Mechanistic Studies of Antimicrobial Peptides in Lipid Membranes

by

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Abstract

Antibiotics with unexplored mechanisms are needed to fight the alarming rise in antimicrobial resistance. Antimicrobial compounds targeting peptidoglycan precursor lipid II, the Achilles' heel of bacteria, can be a suitable template. Therefore, structural studies of membrane mimetic models may provide insights regarding peptide-lipid interactions at atomic resolution. Highlighted in this thesis are the syntheses of peptidoglycan precursor lipid II and its analogues as well as various phospholipids, with the aim of using these synthetic analogues for structural studies on peptides. Some smaller compounds with antimicrobial properties are also explored in this thesis.

Tridecaptin A_1 is part of a class of peptides that are potent against Gram-negative bacteria. They are known to bind to lipid II, the same peptidoglycan precursor targeted by certain other antimicrobial peptides. In order to better understand the interaction between tridecaptin A_1 and its target, insights into the physiological mode of action require a liposomal solvent to mimic the native setting. I addressed this by synthesizing native Gram-negative lipid II and tridecaptin A_1 containing fully labelled amino acids which appear to be in close proximity to its lipid II target. Solid state NMR spectroscopic studies of the peptide and lipid II in liposomes was measured to understand how tridecaptin interacts with the cell wall precursor lipid II.

Cycloalanopine is a cyclic opine with reported antimicrobial activity identified in 2019. I synthesized a series of analogues to determine the stereochemistry of the active cycloalanopine. Additionally, a series of cycloalanopine analogues were also

synthesized and a cyclic glycine derivative was identified with activity against both Gram-negative and Gram-positive bacteria.

With our ongoing research in understanding the interaction of tridecaptin A1 and its targets, I synthesized a series of membrane mimic phospholipids with heteroatoms replacing specific methylene groups along the lipid tail of dodecylphosphocholine (DPC). This approach was used to understand how peptides can interact within the lipid membrane, by analyzing the influence heteroatoms exert at different positions along the lipid tail had on the structure of tridecaptin A1 in these micelles. This was accomplished with the use of solution state NMR spectroscopy. This idea will be extended to other membrane-interacting antimicrobial peptides, like leucocin A, and so I synthesized undeuterated analogues of DPC to enhance further studies.

Preface

At this point, the majority of Chapter 2 remain unpublished. Sections of Chapter 2 were a collaborative effort with Dr. Joao Medeiros Silver and Dr. Markus Weingarth at Utrecht University. Syntheses of labelled Oct-TriA_1 and native lipid-DAP were performed by me, and solid-state NMR studies were performed by our collaborators. Part of this chapter has been published as Chiorean S, Antwi I, Carney DW, Kotsogianni I, Giltrap AM, Alexander FM, Cochrane SA, Payne RJ, Martin NI, Henninot A, and Vederas JC. Dissecting the binding interactions of teixobactin with the bacterial cell wall precursor lipid II. *ChemBioChem*, **2020**, *21*, 789-792. I synthesized the C_{55} mDAP-lipid II and C_{55} lys-lipid II for the studies.

Chapter 3 has been published as Antwi I, Chiorean S, van Belkum MJ and Vederas JC. Unveiling the active isomer of cycloalanopine, a cyclic opine from *Lactobacillus rhamnosus LS8,* through synthesis and analog production. *RSC Med. Chem*, **2020**, *11*(4), 528-531. The research was performed by me (75%) and Dr. Sorina Chiorean (20%) and Dr. Marco van Belkum (5%) who assisted with antimicrobial activity assays.

Chapter 4 of this thesis remains unpublished as at the time of this thesis presentation. Chapter 4 was a collaborative effort with Dr. Sorina Chiorean. Synthesis of the deuterated O-DPC-d_{36}, Oct-TriA_1 and lipid II-DAP analogues were performed by me, and NMR structural elucidation experiments and data analysis were performed by Dr. Sorina Chiorean. Undeuterated O-DPC and F_2-DPC analogues and deuterated 6-F_2-DPCd_{36} analogue were performed by myself. At the time of writing this thesis, Miss Cherry Ibarraro Romero, Miss Tess Lamer and Dr. van Belkum are performing experiments for the purification of leucocin A.

Dedication

I would like to dedicate this thesis to my mother, Mrs. Grace Antwi who has been the backbone of my academic career.

Acknowledgements

“If I have seen further, it is by standing upon the shoulders of giants.”

– **Isaac Newton**

This thesis would not have been possible without the support, guidance, and advice from several individuals. First and foremost, I am thankful to my supervisor, Prof. John C. Vederas, for providing me with the platform to pursue my research. His advice, help and guidance has proved invaluable during my years as a graduate student and as a scientist has inspired me to succeed. Thanks also to Dr. Sorina Chiorean who I collaborated with on several projects and proofreading this thesis and the rest of the Vederas group both past and present. Thanks to Wayne Vuoung and Tess Lamer for proofreading my thesis. I would also like to thank Dr. Randy Sanichar for his advice in the early stages of my PhD program.

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Outside of work there have been multiple people supporting me to reach my hopes dreams. I want to thank my family and friends both in Ghana and Canada for their encouragement and support, but in particular my mother, who has always been there for me both in hard times and in good times.

Finally, I want to thank God for blessing me with such a wonderful life and career. His grace throughout this journey has sustained me. He made a way where there seemed to be no way. It is only by his grace that I have achieved all that I have, and I am honoured to have a career in which I study his creation.

"Not that we are adequate in ourselves to consider anything as coming from ourselves, but our adequacy is from God."

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List of Symbols, Units & Abbreviations

$[\alpha]^{25}_{D}$	specific rotation
Å	Ångström
Ac	Acetyl
Ac_2O	acetic anhydride
AcOH	acetic acid
ACN	acetonitrile
AMP	antimicrobial peptide(s)
AMR	antimicrobial resistance
ATP	adenosine triphosphate
Boc	tert-butyloxycarbonyl
CDMT	2-chloro-4,6-dimethoxy-1,3,5-triazine
c	concentration in g mL^{-1}
CD	circular dichroism
CDI	*N,N'*-carbonyldiimidazole
CP	cross-polarisation
d	doublet (in NMR)
DAP	diaminopimelic acid
DBU	diazabicycloundec-7-ene
DIC	*N,N'*-diisoproprylcarbodiimide
DCM	dichloromethane
DIBAL	diisobutylaluminum hydride
DIPEA	N, N-diisopropylethylamine

DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DMPC	dimyristoylphosphatidylcholine
DOPC	1,2-dioleoyl-sn-glycerol-3-phosphocholine
DPC	dodecylphosphocholine
EEA	European Economic Area
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EM	electron microscopy
equiv	equivalent(s)
ESI	electrospray ionization
Et	ethyl
EtOAc	ethyl acetate
EtOH	ethanol
Fmoc	fluorenylmethoxycarbonyl
FTIR	Fourier transform infrared spectroscopy
HATU	1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate,
HBTU	2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
Hex	hexanes
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	hydroxybenzotriazole
HPLC	high performance liquid chromotography
HRMS	high-resolution mass spectrometry

IDSA	Infectious Disease Society of America
Ile	isoleucine
IR	infrared
J	coupling constant, in Hertz
LUV	large unilamellar vesicle
LCMS	liquid chromatography mass spectrometry
LCMS/MS	liquid chromatography with tandem mass spectrometry
LII	lipid II
m	multiplet
m/z	mass to charge ratio
MAS	magic-angle spinning
MALDI	matrix assisted laser desorption/ionization
MALDI-TOF	MALDI-time-of-flight
Me	methyl
MS	mass spectrometry
MQ	Milli-Q, Millipore Quality filtered
n-BuLi	n-butyllithium
NMM	methylmorpholine
NMR	nuclear magnetic resonance
NRAP	nonribosomal antimicrobial peptide
NRP	nonribosomal peptide
Ph	phenyl
Pt/C	platinum on activated carbon

Ppm	parts per million
PGs	phosphatidylglycerols
PyBOP	(benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate)
q	quartet
quant.	quantitative yield
MFS	major facilitator superfamily
MATE	membrane fusion protein
MFP	
mRNA	messenger ribonucleic acid
OMP	outer membrane protein
rSAP	shrimp alkaline phosphatase
RND	resistant-nodulation-cell division
RP-HPLC	reverse phase-high performance liquid chromatography
s	singlet
SDS	sodium dodecyl sulfate
SPPS	solid phase peptide synthesis
SMR	small multidrug resistance
t	triplet
t-BuOH	*tert*-butanol
TBAP	tetra-n-butyl ammonium dihydrogen phosphate
TES	triethylsilane

TCA	trichloroacetonitrile
TFA	trifluoroacetic acid
TFE	2,2,2-trifluoroethanol
TMSOTf	trimethylsilyl triflate
THF	tetrahydrofuran
THP	tetrahydropyran
TIPS	triisopropylsilane
Ti	tumour inducing
TLC	thin layer chromatography
Troc	trichloroethyoxycarbonyl chloride
UV	ultraviolet spectroscopy
VRE	vancomycin resistant enterococci
WHO	World Health Organization
δ	chemical shift in parts per million

Chapter 1. Introduction

1.1. Bacterial Resistance to Antibiotics

Bacteria live in a wide range of environments including soil, the ocean, as well as in humans.[1] The human-bacterial relationship has always been complicated due to the fact that some are helpful to humans while others are harmful.[2] Bacteria can assist humans in a variety of ways, such as curdling milk into yoghurt or assisting with digestion. Children are exposed to bacteria from their mother's microbiome during natural childbirth. These bacteria may stay with us throughout our lives and play significant roles in our health such as defence against invading pathogens and regulating mood.[1] Bacteria may also be harmful and some are classified as pathogenic microorganisms. Cancer, diabetes, cardiovascular disease, and obesity are all illnesses that may be exacerbated by bacteria in our body.[3] Throughout history, pandemics resulting from bacterial infections have devastated our population. In 1347, a bacterial infection probably caused by *Yersina pestis* resulted in 75 million to 200 million deaths within seven years.[4] Bacterial pneumonia has been suggested in a recent study as the primary cause of death in the 1918 influenza pandemic rather than the H1N1 influenza virus itself.[5] Progress in science and our society has helped shape our approach to preventing and treating these infections, one of which is the use of antibiotics. However, the development of bacterial resistance against our current antibiotics has necessitated further exploration for agents against these microbes.[6]

1.2. An Urgent Need for New and Robust Antibiotics

The bedrock of modern medicine is antibiotics, yet the globe is running out of these "miracle drugs" at a swift pace. The first antibiotic, penicillin, was discovered in 1928 by Alexander Fleming.[7] This serendipitous discovery initiated an era of antibiotic development lasting for 40 years. Since then, there has been a steady increase in the rate of antibiotic resistance in bacteria. All clinically-used antibiotics have shown some degree of antimicrobial resistance (AMR).[8] Antibiotic resistance has been observed for many important antibiotics such as penicillin G (**1**), vancomycin (**2**), methicillin (**3**), azithromycin (**4**) and ceftriaxone (**5**) (Figure 1.1).

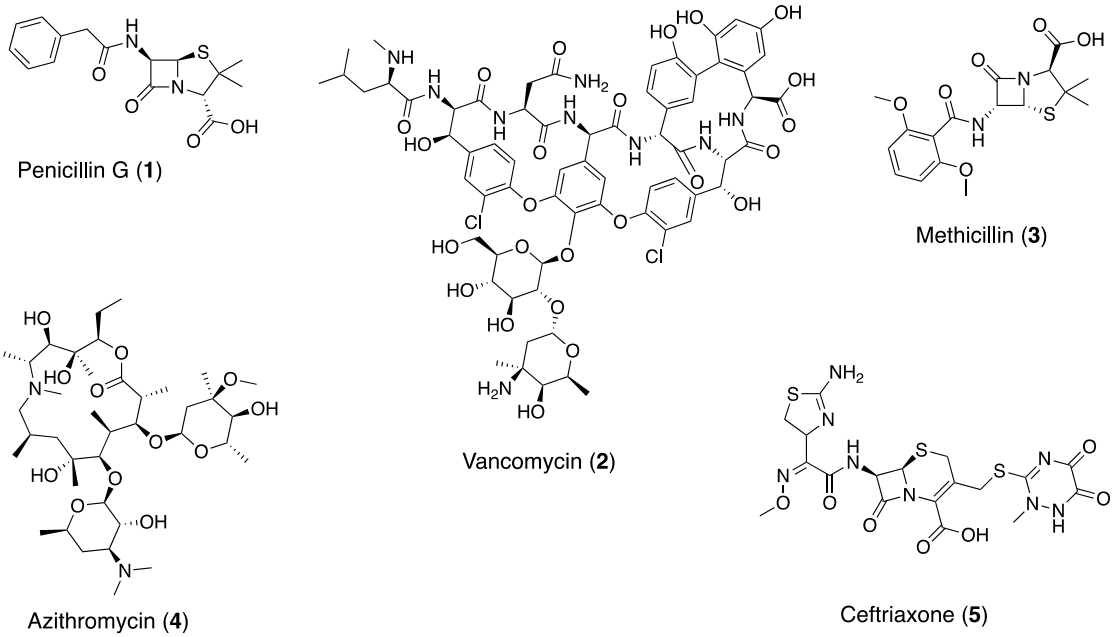


Figure 1.1. Antibiotics on the market that have been linked to reported resistance.

It has been projected that by 2050 10 million people will die each year due to AMR.[9] AMR in the European Economic Area (EEA) is now on the rise, and 33,000 people in the EEA die annually as a result of AMR.[10] The cost that will be associated with antimicrobial resistance is estimated to be in the billions of euros for the European economy.[11] There is an unmet demand for compounds that are effective for bacteria that display resistance.

One avenue of research involves naturally occurring antimicrobial peptides (AMPs). An exciting sub-class of these are those that target lipid II (Figure 1.2), the precursor to bacterial peptidoglycan, as some can kill even the most refractory bacteria at a nanomolar concentration.[12, 13] AMPs that bind to the pyrophosphate motif of lipid II are less prone to develop resistance mechanisms since this group may be difficult to replace.[12] Hence, lipid II may be an excellent target for new antibiotics.[12, 14]

1.3. Targets for Antibiotics

Antibiotics can target the cell wall biosynthesis, protein-synthesis, nucleic acid synthesis, and the cell membrane. Of focus in this thesis are antimicrobial peptides that target the cell wall biosynthesis.

1.4. Antibiotics Targeting the Cell Wall

A main component of the bacterial cell wall is the essential glycopeptide polymer peptidoglycan.[14] It consists of repeating units of *N*-acetylglucosamine (GlcNAc) which are linked to *N*-acetylmuramic acid (MurNAc) through a 1,4 glycosidic linkage. These polysaccharide chains are crossed linked through peptide bridges. Peptidoglycan precursors are biosynthesized in the cytoplasm.[15] One complete peptidoglycan subunit,

containing the two sugars and five amino acids, is attached to a polyisoprenoid C_{55} moiety by a pyrophosphate group to form lipid II. Lipid II is constantly recycled within the cell membrane to ensure efficient peptidoglycan synthesis.[12] A compound that inhibits the synthesis of peptidoglycan or sequesters lipid II is a potential antibiotic.[12, 14] Some lipophilic peptides can access the plasma membrane of Gram-positive bacteria and bind specifically at the pyrophosphate group of lipid II.[16] Peptides with this mode of action have the ability to kill even the most resistant strains, such as methicillin-resistant *Staphylococcus aureus* (MRSA), at nanomolar concentrations.[12] As human cells do not manufacture lipid II, unwanted toxicity may be low. Differences in the cell wall structure between Gram-positive and Gram-negative bacteria affect the activity of these types of antibiotics. The pentapeptide of the cell wall precursor in Gram-positive bacteria is often L-Ala-D-Glu-L-Lys-D-Ala-D-Ala, whereas in Gram-negative bacteria, the third amino acid of the pentapeptide is generally *meso*-diaminopimelic acid (*meso*-DAP). However, the sequences of these stem peptides are variable between species, as are the secondary modifications present in the glycan chains and peptides.[17]

β-Lactams such as penicillins (**6**), cephalosporins (**7**), monobactems (**8**), and carbapenems (**9**) (Figure 1.2) are antibiotics that target the cell wall.[18] Their primary targets are penicillin binding proteins (PBPs), which are responsible for crosslinking the peptide portions of peptidoglycan units. Other antibiotics that target cell wall biosynthesis include cyclic peptides such as bacitracin (**10**), lantibiotics such as nisin (**11**), and glycopeptides such as vancomycin (**2**) (Figure 1.2). The D-alanyl-D-alanine moiety is the binding site of vancomycin (**2**), which can prevent the crosslinking by PBP and thereby impair cell wall synthesis.[18]

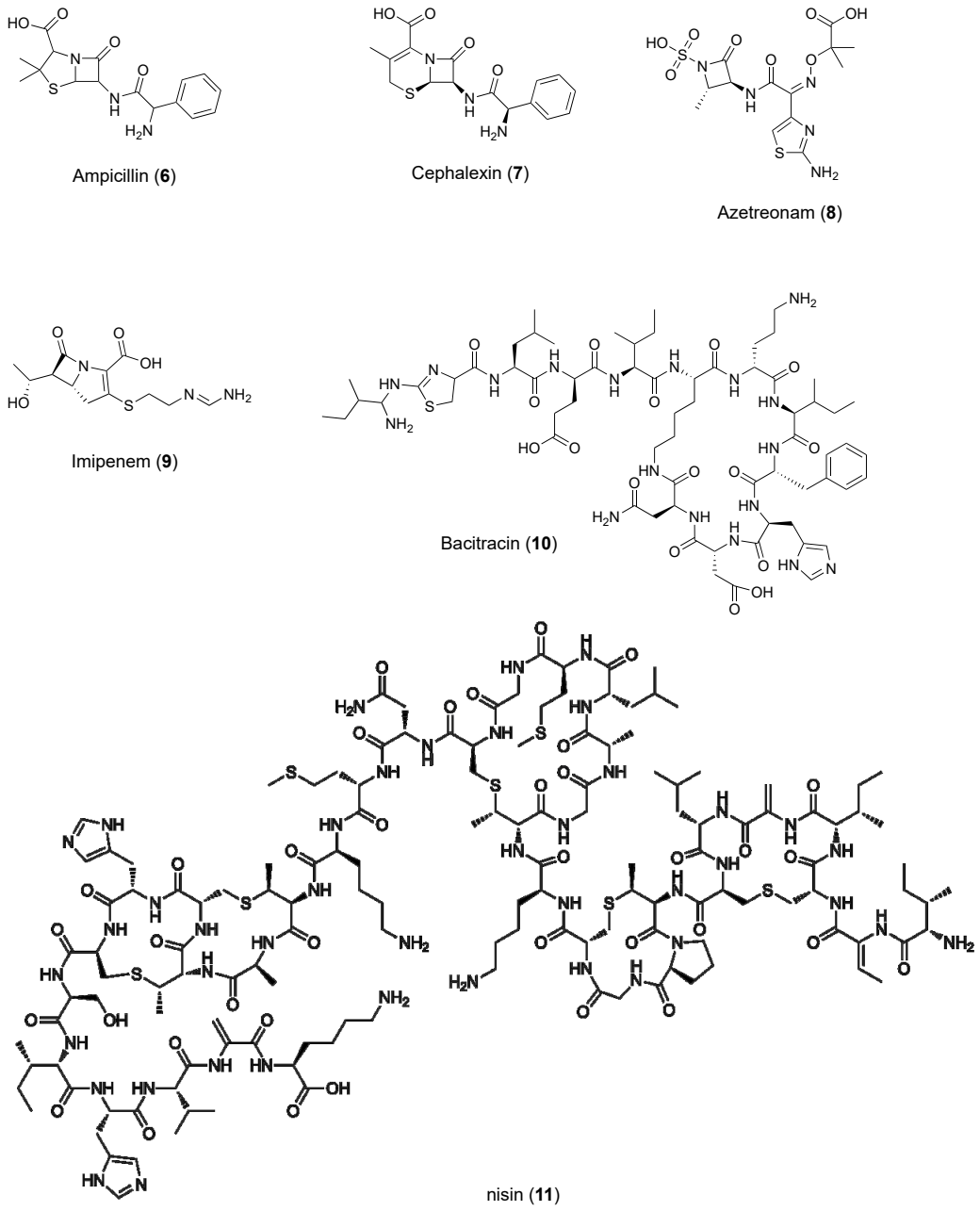


Figure 1.2 Antibiotics that target the cell wall

1.5. Addressing the Issue of Antimicrobial Resistance: The Search for New Antimicrobial Agents

Only six first-in-class antibiotics with novel scaffolds have been approved since the 1960s, and these have been introduced in the past fifteen years. They were primarily

developed to fight Gram-positive bacteria, and most appear to have little or no activity against Gram-negative bacteria. Development of antibiotics with potency against Gram-negative bacteria is lacking. Recent antibiotics include the streptogramin combination quinupristin (**12**) and dalfopristin (**13**) in 1999, the oxazolidinone linezolid (**14**) in 2000, the lipopeptide daptomycin (**15**) in 2003, retapamulin (**16**) in 2007, fidaxomicin (**17**) in 2011 and bedaquiline (**18**) in 2012 (Figure 1.3). Two of these, linezolid and bedaquiline, are synthetic molecules and the rest are natural products.

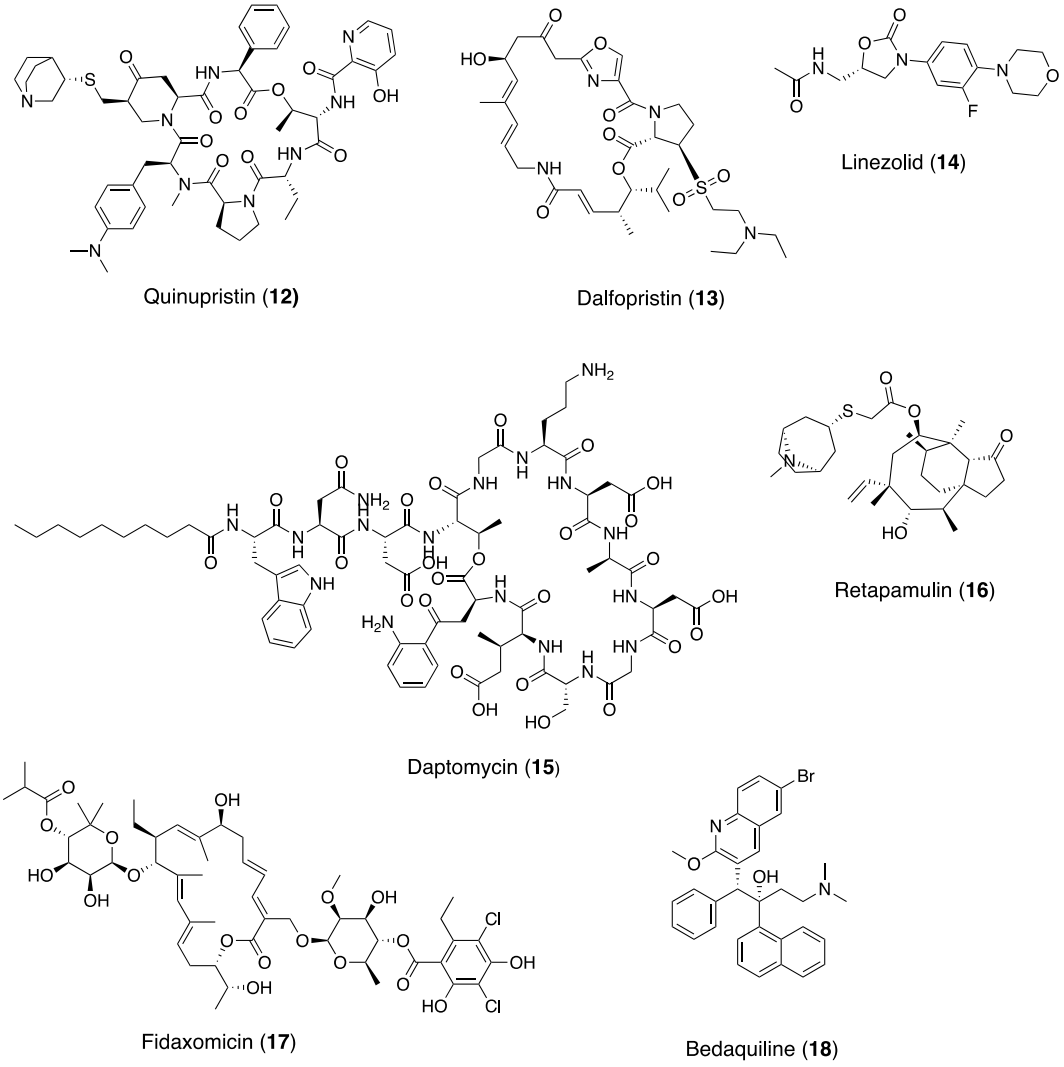
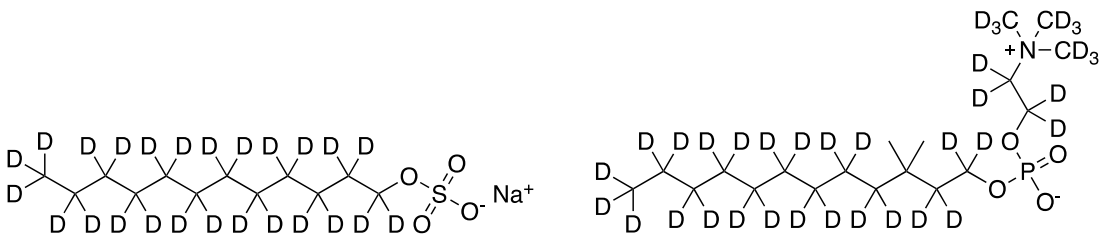


Figure 1.3 New first-in-class antibiotics introduced for human use

1.6. Antimicrobial Peptide in Phospholipid Model Membrane

AMPs are often small peptides, and some play a vital role in many animals' innate immune systems.[19, 20] These molecules act to rapidly eliminate bacteria or other pathogens. They may also act as signalling molecules that instruct the adaptive immune system in vertebrates to initiate a second round of attacks on invading microbes.[19, 20] Evidence suggests that bacteria are less likely to develop resistance to AMPs than traditional antibiotics, and there is increasing interest in the development of drugs based on natural AMPs.[21, 22] They have been identified in bacteria, fungi, amphibians, insects and mammals, including humans.[23] Some have a broad spectrum of toxicity to bacteria, viruses and fungi, whereas others are selective towards specific bacteria with negligible effects on mammalian cells.[24] A key reason for their selectivity is the different membrane composition and structure of bacterial versus human cells.[24] Several studies indicate that electrostatic interactions between anionic lipids in bacterial membranes and cationic peptides are crucial for the selectivity of AMPs.[25, 26] Research on AMP structure and function is essential for a detailed understanding of their mechanism. NMR studies investigating membrane-peptide structures often utilize mimics, such as detergent micelles. Both deuterated sodium dodecyl sulfate (SDS_{d25}) (**19**) and dodecylphosphocholine ($DPCd_{38}$) (**20**) micelles (Figure 1.4)[26-28] are commonly used. SDS has a negatively charged sulfate group, whereas DPC has a phosphatidylcholine head group.



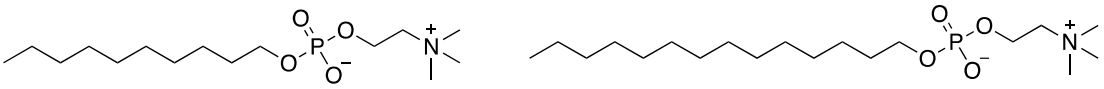
Sodium dodecyl sulfate-d_{25} (**19**) Dodecylphosphocholine-d_{38} (**20**)

Figure 1.4 Examples of deuterated detergents for solution NMR studies of peptides

1.7. Solution NMR of Peptides in Detergent Micelles

NMR studies on lipid-binding peptides often used models such as micelles or vesicles (or liposomes).[29-32] The medium may make a difference in the structures of small peptides, which are often flexible and prone to conformational changes. For solution NMR analyses, the compound must also reorient sufficiently rapidly to achieve a good spectral resolution; this imposes a restriction on the types of lipid models of membranes. NMR is employed to study not only the three-dimensional structures of peptides, but also the intramolecular dynamics, kinetics and thermodynamics of binding to ligands. One of the major restraints of solution NMR spectroscopy is the size of the molecule or complex. NMR spectra of large molecules show enhanced transverse relaxation, broad lines, decreased sensitivity, and low resolution because large molecules tumble slowly in solution.[33] Also, the number of nuclei in large molecules often results in signals that overlap in the NMR spectra.[34, 35] Thus, to study peptides in membrane mimetics, the overall size should be relatively small. A micellar membrane model is useful because it is often relatively small of (5 – 50 nm)[36] and rotates rapidly on the timescale (2.0 ms)[37] required. Numerous NMR studies of peptides and proteins are conducted in sodium dodecylsulfate (SDS) (**19**), and

dodecylphosphocholine (DPC) (**20**).[38, 39] Perdeuterated dodecylphosphocholine (DPC-d38) has been used extensively since the pioneering work of Lauterwein and coworkers in 1979.[40] NMR conformational studies of peripheral proteins[41], signal peptides, effector peptides, protein fragments,[42-46] as well as of lipopeptides have been reported.[47-49] The presence of phosphocholine head groups that resemble a membrane surface contributes to why DPC micelles have been widely used. Unconventional DPC analogues with altered carbon chain lengths like FOS-10 (**21**) or FOS-14 (**22**) (Figure 1.5) reveal a better performance to dissolve some membrane proteins.[50]



FOS-10 (**21**) FOS-14 (**22**)

Figure 1.5 Unconventional DPC analogues

Structural modification of phospholipids has been employed to understand the interaction of antimicrobial peptides (AMPs) with lipid membrane. Monofluorinated analogues of dimyristoylphosphatidylcholine (F-DMPC) **(23a**), (**23b**), (**23c**), (**23d**), (**23e**), and (**23f**) (Figure 1.6) have been used to understand the influence of the heteroatom on DMPC (**24**) properties.[51] Such modification can potentially be applied to DPC for solution NMR studies of lipophilic peptides.

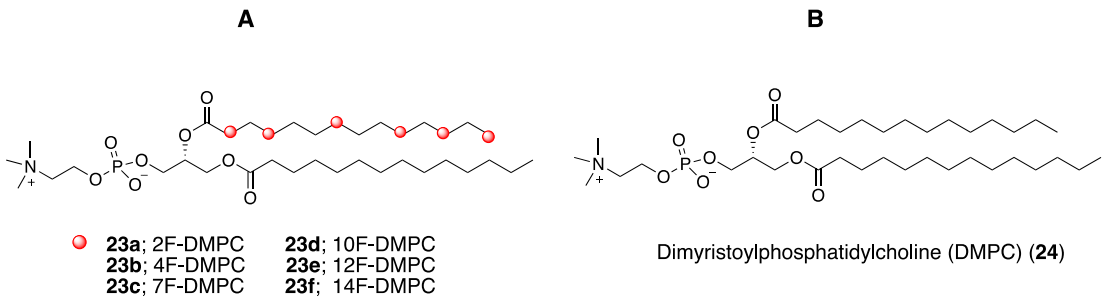
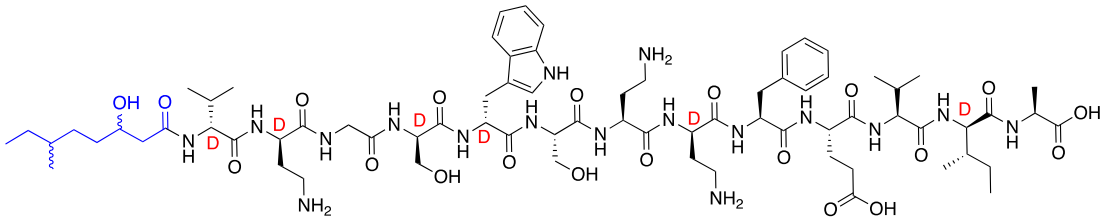


Figure 1.6 A) Structure of monofluorinated DMPC analogues, B) Structure of DMPC

1.8. Linear Antimicrobial Lipopeptides

In the World Health Organization's "Priority Pathogen List for Research and Development on Antibiotics" there is emphasis on the need for antibiotics against Gram-negative bacteria.[52] The tridecaptins are a class of linear lipopeptides isolated from several strains of *Paenibacillus polymyxa* in the 1970's, and are made of a combination of L-and D- amino acids with an *N*-terminal acylated lipid tail that usually contains a β-hydroxy group (Figure 1.7). The promising aspect of tridecaptins is that they have been shown to have a potent antimicrobial effect against Gram-negative bacteria by binding to lipid II.[49] Furthermore, they have low mammalian toxicity because they do not appear to cause general membrane lysis.[49] AMPs that bind lipid II may be considered for next generation antibiotics, but data on their mode of action is critical for development into clinical drugs. Further understanding of their interaction with their target lipid II may be possible via NMR analyses .



Tridecaptin A (**25**)

Figure 1.7 Example of tridecaptin lipopeptide

1.9. Thesis Overview

Identification and synthesis of new antibiotics are of great importance, together with an understanding of the mechanisms of action, in the quest to combat antimicrobial resistance. The work in the projects outlined in this thesis provides strategies for understanding the mode of action of antimicrobial peptides.

Chapter 2 will discuss the binding mode of antibiotic tridecaptin that targets lipid II through a solid-state NMR strategy. This work describes the syntheses of cell wall precursor lipid II and labelled tridecaptin A_1 and, in collaboration with the group of Dr. Weingarth (University of Utrecht), demonstrates that the bound state of the peptide is highly heterogenous and does not adopt a well-defined conformation. The effect of pH on the structure of the peptide was also examined by our collaborators.

Chapter 3 will outline the preparation and testing of pure stereochemical isomers of a cyclic opine reported to have antibacterial activity. Potent analogues were also synthesized and the method for their preparation is detailed.

Chapter 4 will focus on the preparation of dodecylphosphocholine (DPC) analogues for NMR studies with antimicrobial peptide tridecaptin A_1 and lipid II. The DPC analogues will also be used as membrane mimics to determine the structure of other antimicrobial peptides. The synthesis of both deuterated and undeuterated analogues is outlined.

Chapter 2. Synthesis of Labelled Octyl-Tridecaptin A_1 and Native Gram-Negative and Gram-Positive Lipid II

2.1. Introduction

One of the challenges of targeting Gram-negative bacteria is the obstacle imposed by the outer membrane, which blocks entry of many molecules including some antibiotics.[53, 54] Several priority Gram-negative pathogens targeted for the development of new antibiotics by WHO are carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.[55] Among the Gram-positive pathogens are vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus*.[55] Of special interest would be broad spectrum antibiotics for treating infections caused by both Gram-negative and Gram-positive organisms.

Mechanistic understanding is helpful for developing novel antibiotics and other therapeutic methods to revitalize existing antibiotics.[56] For example, during the treatment of *Enterobacteriaceae*, the fungal natural product aspergillomarasmin A has been shown to remove the zinc ion selectively from metallo-ß-lactamases such as VIM-2 and NSM-1, restoring their susceptibility to carbapenems.[57]

Non-ribosomal peptides (NRPs) are attractive for the development of new antibiotics because of their structural diversity, and several are therapeutics.[56, 58] An example is penicillin, which ushered in the age of modern medicine.[7, 59, 60] Other examples include colistin and vancomycin, which have been classified as last-resort antibiotics for infections by Gram-negative and Gram-positive pathogens, respectively.[61]

2.2. Tridecaptins

Tridecaptins are linear NRPs that have 13 amino acids and are acylated at the *N*-terminus. They display selective activity against Gram-negative bacteria.[62, 63] The first of this class of compounds was tridecaptin A_1 (Figure 2.1), which was isolated in the 1970's from *Paenibacillus polymyxa*.[64] These compounds consist of combinations of L- and D- amino acids with an N-terminal lipid tail. The stereochemistry of the most studied compound in this class, tridecaptin A_1 ($TriA_1$), was fully determined by our group.[65]

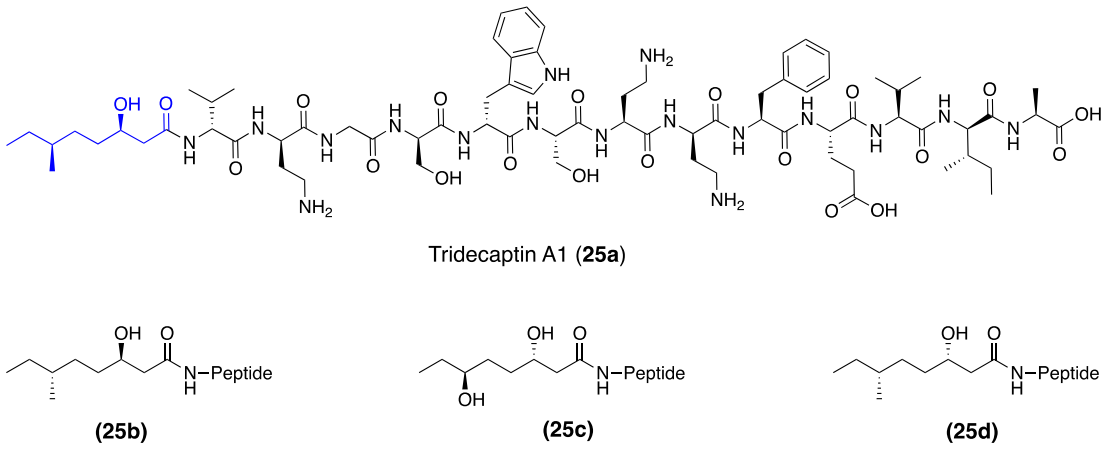
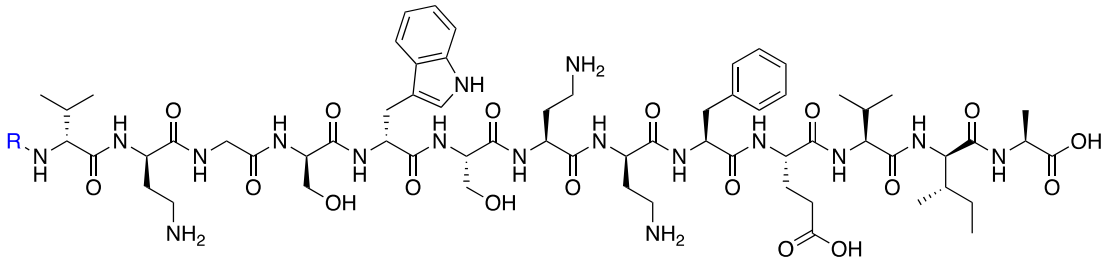


Figure 2.1 Structure of four stereoisomers of Tri A1

Dr. Stephen Cochrane of our group synthesized all configurations. However, the use of expensive chiral starting materials and several reaction steps necessary for the synthesis of the lipid tail hindered access to this compound. Our group synthesized a small library of analogues with different lipid tails (Figure 2.2) using solid phase peptide synthesis (SPPS) and determined the antimicrobial activities.[66]



(26): R = H

(27): R = $C(O)C_5H_{11}$

(28): R = $C(O)C6H_{13}$

(29): R = $C(O)C_7H_{15}$

(30): R = $C(O)C_8H_{17}$

(31): R = $C(O)C_9H_{19}$

(32): R = $C(O)C_{10}H_{21}$

(33): R = $C(O)C_{11}H_{23}$

(34): R = $C(O)C_{13}H_{27}$

(35): R = $C(O)C_{15}H_{31}$

(36): R = Fmoc

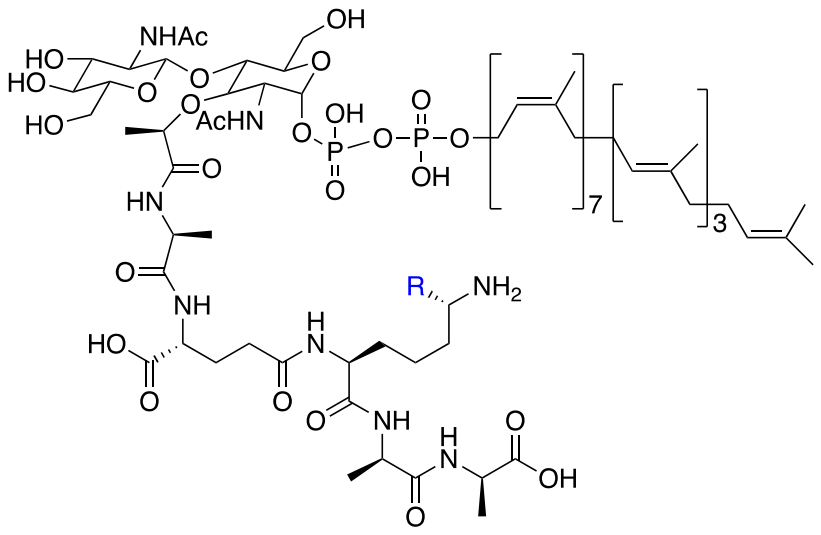
(37): R = PEG

Figure 2.2 Structure of analogs of Tri A1 with various lipid tails

The absence of the lipid tail in H-TriA_1 (**26**) resulted in a substantial decrease in the compound's activity.[66] This effect has also been observed in unacylated polymyxins, which showed no antibacterial effect due to the lack of the lipid tail required for insertion into the phospholipid bilayer.[67] It is known that lipopeptides' bactericidal effects occur through lysis of the bacterial membrane, which could explain why H-TriA_1 (no lipid tail) has greatly reduced antimicrobial activity compared to the native tridecaptin A_1.[66] When the natural lipid tail was replaced with a simple octyl chain to yield Oct-TriA_1 (**29**), the activity of the compound was retained.

The length of the lipid tail was important for antimicrobial activity as chains that were shorter than C_6 or longer than C_{12} were less active. The best tails ranged from C_8-C_{11}. It also appeared that the peptide's aqueous solubility played a crucial role, which could explain why a longer alkyl chain resulted in less activity. These observations supported the initial proposal that the cell membrane is a target for TriA_1.[49] The differences in the

activity observed for the four hydroxy isomers of $TriA_1$ (**25a-d**, Figure 2.2)suggested that it requires a specific target molecule rather than operating by general membrane distruption. The same studies showed that $TriA_1$ targets the peptidoglycan layer precursor lipid II (Figure 2.3) and selectively binds to the *m*DAP moiety found in most Gram-negative organisms.[49] In many Gram-positive bacteria, the *meso*-diaminopimelic acid in lipid II (**38**) is replaced by the amino acid lysine (**39**).



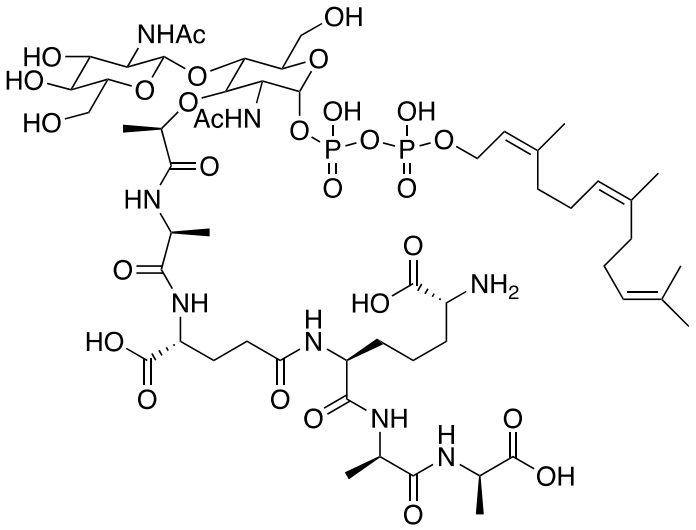
Gram-negative lipid II (**38**): R = COOH

Gram-positive lipid II (**39**): R = H

Figure 2.3 Structure of many Gram-negative and Gram-positive lipid II variants

Natural lipid II contains a C_{55} lipid chain which is problematic for solution NMR studies due to its poor solubility in aqueous systems. The amphiphilic nature of the C_{55} lipid II makes it prone to the formation of micelles, which results in line broadening. NMR spectra of $TriA_1$ and lipid II would be overwhelmed by the numerous signals from the C_{55} chain, making a complete spectral assignment more difficult. To overcome this problem, the C_{55} tail was replaced with a shorter C_{15} chain.[49] This approach was

first reported by Breukink and coworkers who used a biosynthetic strategy to prepare a Gram-positive lipid II analogue that had the C_{55} tail replaced with an *(E,E)*-farnesyl chain.[16] They were then able to determine the solution structure of nisin A in the presence of this lipid II analogue.[16] The authors observed that the ability of nisin to bind to lipid II was not affected by the length of the terpene portion, possibly because it is embedded in the membrane. Our group previously synthesized a C_{15} *m*DAP-lipid II with *Z,Z* stereochemistry (**40**) (Figure 2.4) based on the approach reported by VanNieuwenhze and coworkers.[68, 69] The structure of Oct-TriA_1 with C_{15} *m*DAP-lipid II in DPC-d_{38} micelles was then determined.[49]



C_{15}-Z,Z-*m*DAP-lipid II (**40**)

Figure 2.4 Structure of C15-Z, Z-mDAP-lipid II (40)

Solution NMR studies of Oct-TriA_1 with compound **40** in DPC-d_{38} micelles revealed that three amino acids, Trp5 and Phe9 and Gly3, were in close proximity and could engage in pi-stacking.[49] These studies[49] also suggest that the alpha hydrogen of Gly3 and the *para* carbon of Phe9 are in close proximity (ca. 2.2 Å) (Figure 2.5).

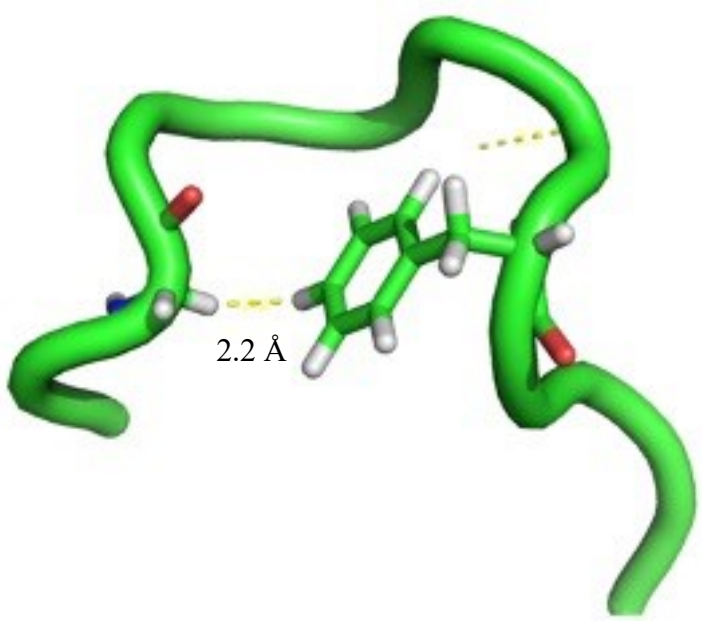


Figure 2.5 3D solution structure of Oct-TriA1 in C15-Z, Z-mDAP-lipid II bound state

To better understand how the 3D structure of this peptide bound to lipid II causes pore formation in lipid bilayers, a collaboration using solid state NMR spectroscopy was undertakeN with Professor Markus Weingarth at the University of Utrecht. It was decided to synthesize Oct-TriA$_1$ (**41**) (Figure 2.6) with Gly3 and Phe9 amino acids fully labelled with ^{13}C and ^{15}N to help define the peptide 3D structure with solid state NMR spectroscopy. The solid-state NMR experiments discussed in this chapter of the thesis were performed by Dr. Weingarth and coworkers at Utrecht University using the materials I prepared.

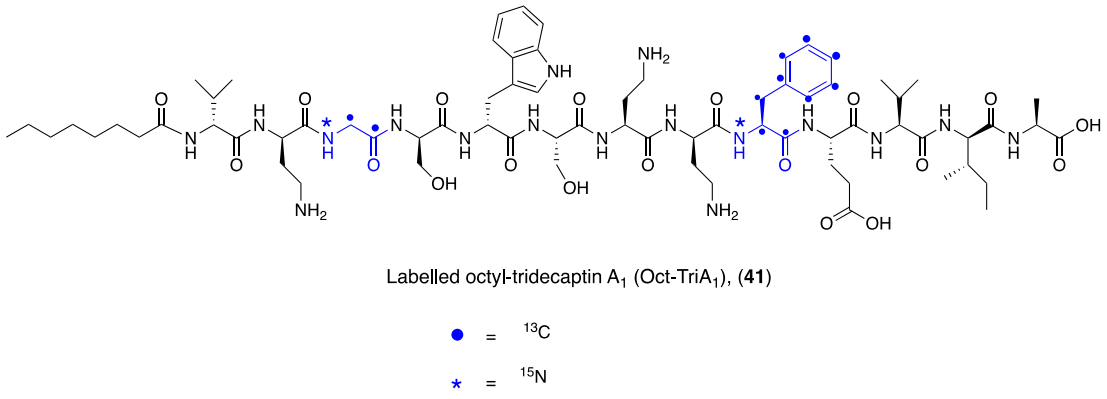
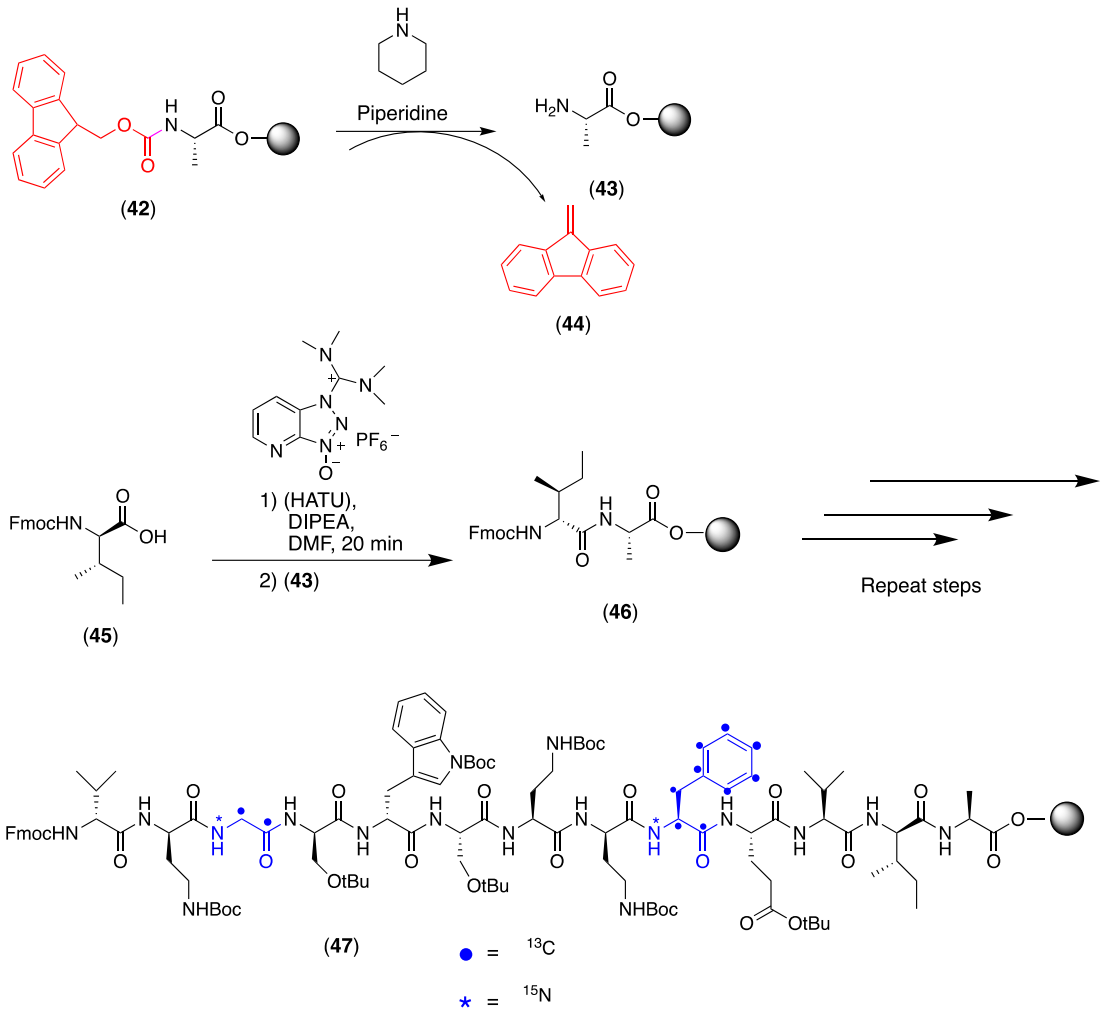


*Figure 2.6 Structure of labelled Oct-triA$_1$ (**41**)*

2.3. Results and Discussion of Syntheses

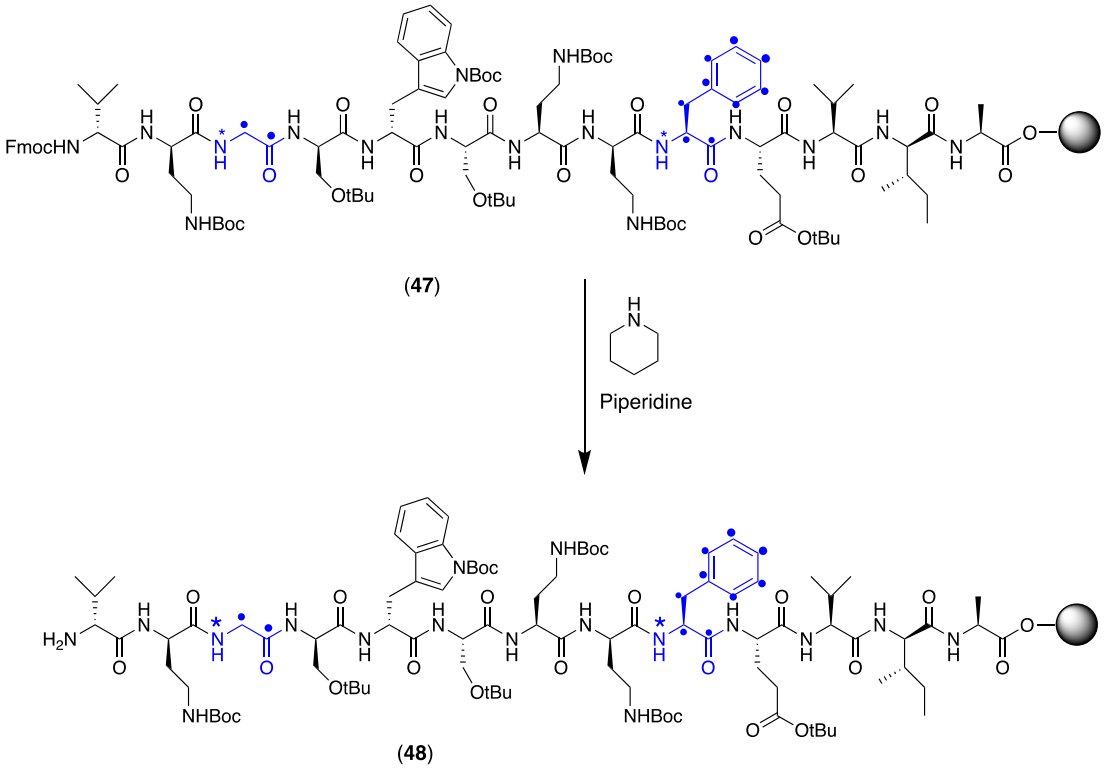
2.3.1. Synthesis of Labelled Octyl-Tridecaptin A$_1$

The synthesis of the ^{13}C, ^{15}N Oct-TriA$_1$ (**41**) was achieved following previously developed methods.[49] The TriA$_1$ peptide chain was synthesized using solid-phase peptide synthesis (Scheme 2.1). For the synthesis of the TriA$_1$ peptide chain, the first step is the Fmoc group removal on the resin-bound alanine **42** using piperidine to yield amine **43** and dibenzofulvene **44**. The carboxylic acid of the second amino acid, Fmoc-D-*allo*-Ile-OH (**45**), is reacted with 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium-3-oxide hexafluorophosphate (HATU) and diisopropylethylamine (DIPEA) to give the activated ester. This activated ester is then added to the free amine **44**, which results in the formation of an amide bond to give the dipeptide **46**. To obtain the full peptide **47**, the Fmoc deprotection and the couplings of the subsequent activated amino acid were repeated using the appropriate Fmoc amino acid in place of **45**.



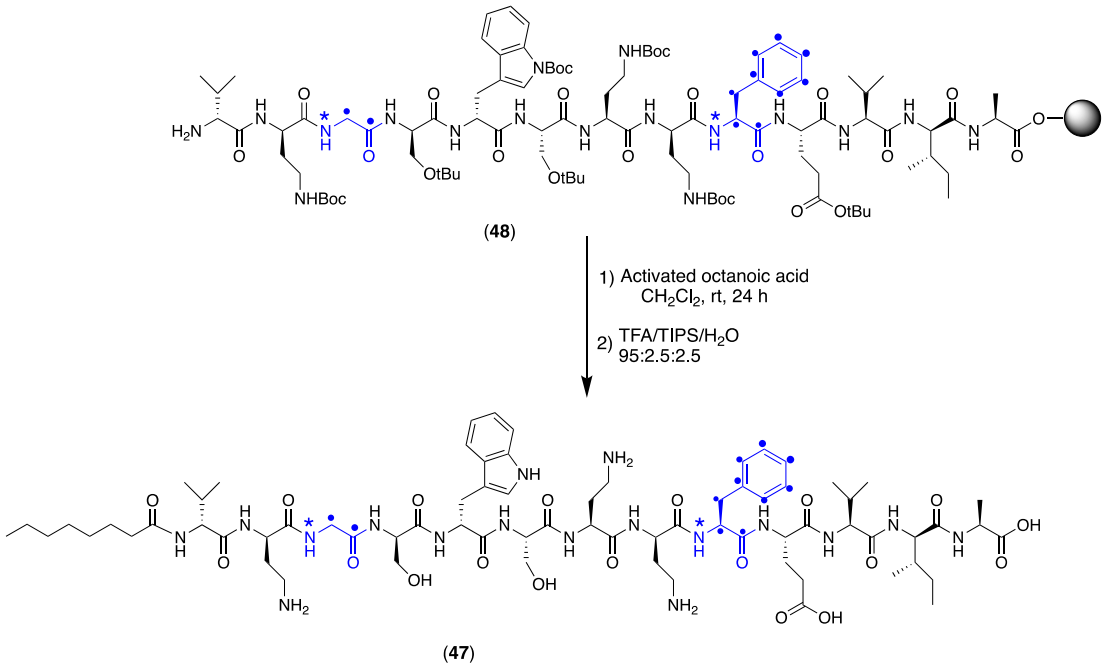
Scheme 2.1 Fmoc-Solid Phase Peptide Synthesis (SPPS) strategy for TriA$_1$ synthesis

The final step in the synthesis of **41** is N-terminal acylation with octanoic acid, which was accomplished by removing the final Fmoc group on the peptide chain to give the peptide with a free amine **48** and coupling to activated octanoic acid (Scheme 2.2).



Scheme 2.2 Fmoc deprotection of full peptide chain

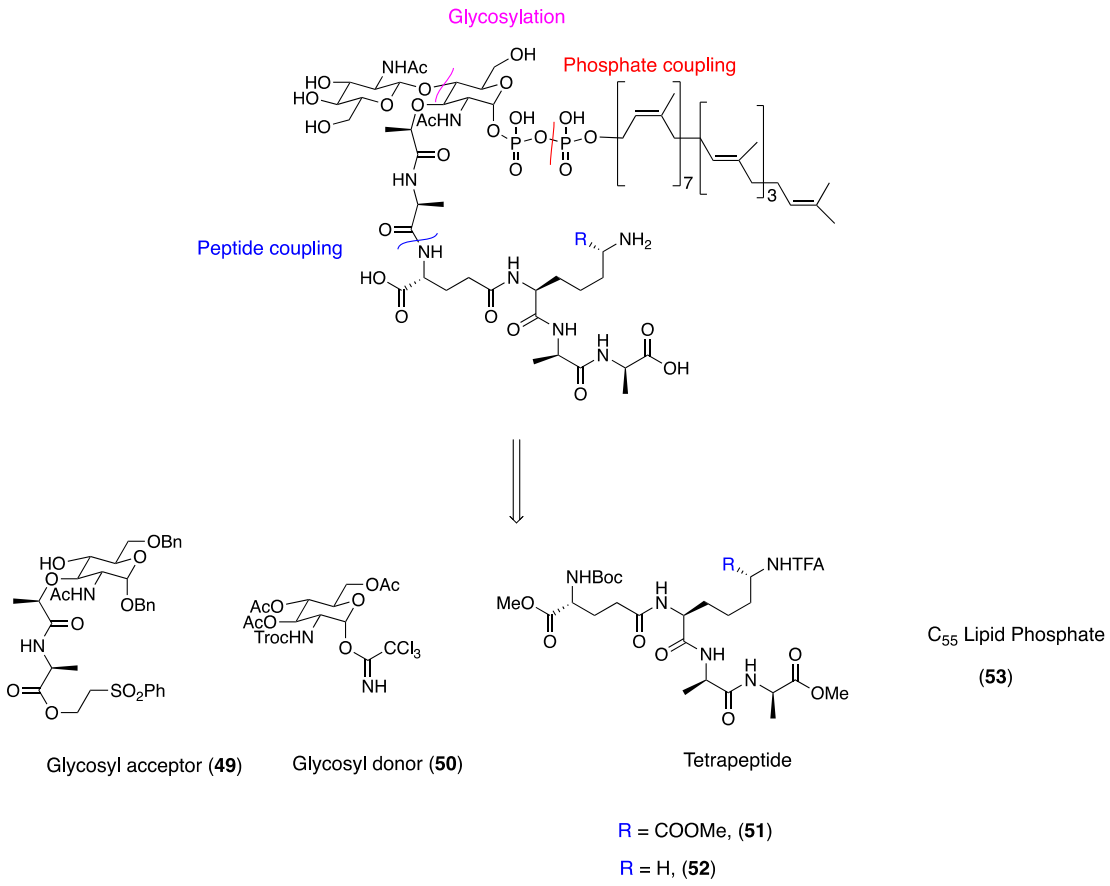
The resin-bound *N*-acylated peptide was then cleaved from the resin and purified by HPLC to give ^{13}C ^{15}N labelled Oct-TriA$_1$ (**47**) in 28% yield (Scheme 2.3).



Scheme 2.3 Acylation, cleavage from resin, and side chain deprotection

2.3.2. Synthesis of C_{55} Gram-Negative and Gram-Positive Lipid II

This study required native C_{55} *m*DAP-lipid II, so both the C_{55} *m*DAP-lipid II (**38**) and C_{55} Lys-lipid II (**39**) were synthesized by adapting procedures of Van Nieuwenze and coworkers.[68, 69] [49] The retrosynthetic approach is shown in (Scheme 2.4).



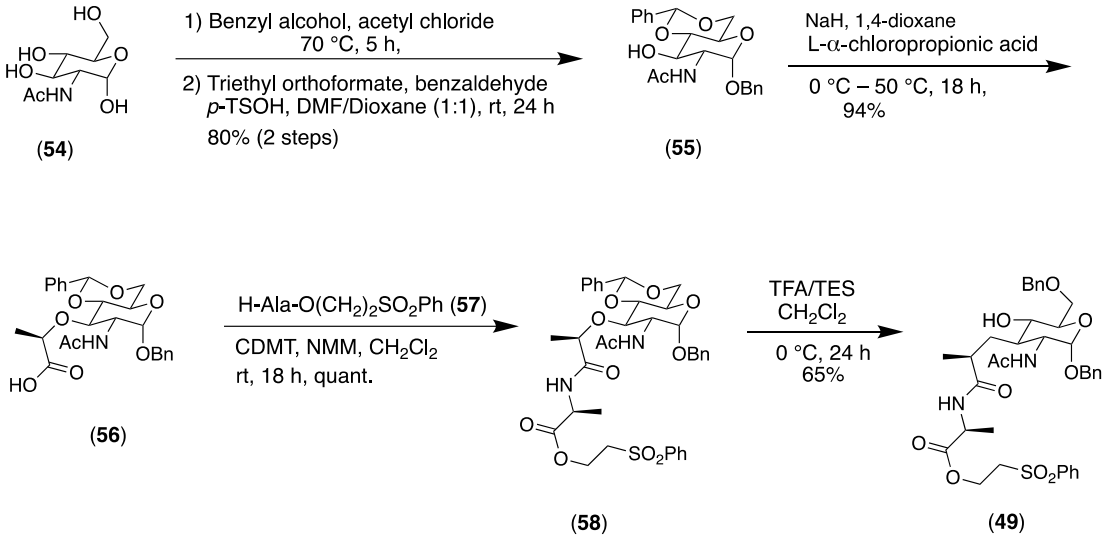
Scheme 2.4 Retrosynthetic analysis of C_{55} lipid II

The synthesis involves the reaction of the glycosyl acceptor **49** with the glycosyl donor **50** to give the desired disaccharide. Protecting group manipulation on the disaccharide was done to aid in coupling to the tetrapeptide **51** or **52**. The last step is adding the C_{55} lipid phosphate **53** followed by hydrolysis of the ester and TFA groups to give the final lipid II compound **38** or **39**.

2.3.3. Synthesis of Glycosyl Acceptor (49)

Glycosyl acceptor **49** was synthesized in five steps using a modified literature procedure[49] from *N*-acetylglucosamine (**54**) (Scheme 2.5). Treatment of **54** with benzyl alcohol and acetyl chloride and then reacting the resultant product with triethyl

orthoformate, benzaldehyde, and *p*-toluene sulfonic acid in DMF/dioxane gives **55** in good yield. Reaction of **55** with L-α-chloropropionic acid *via* deprotonation by NaH affords **56** in excellent yield. Coupling with **57** using 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) and N-methylmorpholine (NMM) as the activating reagents furnishes benzylidene **58** in excellent yield. TFA/TES was used to selectively remove the 4,6-OH benzylidene protecting group to give the free 4-OH product **49** in 65% yield.

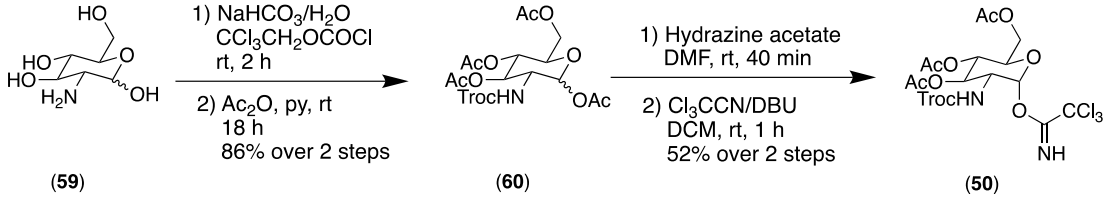


*Scheme 2.5 Synthesis of glycosyl acceptor (**49**)*

2.3.4. Synthesis of Glycosyl Donor (50)

The glycosyl donor **50** was synthesized in four steps using a modified literature procedure[49] from D-glucosamine (**59**) (Scheme 2.6). Treatment of **59** with $NaHCO_3$ followed by 2,2,2,-trichloroethoxycarbonyl chloride gave the protected amine. The hydroxyl groups were globally acetylated to yield intermediate **60** in excellent yield. The anomeric acetyl group was selectively removed using hydrazine acetate to give a free alcohol at the anomeric position, which was reacted with trichloroacetonitrile

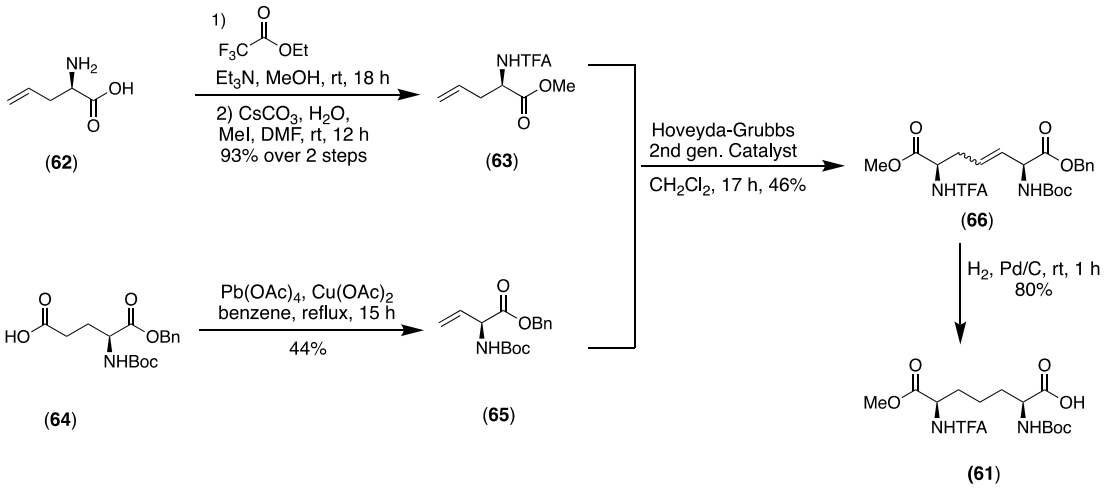
(Cl_3CCN) with diazabicycloundec-7-ene (DBU) as the base to give the glycosyl donor **50** in 53% yield over two steps.



*Scheme 2.6 Synthesis of glycosyl donor (**50**)*

2.3.5. Synthesis of *meso*-DAP (61)

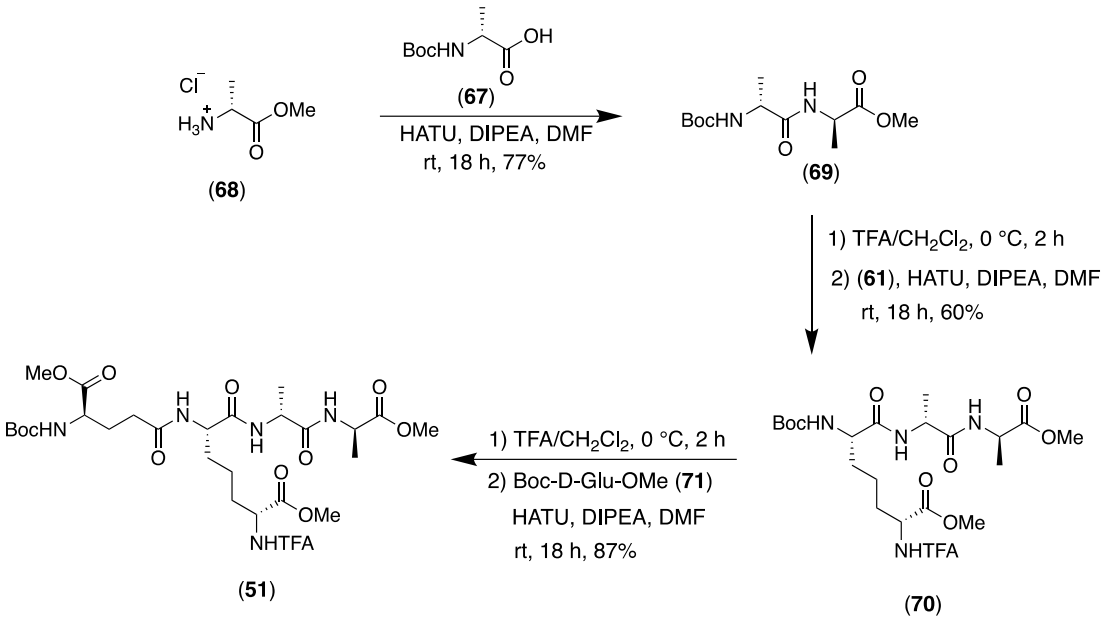
For Gram-negative lipid II, the tetrapeptide contains *meso*-DAP **61** which was prepared in five steps following literature procedures (Scheme 2.7).[49,70] D-Allylglycine (**62**) was treated with ethyl trifluoroacetate to give the protected amine in good yield. In a previously reported method[49], the esterification proceeded in 42% yield with the use of trimethylsilyl diazomethane. However, the use of 20% cesium carbonate and MeI resulted in a significant increase in the yield to give the alkene **62** in a 93% yield over two steps. *N*-Boc-L-glutamic acid α-benzyl ester **64** was treated with $Pb(OAc)_2$ and $Cu(OAc)_2$ in benzene under reflux to yield the dehydro decarboxylated product **65** in 44% yield. The alkenes **63** and **65** were treated with Hoveyda-Grubbs 2nd generation catalyst to give the benzyl ester **66** as a mixture of isomers in 46% yield, and the double bond was subsequently reduced with hydrogen and Pd catalyst to give **61** in 80% yield.



Scheme 2.7 Synthesis of orthogonally protected meso-*DAP* ***(61)***

2.3.6. Synthesis of Protected D-Glu-*meso*-DAP-D-Ala-D-Ala Tetrapeptide (51)

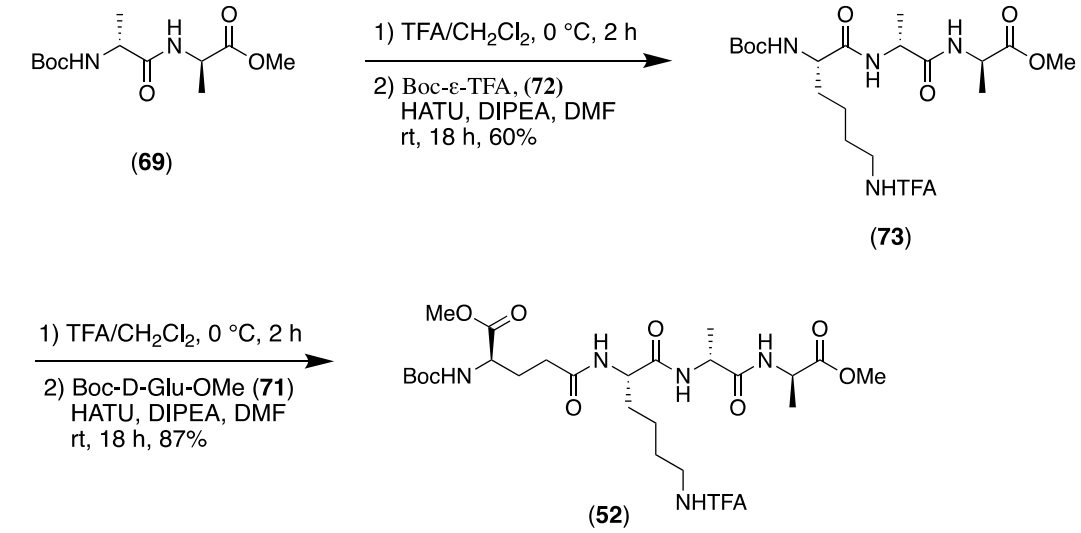
With the *meso*-DAP **(61)** available, the next step was to complete the synthesis of tetrapeptide **51**, which was achieved following a literature procedure[49] (Scheme 2.8). Boc-D-Ala-OH (**67**) was activated with HATU, and this solution was added to the amine **68** to yield the dipeptide **69** in 77% yield. The Boc protecting group was removed, and the amine compound was coupled to the activated compound **61** to give the tripeptide **70**, which was then coupled to Boc-D-Glu-OMe (**71**) to yield the tetrapeptide **51** in 87% yield. Deprotection of dipeptide **69** and tripeptide **70** was achieved using trifluoroacetic acid (TFA).



*Scheme 2.8 Synthesis of Protected D-Glu-*meso*-DAP-D-Ala-D-Ala (**51**)*

2.3.7. Synthesis of D-Glu-Lys-D-Ala-D-Ala (52)

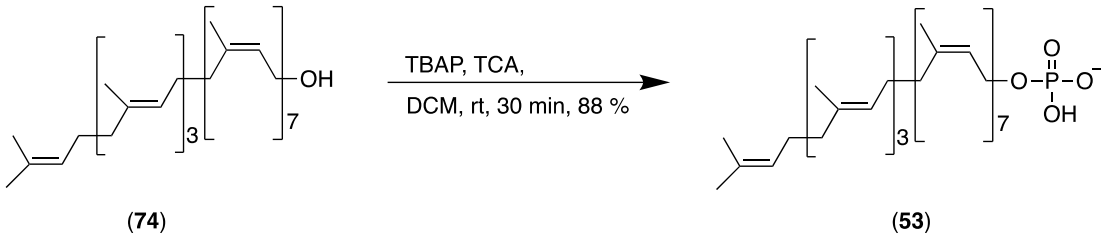
The synthesis of the tetrapeptide **52** (Scheme 2.9) was achieved following a literature procedure[49] (Scheme 2.8), except that Boc-ε-TFA-lysine (**72**) was used. Deprotection of dipeptide **69** and tripeptide **73** was achieved using TFA.



*Scheme 2.9 Synthesis of D-Glu-Lys-D-Ala-D-Ala (**52**)*

2.3.8. Synthesis of C_{55} Lipid Phosphate (**53**)

The synthesis of **53** was achieved using natural undecaprenol (**74**) extracted by Dr. Stephen Cochrane and coworkers[71] (Scheme 2.10). The undecaprenol (**74**) was treated with trichloroacetonitrile (TCA) and tetra-*n*-butyl ammonium dihydrogen phosphate (TBAP), and after ion exchange purification, yielded **53** in 56% yield.



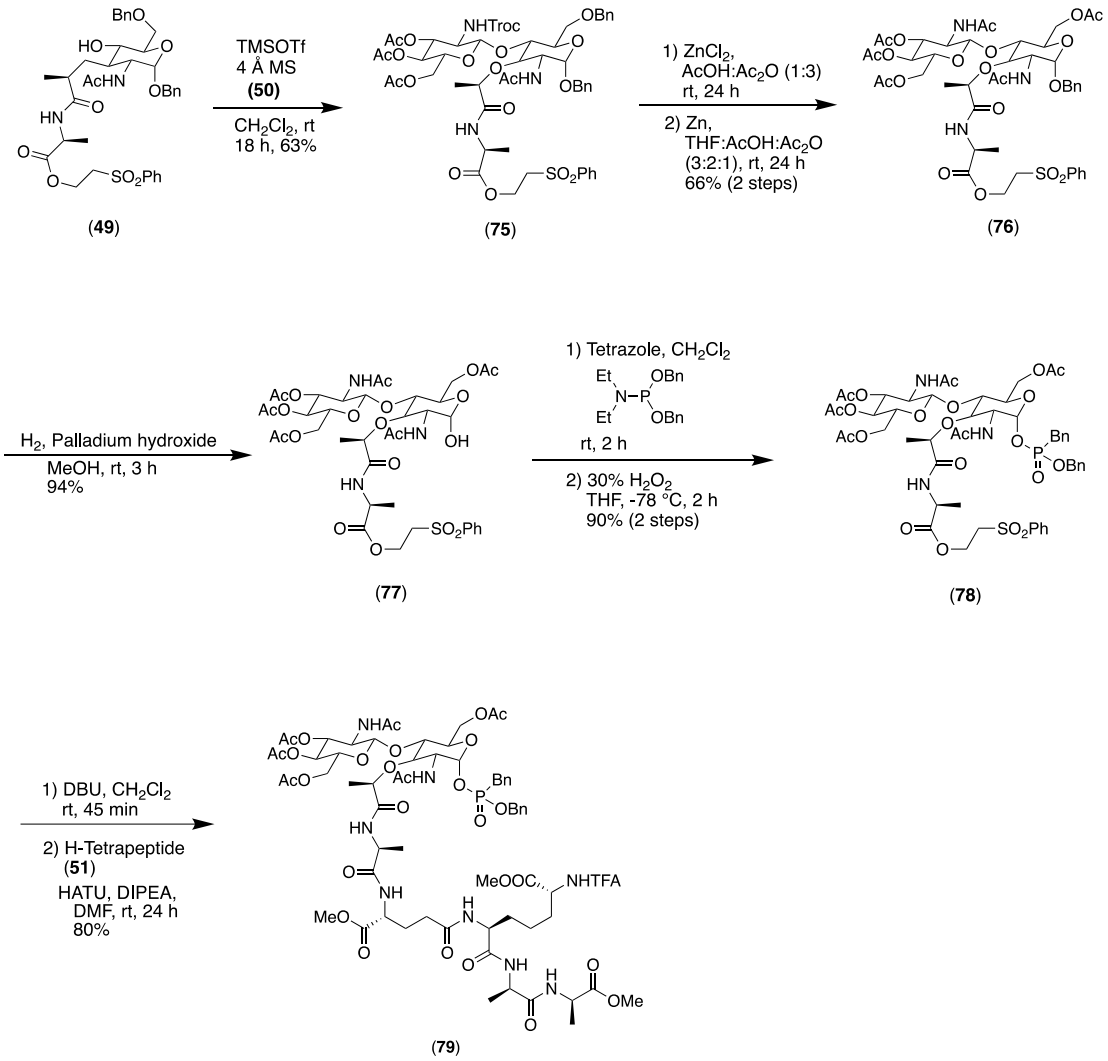
(**74**) (**53**)

*Scheme 2.10 Synthesis of Undecaprenol Phosphate (**53**)*

2.3.9. Synthesis of Gram-negative Lipid II

The synthesis of the Gram-negative lipid II **38** was executed following literature procedures[49,68,69] (Scheme 2.11). A mixture of the glycosyl acceptor **49** and the glycosyl donor **50** after treatment with trimethylsilyl triflate (TMSOTf) gave the disaccharide **75** in good yield. Deprotection of the benzyl protecting group at the 6-position on the glycosyl acceptor was achieved using zinc chloride to give the free 6-OH, which was protected using acetic anhydride and acetic acid. In the same reaction, the Troc protecting group on the amine of the glycosyl donor was removed using zinc powder, and the free amine was protected as an acetamide to give the disaccharide **76** in 66% yield over two steps. The anomeric benzyl group of **76** was removed using hydrogen over palladium hydroxide catalyst to give the free alcohol **77** in 94% yield. The alcohol was reacted with dibenzyl *N*,*N*-diethylphosphoramidite followed by phosphite oxidation using 30% H_2O_2, which gave the phosphate **78** in 90% yield over

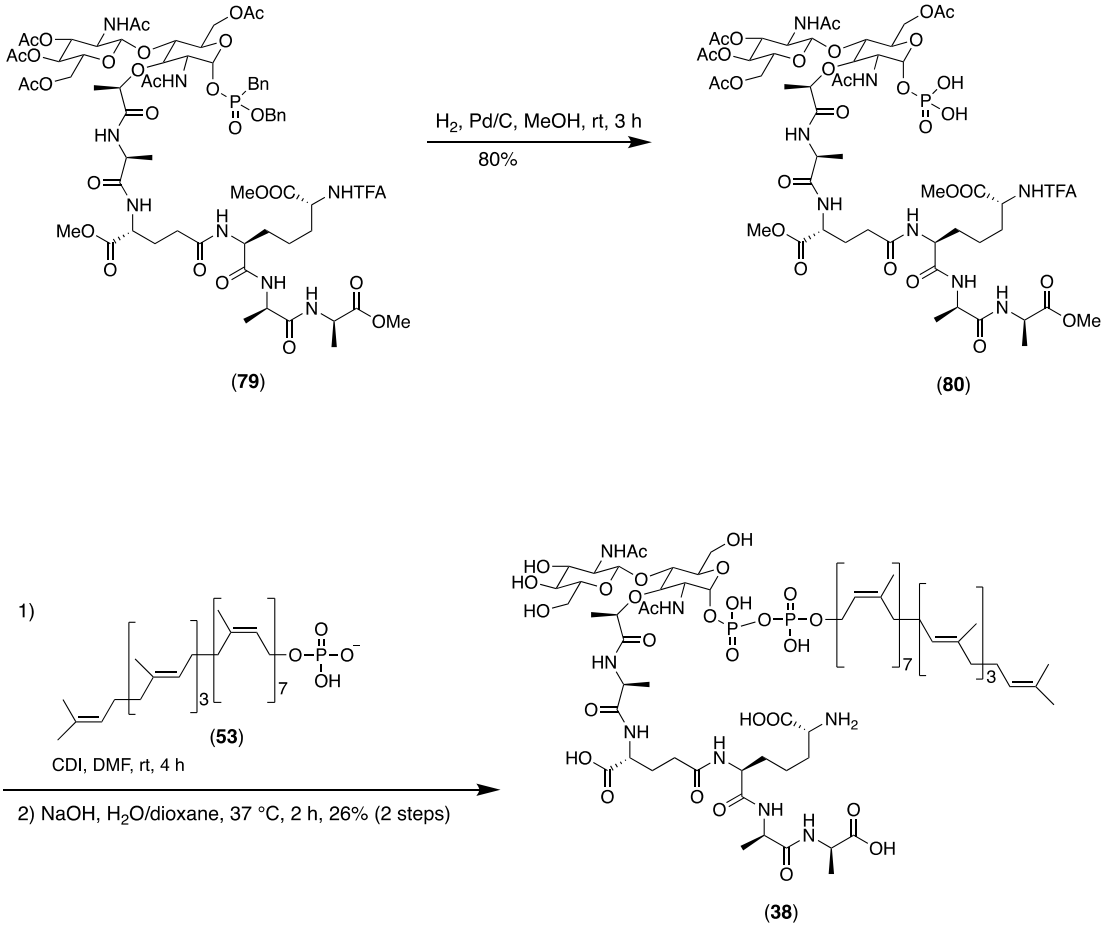
two steps. Deprotection of **78** using DBU gave the carboxylic acid, which was subsequently coupled to the Boc-deprotected tetrapeptide of **51** to give the pentapeptide disaccharide **79** in 80% yield over two steps.



*Scheme 2.11 Synthesis of disaccharide-pentapeptide moiety (**79**)*

In the final step, the dibenzyl groups on **79** were removed using hydrogen over palladium catalyst to give **80** in 80% yield (Scheme 2.12). The resulting phosphate was

activated using *N*,*N*'-carbonyldiimidazole (CDI), followed by coupling with the undecaprenol phosphate (**53**). The esters on the tetrapeptide, the amine TFA, and the OH-acetyl protecting groups were removed using NaOH to yield C_{55} *m*DAP-lipid II (**38**) in 26% over two steps.

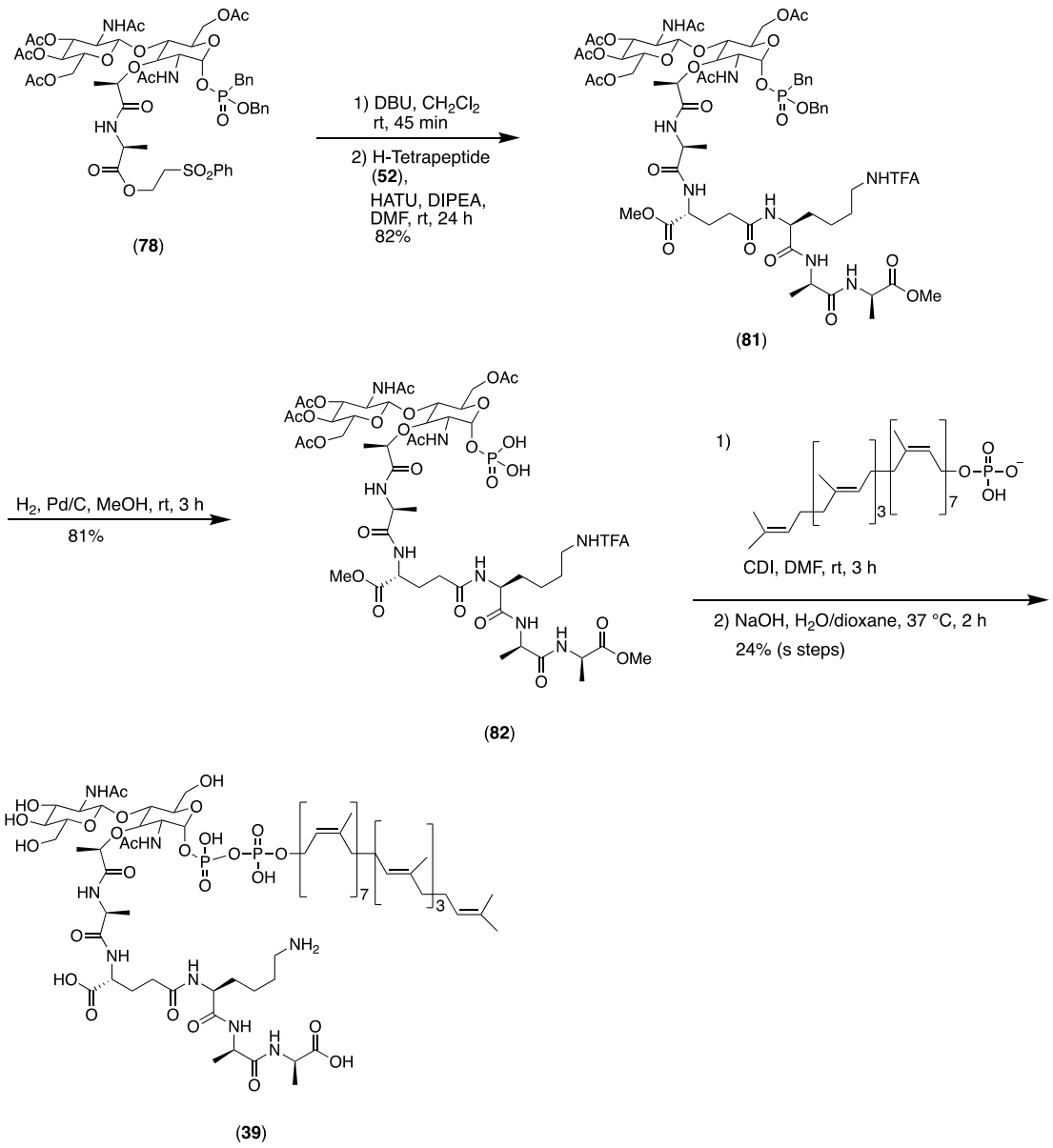


Scheme 2.12 Synthesis of C_{55} m*DAP-lipid II (**38**)*

2.3.10. Synthesis of Gram-Positive Lipid II

The synthesis of the C_{55} Lys-lipid II variant **39** was achieved using the same protocol as for the Gram-negative variant except that tetrapeptide **52** was used (Scheme 2.13).

The disaccharide phosphate **78** was synthesized following the same procedure as the Gram-negative lipid II. Deprotection of **78** using DBU gave the carboxylic acid, which was then coupled to the desired Boc-deprotected tetrapeptide **52** to give the pentapeptide disaccharide **81** in 82% yield over two steps. In the final step, the dibenzyl groups on **81** were removed using hydrogen over palladium catalyst to give **82** in 81% yield. The resulting phosphate was activated using CDI followed by coupling with the undecaprenol phosphate (**53**). The esters on the tetrapeptide, the amine TFA, and the OH-acetyl protecting groups were removed using NaOH to yield C_{55} Lys-lipid II (**39**) in 24% yield over two steps.

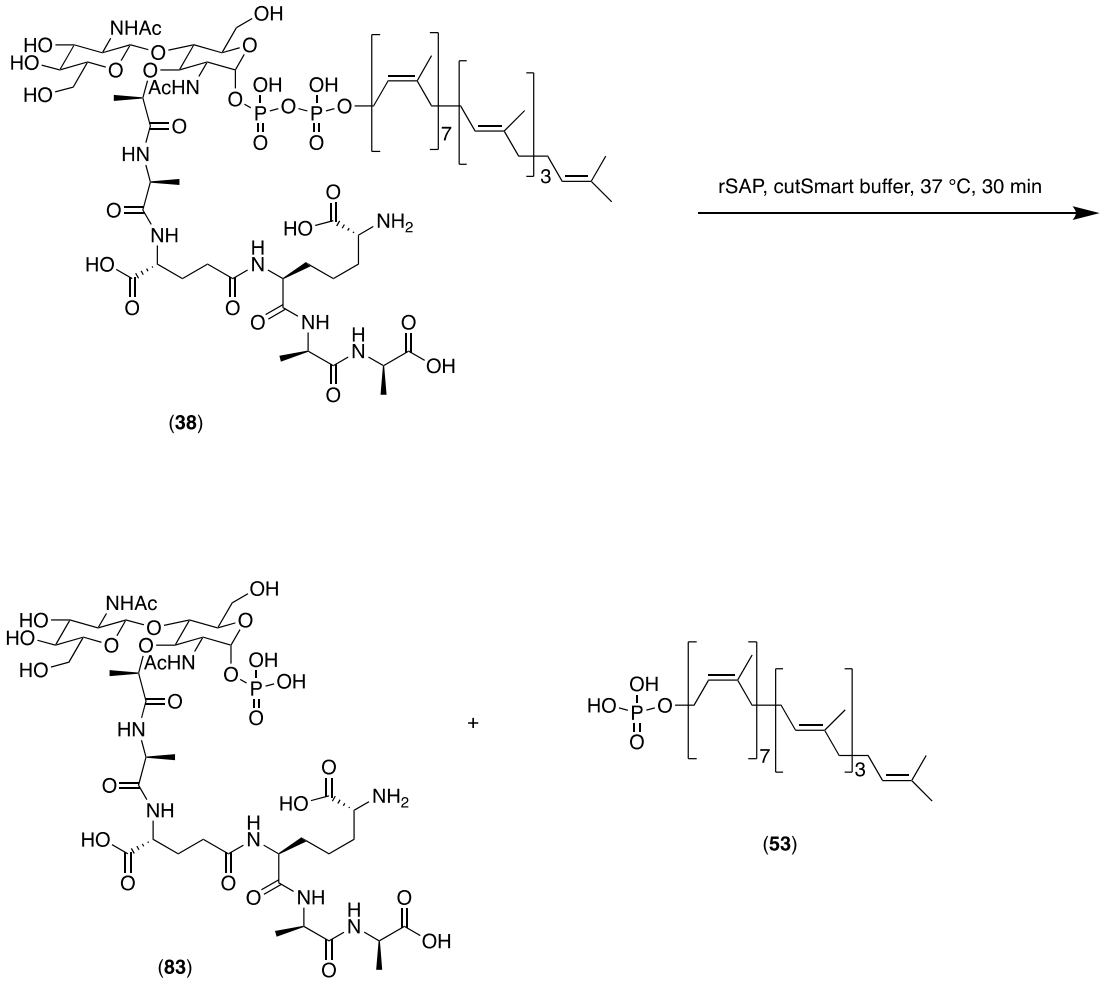


*Scheme 2.13 Synthesis of C_{55} Lys-lipid II (**39**)*

2.3.11. Shrimp Alkaline Phosphatase Cleavage of Lipid II Pyrophosphate

Although it was not possible to get solution ^{1}H-NMR data on the lipid II, probably due to aggregation, we did see the pyrophosphate in the ^{31}P NMR. Cleavage of the lipid II **38** by shrimp alkaline phosphatase enzyme rSAP (MO371) and cutSmart buffer gave the expected fragments **83** and **53** (Scheme 2.14). These were fully characterized by

solution-phase NMR and HRMS-ESI analysis, confirming that lipid II **38** was successfully synthesized.



*Scheme 2.14 Alkaline phosphatase hydrolysis of lipid II (**39**)*

2.4. Results and Discussion of Solid-State NMR Studies

2.4.1. Solid State NMR of Oct-TriA$_1$ (47) and C$_{55}$ *m*DAP-lipid II (38) in DOPC

These experiments were conducted by Dr. Markus Weingarth and coworkers at Utrecht University. 1,2-Dioleoyl-sn-glycerol-3-phosphocholine (DOPC) vesicles with 2% C_{55}

*m*DAP-lipid II were prepared, and ^{31}P solid state NMR measurements were recorded with and without selectively labelled Oct-TriA$_1$ at 285-305 K. There was no chemical shift changes observed in the ^{31}P solid state NMR spectra of *m*DAP-lipid II upon the addition of Oct-TriA$_1$. This suggests that the peptide does not bind to the pyrophosphate moiety of lipid II.This observation is consistent with previous studies in our group, which suggest that the portion of lipid II recognized by tridecaptin is the pentapeptide moiety.[49] Additionally, the preliminary 2D ^{13}C-^{13}C experiments conducted on the *m*DAP-lipid II and tridecaptin contained numerous peaks corresponding to the carbons of the ^{13}C labelled amino acid residues Gly3 and Phe9. This led us to postulate that the peptide binds to lipid II in many different conformations, due to the lack of individual, distinct peaks for Gly3 and Phe9 carbons.

2.5. Conclusion and Future Work

The synthesis of Oct-TriA$_1$ (**47**) (^{13}C, ^{15}N, Gly3 and Phe9) was successfully completed using standard solid-phase peptide synthesis in 25% overall yield. Both C_{55} *m*DAP-lipid II (**38**) and C_{55} Lys-lipid II (**39**) were successfully synthesized over a series of chemical steps in 26% and 24% yield, respectively. A series of multi-dimesional solid-state NMR experiments were conducted by our collaborators and a few conclusions can be drawn from this data: the bound state of tridecaptin does not adopt a well-defined conformation and tridecaptin does not interact with the pyrophosphate moiety of lipid II. Having determined that Oct-TriA$_1$ does not interact with the pyrophosphate of lipid II, we will next want to study the behaviour of this peptide to liposomes in the absence of lipid II to conclusively demonstrate that TriA1 does not bind to lipid II-free liposomes.

Chapter 3. Synthesis of Cycloalanopine and its Analogues as Potential Antimicrobial Agents

3.1. Introduction

Opines are natural products that result when an amino acid undergoes reductive condensation with the carbonyl of a sugar or α-keto acid.[72] These compounds have relevant biological roles in fungi, bacteria, higher plants, and mammals, including humans. These small metabolites were initially found by analyzing the products of an *Agrobacterium* strain.[73] The tumour inducing plasmid (Ti plasmid) of each unique *Agrobacterium* strain is distinguishable by the family of opines whose biosynthetic gene cluster it encodes.[74] There are over 20 opines known in *Agrobacterium*-induced plant tumours that can be classified into distinct families according to their precursors, encompassing amino acids, sugars, and keto acids.[75] Structurally, opines can also be classified into various groups. One subcategory has been categorized as the (*N'*-1-D-carboxyethyl) derivative of arginine (octopine) (**84**). A recent study by Stephen Winans and coworkers revealed that octopine synthase (OCS) does not only utilize L-arginine (**85**) for reductive condensation, but also other amino acids such as L-lysine, L-methionine, L-ornithine, and L-histidine[76-79] to yield the corresponding members of this family, namely lysopine (**86**), methiopine (**87**), octopinic acid, (**88**), and histopine (**89**), respectively (Figure 3.1*Figure 3.1 Structure of other family of opine produced by the utilization of octopine synthase*). The second subcategory has been characterized as the *N*'-(1,3-D-dicarboxypropyl) derivatives of L-arginine (nopaline) (**90**). Other amino acids such as L-leucine, L-asparagine, and L-ornithine are utilized by nopaline synthase (NOS) to yield the corresponding members of the family leucinopine (**91**),

succinamopine (**92**), and nopalinic acid (**93**), respectively (Figure 3.2).[80] Among these classes of compounds, octopine (**84**) and nopaline (**90**) are synthesized through a reversible process of reductive condensation of L-arginine with pyruvate (for octopine) or α-ketoglutarate (for nopaline) through the action of the NADH-dependant dehydrogenase (Scheme 3.1).[81, 82]

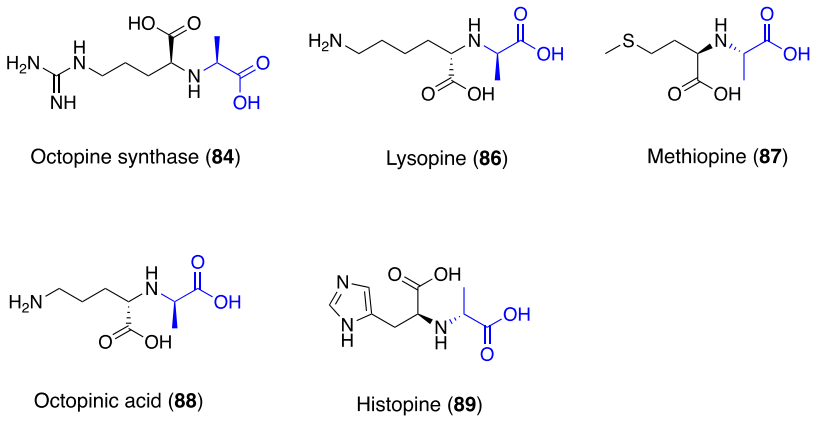


Figure 3.1 Structure of other family of opine produced by the utilization of octopine synthase

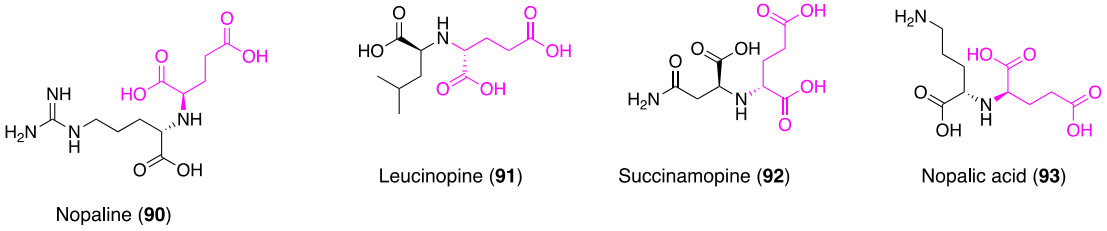
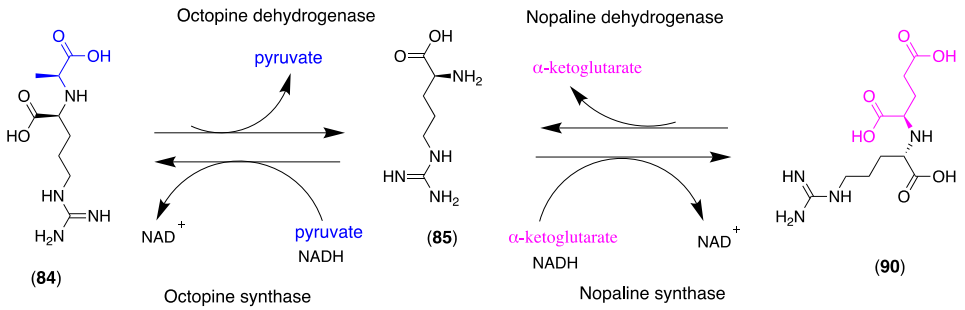
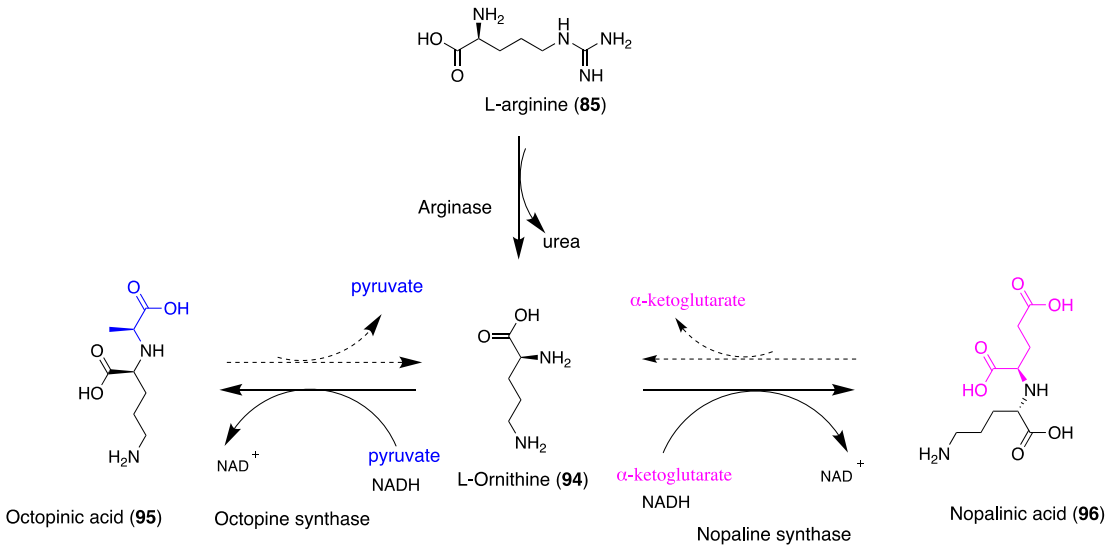


Figure 3.2 Structure of other opines produced by the utilization of nopaline synthase



*Scheme 3.1 Biosynthetic pathway for octopine (**84**) and nopaline (**90**) from L-arginine (**121**) using octopine synthase and nopaline synthase, respectively*

L-ornithine (**94**) as a starting amino acid for the formation of octopinic acid and nopalinic acid can be obtained through metabolism of L-arginine (**85**) by the arginase enzyme.[74] It has been shown that reductive condensation of L-ornithine (**94**) with pyruvate (for octopinic acid) (**95**), or α-ketoglutarate (for nopallinic acid) (**96**), can be achieved by octopine synthase and nopaline synthase, respectively (Scheme 3.2).[79]



*Scheme 3.2 Biosynthetic pathway for octopinic acid (**124**) and nopalinic acid (**125**) from L-arginine (**121**) using octopine synthase and nopaline synthase, respectively*

There has been an increase in research on opines, including a recent report of an opine operon in *Staphylococcus aureus*.[83] Our group was interested in the recent discovery of cycloalanopine (**97**) (Figure 3.3A), an antimicrobial opine, produced by *Lactobacillus rhamnosus*.[84] This compound was isolated from fermented milk in Xinjiang, China. It was purified and subsequently characterized by HRMS and NMR as 4,6-dimethyl-1,2,5-triazepane-3,7-dione. However, the authors did not report which isomer of cycloalanopine (**97**) was the active antimicrobial. Our interest in cycloalanopine's antimicrobial potential, due to its reported activity against both Gram-negative and Gram-positive bacteria, led us to explore synthetic approaches to make all the possible isomers of **97**. Structurally, cycloalanopine can be viewed as a cyclic bis-hydrazide of alanopine **98** (Figure 3.3B), which is produced by mollusks and squids in *meso* form through the reductive condensation of alanine and pyruvate.[85] Cycloalanopine (**97**) contains a unique 7-membered heterocyclic ring with an N-N bond that is relatively uncommon in nature. Biosynthetic formation of N-N bonds in other systems has been actively studied.[86-90] We decided to develop a synthetic route to prepare all possible isomers of cycloalanopine and test the antimicrobial activity of each. Key objectives were to determine which isomers is the active antimicrobial agent, to perform structure-activity studies, and to examine which organisms are sensitive to this compound.

Cycloalanopine (**97**) Alanopine (**98**)

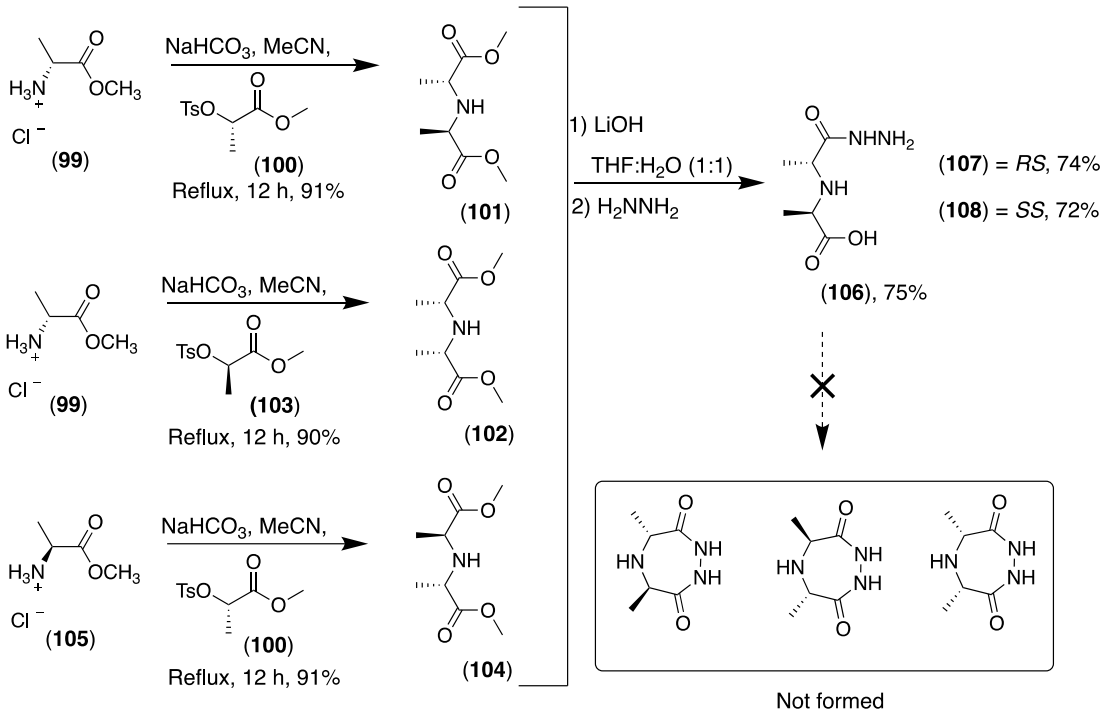
*Figure 3.3 A) Structure of Cycloalanopine (**97**), B) Structure of Alanopine (**98**)*

3.2. Results and Discussion

3.2.1. Initial Synthetic Trials for Cycloalanopine

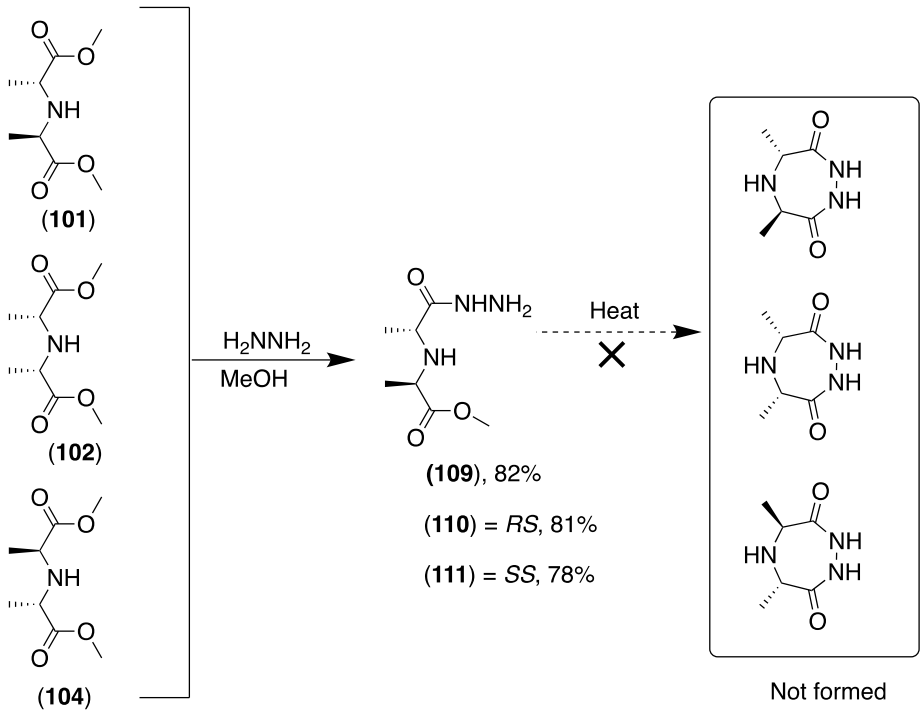
The synthesis of cycloalanopine initially appeared to be a matter of constructing the dimethyl ester isomers of alanopine **98**, followed by monohydrolysis and subsequent formation of the mono-hydrazide, which could then be cyclized onto the remaining carboxylic acid moiety (Scheme 3.3). Syntheses of the alanopine dimethyl esters were achieved using a modified literature procedure.[91] D-Alanine methyl ester hydrochloride (**99**) was reacted with $NaHCO_3$ and methyl (*S*)-2-(tosyloxy)propanoate (**100**) in MeCN to give the (*R,R*) dimethyl alanopine **101**. The formation of the (*R,S*) dimethyl alanopine isomer **102** required D-alanine methyl ester hydrochloride with methyl (*R*)-2-(tosyloxy) propanoate **103** whereas the (*S,S*) isomer **104** required L-alanine methyl ester hydrochloride (**105**) and **100**. The corresponding dimethyl alanopines **101**, **102**, and **104** were then treated with LiOH to selectively hydrolyse one ester to the corresponding monoester, followed by treatment with hydrazine monohydrate to give the corresponding mono-hydrazide **106**, **107**, and **108** in 75%, 74%, and 72% yield, respectively.[92] Surprisingly, the final cyclization step on the carboxylic acid proved

impossible, even with a large selection of conventional peptide coupling reagents such as HATU, N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole (HOBT), (CDI), (7-azabenzotriazol-1-yloxy)trispyrrolidinophosphonium hexafluorophosphate (PyAOP), N,N,N′,N′-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU), 3H-[1,2,3]-triazolo[4,5-b]pyridin-3-ol (HOAt). In all instances, the unreacted starting material was recovered after quenching with water.



*Scheme 3.3 First attempted synthetic route for the synthesis of pure isomers of cycloalanopine (**97**)*

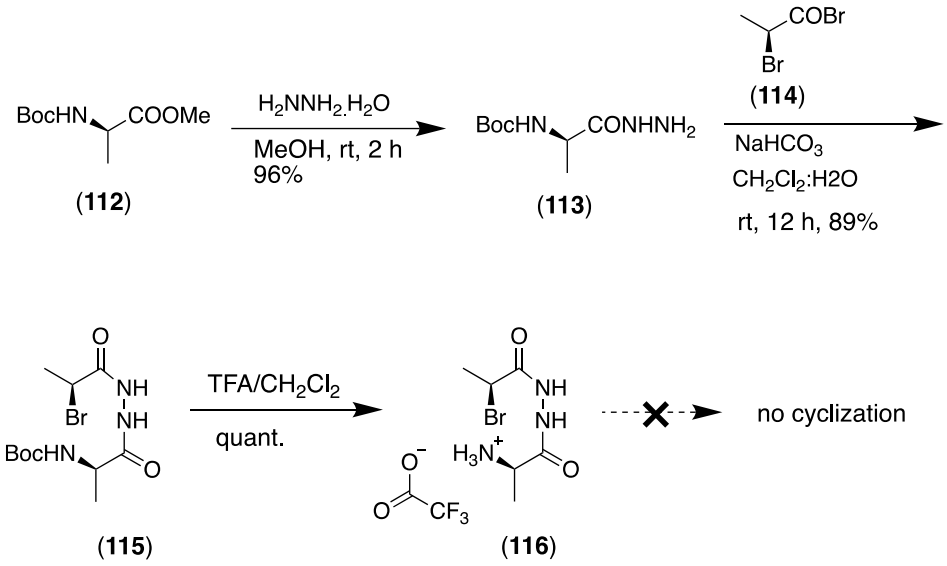
I also attempted to obtain the cyclized products by forming the mono-hydrazides **109**, **110**, and **111** with the expectation of intramolecular cyclization of the terminal nitrogen onto the carbonyl of the ester. However, the expected product was not achieved even over prolonged reaction time and the majority of the starting material was recovered with no trace of product (Scheme 3.4).



*Scheme 3.4 Second attempted synthetic pathway towards the synthesis of pure isomers of cycloalanopine (**97**)*

An alternative route for the synthesis of cycloalanopine was attempted (Scheme 3.5). The first step involved the reaction of Boc-D-alanine methyl ester (**112**) with hydrazine monohydrate to form **113** in 96% yield, which was subsequently reacted with (*S*)-2-bromopropionyl bromide **114** to give **115** in good yield. The Boc protecting group was subsequently deprotected using TFA:CH_2Cl_2 (1:1) to give (**116**). However

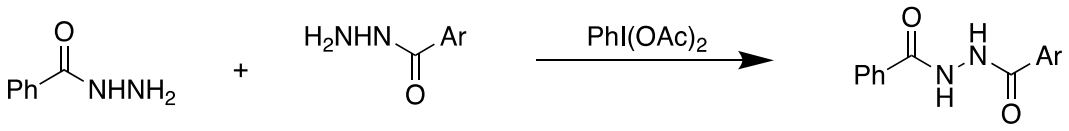
intramolecular cyclization of **116** by alkylation using $NaHCO_3$ as the base failed to occur (Scheme 3.5). Several other organic bases like triethylamine, pyridine, and imidazole were used in different solvent systems with varying durations and temperatures, but in all instances no trace of the cyclic product was observed and majority of starting materials was recovered. We proposed that these unexpected results may be the result of conformational preferences of diacyl hydrazide. In a previous study by our group on a 15-membered steroid ring system, crystallographic studies on a cyclic diacyl hydrazide within the compound indicate that the torsional angle about the N-N bond (i.e. C-N-N-C) prefers to have a dihedral angle approaching 90° if possible.[93] We believe this kind of effect may be hindering the cyclization to the seven-membered ring of cycloalanopine.



*Scheme 3.5 Third attempted synthetic route for the synthesis of pure isomers of cycloalanopine (**97**)*

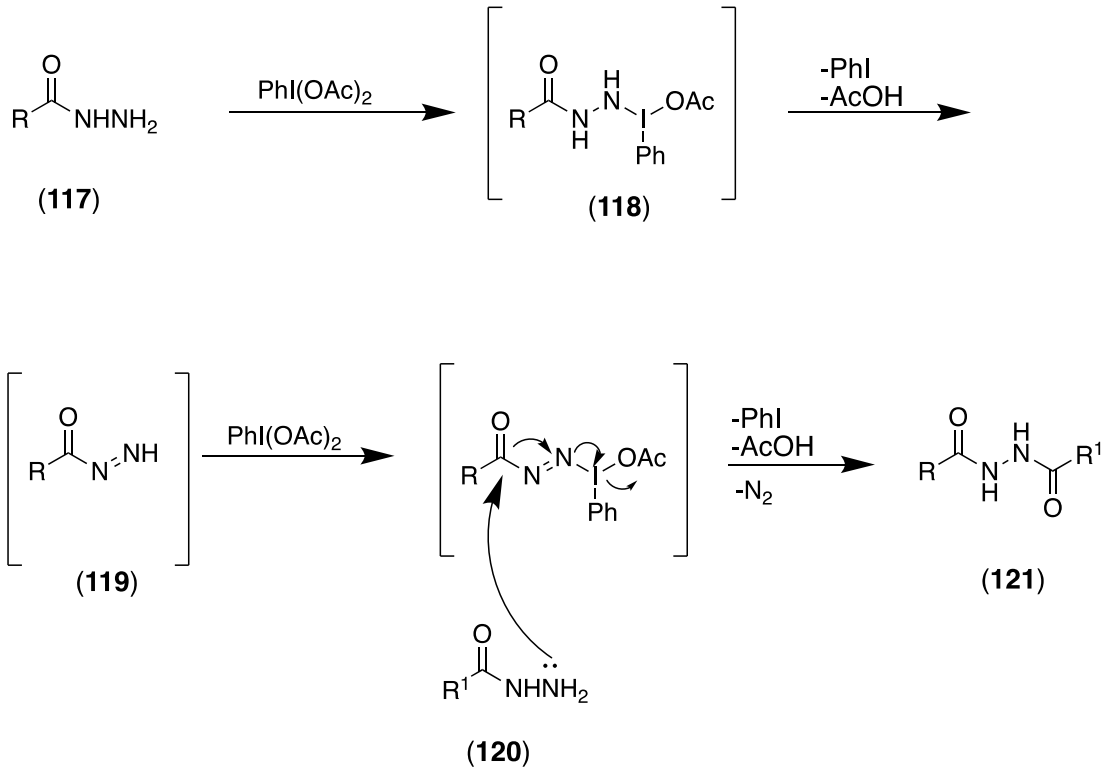
3.2.2. Synthesis of Cycloalanopines using Hypervalent Iodine Reagent

The unsuccessful attempts in synthesizing cycloalanopine spurred us to explore other possible synthetic routes and reagents that can facilitate this unfavourable cyclization. Hypervalent iodine reagents have been widely used to affect many chemical reactions and are well studied in literature.[94-96] Sadana and co-workers showed that two hydrazides having an aromatic acyl group can be coupled intermolecularly to the corresponding diacyl hydrazides using phenyl iododiacetate with the loss of nitrogen gas (Scheme 3.6).[98]



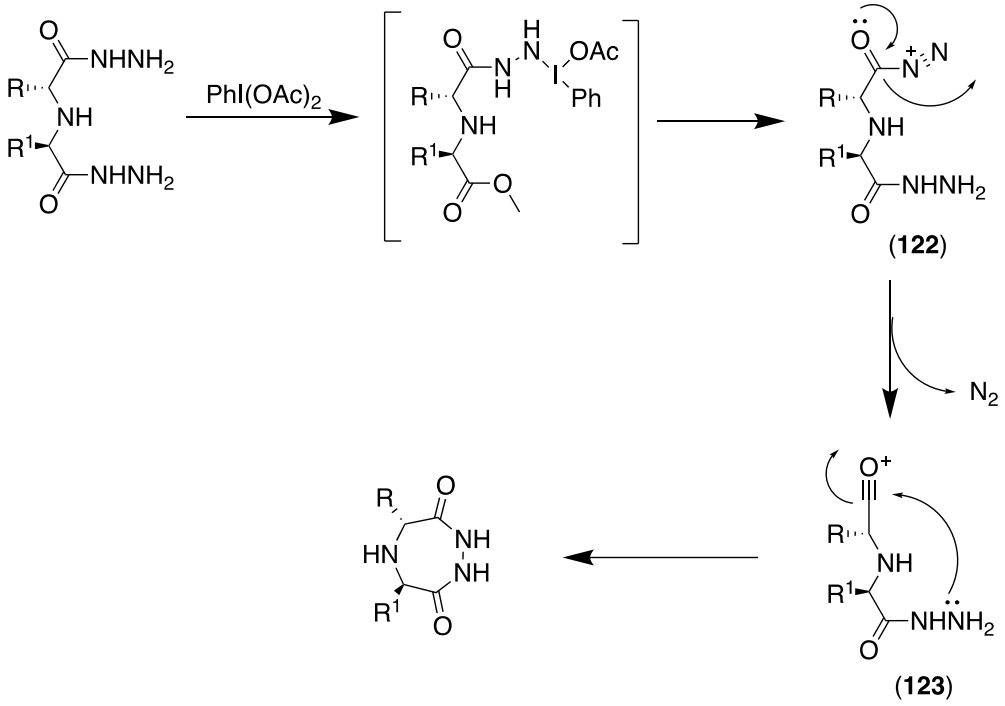
Scheme 3.6 Formation of diacyl hydrazides using $PhI(OAc)_2$

The authors proposed a mechanism involving a nucleophilic attack on $PhI(OAc)_2$ by the lone pair of the terminal nitrogen of **117** to give intermediate **118**, which then undergoes loss of iodobenzene along with the release of a molecule of acetic acid to generate acyldiimide **119**. Compound **119** may then undergo oxidation with a second molecule of $PhI(OAc)_2$ followed by nucleophilic attack of the second hydrazide **120** at the carbonyl, resulting in the formation of product **121** (Scheme 3.6).[98]



Scheme 3.7 Proposed mechanism for the formation of diacyl hydrazine using $PhI(OAc)_2$

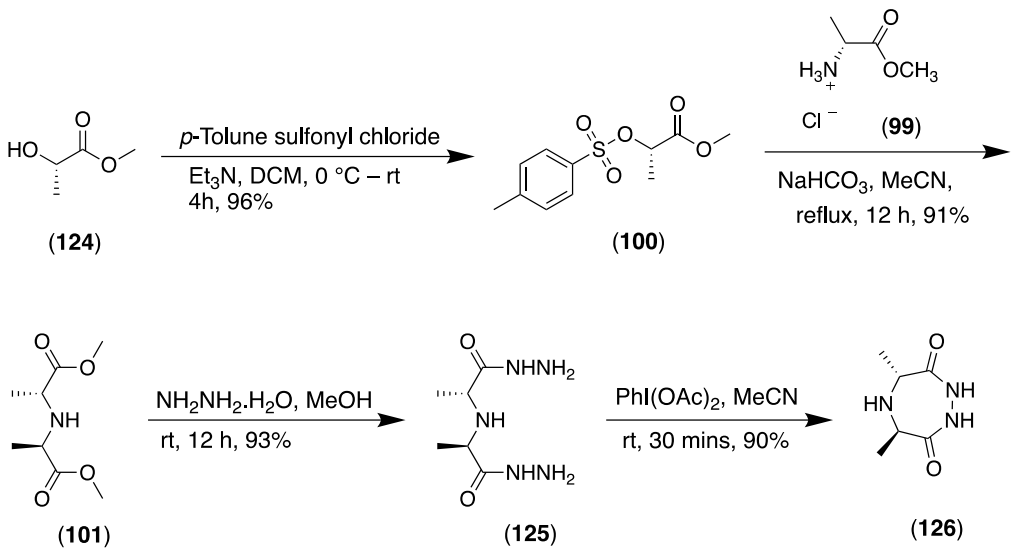
I decided to attempt this strategy in the preparation of all the isomers of cycloalanopine for our studies. The success of this reaction may be attributed to one of the acyl hydrazides generating a highly reactive acylating moiety *in situ*, perhaps an acyl diazonium species **122**, or even an acylium ion **123** (Scheme 3.8).



*Scheme 3.8 Proposed mechanism for the formation of cycloalanopine (**97**)*

3.2.2.1. Synthesis of (*R*, *R*)-Cycloalanopine (126)

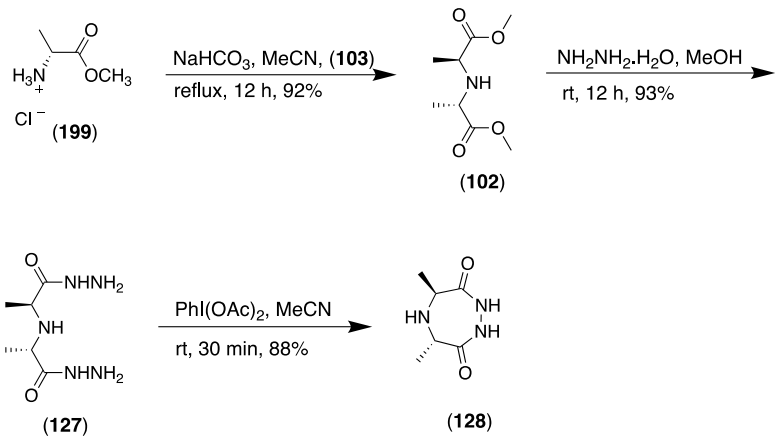
The synthesis of **126** was achieved with some modifications (Scheme 3.9).[91, 92, 98] Commercially available (*S*)-lactate methyl ester (**124**) was treated with *p*-toluenesulfonyl chloride and triethylamine to form the tosylated compound **100** in excellent yield. This was then reacted with D-alanine methyl ester hydrochloride (**99**) in the presence of $NaHCO_3$ to give (*R,R*)-alanopine dimethyl ester **101** in 91% yield. Treatment of **101** with hydrazine monohydrate in MeOH gave the bis-hydrazide compound **125**, which upon treatment with phenyl iododiacetate provided cycloalanopine **126** in 90% yield.



*Scheme 3.9 Synthesis of (4R,6R)-4,6-dimethyl-1,2,5-triazepane-3,7-dione (**126**)*

3.2.2.2. Synthesis of (*R*, *S*)-4,6-Cycloalanopine (128)

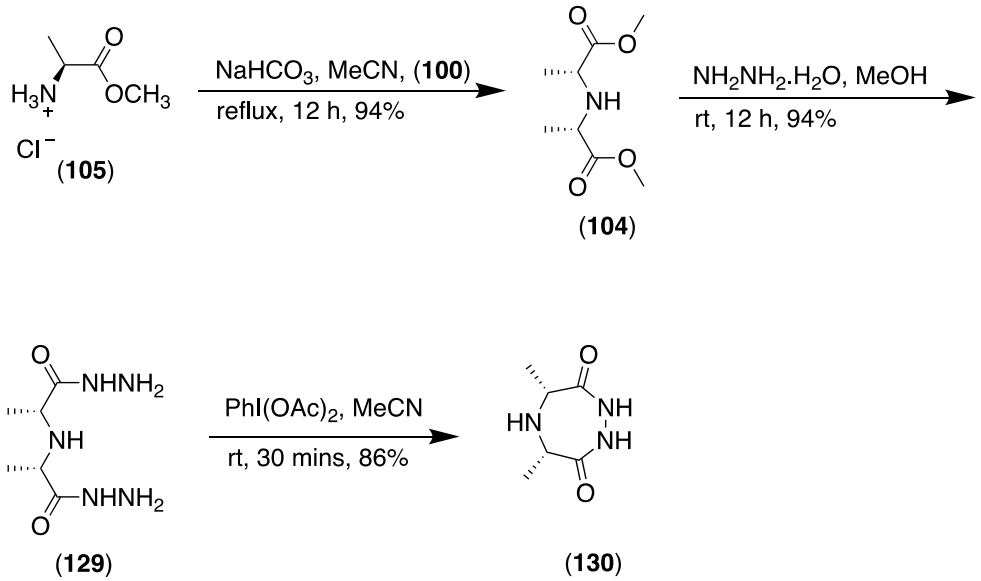
The tosylated compound **103** was reacted with D-alanine methyl ester hydrochloride (**99**) in the presence of $NaHCO_3$ to give the alanopine dimethyl ester **102** in 92% yield. Treatment of **102** with hydrazine monohydrate in MeOH gave the bis-hydrazide **127** in 93% yield, and upon treatment with phenyliododiacetate gave the final cycloalanopine **128** in 88% yield (Scheme 3.10).



*Scheme 3.10 Synthesis of (4R,6S)-4,6-dimethyl-1,2,5-triazepane-3,7-dione (**128**)*

3.2.2.3. Synthesis of (*S*, *S*)-4,6-Cycloalanopine (130)

The tosylated compound **100** was reacted with L-alanine methyl ester hydrochloride (**105**) in the presence of $NaHCO_3$ to give the alanopine dimethyl ester **104** in 94% yield. Treatment of **104** with hydrazine monohydrate in MeOH gave the bis-hydrazide **129** in 94% yield and upon treatment with phenyliododiacetate gave the cycloalanopine (**130**) in 86% yield (Scheme 3.11).



*Scheme 3.11 Preparation of (4S,6S)-4,6-dimethyl-1,2,5-triazepane-3,7-dione (**130**)*

3.2.3. Activity Assays of Cycloalanopine Isomers

These experiments were done by Dr. Sorina Chiorean and Dr. Marco J. van Belkum of our group. They performed spot-on-lawn assays to determine the antibacterial activity of the individual isomers of cycloalanopine **126**, **128**, and **130** against several Gram-negative and Gram-positive bacterial strains. These tests revealed that the *meso* compound **130** was the only active isomer, with the strongest potency against *E. coli* (3.2 mg mL^{-1}) (Table 3.2). However, the inhibitory activity of **130** against other strains of bacteria was much lower. The other two isomers, **126** and **128**, showed no antimicrobial activity with these organisms at the highest tested concentration of 250 mg mL^{-1}. The results of the modest minimum inhibitory concentration of **130** indicated that this compound is not potent enough for use as an antimicrobial. Based on the results of the antimicrobial assays, we became interested in whether the replacement of a D-alanine moiety in these compounds could potentially improve antimicrobial activity.

Table 3.1 Minimum inhibitory concentrations (MIC) obtained by spot-on-lawn assays (triplicate) reported in mg mL^{-1} for active isomer (**130**)

Organism	**Strain**	(**130**)
Salmonella typhimurium	ATCC 13311	26
Escherichia coli	ATCC 25922	3.2
Pseudomonas aeruginosa	ATCC 14207	52

Enterococcus faecalis	ATCC 7080	26
Listeria monocytogenes	ATCC 15313	26
Staphylococcus aureus	ATCC 25923	26

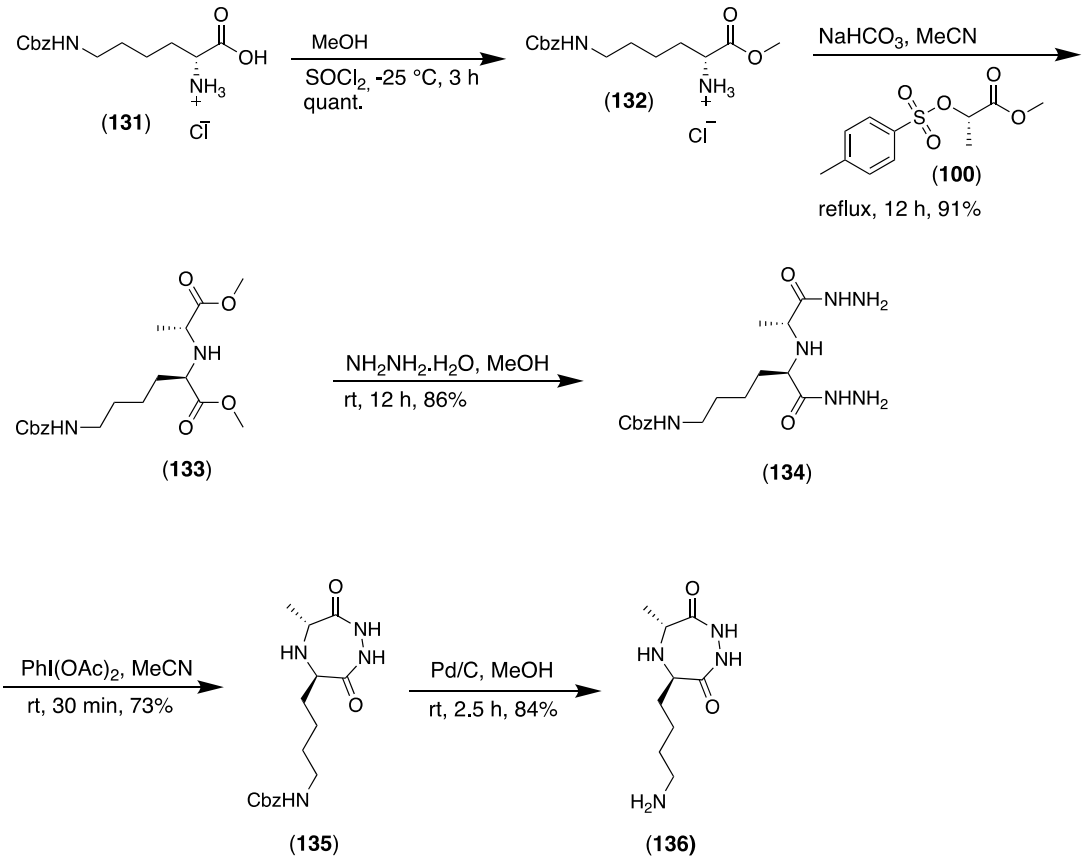
3.2.4. Investigating Analogues of Cycloalanopine

I embarked on the synthesis of analogues of **130** in hopes of increasing antimicrobial activity. Of particular interest was to determine whether substituents on the cycloalanopine could be extended to larger groups and still maintain or improve antibacterial activity. It is well known that many antimicrobial peptides that target negatively charged bacterial cell membranes are cationic[99-101], so we selected lysine as one of the substituents for this investigation. We also wanted to clarify the importance of the substituents on the cycloalanopine, so two analogues were also synthesized using glycine methyl ester in place of the D-alanine methyl ester to eventually have only one methyl substituent on the ring with well-defined stereochemistry. These four analogues could give us a better understanding of the structure-activity relationship of cycloalanopine's antimicrobial activity.

3.2.4.1. Synthesis of (4*S*,6*R*)-4-(4-aminobutyl)-6-methyl-1,2,5-triazepane-3,7-dione (136)

This compound was synthesized in four chemical steps from commercially available *N*-Cbz-D-Lys-OH (**131**) (Scheme 3.12). Esterification of **131** followed by reaction with methyl (*S*)-2-(tosyloxy)propanoate (**100)** yields (**133)** in 90% yield. Treatment of **133** with hydrazine monohydrate in MeOH gave the bis-hydrazide **134** in good yield,

which, upon treatment with phenyl iododiacetate, gave the cyclic compound **135** in 73% yield. The final step was to remove the Cbz protecting group, which was achieved using hydrogen over a palladium catalyst, to give **166** in 84% yield.

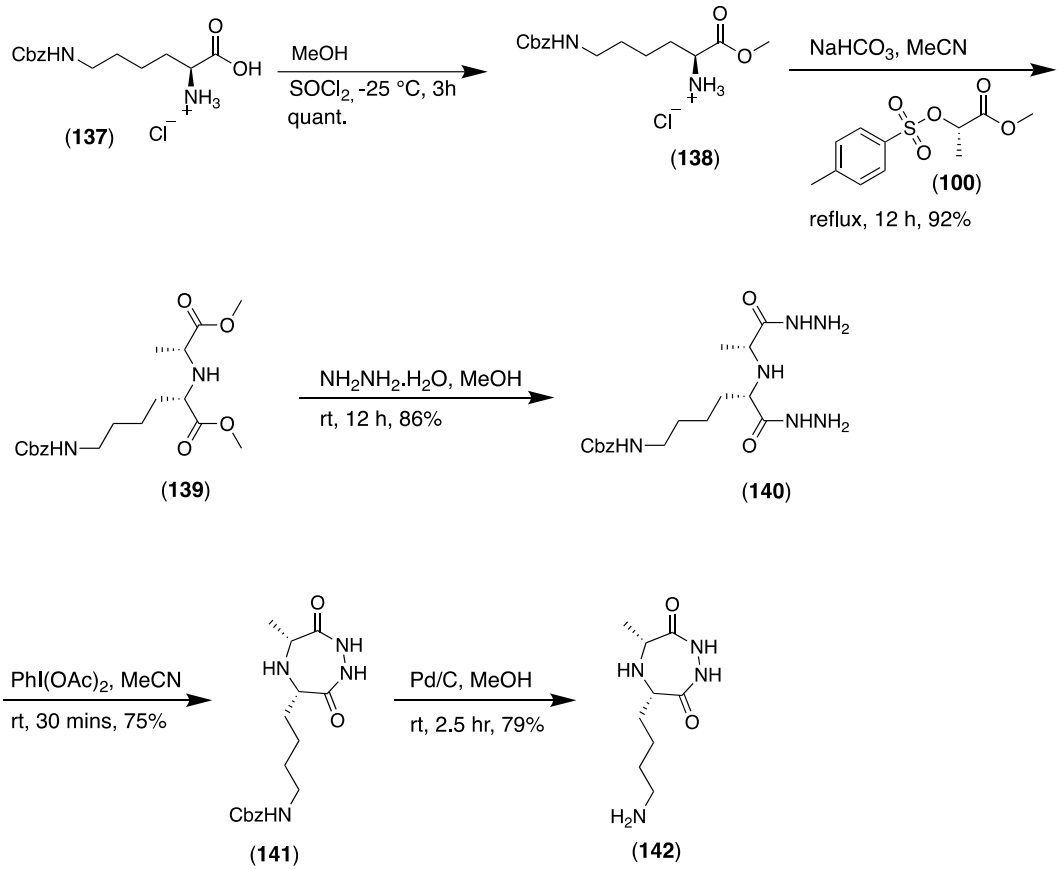


*Scheme 3.12 Synthesis of (**136**)*

3.2.4.2. Synthesis of (*R*,*R*)-Lysine Analogue (142)

This compound was synthesized in four chemical steps from commercially available *N*-Cbz-L-Lys-OH (**137**) (Scheme 3.13). Esterification followed by reaction with (**100**) yields **139** in 92% yield. Treatment of **139** with hydrazine monohydrate in MeOH gave the bis-hydrazide compound **140** in good yield which, upon treatment with phenyliododiacetate, gave the cyclic compound **141** in 75% yield. The final step was

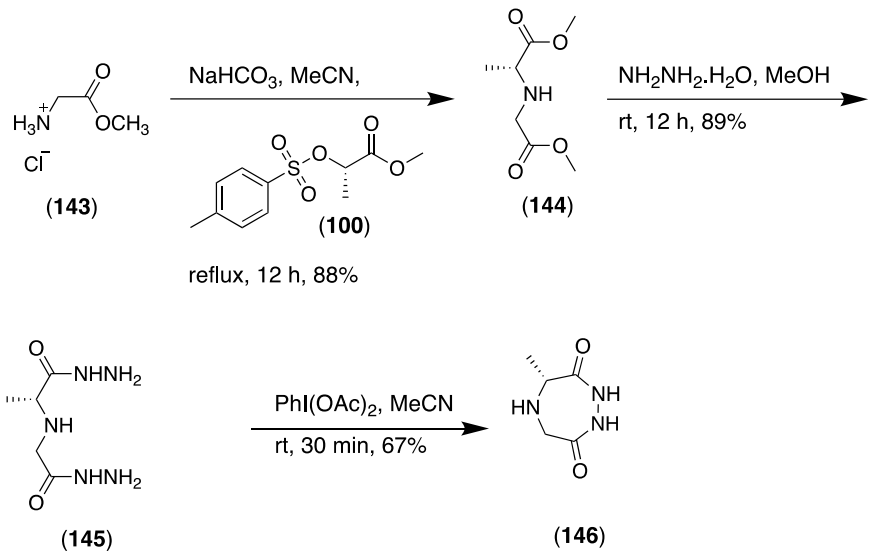
to remove the Cbz protecting group, which was achieved using hydrogen over a palladium catalyst to give **142** in 79% yield.



*Scheme 3.13 Synthesis of (4R,6R)-4-(4-aminobutyl)-6-methyl-1,2,5-triazepane-3,7-dione (**142**)*

3.2.4.3. Synthesis of (*R*)-Glycine Analogue (146)

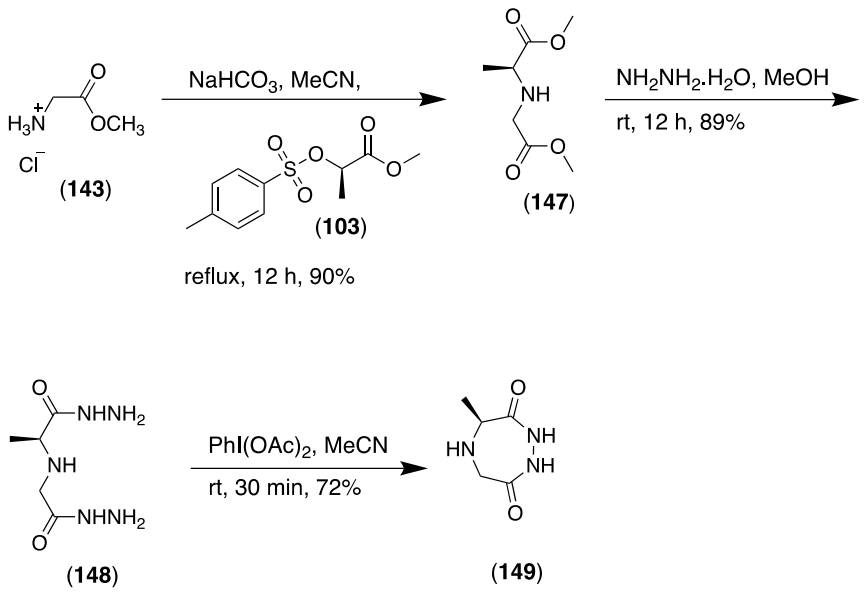
The synthesis of this compound was accomplished in three steps from commercially available glycine methyl ester **143** (Scheme 3.14). Reaction of **143** with **100** gives the dimethyl ester product **144** in 85% yield. This step was followed by treatment with hydrazine monohydrate to give the bis-hydrazide **145** in good yields. The final step is the reaction of **145** with $PhI(OAc)_2$ to give the cyclic product **146** in 89% yield.



*Scheme 3.14 Synthesis of (R)-4-methyl-1,2,5-triazepane-3,7-dione (**146**)*

3.2.4.4. Synthesis of (*S*)-Glycine Analogue (**149**)

The synthesis of this compound was accomplished in three steps from commercially available glycine methyl ester **143** (Scheme 3.15). Reaction of **143** with compound **103** gives the dimethyl ester product **147** in 87% yield. This is followed by treatment with hydrazine monohydrate to give the bis-hydrazide **148** in excellent yields. The final step is the reaction of **148** with $PhI(OAc)_2$ to give the cyclic product **149** in 85% yield.



*Scheme 3.15 Synthesis of (S)-4-methyl-1,2,5-triazepane-3,7-dione (**179**)*

3.2.5. Activity Assays for Lysine and Glycine analogues

Spot-on-lawn assays experiments of compound **136**, **142**, **146** and **149** were performed by Dr. Sorina Chiorean (Table 3.2). These tests revealed that compound **142**, with L-lysine moiety was active against most organisms, with the strongest potency against *Escherichia coli* and *Acinetobacter baumannii* (490 µg mL^{-1} and 980 µg mL^{-1}, respectively) and least activity against *Pseudomonas aeruginosa* and *Enterococcus faecalis* (62 mg mL^{-1}). Compound **142** appeared somewhat more potent against certain organisms than the original compound **130**, which we predict is the naturally occurring material produced by *Lactobacillus rhamnosus LS8*. However, we were unable to obtain this strain and there were no comparative studies of **130** to natural material. Surprisingly, compound **136**, which has the L-lysine replaced with D-lysine, showed a decrease in activity against Gram-negative organisms, although this compound also has some weak activity. Due to solubility limitations of **136** and **142**, these compounds

were dissolved in dimethyl sulfoxide rather than water for activity testing. For glycine analogues, the *S*-isomer **149** was somewhat active with a stronger potency against Gram-negative organisms and little potency towards Gram-positive organisms. Compound **146** was the most active analogue, with the strongest potency against *E. coli* and *A. baumannii* (60 μg mL^{-1} and 120 μg mL^{-1}, respectively). It was the most active compound for both Gram-negative and Gram-positive organisms. From the results obtained, the activity of **146** appears to be stronger than the parent *meso* cycloalanopine **130**.

Table 3.2 Minimum inhibitory concentrations (MIC) obtained by spot-on-lawn (triplicate) reported in mg mL^{-1} for active isomer **130** and its analogues

Organism	**Strain**	**130**	**136**	**142**	**146**	**149**
Salmonella typhimurium	ATCC 13311	26	62	3.9	0.98	19
Escherichia coli	ATCC 25922	3.2	31	0.49	0.06	2.3
Acinetobacter baumannii	ATCC 19606	not available	125	62	0.12	19
Pseudomonas aeruginosa	ATCC 14207	52	16	62	3.9	38
Enterococcus faecalis	ATCC 7080	26	16	62	2.0	>250

Listeria monocytogenes	ATCC 15313	26	16	7.8	2.0	38
Staphylococcus aureus	ATCC 25923	26	62	31	2.0	75

3.3. Conclusion and Future Work

In this project, I was able to synthesize all possible isomers of a previously reported cycloalanopine, and through antimicrobial activity testing, we found that only the *meso* isomer (130) was active against certain Gram-negative and Gram-positive bacteria, with the strongest potency being against *E. coli* (3.2 mg mL^{-1}). I synthesized other analogues of cycloalanopine, with structural modifications which extended or shortened the methyl moiety, and we found that the glycine analogue (146) was the most active compound for both Gram-negative and Gram-positive organisms, with the greatest potency against *E. coli* and *A. baumannii* (60 μg mL^{-1} and 120 μg mL^{-1} respectively). As the class of opine molecules continues to expand, $PhI(OAc)_2$ may be used as a reagent for cyclization and may allow for synthetic access to conformationally locked derivatives. This may lead to better understanding of these compounds' native functions and for the synthesis of analogues with practical applications**.**

Chapter 4. Synthesis of Dodecylphosphocholine Analogues for Solution NMR Studies of Membrane-Interacting Peptides

4.1. Introduction to Dodecylphosphocholine

Per-deuterated dodecylphosphocholine (DPC-d_{38}) micelles have been used for solution NMR studies of various peptides and proteins since the pioneering work of Lauterwein and co-workers in 1979.[40] Importantly for solution NMR experiments, it forms relatively stable small micelles that rapidly rotate in solution.[40,102] DPC is comprised of hydrophilic and hydrophobic components that can mimic those present in cell membrane.[103] Micelles, as shown by numerous examples in the literature, provide an environment that can indicate structural arrangements that proteins or peptides can assume in lipid membranes.[104-106] The majority of known bacteriocins and lipopeptides are thought to target the bacterial membrane or membrane-bound receptor proteins.[107]

Many antimicrobial peptides (AMPs) act against bacteria through the process of pore formation in the cell membrane.[108-112] AMPs such as plectasin[113], tridecapin[49], and texobactin[114] target lipid II and can kill multidrug-resistant bacteria. As described previously, tridecaptin A_1 has been shown by our group to selectively bind C_{15} *m*DAP-lipid II analogue (**40**).[49] It remains unclear how this peptide-target complex is oriented within the membrane. We decided to determine the positioning of Oct-TriA_1 (**29**) C_{15} *m*DAP-lipid II (**40**) with DPC micelles by synthesizing analogs of DPC. Our strategy was to install heteroatoms at certain positions on the DPC lipid tail, thereby allowing formation of micelles with modifications at varying depths (Figure 4.1 B). Oxygen and a CF_2 group were chosen to replace selected methylene carbons. NMR spectroscopy

could then detect chemical shift changes experienced by specific protons on the Oct-TriA$_1$-*m*DAP-lipid II complex. For solution NMR, a *m*DAP-Lipid II (**40**) analogue wherein the C_{55} lipid tail was replaced with a *Z,Z*-C_{15} tail was desired as it would permit soluton NMR studies.

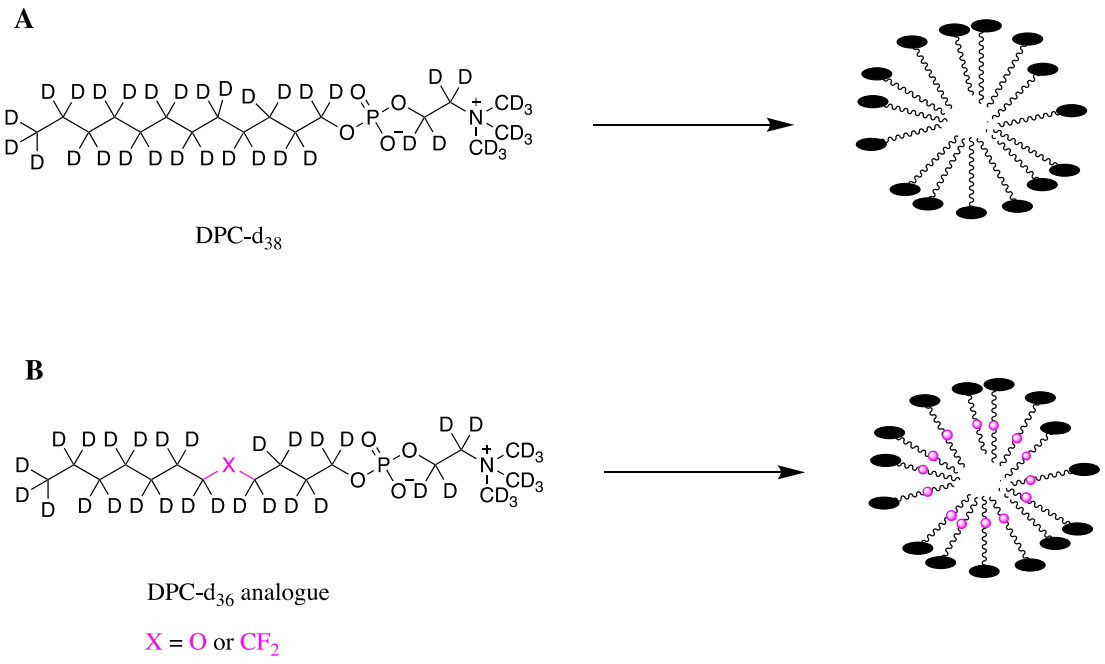


Figure 4.1 DPC forming micelles A) without and B) with heteroatom depth gage

4.2. Synthesis of Undeuterated and Deuterated DPC Analogues for NMR Studies with the Antimicrobial Peptide Leucocin A

Our group had previously determined the three-dimensional structure of leucocin A, a YGNG-motif containing bacteriocin from lactic acid bacteria, in 90% trifluoroethanol (TFE)-water and in dodecylphosphocholine (DPC-d$_{38}$) micelles using two-dimensional NMR techniques.[115] Circular dichroism spectra, NMR chemical shift indices and long-range nuclear Overhauser effects showed that leucocin A adopts a well defined structure in both TFE and the DPC-d$_{38}$ micelle environment. However, in water or

aqueous DMSO, leucocin A exists as a random coil.[115] I developed a synthetic route to synthesize deuterated oxo-DPC$_{d36}$ analogues for solution-phase NMR studies with the antimicrobial lipopeptide Oct-TriA$_1$. Dr. Marco van Belkum, Ms. Cherry Ibarra Romero, and Ms. Tess Lamer in our group are preparing both labelled (^{13}C, ^{15}N) and unlabelled leucocin A by cloning and expression in a heterologous host. The objective is to verify the 3D structure of leucocin A in the DPC analogues and to analyze its interaction with the lipid environment. The O-DPC-d$_{36}$ analogues, whose syntheses are discussed below, and the F$_2$-DPCd$_{36}$ analogues will be used for the NMR studies of leucocin A. With undeuterated DPC analogues, labelled leucocin A will be employed to enable rapid analysis of the structure. The description below outlines the synthetic routes I have developed to prepare these analogues for our future studies.

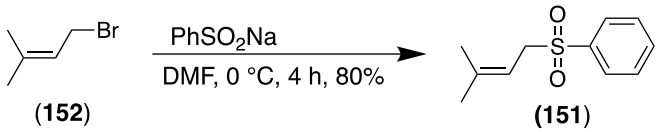
4.3. Results and Discussion on the Synthesis of Required Compounds

4.3.1. Synthesis of C$_{15}$ *m*DAP-lipid II Analogue

The synthesis of the C$_{15}$ *m*DAP-lipid II analogue was prepared following a literature procedure, as described in Chapter 2 of this thesis, except the lipid phosphate for the last step involved the use of (2*Z*,6*Z*)-3,7,11-Trimethyldodeca-2,6,10-trien-1-yl hydrogen phosphate **150**. This analogue was used because it is soluble in water for solution NMR studies. Our research group has previously synthesized a C$_{15}$ *m*DAP-lipid II **40** with *Z,Z* stereochemistry on the terpene tail and determined the structure of Oct-TriA$_1$ within a DPC-d$_{38}$ micelle.[49] However, a large quantity of lipid II **40** was required for NMR studies and therefore a modified procedure was developed.

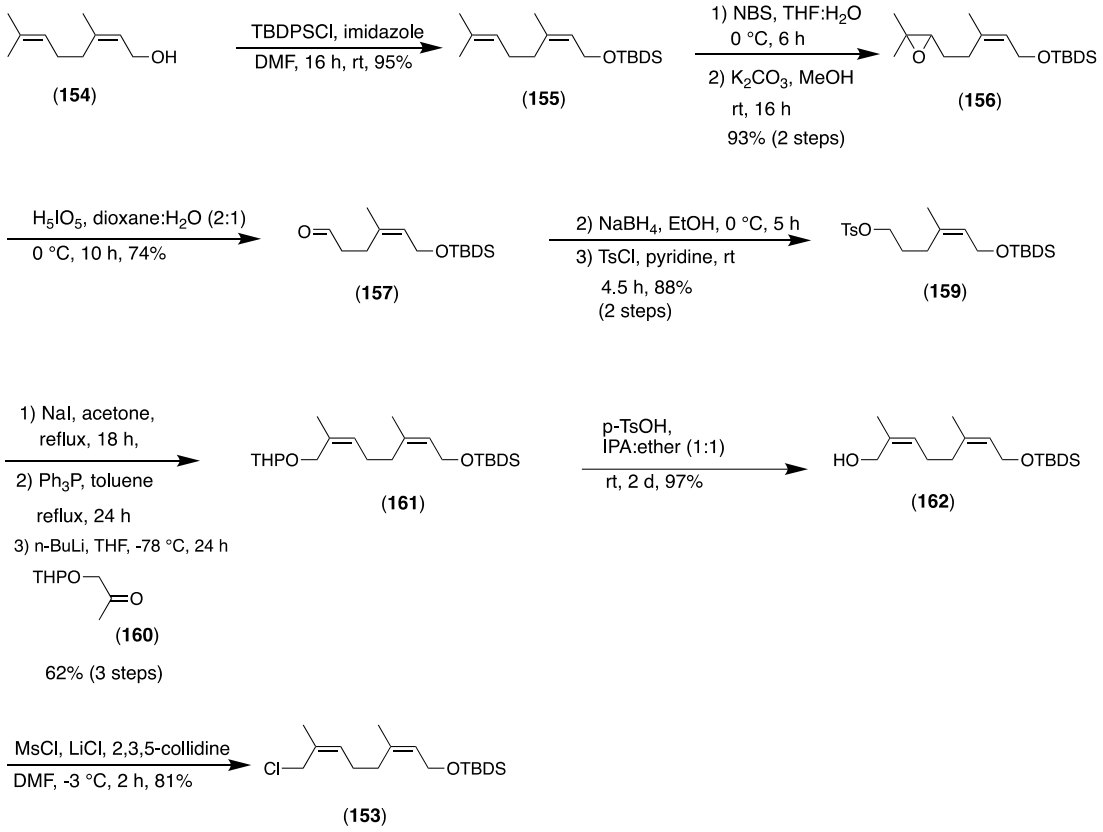
4.3.2. Synthesis of C_{15}-(*Z,Z*)-lipid phosphate

The synthesis of **151** was accomplished following a literature procedure[116] with some modifications (Scheme 4.1) starting from commercially available 3,3-dimethylallyl bromide. Treatment of **152** with $NaSO_2Ph$ in DMF yields **151** in 80% yield.



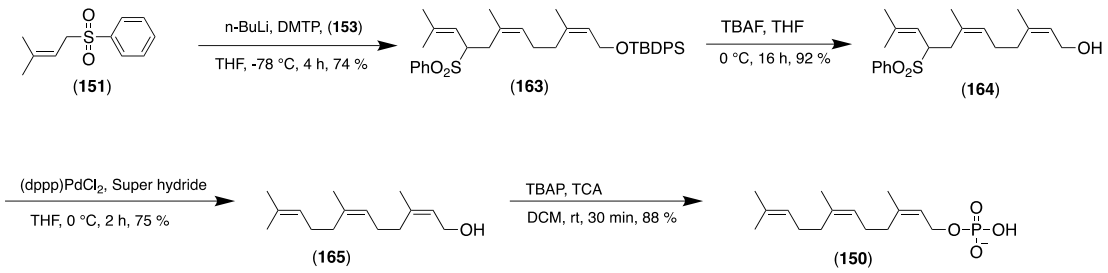
*Scheme 4.1 Preparation of 1-benzenesulfonyl-3-methyl-2-butene (**151**)*

The synthesis of **153** was accomplished in eleven steps from nerol (**154**) (Scheme 4.2). Treatment of **154** with *tert*-butyldiphenylsilyl chloride yields the protected nerol **155** in 95% yield, which was reacted with *N*-bromosuccinimide to give a bromohydrin, followed by treatment with K_2CO_3 to give the epoxide **156** in 93% yield over two steps. The epoxide **156** was treated with H_5IO_5 in a dioxane:water (2:1) solvent system to give the aldehyde **157** in 74% yield. The aldehyde was reduced to the corresponding alcohol **158** when treated with $NaBH_4$, followed by reaction with toluenesulfonyl chloride to yield **159** in 88% over two steps. Treatment of **159** with NaI followed by reaction with triphenylphosphine gave the phosphonium compound, which upon reaction with THP-acetol (**160**) in the presence of *n*-BuLi yields **161** in 62% over three steps. The THP protecting group was removed using *p*-toluenesulfonic acid to give the corresponding alcohol **162** in 97% yield. The final step involved treatment of **163** with LiCl and methanesulfonyl chloride in the presence of 2,4,5-collidine to give **153** in 81% yield.



*Scheme 4.2 Preparation of chlorinated C_{10} lipid tail intermediate (**153**)*

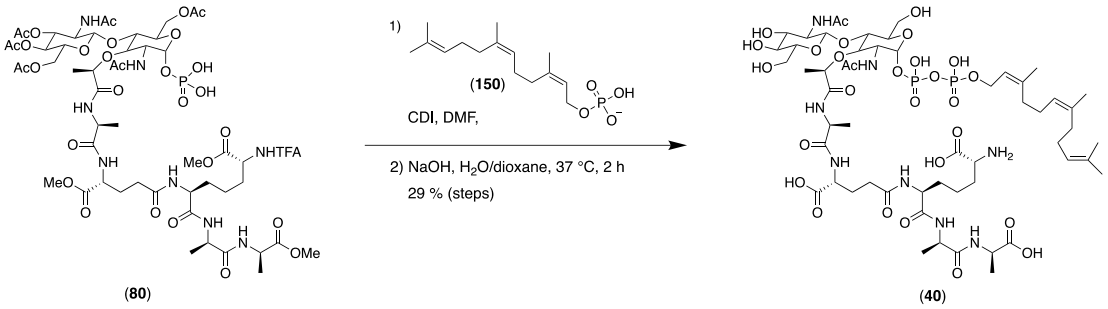
Condensation of **151** with **153** in the presence of 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidone (DMTP) and *n*-butyl lithium yields **163** in 74% yield (Scheme 4.3). The silyl ether protecting group was removed using tetrabutylammonium fluoride (TBAF) to give the corresponding alcohol **164** in 92% yield. This upon treatment with (bis(diphenylphosphino)propane)palladium(II) dichloride ((dppp)$PdCl_2$) yields the C_{15}-(*Z,Z*) terpene alcohol **165** in 75% yield. The alcohol was reacted with trichloroacetonitrile and tetrabutylammonium dihydrogen phosphate and, after ion exchange chromatography and HPLC purification, gave the phosphate **150** in 86%.



*Scheme 4.3 Preparation of C_{15}-Z,Z-lipid phosphate (**150**)*

4.3.2.1. Synthesis of C_{15} *m*DAP-lipid II (40)

With the lipid phosphate **150** available, the synthesis of mDAP-lipid II **40** was completed (Scheme 4.4). This involves the activation of the phosphate **80**, whose synthesis is outlined in Chapter 2 of this thesis, with CDI followed by coupling with *(Z,Z)*-farnesyl phosphate **150**. The final step is the global deprotection which was achieved with NaOH to yield **40** in 29% yield over two steps.



Scheme 4.4 *Preparation of C_{15}-Z,Z-lipid II (**40**)*

4.3.3. Synthesis of Octyl-Tridecaptin A_1 (29) for Solution NMR Studies

The synthesis of Oc-tri A_1 (**29**) (Figure 4.2) has been previously discussed in Chapter 2 of this thesis, except that all amino acids used for the peptide chain were unlabelled amino acids.

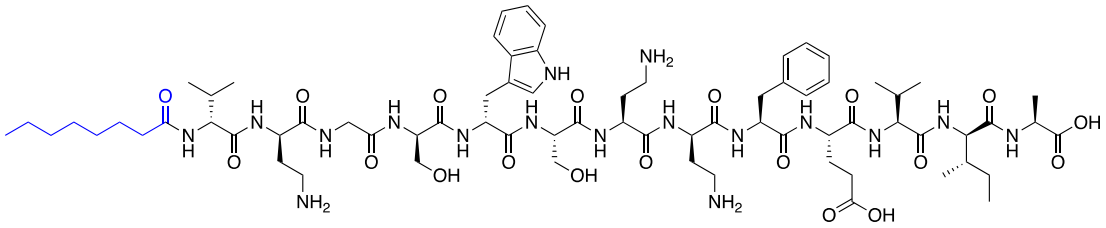
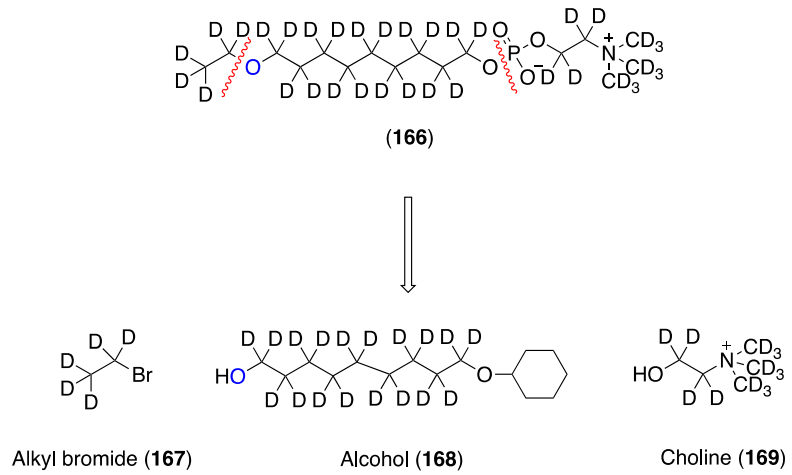


*Figure 4.2 Structure of octyl-tridecaptin A_1 **(29)***

4.3.4. Synthesis of Deuterated DPC Analogues for Solution NMR Studies

The synthesis of DPC-d_{38} is well precedented in the literature, however there is no reported literature on the synthesis of oxo analogues of DPC, and so we designed a synthetic approach to obtain the four desired analogues. The approach involved using a procedure for preparation of per-deuterated alkyl chains, starting from a commercially available aliphatic acid or alcohol.[117-120] Gas-phase exchange with deuterium gas has been used for the preparation of fully deuterated hydrocarbons[121] and fatty acids[122], however the use of long reaction times, low percentage of deuterium incorporation, and the use of deuterium gas makes this strategy difficult. The use of a combination of deuterium source and a catalytic or excess transition metal such as $LiAlD_4$[123, 124], deuterium (D_2) gas[123-126] in the presence of Wilkinson's catalyst, and D_2O with SmI_2[127,128] have been employed in the synthesis of partially-deuterated saturated fatty acids through reduction of the corresponding unsaturated substrates. However, there are limited methods for the preparation of multi- and fully deuterated saturated fatty acids from non-labeled natural substrates. Direct and full deuteration of saturated fatty acids under hydrothermal conditions and a strongly basic solution of D_2O in the presence of a platinum catalyst have been reported.[117]

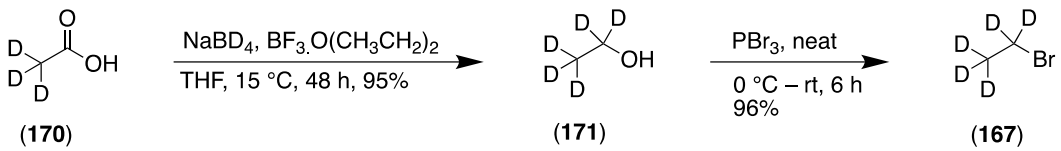
I embarked on the synthesis of the four desired O-DPC-d_{36} analogues. The total synthesis of O-DPC-d_{36} can be broken down into three linear syntheses with each product then used to make O-DPC-d_{36} (Scheme 4.5). The scheme represents one analogue and, depending on the position of the desired oxygen on the lipid tail, the appropriate starting materials were used.



*Scheme 4.5 Retrosynthetic analysis of O-DPC-d_{36} analogue (**166**)*

4.3.4.1. Synthesis of 10-O-DPC-d_{36} (166)

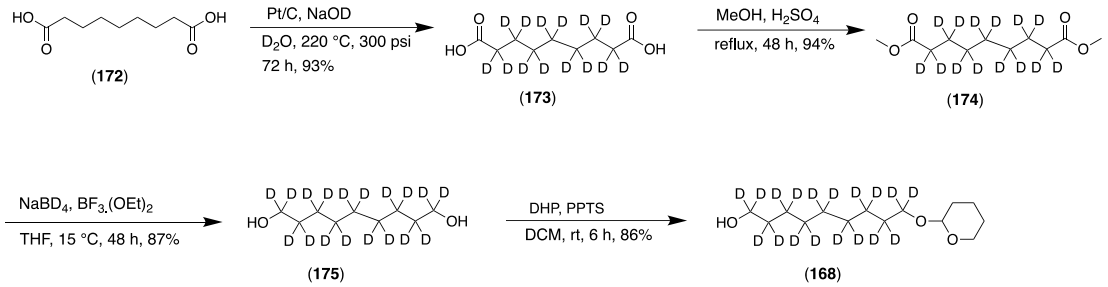
The synthesis of 10-O-DPC-d_{36} (**166**) required the preparation of the alkyl bromide **167**, the alcohol **168**, and the choline (**169**). The alkyl bromide **167** was synthesized in two steps from commercial acetic acid 2,2,2-d_3 (**170**) (Scheme 4.6). Treatment of **170** with $NaBD_4$ and boron trifluoride diethyl etherate, followed by treatment of the resulting ethanol-d_5 (**171**) with PBr_3 yields the alkyl bromide **167** in 98% yield.



*Scheme 4.6 Preparation of alkyl bromide (**167**)*

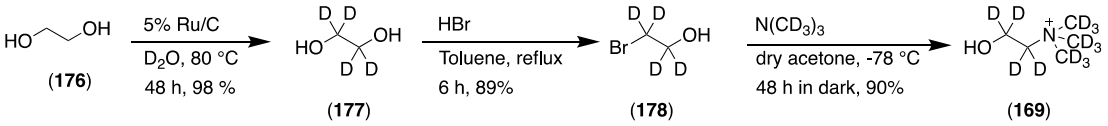
The diol **168** was synthesized in four steps from azelaic acid (**172**) following a literature procedure (Scheme 4.7).[117,129,130] Deuteration of **172** was achieved using D_2O and NaOD in the presence of Pt/C catalyst, which yields **173** in 87% yield and 93% deterium incorporation (D). Crude **173** was reacted with fresh reagent and the above method was repeated. The final percentage of deuteration was calculated to be 98.1% D. The deuterated azelaic acid was treated with MeOH and a catalytic amount of H_2SO_4 to form the diester **174**, followed by reaction with $NaBD_4$ and boron trifluoride diethyl etherate ($BF_3.OEt_2$ to yield the diol **175** in 87% yield and 98.3% D. This was then followed by mono protection of the alcohol using dihydropyran to give the protected alcohol **168** in 86% yield.

The percentage of deuterium incorporation was determined using NMR and mass spectrometry. For NMR analysis, an internal standard (CH_2Cl_2) was used, and the integration of the residual protons was determined, which allowed for calculation of the percentage of deuterium at each position along the lipid chain. With mass spectrometry, the percentage of overall deuterium exchange was calculated from the relative abundance and isotopic distribution of the different isotopologues.



*Scheme 4.7 Preparation of alcohol (**168**)*

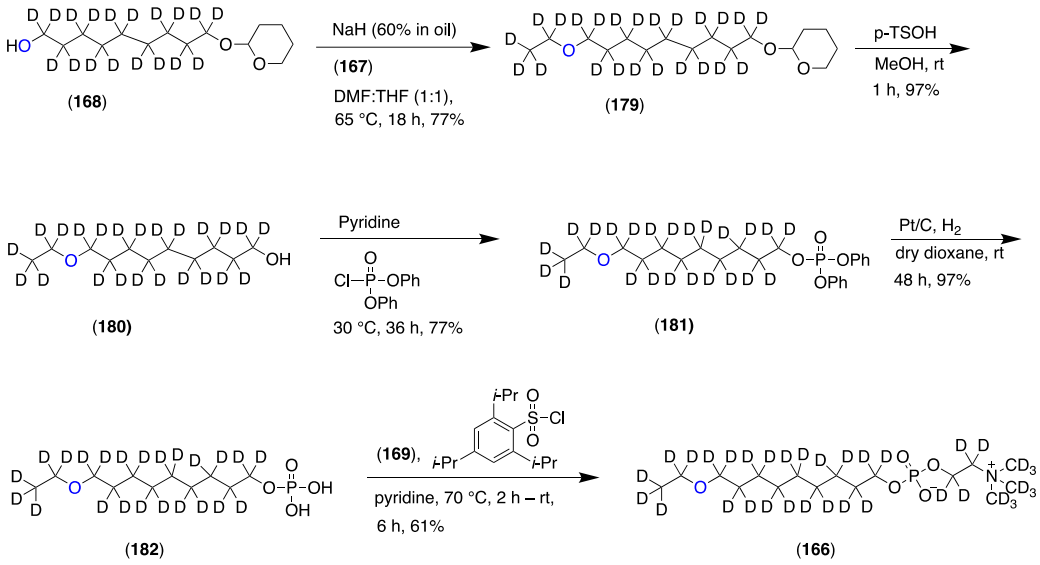
The choline (**169**) was synthesized from commercially available ethylene glycol (**176**) following a literature procedure with some modification (Scheme 4.8).[131, 132] Deuteration of the diol was achieved by treatment of **176** with 5% Ru/C to give **177**, followed by treatment with HBr to give **178**. Treatment of **178** with trimethylamine-d_9 at -78°C in the absence of light yields **169** in 90% yield and 98% D, according to NMR analysis.



*Scheme 4.8 Preparation of choline (**169**)*

With the key building blocks in hand, 10-O-DPC-d_{36} (**166**) was then synthesized (Scheme 4.9). The protected alcohol **168** was coupled to the alkyl bromide (**167**) using NaH to yield **179** in 77% yield, followed by deprotection of the THP protecting group using *p*-toluenesulfonic acid to give the C_{11} alcohol **180** in good yield. Treatment of the alcohol **180** with diphenyl phosphoryl chloride yields **212** in 77% yield, followed by treatment of **181** with Pt/activated carbon under hydrogen gas to yield the lipid

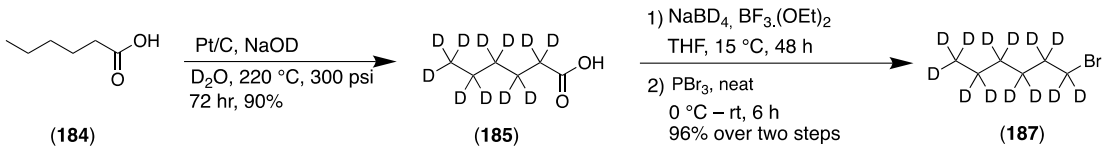
phosphate **182** in 97% yield. The final step involves coupling of **182** with choline **169** to yield the 10-O-DPC-d_{36} (**166**) in 61% yield and 97.9% D.



*Scheme 4.9 Preparation of 10-O-DPC-d_{36} (**166**)*

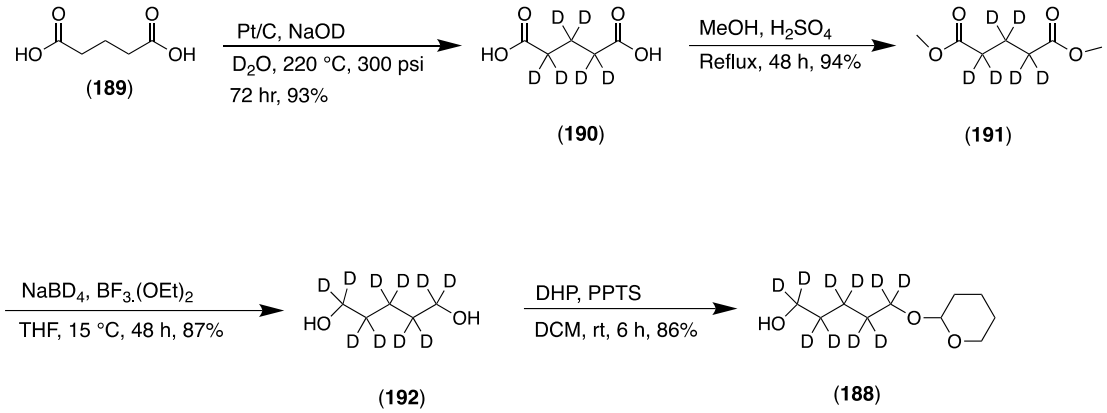
4.3.4.2. Synthesis of 6-O-DPC-d_{36} (183)

The alkyl bromide **187** was synthesized in three steps from pentanoic acid **184** (Scheme 4.10). Deuteration of **184** was achieved using D_2O and NaOD in the presence of Pt/C catalyst, which yields **185** in 90% product yield and 93% D. To achieve a high percentage of deuteration, crude **185** was subjected to fresh reagents, resulting in a final percentage of deuteration of 98% D. Treatment of **185** with $NaBD_4$ and boron trifluoride diethyl etherate, followed by treatment of the resulting pentanol-d_{13} (**186**) with PBr_3 yields the alkyl bromide **187** in 94% yield.



Scheme 4.10 Synthesis of hexyl bromide-d_{13} (**187**)

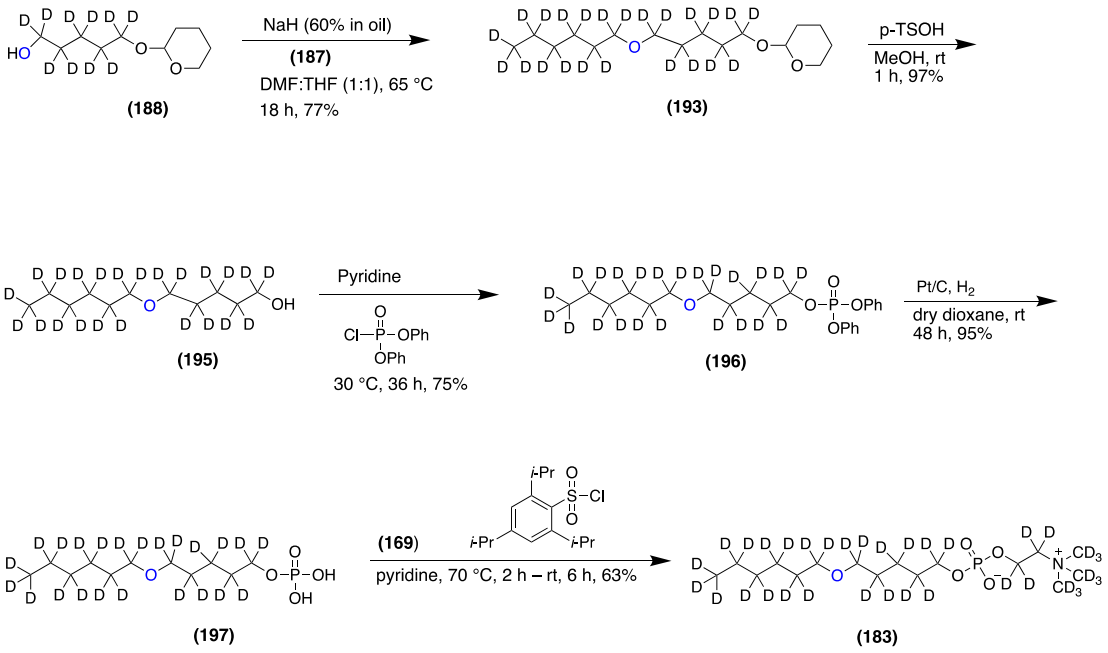
The alcohol **188** was synthesized in four steps from glutaric acid (**189**) following a literature procedure (Scheme 4.11).[117, 129, 130] Deuteration of **189** was achieved using D_2O and NaOD in the presence of Pt/C catalyst, which yields **190** in 97% yield and 98% D. The deuterated glutaric acid was treated with MeOH and a catalytic amount of H_2SO_4, which yields **191** in 94% yield. This was followed by reaction with $NaBD_4$ and ($BF_3O(C_2H_5)_2$) to yield the diol **192** in 87% yield. Subsequent mono protection of the alcohol using dihydropyran gives the protected alcohol **188** in 84% yield.



*Scheme 4.11 Preparation of alcohol (**188**)*

With the key building blocks available, 6-O-DPC-d_{36} (**183**) was then synthesized (Scheme 4.12). The protected alcohol **188** was coupled to the alkyl bromide **187** using NaH to yield **193** in 75%, followed by deprotection of the THP protecting group with *p*-toluenesulfonic acid to give the alcohol **195** in good yield. Treatment of the alcohol

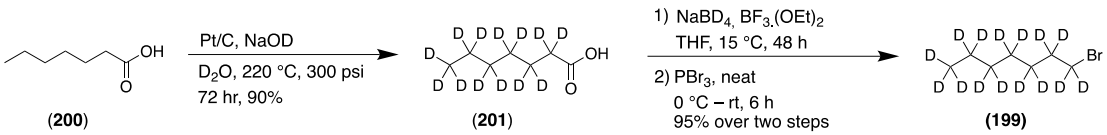
195 with diphenyl phosphoryl chloride yields compound **196**, followed by treatment with Pt/C under hydrogen gas yields the lipid phosphate **197** in 95%. The final step involves coupling of the lipid phosphate with choline **169** to yield the 6-O-DPC-d_{36} (**183**) in 63% and 97.8% D.



*Scheme 4.12 Preparation of 6-O-DPC-d_{36} (**183**)*

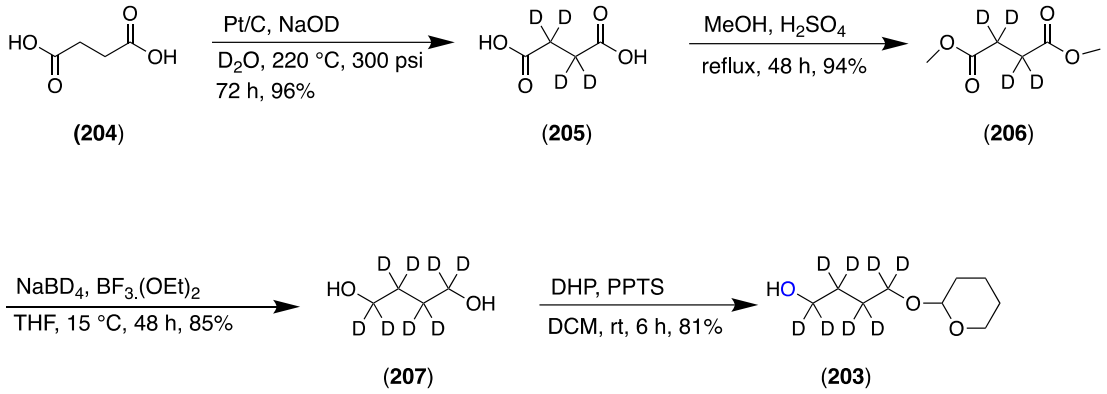
4.3.4.3. Synthesis of 5-O-DPC-d_{36} (198)

The alkyl bromide **199** was synthesized in three steps from heptanoic acid (**230**) (Scheme 4.13). Deuteration of **200** was achieved using D_2O and NaOD in the presence of Pt/C catalyst which gives **201** in 90% yield and 92% D. To achieve a higher percentage of deuteration, the 92% D material was reacted with fresh reagents to give a calculated deuteration of 98.6%. Treatment of **201** with $NaBD_4$ and boron trifluoride diethyl etherate gave the alcohol **202**, followed by treatment of the resulting heptanol-d_{15} with PBr_3 yields the alkyl bromide **199** in 96% yield.



*Scheme 4.13 Preparation of alkyl bromide (**199**)*

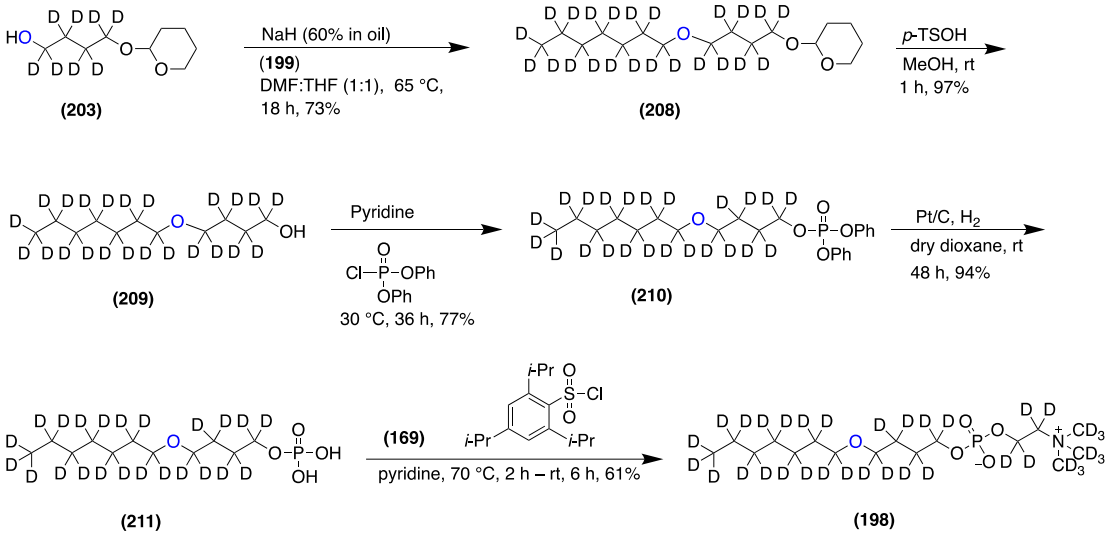
The alcohol **203** was synthesized in four steps from succinic acid (**204**) following a literature procedure (Scheme 4.14).[117, 129, 130] Deuteration of **204** was achieved using D_2O and NaOD in the presence of Pt/C catalyst, which yields **205** in 96% yield and 99% D incorporation. The deuterated succinic acid was treated with MeOH and a catalytic amount of H_2SO_4 to give **206**, followed by reaction with $NaBD_4$ and $BF_3O(C_2H_5)_2$ yields the diol **207** in 85%. This was followed by mono protection of the alcohol using dihydropyran to give the protected alcohol **203** in 81% yield.



*Scheme 4.14 Preparation of mono-protected diol (**203**)*

With the key building blocks available, 5-O-DPC-d_{36} was then synthesized (Scheme 4.15). The protected alcohol **203** was coupled to the alkyl bromide **199** using NaH to give **208** in 73% yield, followed by deprotection of the THP protecting group with *p*-toluenesulfonic acid to give the free alcohol **209** in good yield. Treatment of the free alcohol **209** with diphenyl phosphoryl chloride gives compound **210**, which upon

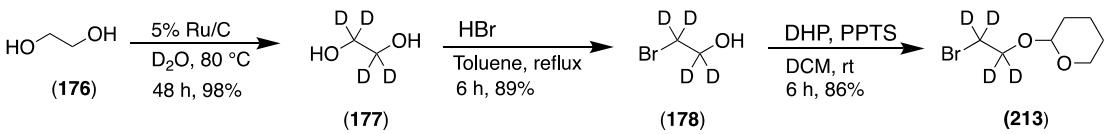
treatment with Pt/C under hydrogen gas yields the lipid phosphate **211** in 94% yield. The final step involves coupling of the lipid phosphate with choline (**169**) to yield the 5-O-DPC-d_{36} (**198**) in 62% yield and 98.1% D.



*Scheme 4.15 Preparation of 5-O-DPC-d_{36} (**198**)*

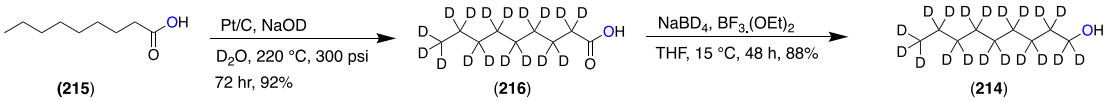
4.3.4.4. Synthesis of 3-O-DPC-d_{36} (212)

The synthesis of the 3-O-DPC-d_{36} (**212**) involves the preparation of compound **213** from ethylene glycol (**176**) (Scheme 4.16). Deuteration of the diol was achieved by treatment with 5% Ru/C to give **177**, followed by treatment of the diol with HBr to give **179**. The alcohol was protected using dihydropyran to give the final product **213** in 86% yield and 98% D.



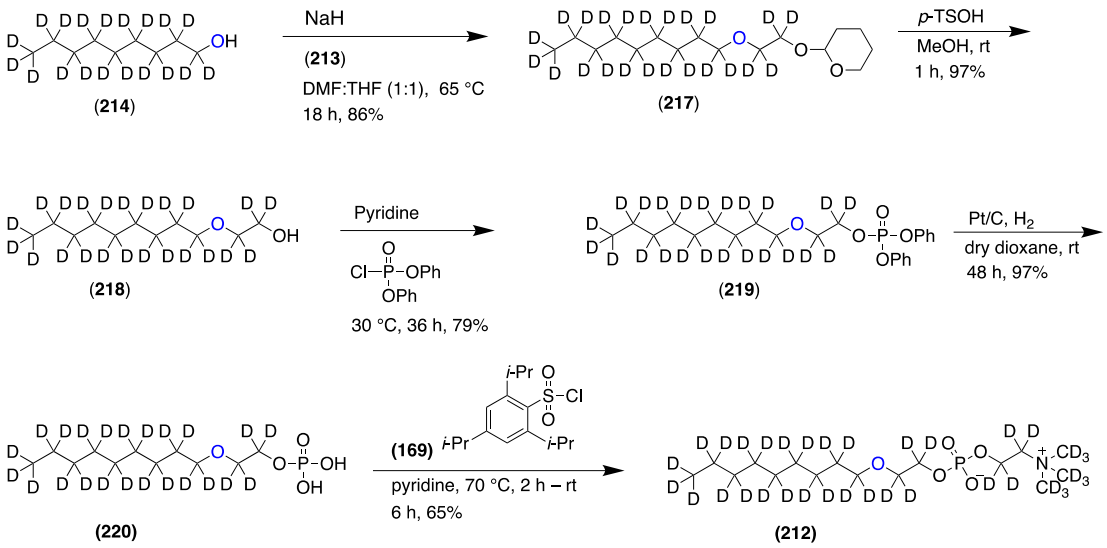
*Scheme 4.16 Preparation of (**213**)*

The alcohol **214** was synthesized in two steps from nonanoic acid (**245**) (Scheme 4.17). Deuteration of **215** was achieved using D_2O and NaOD in the presence of Pt/C catalyst, which gives **216** in 92% yield and 92% D. To achieve a higher percentage of deuteration, the above method was repeated with fresh reagents and the final percentage of deuteration was calculated to be 98% D. The acid was treated with $NaBD_4$ and boron trifluoride diethyl etherate to yield nonanol-d_{19} (**214**) in 88% yield.



*Scheme 4.17 Preparation of nonanol-d_{19} (**214**)*

Next, 3-O-DPC-d_{36} (**212**) was synthesized (Scheme 4.18). Compound **214** was coupled to the alkyl bromide **213** using NaH to yield **217** in 86%, followed by deprotection of the THP protecting group with *p*-toluenesulfonic acid to give the alcohol **218** in good yield. Treatment of **218** with diphenyl phosphoryl chloride yields **219** and followed by treatment with Pt/C under hydrogen gas yields the lipid phosphate **220** in 97% yield. The final step involves coupling of the lipid phosphate with choline (**169**), which yields the 3-O-DPC-d_{36} (**212**) in 65% yield and 97.7% D.



*Scheme 4.18 Preparation of 3-O-DPC-d_{36} (**212**)*

4.4. Circular Dichroism of Oct-TriA$_1$ in DPC Analogues

To further probe whether octyl-tridecaptin A_1 maintains a defined structure in these DPC analogues, circular dichroism (CD) experiments were performed. This also gave us an insight into the relative ratio of peptide to lipid needed to maintain a structured peptide in O-DPC-d_{36} micelles. TriA_1 is unstructured in aqueous buffer, in 50% aqueous trifluorethanol (a structure-inducing solvent), and in sodiun dodecyl sulfate micelles.[49] Previous solution NMR experiments of Oct-TriA_1 in DPC-d_{38} by our group were performed in an aqueous buffer system (pH 6.1), with a peptide concentration of 4 mM, DPC concentration of 180 mM, and a ratio of 1:45 peptide:lipid. I performed CD experiments of the four oxo-DPC-d_{36} under the same conditions, and the data showed similar spectra compared to the commercial DPC-d_{38} (Figure 4.3).

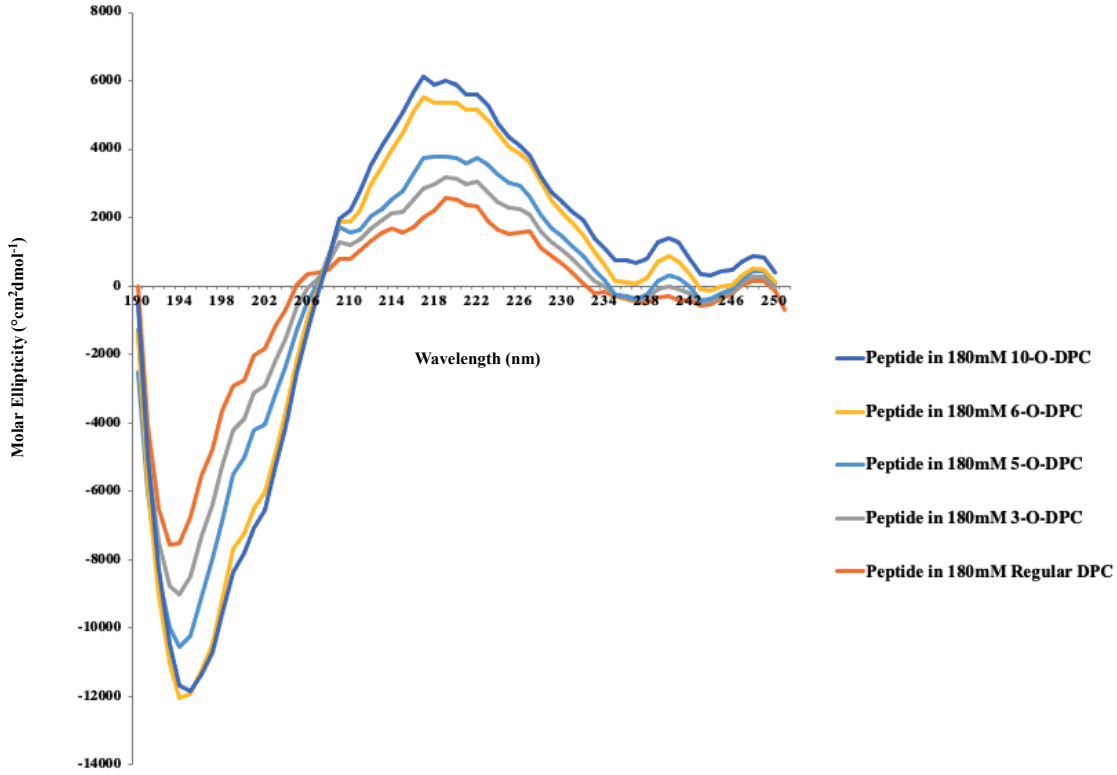


*Figure 4.3 Circular dichroism for Oct-TriA$_1$ 4 mM (**29**) in all four O-DPC-d$_{36}$ analogues at 180 mM*

4.5. Solution NMR Studies of Oct-TriA$_1$ in O-DPC-d$_{36}$ Analogues

Dr. Sorina Chiorean conducted initial solution NMR studies of the O-DPC-d$_{36}$ analogues under the above conditions and peptide:lipid ratio, however, the NMR samples formed a gel over time upon the addition of Oct-TriA$_1$. This problem was predominantly seen with the 3-O-DPC-d$_{36}$ analogue, but was also noticed for the other three analogues. This observation led us to lower the DPC concentration. CD experiments of Oct-TriA$_1$ in various O-DPC-d$_{36}$ concentrations of 145 mM (Figure 4.4), 120 mM (Figure 4.5), 90 mM (Figure 4.6) and 70 mM (Figure 4.7) were conducted. However, we observed changes in the Oct-TriA$_1$ CD spectra at 70 mM

(Figure 4.7), indicating that a change had occurred in the peptide conformation. Thus, we concluded that a DPC concentration above this threshold would be more suited for solution NMR studies of Oct-TriA$_1$ in these O-DPC-d$_{36}$ micelles. There were no significant differences in the CD spectra shape of Oct-TriA$_1$ at 145 mM, 120 mM, and 90 mM O-DPC-d$_{36}$ concentrations.

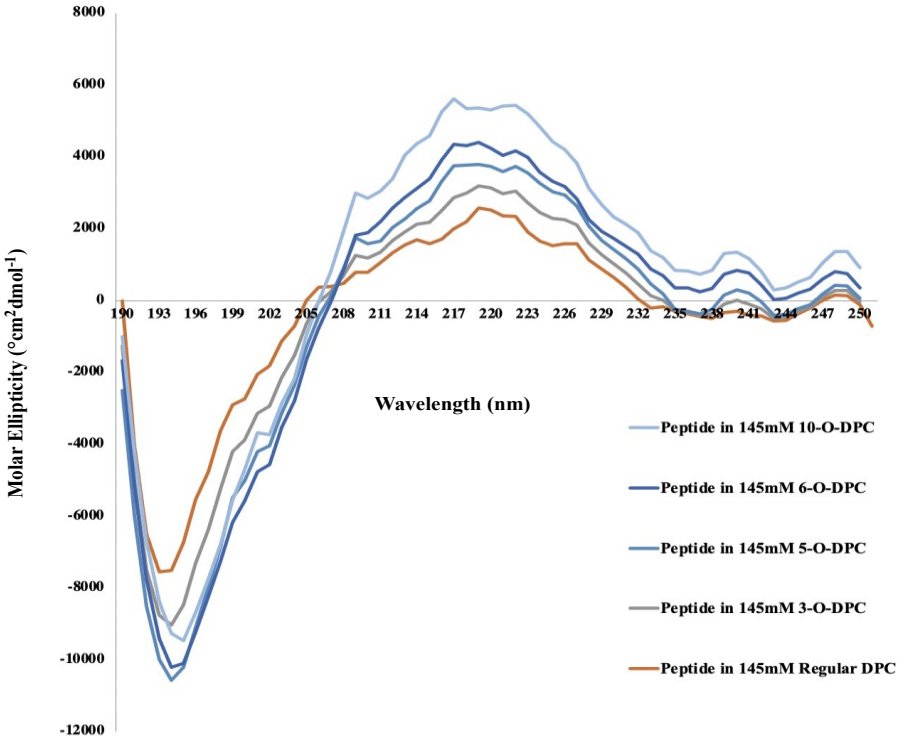


Figure 4.4 CD experiments of Oct-TriA$_1$ 4mM in O-DPC-d$_{36}$ analogues at 145 mM

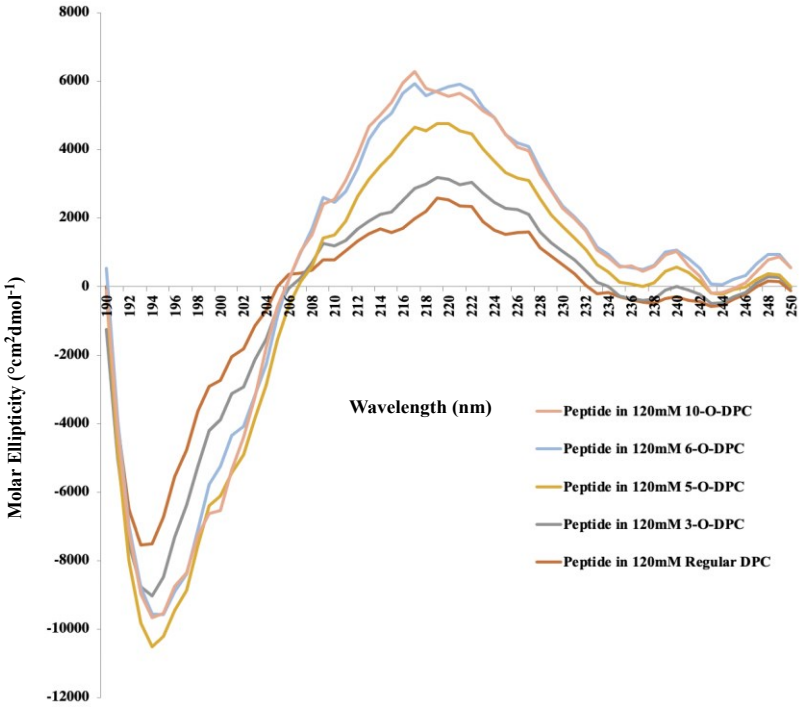


Figure 4.5 CD experiments of Oct-TriA$_1$ 4 mM in O-DPC-d$_{36}$ analogues at 120 mM

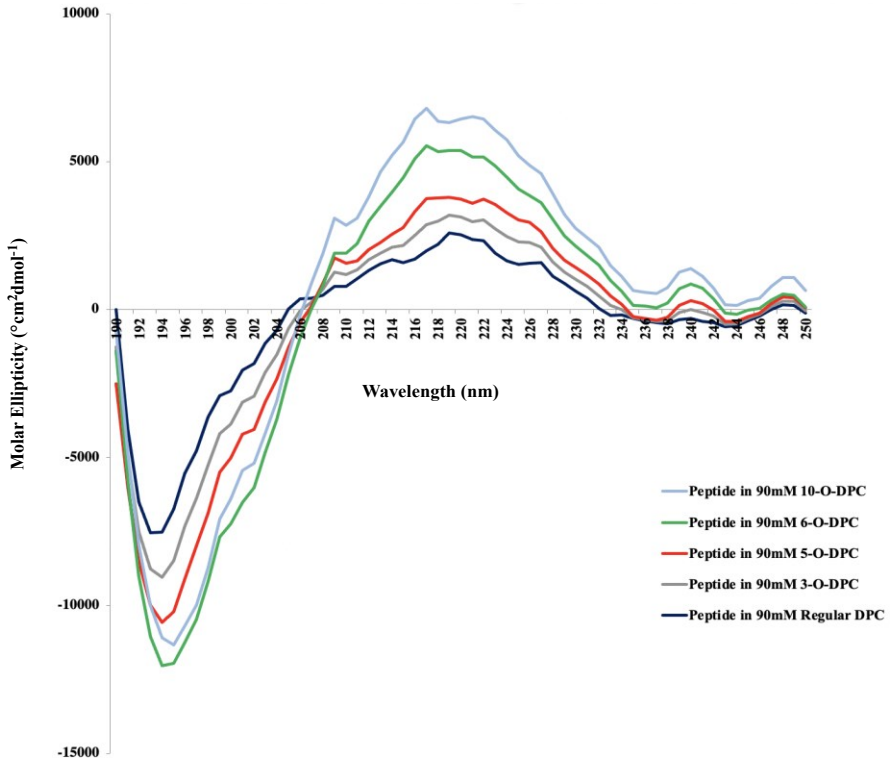


Figure 4.6 CD experiments of Oct-TriA1 4 mM in O-DPC-d$_{36}$ analogue at 90 mM

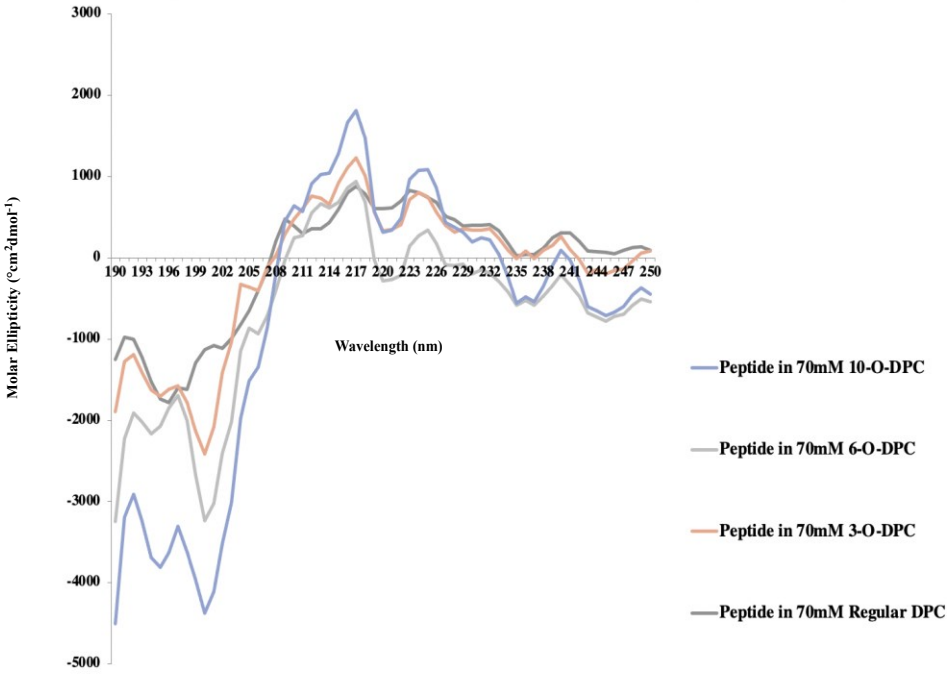


Figure 4.7 CD experiments of Oct-TriA$_1$ 4mM in O-DPC-d$_{36}$ analogues at 70 mM

Dr. Sorina Chiorean of our group performed solution-phase NMR studies using a 120 mM lipid concentration with a peptide:lipid ratio of 1:30 and compiled full chemical shift assignments of Oct-TriA$_1$ in regular DPC$_{d38}$ and all four O-DPC-d$_{36}$ analogues (3-O-DPC-d$_{36}$, 5-O-DPC-d$_{36}$, 6-O-DPC-d$_{36}$, and 10-O-DPC-d$_{36}$).

The chemical shifts of Oct-TriA$_1$ in DPC$_{d38}$ was the reference to which we compared the chemical shifts of all the O-DPC-d$_{36}$ analogues. In this chapter, the observed chemical shifts for Oct-TriA$_1$ in all four O-DPC-d$_{36}$ analogues are represented with shades of red, which are used to indicate the various degree of chemical shift changes (Figure 4.8). For a chemical shift difference of 0.01 ppm, a bright maraschino red used as the indication, chemical shift differences of 0.01 – 0.04 ppm are indicated by strawberry pink-red, and 0.05 ppm or higher chemical shift differences are indicated

by a dark maroon. The lipid tail of Oct-TriA$_1$ (Figure 4.8) experienced moderate differences in chemical shift in all the O-DPC-d$_{36}$ micelles, which posed a challenge to determine how deep the octyl chain penetrates the micelle. When comparing the chemical shifts of Oct-TriA$_1$ in each of the O-DPC-d$_{36}$ to the chemical shifts in regular DPC-d$_{38}$, the chemical shifts of Oct-TriA$_1$ in the 5-O-DPC-d$_{36}$ micelle are most different.

The 3-O-DPC-d$_{36}$ showed the greatest tendency of gel formation, and as a result only a partial assignment was possible. It was not possible to recognize the alpha protons of Ser4, Dab8, Phe9 and, Val11, and the full lipid tail, Gly3, and Glu10 residues were not assigned. A possible reason for this observation could be that these residues are involved in some kind of interaction leading to the gel formation, which makes it difficult to detect protons by solution NMR. The Val1 and Dab2 residues are close to the lipid tail and could be the segment of Oct-TriA$_1$ with the closest DPC-interaction as they experienced the biggest changes in chemical shift at their alpha protons, while the remaining side chains observed only modest changes.

The observed chemical shift changes for the peptide in 5-O-DPC-d$_{36}$ are highlighted in (Figure 4.8B). As shown in the figure, Oct-TriA$_1$ observed the greatest overall chemical shift changes in this DPC analogue compared to the remaining analogues. The alpha proton of Glu10, the backbone amides of Trp5 and Phe9, the Gly3 residue, and the sidechain of Ile12 observed greater chemical shift changes when compared to the 3-O-DPC-d$_{36}$ data, in addition to that of Val1 and Dab3 previously discussed. Residues like Ser4 and Dab8 in areas surrounding the amide protons of Trp5 and Phe9 observed modest chemical shift changes.

Though the 6-O-DPC-d_{36} contains an oxygen atom modification just one position further into the micelle core than the 5-O-DPC-d_{36} analogue, it showed different results. There was not a significant proton chemical shift noticed for Oct-TriA_1 in 6-O-DPC-d_{36} as was observed in the 3-O-DPC-d_{36} and 5-O-DPC-d_{36} analogues, highlighted in (Figure 4.8 A and B). There were modest changes observed for the amide and alpha protons of Trp5 and Phe9 as well as the backbone protons of the neighbouring residues, which also showed modest changes (0.01 – 0.04 ppm). In addition, there were smaller chemical shift differences of residues Glu10 and Ile12, when compared with those in the 5-O-DPC-d_{36} analogue.

Even though the oxygen atom is further from the phosphate head group in the 10-O-DPC-d_{36} analogue, and it is likely closer to the surface than the center of the micelle, there were moderate chemical shifts for Oct-TriA_1 in this analogue when compared to Oct-TriA_1 in regular DPC-d_{38}. The overall chemical shift changes observed for Oct-TriA_1 in the 10-O-DPC-d_{36} analogue (Figure 4.8 D) is relatively similar to that observed for the peptide in 6-O-DPC-d_{36}.

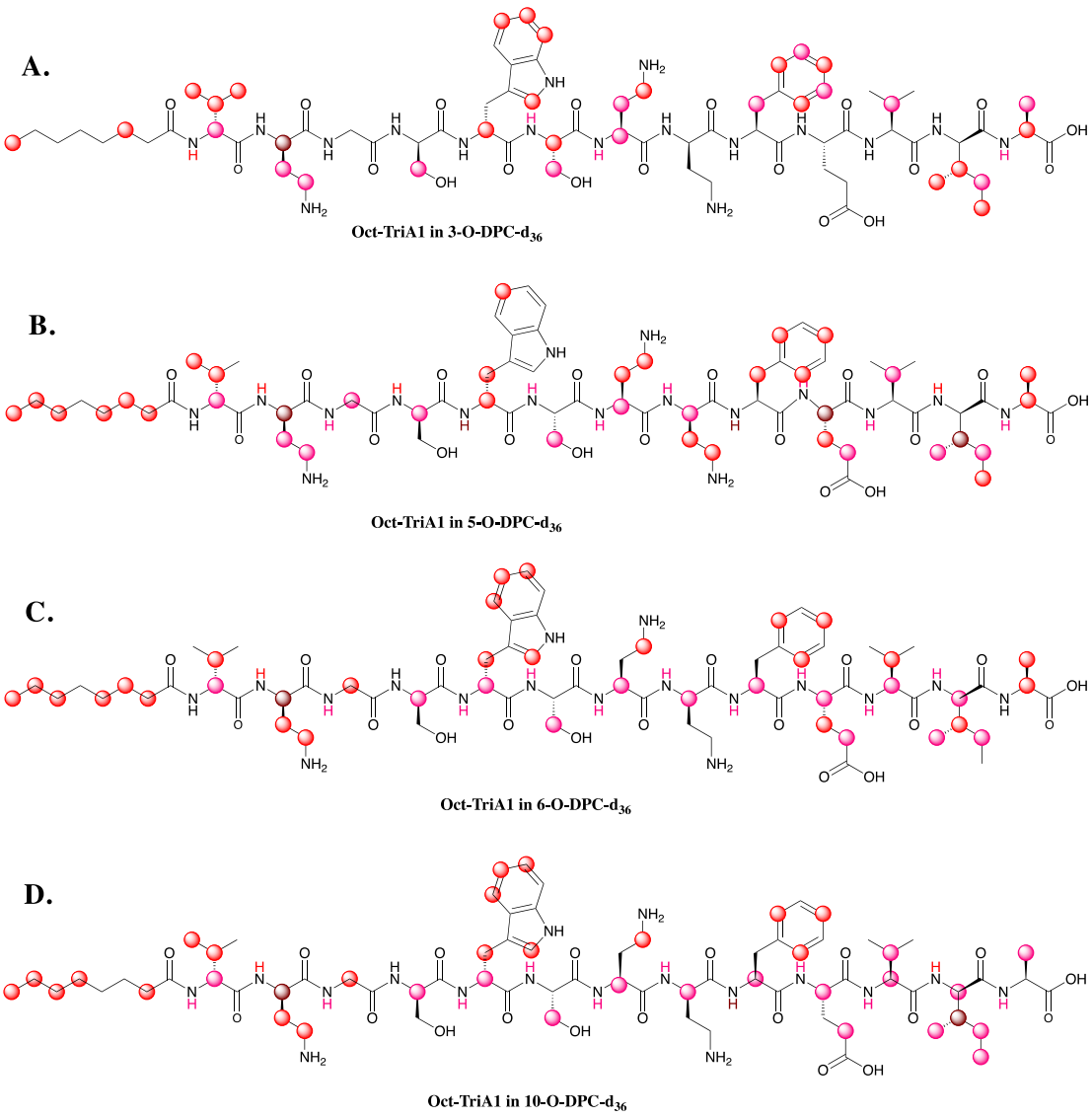


Figure 4.8 Chemical shift differences observed for Oct-TriA$_1$ in the four O-DPC-d$_{36}$ analogues

4.6. Solution NMR Studies of Lipid II in O-DPC-d$_{36}$ Analogues

Solution phase NMR studies of C_{15} *m*DAP-lipid II with all four O-DPC-d$_{36}$ analogues, as well as NMR studies of Oct-TriA$_1$ with C_{15} *m*DAP-lipid II in each of the analogues were also performed by Dr. Sorina Chiorean. The chemical shift observed for each amino acid and the lipid II residues are published in Chapter 4 of her PhD thesis. The

final set of NMR experiments conducted were titration experiments of Oct-TriA_1 into O-DPC-d_{36} micelles containing C_{15} *m*DAP-lipid II . Data acquisition and chemical shift assignments were completed by Dr. Sorina Chiorean, and the results obtained are published in Chapter 4 of her PhD thesis.

4.6.1. Synthesis of Undeuterated O-DPC Analogues for NMR Studies with Leucocin A

As a continuation of work previously done within our group, we next sought to determine the structure of leucocin A (Figure 4.9) with analogues of DPC (3-O-DPC, 5-O-DPC, 6-O-DPC and 10-O-DPC). We envisioned that these modifications would have some effect in the local environment experienced by this membrane-interacting peptide embedded within the micelle.

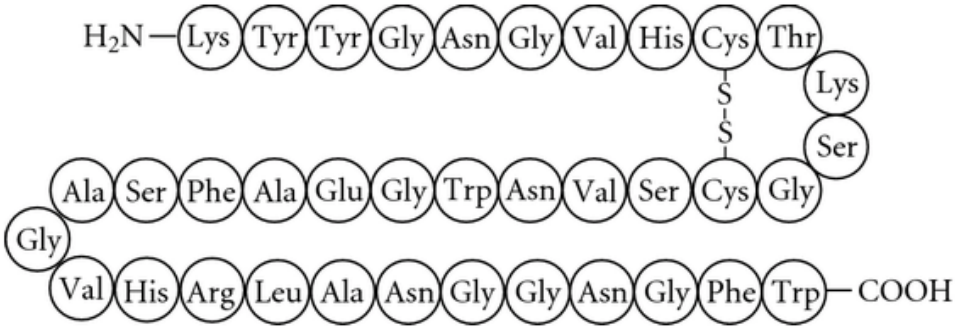
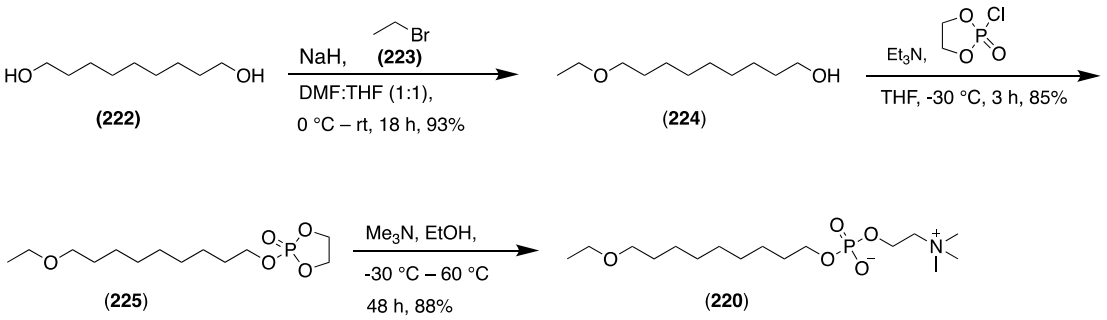


Figure 4.9 Structure of Leucocin A

4.6.1.1. Synthesis of 10-O-DPC analogue

The synthesis of 10-O-DPC (**221**) was accomplished in three steps from commercially available 1,9-nonanediol (**222**) (Scheme 4.19). Treatment of **222** with 1-bromoethane (**223**) in the presence of NaH yields the C_{11} alcohol **224** in 93% yield. The alcohol was

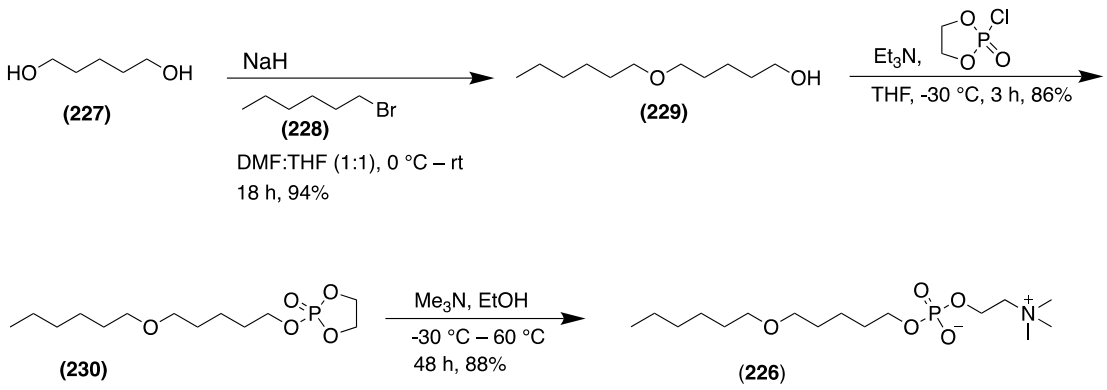
reacted with 2-chloro-1,2,3-dioxaphopholane-2-oxide in the presence of triethylamine to give the phosphorane compound **225** in 85% yield. The phosphorane compound was treated with trimethylamine under anhydrous condition at -30 °C to yield 10-O-DPC (**220**) in 88% yield. Ion exchange chromatography and silica column chromatography were employed to obtain the pure compound.



*Scheme 4.19 Synthesis of undeuterated 10-O-DPC analogue (**221**)*

4.6.1.2. Synthesis of 6-O-DPC Analogue

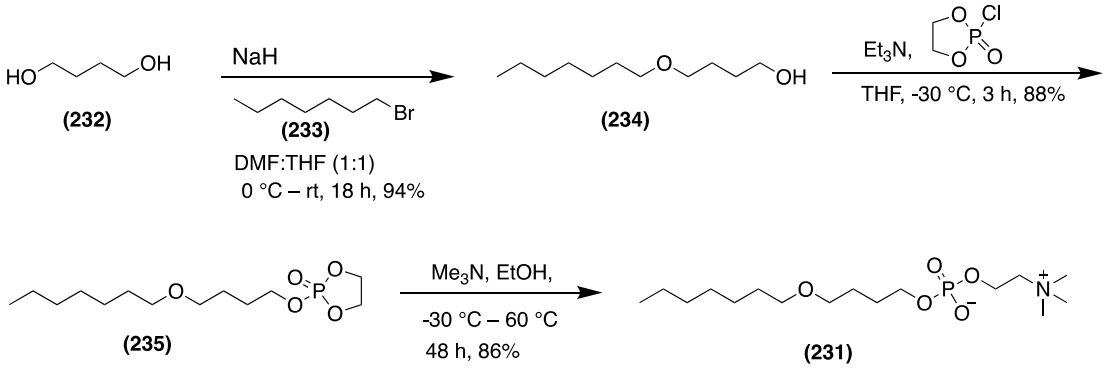
The synthesis of 6-O-DPC (**226**) was accomplished in three steps from commercially available 1,5-pentanediol (**227**) (Scheme 4.20). The first step involves the formation of the alkoxide, which was done using NaH, followed by dropwise addition of 1-bromohexane (**228**) to form the C_{11} alcohol **229** in 94% yield. The alcohol was reacted with 2-chloro-1,2,3-dioxaphopholane-2-oxide in the presence of triethylamine to give compound **230** which was used immediately in the next step. The phosphorane compound was treated with trimethylamine under anhydrous conditions at -30 °C to give the 6-O-DPC (**226**) in 88% yield after ion exchange chromatography and silica column chromatography purification.



*Scheme 4.20 Synthesis of undeuterated 6-O-DPC analogue (**226**)*

4.6.1.3. Synthesis of 5-O-DPC Analogue

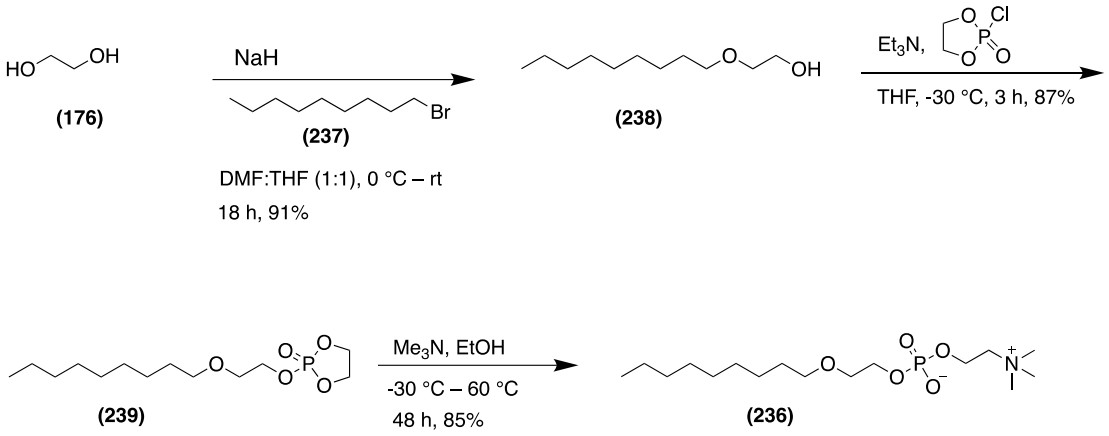
The synthesis of 5-O-DPC (**231**) was accomplished in three steps from 1,4-butanediol (**232**) (Scheme 4.21). The diol was treated with NaH to form the alkoxide, which was then reacted with 1-bromoheptane (**233**) to form the C_{11} alcohol **234** in 94% yield. The alcohol was reacted with 2-chloro-1,2,3-dioxaphopholane-2-oxide in the presence of triethylamine to give the phosphorane compound **235** which was used immediately in the next step. Compound **235** was treated with trimethylamine under anhydrous conditions at -30 °C to give the 5-O-DPC (**231**) in 86% yield after ion exchange chromatography and silica column chromatography purification.



*Scheme 4.21 Synthesis of undeuterated 5-O-DPC analogue (**231**)*

4.6.1.4. Synthesis of 3-O-DPC Analogue

The synthesis of 3-O-DPC (**236**) was accomplished in three steps from commercially available ethyleneglycol (**176**) (Scheme 4.22). Compound **176** was reacted with 1-bromononane (**237**) in the presence of NaH yields the C_{11} alcohol **238** in 91%. The alcohol was reacted with 2-chloro-1,2,3-dioxaphopholane-2-oxide in the presence of triethylamine to give the phosphorane compound **239** which was used immediately in the next step. The phosphorane compound **239** was treated with trimethylamine under anhydrous conditions at -30 °C to yield 3-O-DPC (**236**) in 85% yield after ion exchange chromatography and silica column chromatography purification.

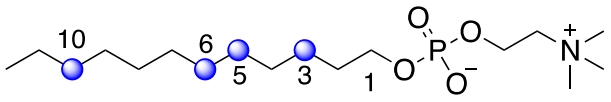


*Scheme 4.22 Synthesis of undeuterated 3-O-DPC analogue (**270**)*

4.6.2. Synthesis of Undeuterated Polyfluorinated DPC Analogues for Solution NMR Studies with ^{13}C and ^{15}N Labelled Leucocin A

Fluorine has been shown to be an efficient NMR reporter group for the study of biomolecules such as proteins or peptides in the presence of phospholipid model membranes.[51] There have been many reports of incorporation of single fluorine atoms or trifluoromethyl groups into peptides or proteins, while still maintaining native

function and conformation of the biomolecule.[133-136] Investigation of structural changes and peptide-lipid interactions can be achieved with the use of these modified biomolecules.[137-142] Fatty acids that are monofluorinated have been shown to be promising ^{19}F NMR reporters when they are incorporated biosynthetically into cell membranes.[143, 144] The use of dimyristoylphosphatidylcholine (DMPC) in model membranes for NMR to mimic the eukaryotic plasma membrane is well studied.[145, 146] Using solid state NMR, monofluorinated and polyfluorinated DMPC analogues have been used to understand their impact on the orientation of several antimicrobial peptides (AMP) in a membrane, as well as the impact of the presence of an AMP on the lipid fluorine local environment.[51, 147] To the best of our knowledge, there is no reported literature on the use of polyfluorinated DPC as a membrane mimic to understand the orientation and structural dynamics of AMPs. We decided to synthesize DPC analogues having a CF_2 at a specific site on the chain and examine these with universally labelled [^{13}C,^{15}N]leucocin. The target compounds are designated 3-F_2-DPC, 5-F_2-DPC, 6-F_2-DPC, and 10-F_2-DPC. (Figure 4.10).



● = CF_2

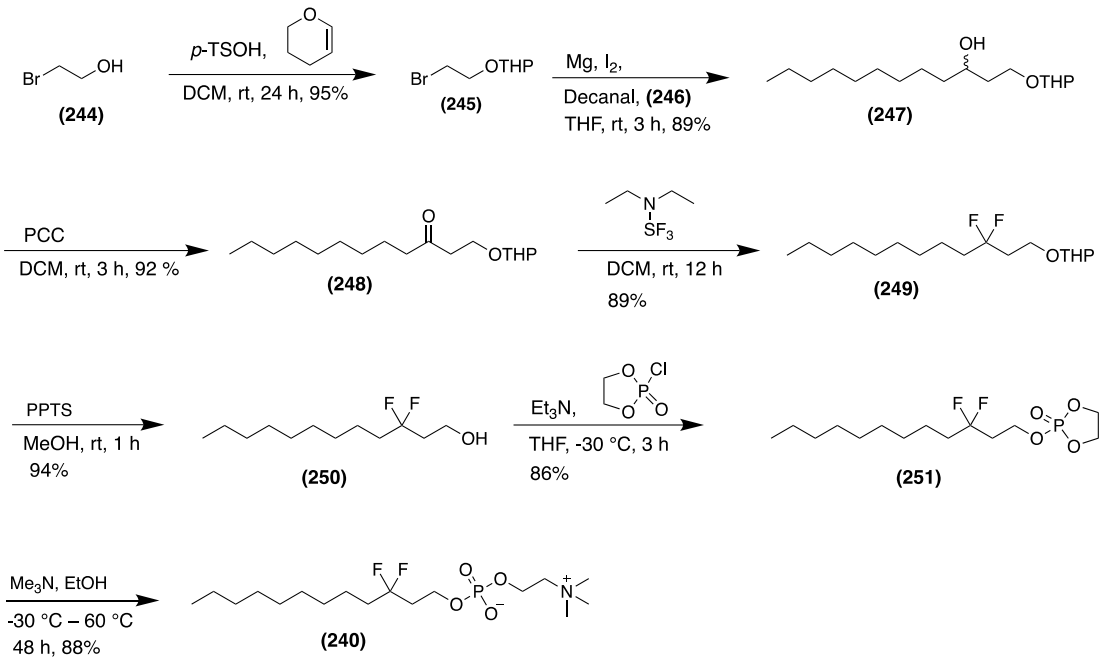
(240); 3-F_2-DPC **(242)**; 6-F_2-DPC

(241); 5-F_2-DPC **(243)**; 10-F_2-DPC

Figure 4.10 Structure of F_2-DPC analogues

4.6.2.1. Synthesis of 3-F_2-DPC Analogue

The synthesis of 3-F_2-DPC (**240**) was accomplished in seven steps from commercially available 2-bromoethanol (**244**) following literature procedures (Scheme 4.23).[148-150] The alcohol of **244** was protected using dihydropyran, which yields **245** in 95% yield followed by a Grignard reaction with decanal (**246**) to give the alcohol **247** as a mixture of isomers. Oxidation of the secondary alcohol to a ketone was done using pyridinium chlorochromate (PCC) to give **248** in 92% yield. Treatment of the ketone with diethylaminosulfur trifluoride (DAST) in CH_2Cl_2 gave the polyfluorinated compound **249** in 89% yield, which was treated with pyridinium *p*-toluenesulfonate to deprotect the alcohol protecting group yielding **250** in 94% yield. The alcohol **250** was reacted with 2-chloro-1,2,3-dioxaphopholane-2-oxide in the presence of triethylamine to give the phosphorane compound **251**, which was used immediately in the next step. The phosphorane compound was treated with trimethylamine under anhydrous conditions at -30 °C giving 3-F_2-DPC (**240**) in 88% yield.

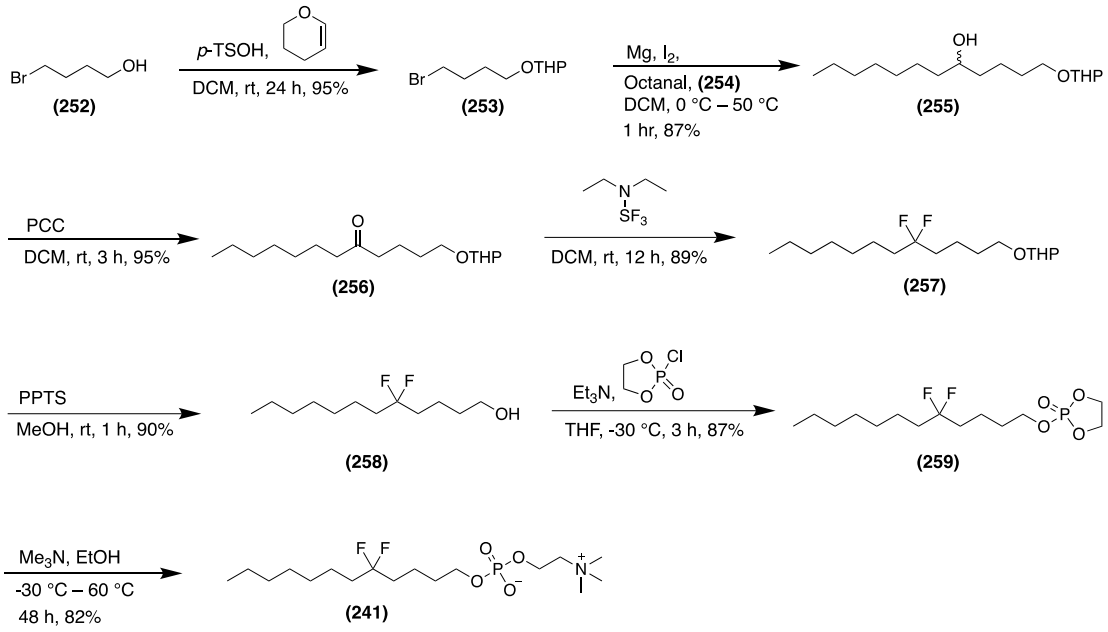


*Scheme 4.23 Preparation of 3-F_2-DPC (**240**)*

4.6.2.2. Synthesis of 5-F_2-DPC Analogue

The synthesis of 5-F_2-DPC (**241**) was accomplished in seven steps from commercially available 2-bromobutanol (**252**) (Scheme 4.24). The alcohol of **252** was protected using dihydropyran to yield **253** in 95%, followed by a Grignard reaction with octanal (**254**) to give the alcohol **255** as a mixture of isomers in good yields. The alcohol was oxidized to the ketone **256** with the use of PCC in 95% yield. Treatment of **256** with DAST in CH_2Cl_2 gave the polyfluorinated compound **257,** which was treated with pyridinium *p*-toluenesulfonate to deprotect the alcohol protecting group to give **258** in 90% yield. The alcohol was reacted with 2-chloro-1,2,3-dioxaphopholane-2-oxide in the presence of triethylamine to give the phosphorane compound **259**, which was used immediately in the next step. The final step involves treatment of the phosphorane compound **259**

with trimethylamine under anhydrous conditions at -30 °C to give 5-F_2-DPC (**241**) in 82% yield.

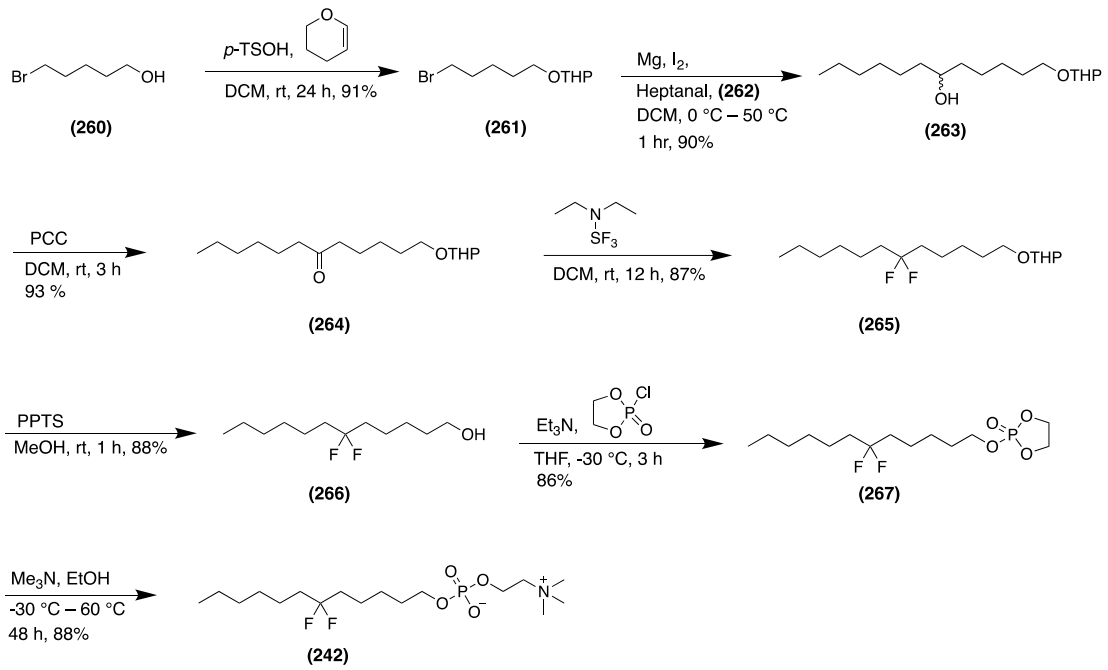


*Scheme 4.24 Preparation of 5-F_2-DPC analogue (**241**)*

4.6.2.3. Synthesis of 6-F_2-DPC Analogue

The synthesis of 6-F_2-DPC (**242**) was accomplished in seven steps from commercially available 5-bromopentanol (**260**) (Scheme 4.25). The alcohol was protected using dihydropyran which yields **261** in 91%, followed by a Grignard reaction with heptanal (**262**)**,** which gave the alcohol **263** as a mixture of isomers in good yields. The secondary alcohol was oxidized to the corresponding ketone **264** in 93% yield using PCC. The ketone **264** was treated with DAST in CH_2Cl_2, which yields the polyfluorinated compound **265** in 87%. The THP protecting group was removed with the use pyridinium *p*-toluenesulfonate, which gives **266** in 88% yield. The alcohol was reacted with 2-chloro-1,2,3-dioxaphopholane-2-oxide in the presence of triethylamine

to give the phosphorane compound **267**. In the final step, the phosphorane compound **267** was treated with trimethylamine under anhydrous conditions at -30 °C to give 6-F_2-DPC **(243)** in 88% yield.

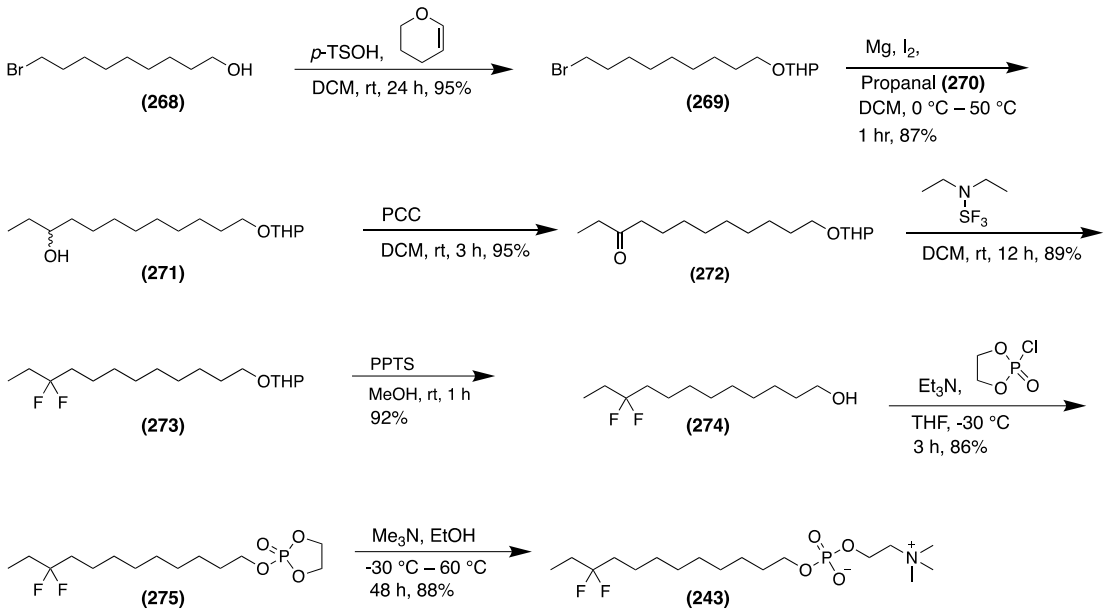


*Scheme 4.25 Preparation of 6-F_2-DPC analogue (**242**)*

4.6.2.4. Synthesis of 10-F_2-DPC Analogue

The synthesis of 10-F_2-DPC (**243**) was accomplished in seven steps from 9-bromo-1-nonanol (**268**) and propanal (**270**) (Scheme 4.26). Protection of the hydroxy group of **268** was done using dihydropyran which yields **369** in 95%, followed by a Grignard reaction with propanal (**270**) to give **271** as a mixture of isomers. Oxidation of the secondary alcohol to ketone was done using PCC to give the ketone compound (**272**) in 95% yield. Treatment of **272** with DAST in CH_2Cl_2 gave the polyfluorinated compound **273**, which upon treatment with pyridinium *p*-toluenesulfonate deprotected the alcohol to give **274** in 92% yield. The alcohol was reacted with 2-chloro-1,2,3-

dioxaphopholane-2-oxide in the presence of triethylamine to give the phosphorane compound **275**, which was used immediately in the next step. The phosphorane compound **275** was treated with trimethylamine under anhydrous conditions at -30 °C to give 10-F_2-DPC (**243**) in 88% yield.

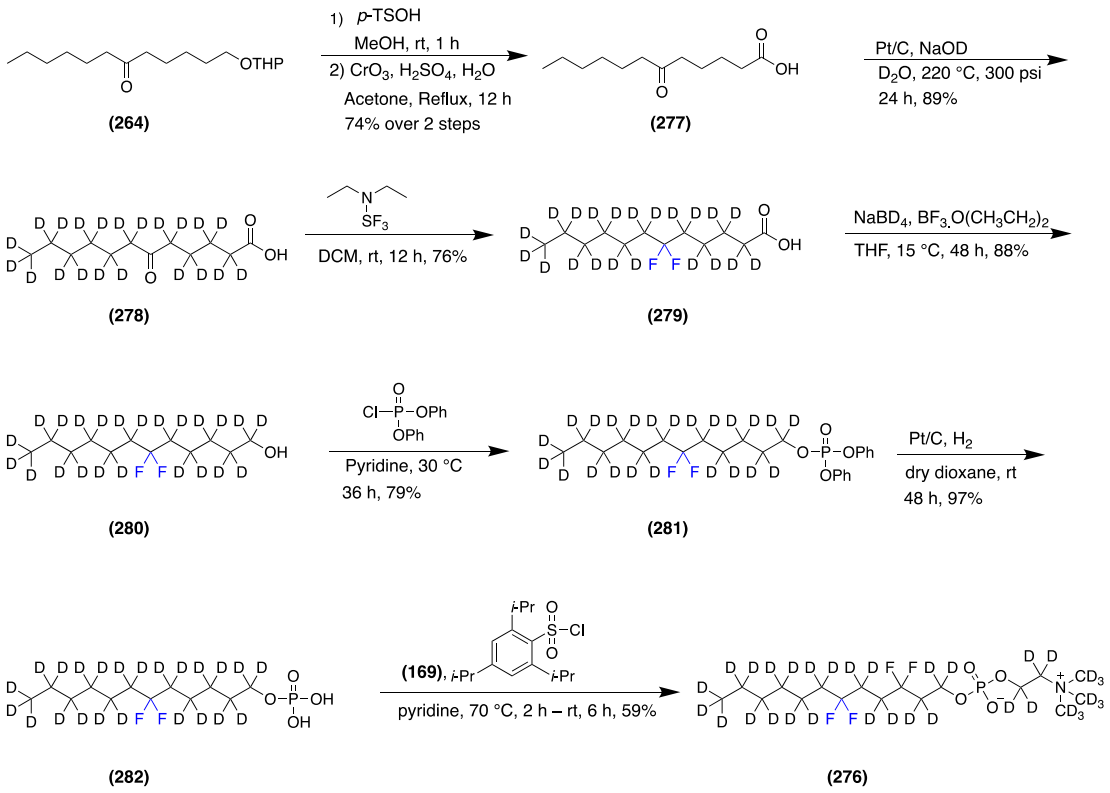


*Scheme 4.26 Preparation of 10-F_2-DPC analogue (**243**)*

4.6.3. Synthesis of Deuterated Polyfluorinated-DPC$_{d36}$ Analogues

There has been an increasing use of ^{19}F in NMR studies of biomolecular complexes in the past decades.[139] Due to the high electronegativity of fluorine, it can have a significant electronic impact on a molecule when it replaces hydrogen atoms.[51] Perfluorine-labeled lipids have been used to develop highly stable, less hemolytic, and surface-active vesicles in the past years.[151, 152] As a continuation of our ongoing research in understanding the mechanism of action of unlabeled antimicrobial peptides that are not labelled and to determine their structures in lipid micelles, we also chose to prepare deuterated F_2-DPC-d_{36} analogues for solution NMR studies. Four different

F_2-DPC analogues (3-F_2-DPC_{d36}, 5-F_2-DPC_{d36}, 6-F_2-DPC_{d36}**,** and 10-F_2-$DPCd_{36}$) were considered. I have synthesized one of these analogues, 6-F_2-DPC_{d36} (**276**), as a proof of concept. The synthesis of 6-F_2-DPC_{d36} (**276**) was accomplished following a literature procedure starting from **264** (Scheme 4.27).[153-155] The protecting group on the alcohol of **264** was removed using *p*-toluenesulfonic acid to give the alcohol, which was then oxidized to the corresponding carboxylic acid **277** in 74% yield using Jones reagent. Deuteration of the acid was done using D_2O and NaOD in the presence of Pt/C catalyst, which gives **278** in 89% yield and 93% D. To achieve a higher percentage of deuteration, the above method was repeated with fresh reagents and the final percentage of deuteration was calculated to be 98.6% D. Treatment of **278** with DAST yields the difluoro compound **279** in 76%, which was treated with $NaBD_4$ and boron trifluoride diethyl etherate to yield the alcohol **280** in 88%. The alcohol was reacted with diphenyl phosphoryl chloride to yield **281** in 79%, followed by treatment with Pt/C under hydrogen gas to yield the lipid phosphate **282** in 95%. The final step is coupling of the lipid phosphate with choline (**169**) to give the 6-F_2-DPC-d_{36} (**276**) in 59% yield.



*Scheme 4.27 Preparation of 6-F_2-DPC-d_{36} (**276**)*

4.7. Conclusions and Future Directions

Tridecaptin A_1 is an antimicrobial lipopeptide that selectively interacts with Gram-negative bacteria. It first binds to the lipopolysaccharide layer, which then permeates the outer membrane and subsequently allows the peptide to bind to *m*DAP-lipid II on the surface of the inner-membrane, thereby disrupting the proton-motive force leading to cell death. Although the presence of the N-terminal lipid tail of tridecaptin A_1 is not critical for crossing the outer membrane, it still plays a role in the compound's antimicrobial activity. It has been previously shown by our group that a key interaction exists between the γ-amino group of D-Dab8 of tridecaptin A_1 and the ε-carboxylate of *meso*-diaminopimelic acid of *m*DAP-lipid II, which is essential for antimicrobial activity. The selective antimicrobial effect of tridecaptin A_1 against Gram-negative

bacteria is mainly due to the selective binding to the Gram-negative variant of lipid II. When tridecaptin A_1 is exposed to lipid II in the presence of DPC micelles, it undergoes a conformational change. In this chapter, the synthesis of four DPC analogues (3-O-DPC-d_{36}, 5-O-DPC-d_{36}, 6-O-DPC-d_{36}, and 10-O-DPC-d_{36}) where a methylene on the lipid tail of DPC-d_{38} is replaced with an oxygen atom yielding O-DPC_{d36} was successful and has been used to study Oct-TriA_1 in micelles formed by these analogues. The structure of *m*DAP-lipid II in these O-DPC-d_{36} analogues was also determined by solution NMR. As part of our ongoing research to determine the structure of antimicrobial peptides in the lipid membrane, undeuterated O-DPC and F_2-DPC analogues have been synthesized for future work using a membrane-interacting peptide, such as leucocin A, in isotopically form. The structure of leucocin A has been previously determined in commercially available DPC micelles by our group.[115] Furthermore, unlabelled leucocin A will require deuterated O-DPC-d_{36} analogues whose synthesis I have completed. The synthesis of F_2-DPC-d_{36} analogues is underway, with one analogue prepared. The synthesis described here could be adapted to make various analogues of phospholipids for structural studies of a wide variety of antimicrobial peptides, a large number of which are found or believed to interact with cellular membranes.

Chapter 5. Summary and Conclusion

There has been considerable interest in antimicrobial peptides that bind lipid II due to the hypothesis that these compouds tend to to avoid development of bacterial resistance. Detailed understanding of the binding of these peptides to lipid II is useful when considering the use of these compounds for the design of next generation antibiotics. Structural dynamics and conformational studies of antimicrobial peptides with membranes have been mostly studied in membrane mimics; however, there is still uncertainty to the extent to which these artificial media enable native binding mode[48, 156]. Tridecaptin A_1 targets lipid II of Gram-negative bacteria. This version of lipid II usually possesses a *meso*-DAP residue on the pentapeptide as opposed to the lysine residue often found in Gram-positive lipid II. Liposomal experiments are a way to have a better insight into the physiological mode of action of tridecaptin A_1. This experiment would require native Gram-negative lipid II and solid-state NMR due to issues with solubility, large size and low tumbling of the vesicles on the NMR timescale of solution-phase NMR. Chapter 2 of this thesis outlined the use of solid-state NMR to try to understand the physiological mode of action of tridecaptin A_1 in a liposomal environment. The synthesis of Gly3 and Phe9 labelled Oct-TriA_1 and the synthesis of native Gram-negative lipid II was successful. Our collaborators successfully performed solid-state NMR experiments of the lipid II bound state of Oct-TriA_1 in liposomes. The bound state is high on the microsecond timescale and displays intriguingly fast relaxation time. The bound state is highly heterogenous and does not adopt a well-defined conformation. We found that the buffer's pH has a drastic effect on the peptide

conformation, and there was no shift on the PPi from ^{31}P NMR, confirming that Oct-TriA_1 does not interact with PPi.

Recently identified cycloalanopine led us to develop a synthetic route to obtain all isomers of this compound. Activity assay experiments revealed that only the *meso* isomer was active against certain strains of bacteria. Through structural modification studies, the synthesis of four analogues of this compound revealed that a glycine analogue was the most active when compared to the isomer of the initially reported cycloalanopine. The strategy of preparing these kinds of cyclic opines may be extended to the synthesis of other amino acid-derived opines.

Phospholipids are a class of essential biomolecules that are an integral part of biological membranes and are also involved in a variety of signalling pathways.[157] The nature of the lipid molecule structure in biological membranes defines the biophysical properties of the membrane and provides a particular environment for various membrane-anchored molecules such protein receptors.[158] DPC is widely used as a membrane-mimic model in the determination of structures of various antimicrobial peptides.[156] Analogues of DPC where the methylene on the lipid tail is replaced with oxygen were successfully synthesized. Structural elucidation and chemical shift differences of Oct-TriA_1 and C_{15}-*Z,Z-m*DAP-lipid II were completed in micelles formed by each of the four O-DPC-d_{36} analogues by Dr. Sorina Chiorean. Solution NMR studies of other antimicrobial peptides, such as leucocin A, in this oxygen or fluorinated DPC analogues may allow for further understanding of how these antimicrobial peptides are able to exert their membrane pore-forming effects.

Chapter 6. Experimental Procedures

6.1. General Synthetic Details

6.1.1. Reagents, solvents, and purification

Reactions involving either air or moisture sensitive reagents were conducted under a positive pressure of dry Ar gas. All solvents and chemicals were reagent grade and used as supplied unless otherwise stated. ^{13}C labelled and deuterium reagents were purchased from Cambridge Isotope Laboratories. All other chemical reagents were purchased from Sigma–Aldrich Chemical Company, Alfa Aesar, AK Scientific, Tokyo Chemical Industry, Carbosynth Limited, and Chem Impex International. Anhydrous solvents required were dried according to the procedures outlined in Perrin and Armarego.[159] Both tetrahydrofuran and Et_2O were distilled over sodium and benzophenone under a dry Ar atmosphere. Acetonitrile, dichloromethane, methanol, pyridine, and triethylamine were distilled over calcium hydride and used immediately or stored over activated molecular sieves. 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 refer to aqueous solutions. Brine refers to a saturated solution of NaCl. Removal of organic solvents was performed under reduced pressure, below 40 °C, using a Büchi rotary evaporator. Water was removed by lyophilization. All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC). Analytical TLC was done on glass plates (5 × 3 cm) pre-coated (0.25 mm) with silica gel (normal SiO_2, Merck 60 F254). Compounds were visualized by exposure to UV light and/or by exposing the plates to a $KMnO_4$ solution, followed by heating. Flash chromatography was performed on silica

gel (EM Science, 60 Å pore size, 230 – 400 mesh). Compounds were visualized by exposure to UV light and by staining with $KMnO_4$, followed by heating with a heat gun.

6.1.2. Compound Characterization

Nuclear magnetic resonance (NMR) spectra were obtained on a Varian Inova 400, Varian Mercury 400, Varian Inova 500, Varian Inova 600 or an Agilent VNMRS 700 MHz spectrometer. ^{1}H NMR chemical shifts are reported in parts per million (ppm) using the residual proton resonance of the solvent as reference: $CDCl_3$ δ 7.26, CD_2Cl_2 δ 5.32, and CD_3OD δ 3.31. ^{13}C NMR chemical shifts are reported relative to $CDCl_3$ δ 77.06, CD_2Cl_2 δ 53.8, and CD_3OD δ 49.0. Infrared spectra (IR) were recorded on a Nicolet Magna 750. Cast film refers to the evaporation of a solution on a NaCl plate. Gas Phase IR spectra were obtained using a 10 cm gas cell, with KBr window on a Thermo Nicolet 8700 (Madison WI) equipped with a liquid nitrogen cooled MCT/B detector. The spectral resolution was 0.250 wavenumbers from 400 to 4000 wavenumbers with 128 co-added scans for both the sample and background. Optical rotations were measured on Perkin Elmer 241 polarimeter with a microcell (10 cm, 1 mL) at 23 °C. Mass spectra were recorded on a Kratos IMS-50 (high resolution, electron impact ionization (EI)) or by using an Agilent Technologies 6220 orthogonal acceleration TOF instrument equipped with +ve and –ve ion ESI ionization source, and full-scan MS (high resolution analysis) with two-point lock mass correction operating mode. The instrument inlet was an Agilent Technologies 1200 SL HPLC system. GC-MS analysis of headspace gas was performed using a Bruker Scion 456-GC-TQ GC-MS instrument (Billerica, Massachusetts, United States). The column used was a

Phenomenex (Zebron ZB-5 fused silica capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness). The method used was as follows: manual injection of 100 μL headspace gas, injector at 200 °C, split rate 50:1, constant flow rate at 1 mL/min, helium as carrier gas, isocratic column oven temperature at 50 °C; mass range 10 – 200 Da, total run time 10 min.

6.2. General Method for Manual Fmoc Solid Phase Peptide Synthesis

Solid-phase peptide synthesis was performed on a 25 or 50 mmol scale using Fmoc chemistry on preloaded trityl resin (100-200 mesh) (Chem Impex). Reactions were performed in a custom-built 20 mL glass fritted column fitted with a T-joint and three-way T-bore Teflon stopcock. The resin was pre-swollen in DMF (5 mL, 10 min) by bubbling with argon. Between deprotections and couplings the vessel was drained under argon pressure and washed with DMF (3 × 5 mL). The Fmoc group was removed by bubbling with 20% piperidine in DMF (3 × 5 mL × 5 min). The deprotection steps were monitored by UV absorbance. The next Fmoc-amino acid (5 equiv) was pre-activated by shaking with HATU (5 equiv) and DIPEA (10 equiv) in DMF (5 mL) for 5 min. The resin was bubbled with argon in the coupling solution for 1 h, drained, and washed with DMF (3 × 5 mL). Appropriate deprotection and coupling steps were continued to complete the peptide synthesis. The resin-bound peptide was washed with CH_2Cl_2 (3 × 5 mL) and dried under argon for 20 min. The resin was transferred to a screw top vial containing trifluoroacetic acid TFA:TIPS:H_2O (95:2.5:2.5, 5 mL) and gently shaken for 2 h. The cleavage solution was filtered and concentrated *in vacuo* and the crude peptide was precipitated with cold Et_2O. The crude peptide was dissolved in

H_2O:MeCN (1:1, 5 mL) and purified by HPLC. The product-containing fractions were lyophilized to yield pure products.

6.3. HPLC Purification Methods

Method 1: Gilson preparative system, Phenomenex C_{18} column, 150 x 2.1 mm, flow rate 10 mL/min, detected at 220 nm. Gradient: starting from 20% MeCN (0.1% TFA) and 80% water (0.1% TFA) for 5 min, ramping up to 50% MeCN over 25 min, then ramping up to 95% MeCN over 5 min, staying at 95% MeCN for 5 min, ramping down to 30% MeCN over 3 min, then staying at 30% MeCN for 5 min.

Method 2: Gilson analytical system, Vydac C_{18} column, 150 x 2.1 mm, flow rate 10 mL/min, detected at 220 nm. Gradient: starting from 15% MeCN (0.1% TFA) and 85% water (0.1% TFA) for 5 min, ramping up to 70% MeCN over 30 min, then ramping up to 90% MeCN over 4 min, staying at 90% MeCN for 3 min, ramping down to 15% MeCN over 3 min, then staying at 15% MeCN for 5 min.

Method 3: Gilson preparative system, Phenomenex C_{18} column, 150 x 2.1 mm, flow rate 10 mL/min, detected at 220 nm. Gradient: starting from 0% MeOH and 100% 50 mM NH_4HCO_3 5min, ramping up to 100% MeOH over 30 min, ramping down to 30% MeOH over 2 min, then staying at 30% MeOH for 2 min.

6.4. Ion-Exchange Chromatography

Compounds with phosphate or pyrophosphate functional groups were purified using an ion-exchange chromatography technique in lieu of silica flash chromatography. Resin (DOWEX 50WX8, 100-200 mesh resin) was presoaked for 1 h in deionized water; approximately 1 g of resin was used for 10 mg of the crude mixture to be separated.

The resin was then transferred to a glass column with a faucet bottom and peristaltic pump. The column was flushed with water (5 mL/min flow rate) until eluent was clear and reached a neutral pH of 7. The resin was charged with ammonium counter ions by flushing with NH_4OH (4 × column volume) and then brought back to neutral pH using water. The elution buffer (49:1 25 mM NH_4HCO_3:IPA) was used to prime the column (2 × column volume) and the flow rate was adjusted to 1 mL/min. Crude sample was loaded and appropriately sized fractions were collected. Fractions containing compound of interest were frozen and lyophilized to yield the ammonium salt of the desired compound.

6.5. Preparation of Oct-TriA1 and Lipid II in DOPC Liposomes for Solid State NMR Studies

These experiments were performed by Dr. Markus Weingarth and co-workers at Utrecht University, Netherlands. Oct-$TriA_1$ **(47)** and Lipid II-DAP **38** stocks were prepared using MilliQ water. The stock solution of Tridecaptin was prepared at 3.0 mM in H_2O. Lipid II-DAP stock solution was prepared in 1:1 H_2O:MeOH to a final concentration of 2.6 mM. 50 nmol (19.2 µL from stock) of Lipid II, 100 nmol (33.0 µL from stock) of Oct-$TriA_1$, and 2450 nmol of DOPC were added and everything was mixed in 1.2 mL MeOH, 0.8 mL $CHCl_3$, and 0.4 mL buffered H_2O (solvent ratio is 3:2:1 MeOH:$CHCl_3$:H_2O). The buffer was 15 mM Tris, 25 mM NaCl, pH 7. $CHCl_3$ and MeOH were slowly evaporated using dry N_2 gas flux to obtain liposomes in ~ 400 µL buffer with unchanged pH at 7. Liposomes were spun-down and filled into an NMR rotor and measurements were taken at 11.75 T under 12 MAS and 250 K.

6.6. General Microbiology Procedures

6.6.1. Antimicrobial Activity Studies

These experiments were conducted by Dr. Sorina Chiorean and Dr. Marco van Belkum. Spot-on-lawn assays were performed to determine the antibacterial activity of the various opine analogs. *Salmonella typhimurium*, *Escherichia coli*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were grown in Luria broth medium at 37 °C. *Enterococcus faecalis* and *Listeria monocytogenes* were grown in All Purpose Tween medium at 25 °C, whereas Staphylococcus aureus was grown in Tryptic Soy Broth medium at 25 °C. Overnight cultures were used to inoculate 5 mL of soft agar (0.75% agar) containing the appropriate medium and poured onto hard agar media (1.5% agar) plates. Compounds to be tested for inhibitory activity were dissolved in MQ-H_2O or DMSO and various concentrations were made by series of two-fold dilution of the opine stock solutions. An aliquot of 10 µL from each concentration was spotted onto the plates and, after drying, plates were incubated overnight at the appropriate temperature. Minimum inhibitory concentration was determined from triplicates of this assay based on inhibited growth observed at the location of opine solution spotting.

6.7. Spectroscopy Analysis

6.7.1. CD Spectroscopy

CD spectra were recorded on an OLIS DSM 17 CD spectrometer. Samples were added to a 0.4 mL quartz cuvette with 0.1 cm path length, measured from 190 – 250 nm at 20 °C and averaged over 5 scans. The peptide concentration remained constant at 20 µM for all experiments. The CD spectrum of phosphate buffer was subtracted from all spectra. For the vesicle experiments, 10 lipid equivalents (by phosphate concentration)

were mixed thoroughly with the peptide solution before measuring. A digital filter of 15 was applied to CD spectra, which were converted to molar ellipticity units.

6.7.2. MALDI-TOF Mass Spectrometry

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed using an AB Sciex Voyager Elite System (Voyager, Foster City, CA) with delayed extractions. Both positive and negative mode as well as reflectron and linear methods were used. Matrices chosen were either 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid) or 4-hydroxy-α-cyanocinnamic acid (HCCA) and applied in two layers.

6.7.3. ESI MS Spectrometry

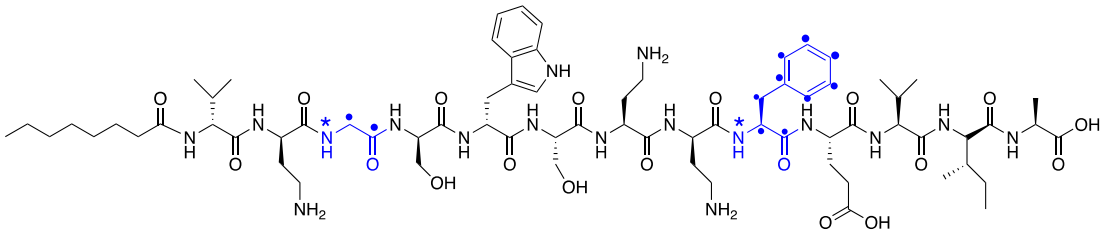
Electrospray ionization-time of flight mass spectrometry (ESI-TOF MS) was performed using an Agilent Technologies 6220 oaTOF (Agilent Technologies, Santa Clara, CA, USA). Orthogonal acceleration TOF (oaTOF) was used to collect MS data, and Fullscan MS (high-resolution analysis) with two-point lock mass correction mode was used.

6.7.4. LC-MS/MS

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed using a nanoAcquity column (100 pore Å size, 75 μm x 15 cm, 3 μm Atlantis dC18; Waters, MA). Quadrupole-time of flight Premier MS (Micromass, UK) was used to collect MS/MS data, which were processed using PEAKS 5.1 software (Bioinformatics Solutions, Waterloo, ON, Canada).

6.8. Experimental Procedure for Synthesis of labelled Oct-TriA$_1$ and Native Gram-negative and Gram-positive Lipid II

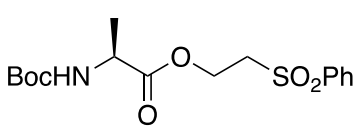
6.8.1. Labelled Octyl-Tridecaptin A$_1$ (47)



(**47**)

Resin-bound H-TriA$_1$ (100 mg, 0.0310 mmol) was stirred gently in a solution of the activated lipid (15 mg, 0.041 mmol) in CH_2Cl_2 (1 mL) at ambient temperature under argon for 24 h. The suspension was then filtered, washed with CH_2Cl_2 (5 x 5 mL) and dried under vacuum for 5 min. To simultaneously cleave the peptide from resin and remove side chain protecting groups, the dry resin was transferred to a screw-top vial containing TFA:TIPS:H_2O (95:2.5:2.5, 1 mL), and shaken for 2 h. The resin beads were removed *via* filtration through glass wool and the filtrate was concentrated *in vacuo*. The crude peptide was obtained by precipitation with cold Et_2O. The crude peptide was re-dissolved in H_2O/MeCN (3:1) containing 0.1% TFA (aq) and purified by HPLC method 1. HRMS (ESI) Calcd for $C_{61}[13C]_{11}H_{115}N_{15}[15N]_2O_{19}$ $[M+2H]^{+2}$ 1534.8722, found 1534.8725.

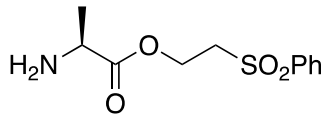
6.8.2. Boc-alanine-2-(phenylsulfonyl)-ether ester (57a)



(**57a**)

This known compound was prepared following a modified literature protocol.[49] To a stirred solution of Boc-L-alanine (7.20 g, 37.9 mmol) in dry CH_2Cl_2 (120 mL) was added 2-phenylsulfonylethanol (7.00 g, 37.9 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (7.37 g, 37.9 mmol) and 4-dimethylaminopyridne (0.470 g, 3.79 mmol) and stirred at room temperature under an argon atmosphere for 24 h. After this time, the reaction mixture was washed with 0.5 M HCl (100 mL) and saturated sodium bicarbonate (100 mL). The organic layer was washed with brine (100 mL) and dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography (SiO_2, 1:2 EtOAc: hexane), yielding the product as a light yellow oil (6.2 g, quant.) $[\alpha]_D^{25}$ = - 99.7 (*c* = 1.10, CH_2Cl_2); IR ($CHCl_3$ cast film, cm^{-1}) 3373, 3321, 3063, 2969, 2931, 1736, 1523, 1120, 724; ^1H NMR (500 MHz, $CDCl_3$) δ 7.96 – 7.89 (m, 2H), 7.76 – 7.63 (m, 1H), 7.60 – 7.54 (m, 2H), 4.51 – 4.4 (m, 2H,), 3.51 – 3.43 (m, 2H), 3.31 (q, *J* = 7.0 Hz, 1H), 1.20 (s, 9H), 1.07 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$,) δ 172.5, 154.9, 139.1, 133.9, 129.3, 127.9, 79.3, 58.9, 54.6, 48.9, 28.1, 17.5; HRMS (ESI) Calcd for $C_{16}H_{24}NO_6S$ $[M+H]^+$ 358.1246, found 358.1244.

6.8.3. Alanine-2-(phenylsulfonyl)-ether ester (87)

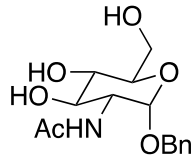


(**57**)

This known compound was prepared following a modified literature protocol.[49] The Boc-protected compound (**57a**) (6.10 g, 17.00 mmol) was dissolved in CH_2Cl_2 (30 mL) and cooled to 0 °C, and then trifluoroacetic acid (30 mL) was slowly added to the

reaction mixture and stirred for 2 h at room temperature. The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene several times to remove traces of TFA, and then dried under high vac for 24 h to give the crude product as a light-yellow oil. The crude product was purified by flash column chromatography (SiO_2, 1:4 EtOAc:hexane), yielding the product as a clear oil (4.5 g, 87% yield). $[\alpha]_D^{25}$ = - 106 (*c* = 0.55, CH_2Cl_2); IR ($CHCl_3$ cast film, cm^{-1}) 3473, 3328, 3067, 2959, 2931, 1734, 1534, 1324, 1115, 742; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.96 – 7.89 (m, 2H), 7.76 – 7.63 (m, 1H), 7.60 – 7.54 (m, 2H), 4.51 – 4.4- (m, 2H), 3.51 – 3.43 (m, 2H), 3.31 (q, *J* = 7.0 Hz, 1H), 1.19 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$,) δ 175.9, 139.5, 134.1, 129.5, 129.4, 128.2, 128.0, 58.0, 56.3, 55.2, 49.9, 20.3; HRMS (ESI) Calcd for $C_{11}H_{15}NO_4S$ $[M+H]^+$ 258.0795 found 258.0793.

6.8.4. 1-*O*-Benzyl-*N*-Acetyl- α -D-Glucosamine (55a)

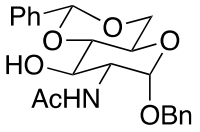


(**55a**)

This known compound was prepared following a modified literature protocol.[160] *N*-acetylglucosamine (**54**) (10.0 g, 45.2 mmol) was dissolved in benzyl alcohol (125 mL). The suspension was cooled to 0 °C and acetyl chloride (10.9 mL, 152 mmol) was added dropwise over 45 minutes, and then the reaction mixture was stirred for another 5 h at 70 °C. The reaction mixture was then cooled to 0 °C and $NaHCO_3$ was added with continuous stirring until a pH 7 was attained. The suspension was filtered through Celite and subsequently washed with MeOH (250 mL). The solvent was then removed

and Et_2O (200 mL) was added to the residue and the precipitate was filtered and dried *in vacuo*. The crude product was recrystallized from EtOH to yield a white solid as a product (12.0 g, 86% yield). $[\alpha]_D^{25}$ = + 115 (*c* = 0.53, MeOH); IR (CH_2Cl_2 cast film, cm^{-1}) 3397, 3027, 2930, 1649, 1636, 1550, 1452, 1376, 1120, 1089, 1036, 732, 693; ^1H NMR (500 MHz, CD_3OD) δ 7.38 –7.22 (m, 5H), 4.65 (s, 2H), 4.53 (d, *J* = 2.6 Hz, 1H), 3.85 (d, *J* = 6.2 Hz, 2H), 3.52 (m, 2H), 3.32 (dd, *J* = 3.4, 2.1, 2H), 3.20 (dd, *J* = 2.6, 2.18 Hz, 2H), 1.91 (s, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 173.6, 138.9, 129.3, 129.2, 128.7, 97.4, 74.0, 72.6, 72.3, 70.04, 62.6, 55.3, 22.5; HRMS (ESI) Calcd for $C_{15}H_{21}NNaO_6$ $[M+Na]^+$ 334.1261 found 334.1264.

6.8.5. 1-*O*-Benzyl-4,6-O-benzylylidene-*N*-Acetyl-a-D- glucosamine (55)

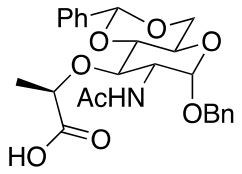


(55)

This known compound was prepared following a modified literature protocol.[160] 1-*O*-Benzyl-*N*-acetyl- α- D-glucosamine (**55a**) (10.0 g, 32.1 mmol) was co-evaporated with dry EtOH (20 mL) and dry toluene (65 mL). The compound was dried *in vacuo* for 1 h and was then dissolved in a mixture of dry DMF and dry dioxane 1:1 (60 mL). To this suspension was added triethyl orthoformate (16.0 mL, 96.0 mmol), benzaldehyde (13.0 mL, 129 mmol), and *p*-toluene sulfonic acid monohydrate (1.66 g, 9.60 mmol). The reaction mixture was stirred at ambient temperature for 24 h. Et_2O (80 mL) was then added to the suspension and was stirred for 3 h at 0 °C. The colorless precipitate was filtered off, washed with Et_2O (200 mL) and dried *in vacuo* to yield the product as a white solid (10.3 g, 80% yield). $[\alpha]_D^{25}$ = + 118 (*c* =

1.10, DMSO-d_6); IR (CH_2Cl_2 cast film, cm^{-1}) 3027, 2930, 1649, 1636, 1550, 1452, 1376, 1120, 1089, 1036, 732, 693; ^1H NMR (500 MHz, DMSO-d_6) δ 7.45 – 7.26 (m, 10H), 5.60 (s, 1H), 5.13 (d, J = 5.5 Hz, 1H), 4.80 (d, J = 3.2 Hz, 1H), 4.04 (dd, J = 14.5, 10.2 Hz, 2H), 3.84 (dd, J = 3.5, 3.3 Hz, 2H), 4.13 (d, J = 4.8 Hz, 1H), 3.94 – 3.49 (m, 5H), 1.88 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.1, 137.5, 137.4, 128.5, 128.0, 127.7, 127.4, 127.2, 126.1, 100.7, 96.8, 81.9, 68.5, 67.8, 67.1, 62.7, 54.1, 22.4; HRMS (ESI) Calcd for $C_{22}H_{25}NaNO_6$ $[M+Na]^+$ 422.1574 found 422.1573.

6.8.6. 1-O-Benzyl-4,6-*O*-benzylidene-3-*O*-((*R*)-propion-2-yl)-*N*-acetyl-a-D-glucosamine (56)

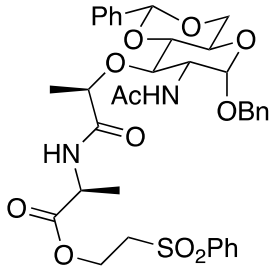


(**56**)

This known compound was prepared following a modified literature protocol.[68] The reaction was carried out under an inert atmosphere of argon. 1-O-Benzyl-4,6-O-benzylidene-3-O-((*R*)-propion-2-yl)-*N*-acetyl- α- D-glucosamine (**55**) (8.30 g, 20.7 mmol) was dissolved in dry dioxane (600 ml) and was heated to 60 °C. NaH (60% dispersion in oil, (1.47 g, 61.3 mmol)) was added to the suspension and was heated under reflux for 10 min. (*S*)-2-chloropropionic acid (12.4 g, 104 mmol) was added and the reaction mixture was stirred for 50 min. After this time, a second portion of NaH (60% dispersion in oil, (5.87 g, 245 mmol)) was added and the reaction mixture was stirred for 20 h at 60 °C. The reaction mixture was then allowed to cool to 0 °C and was slowly quenched by adding ice water (100 mL). The reaction mixture was acidified with ice-cold aq. HCl (6 M) until a pH 2 was attained and was poured into ice

water (1000 mL). The mixture was allowed to stand in an ice bath and then the precipitate formed was filtered, washed with water (3 × 100 mL) and petroleum ether (2 × 100 mL), and dried *in vacuo* to yield the product as a white solid (9.24 g, 94% yield). $[\alpha]_D^{25} = +77.5$ (*c* = 1.20, DMSO); IR ($CHCl_3$ cast film, cm^{-1}) 3298, 3032, 2925, 1654, 1560, 1121, 1089, 1079, 1052, 1021, 694; ^1H NMR (500 MHz, DMSO-*d*$_6$) δ 12.78 (s, 1H), 7.45 – 7.26 (m, 10H), 5.60 (s, 1H), 5.13 (d, *J* = 5.5 Hz, 1H), 4.80 (d, *J* = 3.2 Hz, 1H), 4.15 (t, *J* = 7.1 Hz, 2H), 4.04 – 3.98 (m, 2H), 3.84 (dd, *J* = 3.5, 3.3 Hz, 2H), 4.13 (d, *J* = 4.8 Hz, 1H), 3.94 – 3.83 (m, 1H), 3.79 – 3.66 (m, 3H), 3.54 – 3.49 (m, 1H), 1.88 (s, 3H), 1.35 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 174.8, 169.0, 137.3, 137.3, 128.5, 128.0, 127.8, 127.4, 127.3, 125.5, 100.1, 96.5, 81.4, 74.9, 74.9, 68.8, 67.7, 62.7, 53.4, 22.5, 18. 6; HRMS (ESI) Calcd for $C_{25}H_{28}NO_8$ $[M-H]^-$ 470.1484 found 470.1486.

6.8.7. Benzyl *N*-acetyl-4,6-benzylidenemuramic acid monopeptide (58)

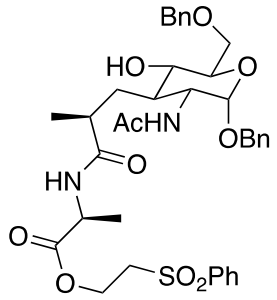


(58)

This known compound was prepared following a modified literature protocol.[68] Benzyl *N*-acetyl-4,6-benzylidine muramic acid (5.00 g, 10.5 mmol) was dissolved in 200 mL of CH_2Cl_2 and the suspension was cooled to 0 °C. *N*-methyl morpholine (NMM) (0.970 mL, 8.73 mmol) and 2-chloro-4,6-dimethoxy-1,35-triazine (1.80 g, 10.4 mmol) was added and the reaction was stirred for 1 hr. L-alanine (phenylsulfonyl ethyl ester) (**57**)

(2.70 g, 10.4 mmol) in 20 ml of CH_2Cl_2 was added to the above reaction mixture and the mixture was allowed to warm slowly to room temperature and stirred for 24 h. After this time TLC (EtOAc:hexane, 9:1, R_f = 0.43) showed the formation of a single product. The reaction mixture was washed with 1N HCl, brine, and then dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting crude product was purified by column chromatography (SiO_2, 7:3 EtOAc:hexane), yielding the product as a white solid (quant.) $[\alpha]_D^{25}$ = + 52.6 (*c* = 1.00, $CHCl_3$); IR (CH_2Cl_2 cast film, cm^{-1}) 3294, 3068, 2980, 1744, 1652, 1551, 1371, 1130, 1087, 748; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 – 7.85 (m, 2H, ArH), 7.68 – 7.62 (m, 1H, ArH), 7.54 (t, *J* = 7.7 Hz, 2H, ArH), 7.49 – 7.42 (m, 2H, ArH), 7.42 – 7.23 (m, 8H, ArH), 6.95 (d, *J* = 7.0 Hz, 1H, Ala-NH), 6.17 (d, *J* = 9.0 Hz, 2H, AcNH), 5.59 (s, 1H, OCH), 4.99 (d, *J* = 3.7 Hz, 1H, H1), 4.69 (d, *J* = 12.1 Hz, 1H, PhCHH), 4.50 – 4.37 (m, 3H, PhCHH + OCH_2), 4.29 (td, *J* = 9.3, 3.9 Hz, 1H, H2), 4.20 (dd, *J* = 10.2, 4.9 Hz, 1H, H6), 4.15 (t, *J* = 7.1 Hz, 1H, Ala1-Hα), 4.04 (q, *J* = 6.8 Hz, 1H, OCH), 3.85 – 3.81 (m, 1H, H5), 3.75 (t, *J* = Hz, 1H 2H, H6), 3.72 – 3.60 (m, 2H, H3 + H4), 3.41 (ddd, *J* = 14.6, 6.5, 5.3 Hz, 2H, SCH_2), 1.92 (s, 3H, Ac), 1.35 (d, *J* = 6.9 Hz, 3H, MurNAc-CH_3), 1.26 (d, *J* = 7.1 Hz, 3H, Ala1-Hβ); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.8, 171.8, 170.6, 137.1, 136.8, 134.2, 129.4, 128.9, 128.7, 128.4, 128.4, 128.64, 126.0, 101.2, 97.2, 81.4, 78.2, 78.6, 77.3, 70.2, 68.9, 63.2, 58.4, 55.46, 53.6, 48.2, 23.5, 19.2, 17.4; HRMS (ESI) Calcd for $C_{36}H_{46}N_2NaO_{10}S$ $[M+Na]^+$ 733.2739 found 733.2742.

6.8.8. Benzyl *N*-acetyl-4,6-benzylidenemuramic acid monopeptide ester (49)

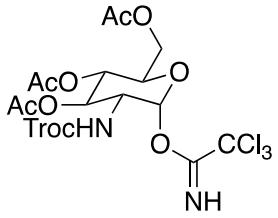


(**49**)

This known compound was prepared following a modified literature protocol.[68] Benzyl *N*-acetyl-4,6-benzylidenemuramic acid monopeptide (**58**) (4.50 g, 6.33 mmol) was dissolved in CH_2Cl_2 (70 ml) and was cooled to 0 °C. Triethylsilane (4.70 mL, 31.6 mmol) was added to this solution followed by dropwise addition of TFA (2.41 mL, 31.6 mmol). The reaction mixture was stirred for 5 hours at 0 °C after which additional TFA (1.46 mL, 18.9 mmol) was added dropwise and stirring was continued for an additional 24 h. After this time TLC (100% EtOAc, R_f = 0.41) showed a completion of the reaction and the reaction mixture was diluted with CH_2Cl_2 (60 mL). $NaHCO_3$ was added slowly to neutralize any excess TFA, followed by extraction of the aqueous layer with CH_2Cl_2. The organic layer was washed with brine (30 mL), dried with Na_2SO_4, filtered, and concentrated in vacuo, and purified by column chromatography (SiO_2, gradient: 0% – 3% MeOH in CH_2Cl_2) yielding the product as a white solid (2.5 g, 63% yield). $[\alpha]_D^{25}$ = + 82.4 (*c* = 0.80, CH_2Cl_2); IR (CH_2Cl_2 cast film, cm^{-1}) 3288, 3065, 3032, 2984, 2928, 1749, 1658, 1087, 734; ^1H NMR (500 MHz, $CDCl_3$) δ 7.94 – 7.85 (m, 2H, ArH), 7.69 – 7.63 (m, 1H, ArH), 7.57 (t, *J* = 7.7 Hz, 2H, ArH), 7.41 – 7.22 (m, 9H, ArH), 6.92 (d, *J* = 7.2 Hz, 1H, Ala-NH), 6.10 (d, *J* = 9.0 Hz, 1H, MurNAc-NH), 4.92 (d, *J* = 3.6 Hz, 1H, H1), 4.71 (d, *J* = 11.8 Hz, 1H, OCHH), 4.65 – 4.54 (m, 2H, OCHH

+ OCHH), 4.49 – 4.34 (m, 3H, OCHH + OCH_2), 4.29 – 4.17 (m, 2H, H2 + Ala1-Hα), 4.13 (t, *J* = 6.7 Hz, 1H, MurNAc-OCH), 3.80 (dd, *J* = 9.5, 4.6 Hz, 1H, H5), 3.74 (dd, *J* = 10.3, 4.5 Hz, 1H, H6), 3.72 – 3.65 (m, 2H, H3 + H4), 3.53 (dd, *J* = 10.5, 8.7 Hz, 1H, H6), 3.39 (ddd, *J* = 9.2, 6.6, 5.5 Hz, 2H, S-CH_2), 3.01 (s, 1H, OH), 1.90 (s, 3H, NHAc-CH_3), 1.40 (d, *J* = 6.7 Hz, 3H, MurNAc-CH_3), 1.30 (d, *J* = 7.2 Hz, 3H, Ala1-Hβ); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.09, 171.9, 170.4, 139.2, 137.9, 137.1, 134.2, 129.6, 128.7, 128.5, 128.3, 128.3, 128.1, 127.9, 127.8, 97.2, 80.6, 77.9, 73.8, 71.7, 70.5, 70.3, 69.9, 58.1, 55.0, 52.6, 48.1, 23.5, 19.1, 17.2; HRMS (ES) Calcd for $C_{36}H_{44}N_2O_{11}S$ $[M+H]^+$ 733.2558, found 733.2546.

6.8.9. Glycosyl Donor (50)

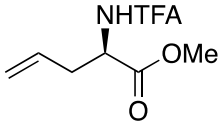


(50)

This known compound was prepared following a modified literature protocol.[49] To a vigorously stirred solution of D-glucosamine (**59**) (12.0 g, 55.6 mmol) and sodium bicarbonate (9.36 g, 111 mmol) in water was added 2,2,2-trichloroethoxycarbonyl chloride (9.18 mL, 66.7 mmol) dropwise and the solution was stirred at room temperature for 3 h, and during this time led to the formation of a white precipitate. The precipitate was filtered and washed with water several times and dried by lyophilization for 48 h to give a white solid. This product obtained was dissolved in pyridine (150 mL) and acetic anhydride (70 mL) and stirred vigorously at ambient temperature for 24 h. After this time, the reaction mixture was concentrated *in vacuo*

followed by subsequent co-evaporation with toluene (3 × 50 mL) to give the crude compound as a light-yellow oil. The crude compound was dissolved in $CHCl_3$ (150 mL) and was washed with 0.5 M HCl, brine, dried over anhydrous sodium sulfate, filtered, and concentrated in *vacuo* to give 1,3,4,6-tetra-O-acetyl-2-troc-D-glucosamine (**60**) (20.1 g) as a white solid. To a solution of the acetate **60** (14 g, 27.1 mmol) in dry DMF was added hydrazine acetate (2.95 g, 32.2 mmol), and stirring was continued for 1 h at ambient temperature. The reaction mixture was diluted with EtOAc, and the organic layer was washed with water, saturated sodium bicarbonate, brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to give a yellow oil. The resulting oil was dissolved in CH_2Cl_2 (120 mL) and trichloroacetonitrile (21.4 mL) and stirred for 10 min. To this solution was added 1,8-diazabicycloundec-7-ene (0.67 mL, 4.1 mmol) and then the mixture was stirred at room temperature for 2 h and then concentrated *in vacuo*. The crude product was purified by flash column chromatography (SiO_2, 1:2 EtOAc:hexane + 0.1% triethylamine), yielding the product as a white foam (6.9 g, 53% yield), $[\alpha]_D^{25}$ = + 76.5 (*c* = 1.00, CH_2Cl_2); IR ($CHCl_3$ cast film, cm^{-1}) 3316, 2954, 1748; ^1H NMR (500 MHz, $CDCl_3$) δ 8.80 (s, 1H, -N<u>H</u>), 6.43 (d, *J* = 3.7 Hz, 1H, H1), 5.35 (dd, *J* = 10.9 Hz, 9.6 Hz, 1H, H3), 5.25 (t, *J* = 9.8 Hz, 1H, H4), 5.16 (d, *J* = 9.4 Hz, Troc-NH), 4.76 – 4.64 (m, 2H, Troc-C<u>H</u>$_2$), 4.27 (m, 2H, H2, H6), 4.15 – 4.06 (m, 2H, H5, H6), 2.06 (m, 9H, 3 × OCH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.0, 170.4, 169.2, 160.3, 154.0, 95.5, 94.5, 90.4, 74.5, 67.2, 53.8, 20.6, 20.3; HRMS (ESI) Calcd for $C_{17}H_{20}N_2NaO_{10}$ $[M+Na]^+$ 644.9143, found 644.9141.

6.8.10. TFA-D-Agl-OMe (63)

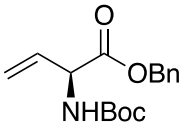


(63)

This known compound was prepared following a modified literature protocol.[70] D-allylglycine (**62**) (5.00 g, 43.3 mmol) was dissolved in dry MeOH (40 mL) and trifluoroacetate (10.0 mL, 83.6 mmol) followed by the addition of triethylamine (4.61 mL, 42.7 mmol). Stirring was continued for 24 h under argon at ambient temperature, and then the mixture was concentrated *in vacuo* after completion of the reaction. The crude oil was dissolved in EtOAc (300 mL) and the organic layer was washed with 0.1 M citric acid, brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to yield the acid as colorless oil, which was used in the next step without further purification. The acid (6.40 g, 30.3 mmol) was dissolved in 20% Cesium carbonate (140 mL) and stirred for 2 h, and then the solvent was removed *in vacuo*. To a solution of the carboxylate (5.8 g) in DMF was added MeI (2.90 mL, 28.7 mmol), and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was then concentrated *in vacuo* and dissolved in Et_2O (100 mL), washed with 5% sodium bicarbonate and brine, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to yield the crude product as a clear yellow oil. The crude product was purified by flash column chromatography (SiO_2, 1:2 EtOAc:hexane), yielding the product as a light-yellow oil (4.6 g, 93% over two steps). $[\alpha]_D^{25}$ = - 12.8 (*c* = 1.00, CH_2Cl_2); IR ($CHCl_3$ cast film, cm^{-1}) 3327, 3085, 3006, 2989, 2958, 1722, 1555, 1442, 1211, 1118; 1H NMR (500 MHz, $CDCl_3$) δ 6.89 (br, s, 1H, N<u>H</u>), 5.67 (m, 1H, -CH_2CHCH_2-), 5.18 (m, 2H, -CH_2-

CH), 4.71 (m, 1H, C<u>H</u>NH), 3.83 (s, 3H, OC<u>H</u>$_3$), 2.72 – 2.63 (m, 2H, -CH-C<u>H</u>$_2$-CH-); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6, 156.6, 130.8, 120.4, 115.6, 52.9, 52.0, 35.9; HRMS (ESI) Calcd for $C_8H_{10}F_3NNaO_3$ [M+Na]+ 248.0505 found 248.0508.

6.8.11. Boc-Vgl-OBn (65)

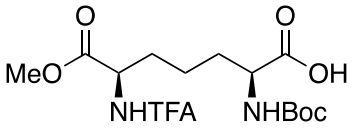


(65)

This known compound was prepared following a modified literature protocol.[161] *N*-Boc-L-glutamic acid– α-benzyl ester (**64**) (9.00 g, 24.3 mmol) and cupric acetate monohydrate (1.20 g, 6.00 mmol) was dissolved in dry benzene (250 mL) and stirred for 1.5 h at room temperature under an Argon atmosphere. After this time, lead tetraacetate (21.5 g, 48.6 mmol) was added and the resulting mixture was refluxed for 20 h, and then was cooled to room temperature after completion of the reaction. The reaction mixture was filtered through Celite and was washed with EtOAc (3 × 100 mL). The organic layer was washed with water (3 × 150 mL), brine, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to give a brown oil. The crude product was purified by flash column chromatography (SiO_2, 2:8 EtOAc:hexane), yielding the product as a colorless oil (3.8 g, 54% yield). $[\alpha]_D^{25}$ = - 15.5 (*c* = 1.40, CH_2Cl_2); IR (EtOAc cast film, cm^{-1}) 3365, 3064, 3029, 2976, 1714, 1645, 1496; ^{1}H NMR ($CDCl_3$, 500 MHz,) δ 7.34 (m, 5H, Ar-H), 5.96 (m, 1H, NH), 5.33 (dd, *J* = 17.1 Hz, 1.8 Hz, 1H, -CH-C<u>H</u>$_2$-CH), 5.24 (dd, *J* = 10.4 Hz, 1.8 Hz, 1H, -C<u>H</u>$_2$-CH-CH$_2$), 5.20 (m, 3H, -C<u>H</u>$_2$-CH-CH$_2$, C<u>H</u>$_2$Ph), 4.91 (s, 1H, -C<u>H</u>-NH), 1.44 (s, 9H, Boc-H); ^{13}C NMR

($CDCl_3$, 126 MHz) δ 170.6, 155.0, 135.3, 132.6, 128.6, 128.4, 128.2, 117.5, 80.2, 67.5, 55.9, 28.3; HRMS (ESI) Calcd for $C_{16}H_{22}NO_4$ $[M+H]^+$ 292.1545 found 292.1542.

6.8.12. Orthogonally Protected *meso* DAP (61)

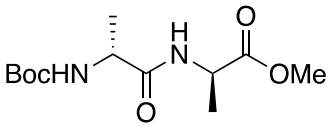


(61)

This known compound was prepared following a modified literature protocol.[161] Boc-Vgl-OBn (**65**) (3.01 g, 13.7 mmol) and TFA- D-Agl-OMe (**63**) (5.80 g, 19.8 mmol) was dissolved in dry CH_2Cl_2 (50 mL) and the reaction mixture was flushed with Argon bubbling for 5 min, after which Hoyveda-Grubbs 2nd generation catalyst (1.14 g, 1.33 mmol) was added. The reaction mixture was refluxed under argon for 24 h, then concentrated *in vacuo* to yield a black oil. The crude product was purified by flash column chromatography (SiO_2, 1:9 EtOAc:hexane), yielding the product **66** as a colorless oil (3.1 g, 46% yield). The alkene **66** (2.9 g, 5.8 mmol) and 10% palladium on carbon (350 mg) was added to a flask containing dry MeOH (65 mL) and was stirred under a hydrogen atmosphere for 1 h. The suspension was then filtered through Celite, washed with methanol, and the filtrate concentrated *in vacuo* to afford orthogonally protected *meso*-DAP as a white solid (2.0 g, 82% yield). $[\alpha]_D^{25}$ = +11.8 (*c* = 1.40, MeOH); IR (MeOH cast film, cm^{-1}) 3317, 3096, 2976, 1719, 1530, 1454; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.46 (m, 1H, Hε), 4.09 (m, 1H, Hα), 3.75 (s, 3H, OCH_3), 1.96 (m, 1H, Hδ), 1.82 (m, 2H, Hβ), 1.65 (m, 1H, Hδ), 1.48 (m, 2H, Hγ), 1.44 (s, 9H, Boc-H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 177.1, 172.6, 159.1, 158.0, 117.4, 80.5, 54.3, 53.9,

53.0, 32.6, 31.3, 31.2, 28.7, 23.4; HRMS (ESI) Calcd for $C_{15}H_{22}F_3N_2O_7$ $[M-H]^-$ 399.1385 found 399.1384.

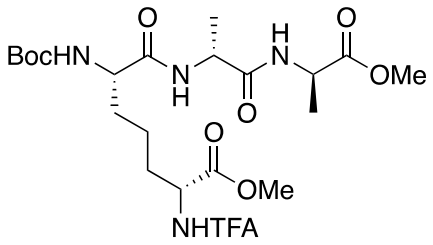
6.8.13. Boc-D-Ala-D-Ala-OMe (69)



(69)

This known compound was prepared following a modified literature protocol.[49] To a stirred solution of H-D-Ala-OMe (**68**) (4.20 g, 29.9 mmol) and Boc-D-Ala-OH (**67**) (5.69 g, 30.1 mmol) in dry DMF (180 mL) was added HATU (11.4 g, 30.1 mmol), and then the reaction mixture was cooled to 0 °C. DIPEA (5.21 mL, 90.2 mmol) was added to the reaction mixture and stirred at room temperature for 24 h. After completion of reaction, the reaction mixture was then concentrated *in vacuo*, and was dissolved in EtOAc (150 mL), and then the organic layer was washed with 0.5 M HCl, saturated sodium bicarbonate, and brine, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to yield the Boc-dipeptide as a white foam (6.4 g, 77% yield). $[\alpha]_D^{25} = +26.2$ ($c = 0.80$, CH_2Cl_2); IR (CH_2Cl_2 cast film, cm^{-1}) 3312, 3075, 2980, 2938, 1748, 1665; ^1H NMR ($CDCl_3$, 700 MHz) δ 6.68 (m, 1H, D-AlaNH), 5.05 (m, 1H, D-Ala-NH), 4.57 (m, 1H, D-Ala-Hα), 4.15 (m, 1H, D-Ala-Hα), 3.75 (s, 3H, OCH_3), 1.46 (m, 9H, Boc-H), 1.42 (d, $J = 7.2$ Hz, 3H, D-Ala-Hβ), 1.33 (d, $J = 7.2$ Hz, 3H, D-Ala-Hβ); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 173.3, 172.3, 52.6, 48.1, 28.4, 18.5,18.4; HRMS (ESI) Calcd for $C_{12}H_{22}N_2NaO_5$ $[M+Na]^+$ 297.1422, found 297.1420.

6.8.14. Boc-DAP (TFA, OMe)-D-Ala-D-Ala-OMe (70)

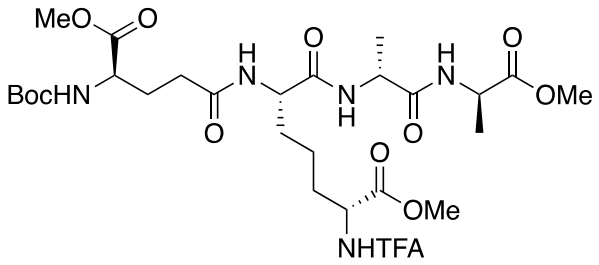


(70)

This known compound was prepared following a modified literature protocol.[49] The Boc-dipeptide **69** (850 mg, 3.10 mmol) was dissolved in CH_2Cl_2 (20 mL) and was cooled to 0 °C, TFA (20 mL) was added, and the reaction mixture was stirred for 2 h and concentrated *in vacuo*. Excess TFA was removed by co-evaporation with toluene and dried under high vac for 45 min. During this period, *meso*-DAP (**61**) (1.24 g, 3.10 mmol) and HATU (1.17 g, 3.10 mmol) were dissolved in dry DMF (15 mL), and then the solution was cooled to 0 °C and DIPEA (0.7 mL, 3.17 mmol) was added. The resulting light-yellow mixture was stirred at 0 °C for 20 min. After this time, the deprotected dipeptide in dry DMF (10 mL) and DIPEA (0.700 mL, 3.17 mmol) was added to the activated *meso*-DAP reaction mixture. The resultant reaction mixture was stirred at room temperature for 20 h and concentrated in *vacuo*. The crude oil residue was re-dissolved in EtOAc (30 mL) and washed with 0.5 M HCl, saturated sodium bicarbonate, and brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to yield the Boc-tripeptide as a white solid (1.1 g, 87% yield). $[\alpha]_D^{25}$ = - 3.30 (*c* = 0.70, CH_2Cl_2); IR (CH_2Cl_2 cast film, cm^{-1}) 3299, 3079, 2981, 2929, 1747, 1724, 1658; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.71 (d, *J* = 7.5 Hz, 3H, D-AlaNH), 6.68 – 6.56 (m, 1H, D-AlaNH), 5.08 (m, 1H, DAPγNH), 4.67 – 4.45 (m,

3H, DAP-Hε + D-AlaNH, D-Ala-NHα), 4.08 (m, 1H, DAP-Hα), 3.78 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 2.01 – 1.93 (m, 1H, DAP-Hδ), 1.92 – 1.72 (m, 2H, DAP-Hδ + DAP-Hβ), 1.70 – 1.53 (m, 2H, DAP-Hβ + DAP-Hγ), 1.43 (m, 16H, DAP-Hβ + Boc-H + D-Ala-Hβ + D-Ala-H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 173.2, 171.5, 171.2, 53.1, 53.6, 52.5, 49.0, 48.2, 31.9, 31.4, 28.3, 21.3, 18.30; HRMS (ESI) Calcd for $C_{22}H_{35}F_3N_4NaO_9$ $[M+Na]^+$ 579.2247, found 579.2243.

6.8.15. Boc-D-γ-Glu(OMe)-*meso* DAP(TFA, OMe)-D-Ala-D-Ala-OMe (51)

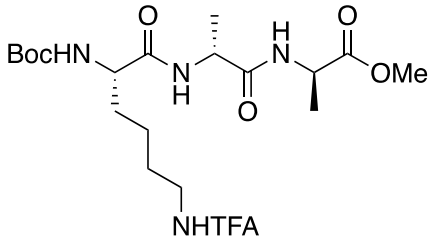


(51)

This known compound was prepared following a modified literature protocol.[49] The Boc-tripeptide **70** (800 mg, 1.4 mmol) was dissolved in CH_2Cl_2 (15 mL) and was cooled to 0 °C. TFA (15 mL) was added, and the reaction mixture was stirred for 2 h and then concentrated *in vacuo*. Excess TFA was removed by co-evaporation with toluene and dried under high vac for 1 h. Boc-D-Glu-OMe (377 mg, 1.40 mmol) and HATU (548 mg, 1.40 mmol) were dissolved in dry DMF (15 mL) and the solution was cooled to 0 °C. DIPEA (0.390 mL, 2.16 mmol) was added to the reaction mixture and the resulting yellow solution was stirred at 0 °C for 15 min. After this time, the deprotected tripeptide in dry DMF (10 mL) and DIPEA (0.390 mL, 2.16 mmol) was added to the activated Boc-D-Glu-OMe reaction mixture. The resultant reaction mixture was stirred at room temperature for 24 h and concentrated *in vacuo*. The crude oil residue was re-dissolved

in EtOAc (25 mL) and washed with 0.5 M HCl, saturated sodium bicarbonate and brine, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to yield the Boc-tetrapeptide as a white solid (902 mg, 90% yield). $[\alpha]_D^{25}$ = - 4.41 (*c* = 1.10, CH_2Cl_2); IR (CH_2Cl_2 cast film, cm^{-1}) 3299, 3079, 2981, 2929, 1747, 1724, 1658; ^1H NMR (DMSO-d_6, 700 MHz) δ 9.80 (d, *J* = 7.5 Hz, 3H, DAPε-NH), 8.20 (m, 2H, D-Ala-NH + D-Ala-NH), 7.99 (d, *J* = 7.6 Hz, 3H, DAPε-NH), 7.23 (d, *J* = 7.7 Hz, 3H, D-Glu-NH), 4.37 – 4.18 (m, 4H, DAP-Hα + DAP-Hε + D-Ala-Hα-D-Ala-Hα), 3.96 (m, 1H, Glu-Hα), 3.71 – 3.58 (m, 9H, 3 × OCH_3), 2.27 – 2.13 (m, 2H, D-Glu-Hγ), 1.95 – 1.68 (m, 4H, D-Glu-Hβ + DAP-Hδ), 1.57 (m, 1H, DAP-Hβ), 1.49 (m, 1H, DAP-Hβ), 1.43 – 1.14 (m, 17H, DAP-Hγ + Boc-H + D-Ala-Hβ + D-Ala-Hβ); HRMS (ESI) Calcd for $C_{28}H_{44}F_3N_5NaO_{12}$ $[M+Na]^+$ 722.2987, found 722.2989.

6.8.16. Boc-ε-TFA-Lys -D-Ala-D-Ala-OMe (73)

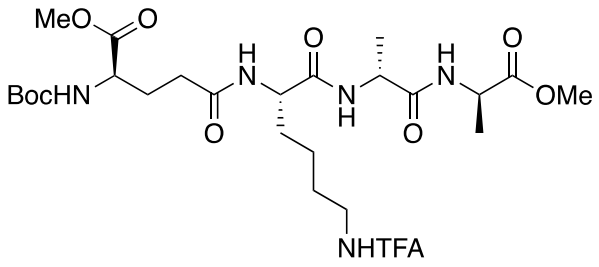


(73)

This known compound was prepared following a modified literature protocol.[49] The Boc-dipeptide **69** (507 mg, 1.85 mmol) was dissolved in CH_2Cl_2 (7 mL) and was cooled to 0 °C. TFA (7 mL) was added, and the reaction mixture was stirred for 2 h and concentrated *in vacuo*. Excess TFA was removed by co-evaporation with toluene and dried under high vac for 45 min. During this period, Boc-ε-TFA-lysine (740 mg, 1.85 mmol) and HATU (703 mg, 1.85 mmol) were dissolved in dry DMF (8 mL) and the

solution was cooled to 0 °C. DIPEA (0.500 mL, 2.87) was added to the reaction mixture and the resulting yellow solution was stirred at 0 °C for 20 min. After this time, the deprotected dipeptide (1.85 mmol) in dry DMF (5 mL) and DIPEA (0.5 mL, 2.87 mmol) was added to the activated *meso*-DAP reaction mixture. The resultant reaction mixture was stirred at room temperature for 20 h and concentrated *in vacuo*. The crude oil residue was re-dissolved in EtOAc (25 mL) and washed with 0.5 M HCl (5 mL), saturated sodium bicarbonate and brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to yield the Boc-tripeptide as a white solid (628 mg, 82% yield). $[\alpha]_D^{25} = -4.52$ (*c* = 0.60, CH_2Cl_2); IR (CH_2Cl_2 cast film, cm^{-1}) 3299, 3079, 2981, 2929, 1747, 1724, 1658; ^1H NMR ($CDCl_3$, 700 MHz) δ 6.71 (d, *J* = 7.5 Hz, 3H, D-AlaNH), 6.68 – 6.56 (m, 1H, D-AlaNH), 5.08 (m, 1H, lysγN<u>H</u>), 4.67 – 4.45 (m, 3H, lys-<u>H</u>ε + D-AlaN<u>H</u>, D-Ala-N<u>H</u>α), 4.08 (m, 1H, lys-<u>H</u>α), 3.78 3.75 (s, 3H, OC<u>H</u>$_3$), 2.01 – 1.93 (m, 2H, lys-<u>H</u>δ), 1.92 – 1.72 (m, 2H, lys-<u>H</u>δ + lys-<u>H</u>β), 1.70 – 1.53 (m, 2H, lys-<u>H</u>β + lys-<u>H</u>γ), 1.43 (m, 16H, lys-<u>H</u>β + Boc-<u>H</u> + D-Ala-<u>H</u>β + D-Ala-<u>H</u>); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 173.2, 171.5, 53.0, 53.6, 52.5, 49.0, 48.2, 31.9, 31.4, 28.3, 21.3, 18.3; HRMS (ESI) Calcd for $C_{20}H_{33}F_3N_4NaO_7$ $[M+Na]^+$ 521.2847, found 521.2849.

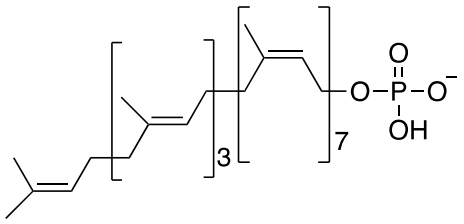
6.8.17. Boc-D-γ-Glu(OMe)-ε-TFA-lys -D-Ala-D-Ala-OMe (52)



(52)

This known compound was prepared following a modified literature protocol.[49] The Boc-tripeptide **73** (600 mg, 1.20 mmol) was dissolved in CH_2Cl_2 (7 mL) and was cooled to 0 °C, TFA (7 mL) was added, and the reaction mixture was stirred for 2 h and concentrated *in vacuo*. Excess TFA was removed by co-evaporation with toluene and dried under high vac for 45 min. During this period, Boc-D-Glu-OMe (286 mg, 1.10 mmol) and HATU (418 mg, 1.1 mmol) were dissolved in dry DMF (8 mL) and the solution was cooled to 0 °C. DIPEA (0.29 mL, 1.65 mmol) was added to the reaction mixture and the resulting yellow solution was stirred at 0 °C for 20 min. After this time, the deprotected tripeptide (1.1 mmol) in dry DMF (5 mL) and DIPEA (0.29 mL, 1.65 mmol) was added to the activated Boc-D-Glu-OMe reaction mixture. The resultant reaction mixture was stirred at room temperature for 20 h and then concentrated *in vacuo*. The crude oil residue was re-dissolved in EtOAc (25 mL) and washed with 0.5 M HCl (5 mL), saturated sodium bicarbonate (5 mL) and brine (5 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to yield the Boc-tetrapeptide as a white solid (688 mg, 90% yield). $[\alpha]_D^{25}$ = - 4.72 (*c* = 1.10, CH_2Cl_2); ^{1}H NMR (DMSO-d_6, 700 MHz) δ 9.80 (d, *J* = 7.5 Hz, 3H, lysε-NH), 8.20 (m, 2H, D-Ala-NH + D-Ala-NH), 7.99 (d, *J* = 7.6 Hz, 3H, lysε-NH), 7.23 (d, *J* = 7.7 Hz, 3H, D-Glu-NH), 4.37 – 4.18 (m, 4H, lys-Hα + lys-Hε + D-Ala-Hα-D-Ala-Hα), 3.96 (m, 1H, Glu-Hα), 3.71 – 3.58 (m, 3H, OCH_3), 2.27 – 2.13 (m, 2H, D-Glu-Hγ), 1.95 – 1.68 (m, 4H, D-Glu-Hβ + lys-Hδ), 1.57 (m, 1H, lys-Hβ), 1.49 (m, 1H, lys-Hβ), 1.43 – 1.14 (m, 17H, lys-Hγ + Boc-H + D-Ala-Hβ + D-Ala-Hβ); HRMS (ESI) Calcd for $C_{26}H_{42}F_3N_5NaO_{10}$ $[M+Na]^+$ 664.2775, found 664.2776.

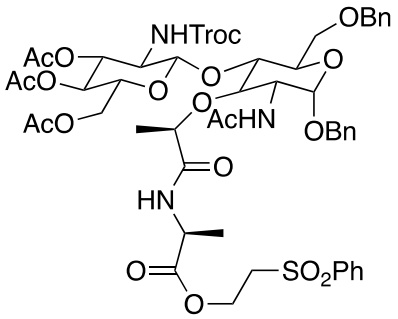
6.8.18. Undecaprenyl Phosphate (53)



(**53**)

This known compound was prepared following a modified literature protocol.[116] Trichloroacetonitrile (11.0 μL, 0.410 mmol) was added to a stirred solution of undecaprenol (**74**) (30.0 mg, 0.0100 mmol) and tetra-*n*-butylammonium dihydrogen phosphate (24.1 mg, 0.0210 mmol) in dichloromethane (5 mL). The reaction mixture was stirred at room temperature for 10 min before the solvent was removed *in vacuo*. The residue was dissolved in THF (1 mL) and 25% ammonium hydroxide solution in water (0.10 mL) was added. After 30 min, 1:1 toluene:methanol (5 mL) was added, and the mixture was stirred for 20 min and the resultant precipitate removed by filtration. The solvent was removed *in vacuo* and the residue was washed with petroleum ether (5 mL × 3), dissolved in methanol (2 mL), and filtered. Excess Dowex 50WX8 ($NH4^+$ form) was added and the mixture was stirred for 30 min and then filtered. The solvent was removed *in vacuo* to give the crude lipid phosphate **53** as a white solid (water:*iso*-propanol:ethyl acetate 1:2:4, R_f = 0.27). This crude product was purified by HPLC: column = GraceVydac Protein and Peptide C_{18} 100 mm column; flow-rate =10 mL/min, UV =220 nm, method = 100% 50 mM NH_4HCO_3 (aq) to 100 % MeOH over 35 min. Fractions containing products (determine by ESI-MS) were combined and lyophilized to yield the final product as a white powder (14 mg, 56% yield). HRMS (ESI) Calcd for $C_{55}H_{90}O_4P$ $[M-H]^-$ 845.6582, found 845.6591.

6.8.19. Troc-Disaccharide (75)

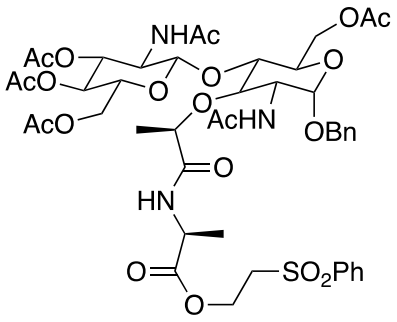


(**75**)

This known compound was prepared following a modified literature protocol.[68, 69] To a round-bottom flask was added 4 Å molecular sieves (20 g) and heated for approximately 10 minutes. The round-bottom flask was allowed to cool to room temperature and was depressurized with argon. Benzyl *N*-acetyl-4,6-benzylidenemuramic acid monopeptide ester **49** (2.50 g, 3.51 mmol) was dissolved in 40 mL of dry CH_2Cl_2 and was added to the flask containing the 4 Å MS under argon with gentle stirring. To the above reaction mixture was added trimethylsilyl triflate (0.410 mL, 3.72 mmol), followed by a solution of acetimidate **50** (6.61 g, 10.5 mmol) in 50 mL of dry CH_2Cl_2. The resulting mixture was stirred under argon at room temperature for 24 h. The reaction mixture was decanted, and the filtrate was diluted with CH_2Cl_2 (30 mL). The organic layer was washed with saturated sodium bicarbonate, brine, dried with Na_2SO_4, filtered, and concentrated *in vacuo*, and purified by column chromatography (SiO_2, 4:6 EtOAc:hexane), yielding the product as a white solid (2.4 g, 64% yield). $[\alpha]_D^{25}$ = + 28.7 (*c* = 0.8 g/100mL, CH_2Cl_2); IR (CH_2Cl_2 cast film, cm^{-1}) 3352, 3287, 3072, 2941, 1755, 1673; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.93 – 7.81 (m, 2H, ArH), 7.68 – 7.63 (m, 1H, ArH), 7.59 – 7.44 (m, 6H, ArH), 7.39 – 7.26

(m, 6H, ArH), 6.91 (d, *J* = 7.6 Hz, 1H, Ala1NH), 6.61 (d, *J* = 7.3 Hz, 1H, MurNAc-NH), 5.11 (d, *J* = 3.7 Hz, 1H, MurNAc-H), 4.94 (t, *J* = 9.8 Hz, 1H, GlcNAc-H), 4.84 (d, *J* = 12.2 Hz, 1H, MurNAc-1-CHHPh), 4.73 (m, 2H, GlcNAc-H3 + Troc-CHH), 4.58 (m, 2H, Troc-CHH + MurNAc-6-CHHPh), 4.52 – 4.43 (m, 2H, OCHH + MurNAc-6-CHHPh), 4.31 (m, 2H, OCHH + MurNAc-1-CHHPh), 4.26 – 4.01 (m, 5H, MurNAc-H + MurNAc-CHO + GlcNAc-H + GlcNAc-H + Ala1Hα), 3.96 (dd, *J* = 12.5, 2.4 Hz, 1H, GlcNAc-H), 3.89 (t, *J* = 9.7 Hz, 1H, MurNAc-H), 3.66 (dd, *J* = 10.5, 2.4 Hz, 1H, MurNAc-H), 3.59 (dt, *J* = 10.3, 2.5 Hz, 1H, MurNAc-H), 3.55 (td, *J* = 10.5, 8.6 Hz, 1H, MurNAc-H), 3.46 – 3.31 (m, 4H, CH_2S + GlcNAc-H + GlcNAc-H), 2.052 – 1.91 (m, 9H, 3 × Ac), 1.89 (s, 3H, Ac), 1.31 (d, *J* = 6.8 Hz, 3H, Ala1Hβ), 1.23 (dd, *J* = 9.5, 7.4 Hz, 3H, MurNAc-CH_3); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 173.5, 171.8, 170.7, 170.6, 170.5, 169.5, 154.2, 139.3, 137.4, 137.2, 134.1, 129.4, 129.1, 128.5, 128.2, 128.1, 100.1, 97.2, 95.8, 77.8, 75.7, 74.5, 73.8, 72.2, 71.2, 70.4, 70.3, 68.3, 67.1, 61.2, 58.3, 56.4, 55.1, 53.6, 47.7, 23.1, 20.4, 18.2, 17.5; HRMS (ES) Calcd for $C_{51}H_{62}Cl_3N_3NaO_{20}S$ $[M+Na]^+$ 1196.2605, found 1196.2585.

6.8.20. Acetyl-Disaccharide (76)

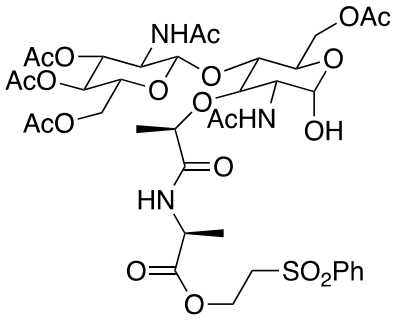


(**76**)

This known compound was prepared following a modified literature protocol.[68, 69] To a mixture of Ac_2O and AcOH (3:1, 21 mL) was added the troc-disaccharide **75** (3.50 g 2.91 mmol) and the reaction mixture. was stirred for 5 mins. A solution of anhydrous $ZnCl_2$ (4.2 g, 32.0 mmol) in AC_2O and AcOH (3:1, 9 mL) was added to the solution. The reaction mixture was stirred for 24 h at room temperature, after which TLC (100% EtOAc, R_f = 0.3) showed a completion of reaction. Zinc powder (8.9 g, 136.0 mmol) and a mixture of THF, AcOH, and Ac_2O (3:2:1 54 mL) were added and the reaction was stirred for further 24 h at room temperature and after TLC (100% EtOAc, R_f = 0.22) showed completion of reaction, the reaction mixture was filtered through Celite and washed with EtOAc (100 mL) and concentrated *in vacuo*. The resulting oil residue was co-evaporated with toluene (4 × 20 mL) and the crude was redissolved in EtOAc, washed with saturated sodium bicarbonate, water, and brine, dried with Na_2SO_4, filtered, and concentrated *in vacuo*, and purified by column chromatography (SiO_2, 2% MeOH in EtOAc), yielding the product as a white solid (1.88 g, 66% yield). $[\alpha]_D^{25}$ = + 28.9 (*c* = 0.8, CH_2Cl_2); IR (CH_2Cl_2 cast film, cm^{-1}) 3360, 3279, 3058, 2945, 1755, 1669; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.91 – 7.82 (m, 2H, ortho-ArH), 7.68 – 7.57 (m, 1H, para-ArH), 7.52 – 7.43 (m, 2H, meta-ArH), 7.39 – 7.20 (m, 5H, Bn-ArH), 7.11 (d, *J* = 7.4 Hz, 1H, MurNAc-NH), 6.78 (d, *J* = 6.6 Hz, 1H, Ala1NH), 6.05 (d, *J* = 9.6 Hz, 1H, GlcNAc-NH), 5.12 – 5.01 (m, 2H, MurNAcH + GlcNAc-H), 4.59 (d, *J* = 12.2 Hz, 1H, MurNAc-CHHPh), 4.39 (d, *J* = 12.4 Hz, 1H, MurNAc-CHHPh), 4.39 – 4.20 (m, 6H, OCH_2 + MurNAc-CHO + GlcNAc-H + GlcNAc-H + MurNAc-H), 4.16 – 4.11 (m, 1H, MurNAc-H), 3.98 (q, *J* = 7.3 Hz, 1H, GlcNAc-H), 4.04 – 3.89 (m, 3H, GlcNAc-H + MurNAc-H + MurNAc-H), 3.69 (d, *J* = 5.5 Hz, MurNAc-H), 3.59– 3.47 (m, 2H,

GlcNAc-H + MurNAc-H), 3.32 (m, 2H, CH_2S), 2.09 (s, 3H), 2.01 – 1.89 (m, 6H), 1.89 (s, 3H), 1.85 (s, 3H), 1.25 (d, *J* = 6.5 Hz, 3H, MurNAc-CH_3), 1.23 (d, *J* = 7.5 Hz, 3H, Ala1Hβ); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 173.7, 172.2, 171.5, 171.1, 170.8, 170.6, 170.6, 169.3, 139.2, 137.3, 134.1, 129.4, 128.4, 128.1, 128.0, 127.1, 100.5, 97.1, 76.2, 75.6, 72.5, 71.8, 70.3, 69.5, 68.1, 62.3, 61.6, 60.4, 58.0, 55.2, 54.6, 53.6, 47.8, 23.2, 23.1, 21.2, 20.2, 20.0, 19.7, 18.2, 17.3; HRMS (ES) Calcd for $C_{45}H_{59}N_3NaO_{20}S$ $[M+Na]^+$ 1016.3305, found 1016.3293.

6.8.21. Hydroxy-Disaccharide (77)

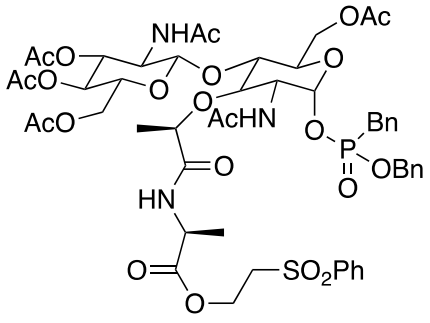


(**77**)

This known compound was prepared following a modified literature protocol.[69] Acetyl-disaccharide **76** (2.00 g, 2.00 mmol) was dissolved in THF and MeOH (4:1, 30 mL) and this solution was added to a degassed suspension of Palladium Hydroxide on charcoal (1.4 g) in the same solvent (10 mL). The reaction mixture was stirred under a hydrogen atmosphere for 3 h after which TLC (100% EtOAc, R_f = 0.5) showed the formation of a single product, the solution was filtered through Celite and was washed with CH_2Cl_2 (20 mL), the filtrate was concentrated *in vacuo.* Upon the addition of ether and hexane (1:1) the product precipitated out and was filtered, dried under high vacuum for 2 h to yield the alcohol as a white solid (1.7 g, 94%). $[\alpha]_D^{25}$ = + 23.9 (*c* = 0.6,

CH_2Cl_2), IR (CH_2Cl_2 cast film, cm^{-1}) 3278, 3066, 2982, S44 1747, 1666; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.67 (d, J = 4.6 Hz, 1H, NHAc), 8.40 (d, J = 7.0 Hz, 1H, NHAc), 8.07 (d, J = 9.0 Hz, 1H, NHAc), 7.92 – 7.80 (m, 2H, ortho-ArH), 7.76 – 7.72 (m, 1H, para-ArH), 7.67 – 7.59 (m, 2H, meta-ArH), 5.81 (dd, J = 6.4, 3.1 Hz, 1H, MurNAc-H1), 5.24 (t, J = 9.9 Hz, 1H, GlcNAc-H3), 5.13 – 4.95 (m, 4H, 2 × CH_2Ph), 4.91 (t, J = 9.8 Hz, 1H, GlcNAc-H4), 4.72 (dd, J = 16.2, 7.5 Hz, 1H, GlcNAc-H1), 4.60 (d, J = 6.7 Hz, 1H, MurNAc-CHO), 4.40 – 4.18 (m, 3H, MurNAc-H6 + GlcNAc-H6 + OCHH), 4.13 – 3.95 (m, 4H, MurNAc-H6 + GlcNAcH6 + OCHH + Ala1Hα), 3.87 – 3.71 (m, 4H, GlcNAc-H2 + GlcNAc-H5 + MurNAc-H3 + MurNAc-H5), 3.65 – 3.56 (m, 3H, MurNAc-H2 + SCH_2), 3.42 (dd, J = 10.9, 8.7 Hz, 1H, MurNAc-H4), 2.03 – 1.87 (m, 12H, 4 × Ac), 1.75 (s, 3H, Ac), 1.69 (s, 3H, Ac), 1.29 (d, J = 6.7 Hz, 3H, MurNAc-CH3), 1.11 (d, J = 7.3 Hz, 3H, Ala-Hβ); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 174.4, 171.3, 169.9, 169.8, 169.5, 169.3, 139.2, 135.7, 133.9, 129.3, 128.4, 128.3, 128.3, 128.3, 127.9, 127.8, 127.7, 127.6, 127.6, 99.6, 75.9, 75.7, 73.8, 72.3, 70.7, 70.4, 68.6, 68.4, 68.4, 68.3, 66.3, 61.6, 57.9, 53.6, 47.3, 40.0, 39.9, 39.8, 39.7, 39.6, 39.6, 39.5, 39.4, 39.3, 39.1, 39.0, 22.6, 22.3, 20.5, 20.3, 20.2, 18.9, 16.5; HRMS (ES) Calcd for $C_{38}H_{53}N_3NaO_{20}S$ $[M+Na]^+$ 926.2835, found 926.2837.

6.8.22. Disaccharide Dibenzylphosphate (78)

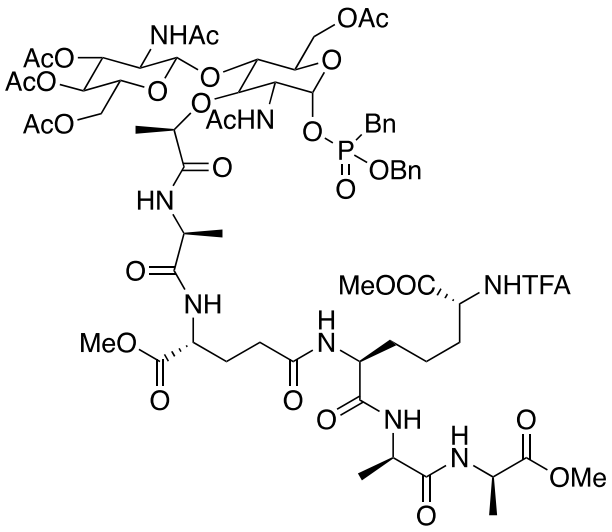


(**78**)

This known compound was prepared following a modified literature protocol.[69] The lactol compound **77** (1.40 g, 1.55 mmol) in anhydrous CH_2Cl_2 (10mL) was rapidly added to a vigorously stirred suspension of tetrazole solution (2.1 mL) and dibenzyl *N,N*'-dimethylphosphoramidite (1.4 mL, 4.70 mmol) in anhydrous CH_2Cl_2 (10 mL) under argon at room temperature. Within a few minutes, the reaction mixture became homogenous. After 2 h, TLC (10% MeOH/$CHCl_3$, R_f = 0.34) showed a complete reaction, and the reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with saturated sodium bicarbonate (10 mL), water (10 mL) and brine (10 mL), dried with Na_2SO_4, filtered, and concentrated *in vacu*o to give a colorless oil, which crystallized upon trituration with 1:1 diethyl ether/hexanes. The product was dissolved in THF (10 mL) and cooled to -78 °C. Hydrogen peroxide (30%, 1.9 mL) was added dropwise via a syringe to the vigorously stirred solution. After the addition was complete, the ice bath was removed, and the reaction mixture was allowed to warm to room temperature over 2.5 h. A TLC (100% EtOAc, R_f = 0.32) showed a complete reaction and the reaction mixture was diluted with ice-cold saturated sodium sulfite (5 mL), followed by EtOAc (10 mL), and was stirred for 10 min. The organic layer was dried over

Na_2SO_4 and concentrated *in vacuo* to give a colorless oil, which upon trituration with 1:1 diethyl ether/hexane produced a white solid. The product was dried under high vacuum for 6 h to provide (1.4 g, 90% yield) the desired phosphate compound. $[\alpha]_D^{25}$ = + 2.98 (c = 0.70, CH_2Cl_2); IR (CH_2Cl_2 cast film, cm^{-1}) 3275, 3062, 2989, 1748, 1669; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.62 (d, *J* = 4.7 Hz, 1H, NHAc), 8.39 (d, *J* = 7.2 Hz, 1H, NHAc), 8.02 (d, *J* = 9.2 Hz, 1H, NHAc), 7.94 – 7.79 (m, 2H, *o*-ArH), 7.71 – 7.69 (m, 1H, *p*-ArH), 7.62 – 7.48 (m, 2H, *m*-ArH), 7.44 – 7.27 (m, 10H, 2 × Bn-ArH), 5.86 (dd, *J* = 6.4, 3.1 Hz, 1H, MurNAc-H), 5.27 (t, *J* = 9.6 Hz, 1H, GlcNAc-H), 5.18 – 4.89 (m, 4H, 2 × CH_2Ph), 4.91 (t, *J* = 9.6 Hz, 1H, GlcNAc-H), 4.68 (dd, *J* = 16.8, 7.1 Hz, 1H, GlcNAc-H), 4.59 (d, *J* = 6.9 Hz, 1H, MurNAc-CHO), 4.39 – 4.12 (m, 3H, MurNAc-H + GlcNAc-H + OCHH), 4.05 – 3.89 (m, 4H, MurNAc-H + GlcNAcH + OCHH + Ala1Hα), 3.85 – 3.77 (m, 4H, GlcNAc-H + GlcNAc-H + MurNAcH + MurNAc-H), 3.61 – 3.52 (m, 3H, MurNAc-H + SCH_2), 3.39 (dd, *J* = 10.6, 8.5 Hz, 1H, MurNAc-H), 2.01 – 1.85 (m, 12H, 4 × Ac), 1.69 (s, 3H, Ac), 1.58 (s, 3H, Ac), 1.22 (d, *J* = 6.5 Hz, 3H, MurNAc-CH_3), 1.01 (d, *J* = 7.5 Hz, 3H, AlaHβ); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 174.5, 171.4, 169.8, 169.2, 169.4, 169.2, 139.4, 135.6, 133.8, 129.4, 128.2, 128.0, 128.0, 128.0, 127.8, 127.7, 127.6, 127.5, 127.5, 99.5, 75.8, 75.7, 73.7, 72.3, 70.6, 70.5, 68.6, 68.4, 68.3, 68.1, 66.2, 61.4, 57.8, 53.5, 47.3, 40.0, 39.8, 39.7, 39.6, 39.6, 39.5, 39.4, 39.4, 39.2, 39.1, 38.4, 22.7, 22.3, 20.4, 20.2, 20.1, 18.9, 16.4; HRMS (ES) Calcd for $C_{52}H_{66}N_3NaO_{23}PS$ $[M+Na]^+$ 1186.3438, found 1186.3416.

6.8.23. Pentapeptide Disaccharide (79)

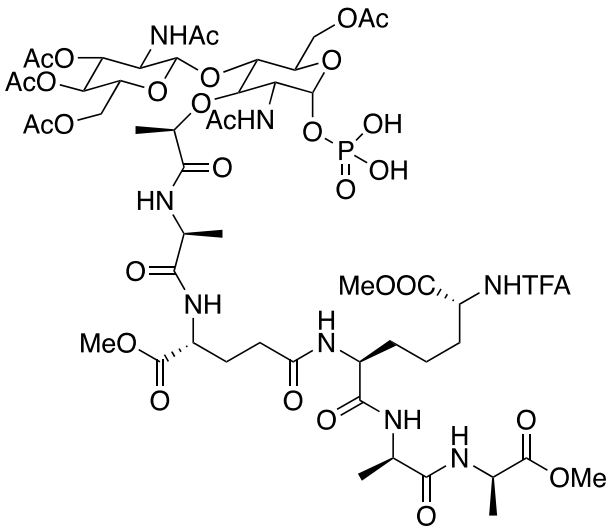


(**79**)

This known compound was prepared following a modified literature protocol.[69] To a mixture of CH_2Cl_2 (5 mL) and TFA (5 mL) was added the Boc-Tetrapeptide **51** (220 mg, 0.320 mmol) and the mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene (3 × 3 mL) and was precipitated with cold ether (10 mL) to yield the deprotected peptide TFA salt as an off-white solid (221 mg, 0.32 mmol). The disaccharide phosphate **78** (221 mg, 0.32 mmol) was dissolved in dry CH_2Cl_2 (6 mL) and stirred under an Argon atmosphere. To this solution was added a diazabicycloundec-7-ene (40.0 µL, 0.290 mmol) in dry CH_2Cl_2 (6 mL) dropwise over 3 min and stirred for a further 40 min. The reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with 1 M HCl (5 mL), brine (5 mL), dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. A colorless oil was obtained and was dried under high vac for approximately 3 h to yield an off-white solid (294 mg, 0.289 mmol). The acid was dissolved in dry DMF (8 mL) and was cooled to 0 °C, HATU (122 mg, 0.320 mmol), followed by DIPEA (50 µL, 0.292

mmol) was added and the resulting yellow solution was stirred for 5 min. The Boc-deprotected peptide TFA salt was dissolved in dry DMF (2 mL) and DIPEA (101 μL, 0.580 mmol)) and the resulting solution was added at once to the activated ester. Another 2 equivalents of DIPEA (101 μL, 0.580 mmol) was added to the reaction mixture to give a pH of 8.0. The reaction was allowed to warm to room temperature and stirring continued for 24 h. The reaction mixture was concentrated *in vacuo* and redissolved in $CHCl_3$ and IPA ((9:1), 15mL) and was washed with 1 M HCl and saturated sodium bicarbonate. The aqueous layers were back extracted with $CHCl_3$ (10 mL) and the combined organic extract was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude oil product was precipitated from Et_2O to give the product as an off-white solid (358 mg, 80% yield), which was used in the next step without further purification. HRMS (ES) Calcd for $C_{67}H_{92}F_3N_8NaO_{30}P$ $[M+Na]^+$ 1599.5501 found 1599.5518.

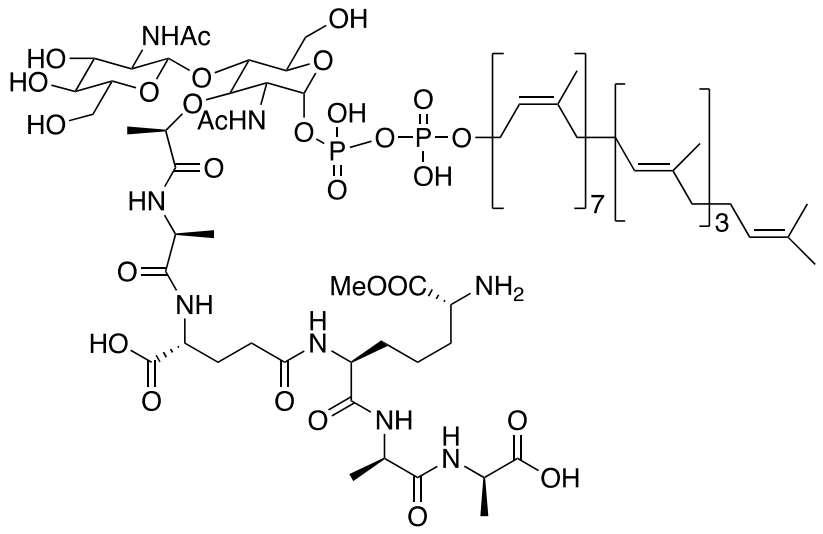
6.8.24. Disaccharide Phosphate-Gram-negative Variant (80)



(**80**)

This known compound was prepared following a modified literature protocol.[68, 69] A flask containing dibenzyl phosphate **79** (100 mg, 63.29 μmol) and anhydrous MeOH (10ml) was flushed with argon for 5 min. Pd/C (10% w/w, 180 mg) was added and the resulting suspension was stirred under a H_2 atmosphere for 3 h. After this time TLC showed completion of reaction (2% DCM in MeOH, R_f = 0.42) and the suspension was filtered through a pad of Celite and washed with MeOH (2 × 5 mL). To this filtrate was added pyridine (2 mL) and the filtrate was concentrated *in vacuo* and dried under high vacuum for 24 h to yield the sugar phosphate as a white solid. (92 mg, quant.), which was used in the nest step without further purification. HRMS (ES) Calcd for $C_{53}H_{80}F_3N_8NaO_{30}P$ $[M+Na]^+$ 1419.4647 found 1419.4649.

6.8.25. Synthesis of C_{55} *m*DAP-lipid II (38)

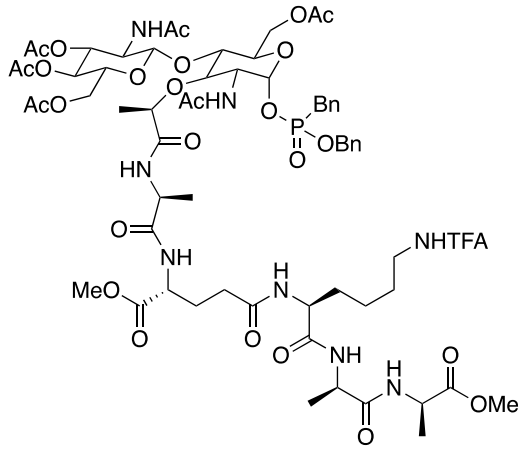


(**38**)

This known compound was prepared following a modified literature protocol.[69] Undecaprenyl phosphate (**53**) (20 mg, 0.010 mmol) was dissolved in dry DMF (5 ml), which formed a cloudy suspension. To this suspension was added CDI (50 mg, 0.30

mmol) and the resulting solution became clear, and was subsequently stirred for 2 h. Anhydrous MeOH (8 μL) was added to destroy any excess CDI after 45 min of stirring. Excess MeOH was removed by rotary evaporator and a solution of the sugar phosphate **80** (35 mg) in dry DMF (2 mL) was added to this solution. The resulting solution was stirred for 3 days under argon at room temperature and concentrated *in vacuo.* The crude diphosphate was dissolved in dioxane (1 mL) and 1 M NaOH (1 mL) was added, and the reaction mixture was stirred at 37 °C for 2 h. The reaction mixture was filtered through an aqueous filter paper and the crude compound was purified by HPLC: column = GraceVydac Protein and Peptide C_{18} 100 mm column; flow-rate = 10 mL/min, UV = 220 nm, method = 100% 50 mM NH_4HCO_3 (aq) to 100% MeOH over 35 min. Fractions containing products (determined by ESI-MS) were combined and lyophilized to yield C_{55} Gram-negative lipid II as a fluffy white powder (6 mg, 26% yield).

6.8.26. Disaccharide Dibenzyl Phosphate-Gram-positive Variant (81)



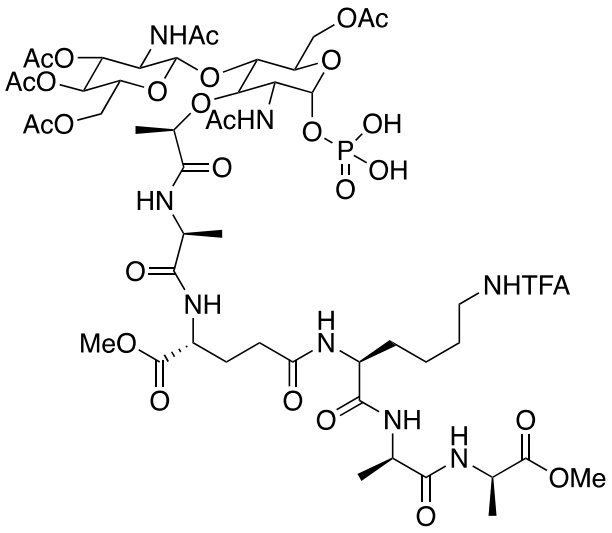
(**81**)

This known compound was prepared following a modified literature protocol.[69] To a mixture of CH_2Cl_2 (5 mL) and TFA (5 mL) was added the Boc-Tetrapeptide (**52**) (198

mg, 0.300 mmol) and the mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene (3 × 3 mL) and was precipitated with cold ether (10 mL) to yield the deprotected peptide TFA salt as an off-white solid (221 mg, 0.320 mmol). The disaccharide phosphate **78** (198 mg, 0.310 mmol) was dissolved in dry CH_2Cl_2 (6 mL) and stirred under argon atmosphere. To this solution was added a diazabicycloundec-7-ene (35.0 µL, 0.260 mmol) in dry CH_2Cl_2 (6 mL) dropwise over 3 min, and then the mixture was stirred for a further 40 min. The reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with 1 M HCl (5 mL), brine (5 mL), dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. A colorless oil obtained was dried under high vac for approximately 3 h and an off-white solid was obtained (294 mg, 0.289 mmol). The acid was dissolved in dry DMF (8 mL) and was cooled to 0 °C, and then HATU (122mg, 0.320 mmol), followed by DIPEA (45.0 µL, 0.280 mmol) was added and the resulting yellow solution was stirred for 5 min. The Boc-deprotected peptide TFA salt was dissolved in dry DMF (2 mL) and DIPEA (98.7 µL, 0.580 mmol), and the resulting solution was added at once to the activated ester. Another 2 equivalents of DIPEA (98.7 µL, 0.580 mmol) was added to the reaction mixture to make a pH of 8.0. The reaction was allowed to warm to room temperature and stirring continued for 24 h. The reaction mixture was concentrated *in vacuo* and redissolved in $CHCl_3$ and IPA ((9:1), 15mL), and then was washed with 1 M HCl (7 mL) and saturated sodium bicarbonate (7 mL). The aqueous layers were back extracted with $CHCl_3$ (10 mL) and the combined organic extracts was washed with brine (2 × 7 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude oil product was precipitated from Et_2O to give the product as an

off-white solid (379 mg, 82% yield), which was used in the next step without further purification. HRMS (ES) Calcd for $C_{65}H_{90}F_3N_8NaO_{28}P$ $[M+Na]^+$ 1541.5446 found 1541.5483.

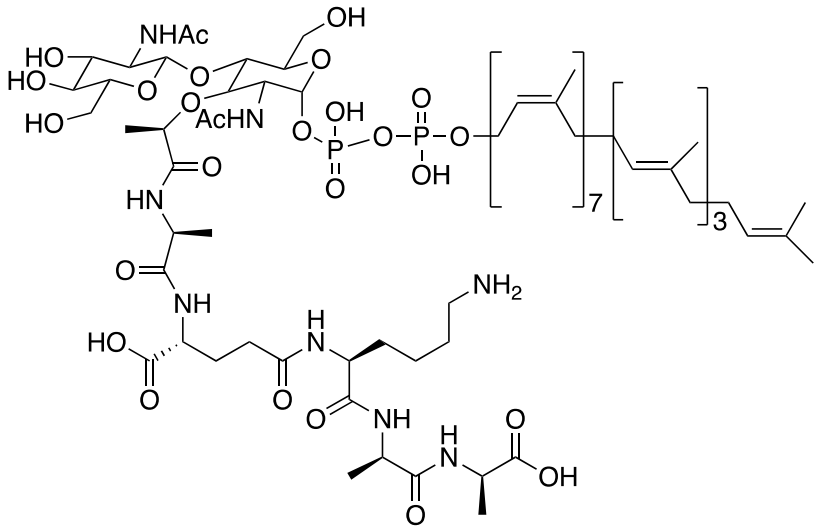
6.8.27. Disaccharide Phosphate-Gram-positive Variant (82)



(**82**)

This known compound was prepared following a modified literature protocol.[69] A flask containing dibenzyl phosphate **81** (98 mg, 65.2 μmol) and anhydrous MeOH (10mL) was flushed with argon for 5 min. Pd/C (10% w/w, 180 mg) was added and the resulting suspension was stirred under a H_2 atmosphere for 3 h. After this time TLC showed completion of reaction (2% CH_2Cl_2 in MeOH, R_f = 0.42) and the suspension was filtered through a pad of Celite (7 g) and washed with MeOH (2 × 5 mL). To this filtrate was added pyridine (2 mL) and the filtrate was concentrated *in vacuo* and dried under high vacuum for 24 h to yield the sugar phosphate as a white solid (89 mg quant.), which was used in the next step without further purification. HRMS (ES) Calcd for $C_{51}H_{78}F_3N_8NaO_{28}P$ $[M+Na]^+$ 1361.4621 found 1361.4626.

6.8.28. Synthesis of C_{55} Lys-lipid II (39)



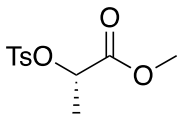
(**39**)

This known compound was prepared following a modified literature protocol.[69] Undecaprenyl phosphate (**53**) (20.0 mg, 0.0100 mmol) was dissolved in dry DMF (5 mL), which formed a cloudy suspension. To this suspension was added CDI (50 mg, 0.30 mmol) and the resulting solution became clear and then was stirred for 2 h. Anhydrous MeOH (8 µL) was added to destroy any excess CDI after 45 min of stirring. Excess MeOH was removed by rotary evaporator and a solution of the sugar phosphate **82** (45 mg) in dry DMF (2 mL) was added to this solution. The resulting solution was stirred for 3 days under argon at room temperature and then concentrated *in vacuo*. The crude diphosphate was dissolved in dioxane (1 mL) and 1 M NaOH (1 mL) was added, and the reaction mixture was stirred at 37 °C for 2 h. The reaction mixture was filtered through an aqueous filter paper and the crude was purified by HPLC: column = GraceVydac Protein and Peptide C_{18} 100 mm column; flow-rate = 10 mL/min, UV = 220 nm, method = 100% 50 mM NH_4HCO_3 (aq) to 100% MeOH over 35 min. Fractions

containing products (determined by ESI-MS) were combined and lyophilized to yield C_{55} Gram-negative lipid II as a fluffy white powder (6 mg, 26% yield).

6.9. Experimental Procedure for the Synthesis of Cycloalanopine and its Analogues

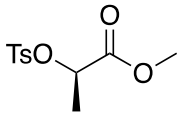
6.9.1. Methyl (*S*)-2-(tosyloxy) propanoate (100)



(**100**)

This known compound was prepared following a modified literature protocol.[162] (*S*)-methyl lactate (15.5 g, 149 mmol), CH_2Cl_2 (35 ml), and triethylamine (16.6 g, 164 mmol) were put into a three-neck round bottom flask in turn, and then stirred for 10 min at 0°C. After this time, 1 M *p*-toluene sulfonyl chloride in CH_2Cl_2 (164 mL) was added dropwise over 1 h to the reaction mixture. The mixture was stirred for 2 h and then continued for 4 h with stirring at room temperature. The reaction mixture was washed with water, 5% HCl, and saturated aqueous $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered, and the solvent was removed *in vacuo,* and purified by column chromatography (SiO_2, 10 – 20% EtOAc in hexanes), yielding the product as a pale-yellow oil, R_f = 0.34 in 10% EtOAc in hexanes, (30.5 g, 96% yield). $[\alpha]_D^{25}$ = - 29.5 (*c* = 1.00, $CHCl_3$); IR ($CHCl_3$, cast film, cm^{-1}) 2996, 2956, 1761, 1595, 1190, 1176, 1082, 664; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.79 (d, *J* = 7.9 Hz, 2H), 7.36 (d, *J* = 7.9 Hz, 2H), 4.96 (q, *J* = 7.2 Hz, 1H), 3.67 (s, 3H), 2.49 (s, 3H), 1.51 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 169.5, 145.1, 133.4, 130.3, 127.9, 74.1, 52.6, 21.7, 18.4; HRMS (ESI) Calcd for $C_6H_9NNaOS_2$ $[M+Na]^+$ 281.0454, found 281.0453.

6.9.2. Methyl (*R*)-2-(tosyloxy)propanoate (103)



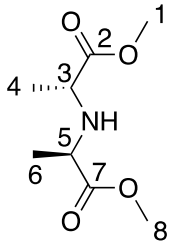
(**103**)

This known compound was prepared following a modified literature protocol.[162] (*R*)-methyl lactate (15.5 g, 149 mmol), CH_2Cl_2 (35 ml), and triethylamine (16.6 g, 164 mmol) were put into a three-neck round bottom flask in turn, stirred for 10 min at 0°C. After this time, 1 M *p*-toluene sulfonyl chloride in CH_2Cl_2 (164 mL) was added dropwise over 1 h to the reaction mixture and stirred for 2h and then continued for 4 h with stirring at room temperature. The reaction mixture was washed with water, 5% HCl, and saturated aqueous $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered, and the solvent was removed *in vacuo,* and purified by column chromatography (SiO_2, 10 – 20% EtOAc in hexanes), yielding the product as a pale-yellow oil, R_f = 0.35 in 10% EtOAc in hexanes, (30.5 g, 96% yield). $[\alpha]_D^{25}$ = + 27.8 (*c* = 1.00, $CHCl_3$); IR ($CHCl_3$, cast film, cm^{-1}) 2930, 2850, 1759, 1465, 1368, 1180, 1085, 820, 555; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.79 (d, *J* = 7.9 Hz, 2H), 7.33 (d, *J* = 7.9 Hz, 2H), 4.93 (q, *J* = 7.0 Hz), 3.64 (s, 3H), 2.43 (s, 3H), 1.48 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 169.5, 145.2, 133.3, 129.8, 127.9, 74.1, 52.6, 21.6 , 18.3; HRMS (ESI) Calcd for $C_{11}H_{14}NaO_5S_2$ $[M+Na]^+$ 281.0454, found 281.0453.

6.10. General Procedure for the Preparation of the Diesters

The synthesis of the desired diester was accomplished following a modified literature protocol.[91] To a solution of D-alanine methyl ester hydrochloride (5.00 g, 35.8 mmol) in MeCN (30 mL) was added $NaHCO_3$ (6.00 g, 71.6 mmol), and then the reaction mixture was stirred for 1 h at room temperature. Compound **101** (9.20 g, 35.8 mmol) in MeCN (10 mL) was added and the reaction mixture was stirred under reflux for 12 h. TLC analysis of the reaction mixture (30% EtOAc in hexanes, R_f = 0.33) showed completion of reaction. The reaction mixture was allowed to cool to room temperature, filtered, and the filtrate was concentrated *in vacuo* to give a pale yellow oil. The crude compound was purified by column chromatography (SiO_2, 40% EtOAc in hexanes), yielding the product as a pale-yellow oil (6.2 g, 91% yield). This same procedure was used for the synthesis of **102**, **104**, **133**, **139**, **144** and **147** starting from the appropriate amino acid and the corresponding tosylated lactic acid compound.

6.10.1. Dimethyl 2,2'-azanediyl(2*R*,2'*R*)-dipropionate (101)

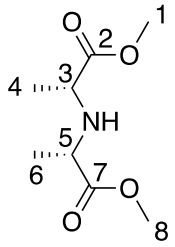


(**101**)

$[\alpha]_D^{25}$ = + 48.9 (*c* = 1.00, $CHCl_3$); IR ($CHCl_3$, cast film, cm^{-1}) 3454, 3341, 2981, 2954, 2876, 1737, 1453, 1374, 1202, 1095, 983, 743; ^1H NMR ($CDCl_3$, 700 MHz) δ 3.71 (s, 6H, H1, H8), 3.41 (q, *J* = 7.0 Hz, 2H, H3, H5) 1.31 (d, *J* = 7.0 Hz, 6H, H4, H6); ^{13}C

NMR ($CDCl_3$, 176 MHz) δ 175.5 (C2, C7), 55.1 (C3, C5), 51.9 (C1, C8), 19.3 (C4, C6); HRMS (ESI) Calcd for $C_8H_{16}NO_4$ $[M + H]^+$ 190.1074, found 190.1072.

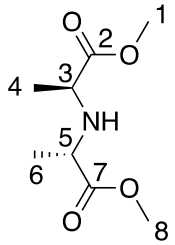
6.10.2. Dimethyl 2,2'-azanediyl(2*R*,2'*S*)-dipropionate (102)



(**102**)

Starting from D-alanine methyl ester hydrochloride and (**103)** and following the general procedure as described above yields **102** as a yellow oil (6.8 g, 92% yield), R_f = 0.41 in 30% EtOAc in hexanes); $[\alpha]_D^{25}$ = 0.0 (*c* = 1.0, $CHCl_3$); IR ($CHCl_3$, cast film, cm^{-1}) 3461, 3335, 2981, 2954, 2873, 1740, 1453, 1376, 1209, 1056, 982, 755; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.70 (s, 6H, H1, H8), 3.41 (q, *J* = 7.0 Hz, 2H, H3, H5) 1.31 (d, *J* = 7.0 Hz, 6H, H4, H6); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 175.2 (C2, C7), 54.4 (C3), 51.8 (C5), 18.7 (C4, C6); HRMS (ESI) Calcd for $C_8H_{16}NO_4$ $[M + H]^+$ 190.1074, found 190.1081.

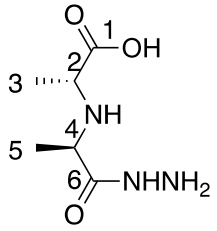
6.10.3. Dimethyl 2,2'-azanediyl(2*R*,2'*S*)-dipropionate (104)



(**104**)

This compound was synthesized following the general procedure as described above starting from L-alanine methyl ester hydrochloride (**100)** yielding the final product as a yellow oil (6.3 g, 94% yield); R_f = 0.34 in 30% EtOAc in hexanes; $[\alpha]_D^{25}$ = -47.8 (*c* = 0.86, $CHCl_3$); IR ($CHCl_3$, cast film, cm^{-1}) 3453, 3321, 2986, 2962, 2886, 1745, 1452, 1369, 1202, 1098, 986, 746; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.72 (s, 6H, H1, H8), 3.42 (q, *J* = 7.0 Hz, 2H, H3, H5) 1.32 (d, *J* = 7.0 Hz, 6H, H4, H6); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 175.5 (C2, C7), 55.0 (C3), 51.9 (C5), 19.2 (C4, C6); HRMS (ESI) Calcd for $C_8H_{16}NO_4$ $[M + H]^+$ 190.1074, found 190.1082.

6.10.4. ((*R*)-1-Hydrazineyl-1-oxopropane-2-yl)-D-alanine (106)

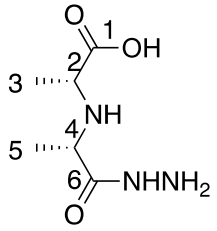


(**106**)

This new compound was prepared following a modified literature protocol.[163] A solution of LiOH (0.24 g, 0.010 mol) in MeOH (10 mL) was added to **101** (2.00 g, 0.010 mmol) and the mixture was stirred for 1 h at 0 °C. The solvent was then removed *in vacuo*, and Et_2O (20 mL) and H_2O (30 mL) were added. The aqueous layer was acidified with concentrated HCl to pH 3 and extracted with Et_2O (3 × 20 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and filtered. The solvent was removed *in vacuo* to give the acid as a light-yellow oil (1.5 g), which was used in the next step without further purification. The acid (1.2 g, 0.0068 mol) was dissolved in MeOH (10 mL) and hydrazine monohydrate (0.33 mL, 0.0068 mol) was

added, and the reaction was stirred at room temperature for 6 h. After this time, a white precipitated formed, which was filtered and washed with Et_2O (5 × 20 mL). This solid was allowed to dry and was recrystallized from Et_2O to give the product as white powder (0.92 g, 76% yield). $[\alpha]_D^{25}$ = + 41.1 (*c* = 1.00, MeOH); IR (MeOH, cast film, cm^{-1}) 3320, 2977, 1714, 1552, 1101; ^1H NMR (D_2O, 500 MHz) δ 2.95 (q, *J* = 7.0 Hz, 2H, H2, H4) 1.31 (d, *J* = 7.0 Hz, 3H, H3), 1.28 (d, *J* = 7.0 Hz, 3H, H5); ^{13}C NMR (D_2O, 126 MHz) δ 175.3 (C1), 170.3 (C6), 51.1 (C2), 49.1 (C4), 18.86 (C3), 17.6 (C5); HRMS (ESI) Calcd for $C_6H_{12}N_3O_3$ $[M - H]^-$ 174.0884 found 174.0887.

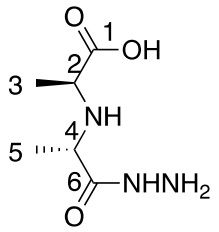
6.10.5. ((*R*)-1-Hydrazineyl-1-oxopropane-2-yl)-D-alanine (107)



(**107**)

Starting from compound **102** and using the same procedure as described for the synthesis of **106** yields compound **107** as a white powder (0.86 g, 74% yield). $[\alpha]_D^{25}$ = +11.1 (*c* = 1.00, MeOH); IR (MeOH, cast film, cm^{-1}) 3312, 2977, 1719, 1542, 1104; ^1H NMR (D_2O, 500 MHz) δ 2.95 (d, *J* = 7.0 Hz, 2H, H2), 2.32 (d, *J* = 7.1 Hz, 2H, H4) 1.31 (d, *J* = 7.0 Hz, 3H, H3), 1.29 (d, *J* = 7.0 Hz, 3H, H5); ^{13}C NMR (D_2O, 126 MHz) δ 175.9 (C1), 170.7 (C6), 51.3 (C2), 49.4 (C4), 18.9 (C3), 17.5 (C5); HRMS (ESI) Calcd for $C_6H_{12}N_3O_3$ $[M - H]^-$ 174.0873 found 174.0876.

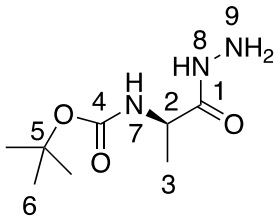
6.10.6. ((*R*)-1-Hydrazineyl-1-oxopropane-2-yl)-D-alanine (108)



(**108**)

Starting from compound **104**: white powder (0.83 g, 72% yield). $[\alpha]_D^{25}$ = + 36.1 (*c* = 1.00, MeOH); IR (MeOH, cast film, cm^{-1}) 3322, 2987, 1719, 1552, 1104; ^1H NMR (D_2O, 500 MHz) δ 2.96 (q, *J* = 7.0 Hz, 2H, H2, H4) 1.30 (d, *J* = 7.0 Hz, 3H, H3), 1.27 (d, *J* = 7.0 Hz, 3H, H5); ^{13}C NMR (D_2O, 126 MHz) δ 175.5 (C1), 170.3 (C6), 51.3 (C2), 49.2 (C4), 18.7 (C3), 17.5 (C5); HRMS (ESI) Calcd for $C_6H_{12}N_3O_3$ $[M - H]^-$ 174.0892 found 174.0895.

6.10.7. Tert-butyl (*R*)-(1-hydrazineyl-1-oxopropan-2-yl)carbamate (113)

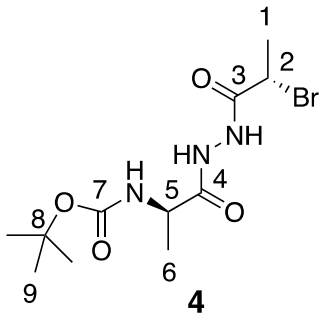


(**113**)

This new compound was prepared following literature protocol.[164] To a solution of Boc-D-Ala-OMe (**112**) (5.50 g, 27.0 mmol) in MeOH (40 mL) was added hydrazine monohydrate (2.50 mL, 54.2 mmol) and stirred for 12 h. A white precipitate formed and was filtered, washed with Et_2O (5 × 20 mL), and dried to give a white solid which was recrystallized from Et_2O to give the product as white powder (5.2 g, 95%). $[\alpha]_D^{25}$ = + 36.5 (*c* = 0.94, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 3316, 2979, 1701, 1671, 1525, 1169,

1046, 757; ^{1}H NMR (DMSO-*d6*, 500 MHz) δ 8.93 (s, 1H, H7), 4.09 (s, 2H, H9), 3.91 (q, *J* = 7.14Hz, 1H, H2), 1.35 (s, 9H, H6), 1.12 (d, *J* = 7.1 Hz, 3H, H3); ^{13}C NMR (DMSO-*d6*, 126 MHz) δ 172.1 (C1), 154.6 (C4), 77.8 (C5), 48.3 (C2), 28.1 (C6), 18.5 (C3); HRMS (ESI) Calcd for $C_8H_{17}N_3NaO_3$ $[M + Na]^+$ 226.1162, found 226.1165.

6.10.8. Tert-butyl((*R*)-1-(2-(*S*)-2-bromopropanoyl)hydrazineyl)-1-oxopropan-2-yl)carbamate (115)



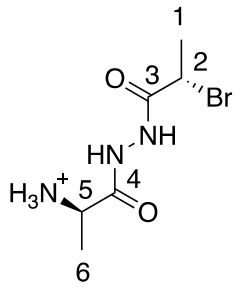
4

(**115**)

This new compound was prepared following literature protocol.[91] To a solution of *tert*-butyl (*R*)-(1-hydrazineyl-1-oxopropan-2-yl)carbamate L-alanine methyl ester hydrochloride (**113**) (4.90 g, 24.3 mmol) in MeCN (30 mL) was added $NaHCO_3$ (6.0 g, 71.6 mmol), and the reaction mixture was stirred for 1 h at room temperature. *(S)*-2-Bromopropionylbromide (**114**) (1.70 mL, 16.2 mmol) in MeCN (10 mL) was added and the mixture was stirred under reflux for 12 h. TLC analysis of the reaction mixture (30% EtOAc in hexanes, R_f = 0.41) showed completion of reaction. The reaction mixture was allowed to cool to room temperature, filtered, and then the filtrate was concentrated *in vacuo*. The crude compound was purified by column chromatography (SiO_2, 40% EtOAc in hexanes), yielding the product as a pale yellow oil (4.2 g, 77% yield); R_f = 0.41 in 30% EtOAc in hexanes; $[\alpha]_D^{25}$ = + 25.2 (*c* = 0.94, MeOH); IR

(MeOH, cast film, cm^{-1}) 3313, 2959, 1721, 1681, 1555, 1159, 1041, 755; ^{1}H NMR (CD_3OD, 500 MHz) δ 4.52 (q, *J* = 7.1 Hz, 1H, H5), 4.14 (q, *J* = 6.8 Hz, 1H, H2), 1.68 (d, *J* = 6.8 Hz, 3H, H1), 1.21 (d, *J* = 7.1 Hz, 3H, H6); ^{13}C NMR (CD_3OD, 126 MHz) δ 171.1 (C3), 167.4 (C4), 154.9 (C7), 80.5 (C8), 49.3 (C5), 41. 3 (C2), 28.3 (C9), 21. 6 (C1), 17.5 (C6); HRMS (EI) Calcd for $C_{11}H_{20}BrN_3NaO_4$ $[M + Na]^+$ 360.0528, found 360.0529.

6.10.9. *(R)*-1-(2-((*S*)-2-Bromopropanoyl)hydrazineyl)-1-oxopropan-2-aminium (116)



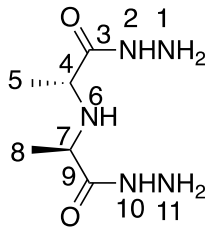
(**116**)

This new compound was prepared following literature protocol.[165] To solution of compound **115** (4.2 g, 0.012 mol) in CH_2Cl_2 (20 mL) at 0 °C was added TFA (20 mL), and the reaction mixture was stirred for 4 h. The solvent was removed and co-evaporated with toluene (3 × 50 mL) to give compound **116** as a white solid (2.7 g, 93% yield) which was used without further purification. $[\alpha]_D^{25}$ = +29.4 (*c* = 0.94, MeOH); IR (MeOH, cast film, cm^{-1}) 3316, 2979, 1701, 1671, 1525, 1169, 1046, 757; ^{1}H NMR (CD_3OD, 500 MHz) δ 4.59 (q, *J* = 7.1 Hz, 1H, H5), 3.89 (q, *J* = 6.8 Hz, 1H, H2), 1.68 (d, *J* = 6.8 Hz, 3H, H1), 1.21 (d, *J* = 7.1 Hz, 3H, H6); ^{13}C NMR (CD_3OD, 126 MHz) δ 171.1 (C3), 167.4 (C4), 154.9 (C7), 49.3 (C5), 41. 3 (C2), 21. 6 (C1), 17.5 (C6); HRMS (EI) Calcd for $C_6H_{14}BrN_3O_2$ $[M + H]^+$ 239.0850, found 239.0852.

6.11. General Procedure for the Preparation of the Bishydrazides

The synthesis of the bishydrazides was achieved following literature protocol.[164] To a solution of **101** (3.00 g, 15.9 mmol) in MeOH (10 mL) was added hydrazine monohydrate (1.50 mL, 31.8 mmol), and the reaction mixture was stirred for 12 h. A white precipitate formed and was filtered, washed with Et_2O (5 × 20 mL), and dried to give a white solid which was recrystallized from Et_2O to give the product as white powder (2.8 g, 93% yield). Using the same procedure, compounds **127**, **129**, **134**, (**140**), **145** and **148** were prepared.

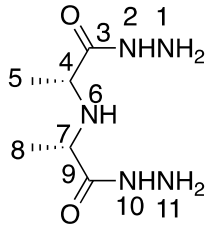
6.11.1. (2*R*,2'*R*)-2,2'-Azanediyldi(propanehydrazide) (125)



(**125**)

$[\alpha]_D^{25}$ = + 37.2 (*c* = 1.00, MeOH); IR (MeOH, cast film, cm^{-1}) 3301, 3208, 3041, 2991, 2979, 1653, 1534, 1322, 971, 689; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.92 (s, 2H, H2, H10), 4.15 (s, 4H, H1, H11), 3.31 (s, 1H, H6), 2.95 (q, *J* = 7.0 Hz, 2H, H4, H7) 1.28 (d, *J* = 7.0 Hz, 6H, H5, H8); ^{13}C NMR (DMSO-*d6*, 126 MHz) δ 173.3 (C3, C9), 54.7 (C4, C7), 19.6 (C5, C8); HRMS (ESI) Calcd for $C_6H_{16}N_5O_2$ $[M + H]^+$ 190.1299, found 190.1275.

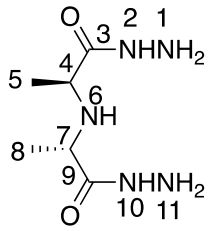
6.11.2. (*R*)-2-(((S)-1-Hydrazinyl-1-oxopropan-2-yl)amino)propanehydrazide (127)



(**127**)

Starting from compound **102** and following the procedure as described for the preparation of **125**: white powder (2.8 g, 93% yield); $[\alpha]_D^{25}$ = 0.03 (*c* = 1.00, MeOH); IR (MeOH, cast film, cm^{-1}) 3301, 3208, 3041, 2991, 2979, 1653, 1534, 1322, 971, 689; ^1H NMR (DMSO-*d*$_6$, 500 MHz) δ 8.93 (s, 2H, H2, H10), 4.21 (s, 4H, H1, H11), 3.32 (s, 1H, H6), 3.01 (q, *J* = 7.0 Hz, 2H, H4, H9), 1.31 (d, *J* = 7.0 Hz, 6H, H5, H8); ^{13}C NMR (DMSO-*d*$_6$, 126 MHz) δ 174.2 (C3, C9), 54.9 (C4, C7), 19.7 (C5, C8); HRMS (ESI) Calcd for $C_6H_{16}N_5O_2$ $[M + H]^+$ 190.1299, found 190.1300.

6.11.3. (2*S*,2'*S*)-2,2'-Azanediyldi(propanehydrazide) (129)



(**129**)

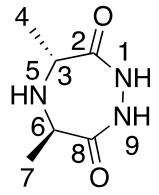
Starting from compound **104** and following the procedure as described for the preparation of **125**: white powder (2.8 g, 94% yield); $[\alpha]_D^{25}$ = - 36.9 (*c* = 0.94, MeOH); IR (MeOH, cast film, cm^{-1}) 3312, 3207, 3032, 2988, 2974, 1662, 1553, 1352, 963, 679;

^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.11 (s, 2H, H2, H10), 3.32 (s, 4H, H1, H11), 3.13 (q, *J* = 7.0 Hz, 2H, H4, H7) 1.27 (d, *J* = 7.0 Hz, 6H, H5, H8); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 173.1 (C3, C9), 54.5 (C4, C7), 19.3 (C5, C8); HRMS (EI) Calcd for $C_6H_{16}N_5O_2$ $[M + H]^+$ 190.1299, found 190.1301.

6.12. General Procedure for Formation of the Cyclized Product Using PhI(OAc)$_2$

The synthesis of the cyclized product was achieved following literature protocol.[98] To a solution of **125** (1.2 g, 6.3 mmol) in dry MeCN (5 mL) was added $PhI(OAc)_2$ (2.1 g, 6.3 mmol) in four portions. The reaction became exothermic with the evolution of gas. After stirring for 10 min the reaction became homogeneous and stirring was continued for an additional 20 mins at room temperature. TLC monitoring of the reaction (10% MeOH in EtOAc, R_f = 0.41) showed completion. The solvent was removed *in vacuo* to a volume of about 1 mL and the crude was purified by column chromatography (SiO_2, 10% MeOH in EtOAc), yielding the product as a yellow solid (0.89 g, 90% yield). The same procedure was used to prepare compounds **128**, **130**, **135**, **141**, **146** and **149**.

6.12.1. (4*R*,6*R*)-4,6-Dimethyl-1,2,5-triazepane-3,7-dione (126)

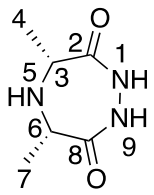


(**126**)

$[\alpha]_D^{25}$ = 43.23 (*c* = 0.89, MeOH); IR (MeOH, cast film, cm^{-1}) 3354, 2991, 2944, 2843, 1714, 1423, 1109, 1056, 755; ^{1}H NMR (D_2O, 500 MHz) δ 4.06 (q, *J* = 7.0 Hz, 2H, H3,

H6) 1.51 (d, J = 7.0 Hz, H4, H7); ^{13}C NMR (D_2O, 126 MHz) δ 174.9 (C2, C8), 51.2 (C3, C6), 16.1 (C4, C7); HRMS (ESI) Calcd for $C_6H_{12}N_3O_2$ $[M + H]^+$ 158.0924, found 158.0926.

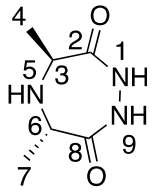
6.12.2. (4*R*,6*S*)-4,6-Dimethyl-1,2,5-triazepane-3,7-dione (128)



(**128**)

This new compound was prepared from compound **127** and following procedure as described for the synthesis of **126**: yellow solid (0.87 g, 88% yield); R_f = 0.48 in 10% MeOH in EtOAc; $[\alpha]_D^{25}$ = 0.00 (c = 0.89, MeOH); IR (MeOH, cast film, cm^{-1}) 3349, 2998, 2954, 2863, 1721, 1424, 1121, 1054, 746; ^{1}H NMR (D_2O, 500 MHz) δ 3.14 (q, J = 7.0 Hz, 2H, H3, H6) 1.21 (d, J = 7.0 Hz, 6H, H4, H7); ^{13}C NMR (D_2O, 126 MHz) δ 176.4 (C2, C8), 55.4 (C3, C6), 18.8 (C4, C7); HRMS (ESI) Calcd for $C_6H_{12}N_3O_2$ $[M + H]^+$ 158.0924, found 158.0921.

6.12.3. (4*S*,6*S*)-4,6-Dimethyl-1,2,5-triazepane-3,7-dione (130)

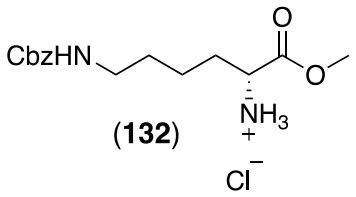


(**130**)

This new compound was prepared following same procedure as described for the synthesis of **126** except that the staring material used was **129**: yellow solid (0.80 g,

86% yield); R_f = 0.42 in 10% MeOH in EtOAc; $[\alpha]_D^{25}$ = - 43.2 (*c* = 0.92, MeOH); IR (MeOH, cast film, cm^{-1}) 3364, 2988, 2943, 2844, 1716, 1427, 1118, 1054, 753; ^1H NMR (D_2O, 500 MHz) δ 4.06 (q, *J* = 7.0 Hz, 2H, H3, H6), 1.52 (d, *J* = 7.02 Hz, 6H, H4, H7); ^{13}C NMR (D_2O, 126 MHz) δ 174.9 (C2, C8), 51.2 (C3, C6), 16.2 (C4, C7); HRMS (ESI) Calcd for $C_6H_{12}N_3O_2$ $[M + H]^+$ 158.0924, found 158.0927.

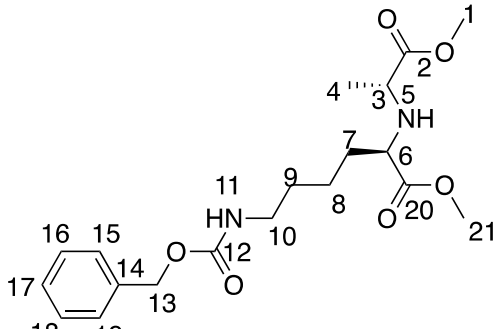
6.12.4. N^{ε}-benzyloxycarbonyl-L-lysine methyl ester hydrochloride (132)



(**132**)

N-Cbz-D-lys-OH (**131**) (10.0 g, 35.7 mmol) was dissolved in MeOH (150 mL) at -25 °C and stirred for 5 minutes. Thionyl chloride (5.0 mL, 0.35 mol) was added dropwise to the reaction mixture and stirring continued for 3 hours. The solvent was evaporated, and the compound was lyophilized to afford **132** as a white solid (quant.). $[\alpha]_D^{25}$ = +15.6 (*c* = 1.0, MeOH); IR (MeOH, cast film, cm^{-1}) 3309, 3032, 2951, 1749, 1697, 1527, 1253, 1135, 776; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.65 (s, 1H), 7.31 – 7.27 (m, 5H), 5.01 (s, 2H), 4.02 (appt, *J* = 6.2 Hz, 1H), 3.8 (s, 3H), 3.33 (s, 3H), 2.98 – 2.96 (m, 2H), 1.81 – 1.78 (m, 2 H), 1.41 – 1.38 (m, 4H), 1.28 – 1.26 (m, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 169.9, 156.1, 137.2, 128.3, 127.7, 127.6, 65.1, 52.6, 51.7, 29.6, 28.7, 21.4; HRMS (ESI) Calcd for $C_{15}H_{23}N_2O_4$ $[M + H]^+$ 295.1580 found 295.1581.

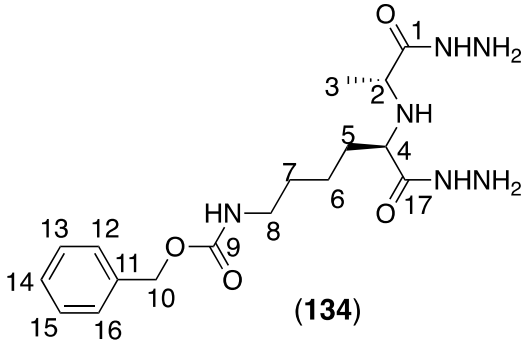
6.12.5. Methyl N^6-((benzyloxy)carbonyl)-N^2-((*R*)-1-methoxy-1-oxopropan-2-yl)-L-lysinate (133)



(**133**)

This new compound was prepared from N^{ε}-benzyloxycarbonyl-L-lysine methyl ester hydrochloride **(132)** and **100** and using the general procedure for synthesis of dimethyl esters yields the final product as a pale yellow oil (9.1 g, 91% yield); R_f = 0.60 in 50% EtOAc in hexanes; $[\alpha]_D^{25}$ = + 3.9 (*c* = 1.5, $CHCl_3$); IR ($CHCl_3$, cast film, cm^{-1}) 3336, 2950, 1731, 1527, 1239, 1057, 697; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.33 – 7.24 (m, 5H, H15, H16, H17, H18, H19), 5.02 (s, 2H, H13), 3.64 (s, 6H, H1, H21), 3.23 (q, *J* = 7.0 Hz, 1H, H3), 3.19 (t, *J* = 7.6 Hz, 1H, H3), 3.17 – 3.11 (m, 2H, H9), 1.67 (td, *J* = 7.7, 7.3 Hz, 2H, H7), 1.51 – 1.45 (m, 2H, H8), 1.40 – 1.34 (m, 2H, H10), 1.27 (d, *J* = 7.0 Hz, 3H, H4); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 175.1 (C2), 175.0 (C20), 156.4 (C12), 136.7 (C14), 129.6 (C16), 128.5 (C18), 128.0 (C17), 127.2 (C15, C19), 66.5 (C13), 59.2 (C6), 55.0 (C5), 51.9 (C1), 51.8 (C21), 40.8 (C10), 33.0 (C7), 29.6 (C9), 22.7 (C8), 18.6 (C4); HRMS (ESI) Calcd for $C_{19}H_{29}N_2O_6$ $[M + H]^+$ 381.2020, found 381.2016.

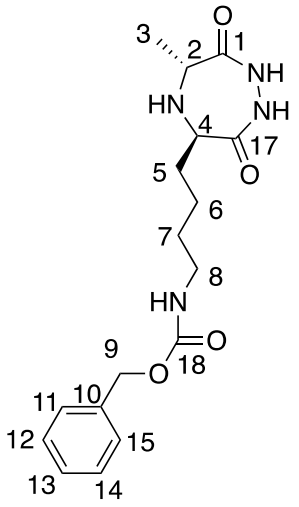
6.12.6. Benzyl((*S*)-6-hydrazineyl-5-(((*R*)-1-hydrazineyl-1-oxopropan-2-yl)amino)-6-oxohexyl)carbamate (134)



(**134**)

This new compound was prepared from **133** and following the general procedure for the synthesis of bishydrazides yields the product as white powder (2.6 g, 86% yield); $[\alpha]_D^{25}$ = + 3.8 (*c* = 1.4, MeOH); IR (MeOH, cast film, cm^{-1}) 3445, 3339, 2961, 1741, 1537, 1236, 1057, 695; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.36 – 7.29 (m, 5H, H12, H13, H14, H15, H16), 4.99 (s, 2H, H10), 3.01 – 2.98 (m, 2H, H2, H4), 1.67 (td, *J* = 7.7 Hz, 7.30 Hz, 2H, H5), 1.51 – 1.45 (m, 6H, H6, H7, H8), 1.27 (d, *J* = 7.0 Hz, 3H, H3); ^{13}C NMR (DMSO-*d*$_6$, 126 MHz) δ 173.2 (C10), 172.8 (C12), 156.1 (C9), 137.2 (C11), 128.3 (C13, C15), 127.7 (C12, C16), 127.6 (C14), 65.0 (C10), 58.6 (C4), 54.2 (C2), 40.1 (C8), 32.8 (C5), 29.3 (C7), 22.5 (C6), 18.5 (C3); HRMS (ESI) Calcd for $C_{17}H_{29}N_6O_4$ $[M + H]^+$ 381.2245, found 381.2243.

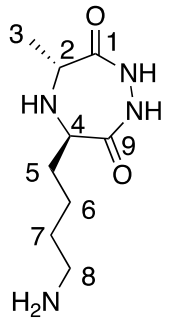
6.12.7. Benzyl (4-((4*S*,6*R*)-6-methyl-3,7-dioxo-1,2,5-triazepan-4-yl)butyl) carbamate (135)



(**135**)

This new compound was prepared from **134** and using the general procedure for the preparation of cyclizied compound with the use of $PhI(OAc)_2$ yields the final product as a yellow solid (1.0 g, 71% yield); R_f = 0.31 in 10% MeOH in EtOAc; $[\alpha]_D^{25}$ = + 3.9 (*c* = 1.3, MeOH); IR (MeOH, cast film, cm^{-1}) 3339, 2971, 1724, 1545, 1238, 1061, 698; ^1H NMR (D_2O, 500 MHz) δ 7.36 – 7.29 (m, 5H, H10, H11, H12, H13, H14, H15), 4.99 (s, 2H, H9), 3.23 (q, *J* = 7.0 Hz, 1H, H2), 3.03 – 3.29 (m, 3H, H4, H8), 1.47 (td, *J* = 7.7, 7.3 Hz, 2H, H5), 1.38 – 1.36 (m, 2H, H6), 1.24 – 1.22 (m, 2H, H7), 1.10 (d, *J* = 7.0 Hz, 3H, H3); ^{13}C NMR (D_2O, 126 MHz) δ 173.3 (C1), 172.7 (C17), 156.4 (C18), 137.6 (C10), 128.4 (C12, C14), 127.8 (C13), 127.6 (C11, C15), 65.2 (C9), 58.8 (C4), 54.3 (C2), 40.4 (C8), 32.6 (C5), 29.4 (C7), 22.7 (C6), 18.6 (C3); HRMS (ESI) Calcd for $C_{17}H_{24}N_4NaO_4$ $[M + Na]^+$ 371.1690, found 371.1689.

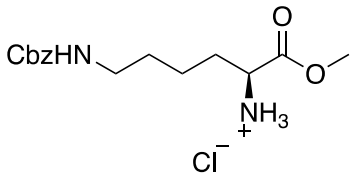
6.12.8. (4*S*,6*R*)-4-(4-Aminobutyl)-6-methyl-1,2,5-triazepane-3,7-dione (136)



(**136**)

This new compound was prepared following literature procedure.[166] A solution of **135** (0.50 g, 1.4 mmol) in dry MeOH (10 mL) was degassed by flushing with Argon for 5 min and palladium on carbon 10% w/w (640 mg) was added. The reaction was stirred under a hydrogen atmosphere at room temperature for 2.5 h. The reaction was filtered through a pad of Celite and washed with MeOH (10 mL), and the solvent was removed *in vacuo* and the crude was purified by column chromatography (SiO_2, 10% MeOH in EtOAc), yielding the product as a yellow solid (0.36 g, 84% yield); R_f = 0.4 in 10% MeOH in EtOAc; $[\alpha]_D^{25}$ = + 4.1 (*c* = 1.6, MeOH); IR (MeOH, cast film, cm^{-1}) 3339, 2971, 1724, 1545, 1238, 1061, 698; ^1H NMR (D_2O, 500 MHz) δ 3.96 (t, *J* = 7.0 Hz, 1H, H4), 3.66 (q, *J* = 7.0 Hz, 1H), 2.64 (t, *J* = 7.3 Hz, 2H, H8), 1.47 (td, *J* = 7.7, 7.3 Hz, 2H, H5), 1.38 – 1.36 (m, 2H, H6), 1.24 – 1.22 (m, 2H, H7), 1.10 (d, *J* = 7.0 Hz, 3H, H3); ^{13}C NMR (D_2O, 126 MHz) δ 164.7 (C1), 164.7 (C9), 60.3 (C4), 55.7 (C2), 41.1 (C8), 29.3 (C5), 27.7 (C7), 22.1 (C6),17.5 (C3); HRMS (ESI) Calcd for $C_9H_{19}N_4O_2$ $[M + H]^+$ 215.1430 found 215.1434.

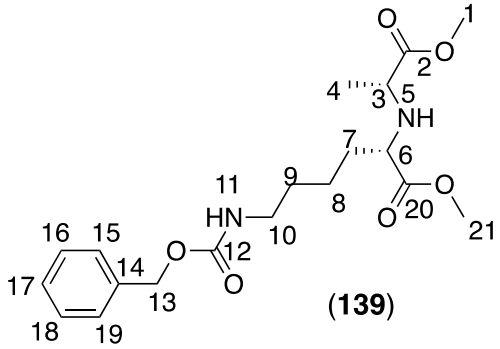
6.12.9. N$^{\varepsilon}$-benzyloxycarbonyl-D-lysine methyl ester hydrochloride (138)



(**138**)

Using the same procedure as described for the preparation of **132**, this known compound was prepared starting from *N*-Cbz-D-lys-OH **(137)**: white solid (quant.); $[\alpha]_D^{25}$ = - 14.3 (*c* = 1.0, MeOH); IR (MeOH, cast film, cm^{-1}) 3307, 3034, 2949, 1749, 1697, 1528, 1252,1135, 736; ^{1}H NMR, (DMSO-d_6, 500 MHz) δ 8.65 (s, 1H, H8), 7.37 – 7.26 (m, 5H, H12, H13, H14, H15, H16), 4.99 (s, 2H, H10), 3.95 (t, *J* = 6.2 Hz,1H, H3), 3.72 (s, 3H, H1), 3.35 (s, 3H, H17), 2.98 – 2.96 (m, 2H, H7), 1.81 – 1.78 (m, 2H, H4), 1.42 – 1.38 (m, 4H, H5, H6), 1.28 – 1.26 1 (m, 1H, H3); ^{13}C NMR (DMSO-d_s, 126 MHz) δ 169.9, 156.1, 137.2, 128.3, 127.7, 127.6, 65.1, 52.6, 51.7, 29.6, 28.7, 21.4; HRMS (ESI) Calcd for $C_{15}H_{23}N_2O_4$ $[M + H]^+$ 295.1580 found 295.1579.

6.12.10. Methyl*N*^{6}-((benzyloxy)carbonyl)-*N*^{2}-((*R*)-1-methoxy-1-oxopropan-2-yl)-*D*-lysinate (139)

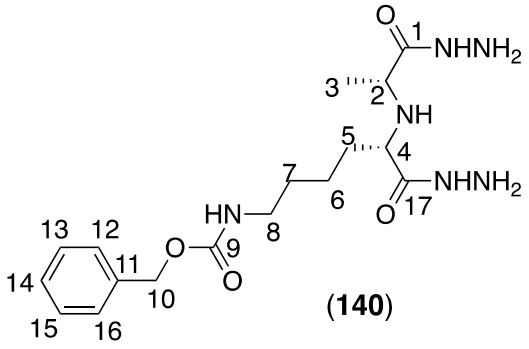


(**139**)

This new compound was synthesized starting from N$^{\varepsilon}$-benzyloxycarbonyl-D-lysine methyl ester hydrochloride **138** and **100** and using the general procedure for the

preparation of dimethyl ester yields the product as a pale yellow oil (8.9 g, 89% yield). R_f = 0.54 in 50% EtOAc in hexanes; $[\alpha]_D^{25}$ = + 18.5 (*c* = 1.5, $CHCl_3$); IR ($CHCl_3$, cast film, cm^{-1}) 3346, 2950, 1730, 1528, 1244, 1202, 1053, 776; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.21 – 7.16 (m, 5H, H15, H16, H17, H18, H19), 5.19 (s, 2H, H13), 3.59 (s, 6H, H1, H21), 3.25 (q, *J* = 7.0 Hz, 1H, H3), 3.19 (t, *J* = 7.6 Hz, 1H, H3), 3.17 – 3.11 (m, 2H, H9), 1.67 (td, *J* = 7.7 Hz, 7.30 Hz, 2H, H7), 1.57 – 1.55 (m, 2H, H8), 1.30 – 1.27 (m, 2H, H10), 1.19 (d, *J* = 7.0 Hz, 3H, H4); ^{13}C NMR ($CDCl_3$, 126 MHz) δ δ 175.1 (C2), 175.0 (C20), 156.4 (C12), 136.7 (C14), 129.6 (C16), 128.5 (C18), 128.0 (C17), 127.2 (C15, C19), 66.5 (C13), 59.2 (C6), 55.0 (C5), 51.9 (C1), 51.8 (C21), 40.8 (C10), 33.0 (C7), 29.6 (C9), 22.7 (C8), 19.0 (C4); HRMS (EI) Calcd for $C_{19}H_{29}N_2O_6$ $[M + H]^+$ 381.2020, found 381.2017.

6.12.11. Benzyl((*R*)-6-hydrazineyl-5-(((*R*)-1-hydrazineyl-1-oxopropan-2-yl)amino)-6-oxohexyl)carbamate (140)

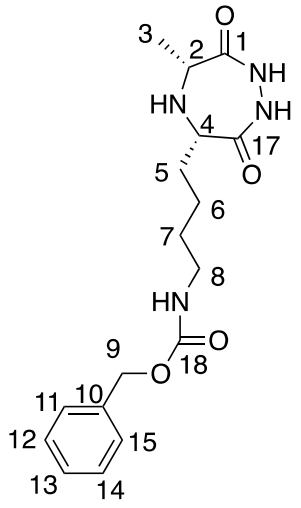


(**140**)

This new compound was prepared starting from **139** and using the general procedure for the preparation of bishydrazides yields the product as a white powder (2.8 g, 88% yield); $[\alpha]_D^{25}$ = + 18.3 (*c* = 1.4, MeOH); IR (MeOH, cast film, cm^{-1}) 3443, 3337, 2959, 1739, 1567, 1236, 1057, 695; ^1H NMR (DMSO-d_6, 500 MHz) δ 7.38 – 7.29 (m, 5H, H12, H13, H14, H15, H16), 5.00 (s, 2H, H10), 3.02 – 2.98 (m, 2H, H2, H4), 1.67 (td,

J = 7.7, 7.3 Hz, 2H, H5), 1.51 – 1.45 (m, 6H, H6, H7, H8), 1.27 (d, *J* = 7.0 Hz, 3H, H3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 173.2 (C10, 172.8 (C12), 156.1 (C9), 137.2 (C11), 128.3 (C13, C15), 127.7 (C12, C16), 127.6 (C14), 65.0 (C10), 58.6 (C4), 54.2 (C2), 40.1 (C8), 32.8 (C5), 29.3 (C7), 22.5 (C6), 18.5 (C3); HRMS (ESI) Calcd for $C_{17}H_{28}N_6NaO_4$ $[M + Na]^+$ 403.2064, found 403.2059.

6.12.12. Benzyl (4-((4*R*,6*R*)-6-methyl-3,7-dioxo-1,2,5-triazepan-4-yl)butyl) carbamate (141)

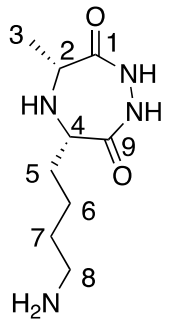


(**141**)

This new compound was prepared following the general procedure for the preparation of cyclized compounds with the use of $PhI(OAc)_2$ starting from **140** to yield the product as yellow solid (1.1 g, 73% yield); R_f = 0.50 in 20% MeOH in EtOAc; $[\alpha]_D^{25}$ = +18.2 (*c* = 1.3, MeOH); IR (MeOH, cast film, cm^{-1}) 3339, 2971, 1724, 1545, 1238, 1061, 698; ^{1}H NMR (D_2O, 500 MHz) δ 7.36 – 7.29 (m, 5H, H10, H11, H12, H13, H14, H15), 4.99 (s, 2H, H9), 3.21 (q, *J* = 7.0 Hz, 1H, H2), 3.04 – 3.01 (m, 3H, H4, H8), 1.44 (td, *J* = 7.7, 7.3 Hz, 2H, H5), 1.38 – 1.36 (m, 2H, H6), 1.24 – 1.22 (m, 2H, H7), 1.10 (d, *J* = 7.0 Hz, 3H, H3); ^{13}C NMR (D_2O, 126 MHz) δ 173.3 (C1), 172.7 (C17), 156.4 (C18),

137.6 (C10), 128.4 (C12, C14), 127.8 (C13), 127.6 (C11, C15), 65.2 (C9), 58.8 (C4), 54.3 (C2), 40.4 (C8), 32.6 (C5), 29.4 (C7), 22.7 (C6), 18.6 (C3); HRMS (ESI) Calcd for $C_{17}H_{24}N_4NaO_4$ $[M + Na]^+$ 371.1690, found 371.1688.

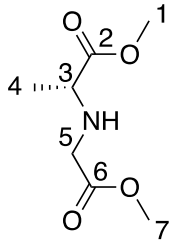
6.12.13. (4*R*,6*R*)-4-(4-Aminobutyl)-6-methyl-1,2,5-triazepane-3,7-dione (142)



(**142**)

This new compound was prepared from compound **141** and following same procedure as described for the synthesis of compound **136** to yield the final product as a yellow solid (0.38 g, 86% yield); R_f = 0.43 in 10% MeOH in EtOAc; $[\alpha]_D^{25}$ = +20.1 (*c* = 1.6, MeOH); IR (MeOH, cast film, cm^{-1}) 3339, 2971, 1724, 1545, 1238, 1061, 698; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.96 (t, *J* = 7.0 Hz, 1H, H4), 3.66 (q, *J* = 7.0 Hz, 1H), 2.64 (t, *J* = 7.3 Hz, 2H, H8), 1.47 (td, *J* = 7.7, 7.3 Hz, 2H, H5), 1.38 – 1.36 (m, 2H, H6), 1.24 – 1.22 (m, 2H, H7), 1.10 (d, *J* = 7.0 Hz, 3H, H3); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 164.7 (C1), 164.7 (C9), 60.3 (C4), 55.7 (C2), 41.1 (C8), 29.3 (C5), 27.7 (C7), 22.1 (C6),17.5 (C3); HRMS (ESI) Calcd for $C_9H_{18}N_4NaO_2$ $[M + Na]^+$ 214.1430 found 214.1438.

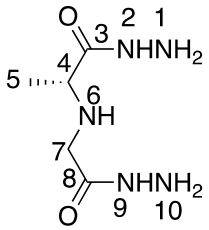
6.12.14. Methyl (2-methoxy-2-oxoethyl)-D-alaninate (144)



(**144**)

Starting from glycine methyl ester hydrochloride **(143)** and using the same procedure as described for the synthesis of **101** yields compound this known compound **144** as a pale yellow oil (3.4 g, 85% yield); R_f = 0.36 in 30% EtOAc in hexanes; $[\alpha]_D^{25}$ = +27.3 (*c* = 1.0, $CHCl_3$); IR ($CHCl_3$, cast film, cm^{-1}) 3623, 3342, 2955, 1739, 1437, 1205, 1157, 978, 754; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.70 (s, 6H, H1, H7), 3.47 – 3.33 (m, 3H, H3, H5) 1.32 (d, *J* = 7.0 Hz, 3H, H4); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.1 (C2), 172.2 (C6), 55.8 (C3), 51.9 (C1), 51.8 (C7), 48.8 (C5), 18.7 (C4); HRMS (ESI) Calcd for $C_7H_{14}NO_4$ $[M + H]^+$ 176.0917, found 176.0920.

6.12.15. (*R*)-2-((2-Hydrazineyl-2-oxoethyl)amino)propanehydrazide (145)

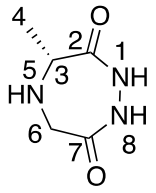


(**145**)

This new compound was prepared using the general procure for the preparation of bishydrazides starting from (174) to yield the final product as a white powder (2.7 g, 90% yield); $[\alpha]_D^{25}$ = + 26.8 (*c* = 0.90, MeOH); IR (MeOH, cast film, cm^{-1}) 3424, 3332,

3206, 3031, 2988, 2971, 1714, 1550, 1358, 958, 674; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.01 – 2.98 (m, 3H, H4, H7), 1.09 (d, J = 7.0 Hz, 3 H, H5); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 173.2 (C3), 170.1 (C8), 55.8 (C4), 49.2 (C7), 18.8 (C5); HRMS (ESI) Calcd for $C_5H_{14}N_5O_2$ $[M + H]^+$ 176.1142, found 176.1141.

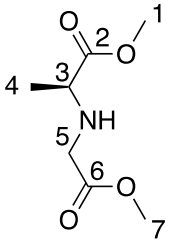
6.12.16. (*R*)-4-Methyl-1,2,5-triazepane-3,7-dione (146)



(**146**)

This new compound was prepared using the general procedure for the preparation of cyclized compound with the use of $PhI(OAc)_2$ starting from **175** yields the final product as a yellow solid (1.79 g, 67% yield); R_f = 0.44 in 10% MeOH in EtOAc; $[\alpha]_D^{25}$ = + 25.5 (c = 1.0, MeOH); IR (MeOH, cast film, cm^{-1}) 3354, 2978, 2953, 2832, 1721, 1422, 1128, 1051, 763; ^{1}H NMR (D_2O, 500 MHz) δ 3.28 – 3.24 (m, 3H, H3, H6) 1.24 (d, J = 7.0 Hz, 3H, H4); ^{13}C NMR (D_2O, 126 MHz) δ 173.2 (C2), 170.1 (C7), 55.8 (C3), 49.2 (C6), 18.8 (C4); HRMS (ESI) Calcd for $C_5H_{10}N_3O_2$ $[M + H]^+$ 144.0697, found 144.0694.

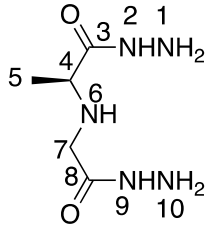
6.12.17. Methyl (2-methoxy-2-oxoethyl)-L-alaninate (147)



(**147**)

Starting from glycine methyl ester hydrochloride **(143)** and **103** and using the same procedure as describe for the preparation of the diester, yields this known compound **147** as a pale-yellow oil. The crude compound was purified by column chromatography (SiO_2, 40% EtOAc in hexanes), yielding the product as a pale yellow oil (3.2 g, 82% yield); R_f = 0.38 in 30% EtOAc in hexanes; $[\alpha]_D^{25}$ = + 27.3 (*c* = 1.0, $CHCl_3$); IR ($CHCl_3$, cast film, cm^{-1}) 3623, 3342, 2955, 1739, 1437, 1205, 1157, 978, 754; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.63 (s, 6H, H1, H7), 3.38 – 3.28 (m, 3H, H3, H6) 1.25 (d, *J* = 7.0, 3H, H4); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 175.0 (C2), 172.1 (C7), 55.8 (C3), 51.8 (C1), 51.7 (C8), 48.8 (C7), 18.6 (C4); HRMS (ESI) Calcd for $C_7H_{14}NO_4$ $[M + H]^+$ 176.0917, found 176.0921.

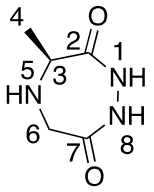
6.12.18. (*S*)-2-((2-Hydrazineyl-2-oxoethyl)amino)propanehydrazide (148)



(**148**)

This new compound was prepared using the general procedure for the preparation of bishydrazides starting from **147** yields compound **148** as a white powder (2.6 g, 89% yield); $[\alpha]_D^{25}$ = +26.8 (*c* = 0.90, MeOH); IR (MeOH, cast film, cm^{-1}) 3434, 3312, 3207, 2998, 2974, 1721, 1554, 1348, 953, 676; ^1H NMR (DMSO-d_6, 500 MHz) δ 3.04 – 2.98 (m, 3H, H4, H7) 1.07 (d, *J* = 7.0, 3H, H5); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 174.2 (C3), 171.1 (C8), 55.7 (C4), 49.5 (C7), 18.6 (C5); HRMS (ESI) Calcd for $C_5H_{14}N_5O_2$ $[M + H]^+$ 176.1142, found 176.1140.

6.12.19. (*S*)-4-Methyl-1,2,5-triazepane-3,7-dione (149)

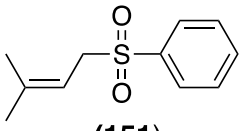


(**149**)

The same procedure for the preparation of the cyclized product using $PhI(OAc)_2$ was used to prepare this new compound **149** to give the final product as a yellow solid (1.8 g, 68% yield); R_f = 0.46 in 10% MeOH in EtOAc; $[\alpha]_D^{25}$ = + 25.5 (*c* = 1.0, MeOH); IR (MeOH, cast film, cm^{-1}) 3352, 2988, 2962, 2835, 1719, 1411, 1127, 1054, 765; ^1H NMR (D_2O, 500 MHz) δ 3.26 – 3.22 (m, 3H, H3, H6) 1.23 (d, *J* = 7.0, 3H, H4); ^{13}C NMR (D_2O, 126 MHz) δ 173.4 (C2), 170.5 (C7), 55.9 (C3), 49.4 (C6), 18.7 (C4); HRMS (EI) Calcd for $C_5H_{10}N_3O_2$ $[M + H]^+$ 144.0697, found 144.0695.

6.13. Experimental Procedures for the Synthesis of C15-*Z, Z*- Gram-negative and Gram-positive Lipid II

6.13.1. 1-Benzenesulfonyl-3-methyl-2-butene (151)

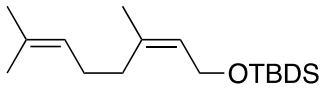


(151)

This known compound was prepared following a modified literature protocol.[116] To a solution of $NaSO_2Ph$ (4.0 g, 24 mmol) in DMF (40 mL) at 0 °C was added 3,3-dimethylallyl bromide (2.5 g, 17 mmol). The reaction was stirred for 4 h at 0 °C and after completion as indicated by TLC (20% EtOAc in Hexanes (R_f = 0.3)), the reaction mixture was diluted with EtOAc. The organic phase was washed with water (15 mL),

brine (15 mL), dried with Na_2SO_4, filtered, and concentrated *in vacuo*, and purified by column chromatography (SiO_2, 20% EtOAc in hexanes,), yielding the product as a colorless oil (2.7 g, 80% yield). IR ($CHCl_3$, cast film, cm^{-1}) 3062, 2975, 2916, 1667, 1585, 1479, 1447, 1306, 1150, 1131, 1106, 1085, 898, 773, 739, 689; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.74 – 7.73 (m, 2H,), 7.52 – 7.50 (m, 1H), 7.42 – 7.40 (m, 2H), 5.16 (qqt, 1H, J = 7.9, 1.4, 1.4 Hz), 3.67 (d, 2H, J = 7.8 Hz), 1.69 (s, 3H), 1.29 (s, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 143.0, 138.8, 133.6, 129.0, 128.5, 110.5, 56.2, 25.9, 17.8; HRMS (ESI) Calcd for $C_{11}H_{15}O_2S$ $[M+H]^+$ 211.0711, found 211.0714.

6.13.2. 1,1′-[(1,1-Dimethylethyl)[[(2z)-3,7-dimethyl-2,6-octadien-1-yl]oxy]silylene]bis[benzene (155)

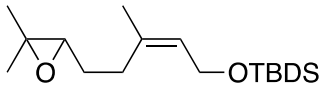


(**155**)

This known compound was prepared following a modified literature protocol.[116] To a solution of commercially available nerol **(154)** (10 mL, 0.057 mol) in DMF was added imidazole (9.7 g, 0.14 mol) and stirred for 5 min at rt. *Tert*-butyl diphenyl silylchloride (18 mL, 0.070 mol) was added and the reaction mixture was stirred at rt for 16 h, after which TLC analysis indicated a completion of reaction (5% EtOAc in Hexane, R_f = 0.2). The reaction mixture was diluted with EtOAc (300 mL) and the organic phase was washed with 0.5 M HCl, saturated $NaHCO_3$, water, and brine and then dried over $NaSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2, 10% EtOAc in hexane), yielding the product as a colorless oil (quant.). IR ($CHCl_3$, cast film, cm^{-1}) 3062, 2975,

2916, 1667, 1585, 1479, 1447, 1306, 1150, 1131, 1106, 1085, 898, 773, 739, 689; ^{1}H NMR ($CDCl_3$, 700 MHz) δ 7.73 – 7.71 (m, 4H), 7.45 – 7.38 (m, 6H), 5.44 (t, 1H, *J* = 6.4 Hz), 5.02 (t, 1H, *J* = 7.2 Hz), 4.32 (d, 2H, *J* = 6.7 Hz), 2.21 (td, 2H, *J* = 7.4, 7.15 Hz), 2.13 (t, 2H, J = 7.4 Hz), 1.72 (s, 3H), 1.62 (s, 3H), 1.53 (s, 3H), 1.06 (s, 9H); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 137.5, 135.7, 134.1, 131.3, 129.6, 127.7, 124.2, 123.6, 60.9, 38.3, 26.9, 26.7, 21.8, 21.8, 19.2, 17.6; HRMS (ESI) Calcd for $C_{26}H_{36}NaOSi$ $[M+Na]^+$ 415.2535, found 415.2539.

6.13.3. (*Z*)-Tert-butyl((5-(3,3-dimethyloxiran-2-yl)-3-methylpent-2-en-1-yl)oxy)diphenylsilane (156)

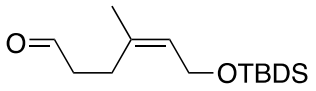


(**156**)

This known compound was prepared following a modified literature protocol.[116] To a stirred solution of the protected nerol **155** (20 g, 0.05 mol) in THF/H_2O (110:35 mL) at 0 °C, was added N-bromosuccinimide (10 g, 0.057 mol). The reaction mixture was stirred at 0 °C for 8 h and TLC analysis indicated the formation of a single product (3% EtOAc in hexane, R_f = 0.25). The reaction mixture was concentrated *in vacuo* to give a light-yellow oil which was extracted with diethyl ether (2 × 100 mL). The combined organic layer was washed with H_2O (30 mL), saturated NH_4CO_3 (30 mL), and brine (30 mL), then dried over $NaSO_4$, filtered, and concentrated *in vacuo* to give a yellow oil which was used directly without further purification. This compound was re-dissolved in methanol (30 mL) and potassium carbonate (14 g, 0.10 mol) was added and the mixture was stirred at rt for 24 h. A TLC after this time indicated a completion

of reaction (6% EtOAc in hexane, R_f = 0.23). The reaction mixture was filtered, and the solvent was concentrated *in vacuo*, and the residue was dissolved in petroleum ether, filtered, and the solvent was removed *in vacuo* to give a light-yellow oil. Purification by column chromatography (SiO_2, 10% EtOAc in hexane), gave the desired product as colorless oil (quant.). IR ($CHCl_3$, cast film, cm^{-1}) 3065, 2979, 2926, 1668, 1587, 1489, 1456, 1309, 1161, 1137, 1108, 1083, 896, 774, 734, 689; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.73 – 7.71 (m, 4H), 7.45 – 7.38 (m, 6H), 5.50 (t, 1H, *J* = 6.1 Hz), 4.32 (d, 2H, *J* = 6.5 Hz), 2.61 (t, 2H, *J* = 7.3 Hz), 2.07 – 2.01 (m, 2H), 1.75 (s, 3H), 1.63 – 1.59 (m, 1H), 1.50 – 1.46 (m, 1H), 1.25 (s, 3H), 1.19 (s, 3H), 1.09 (s, 9H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 136.6, 135.6, 134.0, 129.6, 127.7, 125.5, 63.8, 60.7, 58.2, 28.8, 27.5, 26.9, 24.8, 23.4, 19.2, 18.6; HRMS (ESI) Calcd for $C_{26}H_{36}NaO_2Si$ $[M+Na]^+$ 431.2377, found 431.2378.

6.13.4. (*Z*)-6-((Tert-butyldiphenylsilyl)oxy)-4-methylhex-4-enal (157)

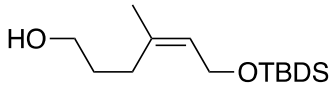


(**157**)

This known compound was prepared following a modified literature protocol.[116] To a solution of dioxane/H_2O (40 mL/20 mL) at 0 °C was added the epoxide **156** (20 g, 0.049 mmol). The reaction mixture was stirred at 0 °C for 10 h, after which time a TLC analysis indicated a completion of reaction (15% EtOAc in hexane, R_f = 0.32), and then the reaction mixture was filtered through Celite and washed with dioxane (30 mL). The organic layer was extracted with ethyl acetate (3 × 60 mL) and the combined organic layer was washed with saturated NH_4CO_3 (30 mL) and dried over Na_2SO_4, filtered, and

concentrated *in vacuo.* Purification by column chromatography (SiO_2, 10% EtOAc in hexane) gave the desired product as colorless oil (14.9 g, 74% yield). IR ($CHCl_3$, cast film, cm^{-1}) 3071, 2959, 2857, 1725, 1472, 1428, 1150, 1112, 1064, 823, 703; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.65 (s, 1H), 7.75 – 7.73 (m, 4H), 7.44 – 7.39 (m, 6H), 5.49 (t, 1H, *J* = 6.1 Hz), 4.23 (d, 2H, *J* = 5.0 Hz), 2.39 – 2.36 (m, 2H), 2.21 – 2.18 (m, 2H), 1.54 (s, 3H), 1.09 (s, 9H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 201.8, 140.1, 135.6, 134.8, 133.8, 129.6, 127.6, 126.1, 60.5, 42.1, 30.1, 26.88, 19.2, 17.2; HRMS (ESI) Calcd for $C_{23}H_{30}NaO_2Si$ $[M+Na]^+$ 389.1907, found 389.1910.

6.13.5. (*Z*)-6-((Tert-butyldiphenylsilyl)oxy)-4-methylhex-4-en-1-ol (158)

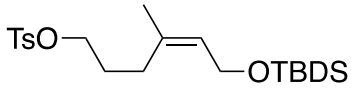


(**158**)

This known compound was prepared following a modified literature protocol.[116] To a stirred solution of the aldehyde **157** (15 g, 0.040 mmol) in EtOH (60 mL) at 0 °C, was added $NaBH_4$ (2.9 g, 0.078 mmol). The reaction mixture was stirred at 0 °C for 4 h and 0.5 M HCl was carefully added to quench the reaction, and then the reaction mixture was filtered through Celite. The filtrate was evaporated *in vacuo*, and the oil residue was re-dissolved in diethyl ether. This ether solution was washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo.* Purification by column chromatography (SiO_2, 10% EtOAc in Hexane) gave the desired product as a colorless oil (14.1 g, 95% yield). IR ($CHCl_3$, cast film, cm^{-1}) 3345, 3071, 2932, 2857, 1472, 1447, 1112, 1061, 823, 702; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.65 (s, 1H), 7.73 – 7.70 (m, 4H), 7.44 – 7.40 (m, 6H), 5.45 (t, 1H, *J* = 6.2 Hz), 3.71 (d, 2H, *J* = 7.0 Hz), 3.34 (t,

2H, *J* = 6.3 Hz), 1.95 (t, 2H, *J* = 7.4 Hz), 1.88 (tt, *J* = 7.5, 5.3 Hz), 1.54 (s, 3H), 1.09 (s, 9H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 140.1, 135.6, 134.8, 133.8, 129.6, 127.6, 126.1, 60.5, 60.4, 42.1, 30.1, 26.8, 19.20, 17.2; HRMS (ESI) Calcd for $C_{23}H_{32}NaO_2Si$ $[M+Na]^+$ 391.2064, found 391.2068.

6.13.6. (*Z*)-6-((Tert-butyldiphenylsilyl)oxy)-4-methylhex-4-en-1-yl- 4-methylbenzenesulfonate (159)

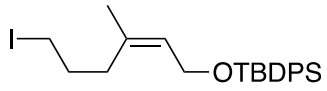


(**159**)

This known compound was prepared following a modified literature protocol.[116] The alcohol **158** (10 g, 0.027 mmol) was dissolved in pyridine (20 mL) and TsCl (6.3 g, 0.033 mmol) was added, and the reaction mixture was stirred at rt for 4.5 h. The reaction mixture was concentrated *in vacuo*, and the yellow oil residue was re-dissolved in petroleum ether and the insoluble pyridinium salt was removed by filtration. The organic layer was washed with $CuSO_4$ solution (20 mL), water (15 mL), saturated NH_4CO_3 (20 mL), and then dried over Na_2SO_4, filtered, and concentrated *in vacuo.* Purification by column chromatography (SiO_2, 7% EtOAc in hexane), gave the desired product as colorless oil (13.3 g, 88% yield). IR ($CHCl_3$, cast film, cm^{-1}) 3243, 3065, 2912, 2857, 1477, 1427, 1117, 1063, 823, 702; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.73 (d, 2H, *J* = 8.4Hz), 6.68 – 6.67 (m, 4H), 7.44 – 7.40 (m, 6H), 7.27 (d, 2H, *J* = 8.4Hz), 5.38 (t, 1H, *J* = 6.2 Hz), 4.12 (d, 2H, *J* = 6.5 Hz), 3.90 – 3.89 (t, 2H, *J* = 6.5), 2.42 (s, 3H), 1.87 (m, 2H), 1.61 (m, 5H), 1.08 (s, 9H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 144.7, 135.9, 135.7, 134.0, 133.3, 129.7, 129.7, 127.9, 127.78, 126.1, 70.2, 60.5, 27.8, 27.3, 26.9,

23.2, 21.7, 19.2; HRMS (ESI) calcd for $C_{30}H_{38}NaO_4SSi$ $[M+Na]^+$ 545.2148 found 545.2154.

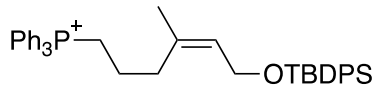
6.13.7. (*Z*)-Tert-butyl((6-iodo-3-methylhex-2-en-1-yl)oxy)diphenylsilane (159a)



(159a)

This known compound was prepared following a modified literature protocol.[116] The tosylated compound **159** (11.2 g, 21.9 mmol) and NaI (8.2 g, 56 mmol) were dissolved in acetone (40 mL) and the reaction mixture was heated at reflux for 18 h. A TLC analysis indicted the formation of a single product and a total consumption of the starting material (10% EtOAc in Hexane, R_f = 0.32). The reaction mixture was poured into a solution of $Na_2S_2O_3$, and the organic layer was extracted with petroleum ether, and then the organic layer was dried over $NaSO_4$, filtered, and concentrated *in vacuo.* Purification by column chromatography (SiO_2, 7% EtOAc in hexane), gave the desired product as colorless oil (10.3 g, 97% yield). IR ($CHCl_3$, cast film, cm^{-1}) 3345, 3071, 2932, 2857, 1472, 1447, 1112, 1061, 823, 702; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.65 (s, 1H), 7.73 – 7.7 (m, 4H), 7.44 – 7.40 (m, 6H), 5.45 (t, 1H, *J* = 6.2 Hz), 3.71 (d, 2H, *J* = 7.0 Hz), 2.95 (t, 2H, *J* = 6.3 Hz), 1.95 (t, 2H, *J* = 7.5 Hz), 1.88 (tt, *J* = 7.5, 5.3 Hz, 2H),1.54 (s, 3H), 1.09 (s, 9H); ^{13}C NMR ($CDCl_3$, 126 MHz) 140.1, 135.6, 134.8, 133.8, 129.6, 127.6, 126.1, 60.5, 42.1, 30.1, 26.8, 19.2, 17.2, 6.7; HRMS (ESI) calcd for $C_{23}H_{31}INaOSi$ $[M+Na]^+$ 501.1187 found 501.1189.

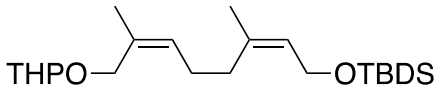
6.13.8. (*Z*)-(6-((Tert-butyldiphenylsilyl)oxy)-4-methylhex-4-en-1-yl)triphenylphosphonium (159b)



(159b)

This known compound was prepared following a modified literature protocol.[116] PPh_3 (7.6 g, 29 mmol) was added to a solution of the iodinated compound **159a** (10.00 g, 26.01 mmol) in dry toluene (50 mL) and the reaction mixture was heated at reflux under Argon atmosphere for 24 h. After this time, the reaction mixture was cooled to rt and was diluted with petroleum ether (180 mL) and a precipitate was formed. The precipitated was collected by filtration and dried for 5 min. The precipitate was dissolved in acetone (20 mL) and concentrated to a volume around 10 mL *in vacuo*. Boiling petroleum ether (150 mL) was added to the concentrated mixture, and then the solution was cooled to 0 °C and left to recrystallize. The product was obtained as an off-white crystalline solid (9.2 g, 71% yield).

6.13.9. Tert-butyl(((2*Z*,6*Z*)-3,7-dimethyl-8-((tetrahydro-2*H*-pyran-2-yl)oxy)octa-2,6-dien-1-yl)oxy)diphenylsilane (161)

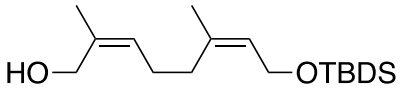


(161)

This known compound was prepared following a modified literature protocol.[116] Compound **159b** (9.0 g, 12.1 mmol) was dissolved in dry THF (50 mL) and BuLi (2.5 M, solution in hexane, 45 mL) was added. The reaction was stirred at rt for 1 h after

which the mixture was cooled to -78 °C and a solution of THP-acetol **160** (2.6 g, 16 mmol) in dry THF (15 mL) was added. The reaction mixture was allowed to slowly warm to rt over 24 h and was poured onto water, then the mixture was then extracted with petroleum ether and the organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by column chromatography (SiO_2, 10% EtOAc in hexane), gave the desired product as a colorless oil (5.5 g, 91% yield). IR (neat, cm^{-1}) 2931, 2857, 1427, 1258, 1108, 1055, 823, 738, 700; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.78 – 7.76 (m, 4H), 7.48 – 7.42 (m, 6H), 5.51 (t, 1H, J = 6.5 Hz), 5.32 (t, 1H, J = 7.0 Hz), 4.59 (t, 1H, J = 3.5 Hz), 4.29 (d, 2H, J = 6.5 Hz), 4.12 – 4.06 (m, 2H), 3.90 – 3.86 (m, 1H), 3.52 – 3.49 (m, 1H), 2.16 – 2.11 (m, 2H), 1.98 – 1.97 (m, 2H), 1.93–1.85 (m, 1H), 1.77 (s, 6H), 1.75-1.53 (m, 5H), 1.13 (s, 9H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 137.0, 135.7, 134.1, 132.2, 129.6, 128.9, 127.7, 125.3, 97.4, 65.3, 62.1, 60.88, 32.4, 30.7, 26.9, 26.1, 25.6, 23.4, 21.7, 19.5, 19.2; HRMS (ESI^+) m/z 515.2942 $[M+Na]^+$, calculated 515.2957 for $C_{31}H_{44}NaO_3Si$.

6.13.10. (2*Z*,6*Z*)-8-((Tert-butyldiphenylsilyl)oxy)-2,6-dimethylocta-2,6-dien-1-ol (162)

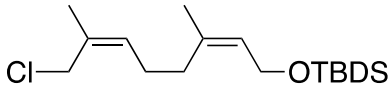


(**162**)

This known compound was prepared following a modified literature protocol.[116] *p*-Toluene sulfonic acid (44 mg, 0.22 mmol) was added to a solution of the acetol product **161** (5.00 g, 10.2 mmol) in isopropanol/Et_2O (25 mL/13mL), and the reaction mixture was stirred at rt for 3 days. The reaction mixture was diluted with Et_2O (300 mL),

washed with saturated $NaHCO_3$, brine, and then dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by column chromatography (SiO_2, 10% EtOAc in Hexane), gave the desired product as colorless oil (3.6 g, 91% yield). IR (neat, cm^{-1}) 3345, 2936, 2847, 1422, 1258, 1108, 1065, 833, 732, 698; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.70 – 7.68 (m, 4H), 7.44 – 7.37 (m, 6H), 5.44 (t, 1H, J = 6.0 Hz), 5.16 (t, 1H, J = 7.5 Hz), 4.17 (d, 2H, J = 6.5 Hz), 3.99 (s, 2H), 2.05 – 2.01 (m, 2H), 1.92 (t, J = 7.5 Hz, 2H), 1.71 (s, 6H), 1.05 (s, 9H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 137.2, 135.7, 135.1, 134.0, 129.6, 127.7, 127.7, 125.4, 61.5, 60.9, 32.4, 26.9, 26.1, 23.6, 21.3, 19.2; HRMS (ESI) calcd for $C_{26}H_{36}NaO_2Si$ $[M+Na]^+$ 431.2366 found 431.2377.

6.13.11. Tert-butyl(((2*Z*,6*Z*)-8-chloro-3,7-dimethylocta-2,6-dien-1-yl)oxy)diphenylsilane (153)

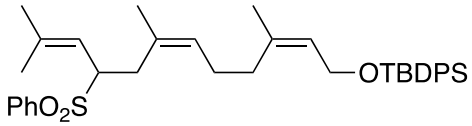


(**153**)

This known compound was prepared following a modified literature protocol.[116] To a solution of the alcohol compound **162** (2.00 g, 4.89 mmol), LiCl (0.64 g, 15 mmol) and 2,4,5-collidine (2.6 mL, 20 mmol) in DMF at -3 °C was added methanesulfonyl chloride (1.2 mL, 15 mmol). The reaction was stirred for 3 h and after this time, the reaction mixture was poured into an ice cold saturated $NaHCO_3$ solution (55 mL) and extracted with 1:1 hexane/Et_2O (3 × 50 mL). The combined organic layers were washed with saturated NH_4Cl, brine, and then dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by column chromatography (SiO_2, 10% EtOAc in hexane), gave the desired product as colorless oil (1.8 g, 81% yield). IR (neat, cm^{-1})

2938, 2853, 1432, 1248, 1118, 1055, 823, 712, 699; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.71 – 7.70 (m, 4H), 7.45 – 7.38 (m, 6H), 5.45 (t, 1H, *J* = 6.1 Hz), 5.16 (t, 1H, *J* = 7.6 Hz), 4.17 (d, 2H, *J* = 6.5 Hz), 3.99 (s, 2H), 2.09 – 2.04 (m, 2H), 1.96 – 1.93 (m, 2H), 1.75 (s, H), 1.72 (s, 3H), 1.06 (s, 9H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 136, 135.62, 133.97, 130.41, 129.54, 127.60, 125.57, 60.73, 43.49, 31.88, 26.85, 26.31, 23.32, 21.50, 19.17; HRMS (ESI) calcd for $C_{26}H_{35}NaOClSi$ $[M+Na]^+$ 449.2035 found 449.2043.

6.13.12. Tert-butyldiphenyl(((2*Z*,6*Z*)-3,7,11-trimethyl-9-(phenylsulfonyl)dodeca-2,6,10-trien-1-yl)oxy)silane (163)

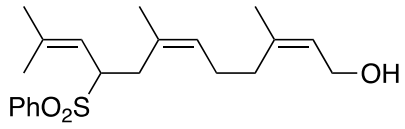


(**163**)

This known compound was prepared following a modified literature protocol.[116] To a solution of 2,2-dimethylallyl phenylsulfone **(151)** (130 mg, 0.620 mmol) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidone (DMTP) (0.12 mL) in THF (1.2 mL) at -78 °C, was added Butyl lithium (1.5 M solution in hexane, 0.54 mL, 0.81 mmol). After stirring for 2h at -78 °C, a solution of compound **153** (132 mg, 0.31 mmol) in THF (15 mL) was added dropwise over 90 min. The reaction mixture was allowed to warm to -30 °C over 2.5 h and was stirred at this temperature for a further 2 h. After this time, the reaction was quenched by pouring onto saturated NH_4Cl solution (25 mL) and the aqueous phase was extracted with 1:1 hexane/Et_2O (3 × 60 mL). The organic phase was washed with brine (3 × 60 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by column chromatography (SiO_2, 10% EtOAc in

hexane), gave the desired product as a colorless oil (139 mg, 75% yield). IR (neat, cm^{-1}) 1665, 1470, 1428, 1362, 1113, 700; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.85 – 7.79 (m, 2H), 7.74 – 7.64 (m, 4H), 7.64 – 7.56 (m, 1H), 7.56 – 7.45 (m, 2H), 7.45 – 7.35 (m, 6H), 5.40 (dt, 1H, *J* = 6.3, 1.0 Hz), 5.06 (t, 1H, *J* = 7.0 Hz), 4.94 – 4.85 (6, 1H, *J* = 10.4 Hz), 4.17 (d, 2H, *J* = 6.3), 3.80 (ddd, 1H, *J* = 11.3, 10.5, 2.6 Hz), 2.67 (dd, 1H, *J* =13.3, 2.6 Hz), 2.47 (dd,1H, *J* = 13.2, 11.3 Hz), 1.94 (m, 2H), 1.87 (m, 2H), 1.69 (d, 3H, *J* = 1.1 Hz), 1.60 (d, 3H, *J* = 1.0 Hz), 1.53 (d, 3H, *J* = 0.6 Hz), 1.12 (d, 3H, *J* = 1.1 Hz), 1.05 (s, 9H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 142.2, 138.2, 137.2, 136.0, 134.4, 133.8, 130.6, 129.9, 129.5, 129.1, 128.6, 128.0, 125.7, 117.5, 64.1, 61.1, 32.4, 30.0, 27.2, 26.7, 26.2, 23.7, 23.7, 19.61, 18.2; HRMS (ESI) calcd for $C_{37}H_{48}NaO_3SSi$ $[M+Na]^+$ 623.2988 found 623.2986.

6.13.13. (2*Z*,6*Z*)-3,7,11-Trimethyl-9-(phenylsulfonyl)dodeca-2,6,10-trien-1-ol (164)

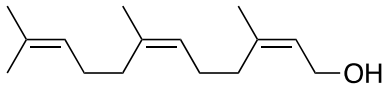


(**164**)

This known compound was prepared following a modified literature protocol.[116] Compound **163** (100 mg, 0.170 mmol) was dissolved in THF (100 mL) and the solution was cooled to 0 °C, and a solution of tetrabutylammonium fluoride (1 M in THF, 0.3 mL, 0.3 mmol) was added. The reaction mixture was stirred at 0 °C for 16 h and was diluted with diethyl ether (100 mL) and washed with brine (3 × 40 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by column chromatography (SiO_2, 10% EtOAc in hexane) gave the desired product as a colorless

oil (87 mg, 75% yield). IR (neat, cm^{-1}) 1664, 1472, 1427, 1362, 1113; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.85 – 7.80 (m, 2H), 7.68 – 7.67 (m, 4H), 7.61 – 7.58 (m, 1H), 7.50 – 7.47 (m, 2H), 5.39 (dt, 1H, *J* = 6.0 Hz), 5.07 (t, 1H, *J* = 6.5 Hz), 4.89 (d, 1H, *J* = 10.5 Hz), 4.16 (d, 2H, *J* = 6.5 Hz), 3.80 (ddd, 1H, *J* = 2.6, 11.3, 10.55 Hz), 2.67 (dd, 1H, *J* = 2.6, 13.3 Hz), 2.48 (dd, 1H, *J* = 11.3, 13.3 Hz) 1.94 (m, 2H), 1.86 (m, 2H), 1.67 (s, 3H), 1.58 (s, 3H), 1.55 (s, 3H), 1.12 (d, 3H, *J* = 1.1 Hz, H12) 1.05 (s, 9H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 140.1, 138.2, 136.1, 133.8, 131.8, 129.5, 129.15, 124.6, 124.5, 124.3, 59.1, 32.3, 32.0, 26.7, 26.4, 25.8, 23.5, 23.4, 17.8; HRMS (ESI) Calcd for $C_{26}H_{36}NaO_2Si$ $[M+Na]^+$ 385.1915 found 385.1918.

6.13.14. (2*Z*,6*Z*)-3,7,11-Trimethyldodeca-2,6,10-trien-1-ol (165)

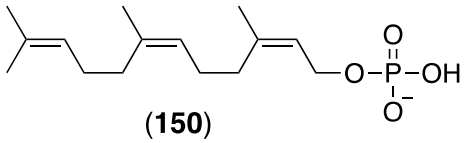


(**165**)

This known compound was prepared following a modified literature protocol.[116] The alcohol **164** was dissolved in in THF (4 mL) and (bis(diphenylphosphino)propane)palladium(II) dichloride ((dppp)$PdCl_2$, (13.0 mg, 0.120 mmol)) was added. Super-hydride (1 M solution in THF, 0.7 mL, 0.7 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 2 h. Saturated NH_4Cl solution (15 mL) was then added, and the mixture was extracted with petroleum ether (3 × 20 mL). The combined organic layer was washed with brine (3 × 20 mL), dried over Mg_2SO_4, filtered, and concentrated *in vacuo*. Purification by column chromatography (SiO_2, 10% EtOAc in hexane), gave the desired product as colorless oil (70 mg, 75% yield). IR (neat, cm^{-1}) 3326, 2963, 2928, 2855, 1667, 1442, 1375,

1001, 826; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.49 (dt, 1H, *J* = 6.0 Hz), 5.13 – 5.08 (m, 2H), 4.11 (d, 1H, *J* = 7.1 Hz), 2.17 – 2.06 (m, 4H), 2.05 – 2.01 (m, 4H), 1.76 (s, 3H), 1.69 – 1.65 (m, 6H), 1.61 (s, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 140.1, 136.1, 131.8, 124.6, 124.5, 124.3, 59.1, 32.3, 32.0, 26.7, 26.4, 25.8, 23.5, 23.4, 17.8; HRMS (ESI) Calcd for $C_{15}H_{26}NaO$ $[M+Na]^+$ 245.1876, found 245.1878.

6.13.15. (2*Z*,6*Z*)-3,7,11-Trimethyldodeca-2,6,10-trien-1-yl hydrogen phosphate (150)

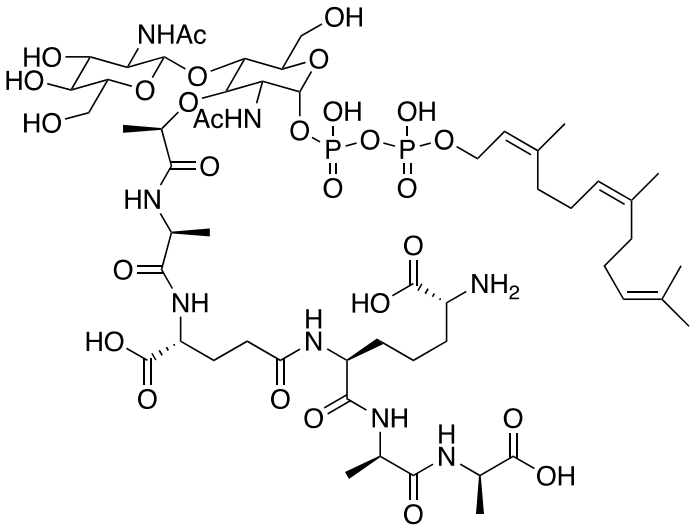


(**150**)

This known compound was prepared following a modified literature protocol.[116] Trichloroacetonitrile (11 μL, 0.40 mmol) was added to a stirred solution of prenol **165** (200 mg, 0.930 mmol) and tetra-*n*-butylammonium dihydrogen phosphate (64 mg, 1.7 mmol) in dichloromethane (20 mL). The reaction mixture was stirred at rt for 10 min before the solvent was removed *in vacuo*. The residue was dissolved in THF (1 mL) and 25% ammonium hydroxide solution in water (0.1 mL) was added. After 30 min, 1:1 toluene:methanol (5 mL) was added, the mixture stirred for 20 min and the resultant precipitate removed by filtration. The solvent was removed *in vacuo* and the residue was washed with petroleum ether (3 × 5 mL), dissolved in methanol (2 mL), and filtered. Excess Dowex 50WX8 ($NH4^+$ form) was added and the mixture stirred for 30 min and then filtered. The solvent was removed *in vacuo* to give lipid phosphate **180** as a white solid (water:isopropanol:ethyl acetate = 1:2:4, R_f = 0.47). This crude product was purified by HPLC: column = GraceVydac Protein and Peptide C_{18} 100 mm

column; flow-rate =10 mL/min, UV =220 nm, method = 100% 50 mM NH_4HCO_3 (aq.) to 100% MeOH over 35 min. Fractions containing products (determine by ESI-MS) were combined and lyophilized to yield *Z, Z*-farnesyl monophosphate **150** (120 mg, 44% yield). IR (neat, cm^{-1}) 3327, 2965, 2928, 2855, 1667, 1442, 1375, 1011, 826; ^1H NMR (500 MHz, CD_3OD) δ 5.44 (t, 1H, *J* = 6.6, 6.6 Hz), 5.16 – 5.20 (m, 2H), 4.50 (t, 2H, *J* = 7.0, 7.0 Hz), 2.15 – 2.00 (m, 8H), 1.92 (s, 3H), 1.76 (s, 3H), 1.70 (s, 6H), 1.63 (s, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 136.6, 132.4, 130.8, 125.7, 125.5, 33.3, 32.9, 27.6, 27.5, 25.9, 23.7, 23.6, 22.8; HRMS (ESI) Calcd for $C_{15}H_{25}O_4P$ $[M-H]^-$ 300.1573, found 300.1574.

6.13.16. C_{15}-*Z, Z*-lipid II- Gram-negative Analogue (40)

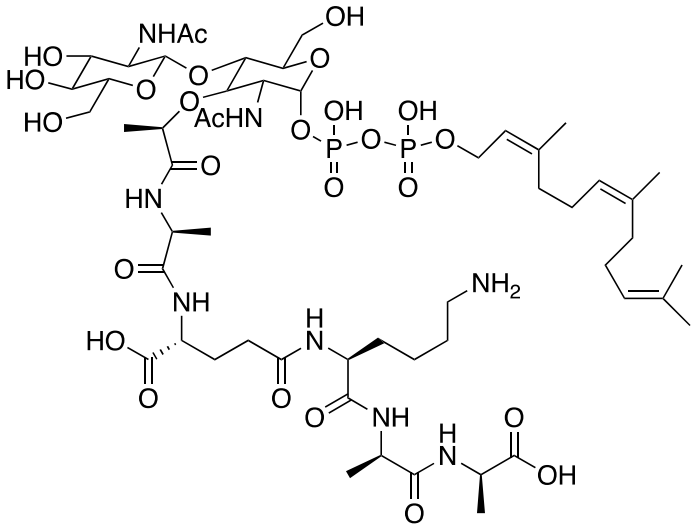


(**40**)

This known compound was prepared following a modified literature protocol.[49, 69] (*Z, Z*)-farnesyl phosphate **150** (30.0 mg, 0.00620 mmol) was dissolved in dry DMF (2ml), which formed a cloudy suspension. To this suspension was added CDI (50.0 mg, 0.300 mmol), and the resulting solution became clear and then was stirred for 2 h. Anhydrous MeOH (8 μL) was added to destroy any excess CDI after 45 min of stirring. Excess

MeOH was removed *in vacuo* and a solution of the disaccharide phosphate **80** (89 mg) in dry DMF (2 mL) was added to this solution. The resulting solution was stirred for 3 days under Argon at room temperature and concentrated *in vacuo*. The crude diphosphate was dissolved in dioxane (1 mL) and 1 M NaOH (1 mL) was added, and the reaction mixture was stirred at 37 °C for 2 h. The reaction mixture was filtered through an aqueous filter paper and the crude was purified by HPLC: column = GraceVydac Protein and Peptide C_{18} 100 mm column; flow-rate = 10 mL/min, UV = 220 nm, method = 100% 50 mM NH_4HCO_3 (aq) to 100% MeOH over 35 min. Fractions containing products (determined by ESI-MS) were combined and lyophilized to yield C_{15}-(*Z, Z)*-farnesyl lipid II-DAP as a fluffy white powder (23 mg, 29% yield). HRMS (ESI) Calcd for $C_{55}H_{91}N_8O_{28}P_2$ $[M-H]^-$ 1373.5569, found 1373.5571.

6.13.17. C_{15}-*Z, Z*-Lipid II- Gram-positive Analogue (275)

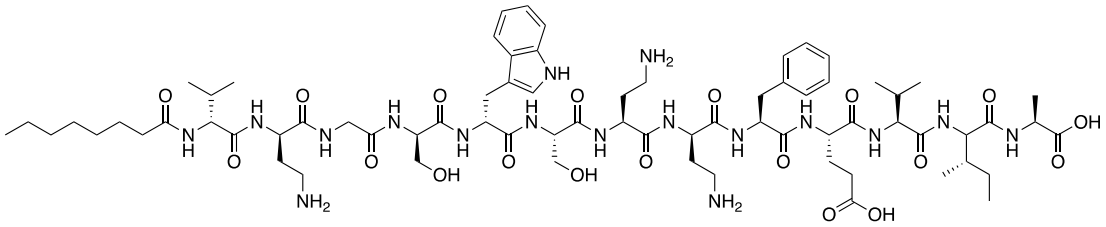


(**275**)

This known compound was prepared following a modified literature protocol.[49, 69] (*Z, Z*)-farnesyl phosphate **150** (30 mg, 0.0062 mmol) was dissolved in dry DMF (2ml), which formed a cloudy suspension. To this suspension was added CDI (50 mg, 0.30

mmol) and the resulting solution became clear, and then was stirred for 2 h. Anhydrous MeOH (8 μL) was added to destroy any excess CDI after 45 min of stirring. Excess MeOH was removed *in vacuo* and a solution of the disaccharide phosphate **82** (85 mg) in dry DMF (2 mL) was added to this solution. The resulting solution was stirred for 3 days under Argon at room temperature and concentrated *in vacuo*. The crude diphosphate was dissolved in dioxane (1 mL) and 1 M NaOH (1 mL) was added, and the reaction mixture was stirred at 37 °C for 2 h. The reaction mixture was filtered through an aqueous filter paper and the crude was purified by HPLC: column = GraceVydac Protein and Peptide C_{18} 100 mm column; flow-rate = 10 mL/min, UV = 220 nm, method = 100% 50 mM NH_4HCO_3 (aq) to 100% MeOH over 35 min. Fractions containing products (determined by ESI-MS) were combined and lyophilized to yield (*Z,Z*)-farnesyl Gram-positive lipid II as a fluffy white powder (21 mg, 23% yield). HRMS (ESI) Calcd for $C_{54}H_{92}N_8O_{26}P_2$ $[M-H]^-$ 1329.5525 found 1329.5447.

6.13.18. Octyl-Tridecaptin A_1 (29)



(**29**)

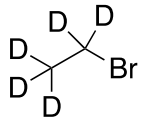
Product eluted at 38.0 min (HPLC method 1) and was isolated as a white powder (20 mg). HRMS (ES) Calcd for $C_{72}H_{114}N_{17}O_{19}$ $[M+H]^+$ 1520.8345 found 1520.8349.

6.14. Experimental Procedures for the Preparation of O-DPC-d_{36} Analogues for NMR Studies with Oct-TriA_1 and Lipid II

6.15. General Procedure for the Formation of Alkyl Bromides

The synthesis of the alkyl bromides was achieved following a modified literature protocol.[130] The alcohol **171** (4.8 g, 0.090 mol) was transferred into a 50 mL round bottom flask and cooled to 0 °C, and then PBr_3 (3.1 mL) was added and the reaction mixture was allowed to warm to room temperature, followed by stirring for 6 h. Ice cold water (10 mL) was added and extracted into diethyl ether. The organic layer was dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude oil was passed through a plug of silica gel and eluted with hexane to give the final product **167** as a colorless oil (10.1 g, 96% yield, 98.8% D).

6.15.1. 1-Bromoethane-1,1,2,2,2-d_5 (167)



(**167**)

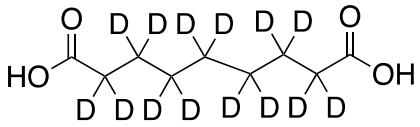
^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.29 (s, 0.025H, residual C$\underline{H}_2$-Br), 1.22 (s, 0.038H, residual C$\underline{H}_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.29 (br s, 2D, C$\underline{D}_2$-Br), 31.23 (br s, 3D, CD_3); HRMS (ESI) $[M]^+$ calcd for 112.9888 found 112.9876. Isotopic distribution (ESI-MS, -ve mode): 69% d_5, 31% d_4.

6.16. General Procedure for the Deuteration of Aliphatic Carboxylic Acid

The preparation of this known deuterated fatty acid was accomplished following a modified literature protocol.[117] A mixture of azelaic acid **172** (12.0 g, 63.0 mmol),

Pt/activated carbon (10% Pt) (0.380 g, 1.90 mmol) and 40% w/w NaOD (12.0 g, 130 mmol) in D_2O (150 mL) was loaded into a stainless-steel Parr pressure reactor and then heated to 220 °C (330 psi), with constant stirring for 72 h. The reactor was cooled to room temperature and the reaction mixture was filtered through a plug of Celite to remove the catalyst and was further washed with H_2O (100 mL). The filtrate was acidified to pH 2 using 2M HCl. The white solid that was formed upon acidification was extracted from the aqueous solution with ethyl acetate (3 × 100 mL), and the combined organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give 11.8 g of a white solid of azelaic acid [D_{14}] (93.2% D). The 93% D compound was treated with fresh reagent and the method was repeated to give the final product **173** as a white solid (10.2 g, 87% based on original amount of protonated azelaic acid used, 98.6% D).

6.16.1. Nonanedioic-d_{14} acid (173)



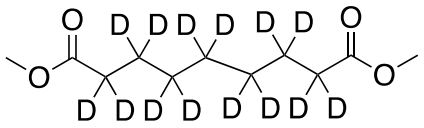
(**173**)

^{1}H NMR ($CDCl_3$, 400 MHz, dichloromethane as internal standard) δ 5.29 (s, 2H, CH_2, internal standard), 2.26 (br s, 0.043H, 2 × C<u>H</u>$_2$-COOH), 1.55 (br s, 0.045H, 2 × residual CH_2), 1.26 (s, 0.15H, 3 × residual CH_2); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 2.27 (s, 4D, 2 × C<u>D</u>$_2$-COOH), 1.56 (s, 4D, 2 × C<u>D</u>$_2$), 1.26 (s, 6D, 3 × CDs_2); HRMS (ES) Calcd for $C_9HD_{14}O_4$ $[M-H]^-$ 201.1855 found 201.1856. Isotopic distribution (ESI-MS, -ve mode): 55.8% d_{14}, 22.4% d_{13}, 19.5% d_{12}, 2.3% d_{11}.

6.17. General Procedure for Esterification of Diacids

The synthesis of this known diester was accomplished following a modified literature protocol.[117] Azelaic acid [D_{14}] **(173)** (10.0 g, 49.0 mmol), anhydrous MeOH (100 mL), and concentrated H_2SO_4 (2 mL) was refluxed under argon for 48 h. The reaction was cooled to room temperature and the solvent was evaporated until a small amount of MeOH remained. This was transferred into a flask containing ice cold water and extracted three times with diethyl ether. The organic layer was washed with 10% $NaHCO_3$, brine, and then dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by column chromatography (SiO_2, 20% EtOAc in hexane), gave the desired product **174** as colorless oil, [D_{14}] dimethyl azelate (10.7 g, 94% yield, 98.6 D).

6.17.1. Dimethyl nonanedioate-d_{14} (174)



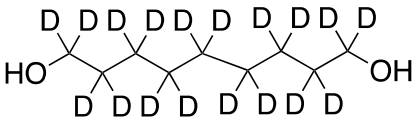
(**174**)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.64 (s, 6.00H, 2 × CH_3), 2.26 (br s, 0.043H, 2 × C$\underline{H}_2$-COOMe), 1.55 (br s, 0.045H, 2 × residual CH_2), 1.26 (s, 0.15H, 3 × residual CH_2). ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 2.27 (s, 4D, 2 × CD_2-COOH), 1.56 (s, 4D, 2 × C$\underline{D}_2$), 1.26(s, 6D, 3 × CD_2); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 173.5 (2 × Carbonyl C), 51.9 (2 × CH_3), 33.9 – 25.7 (all C$\underline{D}_2$); HRMS (ES) Calcd for $C_{11}H_6D_{14}NaO_4$ $[M+Na]^+$ 253.2133 found 253.2135. Isotopic distribution (ESI-MS, +ve mode): 55.7% d_{14}, 22.2% d_{13}, 19.4% d_{12}, 2.7% d_{11}.

6.18. General Procedure for Reduction of Esters

The synthesis of the diester was accomplished following modified literature protocol.[129] Under a stream of Argon, dimethyl azelate [D_{14}] **174** (9.20 g, 40.0 mmol) and $NaBD_4$ (4.90 g, 120 mmol) were dissolved in freshly distilled THF (200 mL). Boron trifluoride diethyl etherate (25 mL, 0.21 mol) was added dropwise and the closed reaction mixture was stirred for 72 h. After completion of the reaction as monitored by TLC (hexane: Ethyl acetate, 1:5) R_f = 0.21, absolute ethanol was added to destroy any excess diborane and the bulk of the solvent was removed *in vacuo*. The mixture was acidified with 2 M HCl, and then water was added to dissolve all salts formed. 1,9-Nonanaediol [D_{14}] was extracted into ethyl acetate (3 × 100 mL). The organic layer was washed with $NaHCO_3$, brine, and then dried over $MgSO_4$, filtered, and the solvent was removed by rotary evaporation to give the product **(175)** as a colorless thick oil which was used in the next step without further purification (6.1 g, 87% yield, 98.5% D).

6.18.1. Nonane-d_{18}-1,9-diol (175)



(**175**)

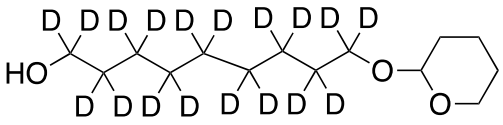
^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.34 (s, 0.025H, 2 × residual C$\underline{H}_2$-OH), 1.74 (br s, 0.044H, 2 × C$\underline{H}_2$-CH_2OH), 1.35 (br s, 0.17H, 5 × residual CH_2); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.33 (s, 4D, 2 × C$\underline{D}_2$-OH), 1.76 (s, 4D, 2 × C$\underline{D}_2$-CD_2OH), 1.36 (s, 10D, 5 ×

CD_2); HRMS (ES) Calcd for $C_9HD_{18}O_2$ $[M-H]^-$ 177.2547 found 177.2548. Isotopic distribution (ESI-MS, -ve mode): 60.3% d_{18}, 21.7% d_{17}, 16.6% d_{16}, 1.5% d_{15}.

6.19. General Procedure for Protection of Alcohols with Dihydropyran

The synthesis of the THP protected alcohols was accomplished following modified literature protocol.[130] To a solution of 1,9-nonanediol-d_{18} **(175)** (5.20 g, 29.2 mmol) in CH_2Cl_2 was added pyridinium *p*-toluenesulfonate (0.33 g, 2.6 mmol) at 0 °C, then dihydropyran (3.4 mL, 37 mmol) was added and the mixture was stirred at rt for 6 h. After completion of the reaction as monitored by TLC (hexane:ethyl acetate, 2:1, R_f = 0.2), the reaction mixture was hydrolyzed by addition of water (10 mL), and the product was extracted with dichloromethane (3 × 50 mL). The organic layers were dried with $MgSO_4$ and filtered, and the solvent was removed *in vacuo* to give the crude compound as a light-yellow oil. The crude oil was purified by column chromatography (SiO_2, hexane:ethyl acetate, 4:1) to give the desired product **168** as a colorless oil (6.5 g, 86% yield, 98.5% D).

6.19.1. 9-((tetrahydro-2*H*-pyran-2-yl)oxy)nonan-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-d_{18}-1-ol (168)

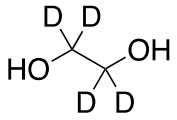


(**168**)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.71 (dd, *J* = 10.2, 2.6 Hz, 1H, O-CH-O), 3.81 (br s, 0.012H, residual CH_2-O-THP), 3.33 (s, 0.011H, residual $C\underline{H}_2$-OH), 1.90 – 1.84 (m, 6H,

3 × CH_2), 1.72 (br s, 0.043H, 2 × residual C<u>H</u>$_2$-CH_2-OH), 1.35 (br s, 0.16H, 5 × residual C<u>H</u>$_2$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.81 (br s, 2D, CD_2-O-THP), 3.34 (br s, 2D, 2 × C<u>D</u>$_2$-OH), 1.76 (s, 4D, 2 × C<u>D</u>$_2$-CD_2O, 1.36 (s, 10D, 5 × C<u>D</u>$_2$); HRMS (ES) Calcd for $C_{14}H_{10}D_{18}O_2$ $[M-H]^-$ 261.3456 found 261.3454. Isotopic distribution (ESI-MS, -ve mode): 60.2% d_{18}, 21.8% d_{17}, 16.5% d_{16}, 1.6% d_{15}.

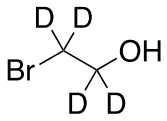
6.19.2. Ethane-d_4-1,2-diol (177)



(**177**)

This known compound was prepared following literature protocol.[132] To a solution of ethylene glycol (**176**) (5.00 g, 80.6 mmol) in D_2O (150 mL) was added 5% Ru/C (20 wt% of starting material). The flask was sealed and stirred at 80 °C for 48 h. After completion of the reaction, the reaction mixture was cooled to room temperature and filtered through Celite, and then the solvent was removed *in vacuo* to give the deuterated diol [D_4] (5.20 g, 98% yield, 99.2% D) as a colorless thick oil. ^{1}H NMR ($CDCl_3$, 400 MHz dichloromethane was used as an internal standard) δ 5.29 (s, 2H, CH_2, internal standard), 3.58 (br s, 0.042H, 2 × C<u>H</u>$_2$-OH), 1.22 (s, 1H, OH); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.60 (br s, 4D, 2 × C<u>D</u>$_2$-OH). HRMS (ESI) $[M]^+$ 66.0748. Isotopic distribution (ESI-MS, -ve mode): 90.3% d_4, 9.7% d_3.

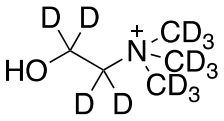
6.19.3. 2-Bromoethan-1,1,2,2-d_4-1-ol (178)



(**178**)

This known compound was synthesized following a modified literature protocol.[167] To a solution of ethylene glycol [D_4] **(177)** (5.00 g, 75.7 mmol) in toluene (30 mL) was added hydrobromic acid (1.20 mL), and the mixture was stirred at reflux for 6 h. Ice cold water (10 mL) was added, and the bulk of the solvent was removed *in vacuo,* and the remaining solution was extracted into diethyl ether. The ether layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation to give the desired product as a colorless oil (8.6 g, 89% yield, 99.1% D), which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.61 (br s, 0.021H, residual C$\underline{H}_2$-OH), 3.30 (br s, 0.022H, residual C$\underline{H}_2$-Br), 1.22 (s, 1H, O$\underline{H}$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.60 (br s, 2D, C$\underline{D}_2$-OH), 3.30 (br s, 2D, CD_2-Br). HRMS (ESI) $[M]^+$ 127.9842. Isotopic distribution (ESI-MS, -ve mode): 90.3% d_4, 9.7% d_3.

6.19.4. 2-Hydroxy-*N*,*N*,*N*-tris(methyl-d_3)ethan-1-aminium-1,1,2,2-d_4 (169)



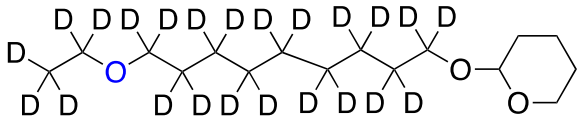
This known compound was synthesized following a modified literature protocol.[131] $N(CD_3)_3$ (5.00 g, 74.1 mmol) was transferred under vacuum to a round bottom flask containing 150 mL of dry acetone, and then cooled to -78 °C. 2-bromoethane-1-ol [D_4] **(178)** (5.00 g, 39.1 mmol) in 50 mL of cold dry acetone was added dropwise. The

reaction mixture was allowed to warm to room temperature and then stirred for 48 h in the dark. The white precipitate formed after this period was filtered and dried under vacuum. The material was dissolved in $CHCl_3/CH_3OH/H_2O$ (65:25:5) and washed through a column of Amberlite IRA-93 in acetate form. The collected fractions were combined, and the solvent was removed by rotary evaporation and dried under vacuum over P_2O_5 to give the final product as a white powder (6.2 g, 90% yield, 98.8% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.48 (s, 0.021H, residual C$\underline{H}_2$-OH), 2.68 (s, 0.21H, residual C$\underline{H}_2$-N), 2.28 (s, 0.10H, residual 3 × C$\underline{H}_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.49 (br s, 2D, C$\underline{D}_2$-OH), 2.66 (br s, 2D, CD_2-N), 2.26 (br s, 9D, 3 × C$\underline{D}_3$); HRMS (ESI) $C_5H_2D_{13}NO$ $[M + H]^+$ Calcd for 118.1914, found 118.1915. Isotopic distribution (ESI-MS, +ve mode): 88.4% d_{13}, 10.2% d_{12}, 1.4% d_{11}.

6.20. General Procedure for Williamson Ether Synthesis

The synthesis of the ether compounds was accomplished following a modified literature protocol.[168] To a solution of compound **168** (4.90 g, 18.7 mmol) in dry THF (50mL) was added NaH (60% in mineral oil) (3.70 g, 33.0 mmol) and 18-crown ether (8.80 g, 33.0 mmol), and then the reaction mixture was stirred for 30 min at 50 °C. After stirring at this temperature for 2 h, the reaction mixture was allowed to cool to room temperature. Compound **167** (2.10 g, 33.0 mol) in THF (20 mL) was added slowly and the mixture was stirred for 12 h at room temperature. After completion of the reaction as monitored by TLC (20% ethyl acetate in hexane, R_f = 0.4), 1 M HCl was added and then the solvent was removed by evaporation. The crude compound was purified by column chromatography (SiO_2, 20% ethyl acetate in hexane) to give the final product **179** as a colorless oil (4.7 g, 85% yield, 98.4% D).

6.20.1. 2-((9-(Ethoxy-d_5)nonyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-d_{18})oxy)tetrahydro-2*H*-pyran (179)



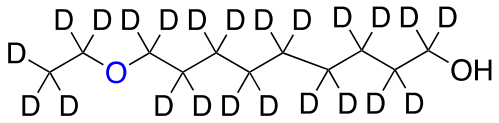
(**179**)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.71 (dd, J = 10.2, 2.6 Hz, 1H, O-C<u>H</u>-O), 3.81 (br s, 0.041H, residual C<u>H</u>$_2$-OTHP), 3.74 – 3.69 (m, 2H, OC<u>H</u>$_2$-CH$_2$), 3.58 (br s, 0.022H, residual C<u>H</u>$_2$-OCH$_2$CH$_2$), 3.40 (br s, 0.021H, residual C<u>H</u>$_2$-OCH$_2$CH$_3$), 1.90 – 1.84 (m, 6H, 3 × CH$_2$), 1.54 (br s, 0.043H, residual CH$_2$), 1.22 (s, 1H, OH), 1.27 (br s, 0.18H, 6 × residual CH$_2$) 0.84 (br s, 0.038H, residual CH$_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.60 (br s, 2D, CD$_2$-O-CD$_2$), 3.57 (br s, 2D, CD$_2$-OTHP), 1.49 (br s, 2D, CD$_2$), 1.24 (m, 12D, 6 × CD$_2$), 0.82 (s, 3D, CD$_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 97.4 (O-<u>C</u>H-O), 70.2 – 65.2 (all <u>C</u>'D$_2$-O), 62.1 (<u>C</u>H$_2$-O-CHO), 32. 4 – 30.4 (all CD$_2$), 30.1 (<u>C</u>H$_2$-CH-O), 25.4 (<u>C</u>H$_2$-CH$_2$-O), 19.0 (<u>C</u>H$_2$-CH$_2$-CH-O), 14.0 (<u>C</u>D$_3$). HRMS (ES) Calcd for $C_{16}H_9D_{23}NaO_3$ $[M+Na]^+$ 318.3687 found 318.3678. Isotopic distribution (ESI-MS, +ve mode): 67.4% d_{23}, 16.5% d_{22}, 12.3% d_{21}, 3.8% d_{20}.

6.21. General Procedure for Deprotection of THP group

The deprotection of the THP protecting group was accomplished following a literature protocol.[130] To a solution of **179** (3.20 g, 10.8 mmol) in MeOH (20 mL) was added pyridinium *p*-toluenesulfonate (280 mg, 1.10 mmol) and the reaction mixture was stirred at room temperature for 1 h. The solvent was removed *in vacuo* and the crude was purified by column chromatography (SiO_2, 5:1 hexane:ethyl acetate) to give the product **180** as a light-yellow oil (2.0 g, 91% yield, 98.3% D).

6.21.1. 9-(Ethoxy-d_5)nonan-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-d_{18}-1-ol (180)



(**180**)

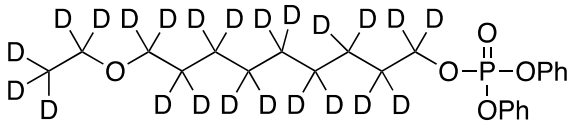
^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.37 (s, 0.075H, 3 × residual CH_2-O), 1.74 (br s, 0.08H, 2 × $C\underline{H}_2$-CH_2O), 1.35 (br s, 0.16H, 5 × residual $C\underline{H}_2$), 1.20 (br s, 0.04H, residual $C\underline{H}_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.38 (s, 6D, 3 × $C\underline{D}_2$-O), 1.75 (s, 4D, 2 × $C\underline{D}_2$-CD_2O), 1.37 (s, 10D, 5 × $C\underline{D}_2$), 1.21 (br s, 3D, $C\underline{D}_3$); HRMS (ES) Calcd for $C_{11}HD_{23}NaO_2$ $[M+Na]^+$ 234.3112 found 234.3098. Isotopic distribution (ESI-MS, +ve mode): 67.6% d_{23}, 16.2% $d_{22,}$ 12.6% $d_{21,}$ 3.5% $d_{20.}$

6.22. General Procedure for Formation of Lipid Dibenzyl Phosphate

The synthesis of the lipid dibenzyl phosphate compounds was achieved following a modified literature protocol.[131] The alcohol **180** (3.00 g, 14.2 mol) was dissolved in dry pyridine (50 mL) and diphenyl phosphoryl chloride (3.6 mL, 17 mol) was added dropwise. The reaction mixture was stirred at 30 °C for 24 h, then a further 1 mL of diphenyl phosphoryl chloride was added and stirring continued for another 12 h. Water (10 mL) was added to destroy excess diphenyl phosphoryl chloride and the bulk of the pyridine was removed *in vacuo*, and then the oily residue was dissolved in diethyl ether. The organic phase was washed with 2 M H_2SO_4, water, and then dried over $MgSO_4$, filtrered, and the solvent was removed *in vacuo* to give the crude product as a thick light-yellow oil. The crude compound was purified by column chromatography (SiO_2,

$CHCl_3$:MeOH, 9:1) to give the final product **(181)** as a light-yellow thick oil (4.8 g, 79% yield, 98.3% D).

6.22.1. 9-(Ethoxy-d_5)nonyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-d_{18} diphenyl phosphate (181)



(**181**)

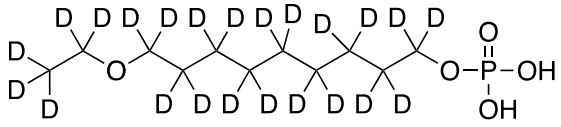
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38 – 7.02 (m, 10H, Ar-H), 4.15 (br s, 0.02H, residual CH_2-OP), 3.37 (s, 0.04H, 2 × residual CH_2-O), 1.74 (br s, 0.08H, 2 × CH_2-CH_2O), 1.35 (br s, 0.17H, 5 × residual CH_2), 1.20 (br s, 0.04H, residual CH_3); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 4.14 (s, 2D, CD_2-OP), 3.38 (s, 4D, 2 × CD_2-O), 1.75 (s, 4D, 2 × CD_2-CD_2O), 1.37 (s, 10D, 5 × CD_2), 1.21 (br s, 3D, CD_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 151.2 (2 × ArC-OP), 129.9 (4 × *m*-ArC), 123.7 (2 × *p*-ArC), 120.7 (4 × *o*-ArC), 68.7 (2 × CD_2-O), 61.4 (CD_2-OP), 32. 1 – 22.4 (all CD_2), 14.2 (CD_3); ^{31}P NMR ($CDCl_3$, 162 MHz) δ -2.39 (s); HRMS (ES) Calcd for $C_{23}H_{11}D_{23}O_5P$ $[M+H]^+$ 444.3582 found 444.3576. Isotopic distribution (ESI-MS, +ve mode): 67.6% d_{23}, 16.1% d_{22}, 12.5% d_{21}, 3.7% d_{20}.

6.23. General Procedure for Deprotection of Benzyl Group

These new compounds were synthesized following a modified literature protocol.[131] To a solution of the diphenyl phosphoric acid **181** (1.80 g, 4.10 mmol) in dry dioxane (50 mL) was added 0.1 g of 10% Pt/activated carbon, and the reaction mixture was stirred under an H_2 atmosphere for 48 h at room temperature. After completion of the reaction by TLC (hexane:ethyl acetate, 3:1), the reaction mixture was filtered through

Celite to remove the catalyst, washed with warm $CHCl_3$ and MeOH, and then the solvent was removed by rotary evaporation. Purification by column chromatography (SiO_2, 10% EtOAc in hexane) gave the desired product **182** as colorless oil, (1.02 g, 89% yield, 98.1% D).

6.23.1. 9-(Ethoxy-d_5)nonyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-d_{18} dihydrogen phosphate (182)



(**182**)

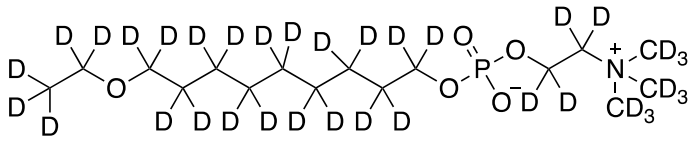
^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.12 (br s, 0.02H, residual C$\underline{H}_2$-OP), 3.36 (s, 0.04H, 2 × residual CH_2-O), 1.75 (br s, 0.08H, 2 × C$\underline{H}_2$-CH_2O), 1.33 (br s, 0.21H, 5 × residual C$\underline{H}_2$), 1.21 (br s, 0.04H, residual CH_3); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 4.16 (s, 2D, C$\underline{D}_2$-OP), 3.38 (s, 4D, 2 × C$\underline{D}_2$-O), 1.77 (s, 4D, 2 × C$\underline{D}_2$-CD_2O), 1.35 (s, 10D, 5 × C$\underline{D}_2$), 1.22 (br s, 3D, C$\underline{D}_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 68.9 (2 × CD_2-O), 61.7 (CD_2-OP), 33. 2 – 22.3 (all CD_2), 14.1 ($\underline{C}D_3$); ^{31}P NMR ($CDCl_3$, 162 MHz) δ -0.38 (s); HRMS (ESI) $C_{11}HD_{23}O_5P$ $[M - H]^-$ Calcd for 290.2882, found 290.2884. Isotopic distribution (ESI-MS, +ve mode): 67.5% d_{23}, 16.3% $d_{22,}$ 12.2% $d_{21,}$ 3.9% $d_{20.}$

6.24. General Procedure for Coupling of Choline to Lipid Phosphate

These new compounds were prepared following a modified literature protocol.[131] Compound **182** (0.20 g, 6.8 mol) and choline **(169)** (0.20 g, 1.7 mol) were mixed and dried overnight *in vacuo* over P_2O_5. Pyridine (10 mL) was added, followed by

triisopropylbenzenesulfonyl chloride (0.62 g, 0.0021 mol). The reaction mixture was stirred at 70 °C for 2 h and then at room temperature for 6 h. After this time, the mixture was cooled in an ice bath and the unreacted choline was filtered off. Water (1 mL) was added to destroy excess triisopropylbenzenesulfonyl chloride and the solvent was removed by rotary evaporation. The crude solid was extracted with $CHCl_3$, and the solvent was removed *in vacuo*. The solid material was purified by column chromatography (SiO_2, $CHCl_3$:CH_3OH:H_2O 65:25:5) to give the product as a white solid (0.36 g, 61% yield, 97.9% D).

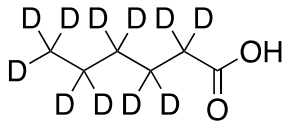
6.24.1. 9-(Ethoxy-d5)nonyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-d18 (2-(tris(methyl-d3)ammonio)ethyl-1,1,2,2-d4) phosphate (166)



(**166**)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.12 (br s, 0.04H, 2 × residual C$\underline{H}_2$-OP), 3.36 (s, 0.04H, 2 × residual C$\underline{H}_2$-O), 2.77 (br s, 0.04H, residual C$\underline{H}_2$-N), 2.29 (br s, 0.09H, 3 × residual C$\underline{H}_3$), 1.75 (br s, 0.08H, 2 × C$\underline{H}_2$-CH_2O), 1.34 (br s, 0.21H, 5 × residual C$\underline{H}_2$), 1.23 (br s, 0.04H, residual C$\underline{H}_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 4.14 (s, 4D, 2 × C$\underline{D}_2$-OP), 3.37 (s, 4D, 2 × C$\underline{D}_2$-O), 2.77 (s, 3D, C$\underline{D}_2$-N), 2.29 (s, 9D, 3 × C$\underline{D}_3$), 1.76 (s, 4D, 2 × C$\underline{D}_2$-CD_2O), 1.33 (s, 10D, 5 × C$\underline{D}_2$), 1.23 (br s, 3D, C$\underline{D}_3$); ^{31}P NMR ($CDCl_3$, 162 MHz) δ 0.48 (s); HRMS (ESI) $C_{16}HD_{36}NO_5P$ $[M - H]^-$ Calcd for 388.4593, found 388.4595. Isotopic distribution (ESI-MS, +ve mode): 62.2% d_{36}, 16.5% d_{35}, 8.4% d_{34}, 5.2% d_{33}, 4.7% d_{32}, 3.4% d_{31}.

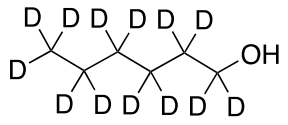
6.24.2. Hexanoic-d11 acid (185)



(**185**)

This known compound was prepared using the general procedure for the deuteration of of fatty acid starting from hexanoic acid **(184)** to give the final product as a colorless oil (11.4 g, 90% yield based on original amount of protonated nonanoic acid used, 98.5% D). ^{1}H NMR ($CDCl_3$, 400 MHz, dichloromethane as internal standard) δ 5.29 (s, 2H, CH_2, internal standard), 2.33 (br s, 0.02H, C$\underline{H}_2$-COOH), 1.54 (br s, 0.03H, residual C$\underline{H}_2$), 1.23 (br s, 0.072H, residual 2× C$\underline{H}_2$), 0.82 (br s, 0.07H, residual C$\underline{H}_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 2.26 (s, 2D, C$\underline{D}_2$-COOH), 1.58 (s, 2D, C$\underline{D}_2$), 1.25 (s, 4D, 2 × C$\underline{D}_2$), 0.84 (s, 3D, C$\underline{D}_3$); HRMS (ESI) $C_6H_2D_{11}O_2$ $[M + H]^+$ Calcd for 128.1526, found 128.1528. Isotopic distribution (ESI-MS, +ve mode): 68.8% d_{11}, 31.2% d_{10}.

6.24.3. Hexan-d13-1-ol (186)

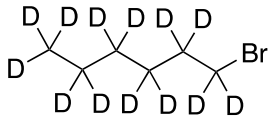


(**186**)

This known compound was prepared using the general procdure for the reduction of esters using NaBD4 and boron trifluoride diethyl etherate starting from **185** to give the final product as a colorless oil (8.3 g, 92% yield, 98.4% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.56 (br s, 0.01H, C$\underline{H}_2$-OH), 1.57 (br s, 0.02H, residual C$\underline{H}_2$), 1.22 (s, 1H, O$\underline{H}$), 1.27 (br s, 0.10H, 3 × C$\underline{H}_2$), 0.84 (br s, 0.07H, residual C$\underline{H}_3$). ^{2}H NMR ($CHCl_3$, 400

MHz,) δ 3.60 (br s, 2D, C<u>D</u>$_2$-OH), 1.56 (br s, 2D, C<u>D</u>$_2$), 1.24 (m, 6D, 3× C<u>D</u>$_2$), 0.85 (s, 3D, C<u>D</u>$_3$); HRMS (ESI) Calcd for 115.1862, found 115.1864. Isotopic distribution (ESI-MS, -ve mode): 67.5% d_{13}, 28.4% d_{12}, 4.1% d_{11}.

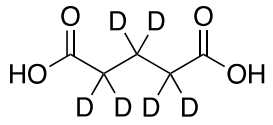
6.24.4. 1-Bromohexane-1,1,2,2,3,3,4,4,5,5,6,6,6-d_{13} (187)



(**187**)

This new compound was prepared following the general procedure for the preparation of alkyl bromides starting from **186** to give the final product as a colorless oil (10.9 g, 96% yield, 98.4% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.34 (br s, 0.01H, residual C<u>H</u>$_2$-Br), 1.54 (br s, 0.02H, residual C<u>H</u>$_2$), 1.22 (s, 1H, O<u>H</u>), 1.27 (br s, 0.10H, 3 × C<u>H</u>$_2$) 0.86 (br s, 0.07H, residual C<u>H</u>$_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.35 (br s, 2D, C<u>D</u>$_2$-Br), 1.59 (br s, 2D, C<u>D</u>$_2$), 1.25 (s, 6D, 3 × C<u>D</u>$_2$), 0.85 (s, 3D, C<u>D</u>$_3$); HRMS (ESI) Calcd for 177.1018, found 177.1020. Isotopic distribution (ESI-MS, -ve mode): 67.3% d_{13}, 28.3% d_{12}, 4.4% d_{11}.

6.24.5. Pentanedioic-d_6 acid (190)

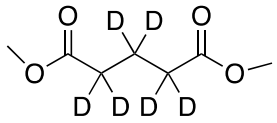


(**190**)

This known compound was prepared using the general procedure for deuteration of fatty acid starting from glutaric acid **(189)** to give the final product as a white solid (10.2 g, 96% yield, 98.8% D). ^{1}H NMR ($CDCl_3$, 400 MHz, dichloromethane as internal

standard) δ 5.29 (s, 2H, CH_2, internal standard), 2.36 (br s, 0.04H, 2 × C<u>H</u>$_2$-COOH, 99.2% D), 1.84 (br s, 0.03H); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 2.34 (s, 4D, 2 × C<u>D</u>$_2$-COOH), 1.82 (s, 2D, C<u>D</u>$_2$); HRMS (ESI) $C_5HD_6O_4$ $[M - H]^-$ Calcd for 137.0726, found 137.0726. Isotopic distribution (ESI-MS, -ve mode): 89.4% d_6, 10.6% d_5.

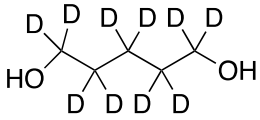
6.24.6. Dimethyl pentanedioate-d_6 (191)



(**191**)

This compound was synthesized using the general procedure for esterification of acids starting from **190** to give the final product as a light-yellow oil (10.9 g, 94% yield, 99.1% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.63 (s, 6H, 2 × C<u>H</u>$_3$), 2.31 (br s, 0.06H, 2 × C<u>H</u>$_2$-$COOCH_3$), 1.82 (br s, 0.04H, residual C<u>H</u>$_2$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 2.32 (s, 4D, 2 × C<u>D</u>$_2$-$COOCH_3$), 1.81 (s, 2D, C<u>D</u>$_2$). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 173.5 (2 × Carbonyl C), 51.7 (2 × CH_3), 32.9 – 20.7 (3 × CD_2); HRMS (ESI) $C_7H_7D_6NaO_4$ $[M + Na]^+$ Calcd for 189.1004, found 189.1002. . Isotopic distribution (ESI-MS, +ve mode): 89.2% d_6, 10.8% d_5.

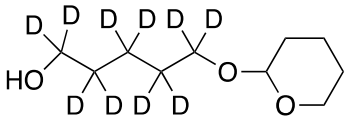
6.24.7. Pentane-d_{10}-1,5-diol (192)



(**192**)

This compound was prepared following the general procedure for reduction of esters starting from **191** to give the final product as a light yellow thick oil (5.1 g, 86% yield, 98.6% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.33 (s, 0.06H, 2 × residual C$\underline{H}_2$-OH), 1.72 (br s, 0.06H, 2 × residual C$\underline{H}_2$-CH_2-OH), 1.32 (br s, 0.04H, residual CH_2); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.34 (s, 4.00D, 2 × C$\underline{D}_2$-OH, 98.5% D), 2.31 (s, 4D, 2 × C$\underline{D}_2$-CD_2-OH), 1.31 (s, 2D, C$\underline{D}_2$); HRMS (ESI) Calcd for 114.1465, found 114.1467. Isotopic distribution (ESI-MS, -ve mode): 78.8% d_{10}, 14.3% d_9, 6.9% d_8.

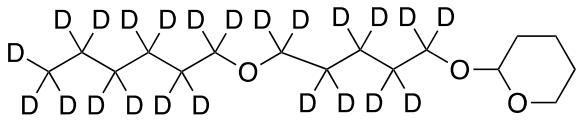
6.24.8. 5-((Tetrahydro-2*H*-pyran-2-yl)oxy)pentan-1,1,2,2,3,3,4,4,5,5-d_{10}-1-ol (188)



(**188**)

This new compound was synthesized following the general procedure for the protection of alcohol using DHP starting from **192** to give the final product as a colorless oil (6.2 g, 86% yield, 98.4% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.71 (dd, J = 10.2, 2.6 Hz, 1H, O-CH-O), 3.81 (br s, 0.04H, residual CH_2-O-THP), 3.33 (s, 0.06H, residual C$\underline{H}_2$-OH), 1.90 – 1.84 (m, 6H, 3 × CH_2), 1.72 (br s, 0.06H, 2 × residual C$\underline{H}_2$-CH_2-OH), 1.32 (br s, 0.04H, residual CH_2); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.81 (br s, 2D, CD_2-O-THP), 3.34 (br s, 2D, 2 × C$\underline{D}_2$-OH), 2.31 (s, 4D, 2 × C$\underline{D}_2$-CD_2-OH), 1.31 (s, 2D, C$\underline{D}_2$); HRMS (ESI) Calcd for 196.2344, found 196.2345. Isotopic distribution (ESI-MS, -ve mode): 78.7% d_{10}, 14.2% d_9, 7.1% d_8.

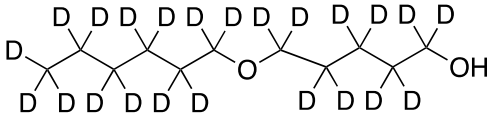
6.24.9. 2-((5-((Hexyl-d_{13})oxy)pentyl-1,1,2,2,3,3,4,4,5,5-d_{10})oxy)tetrahydro-2*H*-pyran (193)



(193)

This new compound was prepared using the general procedure for the Williamson ether synthesis from alkyl bromide **187** and alcohol **188** to give the final product as a colorless oil (7.1 g, 77% yield, 98.2% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.71 (dd, *J* = 10.2, 2.6 Hz, 1H, O-CH-O), 3.81 (br s, 0.04H, residual CH_2-OTHP), 3.74 – 3.69 (m, 2H, OCH_2-CH_2), 3.58 (br s, 0.01H, residual C$\underline{H}_2$-OCH_2), 3.40 (br s, 0.06H, residual C$\underline{H}_2$-OCH_2), 1.90 – 1.84 (m, 6H, 2 × CH_2), 1.54 (br s, 0.029H, residual CH_2), 1.22 (s, 1H, OH), 1.27 (br s, 0.25H, 6 × CH_2) 0.84 (br s, 0.07H, residual CH_3); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.60 (br s, 2D, CD_2-O-CD2), 3.57 (br s, 2D, CD_2-OTHP), 1.49 (br s, 2D, CD_2), 1.24 (m, 12D, 6 × CD_2), 0.82 (s, 3D, CD_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 97.4 (O-$\underline{C}$H-O), 70.2 – 65.2 (all CD_2-O), 62.1 ($\underline{C}H_2$-O-CHO), 32. 4 – 30.4 (all CD_2), 30.1 ($\underline{C}H_2$-CH-O), 25.4 ($\underline{C}H_2$-CH_2-O), 19.0 ($\underline{C}H_2$-CH_2-CH-O), 14.0 ($\underline{C}D_3$); HRMS (ES) Cald for $C_{16}H_9D_{23}NaO_3$ $[M+Na]^+$ 318.3687 found 318.3678. Isotopic distribution (ESI-MS, +ve mode): 65.2% d_{23}, 15.7% d_{22}, 10.5% d_{21}, 5.3% d_{20}, 3.3% d_{19}.

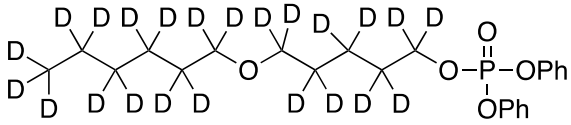
6.24.10. 5-((Hexyl-d_{13})oxy)pentan-1,1,2,2,3,3,4,4,5,5-d_{10}-1-ol (195)



(195)

This new compound was synthesized using the general procedure for THP deprotection using **193** as the starting material to give the final product as a light yellow oil (3.8 g, 97% yield, 98.2% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.36 (br s, 0.09H, 3 × residual C$\underline{H}_2$-O), 1.72 (br s, 0.08H, 3 × residual C$\underline{H}_2$), 1.35 (br s, 0.07H, 2 × residual C$\underline{H}_2$), 1.27 (br s, 0.08H, 2 × residual C$\underline{H}_2$), 0.87 (br s, 0.07H, residual C$\underline{H}_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.37 (br s, 6D, 3 C$\underline{D}_2$-O), 1.74 (br s, 6D, 3 × C$\underline{D}_2$), 1.37 (br s, 4D, 2 × C$\underline{D}_2$), 1.28 (br s, 4D, 2 × C$\underline{D}_2$), 0.85 (br s, 3D, C$\underline{D}_3$); HRMS (ES) Calcd for $C_{11}HD_{23}NaO_2$ $[M+Na]^+$ 234.3112 found 234.3098. . Isotopic distribution (ESI-MS, +ve mode): 65.3% d_{23}, 15.6% $d_{22,}$ 10.2% $d_{21,}$ 5.5% d_{20}, 3.4% d_{19}.

6.24.11. 5-((Hexyl-d_{13})oxy)pentyl-1,1,2,2,3,3,4,4,5,5-d_{10} diphenyl phosphate (196)

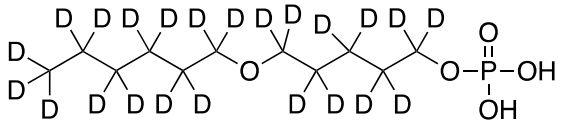


(196)

This new compound was prepared using the general procedure for the preparation of of lipid dibenzyl phosphate using **195** as the starting material to give yield the final product as a light yellow thick oil (4.5 g, 73% yield, 98.1% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.48 – 7.34 (m, 10 H, Ar-H), 4.15 (br s, 0.03H, residual CH_2-OP), 3.37 (br s, 0.06H, 2 × residual C$\underline{H}_2$-O), 1.84 (br s, 0.08H, 3 × residual C$\underline{H}_2$), 1.33 (br s, 0.15H, 4

× residual C<u>H</u>$_2$), 0.85 (br s, 0.07H, residual C<u>H</u>$_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 4.16 (br s, 2D, C<u>D</u>$_2$-OP), 3.36 (br s, 4D, 2 × C<u>D</u>$_2$-O), 1.82 (br s, 6D, 3 × C<u>D</u>$_2$), 1.34 (br s, 8D, 4 × C<u>D</u>$_2$), 0.86 (s, 3D, C<u>D</u>$_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 150.6 (2 × ArC-OP), 129.7 (4 × *m*-ArC), 123.8 (2 × *p*-ArC), 120.3 (4 × *o*-ArC), 68.8 (2 × CD_2-O), 61.6 (CD_2-OP), 32.1 – 22.4 (all CD_2), 14.0 (<u>C</u>D_3); ^{31}P NMR ($CDCl_3$, 162 MHz) δ -2.37 (s); HRMS (ESI) $C_{23}H_{11}D_{23}O_5P$ $[M + H]^+$ Calcd for 444.3507, found 444.3508. Isotopic distribution (ESI-MS, +ve mode): 65.3% d_{23}, 15.6% d_{22}, 10.2% d_{21}, 5.5% d_{20}, 3.4% d_{19}.

6.24.12. 5-((Hexyl-d_{13})oxy)pentyl-1,1,2,2,3,3,4,4,5,5-d_{10} dihydrogen phosphate (197)

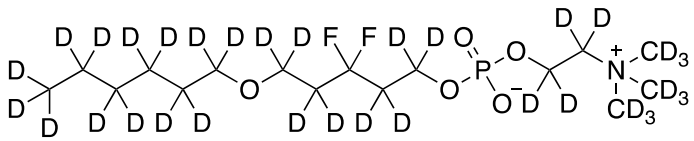


(197)

The general procedure for deprotection of benzyl group was employed to prepare this new compound starting with **196** to give the final product as a colorless oil, (1.3 g, 95% yield, 98.1% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.15 (br s, 0.03H, residual C<u>H</u>$_2$-OP), 3.36 (br s, 0.06H, 2 × residual C<u>H</u>$_2$-O), 1.86 (br s, 0.08H, 3 × residual C<u>H</u>$_2$), 1.35 (br s, 0.15H, × residual C<u>H</u>$_2$), 0.86 (br s, 0.07H, residual C<u>H</u>$_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 4.16 (br s, 2D, C<u>D</u>$_2$-OP), 3.35 (br s, 3.98D, 2 × C<u>D</u>$_2$-O), 1.84 (br s, 5.99D, 3 × C<u>D</u>$_2$), 1.35 (br s, 8.00D, 4 × C<u>D</u>$_2$), 0.86 (s, 3.00D, C<u>D</u>$_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 68.8 (2 × CD_2-O), 61.6 (CD_2-OP), 33. 4 – 22.6 (all CD_2), 14.2 (<u>C</u>D_3); ^{31}P NMR ($CDCl_3$, 162 MHz) δ -0.38 (s); HRMS (ESI) $C_{11}HD_{23}O_5P$ $[M - H]^-$ Calcd for 290.2883, found

290.2886. Isotopic distribution (ESI-MS, +ve mode): 65.8% d_{23}, 14.9% d_{22}, 10.7% d_{21}, 4.8% d_{20}, 3.8% d_{19}.

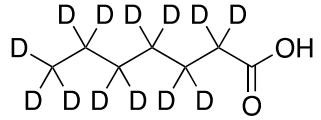
6.24.13. Synthesis of 6-O-DPC-d$_{36}$ (183)



(183)

This new compound was prepared following the general procedure for coupling of choline with lipid phosphate using choline **(169)** and lipid phosphate **197** to give the final product as a white solid (0.52 g, 65% yield, 97.8% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.17 (br s, 0.06H, 2 × residual C$\underline{H}_2$-OP), 3.36 (br s, 0.06H, residual C$\underline{H}_2$-O-C$\underline{H}_2$), 2.87 (s, 0,04H, residual C$\underline{H}_2$-N), 2.3 (s, 0.14H, 3 × residual C$\underline{H}_3$), 1.78 (br s, 0.13H, 3 × residual CH_2), 1.27 (br s, 0.13H, 4 × residual C$\underline{H}_2$), 0.86 (br s, 0.07H, residual C$\underline{H}_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 4.18 (br s, 4D, 2 × C$\underline{D}_2$-OP), 3.33 (br s, 3.98D, C$\underline{D}_2$-O-C$\underline{D}_2$), 2.74 (s, 2.99D, C$\underline{D}_2$-N), 2.26 (s, 8.99D, 3 × C$\underline{D}_3$), 1.86 (br s, 5.99D, 3 × C$\underline{D}_2$), 1.27 (br s, 8.00D, 4 × C$\underline{D}_2$), 0.86 (br s, D, C$\underline{D}_3$); ^{31}P NMR ($CDCl_3$, 162 MHz) δ 0.46 (s); HRMS (ESI) $C_{16}D_{36}O_5P$ $[M - H]^-$ Calcd for 388.4592, found 388.4594. Isotopic distribution (ESI-MS, +ve mode): 62.1% d_{36}, 16.6% d_{35}, 8.2% d_{34}, 5.4% d_{33}, 5.1% d_{32}, 2.6% d_{31}.

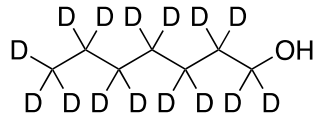
6.24.14. Heptanoic-d_{13} acid (201)



(**201**)

This known compound was prepared using the general method for deuteration of fatty acid starting with heptanoic acid **(200)** to give the final product as a colorless oil (12.1 g, 90% yield based on original amount of protonated nonanoic acid used, 98.6% D). ^{1}H NMR ($CDCl_3$, 400 MHz, dichloromethane as internal standard) δ 5.29 (s, 2H, C$\underline{H}_2$, internal standard), 2.30 (br s, 0.025H, residual C$\underline{H}_2$-COOH), 1.58 (br s, 0.034H, residual C$\underline{H}_2$), 1.23 (br m, 0.21H, residual 3× C$\underline{H}_2$), 0.82 (br s, 0.08H, residual C$\underline{H}_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 2.28 (s, 2D, C$\underline{D}_2$-COOH), 1.56 (s, 2D, C$\underline{D}_2$), 1.23 (m, 6D, 3× C$\underline{D}_2$), 0.82 (s, 3D, C$\underline{D}_3$); HRMS (ESI) $C_7D_{13}O_2$ $[M - H]^-$ Calcd for 142.1811, found 143.1813. Isotopic distribution (ESI-MS, -ve mode): 72.5% d_{13}, 23.3% d_{12}, 4.2% d_{11}.

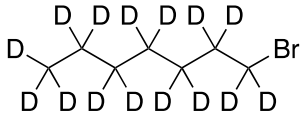
6.24.15. Heptan-d_{15}-1-ol (202)



(202)

The general procedure for the reduction of esters was employed for the preparation of this known compound starting with **201** to yield the final product as a colorless oil (9.4 g, 92% yield, 98.5% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.58 (br s, 0.01 H, C$\underline{H}_2$-OH), 1.54 (br s, 0.025H, residual C$\underline{H}_2$-CH_2OH), 1.22 (s, 1H, O$\underline{H}$), 1.27 (br s, 0.25H, 4 × C$\underline{H}_2$) 0.84 (br s, 0.08 H, residual C$\underline{H}_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.60 (br s, 2D, C$\underline{D}_2$-OH), 1.49 (br s, 2D, C$\underline{D}_2$-CD_2OH), 1.24 (br s, 8D, 4× C$\underline{D}_2$), 0.82 (s, 3D, C$\underline{D}_3$); HRMS (ESI) Calcd for 131.2144, found 131.2146. Isotopic distribution (ESI-MS, -ve mode): 68.5% d_{15}, 21.4% d_{14}, 7.5% d_{13}, 2.6% d_{12}.

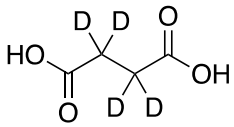
6.24.16. 1-Bromoheptane-1,1,2,2,3,3,4,4,5,5,6,6,7,7,7-d_{15} (199)



(199)

This new compound was prepared using the general procedure for the preparation of alkyl bromide starting with **202** to yield the final product as a colorless oil (8.6 g, 95% yield, 98.4% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.32 (br s, 0.01H, C$\underline{H}_2$-Br), 1.54 (br s, 0.025H, residual C$\underline{H}_2$-CH_2OH), 1.22 (s, 1H, O$\underline{H}$), 1.27 (br s, 0.24H, 4 × C$\underline{H}_2$) 0.84 (br s, 0.08H, residual C$\underline{H}_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.34 (br s, 2D, C$\underline{D}_2$-Br), 1.49 (br s, 2D, C$\underline{D}_2$-CD_2OH), 1.24 (br s, 8D, 4× C$\underline{D}_2$), 0.82 (s, 3D, C$\underline{D}_3$); HRMS (ESI) Calcd for 193.1298, found 193.1299. Isotopic distribution (ESI-MS, -ve mode): 68.4% d_{15}, 21.3% d_{14}, 7.4% d_{13}, 2.9% d_{12}.

6.24.17. Succinic-2,2,3,3-d_4 acid (205)

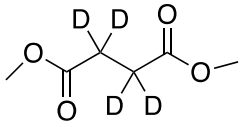


(205)

This known compound was synthesized following the general procedure for deuteration of fatty acids starting with succinic acid (**204**) to give the final product as a white solid (8.9 g, 96% yield, 99.2% D). ^{1}H NMR ($CDCl_3$, 400 MHz, dichloromethane as internal standard) δ 5.29 (s, 2H, CH_2, internal standard), 2.87(br s, 0.10 H, 2 × residual C$\underline{H}_2$-COOH); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 2.88 (br s, 4D, 2 × C$\underline{D}_2$-COOH);

HRMS (ESI) $C_4HD_40_4$ $[M - H]^-$ Calcd for 121.0513, found 121.0516. Isotopic distribution (ESI-MS, -ve mode): 97.6% d_3, 2.4% d_2.

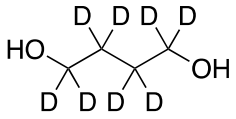
6.24.18. Dimethyl succinate-d_4 (206)



(**206**)

This known compound was prepared using the general procedure for esterification of acids starting with **20)** to yield the final product as a light-yellow oil (8.5 g, 94% yield, 99.1% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.62 (s, 6H, 2 × C<u>H</u>$_3$), 2.87(br s, 0.10H, 2 × residual CH_2-$COOCH_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 2.73 (br s, 4D, 2 × CD_2-$COOCH_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 173.1 (2 × Carbonyl C), 51.8 (2 × CH_3), 29.1 (2 × CD_2); HRMS (ESI) $C_6H_7D_4O_4$ $[M + H]^+$ Calcd for 151.0830, found 150.0832. Isotopic distribution (ESI-MS, +ve mode): 97.4% d_3, 2.6% d_2.

6.24.19. Butane-d_8-1,4-diol (207)

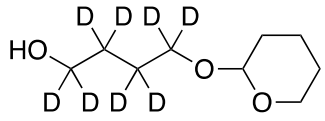


(**207**)

This known compound was prepared following the general procedure for reduction of esters starting with **206** to give the final product as a light-yellow thick oil (6.6 g, 85% yield, 98.8% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.32 (br s, 0.08H, 2 × residual C<u>H</u>$_2$-OH), 1.75 (br s, 0.10H, 2 × residual C<u>H</u>$_2$-CH_2-OH); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.34 (br s, 4D, 2 × C<u>D</u>$_2$-OH), 1.73 (br s, 4D, 2 × C<u>D</u>$_2$-CD_2-OH); HRMS (ESI) Calcd

for 98.1184, found 98.1185. Isotopic distribution (ESI-MS, -ve mode): 95.3% d_8, 3.3% d_7, 1.4% d_6.

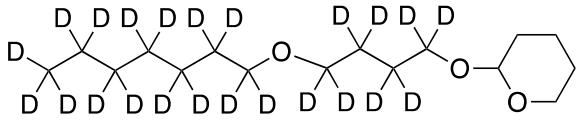
6.24.20. 4-((Tetrahydro-2*H*-pyran-2-yl)oxy)butan-1,1,2,2,3,3,4,4-d_8-1-ol (203)



(**203**)

This new compound was prepared following the general for the protection of alcohol using DHP starting with **207** to give the final product as a colorless oil (6.2 g, 85% yield, 98.8% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.71 (dd, J = 10.2, 2.6 Hz, 1H, -OC<u>H</u>-O), 3.81 (br s, 0.04H, residual C<u>H</u>$_2$-O-THP), 3.74 – 3.69 (m, 2H, OC<u>H</u>$_2$-CH$_2$), 3.32 (br s, 0.08H, residual C<u>H</u>$_2$-OH), 1.90 – 1.84 (m, 6H, 3 × C<u>H</u>$_2$), 1.75 (br s, 0.10H, 2 × residual C<u>H</u>$_2$-CH$_2$-OH); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.81 (br s, 2D, C<u>D</u>$_2$-O-THP), 3.34 (br s, 2D, 2 × C<u>D</u>$_2$-OH), 1.73 (br s, 4D, 2 × C<u>D</u>$_2$-CD$_2$-O); HRMS (ESI) Calcd for 180.1762, found 180.1763. Isotopic distribution (ESI-MS, -ve mode): 95.1% d_8, 3.2% d_7, 1.7% d_6.

6.24.21. 2-(4-((Heptyl-d_{15})oxy)butoxy-1,1,2,2,3,3,4,4-d_8)tetrahydro-2*H*-pyran (208)

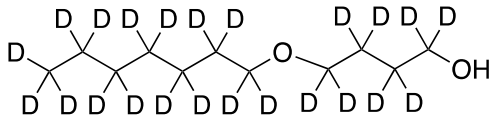


(208)

This new compound was prepared using the general procedure for williamson ether synthesis starting with alkyl bromide **199** and alcohol **203** to give the final product as

a colorless oil (7.2 g, 87% yield, 98.5% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.71 (dd, *J* = 10.2, 2.6 Hz, 1H, O-C<u>H</u>-O), 3.81 (br s, 0.04H, residual C<u>H</u>$_2$-OTHP), 3.74 – 3.69 (m, 2H, OC<u>H</u>$_2$-CH$_2$), 3.58 (br s, 0.01H, residual C<u>H</u>$_2$-OCH$_2$), 3.40 (br s, 0.06H, residual C<u>H</u>$_2$-OCH$_2$), 1.90 – 1.84 (m, 6H, 3 × C<u>H</u>$_2$), 1.54 (br s, 0.029H, residual C<u>H</u>$_2$), 1.22 (s, 1H, O<u>H</u>), 1.27 (br s, 0.25H, 6 × residual C<u>H</u>$_2$), 0.84 (br s, 0.07H, residual C<u>H</u>$_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.60 (br s, 2D, C<u>D</u>$_2$-O-CD2), 3.57 (br s, 2D, C<u>D</u>$_2$-OTHP), 1.49 (br s, 2D, C<u>D</u>$_2$), 1.24 (m, 12D, 6 × C<u>D</u>$_2$), 0.82 (s, 3D, C<u>D</u>$_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 97.4 (O-<u>C</u>H-O), 70.2 – 65.2 (all CD$_2$-O), 62.1 (<u>C</u>H$_2$-O-CHO), 32.4 – 30.4 (all CD$_2$), 30.1 (<u>C</u>H$_2$-CH-O), 25.4 (<u>C</u>H$_2$-CH$_2$-O), 19.0 (<u>C</u>H$_2$-CH$_2$-CH-O), 14.0 (<u>C</u>D$_3$); HRMS (ES) Calcd for $C_{16}H_9D_{23}NaO_3$ $[M+Na]^+$ 318.3687 found 318.3678. Isotopic distribution (ESI-MS, +ve mode): 67.2% d_{23}, 16.6% d_{22}, 12.3% d_{21}, 3.8% d_{20}.

6.24.22. 4-((Heptyl-d_{15})oxy)butan-1,1,2,2,3,3,4,4-d_8-1-ol (209)

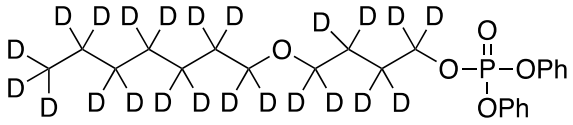


(209)

This new compound was prepared following the general procedure for deprotection of THP protecting group starting with **208** to give the final product as a light yellow oil (6.5 g, 97% yield, 98.5% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.39 (br s, 0.09H, 3 × residual C<u>H</u>$_2$-O), 1.82 (br s, 0.025H, residual C<u>H</u>$_2$), 1.73 (br s, 0.08H, 2 × residual C<u>H</u>$_2$), 1.34 (br s, 0.04H, residual C<u>H</u>$_2$), 1.27 (br s, 0.20H, 3 × residual C<u>H</u>$_2$), 0.87 (br s, 0.08H, residual C<u>H</u>$_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.36 (br s, 6D, OC<u>D</u>$_2$-O), 1.84 (br s, 2D, C<u>D</u>$_2$), 1.73 (br s, 4D, 2 × C<u>D</u>$_2$), 1.39 (br s, 2D, C<u>D</u>$_2$), 1.26 (br s, 6D, 3 × C<u>D</u>$_2$), 0.85 (s,

3D, C<u>D</u>$_3$); HRMS (ES) Calcd for $C_{11}HD_{23}NaO_2$ $[M+Na]^+$ 234.3112 found 234.3098. Isotopic distribution (ESI-MS, +ve mode): 67.4% d_{23}, 16.3% $d_{22,}$ 12.7% $d_{21,}$ 3.6% d_{20}.

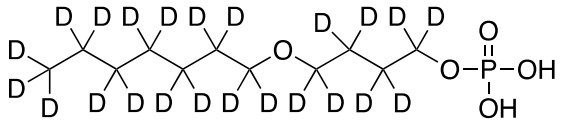
6.24.23. 4-((Heptyl-d_{15})oxy)butyl-1,1,2,2,3,3,4,4-d_8 diphenyl phosphate (210)



(210)

This new compound was prepared using the general procedure for the preparation of lipid dibenzyl phosphate starting with **209** to give the final product as a light yellow thick oil (4.5 g, 77% yield, 98.4% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.49 – 7.36 (m, 10H, Ar-H), 3.58 (br s, 0.04H, residual C<u>H</u>$_2$-OP), 3.36 (br s, 0.05H, 2 × residual C<u>H</u>$_2$-O), 1.82 (br s, 0.12H, 3 × residual C<u>H</u>$_2$), 1.23 (br s, 0.24H, 4 × residual C<u>H</u>$_2$), 0.87 (br s, 0.08H, residual C<u>H</u>$_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.56 (br s, 2D, C<u>D</u>$_2$-OP), 3.37 (br s, 4D, 2 × C<u>D</u>$_2$-O), 1.73 (br s, 6D, 3 × C<u>D</u>$_2$), 1.29 (br s, 8D, 4 × C<u>D</u>$_2$), 0.85 (s, 3D, C<u>D</u>$_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 150.8 (2 × ArC-OP), 129.9 (4 × *m*-ArC), 123.6 (2 × *p*-ArC), 120.1 (4 × *o*-ArC), 68.6 (2 × CD_2-O), 61.8 (CD_2-OP), 32. 4 – 22.6 (all CD_2), 14.0 (<u>C</u>D$_3$); ^{31}P NMR ($CDCl_3$, 162 MHz) δ -2.37 (s); HRMS (ESI) $C_{23}H_{11}D_{23}O_5P$ $[M + H]^+$ Calcd for 444.3522, found 443.3525. Isotopic distribution (ESI-MS, +ve mode): 67.6% d_{23}, 16.5% $d_{22,}$ 12.4% $d_{21,}$ 3.5% d_{20}.

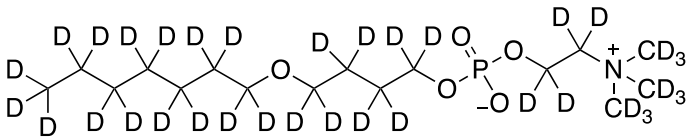
6.24.24. 4-((Heptyl-d_{15})oxy)butyl-1,1,2,2,3,3,4,4-d_8 dihydrogen phosphate (211)



(211)

This new compound was prepared using the general procedure for deprotection of benzyl group starting with **210** to give the final product as a light-yellow oil (3.8 g, 94% yield, 98.3% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.18 (br s, 0.04H, residual C$\underline{H}_2$-OP), 3.39 (br s, 0.08H, 2 × residual C$\underline{H}_2$-O), 1.84 (br s, 0.05H, 2 × residual C$\underline{H}_2$), 1.72 (br s, 0.02H, residual C$\underline{H}_2$), 1.35 (br s, 0.02H, residual C$\underline{H}_2$), 1.27 (br s, 0.11H, 3 × residual C$\underline{H}_2$), 0.85 (br s, 0.08H, residual C$\underline{H}_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 4.18 (br s, 2D, C$\underline{D}_2$-OP), 3.39 (br s, 4D, 2 × C$\underline{D}_2$-O), 1.84 (br s, 4D, 2 × C$\underline{D}_2$), 1.74 (br s, 2D, C$\underline{D}_2$), 1.36 (br s, 2D, C$\underline{D}_2$), 1.29 (br s, 6D, 3 × C$\underline{D}_2$), 0.85 (br s, 3D, C$\underline{D}_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 68.8 (2 × CD_2-O), 61.6 (CD_2-OP), 33. 4 – 22.6 (all CD_2), 14.2 ($\underline{C}D_3$); ^{31}P NMR ($CDCl_3$, 162 MHz) δ -0.38 (s); HRMS (ESI) $C_{11}H_1D_{23}O_5P$ [M - H]$^-$ Calcd for 290.2872, found 290.2875. Isotopic distribution (ESI-MS, -ve mode): 67.8% *d*$_{23}$, 16.1% *d*$_{22}$, 12.4% *d*$_{21}$, 3.7% *d*$_{20}$.

6.24.25. Synthesis of 5-O-DPC-d$_{36}$ (198)

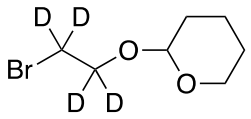


(198)

This new compound was prepared using the general procedure for coupling choline with lipid phosphates starting with choline **(169)** and lipid phosphate **211** to give the final product as a white solid (2.3 g, 61% yield, 98.1% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.17 (br s, 0.04H, 2 × residual C$\underline{H}_2$-OP), 3.32 (br s, 0.05H, residual C$\underline{H}_2$-O-C$\underline{H}_2$), 2.72 (s, 0.04H, residual C$\underline{H}_2$-N), 2.28 (s, 0.14H, 3 × residual C$\underline{H}_3$), 1.89 (br s, 0.19H, 3 × residual C$\underline{H}_2$), 1.25 (br s, 0.24H, 4 × residual C$\underline{H}_2$), 0.82 (br s, 0.08H, residual C$\underline{H}_3$);

^{2}H NMR ($CHCl_3$, 400 MHz,) δ 4.18 (br s, 4, 2 × C<u>D</u>$_2$-OP), 3.33 (br s, 4D, C<u>D</u>$_2$-O-C<u>D</u>$_2$), 2.74 (s, 2D, C<u>D</u>$_2$-N), 2.26 (s, 9D, 3 × C<u>D</u>$_3$), 1.86 (br s, 6D, 3 × C<u>D</u>$_2$), 1.27 (br s, 8D, 4 × C<u>D</u>$_2$), 0.86 (br s, 3D, C<u>D</u>$_3$); ^{31}P NMR ($CDCl_3$, 162 MHz) δ 0.46 (s); HRMS (ESI) $C_{16}D_{36}0_5P$ $[M - H]^-$ Calcd for 388.4592, found 388.4594. Isotopic distribution (ESI-MS, +ve mode): 62.1% *d*$_{36}$, 16.1% *d*$_{35}$, 8.6% *d*$_{34}$, 5.1% *d*$_{33}$, 4.8% *d*$_{32}$, 3.3% *d*$_{31}$.

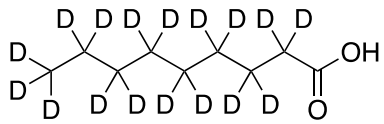
6.24.26. 2-(2-Bromoethoxy-1,1,2,2-*d*$_4$)tetrahydro-2*H*-pyran (213)



(**213**)

This new compound was prepared following using the general procedure for the protection of alcohol using DHP starting with **178** to give the final product as a a colorless oil (8.5 g, 89% yield, 98.3% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.71 (dd, *J* = 10.2, 2.6 Hz, 1H, -OC<u>H</u>-O), 3.81 (br s, 0.04H, residual C<u>H</u>$_2$-O), 3.74 – 3.69 (m, 2H, OC<u>H</u>$_2$-CH$_2$-), 3.40 (br s, 0.06H, residual C<u>H</u>$_2$-Br), 1.90 – 1.84 (m, 6H, 3 × C<u>H</u>$_2$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.60 (br s, 2D, 2 × CD_2-OH), 3.30 (br s, 2D, CD_2-Br); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 97.3 (O-<u>C</u>H-O), 68.0 (<u>C</u>D$_2$-OTHP), 62.0 (<u>C</u>H$_2$-O-CHO), 30.2 (<u>C</u>H$_2$-CHO), 29.4 (<u>C</u>D$_2$-Br), 25.4 (<u>C</u>H$_2$-CH$_2$-O), 19.0 (<u>C</u>H$_2$-CH$_2$-CH-O); HRMS (ESI) Calcd for 212.0352, found 212.0354. Isotopic distribution (ESI-MS, -ve mode): 89.8% *d*$_4$, 10.2% *d*$_3$.

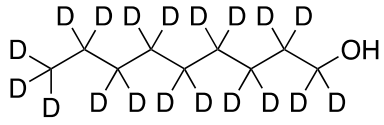
6.24.27. Nonanoic-*d*$_{17}$ acid (216)



(**216**)

This known compound was prepared using the general procedure for deuteration of fatty acids starting with nonanoic acid **(215)** to give the final product as a colorless oil (9.4 g, 92% yield based on original amount of protonated nonanoic acid used, 98.6% D). ^{1}H NMR ($CDCl_3$, 400 MHz, dichloromethane as internal standard) δ 5.29 (s, 2.00H, C$\underline{H}_2$, internal standard), 2.30 (br s, 0.029H, C$\underline{H}_2$-COOH), 1.58 (br s, 0.036H, residual C$\underline{H}_2$), 1.23 (br m, 0.21H, residual 5 × C$\underline{H}_2$), 0.82 (br s, 0.07H, residual C$\underline{H}_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 2.28 (s, 2D, C$\underline{D}_2$-COOH), 1.56 (s, 2D, C$\underline{D}_2$), 1.23 (s, 10D, 5× C$\underline{D}_2$), 0.82 (s, 3D, C$\underline{D}_3$); HRMS (ESI) Calcd for $C_9D_{17}O_2$ $[M-H]^-$ 174.2373, found 174.2375. Isotopic distribution (ESI-MS, -ve mode): 63.3% d_{17}, 21.3% d_{16}, 12.5% d_{15}, 2.9% d_{14}.

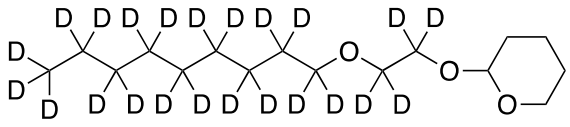
6.24.28. Nonan-d_{19}-1-ol (214)



(**214**)

This new compound was prepared using the general procedure for reduction of esters starting with **216** to give the final protect as a colorless oil (8.8 g, 95% yield, 98.3% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.58 (br s, 0.011H, CH_2-OH), 1.54 (br s, 0.029H, residual CH_2), 1.22 (s, 1H, OH), 1.27 (br s, 0.25H, 6 × CH_2), 0.84 (br s, 0.07H, residual CH_3); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.60 (br s, 2D, C$\underline{D}_2$-OH), 1.49 (br s, 2D, C$\underline{D}_2$-CD_2-OH), 1.24 (m, 12D, 6× C$\underline{D}_2$), 0.82 (s, 3D, C$\underline{D}_3$); HRMS (ESI) Calcd for 163.2707, found 163.2709. 65.2% d_{19}, 21.2% d_{18}, 12.2% d_{17}, 1.4% d_{16}.

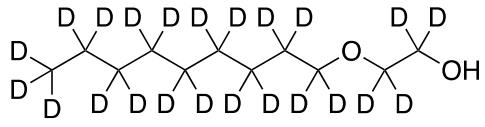
6.24.29. 2-(2-((Nonyl-d_{19})oxy)ethoxy-1,1,2,2-d_4)tetrahydro-2*H*-pyran (217)



(**217**)

This new compound was prepared using the general procedure for williamson ether synthesis starting with alkyl bromide **213** and alcohol **214** to give the final product as a colorless oil (8.2 g, 86% yield, 98.1% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.71 (dd, *J* = 10.2, 2.6 Hz, 1H, O-C<u>H</u>-O), 3.81 (br s, 0.04H, residual C<u>H</u>$_2$-OTHP), 3.74 – 3.69 (m, 2H, OC<u>H</u>$_2$-CH$_2$), 3.58 (br s, 0.01H, residual C<u>H</u>$_2$-OCH$_2$), 3.40 (br s, 0.06H, residual C<u>H</u>$_2$-OCH$_2$), 1.90 – 1.84 (m, 6H, 3 × C<u>H</u>$_2$), 1.54 (br s, 0.029H, residual C<u>H</u>$_2$), 1.22 (s, 1H, O<u>H</u>), 1.27 (br s, 0.25H, 6 × C<u>H</u>$_2$) 0.84 (br s, 0.07H, residual C<u>H</u>$_3$). ^{2}H NMR ($CHCl_3$, 400 MHz,) δ_D 3.60 (br s, 2D, -C<u>D</u>$_2$-O-CD$_2$-), 3.57 (br s, 2D, C<u>D</u>$_2$-OTHP), 1.49 (br s, 2D, C<u>D</u>$_2$), 1.24 (m, 12D, 6× C<u>D</u>$_2$), 0.82 (s, 3D, C<u>D</u>$_3$). ^{13}C [H] NMR (100.6 MHz, $CDCl_3$): δ_C 97.4 (O-<u>C</u>H-O), 70.2 – 65.2 (all CD_2-O), 62.1 (<u>C</u>H$_2$-O-CHO), 32. 4 – 30.4 (all CD_2), 30.1 (<u>C</u>H$_2$-CH-O), 25.4 (<u>C</u>H$_2$-CH$_2$-O), 19.0 (<u>C</u>H$_2$-CH$_2$-CH-O), 14.0 (<u>C</u>D$_3$); HRMS (ES) Cald for $C_{16}H_9D_{23}NaO_3$ $[M+Na]^+$ 318.3687 found 318.3678. Isotopic distribution (ESI-MS, +ve mode): 67.7% d_{23}, 16.2% $d_{22,}$ 12.8% $d_{21,}$ 3.3% $d_{20.}$

6.24.30. 2-((Nonyl-d_{19})oxy)ethan-1,1,2,2-d_4-1-ol (218)

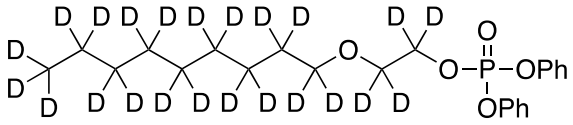


(**218**)

This new compound was prepared following the general procedure for THP deprotection starting with **217** to give the final product as a light yellow oil (7.7 g, 97%

yield, 98.1% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.62 (br s, 0.06H, residual OC$\underline{H}_2$-CH_2OH), 3.58 (br s, 0.04H, residual C$\underline{H}_2$-OH), 3.40 (br s, 0.01H, residual C$\underline{H}_2$-O-CH_2), 1.54 (br s, 0.03H, residual C$\underline{H}_2$), 1.22 (s, 1H, O$\underline{H}$), 1.27 (br s, 0.25H, 6 × residual C$\underline{H}_2$) 0.84 (br s, 0.07H, residual C$\underline{H}_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.60 (br s, 2D, OC$\underline{D}_2$-CD_2), 3.57 (br s, 2D, C$\underline{D}_2$-OH), 3.40 (br s, 2D, C$\underline{D}_2$-O-CD_2), 1.49 (br s, 2D, C$\underline{D}_2$), 1.24 (m, 12D, 6× C$\underline{D}_2$), 0.82 (s, 3D, C$\underline{D}_3$); HRMS (ES) Cald for $C_{11}HD_{23}NaO_2$ $[M+Na]^+$ 234.3112 found 234.3098. Isotopic distribution (ESI-MS, +ve mode): 67.6% d_{23}, 16.9% d_{22}, 12.4% d_{21}, 3.3.1% d_{20}.

6.24.31. 2-((Nonyl-d_{19})oxy)ethyl-1,1,2,2-d_4 diphenyl phosphate (219)

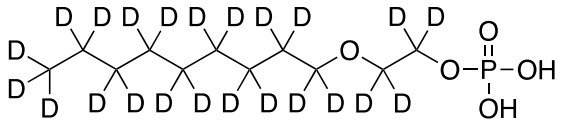


(**219**)

This new compound was prepared using the general procedure for preparation of lipid dibenzyl phosphate starting with **218** to give the final product as a light yellow thick oil (5.4 g, 79% yield, 98.1% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74 – 7.43 (m, 10H, Ar-H), 3.59 (br s, 0.03H, residual OC$\underline{H}_2$-CH_2OH), 3.56 (br s, 0.04H, residual C$\underline{H}_2$-O-P), 3.38 (br s, 0.04H, residual C$\underline{H}_2$-O-CH_2), 1.49 (br s, 0.03H, residual CH_2), 1.20 (s, 1H, OH), 1.25 (br s, 0.25H, 6 × residual C$\underline{H}_2$), 1.20 (s, 1H, O$\underline{H}$), 0.82 (br s, 0.07H, residual C$\underline{H}_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.64 (br s, 2D, OC$\underline{D}_2$-CD_2), 3.59 (br s, 2D, C$\underline{D}_2$-O-P), 3.42 (br s, 2D, C$\underline{D}_2$-O-CD_2), 1.51 (br s, 2D, C$\underline{D}_2$), 1.27 (m, 12D, 6× C$\underline{D}_2$), 0.84 (s, 3D, C$\underline{D}_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 150.8 (2 × ArC-OP), 129.9 (4 × *m*-ArC), 123.6 (2 × *p*-ArC), 120.1 (4 × *o*-ArC), 68.6 (2 × CD_2-O), 61.8 (CD_2-OP), 32.4 – 22.6 (all CD_2), 14.0 ($\underline{C}D_3$); ^{31}P NMR ($CDCl_3$, 176 MHz) δ -2.35 (s); HRMS

(ESI) $C_{23}H_{10}D_{23}O_5P$ [M + H]$^+$ Calcd for 443.3510, found 443.3512. Isotopic distribution (ESI-MS, +ve mode): 67.2% d_{23}, 16.3% d_{22}, 12.6% d_{21}, 3.9% d_{20}.

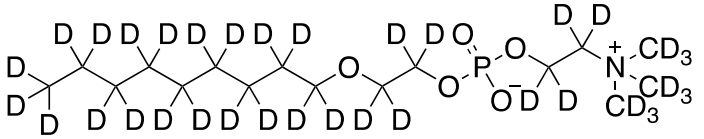
6.24.32. 2-((Nonyl-d_{19})oxy)ethyl-1,1,2,2-d_4 dihydrogen phosphate (220)



(220)

This new compound was prepared using the general procedure for deprotection of benzyl groups starting with **219** to give the final product as a light yellow thick oil (3.1 g, 92% yield, 97.9% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.59 (br s, 0.03H, residual O$C\underline{H}_2$-CH_2OH), 3.56 (br s, 0.04H, residual $C\underline{H}_2$-O-P), 3.38 (br s, 0.04H, residual $C\underline{H}_2$-O-CH_2), 1.49 (br s, 0.03H, residual CH_2), 1.20 (s, 1H, OH), 1.25 (br s, 0.25H, 6 × CH_2), 1.20 (s, 1H, OH), 0.82 (br s, 0.07H, residual CH_3); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.64 (br s, 2D, O$C\underline{D}_2$-CD_2), 3.59 (br s, 2D, CD_2-O-P), 3.42 (br s, 2D, $C\underline{D}_2$-O-CD_2), 1.51 (br s, 2D, $C\underline{D}_2$), 1.27 (m, 12D, 6× $C\underline{D}_2$), 0.84 (s, 3D, $C\underline{D}_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 68.7 (2 × $C\underline{D}_2$-O), 61.6 (CD_2-OP), 32.4 – 22.6 (all $\underline{C}D_2$), 14.1 ($\underline{C}D_3$); ^{31}P NMR ($CDCl_3$, 162 MHz) δ -0.42 (s); HRMS (ESI) $C_{11}H_1D_{23}O_5P$ [M - H]$^-$ Calcd for 291.2881, found 291.2884. Isotopic distribution (ESI-MS, -ve mode): 67.3% d_{23}, 16.2% d_{22}, 12.8% d_{21}, 3.7% d_{20}.

6.24.33. Synthesis of 3-O-DPC-d$_{36}$ (212)



(251)

This new comppund was prepared following the general procedure for coupling of choline to lipid phosphate starting with choline **(169)** and lipid phosphate **220** to give the final product as a white solid (1.9 g, 65% yield, 97.7% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.59 (br s, 0.03H, residual OC$\underline{H}_2$-CH_2OH), 3.56 (br s, 0.04H, residual C$\underline{H}_2$-O-P), 3.48 (s, 0,04H, residual C$\underline{H}_2$-OP), 3.38 (br s, 0.04H, residual C$\underline{H}_2$-O-CH_2), 2.68 (s, 0.04H, residual C$\underline{H}_2$-N), 2.28 (s, 0.10H, residual 3 × C$\underline{H}_3$), 1.49 (br s, 0.03H, residual C$\underline{H}_2$), 1.20 (s, 1H, O$\underline{H}$), 1.25 (br s, 0.25H, 6 × C$\underline{H}_2$), 1.20 (s, 1H, OH), 0.82 (br s, 0.07H, residual C$\underline{H}_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ 3.64 (br s, 2D, OC$\underline{D}_2$-CD_2), 3.59 (br s, 2D, C$\underline{D}_2$-O-P), 3.42 (br s, 2D, C$\underline{D}_2$-O-CD_2), 2.66 (br s, 2D, C$\underline{D}_2$-N), 2.26 (br s, 9D, 3 × C$\underline{D}_3$), 1.51 (br s, 2D, C$\underline{D}_2$), 1.27 (m, 12D, 6 × C$\underline{D}_2$), 0.84 (s, 3D, C$\underline{D}_3$); ^{31}P NMR ($CDCl_3$, 176 MHz) δ 0.49 (s); HRMS (ESI) $C_{16}D_{36}NO_5P$ $[M - H]^-$ Calcd for 291.2881, found 291.2884. Isotopic distribution (ESI-MS, +ve mode): 62.3% d_{36}, 16.2% d_{35}, 8.6% d_{34}, 5.3% d_{33}, 4.9% d_{32}, 2.7% d_{31}.

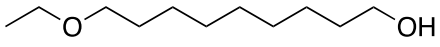
6.25. Synthesis of Undeuterated O-DPC Analogues for NMR Studies with Leucocin A

6.26. General Procedure for Williamson Ether Synthesis

These compounds were prepared following a modified literature protocol.[169] To a solution of 1,9-nonanediol **(222)** (10.0 g, 60.0 mmol) in N,N-dimethylformamide:tetrahydrofuran (1:1, 100 mL) was added sodium hydride (60% mineral oil suspension, 4.90 g, 100 mmol) at 0 °C. The mixture was stirred for 20 minutes at 0 °C, and then 1-bromoethane **(223)** (4.10 mL, 62.0 mmol) in THF (20 mL) was added dropwise to the reaction mixture and stirring continued at room temperature

for 18 hr. After completion of reaction monitored by TLC (hexane:ethyl acetate, 4:1), R_f = 0.34, water was added, and the mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layer was washed with water, brine, and then dried over anhydrous sodium sulfate, filtered, and the solvent was removed *in vacuo*. The crude product was purified by column chromatography (SiO_2, 7:3 hexane:ethyl acetate) to give the product **224** as a colorless oil (10.8 g, 93% yield). Using the same procedure, these known compounds **229**, **234**, and **238** were synthesized using the appropriate diol and alkyl bromide.

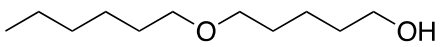
6.26.1. 9-Ethoxynonan-1-ol (224)



(**224**)

IR ($CHCl_3$ cast film, cm^{-1}) 3365, 2933, 2853, 2795, 1462, 1375, 1306, 1119, 1055, 727; ^{1}H NMR ($CDCl_3$, 700 MHz) δ 3.48 (q, *J* = 7.0 Hz, 2H, CH_3-C<u>H</u>$_2$-O), 3.37 (t, *J* = 7.2 Hz, 2H, CH_2-O), 3.33 (t, *J* = 7.2 Hz, 2 H, C<u>H</u>$_2$-OH), 1.83 (m, 4H, 2 × C<u>H</u>$_2$-CH_2-O), 1.77 – 1.32 (m, 10H, 5 × CH_2), 0.86 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 72.5, 70.8, 62.9, 32.7, 29.7, 29.3, 29.3, 26.1, 25.6, 22.8, 10.5; HRMS (ESI) calcd for 189.1845, found 189.1847.

6.26.2. 5-(Hexyloxy)pentan-1-ol (229)

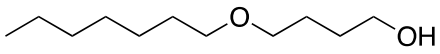


(229)

Starting from 1,5-pentanediol **(227)** and 1-bromohexane **(228)** gave **229** as a colorless oil (10.2 g, 94% yield). IR ($CHCl_3$ cast film, cm^{-1}) 3362, 2929, 2857, 2796, 1466, 1376,

1115, 1058, 954, 724; 1H NMR ($CDCl_3$, 500 MHz) δ 3.45 (q, J = 7.0 Hz, 2H, CH_3-$C\underline{H}_2$-O), 3.38 (t, J = 7.2 Hz, 2H, CH_2-O), 3.33 (t, J = 7.2 Hz, 2 H, $C\underline{H}_2$-OH),1.87 (m, 6H, 3 × $C\underline{H}_2$-CH_2-O), 1.77 – 1.32 (m, 8H, 5 × CH_2), 0.89 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 71.1, 62.5, 32.4, 31.5, 29.7, 29.2, 25.7, 23.2, 22.6, 14.0; HRMS (ESI) m/z Calcd for 189.1773, found 189.1776.

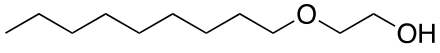
6.26.3. 4-(Heptyloxy)butan-1-ol (234)



(234)

Starting from 1,4-butanediol **(232)** and 1-bromoheptane **(233)** gave **234** as a colorless oil (9.7 g, 95% yield). IR ($CHCl_3$ cast film, cm^{-1}) 3300, 3027, 2930, 1452, 1376, 1120, 1089, 1036, 732, 693 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.46 (q, J = 7.0 Hz, 2H, CH_3-$C\underline{H}_2$-O), 3.37 (t, J = 7.2 Hz, 2H, CH_2-O), 3.33 (t, J = 7.2 Hz, 2 H, $C\underline{H}_2$-OH), 1.82 – 1.73 (m, 6H, 3 × $C\underline{H}_2$-CH_2-O), 1.35 – 1.27 (m, 8H, 5 × CH_2), 0.87 (t, J = 7.0 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 71.2, 71.1, 62.0, 32.4, 29.7, 29.1, 29.0, 27.5, 26.2, 22.6, 14.0; HRMS (ESI) m/z Calcd for 189.1782, found 189.1785.

6.26.4. 2-(Nonyloxy)ethan-1-ol (238)



(238)

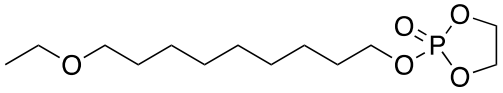
Starting from ethylene glycol **(176)** and 1-bromononane **(237)** gave **238** Colorless oil (9.5 g, 91% yield). IR ($CHCl_3$ cast film, cm^{-1}) 3306, 3029, 2933, 1452, 1376, 1120, 1087, 1038, 732, 693; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.61 (t, 2H, J = 4.24 Hz, CH_2-O), 3.56 (t, 2H, J = 4.24 Hz, CH_2-OH), 3.36 (t, 2H, J = 7.24 Hz, CH_2-$C\underline{H}_2$-O) (m, 6H, 3 ×

C$\underline{H}_2$), 1.83 (tt, J = 7.5, 7.2 Hz, 2H, C$\underline{H}_2$-CH$_2$), 1.73 (tt, 2H, J = 7.21, 5.63 Hz, C$\underline{H}_2$-CH$_2$-O), 1.36 (quint, J = 7.0 Hz, 2H, CH$_2$), 1.20 – 1.32 (m, 1H, 2 × CH$_2$), 0.86 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 68.1, 65.5, 61.7, 32.4, 29.7, 29.4, 29.4, 29.0, 26.2, 22.6, 14.0; HRMS (ESI) m/z Calcd for 189.1772, found 189.1775.

6.27. General Procedure for Preparation of Phosphorane Compounds

These new compounds were prepared following a modified literature protocol.[148] To a solution of the alcohol **224** (10.0 g, 52.2 mmol) in dry THF (60 mL) was added triethylamine (9.50 mL, 68.1 mmol), and then the solution was cooled to -30 °C. 2-chloro-1,2,3-dioxaphospholane-2-oxide (4.75 mL, 52.2 mmol) was added under vigorous stirring via a glass syringe over a period of 45 min. After completion of addition a white precipitate formed and stirring was continued at -30 °C for 3 h. The temperature was then increased to 5 °C stepwise over 2 h. The white precipitate was filtered, and the solvent was removed *in vacuo*. To remove further residues of trimethyl hydrochloride, 20 mL of diethyl ether was added, and the precipitate was filtered off and the solvent was removed *in vacuo*. The product was dried under vacuum for 45 min to give **225** (13.1 g) as a light-yellow viscous oil which was used immediately in the next step. Using the same procedure, these new compounds compounds, **230**, **235**, and **239** were synthesized using the respective starting material.

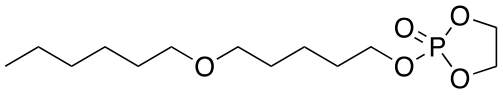
6.27.1. 2-((9-Ethoxynonyl)oxy)-1,3,2-dioxaphospholane 2-oxide (225)



(**225**)

IR ($CHCl_3$ cast film, cm^{-1}) 2927, 2852, 2797, 1464, 1377, 1302, 1118, 1057, 723; ^1H NMR (CDCl3, 400 MHz) δ 4.58 (ddd, *J* = 15.9, 9.7, 4.7 Hz, 2H, cyclic CH_2-OP), 4.43 (ddd, *J* = 15.9, 4.8, 1.5 Hz, 2H, cyclic CH_2-OP), 4.17 (t, *J* = 7.2 Hz, 2H, CH_2-OP), 3.37 (t, *J* = 7.0 Hz, 2H, CH_3-C<u>H</u>$_2$-O), 3.36 (t, *J* = 7.2 Hz, 2H, CH_2-C<u>H</u>$_2$-O), 1.77 – 1.68 (m, 4H, 2 × OCH_2-CH_2), 1.37 – 1.36 (m, 4H, 2 × CH_2), 1.28 – 1.24 (m, 6H, 3 × CH_2), 0.86 (t, *J* = 7.0 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 71.2, 67.5, 66.1, 66.0, 66.0, 30.2, 29.7, 29.4, 29.0, 26.2, 25.6, 15.1; ^{31}P NMR ($CDCl_3$, 162 MHz) δ -3.52 (s); HRMS (ESI) Calcd for $C_{13}H_{28}O_5P$ $[M+H]^+$ 295.1643, found 295.1645.

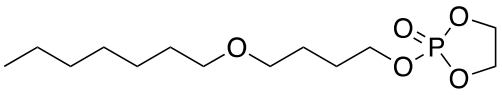
6.27.2. 2-((5-(Hexyloxy)pentyl)oxy)-1,3,2-dioxaphospholane 2-oxide (230)



(230)

Starting from compound **229** gave **230** as a light-yellow viscous oil (7.5 g). IR (CHCl3 cast, cm^{-1}) 2929, 2852, 2797, 1464, 1377, 1302, 1118, 1057, 723; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.57 (ddd, *J* = 15.9, 9.7, 4.7 Hz, 2H, cyclic CH_2-OP), 4.46 (ddd, *J* = 15.9, 4.8, 1.5 Hz, 2H, cyclic CH_2-OP), 4.19 (t, *J* = 7.3 Hz, 2H, CH_2-OP), 3.36 (t, *J* = 7.2 Hz, 2H, CH_2-C<u>H</u>$_2$-O), 3.36 (t, *J* = 7.2 Hz, 2H, CH_2-C<u>H</u>$_2$-O), 1.76 – 1.63 (m, 6H, 3 × OCH_2-C<u>H</u>$_2$), 1.41 – 1.26 (m, 8H, 4 × CH_2), 0.86 (t, *J* = 7.0 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 71.1, 66.4, 66.4, 62.5, 32.4, 31.5, 29.7, 29.2, 25.7, 23.2, 22.6, 14.0, 10.8; ^{31}P NMR ($CDCl_3$, 162 MHz) δ -3.54 (s); HRMS (ESI) Calcd for $C_{13}H_{28}O_5P$ $[M+H]^+$ 295.1645, found 295.1646.

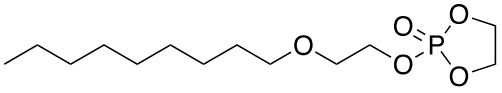
6.27.3. 2-(4-(Heptyloxy)butoxy)-1,3,2-dioxaphospholane 2-oxide (235)



(235)

Starting from compound **234** gave **235** as light-yellow viscous oil (7.9 g). IR ($CHCl_3$ cast film, cm^{-1}) 2927, 2852, 2797, 1464, 1377, 1302, 1118, 1057, 723; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.58 (ddd, *J* = 15.9, 9.7, 4.7 Hz, 2H, cyclic CH_2-OP), 4.43 (ddd, *J* = 15.9, 4.8, 1.5 Hz, 2H, cyclic CH_2-OP), 4.17 (t, *J* = 7.2 Hz, 2H, CH_2-OP), 3.31 – 3.39 (m, 4H, 2 × CH_2-O), 1.75 – 1.71 (m, 6H, 3 × CH_2-CH_2-O), 1.41 – 1.27 (m, 8H, 4 × CH_2), 0.86 (t, *J* = 7.0 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 71.2, 71.1 67.5, 66.1, 32.4, 29.7, 29.0, 28.9, 27.5, 26.2, 23.6, 22.6, 14.0, 7.8; ^{31}P NMR ($CDCl_3$, 162 MHz) δ -3.47 (s); HRMS (ESI) Calcd for $C_{13}H_{28}O_5P$ $[M+H]^+$ 295.1645, found 295.1646.

6.27.4. 2-(2-(Nonyloxy)ethoxy)-1,3,2-dioxaphospholane 2-oxide (239)



(239)

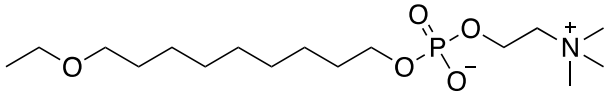
Starting from compound **238** gave **239** as light-yellow viscous oil (8.1 g). IR ($CHCl_3$ cast film, cm^{-1}) 2927, 2852, 2797, 1464, 1377, 1302, 1118, 1057, 723; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.58 (ddd, *J* = 15.8, 9.7, 4.6 Hz, 2H, cyclic CH_2-OP), 4.43 (ddd, *J* = 15.7, 4.6, 1.5 Hz, 2H, cyclic CH_2-OP), 4.17 (t, *J* = 7.2 Hz, 2H, CH_2-OP), 3.31 – 3.39 (m, 4H, 2 × CH_2-O), 1.73 (tt, *J* = 7.2, 6.9 Hz, 2H, C<u>H</u>$_2$-CH_2-O), 1.39 (tt, *J* = 7.1, 6.9 Hz, 2H, C<u>H</u>$_2$-CH_2CH_2-O), 1.39 – 1.27 (m, 10H, 5 × CH_2), 0.86 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 68.6, 68.1, 66.0, 66.0, 61.8, 32.4, 29.4, 29.4, 29.0, 26.2,

14.0; ^{31}P NMR ($CDCl_3$, 162 MHz) δ -3.51 (s); HRMS (ESI) Calcd for $C_{13}H_{28}O_5P$ $[M+H]^+$ 295.1594, found 295.1597.

6.28. General Procedure for Preparation of O-DPC

These new compounds were prepared following a modified literature protocol.[148] A dry 100 mL Schlenk-flask was filled with compound **(225)** (5.00 g, 16.0 mmol) and dry acetonitrile (20 mL), and the solution was cooled to -30 °C. 4.2 M Trimethylamine in ethanol (12.1 mL, 51.0 mmol) was added quickly *via* a syringe and the reaction mixture was stirred vigorously for 10 min at -30 °C. The temperature was increased to 60 °C and stirring was continued for 48 h. After this time, a turbid solution was obtained and was allowed to cool to room temperature, followed by storage at 4 °C for 24 h and another 24 hours at -20 °C. A white precipitate formed and was filtered under argon and washed with a small amount of cold acetonitrile to give the crude product **(220)** as a white solid. The solid material was purified by column chromatography (SiO_2, $CHCl_3/CH_3OH/H_2O$ (65:25:5) to give the product as a white solid (5.7 g, 85% yield). Using the same procedure, these new compounds, **(226)**, **(231)**, and **(236)** were prepared using the appropriate starting material.

6.28.1. 9-Ethoxynonyl (2-(trimethylammonio)ethyl) phosphate (220)

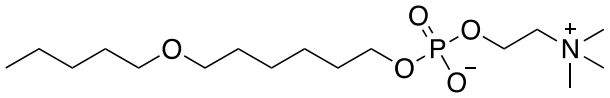


(**220**)

White solid, IR ($CHCl_3$ cast film, cm^{-1}) 2927, 2852, 2797, 1464, 1377, 1302, 1118, 1057, 723; ^{1}H NMR (D_2O, 400 MHz) δ 4.18 (t, J = 6.1 Hz, 2H, Choline C<u>H</u>$_2$-OP), 4.15 (t, J = 7.5 Hz, 2H, CH_2-OP), 3.37 (q, J = 7.0 Hz, 2H, CH_3-CH_2-O), 3.36 (t, J = 7.2 Hz,

2H, CH_2-C<u>H</u>$_2$-O), 2.76 (t, J = 6.15 Hz, 2H, CH_2-N), 2.29 (s, 9H, 3 × CH_3), 1.82 – 1.78 (m, 6H, 3 × CH_2), 1.39 – 1.29 (m, 8H, 4 × CH_2), 0.86 (t, J = 7.0 Hz, 3H, CH_3); ^{13}C NMR (D_2O, 126 MHz) δ 71.2, 67.7, 66.1, 62.3, 49.3, 37.3, 37.3, 37.3, 30.2, 29.7, 29.4, 29.0, 26.2, 25.6, 15.1; ^{31}P NMR (D_2O, 162 MHz) δ 0.53 (s); HRMS (ESI) Calcd for $C_{16}H_{37}NO_5P$ $[M+H]^+$ 354.2404, found 354.2406.

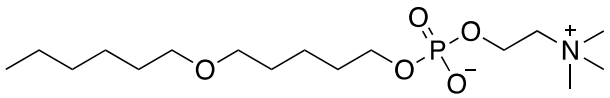
6.28.2. 5-(Hexyloxy)pentyl (2-(trimethylammonio)ethyl) phosphate (226)



(**226**)

White solid (88% yield). IR ($CHCl_3$ cast film, cm^{-1}) 2927, 2852, 2797, 1464, 1377, 1302, 1118, 1057, 723; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.19 (t, J = 6.1 Hz, 2H, Choline C<u>H</u>$_2$-OP), 4.16 (t, J = 7.5 Hz, 2H, CH_2-OP), 3.38 (q, J = 7.0 Hz, 2H, CH_3-CH_2-O), 3.37 (t, J = 7.2 Hz, 2H, CH_2-C<u>H</u>$_2$-O), 2.77 (t, J = 6.15 Hz, 2H, CH_2-N), 2.29 (s, 9H, 3 × CH_3), 1.82 – 1.78 (m, 6H, 3 × CH_2), 1.39 – 1.29 (m, 8H, 4 × CH_2), 0.89 (t, J = 7.0 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 71.2, 71.1, 62.5, 62.3, 49.3, 37.3, 37.3, 37.3, 32.4, 31.5, 29.7, 29.2, 25.7, 23.2, 22.6, 14.0, 10.8; ^{31}P NMR ($CDCl_3$, 162 MHz) δ 0.49 (s); HRMS (ESI) Calcd for $C_{16}H_{36}NNaO_5P$ $[M+Na]^+$ 376.2223, found 376.2219.

6.28.3. 4-(Heptyloxy)butyl (2-(trimethylammonio)ethyl) phosphate (231)

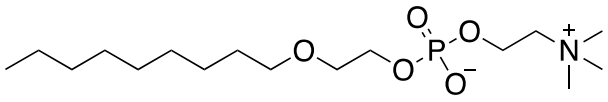


(231)

White solid (86% yield). IR ($CHCl_3$ cast film, cm^{-1}) 2929, 2857, 2795, 1464, 1373, 1304, 1118, 1058, 726; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.23 – 4.15 (m, 4H, 2 × CH_2-

OP), 3.41 – 3.33 (m, 4H, 2 × CH_2-O), 2.76 (t, J = 6.1 Hz, 2H, CH_2-N), 2.29 (s, 9H, 3 × CH_3-N), 1.82 – 1.78 (m, 6H, 3 × C$\underline{H}_2$-CH_2-O), 1.39 – 1.29 (m, 8H, 4 × CH_2), 0.86 (t, J = 7.0 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 71.2, 71.1, 67.5, 49.3, 37.3, 37.3, 37.3, 32.4, 29.0, 28.9, 27.5, 26.2, 22.6, 21.6, 14.0, 11.5; ^{31}P NMR ($CDCl_3$, 162 MHz) δ 0.51 (s); HRMS (ESI) Calcd for $C_{16}H_{37}NO_5P$ $[M+H]^+$ 354.2314, found 354.2317.

6.28.4. 2-(Nonyloxy)ethyl (2-(trimethylammonio)ethyl) phosphate (236)

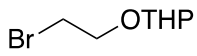


(236)

White powder (85% yield). IR ($CHCl_3$ cast film, cm^{-1}) 2927, 2852, 2797, 1464, 1377, 1302, 1118, 1057, 723; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.23 – 4.15 (m, 4H, 2 × CH_2-OP), 3.41 – 3.33 (m, 4H, 2 × CH_2-O), 2.76 (t, J = 6.15 Hz, 2H, CH_2-N), 2.29 (s, 9H, 3 × CH_3-N), 1.82 – 1.78 (m, 6H, 3 × CH_2-CH_2-O), 1.39 – 1.29 (m, 8H, 4 × CH_2), 0.86 (t, J = 7.0 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 68.8, 68.1, 62.3, 61.8, 49.3, 37.3, 37.3, 37.3, 32.4, 29.7, 29.4, 29.4, 29.0, 26.2, 22.6, 14.0; ^{31}P NMR ($CDCl_3$, 162 MHz) δ -3.56 (s); HRMS (ESI) Calcd for $C_{16}H_{36}NNaO_5P$ $[M+Na]^+$ 376.2223, found 376.2219.

6.29. Experimental Procedures for the Synthesis of Undeuterated F_2-DPC Analogues for NMR Studies

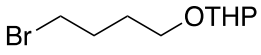
6.29.1. 2-(2-Bromoethoxy)tetrahydro-2*H*-pyran (245)



(245)

This known compound was prepared using the general procedure for protection of alcohol with dihydropyran starting from 2-bromoethanol (**244**) to give the final product as a colorless oil (95% yield). IR ($CHCl_3$ cast film, cm^{-1}) 3300, 3027, 2930, 1452, 1376, 1120, 1089, 1036, 732, 693; ^1H NMR ($CDCl_3$, 500 MHz): δ 4.69 (dd, J = 10.3, 2.7 Hz, 1H, cyclic CH-O), 3.74 – 3.54 (m, 4H, cyclic CH_2-O + linear CH_2-O), 3.27 (t, J = 6.9 Hz, 2H, CH_2Br), 1.92 – 1.71 (m, 10H, 3 × cyclic CH_2 + 2 × linear CH_2); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 97.2, 68.5, 62.0, 34.2, 30.4, 30.2, 29.7, 25.4, 19.0 ppm; HRMS (ESI) Calcd for $C_7H_{13}BrO_2$ $[M+H]^+$ 209.0098 found 209.0099.

6.29.2. 2-(4-Bromobutoxy)tetrahydro-2*H*-pyran (253)

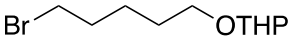


(253)

This known compound was prepared using the general procedure for protection of alcohol with dihydropyran starting from 4-bromobutanol (**252**) to give the final product as colorless oil (95% yield). IR ($CHCl_3$ cast film, cm^{-1}) 2934, 2853, 1467, 1352, 1137, 1122, 1036, 986, 865, 726; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.69 (dd, J = 10.2, 2.6 Hz, 1H, cyclic C<u>H</u>-O), 3.74 – 3.54 (m, 4H, cyclic C<u>H</u>$_2$-O + linear CH_2-O), 3.27 (t, J = 6.8 Hz, 2H, C<u>H</u>$_2$Br), 1.92 – 1.71 (m, 10H, 3 × cyclic C<u>H</u>$_2$ + 2 × linear C<u>H</u>$_2$); ^{13}C NMR

($CDCl_3$, 125 MHz) δ 97.2, 68.5, 62.0, 34.2, 30.4, 30.2, 29.7, 25.4, 19.0; HRMS (ESI) Calcd for $C_9H_{17}BrO_2$ $[M+H]^+$ 236.0412 found 237.0421.

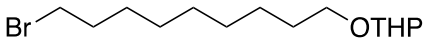
6.29.3. 2-((5-Bromopentyl)oxy)tetrahydro-2*H*-pyran (261)



(261)

This known compound was prepared using the general procedure for protection of alcohol with dihydropyran starting from 5-bromopentanol (**260**) to give the final product as colorless oil (6.4 g, 91% yield). IR ($CHCl_3$ cast film, cm^{-1}) 2932, 2856, 1452, 1376, 1125, 1086, 1033, 738, 697; ^1H NMR ($CDCl_3$, 700 MHz) δ 4.69 (dd, J = 10.2, 2.6 Hz, 1H, cyclic C<u>H</u>-O-), 3.74 – 3.54 (m, 4H, cyclic C<u>H</u>$_2$-O + linear C<u>H</u>$_2$-O-), 3.26 (t, J = 6.8 Hz, 2H, CH_2Br), 1.92 – 1.71 (m, 12H, 3 × cyclic C<u>H</u>$_2$ + 3 × linear C<u>H</u>$_2$); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 97.2, 68.5, 62.0, 33.4, 32.4, 30.2, 29.2, 26.0, 25.4, 19.0; HRMS (ESI) Calcd for $C_{10}H_{19}BrNaO$ $[M + Na]^+$ 273.0568, found 273.0571.

6.29.4. 2-((9-Bromononyl)oxy)tetrahydro-2*H*-pyran (269)



(269)

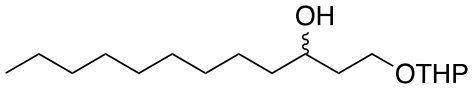
This known compound was prepared using the general procedure for protection of alcohol with dihydropyran starting from 9-bromononan-1-ol (**268**) to give the final product as colorless oil (6.8, 95% yield). IR ($CHCl_3$ cast film, cm^{-1}) 2987, 2923, 14662, 1372, 1118, 1084, 1034, 737, 692; ^1H NMR ($CDCl_3$, 700 MHz) δ 4.69 (dd, J = 10.3, 2.7 Hz, 1H, C<u>H</u>-OCH_2), 3.74 – 3.55 (m, 4H, cyclic CH_2-O + linear CH_2-O), 3.25 (t, J = 6.9 Hz, 2H, C<u>H</u>$_2$-Br), 1.92 – 1.27 (m, 20H, 3 × cyclic C<u>H</u>$_2$, 7 × linear C<u>H</u>$_2$); ^{13}C NMR

($CDCl_3$, 176 MHz) δ 97.2, 68.5, 62.0, 33.4, 32.6, 30.2, 29.4, 29.1, 29.0, 28.8, 28.5, 26.5, 25.4 19.0; HRMS (ESI) Calcd for $C_{14}H_{27}BrNaO_2$ $[M + Na]^+$ 329.1231, found 329.1234.

6.30. General Procedure for Grignard Reactions

These new compounds were prepared following a modified literature protocol.[150] A 100 mL round bottom flask containing Mg (1.40 g, 56.3 mmol) was flame dried for 10 minutes and allowed to cool to room temperature. The flask was purged with argon, and then dry THF (100 mL) was added followed by the addition of compound **245** (10.0 g, 42.2 mmol). A small amount of iodine (ca. 20 mg) was added as the initiator and the mixture was stirred at reflux for 30 min. After this period, decanal **(246)** (5.38 mL, 34.3 mmol) in THF (50 mL) was added dropwise via a cannula syringe to the stirred Grignard reagent at 0 °C, and then the mixture was stirred and heated at 50 °C for 30 min. After cooling, the mixture was poured into an ice-cold mixture of dilute HCl and NH_4Cl and extracted with hexane. The organic layer was washed with water, $NaHCO_3$, brine, and then dried over $MgSO_4$ and filtered. The solvent was removed by evaporation and the crude product was purified by column chromatography (SiO_2, 20:1 hexane:ethyl acetate) to give the product **247** as a colorless oil (8.7 g, 89% yield). Using the same method, compounds **255**, **263**, and **271** were synthesized using the appropriate starting materials.

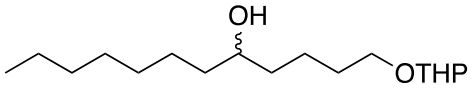
6.30.1. 1-((Tetrahydro-2*H*-pyran-2-yl)oxy)dodecan-3-ol (247)



(247)

IR ($CHCl_3$ cast film, cm^{-1}) 3327, 2932, 1456, 1376, 1123, 1089, 1036, 736, 694 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.69 (dd, *J* = 10.3, 2.7 Hz, 1H, cyclic C<u>H</u>-O), 3.74 – 3.55 (m, 5H, cyclic C<u>H</u>$_2$-O + linear C<u>H</u>$_2$-O +C<u>H</u>-OH), 1.86 – 1.74 (m, 8H, 2 × cyclic C<u>H</u>$_2$, 2 × linear C<u>H</u>$_2$), 1.52 – 1.28 (m, 16H, 2 × cyclic C<u>H</u>$_2$, 7 × linear C<u>H</u>$_2$), 0.93 (t, *J* = 7.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 97.2, 71.4, 68.5, 62.0, 38.3, 36.5, 32.4, 30.2, 29.4, 29.2, 28.5, 25.4, 24.9, 24.8, 22.6, 19.0, 14.0; HRMS (ESI) Calcd for $C_{17}H_{34}NaO_3$ $[M+Na]^+$ 309.2400 found 309.2396.

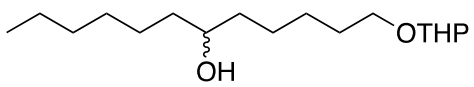
6.30.2. 1-((Tetrahydro-2*H*-pyran-2-yl)oxy)dodecan-5-ol (255)



(255)

Starting from compound **253** and octanal (**254**) gives the final product as a colorless oil (7.4 g, 85% yield). IR ($CHCl_3$ cast film, cm^{-1}) 3325, 2962, 1464, 1373, 1125, 1086, 1032, 735, 694; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.69 (dd, *J* = 10.3, 2.7 Hz, 1H, cyclic C<u>H</u>-O), 3.74 – 3.55 (m, 5H, cyclic C<u>H</u>$_2$-O + linear C<u>H</u>$_2$-O + C<u>H</u>-OH), 1.86 – 1.74 (m, 8H, 2 × cyclic C<u>H</u>$_2$, 2 × linear C<u>H</u>$_2$), 1.52 – 1.28 (m, 16H, 2 × cyclic C<u>H</u>$_2$, 7 × linear C<u>H</u>$_2$), 0.93 (t, *J* = 7.6 Hz, 3H, C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 97.2, 71.4, 68.5, 62.0, 38.3, 36.5, 32.4, 30.2, 29.4, 29.2, 28.5, 25.4, 24.9, 24.8, 22.6, 19.0, 14.0; HRMS (ESI) Calcd for $C_{17}H_{34}NaO_3$ $[M+Na]^+$ 309.2401 found 309.2398.

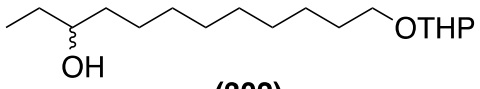
6.30.3. 1-((Tetrahydro-2*H*-pyran-2-yl)oxy)dodecan-6-ol (263)



(263)

Starting from compound **261** and **262** gives the final product as a colorless oil (6.7 g, 90% yield). IR ($CHCl_3$ cast fim, cm^{-1}) 3229, 2923, 1462, 1368, 1131, 1076, 1038, 752, 695; ^1H NMR ($CDCl_3$, 700 MHz) δ 4.69 (dd, *J* = 10.2, 2.6 Hz, 1H, cyclic C<u>H</u>-O), 3.74 – 3.55 (m, 5H, cyclic C<u>H</u>$_2$-O + linear C<u>H</u>$_2$-O +C<u>H</u>-OH), 1.86 – 1.74 (m, 8H, 2 × cyclic C<u>H</u>$_2$, 2 × linear C<u>H</u>$_2$), 1.52 – 1.28 (m, 16H, 2 × cyclic C<u>H</u>$_2$, 7 × linear C<u>H</u>$_2$), 0.93 (t *J* = 7.5 Hz, 3H, C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 97.2, 71.4, 68.5, 62.0, 38.5, 32.4, 30.2, 29.2, 29.1, 25.9, 25.4, 24.9, 24.8, 22.6, 19.0, 14.0; HRMS (ESI) Calcd for $C_{17}H_{34}NaO_3$ $[M+Na]^+$ 309.2410 found 309.24223.

6.30.4. 12-((Tetrahydro-2*H*-pyran-2-yl)oxy)dodecan-3-ol (271)



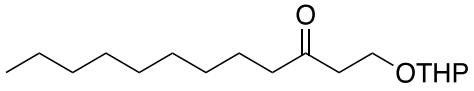
(302)

Staring from compounds **269** and **270** gave the final product as colorless oil (6.5 g, 87% yield). IR ($CHCl_3$ cast film, cm^{-1}) 3345, 3027, 2930, 1452, 1376, 1120, 1089, 1036, 732, 693; ^1H NMR ($CDCl_3$, 700 MHz) δ 4.69 (dd, *J* = 10.2, 2.6 Hz, 1H, cyclic C<u>H</u>-O), 3.74 – 3.55 (m, 5H, cyclic CH$_2$-O + linear C<u>H</u>$_2$-O +C<u>H</u>-OH), 1.86 – 1.74 (m, 8H, 2 × cyclic C<u>H</u>$_2$, 2 × linear C<u>H</u>$_2$), 1.52 – 1.28 (m, 16H, 2 × cyclic C<u>H</u>$_2$, 7 × linear C<u>H</u>$_2$), 0.93 (t, *J* = 7.5 Hz, 3H, C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 97.2, 73.1, 68.5, 62.0, 36.5, 30.2, 30.2, 29.4, 29.1, 29.0, 28.5, 26.2, 25.4, 24.9, 19.0, 9.9; HRMS (ESI) Calcd for $C_{17}H_{34}NaO_3$ $[M+Na]^+$ 309.2412 found 309.2416.

6.31. General Procedure for PCC Oxidation of the Alcohols

These new compounds were prepared following a modified literature protocol.[170] To a stirring solution of pyridinium chlorochromate (9.10 g, 42.3 mol) and Celite (8.5 g) in DCM (100 mL), was added a solution of compound **247** (6.00 g, 21.4 mol) in CH_2Cl_2 (50 mL) at 0 °C. The reaction mixture was stirred at room temperature for 3 h. After completion of the reaction as monitored by TLC (hexane:ethyl acetate 4:1, R_f = 0.32), the reaction was filtered and the filtrate was washed with saturated $NaHCO_3$ solution, water, and brine, and then dried over anhydrous $MgSO_4$ and filtered. The solvent was removed *in vacuo* and the crude oil was purified by column chromatography (SiO2, 4:1 hexane: ethyl acetate) to give the product **248** as a colorless oil (5.5 g, 92% yield). The same procedure was used to prepare these new compounds **256**, **264**, and **272**.

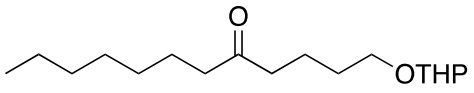
6.31.1. 1-((Tetrahydro-2*H*-pyran-2-yl)oxy)dodecan-3-one (248)



(248)

IR ($CHCl_3$ cast film, cm^{-1}) 3029, 2932, 1735, 1452, 1376, 1120, 1089, 1036, 732, 693; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.68 (dd, *J* = 10.3, 2.7 Hz, 1H, cyclic C<u>H</u>-O), 3.74 – 3.55 (m, 4H, cyclic C<u>H</u>$_2$-O + linear C<u>H</u>$_2$-O), 2.46 (t, *J* = 7.44 Hz, 2 H, C<u>H</u>α-C<u>H</u>$_2$), 2.44 (t, *J* = 7.44, 2H, C<u>H</u>α-C<u>H</u>$_2$), 1.84 – 1.74 (m, 12H, 3 × cyclic C<u>H</u>$_2$, 3 × linear C<u>H</u>$_2$), 1.28 – 1.24 (m, 8H, 4 × linear C<u>H</u>$_2$), 0.86 (t, *J* = 7.44 Hz, 3 H, CH_3); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 192.4, 97.2, 68.5, 62.0, 41.8, 41.6, 32.4, 30.2, 29.4, 29.2, 27.3, 25.4, 25.3, 22.6, 19.0, 14.0; HRMS (ESI) Calcd for $C_{17}H_{33}O$ $[M + H]^+$ 285.2349, found 285.2351.

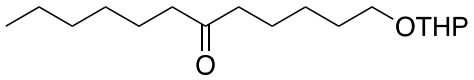
6.31.2. 1-((Tetrahydro-2*H*-pyran-2-yl)oxy)dodecan-5-one (256)



(256)

Starting from compound alcohol **255** gave the desired compound **256** as a colorless oil (5.5 g, 93% yield). IR ($CHCl_3$ cast film, cm^{-1}) 2966, 2932, 1463, 1713, 1357, 1131, 1075, 1038, 742, 689; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.68 (dd, J = 10.2, 2.7 Hz, 1H, cyclic C<u>H</u>-O), 3.74 – 3.55 (m, 4H, cyclic C<u>H</u>$_2$-O + linear C<u>H</u>$_2$-O), 2.46 (t, J = 7.4 Hz, 2H, C<u>H</u>α-CH), 2.44 (t, J = 7.4, 2H, C<u>H</u>α-CH_2), 1.84 – 1.74 (m, 12H, 3 × cyclic C<u>H</u>$_2$, 3 × linear C<u>H</u>$_2$), 1.28 – 1.24 (m, 8H, 4 × linear C<u>H</u>$_2$), 0.86 (t, J = 7.4 Hz, 3 H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 192.4, 97.2, 68.5, 62.0, 41.8, 41.6, 32.4, 30.2, 29.4, 29.2, 27.3, 25.4, 25.3, 22.6, 19.0, 14.0; HRMS (ESI) Calcd for $C_{17}H_{33}O_3$ $[M + H]^+$ 285.2354, found 285.2357.

6.31.3. 1-((Tetrahydro-2*H*-pyran-2-yl)oxy)dodecan-6-one (264)

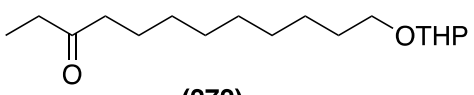


(264)

Starting from compound **263** gave the desired compound **264** as a colorless oil (5.1 g, 93% yield). IR ($CHCl_3$ cast film, cm^{-1}) 2942, 1721, 1453, 1381, 1133, 1074, 1034, 756, 693; ^1H NMR ($CDCl_3$, 700 MHz) δ 4.68 (dd, J = 10.3, 2.6 Hz, 1H, cyclic C<u>H</u>-O-), 3.74 – 3.55 (m, 4H, cyclic CH_2-O- + linear CH_2-O-), 2.46 (t, J = 7.4 Hz, 2H, C<u>H</u>α-CH_2), 2.44 (t, J = 7.4, 2H, C<u>H</u>α-CH_2), 1.84 – 1.74 (m, 12H, 3 × cyclic C<u>H</u>$_2$, 3 × linear C<u>H</u>$_2$), 1.28 – 1.24 (m, 8H, 4 × linear C<u>H</u>$_2$), 0.86 (t, J = 7.4 Hz, 3H, C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$,

176 MHz) δ 97.2, 68.5, 62.0, 33.4, 32.6, 30.2, 29.4, 26.2, 25.4 19.0; HRMS (ESI) Calcd for $C_{17}H_{33}O_3$ $[M + H]^+$ 285.2348, found 285.2351.

6.31.4. 12-((Tetrahydro-2*H*-pyran-2-yl)oxy)dodecan-3-one (272)



(272)

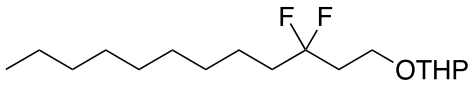
Starting from compound (**271**) gave the desired compound **272** as a colorless oil (5.3 g, 95% yield). IR ($CHCl_3$ cast film, cm^{-1}) 2928, 1721, 1455, 1386, 1124, 1077, 1032, 734, 698; ^1H NMR ($CDCl_3$, 700 MHz) δ 4.69 (dd, J = 10.2, 2.6 Hz, 1H, cyclic C<u>H</u>-O), 3.74 – 3.55 (m, 4H, cyclic CH_2-O + linear CH_2-O), 2.46 (q, J = 7.4 Hz, 2H, CHα-CH_3), 2.44 (t, J = 7.4, 2H, CHα-CH_2), 1.86 – 1.71 (m, 6H, 2 × cyclic CH_2, linear CH_2), 1.28 – 1.27 (m, 14H, cyclic CH_2, 6 × linear CH_2), 1.01 (t, J = 7.4 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 210.9, 97.2, 68.5, 62.0, 42.5, 35.4, 30.2, 29.4, 29.1, 29.0, 27.3, 26.2, 25.4, 19.0, 7.7; HRMS (ESI) Calcd for $C_{17}H_{33}O_3$ $[M + H]+$ 284.2353, found 284.2356.

6.32. General Procedure for Fluorinating of the Ketone Using DAST

These new compounds were prepared following a modified literature protocol.[171] To a solution of the ketone **248** (5.00 g, 18.1 mmol) in dry CH_2Cl_2 (60 mL) was added diethylaminosulfurtrifluoride (4.80 mL, 36.1 mmol) at -78 °C, and the reaction mixture was stirred for 12 hr at 80 °C. Saturated $NaHCO_3$ (50 mL) was added to terminate the reaction at 0 °C and the organic layer was extracted with CH_2Cl_2. The combined organic layers were washed twice with brine, dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo.* The crude oil was purified by column chromatography

(SiO_2, 5:1 hexane:ethyl acetate) to give the product **249** as a light-yellow oil (4.9 g, 89% yield). Using the same procedure, these new compounds **257**, **265**, and **273** were prepared.

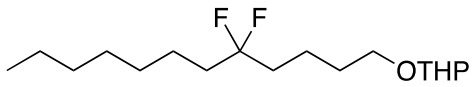
6.32.1. 2-((3,3-Difluorododecyl)oxy)tetrahydro-2*H*-pyran (249)



(249)

IR ($CHCl_3$ cast film, cm^{-1}) 3028, 2929, 2857, 1465, 1376, 1120, 1089, 1036, 732, 693 cm^{-1}; ^1H NMR ($CDCl_3$, 700 MHz) δ 4.69 (dd, J = 10.3, 2.7 Hz, 1H, cyclic C<u>H</u>-O), 3.74 – 3.55 (m, 4H, cyclic CH_2-O + linear CH_2-O), 1.92 – 1.78 (m, 24H, 3 × cyclic CH_2, 9 × linear CH_2), 0.87 (t, J = 7.0 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 123.2, 97.2, 68.5, 62.0, 33.3, 32.4, 30.2, 30.0, 29.4, 29.2, 26.0, 25.4, 22.6, 19.0, 14.0; ^{19}F NMR ($CDCl_3$, 658 MHz) δ 98.42 (pentet, J = 16.8 Hz); HRMS (ESI) Calcd for $C_{17}H_{32}F_2NaO_2$ $[M + Na]^+$ 329.2471, found 329.2473.

6.32.2. 2-((5,5-Difluorododecyl)oxy)tetrahydro-2*H*-pyran (257)

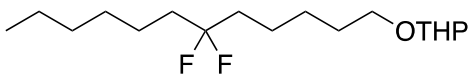


(257)

Starting from compound **256** gave the desired compound **257** as a light-yellow oil (4.8 g, 87% yield). IR ($CHCl_3$ cast film, cm^{-1}) 2931, 2857, 1467, 1384, 1352, 1221, 1123, 1079, 1033, 722, 696; ^1H NMR ($CDCl_3$, 700 MHz) δ 4.69 (dd, J = 10.2, 2.6 Hz, 1H, cyclic C<u>H</u>-O), 3.74 – 3.55 (m, 4H, cyclic C<u>H</u>$_2$-O + linear C<u>H</u>$_2$-O), 1.92 – 1.78 (m, 24H, 3 × cyclic C<u>H</u>$_2$, 9 × linear C<u>H</u>$_2$), 0.87 (t, J = 7.0 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 176

MHz) δ 123.2, 97.2, 68.5, 62.0, 33.3, 32.4, 30.2, 30.0, 29.4, 29.2, 26.0, 25.4, 22.6, 19.0, 14.0; ^{19}F NMR ($CDCl_3$, 658 MHz) δ 98.24 (pentet, *J* = 16.7 Hz); HRMS (ESI) Calcd for $C_{17}H_{32}F_2NaO_2$ $[M + Na]^+$ 329.2381, found 329.2384.

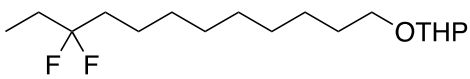
6.32.3. 2-((6,6-Difluorododecyl)oxy)tetrahydro-2*H*-pyran (265)



(265)

Starting from compound **264** gave the desired compound **265** as a light-yellow oil (4.8 g, 87% yield). IR ($CHCl_3$ cast film, cm^{-1}) 2929, 2824, 1435, 1358, 1145, 1073, 1031, 756, 685; ^{1}H NMR ($CDCl_3$, 700 MHz) δ 4.69 (dd, *J* = 10.3, 2.7 Hz, 1H, cyclic C<u>H</u>-O), 3.74 – 3.55 (m, 4H, cyclic CH_2-O + linear CH_2-O), 1.92 – 1.78 (m, 24H, 3 × cyclic CH_2, 9 × linear CH_2), 0.87 (t, *J* = 7.0 Hz, 3 H, CH_3); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 123.2, 97.2, 68.5, 62.0, 33.3, 32.4, 30.4, 30.0, 39.1, 26.0, 25.9, 25.4, 22.6, 19.0, 14.0; ^{19}F NMR ($CDCl_3$, 658MHz) δ 98.22 (pentet, *J* = 16.7 Hz); HRMS (ESI) Calcd for $C_{17}H_{32}F_2NaO_2$ $[M + Na]^+$ 329.2369, found 329.2373.

6.32.4. 2-((10,10-Difluorododecyl)oxy)tetrahydro-2*H*-pyran (273)

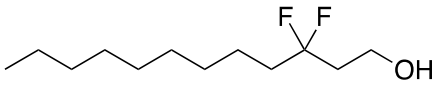


(273)

Starting from compound **272** gave the desired compound **273** as a light-yellow oil (4.9 g, 89% yield). IR ($CHCl_3$ cast film, cm^{-1}) 2946, 2928, 1436, 1378, 1124, 1084, 1037, 746, 694; ^{1}H NMR ($CDCl_3$, 700 MHz) δ 4.69 (dd, *J* = 10.2, 2.6 Hz, 1H, cyclic C<u>H</u>-O), 3.74 – 3.55 (m, 4H, cyclic CH_2-O + linear CH_2-O), 1.92 – 1.28 (m, 24H, 3 × cyclic CH_2, 9 × linear CH_2), 0.97 (t, *J* = 6.9 Hz, 3 H, CH_3); ^{13}C NMR ($CDCl_3$, 176 MHz) δ

123.2, 97.2, 68.5, 62.0, 33.4, 30.5, 30.2, 30.0, 29.4, 29.1, 29.0, 26.2, 26.0, 25.4, 19.0, 12.6; ^{19}F NMR ($CDCl_3$, 658 MHz) δ 98.43 (pentet, J = 16.8 Hz); HRMS (ESI) Calcd for $C_{17}H_{32}F_2NaO_2$ $[M + Na]^+$ 329.2373, found 329.2376.

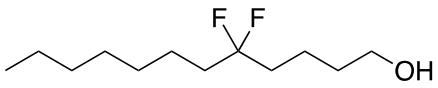
6.32.5. 3,3-Difluorododecan-1-ol (250)



(250)

This new compound was prepared using the general procedure for the deprotection of THP protecting group starting from compound **249** gave **250** as a light-yellow oil (3.1 g, 94% yield). IR (CHCl3 cast film, cm^{-1}) 3292, 2953, 1456, 1375, 1120, 1089, 1036, 732, 694 cm^{-1}; ^{1}H NMR ($CDCl_3$, 700 MHz) δ 3.33 (t, J = 7.2 Hz, 2H, CH_2-OH), 1.32 (tt, J = 7.5, 7.2 Hz, 2H, C$\underline{H}_2$-CH_2-OH), 1.48 – 1.44 (m, 8H, 4 × C$\underline{H}_2$), 1.32 – 1.27 (m, 8H, 4 × C$\underline{H}_2$), 0.87 (t, J = 7.0 Hz, 3H, C$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 123.3, 62.5, 33.3, 32.4, 32.4, 30.0, 29.4, 26.0, 22.6, 14.0; ^{19}F NMR (CDCl3, 659 MHz) δ 98.16 (pentet, J = 16.8 Hz); HRMS (ESI) Calcd for $C_{12}H_{24}F_2NaO_2$ $[M + Na]^+$ 245.1789, found 245.1791.

6.32.6. 5,5-Difluorododecan-1-ol (258)

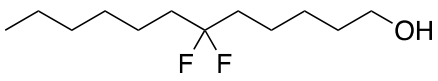


(258)

This new compound was prepared using the general procedure for the deprotection of THP protecting group starting from compound **257** gave **258** as a light-yellow oil Light yellow oil (3.2 g, 96% yield). IR ($CHCl_3$ cast film, cm^{-1}) 3294, 2951, 2852, 1457, 1427,

1393, 1221, 1143, 1071, 938, 726, 687; ^{1}H NMR ($CDCl_3$, 700 MHz) δ 3.33 (t, J = 7.2 Hz, 2H, C<u>H</u>$_2$-OH), 1.32 (tt, J = 7.5, 7.2 Hz, 2H, C<u>H</u>$_2$-CH$_2$-OH), 1.48 – 1.44 (m, 8H, 4 × C<u>H</u>$_2$), 1.32 – 1.27 (m, 8H, 4 × C<u>H</u>$_2$), 0.87 (t, J = 7.0 Hz, 3H, C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 123.3, 62.5, 33.3, 32.4, 32.4, 30.0, 29.4, 26.0, 22.6, 14.0; ^{19}F NMR (CDCl3, 658 MHz) δ 98.18 (pentet, J = 16.8 Hz) ppm; HRMS (ESI) Calcd for $C_{12}H_{24}F_2NaO_2$ $[M + Na]^+$ 245.1793, found 245.1796.

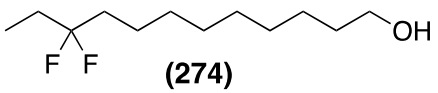
6.32.7. 6,6-Difluorododecan-1-ol (266)



(266)

This new compound was prepared using the general procedure for the deprotection of THP protecting group starting from compound **265** gave **266** as a light-yellow oil (2.9 g, 89% yield). IR ($CHCl_3$ cast film, cm^{-1}) 3327, 2924, 2827, 1465, 1346, 1154, 1078, 1037, 754, 682; ^{1}H NMR ($CDCl_3$, 700 MHz) δ 3.33 (t, J = 7.2 Hz, 2H, CH_2-OH), 1.32 (tt, J = 7.5, 7.2 Hz, 2 H, C<u>H</u>$_2$-CH$_2$-OH), 1.48 – 1.44 (m, 8H, 4 × CH_2), 1.32 – 1.27 (m, 8H, 4 × CH2), 0.87 (t, J = 7.0 Hz, 3 H, CH_3); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 123.2, 62.5, 33.3, 32.5, 30.0, 26.0, 25.9, 22.6, 14.0; ^{19}F NMR ($CDCl_3$, 658 MHz) δ 98.16 (pentet, J = 16.8 Hz); HRMS (ESI) Calcd for $C_{12}H_{24}F_2NaO$ $[M + Na]^+$ 245.1793, found 245.1795.

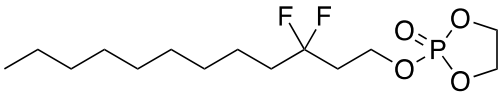
6.32.8. 10,10-Difluorododecan-1-ol (274)



(274)

This new compound was prepared using the general procedure for the deprotection of THP protecting group starting from compound **273** gave **274** as a light-yellow oil (92% yield). IR ($CHCl_3$ cast film, cm^{-1}) 3268, 2927, 1457, 1372, 1124, 1086, 1029, 727, 689; ^1H NMR ($CDCl_3$, 700 MHz) δ 3.34 (t, J = 7.2 Hz, 2H, CH_2-OH), 1.35 (tt, J = 7.5, 7.2 Hz, 2H, $C\underline{H}_2$-CH_2-OH), 1.47 – 1.44 (m, 8H, 4 × CH_2), 1.33 – 1.26 (m, 8H, 4 × CH2), 0.86 (t, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 123.2, 62.5, 33.3, 32.5, 30.5, 30.0, 29.4, 29.0, 26.0, 25.9, 12.6; ^{19}F NMR ($CDCl_3$, 658 MHz) δ 98.16 (pentet, J = 16.8 Hz); HRMS (ESI) Calcd for $C_{12}H_{25}F_2NaO$ $[M + Na]^+$ 245.1793, found 245.1795.

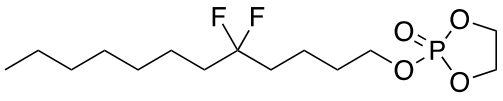
6.32.9. 2-((3,3-Difluorododecyl)oxy)-1,3,2-dioxaphospholane 2-oxide (251)



(251)

This new compound was prepared using the general procedure for the preparation of phosphorane compounds starting from **250** gave **251** as thick viscous oil (2.2 g, 86% yield). IR ($CHCl_3$ cast film, cm^{-1}) 2927, 2852, 2797, 1464, 1377, 1302, 1118, 1057, 723; ^1H NMR ($CDCl_3$, 700 MHz) δ 4.60 (ddd, J = 16.4, 9.7, 4.4 Hz, 2H, cyclic $C\underline{H}_2$-OP), 4.41 (ddd, J = 16.4, 4.7, 1.5 Hz, 2H, cyclic $C\underline{H}_2$-OP), 4.16 (t, J = 7.5 Hz, 2H, $C\underline{H}_2$-OP), 1.75 (quint, J = 7.50 Hz, 2 H, $C\underline{H}_2$-CH_2-O), 1.48 – 1.25 (m, 16H, 8 × $C\underline{H}_2$), 0.86 (t, J = 7.0 Hz, 3 H, $C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 123.2, 67.5, 66.0, 66.0, 33.3, 32.4, 31.0, 30.0, 29.4, 26.0, 22.6, 14.0 ppm; ^{19}F NMR (CDCl3, 658 MHz) δ 98.16 (pentet, J = 16.8 Hz); ^{31}P NMR ($CDCl_3$, 283 MHz) δ 0.48 (s); HRMS (ESI) Calcd for $C_{14}H_{27}F_2NaO_4P$ $[M + Na]^+$ 351.1721, found 351.1724.

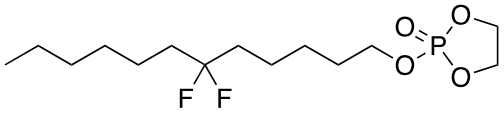
6.32.10. 2-((5,5-Difluorododecyl)oxy)-1,3,2-dioxaphospholane 2-oxide (259)



(259)

This new compound was prepared using the general procedure for the preparation of phosphorane compounds starting from **258** gave **259** as thick viscous oil (2.5 g, 87% yield). IR ($CHCl_3$ cast film, cm^{-1}) 2927, 2852, 2797, 1464, 1377, 1302, 1118, 1057, 723; ^1H NMR ($CDCl_3$, 700 MHz) δ 4.60 (ddd, J = 16.4, 9.7, 4.4 Hz, 2H, cyclic C$\underline{H}_2$-OP), 4.41 (ddd, J = 16.4, 4.7, 1.5 Hz, 2H, cyclic C$\underline{H}_2$-OP), 4.16 (t, J = 7.5 Hz, 2H, CH_2-OP), 1.75 (quint, J = 7.5 Hz, 2H, C$\underline{H}_2$-CH_2-O), 1.48 – 1.25 (m, 16H, 8 × C$\underline{H}_2$), 0.86 (t, J = 7.0 Hz, 3H, C$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 123.2, 67.5, 66.0, 66.0, 33.3, 32.4, 31.0, 30.0, 29.4, 26.0, 22.6, 14.0; ^{19}F NMR (CDCl3, 658 MHz) δ 98.16 (pentet, J = 16.8 Hz); ^{31}P NMR ($CDCl_3$, 283 MHz) δ 0.48 (s); HRMS (ESI) Calcd for $C_{14}H_{27}F_2NaO_4P$ $[M + Na]^+$ 351.1721, found 351.1725.

6.32.11. 2-((6,6-Difluorododecyl)oxy)-1,3,2-dioxaphospholane 2-oxide (267)

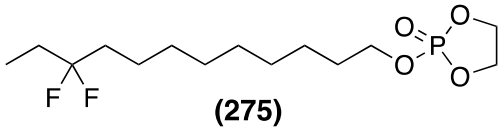


(267)

This new compound was prepared using the general procedure for the preparation of phosphorane compounds starting from **266** gave **267** as thick viscous oil (2.4 g, 86% yield). IR ($CHCl_3$ cast film, cm^{-1}) 2945, 2833, 1453, 1354, 1166, 1074, 1036, 762, 684; ^1H NMR ($CDCl_3$, 700 MHz) δ 4.60 (ddd, J = 16.3, 9.6, 4.4 Hz, 2H, cyclic C$\underline{H}_2$-OP), 4.41 (ddd, J = 16.3, 4.7, 1.4 Hz, 2H, cyclic C$\underline{H}_2$-OP), 4.16 (t, J = 7.5 Hz, 2H, C$\underline{H}_2$-OP),

1.75 (quint, J = 7.5 Hz, 2H, C<u>H</u>$_2$-CH$_2$-O), 1.48 – 1.25 (m, 16H, 8 × CH$_2$), 0.86 (t, J = 7.0 Hz, 3H, CH$_3$); ^{13}C NMR (CDCl$_3$, 176 MHz) δ 123.2, 67.5, 66.0, 66.0, 33.3, 32.4, 30.2, 30.0, 26.0, 25.9, 22.6, 14.0; ^{19}F NMR (CDCl$_3$, 658 MHz) δ 98.16 (pentet, J = 16.8 Hz) ppm; ^{31}P NMR (CDCl$_3$, 283MHz) δ -3.54 (s); HRMS (ESI) Calcd for $C_{14}H_{27}F_2NaO_4P$ [M + Na]$^+$ 351.1619, found 351.1622.

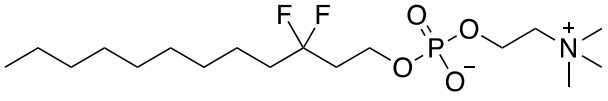
6.32.12. 2-((10,10-Difluorododecyl)oxy)-1,3,2-dioxaphospholane 2-oxide (275)



(275)

This new compound was prepared using the general procedure for the preparation of phosphorane compounds starting from **274** gave **275** as thick viscous oil (2.3 g, 86% yield). IR (CHCl$_3$ cast film, cm^{-1}) 2927, 2852, 2797, 1464, 1377, 1302, 1118, 1057, 723; ^{1}H NMR (CDCl$_3$, 700 MHz) δ 4.58 (ddd, J = 15.8, 9.7, 4.6 Hz, 2H, cyclic C<u>H</u>$_2$-OP), 4.43 (ddd, J = 15.7, 4.6, 1.5 Hz, 2H, cyclic CH$_2$-OP), 4.16 (t, J = 7.2 Hz, 2H, CH$_2$-OP), 1.77 (quint, J = 7.5 Hz, 2H, C<u>H</u>$_2$-CH$_2$-O), 1.49 – 1.23 (m, 16H, 8 × CH$_2$), 0.96 (t, J = 7.0 Hz, 3H, CH$_3$); ^{13}C NMR (CDCl$_3$, 176 MHz) δ 123.2, 69.0, 63.1, 33.3, 32.7, 30.5, 30.0, 29.4, 29.0, 26.0, 25.9, 12.6; ^{19}F NMR (CDCl3, 658 MHz) δ 98.16 (pentet, J = 16.8 Hz); ^{31}P NMR (CDCl$_3$, 283 MHz) δ -3.62 (s); HRMS (ESI) Calcd for $C_{14}H_{25}F_2NaO_4P$ [M + Na]$^+$ 351.1618, found 351.1620.

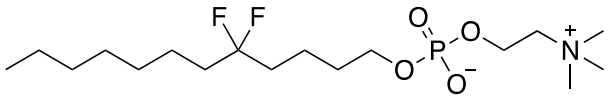
6.32.13. 3,3-Difluorododecyl (2-(trimethylammonio)ethyl) phosphate (240)



(240)

This new compound was prepared using the general procedure for the preparation of O-DPC starting from **251** and triemethyl amine gave **240** as a white powder (1.8 g, 88% yield). IR ($CHCl_3$ cast, cm^{-1}) 3022, 2962, 1447, 1383, 1126, 1086, 1046, 762, 696; ^1H NMR ($CDCl_3$, 700 MHz) δ 4.18 – 4.14 (m, 4H, 2 × CH_2-OP), 2.77 (t, J = 6.1 Hz, 2H, CH_2-N), 2.29 (s, 9H, 3 × CH_3), 1.77 (quint, J = 7.5 Hz, 2 H, C$\underline{H}_2$-CH_2-O), 1.45 – 1.21 (m, 16H, 8 × CH_2), 0.88 (t, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 123.2, 67.7, 62.3, 49.3, 37.3, 37.3, 37.3, 33.3, 32.4, 31.0, 30.0, 29.4, 26.0, 22.6, 14.0; ^{19}F NMR ($CDCl_3$, 568 MHz) δ 98.36 (pentet, J = 16.6 Hz); ^{31}P NMR ($CDCl_3$, 283 MHz) δ 0.47 (s); HRMS (ESI) Calcd for $C_{17}H_{36}F_2NNaO_4P$ $[M + Na]^+$ 410.2413, found 410.2417.

6.32.14. 5,5-Difluorododecyl (2-(trimethylammonio)ethyl) phosphate (241)

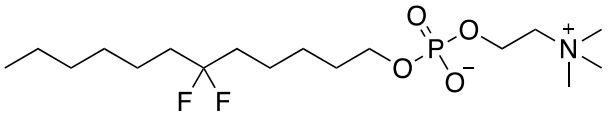


(241)

This new compound was prepared using the general procedure for the preparation of O-DPC starting from **259** and triemethyl amine gave **241** as a white powder (1.9 g, 90% yield). IR ($CHCl_3$ cast film, cm^{-1}) 3024, 2936, 1463, 1374, 1124, 1089, 1034, 742, 694; ^1H NMR ($CDCl_3$, 700 MHz) δ 4.18 – 4.14 (m, 4H, 2 × CH_2-OP), 2.77 (t, J = 6.2 Hz, 2H, CH_2-N), 2.29 (s, 9H, 3 × CH_3), 1.77 (quint, J = 7.5 Hz, 2 H, C$\underline{H}_2$-CH_2-O), 1.45 – 1.21 (m, 16H, 8 × CH_2), 0.88 (t, J = 7.0 Hz, 3 H, CH_3); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 123.2, 67.7, 62.3, 49.3, 37.3, 37.3, 37.3, 33.3, 32.4, 31.0, 30.0, 29.4, 26.0, 22.6, 14.0 ppm; ^{19}F NMR ($CDCl_3$, 658 MHz) δ 98.36 (pentet, J = 16.6 Hz); ^{31}P NMR ($CDCl_3$,

283 MHz) δ 0.47 (s); HRMS (ESI) Calcd for $C_{17}H_{36}F_2NNaO_4P$ $[M + Na]^+$ 410.2352, found 410.2354.

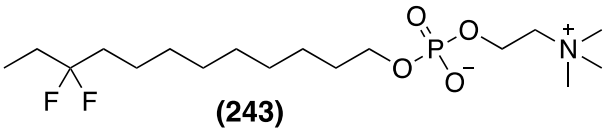
6.32.15. 6,6-Difluorododecyl (2-(trimethylammonio)ethyl) phosphate (242)



(242)

This new compound was prepared using the general procedure for the preparation of O-DPC starting from **267** and triemethyl amine gave **242** as a White powder (88% yield). IR ($CHCl_3$ cast film, cm^{-1}) 2955, 2823, 1456, 1353, 1164, 1077, 1032, 766, 685; ^1H NMR ($CDCl_3$, 700 MHz) δ 4.18 – 4.14 (m, 4H, 2 × CH_2-OP), 2.77 (t, J = 6.1 Hz, 2H, C<u>H</u>$_2$-N), 2.29 (s, 9H, 3 × C<u>H</u>$_3$), 1.77 (quint, J = 7.5 Hz, 2H, C<u>H</u>$_2$-CH_2-O), 1.45 – 1.21 (m, 16H, 8 × C<u>H</u>$_2$), 0.88 (t, J = 6.9 Hz, 3H, C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 123.2, 67.7, 62.3, 49.3, 37.3, 37.3, 37.3, 33.3, 32.4, 30.2, 30.0, 26.0, 25.9, 22.6, 14.0; ^{19}F NMR ($CDCl_3$, 658 MHz) δ 98.36 (pentet, J = 16.6 Hz); ^{31}P NMR ($CDCl_3$, 283 MHz) δ 0.47 (s); HRMS (ESI) Calcd for $C_{17}H_{36}F_2NNaO_4P$ $[M + Na]^+$ 410.2354, found 410.2357.

6.32.16. 10,10-Difluorododecyl (2-(trimethylammonio)ethyl) phosphate (243)



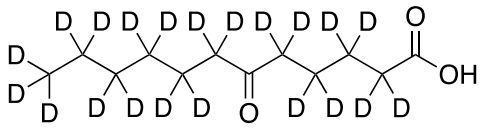
(243)

This new compound was prepared using the general procedure for the preparation of O-DPC starting from **275** and triemethyl amine gave **243** as a White powder (88% yield). IR ($CHCl_3$ cast film, cm^{-1}) 2927, 2852, 2797, 1464, 1377, 1302, 1118, 1057,

723; ^{1}H NMR ($CDCl_3$, 700 MHz) δ 4.23 – 4.15 (m, 4H, 2 × CH_2-OP), 2.77 (t, J = 6.1 Hz, 2H, C<u>H</u>$_2$-N), 2.29 (s, 9 H, 3 × C<u>H</u>$_3$), 1.77 (quint, J = 7.4 Hz, 2 H, C<u>H</u>$_2$-CH_2-O), 1.48 – 1.21 (m, 16H, 8 × C<u>H</u>$_2$), 0.98 (t, J = 6.9 Hz, 3H, C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 123.3, 67.7, 62.3, 49.3, 37.3, 37.3, 37.3, 33.3, 30.5, 30.2, 30.0, 29.4, 29.0, 26.0, 25.6, 12.6; ^{19}F NMR ($CDCl_3$, 658 MHz) δ 98.36 (pentet, J = 16.6 Hz); ^{31}P NMR ($CDCl_3$, 283 MHz) δ 0.49 (s); HRMS (ESI) Calcd for $C_{17}H_{36}F_2NNaO$ $[M + Na]^+$ 401.2352, found 401.2354.

6.33. Experimental Procedure for the Synthesis of Deuterated F_2-DPC-d_{36} Analogues

6.33.1. 6-Oxododecanoic-2,2,3,3,4,4,5,5,7,7,8,8,9,9,10,10,11,11,12,12,12-d_{21} acid (278)

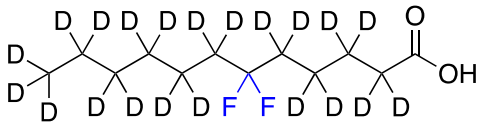


(278)

This new compound was prepared using the general procedure for the preparation of deuterated acids starting from **277** gave **278** as colorless oil (7.5 g, 89% yield, 98.5% D). ^{1}H NMR ($CDCl_3$, 400 MHz, dichloromethane as internal standard) δ_H 5.29 (s, 2H, CH_2, internal standard), 2.43 (br s, 0.018H, 2 × residual C<u>H</u>$_2$-CO), 2.30 (br s, 0.02H, residual C<u>H</u>$_2$-COOH), 1.56 (s, 0.032H, 3 × residual C<u>H</u>$_2$), 1.26 (s, 0.031H, 3 × residual C<u>H</u>$_2$), 0.86 (s, 0.022H, residual C<u>H</u>$_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ_D 2.42 (s, 4D, 2 × CD_2-CO), 2.29 (s, 2D, C<u>D</u>$_2$-COOH), 1.58 (s, 6D, 3 × CD_2), 1.24 (s, 6D, 3 × CD_2), 0.87 (s, 3D, CD_3); HRMS (ESI) $C_{12}D_{21}O_3$ $[M - H]^-$ Calcd for 234.2867, found 234.2864.

Isotopic distribution (ESI-MS, -ve mode): 59.7% d_{21}, 23.5% d_{20}, 10.4% d_{19}, 4.3% d_{18}, 2.1% d_{17}.

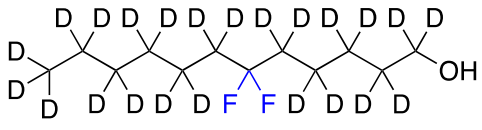
6.33.2. 6,6-Difluorododecanoic-2,2,3,3,4,4,5,5,7,7,8,8,9,9,10,10,11,11,12,12,12-d_{21} acid (279)



(279)

This new compound was prepared using the general procedure for the fluorination of ketone with DAST staring from **278** gave **279** as a colorless oil (6.3 g, 77% yield, 98.2% D). ^{1}H NMR ($CDCl_3$, 400 MHz, dichloromethane as internal standard) δ_H 5.29 (s, 2H, CH_2, internal standard), 2.30 (br s, 0.013H, residual C$\underline{H}_2$-COOH), 1.48 (br s, 0.08H, residual 5 × C$\underline{H}_2$), 1.26 (s, 0.032H, 3 × residual C$\underline{H}_2$), 0.86 (s, 0.022H, residual C$\underline{H}_3$); ^{2}H NMR ($CHCl_3$, 400 MHz,) δ_D 2.31 (s, 2D, CD_2-COOH), 1.47 (s, 10D, 5 × C$\underline{D}_2$), 1.26 (s, 6D, 3 × CD_2), 0.87 (s, 3D, CD_3); HRMS (ESI) $C_{12}D_{21}F_2O_2$ $[M - H]^-$ Calcd for 256.2905, found 256.2902. Isotopic distribution (ESI-MS, -ve mode): 59.6% d_{21}, 22.6% d_{20}, 11.5% d_{19}, 4.4% d_{18}, 1.9% d_{17}.

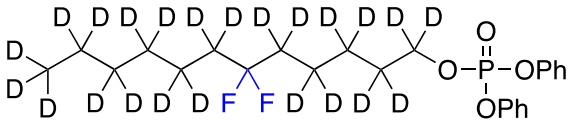
6.33.3. 6,6-Difluorododecan-1,1,2,2,3,3,4,4,5,5,7,7,8,8,9,9,10,10,11,11,12,12,12-d_{23}-1-ol (280)



(280)

This new compound was prepared using the general procedure for the reduction of esters with $NaBD_4$ starting from **279** gave **280** as a light-yellow oil (5.5 g, 88% yield, 98.2% D). ^1H NMR ($CDCl_3$, 400 MHz, dichloromethane as internal standard) δ_H 5.29 (s, 2H, CH_2, internal standard), 3.33 (br s, 0.014H, residual C$\underline{H}_2$-OH), 1.73 (br s, 0.013H, residual C$\underline{H}_2$-CH_2-OH), 1.48 (br s, 0.12H, residual 2 × C$\underline{H}_2$), 1.26 (s, 0.032H, 4 × residual C$\underline{H}_2$), 0.86 (s, 0.022H, residual C$\underline{H}_3$); ^2H NMR ($CHCl_3$, 400 MHz,) δ_D 3.34 (s, 2D, CD_2-OH), 1.73 (s, 2D, C$\underline{D}_2$-CD_2-OH), 1.47 (s, 8D, 4 × C$\underline{D}_2$), 1.26 (s, 8D, 4 × CD_2), 0.87 (s, 6D, CD_3); HRMS (ESI) m/z = 245.3229. Isotopic distribution (ESI-MS, -ve mode): 55.7% d_{23}, 19.5% d_{22}, 10.2% d_{21}, 8.6% d_{20}, 4.1% d_{19}, 1.9% d_{18}.

6.33.4. 6,6-Difluorododecyl-1,1,2,2,3,3,4,4,5,5,7,7,8,8,9,9,10,10,11,11,12,12,12-d_{23} diphenyl phosphate (281)

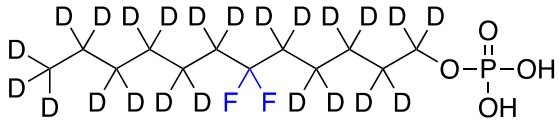


(281)

This new compound was prepared using the general procedure for the preparation of dibenzylphosphite compounds starting from **280** gave **281** as a light-yellow (4.9 g, 79% yield, 98.1% D). ^1H NMR ($CDCl_3$, 400 MHz) δ_H 7.49 – 7.36 (m, 10H, Ar-H), 4.17 (br s, 0.014H, residual C$\underline{H}_2$-OP), 1.73 (br s, 0.013H, residual C$\underline{H}_2$-CH_2-OH), 1.48 (br s, 0.15H, residual 4 × C$\underline{H}_2$), 1.26 (s, 0.032H, 4 × residual C$\underline{H}_2$), 0.87 (br s, 0.08H, residual C$\underline{H}_3$); ^2H NMR ($CHCl_3$, 400 MHz,) δ_D 4.17 (s, 2D, CD_2-0P), 1.73 (br s, 2.00D, C$\underline{D}_2$-CD_2-OP), 1.47 – 1.43 (br s, 8D, 2 × C$\underline{D}_2$), 1.36 – 1.25 (br s, 7.98D, 4 × C$\underline{D}_2$), 0.85 (s, 3.00D, C$\underline{D}_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 150.8 (2 × ArC-OP), 129.9 (4 × *m*-

ArC), 123.6 (2 × *p*-ArC), 123.03 ($\underline{C}F_2$), 120.1 (4 × *o*-ArC), 68.6 (residual $\underline{C}H_2$-OP), 32. 4 – 22.6 (all residual $C\underline{H}_2$), 14.0 (residual $\underline{C}H_3$); ^{31}P NMR ($CDCl_3$, 162 MHz) δ -3.46 (s); HRMS (ESI) $C_{24}H_{11}D_{23}F_2O_4P$ $[M + H]^+$ Calcd for 478.3502, found 478.3503. Isotopic distribution (ESI-MS, -ve mode): 55.7% d_{23}, 19.4% d_{22}, 10.3% d_{21}, 8.5% d_{20}, 4.3% d_{19}, 1.8% d_{18}.

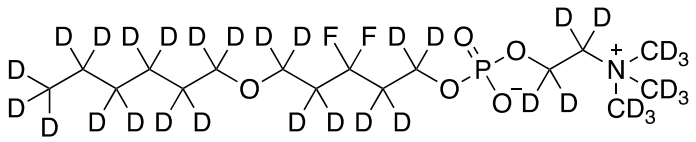
6.33.5. 6,6-Difluorododecyl-1,1,2,2,3,3,4,4,5,5,7,7,8,8,9,9,10,10,11,11,12,12,12-d_{23} dihydrogen phosphate (282)



(282)

This new compound was prepared using the general procedure for the deprotection of bezyl group starting from **281** gave **282** as a light-yellow oil 94.2 g, 97% yield, 98.1% D). (^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 4.17 (br s, 0.014H, residual $C\underline{H}_2$-OP), 1.73 (br s, 0.013H, residual $C\underline{H}_2$-CH_2-OH), 1.48 (br s, 0.15H, residual 4 × $C\underline{H}_2$), 1.26 (s, 0.032H, 4 × residual $C\underline{H}_2$), 0.87 (br s, 0.08H, residual $C\underline{H}_3$). ^{2}H NMR ($CHCl_3$, 400 MHz,) δ_D 4.17 (s, 2D, CD_2-0P), 1.73 (br s, 2.00D, $C\underline{D}_2$-CD_2-OP), 1.47 – 1.43 (br s, 8D, 2 × $C\underline{D}_2$), 1.36 – 1.25 (br s, 7.98D, 4 × $C\underline{D}_2$), 0.85 (s, 3.00D, $C\underline{D}_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 123.2 (CF_2), 68.6 (Residual $\underline{C}H_2$-OP), 32. 4 – 22.6 (all residual $C\underline{H}_2$), 14.0 (Residual $\underline{C}H_3$); ^{31}P NMR ($CDCl_3$, 162 MHz) δ -0.42 (s); HRMS (ESI) $C_{12}HD_{23}F_2O_4P$ $[M - H]^-$ Calcd for 324.2904, found 324.2912. . Isotopic distribution (ESI-MS, -ve mode): 55.9% d_{23}, 19.2% d_{22}, 10.1% d_{21}, 8.7% d_{20}, 4.1% d_{19}, 2.0% d_{18}.

6.33.6. Synthesis of 6-F2-DPC-d_{36} (183)



(183)

This new compound was prepared using the general procedure for the coupling of choline to lipid phosphate starting from lipid phosphate **282** and choline (**169**) gave (309) as a white solid (2.3 g, 59% yield, 97.7% D). ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 4.17 (br s, 0.018H, residual 2 × C$\underline{H}_2$-OP), 2.76 (br s, residual CH_2-N), 2.29 (br s, 0.15H, residual 3 × CH_2), 1.78 (br s, 0.013H, residual C$\underline{H}_2$-CH_2-OH), 1.47 (br s, 0.15H, residual 4 × C$\underline{H}_2$), 1.28 (s, 0.032H, 4 × residual C$\underline{H}_2$), 0.87 (br s, 0.08H, residual C$\underline{H}_3$). ^{2}H NMR ($CHCl_3$, 400 MHz,) δ_D 4.17 (s, 2D, CD_2-0P), 2.76 (s, CD_2-N), 2.28 (s, 6D, 3 × CD_2), 1.75 (br s, 2.00D, C$\underline{D}_2$-CD_2-OP), 1.49 – 1.44 (br s, 8D, 2 × C$\underline{D}_2$), 1.38 – 1.26 (br s, 7.98D, 4 × C$\underline{D}_2$), 0.85 (s, 3.00D, C$\underline{D}_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ_C 123.2 (CF_2), 68.6 (residual $\underline{C}H_2$-OP), 62.4 (residual CH_2-OP), 49.4 (residual CH_2-N), 45.6 (residual 3 × CH_3), 32. 4 – 22.6 (all residual C$\underline{H}_2$), 14.0 (residual $\underline{C}H_3$); ^{31}P NMR ($CDCl_3$, 162 MHz) δ 0.46 (s); HRMS (ESI) $C_{17}HD_{36}F_2O_4P$ $[M + H]^+$ Calcd for 424.4623, found 424.4626. Isotopic distribution (ESI-MS, +ve mode): 62.3% d_{36}, 16.4% d_{35}, 8.1% d_{34}, 5.5% d_{33}, 5.3% d_{32}, 2.4% d_{31}.

References

1. Lynch, D. B.; Jeffery, I. B.; Cusack, S.; O'Connor, E. M.; O'Toole, P. W., Diet-microbiota-health interactions in older subjects: implications for healthy aging. *Interdiscip Top Gerontol* **2015,** *40*, 141-54.

2. Bushman, F. D.; Lewis, J. D.; Wu, G. D., Diet, gut enterotypes and health: is there a link? *Nestle Nutr Inst Workshop Ser* **2013,** *77*, 65-73.

3. Tlaskalová-Hogenová, H.; Stěpánková, R.; Kozáková, H.; Hudcovic, T.; Vannucci, L.; Tučková, L.; Rossmann, P.; Hrnčíř, T.; Kverka, M.; Zákostelská, Z.; Klimešová, K.; Přibylová, J.; Bártová, J.; Sanchez, D.; Fundová, P.; Borovská, D.; Srůtková, D.; Zídek, Z.; Schwarzer, M.; Drastich, P.; Funda, D. P., The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: contribution of germ-free and gnotobiotic animal models of human diseases. *Cell Mol Immunol* **2011,** *8* (2), 110-20.

4. Wei, S.; Kenneth, L. R.; Roy, C., III, Developing live vaccines against plague. *The Journal of Infection in Developing Countries* **2011,** *5* (09), 2135-2139.

5. Morens, D. M.; Taubenberger, J. K.; Fauci, A. S., Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. *Journal of Infecteous Disease* **2008,** *198* (7), 962-70.

6. Bérdy, J., Thoughts and facts about antibiotics: Where we are now and where we are heading. *The Journal of Antibiotics* **2012,** *65* (8), 385-395.

7. Fleming, A., On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to their Use in the Isolation of B. influenzæ. *Br J Exp Pathol* **1929,** *10* (3), 226-236.

8. Ventola, C. L., The antibiotic resistance crisis: part 1: causes and threats. *P t* **2015,** *40* (4), 277-83.

9. Price, R., O'Neill report on antimicrobial resistance: funding for antimicrobial specialists should be improved. *European Journal of Hospital Pharmacy* **2016,** *23* (4), 245-247.

10. Cassini, A.; Högberg, L. D.; Plachouras, D.; Quattrocchi, A.; Hoxha, A.; Simonsen, G. S.; Colomb-Cotinat, M.; Kretzschmar, M. E.; Devleesschauwer, B.; Cecchini, M.; Ouakrim, D. A.; Oliveira, T. C.; Struelens, M. J.; Suetens, C.; Monnet, D. L., Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *Lancet Infectious Disease* **2019,** *19* (1), 56-66.

11. Gandra, S.; Barter, D. M.; Laxminarayan, R., Economic burden of antibiotic resistance: how much do we really know? *Clinical Microbiology Infectious* **2014,** *20* (10), 973-80.

12. Breukink, E.; de Kruijff, B., Lipid II as a target for antibiotics. *Nature Review Drug Discovery* **2006,** *5* (4), 321-32.

13. Scheffers, D. J.; Tol, M. B., LipidII: Just Another Brick in the Wall? *PLoS Pathog* **2015,** *11* (12), e1005213.

14. Müller, A.; Klöckner, A.; Schneider, T., Targeting a cell wall biosynthesis hot spot. *Natural Product Reports* **2017,** *34* (7), 909-932.

15. Kahne, D.; Leimkuhler, C.; Lu, W.; Walsh, C., Glycopeptide and lipoglycopeptide antibiotics. *Chemical Review* **2005,** *105* (2), 425-48.

16. Hsu, S.-T. D.; Breukink, E.; Tischenko, E.; Lutters, M. A. G.; de Kruijff, B.; Kaptein, R.; Bonvin, A. M. J. J.; van Nuland, N. A. J., The nisin–lipid II complex reveals a pyrophosphate cage that provides a blueprint for novel antibiotics. *Nature Structural & Molecular Biology* **2004,** *11* (10), 963-967.

17. Vollmer, W.; Blanot, D.; De Pedro, M. A., Peptidoglycan structure and architecture. *FEMS Microbiology Reviews* **2008,** *32* (2), 149-167.

18. Kong, K.-F.; Schneper, L.; Mathee, K., Beta-lactam antibiotics: from antibiosis to resistance and bacteriology. *APMIS* **2010,** *118* (1), 1-36.

19. Sambhara, S.; Lehrer, R. I., The innate immune system: a repository for future drugs? *Expert Rev Anti Infect Ther* **2007,** *5* (1), 1-5.

20. Brown, K. L.; Cosseau, C.; Gardy, J. L.; Hancock, R. E. W., Complexities of targeting innate immunity to treat infection. *Trends Immunol* **2007,** *28* (6), 260-266.

21. Boman, H. G., Peptide antibiotics and their role in innate immunity. *Annu Rev Immunol* **1995,** *13*, 61-92.

22. Zasloff, M., Antimicrobial peptides of multicellular organisms. *Nature* **2002,** *415* (6870), 389-395.

23. Wang, Z.; Wang, G., APD: the Antimicrobial Peptide Database. *Nucleic Acids Research* **2004,** *32* (suppl_1), D590-D592.

24. Huan, Y.; Kong, Q.; Mou, H.; Yi, H., Antimicrobial Peptides: Classification, Design, Application and Research Progress in Multiple Fields. *Frontiers in Microbiology* **2020,** *11* (2559).

25. Wang, G., Determination of solution structure and lipid micelle location of an engineered membrane peptide by using one NMR experiment and one sample. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2007,** *1768* (12), 3271-3281.

26. Wang, G., Structural Biology of Antimicrobial Peptides by NMR Spectroscopy. *Current Organic Chemistry* **2006,** *10*, 569-581.

27. Gesell, J.; Zasloff, M.; Opella, S. J., Two-dimensional 1H NMR experiments show that the 23-residue magainin antibiotic peptide is an α-helix in dodecylphosphocholine micelles, sodium dodecylsulfate micelles, and trifluoroethanol/water solution. *Journal of Biomolecular NMR* **1997,** *9* (2), 127-135.

28. Rozek, A.; Friedrich, C. L.; Hancock, R. E., Structure of the bovine antimicrobial peptide indolicidin bound to dodecylphosphocholine and sodium dodecyl sulfate micelles. *Biochemistry* **2000,** *39* (51), 15765-74.

29. Sanders, C. R.; Prosser, R. S., Bicelles: a model membrane system for all seasons? *Structure* **1998,** *6* (10), 1227-34.

30. Sanders, C. R.; Oxenoid, K., Customizing model membranes and samples for NMR spectroscopic studies of complex membrane proteins11This review is dedicated to the fond memory of Professor Gitte Vold of the University of California, San Diego, CA, USA. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2000,** *1508* (1), 129-145.

31. Marcotte, I.; Auger, M., Bicelles as model membranes for solid- and solution-state NMR studies of membrane peptides and proteins. *Concepts in Magnetic Resonance Part A* **2005,** *24A* (1), 17-37.

32. Medeiros-Silva, J.; Jekhmane, S.; Paioni, A. L.; Gawarecka, K.; Baldus, M.; Swiezewska, E.; Breukink, E.; Weingarth, M., High-resolution NMR studies of antibiotics in cellular membranes. *Nature Communications* **2018,** *9* (1), 3963.

33. Vitali Tugarinov; Hwang, P. M.; Kay, L. E., Nuclear Magnetic Resonance Spectroscopy of High-Molecular-Weight Proteins. *Annual Review of Biochemistry* **2004,** *73* (1), 107-146.

34. Tugarinov, V.; Kay, L. E., Ile, Leu, and Val Methyl Assignments of the 723-Residue Malate Synthase G Using a New Labeling Strategy and Novel NMR Methods. *Journal of the American Chemical Society* **2003,** *125* (45), 13868-13878.

35. Religa, T. L.; Sprangers, R.; Kay, L. E., Dynamic Regulation of Archaeal Proteasome Gate Opening As Studied by TROSY NMR. *Science* **2010,** *328* (5974), 98-102.

36. Stanczak, P.; Horst, R.; Serrano, P.; Wüthrich, K., NMR characterization of membrane protein-detergent micelle solutions by use of microcoil equipment. *Journal of the American Chemical Society* **2009,** *131* (51), 18450-18456.

37. Kondo, Y.; Miyazawa, H.; Sakai, H.; Abe, M.; Yoshino, N., First Anionic Micelle with Unusually Long Lifetime: Self-Assembly of Fluorocarbon−Hydrocarbon Hybrid Surfactant. *Journal of the American Chemical Society* **2002,** *124* (23), 6516-6517.

38. Fernández, C.; Wüthrich, K., NMR solution structure determination of membrane proteins reconstituted in detergent micelles. *FEBS Letters* **2003,** *555* (1), 144-150.

39. Liang, B.; Tamm, L. K., NMR as a tool to investigate the structure, dynamics and function of membrane proteins. *Nature structural & molecular biology* **2016,** *23* (6), 468.

40. Lauterwein, J.; Bösch, C.; Brown, L. R.; Wüthrich, K., Physicochemical studies of the protein-lipid interactions in melittin-containing micelles. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1979,** *556* (2), 244-264.

41. den Berg, B. v.; Tessari, M.; Boelens, R.; Dijkman, R.; de Hass, G. H.; Kaptein, R.; Verheij, H. M., NMR structures of phospholipase A2 reveal conformational changes during interfacial activation. *Nature Structural Biology* **1995,** *2* (5), 402-406.

42. Brown, L. R.; Bösch, C.; Wüthrich, K., Location and orientation relative to the micelle surface for glucagon in mixed micelles with dodecylphosphocholine: EPR and NMR studies. *Biochim Biophys Acta* **1981,** *642* (2), 296-312.

43. Wider, G.; Lee, K. H.; Wüthrich, K., Sequential resonance assignments in protein 1H nuclear magnetic resonance spectra. Glucagon bound to perdeuterated dodecylphosphocholine micelles. *J Mol Biol* **1982,** *155* (3), 367-88.

44. Inagaki, F.; Shimada, I.; Kawaguchi, K.; Hirano, M.; Terasawa, I.; Ikura, T.; Go, N., Structure of melittin bound to perdeuterated dodecylphosphocholine micelles as studied by two-dimensional NMR and distance geometry calculations. *Biochemistry* **1989,** *28* (14), 5985-5991.

45. Malikayil, J. A.; Edwards, J. V.; McLean, L. R., Micelle-bound conformations of a bombesin/gastrin releasing peptide receptor agonist and an antagonist by two-

dimensional NMR and restrained molecular dynamics. *Biochemistry* **1992,** *31* (31), 7043-9.

46. Fregeau Gallagher, N. L.; Sailer, M.; Niemczura, W. P.; Nakashima, T. T.; Stiles, M. E.; Vederas, J. C., Three-dimensional structure of leucocin A in trifluoroethanol and dodecylphosphocholine micelles: spatial location of residues critical for biological activity in type IIa bacteriocins from lactic acid bacteria. *Biochemistry* **1997,** *36* (49), 15062-72.

47. Macquaire, F.; Baleux, F.; Giaccobi, E.; Huynh-Dinh, T.; Neumann, J. M.; Sanson, A., Peptide secondary structure induced by a micellar phospholipidic interface: proton NMR conformational study of a lipopeptide. *Biochemistry* **1992,** *31* (9), 2576-2582.

48. Tsan, P.; Volpon, L.; Besson, F.; Lancelin, J. M., Structure and dynamics of surfactin studied by NMR in micellar media. *J Am Chem Soc* **2007,** *129* (7), 1968-77.

49. Cochrane, S. A.; Findlay, B.; Bakhtiary, A.; Acedo, J. Z.; Rodriguez-Lopez, E. M.; Mercier, P.; Vederas, J. C., Antimicrobial lipopeptide tridecaptin A_1 selectively binds to Gram-negative lipid II. *Proceedings of the National Academy of Sciences* **2016,** *113* (41), 11561-11566.

50. Zhang, Q.; Horst, R.; Geralt, M.; Ma, X.; Hong, W.-X.; Finn, M.; Stevens, R. C., Microscale NMR screening of new detergents for membrane protein structural biology. *Journal of the American Chemical Society* **2008,** *130* (23), 7357-7363.

51. Gagnon, M. C.; Strandberg, E.; Ulrich, A. S.; Paquin, J. F.; Auger, M., New insights into the influence of monofluorination on dimyristoylphosphatidylcholine

membrane properties: A solid-state NMR study. *Biochim Biophys Acta Biomembr* **2018,** *1860* (3), 654-663.

52. Theuretzbacher, U.; Bush, K.; Harbarth, S.; Paul, M.; Rex, J. H.; Tacconelli, E.; Thwaites, G. E., Critical analysis of antibacterial agents in clinical development. *Nature Reviews Microbiology* **2020,** *18* (5), 286-298.

53. O'Shea, R.; Moser, H. E., Physicochemical properties of antibacterial compounds: Implications for drug discovery. *Journal of Medicinal Chemistry* **2008,** *51* (10), 2871-2878.

54. Silver, L. L., Are natural products still the best source for antibacterial discovery? The bacterial entry factor. *Expert Opin Drug Discov* **2008,** *3* (5), 487-500.

55. Zitterbart, R.; Berger, N.; Reimann, O.; Noble, G. T.; Lüdtke, S.; Sarma, D.; Seitz, O., Traceless parallel peptide purification by a first-in-class reductively cleavable linker system featuring a safety-release. *Chemical Science* **2021,** *12* (7), 2389-2396.

56. Liu, Y.; Ding, S.; Shen, J.; Zhu, K., Nonribosomal antibacterial peptides that target multidrug-resistant bacteria. *Natural Product Reports* **2019,** *36* (4), 573-592.

57. King, A. M.; Reid-Yu, S. A.; Wang, W.; King, D. T.; De Pascale, G.; Strynadka, N. C.; Walsh, T. R.; Coombes, B. K.; Wright, G. D., Aspergillomarasmine A overcomes metallo-β-lactamase antibiotic resistance. *Nature* **2014,** *510* (7506), 503-506.

58. Cochrane, S. A.; Vederas, J. C., Lipopeptides from Bacillus and Paenibacillus spp.: A Gold Mine of Antibiotic Candidates. *Med Res Rev* **2016,** *36* (1), 4-31.

59. Banko, G.; Demain, A. L.; Wolfe, S., δ -(L-α-Aminoadipyl)-L-cysteinyl-D-valine synthetase (ACV synthetase): a multifunctional enzyme with broad substrate

specificity for the synthesis of penicillin and cephalosporin precursors. *Journal of the American Chemical Society* **1987,** *109* (9), 2858-2860.

60. Awan, A. R.; Blount, B. A.; Bell, D. J.; Shaw, W. M.; Ho, J. C. H.; McKiernan, R. M.; Ellis, T., Biosynthesis of the antibiotic nonribosomal peptide penicillin in baker's yeast. *Nature Communications* **2017,** *8* (1), 15202.

61. Corona, A.; Cattaneo, D., Dosing Colistin Properly: Let's Save "Our Last Resort Old Drug!". *Clinical Infectious Diseases* **2017,** *65* (5), 870-870.

62. Kato, T.; Hinoo, H.; Shoji, J., The structure of tridecaptin A (studies on antibiotics from the genus Bacillus. XXIV). *J Antibiot (Tokyo)* **1978,** *31* (7), 652-61.

63. Kato, T.; Sakazaki, R.; Hinoo, H.; Shoji, J., The structures of tridecaptins B and C (studies on antibiotics from the genus Bacillus. XXV). *J Antibiot (Tokyo)* **1979,** *32* (4), 305-12.

64. Shoji, J.; Hinoo, H.; Sakazaki, R.; Kato, T.; Wakisaka, Y.; Mayama, M.; Matsuura, S.; Miwa, H., Isolation of tridecaptins A, B and C (studies on antibiotics from the genus Bacillus. XXIII). *J Antibiot (Tokyo)* **1978,** *31* (7), 646-51.

65. Lohans, C. T.; van Belkum, M. J.; Cochrane, S. A.; Huang, Z.; Sit, C. S.; McMullen, L. M.; Vederas, J. C., Biochemical, structural, and genetic characterization of tridecaptin A_1, an antagonist of Campylobacter jejuni. *Chembiochem* **2014,** *15* (2), 243-9.

66. Cochrane, S. A.; Lohans, C. T.; Brandelli, J. R.; Mulvey, G.; Armstrong, G. D.; Vederas, J. C., Synthesis and Structure–Activity Relationship Studies of N-Terminal Analogues of the Antimicrobial Peptide Tridecaptin A1. *Journal of Medicinal Chemistry* **2014,** *57* (3), 1127-1131.

67. Velkov, T.; Thompson, P. E.; Nation, R. L.; Li, J., Structure−Activity Relationships of Polymyxin Antibiotics. *Journal of Medicinal Chemistry* **2010,** *53* (5), 1898-1916.

68. Saha, S. L.; Van Nieuwenhze, M. S.; Hornback, W. J.; Aikins, J. A.; Blaszczak, L. C., Synthesis of an Orthogonally Protected Precursor to the Glycan Repeating Unit of the Bacterial Cell Wall. *Organic Letters* **2001,** *3* (22), 3575-3577.

69. VanNieuwenhze, M. S.; Mauldin, S. C.; Zia-Ebrahimi, M.; Winger, B. E.; Hornback, W. J.; Saha, S. L.; Aikins, J. A.; Blaszczak, L. C., The First Total Synthesis of Lipid II: The Final Monomeric Intermediate in Bacterial Cell Wall Biosynthesis. *Journal of the American Chemical Society* **2002,** *124* (14), 3656-3660.

70. Lebar, M. D.; Lupoli, T. J.; Tsukamoto, H.; May, J. M.; Walker, S.; Kahne, D., Forming Cross-Linked Peptidoglycan from Synthetic Gram-Negative Lipid II. *Journal of the American Chemical Society* **2013,** *135* (12), 4632-4635.

71. Cochrane, R. V. K.; Alexander, F. M.; Boland, C.; Fetics, S. K.; Caffrey, M.; Cochrane, S. A., From plant to probe: semi-synthesis of labelled undecaprenol analogues allows rapid access to probes for antibiotic targets. *Chemical Communications* **2020,** *56* (61), 8603-8606.

72. Antwi, I.; Chiorean, S.; van Belkum, M. J.; Vederas, J. C., Unveiling the active isomer of cycloalanopine, a cyclic opine from Lactobacillus rhamnosus LS8, through synthesis and analog production. *Rsc Medicinal Chemistry* **2020,** *11* (4), 528-531.

73. Moore, L. W.; Chilton, W. S.; Canfield, M. L., Diversity of opines and opine-catabolizing bacteria isolated from naturally occurring crown gall tumors. *Applied and Environmental Microbiology* **1997,** *63* (1), 201-207.

74. Montoya, A. L.; Chilton, M. D.; Gordon, M. P.; Sciaky, D.; Nester, E. W., Octopine and nopaline metabolism in Agrobacterium tumefaciens and crown gall tumor cells: role of plasmid genes. *J Bacteriol* **1977,** *129* (1), 101-7.

75. Marty, L.; Vigouroux, A.; Aumont-Nicaise, M.; Pelissier, F.; Meyer, T.; Lavire, C.; Dessaux, Y.; Morera, S., Structural basis for two efficient modes of agropinic acid opine import into the bacterial pathogen Agrobacterium tumefaciens. *Biochemical Journal* **2019,** *476*, 165-178.

76. Hack, E.; Kemp, J. D., Comparison of octopine, histopine, lysopine, and octopinic acid synthesizing activities in sunflower crown gall tissues. *Biochem Biophys Res Commun* **1977,** *78* (2), 785-91.

77. Firmin, J. L.; Stewart, I. M.; Wilson, K. E., N2-(1-carboxyethyl)methionine. A 'pseudo-opine' in octopine-type crown-gall tumours. *Biochem J* **1985,** *232* (2), 431-4.

78. Fushiya, S.; Matsuda, M.; Yamada, S.; Nozoe, S., New opine type amino acids from a poisonous mushroom, Clitocybe acromelalga. *Tetrahedron* **1996,** *52* (3), 877-886.

79. Flores-Mireles, A. L.; Eberhard, A.; Winans, S. C., Agrobacterium tumefaciens can obtain sulphur from an opine that is synthesized by octopine synthase using S-methylmethionine as a substrate. *Mol Microbiol* **2012,** *84* (5), 845-56.

80. Sutton, D. W.; Kemp, J. D.; Hack, E., Characterization of the enzyme responsible for nopaline and ornaline synthesis in sunflower crown gall tissues. *Plant Physiol* **1978,** *62* (3), 363-7.

81. Chang, C. C.; Jayaswal, R. K.; Chen, C. M.; Gelvin, S. B., Altered imino diacid synthesis and transcription in crown gall tumors with transposon Tn5 insertions in the

3' end of the octopine synthase gene. *Journal of bacteriology* **1989,** *171* (11), 5922-5927.

82. Smits, S. H. J.; Mueller, A.; Schmitt, L.; Grieshaber, M. K., A Structural Basis for Substrate Selectivity and Stereoselectivity in Octopine Dehydrogenase from Pecten maximus. *Journal of Molecular Biology* **2008,** *381* (1), 200-211.

83. Ghssein, G.; Brutesco, C.; Ouerdane, L.; Fojcik, C.; Izaute, A.; Wang, S.; Hajjar, C.; Lobinski, R.; Lemaire, D.; Richaud, P.; Voulhoux, R.; Espaillat, A.; Cava, F.; Pignol, D.; Borezée-Durant, E.; Arnoux, P., Biosynthesis of a broad-spectrum nicotianamine-like metallophore in Staphylococcus aureus. *Science* **2016,** *352* (6289), 1105.

84. Zhang, L.; Wang, L.; Yi, L.; Wang, X.; Zhang, Y.; Liu, J.; Guo, X.; Liu, L.; Shao, C.; Lü, X., A novel antimicrobial substance produced by Lactobacillus rhamnous LS8. *Food Control* **2017,** *73*, 754-760.

85. Pörtner, H. O.; Kreutzer, U.; Siegmund, B.; Heisler, N.; Grieshaber, M. K., Metabolic adaptation of the intertidal worm Sipunculus nudus to functional and environmental hypoxia. *Marine Biology* **1984,** *79* (3), 237-247.

86. Twigg, F. F.; Cai, W.; Huang, W.; Liu, J.; Sato, M.; Perez, T. J.; Geng, J.; Dror, M. J.; Montanez, I.; Tong, T. L.; Lee, H.; Zhang, W., Identifying the Biosynthetic Gene Cluster for Triacsins with an N-Hydroxytriazene Moiety. *Chembiochem* **2019,** *20* (9), 1145-1149.

87. Wang, K.-K. A.; Ng, T. L.; Wang, P.; Huang, Z.; Balskus, E. P.; van der Donk, W. A., Glutamic acid is a carrier for hydrazine during the biosyntheses of fosfazinomycin and kinamycin. *Nature Communications* **2018,** *9* (1), 3687.

88. Morgan, K. D.; Andersen, R. J.; Ryan, K. S., Piperazic acid-containing natural products: structures and biosynthesis. *Natural Product Reports* **2019,** *36* (12), 1628-1653.

89. Matsuda, K.; Tomita, T.; Shin-ya, K.; Wakimoto, T.; Kuzuyama, T.; Nishiyama, M., Discovery of Unprecedented Hydrazine-Forming Machinery in Bacteria. *Journal of the American Chemical Society* **2018,** *140* (29), 9083-9086.

90. Le Goff, G.; Ouazzani, J., Natural hydrazine-containing compounds: Biosynthesis, isolation, biological activities and synthesis. *Bioorg Med Chem* **2014,** *22* (23), 6529-6544.

91. Li, J.; Sha, Y., A Convenient Synthesis of Amino Acid Methyl Esters. *Molecules* **2008,** *13* (5), 1111-1119.

92. Niwayama, S., Highly Efficient Selective Monohydrolysis of Symmetric Diesters. *The Journal of Organic Chemistry* **2000,** *65* (18), 5834-5836.

93. Harris, J. M.; Bolessa, E. A.; Vederas, J. C., Synthesis of macrocyclic diazanedicarboxylate and diazenedicarboxylate esters containing a steroid skeleton: an unusual oxidation of bromide to bromine by a strained diazenedicarboxylate ester. X-Ray molecular structure of 3α-(3-hydroxypropyl)-24-nor-5β-cholan-7α-ol diazane-1,2-dicarboxylate cyclic diester. *Journal of the Chemical Society, Perkin Transactions 1* **1995,** (15), 1951-1959.

94. Kitamura, T.; Fujiwara, Y., Recent progress in the use of hypervalent iodine reagents in organic synthesis. a review. *Organic preparations and procedures international* **1997,** *29* (4), 409-458.

95. Dohi, T.; Kita, Y., Hypervalent iodine reagents as a new entrance to organocatalysts. *Chemical Communications* **2009,** (16), 2073-2085.

96. Zheng, Z.; Zhang-Negrerie, D.; Du, Y.; Zhao, K., The applications of hypervalent iodine(III) reagents in the constructions of heterocyclic compounds through oxidative coupling reactions. *Science China Chemistry* **2014,** *57* (2), 189-214.

97. Zhdankin, V. V.; Stang, P. J., Recent Developments in the Chemistry of Polyvalent Iodine Compounds. *Chemical Reviews* **2002,** *102* (7), 2523-2584.

98. Prakash, O.; Sharma, V.; Sadana, A., Hypervalent Iodine Oxidation of Acid Hydrazides: A New Synthesis of N,N′-Diacylhydrazines. *Synthetic Communications* **1997,** *27* (19), 3371-3377.

99. Da Costa, C. F.; Pinheiro, A. C.; De Almeida, M. V.; Lourenço, M. C.; De Souza, M. V., Synthesis and antitubercular activity of novel amino acid derivatives. *Chem Biol Drug Des* **2012,** *79* (2), 216-22.

100. Ageitos, J. M.; Sánchez-Pérez, A.; Calo-Mata, P.; Villa, T. G., Antimicrobial peptides (AMPs): Ancient compounds that represent novel weapons in the fight against bacteria. *Biochem Pharmacol* **2017,** *133*, 117-138.

101. Wiesner, J.; Vilcinskas, A., Antimicrobial peptides: the ancient arm of the human immune system. *Virulence* **2010,** *1* (5), 440-64.

102. Langham, A. A.; Waring, A. J.; Kaznessis, Y. N., Comparison of interactions between beta-hairpin decapeptides and SDS/DPC micelles from experimental and simulation data. *BMC Biochemistry* **2007,** *8* (1), 11.

103. Tieleman, D. P.; van der Spoel, D.; Berendsen, H. J. C., Molecular Dynamics Simulations of Dodecylphosphocholine Micelles at Three Different Aggregate Sizes:

Micellar Structure and Chain Relaxation. *The Journal of Physical Chemistry B* **2000,** *104* (27), 6380-6388.

104. Beswick, V.; Guerois, R.; Cordier-Ochsenbein, F.; Coïc, Y.-M.; Huynh-Dinh, T.; Tostain, J.; Noël, J.-P.; Sanson, A.; Neumann, J. M., Dodecylphosphocholine micelles as a membrane-like environment: new results from NMR relaxation and paramagnetic relaxation enhancement analysis. *European Biophysics Journal* **1998,** *28* (1), 48-58.

105. Sinha, S.; Ng, W. J.; Bhattacharjya, S., NMR structure and localization of the host defense antimicrobial peptide thanatin in zwitterionic dodecylphosphocholine micelle: Implications in antimicrobial activity. *Biochim Biophys Acta Biomembr* **2020,** *1862* (11), 183432.

106. Pervushin, K. V.; Orekhov, V.; Popov, A. I.; Musina, L.; Arseniev, A. S., Three-dimensional structure of (1-71)bacterioopsin solubilized in methanol/chloroform and SDS micelles determined by 15N-1H heteronuclear NMR spectroscopy. *European Journal of Biochemistry* **1994,** *219* (1-2), 571-83.

107. Simons, A.; Alhanout, K.; Duval, R. E., Bacteriocins, Antimicrobial Peptides from Bacterial Origin: Overview of Their Biology and Their Impact against Multidrug-Resistant Bacteria. *Microorganisms* **2020,** *8* (5), 639.

108. Brogden, K. A., Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nature Reviews Microbiology* **2005,** *3* (3), 238-250.

109. Shai, Y., Mode of action of membrane active antimicrobial peptides. *Peptide Science* **2002,** *66* (4), 236-248.

110. Lee, T.-H.; Hofferek, V.; Separovic, F.; Reid, G. E.; Aguilar, M.-I., The role of bacterial lipid diversity and membrane properties in modulating antimicrobial peptide activity and drug resistance. *Current Opinion in Chemical Biology* **2019,** *52*, 85-92.

111. Sani, M.-A.; Separovic, F., How Membrane-Active Peptides Get into Lipid Membranes. *Accounts of Chemical Research* **2016,** *49* (6), 1130-1138.

112. Strömstedt, A. A.; Ringstad, L.; Schmidtchen, A.; Malmsten, M., Interaction between amphiphilic peptides and phospholipid membranes. *Current Opinion in Colloid & Interface Science* **2010,** *15* (6), 467-478.

113. Schneider, T.; Kruse, T.; Wimmer, R.; Wiedemann, I.; Sass, V.; Pag, U.; Jansen, A.; Nielsen, A. K.; Mygind, P. H.; Raventós, D. S.; Neve, S.; Ravn, B.; Bonvin, A. M. J. J.; De Maria, L.; Andersen, A. S.; Gammelgaard, L. K.; Sahl, H.-G.; Kristensen, H.-H., Plectasin, a Fungal Defensin, Targets the Bacterial Cell Wall Precursor Lipid II. *Science* **2010,** *328* (5982), 1168-1172.

114. Ling, L. L.; Schneider, T.; Peoples, A. J.; Spoering, A. L.; Engels, I.; Conlon, B. P.; Mueller, A.; Schäberle, T. F.; Hughes, D. E.; Epstein, S.; Jones, M.; Lazarides, L.; Steadman, V. A.; Cohen, D. R.; Felix, C. R.; Fetterman, K. A.; Millett, W. P.; Nitti, A. G.; Zullo, A. M.; Chen, C.; Lewis, K., A new antibiotic kills pathogens without detectable resistance. *Nature* **2015,** *517* (7535), 455-459.

115. Fregeau Gallagher, N. L.; Sailer, M.; Niemczura, W. P.; Nakashima, T. T.; Stiles, M. E.; Vederas, J. C., Three-Dimensional Structure of Leucocin A in Trifluoroethanol and Dodecylphosphocholine Micelles: Spatial Location of Residues

Critical for Biological Activity in Type IIa Bacteriocins from Lactic Acid Bacteria. *Biochemistry* **1997,** *36* (49), 15062-15072.

116. Liu, F.; Vijayakrishnan, B.; Faridmoayer, A.; Taylor, T. A.; Parsons, T. B.; Bernardes, G. J. L.; Kowarik, M.; Davis, B. G., Rationally Designed Short Polyisoprenol-Linked PglB Substrates for Engineered Polypeptide and Protein N-Glycosylation. *Journal of the American Chemical Society* **2014,** *136* (2), 566-569.

117. Darwish, T. A.; Luks, E.; Moraes, G.; Yepuri, N. R.; Holden, P. J.; James, M., Synthesis of deuterated [D32]oleic acid and its phospholipid derivative [D64]dioleoyl-sn-glycero-3-phosphocholine. *J Labelled Comp Radiopharm* **2013,** *56* (9-10), 520-9.

118. Yamada, T.; Park, K.; Yasukawa, N.; Morita, K.; Monguchi, Y.; Sawama, Y.; Sajiki, H., Mild and Direct Multiple Deuterium-Labeling of Saturated Fatty Acids. *Advanced Synthesis & Catalysis* **2016,** *358* (20), 3277-3282.

119. Bai, W.; Lee, K.-H.; Tse, S. K. S.; Chan, K. W.; Lin, Z.; Jia, G., Ruthenium-Catalyzed Deuteration of Alcohols with Deuterium Oxide. *Organometallics* **2015,** *34* (15), 3686-3698.

120. Chatterjee, B.; Gunanathan, C., Ruthenium Catalyzed Selective α- and α,β-Deuteration of Alcohols Using D2O. *Organic Letters* **2015,** *17* (19), 4794-4797.

121. Atkinson, J. G.; Luke, M. O.; Stuart, R. S., A direct preparation of fully deuterated high-molecular-weight hydrocarbons. *Chemical Communications (London)* **1967,** (10), 474-475.

122. Hsiao, C. Y. Y.; Ottaway, C. A.; Wetlaufer, D. B., Preparation of fully deuterated fatty acids by simple method. *Lipids* **1974,** *9* (11), 913-915.

123. Abad, J.-L.; Villorbina, G.; Fabriàs, G.; Camps, F., Synthesis and Use of Stereospecifically Deuterated Analogues of Palmitic Acid To Investigate the Stereochemical Course of the Δ11 Desaturase of the Processionary Moth. *The Journal of Organic Chemistry* **2004,** *69* (21), 7108-7113.

124. Abad, J.-L.; Camps, F.; Fabriàs, G., Substrate-Dependent Stereochemical Course of the (Z)-13-Desaturation Catalyzed by the Processionary Moth Multifunctional Desaturase. *Journal of the American Chemical Society* **2007,** *129* (48), 15007-15012.

125. Abad, J.-L.; Fabriàs, G.; Camps, F., Synthesis of deuterated fatty acids to investigate the biosynthetic pathway of disparlure, the sex pheromone of the gypsy moth, Lymantria dispar. *Lipids* **2004,** *39* (4), 397-401.

126. Horner, J. H.; Newcomb, M., (ω-2,ω-2,ω-3,ω-3)-Tetradeuterio-fatty acids for mechanistic studies of enzyme-catalyzed hydroxylation reactions. *Journal of Labelled Compounds and Radiopharmaceuticals* **2012,** *55* (11), 406-410.

127. Concellón, J. M.; Rodríguez-Solla, H., Synthesis of Different Deuterated Carboxylic Acids from Unsaturated Acids Promoted by Samarium Diiodide and D2O. *Chemistry – A European Journal* **2002,** *8* (19), 4493-4497.

128. Concellón, J. M.; Rodríguez-Solla, H.; Concellón, C., Deuteration of α,β-acetylenic esters, amides, or carboxylic acids without using deuterium gas: synthesis of 2,2,3,3-tetradeuterioesters, amides, or acids. *Tetrahedron Letters* **2004,** *45* (10), 2129-2131.

129. Cho, S.-D.; Park, Y.; Kim, J.-J.; Falck, J.; Yoon, Y.-J., Facile Reduction of Carboxylic Acids, Esters, Acid Chlorides, Amides and Nitriles to Alcohols or Amines Using $NaBH_4/BF_3.Et_2O$. *Cheminform* **2004,** *35*.

130. Sonnenberger, S.; Lange, S.; Langner, A.; Neubert, R. H.; Dobner, B., Synthesis of ceramides NS and NP with perdeuterated and specifically ω deuterated N-acyl residues. *J Labelled Comp Radiopharm* **2016,** *59* (12), 531-542.

131. Brown, L. R., Use of fully deuterated micelles for conformational studies of membrane proteins by high resolution 1H nuclear magnetic resonance. *Biochim Biophys Acta* **1979,** *557* (1), 135-48.

132. Maegawa, T.; Fujiwara, Y.; Inagaki, Y.; Monguchi, Y.; Sajiki, H., A Convenient and Effective Method for the Regioselective Deuteration of Alcohols. *Advanced Synthesis & Catalysis* **2008,** *350* (14-15), 2215-2218.

133. Ulrich, A. S., Solid state 19F NMR methods for studying biomembranes. *Progress in Nuclear Magnetic Resonance Spectroscopy* **2005,** *46* (1), 1-21.

134. Kitevski-LeBlanc, J. L.; Prosser, R. S., Current applications of 19F NMR to studies of protein structure and dynamics. *Prog Nucl Magn Reson Spectrosc* **2012,** *62*, 1-33.

135. Grage, S. L.; Dürr, U. H.; Afonin, S.; Mikhailiuk, P. K.; Komarov, I. V.; Ulrich, A. S., Solid state 19F NMR parameters of fluorine-labeled amino acids. Part II: aliphatic substituents. *J Magn Reson* **2008,** *191* (1), 16-23.

136. Kubyshkin, V.; Afonin, S.; Kara, S.; Budisa, N.; Mykhailiuk, P. K.; Ulrich, A. S., γ-(S)-Trifluoromethyl proline: evaluation as a structural substitute of proline for

solid state 19F-NMR peptide studies. *Organic & Biomolecular Chemistry* **2015,** *13* (11), 3171-3181.

137. Strandberg, E.; Wadhwani, P.; Tremouilhac, P.; Dürr, U. H. N.; Ulrich, A. S., Solid-State NMR Analysis of the PGLa Peptide Orientation in DMPC Bilayers: Structural Fidelity of 2H-Labels versus High Sensitivity of 19F-NMR. *Biophysical Journal* **2006,** *90* (5), 1676-1686.

138. Gottler, L. M.; de la Salud Bea, R.; Shelburne, C. E.; Ramamoorthy, A.; Marsh, E. N. G., Using Fluorous Amino Acids To Probe the Effects of Changing Hydrophobicity on the Physical and Biological Properties of the β-Hairpin Antimicrobial Peptide Protegrin-1. *Biochemistry* **2008,** *47* (35), 9243-9250.

139. Cobb, S. L.; Murphy, C. D., 19F NMR applications in chemical biology. *Journal of Fluorine Chemistry* **2009,** *130* (2), 132-143.

140. Wadhwani, P.; Strandberg, E.; Heidenreich, N.; Bürck, J.; Fanghänel, S.; Ulrich, A. S., Self-assembly of flexible β-strands into immobile amyloid-like β-sheets in membranes as revealed by solid-state 19F NMR. *Journal of American Chemical Society* **2012,** *134* (15), 6512-5.

141. Marsh, E. N. G.; Suzuki, Y., Using 19F NMR to Probe Biological Interactions of Proteins and Peptides. *ACS Chemical Biology* **2014,** *9* (6), 1242-1250.

142. Kokhan, S. O.; Tymtsunik, A. V.; Grage, S. L.; Afonin, S.; Babii, O.; Berditsch, M.; Strizhak, A. V.; Bandak, D.; Platonov, M. O.; Komarov, I. V.; Ulrich, A. S.; Mykhailiuk, P. K., Design, Synthesis, and Application of an Optimized Monofluorinated Aliphatic Label for Peptide Studies by Solid-State 19F NMR Spectroscopy. *Angewandte Chemie International Edition* **2016,** *55* (47), 14788-14792.

143. Birdsall, N. J. M.; Lee, A. G.; Levine, Y. K.; Metcalfe, J. C., 19F NMR of monofluorostearic acids in lecithin vesicles. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1971,** *241* (2), 693-696.

144. Esfahani, M.; Cavanaugh, J. R.; Pfeffer, P. E.; Luken, D. W.; Devlin, T. M., 19F-NMR and fluorescence polarization of yeast plasma membrane and isolated lipids. *Biochemical and Biophysical Research Communications* **1981,** *101* (1), 306-311.

145. Orädd, G.; Lindblom, G., NMR Studies of lipid lateral diffusion in the DMPC/gramicidin D/water system: peptide aggregation and obstruction effects. *Biophysical journal* **2004,** *87* (2), 980-987.

146. Michurin, O. M.; Afonin, S.; Berditsch, M.; Daniliuc, C. G.; Ulrich, A. S.; Komarov, I. V.; Radchenko, D. S., Delivering Structural Information on the Polar Face of Membrane-Active Peptides: (19) F-NMR Labels with a Cationic Side Chain. *Angew Chem Int Ed Engl* **2016,** *55* (47), 14595-14599.

147. Gagnon, M.-C.; Auger, M.; Paquin, J.-F., Progress in the synthesis of fluorinated phosphatidylcholines for biological applications. *Organic & Biomolecular Chemistry* **2018,** *16* (27), 4925-4941.

148. Kang, E.-C.; Kataoka, S.; Kato, K., Synthesis and Properties of Alkyl Phosphorylcholine Amphiphiles with a Linear and an Asymmetrically Branched Alkyl Chain. *Bulletin of the Chemical Society of Japan* **2005,** *78* (8), 1558-1564.

149. Zhang, Q.; Dong, J.; Meng, Q.; Huang, G.; Li, S., Synthesis and Cytotoxicity of 1,4-Naphthoquinone Oxime Derivatives. *Russian Journal of General Chemistry* **2018,** *88* (11), 2388-2393.

150. Rao, B. V.; Kumar, V. S., Synthesis of azamacrolides — (±)Epilachnadiene, (±)Norepilachnene and (±)Homoepilachnene. *Tetrahedron Letters* **1995,** *36* (1), 147-150.

151. Krafft, M. P.; Riess, J. G., Highly fluorinated amphiphiles and colloidal systems, and their applications in the biomedical field. A contribution. *Biochimie* **1998,** *80* (5-6), 489-514.

152. Riess, J. G.; Krafft, M. P., Fluorinated materials for in vivo oxygen transport (blood substitutes), diagnosis and drug delivery. *Biomaterials* **1998,** *19* (16), 1529-39.

153. Qiang, W.; Sun, Y.; Weliky, D. P., A strong correlation between fusogenicity and membrane insertion depth of the HIV fusion peptide. *Proceedings of the National Academy of Sciences* **2009,** *106* (36), 15314-15319.

154. Guimond-Tremblay, J.; Gagnon, M.-C.; Pineault-Maltais, J.-A.; Turcotte, V.; Auger, M.; Paquin, J.-F., Synthesis and properties of monofluorinated dimyristoylphosphatidylcholine derivatives: Potential fluorinated probes for the study of membrane topology. *Organic & Biomolecular Chemistry* **2012,** *10* (6), 1145-1148.

155. Gagnon, M.-C.; Turgeon, B.; Savoie, J.-D.; Parent, J.-F.; Auger, M.; Paquin, J.-F., Evaluation of the effect of fluorination on the property of monofluorinated dimyristoylphosphatidylcholines. *Organic & Biomolecular Chemistry* **2014,** *12* (28), 5126-5135.

156. Kallick, D. A.; Tessmer, M. R.; Watts, C. R.; Li, C. Y., The use of dodecylphosphocholine micelles in solution NMR. *J Magn Reson B* **1995,** *109* (1), 60-65.

157. Sohlenkamp, C.; Geiger, O., Bacterial membrane lipids: diversity in structures and pathways. *FEMS Microbiology Reviews* **2015,** *40* (1), 133-159.

158. Epand, R. M., Introduction to membrane lipids. *Methods Mol Biol* **2015,** *1232*, 1-6.

159. Kieboom, A. P. G., Purification of Laboratory Chemicals, 3rd edition. D.D. Perrin and W. L. F. Armarego. Pergamon Press, Oxford, 1988, X + 392 pp. ISBN 0-08-034714-2, Flexicover, ISBN 0-08-034715-0, **1988,** *107* (12), 685-685.

160. Wasonga, G.; Tatara, Y.; Kakizaki, I.; Huang, X., Synthesis of N-acetyl Glucosamine Analogs as Inhibitors for Hyaluronan Biosynthesis. *J Carbohydr Chem* **2013,** *32* (5-6), 392-409.

161. Hanessian, S.; Sahoo, S. P., A novel and efficient synthesis of L-vinylglycine. *Tetrahedron Letters* **1984,** *25* (14), 1425-1428.

162. Perrone, M. G.; Santandrea, E.; Bleve, L.; Vitale, P.; Colabufo, N. A.; Jockers, R.; Milazzo, F. M.; Sciarroni, A. F.; Scilimati, A., Stereospecific synthesis and bio-activity of novel β3-adrenoceptor agonists and inverse agonists. *Bioorganic & Medicinal Chemistry* **2008,** *16* (5), 2473-2488.

163. Guan, P.; Sun, F. e.; Hou, X.; Wang, F.; Yi, F.; Xu, W.; Fang, H., Design, synthesis and preliminary bioactivity studies of 1,3,4-thiadiazole hydroxamic acid derivatives as novel histone deacetylase inhibitors. *Bioorg Med Chem* **2012,** *20* (12), 3865-3872.

164. Khan, K. M.; Rasheed, M.; Ullah, Z.; Hayat, S.; Kaukab, F.; Choudhary, M. I.; ur-Rahman, A.; Perveen, S., Synthesis and in vitro leishmanicidal activity of some

hydrazides and their analogues. *Bioorganic & Medicinal Chemistry* **2003,** *11* (7), 1381-1387.

165. Shendage, D. M.; Fröhlich, R.; Haufe, G., Highly Efficient Stereoconservative Amidation and Deamidation of α-Amino Acids. *Organic Letters* **2004,** *6* (21), 3675-3678.

166. Guo-Qiang, S.; Feng, Q.; Xian-Feng, H.; Xiao-Bing, L.; Bei, Y., Easy Removal of N-carboxybenzyl (Cbz) Protective Group by Low- Carbon Alcohol. *Letters in Organic Chemistry* **2016,** *13* (3), 177-180.

167. Golding, B. T.; Hall, D. R.; Sakrikar, S., Reaction between vicinal diols and hydrogen bromide in acetic acid; synthesis of chiral propylene oxide. *Journal of the Chemical Society, Perkin Transactions 1* **1973,** (0), 1214-1220.

168. Agresta, M.; D'Arrigo, P.; Fasoli, E.; Losi, D.; Pedrocchi-Fantoni, G.; Riva, S.; Servi, S.; Tessaro, D., Synthesis and antiproliferative activity of alkylphosphocholines. *Chem Phys Lipids* **2003,** *126* (2), 201-10.

169. Marshall, A. J.; Lin, J.-M.; Grey, A.; Reid, I. R.; Cornish, J.; Denny, W. A., Long-chain triazolyl acids as inhibitors of osteoclastogenesis. *Bioorganic & Medicinal Chemistry* **2013,** *21* (14), 4112-4119.

170. Moreira, J. A.; Corrêa, A. G., Enantioselective synthesis of three stereoisomers of 5,9-dimethylpentadecane, sex pheromone component of Leucoptera coffeella, from (−)-isopulegol. *Tetrahedron: Asymmetry* **2003,** *14* (23), 3787-3795.

171. Park, C. M.; Baek, S.; Kim, S.; Song, J.-H.; Lee, S.; Kim, M., Trans-fused 5-[(tert-Butoxtycarbonyl)amino]octahydroindenes as a protease activated receptor-1 (PAR1) antagonist. *Archives of Pharmacal Research* **2016,** *39* (9), 1275-1295.