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The Total Synthesis of Depsipeptide Antibiotics

A dissertation submitted in partial satisfaction of the  
requirements for the degree Doctor of Philosophy

in

Chemistry

by

Aaron M. Wohlrab

Committee in charge:

Professor Yitzhak Tor, Chair  
Professor Michael S. VanNieuwenhze, Co-Chair  
Professor K. C. Nicolaou  
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Professor Wei Wang

2007

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Co-Chair

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University of California, San Diego

2007

## **DEDICATION**

To all my family and friends who have supported  
me throughout this journey

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## LIST OF ABBREVIATIONS

Å	angstrom
Ac	acetyl
AcOH	acetic acid
All	allyl
Aq	aqueous
Ar	aromatic
Boc	<i>tert</i> -butoxycarbonyl
<i>t</i> -Bu	<i>tert</i> -butyl
Bn	benzyl
°C	degrees celius
Calcd	calculated
cat.	catalytic
Cbz	benzyloxycarbonyl
CDCl <sub>3</sub>	deuterated chloroform
CHCl <sub>3</sub>	chloroform
CH <sub>2</sub> Cl <sub>2</sub>	dichloromethane
Cy	cyclohexyl
%de	percent of diastereomeric excess
DEPBT	3-(diethoxyphophoryloxy)-1,2,3-benzotriazin-4(3H)-one
DIEA	Diisopropylethylamine

DG	Distance Geometry
DMAP	4-dimetheylaminopyridine
DMF	<i>N,N'</i> -dimethylformamide
DMSO- <i>d</i> <sub>6</sub>	fully deuterated dimethyl sulfoxide
dr	diastereomeric ration
%ee	enantiomeric excess
ED <sub>50</sub>	the dose which results 50% effect
equiv.	equivalent
ESI	electrospray
Ether	diethyl ether
EtOAc	ethyl acetate
EtOH	ethyl alcohol
Fmoc	fluorenylmethoxycarbonyl
h	hours
HCl	hydrogen chloride
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzatriazole monohydrate
HRMS	high resolution mass spectroscopy
IC <sub>50</sub>	then concentration which produces a half-maximal inhibition
iPr	isopropyl
IR	infrared
LHMDS	lithium hexamethyldisilazide

M	molar
MALDI	matrix-assisted laser desorption/ionization
Me	methyl
MeI	methyl iodide
MHz	megahertz
min	minutes
mL	milliliter
$\mu\text{L}$	microliter
$\mu\text{M}$	micromolar
mM	millimolar
mol	mole
mmol	millimole
nM	nanomolar
NMR	nuclear magnetic resonance
NOE	nuclear overhauser effect
Ph	phenyl
$\text{Pd}(\text{PPh}_3)_4$	tetrakis(triphenylphosphine)palladium(0)
$\text{Ph}_3\text{P}$	triphenylphosphine
ppm	part per million
quant.	quantitative yield
$R_f$	retention factor
RP-HPLC	reverse phase high performance liquid chromatography

SAR	structure-activity relationship
rt	room temperature
TBDMS	<i>tert</i> -butyldimethylsilyl
TEA	triethylamine
Tf	triflate
TFA	trifluoroacetic acid
TFE	trifluoroethanol
THF	tetrahydrofuran
TMS	trimethylsilyl
tlc	thin layer chromatography
Trt	triphenylmethyl
v/v	volume per volume

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## PUBLICATIONS

Smith, N; Wohlrab, A.; Goodman, M. "Enantiocontrolled Synthesis of  $\alpha$ -Methyl Amino Acids via Bn<sub>2</sub>N- $\alpha$ -Methylserine- $\beta$ -lactone", *Org. Lett.*, **2005**, 7(2), 255-258

Perelman, Loren A.; Schwartz, Michael P.; Wohlrab, Aaron M.; VanNieuwenhze, Michael S.; Sailor, Michael J. "A Simplified Biomolecule Attachment Strategy for Biosensing Using a Porous Si Oxide Interferometer", *Phys. Stat. Sol.*, **2007**, 204, No. 5, 1394–1398

Wohlrab A.; Lamer R.; VanNieuwenhze M. S. "Total Synthesis of Plusbacin A3: A Depsipeptide Antibiotic Active Against Vancomycin-Resistant Bacteria", *J. Am. Chem. Soc.*, **2007**, 129, 4175-4177

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## **ABSTRACT OF DISSERTATION**

### **The Total Synthesis of Depsipeptide Antibiotics**

by

Aaron M. Wohlrab

Doctor of Philosophy in Chemistry

University of California, San Diego, 2007

Professor Yitzhak Tor, Chair

Professor Michael S. VanNieuwenhze, Co-Chair

The use of antibiotics has greatly impacted the treatment of bacterial infections since their introduction in the 1940's. However, the selective pressures caused by the widespread use of antibiotics have resulted in the emergence of resistant bacteria, decreasing the therapeutic efficiency of many classes of chemotherapeutic agents. Nature's ability to select for resistance pathogens has resulted in the need to replace obsolete antibiotics with new ones functioning via different modes of action. In recent years, we have seen a renewed interest in the isolation of natural products with antibiotic activity and has led to the discovery of new molecular platforms for development of potent



analogs and/or semi-synthetic derivatives. Our focus has been directed toward the chemical synthesis and mechanistic studies of new peptide antibiotics that show potential as alternative treatments for bacterial infections arising from resistant bacteria.

Plusbacin A3 and WAP-8294A2 are lipodepsipeptide antibiotics isolated from the culture broths of *Psuedomonas* and *Lysobacter*, respectively. Both natural products are active against a diverse panel of gram positive bacteria. In particular they have been shown to exhibit potent *in vitro* and *in vivo* activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant Enterococci. Our goal was to develop a convergent total synthesis for each antibiotic and to carry out structure activity relationships in order to study their mechanism of action.

We have successfully completed the first total synthesis and stereochemical assignment of plusbacin A3. We accomplished the stereoselective synthesis of a variety of non-proteogenic amino acids including an innovative route for preparation of both Boc- $\beta$ OBn-D-Asp(OCy)-OH and Boc- $\beta$ OBn-L-Asp(OCy)-OH. An efficient synthesis of Boc-D-*allo*-Thr-OH was utilized to provide ample material for our synthetic effort. Both stereochemical configurations of the allyl 3-hydroxy-14-methylpentadecanoate were accessed through the key lipase resolution.

With the total synthesis of plusbacin A3 complete, our attention was then directed to a mechanistic analysis of this promising antibiotic. A conformational analysis using 2D NMR with molecular modeling provided a solution structure of plusbacin A3 and was used to design a series of analogs to analyze structure activity relationships (SARs). Several analogs were synthesized to examine the importance of key structural elements. Biological assays of the analogs determining the minimum inhibitory concentrations

revealed that the hydrophobic lipid chain of plusbacin was essential for antibacterial activity. The arginine moiety was determined to play an important role in the stabilization of the plusbacin A3 active conformation. The modification of several residues that were believed to participate in stabilizing hydrogen bonding interactions were also examined. Although many of the analogs showed a decreased, or complete loss of activity, the initial SAR studies provided important mechanistic information about the mode of action.

The first total synthesis of WAP-8294A2 was accomplished via the enantioselective synthesis of a several non-proteogenic amino acids and their incorporation into the peptide backbone. The later stages of the synthesis employed solid phase peptide synthesis (SPPS) which allowed for the efficient acylations of a variety of *N*-methyl amino acids and a strategic on-resin macrocyclization reaction. The convergent total synthesis of the promising antibiotic has set the stage for important mechanistic and SAR studies.

# CHAPTER I

Antibiotic: 1. tending to prevent, inhibit, or destroy life; 2. a substance produced by or a semisynthetic substance derived from a microorganism and able in dilute solution to inhibit or kill another microorganism.<sup>1</sup>

## INTRODUCTION

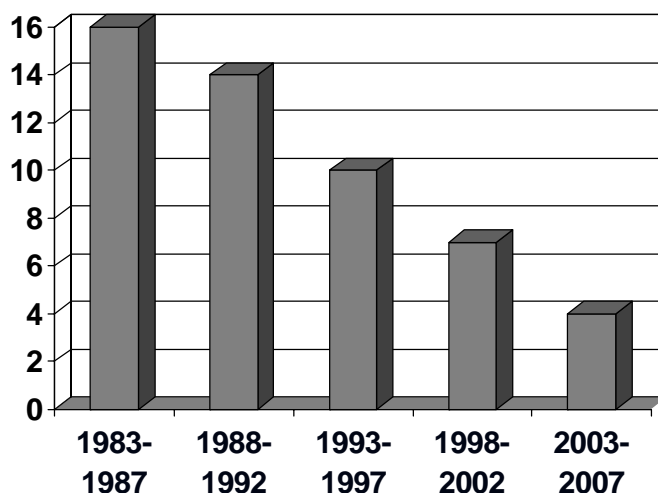
### I.1 The Need for New Antibiotics

The treatment of microbial infections is one of modern medicine's greatest accomplishments. Since their introduction in the 1940s and 1950s, the use of antibiotics lowered death rates from microbial infections from 797 per hundred thousand in 1900 to 36 per hundred thousand in 1980.<sup>2,3</sup>

The treatment of infectious diseases is a perpetual battle that has demanded the constant renewal of effective therapeutics. The onset of multi-drug resistant bacteria, mainly methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and vancomycin-resistant *Staphylococcus aureus* (VRSA) has prompted the scientific community to seek alternative antibiotics functioning via different modes of action.<sup>4</sup>

The US market for antibiotics has grown to more than \$25 billion per year<sup>5</sup>. However, due to bacterial resistance, which has limited the effectiveness of clinically used therapeutics, major drug companies have cut back their antibiotic research programs

to concentrate on treatments for chronic illnesses instead.<sup>6,7</sup> John Edwards, head of policy at the Infectious Diseases Society of America stated “There’s unequivocal evidence that antimicrobial research is on a steep downward slope”. The evidence is overwhelming as the industry sees fewer new drugs in the pipeline every year<sup>8</sup> (**Figure I.1**)

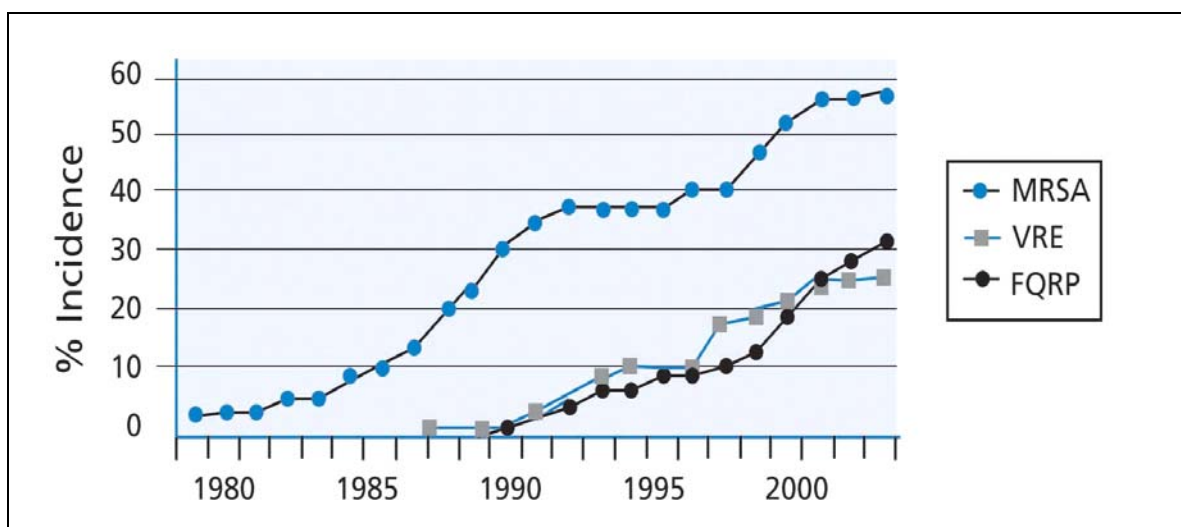


**Figure I.1: Total number of approved antibacterial agents in the United States since 1983<sup>9</sup>**

Infectious diseases are the second leading cause of death worldwide and the third leading cause of death in developed countries.<sup>10,11</sup> It has been recently estimated that 20% of hospital patients have or will develop an infection, and 70% of the bacteria that give rise to these infections are resistant to one or more of the main antibiotics.<sup>5</sup> The incidence of healthcare-associated staphylococcal infections that are due to MRSA has been increasing: 2% of *Staph. aureus* infections in U.S. intensive-care units were MRSA in 1974, 22% in 1995, and 64% in 2004.<sup>12</sup> Since the first observed case of VRE in 1989,

the incidence of enterococcal isolates resistant to vancomycin reported to the National Nosocomial Infections Surveillance System increased 20-fold.<sup>13</sup> These statistics illustrate the alarming rise in resistant strains since the early 1980's. Due to antimicrobial resistance, the decreasing arsenal of useful antibiotics is a significant threat to public health, underlining the pressing need for the development of new antibiotics.<sup>14,15</sup>

Since 1928 when Scottish scientist Alexander Fleming first suggested that penicillin could be used for its antibiotic properties, the successful use of antimicrobials for the treatment of bacterial infections has been one of the greatest achievements of modern medicine.<sup>16</sup> With the discovery of and introduction of penicillin and the sulfa drugs more than 75 years ago the widespread use of antibiotics has saved countless lives and has prevented the outbreak of many feared diseases and infections. However, this widespread use, and often misuse, of antibiotics has rendered many useful drugs ineffective due to increasing resistance development (**Figure I.2**).



**Figure I.2: Incidence of different resistant bacterial strains in clinical settings since 1980's.** MRSA (methicillin-resistant *Staphylococcus aureus*), VRE (vancomycin-resistant *enterococci*, FQRP (FQRP = Fluoroquinolone-resistant *Pseudomonas aeruginosa*).<sup>17</sup>

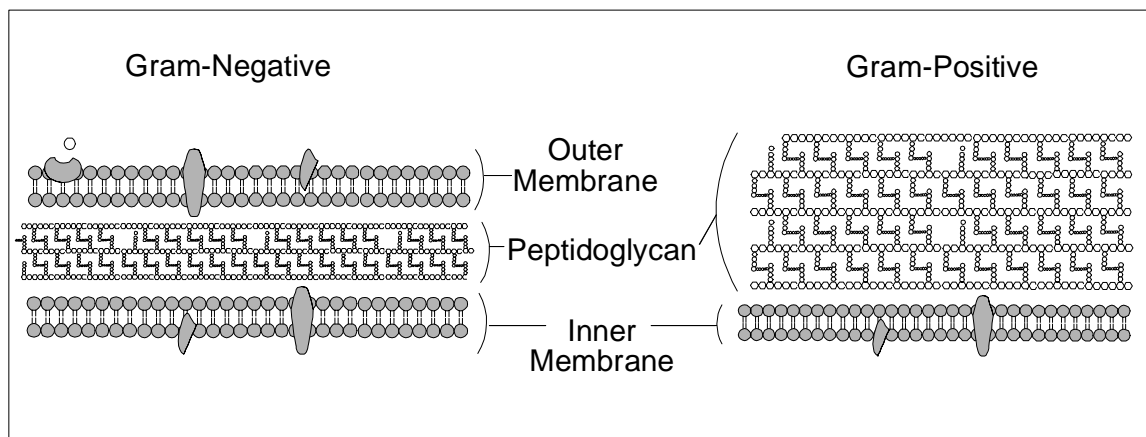
Drug resistance is a natural process which has allowed microbes to grow in the presence of a chemical that would normally kill it. Although the impact of resistance has only been felt by our society in recent years, bacteria themselves have been dealing with the issue for hundreds of millions of years. Bacterial antibiotic producers use these secondary metabolites for protection against other pathogens and for competition for space and nutrients. In addition to mechanisms of autoimmunity against antibiotics, evolutionary pressures are also at work to provide resistance mechanisms for organisms under attack. Christopher Walsh, Professor at the Harvard Medical School, provides an interesting analysis for the likelihood of resistance based solely on bacterial genetic mutations, where he states: “The large numbers of bacterial cells in a population and the short generation times facilitate the development of mutants. Bacterial DNA replication machinery may produce one error in  $10^7$ ; in replication of a  $3 \times 10^6$  –bp genome containing about 3,000 genes, that is 0.3 errors per generation. If there are  $10^{11}$  bacteria in a population, e.g., in a patient being treated for a blood-borne bacterial infection, then there may be 1,000 mutant variants. If the mutations are randomly distributed throughout the bacterial genome then 1,000 genes, one out of every three, will have a mutation. If one of these confers a selective advantage for survival, e.g., in the presence of a given antibiotic, then the resistant bacterium will be selected for, grow up as its neighbors perish, and take over the culture”.<sup>18</sup>

## I.2 Bacteria and Killing Targets

### I.2.1 Gram-Positive and Gram-Negative Bacteria

This section will examine the different types of pathogens causing bacterial infections, the primary targets for antibiotic action, and an overview of the major antibiotic classes.

A closer examination of these prokaryotic organisms reveals a wide variety of morphological features. The size and shape of bacteria is a result of genetic variations which present themselves most commonly as rods (bacilli) and spheres (cocci). Additional morphologies include vibrios (curved rods or ‘bananas’) helical (corkscrew), and miscellaneous shapes (triangular, square, annular, branched, filamentous *etc*).



**Figure I.3: Representation of the differences in gram-negative and gram-positive bacterial cell walls**

Within these groups there are hundreds of variations, however, most bacteria can be assigned to one of two major classification categories. The categories are based on the gram-staining status of the organism. Gram-positive bacteria maintain the crystal-violet dye during the staining process resulting in their blue appearance when visualized under a

microscope.<sup>19</sup> Gram-negative bacteria on the other hand, do not retain the dye and are recognized by their red/pink color when visualized under a microscope. The staining status of bacteria reflects differences in their cell wall compositions<sup>20</sup> (**Figure I.3**). **Table I.1** illustrates a comparison of the major chemical components of gram-positive and gram-negative cellular envelopes.

**Table I.1: Major Classes of chemical components in bacterial envelopes<sup>20</sup>**

Major Chemical Components	
Gram-positive cell walls	Gram-negative cell walls
Peptidoglycan	Peptidoglycan
Polysaccharides	Lipopolysaccharide
Techoic Acids	Lipoproteins
Lipotechoic Acids	Porins
Peptidoglycolipids	Phospholipids
Glycolipids	

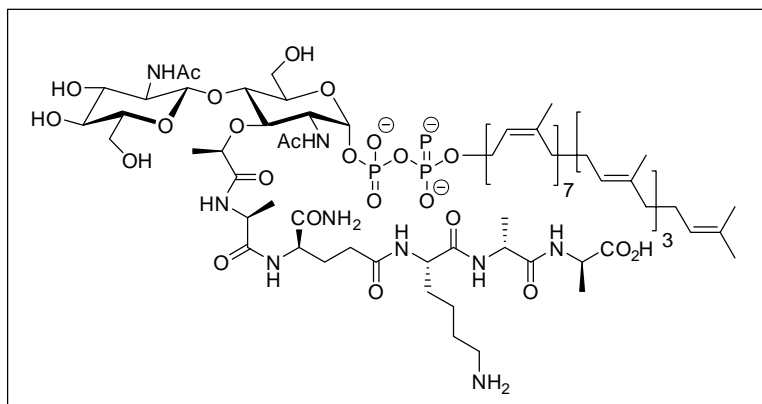
Both types of cells contain a cytoplasmic membrane and are supported by a cell wall which provides shape and strength. The cell wall consists of covalently linked polysaccharide and polypeptide chains forming a cage-like capsule called peptidoglycan (PG). The polysaccharide framework of peptidoglycan consists of linear strands of alternating  $\beta$ -(1,4) linked *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) as represented in **Figure I.4**, illustrating lipid II, the monomeric peptidoglycan precursor.<sup>21</sup>

The lactic acid group of the MurNAc forms a peptide bond with the peptide chain. This peptide chain is responsible for the covalent cross-linking present in the bacterial cell wall. It not only provides mechanical strength, but is also a major barrier to



resist osmotic pressure forces that could kill the bacteria. Although both Gram-positive and Gram-negative bacteria have a PG layer, it is much thicker in the Gram-positives.<sup>21,</sup>

22



**Figure I.4: Representation of Lipid II, the monomeric unit of peptidoglycan**

Gram negative bacteria have a thin cell wall (30 Å) which is surrounded by a complex outer membrane.<sup>23</sup> This outer membrane serves as a permeability barrier which functions to exclude toxic substances.<sup>18</sup> It is primarily made up of lipids, proteins and lipopolysaccharides (LPS). The inner leaflet contains mostly phospholipids while the outer is comprised of Lipid A.<sup>24</sup> Additional components of Gram-negative cell walls include porins and lipoproteins. Unlike Gram-positive bacteria, there are no teichoic acids or lipoteichoic acids present in Gram-negative organisms. Common examples of Gram-negative bacteria are *Escherichia coli*, *Salmonella*, *Pseudomonas*, and cyanobacteria.<sup>25</sup>

Gram-positive bacteria have a thicker PG layer (250 Å) yet lack the additional outer membrane found in Gram-negative bacteria.<sup>26</sup> Other cell wall components can also be found in Gram-positive bacteria such as teichoic acids, polysaccharides, and peptidoglycolipids. These compounds are covalently attached to the peptidoglycan and

provide additional protection and points of adhesion for proteins and other key recognition processes.<sup>27,28</sup> Common examples of Gram-positive bacteria are *Streptococcus Pneumoniae* , *Staphylococcus aureus*, *Bacillus anthracis* (Anthrax), *Enterococcus*, and *Actinomycetes*.

## **I.2.2 Major Targets and Antibiotics Used Against Them**

With such a large number of antibiotics available, there are surprisingly few targets. All antibiotics used to treat human infections are grouped based on their structure or by their targets, either at the membrane surface or inside the cell. Although many subcategories exist, four major targets will be examined. The major targets are as follows:

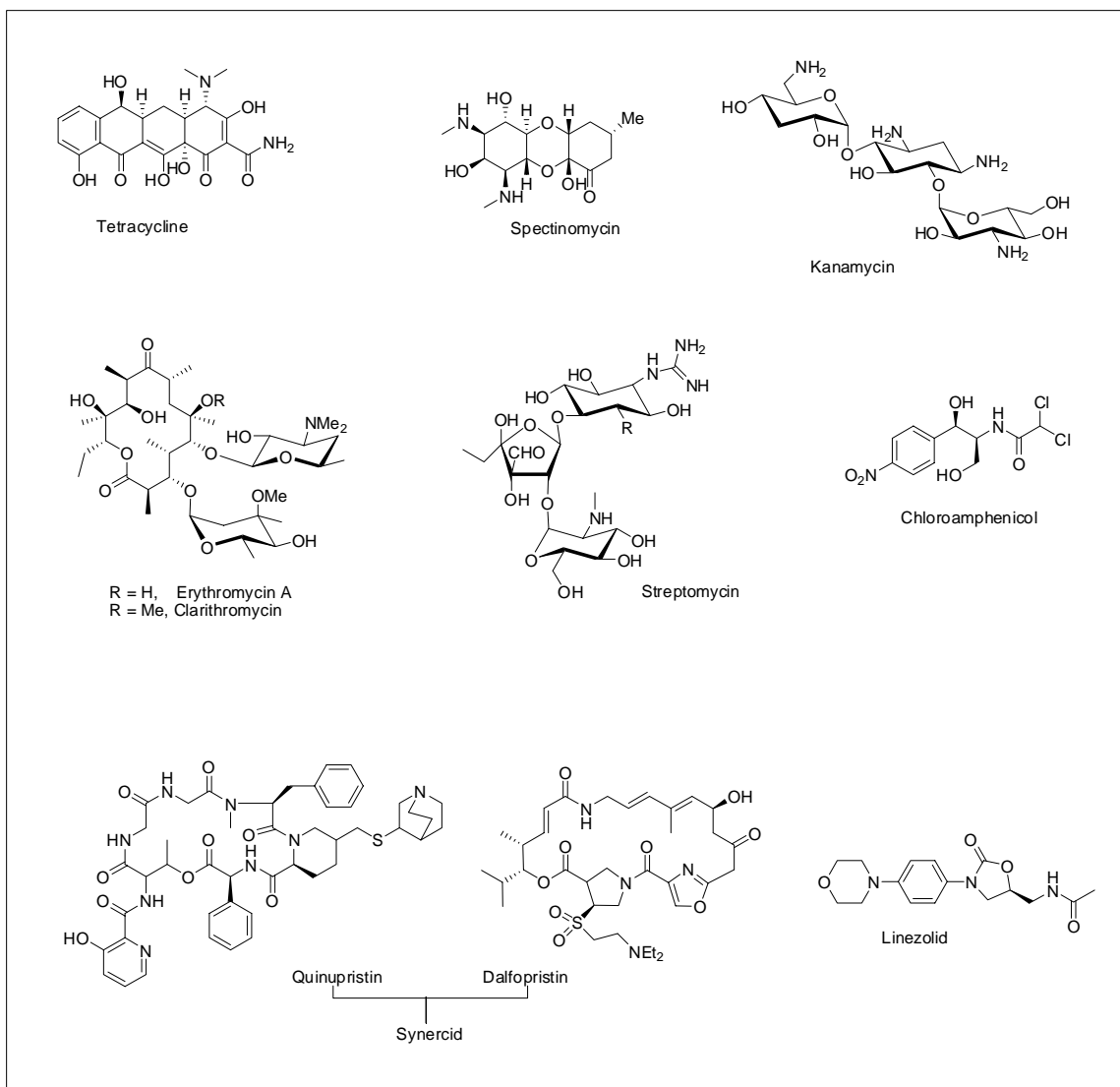
- 1) Inhibition of bacterial cell wall biosynthesis
- 2) Inhibition of protein synthesis
- 3) Inhibition of DNA or RNA synthesis
- 4) Inhibition of the folate biosynthetic pathway<sup>29</sup>

This section will briefly summarize these major targets. A closer examination of the cell wall inhibitors will follow in subsequent sections of this thesis.

Antibiotics that selectively kill bacterial cells, leaving human cells unharmed, would require the target to be present in bacteria but not in humans. The most desirable targets therefore would be the inhibition of bacterial cell wall biosynthesis and the inhibition of the folic acid pathway. Although mammals do possess the machinery for

protein synthesis (ribosome) and DNA replication, the structural differences have been taken advantage of and have allowed for the development of selective therapeutics.<sup>18</sup>

One of the first recognized molecular targets for antibiotics was protein biosynthesis, in particular the ribosome, a 2.6 MDa nucleoprotein particle, which presents a range of inhibition possibilities.<sup>30-32</sup> These antibiotics interfere with one or more steps carried out by the 50S or 30S ribosomal subunits during the processes of protein biosynthesis.<sup>33</sup> Since their introduction more than 50 years ago, many of these antimicrobial agents have been clinically useful. Protein biosynthesis is an obvious target due to its crucial role in cellular function. There are numerous points of intervention for the development of antibiotics such as the activation of the 20 proteogenic amino acids, protein chain initiation, chain elongation, chain termination, and posttranslational modifications. The inhibition of protein translation has also gained much attention due primarily to the high resolution structural data available for the ribosome and co-structures with targeted antibiotics.<sup>34-37</sup>



**Figure I.5: Molecular structure of common antibiotics that inhibit protein biosynthesis**

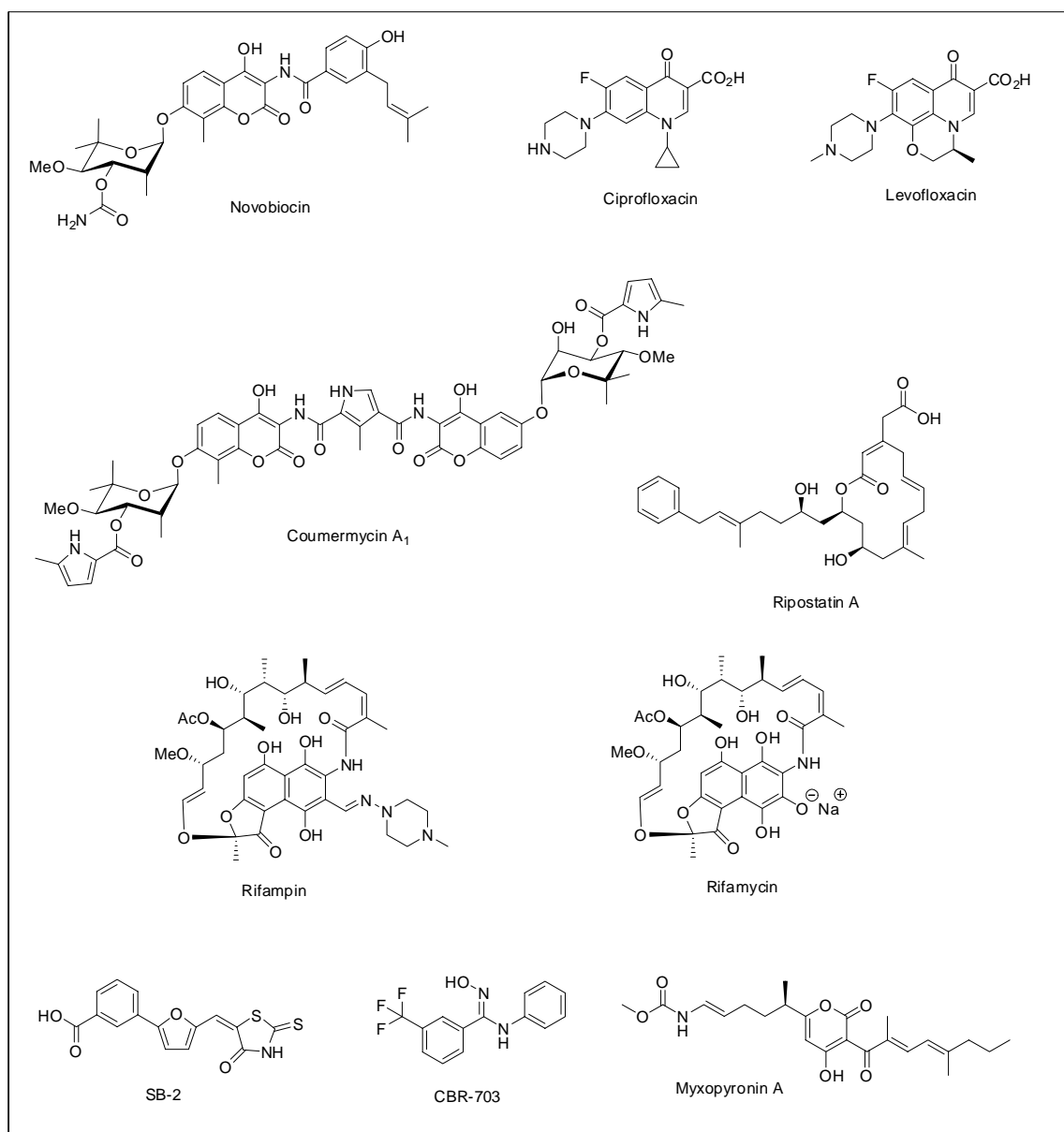
This information has facilitated structure based drug design and has allowed a wide range of chemically diverse compounds to be developed. Examples of the diverse array of chemicals known to bind ribosomal targets and ancillary proteins include cationic aminoglycosides<sup>38</sup> and neutral carbohydrates, macrolides<sup>39</sup>, peptides<sup>40</sup>, and diverse small molecules (**Figure I.5**).

The inhibition of DNA and RNA replication and repair is also a prime target for the development of antibiotics. Many of the antibiotics of this class are produced by bacteria themselves to ward off and kill their adversaries.<sup>41</sup> DNA replication processes are attractive targets not only for their critical role in cell viability, but also because the replication machinery is highly conserved among bacteria.<sup>42</sup> The main inhibition pathways are via the blockade of the DNA topoisomerases and RNA polymerase as achieved by the quinolone and rifamycin type antibiotics, respectively.

Most antibiotics that shut down the replisome inhibit one or more topoisomerases, enzymes that are responsible for unwinding DNA super-coils.<sup>15</sup> These enzymes are generally classified as type I or type II topoisomerases. Type I topoisomerases break only one DNA strand at a time. Type II topoisomerases, specifically, DNA gyrase, are able to break up to two DNA strands at a time. This unknotting process is a prerequisite to replication, as it is essential for the super-coil to be relaxed before replication, transcription, recombination, repair and encoding can take place.<sup>43,44</sup> The most common inhibitors of DNA topoisomerases are the coumarins, represented by novobiocin and coumermycin, as well as the flouroquinolones, levofloxacin and ciprofloxacin, (**Figure I.6**).

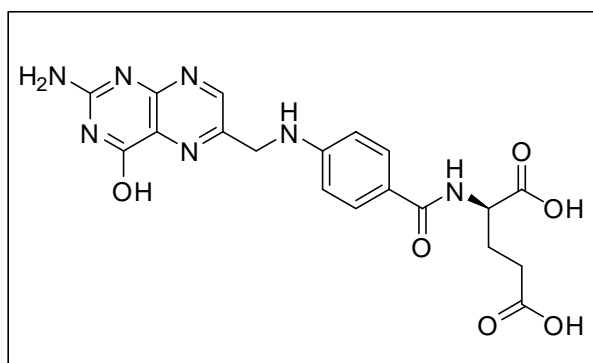
Yet another established target for antibiotics is the RNA polymerase (RNAP).<sup>45</sup> The RNAP consists of five subunits and is highly conserved in bacteria.<sup>46</sup> It is essential for transcription from which the loss of this process results in cellular death. A common RNAP inhibitor, rifampin, is a semisynthetic version of rifamycin B and is used clinically to treat tuberculosis, leprosy and staphylococcal diseases.<sup>47</sup> The rifamycin class of antibiotics function by binding an allosteric site of the  $\beta$ -subunit of the RNAP thus

blocking the elongating RNA chain.<sup>48,49</sup> The ability to selectively disrupt bacterial RNAP is due to the differences between eukaryotic and prokaryotic RNAP beta subunits.<sup>45, 49,50,51</sup>



**Figure I.6: Molecular structures of common antibiotics that inhibit DNA replication and repair**

The inhibition of folate (vitamin B<sub>9</sub>) biosynthesis has been a long standing target for the development of antibiotics. Folic acid (**Figure I.7**) is critical for cell survival as it is necessary for the production and maintenance of new cells, in particular, DNA replication and cell division.

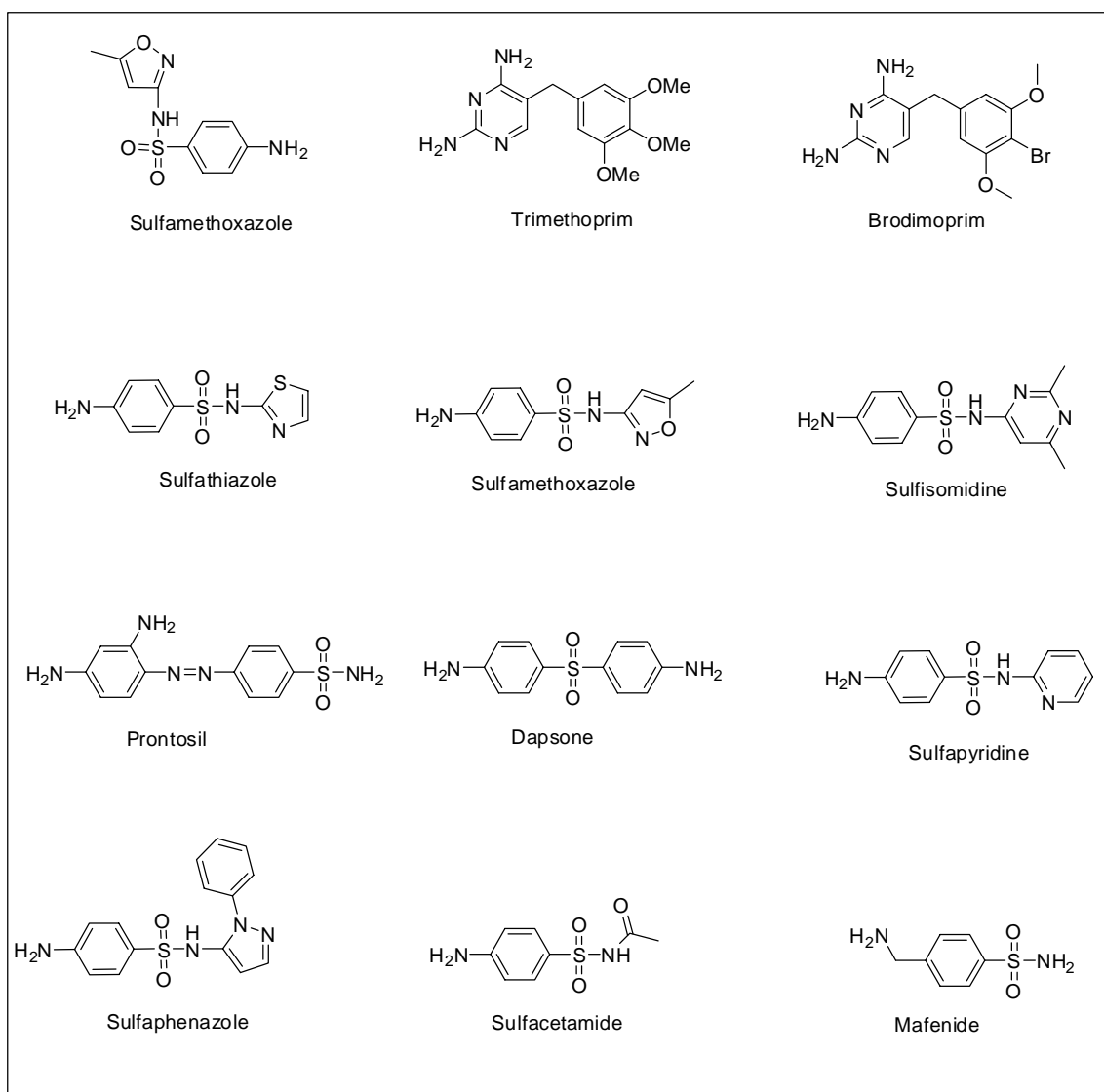


**Figure I.7: Molecular structure of folic acid**

Selective toxicity towards bacteria makes folic acid metabolism an attractive target because prokaryotes must make their own folate while eukaryotes obtain it through dietary sources.<sup>52</sup> A variety of steps along this biosynthetic pathway have been exploited for inhibition and include the enzymes DHNA (dihydroneopterin adolase), DHFR (dihydrofolate reductase), DHPS (dihydropteroate synthase), and HPPK (hydroxymethyldihydropterin pyrophosphokinase).<sup>53</sup> Although the DHFR target is present in humans, there are enough structural differences that have allowed selective inhibition to be achieved.<sup>47</sup> The sulfa drugs or sulfonamides are amongst the oldest clinically used antibiotic and are often used in combination with other inhibitors.<sup>18</sup> Sulfamethoxazole for example is used with trimethoprim, (**Figure I.8**). When used in combination, they act as bactericidal agents, however, when used individually, they maintain bacteriostatic action. Each of these drugs works to interfere with different steps

in folic acid metabolism. Sulfamethoxazole inhabits the enzyme DHPS, functioning as a mimic of *p*-aminobenzoic acid (PABA). Trimethoprim blocks DHFR, inhibiting the production of tetrahydrofolate and is 50,000 to 100,000 more active against prokaryotes than eukaryotes, thus allowing for high selectivity towards bacterial cell death.<sup>54</sup> Interestingly, the synergistic effect of this powerful drug combination has shown a reduced mutation rate for resistance when compared to the use of each inhibitor alone.<sup>55,56</sup>





**Figure I.8: Molecular structures of common antibiotics that inhibit the folic acid biosynthetic pathway**

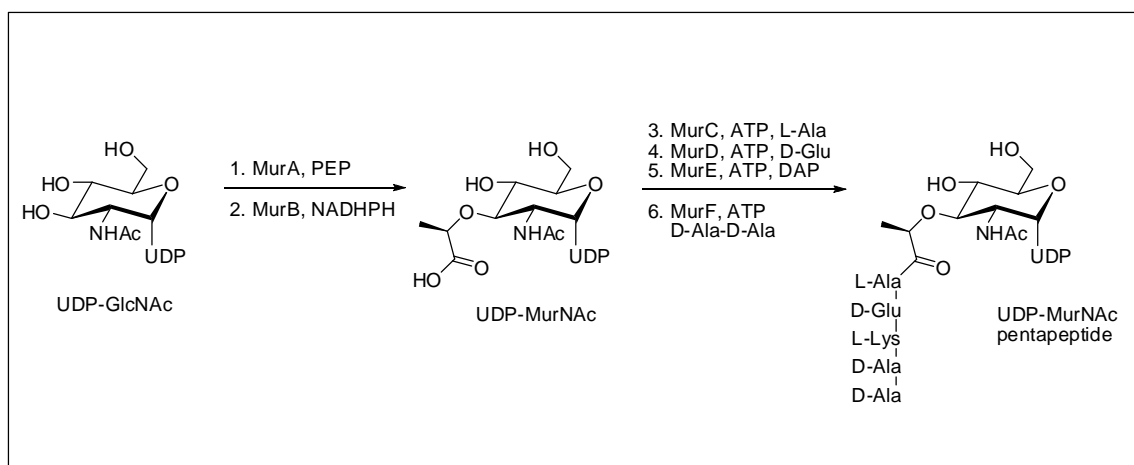
### I.3 The Bacterial Cell Wall

The bacterial cell wall is often considered the ideal target for antibacterial chemotherapy. As mentioned previously, the enzymatic machinery required for bacterial cell wall biosynthesis does not have a eukaryotic counterpart and allows for the selective killing of bacterial cells. Also, cellular penetration is not a prerequisite for antimicrobial

action. Although the bacterial cell wall is an attractive target, certain antibiotics have been found to perform much differently on Gram-positive bacteria when compared to Gram-negative bacteria. The outer membrane of Gram-negative bacteria has been shown to limit the diffusion of antibiotics. This is due to the limited pore size of the porin proteins found in the membrane.<sup>57</sup> Due to the absence of the outer membrane, Gram-positive bacteria are not subject to this limitation therefore a broader spectrum of antibiotic platforms can be utilized. Regardless of the limitations on size and polarity of effective antibiotics, there are several possible steps in cell wall assembly, membrane translocation, and extracellular cross-linking that can be inhibited. The following sections will provide a detailed elaboration of the bacterial cell wall, its construction, the major targets and important cell wall antibiotics.

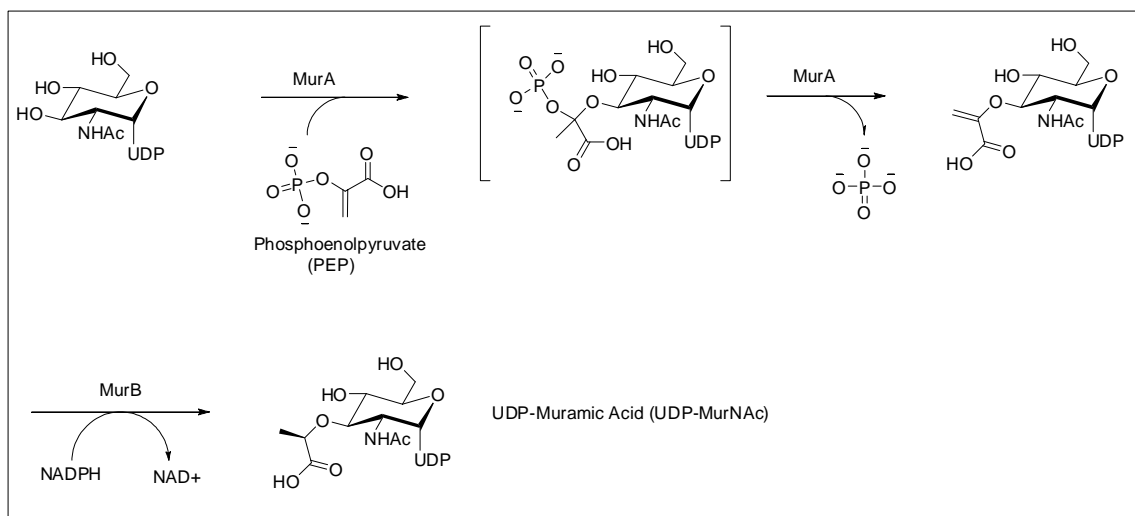
### **I.3.1 Peptidoglycan Biosynthesis**

In order to protect themselves from osmolysis due to high intracellular pressure, bacteria (both Gram-positive and negative) utilize peptidoglycan (PG, also referred to as murein), the covalently cross-linked mesh of glycan and peptide strands.<sup>21,22</sup> The biosynthetic pathway leading to the construction of this network occurs in three distinct phases, the cytoplasmic, membrane-associated, and extracytoplasmic.<sup>18</sup> The first stage of murein assembly is initiated by the action of six critical enzymes, MurA-F. The overall transformation results in the conversion of UDP-N-acetylglucosamine into UDP-N-acetyl muramic acid pentapeptide (Park nucleotide).<sup>22</sup> The overall transformation is summarized in **Scheme I.1**.



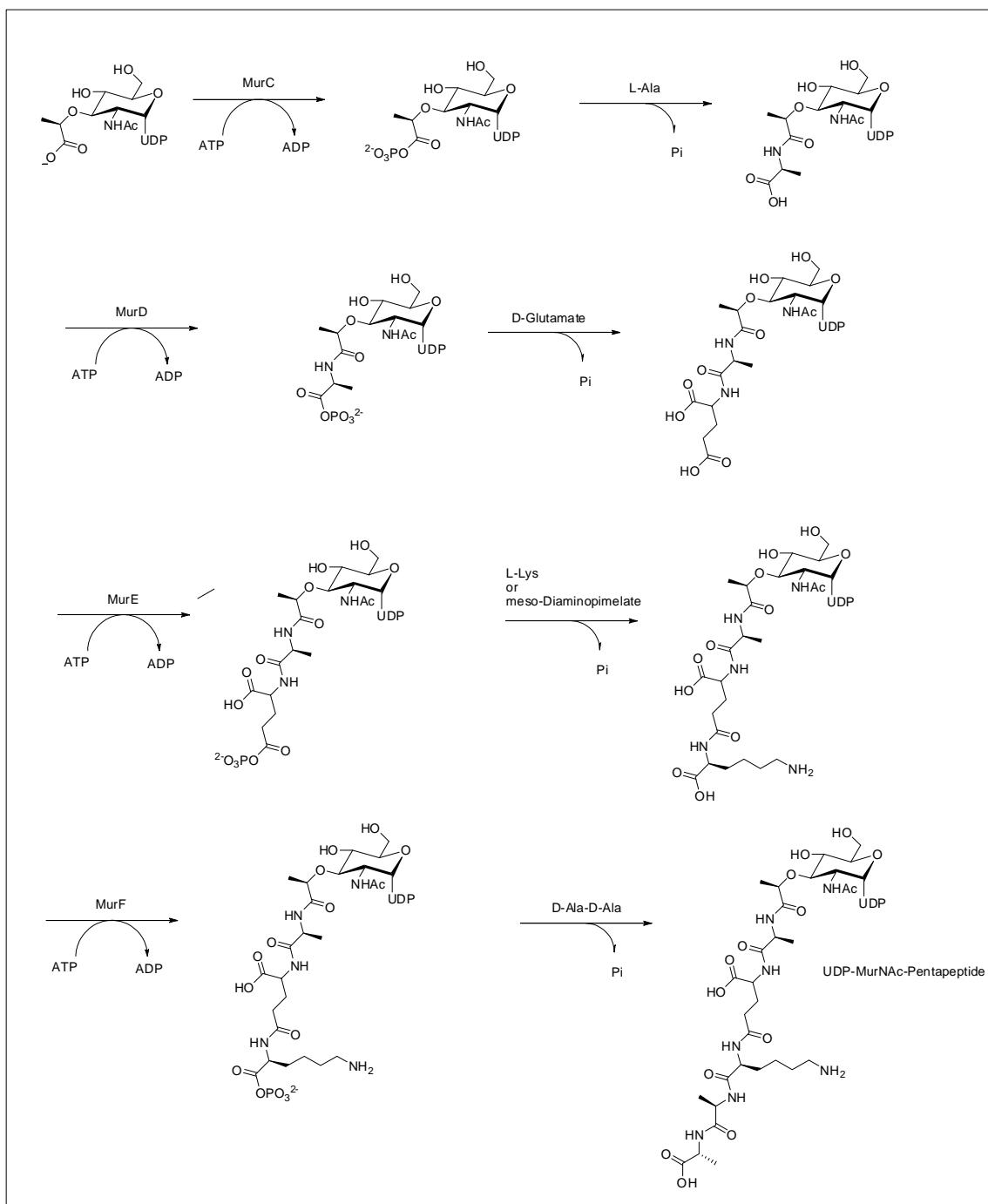
**Scheme I.1: Stage 1 of PG assembly via enzymes MurA-F**

UDP-GlcNAc is initially converted to the 3'-O-lactyl ether by a two step process involving MurA and MurB. **Scheme I.2** illustrates this transformation. MurA facilitates the regioselective nucleophilic addition of GlcNAc C(3) hydroxyl onto a PEP group. MurA then catalyzes the elimination of H<sup>+</sup> and phosphate to generate the enol ether.<sup>22,58</sup> A stereoselective reduction of the enol double bond is then carried out by the NADPH-oxidizing flavoprotein MurB to produce the UDP-N-acetyl muramic acid<sup>59</sup>.

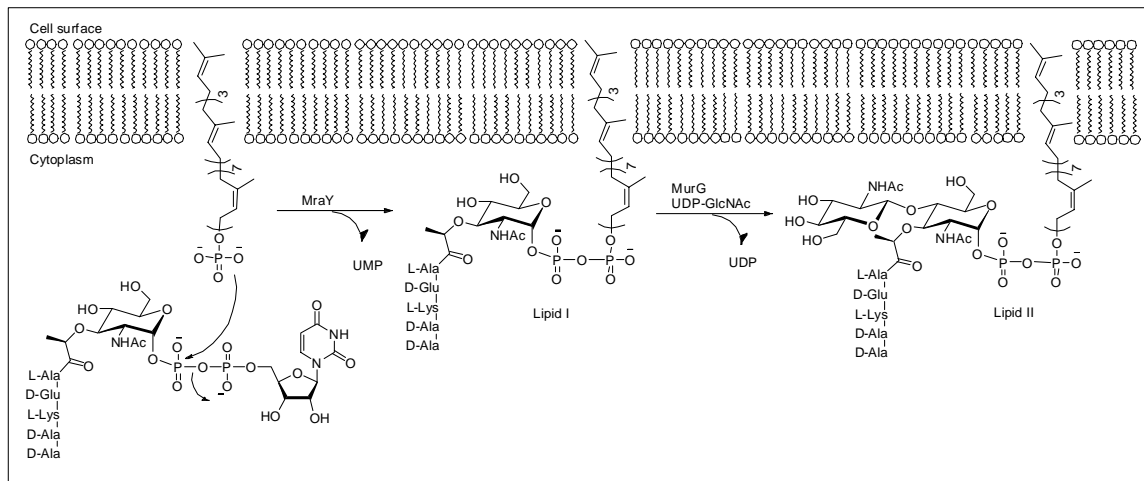


**Scheme I.2: Biosynthetic conversion of UDP-GlcNAc to UDP-MurNAc**

The carboxylate of the lactyl ether previously installed serves as the foundation for the introduction of the pentapeptide chain. **Scheme I.3** illustrates the elaboration of the peptide sidechain through the action of MurC-F, ATP dependent amino acid ligases. The sequential addition of the amino acids is accomplished via the enzymatic activation of the C-terminal carboxylates as the mixed anhydride using ATP as the cosubstrate.<sup>60</sup> This highly reactive acyl-phosphate intermediate is then captured by the appropriate amino functionality generating an amide bond. The sequential incorporation of L-Ala, D-Glu, L-Lys (or meso-DAP in Gram-negative and some Gram-positive bacteria), and D-Ala-D-Ala results in the UDP-MurNAc pentapeptide also referred to as the “Park nucleotide”.<sup>61,62</sup> The completion of the UDP-MurNAc pentapeptide concludes phase one of the PG biosynthetic pathway. Inhibitors of the enzymatic events in this process will be discussed in later sections.

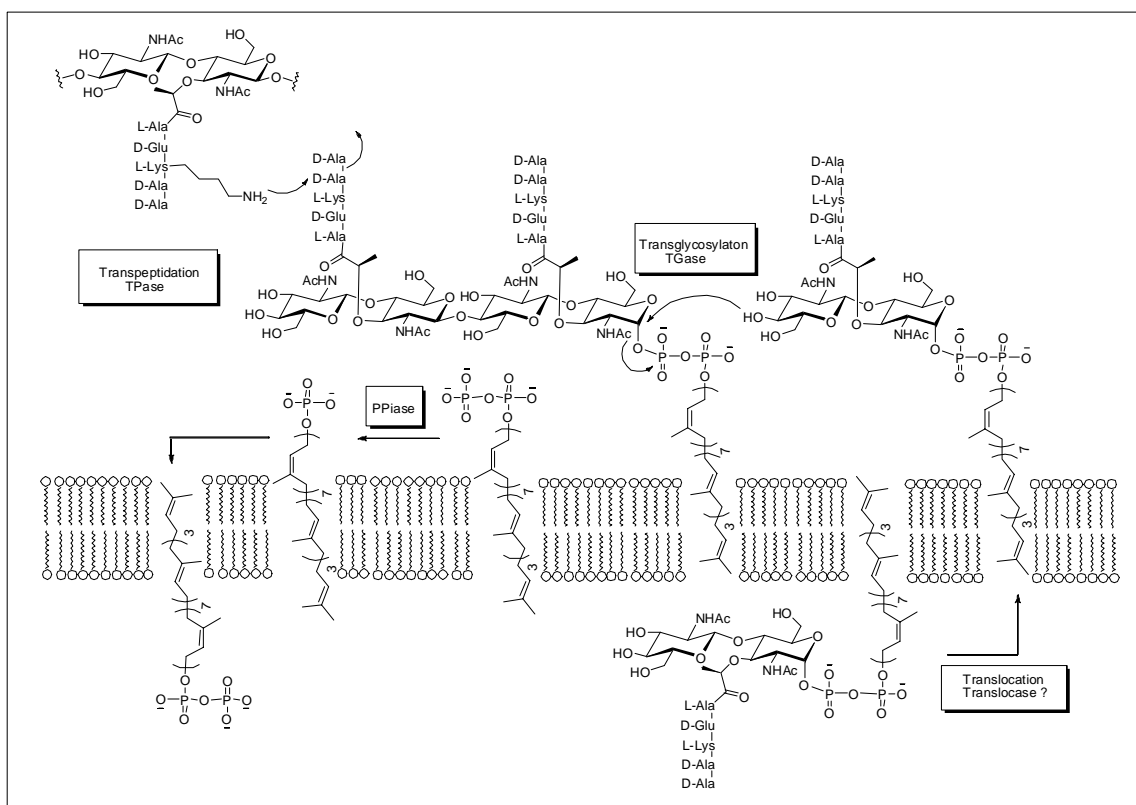


**Scheme I.3: Enzymatic formation of UDP-MurNAc pentapeptide by action of MurC-F**



**Scheme I.4: Phase two of peptidoglycan biosynthesis**

Stage two of bacterial cell wall biosynthesis occurs on the cytoplasmic membrane surface (**Scheme I.4**). The sequence begins with the attachment of a  $C_{55}$  undecaprenyl lipid carrier to the Park nucleotide, via an enzyme catalyzed phosphate exchange reaction. MraY mediates the attack of the lipid phosphate oxygen onto the phosphate linkage of the UDP group, releasing a uridine monophosphate, and generating a new pyrophosphate linkage.<sup>63</sup> The following step of phase two involves the glycosylation of a second sugar moiety, GlcNAc, onto Lipid I. MurG is a  $\beta$ -(1,4) glycotransferase and catalyzes the addition of the  $C_4$ -OH of the muramyl group onto the  $C_1$  position of UDP-GlcNAc, releasing UDP to afford Lipid II.<sup>21</sup>



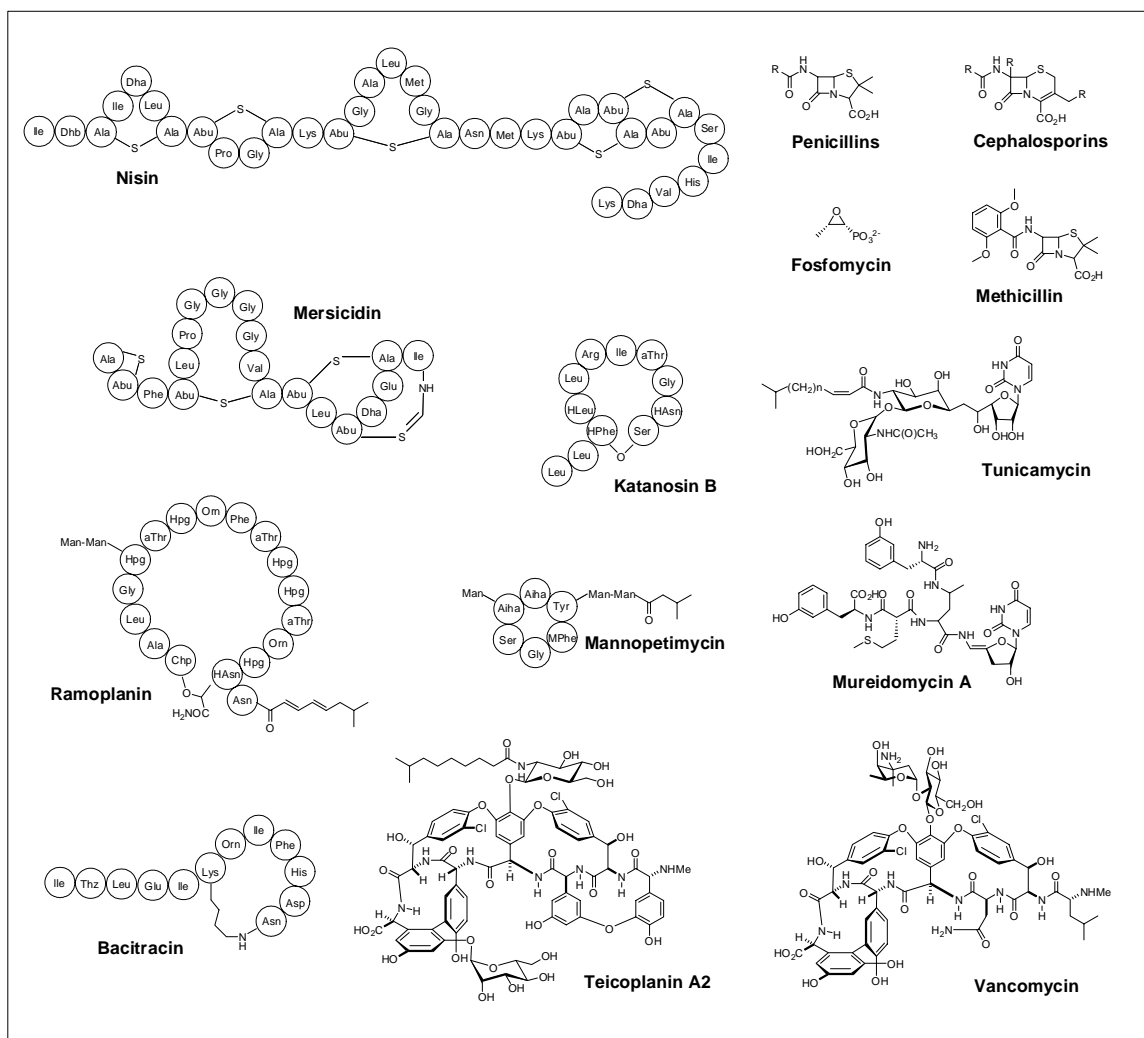
**Scheme I.5: Stage three of the bacterial biosynthetic pathway**

**Scheme I.5** illustrates the third and final stage of cell wall biosynthesis which occurs on the extracellular surface of the bacterial membrane. The action of MurG provided the final transformation in constructing the monomeric peptidoglycan unit Lipid II. The transfer of Lipid II across the cellular membrane to the periplasmic face, also called translocation, is believed to be effected by the action of a translocase carrier protein. However, there have not been any reports identifying such protein. After the transfer process, the membrane anchored dissacharyl pentapeptide then becomes a substrate for a series of polymerization reactions to link the glycan and peptide strands. First, membrane bound transglycosylases facilitate polysaccharide polymerization by making a  $\beta$ -(1,4) linkage between the C(4) OH of the terminal GlcNAc and the muramyl

C(1)-O-PO<sub>3</sub>, releasing the undecaprenyl carrier lipid<sup>60</sup>. The C<sub>55</sub> carrier lipid pyrophosphate is then hydrolyzed back to the phosphate via membrane bound phosphatases and subsequently translocated across the membrane for use in another cycle of lipid II synthesis. The final step to generate the fully mature peptidoglycan network involves the transpeptidases, also called *penicillin-binding proteins* (PBPs) which catalyze the amide bond formation between the terminal amino group of the Lys (DAP or attached peptide chain) residue and the penultimate D-Ala residue of an adjacent peptide strand.<sup>64-67</sup> The reaction results in the final cross-link for mature PG and the loss of a D-Ala residue.



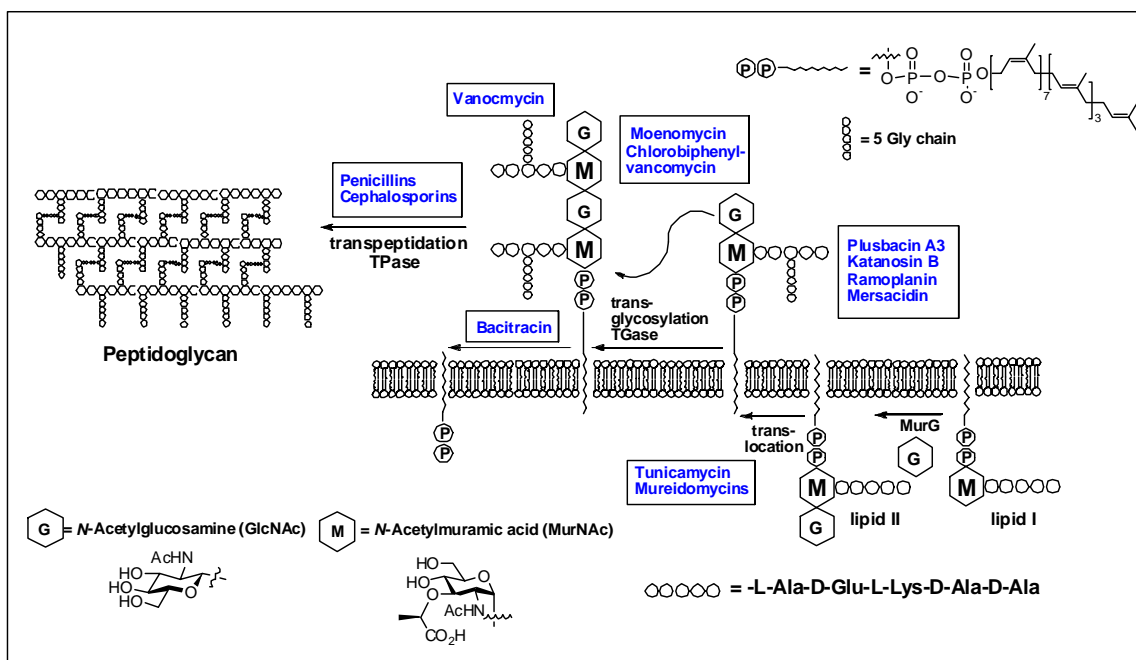
### I.3.2 Cell Wall Antibiotics



**Figure I.9: Structures of antibiotics that inhibit bacterial cell wall biosynthesis**

Antibacterial agents that inhibit bacterial cell wall biosynthesis are among the most important class of compounds used to treat bacterial infections. **Figure I.9** illustrates some of the most potent cell wall antibiotics known to date. These antibiotics consist of a unique variety of structurally different molecules such as  $\beta$ -lactams,

glycopeptides, lipoglycopeptides, lantibiotics and lipodepsipetides. Each of these antibiotics exert their activity at one or more steps in PG biosynthesis.

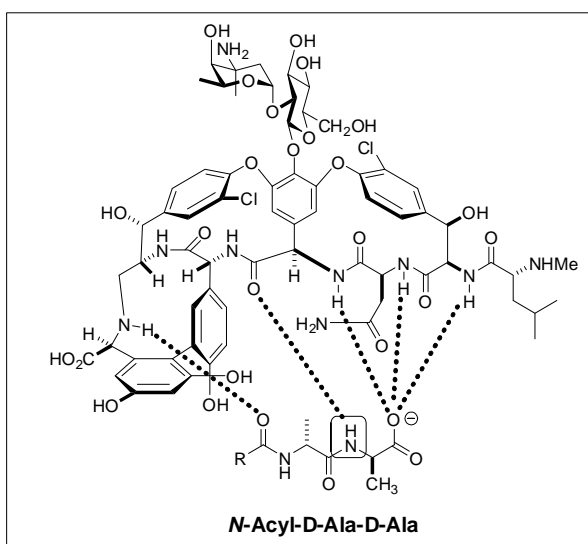


**Figure I.10: Representation of cell wall biosynthesis where cell wall antibiotics function**

The  $\beta$ -lactams are the most famous chemotherapeutics of this class dating back to the discovery of penicillin and its use during World War II.<sup>68</sup> This class of antimicrobials exert their mechanism of action by blocking the critical transpeptidation step in cell wall biosynthesis, **Figure I.10**. The defining structural features of these compounds include the four-membered  $\beta$ -Lactam ring fused to the five-membered ring system. The cephalosporins contain a six-membered ring system and function analogously to the penicillins. The  $\beta$ -lactams function as D-Ala-D-Ala mimics causing the acylation of the transpeptidase (PBP) active site serine. The acyl-enzyme intermediate is then rendered unable to participate in subsequent transpeptidation reactions. The rate at which the acyl

complex is hydrolyzed is slow enough to cause the pile up of inactive enzyme, shutting down the transpeptidation process, thus resulting in the bacterial cell lysis.<sup>69</sup>

Glycopeptides are an important class of antibiotics used to kill bacteria by inhibition of PG biosynthesis. Antibiotics in this class such as vancomycin and teichoplanin are often used as a last resort for the treatment of resistant Gram-positive pathogens.<sup>70</sup> Vancomycin was the first glycopeptide discovered and the first antibiotic identified to target lipid II.<sup>71</sup> Vancomycin exerts its activity by binding the terminal D-Ala-D-Ala dipeptide unit of the lipid II pentapeptide chain.<sup>72,73</sup> The sequestration of the PG precursor shuts down the transpeptidation process by preventing access of the transpeptidase. NMR studies have demonstrated that the molecular recognition process involves five key hydrogen bonds in the complex, **Figure I.10**.<sup>74,75</sup> Recent studies have suggested that vancomycin may also inhibit the transglycosylation process.<sup>18</sup>



**Figure I.11: Representation of the vancomycin binding interaction with the D-Ala-D-Ala PG subunit**

Ramoplanin is a lipoglycopeptide isolated from a bacterial strain of *Actinoplanes* spp.<sup>76</sup> The cyclic peptide is 2-10 times more active than vancomycin against Gram positive bacterial and a variety of resistant strains.<sup>77</sup> This compound has not yet been used clinically, however it is in phase III clinical trials in the US. Initial studies suggested that ramoplanin exerts its antibiotic activity by inhibiting the intracellular UDP-GlcNAc transferase (MurG) and the conversion of the lipid I intermediate to lipid II.<sup>78,79,80</sup> However, more recent studies have shown that it inhibits the polymerization of the lipid II glycan strands at the extracellular membrane face.<sup>81,82,83</sup> NMR analysis has demonstrated that ramoplanin achieves its activity through complexation with nascent peptidoglycan intermediates, lipid I and II<sup>84</sup>. The synthesis of ramoplanin analogs and semi-synthetic modifications have revealed key structure-activity relationships, uncovering the minimal structural features required for activity.<sup>85</sup> This work has shown the participation of the Hpg3-Orn10 residues at the ramoplanin-PG intermediate binding interface.<sup>86</sup> Since ramoplanin inhibits cell wall biosynthesis by sequestering lipid intermediates at a location different than that of vancomycin, it has become a promising candidate for the treatment of antibiotic resistant Gram-positive bacteria.

The lantibiotics are a large family of potent antibiotics active against cell wall biosynthesis. They often contain a variety of post-translationally modified non-proteogenic amino acids including lanthionines and dehydrated serines. Almost 50 lantibiotics have been discovered from approximately 30 different types of bacteria. Although none of the lantibiotics isolated have been successfully employed in the clinic, the mechanism by which they exert their activity has drawn considerable attention. Nisin

is one of the more extensively studied lantibiotics and is believed to exert its action via sequestration of lipid II and its ability to form pores in bacterial membranes.<sup>87,88,89,90</sup>

Another important lantibiotic is mersacidin, **Figure I.9**. Mersacidin is another member of the lantibiotic family of antibiotics that effect bacterial cell death by an interaction with lipid II.<sup>91</sup> The active form of the antibiotic consists of a unique polycyclic framework including four cross-linked lanthionine bridges. Extensive studies have shown that the flexible peptide backbone interacts with the sugar-pyrophosphate and lipid groups of Lipid II via electrostatic interactions, therefore shutting down the transglycosylation process. However, unlike ramoplanin, mersacidin does not bind lipid I<sup>92,93</sup>.

Due to the need for new antibiotics there has been a renewed interest in natural products. The mannopeptomycins were isolated from a *Streptomyces* strain during a set of screening experiments in the 1950's.<sup>94</sup> However, these compounds had not been re-examined until the beginning of this decade. This new class of antibiotics is characterized by a glycosylated cyclic peptide backbone containing a variety of post-translationally modified amino acids. This family of peptides is active against Gram-positive infections, in particular MRSA, making them a promising candidate for development and clinical use. Although detailed information on the mechanism of the mannopeptomycins is scarce, recent studies have shown that the antibiotic activity arises due to the binding of peptidoglycan intermediate, specifically lipid II. Additional experiments have demonstrated that they do not bind lipid I and may potentially be involved in the direct inhibition of the transglycosylation reaction mediated by high molecular-mass penicillin-binding.<sup>95,96</sup>

Moenomycin is a natural product glycolipid that inhibits the growth of a broad spectrum of Gram-positive bacteria as well as some Gram-negative bacteria.<sup>97</sup> Its structure is illustrated in **Figure I.9**. It consists of a pentasaccharide chain linked to a C<sub>25</sub> hydrophobic tail, moenocinol, via a phosphoric acid diester, and a glyceric acid group.<sup>98</sup> Moenomycin inhibits peptidoglycan synthesis at the transglycosylation stage, causing an accumulation of cell-wall intermediates, that leads to lysis and cell death. In contrast to the mechanisms previously discussed, moenomycin selectively targets the transglycosylase active site of the multifunctional high-molecular weight PBPs. Recent NMR studies have shown that the carbohydrate moiety of moenomycin functions as a lipid II substrate mimic, thus shutting down the glycan polymerization process.

The plusbacins and lysobactins comprise a promising new class of inhibitors of cell wall biosynthesis.<sup>99,100</sup> Both of these compounds are cyclic depsipeptides isolated from *Cytophaga* and *Pseudomonas*, respectively. Structurally, both compounds are similar to the ramoplanins and mannopeptomycins, containing numerous non-proteogenic amino acids with peptide backbones of alternating D and L amino acid residues. Both peptides are believed to exert the biological activity via complexation with PG intermediates, in particular lipid II. Although they are only active against Gram-positive bacteria, both demonstrate potent activity against MRSA and VRE<sup>101</sup>. A detailed explanation of plusbacin A3 and its activity will follow in subsequent chapters.

## I. 4 Antibiotic Resistance

As mentioned in previous chapters, the wide-spread use of antibiotics has caused the strong selective pressure for the evolution of antibiotic resistance. The obvious threat to public health has prompted not only the development of new antimicrobial agents, but the extensive investigation into the mechanisms of resistance. Although many antibiotic producing bacteria have their own intrinsic resistance mechanisms for self-immunity, this section will only cover those mechanisms which have resulted from the use of cell wall antibiotics.

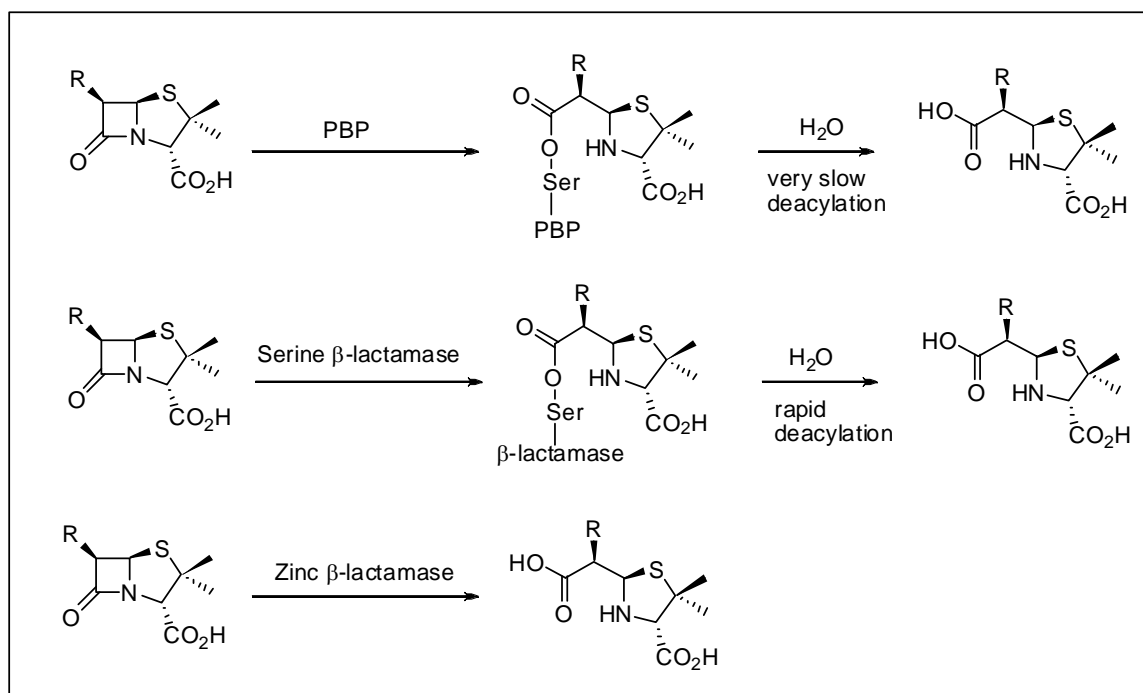
### I.4.1 General Resistance Mechanisms for Cell Wall Antibiotics

Antibiotic resistance is either natural or acquired. Acquired resistance is a direct result of bacteria's ability to accept plasmids and transposons that facilitate the genetic determinants of resistance. It has also been found to result from chromosomal mutations.<sup>5,102</sup> Natural resistance lies in the bacteria's intrinsic machinery that prevent the action of antibiotic agents. For example, *Pseudomonas aeruginosa* has been recognized as a "successful pathogen" in part by its ability to maintain minimum intracellular antibiotic concentrations.<sup>103</sup> This advantageous permeability property is maintained through the action of membrane porins.<sup>104</sup>

The main mechanisms of resistance in pathogenic bacteria are (i) modulation and prevention of antibiotic uptake through efflux pumps, (ii) the inactivation of antibiotics through modification, and (iii) modification or overproduction of the antibiotic target.<sup>102,105,106</sup> Resistance mechanisms involving efflux pumps will not be discussed since they are not known to inactivate antibiotics functioning at the cell wall interface.

Resistance mechanisms that involve the inactivation of antibiotics can be observed for several natural products classes, however, it has not occurred for many synthetic agents.<sup>18</sup> This evidence suggests that inactivation of antibiotics derived from synthetic libraries may be the most effective way of combating this resistance mechanism. One of the more famous example of this mechanism can be seen with  $\beta$ -lactams such as the penicillins and cephalosporins.<sup>107,108</sup> Bacteria render these antibiotics inactive through the hydrolysis and opening of the  $\beta$ -lactam four membered ring by hydrolytic enzymes called  $\beta$ -lactamases.<sup>109</sup> The result is a modified antibiotic that is unable to acylate its target, the active site serines of PBPs, **Figure I.12**.<sup>110</sup> The group A, C, and D lactamases also contain active site serines and generate an acyl-enzyme intermediate similar those formed from PBPs.<sup>111</sup> However, in contrast to the long lifetime of acyl-enzyme intermediates of PBPs, which deactivate them towards subsequent transpeptidation/transglycosylation reactions, the penicilloyl-lactamase intermediate lifetime is significantly shorter.<sup>111</sup> The rapid deacylation rate results in an inactive ring-opened penicillic acid derivative.



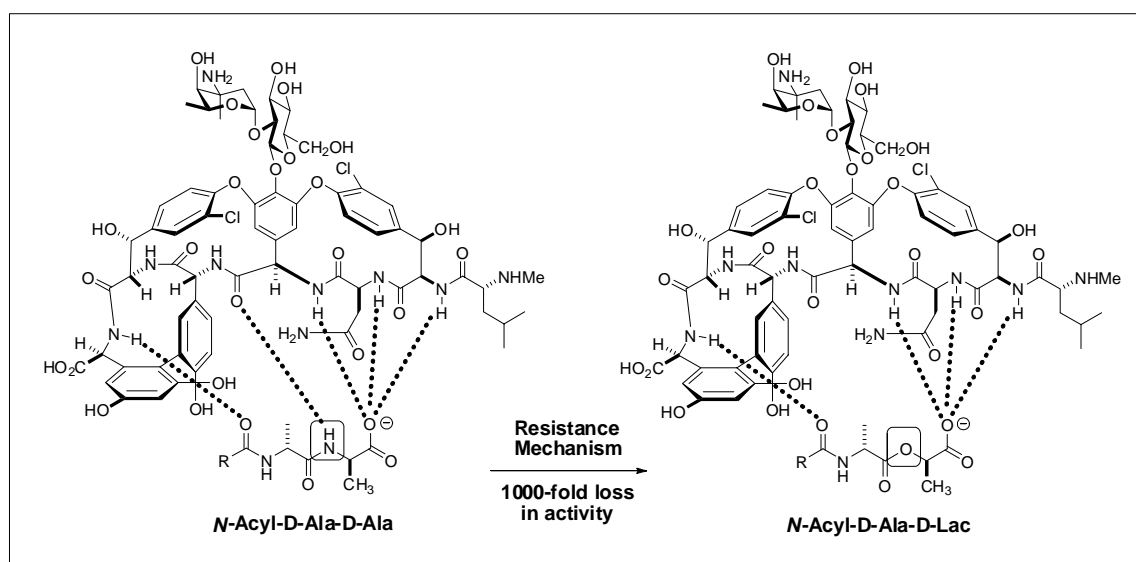


**Figure I.12: General representation of penicillin resistance mechanisms**

Fosfomycin is analogous to the  $\beta$ -lactams in that it also acylates its enzymatic target via a reactive epoxide. In contrast however, fosfomycin exerts its activity against MurA, as it functions as a PEP (phosphoenolpyruvate) mimic.<sup>112</sup> The epoxide is attacked by an active site cysteine (SH), inhibiting the initial cytoplasmic step in phase one of peptidoglycan biosynthesis. The acylation of MurA renders the enzyme unable to generate the enolpyruvyl ether of UDP-MurNAc.<sup>113</sup> The inactivation of fosfomycin is accomplished via capture of the epoxide with a soluble cosubstrate, a tripeptide glutathione.<sup>114</sup> Fosfomycin glutathione S-transferase (FosA), a metalloenzyme, is responsible for generating the ring opened thioether product.<sup>115</sup> This deactivation is not uncommon, as glutathione is abundant in bacteria where it is used in protection and detoxification processes.

The enzymatic deactivation of antibiotics is one of the most common acquired resistance mechanisms. Most clinical isolates are believed to receive their resistance genes on plasmids inherited from previous generations or from other microbial genera. Interestingly, the resistance genes for the  $\beta$ -lactams were discovered in 1940, several years before the introduction of penicillin into clinical use.<sup>116</sup> This suggests that nature had already selected for, and developed, resistance mechanisms for penicillin type compounds

Alteration of the antibiotic target is a major route to clinically significant bacterial resistance. This mechanism is used in situations where the bacteria cannot modify the antibiotic, for example, as seen for the synthetic sulfa-drugs and quinolones.<sup>106</sup> In general, pathogens modify the target so that it does not bind antibiotics, yet still allowing it to maintain regular cellular function. This resistance mechanism is accomplished by either gene transfer, which codes for new or replacement enzymes, or by the mutation of the target gene itself.



**Figure I.13: Representation of vancomycin resistance and loss of antibiotic activity**

Perhaps the best example of target modification is observed in vancomycin-resistant enterococci (VRE). The treatment of MRSA infections in the 1980s and 1990s with vancomycin selected for resistance in clinical settings. VRE has resulted in the identification of five resistant phenotypes for clinical isolates. These phenotypes have been termed VanA, VanB, VanC, VanD, and Van E.<sup>117</sup> Only VanC is an intrinsic resistance phenotype, while all other are acquired.<sup>118</sup> The acquired phenotypes are plasmid borne from which the determinant genes are have spread through transposable elements. The five-gene cassette required for phenotypes VanA, VanB and VanD produce enzymes that alter the peptidoglycan peptide terminus from N-acyl-D-Ala-D-Ala to N-acyl-D-Ala-D-lactate, **Figure I.12**.<sup>119</sup> The modification to the depsipeptide at the stem peptide terminus results in a decreased binding affinity of vancomycin to its target by thousand-fold. The phenotypes VanC and VanE cause a similar resistance mechanism by replacing the N-acyl-D-Ala-D-Ala to N-acyl-D-Ala-D-Ser, inducing a lower, yet still effective 10-fold drop in binding affinity.<sup>120</sup>

$\beta$ -lactam resistance has also been caused by the alteration of the antibiotic target. As mentioned earlier,  $\beta$ -lactamases produced by resistant strains, cause the inactivation and alteration of the antibiotic.<sup>106,108</sup> Additional resistance mechanisms have been identified in which the bacteria mutate the PBPs or acquire new PBPs with decreased affinities for the antibiotics that target them. For example, *Streptococcus pneumoniae*, that unlike other resistant strains, uses altered high molecular weight PBPs (1A, 1B, 2A, 2B, and 2X).<sup>121-124</sup> It has been determined that these alterations to low affinity PBPs are the result of interspecies homologous recombination.<sup>125,126</sup> MRSA also uses this

mechanism through an acquired *mecA* gene that encodes for altered PBPs termed PBP2A. Although the origin of *mecA* and the molecular basis of this decreased binding affinity are unknown, researchers have postulated that this phenotype arises from the horizontal gene transfer from other *Staphylococcus* species.<sup>106,127,128,129</sup>

Antibiotic resistance has caused a significant threat to public health and has underscored the need for new antibiotics functioning by different mechanisms. With only four major targets and several minor targets validated, the discovery of new inhibition pathways is crucial to the development of new clinically useful antimicrobials. Although, the primary strategy of the pharmaceutical industry has been to elaborate on existing drugs through the synthesis of 2<sup>nd</sup> and 3<sup>rd</sup> generation antibiotics, this approach is lengthy, expensive, and will only lead to the same resistance problems.<sup>130</sup> The interest in bioinformatics and genomic sequencing may help to identify new targets allowing for development of new screening methods.<sup>131-133</sup> Combinatorial chemical synthesis may also aid in the discovery of new antibiotics.<sup>134</sup> The isolation, identification and screening of natural products has gained considerable attention and may provide new molecular scaffolds with different mechanisms of action. In addition, perhaps most importantly, society will need to rethink its strategies for extending the clinically useful lifetime of the antibiotic arsenal.<sup>135,130,</sup>

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# CHAPTER II

## TOTAL SYNTHESIS OF PLUSBACIN A3

### II.1 INTRODUCTION

With the discovery of penicillin more than 75 years ago, the use of antibiotics has greatly impacted the treatment of bacterial infections. The widespread use, and often misuse, of antibiotics has resulted in the emergence of a variety of multi-drug resistant bacteria. With this in mind, it should be noted that at present, there are no clinically used antibiotics to which resistance has not been developed.<sup>1</sup> The incidence of healthcare-associated staphylococcal infections that are due to MRSA has been increasing: 2% of *S. aureus* infections in U.S. intensive-care units were MRSA in 1974, 22% in 1995, and 64% in 2004.<sup>2</sup> Since the first observed case of VRE in 1989, the incidence of enterococcal isolates resistant to vancomycin reported to the National Nosocomial Infections Surveillance System increased 20-fold.<sup>3</sup> These statistics illustrate the alarming rise in resistant strains since the early 1980's. It is overwhelming that the decreasing arsenal of useful antibiotics is a significant threat to public health, underlining the pressing need for the development of new antibiotics.

New drug candidates must function via different modes of action than those to which bacterial resistance has developed. Although several targets exist, perhaps one of the most attractive is bacterial cell wall biosynthesis.<sup>4</sup> Cell wall biosynthesis has drawn

attention from pharmaceutical developers for a variety of reasons. First, cellular penetration and diffusion is not a requirement for biological activity. Second, there are no mammalian counterparts to the enzymatic reactions involved in peptidoglycan biosynthesis. This allows for selective toxicity towards bacterial cells.

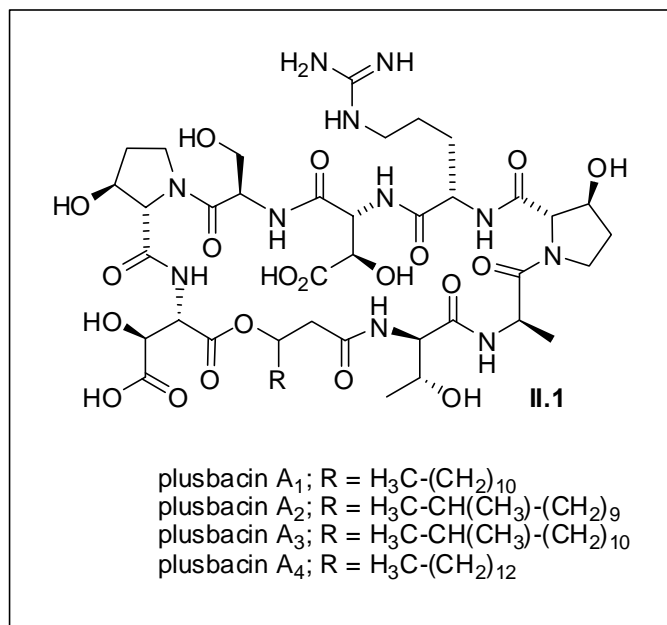
There are a variety of ways antibiotics can disrupt peptidoglycan synthesis. For example, tunicamycin and mureidomycin act as UDP-muramyl pentapeptide mimics, inhibiting *MraY* enzyme. They prevent the cytoplasmic translocation of membrane bound peptidoglycan intermediates across the cellular surface thereby suppressing the formation of lipid I and II.<sup>5</sup> Ramoplanin and mersacidin have been shown to act upstream of the cytoplasmic *MurG* glycotransferase reaction, functioning at the periplasmic membrane face via complexation of the membrane anchored lipid II substrate, therefore preventing its participation in the subsequent transglycosylation reaction.<sup>6-11</sup> Moenomycin and chlorobiphenyl-vancomycin also act to block the formation of nascent peptidoglycan.<sup>12-13</sup> Yet another glycopeptide, vancomycin, acts through binding the terminal D-Ala-D-Ala subunit of the peptidoglycan pentapeptide chain.<sup>14</sup> This blocks the access to the transpeptidase enzymes (recent studies also support evidence of transglycosylation suppression), and therefore inhibits the cross-linking transpeptidation reaction. The penicillins and cephalosporins also inhibit this step of bacterial cell wall biosynthesis; however, their mode of action differs, as they function as “suicide agents” acylating the transpeptidase active site, rendering it incapable of performing the transpeptidation reaction.<sup>14</sup>

Most of the cell wall drugs being clinically used or in clinical trials are natural products or derivatives of them. The advantage of these drugs is that nature has already

done much of the R&D work. However, a major disadvantage is that many of organisms producing the molecules, often bacteria, have evolved an innate immunity towards the toxins.<sup>14</sup> The danger of bacteria transferring the genetic information required for resistance/immunity is a risk we take in order to combat bacterial infections.

Vancomycin has long been the drug of last resort for treating infections caused by resistant Gram-positive bacteria such as methicillin-resistant *Staph. aureus*. However, vancomycin resistance is now common.<sup>15</sup> As mentioned above, vancomycin achieves its activity by complexing the D-Ala-D-Ala subunit of peptidoglycan and blocking the transpeptidation reaction<sup>16</sup>. Bacteria have evolved a resistance mechanism that results in a 1000-fold loss in antibiotic activity.<sup>17</sup> Given the increasing prevalence of these pathogens, a vancomycin alternative is of the utmost importance.

Plusbacin A3 (**II.1**) is a lipodepsipeptide antibiotic isolated from a strain of *Pseudomonas* sp. PB-6250 in the Okinawa prefecture of Japan.<sup>18</sup> It is one of eight cyclic peptides isolated from a family of compounds which differ only in the structure of their fatty acid side chains.<sup>19</sup> It has been shown to exhibit potent *in vitro* activity upon subcutaneous administration to mice infected with *Staphylococcus aureus*. Plusbacin A3 showed inhibitory activities of 0.78-3.13 µg/mL against vancomycin resistant enterococci and methicillin-resistant *staphylococcus aureus* with IC<sub>50</sub> values close to their MIC values.<sup>20</sup>



**Figure II.1: Structure of plusbacins A1-A4.**

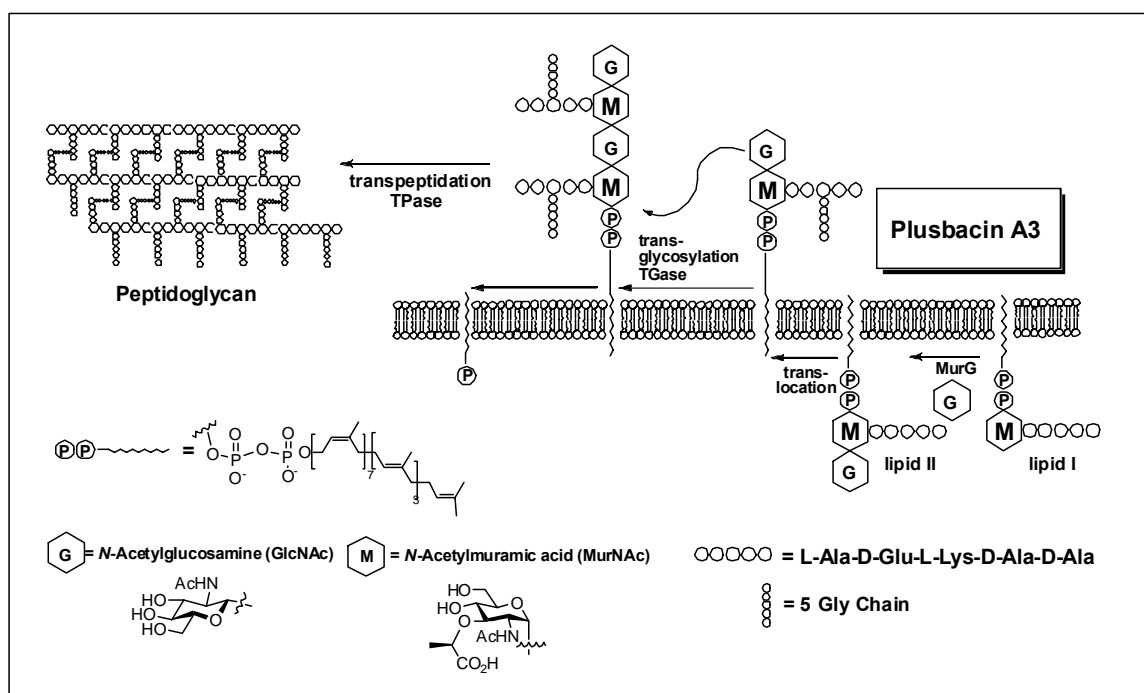
A previous biological evaluation demonstrated the antibiotic activity of plusbacin A3 as judged by its ability to inhibit the incorporation of radio-labeled meso-diaminopimeolic acid into the peptidoglycan layer of a *Bacillus* strain. Plusbacin A3 also demonstrated the ability to inhibit the incorporation of radio-labeled [<sup>14</sup>C]GlcNAc into the peptidoglycan layer of whole cells with an IC<sub>50</sub> of 0.4 µg/mL. In the same study, plusbacin A3 inhibited the formation of nascent peptidoglycan as did vancomycin, whereas it also inhibited the formation of lipid intermediates, unlike vancomycin.<sup>20</sup>



**Table II.1: MIC values for plusbacin A3 and vancomycin**

Strain	Phenotype	MIC (µg/ml)	
		Plusbacin A3	Vancomycin
<b><i>Staphylococcus aureus</i></b>			
NCTC8325	Mc <sup>s</sup>	0.78	1.56
Smith(diffuse)	Mc <sup>s</sup>	0.78	0.78
SRM133	Mc <sup>s</sup>	0.78	1.56
SR3637	Mc <sup>s</sup>	1.56	1.56
Mu50	Mc <sup>s</sup> Vm <sup>i</sup>	1.56	12.5
<b><i>Enterococcus faecalis</i></b>			
SR1004	Vm <sup>s</sup>	3.13	1.56
SR7914	Vm <sup>r</sup> (VanA)	1.56	>50
<b><i>Enterococcus faecium</i></b>			
SR15941	Vm <sup>s</sup>	3.13	1.56
SR7917	Vm <sup>r</sup> (VanA)	3.13	>50

In contrast to the inhibitory effects of vancomycin, the addition Ac-Lys-D-Ala-D-Ala (50 µg/mL) to whole cells neither suppressed the formation of nascent peptidoglycan nor did it reduce its efficacy. This experiment indicated that plusbacin A3 inhibits peptidoglycan biosynthesis via a mechanism different from that of vancomycin. This differing mode of action would mean that mechanisms of resistance to vancomycin would be rendered ineffective against plusbacin A3. This is supported by the antibiotic activity against various VRE<sup>20</sup>. Although its antimicrobial mechanism is not understood in full detail, the activity is believed to arise via its complexation to lipid II intermediates. This would prevent successive enzymatic reactions, disrupting the bacterial cell wall, causing cellular rupture due to osmotic pressure.



**Figure II.2: Late stages of bacterial cell wall biosynthesis**

## II.2 RESEARCH DESIGN AND SYNTHESIS

The objective of our antibiotic program is to learn as much about the antibiotic as possible, encompassing all areas synthetic, structural, and biological. The first goal was to design and carry out a convergent total synthesis of plusbacin A3 which would be amenable to analogs. From here we could then pursue a mechanistic evaluation through SARs, ultimately aiding in the development of novel antibiotics with enhanced activity.

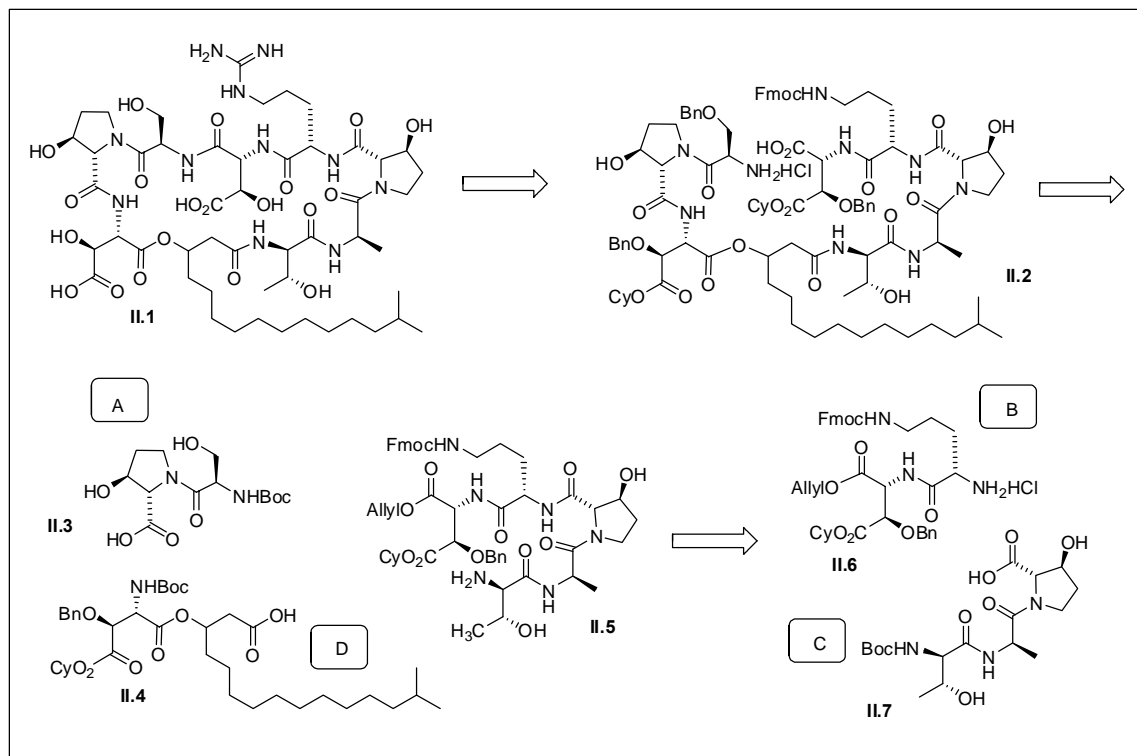
Edman degradation of the acidic hydrolysate, (deacylated products), supported by mass spectrometric studies, confirmed the amino acid sequence of plusbacin A3. The chiralities of the amino acids were determined by direct comparison with reference amino acids via chiral HPLC. Degradation studies have also shown a depsipeptide linkage between an *L-threo*- $\beta$ -hydroxy-aspartic acid and a 3-hydroxyisopentanoic acid.

However, the stereochemical configuration at this carbon stereocenter remains unassigned.

A survey of the plusbacin A3 amino acid constitution shows a backbone decorated with a variety of non-proteogenic amino acids. The cyclic peptide contains an *L-threo*- $\beta$ -hydroxy-aspartic acid, *D-threo*- $\beta$ -hydroxy-aspartic acid, *D-allo*-Threonine, and two *trans*-3-hydroxy-L-prolines. The synthesis of these unnatural amino acids coupled with the sensitivity of the lactone linkage (which is prone to base catalyzed  $\beta$ -elimination) makes plusbacin A3 a challenging molecule for total synthesis.

## II.2.1 RETROSYNTHETIC ANALYSIS

The retrosynthetic analysis for plusbacin A3 is presented in **Figure II.1**. The amide bond to be made at the D-Ser/ D- $\beta$ OH-Asp junction for the macrocyclization was chosen based on preliminary data from a simplified model system. From here, the linear peptide was assembled from four key fragments (**II.3**, **II.4**, **II.6**, **II.7**) each of equal complexity. The analysis was guided by the necessity for flexibility in amide bond formation and amenability to analog synthesis. For example, we were drawn to the possibility that the fatty acid side chain and arginine residue may play an important role in biological activity. Therefore, we desired a strategy that would easily facilitate modifications in the lipophilic moiety and guanidine functionality.



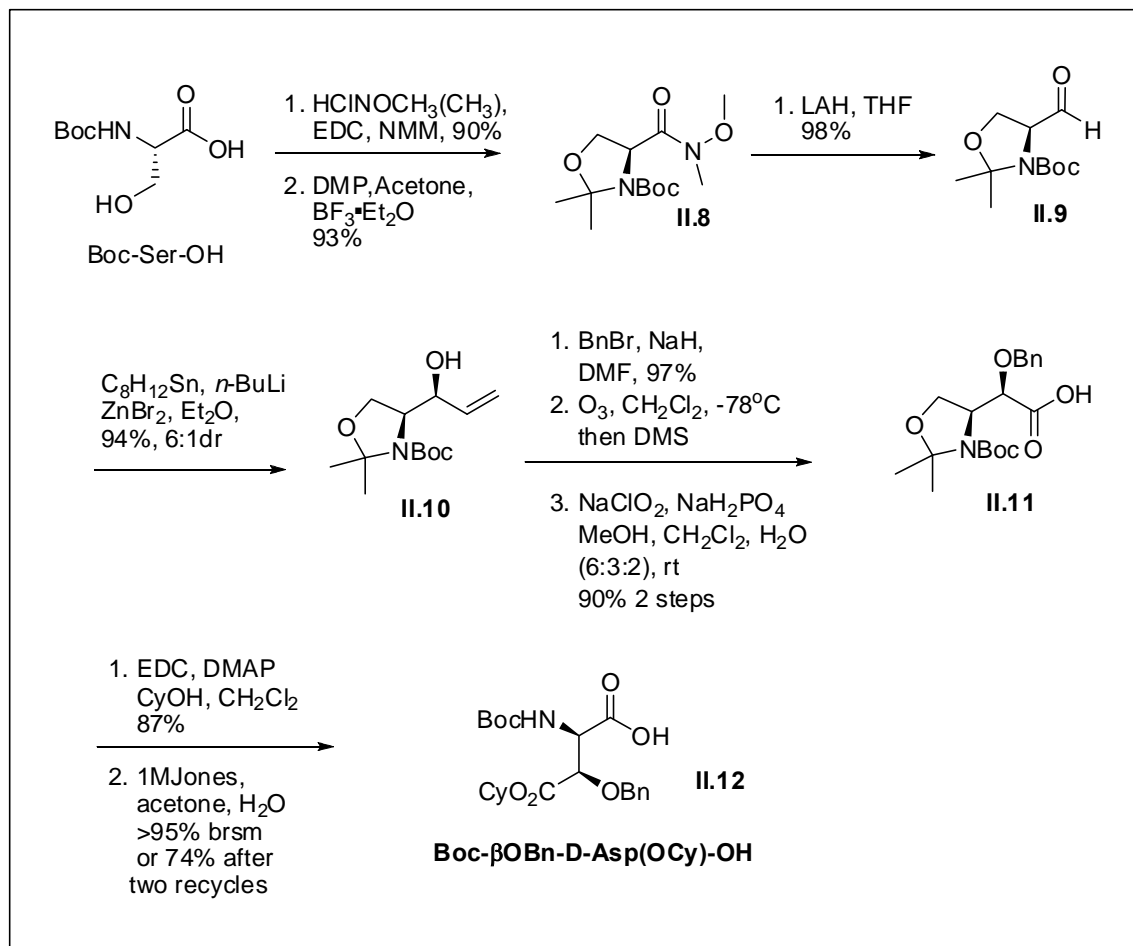
**Scheme II.1: Retrosynthetic analysis for plusbacin A3**

## II.2.2 SYNTHESIS OF NON-PROTEOGENIC AMINO ACIDS

The synthesis of  $\beta$ -hydroxy amino acids is of current interest due to their presence in a variety of biologically active peptides, their synthetic utility, and pharmacological profiles. In recent years, various methods have been reported for the construction of  $\beta$ -hydroxy amino acids, such as asymmetric aminohydroxylations<sup>21</sup>, Sharpless asymmetric dihydroxylations<sup>22</sup>, asymmetric epoxidations<sup>23</sup>, sulfonamide mediated asymmetric Strecker reactions<sup>24</sup>, cycloaddition of chiral azomethine ylides<sup>25</sup>, asymmetric aldol reactions<sup>26</sup>, alkylation of chiral enolates from oxazolidines<sup>27</sup>, oxazolidinones<sup>28</sup>, bis-lactam ethers<sup>29</sup> and imiazolidines<sup>30</sup>, enzymatic transformations<sup>31</sup>, and a variety of others.

### II.2.2.a SYNTHESIS OF $\beta$ -OH-ASPARTIC ACID

Our synthesis of plusbacin A3 (**II.1**) required an efficient, stereoselective synthesis of an orthogonally protected  $\beta$ -hydroxy aspartic acid. Additionally, the synthetic route required access to both enantiomeric forms of the aspartate derivative. Several reports of both syn (erythro) and anti (threo) aspartates have been published<sup>32-39</sup>, however, none provided a stereoselective, orthogonally protected aspartate derivative suitable for both solid phase and solution chemistry. The utility of certain side chain protecting groups, for example  $\beta$  benzyl esters<sup>40</sup>, is limited due to the need to suppress of aspartamide formation during standard peptide chain elongation protocols. As beta-*t*-Bu esters have been the standard in Fmoc based peptide synthesis, our aim was to utilize an aspartate derivative with side chain protection stable both acidic and basic conditions. The use of a cyclohexyl ester became the obvious choice for this objective.<sup>41</sup>




**Scheme II.2: Preparation of Boc-βOBn-D-Asp(OCy)-OH**

**Scheme II,2** presents the synthesis of the Boc-βOBn-D-Asp(OCy)-OH (**II.12**) derivative used in our total synthesis. The synthetic strategy is based on a previous report by Shimamoto in 2000<sup>42</sup>, from which several modifications and optimizations were made. The commercially available Boc-Ser-OH was taken from the chiral pool and used as a starting point for our synthesis. The oxazolidine (**II.8**) was prepared via coupling Boc-Ser-OH with *N,O*-dimethylhydroxylamine hydrochloride (EDCI, NMM, CH<sub>2</sub>Cl<sub>2</sub>, 90%) and subsequent protection (DMP, Acetone, BF<sub>3</sub>Et<sub>2</sub>O, 93%). Hydride reduction of the Weinreb amide afforded “Garners Aldehyde” (**II.9**) (LAH, THF, 98%), a chiral synthon,

which set the stage for the installation of the second stereocenter<sup>43-44</sup>. A substrate controlled, diastereoselective alkylzinc mediated vinyl addition, ((vinyl)<sub>4</sub>Sn, n-Buli, ZnBr<sub>2</sub>, Et<sub>2</sub>O, 94%, 6:1dr) gave the desired syn-allylic alcohol (**II.10**) in excellent yields and good diastereomeric ratio.<sup>42,45</sup> It should be noted, that although other coordination metals (MgBr-Et<sub>2</sub>O) offered superior diastereoselectivities (dr = 9:1), only the alkyl zinc reagents provided acceptable yields<sup>46</sup>. The allylic alcohol was then protected as the benzyl ether (BnBr, NaH, DMF, 97%) in excellent yield.

Oxidation of the olefin to the carboxylic (**II.11**) acid proved troublesome. **Table II.2** presents the various conditions for the oxidation reaction. Experimentation with different oxidation conditions resulted in low, capricious yields and unexpected side reactions. One step procedures to oxidize the olefin failed to produce acceptable yields. Further experimentation with olefin oxidations revealed that the two step sequence (1. O<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, -78°C, DMS, 90%, 2. NaClO<sub>2</sub>, NaH<sub>2</sub>PO<sub>4</sub>, MeOH, CH<sub>2</sub>Cl<sub>2</sub>, H<sub>2</sub>O, 98%) afforded the desired carboxylic acid (**II.2**) with excellent yields and purity.

Table II.2: Oxidation conditions in preparation of carboxylic acid II.11

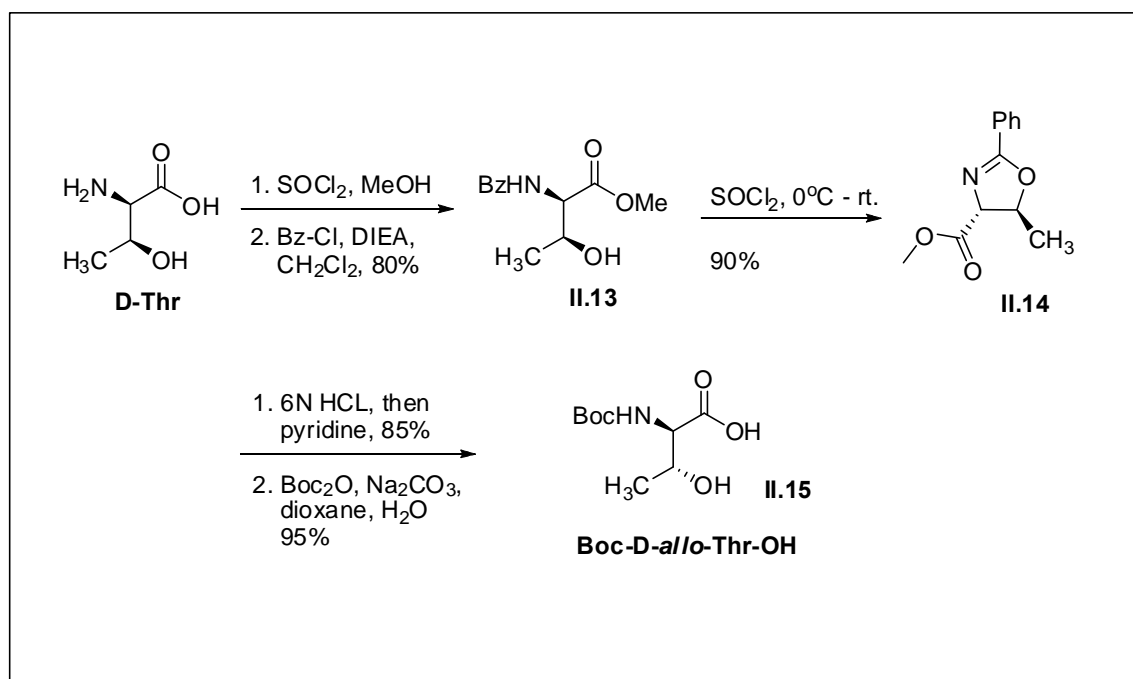
		
R	Conditions	Yield
vinyl	OsO <sub>4</sub> (1 mol%), Oxone, DMF, 3 hr	<5
vinyl	OsO <sub>4</sub> (1 mol%), Oxone, DMF, NaHCO <sub>3</sub> , 3 hr	<5
vinyl	1. O <sub>3</sub> , CH <sub>2</sub> Cl <sub>2</sub> , -78 °C 2. H <sub>2</sub> O <sub>2</sub>	7
vinyl	1. O <sub>3</sub> , CH <sub>2</sub> Cl <sub>2</sub> , -78 °C DMS, 90% 2. NaClO <sub>2</sub> , NaH <sub>2</sub> PO <sub>4</sub> , MeOH CH <sub>2</sub> Cl <sub>2</sub> , H <sub>2</sub> O, 98%	90
vinyl	1. O <sub>3</sub> , CH <sub>2</sub> Cl <sub>2</sub> , -78 °C DMS, 70% 2. NaClO <sub>2</sub> , NaH <sub>2</sub> PO <sub>4</sub> <i>t</i> -BuOH, cyclohexene	63

The carboxylic acid (**II.11**) was then subjected to esterification conditions (EDCI, DMAP, CyOH, 87%) to afford the suitably protected cyclohexyl ester. A one-pot deprotection and subsequent oxidation of the resulting primary alcohol (1M Jones, acetone, H<sub>2</sub>O, 74%) provided the orthogonally protected Boc-*D-threo*-βOBn-Asp(OCy)-OH (**II.12**) needed for our total synthesis<sup>47</sup>.



### II.2.2.b SYNTHESIS OF D-*ALLO*-THREONINE

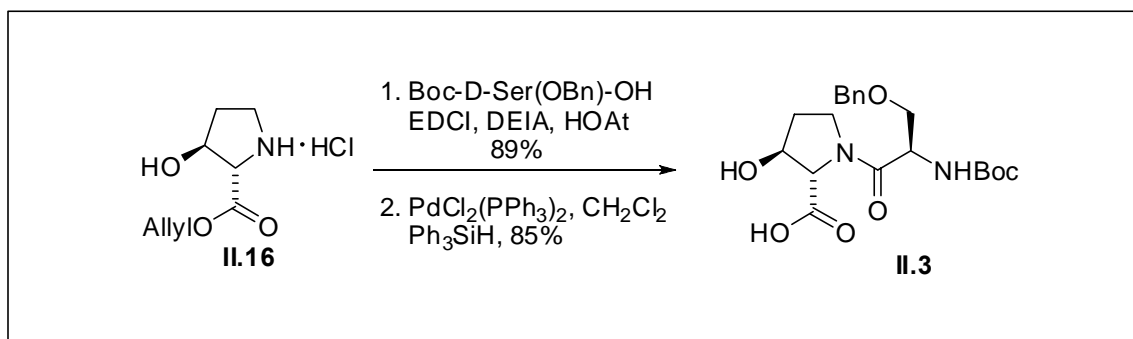
Although a variety of syntheses for D-*allo*-threonine had been reported in the literature, many were often long, or required expensive reagents. The necessary D-*allo*-threonine derivative (**II.15**) was prepared according to the Elliot method<sup>48</sup>, which was very scalable and efficient, **Scheme II.3**. The synthesis initiated with the esterification of D-threonine (SOCl<sub>2</sub>, MeOH, quant.) and subsequent protection of the amine (BzCl, DIEA, CH<sub>2</sub>Cl<sub>2</sub>, 80%). Inversion of the βOH stereocenter (SOCl<sub>2</sub>, 90%) afforded the oxazoline (**II.14**) in good yield. Hydrolysis (6N HCl, reflux, 85%) of the ring generated the amino acid intermediate which was then protected (Boc<sub>2</sub>O, Na<sub>2</sub>CO<sub>3</sub>, dioxane, H<sub>2</sub>O, 95%) to afford the synthetically useful Boc-D-*allo*-Threonine derivative.



**Scheme II.3: Synthesis of Boc-D-*allo*-Threonine**

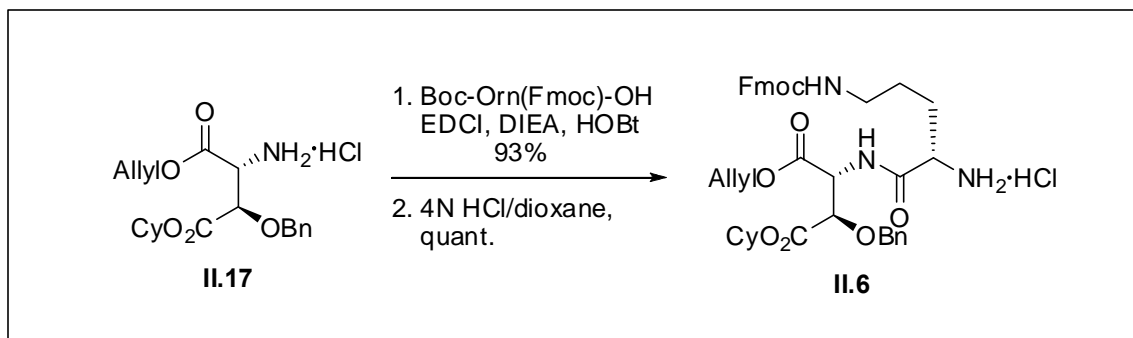
### II.2.3 SYNTHESIS OF FRAGMENTS II.3 and II.6

The synthesis of dipeptide fragment **II.3** is illustrated in **Scheme II.4**. The synthesis began with the commercially available *trans*-3-hydroxy-L-proline which, after protecting group manipulations (**II.16**), was coupled to the suitably protected Boc-D-Ser(OBn)-OH (EDCI, DIEA, HOAt, 89%) to afford the dipeptide. The allyl ester protecting group was then removed under standard deprotection conditions to afford the carboxylic acid **II.3** in overall high yield.



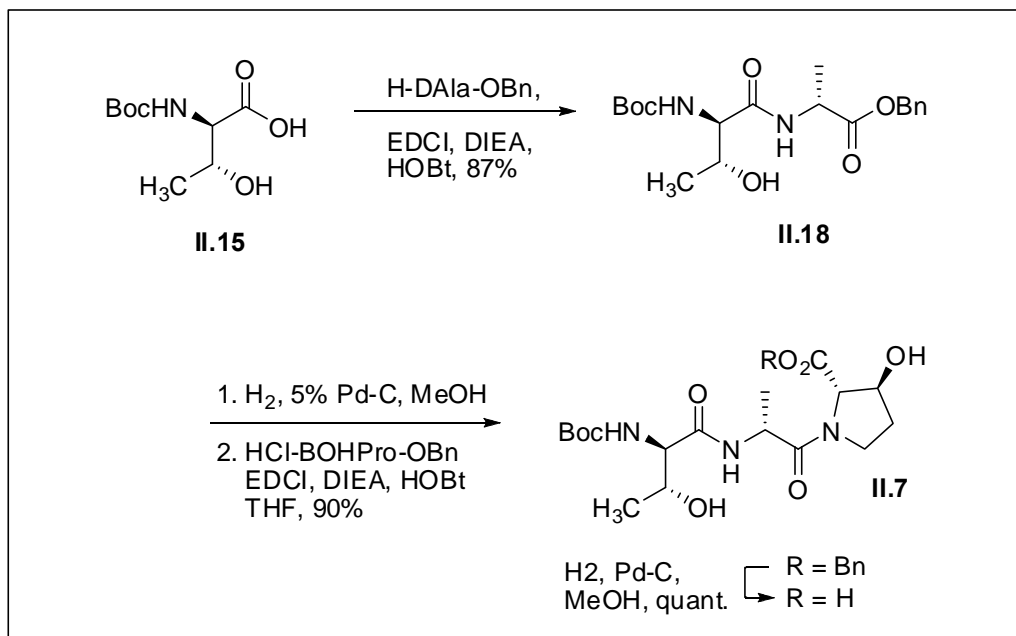
**Scheme II.4: Synthesis of dipeptide fragment II.3**

The synthesis of dipeptide fragment **II.6** is represented in **Scheme II.5**. The previously prepared *D-threo*-hydroxy-aspartate derivative (**II.17**) was coupled to the orthogonally protected Boc-Orn(Fmoc)-OH (EDC, DIEA, HOBt, 93%) to afford the corresponding dipeptide. Clean deprotection of the Boc protecting group (4N HCl, Dioxane, quant.) afforded dipeptide **II.6** as its hydrochloride salt.



**Scheme II.5 Preparation of dipeptide fragment II.6**

The preparation of tripeptide **II.7** (**Scheme II.7**) was initiated from the previously synthesized Boc-D-*allo*-Thr-OH (**II.15**). Coupling of this residue with H-D-Ala-OBn (EDCI, DIEA, HOBT, 87%) afforded the dipeptide (**II.18**) in good yield. The C-terminal benzyl ester was removed via hydrogenolysis (H<sub>2</sub>, 5% Pd/C, quant.) to afford the carboxylic acid which was then coupled with HCl-*trans*-β-OH-Pro-OBn (EDCI, DIEA, HOBT, 90%). The hydrogenolysis of the C-terminal benzyl ester (H<sub>2</sub>, 5% Pd/C, quant.) cleanly afforded the desired carboxylic acid, tripeptide **II.7**.

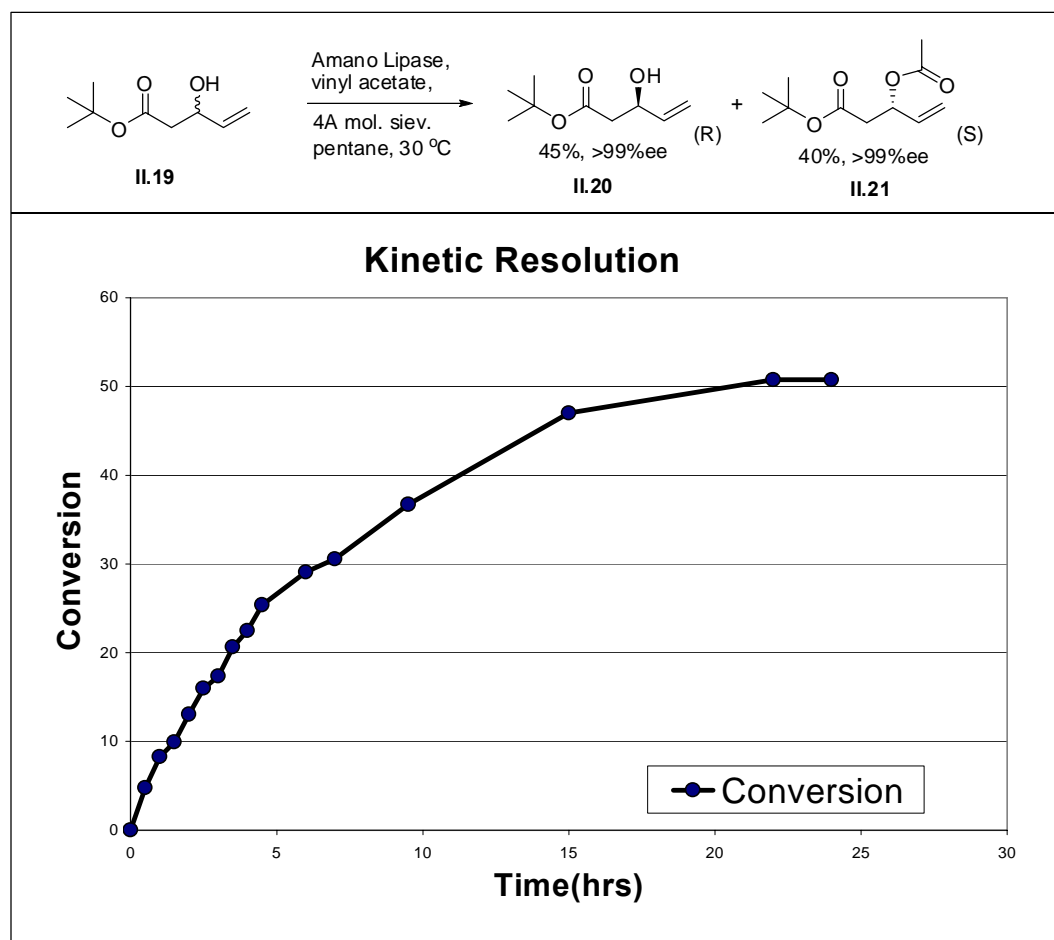


**Scheme II.6: Synthesis of tripeptide II.7**

## II.2.4 SYNTHESIS OF 3-HYDROXY FATTY ACID SUBUNIT AND DEPSIPEPTIDE FRAGMENT

As stated above, the stereochemistry at the lactone stereocenter was previously unassigned, and given this uncertainty, our synthetic route to the suitably protected 3-acetoxy-isopentanoate intermediate required access to both enantiomeric forms. Given the utility of the alkene functionality, we envisioned it as a handle for an olefin cross metathesis reaction. This would allow for the incorporation of the rest of the fatty acid side chain as well as a variety of other side chain modifications. The kinetic resolution illustrated in **Figure II.3** was accomplished utilizing the Amano PS Lipase to afford the (R)-allylic alcohol (**II.20**) and (S)-allylic (**II.21**) acetate in excellent enantiomeric purities (ee's determined by chiral HPLC analysis of the respective p-nitrobenzoate derivatives)<sup>49-</sup><sup>51</sup>. The efficiency of the resolution lies in the ability to closely monitor and control the conversion. **Figure II.3** represents a typical resolution from which the % conversion can

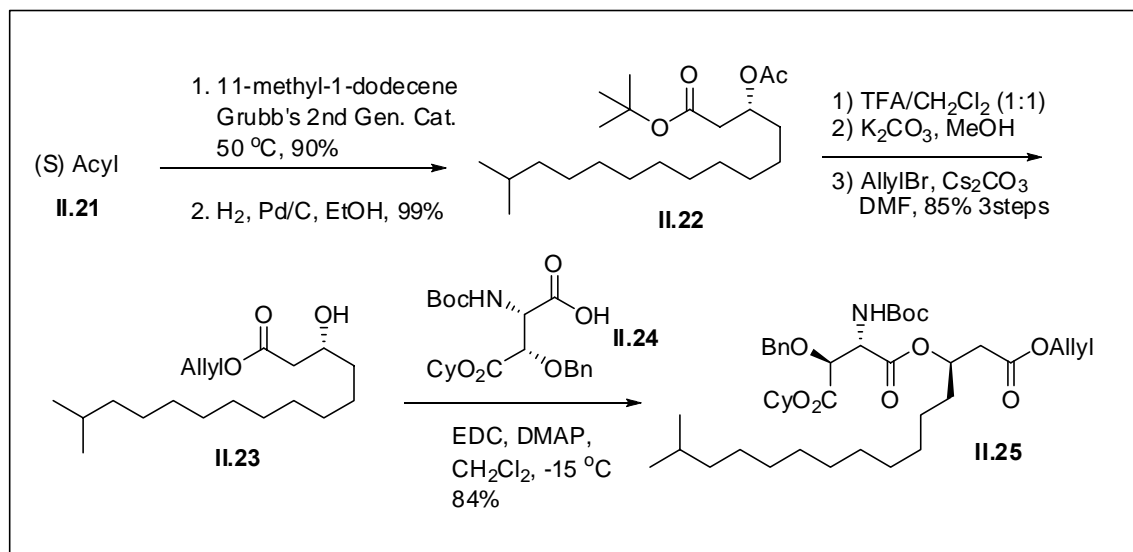
be followed by comparative integrations of the  $\beta$ -protons from  $^1\text{H}$  NMR at various reaction time points.



**Figure II.3: Enzymatic kinetic resolution of racemic alcohol**

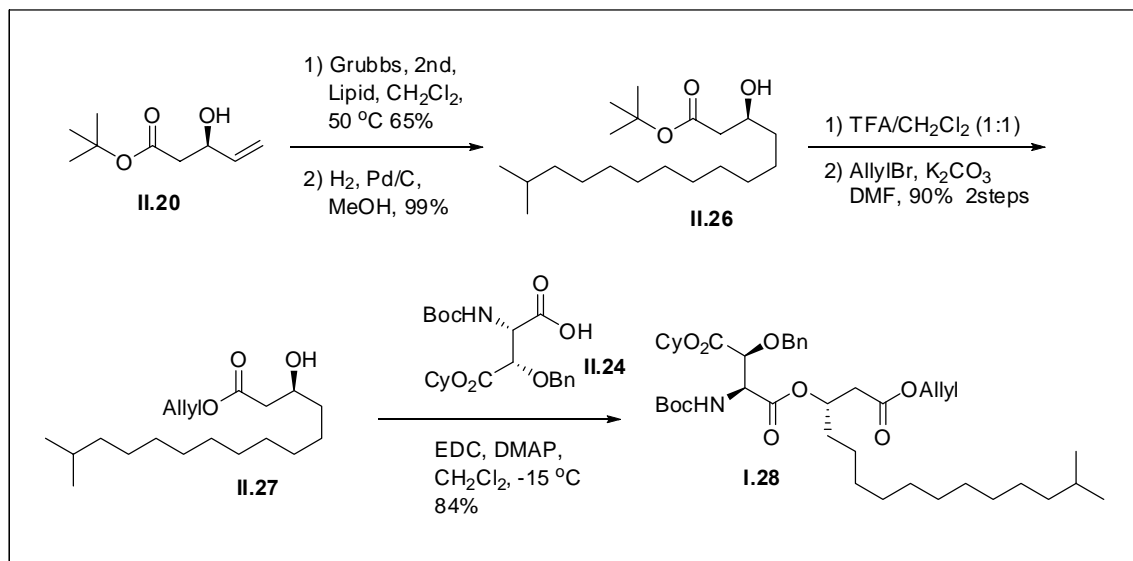
**Scheme II.8** presents the synthesis of the depsipeptide fragment (**II.25**) containing an (R)-configuration at the lactone stereocenter. An olefin cross metathesis reaction between (S)-allylic acetate (**II.21**) and 11-methyl-1-dodecene using Grubb's 2<sup>nd</sup> generation catalyst followed by hydrogenolysis ( $\text{H}_2$ , Pd/C, MeOH) of the resulting alkene afforded the acetoxyester (**II.22**) in good overall yield<sup>52</sup>. A series of protecting group

manipulations including the removal of the tert-butyl ester (TFA/CH<sub>2</sub>Cl<sub>2</sub>), deprotection of the acetate (K<sub>2</sub>CO<sub>3</sub>, MeOH) and protection of the carboxylic acid (AllylBr, K<sub>2</sub>CO<sub>3</sub>, DMF,) provided the desired allyl ester (**II.23**) in 90% overall yield. Coupling this alcohol with Boc-L-*threo*-βOH-Asp(OCy)-OH (**II.24**) (EDCI, DMAP, 84%) cleanly afforded the orthogonally protected target fragment **II.25**.



**Scheme II.7: Synthesis of depsipeptide fragment II.25**

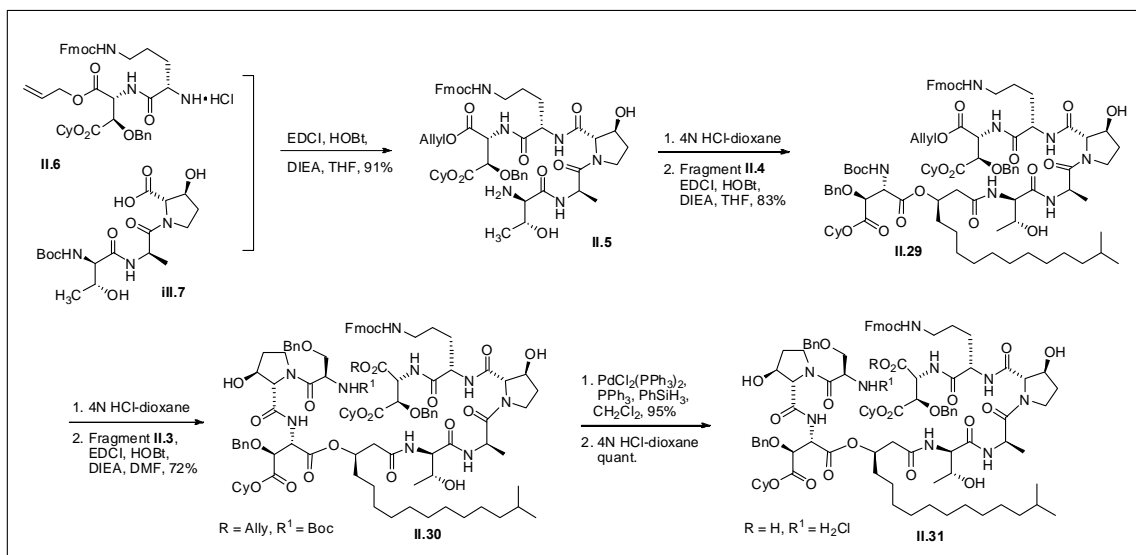
**Scheme II.9** illustrates the synthesis of fragment **II.28** containing an (S)-configuration at the lactone stereocenter. An olefin cross metathesis reaction between (R)-allylic alcohol and 11-methyl-1dodecene using Grubb's 2<sup>nd</sup> generation catalyst followed by hydrogenolysis (H<sub>2</sub>, Pd/C, MeOH, quant.) of the resulting alkene provided the hydroxy-ester. Deprotection of the tert-butyl ester (TFA/CH<sub>2</sub>Cl<sub>2</sub>) and esterification (allyl-Br, K<sub>2</sub>CO<sub>3</sub>, DMF) gave the hydroxyl ester in good overall yield. Coupling of this intermediate to Boc-L-*threo*-βOH-Asp(OCy)-OH afforded fragment **II.28** with the desired (S) stereochemical configuration.



**Scheme II.8** Synthesis of depsipeptide fragment **II.28**

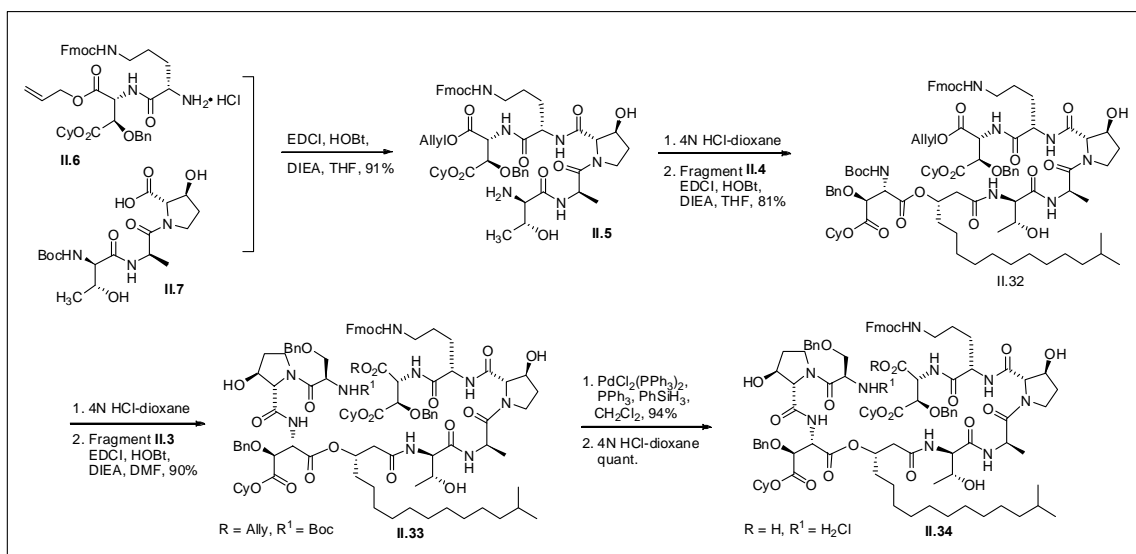
## II.2.5 SCAFFOLD ASSEMBLY AND FINAL STEPS

With the four fragments complete the stage was now set for their assembly and incorporation into the plusbacin A3 backbone (**Scheme II.10**). The coupling of fragments **II.6** and **II.7** (EDCI, DIEA, HOBt, 91%) gave the pentapeptide (**II.5**) in excellent yield. Removal of the N-terminal Boc protecting group (4N HCl-dioxane, quant.) followed by its coupling with the carboxylic acid of fragment **II.4** (EDCI, HOBt, DIEA, 83%) cleanly provided the septapeptide (**II.29**). Boc removal (4N HCl-dioxane) and coupling of the dipeptide fragment (**II.3**) (EDCI, HOBt, DIEA, 72%) afforded the fully protected linear depsipeptide (**II.30**). Removal of the allyl ester and cleavage of the N-terminal Boc group gave peptide **II.31**, precursor to the key macrocyclization reaction.



**Scheme II.9: Incorporation of key fragments and assembly of linear peptide II.31**

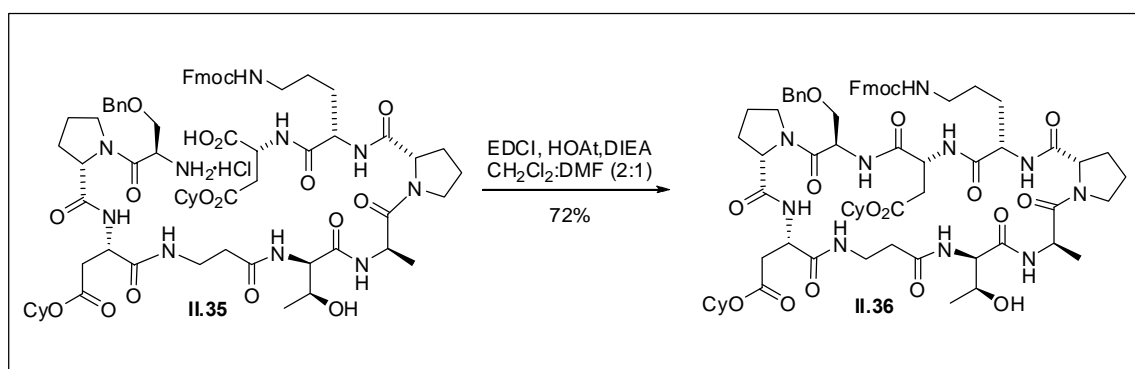
The preparation of the linear peptide (**II.34**) containing the (S)-configuration at the depsipeptide stereocenter is presented in **Scheme II.11**. This synthesis was carried out identically to that of the (R)-configuration with only slight variations in reaction yields.



**Scheme II.10: Incorporation of key fragments and assembly of linear peptide II.34**

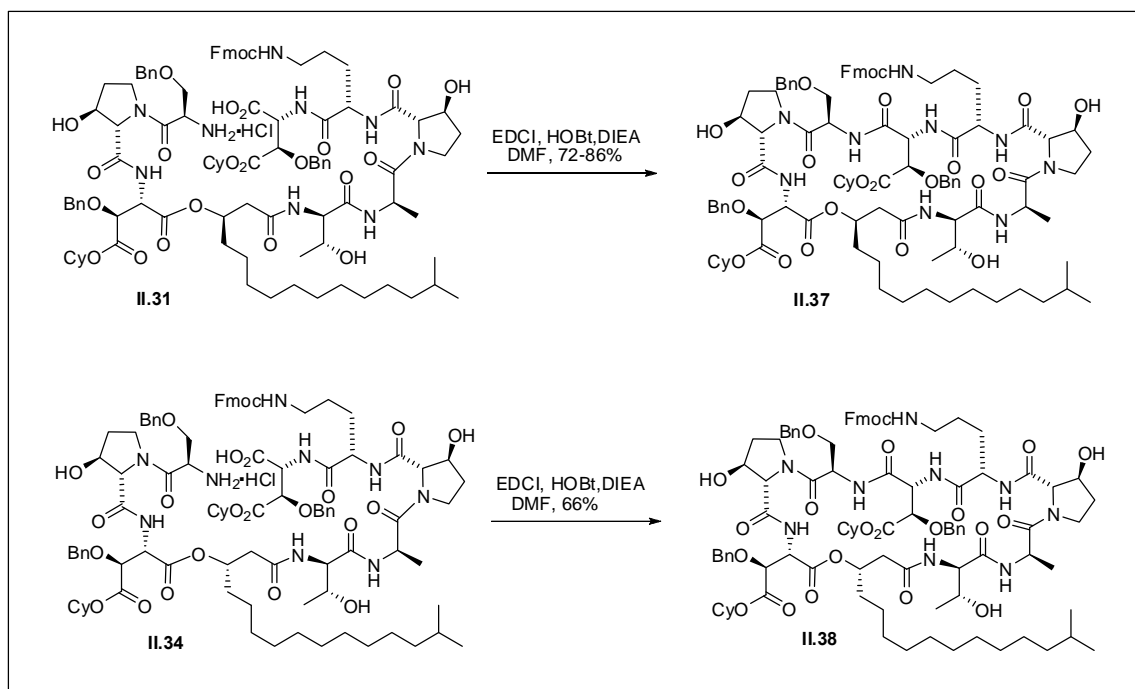


Our decision for amide bond formation between the N-terminal D-Ser residue and  $\beta$ -hydroxy aspartate residue in the macrocyclization step was driven by the data gathered from a simplified model system (**II.35**). The linear precursor was assembled in a similar manner as in our total synthesis, using standard protection/deprotection and coupling procedures. With non-hydroxy residues and an amide in place of the depsipeptide moiety, cyclization was accomplished efficiently (EDCI, HOAt, DIEA, 72%) (**Scheme II.12**)



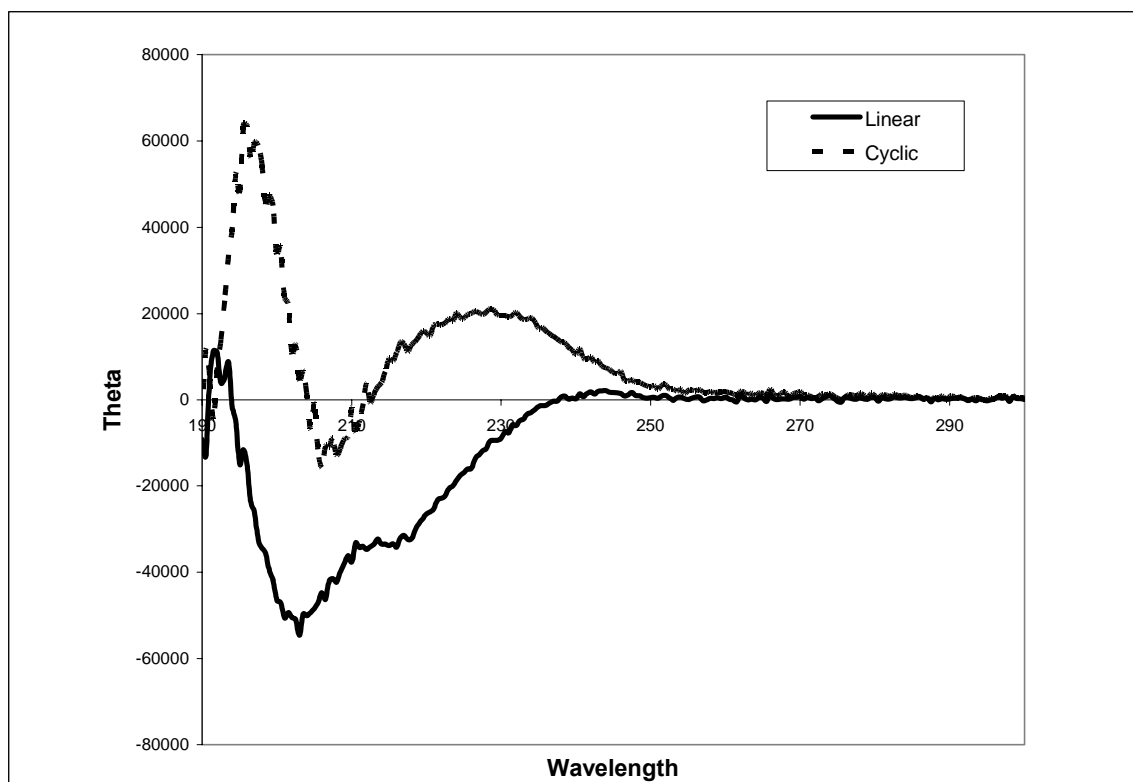
**Scheme II.11: Model system macrocyclization study**

**Schemes II.13** shows the macrocyclization reaction for both the (R) and (S)-diastereomers of the corresponding linear peptides. Activation and subsequent cyclization provided the cyclic peptides in 72-86% and 66% yields, respectively.



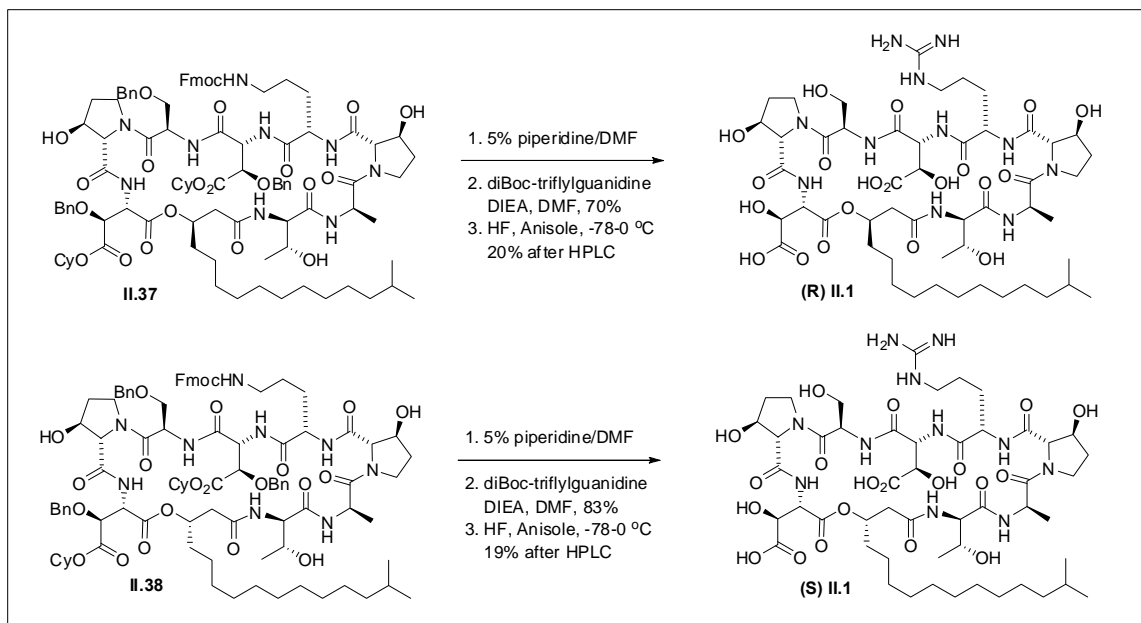
**Scheme II.12: Macrocyclization of linear substrates towards plusbacin A3**

Early on in the planning and development stages of our synthetic route we had hypothesized that the hydroxyproline residues located at opposite ends of plusbacin A3 would enforce a stabilized conformation of the linear peptide. Our hope was that the presence of a pre-organized conformation could be exploited to facilitate the key macrocyclization event. Subsequent to the successful macrocyclization reactions we further investigated our hypothesis through a comparative CD (circular dichroism) analysis of the linear (**II.31**) and cyclic peptide (**II.37**). **Figure II.4** illustrated the CD data which suggests that, although the cyclic peptide's curve has shifted, the relative shape has been maintained. This data suggests a conformational similarity between the cyclic and linear peptides, therefore supporting our original hypothesis.



**Figure II.4: Comparative CD analysis of linear and cyclic peptides II.31 and II.37 (H<sub>2</sub>O, 0.2 mg/mL)**

The final steps towards plusbacin A3 are represented in **Scheme II.14**. Finally, removal of the side chain Fmoc protecting group (5% piperidine/DMF, quant.) followed by guanidinylation using Goodman's protocol, (N, N'-diboc-N''-triflylguanidine, DIEA, DMF) afforded the depsipeptides in 70% and 83% yields respectively<sup>53</sup>. A global deprotection, i.e., removing of the cyclohexyl esters, benzyl ethers, and Boc groups (HF, Anisole), provided the target compounds in both diastereomeric forms.

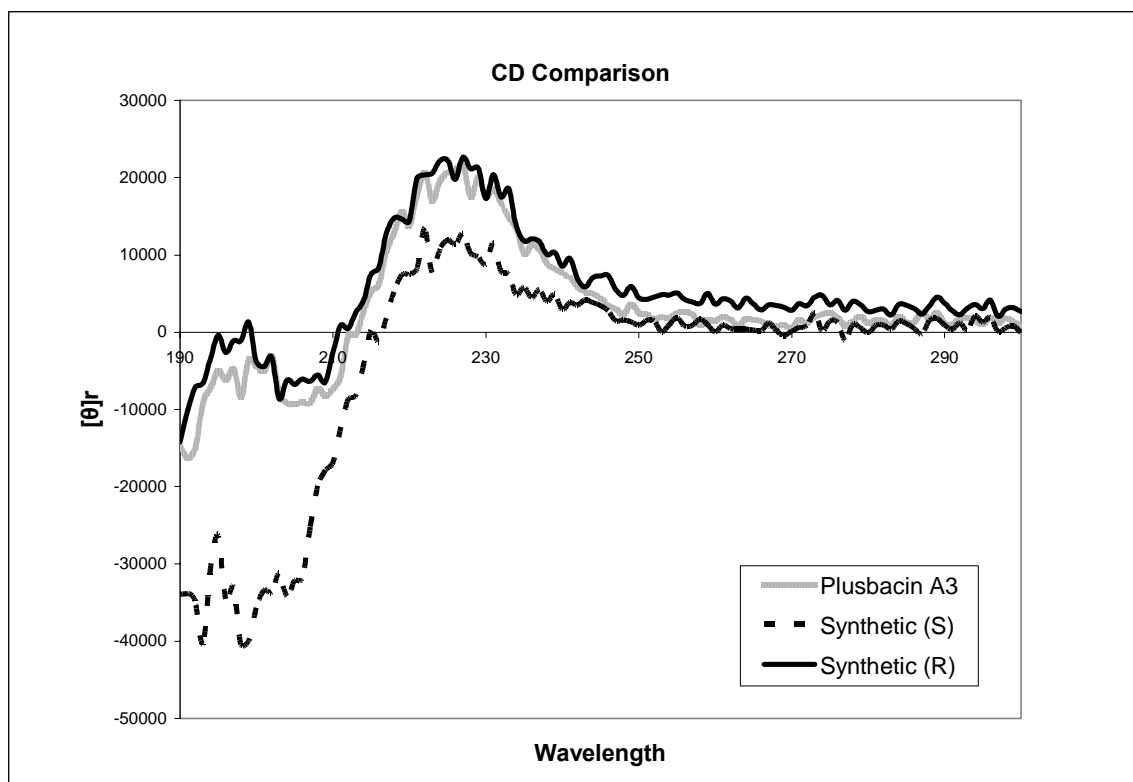


**Scheme II.13: Final steps towards target compound, plusbacin A3**

## II.2.6 STEREOCHEMICAL ASSIGNMENT

Both synthetic samples were compared with an authentic sample of the natural product provided by Shionogi Corporation. Extensive analysis revealed that the synthetic sample containing the (R)-3-hydroxyisopentanoic acid fragment in the peptide matched the analytical data for plusbacin A3 in all respects, thus establishing that the stereochemistry at the lactone stereocenter has the (R)-configuration.

Circular dichroism proved to be an invaluable tool for the identification of the natural product. **Figure II.5** presents the data for both synthetic isomer and the natural product. Samples were prepared at 0.2 mg/mL in spectroscopic grade H<sub>2</sub>O and spectra were collected at ambient temperature. From the graph, the synthetic (R)-configuration clearly matched the natural product curve while synthetic (S)-isomer did not.



**Figure II.5:** Comparative CD analysis of both plusbacin A3 stereoisomers (H<sub>2</sub>O, 0.2 mg/mL)

**Table II.3** illustrates the biological data collected for both synthetic compounds and the authentic sample of plusbacin A3. The synthetic sample containing the (R)-3-hydroxyisopentanoic acid residue was active against the panel of bacteria with MIC values of 0.2-6.25, which were identical to those established for the natural product. The values for the synthetic sample containing the (S)-configuration did not match, however did retain moderate activity for Group A streptococcus and methicillin-resistant *staphylococcus aureus*. Two gram negative strains, *E. coli* and *Pseudomonas* were assayed for control purposes.

**Table II.3: Activity data for synthetic stereoisomers and plusbacin A3 (see experimental section for assay conditions)**

	MIC ( $\mu\text{g/mL}$ )					
	GAS	GAB	MRSA	VRE	E. Coli	Pseudomonas
Plusbacin A3	0.2	6.25	1.56	6.25	>50	>50
Synthetic R	0.2	6.25	1.56	6.25	>50	>50
Synthetic S	3.13	>50	25	50	>50	>50

In addition to the CD and MIC data collected, determination of the correct stereoisomer was supported by NMR, and HPLC Data (see experimental section).

## II.3 CONCLUSIONS

The onset of resistant bacteria in the clinical setting, becoming an increasing threat to public health, has prompted the development of a new antibiotic arsenal. Plusbacin A3 has recently been evaluated and shows promise as an agent for the treatment of infections caused by resistant bacteria. We have successfully completed the first total synthesis and stereochemical assignment of plusbacin A3, a potent depsipeptide active against vancomycin and methicillin-resistant pathogens.

We successfully carried out the synthesis of a variety of non-proteogenic amino acids. An innovative route for preparation of both Boc- $\beta$ OBn-D-Asp(OCy)-OH and Boc- $\beta$ OBn-L-Asp(OCy)-OH was developed from which these building blocks were then incorporated into the plusbacin A3 backbone. An efficient synthesis of Boc-D-*allo*-Thr-OH was utilized to provide ample material for our synthetic effort. Both stereochemical configurations of the allyl 3-hydroxy-14-methylpentadecanoate were accessed through the key lipase resolution. The synthetic route to this intermediate is also highly amenable to analogs from which numerous fatty acid side chain variations could be envisioned.

The assembly of four key fragments was carried out and set the stage for the assembly of the plusbacin A3 backbone. Elaboration of these intermediates into the linear peptide provided the platform for the key macrocyclization event forming the plusbacin A3's 28-membered ring. Evidence supporting our initial hypothesis of a preorganized linear peptide was validated experimentally from CD conformational analysis and the efficiency of the macrocyclization reaction.

The final steps toward both diastereomeric forms of plusbacin A3 were achieved cleanly from which analytical data of the target compounds revealed the stereochemical configuration at the lactone stereocenter. With the total synthesis complete our focus was then shifted towards understanding the plusbacin A3 mechanism of biological activity.

## II.4 EXPERIMENTAL SECTION

General. All reactions were carried out in flame-dried glassware under an atmosphere of dry nitrogen or argon. Unless otherwise mentioned, solvents were purified as follows. All solvents were dried over activated alumina using a Seca Solvent System (Glass Contour). All other commercially available reagents were used as received.  $^1\text{H}$

NMR spectra were measured at 300 MHz on a Varian Mercury instrument, at 400 MHz on a Varian Gemini-400, or at 500 MHz on a Varian VXR-500 instrument.  $^{13}\text{C}$  NMR spectra were measured at 100 MHz or 75 MHz on a Varian Gemini spectrometer. Chemical shifts are reported relative to the central line of residual solvent. Infrared spectra were recorded using a Nicolet IR/42 spectrometer FT-IR (thin film, NaCl cells). High resolution mass spectra were obtained via electrospray ionization on an Agilent

ESI-TOF spectrometer. Optical rotations were measured on a Perkin–Elmer polarimeter (Model 241) using a 1 mL capacity quartz cell with a 10 cm path length.

Analytical thin layer chromatography (TLC) was performed using Whatman glass plates coated with a 0.25 mm thickness of silica gel containing PF 254 indicator, and compounds were visualized with UV light, potassium permanganate stain, cerium molybdate stain or ninhydrin stain. Analytical high performance liquid chromatography (HPLC) was performed on a Beckman-Coulter instrument (System Gold) with diode array detection. Analysis was carried out using Phenomenex Jupiter reverse-phase (C18) column (10 $\mu$  particle size, 300 Å pore size, 250 mm length x 4.6 mm diameter) with mobile phases consisting of 1% trifluoroacetic acid in water and acetonitrile. Preparatory HPLC purifications (Phenomenex Jupiter C<sub>18</sub> reverse-phase column, 10 $\mu$  particle size, 300 Å pore size, 250 mm length x 21.2 mm diameter) were performed with a Waters S3 Millipore Model 510 System with a Model 2487 Dual Absorbance Detector. Flash chromatography purifications were performed using Silicycle 60 Å, 35-75  $\mu$ m silica gel or Biotage purification system (SP1 HPFC system). All compounds purified by chromatography were sufficiently pure for use in further experiments, unless otherwise noted.

#### **II.4.1 Synthesis of $\beta$ OH-Aspartic acid**

The experimental below represents the synthesis of HCl-H<sub>2</sub>N- $\beta$ OBn-D-Asp(OCy)-OAllyl (**II.17**). The synthesis of Boc- $\beta$ OBn-L-Asp(OCy)-OH (**II.24**) was carried out



identically with the exception of the last protection / deprotection sequence which was omitted. Both enantiomeric forms of Aldehyde (**II.9**) were synthesized according to the procedure elaborated in reference 44. All intermediate spectroscopic data was identical to that provided in the literature.

**(S)-tert-butyl 4-((S)-1-hydroxyallyl)-2,2-dimethyloxazolidine-3-carboxylate (II.10)**

To a stirring solution of tetravinyl tin (0.905 mL, 4.80 mmol) in dry ether (36 mL) at 0 °C under an atmosphere of argon, was added *n*-butyllithium (2.34 M, 8.20 mL, 19.19 mmol) drop-wise via syringe. The solution stirred for 15 minutes at this temperature. Zinc Bromide (4.32 g, 19.19 mmol) was added as a steady stream of argon was passed through a schlenk line into the reaction flask. The mixture was stirred for 1 hour at room temperature and then recooled to 0 °C. This solution was then added via cannula, over a period of 45 minutes, to a vigorously stirring solution of Garner's Aldehyde **II.9** (1.00 g, 4.36mmol) and zinc bromide (0.982 g, 4.36 mmol) in dry Ether (11.0 mL) at -78 °C . The cold bath was removed and the mixture was stirred at room temperature for an additional 2 hours, or until completion as indicated by TLC (hexanes / EtOAc, 7:3). The reaction was then cooled to 0 °C and carefully quenched by the addition of sat. aq. NH<sub>4</sub>Cl (30 mL). The organic layer was separated and the aq. phase was extracted with ether (3 x 50 mL). The combined organic extracts were then washed with brine (40 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and condensed. The crude residue was purified by column chromatography (10 -25% EtOAc/Hexanes) to afford a mixture of diastereomers (syn : anti = 6:1) ( 1.053 g, 4.09 mmol, 94%) as a white crystalline solid. m.p. 77-79 °C (lit.

80-80.5 °C);  $[\alpha]^{23}_D = -48.3$  (c 1.0,  $\text{CHCl}_3$ ) (lit. - 48.9 (c 1.25,  $\text{CHCl}_3$ ));  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.80 (m, 1H), 5.32 (d,  $J=17.2$  Hz, 1H), 5.21 (d,  $J=10.0$  Hz, 1H), 4.34 (br, 1H), 4.20 (br, 1H), 3.96 (m, 1H), 3.91- 3.87 (m, 2H), 1.56 (s, 3H), 1.48 (s, 12H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  154.7, 137.4, 117.7, 94.1, 81.2, 75.4, 64.2, 61.6, 28.2, 26.9, 24.1; IR (neat)  $\nu_{\text{max}}$  3453, 2979, 2937, 1699, 1457, 1393, 1258, 1173, 1097, 1053, 991, 924, 852, 769, 668; ESI  $m/z$  280.02  $[\text{M}+\text{Na}]^+$

**(*R*)-2-(benzyloxy)-2-((*S*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyloxazolidin-4-yl)acetic acid (**II.1**)**

To a stirring solution of the alcohol **II.10** (0.378 g, 1.47 mmol) and benzyl bromide (0.21 mL, 1.76 mmol) in dry DMF at 0 °C was added NaH (60% dispersion, 0.062 g, 1.16 mmol) in one portion. The solution was stirred under argon for 4 hours while slowly warming to room temperature. The reaction was quenched by the addition of  $\text{H}_2\text{O}$  (2 mL) and the solvent was removed in vacuo. The crude residue was partitioned between EtOAc (20 mL) and  $\text{H}_2\text{O}$  (20 mL). The aqueous phase was extracted with EtOAc (3 x 20 mL) and the combined organic extracts were dried over  $\text{MgSO}_4$ . Column chromatography (Hexanes/EtOAc, 9.5:0.5) provided the benzyl ether (0.490 g, 1.41 mmol, 96%) as a colorless oil.  $[\alpha]^{25}_D = -11.4$  (c 1.0,  $\text{CHCl}_3$ )  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.38-7.24 (m, 5H), 5.84 (td,  $J=18.09, 9.18, 9.18$  Hz, 1H), 5.41-5.21 (m, 2H), 4.61 (m, 1H), 4.44-4.31 (m, 1H), 4.23 (t,  $J=5.04, 5.04$  Hz, 1H), 4.20-4.06 (m, 1H), 4.02 (t,  $J=5.29, 5.29$  Hz, 1H), 3.97-3.86 (m, 1H), 1.65-1.30 (m, 15H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  152.48, 151.80, 138.52, 138.23, 134.87, 134.39, 128.30, 128.18, 127.57, 127.51, 127.45, 127.32, 120.29, 119.79, 94.23, 93.74, 80.11, 79.63, 79.43, 78.92, 70.75,

70.1, 63.61, 63.44, 59.53, 59.32, 28.29, 26.50, 25.65, 24.27, 22.83; IR (neat)  $\nu_{\max}$  3369, 2982, 2935, 2880, 1704, 1452, 1389, 1263, 1168, 1089; ESI MS  $m/z$  370.06  $[M+Na]^+$ , 347.92  $[M+H]^+$

Ozone was gently bubbled through a solution stirring solution of the olefin (1.21 g, 3.49 mmol) at -78 °C until a light blue color persisted (approximately 20 min). Nitrogen was then passed through the solution for 30 min followed by the addition of dimethylsulfide (7.3 ml, 82.0 mmol). The solution was stirred at this temperature for 15 min then slowly warmed to room temperature. After stirring for 2 hrs at this temperature the solvent was removed in vacuo. The residue was dissolved in MeOH/CH<sub>2</sub>Cl<sub>2</sub>/H<sub>2</sub>O (115 ml, 6:3:2) followed by the addition of NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (1.93 g, 13.98 mmol) and NaClO<sub>2</sub> (0.790 g, 6.99 mmol). The reaction was stirred for 90 min and the volatiles were removed in vacuo. The aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 30 ml). The combined CH<sub>2</sub>Cl<sub>2</sub> was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Silica gel chromatography (1-10% MeOH/CHCl<sub>3</sub>) afforded the carboxylic acid (1.15 g, 3.14 mmol, 90%) as a hygroscopic foam.  $[\alpha]_D^{22} + 1.8$  (c = 1.0, CHCl<sub>3</sub>) <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  7.36-7.23 (m, 5H), 4.61 (t, *J* = 15.06, 15.06 Hz, 1H), 4.35 (t, *J* = 12.14, 12.14 Hz, 1H), 4.17-3.98 (m, 3H), 3.89 (m, 1H), 1.48-1.19 (m, 15H), <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) 175.607, 175.015 (rotamer), 152.528, 151.492 (rotamer), 137.150, 136.812 (rotamer), 128.212, 128.078, 127.793, 127.560, 94.463, 94.168 (rotamer), 80.825, 80.197 (rotamer), 77.204, 76.328 (rotamer), 72.723, 72.444 72.194, 64.188, 63.785 (rotamer), 58.826, 29.675, 28.363, 26.374, 25.768 (rotamer), 24.303, 22.849 (rotamer); IR (neat)  $\nu_{\max}$  3432, 2975, 2927, 2359, 1704, 1389, 1263, 1168, 1097; ESI MS  $m/z$  388.04  $[M+Na]^+$ , 365.82  $[M+H]^+$

### Boc- $\beta$ OBn-D-Asp(OCy)-OH (II.12)

To a stirring solution of the carboxylic acid (4.846 g, 13.26 mmol), DMAP (0.810 g, 6.63 mmol) and cyclohexanol (4.25 mL, 39.8 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (44 mL) at 0 °C was added EDCI (2.80 g, 14.59 mmol) in one portion. The reaction mixture was stirred under argon for 7 hours while slowly warming to room temperature. The solution was diluted with  $\text{CH}_2\text{Cl}_2$  (50 mL) and  $\text{H}_2\text{O}$  (50 mL). The aqueous phase was extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 50 mL). The combined organic extracts were dried over  $\text{Na}_2\text{SO}_4$ , filtered and condensed. Column chromatography (Hexanes/EtOAc, 9:1) afforded the ester (5.123 g, 11.45 mmol, 86%) as a colorless oil.  $[\alpha]_D^{22}$  - 14.4 ( $c$  = 2.43,  $\text{CHCl}_3$ )  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.32 (d,  $J$  = 6.42 Hz, 1H), 4.85 (m, 1H), 4.63 (dd,  $J$  = 29.85, 11.45 Hz, 1H), 4.47-4.41 (m, 1H), 4.39-4.23 (m, 2H), 4.04 (t,  $J$  = 5.26, 5.26 Hz, 1H), 3.90 (dd,  $J$  = 9.45, 6.61 Hz, 1H), 1.99-1.81 (m, 2H), 1.78-1.67 (m, 2H), 1.57-1.31 (m, 20H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100MHz)  $\delta$  169.5, 169.3 (rotamer), 152.2, 151.4 (rotamer), 137.3, 136.8 (rotamer), 128.2, 128.0 (rotamer), 127.8, 127.7 (rotamer), 127.6, 127.5 (rotamer), 94.2, 93.8 (rotamer), 80.2, 79.8 (rotamer), 77.1, 76.7 (rotamer), 73.5, 73.3 (rotamer), 72.3, 72.0 (rotamer), 63.4, 63.4 (rotamer), 59.0, 58.7, 58.7 (rotamer), 31.6, 31.5 (rotamer), 31.4, 28.3, 28.2 (rotamer), 26.506, 25.8 (rotamer), 25.3, 24.1, 23.7, 22.7 (rotamer); IR (Neat)  $\nu_{\text{max}}$ ; ESI MS  $m/z$  470.09  $[\text{M}+\text{Na}]^+$

The oxazolidine (6.302 g, 14.08 mmol) was dissolved in dry acetone (280 mL) and cooled to 0 °C. To this solution was added a freshly prepared solution of 1M Jones reagent (43 mL) drop-wise via addition funnel over a period of 30 min. The reaction was

stirred vigorously at this temperature for 30 min then at room temperature for 12 hours. Isopropanol was added drop-wise until the solution turned dark green. The acetone was removed in vacuo and the mixture was diluted with EtOAc (200 mL) and water (100 mL). The aqueous phase was extracted with EtOAc (3 x 100 mL) and the combined organic extracts were washed with brine (200 mL). The EtOAc was dried over  $\text{Mg}_2\text{SO}_4$ , filtered and condensed. Column chromatography (1-15% MeOH/ $\text{CHCl}_3$ ) afforded the carboxylic acid (2.48 g, 5.89 mmol, 42%, 88% based on recovered starting material) as a white foam. The unreacted starting material (3.30 g, 7.37 mmol, 53%) was recovered and recycled. m.p. 55-58 °C,  $[\alpha]_D^{22} + 13.4$  (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  7.35-7.23 (m, 5H), 6.21 (s, 1H), 4.70 (m, 1H), 4.63 (d,  $J = 11.93$  Hz, 1H), 4.52 (d,  $J = 2.09$  Hz, 1H), 4.41 (d,  $J = 11.03$  Hz, 2H), 1.82-1.71 (m, 2H), 1.70-1.59 (m, 2H), 1.52-1.18 (m, 16H);  $^{13}\text{C}$  NMR (100MHz,  $\text{CDCl}_3$ )  $\delta$  175.003, 169.258, 155.700, 136.677, 128.277, 127.941, 80.079, 77.246, 74.222, 73.017, 56.305, 31.356, 31.116, 28.143, 25.176, 23.562, 23.470, IR (neat)  $\nu_{\text{max}}$  3443, 2978, 2862, 1747, 1499, 1453, 1391, 1213, 1174, 1112; ESI  $m/z$  444.03  $[\text{M}+\text{Na}]^+$

### **HCl- $\text{H}_2\text{N}$ - $\beta\text{OBn}$ -D-Asp(OCy)-OAllyl (II.17)**

To a stirring solution of the carboxylic acid (5.00 g, 11.91 mmol) and allyl bromide (2.061 mL, 23.82 mmol) in dry DMF (60 mL) at 0°C was added  $\text{K}_2\text{CO}_3$  (3.29 g, 23.82 mmol). The mixture was stirred under argon for 12 hours while slowly warming to room temperature. The solvent was then removed and to the crude residue was added  $\text{H}_2\text{O}$  (100 mL) and EtOAc (100 mL). The aqueous phase was extracted with EtOAc (3 x 100 mL) and the combined organic extracts were dried over  $\text{Mg}_2\text{SO}_4$ , filtered and condensed.

Silica gel chromatography (10% EtOAc/Hexanes) afforded Boc- $\beta$ OBn-D-Asp(OCy)-OAllyl (4.984g, 10.80mmol, 91%) as a colorless oil.  $[\alpha]_D^{22} +29.5$ ;  $^1\text{H}$  NMR (400 MHz, *DMSO-d*<sub>6</sub>)  $\delta$  7.40-7.25 (m, 1H), 7.05 (d,  $J$  = 9.71 Hz, 1H), 5.83 (tdd,  $J$  = 15.90, 10.58, 5.35, 5.35 Hz, 1H), 5.29 (dd,  $J$  = 17.24, 1.51 Hz, 1H), 5.19 (d,  $J$  = 10.43 Hz, 1H), 4.74 (m, 1H), 4.72-4.63 (m, 2H), 4.60 (d,  $J$  = 5.35 Hz, 1H), 4.55 (dd,  $J$  = 14.07, 5.28 Hz, 2H), 4.49 (d,  $J$  = 4.20 Hz, 1H), 4.43 (d,  $J$  = 11.77 Hz, 1H), 1.83-1.71 (m, 2H), 1.70-1.58 (m, 2H), 1.51-1.19 (m, 15H)  $^{13}\text{C}$  NMR (100MHz, *CDCl*<sub>3</sub>)  $\delta$  169.1, 168.5, 155.3, 136.6, 131.4, 128.3, 128.1, 128.0, 118.8, 79.9, 77.0, 74.2, 72.8, 66.2, 56.0, 31.4, 31.1, 28.1, 25.1, 23.5, 23.4; IR (neat)  $\nu_{\text{max}}$  3453, 2937, 2859, 1757, 1726, 1499, 1452, 1366, 1257, 1210, 1155, 1116, 1014; ESI  $m/z$  484.03  $[\text{M}+\text{Na}]^+$ . The product was then treated with 4N HCl-Dioxane (15mL) and stirred at room temperature for 90 min. The volatiles were removed in vacuo and to the residue was added  $\text{CH}_2\text{Cl}_2$ /Hexanes (1:1, 20 mL). The solvent was removed in vacuo to drive off any residual HCl to afford HCl- $\text{H}_2\text{N}$ - $\beta$ OBn-D-Asp(OCy)-OAllyl (4.30 g, 10.80 mmol, 100%) as white solid. The hydrochloride was left under high vacuum for several hours and then used without further purification.

## II.4.2 Synthesis of D-*allo*-Threonine

### Boc-D-*allo*-Thr-OH (II.15)

The synthesis of the D-*allo*-Threonine derivative was carried out according to the original literature procedure. Spectroscopic data for all intermediates was identical to that reported in the literature.

### II.4.3 Synthesis of Plusbacin *R* isomer experimental

#### Boc-D-Ser(OBn)- $\beta$ OHPro-OH (II.3)

Boc- $\beta$ OHPro-OAllyl (0.457 g, 1.68 mmol) was treated with 4N HCl-Dioxane (3 mL) and the resulting mixture was stirred at room temperature for 90 minutes. The volatiles were removed in vacuo. The residual HCl was further removed by adding Et<sub>2</sub>O (3 mL) to the hydrochloride salt followed by its removal in vacuo. This residue and Boc-D-Ser(OBn)-OH (0.500 g, 1.69 mmol) were dissolved in THF (5.11 mL). The mixture was then treated sequentially at 0°C with DIEA (0.587 mL, 3.37 mmol), HOAt (0.298 g, 2.19 mmol), and EDCI (0.420 g, 2.19 mmol). The reaction mixture was stirred for 8 hrs while slowly warming to room temperature. The reaction was quenched with H<sub>2</sub>O (2 mL) and the solvent was condensed. The residue was redissolved in EtOAc (30 mL), washed with 1N HCl (1 x 20 mL), saturated NaHCO<sub>3</sub> (1 x 20 mL), and brine (1x 20 mL), dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and concentrated in vacuo. Chromatography (10% EtOAc-Hexanes) provided Boc-D-Ser(OBn)- $\beta$ OH-Pro-OAllyl as a white hygroscopic foam (0.676 g, 1.506 mmol, 89%).  $[\alpha]_D^{22}$  -34.8 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.33-7.22 (m, 1H), 5.87 (tdd, *J* = 16.14, 10.56, 5.68, 5.68 Hz, 1H), 5.42 (d, *J* = 8.32 Hz, 1H), 5.34-5.26 (m, 1H), 5.22 (dd, *J* = 10.45, 1.23 Hz, 1H), 4.75 (dd, *J* = 13.51, 7.54 Hz, 1H), 4.60 (td, *J* = 5.64, 1.22, 1.22 Hz, 2H), 4.49 (d, *J* = 9.31 Hz, 2H), 4.45 (t, *J* = 4.47, 4.47 Hz, 1H), 4.41 (s, 1H), 3.90-3.77 (m, 2H), 3.68 (dd, *J* = 9.27, 5.41 Hz, 1H), 3.58 (dd, *J* = 9.24, 7.36 Hz, 1H), 2.11 (m, 1H), 1.99-1.87 (m, 1H), 1.45-1.36 (m, 9H); <sup>13</sup>C NMR (100MHz, CDCl<sub>3</sub>)  $\delta$  169.8, 169.4, 155.0, 137.6, 131.5, 128.3, 127.6, 127.5, 118.6, 79.8, 73.2, 73.1, 70.5, 68.0, 65.9, 51.6, 45.0, 32.8, 28.3; IR (neat)  $\nu_{max}$  3409, 2980, 2934, 2873, 1745, 1707, 1645, 1507, 1454, 1362, 1247, 1170, 1108; ESI MS *m/z* 470.99 [M+Na]<sup>+</sup>

$\text{PdCl}_2(\text{PPh}_3)_2$  (0.067 g, 0.096 mmol) and  $\text{PPh}_3$  (0.075 g, 0.287 mmol) were dissolved in dry  $\text{CH}_2\text{Cl}_2$  (2 mL) and stirred under an atmosphere of argon for 15 minutes. This solution was transferred via syringe to a solution of Boc-D-Ser(OBn)- $\beta$ OHPro-OAllyl (1.716 g, 3.83 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (17.0 mL) under argon.  $\text{PhSiH}_3$  (0.944 mL, 7.65 mmol) was added via syringe and the reaction was stirred at room temperature for 12 hours. The solvent was condensed and the crude residue was loaded directly onto a silica gel column. Chromatography (1-30% MeOH- $\text{CHCl}_3$ ) afforded **2** (1.33 g, 3.26 mmol, 85%) as a white solid. mp 88-90 °C;  $[\alpha]_D^{22}$  - 12.6 (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz, 45 °C,  $\text{DMSO}-d_6$ )  $\delta$  7.35-7.25 (m, 5H), 4.60 (dd,  $J$  = 13.51, 6.58 Hz, 1H), 4.49 (d,  $J$  = 3.52 Hz, 1H), 4.47-4.43 (m, 1H), 4.41-4.38 (m, 1H), 4.36 (d,  $J$  = 11.34 Hz, 1H), 4.28-4.23 (m, 1H), 4.14 (s, 1H), 3.71 (ddd,  $J$  = 27.00, 17.53, 9.09 Hz, 2H), 3.63-3.47 (m, 1H), 3.45-3.38 (m, 1H), 2.04-1.82 (m, 1H), 1.84-1.63 (m, 1H), 1.40-1.36 (m, 9H), 6.71 (d,  $J$  = 42.74 Hz, 1H);  $^{13}\text{C}$  NMR (100MHz,  $\text{CDCl}_3$ )  $\delta$  174.457, 169.946, 155.298, 137.626, 128.243, 127.484, 127.418, 79.771, 72.915, 69.872, 69.107, 64.481, 51.550, 45.490, 32.471, 28.249, IR (neat)  $\nu_{\text{max}}$  3386, 2983, 2928, 1717, 1631, 1515, 1452, 1367, 1165, 1103; ESI MS  $m/z$  431.08  $[\text{M}+\text{Na}]^+$

#### Boc-D- $\alpha$ Thr-DAla-OBn (II.18)

Boc-D-Ala-OBn (7.08 g, 25.3 mmol) was treated with a solution of 20% TFA in  $\text{CH}_2\text{Cl}_2$  (48.7 mL, 127 mmol) and stirred for 1 hr at room temperature. The solvent was removed in vacuo and the residue was diluted with sat. aq.  $\text{NaHCO}_3$  (75 mL) and  $\text{CH}_2\text{Cl}_2$  (75 mL).



The aq. phase was extracted with  $\text{CH}_2\text{Cl}_2$  (4 x 75 mL). The combined organic extracts were dried over  $\text{Na}_2\text{SO}_4$ , filtered and condensed to afford  $\text{H}_2\text{N-D-Ala-OBn}$  as a colorless, viscous oil which was used without further purification. This residue, Boc-D- $\alpha$ Thr-OH (4.5 g, 20.53 mmol), HOBt (5.55 g, 41.1 mmol) and DIEA (3.60 mL, 20.53 mmol) were dissolved in THF (62 mL). The solution was cooled to  $0^\circ\text{C}$  and EDCI (7.87 g, 41.1 mmol) was added. The reaction mixture was stirred 36 hrs and then quenched with  $\text{H}_2\text{O}$  (3 mL). The THF was removed in vacuo and to the residue was added EtOAc (100 mL). This solution was washed with 1N HCl (1 x 75 mL), sat. aq.  $\text{NaHCO}_3$  (1 x 75 mL), and brine (1 x 75mL). The EtOAc was dried over  $\text{Mg}_2\text{SO}_4$ , filtered and condensed. Chromatography ( 35% EtOAc/Hexanes) provided **9** (6.815 g, 17.91 mmol, 87%) as a white solid. mp  $79-81^\circ\text{C}$ ;  $[\alpha]^{22}_{\text{D}} + 36.1$  (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.24 (d,  $J = 6.87$  Hz, 1H), 7.41-7.29 (m, 5H), 6.58 (d,  $J = 8.95$  Hz, 1H), 5.11 (s, 2H), 4.69 (d,  $J = 5.08$  Hz, 1H), 4.34 (p,  $J = 7.17, 7.17, 7.09, 7.09$  Hz, 1H), 3.98 (dd,  $J = 8.86, 5.82$  Hz, 1H), 3.83 (dd,  $J = 11.61, 5.78$  Hz, 1H), 1.38 (s, 9H), 1.30 (d,  $J = 7.26$  Hz, 3H), 1.00 (d,  $J = 6.33$  Hz, 3H);  $^{13}\text{C}$  NMR (100MHz,  $\text{CDCl}_3$ ) 172.7, 171.0, 156.0, 135.1, 128.5, 128.4, 128.1, 80.2, 69.3, 67.7, 58.6, 48.3, 28.2, 19.4, 17.4; IR (neat)  $\nu_{\text{max}}$  3308, 3072, 2981, 2933, 1713, 1658, 1522, 1456, 1394, 1367, 1293, 1247, 1204, 1161, 1049, 1017, 885, 750, 699; ESI MS  $m/z$  403.04  $[\text{M}+\text{Na}]^+$ , 380.85  $[\text{M}+\text{H}]^+$

### **Boc-D- $\alpha$ Thr-D-Ala- $\beta$ OHPro-OH (II.17)**

Boc-D- $\alpha$ Thr-D-Ala-OBn (6.51 g, 17.11 mmol) was dissolved in MeOH (115 mL). 10% palladium on carbon (0.651 g, 0.612 mmol) was added and the mixture was purged with  $\text{H}_2$  three times. The reaction was stirred vigorously under an atmosphere of  $\text{H}_2$  for 5

hours. The mixture was then filtered through a pad of celite and the solvent was removed to afford Boc-D-*a*Thr-D-Ala-OH (4.91 g, 16.91 mmol, 99%) as a white solid. The crude product was used without further purification. mp 113-115 °C ;  $[\alpha]_D^{22}$  -11.7 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.04 (d, *J* = 7.05 Hz, 1H), 6.64 (d, *J* = 8.90 Hz, 1H), 4.21 (p, *J* = 7.16, 7.16, 7.15, 7.15 Hz, 1H), 3.93 (dd, *J* = 8.70, 6.21 Hz, 1H), 3.82 (p, *J* = 6.05, 6.05, 6.01, 6.01 Hz, 1H), 3.35 (s, 1H), 1.38 (s, 9H), 1.26 (d, *J* = 7.27 Hz, 3H), 1.02 (d, *J* = 6.28 Hz, 3H); <sup>13</sup>C NMR (100MHz, CDCl<sub>3</sub>) δ 175.601, 171.213, 156.262, 80.520, 69.404, 59.273, 48.395, 28.254, 19.062, 17.202; IR (neat)  $\nu_{max}$  3308, 2984, 2933, 1697, 1654, 1530, 1456, 1394, 1367, 1297, 1243, 1161, 1041, 1017, 937, 870, 781; ESI MS *m/z* 288.86 [M+Na]<sup>+</sup>

Boc-D-βOHPro-OBn (6.455 g, 20.09 mmol) was treated with a solution of 4N HCl-Dioxane (25 mL 100mmol) and stirred for 1hr at room temperature. The solvent was condensed and hexanes (50 mL) was added then condensed in vacuo two times to remove any residual HCl. The white solid was left under high vacuum for several hours to afford HCl-βOHPro-OBn (5.17 g, 20.06 mmol, 99%) which was used without further purification. To this solid, Boc-D-*a*Thr-D-Ala-OH (4.87 g, 16.78 mmol), HOBT (4.53 g, 33.6 mmol) and DIEA (4.34 mL, 33.6 mmol) in DMF (51mL) at 0 °C was added EDCI (6.43 g, 33.6 mmol). The reaction mixture was stirred 36 hrs and then quenched with H<sub>2</sub>O (2 mL). The DMF was removed in vacuo and to the residue was added EtOAc (100 mL). This solution was washed with 1N HCl (1 x 75 mL), sat. aq. NaHCO<sub>3</sub> (1 x 75 mL), and brine (1 x 75 mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Chromatography ( 35% Acetone-CH<sub>2</sub>Cl<sub>2</sub>) provided **10** (7.41 g, 15.0 mmol, 90%) as a

white solid. mp 71-73 °C;  $[\alpha]_D^{22}$  - 3.9 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 8.02 (d, *J* = 7.35 Hz, 1H), 7.86 (d, *J* = 7.61 Hz, 1H), 7.41-7.31 (m, 5H), 6.76 (d, *J* = 8.82 Hz, 1H), 5.57 (d, *J* = 3.61 Hz, 1H), 5.20 (s, 1H), 5.10 (dd, *J* = 30.20, 12.57 Hz, 2H), 4.79 (d, *J* = 4.82 Hz, 1H), 4.65 (p, *J* = 6.75, 6.75, 6.67, 6.67 Hz, 1H), 4.22 (s, 1H), 4.18 (s, 1H), 3.91 (dd, *J* = 8.45, 6.23 Hz, 1H), 3.86-3.74 (m, 2H), 3.63 (dd, *J* = 16.66, 9.63 Hz, 1H), 3.47 (m, 1H), 1.97 (dq, *J* = 9.22, 9.10, 9.10, 4.34 Hz, 1H), 1.91-1.73 (m, 1H), 1.37 (s, 9H), 1.20 (d, *J* = 6.79 Hz, 3H), 1.04-0.96 (m, 3H); <sup>13</sup>C NMR (100MHz, CDCl<sub>3</sub>) δ 172.4, 172.0, 171.4, 170.8, 169.7, 169.5, 155.8, 155.8, 135.2, 134.9, 128.6, 128.5, 128.3, 128.3, 128.0, 80.0, 74.6, 72.7, 69.2, 68.7, 68.1, 68.1, 67.5, 67.1, 59.5, 58.8, 47.2, 47.1, 44.9, 32.6, 30.5, 28.2, 19.7, 19.3, 17.1, 16.9; IR (neat)  $\nu_{max}$  3312, 3070, 2976, 2929, 1740, 1694, 1632, 1519, 1454, 1255, 1170, 1102, 1044; ESI MS *m/z* 516.09 [M+Na]<sup>+</sup>

To a solution of Boc-D-*α*Thr-D-Ala-βOH-Pro-OBn (4.524 g, 9.17 mmol) in MeOH (45 mL) was added 5% Pd-C (0.500 g). The flask was purged with H<sub>2</sub> three times and stirred for 8hr under an H<sub>2</sub> atmosphere. The mixture was then filtered through a pad of celite and the solvent was removed to afford the carboxylic acid (3.68 g, 9.12 mmol, 100%) as a white solid. The product was used without further purification. mp 92-95 °C;  $[\alpha]_D^{22}$  + 25.2 (c 1.0, MeOH); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.00 (d, *J* = 7.32 Hz, 1H), 7.84 (d, *J* = 7.61 Hz, 1H), 6.78 (d, *J* = 8.79 Hz, 1H), 5.49 (s, 1H), 4.84-4.72 (m, 1H), 4.62 (p, *J* = 6.58, 6.58, 6.58, 6.58 Hz, 1H), 4.52 (s, 1H), 4.39 (s, 1H), 4.31 (p, *J* = 7.33, 7.33, 7.15, 7.15 Hz, 1H), 4.22 (s, 1H), 4.08 (s, 1H), 3.94-3.86 (m, 1H), 3.85-3.69 (m, 2H), 3.60 (q, *J* = 9.23, 9.23, 9.00 Hz, 1H), 3.49-3.40 (m, 1H), 3.16 (s, 1H), 2.03-1.90 (m, 1H), 1.90-1.70 (m, 1H), 1.37 (s, 9H), 1.18 (d, *J* = 6.70 Hz, 2H), 1.11 (d, *J* = 6.67 Hz, 1H), 1.01 (d, *J* =

5.87 Hz, 1H);  $^{13}\text{C}$  NMR (100MHz,  $\text{CDCl}_3$ )  $\delta$  174.0, 173.2, 172.4, 157.7, 80.7, 76.0, 74.0, 68.8, 61.2, 48.2, 46.0, 33.8, 31.6, 28.7, 19.5, 17.4, IR (neat)  $\nu_{\text{max}}$  3351, 2984, 2929, 1692, 1633, 1529, 1464, 1393, 1367, 1250, 1156; ESI MS  $m/z$  426.02  $[\text{M}+\text{Na}]^+$

**(S)-tert-butyl 3-acetoxypent-4-enoate (II.21)** To a solution of the racemic allylic alcohol **II.19** (7.95 g, 46.2 mmol) and vinyl acetate (21.3 mL, 231 mmol) in pentane (230 mL) was added Amano Lipase PS (4.62 g) and 4 Angstrom molecular Sieves (7.62 g). The reaction was stirred at 30 °C for 10 hours and filtered through a short plug of silica rinsing with Ether. (Reaction times varied between 10 – 24 hrs. Conversion to the acetate was monitored following  $\beta$ -proton signals in  $^1\text{H}$  NMR.) The mixture was condensed and column chromatography (PE-Et<sub>2</sub>O,8:2) afforded the (S) allylic acetate product **II.21** (3.88 g, 18.1 mmol, 40%) and (R) alcohol (3.56 g, 20.7 mmol, 45%) as colorless oils. Analytical data for **(S)-tert-butyl 3-acetoxypent-4-enoate**:  $[\alpha]_{\text{D}}^{22}$  - 4.4 (c 1.0,  $\text{CHCl}_3$ )  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  5.82 (ddd,  $J$  = 17.01, 10.50, 6.18 Hz, 1H), 5.59 (dd,  $J$  = 13.53, 6.35 Hz, 1H), 5.29 (d,  $J$  = 17.31 Hz, 1H), 5.19 (d,  $J$  = 10.21 Hz, 1H), 2.55 (ddd,  $J$  = 20.99, 15.25, 6.89 Hz, 2H), 2.04 (s, 3H), 1.43 (s, 9H);  $^{13}\text{C}$  NMR (100MHz,  $\text{CDCl}_3$ )  $\delta$  169.5, 168.7, 135.0, 117.2, 81.0, 71.1.0, 40.7, 28.1.0, 21.1; IR (Neat)  $\nu_{\text{max}}$  2976, 2937, 2367, 1741, 1366, 1233, 1155; ES MS  $m/z$  236.90  $[\text{M}+\text{Na}]^+$

Analytical data for **(R)-tert-butyl 3-hydroxypent-4-enoate (II.20)**:  $[\alpha]_{\text{D}}^{22}$  + 8.4 (c 1.0,  $\text{CHCl}_3$ )  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.83 (ddd,  $J$  = 17.16, 10.51, 5.48 Hz, 1H), 5.26 (td,  $J$  = 17.22, 1.43, 1.43 Hz, 1H), 5.09 (td,  $J$  = 10.52, 1.35, 1.35 Hz, 1H), 4.45 (s, 1H), 3.25 (s, 1H), 2.43 (dq,  $J$  = 16.07, 16.07, 16.07, 6.20 Hz, 2H), 1.42 (s, 9H);  $^{13}\text{C}$  NMR

(100MHz,  $CDCl_3$ )  $\delta$  171.3, 138.7, 114.9, 81.3, 69.0, 42.2, 28.1; IR (Neat)  $\nu_{max}$  3425, 2983, 2920, 2105, 1647, 1367, 1258, 1157; ES MS  $m/z$  194.91  $[M+Na]^+$

**(R)-tert-butyl 3-acetoxy-14-methylpentadecanoate (II.22)**

To a stirring solution of the allylic acetate (**II.21**) (5.00 g, 23.3 mmol) and 11-methyl-1-dodecene (16.71g, 92.0 mmol) in dry  $CH_2Cl_2$  (117 mL) was added Grubb's 2<sup>nd</sup> Generation catalyst (0.992 g, 1.17 mmol). The flask was fitted with condenser and stirred at 50 °C under a steady stream of argon for 24 hours. The reaction mixture was then reduced in volume and loaded directly onto a silica gel column. Chromatography (0-10% EtOAc-Hexanes) afforded the hydroxy lipid (7.74g, 21.0mmol, 90%) as a pale yellow oil. The unreacted lipid was recovered quantitatively and recycled.  $[\alpha]_D^{22}$  -23.9 (c 1.0,  $CHCl_3$ )  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  5.73 (m, 1H), 5.52 (dd,  $J$  = 13.78, 7.28 Hz, 1H), 5.37 (dd,  $J$  = 15.35, 7.28 Hz, 1H), 2.50 (ddd,  $J$  = 20.92, 15.08, 6.93 Hz, 2H), 1.98 (s, 3H), 1.54-1.41 (m, 3H), 1.39 (s, 9H), 1.35-1.26 (m, 4H), 1.26-1.17 (m, 11H), 1.15-1.06 (m, 3H), 0.82 (d,  $J$  = 6.60 Hz, 6H)  $^{13}C$  NMR (75MHz,  $CDCl_3$ )  $\delta$  169.7, 169.0, 135.2, 126.7, 80.7, 71.2, 41.1, 38.9, 32.1, 29.8, 29.5, 29.4, 29.0, 28.7, 27.9, 27.9, 27.3, 22.6, 21.1; IR (neat)  $\nu_{max}$  3441, 2928, 2850, 2361, 2097, 1732, 1639, 1468, 11367, 1235, 1157, 1017, 963; ESI MS  $m/z$  391.05  $[M+Na]^+$ , 385.86  $[M+NH_4]^+$

(S)-tert-butyl 3-acetoxy-14-methylpentadec-4-enoate (1.03g, 2.79mmol) was dissolved in ethanol (27 mL) and 10% Pd/C (0.155g) was added. The flask was sealed and purged with hydrogen. The mixture was stirred for 24 hours, and upon completion filtered through a celite pad. The solvent was removed and silica gel chromatography (10%

EtOAc-Hexanes) afforded **II.22** (1.0181 g, 2.75 mmol, 98%) as a colorless oil.  $[\alpha]_D^{22} + 2.89$  (c 1.625,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  5.14 (m, 1H), 2.40 (dd,  $J = 6.56$ , 2.28 Hz, 2H), 1.96 (s, 3H), 1.57-1.41 (m, 6H), 1.37 (s, 1H), 1.24-1.16 (m, 15H), 0.80 (d,  $J = 6.61$  Hz, 6H);  $^{13}\text{C}$  NMR (75MHz,  $\text{CDCl}_3$ )  $\delta$  170.1, 169.5, 80.5, 70.7, 40.5, 38.9, 33.9, 29.8, 29.6, 29.5, 29.5, 29.4, 29.3, 29.3, 27.9, 27.3, 25.0, 22.5, 20.9; IR (neat) 2930, 2857, 1743, 1466, 1371, 1240, 1152; ESI MS  $m/z$  393.09  $[\text{M}+\text{Na}]^+$

**(R)-allyl 3-hydroxy-14-methylpentadecanoate (II.23)**

(R)-tert-butyl 3-acetoxy-14-methylpentadecanoate (**II.22**) (9.25 g, 24.95 mmol) was treated with a solution of TFA/ $\text{CH}_2\text{Cl}_2$  (1:1, 100 mL) and allowed to stir at room temperature overnight. The volatiles were removed in vacuo and the residue was left under high vacuum for several hours. The crude material was dissolved in MeOH (125 mL) and  $\text{K}_2\text{CO}_3$  (17.3 g, 125 mmol) was added. The mixture was stirred for 12 hours and the solvent was removed. EtOAc (50 mL) was added followed by the careful addition of 1N HCl while stirring. The aq. phase was extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with brine (1x 50 mL), dried over  $\text{Mg}_2\text{SO}_4$ , filtered and condensed. Silica gel chromatography (EtOAc) afforded (R)-3-hydroxy-14-methylpentadecanoic acid (6.12 g, 22.47 mmol, 90%) as a white solid. mp 59-60 °C;  $[\alpha]_D^{22} - 13.6$  (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  4.03 (tt,  $J = 8.04$ , 8.04, 4.08, 4.08 Hz, 1H), 2.51 (ddd,  $J = 25.53$ , 16.54, 6.07 Hz, 2H), 1.59-1.38 (m, 4H), 1.34-1.19 (m, 15H), 1.14 (dd,  $J = 13.18$ , 6.47 Hz, 2H), 0.85 (d,  $J = 6.62$  Hz, 1H);  $^{13}\text{C}$  NMR (100MHz,  $\text{CDCl}_3$ )  $\delta$  178.080, 68.323, 41.3, 39.3, 36.7, 30.2, 29.9, 29.9, 29.8, 29.8, 29.7,

28.2, 27.6, 25.7, 22.9; IR (neat)  $\nu_{\max}$  3228, 2922, 2850, 2689, 2592, 1713, 1467, 1446, 1363, 1316, 1301, 1189; ESI MS  $m/z$  271.16 [M-H]<sup>-</sup>

To a solution of (R)-3-hydroxy-14-methylpentadecanoic acid (1.50 g, 5.51 mmol) in MeOH (23 mL) was added Cs<sub>2</sub>CO<sub>3</sub> (1.83 g, 5.62 mmol) dissolved in H<sub>2</sub>O (2.3 mL) drop wise. The mixture was stirred at room temperature for 15 minutes and the solvent was removed in vacuo. To the residue was added DMF (5 mL). The solvent was removed in vacuo and placed under high vacuum for several hours. DMF (25 mL) was added followed by allyl bromide (4.76 mL, 55.1 mmol) and the mixture was stirred under argon for 12 hours. The DMF was removed and the crude residue was dissolved in EtOAc (30 mL) and washed with water (1 x 20 mL) and brine (1 x 20 mL). The organic phase was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Column chromatography (10% EtOAc-Hexanes) afforded the allyl ester (**II.23**) (1.55 g, 4.96 mmol, 90%) as a colorless oil.  $[\alpha]_D^{22}$  -13.7 (c 1.0); <sup>1</sup>H NMR (400 MHz, CHCl<sub>3</sub>)  $\delta$  5.85 (qd,  $J$  = 10.44, 5.73, 5.73, 5.73 Hz, 1H), 5.23 (m, 2H), 4.54 (dd,  $J$  = 5.75, 1.24 Hz, 1H), 3.95 (s, 1H), 3.13 (s, 1H), 2.42 (dq,  $J$  = 16.23, 16.19, 16.19, 6.13 Hz, 2H), 1.54-1.33 (m, 1H), 1.28-1.16 (m, 15H), 1.12-1.05 (m, 2H), 0.80 (dd,  $J$  = 6.63, 1.87 Hz, 1H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  172.9, 132.1, 118.8, 68.2, 65.5, 41.5, 39.270, 36.8, 30.2, 29.9, 29.9, 29.8, 29.8, 29.7, 28.2, 27.6, 25.7, 22.9; IR (neat)  $\nu_{\max}$  3456, 2922, 2854, 2364, 1731, 1648, 1464, 1411, 1384, 1363, 1168; ESI MS  $m/z$  313.00 [M+H]<sup>+</sup>

**(2S,3S)-1-((R)-1-(allyloxy)-14-methyl-1-oxopentadecan-3-yl) 4-cyclohexyl 3-(benzyloxy)-2-(tert-butoxycarbonylamino)succinate (II.25)**

To a stirring solution of Boc- $\beta$ OBn-Asp(OCy)-OH (0.141 g, 0.335 mmol), the alcohol (0.209 g, 0.670 mmol), and DMAP (0.020 g, 0.168 mmol) dissolved in CH<sub>2</sub>Cl<sub>2</sub> (1.1ml) at -15 °C was added EDCI (0.066 g, 0.342 mmol) in five portions over a period of 1 hour. The solution was stirred at this temperature under argon for 2 hours then slowly warmed to room temperature. The reaction was stirred 12hr then quenched with EtOAc (1ml). The solvent was removed in vacuo and the residue was dissolved in EtOAc (20 mL) then washed with 1N HCl (1 x 10 mL) and brine (1 x 10 mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Column chromatography (15% Pet. Ether-Ether) afforded (2*S*,3*S*)-1-((*R*)-1-(allyloxy)-14-methyl-1-oxopentadecan-3-yl) 4-cyclohexyl 3-(benzyloxy)-2-(tert-butoxycarbonylamino)succinate (0.201g, 0.280 mmol, 84%) as a colorless oil.  $[\alpha]_D^{22}$  - 16.0 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.33-7.24 (m, 5H), 5.88 (qd, *J* = 10.50, 5.75, 5.73, 5.73 Hz, 1H), 5.26 (q4 4H), 4.89-4.82 (m, 1H), 4.84-4.76 (m, 2H), 4.58-4.50 (m, 1H), 4.39 (d, *J* = 10.97 Hz, 1H), 2.59 (dq, *J* = 15.62, 15.54, 15.54, 6.24 Hz, 1H), 1.90-1.78 (m, 2H), 1.77-1.65 (m, 2H), 1.63-1.44 (m, 5H), 1.45-1.08 (m, 33H), 0.85 (d, *J* = 6.61 Hz, 1H); <sup>13</sup>C (100MHz, CDCl<sub>3</sub>)  $\delta$  169.6, 168.8, 168.5, 155.1, 136.7, 131.8, 128.2, 127.8, 127.8, 118.5, 79.7, 77.7, 74.1, 72.9, 72.3, 65.3, 56.022, 38.9, 38.8, 33.7, 31.4, 31.1, 29.8, 29.5, 29.5, 29.5, 29.3, 29.2, 28.0, 27.8, 27.3, 25.1, 24.9, 23.5, 23.4, 22.6, IR (neat)  $\nu_{max}$  3450, 2926, 2857, 1741, 1501, 1456, 1390, 1365, 1336, 1258, 1208, 1163, 1126, 1064; ESI MS *m/z* 738.28 [M+Na]<sup>+</sup>

**Boc-D- $\alpha$ Thr-D-Ala- $\beta$ OH-Pro-Orn(Fmoc)- $\beta$ OBn-D- $\alpha$ Asp(OCy)-OAllyl (11.5).**

Boc-Orn(Fmoc)- $\beta$ OBn-D- $\alpha$ Asp(OCy)-OAllyl (5.87 g, 7.37 mmol) was treated with 4N HCl-Dioxane (15 mL) and the resulting mixture was stirred at room temperature for



90min. The volatiles were removed in vacuo. The residual HCl was further removed by adding Et<sub>2</sub>O (3 mL) to the hydrochloride salt followed by its removal in vacuo. This residue, Boc-D-*α*Thr-D-Ala-βOH-Pro-OH (2.70 g, 6.70 mmol), and DIEA (1.4 mL, 8.04 mmol) were dissolved in THF (22 mL). The solution was cooled to 0 °C and EDCI (1.54 g, 8.04 mmol) was added. The reaction mixture was stirred under argon for 18hr and then quenched with H<sub>2</sub>O (3 mL). The THF was removed in vacuo and to the residue was added EtOAc (100 mL). This solution was extracted with 1N HCl (1 x 50ml), sat. aq. NaHCO<sub>3</sub> (1 x 50 mL), and brine (1 x 50 mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Chromatography (EtOAc) provided the pentapeptide (6.60 g, 6.10 mmol, 91%) as a white solid. mp 84-86°C,  $[\alpha]^{22}_D$  - 2.2 (c 1.0, CHCl<sub>3</sub>) <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.41 (d, *J* = 7.53 Hz, 1H), 8.39 (d, *J* = 9.08 Hz, 1H), 8.23 (d, *J* = 9.37 Hz, 1H), 8.03 (d, *J* = 8.21 Hz, 1H), 7.94-7.86 (m, 4H), 7.71-7.64 (m, 2H), 7.41 (t, *J* = 7.46, 7.46 Hz, 1H), 7.36-7.22 (m, 13H), 6.73 (t, *J* = 9.32, 9.32 Hz, 1H), 5.81 (tdd, *J* = 16.00, 10.71, 5.49, 5.49 Hz, 1H), 5.32-5.15 (m, 3H), 4.99-4.89 (m, 1H), 4.79 (dd, *J* = 18.04, 4.81 Hz, 2H), 4.75-4.66 (m, 2H), 4.61-4.52 (m, 4H), 4.50 (m, 1H), 4.46 (m, , 1H), 4.43 (d, *J* = 11.85 Hz, 1H), 4.35 (dd, *J* = 13.78, 6.59 Hz, 1H), 4.31-4.25 (m, 3H), 4.24-4.15 (m, 4H), 3.90 (t, *J* = 7.56, 7.56 Hz, 1H), 3.83-3.75 (m, 1H), 3.74 (t, *J* = 10.02, 10.02 Hz, 1H), 3.57 (m , 1H), 3.46 (dd, *J* = 9.87, 4.17 Hz, 1H), 3.02-2.88 (m, 2H), 2.03-1.83 (m, 1H), 1.84-1.55 (m, 9H), 1.55-1.22 (m, 18H), 1.24-1.11 (m, 3H), 1.07-0.98 (m, 3H); <sup>13</sup>C (100MHz, CDCl<sub>3</sub>) δ 172.5, 171.941, 171.3, 169.8, 168.3, 156.7, 155.7, 144.0, 143.9, 141.1, 136.3, 131.2, 128.3, 128.e, 128.1, 127.5, 126.9, 125.1, 119.8, 119.1, 79.8, 76.4, 74.5, 73.0, 72.7, 69.0, 68.9, 66.5, 59.2, 54.2, 52.8, 47.5, 47.2, 45.2, 40.3, 32.3, 31.4, 31.1, 30.5, 28.7, 28.2, 25.7, 25.1, 23.496, 19.7, 19.6, 16.4; IR (neat)  $\nu_{max}$  3306, 3068, 2981,

2937, 2859, 2364, 1743, 1699, 1650, 1527, 1454, 1393, 1367, 1163, 1104; ESI MS  $m/z$  1105.27  $[M+Na]^+$

### Septapeptide (II.29)

Boc-D-*α*Thr-D-Ala-βOH-Pro-Orn(Fmoc)-βOBn-D-*α*Asp(OCy)-OAllyl (0.860 g, 0.794 mmol) was treated with a solution of 4N HCl-Dioxane (4.0 mL, 15.89 mmol) and stirred for 90 minutes. The volatiles were removed in vacuo. The residual HCl was further removed by adding Et<sub>2</sub>O (5 mL) to the hydrochloride salt followed by its removal in vacuo. The residue was then dissolved in EtOAc (30 mL) and washed with sat. aq. NaHCO<sub>3</sub> (2 x 20 mL). The EtOAc was dried over MgSO<sub>4</sub>, filtered and condensed. This residue, the carboxylic acid (0.486 g, 0.719 mmol), and HOBt (0.117 g, 0.863 mmol) were dissolved in THF (2.4 mL) and cooled to 0 °C. EDCI (0.165 g, 0.863 mmol) was added and the mixture was stirred under argon for 18 hrs while slowly warming to room temperature. The reaction was quenched with EtOAc (5 mL) and the THF removed in vacuo. This residue was dissolved in EtOAc (50 mL) and washed with 1N HCl (1 x 30 mL), sat. aq. NaHCO<sub>3</sub> (1 x 30 mL), and brine (1 x 30 mL). The organic phase was dried over Mg<sup>2</sup>SO<sub>4</sub>, filtered and condensed. Column chromatography (EtOAc) afforded the septapeptide (0.980 g, 0.597 mmol, 83%) as a white solid. mp 70-72 °C;  $[\alpha]_D^{22}$  - 1.0 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.38 (d,  $J$  = 9.31 Hz, 1H), 8.23 (d,  $J$  = 9.13 Hz, 1H), 8.06 (d,  $J$  = 9.10 Hz, 1H), 8.04 (d,  $J$  = 9.65 Hz, 1H), 8.00 (d,  $J$  = 8.21 Hz, 1H), 7.86 (d,  $J$  = 7.47 Hz, 2H), 7.69-7.61 (m, 2H), 7.38 (t,  $J$  = 7.40, 7.40 Hz, 2H), 7.34-7.21 (m, 14H), 6.83 (dd,  $J$  = 17.39, 9.66 Hz, 1H), 5.79 (tdd,  $J$  = 16.10, 10.95, 5.47, 5.47 Hz, 1H), 5.21 (dd,  $J$  = 42.63, 13.72 Hz, 2H), 5.08 (m, 1H), 4.97-4.87 (m, 1H), 4.82-4.60

(m, 6H), 4.60-4.32 (m, 9H), 4.24 (t,  $J = 6.66, 6.66$  Hz, 3H), 4.22-4.13 (m, 3H), 3.75 (dd,  $J = 16.31, 7.92$  Hz, 1H), 3.50 (dd,  $J = 46.41, 6.44$  Hz, 1H), 3.01-2.86 (m, 1H), 2.47-2.35 (m, 1H), 1.82-1.53 (m, 11H), 1.52-1.25 (m, 29H), 1.25-1.02 (m, 31H), 0.99 (d,  $J = 5.66$  Hz, 1H), 0.81 (d,  $J = 6.57$  Hz, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  173.3, 172.6, 172.2, 170.9, 170.1, 169.9, 169.2, 168.6, 157.6, 157.1, 155.7, 144.3, 141.5, 137.0, 136.7, 131.6, 128.636, 128.5, 128.2, 127.9, 127.8, 127.3, 125.4, 125.2, 120.1, 119.4, 78.0, 78.4, 78.2, 76.8, 74.7, 74.4, 73.7, 73.5, 73.2, 73.0, 69.0, 68.2, 67.5, 66.8, 58.8, 58.2, 56.4, 54.6, 53.1, 48.2, 47.9, 47.5, 45.5, 41.2, 40.7, 39.3, 34.1, 33.9, 32.6, 31.7, 31.4, 30.2, 29.9, 29.7, 28.4, 28.183, 27.635, 26.2, 25.4, 23.8, 22.9, 20.3, 20.1, 16.7, 15.9; IR (Neat)  $\nu_{\text{max}}$  3300, 3069, 2932, 2857, 2361, 1749, 1731, 1713, 1655, 1634, 1540, 1522, 1507, 1456, 1367, 1339, 1257, 1206, 1160, 1104; ESI MS  $m/z$  1662.55  $[\text{M}+\text{Na}]^+$

### Linear Peptide (II.30)

The septapeptide (0.8759 g, 0.534 mmol) was treated with 4N HCl-Dioxane (4 mL) and the resulting mixture was stirred at room temperature for 90 minutes. The volatiles were removed in vacuo. The residual HCl was further removed by adding  $\text{Et}_2\text{O}$  (5 mL) to the hydrochloride salt followed by its removal in vacuo. This residue, Boc-D-Ser(OBn)- $\beta$ OHPro-OH (0.216 g, 0.529 mmol), HOBt (0.086 g, 0.634 mmol) and DIEA (0.110 mL, 0.634 mmol) were dissolved in THF (1.7 mL). The solution was cooled to 0 °C and EDCI (0.122 g, 0.634 mmol) was added. The reaction mixture was stirred under argon for 18 hrs and then quenched with EtOAc (1 mL). The THF was removed in vacuo and to the residue was added EtOAc (50 mL). This solution was extracted with 1N HCl (1 x 25 mL), sat. aq.  $\text{NaHCO}_3$  (1 x 25 mL), and brine (1 x 25 mL). The EtOAc was dried over

Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Chromatography (10% Acetone-EtOAc) provided the linear peptide (0.736 g, 0.381 mmol, 72%) as a white solid. mp 78-81 °C;  $[\alpha]_D^{22} + 1.2$  (c 1.0, CHCl<sub>3</sub>) <sup>1</sup>H NMR (500 MHz DMSO-*d*<sub>6</sub>, 45°C)  $\delta$  8.29 (t, *J* = 9.97, 9.97 Hz, 1H), 8.14 (d, *J* = 8.96 Hz, 1H), 8.06-7.91 (m, 3H), 7.87 (d, *J* = 7.53 Hz, 2H), 7.69-7.64 (m, 2H), 7.40 (t, *J* = 7.43, 7.43 Hz, 2H), 7.36-7.23 (m, 19H), 5.88-5.76 (m, 1H), 5.29 (dd, *J* = 17.15, 1.22 Hz, 1H), 5.18 (dd, *J* = 10.52, 1.35 Hz, 1H), 5.12 (d, *J* = 3.86 Hz, 1H), 5.09 (d, *J* = 3.77 Hz, 1H), 5.00 (s, 1H), 4.98-4.85 (m, 1H), 4.76-4.66 (m, 6H), 4.63-4.40 (m, 11H), 4.36 (s, 2H), 4.34-4.15 (m, 8H), 3.86-3.64 (m, 1H), 3.65-3.38 (m, 2H), 3.02-2.91 (m, 2H), 2.47-2.39 (m, 1H), 2.07-1.86 (m, 2H), 1.85-1.56 (m, 11H), 1.55-1.05 (m, 57H), 1.03 (d, *J* = 6.01 Hz, 3H), 0.84 (d, *J* = 1.63 Hz, 1H), 0.83 (d, *J* = 1.64 Hz, 3H); <sup>13</sup>C (100 MHz, CDCl<sub>3</sub>)  $\delta$  173.5, 172.8, 172.0, 171.8, 170.0, 169.5, 168.6, 168.5, 168.4, 168.2, 157.1, 156.896, 155.1, 144.2, 141.4, 138.2, 137.4, 136.9, 136.6, 131.5, 131.4, 130.0, 128.6, 128.5, 128.4, 128.2, 127.9, 127.8, 127.2, 125.2, 120.1, 119.4, 119.2, 79.6, 76.6, 76.1, 74.8, 74.6, 74.5, 74.1, 73.7, 73.5, 73.2, 72.9, 71.2, 70.6, 69.2, 68.7, 68.2, 66.8, 66.5, 58.3, 54.7, 53.0, 51.8, 51.1, 48.8, 48.4, 47.4, 46.2, 45.8, 41.6, 40.6, 39.2, 34.1, 33.1, 31.7, 31.5, 31.4, 31.3, 30.9, 30.1, 29.8, 29.7, 28.5, 28.1, 27.6, 26.0, 25.5, 25.3, 24.7, 23.8, 22.9, 19.9, 16.4; IR (neat)  $\nu_{max}$  3314, 3089, 3066, 3034, 2932, 2862, 1740, 1691, 1642, 1529, 1450, 1260, 1104; ESI MS *m/z* 1953.55 [M+Na]<sup>+</sup>

### Carboxylic acid of II.30

PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> (6.5 mg, 0.00921 mmol) and PPh<sub>3</sub> (7.25 mg, 0.028 mmol) were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (1.85 mL) and stirred under an atmosphere of argon for 15 min. This solution was then added to the allyl ester (0.712 g, 0.368 mmol) via syringe and

phenylsilane (0.091 mL, 0.737 mmol) was added drop wise. The solution was stirred at room temperature under an atmosphere of argon for 6 hrs. The solvent was condensed and the crude material was loaded directly onto a silica gel column. Chromatography (1-15%EtOH-CHCl<sub>3</sub>) afforded the carboxylic acid (0.664 g, 0.351 mmol, 95%) as a white solid. mp 109-111 °C;  $[\alpha]_D^{22}$  - 12.9 (c 1.0, CHCl<sub>3</sub>) <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>, 75°C) δ 7.83 (d, *J* = 1.87 Hz, 1H), 7.43-7.15 (m, 19H), 7.64 (d, *J* = 1.87 Hz, 2H), 5.16 (d, *J* = 1.87 Hz, 1H), 4.87 (d, *J* = 1.87 Hz, 1H), 4.72-4.61 (m, 3H), 4.60-4.39 (m, 9H), 4.38-4.31 (m, 2H), 4.30-4.20 (m, 6H), 4.18 (dd, *J* = 6.63, 1.87 Hz, 1H), 4.14 (s, 1H), 3.82-3.64 (m, 1H), 3.64-3.50 (m, 1H), 2.96 (d, *J* = 1.87 Hz, 2H), 2.44-2.34 (m, 1H), 2.03-1.68 (m, 8H), 1.63 (s, 4H), 1.56-1.06 (m, 57H), 1.03 (d, *J* = 1.87 Hz, 3H), 0.82 (d, *J* = 1.87 Hz, 1H) <sup>13</sup>C NMR(100 MHz, CDCl<sub>3</sub>) δ 174.4, 173.69, 170.71, 170.9, 170.7, 170.2, 169.7, 168.7, 166.6, 163.0, 157.6, 156.8, 155.7, 155.3, 144.2, 141.5, 138.1, 136.9, 134.3, 133.2, 130.1, 128.2, 127.8, 127.3, 125.4, 120.2, 79.7, 76.05, 74.5, 73.3, 72.7, 71.7, 70.7, 69.7, 68.7, 68.4, 67.8, 67.5, 66.8, 66.2, 66.0, 65.2, 63.9, 62.0, 61.2, 59.4, 58.9, 58.1, 56.4, 56.1, 55.9, 54.7, 53.2, 53.0, 51.9, 49.2, 48.1, 47.5, 45.7, 41.1, 40.8, 39.3, 36.7, 34.3, 32.7, 31.7, 30.1, 29.9, 28.6, 28.2, 27.6, 26.4, 25.5, 23.9, 22.9, 21.6, 20.9, 20.0, 17.8, 15.4, 14.2, 13.7; IR (neat)  $\nu_{max}$  3308, 3065, 2929, 2857, 1706, 1642, 1527, 1451, 1255, 1200, 1158; ESI MS *m/z* 1913.57 [M+Na]<sup>+</sup>

### Cyclic peptide (II.37)

The linear peptide (0.276 g, 0.146 mmol) was treated with a 4N HCl-Dioxane solution (2.0 mL) and stirred under an atmosphere of argon for 1 hr. The volatiles were then removed in vacuo and to the residue was added CH<sub>2</sub>Cl<sub>2</sub>/Hexanes (1:1, 10 mL). The

solvent was condensed to remove any residual HCl and the crude residue was placed under high vacuum for several hours. The product was used without further purification. To this residue in dry DMF (73 mL) at 0 °C was added HOBt (0.099 g, 0.730 mmol), DIEA (0.51 mL, 0.292 mmol) and finally EDCI (0.140 g, 0.730 mmol). The mixture was stirred under an atmosphere of argon at this temperature for 48 hours. The solvent was then removed and the crude material was diluted with EtOAc (30 mL). The organic phase was washed with 1N HCl (1 x 20 mL), sat. aq. NaHCO<sub>3</sub> (1 x 20 mL) and brine (1 x 20 mL). The EtOAc was dried over MgSO<sub>4</sub>, filtered and condensed. Silica gel chromatography (1-10% EtOH-CHCl<sub>3</sub>) afforded the cyclic peptide as a white solid. mp 93-95 °C; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 7.89-7.81 (m, 4H), 7.81-7.76 (m, 1H), 7.64 (d, *J* = 7.02 Hz, 2H), 7.60-7.53 (m, 2H), 7.39 (t, *J* = 7.45, 7.45 Hz, 3H), 7.36-7.18 (m, 17H), 5.23 (s, 1H), 5.05-4.89 (m, 1H), 4.86 (d, *J* = 7.85 Hz, 1H), 4.79-4.68 (m, 4H), 4.67-4.30 (m, 13H), 4.31-4.09 (m, 8H), 3.95-3.88 (m, 1H), 3.88-3.77 (m, 1H), 3.76-3.70 (m, 1H), 3.64 (dd, *J* = 9.45, 6.54 Hz, 1H), 3.61-3.52 (m, 2H), 2.93 (m, 2H), 2.61 (dd, *J* = 19.11, 11.29 Hz, 1H), 2.47-2.44 (m, 1H), 2.34 (dd, *J* = 14.25, 5.38 Hz, 2H), 2.00-1.87 (m, 1H), 1.85-1.56 (m, 14H), 1.57-0.99 (m, 51H), 0.88-0.76 (m, 6H); <sup>13</sup>C (100MHz, CDCl<sub>3</sub>) δ 175.0, 173.4, 172.4, 171.5, 170.9, 170.5, 170.0, 169.6, 169.2, 168.3, 167.63, 157.0, 144.2, 141.5, 137.9, 137.2, 136.7, 128.7, 128.7, 128.6, 128.6, 128.4, 128.3, 128.1, 127.9, 127.2, 126.2, 125.7, 125.4, 120.1, 117.8, 111.3, 74.6, 73.5, 73.4, 69.9, 68.9, 68.3, 67.4, 67.2, 66.8, 54.6, 54.1, 52.1, 47.5, 47.4, 45.5, 41.5, 40.9, 39.8, 34.5, 33.3, 32.0, 31.8, 31.7, 31.5, 31.2, 30.9, 30.3, 30.1, 29.9, 29.9, 29.8, 29.8, 29.7, 29.6, 29.5, 28.2, 27.6, 26.4, 25.5, 25.3, 24.8, 24.0, 23.7, 23.7, 22.9, 20.2, 19.3, 16.8; IR (neat) *v*<sub>max</sub> 3303, 3066, 3035,

2926, 2855, 1741, 1650, 1546, 1528, 1467, 1450, 1260, 1202; ESI MS  $m/z$  1795.65  $[M+Na]^+$ .

### Plusbacin A3 ((*R*)-II.1)

The cyclic peptide (0.117 g, 0.066 mmol) was treated with a 5% piperidine-DMF solution (0.5 mL) and stirred at room temperature for 15 minutes. The reaction was diluted with DMF 5ml and the solvent was removed in vacuo. To the residue was added DMF (5 mL) and the solvent was again removed in vacuo. The crude material was then left under high vacuum for several hours (4-8). To a stirring solution of the deprotected amine in dry DMF (0.75 mL) was added Boc-triflylguanidine (0.387g, 0.990mmol) DIEA (0.172 mL, 0.990 mmol). The reaction was stirred at room temperature for 18hrs then the DMF was removed in vacuo. Column chromatography (1-20% EtOH -  $CHCl_3$ ) afforded the guanidinylated peptide (0.107 g, 0.060 mmol, 91%) as a pale yellow solid. mp 89-91 °C;  $[\alpha]_D^{22}$  - 36.8 (c 1.0,  $CHCl_3$ );  $^1H$  NMR (500 MHz,  $DMSO-d_6$ )  $\delta$  8.80 (s, 1H), 8.23 (t,  $J$  = 4.84, 4.84 Hz, 1H), 8.10 (d,  $J$  = 12.10 Hz, 1H), 7.92 (d,  $J$  = 7.08 Hz, 1H), 7.86 (d,  $J$  = 5.39 Hz, 1H), 7.61 (d,  $J$  = 5.35 Hz, 1H), 7.38-7.17 (m, 1H), 5.33 (d,  $J$  = 6.32 Hz, 1H), 5.02 (s, 1H), 4.96-4.82 (m, 2H), 4.82-4.72 (m, 2H), 4.70 (dd,  $J$  = 15.65, 11.77 Hz, 3H), 4.65-4.08 (m, 12H), 3.85 (m, 1H), 3.73 (ddd,  $J$  = 25.55, 14.57, 7.93 Hz, 1H), 3.62 (dd,  $J$  = 8.83, 6.00 Hz, 1H), 3.56 (dd,  $J$  = 10.73, 6.55 Hz, 1H), 2.58 (d,  $J$  = 4.73 Hz, 1H), 2.46 (d,  $J$  = 10.88 Hz, 1H), 2.35 (dd,  $J$  = 12.91, 5.83 Hz, 1H), 1.98-1.87 (m, 1H), 1.87-1.56 (m, 12H), 1.56-1.08 (m, 70H), 1.05 (d,  $J$  = 6.03 Hz, 1H), 0.99 (d,  $J$  = 6.17 Hz, 1H), 0.84 (d,  $J$  = 6.26 Hz, 1H), 0.83 (d,  $J$  = 6.58 Hz, 6H);  $^{13}C$  NMR (100MHz,  $CDCl_3$ )  $\delta$  173.7, 172.4, 170.9, 170.388, 170.0, 170.0, 169.6, 169.3, 168.4, 167.4, 167.7, 163.6, 163.2,

156.3, 153.3, 137.2, 136.5, 129.9, 128.849, 128.7, 128.6, 128.6, 128.5, 128.3, 128.3, 128.2, 128.1, 127.8, 127.8, 121.3, 118.2, 115.0, 83.3, 79.6, 76.3, 74.6, 73.6, 73.4, 73.3, 73.3, 68.1, 54.3, 53.7, 52.9, 52.431, 52.3, 48.0, 45.7, 41.6, 40.9, 40.6, 39.2, 34.4, 33.0, 32.1, 31.6, 31.7, 31.4, 31.3, 30.9, 30.5, 30.108, 29.8, 29.7, 29.6, 29.6, 28.4, 28.2, 27.6, 25.7, 25.4, 25.4, 24.8, 23.8, 23.7, 23.6, 22.9, 22.7, 20.5, 19.6, 16.9, 14.3; IR (neat)  $\nu_{max}$  3337, 3068, 3028, 2930, 2853, 1740, 1642, 1452, 1415, 1386, 1365, 1328, 1258, 1233, 1196, 1155, 1134, 1101; ESI MS  $m/z$  1814.26  $[M+Na]^+$

The guanidinylated peptide (0.091g, 0.051mmol) and anisole (1ml) in an HF reaction apparatus were purged with N<sub>2</sub> and cooled to -78 °C. HF gas was distilled into the reaction vessel to a total volume of approximately 5 mL. The reaction was warmed to 0 °C and stirred at this temperature for 3hrs. The HF was then evaporated under a steady stream of N<sub>2</sub> and the crude material was transferred to a round bottom flask using EtOH. The volatiles were removed in vacuo and the crude material was triturated with Et<sub>2</sub>O and centrifuged (3 x). The crude material (0.059 g) was HPLC purified (35-65% CH<sub>3</sub>CN/H<sub>2</sub>O over 60 minutes) to afford (0.011 g, 0.0103 mmol, 20%) of Plusbacin A3 as a white powder. mp >250 °C decomposition;  $[\alpha]_D^{22} + 21.1$  (c 0.052, EtOH); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>CN/D<sub>2</sub>O/TFA, 500:500:1)  $\delta$  5.20-5.13 (m, 1H), 5.10 (t,  $J$  = 5.99, 5.99 Hz, 1H), 5.04 (d,  $J$  = 2.80 Hz, 1H), 4.92 (d,  $J$  = 2.31 Hz, 1H), 4.91 (m, 1H), 4.85 (d,  $J$  = 2.92 Hz, 1H), 4.78 (dd,  $J$  = 9.22, 2.04 Hz, 1H), 4.75 (d,  $J$  = 2.07 Hz, 1H), 4.73 (d,  $J$  = 2.25 Hz, 1H), 4.71-4.67 (m, 3H), 4.64 (d,  $J$  = 2.90 Hz, 1H), 4.61-4.57 (m, 3H), 4.49 (t,  $J$  = 7.16, 7.16 Hz, 1H), 4.41-4.36 (m, 1H), 4.34 (s, 1H), 4.33-4.30 (m, 1H), 4.28 (d,  $J$  = 2.13 Hz, 1H), 4.19-4.18 (m, 1H), 4.15-4.11 (m, 1H), 4.02-3.80 (m, 2H), 3.81-3.70 (m,



1H), 3.70-3.49 (m, 1H), 3.07 (dd,  $J = 14.19, 7.16$  Hz, 2H), 2.64-2.41 (m, 2H), 2.12-1.99 (m, 1H), 1.90-1.85 (m, 1H), 1.82-1.77 (m, 1H), 1.66 (s, 1H), 1.55 (s, 3H), 1.44 (dd,  $J = 13.36, 6.72$  Hz, 2H), 1.42-1.38 (m, 1H), 1.28-1.16 (m, 19H), 1.14 (d,  $J = 6.77$  Hz, 1H), 1.12-1.07 (m, 3H), 1.03 (d,  $J = 6.43$  Hz, 1H), 0.80 (d,  $J = 6.62$  Hz, 6H);  $^{13}\text{C}$  NMR (100MHz,  $\text{CD}_3\text{CN}/\text{D}_2\text{O}/\text{TFA}$ , 500:500:1)  $\delta$  175.2, 174.6, 174.1, 173.8, 173.3, 172.6, 172.2, 171.7, 171.4, 170.3, 169.7, 157.5, 76.3, 74.7, 74.3, 74.0, 70.7, 70.2, 69.7, 69.5, 68.7, 68.1, 61.2, 61.0, 56.2, 55.9, 54.8, 54.1, 53.8, 48.6, 46.2, 41.2, 40.8, 39.5, 34.5, 34.0, 33.2, 31.1, 30.3, 30.0, 29.1, 28.4, 27.8, 25.4, 25.2, 22.8, 19.5, 18.6, 16.8, 16.5; IR (KBr pellet)  $\nu_{\text{max}}$  3432, 2959, 2926, 2860, 1735, 1674, 1535, 1441, 1204, 1186, 1127; HR ESI TOF  $m/z$  1158.5873  $[\text{M}+\text{H}]^+$

## II.4.4 Synthesis of Plusbacin S isomer

### (S)-*tert*-butyl 3-hydroxy-14-methylpentadecanoate (II.26)

To a stirring solution of the allylic alcohol (2.312 g, 13.42 mmol) and lipid (9.79g, 53.7 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (65 mL) was added Grubb's 2<sup>nd</sup> Generation catalyst (0.571 g, 0.671 mmol). The flask was fitted with condenser and stirred at 50°C under a steady stream of argon for 24 hours. The reaction mixture was then reduced in volume and loaded directly onto silica gel column. Chromatography (0-10% EtOAc / Hexanes) afforded the hydroxy lipid (3.207 g, 9.82 mmol, 73%) as a pale yellow oil. The unreacted lipid was recovered quantitatively and recycled.  $[\alpha]_{\text{D}}^{22} + 10.8$  (c 1.0,  $\text{CHCl}_3$ )  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  5.66 (td,  $J = 13.47, 6.67, 6.67$  Hz, 1H), 5.42 (dd,  $J = 15.40, 6.37$  Hz, 1H), 4.39 (q,  $J = 6.13, 6.13, 6.08$  Hz, 1H), 3.13 (s, 1H), 2.40 (d,  $J = 6.72$  Hz, 2H), 1.97 (q,  $J = 7.06, 6.82, 6.82$  Hz, 2H), 1.53-1.44 (m, 2H), 1.43-1.40 (m, 10H), 1.38-1.27 (m, 4H), 1.25-1.18 (m,

11H), 1.16-1.06 (m, 3H), 0.85-0.78 (m, 6H);  $^{13}\text{C}$  NMR (75MHz,  $\text{CDCl}_3$ ) 171.7, 132.3, 130.5, 81.0, 68.9, 42.6, 39.0, 32.1, 29.8, 29.5, 29.4, 29.1, 29.0, 28.0, 27.9, 27.3, 22.6; IR (neat)  $\nu_{\text{max}}$  3441, 2928, 2850, 1740, 1468, 1367, 1258, 1157; ESI MS  $m/z$  349.09  $[\text{M}+\text{Na}]^+$

The olefin (3.03g, 9.29 mmol) was dissolved in Ethanol (93 mL) and 5% Pd/C (0.300 g) was added. The flask was sealed and purged with hydrogen. The mixture was stirred for 24 hours, and upon completion filtered through a celite pad. The solvent was removed and silica gel column chromatography (5% EtOAc / Hexanes) afforded the product (2.90 g, 8.83 mmol, 95%) as a colorless oil.  $[\alpha]_{\text{D}}^{22} + 13.1$  (c 1.0,  $\text{CHCl}_3$ )  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  3.96-3.85 (m, 1H), 3.19 (d,  $J = 3.10$  Hz, 1H), 2.37 (dd,  $J = 16.26, 3.24$  Hz, 1H), 2.27 (dd,  $J = 16.27, 8.90$  Hz, 1H), 1.52-1.43 (m, 2H), 1.42 (s, 9H), 1.40-1.34 (m, 2H), 1.28-1.17 (m, 15H), 1.14-1.06 (m, 2H), 0.81 (d,  $J = 6.62$  Hz, 1H);  $^{13}\text{C}$  NMR (100MHz,  $\text{CDCl}_3$ )  $\delta$  172.5, 81.0, 68.0, 42.2, 39.0, 36.43, 29.9, 29.6, 29.6, 29.582, 29.5, 29.5, 28.0, 27.9, 27.3, 25.9, 22.6; IR (Neat)  $\nu_{\text{max}}$  3458, 2853, 1730, 1712, 1466, 1366, 1254, 1154; ESI MS  $m/z$  351.10  $[\text{M}+\text{Na}]^+$

### **(S)-allyl 3-hydroxy-14-methylpentadecanoate (II.27)**

(S)-tert-butyl 3-hydroxy-14-methylpentadecanoate (2.40 g, 7.31mmol) was treated with a solution of TFA /  $\text{CH}_2\text{Cl}_2$  (1:1, 20 mL) and allowed to stir at room temperature overnight. The volatiles were removed in vacuo and the residue was left under high vacuum for several hours. Chromatography (EtOAc) afforded (S)-3-hydroxy-14-methylpentadecanoic acid (1.95 g, 7.16 mmol, 98%) as a white solid. m.p. 59-60 °C;

$[\alpha]^{22}_{\text{D}} + 13.5$  (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  4.03 (tt,  $J = 8.04, 8.04, 4.08, 4.08$  Hz, 1H), 2.51 (ddd,  $J = 25.53, 16.54, 6.07$  Hz, 2H), 1.59-1.38 (m, 4H), 1.34-1.19 (m, 15H), 1.14 (dd,  $J = 13.18, 6.47$  Hz, 2H), 0.85 (d,  $J = 6.62$  Hz, 1H);  $^{13}\text{C}$  NMR (100MHz,  $\text{CDCl}_3$ )  $\delta$  178.080, 68.323, 41.3, 39.3, 36.7, 30.2, 29.9, 29.9, 29.8, 29.8, 29.7, 28.2, 27.6, 25.7, 22.9; IR (Neat)  $\nu_{\text{max}}$  3228, 2922, 2850, 2689, 2592, 1713, 1467, 1446, 1363, 1316, 1301, 1189; ESI MS  $m/z$  271.16; ESI MS  $m/z$  271.16  $[\text{M}+\text{Na}]^+$

To a solution of the carboxylic acid (1.078 g, 3.96 mmol) in MeOH (17 mL) was added  $\text{Cs}_2\text{CO}_3$  (1.32 g, 4.04 mmol) dissolved in  $\text{H}_2\text{O}$  (1.7ml) drop-wise. The mixture was stirred at room temperature for 15 minutes and the solvent was removed in vacuo. To the residue was added DMF (5 mL). The solvent was removed in vacuo and placed under high vacuum for several hours. DMF (20 mL) was added followed by allyl bromide (3.43 mL, 39.6 mmol) and the mixture was stirred under argon for 12 hours. The DMF was removed and the residue was dissolved in EtOAc (30 mL) and washed with water (20 mL) and brine (20 mL). The organic phase was dried over  $\text{Mg}_2\text{SO}_4$ , filtered and condensed. Column chromatography (10% EtOAc / Hexanes) afforded the allyl ester (1.10 g, 3.52 mmol, 89%) as a colorless oil.  $[\alpha]^{22}_{\text{D}} + 12.1$  (c 1.0);  $^1\text{H}$  NMR (400 MHz,  $\text{CHCl}_3$ )  $\delta$  5.85 (qd,  $J = 10.44, 5.73, 5.73, 5.73$  Hz, 1H), 5.23 (m, 2H), 4.54 (dd,  $J = 5.75, 1.24$  Hz, 1H), 3.95 (s, 1H), 3.13 (s, 1H), 2.42 (dq,  $J = 16.23, 16.19, 16.19, 6.13$  Hz, 2H), 1.54-1.33 (m, 1H), 1.28-1.16 (m, 15H), 1.12-1.05 (m, 2H), 0.80 (dd,  $J = 6.63, 1.87$  Hz, 1H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  172.9, 132.1, 118.8, 68.2, 65.5, 41.5, 39.270, 36.8, 30.2, 29.9, 29.9, 29.8, 29.8, 29.7, 28.2, 27.6, 25.7, 22.9; IR (neat)  $\nu_{\text{max}}$  3456, 2922, 2854, 2364, 1731, 1648, 1464, 1411, 1384, 1363, 1168; ESI MS  $m/z$  313.00  $[\text{M}+\text{H}]^+$

**(2*S*,3*S*)-1-((*S*)-1-(allyloxy)-14-methyl-1-oxopentadecan-3-yl) 4-cyclohexyl 3-(benzyloxy)-2-(*tert*-butoxycarbonylamino)succinate (II.28)**

To a stirring solution of the carboxylic acid (**II.24**) (0.5771 g, 1.37 mmol), the alcohol (0.723 g, 2.31 mmol), and DMAP (0.084 g, 0.685 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (4.6 mL) at -15°C was added EDCI (0.289 g, 1.506 mmol) in five portions over a period of 1 hr. The solution was stirred at this temperature under argon for 2 hr then slowly warmed to room temperature. The reaction was stirred 12 hr then quenched with EtOAc (2 mL). The solvent was removed and the residue was dissolved in EtOAc (50 mL) then washed with 1N HCl (1x40 mL) and brine (1x40 mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Column chromatography (Pet. Ether / Ether, 8.5:1.5) afforded the product (0.771 g, 1.077 mmol, 79%) as a colorless oil.  $[\alpha]_D^{22}$  - 18.1 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.31-7.21 (m, 1H), 5.83 (m, 1H), 5.31-5.15 (m, 4H), 4.84 (m, 1H), 4.79 (dd, *J* = 10.23, 2.34 Hz, 1H), 4.74 (d, *J* = 10.94 Hz, 1H), 4.53-4.45 (m, 3H), 4.41 (d, *J* = 10.94 Hz, 1H), 2.48 (dq, *J* = 15.78, 15.78, 15.76, 6.34 Hz, 1H), 1.89-1.78 (m, 2H), 1.79-1.63 (m, 2H), 1.63-1.55 (m, 2H), 1.55-1.42 (m, 5H), 1.42-1.16 (m, 33H), 1.13 (m, 2H), 0.83 (d, *J* = 6.62 Hz, 1H); <sup>13</sup>C (CDCl<sub>3</sub>, 100MHz) δ 169.5, 168.8, 168.5, 155.1, 136.8, 131.8, 128.2, 127.9, 127.8, 118.3, 79.7, 77.5, 74.1, 72.8, 72.1, 65.1, 56.0, 38.9, 38.5, 33.6, 31.3, 31.0, 29.8, 29.5, 29.5, 29.4, 29.2, 29.2, 28.0, 27.8, 27.3, 25.1, 24.9, 23.5, 23.4, 22.5; IR (neat)  $\nu_{\max}$  3450, 2926, 2857, 1741, 1501, 1456, 1390, 1365, 1336, 1258, 1208, 1163, 1126, 1064; ESI MS *m/z* 738.27 [M+Na]<sup>+</sup>

### Septapeptide (II.29)

Boc-D-allo-Thr-D-Ala- $\beta$ OH-Pro-Orn(Fmoc)- $\beta$ -OBn-D-Asp(OCy)-OAllyl (1.289 g, 1.19 mmol) was treated with a solution of 4N HCl/Dioxane (5.95 mL, 23.8 mmol) and stirred for 90 minutes. The volatiles were removed in vacuo. The residual HCl was further removed by adding Et<sub>2</sub>O (5 mL) to the hydrochloride salt followed by its removal in vacuo. The residue was then dissolved in EtOAc (30 mL) and washed with sat. aq. NaHCO<sub>3</sub> (2 x 15 mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. This residue, the carboxylic acid (0.700 g, 1.036 mmol), and HOBt (0.210 g, 1.554 mmol) were dissolved in THF (3.45 mL) and cooled to 0°C. EDCI (0.298 g, 1.554 mmol) was added and the mixture was stirred under argon for 24 hrs while slowly warming to room temperature. The reaction was quenched with EtOAc (5 mL) and the THF removed in vacuo. This residue was dissolved in EtOAc (50 mL) and washed with 1N HCl (1 x 30 mL), sat. aq. NaHCO<sub>3</sub> (1 x 30 mL), and brine (1 x 30 mL). The organic phase was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Column chromatography (EtOAc) afforded the septapeptide (1.4206 g, 0.866 mmol, 84%) as a white solid. mp. 66-69 °C; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.21 (d, *J* = 8.44 Hz, 1H), 8.14 (d, *J* = 8.78 Hz, 1H), 8.01 (d, *J* = 9.08 Hz, 1H), 7.88-7.82 (m, 3H), 7.82-7.75 (m, 2H), 7.64 (m, 2H), 7.38 (t, *J* = 7.40, 7.40 Hz, 2H), 7.35-7.22 (m, 14H), 7.05 (s, 1H), 6.40 (s, 1H), 5.81 (tdd, *J* = 16.18, 10.72, 5.48, 5.48 Hz, 1H), 5.27 (dd, *J* = 17.22, 1.24 Hz, 1H), 5.17 (dd, *J* = 10.45, 0.70 Hz, 1H), 5.09 (m, 1H), 5.05-4.97 (m, 1H), 4.91 (ddd, *J* = 13.06, 9.30, 3.62 Hz, 1H), 4.78-4.66 (m, 3H), 4.66-4.40 (m, 11H), 4.33 (dd, *J* = 13.76, 7.51 Hz, 1H), 4.30-4.16 (m, 6H), 3.80 (m, 1H), , 3.76-3.65 (m, 1H), 3.63-3.40 (m, 2H), 2.97 (d, *J* = 4.44 Hz, 2H), 2.47-2.36 (m, 2H), 2.05-1.95 (m, 1H), 1.94-1.84 (m, 1H), 1.82-1.71 (m, 4H), 1.71-1.56 (m, 7H), 1.57-1.08

(m, 57H), 1.06 (d,  $J = 6.59$  Hz, 2H), 1.02 (d,  $J = 6.27$  Hz, 3H), 0.82 (d,  $J = 6.61$  Hz, 6H);  $^{13}\text{C}$  NMR (100MHz,  $\text{CDCl}_3$ )  $\delta$  171.8, 169.7, 169.5, 169.365, 168.7, 168.5, 168.0, 157.1, 156.5, 155.1, 155.0, 143.7, 140.9, 136.7, 136.1, 131.0, 128.1, 128.1, 128.0, 127.9, 127.7, 127.7, 127.4, 127.3, 126.7, 124.9, 124.7, 119.7, 119.7, 118.8, 79.6, 79.4, 76.2, 74.1, 73.9, 72.9, 72.8, 72.4, 68.5, 67.6, 66.8, 66.3, 55.8, 54.1, 52.3, 47.7, 47.4, 46.8, 45.0, 40.6, 40.4, 40.1, 38.7, 33.6, 32.1, 31.2, 30.9, 30.31, 29.6, 29.4, 29.2, 27.9, 27.7, 27.1, 24.9, 24.8, 23.2, 22.4, 20.7, 19.8, 19.3, 16.2, 15.3, IR (Neat)  $\nu_{\text{max}}$  3312, 3068, 2933, 2857, 1749, 1724, 1650, 1530, 1452, 1369, 1336, 1266, 1159, 1114; ESI MS  $m/z$  1662.51  $[\text{M}+\text{Na}]^+$ , 1540.55  $[\text{M}+\text{H}]^+$

### Linear Peptide (II.33)

The septapeptide **II.29** (1.396 g, 0.534 mmol) was treated with 4N HCl-Dioxane (6 mL) and the resulting mixture was stirred at room temperature for 90 min. The volatiles were removed in vacuo. The residual HCl was further removed by adding  $\text{Et}_2\text{O}$  (5 mL) to the hydrochloride salt followed by its removal in vacuo. This residue, Boc-D-Ser(OBn)- $\beta$ OHPro-OH (0.327 g, 0.801 mmol), HOBt (0.162 g, 1.202 mmol) and DIEA (0.209 mL, 1.201 mmol) were dissolved in THF (2.67 mL). The solution was cooled to  $0^\circ\text{C}$  and EDCI (0.230 g, 1.201 mmol) was added. The reaction mixture was stirred under argon for 36 hr and then quenched with EtOAc (1 mL). The THF was removed in vacuo and to the residue was added EtOAc (50 mL). This solution was extracted with 1N HCl (1 x 25 mL), Sat. aq.  $\text{NaHCO}_3$  (1 x 25 mL), and brine (1 x 25 mL). The EtOAc was dried over  $\text{Mg}_2\text{SO}_4$ , filtered and condensed. Chromatography (10% Acetone / EtOAc) provided the linear peptide (1.3947 g, 0.722 mmol, 90%) as a white solid. mp  $66\text{--}69^\circ\text{C}$ ,  $[\alpha]_{\text{D}}^{22}$  -

16.5 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>, 45°C) δ 8.31 (d, *J* = 8.31 Hz, 1H), 8.27 (d, *J* = 9.47 Hz, 1H), 8.13 (d, *J* = 9.12 Hz, 1H), 8.03 (d, *J* = 8.72 Hz, 1H), 7.95 (d, *J* = 7.78 Hz, 2H), 7.87 (d, *J* = 7.42 Hz, 2H), 7.70-7.62 (m, 2H), 7.40 (t, *J* = 7.45, 7.45 Hz, 2H), 7.38-7.24 (m, 19H), 7.17 (d, *J* = 4.83 Hz, 1H), 6.43 (s, 1H), 5.82 (m, 1H), 5.29 (d, *J* = 17.30 Hz, 1H), 5.18 (d, *J* = 10.44 Hz, 1H), 5.16-5.05 (m, 2H), 5.05-5.00 (m, 1H), 4.99-4.90 (m, 1H), 4.83-4.61 (m, 6H), 4.62-4.41 (m, 10H), 4.39-4.14 (m, 9H), 3.85-3.64 (m, 2H), 3.64-3.38 (m, 3H), 2.97 (m, 1H), 2.45-2.40 (m, 1H), 2.06-1.87 (m, 2H), 1.84-1.58 (m, 12H), 1.56-1.09 (m, 57H), 1.06 (d, *J* = 6.65 Hz, 2H), 1.03 (d, *J* = 6.11 Hz, 3H), 0.83 (d, *J* = 6.43 Hz, 6H); <sup>13</sup>C NMR (100MHz, CDCl<sub>3</sub>) δ 173.4, 172.8, 172.4, 172.0, 171.8, 170.9, 170.3, 168.5, 168.2, 157.6, 157.2, 157.0, 155.6, 144.2, 141.4, 138.1, 138.0, 137.1, 136.8, 136.7, 136.6, 131.5, 131.5, 130.1, 129.4, 128.6, 128.6, 128.2, 128.1, 128.0, 127.8, 127.3, 125.404, 125.2, 120.1, 119.4, 79.9, 76.6, 74.6, 74.5, 74.0, 73.3, 72.8, 70.6, 70.4, 68.971, 68.5, 67.2, 66.8, 64.6, 54.6, 54.3, 53.0, 52.6, 52.1, 52.0, 48.1, 47.4, 45.8, 40.8, 39.3, 34.2, 32.665, 32.1, 31.7, 31.4, 31.3, 30.8, 30.2, 29.9, 29.7, 29.7, 29.5, 28.8, 28.6, 28.2, 27.662, 25.4, 23.8, 22.9, 21.3, 20.1, 19.3, 17.6, 14.4; IR (Neat) ν<sub>max</sub> 3312, 3064, 2926, 2857, 1741, 1646, 1534, 1452, 1369, 1332, 1262, 1200, 1163, 1101; ESI MS *m/z* 1953.56 [M+Na]<sup>+</sup>

### Carboxylic Acid of II.33

PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> (0.012 g, 0.0170 mmol) and PPh<sub>3</sub> (0.013 g, 0.05 mmol) were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (3.4 mL) and stirred under an atmosphere of argon for 15 min. This solution was then added to the linear peptide (1.295 g, 0.671 mmol) via syringe and phenylsilane (0.166 mL, 1.342 mmol) was added drop-wise. The solution was stirred at room

temperature under an atmosphere of argon for 6 hr. The solvent was condensed and the residue immediately loaded onto a silica gel column. Column chromatography (1 -15% EtOH / CHCl<sub>3</sub>) afforded the carboxylic acid (1.189 g , 0.628 mmol, 94%) as a white solid. mp 102-105 °C,  $[\alpha]_D^{22}$  - 26.8 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>, 45°C) δ 8.61 (s, 1H), 8.45 (s, 1H), 8.04 (d, *J* = 8.33 Hz, 1H), 8.01-7.93 (m, 2H), 7.87 (d, *J* = 7.51 Hz, 2H), 7.67 (d, *J* = 6.94 Hz, 2H), 7.40 (t, *J* = 7.43, 7.43 Hz, 2H), 7.37-7.15 (m, 17H), 5.11 (s, 1H), 4.86 (d, *J* = 7.43 Hz, 1H), 4.84-4.77 (m, 1H), 4.73 (s, 1H), 4.69-4.61 (m, 2H), 4.61-4.43 (m, 9H), 4.43-4.14 (m, 10H), 3.85-3.66 (m, 2H), 3.64-3.48 (m, 3H), 3.47-3.37 (m, 1H), 2.97 (d, *J* = 4.46 Hz, 1H), 2.41 (s, 2H), 2.02-1.88 (m, 1H), 1.88-1.70 (m, 7H), 1.69-1.58 (m, 4H), 1.58-1.06 (m, 56H), 1.03 (d, *J* = 5.85 Hz, 3H), 0.83 (d, *J* = 5.85 Hz, 6H); <sup>13</sup>C NMR (100MHz, CDCl<sub>3</sub>) δ 173.244, 172.973, 172.6(2C), 172., 172.1, 171.6, 169.8, 168.8, 168.0, 166.1, 157.3, 156.5, 155.0, 143.6, 141.0, 137.6, 136.6, 134.1, 132.9, 129.6, 128.0, 127.8, 127.6, 127.3, 126.8, 124.9, 124.7, 119.8, 119.7, 79.2, 77.2, 74.0, 72.9, 72.4, 70.1, 69.3, 68.1, 67.1, 66.4, 58.0, 55.8, 54.2, 51.4, 47.9, 46.9, 45.1, 40.5, 38.8, 33.7, 33.4, 32.5, 31.6, 31., 29.7, 29.5, 29.4, 29.2, 28.0, 28.0, 27.7, 27.2, 26.2, 24.9, 24.3, 23.326, 22.4, 21.4, 20.0, 17.7, 15.0, 13.9; IR (Neat)  $\nu_{\max}$  3312, 3064, 2933, 2857, 1708, 1646, 1534, 1452, 1365, 1266, 200, 1159, 1109; ESI MS *m/z* 1913.50 [M+Na]<sup>+</sup>

### Cyclic Peptide (II.38)

The peptide (0.518 g, 0.274 mmol) was treated with a 4N HCl / Dioxane solution (2.0 ml) and stirred under an atmosphere of argon for 1 hr. The volatiles were then removed in vacuo and to the residue was added CH<sub>2</sub>Cl<sub>2</sub>/Hex (1:1, 5 mL). The solvent was



condensed to remove any residual HCl and the crude residue was placed under high vacuum for several hours. The product was used with no further purification. To this residue in dry DMF (137 mL) at 0°C was added HOBt (0.185 g, 1.37 mmol), DIEA (0.95 mL, 0.547 mmol) and finally EDC (0.262 g, 1.37 mmol). The mixture was stirred under and atmosphere of argon at this temp for 48 hours. The solvent was removed and the crude material was diluted with EtOAc (30 mL). The organic phase was washed with 1N HCl (1 x 20 mL), sat. aq. NaHCO<sub>3</sub> (1 x 20 mL) and brine (1 x 20 mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Column chromatography (5-100% Acetone / EtOAc) afforded the cyclic peptide (0.319 g, 0.180 mmol, 65%) as a white solid. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 8.03 (d, *J* = 7.44 Hz, 1H), 7.93 (d, *J* = 9.80 Hz, 1H), 7.89 (d, *J* = 7.53 Hz, 2H), 7.84 (d, *J* = 8.57 Hz, 1H), 7.78 (d, *J* = 8.22 Hz, 1H), 7.68 (d, *J* = 7.44 Hz, 2H), 7.55 (d, *J* = 9.09 Hz, 1H), 7.41 (t, *J* = 7.35, 7.35 Hz, 2H), 7.36-7.16 (m, 17H), 5.51 (d, *J* = 2.26 Hz, 1H), 5.40 (d, *J* = 3.37 Hz, 1H), 5.01 (m, 1H), 4.92 (d, *J* = 10.17 Hz, 1H), 4.86 (dd, *J* = 15.22, 8.97 Hz, 1H), 4.81 (dd, *J* = 8.90, 4.41 Hz, 1H), 4.75 (d, *J* = 5.28 Hz, 1H), 4.69-4.59 (m, 4H), 4.57 (d, *J* = 1.59 Hz, 1H), 4.55-4.38 (m, 6H), 4.36-4.30 (m, 1H), 4.30-4.25 (m, 2H), 4.24-4.16 (m, 3H), 4.14 (s, 1H), 4.13-4.04 (m, 2H), 4.00-3.91 (m, 1H), 3.81 (dd, *J* = 10.96, 6.21 Hz, 1H), 3.69 (d, *J* = 6.01 Hz, 1H), 3.52 (dd, *J* = 16.47, 8.41 Hz, 1H), 3.02-2.83 (m, 2H), 2.34-2.17 (m, 1H), 2.05-1.95 (m, 1H), 1.91-1.70 (m, 6H), 1.69-1.56 (m, 6H), 1.53-1.01 (m, 46H), 0.95 (d, *J* = 6.02 Hz, 3H), 0.81 (d, *J* = 6.55 Hz, 6H); <sup>13</sup>C NMR (100MHz, CDCl<sub>3</sub>) δ 172.9, 172.7, 171.5, 171.1, 170.1, 169.8, 169.4, 169.3, 168.1, 167.9, 166.7, 156.6, 143.9, 143.8, 143.6, 140.9, 137.3, 136.9, 135.9, 128.2, 128.1, 127.8, 127.8, 127.5, 127.3, 127.3, 126.8, 125.2, 125.1, 124.8, 119.6, 119.5, 78.4, 77.2, 75.2, 74.9, 74.2, 74.2, 73.4, 72.9, 72.7, 72.4, 71.7, 69.8,

67.6, 66.8, 66.3, 58.8, 56.0, 53.7, 51.7, 47.0, 45.6, 41.3, 40.3, 38.8, 34.2, 32.4, 32.2, 31.6, 31.4, 31.2, 31.0, 30.8, 29.7, 29.7, 29.5, 29.5, 29.4, 29.2, 29.2, 27.7, 27.1, 26.3, 25.5, 24.9, 23.7, 23.2, 22.4, 19.6, 19.1, 15.8, 15.3, 14.9, 13.877; IR (Neat)  $\nu_{\max}$  3334, 3068, 3032, 2933, 2850, 1738, 1648, 1534, 1455, 1254, 1205, 1100; ESI MS  $m/z$  1794.66  $[M+Na]^+$ , 1809.52  $[M+K]^+$

### Plusbacin Stereoisomer ((S)-II.1)

To a stirring solution of the amine (0.260 g, 0.147 mmol) in dry DMF (1.50 mL) was added DIEA (0.383 mL, 2.20 mmol) followed by the portion-wise addition of Boc-triflylguanidine (0.861 g, 2.20 mmol). The reaction was stirred at room temperature for 18 hrs then the DMF was removed in vacuo. Column chromatography (1-20% EtOH /  $CHCl_3$ ) afforded the guanidinylated peptide (0.218 g, 0.121 mmol, 83%) as a pale white solid. m.p. 83-86 °C;  $[\alpha]^{22}_D$  -46.5 (c 1.0,  $CHCl_3$ );  $^1H$  NMR (500 MHz,  $DMSO-d_6$ )  $\delta$  8.27 (t,  $J = 5.31, 5.31$  Hz, 1H), 8.03 (d,  $J = 7.86$  Hz, 1H), 7.98 (d,  $J = 9.70$  Hz, 1H), 7.95 (d,  $J = 7.47$  Hz, 1H), 7.84 (d,  $J = 6.90$  Hz, 1H), 7.78 (d,  $J = 8.10$  Hz, 1H), 7.52 (d,  $J = 9.09$  Hz, 1H), 7.40 (d,  $J = 6.01$  Hz, 1H), 7.35-7.21 (m, 15H), 5.51 (d,  $J = 2.66$  Hz, 1H), 5.39 (d,  $J = 3.72$  Hz, 1H), 4.98 (dd,  $J = 13.27, 6.66$  Hz, 1H), 4.93 (d,  $J = 11.05$  Hz, 1H), 4.86 (dd,  $J = 14.58, 8.82$  Hz, 1H), 4.80 (dd,  $J = 8.90, 4.67$  Hz, 1H), 4.74 (d,  $J = 5.31$  Hz, 1H), 4.69-4.59 (m, 4H), 4.57 (d,  $J = 1.89$  Hz, 1H), 4.55-4.39 (m, 6H), 4.35 (dd,  $J = 13.74, 8.22$  Hz, 1H), 4.20 (s, 2H), 4.14 (s, 1H), 4.12-4.04 (m, 2H), 3.95 (t,  $J = 8.84, 8.84$  Hz, 1H), 3.81 (m, , 1H), 3.69 (d,  $J = 6.32$  Hz, 1H), 3.52 (dd,  $J = 16.34, 8.28$  Hz, 1H), 3.25 (m, 2H), 2.33-2.19 (m, 2H), 2.07-1.95 (m, 1H), 1.92-1.71 (m, 7H), 1.70-1.58 (m, 6H), 1.52-1.43 (m, 1H), 1.41-1.37 (m, 14H), 1.35-1.25 (m, 8H), 1.25-1.05 (m, 29H), 0.95

(d,  $J = 6.13$  Hz, 1H), 0.85 (dd,  $J = 6.73, 0.48$  Hz, 1H), 0.82 (d,  $J = 6.58$  Hz, 1H);  $^{13}\text{C}$  NMR (100MHz,  $\text{CDCl}_3$ )  $\delta$  172.7, 171.4, 171.1, 170.7, 169.9, 169.7, 169.4, 169.0, 168.4, 168.2, 167.0, 163.4, 155.9, 152.9, 137.2, 136.3, 136.0, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.4, 82.6, 78.9, 77.7, 77.6, 75.1, 74.3, 74.2, 73.6, 73.0, 72.9, 72.6, 72.5, 71.8, 70.1, 69.4, 67.8, 59.0, 56.0, 53.9, 52.8, 52.6, 52.3, 46.9, 45.5, 41.2, 40.3, 38.9, 34.3, 32.5, 32.3, 31.5, 31.3, 31.2, 31.1, 30.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.1, 27.9, 27.2, 26.9, 25.8, 24.9, 24.7, 23.6, 23.4, 22.5, 22.3, 19.7, 15.9; ESI MS  $m/z$  1815.21  $[\text{M}+\text{Na}]^+$

The cyclic peptide (0.091 g, 0.051 mmol) and anisole (1 mL) in an HF reaction apparatus were purged with  $\text{N}_2$  and cooled to  $-78^\circ\text{C}$ . HF gas was distilled into the reaction vessel to a total volume of approximately 5 mL. The reaction was warmed to  $0^\circ\text{C}$  and stirred at this temperature for 3hrs. The HF was then evaporated under a steady stream of argon. The volatiles were removed in vacuo and the crude material was triturated with  $\text{Et}_2\text{O}$  and centrifuged (3 x). The crude material was HPLC purified to afford (10.9 mg, mmol) of plusbacin A3 stereoisomer as a white solid. mp  $>250^\circ\text{C}$  decomposition;  $[\alpha]_{\text{D}}^{22} + 10.1$  (c 0.034, EtOH);  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{CN}/\text{D}_2\text{O}/\text{TFA}$ , 500:500:1)  $\delta$  5.20-5.13 (m, 1H), 5.10 (t,  $J = 5.99, 5.99$  Hz, 1H), 5.04 (d,  $J = 2.80$  Hz, 1H), 4.92 (d,  $J = 2.31$  Hz, 1H), 4.91 (m, 1H), 4.85 (d,  $J = 2.92$  Hz, 1H), 4.78 (dd,  $J = 9.22, 2.04$  Hz, 1H), 4.75 (d,  $J = 2.07$  Hz, 1H), 4.73 (d,  $J = 2.25$  Hz, 1H), 4.71-4.67 (m, 3H), 4.64 (d,  $J = 2.90$  Hz, 1H), 4.61-4.57 (m, 3H), 4.49 (t,  $J = 7.16, 7.16$  Hz, 1H), 4.41-4.36 (m, 1H), 4.34 (s, 1H), 4.33-4.30 (m, 1H), 4.28 (d,  $J = 2.13$  Hz, 1H), 4.19-4.18 (m, 1H), 4.15-4.11 (m, 1H), 4.02-3.80 (m, 2H), 3.81-3.70 (m, 1H), 3.70-3.49 (m, 1H), 3.07 (dd,  $J = 14.19, 7.16$  Hz, 2H), 2.64-2.41 (m, 2H), 2.12-1.99 (m, 1H), 1.90-1.85 (m, 1H), 1.82-1.77 (m, 1H), 1.66 (s, 1H), 1.55

(s, 3H), 1.44 (dd,  $J = 13.36, 6.72$  Hz, 2H), 1.42-1.38 (m, 1H), 1.28-1.16 (m, 19H), 1.14 (d,  $J = 6.77$  Hz, 1H), 1.12-1.07 (m, 3H), 1.03 (d,  $J = 6.43$  Hz, 1H), 0.80 (d,  $J = 6.62$  Hz, 6H); (KBr pellet)  $\nu_{max}$  3432, 2959, 2926, 2860, 1735, 1674, 1535, 1441, 1204, 1186, 1127; HR ESI TOF  $m/z$  1158.5873  $[M+H]^+$

## MIC ASSAYS

Todd-Hewitt base (Hardy Diagnostics, Santa Maria, CA) was used to prepare Todd-Hewitt broth (THB) and agar (THA) per standard protocol. Microorganisms used in the screening included methicillin-resistant *Staphylococcus aureus* (ATCC # 33591), vancomycin-resistant *Enterococcus faecalis* (ATCC # 51299), *Streptococcus pyogenes* M49 strain NZ131 (Datta, V, et al. 2005. Mol. Micro. 56: 681), *Streptococcus agalactiae* strain A909 (Doran, KS, et al. 2002. J. Infect. Dis. 185: 196), *Escherichia coli* (ATCC # 25922), and *Pseudomonas aeruginosa* (ATCC # 27853). Compound dilutions were prepared in THB in sterile flat-bottom 96 well polystyrene plates (Costar # 7593, Corning Life Sciences, Inc. Lowell, MA). Screening controls for every assay included vancomycin (Abbott Laboratories, Chicago, IL) at the same concentration as the compounds/mixtures, vehicle alone, and no bacteria. Screening was done by inoculating 200  $\mu$ l THB containing the diluted compound mixtures with 2  $\mu$ l of an overnight culture of the desired bacterial strain using a Boekel replicator (Boekel Scientific, Feasterville, PA). The inoculated plates were incubated at 37°C for 24 hours. The minimal inhibitory concentration (MIC, in  $\mu$ g/ml), the lowest concentration of the inhibitor that completely prevented visible growth, was then determined turbidimetrically at  $A_{595}$ . To determine minimal bactericidal concentration (MBC, in  $\mu$ g/ml), Todd-Hewitt agar plates in the

absence of antibiotic were inoculated with bacteria from the MIC assay using the Boeckel replicator and were incubated at 37°C for 24 hours. The MBC was determined by careful visual examination of the agar plates for evidence of growth and was calculated as the lowest concentration of compound that completely inhibited growth in the agar. Each set of screening assays was repeated three times.

## CIRCULAR DICHROISM

The CD spectra were recorded with an Aviv Instruments Model 215 with a path cell length of 2.0 mm. The spectra were acquired between 300 and 190 nm sampling every 0.5 nm. Ellipticity measurements were expressed as mean residue ellipticity  $[\theta]$  in deg cm<sup>2</sup>/dmol. All the samples were prepared in an analogous manner first dissolving peptide as a 1.0 mg/mL solution and then preparing the appropriate sample dilution using Fischer HPLC grade H<sub>2</sub>O. In each sample the peptide concentration was 0.2 mg/mL.

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# CHAPTER III

## DESIGN SYNTHESIS AND BIOLOGICAL EVALUATION OF PLUSBACIN A3 ANALOGS

### III.1 INTRODUCTION

Plusbacin A3 is predicted to exert its antimicrobial action via the sequestration of peptidoglycan intermediates such as lipid II<sup>1-3</sup>. We have completed the first total synthesis of plusbacin A3 and shifted our efforts to pursue a detailed understanding of its mode of action and pharmacological properties.<sup>4</sup>

As a first step towards improving its pharmacologic properties, and with NMR as one of the most useful methods for determining the structure of peptides and proteins in solution, we have performed a conformational and functional analysis of this antibiotic peptide to determine the structural features responsible for its antibiotic activity. We report here the initial solution model of plusbacin A3, as derived from two-dimensional nuclear magnetic resonance (NMR) restraints and molecular modeling. Distance and dihedral angle restraints, generated from Nuclear Overhauser Effect Spectroscopy (NOESY) and one-dimensional NMR experiments, were used to generate an ensemble of structures using distance geometry.

## III.2 CONFORMATIONAL ANALYSIS OF PLUSBACIN A3

### III.2.1 NMR Analysis of Plusbacin A3

In order to gain a better understanding of the relationship between structure and biological activity, the conformational analysis of Plusbacin A3 was investigated in collaboration with Professor Dale. Mierke of Dartmouth College, using  $^1\text{H}$  NMR spectroscopy and computer simulations *in vacuo*.

The conformational analysis of a ligand can provide insight into the relationship between three dimensional structure of a ligand in solution and its biological activity. Utilizing a combination of  $^1\text{H}$  NMR and molecular modeling, plusbacin A3, has been shown to exhibit specific spatial arrangements of the peptide backbone and amino acid side chains which are critical in defining the antibiotic activity.

$^1\text{H}$  NMR assignments of the peptide in DMSO- $\text{d}_6$  were accomplished by means of TOCSY and NOESY experiments according to the standard procedures<sup>5</sup>. Complete chemical shift assignments in DMSO are reported in **Table III.1**. The presence of intramolecular hydrogen bonds was deduced from the initial solution structure and from the temperature dependence of the amide proton resonances. In DMSO solution, amide proton temperature coefficients ( $-\delta$  ppb/K) in the range of 0-2.0 are considered to be involved in an intramolecular hydrogen bond or solvent shielded. Values greater than 4.0 –ppb/K are considered to be completely solvent exposed. Intermediate values, 2.0-4.0 are involved in a hydrogen bond for a small fraction of time. Temperature coefficients are reported in **Table III.2** and the relevant NOE-derived distances are reported in **Table III.3**.

The spectrum of plusbacin A3 in aqueous solution did not display narrow lines, but instead broad dispersed signals. Experimentation with variable temperature did not resolve the problem. Similar results were obtained for samples containing 25% DMSO- $d_6$ . However, spectra recorded in DMSO only, resulted in narrow lines and desirable amide resolution.

**Table III.1: Proton chemical shifts for plusbacin A3 in DMSO at 35°C, 600MHz**

AA	NH	$\alpha$ CH	$\beta$ CH	$\gamma$ CH	$\delta$ CH	other
<b>14-MePent. Acid<sup>1</sup></b>		2.35, 2.70	4.9	1.25	1.24	C7-13 1.24 C14 1.14 C15 0.84
<b>D-aThr<sup>2</sup></b>	8.01	4.37	3.832	0.955		
<b>Ala<sup>3</sup></b>	8.15	4.26	1.03			
<b><math>\beta</math>OH-Pro<sup>4</sup></b>		4.438	4.406	1.795, 1.922	3.28, 3.423	
<b>Arg<sup>5</sup></b>	7.77	4.27	1.66	1.414	3.08	NH' 10.64; NH'' 6.8-7.2
<b><math>\beta</math>OH-D-Asp<sup>6</sup></b>	7.75	4.66	4.31			
<b>D-Ser<sup>7</sup></b>	8.27	4.53	3.52, 3.66			
<b><math>\beta</math>OH-Pro<sup>8</sup></b>		4.52	4.365	1.604, 1.945	3.508, 3.270	
<b><math>\beta</math>OH-Asp<sup>9</sup></b>	8.22	4.27	3.84			

**Table III.2: Calculated temperature coefficient (-ppb/K) of the amide protons for plusbacin A3 in DMSO**

Residue	-ppb/K
14-MePent. Acid <sup>1</sup>	-
DaThr <sup>2</sup>	3
Ala <sup>3</sup>	2.5
OHPro <sup>4</sup>	-
Arg <sup>5</sup>	0.5
OHDas <sup>6</sup>	1
Dser <sup>7</sup>	2.5
OHPro <sup>8</sup>	-
OHAsp <sup>9</sup>	0

**Table III.3: Relevant NOE-derived distances (in Angstroms) and corresponding average calculated distances derived from distance geometry (DG)**

Residue	Atom	Residue	Atom	Upper	Average
5 arg	$\gamma$ C	5 arg	NH	3.74	3.77
5 arg	NH	5 arg	$\alpha$ H	2.71	2.77
5 arg	NH	9 bha	NH	2.63	2.63
3 ala	$\beta$ C	5 arg	NH	5.2	3.55
4 bhp	$\alpha$ H	5 arg	NH	2.8	2.23
7 ser	NH	7 ser	$\alpha$ H	2.84	2.44
7 ser	$\beta$ C	7 ser	NH	3.45	3.37
6 bha	NH	7 ser	NH	4	3.73
5 arg	$\gamma$ C	7 ser	NH	4.69	4.15
5 arg	$\gamma$ C	9 bha	NH	4.03	3.98
5 arg	$\beta$ C	9 bha	NH	4.69	3.28
9 bha	NH	9 bha	$\alpha$ H	2.58	2.42
3 ala	$\beta$ C	9 bha	NH	5.1	4.56
9 bha	NH	9 bha	$\beta$ H	2.97	2.72
2 thr	$\gamma$ H1	3 ala	NH	3.7	3.06
2 thr	$\alpha$ H	3 ala	NH	2.38	2.4
2 thr	$\beta$ H	3 ala	NH	2.8	2.8
3 ala	NH	4 bhp	$\alpha$ H	4	3.95
1 lac	C4	2 thr	NH	4.34	3.27
1 lac	C5	2 thr	NH	5	3.99
2 thr	$\gamma$ C2	2 thr	NH	4.18	2.35
2 thr	NH	2 thr	$\alpha$ H	3.01	2.73
2 thr	NH	2 thr	$\beta$ H	3.1	2.82
1 lac	h3	2 thr	NH	3.79	3.8
2 thr	NH	2 thr	$\gamma$ H1	4.06	4.18
5 arg	$\beta$ C	5 arg	$\epsilon$ H	4.15	3.72
5 arg	NH	6 bha	NH	4	3.07
6 bha	NH	6 bha	$\alpha$ H	3.05	2.98
6 bha	NH	6 bha	hw	4.26	3.58
2 thr	NH	2 thr	$\alpha$ H	2.82	2.73
2 thr	NH	2 thr	$\beta$ H	3.1	2.82
5 arg	$\delta$ C	5 arg	$\alpha$ H	4.6	3.47
1 lac	C5	1 lac	H3	3.61	3.03
2 thr	$\alpha$ H	2 thr	$\gamma$ H1	2.84	2.88
5 arg	$\delta$ C	6 bha	$\alpha$ H	4.96	4.78
6 bha	$\alpha$ H	6 bha	$\beta$ H	2.38	2.36
5 arg	$\gamma$ C	6 bha	$\alpha$ H	4.51	4.32
6 bha	$\alpha$ H	6 bha	$\omega$ H	3.91	3.71
3 ala	$\alpha$ H	4 bhp	$\alpha$ H	2.3	2.16
3 ala	$\beta$ C	4 bhp	$\alpha$ H	4.42	4.13
2 thr	$\gamma$ C2	2 thr	$\alpha$ H	2.82	2.86
2 thr	$\alpha$ H	2 thr	$\beta$ H	2.57	2.65
9 bha	$\alpha$ H	9 bha	$\beta$ H	2.49	2.44
5 arg	$\delta$ C	5 arg	$\alpha$ H	3.95	3.47
5 arg	$\gamma$ C	5 arg	$\alpha$ H	3.13	3.1
3 ala	$\beta$ C	5 arg	$\delta$ C	6.26	6.25
1 lac	C5	1 lac	C2	3.99	3.45
H3 = proton on carbon 3		C# = carbon atom			

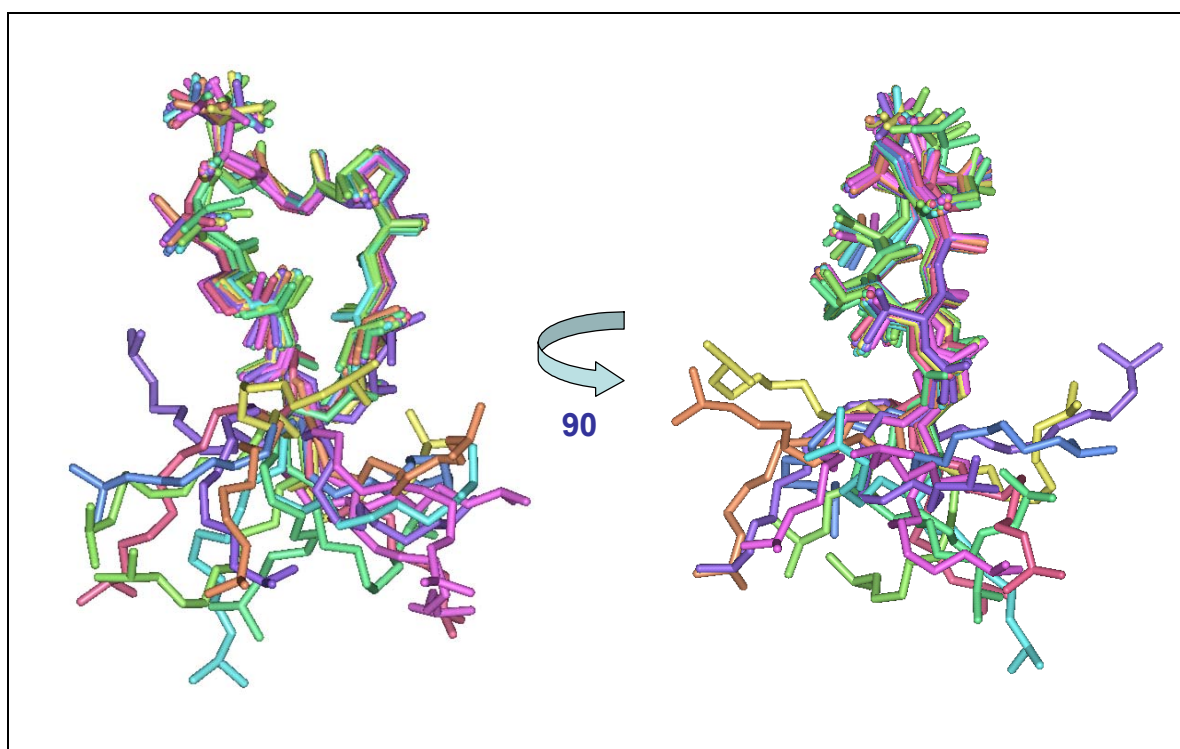
### III.2.2 Molecular Modeling

To obtain structural information on the conformational preferences of plusbacin A3, Distance Geometry (DG)<sup>6-9</sup> calculations were carried out for the NMR data in DMSO media.<sup>10</sup> These calculations represent a rough approximation of the complex conformational equilibrium existing in solution and, in addition, allow for a thorough searching of the conformational space consistent with the experimental observations. **Figure III.1** represents an overlay of the 29 lowest energy backbone formations refined with DG. The analysis of the structures obtained underlines that a large flexibility in the fatty acid side chain exists. The DG calculations of 100 starting structures produced resulting conformations with low penalties with respect to the distance derived from the molecular constitution and experimental constraints.

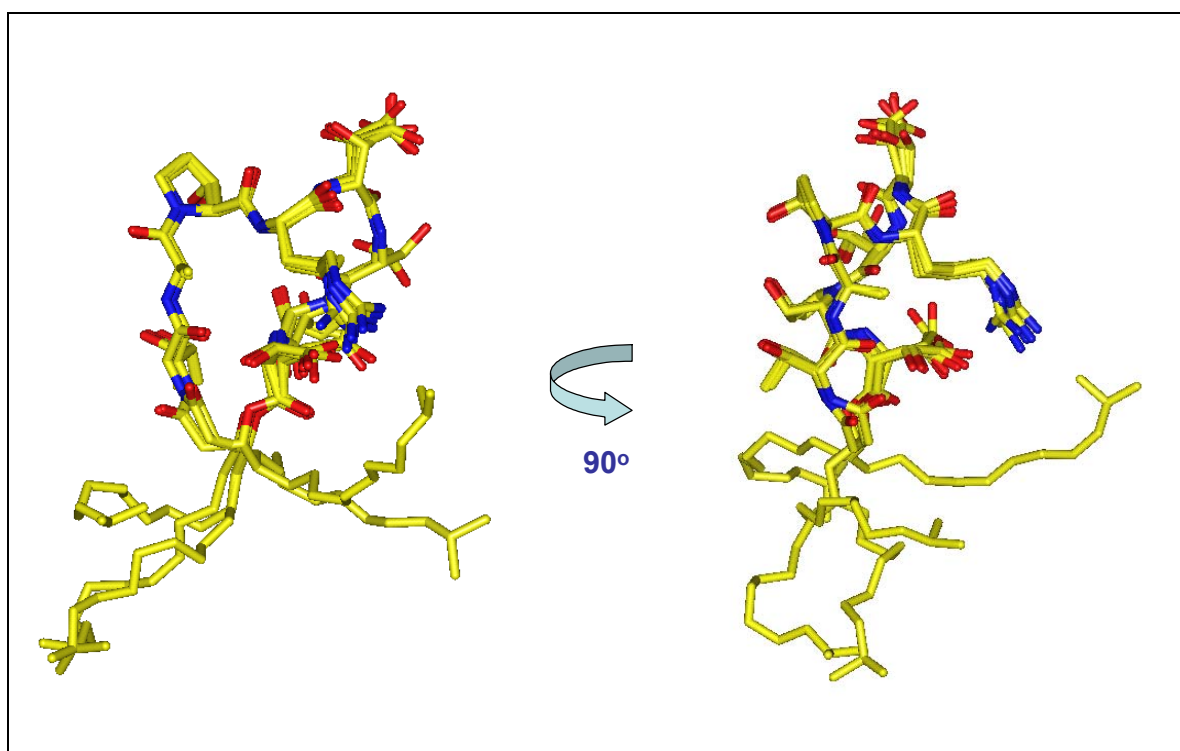
The analysis of the peptide conformation in DMSO showed a well-defined secondary structure for the cyclic backbone of plusbacin A3. The side chain constraints resulted in an interesting intramolecular interaction between the Arg guanidine and the  $\beta$ OH-L-Asp side chain, suggesting the possibility of a defined salt bridge, **Figure III.2**.

The 29 low energy conformations derived from DG also indicate the possibility of four key intramolecular hydrogen bonds in addition to salt bridge mentioned above. **Table III.4** presents the hydrogen bonded atoms as derived from molecular modeling. Interestingly, these calculations correlate well with the temperature coefficient data from **Table III.2**.

Based on the molecular modeling data, **Figures III.1-III.3**, we envisioned a series of analogs that could probe the importance of key structural features and select non-covalent interactions for antibiotic activity.



**Figure III.1:** Superposition of 29 lowest energy structures derived from DG calculations

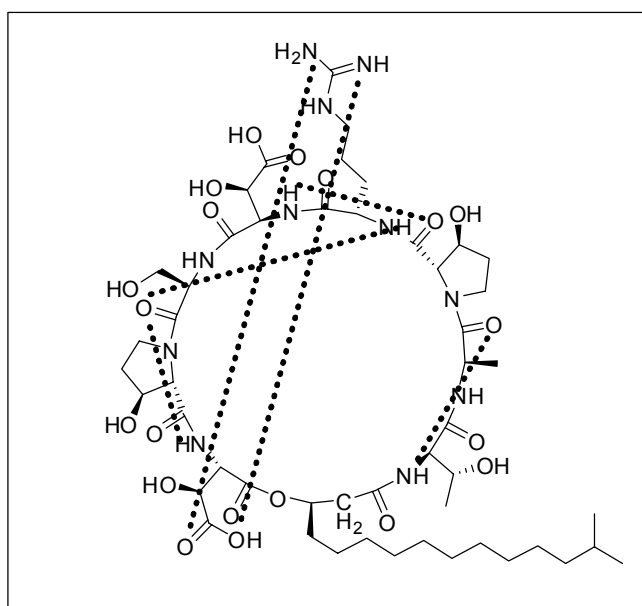


**Figure III.2: Five representative structures illustrating Arg<sup>5</sup>-Bha<sup>9</sup> Salt Bridge**



**Table III.4: Hydrogen Bonds derived from DG calculations**

	Atom 1	Atom 2
1	D-a Thr <sup>2</sup> <b>HN</b>	D-Ala <sup>3</sup> <b>CO</b>
2	Arg <sup>5</sup> <b>HN</b>	D-Ser <sup>7</sup> <b>CO</b>
3	$\beta$ OH-D-Asp <sup>6</sup> <b>HN</b>	$\beta$ OH-Pro <sup>4</sup> <b>CO</b>
4	$\beta$ OH-Asp <sup>9</sup> <b>HN</b>	D-Ser <sup>7</sup> <b>CO</b>
5	Arg <sup>5</sup> (NH <sub>2</sub> ) <sub>2</sub> <b>HN</b>	$\beta$ OH-Asp <sup>9</sup> <b>CO</b>

**Figure III.3. Illustration of hydrogen bonds derived from molecular modeling**

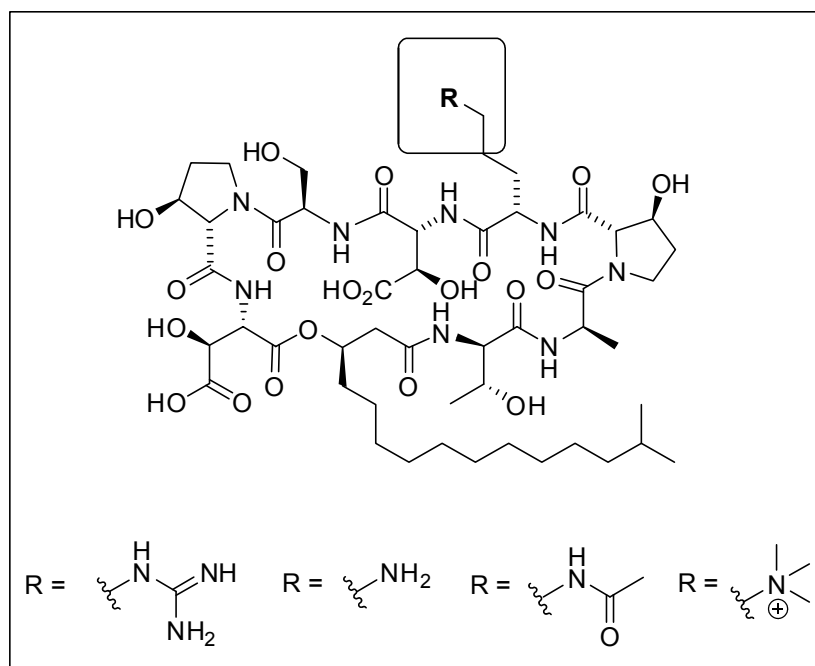
### III.3 ARGININE MODIFICATIONS

In our initial SAR studies we hoped to study two principle interactions that may play important roles in the biological activity of plusbacin A3. First we had hoped to disrupt the salt-bridge between the terminal guanidine group of the arginine and the carboxylate of the  $\beta$ OH-L-aspartic acid. By reducing, then completely removing the ability of the basic guanidinium functionality to participate in hydrogen bonding, we hypothesized that an ornithine derivative could influence and destabilize the peptide's active conformation. Second, one is drawn to the possibility that the guanidine functionality could be important for binding interactions with diphosphate or carboxylate groups present in lipid intermediates and/or nascent peptidoglycan<sup>11,12</sup>. Modification of the cationic species through a similar series of analogs could effectively provide insight into these binding interactions.

#### III.3.2 Synthesis of Arginine Analogs

In order to compare the effects of electronic and steric interactions about the arginine residue of plusbacin A3, a series of unnatural ornithine derivatives were incorporated into the peptide backbone (**Figure III.4**). The other non-proteogenic amino acids were prepared in our laboratory as previously reported. The synthesis of three analogs was designed based on the previously reported total synthesis. The key intermediates were formed via segment couplings of common peptide fragments, thereby reducing the number of intermediates that had to be synthesized. Also, the protecting

groups were chosen so that the global deprotection could occur subsequent to the installation of the key ornithine side chain functionalities.



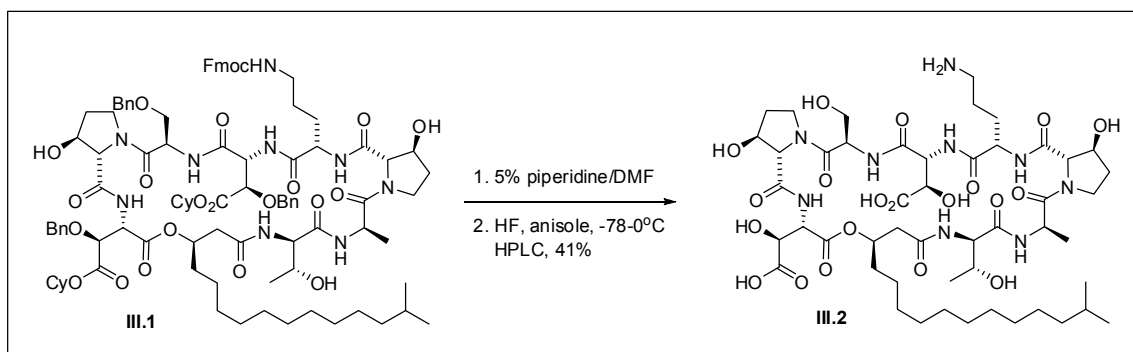
**Figure III.4: Modifications to be made at the arginine residue**

**Scheme III.1.** represents the preparation of our ornithine substituted analog. The core cyclic peptide intermediate **III.1** is identical to that prepared during our synthesis of the natural product. The ornithine side chain protection was cleanly removed (5% piperidine, DMF) to afford the free amine. Global deprotection of the benzyl ethers and cyclohexyl ester under our standard conditions (HF, anisole,  $-78^\circ\text{C}$ , 41%) afforded the target analog (**III.2**) in good yield after HPLC purification.

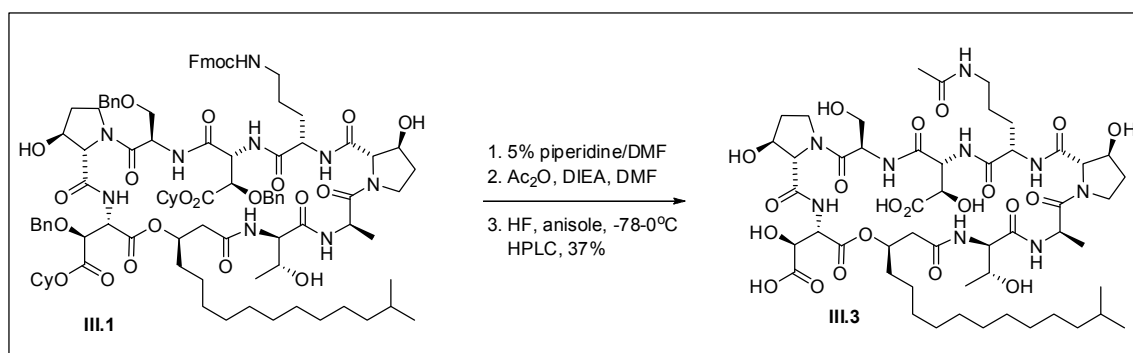
A similar route was taken for the synthesis the N-acyl-ornithine derivative (**Scheme III.2**). The Fmoc protecting group was removed (5% piperidine, DMF) under

standard conditions, and subsequent acylation ( $\text{Ac}_2\text{O}$ , DIEA, DMF) of the free amine provided the cyclic peptide. Deprotection of the peptide (HF, anisole,  $-78^\circ\text{C}$ , 37%) gave the desired N-acylated analog (**III.3**).

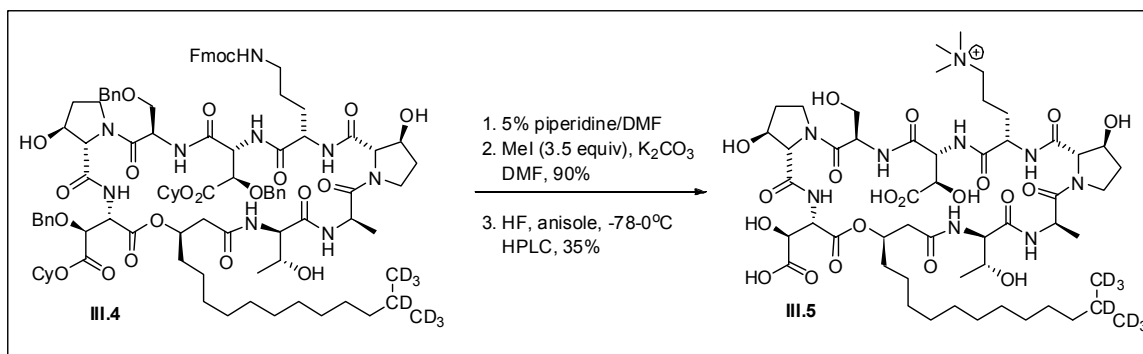
The alkylated ornithine analog was prepared according to **Scheme III.3**. As above with the previous derivatives, the Fmoc group was removed quantitatively upon treatment with piperidine in DMF. The resulting free amine was alkylated (MeI,  $\text{K}_2\text{CO}_3$ , DMF) to afford the ammonium salt in 90% yield. Global deprotection (HF, anisole,  $-78^\circ\text{C}$ , 35%) provided the cationic analog (**III.5**) in acceptable yield.



**Scheme III.1: Preparation of ornithine analog**



**Scheme III.2: Synthesis of N-acyl-ornithine derivative**



**Scheme III.3: Preparation of cationic ornithine residue**

### III.3.2 Biological and Conformational Evaluation

All three of the plusbacin A3 analogs were evaluated for their in vitro antibiotic activity by measuring the minimum inhibitory concentrations for panel of gram positive and gram negative bacteria (**Table III.5**). Although we did not expect activity for gram negative strains, *E. coli* and *Pseudomonas* sp. were used as controls. Plusbacin A3 was used as a reference compound. The results are reported in  $\mu\text{g/mL}$  and reflect the dose necessary to completely inhibit bacterial cell growth. Concentrations greater than 50  $\mu\text{g/mL}$  were not measured.

**Table III.5: Biological evaluation for arginine modified plusbacin A3 analogs (see experimental section for assay conditions)**

	MIC ( $\mu\text{g/mL}$ )					
	GAS	GAB	MRSA	VRE	E. Coli	Pseudomonas
<b>Plusbacin A3</b>	0.2	6.25	1.56	6.25	>50	>50
<b>III.2</b>	3.13	25	50	>50	>50	>50
<b>III.3</b>	25	>50	>50	>50	>50	>50
<b>III.5</b>	25	>50	>50	>50	>50	>50

As mentioned previously, molecular modeling studies have determined that the arginine guanidine functionality was in close proximity to the carboxylate of the  $\beta\text{OH-L-}$

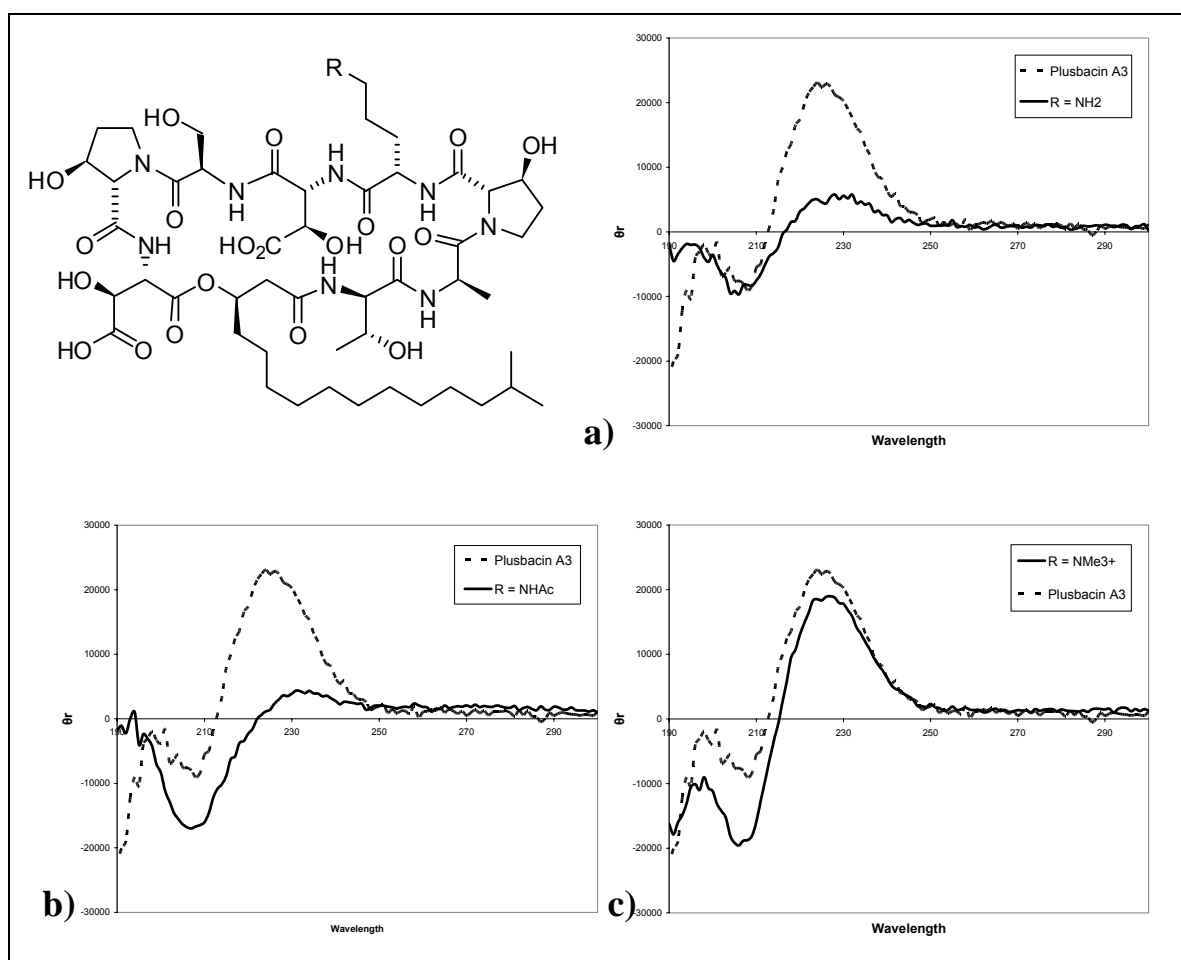
aspartic acid, therefore suggesting possible presence of a conformationally stabilizing salt-bridge. We therefore sought to perturb this interaction while maintaining some degree of basicity (arginine side chain  $pK_a = 12.5$ , ornithine side chain  $pK_a = 10.8$ ) and hydrogen bonding capability. Also, maintaining the cationic group could induce similar conformational restraints to the peptide backbone. However, it would be less sterically demanding.

Analog (**III.2**), when evaluated for antibiotic activity (**Table III.5**) showed a significant decrease in potency. The MIC for Group A Streptococcus was 3.13  $\mu\text{g/mL}$  as compared to 0.2  $\mu\text{g/mL}$  for plusbacin A3. The MIC against Group B Streptococcus was 25  $\mu\text{g/mL}$ , considerably higher than for plusbacin A3 at 6.25  $\mu\text{g/mL}$ . When tested against methicillin resistant *Staph.aureus*, the ornithine analog almost completely lost activity with an MIC at 50  $\mu\text{g/mL}$  as compared to that of 1.56  $\mu\text{g/mL}$  for plusbacin A3. Unfortunately, the analog lost all activity against vancomycin resistant enterococci.

Additional efforts to study the role of the salt-bridge were carried out with the N-acyl-ornithine derivative (**III.3**). This analog was derivatized such that the basicity of the side chain functionality was significantly increased ( $pK_a > 20$  for amide NH). Furthermore, the ability to form hydrogen bonds with the carbonyl group of  $\beta\text{-OH Asp}$  was maintained, however, the charge of the side chain decreased from +1 to 0. This analog was evaluated for its biological activity and was found to display only moderate activity against Group A Streptococcus with an MIC of 25  $\mu\text{g/mL}$ , compared to 0.2  $\mu\text{g/mL}$  for plusbacin A3. The analog was completely inactive against all other strains in our panel.

The alkylated ornithine analog (**III.5**) was designed to maintain a fixed positive charge yet to completely diminish the ability to form a definitive hydrogen bond across the ring with the  $\beta$ -OH-Asp carboxylate residue. The loss of hydrogen bonding however does not completely eliminate the possibility of an ionic interaction with carboxylates at physiological pH (7.4) since the aspartic acid carboxylic acid pKa is approximately 4.5. The MIC data for this analog was identical to that for the N-Acyl ornithine analog, with only weak activity against Group A Streptococcus (25  $\mu$ g/mL).

**Figure III.5** represents the CD data collected for each of the plusbacin A3 analogs. Although a direct correlation between CD spectrum and biological activity can not be made, each analog seems to maintain a curve shape similar to that of the natural product. Interestingly, the cationic analog bears a striking similarity to plusbacin A3 with an almost identical curve (**Figure III.5.c**), suggesting an analogous conformation to the natural product.



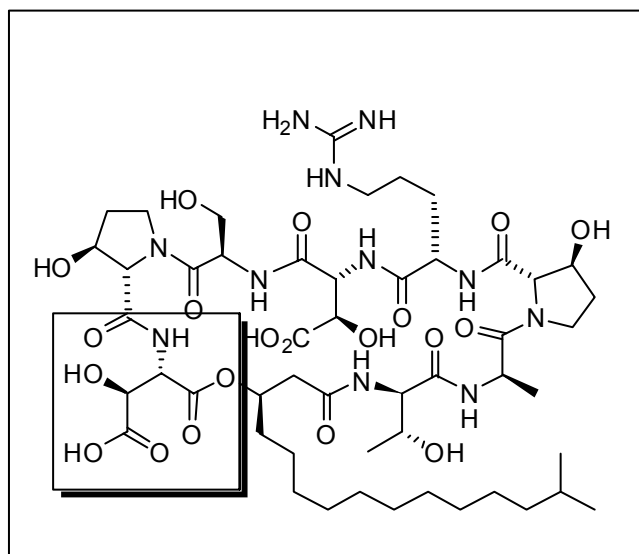
**Figure III.5: Circular Dichroism data collected for plusbacin A3 analogs. a) III.2, b) III.3, c) III.5 (all samples were prepared in H<sub>2</sub>O at 0.2 mg/mL)**

### III.4 HYDROXY-ASPARTIC ACID MODIFICATIONS

It was apparent from the previous analogs that the arginine residue is very important in maintaining antibiotic activity against MRSA and VRE. However, this data was not conclusive in determining the role of the aspartic acid carboxylate or the importance the salt bridge. A new analog was synthesized and designed to replace the  $\beta$ OH aspartate with an alanine residue in order to completely eliminate any possible salt



bridge interactions, yet maintaining the key hydrogen bonding interaction between the amide NH and the D-Serine carbonyl oxygen. **Figure III.6** illustrates the hydroxy-aspartic acid residue to be modified.



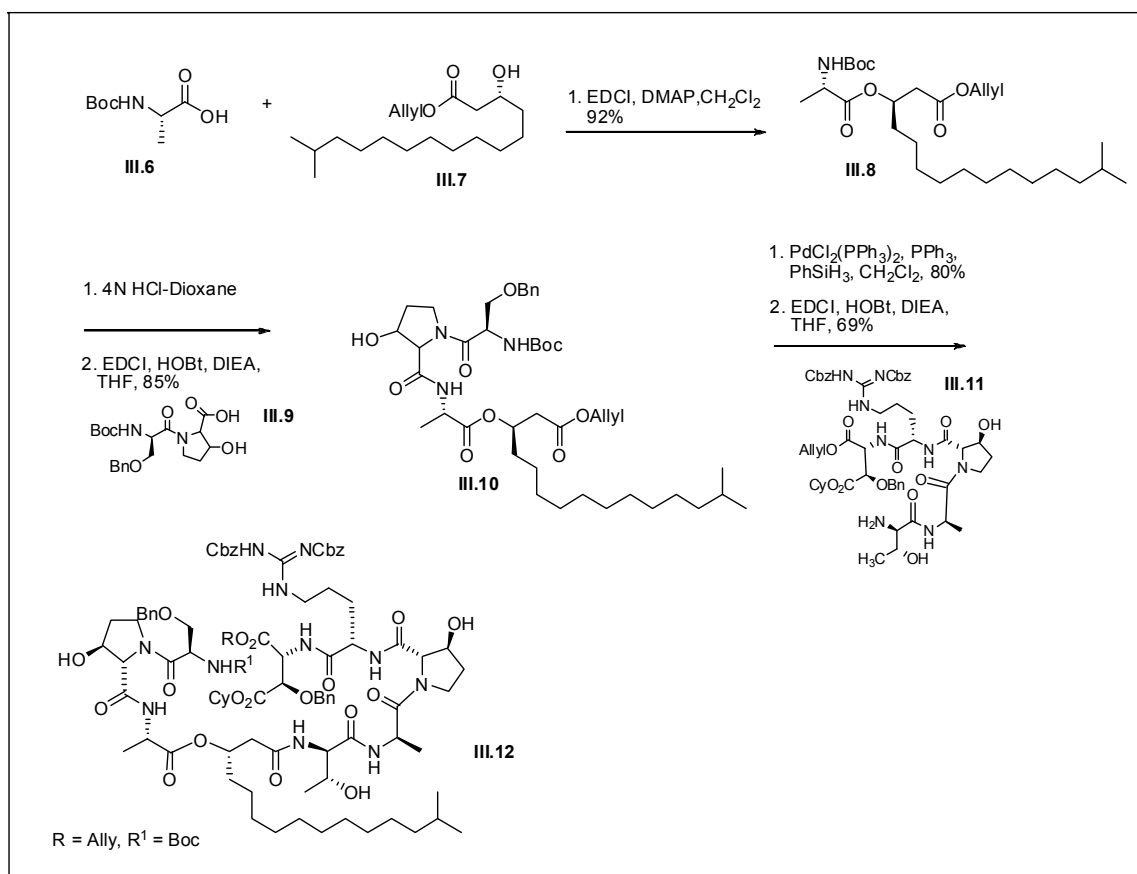
**Figure III.6: Plusbacin A3 highlighted at the aspartic acid residue to be modified**

### III.4.1 Synthesis of Alanine Analog

The synthetic route used to prepare the target analog was similar to that used for the natural product<sup>4</sup>. The key intermediates were prepared via coupling of peptide fragments and protecting groups were chosen in order to take advantage of a final global deprotection.

**Scheme III.4** presents the synthesis of the key linear peptide **III.12**. Due to the ability of sensitive DMAP activated esters to undergo racemization, careful control of temperature and activating reagent stoichiometry was used in to prepare the diester **III.8** (EDCI, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, -15°C). Removal of the N-terminal Boc protecting group

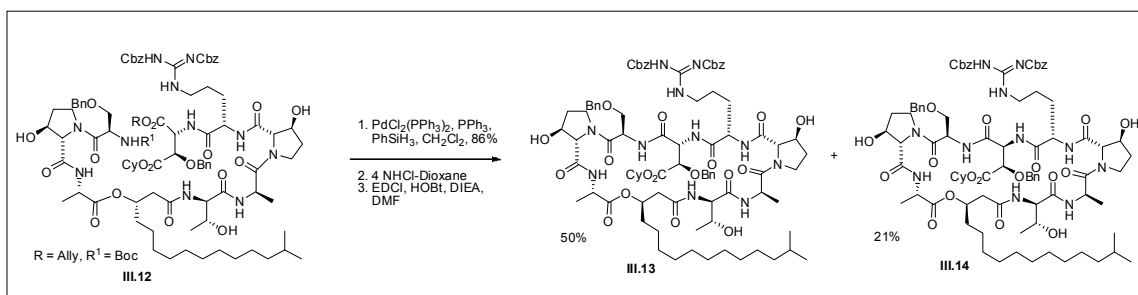
followed by its coupling with carboxylic acid **III.9** (EDCI, HOBt, THF, 85%) provided the depsipeptide (**III.10**) in excellent yield. Removal of the allyl ester under standard conditions ( $\text{PdCl}_2(\text{PPh}_3)_2$ ,  $\text{PPh}_3$ ,  $\text{PhSiH}_3$ ,  $\text{CH}_2\text{Cl}_2$ , 80%) afforded the carboxylic acid. Pentapeptide **III.11** was coupled to the carboxylic acid (EDCI, HOBt, DIEA, 69%) in acceptable yield providing the linear peptide (**III.12**).



**Scheme III.4: Synthesis of key linear peptide III.12**

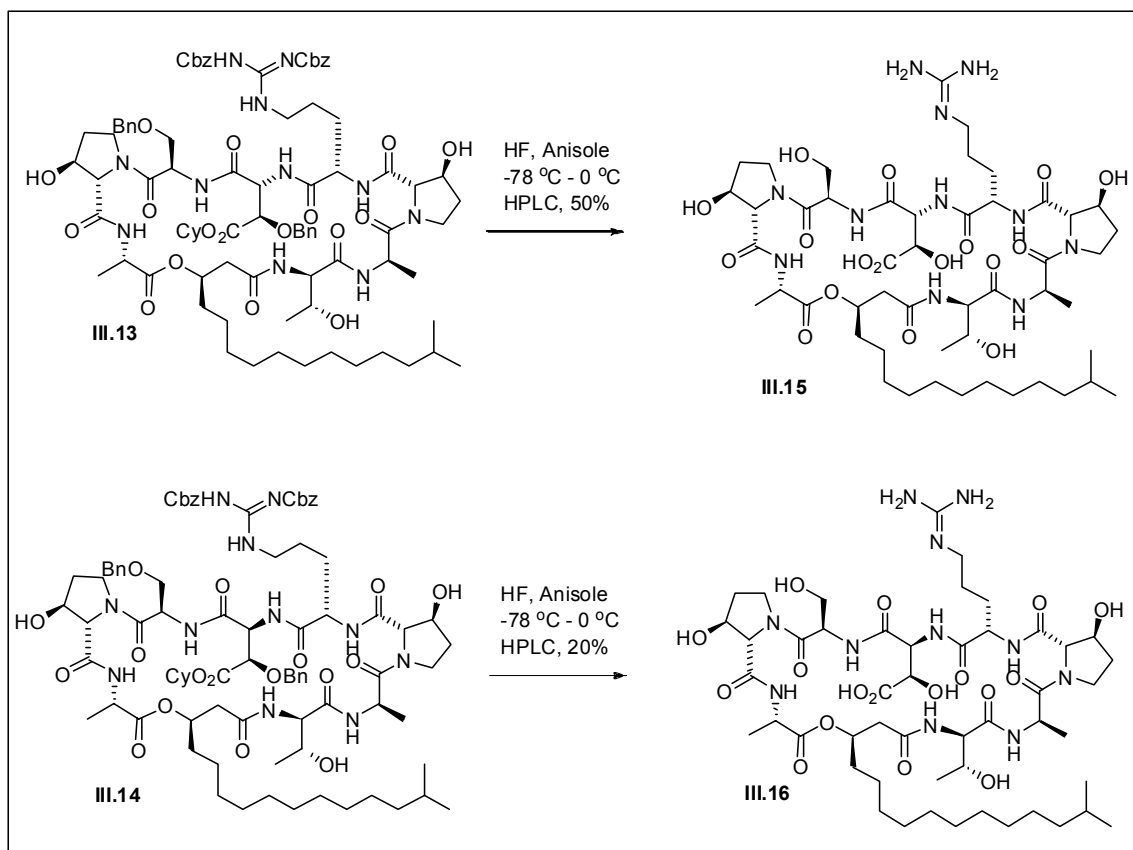
To set the stage for the final macrocyclization, the C-terminal carboxyl group of compound **III.12** was unmasked ( $\text{PdCl}_2(\text{PPh}_3)_2$ ,  $\text{PPh}_3$ ,  $\text{PhSiH}_3$ ,  $\text{CH}_2\text{Cl}_2$ , 86%) (**Scheme III.5**). Cleavage of the N-terminal Boc group (4N HCl, Dioxane) provided the amino

acid cyclization precursor. Activation and cyclization afforded the macrocycle (**III.13**) in good yield. Unlike the macrocyclization event from our total synthesis, an undesired diastereomer (**III.14**) was isolated as a result of epimerization. This material was also brought forward and utilized as an additional analog for biological testing.



**Scheme III.5: Synthesis of cyclic peptide III.13**

**Scheme III.6** illustrates the final steps towards both plusbacin A3 alanine analogs. A global deprotection of the side chain protection was accomplished to afford the desired depsipeptide analog (**III.15**) in 50% yield along with a 20% yield of the diastereomeric product **III.16**.



**Scheme III.6: Final steps toward target alanine analogs**

### III.4.2 Biological and Conformational Evaluation

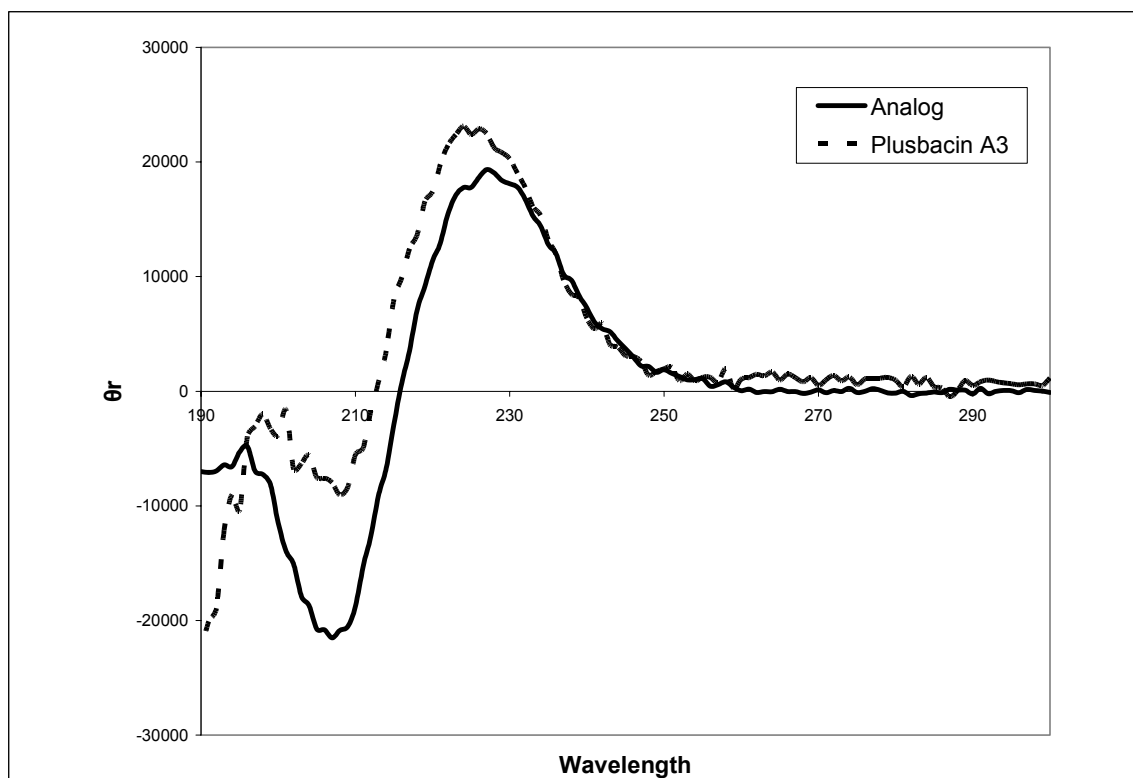
Two plusbacin analogs were synthesized containing an alanine replacement at the L-βOH-Asp position. Both cyclic peptides were evaluated for their antibiotic activity against a panel of Gram-positive and Gram-negative bacteria by measuring the minimum inhibitory concentrations for bacterial cell growth. Plusbacin A3 was used as a reference. *E. coli* and *Pseudomonas* sp. were used as controls.

**Table III.6 Biological evaluation of alanine analogs (see experimental section for assay conditions)**

	MIC ( $\mu\text{g/mL}$ )					
	GAS	GAB	MRSA	VRE	E. Coli	Pseudomonas
<b>Plusbacin A3</b>	0.2	6.25	1.56	6.25	>50	>50
<b>III.15</b>	>50	>50	>50	>50	>50	>50
<b>III.16</b>	>50	>50	>50	>50	>50	>50

The results of the biological assay are presented in **Table III.6**. Surprisingly, both peptides showed a complete loss in activity upon replacement of the  $\beta\text{OH-L-Asp}$  with an L-alanine residue. MIC values were all >50  $\mu\text{g/mL}$  for all strains tested. This loss in activity points to the importance of the  $\beta\text{OHAsp}$  residue as well and the possible intramolecular salt-bridge.

**Figure III.7.** illustrates the CD data collected for the target alanine analog **III.15** in direct comparison with the natural product. Surprisingly, the CD curve for the analog shows a striking similarity compared to the natural product. As with previous experiments there is no correlation between CD curve shape and antibiotic activity. However, since the CD curve shape is representative of peptide backbone configuration one is drawn to several possibilities: i) the guanidinium is interacting with different amino acid residues, ii) the aspartic acid carboxylate is important for activity, yet plays a minimal role in stabilizing the peptide conformation. iii) The CD conditions may not reflect the active conformation presented by the antibiotic at the bacterial cell surface.



**Figure III.7: CD analysis of alanine replacement analog (H<sub>2</sub>O, 0.2 mg/mL)**

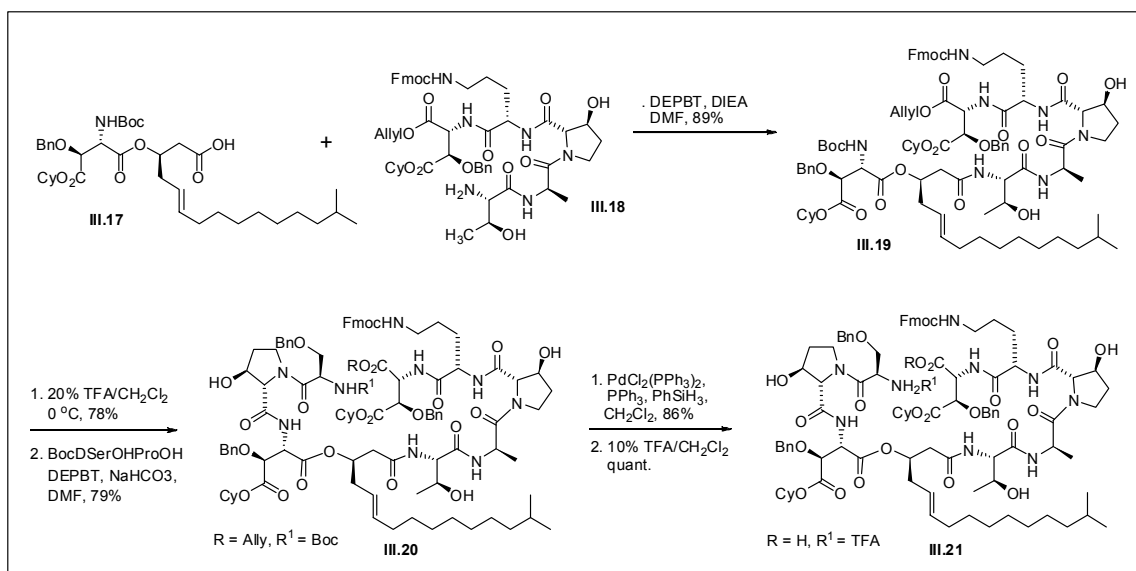
### III.5 D-*ALLO*-THREONINE MODIFICATIONS

Based on previous the NMR structure and SAR data that indicated the presence and importance of the cationic ornithine residue and the βOH-L-Aspartate residue, we shifted our attention to yet another intramolecular hydrogen bond between the D-*a*Thr NH and the D-Ala CO. As one of five observable intramolecular hydrogen bonds, we hoped to perturb this interaction via installation of an L-*allo*-Threonine in its place. Therefore analogs were designed and synthesized containing an L-*allo*-Thr in place of the D-*allo*-threonine residue. Since we had access to both (R) and (S) configurations of the 3-hydroxyisopentanoic acid residue, each of the diastereomeric forms were assayed for antibiotic activity.

### III.5.1 Synthesis of Threonine Analogs

The synthetic route used to prepare the target analog was similar to that described for our total synthesis of plusbacin A3.<sup>4</sup> The key intermediates were prepared via coupling of peptide fragments and protecting groups were chosen in order to take advantage of a final global deprotection.

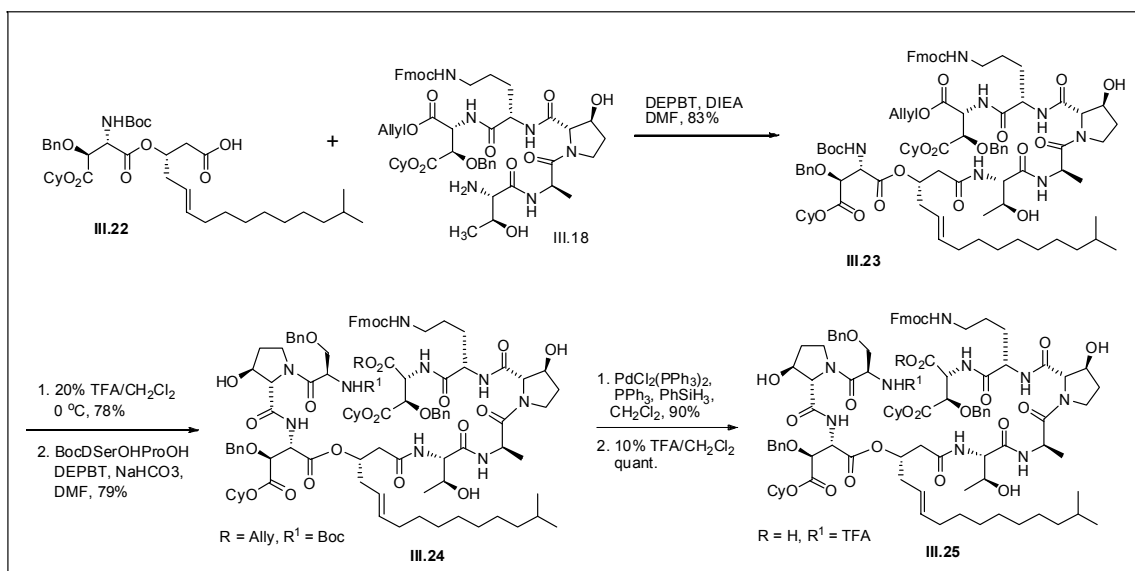
**Scheme III.7.** illustrates the synthesis of the key linear peptide **III.21**. Fragment **III.17** was coupled to the pentapeptide (**III.18**) containing the modified threonine residue to afford the septapeptide (**III.19**) in good yield. Removal of the N-terminal Boc protecting group gave the free amine which was efficiently coupled to the carboxylic acid Boc-D-Ser(OBn)- $\beta$ OH-Pro-OH. With the linear peptide (**III.20**) in hand, the C-terminal allyl ester was deprotected under standard conditions. Removal of the Boc group set the stage for the key macrocyclization event.



**Scheme III.7:** Synthesis of linear macrocyclization precursor with (R)-configuration at lactone stereocenter towards the target threonine analogs.

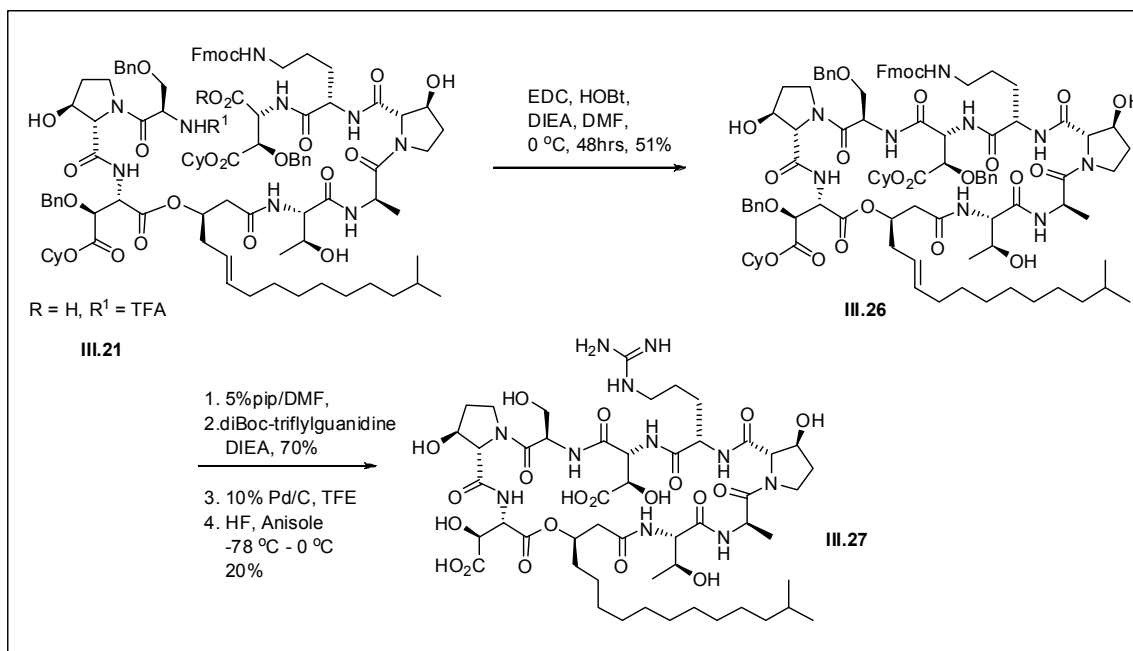
The preparation of the linear peptide (**II.34**) containing the (S)-configuration at the depsipeptide stereocenter is presented in **Scheme III.8**. This synthesis was carried out identically to that of the compound having the (R)-configuration with only slight variations in reaction yields.





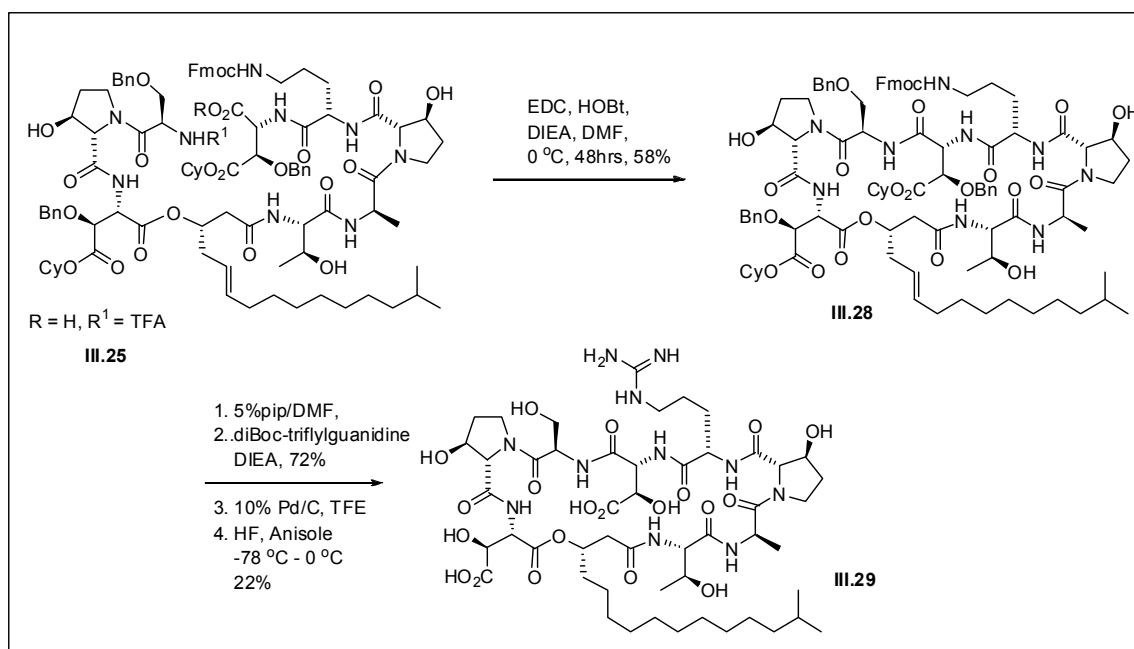
**Scheme III.8: Preparation of the linear peptide containing the (S)-configuration at the depsipeptide stereocenter toward the target threonine analog**

The final steps toward the target analog are presented **Scheme III.9**. Macrocyclization of the linear peptide (**III.21**) (EDCI, HOBT, DIEA, DMF, 51%) afforded the cyclic peptide in acceptable yields. Removal of the Fmoc protecting group allowed for the late stage installation of the guanidine functionality. Hydrogenolysis of the alkene set the stage for the global deprotection to remove the remaining protecting groups. HPLC purification of the crude material afforded the desired threonine analog in modest yield.



**Scheme III.9: Final steps towards the *L*-allo-threonine analog with (R)-configuration at lactone stereocenter**

**Scheme III.10** illustrates the final steps toward the “(S) analog” (**III.27**). This sequence was carried out identically to that presented in scheme however; the yields obtained were slightly higher. With both analogs in hand we the moved forward to evaluate their biological activity.



**Scheme III.10: Final steps towards the *L*-allo-threonine analog with (S)-configuration at lactone stereocenter**

### III.5.2 Biological and Conformational Evaluation

Both cyclic peptides were evaluated for their antibiotic activity against a panel of Gram-positive and Gram-negative bacteria by measuring the minimum inhibitory concentrations (MIC) for bacterial cell growth. Plusbacin A3 was used as a reference compound. *E. coli* and *Pseudomonas* sp. were used as controls.

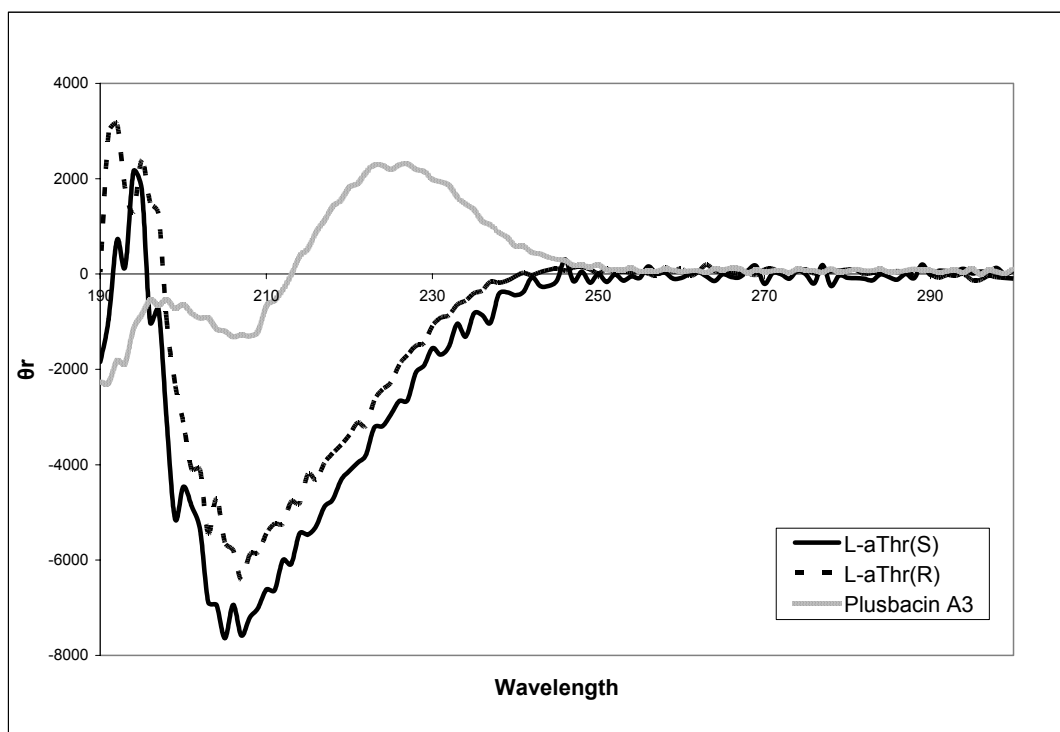
The results of the biological assay are reported in **Table III.7**. Both threonine analogs, containing the R and S configurations at the lactone stereocenter were completely inactive against the panel of Gram-positive bacteria tested. All MIC values were >50 µg/mL. Although activity was lost, the results indicate the importance of the D-configuration at the *allo*-threonine position. The results also verify our hypothesis that

the destroying this key intramolecular hydrogen bonding interaction could destabilize the peptide backbone towards a biologically active conformation.

**Table III.7: Biological evaluation of threonine analogs (see experimental section for assay conditions)**

	MIC ( $\mu\text{g/mL}$ )					
	GAS	GAB	MRSA	VRE	E. Coli	Pseudomonas
<b>Plusbacin A3</b>	0.2	6.25	1.56	6.25	>50	>50
<b>III.27</b>	>50	>50	>50	>50	>50	>50
<b>III.29</b>	>50	>50	>50	>50	>50	>50

**Figure III.8** represents the CD data collected for both analogs (**III.27**, **III.29**) in comparison the natural product. Although both analogs appear to adopt a similar conformation, they are nevertheless quite different in comparison the plusbacin A3 as indicated by the negative absorption between 220 nm and 205 nm.



**Figure III.8: CD analysis of Threonine analogs (H<sub>2</sub>O, 0.2 mg/mL)**

### III.6 LACTONE MODIFICATIONS: REPLACEMENT OF THE LACTONE WITH AN AMIDE

With the completion of our total synthesis of plusbacin A3, we wished to extend a similar approach to the synthesis of a key analog containing an amide bond in place of the sensitive depsipeptide linkage between the L-βOH-Asp and the 3-OH-isopentadecanoic acid residues. This derivative would be considerably more stable compared to the natural product since the possibility of beta-elimination of an acyloxy group would not exist. For this reason, the amide linkage would allow for a much more accessible synthesis.

With this analog in hand we hypothesized that the amide replacement would not destroy its activity, but instead provide a more stable scaffold for the preparation of

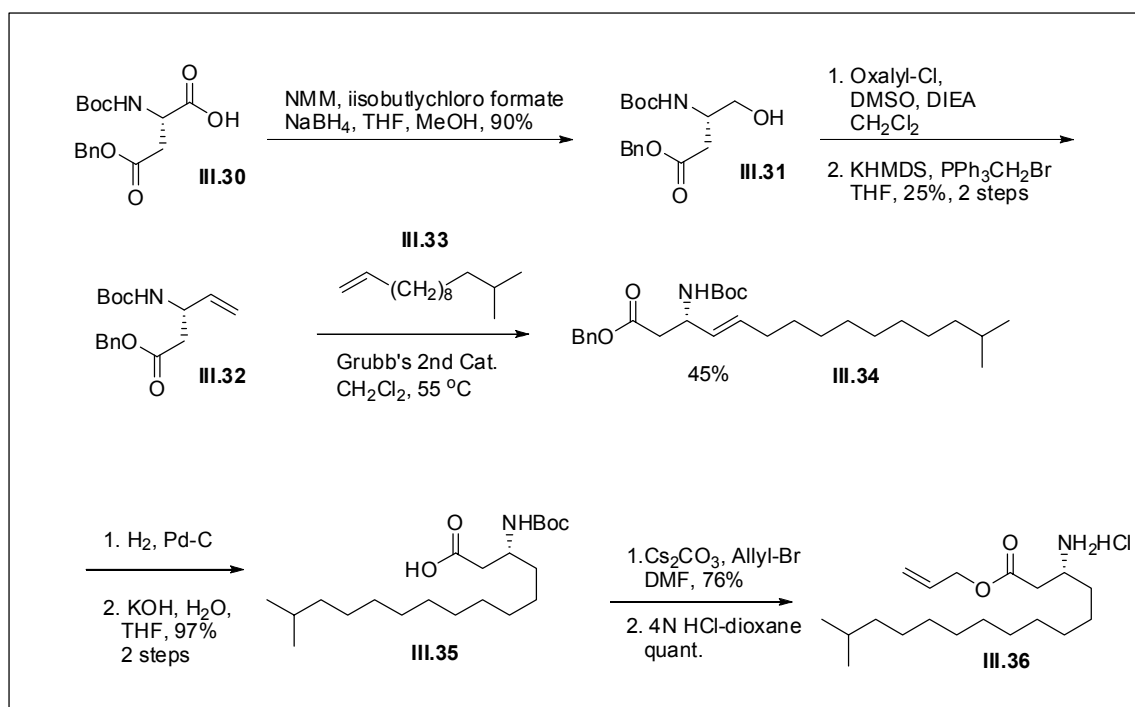
additional analogs. For example, the development of a solid phase synthesis incorporating the amide replacement would allow easy access to an alanine scan of plusbacin A3 from which the resulting antibiotic properties could be used to define the importance of individual residues and regions of the molecule. Boger and coworkers recently published a similar experiment with ramoplanin aglycon where the replacement of the lactone linkage resulted in a highly active antibiotic<sup>13</sup>. They subsequently conducted an alanine scan which provided valuable insight into the importance of each residue in the ramoplanin sequence.

### III.6.1 Synthesis of Amide Analog

The synthesis and assembly of the key fragments for the amide analog were facilitated by the convergent nature of our total synthesis<sup>4</sup>. In order to access the target analog, we first required a synthetic route to a suitable protected (R)-3-amino-14-methylpentadecanoic acid intermediate. Based on the successful olefin metathesis protocols described previously (Chapter II), a retrosynthetic analysis suggested that an (S)-benzyl 3-aminopent-4-enoate could be utilized as a key coupling partner. A survey of literature protocols provided a reasonable synthetic route starting from a commercially available aspartic acid derivative.<sup>14</sup>

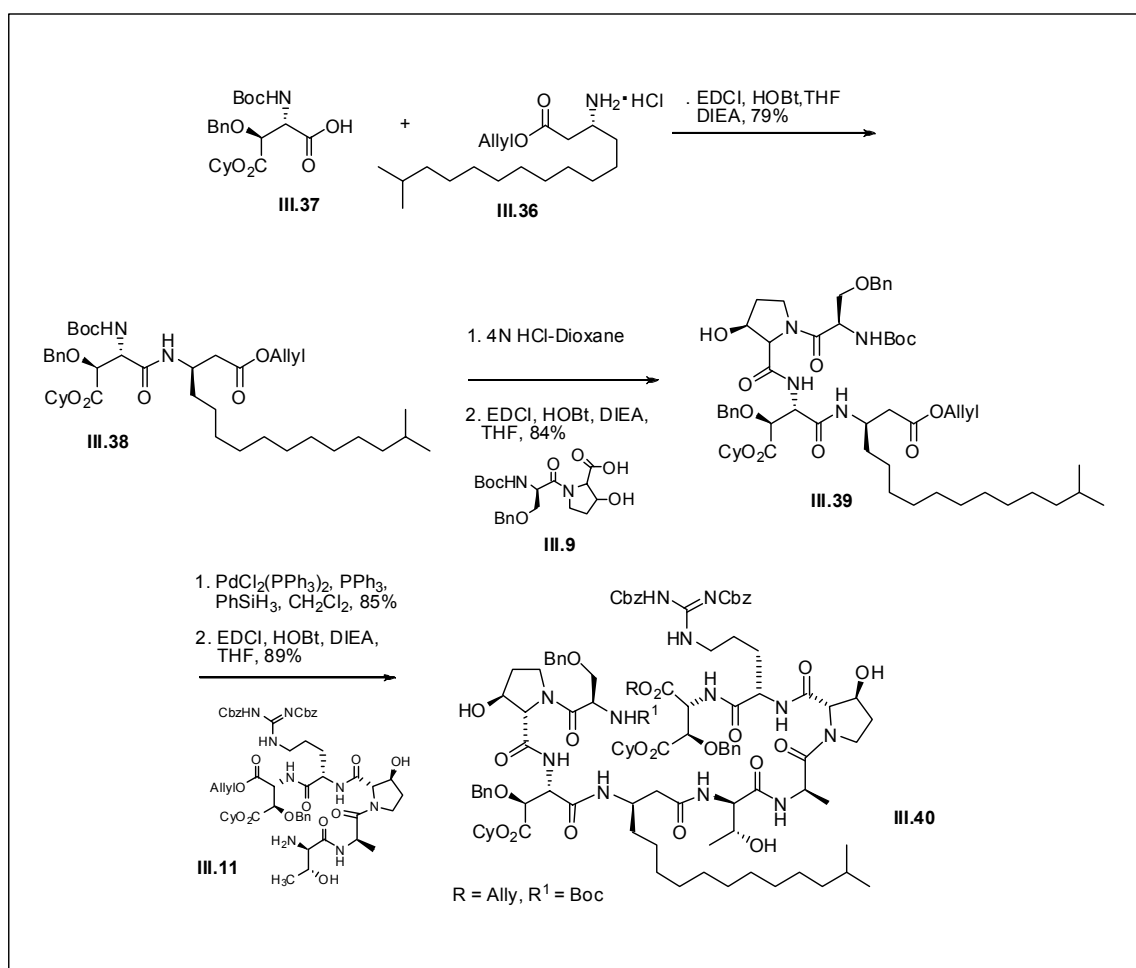
The synthesis of key amine intermediate is presented in **Scheme III.11**. A reduction of aspartic acid affords the alcohol (**III.31**) in good yields (isobutylchloroformate, NMM, NaBH<sub>4</sub>, THF, MeOH, 90%).<sup>15</sup> The olefin functionality (**III.32**) was prepared via a two step Swern / Wittig sequence (1. Oxalyl-Cl, DMSO,

DIEA; 2. KHMDS,  $\text{PPh}_3\text{CH}_2\text{Br}$ , THF, 25%) in modest yields<sup>14</sup>. This alkene then served as a handle for the incorporation of 11-methyl-1-dodecene (**III.33**) (Grubb's 2<sup>nd</sup> generation. Catalyst,  $\text{CH}_2\text{Cl}_2$ , 45%). Hydrogenolysis ( $\text{H}_2$ , Pd-C, EtOH, quant.) of intermediate **III.34** afforded only the alkene reduction product, and surprisingly left the ester intact. Saponification of the benzyl ester afforded the carboxylic acid (**III.35**) in 97% overall yield (KOH,  $\text{H}_2\text{O}$ , THF). Allyl ester protection under standard conditions ( $\text{Cs}_2\text{CO}_3$ , Allyl-Br, DMF, 76%) followed by cleavage of the N-terminal Boc group (4N HCl-dioxane, quant.) provided the (R)-allyl 3-amino-14-methylpentadecanoate hydrochloride intermediate (**III.36**).



**Scheme III.11: Synthesis of (R)-allyl 3-amino-14-methylpentadecanoate hydrochloride. A key fragment for the assembly of plusbacin A3 amide analog**

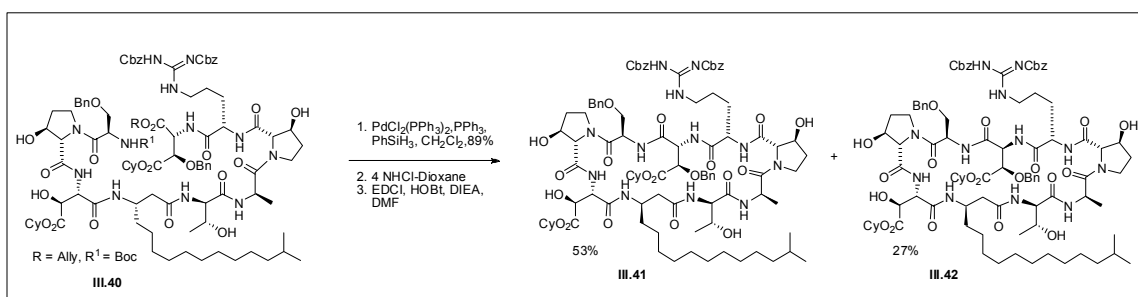
**Scheme III.12.** illustrates the incorporation of the key intermediate **III.36** into the plusbacin A3 backbone. Coupling of the hydrochloride (**III.36**) with Boc-L- $\beta$ OH-Asp(OCy)-OH afforded the dipeptide (**III.38**) (EDCI, HOBt, DIEA, THF, 79%). Boc cleavage (4N HCl-Dioxane) followed by coupling to dipeptide **III.9** (EDCI, HOBt, DIEA, THF, 84%) provided the tetrapeptide in good overall yield. Deprotection of the C-terminal allyl ester (PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub>, PPh<sub>3</sub>, PhSiH<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 85%) gave the carboxylic acid which was then efficiently coupled to the key pentapeptide fragment (EDCI, HOBt, DIEA, THF, 89%) to afford the linear peptide **III.40**.



**Scheme III.12: Synthesis of linear peptide containing amide replacement**

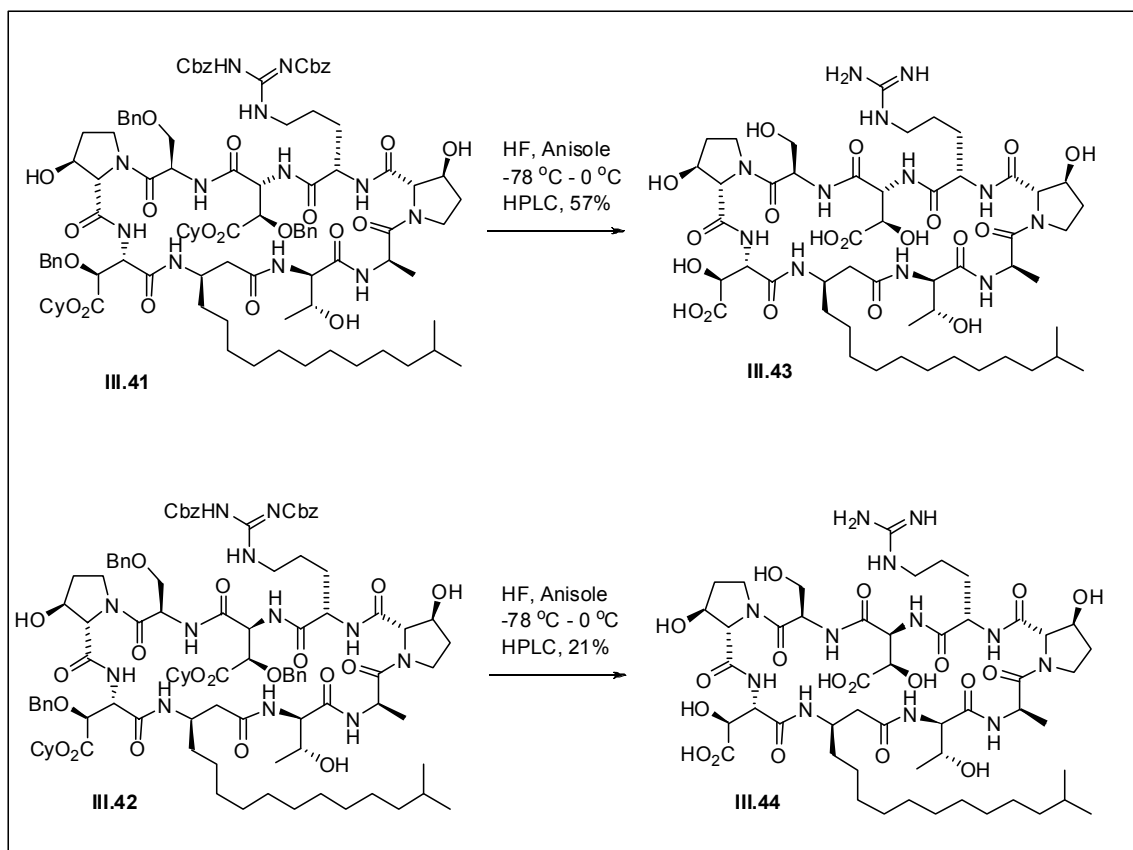


To set the stage for the final macrocyclization reaction, (**Scheme III.13**) the allyl ester was removed from **III.40** ( $\text{PdCl}_2(\text{PPh}_3)_2$ ,  $\text{PPh}_3$ ,  $\text{PhSiH}_3$ ,  $\text{CH}_2\text{Cl}_2$ , 89%). Cleavage of the Boc protecting group afforded the macrocyclization precursor. Using our optimized conditions, the cyclic peptide (**III.41**) was isolated in good yield. In addition to the desired product, the C-terminal  $\alpha$ -carbon epimerized, generating 27% of an undesired diastereomer (**III.42**).



**Scheme III.13: Macrocyclization of plusbacin A3 amide analog**

The final steps toward both analogs are presented in **Scheme III.14**. Global deprotection of both macrocyclization products afforded the amide replacement analogs in good yields. With the completion of the target analog having replaced the lactone with an amide, the compounds were then tested for their biological activity.



**Scheme III.14: Global deprotection of cyclic peptides affording target amide analogs**

### III.6.2 Biological Evaluation and Characterization

Two plusbacin analogs were synthesized containing an amide replacement at the lactone linkage. Both cyclic peptides were evaluated for their antibiotic activity against a panel of Gram-positive and Gram-negative bacteria by measuring the minimum inhibitory concentrations (MIC) for bacterial cell growth. Plusbacin was used as a reference compound. *E. coli* and *Pseudomonas* sp. were used as controls.

The results of the biological assay are reported in **Table III.7**. The macrocyclization epimer (**III.44**) was analogous to the previously isolated epimers in that it proved to be completely inactive against the panel of bacteria (50  $\mu\text{g/mL}$ ). Surprisingly, the target analog (**III.43**) displayed good activity against Group A

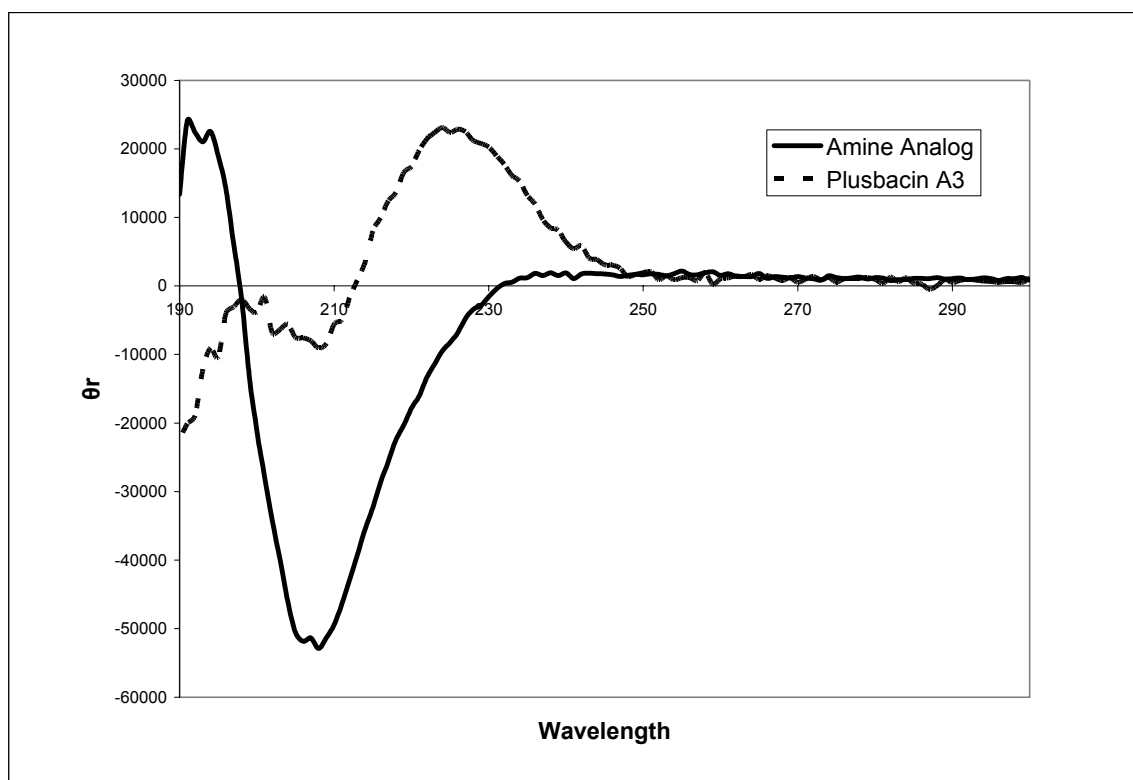
Streptococcus (3.13  $\mu\text{g/mL}$ ). The analog resulted in a modest 4 fold reduction in activity against Group B Streptococcus (25  $\mu\text{g/mL}$ ) as compared to plusbacin A3. Although it maintained some activity against MRSA and VRE (25  $\mu\text{g/mL}$  and 50  $\mu\text{g/mL}$ ), plusbacin A3 still proved to be more potent.

**Table III.8: Biological Evaluation of plusbacin A3 amide analogs (see experimental section for assay conditions)**

	MIC ( $\mu\text{g/mL}$ )					
	GAS	GAB	MRSA	VRE	E. Coli	Pseudomonas
<b>Plusbacin A3</b>	0.2	6.25	1.56	6.25	>50	>50
<b>III.43</b>	3.13	25	25	50	>50	>50
<b>III.44</b>	>50	>50	>50	>50	>50	>50

In summary, two plusbacin A3 analogs were synthesized containing an amide replacement for the lactone linkage. Our hope was to instill conformational stability and resistance to degradation. To our delight, the amine analog did maintain a degree of activity. However, the modest alteration in plusbacin A3's structure led to significant losses in potency.

**Figure III.9** represents the CD analysis of the amide analog from which the conformation is clearly different than that of the natural product. Although the addition of a single amide in place of the lactone may appear to be a small structural change, the new NH allow for new hydrogen bonds to be formed with neighboring carbonyl oxygens. Backbone perturbations could also arise from cis/trans isomerizations of the new amide resulting in a different preferred conformation compared to that of the natural product.

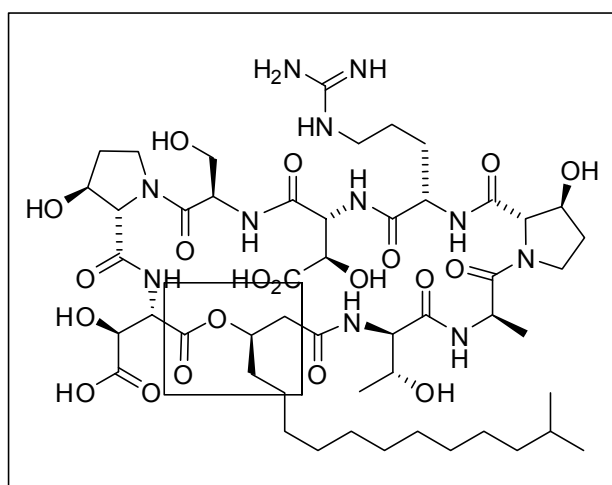


**Figure III.9: CD analysis of amide analog III.43 (H<sub>2</sub>O, 0.2 mg/mL)**

### III.7 PLUSBACIN A3 HYDROLYSIS

In 1993, Williams and coworkers demonstrated by NMR and molecular modeling that ramoplanin, an antibiotic with similar mode of action, maintained a native-like beta sheet structure after hydrolysis.<sup>16</sup> McCafferty and coworkers found that the linearized ramoplanin, possessed an MIC with a 2133 fold increase compared to that of the wild type.<sup>12</sup> They determined that although a similar conformation was maintained, almost all of the antibiotic activity was lost when the lactone was hydrolyzed, suggesting that the capture of nascent peptidoglycan required the presentation of ramoplanin in a highly specific three dimensional conformation.

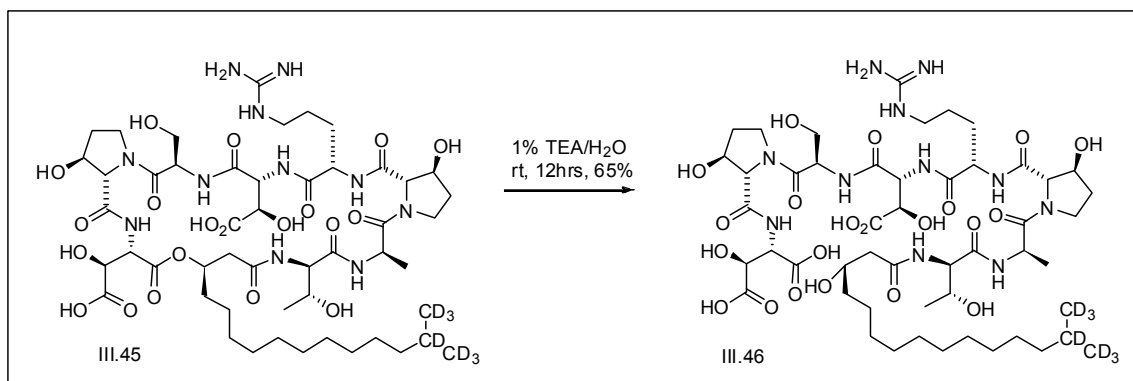
In our efforts to elucidate the molecular basis for the mode of antibacterial action utilized by plusbacin A3, we aimed to define a bioactive pharmacophore, or at least to minimize the structural elements required for activity. Based on the hypothesis that plusbacin A3 was capable of sequestering lipid I and II, it became an important task to determine if the peptide could exert its action when presented in its linear form. Figure **III.10** illustrates plusbacin A3, highlighting the lactone linkage to be hydrolyzed.



**Figure III.10: Representation of lactone to be hydrolyzed**

### III.7.1 Synthesis of Hydrolyzed Plusbacin A3

**Scheme III.15** illustrates the preparation of the linearized plusbacin A3. Due to the sensitivity of the lactone linkage, a mild hydrolysis procedure was used to generate the linear peptide (**III.46**).<sup>17</sup> HPLC purification of the crude material afforded the target analog in 65%.



**Scheme III.15: Hydrolysis of plusbacin A3**

The peptide was evaluated for its antibiotic activity against a panel of Gram-positive and Gram-negative bacteria by measuring the minimum inhibitory concentrations (MIC) for bacterial cell growth. Plusbacin A3 was used as a reference compound. *E. coli* and *Pseudomonas* sp. were used as controls.

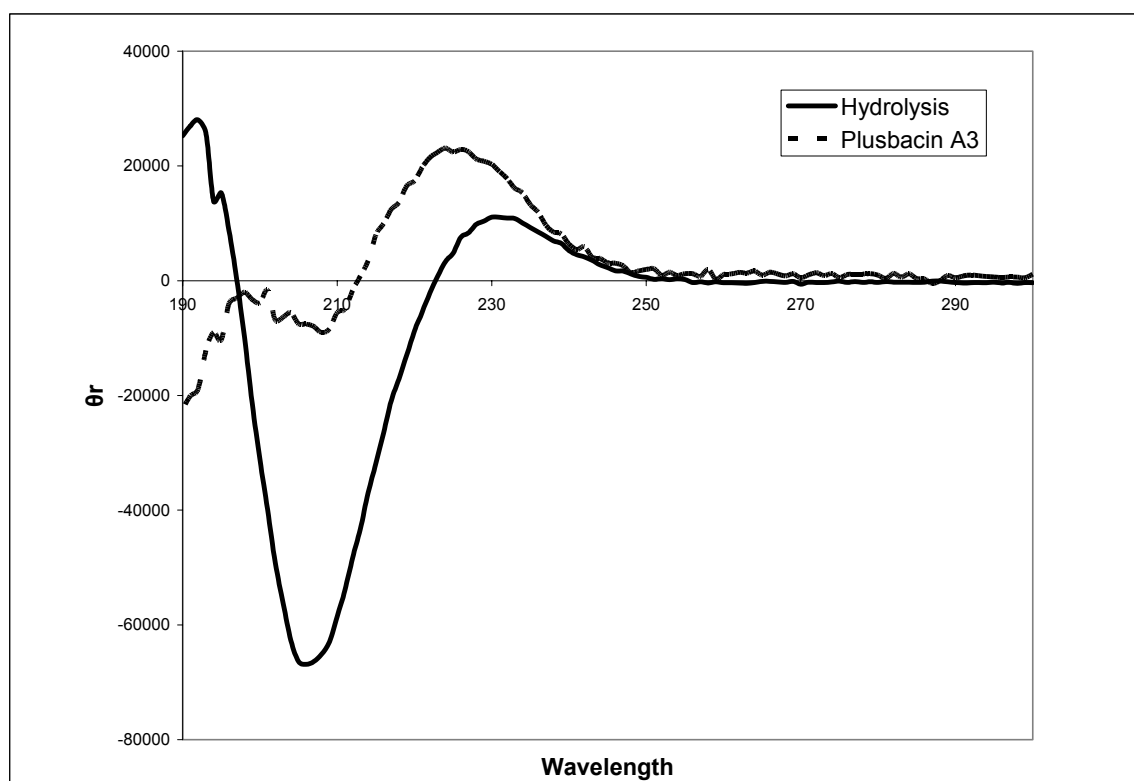
### III.7.2 Biological and Conformational Evaluation

The results of the biological assay are reported in **Table III.9**. Similar to the results obtained for ramoplanin, the biological data shows a complete loss in activity for the open chain plusbacin A3. MIC values for all bacterial strains tested were above 50  $\mu\text{g/mL}$ , the highest concentration tested.

**Table III.9: MIC data for plusbacin hydrolysis analog (see experimental section for assay conditions)**

	MIC ( $\mu\text{g/mL}$ )					
	GAS	GAB	MRSA	VRE	E. Coli	Pseudomonas
<b>Plusbacin A3</b>	0.2	6.25	1.56	6.25	>50	>50
<b>III.46</b>	>50	>50	>50	>50	>50	>50

**Figure III.11.** represents the CD data for the plusbacin A3 linear analog. There is a significant difference in curve shape suggesting a different spatial configuration as compared to the natural product. Although all antibiotic activity was lost, this analog provided a key piece of information for our effort to uncover the molecular basis of action. The linearized analog established that the cyclic macrolactone is a requirement for the presentation of plusbacin A3 in its active form.



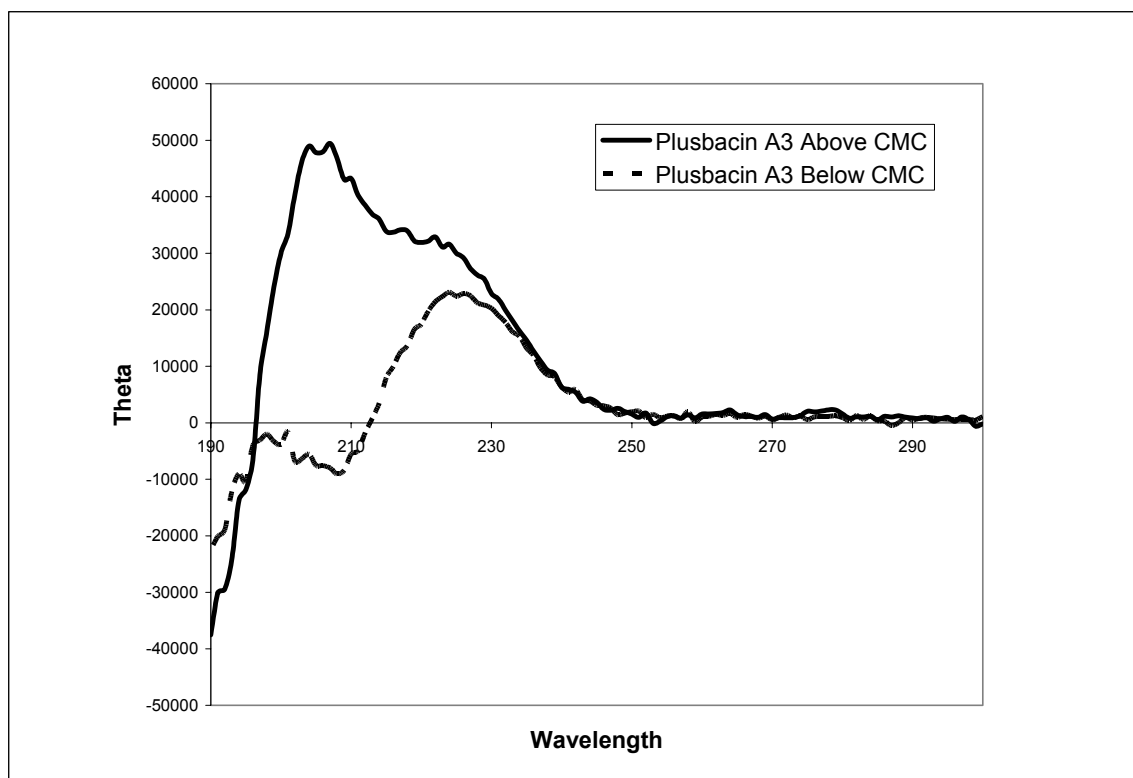
**Figure III.11:** CD analysis of hydrolyzed plusbacin A3, III.46 (H<sub>2</sub>O, 0.2 mg/mL)

### III.9 REMOVAL OF FATTY ACID LIPID SIDE CHAIN

Upon examination of the structure of plusbacin A3 one is drawn to the possibility that the fatty acid side chain of the 3-hydroxyisopentadecanoic acid residue may play an important role in its biological activity. Currently, there are a number of antibiotics that possess similar lipid moieties, such as ramoplanin, the enduracidins, teicoplanin, WAP-8294A2, moenomycin, tunicamycin, and lipsidomycin. The role of this unique structural element is believed to function as a membrane anchor for localization of the antibiotic to the cell membrane. This phenomenon has often been referred to as the “Gulliver effect”<sup>18,19</sup>.

The first task in evaluating the role of the lipid side chain was to determine the conformational stability in the presence of a membrane environment. A circular dichroism experiment was designed to determine if the core structure of plusbacin A3 would undergo a perturbation in the presence of micelles. Sodium dodecyl sulfate (SDS) micelles were chosen as a crude mimic of the bacterial cell membrane, only to replicate a lipophilic surface for plusbacin A3 to interact<sup>20</sup>. Samples were prepared below (0.9mM SDS) the SDS critical micelle concentration of 8.3 mM and above (9.0mM SDS)<sup>20</sup>. **Figure III.12** represents the CD curves for plusbacin A3 (0.2mg/mL) at and below the CMC (critical micelle concentration). The CD curve for plusbacin A3 in the membrane environment indicates a significant conformational change as seen with the positive shift at 220 nm, as compared to that below the CMC. This observation strongly suggests the possibility of a conformational change at the bacterial cell wall interface and that the active conformation could be influenced by the presence of the fatty acid side chain.





**Figure III.12: CD analysis of plusbacin ( $\text{H}_2\text{O}$ , 0.2 mg/mL) above (9.0 mM SDS) and below (0.9 mM SDS) SDS critical micelle concentration**

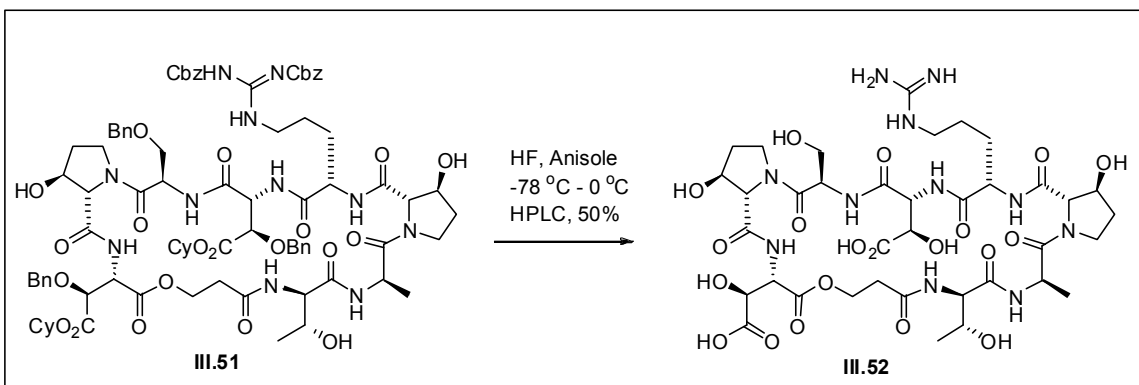
Based on our recent observations we wished to establish whether the lipophilic side chain of plusbacin A3 was a key structural feature required for antibiotic activity. Ciabatti and coworkers reported that a modified side chain of ramoplanin did not affect the overall efficacy of the antibiotic, however, there are no reports of analogs in which the lipid tail was completely removed.<sup>12b-12d</sup> Our efforts to elucidate the role of this structural feature gave rise to two important questions. First, how would the activity of plusbacin A3 be affected by removing the lipid side chain, and second, would this chemical modification allow for the same conformational shift observed for the natural product in a membrane environment?

### **III.8.1 Synthesis of Plusbacin A3 Lacking Lipid Side Chain**

To answer these questions, an analog was synthesized that incorporated a 3-hydroxypropanoic acid residue in place of the 3-hydroxyisopentadecanoic acid found in the natural product. **Scheme III.16** illustrates the preparation of the key linear peptide **III.50**. To begin the synthesis, the alcohol (**III.47**) was coupled to the Boc- $\beta$ OH-L-Asp(OCy)-OH (EDCI, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 60%). Removal of the Boc protecting group (4N HCl, dioxane, quant.) provided the hydrochloride, which was then coupled under standard conditions to Boc-D-Ser(OBn)- $\beta$ OH-Pro-OAllyl (EDCI, HOBt, DIEA, THF, 80%). Removal of the allyl ester (PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub>, PPh<sub>3</sub>, PhSiH<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 95%) afforded the carboxylic acid in excellent yield. Activation of the carboxylate and coupling to the pentapeptide (**III.11**) generated the fully protected linear peptide (**III.50**) (EDCI, HOBt, DIEA, THF, 77%).

To set the stage for the key macrocyclization event the C-terminal carboxyl of (**III.50**) group was deprotected ( $\text{PdCl}_2(\text{PPh}_3)_2$ ,  $\text{PPh}_3$ ,  $\text{PhSiH}_3$ ,  $\text{CH}_2\text{Cl}_2$ , 85%) (**Scheme III.17**). The N-terminal Boc protecting group was removed (4N HCl-dioxane, qaunt.) to give the macrocyclization precursor. The macrocyclization was accomplished efficiently (EDCI, HOBt, DIEA, DMF, 60%) and afforded the cyclic peptide (**III.51**) in good yield.

Finally, a global deprotection provided the desired target peptide (**III.52**) in 50% yield after HPLC purification (**Scheme III.18**). Our synthetic route to this analog took advantage of the convergent strategy established by the total synthesis of plusbacin A3. With the analog in hand we then turned our attention to a biological evaluation and CD analysis.



### III.8.2 Biological and Conformational Evaluation

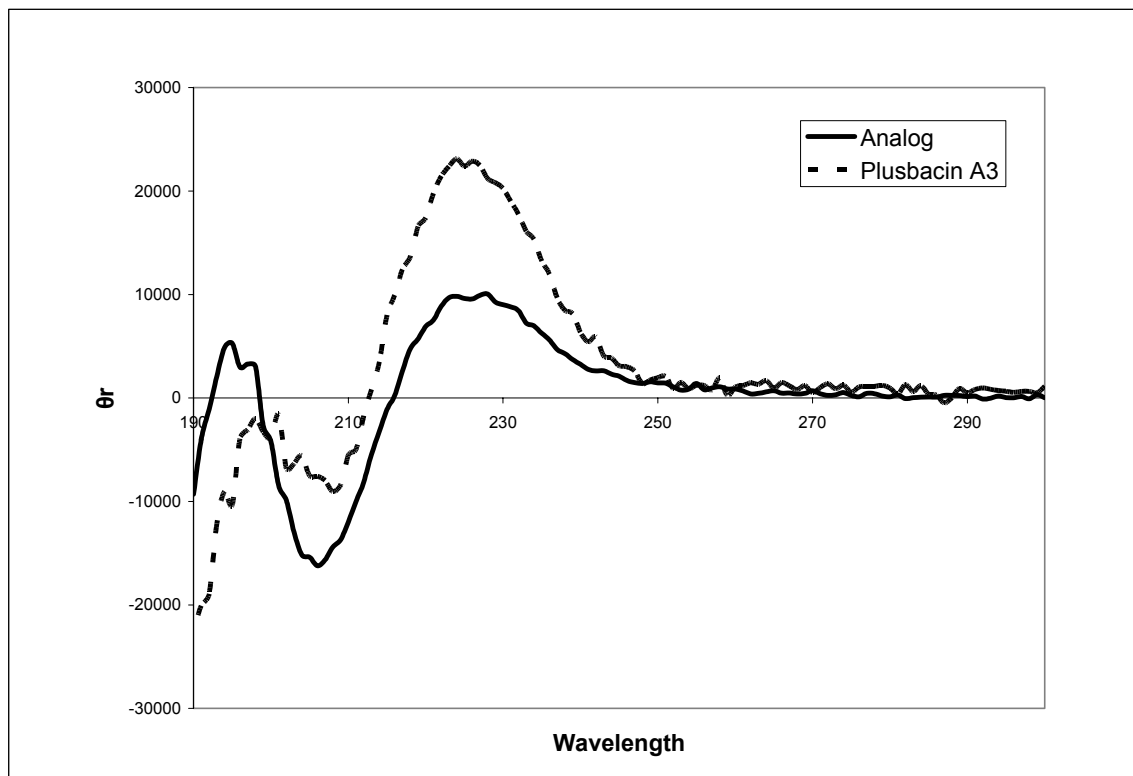
The cyclic peptide containing the 3-hydroxypropanoic acid residue in place of the 3-hydroxyisopentadecanoic acid was evaluated for its antibiotic activity against a panel

of Gram-positive and Gram-negative bacteria by measuring the minimum inhibitory concentration (MIC) for bacterial cell growth. Plusbacin A3 was used as a reference compound. *E. coli* and *Pseudomonas* sp. were used as controls.

**Table III.10: MIC values for plusbacin A3 analog with lipid tail removed (see experimental section for assay conditions)**

	MIC ( $\mu\text{g/mL}$ )					
	GAS	GAB	MRSA	VRE	E. Coli	Pseudomonas
<b>Plusbacin A3</b>	0.2	6.25	1.56	6.25	>50	>50
<b>III.52</b>	>50	>50	>50	>50	>50	>50

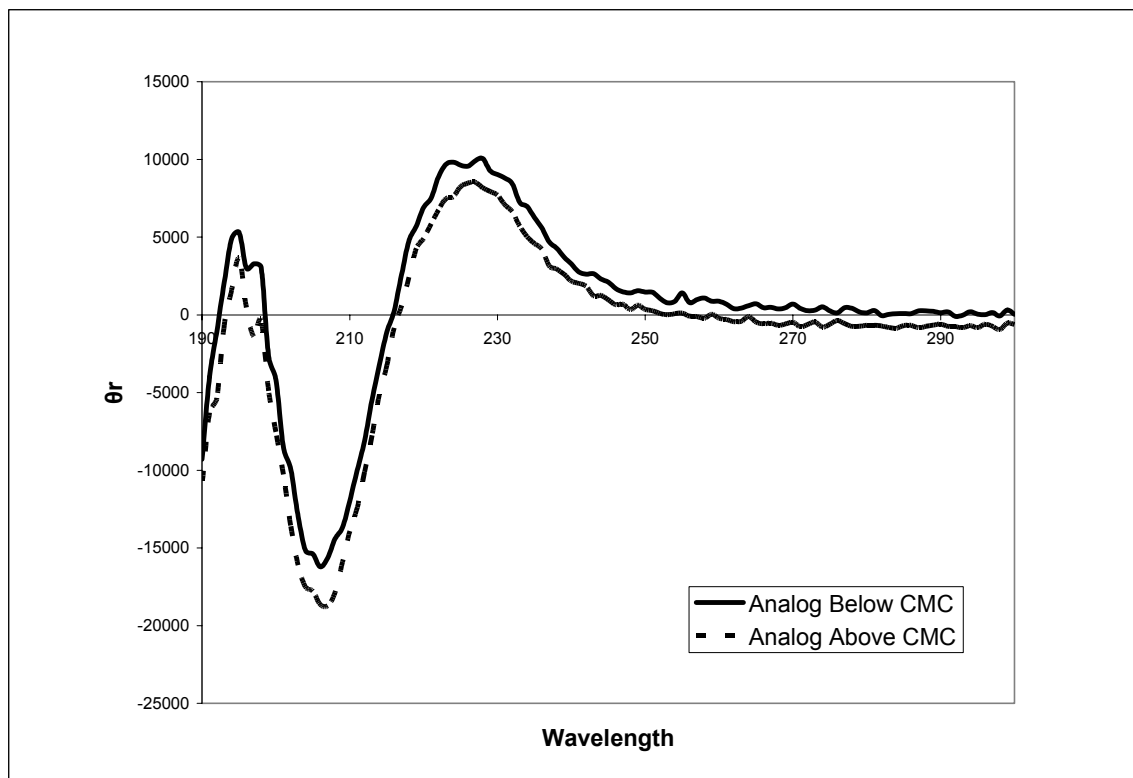
The results of the biological assay are reported in **Table III.10**. Removal of plusbacin A3's fatty acid side chain resulted in a complete loss in antibiotic activity. MIC values for all bacterial strains tested were above 50  $\mu\text{g/mL}$ . This data clearly indicates that the presence of the lipid tail is a required structural element for activity, however it has yet to verify whether or not is involved in membrane localization or simply to stabilize plusbacin to present itself in the active form.



**Figure III.13: CD comparison of plusbacin A3 and analog (III.52) with lipid chain removed (H<sub>2</sub>O, 0.2 mg/mL)**

In order to further probe the role of the lipid side chain a series of CD experiments analogous to those conducted for plusbacin A3 were carried out with the analog. A CD comparison was made between the peptide (**III.52**) and plusbacin A3, above and below CMC. **Figure III.13** represents the CD comparison with plusbacin A3 from which we observed a curve shape suggestive of a similar solution conformation. However, when the analog was studied in the presence of micelles, a conformational change was not observed, **Figure III.14**, indicating the lack of any significant membrane interactions. In conjunction with the MIC values the CD data indicates that the lipid chain is a key

structural element required for membrane interaction, possibly inducing a conformational shift necessary for activity.



**Figure III.14: CD analysis of plusbacin A3 analog above and below CMC for SDS (above cmc = 9.0 mM, below cmc = 0.9 mM, peptides in H<sub>2</sub>O at 0.2 mg/mL)**

### III.9 DEUTERIUM LABELED PLUSBACIN A3

In our efforts to elucidate the mode of action of plusbacin A3, a deuterated analog was designed and synthesized for rotational-echo double resonance (REDOR) NMR studies with whole cells of *S. aureus* and cell wall isolates<sup>21</sup>. We hoped to utilize REDOR NMR, to provide internuclear distances from the <sup>2</sup>H of the peptide antibiotic to <sup>31</sup>P, <sup>13</sup>C and <sup>15</sup>N labels biosynthetically incorporated into the bacteria from isotopically enriched

growth medium. This work was a collaborative effort with Professor Jacob Schaefer (co-inventor of REDOR) and coworkers of Washington University in St. Louis

Rotational-echo double resonance (REDOR) is a high resolution solid NMR experiment which utilizes magic angle spinning and cross polarization to measure the distance between two distinct labeled hetero-nuclei. For example, nuclei such as  $^{13}\text{C}$  -  $^{15}\text{N}$ ,  $^{13}\text{C}$ - $^{17}\text{O}$ ,  $^2\text{H}$ - $^{15}\text{N}$ ,  $^2\text{H}$ - $^{13}\text{C}$  and  $^{31}\text{P}$ - $^2\text{H}$  in the molecule can be used to provide important data about protein structures at a membrane interface, complicated heterogeneous solids such as amyloid plaques<sup>22</sup>, biomaterials<sup>23,24</sup>, ligand-substrate interactions and polymers<sup>25,26</sup> that are not accessible with solution state NMR techniques or X-Ray diffraction. REDOR has also been used previously for the compositional and structural analysis of the  $^{13}\text{C}$ - and  $^{15}\text{N}$ -labeled peptidoglycan of *Staph. aureus*<sup>27</sup>.

Schaefer and coworkers recently demonstrated the use of REDOR to characterize vancomycin binding sites in *Staph. aureus*<sup>28</sup>. Their study employed a vancomycin derivative (LY329332, Eli Lilly and Co.) with intact bacteria as well as cell wall isolates. The results of their study provided binding stoichiometry, and important information regarding the glycopeptide's membrane affinity. This study ultimately generated a model of the binding site which positioned the antibiotic core around and un-cross-linked D-Ala-D-Ala peptide consistent with current theory.

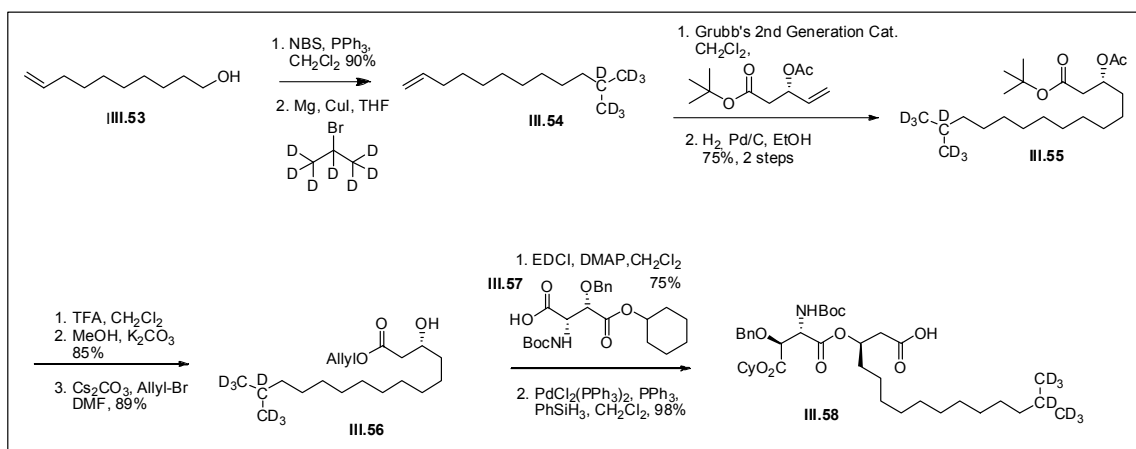
Although studies have shown that vancomycin can enhance its affinity for the bacterial cell wall functioning as a dimeric unit, other derivatives such as the teicoplanins, which carry a glucosamine acylated by a  $\text{C}_{10}$  or  $\text{C}_{11}$  fatty acid, have shown no evidence of dimerization. Williams and coworkers have postulated that the lipophilic side chain can act as a membrane anchor, therefore rendering it more difficult to



antagonize teicoplanin by the addition of lipid intermediates<sup>18,19</sup>. This phenomenon of reduced overall mobility in the site of cell wall attachment, which improves the electrostatics of binding, has been referred to as the “Gulliver effect”. Bearing in mind the structural and functional similarities between plusbacin A and other fatty acid containing cell wall antibiotics we hoped to demonstrate the role of this structural feature through REDOR NMR.

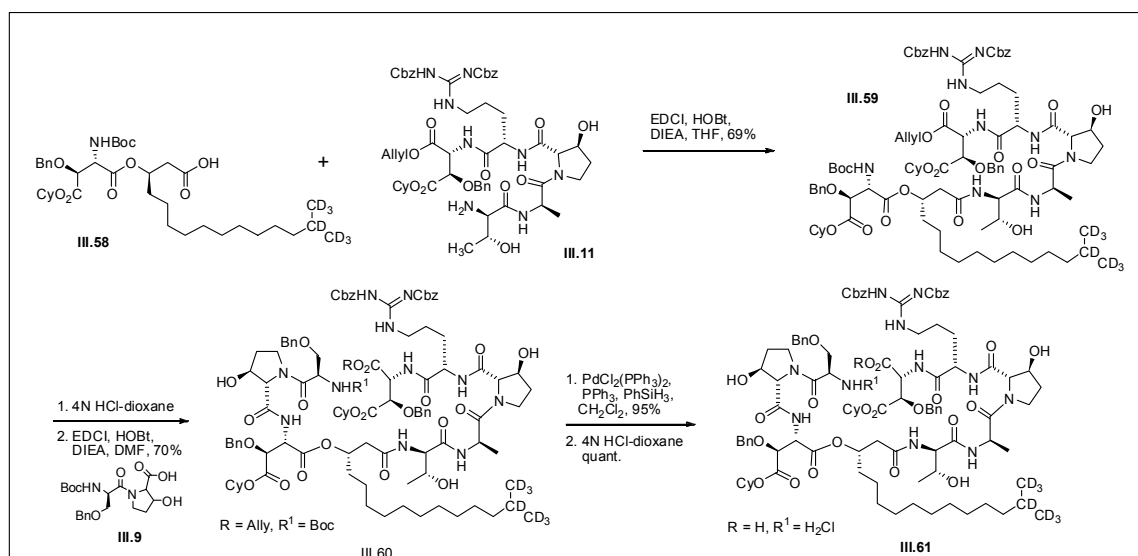
### III.9.1 Synthesis of Deuterated Plusbacin A3

In order to study the heteronuclear couplings via REDOR, a deuterated plusbacin A3 analog was designed and synthesized. Since our intentions were to probe the role of the fatty acid side chain, a D<sub>7</sub> isopropyl unit was installed. The synthesis of the deuterated analog was designed based on the previously reported total synthesis. The key intermediates were formed via segment couplings of common peptide fragments, thereby reducing the number of intermediates that had to be synthesized.



**Scheme III.19: Incorporation of deuterated side chain into depsipeptide fragment**

**Scheme III.19.** presents the incorporation of the deuterium label into the key depsipeptide fragment **III.58**. Bromination of the primary alcohol (NBS, PPh<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 90%) provided the alkyl halide in good yield. Formation of the deuterated Grignard reagent followed by the copper mediated substitution reaction afforded the labeled intermediate **III.54**. Olefin metathesis (Grubb's 2<sup>nd</sup> Gen. Cat., CH<sub>2</sub>Cl<sub>2</sub>) followed by hydrogenolysis of the resulting alkene (H<sub>2</sub>, Pd-C, EtOH) afforded the ester **III.55** in 75% overall yield. Removal of the *t*-Bu ester (CH<sub>2</sub>Cl<sub>2</sub>/TFA 1:1) and acetate protecting groups (MeOH, THF, K<sub>2</sub>CO<sub>3</sub>, 55%) provided the 3-hydroxyisopentadecanoic acid intermediate in moderate yield.



**Scheme III.20: Synthesis of linear peptide toward deuterated plusbacin A3**

**Scheme III.20** illustrates the incorporation of the key deuterated fragment into the peptide backbone and the elaboration of the linear peptide. Coupling of fragments **III.58** and **III.11** afforded the septapeptide (**III.59**) in good yield. Removal of the N-terminal Boc group (4N HCl-dioxane, quant.) followed by an EDCI coupling with Boc-D-Ser(OBn)-βOH-Pro-OH afforded the linear peptide (**III.60**). To set the stage for the



### III.10 CONCLUSIONS

The conformation of plusbacin A3, a cyclic depsipeptide antibiotic, in DMSO has been determined using NMR and DG. The analysis for the peptide conformation shows a well-defined structure for the cyclic backbone, with areas of maximum flexibility around the depsipeptide lactone linkage and fatty acid side chain.

The analysis of the peptide conformation showed an interesting variety conformationally stabilizing hydrogen bonds. Additionally, the molecular modeling data provided evidence suggestive of an intramolecular salt-bridge between the arginine and hydroxyl aspartic acid residues. These interactions provided a starting point for our SAR studies.

We first examined the contribution of the arginine residue, the only charged species in the plusbacin A3 sequence and thus a candidate for interacting with the anionic Lipid I/II pyrophosphate and /or peptidyl carboxylates. Based on plusbacin A3's proposed mode of action, our results indicate a correlation between the presence of a cationic charge at the Arg position and the affinity of the peptide toward peptidoglycan precursors, thus supporting the hypothesis that that these residues anchor the nascent peptidoglycan in a proper orientation for binding using either electrostatic or hydrogen bonding interactions. Additionally, this data supports the need for hydrogen bonding capability at this residue to stabilize the proposed salt bridge interaction.

We further probed the role of the salt-bridge with the installation of an alanine residue at the L-hydroxy-aspartic acid position. This analog directly removed the salt bridge interaction while allowing the interaction with the Ser CO to be maintained. The results showed a complete loss in activity and suggest the importance of the carboxylate to influence an active antibiotic conformation.

The role of the depsipeptide was evaluated by an amide replacement. The results showed a slight loss in activity for Group A and B *Streptococcus* and almost complete inactivation toward the resistance strains. In correlation with the molecular modeling data which suggest the flexibility of the lactone region, the rigidification at this residue could account for the loss in activity.

One of plusbacin A3's most striking features is the fatty acid functionality. We hypothesized that this side chain plays an important role as a membrane anchor, enhancing the peptides ability to sequester lipid intermediates through a phenomena known as the "Gulliver effect". Our studies showed that the removal of this functionality rendered plusbacin A3 completely inactive. Additional support for our hypothesis was demonstrated with a series of circular dichroism experiments which examined changes in peptide conformation in the presence or absence of a membrane environment. Also, a deuterated analog was synthesized and in due course will provide detail evident to support this theory.

As a first step toward improving the pharmacological properties of plusbacin A3, we utilized NMR/ molecular modeling coupled with chemical synthesis to uncover important information into its mechanism of action. Several key structural features were determined which will aid future studies toward understanding the activity of this potent antibiotic active against MRSE and VRE.

### **III.11 EXPERIMENTAL SECTION**

General. All reactions were carried out in flame-dried glassware under an atmosphere of dry nitrogen or argon. Unless otherwise mentioned, solvents were purified as follows. All solvents were dried over activated alumina using a Seca Solvent System (Glass Contour). All other commercially available reagents were used as received.  $^1\text{H}$

NMR spectra were measured at 300 MHz on a Varian Mercury instrument, at 400 MHz on a Varian Gemini-400, or at 500 MHz on a Varian VXR-500 instrument.  $^{13}\text{C}$  NMR spectra were measured at 100 MHz or 75 MHz on a Varian Gemini spectrometer. Chemical shifts are reported relative to the central line of residual solvent. Infrared spectra were recorded using a Nicolet IR/42 spectrometer FT-IR (thin film, NaCl cells). High resolution mass spectra were obtained via electrospray ionization on an Agilent ESI-TOF spectrometer. Optical rotations were measured on a Perkin–Elmer polarimeter (Model 241) using a 1 mL capacity quartz cell with a 10 cm path length.

Analytical thin layer chromatography (TLC) was performed using Whatman glass plates coated with a 0.25 mm thickness of silica gel containing PF 254 indicator, and compounds were visualized with UV light, potassium permanganate stain, cerium molybdate stain or ninhydrin stain. Analytical high performance liquid chromatography (HPLC) was performed on a Beckman-Coulter instrument (System Gold) with diode array detection. Analysis was carried out using Phenomenex Jupiter reverse-phase (C18) column (10 $\mu$  particle size, 300 Å pore size, 250 mm length x 4.6 mm diameter) with mobile phases consisting of 1% trifluoroacetic acid in water and acetonitrile. Preparatory HPLC purifications (Phenomenex Jupiter C<sub>18</sub> reverse-phase column, 10 $\mu$  particle size, 300 Å pore size, 250 mm length x 21.2 mm diameter) were performed with a Waters S3 Millipore Model 510 System with a Model 2487 Dual Absorbance Detector. Flash

chromatography purifications were performed using Silicycle 60 Å, 35-75 µm silica gel or Biotage purification system (SP1 HPFC system). All compounds purified by chromatography were sufficiently pure for use in further experiments, unless otherwise noted

### **III.11.1 NMR, MOLECULAR MODELING**

Proton NMR spectra were recorded with a Bruker DRXL 600 spectrometer equipped with Hewlett Packer computers. The raw data was processed on MestreC version 4.86 NMR processing software. Samples of Plusbacin A3 (5-10 mg) were dissolved in DMSO-d<sub>6</sub> (500 µL; 99.96 atom%, Cambridge Isotope Laboratories Inc.). Spectra were recorded over spectral widths of 4000 - 6000 Hz. The TOCSY and NOESY spectra were acquired with mixing times of 70, 500 ms, respectively, at 35°C, and for 64 scans each. Cross-peak intensities in the NOESY spectra were measured by volume integration using the MestreC software and converted into distance restraints using the correlation between Ser βCH<sub>2</sub> protons for calibration (1.78 Å<sup>3</sup>). To determine temperature coefficients, three temperatures (318K, 308K, and 298K) were covered from which the ppm shift differences applied to calculate ppb/K for each amide.

### **III.11.2 CIRCULAR DICHROISM**

The CD spectra were recorded with an Aviv Instruments Model 215 with a path cell length of 2.0 mm. The spectra were acquired between 300 and 190 nm sampling every 0.5 nm. Ellipticity measurements were expressed as mean residue ellipticity [θ] in deg cm<sup>2</sup>/dmol. All the samples were prepared in an analogous manner first dissolving peptide

as a 1.0 mg/mL solution and then preparing the appropriate sample dilution using Fischer HPLC grade H<sub>2</sub>O. In each sample the peptide concentration was 0.2 mg/mL.

### III.11.3 ARGININE MODIFICATIONS

#### III.11.3a Ornithine Analogs

##### Ornithine analog (III.2)

The cyclic peptide (0.043 g, 0.024 mmol) was treated with a solution of 5% piperidine/DMF (0.5 mL) and stirred at room temperature for 15 minutes. The solvent was removed in vacuo and the residue was triturated with hexanes. The product was isolated by centrifugation and used without further purification. The amine and anisole (1.0 mL) in an HF reaction apparatus were purged with N<sub>2</sub> and cooled to -78 °C. HF gas was distilled into the reaction vessel to a total volume of approximately 5 mL. The reaction was warmed to 0°C and stirred at this temperature for 1hr. The HF was then evaporated under a steady stream of N<sub>2</sub> and the crude material was transferred to a round bottom flask using EtOH. The volatiles were removed in vacuo and the crude material was triturated with Et<sub>2</sub>O and centrifuged (3x). The crude material was HPLC purified (30-75% CH<sub>3</sub>CN/H<sub>2</sub>O 0.1%TFA over 60 minutes) to afford (0.0110 g, 0.0099 mmol, 41%) of the plusbacin ornithine analog as a white powder. mp >200 °C decomposition;  $[\alpha]_D^{22} + 1.40$  (c 0.32, EtOH); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>CN/D<sub>2</sub>O/TFA, 500:500:1) δ 5.21-5.08 (m, 1H), 5.05 (d, *J* = 1.32 Hz, 1H), 4.93 (d, *J* = 5.02 Hz, 1H), 4.89 (s, 1H), 4.85-4.72 (m, 2H), 4.71-4.63 (m, 2H), 4.59 (dd, *J* = 13.42, 6.25 Hz, 1H), 4.51-4.46 (m, 1H), 4.43-4.32 (m, 6H), 4.13 (d, *J* = 6.09 Hz, 1H), 4.01 (t, *J* = 7.21, 7.21 Hz, 1H), 3.98-3.84



(m, 2H), 3.83-3.72 (m, 3H), 3.72-3.50 (m, 1H), 2.97-2.80 (m, 2H), 2.62-2.41 (m, 2H), 2.17-2.01 (m, 1H), 1.88 (d,  $J = 6.63$  Hz, 1H), 1.78-1.65 (m, 1H), 1.61-1.43 (m, 6H), 1.28-1.13 (m, 27H), 1.12-1.07 (m, 4H), 1.03 (d,  $J = 6.36$  Hz, 1H), 0.80 (d,  $J = 6.58$  Hz, 6H); IR (KBr pellet)  $\nu_{max}$  3378, 2921, 2855, 1741, 1659, 1459, 1204, 1131, 1098, 991; HR ESI TOF  $m/z$  1116.5696  $[M+H]^+$

### III.11.3b N-Acyl Ornithine Analog

#### N-Acyl Ornithine analog (III.3)

The Fmoc protected cyclic peptide (0.035 g, 0.020 mmol) was treated with a solution of 5% piperidine/DMF (0.5 mL) and stirred at room temperature for 15 minutes. The solvent was removed in vacuo and the residue was triturated with hexanes. The product was isolated by centrifugation and used without further purification. The amine was then treated with a freshly prepared solution of DMF/DIEA/acetic anhydride (0.392 mL/ 0.00341 mL/ 0.0019 mL) and stirred at room temperature for 8 hours. The solvent was removed in vacuo and the product was purified by column chromatography (2 - 30% EtOH/ $CHCl_3$ ). The acylated peptide and anisole (1.0 mL) in an HF reaction apparatus were purged with  $N_2$  and cooled to  $-78^\circ C$ . HF gas was distilled into the reaction vessel to a total volume of approximately 5 mL. The reaction was warmed to  $0^\circ C$  and stirred at this temperature for 1 hr. The HF was then evaporated under a steady stream of  $N_2$  and the crude material was transferred to a round bottom flask using EtOH. The volatiles were removed in vacuo and the crude material was triturated with  $Et_2O$  and centrifuged (3 x). The crude material was HPLC purified (30-75%  $CH_3CN/H_2O$  0.1% TFA over 60 minutes) to afford (0.0110 g, 0.0099 mmol, 41%) of the plusbacin ornithine analog as a

white powder. mp > 200 °C decomposition;  $[\alpha]_D^{22} + 2.1$  (c 0.45, EtOH);  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{CN}/\text{D}_2\text{O}/\text{TFA}$ , 500:500:1);  $\delta$  5.25-5.05 (m, 1H), 5.03 (s, 1H), 4.98-4.89 (m, 1H), 4.88-4.83 (m, 1H), 4.83-4.74 (m, 2H), 4.72 (s, 1H), 4.70-4.66 (m, 1H), 4.65-4.55 (m, 1H), 4.50 (t,  $J = 6.44, 6.44$  Hz, 1H), 4.47-4.31 (m, 1H), 3.98 (t,  $J = 7.22, 7.22$  Hz, 1H), 3.97-3.83 (m, 2H), 3.83-3.51 (m, 5H), 3.13-3.02 (m, 2H), 2.64-2.42 (m, 1H), 1.91-1.81 (m, 3H), 1.75-1.68 (m, 1H), 1.69-1.51 (m, 4H), 1.45 (td,  $J = 13.28, 6.59, 6.59$  Hz, 1H), 1.37-1.13 (m, 28H), 1.13-1.06 (m, 4H), 1.02 (d,  $J = 6.08$  Hz, 1H), 0.80 (d,  $J = 6.49$  Hz, 1H); IR (KBr pellet)  $\nu_{\text{max}}$  3374, 2928, 2859, 1743, 1662, 1457, 1199, 1131, 1099, 993; HR ESI TOF  $m/z$  1158.5819  $[\text{M}+\text{H}]^+$

### III.11.3c Cationic Ornithine Analog

#### Cyclic peptide (III.4)

Boc-D-*α*-Thr-D-Ala-βOH-Pro-Orn(Fmoc)-βOBn-D-*α*-Asp(OCy)-OAllyl (0.509 g, 0.470 mmol) was treated with a solution of 4N HCl-Dioxane (4.0 mL, 15.89 mmol) and stirred for 90 minutes. The volatiles were removed in vacuo. The residual HCl was further removed by adding  $\text{Et}_2\text{O}$  (5 mL) to the hydrochloride salt followed by its removal in vacuo. The residue was then dissolved in EtOAc (30 mL) and washed with sat. aq.  $\text{NaHCO}_3$  (2 x 20 mL). The EtOAc was dried over  $\text{MgSO}_4$ , filtered and condensed. This residue, the carboxylic acid (0.292 g, 0.428 mmol), and HOBt (0.069 g, 0.513 mmol) were dissolved in THF (1.7 mL) and cooled to 0 °C. EDCI (0.098 g, 0.513 mmol) was added and the mixture was stirred under argon for 18 hrs while slowly warming to room temperature. The reaction was quenched with EtOAc (1 mL) and the THF removed in vacuo. This residue was dissolved in EtOAc (20 mL) and washed with 1N HCl (1 x 10

mL), sat. aq.  $\text{NaHCO}_3$  (1 x 10 mL), and brine (1 x 10mL). The organic phase was dried over  $\text{Mg}_2\text{SO}_4$ , filtered and condensed. Chromatography using a Biotage SP1 Flash system (7-60% Acetone-EtOAc) afforded the septapeptide (0.539 g, 0.327 mmol, 77%) as a white solid. mp 70-72 °C;  $[\alpha]_D^{22} - 1.0$  (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.38 (d,  $J = 9.31$  Hz, 1H), 8.23 (d,  $J = 9.13$  Hz, 1H), 8.06 (d,  $J = 9.10$  Hz, 1H), 8.04 (d,  $J = 9.65$  Hz, 1H), 8.00 (d,  $J = 8.21$  Hz, 1H), 7.86 (d,  $J = 7.47$  Hz, 2H), 7.69-7.61 (m, 2H), 7.38 (t,  $J = 7.40, 7.40$  Hz, 2H), 7.34-7.21 (m, 14H), 6.83 (dd,  $J = 17.39, 9.66$  Hz, 1H), 5.79 (tdd,  $J = 16.10, 10.95, 5.47, 5.47$  Hz, 1H), 5.21 (dd,  $J = 42.63, 13.72$  Hz, 2H), 5.08 (m, 1H), 4.97-4.87 (m, 1H), 4.82-4.60 (m, 6H), 4.60-4.32 (m, 9H), 4.24 (t,  $J = 6.66, 6.66$  Hz, 3H), 4.22-4.13 (m, 3H), 3.75 (dd,  $J = 16.31, 7.92$  Hz, 1H), 3.50 (dd,  $J = 46.41, 6.44$  Hz, 1H), 3.01-2.86 (m, 1H), 2.47-2.35 (m, 1H), 1.82-1.53 (m, 11H), 1.52-1.25 (m, 29H), 1.25-1.02 (m, 31H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  173.3, 172.6, 172.2, 170.9, 170.1, 169.9, 169.2, 168.6, 157.6, 157.1, 155.7, 144.3, 141.5, 137.0, 136.7, 131.6, 128.636, 128.5, 128.2, 127.9, 127.8, 127.3, 125.4, 125.2, 120.1, 119.4, 78.0, 78.4, 78.2, 76.8, 74.7, 74.4, 73.7, 73.5, 73.2, 73.0, 69.0, 68.2, 67.5, 66.8, 58.8, 58.2, 56.4, 54.6, 53.1, 48.2, 47.9, 47.5, 45.5, 41.2, 40.7, 39.3, 34.1, 33.9, 32.6, 31.7, 31.4, 30.2, 29.9, 29.7, 28.4, 28.183, 27.635, 26.2, 25.4, 23.8, 22.9, 20.3, 20.1, 16.7, 15.9; IR (Film)  $\nu_{\text{max}}$  3300, 3069, 2932, 2857, 2361, 1749, 1731, 1713, 1655, 1634, 1540, 1522, 1507, 1456, 1367, 1339, 1257, 1206, 1160, 1104; ESI MS  $m/z$  1670.70  $[\text{M}+\text{Na}]^+$

The septapeptide (0.520 g, 0.319 mmol) was treated with 4N HCl-Dioxane (4 mL) and the resulting mixture was stirred at room temperature for 90 minutes. The volatiles were removed in vacuo. The residual HCl was further removed by adding  $\text{Et}_2\text{O}$  (5 mL) to the

hydrochloride salt followed by its removal in vacuo. This residue, Boc-D-Ser(OBn)- $\beta$ OH-Pro-OH (0.123 g, 0.301 mmol), HOBt (0.061 g, 0.451 mmol) and DIEA (0.058 mL, 0.451 mmol) were dissolved in THF (1.0 mL). The solution was cooled to 0 °C and EDCI (0.086 g, 0.451 mmol) was added. The reaction mixture was stirred under argon for 18 hrs and then quenched with EtOAc (1 mL). The THF was removed in vacuo and to the residue was added EtOAc (50 mL). This solution was extracted with 1N HCl (1 x 25 mL), sat. aq. NaHCO<sub>3</sub> (1 x 25 mL), and brine (1 x 25 mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Chromatography (10% Acetone-EtOAc) provided the linear peptide (0.445 g, 0.230 mmol, 76%) as a white solid. mp 78-81 °C;  $[\alpha]_D^{22} +1.2$  (c 1.0, CHCl<sub>3</sub>) <sup>1</sup>H NMR (500 MHz DMSO-*d*<sub>6</sub>, 45°C)  $\delta$  8.29 (t, *J* = 9.97, 9.97 Hz, 1H), 8.14 (d, *J* = 8.96 Hz, 1H), 8.06-7.91 (m, 3H), 7.87 (d, *J* = 7.53 Hz, 2H), 7.69-7.64 (m, 2H), 7.40 (t, *J* = 7.43, 7.43 Hz, 2H), 7.36-7.23 (m, 19H), 5.88-5.76 (m, 1H), 5.29 (dd, *J* = 17.15, 1.22 Hz, 1H), 5.18 (dd, *J* = 10.52, 1.35 Hz, 1H), 5.12 (d, *J* = 3.86 Hz, 1H), 5.09 (d, *J* = 3.77 Hz, 1H), 5.00 (s, 1H), 4.98-4.85 (m, 1H), 4.76-4.66 (m, 6H), 4.63-4.40 (m, 11H), 4.36 (s, 2H), 4.34-4.15 (m, 8H), 3.86-3.64 (m, 1H), 3.65-3.38 (m, 2H), 3.02-2.91 (m, 2H), 2.47-2.39 (m, 1H), 2.07-1.86 (m, 2H), 1.85-1.56 (m, 11H), 1.55-1.05 (m, 57H); <sup>13</sup>C (100 MHz, CDCl<sub>3</sub>)  $\delta$  173.5, 172.8, 172.0, 171.8, 170.0, 169.5, 168.6, 168.5, 168.4, 168.2, 157.1, 156.896, 155.1, 144.2, 141.4, 138.2, 137.4, 136.9, 136.6, 131.5, 131.4, 130.0, 128.6, 128.5, 128.4, 128.2, 127.9, 127.8, 127.2, 125.2, 120.1, 119.4, 119.2, 79.6, 76.6, 76.1, 74.8, 74.6, 74.5, 74.1, 73.7, 73.5, 73.2, 72.9, 71.2, 70.6, 69.2, 68.7, 68.2, 66.8, 66.5, 58.3, 54.7, 53.0, 51.8, 51.1, 48.8, 48.4, 47.4, 46.2, 45.8, 41.6, 40.6, 39.2, 34.1, 33.1, 31.7, 31.5, 31.4, 31.3, 30.9, 30.1, 29.8, 29.7, 28.5, 28.1, 27.6, 26.0, 25.5, 25.3, 24.7, 23.8,

22.9, 19.9, 16.4; IR (*neat*)  $\nu_{\max}$  3314, 3089, 3066, 3034, 2932, 2862, 1740, 1691, 1642, 1529, 1450, 1260, 1104; ESI MS  $m/z$  1960.84  $[M+Na]^+$

$PdCl_2(PPh_3)_2$  (3.9 mg, 0.0056 mmol) and  $PPh_3$  (4.4 mg, 0.017 mmol) were dissolved in dry  $CH_2Cl_2$  (1.10 mL) and stirred under an atmosphere of argon for 15 min. This solution was then added to the allyl ester (0.435 g, 0.224 mmol) via syringe and phenylsilane (0.055 mL, 0.449 mmol) was added drop wise. The solution was stirred at room temperature under an atmosphere of argon for 6 hrs. The solvent was condensed and the crude material was loaded directly onto a silica gel column. Chromatography (1-15% EtOH- $CHCl_3$ ) afforded the carboxylic acid (0.395 g, 0.208 mmol, 93%) as a white solid. mp 109-111 °C;  $[\alpha]_D^{22}$  - 12.9 (c 1.0,  $CHCl_3$ )  $^1H$  NMR (500 MHz,  $DMSO-d_6$ , 75°C)  $\delta$  7.83 (d,  $J$  = 1.87 Hz, 1H), 7.43-7.15 (m, 19H), 7.64 (d,  $J$  = 1.87 Hz, 2H), 5.16 (d,  $J$  = 1.87 Hz, 1H), 4.87 (d,  $J$  = 1.87 Hz, 1H), 4.72-4.61 (m, 3H), 4.60-4.39 (m, 9H), 4.38-4.31 (m, 2H), 4.30-4.20 (m, 6H), 4.18 (dd,  $J$  = 6.63, 1.87 Hz, 1H), 4.14 (s, 1H), 3.82-3.64 (m, 1H), 3.64-3.50 (m, 1H), 2.96 (d,  $J$  = 1.87 Hz, 2H), 2.44-2.34 (m, 1H), 2.03-1.68 (m, 8H), 1.63 (s, 4H), 1.56-1.06 (m, 57H), 1.03 (d,  $J$  = 1.87 Hz, 3H), 0.82 (d,  $J$  = 1.87 Hz, 1H)  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  174.4, 173.69, 170.71, 170.9, 170.7, 170.2, 169.7, 168.7, 166.6, 163.0, 157.6, 156.8, 155.7, 155.3, 144.2, 141.5, 138.1, 136.9, 134.3, 133.2, 130.1, 128.2, 127.8, 127.3, 125.4, 120.2, 79.7, 76.05, 74.5, 73.3, 72.7, 71.7, 70.7, 69.7, 68.7, 68.4, 67.8, 67.5, 66.8, 66.2, 66.0, 65.2, 63.9, 62.0, 61.2, 59.4, 58.9, 58.1, 56.4, 56.1, 55.9, 54.7, 53.2, 53.0, 51.9, 49.2, 48.1, 47.5, 45.7, 41.1, 40.8, 39.3, 36.7, 34.3, 32.7, 31.7, 30.1, 29.9, 28.6, 28.2, 27.6, 26.4, 25.5, 23.9, 22.9, 21.6, 20.9, 20.0, 17.8, 15.4, 14.2,

13.7; IR (neat)  $\nu_{\max}$  3308, 3065, 2929, 2857, 1706, 1642, 1527, 1451, 1255, 1200, 1158;  
ESI MS  $m/z$  1920.84[M+Na]<sup>+</sup>

The linear peptide (0.370 g, 0.195 mmol) was treated with a 4N HCl-Dioxane solution (2.0 mL) and stirred under an atmosphere of argon for 1 hr. The volatiles were then removed in vacuo and to the residue was added CH<sub>2</sub>Cl<sub>2</sub> / Hexanes (1:1, 10 mL). The solvent was condensed to remove any residual HCl and the crude residue was placed under high vacuum for several hours. The product was used without further purification. To this residue in dry DMF (95 mL) at 0 °C was added HOBt (0.129 g, 0.952 mmol), DIEA (0.066 mL, 0.381 mmol) and finally EDCI (0.183 g, 0.952 mmol). The mixture was stirred under an atmosphere of argon at this temperature for 48 hours. The solvent was then removed and the crude material was diluted with EtOAc (30 mL). The organic phase was washed with 1N HCl (1 x 20 mL), sat. aq. NaHCO<sub>3</sub> (1 x 20 mL) and brine (1 x 20 mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Silica gel chromatography (1 - 10% EtOH-CHCl<sub>3</sub>) afforded the cyclic peptide as a white solid. mp 93-95 °C; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  7.89-7.81 (m, 4H), 7.81-7.76 (m, 1H), 7.64 (d,  $J$  = 7.02 Hz, 2H), 7.60-7.53 (m, 2H), 7.39 (t,  $J$  = 7.45, 7.45 Hz, 3H), 7.36-7.18 (m, 17H), 5.23 (s, 1H), 5.05-4.89 (m, 1H), 4.86 (d,  $J$  = 7.85 Hz, 1H), 4.79-4.68 (m, 4H), 4.67-4.30 (m, 13H), 4.31-4.09 (m, 8H), 3.95-3.88 (m, 1H), 3.88-3.77 (m, 1H), 3.76-3.70 (m, 1H), 3.64 (dd,  $J$  = 9.45, 6.54 Hz, 1H), 3.61-3.52 (m, 2H), 2.93 (m, 2H), 2.61 (dd,  $J$  = 19.11, 11.29 Hz, 1H), 2.47-2.44 (m, 1H), 2.34 (dd,  $J$  = 14.25, 5.38 Hz, 2H), 2.00-1.87 (m, 1H), 1.85-1.56 (m, 14H), 1.57-0.99 (m, 50H); <sup>13</sup>C (100MHz, CDCl<sub>3</sub>)  $\delta$  175.0, 173.4, 172.4, 171.5, 170.9, 170.5, 170.0, 169.6, 169.2, 168.3, 167.63, 157.0, 144.2, 141.5, 137.9,

137.2, 136.7, 128.7, 128.7, 128.6, 128.6, 128.4, 128.3, 128.1, 127.9, 127.2, 126.2, 125.7, 125.4, 120.1, 117.8, 111.3, 74.6, 73.5, 73.4, 69.9, 68.9, 68.3, 67.4, 67.2, 66.8, 54.6, 54.1, 52.1, 47.5, 47.4, 45.5, 41.5, 40.9, 39.8, 34.5, 33.3, 32.0, 31.8, 31.7, 31.5, 31.2, 30.9, 30.3, 30.1, 29.9, 29.9, 29.8, 29.8, 29.7, 29.6, 29.5, 28.2, 27.6, 26.4, 25.5, 25.3, 24.8, 24.0, 23.7, 23.7, 22.9, 20.2, 19.3, 16.8; IR (neat)  $\nu_{max}$  3303, 3066, 3035, 2926, 2855, 1741, 1650, 1546, 1528, 1467, 1450, 1260, 1202; ESI MS  $m/z$  1801.73  $[M+Na]^+$ .

### Cationic Plusbacin Analog (III.5)

The Fmoc protected peptide (0.039 g, 0.022 mmol) was treated with a solution of 5% piperidine/DMF (0.5 mL) and stirred at room temperature for 15 minutes. The solvent was removed and the crude residue was triturated with hexanes (1 x 20 mL). The amine was further purified by column chromatography (2-50% EtOH-CHCl<sub>3</sub>) to afford a white solid (0.030g, 0.019 mmol, 88%). This material was then dissolved in DMF (0.422 mL) and treated with MeI (3.60  $\mu$ L, 0.058 mmol) and K<sub>2</sub>CO<sub>3</sub> (0.0093g, 0.067 mmol) and stirred for 8 hrs at room temperature. The mixture was diluted with EtOAc (10 mL) and filtered through a pad of celite. Removal of the solvent afforded the product (0.032 g, 0.018 mmol) as a tan solid. mp 107-109 °C;  $[\alpha]_D^{22}$  - 46.4 (c 0.5, CHCl<sub>3</sub>). <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.34 (s, 1H), 8.28 (d,  $J$  = 7.10 Hz, 1H), 8.20 (d,  $J$  = 8.83 Hz, 1H), 8.01 (d,  $J$  = 8.74 Hz, 1H), 7.97 (d,  $J$  = 7.66 Hz, 1H), 7.88 (d,  $J$  = 7.33 Hz, 1H), 7.82 (d,  $J$  = 5.86 Hz, 1H), 7.72 (d,  $J$  = 6.37 Hz, 1H), 7.51 (d,  $J$  = 6.22 Hz, 1H), 7.41-7.20 (m, 15H), 5.29 (d,  $J$  = 2.81 Hz, 1H), 5.18 (d,  $J$  = 3.28 Hz, 1H), 5.01 (d,  $J$  = 3.32 Hz, 1H), 4.98-4.90 (m, 1H), 4.88-4.11 (m, 19H), 3.94 (dd,  $J$  = 10.87, 5.73 Hz, 1H), 3.87-3.76 (m, 1H), 3.68 (td,  $J$  = 16.88, 8.47, 8.47 Hz, 1H), 3.65-3.45 (m, 5H), 3.41 (t,  $J$  = 5.24, 5.24 Hz, 1H),

3.07-2.91 (m, 9H), 2.73-2.62 (m, 1H), 2.44-2.27 (m, 1H), 2.08-1.56 (m, 16H), 1.56-1.43 (m, 8H), 1.39-0.81 (m, 60H); IR (neat)  $\nu_{max}$  3454, 2930, 2853, 1746, 1654, 1537, 1455, 1343, 1200, 1103; HR ESI TOF  $m/z$  1599.9531  $[M]^+$

The cyclic peptide (0.0220 g, 0.014 mmol) and anisole (1 mL) in an HF reaction apparatus were purged with N<sub>2</sub> and cooled to -78 °C. HF gas was distilled into the reaction vessel to a total volume of approximately 5 mL. The reaction was warmed to 0 °C and stirred at this temperature for 1hr. The HF was then evaporated under a steady stream of N<sub>2</sub> and the crude material was transferred to a round bottom flask using EtOH. The volatiles were removed in vacuo and the crude material was triturated with Et<sub>2</sub>O and centrifuged (3x). The crude material was HPLC purified (30-65% CH<sub>3</sub>CN/H<sub>2</sub>O 0.1%TFA over 60 minutes) to afford (0.0072 g, 0.0062 mmol, 45%) of the cationic plusbacin analog as a white solid. mp > 200 °C decomposition;  $[\alpha]_D^{22}$  - 1.90 (c 0.27, EtOH); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>CN/D<sub>2</sub>O/TFA, 500:500:1);  $\delta$  5.17 (s, 1H), 5.13-5.06 (m, 1H), 5.06-5.03 (m, 1H), 4.99-4.89 (m, 2H), 4.89-4.72 (m, 2H), 4.72-4.56 (m, 2H), 4.51-4.47 (m, 1H), 4.41 (s, 1H), 4.37 (d,  $J$  = 1.94 Hz, 1H), 4.33 (s, 1H), 4.29 (s, 1H), 4.13 (dd,  $J$  = 8.90, 3.44 Hz, 1H), 4.02 (t,  $J$  = 6.68, 6.68 Hz, 1H), 3.97-3.85 (m, 2H), 3.83-3.73 (m, 2H), 3.72-3.48 (m, 3H), 3.20 (s, 1H), 3.05-2.93 (m, 9H), 2.63-2.41 (m, 1H), 1.76-1.48 (m, 4H), 1.32-1.12 (m, 24H), 1.12-1.05 (m, 3H), 1.02 (d,  $J$  = 6.32 Hz, 1H), 0.80 (d,  $J$  = 6.63 Hz, 1H); IR (KBr pellet)  $\nu_{max}$  3450, 2925, 2855, 1741, 1674, 1649, 1542, 1445, 1205, 1134, 996, 808, 726; HR ESI TOF  $m/z$  1165.6569  $[M]^+$

### III.11.4 HYDROXY-ASPARTIC ACID MODIFICATIONS



**(R)-allyl3-((S)-2-(tert-butoxycarbonylamino)propanoyloxy)-14-methylpentadecanoate (III.8)**

To a stirring solution of Boc-Ala-OH (0.328 g, 1.73 mmol), the alcohol (0.451 g, 1.44 mmol), and DMAP (0.035 g, 0.289 mmol) dissolved in CH<sub>2</sub>Cl<sub>2</sub> (1 mL) at -15 °C was added EDCI (0.332 g, 1.732 mmol) in five portions over a period of 1 hour. The solution was stirred at this temperature under argon for 2 hours then slowly warmed to room temperature. The reaction was stirred 12 hrs then quenched with EtOAc (1 mL). The solvent was removed in vacuo and the residue was dissolved in EtOAc (20 mL) then washed with 1N HCl (1 x 10 mL) and brine (1 x 10 mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Column chromatography (5-50% EtOAc/Hexanes) afforded (R)-allyl 3-((S)-2-(tert-butoxycarbonylamino)propanoyloxy)-14-methylpentadecanoate (0.639 g, 1.32 mmol, 92 %) as a colorless oil.  $[\alpha]_D^{22}$  - 2.4 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.90 (tdd, *J* = 17.15, 10.42, 5.84, 5.84 Hz, 1H), 5.35-5.19 (m, 3H), 5.06 (d, *J* = 7.13 Hz, 1H), 4.57 (td, *J* = 5.72, 1.13, 1.13 Hz, 2H), 4.26 (t, *J* = 6.87, 6.87 Hz, 1H), 2.61 (dq, *J* = 15.60, 15.60, 15.60, 6.50 Hz, 2H), 1.70-1.56 (m, 3H), 1.56-1.40 (m, 10H), 1.36 (d, *J* = 7.15 Hz, 3H), 1.32-1.20 (m, 17H), 1.14 (d, *J* = 6.33 Hz, 1H), 0.90-0.82 (m, 5H); <sup>13</sup>C (100MHz, CDCl<sub>3</sub>) δ 170.080, 167.089, 154.250, 132.038, 118.808, 71.737, 65.672, 49.536, 39.33, 34.14, 32.08, 30.14, 30.12, 29.90, 29.83, 29.78, 29.73, 29.64, 29.53, 29.49, 28.54, 28.18, 27.62, 25.21, 22.89, 19.04; IR (neat)  $\nu_{max}$  2926, 2857, 1746, 1715, 1501, 1455, 1368, 1312, 1246, 1170, 1063, 930; ESI MS *m/z* 506.17 [M+Na]<sup>+</sup>

**Tetrapeptide (III.10)**

The dipeptide (0.622 g, 1.29 mmol) was treated with 4N HCl-Dioxane (4 mL) and the resulting mixture was stirred at room temperature for 90 minutes. The volatiles were removed in vacuo. The residual HCl was further removed by adding Et<sub>2</sub>O (5 mL) to the hydrochloride salt followed by its removal in vacuo. This residue, Boc-D-Ser(OBn)- $\beta$ OH-Pro-OH (0.416 g, 1.02 mmol), HOBt (0.206 g, 1.528 mmol) and DIEA (0.266 mL, 1.528 mmol) were dissolved in THF (3.4 mL). The solution was cooled to 0 °C and EDCI (0.293 g, 1.528 mmol) was added. The reaction mixture was stirred under argon for 18 hrs and then quenched with EtOAc (1 mL). The THF was removed in vacuo and to the residue was added EtOAc (25 mL). This solution was extracted with 1N HCl (1 x 20 mL), sat. aq. NaHCO<sub>3</sub> (1 x 20 mL), and brine (1 x 20 mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Silica gel chromatography on a Biotage SP1 Flash system (20-80 % EtOAc-Hex) afforded the product (0.591 g, 0.764 mmol, 75 %) as a colorless wax [ $\alpha$ ]<sub>D</sub><sup>22</sup> - 39.3 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.30 (d, *J* = 6.46 Hz, 1H), 7.36-7.23 (m, 5H), 6.96 (d, *J* = 8.00 Hz, 1H), 5.95-5.83 (m, 1H), 5.38-5.01 (m, 3H), 4.53 (d, *J* = 5.34 Hz, 2H), 4.49-4.47 (m, 2H), 4.37 (d, *J* = 3.04 Hz, 1H), 4.32-4.26 (m, 1H), 4.23 (s, 1H), 4.17 (d, *J* = 3.35 Hz, 1H), 4.12 (dd, *J* = 15.04, 8.23 Hz, 1H), 3.77 (t, *J* = 8.07, 8.07 Hz, 1H), 3.67 (dd, *J* = 14.44, 9.44 Hz, 1H), 3.63-3.55 (m, 1H), 3.52 (dd, *J* = 9.43, 7.02 Hz, 1H), 3.49-3.42 (m, 2H), 2.74-2.63 (m, 1H), 2.60-2.52 (m, 1H), 1.98 (d, *J* = 10.20 Hz, 1H), 1.86-1.72 (m, 1H), 1.57-1.42 (m, 3H), 1.39-1.35 (m, 9H), 1.26-1.18 (m, 22H), 1.15-1.11 (m, 6H), 0.84 (d, *J* = 6.60 Hz, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  173.665, 171.770, 170.548, 169.636, 155.622, 137.373, 128.327, 127.667, 127.559, 79.941, 73.205, 73.029, 71.889, 69.286, 68.998, 52.333, 48.303, 45.688, 39.318, 38.928, 33.992, 32.026, 29.827, 29.589, 29.541, 29.474, 29.389, 29.287,

28.208, 27.840, 27.303, 25.037, 22.562, 16.617 IR (neat)  $\nu_{max}$  3352, 2930, 2850, 1744, 1693, 1643, 1534, 1390, 1367, 1254, 1169, 1107, 1052, 986, 742; ESI MS  $m/z$  796.37  $[M+Na]^+$

### Linear Peptide (III.12)

$PdCl_2(PPh_3)_2$  (0.0103 g, 0.015 mmol) and  $PPh_3$  (0.012 g, 0.044 mmol) were dissolved in dry  $CH_2Cl_2$  (1.0 mL) and stirred under an atmosphere of argon for 15 min. This solution was transferred via syringe to a solution of the allyl ester (0.456 g, 0.589 mmol) in dry  $CH_2Cl_2$  (1.95 mL) under argon.  $PhSiH_3$  (0.15 mL, 1.18 mmol) was added via syringe and the reaction was stirred at room temperature for 4 hours. The solvent was condensed and the crude residue was loaded directly onto a silica gel column. Silica gel chromatography on a Biotage SP1 Flash system (2-20% EtOH/ $CHCl_3$ ) afforded the product (0.346 g, 0.472 mmol, 80%) as a tan foam; mp. 47- 49 °C;  $[\alpha]^{22}_D$  - 51.8 (c 1.0,  $CHCl_3$ );  $^1H$  NMR (300 MHz,  $DMSO-d_6$ )  $\delta$  8.73 (d,  $J$  = 6.19 Hz, 1H), 8.32 (d,  $J$  = 6.69 Hz, 1H), 7.38-7.24 (m, 5H), 7.13 (d,  $J$  = 7.84 Hz, 1H), 6.95 (d,  $J$  = 7.85 Hz, 1H), 5.05 (dd,  $J$  = 10.82, 5.43 Hz, 1H), 4.64-4.46 (m, 3H), 4.37 (s, 1H), 4.30 (dd,  $J$  = 8.19, 5.52 Hz, 1H), 4.24 (s, 1H), 4.21-4.05 (m, 2H), 3.85-3.39 (m, 5H), 2.48-2.41 (m, 1H), 1.98 (d,  $J$  = 9.87 Hz, 1H), 1.90-1.68 (m, 2H), 1.59-1.42 (m, 5H), 1.43-1.09 (m, 34H), 0.84 (d,  $J$  = 6.59 Hz, 1H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  173.73, 171.77, 170.54, 169.63, 155.62, 137.37, 128.32, 127.66, 127.55, 79.94, 73.20, 73.02, 71.88, 69.28, 68.99, 52.33, 48.30, 45.68, 39.33, 38.92, 33.99, 32.02, 29.82, 29.58, 29.54, 29.47, 29.38, 29.28, 28.20, 27.84, 27.30, 25.03, 22.56, 16.61; IR (film)  $\nu_{max}$  3345, 3068, 2926, 2853, 1746, 1735, 1690, 1639, 1537, 1455, 1368, 1175, 1098, 1058, 1027, 991, 742, 701; ESI MS  $m/z$  756.37  $[M+Na]^+$

This pentapeptide (0.344 g, 0.311 mmol), the tetrapeptide carboxylic acid (0.217 g, 0.296 mmol), HOBt (0.060 g, 0.444 mmol) and DIEA (0.077 mL, 0.444 mmol) were dissolved in THF (1.18 mL). The solution was cooled to 0 °C and EDCI (0.085 g, 0.444 mmol) was added. The reaction mixture was stirred under argon for 12 hrs and then quenched with EtOAc (1 mL). The THF was removed in vacuo and to the residue was added EtOAc (25 mL). This solution was extracted with 1N HCl (1 x 20 mL), sat. aq. NaHCO<sub>3</sub> (1 x 20 mL), and brine (1 x 20mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Silica gel chromatography on a Biotage SP1 Flash system (10-100% Acetone/CHCl<sub>3</sub>) afforded the product (0.426 g, 0.238 mmol, 89%) as a white solid. mp. 76-78 °C;  $[\alpha]_D^{22}$  - 10.4 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 11.63 (d, *J* = 9.39 Hz, 1H), 8.70-8.65 (m, 1H), 8.49 (d, *J* = 9.48 Hz, 1H), 8.42-8.36 (m, 2H), 8.33 (d, *J* = 9.15 Hz, 1H), 8.25 (t, *J* = 6.97, 6.97 Hz, 1H), 8.06 (d, *J* = 8.26 Hz, 1H), 8.04-7.98 (m, 2H), 7.43-7.22 (m, 20H), 6.94 (d, *J* = 7.87 Hz, 1H), 5.86-5.75 (m, 1H), 5.36-5.12 (m, 5H), 5.11-4.99 (m, 3H), 4.93 (ddd, *J* = 13.08, 9.38, 3.52 Hz, 1H), 4.79 (d, *J* = 4.79 Hz, 1H), 4.75-4.63 (m, 2H), 4.63-4.34 (m, 8H), 4.33-4.28 (m, 1H), 4.27-4.09 (m, 5H), 3.83-3.72 (m, 2H), 3.68 (m, 1H), 3.63-3.49 (m, 2H), 3.46 (d, *J* = 6.42 Hz, 2H), 3.31-3.28 (m, 1H), 2.45 (d, *J* = 7.05 Hz, 1H), 2.39 (m, 1H), 1.99 (m, 1H), 1.92-1.84 (m, 1H), 1.84-1.70 (m, 2H), 1.70-1.58 (m, 3H), 1.56-1.43 (m, 1H), 1.41-1.33 (m, 13H), 1.28-1.09 (m, 29H), 1.07-0.97 (m, 4H), 0.83 (dd, *J* = 6.61, 1.88 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 172.45, 171.96, 171.73, 171.26, 170.26, 169.76, 169.47, 169.35, 168.13, 167.91, 167.81, 163.42, 155.73, 153.41, 137.44, 137.31, 136.65, 136.61, 136.22, 136.07, 134.44, 134.41, 131.05, 128.43, 128.38, 128.24, 128.18, 128.12, 128.10, 128.04, 127.88, 127.78, 127.62, 127.56, 127.46, 127.40, 127.35, 118.92, 118.86, 79.63, 79.51, 77.19, 75.93, 75.80, 74.08,

73.24, 72.95, 72.59, 72.32, 69.58, 69.23, 69.01, 68.58, 67.93, 67.86, 66.82, 66.37, 66.20, 57.52, 54.07, 52.44, 52.03, 51.86, 47.94, 47.47, 47.34, 45.40, 45.11, 40.36, 38.79, 33.88, 32.37, 31.74, 31.25, 31.00, 30.16, 29.69, 29.46, 29.41, 29.35, 29.30, 29.19, 29.04, 28.03, 27.70, 27.16, 24.94, 23.31, 23.24, 22.45, 19.65, 17.12, 16.85, 16.22; IR (neat)  $\nu_{\max}$  3327, 2925, 2847, 1741, 1639, 1542, 1450, 1384, 1256, 1205, 1103, 1047, 752, 696; ESI MS  $m/z$  1808.55  $[M+Na]^+$

### Cyclic Peptide (III.13)

$PdCl_2(PPh_3)_2$  (0.0042 g, 0.0060 mmol) and  $PPh_3$  (0.0047 g, 0.018 mmol) were dissolved in dry  $CH_2Cl_2$  (1.0 mL) and stirred under an atmosphere of argon for 15 min. This solution was transferred via syringe to a solution of the allyl ester (0.426 g, 0.238 mmol) in dry  $CH_2Cl_2$  (0.60 mL) under argon.  $PhSiH_3$  (0.050 mL, 0.477 mmol) was added via syringe and the reaction was stirred at room temperature for 4 hours. The solvent was condensed and the crude residue was loaded directly onto a silica gel column. Silica gel chromatography on a Biotage SP1 Flash system (3-30% EtOH/ $CHCl_3$ ) afforded the product (0.358 g, 0.205 mmol, 86 %) as a tan foam; mp. 115-117 °C;  $[\alpha]^{22}_D$  - 22.8 (c 1.0,  $CHCl_3$ );  $^1H$  NMR (500 MHz,  $DMSO-d_6$ )  $\delta$  11.61 (s, 1H), 8.42 (t,  $J$  = 5.07, 5.07 Hz, 1H), 8.39-8.32 (m, 1H), 8.29-8.21 (m, 2H), 8.06-7.98 (m, 1H), 7.46-7.22 (m, 21H), 6.93 (d,  $J$  = 7.26 Hz, 1H), 5.42 (s, 1H), 5.23-5.14 (m, 2H), 5.10-4.98 (m, 3H), 4.63 (m, 1H), 4.60-4.52 (m, 3H), 4.52-4.36 (m, 5H), 4.36-4.16 (m, 6H), 4.12 (s, 1H), 3.75 (m, 2H), 3.61-3.55 (m, 1H), 3.54-3.47 (m, 1H), 3.47-3.40 (m, 1H), 3.30 (d,  $J$  = 2.39 Hz, 1H), 2.41-2.31 (m, 1H), 2.02-1.95 (m, 1H), 1.91-1.82 (m, 2H), 1.82-1.70 (m, 4H), 1.63 (s, 3H), 1.57-1.31 (m, 22H), 1.29-1.10 (m, 32H), 1.06-0.99 (m, 4H), 0.83 (dd,  $J$  = 6.56, 1.23 Hz, 6H);  $^{13}C$

NMR (100 MHz,  $CDCl_3$ )  $\delta$  174.63, 174.47, 173.41, 173.19, 172.32, 171.68, 170.94, 170.29, 169.80, 169.22, 163.40, 155.72, 155.23, 153.41, 137.37, 136.38, 134.30, 134.05, 128.54, 128.41, 128.18, 127.94, 127.92, 127.77, 127.49, 127.36, 79.53, 77.20, 72.99, 69.75, 68.43, 67.94, 66.87, 60.16, 57.49, 55.82, 51.80, 48.78, 48.06, 45.25, 40.44, 38.79, 33.99, 33.79, 33.09, 32.41, 31.66, 31.13, 29.70, 29.46, 29.41, 29.31, 29.21, 28.09, 27.71, 27.18, 25.56, 24.98, 23.28, 22.45, 21.11, 20.80, 20.06, 19.46, 17.05, 15.98, 15.06, 14.37, 13.95, 13.60, 13.47, 12.91; IR (film)  $\nu_{max}$  3337, 2937, 2860, 1745, 1654, 1537, 1460, 1384, 1323, 1261, 1144, 1103, 1047, 762, 696; ESI MS  $m/z$  1768.39 [M+Na]

The linear peptide (0.3073g, 0.183 mmol) was treated with a 4N HCl-Dioxane solution (2.0 mL) and stirred under an atmosphere of argon for 1 hr. The volatiles were then removed in vacuo and to the residue was added  $CH_2Cl_2$ /Hexanes (1:1, 10 mL). The solvent was condensed to remove any residual HCl and the crude residue was placed under high vacuum for several hours. The product was used without further purification. To this residue, in dry DMF (91 mL) at 0 °C, was added HOBt (0.123 g, 0.913 mmol), DIEA (0.064 mL, 0.365 mmol) and finally EDCI (0.175g, 0.913 mmol). The mixture was stirred under an atmosphere of argon at this temperature for 48 hours. The solvent was then removed and the crude material was diluted with EtOAc (30 mL). The organic phase was washed with 1N HCl (1 x 20 mL), sat. aq.  $NaHCO_3$  (1 x 20 mL) and brine (1 x 20 mL). The EtOAc was dried over  $Mg_2SO_4$ , filtered and condensed. Silica gel chromatography (1-15% EtOH- $CHCl_3$ ) afforded the cyclic peptide (g, mmol, %) as a white solid. Silica gel chromatography on a Biotage SP1 Flash system (2-20% EtOH/ $CHCl_3$ ) afforded the product (0.150 g, 0.092 mmol, 50%) as a white foam; mp. 105-107 °C;  $[\alpha]^{22}_D$  -14.4 (c 1.0,  $CHCl_3$ );  $^1H$  NMR (500 MHz,  $CDCl_3$ ),  $\delta$  11.61 (s, 1H),

8.66 (d,  $J = 9.53$  Hz, 1H), 8.55 (s, 1H), 8.44-8.27 (m, 2H), 8.16 (d,  $J = 8.18$  Hz, 1H), 8.07 (d,  $J = 9.28$  Hz, 1H), 8.00-7.90 (m, 2H), 7.82-7.71 (m, 2H), 7.54 (d,  $J = 8.06$  Hz, 1H), 7.45-7.21 (m, 20H), 5.34 (d,  $J = 3.36$  Hz, 1H), 5.29 (d,  $J = 3.67$  Hz, 1H), 5.25-5.21 (m, 2H), 5.20-5.18 (m, 1H), 5.06-4.95 (m, 3H), 4.88-4.72 (m, 1H), 4.70-4.44 (m, 6H), 4.44-4.37 (m, 2H), 4.36-4.30 (m, 1H), 4.27 (s, 1H), 4.23-4.15 (m, 4H), 4.12-4.05 (m, 1H), 3.97-3.83 (m, 2H), 3.82-3.60 (m, 3H), 3.58-3.42 (m, 3H), 3.26 (dd,  $J = 13.07, 6.44$  Hz, 1H), 2.66-2.56 (m, 1H), 2.46-2.33 (m, 1H), 2.30 (d,  $J = 11.61$  Hz, 1H), 2.03 (d,  $J = 8.58$  Hz, 1H), 1.98-1.73 (m, 3H), 1.73-1.42 (m, 12H), 1.42-1.32 (m, 4H), 1.33-1.07 (m, 36H), 1.06-0.97 (m, 4H), 0.84 (dd,  $J = 6.43, 4.81$  Hz, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  172.29, 172.14, 171.60, 171.14, 170.58, 170.34, 169.89, 169.62, 168.97, 168.75, 163.42, 155.77, 153.57, 137.66, 137.00, 136.51, 134.38, 128.58, 128.49, 128.23, 128.04, 127.91, 127.75, 127.68, 127.49, 76.25, 75.92, 74.36, 73.89, 73.14, 72.91, 72.78, 72.46, 69.31, 68.97, 68.48, 68.00, 67.27, 66.93, 59.25, 57.27, 53.58, 51.96, 51.53, 51.33, 48.74, 47.71, 47.34, 45.13, 41.31, 40.61, 40.34, 40.03, 38.87, 34.15, 33.06, 32.48, 32.15, 31.24, 31.09, 30.53, 29.77, 29.50, 29.24, 27.78, 27.24, 25.51, 25.07, 24.95, 23.27, 22.51, 19.57, 19.30, 17.50, 16.76, 16.58, 15.80, 13.98; IR (film)  $\nu_{\text{max}}$  3319, 2933, 2864, 1740, 1647, 1542, 11452, 1383, 1324, 1266, 1212, 1138, 11099, 1056, 7611, 691; ESI MS  $m/z$  1650.38  $[\text{M}+\text{Na}]^+$

Analytical data for macrocyclization epimer (**III.14**): mp. 103-105 °C;  $[\alpha]_{\text{D}}^{22}$  -11.9 (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ),  $\delta$  11.58 (s, 1H), 8.73-8.08 (m, 5H), 8.05-7.72 (m, 3H), 7.45-7.19 (m, 20H), 5.42-5.29 (m, 1H), 5.26-5.13 (m, 3H), 5.06-4.97 (m, 3H), 4.92-4.82 (m, 1H), 4.82-4.72 (m, 1H), 4.69-4.57 (m, 2H), 4.54-4.38 (m, 4H), 4.35-4.29 (m, 1H), 4.29-4.01 (m, 9H), 3.94-3.45 (m, 6H), 2.36-2.23 (m, 1H), 1.98-1.37 (m, 18H), 1.36-

0.94 (m, 43H), 0.91 (d,  $J = 6.03$  Hz, 1H), 0.86-0.81 (m, 6H); IR (film)  $\nu_{max}$  3319, 2933, 2864, 1740, 1647, 1542, 11452, 1383, 1324, 1266, 1212, 1138, 11099, 1056, 7611, 691; ESI MS  $m/z$  1628.60  $[M+H]^+$

### Alanine Analog (III.15)

The cyclic peptide (0.0706 g, 0.0430 mmol) and anisole (1ml) in an HF reaction apparatus were purged with  $N_2$  and cooled to  $-78$  °C. HF gas was distilled into the reaction vessel to a total volume of approximately 5 mL. The reaction was warmed to  $0$  °C and stirred at this temperature for 1hr. The HF was then evaporated under a steady stream of  $N_2$  and the crude material was transferred to a round bottom flask using EtOH. The volatiles were removed in vacuo and the crude material was triturated with  $Et_2O$  and centrifuged (3x). The crude material was HPLC purified (30-65%  $CH_3CN/H_2O$  0.1% TFA over 60 minutes) to afford (0.0210 g, 0.0200 mmol, 50%) of the plusbacin A3 alanine analog as a white powder. mp.  $> 200$  °C decomposition;  $[\alpha]_D^{22} + 3.5$  (c 0.37, EtOH);  $^1H$  NMR (500 MHz,  $CD_3CN/D_2O/TFA$ , 500:500:1)  $\delta$  5.18-4.98 (m, 1H), 4.96 (td,  $J = 8.76, 4.59, 4.59$  Hz, 1H), 4.92-4.90 (m, 1H), 4.82-4.64 (m, 3H), 4.50 (td,  $J = 13.07, 6.40, 6.40$  Hz, 1H), 4.43 (s, 1H), 4.39-4.31 (m, 2H), 4.15-4.08 (m, 2H), 4.05-4.00 (m, 1H), 3.92-3.83 (m, 2H), 3.78-3.71 (m, 1H), 3.71-3.63 (m, 1H), 3.64-3.49 (m, 3H), 3.09 (dd,  $J = 6.81, 4.19$  Hz, 1H), 3.05 (t,  $J = 7.19, 7.19$  Hz, 1H), 2.60-2.46 (m, 3H), 2.33 (dd,  $J = 14.17, 4.33$  Hz, 1H), 2.14-2.00 (m, 1H), 1.69 (s, 1H), 1.58-1.39 (m, 5H), 1.35-1.30 (m, 2H), 1.31-1.14 (m, 30H), 1.13-1.04 (m, 6H), 0.80 (d,  $J = 6.62$  Hz, 1H); IR (KBr pellet)  $\nu_{max}$  3406, 2925, 2855, 1735, 1664, 1537, 1205, 1144, 1098, 996, 843, 803, 721; HR ESI TOF  $m/z$  1098.6051  $[M+H]^+$



### Alanine Isomer Analog (III.16)

The cyclic peptide (0.055 g, 0.034 mmol) and anisole (1ml) in an HF reaction apparatus were purged with N<sub>2</sub> and cooled to -78 °C. HF gas was distilled into the reaction vessel to a total volume of approximately 5 mL. The reaction was warmed to 0 °C and stirred at this temperature for 1hr. The HF was then evaporated under a steady stream of N<sub>2</sub> and the crude material was transferred to a round bottom flask using EtOH. The volatiles were removed in vacuo and the crude material was triturated with Et<sub>2</sub>O and centrifuged (3x). The crude material was HPLC purified (30-65% CH<sub>3</sub>CN/H<sub>2</sub>O 0.1% TFA over 60 minutes) to afford (0.00747 g, 0.0068 mmol, 20%) of the plusbacin A3 amine analog as a white powder. mp >200 °C decomposition;  $[\alpha]^{22}_{\text{D}} - 5.0$  (c 0.38, EtOH); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>CN/D<sub>2</sub>O/TFA, 500:500:1)  $\delta$  5.14-5.01 (m, 1H), 4.93-4.82 (m, 2H), 4.67-4.60 (m, 1H), 4.46 (d,  $J = 5.12$  Hz, 1H), 4.37-4.33 (m, 3H), 4.14 (s, 1H), 4.12-4.05 (m, 1H), 4.00 (m, 1H), 3.91-3.77 (m, 1H), 3.75-3.62 (m, 2H), 3.06 (t,  $J = 6.72, 6.72$  Hz, 1H), 2.49-2.35 (m, 2H), 1.99-1.96 (m, 1H), 1.76-1.65 (m, 1H), 1.61-1.41 (m, 2H), 1.35-1.28 (m, 2H), 1.27-1.15 (m, 28H), 0.80 (d,  $J = 6.63$  Hz, 1H); IR (KBr pellet)  $\nu_{\text{max}}$  3405, 2925, 2855, 1735, 1662, 1535, 1205, 1145, 1098, 996, 843, 803, 721; HR ESI TOF  $m/z$  1098.6059 [M+H]<sup>+</sup>

## III.11.5 D-ALLO-THREONINE MODIFICATIONS

### III.11.5a R-configuration

#### Septapeptide (III.19)

Boc-*α*Thr-D-Ala-βOH-Pro-Orn(Fmoc)-βOBn-D-*α*Asp(OCy)-OAllyl (0.6765 g, 0.623 mmol) was treated with a solution of 4N HCl-Dioxane (4.0 mL) and stirred for 90 minutes. The volatiles were removed in vacuo. The residual HCl was further removed by adding Et<sub>2</sub>O (5 mL) to the hydrochloride salt followed by its removal in vacuo. This residue, the carboxylic acid (0.380g, 0.566 mmol), NaHCO<sub>3</sub> (0.100 g, 1.132 mmol) and DEPBT (0.508 g, 1.699 mmol) were dissolved in DMF (1.7 mL) and cooled to 0 °C. The mixture was stirred under argon for 18 hrs while slowly warming to room temperature. The reaction was quenched with EtOAc (5 mL) and the DMF removed in vacuo. This residue was dissolved in EtOAc (20 mL) and washed with 1N HCl (1 x 20 mL), sat. aq. NaHCO<sub>3</sub> (1 x 20 mL), and brine (1 x 20mL). The organic phase was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Column chromatography (EtOAc) afforded the septapeptide (0.695 g, 0.424 mmol, 75%) as a white solid. mp 71-72 °C;  $[\alpha]_D^{22} - 1.03$  (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.58-8.26 (m, 2H), 8.13-7.83 (m, 6H), 7.72-7.59 (m, 3H), 7.40 (t, *J* = 7.35, 7.35 Hz, 2H), 7.36-7.24 (m, 14H), 6.91-6.54 (m, 2H), 5.89-5.67 (m, 1H), 5.45-4.88 (m, 9H), 4.83-4.35 (m, 16H), 4.33-4.11 (m, 7H), 3.74 (d, *J* = 7.12 Hz, 2H), 3.57 (s, 1H), 3.45 (s, 1H), 2.94 (s, 2H), 2.45-2.36 (m, 2H), 2.35-2.04 (m, 1H), 1.98-1.54 (m, 15H), 1.53-0.94 (m, 48H), 0.82 (d, *J* = 6.56 Hz, 6H); <sup>13</sup>C NMR (75MHz, CDCl<sub>3</sub>) δ 172.21, 171.91, 171.49, 171.34, 171.00, 170.62, 169.05, 168.86, 168.58, 156.92, 156.03, 155.70, 144.45, 144.22, 141.47, 141.32, 137.18, 137.04, 136.67, 135.42, 135.22, 131.58, 131.52, 128.62, 128.57, 128.42, 127.77, 127.21, 125.36, 123.54, 120.03, 119.23, 79.94, 74.90, 74.80, 74.36, 73.36, 73.06, 72.87, 72.81, 69.56, 69.02, 68.58, 66.56, 64.51, 60.59, 56.42, 54.39, 54.20, 53.44, 47.59, 47.43, 46.85, 46.69, 45.600 45.30, 44.86, 41.00, 40.82, 40.56, 39.21, 37.30, 36.65, 32.84, 31.60, 31.39, 30.82,

30.08, 29.72, 29.62, 29.52, 28.42, 28.13, 27.59, 25.40, 23.78, 22.84, 19.68, 19.29, 14.38, 13.88, IR (Neat)  $\nu_{max}$  3300, 3069, 2932, 2857, 2361, 1749, 1731, 1713, 1655, 1634, 1540, 1522, 1507, 1456, 1367, 1339, 1257, 1206, 1160, 1104; ESI MS  $m/z$  1688.55  $[M+Na]^+$

### Linear Peptide (III.20)

The septapeptide (0.210 g, 0.128 mmol) was treated with a solution of 10% TFA/CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and the resulting mixture was stirred at room temperature for 40 min. The solution was diluted with EtOAc (20 mL) and washed with sat. aq. NaHCO<sub>3</sub> (2 x 10 mL). The organic phase was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. This residue, Boc-D-Ser(OBn)- $\beta$ OH-Pro-OH (0.036 g, 0.088 mmol), NaHCO<sub>3</sub> (0.015 g, 0.136 mmol) and DIEA (0.005 mL, 0.018 mmol) were dissolved in DMF (0.300 mL). The solution was cooled to 0 °C and DEPBT (0.053 g, 0.177 mmol) was added. The reaction mixture was stirred under argon for 18 hrs and then quenched with EtOAc (1 mL). The DMF was removed in vacuo and to the residue was added EtOAc (20 mL). This solution was extracted with 1N HCl (1 x 20 mL), sat. aq. NaHCO<sub>3</sub> (1 x 20 mL), and brine (1 x 20 mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Chromatography (20-80% Acetone-EtOAc) provided the linear peptide (0.153 g, 0.079 mmol, 90%) as a white solid. mp 85-89 °C;  $[\alpha]_D^{22} + 1.1$  (c 1.0, CHCl<sub>3</sub>) <sup>1</sup>H NMR (500 MHz DMSO-*d*<sub>6</sub>)  $\delta$  8.72 (d,  $J$  = 7.02 Hz, 1H), 8.48-8.28 (m, 2H), 8.20 (d,  $J$  = 5.23 Hz, 1H), 8.12-7.92 (m, 3H), 7.88 (d,  $J$  = 7.50 Hz, 2H), 7.69-7.63 (m, 1H), 7.40 (t,  $J$  = 7.48, 7.48 Hz, 2H), 7.36-7.22 (m, 18H), 6.91-6.72 (m, 1H), 5.88-5.67 (m, 1H), 5.41-4.82 (m, 5H), 4.80-4.36 (m, 14H), 4.34-4.06 (m, 6H), 3.80-3.70 (m, 1H), 3.68-3.42 (m, 3H), 3.31 (s, 1H), 2.94 (s, 1H), 2.17 (s, 1H), 2.02-1.54 (m, 14H), 1.53-0.97 (m, 47H), 0.82 (dd,  $J$  = 6.54, 3.70 Hz,

6H)  $^{13}\text{C}$  NMR (100MHz,  $\text{CDCl}_3$ )  $\delta$  172.12, 172.01, 171.71, 171.46, 171.32, 170.59, 169.76, 169.49, 169.09, 168.43, 168.26, 167.86, 156.80, 154.95, 143.96, 141.19, 137.94, 137.38, 136.53, 136.32, 135.16, 134.81, 131.19, 129.12, 128.40, 128.33, 128.20, 127.78, 127.53, 126.96, 125.06, 123.45, 119.82, 119.15, 79.81, 74.82, 74.71, 74.64, 74.37, 73.96, 73.72, 73.45, 73.24, 73.07, 72.87, 70.48, 70.06, 68.99, 68.82, 66.55, 65.16, 58.57, 58.17, 54.27, 53.96, 52.60, 52.49, 52.19, 51.73, 47.13, 45.61, 45.40, 45.23, 45.11, 44.91, 40.16, 38.97, 37.10, 32.54, 32.18, 31.43, 31.12, 29.82, 29.49, 29.26, 28.31, 27.88, 27.34, 25.92, 25.10, 23.52, 22.59, 20.18, 19.95, 15.92; IR (neat)  $\nu_{\text{max}}$  3314, 3089, 3066, 3034, 2932, 2862, 1740, 1691, 1642, 1529, 1450, 1260, 1104; ESI MS  $m/z$  1951.49  $[\text{M}+\text{Na}]^+$

### Cyclic peptide (III.21)

$\text{PdCl}_2(\text{PPh}_3)_2$  (2.0 mg, 0.00285 mmol) and  $\text{PPh}_3$  (2.24 mg, 0.00855 mmol) were dissolved in dry  $\text{CH}_2\text{Cl}_2$  (0.5 mL) and stirred under an atmosphere of argon for 15 min. This solution was then added to the allyl ester (0.220 g, 0.114 mmol) via syringe and phenylsilane (0.022 mL, 0.176 mmol) was added drop wise. The solution was stirred at room temperature under an atmosphere of argon for 6 hrs. The solvent was condensed and the crude material was loaded directly onto a silica gel column. Chromatography (1-15% EtOH- $\text{CHCl}_3$ ) afforded the carboxylic acid (0.184 g, 0.097 mmol, 85%) as a white solid. mp 107-110 °C;  $[\alpha]_{\text{D}}^{22}$  - 9.0 (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  ppm 7.88 (d,  $J$  = 7.76 Hz, 1H), 7.82-7.75 (m, 1H), 7.68 (d,  $J$  = 6.85 Hz, 2H), 7.47-7.21 (m, 19H), 5.49-5.00 (m, 1H), 4.86-4.01 (m, 20H), 3.93-3.71 (m, 1H), 2.96 (s, 1H), 2.29-2.11 (m, 1H), 2.04-1.58 (m, 12H), 1.56-0.99 (m, 45H), 0.82 (d,  $J$  = 6.57 Hz, 6H)  $^{13}\text{C}$  NMR(100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.23, 174.52, 172.86, 171.65, 170.68, 170.47, 170.14,

169.67, 168.58, 168.24, 167.74, 156.50, 154.84, 143.94, 141.13, 137.91, 137.08, 136.65, 135.46, 134.98, 133.61, 130.56, 128.24, 126.93, 126.04, 125.04, 123.40, 79.77, 73.04, 70.27, 69.22, 69.10, 67.10, 66.56, 55.61, 54.32, 52.20, 51.67, 48.13, 47.13, 45.33, 40.42, 38.92, 37.16, 32.49, 31.36, 31.12, 29.78, 29.43, 29.32, 29.20, 28.26, 27.83, 27.29, 25.11, 23.50, 22.54, 20.12, 16.24, 14.65, 14.05; IR (neat)  $\nu_{max}$  3308, 3065, 2929, 2857, 1706, 1642, 1527, 1451, 1255, 1200, 1158; ESI MS  $m/z$  1911.51  $[M+Na]^+$

The linear peptide (0.074 g, 0.039 mmol) was treated with a 10% TFA/ $CH_2Cl_2$  solution (2.0 mL) and stirred under an atmosphere of argon for 1 hr. The volatiles were then removed in vacuo and to the residue was added  $CH_2Cl_2$ /Hexanes (1:1, 10 mL). The solvent was condensed to remove any residual HCl and the crude residue was placed under high vacuum for several hours. The product was used without further purification. To this residue in dry DMF (15 mL) at 0 °C was added HOAt (0.027 g, 0.196 mmol), DIEA (0.034 mL, 0.196 mmol), and finally EDCI (0.038 g, 0.196 mmol). The mixture was stirred under an atmosphere of argon at this temperature for 48 hours. The solvent was then removed and the crude material was diluted with EtOAc (30 mL). The organic phase was washed with 1N HCl (1 x 20 mL), sat. aq.  $NaHCO_3$  (1 x 20 mL) and brine (1 x 20 mL). The EtOAc was dried over  $Mg_2SO_4$ , filtered and condensed. Silica gel chromatography (1-10% EtOH- $CHCl_3$ ) afforded the cyclic peptide (0.035 g, 0.020 mmol, 51%) as a white solid. mp 91-94 °C;  $[\alpha]_D^{22}$  -14.4 (c 1.0,  $CHCl_3$ );  $^1H$  NMR (400 MHz,  $DMSO-d_6$ )  $\delta$  ; 8.64 (s, 1H), 8.56 (d,  $J$  = 8.08 Hz, 1H), 7.96 (dd,  $J$  = 18.82, 9.20 Hz, 1H), 7.88 (d,  $J$  = 7.42 Hz, 2H), 7.67 (t,  $J$  = 6.46, 6.46 Hz, 1H), 7.40 (t,  $J$  = 7.27, 7.27 Hz, 1H), 7.36-7.20 (m, 18H), 5.41-5.22 (m, 1H), 5.22-5.06 (m, 1H), 5.03-4.83 (m, 2H), 4.74-4.07

(m, 13H), 4.00 (s, 1H), 3.94 (s, 1H), 3.73-3.47 (m, 1H), 2.95 (d,  $J = 4.62$  Hz, 1H), 2.12 (s, 1H), 2.03-1.55 (m, 15H), 1.55-0.93 (m, 43H), 0.83 (d,  $J = 6.61$  Hz, 6H);  $^{13}\text{C}$  (100MHz,  $\text{CDCl}_3$ )  $\delta$  173.28, 172.48, 171.78, 171.62, 171.18, 170.53, 169.97, 169.59, 168.73, 168.32, 167.83, 156.71, 143.96, 141.25, 138.03, 137.68, 136.77, 136.57, 135.00, 128.32, 127.66, 127.07, 125.12, 124.12, 123.67, 119.92, 74.51, 73.42, 69.26, 68.23, 67.33, 66.68, 54.47, 54.03, 50.79, 48.54, 47.25, 45.98, 45.13, 40.42, 39.03, 37.18, 32.69, 31.46, 29.65, 28.38, 27.96, 27.40, 25.15, 23.62, 22.65, 19.63, 14.10; IR (neat)  $\nu_{\text{max}}$  3303, 3066, 3035, 2926, 2855, 1741, 1650, 1546, 1528, 1467, 1450, 1260, 1202; ESI MS  $m/z$  1793.49  $[\text{M}+\text{Na}]^+$ .

### Threonine Analog (III.27)

Cyclic peptide (0.027 g, 0.015 mmol) was treated with a 5% piperidine-DMF solution (1.5 mL) and stirred at room temperature for 15 minutes. The reaction was diluted with DMF 5ml and the solvent was removed in vacuo. To the residue was added DMF (5 mL) and the solvent was again removed in vacuo. The crude material was then left under high vacuum for several hours (4-8). To a stirring solution of the deprotected amine in dry DMF (0.40 mL) was added Boc-triflylguanidine (0.058 g, 0.148 mmol) DIEA (0.0052 mL, 0.030 mmol). The reaction was stirred at room temperature for 18 hrs then the DMF was removed in vacuo. Column chromatography (1-20% EtOH -  $\text{CHCl}_3$ ) afforded the guanidinylated peptide (0.017 g, 0.0095 mmol, 64 %) as a pale yellow solid. This material was then dissolved in TFE (1 mL) and treated with 10% Pd-C. The flask was purged with  $\text{H}_2$  and stirred at room temperature for 12 hours. The mixture was filtered through a pad of celite and the solvent was removed. The peptide and anisole (1 mL) in

an HF reaction apparatus were purged with N<sub>2</sub> and cooled to -78 °C. HF gas was distilled into the reaction vessel to a total volume of approximately 5 mL. The reaction was warmed to 0 °C and stirred at this temperature for 3hrs. The HF was then evaporated under a steady stream of N<sub>2</sub> and the crude material was transferred to a round bottom flask using EtOH. The volatiles were removed in vacuo and the crude material was triturated with Et<sub>2</sub>O and centrifuged (3 x). The crude material was HPLC purified (35-65% CH<sub>3</sub>CN/H<sub>2</sub>O over 60 minutes) to afford (0.0033 g, 0.00285 mmol, 30%) of plusbacin analog as a white solid. mp >250 °C decomposition;  $[\alpha]_D^{22} + 4.4$  (c 0.090, EtOH); <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 8.78-7.34 (m, 5H), 7.33-6.36 (m, 5H), 5.23 (s, 1H), 5.03 (d, *J* = 40.95 Hz, 1H), 4.83 (s, 1H), 4.77 (d, *J* = 9.58 Hz, 1H), 4.70 (d, *J* = 10.48 Hz, 1H), 4.64 (s, 1H), 4.58-4.46 (m, 2H), 4.43-4.10 (m, 5H), 3.97-3.72 (m, 1H), 3.73-3.58 (m, 2H), 3.11-3.01 (m, 3H), 2.40-2.26 (m, 1H), 1.95-1.70 (m, 3H), 1.55-1.41 (m, 2H), 1.32-1.15 (m, 20H), 1.15-1.08 (m, 6H), 1.06-0.97 (m, 3H), 0.83 (d, *J* = 6.32 Hz, 6H); IR (KBr pellet)  $\nu_{max}$  3432, 2959, 2926, 2860, 1735, 1674, 1535, 1441, 1204, 1186, 1127; HR ESI TOF *m/z* 1158.5874 [M+H]<sup>+</sup>

### III.11.5b S-configuration

#### Septapeptide (III.23)

Boc-*α*Thr-D-Ala-βOH-Pro-Orn(Fmoc)-βOBn-D-*α*Asp(OCy)-OAllyl (0.5563 g, 0.514 mmol) was treated with a solution of 4N HCl-Dioxane (4.0 mL, 15.89 mmol) and stirred for 90 minutes. The volatiles were removed in vacuo. The residual HCl was further removed by adding Et<sub>2</sub>O (5 mL) to the hydrochloride salt followed by its removal in

vacuo. The residue was then dissolved in EtOAc (30 mL) and washed with sat. aq.  $\text{NaHCO}_3$  (2 x 20 mL). The EtOAc was dried over  $\text{Mg}_2\text{SO}_4$ , filtered and condensed. This residue, the carboxylic acid (0.343 g, 0.509 mmol),  $\text{NaHCO}_3$  (0.085 g, 1.017 mmol) and DEPBT (0.304 g, 1.017 mmol) were dissolved in DMF (1.5 mL) and cooled to 0 °C. The mixture was stirred under argon for 18 hrs while slowly warming to room temperature. The reaction was quenched with EtOAc (5 mL) and the DMF removed in vacuo. This residue was dissolved in EtOAc (20 mL) and washed with 1N HCl (1 x 20 mL), sat. aq.  $\text{NaHCO}_3$  (1 x 20 mL), and brine (1 x 20 mL). The organic phase was dried over  $\text{Mg}_2\text{SO}_4$ , filtered and condensed. Column chromatography (EtOAc) afforded the septapeptide (0.742 g, 0.453 mmol, 89%) as a white solid. mp 71-72 °C;  $[\alpha]_D^{22} - 1.4$  (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.45 (d,  $J = 8.92$  Hz, 1H), 8.42-8.27 (m, 2H), 8.13 (d,  $J = 6.40$  Hz, 1H), 8.09-7.92 (m, 2H), 7.88 (d,  $J = 7.53$  Hz, 2H), 7.72-7.63 (m, 2H), 7.40 (t,  $J = 7.45, 7.45$  Hz, 2H), 7.36-7.24 (m, 14H), 6.80 (dd,  $J = 17.84, 13.81$  Hz, 1H), 6.70 (d,  $J = 9.50$  Hz, 1H), 6.62 (d,  $J = 5.40$  Hz, 1H), 5.87-5.71 (m, 1H), 5.53-4.90 (m, 2H), 4.89-4.62 (m, 4H), 4.61-4.35 (m, 7H), 4.31-4.21 (m, 4H), 4.20-4.10 (m, 6H), 3.82-3.67 (m, 1H), 3.62-3.43 (m, 2H), 2.94 (s, 1H), 2.36-2.06 (m, 2H), 1.89 (s, 1H), 1.86-1.72 (m, 4H), 1.71-1.56 (m, 6H), 1.51-0.98 (m, 55H), 0.82 (dd,  $J = 6.51, 2.51$  Hz, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) ppm 171.764, 171.471, 171.106, 170.271, 169.685, 168.790, 168.678, 168.523, 168.398, 168.238, 162.466, 156.627, 155.330, 144.148, 143.912, 143.807, 141.089, 136.809, 136.564, 136.287, 135.150, 134.974, 131.136, 128.258, 128.160, 128.098, 127.980, 127.979, 127.411, 127.405, 126.856, 125.020, 123.491, 123.170, 119.731, 119.666, 118.927, 118.826, 79.824, 79.546, 74.581, 74.021, 73.114, 72.991, 72.785, 72.648, 72.538, 68.865, 68.585, 68.364, 66.380, 66.261, 56.120,



56.047, 54.146, 52.856, 52.450, 47.188, 46.687, 44.899, 40.601, 40.188, 38.862, 36.321, 32.495, 31.288, 31.031, 29.731, 29.377, 29.241, 28.052, 27.791, 27.252, 25.086, 23.443, 22.514, 19.525, 17.806, 17.095, IR (Neat)  $\nu_{max}$  3300, 3069, 2932, 2857, 2361, 1749, 1731, 1713, 1655, 1634, 1540, 1522, 1507, 1456, 1367, 1339, 1257, 1206, 1160, 1104; ESI MS  $m/z$  1688.56  $[M+Na]^+$

### Linear Peptide (III.24)

The septapeptide (0.150 g, 0.092 mmol) was treated with a solution of 10% TFA/ $CH_2Cl_2$  (5 mL) and the resulting mixture was stirred at room temperature for 4 hours. The solution was diluted with EtOAc (20 mL) and washed with sat. aq.  $NaHCO_3$  (2 x 10 mL). The organic phase was dried over  $Mg_2SO_4$ , filtered and condensed. The material was purified via column chromatography (1-20% EtOH- $CHCl_3$ ) to afford the amine (0.1104 g, 78%). This residue, Boc-D-Ser(OBn)- $\beta$ OH-Pro-OH (0.028 g, 0.0689 mmol),  $NaHCO_3$  (0.011 g, 0.136 mmol) and DEPBT (0.041 mL, 0.136 mmol) were dissolved in DMF (0.250 mL). The solution was cooled to 0 °C and the reaction mixture was stirred under argon for 18 hrs and then quenched with EtOAc (1 mL). The DMF was removed in vacuo and to the residue was added EtOAc (20 mL). This solution was extracted with 1N HCl (1 x 20 mL), sat. aq.  $NaHCO_3$  (1 x 20 mL), and brine (1 x 20mL). The EtOAc was dried over  $Mg_2SO_4$ , filtered and condensed. Chromatography ( 2-40% Acetone-EtOAc) provided the linear peptide (0.113 g, 0.059 mmol, 86%) as a white solid. mp 74-76 °C;  $[\alpha]_D^{22} + 1.0$  (c 1.0,  $CHCl_3$ )  $^1H$  NMR (500 MHz  $DMSO-d_6$ )  $\delta$  8.72 (d,  $J$  = 7.02 Hz, 1H), 8.48-8.28 (m, 2H), 8.20 (d,  $J$  = 5.23 Hz, 1H), 8.12-7.92 (m, 3H), 7.88 (d,  $J$  = 7.50 Hz, 2H), 7.69-7.63 (m, 1H), 7.40 (t,  $J$  = 7.48, 7.48 Hz, 2H), 7.36-7.22 (m, 18H), 6.91-6.72

(m, 1H), 5.88-5.67 (m, 1H), 5.41-4.82 (m, 5H), 4.80-4.36 (m, 14H), 4.34-4.06 (m, 6H), 3.80-3.70 (m, 1H), 3.68-3.42 (m, 3H), 3.31 (s, 1H), 2.94 (s, 1H), 2.17 (s, 1H), 2.02-1.54 (m, 14H), 1.53-0.97 (m, 47H), 0.82 (dd,  $J = 6.54, 3.70$  Hz, 6H);  $^{13}\text{C}$  NMR (100MHz,  $\text{CDCl}_3$ )  $\delta$  172.12, 172.01, 171.71, 171.46, 171.32, 170.59, 169.76, 169.49, 169.09, 168.43, 168.26, 167.86, 156.80, 154.95, 143.96, 141.19, 137.94, 137.38, 136.53, 136.32, 135.16, 134.81, 131.19, 129.12, 128.40, 128.33, 128.20, 127.78, 127.53, 126.96, 125.06, 123.45, 119.82, 119.15, 79.81, 74.82, 74.71, 74.64, 74.37, 73.96, 73.72, 73.45, 73.24, 73.07, 72.87, 70.48, 70.06, 68.99, 68.82, 66.55, 65.16, 58.57, 58.17, 54.27, 53.96, 52.60, 52.49, 52.19, 51.73, 47.13, 45.61, 45.40, 45.23, 45.11, 44.91, 40.16, 38.97, 37.10, 32.54, 32.18, 31.43, 31.12, 29.82, 29.49, 29.26, 28.31, 27.88, 27.34, 25.92, 25.10, 23.52, 22.59, 20.18, 19.95, 15.92; IR (neat)  $\nu_{\text{max}}$  3314, 3089, 3066, 3034, 2932, 2862, 1740, 1691, 1642, 1529, 1450, 1260, 1104; ESI MS  $m/z$  1951.55  $[\text{M}+\text{Na}]^+$

### Cyclic peptide (III.28)

$\text{PdCl}_2(\text{PPh}_3)_2$  (1.5 mg, 0.0022 mmol) and  $\text{PPh}_3$  (1.73 mg, 0.00661 mmol) were dissolved in dry  $\text{CH}_2\text{Cl}_2$  (0.45 mL) and stirred under an atmosphere of argon for 15 min. This solution was then added to the allyl ester (0.170 g, 0.088 mmol) via syringe and phenylsilane (0.022 mL, 0.176 mmol) was added drop wise. The solution was stirred at room temperature under an atmosphere of argon for 6 hrs. The solvent was condensed and the crude material was loaded directly onto a silica gel column. Chromatography (1-15% EtOH- $\text{CHCl}_3$ ) afforded the carboxylic acid (0.143 g, 0.076 mmol, 86%) as a white solid. mp 107-110 °C;  $[\alpha]_{\text{D}}^{22} - 15.0$  (c 1.0,  $\text{CHCl}_3$ )  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.72 (d,  $J = 9.82$  Hz, 1H), 8.50 (d,  $J = 6.57$  Hz, 1H), 8.40 (d,  $J = 7.03$  Hz, 1H), 8.14-7.96

(m, 2H), 7.88 (d,  $J = 7.50$  Hz, 2H), 7.68 (d,  $J = 7.34$  Hz, 1H), 7.40 (t,  $J = 7.49, 7.49$  Hz, 2H), 7.36-7.22 (m, 18H), 6.90-6.73 (m, 1H), 5.27 (m, 1H), 5.15-4.88 (m, 1H), 4.78-4.49 (m, 5H), 4.35-4.06 (m, 6H), 3.76 (s, 1H), 3.70-3.44 (m, 2H), 2.94 (s, 1H), 2.15 (s, 1H), 1.90 (s, 1H), 1.78 (s, 7H), 1.63 (s, 4H), 1.54-0.97 (m, 45H), 0.84-0.79 (m, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.23, 174.52, 172.86, 171.65, 170.68, 170.47, 170.14, 169.67, 168.58, 168.24, 167.74, 156.50, 154.84, 143.94, 141.13, 137.91, 137.08, 136.65, 135.46, 134.98, 133.61, 130.56, 128.24, 126.93, 126.04, 125.04, 123.40, 79.77, 73.04, 70.27, 69.22, 69.10, 67.10, 66.56, 55.61, 54.32, 52.20, 51.67, 48.13, 47.13, 45.33, 40.42, 38.92, 37.16, 32.49, 31.36, 31.12, 29.78, 29.43, 29.32, 29.20, 28.26, 27.83, 27.29, 25.11, 23.50, 22.54, 20.12, 16.24, 14.65, 14.05; IR (neat)  $\nu_{\text{max}}$  3308, 3065, 2929, 2857, 1706, 1642, 1527, 1451, 1255, 1200, 1158; ESI MS  $m/z$  1911.52  $[\text{M}+\text{Na}]^+$

The linear peptide (0.043 g, 0.023 mmol) was treated with a 10% TFA/ $\text{CH}_2\text{Cl}_2$  solution (2.0 mL) and stirred under an atmosphere of argon for 1 hr. The volatiles were then removed in vacuo and to the residue was added  $\text{CH}_2\text{Cl}_2$ /Hexanes (1:1, 10 mL). The solvent was condensed to remove any residual HCl and the crude residue was placed under high vacuum for several hours. The product was used without further purification. To this residue in dry DMF (10.6 mL) at 0 °C was added HOBt (0.016 g, 0.117 mmol), DIEA (0.0081 mL, 0.047 mmol),  $\text{NaHCO}_3$  (0.0059 g, 0.070 mmol) and finally EDCI (0.022 g, 0.117 mmol). The mixture was stirred under an atmosphere of argon at this temperature for 48 hours. The solvent was then removed and the crude material was diluted with EtOAc (30 mL). The organic phase was washed with 1N HCl (1 x 20 mL), sat. aq.  $\text{NaHCO}_3$  (1 x 20 mL) and brine (1 x 20 mL). The EtOAc was dried over  $\text{Mg}_2\text{SO}_4$ , filtered and condensed. Silica gel chromatography (1-10% EtOH- $\text{CHCl}_3$ ) afforded the

cyclic peptide (0.024g, 0.014 mmol, 58%) as a white solid. mp 93-95 °C;  $[\alpha]_D^{22}$  - 14.4 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ; 8.64 (s, 1H), 8.56 (d, *J* = 8.08 Hz, 1H), 7.96 (dd, *J* = 18.82, 9.20 Hz, 1H), 7.88 (d, *J* = 7.42 Hz, 2H), 7.67 (t, *J* = 6.46, 6.46 Hz, 1H), 7.40 (t, *J* = 7.27, 7.27 Hz, 1H), 7.36-7.20 (m, 18H), 5.41-5.22 (m, 1H), 5.22-5.06 (m, 1H), 5.03-4.83 (m, 2H), 4.74-4.07 (m, 13H), 4.00 (s, 1H), 3.94 (s, 1H), 3.73-3.47 (m, 1H), 2.95 (d, *J* = 4.62 Hz, 1H), 2.12 (s, 1H), 2.03-1.55 (m, 15H), 1.55-0.93 (m, 43H), 0.83 (d, *J* = 6.61 Hz, 6H); <sup>13</sup>C (100MHz, CDCl<sub>3</sub>)  $\delta$  173.28, 172.48, 171.78, 171.62, 171.18, 170.53, 169.97, 169.59, 168.73, 168.32, 167.83, 156.71, 143.96, 141.25, 138.03, 137.68, 136.77, 136.57, 135.00, 128.32, 127.66, 127.07, 125.12, 124.12, 123.67, 119.92, 74.51, 73.42, 69.26, 68.23, 67.33, 66.68, 54.47, 54.03, 50.79, 48.54, 47.25, 45.98, 45.13, 40.42, 39.03, 37.18, 32.69, 31.46, 29.65, 28.38, 27.96, 27.40, 25.15, 23.62, 22.65, 19.63, 14.10;; IR (neat)  $\nu_{max}$  3303, 3066, 3035, 2926, 2855, 1741, 1650, 1546, 1528, 1467, 1450, 1260, 1202; ESI MS *m/z* 1793.32 [M+Na]<sup>+</sup>.

### Threonine Analog (III.29)

Cyclic peptide (0.017 g, 0.0096 mmol) was treated with a 5% piperidine-DMF solution (1.5 mL) and stirred at room temperature for 15 minutes. The reaction was diluted with DMF 5 mL and the solvent was removed in vacuo. To the residue was added DMF (5 mL) and the solvent was again removed in vacuo. The crude material was then left under high vacuum for several hours (4-8). To a stirring solution of the deprotected amine in dry DMF (0.75 mL) was added Boctriflylguanidine (0.037 g, 0.094 mmol) DIEA (0.0082 mL, 0.047 mmol. The reaction was stirred at room temperature for 18hrs then the DMF was removed in vacuo. Column chromatography (1-20% EtOH - CHCl<sub>3</sub>)

afforded the guanidinylated peptide (0.0114 g, 0.064 mmol, 68%) as a pale yellow solid. This material was then dissolved in TFE (1 mL) and treated with 10% Pd-C. The flask was purged with H<sub>2</sub> and stirred at room temperature for 12 hours. The mixture was filtered through a pad of celite and the solvent was removed. The guanidinylated peptide and anisole (1ml) in an HF reaction apparatus were purged with N<sub>2</sub> and cooled to -78 °C. HF gas was distilled into the reaction vessel to a total volume of approximately 5 mL. The reaction was warmed to 0 °C and stirred at this temperature for 3hrs. The HF was then evaporated under a steady stream of N<sub>2</sub> and the crude material was transferred to a round bottom flask using EtOH. The volatiles were removed in vacuo and the crude material was triturated with Et<sub>2</sub>O and centrifuged (3 x). The crude material was HPLC purified (25-75% CH<sub>3</sub>CN/H<sub>2</sub>O over 40 minutes) to afford (0.00162 g, 0.0014 mmol, 25%) of the plusbacin analog as a white solid. mp >250 °C decomposition;  $[\alpha]_D^{22} + 2.0$  (c 0.10, EtOH); <sup>1</sup>H NMR (500 MHz, *DMSO-d*<sub>6</sub>) δ 9.11-7.58 (m, 5H), 7.62-6.26 (m, 5H), 5.51-4.91 (m, 2H), 4.80 (s, 1H), 4.73-4.34 (m, 5H), 4.31-4.04 (m, 4H), 3.94 (s, 1H), 3.89-3.64 (m, 4H), 3.64-3.50 (m, 3H), 3.09 (s, 1H), 1.92-1.66 (m, 2H), 1.55-1.39 (m, 3H), 1.28-1.16 (m, 17H), 1.14 (s, 1H), 1.08-0.98 (m, 4H), 0.86-0.80 (m, 6H); IR (KBr pellet)  $\nu_{max}$  3432, 2959, 2926, 2860, 1735, 1674, 1535, 1441, 1204, 1186, 1127; HR ESI TOF *m/z* 1158.5869 [M+H]<sup>+</sup>

### III.11.6 LACTONE MODIFICATIONS

#### (*S,E*)-benzyl 3-(tert-butoxycarbonylamino)-14-methylpentadec-4-enoate (III.34)

To a stirring solution of the allylic amine (0.055 g, 0.180 mmol) and 11-methyl-1-dodecene (0.164 g, 0.901 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (0.9 mL) was added Grubb's 2<sup>nd</sup>

Generation catalyst (0.0077 g, 0.0091 mmol). The flask was fitted with condenser and stirred at 50 °C under a steady stream of argon for 24 hours. The reaction mixture was then reduced in volume and loaded directly onto a silica gel column. Chromatography (2-40% EtOAc-Hexanes) afforded the product (0.039 g, 0.180 mmol, 47%, 89% brsm) as a pale yellow oil. The unreacted lipid was recovered quantitatively and recycled.  $[\alpha]_D^{22} + 3.4$  (c 1.0,  $\text{CHCl}_3$ )  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.34-7.23 (m, 5H), 5.56 (m, 1H), 5.37 (dd,  $J = 15.43, 6.28$  Hz, 1H), 5.27-4.98 (m, 3H), 4.45 (s, 1H), 2.58 (s, 2H), 1.93 (d,  $J = 6.21$  Hz, 2H), 1.57-1.34 (m, 12H), 1.52-1.34 (m, 11H), 1.31-1.10 (m, 13H), 0.84 (dd,  $J = 6.59, 1.95$  Hz, 6H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  171.19, 155.21, 135.96, 132.70, 132.29, 128.906, 128.60, 128.50, 128.20, 79.41, 66.50, 49.31, 40.04, 39.25, 32.35, 30.13, 29.85, 29.71, 29.27, 28.79, 28.36, 27.94, 23.02, 22.74; IR (neat)  $\nu_{\text{max}}$  3436, 2955, 2922, 2853, 1715, 1496, 1460, 1394, 1363, 1282, 1165, 1052, 1017, 971, 869, 752, 696; ESI MS  $m/z$  459.93 $[\text{M}+\text{H}]^+$ , 482.20  $[\text{M}+\text{Na}]^+$

**(R)-allyl 3-(tert-butoxycarbonylamino)-14-methylpentadecanoate (III.36)**

The olefin (0.635 g, 1.381 mmol) was dissolved in MeOH (13.8 mL) and 10% Pd/C (0.040 g) in MeOH (13.8 mL). The flask was sealed and purged with hydrogen. The mixture was stirred for 12 hours under  $\text{H}_2$ , and upon completion filtered through a celite pad. The solvent was removed and to the crude residue was added a solution of KOH (0.388 g, 6.91 mmol) in MeOH/ $\text{H}_2\text{O}$  (2:1, 14.0 mL). The reaction was stirred for 2 hours. The MeOH was removed in vacuo and the reaction was diluted with EtOAc (20 mL) then washed with 1N HCl (10 mL). The aq. phase was extracted with EtOAc (3 x 10 mL) and the combined organic portions were dried over  $\text{Mg}_2\text{SO}_4$ , filtered and

condensed. Silica gel chromatography on a Biotage SP1 Flash system (2-20% EtOH/CHCl<sub>3</sub>) afforded the product (0.498 g, 1.340 mmol, 97%) as a colorless oil. To a solution of the carboxylic acid in allyl bromide (2.39 mL, 27.6 mmol) was added DIEA (0.481 mL, 2.76 mmol). The reaction was stirred for 2hrs and the solvent was removed. The residue was diluted with EtOAc (20 mL) and washed with 1 N HCl (1 x 10 mL), sat. aq. NaHCO<sub>3</sub> (1 x 10 mL), and Brine (1 x 10 mL). Silica gel chromatography on a Biotage SP1 Flash system (5-40% EtOAc/Hex) afforded the product (0.432 g, 1.05 mmol, 76%) as a colorless oil.  $[\alpha]_D^{22} + 12.2$  (c 2.41, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.89 (ddd,  $J = 22.80, 10.95, 5.76$  Hz, 1H), 5.25 (ddd,  $J = 13.81, 11.25, 1.10$  Hz, 2H), 4.90 (d,  $J = 8.62$  Hz, 1H), 4.56 (d,  $J = 5.74$  Hz, 1H), 3.96-3.84 (m, 1H), 2.52 (t,  $J = 4.64, 4.64$  Hz, 2H), 1.54-1.37 (m, 14H), 1.29-1.18 (m, 14H), 1.16-1.08 (m, 2H), 0.84 (d,  $J = 6.61$  Hz, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.34, 155.28, 131.95, 118.34, 79.06, 65.12, 47.57, 39.17, 38.99, 34.57, 29.87, 29.64, 29.61, 29.58, 29.50, 29.47, 29.29, 28.32, 27.91, 27.36, 26.07, 22.61; IR (neat)  $\nu_{max}$  3450, 3371, 2925, 2855, 1728, 1518, 1457, 13370, 1256, 1186, 985, 942, 863, 767; ESI MS  $m/z$  411.93[M+H]<sup>+</sup>

### Dipeptide (III.38)

(R)-allyl 3-(tert-butoxycarbonylamino)-14-methylpentadecanoate (0.400 g, 0.971 mmol) was treated with a solution of 4N HCl-Dioxane (2.0 mL) and stirred for 90 minutes. The volatiles were removed in vacuo. The residual HCl was further removed by adding a solution of CH<sub>2</sub>Cl<sub>2</sub>/Hexanes (1:1, 5 mL) to the hydrochloride salt followed by its removal in vacuo. To a stirring solution of Boc- $\beta$ OBn-Asp(OCy)-OH (0.405 g, 0.962 mmol), the hydrochloride (0.971 mmol), HOBt (0.156 g, 1.154 mmol) and DIEA (0.20 mL, 1.154

mmol) dissolved in THF (3.2 mL) at 0 °C was added EDCI (0.221 g, 1.154 mmol) in one portion. The solution was stirred at this temperature under argon for 2 hours then slowly warmed to room temperature. The reaction was stirred 12 hrs then quenched with EtOAc (1 mL). The solvent was removed in vacuo and the residue was dissolved in EtOAc (20 mL) then washed with 1N HCl (1 x 10 mL) and brine (1 x 10 mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Silica gel chromatography on a Biotage SP1 Flash system (5-40% EtOAc/Hex) afforded the product (0.545 g, 0.762 mmol, 79%) as a colorless oil  $[\alpha]^{22}_{\text{D}} + 8.0$  (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  7.34-7.28 (m, 5H), 6.80 (d, *J* = 8.79 Hz, 1H), 5.92-5.80 (m, 1H), 5.36 (d, *J* = 7.92 Hz, 1H), 5.30 (s, 1H), 5.27-5.19 (m, 1H), 4.83 (s, 1H), 4.76-4.70 (m, 2H), 4.64 (d, *J* = 7.64 Hz, 1H), 4.52-4.44 (m, 3H), 4.20 (dd, *J* = 12.85, 7.27 Hz, 1H), 2.50 (dq, *J* = 15.41, 15.10, 15.10, 4.88 Hz, 1H), 1.90-1.64 (m, 4H), 1.57 (s, 4H), 1.55-1.45 (m, 6H), 1.44-1.40 (m, 11H), 1.28-1.19 (m, 21H), 1.17-1.11 (m, 2H), 0.85 (d, *J* = 6.61 Hz, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  ; 170.58, 169.12, 167.87, 154.86, 136.78, 131.63, 127.93, 127.79, 127.61, 118.18, 79.88, 76.92, 73.51, 73.00, 64.79, 56.65, 46.12, 38.71, 38.16, 33.54, 31.08, 30.85, 29.59, 29.55, 29.33, 29.29, 29.23, 29.09, 28.98, 27.79, 27.62, 27.07, 25.61, 24.92, 23.16, 23.09, 22.34 ; IR (neat)  $\nu_{\text{max}}$  3352, 3312, 2919, 2853, 1752, 1690, 1654, 1547, 1521, 1363, 1297, 1175, 1129, 1017; ESI MS *m/z* 737.343[M+Na]<sup>+</sup>

### Tetrapeptide (III.39)

The dipeptide (0.500 g, 0.699 mmol) was treated with 4N HCl-Dioxane (2 mL) and the resulting mixture was stirred at room temperature for 90 minutes. The volatiles were removed in vacuo. The residual HCl was further removed by adding Et<sub>2</sub>O (5 mL) to the



hydrochloride salt followed by its removal in vacuo. This residue, Boc-D-Ser(OBn)- $\beta$ OH-Pro-OH (0.272 g, 0.665 mmol), HOBT (0.135 g, 0.998 mmol) and DIEA (0.174 mL, 0.998 mmol) were dissolved in THF (2.2 mL). The solution was cooled to 0 °C and EDCI (0.191 g, 0.998 mmol) was added. The reaction mixture was stirred under argon for 18 hrs and then quenched with EtOAc (1 mL). The THF was removed in vacuo and to the residue was added EtOAc (25 mL). This solution was extracted with 1N HCl (1 x 20 mL), sat. aq. NaHCO<sub>3</sub> (1 x 20 mL), and brine (1 x 20 mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Silica gel chromatography on a Biotage SP1 Flash system (20-80% EtOAc/Hex) afforded the product (0.561 g, 0.558 mmol, 84%) as a colorless wax  $[\alpha]_D^{22}$  -10.5 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.32-7.19 (m, 10H), 7.02 (d,  $J$  = 9.17 Hz, 1H), 6.92 (d,  $J$  = 8.50 Hz, 1H), 5.91-5.70 (m, 1H), 5.24 (dd,  $J$  = 17.20, 1.35 Hz, 1H), 5.17 (d,  $J$  = 9.65 Hz, 1H), 4.89 (d,  $J$  = 9.22 Hz, 1H), 4.77-4.60 (m, 3H), 4.58-4.45 (m, 3H), 4.44-4.39 (m, 2H), 4.36 (s, 1H), 4.16 (m, 1H), 3.93-3.79 (m, 2H), 3.64 (d,  $J$  = 6.59 Hz, 2H), 2.49 (t,  $J$  = 5.35, 5.35 Hz, 1H), 2.18-2.01 (m, 1H), 1.99-1.85 (m, 1H), 1.79-1.58 (m, 4H), 1.55-1.42 (m, 4H), 1.41-1.29 (m, 13H), 1.29-1.17 (m, 20H), 1.15-1.08 (m, 2H), 0.84 (d,  $J$  = 6.62 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.55, 171.35, 169.70, 169.60, 167.55, 155.77, 137.94, 137.07, 132.14, 128.59, 128.53, 128.19, 128.15, 127.91, 127.83, 118.74, 80.22, 76.11, 74.17, 73.48, 73.39, 72.91, 70.25, 70.18, 65.50, 55.07, 52.04, 47.20, 45.73, 39.26, 38.93, 34.23, 33.02, 31.59, 31.33, 30.17, 29.94, 29.89, 29.87, 29.69, 29.53, 28.50, 28.17, 27.64, 26.05, 25.41, 23.71, 23.67, 22.90, IR (neat)  $\nu_{max}$  3359, 3065, 3033, 2931, 2854, 1746, 1684, 1649, 1511, 1460, 1368, 1261, 1165, 1114, 1017, 747, 701; ESI MS  $m/z$  1027.53[M+Na]<sup>+</sup>

**Linear Peptide (III.40)**

$\text{PdCl}_2(\text{PPh}_3)_2$  (0.0094 g, 0.013 mmol) and  $\text{PPh}_3$  (0.0106 g, 0.040 mmol) were dissolved in dry  $\text{CH}_2\text{Cl}_2$  (1.0 mL) and stirred under an atmosphere of argon for 15 min. This solution was transferred via syringe to a solution of the allyl ester (0.540 g, 0.537 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (1.7 mL) under argon.  $\text{PhSiH}_3$  (0.133 mL, 1.07 mmol) was added via syringe and the reaction was stirred at room temperature for 12 hours. The solvent was condensed and the crude residue was loaded directly onto a silica gel column. Silica gel chromatography on a Biotage SP1 Flash system (2-20%  $\text{MeOH}/\text{CHCl}_3$ ) afforded the product (0.387 g, 0.401 mmol, 75%) as a tan foam; mp. 48-50°C;  $[\alpha]_D^{22}$  - 12.3 (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.74 (d,  $J$  = 9.34 Hz, 1H), 7.37-7.22 (m, 10H), 6.55 (d,  $J$  = 7.25 Hz, 1H), 4.73 (dd,  $J$  = 9.27, 3.87 Hz, 1H), 4.71-4.65 (m, 1H), 4.62 (d,  $J$  = 11.94 Hz, 1H), 4.57 (dd,  $J$  = 13.22, 6.34 Hz, 1H), 4.50 (d,  $J$  = 4.09 Hz, 1H), 4.48 (d,  $J$  = 1.75 Hz, 2H), 4.42 (d,  $J$  = 12.04 Hz, 1H), 4.19 (s, 1H), 4.16 (s, 1H), 3.99 (td,  $J$  = 13.35, 4.30, 4.30 Hz, 1H), 3.81 (t,  $J$  = 9.00, 9.00 Hz, 1H), 3.70 (dd,  $J$  = 16.94, 10.20 Hz, 1H), 3.62-3.52 (m, 2H), 2.38 (dd,  $J$  = 15.65, 4.77 Hz, 1H), 2.30-2.20 (m, 1H), 1.92 (m, 1H), 1.85-1.74 (m, 2H), 1.74-1.68 (m, 1H), 1.68-1.59 (m, 2H), 1.54-1.41 (m, 3H), 1.42-1.28 (m, 13H), 1.25-1.09 (m, 22H), 0.83 (d,  $J$  = 6.62 Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  174.23, 170.59, 169.94, 169.30, 167.51, 155.25, 137.38, 136.80, 128.17, 128.08, 127.74, 127.50, 127.38, 79.92, 76.26, 73.83, 72.98, 72.74, 69.57, 68.94, 54.83, 51.77, 46.44, 45.37, 38.83, 38.13, 33.87, 32.19, 31.13, 30.87, 29.74, 29.52, 29.47, 29.35, 29.20, 28.09, 27.73, 27.21, 25.87, 24.98, 23.23, 22.47; IR (film)  $\nu_{\text{max}}$  3321, 3065, 2932,

2854, 1725, 1649, 1516, 1455, 1368, 1261, 1159, 1098, 1037, 736, 696; ESI MS  $m/z$  987.48  $[M+Na]^+$

This pentapeptide (0.365 g, 0.330 mmol), the tetrapeptide carboxylic acid (0.271 g, 0.281 mmol), HOBT (0.057 g, 0.422 mmol) and DIEA (0.073 mL, 0.054 mmol) were dissolved in THF (1.1 mL). The solution was cooled to 0 °C and EDCI (0.081 g, 0.422 mmol) was added. The reaction mixture was stirred under argon for 18 hrs and then quenched with EtOAc (1 mL). The THF was removed in vacuo and to the residue was added EtOAc (25 mL). This solution was extracted with 1N HCl (1 x 20 mL), sat. aq. NaHCO<sub>3</sub> (1 x 20 mL), and brine (1 x 20mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Silica gel chromatography on a Biotage SP1 Flash system (12-100% Acetone/CHCl<sub>3</sub>) afforded the product (0.505 g, 0.250 mmol, 89%) as a white solid. mp. 64-66 °C;  $[\alpha]_D^{22} + 4.8$  (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 11.63 (d,  $J$  = 9.28 Hz, 1H), 8.49 (d,  $J$  = 8.14 Hz, 1H), 8.43-8.35 (m, 2H), 8.33-8.30 (m, 1H), 8.08 (d,  $J$  = 8.27 Hz, 1H), 8.03-7.94 (m, 1H), 7.72-7.66 (m, 1H), 7.45-7.19 (m, 25H), 6.62 (d,  $J$  = 7.58 Hz, 1H), 5.86-5.77 (m, 1H), 5.30-5.28 (m, 1H), 5.26 (p,  $J$  = 1.56, 1.56, 1.55, 1.55 Hz, 1H), 5.23 (dd,  $J$  = 4.82, 3.42 Hz, 1H), 5.22-5.14 (m, 4H), 5.02 (d,  $J$  = 2.85 Hz, 2H), 4.93 (ddd,  $J$  = 13.06, 9.28, 3.49 Hz, 1H), 4.79 (d,  $J$  = 4.91 Hz, 1H), 4.77-4.64 (m, 4H), 4.63-4.52 (m, 5H), 4.51-4.45 (m, 4H), 4.45-4.39 (m, 3H), 4.33 (d,  $J$  = 3.24 Hz, 1H), 4.28-4.14 (m, 4H), 4.05 (s, 1H), 3.83-3.72 (m, 2H), 3.72-3.66 (m, 1H), 3.64-3.53 (m, 2H), 3.46 (m, 1H), 2.33-2.24 (m, 1H), 2.12 (s, 1H), 1.99-1.86 (m, 1H), 1.84-1.70 (m, 5H), 1.70-1.56 (m, 7H), 1.52-1.43 (m, 5H), 1.43-1.28 (m, 19H), 1.24-0.99 (m, 30H), 0.82 (dd,  $J$  = 6.59, 1.47 Hz, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 172.53, 172.07, 171.35, 171.05, 170.73, 170.41, 169.81, 169.07, 167.94, 167.22, 163.13, 155.93, 154.87, 154.68, 153.25, 137.55, 137.02, 136.80,

136.69, 136.52, 136.37, 136.04, 135.93, 134.22, 130.91, 128.24, 127.98, 127.72, 127.50, 127.31, 127.17, 118.69, 79.26, 77.20, 73.98, 73.38, 73.01, 72.70, 72.35, 69.65, 69.20, 68.44, 68.13, 67.75, 66.67, 66.60, 66.16, 66.04, 57.70, 54.71, 53.98, 53.73, 52.63, 51.40, 47.27, 45.03, 40.19, 38.65, 34.27, 32.63, 32.29, 31.39, 30.96, 30.76, 29.50, 29.31, 28.91, 27.55, 27.02, 25.55, 24.82, 23.11, 22.31, 19.52, 19.33, 16.28, 15.68; IR (neat)  $\nu_{max}$  3359, 3065, 3033, 2931, 2854, 1746, 1684, 1649, 1511, 1460, 1368, 1261, 1165, 1114, 1017, 747, 701; ESI MS  $m/z$  1931.71[M+Na -Bn]<sup>+</sup>

### Cyclic Peptide (III.41)

PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> (0.0045 g, 0.0059 mmol) and PPh<sub>3</sub> (0.0047 g, 0.018 mmol) were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (1.0 mL) and stirred under an atmosphere of argon for 15 min. This solution was transferred via syringe to a solution of the allyl ester (0.477 g, 0.236 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (1.3 mL) under argon. PhSiH<sub>3</sub> (0.058 mL, 0.473 mmol) was added via syringe and the reaction was stirred at room temperature for 4 hours. The solvent was condensed and the crude residue was loaded directly onto a silica gel column. Silica gel chromatography on a Biotage SP1 Flash system (2-20% MeOH/CHCl<sub>3</sub>) afforded the product (0.415 g, 0.210 mmol, 89%) as a tan foam; mp. 101-103 °C;  $[\alpha]_D^{22}$  - 14.7 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  ppm 11.61-11.59 (m, 1H), 8.41-8.35 (m, 1H), 8.21-8.15 (m, 1H), 8.03-7.93 (m, 1H), 7.87-7.82 (m, 1H), 7.73-7.57 (m, 1H), 7.42-7.14 (m, 25H), 6.63-6.49 (m, 2H), 5.58 (s, 1H), 5.50-5.35 (m, 2H), 5.35-5.30 (m, 1H), 5.28 (s, 1H), 5.23 (d,  $J$  = 2.63 Hz, 1H), 5.19-5.14 (m, 2H), 5.01-4.98 (m, 2H), 4.81 (d,  $J$  = 4.67 Hz, 1H), 4.73-4.64 (m, 2H), 4.65-4.50 (m, 5H), 4.50-4.37 (m, 6H), 4.36-4.32 (m, 1H),

4.32-4.25 (m, 2H), 4.24-4.21 (m, 2H), 4.17 (s, 1H), 4.11 (s, 1H), 4.03 (s, 1H), 3.86-3.63 (m, 2H), 3.61-3.49 (m, 2H), 3.46-3.38 (m, 1H), 2.38-2.19 (m, 2H), 1.96-1.83 (m, 1H), 1.83-1.66 (m, 6H), 1.65-1.55 (m, 4H), 1.55-1.37 (m, 6H), 1.39-0.97 (m, 48H), 0.81 (d,  $J$  = 6.61 Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  174.93, 174.79, 172.68, 170.73, 170.54, 170.27, 169.69, 168.51, 167.83, 163.85, 156.22, 155.51, 153.90, 138.04, 137.42, 136.75, 134.72, 129.01, 128.89, 128.80, 128.66, 128.59, 128.30, 127.99, 127.88, 127.74, 79.93, 77.74, 74.12, 73.68, 73.39, 70.66, 68.92, 68.40, 67.36, 57.98, 56.29, 55.46, 51.71, 49.21, 48.94, 47.54, 45.56, 40.88, 39.26, 34.27, 33.32, 32.13, 32.03, 31.88, 31.57, 31.31, 30.18, 29.97, 29.82, 28.57, 28.17, 27.65, 26.42, 25.46, 23.72, 22.927, 21.36, 18.50, 14.39, 13.84, IR (film)  $\nu_{\text{max}}$  3334, 3035, 2933, 2857, 1735, 1644, 1532, 1460, 1384, 1261, 1200, 1108, 1052, 752, 696; ESI MS  $m/z$  1999.53  $[\text{M}+\text{Na}]^+$

The linear peptide (0.400 g, 0.202 mmol) was treated with a 4N HCl-Dioxane solution (2.0 mL) and stirred under an atmosphere of argon for 1 hr. The volatiles were then removed in vacuo and to the residue was added  $\text{CH}_2\text{Cl}_2$ /Hexanes (1:1, 10 mL). The solvent was condensed to remove any residual HCl and the crude residue was placed under high vacuum for several hours. The product was used without further purification. To this residue in dry DMF (100 mL) at 0 °C was added HOBt (0.109 g, 0.808 mmol), DIEA (0.055 mL, 0.323 mmol) and finally EDCI (0.155 g, 0.808 mmol). The mixture was stirred under an atmosphere of argon at this temperature for 48 hours. The solvent was then removed and the crude material was diluted with EtOAc (30 mL). The organic phase was washed with 1N HCl (1 x 20 mL), sat. aq.  $\text{NaHCO}_3$  (1 x 20 mL) and brine (1 x 20 mL). The EtOAc was dried over  $\text{Mg}_2\text{SO}_4$ , filtered and condensed. Silica gel chromatography (1-10% EtOH- $\text{CHCl}_3$ ) afforded the cyclic peptide as a white solid. Silica

gel chromatography on a Biotage SP1 Flash system (2-20% EtOH/CHCl<sub>3</sub>) afforded the product (0.217 g, 0.117 mmol, 59%) as a white foam; mp. 74-76 °C;  $[\alpha]_D^{22}$  - 15.8 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, 45°C),  $\delta$  11.57 (s, 1H), 8.39-8.29 (m, 2H), 7.98-7.92 (m, 2H), 7.78 (d,  $J$  = 5.57 Hz, 1H), 7.50 (d,  $J$  = 8.92 Hz, 1H), 7.46-7.18 (m, 25H), 6.93 (d,  $J$  = 6.89 Hz, 1H), 5.41 (s, 1H), 5.29 (d,  $J$  = 3.32 Hz, 1H), 5.19 (s, 2H), 5.02 (s, 2H), 4.95 (d,  $J$  = 7.56 Hz, 1H), 4.88 (d,  $J$  = 5.00 Hz, 1H), 4.68 (s, 1H), 4.65-4.58 (m, 3H), 4.58-4.41 (m, 8H), 4.33 (d,  $J$  = 11.55 Hz, 1H), 4.26-4.21 (m, 2H), 4.21-4.15 (m, 3H), 4.01 (s, 1H), 3.95 (dd,  $J$  = 11.86, 6.25 Hz, 1H), 3.85 (t,  $J$  = 9.23, 9.23 Hz, 2H), 3.80-3.70 (m, 2H), 3.65 (dd,  $J$  = 9.64, 6.17 Hz, 1H), 3.60-3.47 (m, 2H), 3.00-2.80 (m, 1H), 2.34 (d,  $J$  = 5.84 Hz, 2H), 2.12 (s, 2H), 1.93-1.70 (m, 5H), 1.64 (s, 6H), 1.59-1.51 (m, 1H), 1.51-1.42 (m, 6H), 1.43-1.29 (m, 10H), 1.30-1.08 (m, 38H), 1.06-1.02 (m, 3H), 0.83 (d,  $J$  = 6.57 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  210.46, 173.41, 172.90, 172.00, 171.25, 170.71, 169.25, 168.94, 167.59, 167.31, 166.79, 163.36, 155.79, 153.48, 137.51, 136.90, 136.80, 136.47, 134.31, 128.52, 128.42, 128.14, 127.86, 127.67, 127.54, 74.14, 73.97, 73.48, 73.21, 73.07, 72.84, 70.25, 69.54, 69.43, 69.33, 68.40, 67.91, 67.82, 66.85, 53.73, 40.79, 40.34, 38.80, 35.75, 35.13, 32.73, 32.32, 31.52, 31.24, 31.04, 30.46, 29.71, 29.48, 29.02, 27.70, 27.18, 27.00, 26.70, 25.76, 25.51, 24.94, 23.42, 23.34, 22.44, 19.78, 19.31, 16.75, 15.38, 15.24, 14.84, 14.77, 13.90; IR (film)  $\nu_{max}$  3326, 3064, 2933, 2860, 1735, 1649, 1532, 1460, 1379, 1328, 1261, 1205, 1098, 1058, 757, 701; ESI MS  $m/z$  1881.48 [M+Na]<sup>+</sup>

Analytical data for macrocyclization epimer (**III.42**): mp. 79-82 °C;  $[\alpha]_D^{22}$  - 9.3 (c 1.0, CHCl<sub>3</sub>); IR (film)  $\nu_{max}$  3326, 3064, 2933, 2860, 1735, 1649, 1532, 1460, 1379, 1328, 1261, 1205, 1098, 1058, 757, 701; ESI MS  $m/z$  1881.47 [M+Na]<sup>+</sup>

**Amide Analog (III.43)**

The cyclic peptide (0.135 g, 0.073 mmol) and anisole (1 mL) in an HF reaction apparatus were purged with N<sub>2</sub> and cooled to -78 °C. HF gas was distilled into the reaction vessel to a total volume of approximately 5 mL. The reaction was warmed to 0 °C and stirred at this temperature for 1hr. The HF was then evaporated under a steady stream of N<sub>2</sub> and the crude material was transferred to a round bottom flask using EtOH. The volatiles were removed in vacuo and the crude material was triturated with Et<sub>2</sub>O and centrifuged (3x). The crude material was HPLC purified (30-65% CH<sub>3</sub>CN/H<sub>2</sub>O 0.1% TFA over 60 minutes) to afford (0.0480 g, 0.041 mmol, 57%) of the plusbacin A3 amide analog as a white powder. mp >200 °C decomposition;  $[\alpha]_D^{22} + 1.79$  (c 0.39, EtOH); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>CN/D<sub>2</sub>O/TFA, 500:500:1) δ 4.92 (s, 1H), 4.87 (s, 1H), 4.81-4.76 (m, 1H), 4.76-4.71 (m, 1H), 4.69 (d, *J* = 2.55 Hz, 1H), 4.66 (s, 1H), 4.60 (t, *J* = 6.62, 6.62 Hz, 1H), 4.47-4.43 (m, 1H), 4.43-4.38 (m, 1H), 4.35 (s, 1H), 4.32 (d, *J* = 13.07 Hz, 1H), 4.24 (s, 1H), 4.21 (s, 1H), 4.08-4.02 (m, 1H), 3.99 (dd, *J* = 12.58, 6.16 Hz, 1H), 3.89 (dd, *J* = 11.48, 5.92 Hz, 2H), 3.83-3.71 (m, 2H), 3.68 (dd, *J* = 17.75, 8.33 Hz, 2H), 3.59-3.38 (m, 1H), 3.07 (dd, *J* = 12.44, 6.37 Hz, 2H), 2.50-2.28 (m, 2H), 1.86 (dd, *J* = 12.83, 7.09 Hz, 1H), 1.69 (m, 1H), 1.61 (dd, *J* = 14.90, 7.41 Hz, 1H), 1.50-1.36 (m, 4H), 1.31-1.06 (m, 30H), 0.80 (d, *J* = 6.62 Hz, 1H); IR (KBr pellet)  $\nu_{max}$  3388, 2925, 2855, 1737, 1659, 1450, 1205, 1134, 1098, 996; HR ESI TOF *m/z* 1157.6039 [M+H]<sup>+</sup>

**Amide Stereoisomer Analog (III.44)**

The macrocyclization epimer (0.0843 g, 0.045 mmol) and anisole (1 mL) in an HF reaction apparatus were purged with N<sub>2</sub> and cooled to -78 °C. HF gas was distilled into the reaction vessel to a total volume of approximately 5 mL. The reaction was warmed to 0 °C and stirred at this temperature for 1 hr. The HF was then evaporated under a steady stream of N<sub>2</sub> and the crude material was transferred to a round bottom flask using EtOH. The volatiles were removed in vacuo and the crude material was triturated with Et<sub>2</sub>O and centrifuged (3x). The crude material was HPLC purified (30-65% CH<sub>3</sub>CN/H<sub>2</sub>O 0.1% TFA over 60 minutes) to afford (0.0113 g, 0.0098 mmol, 21%) of the plusbacin A3 amide analog as a white powder. mp >200 °C decomposition;  $[\alpha]_D^{22} + 4.81$  (c 0.39, EtOH); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>CN/D<sub>2</sub>O/TFA, 500:500:1) δ 4.85 (d, *J* = 5.65 Hz, 1H), 4.72 (d, *J* = 2.56 Hz, 2H), 4.68 (t, *J* = 5.64, 5.64 Hz, 1H), 4.52 (q, *J* = 6.79, 6.50, 6.50 Hz, 1H), 4.39 (s, 1H), 4.35 (d, *J* = 5.65 Hz, 1H), 4.29-4.27 (m, 4H), 4.03 (dd, *J* = 12.95, 6.45 Hz, 1H), 3.94 (t, *J* = 7.84, 7.84 Hz, 1H), 3.86 (m, 1H), 3.80-3.66 (m, 4H), 3.07 (qd, *J* = 14.11, 7.15, 7.15, 6.95 Hz, 2H), 2.25 (dd, *J* = 14.33, 8.88 Hz, 1H), 2.15 (dd, *J* = 14.33, 5.37 Hz, 1H), 1.79-1.66 (m, 1H), 1.64-1.54 (m, 1H), 1.51-1.37 (m, 5H), 1.27-1.23 (m, 4H), 1.22-1.16 (m, 17H), 1.14-1.08 (m, 10H), 0.80 (d, *J* = 6.63 Hz, 1H); IR (KBr pellet)  $\nu_{max}$  3388, 2925, 2855, 1737, 1659, 1450, 1205, 1134, 1098, 996; HR ESI TOF *m/z* 1157.6038 [M+H]<sup>+</sup>

### III.11.7 PLUSBACIN HYDROLYSIS

#### Linear Analog (III.46)



A sample of synthetic plusbacin A3 (0.002 g, 0.00172 mmol) was treated with a solution of 1% Et<sub>3</sub>N/H<sub>2</sub>O (1.0 mL) and stirred at room temperature for 12 hours. The sample was diluted with 0.1N HCl (0.5 mL), frozen, and lyophilized. Reverse phase HPLC (30-65% CHCN/H<sub>2</sub>O, 0.1% TFA, over 30 min, 215 nm) afforded the hydrolyzed peptide (1.6 mg, 1.35  $\mu$ mol, 79%) as a white solid. >250 °C decomposition;  $[\alpha]^{22}_{\text{D}} + 1.4$  (c 0.20, EtOH); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>CN/D<sub>2</sub>O/TFA, 500:500:1)  $\delta$  5.01-4.94 (m, 1H), 4.90 (s, 2H), 4.83-4.70 (m, 2H), 4.70-4.52 (m, 2H), 4.45 (s, 1H), 4.38-4.30 (m, 4H), 4.29-4.22 (m, 5H), 4.00-3.83 (m, 4H), 3.83-3.71 (m, 4H), 3.65 (s, 1H), 3.58-3.47 (m, 1H), 3.10 (s, 1H), 2.48-2.21 (m, 1H), 2.22-2.05 (m, 1H), 1.69 (s, 1H), 1.50 (d,  $J = 6.06$  Hz, 1H), 1.42-1.00 (m, 46H); IR (KBr pellet)  $\nu_{\text{max}}$  3432, 2959, 2926, 2860, 1735, 1674, 1535, 1441, 1204, 1186, 1127; HR ESI TOF  $m/z$  1183.6427 [M+H]<sup>+</sup>

### III.11.8 LIPID REMOVAL

**(2S,3S)-1-(3-(allyloxy)-3-oxopropyl) 4-cyclohexyl 3-(benzyloxy)-2-(tert-butoxycarbonylamino)succinate (III.48)**

To a stirring solution of Boc- $\beta$ OBn-Asp(OCy)-OH (0.538 g, 1.276 mmol), the alcohol (0.249 g, 1.914 mmol), and DMAP (0.078 g, 0.638 mmol) dissolved in CH<sub>2</sub>Cl<sub>2</sub> (4.25ml) at -15 °C was added EDCI (0.367 g, 0.1.914 mmol) in five portions over a period of 1 hour. The solution was stirred at this temperature under argon for 2 hours then slowly warmed to room temperature. The reaction was stirred 12hr then quenched with EtOAc (1ml). The solvent was removed in vacuo and the residue was dissolved in EtOAc (20 mL) then washed with 1N HCl (1 x 10 mL) and brine (1 x 10 mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Column chromatography (5-40% EtOAc-

Hexanes) afforded (2S,3S)-1-(3-(allyloxy)-3-oxopropyl) 4-cyclohexyl 3-(benzyloxy)-2-(tert-butoxycarbonylamino)succinate (0.409 g, 0.766 mmol, 84%) as a colorless oil.  $[\alpha]_D^{22}$  - 22.0 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.36-7.21 (m, 1H), 5.86 (qd,  $J$  = 10.62, 5.79, 5.77, 5.77 Hz, 1H), 5.32-5.18 (m, 3H), 4.95-4.72 (m, 3H), 4.54 (t,  $J$  = 5.04, 5.04 Hz, 1H), 4.45 (d,  $J$  = 2.37 Hz, 1H), 4.39-4.33 (m, 2H), 4.29-4.21 (m, 1H), 2.54 (t,  $J$  = 6.48, 6.48 Hz, 2H), 1.89-1.78 (m, 1H), 1.75-1.65 (m, 2H), 1.58-1.19 (m, 16H); <sup>13</sup>C (100MHz, CDCl<sub>3</sub>)  $\delta$  170.07, 169.48, 168.73, 155.49, 137.01, 132.02, 128.60, 128.46, 128.32, 118.79, 80.17, 77.38, 74.52, 73.11, 65.64, 61.12, 56.20, 33.73, 31.68, 31.38, 28.36, 25.43, 23.86, 23.73, IR (neat)  $\nu_{max}$  3437, 2939, 2866, 1740, 1725, 1506, 1450, 1368, 1338, 1267, 1210, 1165, 1124, 1068, 1017, 752, 706; ESI MS  $m/z$  556.15 [M+Na]<sup>+</sup>

#### **Tetrapeptide (III.49)**

(2S,3S)-1-(3-(allyloxy)-3-oxopropyl) 4-cyclohexyl 3-(benzyloxy)-2-(tert-butoxycarbonylamino)succinate (0.342 g, 0.641 mmol) was treated with 4N HCl-Dioxane (2 mL) and the resulting mixture was stirred at room temperature for 90 minutes. The volatiles were removed in vacuo. The residual HCl was further removed by adding Et<sub>2</sub>O (5 mL) to the hydrochloride salt followed by its removal in vacuo. This residue, Boc-D-Ser(OBn)- $\beta$ OH-Pro-OH (0.259 g, 0.634 mmol), HOBt (0.129 g, 0.951 mmol) and DIEA (0.17 mL, 0.951 mmol) were dissolved in THF (2.1 mL). The solution was cooled to 0 °C and EDCI (0.182 g, 0.951 mmol) was added. The reaction mixture was stirred under argon for 18 hrs and then quenched with EtOAc (1 mL). The THF was removed in vacuo and to the residue was added EtOAc (25 mL). This solution was extracted with 1N HCl (1 x 20 mL), sat. aq. NaHCO<sub>3</sub> (1 x 20 mL), and brine (1 x 20mL). The EtOAc was dried

over  $\text{Mg}_2\text{SO}_4$ , filtered and condensed. Silica gel chromatography on a Biotage SP1 Flash system (20-80% EtOAc/Hex) afforded the product (0.411 g, 0.498 mmol, 79%) as a hygroscopic foam.  $[\alpha]_D^{22}$  -35.3 (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.09 (d,  $J = 9.16$  Hz, 1H), 7.37-7.22 (m, 10H), 6.53 (d,  $J = 7.96$  Hz, 1H), 5.92-5.83 (m, 1H), 5.27 (dd,  $J = 17.24, 1.64$  Hz, 1H), 5.18 (dd,  $J = 10.48, 1.35$  Hz, 1H), 5.05 (d,  $J = 3.34$  Hz, 1H), 5.01 (d,  $J = 3.09$  Hz, 1H), 4.89-4.80 (m, 2H), 4.78-4.63 (m, 2H), 4.60-4.40 (m, 9H), 4.39-4.29 (m, 2H), 4.22 (dd,  $J = 11.64, 5.30$  Hz, 1H), 4.19-4.13 (m, 2H), 3.74 (t,  $J = 8.92, 8.92$  Hz, 1H), 3.65 (dd,  $J = 16.85, 10.63$  Hz, 1H), 3.61-3.35 (m, 3H), 2.63 (dd,  $J = 12.37, 6.36$  Hz, 2H), 1.90 (q,  $J = 9.19, 9.19, 9.14$  Hz, 1H), 1.77 (dd,  $J = 12.15, 5.98$  Hz, 2H), 1.70 (d,  $J = 12.28$  Hz, 1H), 1.66-1.54 (m, 2H), 1.45-1.14 (m, 16H);  $^{13}\text{C}$  (100MHz,  $\text{CDCl}_3$ )  $\delta$  169.75, 169.62, 169.53, 168.30, 167.87, 167.75, 154.87, 137.61, 136.47, 131.51, 128.09, 128.00, 127.79, 127.25, 118.22, 79.16, 76.37, 74.08, 72.78, 72.36, 72.09, 70.09, 68.11, 65.13, 60.76, 53.78, 51.39, 44.91, 33.12, 32.41, 31.15, 30.94, 30.75, 28.02, 24.90, 23.31, 23.24, IR (neat)  $\nu_{\text{max}}$  3428, 2981, 2937, 2860, 1751, 1715, 1684, 1649, 1526, 1455, 1374, 1185, 1098, 1027, 752, 701; ESI MS  $m/z$  846.27  $[\text{M}+\text{Na}]^+$

### Linear Peptide (III.50)

$\text{PdCl}_2(\text{PPh}_3)_2$  (0.0084 g, 0.012 mmol) and  $\text{PPh}_3$  (0.0094 g, 0.036 mmol) were dissolved in dry  $\text{CH}_2\text{Cl}_2$  (1.0 mL) and stirred under an atmosphere of argon for 15 min. This solution was transferred via syringe to a solution of the allyl ester (0.392 g, 0.476 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (2.2 mL) under argon.  $\text{PhSiH}_3$  (0.120 mL, 0.953 mmol) was added via syringe and the reaction was stirred at room temperature for 12 hours. The solvent was condensed and the crude residue was loaded directly onto a silica gel column. Silica gel

chromatography on a Biotage SP1 Flash system (2-20% EtOH/CHCl<sub>3</sub>) afforded the product (0.355 g, 0.453 mmol, 95%) as a tan foam; mp. 49-52 °C;  $[\alpha]_D^{22}$  - 31.7 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 8.10 (d, *J* = 9.20 Hz, 1H), 7.40-7.23 (m, 10H), 6.55 (d, *J* = 7.96 Hz, 1H), 5.76 (s, 1H), 4.90-4.82 (m, 1H), 4.79 (s, 1H), 4.77-4.64 (m, 2H), 4.57 (dd, *J* = 13.76, 6.46 Hz, 1H), 4.54 (d, *J* = 3.37 Hz, 1H), 4.52-4.44 (m, 4H), 4.41-4.30 (m, 2H), 4.25-4.10 (m, 3H), 3.76 (t, *J* = 9.35, 9.35 Hz, 1H), 3.67 (dd, *J* = 16.66, 9.58 Hz, 1H), 3.62-3.46 (m, 3H), 3.46-3.22 (m, 5H), 1.92 (dt, *J* = 8.97, 8.83, 5.59 Hz, 1H), 1.79 (dd, *J* = 11.51, 5.67 Hz, 2H), 1.73 (d, *J* = 12.84 Hz, 1H), 1.68-1.55 (m, 2H), 1.49-1.10 (m, 20H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 173.87, 170.08, 169.80, 169.58, 168.37, 155.15, 137.57, 136.47, 134.04, 128.19, 128.17, 128.12, 127.94, 127.43, 127.39, 79.55, 76.37, 74.38, 72.96, 72.63, 69.85, 68.45, 61.24, 54.16, 51.66, 45.30, 33.39, 32.21, 31.25, 31.04, 30.85, 29.46, 28.13, 25.01, 23.40, 23.34; IR (film)  $\nu_{max}$  3421, 3068, 2941, 2857, 1741, 1710, 1644, 1526, 1363, 1251, 1205, 1103, 1022, 757, 696; ESI MS *m/z* 806.26 [M+Na]<sup>+</sup>

This pentapeptide (0.463 g, 0.418 mmol), the tetrapeptide carboxylic acid (0.298 g, 0.380 mmol), HOBT (0.077 g, 0.570 mmol) and DIEA (0.099 mL, 0.0570 mmol) were dissolved in THF (1.5 mL). The solution was cooled to 0 °C and EDCI (0.109 g, 0.570 mmol) was added. The reaction mixture was stirred under argon for 18 hrs and then quenched with EtOAc (1 mL). The THF was removed in vacuo and to the residue was added EtOAc (25 mL). This solution was extracted with 1N HCl (1 x 20 mL), sat. aq. NaHCO<sub>3</sub> (1 x 20 mL), and brine (1 x 20 mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Silica gel chromatography on a Biotage SP1 Flash system (12-100% Acetone/CHCl<sub>3</sub>) afforded the product (0.535 g, 0.291 mmol, 77%) as a white solid. mp. 63-65°C;  $[\alpha]_D^{22}$  -

3.0 (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ )  $\delta$  11.61 (d,  $J = 8.78$  Hz, 1H), 8.46 (d,  $J = 9.35$  Hz, 1H), 8.41-8.33 (m, 2H), 8.29 (d,  $J = 9.23$  Hz, 1H), 8.09-7.95 (m, 1H), 7.45-7.21 (m, 25H), 6.53 (t,  $J = 7.24$ , 7.24 Hz, 1H), 5.79 (qt,  $J = 10.71$ , 10.71, 5.46, 5.46, 5.39 Hz, 1H), 5.28 (d,  $J = 1.36$  Hz, 1H), 5.24 (d,  $J = 1.47$  Hz, 1H), 5.20-5.12 (m, 4H), 5.03-4.98 (m, 3H), 4.95-4.88 (m, 1H), 4.87-4.80 (m, 2H), 4.78 (s, 1H), 4.75 (d,  $J = 5.24$  Hz, 1H), 4.71 (dd,  $J = 11.68$ , 1.83 Hz, 2H), 4.68-4.61 (m, 2H), 4.59-4.49 (m, 5H), 4.50-4.44 (m, 1H), 4.44-4.39 (m, 3H), 4.32 (s, 1H), 4.30-4.24 (m, 1H), 4.24-4.17 (m, 3H), 4.16-4.12 (m, 1H), 3.84-3.70 (m, 1H), 3.65 (dd,  $J = 17.12$ , 8.79 Hz, 1H), 3.59-3.49 (m, 2H), 3.47-3.41 (m, 2H), 2.46 (s, 1H), 2.10 (s, 1H), 1.91 (dt,  $J = 24.92$ , 24.89, 11.09 Hz, 1H), 1.82-1.69 (m, 5H), 1.68-1.52 (m, 8H), 1.51-1.44 (m, 3H), 1.44-1.19 (m, 24H), 1.19-1.14 (m, 1H), 1.13-1.11 (m, 3H), 1.05-0.98 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  172.59, 171.71, 170.91, 170.02, 169.64, 168.26, 167.97, 167.74, 163.16, 155.95, 155.61, 154.83, 154.64, 153.28, 137.61, 137.14, 136.56, 136.38, 136.14, 135.93, 134.30, 134.23, 130.94, 128.38, 128.29, 128.28, 128.23, 128.10, 128.03, 128.02, 127.96, 127.90, 127.86, 127.78, 127.56, 127.50, 127.46, 127.31, 127.22, 118.80, 118.71, 79.21, 79.12, 74.13, 73.98, 73.71, 72.97, 72.83, 72.70, 72.35, 70.35, 70.02, 69.22, 68.59, 68.29, 67.81, 67.73, 66.73, 66.63, 66.22, 66.04, 62.79, 62.03, 58.21, 57.80, 54.04, 53.69, 52.65, 51.38, 51.01, 47.91, 45.19, 40.34, 40.05, 35.21, 32.59, 32.36, 31.44, 31.12, 30.94, 30.78, 30.53, 28.95, 28.00, 24.83, 23.17, 19.46, 15.97; IR (neat)  $\nu_{\text{max}}$  3327, 2934, 2855, 1746, 1641, 1536, 1457, 1379, 1335, 1265, 1205, 1108, 1047, 758, 697; ESI MS  $m/z$  1859.49  $[\text{M}+\text{Na}]^+$

### Cyclic Peptide (III.51)

$\text{PdCl}_2(\text{PPh}_3)_2$  (0.0045 g, 0.0064 mmol) and  $\text{PPh}_3$  (0.0050 g, 0.019 mmol) were dissolved in dry  $\text{CH}_2\text{Cl}_2$  (1.0 mL) and stirred under an atmosphere of argon for 15 min. This solution was transferred via syringe to a solution of the allyl ester (0.468 g, 0.255 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (1.5 mL) under argon.  $\text{PhSiH}_3$  (0.063 mL, 0.510 mmol) was added via syringe and the reaction was stirred at room temperature for 4 hours. The solvent was condensed and the crude residue was loaded directly onto a silica gel column. Silica gel chromatography on a Biotage SP1 Flash system (2-20%  $\text{MeOH}/\text{CHCl}_3$ ) afforded the product (0.455 g, 0.253 mmol, 99%) as a tan foam; mp. 112-115°C;  $[\alpha]_D^{22}$  -21.7 (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.43-8.25 (m, 3H), 8.11-7.92 (m, 4H), 7.47-7.12 (m, 25H), 6.58-6.46 (m, 1H), 5.22-5.13 (m, 2H), 5.00 (s, 1H), 4.89-4.81 (m, 1H), 4.79-4.36 (m, 12H), 4.36-4.08 (m, 8H), 3.81-3.69 (m, 2H), 3.65 (d,  $J = 8.25$  Hz, 1H), 3.60-3.37 (m, 3H), 2.44-2.38 (m, 1H), 1.91 (m, 1H), 1.82-1.65 (m, 1H), 1.64-1.55 (m, 1H), 1.55-1.08 (m, 1H), 1.08-0.95 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  174.79, 172.42, 170.36, 169.92, 169.61, 168.58, 168.14, 163.40, 155.74, 154.97, 153.45, 137.61, 136.36, 134.31, 128.52, 128.43, 128.18, 128.12, 128.04, 127.90, 127.77, 127.37, 127.27, 79.36, 74.15, 73.27, 72.92, 72.49, 70.12, 69.65, 67.97, 66.90, 61.86, 58.13, 57.60, 55.86, 54.03, 51.86, 51.46, 48.70, 45.18, 44.65, 40.50, 35.20, 32.87, 32.72, 32.05, 31.25, 30.86, 29.42, 28.11, 26.31, 25.55, 25.06, 24.99, 23.37, 20.69, 18.04, 13.16; IR (film)  $\nu_{\text{max}}$  3319, 2941, 2860, 1735, 1649, 1537, 1450, 1384, 1333, 1200, 1098, 10047, 757, 696; ESI MS  $m/z$  1819.47  $[\text{M}+\text{Na}]^+$

The linear peptide (0.446 g, 0.248 mmol) was treated with a 4N HCl-Dioxane solution (2.0 mL) and stirred under an atmosphere of argon for 1 hr. The volatiles were then

removed in vacuo and to the residue was added CH<sub>2</sub>Cl<sub>2</sub>/Hexanes (1:1, 10 mL). The solvent was condensed to remove any residual HCl and the crude residue was placed under high vacuum for several hours. The product was used without further purification. To this residue in dry DMF (124 mL) at 0 °C was added HOBt (0.168 g, 1.240 mmol), DIEA (0.086 mL, 0.496 mmol) and finally EDCI (0.238 g, 1.240 mmol). The mixture was stirred under an atmosphere of argon at this temperature for 48 hours. The solvent was then removed and the crude material was diluted with EtOAc (30 mL). The organic phase was washed with 1N HCl (1 x 20 mL), sat. aq. NaHCO<sub>3</sub> (1 x 20 mL) and brine (1 x 20 mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Silica gel chromatography on a Biotage SP1 Flash system (2-20% EtOH/CHCl<sub>3</sub>) afforded the product (0.250 g, 0.149 mmol, 60%) as a white foam; mp. 104-106 °C; [ $\alpha$ ]<sub>D</sub><sup>22</sup> - 32.5 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>, 45°C)  $\delta$  11.58 (s, 1H), 8.34 (d, *J* = 5.76 Hz, 1H), 8.03 (d, *J* = 9.13 Hz, 1H), 7.96 (d, *J* = 8.96 Hz, 1H), 7.85 (d, *J* = 5.40 Hz, 1H), 7.71 (d, *J* = 7.95 Hz, 1H), 7.46-7.20 (m, 25H), 5.34 (s, 1H), 5.27 (d, *J* = 3.10 Hz, 1H), 5.20 (s, 2H), 5.03 (s, 2H), 4.89-4.76 (m, 2H), 4.74-4.66 (m, 2H), 4.67-4.56 (m, 3H), 4.53-4.44 (m, 4H), 4.45-4.39 (m, 2H), 4.32-4.26 (m, 2H), 4.24 (s, 1H), 4.22-4.13 (m, 4H), 3.91 (dd, *J* = 11.34, 5.73 Hz, 1H), 3.82-3.70 (m, 2H), 3.66 (dd, *J* = 9.49, 6.37 Hz, 1H), 3.51 (s, 1H), 2.31 (td, *J* = 14.92, 7.38, 7.38 Hz, 1H), 1.97-1.83 (m, 1H), 1.81-1.73 (m, 3H), 1.73-1.54 (m, 9H), 1.50-1.15 (m, 18H), 1.13 (d, *J* = 6.59 Hz, 1H), 1.05 (d, *J* = 6.29 Hz, 3H), 1.00 (d, *J* = 6.28 Hz, 1H), <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  173.34, 172.73, 172.32, 171.69, 171.09, 170.66, 170.32, 169.87, 169.49, 169.06, 168.24, 163.85, 156.12, 153.82, 137.67, 137.21, 136.95, 136.62, 134.88, 134.81, 128.88, 128.64, 128.50, 128.37, 128.13, 74.62, 74.01, 73.87, 73.34, 73.10, 71.07, 70.76, 70.26, 69.36, 68.38, 68.29, 67.34, 63.22,

63.04, 59.17, 58.09, 57.84, 57.07, 56.20, 55.68, 54.82, 54.39, 53.11, 51.76, 50.77, 47.89, 47.60, 45.54, 40.98, 36.10, 33.69, 32.84, 31.50, 30.66, 29.90, 27.91, 27.12, 26.62, 25.74, 25.41, 23.89, 20.51, 20.05, 19.63, 16.19; IR (film)  $\nu_{max}$  3326, 3064, 2933, 2860, 1735, 1649, 1532, 1460, 1379, 1328, 1261, 1205, 1098, 1058, 757, 701; ESI MS  $m/z$  1700.35  $[M+Na]^+$

### Alipo Plusbacin A3 Analog (III.52)

The cyclic peptide (0.0991 g, 0.059 mmol) and anisole (1 mL) in an HF reaction apparatus were purged with N<sub>2</sub> and cooled to -78 °C. HF gas was distilled into the reaction vessel to a total volume of approximately 5 mL. The reaction was warmed to 0 °C and stirred at this temperature for 1 hr. The HF was then evaporated under a steady stream of N<sub>2</sub> and the crude material was transferred to a round bottom flask using EtOH. The volatiles were removed in vacuo and the crude material was triturated with Et<sub>2</sub>O and centrifuged (3x). The crude material was HPLC purified (2-20% CH<sub>3</sub>CN/H<sub>2</sub>O 0.1% TFA over 120 minutes) to afford (0.0300 g, 0.031 mmol, 52%) of the plusbacin A3 analog as a white powder. mp >200 °C decomposition;  $[\alpha]_D^{22}$  - 1.03 (c 0.39, H<sub>2</sub>O); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>CN/D<sub>2</sub>O/TFA, 500:500:1)  $\delta$  5.02 (d,  $J$  = 3.03 Hz, 1H), 4.87 (d,  $J$  = 1.86 Hz, 1H), 4.82-4.75 (m, 2H), 4.73 (d,  $J$  = 1.94 Hz, 1H), 4.72-4.70 (m, 2H), 4.58 (q,  $J$  = 7.17, 6.92, 6.92 Hz, 1H), 4.47-4.41 (m, 1H), 4.38-4.33 (m, 3H), 4.33-4.30 (m, 2H), 4.02 (m, 1H), 3.92 (dd,  $J$  = 14.09, 7.69 Hz, 1H), 3.84-3.71 (m, 3H), 3.69-3.61 (m, 2H), 3.07 (dd,  $J$  = 9.50, 6.85 Hz, 2H), 2.63-2.46 (m, 1H), 1.70 (m, 1H), 1.57-1.49 (m, 1H), 1.46-1.38 (m, 1H), 1.27 (d,  $J$  = 6.98 Hz, 1H), 1.23 (d,  $J$  = 6.86 Hz, 2H), 1.19 (d,  $J$  = 6.94 Hz, 1H), 1.13 (s, 3H), 1.07 (d,  $J$  = 6.49 Hz, 3H); IR (KBr pellet)  $\nu_{max}$  3423, 2960, 1741, 1654, 1547,



1445, 1384, 1272, 1205, 1134, 1103, 996, 838, 803, 721; HR ESI TOF  $m/z$  976.3846  
 $[M+H]^+$

### III.11.9 DEUTERATED PLUSBACIN A3

#### 11-methyldodec-1-ene ( $d_7$ ) (III.54)

A two neck flask containing Mg turnings and dry ether (19.3 mL) was fitted to a reflux condenser and purged with argon several times. 2-bromopropane (5.00 g, 3.81 mL, 38.5 mmol) was added drop-wise and the mixture was stirred for 1 hour. This solution was added via cannula to a solution of the lipid bromide (5.62 g, 25.6 mmol) and CuI (0.973 g, 5.13 mmol) in dry THF (64 mL) at  $-78^\circ\text{C}$  under an atmosphere of argon. The reaction was stirred for 3 hours at this temperature and then slowly warmed to room temp. Upon completion the reaction was quenched with sat. aq.  $\text{NH}_4\text{Cl}$  (2 mL), diluted with  $\text{CH}_2\text{Cl}_2$ . The aq. phase was extracted with  $\text{CH}_2\text{Cl}_2$  (3x 20 mL) and the combined organic extracts were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , filtered and condensed. Column chromatography (hexanes) afforded the product (3.58g, 18.91mmol, 73% yield) as a colorless oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.82 (tdd,  $J = 16.89, 10.17, 6.68, 6.68$  Hz, 1H), 5.04-4.91 (m, 2H), 2.05 (dd,  $J = 14.26, 6.90$  Hz, 1H), 1.39 (m, 2H), 1.34-1.24 (m, 10H), 1.16 (m, 2H);  $^{13}\text{C}$  (100MHz,  $\text{CDCl}_3$ )  $\delta$  139.205, 114.087, 38.851, 33.884, 29.991, 29.725, 29.584, 29.227, 29.005, 27.437; IR (neat)  $\nu_{\text{max}}$  2954, 2922, 2854, 1468, 1375; ESI MS  $m/z$  190.26  $[M+H]^+$

#### (R)-tert-butyl 3-acetoxy-14-methylpentadecanoate ( $d_7$ ) (III.55)

To a stirring solution of (S)-tert-butyl 3-acetoxypent-4-enoate (3.68 g, 17.18 mmol) and 11-methyldodec-1-ene ( $d_7$ ) (3.58g, 18.90 mmol) in dry  $CH_2Cl_2$  (68.7 mL) was added Grubb's 2<sup>nd</sup> Generation catalyst (0.730 g, 0.859 mmol). The flask was fitted with condenser and stirred at 50 °C under a steady stream of argon for 24 hours. The reaction mixture was then reduced in volume and loaded directly onto a silica gel column. Chromatography (0-10% EtOAc-Hexanes) afforded the product (4.95 g, 13.2mmol, 77%) as a pale yellow oil. The unreacted lipid was recovered quantitatively and recycled.  $[\alpha]^{22}_D$  - 23.9 (c 1.0,  $CHCl_3$ )  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  5.82-5.66 (m, 1H), 5.53 (dd,  $J$  = 13.92, 7.19 Hz, 1H), 5.37 (ddd,  $J$  = 15.35, 7.29, 1.08 Hz, 1H), 2.50 (ddd,  $J$  = 20.95, 15.06, 6.45 Hz, 2H), 2.06-1.91 (m, 4H), 1.39 (s, 1H), 1.36-1.25 (m, 4H), 1.21 (s, 10H), 1.14-1.05 (m, 2H);  $^{13}C$  NMR (75MHz,  $CDCl_3$ )  $\delta$  169.69, 168.99, 135.17, 126.66, 80.69, 71.21, 41.04, 38.68, 32.06, 29.81, 29.51, 29.38, 29.35, 29.02, 28.72, 27.90, 27.27, 21.072; IR (neat)  $\nu_{max}$  3441, 2928, 2850, 2361, 2097, 1732, 1639, 1468, 11367, 1235, 1157, 1017, 963; ESI MS  $m/z$  398.19  $[M+Na]^+$

(S)-tert-butyl 3-acetoxy-14-methylpentadec-4-enoate ( $d_7$ ). (4.77 g, 12.70 mmol) was dissolved in ethanol (160 mL) and 5% Pd/C (0.715 g) was added. The flask was sealed and purged with hydrogen. The mixture was stirred for 24 hours, and upon completion filtered through a celite pad. The solvent was removed and silica gel chromatography (10% EtOAc-Hexanes) afforded (3.48 g, 9.39 mmol, 74%) as a colorless oil.  $[\alpha]^{22}_D$  + 3.0 (c 1.0,  $CHCl_3$ )  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  5.17 (td,  $J$  = 12.98, 6.36, 6.36 Hz, 1H), 2.48-2.37 (m, 2H), 2.00 (s, 3H), 1.54 (s, 3H), 1.41 (s, 1H), 1.26-1.19 (m, 16H);  $^{13}C$  NMR (75MHz,  $CDCl_3$ )  $\delta$  170.224, 169.626, 80.675, 70.758, 40.534, 38.731, 33.994, 29.877,

29.635, 29.576, 29.484, 29.419, 29.340, 27.934, 27.322, 25.059, 21.051; IR (neat) 2930, 2857, 1743, 1466, 1371, 1240, 1152; ESI MS  $m/z$  400.18  $[M+Na]^+$

**(R)-allyl 3-hydroxy-14-methylpentadecanoate (d<sub>7</sub>) (III.56)**

(R)-tert-butyl 3-acetoxy-14-methylpentadecanoate (d<sub>7</sub>) (3.46 g, 9.17 mmol) was treated with a solution of TFA/CH<sub>2</sub>Cl<sub>2</sub> (1:1, 30 mL) and allowed to stir at room temperature overnight. The volatiles were removed in vacuo and the residue was left under high vacuum for several hours. The crude material was dissolved in MeOH (46 mL) and K<sub>2</sub>CO<sub>3</sub> (6.333 g, 45.8) was added. The mixture was stirred for 12 hours and the solvent was removed. EtOAc (50 mL) was added followed by the careful addition of 1N HCl while stirring. The aq. phase was extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with brine (1x 50 mL), dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Silica gel chromatography (EtOAc) afforded (R)-3-hydroxy-14-methylpentadecanoic acid (2.34 g, 8.55 mmol, 93%) as a white solid. mp 59-60 °C;  $[\alpha]_D^{22}$  -13.6 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.02 (s, 1H), 4.03 (s, 1H), 2.56 (dd,  $J$  = 16.42, 2.95 Hz, 1H), 2.45 (dd,  $J$  = 16.48, 8.84 Hz, 1H), 1.65-1.08 (m, 20H); <sup>13</sup>C NMR (100MHz, CDCl<sub>3</sub>) δ 177.79, 68.11, 41.09, 38.77, 36.42, 29.93, 29.70, 29.65, 29.59, 29.55, 29.47, 27.37, 25.44; IR (neat)  $\nu_{max}$  3228, 2922, 2850, 2689, 2592, 1713, 1467, 1446, 1363, 1316, 1301, 1189; ESI MS  $m/z$  278.30  $[M-H]^-$

To a solution of (R)-3-hydroxy-14-methylpentadecanoic acid (2.032 g, 7.27 mmol) in MeOH (30 mL) was added Cs<sub>2</sub>CO<sub>3</sub> (2.42 g, 7.42 mmol) dissolved in H<sub>2</sub>O (3.0 mL) drop wise. The mixture was stirred at room temperature for 15 minutes and the solvent was removed in vacuo. To the residue was added DMF (5 mL). The solvent was removed in

vacuo and placed under high vacuum for several hours. DMF (25 mL) was added followed by allyl bromide (6.3 mL, 72.7 mmol) and the mixture was stirred under argon for 12 hours. The DMF was removed and the crude residue was dissolved in EtOAc (30 mL) and washed with water (1 x 20 mL) and brine (1 x 20 mL). The organic phase was dried over  $\text{Mg}_2\text{SO}_4$ , filtered and condensed. Column chromatography (10% EtOAc-Hexanes) afforded the allyl ester (1.92 g, 6.02 mmol, 83%) as a colorless oil.  $[\alpha]_D^{22}$  - 13.7 (c 1.0);  $^1\text{H}$  NMR (400 MHz,  $\text{CHCl}_3$ )  $\delta$  6.02-5.77 (m, 1H), 5.29 (td,  $J$  = 17.19, 1.47, 1.47 Hz, 1H), 5.24-5.18 (m, 1H), 4.58 (dd,  $J$  = 5.75, 1.29 Hz, 2H), 4.04-3.92 (m, 1H), 2.91 (s, 1H), 2.56-2.34 (m, 2H), 1.60-1.02 (m, 20H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  172.88, 132.06, 118.72, 68.19, 65.49, 41.54, 39.00, 36.77, 30.15, 29.92, 29.87, 29.81, 29.79, 29.74, 27.60, 25.70; IR (neat)  $\nu_{\text{max}}$  3456, 2922, 2854, 2364, 1731, 1648, 1464, 1411, 1384, 1363, 1168; ESI MS  $m/z$  320.32  $[\text{M}+\text{H}]^+$

**(R)-3-((2S,3S)-3-(benzyloxy)-2-(tert-butoxycarbonylamino)-4-(cyclohexyloxy)-4-oxobutanoyloxy)-14-methylpentadecanoic acid ( $d_7$ ) (III.58)**

To a stirring solution of Boc- $\beta$ OBn-Asp(OCy)-OH (1.107 g, 2.63 mmol), the alcohol (1.843 g, 5.77 mmol), and DMAP (0.160 g, 1.31 mmol) dissolved in  $\text{CH}_2\text{Cl}_2$  (10 mL) at -15  $^\circ\text{C}$  was added EDCI (0.554 g, 2.98 mmol) in five portions over a period of 1 hour. The solution was stirred at this temperature under argon for 2 hours then slowly warmed to room temperature. The reaction was stirred 12hr then quenched with EtOAc (1 mL). The solvent was removed in vacuo and the residue was dissolved in EtOAc (20 mL) then washed with 1N HCl (1 x 10 mL) and brine (1 x 10 mL). The EtOAc was dried over  $\text{Mg}_2\text{SO}_4$ , filtered and condensed. Column chromatography (15% Pet. Ether-Ether) afforded (2S,3S)-1-((R)-1-(allyloxy)-14-methyl-1-oxopentadecan-3-yl) 4-cyclohexyl 3-

(benzyloxy)-2-(tert-butoxycarbonylamino)succinate (1.415 g, 1.957 mmol, 75%) as a colorless oil.  $[\alpha]_D^{22}$  -17.0 (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.33-7.24 (m, 6H), 5.95-5.81 (m, 1H), 5.34-5.17 (m, 4H), 4.92-4.75 (m, 3H), 4.57-4.48 (m, 3H), 4.39 (d,  $J$  = 10.97 Hz, 1H), 2.59 (dq,  $J$  = 15.60, 15.60, 15.58, 6.34 Hz, 2H), 1.91-1.04 (m, 40H);  $^{13}\text{C}$  (100MHz,  $\text{CDCl}_3$ )  $\delta$ , 169.64, 168.89, 168.54, 155.18, 136.78, 131.86, 128.26, 127.89, 127.86, 118.59, 79.77, 77.79, 74.22, 72.99, 72.35, 65.35, 56.08, 38.86, 38.73, 33.74, 31.42, 31.12, 29.88, 29.62, 29.54, 29.52, 29.34, 29.28, 28.10, 27.32, 25.17, 24.94, 23.61, 23.48; IR (neat)  $\nu_{\text{max}}$  3450, 2926, 2857, 1741, 1501, 1456, 1390, 1365, 1336, 1258, 1208, 1163, 1126, 1064; ESI MS  $m/z$  745.39  $[\text{M}+\text{Na}]^+$

$\text{PdCl}_2(\text{PPh}_3)_2$  (0.034 g, 0.048 mmol) and  $\text{PPh}_3$  (0.038 g, 0.145 mmol) were dissolved in dry  $\text{CH}_2\text{Cl}_2$  (1.0 mL) and stirred under an atmosphere of argon for 15 min. This solution was transferred via syringe to a solution of the allyl ester (1.40 g, 1.94 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (8.7mL) under argon.  $\text{PhSiH}_3$  (0.48 mL, 3.87 mmol) was added via syringe and the reaction was stirred at room temperature for 12 hours. The solvent was condensed and the crude residue was loaded directly onto a silica gel column. Chromatography (30% EtOAc-Hexanes) afforded the carboxylic acid (1.30 g, 1.90 mmol, 98%) as a colorless oil.  $[\alpha]_D^{22}$  -14.8 (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.41-7.21 (m, 5H), 5.33 (d,  $J$  = 10.17 Hz, 1H), 5.21 (p,  $J$  = 6.43, 6.43, 6.30, 6.30 Hz, 1H), 4.90-4.76 (m, 3H), 4.54 (d,  $J$  = 2.36 Hz, 1H), 2.59 (ddd,  $J$  = 45.71, 16.15, 6.40 Hz, 2H), 2.03 (s, 1H), 1.91-1.07 (m, 40H);  $^{13}\text{C}$  (100MHz,  $\text{CDCl}_3$ )  $\delta$  175.08, 168.90, 168.56, 155.31, 136.70, 128.29, 127.95, 127.87, 79.95, 77.71, 74.27, 72.98, 72.13, 56.12, 38.74, 38.56, 33.72, 31.40, 31.11, 29.88, 29.63, 29.56, 29.38, 29.31, 28.10, 27.32, 25.17, 24.96, 23.60,

23.47; IR (neat)  $\nu_{\max}$  3262, 2936, 2858, 1733, 1506, 1456, 1394, 1365, 1336, 1262, 1208, 1163, 1118, 1019; ESI MS  $m/z$  683.49  $[M+H]^+$

### Septapeptide (III.59)

Boc-D-*a*Thr-D-Ala- $\beta$ OH-Pro-Arg(Z<sub>2</sub>)- $\beta$ OBn-D-*a*Asp(OCy)-OAllyl (0.500 g, 0.427 mmol) was treated with a solution of 4N HCl-Dioxane (4.0 mL) and stirred for 90 minutes. The volatiles were removed in vacuo. The residual HCl was further removed by adding Et<sub>2</sub>O (5 mL) to the hydrochloride salt followed by its removal in vacuo. The residue was then dissolved in EtOAc (30 mL) and washed with sat. aq. NaHCO<sub>3</sub> (2 x 20 mL). The EtOAc was dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. This residue, the carboxylic acid (0.273 g, 0.400 mmol), and HOBt (0.065 g, 0.480 mmol) were dissolved in THF (2.0 mL) and cooled to 0 °C. EDCI (0.092 g, 0.480 mmol) was added and the mixture was stirred under argon for 18 hrs while slowly warming to room temperature. The reaction was quenched with EtOAc (5 mL) and the THF removed in vacuo. This residue was dissolved in EtOAc (50 mL) and washed with 1N HCl (1 x 30 mL), sat. aq. NaHCO<sub>3</sub> (1 x 30 mL), and brine (1 x 30 mL). The organic phase was dried over Mg<sup>2</sup>SO<sub>4</sub>, filtered and condensed. Column chromatography (0-50% Acetone/EtOAc) afforded the septapeptide (0.472 g, 0.275 mmol, 69%) as a white solid. mp 71-73 °C;  $[\alpha]_D^{22}$  -4.8 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.47 (d,  $J$  = 9.33 Hz, 1H), 8.41-8.33 (m, 2H), 8.31 (d,  $J$  = 9.23 Hz, 1H), 8.09-7.95 (m, 2H), 7.45-7.18 (m, 20H), 6.80 (dd,  $J$  = 19.04, 9.86 Hz, 1H), 5.78 (m, 1H), 5.26 (d,  $J$  = 17.28 Hz, 1H), 5.23-5.11 (m, 4H), 5.08 (dd,  $J$  = 11.18, 5.69 Hz, 1H), 5.00 (d,  $J$  = 1.53 Hz, 2H), 4.92 (ddd,  $J$  = 12.95, 9.34, 3.50 Hz, 1H), 4.82-4.61 (m, 6H), 4.60-4.33 (m, 9H), 4.33-4.10 (m, 3H),

3.83-3.67 (m, 1H), 3.55 (d,  $J = 7.69$  Hz, 1H), 3.43 (d,  $J = 8.39$  Hz, 1H), 1.96 (d,  $J = 10.25$  Hz, 1H), 1.86 (d,  $J = 10.95$  Hz, 1H), 1.81-1.69 (m, 4H), 1.65-1.54 (m, 8H), 1.53-1.27 (m, 29H), 1.24-0.96 (m, 35H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  172.09, 171.70, 171.20, 170.67, 169.93, 169.14, 168.84, 168.39, 168.29, 163.39, 156.09, 155.33, 153.50, 136.73, 136.61, 136.24, 134.48, 131.19, 128.53, 128.48, 128.29, 128.24, 128.06, 128.00, 127.87, 127.81, 127.73, 118.98, 79.58, 77.85, 76.45, 74.46, 74.05, 73.36, 72.86, 72.70, 68.63, 68.07, 66.83, 66.47, 57.63, 56.13, 54.14, 52.85, 52.71, 47.60, 47.25, 45.08, 40.53, 40.30, 38.64, 33.47, 32.33, 31.32, 31.05, 30.99, 30.80, 29.79, 29.54, 29.48, 29.34, 28.05, 27.23, 25.77, 25.09, 23.49, 23.42, 23.34, 19.83, 16.54, 16.38; IR (Neat)  $\nu_{\text{max}}$  3327, 3090, 3068, 3032, 2930, 2857, 1735, 1644, 1532, 1455, 1384, 1333, 1267, 1210, 1159, 1114, 1052, 757, 706; ESI MS  $m/z$  1758.71  $[\text{M}+\text{Na}]^+$

### Linear Peptide (III.60)

The septapeptide (0.450 g, 0.259 mmol) was treated with 4N HCl-Dioxane (4 mL) and the resulting mixture was stirred at room temperature for 90 minutes. The volatiles were removed in vacuo. The residual HCl was further removed by adding  $\text{Et}_2\text{O}$  (5 mL) to the hydrochloride salt followed by its removal in vacuo. This residue, Boc-D-Ser(OBn)- $\beta$ OH-Pro-OH (0.101 g, 0.247 mmol), HOBt (0.050 g, 0.371 mmol) and DIEA (0.070 mL, 0.371 mmol) were dissolved in THF (0.82 mL). The solution was cooled to 0 °C and EDCI (0.071 g, 0.371 mmol) was added. The reaction mixture was stirred under argon for 18 hrs and then quenched with EtOAc (1 mL). The THF was removed in vacuo and to the residue was added EtOAc (50 mL). This solution was extracted with 1N HCl (1 x 25 mL), sat. aq.  $\text{NaHCO}_3$  (1 x 25 mL), and brine (1 x 25mL). The EtOAc was dried over

Mg<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Chromatography (10% Acetone-EtOAc) provided the linear peptide (0.353 g, 0.174 mmol, 70%) as a white solid. mp 74-76 °C;  $[\alpha]_D^{22} - 7.1$  (c 1.0, CHCl<sub>3</sub>) <sup>1</sup>H NMR (500 MHz DMSO-*d*<sub>6</sub>, 25°C) δ 11.63 (d, *J* = 9.69 Hz, 1H), 8.49 (d, *J* = 9.38 Hz, 1H), 8.42-8.35 (m, 2H), 8.33 (d, *J* = 9.26 Hz, 1H), 8.11-8.00 (m, 3H), 7.45-7.21 (m, 25H), 6.51 (d, *J* = 7.69 Hz, 1H), 5.85-5.76 (m, 1H), 5.28 (d, *J* = 17.19 Hz, 1H), 5.22-5.13 (m, 4H), 5.12-5.07 (m, 1H), 5.06-5.03 (m, 1H), 5.03-5.01 (m, 2H), 4.97-4.86 (m, 2H), 4.83-4.79 (m, 1H), 4.76-4.65 (m, 5H), 4.63-4.51 (m, 5H), 4.51-4.30 (m, 7H), 4.29-4.18 (m, 3H), 4.16 (s, 1H), 3.76 (s, 2H), 3.68 (dd, *J* = 16.99, 8.82 Hz, 1H), 3.62-3.50 (m, 2H), 3.46 (s, 1H), 2.45-2.36 (m, 1H), 2.02-1.91 (m, 1H), 1.85-1.69 (m, 1H), 1.69-1.54 (m, 7H), 1.52-0.96 (m, 62H); <sup>13</sup>C(100 MHz, CDCl<sub>3</sub>) δ 173.03, 172.95, 172.68, 171.77, 171.02, 169.62, 169.50, 169.14, 167.85, 167.72, 163.43, 155.75, 154.67, 153.45, 136.99, 136.47, 136.24, 134.31, 131.07, 131.02, 128.54, 128.45, 128.35, 128.32, 128.24, 128.18, 128.13, 128.09, 128.06, 128.01, 127.95, 127.83, 127.72, 127.55, 127.35, 118.85, 79.31, 76.06, 75.68, 74.43, 74.25, 74.18, 74.13, 74.05, 73.74, 73.69, 73.54, 73.34, 72.82, 72.74, 70.91, 70.44, 68.99, 68.79, 67.99, 67.88, 67.68, 66.89, 66.63, 66.38, 66.15, 58.00, 54.34, 54.20, 53.73, 52.86, 51.26, 50.65, 48.65, 47.93, 45.83, 45.42, 45.21, 41.28, 40.54, 38.56, 33.82, 32.88, 32.50, 31.32, 31.15, 31.10, 30.89, 30.51, 29.70, 29.45, 29.40, 29.35, 29.29, 29.21, 29.07, 28.18, 28.14, 27.14, 25.21, 25.11, 24.95, 24.21, 23.39, 23.34, 22.85, 22.45, 19.58, 16.09, 15.30; IR (neat)  $\nu_{max}$  3337, 3086, 3064, 3032, 2926, 2857, 1741, 1644, 1532, 1450, 1384, 1328, 1267, 1210, 1108, 1042, 935, 813, 696; ESI MS *m/z* 2027.25 [M+H]<sup>+</sup>



**Cyclic Peptide (III.61)**

$\text{PdCl}_2(\text{PPh}_3)_2$  (2.92 mg, 0.00420 mmol) and  $\text{PPh}_3$  (3.28 mg, 0.012 mmol) were dissolved in dry THF (0.5 mL) and stirred under an atmosphere of argon for 15 min. This solution was then added to the allyl ester (0.338 g, 0.167 mmol) in dry THF (0.5 mL) via syringe and phenylsilane (0.041 mL, 0.333 mmol) was added drop-wise. The solution was stirred at room temperature under an atmosphere of argon for 6 hrs. The solvent was condensed and the crude material was loaded directly onto a silica gel column. Chromatography (2-50% EtOH- $\text{CHCl}_3$ ) afforded the carboxylic acid (0.312 g, 0.157 mmol, 94%) as a white solid. mp 112-115 °C;  $[\alpha]_D^{22}$  - 15.6 (c 1.0,  $\text{CHCl}_3$ )  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ , 25°C)  $\delta$  11.65-11.57 (m, 1H), 8.44-8.26 (m, 2H), 8.15-7.82 (m, 2H), 7.46-7.07 (m, 25H), 5.59-5.26 (m, 1H), 5.23-4.79 (m, 5H), 4.79-4.03 (m, 19H), 3.86-3.62 (m, 1H), 3.61-3.37 (m, 3H), 2.41-2.36 (m, 2H), 2.03-1.54 (m, 11H), 1.55-0.72 (m, 56H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  174.83, 174.58, 173.74, 172.46, 172.20, 170.00, 169.48, 169.07, 168.68, 168.09, 163.37, 155.68, 154.78, 153.38, 137.65, 136.44, 136.00, 134.35, 128.36, 128.14, 128.04, 127.96, 127.89, 127.82, 127.77, 127.67, 127.22, 79.24, 77.20, 74.16, 73.18, 72.85, 70.12, 67.92, 66.78, 55.67, 54.33, 51.87, 51.32, 48.69, 45.14, 40.92, 40.72, 40.34, 38.52, 34.06, 33.63, 33.14, 32.44, 32.30, 32.13, 31.83, 31.64, 31.19, 30.82, 30.27, 29.68, 29.39, 29.26, 28.07, 27.12, 26.51, 26.40, 26.19, 25.90, 24.99, 24.31, 23.31, 22.43, 22.27, 21.48, 21.29, 21.08, 14.86, 13.90, 13.65, 13.39; IR (neat)  $\nu_{\text{max}}$  3319, 2930, 2853, 1741, 1644, 1537, 1450, 1384, 1328, 1256, 1205, 1114, 1047, 752, 701; ESI MS  $m/z$  1913.57  $[\text{M}+\text{Na}]^+$

The linear peptide (0.274 g, 0.138 mmol) was treated with a 4N HCl-Dioxane solution (2.0 mL) and stirred under an atmosphere of argon for 1 hr. The volatiles were then

removed in vacuo and to the residue was added  $\text{CH}_2\text{Cl}_2$ /Hexanes (1:1, 10 mL). The solvent was condensed to remove any residual HCl and the crude residue was placed under high vacuum for several hours. The product was used without further purification. To this residue in dry DMF (67 mL) at 0 °C was added HOBt (0.091 g, 0.672 mmol), DIEA (0.47 mL, 0.267 mmol) and finally EDCI (0.129 g, 0.672 mmol). The mixture was stirred under an atmosphere of argon at this temperature for 48 hours. The solvent was then removed and the crude material was diluted with EtOAc (20 mL). The organic phase was washed with 1N HCl (1 x 10 mL), sat. aq.  $\text{NaHCO}_3$  (1 x 10 mL) and brine (1 x 10 mL). The EtOAc was dried over  $\text{Mg}_2\text{SO}_4$ , filtered and condensed. Silica gel chromatography (3-30% EtOH- $\text{CHCl}_3$ ) afforded the cyclic peptide as a white solid. mp 90-92 °C;  $[\alpha]^{22}_{\text{D}} - 34.6$  (c 1.0,  $\text{CHCl}_3$ ) ;  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ )  $\delta$  11.64-11.58 (m, 1H), 8.37 (t,  $J = 5.51, 5.51$  Hz, 1H), 8.28-8.20 (m, 1H), 8.14-8.01 (m, 2H), 7.97-7.81 (m, 2H), 7.76 (d,  $J = 8.83$  Hz, 1H), 7.71 (d,  $J = 7.78$  Hz, 1H), 7.60 (d,  $J = 5.71$  Hz, 1H), 7.57 (d,  $J = 6.31$  Hz, 1H), 7.47-7.19 (m, 25H), 5.32 (d,  $J = 14.39$  Hz, 1H), 5.21-5.18 (m, 2H), 5.05-4.99 (m, 2H), 4.97-4.90 (m, 1H), 4.88 (d,  $J = 10.95$  Hz, 1H), 4.82-4.75 (m, 1H), 4.75-4.64 (m, 3H), 4.61 (d,  $J = 3.80$  Hz, 1H), 4.59 (d,  $J = 3.82$  Hz, 1H), 4.55 (d,  $J = 4.31$  Hz, 1H), 4.54-4.51 (m, 1H), 4.51-4.37 (m, 5H), 4.34 (d,  $J = 3.48$  Hz, 1H), 4.33-4.29 (m, 1H), 4.26 (s, 1H), 4.24-4.11 (m, 3H), 3.88 (dd,  $J = 11.80, 5.91$  Hz, 1H), 3.82-3.66 (m, 2H), 3.67-3.46 (m, 4H), 2.35 (d,  $J = 8.83$  Hz, 1H), 1.96-1.86 (m, 1H), 1.84-1.53 (m, 15H), 1.50-0.92 (m, 56H);  $^{13}\text{C}$  (100MHz,  $\text{CDCl}_3$ )  $\delta$ ; IR (neat)  $\nu_{\text{max}}$  3323, 2927, 2853, 1735, 1649, 1537, 1455, 1379, 1205, 1108, 1047, 757, 696; ESI MS  $m/z$  1889.43  $[\text{M}+\text{Na}]^+$ .

### Deuterated Plusbacin A3 (III.62)

The cyclic peptide (0.075 g, 0.0401 mmol) and anisole (1 mL) in an HF reaction apparatus were purged with N<sub>2</sub> and cooled to -78 °C. HF gas was distilled into the reaction vessel to a total volume of approximately 5 mL. The reaction was warmed to 0 °C and stirred at this temperature for 1hrs. The HF was then evaporated under a steady stream of N<sub>2</sub> and the crude material was transferred to a round bottom flask using EtOH. The volatiles were removed in vacuo and the crude material was triturated with Et<sub>2</sub>O and centrifuged (3x). The crude material was HPLC purified (30-65% CH<sub>3</sub>CN/H<sub>2</sub>O 0.1% TFA over 120 minutes) to afford (0.0202 g, 0.0170 mmol, 43 %) of deuterated plusbacin A3 as a white solid. mp >250 °C decomposition;  $[\alpha]_D^{22} + 21.1$  (c 0.052, EtOH); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>CN/D<sub>2</sub>O/TFA, 500:500:1) δ 5.20-5.13 (m, 1H), 5.10 (t, *J* = 5.99, 5.99 Hz, 1H), 5.04 (d, *J* = 2.80 Hz, 1H), 4.92 (d, *J* = 2.31 Hz, 1H), 4.91 (m, 1H), 4.85 (d, *J* = 2.92 Hz, 1H), 4.78 (dd, *J* = 9.22, 2.04 Hz, 1H), 4.75 (d, *J* = 2.07 Hz, 1H), 4.73 (d, *J* = 2.25 Hz, 1H), 4.71-4.67 (m, 3H), 4.64 (d, *J* = 2.90 Hz, 1H), 4.61-4.57 (m, 3H), 4.49 (t, *J* = 7.16, 7.16 Hz, 1H), 4.41-4.36 (m, 1H), 4.34 (s, 1H), 4.33-4.30 (m, 1H), 4.28 (d, *J* = 2.13 Hz, 1H), 4.19-4.18 (m, 1H), 4.15-4.11 (m, 1H), 4.02-3.80 (m, 2H), 3.81-3.70 (m, 1H), 3.70-3.49 (m, 1H), 3.07 (dd, *J* = 14.19, 7.16 Hz, 2H), 2.64-2.41 (m, 2H), 2.12-1.99 (m, 1H), 1.90-1.85 (m, 1H), 1.82-1.77 (m, 1H), 1.66 (s, 1H), 1.55 (s, 3H), 1.44 (dd, *J* = 13.36, 6.72 Hz, 2H), 1.42-1.38 (m, 1H), 1.28-1.16 (m, 19H), 1.14 (d, *J* = 6.77 Hz, 1H), 1.12-1.07 (m, 3H); IR (KBr pellet)  $\nu_{max}$  3432, 2959, 2926, 2860, 1735, 1674, 1535, 1441, 1204, 1186, 1127; HR ESI TOF *m/z* 1165.6321[M+H]<sup>+</sup>

### III.11.10 MIC ASSAYS

Todd-Hewitt base (Hardy Diagnostics, Santa Maria, CA) was used to prepare Todd-Hewitt broth (THB) and agar (THA) per standard protocol. Microorganisms used in the screening included methicillin-resistant *Staphylococcus aureus* (ATCC # 33591), vancomycin-resistant *Enterococcus faecalis* (ATCC # 51299), *Streptococcus pyogenes* M49 strain NZ131 (Datta, V, et al. 2005. Mol. Micro. 56: 681), *Streptococcus agalactiae* strain A909 (Doran, KS, et al. 2002. J. Infect. Dis. 185: 196), *Escherichia coli* (ATCC # 25922), and *Pseudomonas aeruginosa* (ATCC # 27853). Compound dilutions were prepared in THB in sterile flat-bottom 96 well polystyrene plates (Costar # 7593, Corning Life Sciences, Inc. Lowell, MA). Screening controls for every assay included vancomycin (Abbott Laboratories, Chicago, IL) at the same concentration as the compounds/mixtures, vehicle alone, and no bacteria. Screening was done by inoculating 200  $\mu$ l THB containing the diluted compound mixtures with 2  $\mu$ l of an overnight culture of the desired bacterial strain using a Boekel replicator (Boekel Scientific, Feasterville, PA). The inoculated plates were incubated at 37°C for 24 hours. The minimal inhibitory concentration (MIC, in  $\mu$ g/ml), the lowest concentration of the inhibitor that completely prevented visible growth, was then determined turbidimetrically at  $A_{595}$ . To determine minimal bactericidal concentration (MBC, in  $\mu$ g/ml), Todd-Hewitt agar plates in the absence of antibiotic were inoculated with bacteria from the MIC assay using the Boekel replicator and were incubated at 37°C for 24 hours. The MBC was determined by careful visual examination of the agar plates for evidence of growth and was calculated as the lowest concentration of compound that completely inhibited growth in the agar. Each set of screening assays was repeated three times.

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# CHAPTER IV

## TOTAL SYNTHESIS OF WAP-8294A2

### IV.1 INTRODUCTION

Nature's inevitable ability to select for resistant pathogens has resulted in the need to replace obsolete antibiotics with new ones functioning via different modes of action. In recent years we have seen a renewed interest in the isolation of natural products with antibiotic activity, this has resulted in the discovery of new molecular platforms for development potent analogs and/or semi-synthetic derivatives.

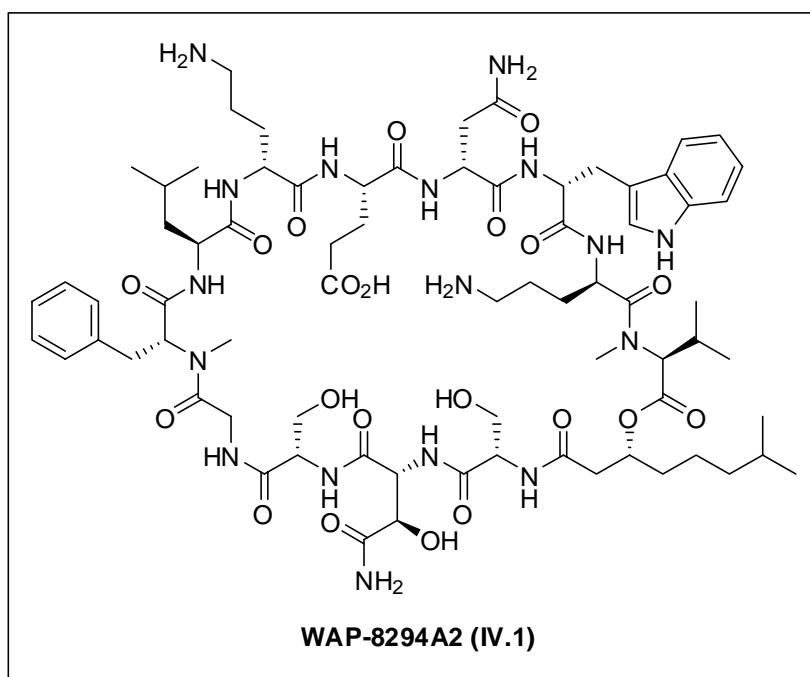


Figure IV.1: Structure of WAP-8294A2



WAP-8294A2 (**IV.1, Figure IV.1**) is a water soluble lipodepsipeptide antibiotic isolated from the culture broth of *Lysobacter* sp. WAP-8294 in the Shizuoka Prefecture, Japan. It is the major component of a complex consisting of 19 antibiotic peptides and is active against a diverse panel of gram-positive bacteria. In particular it has been shown to exhibit potent *in vitro* and *in vivo* activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci.<sup>1,2</sup> The peptide displayed MIC values of 0.1- 0.78 µg/mL for gram-positive bacteria and was inactive against gram-negative bacteria, (**Table IV.1**). *In vivo* efficacies were compared to vancomycin and assessed in the experimental systemic MRSA infection of mice. The ED<sub>50</sub> values of 0.38mg/kg indicated that WAP-8294A2 is 14 times more active than vancomycin. WAP-8294A2 also showed weak cytotoxicity against L1210 cells (mouse leukemia cell line), however, measurement of the acute toxicity revealed that it was not toxic by oral, intravenous, and intraperitoneal administration (200mg/kg, 50mg/kg, 100mg/kg respectively).<sup>2</sup>

**Table IV.1: Antimicrobial activity of WAP-8294A2<sup>2</sup>**

	MIC (µg/ml)
Strain	WAP-8294A2
<i>Staphylococcus aureus</i> JCM 8702 (MRSA)	0.78
w/ 10% FCS	<0.1
<i>S. aureus</i> No. 1 <sup>a</sup>	0.78
w/ 10% FCS	<0.1
<i>S. aureus</i> No. 11 <sup>b</sup>	0.78
w/ 10% FCS	<0.1
<i>S. aureus</i> ATCC 25923	0.78
w/ 10% FCS	<0.1
<i>S. aureus</i> ATCC 12228	0.78
w/ 10% FCS	<0.1
<i>Bacillus subtilis</i> ATCC 6633	0.78
w/ 10% FCS	<0.1
<i>Enterococcus faecium</i> CIP 103510 (VRE)	6.25
w/ 10% FCS	6.25
<i>Streptococcus pyogenes</i> ATCC 19615	6.25
w/ 10% FCS	25
<i>Escherichia coli</i> ATCC 25922	>100
w/ 10% FCS	>100
<i>Pseudomonas aeruginosa</i> ATCC 9027	>100
w/ 10% FCS	>100
<i>Candida albicans</i> TIMM 0239	>100
w/ 10% FCS	>100
<i>Aspergillus fumigatus</i> IAM 2004	>100
w/ 10% FCS	>100
<sup>a</sup> clinical isolate, FCS: Fetal calf serum	

A recent biological evaluation investigated the mode of action of WAP-8294A2 and determined that it functions by compromising the integrity of the bacterial cell membrane. The bactericidal activity was compared to that of vancomycin and showed clear activity within 2 hours. However, after 6 hours vancomycin displayed only weak bactericidal action. Furthermore, the same study demonstrated that in contrast to the

inhibitory effects of vancomycin, the addition of diacetyl-Lys-D-Ala-D-Ala to whole cells, did not suppress the depsipeptide's activity.<sup>2</sup> This experiment indicated that WAP-8294A2 inhibited bacterial cell growth via a different mode of action from that of vancomycin and suggests that pathogenic mechanisms of resistance to vancomycin would be ineffective against WAP-8294A2.

Additional experiments of interest include a disc diffusion test performed in the presence of MRSA. The results demonstrated that the antibiotic activity was inhibited by the addition of phosphatidyl glycerol or cardiolipin to the disc, suggesting that the antibiotic interacts selectively with phospholipids in the target cell causing membrane damage.<sup>2</sup>

## IV.2 RESEARCH DESIGN AND SYNTHESIS

As mentioned previously in Chapter II, the objective of our laboratory is focused on the development of new antimicrobials active against drug resistant pathogens, in particular MRSA and VRE. With its promising biological profile, we believe that WAP-8294A2 makes an excellent candidate for total synthesis.

\ An amino acid analysis of the acidic hydrolysate supported by HPLC, M.S. and NMR revealed the presence of 12 amino acids and one hydroxy fatty acid residue. The chiralities of the amino acids were determined by direct comparison with reference amino acids via chiral HPLC and by chiral TLC.

A survey of the WAP-8294A2 amino acid constitution shows a backbone decorated with unusual amino acids residues. The cyclic peptide contains two D-ornithines, glutamic acid, D-asparagine, D-*threo*- $\beta$ -hydroxy-asparagine, D-tryptophan, *N*-

Me-valine, serine, glycine, D-*N*-Me-phenylalanine, leucine, and an (R)-3-OH-7-Me-octanoic acid residue. Although other isolated cyclic peptide antibiotics contain 3-hydroxy fatty acids, the 3-OH-7-Me-octanoic acid of WAP-8294A2 is the first to be isolated from an antibiotic natural product.

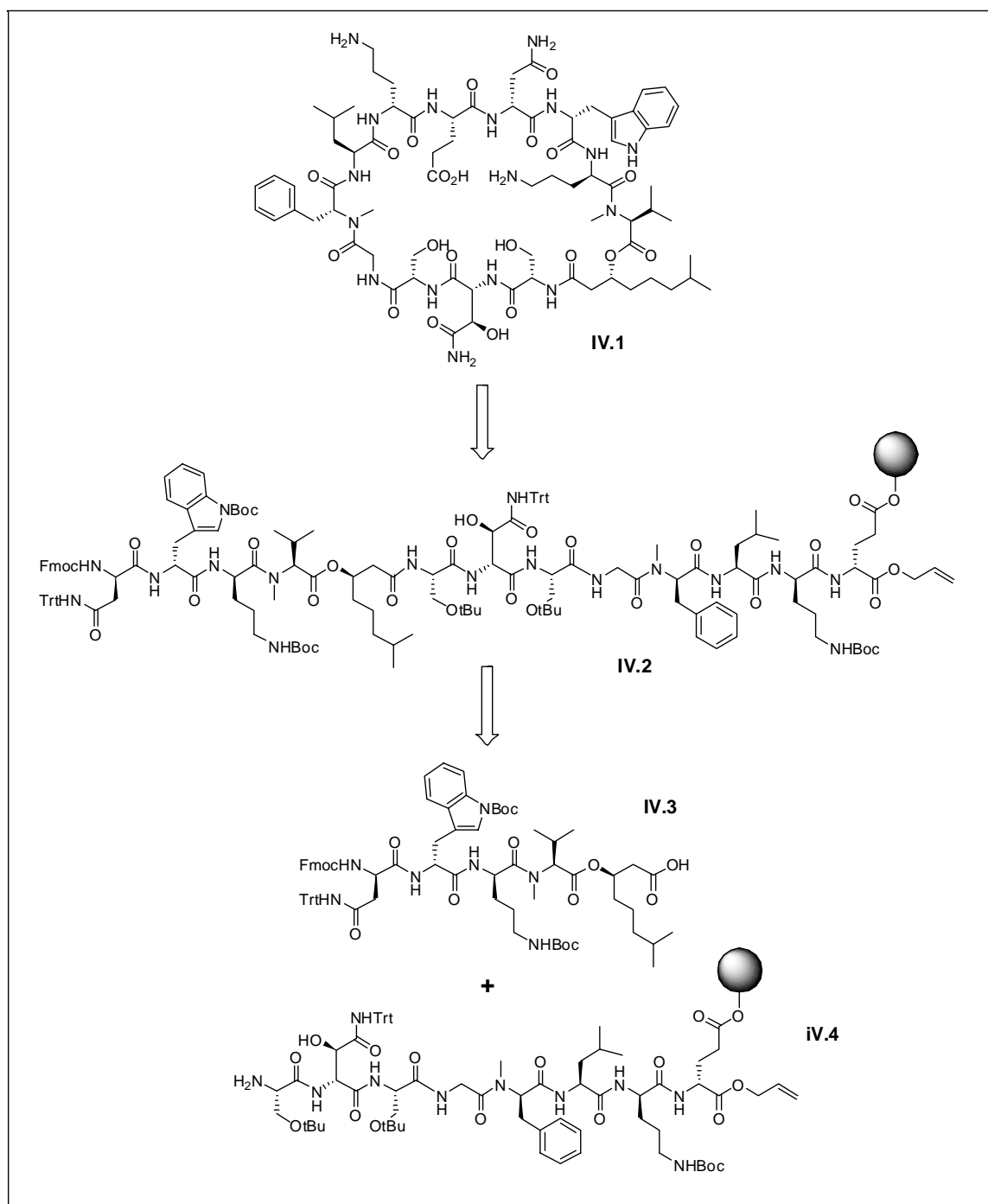
The synthetic challenges associated with the total synthesis of WAP-8294A2 (**IV.1**) include the presence of non-proteogenic amino acids, the sensitive depsipeptide lactone linkage<sup>3</sup>, the sterically hindered *N*-methyl amino acids<sup>4</sup> and the 40-membered macrocyclic peptide backbone.

#### IV.2.1 RETROSYNTHETIC ANALYSIS

The retrosynthetic analysis for our synthesis is outlined in **Figure IV.2**. The sites selected for backbone bond formation and macrocyclization were driven by the complexity of each fragment as well as the utility of an on-resin macrocyclization. The first disconnection illustrates the key macrocyclization event which would generate the 40-membered macrocycle from a linear precursor (**IV.2**). Although one could envision a variety of possible sites for this amide bond formation, we hypothesized that the Glu/D-Asn junction would prove ideal for two main reasons. First, the side chain anchored Glu would easily allow for the solid phase elaboration of the linear peptide while maintaining the C-terminal allyl ester for facile deprotection. Second, literature precedents have shown an increased efficiency for the D-L couplings relative to identical coupling reactions involving D,D or L,L coupling partners<sup>5</sup>. The clear disadvantage of this strategy is that only a portion of the scaffold could be installed in a linear fashion using

standard Fmoc Solid phase protocols. Due to the sensitivity of the depsipeptide bond, known to readily undergo base catalyzed beta-elimination, we chose to take a convergent approach via coupling of the two key fragments. We envisioned a solution synthesis of the ester containing fragment **IV.3** which would be coupled to the resin bound amine, fragment **IV.4**. From here, the strategy included a microwave assisted solid phase synthesis of the resin bound hexapeptide using commercially available intermediates. The depsipeptide fragment however would require the incorporation of residues not commercially available, therefore making it the limiting component of the synthesis.

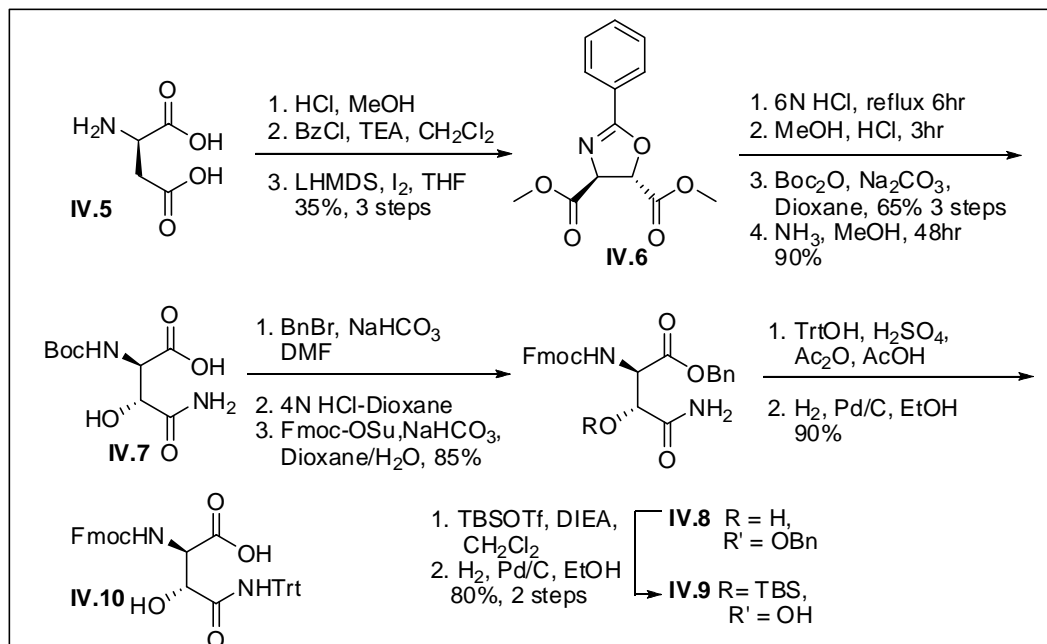
Additionally, the use of a solid phase strategy would allow easy access to a variety of analogs required for future SAR studies. With a synthetic plan in mind we then moved forward with the synthesis of the non-natural amino acids and their elaboration into the peptide backbone of WAP-8294A2.



**Figure IV.2: Retrosynthetic analysis of WAP-8294A2**

## IV.2.2 SYNTHESIS OF NON-PROTEOGENIC AMINO ACIDS

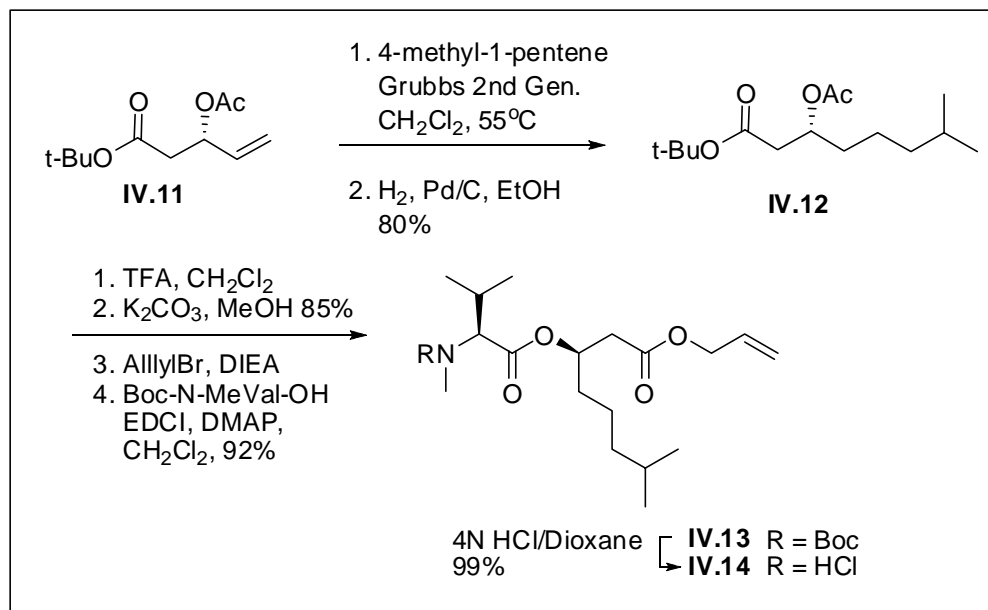
Synthesis of the orthogonally protected Fmoc-D-*threo*- $\beta$ OH-Asn(Trt)-OH (**IV.10**) was accomplished according to a procedure previously developed in our laboratory<sup>6</sup>, **Scheme IV.1**. The key feature of this route is the stereoselective  $\beta$ -hydroxylation via displacement of a  $\beta$ -iodo aspartic acid derivative generated *in-situ* from (**IV.5**) (LHMDS, I<sub>2</sub>, THF, 35% 3 steps). Following a series of protecting group manipulations, removal of the benzyl ester (H<sub>2</sub>, Pd/C, EtOH, 90%) provided an orthogonally protected acid (**IV.10**). Although the  $\beta$ -OH functionality was left unprotected, it was suitable for Fmoc solid phase peptide synthesis (SPPS) when utilized with DEPBT<sup>7</sup> for amide bond formation. Alternatively, protection of the hydroxyl (**IV.8**) as the silyl ether (TBDMSOTf, DIEA, CH<sub>2</sub>Cl<sub>2</sub>) and subsequent removal of the benzyl ester (H<sub>2</sub>, Pd/C, EtOH, 90% 2 steps) afforded intermediate (**IV.9**)



**Scheme IV.1: Preparation of the orthogonally protected Fmoc-D-threo-βOH-Asn(Trt)-OH**

The assembly of the 3-hydroxyoctanoic acid residue is depicted in **Scheme IV.2**. The synthesis of this residue utilized an olefin cross metathesis<sup>8</sup> reaction between the allylic acetate<sup>9-12</sup>(**IV.11**) and commercially available 4-methyl-1-pentene (Grubbs catalyst, CH<sub>2</sub>Cl<sub>2</sub>, reflux). Subsequent hydrogenation (H<sub>2</sub>, Pd/C, EtOH, 80% 2 steps) provided the β-acetoxy ester (**IV.12**) in excellent yields. Following a series of protecting group manipulations, the alcohol was coupled to Boc-*N*-Me-Val-OH (EDCI, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, -15°C-rt, 92%), providing the depsipeptide **IV.13**. Cleavage of the N-terminal Boc protecting group gave the key fragment **IV.14** poised for incorporation into the WAP-8294A2 backbone.



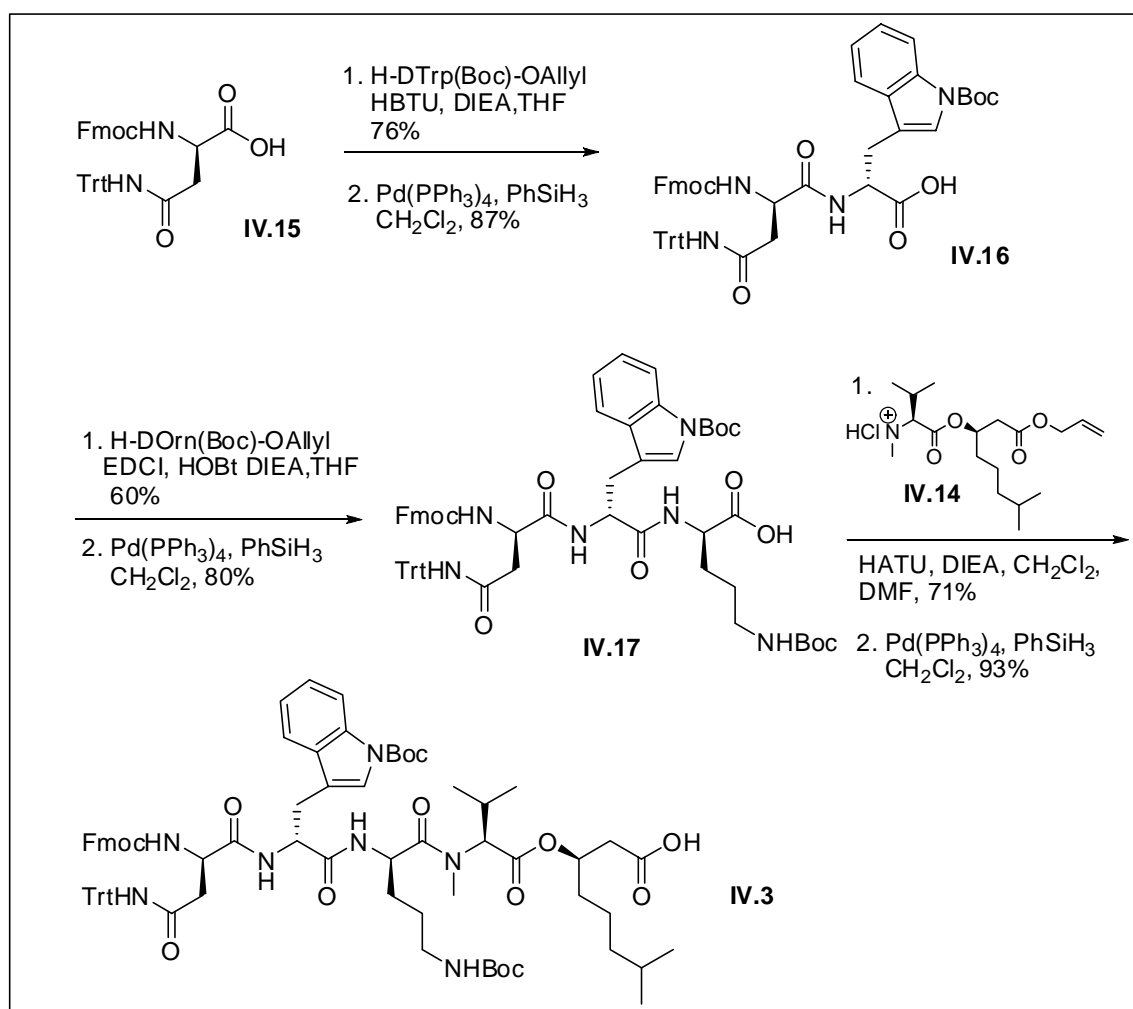


**Scheme IV.2:** Synthesis of 3-OH-Me-Octanoic acid residue and coupling to Boc-NMeVal-OH

### IV.2.3 SCAFFOLD ASSEMBLY

The synthesis of pentapeptide fragment (**IV.3**) is shown in **Scheme IV.3**. Our strategy was to carry out its assembly in solution, to avoid complications on the solid phase, such as the potential of a base promoted  $\beta$ -elimination at the depsipeptide linkage and the difficulties associated with the acylation of *N*-Me-amino acids<sup>4</sup> (steric and racemization issues). Coupling of a suitably protected Asn derivative (**IV.15**) with H-D-Trp(Boc)-OAllyl (HBTU, DIEA, THF, 76%) followed by a clean allyl ester deprotection ( $\text{Pd}(\text{Ph}_3)_4$ ,  $\text{PhSiH}_3$ ,  $\text{CH}_2\text{Cl}_2$ , 87%) afforded **IV.16**. This dipeptide fragment was then condensed with H-D-Orn(Boc)-OAllyl and the allyl ester was removed under standard conditions. The condensation of tripeptide (**IV.17**) with the sterically hindered *N*-Me

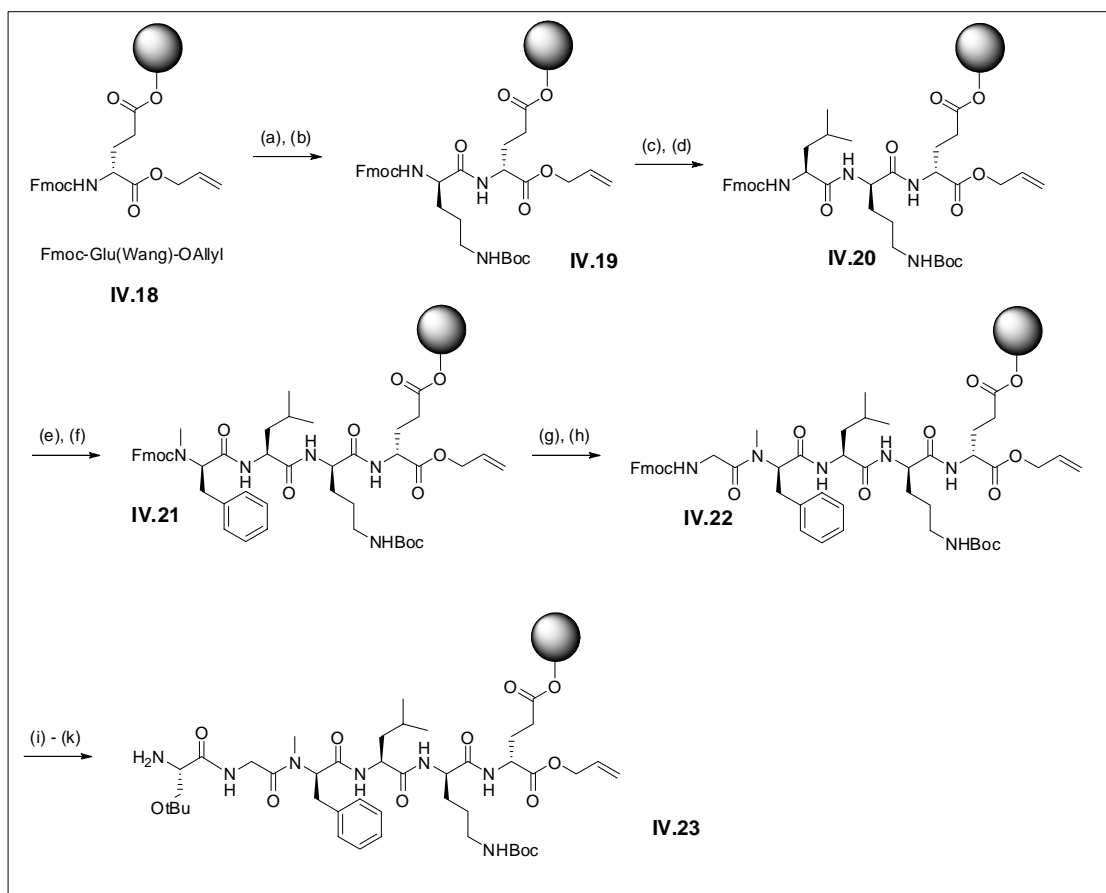
amino functionality of the depsipeptide fragment (**IV.14**) afforded the fully protected pentapeptide in an good yield (HATU, DIEA,  $\text{CH}_2\text{Cl}_2$ , DMF, 71%). Allyl ester deprotection provided the necessary carboxylic acid (**IV.3**) for subsequent attachment to the resin bound peptide.



**Scheme IV.3: Solution Synthesis of Pentapeptide Fragment**

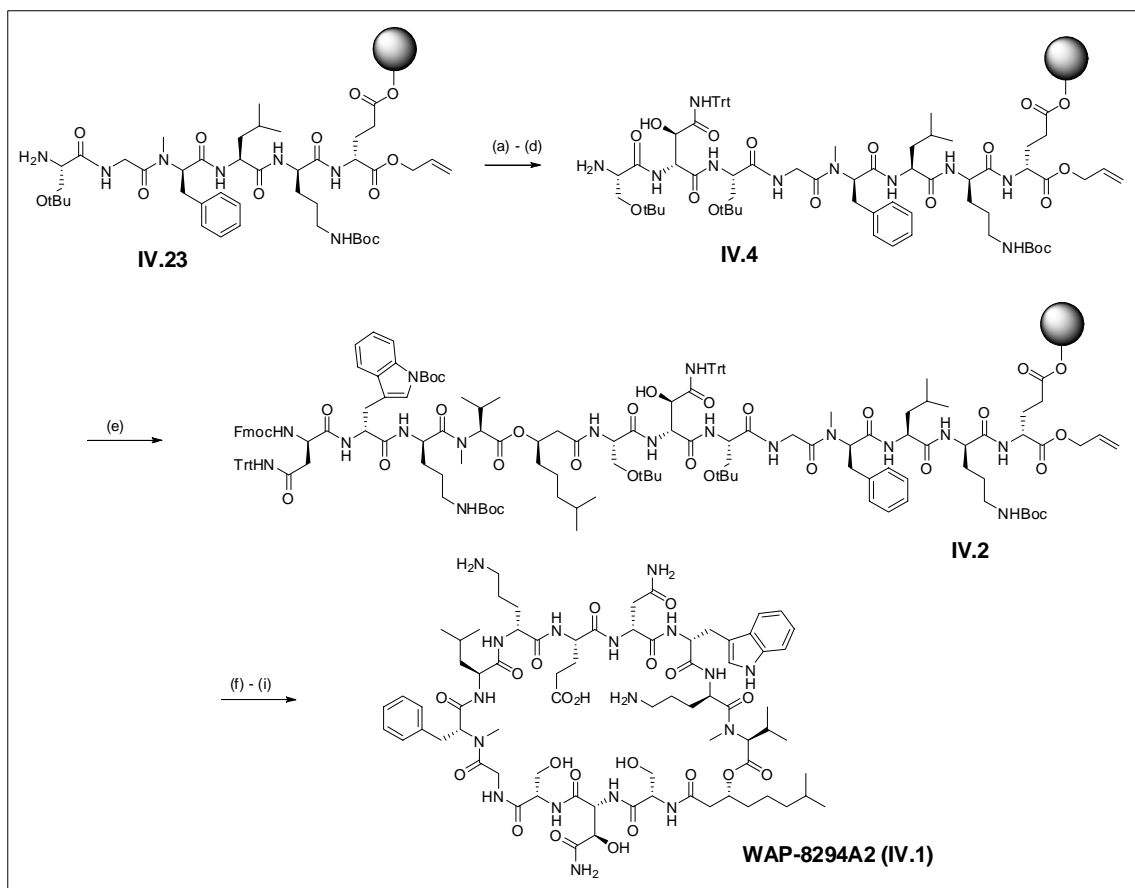
Consideration of the many possible macrocyclization options led us to take advantage of an on resin, L-D coupling at the Glu-Asn site.<sup>13</sup> The C-terminal hexamer (**IV.23**) was assembled on a CEM automated peptide synthesizer (**Scheme IV.4**) via a

commercially available side chain anchored Fmoc-Glu(Wang)-OAllyl residue using standard Fmoc SPPS protocols, (HATU activation with 20% piperidine/DMF/0.1M HOBT deprotection).



**Scheme IV.4: Solid Phase Synthesis of C-Terminal Hexamer.** (a) 20% piperidine/DMF/0.1M HOBT; (b) Fmoc-D-Orn(Boc)-OH, HATU, DIEA, NMP; (c) 20% piperidine/DMF/0.1M HOBT; (d) Fmoc-Val-OH, HATU, DIEA, NMP; (e) 20% piperidine/DMF/0.1M HOBT; (f) Fmoc-D-N-Me-Phe-OH, HATU, DIEA, NMP; (g) 20% piperidine/DMF/0.1M HOBT; (h) Fmoc-Gly-OH, HATU, DIEA, NMP; (i) 20% piperidine/DMF/0.1M HOBT; (j) Fmoc-Ser(t-Bu)-OH, HATU, DIEA, NMP (k); 20% piperidine/DMF/0.1M HOBT

**Scheme IV.5** presents the final step of the total synthesis of WAP-8294A2 (**IV.1**). The Fmoc-D-*threo*- $\beta$ OH-Asn(Trt)-OH (**IV.10**, 1.1 equiv. ) and Fmoc-Ser(Ot-Bu)-OH (1.1 equiv.) residues were installed manually. (DEPBT, DIEA, DMF, 12hrs). The peptapeptide (**IV.3**, 1.01 equiv.), previously prepared in solution, was coupled to a resin bound amine (**IV.23**) (DEPBT, DIEA, DMF). To set the stage for the key macrocyclization event the C-terminal allyl ester was deprotected ( $\text{Pd}(\text{Ph}_3)_4$ ,  $\text{PhSiH}_3$ ,  $\text{CH}_2\text{Cl}_2$ ) under standard conditions. The amino functionality was unmasked utilizing a modified Fmoc deprotection protocol to afford the macrocyclization precursor, **IV.2**. (10% piperidine / DMF / 0.05M HOBt). A head to tail macrocyclization was then carried out according to the conditions developed by Carpino and coworkers<sup>13</sup> (DIC, HOAt, DIEA,  $\text{CH}_2\text{Cl}_2$ , DMF). Finally a global deprotection afforded the target WAP-8294A2 (**IV.1**), which after HPLC purification was identical in all respects with the reported spectral data for the authentic compound.



**Scheme IV.5: Final Steps toward WAP-8294A2.** (a) Fmoc- $\beta$ OH-D-Asn(Trt)-OH, DEPBT, DIEA, DMF; (b) 20% piperidine/DMF; (c) Fmoc-Ser(Ot-Bu)-OH, DEPBT, DIEA, DMF; (d) 20% piperidine/DMF (e) Pentapeptide **IV.3**, DEPBT, DIEA (f). Pd(PPh<sub>3</sub>)<sub>4</sub>, PhSiH<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub> (g) 10% Piperidine/DMF/0.05% HOBT (h) DIC, HOAt, DIEA, CH<sub>2</sub>Cl<sub>2</sub>, DMF(1:1); (i) TFA/TES/H<sub>2</sub>O, 20% yield of the final product

It should be noted that an alternate route employing the TBS protected  $\beta$ -OHAsn (**IV.9**) for the fully automated assembly of octamer (**IV.4**), resulted in considerably lower yields (c.a. 5%) of the target compound. Furthermore, this route generated impurities which resulted in a less efficient and more difficult final purification.

### IV.3 CONCLUSIONS

The first total synthesis of the potent depsipeptide antibiotic WAP-8294A2 was completed. Our synthesis utilized a convergent approach which took advantage of solution and solid phase chemistry. We carried out the asymmetric synthesis of non-proteogenic amino acids and their elaboration into the peptide backbone. Our design element and strategy allowed a successful formation of a 40-membered macrocycle through a solid phase on resin macrocyclization event. Also of interest was the rapid assembly and incorporation of the *N*-Methyl amino acids employing microwave SPPS technology. In light of the difficulties associated with solid phase synthesis depsipeptides, it is highly unlikely that a fully automated solid phase synthesis of WAP-8294A2 is possible. However, our strategy will allow rapid systematic preparation of analogs from which the role of each residue and side chain may be assessed.

With the total synthesis of WAP-8294A2 complete, future efforts will be directed toward the elucidation of its mechanism of activity. We hope to achieve this understanding through SAR studies via rational analog design as well as NMR binding studies with a diverse library of membrane phospholipids and bacterial cell wall biosynthetic precursors.

## IV.4 EXPERIMENTAL SECTION

**General.** All reactions were carried out in flame-dried glassware under an atmosphere of dry nitrogen or argon. Unless otherwise mentioned, solvents were purified as follows. All solvents were dried using a Seca Solvent System (Glass Contour). All other commercially available reagents were used as received.

$^1\text{H}$  NMR spectra were measured at 300 MHz on a Varian Mercury instrument, at 400 MHz on a Varian Gemini-400, or at 500 MHz on a Varian VXR-500 instrument.  $^{13}\text{C}$  NMR spectra were measured at 100 MHz on a Varian Gemini spectrometer. Chemical shifts are reported relative to the central line of residual solvent. Infrared spectra were recorded using a Nicolet IR/42 spectrometer FT-IR (thin film, NaCl cells). High-resolution mass spectra were obtained via electrospray ionization on an Agilent ESI-TOF spectrometer. Optical rotations were measured on a Perkin–Elmer polarimeter (Model 241) using a 1 mL capacity quartz cell with a 10 cm path length.

Analytical thin layer chromatography (TLC) was performed using Whatman glass plates coated with a 0.25 mm thickness of silica gel containing PF 254 indicator, and compounds were visualized with UV light, cerium molybdate stain or ninhydrin stain. Analytical high performance liquid chromatography (HPLC) was performed on a Beckman-Coulter instrument (System Gold) with diode array detection. Analysis was carried out using Phenomenex Jupiter reverse-phase ( $\text{C}_{18}$ ) column (10m particle size, 300 Å pore size, 250 mm length x 4.6 mm diameter) with mobile phases consisting of either 0.05% trifluoroacetic acid in either water or acetonitrile. Preparatory HPLC purifications (Phenomenex Jupiter  $\text{C}_{18}$  reverse-phase column, 10m particle size, 300 Å

pore size, 250 mm length x 21.2 mm diameter) were performed with a Waters Millipore Model 510 System with an automated gradient collector and a Model 2487 Dual Absorbance Detector. Flash chromatography purifications were performed using Silicycle 60 Å, 35-75 mm silica gel or Biotage purification system (SP1 HPFC system). All compounds purified by chromatography were sufficiently pure for use in further experiments, unless otherwise noted.

**(S)-tert-butyl 3-acetoxy-7-methyloctanoate (IV.12)**

To a stirring solution of the allylic acetate (1.00g, 4.67mmol) and 4-methyl pentene (2.95 mL, 23.1 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (22 mL) was added Grubb's 2<sup>nd</sup> Generation catalyst (0.247 g, 0.291 mmol). The flask was fitted with condenser and stirred at 50°C under a steady stream of argon for 24 hours. The reaction mixture was then reduced in volume and loaded directly onto a silica gel column. Chromatography (0-10 % EtOAc / Hexanes) afforded the product (1.034 g, 3.82 mmol, 82.0 %) as a pale yellow oil. The unreacted lipid was recovered quantitatively and recycled.  $[\alpha]_{\text{D}}^{22} +3.0$  (c=1,  $\text{CHCl}_3$ )  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.75 (m, 1H), 5.56 (dd,  $J = 13.66, 7.24$  Hz, 1H), 5.40 (dd,  $J = 15.33, 7.32$  Hz, 1H), 5.29 (s, 1H), 4.11 (q,  $J = 7.15, 7.14, 7.14$  Hz, 1H), 2.54 (ddd,  $J = 20.99, 15.15, 6.95$  Hz, 2H), 1.90 (t,  $J = 6.87, 6.87$  Hz, 2H), 1.66-1.55 (m, 2H), 1.55-1.45 (m, 1H), 1.42 (s, 9H), 1.30-1.21 (m, 8H);  $^{13}\text{C}$  NMR (400MHz,  $\text{CDCl}_3$ )  $\delta$  170.1, 169.3, 134.213, 128.201, 81.090, 71.526, 41.704, 41.400, 31.820, 28.226, 22.886, 22.466, 22.427, 21.400, 14.348; IR (Neat)  $\nu_{\text{max}}$  3006, 2979, 2953, 2932, 2868, 2361, 1736, 1472, 1390, 1363, 1239, 1157; ESI MS  $m/z$  295.01  $[\text{M}+\text{Na}]^+$



The olefin (2.96 g, 10.9 mmol) was dissolved in Ethanol (13.6 mL) and 5% Pd/C (0.44 g) was added. The flask was sealed and purged with hydrogen. The mixture was stirred for 24 hours, and upon completion filtered through a celite pad. The solvent was removed and silica gel chromatography (10% EtOAc / Hexanes) afforded (S)-tert-butyl 3-acetoxy-7-methyloctanoate (2.139 g, 7.86 mmol, 72.1 %) as a colorless oil.  $[\alpha]_D^{22} +2.90$  (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  5.19 (m, 1H), 2.46 (m, 2H), 2.02 (d, J = 0.70 Hz, 3H), 1.54 (m, 3H), 1.42 (t, J = 1.29, 1.29 Hz, 9H), 1.27 (s, 2H), 1.16 (s, 2H), 0.86 (t, J = 5.66, 5.66 Hz, 6H); <sup>13</sup>C NMR (100MHz, CDCl<sub>3</sub>)  $\delta$  170.30, 169.68, 80.75, 70.79, 40.53, 38.58, 34.21, 27.97, 27.77, 22.86, 22.58, 22.55, 22.51, 22.47, 21.10; IR (Neat)  $\nu_{\max}$  3004, 2950, 2934, 2870, 1744, 1461, 1431, 1365, 1290, 1231, 1156, 1023; ESI MS  $m/z$  393.09 [M+NH<sub>4</sub>]<sup>+</sup>

**(R)-allyl 7-methyl-3-((S)-3-methyl-2-(methylamino)butanoyloxy)octanoate hydrochloride (IV.14)**

(R)-tert-butyl 3-acetoxy-14-methylpentadecanoate (2.135 g, 7.84 mmol) was treated with a solution of TFA / CH<sub>2</sub>Cl<sub>2</sub> (1:1, 24 mL) and allowed to stir at room temperature overnight. The volatiles were removed in vacuo and the residue was left under high vacuum for several hours. The crude material was dissolved in MeOH (113 mL) and K<sub>2</sub>CO<sub>3</sub> (20.69 g, 150 mmol) was added. The mixture was stirred for 12 hours and the solvent was removed. EtOAc (50ml) was added followed by the careful addition of 1N HCl while stirring. The aq. phase was extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with brine, dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and

condensed. Chromatography (EtOAc) afforded (S) 3-hydroxy-7-methyloctanoic acid (1.098 g, 6.30 mmol, 80 %) as a yellow oil.  $[\alpha]_D^{22}$  -25.8 (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ),  $\delta$  5.35 (s, 1H), 4.04 (s, 1H), 2.51 (td,  $J$  = 15.71, 12.02, 12.02 Hz, 2H), 1.51 (m, 5H), 1.34 (s, 1H), 1.20 (dd,  $J$  = 7.12, 4.45 Hz, 2H), 0.88 (dd,  $J$  = 6.58, 2.94 Hz, 6H);  $^{13}\text{C}$  NMR (100MHz,  $\text{CDCl}_3$ )  $\delta$  177.32, 41.21, 38.66, 36.63, 27.84, 27.19, 25.68, 23.20, 22.51; IR (Neat)  $\nu_{\text{max}}$  2955, 2932, 2901, 2846, 1710, 1465, 1408, 1382, 1280, 1240, 1199, 1168, 1135, 1083; ESI MS  $m/z$  196.97  $[\text{M}+\text{Na}]^+$

(R)-3-hydroxy-7-methyloctanoic acid (0.301 g, 1.73 mmol) was treated with allyl bromide (3.00 mL, 34.6 mmol) and DIEA (0.60 mL, 3.46 mmol) and stirred at room temperature for 3 hours. The mixture was diluted with  $\text{Et}_2\text{O}$  (20mL) and washed with 1N HCl (20 mL), sat. aq.  $\text{NaHCO}_3$  (20 mL), and brine (20 mL). The organic phase was dried over  $\text{Mg}_2\text{SO}_4$ , filtered and condensed. Purification on a Biotage SP1 flash chromatography system afforded (R)-allyl 3-hydroxy-7-methyloctanoate (0.359 g, 1.68 mmol, 97 %) as a colorless oil.  $[\alpha]_D^{22}$  -17.7 (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  5.87 (qd,  $J$  = 10.81, 5.79, 5.77, 5.77 Hz, 1H), 5.24 (ddd,  $J$  = 13.78, 11.08, 0.96 Hz, 2H), 4.57 (d,  $J$  = 5.77 Hz, 2H), 3.97 (ddd,  $J$  = 11.79, 7.86, 3.80 Hz, 1H), 2.95 (s, 1H), 2.45 (ddd,  $J$  = 25.25, 16.31, 6.09 Hz, 2H), 1.56-1.43 (m, 1H), 1.42-1.36 (m, 2H), 1.32-1.24 (m, 1H), 1.22-1.07 (m, 2H), 0.83 (d,  $J$  = 6.67 Hz, 6H);  $^{13}\text{C}$  NMR (100MHz,  $\text{CDCl}_3$ )  $\delta$  172.58, 131.78, 118.45, 67.92, 65.20, 41.26, 38.70, 36.70, 27.81, 23.17, 22.47; IR (film)  $\nu_{\text{max}}$  3484, 2960, 2873, 1737, 1475, 1414, 1368, 1287, 1170, 991, 930 ; ESI MS  $m/z$  215.02  $[\text{M}+\text{H}]^+$

To a solution of (R)-allyl 3-hydroxy-7-methyloctanoate (0.716 g, 3.34 mmol), Boc-*N*-Me-Val-OH (0.500 g, 2.16 mmol), and DMAP (0.130 g, 1.08 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (7.2 mL) at -15°C was added EDCI (0.830 g, 4.32 mmol) in one portion. The reaction was purged with argon then slowly warmed to room temperature. The reaction was stirred for 8 hrs then diluted with EtOAc (20 mL) and washed with 1N HCl (20 mL), sat. aq. NaHCO<sub>3</sub> (20 mL), and brine (20 mL). The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and condensed. Purification on a Biotage SP1 Flash system (3-40 % EtOAc-Hexanes) afforded the Boc protected intermediate (0.854 g, 2.00 mmol, 92 %) as a colorless oil.  $[\alpha]_D^{22}$  -58.1 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 5.81 (qd, *J* = 10.95, 5.75, 5.75, 5.74 Hz, 1H), 5.18 (m, 3H), 4.48 (d, *J* = 5.76 Hz, 2H), 2.70 (d, *J* = 7.58 Hz, 3H), 2.61-2.41 (m, 2H), 2.08 (m, 1H), 1.61-1.42 (m, 3H), 1.37 (s, 9H), 1.30-1.15 (m, 3H), 1.14-1.03 (m, 2H), 0.91-0.85 (m, 3H), 0.82-0.74 (m, 10H); <sup>13</sup>C NMR (100MHz, CDCl<sub>3</sub>) δ 170.42, 169.96, 169.67, 169.58, 155.95, 155.35, 131.76, 131.72, 118.26, 118.24, 79.82, 79.56, 70.78, 70.66, 65.20, 65.09, 64.69, 63.11, 38.84, 38.29, 33.95, 33.83, 33.74, 30.10, 29.68, 28.14, 27.60, 27.21, 27.17, 22.67, 22.39, 22.34, 22.30, 19.66, 19.54, 18.76, 18.47; IR (film)  $\nu_{max}$  2960, 2873, 1754, 1702, 1470, 1389, 1363, 1312, 1261, 1175, 1144, 991, 940, 889, 772 ; ESI MS *m/z* 450.20 [M+Na]<sup>+</sup> This material was then treated with a 4N HCl-Dioxane solution and stirred for 1 hour at room temperature. The volatiles were removed in vacuo. A solution of Hexanes/CH<sub>2</sub>Cl<sub>2</sub> (1:1, 5 mL) was added and removed in vacuo to get rid of any residual HCl. The hydrochloride was left under high vacuum for several hours and used without further purification.

**Fmoc-D-Asn(Trt)-D-Trp(Boc)-OH (IV.16)**

To a solution of Fmoc-D-Asn(Trt)-OH (1.96 g, 3.28 mmol), H-D-Trp(Boc)-OAllyl (1.30 g, 3.77 mmol), and DIEA (1.14 mL, 6.57 mmol) in THF/DMF (3:2, 10 mL) at 0°C was added HBTU (1.50 g, 3.94 mmol). The reaction was allowed to 12 hours while slowly warming to room temperature then quenched with EtOAc (50 mL). The solution was then washed with 1N HCl (20 mL), sat. aq. NaHCO<sub>3</sub> (20 mL), and brine (20 mL). The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Purification on a biotage SP1 flash system (12-100% EtOAc-Hexanes) afforded the dipeptide (2.30 g, 2.49 mmol, 76%) as a tan foam. mp. 98-99°C;  $[\alpha]_D^{22}$  -22.3 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.11 (d, *J* = 6.07 Hz, 1H), 7.75 (d, *J* = 7.34 Hz, 1H), 7.58-7.47 (m, 3H), 7.44-7.32 (m, 4H), 7.30-7.17 (m, 20H), 6.92 (s, 1H), 6.48 (d, *J* = 7.65 Hz, 1H), 5.75 (qd, *J* = 10.51, 5.92, 5.92, 5.89 Hz, 1H), 5.25-5.11 (m, 2H), 4.81 (q, *J* = 6.49, 6.49, 6.48 Hz, 1H), 4.57 (s, 1H), 4.49 (d, *J* = 5.77 Hz, 1H), 4.33-4.24 (m, 2H), 4.20-4.10 (m, 1H), 3.23-2.92 (m, 3H), 2.68 (dd, *J* = 15.54, 5.41 Hz, 1H), 1.69 (s, 1H), 1.64-1.55 (m, 10H); <sup>13</sup>C NMR (100MHz, CDCl<sub>3</sub>) δ 170.764, 170.636, 170.437, 156.229, 149.491, 144.255, 143.825, 143.579, 141.217, 141.204, 135.328, 131.273, 130.198, 128.675, 127.963, 127.669, 127.098, 127.060, 125.160, 124.444, 124.370, 122.579, 119.927, 118.937, 115.174, 83.497, 70.865, 67.364, 66.033, 60.370, 53.027, 51.339, 46.976, 38.090, 28.131, 27.492, 21.025, 14.175; IR (film)  $\nu_{max}$  3340, 3051, 2985, 2919, 1731, 1681, 1483, 1450, 1359, 1318, 1244, 1153, 1087, 740, 675 ; ESI MS *m/z* 945.22 [M+Na]<sup>+</sup>

To a stirring solution of Fmoc-D-Asn(Trt)-D-Trp(Boc)-OAllyl (2.17 g, 2.35 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (9.4 mL) was added  $\text{Pd}(\text{PPh}_3)_4$  (0.068 g, 0.059 mmol). The reaction was purged with argon and to this was added  $\text{PhSiH}_3$  (0.58 mL, 4.71 mmol) via syringe. The reaction was stirred 2 hours at room temperature and the purified immediately on a Biotage SP1 flash system (2-20%  $\text{MeOH}-\text{CHCl}_3$ ) to afford the carboxylic acid (1.804 g, 2.04 mmol, 87 %), as a white solid. mp. 131-133 °C;  $[\alpha]_D^{22}$  - 7.9 (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.50 (s, 1H), 8.01 (d,  $J$  = 7.05 Hz, 1H), 7.89 (d,  $J$  = 7.57 Hz, 1H), 7.71-7.68 (m, 2H), 7.67-7.58 (m, 3H), 7.58-7.52 (m, 1H), 7.43 (s, 1H), 7.40 (dd,  $J$  = 15.28, 7.78 Hz, 2H), 7.29 (t,  $J$  = 7.31, 7.31 Hz, 2H), 7.25-7.12 (m, 20H), 4.33 (s, 1H), 4.28 (dd,  $J$  = 9.94, 6.98 Hz, 1H), 4.23-4.13 (m, 2H), 3.16 (m, 1H), 3.04 (dd,  $J$  = 14.18, 7.23 Hz, 1H), 2.61 (m, 1H), 1.56 (s, 1H);  $^{13}\text{C}$  NMR (100MHz,  $\text{CDCl}_3$ )  $\delta$  171.544, 170.468, 157.559, 156.970, 156.318, 149.633, 144.194, 143.798, 143.559, 141.156, 135.227, 134.226, 132.101, 130.300, 127.632, 127.020, 125.177, 124.356, 122.589, 119.843, 119.010, 115.160, 83.594, 70.825, 67.351, 53.336, 51.520, 46.857, 38.190, 28.089, 26.926; IR (film)  $\nu_{\text{max}}$  3327, 3056, 3013, 2969, 2925, 1728, 1658, 1510, 1492, 1449, 1361, 1326, 1256, 1143, 1082, 758, 697; ESI MS  $m/z$  905.22  $[\text{M}+\text{Na}]^+$

#### **Fmoc-D-Asn(Trt)-D-Trp(Boc)-D-Orn(Boc)-OH (IV.17)**

To a solution of Fmoc-D-Asn(Trt)-D-Trp(Boc)-OH (1.76 g, 1.99 mmol), H-D-Orn(Boc)-OAllyl (1.50 g, 5.5 mmol), DIEA (0.35 mL, 1.93 mmol), and HOBt (0.404 g, 2.99 mmol) in dry THF (6.0 mL) at 0°C was added EDCI (0.573 g, 2.99 mmol). The reaction was purged with argon and slowly warmed to room temperature. After stirring 12 hours the reaction was quenched with EtOAc (50 mL) and washed with 1N HCl (20

mL), sat. aq.  $\text{NaHCO}_3$  (20 mL), and brine (20 mL). The organic phase was dried over  $\text{Na}_2\text{SO}_4$ , filtered and condensed. Purification on a biotage SP1 flash system (12-100% EtOAc-Hexanes) afforded the tripeptide (1.36 g, 1.20 mmol, 60%) as a white solid. mp. 145-149°C  $[\alpha]_D^{22} + 12.8$  (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.53 (s, 1H), 8.45 (d,  $J = 6.92$  Hz, 1H), 8.06 (d,  $J = 8.03$  Hz, 1H), 8.01 (d,  $J = 8.15$  Hz, 1H), 7.89 (d,  $J = 7.43$  Hz, 1H), 7.70 (d,  $J = 6.58$  Hz, 1H), 7.66 (d,  $J = 7.81$  Hz, 1H), 7.60 (d,  $J = 8.35$  Hz, 1H), 7.49 (s, 1H), 7.40 (q,  $J = 7.45, 7.45, 7.40$  Hz, 1H), 7.29 (t,  $J = 7.54, 7.54$  Hz, 2H), 7.26-7.11 (m, 18H), 6.85-6.77 (m, 1H), 6.42 (d,  $J = 8.06$  Hz, 1H), 5.88 (m, =1H), 5.35-5.13 (m, 2H), 4.62 (dd,  $J = 13.16, 7.80$  Hz, 1H), 4.58-4.51 (m, 3H), 4.33 (m, 1H), 4.30-4.10 (m, 4H), 4.03 (dq,  $J = 7.15, 7.12, 7.12, 0.92$  Hz, 1H), 3.05 (dd,  $J = 14.87, 4.58$  Hz, 1H), 2.97-2.85 (m, 4H), 2.60 (dd,  $J = 13.98, 10.80$  Hz, 1H), 1.75-1.65 (m, 1H), 1.63-1.50 (m, 12H), 1.48 (s, 1H), 1.44-1.32 (m, 17H);  $^{13}\text{C}$  NMR (100MHz,  $\text{CDCl}_3$ )  $\delta$  173.302, 171.018, 170.871, 170.194, 157.080, 156.153, 155.858, 149.365, 144.076, 143.904, 143.577, 143.319, 140.938, 135.110, 131.324, 131.256, 130.036, 128.480, 127.655, 127.471, 126.868, 126.754, 124.975, 124.341, 124.206, 122.364, 119.703, 118.883, 118.574, 118.508, 115.034, 114.952, 83.303, 78.737, 70.481, 67.155, 65.594, 60.155, 53.221, 52.269, 51.812, 51.693, 46.699, 39.724, 39.568, 37.742, 30.033, 28.870, 28.403, 27.202, 25.657, 20.794, 13.957; ; IR (film)  $\nu_{\text{max}}$  3318, 3074, 2978, 2925, 1737, 1690, 1659, 1521, 1455, 1374, 1251, 1165, 1088, 757, 701 ; ESI MS  $m/z$  593.32  $[\text{M}+2\text{Na}]^{2+}$

To a stirring solution of Fmoc-D-Asn(Trt)-D-Trp(Boc)-D-Orn(Boc)-OAllyl (1.147 g, 1.01 mmol) in dry  $\text{CH}_2\text{Cl}_2$  ( 5.0 mL) was added  $\text{Pd}(\text{PPh}_3)_4$  (0.029 g, 0.025 mmol). The reaction was purged with argon and to this was added  $\text{PhSiH}_3$  (0.25 mL, 2.02 mmol) via

syringe. The reaction was stirred 2 hours at room temperature and the purified immediately on a Biotage SP1 flash system (2-20% MeOH-CHCl<sub>3</sub>) to afford the carboxylic acid (0.853 g, 0.778 mmol, 77%), as a white solid. mp. 150-153 °C;  $[\alpha]_D^{22} + 10.9$  (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 8.56 (s, 1H), 8.26 (s, 1H), 8.00 (d, *J* = 8.48 Hz, 1H), 7.88 (d, *J* = 7.60 Hz, 2H), 7.68 (d, *J* = 7.65 Hz, 2H), 7.63 (d, *J* = 7.13 Hz, 1H), 7.56 (s, 1H), 7.46 (s, 1H), 7.40 (dd, *J* = 16.31, 7.92 Hz, 2H), 7.28 (t, *J* = 7.49, 7.49 Hz, 2H), 7.24-7.13 (m, 21H), 6.75 (t, *J* = 5.25, 5.25 Hz, 1H), 4.52 (s, 1H), 4.32 (t, *J* = 7.85, 7.85 Hz, 1H), 4.28-4.11 (m, 2H), 3.82 (s, 1H), 3.53 (s, 1H), 3.13 (d, *J* = 11.81 Hz, 1H), 2.94 (dd, *J* = 14.37, 7.85 Hz, 1H), 2.85 (m, 2H), 2.70-2.62 (m, 1H), 2.61-2.55 (m, 1H), 1.66 (s, 1H), 1.60-1.50 (m, 13H), 1.45 (s, 1H), 1.37-1.33 (m, 12H); <sup>13</sup>C NMR (100MHz, CDCl<sub>3</sub>) δ 171.419, 171.003, 170.783, 170.572, 156.775, 156.463, 156.335, 149.809, 144.621, 141.386, 135.493, 135.007, 134.605, 132.404, 132.302, 130.905, 130.470, 129.018, 128.045, 127.334, 127.136, 125.495, 124.689, 122.864, 120.080, 119.428, 118.948, 115.367, 84.037, 79.092, 70.917, 67.637, 60.658, 53.864, 47.088, 40.239, 38.093, 26.207, 21.311, 14.461; IR (film)  $\nu_{max}$  3362, 3057, 2976, 2922, 1710, 1678, 1523, 1453, 1373, 1154, 1090, 754, 700; ESI MS *m/z* 1119.37 [M+Na]<sup>+</sup>

### Carboxylic Acid (IV.3)

To a solution of Fmoc-D-Asn(Trt)-D-Trp(Boc)-D-Orn(Boc)-OH (0.838 g, 0.764 mmol), the hydrochloride (0.385 g, 1.06 mmol), and DIEA (0.19 mL, 1.07 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub>/DMF (1:1, 3.0 mL) at 0°C was added HATU (0.293 g, 0.771 mmol). The reaction was purged with argon and slowly warmed to room temperature. After stirring

12 hours the reaction was quenched with EtOAc (50 mL) and washed with 1N HCl (20 mL), sat. aq. NaHCO<sub>3</sub> (20 mL), and brine (20 mL). The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and condensed. Purification on a biotage SP1 flash system (12-100% EtOAc-Hexanes) afforded the pentapeptide (0.752 g, 0.539 mmol, 71%) as a white solid. mp. 65-69 °C;  $[\alpha]_D^{22} + 9.3$  (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.50 (s, 1H), 8.11-7.95 (m, 2H), 7.89 (d, *J* = 7.53 Hz, 2H), 7.74-7.65 (m, 3H), 7.60 (d, *J* = 5.19 Hz, 1H), 7.48 (d, *J* = 6.24 Hz, 1H), 7.40 (dd, *J* = 13.65, 6.55 Hz, 1H), 7.29 (t, *J* = 7.64, 7.64 Hz, 2H), 7.24-7.12 (m, 21H), 6.81-6.60 (m, 1H), 5.85 (m, 1H), 5.35-5.06 (m, 2H), 4.67 (dd, *J* = 10.42, 3.10 Hz, 1H), 4.62-4.55 (m, 1H), 4.53-4.44 (m, 2H), 4.34-4.12 (m, 3H), 3.06-2.95 (m, 2H), 2.94-2.77 (m, 4H), 2.66 (s, 1H), 2.62-2.54 (m, 2H), 2.17 (dd, *J* = 13.48, 7.05 Hz, 1H), 1.57 (s, 1H), 1.53-1.42 (m, 6H), 1.37-1.32 (m, 13H), 1.25-1.21 (m, 10H), 0.94-0.78 (m, 9H), 0.77-0.73 (m, 5H), 0.69-0.58 (m, 2H); <sup>13</sup>C NMR (100MHz, CDCl<sub>3</sub>) δ 171.932, 170.902, 170.258, 169.732, 169.537, 169.436, 168.527, 156.101, 155.693, 144.149, 143.631, 143.367, 140.983, 135.118, 131.613, 129.970, 128.493, 127.664, 127.433, 126.782, 124.951, 124.193, 122.538, 122.378, 119.671, 119.147, 118.925, 118.592, 118.269, 117.985, 114.979, 83.536, 83.252, 78.574, 71.628, 71.254, 70.949, 70.524, 67.205, 67.084, 65.142, 61.712, 60.097, 53.477, 51.808, 51.281, 49.180, 48.881, 46.757, 39.790, 38.531, 38.131, 33.832, 31.192, 30.806, 29.416, 28.914, 28.175, 27.910, 27.529, 26.918, 25.125, 22.759, 22.632, 22.544, 22.352, 22.249, 20.755, 19.574, 19.530, 19.435, 19.147, 18.858, 18.598, 18.476, 18.032, 17.867, 13.953; IR (film)  $\nu_{max}$  3380, 3056, 2960, 2925, 2864, 1737, 1684, 1644, 1516, 1455, 1368, 1256, 1165, 108, 762, 701; ESI MS *m/z* 1428.50 [M+Na]<sup>+</sup>



To a stirring solution of the pentapeptide (0.200 g, 0.142 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (0.70 mL) was added  $\text{Pd}(\text{PPh}_3)_4$  (0.0041 g, 0.0036 mmol). The reaction was purged with argon and  $\text{PhSiH}_3$  (0.035 mL, 0.284 mmol) was added via syringe. The reaction was stirred 2 hours at room temperature and the purified immediately on a Biotage SP1 flash system (2-20% EtOH- $\text{CHCl}_3$ ) to afford the carboxylic acid (0.187 g, 0.137 mmol, 96%), as a white solid. mp. 95-97°C;  $[\alpha]_D^{22} + 3.7$  (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.50 (s, 1H), 8.45 (d,  $J = 7.09$  Hz, 1H), 8.00 (d,  $J = 8.25$  Hz, 1H), 7.89 (d,  $J = 6.55$  Hz, 2H), 7.75 (s, 1H), 7.70 (t,  $J = 7.11, 7.11$  Hz, 1H), 7.67 (d,  $J = 7.38$  Hz, 1H), 7.61 (d,  $J = 7.31$  Hz, 1H), 7.48 (s, 1H), 7.46-7.36 (m, 2H), 7.29 (t,  $J = 7.48, 7.48$  Hz, 2H), 7.25-7.11 (m, 21H), 6.64 (s, 1H), 5.12 (s, 1H), 4.65 (d,  $J = 7.86$  Hz, 1H), 4.57 (d,  $J = 5.36$  Hz, 1H), 4.38-4.24 (m, 2H), 4.19 (dd,  $J = 17.80, 7.26$  Hz, 1H), 3.04 (dd,  $J = 14.19, 5.07$  Hz, 1H), 2.93-2.80 (m, 6H), 2.68 (t,  $J = 21.48, 21.48$  Hz, 1H), 2.46-2.38 (m, 1H), 2.07 (m, 1H), 1.62-1.53 (m, 11H), 1.52-1.45 (m, 4H), 1.37-1.32 (m, 13H), 1.28-1.17 (m, 5H), 1.15-1.06 (m, 2H), 0.91 (d,  $J = 6.39$  Hz, 1H), 0.86 (d,  $J = 6.30$  Hz, 1H), 0.82 (d,  $J = 2.66$  Hz, 3H), 0.81 (d,  $J = 2.54$  Hz, 1H), 0.75 (d,  $J = 6.64$  Hz, 3H), 0.72 (d,  $J = 6.46$  Hz, 1H), 0.66 (d,  $J = 6.60$  Hz, 1H);  $^{13}\text{C}$  NMR (100MHz,  $\text{CDCl}_3$ )  $\delta$  172.312, 171.884, 171.705, 170.905, 170.734, 170.110, 169.539, 156.185, 155.793, 149.404, 149.290, 144.129, 143.642, 143.373, 140.935, 134.966, 131.776, 130.222, 128.481, 127.627, 127.419, 126.840, 126.741, 125.036, 124.259, 124.110, 123.993, 122.456, 119.643, 119.076, 115.462, 114.916, 83.305, 80.627, 79.457, 78.588, 71.969, 71.060, 70.519, 67.195, 65.029, 62.368, 61.596, 54.195, 53.375, 52.048, 49.501, 49.005, 48.903, 46.908, 39.804, 38.239, 37.768, 34.156, 31.230, 30.431, 29.411, 29.189, 28.165, 27.905, 27.530, 26.565, 25.184, 22.717, 22.304, 19.493, 19.380,

18.193; IR (film)  $\nu_{max}$  3318, 3056, 3021, 2969, 1735, 1654, 1521, 1450, 1368, 1256, 1159, 1088, 762, 696; ESI MS  $m/z$  1388.50  $[M+Na]^+$

### Resin Bound Hexapeptide (IV.23)

The solid phase synthesis of peptide x was carried out on a 0.1 mmol scale using a CEM automated microwave assisted peptide synthesizer employing the Fmoc/t-Bu/Trt strategy<sup>11</sup>. A preloaded Fmoc-Glu(Wang)-OAlly resin (0.34 mmol/g loading) was used as the resin bound amino acid. Typical coupling cycles consisted of 20% piperidine / DMF / 0.1M HOBt Fmoc deprotections followed by HATU / DIEA / NMP double couplings for each amino acid residue.

### Octapeptide (IV.4)

To a solid phase reaction vessel containing the hexapeptide in DMF (1.0 mL) was added Fmoc- $\beta$ OH-D-Asn(Trt)-OH (0.074 g, 0.120 mmol), DIEA (0.021 mL, 0.120 mmol) and DEPBT (0.072 g, 0.240 mmol). The resin was agitated on a wrist action shaker for 12 hours and then rinsed with DMF (4 x 5 mL) and CH<sub>2</sub>Cl<sub>2</sub> (4 x 5 mL). A solution of 20% piperidine/ DMF (1.0 mL) was added and the resin was agitated for 7 min. The resin was filtered and the deprotection repeated. At this point the resin was again washed with DMF (4 x 5 mL) and CH<sub>2</sub>Cl<sub>2</sub> (4 x 5 mL) and DMF (1.0 mL) was added followed by Fmoc-Ser(Ot-Bu)OH (0.050 g, 0.120 mmol), DIEA (0.021 mL, 0.120 mmol) and DEPBT (0.072 g, 0.240 mmol). The resin was agitated on a wrist action shaker for 12 hours and then rinsed with DMF (4 x 5 mL) and CH<sub>2</sub>Cl<sub>2</sub> (4 x 5

mL). A solution of 20% piperidine / DMF (1.0 mL) was added and the resin was agitated for 7mins. The resin was filtered and the deprotection repeated.

### **Linear Peptide (IV.2)**

The resin bound peptide was first washed with DMF (4 x 5 mL) and CH<sub>2</sub>Cl<sub>2</sub> (4 x 5 mL). To the reaction vessel was added DMF (1.5 mL), pentapeptide (0.176 g, 0.129 mmol), DIEA (0.023 mL, 0.130 mmol), and DEPBT (0.078 g, 0.260 mmol). The resin was agitated on a wrist action shaker for 12 hours then washed with DMF (4 x 5 mL) and CH<sub>2</sub>Cl<sub>2</sub> (4 x 5 mL).

### **WAP-8294A2 (IV.1)**

The solid phase reaction vessel containing the linear peptide (0.1 mmol) was capped and purged with argon. The resin was then washed with dry CH<sub>2</sub>Cl<sub>2</sub> ( 5 x 5 mL) while under an atmosphere of argon. To the resin was then added a previously prepared solution of Pd(PPh<sub>3</sub>)<sub>4</sub> (0.029 g, 0.025 mmol ) in dry CH<sub>2</sub>Cl<sub>2</sub> (2.0 mL) via syringe. PhSiH<sub>3</sub> (0.12 mL, 1.00 mmol) was added via syringe and the reaction was agitated for 2hrs. The resin was then washed with DMF (4 x 5 mL) and CH<sub>2</sub>Cl<sub>2</sub> (4 x 5 mL). A solution of 10% piperidine/ DMF/ 0.05% HOBt (1.5mL) was added and the resin was agitated 10 minutes then filtered. After washing with DMF/0.1M HOBt (4 x 5 mL) and CH<sub>2</sub>Cl<sub>2</sub> (4 x 5 mL), a solution of DIC (0.078 mL, 0.500 mmol), HOAt (0.068 g, 0.500 mmol), and DIEA (0.052 mL, 0.300 mmol) in CH<sub>2</sub>Cl<sub>2</sub>/DMF (1:1, 3.0 mL) was added. The resin was agitated for 24 hours then washed with DMF (4 x 5 mL) and CH<sub>2</sub>Cl<sub>2</sub> (4 x 5 mL). The peptide was deprotected and cleaved from the resin using TFA / TES / H<sub>2</sub>O (9:1:1, 10 mL) over 3 hours with agitation. The resin was filtered and the TFA was

reduced to 1/3 the original volume in vacuo. The product was precipitated using ice cold Et<sub>2</sub>O and collect via centrifugation. The crude material was dissolved in H<sub>2</sub>O/CH<sub>3</sub>CN (9:1, 10 mL) and lyophilized. Reverse phase HPLC (25-75% CH<sub>3</sub>CN/H<sub>2</sub>O with a 0.05% TFA buffer over 60 minutes) afforded WAP-8294A2 (0.031g, 0.20 mmol, 20%) as a white solid. mp. >200°C decomposition;  $[\alpha]_D^{22} + 36.0$  (c 0.5, H<sub>2</sub>O); <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>, 45°C)  $\delta$  12.01 (s, 1H), 10.26 (s, 1H), 8.68 (d, *J* = 6.39 Hz, 1H), 8.48 (d, *J* = 8.77 Hz, 1H), 8.26 (d, *J* = 7.62 Hz, 1H), 8.17 (d, *J* = 7.09 Hz, 1H), 8.06 (d, *J* = 9.30 Hz, 1H), 7.98 (d, *J* = 8.06 Hz, 1H), 7.66 (s, 2H), 7.62 (s, 2H), 7.91 (d, *J* = 7.30 Hz, 1H), 7.89 (d, *J* = 7.03 Hz, 1H), 7.57 (s, 1H), 7.46 (d, *J* = 4.79 Hz, 1H), 7.45 (d, *J* = 7.56 Hz, 1H), 7.37 (s, 1H), 7.33 (s, 1H), 7.32 (s, 1H), 7.30 (d, *J* = 7.94 Hz, 1H), 7.27 (s, 1H), 7.26 (s, 1H), 7.21 (s, 1H), 7.18-7.13 (m, 1H), 7.10 (s, 1H), 6.98 (d, *J* = 16.17 Hz, 1H), 6.97 (d, *J* = 15.36 Hz, 1H), 6.89 (d, *J* = 7.08 Hz, 1H), 5.64 (d, *J* = 5.20 Hz, 1H), 4.98 (d, *J* = 9.26 Hz, 1H), 4.93 (dd, *J* = 8.83, 5.28 Hz, 1H), 4.88-4.82 (m, 2H), 4.79 (dd, *J* = 15.51, 7.15 Hz, 1H), 4.75 (d, *J* = 8.02 Hz, 1H), 4.67 (d, *J* = 8.72 Hz, 1H), 4.65 (d, *J* = 6.84 Hz, 1H), 4.63 (d, *J* = 11.25 Hz, 1H), 4.58 (d, *J* = 6.76 Hz, 1H), 4.40 (dd, *J* = 8.72, 4.59 Hz, 1H), 4.33 (s, 1H), 4.30-4.25 (m, 1H), 3.90 (s, 1H), 3.64 (d, *J* = 6.48 Hz, 2H), 3.56 (dd, *J* = 10.29, 5.21 Hz, 1H), 3.45 (dd, *J* = 10.12, 5.60 Hz, 1H), 3.26 (m, 1H), 3.13 (dd, *J* = 14.42, 7.14 Hz, 1H), 2.97 (dd, *J* = 25.70, 15.45 Hz, 1H), 2.88-2.71 (m, 6H), 2.66-2.60 (m, 1H), 2.58 (s, 3H), 2.55-2.53 (m, 1H), 2.53-2.49 (m, 3H), 2.44 (dd, *J* = 15.10, 6.63 Hz, 1H), 2.37-2.28 (m, 1H), 2.19 (dd, *J* = 8.91, 3.40 Hz, 2H), 2.06 (m, 1H), 1.94-1.79 (m, 1H), 1.78-1.64 (m, 3H), 1.64-1.33 (m, 12H), 1.28-1.19 (m, 2H), 1.17-1.09 (m, 2H), 0.97-0.75 (m, 9H), 0.74-0.67 (m, 9H); <sup>13</sup>C NMR (100MHz, DMSO-*d*<sub>6</sub>)  $\delta$  174.03, 173.48, 171.79, 171.47, 170.88, 170.63, 170.44, 170.19, 169.75, 169.57,

169.32, 169.19, 169.05, 168.94, 158.11, 157.80, 157.52, 138.28, 135.78, 129.00, 128.25, 128.10, 127.12, 126.23, 123.60, 120.64, 118.14, 115.95, 111.26, 109.27, 71.26, 70.93, 62.81, 62.01, 61.57, 60.88, 55.55, 55.06, 54.91, 54.57, 53.81, 51.71, 51.55, 51.26, 51.13, 49.14, 48.16, 42.12, 39.43, 38.43, 38.11, 37.95, 35.16, 33.86, 33.40, 30.31, 29.84, 29.50, 29.14, 28.91, 28.54, 28.07, 27.21, 25.53, 24.00, 23.51, 22.94, 22.29, 21.93, 21.27, 20.76, 19.63, 19.04, 18.60, 18.16, 17.96; IR (KBr)  $\nu_{max}$  3441, 3310, 3083, 2960, 2934, 2873, 1674, 1639, 1542, 1460, 1414, 1205, 1139, 1027, 1001, 843, 808, 747, 721, 711 ; HR ESI TOFS  $m/z$  1562.8235  $[M+H]^+$

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