STRUCTURE –ACTIVITY STUDIES ON BACTERIAL EFFLUX INHIBITORS

By

GIFTY AYENSU BLANKSON

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Edmond J. LaVoie

And approved by

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ABSTRACT OF THE DISSERTATION

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Antibiotic resistance poses a significant challenge in anti-infective therapy. There are several mechanisms involved in antibiotic resistance with efflux being a major determinant. Overcoming efflux will allow for the reintroduction of old antibiotics and prevent the development of resistance to new antibiotics. Several bacterial efflux pump inhibitors (EPIs) have been discovered, but none of these compounds have been FDA-approved for use in humans. There is a need for a systematic study of some of these inhibitors to gain a better understanding of their structure activity relationship (SAR). This work focuses on the SAR studies conducted on two classes of known EPI inhibitors.

Analogues of aryl piperazines (biphenyl piperazines and naphthalene amines) were synthesized and tested for their EPI activity. None of the compounds in this series exhibited EPI activity and thus no further exploration was done on their SAR. Structurally simpler analogues of phenylalanine-arginine β-naphthylamide (PAβN) were also designed and an SAR study conducted on the three different scaffolds proposed. The three scaffolds include the "normal" amides, the "reverse" amides and the secondary amine series. Although these scaffolds exhibited some similarities in their SAR, there were some differences. In all cases, the 1,5-diphenylpentane core was the preferred substituent on the

right hand side of the molecules. For the left hand side, diaminopentane was the preferred substituent. Further studies revealed that N^5 substitution was detrimental to EPI activity.

The "reverse" amides had better activity when compared against their analogous "normal" amides. The data indicated that a bis-alkylaryl was necessary for activity and that there was preference for the aryl to be hydrophobic. A methylene insertion in the reverse amides series gave a compound that caused 512-fold reduction in the minimum inhibitory concentration (MIC) of clarithromycin (PAβN, the historical EPI standard offered a 4-fold reduction in MIC). The amine analogues also offered some active compounds with one of these compounds causing a 128-fold reduction in the MIC of clarithromycin. (These tests were done in *E. coli*). Some success was achieved in *P. aeruginosa*; two compounds when independently co-administered with levofloxacin resulted in a 32-fold decrease in the MIC of levofloxacin.

DEDICATION

To my parents,

Robert A. Blankson and Christina E. Blankson

I have seen further because I have stood on your shoulders.

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INTRODUCTION

The mainstay of anti-infective therapy is antibiotics. The advent of penicillin brought about a dramatic increase in the life expectancy.[1-2] Forthwith, soldiers wounded in war had an improved chance at survival as the antibiotics could stop the spread of infections which would usually lead to deaths. It initially appeared that mankind had successfully developed weapons to win the arms race against bacteria. However, a few years after the introduction of penicillin, widespread resistance by some bacteria developed.[3-5] The use of this antibiotic had created a natural selection of these resistant strains. The situation has progressed with antibiotic resistance further occurring in several alternative antibiotics that have been introduced. A reduction in the numbers of new and effective antibiotic introduced into the clinic has resulted in antibiotic resistance being a global issue. The issue is further complicated by the abuse of antibiotics in both humans and in veterinary and agricultural use. An estimated 2 million people in the United States become infected each year with antibiotic-resistant bacteria and 23,000 of these die.[6-7] These resistant strains are found in the community but are most commonly associated with hospitals and nursing homes. These nosocomial infections have been caused by six microbes which pose serious threats to humans due to their resistance to existing antibiotics. These include; *Enterococcus faecium, Staphylococcus aureus, Kleibsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter species* collectively referred to as ESKAPE.[8-9] Mankind is under a constant threat of life-threatening infections as these organisms are constantly going above the minimum inhibitory concentration threshold and becoming resistant to newly introduced antibiotics.

Combating these organisms will require a firm grip on the understanding that any organism that survives a wave of antibacterial therapy could potentially develop resistance. It is an all or none attack and it is necessary to constantly find new ways to solve the problem of antibiotic resistance.

1.1. ANTIBIOTIC RESISTANCE

Antibiotic resistance occurs when microbes lose their susceptibility to drugs/antibiotics that they were susceptible.[10] The microbes are able to develop resistance and survive in the presence of known antimicrobial agents. Although it had been previously thought that intrinsic resistance may be due to antibiotic use, this knowledge has proved to be false.[11] Resistance predates the antibiotic era; microbes that produce antibiotics may have evolved self-protective agents to protect themselves against the toxic substances that they produced.[12,13] The collection of all genes that bestow antibiotic resistance to microorganisms are referred to as the antibiotic resistome. These genes may work directly or indirectly to confer resistance on these microbes. There are three main types of antibiotic resistance; intrinsic, acquired and adaptive.[14]

Intrinsic resistance refers to all mechanisms associated to the characteristics of a particular organism that prevent the action of antibiotics.[11,14] An example is the semipermeable outer membrane, which is found in Gram-negative bacteria. This outer membrane has a low permeability disallowing the entry of some xenobiotics. Another example of intrinsic resistance is the presence of constitutive efflux pumps observed in bacteria.[14]

Acquired resistance refers to the type of resistance that is gained by a once susceptible bacterium by accepting new genetic material such as plasmids, transposons, integrons and naked DNA.[14-16] Also, when resistance is gained by mutations, it falls under the category of acquired resistance. Acquired resistance can confer either a breakthrough resistance or a low-level resistance. Breakthrough resistance results in a large increase in MIC which makes clinically-susceptible organisms, clinically resistant. The more common of the two types of acquired resistance is the low-level resistance; which is the mutation that often to leads to small and inconsequential change in MIC.[14,17] Over time, however, these negligible changes in MIC leads to a significant world-wide resistance of microbes to the antibiotic. Also the additive effect of this low-level resistance can sometimes result in a breakthrough resistance.

Adaptive resistance is a temporal ability of bacteria to overcome an antibiotic due to changes in genetic material and protein expression. These changes may be due to stress, nutrient conditions and growth state or a subinhibitory concentration of the antimicrobial present in the environment of the bacteria. Studies show that low concentration of quinolones increase the frequency of resistance mutations. For example, ciprofloxacin increases the frequency of rifampicin resistance mutations by up to 5-fold possible by the induction of error prone DNA polymerase in an SOS-dependent or independent response. [17-19]

A comparison of the different types of antibiotic resistance is shown in Table 1. The comparison is with their mode of acquisition, their characteristics and the effect of the environment on these three types of resistance.

Table 1: Comparison between the three major types of antibiotic resistance – A summary (Reproduced with permission[17])

Type of Resistance	Intrinsic	Acquired	Adaptive
Acquisition	Not acquired, part of the genetic make-up of strain or species	Mutation Horizontal transfer	Changes in gene expression triggered by environmental factors or presence of antimicrobials
Characteristics	Inheritable Stable Irreversible	Inheritable Stable Irreversible	Not inheritable Transient Generally reverts upon removal of inducing signal
Effect of Environment	Independent	Independent	Dependent

Public health officials have proposed several methods to battle antibiotic resistance. These methods include: education, appropriate antibiotic prescribing, antimicrobial stewardship programs, hygiene as well as appropriate disinfection.[20] Examining this problem from a drug discovery point of view requires an understanding of the cellular mechanisms involved in antibiotic resistance. These mechanisms that have been found to be responsible for the resistance to antibiotics are discussed below:

1). <u>Antimicrobial inactivation by enzymes.</u> A classic example is the inactivation of β-lactam antibiotics by the enzyme β-lactamase. The β-lactamase can produce antibiotic resistance by hydrolyzing the lactam ring of β-lactams antibiotics. Despite the development of β-lactamase inhibitors, resistance has also developed to inhibition to such compounds as clavulanic acid. There are also aminoglycoside-modifying enzymes. There are a least 30 different genes that are implicated in aminoglycoside resistance. Microbes respond to the introduction of new aminoglycosides with the concomitant introduction of an unknown antibiotic-inactivating enzyme. These enzymes could be acetyltransferases,

phosphotransferases and adenylyl transferases. Chloramphenicol acetyltransferases also belongs in this class and works by acylating chloramphenicol and forming a complex with fusidic acid which renders the drugs inactive.[14,15]

2). Intracellular target mutation. In this case the intracellular target of the antimicrobial becomes changed due to mutations. Thus, although the antimicrobial reaches its target, it is rendered ineffective. Mutation in certain domains of bacterial type II topoisomerase enzymes: DNA gyrase and DNA topoisomerase IV have led to microbial resistance to quinolones.[21-23] Mutation in the 30S ribosomal subunit as well as methylation of the aminoglycoside binding site are also sources of resistance in aminoglycosides. Methylation of nucleotides A1408 and G1405 on 16S rRNA of bacteria eliminate the intermolecular interactions between the nucleotides and the drugs. These interactions are necessary for the activity of these aminoglycosides.[24] A dimethylation of the 23S ribosomal RNA near or within the macrolide binding site has also been shown to lead to resistance in the macrolide, lincosamide and streptogramin B classes by staphylococcal, streptococcal and enterococcal strains.[25]

3). Reduced Entry of Antibiotic. This could be due to a reduction in the number of entry portal (porins) on the surface of the bacteria.[26] It could also be due to the alterations in the cell surface, which affect interactions leading to reduced entry of foreign substances into the bacteria cell.[27] An example of these cell surface alterations is found in Lipid A: PhoPQ-regulated modification (acylation) of salmonella lipopolysaccharides, which leads to resistance to novobiocin.[14,28] *Klebsiella* and *Pseudomonas* resistance has been studied to be due to several factors, one of which is the presence of an impermeability barrier that

reduces antibiotic uptake.[29,30] The permeability barrier mostly acts in synergy with another resistance mechanism called efflux.[31]

4). Efflux. This is the process by which bacteria actively expel xenobiotics from within the cell. This leads to a reduced amount of antibiotic in the cells leading to a subinhibitory concentration, which is ineffective in killing bacteria. Efflux is currently recognized as the major source of antibiotic resistance. The focus of the succeeding sections will be an in depth discussion on bacterial efflux mechanisms.

1.2. EFFLUX PUMPS IN ANTIBIOTIC RESISTANCE

Efflux is a major determinant of antibiotic levels in the cells of bacteria and as such it presents a promising target for reviving the activity of drugs that are substrates for these pumps. Efflux pumps can be single or multi-component energy-dependent systems that remove toxic compounds from cells. In this process there is no metabolism of the xenobiotic; the compound is expelled unchanged out of the cell through the pump. In 1978, Levy and McMurry first discovered the presence of a mechanism that reduced the amount of tetracycline in *E. coli*.[14,32] It was initially thought to be due to reduce influx, but was later found to be actually due to increased efflux. Efflux pumps can transport a large array of substrates out of bacterial cells allowing for multiple drug resistance. There are two mechanisms by which bacteria use efflux pump to remove antibiotics from the cells. The first is a simple overexpression of pumps to override the effect of increasing concentration whilst the second involves an accumulation of mutations that permit a more efficient expulsion of the toxic substance.[26]

Numerous efflux systems have been characterized in both Gram-negative and Gram-positive bacteria. However, efflux pumps of Gram-negative bacteria and mycobacteria pose the greater challenge in antimicrobial resistance. This is because these bacteria also possess cell envelope which has low cell permeability. In recent times, it has been uncovered that efflux pumps play a large role in acquired clinical resistance.[14] Efflux is now recognized as a major cause of resistance in microbes.[33] As an example, Enterobacteriaceae has become resistant to carbepenem largely due to efflux pumps.[34] Efflux mediated resistance appears to be the most basic of all resistance and works in synergy with other resistance mechanisms.

1.3.   MAIN CLASSES OF EFFLUX PUMPS

Efflux pumps can be divided in to two main classes based on the energy required for transportation; ATP-binding cassette (ABC) transporters uses energy obtained from ATP hydrolysis while secondary multidrug transporters uses proton motive force. The secondary multidrug transporters which are clinically significant can be further divided into superfamilies based on the homology at the primary and secondary structure levels.[14,35] In addition to ABC transporters, there are four superfamilies of secondary multidrug transporters. These are:

a. Major Facilitator Superfamily (MFS)

b. Small Multidrug Resistance (SMR)

c. Multidrug And Toxic compound Extrusion

d. Resistance-Nodulation-cell Division (RND)

ATP-Binding Cassette (ABC) – These transporters are well characterized in eukaryotes with the commonest example being P-glycoprotein.[26] P-glycoprotein confers resistance to chemotherapy in eukaryotes. ABC transporters are involved in both uptake and efflux. They transport a large variety of substrates including sugars, amino acids, ions, drugs, polysaccharides and proteins.[14] These ubiquitous transporters are also found in bacteria.[36] MacAB pump is an example of this system and it confers macrolide specific resistance to *E. coli*.[37]

Major Facilitator Superfamily (MFS) – This is the most diverse family of transporters. Its members can carry out a uniport, symport or an antiport mode of transportation.[14] Uniport is when a substrate is transported without being coupled to ion movement. With symport, there is an ion movement in the same direction whilst in antiport the coupled ion moves in opposite directions. The proteins in this family consist of either 12 or 14 transmembrane and all the transporters involved in antibiotic efflux are Drug/Proton antiporters (DHA). These DHAs can be subdivided into 3 classes: DHA1, DHA2 and DHA3. DHA1 and 2 are involved in the extrusion of several different types of drugs, examples of which are Bmr of *Bacillus subtilis* and QacA of *S. aureus,* respectively. DHA3 specializes in antibiotics such as macrolides and tetracyclines. DHA3 can be found only in bacteria, an example of which is MefA of *Streptococcus pyogenes*.[14,25]

Small Multidrug Resistance (SMR) – They are small proteins containing approximately 107 to 110 amino acids. They contain 4 transmembrane segments which usually form tetramers in the cytoplasmic membrane.[14] The EmrE of *E. coli,* AbeS of *A. baumannii* and Smr and QacE from *Staphylococcus aureus* are examples of this family.[38,39] Generally, however, only a small group in this family is responsible for clinical resistance.[14]

<u>Multidrug And Toxic compound Extrusion</u> – These protein share a similar topology with proteins of MFS, however, the homology between the amino acid sequences is very low. They have 12 transmembrane regions and use a sodium gradient for transport. The MATE transporters in bacteria export drugs through sodium ion exchange, the human MATE is by proton ion exchange and the plant MATE is a combination of both.[40] Examples include NorM of *Vibro parahaemolyticus, Vibro cholera* and *Neisseria gonorrhoe* and YdhE of *E. coli.*[40,41]

<u>Resistance-Nodulation-cell Division (RND)</u> – This family contains the most clinically important efflux pumps. It is well characterized especially in Gram-negative bacteria. These pumps consist of 3 elements: an inner membrane pump protein, two large periplasmic loops and an outer membrane. The inner membrane contains 12 transmembrane regions and the outer membrane forms a channel-tunnel.[14] The substrates of RND pumps are very diverse and comprise antibiotics, biocides, toxic fatty acids, bile salts, aromatic hydrocarbons, inhibitors of fatty acid biosynthesis, detergents, homoserine lactones and dyes.[14] Two common examples of this pump are the AcrAB-TolC of *E. coli* and MexAB-OprM transporters of *P. aeruginosa.*

The best known role of EPIs is in the efflux of antibiotics. However, given the diversity and ubiquitous nature of efflux pumps, it hints at the fact that these pumps play a more central role in the survival of the organism other than the efflux of antibiotics. The pumps serve as defensive barriers against the different toxic substances that bacteria encounter in their environment. As an example; the natural substrates of ArcAB-TolC (*E. coli*) are bile salts which are found in the gastrointestinal tract, which is the natural habitat of *E. coli.*[14, 26]

1.4. GRAM-POSITIVE BACTERIA AND EFFLUX PUMPS

Gram-positive bacteria have a thick outer peptidoglycan layer with teichoic acid polymers and covalently bond proteins. This outer layer mostly serves as a structural barrier providing counteraction against the osmotic pressure of the bacterial cytoplasm. It allows molecules of up to 30-57 kDa to pass through indicative of its large permeability threshold.[11] Thus, Gram-positive bacteria are intrinsically susceptible to most antibiotics: an exception is the mycobacteria-nocardia corynebacteria, which possess a mycolate-containing cell wall making it a highly resistant target.[31] The emergence of resistant strains has been correlated with the overexpression of clinically relevant MDR pumps. With the exception of pumps from the RND family, Gram-positive bacteria possess efflux pumps from all the other families.[42] The pumps of these bacteria which are clinically significant are those of the MFS family of which Nor A in *Staphylococcus aureus* and PmrA of *Streptococcus pneumoniae* are members. The overexpression of NorA leads to resistance to fluoroquinolones, chloramphenicol, antiseptics, dyes and disinfectants.[43] NorA has been found to be overexpressed in methicillin-resistant *S. aureus* (MRSA).[44] Notable is the fact that there are still several antibiotics that are active against MRSA. Among the agents used to treat MRSA currently are ciprofloxacin, vancomycin, daptomycin, ceftazoline and linezolid.[43]

1.5. GRAM-NEGATIVE BACTERIA AND EFFLUX PUMPS

Most of the antibiotics currently used in the clinic are ineffective against Gram-negative bacteria.[45] The innate characteristics of Gram-negative bacteria provide them

with a high level of resistance to antibiotic agents. These bacteria commonly display the multi-drug resistant (MDR) phenotype.[11] The features of these organisms responsible for making intrinsically resistant is the presence of the outer membrane and efflux pumps. The outer membrane of Gram-negative bacteria is made up of a bilayer of phospholipid and lipopolysaccharides. The lipopolysaccharides is only found in the outermost part of the membrane and made up of lipid A, oligosaccharide and distal polysaccharide. The phospholipids are made up of phosphatidylethanolamine, phosphatidylglycerol and cardiolipin.[27] The permeability threshold of this outer membrane is very low and thus slows down the diffusion of toxic substances across the membrane into the cytoplasm. The efflux systems found in Gram-negative bacteria are usually tripartite with portions located in the inner and outer membrane and the periplasm. This tripartite system consists of an inner membrane, an outer membrane and a membrane fusion protein (MFP) located in the periplasm.

The search for Gram-negative susceptible antibiotics has been very slow. Daptomycin, gemifloxacin, telithromycin and telavancin, which were recently introduced into the clinic, have no activity against Gram-negative bacteria.[46] Tigecycline and doripenem have been FDA approved for Gram-negative therapy. Due to efflux pumps, tigecycline's use is limited to urinary tract infection and blood stream infections. Efflux pumps play an enormous role in the resistance of Gram-negative bacteria to antibiotics. To support this is that fact that removal of the expressed multidrug resistant (MDR) efflux pumps in *P. aeruginosa* was shown to permit susceptibility of highly-resistant bacteria to numerous antibiotics that are typically or more commonly used for Gram-positive organisms.[45]

Two well characterized Gram-negative pumps of clinical relevance belong to the RND superfamily and as they are pertinent to our study will be described in more detail below.

AcrAB-TolC of *E. coli* – *E. coli* is a rod-shaped facultative anaerobe usually present in the intestines. Although it is usually harmless there are certain strains that are harmful to human health. Its most famous efflux pump is the AcrAB-TolC pump. The acridine resistance complex, as it can be referred to, is a well-defined pump that exists in *E. coli*. It is made up of the outer membrane TolC, the inner membrane AcrB and the periplasmic AcrA. These come together to form a reversible assembly pump. The TolC serves as the outer channel whilst AcrA serves as the connecter between the outer pore and AcrB.[47] Recent studies indicate that there is no direct interaction between TolC and AcrB as had been previously suggested in earlier models.[47-50] The bridge AcrA interacts independently with TolC and AcrB. AcrB and TolC exist as a trimer in the assembly while AcrA is a hexamer. TolC and AcrA interaction occurs with each one using their α-helical coiled coils. The α-helical coils of AcrA are found in the hairpin region as shown in Figure 1. TolC uses its periplasmic portion for this interaction. When interacting with AcrB, AcrA uses its membrane proximal and β-barrel domains. The assembly of these components occurs when there is an antibiotic present in the periplasmic space.[51] The more efficient strategy used by bacteria is to assemble the AcrAB-TolC pump system in the presence of stress and disassemble it in the absence of antibiotic stress so that other efflux systems can use the TolC. AcrB is an antiporter that derives its energy from protons from the proton motive force present across the cytoplasmic membrane.[52]
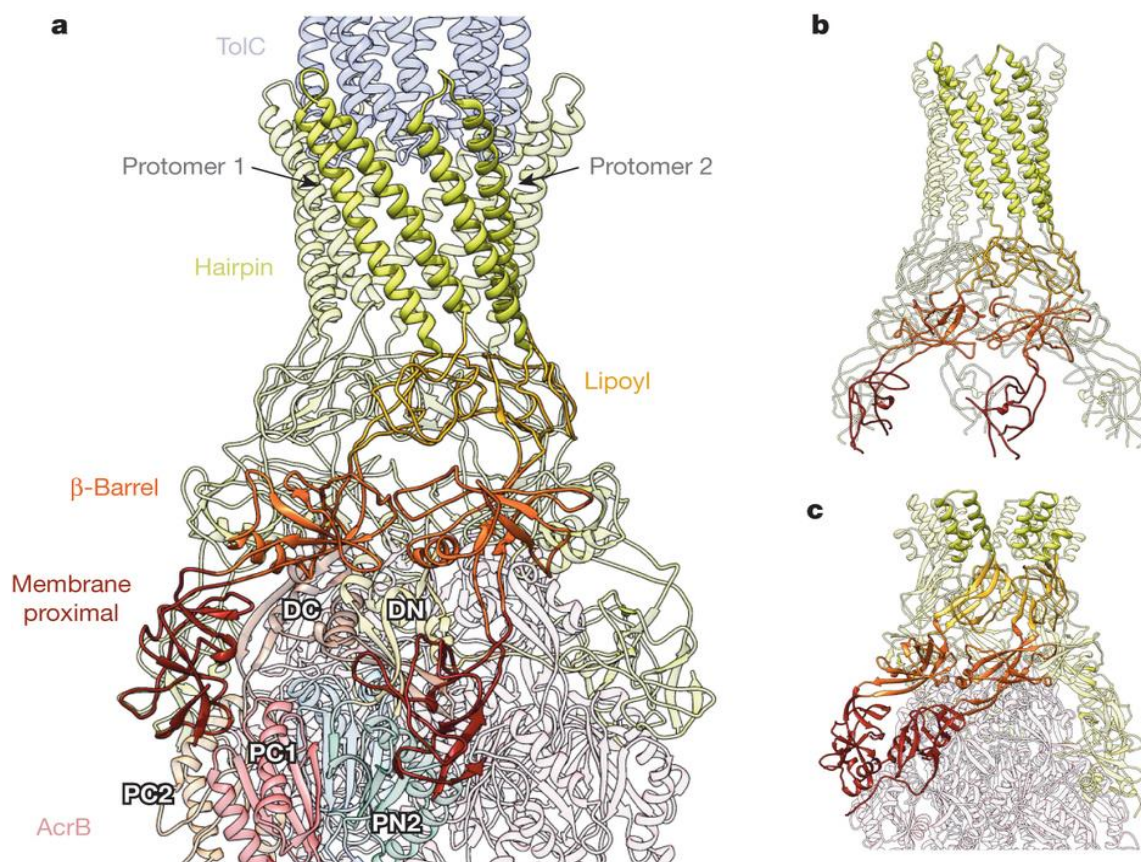
Figure 1: Modelled interaction of efflux pump components.

(a) Interactions of the AcrA hairpin domain with TolC and the AcrA β-barrel and membrane-proximal domains with AcrB. The lipoyl domains principally interact with each other and make no interactions with AcrB or TolC. Two AcrA protomers in the homohexameric ring are shown in color for the hairpin, lipoyl, β-barrel and membrane-proximal domains to illustrate the domain–domain interactions. Subdomains of one protomer of the AcrB trimer are labelled with white labels. The membrane-proximal domains of the two contiguous AcrA protomers make different contacts with the same protomer of AcrB. **(b)** Homohexameric subunit organization in the structure of MacA, a homologue of AcrA from *Actinobacillus actinomycetemcomitans* (Protein Data Bank (PDB) accession 4DK0). **(c)** Interactions between CusA and CusB in the metal-efflux pump from *E. coli.* (**Reproduced with permission from Nature**[47])

MexAB-OprM of *Pseudomonas aeruginosa-* *P. aeruginosa* is an opportunistic rod-shaped bacterium which usually causes serious infections in immunocompromised patients such as HIV/AIDS, cancer and cystic fibrosis. It has been found to have 12 pumps with 8

of them playing a role in antibiotic resistance. These pumps confer resistance to β-lactams, quinolones, aminoglycosides, trimethoprim-sulphamides, tetracycline, chloramphenicol, erythromycin and triclosan. Of the 8 pumps, only the MexAB-OprM is expressed constitutively. This pump system is illustrated in Figure 2.[53]
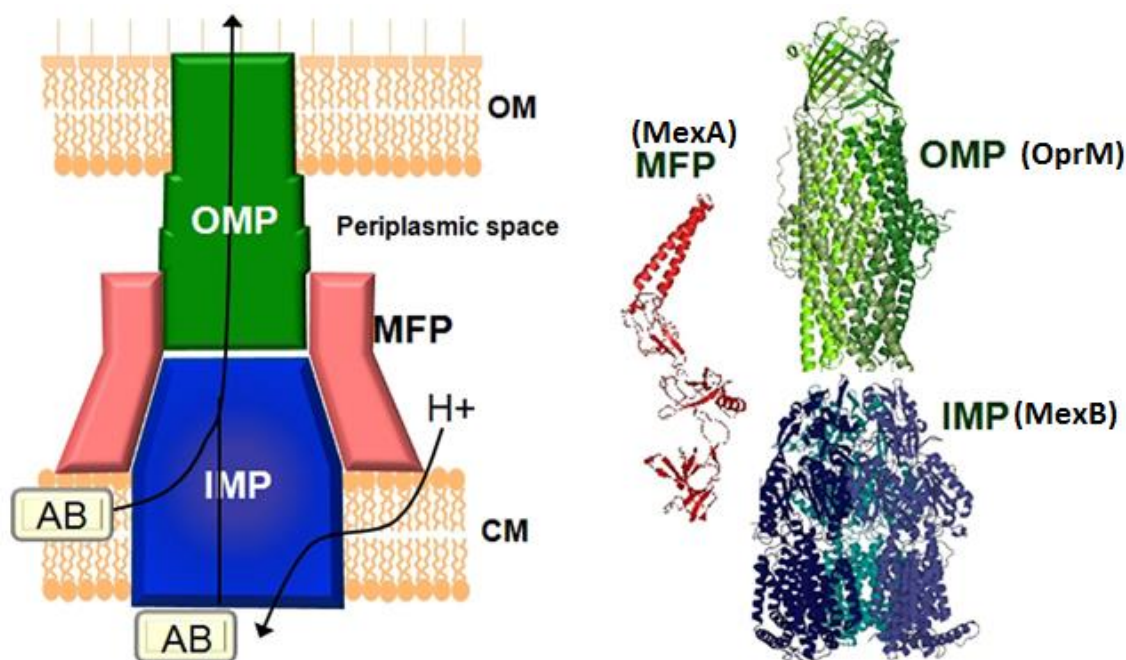
Figure 2: Schematic Representation of MexAB-OprM of *P. aeruginosa*

Positions of the three proteins in the inner membrane (CM), outer membrane (OM) and the periplasmic space (AB – drug, IMP – inner membrane protein, MFP – membrane fusion protein, OMP – outer membrane protein)

The OprM is the outer membrane protein channel, and although it shares only 21% sequence identity with TolC, it folds in a similar pattern as TolC.[54] MexB is the inner membrane protein and can be used to replace AcrB as these two inner membrane proteins are 86% similar.[53] This inner membrane protein is responsible for the substrate specificity

of this pump complex and recognizing a wide variety of compounds. MexB has been shown to have both a cytoplasmic and a periplasmic binding site that aid in the recognition and extrusion of foreign substances.[55] Absence of this cytoplasmic binding site by mutation (phenylalanine to alanine conversion) prevented resistance of *P. aeruginosa* to antibiotics that have binding sites in the cytoplasm. MexA is the membrane fusion protein. It is a lipoprotein which forms a hexameric complex to interact with OprM; the α-barrel of MexA interacts with α-barrel of OprM.[56]

1.6. STRATEGIES TO OVERCOME RESISTANCE BY EFFLUX

It has been demonstrated that inhibition of efflux results in a decrease in emergence of resistant strains of bacteria, decrease in innate resistance and a reversal of acquired resistance.[57] With efflux playing such a vital role in antibiotic resistance, it is necessary to come up with strategies to overcome efflux in bacteria. The problem of efflux could possibly be solved in four main ways: 1) by-passing efflux activity, 2) direct action on the permeability of the bacterial cell envelope and 3) blocking the efflux capacity of the bacterial cell.[58]

1). By-passing efflux activity – This can primarily be done by redesigning old antibiotics to reduce their susceptibility to the efflux pumps. By making structural changes that decrease their susceptibility to efflux, it could be possible to modify existing antibiotics such that they will not be susceptible to efflux activity.

2). Direct action on the permeability of the bacterial cell envelope – This can be achieved by reducing the efficiency of the membrane barrier. This can be achieved in one of two ways. Firstly, the permeability of the membrane can be increase through the action of 'surfactant-like' agents. These agents make the membrane readily permeable allowing more antibiotics to enter the cell. The second route is by blocking the channel on the outer membrane to prevent the exit of drug molecules after they enter the cell.[26] These drugs could interfere with the assembly of the pump making them dysfunctional. Blockage of these channels ensures that a high intracellular concentration is maintained.

3). Blocking efflux capacity of the bacterial cell through competition with the antibiotics for the inner membrane pump component - This can be a competitive inhibition or a non-competitive inhibition. There can also be the inhibition of the energy source used by the pump. These are also called energy decouplers. These compounds dissipates the energy in the Proton Motive Force (PMF).[26] These compounds are effective because most efflux pumps rely on the proton motive force as their source of energy. They do not bind directly to the efflux pumps. However they affect the transmembrane electrochemical potential thus affecting the efflux system.

4). Biological inhibition – This is an emerging strategy that is currently gaining interest. In biological inhibition, there is an alteration of the transcriptional regulation of the pump-encoding genes that is affecting the efflux pump protein or gene.[26] Also, there can be interference of any of the regulatory steps in efflux pump expression. Antibodies have been patented that deactivate the MexAB-OprM pumps of *Pseudomonas*.[59] An antisense approach towards the AcrAB pump of *E. coli* has also been described.[60,61]

1.7. EFFLUX PUMP INHIBITORS

Efflux pump inhibitors are structurally diverse with a wide variety of compounds capable of acting as inhibitors. These compounds included diamines like phenylalanyl-arginyl-β-naphthylamide (PAβN) also called MC-207,110, the energy uncoupler carbonyl cyanide m-chlorophenylhydrazone (CCCP), globomycin, pyridopyrimidines, arylpiperazines derivatives, tetracycline analogues and quinolone derivatives.[14] The different types of efflux inhibitors can be classified under broad groups based on the origins of their discovery. These compounds could be obtained through screenings, testing of already known inhibitors of efflux to expand their therapeutic potential, evaluation of current drugs other than antibiotics for efflux inhibition and through rational drug design.

1. **SCREENINGS –** The drugs derived from screenings can be categorized into those that are obtained from synthetic compounds libraries and those that are from natural sources such as plants and insects. The chemical structures of some of these compounds are illustrated in Figure 3 (from synthetic compound libraries) and Figure 4 (from plants)

A). SCREENINGS OF SYNTHETIC COMPOUND LIBRARIES

Phenylalanyl-Arginyl-β-Naphthylamide (MC-207,110) – This was the first described EPI.[50] It is a broad spectrum EPI found to work in *P. aeruginosa* and several other Gram-negative bacteria including *K. pneumonia, E. coli, S. enterica* and *E. aerogenes*.[62] It works to improve the activity of fluoroquinolones, chloramphenicol, macrolides/ketolides, oxazolidinones and rifampicin.[62] It, however, does not have a beneficial effect when used with β-lactams and aminoglycosides. Its mechanism of action studies indicate that it is

itself a substrate of the pumps and is in competition with the antibiotic that it potentiates for the binding site associated with the efflux pump. Attempts to discover mutations responsible for its activities proved futile.[50] It has the disadvantage of being significantly cytotoxic and thus cannot be used in humans.

IRON CHELATORS – An example of this is nocardamine. These compounds have been found to be non-specific antagonist of tetracycline efflux pumps which may work directly or indirectly to inhibit the pump.[63] It can interact indirectly with the pump by chelating iron which is a cofactor important for maintaining the proton motive force (pmf). TetB and TetK are pmf-dependent.[64]

MP-601,205 – This is the only EPI that was reported to be in Phase II clinical trials. The data remain unknown, but is currently on hold due to issues with drug tolerability. Its use is being examined together with ciprofloxacin for the treatment of pulmonary infections.[45,50,62]

ARYL PIPERIDINES AND PIPERAZINES- These are inhibitors of AcrAB-Tolc pump and were discovered in a high-throughput screen.[65] The most active is 1-(1-naphthylmethyl)-piperazine (NMP), which has activity against fluoroquinolone susceptible *E. coli*.[66] The amino acid residues G141 (mutated to D), N282 (mutated to Y), G288 and A279 have be found to be the amino acids critically associated with retention of necessary efflux inhibition.[67]

MBX2319 – Discovered through high-throughput screening using *E. coli*.[46] This research was performed to identify compound that work to increase the potency of ciprofloxacin against enterobacteriaceae. SAR studies were done on this lead compound to obtain

compounds capable of producing higher potentiation of both levofloxacin and piperacillin.[68]
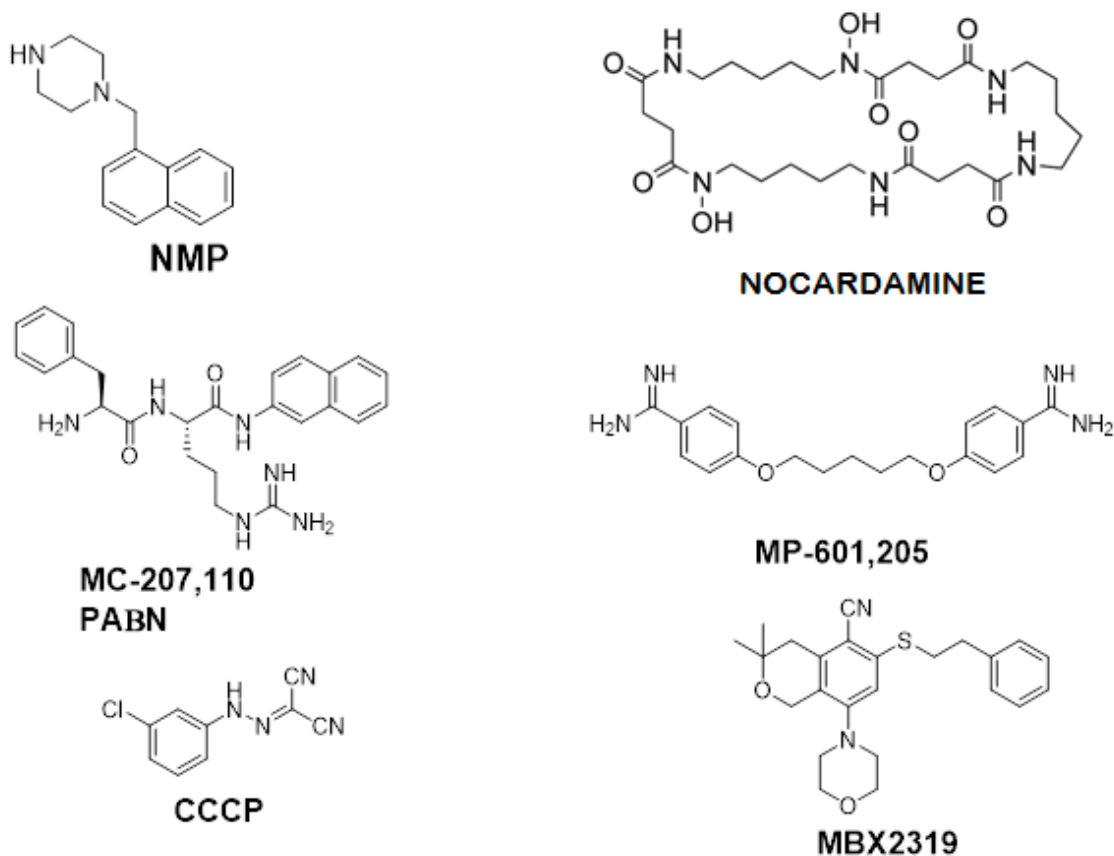
Figure 3: Efflux Pump Inhibitors Obtained from Screenings of Synthetic Compound Libraries

B). PLANT DERIVED EFFLUX PUMP INHIBITORS.

Natural products have always been a major source for the discovery of new medicinal agents. Plants have had to guard themselves against a plethora of bacteria found in their environment. By selection, certain plants have developed agents that can inhibit bacterial

efflux pumps and effectively kill bacteria. Natural product research has provided new agents as efflux pump inhibitors.[8] The Berberis plants produce two pump inhibitors pheophorbide A and 5'-methoxyhydnocarpins (5'-MHC). They potentiate the activity of the antibiotic berberine against the NorA pump of *S. aureus*. *Silybum marianum* and *Jatropha elliptica* plant species produce several NorA inhibitors. The diterpenes, carnosic acid and isopimarane also potentiate the activity of erythromycin in resistant strains of *S. aureus*.[69]

PHEOPHORBIDE A

5'-METHOXYHYDNOCARPIN

ISOPIMARANE DERIVATIVE

CARNOSIC ACID
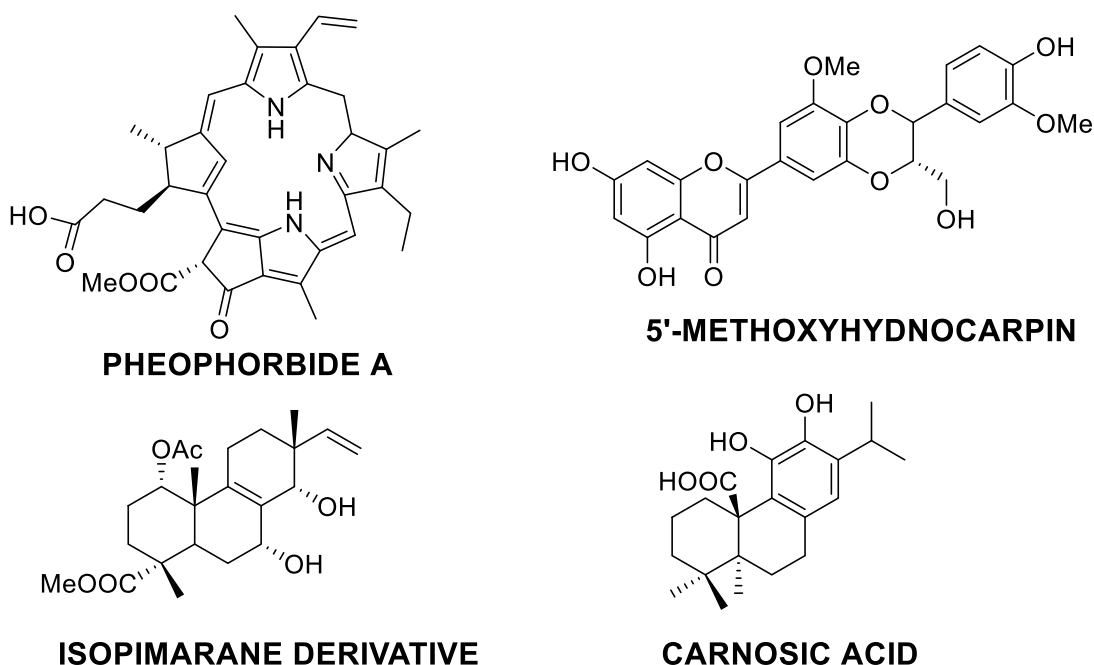
Figure 4: Plant Derived Efflux Pump Inhibitors

2. DRUGS OTHER THAN ANTIBIOTICS

PHENOTHIAZINES – The working hypothesis is that these drugs work to inhibit the proton motive force.[69] Phenothiazine, an example of which is chlorpromazine (Figure 5),

is used as antipsychotic agents or antihistamines. They potentiate the activity of some anti-tubercular drugs.

SELECTIVE SEROTONIN REUPTAKE INHIBITORS – These are antidepressants, an example of which is paroxetine (Figure 5). They potentiate the activity of several substrates that would otherwise have limited bacterial penetration as a result of S. *aureus* efflux pumps.[69]

CHLORPROMAZINE   **PAROXETINE**
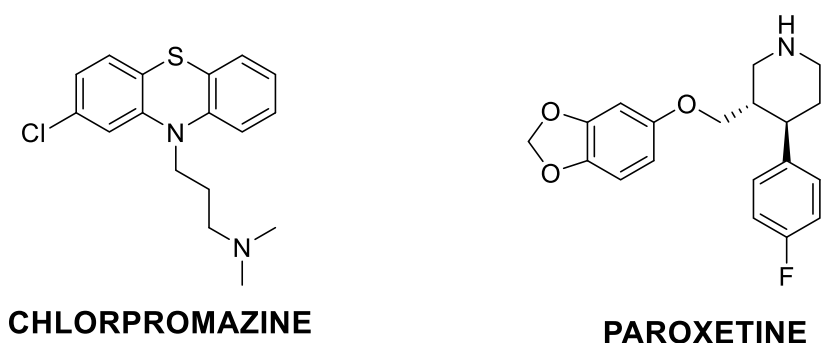
Figure 5: Drugs Other than Antibiotics as Efflux Pump Inhibitors

3. **RATIONAL DRUG DESIGN**

D13-9001 – This is a pyridopyrimidine derivative. The structure of D13-9001 is shown in Figure 6. It has been shown to have *in vivo* activity and a good safety profile. It is active against MexAB-OprM being specific to this pump.[62] In other words, it is able to potentiate all the substrates of this pump.[50] The pyridopyrimidine series are known to have poor stability due to photoisomerization. Modification of their structure led to D13-9001. Discussion on the activity of these analogs in the literature indicate that there is a clear

need to improve the solubility of these compounds in order for them to further developed into clinical lead compounds.[69-75]

13-CYCLOPENTYLTHIOTETRACYCLINE- This is a potent specific TetB EPI developed from doxycycline. Its structure is shown in Figure 6. It has synergistic activity with doxycycline against resistant *E.coli* strains.[76-78] However, development of a universal tetracycline-efflux pump has proved challenging due to the many sub families of efflux pumps.

D13-9001

13-CYCLOPENTYLTHIO-TETRACYLINE
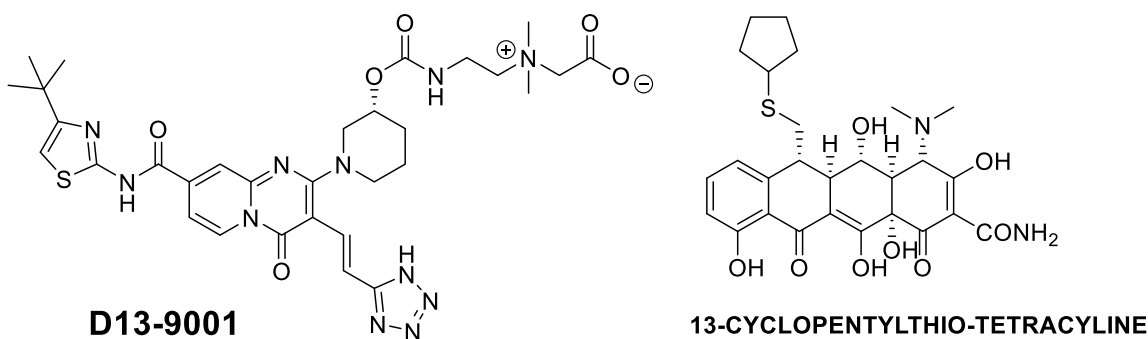
Figure 6: Efflux Pump Inhibitors obtained from Rational Drug Design

4. KNOWN INHIBITORS OF OTHER EFFLUX SYSTEMS

RESERPINE – This is an antihypertensive plant alkaloid which also acts an inhibitor of the eukaryotic ABC transporter, P-glycoprotein. It works at high concentrations to inhibit the Bmr pump of *B. subtilis*. However, this inhibition is not clinically relevant due to its neurotoxicity.[69] It potentiates the activity of fluoroquinolones in Gram-positive bacteria and tetracycline. The structure of reserpine is shown in Figure 7.

VERAPAMIL – This is a calcium channel antagonist which is also an inhibitor of P-glycoprotein.[69] Its structure is shown in Figure 7. It also inhibits bacterial ABC pumps such as LmrA of *Lactococcus lactis* and EfrAB in *Enterococcus faecalis*.[79,80]

EPICATHECHIN GALLATE AND EPIGALLOCATECHIN-GALLATE – These are shown in Figure 7. They are active against tetracycline resistance in *Staphylococcus* strains that overexpress TetB or TetK pumps. They also work against NorA overexpressing *S. aureus*. The NorA efflux is known to limit the cellular level of norfloxacin in these strains.[69]

GG918, BIRICODAR AND TIMCODAR – These are also inhibitors of the mammalian efflux pump. Timcodar and Biricordar work specifically against P-glycoprotein and multidrug-resistance associated protein (MRP-1).[81] Their structures are shown in Figure 7. They potentiate the activity of fluoroquinolones and three other antibiotics in Gram-positive bacteria (specifically *S. aureus, E. faecalis* and *S. pneumoniae*).[69,82] Timcodar has also been shown to have some efflux inhibitory activity for the drugs, isoniazid and rifampicin, that are used against *Mycobacterium tuberculosis*.[83]
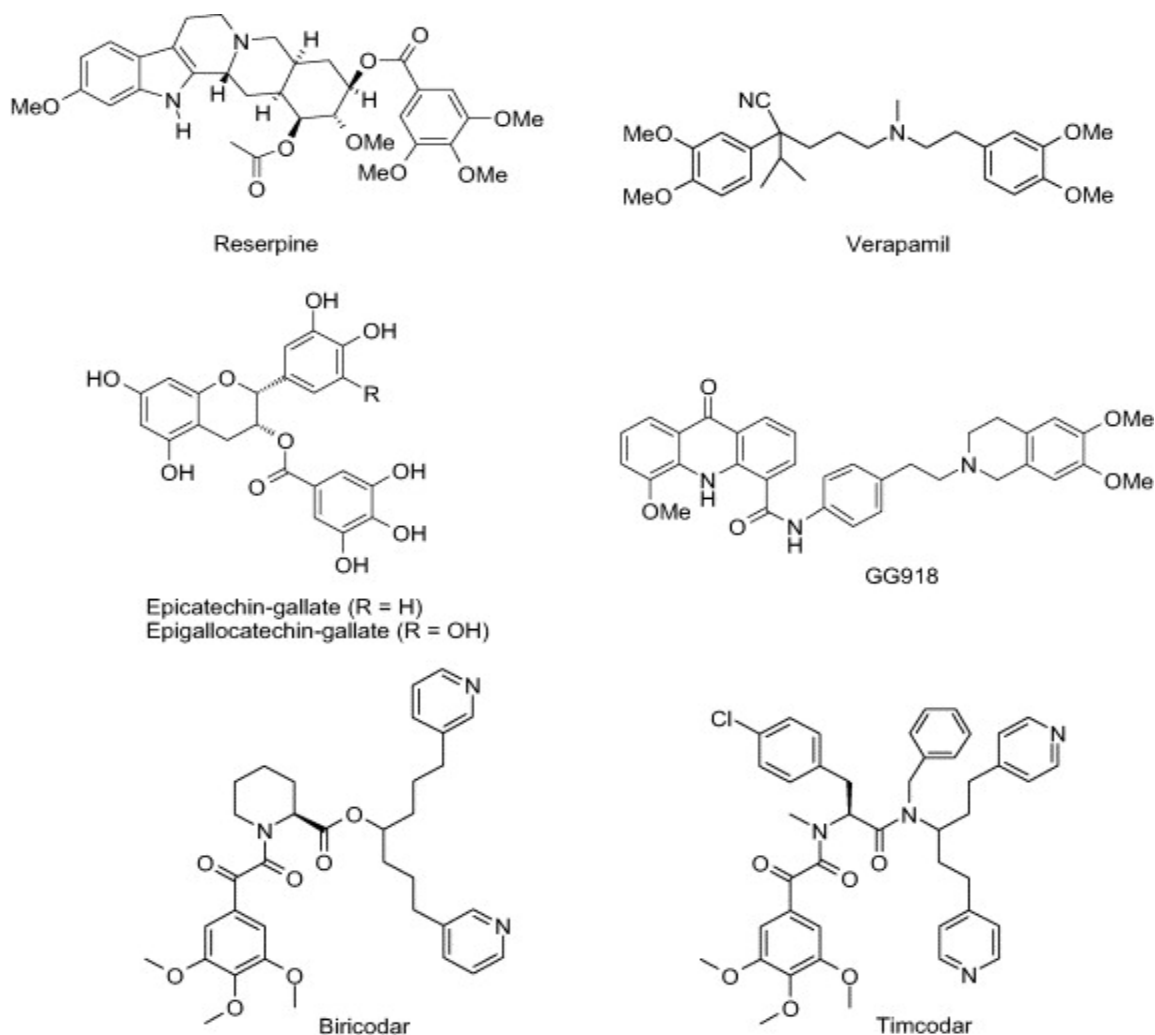
Figure 7: Known Inhibitors of Mammalian efflux systems

1.8. CURRENT AND FUTURE DEVELOPMENTS

Several challenges have arisen is the search for clinical suitable EPIs. First and foremost, most EPIs developed are not broad spectrum (in antibiotic therapy it is preferably to have broad spectrum activity).   Also, efficient animal models have not been developed

that allow for efficacy, safety and toxicity profiles to be readily established. There is a need to develop models that can specifically elucidate the mechanism of action of these pumps. The models must be able to judge which binding domains and which interactions are necessary to be inhibited to elicit an efflux pump inhibitory effect. Furthermore, there is a possibility of the development of resistance to these EPIs themselves. It seems paradoxical that a compound may exist that could potentially block efflux in view of the fact that these pumps are well crafted to handle a wide variety of compounds.[47] An area that can be looked into is to develop small molecules that affect protein-protein interactions

The search for a clinical MDR candidate has become long and arduous. It seems that after so many years of the discovery of these pumps, there has not been significant progress in the attainment of a clinical drug. Much of the effort has been in the synthesis and *in vitro* evaluation of large libraries of compounds. With this major gap in antibacterial therapy, there is a need, at this juncture, for a systematic study of the structure-activity relationship of EPIs. This will hopefully aid the discovery and development of a clinically-useful efflux pump inhibitor as an effective adjuvant in anti-infective therapy.

RATIONALE

Antibiotics have served mankind well in providing a means of defense against infectious diseases. The emergence of antibiotic resistance within a few years of the introduction of a novel antibiotic poses a constant challenge in antibacterial therapy.

Combined with this menace, is the reduction in the number antibiotics that are being introduced each year into the market. With the increase in clinical isolates that are resistant to vancomycin (which approaches the last line therapy in MRSA), there is a need to come up with novel strategies to combat antimicrobial resistance.[69] The data show that a lack of efflux activity causes not only lower intrinsic resistance, but also a reduced risk of selection of resistant mutant strains.[14,15] The introduction of a clinically-effective efflux pump inhibitor has the potential to enable the reintroduction of old antibiotics that have been discarded due to the issue of resistance. The effective use of existing antibiotic that may act in synergy with these EPIs could allow for a reduction in the appearance of drug resistant pathogens and prevent the development of resistant biofilms.[53] Efflux pump inhibitors could be a means to combat several of the resistance issues that have arisen in anti-infective therapy.

The goal of the research was to propose novel efflux pump inhibitors. We looked into the SAR of these novel EPIs to increase the pharmacologic activity and reduce toxicity. The SAR focused predominantly on the Gram-negative bacteria; *E. coli*. However, we did examine the potential for success in *P. aeruginosa*. Our efforts were focused on improving upon the discovered efflux pump inhibitors and to provide a better understanding of the influence of structure on activity as an EPI. In the long term, it is the hope that establishing this ground work will enable the discovery and development of EPI's into the clinic.

RESULTS AND DISCUSSION

2.1 INITIAL EFFORTS TOWARDS THE DISCOVERY OF A NOVEL EPI

Certain requisites should be satisfied by a compound to be an EPI.[26] These are:

1. The EPI candidate should not have any adverse effect on mammalian cells. As this therapy is directed against microbes living in humans, it stands to reason that these candidates need to be relatively non-toxic at effective concentrations.

2. The synthesis of these compounds should not be cumbersome and expensive as that reduces their potential to be developed into the clinic. For an ideal EPI, the synthesis should be straight-forward and one that could ultimately be scalable.

3. The candidates should be stable to mammalian metabolism allowing them to reach a high enough concentration in bacteria to exert their effect on the microbial pump.

Therefore, it would be highly desirable to have a large therapeutic index, as well as a good pharmacokinetic profile, to ensure maximal activity and specificity. These factors were taken into consideration in the selection of our EPI lead compound for further exploration. Aryl piperazine derivatives fulfilled most of these requisites and thus our efforts to create a novel EPI begun from within these series of compounds. It had been indicated that compounds in this series are able to reverse multidrug resistance in *E. coli*.[84] Work on the aryl piperazine series began in our laboratory by synthesizing biphenyl analogues as illustrated in Scheme 1. Bromobenzene was coupled to 4-methyl phenyl

boronic acid using the Suzuki reaction to give the biphenyl intermediate, **1**, as illustrated

in Scheme 1. The catalyst used for the reaction was palladium acetate and Xphos with the

solvents acetonitrile and water (2:1) to give product in an approximately quantitative yield.

Free radical bromination of 4-methyl-1,1´-biphenyl, **1**, with AIBN afforded the 4-

(bromomethyl)-1,1'-biphenyl, **2**. This product was independently reacted with methyl

piperazine and piperazine to obtain 1-([1,1'-biphenyl]-4-ylmethyl)-4-methylpiperazine and

1-([1,1'-biphenyl]-4-ylmethyl)piperazine, **3** and **4** respectively. 1,4-bis([1,1'-biphenyl]-4-

ylmethyl)piperazine, **5**, was formed as a byproduct of the reaction between 4-

(bromomethyl)-1,1'-biphenyl, **2**, and piperazine.
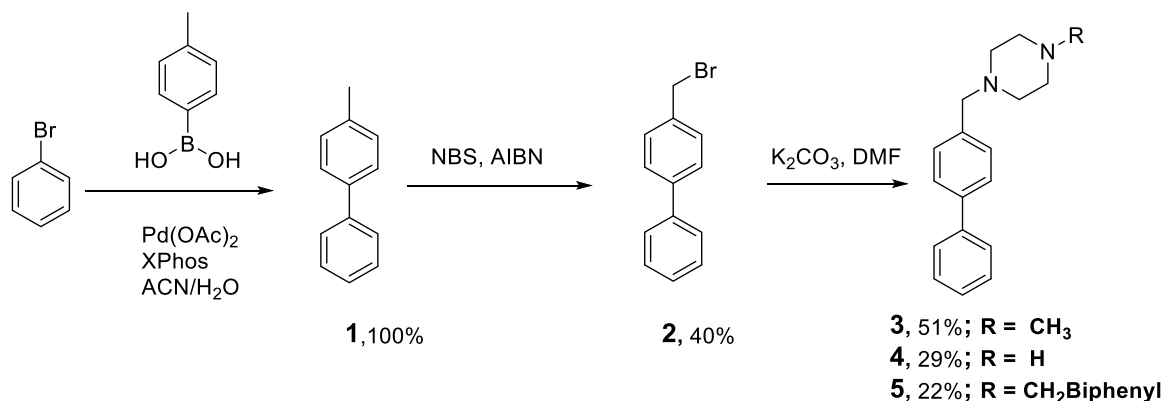
Scheme 1: Synthesis of Compound **3-5**

Having obtained these compounds, they were isolated, purified and tested for their

efficacy as EPI agents. All the bacterial assays presented here and in subsequent pages

were done by Dr. Malvika Kaul, Dr. Yevgeniy Turovskiy and Prof. Daniel Pilch of the

Department of Pharmacology at Rutgers-UMDNJ. Initial assays sought to identify if these

compounds were EPIs by answering a simple yes or no to the question as to whether they

exhibited activity as an EPI. The biological assays first found the Minimum Inhibitory Concentration (MIC in µg/mL) of the test compound by alone and concurrently the MIC of the compound when tested with 16 µg/mL CK-1-12 in wild type *E. coli*. We sought after a reduction in the MIC of the synthesized compound when it was tested in the presence of CK-1-12.
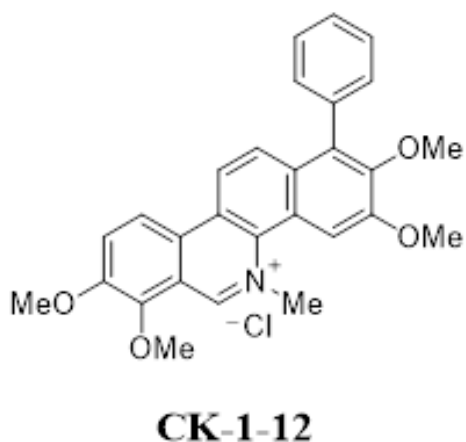
Figure 8: Structure of efflux pump substrate **CK-1-12**

The rationale for this assay is based on the inherent activity of CK-1-12.[85] This compound was synthesized by Dr. Cody Kelley (a former member of the LaVoie group). CK-1-12 had been found to be an efflux pump substrate. Although it has activity in mutant *E. coli* with no efflux pump (N43) (MIC = 1 µg/mL), it shows no activity in the wild type *E. coli* (W4573) (MIC > 64 µg/mL). The two *E. coli* strains are genetically identical, with the only difference being a mutation in the *acrA* gene in N43, which knocks out the function of the AcrAB efflux pump. The fact that CK-1-12 is active against the mutant N43 strain but lacks activity against the wildtype W4573 strain indicates that CK-1-12 is a substrate for the AcrAB pump.

Armed with the knowledge that CK-1-12 is a substrate of the efflux pump, we developed an assay to screen for efflux pump inhibition activity using CK-1-12. We added CK-1-12 at a constant concentration of 16 µg/mL to wildtype W4573 bacteria. In the absence of any inhibitors, the bacteria grew well at this concentration of CK-1-12. This is due to the fact that the wild type *E. coli* have an active efflux pump (as stated in the previous

section, the MIC of CK-1-12 against wildtype W4573 is > 64 µg/mL). It was anticipated that in the presence of an active efflux pump inhibitor (EPI), CK-1-12 will be active against the wild type *E. coli*. In other words, a lower minimal inhibitory concentration (MIC) against the wildtype bacteria with the EPI in the presence of CK-1-12 versus in the absence of CK-1-12 would be indicative of efflux pump inhibition activity. While PAβN did prove to be active under the assay condition as an EPI, the data obtained under these same conditions and illustrated in Table 2 indicated that none of the piperazine derivatives, **3-5**, exhibited EPI activity.
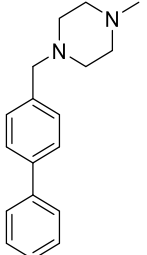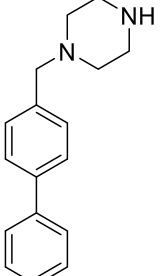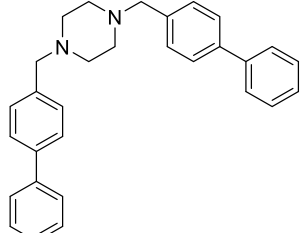
Code	Structure	efflux pump inhibitor activity	MIC (µg/mL)	MIC with 16µg/mL CK-1-12 (µg/mL)
PABN		Yes	>400	25/12.5
3		No	>400	>400
4		No	>400	>400
5		No	>400	>400

Table 2: EPI activity of Biphenylpiperazine

Naphthyl piperazines, as discussed previously, have also been shown to have EPI activity. The leading example 1-(1-naphthylmethyl)piperazine is able to moderately reverse the efflux pump activity of some members of Enterobacteriaceae which includes *E. coli.*[65] We synthesized these compounds as we searched for new leads in the discovery of efflux pump inhibitors. Commercially-available 1-methylnaphthalene was brominated to give 1-(bromomethyl)naphthalene, **6** as illustrated in Scheme 2. The synthesis of 1-methyl-4-(naphthalen-1-ylmethyl)piperazine, **7**, proceeded in a similar manner to the synthesis of 1-([1,1'-biphenyl]-4-ylmethyl)-4-methylpiperazine, **3**. 1-(bromomethyl)-naphthalene, **6** was treated with 1-methylpiperazine to provide 1-methyl-4-(naphthalen-1-ylmethyl)piperazine in 56% yield.

The synthesis of 1-(naphthalen-1-ylmethyl)piperazine, **9**, followed a two-step sequence to avoid the by-product obtained previously in the synthesis of 1,4-bis([1,1'-biphenyl]-4-ylmethyl)piperazine, **4**. 1-(Bromomethyl)naphthalene, **6**, was displaced with boc-piperazine to obtain *tert*-butyl 4-(naphthalen-1-ylmethyl)piperazine-1-carboxylate, **8**. The boc-protected, **8**, compound was deprotected with trifluoroacetic acid and dichloromethane to give the final compound, 1-(naphthalen-1-ylmethyl)piperazine, **9**, in 75% yield**.**
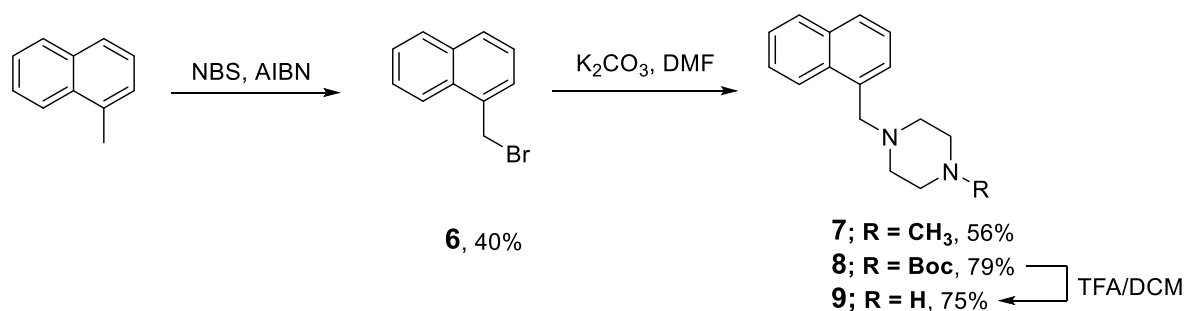
Scheme 2: Synthetic Route for Compounds **7-9**

Analogues of the naphthylmethyl compound with a longer chain length were synthesized to explore the possible effect of increased chain length on the activity of these piperazines derivatives as efflux pump inhibitors. Chain elongation of the spacer between the naphthalene and the piperazine could potential improve potency as had been discussed in literature.[84] We used the organomagnesium reagent as nucleophile to synthesize 1-allylnaphthalene, **10,** from commercially-available 1-bromonaphthalene. We had previously tried to use the Stille procedure in which 1-(Bromomethyl)naphthalene, **6,** was reacted with vinyl tributyltin in the presence of Pd₂dba₃ and tri(2-furyl)phosphine to form allylnapthalene. While the reaction had worked, it was not possible to purify the product due to contamination with tributyltin. The Grignard reagent was, therefore, selected as the alternative to synthesize intermediate **10** as illustrated in Scheme 3. Hydroboration-oxidation was used to form the alcohol, **11**, which was reacted with mesyl chloride to give the mesylate, **12**. Displacement of the mesylate with potassium cyanide gave rise to 4-(naphthalen-1-yl)butanenitrile, **13.** The LAH reduction of the cyano group gave the 4-(naphthalen-1-yl)butan-1-amine, **13.** Reaction of this amine, **13**, with 1,3-diboc-2-(trifluoromethyl sulfonyl) guanidine in the presence of triethylamine and dichloromethane

at room temperature afforded the diboc-protected guanidine, **15**, which was deprotected with trifluoroacetic acid and dichloromethane to give 2-(4-(naphthalen-1-yl)butyl)guanidine, **16**.
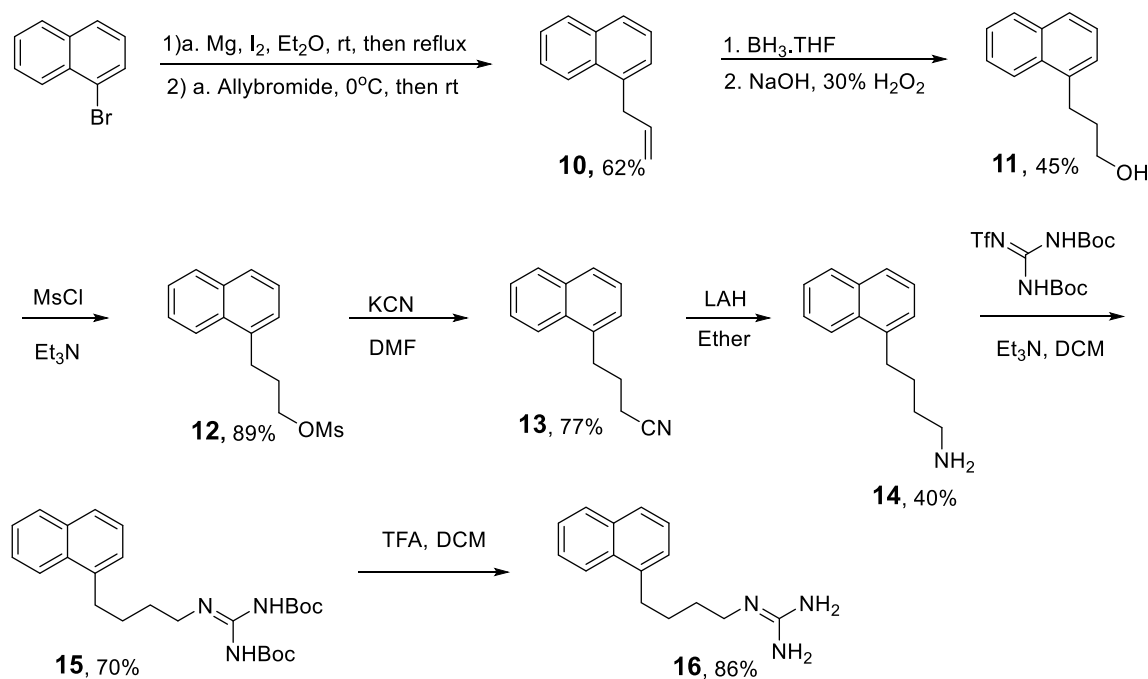
Scheme 3: Synthetic Route to Compounds **13-16**

The compounds **7-9** and **13-16** were tested for EPI activity using the same biological assay as outlined previously for the biphenyl piperazines. The results obtained from the assay are shown in Table 3. Unfortunately, none of the compounds synthesized displayed efflux pump activity. All the compounds failed to improve the activity of CK-1-12 indicating that the synthesized compounds were not inhibiting the AcrAB-TolC pump in *E. coli*. The search for a lead EPI shifted away from this series of compounds.
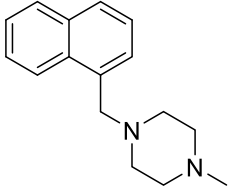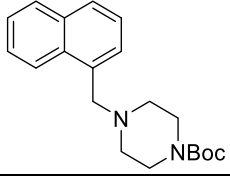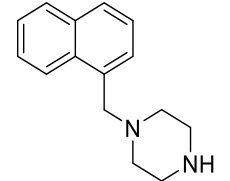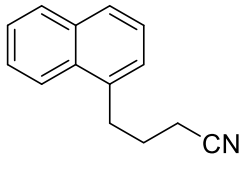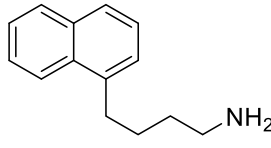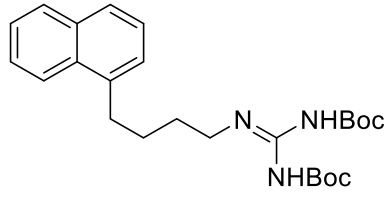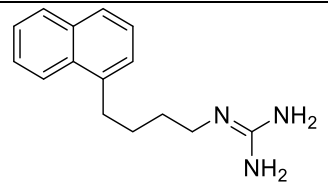
Compound code	Structure	efflux pump inhibitor activity	MIC (µg/mL)	MIC with 16µg/mL CK-1-12 (µg/mL)
PABN		Yes	>400	25/12.5
7		No	>400	>400
8		No	>400	>400
9		No	>400	>400
13		No	>400	>400
14		No	>400	>400
15		No	>400	>400
16		No	>400	>400

Table 3: EPI activity of Naphthalene amines

2.2. STRUCTURE ACTIVITY RELATIONSHIP OF "NORMAL" AMIDES

Prior structural studies on PAβN as reported in the literature with had indicated that the guanidinium moiety was not essential in EPI activity.[86] The removal of the guanidine led to a compound with an MPC_8 two-fold more potent than the original compound PAβN as illustrated in Figure 9.[86] MPC_8 is the minimum concentration of the inhibitor required to decrease the MIC of levofloxacin by 8-fold (Figure 9).

PAβN

MPC_8 = 10 μg/mL

17

MPC_8 = 5 μg/mL

L-phenylalanine-L-ornithine-β-naphthylamine
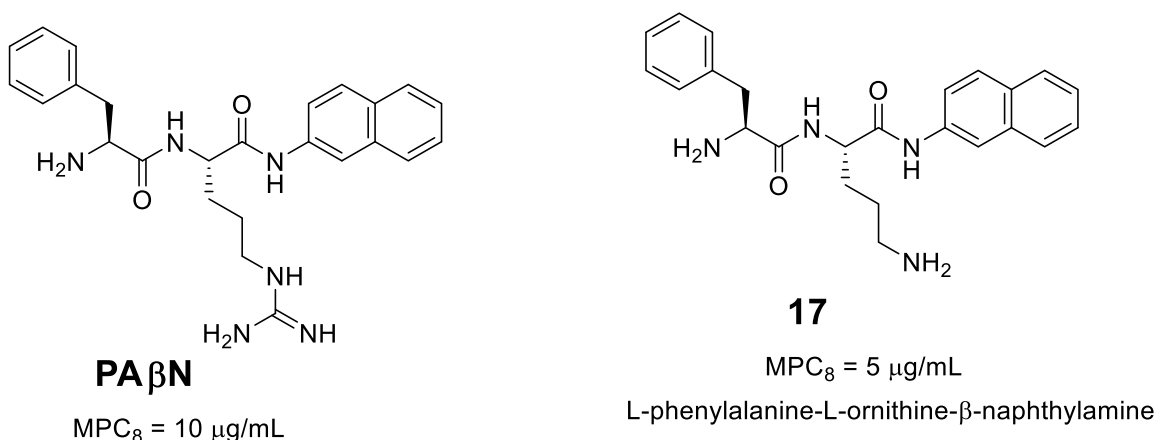
Figure 9: Development of PAβN - Step 1

L-phenylalanine-L-ornithine-β-naphthylamine, **17** was stable in growth media but was highly unstable in biological systems such as mouse and rat human serum.[33] The product of metabolic degradation was found to be L-ornithine-β-naphthylamine, **18** (Figure 10). They sought to make the amide, **17**, more stable by forming the N-methyl amide derivative, **19**.

17   **18**

Product of Metabolism
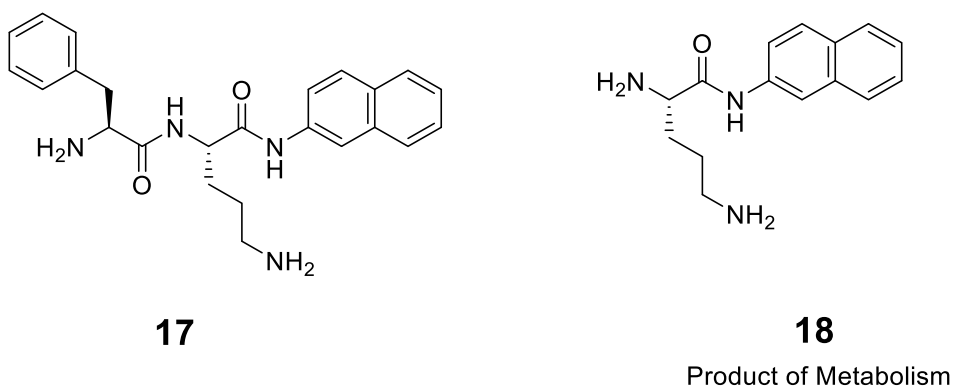
Figure 10: Developing the SAR of PAβN - Step 2

However, the N-methyl amide derivative, **19**, also presented stability issues. It cyclized to form the lactam, **20**, as a metabolite which was not active as an EPI (Figure 11).[33] The β-naphthylamine is also a known human bladder carcinogen. Chronic exposure to this compound either by itself or as an impurity has been linked to an increased frequency in the occurrence of bladder cancer.

19   **20**
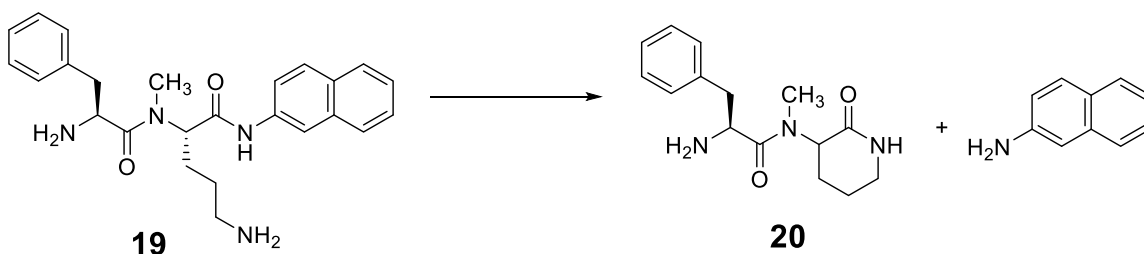
Figure 11: Development of PAβN - Step 3

The most logical next step, which was replacement of the ornithine, had already proven to be ineffective. A strategy which was then tried was switching the positions of amino acids in the dipeptides sequence. This led to the synthesis of L-ornithine-L-phenylalanine-β-naphthylamine, **21**, which did display some EPI activity although it was

not as potent as **17**. However, it also did not display any propensity to form the lactam ring, which was an improvement over, **19**. SAR studies of this new compound led the L-ornithine-L-homophenylalanine-3-aminoquinoline, **22**, which had a good balance between its potency and intrinsic antibacterial activity.[33] The structures of **21** and **22** are illustrated in Figure 12.

L-Ornithine-L-phenylalanine-β−naphthylamine

21

L-Ornithine-L-homophenylalanine-3-aminoquinoline

22
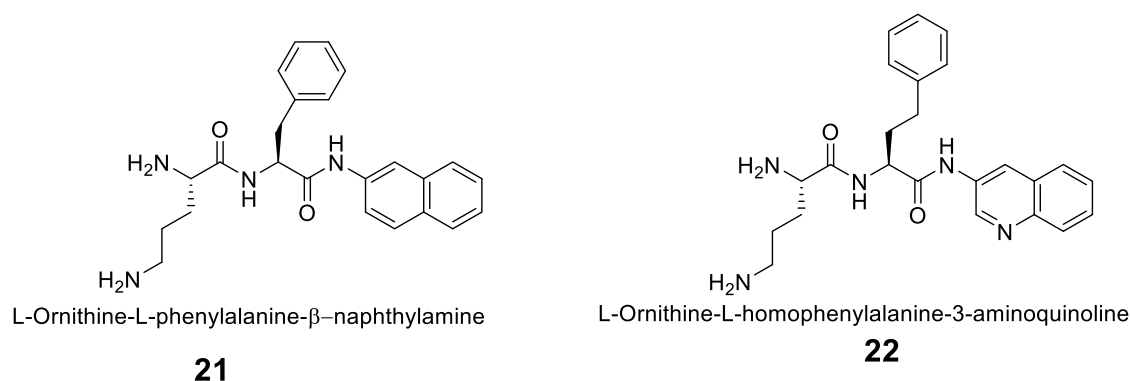
Figure 12: Development of PAβN - Step 4

Conversion of the L-amino acids to the D-isomers gave final compounds which were very stable in biological systems. The D-isomer, **23**, was used as lead in the synthesis of structurally simpler analogues, which maintained the peptide backbone (Figure 13). These modified compounds would feature either an ether and thioether bond whilst also replacing the aminoquinoline with other heterocycles.[87] Some of the more potent of these new compounds, which also had relatively easier routes of synthesis were the ether, **24a**, and thioether, **24b**.[87]

L-Ornithine-L-homophenylalanine-3–aminoquinoline
22

D-Ornithine-D-homophenylalanine-3–aminoquinoline
MC-02,595 (23)

24a (X = O)
24b (X = S)
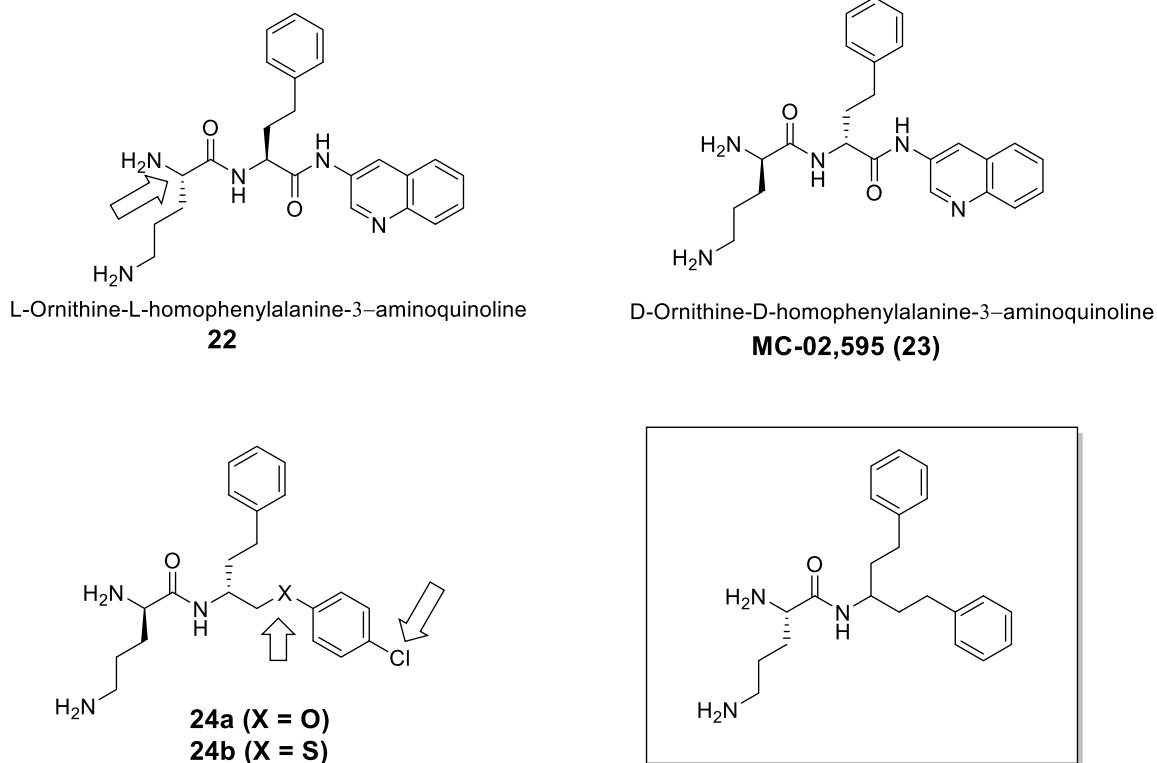
Figure 13: Development of PAβN - Step 5

The insights we had gleamed from the prior studies since the discovery of PAβN

up to the ether, **24a**, and thioether analogues, **24b**, enabled us to propose a simpler structure

as lead for the synthesis of novel EPI analogues. We decided to replace the oxygen with a

carbon whilst removing the chlorine substituent. This gave a compound with only one

stereocenter and reduced the number of steps to its synthesis providing a relatively

symmetrical compound. Using the L-amino acids for our initial SAR studies and the later

evaluation of their enantiomer by using D-amino acids proved economical as the L-isomers

are less expensive. Using L-ornithine, which is commercially available, we initially

decided to explore varied lipophilic substituents that could be associated with increased

activity as an EPI. With this new lead in mind, we decided to explore the SAR of this

target compound. Our first objective was to synthesize the varying alkyl chain length analogues as well as assessing the relative activity of its D-enantiomer. Keeping the ornithine constant, the right hand portion of the molecule was coupled with various acids.

Peptide coupling of L-bis-boc-ornithine with commercially-available diphenylmethanamine was used to form the amide, di-*tert*-butyl (5-(benzhydrylamino)-5-oxopentane-1,4-diyl)(S)-dicarbamate, **25**. No base was used in the peptide coupling step. Deprotection with trifluoroacetic acid and dichloromethane (1:1) provided the final product; (S)-2,5-diamino-N-benzhydrylpentanamide, **26** in quantitative yield. The synthesis of **26** is outlined in Scheme 4.
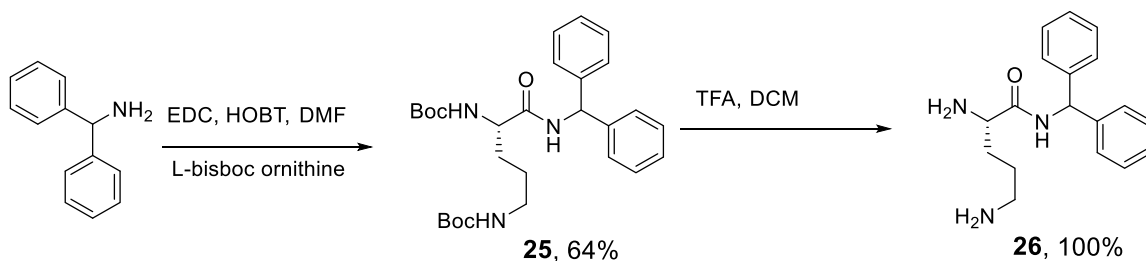
Scheme 4: Synthetic Route to Compound **26**

Commercially-available 1,3-diphenylpropan-2-one was condensed with hydroxylamine hydrochloride under reflux as illustrated in Scheme 5.[88] The 1,3-diphenylpropan-2-one oxime, **27**, was reduced with LAH to give desired product in low yield to provide the amine, 1,3-diphenylpropan-2-amine, **28**, as it was not commercially available Condensation of this amine, **28**, with L-bis-boc ornithine led to di-*tert*-butyl (5-((1,3-diphenylpropan-2-yl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate, **29**, which was

deprotected to give (S)-2,5-diamino-N-(1,3-diphenylpropan-2-yl)pentanamide, **30**, as shown in Scheme 5.[89]
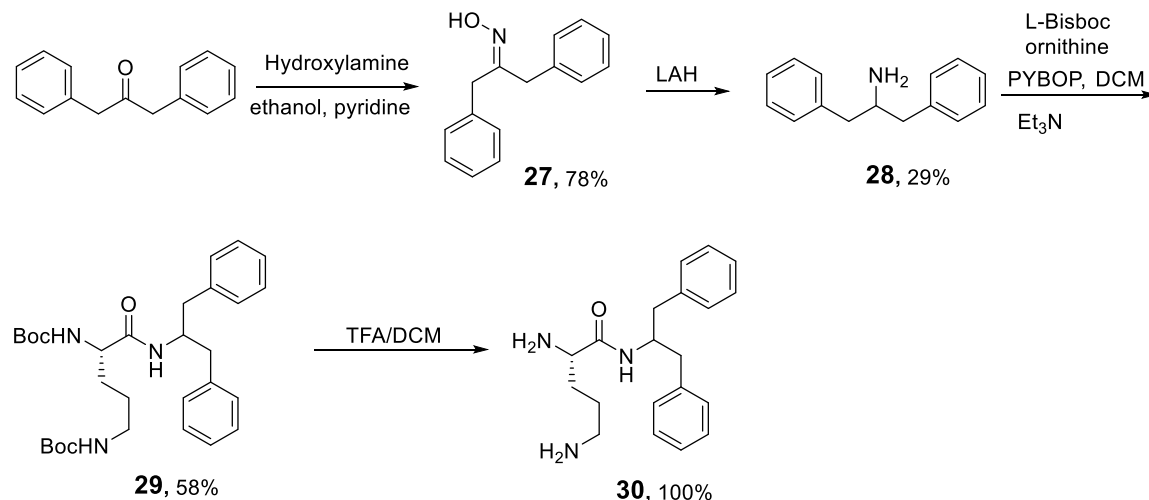
Scheme 5: Synthetic Route to Compound **30**

The synthesis of (S)-2,5-diamino-N-(1,5-diphenylpentan-3-yl)pentanamide, **36**, is illustrated in Scheme 6. Cross aldol condensation of commercially-available benzaldehyde with acetone afforded 1,5-diphenylpenta-1,4-dien-3-one, **31**.[90] The Palladium on carbon catalyzed hydrogenation of this diene-one led to the versatile ketone intermediate; 1,5-diphenylpentan-3-one, **32**. Condensation of this ketone, **32**, with hydroxylamine hydrochloride at reflux in the presence of pyridine and ethanol provided 1,5-diphenylpentan-3-one oxime, **33**. LAH reduction of the oxime, **33**, afforded 1,5-diphenylpentan-3-amine, **34**, which was condensed with L-bis-boc ornithine to give the amide, **35**. BOP was used as the peptide coupling agent in this condensation, however, there was a need for a better coupling agent as BOP reacts to give carcinogenic side products. Deprotection of di-*tert*-butyl (5-(((1,5-diphenylpentan-3-yl)amino)-5-

oxopentane-1,4-diyl)(S)-dicarbamate, **35**, with trifluoroacetic acid and dichloromethane led to the final compound, **36**.

Scheme 6: Synthetic Route to Compound **36**

The D-isomer of (S)-2,5-diamino-N-(1,5-diphenylpentan-3-yl)pentanamide, **36**, was synthesized through the condensation of **34** with commercially-available D-bis-boc-ornithine as illustrated in Scheme 7. When conducting the condensation reaction, the coupling agent used was PyBop which provided a better alternate to the BOP reagent. There is no carcinogenic side products associated with PyBOP. The amide, **37**, formed

was deprotected using trifluoroacetic acid and dichloromethane (1:1) to obtain R-2,5-diamino-N-(1,5-diphenylpentan-3-yl)pentanamide, **38** in quantitative yield.
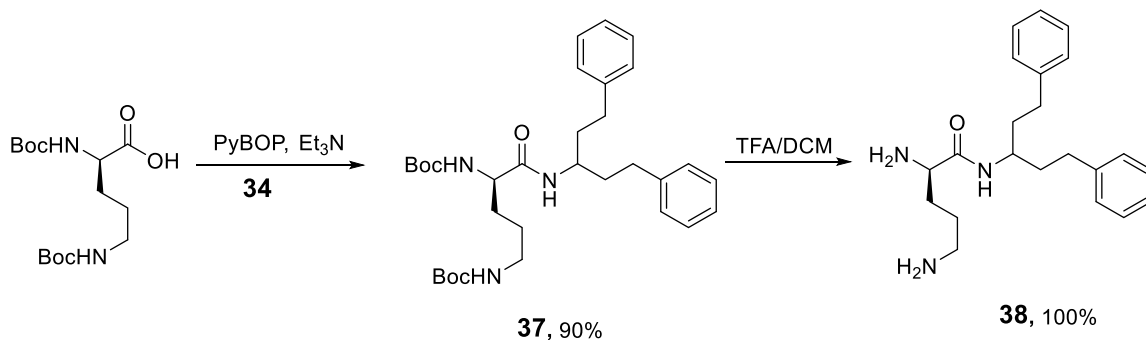
Scheme 7: Synthetic Route to Compound **38**

A preliminary biological assay was done of intermediates to determine if any of the intermediates had any EPI activity (Data not shown). This initial screen affirmed some of the previous data in literature and also brought some new insights.

1. The diamino group appears to be necessary for efflux pump inhibition. All the intermediates that lacked the two primary diamine groups showed no activity. The presence of a protecting group on the diamine rendered them inactive.

2. The bis-alkylphenyl derivative demonstrated that an aryl heterocycle was not essential.

3. The hypothesized carbon analogues of both the ether and sulfide side chains linked to the aryl group had comparable activity to the PAβN. There was no loss of activity with the replacement of the oxygen or sulfur with the carbon in the alkyl aryl side chain.

These initial screens where done using the qualitative assay described, with CK-1-12 as the test compound. A new quantitative screen was used henceforth for our SAR studies. The compounds used as part of these test included clarithromycin (for *E. coli*) and levofloxacin (for *P. aeruginosa*). These two compounds are illustrated in Figure 14. The assay performed with *E. coli* first measured the MIC of the potential EPIs that were synthesized, then measured the activity of clarithromycin in the presence of each compound. The synthesized compounds were used at a fixed concentration (12.5 µg/mL). The positive control was 50 µg/mL of PAβN while the negative control had no PAβN or experimental test compound. The MIC of clarithromycin was measured and compared against the negative control. The same experiment was done in *Pseudomonas aeruginosa*. In these studies levofloxacin was used as the antibiotic instead of clarithromycin. (The data obtained in *P. aeruginosa* will be discussed in a different section).
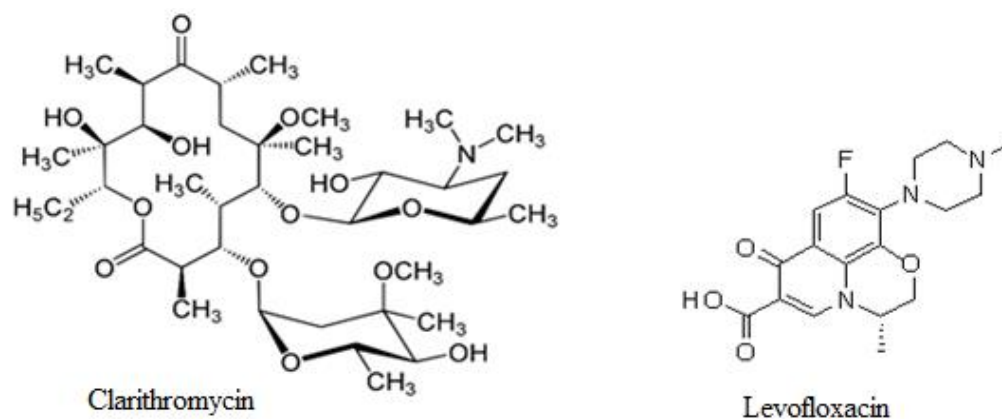
Figure 14: Antibacterial Agents Used in Efflux Pump Inhibition Assay

The MICs observed with clarithromycin in the presence of the test compounds are summarized in Table 4. For easier comparison of the MIC, the fold reduction in MIC

induced by the test compound was calculated and included in this table. The higher the number for the fold reduction, the better the test compound is as an EPI.

Table 4 shows the data obtained from the biological assays done on compounds **30**, **36** and **38**. The results indicated that **36** had comparable activity to PABN. All the other compounds exhibited less activity as EPIs as compared to PABN. These data suggest that the best chain length for the diphenyl moiety was a 2-carbon linker. It is of interest to note that the R-isomer (derived from D-amino acids) was not as active as the S-isomer (derived from L-amino acids). Renau et al had also reported stereo chemical differences in activity.[33] These data are very interesting as they do suggest that their mode of interaction is very specific and that their relative activity is unlikely to be due to nonspecific membrane binding.

Code	Structure	Concentration of EPI (μM) (12.5 μg/mL)	MIC of Clarithromy -cin (μg/mL)	Fold Reduction in MIC Induced by the Compound
--		--	64	--
PAβN		--	16	4
26		42	64	1
30		38	32	2
36		35	16	4
38		35	32	2

Table 4: Effect of Chain Length and Enantiomers on Bis-AlkylAryl Amides

The next step was to determine if a tertiary amide could be tolerated or even potentially be associated with enhanced activity. N-methyl amides have been used in the past as isosteres of regular amide groups. They have been found to promote *cis-trans*

isomerization, prevent hydrogen bonding and also project a hydrophobic group into an area previously occupied by a hydrogen.[91] N- Methyl amides encompass three chemical reactivity; steric, conformational and a removal of an H-bond donor. They may also confer on a compound enhanced metabolic stability.

The synthesis of these compounds followed the condensation of the appropriately substituted N-methylamino alkylaryl derivative with L-bis-boc ornithine. The product of this reaction was then deprotected to obtain the final compounds. The peptide coupling of these N-methyl amines required the use of a specialized peptide coupling agent, PyBrop.[92] Commercially-available N-methyl-1,1-diphenylmethanamine was reacted with L-bis-boc ornithine to obtain amide, **39**. Di-*tert*-butyl (5-(benzhydryl(methyl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate, **39**, was treated with trifluoroacetic acid and dichloromethane to obtain (S)-2,5-diamino-N-benzhydryl-N-methylpentanamide, **40**, as outlined in Scheme 8.

Reductive amination with methylamine and commercially-available, 1,3-diphenylpropan-2-one led to N-methyl-1,3-diphenylpropan-2-amine, **41** illustrated in Scheme 9.[93]
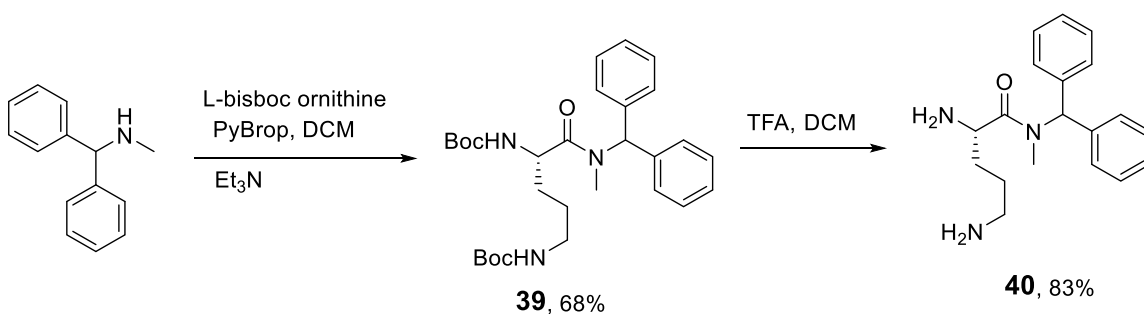
Scheme 8: Synthetic Route to Compound **40**

Reductive amination was performed by initial treatment of the ketone with methylamine, followed by reduction with sodium triacetoxyborohydride. N-methyl-1,3-diphenylpropan-2-amine, **41** was condensed with L-bis-boc ornithine to give di-*tert*-butyl (5-((1,3-diphenylpropan-2-yl)(methyl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate, **42**. Compound **42** was then treated with trifluoroacetic acid and dichloromethane to afford, (S)-2,5-Diamino-N-(1,3-diphenylpropan-2-yl)-N-methylpentanamide, **43**, as shown in Scheme 9.
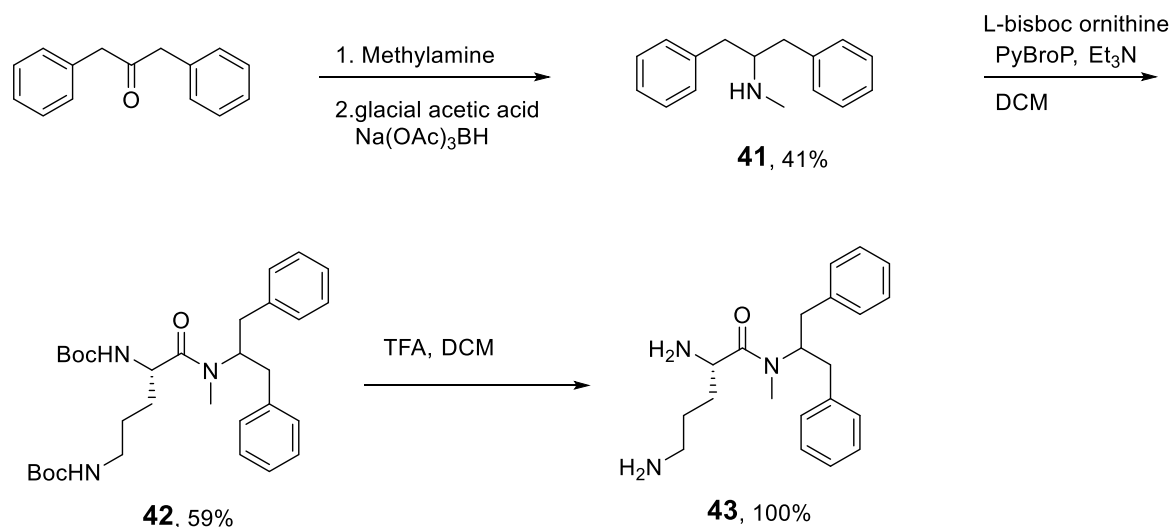
Scheme 9: Synthetic Route to Compound **43**

Reductive amination of the 1,5-diphenylpentan-3-one, **32**, with monomethyl amine provided N-methyl-1,5-diphenylpentan-3-amine, **44** as illustrated in Scheme 10. Compound **44** was condensed with L-bis-boc ornithine to afford di-*tert*-butyl (5-((1,5-diphenylpentan-3-yl)(methyl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate, **45**. Deprotection with trifluoroacetic acid and dichloromethane of this protected amide, **45**,

gave   (S)-2,5-diamino-N-(1,5-diphenylpentan-3-yl)-N-methylpentanamide,   **46**,   as
summarized in Scheme 10.
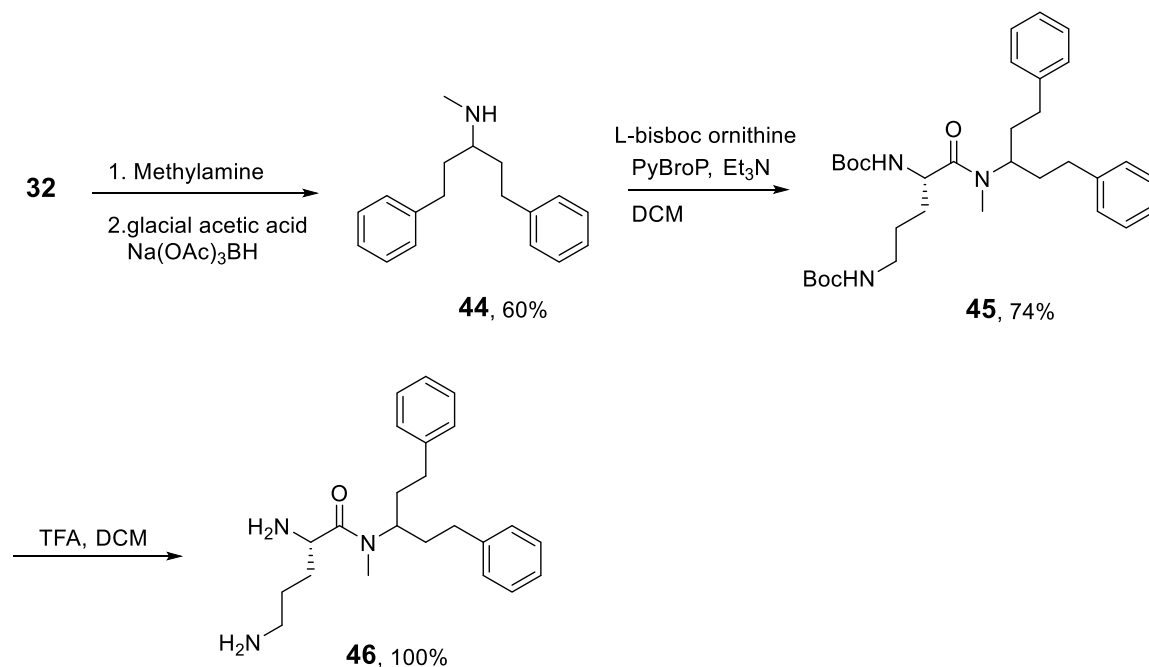
Scheme 10: Synthetic Route to Compound **46**

Table 5 lists the data obtained from the assay of the compounds (**40, 43** and **46**). There
was a reduction in potency observed for all the analogues of N-methyl amide derivatives.
None of the compounds tested exhibited EPI active comparable to PAβN or our new lead
EPI compound, **36**. Due to this finding, it was decided that the 1,5-diphenylpentan-3-
amine, **34**, and the secondary amide be maintained as constants in the subsequent near term
SAR studies.
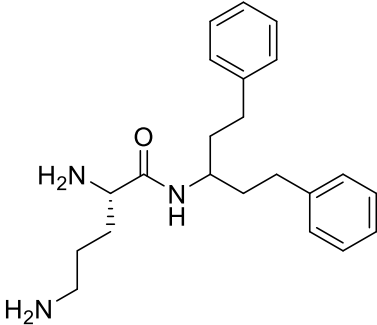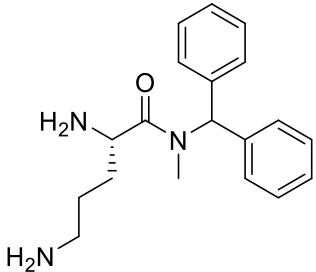
Code	Structure	Concentration of EPI (μM) (12.5 μg/mL)	MIC of Clarithro-mycin (μg/mL)	Fold Reduction in MIC Induced by the Compound
--		--	64	--
PAβN		--	16	4
36		35	16	4
40		40	32	2
43		37	32	2
46		34	32	2

Table 5: Effect of N-Methylation on the EPI Activity of Varied Alkylaryl Amides

The next step in our SAR was to decide which group will be the best to replace the L-ornithine group. Aside from looking to improve the activity, the N^5 primary amine could potentially be easily metabolized. To possibly circumvent this problem and to further the SAR, an N-monomethyl, **47**, N,N-dimethyl, **48**, and an N- guanidine, **49**, were substituted in place of this primary amine.

The synthesis of the monomethyl compound began with the coupling of commercially-available (S)-2-(((benzyloxy)carbonyl)amino)-5-((*tert*-butoxycarbonyl)-amino)pentanoic acid as illustrated in Scheme 11. This acid was coupled with 1,5-diphenylpentan-3-amine, **34**, to form the benzyl *tert*-butyl (5-((1,5-diphenylpentan-3-yl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate, **50**. The boc-protecting group was deprotected, then followed by reductive amination of this amine, **51**, with benzaldehyde and sodium cyanoborohydride. The product of this reaction was treated with formaldehyde and reduced with sodium cyanoborohydride to provide benzyl (S)-(5-(benzyl(methyl)amino)-1-((1,5-diphenylpentan-3-yl)amino)-1-oxopentan-2-yl)carbamate, **52**. The cbz and benzyl groups were removed by hydrogenation using 20% Pd(OH)$_2$/C (Pearlman's catalyst) with ethanol as solvent. The addition of boc anhydride during the hydrogenation step allowed the formation of a bis-boc-protected intermediate. The use of the boc-protected derivative simplified the purification of the diamino compound. The boc-protected intermediate is significantly less polar than the diamino compound. The Boc protected compound was deprotected with trifluoroacetic acid and dichloromethane to afford (S)-2-amino-N-(1,5-diphenylpentan-3-yl)-5-(methylamino)pentanamide, **47** as a trifluoroacetic acid salt.
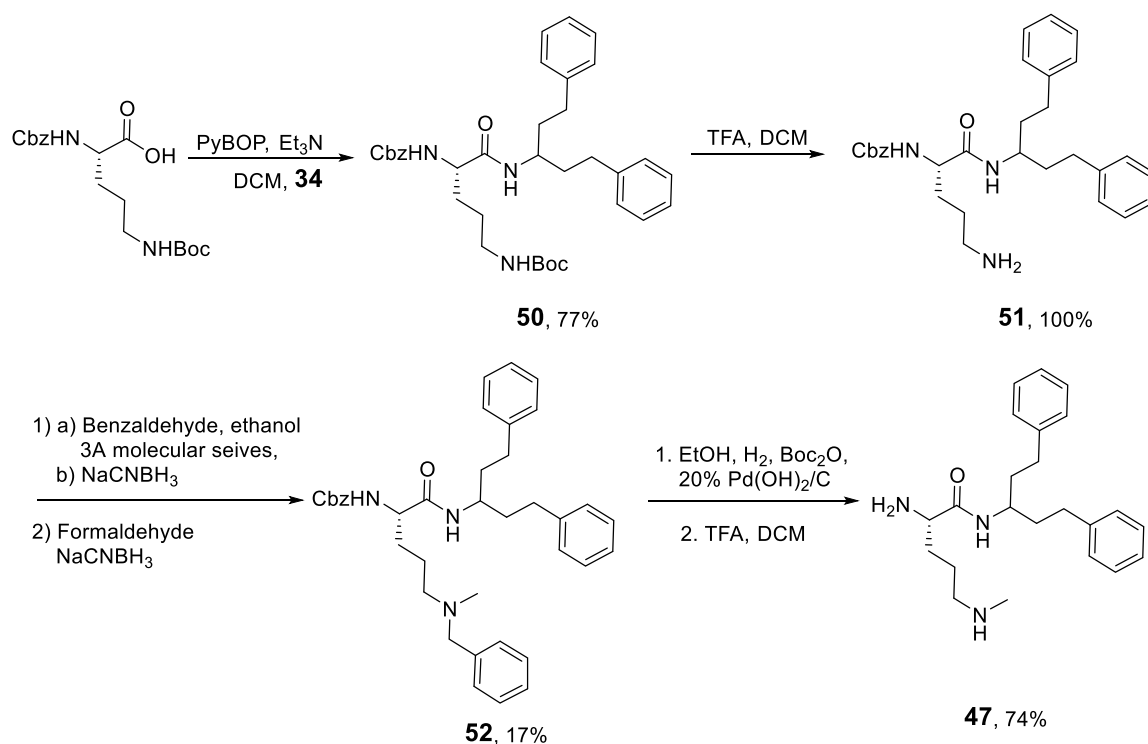
Scheme 11: Synthetic Route to compound **47**

The synthesis of (S)-2-amino-5-(dimethylamino)-N-(1,5-diphenylpentan-3-yl)pentanamide, **48**, also required the use of benzyl (S)-(5-amino-1-((1,5-diphenylpentan-3-yl)amino)-1-oxopentan-2-yl)carbamate, **51** as shown in Scheme 12. Compound **51** underwent a reductive amination with formaldehyde and sodium triacetoxyborohydride to give benzyl (S)-(5-(dimethylamino)-1-((1,5-diphenylpentan-3-yl)amino)-1-oxopentan-2-yl)carbamate, **53**. Deprotection of the cbz protecting groups of **53** by hydrogenation using the Pearlman's catalyst under a hydrogen atmosphere, with ethanol as solvent gave **48** in a 97% yield.
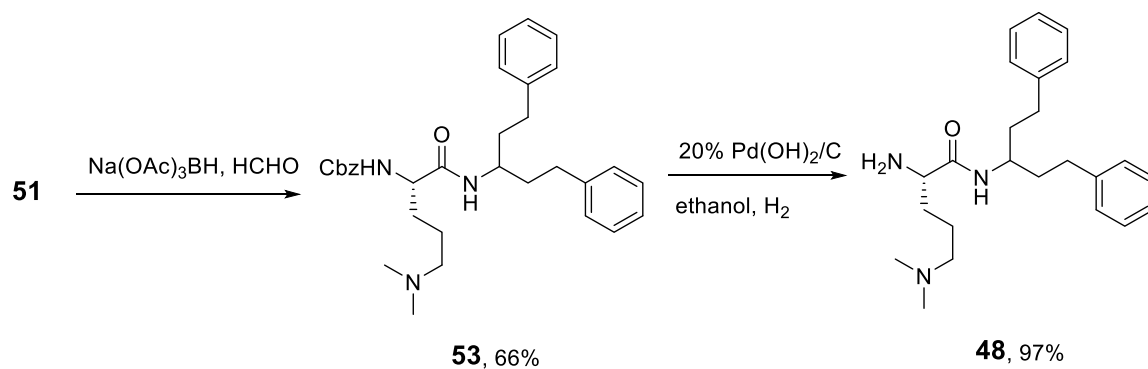
Scheme 12: Synthetic Route to Compound **48**

(S)-2-Amino-N-(1,5-diphenylpentan-3-yl)-5-guanidinopentanamide, **49**, was synthesized, as shown in Scheme 13, by condensing commercially-available Nα, Nω, Nω-tris-Boc-L-arginine with 1,5-diphenyl-3-aminopentane, **34**, to provide **54**. The boc-protecting groups of **54** were removed with trifluoroacetic acid and dichloromethane to afford **49**.
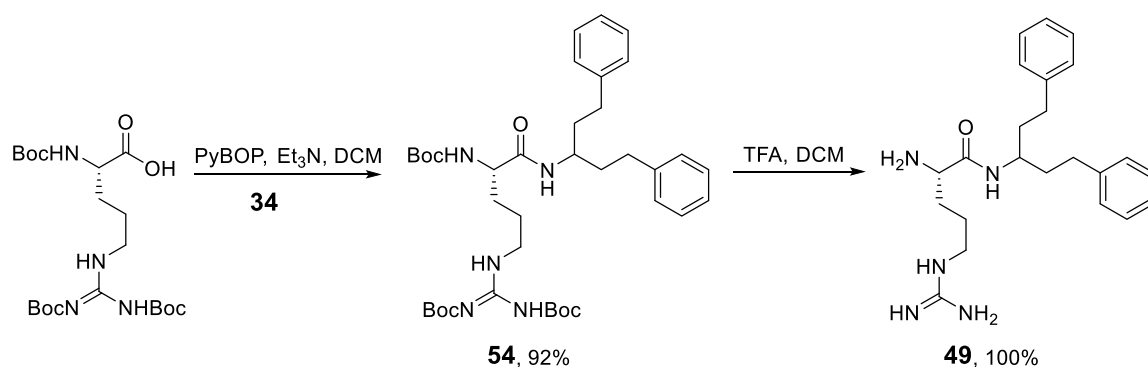
Scheme 13: Synthetic Route to Compound **49**

The data obtained shown in Table 6 indicated that an N^5 substitution was detrimental to EPI activity. None of the compounds synthesized, **47-49**, showed an improvement in EPI activity over PAβN and our recent EPI reference compound **36**. A primary amine appears to be the preferred substituent at the N^5 position.
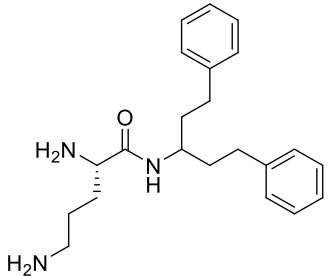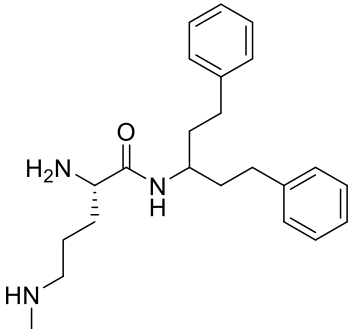
Code	Structure	Concentration of EPI (µM) (12.5 µg/mL)	MIC of Clarithro-mycin (µg/mL)	Fold Reduction in MIC Induced by the Compound
--		--	64	--
PAβN		--	16	4
36		35	16	4
47		34	64	1
48		33	64	1
49		32	32	2

Table 6: Effect N-substitution of the C5 of Diaminopentane in Normal Amides Series

Further studies were done to investigate the EPI activity of different isomeric diaminopentanes. Our reference compound had a 2,5-diaminosubstitution, thus 2 compounds additional isomers were added to our SAR studies. These two compounds were the (S)-3,5-diamino-N-(1,5-diphenylpentan-3-yl)pentanamide, **55**, and 4,5-diamino-N-(1,5-diphenylpentan-3-yl)pentanamide, **56**.

The synthesis of (S)-3,5-diamino-N-(1,5-diphenylpentan-3-yl)pentanamide, **55**, is outlined in Scheme 14. The commercially-available (S)-2,4-bis(((benzyloxy)-carbonyl)amino)butanoic acid was converted to methyl (S)-3,5-bis(((benzyloxy)-carbonyl)amino)pentanoate, **58**, using the Arndt-Eistert reaction.[94] The two-step sequence resulted in the formation of the ester, **58**, in 38% overall yield. The ester, **58**, thus formed underwent saponification with lithium hydroxide to form (S)-3,5-bis(((benzyloxy)-carbonyl)amino)pentanoic acid, **59**. The acid, **59**, formed was used in a peptide coupling reaction to form dibenzyl (5-((1,5-diphenylpentan-3-yl)amino)-5-oxopentane-1,3-diyl)(S)-dicarbamate, **60**. EDC and HOBT were used as the peptide coupling agents. The cbz protecting groups were removed using hydrogenation using the Pearlman's catalyst in the presence of boc-anhydride to provide di-*tert*-butyl (5-((1,5-diphenylpentan-3-yl)amino)-5-oxopentane-1,3-diyl)(S)-dicarbamate, **61**. This was done to obtain a pure final compound. Compound **61** was deprotected with trifluoroacetic acid and dichloromethane to give (S)-3,5-diamino-N-(1,5-diphenylpentan-3-yl)pentanamide, **55**.
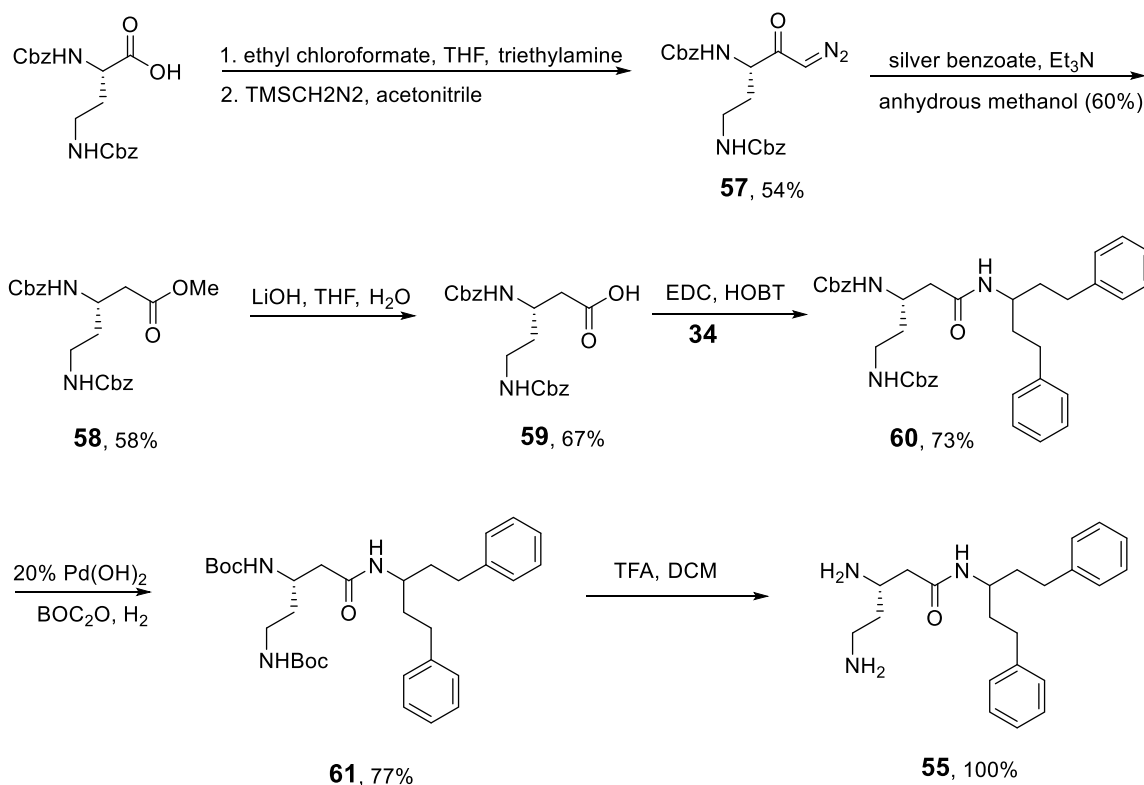
Scheme 14: Synthetic Route of Compound **55**

The Arndt-Eistert reaction is a reaction sequence that consists of the conversion of activated carboxylic acids to diazoketones, then a Wolff rearrangement to form an extended ester or acid as shown in Figure 15. The formation of an acid or an ester depends on the solvent used which supplies the nucleophile. The diazoketone formation step is usually done with diazomethane but this reagent is very toxic, unstable and can easily explode. A less toxic alternative had been introduced which is trimethylsilyldiazomethane. The Wolff rearrangement features the catalysis of a diazoketone to a ketene through a 1,2-rearrangement. The catalysis can be thermal, photolytic or through transition metals with the most common metal being silver. The ketene formation has been shown to proceed both in stepwise or a concerted manner.

The commercially-available (S)-2,4-bis(((benzyloxy)carbonyl)amino)butanoic acid was converted its mixed anhydride using ethyl chloroformate. This mixed anhydride reacts with the in situ generated diazomethane to form dibenzyl (5-diazo-4-oxopentane-1,3-diyl)(S)-dicarbamate, **57**. Compound **57** undergoes catalysis with silver benzoate to form the ketene. The ketene in the presence of anhydrous methanol (as nucleophile) reacts to form ester, **58**.
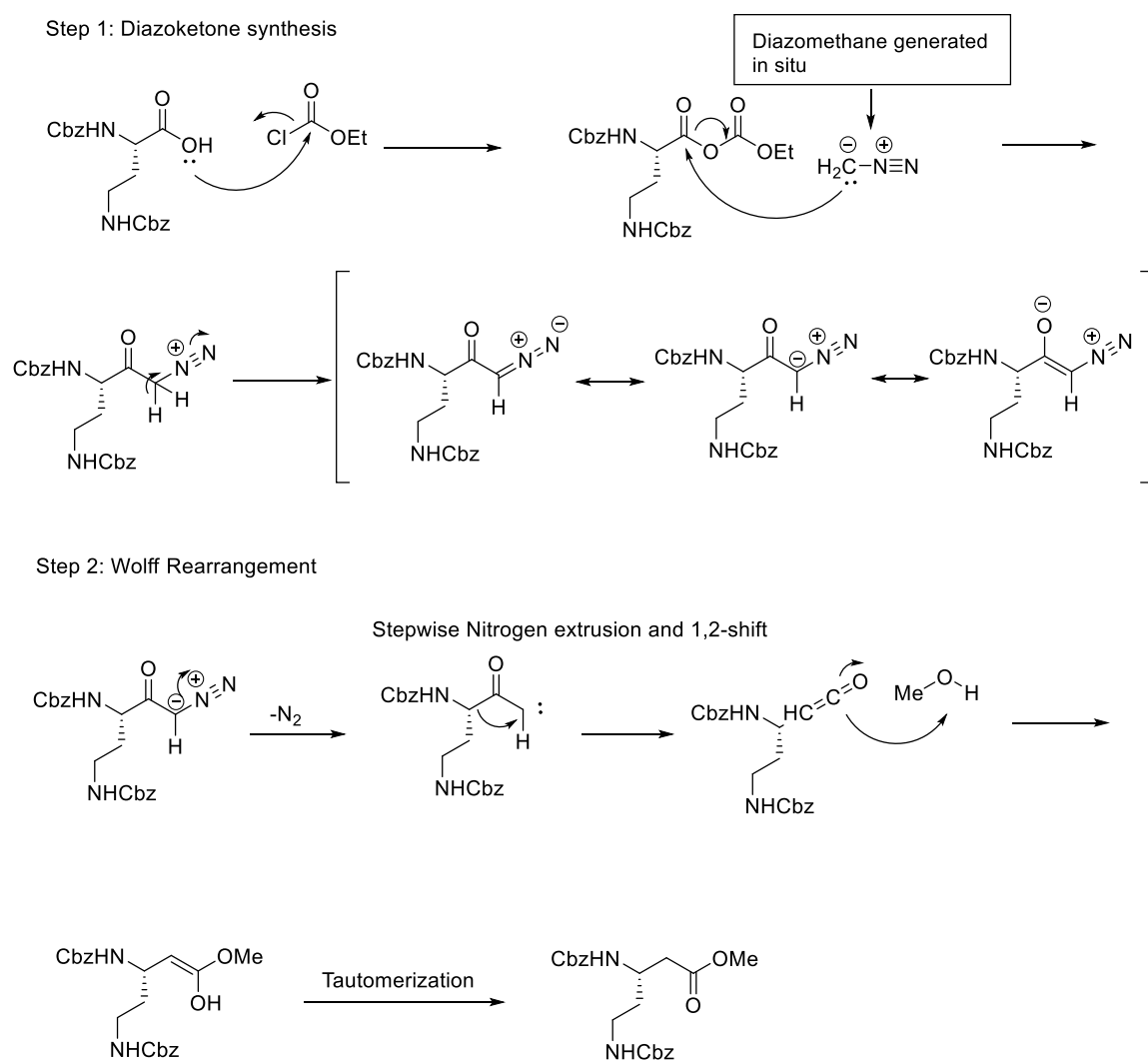
Figure 15: Mechanism for Arndt-Eistert Reaction

The preparation of 4,5-diamino-N-(1,5-diphenylpentan-3-yl)pentanamide, **56**, was accomplished as outlined in Scheme 15 and 16. Commercially-available (S)-4-((((benzyloxy)carbonyl)amino)-5-methoxy-5-oxopentanoic acid (L-glutamic acid α-methyl ester) was condensed with 1,5-diphenylpentan-3-amine, **34**, to give methyl N^2-((benzyloxy)carbonyl)-N^5-(1,5-diphenylpentan-3-yl)-L-glutaminate, **62**. The ester group was reduced to the alcohol, **63**, using lithium borohydride. The original plan for the synthesis of **56** was to use the Mitsunobu reaction to convert the alcohol, **63** to the phthalimide, **64**. Once the phthalimide is obtained, it would have been converted to the amine using hydrazine hydrate. This hypothetical plan is outlined in Scheme 15. However, the Mitsunobu reaction did not work and an alternate procedure was explored.
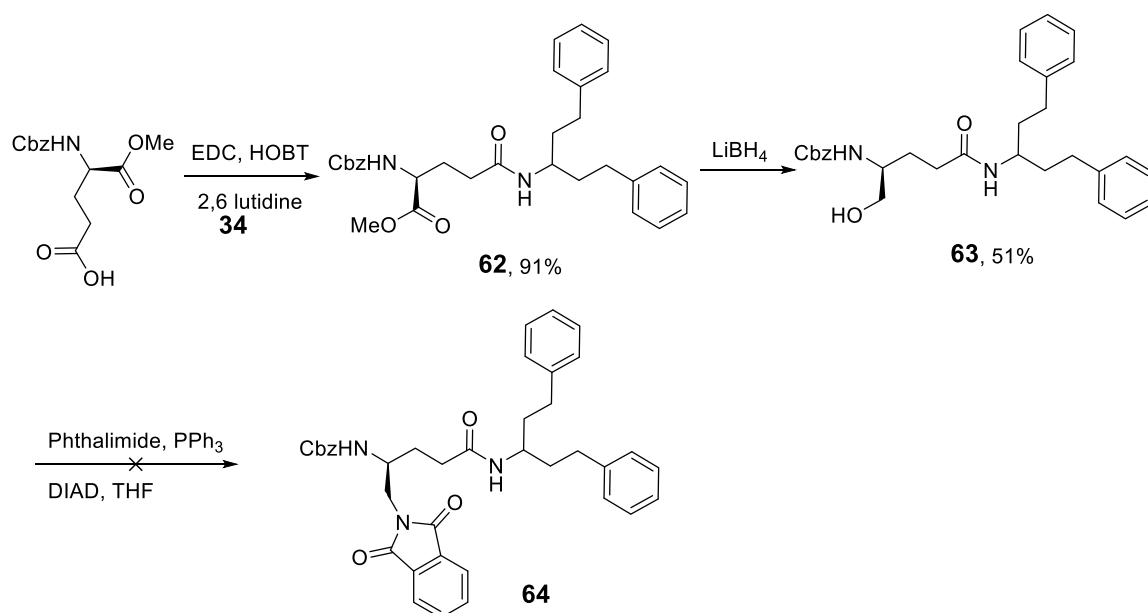
Scheme 15: Original scheme proposed for the synthesis of Compound **56**

The alternate synthetic route proposed involved a one-pot conversion of alcohol to azide using diphenylphosphoryl azide, DPPA.[95] However, we were able to obtain the desired intermediate by a two-step reaction. After an overnight reaction of DPPA and

DBU, the product of the reaction was the stable benzyl (S)-(1-((diphenoxyphosphoryl)oxy)-5-((1,5-diphenylpentan-3-yl)amino)-5-oxopentan-2-yl)carbamate, **65**. It was reasoned that this stable intermediate could be heated with sodium azide to give the azido compound, **65**. The hypothesis proved right giving a clean conversion of the phosphoryl compound, **65**, to the azide, **66**. Hydrogenation using Pearlman's catalyst led to the reduction of the azide and the removal of the cbz group to give (S)-4,5-diamino-N-(1,5-diphenylpentan-3-yl)pentanamide, **56**.
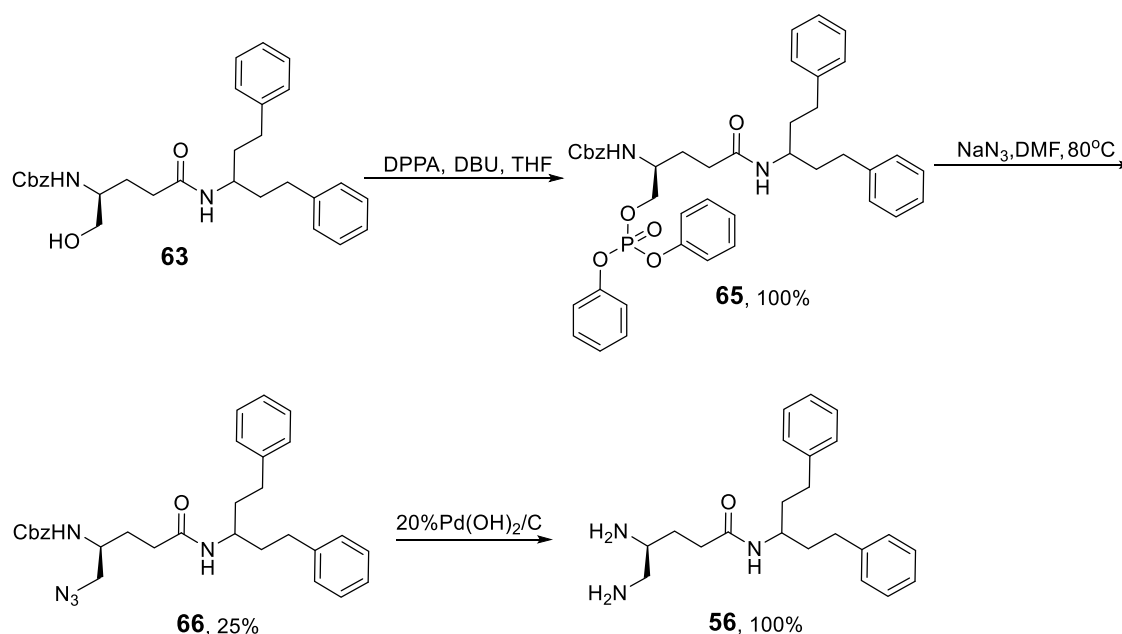
Scheme 16: Synthetic Route to Compound **56**

The results obtained from the biological assay of diaminopentane isomers, **55** and **56** are summarized in Table 7. Of all the pentanamide analogues synthesized, the 3,5-diamino derivative, **55**, had the best activity, reducing the MIC of clarithromycin by 16-fold, which was 4 times greater than either PAβN or **36.** The 4,5-diamino derivative, **56**, was not as effective and proved less active than PAβN.
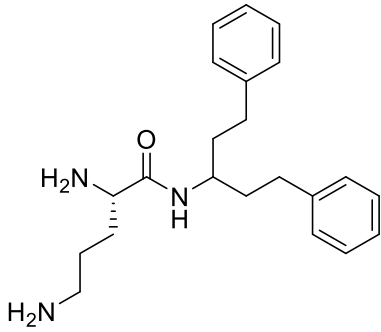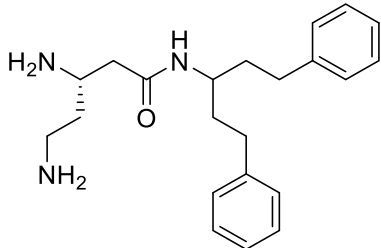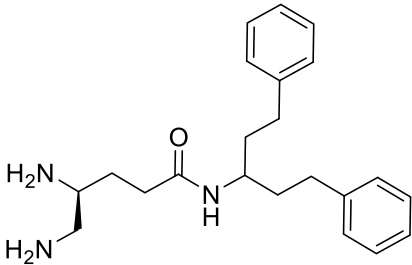
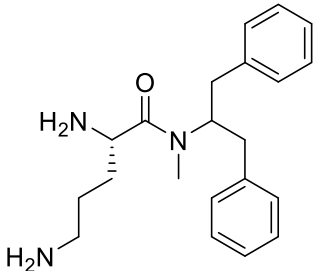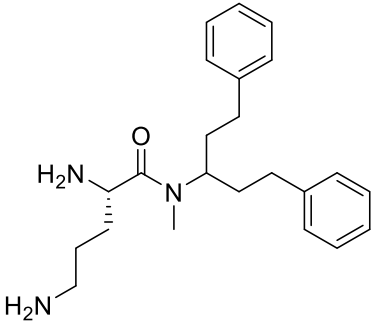| Code	Structure	Concentration of EPI (μM) (12.5 μg/mL)	MIC of Clarithro-mycin (μg/mL)	Fold Reduction in MIC Induced by the Compound
--		--	64	--
PAβN		--	16	4
36		35	16	4
55		35	4	16
56		35	32	2

Table 7: Effect of Diaminopentanoic Acid Isomers on Normal Amides Series

Butanamide analogues were also synthesized to ascertain the effect of a reduction in the number of carbons in the diamino portion of these molecules on EPI activity. The two possible butanamide analogues ((S)-2,4-diamino-N-(1,5-diphenylpentan-3-yl)butanamide, **67**, and (S)-3,4-diamino-N-(1,5-diphenylpentan-3-yl)butanamide, **68**, were synthesized and EPI activity assayed as part of the SAR. The synthesis of the butanamide analogue, **67** is illustrated in Scheme 17. The condensation of (S)-2,4-bis(((benzyloxy)carbonyl)amino)butanoic acid with 1,5-diphenylpentan-3-amine, **34**, provided dibenzyl (4-((1,5-diphenylpentan-3-yl)amino)-4-oxobutane-1,3-diyl)(S)-dicarbamate, **69**. Removal of the cbz groups with hydrogenation led to (S)-2,4-diamino-N-(1,5-diphenylpentan-3-yl)butanamide, **67**.
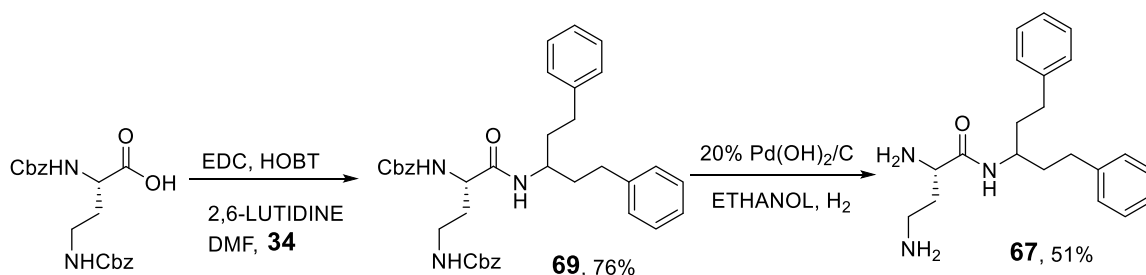
Scheme 17: Synthesis of Compound **67**

The (S)-3,4-diamino-N-(1,5-diphenylpentan-3-yl)butanamide, **68**, was synthesized following a procedure similar to the synthesis of **56** with some differences as shown in Scheme 18. The acid used was the commercially-available (S)-3-((*tert*-butoxycarbonyl)amino)-4-methoxy-4-oxobutanoic acid. This acid was condensed with 1,5-diphenylpentan-3-amine, **34,** to form, **70**. The ester of **70** was reduced with lithium borohydride to give *tert*-butyl (S)-(4-((1,5-diphenylpentan-3-yl)amino)-1-hydroxy-4-

oxobutan-2-yl)carbamate, **71**. Instead of using DPPA, the alcohol, **71**, was converted to the mesylate, which was not isolated, and the crude mesylate reacted with sodium azide to give *tert*-butyl (S)-(1-azido-4-((1,5-diphenylpentan-3-yl)amino)-4-oxobutan-2-yl)carbamate, **72**. The azide, **72**, was reduced to the amine which was protected with boc anhydride to aid purification. Di-*tert*-butyl (4-((1,5-diphenylpentan-3-yl)amino)-4-oxobutane-1,2-diyl)(S)-dicarbamate, **73**, after it had been purified, was stirred in trifluoroacetic acid and dichloromethane to give (S)-3,4-diamino-N-(1,5-diphenylpentan-3-yl)butanamide, **68**.
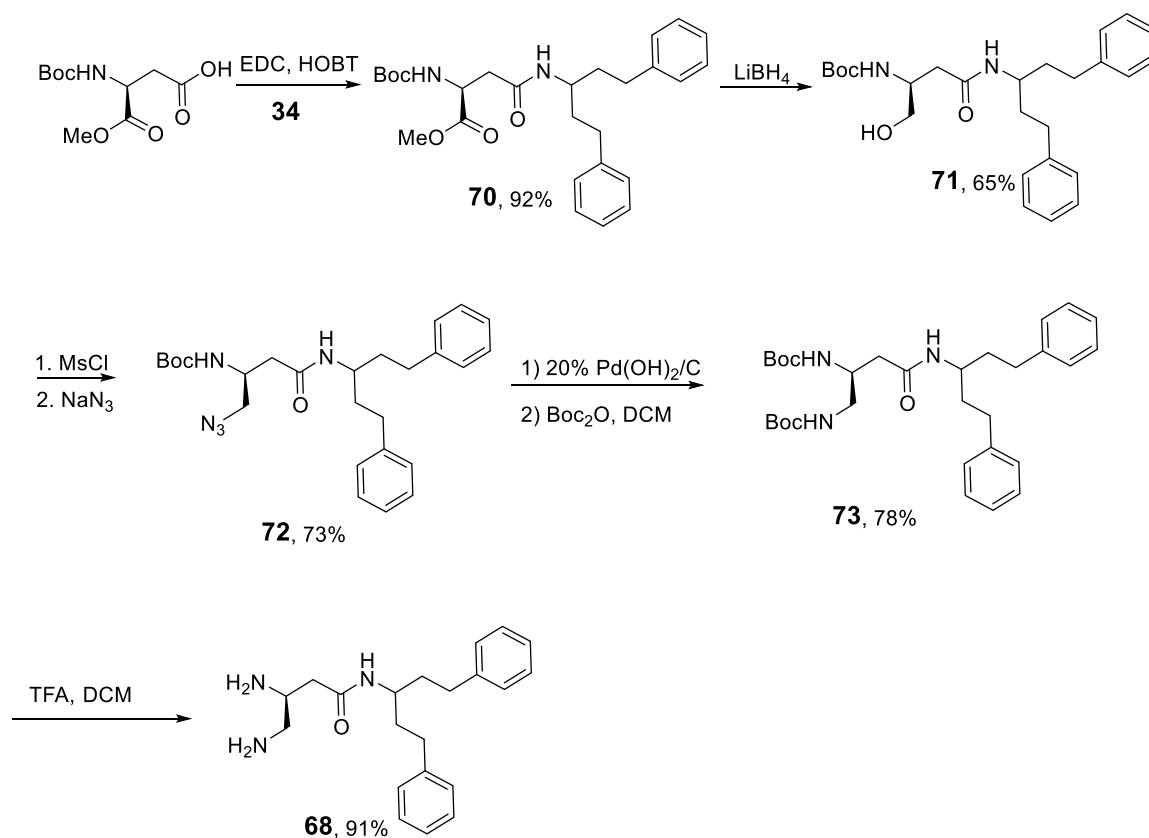
Scheme 18: Synthetic Route to Compound **68**

Table 8 summarizes the data obtained from the assay of the butanamide analogues, **67** and **68**. None of the butanamide compounds showed any improved activity over the structurally similar pentanamide derivative, **55**. They were inactive as EPI agents and thus further studies were not conducted on this series
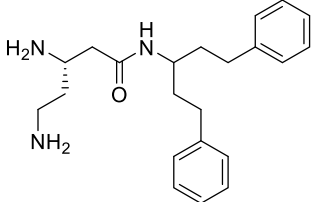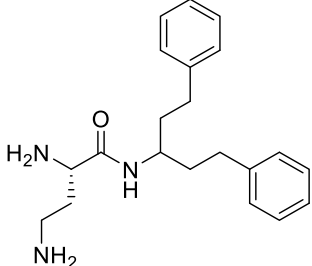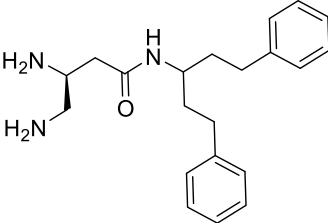
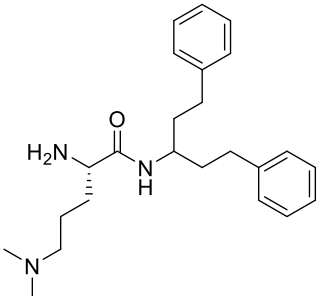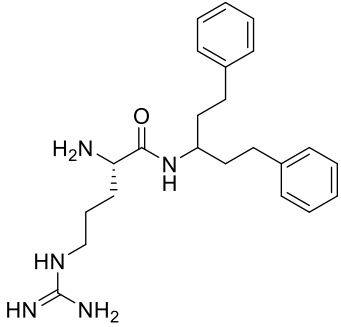| Code	Structure	Concentration of EPI (µM) (12.5 µg/mL)	MIC of Clarithro-mycin (µg/mL)	Fold Reduction in MIC Induced by the Compound
--		--	64	--
PAβN		--	16	4
55		35	4	16
67		37	32	2
68		37	64	1

Table 8: Effect of Diaminobutanoic Acid Isomers on EPI Activity of Normal Amides.

2.3. STRUCTURE ACTIVITY RELATIONSHIP OF "REVERSE" AMIDES

The SAR of the series of compounds that we classified as "the normal amides" did not reveal particularly active EPIs (the best compound showed a four-fold increase in activity over PAβN, with most of the other compounds showing no improvement in activity or a decreased activity). A new scaffold was sought that potentially could be associated with an increased EPI activity. Two scaffolds were proposed; 1) reverse amide derivatives and 2) secondary amine derivatives.

Normal Amides   Reverse Amides   Secondary Amines
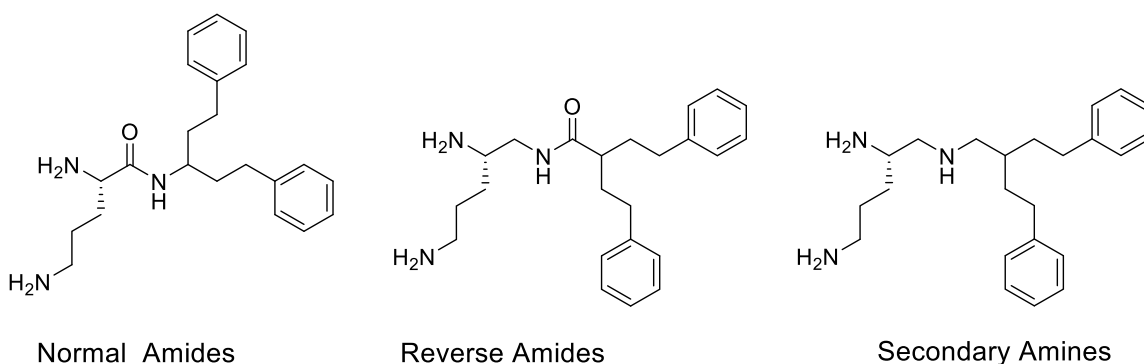
Figure 16: Proposed New Scaffolds

The initial emphasis was to explore the SAR of the reverse amides. Reverse amides also called retro-amides have been shown to improve activity of some compounds verses the amides of compounds with known biological activity.[96] These derivatives were not exact "reverse amides" as an extra carbon had been inserted to avoid the instability associated with the exact reverse amide. The exact reverse amide would have an instable aminal functionality by virtue of the two amine substituents being on the same carbon atom.
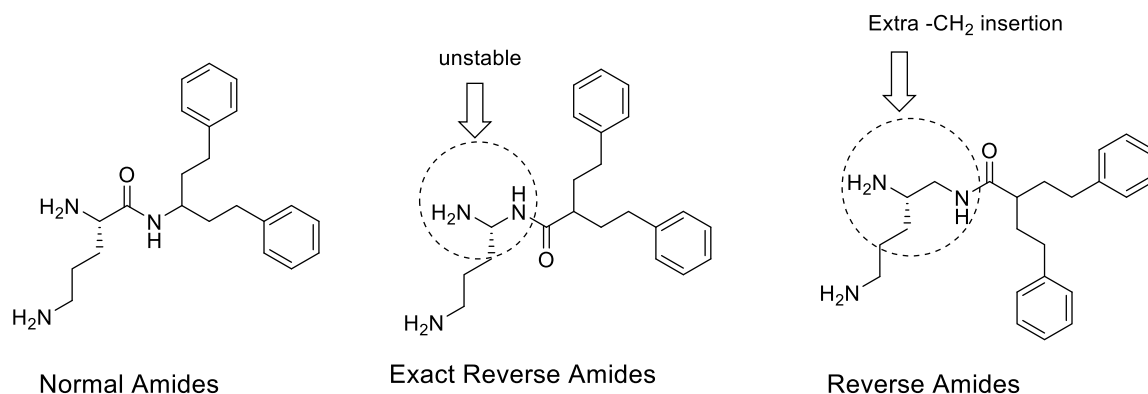
Figure 17: Insertion of -CH$_2$- to improve Stability of Reverse Amide Series

To obtain a proof of concept, a series of compounds were synthesized. As with the normal amides series, the SAR began with the right hand portion based upon the core structures listed in Figure 17. The goal was to determine if changes in the chain length of the bis-alkylaryl had any effect on EPI activity. The objective here was to examine whether some of the earlier SAR observation would also apply to the new "reversed amide" series. Using convergent synthesis, the synthesis of the reverse amide series required an amine (on the left hand side) and the appropriate acid (on the right hand side). The synthetic approach to the formation of (S)-N-(2,5-diaminopentyl)-2,2-diphenylacetamide, **74**, and (S)-2-benzyl-N-(2,5-diaminopentyl)-3-phenylpropanamide, **75** is illustrated in Scheme 19. Commercially-available (S)-5-(((benzyloxy)carbonyl)amino)-2-((*tert*-butoxycarbonyl)-amino)pentanoic acid was converted to the mixed anhydride using isobutyl chloroformate and reducing the mixed anhydride with sodium borohydride to obtain alcohol, **76**.

The original intent was to convert the alcohol to a leaving group, then displacement of this leaving group to from the azide. This azide would then be converted to the amine

using the Staudinger reaction. The Staudinger reaction involves the conversion of an azide to an amine in the presence of triphenylphosphine and water. Our early exploratory studies indicated that the conversion of the alcohol to the bromo- did not yield expected product.[97] Conversion of this alcohol to a mesylate did provide a moderately stable compound. We considered the option of using a Gabriel synthesis which had the possibility of reducing the number of steps in the synthetic route and also avoided the formation of any unstable intermediates. In the Gabriel synthesis, an alkyl halide is converted to a phthalimide using potassium phthalimide and then cleavage of the phthalimide with hydrazine to obtain the amine. Using this modified Gabriel synthesis, proved to be the preferred synthetic route.

The alcohol, **76**, was first converted to the phthalimide using a Mitsunobu reaction in this modified Gabriel synthesis. Benzyl *tert*-butyl (5-(1,3-dioxoisoindolin-2-yl)pentane-1,4-diyl)(S)-dicarbamate, **77**, was formed by stirring alcohol, **76**, in the presence of triphenylphosphine, phthalimide and diisopropyl azodicarboxylate. In the Mitsunobu reaction, the triphenylphosphine forms a complex with diisopropyl azodicarboxylate as illustrated in Figure 18. This complex facilitates that formation of the alcohol-triphenylphosphine complex which allows the phthalimide to act as the nucleophile. This nucleophile then attacks the carbon expelling the oxygen-triphenylphosphine leaving group to form the phthalimide derivative, **77**.
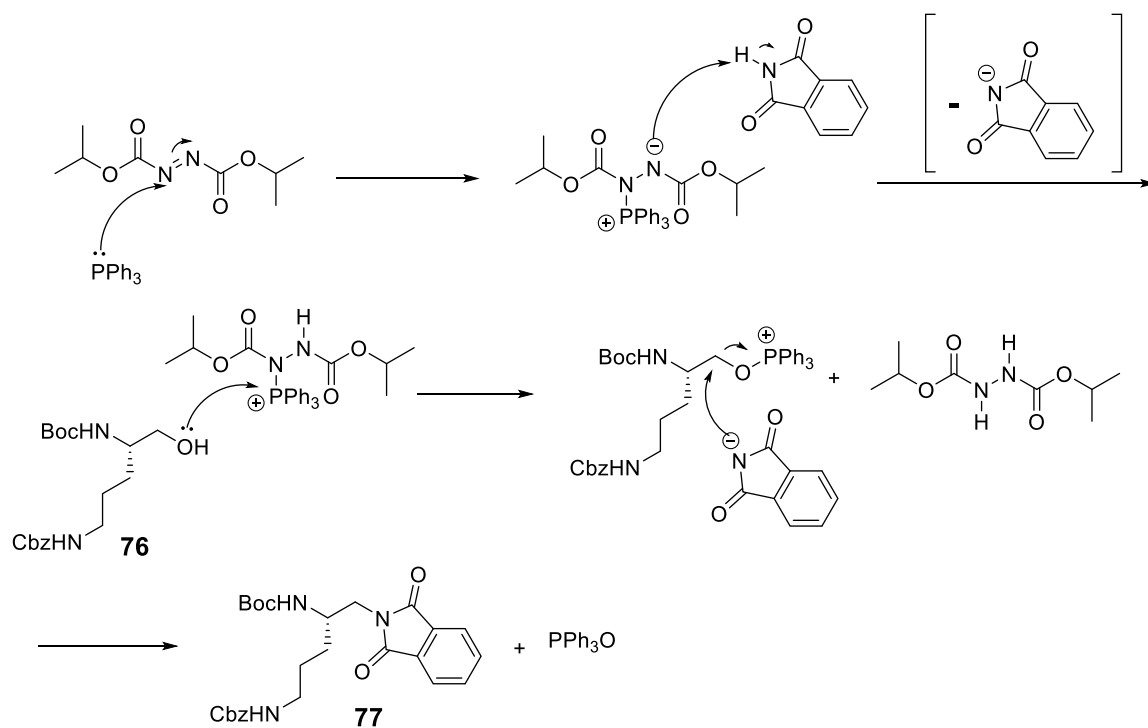
Figure 18: Mechanism for the Mitsunobu Synthesis (Intermediate **77**)

The phthalimide was then converted to benzyl *tert*-butyl (5-aminopentane-1,4-diyl)(S)-dicarbamate, **78** as shown in Scheme 19. The amine, **78**, thus obtained was independently reacted with the acids: 2,2-diphenylacetic acid and 2-benzyl-3-phenylpropanoic acid to obtain benzyl *tert*-butyl (5-(2,2-diphenylacetamido)pentane-1,4-diyl)(S)-dicarbamate, **79**, and benzyl *tert*-butyl (5-(2-benzyl-3-phenylpropanamido)-pentane-1,4-diyl)(S)-dicarbamate, **81**, respectively. The boc and cbz protecting groups of the amide intermediates, **79** and **81**, were removed by acid hydrolysis and hydrogenation sequentially to provide **74** and **75** respectively.
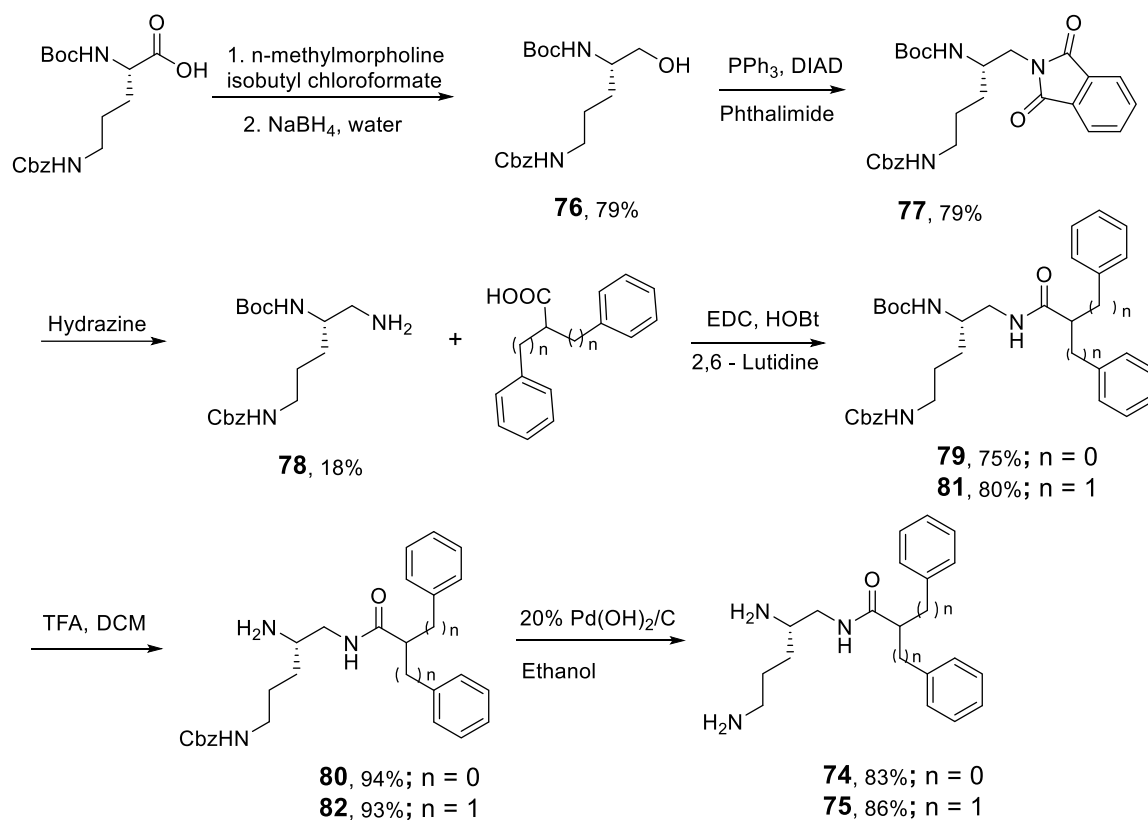
Scheme 19: Synthetic Route for the Preparation of Compounds **74-75**

The synthesis of (S)-N-(2,5-diaminopentyl)-2-phenethyl-4-phenylbutanamide, **83**, required the synthesis of the acid, **84**, as this acid was not commercially available. The synthetic route to this acid is outlined in Scheme 20. The initial step involved the conversion of diethyl malonate to diethyl 2,2-diphenethylmalonate, **85**. The hydrolysis of one ester group led to the mono acid, **86**, which was decarboxylated at reflux to give ethyl 2-phenethyl-4-phenylbutanoate, **87**.[98] Saponification of this ester led to the desired intermediate, 2-phenethyl-4-phenylbutanoic acid, **84**.
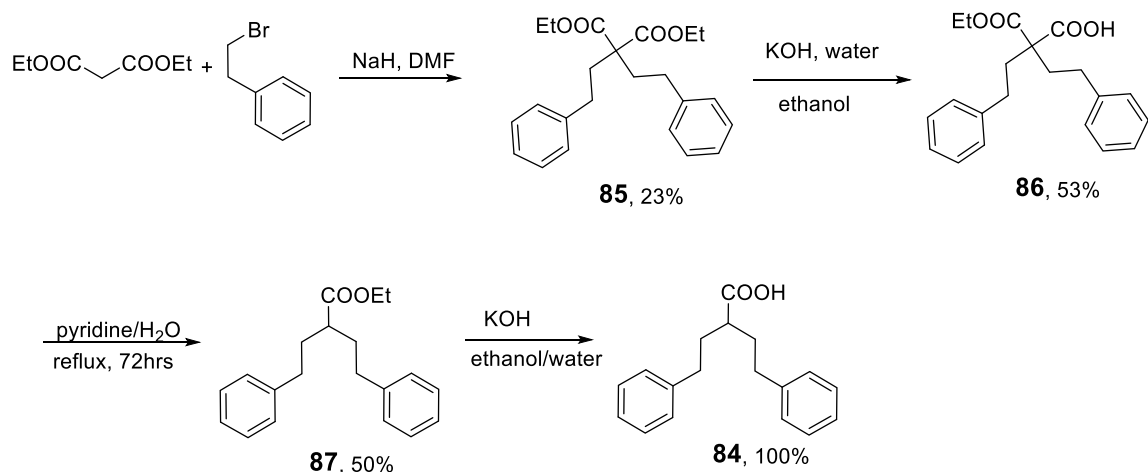
Scheme 20: Synthetic Route to Intermediate **84**

The amine intermediate, **88**, used for the synthesis of **83**, was synthesized in 3 steps as outline in Scheme 21 (using a similar procedure discussed for the synthesis of benzyl *tert*-butyl (5-aminopentane-1,4-diyl)(S)-dicarbamate, **78**). We changed the amine intermediate because we reasoned that, using an amine with one type of protecting group will reduce the number of deprotection steps. This will ultimately reduce the number of steps in our synthetic scheme. The commercially-available (S)-2,5-bis(((benzyloxy)-carbonyl)amino)pentanoic acid was reacted to form the mixed anhydride which was reduced with sodium borohydride to provide the alcohol, **89**. This alcohol, **89**, was reacted in the presence of triphenylphosphine, phthalimide and diisopropyl azodicarboxylate at 0 ºC for 30 minutes and at room temperature overnight to obtain dibenzyl (5-(1,3-dioxoisoindolin-2-yl)pentane-1,4-diyl)(S)-dicarbamate, **90**. This phthalimide, **90**, was reduced to afford amine intermediate, **88**. 2-Phenethyl-4-phenylbutanoic acid, **84**, was converted to the acid chloride using oxalyl chloride and reacted with amine, **88** to provide dibenzyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,4-diyl)(S)-dicarbamate, **91**. Hydrogenation using the Pearlman's catalyst at room temperature of the cbz protected

amide, **91**, provided the final compound, (S)-N-(2,5-diaminopentyl)-2-phenethyl-4-phenylbutanamide, **83**.
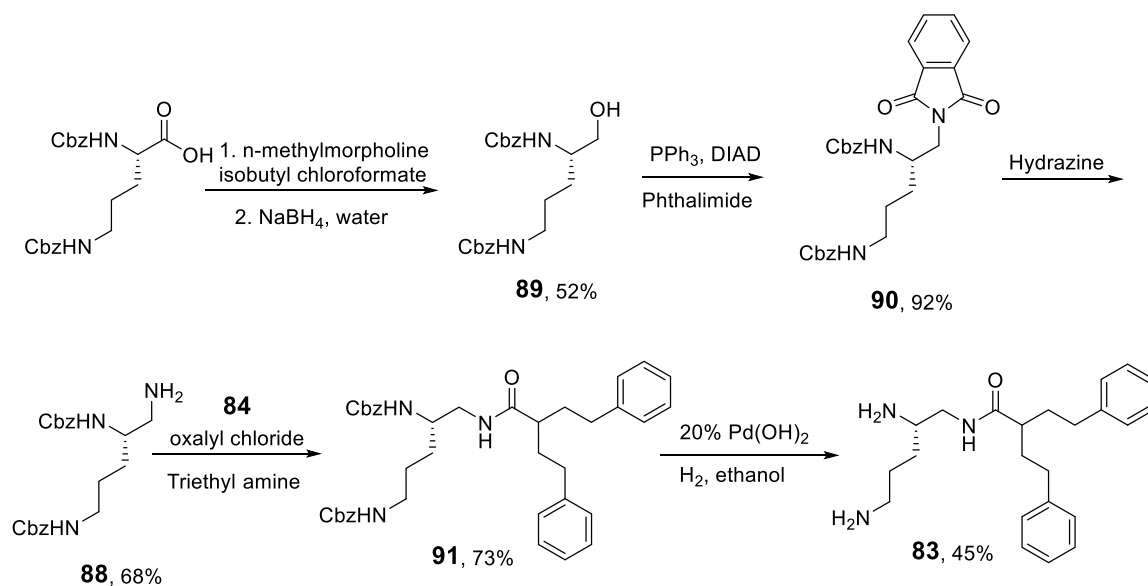
\Scheme 21: Synthetic Route to Compound **83**

As done with the normal amides, the R-enantiomer, (R)-N-(2,5-diaminopentyl)-2-phenethyl-4-phenylbutanamide, **92**, was also synthesized to examine possible difference in the EPI activity of this pair of enantiomers as illustrated in Scheme 22. The commercially-available (R)-2-(((benzyloxy)carbonyl)amino)-5-((*tert*-butoxycarbonyl)amino)pentanoic acid was reduced to the alcohol, **93**, through the formation of the mixed anhydride using isobutyl chloroformate and reduction of the anhydride with sodium borohydride. Benzyl *tert*-butyl (5-hydroxypentane-1,4-diyl)(R)-dicarbamate, **93**, underwent a Mitsunobu to obtain the phthalimide, **94**. This phthalimide, **93**, was reduced with hydrazine to afford benzyl *tert*-butyl (5-aminopentane-1,4-diyl)(R)-dicarbamate, **95**. This amine intermediate, **95**, was condensed with 2-phenethyl-4-phenylbutanoic acid, **84**, in the presence of EDC,

HOBT and 2,6-lutidine to provide the amide, **97**. Hydrogenation of benzyl (R)-(5-amino-1-(2-phenethyl-4-phenylbutanamido)pentan-2-yl)carbamate, **97**, provided **92**.
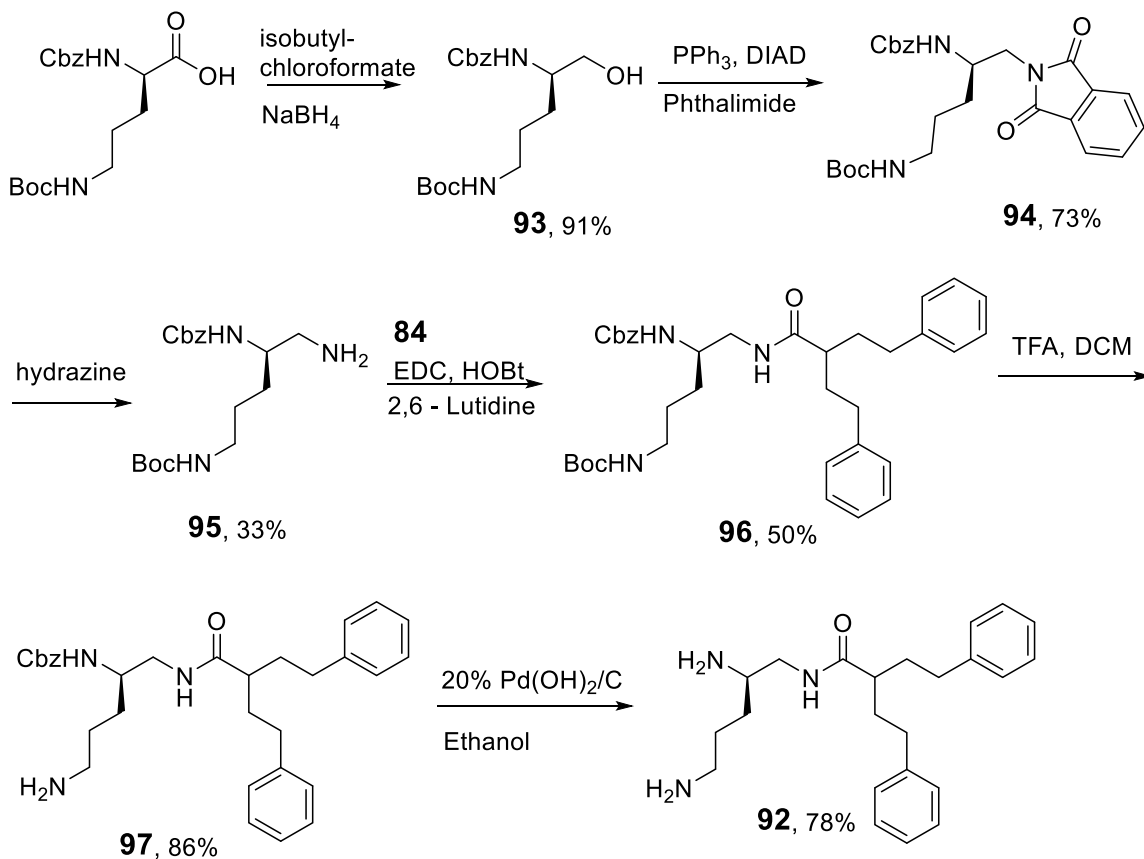
Scheme 22: Synthetic Route to Compound **92**

Data from the *in vitro* biological assays are summarized in Table 9. These data indicate that (S)-N-(2,5-diaminopentyl)-2-phenethyl-4-phenylbutanamide, **83**, and its R-enantiomer, (R)-N-(2,5-diaminopentyl)-2-phenethyl-4-phenylbutanamide, **92** were among the more potent EPIs. Most exciting was the fact that these compounds increased by 16-fold the antimicrobial activity with clarithromycin alone and had a 4-fold greater

enhancement than either PAβN or **36**. These data supported efforts to further explore the

SAR of this new series of alkyl aryl amides.
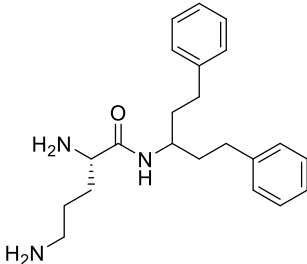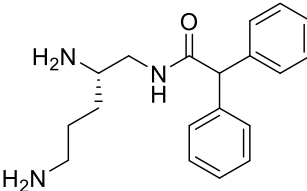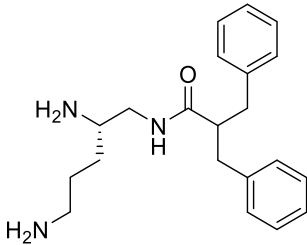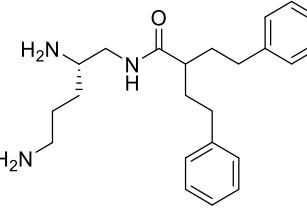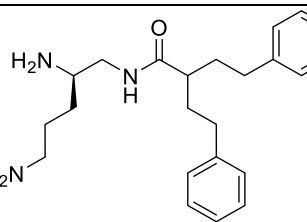
Code	Structure	Concentration of EPI (μM) (12.5 μg/mL)	MIC of Clarithro-mycin (μg/mL)	Fold Reduction in MIC Induced by the Compound
--		--	64	--
PAβN		--	16	4
36		35	16	4
74		40	32	2
75		37	64	1
83		34	4	16
92		34	4	16

Table 9: Effect of chain length on diphenyl substitution on Reverse Amides Series

The SAR associated with compounds related to **83** was further expanded in an effort to improve its activity as an EPI agent. Keeping (S)-pentane-1,2,5-triamine side as a constant, as illustrated in Figure 19, the influence of structural variation on the right-hand portion of the molecule was explored.
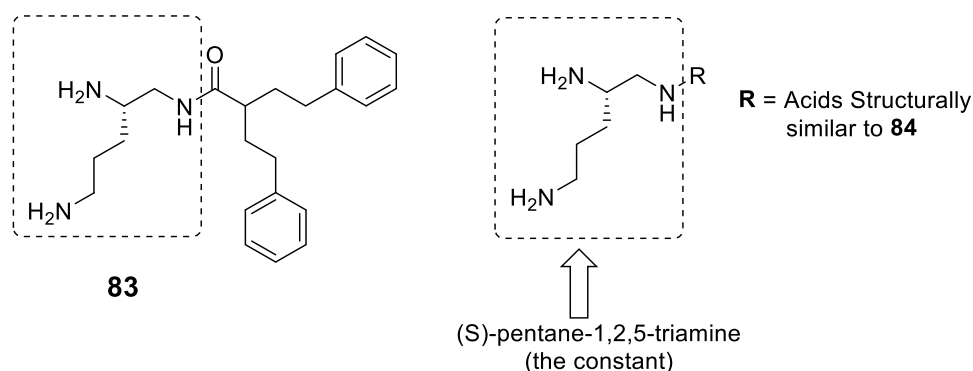
Figure 19: Further Expansion of the SAR of Compound **83**

The effect of bis-4-pyridyl moieties instead of the bis-phenyl was investigated. One reason for exploring this particular variation was based on Timcodar, which a known mammalian efflux pump inhibitor. The bis-4-pyridyl substitution also allowed us to examine the effect of reduced lipophilicity on this portion of the molecule on EPI activity.
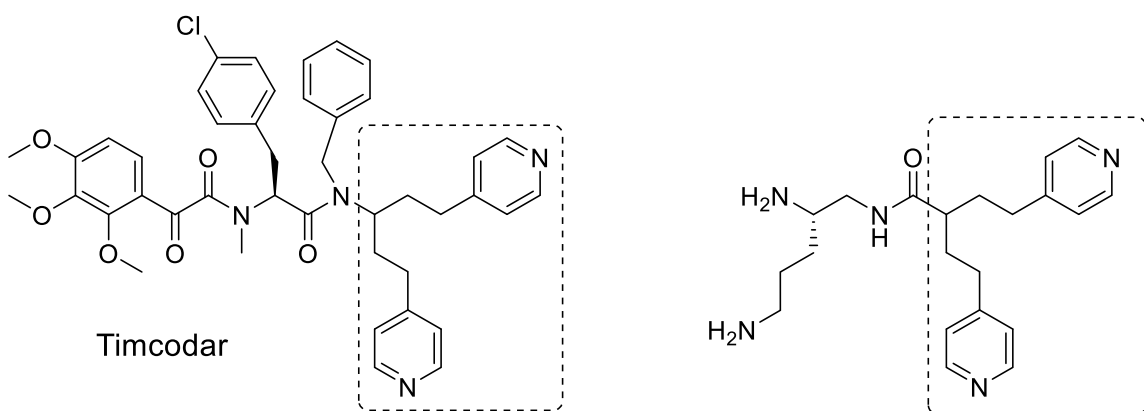
Figure 20: Structural Similarities between Timcodar and Proposed Analogue of **83**

The commercially-available vinylpyridine and diethyl malonate was reacted to form diethyl 2,2-bis(2-(pyridin-4-yl)ethyl)malonate, **98** as outlined in Scheme 23.[99] A one-step decarboxylation of **98** and hydrolysis of ester led to the 4-(pyridin-4-yl)-2-(2-(pyridin-4-yl)ethyl)butanoic acid, **99** as illustrated in Scheme 23. Condensation of this acid with dibenzyl (5-aminopentane-1,4-diyl)(S)-dicarbamate, **88**, and deprotection of the cbz protecting groups afforded N-((S)-2,5-diaminopentyl)-4-(pyridin-4-yl)-2-(2-(pyridin-4-yl)ethyl)butanamide, **101**.
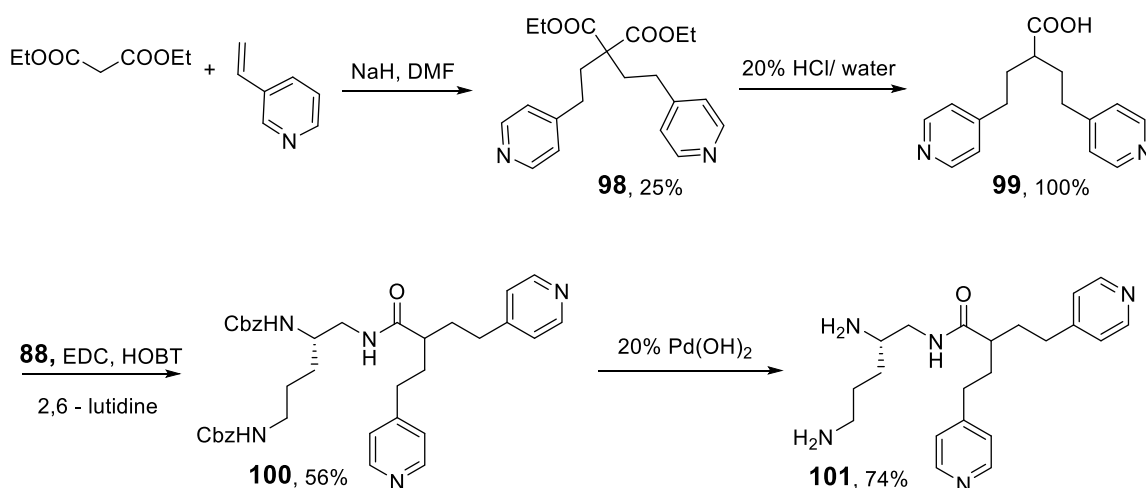
Scheme 23: Synthetic Route to Compound **100**

The mono-alkylaryl derivatives of **83** with varied chain lengths were also synthesized as illustrated in Scheme 24. These compounds were designed to probe whether the bis-alkylaryl substituents on these amides were required for good EPI potency. The acids used were commercially available; 4-phenylbutanoic acid and 3-phenylpropanoic acid. These were coupled independently to dibenzyl (5-aminopentane-1,4-diyl)(S)-dicarbamate, **88**. Deprotection of the cbz groups afforded (S)-N-(2,5-diaminopentyl)-3-

phenylpropanamide, **103**, and (S)-N-(2,5-diaminopentyl)-4-phenylbutanamide, **105**

respectively.
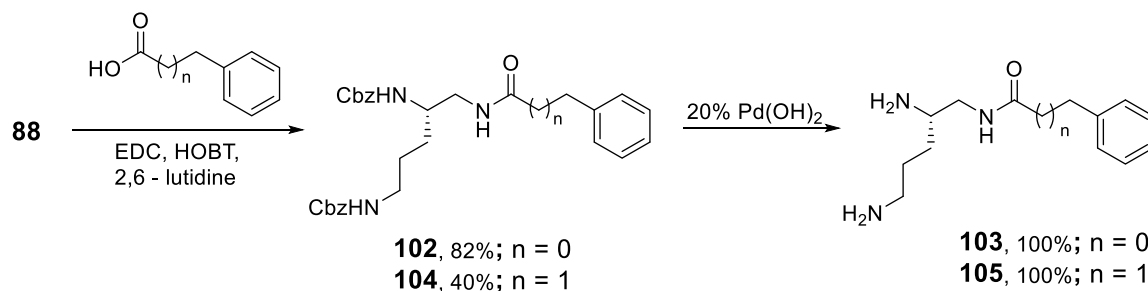
Scheme 24: Synthetic Route to Compounds **103** and **105**

The SAR associated with the EPI activity of **101, 103** and **105** are summarized in

the Table 10. The data for compounds **103** and **105** indicated that the second alkylaryl was

necessary for EPI activity. Loss of activity with the pyridyl, **101**, also showed that the site

of action for the synthesized EPI had a hydrophobic pocket and a hydrophilic component.

The presence of a pyridyl in the hydrophobic pocket may have led to a loss of activity.
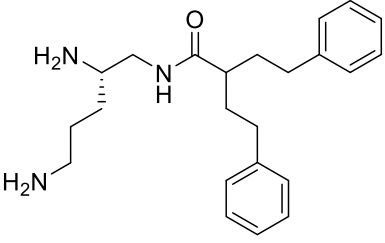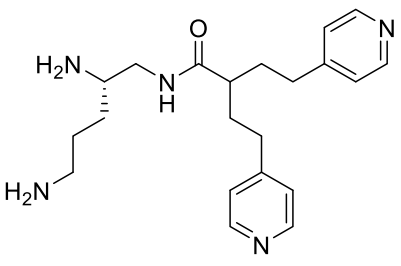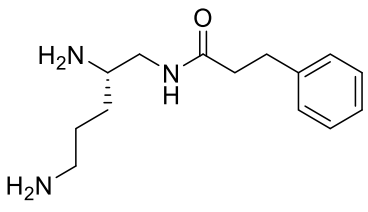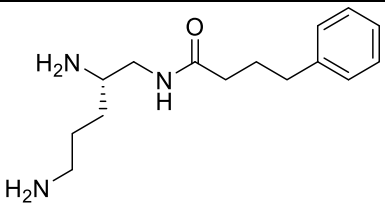
Code	Structure	Concentration of EPI (μM) (12.5 μg/mL)	MIC of Clarithro-mycin (μg/mL)	Fold Reduction in MIC Induced by the Compound
--		--	64	--
PAβN		--	16	4
83		34	4	16
101		34	64	1
103		50	64	1
105		47	64	1

Table 10: Effect of Pyridyl and Mono-Akylaryl on Reverse Amides

Having gained insight into a preferred substituent for the right hand portion of the molecule, we initiated studies to explore varied diamino alkyl substituents on the left hand portion of the molecule that could potentially enhance EPI activity. This concept is illustrated in Figure 21.
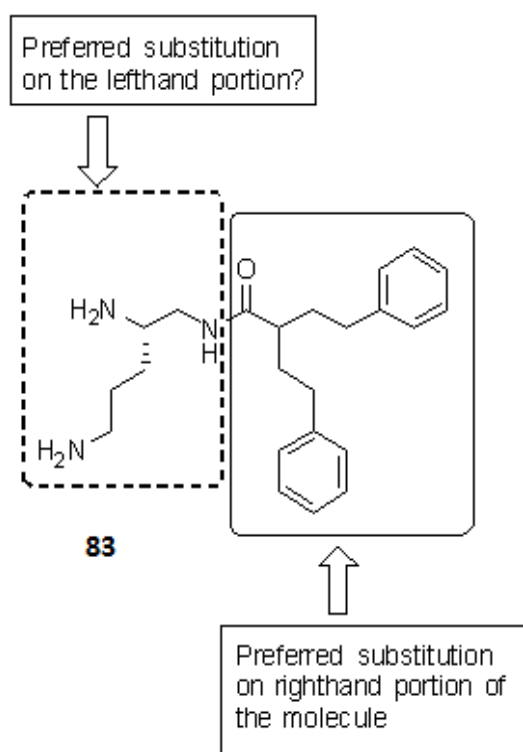
Figure 21: Preferred Substitution on Righthand Portion of "Reverse Amide" Series

Our investigations started with different isomers of the diaminopentane core; 3,5-diaminopentamide, **106**, and 4,5-diaminopentamide, **107**. For the synthesis of (S)-N-(3,5-diaminopentyl)-2-phenethyl-4-phenylbutanamide, **106**, required the amine, **108**. We envisioned that we could convert (S)-2,4-bis(((benzyloxy)carbonyl)amino)-butanoic acid to the alcohol, **109**. This alcohol was then converted to the mesylate, **110**. The mesylate thus obtained was reacted with potassium cyanide to obtain the organic nitrile, **111**. However, under the reduction conditions employed, the nitrile could not be converted to the amine to afford the 2 carbon extended amine, **108**. We therefore explored alternated synthetic routes. We tried to oxidize the alcohol, **109**, to the aldehyde, **112** with the intention of converting the aldehyde intermediate to the nitroalkene, **113**, which could be reduced to provide the amine, **109**. The aldehyde could not be isolated and thus we had to use a much longer synthetic

approach to obtain the amine. These failed routes of synthesis are illustrated in Scheme 25.
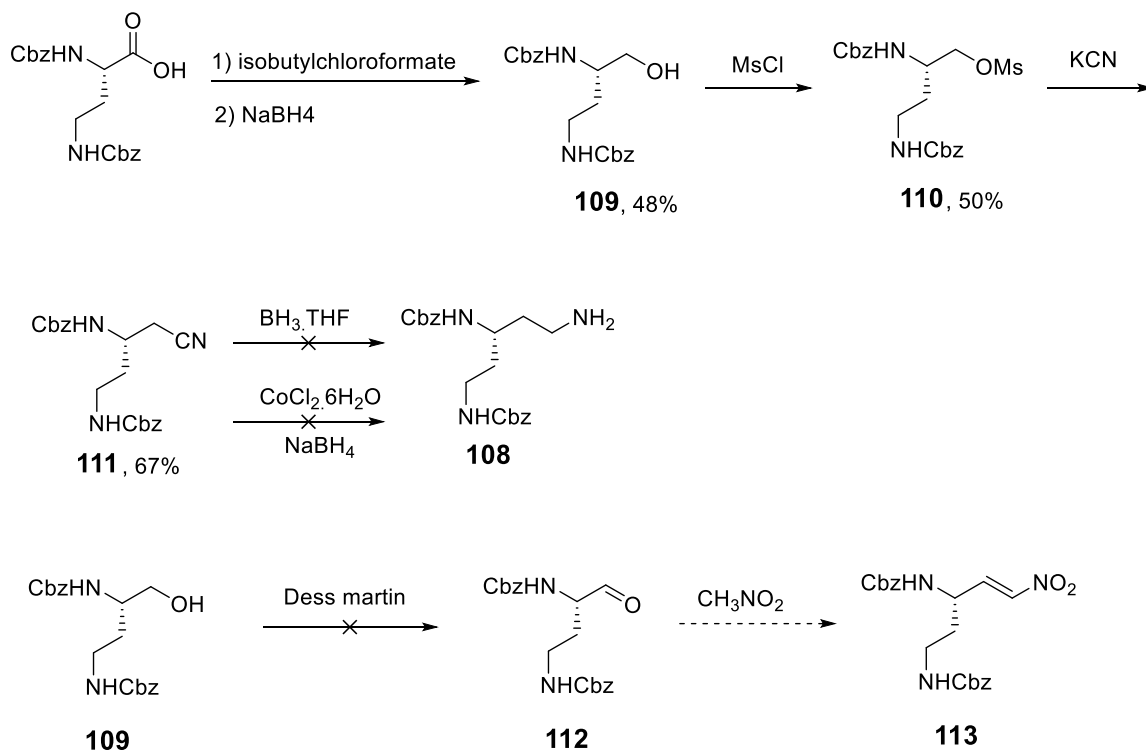
Scheme 25: Failed Routes to the Synthesis of Intermediate **108**

The synthetic pathway that ultimately provided the desired compound **106** is summarized in Scheme 26. Methyl (S)-3,5-bis(((benzyloxy)carbonyl)amino)pentanoate, **58**, whose synthesis has been previously described served as our initial intermediate for this synthesis. The ester, **58**, was reduced to the alcohol, **114**, using isobutyl chloroformate and sodium borohydride as illustrated in Scheme 26. The alcohol, **114**, was reacted with phthalimide under Mitsunobu conditions to obtain dibenzyl (5-(1,3-dioxoisoindolin-2-yl)pentane-1,3-diyl)(S)-dicarbamate, **115**. The phthalimide, **115**, was reduced to the amine, **108**. Condensation of dibenzyl (5-aminopentane-1,3-diyl)(R)-dicarbamate, **108**, with acid, **84**, provided the amide, **116**. This amide was deprotected by hydrogenation to afford **106**.
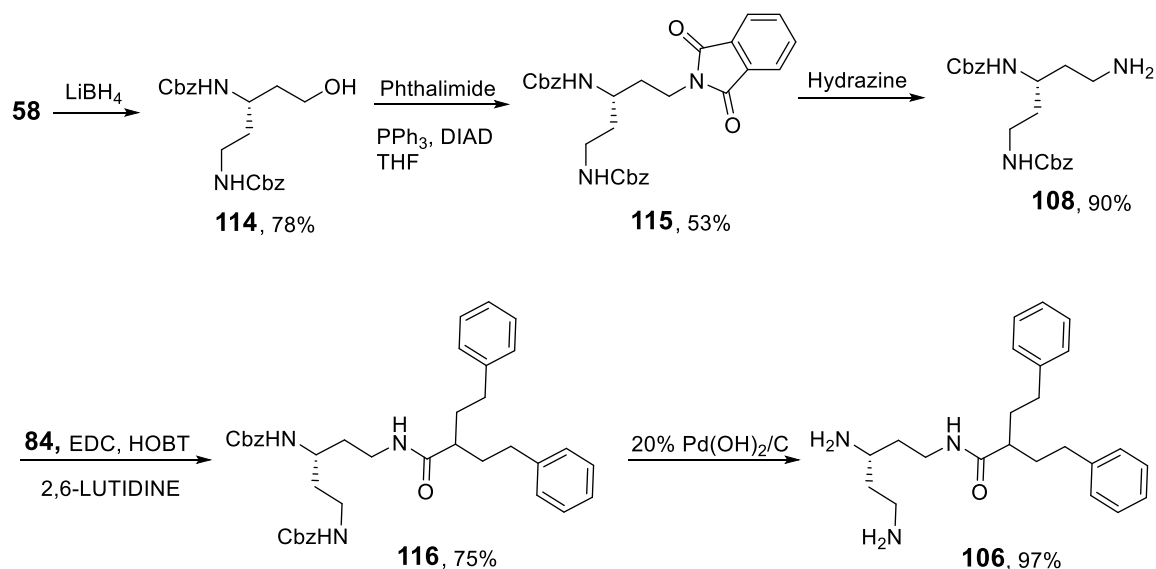
Scheme 26: Synthetic Route to Compound **106**

The synthesis of (S)-N-(4,5-diaminopentyl)-2-phenethyl-4-phenylbutanamide, **107**, as shown in Scheme 27 started from the commercially-available (S)-2-(((benzyloxy)carbonyl)amino)-5-((*tert*-butoxycarbonyl)amino)pentanoic acid. This acid was converted to methyl (S)-2-(((benzyloxy)carbonyl)amino)-5-((*tert*-butoxycarbonyl)amino)pentanoate, **117**, using potassium carbonate and methyl iodide. This ester, **117**, was reduced to the alcohol, **118**, and converted to the phthalimide, **119**. This phthalimide, **119**, was reduced with hydrazine monohydrate to the amine, **120**. The free amine of benzyl *tert*-butyl (5-aminopentane-1,4-diyl)(S)-dicarbamate, **120**, was protected using CbzCl to obtain dibenzyl *tert*-butyl pentane-1,2,5-triyl(S)-tricarbamate, **121**. The boc-protecting group of **121** was removed using trifluoroacetic acid and dichloromethane. The amine, **122**, was condensed with acid, **84**, to give dibenzyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,2-diyl)(S)-dicarbamate, **123**. Deprotection of the cbz groups using the Pearlman's catalyst under a hydrogen atmosphere afforded **107.**
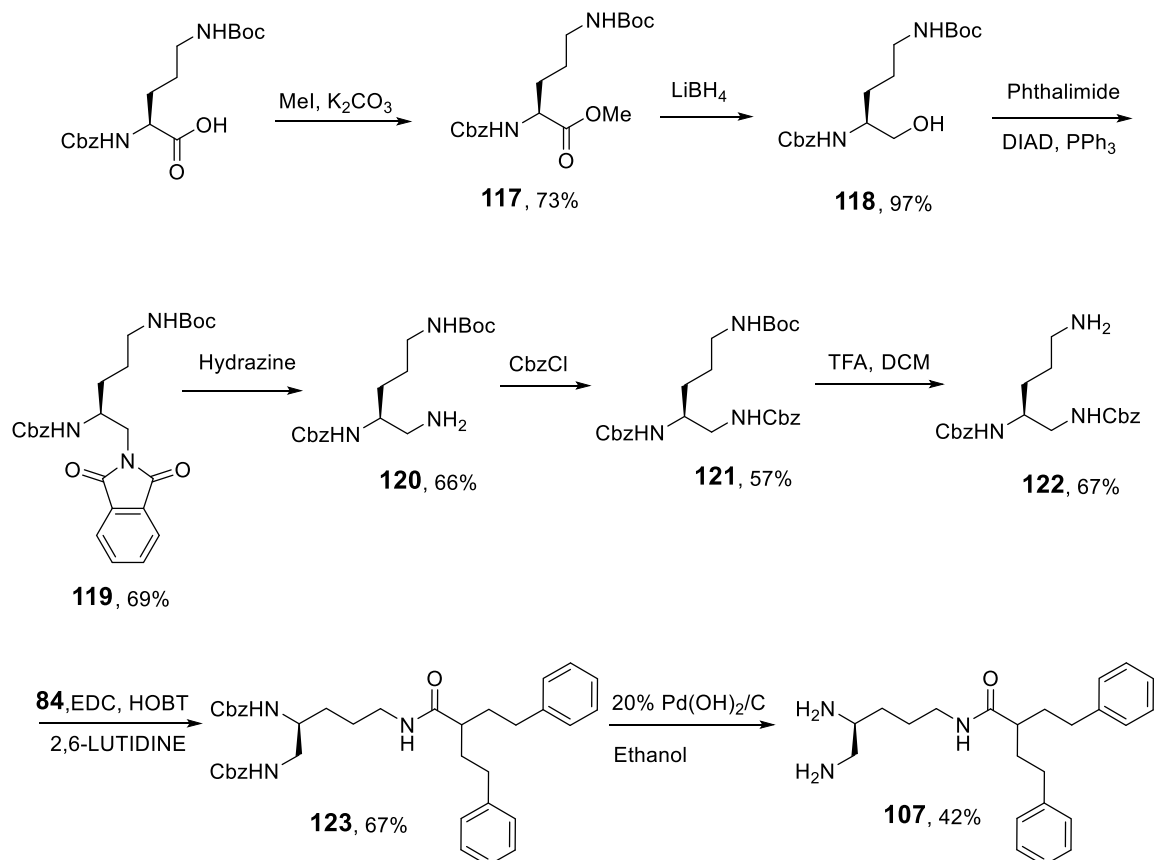
Scheme 27: Synthetic Route to Compound **107**

The data obtained from the testing of **106** and **107** are presented in Table 11. They indicate that all the pentanamide isomers are active as EPIs. The 4,5-diaminopentanamide, **107**, was the most active as an EPI agent enhancing by 32-fold the activity of clarithromycin. The 3,5-diaminopentanamide, **106**, had a similar activity to the reference **83.**
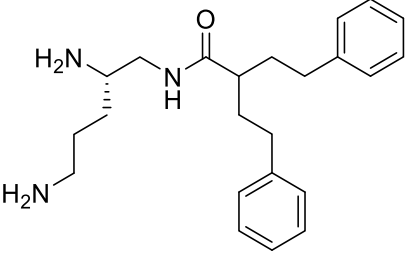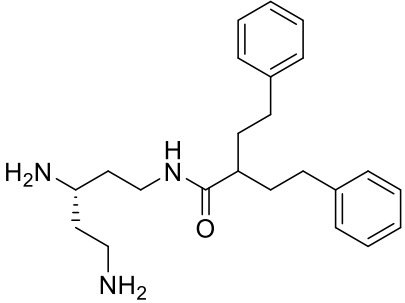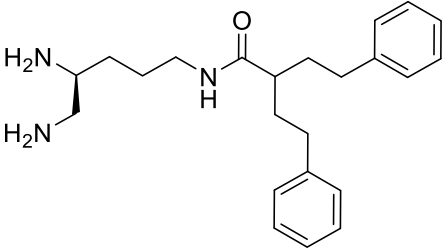
Code	Structure	Concentration of EPI (μM) (12.5 μg/mL)	MIC of Clarithro-mycin (μg/mL)	Fold Reduction in MIC Induced by the Compound
--		--	64	--
PAβN		--	16	4
83		34	4	16
106		34	4	16
107		34	2	32

Table 11: Pentanamide Analogues within the Reverse Amide Series

The effect of diamino alkyl chain length was also examined. The synthesis of (S)-N-(2,3-diaminopropyl)-2-phenethyl-4-phenylbutanamide, **124**, and (S)-N-(2,4-diaminobutyl)-2-phenethyl-4-phenylbutanamide, **125**, was accomplished as outlined in Schemes 28 and 29 respectively.

The commercially-available (S)-2-((((benzyloxy)carbonyl)amino)-3-((*tert*-butoxycarbonyl)amino)propanoic acid was reduced to the alcohol, **126** as shown in Scheme 28. This alcohol, **126**, was reacted under Mitsunobu conditions to afford phthalimide derivative, **127**. Benzyl *tert*-butyl (3-(1,3-dioxoisoindolin-2-yl)propane-1,2-diyl)(S)-dicarbamate, **127**, was then converted to the amine, **128**. Peptide coupling of benzyl *tert*-butyl (3-aminopropane-1,2-diyl)(R)-dicarbamate, **128**, to the acid, **84**, provided the amide, **129**. Deprotection of this amide, **129**, afforded **124**.

The previously synthesized dibenzyl (4-hydroxybutane-1,3-diyl)(S)-dicarbamate, **109**, was used as starting material for the synthesis of **125** as outlined in Scheme 29. The alcohol, **109**, was reacted with phthalimide in the presence of triphenylphosphine and diisopropyl azodicarboxylate to afford dibenzyl (4-(1,3-dioxoisoindolin-2-yl)butane-1,3-diyl)(S)-dicarbamate, **131**. This phthalimide derivative, **131**, was reacted with hydrazine to provide the amine, **132**. Amine, **132** underwent condensation with the acid, **84**, to afford dibenzyl (4-(2-phenethyl-4-phenylbutanamido)butane-1,3-diyl)(S)-dicarbamate, **133**, as product. The last step in this synthesis was hydrogenation to remove the cbz protecting groups to obtain **125**.
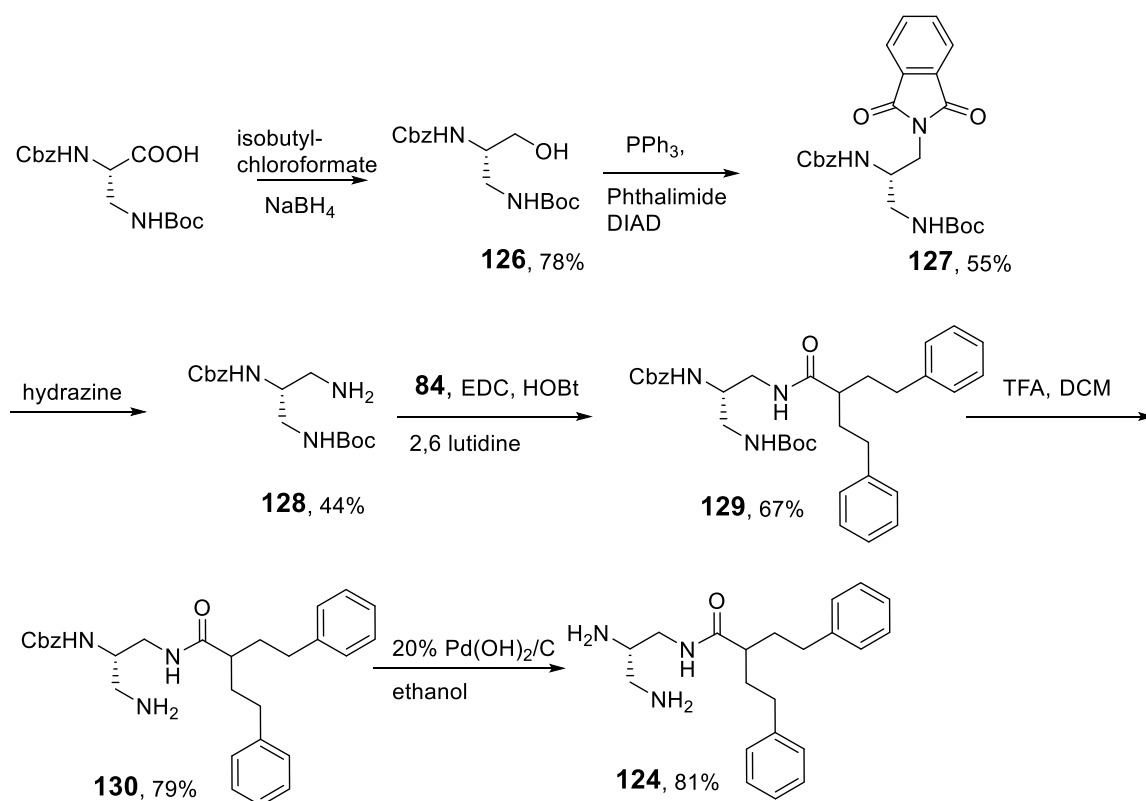
Scheme 28: Synthetic Route to Compound **124**
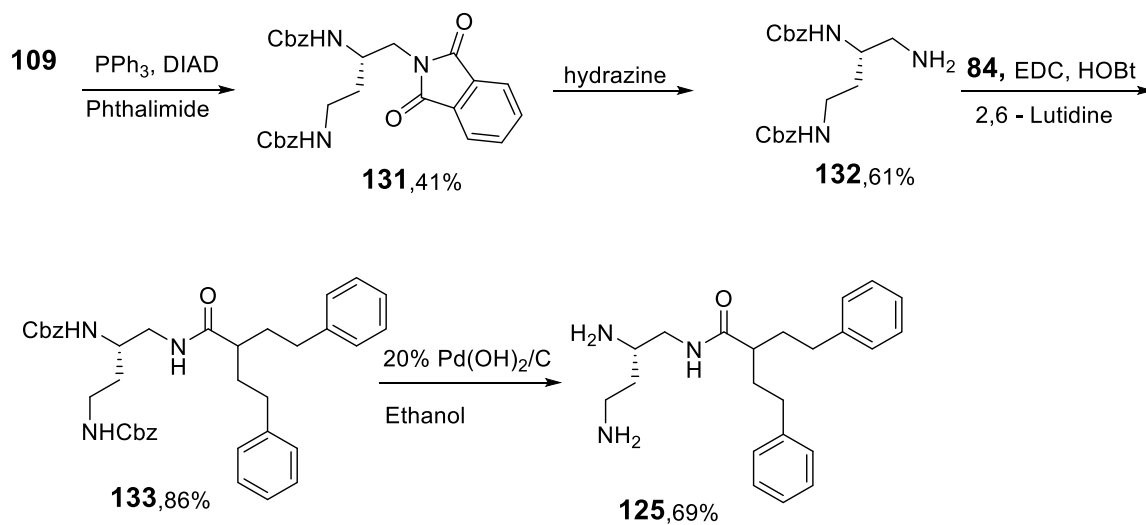
Scheme 29: Synthetic Route to Compound **125**

The data obtained from the assay of **124** and **125** is summarized in Table 12. The butanamide derivative, **125**, had a similar activity to the pentanamide derivative, **83**. They both increased the MIC of clarithromycin by 16-fold. The propanamide derivative, **124** had the least activity of the set. Its EPI activity was even having a less than that of PAβN.
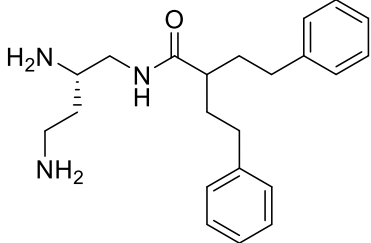
Code	Structure	Concentration of EPI (µM) (12.5 µg/mL)	MIC of Clarithro-mycin (µg/mL)	Fold Reduction in MIC Induced by the Compound
--		-	64	--
PAβN		-	16	4
83		34	4	16
124		37	32	2
125		35	4	16

Table 12: Effect of chain length of diamino- portion on EPI activity of Reverse Amides

The effect of methyl substitutions at different positions of (S)-N-(2,5-diaminopentyl)-2-phenethyl-4-phenylbutanamide, **83**, was also investigated. We targeted for synthesis; (S)-N-(2,5-diaminopentyl)-N-methyl-2-phenethyl-4-phenylbutanamide, **134**, (S)-N-(5-amino-2-(methylamino)pentyl)-2-phenethyl-4-phenylbutanamide, **135**, and (S)-N-(2-amino-5-(methylamino)pentyl)-2-phenethyl-4-phenylbutanamide, **136**.

The synthesis of (S)-N-(2,5-diaminopentyl)-N-methyl-2-phenethyl-4-phenylbutanamide, **134**, presented some challenges. We had hoped to convert the mesyl compound, **137**, to the monomethyl amine, **138**, and use this amine to form our desired final compound. However, this procedure was not successful as illustrated in Scheme 30.[97]
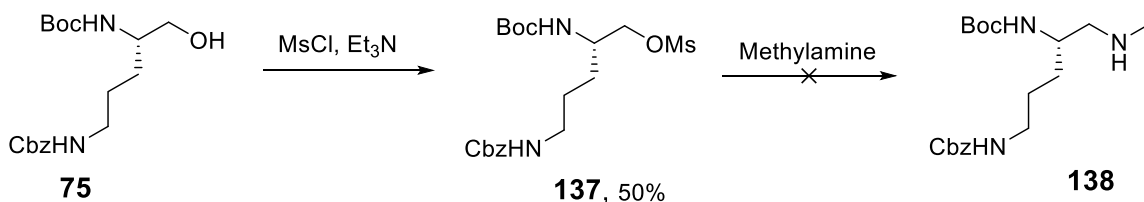
Scheme 30: Unsuccessful Attempts in the Synthesis of Compound **138** - 1

We also attempted to convert commercially available (S)-5-(((benzyloxy)-carbonyl)amino)-2-((*tert*-butoxycarbonyl)amino)pentanoic acid to benzyl *tert*-butyl (5-(methylamino)-5-oxopentane-1,4-diyl)(S)-dicarbamate, **139**.[100] We had hoped to reduce the amide intermediate, **139**, to the amine, **138**. However, irrespective of the reducing conditions used, the expected benzyl *tert*-butyl (5-(methylamino)pentane-1,4-diyl)(S)-dicarbamate, **138**, could not be obtained. We initially tried to use the milder REDAL reducing agent, which has be proposed in literature to be useful for executing such chemistry.[100] Borane-THF was also tried but both attempts proved unsucessful.[100,101]
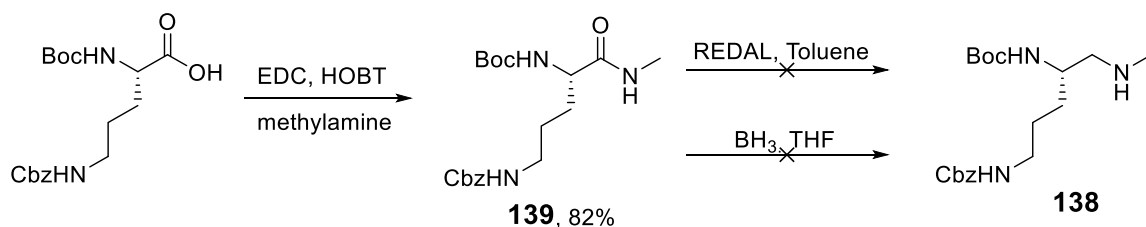
Scheme 31: Unsuccessful attempts in the Synthesis of Compound **134** - 2

Serendipitously, we found out that borane-THF could cause the reduction of an amide bond successfully, when there were no protecting groups present on the compound. To illustrate this (shown in Figure 22), benzyl *tert*-butyl (5-(methylamino)-5-oxopentane-1,4-diyl)(S)-dicarbamate, **139**, could not be converted to the amine derivative, **138**, in the presence of borane-THF. However, (S)-2,5-diamino-N-methylpentanamide, **140**, could be converted to its amine derivative, **141**, under the same conditions. We reasoned that if the N^1 of (S)-N1-methylpentane-1,2,5-triamine was protected with a second cleavable substituent such as a benzyl group, and this could prove to be a useful alternative approach.
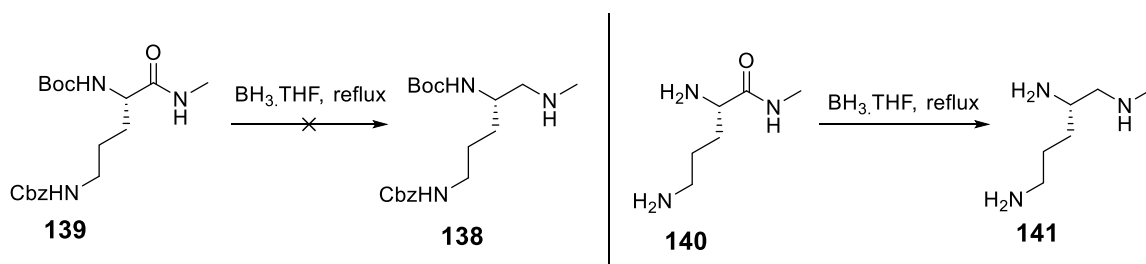
Figure 22: Working out Conditions for Borane-THF Reduction

Thus, commercially-available (S)-2,5-bis((*tert*-butoxycarbonyl)amino)pentanoic acid was coupled with N-methyl-1-phenylmethanamine using EDC and HOBT to obtain di-*tert*-butyl (5-(benzyl(methyl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate, **142**, as illustrated in Scheme 32. Compound **142** was deprotected with trifluoroacetic acid and

dichloromethane to obtain the diamine, **143**. The amide bond of **143** was reduced with borane-THF to give the amine, which was protected with boc-anhydride to give di-*tert*-butyl (5-(benzyl(methyl)amino)pentane-1,4-diyl)(S)-dicarbamate, **144**. The benzyl group of **144** was removed by hydrogenation to give di-*tert*-butyl (5-(methylamino)pentane-1,4-diyl)(S)-dicarbamate, **145**. Coupling of **145** and **84** using PyBrop gave **146** which was deprotected with trifluoroacetic acid and dichloromethane to give **134**.
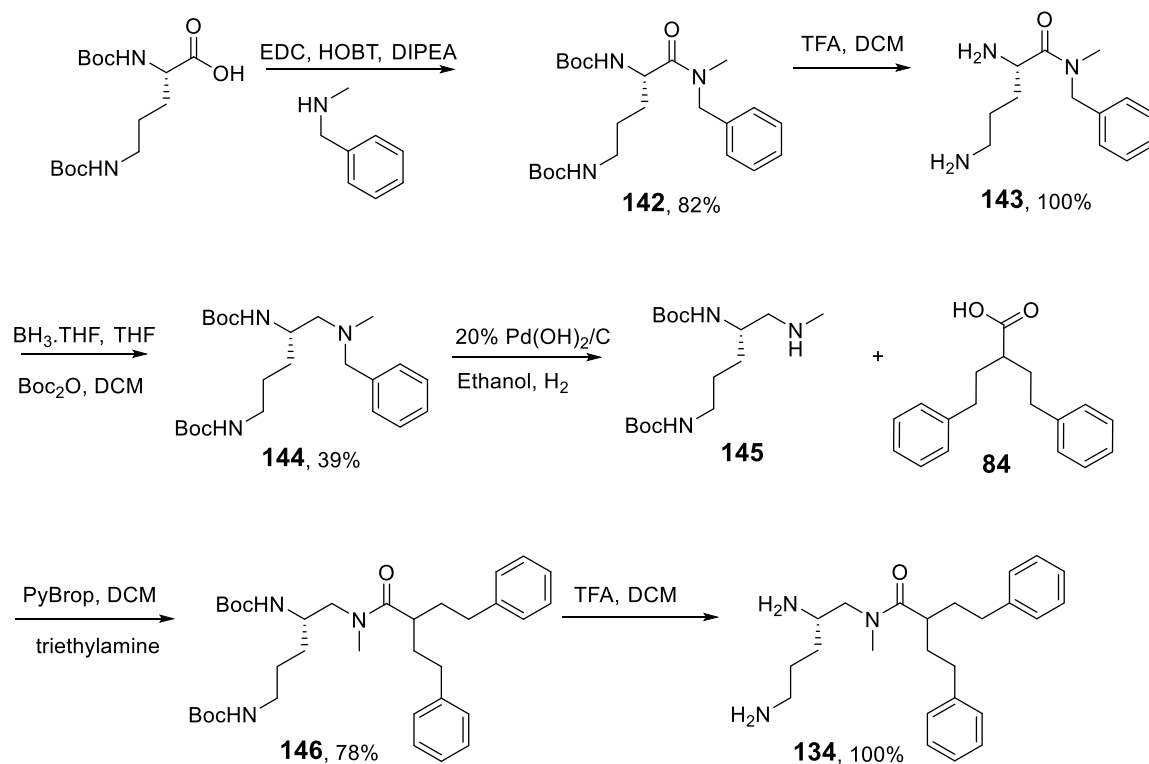
Scheme 32: Synthetic Route to Compound **134**

The synthesis of (S)-N-(5-amino-2-(methylamino)pentyl)-2-phenethyl-4-phenyl-butanamide, **135**, is shown in scheme 33. Coupling of amine, **78**, and acid, **84** provided benzyl *tert*-butyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,4-diyl)(S)-dicarbamate, **147**. This intermediate was deprotected to obtain benzyl (S)-(4-amino-5-(2-phenethyl-4-

phenylbutanamido)pentyl)carbamate, **148**. Reductive amine of **148** with benzaldehyde for overnight followed by a second reductive amination with formaldehyde gave benzyl (S)-(5-(benzyl(methyl)amino)-1-(2-phenethyl-4-phenylbutanamido)pentan-2-yl)carbamate, **149**. Hydrogenation of **149** and protection with boc anhydride afforded **150**. The boc-protection provided both the diboc protected compound and the monoboc-protected compound, **150**, with **150** being the major product. Intermediate **150** was purified and deprotected with trifluoroacetic acid and dichloromethane to obtain **135**. The deprotection of cbz and protection with boc-anhydride was done, as in the past, facilitate purification of the final diamine product.
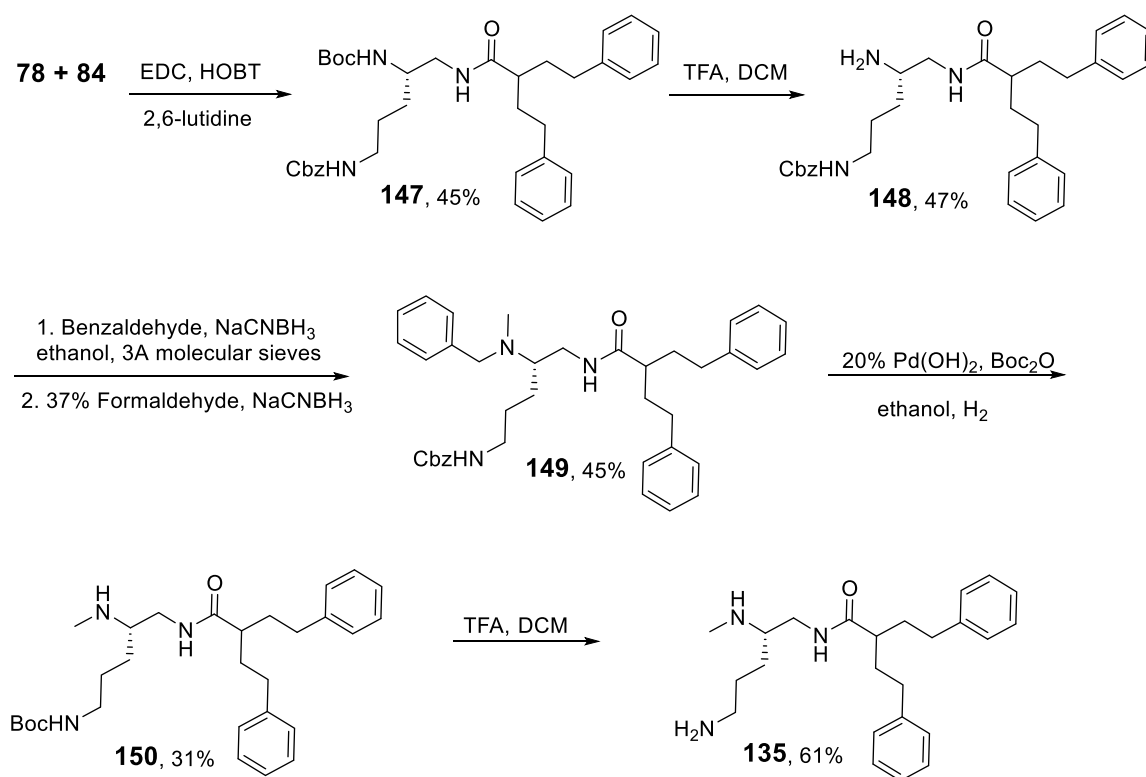
Scheme 33: Synthetic Route to Compound **135**

A similar procedure was used in the synthesis of (S)-N-(2-amino-5-(methyl-amino)pentyl)-2-phenethyl-4-phenylbutanamide, **136**. Scheme 34 summarizes the approach that was employed for its synthesis. The synthetic route to **136**, used a different starting amine, **120**, to allow for the selective monomethylation at the 2-position. The amine, **120**, was coupled with acid, **84**, to obtain benzyl *tert*-butyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,4-diyl)(S)-dicarbamate, **151**. The boc of **151** was deprotected with trifluoroacetic acid and dichloromethane to obtain the intermediate, **152**. Reductive amination of **152** with benzaldehyde overnight followed by a second reductive amination with formaldehyde for 1 hour in the presence of sodium cyanoborohydride afforded benzyl (S)-(5-(benzyl(methyl)amino)-1-(2-phenethyl-4-phenylbutanamido)-pentan-2-yl)carbamate, **153**. The cbz and benzyl groups were converted to the boc-protecting groups to facilitate ease of purification. This was done in one step to provide *tert*-butyl (S)-(4-((*tert*-butoxycarbonyl)amino)-5-(2-phenethyl-4-phenylbutanamido)-pentyl)(methyl)carbamate, **154**. In this case, **154** was the only observed product; no mono protected compound was observed. Compound **154** was treated with trifluoroacetic acid and dichloromethane to provide **136**.
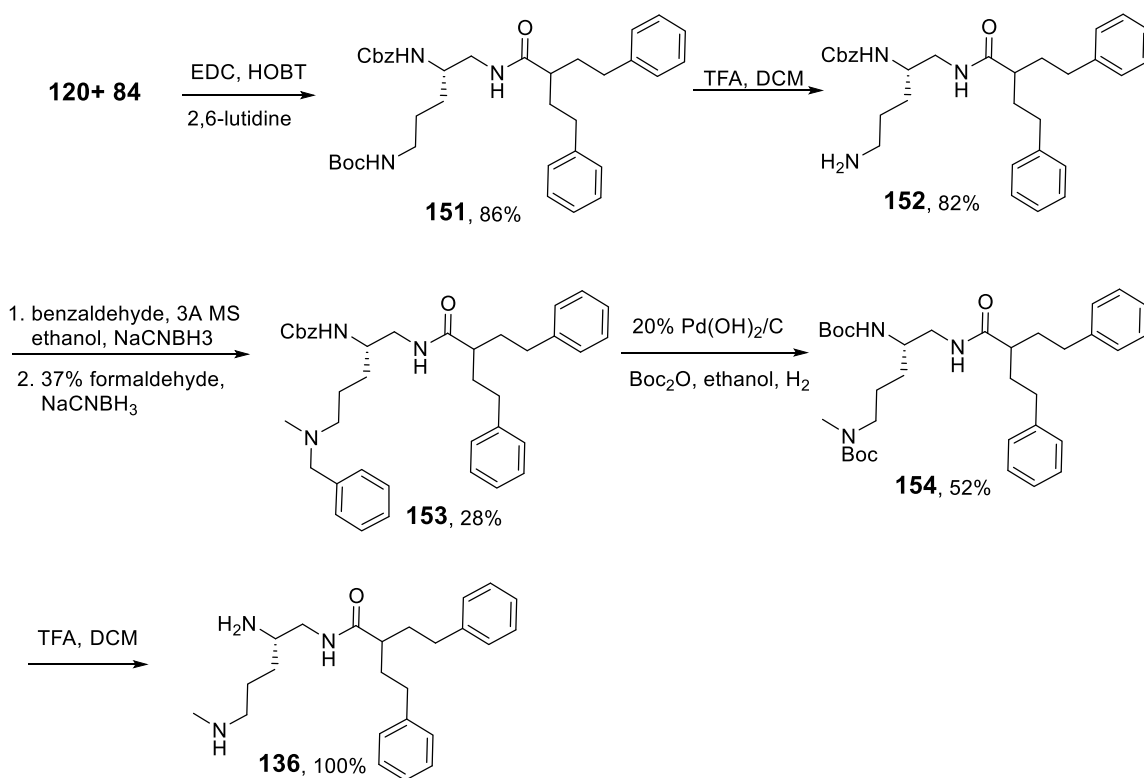
Scheme 34: Synthetic Route to Compound **136**

The biological assay data for **134-136** are summarized in Table 13. The methyl substitution did not give any improvement in activity. Each of the N-methyl derivatives, **134-136** were less active as EPIs when compared with our new reference, **83**. The conversion of the amide to a tertiary amide as in **134** resulted in a derivative with greater activity than PAβN. The presence of a methyl group on N^2 as in **135** provides an analogue with comparative activity to PAβN. The addition of a methyl substituent on the N^5 led to a significant decrease in activity. These SAR data for these "reverse amide" derivatives are consistent with the activity observed in the SAR for the "normal" amides.
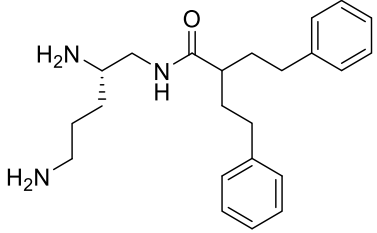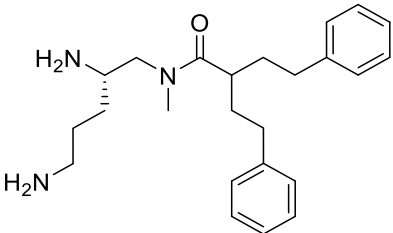
Code	Structure	Concentration of EPI (μM) (12.5 μg/mL)	MIC of Clarithro-mycin (μg/mL)	Fold Reduction in MIC Induced by the Compound
--		--	64	--
PAβN		--	16	4
83		34	4	16
134		33	8	8
135		33	16	4
136		33	32	2

Table 13: Effect of Methyl Substitution within the Reverse Amide Series

The effect of N,N-dimethyl substitutions was investigated with the synthesis of (S)-N-(5-amino-2-(dimethylamino)pentyl)-2-phenethyl-4-phenylbutanamide, **155**, and (S)-N-(2-amino-5-(dimethylamino)pentyl)-2-phenethyl-4-phenylbutanamide, **156**. These compounds were synthesized as outlined in Scheme 35 using intermediates **148** and **152** as starting materials for **155** and **156**, respectively. These intermediates underwent reductive amination with 10 equivalents of sodium triacetoxyborohydride and formaldehyde to obtain **157** and **158** which were deprotected to provide the desired final products.
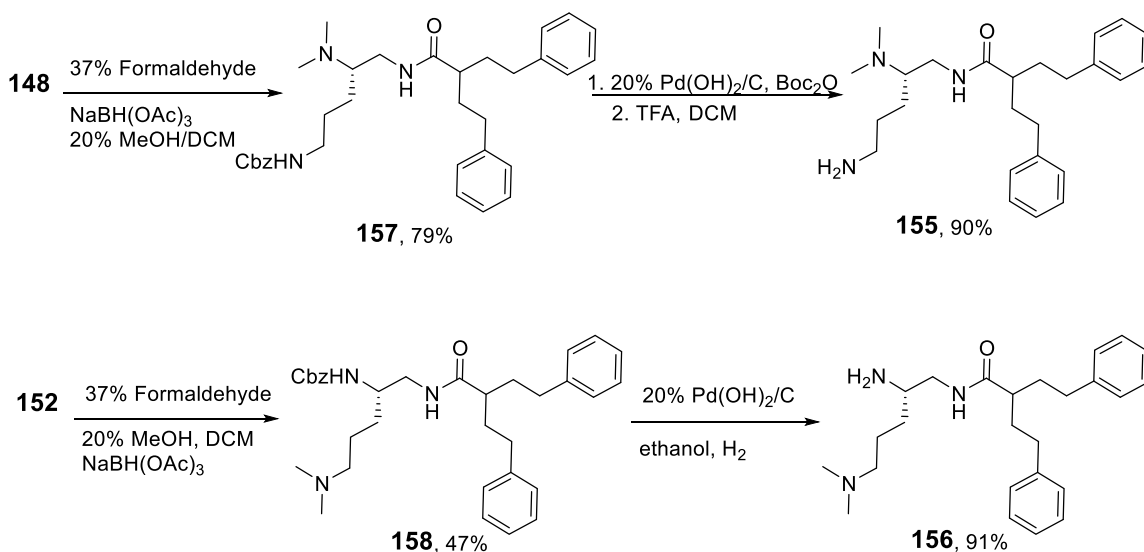
Scheme 35: Synthetic Route to Compounds **155** and **156**

Table 14 summarizes the effect of N,N-dimethyl substitution within the "reversed amide" series. None of the dimethyl substitutions presented an advantageous increase to the activity of our lead compound, **83**, or PAβN. Both compounds had very low activity as EPI agents. These data point to the preference for a non-substituted primary amine at the N^5 position. The N^2 position appears to moderately tolerate monomethyl substitution, but cannot tolerate N,N-dimethyl substitution (see **135** vs **83** in Table 13).
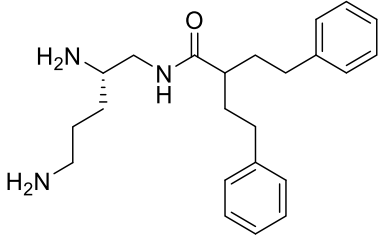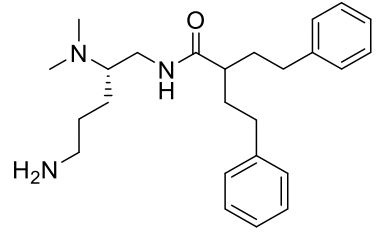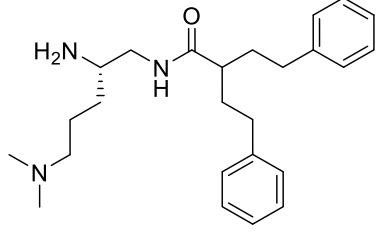
Code	Structure	Concentration of EPI (µM) (12.5 µg/mL)	MIC of Clarithro-mycin (µg/mL)	Fold Reduction in MIC Induced by the Compound
--		--	64	--
PAβN		--	16	4
83		34	4	16
155		32	32	2
156		32	32	2

Table 14: Effect of N,N-dimethyl Substitution on the Reverse Amide Series

The effect of substitution on the 5-position of the pentyl with hydrazine and guanidine was also investigated. (S)-N-(2-Amino-5-hydrazinylpentyl)-2-phenethyl-4-phenyl-butanamide, **159**, was expected to be synthesized from **147,** as shown in Scheme 36. The cbz protecting group was removed by hydrogenation and the free amine, **160**, was reacted with BocNHOTs expecting to obtain the boc-protected hydrazine, **161**.[102] However, this reaction lead to the formation of *tert*-butyl (S)-(2,2-dimethyl-5,13-dioxo-14-

phenethyl-16-phenyl-3-oxa-4,6,12-triazahexadecan-10-yl)carbamate, **162**.[103] It was deprotected to give the urea derivative as final compound, **163**.
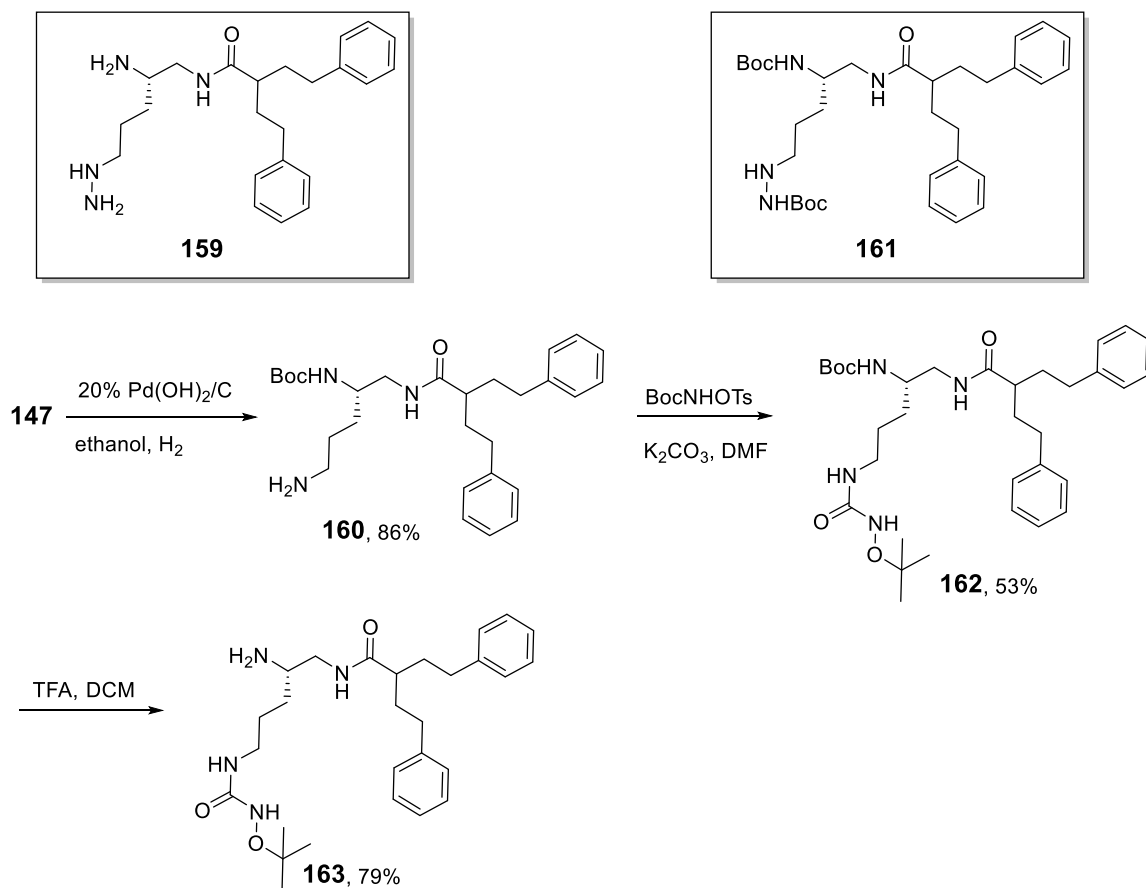
Scheme 36: Synthetic Route to Compound **163**

The formation of intermediate, **162**, was due to a Lossen rearrangement which is illustrated in Figure 23. The N-Boc-O-tosyl hydroxylamine is converted to the isocyanate. The presence of the amine nucleophile, **160**, induces the formation of the isomeric urea derivative, **162**, instead of the expected boc-protected hydrazine compound, **161**. The hydrazine compound is currently being synthesized to obtain its activity.
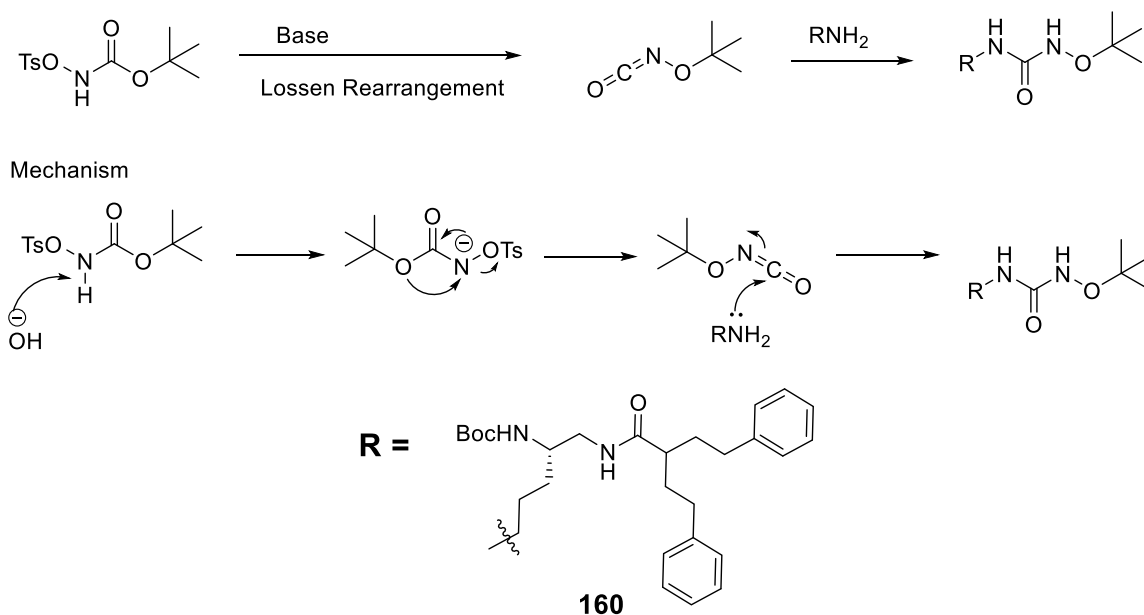
Figure 23: Mechanism of Lossen Rearrangement

The guanidine compound, **164**, was synthesized as illustrated in Scheme 37 by reacting **160** with 1,3-diboc-2-(trifluoromethylsulfonyl)guanidine at room temperature to yield **165.** The final product, **164**, was obtained after deprotection of this tris-boc derivative with trifluoroacetic acid and dichloromethane.
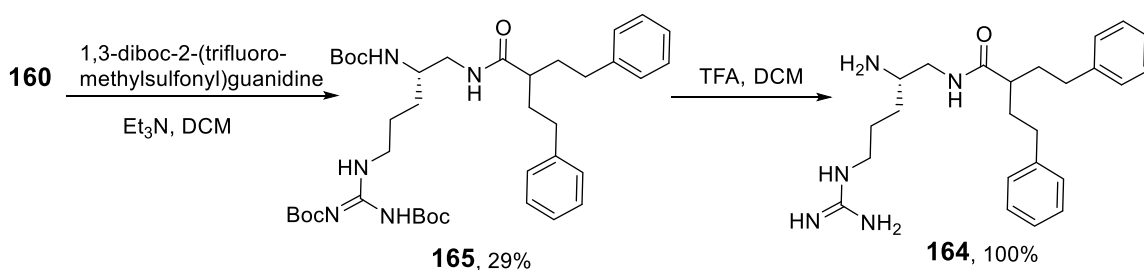
Scheme 37: Synthetic Route to Compound **164**

The results obtained from the assay of **163** and **164** are presented in Table 15. The guanidine showed similar activity to PAβN but was less active than **83**. The urea derivative, **163**, showed no activity as an EPI. On the whole, substitution on the nitrogen at the 5-position of the diaminopentyl appears to be associated with a reduction in EPI activity.
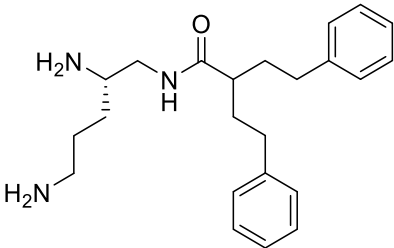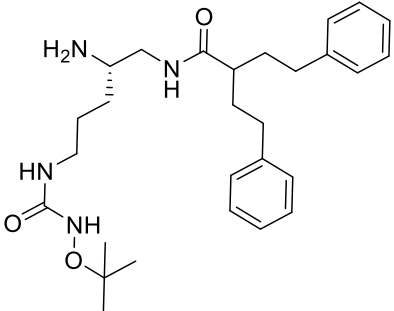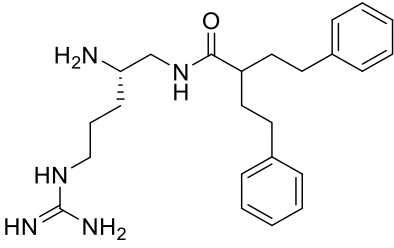
Code	Structure	Concentration of EPI (µM) (12.5 µg/mL)	MIC of Clarithro-mycin (µg/mL)	Fold Reduction in MIC Induced by the Compound
--		-	64	--
PAβN		-	16	4
83		34	4	16
163		26	64	1
164		31	16	4

Table 15: Effect of Urea Derivative and Guanidine on Reverse Amides Series

The effect of cyclic amines on the SAR within this "reverse amide" series was also explored. Conformationally-restricted amines have been reported to potentiate activity of various EPIs and to reduce mammalian toxicity.[104,105] The two cyclic amines intermediates that were chosen were benzyl (4S)-2-(aminomethyl)-4-((*tert*-butoxycarbonyl)-amino)pyrrolidine-1-carboxylate, **166**, and dibenzyl 2-(aminomethyl)piperazine-1,4-dicarboxylate, **167**.[105] Both of these cyclic amines, **166** and **167**, were synthesized by Liping Wang and Michelle Chen of Taxis Pharmaceuticals. The amines were independently coupled with **84** as summarized in Scheme 38 to obtain **168** and **169** respectively. These intermediates were deprotected to give **170** and **171**.
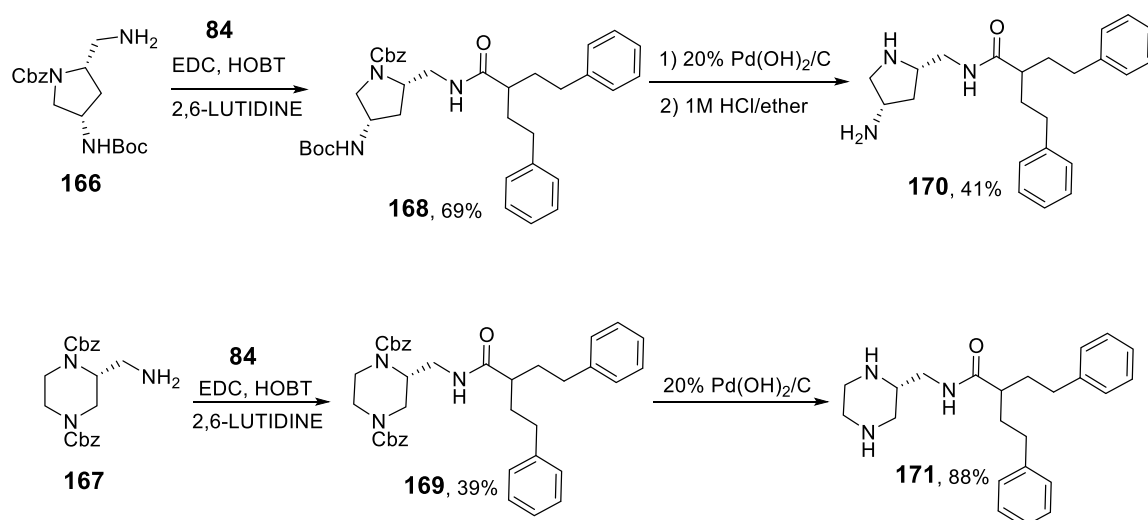
Scheme 38: Synthetic Route to Compound **170** and **171**

Despite the fact that these amines had been previously used to increase potency and decrease the toxicity of other EPIs, improved activity of clarithromycin in wild-type *E. coli* was not observed. In fact both compounds failed to exhibit any significant activity as efflux pump inhibitors.
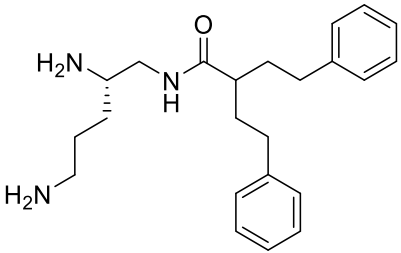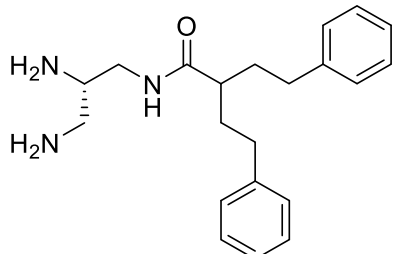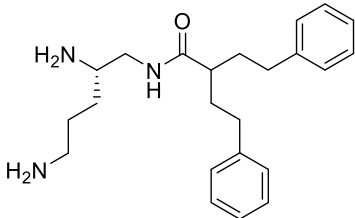
Code	Structure	Concentration of EPI (µM) (12.5 µg/mL)	MIC of Clarithro-mycin (µg/mL)	Fold Reduction in MIC Induced by the Compound
--		--	64	--
PAβN		--	16	4
83		34	4	16
170		34	64	1
171		34	64	1

Table 16: Effect of Cyclic Amines on the Reverse Amide Series

To conclude our studies the amine series, we synthesized two amine compounds which had not been synthesized from amino acids. Our objective was to achieve enhanced potency by combining these amines to our core pentanoic acid, **84**. These two amines were dibenzyl (azanediylbis(ethane-2,1-diyl))dicarbamate (which was commercially available) and dibenzyl (2-aminopropane-1,3-diyl)dicarbamate, **172**, which had to be synthesized.

The synthesis of N,N-bis(2-aminoethyl)-2-phenethyl-4-phenylbutanamide, **173**, as outlined in Scheme 39, followed the coupling of dibenzyl (azanediylbis(ethane-2,1-diyl))dicarbamate with **84** to afford **174**. Deprotection of the cbz protecting groups of **174** by hydrogenation provided the desired final compound, **173**.
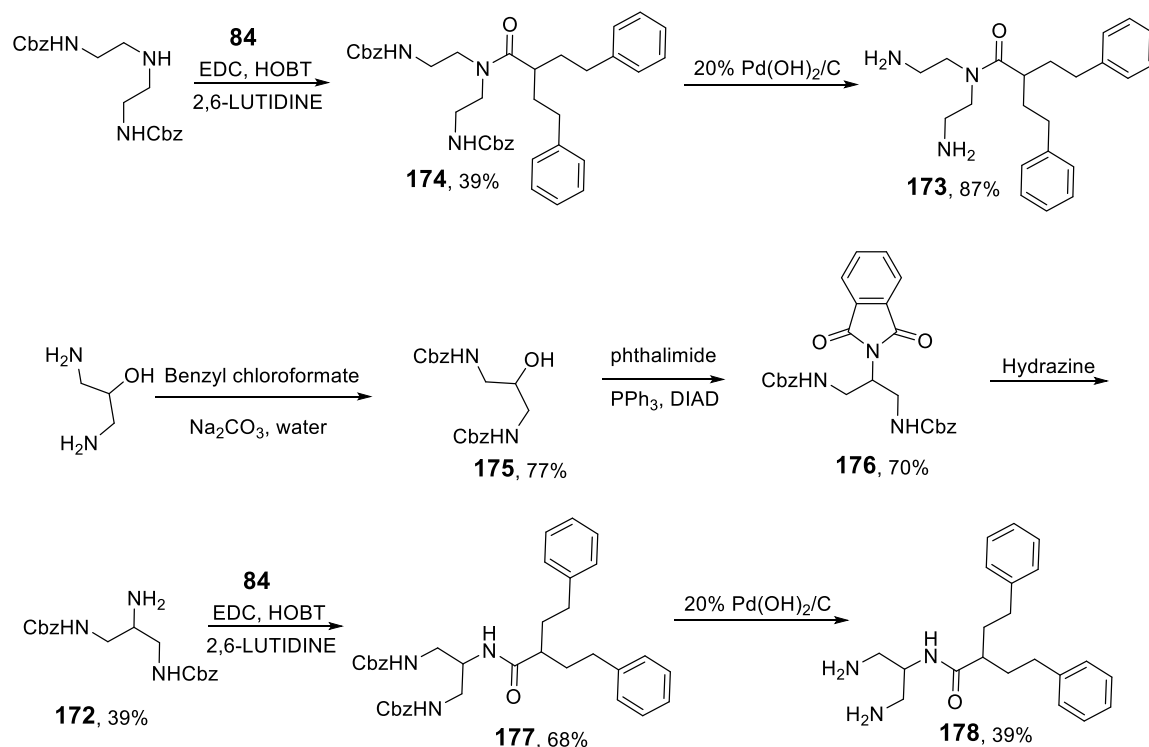
Scheme 39: Synthesis of Compound **173** and **178**

The synthesis of dibenzyl (2-aminopropane-1,3-diyl)dicarbamate, **172**, illustrated in Scheme 39, employed the commercially available benzyl (3-amino-2-hydroxypropyl)-carbamate. Benzyl chloroformate was used to protect the amino groups of this alcohol. This protected alcohol, **175**, was then converted to its phthalimide derivative under Mitsunobu conditions. The phthalimide derivative, **176**, was reduced with hydrazine monohydrate to afford the amine, **172**. This amine, **172**, was reacted with **84** to obtain

dibenzyl (2-(2-phenethyl-4-phenylbutanamido)propane-1,3-diyl)dicarbamate, **177**, which was deprotected to give **178**.

These compounds were tested for their EPI activity which is presented in Table 17. Although these new compounds showed some activity as EPI agents, they were not as active as the lead we had been working with **83.** At similar doses, **173** and **178** had activity only one-half and one quarter that of **83**.
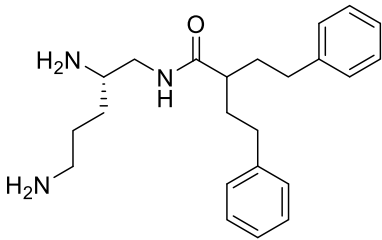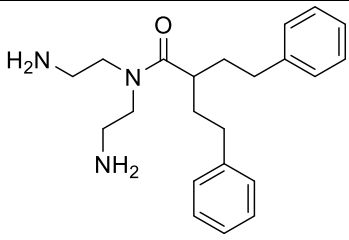
Code	Structure	Concentration of EPI (μM) (12.5 μg/mL)	MIC of Clarithro-mycin (μg/mL)	Fold Reduction in MIC Induced by the Compound
--		--	64	--
PAβN		--	16	4
83		34	4	16
173		35	8	8
178		37	16	4

Table 17: Effect of Non-Amino acid Amines on EPI activity of Reverse Amides

Modification of the amines had not brought any significant increase in activity. Thus we redirected our efforts to work on the right hand portion to see if we could improve the activity by the addition of a methylene group between the acid and the junction of the bis-alkylaryl branching. We hypothesized that increasing the chain length could possible lead to an increase in potency. We decided to apply this concept to all the compound synthesized in Table 8. (The R-enantiomers were excluded from the series). The compounds targeted for synthesis were; (S)-N-(2,5-diaminopentyl)-3,3-diphenyl-propanamide, **179**, (S)-3-benzyl-N-(2,5-diaminopentyl)-4-phenylbutanamide, **180**, and (S)-N-(2,5-diaminopentyl)-3-phenethyl-5-phenylpentanamide, **181**.

The synthesis of (S)-N-(2,5-diaminopentyl)-3,3-diphenylpropanamide, **179**, as shown in Scheme 40 involved the use of the commercially-available 3,3-diphenylpropanoic acid. Coupling of this acid to amine, **78**, and deprotection afforded **179**.
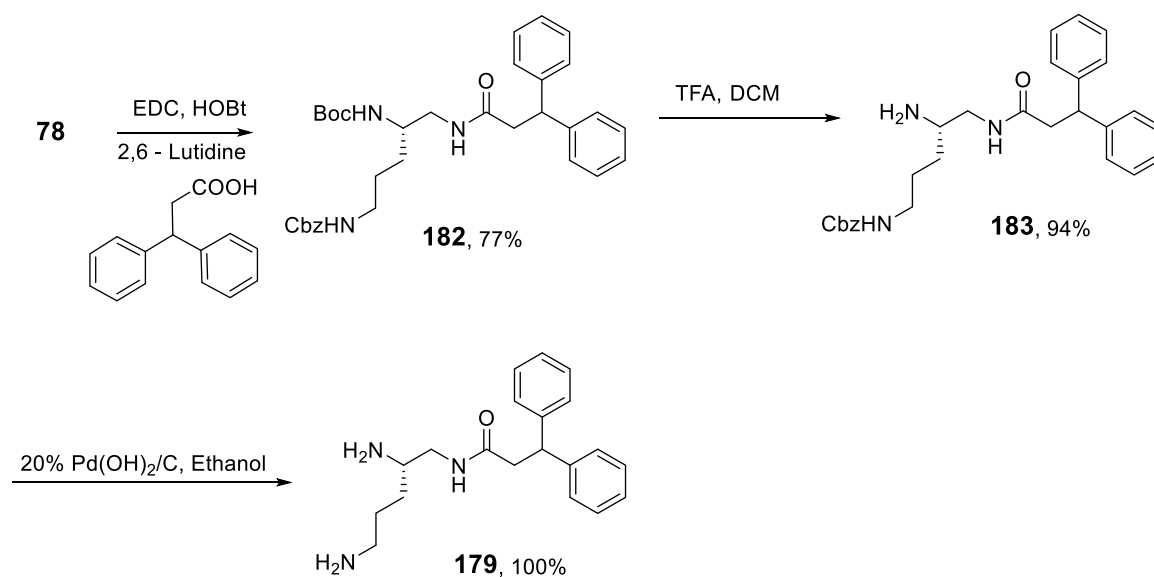
Scheme 40: Synthetic Route to Compound **179**

The synthesis of (S)-3-benzyl-N-(2,5-diaminopentyl)-4-phenylbutanamide, **180**, and (S)-N-(2,5-diaminopentyl)-3-phenethyl-5-phenylpentanamide, **181**, required several synthetic steps. Using the Wittig - Horner- reaction, 1,3-diphenylpropan-2-one and 1,5-diphenylpentan-3-one, **32**, the ketones were converted to their olefins, **184** and **185**, respectively.[106] Reduction of the double bond and saponification of the esters, **186** and **187**, led to acids: 3-benzyl-4-phenylbutanoic acid, **188**, and 3-phenethyl-5-phenylpentanoic acid, **189**. These acids were coupled with amine, **88** and deprotected to obtain the desired compounds, **180** and **181**.

184, 83%; n = 1
185, 25%; n = 2

186, 89%; n = 1
187, 69%; n = 2

188, 75%; n = 1
189, 74%; n = 2

190, 60%; n = 1
191, 48%; n = 2

180, 73%; n = 1
181, 73%; n = 2
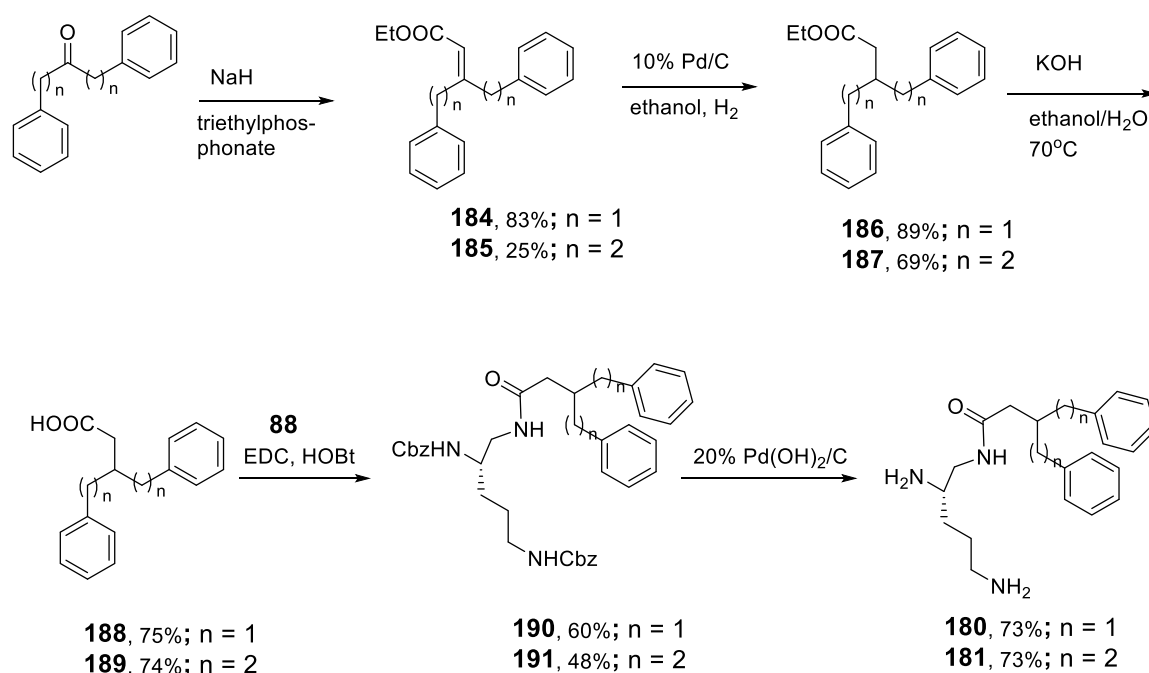
Scheme 41: Synthetic Route to Compounds **180** and **181**

Table 18 summarizes the data obtained from the *in vitro* EPI biological assays. Compound **179** did not exhibit activity as an efflux pump inhibitor. However, the 1C homologue, **180**, exhibited good EPI activity. Compound **181** showed a dramatic enhancement in EPI activity resulting in a 512-fold reduction in the MIC of clarithromycin under these assay

conditions. This is the best activity of all the compounds synthesized so far. Compound **181** was also evaluated for toxicity in mice. This compound was toxic at a dose of ≥ 200 µg (8mg/kg) when administered i.v to mice.
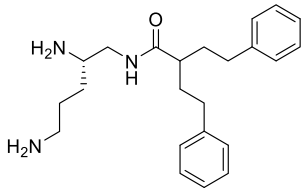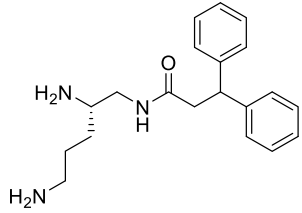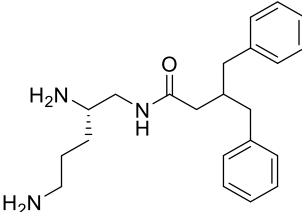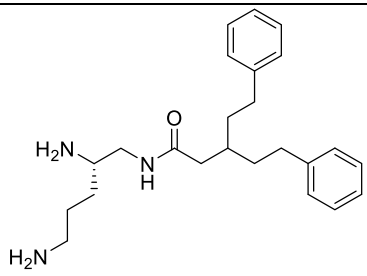
Code	Structure	Concentration of EPI (µM) (12.5 µg/mL)	MIC of Clarithro-mycin (µg/mL)	Fold Reduction in MIC Induced by the Compound
--		--	64	--
PAβN		--	16	4
83		34	4	16
179		38	64	1
180		35	4	16
181		33	0.125	512

Table 18: Effect of Methylene Insertion on EPI activity in the Reverse Amides Series

2.4. STRUCTURE ACTIVITY RELATIONSHIP OF THE AMINE SERIES

The alkyl amines series was the final group of EPIs that we investigated. We initially made compounds structurally similar to the normal amides and assayed them for their activity. The difference between these compounds was the carbonyl of the amide was replaced by $-CH_2-$. The compounds in this set included (S)-N^1-(1,5-diphenylpentan-3-yl)pentane-1,2,5-triamine, **192**, (S)-N^1-(1,5-diphenylpentan-3-yl)pentane-1,3,5-triamine, **193**, and (S)-N^5-(1,5-diphenylpentan-3-yl)pentane-1,2,5-triamine, **194**. These compounds were pentane triamine isomers which had a secondary amine linked to a diaminoalkyl moiety. This SAR would enable us to determine the effect of the position of the diamines on EPI activity.

(S)-N^1-(1,5-diphenylpentan-3-yl)pentane-1,2,5-triamine, **192**, was synthesized as shown in Scheme 42. Reductive amination of amine, **88**, and ketone, **32**, in the presence of sodium cyanoborohydride gave intermediate dibenzyl (5-(((1,5-diphenylpentan-3-yl)amino)pentane-1,4-diyl)(S)-dicarbamate, **195**.[107] The cbz of the amine intermediate, **195**, was deprotected by hydrogenation. It had been hoped that this deprotection will afford a clean compound requiring no further purification. However, the triamine product obtained was very impure. To facilitate purification, the impure triamine was stirred in boc anhydride and dichloromethane to afford the triboc-protected derivative. This was purified and the pure compound treated with trifluoroacetic acid and dichloromethane to provide **192**.
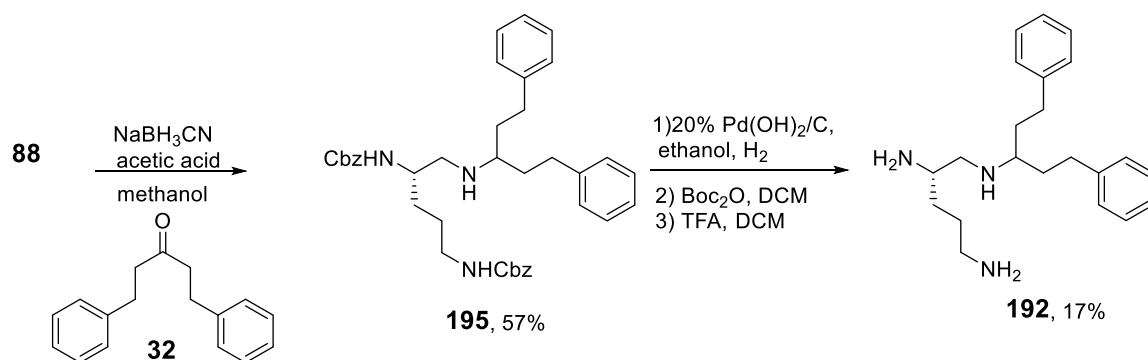
Scheme 42: Synthetic Route to Compound **192**

(S)-N^1-(1,5-Diphenylpentan-3-yl)pentane-1,3,5-triamine, **193**, was synthesized as illustrated in Scheme 43, from (S)-3,5-diamino-N-(1,5-diphenylpentan-3-yl)pentanamide, **55**, in three steps. Compound **55** was reduced to the secondary amine with borane-tetrahydrofuran. However, the borane reduction was very impure and poor yielding. The protection with boc, followed by purification and deprotection enabled us to as to isolate as a pure compound this highly polar triamine as its triflate salt.
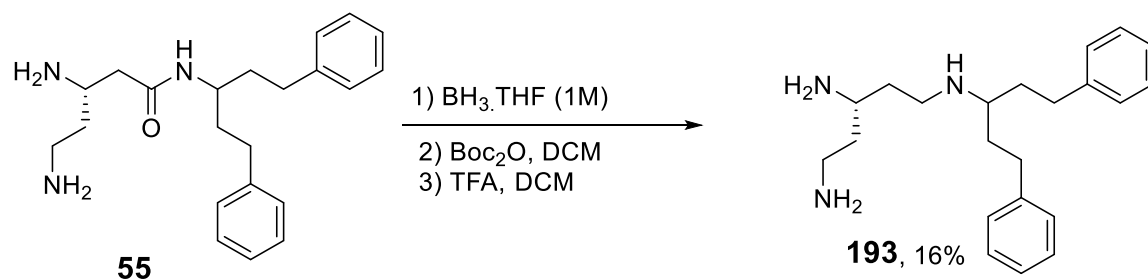
Scheme 43: Synthetic Route to Compound **193**

The third triamine (S)-N^5-(1,5-diphenylpentan-3-yl)pentane-1,2,5-triamine, **194**, was synthesized as outlined in Scheme 44 in a procedure similar to the synthesis of **192**. Amine, **122** and 1,5-diphenylpentan-3-one, **32**, underwent reductive amination to form **196**. The

cbz protecting groups were removed and the tris-boc protected derivative formed was purified. The pure tris-boc derivative was deprotected to provide the triamine, **194.**
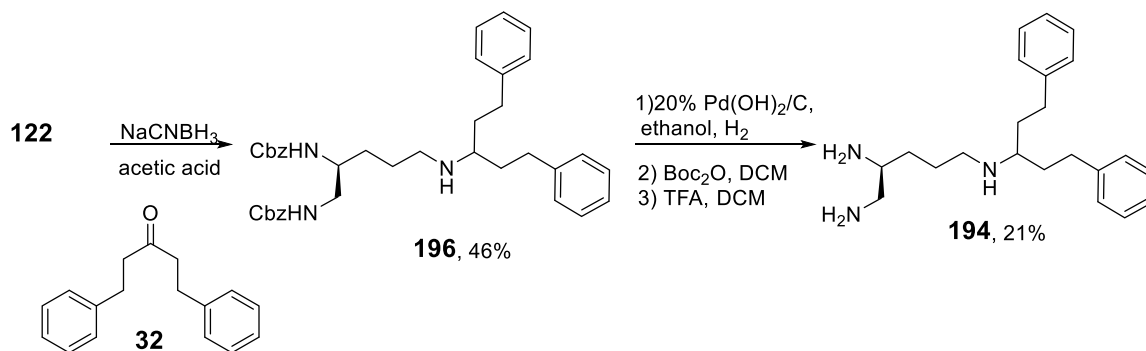
Scheme 44: Synthetic Route to Compound **194**

The data obtained from the EPI assay indicated that all three triamine derivatives were active. These data are listed in Table 19. (S)-N[1]-(1,5-diphenylpentan-3-yl)pentane-1,3,5-triamine, **193,** had the best activity within this set of compounds. It was 8-fold more effective than PAβN. The worst compound in this set, **192,** still had a better EPI activity than that of the reference compound, **36.** It caused an 8 fold reduction in the MIC of clarithromycin which was twice that of the reference compound or PAβN.
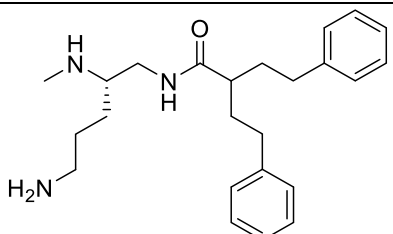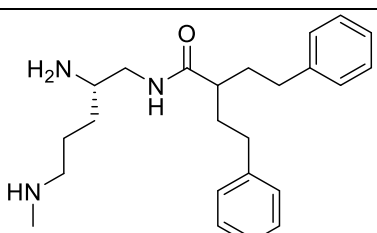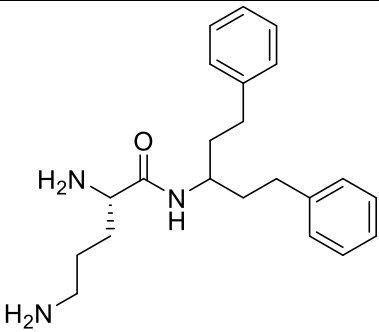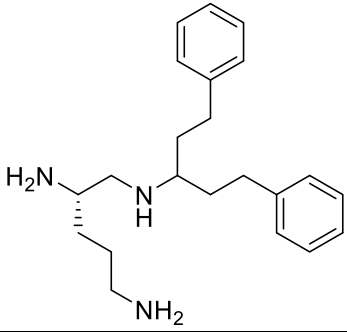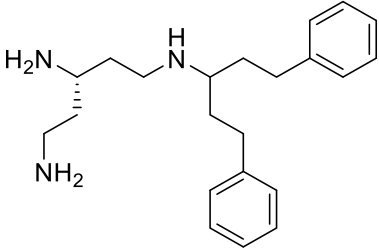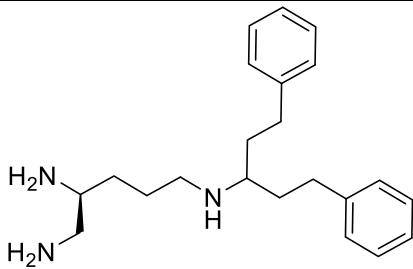
Code	Structure	Concentration of EPI (µM) (12.5 µg/mL)	MIC of Clarithro-mycin (µg/mL)	Fold Reduction in MIC Induced by the Compound
--		--	64	--
PAβN		--	16	4
36		35	16	4
192		37	8	8
193		37	2	32
194		37	4	16

Table 19: Effect of Pentane Triamine Isomers on the EPI Activity of the Amine Series.

We were interested in demonstrating that the compounds being synthesized inhibited efflux pump activity, and not some non-specific type of binding with the bacteria membrane that might account for the reactivation of clarithromycin against the wildtype bacteria. We chose compound **192** and **194** for these more detailed studies; these compounds had been shown to be have low intrinsic MICs (25 µg/mL) (Appendix). Our goal was to directly monitor inhibition of efflux activity by the compounds using a fluorescent substrate of the efflux pump.

We confirmed that our test protocols were working as they should, by running the test in the presence of N43. N43 is an *E. coli* which is mutated on acrA1 thus assembly of the AcrAB-TolC pump is defective. This mutant is, in essence, devoid of the pump activity of AcrAB-TolC. Examining the data in Table 20, it can be seen the **192** and **194** are working in a manner to inhibit pump activity. Table 20 shows that the MIC of clarithromycin against the wildtype E. coli strain W4573 in the presence of **192** and **194** is in a range similar to that observed against N43, which is genetically identical to the wildtype strain with the exception of the acrA gene mutation that renders the pump inactive. Thus the compounds **192** and **194** induce a similar effect on the bacteria as is induced by an inactivation of their efflux pump.

Strain	Compound*	MIC of Clarithromycin (µg/mL)	Fold Reduction in MIC Induced by the Compound
W4573 (Wildtype)	None	32	--
W4573 (Wildtype)	**192**	4	8
W4573 (Wildtype)	**194**	2	16
W4573 (Wildtype)	PAβN	8	4
N43 (*acrA1* mutant)	None	2	--
*When present, all compounds were used at a concentration of 12.5 µg/mL.			

Table 20: Comparison of the reduction in clarithromycin MIC against *E. coli*

We then used a fluorescence-based cellular assay developed by Prof. Pilch and Dr. Kaul to monitor efflux pump activity. The assay monitors the impact of EPIs on drug efflux out of bacteria. The Hoechst 33352 dye, used in this assay, is a known efflux pump substrate. This dye has a much higher fluorescence when bound to DNA inside the cell as compared to when it is free in solution outside the cell. In the assay, the ATP source is depleted in the bacterial cells by poisoning with CCCP, which turns off the efflux pump machinery and allows the influx of the dye into the cell without efflux resulting in high fluorescence. Then CCCP is washed out and the pump rejuvenated with glucose; this restarts the pump and leads to an efflux of the dye leading to a lower fluorescence. However in the presence of a pump inhibitor, the rejuvenation does not lead to an efflux of the dye thus the high fluorescence is maintained. Figure 24 show that **192** and **194** maintained fluorescence over a 45 minute period due to their ability to inhibit the bacterial efflux pump. PAβN is included as a positive EPI control agent. Note that **192** and **194**

inhibited the pump at a much lower concentration (12.5 µg/mL) relative to PAβN, which exhibited very little efflux pump inhibition activity at 12.5 µg/ml, instead requiring higher concentrations of up to 200 µg/mL to exhibit activity. These results suggest that **192** and **194** are more potent inhibitors of the efflux pump in E. coli than PAβN. The compound vehicle was DMSO. Glc denotes glucose.
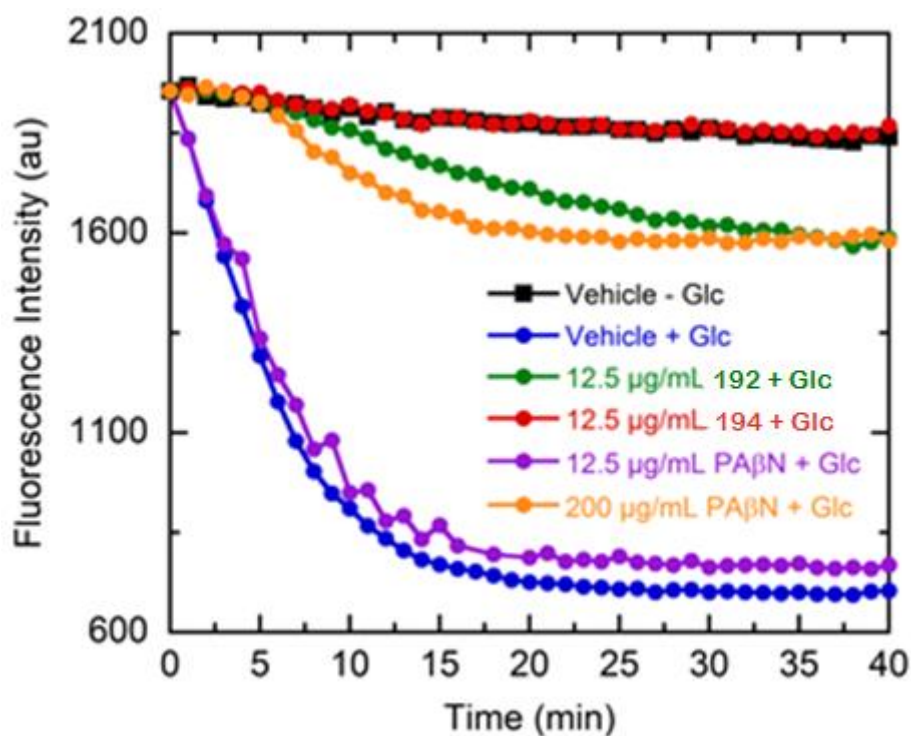
Figure 24: Compounds **192** and **194** inhibit the pump-mediated efflux of Hoechst 33342 in *E. coli* ATCC 25922.

The effect of using butane triamines also explored as had been done previously with the normal amides. The two isomers of butane triamine; (S)-N^1-(1,5-diphenylpentan-3-yl)butane-1,2,4-triamine, **197**, and (S)-N^4-(1,5-diphenylpentan-3-yl)butane-1,2,4-triamine, **198**, were synthesized and assayed for their EPI activity. Reductive amination was

performed using benzyl *tert*-butyl (4-aminobutane-1,3-diyl)(S)-dicarbamate, **199**, and 1,5-diphenylpentan-3-one, **32**, together with sodium cyanoborohydride as illustrated in Scheme 45. The reaction was stirred at room temperature overnight, but no product formed. The reaction was then heated at 70 °C for 7 hours providing the desired product, **200**. The product of this reaction, benzyl *tert*-butyl (4-((1,5-diphenylpentan-3-yl)amino)butane-1,3-diyl)(S)-dicarbamate, **200**, was then deprotected to afford the diboc-protected compound, **201**. This was done to provide a pure triamine, **197**, after treatment of **201** with trifluoroacetic acid and dichloromethane.
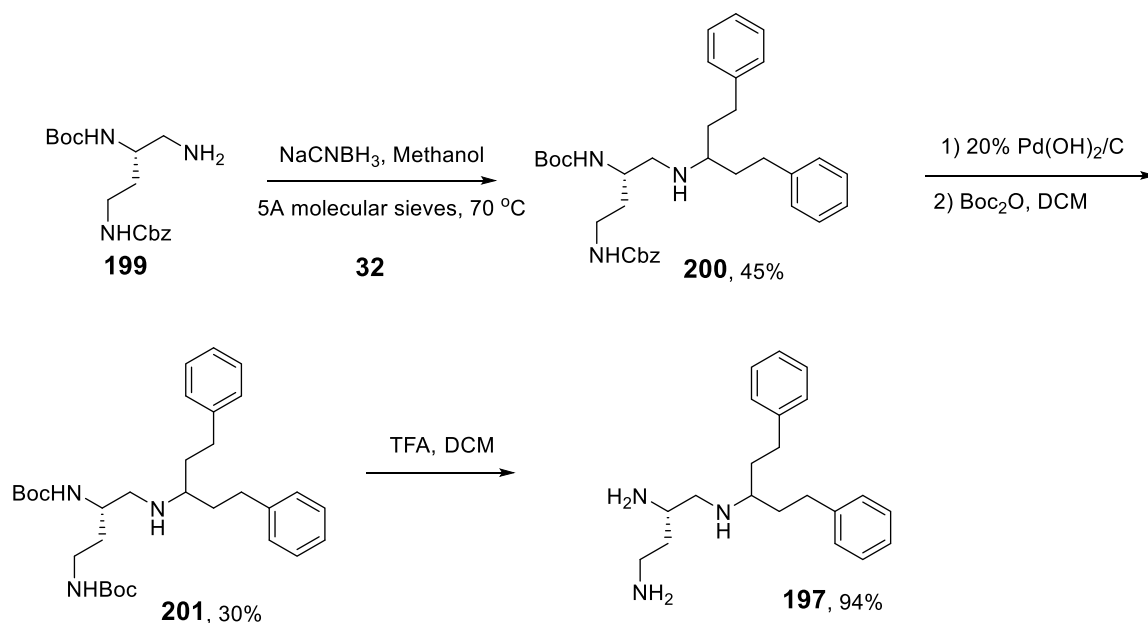
Scheme 45: Synthetic Route to Compound **197**

The synthetic approach employed for the preparation of **198** is illustrated in Scheme 46. Benzyl *tert*-butyl (4-aminobutane-1,3-diyl)(S)-dicarbamate, **199**, was protected with boc on the free amine to afford benzyl di-*tert*-butyl butane-1,2,4-triyl(S)-tricarbamate, **194**. The cbz protecting groups were removed by hydrogenation and the unisolated amine was

condensed with 1,5-diphenylpentan-3-one, **32**, under reductive amination conditions. The product obtained; di-*tert*-butyl (4-((1,5-diphenylpentan-3-yl)amino)butane-1,2-diyl)(R)-dicarbamate**, 203**, was deprotected with trifluoroacetic acid and dichloromethane to give (S)-N^4-(1,5-diphenylpentan-3-yl)butane-1,2,4-triamine, **198**.
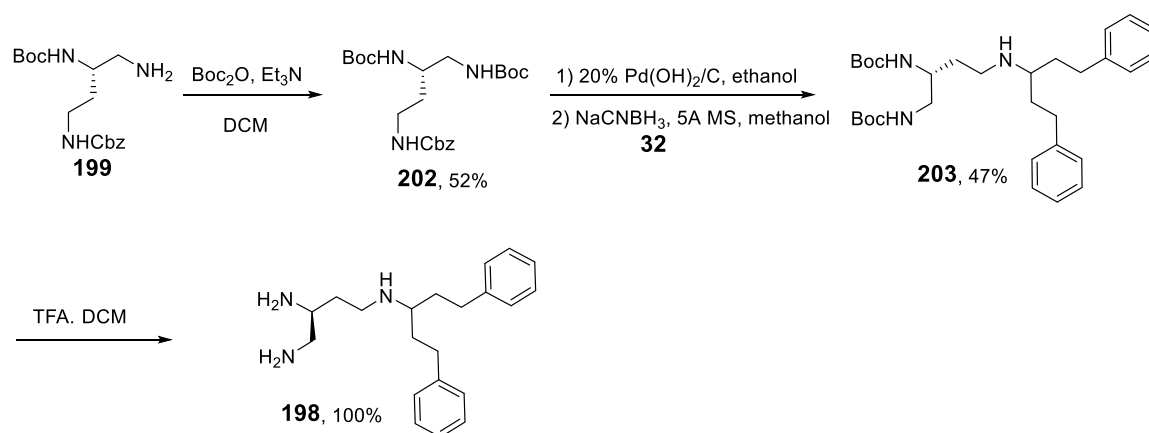
Scheme 46: Synthetic Route to Compound **198**

Compound **197** and **198** were tested for EPI activity and the data is shown in Table 21. The butane triamines did not reveal an increase in efflux pump inhibiting activity, which parallels the earlier results obtained within the butanamide series of the normal amides. Both **197** and **198** exhibited only modest activity as efflux pump inhibitors. These results are consistent with our earlier observation that the pentane analogues tended to have greater EPI activity.
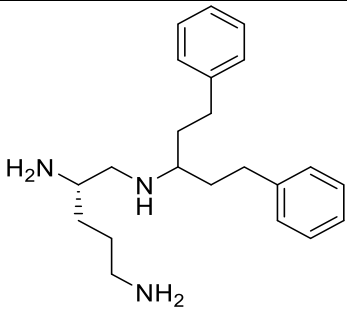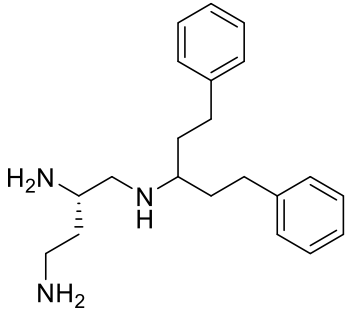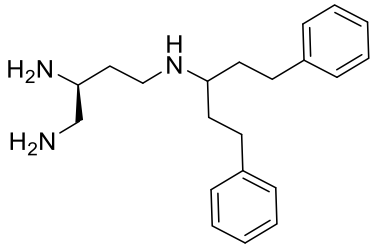
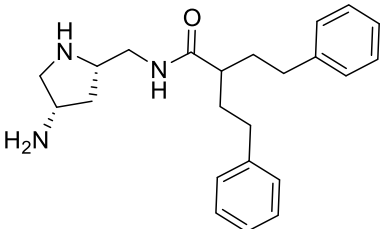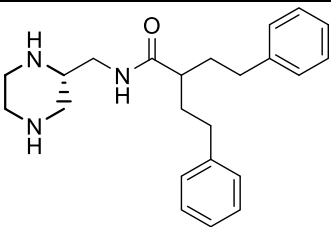| Code	Structure	Concentration of EPI (µM) (12.5 µg/mL)	MIC of Clarithro-mycin (µg/mL)	Fold Reduction in MIC Induced by the Compound
--		--	64	--
PAβN		--	16	4
192		37	8	8
197		38	16	4
198		38	32	2

Table 21: Effect of Isomers of Butane Triamine on EPI Activity within the Amine Series

The effect of amines which are not amino acids by origin were also investigated. The three compounds synthesized were; N^1-(2-aminoethyl)-N^2-(1,5-diphenylpentan-3-yl)ethane-1,2-diamine, **204**, 1-(1,5-diphenylpentan-3-yl)-4-methylpiperazine, **205**, and 1-(1,5-diphenylpentan-3-yl)-4-(4-methoxybenzyl)piperazine, **206.**

Commercially-available 2-((2-aminoethyl)amino)ethan-1-ol was treated with benzyl chloroformate in the presence of 10M NaOH, sodium bicarbonate water and THF to provide benzyl (2-(((benzyloxy)carbonyl)amino)ethyl)(2-hydroxyethyl)carbamate, **207**. We tried to convert this alcohol, **207**, to the aldehyde with the hope that it could be used as an intermediate in a reductive amination with 1,5-diphenylpentan-3-amine, **34**. The attempt to form this aldehyde intermediate, however, was unsuccessful. We also considered converting the alcohol intermediate to the mesylate which could be displaced with 1,5-diphenylpentan-3-amine, **34**, in the presence of potassium carbonate. This attempt led to the formation of several with a lack of consistent results. This alcohol intermediate, **207**, was then reacted with phthalimide, triphenylphosphine and diisopropyl azodicarboxylate to give **208**. Reflux of this phthalimide derivate, **208**, with hydrazine monohydrate afforded the amine **209**. The condition for the converting the phthalimide derivative to the amine required some optimization. The initial temperature which had been used for most of the phthalimide reduction reactions had been 100 ºC. However, when this temperature was used in this synthesis there was a decomposition of the product. The temperature was lowered to 75 ºC and that worked to produce the amine, **209**, in a good yield (72%). Reductive amination of benzyl (2-aminoethyl)(2-(((benzyloxy)-carbonyl)amino)ethyl)carbamate, **209**, with 1,5-diphenylpentan-3-one, **32**, as shown in Scheme 47, gave benzyl (2-(((benzyloxy)carbonyl)amino)ethyl)(2-((1,5-diphenylpentan-

3-yl)amino)ethyl)carbamate, **210**. Deprotection of **210** in the presence of boc anhydride provided the diboc compound, **211.** Treatment of **211** with trifluoroacetic acid and dichloromethane afforded N^1-(2-aminoethyl)-N^2-(1,5-diphenylpentan-3-yl)ethane-1,2-diamine, **204**. .
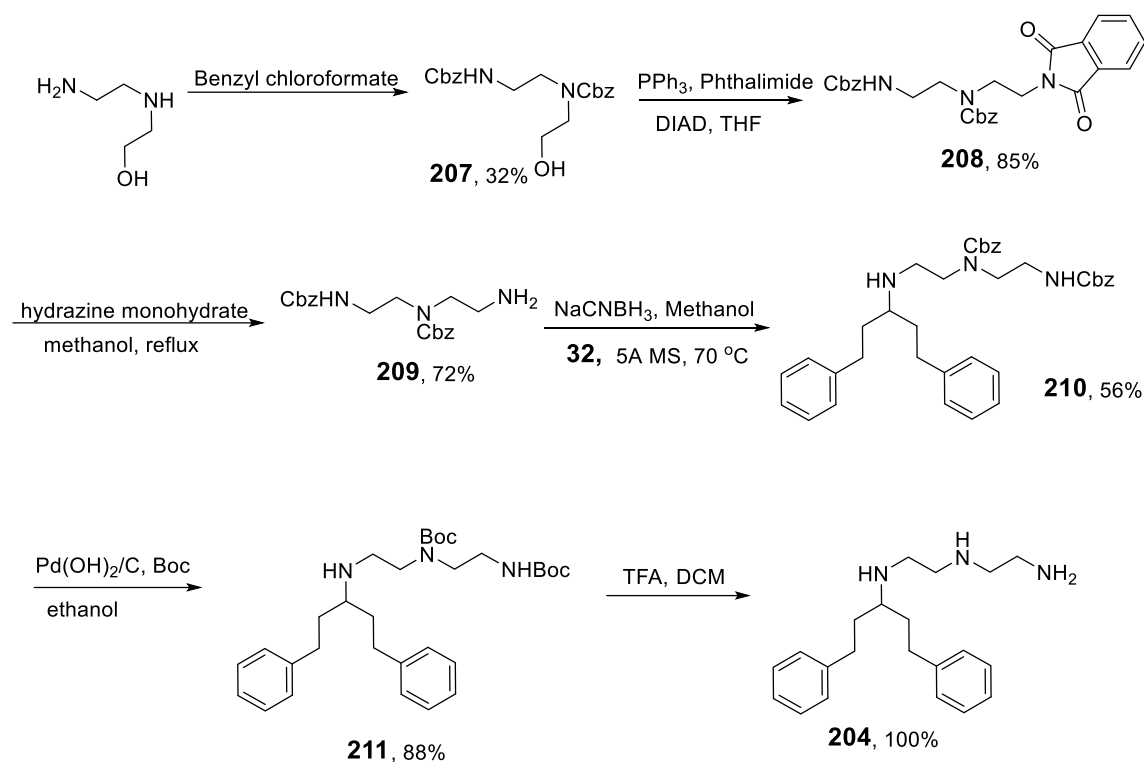
Scheme 47: Synthetic Route to Compound **204**

The method used for the preparation of the N-methylpiperazine derivative, **205**, is outlined in Scheme 48. Using reductive amination, 1-(1,5-diphenylpentan-3-yl)-4-methylpiperazine, **205**, was synthesized from 1,5-diphenylpentan-3-one, **32**, and 1-methyl piperazine in the presence of sodium triacetoxyborohydride.[108]
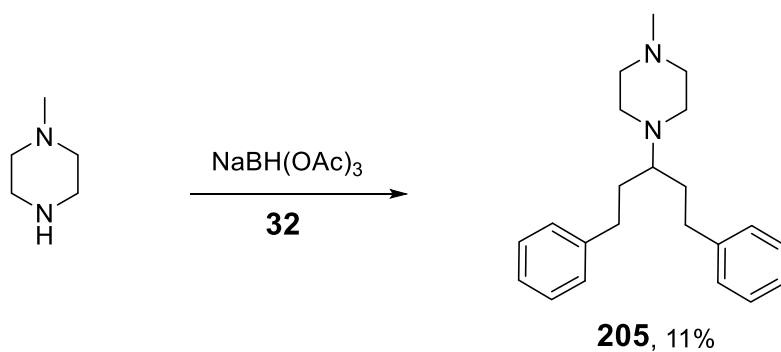
Scheme 48: Synthetic Route to Compound **205**

1-(1,5-Diphenylpentan-3-yl)-4-(4-methoxybenzyl)piperazine, **206**, was synthesized as outlined in Scheme 49, by reaction of 1-(4-methoxybenzyl)piperazine, **212**, and 1,5-diphenylpentan-3-yl methanesulfonate, **213**.[109] Intermediate **197**, was synthesized by reacting 1-(chloromethyl)-4-methoxybenzene with piperazine. The versatile ketone, **32**, was reduced with sodium borohydride to afford the alcohol, **214**. The alcohol, **214**, was reacted with mesyl chloride to provide the mesylate, **213**.
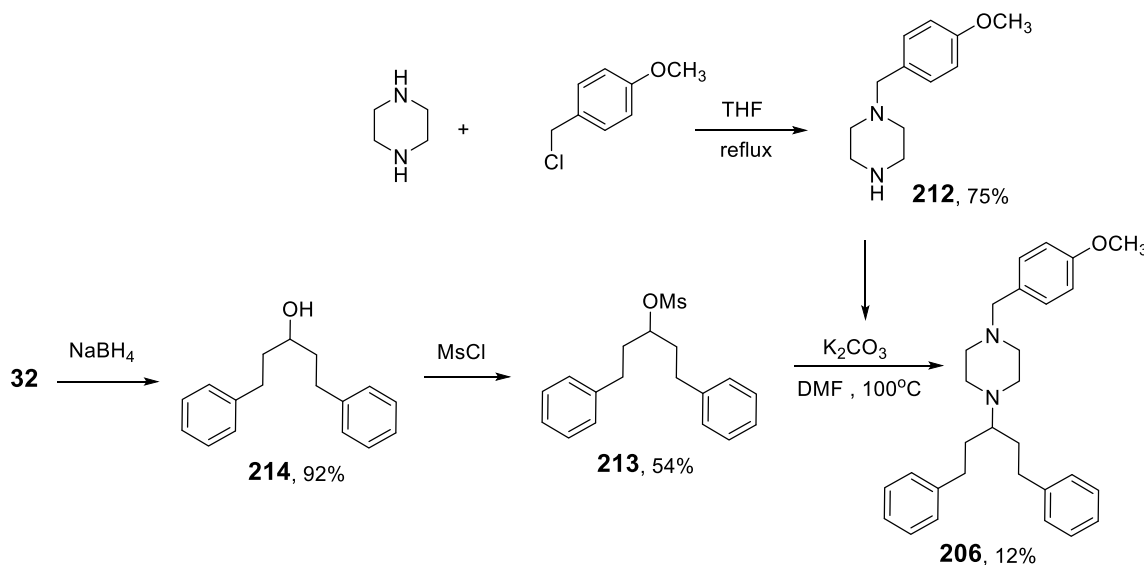
Scheme 49: Synthetic Route to Compound **206**

The data obtained from the EPI assay of compounds **204-206** is shown in Table 22. None of the compounds provided an observable increase in the MIC of clarithromycin. All the compounds maintained the MIC of clarithromycin at 64.
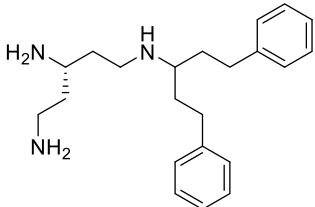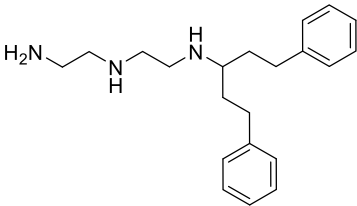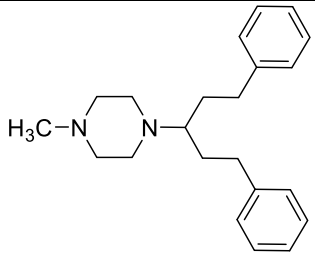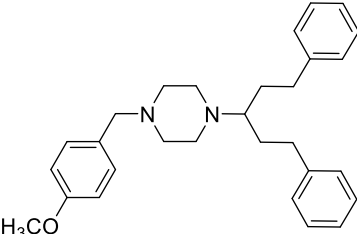
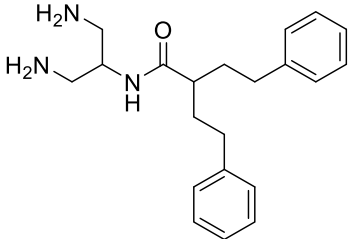| Code	Structure	Concentration of EPI (µM) (12.5 µg/mL)	MIC of Clarithro-mycin (µg/mL)	Fold Reduction in MIC Induced by the Compound
--		--	64	--
PAβN		--	16	4
193		37	2	32
204		38	64	1
205		39	64	1
206		29	64	1

Table 22: Effect of Amino ethyl derivatives on EPI activity of the Amine Series

The focus of the research was then switched to making amine analogues which are structurally similar to reversed amides series. These compounds included the isomers of pentane triamine: (S)-N^1-(2-phenethyl-4-phenylbutyl)pentane-1,2,5-triamine, **215**, (S)-N^1-(2-phenethyl-4-phenylbutyl)pentane-1,3,5-triamine, **216**, and (S)-N^5-(2-phenethyl-4-phenylbutyl)pentane-1,2,5-triamine, **217**. These isomers have consistently proved to be among the better compounds within any given series.

Compound **215** was prepared as outline in Scheme 50. The previously synthesized dicbz protected intermediate, **91**, was reacted under hydrogen atmosphere in the presence of the Pearlman's catalyst and boc anhydride to afford the diboc-protected derivative, **218**. (Compound **91** was used in the synthesis of our lead compound, **83)**. Di-*tert*-butyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,4-diyl)(S)-dicarbamate, **218**, was deprotected and converted to the triamine. This impure and unisolated triamine was protected again with boc-anhydride and purified to obtain pure compound, **220**. Di-*tert*-butyl (5-((*tert*-butoxycarbonyl)(2-phenethyl-4-phenylbutyl)amino)pentane-1,4-diyl)(S)-dicarbamate, **220**, was deprotected with trifluoroacetic acid and dichloromethane to obtain **215**.
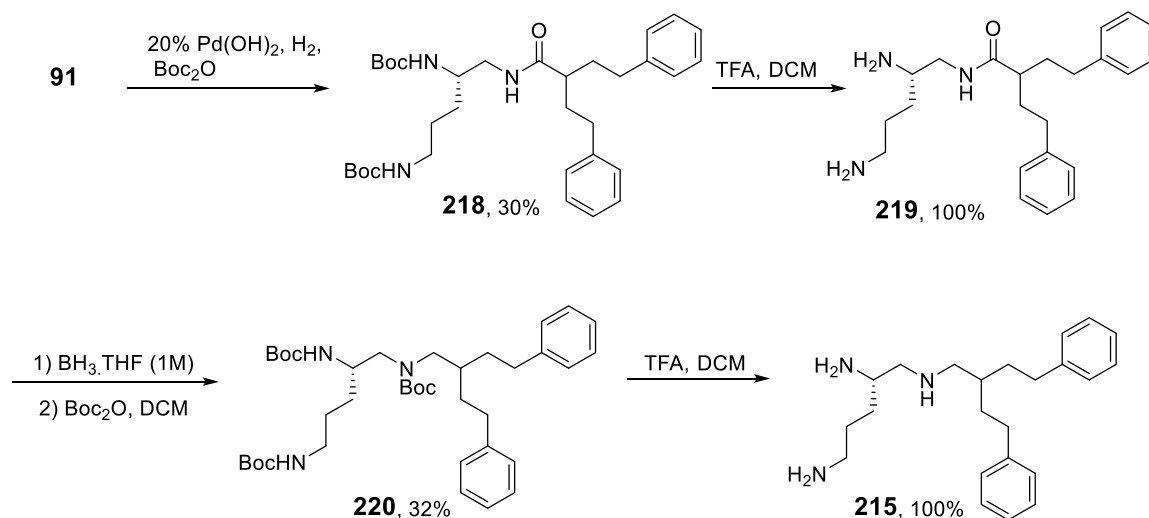
Scheme 50: Synthetic Route to Synthesis of Compound **215**

The synthesis of (S)-N^1-(2-phenethyl-4-phenylbutyl)pentane-1,3,5-triamine, **216**, as illustrated in Scheme 51, begun with the versatile intermediate, 1,5-diphenylpentan-3-one, **32**. Compound **32** was converted to the olefin, **221**, using the Wittig reaction. Hydroboration and oxidation led to the 2-phenethyl-4-phenylbutan-1-ol, **222**, which was converted to 2-phenethyl-4-phenylbutan-1-amine, **224**, in two steps. The amine was condensed with (S)-3,5-bis(((benzyloxy)carbonyl)amino)pentanoic acid, **59**, to give dibenzyl (5-oxo-5-((2-phenethyl-4-phenylbutyl)amino)pentane-1,3-diyl)(S)-dicarbamate, **225**. The cbz protecting groups were removed by hydrogenation and the diamino compound, **226**, was converted to the triamine, **216**. As performed previously, the formation of the diboc-protected derivative allowed for a more facile purification. Deprotection with trifluoroacetic acid, provided the tri-triflate salt, **216**.
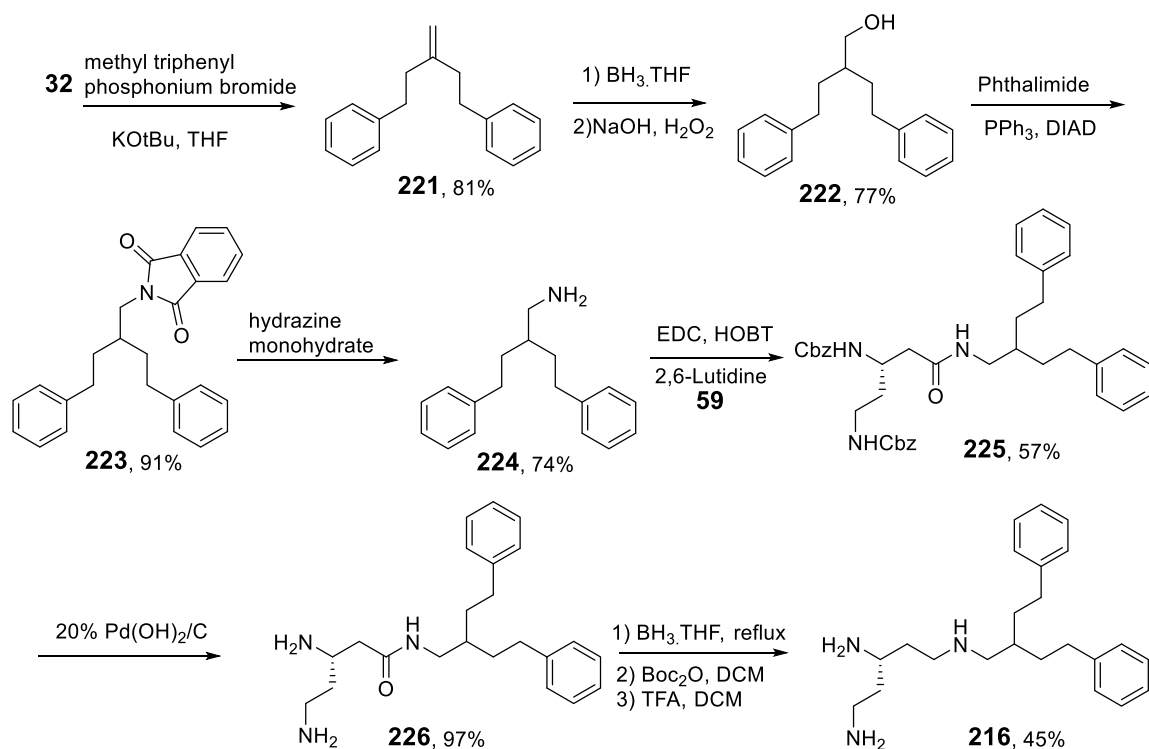
Scheme 51: Synthetic Route to Compound **216**

The synthesis of **217** was performed as illustrated in Scheme 52. Benzyl *tert*-butyl (5-aminopentane-1,4-diyl)(S)-dicarbamate, **78**, was boc-protected and the cbz protecting group removed. The diboc-protected amine, di-*tert*-butyl (5-aminopentane-1,2-diyl)(S)-dicarbamate, **228**, was condensed then with 2-phenethyl-4-phenylbutanoic acid, **84**, to form the amide, di-*tert*-butyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,2-diyl)(S)-dicarbamate, **229**. Deprotection of the boc groups and reduction with borane-tetrahydrofuran, followed by a two-step synthetic purification procedure led to (S)-N^5-(2-phenethyl-4-phenylbutyl)pentane-1,2,5-triamine, **217**.
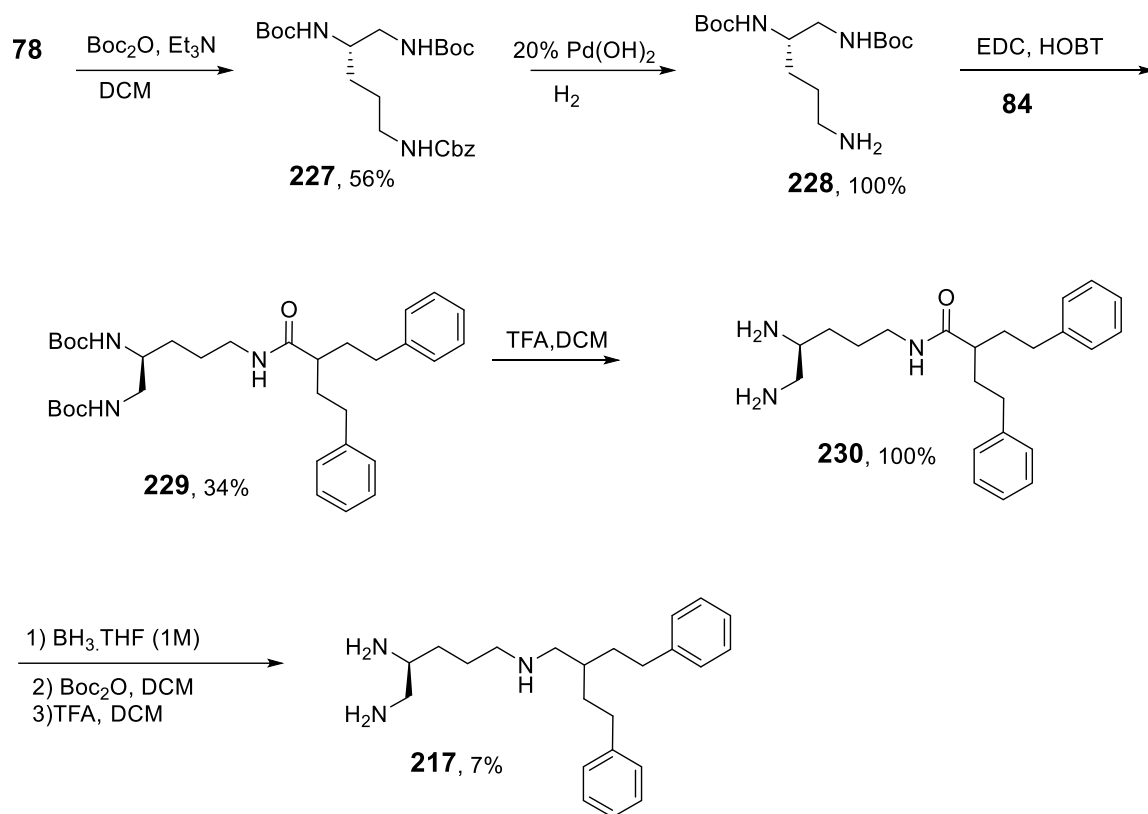
Scheme 52: Synthetic Route to Compound **217**

The EPI data for compound **215-217** is summarized in Table 23. These compounds proved to be among the better compounds in the amine series. All three of these pentane triamine derivatives were more effective than our earlier lead compound, **83**. Compound **215** was the best compound in this series providing a 128-fold reduction in the MIC of clarithromycin by virtue of its potency as a bacterial efflux inhibitor
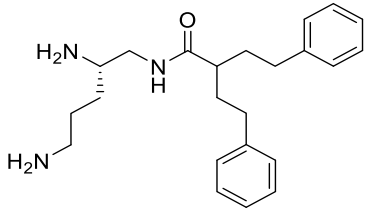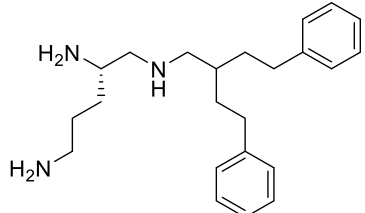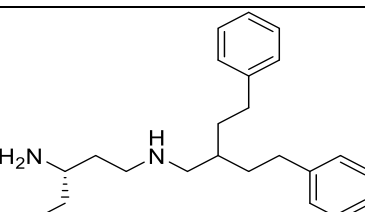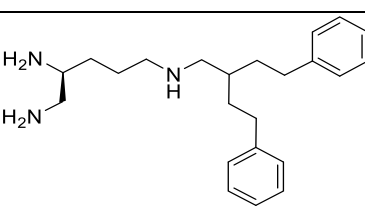
Code	Structure	Concentration of EPI (μM) (12.5 μg/mL)	MIC of Clarithro-mycin (μg/mL)	Fold Reduction in MIC Induced by the Compound
--		--	64	--
PAβN		--	16	4
83		34	4	16
215		35	0.5	128
216		35	1	64
217		35	2	32

Table 23: Effect of Isomers of Pentane Triamine on EPI activity in the Amine Series (2)

2.5. EFFLUX PUMP INHIBITORY ACTIVITY IN *P. AERUGINOSA*

Every compound synthesized were tested for their activity against the MexAB-OprM in *P. aeruginosa*. Most of the compounds had no activity in this bacteria. However a few compounds exhibited activity against this pump and are shown in the Table 24 and Figure 25.

Code	Series	MIC of Levofloxacin (µg/mL)	Fold Reduction in MIC Induced by the Compound
--		1	--
55	Reversed Amides	0.06	16
193	Amine	0.06	16
194	Amine	0.25	4
197	Amine	0.5	2
198	Amine	0.5	2
215	Amine	0.03	32
216	Amine	0.06	16
217	Amine	0.03	32

Table 24: Compounds having activity against *P. aeruginosa*

197
Fold Reduction = 2

198
Fold Reduction = 2

194
Fold Reduction = 4

55
Fold Reduction = 16

193
Fold Reduction = 16

216
Fold Reduction = 16

215
Fold Reduction = 32

217
Fold Reduction = 32
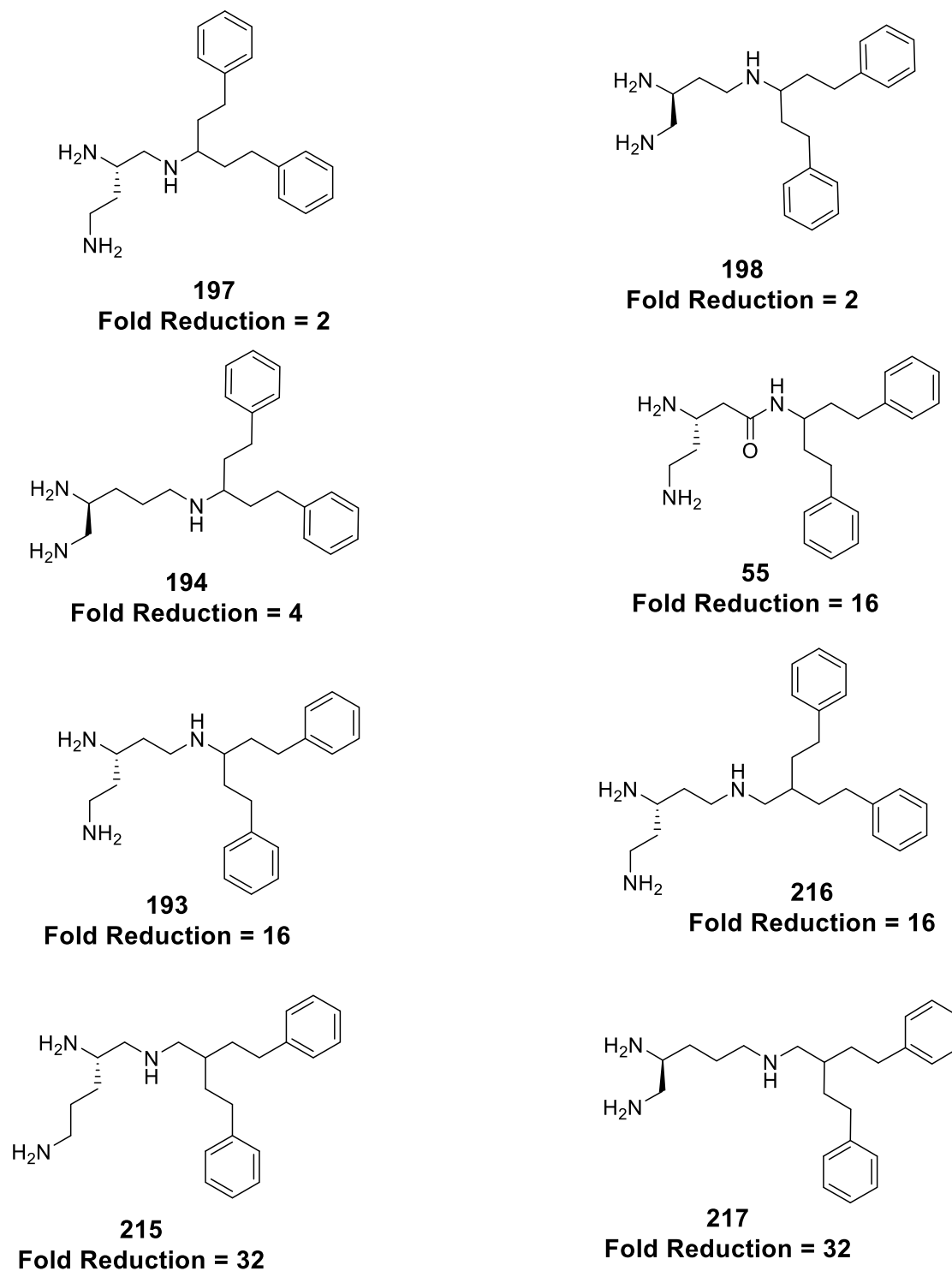
Figure 25: Structures of Compounds with Activity against *P. aeruginosa* in order of increasing EPI activity

It can be seen that most of the compounds exhibiting efflux pump activity against *P. aeruginosa* were of the amine scaffold. All compounds in the amine series that had activity against *E. coli*, typically exhibited potency as an EPI when used with levofloxacin against *P. aeruginosa*. The only exception was **192** which appears not to be active as an EPI under these assay conditions against *P. aeruginosa*. The compounds which mimicked the reverse amide series were on the whole some of the better compounds in the series, as shown for **215** and **217**. An anomaly was observed in the case of the 3,5-diamino compounds. For this set of compounds, its reverse amide, normal amines and "reverse amide" amine analogues were all active.

As the *P. aeruginosa* presents a fatal challenge in infected and immunocompromised patients, these findings offer a chance at further investigations. This is because in *P. aeruginosa*, efflux pumps have been found to be responsible for the secretion of virulence factors which are necessary for infection of a host.[110] Blocking these pumps offers a chance at reducing host infection. These compounds provide a starting point that will enable the discovery of much better and more improved compounds with EPI activity against this organism.

SUMMARY

Antibiotic resistance is a serious issue that is fast becoming a global crisis. Once susceptible bacteria are becoming resistant to antibiotic that they were previously resistant to. Compounding this problem, is the slow rate in the introduction of novel antibiotics into the clinic. There is the need to find better and improved ways of curtailing this problem.

There are several mechanism of antibiotic resistance and these include; antimicrobial inactivation by enzymes, intracellular target mutations, reduced entry of antibiotics and efflux. Efflux is a major determinant of antibiotics levels in bacteria and have been implicated in antibiotic resistance. Efflux pumps are energy-dependent systems that remove toxic compounds from cells. Numerous efflux systems have been characterized in both Gram-negative and Gram-positive.

There are two main classes of efflux pumps; this grouping occurs based on the energy used for transportation. These two classes are the ATP-binding cassette (ABC) transporters and the secondary multidrug transporters. The secondary multidrug transporters can be grouped in four main families: major facilitate superfamily (MFS), small multidrug resistance (SMR), multidrug and toxic compound extrusion (MATE) and resistance nodulation cell division (RND). The MFS is important in Gram-positive and includes the NorA of *S. aureus*.

The RND superfamily is clinically relevant in Gram-negative bacteria such as *P. aeruginosa* and *E. coli*. The presence of the outer membrane and efflux pumps in these bacteria makes them intrinsically resistant to antibiotics. Two well characterized efflux

pumps are the AcrAB-TolC of *E. coli* and MexAB-OprM of *P. aeruginosa*. Several strategies exist to overcome efflux which involve the by-passing of efflux activity by redesigning old antibiotics, increase permeability of the outer cell wall, block the outer pores to prevent exit of drug molecules, use competitive or non-competitive inhibition to block efflux capacity or by biological inhibition.

Several inhibitors of efflux pumps have been discovered but as yet none of these compounds have made it into the clinic. The focus of our work was to design novel efflux pump inhibitors and explore the structure-activity relationships of these new EPIs. We first tried to develop EPI from the aryl piperazine series. We worked first on the biphenyl piperazines and switched to make napthyl piperazines. None of the compounds in these two series of compounds exhibited efflux pump inhibiting activity. Due to this, no further work was done on the arylpiperazines series.

PAβN is a synthetic compound which was discovered in screening assay as having efflux pump inhibiting activity on *P. aeruginosa*. Studies on PAβN led to an ether or thioether linked analogues. From these analogues we proposed bis-alkylaryl analogue which we used as lead for our SAR. We worked on three main scaffolds, the "normal amides", the "reverse amides" and the secondary amines. Most of our SAR was done using E. coli as the bacteria and with clarithromycin as the test drug. The data is given in comparison with the MIC of clarithromycin in the absence of an inhibitors.

Although, there were some similarities in the SAR, there were some differences as well. In the normal amide series, we gleaned that 1,5-diphenylpentan-3-amine was the preferred amine for the right hand side of the molecule. We also observed a stereo chemical

difference in activity between the R and S-isomers of the 1,5-diphenylpentan-3-amine analogue. We also found out the N-methylamino alkylaryl derivatives of the normal amines were detrimental to the EPI activity of these normal amides. Monomethyl, dimethyl and guanidine N^5 substitutions were all inimical to EPI activity of these normal amides. A primary amine appears to be the best substituent at the N^5 position. The pentanamide derivatives appeared to be the best for activity as compared to the butanamide analogues. In the pentanamide series, the 3,5-diaminopentanamide is the best compound offering a 16-fold decrease in activity of clarithromycin. The data suggests that the mode of binding of these EPI agents is very specific. Their relative activity is unlikely to be due to nonspecific membrane binding.

The SAR of the normal amides did not disclose any more active compounds except for the 3,5-diaminopentanamide. Most of the compounds in this series displayed a similar activity to PAβN or even a reduced activity.

The "reverse amide" series which we subsequently explored also indicated that the 2-phenethyl-4-phenylbutanoic acid (which is analogous to 1,5-diphenylpentan-3-amine) is preferred for EPI activity. This analogue caused a 16-fold reduction in the MIC of clarithromycin. This series displayed no stereo chemical differences in activity. We also discovered that the phenyl substituents and the bis-alkylaryl were necessary for activity as the pyridyl and the mono alkylaryl showed no EPI activity. All the pentanamide derivatives displayed potent EPI activity with the 4,5-diamino compound inducing a 32-fold reduction in the MIC of clarithromycin. The effect of diamino alkyl chain length revealed that the propanamide analogue drastically reduced EPI activity. In this series however, the butanamide analogue had a similar activity to the analogous pentanamide

derivative. The N-methyl amino derivative reduce the activity of its analogous reverse amide by 2-fold. Although, an N^2 monomethyl was partially tolerated, an N^2 dimethyl substitution led to a drop in EPI activity.

N^5 substitution in this series, as had been observed for the normal amide series produced a loss of activity. This effect was observed for a monomethyl, dimethyl, guanidine and urea derivatives. The use of cyclic amines was unfavorable to our series although they had been used in other EPIs to increase potency and reduce toxicity. We extended our efforts to amines that had not been synthesized from amino acids. At similar doses, these amines were active at one-half and one-quarter our lead compound. We extended some of compounds by one methylene chain, and found that one of those compounds, 3-phenethyl-5-phenylpentanoic acid derivative reduced the MIC of clarithromycin by 512-fold. However, this compound was toxic when dosed to mice iv.

The last scaffold can be divided into three main groups. These are the amines that mimic the normal amides, those whose amine components are not synthesized from amino acids and amines that mimic the reverse amides. In the amines the mimicked the normal amides, the pentane triamine analogues had better activity than the butane triamines. The 1,4,5-pentane triamine decrease the MIC of clarithromycin by 32-fold. We used a fluorescence-based cellular assay to evaluate if the effect of two of these amines were due to a non-specific type of binding with the bacterial membrane or the compounds activity on the pump. This assay proved that the effect observed was due to inhibition of the bacterial efflux pump. For the secondary amines that had been developed from amines not synthesized from amino acids, there was observed a lack of efflux pump inhibition activity. The amines that mimicked the "reverse amide" scaffold had the best activity of all the

amines synthesized. The best in class compound decreased the MIC of clarithromycin by 128-fold. The least potent in the group caused a 32-fold decrease in the activity of clarithromycin when compared to the activity of clarithromycin in the absence of an inhibitor.

The compounds were also tested in P. aeruginosa using levofloxacin as the test compound. Of all the compounds synthesized only eight had activity against this organism. Two of the pentane triamine analogues that mimicked the reverse amides, were able to reduce the MIC of levofloxacin by 32 fold in comparison with the data with no inhibitor.

Herein in this work is presented a systematic structure activity relationship study on novel EPI compounds. We designed and proposed novel compounds and developed these compounds to achieve potency in *E. coli*. We have also able to achieve a modest activity in P. aeruginosa with some of the compounds synthesized.

EXPERIMENTAL

General

All reactions, unless otherwise stated, were done under nitrogen atmosphere. Reaction monitoring and follow-up were done using aluminum backed Silica G TLC plates with UV254 (Sorbent Technologies), visualizing with ultraviolet light. Flash column chromatography was done on a Combi Flash Rf Teledyne ISCO using hexane, ethyl acetate, dichloromethane, and methanol. The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were done in CDCl$_3$, methanol-d$_4$, and DMSO-d$_6$ and recorded on a Bruker Avance III (400 MHz) Multinuclear NMR Spectrometer. Data is expressed in parts per million relative to the residual nondeuterated solvent signals, spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), dt (doublet of triplets), q (quartet), m (multiplet), and brs (broad singlet), and coupling constants (J) are reported in Hertz. Melting points were determined using Mel-temp II apparatus and are uncorrected. Infrared spectra was obtained using a Thermo-Nicolet Avatar 360 Fourier transform spectrophotometer and reported in cm-1. HRMS experiments were conducted by Washington University Resource for Biomedical and Bioorganic Mass Spectrometry Department of Chemistry.

4-Methyl-1,1'-biphenyl (1).

Bromobenzene (500 mg, 3.2 mmol), 4- methylphenylboronic acid (519.7 mg, 3.8 mmol), palladium acetate (72 mg, 0.32 mmol), XPhos (304 mg, 0.64 mmol) and potassium carbonate (1500 mg, 11.2 mmol) were dissolved in a mixture of acetonitrile/ water (2:1) (15 mL) and was degassed. The mixture was heated under nitrogen for 2 hours. The resulting mixture was washed with saturated NH_4Cl solution and extracted with dichloromethane. The organic layer was dried over sodium sulfate and solvent evaporated. The residue was purified on flash column using silica gel hexane. Product was obtained as a pale yellow oil (555 mg, 100%) ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.75 (m, 2H), 7.66 (m, 2H), 7.58 (m, 2H), 7.48 (m, 1H), 7.40 (m, 2H), 2.56 (s, 3H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 141.3, 138.5, 137.1, 129.6, 129.5, 128.8, 127.13, 127.1, 21.2

4-(Bromomethyl)-1,1'-biphenyl (2).

4-Methyl-1,1'-biphenyl, **1**, (483 mg, 2.9 mmol) was dissolved in carbon tetrachloride (5 mL). N-Bromosuccinimide (537 mg, 3 mmol) and AIBN (48mg, 0.29 mmol) was added and reaction was stirred at reflux (85 °C) for 2 hours. The mixture was allowed to cool and hexane added. The solid precipitate was filtered off and the filtrate concentrated. Purification was done by flash chromatography (silica gel with hexane as eluent). Product was obtained as a white solid. (289 mg, 40%); MP 70-72 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.67 (m, 4H), 7.54 (m, 4H), 7.46 (m, 1H), 4.62 (s, 2H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 141.4, 140.5, 136.8, 129.6, 128.9, 127.67, 127.60, 127.1, 33.4

1-([1,1'-Biphenyl]-4-ylmethyl)-4-methylpiperazine (3).

4-(Bromomethyl)-1,1ʼ–biphenyl, **2**, (88 mg, 0.36 mmol) was dissolved in DMF (2 mL) and potassium carbonate (99 mg, 0.72 mmol) and 1-methylpiperazine (43.2 mg, 0.43 mmol) added. The mixture was stirred at room temperature for overnight. The mixture was then dissolved in saturated sodium bicarbonate solution and extracted with ethyl acetate. The organic layer was dried over sodium sulfate and concentrated. Residue was purified by flash chromatography using silica gel and 0-10% methanol/dichloromethane to give product as a colorless oil. (58.7 mg, 51%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.60 (m, 4H), 7.43 (m, 4H), 7.36 (m, 1H), 3.56 (s, 2H), 2.54 (brs, 4H), 2.51 (brs, 4H), 2.32 (s, 3H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 141.0, 140.0, 137.3, 129.5, 128.7, 127.1, 127.0, 126.9, 62.7, 55.2, 53.1, 46.0; HRMS (ESI) Calculated for C$_{18}$H$_{22}$N$_2$ (M+H)$^+$ 267.1856, found 267.1855

1-([1,1'-Biphenyl]-4-ylmethyl)piperazine (4).

4-(Bromomethyl)-1,1ʼ–biphenyl, **2**, (84 mg, 0.34 mmol) was dissolved in DMF (1 mL) and potassium carbonate (94 mg, 0.68 mmol) and piperazine (35 mg, 0.41 mmol) added. The mixture was stirred at room temperature for overnight. The mixture was then dissolved in saturated sodium bicarbonate solution and extracted with ethyl acetate. The organic layer was dried over sodium sulfate and concentrated. Residue was purified by flash chromatography using silica gel and 0-10% methanol/dichloromethane with 1% NH$_4$OH to give an off white solid (24.6 mg, 29%); MP 89-90 ºC; ^{1}H NMR (400 MHz) (CDCl$_3$) δ7.60 (m, 4H), 7.41 (m, 5H), 3.56 (s, 2H), 2.93 (t, 4H, *J=8)*, 2.48 (brs, 4H), 1.73 (brs, 1H);

^{13}C NMR (100 MHz) (CDCl$_3$) δ 141.0, 139.9, 137.2, 129.6, 128.7, 127.1, 127.0, 126.9, 63.3, 54.5, 46.1; HRMS (ESI) Calculated for C$_{17}$H$_{20}$N$_2$ (M+H)$^+$ 253.1699, found 253.1702

1,4-Bis([1,1'-biphenyl]-4-ylmethyl)piperazine (5).

This was formed as a side product of the reaction to form 1-([1,1'-biphenyl]-4-ylmethyl)piperazine, **4**. Product eluted on silica gel with 60% ethyl acetate/hexane. 1,4-bis([1,1'-biphenyl]-4-ylmethyl)piperazine, **5**, was obtained as white solid (31.2 mg, 22%); MP 171-173 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.61 (m, 8H), 7.43 (m, 10H), 3.61 (s, 4H), 2.58 (brs, 8H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 141.0, 139.9, 137.3, 129.6, 128.74, 128.71, 127.1, 127.0, 126.9, 62.7, 53.1; HRMS (ESI) Calculated for C$_{30}$H$_{30}$N$_2$ (M+H)$^+$ 419.2482, found 419.2475

1-(Bromomethyl)naphthalene (6).

1-Methyl naphthalene (1000 mg, 7 mmol) was dissolved in carbon tetrachloride (10 mL). N-bromosuccinimide (1.3 g, 7.4 mmol) and AIBN (114 mg, 0.7 mmol) was added and reaction was stirred at reflux (85 °C) for 2 hours. The mixture was allowed to cool and hexane added. The solid precipitate was filtered off and the filtrate concentrated. Purification was done by flash chromatography (silica gel with hexane as eluent). Product was obtained as a colorless oil (605 mg, 40%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 8.28 (d, 1H, *J=8)*, 7.99 (d, 1H, *J=8)*, 7.93 (d, 1H, *J=8), *7.73 (t, 1H, *J* =8), 7.63 (m, 2H), 7.49 (t,

1H, $J = 8$), 5.03 (s, 2H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 134.1, 133.4, 131.2, 129.9, 129.0, 127.9, 126.7, 126.2, 125.5, 123.8, 31.9

1-Methyl-4-(naphthalen-1-ylmethyl)piperazine (7)

1-(Bromomethyl)naphthalene, **6**, (100 mg, 0.45 mmol) was dissolved in DMF (2 mL) and potassium carbonate (124 mg, 0.9 mmol) and 1-methylpiperazine (54 mg, 0.54 mmol) added. The mixture was stirred at room temperature for overnight. The mixture was then dissolved in saturated sodium bicarbonate solution and extracted with ethyl acetated. The organic layer was dried over sodium sulfate and concentrated. Residue was purified by flash chromatography using silica gel and 0-10% methanol/dichloromethane to give product as a yellow colored oil. (60.4 mg, 56%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 8.34 (m, 1H), 7.87 (t, 1H, $J = 8$), 7.79 7.87 (t, 1H, $J = 8$), 7.48 (m, 4H), 3.94 (s, 2H), 2.59 (brs, 4H), 2.47 (brs, 4H), 2.31 (s, 3H); ^{13}C NMR (400 MHz) (CDCl$_3$) δ 134.1, 133.8, 132.6, 128.3, 127.8, 127.3, 125.6, 125.5, 125.1, 124.8, 61.1, 55.2, 53.3, 46.0; HRMS (ESI) Calculated for C$_{16}$H$_{20}$N$_2$ (M+H)$^+$ 241.1699, found 241.1691

tert-Butyl 4-(naphthalen-1-ylmethyl)piperazine-1-carboxylate (8).

1-(Bromomethyl) naphthalene, **6**, (100mg, 0.45 mmol) was dissolved in DMF (2 mL) and potassium carbonate (125 mg, 0.90 mmol) and 1-bocpiperazine (100 mg, 0.54 mmol) added. The mixture was stirred at room temperature for overnight. The mixture was then dissolved in saturated sodium bicarbonate solution and extracted with ethyl acetate. The

organic layer was dried over sodium sulfate and concentrated. Residue was purified by flash chromatography using silica gel and 0-5% ethyl acetate/hexane to give product which was a colorless oil. (117 mg , 79%); ^{1}H (400 MHz) (CDCl$_3$) δ 8.33 (m, 1H), 7.88 (m, 1H), 7.82 (m, 1H), 7.54 (m, 2H),7.44 (m, 2H), 3.94 (s, 2H), 3.46 (brs, 4H), 2.49 (brs, 4H), 1.50 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 154.8, 133.9, 133.7, 132.5, 128.4, 128.1, 127.4, 125.7, 125.6, 125.1, 124.7, 79.5, 61.2, 53.1, 43.8, 28.4; HRMS (ESI) Calculated for C$_{20}$H$_{26}$N$_2$O$_2$ (M+H)$^{+}$ 327.2067, found 327.2064

1-(Naphthalen-1-ylmethyl)piperazine (9)

Tert-butyl 4-(naphthalen-1-ylmethyl)piperazine-1-carboxylate, **8**, (83 mg, 0.26 mmol) was dissolved in dichloromethane (1 mL) at 0 °C under nitrogen. Trifluoroacetic acid (1mL) was added and reaction stirred at that temperature for 2hours. The completed reaction was washed with saturated NaHCO$_3$ solution and extracted with dichloromethane. The organic layer was dried over sodium sulfate and purified by flash chromatography with silica gel (0-10% MeOH/ dichloromethane with 1% NH$_4$OH) to give product as a white solid. (43.4 mg, 75%); MP 54-56 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 8.34 (d, 1H, J = 8), 7.87 7.87 (d, 1H, J =8), 7.80 7.87 (d, 1H, J =8), 7.54 (m, 2H), 7.45 (m, 2H), 3.91 (s, 2H), 2.89 (t, 4H, J=4), 2.51 (brs, 4H), 1.73 (brs, 1H); ^{13}C (100 MHz) (CDCl$_3$) δ134.0,133.8, 132.6, 128.3, 127.8, 127.3, 125.6, 125.5, 125.0, 124.8, 61.7, 54.8, 46.2; HRMS (ESI) Calculated for C$_{15}$H$_{18}$N$_2$ (M+H)$^{+}$ 227.1543, found 227.1541

1-Allylnaphthalene (10)

A dry round bottom flash was charged with a stir bar and Mg (288 mg, 12 mmol) under nitrogen. 1-Bromonaphthalene (2 g, 10 mmol) and an iodide grain were dissolved in diethyl ether (7 mL, 1.6M) in a dry conical flask. Part of the solution was added to the Mg flash and stirred until a color change occurred. The rest of the solution was added and the reaction stirred under reflux for 2 hours. The reaction solution was decanted into another dry flask and allyl bromide (1.3 mL, 15 mmol) was carefully added in an ice bath. Reaction mixture was allowed to reach room temperature and carefully hydrolyzed with saturated NH_4Cl solution. The aqueous phase was removed and extracted with Et_2O. The combined organic phases were dried with sodium sulfate and concentrated under reduced pressure. The residue was purified with flash chromatography (silica gel with hexane as eluent) to obtain a colorless oil. (1 g, 62%); ^1H NMR (400 MHz) (CDCl$_3$) δ 7.95 (d, 1H, *J=8)*; 7.76 (dd, 1H, *J=4,8), 7.65 (d, 1H, J=8),* 7.34 (m, 4H), 6.03 (m, 1H), 5.01 (m, 2H), 3.75 (d, 2H, *J=8*); ^{13}C NMR (100 MHz) (CDCl$_3$) δ137.8, 136.1, 133.9, 132.0. 128.7, 127.0, 126.3, 125.8, 125.6, 125.5, 124.0, 116.1, 37.3

3-(Naphthalen-1-yl)propan-1-ol (11)

To a solution of 1-allylnaphthalene, **10**, (478 mg, 2.84 mmol) in THF cooled to 0 °C under nitrogen was added BH$_3$.THF solution (4.2 mL, 3.17 mmol). The mixture was stirred at that temperature for 30 minutes, warmed to room temperature and stirred at that temperature for an additional 2 hours. After the 2 hours, the reaction mixture was cooled to 0 °C and 3M NaOH (3.6 mL) and 30% H$_2$O$_2$ (1.2mL) were added. The reaction mixture

was stirred at 0 °C for 30 minutes and heated at 60 °C for an additional 1 hour. The solvent was evaporated under reduced pressure and the resulting residue diluted with water and extracted with ethyl acetate. The combined organic layers were washed with water, brine and dried over sodium sulfate and concentrated. Purification was done using flash chromatography (silica gel with 0-30% ethyl acetate/ hexane) to give product as a colorless oil. (237.5 mg, 45%); ^{1}H (400 MHz) (CDCl$_3$) δ 8.12 (dd, 1H, *J=4,8)* , 7.90 (dd, 1H, *J=4,8)*, 7.78 (d, 1H, *J = 8),* 7.48 (m, 4H), 3.77 (t, 2H, *J=8),* 3.22 (t, 2H, *J=8),* 2.07 (m, 2H); ^{13}C (100 MHz) (CDCl$_3$) δ 138.0, 133.9, 131.9, 128.8, 126.7, 126.0, 125.8, 125.6, 125.5, 123.8, 62.4, 33.5, 29.2

3-(Naphthalen-1-yl)propyl methane sulfonate (12)

To a solution of 3-(naphthalen-1-yl)propan-1-ol, **11**, (200 mg, 1.0 mmol) in dichloromethane (5 mL) under nitrogen was added triethylamine (0.3 mL, 2.15 mmol) then methane sulfonyl chloride (185 mg, 1.61 mmol). Reaction was stirred at room temperature overnight. Reaction mixture was washed with saturated sodium bicarbonate solution and purified on flash chromatography (silica gel with 0-20% ethyl acetate/ hexane) to give a colorless oil. (253.1 mg, 89%); ^{1}H (400 MHz) (CDCl$_3$) δ 8.06 (dd, 1H, *J=4,8)*, 7.92 (dd, 1H, *J=4,8)* , 7.80 (dd, 1H, *J=4,8),* 7.47 (m, 4H), 4.31 (t, 2H, *J=8),* 3.25 (t, 2H, *J=8),* ,3.02 (s, 3H), 2.24 (m, 2H); ^{13}C (100 MHz) (CDCl$_3$) δ 136.4, 134.0, 131.7, 128.9, 127.2, 126.3, 126.1, 125.6, 125.5, 123.4, 69.4, 37.3,29.9, 28.7

4-(Naphthalen-1-yl)butanenitrile (13)

 To a solution of 3-(naphthalen-1-yl)propyl methane sulfonate, **12**, (190 mg, 0.72 mmol) in DMF (5mL) was added KCN (108 mg, 1.66 mmol) and stirred at room temperature overnight. The reaction mixture was then washed with 10% LiCl to remove DMF and purified on an ISCO chromatograph (silica gel 0-20% ethyl acetate/hexane) to give product as a colorless oil. (108 mg, 77%); ^{1}H (400 MHz) (CDCl$_3$) δ 8.02 (dd, 1H, *J=4,8)* , 7.91 (dd, 1H, *J=4,8),* 7.79 (dd, 1H, *J=4,8),* 7.48 (m, 4H), 3.25 (t, 2H, *J=8),* 2.36 (t, 2H, *J=8),*2.12 (m, 2H); ^{13}C (100 MHz) (CDCl$_3$) δ 135.8, 134.0, 131.6, 129.0, 127.4, 126.4, 126.2, 125.7, 125.5, 123.3, 119.6, 31.5, 26.2, 16.7; HRMS (ESI) Calculated for C$_{14}$H$_{13}$N (M+Na)$^+$ 218.0940, found 218.0928

4-(Naphthalen-1-yl)butan-1-amine (14)

To a flask charged with LAH (38 mg, 1.0 mmol) in dry ether (2 mL) was added 4-(naphthalen-1-yl)butanenitrile, **13**, (80 mg, 0.41 mmol) in dry ether (3 mL) dropwise. The reaction was stirred for 30 minutes at room temperature then placed on an ice bath. Water (5 μL), 1M NaOH (5 μL) and water (15 μL) and resulting mixture filtered on celite and washed with 10% MeOH/ dichloromethane. The filtrate was dried over sodium sulfate and organic layer concentrated. Purification was done using an ISCO chromatograph (silica gel – 0-10% MeOH/ dichloromethane with 1% NH$_4$OH) to obtain a white solid. (41 mg, 50%); MP 96-98 °C; ^{1}H (400 MHz) (CDCl$_3$) δ 7.96 (dd, 1H, *J=4,8)* , 7.78 (dd, 1H, *J=4,8),* 7.63 (dd, 1H, *J=4,8),* 7.34 (m, 4H), 3.02 (t, 2H, *J=8),* 2.67 (t, 2H, *J=8),* 1.72 (m, 2H), 1.50 (m, 2H); ^{13}C (100 MHz) (CDCl$_3$) δ 138.5, 133.9, 131.8, 128.7, 126.5, 125.9, 125.6, 125.5,

125.3, 123.7, 42.1, 33.8, 32.8, 28.0; HRMS (ESI) Calculated for $C_{14}H_{17}N$ $(M+H)^+$ 200.1434, found 200.1432

Bis-Boc-protected 2-(4-(naphthalen-1-yl)butyl)guanidine (15)

4-(Naphthalen-1-yl)butan-1-amine, **14**, (30 mg, 0.15mmol) was dissolved in dichloromethane (3 mL) and trimethylamine (30 μL) was added. 1,3-diboc-2-(Trifluoromethyl sulfonyl) guanidine (71 mg, 0.18 mmol) and stirred at room temperature overnight. The reaction mixture was washed with $NaHCO_3$ and extracted with dichloromethane. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified on an ISCO chromatograph (0-20% ethyl acetate/hexane) to give a colorless oil (46.5 mg, 70%); ^1H (400 MHz) (CDCl$_3$) δ 11.59 (s, 1H), 8.37 (brs, 1H), 8.05 (dd, 1H, *J=4,8)* , 7.88 (dd, 1H, *J=4,8), 7.74 (dd, 1H, J=4,8),* 7.34 (m, 4H), 3.53 (m, 2H), 3.14 (m, 2H, 1.87 (m, 2H), 1.74 (m, 2H), 1.32 (s, 18H); ^{13}C (100 MHz) (CDCl$_3$) δ 163.6, 156.1, 153.3, 138.1, 133.9, 131.8, 128.2, 126.6, 125.9, 125.7, 125.5, 125.4, 123.7, 40.6, 32.5, 31.5, 29.1, 28.3, 28.0, 27.8; HRMS (ESI) Calculated for $C_{25}H_{35}N_3O_4$ $(M+H)^+$ 442.2700, found 442.2701

2-(4-(Naphthalen-1-yl)butyl)guanidine (16)

The boc–protected guanidine, **15**, (37 mg, 0.08 mmol) was dissolved in dichloromethane (1 mL) and cooled to 0 °C under nitrogen. Trifluoroacetic acid (1 mL) was added and the reaction was stirred at that temperature for 2 hours. The solution was dissolved in saturated

sodium bicarbonate solution and extracted with dichloromethane. The organic layer was dried over sodium sulfate and concentrated. The concentrate was purified on an ISCO chromatograph (0-10% MeOH/ dichloromethane with 1% NH_4OH) to give product as a colorless oil. (17.2 mg, 86%); ^1H (400 MHz) ($CDCl_3$) δ 7.94 (dd, 1H, *J=4,8)* , 7.79 (dd, 1H, *J=4,8),* 7.65 (dd, 1H, *J=4,8),* 7.36 (m, 4H), 3.00 (m, 2H), 2.89 (m, 2H), 1.68 (m, 2H), 1.59 (m, 2H); ^{13}C (100 MHz) ($CDCl_3$) δ 157.1, 137.6, 133.8, 131.6, 128.7, 126.7, 125.9. 125.8, 125.6, 123.6, 41.2, 32.2, 28.3, 27.4; HRMS (ESI) Calculated for $C_{15}H_{19}N_3$ $(M+H)^+$ 242.1652, found 242.1651

Di-*tert*-butyl (5-(benzhydrylamino)-5-oxopentane-1,4-diyl)(S)-dicarbamate (25)

Bis- Boc–L- ornithine (906 mg, 2.73 mmol) was dissolved in DMF (5mL), EDC (1040 mg, 5.46 mmol) and HOBt (736 mg, 5.46 mmol) was added and stirred until a clear solution was formed. Diphenyl methamine (500 mg, 2.73 mmol) was added and reaction was stirred overnight at room temperature. The DMF was removed on the kugelrohr and the residue purified on an ISCO chromatograph (0-50% Ethyl acetate/Hexane) to give a white product (874 mg , 64%); MP- 98-99 °C; ^1H NMR (400 MHz) (MeOD) δ 8.62 (d, 1H, *J = 7.84*) , 7.30 (m, 10 H), 6.74 (brs, 1H), 6.56 (brs, 1H), 6.18 (d, 1H, *J = 7.76*) , 4.17 (m, 1H), 3.05 (m, 2H), 1.78 (m, 4H) 1.61 (m, 18H); 13 C NMR (100 MHz) (MeOD) δ 174.4, 158.5, 157.8, 143.0, 142.8, 129.8, 129.5, 128.7, 128.5, 128.4, 128.3, 80.6, 79.9, 58.3, 55.9, 40.8, 30.6, 28.89, 28.81, 27.5

(S)-2,5-Diamino-N-benzhydrylpentanamide (26).

Di-*tert*-butyl (5-(benzhydrylamino)-5-oxopentane-1,4-diyl)dicarbamate, **25**, (200 mg, 0.40 mmol) was dissolved in dichloromethane (3 mL) and the reaction mixture cooled to 0 °C under nitrogen. Trifluoroacetic acid (2 mL) was added and the reaction stirred at that temperature for 3 hours. On completion of the reaction , the solvents were removed and residue purified on an ISCO chromatograph (0–10% methanol/ dichloromethane with 0.1% NH$_4$OH) to give product as a colorless oil (118 mg, 100%); ^{1}H NMR (CD$_3$OD) (400 MHz) δ 7.33 (m, 11H), 6.21 (d, 1H, *J= 8)*, 4.07 (m, 1H), 2.96 (m, 2H), 1.97 (m, 2H), 1.78 (m, 2H); ^{13}C NMR (CD$_3$OD) (100 MHz) δ 169.1, 142,5, 142.4, 129.82, 129.80, 129.66, 129.63, 128.7, 128.65, 128.61, 58.7, 58.9, 40,0, 29.8, 24.1; HRMS (ESI) Calculated for C$_{18}$H$_{23}$N$_3$O (M+H)$^+$ 298.1914, found 298.1913

1,3-Diphenylpropan-2-one oxime (27).

1,3-Diphenylacetone (1 g, 4.76 mmol), hydroxylamine hydrochloride (826 mg, 11.89 mmol) was dissolved in a mixture of pyridine (12 mL) and ethanol (20 mL). The reaction mixture was refluxed at 100 °C for 2hours and then cooled to room temperature. The reaction mixture was acidified with 10% HCl solution and extracted with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate and solvent was evaporated in vacuo and purified on an ISCO chromatograph using 20% ethyl acetate/hexane to give product as a white flaky solid. (833.7 mg, 78%); MP = 113-115 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.30 (m, 10H), 3.74 (s, 2H), 3.52 (s, 2H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ159.0, 136.5, 136.4, 129.3, 129.2, 128.6, 126.8, 126.5, 39.6, 32.7;

1,3-Diphenylpropan-2-amine (28).

To a solution of lithium aluminium hydride (538.0 mg, 14.18 mmol) in THF (10 mL) was added 1,3-diphenylpropan-2-one oxime, **27**, (1000 mg, 4.73 mmol) in THF (10 mL) dropwise. The mixture was heated at reflux for 3 hours under nitrogen. The mixture was quenched with 0.5 mL of water, 0.5 mL of 3N NaOH, and 1.5 mL of water. The precipitate was filtered out and the filtrate dried over sodium sulfate, concentrated in vacuo and purified on an ISCO chromatograph using 0-10% methanol/ dichloromethane with 1% ammonium hydroxide to give 1,3-diphenylpropan-2-amine as a yellow solid. (289.8 mg, 29%); MP 44-45 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.31 (m, 10H), 3.34 (m, 1H), 2.90 (dd, 2H, $J = 8, 16$), 2.61 (dd, 2H, $J= 8, 16$), 1.30 (brs, 2H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 139.5, 129.3, 128.5, 126.3, 54.2, 44.3.

Di-*tert*-butyl (5-((1,3-diphenylpropan-2-yl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate (29).

N$^{\alpha,\delta}$-Bis-boc-L-ornithine (143 mg, 0.43 mmol) was dissolved in dichloromethane (5 mL) and PyBOP (223 mg, 0.43 mmol) was added and stirred for 5 minutes. 1,3-Diphenylpropan-2-amine, **28**, (100 mg, 0.47 mmol) was added followed by triethylamine (0.12 mL, 0.86 mmol) and reaction was stirred at room temperature overnight to give di-*tert*-butyl (5-((1,3-diphenylpropan-2-yl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate, **29**, as a colorless oil; (130.3 mg, 58%); ^{1}H (CDCl$_3$) (400 MHz) δ 7.06(m, 11H), 5.87 (d, 1H, $J = 8$), 4.81 (brs, 1H), 4.29 (m, 1H), 3.80 (m, 1H), 2.71 (m, 6H), 1.38 (m, 4H), 1.27 (s, 9H), 1.25 (s, 9H), 0.99 (m, 2H); ^{13}C (100 MHz) (CDCl$_3$) δ 171,1, 156.1, 155.5, 138.0, 137.3, 129.3, 129.2, 128.4, 126.5, 79.9, 79.1, 53.8, 51.4, 40.4, 40.1, 29.8, 28.4, 28.3, 25.8;

(S)-2,5-Diamino-N-(1,3-diphenylpropan-2-yl)pentanamide (30).

Di-*tert*-butyl (5-((1,3-diphenylpropan-2-yl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate, **29**, (100 mg, 0.19 mmol) was dissolved in dichloromethane (3 mL) and cooled to 0 ºC. Trifluoroacetic acid (1.5 mL) was added and reaction stirred under nitrogen at same temperature for 2 hours. The reaction mixture was concentrated under reduced pressure to remove solvent and the residue purified on an ISCO chromatograph with 0-10% methanol/dichloromethane with 1% NH_4OH to give (S)-2,5-diamino-N-(1,3-diphenylpropan-2-yl)pentanamide as a colorless oil (100 mg, 100%); ^{1}H NMR (400 MHz) ($CDOD_3$) δ 7.22 (m, 11H), 4.41 (m, 1H), 3.48 (m, 1H), 2.76 (m, 6H), 1.49 (m, 4H); ^{13}C NMR (100 MHz) ($CDOD_3$) δ 172.5, 140.0, 139.7, 130.4, 130.3, 129.5, 129.4, 127.5, 127.4, 55.4, 53.8, 49.9, 41.9, 42.2, 40.1, 31.3, 24.2; HRMS (ESI) calculated for $C_{20}H_{27}N_3O$ (M+H)[+] 326.2227, found 326.2251

1,5-Diphenylpenta-1,4-dien-3-one (31).

To a solution of sodium hydroxide (1.9 g, 47 mmol) in water/ethanol (1:1) (40 mL) was added a solution of benzaldehyde (1.9 mL, 18.8 mmol) in acetone (0.8 mL, 9.4 mmol) at room temperature. The reaction was allowed to go for 1 hour and the solution then became a yellow suspension. The suspension was filtered and the residue washed with water to give the 1,5-diphenylpenta-1,4-dien-3-one as a pale yellow solid. (2.3 g, 100%); MP 95-98 ºC; ^{1}H NMR ($CDCl_3$) (400 MHz) δ 7.79 (s, 1H), 7.75 (s, 1H), 7.65 (m, 4H), 7.45 (m, 6H), 7.14 (s, 1H), 7.10 (s, 1H); [13]C ($CDCl_3$) (100 MHZ) δ 188.9, 143.3, 134.8, 130.4, 128.9, 128.3, 125.4

1,5-Diphenylpentan-3-one (32).

To a solution of 1,5-diphenylpenta-1,4-dien-3-one, **31**, (1 g, 4.27 mmol) in ethyl acetate was added palladium/carbon (10%) (120 mg) and stirred at room temperature overnight. The catalyst was filtered off and concentrated. The filtrate was purified on an ISCO chromatograph (0-10% ethyl acetate/hexane) to give 1,5-diphenylpentan-3-one, **32**, as a colorless oil. (800 mg, 79%); ^{1}H NMR (CDCl$_3$) (400 MHZ) δ 7.39 (m, 4H), 7.28 (m, 6H), 3.00 (t, 4H, *J = 8),* 2.79 (t, 4H, *J = 8);* ^{13}C NMR (CDCl$_3$) (100 MHZ) δ 209.0, 141.1, 128.6, 128.4, 126.2, 44.5, 29.8

1,5-Diphenylpentan-3-one oxime (33).

1,5-Diphenylpentan-3-one, **32**, (919 mg, 3.86 mmol), hydroxylamine hydrochloride (669 mg , 9.64 mmol), pyridine (10 mL) and ethanol (16 mL) were refluxed for 2 hours and cooled to room temperature. The reaction mixture was then acidified with 2N HCl and extracted with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate and solvent was evaporated under reduced pressure and the residue purified on an ISCO chromatograph using 0 - 20% ethyl acetate/hexane to give product as a white flaky solid. (850 mg, 87%); MP 82-84 °C; ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.37 (m, 10H), 3.99 (m, 4H), 2.84 (dd, 2H, *J* = 7.92, 10.28), 2.59 (dd, 2H, *J* = 7.92, 10.28); ^{13}C NMR (CDCl$_3$) (100 MHz) δ 160.6, 142.4, 142.3, 128.62, 128.60, 128.56, 128.48, 128.45, 126.3, 126.2, 36.86, 32.7, 31.7, 30.4

1,5-Diphenylpentan-3-amine (34).

To a solution of lithium aluminium hydride (224 mg, 5.92 mmol) in THF (5 mL) was added 1,5-diphenylpentan-3-one oxime, **33**, (500 mg, 1.97 mmol) in THF (10 mL) dropwise. The mixture was heated at reflux for 3 hours under nitrogen. The mixture was quenched with 0.23 mL of water, 0.23 mL of 3N NaOH, and 0.7 mL of water. The precipitate was filtered out and the filtrate dried over sodium sulfate, concentrated under reduced pressure and the residue purified on an ISCO chromatograph using 0-10% methanol/ dichloromethane with 1% ammonium hydroxide to give product as a colorless oil. (145 mg, 31%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.32 (m, 4H), 7.22 (m, 6H), 2.79 (m, 3H), 2.67 (m, 2H), 1.84 (m, 2H), 1.67 (m, 2H), 1.47 (s, 2H); ^{13}C NMR (CDCl$_3$) (100 MHz) δ 142.3, 128.4, 128.2, 125.8, 50.5, 39.9, 32.5

Di-*tert*-butyl (5-((1,5-diphenylpentan-3-yl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate (35)*.

N^{α,δ}-Bis-boc-L-ornithine (143 mg, 0.43 mmol) was dissolved in dichloromethane (5 mL) and Bop (190 mg, 0.43 mmol) was added and stirred for 5 minutes. 1,5-Diphenylpentan-3-amine, **34**, (114 mg, 0.48 mmol) was added followed by triethylamine (0.12 mL, 0.86 mmol) and reaction was stirred at room temperature overnight. The solvent was evaporated and residue redissolved in ethyl acetate. The organic layer was washed with 1 M HCl, 5% NaHCO$_3$ brine, dried over sodium sulfate and concentrated. This was purified on ISCO with 10% MeOH/ dichloromethane to give product as a white powder. (120 mg, 45%); MP 158-160 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.21 (m, 10H), 6.38 (s, 1H), 5.25 (brs, 1H),

4.79 (brs, 1H), 4.11 (m, 2H), 3.30 (s, 1H), 3.14 (m, 1H), 2.64 (m, 4H), 1.75 (m, 8H), 1.46 (s, 9H), 1.45 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 171.8, 156.4, 155.8, 141.8, 141.7, 128.5, 128.4, 128.3, 128.3, 125.8, 79.9, 79.2, 49.1, 39.5, 37.2, 37.0, 36.9, 32.3, 32.3, 29.9, 28.4, 28.3, 26.5

(S)-2,5-Diamino-N-(1,5-diphenylpentan-3-yl)pentanamide (36)

Di-*tert*-butyl   (5-((1,5-diphenylpentan-3-yl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate, **35**, (100 mg, 0.18 mmol) was dissolved in dichloromethane (3 mL) and the reaction mixture cooled to 0 °C under nitrogen. Trifluoroacetic acid (2 mL) was added and the reaction stirred at that temperature for 3 hours. On completion of the reaction, the solvents were removed and residue purified on an ISCO chromatograph (0–10% methanol/dichloromethane with 0.1% NH$_4$OH) to give product as a colorless oil. (63.7 mg, 100%); ^{1}H NMR (CD$_3$OD) (400 MHz) δ 7.07 (m, 11H), 3.82 (m, 2H), 2.89 (t, 2H, *J = 8)*, 2.54 (m, 4H), 1.72 (m, 8H); ^{13}C NMR (CD$_3$OD) (100 MHz) δ 169.6, 143.1, 142.9, 129.5, 129.4, 126.9, 126.8, 54.0, 50.8, 40.0, 37.8, 37.7, 33.5, 33.3, 30.0, 24.3; HRMS (ESI) Calculated for C$_{22}$H$_{31}$N$_3$O (M+Na)$^+$ 376.2359, found 376.2379

Di-*tert*-butyl (5-((1,5-diphenylpentan-3-yl)amino)-5-oxopentane-1,4-diyl)(R)-dicarbamate (37).

Bis-boc-D-ornithine (138 mg, 0.42 mmol) was dissolved in dichloromethane (5 mL) and PyBop (218 mg, 0.42 mmol) was added and stirred for 5 minutes. 1,5-Diphenylpentan-3-

amine, **34**, (100 mg, 0.42 mmol) was added followed by triethylamine (0.12 mL, 0.84 mmol) and reaction was stirred at room temperature overnight. The solvent was evaporated and residue redissolved in ethyl acetate. The organic layer was washed with 5% HCl, sat. NaHCO$_3$ brine, dried over sodium sulfate and concentrated. This was purified on an ISCO chromatograph with 10% methanol/ dichloromethane to give product as a white powder. (210 mg, 90%); MP 158-160°C; ^{1}H NMR (400 MHz) (CDCl$_3$ + MeOD) δ 7.35 (m, 10H), 6.38 (brs, 1H), 4.79 (brs, 1H), 4.20 (m, 1H), 4.08 (m, 1H), 2.77 (m, 5H), 1.75 (m, 8H), 1.60 (s, 9H), 1.57 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$ + MeOD) δ 177.8, 161.0, 145.7, 145.6, 132.4, 132.2, 132.1, 129.5, 83.6, 82.9, 43.6, 40.8, 36.1, 36.0, 33.5, 31.9, 31.8, 30.1, 30.0

(R)-2,5-Diamino-N-(1,5-diphenylpentan-3-yl)pentanamide (38).

Di-*tert*-butyl   (5-((1,5-diphenylpentan-3-yl)amino)-5-oxopentane-1,4-diyl)(R)-dicarbamate, **37**, (115 mg, 0.20 mmol) was dissolved in dichloromethane (3 mL) and the reaction mixture cooled to 0 $^{\circ}$C under nitrogen. Trifluoroacetic acid (2 mL) was added and the reaction stirred at that temperature for 3 hours. On completion of the reaction, the solvents were removed and residue purified on an ISCO chromatograph (0–10% methanol/dichloromethane with 0.1% NH$_4$OH) to give product as a colorless oil. (73 mg, 100%); ^{1}H NMR (CD$_3$OD) (400 MHz) δ 7.22 (m, 11H), 3.83 (m, 2H), 2.89 (t, 2H, *J = 8),* 2.52 (m, 4H), 1.88 (m, 8H); ^{13}C δ 169.6, 143.1, 142.9, 129.5, 129.45, 129.41, 126.98, 126.90, 54.0, 50.8, 40.0, 37.8, 37.7, 33.5, 33.3, 30.0, 24.3; HRMS (ESI) Calculated for C$_{22}$H$_{31}$N$_3$O (M+Na)$^+$ 376.2359, found 376.2380

Di-*tert*-butyl   (5-(benzhydryl(methyl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate (39).

Bis-boc-L-ornithine (305 mg, 0.92 mmol) was dissolved in dichloromethane (5 mL) and PyBrop (430 mg, 0.92 mmol) was added and stirred for 5 minutes.   N-methyl(diphenyl)methylamine (200 mg, 1.01 mmol) was added followed by Triethylamine (0.26 mL, 1.84 mmol) and reaction was stirred at room temperature overnight to give product as a colorless oil; (349.6 mg, 68%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.33(m, 6H), 7.17 (m, 4H), 7.10 (s,1H), 5.47 (d, 1H, $J = 8$), 4.73 (brs, 1H), 4.60 (m, 1H), 3.15 (m, 2H), 2.89 (s, 3H), 1.66 (m, 4H), 1.44 (s, 9H), 1.43 (s, 9H); ^{13}C NMR (CDCl$_3$) (100 MHz) δ 172.7. 155.9, 155.6, 138.8, 138.2, 129.2, 128.7, 128.6, 128.5, 128.4, 127.9, 127.6, 127.4, 79.6, 79.1, 60.8, 50.3, 40.1, 31.9, 30.8, 30.4, 28.4, 28.3, 25.8

(S)-2,5-Diamino-N-benzhydryl-N-methylpentanamide (40).

Di-*tert*-butyl (5-(benzhydryl(methyl)amino)-5-oxopentane-1,4-diyl)dicarbamate, **39**, (200 mg , 0.39 mmol) was dissolved in dichloromethane (3 mL) and cooled to 0 °C. Trifluoroacetic acid (2 mL) was added and reaction stirred under nitrogen at same temperature for 2 hours. The solvent was removed under reduced pressure and the residue purified on an ISCO chromatograph using 0-10% methanol/dichloromethane with 1% NH$_4$OH to give product as a colorless oil (100 mg, 83%); ^{1}H NMR (CD$_3$OD) (400 MHz) δ 7.40 (m, 6H), 7.21 (m, 4H), 7.03 (s, 1H), 4.60 (m, 1H), 3.01 (t, 2H, $J = 8$), 2.94 (s, 3H), 1.85 (m, 4H); ^{13}C NMR (CD$_3$OD) (100 MHz) δ 170.4, 139.8, 139.8, 139.7, 139.3,

130.1, 129.9, 129.7, 129.6, 129.4, 129.3, 129.0, 128.9, 63.0, 51.8, 40.0, 32.6, 28.5, 24.2, 23.9; HRMS (ESI) calculated for $C_{19}H_{26}N_3O$ $(M+H)^+$ 312.2070. Found 312.2092

N-Methyl-1,3-diphenylpropan-2-amine (41)

To a stirred solution of 1.3-diphenylacetone (1000 mg, 4.76 mmol) in dry dichloromethane (10 mL) cooled to -5 °C was added a 2M solution of methylamine in THF (10.7 mL). The reaction was allowed to stir at room temperature for 1 hour. Glacial acetic acid was added, followed by sodium triacetoxyborohydride (1500 mg, 7.14 mmol) in small portions. The reaction was stirred at room temperature for 3 hours and quenched with 3M solution of sodium hydroxide. The aqueous layer was extracted with diethyl ether and the combined ether fractions washed with 3M NaOH, dried over sodium sulfate and evaporated to dryness to give product as a pale yellow colored oil. (151.9 mg, 14%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.28 (m, 10H), 2.98 (m, 1H), 2.77 (m, 4H), 2.45 (s, 3H), 1.55 (brs, 1H); ^{13}C NMR (CDCl$_3$) (100 MHz) δ 139.4, 129.3, 128.4, 126.2, 62.7, 40.2, 34.1

Di-*tert*-butyl (5-((1,3-diphenylpropan-2-yl)(methyl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate (42).

$N^{\alpha,\delta}$-Bis-boc-L-ornithine (148 mg, 0.45 mmol) was dissolved in dichloromethane (5 mL) and PyBrop (209 mg, 0.92 mmol) was added and stirred for 5 minutes. N-Methyl-1,3-diphenylpropan-2-amine, **41**, (109 mg, 0.49 mmol) was added followed by triethylamine (0.14 mL, 0.97 mmol) and reaction was stirred at room temperature overnight. The solvent

was removed to give product as a colorless oil. (144 mg, 59%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.22 (m, 10H), 5.15 (m, 2H), 4.35 (m, 2H), 2.89 (m, 9H), 1.46 (s, 9H), 1.42 (s, 9H), 1.07 (m, 4H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 172.7, 172.3, 155.8, 155.4, 138.0, 137.2, 129.1, 129.0, 128.9, 128.8, 128.4, 128.3, 126.8, 126.7, 126.5, 126.4, 79.4, 79.2, 61.0, 50.1, 39.8, 38.6, 37.6, 30.6, 30.1, 28.4, 28.3, 25.1

(S)-2,5-Diamino-N-(1,3-diphenylpropan-2-yl)-N-methylpentanamide (43).

Di-*tert*-butyl (5-((1,3-diphenylpropan-2-yl)(methyl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate, **42**, (96 mg, 0.18 mmol) was dissolved in dichloromethane (3mL) and the reaction mixture cooled to 0 ^{o}C under nitrogen. Trifluoroacetic acid (2mL) was added and the reaction stirred at that temperature for 3 hours. On completion of the reaction, the solvents were removed and residue purified on an ISCO chromatograph (0–10% methanol/dichloromethane with 0.1% NH$_4$OH) to give product as a colorless oil. (60 mg, 100%); ^{1}H NMR (CD$_3$OD) (400 MHz) δ 7.27 (m, 10H), 4.12 (m, 1H), 2.70 (m, 10H), 1.33 (m, 4H); ^{13}C NMR (CD$_3$OD) (100 MHz) δ 170.6, 139.7, 139.5, 130.5, 130.3, 130.2, 130.1, 130.0, 129.6, 129.5, 128.0, 127.6, 127.5, 63.8, 51.4, 40.0, 39.1, 38.4, 28.7, 23.9, 23.6; HRMS (ESI) Calculated for C$_{21}$H$_{29}$N$_3$ONa (M+Na)$^+$ 362.2203, found 362.2225

N-Methyl-1,5-diphenylpentan-3-amine (44).

To a stirred solution of 1,5-diphenylpentan-3-one, **32**, (872 mg, 3.66 mmol) in dry dichloromethane (10 mL) cooled to -5 ^{o}C was added a 2M solution of methylamine in THF

(8.2 mL, 16.47 mmol). The reaction was allowed to stir at room temperature for 1 hour. Glacial acetic acid (0.94 mL, 16.47 mmol) was added, followed by sodium triacetoxyborohydride (989 mg, 16.47 mmol) in small portions. The reaction was stirred at room temperature for 3 hours and quenched with 3M solution of sodium hydroxide. The aqueous layer was extracted with diethyl ether and the combined ether fractions washed with 3M NaOH, dried over sodium sulfate and evaporated to dryness purified on an ISCO chromatograph (0-100% ethyl acetate/hexane) to give product as a pale yellow colored oil. (564 mg, 60%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.29 (m, 10H), 2.83 (d, 1H, *J = 4),* 2.70 (m, 4H), 2.58 (m, 1H), 2.45 (s, 3H), 1.83 (m, 4H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 142.48, 128.4, 128.3, 125.7, 58.2, 35.2, 33.2, 32.0

Di-*tert*-butyl (5-((1,5-diphenylpentan-3-yl)(methyl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate (45).

$N^{\alpha,\delta}$-Bis-boc-L-ornithine (179 mg, 0.54 mmol) was dissolved in dichloromethane (5 mL) and PyBrop (251 mg, 0.54 mmol) was added and stirred for 5 minutes. N-Methyl-1,5-diphenylpentan-3-amine, **44**, (150 mg, 0.59 mmol) was added followed by triethylamine (0.15 mL, 1.08 mmol) and reaction was stirred at room temperature overnight. The dichloromethane was concentrated under reduced pressure and the residue purified on an ISCO chromatograph (0-10% MeOH/dichloromethane) to give product as a colorless oil. (249 mg, 74%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.23 (m, 10H), 5.48 (m, 2H), 4.75 (m, 1H), 4.47 (m, 1H), 3.18 (m, 2H), 2.92 (s, 3H), 2.52 (m, 4H), 1.77 (m, 4H), 1.62 (m, 4H), 1.46 (s, 9H), 1.44 (s, 9H); ^{13}C NMR (CDCl$_3$) (100 MHz) δ 173.2, 156.0, 155.7, 141.7, 141.5,

128.7, 128.5, 128.4, 128.3, 128.2, 126.0, 125.9, 60.6, 50.5, 34.3, 34.1, 32.7, 30.5, 28.4, 28.3, 26.5

(S)-2,5-Diamino-N-(1,5-diphenylpentan-3-yl)-N-methylpentanamide (46).

Di-*tert*-butyl(5-((1,5-diphenylpentan-3-yl)(methyl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate, **45**, (146 mg , 0.26 mmol) was dissolved in dichloromethane (3 mL) and the reaction mixture cooled to 0 $^\circ$C under nitrogen. Trifluoroacetic acid (2 mL) was added and the reaction stirred at that temperature for 3 hours. On completion of the reaction, the solvents were removed and residue purified on an ISCO chromatograph (0–10% methanol/ dichloromethane with 0.1% NH_4OH) to give product as a colorless oil. (95 mg, 100%); ^{1}H NMR (400 MHz) (MeOD) δ 7.23 (m, 10H), 4.67 (m, 1H), 4.51 (m, 1H), 3.01 (s, 3H), 3.00 (m,1H), 2.54 (m, 5H), 1.85 (m, 8H); ^{13}C NMR (100 MHz) (MeOD) δ 170.4, 143.0, 142.8, 129.8, 129.6, 129.5, 129.43, 129.41, 127.4, 127.0, 126.9, 60.6, 52.0, 40.0, 35.1, 34.9, 33.8, 33.6, 28.7, 24.1; HRMS (ESI) Calculated for $C_{23}H_{34}N_3O$ (M+Na)[+] 390.2516, found 390.2536

(S)-2-Amino-N-(1,5-diphenylpentan-3-yl)-5-(methylamino)pentanamide (47).

Benzyl (S)-(5-(benzyl(methyl)amino)-1-((1,5-diphenylpentan-3-yl)amino)-1-oxopentan-2-yl)carbamate, **52**, (31.5 mg, 0.05 mmol) was dissolved in ethanol (5 mL), then Boc_2O (29 mg, 0.13 mmol) and $Pd(OH)_2/C$ (20 mg) was added. The reaction was stirred under a hydrogen atmosphere overnight. Upon completion of the reaction, the catalyst was filtered

and the residue washed with 20% MeOH/dichloromethane. The solvents were removed under reduced pressure and the product was obtained after purification on an ISCO chromatograph (0-30% ethyl acetate/hexane) to give a colorless oil (23 mg, 77%). The oil was dissolved in dichloromethane (1 mL) and trifluoroacetic acid (0.5 mL) and reaction mixture stirred at room temperature for 2 hours. Product obtained as a colorless oil (trifluoroacetic acid salt) (17 mg, 96%)(overall yield = 74%); ^{1}H NMR (MeOD) (400 MHz) δ 7.21 (m, 11H), 3.96 (m, 2H), 3.08 (m, 2H), 2.66 (m, 7H), 1.90 (m, 9H); ^{13}C NMR (MeOD) (100 MHz) δ169.2, 143.0, 142.9, 129.5, 129.4, 127.0, 126.9, 53.9, 50.8, 37.8, 37.7, 33.6. 33.5, 33.3. 29.8, 22.8; HRMS (ESI) Calculated for $C_{23}H_{33}N_3O$ (M+H)$^+$ 368.2696, found 368.2696

(S)-2-Amino-5-(dimethylamino)-N-(1,5-diphenylpentan-3-yl)pentanamide (48)

Benzyl (5-(dimethylamino)-1-((1,5-diphenylpentan-3-yl)amino)-1-oxopentan-2-yl)-carbamate, **53**, (98.1 mg, 0.19 mmol) was dissolved in ethanol (15 mL) and treated with 20% Pd(OH)$_2$/C (15 mg) and stirred under a H$_2$ balloon for overnight. The catalyst was then filtered out and washed with ethanol to afford product as a colorless oil. (70.8 mg, 97.5%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.14 (m, 11H), 3.98 (m, 1H), 3.30 (m, 1H), 2.72 (m, 2H), 2.56 (m, 4H), 2.29 (m, 2H), 2.18 (s, 6H), 1.74 (m, 2H), 1.53 (m, 2H); ^{13}C NMR (CDCl$_3$) (400 MHz) δ 174.3. 142.0, 141.9, 128.3, 128.5, 125.84, 125.82, 58.9, 54.7, 48.8, 46.3, 46.2, 45.0, 37.2, 36.9, 32.9, 32.5, 32.4, 26.4, 26.3, 23.6; HRMS (ESI) Calculated for $C_{24}H_{35}N_3O$ (M+H)$^+$ 382.2853, found 382.2876

(S)-2-Amino-N-(1,5-diphenylpentan-3-yl)-5-guanidinopentanamide (49).

The tris boc-(S)-2-amino-N-(1,5-diphenylpentan-3-yl)-5-guanidinopentanamide, **54**, formed (198 mg, 0.29 mmol) was dissolved in dichloromethane (3 mL) and the reaction mixture cooled to 0 °C under nitrogen. Trifluoroacetic acid (2 mL) was added and the reaction stirred at that temperature for 3 hours. On completion of the reaction, the solvents were removed and residue purified on an ISCO chromatograph (0–10% methanol/dichloromethane with 0.1% NH$_4$OH) to give product as a colorless oil. (112.8 mg, 100%); ^{1}H NMR (MeOD) (400 MHz) δ 7.26 (m, 10H), 3.93 (m, 2H), 3.28 (m, 2H), 2.57 (m, 4H), 1.88 (m, 8H); ^{13}C NMR (MeOD) (100 MHz) δ 169.89, 143.1, 142.9, 129.5, 129.44, 129.41, 127.0, 126.9, 54.3, 50.7, 41.8, 37.8, 33.5, 33.3, 30.2, 25.7; HRMS (ESI) Calculated for C$_{23}$H$_{33}$N$_5$O (M+Na)$^+$ 418.2577, found 418.2602

Benzyl *tert*-butyl (5-((1,5-diphenylpentan-3-yl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate (50).

Nα-Cbz-Nδ-Boc-L-ornithine (458 mg, 1.25 mmol) was dissolved in dichloromethane (5 mL) and PyBop (650 mg, 1.25 mmol) was added and stirred for 5 minutes. 1,5-Diphenylpentan-3-amine, **34**, (300 mg, 1.25 mmol) was added followed by triethylamine (0.35 mL, 2.51 mmol) and the reaction was stirred at room temperature overnight. The solvent was evaporated and residue re-dissolved in ethyl acetate. The organic layer was washed with 5% HCl, sat. NaHCO$_3$ brine, dried over sodium sulfate and concentrated. The residue was purified on an ISCO chromatograph with 10% MeOH/dichloromethane to give product as a white powder. (566 mg, 77%); MP 133 -135 ^{0}C; ^{1}H NMR (400 MHz) (CDCl$_3$)

δ 7.27 (m, 15H), 6.38 (s, 1H), 5.77 (m, 1H), 5.12 (s, 2H), 4.84 (brs, 1H), 4.36 (m, 1H), 4.04 (m, 1H), 2.63 (m, 4H), 1.75 (m, 10H), 1.45 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 171.5, 156.6, 156.4, 141.8, 141.7, 136.3, 128.5, 128.4, 128.3, 128.1, 128.0, 125.9, 125.8, 79.2, 66.9, 53.9, 49.2, 46.3, 39.3, 37.1, 36.9, 32.3, 30.3, 28.4, 26.5, 26.4, 26.3

Benzyl (S)-(5-amino-1-((1,5-diphenylpentan-3-yl)amino)-1-oxopentan-2-yl)-carbamate (51).

 Benzyl   *tert*-butyl   (5-((1,5-diphenylpentan-3-yl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate, **50**, (200 mg, 0.34 mmol) was dissolved in dichloromethane (3 mL) and the reaction mixture cooled to 0 °C under nitrogen. Trifluoroacetic acid (2 mL) was added and the reaction stirred at that temperature for 3 hours. On completion of the reaction, the solvents were removed and residue purified on an ISCO chromatograph (0–10% methanol/ dichloromethane with 0.1% NH$_4$OH) to give product as a colorless oil. (165 mg, 100%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 8.24 (s, 2H), 7.21 (m, 16H), 6.24 (m, 1H), 5.08 (m, 2H), 4.25 (m 1H), 3.98 (m, 1H), 2.57 (m, 4H), 1.80 (m, 8H); ^{13}C NMR (CDCl$_3$) (100 MHz) δ 171.3, 157.2, 141.7, 141.6, 135.9, 128.5, 128.4, 128.36, 128.34, 128.2, 127.8, 125.9, 125.8, 67.2, 53.9, 49.3, 46.3, 46.2, 39.4, 36.6, 36.5, 32.3, 30.5, 25.4, 26.3, 23.1

Benzyl (S)-(5-(benzyl(methyl)amino)-1-((1,5-diphenylpentan-3-yl)amino)-1-oxopentan-2-yl)carbamate (52).

To a stirred solution of benzyl (S)-(5-amino-1-((1,5-diphenylpentan-3-yl)amino)-1-oxopentan-2-yl)carbamate, **51**, (198 mg, 0.41 mmol) in ethanol (10 mL) in the presence of 3Å molecular sieves at room temperature was added freshly distilled benzaldehyde (0.05 mL, 0.49 mmol). After stirring for 30 minutes, $NaCNBH_3$ (30 mg, 0.49 mmol) was added and the reaction was allowed to stir overnight. Then 37% aqueous formaldehyde (0.06 mL, 0.82 mmol) was added followed by $NaCNBH_3$ (36 mg, 0.57 mmol). Reaction was stirred for 1 hour under nitrogen. Reaction was then filtered to remove the molecular sieves and washed with methanol. The methanol was removed by evaporation and residue was dissolved in ethyl acetate. The ethyl acetate was quenched with sat NH_4Cl. Phase separation was used to remove the ammonium chloride. The organic layer was washed with saturated $NaHCO_3$ and brine. The organic layer was dried over sodium sulfate and filtrate concentrated and purified on an ISCO chromatograph (0–10% MeOH/dichloromethane) to give product as a white powder. (40 mg, 17%); MP 101-102 °C ; ^{1}H NMR (400 MHz) ($CDCl_3$) δ 7.25 (m, 20H), 6.77 (s, 1H), 6.11 (brs, 1H), 5.15 (m, 2H), 4.08 (m, 2H), 3.54 (m, 2H), 2.62 (m, 4H), 2.44 (m, 2H), 2.21 (s, 3H), 1.75 (m, 8H); ^{13}C NMR (100 MHz) ($CDCl_3$) δ 171.5, 156.6, 141.7, 138.1, 136.3, 129.2, 128.5, 128.4, 128.39, 128.37, 128.32, 128.1, 128.0, 127.2, 125.9, 66.9, 62.4, 56.6, 55.1, 49.1, 41.7, 37.2, 37.1, 32.4, 32.3, 30.4, 23.2

Benzyl (S)-(5-(dimethylamino)-1-((1,5-diphenylpentan-3-yl)amino)-1-oxopentan-2-yl)carbamate (53).

Benzyl (5-amino-1-((1,5-diphenylpentan-3-yl)amino)-1-oxopentan-2-yl)carbamate, **51**, (140 mg, 0.29 mmol) was dissolved in a 20% methanol/dichloromethane (5 mL) solution and aqueous 37% formaldehyde (1 mL) was added and allowed to stir at room temperature for 10 minutes. Sodium triacetoxyborohydride (609 mg, 2.87 mmol) was added and reaction was stirred at room temperature overnight. The mixture was then dissolved in saturated sodium bicarbonate and extracted with dichloromethane. The combined organic extracts were dried over sodium sulfate, concentrated and purified on ISCO with (10% MeOH/dichloromethane + 1% NH_4OH) to give 98 mg of product as a white solid; (66%); MP 98–100 °C; ^1H NMR ($CDCl_3$) (400 MHz) δ 7.15 (m, 16H), 6.38 (m, 1H), 5.05 (m, 2H), 3.97 (m, 2H), 2.42 (m, 4H), 2.26 (m, 2H), 2.15 (m, 6H), 1.57 (m, 8H); ^{13}C NMR ($CDCl_3$) (100 MHz) δ 171.6, 141.8, 136.5, 128.5, 128.45, 128.41, 128.38, 128.34, 128.1, 127.9, 125.9, 125.8, 66.8, 58.8, 49.0, 45.0, 37.3, 37.2, 32.4, 32.3, 23.5

Tris Boc -(S)-2-amino-N-(1,5-diphenylpentan-3-yl)-5-guanidinopentanamide (54)

$N^\alpha,N^\omega,N^\omega$ Tris Boc- L- arginine (199 mg, 0.42 mmol) was dissolved in dichloromethane (5 mL) and PyBop (218 mg, 0.42 mmol) was added and stirred for 5 minutes. 1,5-Diphenylpentan-3-amine (100 mg, 0.42 mmol) was added followed by triethylamine (0.12 mL, 0.84 mmol) and reaction was stirred at room temperature overnight. The solvent was evaporated and purified on an ISCO chromatograph using 0-50% ethyl acetate/hexane to give product as a white solid. (271 mg, 92%); MP 50-52 °C; ^1H NMR (400 MHz) ($CDCl_3$)

δ 9.32 (brs, 1H), 7.24 (m, 10H), 6.44 (d, 1H, *J = 4*), 5.84 (d, 1H, *J = 4*), 4.26 (m, 1H), 4.08 (m, 1H), 3.93 (m, 1H), 3.84 (m,1H), 2.64 (m, 4H), 1.84 (m, 8H), 1.53 (s, 9H), 1.52 (s,1H), 1.47 (s, 1H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 172.0, 163.4, 160.7, 155.9, 154.8, 141.9, 141.7, 128.4, 128.5, 125.9. 128.8, 84.0, 79.8, 79.1, 54.7, 49.2, 44.1, 37.2, 37.1, 32.5, 32.4, 31.5, 28.4, 28.3, 28.0, 25.2, 24.9, 22.6

(S)-3,5-Diamino-N-(1,5-diphenylpentan-3-yl)pentanamide (55)

Di-*tert*-butyl   (5-((1,5-diphenylpentan-3-yl)amino)-5-oxopentane-1,3-diyl)(S)-dicarbamate, **61**, (19 mg, 0.034 mmol) was dissolved in dichloromethane (1 mL) and trifluoroacetic acid (0.5 mL) at room temperature. Reaction was stirred at that temperature for 2 hours under nitrogen.   The solvents were removed to obtain product as a trifluoroacetic acid salt, colorless oil. (18 mg, 100%); ^{1}H NMR (400 MHz) (MeOD) δ 7.09 (m, 10H), 3.84 (m, 1H), 3.58 (m, 1H), 3.01 (m, 2H), 2.54 (m, 6H), 1.98 (m, 2H), 1.70 (m, 4H); ^{13}C NMR (100 MHz) (MeOD) δ 171.2, 143.2, 143.1, 129.4, 126.8, 50.4, 47.7, 37.9, 37.8, 37.2, 37.0, 33.4, 31.7; HRMS (ESI) Calculated for C$_{22}$H$_{32}$N$_3$O (M+H)$^+$ 354.2540, found 354.2541

(S)-4,5-Diamino-N-(1,5-diphenylpentan-3-yl)pentanamide (56)

Benzyl (S)-(1-azido-5-((1,5-diphenylpentan-3-yl)amino)-5-oxopentan-2-yl)carbamate, **66**, (27.9 mg, 0.054 mmol) was dissolved in ethanol (5 mL) and 20% Pd (OH)$_2$/C (15 mg) was added. Reaction was stirred under hydrogen overnight. The catalyst was filtered off and

washed with 10% MeOH/dichloromethane. Solvent was removed under reduced pressure to give product as a colorless oil. (19.2 mg, 100%). ^{1}H NMR (CDCl$_3$) δ 7.14 (m, 10H), 5.85 (d, 1H, $J = 8$), 4.00 (m, 1H), 2.63 (m, 6H), 2.41 (m, 1H), 2.19 (m, 2H), 1.98 (brs, 4H), 1.70 (m, 5H), 1.44 (m, 1H); ^{13}C NMR (100 MHz) 172.5, 141.8, 128.4, 128.3, 125.9, 53.16, 49.1, 48.5, 37.0, 33.6, 32.4, 30.8. HRMS (ESI) Calculated for C$_{22}$H$_{31}$N$_3$O (M+H)$^+$ 354.2540, found 254.2545

Dibenzyl (5-diazo-4-oxopentane-1,3-diyl)(S)-dicarbamate (57)

(S)-2,4-Bis(((benzyloxy)carbonyl)amino)butanoic acid (1000 mg, 2.59 mmol) was dissolved in THF (15 mL) and triethylamine (0.38 mL, 2.72 mmol) and reaction mixture cooled to -15 ℃. Ethyl chloroformate (0.26 mL, 1.05 mmol) was added and the mixture was stirred for 30 minutes at -5 ℃ and the precipitate formed was filtered off. Acetonitrile (dry) (10 mL) and trimethylsilyldiazomethane (2.0 M solution in hexane) (2.6 mL, 5.18 mmol) were added to the filtrate and the mixture stirred at +4 ℃ for 24 hours. Diethyl ether was added to the mixture and extracted with 10% HCl, sat NaHCO$_3$ and brine. The organic layer was dried over sodium sulfate and solvents evaporated to obtain the crude diazoketone. This was purified on an ISCO chromatograph (0-70%) ethyl acetate/hexane to obtain pure product as a yellow colored oil. (578 mg, 54%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.37 (m, 10H), 5.72 (brs, 1H), 5.37 (brs, 1H), 5.30 (brs, 1H), 5.03 (s, 4H), 4.21(brs, 1H), 3.48 (m, 1H), 3.09 (m, 1H), 2.00 (m, 1H), 1.74 (m, 1H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 192.8, 156.6, 156.4, 136.5, 136.1, 128.5, 128.2, 128.1, 128.0, 67.2, 66.7, 55.3, 54.2, 37.1, 33.1

Methyl (S)-3,5-bis(((benzyloxy)carbonyl)amino)pentanoate (58)

The diazoketone, **57**, (560 mg, 1.36 mmol) was suspended in methanol and a solution of

silver benzoate (62 mg, 0.27 mmol) in triethylamine (0.8 mL) was gradually added while

the mixture was sonicated in an ultrasound bath until completion of the reaction (30

minutes). The methanol was removed unde reduced pressure and the residue dissolved in

ethyl acetate and extracted with sat. $NaHCO_3$, 5% HCl and brine and dried over sodium

sulfate, concentrated and purified using anISCO chromatograph (0-50% ethyl acetate/

hexane) to give product as a colorless oil. (312 mg, 55%); ^1H NMR ($CDCl_3$) (400 MHz) δ

7.33 (m, 10H), 5.70 (m, 2H), 5.10 (s, 4H), 4.06 (m, 1H), 3.63 (s, 3H), 3.41 (m, 1H), 3.03

(m, 1H), 2.54 (d, 2H, $J = 4$), 1.68 (m, 2H); ^{13}C NMR (100 MHz) ($CDCl_3$) 171.8, 156.5,

156.4, 136.7, 136.5, 128.5, 128.4, 128.2, 128.1, 128.0, 66.7, 66.5, 51.7, 45.6, 38.9, 37.6,

37.1, 34.6

(S)-3,5-Bis(((benzyloxy)carbonyl)amino)pentanoic acid (59).

Methyl (S)-3,5-bis(((benzyloxy)carbonyl)amino)pentanoate, **59**, (236 mg, 0.56 mmol) was

dissolved in THF/ water (12 mL) (5:1) and treated with $LiOH \cdot H_2O$ (28 mg, 0.67 mmol) at

0 °C under nitrogen. Reaction mixture was stirred at room temperature overnight. Then

1N HCl was added and the THF removed under reduced pressure. A white solid which

had precipitated was filtered. The residue was saved and the filtrate was extracted with

ethyl acetate. The organic layer was dried over sodium sulfate and solvent evaporated.

The precipitated solid was added and the combined residue was dried on a vacuum pump

to give product as a white solid. (150 mg, 67%); MP 110-112 °C; ^1H NMR (400 MHz)

(CD$_3$OD) δ 7.24 (m, 10H), 4.96 (s, 4H), 3.92 (m, 1H); 3.13 (m, 1H), 2.99 (m, 1H), 2.40 (d, 2H, *J = 4);* 1.67 (m, 1H), 1.55 (m, 1H); ^{13}C NMR (100 MHz) (CD$_3$OD) δ 158.8, 158.4, 138.4, 129.4, 128.9, 128.8, 128.77, 128.72, 67.4, 47.5, 40.6, 38.7, 35.6

Dibenzyl (5-((1,5-diphenylpentan-3-yl)amino)-5-oxopentane-1,3-diyl)(S)-dicarbamate (60).

(S)-3,5-Bis(((benzyloxy)carbonyl)amino)pentanoic acid, **59**, (150 mg, 0.375 mmol) was dissolved in DMF (5 mL), then EDC (143 mg, 0.75 mmol) and HOBT (101 mg, 0.75 mmol) was added. Reaction was stirred under nitrogen for 5 minutes. Then 1,5-diphenylpentan-3-amine, **34**, (99 mg, 0.41 mmol) and 2,6-lutidine (0.14 mL, 1.24 mmol) was added sequentially. The reaction was stirred at room temperature overnight. On completion of the reaction, the reaction mixture was diluted with ethyl acetate and the organic layer washed with water, saturated sodium bicarbonate, 5% hydrochloric acid solution, water and brine. The organic layer was dried over sodium sulfate, concentrated to give product as a yellow solid. (171 mg, 73%);; MP 182-183 ºC; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.17 (m, 20H), 6.07 (d, 1H, *J = 8)*; 5.44 (m, 2H), 5.00 (m, 4H), 3.94 (m, 2H), 3.38 (m, 1H), 2.95 (m, 1H), 2.54 (m, 5H), 2.29 (m, 1H), 1.70 (m, 6H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 156.7, 141.5, 136.6, 136.4, 128.5, 128.4, 128.3, 128.0, 127.9, 126.0, 66.7, 66.6, 49.3, 37.0, 36.8, 32.4, 32.3

Di-*tert*-butyl (5-((1,5-diphenylpentan-3-yl)amino)-5-oxopentane-1,3-diyl)(S)-dicarbamate (61).

Dibenzyl (5-((1,5-diphenylpentan-3-yl)amino)-5-oxopentane-1,3-diyl)(S)-dicarbamate, **60**, (170 mg, 0.27 mmol) was dissolved in ethanol (15 mL), then 20% Pd(OH)$_2$/C (50 mg) and Boc$_2$O (176 mg, 0.81 mmol) was added. Reaction was stirred under a hydrogen atmosphere for overnight. Then the catalyst was filter and the filtrate concentrated and purified on an ISCO chromatograph (0-10% MeOH/dichloromethane) to give product as a white solid (115 mg, 77%); MP 147 -149 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.24 (m, 10H), 5.83 (m, 2H), 5.31 (brs, 1H), 4.07 (m, 1H), 3.91 (m, 1H), 3.40 (m, 1H), 2.97 (m, 1H), 2.58 (m, 5H), 2.29 (dd, 1H, *J = 8, 16*), 1.77 (m, 6H), 1.46 (s, 9H), 1.42 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 170.7, 156.1, 141.7, 141.6, 128.5, 128.4, 128.38, 128.33, 125.9, 79.3, 79.1, 49.2, 45.5, 37.1, 36.9, 35.0, 32.5, 32.4, 28.5, 28.4, 28.3

Methyl N^2-((benzyloxy)carbonyl)-N5-(1,5-diphenylpentan-3-yl)-L-glutaminate (62).

Z-L-glutamic acid α-methyl ester (500 mg, 1.69 mmol) was dissolved in DMF (5mL) and EDC (646 mg, 3.38 mmol) and HOBT (455 mg, 3.38 mmol) were added. The reaction was stirred at room temperature for 5 minutes under nitrogen. 1,5-Diphenylpentan-3-amine, **34**, (368 mg, 1.54 mmol) was added followed by 2,6-lutidine (0.5 mL, 4.62 mmol). The reaction was stirred overnight at that same temperature. On completion of the reaction, the reaction mixture was diluted with ethyl acetate and the organic layer washed with water, saturated sodium bicarbonate, 5% hydrochloric acid solution, water and brine. The organic layer was dried over sodium sulfate, concentrated and purified on an ISCO

chromatographusing 0-10% MeOH/dichloromethane to obtain product as a white solid. (730 mg, 91%). MP 102 -104 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.16 (m, 15H), 5.60 (m, 2H), 5.02 (s, 2H), 4.30 (m, 1H), 4.00 (m, 1H), 3.66 (s, 3H), 2.56 (m, 4H), 2.12 (m, 3H), 1.75 (m, 5H); ^{13}C NMR (100 MHz) δ 172.3, 141.8, 136.1, 128.5, 128.4, 128.3, 128.2, 128.1, 125.9, 67.1, 53.5, 52.5, 49.4, 37.0, 36.9, 32.6, 32.4, 32.3, 28.9

Benzyl (S)-(5-((1,5-diphenylpentan-3-yl)amino)-1-hydroxy-5-oxopentan-2-yl)-carbamate (63).

To a solution of Methyl N^2-((benzyloxy)carbonyl)-N5-(1,5-diphenylpentan-3-yl)-L-glutaminate, **62**, (330 mg, 0.64 mmol) in THF (15 mL) and ethanol (1 mL) was added LiBH$_4$ (21 mg, 0.96 mmol) at 0 °C. The mixture was stirred at that temperature for 30 minutes and warmed to room temperature overnight. The reaction mixture on completion of the reaction, was poured into water and extracted with ethyl acetate. The combine organic layers were washed with brine, dried over sodium sulfate and concentrated. It was purified on an ISCO chromatograph (0-10% MeOH/dichloromethane) to give product as a white crystalline solid. (159 mg, 51%); MP 130-132 °C; ^{1}H NMR (CDCl3) (400 MHz) δ 7.26 (m, 15H), 6.08 (d, 1H, *J* = *8*), 5.66 (d, 1H, *J* = *8*), 5.09 (m, 2H), 4.06 (m, 1H), 3.64 (m, 3H), 2.67 (m, 4H), 2.20 (m, 2H), 1.86 (m, 6H); ^{13}C NMR (CDCl3) (100 MHz) δ 172.8, 156.9, 141.8, 136.4, 128.5, 128.4, 128.3, 128.1, 127.9, 125.9, 66.8, 64.3, 52.8, 49.5, 36.9, 36.8, 32.9, 32.4, 32.3, 27.1

Benzyl (S)-(1-((diphenoxyphosphoryl)oxy)-5-((1,5-diphenylpentan-3-yl)amino)-5-oxopentan-2-yl)carbamate (65).

A mixture of benzyl (S)-(5-((1,5-diphenylpentan-3-yl)amino)-1-hydroxy-5-oxopentan-2-yl)carbamate, **63**, (119.8 mg, 0.25 mmol) was dissolved in dry THF (10 mL) and mixture cooled to 0 °C. DPPA (79 µL, 0.37 mmol) and neat DBU (55 µL, 0.37 mmol) was added and reaction stirred at 0 °C for 2 hours and 20 °C overnight. The solvent was removed under reduced pressure and the residue diluted with ethyl acetate and washed with water. The organic layer was extracted and dried over sodium sulfate and concentrated. The concentrate was purified on an ISCO chromatograph (0-50% ethyl acetate/hexane) to obtain product as a colorless solid. (180 mg, 100%). MP 63-64 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.16 (m, 25H), 5.61 (d, 1H, J = 8), 5.29 (d, 1H, J = 8), 4.97 (s, 2H), 4.20 (m, 2H), 3.98 (m, 1H), 3.84 (m, 1H), 2.54 (m, 4H), 2.08 (m, 2H), 1.69 (m, 6H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 171.6, 150.4, 150.3, 141.8, 136.3, 129.9, 129.8, 128.5, 128.4, 128.35, 128.33, 128.1, 128.0, 125.9, 125.5, 120.0, 119.9, 70.3, 66.9, 49.4, 37.0, 36.9, 32.7, 32.4, 32.3, 27.2

Benzyl (S)-(1-azido-5-((1,5-diphenylpentan-3-yl)amino)-5-oxopentan-2-yl)carbamate (66).

To a solution of **65** (180 mg, 0.25 mmol) was dissolved in dry DMF (3 mL) and sodium azide (65 mg, 1 mmol) was added. Reaction mixture was stirred at 80 °C for 4 hours. Mixture was dissolved in water and product extracted with ethyl acetate. The organic layer was dried over sodium sulfate, concentrated and purified on an ISCO chromatograph using

0-40% ethyl acetate hexane to give product as a white solid. (32.8 mg, 25%); MP 122-124

°C; ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.26 (m, 15H), 5.63 (d, 1H, $J = 8$), 5.34 (d, 1H, $J = 8$),

5. 11 (dd, 2H, $J = 12$, 32), 4.09 (m, 1H), 3.82 (m, 1H), 3.44 (m, 2H), 2.65 (m, 4H), 2.19

(m, 2H), 1.83 (m, 6H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 171.8, 156.3, 141.8, 136.3, 128.5,

128.4, 128.3, 128.1, 128.0, 125.9, 66.9, 54.9, 50.7, 49.4, 49.3, 37.0, 36.9, 32.9, 32.4, 32.3,

28.1

(S)-2,4-Diamino-N-(1,5-diphenylpentan-3-yl)butanamide (67).

Compound **69** (74.4 mg, 0.12 mmol) was dissolved in ethanol (10 mL) and 20% Pd(OH)$_2$/C

(40 mg) was added. The reaction was stirred under a hydrogen atmosphere overnight. The

catalyst was removed by filtration and washed with 20% methanol/dichloromethane. The

solvent was removed under reduced pressure and the residue purified on an ISCO

chromatograph (0-15% methanol/dichloromethane with 0.01% ammonium hydroxide) to

give product as a colorless oil. (20 mg, 51%); ^{1}H NMR (CD$_3$OD) (400 MHz) δ 7.12 (m,

11H), 3.40 (m, 1H), 2.88 (m, 1H), 2.64 (m, 1H), 1.85 (m, 6H), 1.35 (m, 1H); ^{13}C NMR

176.9, 143.3, 143.2, 129.4, 129.3, 126.9, 126.8, 54.8, 54.6, 50.2, 50.0, 39.3, 38.2, 38.0,

37.7, 33.5, 33.4, 33.3, 31.8; HRMS (ESI) Calculated for C$_{21}$H$_{29}$N$_3$O$_2$ (M+H)$^+$ 340.2383,

found 340.2383

(S)-3,4-Diamino-N-(1,5-diphenylpentan-3-yl)butanamide (68).

Compound **73** (22.1 mg, 0.04) was dissolved in dichloromethane (1 mL) and trifluoroacetic acid (0.5 mL) was added. Reaction mixture was stirred at room temperature for two hours. Solvents were removed by evaporation, subsequent addition and evaporation to reaction mixture with methanol (3 times) allowed for product to be obtained as a colorless oil. The oil was dried under vacuum. (20 mg, 91%); ^{1}H NMR (400 MHz) (CD$_3$OD) δ 7.11 (m, 1H), 3.82 (m, 2H), 2.71 (m, 2H), 2.54 (m, 4H), 1.72 (m, 4H), 1.23 (m, 2H); ^{13}C NMR (100 MHz) (CD$_3$OD) δ 170.3, 143.1, 129.4, 126.9, 50.5, 48.0, 42.3, 37.9, 39.8, 36.1, 33.4; HRMS (ESI) Calculated for C$_{21}$H$_{29}$N$_3$O (M+H)$^+$ 340.2383, found 340.2388

Dibenzyl (4-((1,5-diphenylpentan-3-yl)amino)-4-oxobutane-1,3-diyl)(S)-dicarbamate (69)

(S)-2,4-Bis(((benzyloxy)carbonyl)amino)butanoic acid (357 mg, 0.92 mmol) was dissolved in DMF (5mL) and EDC (353 mg, 1.85 mmol) and HOBT (249 mg, 1.85 mmol) were added. The reaction was stirred at room temperature for 5 minutes under nitrogen. 1,5-diphenylpentan-3-amine, **34**, (200 mg, 0.84 mmol) was added followed by 2,6-lutidine (0.27 mL, 2.51 mmol). The reaction was stirred overnight at that same temperature. On completion of the reaction, the reaction mixture was diluted with ethyl acetate and the organic layer washed with water, saturated sodium bicarbonate, 5% hydrochloric acid solution, water and brine. The organic layer was dried over sodium sulfate, concentrated and purified on an ISCO chromatograph using 0-10% MeOH/dichloromethane to obtain product as a white solid. (389 mg, 76%); MP 134 – 136 °C; ^{1}H NMR (400 MHz) (CDCl$_3$)

δ 7.24 (m, 20H), 6.05 (d, 1H, $J = 8$), 5.85 (brs, 1H); 5.15 (m, 4H), 4.32 (m,1H), 4.10 (m, 1H), 3.55 (m, 1H), 3.14 (m, 1H), 2.67 (m, 4H), 2.03 (m, 1H), 1.85 (m, 5H); ^{13}C NMR (CDCl₃) (100 MHz) δ 170.8, 157.2, 156.4, 141.8, 141.7, 136.5, 136.2, 128.6, 128.57, 128.52, 128.48, 128.43, 128.3, 128.2, 128.17, 128.11, 128.0, 126.0, 125.9, 67.0, 66.9, 52.3, 49.5, 37.5, 37.0, 36.8, 34.4, 32.4, 32.3

Methyl N2-(*tert*-butoxycarbonyl)-N4-(1,5-diphenylpentan-3-yl)-L-asparaginate (70)

Boc-L-aspartate α-methyl ester (242 mg, 0.98 mmol) was dissolved in DMF (5 mL), EDC (374 mg, 1.96 mmol) and HOBT (263 mg, 1.96 mmol) were added. The reaction was stirred at room temperature for 5 minutes under nitrogen. Then 1,5-diphenylpentan-3-amine, **34**, (213 mg, 0.89 mmol) was added followed by 2,6-lutidine (0.31 mL, 2.67 mmol). The reaction was stirred at room temperature overnight. . On completion of the reaction, the reaction mixture was diluted with ethyl acetate and the organic layer washed with water, saturated sodium bicarbonate, 5% hydrochloric acid solution, water and brine. The organic layer was dried over sodium sulfate, concentrated and purified on an ISCO chromatograph using 0-10% MeOH/dichloromethane to obtain product as a white solid. (385 mg, 92%); MP 113-115 °C; ^{1}H NMR (CDCl₃) (400 MHz) δ 7.19 (m, 4H), 7.09 (m, 6H), 5.74 (d, 1H, $J = 8$), 5.43 (d, 1H, $J = 8$), 4.43 (m, 1H), 3.95 (m, 1H), 3.66 (s, 3H), 2.76 (m, 1H), 2.55 (m, 5H), 1.76 (m, 2H), 1.64 (m, 2H), 1.34 (s, 9H); ^{13}C NMR (CDCl₃) (100 MHz) δ 171.9, 169.5, 141.7, 141.6, 128.4, 128.3, 128.3, 125.9, 80.0, 52.6, 50.6, 49.4, 38.2, 37.1, 37.0, 32.4, 32.2, 28.3, 28.2

tert-Butyl (S)-(4-((1,5-diphenylpentan-3-yl)amino)-1-hydroxy-4-oxobutan-2-yl)carbamate (71)

Methyl N^2-(*tert*-butoxycarbonyl)-N4-(1,5-diphenylpentan-3-yl)-L-asparaginate, **70**, (385 mg, 0.82 mmol) was dissolved in THF (20 mL) and cooled to 0 °C. Ethanol (2 mL) was added followed by LiBH$_4$ (27 mg, 1.23 mmol). Reaction was stirred at room temperature overnight. The reaction mixture on completion of the reaction, was poured into water and extracted with ethyl acetate. The combine organic layers were washed with brine, dried over sodium sulfate and concentrated. It was purified on ISCO (0-10% MeOH/dichloromethane) to give product as a white solid. (237 mg, 65%). MP 145-147 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.24 (m, 10H), 6.32 (d, 1H, J = 8), 5.73 (d, 1H, *J* = 8), 4.37 (brs, 1H), 4.07 (s, 1H), 3.94 (m, 1H), 3.81 (m, 1H), 3.68 (m, 1H), 2.65 (m, 6H), 1.83 (m, 4H), 1.42 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) 171.1, 156.1, 141.7, 141.6, 128.7, 128.5, 128.4, 128.3, 125.9, 79.8, 64.6, 49.7, 49.4, 38.9, 37.0, 36.9, 32.4, 32.3, 28.3

tert-Butyl (S)-(1-azido-4-((1,5-diphenylpentan-3-yl)amino)-4-oxobutan-2-yl)-carbamate (72).

Step 1: (S)-2-((*tert*-Butoxycarbonyl)amino)-4-((1,5-diphenylpentan-3-yl)amino)-4-oxobutyl methane sulfonate - To a stirred solution of *tert*-butyl (S)-(4-((1,5-diphenylpentan-3-yl)amino)-1-hydroxy-4-oxobutan-2-yl)carbamate, **71**, (221 mg, 0.5 mmol) in dichloromethane (10 mL) was added triethylamine (0.13 mL, 0.95 mmol) and methane sulfonyl chloride (70 µL, 0.96 mmol) at 0 °C. The mixture was stirred at that temperature for 30 minutes then 30 minutes at room temperature. Brine was added and the

organic layer was separated. The organic layer was washed with saturated sodium bicarbonate, dried over sodium sulfate. The solvent was evaporated and the crude mixture taken to the next step.

Step 2: *tert*-Butyl (S)-(1-azido-4-((1,5-diphenylpentan-3-yl)amino)-4-oxobutan-2-yl)carbamate **-** The crude (S)-2-((*tert*-butoxycarbonyl)amino)-4-((1,5-diphenylpentan-3-yl)amino)-4-oxobutyl methane sulfonate was dissolved in DMF (5 mL) and sodium azide (105 mg, 1.62 mmol) was added and mixture was heated at 50 ^{0}C for 4 hours. The reaction mixture was then diluted with ethyl acetate and washed with water. The organic layer was dried over sodium sulfate and ethyl acetate, filtered and concnetrated under reduced pressure. Residue was purified on an ISCO chromatograph (10% methanol/dichloromethane) to give product as a white powder. (171 mg, 73%); MP 140-143 ºC; ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.14 (m, 10H), 5.48 (brs,1H), 5.35 (brs, 1H), 3.85 (m, 2H), 3.52 (m, 1H), 3.31 (m, 1H), 2.56 (m, 4H), 2.33 (m, 2H), 1.67 (m, 4H), 1.34 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 169.7, 141.5, 141.5, 128.5, 128.32, 128.30, 126.0, 79.9, 53.4, 49.4, 47.8, 37.7, 37.0, 36.9, 32.4, 32.3, 28.3

Di-*tert*-butyl (4-((1,5-diphenylpentan-3-yl)amino)-4-oxobutane-1,2-diyl)(S)-dicarbamate (73).

Step 1: *tert*-butyl (S)-(1-azido-4-((1,5-diphenylpentan-3-yl)amino)-4-oxobutan-2-yl)carbamate, **72**, (150 mg, 0.32 mmol) was dissolved in ethanol (10 mL) and 20% Pd(OH)$_2$/C (50 mg) was added and reaction was stirred at room temperature overnight. The catalyst was filtered off and solvent removed by under reduced pressure. The crude

tert-butyl (S)-(1-amino-4-((1,5-diphenylpentan-3-yl)amino)-4-oxobutan-2-yl)carbamate was taking to the next step without further purification.

Step 2: The crude compound was dissolved in dichloromethane (10 mL) and Boc$_2$O (140 mg, 0.64 mmol) was added and reaction was stirred at room temperature for 1 hour. The solvent was removed and residue purified on an ISCO chromatograph (0-10% MeOH) to give product as a white powder. (136 mg, 78%); MP 167 -169 °C; ^{1}H NMR (CDCl$_3$) δ 7.16 (m, 10H), 6.62 (brs, 1H), 5.67 (brs, 1H), 4.94 (brs, 1H), 3.98 (m, 1H), 3.71 (m, 1H), 3.22 (m, 2H), 2.59 (m, 4H), 2.31 (m, 2H), 1.80 (m, 4H), 1.38 (s, 9H), 1.34 (s, 1H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 170.0, 141.8, 128.4, 128.38. 128.35, 125.8, 79.9, 49.5, 49.4, 43.3, 36.9, 32.5, 32.3, 28.4, 28.3

(S)-N-(2,5-Diaminopentyl)-2,2-diphenylacetamide (74).

Benzyl (S)-(4-amino-5-(2,2-diphenylacetamido)pentyl)carbamate, **80**, (35 mg, 0.08 mmol) was dissolved in ethanol (10 mL) and 20% Pd(OH)$_2$/C (20 mg) was added. The reaction mixture was then purged and stirred under hydrogen atmosphere overnight. Then the catalyst was filtered and the residue washed with 20% MeOH/dichloromethane. The solvent of the filtrate was removed under reduced pressure and dried on a vacuum pump to give product as a colorless oil. (20 mg, 83%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.21 (m, 10H), 6.64 (m, 1H), 4.87 (s, 1H), 3.25 (m, 1H), 2.95 (m, 1H), 2.84 (brs, 4H), 2.70 (m, 1H), 2.57 (m, 2H), 1.38 (m,4H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 172.5, 139.6, 139.5, 128.9, 128.8, 128.6, 127.2, 58.8, 50.8, 45.5, 41.2, 32.7, 28.5; HRMS (ESI) Calculated for C$_{19}$H$_{25}$N$_3$O (M+H)$^+$ 312.2070, found 312.2065

(S)-2-Benzyl-N-(2,5-diaminopentyl)-3-phenylpropanamide (75).

Benzyl (S)-(4-amino-5-(2-benzyl-3-phenylpropanamido)pentyl)carbamate, **82**, (42 mg, 0.09 mmol) was dissolved in ethanol (10 mL) and 20% Pd(OH)$_2$/C (20 mg) was added. The reaction mixture was then purged and stirred under hydrogen atmosphere for overnight. Then the catalyst was filtered and the residue washed with 20% MeOH/dichloromethane. The solvent of the filtrate was removed under reduced pressure and dried on a vacuum pump to give product as a colorless oil. (26 mg, 86%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.15 (m, 10H), 6.02 (m, 1H), 3.81 (brs, 4H), 3.03 -2.37 (m, 10H), 1.46 (m, 1H), 1.36 (m, 1H), 0.92 (m,1H), 0.80 (m, 1H); ^{13}C NMR (CDCl$_3$) (100 MHz) δ 174.5, 139.7, 139.6, 129.0, 128.9, 128.45, 128.44, 126.3, 52.2, 50.5, 44.8, 40.6, 39.1, 38.8, 32.0, 29.7, 27.5; HRMS (ESI) Calculated for C$_{21}$H$_{29}$N$_3$O (M+H)$^+$ 340.2383, found 340.2380

Benzyl _t_-butyl (5-hydroxypentane-1,4-diyl)(S)-dicarbamate (76).

To a solution of (S)-5-(((benzyloxy)carbonyl)amino)-2-((_t_-butoxycarbonyl)amino)-pentanoic acid (5.0 g, 13.6 mmol) in DME (25 mL) at -15 °C were successively added a solution of N-methyl morpholine (1.7 mL, 15.4 mmol) and isobutyl chloroformate (1.8 mL, 13.65 mmol). The reaction was stirred at -15 °C to -10 °C for 15 minutes. The precipitated N-methyl morpholine HCl was removed by filtration and washed with DME (10 mL), the combine filtrates were chilled to -15 °C in an ice-salt bath. Then a solution of sodium borohydride (1.55 g, 40.95 mmol) in water (10 mL) was added in one portion at -15 °C. This reaction mixture was stirred at this temperature for 10 minutes. The reaction

was quenched by the addition of saturated aq. NH₄Cl and the resulting mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried over sodium sulfate. The solution was then filtered and concentrated under reduced pressure the purified on an ISCO chromatograph (0-70% ethyl acetate/ hexane) to give the product as a colorless oil (3.81g, 79%); ^{1}H NMR (CDCl₃) (400 MHz) δ 7.34 (s, 5H), 5.29 (brs, 1H), 5.07 (s, 2H), 5.04 (brs,1H), 3.55 (m, 3H), 3.18 (m, 2H), 1.53 (m, 4H), 1.43 (s, 9H); ^{13}C NMR (100 MHz) (CDCl₃) δ 156.6, 156.3, 136.6, 128.4, 128.0, 79.4, 66.5, 64.9, 52.1, 40.8, 28.6, 28.4, 26.4.

Benzyl *t*-butyl (5-(1,3-dioxoisoindolin-2-yl)pentane-1,4-diyl)(S)-dicarbamate (77).

Triphenylphosphine (3.26 g 12.44 mmol) and phthalimide (1.83 g, 12.44 mmol) were added to a flask containing dry THF (15 mL). Benzyl *t*-butyl (5-hydroxypentane-1,4-diyl)(S)-dicarbamate, **76**, (3.65g, 10.4 mmol) was added and the flask was cooled to 0ºC. DIAD (2.45 mL, 12.4 mmol) was added dropwise and reaction was allowed to stir for 30 minutes at 0 ºC and then overnight at room temperature. The mixture was concentrated under reduced pressure and the residue purified using an ISCO chromatograph with silica (0–70% ethyl acetate/hexane) to give product as a pale yellow solid. (3.9 g, 79%); MP 131–133 ºC; ^{1}H NMR (CDCl₃) (400 MHz) δ 7.85 (m, 2H), 7.71 (m, 2H), 7.37 (m, 5H), 5.10 (s, 2H), 4.99 (brs, 1H), 4.70 (d, 1H, *J* = 8), 3.98 (m, 1H), 3.70 (m, 2H), 3.24(m, 2H), 1.60 (m, 4H), 1.24 (s, 9H); ^{13}C NMR (100 MHz) (CDCl₃) δ 168.5, 156.4, 155.7, 136.6, 133.9, 132.0, 128.4, 128.0, 123.3, 79.2, 66.5, 49.7, 42.2, 40.7, 30.0, 28.0, 26.4

Benzyl *t*-butyl (5-aminopentane-1,4-diyl)(S)-dicarbamate (78).

Benzyl *t*-butyl (5-(1,3-dioxoisoindolin-2-yl)pentane-1,4-diyl)(S)-dicarbamate, **77**, (3.92 g, 8.1 mmol) was dissolved in methanol (30 mL) and hydrazine monohydrate (0.8 mL, 16.3 mmol) was added. The reaction mixture was then refluxed for 2 hours and cooled to room temperature. The precipitate formed was filtered and methanol used to wash the filtrate. The filtrate was concentrated under reduced pressure and the remaining solid purified using an ISCO chromatograph (0-10% methanol/dichloromethane + 1% NH_4OH) to give product as a yellow oil (500 mg, 18%); ^1H NMR ($CDCl_3$) (400 MHz) δ 7.32 (m, 5H), 5.28 (m, 1H), 5.08 (s, 2H), 4.85 (d, 1H, $J = 8$), 3.50 (m, 1H), 3.19 (m, 2H), 2.71 (m, 1H), 2.60 (m, 1H), 1.51 (m, 4H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$) (100 MHz) δ 156.5, 156.1, 136.6, 128.4, 128.0, 79.1, 66.5, 52.6, 45.9, 40.8, 30.0, 28.4, 26.5.

Benzyl *t*-butyl (5-(2,2-diphenylacetamido)pentane-1,4-diyl)(S)-dicarbamate (79).

2,2-Diphenylacetic acid (63 mg, 0.29 mmol) was dissolved in dry DMF (5 mL) and EDC (113 mg, 0.59 mmol) and HOBt (80 mg, 0.59 mmol) were added and the reaction stirred at room temperature for 5 minutes. Benzyl *t*-butyl (5-aminopentane-1,4-diyl)(S)-dicarbamate, **78**, (95 mg, 0.27 mmol) was added, followed by 2,6-lutidine (0.09 mL, 0.81 mmol). Reaction was stirred at room temperature overnight. The reaction mixture was then diluted with ethyl acetate and washed with water, 1M HCl, saturated $NaHCO_3$, water and brine. The organic layer was dried over sodium sulfate and filtered. The filtrate was then concentrated under reduced pressure and purified using an ISCO chromatograph with silica (0 – 10% MeOH/dichloromethane) to give a white flaky solid (110 mg, 75%); MP

158-160 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.31 (m, 16H), 6.49 (brs, 1H), 5.12 (brs, 2H), 5.10 (s,2H), 4.89 (m, 2H), 3.61 (m, 1H), 3.27 (m, 2H), 3.15 (m, 2H), 1.50 (m,4H), 1.43 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 172.7, 156.5, 156.3, 139.4, 136.6, 128.8, 128.6, 128.5, 128.0, 127.2, 79.50, 66.5, 58.9, 50.7, 44.3, 40.6, 30.0, 23.4, 26.2

Benzyl (S)-(4-amino-5-(2,2-diphenylacetamido)pentyl)carbamate (80).

Benzyl *t*-butyl (5-(2,2-diphenylacetamido)pentane-1,4-diyl)(S)-dicarbamate, **79**, (100 mg, 0.18 mmol) was dissolved in dichloromethane (3mL) and cooled to 0 °C under nitrogen. Trifluoroacetic acid (2 mL) was added and reaction stirred at that temperature for 2 hours. The reaction mixture was dissolved in saturated NaHCO$_3$ and the organic layer separated. The combined organic layers were dried over sodium sulfate and solvent removed under reduced pressure to give product as a colorless oil (76 mg, 94%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.18 (m, 15H), 6.66 (brs, 1H), 5.11 (brs, 1H), 4.97 (s, 2H), 4.82 (s, 1H), 3.17 (m, 3H), 2.98 (m, 3H), 2.70 (brs, 1H), 1.21 (m, 5H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 172.8, 156.6, 139.47, 139.44, 136.6, 128.84, 128.81, 128.7, 128.5, 128.1, 128.0, 127.2, 66.6, 58.7, 50.9, 44.6, 40.7, 31.4, 26.1

Benzyl *t*-butyl (5-(2-benzyl-3-phenylpropanamido)pentane-1,4-diyl)(S)-dicarbamate (81).

2-Benzyl-3-phenylpropanoic acid (68 mg, 0.29 mmol) was dissolved in dry DMF (5 mL) and EDC (109 mg, 0.57 mmol) and HOBt (77 mg, 0.57 mmol) were added and the reaction stirred at room temperature for 5minutes. Benzyl *t*-butyl (5-aminopentane-1,4-diyl)(S)-

dicarbamate, **78**, (91 mg, 0.26 mmol) was added followed by 2,6-lutidine (0.09 mL, 0.78 mmol). Reaction was stirred at room temperature overnight. The reaction mixture was then diluted with ethyl acetate and washed with water, 1M HCl, saturated NaHCO$_3$, water and brine. The organic layer was dried over sodium sulfate and filtered. The filtrate was then concentrated under reduced pressure and purified using an ISCO chromatograph with silica (0–10% MeOH/dichloromethane) to give a white flaky solid, (119 mg, 80%); MP 132-134 ºC; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.26 (m, 15H), 5.59 (brs, 1H), 5.11 (s, 3H), 4.46 (d, 1H, J = 8), 3.32 (m, 1H), 3.05 (m, 6H), 2.80 (m, 2H), 2.61 (m, 1H), 1.44 (s, 9H), 1.41 (m, 2H), 1.07 (m, 2H) ; ^{13}C NMR (400 MHz) (CDCl$_3$) δ 174.8, 156.5, 155.8, 139.69, 139.62, 136.6, 128.9, 128.5, 128.49, 128.41, 128.0, 126.4, 126.3, 79.3, 66.5, 52.3, 50.5, 42.9, 40.6, 38.9, 38.6, 29.1, 28.4, 26.1

Benzyl (S)-(4-amino-5-(2-benzyl-3-phenylpropanamido)pentyl)carbamate (82).

Benzyl *t*-butyl (5-(2-benzyl-3-phenylpropanamido)pentane-1,4-diyl)(S)-dicarbamate, **81**, (81 mg, 0.14 mmol) was dissolved in dichloromethane (3 mL) and cooled to 0 ºC under nitrogen. Trifluoroacetic acid (2mL) was added and reaction stirred at that temperature for 2hrs. The reaction mixture was dissolved in saturated NaHCO$_3$ and the organic layer separated. The combined organic layers were dried over sodium sulfate and solvent removed under reduced pressure to give product as a colorless oil. (62 mg, 93%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.17 (m, 15H), 6.19 (brs, 1H), 5.12 (brs, 1H), 4.98 (s, 2H), 4.85 (brs, 2H), 2.80 (m, 10H), 1.32 (m, 2H), 1.05 (m, 2H); ^{13}C NMR (400 MHz) (CDCl$_3$) δ 175.0, 156.7, 139.58, 139.50, 136.4, 129.0, 128.9, 128.5, 128.49, 128.47, 128.1, 128.0, 126.46, 126.42, 66.7, 52.0, 51.0, 43.1, 40.4, 38.9, 38.8, 29.6, 25.8

(S)-N-(2,5-Diaminopentyl)-2-phenethyl-4-phenylbutanamide (83).

A gel like suspension of dibenzyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,4-diyl)(S)-dicarbamate, **91**, (75 mg, 0.12 mmol), 20% Pd(OH)$_2$/C (20 mg) and ethanol (10 mL) was purged and stirred under a hydrogen atmosphere overnight. The catalyst was then filtered and washed with 20% MeOH/dichloromethane. The solution was then concentrated and purified on ISCO chromatograph (0-20% MeOH/dichloromethane) to give the product as a colorless oil. (19.5 mg, 45%); ^{1}H NMR of triflate salt (400 MHz) (MeOD) δ 7.22 (m, 10H), 3.46 (m, 2H), 2.97 (m, 2H), 2.61 (m, 4H), 2.40 (m, 1H), 1.96 (m, 2H), 1.83 (m, 6H); ^{13}C NMR (100 MHz) (MeOD) δ 180.1, 143.0, 129.4, 129.3, 126.9, 52.7, 47.5, 42.0, 40.1, 35.6, 35.5, 34.7, 28.5, 24.4 ; HRMS (ESI) Calculated for C$_{23}$H$_{34}$N$_3$O (M+H)$^+$ 368.2696, found 368.2687

2-Phenethyl-4-phenylbutanoic acid (84).

A mixture of ethyl 2-phenethyl-4-phenylbutanoate, **87**, (200 mg, 0.44 mmol) and KOH (98 mg, 1.76 mmol) in ethanol/water (3 mL: 2 mL) was heated at 70 °C for 20 hours. The mixture was cooled to room temperature under reduced pressure and residue was extracted with ethyl acetate. The combined extracts were washed with brine and dried over sodium sulfate. This was then filtered and evaporated under reduced pressure to give product was a colorless oil; (117 mg, 100%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 10.74 (brs, 1H), 7.13 (m, 10H), 2.55 (m, 4H), 2.38 (m, 1H), 1.93 (m, 2H), 1.74 (m, 2H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 182.5, 141.4, 128.4, 126.0, 44.5, 33.8, 33.5

Diethyl 2,2-diphenethylmalonate (85)

A 60% dispersion of sodium hydride (1.25 g, 31.25 mmol) was added to a solution of diethyl malonate (1.90 mL, 12.5 mmol) in DMF (20 mL). The mixture was stirred at room temperature for 15 minutes. Then 2-bromoethyl) benzene (7 mL, 52.5 mmol) was added and the reaction mixture was warmed to 50 °C and stirred for 4 hours. The reaction was then allowed to reach room temperature, diluted with brine and extracted with diethyl ether. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The concentrate was purified using an ISCO chromatograph with silica using 0-5% ethyl acetate/ hexane to give a colorless oil (1.06 g, 23%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.42 (m, 4H), 7.34 (m, 6H), 4.34 (q, 4H), 2.72 (m, 4H), 2.47 (m, 4H), 1.41 (t, 6H, $J = 4$); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 171.3, 141.5, 128.6, 128.5, 126.2, 61.2, 57.6, 34.9, 30.9, 14.3

2-(Ethoxycarbonyl)-2-phenethyl-4-phenylbutanoic acid (86).

Diethyl 2,2-diphenethylmalonate, **85**, (1g, 2.9 mmol) was dissolved in 95% ethanol (25 mL) and water (7 mL) and KOH (178 mg, 3.2 mmol) was added. The mixture was refluxed for 4 hours. The ethanol was removed under reduced pressure and water was added. The mixture was washed with ether and the aqueous solution was acidified with conc. HCl at 0 °C. The mixture was extracted with ether and the combined ethereal layers washed with water and dried over anhydrous sodium sulfate to give a colorless oil (520 mg, 53%); ^{1}H NMR (400 MHz) (MeOD) δ 7.21 (m, 10H), 4.17 (q, 2H), 2.53 (m, 4H), 2.24 (m, 4H), 1.25 (t, 3H, $J = 8$); ^{13}C NMR (100 MHz) (MeOD) δ 173.2, 172.7, 142.9, 142.7, 129.6, 129.59,

129.53, 129.47, 129.45, 127.2, 127.1, 62.5, 62.4, 58.97, 58.94, 36.0, 35.9, 31.9, 31.8, 14.64, 14.60

Ethyl 2-phenethyl-4-phenylbutanoate (87).

A solution of 2-(ethoxycarbonyl)-2-phenethyl-4-phenylbutanoic acid, **86**, (520 mg, 1.53 mmol) in pyridine/water solution (14 mL) (6:1) was heated to reflux for 72 hours. The excess solvent was evaporated under reduced pressure and the residue acidified to pH = 2 with 1M HCl. The mixture was extracted with ethyl acetate and the combined organic fractions were washed with brine and dried over sodium sulfate. It was then filtered and evaporated and purified on ISCO chromatograph with silica (0–50% ethyl acetate/ hexane) to give product as a colorless oil (228 mg, 50%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.10 (m, 10H), 4.05 (q, 2H), 2.48 (m, 4H), 2.33 (m, 1H), 1.89 (m, 2H), 1.68 (m, 2H), 1.19 (t, 3H, J = 4); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 175.8, 141.7, 128.5, 128.49, 128.46, 126.0, 60.3, 44.8, 34.2, 33.6, 14.0

Dibenzyl (5-aminopentane-1,4-diyl)(S)-dicarbamate (88).

Dibenzyl (5-(1,3-dioxoisoindolin-2-yl)pentane-1,4-diyl)(S)-dicarbamate, **90**, (400 mg, 0.78 mmol) formed was dissolved in methanol (20 mL) and hydrazine monohydrate (80 µL, 1.55 mmol) was added. The reaction mixture was then refluxed for 2 hours and cooled to room temperature. The precipitate formed was filtered and methanol used to wash the filtrate. The filtrate was concentrated under reduced pressure and the remaining solid

purified using an ISCO chromatograph with silica (0-10% methanol/dichloromethane + 1% NH_4OH) to give product as a white powder. (206 mg, 68%); MP 113-115 ºC; ^{1}H NMR (CDCl3) (400 MHz) δ 7.36 (m, 10H), 5.29 (brs, 1H), 5.22 (brs, 1H), 5.09 (s, 4H), 3.60 (m, 1H), 3.19 (m, 2H), 2.70 (m, 2H), 1.70 (s, 2H), 1.46 (m, 4H); ^{13}C NMR (100 MHz) (CDCl3) δ 156.6, 136.6, 136.5, 128.53, 128.51, 128.1, 128.0, 66.6, 66.5, 53.0, 45.6, 40.7, 29.7. 26.5

Dibenzyl (5-hydroxypentane-1,4-diyl)(S)-dicarbamate (89).

To a solution of (S)-2,5-bis(((benzyloxy)carbonyl)amino)pentanoic acid (1000 mg, 2.5 mmol) in DME (10 mL) at -15 ºC were successively added a solution of N-methyl morpholine (310 µL, 2.82 mmol) and isobutyl chloroformate (320 µL, 2.5 mmol). The reaction was stirred at -15 ºC to -10 ºC for 15 minutes. The precipitated N-methyl morpholine HCl was removed by filtration and washed with DME (10 mL), the combine filtrates were chilled to -15 ºC in an ice-salt bath. Then a solution of sodium borohydride (283 mg, 7.5 mmol) in water (4 mL) was added in one portion at -15 ºC. This reaction mixture was stirred at this temperature for 10 minutes. The reaction was quenched by the addition of saturated aq. NH_4Cl and the resulting mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried over sodium sulfate. The solution was then filtered and concentrated under reduced pressure, purified on column (0-70% ethyl acetate/ hexane) to give product as a white powder (508 mg, 52%); MP 128-129 ºC; ^{1}H NMR (CDCl3) (400 MHz) δ 7.34 (m, 10H), 5.07 (m, 6H), 3.69 (m, 3H), 3.22 (m, 2H),

1.54 (m, 4H) ; ^{13}C NMR (100 MHz) (CDCl$_3$) δ 156.6, 156.5, 136.5, 136.3, 128.54, 128.52, 128.2, 128.1, 66.8, 66.7, 65.1, 52.8, 40.7, 28.5, 26.5

Dibenzyl (5-(1,3-dioxoisoindolin-2-yl)pentane-1,4-diyl)(S)-dicarbamate (90)

Triphenylphosphine (325 mg, 1.24 mmol) and phthalimide (182 mg, 1.24 mmol) were added to a flask containing dry THF (5 mL). Dibenzyl (5-hydroxypentane-1,4-diyl)(S)-dicarbamate, **89**, (400 mg, 1.03 mmol) was added and the flask was cooled to 0 °C. DIAD (250 mg, 1.24 mmol) was added dropwise and reaction allowed to stir for 30 minutes at 0 °C and then overnight at room temperature. The mixture was concentrated under reduced pressure and residue purified using an ISCO chromatograph with silica (0–70% ethyl acetate/hexane) to give product as a white solid. (491 mg, 92%); MP 99-101 °C; ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.83 (m, 2H), 7.72 (m, 2H), 7.32 (m, 10H), 5.22 (brs,1H), 5.09 (s, 2H), 5.04 (brs,1H), 4.97 (s, 2H), 4.03 (m, 1H) 3.76 (m, 2H), 3.24 (m, 2H), 1.57 (m, 4H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 168.5, 156.4, 156.2, 136.6, 136.5, 134.0, 132.1, 123.0, 131.9, 131.8, 128.6, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 123.4, 66.6, 66.5, 50.7, 41.7, 40.6, 30.0, 26.3.

Dibenzyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,4-diyl)(S)-dicarbamate (91).

2-Phenethyl-4-phenylbutanoic acid, **84**, (86.3 mg, 0.28 mmol) was dissolved in dry dichloromethane (5 mL) and oxalyl chloride (48 µL, 0.55 mmol) was added followed by a

catalytic amount of DMF (2 drops). The reaction was stirred at room temperature for 1 hour. The solvent was then evaporated and residue was pumped dry. The residue was redissolved in dichloromethane (5 mL), then dibenzyl (5-aminopentane-1,4-diyl)(S)-dicarbamate, **88**, (87 mg, 0.23 mmol) and triethylamine (60 µL, 0.40 mmol) was added. The reaction was allowed to stir at room temperature overnight. The reaction mixture was then dissolved in saturated sodium bicarbonate and extracted with dichloromethane. The combined organic layers were then dried over sodium sulfate and purified using an ISCO chromatograph (0-10% MeOH/dichloromethane) to give product as a white solid. (106.4 mg, 73%); MP 171-173 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.32 (m, 21H), 5.06 (m, 3H), 4.85 (d, 1H, J = 12), 3.71 (m, 1H), 3.43 (m, 1H), 3.19 (m, 3H), 2. 54 (m, 4H), 2.10 (m, 1H), 1.95 (m, 2H), 1.52 (m, 6H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 176.30, 156.8, 156.5, 141.6, 136.6, 136.2, 128.7, 128.5, 128.46. 128.43, 128.3, 128.0, 125.9, 125.7, 66.8, 66.4, 53.4, 51.8, 46.5, 43.6, 40.6, 34.4, 34.3, 33.6, 33.5, 30.0, 26.3.

(R)-N-(2,5-Diaminopentyl)-2-phenethyl-4-phenylbutanamide (92)

Benzyl (R)-(5-amino-1-(2-phenethyl-4-phenylbutanamido)pentan-2-yl)carbamate, **97**, (45.3 mg, 0.09 mmol) was dissolved in ethanol (10 mL) and 20% Pd(OH)$_2$/C (20 mg) was added. The reaction mixture was then purged and stirred under hydrogen atmosphere for overnight. Then the catalyst was filtered and the residue washed with 20% MeOH/dichloromethane. The solvent of the filtrate was concentrated under reduced pressure and dried under vacuum to give product was a colorless oil. (26.7 mg, 78%); ^{1}H NMR (400 MHz) (MeOD) δ 7.10 (m, 11H), 3.11 (m, 2H), 2.70 (m, 3H), 2.46 (m, 4H), 2.21 (m 1H), 1.80 (m, 2H), 1.59 (m, 4H), 1.18 (m, 2H); ^{13}C NMR (100 MHz) (MeOD) δ 181.2,

145.6, 131.9, 131.8, 129.5, 54.5, 50.9, 50.2, 49.1, 44.0, 38.4, 37.3, 35.4, 30.1, 24.9; HRMS (ESI) Calculated for $C_{23}H_{33}N_3O$ $(M+H)^+$ 368.2696, found 368.2684

Benzyl *t*-butyl (5-hydroxypentane-1,4-diyl)(R)-dicarbamate (93).

To a solution of (R)-2-(((benzyloxy)carbonyl)amino)-5-((*t*-butoxycarbonyl)amino)-pentanoic acid (1000 mg, 2.73 mmol) in DME (10 mL) at -15 °C were successively added a solution of N-methyl morpholine (0.34 mL, 3.08 mmol) and isobutyl chloroformate (0.35 mL, 2.73 mmol). The reaction was stirred at -15 °C to -10 °C for 15 minutes. The precipitated N-methyl morpholine HCl was removed by filtration and washed with DME (10 mL), the combine filtrates were chilled to -15 °C in an ice-salt bath. Then a solution of sodium borohydride (310 mg, 8.19 mmol) in water (4 mL) was added in one portion at -15 °C. This reaction mixture was stirred at this temperature for 10 minutes. The reaction was quenched by the addition of saturated aq. NH₄Cl and the resulting mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried over sodium sulfate. The solution was then filtered and concentrated under reduced pressure, purified on column (0-70% ethyl acetate/ hexane) to give product as a colorless oil (855 mg, 91%); ^{1}H NMR (CDCl₃) (400 MHz) δ 7.28 (s, 5H), 5.46 (brs, 1H), 4.98 (s, 2H), 4.84 (brs, 1H), 4.03 (m, 1H), 3.60 (m, 2H), 2.99 (m, 2H), 1.42 (m, 4H), 1.36 (s, 9H); ^{13}C NMR (CDCl₃) (400 MHz) δ 156.6, 156.1, 136.4, 128.5, 128.1, 128.0, 79.3, 66.8, 65.0, 62.7, 52.9, 52.4, 40.3, 29.8, 28.4, 26.7, 26.0.

Benzyl *t*-butyl (5-(1,3-dioxoisoindolin-2-yl)pentane-1,4-diyl)(R)-dicarbamate (94).

Triphenylphosphine (652 mg, 2.49 mmol) and phthalimide (366 mg, 2.49 mmol) were added to a flask containing dry THF (5 mL). Benzyl *t*-butyl (5-hydroxypentane-1,4-diyl)(R)-dicarbamate, **93**, (730 mg, 2.27 mmol) was added and the flask was cooled to 0 ºC. DIAD (503 mg, 2.49 mmol) was added dropwise and reaction was allowed to stir for 30 minutes at 0 ºC and overnight at room temperature. The mixture was concentrated under reduced pressure and the residue purified on an ISCO chromatograph with silica (0–70% ethyl acetate/hexane) to give product as a yellow solid. (736 mg, 73%); MP 74-76 ºC; ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.82 (m, 2H), 7.71 (m, 2H), 7.27 (m, 5H), 5.22 (brs, 1H), 4.95 (s, 2H), 4.70 (brs, 1H), 4.02 (m, 1H) 3.75 (m, 2H), 3.14 (m, 2H), 1.55 (m, 4H), 1.44 (s, 9H); ^{13}C NMR (CDCl$_3$) (100 MHz) δ 168.4, 156.3, 156.0, 136.6, 133.9, 131.8, 128.4, 128.3, 127.8, 127.7, 123.3, 78.9, 66.3, 50.7, 41.9, 40.2, 29.9, 28.4, 26.4

Benzyl *t*-butyl (5-aminopentane-1,4-diyl)(R)-dicarbamate (95).

Benzyl *t*-butyl (5-(1,3-dioxoisoindolin-2-yl)pentane-1,4-diyl)(R)-dicarbamate, **94**, (700 mg, 1.45 mmol) formed was dissolved in methanol (15 mL) and hydrazine monohydrate (0.14 mL, 2.90 mmol) was added . The reaction mixture was then refluxed for 2 hours and then cooled to room temperature. The precipitate formed was filtered and methanol used to wash the solid. The filtrate was concentrated under reduced pressure and the residue purified using an ISCO chromatograph with silica (0-10% methanol/dichloromethane + 1% NH$_4$OH) to give the desired compound as a yellow oil, (166 mg, 33%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.25 (m, 5H), 5.41 (d, 1H, *J* = 8), 5.00 (s, 2H), 4.76 (brs, 1H);

3.53 (m, 1H), 3.02 (m, 2H), 2.61 (m, 2H), 1.40 (m, 4H), 1.36 (s, 9H); ^{13}C NMR (CDCl$_3$) (100 MHz) δ 156.6, 156.0, 136.6, 128.4, 128.1, 128.0, 78.9, 66.6, 53.2, 50.3, 45.7, 40.2, 29.7, 28.4, 26.6.

Benzyl *t*-butyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,4-diyl)(R)-dicarbamate (96)

2-Phenethyl-4-phenylbutanoic acid, **84**, (75.5 mg, 0.28 mmol) was dissolved in dry DMF (5 mL) and EDC (109 mg, 0.57 mmol) and HOBt (77 mg, 0.57 mmol) were added and the reaction stirred at room temperature for 5 minutes. Benzyl *t*-butyl (5-aminopentane-1,4-diyl)(R)-dicarbamate, **95**, (90 mg, 0.26 mmol) was added followed by 2,6-lutidine (90 µL, 0.78 mmol). Reaction was stirred at room temperature overnight. The reaction mixture was then diluted with ethyl acetate and washed with water, 1M HCl, saturated NaHCO$_3$, water and brine. The organic layer was dried over sodium sulfate and filtered. Filtrate was then concentrated and the residue purified using an ISCO chromatograph with silica (0–10% MeOH/dichloromethane) to give a tan colored solid, (78 mg, 50%); MP 148-150 ºC; ^{1}H NMR (400 MHz) δ 7.11 (m, 15H), 6.09 (s, 1H), 5.30 (d, 1H, *J* = 8), 4.98 (d, 1H, *J* = 12), 4.78 (d, 1H, *J* = 12), 4.63 (s, 1H), 3.67 (m, 1H), 3.34 (m, 1H), 3.19 (m, 1H), 3.03 (m, 2H), 2.46 (m, 4H), 1.93 (m, 3H), 1.66 (m,2H), 1.45 (m, 4H), 1.35 (s, 9H); ^{13}C NMR δ 176.3, 156.9, 156.1, 141.66, 141.64, 136.2, 128.45, 128.43, 128.3, 128.0, 126.1, 125.9, 79.2, 66.8, 51.8, 46.6, 43.7, 40.1, 34.49, 34.4, 34.2, 33.9, 33.6, 30.1, 28.4, 26.5

Benzyl (R)-(5-amino-1-(2-phenethyl-4-phenylbutanamido)pentan-2-yl)carbamate (97).

Benzyl *t*-butyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,4-diyl)(R)-dicarbamate, **96**, (71 mg, 0.12 mmol) was dissolved in dichloromethane (3mL) and cooled to 0 °C under nitrogen. Trifluoroacetic acid was added and reaction stirred at that temperature for 2 hours. The reaction mixture was dissolved in saturated $NaHCO_3$ and the organic layer separated. The combined organic layers were dried over sodium sulfate and solvent removed under reduced pressure to give product as a yellow oil (51 mg, 86%); ^{1}H NMR (MeOD) (400 MHz) δ 7.09 (m, 16H), 4.81 (m, 1H), 3.69 (m, 1H), 3.11 (m, 2H), 2.70 (m, 2H), 2.43 (m, 4H), 2.17 (m, 1H), 1.79 (m, 2H), 1.62 (m, 6H) ; ^{13}C NMR δ (MeOD) (100 MHz) 178.7, 158.8, 143.2, 138.0, 129.4, 128,9, 128.8, 126.9, 67.5, 52.2, 47.7, 44.3, 41.3, 36.0, 34.8, 34.7, 31.1, 27.4

Diethyl 2,2-bis(2-(pyridin-4-yl)ethyl)malonate (98).

Sodium hydride (60% dispersion in oil) was added to a solution of diethyl malonate (2 g. 12.5 mmol) in ethanol (20 mL). The mixture was stirred at 80 °C for 30 minutes. Then vinyl pyridine (5.26g, 50 mmol) was added and the reaction was stirred at that temperature for 4 hours. The reaction was concentrated under reduced pressure and the residue washed with brine and extracted with diethyl ether. The organic layer was dried over sodium sulfate, filtered, concentrated and purified on an ISCO chromatograph (0-100% ethyl acetate/ hexane) to give product as a colorless oil. (1.15g, 25%); ^{1}H NMR (400 MHz) ($CDCl_3$) δ 8.44 (m, 4H), 7.06 (m, 4H), 4.16 (m, 4H), 2.52 (m, 4H), 2.20 (m, 4H), 1.23 (m,

6H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 170.8, 170.7, 150.0, 149.7, 123.6, 61.4, 57.1, 33.7, 30.0, 14.0

4-(Pyridin-4-yl)-2-(2-(pyridin-4-yl)ethyl)butanoic acid (99).

Diethyl 2,2-bis(2-(pyridin-4-yl)ethyl)malonate, **98**, (1.15 g, 3.1 mmol) was dissolved in 20% HCl in water. The reaction mixture was stirred at 100 °C for 16 hours under nitrogen. The solvent was then removed on the kugelrohr and the residue was dried under vacuum to obtain product as a yellow oil (1.13 g, 100%); ^{1}H NMR (400 MHz) (MeOD) δ 8.81 (d, 4H, J = 8), 8.07 (d, 4H, J = 8), 3.07 (m,4H), 2.57 (m, 1H), 2.11 (m, 4H); ^{13}C NMR (100 MHz) (MeOD) δ 177.8, 165.6, 142.1, 128.7, 45.6, 34.7, 33.0

Dibenzyl ((4S)-5-(4-(pyridin-4-yl)-2-(2-(pyridin-4-yl)ethyl)butanamido)pentane-1,4-diyl)dicarbamate (100).

4-(Pyridin-3-yl)-2-(2-(pyridin-4-yl)ethyl)butanoic acid, **99**, (100 mg, 0.29 mmol) was dissolved in DMF (5 mL), EDC (109 mg, 0.57 mmol) and HOBT (77 mg, 0.57 mmol) were added. Reaction mixture was stirred at room temperature for 5 minutes under nitrogen. Then dibenzyl (5-aminopentane-1,4-diyl)(S)-dicarbamate, **88**, (100 mg, 0.26 mmol) was added followed by 2,6-lutidine (90 µL, 0.78 mmol). Reaction was stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate and washed with water, saturated sodium bicarbonate solution, water and brine. The combined organic layers were dried over sodium sulfate and concentrated to give product as a tan

colored solid; (104.7 mg, 56%); MP 180-182 °C; ^{1}H NMR (400 MHz) (MeOD) δ 8.38 (s, 4H), 7.30 (m, 15H), 5.03 (m, 2H), 4.88 (s, 4H), 3.80 (m, 1H), 3.33 (m, 1H), 3.16 (m, 3H), 2.59 (m, 4H), 2.28 (m,1H), 1.94 (m, 2H), 1.77 (m, 2H), 1.58 (m, 4H); ^{13}C NMR (100 MHz) (MeOD) δ 177.6, 158.7, 154.0, 153.9, 149.8, 149.7, 138.4, 138.2, 129.4, 128.9, 128.7, 128.6, 125.6, 67.4, 67.3, 52.4, 47.4, 44.5, 41.5, 34.4, 34.0, 33.9, 31.3, 28.8. 27.4

N-((S)-2,5-Diaminopentyl)-4-(pyridin-4-yl)-2-(2-(pyridin-4-yl)ethyl)butanamide (101)

Dibenzyl   ((4S)-5-(4-(pyridin-3-yl)-2-(2-(pyridin-4-yl)ethyl)butanamido)pentane-1,4-diyl)dicarbamate, **100**, (47.5 mg, 0.074 mmol) was dissolved in ethanol and 20% Pd(OH)$_2$/C was added. Reaction was purged and stirred under hydrogen atmosphere for overnight. The catalyst was filtered and washed with 20% methanol/dichloromethane. The filtrate was concentrated under reduced pressure and dried under vacuum to obtain the product as a yellow oil. (20 mg, 74%); ^{1}H NMR (400 MHz) (MeOD) δ 8.30 (m, 4H), 7.18 (m, 4H), 3.15 (m, 1H), 2.99 (m, 2H), 2.73 (m, 1H), 2.57 (m, 5H), 1.21 (m, 1H), 1.86 (m, 2H), 1.72 (m, 2H), 1.50 (m, 4H); ^{13}C NMR (100 MHz) (MeOD) δ 177.7, 153.6, 150.0, 125.5, 52.0, 51.9, 47.4, 46.7, 46.6, 42.1, 34.3, 33.9, 33.24, 33.20, 29.0, 28.8; HRMS (ESI) Calculated for C$_{21}$H$_{31}$N$_5$O (M+H)$^+$ 370.2601, found 370.2602

Dibenzyl (5-(3-phenylpropanamido)pentane-1,4-diyl)(S)-dicarbamate (102).

3-Phenylpropionic acid (41 mg, 0.28 mmol) was dissolved in DMF (5 mL), EDC (105 mg, 0.55 mmol) and HOBT (74 mg, 0.55 mmol) were added and reaction was stirred at room temperature for 5 minutes under nitrogen.   Dibenzyl (5-aminopentane-1,4-diyl)(S)-dicarbamate, **88**, (97 mg, 0.25 mmol) was added followed by 2,6-lutidine (90 μL, 0.75 mmol). The reaction mixture was then stirred at room temperature overnight. The reaction mixture was then diluted with ethyl acetate and washed with water, 1M HCl, saturated NaHCO$_3$, water and brine. The organic layers were dried over sodium sulfate, concentrated and purified on an ISCO chromatograph (0-10% MeOH/dichloromethane) to give product as a white solid. (106 mg, 82%); MP 159-161 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.22 (m, 12H), 7.09 (m, 3H), 5.91 (brs, 1H), 5.00 (s, 2H), 4.99 (s, 2H), 4.95 (brs, 1H), 4.88 (brs, 1H), 3.55 (m, 1H), 3.13 (m, 4H), 2.82 (m, 2H), 2.34 (m , 2H), 1.72 (m, 1H), 1.44 (m, 2H), 1.30 (m, 2H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 173.0, 156.8, 156.5, 140.7, 136.5, 136.4, 128.5, 128.3, 128.2. 128.1, 128.0, 126.2, 66.7, 66.6, 51.5, 43.7, 40.5, 38.2, 31.6, 29.5, 26.3

(S)-N-(2,5-Diaminopentyl)-3-phenylpropanamide (103)

Dibenzyl (5-(3-phenylpropanamido)pentane-1,4-diyl)(S)-dicarbamate, **102**, (85 mg, 0.16 mmol) was dissolved in ethanol (15 mL)   and 20% Pd(OH)$_2$/C (20mg) was added. Reaction was purged and stirred under hydrogen atmosphere for overnight. The catalyst was filtered and washed with 20% methanol/dichloromethane.   The filtrate was concentrated under reduced pressure and dried under vacuum to obtain the product as a yellow oil. (41 mg, 100%); ^{1}H NMR (400 MHz) (MeOD) δ 7.12 (m, 5H), 3.08 (m, 1H),

2.92 (m, 1H), 2.82 (t, 2H, $J = 8$), 2.56 (m, 3H), 2.41 (t, 2H, $J = 8$), 1.40 (m, 2H), 1.26 (m, 1H), 1.12 (m, 1H); ^{13}C NMR (100 MHz) (MeOD) δ 142.1, 129.5, 129.4, 127.2, 51.8, 46.5, 42.3, 38.9, 32.9, 32.8, 30.7, 29.5; HRMS (ESI) Calculated for $C_{14}H_{24}N_3O$ (M+H)$^+$ 250.1914, found 250.1914

Dibenzyl (5-(4-phenylbutanamido)pentane-1,4-diyl)(S)-dicarbamate (104)

4-Phenylbutanoic acid (47 mg, 0.29 mmol) was dissolved in dry DMF (5mL) and EDC (109 mg, 0.57 mmol) and HOBt (76 mg, 0.57 mmol) were added and the reaction stirred at room temperature for 5 minutes. Benzyl *t*-butyl (5-aminopentane-1,4-diyl)(S)-dicarbamate, **88**, (100 mg, 0.26 mmol) was added followed by 2,6-lutidine (0.09 mL, 0.75 mmol). Reaction was stirred at room temperature overnight. The reaction mixture was then diluted with ethyl acetate and washed with water, 1M HCl, saturated NaHCO₃, water and brine. The organic layer was dried over sodium sulfate and filtered. Filtrate was then concentrated and purified on an ISCO chromatograph (0–10% MeOH/dichloromethane) to give a white solid. (55 mg, 40%); MP 143-145 °C; ^{1}H NMR (100 MHz) (CDCl₃) δ 7.26 (m, 16H), 6.13 (brs, 1H), 5.10 (s, 2H), 5.09 (s, 1H), 5.07 (brs, 1H), 3.72 (m, 1H), 3.27 (m, 4H), 2.63 (t, 2H, $J = 8$), 2.14 (m, 2H), 1.96 (m, 2H), 1.52 (m, 4H); ^{13}C NMR (100 MHz) (CDCl₃) δ 173.6, 156.5, 141.4, 136.5, 136.3, 128.5, 128.4, 128.3, 128.1, 128.08, 128.04, 125.9, 66.8, 66.6, 51.7, 43.9, 40.5, 35.7, 35.1, 29.8, 27.0, 26.4

(S)-N-(2,5-Diaminopentyl)-4-phenylbutanamide (105)

Dibenzyl (5-(4-phenylbutanamido)pentane-1,4-diyl)(S)-dicarbamate, **104**, (57 mg, 0.11 mmol) was dissolved in ethanol (10mL) and 20% Pd(OH)2/C (35 mg) was added. The reaction mixture was then purged and stirred under hydrogen atmosphere overnight. Then the catalyst was filtered and the residue washed with 20% MeOH/dichloromethane. The solvent of the filtrate was removed under reduced pressure and dried under vacuum to give the product as a pale yellow oil, (26 mg, 100%); ^{1}H NMR (MeOD) (400 MHz) δ 7.10 (m, 6H), 3.14 (m, 1H), 2.98 (m, 1H), 2.61 (m, 5H), 2.13 (m, 2H), 1.81 (m, 2H), 1.45 (m, 3H), 1.20 (m, 1H); ^{13}C NMR (MeOD) (100 MHz) δ 176.3, 142.9, 129.5, 129.4, 127.0, 51.9, 46.4, 41.9, 36.5, 36.3, 32.9, 28.7, 28.6; HRMS (ESI) Calculated for $C_{15}H_{25}N_3O$ (M+H)$^+$ 264.2070, found 264.2072

(S)-N-(3,5-Diaminopentyl)-2-phenethyl-4-phenylbutanamide (106)

Dibenzyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,3-diyl)(R)-dicarbamate, **116**, (65.5 mg, 0.103 mmol) was dissolved in ethanol (10 mL) and 20% Pd(OH)$_2$/C (30 mg) was added. Reaction mixture was purged and stirred under a hydrogen atmosphere. The catalyst was removed by filtration and washed residue with 20% methanol/ dichloromethane to give product as a colorless oil. (36.1 mg, 97%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.10 (m, 10H), 2.42 (m, 1H), 3.21 (m, 2H), 2.63 (m, 2H), 2.45 (m, 4H), 2.16 (m, 1H), 1.80 (m, 2H), 1.56 (m, 6H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 178.2, 143.1, 129.4, 129.3, 127.0, 126.9, 68.3, 54.3, 47.6, 45.9, 40.5, 39.5, 38.4, 37.5, 37.1, 36.6, 35.9, 34.8, 32.8; HRMS (ESI) Calculated for $C_{23}H_{33}N_3O$ (M+H)$^+$ 368.2696, found 368.2698

(S)-N-(4,5-Diaminopentyl)-2-phenethyl-4-phenylbutanamide (107).

Dibenzyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,2-diyl)(R)-dicarbamate, **123**, (58 mg, 0.09 mmol) was dissolved in ethanol (10 mL) and 20% Pd(OH)$_2$/C (30 mg) was added. The reaction mixture was then purged and stirred under hydrogen atmosphere for overnight. Then the catalyst was filtered and the residue washed with 20% MeOH/dichloromethane. The solvent of the filtrate was removed under reduced pressure and dried under vacuum to give the product as a colorless oil. (14 mg, 42%); ^{1}H NMR (MeOD) (400 MHz) δ 7.09 (m, 11H), 3.14 (m, 2H), 2.66 (m, 1H), 2.45 (m, 6H), 2.13 (m, 1H), 1.79 (m, 2H), 1.50 (m, 6H); ^{13}C NMR (100 MHz) (MeOD) δ 178.2, 143.1, 129.4, 129.3, 126.9, 53.3, 47.6, 47.5, 40.2, 35.9, 34.8, 32.8, 27.1; HRMS (ESI) Calculated for C$_{23}$H$_{33}$N$_3$O (M+H)$^+$ 368.2696, found 368.2687

Dibenzyl (5-aminopentane-1,3-diyl)(R)-dicarbamate (108).

Dibenzyl (5-(1,3-dioxoisoindolin-2-yl)pentane-1,3-diyl)(S)-dicarbamate, **115**, (120 mg, 0.23 mmol) was dissolved in methanol (10 mL) and hydrazine monohydrate (0.02 mL, 0.47 mmol) was added. The reaction mixture was then refluxed for 2 hours and cooled to room temperature. The precipitate formed was filtered and methanol used to wash the filtrate. The filtrate was concentrated under reduced pressure and the remaining solid purified on an ISCO chromatograph with silica (0-10% methanol/dichloromethane + 1% NH$_4$OH) to give product as a colorless oil. (80 mg, 90%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.30 (m, 10H). 5.05 (s, 4H), 3.68 (m, 1H), 3.19 (m, 2H), 2.62 (t, 2H, J = 8), 1.64 (m, 4H) ; ^{13}C

NMR (100 MHz) (CDCl$_3$) δ 161.4, 161, 3, 141.0, 140.9, 132.0, 131.5, 131.3, 131.2, 69.9, 50.31, 41.9. 41.7, 41.3, 39.0

Dibenzyl (4-hydroxybutane-1,3-diyl)(S)-dicarbamate (109)

To a solution of (S)-2,4-bis(((benzyloxy)carbonyl)amino)butanoic acid (1000 mg, 2.77 mmol) in DME (10 mL) at -15 °C were successively added N-methyl morpholine (340 µL, 3.13 mmol) and isobutyl chloroformate (360 µL, 2.77 mmol). The reaction was stirred at -15 °C to -10 °C for 15 minutes. The precipitated N-methyl morpholine HCl was removed by filtration and washed with DME (10 mL) and the combine filtrates were chilled to -15 °C in an ice-salt bath. Then a solution of sodium borohydride (378 mg, 8.31 mmol) in water (5 mL) was added in one portion at -15 °C. This reaction mixture was stirred at this temperature for 10 minutes. The reaction was quenched by the addition of saturated aq. NH$_4$Cl and the resulting mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried over sodium sulfate. The solution was then filtered and concentrated under reduced pressure, purified on column (0-70% ethyl acetate/ hexane) to give product as a white powder (491 mg, 48%); MP 90-92 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.33 (s, 10H), 5.72 (s, 1H), 5.63 (d, 1H, $J = 8$), 5.08 (s, 4H), 3.48 (m, 4H), 3.02 (m, 1H), 1.71 (m, 1H), 1.57 (m, 1H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 157.0, 156.7, 136.5,136.3, 128.55, 128.50, 128.1, 128.07, 128.02, 66,8, 66.6, 64.6, 50.4, 37.7, 31.7

(S)-2,4-Bis((((benzyloxy)carbonyl)amino)butyl methanesulfonate (110).

Dibenzyl (4-hydroxybutane-1,3-diyl)(S)-dicarbamate, **109**, (300 mg, 0.81 mmol) was dissolved in dichloromethane (5 mL) and triethylamine (0.22 mL, 1.62 mmol) was added. Then mesyl chloride (0.09 mL, 1.21 mmol) was added and reaction stirred at room temperature for 1 hour. Then the organic layer was washed with saturated sodium bicarbonate solution and further extracted with dichloromethane. Reaction was purified on an ISCO chromatograph (60% ethyl acetate/hexane) to produce product as a colorless oil. (184 mg, 52%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.33 (m, 10H), 5.56 (m, 2H), 5.08 (m, 4H), 4.17 (m, 2H), 3.98 (m, 1H), 3.40 (m, 1H), 3.07 (m, 1H), 2.92 (s, 3H), 1.76 (m, 1H), 1.63 (m, 1H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 156.6, 156.4, 136.5, 136.2, 128.6, 128.5, 128.2, 128.13, 128.1, 71.0, 67.0, 66.6, 60.4, 48.0, 37.5, 37.3, 37.1, 31.3

Dibenzyl (4-cyanobutane-1,3-diyl)(S)-dicarbamate (111)

(S)-2,4-Bis((((benzyloxy)carbonyl)amino)butyl methanesulfonate, **110**, (290 mg, 0.65 mmol) was dissolved in DMF (2 mL) under nitrogen atmosphere. Then KCN (46 mg, 0.71 mmol) was added stirred at 70 °C for overnight. The reaction mixture was then dissolved in water and extracted with ethyl acetate. The combined organic layer was washed with water again, then brine and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude residue purified on an ISCO chromatograph to give product as a colorless oil. ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.21 (m, 10H), 5.59 (d, 1H, $J = 8$), 5.32 (brs, 1H), 4.97 (s, 2H), 4.96 (s, 2H), 3.82 (m, 1H), 3.24 (m, 1H), 2.97 (m, 1H), 2.50 (dd, 1H, $J = 4, 16$), 2.39 (dd, 1H, $J = 4, 16$), 1.62 (m, 2H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ

156.5, 155.9, 136.3, 135.9, 128.6, 128.5, 128.3, 128.2, 128.1, 117.0, 67.2, 66.8, 45.6, 37.3, 34.0, 23.7

Dibenzyl (5-hydroxypentane-1,3-diyl)(S)-dicarbamate (114).

Methyl (S)-3,5-bis(((benzyloxy)carbonyl)amino)pentanoate, **58**, was dissolved in ethanol/THF (10:1) (11 mL) under nitrogen. Lithium borohydride was added and reaction stirred at room temperature for overnight. The reaction mixture was dissolved in water and extracted with ethyl acetate and purified on an ISCO chromatograph (0-60% ethyl acetate/hexane) to give product as a colorless oil (216 mg, 78%); ^{1}H NMR (MeOD) (400 MHz) δ 7.35 (m, 12H), 5.10 (s, 4H), 3.80 (s, 1H), 3.22 (m, 4H), 1.69 (m, 4H); ^{13}C NMR (MeOD) (100 MHz) δ 158.94, 158.82, 138.4, 129.5, 129.0, 128.85, 128.8, 67.47. 59.8, 47.4, 39.0, 38.9, 36.4

Dibenzyl (5-(1,3-dioxoisoindolin-2-yl)pentane-1,3-diyl)(S)-dicarbamate (115).

Triphenylphosphine (175 mg, 0.67 mmol) and phthalimide (99 mg, 0.67 mmol) were added to a flask containing dry THF (5 mL). Dibenzyl (5-hydroxypentane-1,3-diyl)(S)-dicarbamate, **114**, (217 mg, 0.56 mmol) was added and the flask was cooled to 0 °C. DIAD (0.13 mL, 0.67 mmol) was added dropwise and the reaction was allowed to stir for 30 minutes at 0 °C and overnight at room temperature. The mixture was concentrated under reduced pressure and the residue purified on an ISCO chromatograph with silica (0 –70% ethyl acetate/hexane) to give product as a white solid. (151 mg, 53%); MP 103-105 °C; [1]H

NMR (400 MHz) (CDCl₃) δ 7.79 (m, 2H), 7.66 (m, 2H), 7.22 (m,10H), 5.61 (m, 1H), 5.33(d, 1H, *J= 12*), 5.08 (m, 4H0, 3.74(m, 3H), 4.43 (m, 1H), 3.02 (m, 1H), 1.83 (m, 3H), 1.51 (m, 1H); ^{13}C NMR (100 MHz) (CDCl₃) δ 168.2, 156.6 156.5, 136.7, 136.4, 133.9, 132.0, 128.5, 128.4, 128.1, 128.0, 127.9, 123.2, 66.7, 66.5, 47.0, 37.4, 35.1. 33.7,

Dibenzyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,3-diyl)(R)-dicarbamate (116).

2-Phenethyl-4-phenylbutanoic acid, **84**, (57.4 mg, 0.21 mmol) was dissolved in dry DMF (5mL) and EDC (80 mg, 0.42 mmol) and HOBt (57 mg , 0.42 mmol) were added and the reaction stirred at room temperature for 5 minutes. Dibenzyl (5-aminopentane-1,3-diyl)(R)-dicarbamate, **108**, (75 mg, 0.19 mmol) was added followed by 2,6-lutidine (0.07 mL, 0.57 mmol). Reaction was stirred at room temperature overnight. The reaction mixture was then diluted with ethyl acetate and washed with water, 1M HCl, saturated NaHCO₃, water and brine. The organic layer was dried over sodium sulfate and filtered. The filtrate was then concentrated and purified with an ISCO chromatograph with silica (0–10% MeOH/dichloromethane) to give a white solid. (90 mg, 75%); MP 152-154 ºC; ^{1}H NMR(CDCl₃) (400 MHz) δ 7.26 (m, 20H), 6.21 (brs, 1H), 5.32 (brs, 1H), 5.06 (m, 5H), 3.75 (m, 1H), 3.63 (m, 1H), 3.39 (m, 1H), 3.06 (m, 2H), 2.58 (m 4H), 2.07 (m. 3H), 1.75 (m, 4H), 1.56 (m, 2H); ^{13}C NMR (100 MHz) (CDCl₃) 175.4, 157.0, 156.5, 141.7, 136.6, 136.3, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 125.9, 66.9, 66.6, 60.4, 46.77, 46.6, 37.7, 36.0, 35.6, 34.5

Methyl (S)-2-(((benzyloxy)carbonyl)amino)-5-((*tert*-butoxycarbonyl)amino)-pentanoate (117).

Cbz ornithine (Boc) acid (1 g, 2.73 mmol) was dissolved in DMF (5 mL) and K_2CO_3 (452.6 mg, 3.26 mmol). The reaction was cooled to 0 ^{0}C and methyl iodide (775 mg, 5.46 mmol) was added. The reaction was allowed to warm to room temperature and stirred at the temperature overnight. Then the reaction mixture was washed with saturated sodium bicarbonate solution and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, concentrated and purified on an ISCO chromatograph (0-60% ethyl acetate/ hexane) to give product as a colorless oil. (761 mg, 73%); ^{1}H NMR δ 7.19 (s, 5H), 6.06 (d, 1H, *J = 8),* 5.12 (brs, 1H), 4.94 (s, 2H), 4.17 (m, 1H), 3.55 (s, 3H), 2.94 (m, 2H), 1.69 (m, 1H), 1.55 (m, 1H), 1.40 (m, 2H), 1.27 (s, 9H); ^{13}C NMR δ 172.7, 156.0, 155.9, 136.3, 128.2, 128.1, 127.9, 127.8, 78.6, 67.2, 66.5, 53.7, 39.8, 29.2, 28.2, 25.9

Benzyl *tert*-butyl (5-hydroxypentane-1,4-diyl)(S)-dicarbamate. (118).

To a solution of methyl (S)-2-(((benzyloxy)carbonyl)amino)-5-((*tert*-butoxycarbonyl)-amino)pentanoate, **117**, (431 mg, 1.13 mmol) in THF (5mL)/ethanol (1 mL) was added LiBH₄ (32 mg, 1.47 mmol) at 0 °C. The mixture was stirred at that temperature for 30 minutes and warmed to room temperature and stirred overnight. The reaction mixture was poured into water and extracted with ethyl acetate. The combine organic layers were washed with brine and dried over sodium sulfate and concentrated. It was purified on an ISCO chromatograph (0-70% ethyl acetate/ hexane) to give product as a colorless oil. (385

mg, 97%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.28 (m, 5H), 5.02 (s, 3H), 3.60 (m, 4H), 3.04 (m, 2H), 1.47 (m, 4H), 1.36 (m, 9H); ^{13}C NMR (CDCl$_3$) (100 MHz)δ 156.6, 156.1, 136.4, 128.5, 128.1, 128.0, 79.3, 66.8, 65.0, 62.7, 52.9, 52.4, 40.3, 29.8, 28.4, 26.7, 26.0

Benzyl *tert*-butyl (5-(1,3-dioxoisoindolin-2-yl)pentane-1,4-diyl)(S)-dicarbamate. (119).

Triphenylphosphine (325 mg, 1.24 mmol) and phthalimide (182 mg, 1.24 mmol) were added to a flask containing dry THF (5 mL). Dibenzyl (5-hydroxypentane-1,4-diyl)(S)-dicarbamate, **118**, (400 mg, 1.03 mmol) was added and the flask was cooled to 0 °C. DIAD (250 mg, 1.24 mmol) was added dropwise and the reaction was allowed to stir for 30 minutes at 0 °C and overnight at room temperature. The mixture was concentrated under reduced pressure and the residue purified on an ISCO chromatograph (0–70% ethyl acetate/hexane) to give product as a white solid. (340 mg, 69%); MP 74-76 °C; ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.82 (m, 2H), 7.71 (m, 2H), 7.27 (m, 5H), 5.18 (brs, 1H), 4.96 (s, 2H), 4.67 (brs, 1H), 4.02 (m, 1H) 3.75 (m, 2H), 3.14 (m, 2H), 1.60 (m, 4H), 1.44 (s, 9H); ^{13}C δ 168.4, 156.3, 156.0, 136.6, 133.9, 131.8, 128.4, 128.3, 127.8, 127.7, 123.3, 78.9, 66.3, 60.3, 50.7, 41.9, 40.2, 29.9, 28.4, 26.4

Benzyl *tert*-butyl (5-aminopentane-1,4-diyl)(S)-dicarbamate (120).

The phthalimide, **119**, (340 mg, 0.71 mmol) formed was dissolved in methanol (20 mL) and hydrazine monohydrate (0.07 mL, 1.41 mmol) was added. The reaction mixture was

then refluxed for 2 hours and then cooled to room temperature. The precipitate formed was filtered and methanol used to wash the filtrate. The filtrate was rotavapped and the remaining solid purified on an ISCO chromatograph (0-10% methanol/dichloromethane + 1% NH₄OH) to give product as a colorless oil. (164 mg, 66%); ^{1}H NMR (CDCl₃) (400 MHz) δ 7.25 (m, 5H), 5.41 (d, 1H, $J = 8$), 5.00 (s, 2H), 4.84 (brs, 1H); 3.50 (m, 1H), 3.01 (m, 2H), 2.61 (m, 2H), 1.40 (m, 4H), 1.36 (s, 9H); ^{13}C NMR (CDCl₃) (100 MHz) δ 156.6, 156.0, 136.6, 128.4, 128.1, 128.0, 78.9, 66.6, 53.2, 45.7, 40.2, 29.7, 28.4, 26.6, 25.0, 24.9

Dibenzyl *tert*-butyl pentane-1,2,5-triyl(S)-tricarbamate (121).

Benzyl *tert*-butyl (5-aminopentane-1,4-diyl)(S)-dicarbamate, **120**, (162.2 mg, 0.46 mmol) was dissolved in dichloromethane (5 mL) under nitrogen atmosphere and triethylamine (.08 mL, 0.55 mmol) was added. CbzCl (0.08 mL, 0.55 mmol) was added and the reaction stirred at room temperature until the reaction was completed as indicated by TLC. The dichloromethane was removed under reduced pressure and the residue purified on an ISCO chromatograph to give product as a white solid. (126 mg, 57%); MP 125-126 ℃; ^{1}H NMR (CDCl₃) (400 MHz) δ 7.34 (m, 10), 5.36 (brs, 1H), 5.23 (d, IH, $J = 4$), 5.09 (s, 4H), 4.72 (m, 1H), 3.72 (m, 1H), 3.21 (m, 4H), 1.45 (m, 4H), 1.42 (s, 9H); ^{13}C NMR (CDCl₃) (100 MHz) δ 157.0, 156.5, 156.1, 136.4, 128.5, 128.1, 128.07, 128.0, 79.1, 66.8, 66.7, 51.7, 45.0, 40.1, 29.5, 28.4, 26.4

Dibenzyl (5-aminopentane-1,2-diyl)(S)-dicarbamate (122).

Dibenzyl *tert*-butyl pentane-1,2,5-triyl(S)-tricarbamate, **121**, (189 mg, 0.39 mmol) was dissolved in dichloromethane (3mL) and the reaction mixture cooled to 0 ^{o}C under nitrogen. Trifluoroacetic acid (2 mL) was added and the reaction stirred at 0 ^{o}C for 3 hours. Upon completion of the reaction, the reaction was quenched with saturated solution of NaHCO$_3$ and extracted with dichloromethane. The organic layer was concentrated under reduced pressure to give product as a yellow solid (101.7 mg, 67%); MP 85-87 ^{o}C ; ^{1}H NMR (CDCl3) (400 MHz) δ 7.17 (m, 10H), 5.51 (m, 1H), 4.90 (m, 4H), 3.54 (m, 4H), 3.05 (m, 2H), 2.84 (m, 2H), 1.43 (m, 4H); 13 C δ 135.7, 128.6, 128.3, 127.7, 67.4, 51.2, 44.6, 39.9, 28.6, 23.4

Dibenzyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,2-diyl)(S)-dicarbamate (123).

2-Phenethyl-4-phenylbutanoic acid, **84**, (46 mg, 0.17 mmol) was dissolved in dry DMF (5 mL) and EDC (63 mg, 0.33 mmol) and HOBt (44 mg, 0.33 mmol) were added and the reaction stirred at room temperature for 5 minutes. Dibenzyl (5-aminopentane-1,2-diyl)(S)-dicarbamate, **122**, (59 mg, 0.15 mmol) was added followed by 2,6-lutidine (0.05 mL, 0.45 mmol). The reaction was stirred at room temperature overnight. The reaction mixture was then diluted with ethyl acetate and washed with water, 1M HCl, saturated NaHCO$_3$, water and brine. The organic layer was dried over sodium sulfate and filtered. The filtrate was then concentrated under reduced pressure and the residue purified using an ISCO chromatograph with silica (0–10% MeOH/dichloromethane) to give a white solid.

(63 mg, 67%); MP 145-147 °C; ^{1}H NMR (400 MHz) δ 7.25 (m, 20H), 5.77 (brs, 1H), 5.32 (m, 2H), 5.09 (s, 2H), 5.08 (s, 2H), 3.77 (m, 1H), 3.32 (m, 4H), 2.60 (m, 4H), 2.03 (m, 3H), 1.78 (m, 2H), 1.58 (m, 4H)

(S)-N-(2,3-Diaminopropyl)-2-phenethyl-4-phenylbutanamide (124).

Benzyl (S)-(1-amino-3-(2-phenethyl-4-phenylbutanamido)propan-2-yl)carbamate, **130**, (51 mg, 0.11 mmol) was dissolved in ethanol (10 mL) and 20% Pd(OH)$_2$/C (20 mg) was added. The reaction mixture was then purged and stirred under a hydrogen atmosphere overnight. Then the catalyst was filtered and the residue washed with 20% MeOH/dichloromethane. The solvent from the filtrate was concentrated under reduced pressure under to give product as a yellow oil. (29.6 mg, 81%); ^{1}H NMR (400 MHz) (MeOD) δ 7.20 (m, 11H), 3.27 (m, 2H), 2.88 (m, 1H), 2.73 (m, 1H), 2.58 (m, 4H), 2.32 (m, 2H), 1.92 (m, 2H), 1.78 (m, 2H); ^{13}C NMR δ 178.8, 143.0, 129.4, 129.3, 126.9, 53.8, 47.6, 45.8, 43.9, 35.8, 34.8; HRMS (ESI) Calculated for C$_{21}$H$_{29}$N$_3$O (M+H)$^+$ 340.2383, found 340.2377

(S)-N-(2,4-Diaminobutyl)-2-phenethyl-4-phenylbutanamide (125).

Dibenzyl (4-(2-phenethyl-4-phenylbutanamido)butane-1,3-diyl)(S)-dicarbamate, **133**, (54 mg, 0.09 mmol) was dissolved in ethanol (10 mL) and 20% Pd(OH)$_2$/C (30 mg) was added. The reaction mixture was then purged and stirred under a hydrogen atmosphere for overnight. Then the catalyst was filtered and the residue washed with 20% MeOH/dichloromethane. The solvent of the filtrate was removed under reduced pressure

and dried under vacuum to give product was a colorless oil. (21.5 mg, 69%); ^{1}H NMR (

MeOD) (400 MHz) δ 7.13 (m, 10H), 6.49 (brs, 1H), 3.30 (m, 1H), 2.92 (m, 2H), 2.49 (m,

6H); 1.97 (m, 3H), 1.54 (m, 4H); ^{13}C NMR (100 MHz) (MeOD) δ 175.6, 141.7, 128.4,

128.3, 125.9, 49.9, 46.5, 45.5, 38.9, 37.6, 34.4, 33; HRMS (ESI) Calculated for $C_{22}H_{31}N_3O$

$(M+H)^+$ 354.2540, found 354.2532

Benzyl *t*-butyl (3-hydroxypropane-1,2-diyl)(S)-dicarbamate (126).

To a solution of (S)-2-(((benzyloxy)carbonyl)amino)-3-((*t*-butoxycarbonyl)amino)-

propanoic acid (900 mg, 2.66 mmol) in DME (10 mL) at -15 °C were successively added

a solution of N-methyl morpholine (0.33 mL, 3 mmol) and isobutyl chloroformate (0.35

mL, 2.66 mmol). The reaction was stirred at -15 °C to -10 °C for 15 minutes. The

precipitated N-methyl morpholine HCl was removed by filtration and washed with DME

(10 mL), the combined filtrates were chilled to -15 °C in an ice-salt bath. Then a solution

of sodium borohydride (301 mg, 7.98 mmol) in water (5 mL) was added in one portion at

-15 °C. This reaction mixture was stirred at this temperature for 10 minutes. The reaction

was quenched by the addition of saturated aq. NH_4Cl and the resulting mixture was

extracted with ethyl acetate. The organic layer was washed with brine and dried over

sodium sulfate. The solution was then filtered and concentrated under reduced pressure,

purified on a ISCO chromatograph (0- 70% ethyl acetate/ hexane) to give product as a

white powder (675 mg, 78%); MP 117-120 °C; ^{1}H NMR (400 MHz) (MeOD) δ 7.34 (m,

5H), 5.09 (s, 2H), 3.73 (m, 1H), 3.24 (m, 4H), 1.44 (s, 9H); ^{13}C NMR (100 MHz) (MeOD)

δ 158.6, 138.3, 129.5, 129.0, 128.9, 80.3, 67.5, 63.0, 54.6, 42.1, 28.8.

Benzyl *t*-butyl (3-(1,3-dioxoisoindolin-2-yl)propane-1,2-diyl)(S)-dicarbamate (127).

Triphenylphosphine (709 mg, 2.71 mmol) and phthalimide (398 mg, 2.71 mmol) were added to a flask containing dry THF (6 mL). Benzyl *t*-butyl (3-hydroxypropane-1,2-diyl)(S)-dicarbamate, **126**, (730 mg, 2.26 mmol) was added and the flask was cooled to 0 °C. DIAD (548 mg, 2.71 mmol) was added dropwise and the reaction was allowed to stir for 30 minutes at 0 °C and overnight at room temperature. The mixture was concentrated under reduced pressure and residue purified on an ISCO chromatograph with silica (0 – 70% ethyl acetate/hexane) to give the product as a white solid. (556 mg, 55%); MP 92-95 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.83 (m, 2H), 7.71 (m, 2H), 7.28 (m, 5H), 5.70 (m, 1H), 5.26 (brs, 1H), 5.02 (s, 2H), 4.06 (m, 1H), 3.84 (m, 2H), 3.31 (m, 2H), 1.44 (s, 9H), ^{13}C NMR (400 MHz) (CDCl$_3$) δ 168.5, 156.6, 156.3, 136.4, 134.1, 131.8, 128.3, 127.9, 123.4, 79.7, 66.6, 51.6, 41.9, 39.2, 28.3, 21.9

Benzyl *t*-butyl (3-aminopropane-1,2-diyl)(S)-dicarbamate (128).

The benzyl *t*-butyl (3-(1,3-dioxoisoindolin-2-yl)propane-1,2-diyl)(S)-dicarbamate, **127**, (450 mg, 0.99 mmol) formed was dissolved in methanol (10 mL) and hydrazine monohydrate (0.1 mL, 1.98 mmol) was added. The reaction mixture was then refluxed for 2 hours and cooled to room temperature. The precipitate formed was filtered and methanol used to wash the filtrate. The filtrate was concentrated under reduced pressure and the remaining solid purified on an ISCO chromatograph with silica (0-10% methanol/dichloromethane + 1% NH$_4$OH) to give product as a colorless oil. (140 mg, 44%); ^{1}H NMR (400 MHz) (MeOD) δ 7.27 (m, 5H), 6.37 (s, 1H), 5.87 (s, 1H), 5.02 (s,

2H), 3.94 (s, 4H), 3.60 (m, 1H), 3.12 (m, 2H), 2.70 (m, 2H), 1.36 (s, 9H); ^{13}C NMR (100

MHz) (MeOD) δ 158.9, 129.4, 129.0, 128.9, 80.3, 67.6, 54.7, 43.4, 2.5, 28.7

Benzyl *t*-butyl (3-(2-phenethyl-4-phenylbutanamido)propane-1,2-diyl)(S)-

dicarbamate (129).

2-Phenethyl-4-phenylbutanoic acid, **84**, (100 mg, 0.37 mmol) was dissolved in dry DMF

(5 mL) and EDC (139 mg, 0.72 mmol) and HOBt (98 mg, 0.72 mmol) were added and the

reaction stirred at room temperature for 5 minutes. Benzyl *t*-butyl (3-aminopropane-1,2-

diyl)(S)-dicarbamate, **128**, (109 mg, 0.33 mmol) was added followed by 2,6-lutidine (0.11

mL, 0.99 mmol). The reaction was stirred at room temperature overnight. The reaction

mixture was then diluted with ethyl acetate and washed with water, 1M HCl, saturated

NaHCO$_3$, water and brine. The organic layer was dried over sodium sulfate and filtered.

The filtrate was then concentrated and purified using an ISCO chromatograph with silica

(0 – 10% MeOH/dichloromethane) to give a white solid. (126 mg, 67%); MP 177-179 ºC;

^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.14 (m, 15 H), 6.60 (brs, 1H), 6.06 (brs, 1H), 5.18 (m,

1H), 4.97 (m, 2H), 3.56 (m, 2H), 3.21 (m, 3H), 2.50 (m, 4H), 2.11 (m, 1H), 1.92 (m, 2H),

1.69 (m, 2H), 1.37 (m, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 177.1, 157.3, 156.6, 141.6,

141.5, 136.4, 128.5, 128.4, 128.3, 128.1, 128.0, 125.9, 80.0, 66.7, 53.6, 46.6, 40.8, 40.0,

34.7, 34.4, 33.7, 33.6, 28.3, 28.2

Benzyl (S)-(1-amino-3-(2-phenethyl-4-phenylbutanamido)propan-2-yl)carbamate (130).

Benzyl *t*-butyl (3-(2-phenethyl-4-phenylbutanamido)propane-1,2-diyl)(R)-dicarbamate, **129**, (114 mg, 0.20 mmol) was dissolved in dichloromethane (3 mL) and cooled to 0 °C under nitrogen. Trifluoroacetic acid (2 mL) was added and reaction stirred at that temperature for 2 hours. The reaction mixture was dissolved in saturated NaHCO$_3$ and the organic layer separated. The combined organic layers were dried over sodium sulfate and solvent removed under reduced pressure to give the product as a white flaky powder. (74.5 mg, 79%); MP 112-115 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.12 (m, 15H), 6.16 (m, 1H), 5.66 (m, 1H), 4.89 (m, 2H), 3.60 (m, 1H), 3.33 (m, 2H), 2.71 (m, 2H), 2.47 (m, 4H), 1.93 (m, 3H), 1.65 (m, 4H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 176.3, 156.8, 141.5, 136.2, 128.5, 128.4, 128.3, 128.1, 128.0, 125.9, 66.8, 52.9, 46.5, 43.0, 41.3, 34.4, 33.6, 33.5

Dibenzyl (4-(1,3-dioxoisoindolin-2-yl)butane-1,3-diyl)(S)-dicarbamate (131).

Triphenylphosphine (365 mg, 1.39 mmol) and phthalimide (204 mg, 1.39 mmol) were added to a flask containing dry THF (6 mL). Dibenzyl (4-hydroxybutane-1,3-diyl)(S)-dicarbamate, **109**, (432 mg, 1.39 mmol) was added and the flask was cooled to 0 °C. DIAD (281 mg, 1.39 mmol) was added dropwise and the reaction was allowed to stir for 30 minutes at 0 °C and then overnight at room temperature. The mixture was concentrated under reduced pressure and the residue purified on an ISCO chromatograph with silica (0–70% ethyl acetate/hexane) to give product as a white solid. (237 mg, 41%); MP 152-154 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.83 (m, 2H), 7.70 (m, 2H), 7.36 (m, 10H), 5.61 (brs,

1H), 5.46 (d, 1H, J = 8), 5.10 (m, 4H), 4.12 (m, 1H), 3.78 (m, 2H), 3.51 (m, 1H), 3.08 (m, 1H), 1.83 (m, 1H), 1.54 (m, 1H) ; ^{13}C NMR (100 MHz) (CDCl$_3$) δ 168.5, 156.7, 156.5, 136.7, 136.4, 134.1, 131.7, 128.46, 128.41, 128.0, 127.9, 127.7, 123.4, 66.6, 66.5, 53.4, 48.8, 41.8, 37.4, 33.2.

Dibenzyl (4-aminobutane-1,3-diyl)(S)-dicarbamate (132).

Dibenzyl (4-(1,3-dioxoisoindolin-2-yl)butane-1,3-diyl)(S)-dicarbamate, **132**, (170 mg, 0.34 mmol) was dissolved in methanol (5 mL) and hydrazine monohydrate (0.03 mL, 0.68 mmol) was added. The reaction mixture was then refluxed for 2 hours and cooled to room temperature. The precipitate formed was filtered and methanol used to wash the filtrate. The filtrate was concentrated under reduced pressure and the remaining solid purified on an ISCO chromatograph with silica (0-10% methanol/dichloromethane + 1% NH$_4$OH) to give product as a colorless oil. (77 mg, 61%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.34 (m, 10H), 5.77 (brs, 1H), 5.56 (d, 1H, J = 8), 5.09 (m, 4H), 3.69 (m, 1H), 3.44 (m, 1H), 3.02 (m, 1H), 2.74 (m, 2H), 2.26 (s, 2H), 1.68 (m, 1H), 1.47 (m, 1H) ; ^{13}C NMR (100 MHz) (CDCl$_3$) δ 157.0, 156.5, 136.7, 136.4, 128.5, 128.4, 128.1, 128.0, 66.8, 66.5, 50.5, 45.5, 37.6, 33.0

Dibenzyl (4-(2-phenethyl-4-phenylbutanamido)butane-1,3-diyl)(S)-dicarbamate (133).

2-Phenethyl-4-phenylbutanoic acid, **84**, (55.6 mg, 0.20 mmol) was dissolved in dry DMF (5mL) and EDC (79 mg, 0.41 mmol) and HOBt (56 mg, 0.41 mmol) were added and the reaction stirred at room temperature for 5 minutes. Dibenzyl (4-aminobutane-1,3-diyl)(S)-dicarbamate, **133**, (70 mg, 0.19 mmol) was added followed by 2,6-lutidine (0.07 mL, 0.56 mmol). Reaction was stirred at room temperature overnight. The reaction mixture was then diluted with ethyl acetate and washed with water, 1M HCl, saturated $NaHCO_3$, water and brine. The organic layer was dried over sodium sulfate and filtered. The filtrate was then concentrated and purified with an ISCO chromatograph with silica (0–10% MeOH/dichloromethane) to give a white solid. (100 mg, 86%); MP 187-189 °C; ^{1}H NMR (400 MHz) ($CDCl_3$) δ 7.13 (m, 20H), 5.89 (m, 1H), 5.61 (m, 1H), 5.54 (m, 1H), 4,98 (m, 3H), 4.76 (m, 1H), 3.68 (m, 1H), 3.38 (m, 2H), 3.14 (m, 2H), 2.93 (m, 1H), 2.43 (m, 4H), 1.87 (m, 3H), 1.64 (m, 3H), 1.42 (m, 1H); ^{13}C NMR (100 MHz) ($CDCl_3$) δ 176.7, 157.2, 156.6, 141.54, 141.50, 136.6, 136.2, 128.5, 128.4, 128.3, 128.07, 128.00, 126.0, 125.9, 66.8, 66.6, 50.1, 46.4, 43.3, 37.4, 34.38, 37.35, 33.6, 33.5.

(S)-N-(2,5-Diaminopentyl)-N-methyl-2-phenethyl-4-phenylbutanamide (134).

Di-*tert*-butyl (5-(N-methyl-2-phenethyl-4-phenylbutanamido)pentane-1,4-diyl)(S)-dicarbamate, **146**, (34 mg, 0.058 mmol) was dissolved in dichloromethane (1 mL) and trifluoroacetic acid (0.5 mL) was added. Reaction was stirred under nitrogen at room temperature for 2 hours. The solvents were removed and the residue dried under vacuum

pump to get product as a salt which was a light brown colored oil. (33 mg, 100%); ^{1}H NMR (400 MHz) (MeOD) δ 7.22 (m, 10H), 3.50 (m, 3H), 2.98 (t, 2H, *J = 8)*, 2.78 (s, 3H), 2.70 (m, 1H), 2.62 (m, 4H), 1.82 (m, 8H); ^{13}C NMR (100 MHz) (MeOD) δ 180.2, 142.8, 142.7, 129.6, 129.5, 127.1, 52.0, 51.8, 40.6, 40.2, 37.3, 34.8, 34.6, 34.3, 28.9, 24.3; HRMS (ESI) Calculated for $C_{24}H_{35}N_3O$ (M+H)$^+$ 382.2853, found 382.2855

(S)-N-(5-Amino-2-(methylamino)pentyl)-2-phenethyl-4-phenylbutanamide (135)

Tert-butyl (S)-(4-(methylamino)-5-(2-phenethyl-4-phenylbutanamido)pentyl)carbamate, **150**, (20 mg, 0.04 mmol) was dissolved in dichloromethane (1 mL) and trifluoroacetic acid (0.5 mL) was added. Reaction was stirred at room temperature for 2 hours. On completion of the reaction, the solvents were removed and residue dried under vacuum to obtain product as colorless oil. (14 mg, 61%). ^{1}H NMR (400 MHz) (MeOD) δ 7.23 (m, 11H), 3.53 (m, 2H), 3.26 (m, 1H), 2.98 (m, 2H), 2.80 (s, 3H), 2.61 (m, 4H), 2.41 (m, 1H), 1.96 (m, 8H); ^{13}C NMR (100 MHz) (MeOD) δ 180.4, 142.9, 129.4, 129.3, 127.0, 60.0, 40.1, 39.6, 35.5, 34.8, 34.7, 31.6, 26.6, 24.4 ; HRMS (ESI) Calculated for $C_{24}H_{35}N_3O$ (M+H)$^+$ 382.2853, found 382.2864

(S)-N-(2-Amino-5-(methylamino)pentyl)-2-phenethyl-4-phenylbutanamide (136).

tert-Butyl (S)-(4-((*tert*-butoxycarbonyl)amino)-5-(2-phenethyl-4-phenylbutanamido)-pentyl)(methyl)carbamate, **155**, (8.8 mg, 0.015 mmol) was dissolved in dichloromethane (1 mL) and trifluoroacetic acid (0.5 mL) was added. The reaction mixture was stirred at

room temperature for 2 hours. Then, the solvents were removed under reduced pressure and the residue dried under vacuum to obtain product as a colorless oil. (8.7 mg, 100%); ^{1}H NMR (400 MHz) (MeOD) δ 7.21 (m, 11H), 3.45 (m, 2H), 3.01 (m, 2H), 3.71 (s, 3H), 2.61 (m, 4H), 2.38 (m, 1H), 1.83 (m, 9H); ^{13}C NMR (100 MHz) (MeOD) δ 180.1, 142.9, 129.4, 129.3, 127.0, 52.7, 47.5, 42.0, 35.6, 35.5, 34.8, 34.7, 33.5, 28.5, 22.9; HRMS (ESI) Calculated for $C_{24}H_{35}N_3O$ (M+H)$^+$ 382.2853, found 382.2846

(S)-5-((((Benzyloxy)carbonyl)amino)-2-((*tert*-butoxycarbonyl)amino)pentyl methanesulfonate (137).

Benzyl *tert*-butyl (5-hydroxypentane-1,4-diyl)(S)-dicarbamate, **75**, (500 mg, 1.42 mmol) was dissolved in dichloromethane (10 mL) and triethylamine (0.4 mL, 2.84 mmol) was added. Then mesyl chloride (0.17 mL, 2.23 mmol) was added and reaction stirred at room temperature for 1 hour. Then the organic layer was washed with saturated sodium bicarbonate solution and further extracted with dichloromethane. Reaction was purified on an ISCO chromatograph (60% ethyl acetate/hexane) to produce product as a colorless oil. (215 mg, 35%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.36 (s, 10H), 5.10 (s, 2H), 5.02 (t, 1H, J = 8), 4.80 (d, 1H, J = 4), 4.19 (m, 2H), 3.85 (m, 1H), 3.23 (d, 2H, J = 4), 3.02 (s, 3H), 1.55 (m, 4H), 1.45 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 156.5, 155.3, 136.5, 128.5, 128.1, 128.0, 79.9, 71.0, 66.6, 49.4, 40.5, 37.3, 28.3, 26.4

Benzyl *tert*-butyl (5-(methylamino)-5-oxopentane-1,4-diyl)(S)-dicarbamate (139)

Boc –L-Ornithine (Z)-OH (1500 mg, 4.1 mmol) was dissolved in DMF (10 mL) and cooled to 0 °C. EDC (1.6g, 8.2 mmol) and HOBt (1.1 g, 8.2 mmol) were added. Methyl amine. HCl (332 mg, 4.92 mmol) and 2,6-lutidine (1.5 g, 14.7 mmol). Reaction was stirred at room temperature overnight. The DMF was then diluted with ethyl acetate and the organic layer washed with water, sat NaHCO$_3$, 1M HCl, water and brine). This gave product as a white powder (1.28 g, 82%); MP 138-140 °C ; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.27 (s, 5H), 6.23 (brs, 1H), 5.03 (m, 2H), 4.87 (brs 1H), 4.11 (m, 1H), 3.31 (m, 1H), 3.10 (m, 1H), 2.69 (d, 3H, *J* = 4), 1.71 (m, 1H)., 1.50 (m, 3H), 1.36 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 172.5, 157.1, 155.8, 136.5, 128.5, 128.1, 127.9, 79.8, 66.6, 52.7, 39.9, 30.20, 28.3, 26.3, 26.1

Di-*tert*-butyl (5-(benzyl(methyl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate (142).

Bis Boc-L- ornithine (1000 mg, 3 mmol) was dissolved in dichloromethane (30 mL), then EDC (631 mg, 3.3 mmol) and HOBT (445 mg, 3.3 mmol) was added. The reaction was stirred at room temperature under nitrogen. Then N-benzylmethyl amine (400 mg, 3.3 mmol) and DIPEA (581.6 mg, 4.5 mmol) was added. Reaction was stirred for overnight and organic layer washed with water, saturated NaHCO$_3$, 1 M HCl, water and brine. The organic layer was dried over sodium sulfate, concentrated under reduced pressure and dried over vacuum to give product as a yellow oil. (1.08 g, 82%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.19 (m, 5H), 5.51 (m, 1H), 4.79 (brs, 1H), 4.50 (m, 3H), 3.01 (m, 2H), 2.90 (s, 3H), 1.56 (m, 4H), 1.36 (s, 9H), 1.34 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 172.3, 155.9, 155.6,

136.6, 136.1, 128.8, 128.6, 127.8, 127.7, 127.4, 126.7, 79.4, 78.9, 53.1, 51.1, 50.0, 40.1, 34.6, 30.7, 30.3, 28.4, 28.3, 28.2, 25.8

(S)-2,5-Diamino-N-benzyl-N-methylpentanamide (143).

Di-*tert*-butyl (5-(benzyl(methyl)amino)-5-oxopentane-1,4-diyl)(S)-dicarbamate, **142**, (524 mg, 1.2 mmol) was dissolved in dichloromethane (6 mL), then trifluoroacetic acid (2 mL) was added. Reaction was stirred at 0 °C for 2 hours. The solvents were then removed under reduced pressure and dried under vacuum to obtain product as a colorless oil. (498 mg, 100%); ^{1}H NMR (400 MHz) (MeOD) δ 7.35 (m, 5H), 4.53 (m, 2H), 3.33 (m, 1H), 2.98 (m, 5H), 1.90 (m, 4H); ^{13}C NMR (100 MHz) (MeOD) δ 170.0, 169.9, 137.5, 137.0, 130.1, 129.8, 129.13, 129.10, 128.8, 128.0, 55.1, 53.1, 53.9, 52.3, 51.5, 51.4, 40.0, 35.0, 34.6, 29.0, 28.5, 23.8

Di-*tert*-butyl (5-(benzyl(methyl)amino)pentane-1,4-diyl)(S)-dicarbamate (144).

(S)-2,5-Diamino-N-benzyl-N-methylpentanamide, **143**, (498 mg, 1.20 mmol) was dissolved in dry THF (10 mL) and BH$_3$.THF (1M in THF) (6 mL, 6 mmol) was added. The reaction mixture was heated at reflux overnight. Then methanol (20 mL) was added and stirred at that temperature for 2 hours and cooled to room temperature. Then water (2 mL) was added. The solvents were removed and redissolved in dichloromethane and dried over sodium sulfate to remove water. The dichloromethane was filtered and the filtrate concentrated to give a colorless oil. The oil was dissolved in dichloromethane (10 mL)

and Boc$_2$O was added. Reaction was stirred at room temperature overnight. Ethyl acetate was added to the reaction mixture and washed with brine. The organic layer was dried over sodium sulfate, concentrated and purified using an ISCO chromatograph to give product as a colorless oil. (195 mg, 39%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.26 (m, 5H), 4.77 (brs, 1H), 4.64 (brs, 1H), 3.67 (m, 1H), 3.53 (d, 1H, *J = 12*), 3.42 (d, 1H, *J = 12*), 3.10 (d, 2H, *J = 4*), 2.32 (m, 2H), 2.20 (s, 3H), 1.59 (m, 4H), 1.44 (s, 9H), 1.43 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 156.0, 139.0, 128.9, 128.1, 126.9, 78.8, 70.7, 62.3, 61.1, 42.6, 40.4, 30.8, 28.4, 26.4, 26.2

Di-*tert*-butyl (5-((1,5-diphenylpentan-3-yl)(methyl)amino)pentane-1,4-diyl)(S)-dicarbamate (145).

Di-*tert*-butyl (5-(benzyl(methyl)amino)pentane-1,4-diyl)(S)-dicarbamate, **144**, (180 mg, 0.42 mmol) was dissolved in ethanol (10 mL) and 20% Pd(OH)$_2$/C (50 mg) was added. The reaction mixture was purged and stirred under hydrogen atmosphere for overnight. The catalyst was filtered off and washed with 20% methanol/dichloromethane, concentrated and dried to give product (di-*tert*-butyl (5-(methylamino)pentane-1,4-diyl)(S)-dicarbamate) which was used in the next step without further purification.

Di-*tert*-butyl (5-(N-methyl-2-phenethyl-4-phenylbutanamido)pentane-1,4-diyl)(S)-dicarbamate (146).

2-Phenethyl-4-phenylbutanoic acid, **84**, (94 mg, 0.35 mmol) was dissolved in dichloromethane (5 mL) and PyBrop (179 mg, 0.38 mmol) was added. Reaction was stirred at room temperature for 5 minutes. Di-*tert*-butyl (5-(methylamino)pentane-1,4-diyl)(S)-dicarbamate, **145**, (105 mg, 0.32 mmol) was added, followed by triethylamine (0.13 mL, 0.95 mmol) and the reaction stirred at room temperature overnight under nitrogen. Then the reaction mixture was dissolved in ethyl acetate, washed with water, saturated NaHCO$_3$, 1 M HCl, water and brine. The organic layer was dried over sodium sulfate, filtered, concentrated and purified on an ISCO chromatograph (0-10% MeOH /dichloromethane) to give product as a colorless oil. (146 mg, 78%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.18 (m, 10H), 5.14 (d, 1H, *J = 8)*, 4.81 (brs, 1H), 3.81 (m, 2H), 3.45 (m, 1H), 3.13 (m, 2H), 2.90 (m, 1H), 2.68 (s, 3H), 2.59 (m, 4H), 2.01 (m, 2H), 1.81 (m, 2H), 1.56 (m, 4H), 1.44 (s, 9H), 1.31 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 176.8, 156.0, 141.7, 141.6, 128.5, 128.4, 128.38, 128.31, 125.9, 79.0, 50.6, 49.2, 40.3, 39.1, 35.2, 34.0, 33.9, 33.8, 33.4, 33.39, 33.34, 30.9, 28.4, 28.36, 28.30, 26.0

Benzyl *tert*-butyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,4-diyl)(S)-dicarbamate (147).

2-Phenethyl-4-phenylbutanoic acid, **84**, (546 mg, 2.04 mmol) was dissolved in dry DMF (5mL) and EDC (816 mg, 4.27 mmol) and HOBt (575mg, 4.27 mmol) were added and the reaction stirred at room temperature for 5 minutes. Benzyl *tert*-butyl (5-aminopentane-1,4-

diyl)(S)-dicarbamate, **78**, (682 mg, 1.94 mmol) was added followed by 2,6-lutidine (0.67 mL, 3 mmol). The reaction was stirred at room temperature overnight. The reaction mixture was then diluted with ethyl acetate and washed with water, 1M HCl, saturated NaHCO$_3$, water and brine. The organic layer was dried over sodium sulfate and filtered. The filtrate was then concentrated and purified on an ISCO chromatograph with silica (0–10% MeOH/dichloromethane) to give a white solid. (520 mg, 45%); MP 155-157 °C ; ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.26 (m, 15H), 6.20 (brs, 1H), 5.10 (s, 3H), 4.95 (d, 1H, J = 8), 3.70 (s, 1H), 3.29 (s, 4H), 2.59 (m, 4H), 2.11 (m, 1H), 2.01 (m, 2H), 1.78 (m, 2H), 1.55 (m, 4), 1.40 (s, 9H) ; ^{13}C NMR (100 MHz) (CDCl$_3$) δ 176.1, 156.5, 156.4, 141.7, 141.6,136.6, 128.5, 128.4, 128.0, 125.9, 79.6, 66.6, 51.0, 46.6, 43.9, 40.7, 34.4, 33.7, 33.6, 30.3, 28.3, 26.3

Benzyl (S)-(4-amino-5-(2-phenethyl-4-phenylbutanamido)pentyl)carbamate (148).

Benzyl *tert*-butyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,4-diyl)(S)-dicarbamate, **147**, (107 mg, 0.18 mmol) was dissolved in dichloromethane (3 mL) and cooled to 0 °C under nitrogen. Trifluoroacetic acid (2 mL) was added and reaction stirred at 0 °C for 2 hours. The solvents were removed under reduced pressure upon completion of the reaction and residue dissolved in saturated sodium bicarbonate. Crude product was extracted with dichloromethane and purified on an ISCO chromatograph (0-10% methanol/ dichloromethane with 1% NH$_4$OH) to give product as a colorless oil. (69 mg, 78%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.09 (m, 10H), 6.69 (s, 1H), 5.28 (s, 2H), 5.05 (s, 1H), 4.89 (s, 2H), 3.28 (m, 1H), 2.97 (s, 4H), 2.41 (m, 4H), 2.05 (m, 1H), 1.82 (m, 2H), 1.61 (m, 2H),

1.40 (m, 4H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 176.8, 156.8, 141.6, 136.4, 128.5, 128.4, 128.3, 128.1, 127.9, 125.9, 66.7, 51.7, 46.5, 43.1, 40.5, 34.2, 33.6, 30.0, 25.9

Benzyl (S)-(4-(benzyl(methyl)amino)-5-(2-phenethyl-4-phenylbutanamido)pentyl)-carbamate (149).

To a stirred solution of benzyl (S)-(4-amino-5-(2-phenethyl-4-phenylbutanamido)-pentyl)carbamate, **148**, (178 mg, 0.35 mmol) in ethanol (15 mL) and 3Å molecular sieves at room temperature was added benzaldehyde (0.043 mL, 0.43 mmol) and reaction was stirred for 1 hour under nitrogen. Then NaCNBH$_3$ (26 mg, 0.425 mmol) was added and the reaction was stirred at room temperature overnight. Formaldehyde (37% aqueous solution) (0.05 ml, 0.7 mmol) was then added and reaction was stirred for 1 hour. A second batch of NaCNBH$_3$ (31 mg, 0.49 mmol) was then added and the reaction mixture stirred for 6 hours. The reaction mixture was filtered to remove the molecular sieves and the residue washed with methanol. The filtrate was concentrated under reduced pressure and the residue redissolved in ethyl acetate. The ethyl acetate layer was quenched with saturated NH$_4$Cl solution and washed with saturated sodium bicarbonate and brine. The combined organic layers were dried over sodium sulfate, concentrated and purified on an ISCO chromatograph (0-60 % ethyl acetate/hexane). This gave product as a colorless oil (96 mg, 45%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.29 (m, 10H), 6.27 (d, 1H, J = 4), 5.14 (s, 2H), 5.04 (brs, 1H), 3.62 (m, 2H), 3.26 (m, 2H), 3.02 (m, 1H), 2.62 (m, 5H), 2.24 (s, 3H), 1.83 (m, 12H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 174.9, 156.5, 141.8, 141.7, 139.3, 136.6,

128.6, 128.6, 128.5, 128.43, 128.40, 128.3, 128.1, 127.2, 125.94, 125.92, 66.7, 61.1, 58.0, 46.5, 41.1, 39.5, 36.0, 34.4, 34.1, 33.6, 33.5, 27.6, 23.3

tert-Butyl (S)-(4-(methylamino)-5-(2-phenethyl-4-phenylbutanamido)pentyl)-carbamate (150).

Benzyl (S)-(4-(benzyl(methyl)amino)-5-(2-phenethyl-4-phenylbutanamido)pentyl)-carbamate, **149**, (81 mg, 0.135 mmol) was dissolved in ethanol (10 mL) and Boc$_2$O (88 mg, 0.41 mmol) was added. The reaction mixture was purged and stirred at room temperature overnight. Then the reaction mixture was filtered to remove catalyst. The filtrate was concentrated and purified on an ISCO chromatograph to obtain product as a colorless oil (20.2 mg, 31%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.22 (m, 11H), 4.82 (brs, 1H), 3.64 (m, 1H), 3.48 (m, 1H), 3.16 (m, 2H), 3.06 (m, 1H), 2.62 (m, 7H), 2.39 (m, 1H), 2.04 (m, 2H), 1.74 (m, 6H), 1.44 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 176.4, 156.3, 141.7, 128.4, 128.3, 125.9, 79.6, 59.9, 46.9, 39.2, 34.6, 34.5, 33.8, 31.4, 28.4, 26.1

Benzyl _tert_-butyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,4-diyl)(S)-dicarbamate (151).

2-Phenethyl-4-phenylbutanoic acid, **84**, (204 mg, 0.76 mmol) was dissolved in dry DMF (5mL) and EDC (290 mg, 1.52 mmol) and HOBt (204 mg, 1.52 mmol) were added and the reaction stirred at room temperature for 5 minutes. Benzyl _tert_-butyl (5-aminopentane-1,4-diyl)(S)-dicarbamate, **120**, (295 mg, 0.83 mmol) was added followed by 2,6-lutidine (0.29

mL, 2.5 mmol). Reaction was stirred at room temperature overnight. The reaction mixture was then diluted with ethyl acetate and washed with water, 1M HCl, saturated NaHCO$_3$, water and brine. The organic layer was dried over sodium sulfate and filtered. The filtrate was then concentrated and purified with an ISCO chromatograph with silica (0–10% MeOH/dichloromethane) to give a white solid. (100 mg, 86%); MP 160-162 °C ; ^{1}H NMR (CDCl$_3$) (400 MHz) (CDCl$_3$) δ 7.12 (m, 15H), 5.94 (brs, 1H), 5.17 (brs, 1H), 4.99 (d, 1H, $J = 12$), 4.80 (d, 1H, $J = 12$), 4.57 (brs, 1H), 3.66 (s, 1H), 3.34 (m, 1H), 3.20 (m, 1H), 3.04 (m, 1H), 2.47 (m, 4H), 1.93 (m, 3H), 1.67 (m, 2H), 1.46 (m, 4H), 1.36 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 176.2, 156.8, 156.1, 141.64, 141.63, 136.2, 128.5, 128.4, 128.3, 128.0, 125.9, 79.2, 66.8, 51.9, 46.6, 43.7, 40.1, 34.4, 34.3, 33.6, 33.5, 30.1, 28.4, 26.5

Benzyl (S)-(5-amino-1-(2-phenethyl-4-phenylbutanamido)pentan-2-yl)carbamate (152).

Benzyl   (S)-5-((*tert*-butoxycarbonyl)amino)-2-((2-phenethyl-4-phenylbutanamido)-methyl)pentanoate, **151**, (145 mg, 0.24 mmol) was dissolved in dichloromethane (3 mL) and cooled to 0 °C under nitrogen. Trifluoroacetic acid (2 mL) was added and reaction stirred at 0 °C for 2 hours. The solvents were rotavapped on completion of the reaction and residue dissolved in saturated sodium bicarbonate. Crude product was extracted with dichloromethane and purified on an ISCO chromatograph (0-10% methanol/ dichloromethane with 1% NH$_4$OH) to give product as a pale yellow solid. (98 mg, 82%); MP 122-123 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.23 (m, 15 H), 6.26 (brs, 1H), 5.63 (d, 1H, J = 8), 5.10 (d, 1H, $J = 12$). 4.91 (d, 1H, $J = 12$), 3.75 (m, 1H), 3.39 (m, 2H), 2.60

(m, 6H), 2.10 (m, 1H), 1.98 (m, 2H), 1.76 (m, 2H), 1.54 (m, 4H), 1.41 (s, 2H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ176.1, 157.0, 141.6, 136.3, 128.5, 128.4, 128.3, 128.0, 125.9, 66.7, 51.8, 46.6, 43.9, 41.7, 34.4, 34.3, 33.6, 33.5, 30.3, 29.3

Benzyl (S)-(5-(benzyl(methyl)amino)-1-(2-phenethyl-4-phenylbutanamido)pentan-2-yl)carbamate (153).

To a stirred solution of benzyl (S)-(5-amino-1-(2-phenethyl-4-phenylbutanamido)pentan-2-yl)carbamate, **152**, (81 mg, 0.16 mmol) in ethanol (10 mL) in 3Å molecular sieves at room temperature was added benzaldehyde (20 μL, 0.20 mmol). This was stirred for 30 minutes, then sodium cyanoborohydride (13 mg, 0.20 mmol) was added and reaction stirred under nitrogen overnight. Then 37% formaldehyde (30 μL, 0.33 mmol) was added, followed by sodium cyanoborohydride (14 mg, 0.23 mmol). Reaction was stirred at room temperature for 1 hour. The reaction mixture was filtered to remove the sieves and washed residue with methanol. The filtrate was concentrated under reduced pressure, the residue redissolved in ethyl acetate and quenched with saturated ammonium chloride solution. The organic layer was washed with saturated NaHCO$_3$ solution and brine, dried over sodium sulfate. It was purified on an ISCO chromatograph (0-10% MeOH/dichloromethane) to give product as a colorless oil. (27 mg, 28%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.24 (m, 20H), 6.34 (brs, 1H), 6.04 (brs, 1H), 5.10 (m, 1H), 4.91 (m, 1H), 3.73 (m, 1H), 3.56 (s, 2H), 3.37 (m, 2H), 2.55 (m, 6H), 2.22 (s, 3H), 1.99 (m, 2H), 1.66 (m, 6H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 176.2, 157.2, 141.7, 136.3, 129.3, 128.8, 128.5, 128.4, 128.3, 128.2,

128.0, 127.9, 127.5, 126.9, 125.9, 66.7, 62.1, 56.6, 51.6, 46.7, 44.3, 41.6, 34.4, 33.6, 30.6, 23.0

tert-Butyl (S)-(4-((_tert_-butoxycarbonyl)amino)-5-(2-phenethyl-4-phenyl-butanamido)-pentyl)(methyl)carbamate (154).

Benzyl (S)-(5-(benzyl(methyl)amino)-1-(2-phenethyl-4-phenylbutanamido)pentan-2-yl)-carbamate, **153**, (25.5 mg, 0.042 mmol) was dissolved in ethanol (5 mL). Boc$_2$O (22.9 mg, 0.11 mmol) and Pd(OH)$_2$/C (20 mg) were added and the reaction mixture stirred under hydrogen for overnight. The reaction mixture was then filtered to remove catalyst. Filtrate was concentrated and purified on an ISCO chromatograph to give product as a colorless oil (12.7 mg, 52%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.23 (m, 10H), 3.72 (m, 1H), 3.35 (m, 2H), 3.24 (m, 2H), 2.84 (s, 3H), 2.64 (m, 2H), 2.55 (m, 2H), 2.12 (m 1H), 2.00 (m, 2H), 1.79 (m, 3H), 1.62 (m, 3H), 1.47(s, 9H), 1.39 (m, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 175.9, 156.5, 141.7, 128.4, 125.9, 79.6, 79.3, 46.6, 44.3, 34.4, 34.1, 33.7, 33.6, 28.4, 28.3

(S)-N-(5-Amino-2-(dimethylamino)pentyl)-2-phenethyl-4-phenylbutanamide (155).

Step 1 : _tert_-Butyl (S)-(4-(dimethylamino)-5-(2-phenethyl-4-phenylbutanamido)pentyl)-carbamate benzyl: (S)-(5-(Dimethylamino)-1-(2-phenethyl-4-phenylbutanamido)pentan-2-yl)carbamate, **157**, (39 mg, 0.074 mmol) was dissolved in ethanol (5 mL), Boc$_2$O (24 mg, 0.112 mmol) and 20% Pd(OH)$_2$/C (20 mg) was added. Reaction mixture was purged and stirred at room temperature under hydrogen overnight. On completion of the reaction,

the reaction was filtered and residue washed with 20% MeOH/dichloromethane. The filtrate was concentrated under reduced pressure and purified on an ISCO chromatograph (0-10% methanol/dichloromethane) to give product as a colorless oil (33 mg, 90%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.13 (m, 10H), 6.21 (brs, 1H), 3.51 (m, 1H), 3.05 (m, 2H), 2.85 (m, 1H), 2.49 (m, 6H), 2.81 (s, 6H), 1.95 (m, 4H), 1.66 (m, 2H), 1.51 (m, 2H), 1.37 (s, 9H) ;^{13}C NMR (100 MHz) (CDCl$_3$) δ 175.0, 156.0, 141.8, 128.4, 128.39, 128.36, 125.8, 79.1, 62.7, 46.3, 40.5, 40.1, 39.4, 34.5, 33.6, 33.3, 31.2, 28.4, 28.0, 27.7, 22.4

Step 2: (S)-N-(2-Amino-5-(dimethylamino)pentyl)-2-phenethyl-4-phenylbutanamide - *tert*-butyl (S)-(4-(Dimethylamino)-5-(2-phenethyl-4-phenylbutanamido)pentyl)carbamate (23 mg, 0.046 mmol) was dissolved in dichloromethane (1 mL) and trifluoroacetic acid (0.5 mL) was added. Reaction was stirred at room temperature for 2 hours under nitrogen. Then solvents were removed and dried under vacuum to give product as a trifluoroacetic acid salt which was a colorless oil. (27 mg, 100%); ^{1}H NMR (400 MHz) (MeOD) δ 7.23 (m, 11H), 3.56 (m, 1H), 3.38 (m, 1H), 3.01 (m, 2H), 2.60 (m, 4H), 2.40 (m, 1H), 1.82 (m, 4H); ^{13}C NMR (100 MHz) (MeOD) δ 180.0, 161.9, 142.9, 129.6, 129.5, 129.4, 129.3, 127.0, 67.3, 47.3, 40.1, 38.9, 35.5, 34.8, 34.7, 25.2; HRMS (ESI) Calculated for C$_{25}$H$_{37}$N$_3$O (M+H)$^+$ 396.3009, found 396.3011

(S)-N-(2-Amino-5-(dimethylamino)pentyl)-2-phenethyl-4-phenylbutanamide (156).

Benzyl   (S)-(5-(dimethylamino)-1-(2-phenethyl-4-phenylbutanamido)pentan-2-yl)-carbamate, **158**, (40 mg, 0.075 mmol) was dissolved in ethanol (10 mL) and 20% Pd(OH)$_2$/C (20 mg)was added. Reaction mixture was purged and stirred at room

temperature under hydrogen. On completion, reaction was filtered and residue washed with 20% MeOH/dichloromethane. The filtrate was concentrated under reduced pressure and dried under vacuum to give product as a yellow oil. (30mg, 91%); ^{1}H NMR (400 MHz) (MeOD) 7.16 (m, 11H), 3.19 (m, 2H), 2.85 (m, 1H), 2.54 (m, 3H), 2.28 (m, 10H), 1.87 (m, 2H), 1.63 (m, 6H); ^{13}C NMR (400 MHz) (MeOD) δ 181.2, 145.6, 131.9, 131.8, 129.4, 63.0, 54.6, 50.2, 48.7, 47.5, 38.4, 38.3, 37.3, 35.8, 27.0; HRMS (ESI) Calculated for $C_{25}H_{37}N_3O$ (M+H)$^+$ 396.3009, found 396.2999

Benzyl (S)-(4-(dimethylamino)-5-(2-phenethyl-4-phenylbutanamido)pentyl)-carbamate (157).

Benzyl (S)-(4-amino-5-(2-phenethyl-4-phenylbutanamido)pentyl)carbamate, **148**, (53 mg, 0.11 mmol) was dissolved in 20% methanol/dichloromethane (10 mL) and a solution of 37% formaldehyde (1 mL) was added and allowed to stir for 10 minutes. Sodium triacetoxyborohydride (226 mg, 1.1 mmol) was added and reaction was stirred at room temperature overnight. The reaction mixture was dissolved in saturated sodium bicarbonate solution and extracted with dichloromethane. The organic layer was dried over sodium sulfate concentrated and purified on an ISCO chromatograph (0-10% MeOH/dichloromethane + 1 NH₄OH) to give product as a colorless oil (44.3 mg, 79%); ^{1}H NMR (400 MHz) (CDCl₃) δ 7.28 (m, 15H), 6.31 (brs, 1H), 5.11 (s, 2H), 5.06 (brs, 1H), 3.61 (m, 1H), 3.24 (m, 2H), 2.97 (m, 1H), 2.65 (m, 2H), 2.56 (m, 3H), 2.28 (s, 6H), 2.07 (m, 3H), 1.77 (m, 2H), 1.58 (m, 3H); ^{13}C NMR (100 MHz) (CDCl₃) δ 175.1, 156.5, 141.8,

136.6, 128.5, 128.42, 128.40, 128.3, 128.1, 125.8, 66.6, 62.7, 46.3, 41.1, 40.1, 39.3, 34.6, 34.5, 33.7, 33.6, 27.5, 22.6

Benzyl (S)-(5-(dimethylamino)-1-(2-phenethyl-4-phenylbutanamido)pentan-2-yl)carbamate (158).

Benzyl (S)-(5-amino-1-(2-phenethyl-4-phenylbutanamido)pentan-2-yl)carbamate, **152**, (82 mg, 0.16 mmol) was dissolved in 20% methanol/dichloromethane (10 mL) and a solution of 37% formaldehyde (0.5 mL) was added and allowed to stir for 10 minutes. Sodium triacetoxyborohydride (345 mg, 1.6 mmol) was added and reaction was stirred at room temperature overnight. The reaction mixture was dissolved in saturated sodium bicarbonate solution and extracted with dichloromethane. It was purified on an ISCO chromatograph (0-10 MeOH/dichloromethane + 1 NH$_4$OH) to give product as a colorless oil (39.9 mg, 47%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.12 (m, 15H), 6.23 (m, 2H), 5.01 (d, 1H, $J = 12$), 4.83 (d, 1H, $J = 12$) 3.63 (m, 1H), 3.30 (d, 2H, $J = 4$), 2.48 (m, 4H), 2.21 (s, 2H0, 2.12 (s, 6H), 2.02 (m, 1H), 1.89 (m, 2H), 1.66 (m, 2H), 1.50 (m, 4H) ; ^{13}C NMR (400 MHz) (CDCl$_3$) δ 175.9, 157.3, 141.7, 136.4, 128.4, 128.3, 128.0, 127.9, 125.8, 66.6, 59.0, 51.59, 46.8, 45.1, 44.3, 34.5, 34.4, 33.7, 33.6, 30.7, 23.5.

tert-Butyl (S)-(5-amino-1-(2-phenethyl-4-phenylbutanamido)pentan-2-yl)carbamate (160).

Benzyl *tert*-butyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,4-diyl)(S)-dicarbamate, **148**, (125, mg, 0.21 mmol) was dissolved in ethanol(15 mL) and 20% Pd(OH)$_2$/C (30 mg) was added. The reaction mixture was then purged and stirred under a hydrogen atmosphere for overnight.   Then the catalyst was filtered and the residue washed with 20% MeOH/dichloromethane. The solvent of the filtrate was removed under reduced pressure and dried under vacuumto give product was a colorless oil. (84 mg, 86%); ^{1}H NMR (400 MHz) (CDCl$_3$)δ 7.06 (m, 10H), 6.43 (s, 1H), 5.14 (d, 1H, J = 4), 3.54 (m, 1H), 3.19 (m, 2H), 2.46 (m, 7H), 1.94 (m, 1H), 1.84 (m, 2H), 1.62 (m, 2H), 1.40 (m, 3H), 1.24 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 176.0, 156.6, 141.8, 141.7, 128.3, 125.8, 79.4, 57.9, 51.1, 46.6, 44.1, 41.4, 34.4, 33.7, 33.6, 30.4, 28.9, 28.3

tert-Butyl (S)-(2,2-dimethyl-5,13-dioxo-14-phenethyl-16-phenyl-3-oxa-4,6,12-triaza-hexadecan-10-yl)carbamate (162).

tert-Butyl (S)-(5-amino-1-(2-phenethyl-4-phenylbutanamido)pentan-2-yl)carbamate, **160**, (99 mg, 0.21 mmol) was dissolved in DMF (4 mL) and reaction was cooled to 0 °C. Potassium carbonate (38 mg, 0.28 mmol) followed by BocNHOTs (73 mg, 0.25 mmol) was added.   The reaction was stirred at room temperature under nitrogen until the completion of the reaction. The reaction mixture was dissolved in water and extracted with ethyl acetate. The organic layer was dried over sodium sulfate and concentrated. Crude product was purified on an ISCO chromatograph (0-10% methanol/dichloromethane) to

give product as a colorless oil (64.9 mg, 53%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ7.22 (m, 10H), 6.73 (s, 1H), 6.35 (s. 1H), 5.80 (s, 1H), 5.07 (d, 1H, $J = 8$), 3.70 (m, 1H), 3.31 (m, 4H), 2.58 (m, 4H), 2.13 (m, 1H), 2.00 (m, 2H), 1.77 (m, 2H), 1.62 (m, 2H), 1.51 (m, 2H), 1.28 (s, 9H), 1.25 (s, 9H); ^{13}C NMR (CDCl$_3$) (100 MHz) δ 176.1, 161.0, 156.4, 141.8, 141.74, 141.70, 128.4, 128.3, 125.8, 80.7, 79.5, 51.1, 46.6, 44.0, 39.22, 34.4, 33.6, 30.2, 28.3, 26.7, 26.5, 26.3

(S)-N-(2-Amino-5-(3-(*tert*-butoxy)ureido)pentyl)-2-phenethyl-4-phenylbutanamide (163).

tert-Butyl (S)-2-(4-((*tert*-butoxycarbonyl)amino)-5-(2-phenethyl-4-phenylbutanamido)-pentyl)hydrazine-1-carboxylate, **163**, (22 mg, 0.038 mmol) was dissolved in dichloromethane (1 mL). Trifluoroacetic acid (0.5 mL) was added and reaction stirred at that temperature for overnight. The solvents were removed and dried under vacuum and the product submitted as the trifluoroacetic acid salt. (20 mg, 79%). ^{1}H NMR (400 MHz) (MeOD) δ 7.21 (m, 10H), 3.41 (m, 2H), 3.23 (m, 2H), 2.60 (m, 4H), 2.36 (m, 1H), 1.94 (m, 2H), 1.82 (m, 2H), 1.65 (m, 4H); ^{13}C NMR (MeOD) (100 MHz) δ 180.1, 143.0, 142.9, 129.4, 129.3, 126.9, 81.4, 52.9, 47.3, 42.5, 39.7, 35.5, 34.7, 28.8, 27.1, 26.7, 26.5

(S)-N-(2-Amino-5-guanidinopentyl)-2-phenethyl-4-phenylbutanamide (164).

Tris-Boc-(S)-N-(2-amino-5-guanidinopentyl)-2-phenethyl-4-phenylbutanamide, **165**, (30 mg, 0.042 mmol) was dissolved in dichloromethane (2 mL) and cooled to 0 ^{o}C.

Trifluoroacetic acid (1 mL) was added and reaction stirred at room temperature for 2 hours. The solvents were removed under reduced pressure and the residue dried under vacuum. Theproduct was submitted as its trifluoroacetic acid salt. (37.6 mg, quantitative). ^{1}H NMR (400 MHz) (MeOD) δ 7.10 (m, 11H), 3.34 (m, 2H), 3.10 (m, 3H), 2.47 (m, 4H), 2.27 (m, 1H), 1.83 (m, 2H), 1.68 (m, 6H); ^{13}C NMR (100 MHz) (MeOD) δ 180.1, 158.7, 143.0, 129.4, 129.3, 126.9, 52.9, 47.4, 42.2, 42.0, 35.5, 34.7, 28.8, 25.6; HRMS (ESI) Calculated for $C_{24}H_{35}N_5O$ (M+H)$^+$ 410.2914, found 410.2919

Tris-Boc-(S)-N-(2-amino-5-guanidinopentyl)-2-phenethyl-4-phenylbutanamide (165).

tert-Butyl (S)-(5-amino-1-(2-phenethyl-4-phenylbutanamido)pentan-2-yl)carbamate, **160**, (80 mg, 0.17 mmol) was dissolved in dichloromethane (5 mL). Triethylamine (0.03 mL, 0.21 mmol) followed by 1,3-diboc-2-(trifluoromethylsulfonyl)guanidine (80 mg, 0.21 mmol) was added. The reaction was stirred at room temperature overnight under nitrogen. The reaction mixture was dissolved in saturated sodium bicarbonate and extracted with dichloromethane. The organic layer was dried over sodium sulfate and concentrated. Crude product was purified on an ISCO chromatograph (0-30% ethyl acetate hexane) to give product as a colorless oil (35.3 mg, 29%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 11.48 (s, 1H), 8.37 (s, 1H), 7.22 (m, 10H), 6.19 (s, 1H), 5.07 (s, 1H), 3.73 (s, 1H), 3.42 (m, 4H), 2.59 (m, 5H), 2.01 (m, 6H), 1.67 (m, 2H), 1.51 (s, 18H), 1.40 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 175.9, 163.5, 165.2, 153.3, 141.6, 128.3, 125.8, 83.1, 79.2, 51.1, 46.6, 40.4, 34.4, 33.6, 30.1, 28.33, 28.31, 28.07, 25.9.

Benzyl (4S)-4-((*tert*-butoxycarbonyl)amino)-2-((2-phenethyl-4-phenylbutanamido)-methyl)pyrrolidine-1-carboxylate (168).

2-Phenethyl-4-phenylbutanoic acid, **84**, (65 mg, 0.22 mmol) was dissolved in DMF (5 mL), EDC (93 mg, 0.48 mmol) and HOBT (65 mg, 0.48 mmol) were added. Reaction was stirred at room temperature for 5 minutes under nitrogen. Benzyl (4S)-2-(aminomethyl)-4-((*tert*-butoxycarbonyl)amino)pyrrolidine-1-carboxylate, **166**, (85 mg, 0.24 mmol) was added followed by 2,6-lutidine (76 μL, 0.66 mmol) and reaction was stirred at room temperature overnight. The reaction mixture was then diluted with ethyl acetate and washed with water, 1M HCl, saturated $NaHCO_3$, water and brine. The organic layers were dried over sodium sulfate, concentrated and purified on an ISCO chromatograph (0-10% MeOH/dichloromethane) to give product as a colorless oil. (92 mg, 69%); ^{1}H NMR (400 MHz) ($CDCl_3$) δ 7.25 (m, 15H), 6.72 (brs, 1H), 5.71 (brs, 1H), 5.12 (q, 2H), 4.12 (m, 1H), 4.00 (m, 1H), 3.92 (m, 1H), 3.70 (m, 1H), 3.33 (m, 2H), 2.60 (m, 4H), 2.34 (m, 1H), 2.19 (m, 1H), 2.02 (m, 2H), 1.78 (m, 3H), 1.47 (s, 9H); ^{13}C NMR (100 MHz) ($CDCl_3$) δ 176.0, 156.4, 156.1, 155.6, 141.7, 136.2, 128.5, 128.4, 128.3, 128.2, 127.9, 125.9, 79.6, 69.9, 67.3, 57.2, 53.1, 49.5, 46.8, 43.9, 34.6, 34.4, 33.7, 28.4;

Dibenzyl 2-((2-phenethyl-4-phenylbutanamido)methyl)piperazine-1,4-dicarboxylate (169).

2-Phenethyl-4-phenylbutanoic acid, **84**, (76 mg, 0.29 mmol) was dissolved in DMF (5 mL), EDC (109 mg, 0.57 mmol) and HOBT (77 mg, 0.57 mmol) were added. The reaction was stirred at room temperature for 5 minutes under nitrogen. Dibenzyl 2-(aminomethyl)-

piperazine-1,4-dicarboxylate, **167**, (100 mg, 0.26 mmol) was added followed by 2,6-lutidine (90 µL, 0.78 mmol) and reaction was stirred at room temperature overnight. The reaction mixture was then diluted with ethyl acetate and washed with water, 1M HCl, saturated NaHCO$_3$, water and brine. The organic layers were dried over sodium sulfate, concentrated and purified on an ISCO chromatograph (0-10% MeOH/dichloromethane) to give product as a white solid. (64 mg, 39%); MP 74-76 °C; ^{1}H NMR (400 MHz) (MeOD) δ 7.24 (m, 21H), 5.14 (s, 2H), 5.00 (m, 1H), 4.85 (s, 2H), 4.45 (s, 1H), 4.05 (m, 3H), 3.65 (m, 1H), 3.12 (m, 2H), 2.89 (m, 1H), 2.50 (m, 4H), 2.22 (m, 1H), 1.97 (m, 2H), 1.72 (m, 2H); ^{13}C NMR (100 MHz) (MeOD) δ 178.4, 178.3, 157.2, 157.0, 143.2, 137.9, 137.6, 129.6, 129.56, 129.50, 129.47, 129.45, 129.2, 129.1, 129.0, 128.9, 126.9, 68.6, 52.1, 47.7, 45.7, 44.7, 40.1, 38.6, 35.7, 34.8

N-(((4S)-4-Aminopyrrolidin-2-yl)methyl)-2-phenethyl-4-phenylbutanamide (170).

Benzyl   (4S)-4-((*tert*-butoxycarbonyl)amino)-2-((2-phenethyl-4-phenylbutanamido)-methyl)pyrrolidine-1-carboxylate, **168**, (75 mg, 0.12 mmol) was dissolved in ethanol (15 mL) and 20% Pd(OH)$_2$/C (40 mg) was added. The reaction was purged and stirred under hydrogen atmosphere overnight. Then the reaction mixture was filtered to remove catalyst and the residue washed with 20% methanol/dichloromethane. The filtrate was concentrated and dissolved in dichloromethane (2 mL) and HCl in ether (1 M) (0.5 mL) was added. This was stirred at room temperature for 30 minutes. The solvent was removed and the residue dried under vacuum to give the product as a dark colored oil. (21.8 mg, 41%); ^{1}H NMR (400 MHz) (MeOD) δ 7.23 (m, 11H), 4.11 (m, 1H), 3.90 (m, 1H), 3.73 (m,

2H), 3.58 (m, 2H), 2.72 (m, 1H), 2.62 (t, 4H, $J = 8$), 2.40 (m, 1H), 1.98 (m, 3H), 1.83 (m, 2H); ^{13}C NMR (100 MHz) (MeOD) δ 180.2, 143.0, 129.4, 126.9, 61.2, 47.3, 41.4, 35.5, 35.4, 34.7, 33.8; HRMS (ESI) Calculated for $C_{23}H_{31}N_3O$ $(M+H)^+$ 366.2540, found 366.2539.

2-Phenethyl-4-phenyl-N-(piperazin-2-ylmethyl)butanamide (171).

Dibenzyl 2-((2-phenethyl-4-phenylbutanamido)methyl)piperazine-1,4-dicarboxylate, **169**, (60 mg, 0.094 mmol) was dissolved in ethanol (10 mL) and 20% Pd(OH)$_2$/C (30 mg) was added. Reaction mixture was purged and stirred at room temperature under hydrogen. Upon completion, the reaction was filtered and the residue washed with 20% MeOH/dichloromethane. The filtrate was concentrated under reduced pressure and dried under vacuum to give product as a yellow solid. (30 mg, 88%); MP 93-95 °C; ^{1}H NMR (400 MHz) (MeOD) 7.08 (m, 11H), 3.08 (m, 1H), 2.73 (m, 5H), 2.44 (m, 4H), 2.31 (m, 2H), 2.18 (m, 2H), 1.78 (m, 2H), 1.63 (m, 2H); ^{13}C NMR (100 MHz) (MeOD) δ 178.5, 143.1, 129.4, 129.3, 126.9, 56.2, 50.2, 47.6, 46.6, 46.2, 43.5, 35.8, 34.8; HRMS (ESI) Calculated for $C_{23}H_{32}N_3O$ $(M+H)^+$ 366.2540, found 366.2542

Dibenzyl (2-aminopropane-1,3-diyl)dicarbamate (172).

Dibenzyl (2-(1,3-dioxoisoindolin-2-yl)propane-1,3-diyl)dicarbamate, **176,** (774 mg, 1.59 mmol) was dissolved in methanol (20 mL) and hydrazine monohydrate (0.15 mL, 3.18 mmol) was added. The reaction mixture was then refluxed for 2 hours and cooled to room

temperature. The precipitate formed was filtered and methanol used to wash the filtrate. The filtrate was concentrated under reduced pressure and the remaining solid purified on an ISCO chromatograph with silica (0-10% methanol/dichloromethane + 1% NH_4OH) to give product as a white flaky solid. (399 mg, 70%); MP 98-100 °C; ^1H NMR ($CDCl_3$) (400 MHz) δ 7.33 (m, 10H), 5.88 (brs, 2H), 5.09 (s, 4H), 3.11 (m, 4H), 2.91 (t, 1H, $J = 4$), 1.35 (brs, 2H); ^{13}C NMR (100 MHz) ($CDCl_3$) δ 157.1, 136.5, 128.5, 128.1, 128.0, 66.7, 51.1, 44.3

N,N-Bis(2-aminoethyl)-2-phenethyl-4-phenylbutanamide (173)

Dibenzyl (((2-phenethyl-4-phenylbutanoyl)azanediyl)bis(ethane-2,1-diyl))dicarbamate, **174**, (55 mg, 0.089 mmol) was dissolved in ethanol (10 mL) and 20% $Pd(OH)_2/C$ (30 mg)was added. Reaction mixture was purged and stirred at room temperature under hydrogen. On completion, reaction was filtered and residue washed with 20% MeOH/dichloromethane. The filtrate was concentrated under reduced pressure and dried under vacuum to give the product as a yellow oil. (27 mg, 87%); ^1H NMR (400 MHz) (MeOD) 7.11 (m, 10H), 3.31 (t, 2H, $J = 8$), 2.97 (t, 2H, $J = 8$), 2.70 (t, 2H, $J = 8$), 2.52 (m, 5H), 2.39 (t, 2H, $J = 8$), 1.80 (m, 4H); ^{13}C NMR (100 MHz) (MeOD) δ 178.5, 143.0, 129.6, 129.5, 127.1, 51.7, 50.0, 41.4, 40.6, 40.2, 35.4, 35.2, 34.3; HRMS (ESI) Calculated for $C_{22}H_{31}N_3O$ (M+H)$^+$ 354.2540, found 354.2541

Dibenzyl (((2-phenethyl-4-phenylbutanoyl)azanediyl)bis(ethane-2,1-diyl))-dicarbamate (174).

2-Phenethyl-4-phenylbutanoic acid, **84**, (79 mg, 0.30 mmol) was dissolved in dichloromethane (5 mL) and PyBrop (151 mg, 0.32 mmol) was added. The reaction was stirred at room temperature for 5 minutes under nitrogen. Dibenzyl (azanediylbis(ethane-2,1-diyl))dicarbamate (100 mg, 0.27 mmol) was added followed by triethylamine (110 μL, 0.81 mmol) and the reaction was stirred at room temperature overnight. The reaction mixture was then diluted with ethyl acetate and washed with water, 1 M HCl, saturated NaHCO₃, water and brine. The organic layers were dried over sodium sulfate, concentrated and purified on an ISCO chromatograph (0-60% ethyl acetate/ hexane) to give product as a colorless oil. (64 mg, 39%); ^{1}H NMR (400 MHz) (CDCl₃) δ 7.25 (m, 20H), 5.61 (brs, 1H), 5.12 (s, 2H), 5.01 (s, 2H), 4.92 (brs, 1H), 3.52 (m, 2H), 3.39 (m, 2H), 3.13 (m, 2H), 3.03 (m, 2H), 2.60(m, 5H), 1.91 (m, 4H) ; ^{13}C NMR (100 MHz) (CDCl₃) δ 177.1, 156.8, 156.3, 141.6, 136.5, 136.2, 128.5, 128.4, 128.2, 128.1, 128.0, 126.0, 66.9, 66.6, 47.4, 45.7, 40.2, 39.7, 39.5, 34.1, 33.3

Dibenzyl (2-hydroxypropane-1,3-diyl)dicarbamate (175).

2-Hydroxy-1,3-diaminopropane (1000 mg, 11.1 mmol) in water at 0 ºC was added sodium carbonate (2.82 g, 26.6 mmol). Benzyl chloroformate (4.54g, 26.6 mmol) was added and the solution stirred at room temperature for 5 hours. Then the reaction mixture was extracted with dichloromethane, dried over sodium sulfate. The solvent was removed under reduced pressureand the residue purified on an ISCO chromatograph (0-60% ethyl

acetate/hexane) to give product as a white solid. (1.53 g, 39%); MP 119-121 °C; ^{1}H NMR

(400 MHz) (MeOD) δ 7.33 (m, 10H), 5.09 (s, 4H), 3.72 (m, 1H), 3.19 (m, 4H); ^{13}C NMR

(100 MHz) (MeOD) δ 159.1, 138.3, 129.4, 128.9, 128.8, 70.7, 67.5, 45.3

Dibenzyl (2-(1,3-dioxoisoindolin-2-yl)propane-1,3-diyl)dicarbamate (176).

Triphenylphosphine (713 mg, 2.72 mmol) and phthalimide (400 mg, 2.72 mmol) were

dissolved in anhydrous THF (20 mL). Dibenzyl (2-hydroxypropane-1,3-diyl)dicarbamate,

175, (812 mg, 2.27 mmol) was added and the flask was cooled to 0 °C. DIAD (0.54 mL,

2.72 mmol) was added dropwise and the reaction was allowed to stir at 0 °C for 30 minutes

and overnight at room temperature under nitrogen. The reaction mixture was concentrated

under reduced pressure and purified on an ISCO chromatograph to give product as a

colorless oil. (848 mg, 77%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.76 (d, 2H, *J = 4*), 7.63 (d,

2H, *J = 4*), 7.27 (m, 10H), 5.69 (brs, 2H), 4.99 (s, 4H), 4.57 (m, 1H), 3.79 (m, 2H), 3.59

(m, 2H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 168.7, 156.6, 136.4, 134.0, 131.6, 128.4, 128.0,

127.9, 123.3, 66.7, 51.7, 40.5

Dibenzyl (2-(2-phenethyl-4-phenylbutanamido)propane-1,3-diyl)dicarbamate (177).

2-Phenethyl-4-phenylbutanoic acid, **84**, (78 mg, 0.29 mmol) was dissolved in dry DMF

(5mL) and EDC (110 mg, 0.58 mmol) and HOBt (78 mg, 0.58 mmol) were added and the

reaction stirred at room temperature for 5 minutes. Dibenzyl (2-aminopropane-1,3-

diyl)dicarbamate, **172**, (93 mg, 0.26 mmol) was added followed by 2,6-lutidine (0.09 mL,

0.78 mmol). Reaction was stirred at room temperature overnight. The reaction mixture was then diluted with ethyl acetate and washed with water, 1 M HCl, saturated $NaHCO_3$, water and brine. The organic layer was dried over sodium sulfate and filtered. The filtrate was then concentrated and purified on an ISCO chromatograph with silica (0–10% MeOH/dichloromethane) to give a white solid. (107 mg, 68%); MP 109-111 °C; ^{1}H NMR($CDCl_3$) (400 MHz) δ 7.23 (m, 21H), 6.05 (brs, 2H), 5.08 (q, 4H), 3.98 (m, 1H), 3.44 (m, 2H), 3.30 (m, 2H), 2.56 (m, 4H), 2.17 (m, 1H), 1.96 (m, 2H), 1,73 (m, 2H) ; ^{13}C NMR (100 MHz) ($CDCl_3$) δ158.0, 141.6, 136.2, 128.7, 128.5, 128.4, 128.3, 128.1, 128.0, 126.4, 125.9, 67.0, 52.0, 47.0, 41.6, 34.5, 33.6, 33.4

N-(1,3-Diaminopropan-2-yl)-2-phenethyl-4-phenylbutanamide as HCl salt (178).

Dibenzyl (2-(2-phenethyl-4-phenylbutanamido)propane-1,3-diyl)dicarbamate, **177**, (51 mg, 0.084 mmol) was dissolved in ethanol (10 mL) and 20% $Pd(OH)_2$/C (30 mg) was added. Reaction mixture was purged and stirred under a hydrogen atmosphere. The catalyst was removed by filtration and the residue washed with 20% methanol/ dichloromethane. The filtrate was concentrated under reduced pressue and dried under vacuum to give product as a colorless oil. This product was then dissolved in dichloromethane (2 mL) and 1 M HCl in ether (0.5 mL) was added. The reaction mixture was stirred at room temperature for 30 minutes. The solvents was removed and triturated with ethyl acetate. The ethyl acetate was removed and residue dried under vacuum to give product as a colorless oil. (11 mg, 39%); ^{1}H NMR (400 MHz) (MeOD) δ 7.12 (m, 11H), 3.22 (m, 5H), 2.62 (m, 5H), 2.01 (m, 2H), 1.87 (m, 2H); [13]C (100 MHz) ($CDCl_3$) δ 180.08,

143.2, 129.5, 129.4, 126.9, 47.9, 47.5, 43.6, 41.9, 35.7; HRMS (ESI) Calculated for $C_{21}H_{29}N_3O$ (M+H)$^+$ 340.2383, found 340.2385

(S)-N-(2,5-Diaminopentyl)-3,3-diphenylpropanamide (179)

Benzyl (S)-(4-amino-5-(3,3-diphenylpropanamido)pentyl)carbamate, **183**, (40 mg, 0.09 mmol) was dissolved in ethanol (10 mL) and 20% Pd(OH)$_2$/C (20 mg) was added. The reaction mixture was then purged and stirred under a hydrogen atmosphere overnight. Then the catalyst was filtered and the residue washed with 20% MeOH/dichloromethane. The solvent of the filtrate was removed under reduced pressure and dried under vacuum to give product as a colorless oil. (31 mg, 100%); ^{1}H NMR (400 MHz) (MeOD) δ 7.13 (m, 11H), 4.43 (m, 1H), 3.25 – 3.09 (m, 2H), 2.89 (m, 3H), 2.70 (m, 2H), 1.53 (m, 2H), 1.23 (m, 2H); ^{13}C NMR (400 MHz) (MeOD) δ 175.2, 145.08, 145.04, 129.6, 128.9, 128.8, 127.6, 52.2, 43.8, 43.1, 42.9, 40.3, 29.2, 24.7; HRMS (ESI) Calculated for $C_{20}H_{27}N_3O$ (M+H)$^+$ 326.2227, found 326.2221

(S)-3-Benzyl-N-(2,5-diaminopentyl)-4-phenylbutanamide (180).

Dibenzyl (5-(3-benzyl-4-phenylbutanamido)pentane-1,4-diyl)(S)-dicarbamate, **190**, (75 mg, 0.12 mmol) was dissolved in ethanol (12 mL) and 20% Pd(OH)$_2$/C (40 mg) was added. The reaction mixture was then purged and stirred under a hydrogen atmosphere overnight, then the catalyst was filtered and the residue washed with 20% MeOH/dichloromethane. The filtrate was concentrated under reduced pressure to give the product as a brown colored

oil. (31 mg, 73%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.13 (m, 11H), 3.07 (m, 1H), 2.93 (m, 1H), 2.57 (m, 8H), 2.04 (d, 2H, J = 4), 1.43 (m, 3H), 1.20 (m, 1H); ^{13}C NMR (400 MHz) (CDCl$_3$) δ 175.7, 141.6, 130.3, 129.4, 127.1, 51.9, 46.5, 41.8, 41.1, 40.5, 32.9, 28.4; HRMS (ESI) Calculated for C$_{22}$H$_{31}$N$_3$O (M+H)$^+$ 354.2540, found 354.2530

(S)-N-(2,5-Diaminopentyl)-3-phenethyl-5-phenylpentanamide (181)

Dibenzyl (5-(3-phenethyl-5-phenylpentanamido)pentane-1,4-diyl)(S)-dicarbamate, **191**, (60 mg, 0.09 mmol) was dissolved in ethanol (10 mL) and 20% Pd(OH)$_2$/C (30 mg) was added. The reaction mixture was then purged and stirred under a hydrogen atmosphere overnight. The catalyst was filtered and washed with 20% MeOH/dichloromethane. The filtrate was concentrated under reduced pressure to give the product as a brown colored oil. (31 mg, 73%); ^{1}H NMR (MeOD) (400 MHz) (trifluoroacetic acid salt) δ 7.11 (m, 11H), 3.24 (m, 2H), 2.89 (m, 3H), 2.53 (t, 4H, J = 8), 2.25 (d, 2H, J = 4), 1.89 (m, 1H), 1.62 (m, 8H); ^{13}C NMR (100 MHz) (MeOD) δ 177.2, 143.7, 129.4, 129.3, 126.8, 52.8, 52.7, 47.6, 44.0, 41.7, 40.1, 36.8, 35.9, 33.8, 30.7, 28.4; HRMS (ESI) Calculated for C$_{24}$H$_{36}$N$_3$O (M+H)$^+$ 382.2853, found 382.2846

Benzyl *t*-butyl (5-(3,3-diphenylpropanamido)pentane-1,4-diyl)(S)-dicarbamate (182).

3,3-Diphenylpropanoic acid (61 mg, 0.27 mmol) was dissolved in dry DMF (5mL) and EDC (105 mg, 0.55 mmol) and HOBt (74 mg, 0.55 mmol) were added and the reaction stirred at room temperature for 5 minutes. Benzyl *t*-butyl (5-aminopentane-1,4-diyl)(S)-

dicarbamate, **78**, (86 mg, 0.25 mmol) was added followed by 2,6-lutidine (0.09 mL, 0.75 mmol). The reaction was stirred at room temperature overnight. The reaction mixture was then diluted with ethyl acetate and washed with water, 1M HCl, saturated $NaHCO_3$, water and brine. The organic layer was dried over sodium sulfate and filtered. The filtrate was then concentrated under reduced pressure and purified using an ISCO chromatograph with silica (0–10% MeOH/dichloromethane) to give a whitish yellow flaky solid. (101 mg, 77%); MP 102-104 °C; ^{1}H NMR (400 MHz) (CDCl₃) δ 7.29 (m, 16H), 6.17 (brs, 1H), 5.12 (s, 3H), 4. 57 (t, 2H, $J = 8$), 3.44 (m, 1H), 3.13 (m, 3H), 2.92 (m, 3H), 1.46 (s, 9H), 1.24 (m, 4H); ^{13}C NMR (100 MHz) (CDCl₃) δ 171.8, 156.6, 156.1, 143.6, 136.6, 128.6, 128.5, 128.1, 128.0, 127.7, 126.5, 79.5, 66.6, 50.5, 47.4, 43.5, 43.1, 40.6, 29.2, 28.4, 26.2.

Benzyl (S)-(4-amino-5-(3,3-diphenylpropanamido)pentyl)carbamate (183)

Benzyl *t*-butyl (5-(3,3-diphenylpropanamido)pentane-1,4-diyl)(S)-dicarbamate, **182**, (74 mg, 0.13 mmol) was dissolved in dichloromethane (3 mL) and cooled to 0 °C under nitrogen. Trifluoroacetic acid (2mL) was added and reaction stirred at that temperature for 2 hours. The reaction mixture was dissolved in saturated $NaHCO_3$ and the organic layer separated. The combined organic layers were dried over sodium sulfate and solvent removed under reduced pressure to give the product as a colorless oil. (56 mg, 94%); ^{1}H NMR (400 MHz) (CDCl₃) δ 7.34 (brs, 1H), 7.15 (m, 15H), 5,20 (m, 1H), 4.94 (s, 2H), 4.42 (t, 1H, $J = 8$), 3.16 (m, 2H), 2.86 (m, 5H), 1.25 (m, 4H); ^{13}C NMR (100 MHz) (CDCl₃) δ 173.1, 157.0, 143.4, 143.3, 136.3, 128.63. 128.61, 128.5, 128.2, 127.8, 127.75, 127.70, 126.6, 66.8, 51.80, 47.4, 42.3, 41.0, 40.1, 27.3, 25.4

Ethyl 3-benzyl-4-phenylbut-2-enoate (184)

To a round bottom flask containing 60% dispersion NaH (571 mg, 14.3 mmol) and anhydrous THF (20 mL) at 0 °C was added triethylphosphonoacetate (3.1 mL, 15.7 mmol) dropwise. The reaction mixture was naturally warmed to room temperature followed by a dropwise addition of 1,3-diphenyl acetone (1.9 mL, 9.5 mmol). The reaction mixture was stirred for 12 hours and then poured in water and extracted with dichloromethane. The combined organic layer was washed with brine and dried over sodium sulfate, filtered and concentrated under reduced pressure. This was purified on an ISCO chromatograph with silica to give the product as a colorless oil. (880 mg, 33%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.29 (m, 10H), 5.84 (s, 1H), 4.27 (q, 2H), 4.09 (s, 2H), 3.42 (s, 2H), 1.36 (t, 3H, J = 8), ^{13}C NMR (100 MHz) (CDCl$_3$) δ 166.5, 159.8, 138.8, 137.7, 129.4, 129.1, 128.6, 128.5, 126.7, 126.4, 118.4, 59.9, 43.4, 36.8, 14.3.

Ethyl 3-phenethyl-5-phenylpent-2-enoate (185).

To a round bottom flask containing 60% dispersion NaH (125 mg, 5.2 mmol) and anhydrous THF (10 mL) at 0 °C was added triethylphosphonoacetate (1.1 mL, 5.78 mmol) dropwise. The reaction mixture was allowed to warm to room temperature. This this reaction mixture was added dropwise 1,5-diphenylpentan-3-one, **32**, (820 mg, 3.5 mmol). The reaction mixture was stirred for 12 hours and then poured into water and extracted with dichloromethane. The combined organic layer was washed with brine and dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified on an ISCO chromatograph to give product as a colorless oil. (270 mg, 25%); ^{1}H NMR

(400 MHz) (CDCl$_3$) δ 7.16 (m, 6H), 7.06 (m, 4H), 5.61 (s, 1H), 4.05 (q, 2H), 2.82 (m, 2H), 2.66 (m, 4H), 2.30 (m, 2H), 1.16 (t, 3H, *J* = 8*)*: ^{13}C NMR (100 MHz) (CDCl$_3$) δ 166.3, 162.3, 142.0, 141.8, 141.1, 128.8, 128.5, 128.4, 128.3, 128.2, 128.0, 126.4, 126.2, 126.0, 116.3, 59.6, 58.4, 44.5, 40.5, 35.1, 34.7, 34.1, 33.2, 29.9, 29.8, 26.6, 26.3, 18.5, 14.4, 14.2.

Ethyl 3-benzyl-4-phenylbutanoate (186)

Ethyl 3-benzyl-4-phenylbut-2-enoate, **184**, (777 mg, 2.77 mmol) was dissolved in ethanol (20 mL) and 10% Pd/C (280 mg) was added. The mixture was purged and stirred overnight under hydrogen atmosphere. The reaction was then filtered to remove catalyst and solvent removed under reduced pressure. The residue was purified on an ISCO chromatograph with silica gel (0-10% ethyl acetate/ hexane) to give the product as a colorless oil. (698 mg, 89%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.31 (m, 4H), 7.22 (m, 6H), 4.08 (q, 2H), 2.62 (m, 5H), 2.25 (d, 2H, *J* = 8), 1.25 (t, 3H, *J* = 8); ^{13}C NMR (100 MHz) (CDCl$_3$) δ172.9, 140.0, 129.3, 128.3, 126.1, 60.2, 40.1, 39.0, 37.9, 14.2.

Ethyl 3-phenethyl-5-phenylpentanoate (187).

Ethyl 3-phenethyl-5-phenylpent-2-enoate, **185**, (270 mg, 0.88 mmol) was dissolved in ethanol (10 mL) and 10% Pd/C (100 mg) was added. The mixture was purged and stirred overnight under a hydrogen atmosphere. The reaction was then filtered to remove catalyst and solvent removed in under reduced pressure. The residue was purified on an ISCO chromatograph with silica gel (0-10% ethyl acetate/hexane) to give the product as a

colorless oil, (188 mg, 69%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.11 (m, 10H), 4.03 (m, 2H), 2.52 (m, 4H), 2,27 (m, 2H), 1.90 (m, 1H), 1.59 (m, 4H), 1.14 (m, 3H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ173.3, 173.1, 142.6, 142,4, 142.1, 128.5, 128.4, 128.2, 126.1, 125.8, 125.5, 60.3, 44.5, 39.2, 39.0, 37.9, 35.8, 35.1, 34.6, 34.1, 33.5, 33.1, 33.0, 30.9, 29.8, 26.8, 26.5, 14.3

3-Benzyl-4-phenylbutanoic acid (188)

A mixture of ethyl 3-benzyl-4-phenylbutanoate, **186**, (260 mg, 0.92 mmol) and KOH (206, 3.68 mmol) in ethanol/water (3:2) (5mL) was heated at 70 °C for 4 hours. The mixture was cooled to room temperature and acidified to pH = 2 with 1 M HCl. The solvent was evaporated under reduced pressure and residue extracted with ethyl acetate. The combined extracts were washed with brine, dried over Na$_2$SO$_4$, filtered and evaporated under reduced pressure to give the product as a colorless oil (176 mg, 75%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 11.73 (brs, 1H), 7.10 (m, 10H), 2.47 (m, 5H), 2.15 (d, 2H, J = 8); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 180.0, 139.9, 129.3, 128.4, 128.3, 126.3, 40.1, 38.9, 37.6.

3-Phenethyl-5-phenylpentanoic acid (189)

A mixture of ethyl 3-phenethyl-5-phenylpentanoate, **187**, (180 mg, 0.58 mmol) and KOH (130, 2.31 mmol) in ethanol/water (3:2) (5mL) was heated at 70 °C for 4 hours. The mixture was cooled to room temperature and acidified to pH = 2 with 1 M HCl. The solvent was evaporated under reduced pressure and residue extracted with ethyl acetate. The combined extracts were washed with brine, dried over Na$_2$SO$_4$, filtered and evaporated under reduced

pressure to give the product as a colorless oil (121 mg, 74%); ^{1}H NMR (CDCl$_3$) (400 MHz)

δ 11.54 (brs, 1H), 7.15 (m, 10H), 2.43 (m, 6H), 1.93 (m, 1H), 1.67 (m, 4H); ^{13}C NMR (100

MHz) (CDCl$_3$) δ 179.9, 142.2, 128.5, 128.4, 125.9, 38.7, 35.7, 34.5, 33.0

Dibenzyl (5-(3-benzyl-4-phenylbutanamido)pentane-1,4-diyl)(S)-dicarbamate (190).

3-Benzyl-4-phenylbutanoic acid, **188**, (119 mg, 0.47 mmol) was dissolved in DMF (5mL).

EDC (179 mg, 0.94 mmol) and HOBt (126 mg, 0.94 mmol) were added and reaction stirred

at room temperature for 5 minutes. Dibenzyl (5-aminopentane-1,4-diyl)(S)-dicarbamate,

88, (150 mg, 0.39 mmol) was added followed by 2,6-lutidine (0.18 mL, 1.55 mmol) and

reaction mixture stirred at room temperature overnight. The mixture was then diluted with

ethyl acetate, washed with water, 1 M HCl, saturated NaHCO$_3$, water and brine, dried over

sodium sulfate and filtered. The filtrate was concentrated under reduced pressure and

purified on an ISCO chromatograph with silica (0-10% methanol/dichloromethane) to give

product as a flaky yellow solid, (176 mg, 60%); MP 134-136 °C; ^{1}H NMR (CDCl$_3$) (400

MHz) δ 7.11 (m, 20H), 6.11 (s, 1H), 5.38 (d, 1H, $J = 8$), 5.08 (s, 1H), 4.87 (m, 4H), 3.54

(s, 1H), 3.09 (m, 4H), 2.45 (m, 5H), 1.92 (m, 2H), 1.35 (m, 4H); ^{13}C NMR (400 MHz)

(CDCl$_3$) δ 173.2, 156.9, 156.6, 140.2, 140.1, 136.6, 136.4, 129.3, 128.53, 128.51, 128.3,

128.1, 128.06, 128.00, 126.0, 66.7, 66.6, 51.6, 43.8, 40.6, 40.03, 39.9, 38.9, 29.8, 26.3.

Dibenzyl (5-(3-phenethyl-5-phenylpentanamido)pentane-1,4-diyl)(S)-dicarbamate (191)

Ethyl 3-phenethyl-5-phenylpentanoate, **189**, (90 mg, 0.32 mmol) was dissolved in DMF (5 mL). EDC (122 mg, 0.64 mmol) and HOBt (86 mg, 0.64 mmol) were added and the reaction stirred at room temperature for 5 minutes. Dibenzyl (5-aminopentane-1,4-diyl)(S)-dicarbamate, **88**, (123 mg, 0.32 mmol) was added followed by 2,6-lutidine (0.12 mL, 0.96 mmol) and reaction mixture stirred at room temperature overnight. The mixture was then diluted with ethyl acetate, washed with water, 1M HCl, saturated $NaHCO_3$, water and brine. The mixture was then dried over sodium sulfate and filtered. The filtrate was then concentrated under reduced pressure and the residue purified on an ISCO chromatograph using silica (0-10% methanol/dichloromethane) to give the product as a flaky white solid. (98 mg, 48%); ^{1}H NMR ($CDCl_3$) (400 MHz) δ 7.27 (m, 10H), 6.11 (s, 1H), 5.16 (m, 6H), 3.69 (s, 1H), 3.25 (m, 4H), 2.62 (m, 3H), 1.99 (m, 3H), 1.51 (m, 9H); ^{13}C NMR (100 MHz) ($CDCl_3$) δ 173.2, 156.9, 156.6, 142.4, 136.6, 136.4, 128.5, 128.4, 128.3, 128.1, 128.07, 128.03, 125.8, 66.7, 66.6, 51.7, 43.9, 41.3, 40.6, 35.5, 34.8, 32.9, 29.7, 26.3.

(S)-N[1]-(1,5-Diphenylpentan-3-yl)pentane-1,2,5-triamine (192).

A solution of dibenzyl (5-((1,5-diphenylpentan-3-yl)amino)pentane-1,4-diyl)(S)-dicarbamate, **195**, (116 mg, 0.19 mmol) in ethanol (10 mL) was treated with 20% Pd(OH)2/C (40 mg) and the mixture was purged. The solution was then stirred under hydrogen gas overnight. The catalyst was then filtered and washed with ethanol which was

concentrated under reduced pressure to give the crude product. The crude product was then dissolved in 5 ml of dichloromethane and Boc$_2$O was added. The reaction mixture was stirred at room temperature for 1 hour and the solvent removed. The residue was purified to give the boc-protected product which was stirred with trifluoroacetic acid (0.5 mL) and dichloromethane (1 mL) for 1 hour to give the product as the trifluoroacetic acid salt. (Colorless oil, 21 mg, 17%); ^{1}H NMR (MeOD) (400 MHz) δ 7.15 (m, 10H), 3.61 (m, 1H), 3.27 (m, 3H), 2.87 (m, 2H), 2.65 (m, 4H), 2.02 (m, 4H), 1.73 (m, 4H); ^{13}C NMR (MeOD) (100 MHz) δ 141.6, 129.7, 129.4, 127.4, 60.6, 39.9, 32.9, 32.7, 31.9, 29.3, 24.2; HRMS (ESI) Calculated for C$_{22}$H$_{33}$N$_3$ (M+H)$^+$ 340.2747, found 340.2747

(S)-N^1-(1,5-Diphenylpentan-3-yl)pentane-1,3,5-triamine (193)

(S)-3,5-Diamino-N-(1,5-diphenylpentan-3-yl)pentanamide, **55**, (77 mg, 0.14 mmol) was dissolved in 10 mL of THF and BH$_3$.THF (2 mL) and the mixture was heated at 83 °C for overnight. Then methanol (10 mL) was added and the reaction mixture was stirred at that same temperature for 2 hours. Then water (1 mL) was added and the reaction cooled to room temperature. Solvents were removed under reduced pressure and the residue redissolved in dichloromethane, dried with sodium sulfate and organic layer filtered and solvent removed to give an oily residue. The oily residue was redissolved in dichloromethane and Boc$_2$O was added and reaction stirred for 2 hours. Workup was done by extraction with water and ethyl acetate. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified on an ISCO chromatograph (0-30% ethyl acetate/hexane) to give di-*tert*-butyl (5-((*tert*-

butoxycarbonyl)(1,5-diphenylpentan-3-yl)amino)pentane-1,3-diyl)(S)-dicarbamate as a colorless oil (15 mg, 16%). The di-*tert*-butyl (5-((*tert*-butoxycarbonyl)(1,5-diphenylpentan-3-yl)amino)pentane-1,3-diyl)(S)-dicarbamate (11 mg, 0.017 mmol) was dissolved in dichloromethane (1 mL) and trifluoroacetic acid (0.5 mL). The reaction mixture was stirred at room temperature for 2 hours. Solvents were removed and residue was vacuum dried to obtain product (trifluoroacetic acid salt) as a colorless oil (10 mg, quantitative); ^{1}H NMR (400 MHz) (MeOD) δ 7.16 (m, 10H), 3.36 (m, 2H), 3.15 (m, 2H), 3.02 (m, 4H), 1.98 (m, 8H); ^{13}C NMR (100 MHz) (MeOD) δ 141.6, 129.7, 129.4, 127.5, 59.5, 42.5, 36.9, 33.0, 32.9, 31.9, 31.6, 30.4; HRMS (ESI) Calculated for $C_{22}H_{33}N_3$ (M+H)$^+$ 340.2747, found 340.2754

(S)-N^5-(1,5-Diphenylpentan-3-yl)pentane-1,2,5-triamine (194).

To a solution of dibenzyl (5-((1,5-diphenylpentan-3-yl)amino)pentane-1,2-diyl)(S)-dicarbamate, **196**, (26 mg, 0.04 mmol) in ethanol (5mL) was added 20% Pd(OH)$_2$/C (20 mg) and (BOC)$_2$O (23 mg, 0.11 mmol) and the mixture was purged. It was then stirred overnight at room temperature under a hydrogen atmosphere. The catalyst was then filtered out by washing with 20% methanol/dichloromethane and the filtrate was concentrated and purified to give a colorless oil (5.7 mg, 21%). The purified product was dissolved in dichloromethane (1 mL) and trifluoroacetic acid (0.5 mL) and stirred at room temperature for 2 hours. Upon completion of the reaction, the solvents were removed and the residue dried on a vacuum to give product as a colorless oil. (6.0 mg, 98%) (overall yield = 21%); ^{1}H NMR (MeOD) (400 MHz) δ 7.16 (m, 10H), 3.50 (m, 1H), 3.16 (m, 4H), 2.96 (m, 2H), 2.64 (m, 4H), 1.98 (m, 4H), 1.73 (m, 4H); ^{13}C δ 1.41.6, 129.7, 129.4, 127.5, 59.1, 50.2,

45.5, 41.9, 33.0, 32.0, 30.7, 28.8, 23.0; HRMS (ESI) Calculated for $C_{22}H_{33}N_3$ $(M+H)^+$ 340.2747, found 340.2748

Dibenzyl (5-((1,5-diphenylpentan-3-yl)amino)pentane-1,4-diyl)(S)-dicarbamate (195).

To a solution of 1,5-diphenylpentan-3-one, **32**, (84 mg, 0.35 mmol), acetic acid (0.1 mL, 1.77 mmol) and dibenzyl (5-aminopentane-1,4-diyl)(S)-dicarbamate, **88**, (150 mg, 0.39 mmol) in methanol (5mL) was added portion wise $NaCNBH_3$ (24.4 mg, 0.39 mmol). The reaction was stirred at room temperature for 17 hours and concentrated under reduced pressure. The residue was partitioned between aqueous K_2CO_3 and dichloromethane. The organic layer was separated and dried over sodium sulfate and the solvent evaporated. The residue was purified on an ISCO chromatograph (10% MeOH/dichloromethane + 1% NH_4OH) to give product as a white solid. (123.3 mg, 57%); MP 75-76 °C; 1H NMR ($CDCl_3$) (400 MHz) δ 7.83 (m, 2H), 7.28 (m, 20H), 5.13 (m, 6H), 3.67(m, 1H), 3.22 (m, 2H), 2.54 (m, 7H), 1.62 (m, 9H); ^{13}C NMR (400 MHz) ($CDCl_3$) δ 156.5, 156.4, 142.3,142.2, 136.7, 136.6, 128.5, 128.44, 128.42, 128.37, 128.35, 128.0, 125.9, 125.8, 66.6, 56.7, 51.0, 49.8, 40.8, 35.9, 35.6, 32.1,30.4, 26.5

Dibenzyl (5-((1,5-diphenylpentan-3-yl)amino)pentane-1,2-diyl)(S)-dicarbamate (196).

To a solution dibenzyl (5-aminopentane-1,2-diyl)(S)-dicarbamate, **122**, (101.7 mg, 0.21 mmol), acetic acid and 1,5-diphenylpentan-3-one, **32**, in methanol was added portionwise

NaBH$_3$CN. The reaction was stirred at room temperature for 17 hours and concentrated under reduced pressure. The residue was partitioned between aqueous K$_2$CO$_3$ and dichloromethane. The organic layer was separated and dried over anhydrous sodium sulfate and solvent evaporated under reduced pressure. The residue was purified on an ISCO chromatograph to give product as a white powder. (30 mg, 46% brsm); MP 82-84 °C; ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.16 (m, 20H), 5.42 (m, 1H), 5. 20 (m, 1H), 4.99 (s, 2H), 4.98 (s, 2H), 3.61 (m, 1H), 3.20 (m, 2H), 2.52 (m, 6H), 1.68 (m, 4H), 1.44 (m, 5H); ^{13}C NMR (CDCl$_3$) (100 MHz) δ 142.3, 136.5, 128.5, 128.4, 128.3, 128.1, 128.5, 125.8, 66.8, 56.7, 51.7, 46.2, 45.3, 35.5, 32.0, 30.4, 26.4

(S)-N^1-(1,5-Diphenylpentan-3-yl)butane-1,2,4-triamine (197).

Di-*tert*-butyl (4-((1,5-diphenylpentan-3-yl)amino)butane-1,3-diyl)(S)-dicarbamate, **201**, (22 mg, 0.03 mmol) was dissolved in dichloromethane (1 mL) and cooled to 0 °C. Trifluoroacetic acid (0.5 mL) was added and reaction stirred at 0 °C for 2 hours. The solvents were evaporated and the residue triturated with methanol. The methanol was evaporated. This tituration was repeated 3 times. The residue was dried under reduced pressure to obtain product as a salt of trifluoroacetic acid (colorless oil, 20.3 mg, 94%). ^{1}H NMR (CD$_3$OD) δ 7.28 (m, 10H), 3.80 (m, 1H), 3.39 (m, 3H), 3.15 (m, 2H), 2.78 (m, 4H), 2.14 (m, 6H); HRMS (ESI) Calculated for C$_{21}$H$_{31}$N$_3$ (M+H)$^+$ 326.2591, found 326.2592

(S)-N4-(1,5-Diphenylpentan-3-yl)butane-1,2,4-triamine (198).

Di-*tert*-butyl (4-((1,5-diphenylpentan-3-yl)amino)butane-1,2-diyl)(R)-dicarbamate, **203**, (19 mg, 0.04 mmol) was dissolved in dichloromethane (1 mL) and trifluoroacetic acid (0.5 mL) was added. Reaction was stirred at room temperature for 2 hours. The solvent was removed and multiple titurations were done with methanol. After removal of the methanol, an oil was obtained that was dried under vacuum. (22 mg, 100%); ^{1}H (400 MHz) (CD$_3$OD) δ 7.15 (m, 10H), 3.65 (m, 1H), 3.16 (m, 5H), 2.64 (t, 4H, $J = 8$), 2.12 (m, 2H), 1.99 (m, 4H); ^{13}CNMR (100 MHz) (CD$_3$OD) δ141.6, 129.7, 129.4, 127.4, 59.6, 42.3, 41.9, 32.9, 32.8, 31.9, 28.6; HRMS (ESI) Calculated for C$_{21}$H$_{31}$N$_3$ (M+H)$^+$ 326.2591, found 326.2592

Benzyl *tert*-butyl (4-((1,5-diphenylpentan-3-yl)amino)butane-1,3-diyl)(S)-dicarbamate (200).

To benzyl *tert*-butyl (4-aminobutane-1,3-diyl)(S)-dicarbamate, **199**, (100 mg, 0.3 mmol) in methanol (10 mL) was added 1,5-diphenylpentan-3-one, **32**, (85 mg, 0.36 mmol) and 5Å molecular sieves. Sodium cyanoborohydride (56 mg, 0.89 mmol) was added. The reaction was heated at 70 °C for 7 hours, then cooled to room temperature. The molecular sieves were filtered off and washed with methanol. The methanol was evaporated and the residue was dissolved in ethyl acetate. The organic layer was quenched with saturated ammonium chloride solution and washed with saturated sodium bicarbonate, then brine. The organic layer was dried over sodium sulfate, concentrated and purified on an ISCO chromatograph (10% methanol/dichloromethane) to give product as a colorless oil. (74 mg, 45%); ^{1}H NMR (CDCl$_3$) δ 7.18 (m, 15H), 5.64 (brs, 1H), 5.01 (m, 3H), 3.60 (brs, 1H), 3.36

(m, 1H), 2.98 (m, 1H), 2.59 (m, 6H), 1.66 (m, 5H), 1.36 (s, 10H), 1.18 (m, 2H); ^{13}C NMR (CDCl$_3$) δ 156.70, 141.8, 141.6, 136.7, 128.53, 128.5, 128.36, 128.33, 128.0, 126.0, 125.9, 80.0, 66.5, 57.0, 50.4, 47.8, 37.6, 35.2, 34.9, 33.6, 31.9, 28.3, 22.0

Di-*tert*-butyl (4-((1,5-diphenylpentan-3-yl)amino)butane-1,3-diyl)(S)-dicarbamate (201).

Benzyl *tert*-butyl (4-((1,5-diphenylpentan-3-yl)amino)butane-1,3-diyl)(S)-dicarbamate, **200**, (128 mg, 0.23 mmol) was dissolved in ethanol (10 mL) and 20% Pd(OH)$_2$/C (60 mg) was added. Reaction was stirred under a hydrogen atmosphere for 2days. The catalyst was removed by filtration and washed with 20% methanol/dichloromethane. The solvent was then rotavapped and the residue redissolved in dichloromethane (10 mL). Boc$_2$O was added and the reaction stirred at room temperature for 1 hour. The solvent was removed by evaporation and the residue purified on an ISCO chromatograph (0-20% ethyl acetate/hexane) to give product as a colorless oil. (22.8 mg, 30%); ^{1}H NMR (CDCl$_3$) δ 5.48 (m, 1H), 3.61 (m, 2H), 3.36 (m, 2H, 2.89 (m, 2H), 2.50 (m, 4H), 1.67 (m, 8H), 1.35 (s, 9H), 1.28 (s, 9H); ^{13}C NMR (CDCl$_3$) δ 156.8, 156.2, 141.2, 128.5, 128.4, 128.3, 125.8, 80.4, 78.8, 36.9, 34.9, 32.7, 28.5, 28.4, 28.3

Benzyl di-*tert*-butyl butane-1,2,4-triyl(S)-tricarbamate (202).

Benzyl *tert*-butyl (4-aminobutane-1,3-diyl)(S)-dicarbamate, **199**, (280 mg, 0.83 mmol) was dissolved in dichloromethane (10 mL) and trimethylamine (0.17 mL, 1.25 mmol) was

added. Boc$_2$O (543 mg, 2.49 mmol) was added and the reaction was stirred at room temperature overnight. The reaction mixture was then dissolved in water and extracted with dichloromethane. The organic layer was dried over sodium sulfate and filtered. The filtrate was rotavapped and the residue purified on an ISCO chromatograph (50% ethyl acetate/hexane) to give product as a colorless oil (190 mg, 52%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.31 (m, 5H), 5.76 (brs, 1H), 5.11 (m, 4H), 3.68 (m, 1H), 3.45 (m, 1H), 3.16 (m, 2H), 3.00 (m, 1H), 1.68 (m, 1H), 1.43 (s, 9H), 1.42 (m, 1H), 1.41 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 156.7, 156.5, 136.7, 128.4, 128.0, 127.9, 79.5, 79.4, 66.4, 49.4, 44.4, 37.5, 33.2, 28.4, 28.3

Di-*tert*-butyl (4-((1,5-diphenylpentan-3-yl)amino)butane-1,2-diyl)(R)-dicarbamate (203).

Step 1: Di-*tert*-butyl (4-aminobutane-1,2-diyl)(S)-dicarbamate - Benzyl di-*tert*-butyl butane-1,2,4-triyl(S)-tricarbamate, **202**, (140 mg, 0.32 mmol) was dissolved in ethanol (10 mL) and 20% Pd(OH)$_2$/C (40 mg) was added. Reaction was stirred under nitrogen atmosphere overnight. The catalyst was filter and the residue washed with 20% methanol/dichloromethane. The solvents were removed by evaporation and reaction taking to the next step with no purification.

Step 2: The crude di-*tert*-butyl (4-aminobutane-1,2-diyl)(S)-dicarbamate was dissolved in methanol (10 mL) and 1,5-diphenylpentan-3-one, **32**, (57 mg, 0.24 mmol) and 5A molecular sieves were added. Then sodium cyanoborohydride (38 mg, 0.6 mmol) was added and reaction was stirred at 70 °C for 7 hours. The molecular sieves were then filtered

off and the residue washed with methanol. The methanol was evaporated and the residue was dissolved in ethyl acetate. The organic layer was quenched with saturated ammonium chloride solution and washed with saturated sodium bicarbonate, then brine. The organic layer was dried over sodium sulfate, concentrated and purified on an ISCO chromatograph (10% methanol/dichloromethane) to give product as a colorless oil. (48.6 mg, 47%); ^{1}H NMR (CDCl$_3$) δ 7.15 (m, 10H), 5.83 (brs, 1H), 5.07 (brs, 1H), 3.60 (brs, 1H), 3.64 (m, 1H), 3.15 (m, 2H), 2.60 (m, 7H), 1.73 (m, 4H), 1.36 (s, 9H), 1.34 (m, 2H), 1.32 (s, 9H); ^{13}C NMR (CDCl$_3$) δ 156.8, 142.0, 128.4, 128.3, 125.8, 79.5, 79.3, 56.8, 50.3, 44.8, 42.9, 35.1, 32.4, 31.9, 32.8, 31.3, 29.6, 28.4, 28.3

N^1-(2-Aminoethyl)-N2-(1,5-diphenylpentan-3-yl)ethane-1,2-diamine (204)

tert-butyl (2-((*tert*-butoxycarbonyl)(2-((*tert*-butoxycarbonyl)amino)ethyl)amino)ethyl)-(1,5-diphenylpentan-3-yl)carbamate, **211**, (19 mg, 0.03 mmol) was dissolved in dichloromethane (2 mL) and trifluoroacetic acid (1 mL). Reaction mixture was stirred at room temperature for 1.5 hours. Solvent was rotavapped to give product as trifluoroacetic acid salt: colorless oil. (18 mg, 100%); ^{1}H NMR (MeOD) (400 MHz) δ 7.28 (m, 10H), 3.37 (m, 9H), 2.76 (m, 4H), 2.12 (m, 4H); ^{13}C NMR (CDCl3) (100 MHz) δ 141.6, 129.7, 129.4, 127.4, 59.8, 46.4, 45.4, 42.9, 37.4, 32.9, 31.9; HRMS (ESI) Calculated for C$_{21}$H$_{31}$N$_3$ (M+H)$^+$ 326.2591, found 326.2584

1-(1,5-Diphenylpentan-3-yl)-4-methylpiperazine (205)

A mixture of 1,5-diphenylpentan-3-one, **32**, (100 mg, 0.42 mmol) and 1-methylpiperazine (0.05 mL, 0.42 mmol) in dichloromethane (3 mL) was stirred at room temperature for 1.5 hours and sodium triacetoxyborohydride (179 mg, 0.85 mmol) was added and the mixture was stirred at room temperature overnight. To this was added 3 M NaOH solution and extracted with dichloromethane. The organic layer was washed with brine and dried over sodium sulfate and concentrated. The concentrate was purified on an ISCO chromatograph (0-10% MeOH, dichloromethane) to give product as a colorless oil. (14.4 mg, 11%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.13 (m, 10H), 2.55 (t, 4H, $J = 8$), 2.49 (t, 4H, $J = 4$), 2.39 (brs, 3H), 2.31 (m, 1H), 2.23 (s, 3H), 1.77 (m, 1H), 1.52 (m, 2H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 142.7, 128.4, 128.2, 125.6, 62.0, 55.7, 47.6, 46.0, 33.2, 31.6; HRMS (ESI) Calculated for C$_{22}$H$_{30}$N$_2$ (M+H)$^+$ 323.2482, found 323.2507

1-(1,5-Diphenylpentan-3-yl)-4-(4-methoxybenzyl)piperazine (206).

1,5-diphenylpentan-3-yl methane sulfonate, **213**, (200 mg, 0.63 mmol) was dissolved in DMF (5 mL) and cooled to 0 °C. 1-(4-Methoxybenzyl)piperazine, **212**, (155.4 mg, 0.75 mmol) was added followed by K$_2$CO$_3$ (174 mg, 1.26 mmol). The reaction was stirred at that temperature for 1 hour then heated at 100 °C for another 1 hour. The reaction mixture was washed with water and extracted with ethyl acetate. The organic layers were washed again with water and then brine, dried over sodium sulfate and concentrated. The concentrate was purified on an ISCO chromatograph (50% ethyl acetate/hexane) to give product as a colorless oil. (33 mg, 12%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.26 (m, 12H),

6.90 (dd , 2H, *J = 4,8)*, 4.95 (m, 1H), 3.83 (s, 3H), 3.50 (s, 6H), 2.69 (m, 4H), 2.43 (brs, 4H), 1.96 (m, 4H) ; ^{13}C NMR (CDCl$_3$) (100 MHz) δ 158.9, 155.2, 141.8, 130.3, 128.4, 128.3, 125.8, 113.7, 74.6, 62.4, 55.2, 52.7, 43.7, 36.1, 31.7; HRMS (ESI) Calculated for C$_{29}$H$_{36}$N$_2$O (M+H)$^+$ 429.2900, found 429.2922

Benzyl (2-(((benzyloxy)carbonyl)amino)ethyl)(2-hydroxyethyl)carbamate (207).

Benzyl chloroformate (7.5 mL, 0.06 mmol) was added in 5 portions with 20 minutes intervals to a cooled (0 °C) and vigorously stirring solution of 2-((2-aminoethyl)amino)ethan-1-ol (2.5 mL, 0.03 mmol), THF (50 mL), water (8.5 mL), NaHCO$_3$ (4.2 g, 0.05 mmol) and 10 M NaOH solution (1.25mL). Stirring was continued for 1 hour at that temperature and 3 hours at room temperature. The organic layer was separated and evaporated to dryness. Residue was dissolved in EtOAc, washed with 1 M HCl, water and dried over sodium sulfate and concentrated. This was purified on an ISCO chromatograph to give product as a colorless oil; (3 g, 32%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.32 (m, 10H), 5.90 (d, 1H, *J = 72)*, 5.09 (s, 2H), 5.05 (s, 2H), 3.75 (m, 3H), 3.42 (m, 6H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 156.9. 156.8, 136.6, 136.5, 136.4, 128.6, 128.5, 128.0, 127.8, 127.7, 67.4, 67.3, 66.6, 60.9, 53.6, 51.4, 50.8, 48.7, 48.3, 40.0

Benzyl (2-(((benzyloxy)carbonyl)amino)ethyl)(2-(1,3-dioxoisoindolin-2-yl)ethyl)-carbamate (208).

Triphenylphosphine (845 mg, 3.22 mmol) and phthalimide (473 mg, 3.22 mmol) was dissolved in THF (6 mL).   Benzyl (2-(((benzyloxy)carbonyl)amino)ethyl)(2-hydroxyethyl)carbamate, **207**, (1000 mg, 2.69 mmol) was added and reaction cooled to 0 ºC under nitrogen.   DIAD (0.63 mL, 3.22 mmol) was added to the reaction mixture dropwise. Reaction was allowed to stir at that temperature for 30 minutes, then overnight at room temperature.   Upon completion of the reaction, the reaction mixture was concentrated and purified on an ISCO chromatograph (0-50% ethyl acetate/hexane) to give product as a yellow colored oil. (1.1 g, 85%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.74 (m, 4H), 7.22 (m, 10H), 5.05 (s, 1H), 5.02 (s, 1H), 4.87 (s, 2H). 4.76 (s, 1H); 3.79 (m, 2H), 3.56 (m, 2H), 3.41 (m, 2H), 3.31 (m, 2H); ^{13}C (CDCl3) (100 MHz) δ 169.8, 169.6, 158.8, 158.2, 157.9, 138.3, 137.9, 137.3, 135.4, 135.3, 133.3, 133.1, 129.5, 129.0, 128.9, 128.7, 128.6, 124.2, 124.1, 68.5, 68.2, 67.4, 47.9, 47.2, 47.1, 40.4, 40.0, 37.1, 36.9, 22.3

Benzyl (2-aminoethyl)(2-(((benzyloxy)carbonyl)amino)ethyl)carbamate (209).

Benzyl   (2-(((benzyloxy)carbonyl)amino)ethyl)(2-(1,3-dioxoisoindolin-2-yl)ethyl)-carbamate, **208**, (1100 mg, 2.19 mmol) was dissolved in methanol (40 mL).   Then hydrazine monohydrate (0.27 mL, 5.38 mmol) was added and reaction mixture heated at 75 ºC for 2 hours. Then the reaction mixture was cooled down and precipitate was filtered off. The residue was washed with cold methanol and the filtrate concentrated and purified on ISCO (0-10% MeOH/dichloromethane + 1% NH$_4$OH) to give product as a colorless oil

(590 mg, 72%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.33 (s, 5H), 7.29 (s, 5H), 5.11 (s, 2H), 5.07 (s, 2H), 3.36 (m, 6H), 2.83 (m, 2H), 1.19 (brs, 2H); ^{13}C NMR(CDCl3) (100 MHz) δ 156.7, 136.7, 136.5, 128.5, 128.4, 128.1, 128.0, 127.9, 67.2, 66.4, 51.3, 50.9, 49.8, 48.0, 40.7, 40.2, 39.9

Benzyl (2-(((benzyloxy)carbonyl)amino)ethyl)(2-((1,5-diphenylpentan-3-yl)amino)-ethyl)carbamate (210).

Benzyl (2-aminoethyl)(2-(((benzyloxy)carbonyl)amino)ethyl)carbamate, **209**, (152 mg, 0.41 mmol) was dissolved in methanol (10 mL), 1,5-diphenylpentan-3-one, **32**, (117 mg, 0.49 mmol) and 5Å molecular sieves was added. Then sodium cyanoborohydride (77 mg, 1.23 mmol) was added and reaction stirred at 70 °C for 7 hours. On completion of the reaction, the molecular sieves were filtered off. The residue was washed with methanol and the filtrate was concentrated. This was quenched with saturated NH$_4$Cl and ethyl acetate was added. Wash organic layer with saturated NaHCO$_3$, brine and purify on an ISCO chromatograph (0-10% MeOH/dichloromethane + 1% NH$_4$OH) to give product as a colorless oil (136 mg, 56%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.27 (m, 20H), 5.12 (m, 4H), 3.41 (m, 6H), 2.89 (s, 1H), 2.76 (s, 1H), 2.63 (m, 5H), 1.77 (m, 4H); ^{13}C NMR(CDCl$_3$) (100 MHz) δ 156.7, 142.2, 136.6, 136.4, 128.6, 128.5, 128.4, 128.3, 128.1, 127.9, 125.8, 67.4, 66.6, 56.6, 49.3, 48.6, 45.2, 40.3, 35.5, 35.2, 32.0

tert-Butyl (2-((_tert_-butoxycarbonyl)(2-((_tert_-butoxycarbonyl)-amino)ethyl)amino)-ethyl)(1,5-diphenylpentan-3-yl)carbamate (211).

Benzyl (2-(((benzyloxy)carbonyl)amino)ethyl)(2-((1,5-diphenylpentan-3-yl)amino)-ethyl)carbamate, **210**, (90 mg, 0.15 mmol) was dissolved in ethanol (10 mL), then Boc$_2$O (106.8 mg, 0.49 mmol) and 20% Pd(OH)$_2$ /C was added. Reaction was stirred at room temperature overnight. On completion of the reaction, the catalyst was filtered off and solvent concentrated and purified on an ISCO chromatograph (0-40% ethyl acetate/ hexane) to give product as a colorless oil. (81.7 mg, 88%); ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.7.24 (m, 10H), 3.29 (m, 8H), 2.58 (m, 4H), 1.85 (m, 4H), 1.56 (s, 9H), 1.46 (s, 9H), 1.44 (s, 9H); ^{13}C NMR(CDCl3) (100 MHz) δ 156.0, 141.8, 128.3, 125.8, 80.0, 79.9, 79.0, 55.4, 47.2, 47.0, 39.7, 35.6, 35.6, 35.1, 32.9, 32.7, 28.4

1-(4-methoxybenzyl)piperazine (212).

Anhydrous piperazine (4 g, 46.4 mmol) was added to THF (80 mL) and the mixture was heated at reflux until the piperazine was fully dissolved. This solution was added _para_-methoxybenzyl chloride (1.05 mL, 7.72 mmol) dropwise. A white precipitate formed and the reaction was refluxed for 3 hours. The stirring mixture was then cooled and filtered and the solids were washed with THF and ethyl acetate. The combined organic layer was concentrated under reduced pressure and washed with 5% KOH in brine (pH >12). The aqueous layer was extracted with ethyl acetate and the combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography (10% MeOH/dichloromethane) to give product as a

white powder. (1.2 g, 75%); MP 85-87 °C; ^{1}H NMR (400 MHz) δ 7.15 (d, 2H, *J = 8*), 6.77 (d, 2H, *J = 8*), 3.71 (s, 3H), 3.62 (s, 2H), 2.83 (t, 4H, *J = 4*), 2.36 (brs, 4H); 13 C δ 158.7, 130.3, 129.6, 113.5, 62.7, 55.1, 53.4, 49.6, 45.4

1,5-Diphenylpentan-3-yl methane sulfonate. (213)

To as solution of 1,5-diphenylpentan-3-ol, **214**, (270 mg, 1.1 mmol) in dichloromethane (5mL) under nitrogen was added triethylamine (0.3 mL, 2.2 mmol) and methane sulfonyl chloride (0.13 mL, 1.69 mmol) and stirred at room temperature overnight. Then the solvent was removed under reduced pressure and purification done on an ISCO chromatograph (0-15% ethyl acetate/hexane) to give product as a colorless oil. (321.5 mg, 92%); 1H NMR (400 MHz) (CDCl$_3$) δ 7.27 (m, 10H), 4.85 (m, 1H), 3.01 (s, 3H), 2.77 (m, 4H), 2.11 (m, 4H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 140.7, 128.5, 128.3, 126.2, 82.4, 38.7, 36.2, 31.2

1,5-Diphenylpentan-3-ol (214)

1,5-Diphenylpentan-3-one, **32**, (500 mg, 2 mmol) was dissolved in THF (6 mL) under a nitrogen atmosphere. Ethanol (5 mL) was added followed by NaBH$_4$ (317 mg, 8 mmol). The reaction was stirred at room temperature for 2 hours. Water was then added to the stirring mix and the reaction extracted with ethyl acetate. The organic layer was washed with brine and dried over sodium sulfate and concentrated. The concentrate was purified on an ISCO chromatograph (0-20% ethyl acetate/hexane) to give product as a white solid.

(270 mg, 54%); MP 39 -40 °C; ^{1}H NMR (400 MHz) δ 7.30 (m, 10H), 3.72 (m, 1H), 2.82 (m, 2H), 2.72 (m, 2H), 1.86 (m, 4H); 13 C δ 142.0, 128.5, 128.4, 125.8, 70.8, 39.2, 32.0

(S)-N^{1}-(2-Phenethyl-4-phenylbutyl)pentane-1,2,5-triamine (215).

Di-*tert*-butyl (5-((*tert*-butoxycarbonyl)(2-phenethyl-4-phenylbutyl)amino)pentane-1,4-diyl)(S)-dicarbamate, **220**, (22 mg, 0.034 mmol) was dissolved in dichloromethane (1 mL) and trifluoroacetic acid (0.5 mL) was added. Reaction mixture was stirred at room temperature for 2 hours. On completion of the reaction, the solvents were removed under reduced pressure. The residue was triturated with methanol three times to obtain product as a white solid. (21 mg, 100%); MP 130-13 °C; ^{1}H NMR (400 MHz) (MeOD) δ 7.12 (m, 10H), 3.59 (m, 1H), 3.25 (m, 1H), 3.03 (m, 2H), 2.87 (m, 2H), 2.55 (m, 4H), 1.68 (m, 10H); ^{13}C NMR (100 MHz) (MeOD) 142.9, 129.5, 129.4, 127.0, 54.0, 51.1, 39.9, 36.3, 33.9, 33.3, 29.4, 24.2; HRMS (ESI) Calculated for $C_{23}H_{35}N_3$ (M+H)$^{+}$ 354.2904, found 354.2906.

(S)-N^{1}-(2-Phenethyl-4-phenylbutyl)pentane-1,3,5-triamine (216)

Step 1: Di-*tert*-butyl (5-((*tert*-butoxycarbonyl)(2-phenethyl-4-phenylbutyl)amino)-pentane-1,3-diyl)(S)-dicarbamate - (S)-3,5-diamino-N-(2-phenethyl-4-phenylbutyl)-pentanamide, **55**, (39 mg, 0.11 mmol) was dissolved in THF (10 mL) and BH$_3$.THF (1 mL) was added. The reaction mixture was heated at reflux for overnight. Then methanol (10 mL) was added and reaction stirred at reflux for 2 hours. The reaction mixture was cooled

down and water (2 mL) was added. This was then dried over sodium sulfate and filtered. The filtrate was concentrated under reduced pressure to provide the product.

Step 2: The crude product was then dissolved in dichloromethane (10 mL), Boc_2O (69 mg, 0.32 mmol) was added and reaction stirred for 2 hours at room temperature under nitrogen. Workup was done by phase extraction with water and ethyl acetate. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified on an ISCO chromatograph (0-30% ethyl acetate/hexane) to give product as a colorless oil. (34 mg, 49%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.14 (m, 10H), 3.10 (m, 6H), 2.54 (m, 4H), 1.28 (m, 37H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 155.7, 142.5, 128.3, 125.7, 79.4, 79.2, 44.6, 43.8, 33.1, 32.9, 32.7, 29.6, 28.5, 28.5, 28.4, 28.0

Step 3; (S)-N^1-(2-Phenethyl-4-phenylbutyl)pentane-1,3,5-triamine

Di-*tert*-butyl (5-(((*tert*-butoxycarbonyl)(2-phenethyl-4-phenylbutyl)amino)pentane-1,3-diyl)(S)-dicarbamate (10.7 mg, 0.016 mmol) was dissolved in dichloromethane (1 mL) and trifluoroacetic acid (0.5 mL). The reaction mixture was stirred at room temperature for 2 hours. Solvents were removed and residue was dried under vacuum to obtain product (trifluoroacetic acid salt) as a colorless oil. (9.4 mg, 91%); ^{1}H NMR (400 MHz) (MeOD) δ 7.14 (m, 10H), 3.41 (m, 1H), 3.06 (m, 6H), 2.59 (m, 4H), 2.02 (m, 3H), 1.70 (m, 5H); ^{13}C NMR (400 MHz) (MeOD) δ 142.9, 129.5, 129.4, 127.0, 53.1, 45.7, 44.3, 44.2, 36.3, 33.9, 33.3, 30.1, 29.8; HRMS (ESI) Calculated for $C_{23}H_{35}N_3$ (M+H)$^+$ 354.2904, found 354.2911

(S)-N5-(2-Phenethyl-4-phenylbutyl)pentane-1,2,5-triamine (217).

Step 1 : di-*tert*-butyl (5-((*tert*-butoxycarbonyl)(2-phenethyl-4-phenylbutyl)amino)-pentane-1,2-diyl)(S)-dicarbamate - (S)-N-(4,5-diaminopentyl)-2-phenethyl-4-phenyl-butanamide, **230**, (80 mg, 0.14 mmol) was dissolved in 10 mL of THF and BH_3.THF (2 mL) and the mixture was heated at 83 °C for overnight. Then methanol (10 mL) was added and the reaction mixture was stirred at that same temperature for 2 hours. Then water (1 mL) was added and the reaction cooled to room temperature. Solvents presents were then rotavapped and redissolved in dichloromethane, dried with sodium sulfate and organic layer filtered and solvent removed to give an oil residue.

Step 2: The residue was redissolved in dichloromethane (5 mL) and Boc_2O (92 mg, 0.42 mmol) was added and reaction stirred for 2 hours. Workup was done by extraction from water with ethyl acetate. The organic layer was dried over sodium sulfate,filtered and concentrated under reduced pressure. The residue was purified on an ISCO chromatograph (0-30% ethyl acetate/hexane) to give product as a colorless oil (9 mg, 10%); ^{1}H NMR (400 MHz) (MeOD) δ 7.24 (m, 10H), 4.72 (m, 2H), 3.62 (brs, 1H), 3.17 (m, 6H), 2.67 (m, 4H), 1.78 (m, 1H), 1.66 (m, 8H), 1.46 (m, 27H); ^{13}C NMR (100 MHz) (MeOD) δ 128.3, 125,7, 79.33, 57.8, 51.2, 46.8, 44.9, 33.2, 32.7, 28.4, 28.3

Step 3: (S)-N5-(2-Phenethyl-4-phenylbutyl)pentane-1,2,5-triamine (GAB-11-78) - Di-*tert*-butyl (5-((*tert*-Butoxycarbonyl)(2-phenethyl-4-phenylbutyl)amino)pentane-1,2-diyl)(S)-dicarbamate (8.7 mg, 0.013 mmol) was dissolved in dichloromethane (1 mL) and trifluoroacetic acid (0.5 mL). The reaction mixture was stirred at room temperature for 2 hours. Solvents were removed and residue was dried under vacuum to obtain product

(trifluoroacetic acid salt) as a white solid. (5.7 mg, 67%); MP 140-142 °C; ^{1}H NMR (400 MHz) (MeOD) δ7.13 (m, 10H); 3.49 (m, 1H), 3.17 (m, 2H), 2.94 (m, 4H), 2.57 (m, 4H), 1.71 (m, 9H); ^{13}C NMR (100 MHz) (MeOD) δ 142.9, 129.5, 129.4, 127.0, 52.7, 50.3, 42.0, 36.0, 33.9, 33.3, 28.8, 22.6; HRMS (ESI) Calculated for $C_{23}H_{36}N_3$ (M+H)$^+$ 354.2904, found 254.2911

Di-*tert*-butyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,4-diyl)(S)-dicarbamate (218).

Compound **91** was dissolved in ethanol (15 mL), 20% Pd (OH)$_2$/C (80 mg) and Boc$_2$O (272 mg, 1.25 mmol) was added. The reaction mixture was stirred under hydrogen at room temperature overnight. On completion of the reaction, the mixture was filtered to remove catalyst and solvent rotavapped. The residue was purified on an ISCO chromatograph (0-10% methanol/dichloromethane) to get product as a white solid. (85 mg, 30%); MP 140–142 °C ; ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.22 (m, 10H), 6.33 (brs, 1H), 5.02 (brs, 1H), 4.80 (m, 1H); 3.70 (m, 1H). 3.35 (m, 2H), 3.14 (m, 2H), 2.59 (m, 4H), 2.14 (m, 1H), 2.02 (m, 2H), 1.78 (m, 2H), 1.57 (m, 4H), 1.45 (s, 9H), 1.39 (m, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 176.0, 156.5, 156.1, 141.7, 141.6, 141.4, 128.3, 125.9, 79.5, 79.1, 51.1, 46.6, 44.0, 40.2, 34.4, 33.6, 30.3, 28.4, 28.3, 26.5

(S)-N-(2,5-Diaminopentyl)-2-phenethyl-4-phenylbutanamide (219).

Di-*tert*-butyl   (5-(2-phenethyl-4-phenylbutanamido)pentane-1,4-diyl)(S)-dicarbamate, **218**, (80 mg, 0.14 mmol) was dissolved in dichloromethane (5 mL) and trifluoroacetic acid (2.5 mL) at 0 ℃. The reaction was stirred at that temperature for 2 hours under nitrogen. The solvents were removed to obtain product as a trifluoroacetic acid salt, colorless oil. (79 mg, 100%); ^{1}H NMR (400 MHz) (MeOD) δ 7.22 (m, 10H), 3.46 (m, 2H), 2.97 (m, 2H), 2.61 (m, 4H), 2.40 (m, 1H), 1.96 (m, 2H), 1.83 (m, 6H); ^{13}C NMR (100 MHz) (MeOD) δ 180.1, 143.0, 129.4, 129.3, 126.9, 52.7, 47.5, 42.0, 40.1, 35.6, 35.5, 34.7, 28.5, 24.4

Di-*tert*-butyl   (5-((*tert*-butoxycarbonyl)(2-phenethyl-4-phenylbutyl)amino)pentane-1,4-diyl)(S)-dicarbamate (220).

(S)-N-(2,5-Diaminopentyl)-2-phenethyl-4-phenylbutanamide, **220**, (79 mg , 0.14 mmol) was dissolved in dry THF (10 mL) and BH$_3$.THF (2 mL) was added. The reaction mixture was stirred under reflux overnight under nitrogen. Methanol (10 mL) was added and the reaction was stirred at reflux for 2 hours. On completion, 2 mL of water was added and the reaction was allowed to cool to room temperature. Then the reaction was dried with sodium sulfate and filtered. The organic layer was concentrated under reduced pressure to remove solvent. The residue was dissolved in dichloromethane (5 mL) and Boc$_2$O (92 mg, 0.42 mmol) and reaction was stirred at room temperature for 2 hours. The organic layer was concentrated under reduced pressure and the residue purified on an ISCO chromatograph (0-30% EtOAc/hexane) to give product as a colorless oil. (30 mg, 32%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.23 (m, 10H), 5.03 (brs, 1H), 4.65 (brs, 1H), 3.71 (m, 1H),

3.43 (m, 2H), 3.14 (m, 2H), 2.84 (m, 1H), 2.63 (m, 4H), 1.66 (m, 6H), 1.47 (s, 18H), 1.43 (s, 9H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 155.5, 128.3, 125.8, 79.9, 79.0, 70.8, 62.7, 49.7, 49.4, 40.3, 35.9, 33.1, 33.0, 32.7, 30.7, 28.43, 28.4, 26.3

(3-Methylenepentane-1,5-diyl)dibenzene (221).

To a suspension of methyl triphenylphosphonium bromide (2397 mg, 6.7 mmol) in THF (8 mL) at room temperature was added portion wise potassium t-butoxide (848 mg, 7.56 mmol) under nitrogen. The mixture was stirred at that temperature for 3 hours. Then a solution of 1,5-diphenylpentan-3-one, **32**, (1000 mg, 4.2 mmol) in THF (2 mL) was added dropwise. The mixture was stirred overnight, then the mixture was concentrated under reduced pressure, then ice water added. The aqueous solution was extracted with ethyl acetate. The organic layer was then washed with brine and dried over sodium sulfate. The filtrate was then concentrated and purified on ISCO chromatograph (0-5% ethyl acetate/hexane) to give product as a colorless oil. (806 mg, 81%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.51 (m, 4H), 7.43 (m, 6H), 5.06 (m, 2H), 3.01 (m, 4H), 2.61 (m, 4H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 148.8, 142.5, 142.3, 128.9, 128.8, 128.5, 128.2, 126.4, 126.2, 126.0, 125.7, 109.9, 38.5, 38.3, 34.6

2-Phenethyl-4-phenylbutan-1-ol (222).

To a solution of (3-methylenepentane-1,5-diyl)dibenzene, **221**, (650 mg, 2.75 mmol) in THF (5 mL), cooled to 0 °C was added BH$_3$.THF (4.1 mL, 4.1 mmol). The reaction was

stirred at that temperature for 30 minutes, then warmed to room temperature and stirred for an additional 2 hours under nitrogen. Then the reaction was cooled again to 0 °C, 3 M NaOH (4.6 mL) and 30% H_2O_2 (1.4 mL) were added. The reaction was stirred at 0 °C for 30 minutes and heated at 60 °C for an additional hour. Solvents were removed under reduced pressure and the resulting residue diluted with water and extracted with ethyl acetate, The combined organic layers were washed with water, brine, dried over sodium sulfate, concentrated and purified on an ISCO gromatograph with 0-40% ethyl acetate/hexane) to give product as a colorless oil. (538 mg, 77%); ^{1}H NMR (400 MHz) (CDCl3) δ 7.29 (m, 10H), 3.69 (d, 2H, *J* = 8), 2.71 (m, 4H, *J* = 8), 1.72 (m, 5H), 1.47 (brs, 1H); ^{13}C NMR (100 MHz) (CDCl3) δ 142.5, 128.41, 128.40, 125.8, 65.3, 39.7, 33.2, 32.8

2-(2-Phenethyl-4-phenylbutyl)isoindoline-1,3-dione (223).

Triphenylphosphine (570 mg, 2.17 mmol) and phthalimide (319 mg, 2.17 mmol) were dissolved in THF (6 mL) at 0 °C under nitrogen. Then 2-phenethyl-4-phenylbutan-1-ol, **222**, (460 mg, 1.80 mmol) in THF (2 mL) and DIAD (0.43 mL, 2.17 mmol) was added dropwise to the reaction mixture. Reaction was allowed to stir for 30 minutes at 0 °C and overnight at room temperature. Then the solvents were rotavapped and residue purified on an ISCO chromatograph (0-30% ethyl acetate/hexane) to give product as a white powder. (630 mg, 91%); MP 75-76 °C; ^{1}H NMR (400 MHz) (CDCl3) δ 7.91 (m, 2H), 7.74 (m, 2H), 7.32 (m, 10H), 3.82 (d, 2H, *J* = 8), 2.83 (m, 4H), 2.15 (m, 1H), 1.81 (m, 4H); ^{13}C NMR (100 MHz) (CDCl3) δ 168.6, 142.7, 142.4, 142.1, 133.9, 132.4, 132.1, 128.9, 128.8, 128.7, 128.5, 128.4, 128.3, 128.2, 126.1, 125.9, 125.5, 123.2, 41.6, 36.9, 33.7, 32.8

2-Phenethyl-4-phenylbutan-1-amine (224).

Hydrazine monohydrate (120 µL, 2.4 mmol) was added to a solution of 2-(2-phenethyl-4-phenylbutyl)isoindoline-1,3-dione, **223**, (460 mg, 1.2 mmol) in methanol (15 mL) at room temperature. Then the reaction stirred under reflux for 2 hours. The completed reaction mixture was cooled to room temperature and the white precipitate filtered off. The residue was washed with cold methanol, the filtrate was concentrated and purified on an ISCO chromatograph (0-10% MeOH/ dichloromethane + 1% NH_4OH) to give product as a colorless oil. (223 mg, 74%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.36 (m, 4H), 7.26 (m, 6H), 2.80 (d, 2H, $J = 4$), 2.71 (t, 4H, $J = 8$), 1.75 (m, 4H), 1.55 (m, 1H), 1.30 (brs, 2H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 142.7, 128.43, 128.41, 125.8, 45.0, 40.2, 33.4, 33.19

Dibenzyl (5-oxo-5-((2-phenethyl-4-phenylbutyl)amino)pentane-1,3-diyl)(S)-dicarbamate (225).

(S)-3,5-Bis(((benzyloxy)carbonyl)amino)pentanoic acid, **59**, (115 mg, 0.29 mmol) was dissolved in DMF (5 mL), EDC (110 mg, 0.58 mmol) and HOBT (78 mg, .58 mmol) were added. The reaction was stirred at room temperature for 5 minutes. Then 2-phenethyl-4-phenylbutan-1-amine, **224**, (84 mg, 0.33 mmol) was added followed by 2,6-lutidine (0.11 mL, 0.99 mmol). The reaction was stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate and washed with water, saturated sodium bicarbonate, 5% HCl, water and brine. The combined organic layers were dried over sodium sulfate, concentrated and purified on an ISCO chromatograph (0-60% ethyl acetate/hexane) to give product as a white solid. (106 mg, 57%). MP 169-170 °C; ^{1}H NMR

(400 MHz) (CDCl$_3$) δ 7.28 (m, 20H), 6.32 (brs, 1H), 5.99 (brs, 1H), 5.69 (brs, 1H), 5.08 (m, 4H), 3.98 (m, 1H), 3.41 (m, 1H), 3.29 (m, 2H), 2.99 (m, 1H), 2.57 (m, 5H), 2.29 (m, 1H), 1.66 (m, 7H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 171.1, 156.6, 142.2, 136.6, 136.5, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 125.9, 66.6, 66.5, 46.0, 42.1, 40.0, 37.5, 37.2, 34.6, 33.6, 33.5, 32.9, 32.8

(S)-3,5-Diamino-N-(2-phenethyl-4-phenylbutyl)pentanamide (226).

Dibenzyl (5-oxo-5-((2-phenethyl-4-phenylbutyl)amino)pentane-1,3-diyl)(S)-dicarbamate, **225**, (90 mg, 0.14 mmol) was dissolved in ethanol (10 mL) and 20% Pd(OH)$_2$/C (40 mg) was added. The reaction mixture was purged and stirred under hydrogen atmosphere for overnight. Then the catalyst was filtered off and washed with 20% methanol/dichloromethane. The solvent was removed under reduced pressure and the residue dried under vacuum to obtain the product as a colorless oil (50.2 mg, 97%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.14 (m, 11H), 3.22 (m, 3H), 2.85 (m, 5H), 2.56 (m, 5H), 2.18 (m, 2H), 1.57 (m, 7H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 171.7, 171.6, 142.4, 142.3, 128.4, 128.3, 125.84, 125.8, 67.2, 53.1, 47.8, 45.3, 43.2, 43.0, 41.9, 41.6, 37.2, 37.1, 33.9, 33.8, 33.7, 33.0, 32.9, 32.6, 22.8; HRMS (ESI) Calculated for C$_{23}$H$_{34}$N$_3$O (M+H)$^+$ 368.2696, found 368.2697

Benzyl di-*tert*-butyl pentane-1,2,5-triyl(S)-tricarbamate (227).

Benzyl *tert*-butyl (5-aminopentane-1,4-diyl)(S)-dicarbamate, **78**, (332 mg, 0.95 mmol) was dissolved in dichloromethane (5 mL) and triethylamine (0.20 mL, 1.42 mmol) was added. Then Boc$_2$O was added and the reaction was stirred at room temperature overnight. The reaction mixture was then washed with water and the organic layer separated. The organic layer was dried with sodium sulfate, concentrated and purified on an ISCO chromatograph (0-50% EtOAc/hexane) to give product as a yellow colored solid (252 mg, 56%); MP 75-77 °C; ^{1}H NMR (400 MHz) (CDCl$_3$) δ 7.33 (m, 5H), 5.13 (brs, 1H), 5.09 (s, 2H), 4.98 (s, 1H), 4.83 (brs, 1H), 3.61 (s, 1H), 3.18 (m, 4H), 1.52 (m, 4H), 1.38 (s, 18H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 156.5, 156.1, 136.6, 128.4, 128.1, 128.0, 79.3, 66.5, 51.1, 44.4, 40.7, 29.9, 28.3, 26.2

Di-*tert*-butyl (5-aminopentane-1,2-diyl)(S)-dicarbamate (228)

Benzyl di-*tert*-butyl pentane-1,2,5-triyl(S)-tricarbamate, **227**, (200 mg, 0.44 mmol) was dissolved in ethanol (10 mL) and 20% Pd(OH)$_2$/C (40 mg) and stirred under hydrogen atmosphere overnight. Then the catalyst was filtered off and filtrate concentrated to give product as an oil (140 mg, 100%); ^{1}H NMR (400 MHz) (CDCl$_3$) δ 5.22 (brs, 1H), 5.13 (brs, 1H), 4.53 (m, 2H), 3.54 (m, 1H), 3.10 (m, 2H), 2.76 (m, 2H), 1.54 (m, 2H), 1.36 (s, 18H); ^{13}C NMR (100 MHz) (CDCl$_3$) δ 156.7, 156.4, 128.4, 127. 9, 79.22, 51.1, 44.5, 40.8, 29.9, 28.3, 27.3

Di-*tert*-butyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,2-diyl)(S)-dicarbamate (229)

2-Phenethyl-4-phenylbutanoic acid, **84**, was dissolved in DMF (5 mL) and EDC (185 mg, 0.96 mmol) and HOBT (185 mg, 0.96 mmol) were added. The reaction was stirred at room temperature for 5 minutes. Then di-*tert*-butyl (5-aminopentane-1,2-diyl)(S)-dicarbamate, **228**, (140 mg, 0.44 mmol) was added followed by 2,6-lutidine (0.15 mL, 1.32 mmol). The reaction was stirred at room temperature overnight under nitrogen. The reaction mixture was then diluted with ethyl acetate, and washed with water, 1 M HCl, saturated NaHCO$_3$, water and brine. The organic layer was dried over sodium sulfate and filtrate was concentrated and purified on an ISCO chromatograph (0-10% methanol/dichloromethane) to obtain a solid. (85 mg, 34%); MP 135-137 °C ; ^{1}H NMR (CDCl$_3$) (400 MHz) δ 7.23 (m, 10H), 5.96 (brs, 1H), 4.97 (brs, 2H), 3.66 (m, 1H), 3.38 (m, 1H), 3.24 (m, 3H), 2.59 (m, 4H), 2.04 (m, 3H), 1.78 (m, 2H), 1.69 (m, 4H), 1.45 (s, 9H), 1.44 (s, 9H); ^{13}C NMR (CDCl$_3$) (100 MHz) δ 175.2, 156.7, 156.3, 141.7, 128.4, 128.3, 125.9, 79.4, 51.2, 46.5, 44.4, 39.0, 34.3, 33.6, 30.4, 28.3, 25.9

(S)-N-(4,5-Diaminopentyl)-2-phenethyl-4-phenylbutanamide (230)

Di-*tert*-butyl (5-(2-phenethyl-4-phenylbutanamido)pentane-1,2-diyl)(S)-dicarbamate, **229**, (80 mg, 0.14 mmol) was dissolved in dichloromethane (1 mL) and trifluoroacetic acid (0.5 mL) and the reaction mixture was stirred at 0 °C for 2 hours. Then the solvents were removed under reduced pressure and dried under vacuum to obtained product as a colorless oil. (80 mg, 100%); ^{1}H NMR (400 MHz) (MeOD) δ 7.10 (m, 10H), 3.52 (m, 1H), 3.18 (m,

4H), 2.47 (m, 4H), 2.20 (m, 1H), 1.83 (m, 2H), 1.69 (m, 6H); ^{13}C NMR (100 MHz) (MeOD) δ 178.7, 143.1, 129.4, 129.3, 126.9, 50.5, 47.6, 42.2, 39.6, 35.8, 34.8, 29.2, 26.2

MIC-Based Assay for Potentiation of the Antibacterial Activity of the Efflux Pump Substrate Antibiotic CK-1-12

In the initial screens, as the concentration of the EPI needed for the assay was unknown, we used a 2-fold range of concentrations of the EPI (from 400 µg/mL to 0.8 µg/mL). The 2-fold concentration of EPI were added in a 96-well plate to W4573 bacteria in media containing 16 µg/mL CK-1-12, and bacterial growth inhibition was monitored at 24 hours. Concurrently we monitored for bacterial growth inhibition by the EPI itself against W4573 in the absence of CK-1-12 to ensure that any growth inhibition observed is because of pump inhibition leading to of CK-1-12 activation, and not due to intrinsic toxicity of the EPI against the wildtype bacteria.

MIC-Based Assay for Potentiation of the Antibacterial Activity of the Efflux Pump Substrate Antibiotic Clarithromycin

An MIC-based assay was used to evaluate the potential of the test compounds as EPIs in *E. coli* strains ATCC 25922 and W4573. In these assays, test compounds were assessed for their ability to reduce the MIC of the macrolide antibiotic clarithromycin, a known substrate of the AcrAB-TolC efflux pump in *E. coli* (5). Log-phase bacteria were added to

96-well microtiter plates (at 5 x 10^5 colony forming units per mL) containing two-fold serial dilutions of clarithromycin in cation-adjusted Mueller-Hinton (CAMH) broth (Becton, Dickinson and Co., Franklin Lakes, NJ) either in the absence or presence of each test compound at a concentration of 12.5 µg/mL. Each serial dilution of clarithromycin was present in duplicate. The final volume in each well was 0.1 mL, and the microtiter plates were incubated aerobically for 24 hours at 37 °C. Bacterial growth was then monitored by measuring the optical density (OD) at 600 nm using a VersaMax® plate reader (Molecular Devices, Inc., Sunnyvale, CA), with the MIC being defined as the lowest compound concentration at which bacterial growth was ≥90% inhibited compared to antibiotic- and compound-free control. For comparative purposes, the MIC of clarithromycin was also assessed in an *E. coli* strain (N43) in which the function of the AcrAB-TolC efflux pump has been genetically compromised through mutation of the *acrA* gene.

Fluorescence-based cellular assay for inhibition of pump-mediated efflux

The potential of the test compounds to serve as EPIs in *E. coli* was also evaluated using a fluorescence-based cellular assay that measures the pump-mediated efflux of the fluorescent dye Hoechst 33342 (6). In this assay, *E. coli* ATCC 25922 bacterial cells were harvested from overnight cultures by centrifugation, and the cell pellets were washed with phosphate-buffered saline containing 1 mM $MgCl_2$ (PBSM). After washing the cells, the cell pellets were resuspended in PBSM to achieve a final OD at 600 nm of 0.8. The ATP

required for efflux pump function was then depleted by addition of carbonyl cyanide 3-chlorophenylhydrazone (CCCP) to a final concentration of 100 µM, and incubating the cells for 20 minutes at 37 °C. The CCCP was removed from the cells by washing the cells twice with equivalent volumes of fresh PBSM. The final cell pellet was resuspended in PBSM to achieve an OD at 600 nm of 0.3, and 200 µL aliquots of the bacterial suspension were added to wells of a black, flat-bottom 96-well plate containing test compounds at a concentration of 12.5 µg/mL or an equivalent volume of the vehicle (DMSO) alone. PAβN was included as a positive EPI control at concentrations ranging from 12.5 to 200 µg/mL. A plate vortexer was used to mix the bacterial cells with the test compounds, and the plates were pre-incubated at 37°C for 5 minutes. After the pre-incubation, Hoechst 33342 was added to each well at a final concentration of 10 µM, and the cells were incubated at 37 °C for 0.5 hours to allow for intracellular accumulation of the Hoechst dye. Efflux pump activity was then initiated by reenergizing the bacterial cells with the addition of glucose to a final concentration of 10 mM. Hoechst 33342 efflux was monitored by using a SpectraMax® 2 fluorescent plate reader (Molecular Devices, Inc., Sunnyvale, CA) to measure the fluorescence of each well at 37°C once per minute for 40 minutes. The excitation and emission wavelengths were set at 355 and 460 nm, respectively.

APPENDIX

Intrinsic MICs of the compounds vs. *E. coli* ATCC 25922

Compound	MIC (µg/ml)
26	>100
30	>100
36	>100
38	>100
40	>100
43	>100
46	>100
47	100
48	>100
49	>100
PAβN	>200

Intrinsic MICs of the compounds vs. *E. coli* ATCC 25922 – Table 2

Compound	MIC (µg/ml)
74	>200
75	>200
83	50
92	50
103	>200
105	>200
106	200
107	100
124	200
125	100
134	>200
135	>200
136	50
155	100
156	200
163	200
164	>200
PAβN	>200

Intrinsic MICs of the compounds vs. *E. coli* ATCC 25922 – Table 3

Compound	MIC (µg/ml)
179	>200
180	200
181	50
AMINES	
192	25
194	25
204	>100
205	>100
206	>100
215	100
216	50
217	100
PAβN	>200

List of Compounds Evaluated as EPIs and their Corresponding GAB Codes

FINAL COMPOUND NUMBER	CORRESPONDING GAB CODE	RESYNTHESIS PROCEDURE CODE
3	GAB-7-4	--
4	GAB-7-3	--
5	GAB-7-3a	--
7	GAB-7-7	--
8	GAB-7-6	--
9	GAB-7-8	--
13	GAB-7-17	--
14	GAB-7-18	--
15	GAB-7-19	--
16	GAB-7-20	--
26	GAB-7-81	--
30	GAB-7-59	--
36	GAB-7-54	--
38	GAB-7-101	--
40	GAB-7-143	--
43	GAB-7-58	--
46	GAB-7-60	--
47	GAB-7-138	GAB-11-60

48	GAB-7-116	--
49	GAB-7-104	--
55	GAB-11-83	--
56	GAB-11-23	--
67	GAB-10-179	--
68	GAB-11-44	--
74	GAB-9-171	--
75	GAB-9-175	--
83	GAB-9-133	GAB-11-68
92	GAB-9-191	--
101	GAB-10-135	--
103	GAB-10-150	--
105	GAB-10-25	--
106	GAB-10-117	--
107	GAB-10-24	--
124	GAB-10-31	--
125	GAB-9-188	--
134	GAB-10-62	GAB-11-129
135	GAB-10-84	GAB-11-134
136	GAB-10-65	GAB-11-114
155	GAB-10-99	GAB-11-122
156	GAB-10-68	--
163	GAB-10-98	--

164	GAB-10-88	--
170	GAB-10-97	--
171	GAB-10-105	--
173	GAB-10-116	--
178	GAB-10-128	--
179	GAB-9-181	--
180	GAB-10-37	--
181	GAB-10-44	--
192	GAB-7-173	GAB-11-52
193	GAB-11-86	--
194	GAB-9-85	GAB-11-58
197	GAB-11-25	--
198	GAB-11-32	--
204	GAB-9-92	GAB-11-100
205	GAB-9-94	--
206	GAB-9-108	--
215	GAB-11-76	--
216	GAB-11-105	--
217	GAB-11-78	--

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