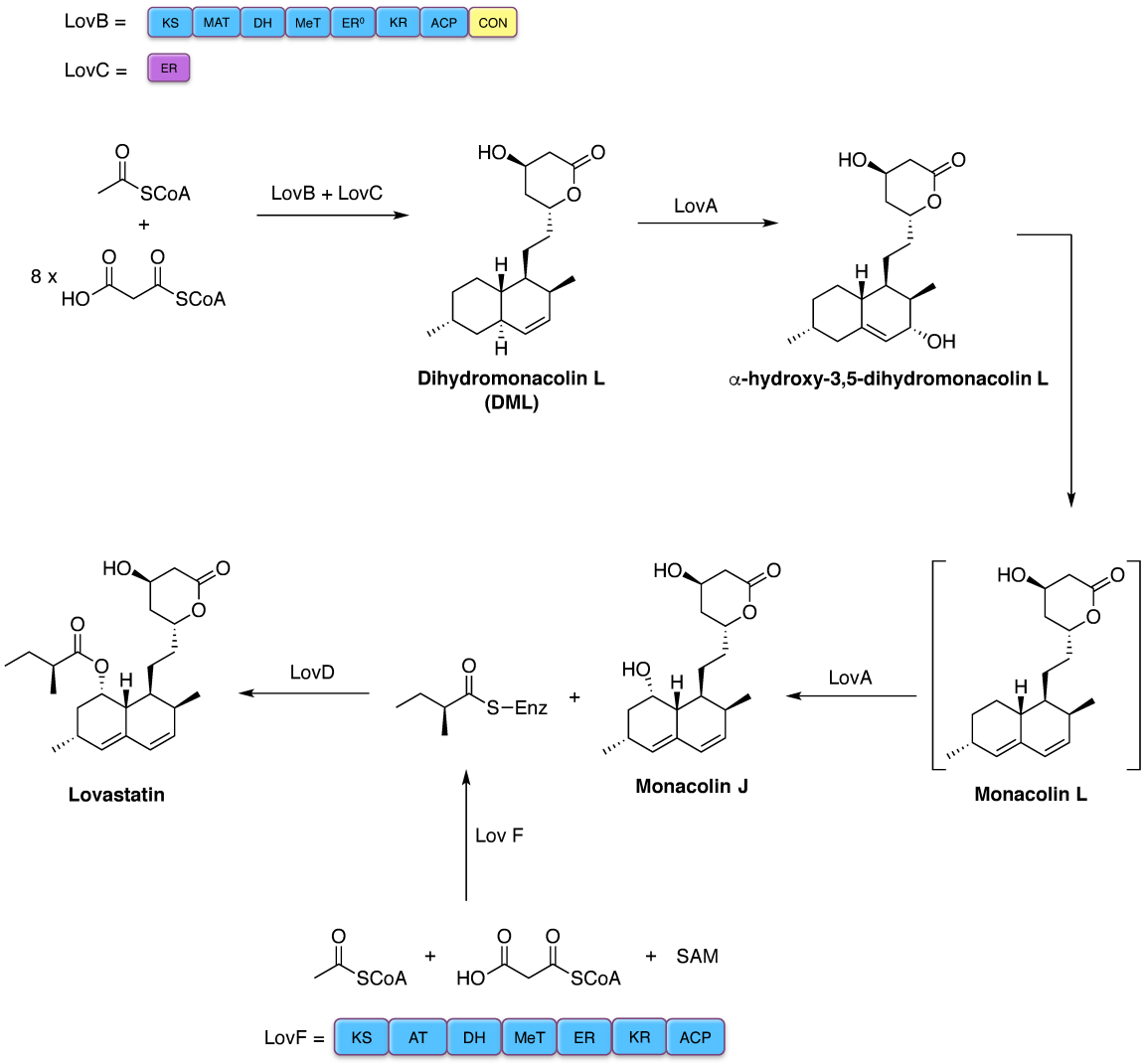


Figure 3.12. Biosynthetic pathway of lovastatin.

3.1.3.2. Function of LovB and LovC

In order to understand the programming steps of LovB in the synthesis of the nonaketide DML, LovB was heterologously expressed in a non-lovastatin producing organism *Aspergillus nidulans* and isolated.[109] Surprisingly, DML was not produced, instead pyrone products were identified. These results suggested

that LovB was not functioning properly, and the enzyme linked triene intermediate cannot be reduced, inducing a shunt pathway.

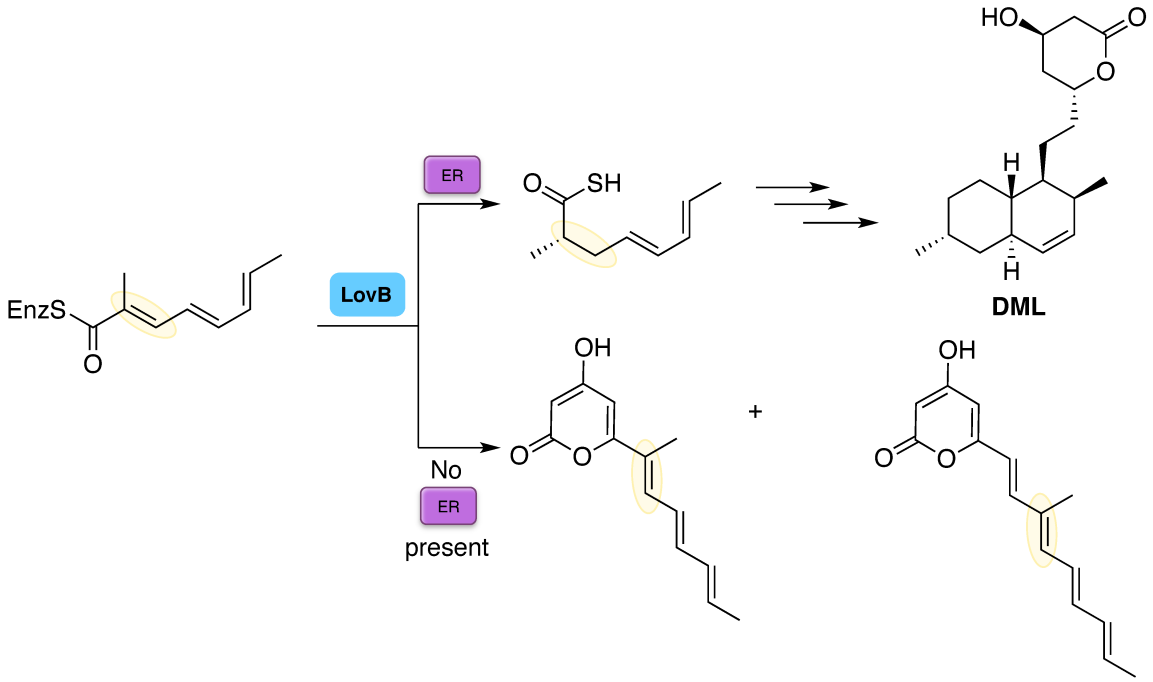


Figure 3.13. Shunt pathway in the absence of a functional enoyl reductase (ER).

The shunt pathway repetitively adds two or three malonyl-CoA units, to afford pyrones. These results suggested that a second protein in the gene cluster must interact with LovB to provide the enoyl reductase activity. Silencing and complementation studies showed that LovC was needed for generation of DML. Other studies have demonstrated that LovC has sequence homology to the ER domain of other PKSs.[109,124-125] Once LovB and LovC were co-expressed DML was produced.[109] This represented one of the first examples where a non-functioning domain of a PKS protein was complemented by an endogenous enzyme.[109]

When one (or several) of the expected transformations are blocked, the PKS LovB will follow a shunt pathway to form undesired products, typically as pyrones. Four substrates/cofactors are required for DML production: malonyl-CoA, SAM, NADPH and LovC. Also, the methylation step plays a critical role in the function of the endogenous ER.[108]

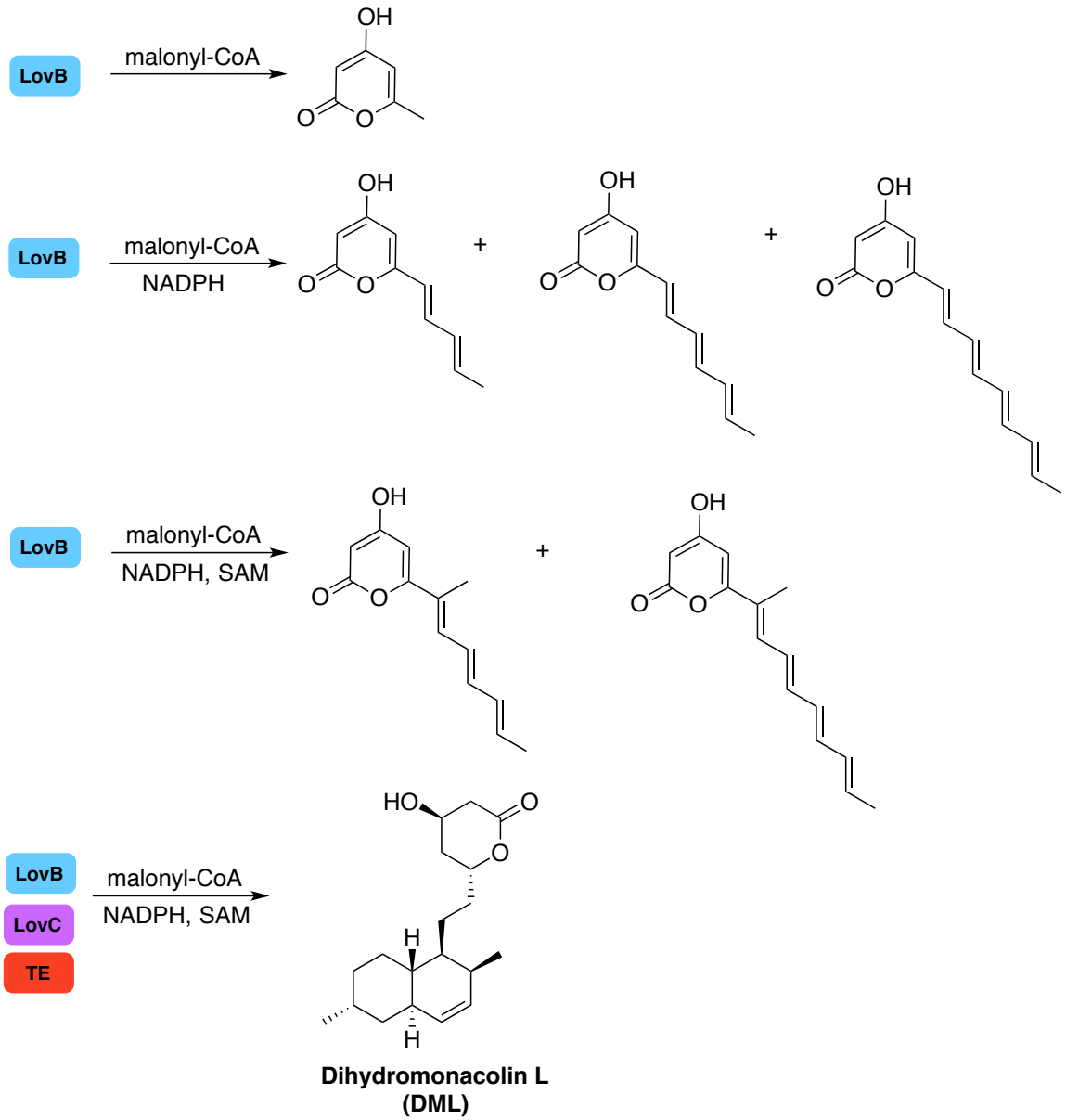


Figure 3.14. Reconstitution of LovB with different substrates or cofactors.[108]

LovB works iteratively to condense nine molecules of malonyl-CoA to yield the nonaketide DML. The KS domain catalyzes each round of Claisen condensation, whereas the growing polyketide is tethered to the phosphopantetheinyl arm of the ACP. The different tailoring permutations after each round of chain extension yield a triene (hexaketide) that can undergo a stereospecific Diels-Alder cyclization to form the decalin moiety (Figure 3.15).

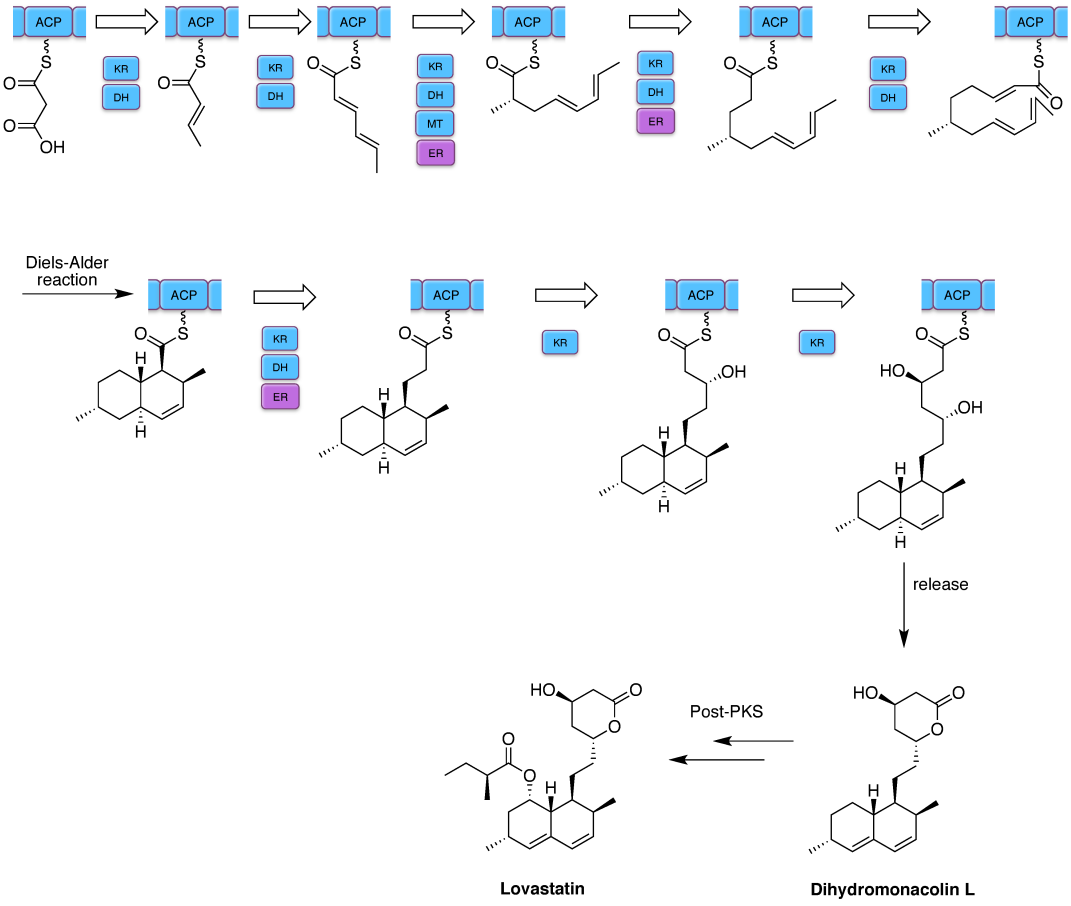


Figure 3.15. Proposed biosynthesis of Lovastatin and detailed synthesis of dihydromonacolin L (DML) by LovB and Lov C.

In order to study the function of the NRPS CON domain that exists at the C-terminus of LovB, a variant of LovB was truncated at the CON domain (ΔCon)

and incubated with malonyl-CoA, NADPH, SAM, LovC, TE and no DML was formed (Figure 3.16).[108]

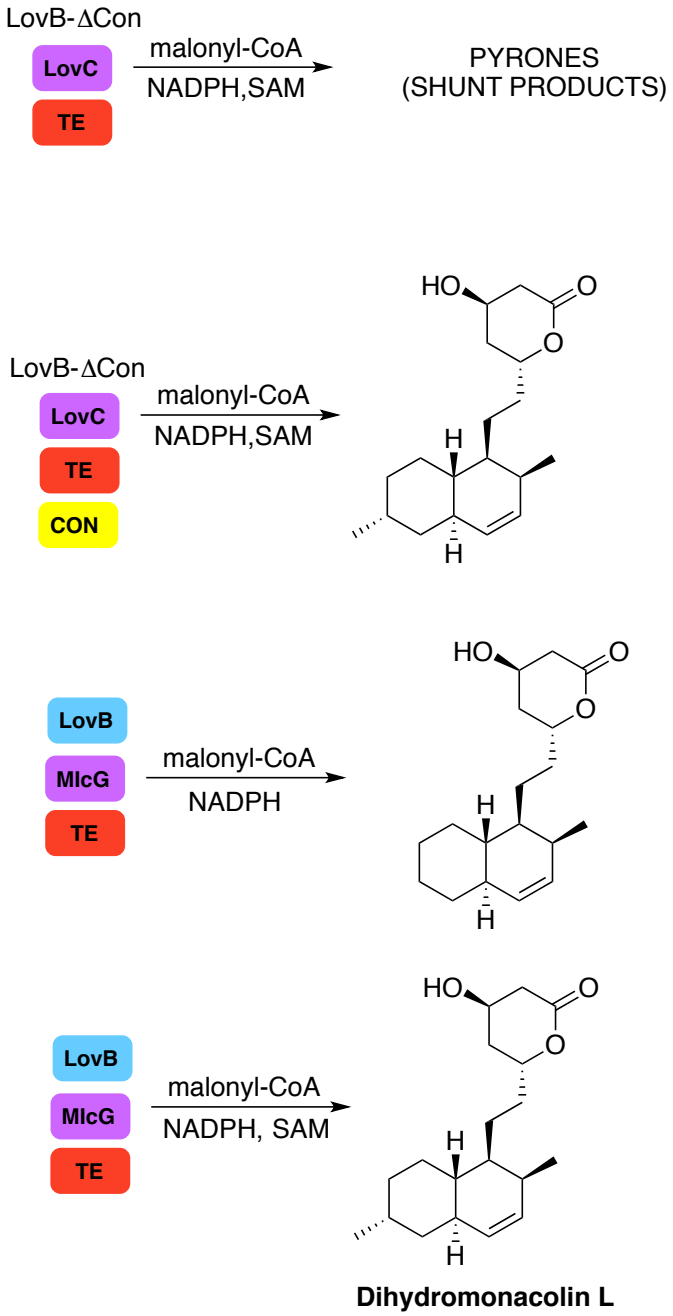


Figure 3.16. Effect of the CON domain in the biosynthesis of DML.[108]

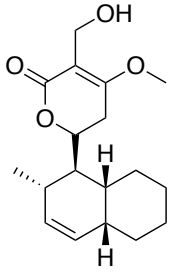
When the fully reconstituted LovB-ΔCon system was supplemented with heterologously expressed CON domain, functional activity was recovered (Figure 3.16). This showed that the CON domain can work in trans with LovB and LovC. This result suggests that the CON domain may be responsible for the stabilization of the later stage steps of DML production, and could be involved in the Diels-Alder cyclization.

In Figure 3.16 LovC was replaced by an exogenous ER (MlcG) to determine whether replacement of LovC would affect the interaction between LovC and MeT during tetraketide biosynthesis. MlcG is a trans-ER from the compactin (**200**) biosynthetic pathway in *Penicillium citrinum*. Since compactin does not contain the methyl group at C-6, the effect of this LovC analog was examined. Surprisingly, MlcG restored the activity of SAM-free LovB and a 6-desmethyl analog of DML was generated. Alternatively, when SAM was added to the mixture, DML was produced. These results imply that the exogenous ER (MlcG) is not affected by the presence of the methyl group. However, LovC will not produce DML in the absence of SAM and the methylation of the tetraketide is a key step for proper function of LovB and LovC.

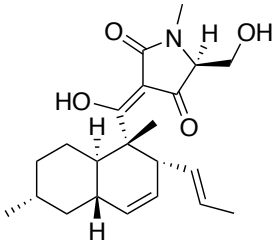
3.1.3.3. Diels Alderase

One interesting aspect of the biosynthetic pathway of lovastatin is the intramolecular Diel-Alder [4+2] cycloaddition reaction, which generates the dehydrodecalin core of DML. This reaction has been suggested to be an enzyme catalyzed process.[121] Other examples of Diels-Alder reactions that are present in

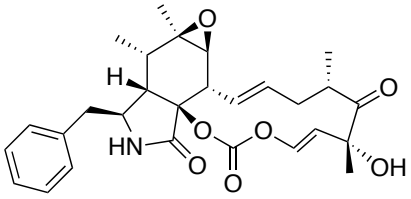
nature include solanapyrone B,[126-127] equisetin[128-129] and cytochalasin E[130-131] (Figure 3.17).



solanopyrone B (206)
Alternaria solani



Equisetin (197)
Fursrium heterosporum



cytochalasin E (207)
Aspergillus clavatus

Figure 3.17. Examples of natural products presumably assembled by Diels-Alder reaction.

However, a biochemical proof of an enzyme-catalyzed reaction on ACP-bound natural substrate has not been demonstrated. Lovastatin biosynthesis provides an unusual example of an enzymatically catalyzed Diels-Alder reaction, as the stereochemical outcome of the cyclization reaction produces a diastereomer with the C-6 were methyl group in an unfavored axial orientation.

In order to investigate whether LovB can catalyze the formation of a decalin ring identical in stereochemistry to DML, a synthetic hexaketide precursor bearing an *N*-acetylcysteamine (SNAC) thioester was synthesized. In aqueous solution in the absence of LovB, the triene substrate produces a 1:1 mixture of diastereomers.[111] When the same triene thioester was incubated with a homogenously purified LovB, a change in the ratio of the diasteromeric products

was observed, and the natural isomer was identified (Figure 3.18).[111] These results suggested the idea that LovB catalyses the Diels-Alder cyclisation in lovastatin biosynthesis.

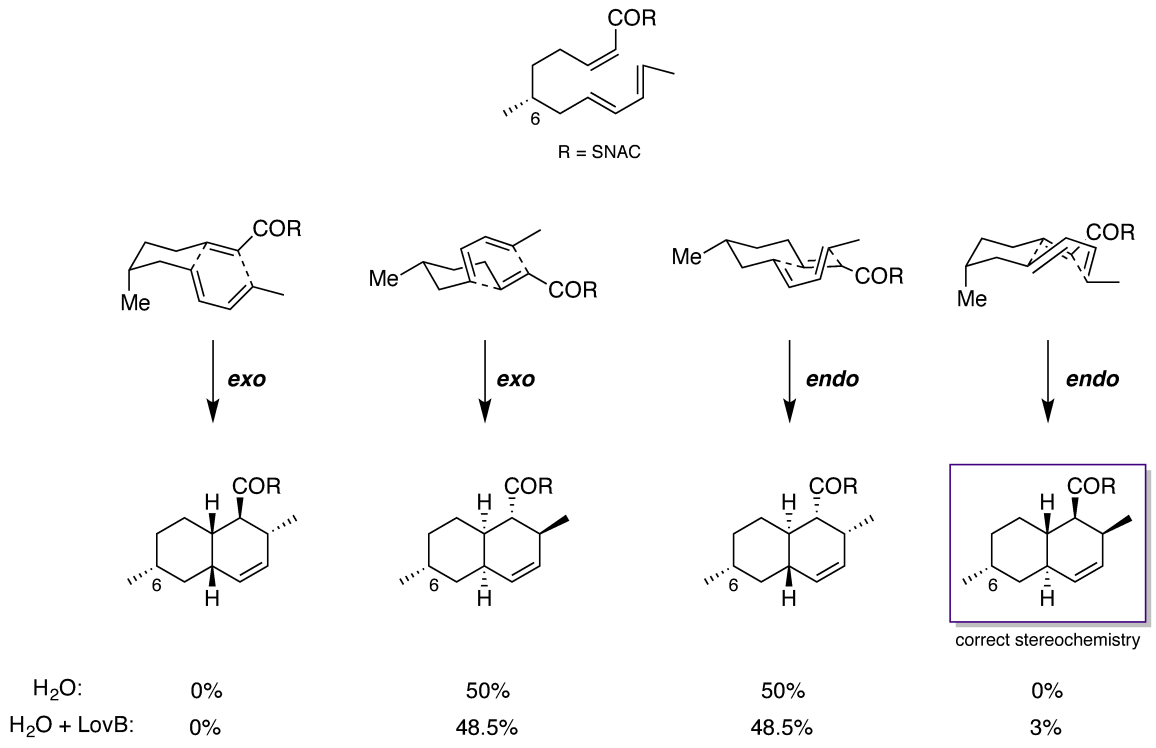


Figure 3.18. Diels-Alder reaction in the presence and absence of LovB.

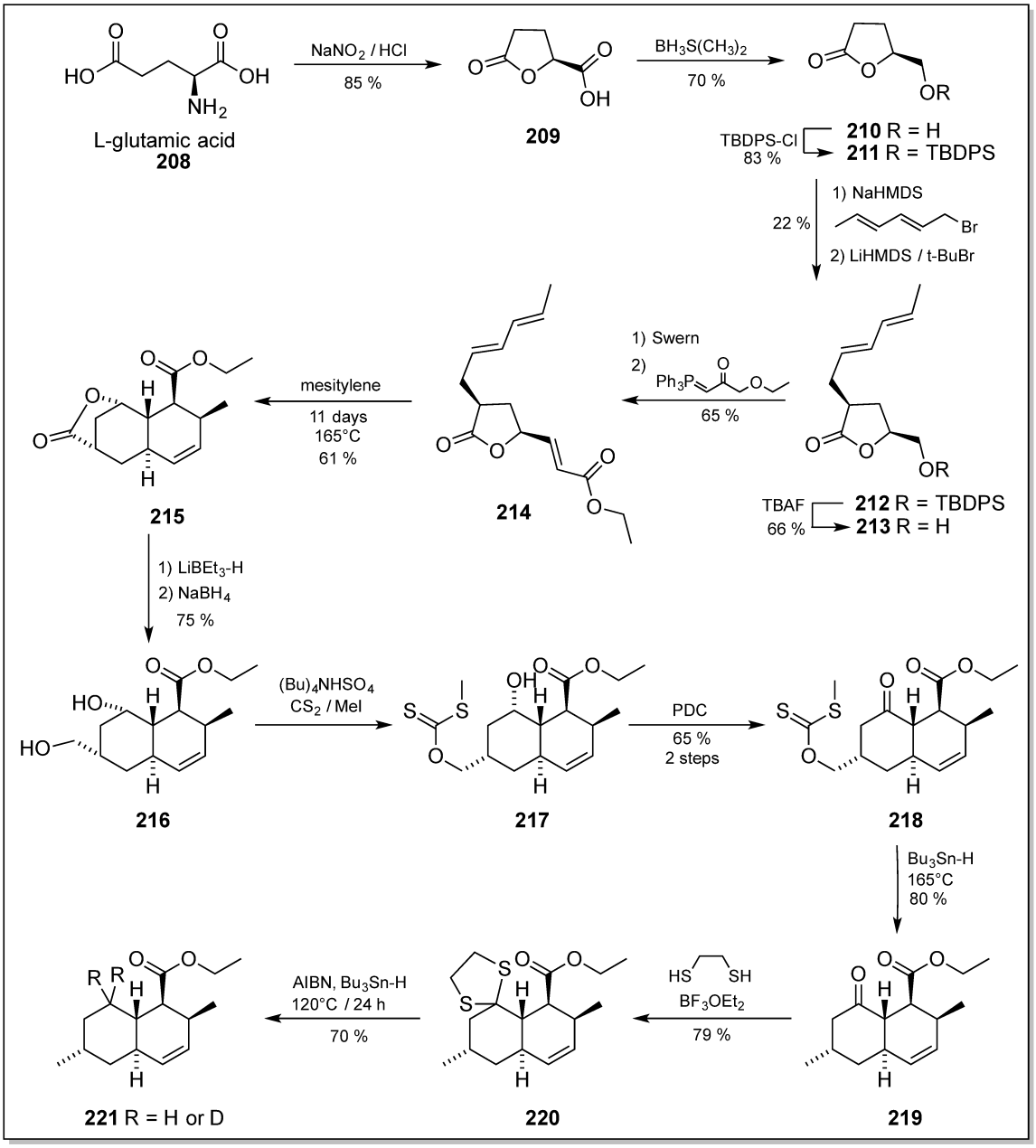
3.2 Results and discussion

3.2.1 Synthesis of hexaketide: ethyl (1*S*,2*S*,4a*R*,6*S*,8a*S*)-2,6-dimethyl-1,2,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylate

Iterative polyketide synthases (PKSs) have only a single copy of each domain (KS, DH, ER), which can be utilized repeatedly for chain elongation and tailoring of diverse functionalities. The limitation in understanding the programming of PKSs has prompted the use of intermediates that are part of the pathway as probes, in order to understand the mechanism that governs PKS enzymes. It has been demonstrated that *N*-acetylcysteamine (SNAC) thioesters of putative intermediates that can be used to probe the mechanism of modular bacterial PKS.[132-133] Another advantage of this approach is that these putative intermediate SNAC thioesters can be studied in cell-free systems.[134-135]

From early studies, it has been suggested that the decalin ring is formed by a [4+2] reaction catalized by a Diels-Alderase enzyme.[109, 111] The SNAC thioester can be used as a probe to study LovB and the CON domain in order to understand the programming of the lovastatin biosynthetic pathway. The objective of this project is to synthesize the SNAC ester of an unlabeled (**223**) and labeled hexaketide with deuterium as standards for GC-MS. These standards will be part of a study conducted by a member of our group, Amy Norquay, in order to investigate the substrate specificity of LovB and to demonstrate if the CON domain of the partial NRPS at the C-terminus of LovB is responsible for catalyzing the Diels-Alder reaction during the biosynthesis of

lovastatin. The SNAC thioester (**223**) can in principle be prepared from the corresponding carboxylic acid (**221**). In order to prepare this compound, the ethyl ester hexaketide can be synthesized following a methodology previously developed in our group,[148] which was suggested and adapted in my study (Scheme 3.1).

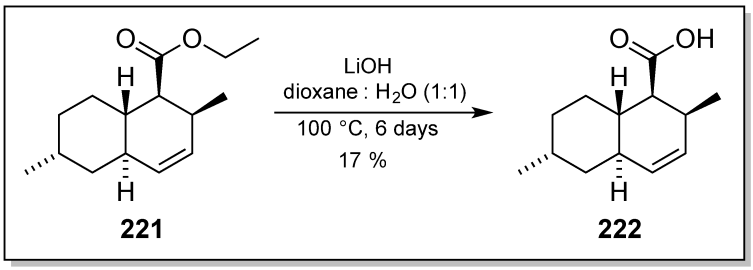


Scheme 3.1. Synthesis of hexaketide **221**.

The starting material is the commercial available L-glutamic acid **208**, which in presence of sodium nitrite and hydrochloric acid affords γ-butyrolactone-γ-carboxylic acid **209** in 85% (Scheme 3.1). Subsequent reduction of the carboxylic acid moiety with borane dimethyl sulfide yields the corresponding primary alcohol **210** in 70%, which is protected as *tert*-butyldiphenylsilyl ether **211** in 83% yield. Treatment of this lactone **211** with sodium hexamethyldisilazide and 1-bromohexa-2,4-diene followed by lithium hexamethyldisilazide and *t*-butyl bromide gave the desired compound **212** in 22% yield. Compound **212** was deprotected with tetrabutylammonium fluoride to yield the primary alcohol **213** in 66%. Swern oxidation of this alcohol **213** afforded the corresponding aldehyde followed by Witting reaction with ethyl (triphenylphophoranylidene)acetate in the same reaction vessel gave the triene **214** in 65% yield in two steps. Diels-Alder reaction of this product **214** produced the functionalized decalin ring **215** in 61% yield.

Further functionalization of the decalin ring **215** is shown in Scheme 3.1. Firstly, the lactone ring of **215** is opened with Super-Hydride followed by sodium borohydride to produce the dialcohol **216**, then the primary alcohol is protected as thiocarbonate **217**. Selective oxidation of the secondary alcohol in **217** with pyridinium dichromate (PDC) afforded the corresponding ketone **218** in 65% yield over 2 steps. Treatment with tri-*n*-butyltin hydride afforded the keto-ester **219** by deoxygenation of the methyl xanthate **218** in 80% yield. Subsequent protection of keto-ester **219** as a dithioketal afforded compound **220** in 79% yield.

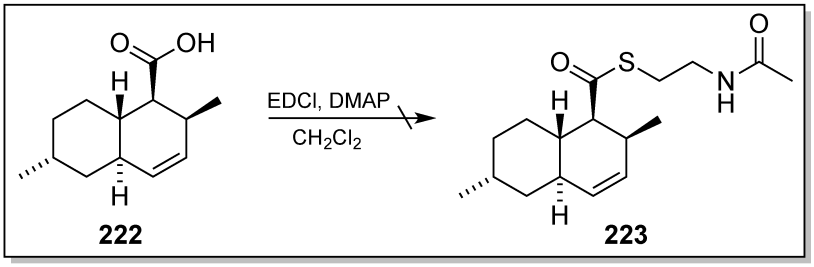
Reaction of the dithioketal **220** in neat tri-*n*-butyltin hydride and catalytic AIBN at high temperature produced the desired compound **221** in 70% (Scheme 3.1). After numerous attempts using different reaction conditions, the hydrolysis of the ethyl ester moiety in **221** was accomplished with lithium hydroxide by heating the reaction mixture over 6 days with 17% yield (Scheme 3.2).



Scheme 3.2. Hydrolysis of ester **221**.

3.2.2 Efforts to synthesize the SNAC thioester: (1*R*,2*S*,4a*R*,6*R*,8a*S*)-*S*-2-acetamidoethyl 2,6-dimethyl-1,2,4a,5,6,7,8,8a-octahydronaphthalene-1-carbothiolate (223)

Some attempts to prepare the SNAC thioester (**223**) were done using the coupling reagent EDCI (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) and DMAP (4-(*N,N*-dimethylamino)pyridine) in dichloromethane without success (Scheme 3.3).

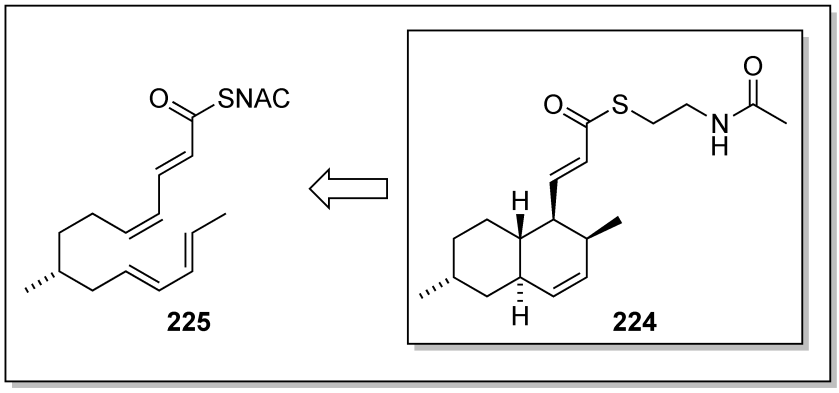


Scheme 3.3. SNAC coupling

3.3 Conclusions and Future work

There has been a great interest in understanding the programming of polyketide synthases. Manipulation or redesign of these enzymes could produce novel drugs. Extensive efforts have been made to decipher how polyketide synthases (PKSs) work and efforts to transform putative substrate analogs (i.e. SNAC esters).

Due to the difficulties faced during the attempts to optimize the hydrolysis of ester **221**, we began to doubt that the hexaketide **223** is a true intermediate in lovastatin biosynthesis. It may be that a heptaketide intermediate (e.g. **224**) could be a possible intermediate. To demonstrate this new hypothesis, it is necessary to synthesize this hypothetical intermediate and conduct further enzymatic experiments (Scheme 3.4).



Scheme 3.4. Hypothetical heptaketide **224**.

Such work is done in our group. The synthetic methodology described here could be potentially adapted to make an aldehyde or vinyl analog of **222** in place of the carboxylic acid. This could be seen useful as quantities of **224** produced by enzymatic cyclization may be very small.

Chapter 4. Experimental Procedures

4.1 General Information

4.1.1 Reagents, solvents and purification

All commercially available reagents were purchased from Sigma-Aldrich Canada Ltd., Fisher Scientific Ltd., Alfa Aesar Ltd., Chem-Impex International Inc., Caledon or VWR International and used without further purification unless otherwise stated. All solvents were of American Chemical Society (ACS) grade and were used without further purification unless otherwise stated. All anhydrous reactions were conducted under a positive pressure of argon using flame-dried glassware. Solvents for anhydrous reactions were distilled prior to use: dichloromethane and dichloroethane were distilled over calcium hydride, tetrahydrofuran and diethyl ether were distilled over sodium with benzophenone as an indicator, and methanol was distilled over magnesium. Triethylamine, DMSO, pyridine were dried over 4Å molecular sieves. HPLC grade acetonitrile, dimethylformamide, isopropyl alcohol, hexanes and methanol were used without further purification. Commercially available ACS grade solvents (>99.0% purity) were used for column chromatography without further purification. Deionized water was obtained from a Milli-Q reagent water system (Millipore Co., Milford, MA). Unless otherwise specified, solutions of NH_4Cl, $NaHCO_3$, HCL, NaOH, and $Na_2S_2O_3$ refer to aqueous solutions. Brine refers to a saturated solution of NaCl. All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC) using glass plates with a UV fluorescent indicator (normal SiO_2, Merck 60 F_{254}). One or more of the following methods were

employed for visualization: UV absorption by fluorescence quenching, staining with phosphomolybdic acid in ethanol (10 g/100 mL), ninhydrin (ninhydrin : acetic acid : *n*-butanol, 0.6 g : 6 mL : 200 mL) potassium permanganate ($KMnO_4$: K_2CO_3 : NaOH : H_2O, 1.5g : 10g : 0.12g : 200 mL). Flash chromatography was performed using Merck type 60, 230 - 400 mesh silica gel. Preparative thin layer chromatography (TLC) purification was performed using plates purchased from Analtech (1000 or 500 microns). The removal of solvents *in vacuo* was performed via evaporation under reduced pressure using a Büchi rotatory evaporator.

4.1.2 Characterization

Optical rotations were measured on a Perkin Elmer 241 polarimeter with a microcell (10 cm, 1 mL) at ambient temperature and are reported in units of 10^{-1} deg cm^2 g^{-1}. All reported optical rotations were referenced against air and measured at the sodium D line (λ = 589.3 nm)

Infrared spectra (IR) were recorded on either a Nicolet Magna 750 FT-IR or a 20SX FT-IR spectrometer. The term cast refers to the evaporation of a solution on a NaCl plate.

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Inova 600, Inova 500, Inova 400, Inova 300, DD2 MR 400, VNMRS 700 or Unity 500 spectrometer at 27 ºC. For ^1H (300, 400, 500, 600 or 700 MHz) spectra, δ values were referenced to $CDCl_3$ (7.26 ppm), CD_3OD (3.30 ppm), or D_2O (4.79

ppm) and for ^{13}C (75, 100, 125 or 150 MHz) spectra, δ values were referenced to $CDCl_3$ (77.0 ppm), CD_3OD (49.0 ppm). Reported splitting patterns are abbreviated as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet.

Mass spectra (MS) were recorded on a Kratos AEIMS-50 (high resolution, electron impact ionization (EI)), and an Agilent Technologies 6220 oaTOF (high resolution, electrospray (ESI)). LCMS Agilent Technologies 6130 LCMS.

4.2 Biological procedures

4.2.1 Expression of DapL orthologs

The cloning of the three DapL orthologs was accomplished by the research group of our collaborator Dr. André Hudson at Rochester Institute of Technology (Rochester, NY, USA). The recombinant expression of each DapL ortholog was done as follow: 10 mL of LB broth containing 50 μg/mL kanamycin and 34 μg/mL chloramphenicol were inoculated and the bacteria was grown overnight at 37 ºC with constant shaking at 225 rpm. These starter cultures were added into 1 L of LB media containing 50 μg/mL kanamycin and 34 μg/mL chloramphenicol and grown at 37 ºC with constant shaking at 225 rpm until an OD_{600} of ~0.5 was achieved. DapL recombinant expression was initiated by the addition of isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.5 mM at 25 ºC for 6 hours with constant shaking at 225 rpm. The bacterial cells were pelleted by centrifugation at 7000 x *g* at 4 ºC for 20 minutes.

4.2.2 Purification of DapL orthologs

For purification, bacterial cells were thawed and resuspended in 30 mL of sonication buffer containing 50 mM sodium phosphate, 300 mM sodium chloride at pH 8.0. This suspension was sonicated on ice at 4 ºC for 4 x 30 s. Cellular debris was pelleted by centrifugation at 11 000 x *g* at 4 ºC for 30 minutes. The decanted supernatant was incubated with 4 mL of Ni-NTA agarose resin (Qiagen, USA) and shaken at 4 ºC for 45 minutes. Upon addition to a biological column, the Ni-NTA agarose resin was packed at a rate of 1 mL/min, and then washed with 3 x 30 mL of wash buffer containing 50 mM sodium phosphate, 300 mM sodium chloride, 10 mM imidazole at pH 8.0 at a rate of 2 mL/min. Recombinant DapL enzymes were eluted with 3 x 10 mL of elution buffer containing 50 mM sodium phosphate, 300 mM sodium chloride, 250 mM imidazole at pH 8.0 at 2 mL/min. Purification of the DapL orthologs was monitored by UV absorption at 220 nm (data not shown). All wash and elution fractions were monitored by SDS-PAGE analysis. Fractions containing His_6-tagged DapL enzymes were concentrated using Amicon Ultra 10,000 molecular weight cutoff (MWCO) filter units by spinning the samples at 7,000 x *g* for 15 minutes at 4 ºC. Concentrated DapL enzymes were diluted to 0.043 mg/mL (*Li*DapL and *Cr*DapL) or 0.430 mg/mL (*Vs*DapL) and stored at 4 ºC in 100 mM HEPES-KOH pH 7.6 buffer containing 20 µM pyridoxal-5'-phosphate (PLP), 2 mM ethylenediaminetetraacetic acid (EDTA pH 7.0), and 1 mM dithiothreitol

(DTT). Overall yields of purified proteins were: 6.2, 20.4, and 6.6 mg/L for *Li*DapL, *Vs*DapL, and *Cr*DapL respectively.

4.2.3 Quantification of purified DapL enzymes

A bicinchoninic acid assay (BCA)[136,137] was used to determine the protein concentration of purified DapL preparation against a bovine serum albumin (BSA) standard curve[138,139] after buffer exchange and concentration.

4.2.4 Preparation of *ortho*-aminobenzaldehyde assay solution to test DapL activity

To test DapL activity the ortho-aminobenzaldehyde (OAB) assay was used according to a literature procedure.[15] A stock solution of 10 mM 2-oxoglutarate and 2.3 mM L,L-DAP in 100 mM HEPES–KOH pH 7.6 was prepared. The stock solution was added to solid OAB, giving a final concentration of 8.3 mM. To prevent polymerization of the OAB, the solution was prepared immediately before each assay. 850 μL of this assay solution was added to a cuvette along with 100 μL of DapL enzyme (0.043 mg/ mL for *Li*DapL and *Cr*DapL, 0.430 mg/mL for VsDapL) in 100 mM HEPES–KOH pH 7.6 containing 20 μM PLP, 2 mM EDTA, and 1 mM DTT. 50 μL of HEPES–KOH pH 7.6 buffer was added to bring the cell volume up to 1 mL. The effective concentration of each component was: 8.5 mM 2-oxoglutarate, 2.0 mM L,L-DAP, 7.0 mM OAB, and either 4.3 μg/mL (LiDapL, CrDapL) or 43 μg/mL (VsDapL) DapL during the assay. Under the enzyme kinetics program in the Varian Cary 100 Bio UV–vis

spectrophotometer, the cells were multi-zeroed, and their absorbance at 440 nm was monitored over 200 min to assess L,L-DAP aminotransferase activity.

4.2.5 Kinetic constants determination

The kinetic constants K_M and k_{cat} for DapL orthologs were calculated using the previously described *ortho*-aminobenzaldehyde (OAB) assay to test L,L-DAP aminotransferase activity, using different concentrations of L,L-DAP. Enzymatic activity was measured in 1.0 mL cells containing 100 mM HEPES–KOH (pH 7.6), 8.5 mM 2-oxoglutarate, 7.0 mM OAB, and DapL enzymes (2 µM of *Li*DapL and *Cr*DapL; 20 µM for *Vs*DapL) in 100 mM HEPES–KOH pH 7.6 containing 20 µM PLP, 2 mM EDTA, and 1 mM DTT. The concentration of L,L-DAP was varied between 0.0625 and 8.0 mM (0.0625, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 mM), monitoring the increase in absorbance at 440 nM every 60 s for 25 min. Runs were conducted in triplicate for each enzyme. Absorbances were converted to molarity using an approximated ε = 316 M^{-1} cm^{-1} for the dihydroquinazolium chromophore at 440 nm. The kinetic constants were calculated by nonlinear regression analysis using GraphPad Prism version 4.0a.

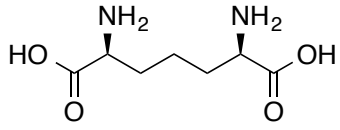
4.2.6. Procedure for inhibitors screening of DapL orthologs

The *ortho*-aminobenzaldehyde assay was employed to test DapL inhibition.[15] Eight separate reaction conditions were run simultaneously using a Varian Cary 100 Bio UV-VIS spectrophotometer. Cell 1 contained a positive control containing the DapL ortholog in the absence of inhibitor. Cells 2 to 7 had increasing concentrations (5, 10, 25, 50, 100, 200 μM respectively) of inhibitor in addition to enzyme and assay solution. Cell 8 served as the negative control, in which no enzyme or inhibitor is added to the assay solution.

To each cell, 850 μL of assay solution containing 10 mM 2-oxoglutarate, 8.3 mM *ortho*-aminobenzaldehyde, and 2.3 mM L,L-DAP were added. 50 μL of DMSO were added to cells 1 and 8. 50 μL of inhibitor solutions dissolved in DMSO were added to cells 2 to 7 in increasing concentrations. 100 μL of DapL enzyme solution (0.043 mg/mL for *Li*DapL and *Cr*DapL, 0.430 mg/mL for *Vs*DapL) in 100 mM HEPES-KOH pH 7.6 containing 20 μM pyridoxal-5'-phosphate (PLP), 2 mM ethylenediaminetetraacetic acid (EDTA pH 7.0), and 1 mM dithiothreitol (DTT) were added to cells 1-7, with 100 μL of 100 mM HEPES-KOH pH 7.6 containing 2 mM EDTA and 1 mM DTT being added to cell 8. Under the enzyme kinetics program in the Varian Cary 100 Bio UV-VIS spectrophotometer, the cells were multi-zeroed and their absorbance at 440 nm was monitored over 200 minutes. After plotting the concentration of inhibitor against the maximal absorbance at 440 nm, IC_{50} values were determined for each of the inhibitors against the three DapL enzymes.

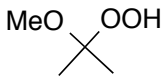
4.3 Tabtoxin: Synthesis and characterization of compounds

(2*R*,6*S*)-2,6-diaminoheptanedioic acid (28)



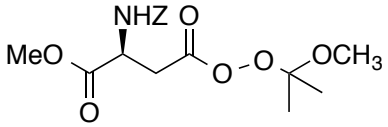
Protected diaminopimelic acid **97** (37 mg, 0.081 mmol) was dissolved in methanol (5 mL) and 10% palladium on charcoal (8.6 mg, 0.081 mmol) was added. The reaction mixture was degasified and stirred under a hydrogen atmosphere for 4 h followed by filtration through a thin layer of celite. The celite was washed with a solution 50 % methanol in water (20 mL), the filtrate was concentrated *in vacuo* and the resulting solid was purified using a DOWEX ion exchange resin to yield **28** as a white solid (15 mg, 99.9%). $[\alpha]_D^{25}$ = 0.0 (c = 0.3 g/100mL, H_2O); IR (cast film): 3198 – 2577 (broad), 3074, 2962, 1630, 1595, 1316 cm^{-1}; ^1H NMR (D_2O, 498 MHz): δ 3.71 (dd, *J* = 6.9, 5.6 Hz, 2H, Hα), 1.96 – 1.78 (m, 4H, Hβ), 1.57 – 1.48 (m, 1H, Hγ), 1.48 – 1.36 (m, 1H, Hγ); ^{13}C NMR (D_2O, 126 MHz): δ 174.6, 54.5, 30.2, 20.8. HRMS (*m/z*) calcd for $C_7H_{13}N_2O_4$ $[M-H]^-$ 189.0881, found 189.0881.

2-Methoxypropane-2-peroxide (91)



The synthesis of this product was adapted from a published procedure.[140] A solution of 2,3-dimethyl-2-butene (0.63 mL, 5.3 mmol) and sodium bicarbonate (~0.2 mg) in a 15% MeOH in DCM solution (15.3 mL) was bubbled with ozone at -78 ºC until the solution turned bright blue indicating the presence of free ozone. The reaction vessel was bubbled with oxygen gas for 30 min to remove excess of ozone, BHT (1.2 mg, 5.3 μmol) was added and the reaction was warmed to room temperature. The reaction mixture was concentrated *in vacuo* and used immediately without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.84 (s, 1H, -OOH), 3.31 (s, 1H, -OCH_3), 1.41 (s, 6H, 2 x CH_3). Spectral data were consisted with the previously reported compound.[140]

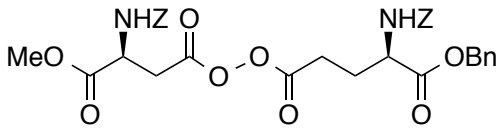
Methyl (*S*)-2-[(benzyloxycarbonyl)amino]-4-[(2-methoxypropan-2-yl)peroxy]-4-oxobutanoate (92)



This compound was made by adapting a published procedure.[66] To a solution of 2-methoxy-2-propyl hydroperoxide **91** (5.3 mmol) in DCM (15 mL) at -20 ºC was added Z-L-aspartic acid-1-methyl ester **90** (1 g, 3.5 mmol), followed by

addition of DCC (1.15 g, 5.6 mmol) and DMAP (43 mg, 0.35 mmol). The reaction mixture was stirred at -20 ºC for 75 min, then warmed to room temperature for 1 h and concentrated *in vacuo*. The residue was purified by flash chromatography (silica, 7:3 hexanes:ethyl acetate) to give compound **92** as colorless oil (1.3 g, 99.9%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.39 (m, 5H, ArH), 5.82 (d, *J* = 8.1 Hz, 1H, NH), 5.16 (s, 2H, $C\underline{H}_2Ph$), 4.73 (d, *J* = 8.2 Hz, 1H, Hα), 3.81 (s, 3H, $-CO_2CH_3$), 3.34 (s, 3H, $-OCH_3$), 3.09 (dd, *J* = 16.6, 4.4 Hz, 1H, Hβ), 2.95 (dd, *J* = 16.6, 4.7 Hz, 1H, Hβ), 1.47 (s, 6H, 2 x CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.1, 170.5, 155.8, 136.1, 128.5, 128.1, 128.0, 107.4, 67.2, 53.0, 50.4, 49.9, 33.9, 22.4.

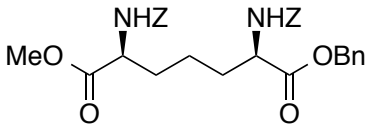
(*R*)-5-(Benzyloxy)-4-(benzyloxycarbonyl)amino-5-oxopentanoic-(*S*)-3-(benzyloxycarbonyl)amino-4-methoxy-4-oxobutanoic peroxyanhydride (95)



This compound was made by adapting a published procedure.[66] To a solution of the perester **92** in $CHCl_3$ (46 mL) was added under vigorous stirring a solution 50% TFA in water over 20 min. The solution was cooled to 0 ºC and a saturated solution of $NaHCO_3$ was added until pH 8 was achieved. The aqueous phase was extracted with Et_2O (3 x 40 mL), and the organic phases were combined, washed sequentially with water (40 mL) and brine (40 mL), dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo* to yield a yellow oil.

The peracid product was dissolved in DCM (46 mL), cooled to 0 ºC and Z-D-Glu-OBn **94** (0.95 g, 2.6 mmol) and DCC (0.613 g, 2.6 mmol) were added. The reaction mixture was stirred for 1 h at 0 ºC and 1.5 h at room temperature. The solids were removed by filtration over a celite pad, and the reaction was concentrated *in vacuo*. The residue was purified by flash chromatography (silica, 7:3 hexanes:ethyl acetate) to give the diacyl peroxide **95** (0.655 g, 34% over 2 steps). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.43 – 7.28 (m, 15H, HAr), 5.74 (s, 1H, NH), 5.40 (s, 1H, NH), 5.2 – 5.11 (m, 6H, 3 x C$\underline{H_2}$Ph), 4.72 (s, 1H, Asp-CHα), 4.47 (s, 1H, Glu-CHα), 3.77 (s, 3H, OCH_3), 3.09 – 3.0 (m, 2H, Asp-CH_2β), 2.55 - 2.41 (m, 2H, Glu-C$\underline{H_2}$γ), 2.40 - 2.20 (m, 1H, Glu-C$\underline{H_2}$β) 2.09 – 2.0 (m, 1H, Glu-C$\underline{H_2}$β).

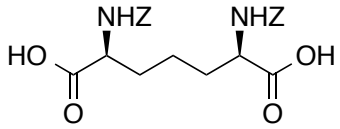
1-Benzyl-7-methyl (2*R*,6*S*)-2,6-bis[(benzyloxycarbonyl)amino] heptanedioate (96)



Orthogonally protected diacyl peroxide **95** (0.59 g, 0.91 mmol) was dissolved in DCM (~5 mL) and transferred to the reaction vessel (a 15 cm diameter recrystallization dish with a quartz lid and two adaptors to allow a flow of argon into the vessel). The solvent was evaporated using a stream of Ar gas, trying to deposit the sample onto the bottom surface of the vessel like a homogenous thin layer. A 2-propanol bath was cooled down to - 80 ºC and maintained at a constant temperature through the use of a Thermo Neslab

Cryotrol/CC-100 cryo-cooler system. The reaction vessel was cooled to -80 ºC under argon, and the diacyl peroxide residue was irradiated at 254 nm with a UV lamp for 5 days in the absence of external light. The product was collected by dissolution in DCM (5 mL), concentrated *in vacuo* and purified by flash chromatography (silica, 8:2 hexanes:ethyl acetate) to give **96** (0.188 g, 37%). $[\alpha]_D^{25}$ = 4.5 (c = 1.0 g/100mL, MeOH); IR (MeOH cast film): 3332, 3089, 2951, 1719, 1586, 1525, 1213 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.38 (m, 1H, ArH), 5.39 (m, 1H, NH), 5.30 (m, 1H, NH), 5.14 – 5.21 (m, 6H, -C$\underline{H}_2$Ph x 3), 4.43 (m, 1H, Hα), 4.36 (m, 1H, Hε), 3.74 (s, 3H, -OCH_3), 1.87 – 1.67 (m, 4H, Hβ + Hδ), 1.43 – 1.32 (m, 2H, Hγ); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.6, 172.0, 155.9, 136.2, 135.2, 128.4, 128.3, 128.1, 128.0, 67.2, 67.1, 53.6, 52.4, 32.1, 20.8; HRMS (*m/z*) calcd for $C_{31}H_{34}N_2NaO_8$ $[M+Na]^+$ 585.2207, found 585.2200.

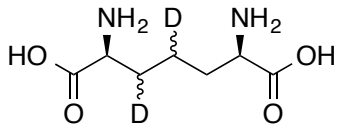
(2*R*,6*S*)-2,6-Bis[(benzyloxycarbonyl)amino]heptanedioic acid (97)



To a solution of **96** (50 mg, 0.088 mmol) in 25 % MeOH in THF (200 μL) a solution of LiOH (7.5 mg, 0.18 mmol) in H_2O (75 μL) was added and reaction mixture was stirred at room temperature. After 2 h, another equivalent of LiOH (3.8 mg 0.088 mmol) in H_2O (40 μL) was added and the reaction mixture was stirred for a further 2 h. The reaction mixture was diluted with H_2O (3 mL),

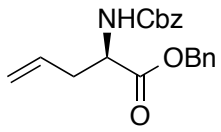
extracted with EtOAc (2 x 3 mL), then the aqueous phase was acidified with HCl (1 M) to pH 2 and extracted with EtOAc (3 x 3 mL), dried over anhydrous sodium sulfate and concentrated *in vacuo* to yield **97** as a white solid (37 mg, 92%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.40-7.26 (m, 10H, ArH), 5.70 (m, 2H, 2 x NH), 5.21 – 5.05 (m, 4H, C$\underline{H}_2$Ph x 2), 1.92 – 1.68 (m, 4H, Hβ), 1.54 – 1.24 (m, 2H, Hγ).

(2*S*,6*R*)-2,6-diaminoheptanedioic-3,4-*d*$_2$ acid (98)



Alkene **103** (50 mg, 0.079 mmol) and 10% palladium on carbon (8 mg) in dry CD_3OD (5 mL) were stirred under a deuterium atmosphere for 2 h. The suspension was then filtered through celite, which was washed with 50% methanol in water. The filtrate was concentrated *in vacuo* and the resulting solid was purified using a DOWEX ion exchange resin to yield **98** as a white solid (10 mg, 67%). ^{1}H NMR (D_2O, 500 MHz) δ 3.74 – 3.64 (m, 2H, Hα + Hε), 1.84 (m, 3H, Hβ + Hδ + Hδ), 1.52 – 1.36 (m, 1H, Hγ); ^{13}C NMR (D_2O, 126 MHz) δ 174.8, 54.5, 30.2, 20.3. HRMS (*m/z*) calcd for $C_7H_{12}D_2N_2O_4Na$ $[M+Na]^+$ 215.0971, found 215.0967.

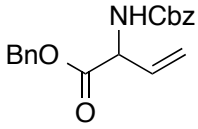
Benzyl (*R*)-2-[(benzyloxycarbonyl)amino]pent-4-enoate (100)



This product was synthesized according to a modified literature procedure.[141] To a suspension of D-allylglycine (**99**) (0.5 g, 4.35 mmol) in water (20 mL) was added $NaHCO_3$ (1.1 g, 13.05 mmol), then benzyl chloroformate (0.93 mL, 6.52 mmol) was added dropwise and stirred under argon over 3 h. The resulting mixture was diluted with diethyl ether and the aqueous phase was acidified to pH 1-2 with HCl (12 N), then extracted with EtOAc, dried over anhydrous magnesium sulfate and concentrated *in vacuo* to yield the carbamate (Cbz-D-Agl-OH) as a yellow oil (0.84 g, 84%), which was used in the next step without further purification. $[\alpha]_D^{25}$ = -17.66 (c = 1.0 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3400-2800 (br.), 3319, 3069, 3033, 2983, 1722, 1587, 1524, 1219 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.77 (br. s, 1H, -$CO_2\underline{H}$), 7.43 – 7.31 (m, 5H, HAr), 5.82 – 5.68 (m, 1H, Hγ), 5.36 (d, *J* = 8.1 Hz, 1H, NH), 5.25 – 5.09 (m, 4H, $C\underline{H}_2Ph$ + 2Hδ), 4.58 – 4.47 (m, 1H, Hα), 2.63 (ddt, *J* = 28.1, 14.3, 7.2 Hz, 2H, Hβ); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.4, 156.0, 136.1, 131.7, 128.6, 128.3, 128.2, 119.8, 67.3, 53.1, 36.4. HRMS (*m/z*) calcd for $C_{13}H_{14}NO_4$ $[M-H]^-$ 248.0928, found 248.0931. To a stirring solution of carbamate (Cbz-D-Agl-OH) (0.8 g, 3.2 mmol) in dry benzene (32 mL) was added benzyl alcohol (3.3 mL, 32.1 mmol), *p*-toluenesulfonic acid (0.31 g, 1.6 mmol), fitted with a dean-stark apparatus and heated under reflux overnight. The solution was allowed to cool to room

temperature, then concentrated *in vacuo* to afford a yellow-brown oil. The residue was purified by flash chromatography (silica, 9:1 hexanes:ethyl acetate) to give **100** as colorless oil (0.963 g, 88%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.45 – 7.30 (m, 10H, HAr), 5.68 (ddt, *J* = 17.3, 10.5, 7.2 Hz, 1H, Hγ), 5.34 (d, *J* = 9.0 Hz, 1H, C$\underline{H}_2$Ph), 5.27 – 5.22 (d, *J* = 12.2 Hz, 1H, C$\underline{H}_2$Ph), 5.22 – 5.17 (d, *J* = 12.2 Hz, 1H, C$\underline{H}_2$Ph), 5.17 – 5.10 (m, 3H, C$\underline{H}_2$Ph + 2Hδ), 4.55 (dt, *J* = 8.2, 5.8 Hz, 1H, Hα), 2.67 – 2.50 (m, 2H, Hβ). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 171.6, 155.7, 136.2, 135.3, 131.9, 128.6, 128.4, 128.2, 128.1, 119.5, 67.2, 53.4, 36.7. Spectral data were consisted with the previously reported compound.[141]

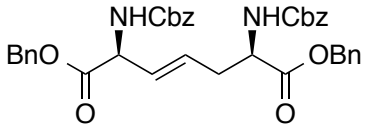
Benzyl (*S*)-2-[(benzyloxycarbonyl)amino]but-3-enoate (102)



This product was synthesized according to a previously reported literature procedure.[75] A solution of Z-L-glutamic acid-α-benzyl ester (**101**) (2.0 g, 5.4 mmol) and cupric acetate monohydrate (0.27 g, 1.35 mmol) in dry benzene (65 mL) was stirred for 1 h at room temperature under argon. Lead tetraacetate (4.8 g, 10.8 mmol) was then added and the resulting suspension refluxed for 14 h, then cooled to room temperature. The mixture was filtered through celite, which was washed with EtOAc (3 x 100 mL). The filtrate was then washed with water (3 x 80 mL) and brine (80 mL), dried over anhydrous Na_2SO_4 and concentrated *in*

vacuo to afford a yellow oil. The residue was purified by flash chromatography (silica, 9:1 hexane:EtOAc) to yield Z-Vgl-OBn (**102**) as colorless oil (0.74 g, 42%). $[\alpha]_D^{25}$ = -12.0 (c = 1.0 g/100mL, MeOH); IR (MeOH cast film): 3400-2800 (br.), 3306, 3090, 3035, 2957, 1743, 1690, 1587, 1540, 1499, 1262, 1083 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.44 – 7.30 (m, 10H, HAr), 5.96 (ddd, *J* = 16.8, 10.3, 5.5 Hz, 1H, Hβ), 5.49 (d, *J* = 8.2 Hz, 1H, NH), 5.40 (dd, *J* = 16.8, 1.8 Hz, 1H, Hγ), 5.31 (dd, *J* = 10.3, 1.7 Hz, 1H, Hγ), 5.23 (s, 2H, C$\underline{H_2}$Ph), 5.16 (s, 2H, C$\underline{H_2}$Ph), 5.03 (m, 1H, Hα); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 170.3, 155.5, 136.2, 135.1, 132.3, 128.7, 128.6, 128.5, 128.2, 128.2, 117.9, 67.5, 67.2, 56.2. HRMS (*m/z*) calcd for $C_{19}H_{20}NO_4$ $[M+H]^+$ 326.1387, found 326.1388.

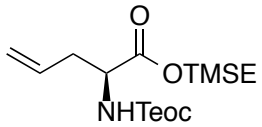
Dibenzyl (2*S*,6*R*,*E*)-2,6-bis[(benzyloxycarbonyl)amino]hept-3-enedioate (103)



This product was synthesized according to a previously reported literature procedure.[72] A solution of Z-Vgl-OBn (**102**) (0.2 g 0.62 mmol) and Z-D-Agl-OBn (**100**) (0.116 g, 0.34 mmol) in dry CH_2Cl_2 (3 mL) was deoxygenated with argon bubbling for 5 min, followed by the addition of Hoyveda-Grubbs 2nd generation catalyst (0.058 g, 0.068 mmol). The reaction mixture was refluxed under argon for 21 h, then concentrated *in vacuo* to give a black oil, which was purified by flash column chromatography (silica, 8:2, hexanes:EtOAc) to yield alkene (**103**)

as a mixture of regioisomers (0.135 g, 62%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.43 – 7.27 (m, 20H), 5.71 – 5.33 (m, 4H, 2NH + Hβ + Hγ), 5.26 – 5.06 (m, 8H, 4 x C$\underline{H}_2$Ph), 4.89 (m, 1H, Hε), 4.57 – 4.43 (m, 1H, Hα), 2.65 – 2.45 (m, 2H, Hδ). HRMS (*m/z*) calcd for $C_{37}H_{36}N_2O_8Na$ $[M+Na]^+$ 659.2364, found 659.2358.

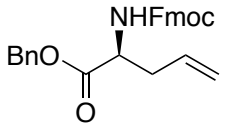
(*S*)-2-(trimethylsilyl)ethyl 2-{[2-(trimethylsilyl)ethoxy]carbonylamino}pent-4-enoate (109)



To a solution of H-allyl-L-gly-OH **99** (2g, 17.4 mmol) in water (17 mL) was added a solution of Et_3N (3.6 mL, 26.1 mmol) in dioxane (17 mL) at room temperature, then Teoc-OSu (4.96 g, 19.1 mmol) was added and was stirred overnight. The reaction mixture was diluted with water (100 mL), acidified with $KHSO_4$ to pH = 1-2, extracted with Et_2O (3 x 100 mL), then organic layers were combined, washed with water (100 mL), dried over anhydrous magnesium sulfate, concentrated *in vacuo*. The crude product NTeoc-allyl-L-gly-OH was left under high vacuum for 1 h and used in the next step without further purification. $[\alpha]_D^{25}$ = +13.91 (c = 1.11 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3400-2800 (br), 3320, 3082, 2955, 2900, 1722, 1523, 1251 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.76 (ddt, *J* = 18.3, 9.5, 7.2 Hz, 1H, Hγ), 5.24 – 5.18 (m, 2H, Hδ), 5.12 (d, *J* = 7.8 Hz, 1H, N$\underline{H}$), 4.47 (dd, *J* = 8.6, 4.5 Hz, 1H, Hα), 4.29 – 4.12 (m, 2H, OC$\underline{H}_2$-Teoc), 2.61 (m, 2H, Hβ), 1.12 – 0.99 (m, 2H, SiC$\underline{H}_2$-Teoc), 0.06 (s, 9H, 3 x CH_3); ^{13}C

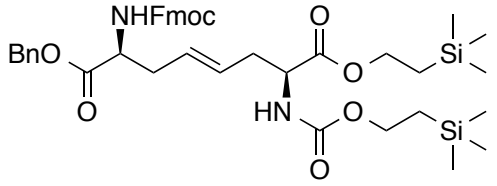
NMR ($CDCl_3$, 126 MHz) δ 175.2, 156.4, 131.9, 119.7, 63.8, 52.9, 36.3, 17.7, -1.5. HRMS (*m/z*) calcd for $C_{11}H_{20}NO_4Si$ $[M-H]^-$ 258.1167, found 258.1167. To a solution of crude NTeoc-allyl-L-gly-OH (17.4 mmol), DMAP (0.319 g, 2.61 mmol), in THF (73 mL) was added 2-(trimethylsilyl)ethanol (5 mL, 34.8 mmol) at 0 ºC, then DCC (4.67 g, 22.62 mmol) was added in one portion. The reaction mixture was allowed to reach room temperature and was stirred 24 h. The resulting suspension was filtered and concentrated *in vacuo*, the crude product was purified by flash chromatography (silica, 9:1 hexanes: EtOAc) to yield **109** as a clear oil (5.96 g, 90 % over two steps). $[\alpha]_D^{25}$ = +4.04 (c = 0.98 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3350, 3320, 3080, 2954, 2900, 2856, 1724, 1642, 1509, 1251 cm^{-1}; ^1H NMR ($CDCl_3$, 700 MHz) δ 5.68 (ddt, *J* = 17.0, 9.7, 7.2 Hz, 1H, Hγ), 5.19 – 5.08 (m, 3H, Hδ + NH), 4.38 (m, 1H, Hα), 4.26 – 4.14 (m, 4H, 2 x OCH_2), 2.52 (m, 2H, Hβ), 1.11 – 0.86 (m, 4H, 2 x $SiCH_2$), 0.03 (s, 9H, 3 x CH_3), 0.02 (s, 9H, 3 x CH_3); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 171.9, 156.0, 132.2, 119.1, 63.8, 63.3, 53.2, 36.8, 17.7, 17.4, -1.5, -1.6. HRMS (*m/z*) calcd for $C_{16}H_{33}NO_4SiNa$ $[M+Na]^+$ 382.1840, found 382.1838.

(*S*)-*tert*-butyl 2-methoxy-2,4-dimethylpentan-3-ylcarbamate (111)



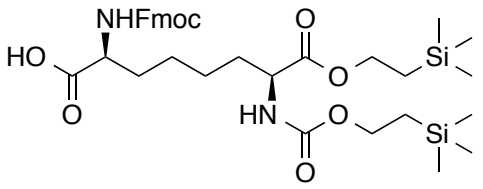
To a solution of Fmoc-L-allyl-gly-OH **110** (3.5 g, 10.4 mmol) in MeOH (52 mL) was added a solution of Cs_2CO_3 (1.69 g, 5.2 mmol) in water (3 mL) at 0 ºC. After warming up to room temperature for 5 min with stirring, the reaction mixture was concentrated *in vacuo*, rinsed with MeOH (2 x 5 mL), and concentrated *in vacuo* again to remove residual water. The salt was dried under high vacuum over night, then, it was suspended in DMF (52 mL) and benzyl bromide (1.24 mL, 10.4 mmol) was added at 0 ºC. The reaction mixture was stirred at room temperature overnight, concentrated *in vacuo*, and the residue was purified by flash chromatography (silica, 8:2 hexanes: EtOAc) to afford **111** as white solid (3.8 g, 85%). $[\alpha]_D^{25}$ = -4.27 (c = 1.04 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3344, 3065, 2950, 1725, 1642, 1515, 1193 cm^{-1}; ^1H NMR ($CDCl_3$, 700 MHz) δ 7.76 (d, *J* = 7.6 Hz, 2H, Fmoc), 7.58 (d, *J* = 7.4 Hz, 2H, HAr-Fmoc), 7.42 – 7.27 (m, 9H, HAr-Fmoc + HAr-Ph), 5.65 (ddt, *J* = 17.2, 10.1, 7.2 Hz, 1H, Hγ), 5.33 (d, *J* = 8.2 Hz, 1H, NH), 5.21 (d, *J* = 12.2 Hz, 1H, 1 x CH_2Ph), 5.16 (d, *J* = 12.2 Hz, 1H, 1 x CH_2Ph), 5.13 – 5.06 (m, 2H, Hδ), 4.52 (dt, *J* = 8.2, 5.7 Hz, 1H, Hα), 4.38 (d, *J* = 7.2 Hz, 2H, CH_2-Fmoc), 4.22 (t, *J* = 7.2 Hz, 1H, CH-Fmoc), 2.61 (m, 1H, 1 x Hβ), 2.53 (m, 1H, 1 x Hβ); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 171.6, 155.7, 143.9, 141.3, 131.9, 128.6, 128.5, 128.4, 127.7, 127.0, 125.1, 120.0, 67.3, 67.0, 47.1, 36.7. HRMS (*m/z*) calcd for $C_{27}H_{25}NO_4Na$ $[M+Na]^+$ 450.1676, found 450.1669.

(2*S*,7*S*,E)-1-benzyl-8-[2-(trimethylsilyl)ethyl]-2-{[(9*H*-fluoren-9-yl) methoxy]carbonylamino}-7-{[2-(trimethylsilyl)ethoxy]carbonylamino}oct-4-enedioate (112)



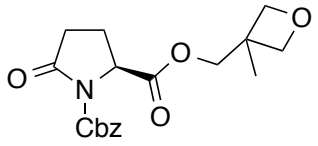
A solution of Fmoc-Agl-OBn **111** (0.564 g 1.32 mmol) and Teoc-Agl-OTMSE **109** (0.522 g, 1.45 mmol) in dry DCM (13 mL) was deoxygenated with argon bubbling for 15 min, followed by the addition of Hoyveda-Grubbs 2nd generation catalyst (0.112 g, 0.132 mmol). The reaction mixture was refluxed under argon for 19 h, then concentrated *in vacuo* to yield a black oil, which was purified by flash column chromatography (silica, 8:2, hexanes:ethyl acetate) to yield alkene **112** as a mixture of regioisomers (0.36 g, 36 %). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 (d, *J* = 7.6 Hz, 2H, HAr-Fmoc), 7.64 (d, *J* = 7.6 Hz, 2H, HAr-Fmoc), 7.45 – 7.28 (m, 9H, HAr-Fmoc + HAr-Ph), 5.51 (m, 1H, Hγ), 5.38 (m, 2H, N<u>H</u>-Fmoc + Hγ), 5.21 (m, 3H, N<u>H</u>-Teoc + C<u>H</u>$_2$Ph), 4.50 (m, 1H, Hα), 4.40 (m, 3H, Hζ + C<u>H</u>$_2$-Fmoc), 4.30 – 4.12 (m, 5H, CH-Fmoc + 2 x OC<u>H</u>$_2$), 2.58 – 2.36 (m, 4H, Hβ + Hε), 1.05 – 0.95 (m, 4H, SiC<u>H</u>$_2$), 0.05 (s, 9H, 3 x C<u>H</u>$_3$), 0.03 (s, 9H, 3 x C<u>H</u>$_3$). HRMS (*m/z*) calcd for $C_{41}H_{54}N_2O_8Si_2Na$ $[M+Na]^+$ 781.3311, found 781.3313.

(2*S*,7*S*,E)-2-{[(9*H*-fluoren-9-yl)methoxy]carbonylamino}-8-oxo-8-[2-(trimethylsilyl)ethoxy]-7-{[2-(trimethylsilyl)ethoxy]carbonylamino}oct-4-enoic acid (113)



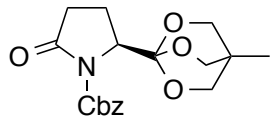
Alkene **112** (0.32 g, 0.42 mmol) and 10 % palladium on carbon (45 mg) in dry MeOH (36 mL) were stirred under a hydrogen atmosphere for 60 min. The suspension was then filtered through celite, which was washed with methanol, and the filtrate concentrated *in vacuo* to afford orthogonally protected suberic acid **113** (0.28 g, 99.9 %). $[\alpha]_D^{25}$ = +14.97 (c = 1.07 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3350, 3321, 2954, 1782, 1522, 1250 cm^{-1}; ^1H NMR ($CDCl_3$, 700 MHz) δ 7.73 (d, *J* = 7.4 Hz, 2H, HAr-Fmoc), 7.57 (d, *J* = 7.4 Hz, 2H, HAr-Fmoc), 7.37 (t, *J* = 7.4 Hz, 2H, HAr-Fmoc), 7.28 (t, *J* = 7.4 Hz, 2H, HAr-Fmoc), 5.55 – 5.39 (m, 1H, NH-Fmoc), 5.21 (m, 1H, NH-Teoc), 4.51 – 4.25 (m, 4H, Hα + Hζ + C$\underline{H}_2$-Fmoc), 4.25 – 4.02 (m, 5H, C$\underline{H}$-Fmoc + 2 x OC$\underline{H}_2$), 1.96 – 1.53 (m, 4H, Hβ + Hε), 1.46 – 1.18 (m, 4H, Hγ + Hδ), 1.02 – 0.89 (m, 4H. SiC$\underline{H}_2$), 0.02 (s, 9H, 3 x C$\underline{H}_3$), 0.01 (s, 9H, 3 x C$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 173.8, 173.4, 157.6, 156.0, 143.8, 141.3, 127.7, 127.0, 125.1, 120.0, 67.0, 63.8, 53.6, 53.4, 47.2, 33.7, 25.5, 17.7, 17.4, -1.5, -1.6. HRMS (*m/z*) calcd for $C_{34}H_{49}N_2O_8Si_2$ $[M-H]^-$ 669.3033, found 669.3033.

(2*S*)-1-Benzyloxycarbonyl-5-oxo-pyrrolidine-2-carboxylic acid-2-(3-methyl-oxetan-3-yl-methyl) ester (125a)



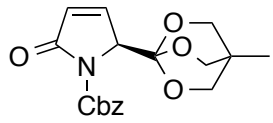
Compound **125a** was prepared by modification of a literature procedure.[79] To a suspension of Z-L-pyroglutamic acid (2 g, 7.6 mmol) in dichloromethane (15 mL) was added 3-hydroxy-methyl-3-oxetane (0.75 mL, 0.78 g, 7.6 mmol) via syringe, then a solution of DMAP (0.046 g, 0.38 mmol) and DCC (1.57 g, 7.6 mmol) in dichloromethane (8 mL) was added slowly over 30 min at room temperature and the reaction mixture was stirred overnight. The white solid was removed by vacuum filtration and washed with dichloromethane. The solution was then concentrated *in vacuo* and dissolved in EtOAc (50 mL), washed with water (30 mL), dried over anhydrous sodium sulfate and concentrated *in vacuo* to give a yellowish oil, which was purified by flash chromatography (silica, 7:3:1 hexane:EtOAc:methanol) to yield **125a** as a colorless oil (2.2 g, 83%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.44 – 7.30 (m, 5H, HAr), 5.36 – 5.22 (m, 2H, C$\underline{H}_2$Ph), 4.74 (dd, *J* = 9.4, 2.7 Hz, 1H, H2), 4.40 (m, 2H, $CH_2OC\underline{H}_2$), 4.33 (m, 2H, $CH_2OC\underline{H}_2$), 4.23 – 4.14 (m, 2H, CO_2CH_2), 2.67 (ddd, *J* = 17.5, 10.5, 9.4 Hz, 1H, H4a), 2.53 (ddd, *J* = 17.5, 9.2, 3.2 Hz, 1H, H4b), 2.39 (ddt, *J* = 13.4, 10.5, 9.3 Hz, 1H, H3a), 2.11 (dddd, *J* = 13.3, 9.4, 3.2, 2.7 Hz, 1H, H3b), 1.26 (s, 3H). HRMS (*m/z*) calcd for $C_{18}H_{21}NO_6Na$ $[M+Na]^+$ 370.1261, found 370.1255.

(2*S*)-2-(4-Methyl-2,6,7-trioxa-bicyclo[2.2.2]oct-1-yl)-5-oxo-pyrrolidine-1-carboxylic acid benzyl ester (126)



Compound **126** was prepared by modification of a literature procedure.[79] To a solution of **125a** (2 g, 5.8 mmol) in dichloromethane (21 mL) was added $BF_3 \cdot OEt_2$ (0.72 mL, 0.81 g, 5.83 mmol) via syringe at 0 ºC under argon. The mixture was allowed to reach room temperature, and was quenched with triethylamine (7.4 mL, 5.4 g, 58 mmol). After 45 min the reaction mixture was concentrated *in vacuo* to give a colorless solid, which was purified by flash chromatography (silica, 7:3:1 hexane:EtOAc:methanol) to yield **126** as a colorless solid (1.8 g, 92%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.47 – 7.28 (m, 5H, ArH), 5.34 – 5.20 (m, 2H, -C$\underline{H_2}$Ph), 4.43 (d, *J* = 8.9, 1H, H2), 3.85 (s, 6H, 3 x OC$\underline{H_2}$), 2.79 (ddd, *J* = 17.5, 11.6, 9.3 Hz, 1H, H4b), 2.30 (ddd, *J* = 17.4, 9.8, 1.2 Hz, 1H, H4a), 2.22 (ddt, *J* = 13.1, 9.3, 1.0 Hz, 1H, H3a), 2.01 (dddd, *J* = 13.2, 11.6, 9.8, 8.9 Hz, 1H, H3b), 0.78 (s, 3H, CH_3). HRMS (*m/z*) calcd for $C_{18}H_{21}NO_6Na$ $[M+Na]^+$ 370.1261, found 370.1257.

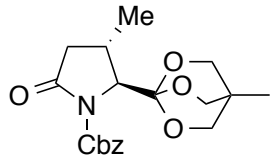
(2*S*)-2-(4-Methyl-2,6,7-trioxa-bicyclo[2.2.2]oct-1-yl)-5-oxo-2,5-dihydro-pyrrole-1-carboxylic acid benzyl ester (129)



This product was synthesized according to a previously reported literature procedure.[79] To a solution of lithium bis(trimethylsilyl)amide (7.3 mL, 7.3 mmol, 1.0 M in THF) diluted in THF (24 mL) at -78 ºC was slowly added a solution of **126** (1.15 g, 3.3 mmol) in THF (8 mL) under argon over a period of 10 min. The reaction was allowed to warm to 0 ºC, then, it was cooled to -78 ºC and a solution of phenylselenyl chloride (0.63 g, 3.3 mmol) in THF (22 mL) was added and the reaction mixture was stirred for a further 2 h. After that, the reaction mixture was allowed to reach room temperature and then quenched with saturated ammonium chloride solution (33 mL) and EtOAc (66 mL), washed with saturated ammonium chloride solution (33 mL) and the organic phase was concentrated *in vacuo* to give a yellow oil. The oily product was dissolved in THF (22 mL) and DABCO was added with vigorous stirring at -20 ºC. After 10 min a solution of *m*CPBA (1.71 g, 9.9 mmol) in DCM (38 mL) was added dropwise to the reaction mixture over 30 min then the reaction was allowed to warm to room temperature. The mixture was diluted with EtOAc (35 mL) and the organic phase was washed consecutively with saturated sodium bisulfite solution, saturated $NaHCO_3$ solution, and brine, concentrated *in vacuo* to give a yellow oil, which was purified by flash chromatography (silica, 1:1 hexane: EtOAc) to yield **129** as colorless solid (0.79 g, 64%). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.51 – 7.27 (m, 5H, HAr), 7.13

(dd, *J* = 6.1, 2.4 Hz, 1H, H-3), 6.11 (dd, *J* = 6.1, 1.7 Hz, 1H, H-4), 5.35 (d, *J* = 12.4 Hz, 1H, C<u>H</u>HPh), 5.27 (d, *J* = 12.4 Hz, 1H, CH<u>H</u>Ph), 4.96 (dd, *J* = 2.3, 1.7 Hz, 1H, H-2), 3.83 (s, 6H, 3 x O<u>CH</u>$_2$), 0.78 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 168.9, 150.5, 146.4, 135.2, 128.0, 127.9, 127.7, 126.5, 107.0, 72.5, 67.7, 64.2, 30.3, 13.8. Spectral data were consisted with the previously reported compound.[79]

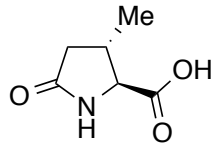
(2*S*, 3*S*)-2-(4-Methyl-2,6,7-trioxa-bicyclo[2.2.2]oct-1-yl)-5-oxo-3-methyl-pyrrolidine-1-carboxylic acid benzyl ester (130)



This product was synthesized according to a previously reported literature procedure.[79] To a suspension of $CuBr{\cdot}S(CH_3)_2$ (0.89 g, 4.34 mmol) in diethyl ether (3.5 mL) was added methyl lithium (5.4 mL, 8.7 mmol, 1.6 M in diethyl ether) at -20 ºC under argon and was stirred for 10 min, then the reaction mixture was cooled to -78 ºC and a solution of trimethylsilyl chloride (0.189 g, 1.74 mmol) and **129** (0.3 g, 0.87 mmol) in THF (4 mL) was added slowly and stirred for 1 h, then it was warmed to -20 ºC and, the suspension was quenched with saturated ammonium chloride solution, diluted with diethyl ether, washed with saturated ammonium chloride solution, brine and concentrated *in vacuo* to give a white solid, which was purified by flash chromatography (silica, 1:1 hexane: EtOAc) to yield **130** as white solid (0.201 g, 64%). $[\alpha]_D^{25}$ = 22.7 (c = 0.5 g/100 mL, DCM); IR

(DCM cast film): 3064, 2958, 1791, 1734, 1499, 1278 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.55 – 7.32 (m, 5H, HAr), 5.35 (d, *J* = 12.5 Hz, 1H, C<u>H</u>HPh), 5.26 (d, *J* = 12.5 Hz, 1H, CH<u>H</u>Ph), 3.82 (s, 6H, 3 x O<u>CH</u>$_2$) 4.06 (s, 1H, H-2), 3.02 (dd, *J* = 17.4, 8.2 Hz, 1H, H-4a), 2.64 – 2.55 (m, 1H, H-3), 1.96 (d, *J* = 17.4 Hz, 1H, H-4b), 1.12 (d, *J* = 7.2 Hz, 3H, CH_3-3), 0.81 (s, 3H, CH_3, *ortho* ester); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 174.7, 151.9, 135.7, 128.6, 128.4, 128.2, 128.1, 128.1, 108.8, 72.9, 68.0, 66.8, 40.1, 30.6, 27.3, 21.2, 14.3. HRMS (*m/z*) calcd for $C_{19}H_{23}NO_6Na$ $[M+Na]^+$ 384.1418, found 384.1413.

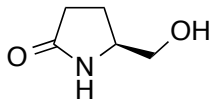
(2*S*,3*S*)-3-methyl-5-oxopyrrolidine-2-carboxylic acid (135)



This product was synthesized by modification of a reported literature procedure.[79] Pyrrolidine **130** (194 mg, 0.54 mmol) was dissolved in methanol (30 mL) and 10 % palladium on charcoal (57 mg, 0.54 mmol) was added. After degasification, the reaction mixture was stirred under a hydrogen atmosphere for 3 h and filtered through a thin layer of celite. The celite was washed with methanol (60 mL), the filtrate concentrated *in vacuo* and DCM (7 mL), TFA (154 μL), and H_2O (100 μL) were added to the residue. After the solution was stirred for 1 h at room temperature, the solvent was removed under vacuum. The residue was dissolved in a solution of 10 % (wt/vol) Cs_2CO_3 (3.7 mL, 1.13 mmol) and stirred for 3 h at room temperature. Subsequently, the reaction mixture was

quenched with a solution of 1 M HCl until pH 2 was reached, and lyophilized. The white solid was recrystallized from EtOH/hexane to yield **135** (38 mg, 49% 2 steps). ^{1}H NMR (CD_3OD, 500 MHz): δ 3.77 (d, *J* = 4.7 Hz, 1H, H-2), 2.60 – 2.49 (m, 2H, H-3 & H-4a), 2.02 – 1.93 (m, 1H, H-4b), 1.27 (d, *J* = 6.6 Hz, 3H, CH_3). HRMS (*m/z*) calcd for $C_6H_8NO_3$ $[M-H]^-$ 142.051, found 142.051.

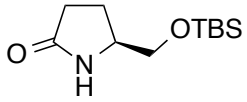
(*S*)-5-Hydroxymethyl-2-pyrrolidinone (137)



This product was synthesized according to a previously reported literature procedure.[77] To a solution of L-pyroglutamic acid **118** (2.0 g, 15.5 mmol) in MeOH (50 mL) was added thionyl chloride (1.25 mL, 17.1 mmol) dropwise at -15 ºC. After 30 min, the reaction mixture was allowed to warm to room temperature over 1 h, and was stirred for another hour. The solution was concentrated *in vacuo*, and the residue was dissolved in EtOH (50 mL), followed by slow addition of $NaBH_4$ (1.18 g, 31.2 mmol) at 0 ºC. Afterwards, the reaction mixture was allowed to warm to room temperature overnight. The reaction mixture was quenched with a 5 % aqueous solution of citric acid (100 mL) and concentrated *in vacuo*. The residue was suspended in a solution 25 % MeOH in EtOAc (200 mL), the solids were filtered off and solvent was removed *in vacuo* to afford and oil that was dissolved in MeOH and filtered. The solution was concentrated *in vacuo* to yield **137** as a white solid (1.6 g, 91%). ^{1}H NMR (CD_3OD, 500 MHz) δ

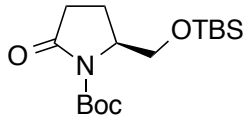
3.78 – 3.70 (m, 1H, H-5), 3.55 (dd, *J* = 11.3, 4.4 Hz, 1H, -C<u>H</u>$_2$-OH), 3.46 (dd, *J* = 11.3, 5.6 Hz, 1H, -C<u>H</u>$_2$-OH), 2.41 – 2.24 (m, 2H, H-3), 2.19 (dddd, *J* = 12.9, 10.0, 8.0, 6.5 Hz, 1H, H-4), 1.87 (dddd, *J* = 12.9, 10.0, 6.2, 4.9 Hz, 1H, H-4). HRMS (*m/z*) calcd for $C_5H_{10}NO_2$ $[M+H]^+$ 116.0706, found 116.0710.

(5*S*)-5-{[(*tert*-Butyl)dimethylsilyloxy]methyl}-pyrrolidin-2-one (138)



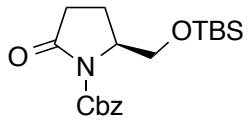
This product was synthesized according to a previously reported literature procedure.[77] To a solution of (*S*)-5-hydroxymethyl-2-pyrrolidinone **137** (1.63 g, 14.2 mmol) in dimethylformamide (100 mL) was added imidazole (2.41 g, 35.4 mmol) and *tert*-butyldimethylsilyl chloride (2.57 g, 17.0 mmol) and stirred for 24 h at room temperature. Then, the solution was diluted with Et_2O (300 mL), washed with water (3 x 100 mL), brine (3 x 100 mL) and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* to give **138** as a colorless oil (3.0 g, 93%). $[\alpha]_D^{25}$ = 39.2 (c = 1.8 g/100mL, DCM); IR (DCM cast film): 3215, 2954, 1703 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.03 (s, 1H, NH), 3.78 (m, 1H, H-2), 3.65 (dd, *J* = 10.1, 4.0 Hz, 1H, C<u>H</u>HO), 3.48 (dd, *J* = 10.1, 7.5 Hz, 1H, CH<u>H</u>O), 2.44 – 2.32 (m, 4H), 2.20 (m, 1H, H-3a), 1.77 (m, 1H, H-3b), 0.93 (s, 9H, -C(C<u>H</u>$_3$)$_3$), 0.07 (s, 6H, 2 x C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 178.0, 66.9, 55.8, 29.8, 25.8, 25.7, 22.8, 18.2, -3.6, -5.4. HRMS (*m/z*) calcd for $C_{11}H_{23}NO_2Si$ $[M+H]^+$ 230.1571, found 230.1566.

***tert*-Butyl (*S*)-2-{[(*tert*-butyldimethylsilyl)oxy]methyl}-5-oxopyrrolidine-1-carboxylate (139)**



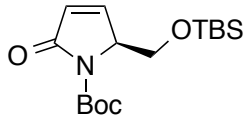
This product was synthesized according to a previously reported literature procedure.[77] To a solution of **138** (2.5 g, 10.9 mmol) in acetonitrile (80 mL) at 0 ºC was added DMAP (0.133 g, 1.09 mmol), di-*tert*-butyl dicarbonate (4.76 g, 21.8 mmol), and the reaction mixture was allowed to warm to room temperature overnight. The solvent was removed *in vacuo* to yield an orange oil, which was purified by flash chromatography (silica, 1:9 EtOAc:DCM) to yield **139** as yellow oil (3.44 g, 96%). $[\alpha]_D^{25}$ = -61.90 (c = 1.2 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 2956, 1791, 1712, 1314 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.20 (dddd, *J* = 8.8, 3.9, 2.3, 1.6 Hz, 1H, H-2), 3.94 (dd, *J* = 10.4, 4.0 Hz, 1H, -C$\underline{H}_2$OTMS), 3.72 (dd, *J* = 10.4, 2.3 Hz, 1H, C$\underline{H}_2$OTMS), 2.73 (ddd, *J* = 17.6, 11.0, 9.9 Hz, 1H, H-3), 2.40 (ddd, *J* = 17.6, 9.9, 2.2 Hz, 1H, H-3), 2.21 – 2.00 (m, 2H, H-4), 1.56 (s, 9H, $CO_2C(C\underline{H}_3)_3$), 0.91 (s, 9H, -SiC(C$\underline{H}_3$)$_3$), 0.07 (d, *J* = 6.6 Hz, 6H, 2 x CH_3). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 174.9, 150.1, 82.7, 64.3, 58.9, 32.4, 28.1, 25.8, 21.1, 18.2, -5.5, -5.6. HRMS (*m/z*) calcd for $C_{16}H_{31}NO_4SiNa$ $[M+Na]^+$ 352.1915, found 352.1909.

Benzyl (*S*)-2-{[(*tert*-butyldimethylsilyl)oxy]methyl}-5-oxopyrrolidine-1-carboxylate (140)



This product was synthesized by modification of a literature procedure.[77, 142] To a solution of **138** (1.6 g, 7.0 mmol) in THF (80 mL) was added *n*-butyl lithium (3.1 mL, 7.7 mmol, 2.5 M in hexanes) at -78 ºC and was stirred for 15 min. Benzyl chloroformate (1.1 mL, 7.7 mmol) was added and the solution was allowed to warm to 0 ºC. After 30 min, Et_3N (1 mL, 7.0 mmol) was added at 0 ºC. Then ice bath was removed and reaction mixture was diluted with Et_2O (20 mL), washed sequentially with water, brine, dried over anhydrous sodium sulfate, concentrated and purified by flash chromatography (silica, 7:3 hexanes: EtOAc) to yield **140** (1.8 g, 71%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.48 – 7.27 (m, 5H, HAr), 5.34 (d, *J* = 12.4 Hz, 1H, 1 x C$\underline{H}_2$Ph), 5.27 (d, *J* = 12.4 Hz, 1H, 1 x C$\underline{H}_2$Ph), 4.26 (dddd, *J* = 8.9, 3.9, 2.3, 1.6 Hz, 1H, H-2), 3.91 (dd, *J* = 10.5, 3.9 Hz, 1H, 1 x C$\underline{H}_2$OTBS), 3.68 (dd, *J* = 10.5, 2.3 Hz, 1H, 1 x C$\underline{H}_2$OTBS), 2.75 (ddd, *J* = 17.7, 11.0, 9.9 Hz, 1H, H-4), 2.42 (ddd, *J* = 17.7, 9.9, 2.2 Hz, 1H, H-4), 2.20 – 2.01 (m, 2H, H-3), 0.87 (s, 9H, SiC(CH_3)$_3$), -0.01 (s, 3H, CH_3), - 0.03 (s, 3H, CH_3).

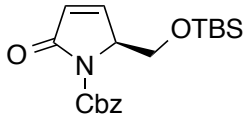
***tert*-Butyl (*S*)-2-{[(*tert*-butyldimethylsilyl)oxy]methyl}-5-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate (141)**



This product was synthesized according to a previously reported literature procedure.[77] To a solution of **139** (4.0 g, 12.1 mmol) in THF (100 mL) at -78 ºC was slowly added LiHMDS (14 mL, 14 mmol, 1 M in hexanes) and stirred for 15 min, then a solution of phenylselenyl bromide (2.3 g, 12.1 mmol) in THF (14 mL) was added via cannula to the reaction mixture and stirred for 1 h at -78 ºC. The reaction was quenched by addition of saturated ammonium chloride solution and diethyl ether, and allowed to warm to room temperature. The solution was washed with saturated ammonium chloride solution and brine, dried over anhydrous sodium sulphate, and concentrated *in vacuo* to yield a red oil, which was then dissolved in dichloromethane (25 mL) and cooled to -78 ºC followed by addition of pyridine (3 mL, 36.3 mmol). After 5 min, hydrogen peroxide (30 %, 4 mL) was added and the temperature was allowed to warm to 0 ºC over 1 h. The solution was diluted with diethyl ether, washed with water, brine, and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* to give a solid, which was purified by flash chromatography (silica, 9:1 hexanes:EtOAc) to yield **141** as white solid (2.77 g, 70%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.29 (dd, *J* = 6.1, 2.1 Hz, 1H, H-3), 6.15 (dd, *J* = 6.1, 1.6 Hz, 1H, H-4), 4.66 – 4.58 (m, 1H, H-2), 4.18 (dd, *J* = 9.7, 3.7 Hz, 1H, C$\underline{H}_2$OTMS), 3.75 (dd, *J* = 9.7, 6.7 Hz, 1H, C$\underline{H}_2$OTMS), 4.23 – 4.13 (m, 1H), 3.94 (dd, *J* = 10.4, 4.0 Hz, 1H) 1.57 (m, 9H,

Boc), 0.90 (m, 9H, -SiC(CH_3)$_3$), 0.07 (m, 6H, CH_3 x 2); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 169.4, 149.7, 127.1, 83.0, 63.6, 62.5, 28.2, 25.8, 18.2, -5.4, -5.5. Spectral data were consisted with the previously reported compound.[77]

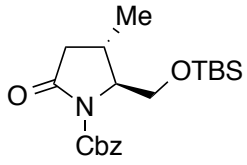
Benzyl (*S*)-2-{[(*tert*-butyldimethylsilyl)oxy]methyl}-5-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate (142)



This product was synthesized by modification of a previously reported literature procedure.[77] To a solution of **140** (1.0 g, 2.75 mmol) in THF (25 mL) was slowly added LiHMDS (5.5 mL, 5.5 mmol, 2 M in THF) at -78 ºC and stirred for 15 min. Subsequently, a solution of phenylselenyl chloride (0.57 g, 3.0 mmol) in THF (4 mL) was added *via* cannula to the reaction mixture and stirred for 1 h at -78 ºC. The reaction was quenched by addition of saturated ammonium chloride solution (12 mL) and diethyl ether (15 mL), and allowed to warm to room temperature. The solution was washed with saturated ammonium chloride solution, brine, dried over anhydrous sodium sulphate, and concentrated *in vacuo* to yield an orange oil, which was then dissolved in DCM (23 mL) and cooled to -78 ºC followed by addition of pyridine (0.7 mL, 8.25 mmol). After 5 min, hydrogen peroxide (30%, 1 mL) was added slowly and the temperature was allowed to warm to 0 ºC over 1 h. The solution was diluted with diethyl ether, washed with water, brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to give a solid, which was purified by flash chromatography (silica, 7:3

hexanes:EtOAc) to yield **142** as a white solid (0.44 g, 44%). $[\alpha]_D^{25}$ = -159.37 (c = 1.0 g/100mL, DCM); IR (DCM cast film): 3080, 3037, 2948, 1763, 1687, 1600, 1499, 1122 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.51 – 7.47 (m, 2H, HAr), 7.43 – 7.34 (m, 3H, HAr), 7.33 (dd, *J* = 6.1, 2.1 Hz, 1H, H-4), 6.19 (dd, *J* = 6.1, 1.6 Hz, 1H, H-3), 5.40 (d, *J* = 12.4 Hz, 1H, 1 x $C\underline{H}_2Ph$), 5.32 (d, *J* = 12.3 Hz, 1H, 1 x $C\underline{H}_2Ph$), 4.70 (dddd, *J* = 6.6, 3.6, 2.1, 1.7 Hz, 1H, H-2), 4.16 (dd, *J* = 9.8, 3.6 Hz, 1H, 1 x $C\underline{H}_2OTBS$), 3.77 (dd, *J* = 9.8, 6.5 Hz, 1H, 1 x $C\underline{H}_2OTBS$), 0.87 (s, 9H, $C(C\underline{H}_3)_3$), 0.02 (s, 3H, 1 x $Si(C\underline{H}_3)_2$), 0.00 (s, 3H, 1 x $Si(C\underline{H}_3)_2$); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 169.0, 151.0, 150.3, 135.3, 128.7, 128.4, 128.3, 126.9, 68.0, 63.7, 62.2, 25.7, 18.1, -5.6, -5.7. HRMS (*m/z*) calcd for $C_{19}H_{28}NO_4Si$ $[M+H]^+$ 362.1782, found 362.1780.

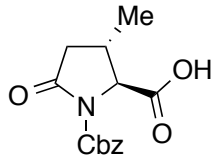
Benzyl (2*S*,3*S*)-2-{[(*tert*-butyldimethylsilyl)oxy]methyl}-3-methyl-5-oxopyrrolidine-1-carboxylate (144)



This product was synthesized by modification of a previously reported literature procedure.[77] To a solution of copper(I) iodide (1.48 g, 7.7 mmol) in diethyl ether (12 mL) was added methyllithium (9.6 mL, 15.4 mmol, 1.6 M in diethyl ether) at 0 ºC under argon and was stirred for 10 min. Afterwards, the reaction mixture was cooled at -78 ºC and a solution of **142** (0.4 g, 1.1 mmol) in

THF (6 mL) was added slowly. After 1 h, the suspension was warmed to 0 ºC and was quenched with saturated ammonium chloride solution, diluted with diethyl ether (20 mL), washed with brine, and concentrated *in vacuo* to give a light yellow oil, which was purified by flash chromatography (silica, 7:3 hexane: EtOAc) to yield **144** as a white solid (0.302 g, 73%). $[\alpha]_D^{25}$ = -55.29 (c = 1.0 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3066, 2956, 1793, 1717, 1600, 1499, 1298 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.50 – 7.32 (m, 5H, HAr), 5.40 – 5.26 (m, 2H, C$\underline{H}_2$Ph), 3.90 (dd, *J* = 10.5, 4.1 Hz, 1H, C$\underline{H}_2$OTBS), 3.83 (ddd, *J* = 4.0, 2.2, 1.3 Hz, 1H, H-2), 3.73 (dd, *J* = 10.5, 2.2 Hz, 1H, -C$\underline{H}_2$OTBS), 2.96 (dd, *J* = 17.6, 8.6 Hz, 1H, H-4a), 2.41 (dqt, *J* = 8.8, 7.2, 1.7 Hz, 1H, H-3), 2.08 (dd, *J* = 17.6, 1.8 Hz, 1H, H-4b), 1.16 (d, *J* = 7.2 Hz, 3H, C$\underline{H}_3$-3), 0.88 (s, 9H, -C(C$\underline{H}_3$)$_3$), -0.01 (s, 3H, SiC$\underline{H}_3$), -0.02 (s, 3H, SiC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 174.2, 151.7, 135.4, 128.6, 128.4, 128.2, 68.0, 66.3, 63.6, 40.4, 28.5, 25.8, 21.4, 18.1, -5.7, -5.7. HRMS (*m/z*) calcd for $C_{20}H_{31}NO_4SiNa$ $[M+Na]^+$ 400.1915, found 400.1905.

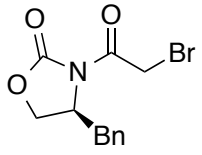
(2*S*,3*S*)-1-[(benzyloxy)carbonyl]-3-methyl-5-oxopyrrolidine-2-carboxylic acid (145)



This product was synthesized by modification of a previously reported literature procedure.[77] To a solution of **144** (0.25 g, 0.7 mmol) in acetone (1.75 mL) at 0 ºC was added dropwise a cooled solution of Jones reagent (0.44 mL,

1.4 mmol) and was stirred 30 min at 0 ºC and 1 h at room temperature. The reaction mixture was quenched by addition of IPA (15 mL) and slow addition of a saturated solution of $NaHCO_3$ (6 mL) at 0 ºC. The solvent was removed *in vacuo* and the residue was dissolved with a mixture of H_2O (9 mL) and EtOAc (15 mL), the solution was extracted with Et_2O (3 x 12 mL). Then, the aqueous layer was separated and acidified with 0.1 N HCl to pH 1-2 at 0 ºC, extracted with EtOAc (3 x 12 mL), and the organic layers were combined and dried over anhydrous sodium sulfate, concentrated *in vacuo* to yield **145** as a clear oil (0.101 g, 55%). $[\alpha]_D^{25}$ = -16.8 (c = 1.0 g/100mL, DCM); IR (DCM cast film): 3189 (br.), 3067, 2972, 1792, 1753, 1725, 1587, 1499, 1301 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 10.45 (s, 1H, $CO_2\underline{H}$), 7.45 – 7.28 (m, 5H, HAr), 5.39 – 5.21 (m, 2H, $C\underline{H}_2Ph$), 4.34 (d, *J* = 3.3 Hz, 1H, H-2), 2.86 (dd, *J* = 17.6, 8.5 Hz, 1H, H-4), 2.59 – 2.48 (m, 1H, H-3), 2.23 (dd, *J* = 17.6, 3.9 Hz, 1H, H-4), 1.29 (d, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 175.3, 172.9, 151.2, 134.8, 128.6, 128.5, 128.0, 68.6, 65.5, 39.3, 30.0, 20.6.

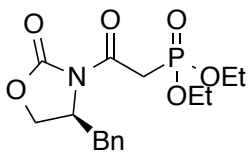
(*S*)-4-benzyl-3-(2-bromoacetyl)oxazolidin-2-one (152)



This product was synthesized according to a previously reported literature procedure.[89] To a solution of (*S*)-oxazolidinone **151** (0.48 g, 1.8 mmol) in THF (6.5 mL) *n*-butyl lithium (0.8 mL, 2.0 mmol, 2.5 M in hexanes) was added over 10 min at -78 ºC, then bromoacetyl bromide (0.24 mL, 2.7 mmol) was added

dropwise. After 1h at -78 ºC the reaction mixture was quenched with an aqueous saturated solution of NH_4Cl (1 mL) followed by addition of an aqueous saturated solution of $NaHCO_3$ (2 mL), then it was allowed to warm to room temperature. The aqueous phase was extracted with Et_2O (2 x 10 mL). The combined organic phases were washed with water, brine, dried over anhydrous sodium sulfate, concentrated *in vacuo* and purified by flash chromatography using a gradient of solvent (silica, from 100 % hexane to 7:3 hexanes:EtOAc) to yield **152** as a clear colorless oil (0.53 g, 99.9%). $[\alpha]_D^{25}$ = +71.57 (c = 2.2 g/100mL, DCM); IR (DCM cast film): 3062, 2971, 1781, 1700, 1604, 1498, 1207 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.42 – 7.22 (m, 5H, HAr), 4.74 (dddd, *J* = 9.6, 7.8, 3.2, 3.2 Hz, 1H, H-4), 4.60 (d, *J* = 12.8 Hz, 1H, 1 x C$\underline{H}_2$Br), 4.57 (d, *J* = 12.8 Hz, 1H, 1 x C$\underline{H}_2$Br), 4.32 (dd, *J* = 9.2, 7.8 Hz, 1H, 1 x H-5a), 4.27 (dd, *J* = 9.2, 3.2 Hz, 1H, 1 x H-5b), 3.37 (dd, *J* = 13.5, 3.2 Hz, 1H, 1 x C$\underline{H}_2$Ph), 2.85 (dd, *J* = 13.5, 9.6 Hz, 1H, 1 x C$\underline{H}_2$Ph); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 166.0, 153.0, 134.7, 129.4, 129.1, 127.6, 66.7, 55.5, 37.6, 28.2. HRMS (*m/z*) calcd for $C_{12}H_{12}BrNO_3Na$ $[M+Na]^+$ 319.9893, found 319.9887.

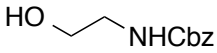
(*S*)-Diethyl 2-(4-benzyl-2-oxoxazolidin-3-yl)-2-oxoethylphosphonate (153)



This product was synthesized according to a previously reported literature procedure.[89] A mixture of oxazolidinone **152** (0.435 g, 1.46 mmol) and

triethylphosphite (1.3 mL, 7.32 mmol) was heated at 50 ºC overnight. The reaction flask was left for one day *in vacuo* to remove the excess of triethylphosphite to give a yellow oil. This residue was purified by flash chromatography (silica, EtOAc) to yield **153** as clear colorless oil (0.5 g, 97%). $[\alpha]_D^{25}$ = +48.94 (c = 2.73 g/100mL, DCM); IR (DCM cast film): 3063, 2984, 1782, 1698, 1604, 1497, 1025 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.41 – 7.23 (m, 5H, HAr), 4.75 (dddd, *J* = 10.5, 7.4, 3.2, 3.2 Hz, 1H, H-4), 4.30 – 4.10 (m, 6H, H-5 + 2 x $OC\underline{H}_2CH_3$), 3.95 – 3.73 (m, 2H, $C\underline{H}_2PO(OEt)_2$), 3.39 (dd, *J* = 13.4, 3.4 Hz, 1H, 1 x $C\underline{H}_2Ph$), 2.79 (dd, *J* = 13.4, 9.9 Hz, 1H, 1 x $C\underline{H}_2Ph$), 1.39 (t, *J* = 7.1 Hz, 6H, 2 x $OCH_2C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 165.1, 153.4, 135.1, 129.4, 129.0, 127.4, 66.0, 62.8, 55.5, 37.7, 34.9, 33.9, 16.4, 16.4. HRMS (*m/z*) calcd for $C_{16}H_{22}NO_6PNa$ $[M+Na]^+$ 378.1077, found 378.1068.

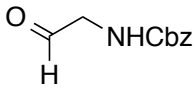
Benzyl 2-hydroxyethylcarbamate (155)



This product was synthesized according to a previously reported literature procedure.[90] To a solution of ethanolamine **154** (1 g, 16.4 mmol) in DCM (15 mL) a solution of benzyl chloroformate (1.84 mL, 13.1 mmol) in DCM (10 mL) was added dropwise over 5 min at 0 ºC. The reaction mixture was stirred for 2 h at 0 ºC, then 18 h at room temperature. After that, the solution was washed with aqueous saturated solution of $NaHCO_3$, and then the aqueous phase was extracted with DCM (3 x 25 mL). The organic phases were combined, dried over

anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by flash chromatography (silica, gradient of solvents from 7:3 hexanes:EtOAc to 100 % EtOAc) to yield **155** (1.38 g, 64%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.59 – 7.33 (m, 5H, HAr), 5.15 (s, 2H, C$\underline{H}_2$Ph), 3.77 (t, *J* = 5.0 Hz, 2H, -C$\underline{H}_2$OH), 3.41 (m, 2H, -C$\underline{H}_2$NH-), 2.0 (br. s, 1H, OH); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 157.6, 136.4, 128.6, 128.2, 128.2, 67.0, 62.1, 43.8. HRMS (*m/z*) calcd for $C_{10}H_{13}NO_3Na$ $[M+Na]^+$ 218.0788, found 218.0782.

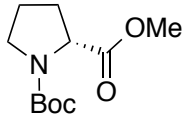
Benzyl (2-oxoethyl)carbamate (156)



This product was synthesized by modification of a previously reported literature procedure.[91] To a solution of ethanolamine **155** (0.3 g, 1.54 mmol) in DCM (4 mL) Dess-Martin periodinane (0.68 g, 1.6 mmol) was added in one portion at 0 ºC. After 90 min, another equivalent of DMP (0.71 g, 1.67 mmol) was added at 0 ºC. The reaction mixture was stirred for 80 min, then quenched with an aqueous saturated solution of $NaHCO_3$:$Na_2S_2O_3$ (1:1, 4 mL) and stirred for 5 min. The aqueous phase was extracted with Et_2O (2 x 10 mL). The combined organic phases were washed with water, brine and dried over anhydrous sodium sulfate and concentrated *in vacuo*. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.66 (s, 1H, C$\underline{H}$O), 7.45 – 7.30 (m, 5H, HAr), 5.53 (br s, 1H, N$\underline{H}$), 5.15 (s, 2H, C$\underline{H}_2$Ph), 4.16 (d, *J* = 5.1 Hz, 2H, -C$\underline{H}_2$NH-); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 196.5, 156.3, 136.1,

128.6, 128.3, 128.2, 67.2, 51.7. HRMS (*m/z*) calcd for $C_{10}H_{11}NO_3Na$ $[M+Na]^+$ 216.0631, found 216.0628.

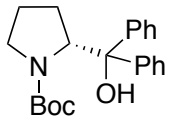
Methyl *N*-(*tert*-butoxycarbonyl)-D-prolinate (161)



This product was synthesized according to a previously reported literature procedure.[92-93] To a flask charged with MeOH (34 mL), acetyl chloride (4.9 mL, 69.3 mmol) was added at 0 ºC, then ice-bath was removed and D-proline **160** (2.8 g, 24.3 mmol) was added in one portion. The reaction mixture was heated under reflux over 2 h, cooled down and concentrated *in vacuo*. The residue was dissolved in THF (70 mL), then a solution of Boc_2O (5.4 g, 24 mmol) in THF (6 mL) was added, followed by addition of Et_3N (7.3 mL, 2.1 mmol) at 0 ºC and stirred at room temperature overnight. The reaction mixture was then concentrated *in vacuo* and dissolved in EtOAc (100 mL), washed with an aqueous saturated solution of $NaHCO_3$ (100 mL), extracted with EtOAc (2 x 100 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to yield the product **161** as a clear colorless oil (5.07 g, 90% over two steps). $[\alpha]_D^{25}$ = +55.56 (c = 1.02 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 2976, 2933, 2881, 1751, 1703, 1162 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.25 (dd, *J* = 8.6, 4.2 Hz, 1H, H-2), 3.75 (s, 3H, $OC\underline{H}_3$), 3.62 – 3.36 (m, 2H, H-5), 2.31 – 2.15 (m, 1H, 1 x H-3a), 2.04 – 1.83 (m, 3H, 1 x H-3b + H-4), 1.52 – 1.39 (m, 9H, Boc); ^{13}C NMR ($CDCl_3$, 126

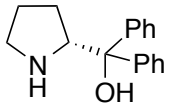
MHz) δ 173.8, 153.8, 79.8, 59.1, 51.9, 46.3, 30.9, 28.4, 28.3, 23.7. HRMS (*m/z*) calcd for $C_{11}H_{19}NO_4Na$ $[M+Na]^+$ 252.1206, found 252.1203.

(*R*)-(+)-2-(Diphenylhydroxymethyl)-*N*-(*tert*-butoxycarbonyl)pyrrolidine (162)



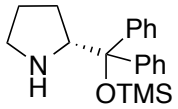
This product was synthesized according to a modified literature procedure.[94] To a solution of phenylmagnesium bromide (8.5 mL, 1 M in THF) was added dropwise a solution of *N*-Boc-D-proline-methyl ester **161** (0.5 g, 2.18 mmol) in THF (1.0 mL) at 0 ºC and was allowed to reach room temperature overnight. The reaction mixture was quenched with an aqueous saturated solution of NH_4Cl (3 mL), the solvent was removed *in vacuo* and the residue was dissolved in DCM, washed with brine, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude product was recrystallized from hexanes-EtOAc to yield **162** as a white solid (0.48 g, 63%). $[\alpha]_D^{25}$ = 131.0 (c = 1.0 g/100mL, DCM); IR (DCM cast film): 3313 (br.), 3087, 2977, 1661, 1600, 1492, 1169 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.45 – 7.26 (m, 10H, HAr), 4.93 (dd, *J* = 9.0, 3.7 Hz, 1H, H-2), 3.38 (m, 1H, H-5a), 2.90 (m, 1H, H-5b), 2.21 – 2.02 (m, 1H, H-3a), 2.00 – 1.88 (m, 1H, H-3b), 1.47 (m, 10H, Boc + H-4a), 082 (m, 1H, H-4b); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 155.4, 146.5, 143.8, 128.2, 127.9, 127.7, 127.3, 127.1, 127.0, 81.7, 80.6, 65.8, 47.9, 29.8, 28.4, 22.9. HRMS (*m/z*) calcd for $C_{22}H_{27}NO_3Na$ $[M+Na]^+$ 376.1883, found 376.1881.

(*R*)-(+)-2-(Diphenylhydroxymethyl)pyrrolidine (163)



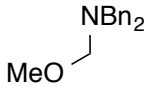
This product was synthesized according to a previously reported literature procedure.[95] To a solution of NaOH (4.5 g, 113 mmol) in EtOH (54 mL), *N*-Boc-pyrrolidine **162** (3.7 g, 10.5 mmol) was added and the resulting light yellow suspension was heated under reflux for 2.5 h. After that, the suspension was cooled to room temperature and concentrated *in vacuo*. To the residue was added H_2O (85 mL) and Et_2O (200 mL) and the suspension was stirred until the solids were dissolved. The aqueous layer was extracted (2 x 90 mL), and the organic layers were combined, dried over K_2CO_3, filtered and concentrated *in vacuo* to yield **163** (2.41 g, 91%). $[\alpha]_D^{25}$ = +64.4 (c = 3.34 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3351, 3085, 3058, 2970, 2871, 1597, 1492, 1174 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.62 – 7.57 (m, 2H, HAr), 7.57 – 7.48 (m, 2H, HAr), 7.40 – 7.24 (m, 4H, HAr), 7.22 – 7.13 (m, 2H, HAr), 4.61 (s, 1H, NH), 4.27 (t, *J* = 7.8 Hz, 1H, H-2), 3.06 (ddd, *J* = 9.3, 6.7, 4.9 Hz, 1H, 1 x H-5a), 2.97 (m, 1H, 1 x H-5b), 1.84 – 1.54 (m, 4H, H-3 + H-4); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 148.2, 145.4, 128.2, 128.0, 126.5, 126.34, 125.9, 125.5, 77.1, 64.50, 46.78, 26.30, 25.53. HRMS (*m/z*) calcd for $C_{17}H_{20}NO$ $[M+H]^+$ 254.1539, found 254.1540.

(*R*)-2-{Diphenyl[(trimethylsilyl)oxy]methyl}pyrrolidine (164)



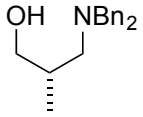
To a solution of pyrrolidine **163** (2.4 g, 9.5 mmol) in DCM (63 mL) was added Et_3N (1.7 mL, 12.3 mmol) followed by addition of TMSOTf (2.2 mL, 12.3 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and after 30 min was quenched with H_2O, extracted with DCM, dried over anhydrous sodium sulfate, concentrated *in vacuo* and purified by flash chromatography (silica, 7:3 hexanes:EtOAc + 1% Et_3N) to afford **164** (2.84 g, 92%). $[\alpha]_D^{25}$ = +75.52 (c = 1.0 g/100mL, DCM); IR (DCM cast film): 3085, 3059, 3024, 2954, 2898, 1598, 1698, 1492, 1072 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.50 – 7.45 (m, 2H, HAr), 7.40 – 7.35 (m, 2H, HAr), 7.33 – 7.19 (m, 6H, HAr), 4.05 (t, *J* = 7.4 Hz, 1H, H-2), 2.94 – 2.73 (m, 2H, H-5), 1.66 – 1.50 (m, 3H, 1 x H-3a + H-4), 1.46 – 1.33 (m, 1H, 1 x H-3b), -0.08 (s, 9H, 3 x $C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 146.9, 145.8, 128.4, 127.6, 127.6, 127.5, 126.9, 126.7, 83.2, 65.4, 47.2, 27.5, 25.1, 2.2. HRMS (*m/z*) calcd for $C_{20}H_{27}NOSi$ $[M+H]^+$ 326.1935, found 326.1934.

_N_,_N_-dibenzyl-1-methoxymethanamine (165)



To a suspension of paraformaldehyde (0.94 g, 31.4 mmol) in MeOH (13 mL) was added dibenzylamine (6 mL, 31.4 mmol) and the reaction mixture was stirred for 5 min at room temperature, then K_2CO_3 (4.3 g, 31.4 mmol) was added at 0 ºC and the ice bath was removed immediately. The reaction mixture was allowed to stand overnight. The solids were filtered off and the filtrate was fractionated. The fraction distilling at 139-140 ºC (~ 8 Torr) was collected (3.36 g, 45%). IR (DCM cast film): 3085, 3062, 3027, 2845, 1602, 1494, 1071 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.45 – 7.24 (m, 10H, HAr), 4.08 (s, 2H, C$\underline{H}_2$OMe), 3.88 (s, 4H, 2 x C$\underline{H}_2$Ph), 3.29 (s, 3H, C$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 139.3, 128.9, 128.2, 127.0, 85.4, 55.7, 55.4. HRMS (EI) calcd for $C_{16}H_{19}ON$ 241.1467, found 241.1472 $[M]^+$.

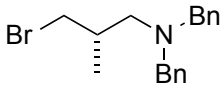
(_S_)-3-(dibenzylamino)-2-methylpropan-1-ol (166)



This product was synthesized by modification of a previously reported literature procedure.[98] To a homogeneous solution of LiBr (0.72 g, 8.29 mmol) in DMF (6 mL) was added sequentially, a solution of catalyst **164** (0.54 g, 1.66 mmol) in DMF (1 mL), AcOH (0.1 mL, 1.66 mmol), and propionaldehyde (0.62 mL, 8.29 mmol) at room temperature. At –25 ºC, a solution of aminomethyl ether

165 (1.0 g, 4.14 mmol) in DMF (1 mL) was added to the bright yellow solution, and was stirred vigorously for 2 h. The reaction mixture was diluted with MeOH (16 mL) and excess of $NaBH_4$ (3 g, 82.9 mmol) was added in small portions at – 25 ºC. After stirring for 5 min at 0 ºC, the solution was poured in a mixture of aqueous saturated solution of $NHCl_4$ (32 mL) and Et_2O (50 mL), extracted with Et_2O (2 x 50 mL), dried over anhydrous sodium sulfate, concentrated *in vacuo*, and purified by flash chromatography (silica, 8:2 hexanes: EtOAc) to yield **166** as a clear oil (0.987 g, 88%). $[\alpha]_D^{25}$ = +81.64 (c = 1.0 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3415, 3086, 3062, 2954, 1602, 1495, 1029 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.46 – 7.16 (m, 10H, HAr), 5.48 (s, 1H, O<u>H</u>), 4.03 (d, *J* = 13.2 Hz, 2H, 2 x C<u>H</u>$_2$Ph), 3.62 (ddd, *J* = 10.5, 3.6, 2.3 Hz, 1H, Hα), 3.27 (dd, *J* = 10.5, 9.4 Hz, 1H, Hα), 3.17 (d, *J* = 13.2 Hz, 2H, 2 x C<u>H</u>$_2$Ph), 2.56 (dd, *J* = 12.7, 11.2 Hz, 1H, Hγ), 2.40 (ddd, *J* = 12.7, 3.5, 2.3 Hz, 1H, Hγ), 2.33 – 2.21 (m, 1H, Hβ), 0.73 (d, *J* = 6.8 Hz, 3H, C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 138.0, 129.3, 128.5, 127.4, 70.5, 61.4, 59.1, 31.5, 15.0. HRMS (*m/z*) calcd for $C_{18}H_{24}NO$ $[M+H]^+$ 270.1852, found 270.1851.

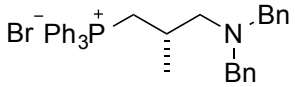
(*S*)-*N,N*-dibenzyl-3-bromo-2-methylpropan-1-amine (167)



This product was synthesized by modification of a previously reported literature procedure.[143] To a solution of γ-amino alcohol **166** (0.6 g, 2.23 mmol) in DCM (7 mL), was added methanesulfonyl chloride (0.21 mL, 2.67 mmol) followed

by triethylamine (0.40 mL, 2.90 mmol) at room temperature. After stirring for 1 h was added LiBr (1.94 g, 22.3 mmol), acetone (7 mL), and the reaction mixture was stirred overnight. The solvent was removed and the residue was diluted with EtOAc (60 mL), washed with water (2 x 30 mL), saturated aqueous solution of $NaHCO_3$ (30 mL), brine (30 mL), concentrated *in vacuo*, and purified by flash chromatography (silica, 9:1 to 8:2 hexanes: EtOAc) to yield **167** as clear oil (0.44 g, 60%). IR ($CHCl_3$ cast film): 3085, 3062, 2960, 1601, 1494 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.40 – 7.23 (m, 10H, HAr), 3.65 (d, *J* = 13.6 Hz, 2H, 2 x C<u>H</u>HPh), 3.57 (dd, *J* = 9.7, 4.2 Hz, 1H, Hα), 3.50 (d, *J* = 13.6 Hz, 2H, 2 x C<u>H</u>HPh), 3.30 (dd, *J* = 9.7, 6.6 Hz, 1H, Hα), 2.47 (dd, *J* = 12.9, 8.5 Hz, 1H, Hγ), 2.29 (dd, *J* = 12.9, 6.0 Hz, 1H, Hγ), 2.06 (m, 1H, Hβ), 0.98 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 139.4, 129.0, 128.3, 127.0, 59.1, 58.6, 39.8, 29.3, 17.4. HRMS (*m/z*) calcd for $C_{18}H_{23}BrN$ $[M+H]^+$ 332.1008, found 332.1002.

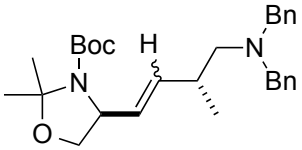
(*S*)-3-(dibenzylamino)-2-(methylpropyl)triphenylphosphonium bromide (168)



This product was synthesized by adaptation of a literature procedure.[143] A solution of **167** (0.3 g, 0.9 mmol) and triphenyl phosphine (0.24 g, 0.9 mmol) in acetonitrile (0.5 mL) was stirred at reflux for 15 h. The reaction mixture was concentrated and triturated with Et_2O giving a beige solid (122 mg, 22%). ^1H NMR ($CDCl_3$, 500 MHz) δ 7.85 – 7.62 (m, 15H, HAr), 7.39 – 7.16 (m, 10H, HAr), 3.72 (ddd, *J* = 16.0, 13.8, 2.4 Hz, 1H, C<u>H</u>$_2$P$^+$Ph$_3$), 3.60 (d, *J* = 13.7 Hz, 2H, 2 x

C<u>H</u>HPh), 3.55 – 3.46 (d, *J* = 13.7 Hz, 2H, 2 x C<u>H</u>HPh), 3.37 (dd, *J* = 16.0, 9.6 Hz, 1H, C<u>H</u>$_2$P$^+$PH$_3$), 2.76 (dd, *J* = 13.3, 8.2 Hz, 1H, C<u>H</u>$_2$NBn$_2$), 2.58 (ddd, *J* = 13.2, 6.2, 4.5 Hz, 1H, C<u>H</u>$_2$NBn$_2$), 1.93 (m, 1H, C<u>H</u>CH$_3$), 0.79 (d, *J* = 6.6 Hz, 3H, C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 138.9, 135.0, 133.7, 133.6, 130.5, 130.4, 129.0, 128.4, 127.1, 119.2, 118.5, 62.0, 59.2, 28.0, 19.1, 15.3. HRMS (*m/z*) calcd for $C_{36}H_{37}NP$ [M$^+$] 514.2658, found 514.2658.

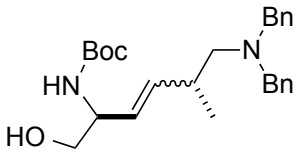
(*S*)-*tert*-Butyl 4-[(*R*,*E*)-4-(dibenzylamino)-3-methylbut-1-enyl]-2,2-dimethyloxazolidine-3-carboxylate (170)



To a solution of **168** (122 mg, 0.21 mmol) in THF (0.4 mL) at -78 ºC was added a solution of KHMDS (373 μL, 0.19 mmol, 0.5 M in Toluene). After 5 min of stirring, the solution was warmed to 0 ºC and was added a solution of garner aldehyde **169** (39 mg, 0.17 mmol) in THF (0.1 mL) and was stirred for 45 min. The reaction mixture was quenched by addition of water, extracted with EtOAc, dried over anhydrous sodium sulfate, concentrated *in vacuo* and purified by preparative TLC (silica, 9:1 hexanes: EtOAc) to yield **170** (40 mg, 51%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41 – 7.29 (m, 8H, HAr), 7.23 (m, 2H, HAr), 5.70 – 5.30 (m, 2H, C<u>H</u>=C<u>H</u>), 4.30 (m, 1H, C<u>H</u>NBoc), 4.00 (dd, *J* = 8.8, 6.1 Hz, 1H, C<u>H</u>$_2$O), 3.68 (dd, *J* = 8.8, 2.2 Hz, 1H, C<u>H</u>$_2$O), 3.65 – 3.40 (m, 4H, 2 x C<u>H</u>$_2$Ph), 2.5 (m, 1H, C<u>H</u>CH$_3$), 2.37 – 2.24 (m, 2H, C<u>H</u>$_2$NBn), 1.71 – 1.35 (m, 15H, C(CH$_3$)$_2$ + Boc), 0.99 (d, *J* = 6.6 Hz, 3H, CHC<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 151.9, 139.4,

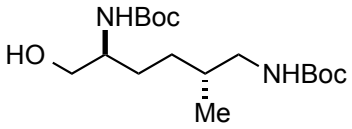
133.8, 133.7, 128.9, 128.3, 127.0, 126.5, 79.8, 68.5, 60.4, 60.1, 58.9, 33.8, 30.8, 28.5, 17.4.

tert-Butyl (2*S*,5*R*,*E*)-6-(dibenzylamino)-1-hydroxy-5-methylhex-3-en-2-ylcarbamate (171)



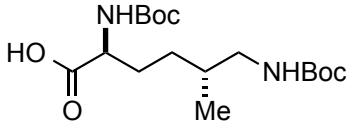
A solution of **170** (50 mg, 0.11 mmol) in 50 % TFA in DCM (1 mL) was stirred at room temperature. After 30 min, the solution was concentrated *in vacuo*, washed with Et_2O and concentrated after every wash. To the residue was added a solution of Boc_2O (24 mg, 0.11 mmol) in DCM:water (1:1, 1 mL), then was added $NaHCO_3$ (26 mg, 0.31 mmol) and the mixture was stirred for 1 h. The mixture was diluted with DCM, washed with water, extracted with DCM, dried over anhydrous sodium sulfate, concentrated *in vacuo* and purified by flash chromatography (silica, 9:1 to 7:3 hexanes: EtOAc) to yield **171** (17 mg, 37%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.39 – 7.20 (m, 10H, HAr), 5.42 – 5.18 (m, 2H, C<u>H</u>=C<u>H</u>), 4.74 (s, 1H, NH), 4.55 – 4.37 (m, 1H, Hβ), 3.83 – 3.38 (m, 6H, C<u>H</u>$_2$OH, 2 x C<u>H</u>$_2$Ph), 2.94 – 2.78 (m, 1H, C<u>H</u>$_2$NBn$_2$), 2.38 – 2.21 (m, 2H, Hε + C<u>H</u>$_2$NBn$_2$), 1.43 (m, 9H, Boc), 0.95 (d, *J* = 6.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 156.6,139.7, 139.2, 133.2, 129.0, 128.2, 126.9, 79.8, 63.4, 59.5, 58.9, 58.7, 31.3, 28.4, 19.0.

***tert*-Butyl (2*R*,5*S*)-6-hydroxy-2-methylhex-1,5-diyldicarbamate (172)**



Alkene **171** (41 mg, 0.097 mmol) and 10 % palladium on carbon in dry MeOH (1 mL) were stirred under a hydrogen atmosphere at 60 psi for 3 days. The suspension was then filtered through celite, which was washed with methanol, and the filtrate concentrated *in vacuo*. The residue was dissolved in water (0.4 mL), followed by addition of a solution of Boc_2O (21 mg, 0.097 mmol) in DCM (0.4 mL), $NaHCO_3$ (9 mg, 0.11 mmol), and the reaction mixture was stirred 3 h at room temperature. The mixture was diluted with DCM, washed with water, extracted with DCM, dried over anhydrous sodium sulfate, concentrated *in vacuo* and purified by flash chromatography (silica, 7:3 hexanes: EtOAc) to yield **172** (3 mg, 22%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.88 (s, 1H, NH), 4.69 (s, 1H, NH), 3.80 – 3.46 (m, 3H, 2Hα + Hβ), 3.26 – 2.76 (m, 2H, Hζ), 1.85 – 1.40 (m, 20 H, Hε + Hγ + Boc), 1.40 – 1.13 (m, 3H, Hγ + Hδ), 0.95 – 0.86 (d, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 156.6, 156.5, 79.4, 65.0, 51.8, 45.3, 33.9, 33.8, 29.7, 17.6. (EI) calcd $C_{17}H_{34}N_2O_5$ $[M+H]^+$ 347.2540 found 347.2.

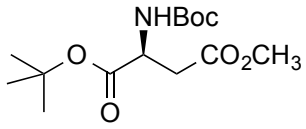
(2*R*,5*S*)-2,6-bis(*tert*-butoxycarbonylamino)-5-methylhexanoic acid (173)



To a solution of **172** (4.8 mg, 13 μmol) in acetone (130 μL) was added a solution of Jones reagent (13 μL, 26 μmol, 2M in H_2O) at 0 ºC and was stirred for

1.5 h. The reaction mixture was quenched with IPA (50 μL) and pH was adjusted to 7 with a saturated solution of $NaHCO_3$. The solids were filtered off and the solution was concentrated *in vacuo*. ^1H NMR ($CDCl_3$, 500 MHz) δ 5.30 (s, 1H, NH), 4.69 (s, 1H, NH), 4.26 (m, 1H, Hα), 3.02 (m, 2H, Hε), 2.0 – 1.53 (m, 3H, Hβ + Hδ), 1.53 -1.39 (m, 18H, 2 x Boc), 1.39 – 1.10 (m, 2H, Hγ), 0.92 (d, *J* = 6.8 Hz, 3H, CH_3). (EI) calcd $C_{17}H_{32}N_2O_5Na$ $[M+Na]^+$ 383.2153 found 383.2.

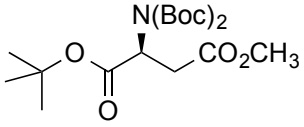
Boc-L-AspOMe-OtBu (176)



This product was synthesized by modification of a literature procedure.[144] To a solution of Boc-L-Asp acid-α-*t*BuO ester **175** (3 g, 10.4 mmol) in DCM (52 mL) was added Et_3N (2.2 mL, 15.6 mmol) under argon. The reaction mixture was cooled to 0 ºC and DMAP (127 mg, 1.04 mmol) followed by methyl chloroformate (1 mL, 12.5 mmol) were added, and stirred over 1 h. After that, the solution was allowed to warm to room temperature and was stirred overnight. The reaction mixture was diluted with EtOAc, washed with a saturated solution of $NaHCO_3$, brine, dried over anhydrous sodium sulfate, concentrated *in vacuo*, and purified by flash chromatography (silica, 7:3 hexanes: EtOAc) to yield **176** as light yellow oil (2.53 g, 80%). $[\alpha]_D^{25}$ = 14.02 (c = 1.0 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3370, 2979, 2935, 1745, 1721, 1157 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.45 (d, *J* = 8.6 Hz, 1H, N<u>H</u>), 4.47 (m, 1H, Hα), 3.71 (s, 3H, $CO_2C\underline{H}_3$), 2.96 (dd, *J* = 16.6,

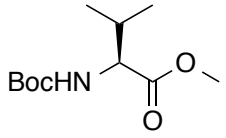
4.7 Hz, 1H, 1 x Hβ), 2.79 (dd, *J* = 16.6, 5.0 Hz, 1H, 1 x Hβ), 1.48 (s, 9H, C(C<u>H</u>$_3$)$_3$), 1.47 (s, 9H, C(C<u>H</u>$_3$)$_3$); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 171.4, 170.0, 155.4, 82.3, 79.9, 51.8, 50.6, 37.0, 28.3, 27.9. HRMS (*m/z*) calcd for $C_{14}H_{26}NO_6$ $[M+H]^+$ 304.1755, found 304.1751.

Boc-L-AspOMe-*t*BuO (177)



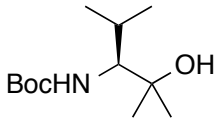
This product was synthesized by modification of a literature procedure.[144] To a solution of Boc-L-AspOMe-α-*t*BuO ester **176** (2.4 g, 7.8 mmol) in acetonitrile (30 mL) was added DMAP (191 mg, 1.56 mmol), and a solution of di-*tert*-butyldicarbonate (3.4 g, 15.6 mmol) in acetonitrile (16 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo* and purified by flash chromatography (silica, 9:1 hexanes: EtOAc) to give **177** as a white solid (2.93 g, 93%). $[\alpha]_D^{25}$ = -44.11 (c = 1.0 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 2980, 2936, 1797, 1741, 1702, 1145 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.35 (dd, *J* = 7.2, 6.5 Hz, 1H, Hα), 3.71 (s, 3H, $CO_2C\underline{H}_3$), 3.24 (dd, *J* = 16.3, 7.2 Hz, 1H, 1 x Hβ), 2.70 (dd, *J* = 16.3, 6.5 Hz, 1H, 1 x Hβ), 1.53 (s, 18H, C(CH_3)$_3$), 1.46 (s, 9H, C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 171.3, 168.6, 152.0, 83.1, 81.9, 55.6, 51.8, 35.5, 28.0, 27.9. HRMS (*m/z*) calcd for $C_{19}H_{33}NO_8Na$ $[M+Na]^+$ 426.2098, found 426.2097.

(*S*)-methyl 2-(*tert*-butoxycarbonylamino)-3-methylbutanoate (180)



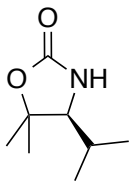
To a solution of Boc-L-Valine **179** (5.3 g, 24.4 mmol) in MeOH (100mL) and Et_2O (100 mL) was added slowly trimethylsilyldiazomethane (35 mL, 69 mmol, 2 M in hexanes) at 0 ºC and the solution became yellow. After stirring 15 min AcOH (0.5 mL, 69 mmol) was added, and the reaction mixture was stirred until the solution became colorless. The solution was then concentrated *in vacuo* and dissolved in Et_2O (150 mL), washed with an aqueous solution of 5 % $NaHCO_3$ (100 mL) and brine (100 mL), dried over anhydrous sodium sulfate and concentrated *in vacuo* to yield the product **180** as a clear colorless oil (5.5 g, 98%). $[\alpha]_D^{25}$ = +11.96 (c = 1.0 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3374, 2969, 2934, 2877, 1745, 1718, 1160 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.08 – 5.02 (m, 1H, NH), 4.25 (m, 1H, Hα), 3.76 (s, 3H, $OC\underline{H}_3$), 2.14 (m, 1H, Hβ), 1.47 (s, 9H, Boc), 0.98 (d, *J* = 6.8 Hz, 3H, CH_3), 0.92 (d, *J* = 6.9 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 172.9, 155.7, 79.8, 58.6, 52.0, 31.3, 28.3, 19.0, 17.6. HRMS (*m/z*) calcd for $C_{11}H_{21}NO_4Na$ $[M+Na]^+$ 254.1363, found 254.1363.

(*S*)-*N*-(*t*-Butylcarboxy)-3-amino-2,4-dimethylpentan-2-ol (181)



This product was synthesized according to a previously reported literature procedure.[99] To a solution of Boc-L-valine methyl ester **180** (5.3 g, 22.9 mmol) in THF (135 mL) was added dropwise a solution of methyl magnesium bromide (31 mL, 92 mmol, 3 M in diethyl ether) over 30 min at 0 ºC and stirred for 20 h at room temperature. The reaction mixture was quenched by slow addition of MeOH (66 mL) at 0 ºC forming a white precipitate. The solids were dissolved by addition of water (15 mL) and concentrated *in vacuo*. The residue was dissolved in Et_2O, filtered through celite and washed with Et_2O, concentrated *in vacuo* to yield **181** as a light yellow oil (3.4 g, 64.2%). $[\alpha]_D^{25}$ = -1.32 (c = 1.0 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3448, 2976, 2933, 2875, 1694, 1175 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.86 (d, *J* = 10.2 Hz, 1H, NH), 3.42 (dd, *J* = 10.3, 2.7 Hz, 1H, Hα), 2.13 (m, 1H, Hβ), 1.48 (s, 9H, Boc), 1.34 (s, 3H, C$\underline{H}_3$), 1.26 (s, 3H, C$\underline{H}_3$), 0.98 (d, *J* = 6.8 Hz, 3H, CH(C$\underline{H}_3$)$_2$), 0.94 (d, *J* = 6.8 Hz, 3H, CH(C$\underline{H}_3$)$_2$); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 157.0, 79.1, 73.8, 61.8, 29.1, 28.4, 28.2, 27.1, 22.3, 16.9. HRMS (*m/z*) calcd for $C_{12}H_{25}NO_3Na$ $[M+Na]^+$ 254.1727, found 254.1725.

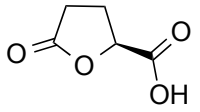
(*S*)-4-*i*-Propyl-5,5-dimethyloxazolidin-2-one (182)



This product was synthesized according to a previously reported literature procedure.[99] To a solution of **181** (3.4 g, 14.7 mmol) in THF (68 mL) at 0 ºC was added a solution of potassium *t*-butoxide (17.6 mL, 17.6 mmol, 1 M in THF) and stirred for 30 min. The reaction mixture was quenched by addition of a saturated solution of ammonium chloride (30 mL), extracted with EtOAc (2 x 30 mL), dried over anhydrous magnesium sulfate, concentrated *in vacuo*, and recrystallized from hexanes / EtOAc to yield **182** as a white solid (0.96 g, 43%). $[\alpha]_D^{25}$ = +16.22 (c = 0.8 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3242, 2977, 2938, 2874, 1743, 1006 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.10 (s, 1H, NH), 3.20 (dd, *J* = 8.6, 0.9 Hz, 1H, H-4), 1.92 – 1.78 (m, 1H, $CH(CH_3)_2$), 1.51 (s, 3H, CH_3-5), 1.41 (s, 3H, CH_3-5), 1.01 (d, *J* = 6.6 Hz, 3H $CH(CH_3)_2$), 0.94 (d, *J* = 6.6 Hz, 3H, $CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.9, 83.9, 68.4, 28.6, 28.5, 21.3, 20.0, 19.9. HRMS (*m/z*) calcd for $C_8H_{16}NO_2$ $[M+H]^+$ 158.1176, found 158.1174.

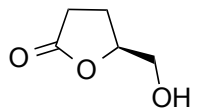
4.4 Lovastatin: synthesis and characterization of compounds

(+)-(2*S*)-5-Oxotetrahydrofuran-2-carboxylic acid (209).



This product was synthesized according to a literature procedure.[145,146] A solution of sodium nitrite (14 g, 200 mmol) in H_2O (30 mL) was added dropwise to a mixture of (*S*)-(+)-glutamic acid sodium salt **208** (20 g, 118 mmol) in H_2O (53 mL) and conc. HCl (28 mL) at 0°C over 70 min and was stirred at room temperature overnight. After that, solvent was removed *in vacuo* to give a light yellow oil with white crystals. The residue was suspended in EtOAc (70 mL), the crystals filtered off and the filtered solution was dried over anhydrous sodium sulfate, and concentrated *in vacuo* to yield **209** as a viscous yellow oil (13.1g, 85 %). $[\alpha]_D^{25}$ = 6.81 (c = 2.09 g/100mL, EtOH); IR (EtOH cast film): 3500-2700 (br.), 2950, 1754, 1177 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.08 – 5.00 (m, 1H, H-2), 2.77 – 2.55 (m, 3H, H-3 + 1 x H-4), 2.50 – 2.39 (m, 1H, 1 x H-4); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 176.7, 174.7, 75.4, 26.8, 25.8. HRMS (*m/z*) calcd for $C_5H_5O_4$ $[M-H]^-$ 129.0193, found 129.0192.

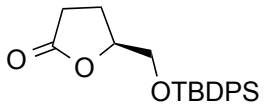
(+)-(5*S*)-5-(Hydroxymethyl)dihydrofuran-2(3*H*)-one (210).



This product was synthesized according to a literature procedure.[145,146] BH_3SMe_2 (10 M, 9.8 mL, 97.8 mmol) was added dropwise via cannula to a

cooled (0 ºC) stirred solution of crude **209** (12 g, 92.2 mol) in dry THF (38 mL) over 1 h. The reaction mixture was allowed to stir and warm to room temperature overnight. Dry MeOH (15 mL) was added dropwise and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography (silica, 7:3 hexanes: EtOAc) to yield **210** (7.5 g, 70%). $[\alpha]_D^{25}$ = +53.8 (c = 1.6 g/100mL, DCM); IR (DCM cast film): 3600-3100 (br.), 2940, 1765, 1186 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.65 (dddd, *J* = 7.6, 6.8, 4.6, 2.9 Hz, 1H, H-5), 3.90 (dd, *J* = 12.5, 2.9 Hz, 1H, 1 x H-6), 3.66 (dd, *J* = 12.5, 4.6 Hz, 1H, 1 x H-6), 3.42 (br. s, 1H, OH), 2.69 – 2.49 (m, 2H, H-3), 2.28 (dddd, *J* = 13.2, 9.9, 7.6, 5.8 Hz, 1H, 1 x H-4), 2.15 (dddd, *J* = 12.8, 10.0, 8.1, 6.8 Hz, 1H, 1 x H-4); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 177.9, 80.9, 64.1, 28.7, 23.2. HRMS (*m/z*) calcd for $C_5H_8O_3Na$ $[M+Na]^+$ 139.0366, found 139.0365.

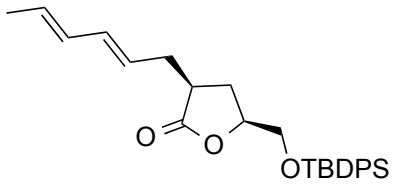
(+)-(5*S*)-5-[(*tert*-Butyldiphenylsilyloxy)methyl]dihydrofuran-2(3*H*)-one (211).



This product was synthesized according to a literature procedure.[147] To a solution of **210** (4.9 g, 42.2 mmol) in DCM (60 mL) was added neat TBDPS-Cl (11 mL, 42.2 mmol), followed by addition of pyridine (14 mL, 170 mmol) dropwise over 10 min at 0 ºC and was stirred at room temperature. After 24 h, the reaction mixture was quenched by addition of 2 N HCl (40 mL), washed with brine, dried over anhydrous magnesium sulfate, concentrated *in vacuo* and recrystallized from diethyl ether / pentane to yield **211** as a white solid (12.4 g, 83%). ^1H NMR ($CDCl_3$, 500 MHz) δ 7.70 – 7.66 (m, 4H, HAr), 7.48 – 7.39 (m, 6H, HAr), 4.62

(dddd, *J* = 7.5, 5.9, 3.4, 3.4 Hz, 1H, H-5), 3.90 (dd, *J* = 11.4, 3.4 Hz, 1H, 1 x H-6), 3.71 (dd, *J* = 11.4, 3.4 Hz, 1H, 1 x H-6), 2.69 (ddd, *J* = 17.8, 10.2, 7.5 Hz, 1H, 1 x H-3), 2.53 (ddd, *J* = 17.8, 10.2, 5.9 Hz, 1H, 1 x H-3), 2.36 – 2.18 (m, 2H, H-4), 1.09 (s, 9H, C(C$\underline{H}_3$)$_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.4, 135.6, 135.5, 133.0, 132.6, 129.9, 127.8, 80.0, 65.5, 28.6, 26.8, 23.7, 19.2. HRMS (*m/z*) calcd for $C_{21}H_{26}O_3NaSi$ $[M+Na]^+$ 377.1543, found 377.1544.

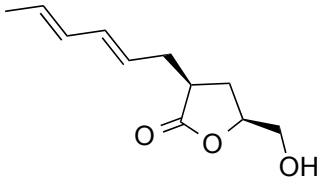
(+)-(3*S*,5*S*)-[(2*E*,4*E*)-Hexa-2,4-dien-1-yl]-5-[(*tert*-butyl-diphenylsilanyloxy)-methyl]dihydrofuran-2(3*H*)-one (212).



This product was synthesized according to a literature procedure.[147] A cooled solution (-78 ºC) of NaHMDS (32 mL, 32 mmol, 1.0 M in THF) was added dropwise to a solution of **211** (11 g, 31 mmol) in dry THF (155 mL) at -78 ºC over 15 min. After stirring for 15 min, a cooled (-78 ºC) crude solution of 2*E*,4*E*-hexadienyl bromide (5 g, mmol) in dry THF (34 mL) was added quickly and the solution was left to stir for 30 min. The resulting mixture was added dropwise *via* cannula to a cooled (-78 ºC) stirred solution of LiHMDS (32 mL, 32 mmol, 1.0 M in THF) and left to stir for 30 min before a cooled (-78 ºC) solution of 2-bromo-2-methylpropane (3.6 mL, 31 mmol) in dry THF (34 mL) was added and the solution was left to stir for 40 min. The reaction mixture was quenched with a saturated solution of ammonium chloride (85 mL) and allowed to warm to room

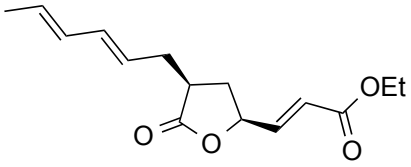
temperature. The solids were dissolved with (85 mL) H_2O and the mixture was extracted with Et_2O (130 mL), washed with saturated solution of ammonium chloride (85 mL) and brine (85 mL). The aqueous layer were combined and extracted with Et_2O (225 mL). The organic layers were combined, dried with anhydrous magnesium sulfate, concentrated *in vacuo* to give a brown oil. The crude mixture was purified by flash chromatography (silica, a gradient of solvents from hexanes to 5 % EtOAc in hexanes) to yield **212** as a clear colorless oil (3 g, 22%). $[\alpha]_D^{25}$ = +40.14 (c = 1.2 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3071, 3049, 3018, 2957, 2931, 2858, 1774, 1126 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.72 – 7.64 (m, 4H, HAr), 7.50 – 7.36 (m, 6H, HAr), 6.13 – 5.93 (m, 2H, H-3' + H-4'), 5.70 – 5.56 (m, 1H, H-5'), 5.49 (dt, *J* = 14.8, 7.1 Hz, 1H, H-2'), 4.48 (ddt, *J* = 9.9, 6.4, 4.0 Hz, 1H, H-5), 3.87 (dd, *J* = 11.5, 3.6 Hz, 1H, 1 x H-6), 3.73 (dd, *J* = 11.5, 4.3 Hz, 1H, 1 x H-6), 2.80 – 2.58 (m, 2H, H-3 + 1 x H-1'), 2.35 – 2.23 (m, 2H, 1 x H-1'+ 1 x H-4), 1.93 (ddd, *J* = 12.7, 11.5, 9.9 Hz, 1H, 1 x H-4), 1.72 (d, *J* = 6.5 Hz, 3H, H-6'), 1.07 (s, 9H, $C(C\underline{H}_3)_3$); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 178.1, 135.7, 135.6, 133.3, 133.1, 132.8, 131.1, 129.9, 129.8, 128.5 127.8, 126.8, 64.6, 40.6, 33.3, 29.3, 26.8, 19.3, 18.0. HRMS (*m/z*) calcd for $C_{27}H_{34}O_3SiNa$ $[M+Na]^+$ 457.2169, found 457.2169.

(+)-(3*S*,5*S*)-3-[(2*E*,4*E*)-Hexa-2,4-dien-1-yl]-5-(hydroxymethyl)dihydrofuran-2(3*H*)-one (213).



This product was synthesized according to a literature procedure.[147] A solution of TBAF (7.6 mL, 7.6 mmol, 1.0 M in THF) was added dropwise to a stirred solution of **212** (3.0 g, 6.9 mmol) in dry THF (35 mL) at 0 ºC. The reaction mixture was allowed to warm to room temperature over 3 h before diluting with Et_2O (30 mL). The reaction mixture was washed with a 3 M solution of citric acid (3 x 12mL), extracted with Et_2O (15 mL), dried over anhydrous magnesium sulfate, concentrated *in vacuo* and purified by flash chromatography (silica, 95% Et_2O in pentane) to yield **213** (0.89 g, 66%). $[\alpha]_D^{25}$ = +83.88 (c = 1.0 g/100mL, MeOH); IR (MeOH cast film): 3363, 3019, 2952, 1758, 1185 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.15 – 5.98 (m, 2H, H-3' + H4'), 5.66 (dq, *J* = 13.6, 6.8 Hz, 1H, H-5'), 5.50 (dt, *J* = 14.7, 7.3 Hz, 1H, H-2'), 4.57 – 4.48 (m, 1H, H-5), 3.92 (dd, *J* = 12.7, 3.0 Hz, 1H, 1 x H-6), 3.65 (dd, *J* = 12.7, 3.3 Hz, 1H, 1 x H-6), 2.78 (dtd, *J* = 11.5, 9.0, 4.4 Hz, 1H, H-3), 2.70 – 2.61 (m, 1H, 1 x H-1'), 2.37 – 2.25 (m, 2H, 1 x H-1' + 1 x H-4), 1.91 – 1.78 (m, 1H, 1 x H-4), 1.78 – 1.73 (d, *J* = 6.5 Hz, 3H, H-6'); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 177.8, 133.4, 131.0, 128.8, 126.5, 78.7, 64.0, 40.7, 33.2, 29.0, 18.0. HRMS (*m/z*) calcd for $C_{11}H_{17}O_3$ $[M+H]^+$ 197.1172, found 197.1172.

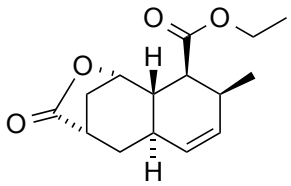
Ethyl (2*E*)-3-{(2*S*,4*S*)-4-[(2*E*,4*E*)-hexa-2,4-dien-1-yl]-5-oxotetrahydrofuran-2-yl}acrylate (214).



This product was synthesized according to a literature procedure.[147] To a solution of oxalyl chloride (0.54 mL, 6.3 mmol) in dry DCM (6.6 mL) was added DMSO at -78 ºC over 25 min. After 20 min, a solution of the alcohol **213** (0.88 g, 4.48 mmol) in dry THF (11.5 mL) was added over 1 h and stirred for another 20 min, then a solution of DIPEA (3.1 mL, 17.92 mmol) was added over 15 min and allowed to stir for 10 min before warning to -5 ºC. This reaction mixture was added *via* cannula to a stirred solution of (carbethoxymethylene)triphenyl-phosphorane (3.12 g, 8.96 mmol) in dry THF (14 mL) at 0 ºC over 1 h and allowed to warm to room temperature for 20 h excluding the light. The solvent was removed *in vacuo*, the residue was dissolved in EtOAc, washed with 2N HCl (2x), extracted with EtOAc, washed with brine, dried with anhydrous magnesium sulphate, concentrated *in vacuo* and, purified by flash chromatography (silica, 1:9 EtOAc:hexanes then switching to 1:3 EtOAc:hexanes) to give **214** (0.77 g, 65%). $[\alpha]_D^{25}$ = 102.86 (c = 1.8 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3019, 2982, 2934 1778, 1720, 1171 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.91 (dd, *J* = 15.7, 5.1 Hz, 1H, C<u>H</u>=CHCO$_2$Et), δ 6.12 (dd, *J* = 15.7, 1.5 Hz, 1H, CH=C<u>H</u>CO$_2$Et), 6.10 – 5.98 (m, 2H, H-3' + H-4'), 5.66 (dq, *J* = 13.3, 6.7 Hz, 1H, H-5'), 5.46 (dt, *J* = 14.9, 7.3 Hz, 1H, H-2'), 4.97 (dddd, *J* = 10.2, 6.4, 5.1, 1.6 Hz, 1H, H-2), 4.24 (q, *J* = 7.1 Hz, 2H, OC<u>H$_2$</u>CH$_3$), 2.78 (dtd, *J* = 11.4, 8.6, 4.5 Hz, 1H, H-4), 2.71 – 2.60 (m, 1H, 1 x

H1'), 2.60 – 2.53 (m, 1H, 1 x H-1'), 2.30 (dt, *J* = 15.3, 8.1 Hz, 1H, 1 x H-3), 1.84 – 1.77 (m, 1H, 1 x H-3), 1.76 (d, *J* = 6.7 Hz, 3H, H-6'), 1.32 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 177.1, 165.7, 143.4, 133.7, 130.9, 129.0, 126.0, 122.3, 76.2, 60.8, 40.6, 34.2, 33.0, 18.0, 14.2. HRMS (*m/z*) calcd for $C_{15}H_{21}O_4$ $[M+H]^+$ 265.1434, found 265.1432.

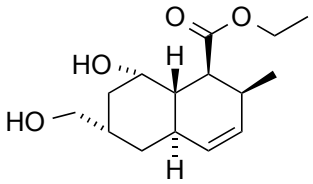
Ethyl (1*S*,2*S*,4a*R*,6*S*,8*S*,8a*S*)-1,2,4a,5,6,7,8,8a-octahydro-2-methyl-6,8-naphthalenecarbolactone-1-carboxylate (215).



This product was synthesized according to a literature procedure.[147] Into a pre-washed round bottom flask with HMDS and dried overnight in the oven (120 ºC), **214** (0.341 g, 1.29 mmol), mesitylene (43 mL) and butylated hydroxytoluene (28 mg) were added and the mixture was refluxed for 11 days under argon before cooling to room temperature and concentrated *in vacuo*. The residue was first partially purified by flash chromatography (silica, 1:1:8 MeCN:DCM:toluene), then purified again by flash chromatography (silica 3:7 Et_2O:pentane) to yield **215** (0.322 g, 61%). $[\alpha]_D^{25}$ = 226.28 (c = 0.97 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3028, 2972, 2931 1781, 1728, 1142 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.57 – 5.45 (m, 2H, H-3 + H-4), 5.06 (d, *J* = 6.1 Hz, 1H, H-8eq), 4.18 (q, *J* = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 2.87 (dd, *J* = 11.9, 6.6 Hz, 2H, H-1), 2.77 – 2.67 (m, 2H, H-6eq + H-2), 2.50 (dddd, *J* = 11.6, 6.1, 5.3, 2.1 Hz, 1H, H-7eq), 2.39 – 2.24 (m, 1H, H-4a),

2.05 (dtd, *J* = 12.3, 5.0, 2.1 Hz, 1H, H-5eq), 1.88 (d, *J* = 11.7 Hz, 1H, H-7ax), 1.81 – 1.70 (m, 2H, H-8a), 1.48 – 1.35 (m, 1H, H-5ax), 1.23 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 0.90 (d, *J* = 7.2 Hz, 3H, $C\underline{H}_3$-2); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 178.8, 173.0, 131.2, 128.0, 77.8, 60.3, 46.0, 39.3, 39.2, 38.7, 35.0, 32.6, 31.8, 17.2, 14.2. HRMS (*m/z*) calcd for $C_{15}H_{21}O_4$ $[M+H]^+$ 265.1434, found 265.1434.

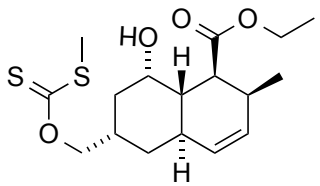
Ethyl (1*S*,2*S*,4a*R*,6*S*,8*S*,8a*S*)-8-hydroxy-6-(hydroxymethyl)-2-methyl-1,2,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylate (216).



This product was synthesized according to a literature procedure.[147,148] A solution of $LiBEt_3H$ (1.2 mL, 1.2 mmol, 1.0 M in THF) was added slowly to a stirred solution of **215** (0.296 g, 1.12 mmol) in dry TFH (16 mL) over 10 min at 0 ºC. After stirring for 30 min, water (0.32 mL), 2 N NaOH (0.62 mL) and 30 % H_2O_2 (0.62 mL) were added and the reaction mixture was stirred for 1 h. The reaction mixture was diluted with Et_2O (16 mL), washed with brine, and the aqueous layer was extracted with Et_2O (3 x 10 mL). The organic layers were combined, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was dissolved in EtOAc (4 mL), $NaBH_4$ (11 mg, 0.28 mmol) was added and the reaction mixture was stirred for 2 h. After that, the reaction mixture was diluted with EtOAc (15 mL), washed with 1 N HCl (10 mL) and aqueous layer was extracted with EtOAc (2 x 15 mL). The organic layers were combined,

dried over anhydrous magnesium sulfate, concentrated *in vacuo* and purified by flash chromatography (silica, 6 % pentane in Et_2O) to give **216** (0.224 g, 75%). $[\alpha]_D^{25}$ = 172.80 (c = 0.53 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3600-3200 (br.), 3012, 2966, 2909 1730, 1140 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.57 (ddd, *J* = 9.8, 4.6, 2.7 Hz, 1H, C<u>H</u>=CH), 5.44 (dd, *J* = 9.9, 1.7 Hz, 1H, C<u>H</u>=CH), 4.31 (ddd, *J* = 2.9 Hz, 1H, H-8eq), 4.25 – 4.12 (m, 2H, OC<u>H</u>$_2$CH$_3$), 3.81 (d, *J* = 5.0 Hz, 2H, C<u>H</u>$_2$OH), 3.00 (br. s, 2H, 2 x O<u>H</u>), 2.86 (dd, *J* = 11.6, 5.9 Hz, 1H, H-1), 2.69 – 2.60 (m, 1H, H-2), 2.61 – 2.48 (m, 1H, H-4a), 2.07 – 1.97 (m, 1H, H-6), 1.96 – 1.90 (m, 2H, H-7), 1.85 (ddd, *J* = 13.4, 3.5, 1.8 Hz, 1H, H-5eq), 1.54 (dd, *J* = 11.3, 11.3, 2.2 Hz, 1H, H-8a), 1.39 (ddd, *J* = 13.4, 13.4, 6.1 Hz, 1H, H-5ax), 1.30 (t, *J* = 7.1 Hz, 3H, OCH$_2$C<u>H</u>$_3$), 0.96 (d, *J* = 7.1 Hz, 3H, C<u>H</u>$_3$-2). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 173.8, 131.2, 130.6, 68.0, 66.0, 60.0, 45.1, 39.9, 35.7, 34.8, 34.1, 32.5, 30.5, 17.6, 14.3. HRMS (*m/z*) calcd for $C_{15}H_{25}O_4$ $[M+H]^+$ 269.1747, found 269.1745.

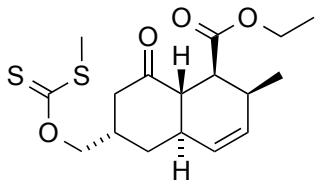
Ethyl (1*S*,2*S*,4a*R*,6*S*,8*S*,8a*S*)-8-hydroxy-2-methyl-6-({[(methylthio)carbonothioyl]oxy}methyl)-1,2,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylate (217).



This product was synthesized according to a literature procedure.[148,149] To a solution of diol **216** (167 mg, 0.62 mmol) and tetrabutylammonium hydrogen

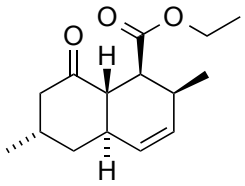
sulfate (232 mg, 0.62 mmol) in benzene (5 mL) was added a solution of 4 N NaOH (5 mL, 20 mmol), followed by addition of CS_2 (75 μL, 1.24 mmol) and MeI (58 μL, 0.93 mmol). After 10 min, ice (10 g) and Et_2O (10 mL) were added and the solution was stirred for another 5 min. The reaction mixture was diluted and extracted with Et_2O (2 x 15 mL), organic layers were combined, washed with brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo* to give 0.22 g of crude product **217**, which was used in the next reaction without further purification. $[\alpha]_D^{25}$ = +115.05 (c = 0.56 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3600-3400 (br.), 3012, 2966, 2922, 2855, 1729, 1712, 1179 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.64 (ddd, *J* = 9.9, 4.6, 2.7 Hz, 1H, C<u>H</u>=CH), 5.46 (dd, *J* = 9.9, 1.7 Hz, 1H, C<u>H</u>=CH), 4.98 (dd, *J* = 10.8, 9.2 Hz, 1H, 1 x C<u>H</u>$_2$OC(S)S), 4.80 (dd, *J* = 10.8, 6.1 Hz, 1H, C<u>H</u>$_2$OC(S)S), 4.38 (m, 1H, H-8eq), 4.21 (m, 2H, OC<u>H</u>$_2$CH$_3$), 2.93 (dd, *J* = 11.6, 5.9 Hz, 1H, -1), 2.75 – 2.66 (m, 1H, H-2), 2.61 (s, 3H, SC<u>H</u>$_3$), 2.58 – 2.38 (m, 2H, H-4a + H-6), 1.98 – 1.81 (m, 3H, H-7 + H-5eq), 1.59 (br. s, 1H, O<u>H</u>), 1.44 – 1.38 (m, 1H, H-8a), 1.36 (ddd, *J* = 13.6, 13.6, 5.2 Hz, 3H, H-5ax), 1.27 (t, *J* = 7.0 Hz, 3H, OCH$_2$C<u>H</u>$_3$), 1.01 (d, *J* = 7.1 Hz, 3H, C<u>H</u>$_3$-2). HRMS (*m/z*) calcd for $C_{17}H_{26}O_4S_2Na$ $[M+Na]^+$ 381.1165, found 381.60.

Ethyl (1*S*,2*S*,4a*R*,6*S*,8*S*,8a*S*)-2-methyl-6-({[(methylthio)carbonothioyl]oxy}methyl)-8-oxo-1,2,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylate (218).



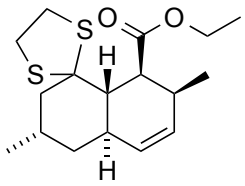
This product was synthesized according to a literature procedure.[148,150] To a solution of **217** (0.22 g, 0.62 mmol) in DCM (5 mL) was added PDC (0.35 g, 0.93 mmol) and the mixture was stirred for 44 h at room temperature before removing the solvent *in vacuo* and filtering the residue through a silica plug (1:1 Et_2O:pentane) to give a yellow oil, which was purified by flash chromatography (silica, 3:7 Et_2O:pentane) to yield **218** as a white waxy solid (0.20 g, 65%). $[\alpha]_D^{25}$ = 174.28 (c = 1.22 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3016, 2967, 2928, 1732, 1718, 1651, 1185 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.69 (ddd, *J* = 9.8, 4.6, 2.7 Hz, 1H, C<u>H</u>=CH), 5.48 (ddd, *J* = 9.8, 1.7, 1.7 Hz, 1H, C<u>H</u>=CH), 4.56 (dd, *J* = 11.1, 7.9 Hz, 1H, 1 x C<u>H</u>$_2$OC(S)S), 4.49 (dd, *J* = 11.1, 7.0 Hz, 1H, 1 x C<u>H</u>$_2$OC(S)S), 4.27 – 4.10 (m, 2H, OC<u>H</u>$_2$CH$_3$), 2.95 – 2.62 (m, 5H, H-1 + H-2 + H-4a + 1 x H-7 + H-8a), 2.58 (s, 3H, SC<u>H</u>$_3$), 2.40 (ddd, *J* = 13.6, 2.0, 2.0 Hz, 1H, 1 x H-7), 2.31 (m, 1H, H-6), 2.00 (ddt, *J* = 14.0, 3.9, 2.0 Hz, 1H, H-5eq), 1.82 (ddd, *J* = 13.5, 13.5, 5.4 Hz, 1H, H-5ax), 1.29 (t, *J* = 7.1 Hz, 3H, OCH$_2$C<u>H</u>$_3$), 0.92 (d, *J* = 7.1 Hz, 3H, C<u>H</u>$_3$-2); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 215.7, 209.0, 173.5, 132.5, 128.0, 74.5, 60.3, 49.24, 43.0, 42.6, 38.0, 35.4, 32.9, 31.2, 19.2, 17.7, 14.3. HRMS (*m/z*) calcd for $C_{17}H_{24}O_4S_2Na$ $[M+Na]^+$ 376.1008, found 376.1006.

Ethyl (1*S*,2*S*,4a*R*,6*S*,8*S*,8a*S*)-2,6-dimethyl-8-oxo-1,2,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylate (219).



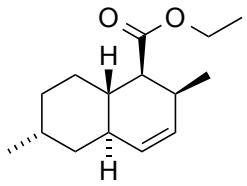
This product was synthesized according to a literature procedure.[148,151] In a 3 neck flask fitted with a condenser, a solution of xanthate **218** (156 mg, 0.44 mmol) in *p*-cymene (15 mL) was bubbled with argon for 15 min before heating to 150 ºC, then a solution of Bu_3SnH (0.93 mL, 3.5 mmol) in *p*-cymene (14 mL), previously bubbled with argon for 15 min, was added with a syringe pump over 110 min at 150 ºC and was allowed to stir overnight at 150 ºC. After cooling, the reaction mixture was concentrated *in vacuo* and purified by flash chromatography (silica, 8:2 pentane:Et_2O) to yield **219** (88 mg, 80%). $[\alpha]_D^{25}$ = 179.81 (c = 1.45 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3016, 2961, 2924, 2874, 1735, 1718, 1650, 1186 cm^{-1}; ^1H NMR ($CDCl_3$, 700 MHz) δ 5.62 (ddd, *J* = 9.8, 4.6, 2.8 Hz, 1H, C<u>H</u>=CH), 5.43 (ddd, *J* = 9.9, 1.8, 1.8 Hz, 1H, C<u>H</u>=CH), 4.20 – 4.08 (m, 2H, OC<u>H</u>$_2$CH$_3$), 2.81 (dd, *J* = 11.5, 6.4, 1H, H-1), 2.73 (dd, *J* = 12.6, 6.9, 1H, H-8a), 2.65 – 2.51 (m, 3H, H-2 + H-7), 2.34 – 2.27 (m, 1H, H-6), 2.14 (ddd, *J* = 12.7, 2.0, 2.0 Hz, 1H, H-4a), 1.77 (ddd, *J* = 13.1, 13.1, 5.0 Hz, 1H, H-5ax), 1.70 – 1.66 (m, 1H, H-5eq), 1.25 (t, *J* = 7.1 Hz, 3H, OCH$_2$C<u>H</u>$_3$), 0.96 (d, *J* = 7.3 Hz, 3H, C<u>H</u>$_3$-6), 0.87 (d, *J* = 7.2 Hz, 3H, C<u>H</u>$_3$-2); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 210.4, 173.7, 132.1, 128.8, 60.1, 49.5, 48.0, 42.7, 37.9, 37.7, 31.3, 31.2, 19.3, 17.7, 14.2. HRMS (*m/z*) calcd for $C_{15}H_{23}O_3$ $[M+H]^+$ 251.1642, found 251.1640.

Ethyl (1*S*,2*S*,4a*R*,6*S*,8a*S*)-1,2,4a,5,6,7,8,8a-octahydro-8-(dimethylenedithio)-2,6-dimethylnaphthalen-1-carboxylate (220).



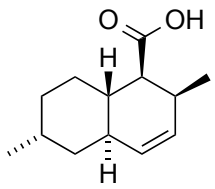
This product was synthesized according to a literature procedure.[148,152] To a solution of **219** (33.4mg, 0.13 mmol), 1,2-ethanedithiol (23 μL, 0.27 mmol) in DCM (1.3 mL) was added freshly distilled BF_3•OEt_2 (16 μL, 0.13 mmol) at 0 ºC and was stirred overnight at room temperature. The reaction mixture was diluted with DCM and washed sequentially with 1 N NaOH, 1 N HCl, water, and brine, then concentrated *in vacuo* and purified by flash chromatography (silica, 5 % Et_2O in pentane) to yield **220** (33 mg, 79%). $[\alpha]_D^{25}$ = 62.22 (c = 1.2 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3027, 2970, 2926, 2877, 1730, 1718, 1637, 1156 cm^{-1}; ^1H NMR ($CDCl_3$, 700 MHz) δ 5.60 (s, 2H, C<u>H</u>=C<u>H</u>), 5.28 (s, 1H), 4.15 – 4.05 (m, 2H, OC<u>H</u>$_2$$CH_3$), 3.40 – 3.17 (m, 4H, SCH_2CH_2S), 3.09 (dd, *J* = 7.9, 6.3 Hz, 1H, H-1), 2.45 (dq, *J* = 7.6, 6.5 Hz, 1H, H-2), 2.29 – 2.21 (m, 2H, H-7), 2.19 (dd, *J* = 10.8, 6.3 Hz, 1H, H-8a), 2.14 (ddtq, *J* = 10.5, 7.6, 5.3, 2.7 Hz, 1H, H-6), 2.09 – 2.01 (m, 1H, H-4a), 1.74 (ddt, *J* = 13.0, 3.6, 2.0 Hz, 1H, H-5eq), 1.61 (ddd, *J* = 13.0, 13.0, 5.5 Hz, 1H, H-5ax), 1.23 (t, *J* = 7.1 Hz, 3H, OCH_2C<u>H</u>$_3$), 1.20 (d, *J* = 7.6 Hz, 3H, C<u>H</u>$_3$-6), 1.09 (d, *J* = 7.4 Hz, 3H, C<u>H</u>$_3$-2); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 176.2, 134.1, 133.3, 72.6, 59.9, 54.2, 50.9, 49.7, 40.9, 38.5, 37.9, 33.7, 31.9, 29.5, 20.4, 17.6, 14.3. HRMS (*m/z*) calcd for $C_{17}H_{27}O_2S_2$ $[M+H]^+$ 327.1447, found 327.1449.

Ethyl (1*S*,2*S*,4a*R*,6*S*,8a*S*)-2,6-dimethyl-1,2,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylate (221).



This product was synthesized according to a literature procedure.[148,153] The dithioacetal **220** (16.5 mg, 0.051 mmol) was bubbled with argon for 30 min, then AIBN (~1 mg) was added and the mixture was bubbled with argon. After 10 min, Bu_3Sn-H (40 μL, 0.152 mmol) was added and the reaction mixture was stirred for 24 h at 120 ºC. The mixture was cooled and filtered through a plug (silica, 1:1 Et_2O:pentane), concentrated *in vacuo* and purified by flash chromatography (silica 2% Et_2O in pentane) to yield **221** (8.4 mg, 70%). $[\alpha]_D^{25}$ = 101.31 (c = 0.36 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3013, 2960, 2914, 2850, 1737, 1650, 1139 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.57 (ddd, *J* = 9.9, 3.6, 3.6 Hz, 1H, H-3), 5.35 (d, *J* = 9.9 Hz, 1H, H-4), 4.17 (q, *J* = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 2.62 – 2.53 (m, 2H, H-1 + H-2), 2.12 – 2.03 (m, 1H, H-6), 1.96 (ddd, *J* = 15.3, 7.6, 5.2 Hz, 1H, H-4a), 1.75 (dddd, *J* = 12.6, 3.6, 3.3, 3.3 Hz, 1H, H-8eq), 1.66 (dddd, *J* = 13.4, 13.4, 4.6, 4.6 Hz, 1H, H-7ax), 1.57 – 1.48 (m, 2H, H-7eq + H-5eq), 1.48 – 1.33 (m, 2H, H-8a + H-5ax), 1.29 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.13 (dddd, *J* = 12.7, 12.7, 12.7, 3.6 Hz, 1H, H-8ax), 1.02 (d, *J* = 7.2 Hz, 3H, $C\underline{H}_3$-6), 0.95 (d, *J* = 6.7 Hz, 3H, $C\underline{H}_3$-2); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 173.9, 131.2, 131.2, 59.8, 49.6, 38.7, 37.2, 35.6, 32.3, 31.9, 27.6, 24.5, 18.4, 17.8, 14.4. HRMS (*m/z*) calcd for $C_{15}H_{24}O_2Na$ $[M+Na]^+$ 259.1669, found 259.1663.

(1*S*,2*S*,4a*R*,6*S*,8a*S*)-2,6-dimethyl-1,2,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylic acid (222).



To a solution of **221** (7.7 mg, 0.029 mmol) in dioxane (200 μL) and water (200 μL) was added LiOH (2.5 mg, 0.058 mmol) and heated to 100 ºC. After six days at 100 ºC, the reaction mixture was cooled and diluted with Et_2O (4 mL) and water (4 mL), extracted with Et_2O (2 x 4 mL), and the layers were separated. The aqueous layer was acidified with 1 N HCl to pH = 1, and extracted with Et_2O (2 x 4 mL). The organic layers were combined, dried with magnesium sulfate and concentrated *in vacuo* to yield **222** (1 mg, 17%). $[\alpha]_D^{25}$ = 80.78 (c = 0.10 g/100mL, $CHCl_3$); IR ($CHCl_3$ cast film): 3400 – 2800 (br), 3014, 2959, 2918, 2852, 1708, 1146 cm^{-1}; ^1H NMR ($CDCl_3$, 700 MHz) δ 5.56 (ddd, *J* = 9.9, 4.3, 2.7 Hz, 1H, H-3), 5.33 (d, *J* = 9.9 Hz, 1H, H-4), 2.69 – 2.57 (m, 2H, H-1 + H-2), 2.09 – 2.02 (m, 1H, H-6), 1.98 – 1.91 (m, 1H, H-4a), 1.79 (dddd, *J* = 12.8, 3.5, 3.5, 3.5 Hz, 1H, H-8eq), 1.62 (dddd, *J* = 13.5, 9.2, 4.6, 4.6 Hz, 1H, H-7ax), 1.55 – 1.48 (m, 2H, H-7eq + H-5eq), 1.39 – 1.31 (m, 2H, H-8a + H-5ax), 1.13 (dddd, *J* = 13.0, 13.0, 13.0, 3.6 Hz, 1H, H-8ax), 0.99 (d, *J* = 7.2 Hz, 3H, CH_3-6), 0.97 (d, *J* = 7.0 Hz, 3H, CH_3-2); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 177.1, 131.2, 130.8, 48.9, 38.6, 36.9, 35.5, 32.1, 31.8, 27.5, 24.4, 18.3, 17.7. HRMS (*m/z*) calcd for $C_{13}H_{19}O_2$ $[M-H]^-$ 207.1391, found 207.1390.

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