Design and Synthesis of Short Amphiphilic Cationic Peptidomimetics Based on Biphenyl Backbone as Antibacterial Agents

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ABSTRACT

Antimicrobial peptides (AMPs) and their synthetic mimics have received recent interest as new alternatives to traditional antibiotics in attempts to overcome the rise of antibiotic resistance in many microbes. AMPs are part of the natural defenses of most living organisms and also they have a unique mechanism of action against bacteria. Herein, a new series of short amphiphilic cationic peptidomimetics were synthesized by incorporating the 3'-amino-[1,1'-biphenyl]-3-carboxylic acid backbone to mimic the essential properties of natural AMPs. By altering hydrophobicity and charge, we identified the most potent analogue **25g** that was active against both Gram-positive *Staphylococcus aureus* (MIC = 15.6 µM) and Gram-negative *Escherichia coli* (MIC = 7.8 µM) bacteria. Cytoplasmic permeability assay results revealed that **25g** acts primarily by depolarization of lipids in cytoplasmic membranes. The active compounds were also investigated for their cytotoxicity to human cells, lysis of lipid bilayers using tethered bilayer lipid membranes (tBLMs) and their activity against pre-established biofilms of *S. aureus* and *E. coli*.

Keywords: antimicrobial peptide, peptidomimetics, membrane disruption, antibiofilm activity.

1. Introduction

Antibiotic resistance in bacteria is a major concern facing global public health. Multidrug resistant strains of Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE), and vancomycin-resistant *Enterococci faecalis* (VRE), and Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, have emerged as major causes of hospital and community-acquired infections [1]. Particularly, Gram-positive bacteria such as MRSA account for a high percentage of hospital-acquired infections [1, 2]. Due to the increasing resistance of bacterial strains against conventional antibiotics, efforts have been made to investigate naturally-occurring antimicrobial peptides (AMPs) and their derivatives as alternative antimicrobial agents [3-5]. AMPs such as PMX30063 and LTX109 are currently in clinical trials [6-8]. Although some promising AMPs are in the pipeline, there is still an urgent need for the development of new antibiotic scaffolds.

AMPs are widespread in nature and serve as the first-line of defense against microbial attack in insects, plants, amphibians, and mammals [9-12]. Unlike conventional antibiotics, AMPs act via non-receptor interactions, which make it difficult for bacteria to develop resistance to AMPs. Most cationic antimicrobial peptides possess a rigid secondary structure and adopt an amphipathic conformation such that their positively-charged face interacts electrostatically with the negatively-charged membrane surface, while their hydrophobic face inserts into the lipophilic interior of the membrane [13, 14]. The broad-spectrum antimicrobial peptide pexiganan, which acts via disruption of bacterial cell membranes, has reached phase III clinical trials [15]. Only a few naturally-occurring AMPs have been used clinically, including polymyxin B and colistin (polymyxin E), due to their *in vivo* toxicity, susceptibility to proteolytic degradation, poor activity in the presence of salts and cytotoxicity to the host cells [16]. Produced through solid-phase synthesis, AMPs also have high manufacturing costs [17].

The drawbacks of conventional AMPs have stimulated the development of peptidomimetics [18], which are synthetic nonpeptidic molecules designed to mimic the properties of peptides. The various kinds of peptidomimetics include α-peptides [19], β-peptides [20], peptoids [21-23], β-turn mimetics [24], cationic β^{3R3}-peptides [25] and lipopeptides [26]. In particular, a number of structurally simple, cationic peptidomimetics possessing natural or unnatural amino acids and with amphipathic character have been investigated as antibacterial agents [27, 28]. Svendsen and co-workers have synthesized a range of peptides of variable length utilizing arginine and tryptophan aminoacids, and showed good minimal inhibitory concentration (MIC) of 2.5 µg mL^{-1} against *Staphylococcus aureus* and 5 µg mL^{-1} against *Escherichia coli* [29]. An ultra-short pyrazole-based peptidomimetics showed a MIC of 4 µg mL^{-1} against MRSA and was four times more potent than melittin [30]. Pyne and co-workers have developed *C2*-symmetric binaphthyl-containing peptidomimetics that showed excellent antimicrobial activity against both Gram-positive and Gram-negative bacterial pathogens [31]. Haldar and co-workers designed aryl-alkyl-lysine-based peptide mimics that mimicked the membrane-active properties of natural AMPs [32]. Their group also investigated cationic small molecules with spatial control of hydrophobicity to minimize toxicity against human erythrocytes while still maintaining antibacterial activity [33].

Our research group has recently synthesized short glyoxamide-based peptidomimetics via the ring-opening reaction of *N*-naphthoylisatins with amines and amino acids [34, 35]. As part of an ongoing program to develop short antimicrobial peptidomimetics, we were interested in utilizing the biphenyl backbone due to its frequent presence in medicinal chemistry [36, 37]. In an analysis of scaffolds of pharmacologically active molecules, biphenyl was found to be present in 2.1% of reference drug molecules [38, 39]. Furthermore, the importance of the biphenyl unit is shown by their presence in several natural products [38-

40], such as the antibacterial compounds MC21-A (**1**) and MC21-B (**2**) isolated from marine bacterium *Pseudoalteromonas phenolica* [41, 42]. The biphenyl-containing antibacterial compounds biphenomycin A (**3**) and B (**4**) were isolated from the cultured broth of *Streptomyces griseorubiginosus* 43608 [43-45]. Antibiotics such as arylomycin A2 (**5**), arylomycin B2 (**6**) and vancomycin also contain biphenyl moieties.

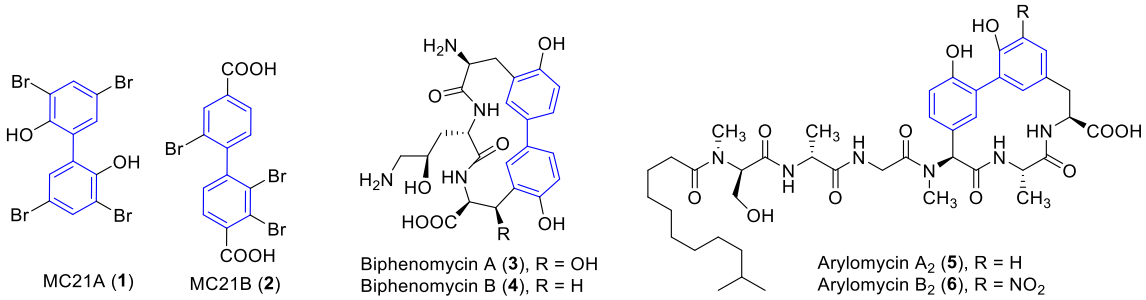


Fig. 1 Biphenyl-containing antibacterial compounds, with the biphenyl motif highlighted in blue.

In this work, we designed a unique scaffold for developing short antimicrobial peptidomimetics by utilizing a 3, 3'-substituted biphenyl unit as the key hydrophobic backbone to mimic the structural and biological properties of many AMPs. The segregation of hydrophobic (R^1) and cationic (R^2) groups via the biphenyl core confers amphipathic character to the entire molecule (**Fig. 2**). Importantly, the modular design of this scaffold allows for ready optimization of the biphenyl-based peptidomimetics simply by varying the nature of the hydrophobic (R^1) and cationic (R^2) groups.

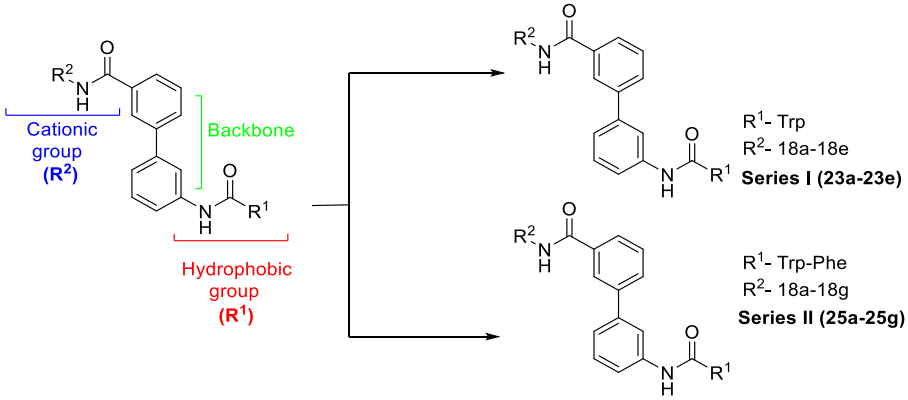


Fig. 2 General structure of biphenyl based peptidomimetics

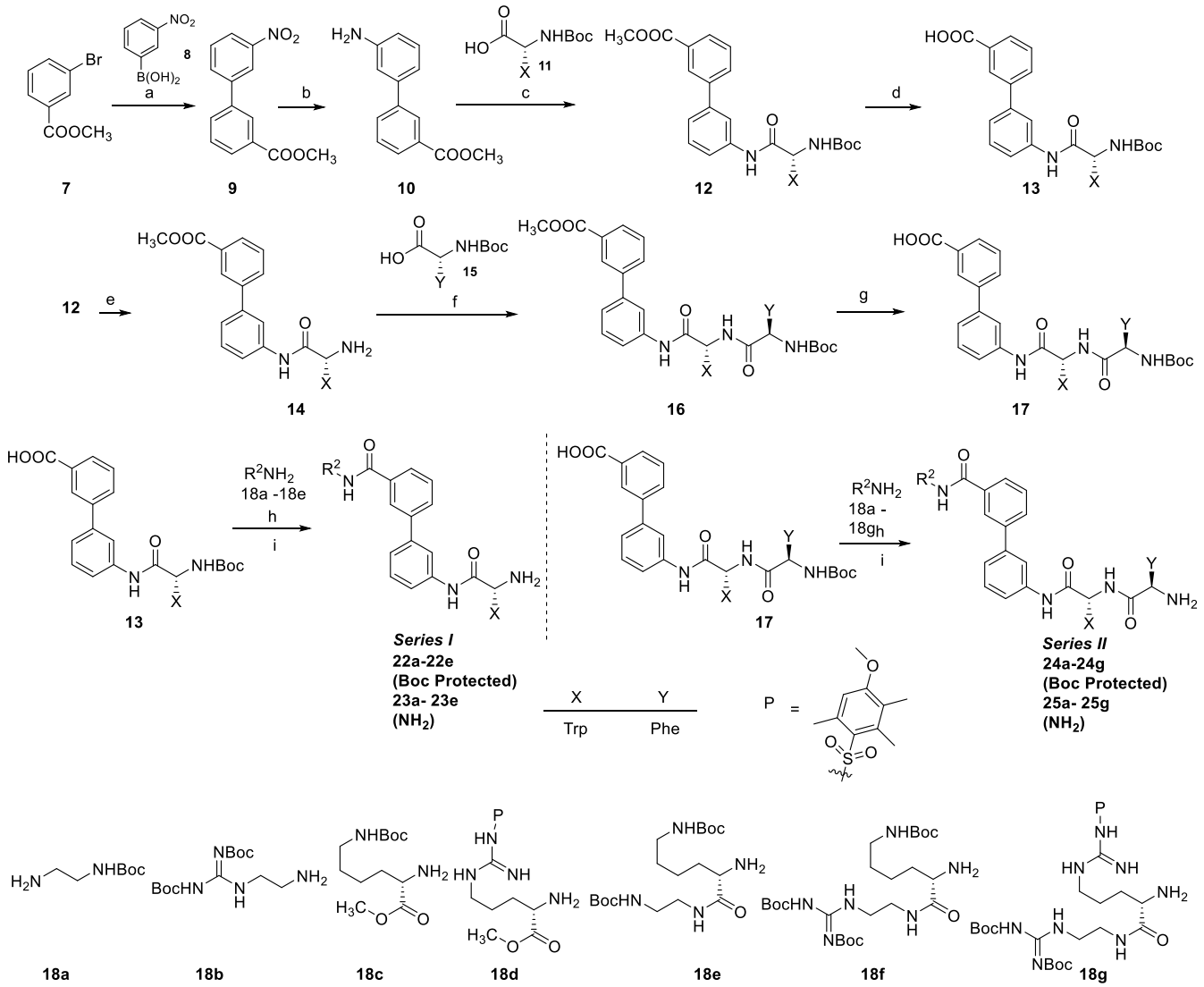
The biphenyl-based peptidomimetics synthesized in this work were classified into two series based on the length of the hydrophobic group (R^1) attached to the molecule. Series I **(23a-23e)** incorporated a single tryptophan (Trp) group at the 3-position of biphenyl, whereas series II **(25a-25g)** incorporated a tryptophan-phenylalanine (Trp-Phe) dipeptide at the same position. The selection of Trp was based on its ability to interact with the interfacial region of the bacterial membranes, thereby anchoring the biphenyl derivatives to lipid bilayers [46]. The cationic groups (R^2) were incorporated at the 3'-position of biphenyl, and they included 1,2-diaminoethane, 1-(2-aminoethyl)guanidine, and amino acids such as arginine and lysine. This demonstrated the importance of short non-natural amine and guanidine groups over cationic amino acids. Arginine and 1-(2-aminoethyl)guanidine were chosen because the guanidine group exhibits a stronger electrostatic interaction and more extensive hydrogen bonding with the negatively-charged phospholipids of the bacterial cell membrane [47].

The synthesized compounds were evaluated for biological activity against Gram-positive and Gram-negative bacterial strains. In addition, cytotoxicity was also investigated for selected potent antimicrobial compounds. To study how the active compounds interacted with lipid membranes, tethered bilayer lipid membranes (tBLMs) in association with electrical impedance spectroscopy was employed. In addition, the ability of the compounds to alter cytoplasmic permeability was assessed by using the membrane potential-sensitive cyanine dye diSC3-5. Finally, the ability of the compounds to inhibit the biofilm formation of *S. aureus* and *E. coli* was also evaluated.

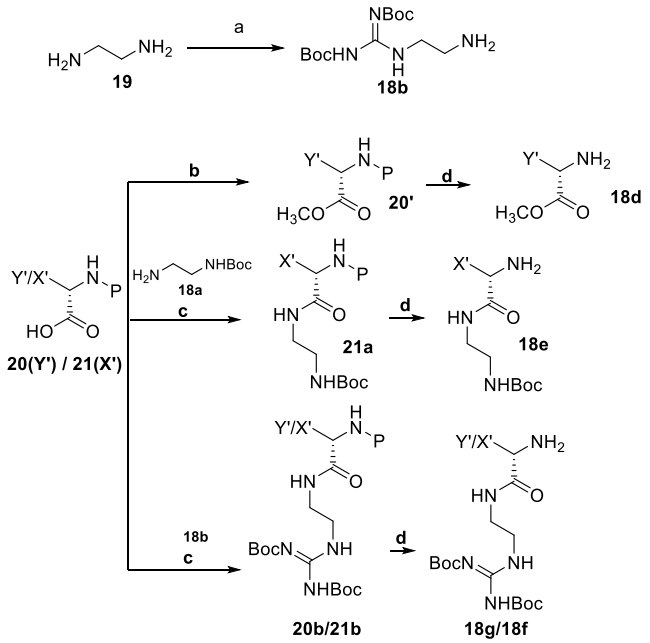
2. Results and discussion

2.1 Design and synthesis of biphenyl derived peptidomimetics

The acid **17** was synthesized by deprotecting the Boc group of **12** with TFA, followed by amide formation with **15** and subsequent ester hydrolysis using aqueous sodium hydroxide. On coupling **17** with different amines (**18a-18g**) respective amides **24a-24g** were yielded and the deprotection of **24a-24g** gave the series II compounds (**25a-25g**). The synthesis of amines **18b**, and **18d-18g** were shown in scheme2. The guanylation reaction using *N*,*N*′-Di-Boc-*1H*-pyrazole-1-carboxamidine provides the corresponding amine **18b**. The amino ester **18d** was prepared from NH_2-Arg(Mtr)-OH using thionyl chloride and methanol. The acids **21(X'), 21(X'), 20(Y')** were used to synthesize the amides **21a**, **20b**, **21b** using PyBOP/CH_2Cl_2 coupling agent, and subsequent Fmoc cleavage using piperidine/DMF to afford **18e-18g**.



Scheme 1. Synthesis of compounds 13 and 17. Reagents and conditions: a) 8 (1.1 equiv), $Pd(PPh_3)_4$ (0.03 equiv), aq. 2M Na_2CO_3 (3.0 equiv), 1,4-dioxane, reflux, overnight, 65%; b) 10% Pd/C, H_2 balloon, THF, rt, overnight, 90%; c) EDCI (1.2 equiv), HOBt (1.0 equiv), DIEA (2.5 equiv), DMF, rt, 8 h, 63%; d) aq. 1N NaOH(2.0 equiv), THF, MeOH, rt, overnight, 90% e) TFA, CH_2Cl_2, RT, 4h, 89%; f) EDCI (1.2 equiv), HOBt (1.0 equiv), DIEA (2.5 equiv), DMF, rt, 8h, 54%; g) aq. 1N NaOH (2.0 equiv), THF, MeOH, rt, overnight, 89%; h) EDCI (1.2 equiv), HOBt (1.0 equiv), DIEA (2.5 equiv), DMF, rt, 8 h-overnight, 52-84%; i) TFA, CH_2Cl_2, RT, 4 h-overnight, 49-89%.

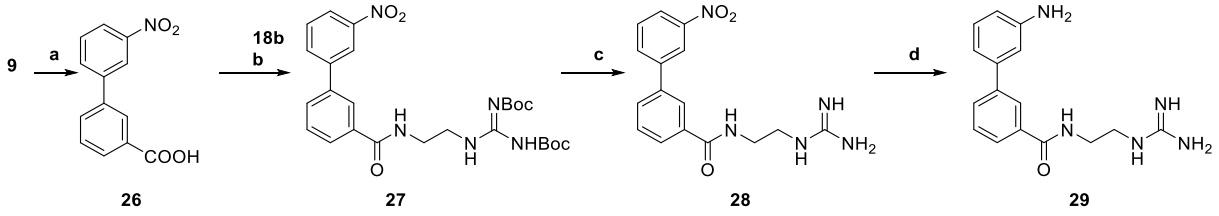


	X'	Y'	P	% Yield
20'		Arg	Fmoc	90
21a	Lys		Fmoc	54
20b		Arg	Fmoc	34
21b	Lys		Fmoc	41

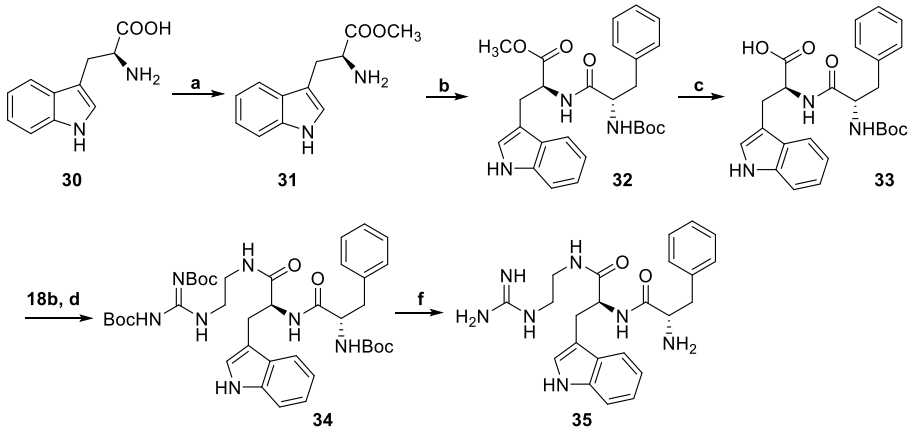
	X'	Y'	% Yield
18d		Arg	85
18e	Lys		89
18g		Arg	78
18f	Lys		79

Scheme 2. Synthesis of **18b**, **18d-18g**. Reagents and conditions: a) *N,N'*-Di-Boc-*1H*-pyrazole-1-carboxamidine (0.1 equiv), THF, rt, 30 min, crude; b) $SOCl_2$ (1.5 equiv), MeOH, rt, overnight; c) PyBOP (1.1 equiv), HOBt (1.0 equiv), DIEA (3.0 equiv), CH_2Cl_2, rt, overnight; d) Piperidine (2.0 equiv), DMF, RT, overnight.

The biphenyl derivative **29** attached with guanidine without any hydrophobic group was synthesized as shown in scheme3. The biphenyl ester **9** was hydrolyzed to acid **26** and coupling with **18b** to afford the amide **27** and, subsequent deprotection with TFA gave **29**. Finally, **35** without the biphenyl backbone but intact with hydrophobic and cationic group was synthesized from L-Tryptophan **30**. The acid **33** was obtained by the sequential formation of dipeptide **32** from **30,** followed by ester hydrolysis. The amide **34** was obtained on coupling **33** with **18b,** and it was deprotected with TFA to yield **35** (Scheme 4).



Scheme 3. Synthesis of compound **29**. Reagents and conditions: a) aq. 1N NaOH (2.0 equiv), THF, MeOH, rt, overnight, 90%; b) EDCI (1.2 equiv), HOBt (1.0 equiv), DIEA (2.5 equiv), DMF, rt, overnight, 80%; c) TFA, CH_2Cl_2, rt, 4h-overnight, 90%; d) *N,N′*-Di-Boc-*1H*-pyrazole-1-carboxamidine (1.0 equiv), Et_3N (2.0 equiv), THF, RT, 6 h, 85% e) 10% Pd/C, H_2 balloon, rt, 5 h, 40%.



Scheme 4. Synthesis of compound 35. Reagents and conditions: a) $SOCl_2$ (1.5 equiv), MeOH, RT, overnight, 90%; b) EDCI (1.2 equiv), HOBt (1.0 equiv), DIEA (2.5 equiv), DMF, rt, overnight, 65%; c) 1N $NaOH_{(aq)}$ (2.0 equiv), THF, MeOH, rt, overnight, 91%; d) EDCI (1.2 equiv), HOBt (1.0 equiv), DIEA (2.5 equiv), DMF, rt, overnight, 45%; e) TFA, CH_2Cl_2, RT, overnight, 47%.

2.2 Antimicrobial activity study

The antimicrobial activities of the newly synthesized peptidomimetics were assessed against the Gram-positive bacterium *Staphylococcus aureus* [SA38], and two Gram-negative bacteria, *Pseudomonas aeruginosa* [PA01], and *Escherichia coli* [K12] are summarized in table1.

The series I compounds **23a-23e** were tested against Gram-positive bacterium *Staphylococcus aureus* [SA38]. The compound **23b** (MIC = 62.5 µM) in which the biphenyl group segregated with tryptophan hydrophobic group and simple guanidine cationic group showed more activity compared to simple amine cationic group **23a** (MIC = 125 µM). The compound **23b** containing simple guanidium cationic moiety which mimic the ariginine amino acid found in natural AMPs displayed the good activity. The MIC values of **23c** (31.2 µM) and **23d** (62.5 µM) attached with the Lys-ester and Arg-ester cationic groups are compared with **23a** and **23b** by maintaining the total net charge and hydrophobicity of the compounds. We noticed that the amine containing cationic group of lysine methyl ester **23d** showed four-fold increase in antibacterial activity compared to the **23a**. Interestingly, the similar antibacterial activity of **23b** and **23d** revealed that the simple guanidine cationic group is enough to mimic the arginine. The antibacterial activity of compound **23e** (MIC = 31.2 µM), and **23c** remains same even though the net cationic charge is increased. This could be due to the imbalance of the peptide hydrophobicity and charge distribution. Although, the series I compounds **23a-23e** found to be active against *S. aureus*, these compounds did not have significant activity against the two Gram-negative bacteria, *Pseudomonas aeruginosa* [PA01], and *Escherichia coli* [K12]. Based on the results from series I, increase in the net cationic charge did not show any profound antibacterial effect against Gram-positive and Gram-negative bacterial strains.

The series II compounds **25a-25g** were made on hypothesis that the increase in hydrophobicity will enhance the antibacterial activity and to understand the effect of hydrophobicity along with the cationic character against the Gram-negative pathogens. All the compounds **25a-25g** were tested against the Gram-positive bacterium *Staphylococcus aureus* [SA38]. As expected **25a** (MIC = 62.5 µM) and **25b** (MIC = 15.6 µM) containing excess hydrophobic phenylalanine group showed excellent activity compared to less hydrophobic compounds **23a** (MIC = 125 µM) and **23b** (MIC = 62.5 µM). The increase in hydrophobicity of **23c** (31.2 µM) did not improve the MIC of **25c.** Whereas, in compound **25d** (31.2 µM) the activity increased two-fold compared to **23d** (62.5 µM)**.** This may be resulted due to the increase in hydrophobic bulkiness along with the extensive hydrogen bonding of guanidine group. **25e** (MIC = 31.2 µM) retained the same activity compared to **23e** (MIC = 31.2 µM) though the hydrophobicity is increased may be due to the net positive charge equals with the number of hydrophobic group. We hypothesized that if the net positive charge is important in the activity of **25e** and if the amine groups are replaced with guanidine groups, the activity should increase.

25f (31.2 µM) showed same activity after replacing with one guanidine of **25e**. As anticipated the compound **25g** (MIC = 15.6 µM) replaced with the two-guanidine by attaching arginine in place of lysine group in **25f** showed excellent activity compared to **25e** (MIC = 31.2 µM) probably due to the strong hydrogen bonding with the negatively charged phospholipids of bacterial cell membrane.

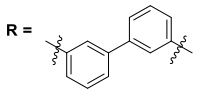
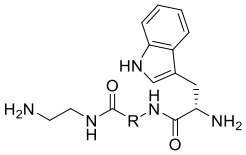
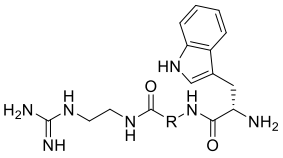
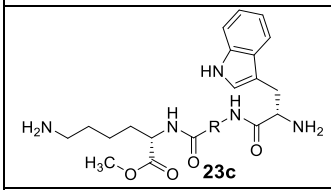
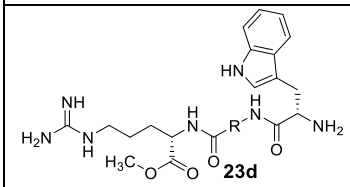
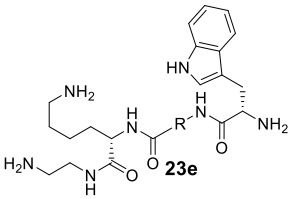
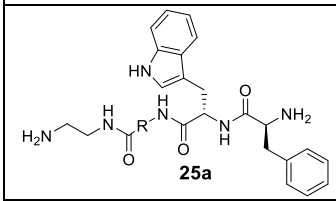
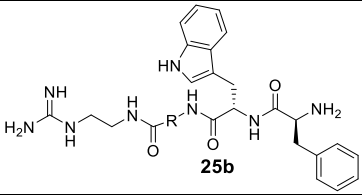
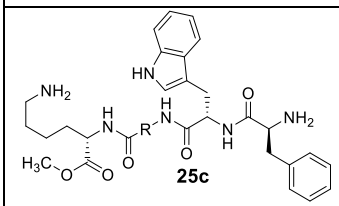
25a-25g were also tested against Gram-negative bacteria *Escherichia coli* [K12]. Compounds **25a**, **25c**, **25d** did not show notable antibacterial activity. The compound **25b** (MIC = 31.2 µM)**,** with the increased hydrophobicity compared to **23e** (MIC = >125 µM) showed dramatically four-fold improvement against *E. coli*. The antibacterial activity of cationic peptides on Gram-negative bacteria is influenced by two steps to overcome the outer-membrane barrier. The high membrane bound concentration of the cationic peptides facilitates the outer-membrane permeabilization and in the final step the inner membrane damage causes the cell lysis [48]. In this step, the hydrophobic interactions become dominant. This clearly suggests the importance of activity of **25b** compared to **23e**. The compounds **25f** (MIC = 15.6 µM), and **25g** (MIC = 7.8 µM) containing guanidine cationic groups displayed good activity against *E. coli* compared to **25c** (MIC = 125 µM) and **25e** (MIC = 31.2 µM) containing the amine cationic groups. These results evidently showed that the electrostatic attraction played the decisive role for the excellent activity against the E. *coli.* The compound **29** (62.5 µM) retains the biphenyl backbone showed the similar activity of **23b** against *S. aureus* and *E. coli* even though the hydrophobic tryptophan group was removed. This could be due to the hydrophobicity of the biphenyl backbone.

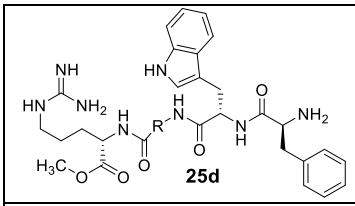
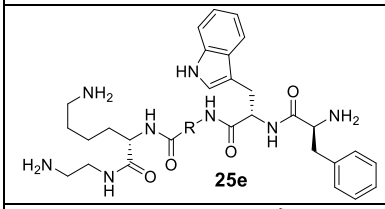
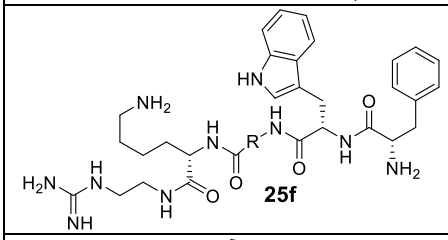
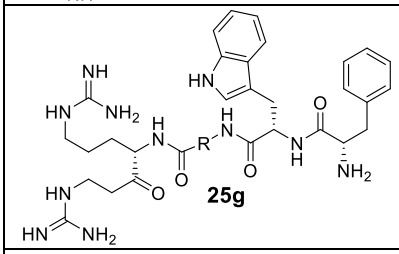
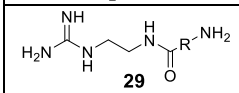
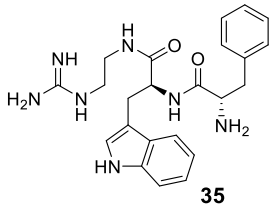
Finally, the MIC results of **35** without the biphenyl backbone (>250 µM) compared with **23b** (62.5 µM) against *Staphylococcus aureus* [SA38]. The antibacterial activity is totally lost after the removal of biphenyl backbone from the active compound **23b**. The MIC results confirms the biphenyl backbone plays a major role in balancing the hydrophobicity and amphipathicity which are considered as the key parameters for the antibacterial activity against Gram-positive and Gram-negative bacterial strains.

Overall, the structure activity relationship (SAR) draw the inference regarding the antibacterial activity of the biphenyl backbone peptidomimetic derivatives against *S. aureus* and *E. coli*. Firstly, the series II compounds with excess hydrophobic group showed good to excellent antimicrobial activity against S. *aureus* and *E. coli*. Secondly, the amphipathic nature played a crucial role in determining the antimicrobial activity. The antibacterial activity of compounds **25c-25f** remain intact against *S. aureus* even after increasing the net positive charge. Among all the synthesized biphenyl derivatives, **25b** and **25g** displayed the most potent antibacterial activity against *S. aureus* (15.6 µM). Finally, **25g** revealed that an increase in cationic charge by utilizing guanidine groups along with increase in hydrophobicity produced excellent activity against Gram-negative *E. coli* (7.6 µM) compared to the corresponding amine compound.

The active peptidomimetic compound **25g** which contains less number of amino-acids is comparable with the MIC of MSI-78. MSI-78 (also known as pexiganan) is a chimera of magainin and melittin peptides [49]. This peptide was chosen as a reference compound as it is currently in Phase-III clinical trials and one of the most well-studied amphipathic antimicrobial peptides. Resistant strains of bacteria cannot be generated against MSI-78 even after repeated exposure to sub-inhibitory concentrations [15], and many studies have shown that the antimicrobial action of the peptide involves disruption of bacterial membranes [50, 51].

Table 1. Antibacterial activities of biphenyl peptidomimetic derivatives.

R = 	MIC (μM)	
	Gram +ve S. aureus	*Gram –ve P. aeruginosa / E. coli*
 **23a**	**125**	**>250 / >125**
 **23b**	**62.5**	**>250 / >125**
 **23c**	**31.2**	**>250 / >125**
 **23d**	**62.5**	**>250 / >125**
 **23e**	**31.2**	**>250 / >125**
 **25a**	**62.5**	**250 / >125**
 **25b**	**15.6**	**250 / 31.2**
 **25c**	**31.2**	**250 / 125**

 25d	**31.2**	**250 / 125**
 25e	**31.2**	**250 / 31.2**
 25f	**31.2**	**NT / 15.6**
 25g	**15.6**	**NT / 7.8**
 29	**62.5**	**>250 / >125**
 35	**>250**	**NT / >125**
MSI-78 [15, 52, 53]	**3.2 – 6.45 (NT)**	***3.2 – 6.45 (NT)**
(NT) – Not tested; MSI-78 [15, 52, 53] – Literature Value **E. coli*		

2.3 Toxicity against human cells

To further evaluate the utility of these biphenyl derivatives as antimicrobial agents, their specificity for bacterial cells over human cells was determined. Therefore, the in vitro toxicity of the most active compounds (**23b, 23d, 25b-25g**) was assessed against MRC-5 normal human lung fibroblasts using the Alamar Blue (Resazurin) assay [54]. A dose-response curve was generated for each compound (shown in supplementary material Fig. S2) at concentrations ranging from 1-350 µM and their IC_{50} values were determined (shown in supplementary material Fig. S3). All of the tested compounds displayed very low toxicity (IC_{50} >300 µM) towards human cells. Although amphipathic antibacterial agents are often cytotoxic, our compounds did not show noticeable cytotoxicity upto the concentration of 350 µM.

2.4 Lipid bilayer membrane interactions

The compounds ability to interact with lipid bilayers was assessed using tethered bilayer lipid membranes (tBLMs) in association with AC electrical impedance spectroscopy.[55, 56]. Compounds which are shown membrane conduction at concentration of 10 µM are only discussed. Membrane conduction responses of a zwitterionic phospholipid membrane to compounds **25b-25e** increased with increasing concentration of compounds (**Fig. 3*A***). Significant conduction changes are evident at concentrations equal to or higher than 10 µM. Interestingly, these responses are more muted in negatively-charged phospholipid membranes which mimic the negatively charged bacterial cell membranes (**Fig. 3*B***).

The changes in membrane conduction are modest when compared to that produced by α-hemolysin or gramicidin-A [56, 57] which produce ion channels in the membranes. This would indicate that it is unlikely that these compounds form membrane

spanning ion channel-like pores. Alternatively, the changes in membrane conduction could be the result of the formation of toroidal pore-like structures in the membrane. Either the compounds form a part of the lipidic toroidal pore themselves, or they independently induce phospholipidic toroidal pore formation by altering the critical packing parameter of the membrane[58].

The membrane capacitance changes increased with compound concentration in both zwitterionic (**Fig. 3*C***) and negatively-charged (**Fig. 3*D***) tBLMs, with the exception of compound **25d** which exhibited a slight decrease in capacitance at high concentrations in negatively-charged membranes. An increase in capacitance is indicative of a thinning of the lipid membrane and/or an increase in membrane dielectric caused by the presence of water molecules. This indicates these compounds may follow mechanism of pore formation like AMPs by membrane thinning effect [59].

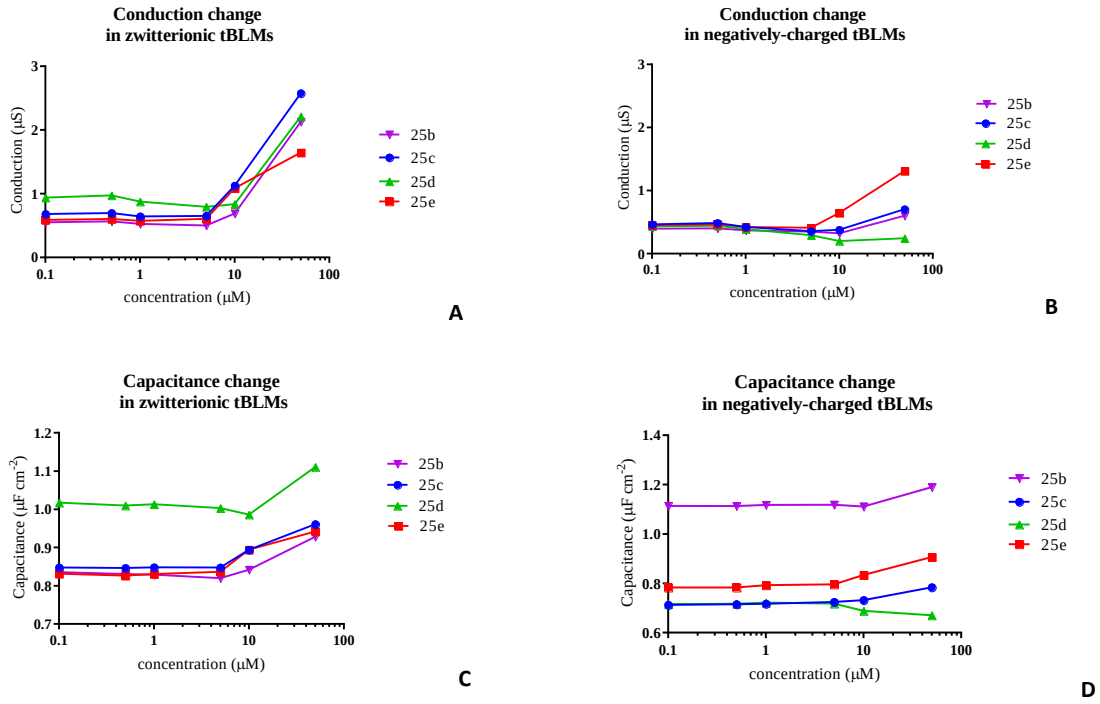


Fig. 3. Change in conduction in (**A**) zwitterionic and (**B**) negatively-charged tBLMs in response to increasing concentrations of compounds **25b-25e.**. Change in membrane capacitance in (**C**) zwitterionic and (**D**) negatively-charged tBLMs in response to increasing concentrations of compounds **25b-25e**.

Notably, the change in membrane conduction at 10 µM was correlated with antimicrobial activity, with compounds having lower MIC values (compounds **25c-25e**) showing greater positive changes in membrane conduction in zwitterionic lipids than compounds with higher MIC values **25b** (**Fig. 4*A***). In negatively-charged lipids, however, the results were mixed and there was no clear relationship between MIC and conductance change (**Fig. 4*B***). This would suggest that the effectiveness of these compounds is not so much related to their overall net cationic charge, rather their ability to insert into the bilayer proper. To test the hypothesis that the tryptophan moiety plays a significant role in membrane disruption, the change of conductance induced by compound **29**, which lacks any Trp residue, was tested. As expected, compound **29** exhibited no change in membrane conduction in either zwitterionic or negatively-charged lipids, demonstrating the importance of the Trp residue.

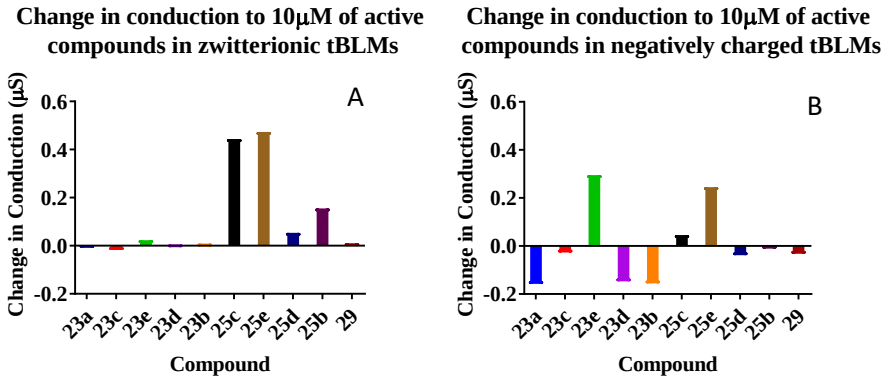


Fig. 4. The change in tBLM conduction of the various antimicrobial compounds at 10 µM in (**A**) zwitterionic and (**B**) negatively-charged membranes.

2.5 Cytoplasmic membrane depolarization

We evaluated the disruption effect of the biphenyl derivatives on the bacterial cytoplasmic membrane using the membrane potential-sensitive dye diSC3-5 (3,3'-dipropylthiadicarbocyanine iodide). The distribution of diSC3-5 between the cell membrane and periphery medium is dependent on the cytoplasmic membrane potential gradient. The dye readily partitions into the bacterial cell membrane and aggregates within the membrane, causing self-quenching. If the antimicrobial compounds perturb the cell membrane, it can lead to the loss of the membrane potential gradient, causing the dye to be released into the medium. As a result,

the fluorescence intensity of the dye increases. As shown in **Fig. 5**, compounds **23b**, **23d**, **25b**, **25e**, **25f**, and **25g** (added at 0.5×, 1×, 2× or 4× MIC) induced disruption of the cytoplasmic membrane of *S. aureus* in a time and concentration-dependent manner. Interestingly, the most active compound **25g** showed an increase in fluorescence intensity even at sub-MIC levels within 3 min. Similarly, increases in fluorescence intensity of **25e** and **25f** also occurred at 1× MIC within 3 minutes.

We also conducted the cytoplasmic permeability assay on Gram-negative bacterium *E. coli* (K12) with the most active compound **25g**. **25g** also perturbs the cell membrane led to similar disruption of diSC3-5 fluorescence intensity (**Fig. 5G**), indicating that the cell membrane of both Gram-positive and Gram-negative bacteria can be disrupted. Taken together, these observations indicate that the biphenyl amphiphilic compounds can readily permeabilize the bacterial membrane, thus resulting in bacterial death.

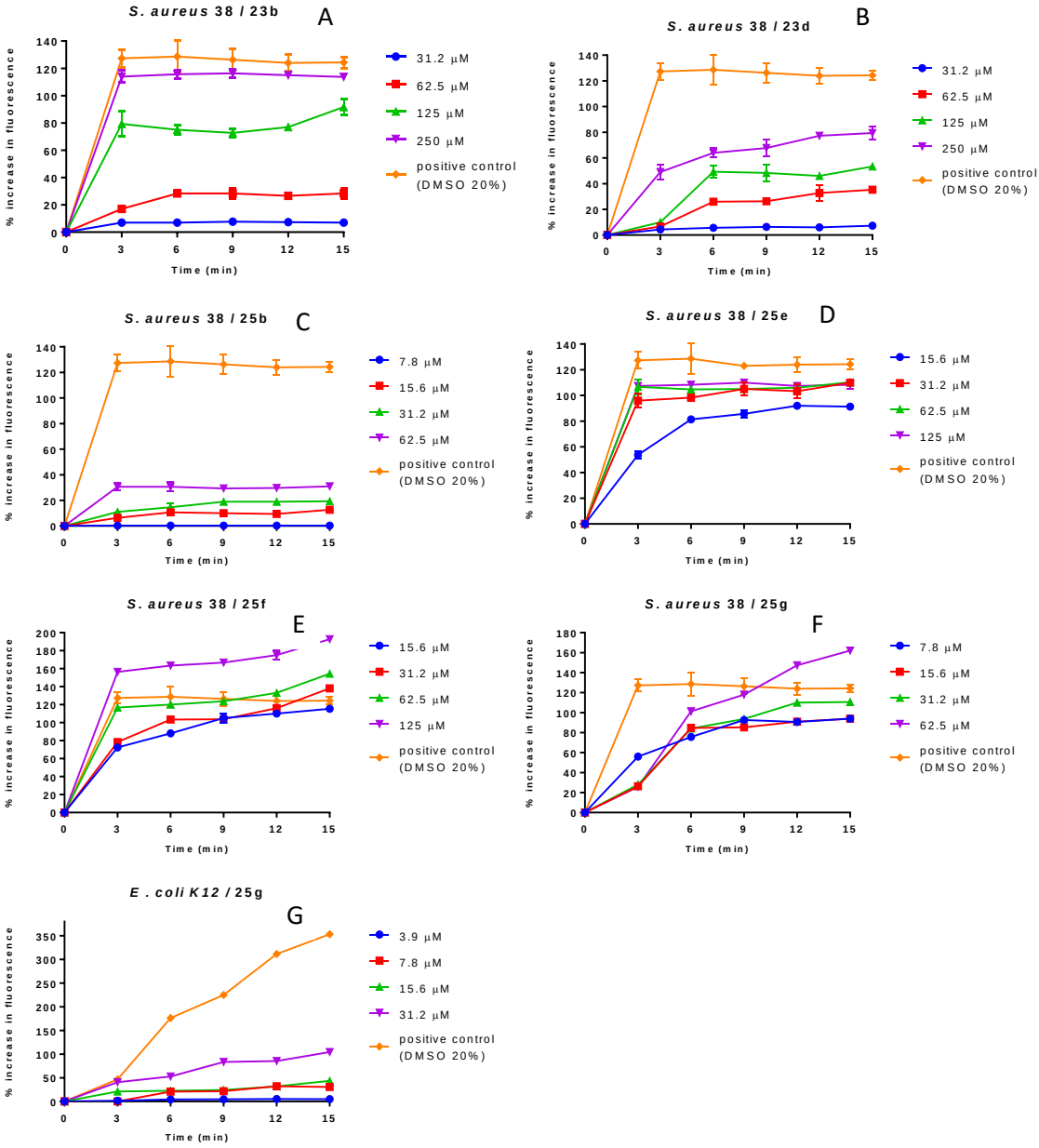


Fig. 5. (**A-F**) *S. aureus* cytoplasmic membrane disruption promoted by **23b, 23d, 25b, 25e, 25f, 25g.** (**G**) *E. coli* cytoplasmic membrane disruption promoted by **25g**. Error bars represent the standard error of triplicates (n = 3).

2.6 Antibiofilm activity

The ability of the amphiphilic biphenyl derivatives to inhibit established *S. aureus* or *E. coli* biofilms was measured at 250 µM (**Fig. 6**). In general, the compounds showed low level of inhibition of biofilm formation. Compound **23b** showed the highest level of disruption against *S. aureus* and *E. coli* of 41% and 39% respectively at 250 µM. Meanwhile, compound **25e** displayed 30% inhibition against *S. aureus* biofilms but only 8% activity against biofilms of *E. coli*. The pre-established biofilms are harder to eradicate and have limited data available on novel AMPs with anti-biofilm properties [60]. Compound **23b** containing less hydrophobic and cationic groups moderately disrupted both the *S. aureus* or *E. coli* biofilms compared to active antibacterial compounds. These results revealed that the increase in cationic charge and hydrophobicity elevates the activity against planktonic cells, but they are unable to disrupt the large aggregates of bacteria surrounded by an extracellular matrix. Previous studies have used a similar method for measuring the antibiofilm properties of cationic peptides, that is allowing the biofilm to form in the presence of the peptide [61]. Cirioni et al.[61] found that at a concentration of half the MIC, MSI-78 (pexiganan) was able to prevent biofilm formation by *P. aeruginosa* by 27.5%. This is higher than the activity of the compounds in the current study

which showed 39% inhibition of biofilm production by another Gram-negative bacterium *E. coli* but at a concentration of 31 times the MIC. In an alternative biofilm study, where biofilms were formed prior to the addition of MSI-78 (pexiganan) was unable to minimize the amount of biofilm produced by with *P. aeruginosa*, *E. coli* or *S. aureus* even at a concentration of 128 times its MIC. However, in a similar preformed biofilm assay, another naturally occurring peptide, LL-37 which is also a membrane disrupting peptide [62], reduced the biofilm mass by approximately 40% at 4 times its MIC [63]. Thus, the current compounds show similar antibiofilm capabilities to cationic antimicrobial peptides, but future studies should examine whether the current compounds are able to disrupt preformed biofilms.

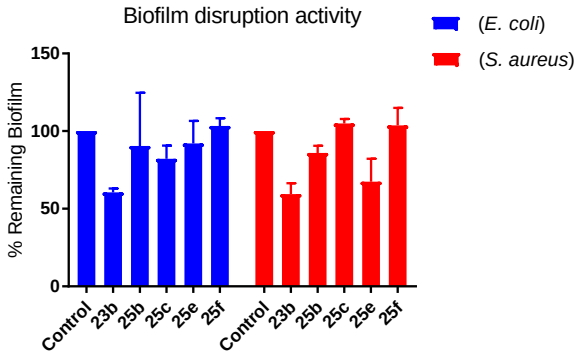


Fig. 6. Percentage of remaining biofilm of *S. aureus* and *E. coli* after 24 h treatment with the synthesized compounds at 250 μM. The control represents the pre-established biofilms without any compounds. Error bars represent the standard error of triplicates (n = 3).

3. Conclusion

In conclusion, we have developed novel peptidomimetics based on the 3,3'-biphenyl structural scaffold. The systematic tuning of hydrophobicity and cationic charge of the peptidomimetics resulted in moderate to excellent antibacterial activities. **25g,** the amphipathic peptidomimetic compound in which the hydrophobic Trp-Phe and cationic Arg-aminoethylguanindine segregated by biphenyl backbone showed excellent antibacterial activity against *S. aureus* (**15.6 μM**) and *E. coli* (**7.8 μM**) without cytotoxicity against mammalian cells. Based on the results of cytoplasmic permeability assay and tBLMs-AC impedance spectroscopy**,** we propose that the biphenyl peptidomimetics exhibit bacterial cell membrane disruption mechanism similar to most AMPs. The importance of tryptophan in mechanism of action was also revealed by compound **29**. In addition, **23b** biphenyl attached with hydrophobic Trp and cationic aminoethyl guanidine could disrupt biofilms of *S. aureus* and *E. coli* at 250 μM. Collectively, our results suggest biphenyl is a versatile core about which hydrophobic and cationic amino acids can be arranged in order to generate short cationic amphipathic peptidomimetics that mimic natural AMPs.

4. Experimental section

4.1 General notes

All chemical reagents were purchased from commercial sources (Combi-Blocks, Chem-Impex and Sigma Aldrich) and used without further purification. Solvents were commercial and used as obtained. Reactions were performed using oven-dried glassware under an atmosphere of nitrogen and in anhydrous conditions (as required). Room temperature refers to the ambient temperature. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) plates pre-coated with Merck silica gel 60 F254. Visualization was accomplished with UV light, a ninhydrin staining solution in n-butanol. Flash chromatography and silica pipette plugs were performed under positive air pressure using Silica Gel 60 of 230–400 mesh (40–63 μm) and also using Grace Davison LC60A 6- μm for reverse phase chromatography. Infrared spectra were recorded using a Cary 630 ATR spectrophotometer. Melting points were obtained using a OptiMelt melting point apparatus and are uncorrected. High-resolution mass spectrometry was performed by the Bioanalytical Mass Spectrometry facility, UNSW. Proton and Carbon NMR spectra were recorded in the solvents specified using a Bruker DPX 300 or a Bruker Avance 400 or 600 MHz spectrometer as designated. Chemical shifts (δ) are quoted in parts per million (ppm), to the nearest 0.01 ppm and internally referenced relative to the solvent nuclei. ^{1}HNMR spectral data are reported as follows [chemical shift in ppm; multiplicity in br, broad; s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; sext, sextet; sept, septet; m, multiplet; or as a combination of these (*e.g*. dd, dt *etc*.)]; coupling constant (*J*) in hertz, integration, proton count and assignment.

4.2 General methods

4.2.1 Procedure 1: peptide formation method a

To a stirred solution of an acid (1 equiv), amine (1.0 equiv), HOBt (1.0 equiv), DIEA (2.5 equiv) in DMF (3 – 5 mL) EDCI (1.2 equiv) was added portion-wise. The reaction was stirred overnight before the solvent was removed under reduced pressure and the resultant residue subjected to flash chromatography (2-5% MeOH/CH_2Cl_2 as the eluent) to afford the desired compound.

4.2.1 Procedure 2: peptide formation method b

To a stirred solution of an acid (1 equiv), amine (1.0 equiv), HOBt (1.0 equiv), PyBOP (1.1 equiv) in CH_2Cl_2 (3 – 5 mL) DIEA (2.0 equiv) was added dropwise. The reaction was stirred overnight before the solvent was removed under reduced pressure and the resultant residue subjected to flash chromatography (2-5% MeOH/CH_2Cl_2 as the eluent) to afford the desired compound.

4.2.2 Procedure 3: N-fmoc deprotection

To a stirred solution of the Fmoc-protected peptide in DMF (3.0 – 5.0 mL)was added piperidine (2.0 equiv). The resultant solution was then stirred at rt for overnight. The solvent was then removed under reduced pressure and purified using flash chromatography (5% of 100:10:1; $CHCl_3$:MeOH: aqueous NH_3 and 95% of CH_2Cl_2) as eluent The resultant compounds isolated as TFA salt.

4.2.3 Procedure 4: N-boc and Pbf deprotection

To a stirred solution of the boc/Pbf protected peptide in CH_2Cl_2 (3.0 mL) was added TFA (3.0 mL). The reaction mixture was stirred at room temperature overnight before the solvent was removed under reduced pressure. After triturating with diethyl ether, the residue was concentrated to dryness. For Pbf and deboc of guanidine the residue was dissolved in CH_2Cl_2 and CH_3CN and precipitated by addition of diethyl ether and filtered and dried. Some of the compounds were purified using reverse phase chromatography with 40% CH_3CN/ H_2O as eluent utilizing GRACE instrument.

4.3 Preparation of derivatives

4.3.1. ^{1}H NMR and ^{13}C NMR spectra of Methyl 3'-nitro-[1,1'-biphenyl]-3-carboxylate (9)

1-Bromo-3-nitrobenzene **7** (5.0 g, 24.7 mmol), (3-(Methoxycarbonyl)phenyl)boronic acid **8** (5.34 g, 29.7 mmol), tetrakistriphenylphosphinepalladium(0) (1.40 g, 1.23 mmol), sodium carbonate (2 M) (37 mL, 74.1 mmol), and 1,4-dioxane (120 mL) were combined and the reaction mixture was heated at reflux under a nitrogen atmosphere for 16 h.The reaction mixture was allowed to cool at room temperature and partitioned between ethyl acetate and water. The organic phase was separated, dried over magnesium sulfate, filtered, and the filtrate was concentrated to give a liquid. The crude product was purified by flash chromatography over silica with a hexanes:ethyl acetate gradient (100:0 to 90:10) to give (4.12 g, 65%) the title compound **9** as a yellow solid; m.p.: 96.5–96.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.45 (t, *J* = 4.00 Hz, 1H), 8.27-8.24 (m, 2H), 8.19 (dd, *J* = 4.00, 8.00 Hz, 1H), 8.08-8.01 (m, 2H), 7.79 (t, *J* = 8.00 Hz, 1H), 7.68 (t, *J* = 8.00 Hz, 1H), 3.90 (s, 3H),; ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.4, 148.9, 141.0, 138.8, 133.9, 132.3, 131.1, 131.0, 130.2, 129.6, 127.9, 123.1, 121.8, 52.8; IR (ATR): vmax 3312, 2953, 2342, 1722, 1520, 1429, 1348, 1301, 1236, 1191, 1104, 963, 883, 839, 801, 690; HRMS (ESI): m/z calcd for $C_{14}H_{11}NO_4$ Na $[M + Na]^+$: 280.0586; found: 280.0579.

4.3.2 ^{1}H NMR and ^{13}C NMR spectra of Methyl 3'-amino-[1,1'-biphenyl]-3-carboxylate (10)

To a stirred solution of 3'-nitro-[1,1'-biphenyl]-3-carboxylic acid methyl ester **9** (4.0 g, 15.5 mmol) in anhydrous THF (100 mL) under nitrogen atmosphere 10% palladium on activated charcoal (1.0 g) was added. The reaction was evacuated and placed under a hydrogen atmosphere and stirred overnight. The reaction mixture was filtered through Celite, and the solvent was removed under reduced pressure to yield gray oil. The residue was chromatographed on silica, eluting with 3:1 hexane/EtOAc. Concentration of the appropriate fractions provided the product as an off-white solid (3.17 g, 90% yield); m.p.: 84.9–85.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (t, *J* = 4.00 Hz, 1H), 8.03 (dt, *J* = 2.00, 5.00 Hz, 1H), 7.79-7.76 (m, 1H), 7.51 (t, *J* = 12.00 Hz, 1H), 7.27 (q, *J* = 4.00 Hz, 1H), 7.06-7.03 (m, 1H), 6.97 (t, *J* = 4.00 Hz, 1H), 6.75-6.73 (m, 1H), 3.96 (s, 5H),; ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.1 146.6, 141.6, 141.3, 131.5, 130.6, 129.9, 128.7, 128.3, 128.2, 117.7, 114.6, 113.9, 52.2; HRMS (ESI): m/z calcd for $C_{14}H_{13}NO_2$ Na $[M + Na]^+$ 250.0844; found: 250.0838.

4.3.3. ^{1}H NMR and ^{13}C NMR spectra of Methyl (S)-3'-(2-((tert-butoxycarbonyl)amino)-3-(1H-indol-3-yl)propanamido)-[1,1'-biphenyl]-3-carboxylate (12)

The title compound was prepared *via* protocol 1, using **10** (1.0 g, 4.4 mmol) and (tert-butoxycarbonyl)-*L*-tryptophan (1.33 g, 4.4 mmol) to afford the coupled product **12** as an off-white solid (1.42 g, 63%); m.p.: 107.3–108.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.82 (s, 1H), 10.17 (s, 1H), 8.17 (s, 1H), 7.98-7.91 (m, 3H), 7.69-7.62 (m, 3H), 7.45-7.38 (m, 2H), 7.32 (d, *J* = 8.00 Hz, 1H), 7.19 (s, 1H), 7.05 (t, *J* = 8.00 Hz, 1H), 6.99-6.97 (m, 2H), 4.41-4.39 (m, 1H), 3.90 (s, 3H), 3.18-2.99 (m, 2H), 1.34 (s, 9H),; ^{13}C NMR (100 MHz, DMSO- d_6): δ 171.8, 166.5, 155.8, 140.9, 140.2, 139.9, 136.5, 131.9, 130.8, 130.8, 128.7, 127.7, 127.4, 124.2, 122.1, 121.3, 119.4, 118.6, 118.1, 111.8, 118.1, 111.8, 110.4, 78.6, 56.4, 52.8, 28.6, 28.3; IR (ATR).vmax 3304, 2330, 2099, 1665, 1605, 1528, 1491, 1423, 1303, 1250, 1160, 1111, 1082, 1010, 856, 733, 690; HRMS (ESI): m/z calcd for $C_{30}H_{31}N_3O_5$ Na $[M + Na]^+$ 536.2161; found: 536.2153.

4.3.4. ^{1}H NMR and ^{13}C NMR spectra of (S)-3'-(2-((tert-butoxycarbonyl)amino)-3-(1H-indol-3-yl)propanamido)-[1,1'-biphenyl]-3-carboxylic acid (13)

To a solution of **12** (1.2 g, 1.81mmol) in THF (10.0 mL) and MeOH (10.0 mL), was added a 1N $NaOH_{(aq)}$ (3.63 mL, 3.63mmol) and stirred at room temperature for 16 h. Ethyl acetate was added and the layers were separated. The aqueous layer was then

acidified with 1N HCl and then extracted with CH_2Cl_2 (2 X 100 mL) and then the solvent was removed under reduced pressure to yield **13** (1.00 g, 85%) as an off-whit solid; m.p.: 140–140.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.10 (s, 1H), 10.82 (s, 1H), 10.17 (s, 1H), 8.18 (t, *J* = 4.00 Hz, 1H), 7.97-7.95 (m, 2H), 7.90-7.87 (m, 1H), 7.69-7.66 (m, 2H), 7.61 (t, *J* = 8.00 Hz, 1H), 7.45-7.38 (m, 2H), 7.32 (d, *J* = 8.00 Hz, 1H), 7.19 (s, 1H), 7.06 (t, *J* = 4.00 Hz, 1H), 6.98 (t, *J* = 8.00 Hz, 2H), 4.41 (q, *J* = 8.00 Hz, 1H), 3.19-3.14 (m, 1H), 3.05-3.00 (m, 1H), 1.34 (s, 9H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 171.7, 167.6, 155.8, 140.7, 140.2, 140.1, 136.5, 132.0, 129.9, 128.8, 127.8, 127.6, 124.3, 122.1, 121.4, 119.3, 119.1, 118.7, 118.1, 111.8, 110.4, 79.6, 78.6, 56.3, 28.6, 28.3; IR (ATR).vmax 3278, 3051, 2946, 2342, 2117, 1917, 1712, 1661, 1589, 1524, 1432, 1310, 1252, 1109, 973, 876, 790, 690; HRMS (ESI): m/z calcd for $C_{29}H_{29}N_3O_5$ Na $[M + Na]^+$ 522.2005; found: 522.1996.

4.3.5. ^{1}H NMR and ^{13}C NMR spectra of Methyl (S)-3'-(2-amino-3-(1H-indol-3-yl)propanamido)-[1,1'-biphenyl]-3-carboxylate (14)

The title compound **14** was prepared *via* protocol 4 using **12** (1.4 g, 2.72 mmol) to yield an off-white solid (1.00 g, 89%); m.p.: 86.4–86.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.06 (s, 1H), 10.73 (s, 1H), 8.33 (br s, 2H), 8.16-8.15 (m, 1H), 8.00-7.97 (m, 1H), 7.92-7.89 (m, 1H), 7.86-7.85 (m, 1H), 7.68-7.60 (m, 3H), 7.48-7.46 (m, 2H), 7.36 (d, *J* = 8.00 Hz, 1H), 7.26 (d, *J* = 4.00 Hz, 1H), 7.09-7.05 (m, 1H), 6.98-6.94 (m, 1H), 4.21 (br s, 1H), 3.89 (s, 3H), 3.40-3.25 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.9, 166.6, 140.7, 140.1, 139.2, 136.7, 131.9, 130.9, 130.3, 130.1, 128.8, 127.5, 127.4, 125.4, 123.0, 121.7, 119.7, 119.0, 118.9, 118.4, 111.9, 107.1, 54.1, 52.8, 27.8; IR (ATR): vmax 3282, 3054, 2947, 2341, 2106, 1911, 1713, 1663, 1589, 1525, 1433, 1310, 1253, 1110, 974, 877, 791, 691; HRMS (ESI): m/z calcd for $C_{25}H_{23}N_3O_3$ $[M + H]^+$: 413.1739; found 414.1810.

4.3.6. ^{1}H NMR and ^{13}C NMR spectra of Methyl 3'-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)-3-(1H-indol-3-yl)propanamido)-[1,1'-biphenyl]-3-carboxylate (16)

The title compound was prepared *via* protocol 1 using **14** and 15 (1.0 g, 3.76 mmol) to afford the coupled product **16** as an off-white solid; m.p.: 116.3–117.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.85 (s, 1H), 10.20 (s, 1H), 8.16-8.14 (m, 2H), 7.98-7.95 (m, 1H), 7.90-7.88 (m, 2H), 7.66-7.62 (m, 3H), 7.44-7.38 (m, 2H), 7.32 (d, *J* = 8.00 Hz, 1H), 7.21-7.11 (m, 6H), 7.07-7.03 (m, 1H), 6.95 (dd, *J* = 8.00, 14.00 Hz, 2H), 4.75 (q, *J* = 8.00 Hz, 1H), 4.22-4.16 (m, 1H), 3.89 (s, 3H), 3.24 (dd, *J* = 8.00, 16.00 Hz, 1H), 3.12 (dd, *J* = 8.00, 14.00 Hz, 1H), 2.93 (dd, *J* = 4.00, 12.00 Hz, 1H), 2.75-2.69 (m, 1H), 1.28 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.9, 170.9, 166.6, 155.7, 140.9, 139.9, 138.4, 136.5, 131.8, 130.8, 130.1, 130.0, 129.7, 128.7, 128.4, 127.8, 127.4, 126.6, 124.1, 122.3, 121.4, 119.4, 119.0, 118.7, 118.1, 111.7, 110.0, 78.7, 56.4, 54.7, 52.8, 37.9, 28.6, 28.4; IR (ATR).vmax 3293, 3055, 2320, 2096, 1649, 1492, 1433, 1310, 1250, 1259, 1110, 1012, 882; HRMS (ESI): m/z calcd for $C_{39}H_{40}N_4O_6$ $[M + H]^+$: 683.2846; found: 683.2841.

4.3.7. ^{1}H NMR and ^{13}C NMR spectra of 3'-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)-3-(1H-indol-3-yl)propanamido)-[1,1'-biphenyl]-3-carboxylic acid (17)

To a solution of **16** (1.2 g, 2.33 mmol) in THF (10.0 mL) and MeOH (10.0 mL), was added a 1N $NaOH_{(aq)}$ (4.66 mL, 4.66 mmol) and stirred at room temperature for 16 h. Ethyl acetate was added and the layers were separated. The aqueous layer was then acidified with 1N HCl and then extracted with CH_2Cl_2 (2 X 100 mL) and then the solvent was removed under reduced pressure to yield **17** (1.05 g, 90%) as an off-white solid; m.p.: 172.6–173.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.08 (br s, 1H), 10.84 (s, 1H), 10.20 (s, 1H), 8.16-8.14 (m, 2H), 7.96-7.85 (m, 3H), 7.69-7.58 (m, 3H), 7.44-7.38 (m, 2H), 7.32 (d, *J* = 4.00 Hz, 1H), 7.21-7.10 (m, 6H), 7.08-7.03 (m, 1H), 6.96 (dd, *J* = 8.00, 12.00 Hz, 2H), 4.75 (q, *J* = 8.00 Hz, 1H), 4.21-4.16 (m, 1H), 3.26-3.21 (m, 1H), 3.14-3.10 (m, 1H), 2.95-2.90 (m, 1H), 2.75-2.69 (m, 1H), 1.28 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.9, 170.8, 167.7, 155.7, 140.7, 140.1, 140.0, 138.4, 136.5, 132.0, 131.4, 130.0, 138.4, 136.5, 132.0, 131.3, 130.0, 129.8, 129.6, 128.8, 128.4, 127.8, 127.6, 126.6, 126.6, 124.1, 122.2, 121.4, 119.3, 119.0, 118.7, 118.1, 111.7, 110.0, 78.7, 56.4, 54.7, 37.9, 28.6, 28.4; IR (ATR).vmax 2048, 1653, 1492, 1434, 1391, 1228, 1158, 1058, 849; HRMS (ESI): m/z calcd for $C_{38}H_{38}N_4O_6Na$ $[M + Na]^+$: 669.2689; found: 669.2689.

4.3.8. ^{1}H NMR and ^{13}C NMR spectra of 1-(2-aminoethyl)2,3-Bis(tert-butoxycarbonyl)guanidine (18b)

The title compound **18b** was prepared by adding the solution of N,N'-bis-(Boc)-1*H*-Pyrazole-1-carboxamidine (2 g, 6.45 mmol) in THF (50 ml) dropwise to ethylene diamine (4 ml, 59.6 mmol) in THF (100 ml). After 30 min of mixing at room temperature solvent was evaporated then toluene (100 ml) was added and evaporated in order to remove remaining traces of the ethylene diamine.The crude reaction mixture taken for next step due to the instability of the compound.^{1}H NMR (400 MHz, $CDCl_3$): δ 11.50 (br s, 1H), 8.63 (br s, 1H), 3.47 (q, *J* = 4.00 Hz, 2H), 2.87 (t, *J* = 4.00 Hz, 2H), 1.49 (s, 9H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.6, 156.5, 153.2, 83.1, 79.3, 43.4, 41.0, 28.3, 28.1; IR (ATR).vmax 3385, 3068, 2972, 2887, 2807, 1613, 1519, 1463, 1347, 1311, 1249, 1090, 1161, 884, 799.

4.3.9. ^{1}H NMR and ^{13}C NMR spectra of Methyl N^w-((4-methoxy-2,3,6-trimethylphenyl)sulfonyl)-L-argininate (18d)

The title compound **18d** was prepared via protocol 3 using **20a** (0.9 g, 1.44 mmol) to yield the desired product as an off-white solid (450 mg, 77%); m.p.: 136.3–137.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.50 (s, 1H), 6.46 (br s, 1H), 6.38 (br s, 2H), 3.80 (s, 3H), 3.67 (s, 3H), 3.47-3.45 (m, 1H), 3.15 (br s, 2H), 2.65 (s, 3H), 2.97 (s, 3H), 2.10 (s, 3H), 1.74-1.55 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.7, 158.4, 156.4, 138.4, 136.4, 133.5, 124.8, 111.7, 55.5, 53.7, 52.2, 40.7, 31.2, 25.5, 24.1, 18.3, 11.9; IR

(ATR).vmax 3428, 3329, 2934, 1733, 1541, 1459, 1398, 1246, 1303, 1098, 1016, 911, 802; HRMS (ESI): m/z calcd for $C_{17}H_{28}N_4O_5S$ $[M + H]^+$: 401.1780; found: 401.1851.

4.3.10.^{1}H NMR and ^{13}C NMR spectra of tert-butyl (S)-(5-amino-6-((2-((tert-butoxycarbonyl)amino)ethyl)amino)-6-oxohexyl)carbamate (18e)

The title compound was prepared via protocol 3, using **20a** (630 mg, 1.03 mmol) to yield the desired product **18e** as an off-white solid (0.32 g, 80%); m.p.: 206.2–207.5 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.63 (s, 1H), 5.09 (s, 1H), 4.67 (s, 1H), 3.34-3.08 (m, 6H), 1.84-1.76 (m, 1H), 1.50-1.46 (m, 2H), 1.42 (s, 18H), 1.37-1.36 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.6, 156.5, 156.1, 79.4,79.1, 55.0, 40.6, 40.1, 39.7, 34.4, 29.8, 28.4, 28.4, 22.8; IR (ATR).vmax 3313, 2930, 1683, 1518, 1452, 1246, 1162, 994, 859, 779; HRMS (ESI): m/z calcd for $C_{18}H_{36}N_4O_5$ $[M + H]^+$: 389.2686; found: 389.2759.

4.3.11. ^{1}H NMR and ^{13}C NMR spectra of tert-butyl (S)-(5-amino-6-((2-(2,3-dibocguanidino)ethyl)amino)-6-oxohexyl)carbamate (18f)

The title compound was prepared via protocol 3, using **20b** (850 mg, 1.07 mmol) to yield the desired product **18f** as an off-white solid (0.45 g, 79%); m.p.: 184.4–185.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.43 (s, 1H), 8.56 (t, J = 4.00 Hz, 1H), 7.91 (t, J = 8.00 Hz, 1H), 4.59 (br s, 1H), 3.59-3.55 (m, 2H), 3.45-3.41 (m, 2H), 3.33-3.30 (m, 1H), 3.13-3.08 (m, 2H), 1.82-1.77 (m, 1H), 1.52-1.51 (m, 1H), 1.49 (s, 18H), 1.49-1.45 (m, 4H), 1.43 (s, 9H), 1.39-1.37 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.6, 163.2, 157.1, 156.0, 153.1, 83.4, 79.5, 55.4, 40.2, 40.1, 40.0, 34.8, 30.0, 34.8, 30.0, 28.4, 28.3, 28.1, 23.0; IR (ATR).vmax 3326, 2931, 1714, 1612, 1521, 1409, 1362, 1324, 1246, 1128, 1047, 854; HRMS (ESI): m/z calcd for $C_{24}H_{46}N_6O_7$ $[M + H]^+$: 531.3428; found: 531.3500.

4.3.12. ^{1}H NMR and ^{13}C NMR spectra of (S)-2-amino-N-(2-(2,3-dibocguanidino)ethyl)-5-(3-((4-methoxy-2,3,6-trimethylphenyl)sulfonyl)guanidino)pentanamide (18g)

The title compound was prepared via protocol 3, using **21b** (730 mg, 0.82 mmol) to yield the desired product **18g** as an off-white solid (0.48 g, 87%); m.p.: 102.2–103.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.39 (br s, 1H), 8.56 (br s, 1H), 8.04 (t, J = 4.00 Hz, 1H), 6.51 (s, 1H), 6.26 (br s, 3H), 3.81 (s, 3H), 3.54-3.39 (m, 5H), 3.24-3.20 (m, 2H), 2.67 (s, 3H), 2.60 (s, 3H), 2.11 (s, 3H), 1.76-1.61 (m, 4H), 1.46 (s, 9H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.1, 163.0, 158.4, 157.1, 156.4, 153.0, 138.5, 136.6, 133.6, 124.7, 111.7, 83.6, 79.8, 55.4, 54.3, 40.1, 31.7, 28.3, 28.0, 25.2, 24.1, 18.3, 11.9; IR (ATR).vmax 3326, 1719, 1611, 1543, 1439, 1409, 1326, 1248, 1115, 1046, 839, 803; HRMS (ESI): m/z calcd for $C_{29}H_{50}N_8O_8S$ $[M + H]^+$: 671.3540; found: 671.3540.

4.3.13. ^{1}H NMR and ^{13}C NMR spectra of Methyl N^2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N^w-((4-methoxy-2,3,6-trimethylphenyl)sulfonyl)-L-argininate (20')

The title compound **20'** was prepared by adding thionyl chloride (0.23 mL, 3.28 mmol) dropwise to N_α-Fmoc-N_ω-MTR-*L*-arginine (1.0 g, 1.64 mmol) in methanol (20.0 mL) at 0° C and then stirred at room temperature for 16 h. The solvents were removed and reduced pressure and the residue was diluted with ethylacetate (100.0 mL) and washed with saturated $NaHCO_3$(40 mL), saturated brine (40 mL), then dried (Na_2SO_4) and concentrated under reduced pressure to afford a white solid (0.95 g, 93%); m.p.: 94.0–94.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76-7.35 (m, 7H), 7.30 – 7.28 (m, 2H), 6.50 (s, 1H), 6.20 (br s, 2H), 5.74 – 5.72 (br d, J = 7.2 Hz), 4.38-4.29 (m, 2H), 4.19-4.14 (m, 2H), 3.79 (s, 3H), 3.71 (s, 3H), 3.22-3.21 (m, 2H), 2.69 (s, 3H), 2.62 (s, 3H), 2.11 (s, 3H), 1.70-1.57 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.7, 158.5, 156.3, 143.7, 143.6, 141.2, 138.5, 136.5, 133.5, 127.8, 127.1, 125.1, 124.8, 120.0, 111.7, 67.13, 55.4, 52.6, 47.1, 40.7, 30.1, 25.2, 24.1, 18.3, 17.9, 11.9; IR (ATR).vmax 2946, 1717, 1542, 1616, 1445, 1244, 1169, 1102; HRMS (ESI): m/z calcd for $C_{32}H_{38}N_4O_7SNa$ $[M + Na]^+$: 645.2359; found: 645.2356.

4.3.14. ^{1}H NMR and ^{13}C NMR spectra of (9H-fluoren-9-yl)methyl (S)-(1-((2-(2,3-diboc guanidino)ethyl)amino)-5-(3-((4-methoxy-2,3,6-trimethylphenyl)sulfonyl)guanidino)-1-oxopentan-2-yl)carbamate (20b)

The title compound **20b** was prepared from compound **18b** (0.5 g, 1.64 mmol) and N_α-Fmoc-N_ω-MTR-*L*-arginine (1.0 g, 1.64 mmol) according to the protocol 2. Off-white solid (0.73 g, 50%); m.p.: 148.0–148.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.40 (br s, 1H), 8.61 (br s, 1H), 7.98 (br s, 1H), 7.73 (d, J = 8.00 Hz, 2H), 7.57 (d, J = 8.00 Hz, 2H), 7.36 (t, J = 8.00 Hz, 2H), 7.30-7.24 (m, 2H), 6.50 (br s, 1H), 6.23 (br s, 2H), 5.99 (d, J = 8.00 Hz, 1H), 4.34-4.22 (m, 2H), 4.18-4.14 (m, 2H), 3.79 (s, 3H), 3.54-3.15 (m, 7H), 2.67 (s, 3H), 2.60 (s, 3H), 2.10 (s, 3H), 1.84-1.57 (m, 4H), 1.45 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.3, 162.8, 159.7, 158.5, 157.2, 156.4, 156.3, 153.0, 143.8, 143.8, 141.3, 138.6, 136.6, 133.4, 127.7, 127.1, 125.2, 124.8, 120.0, 83.6, 80.4, 80.1, 67.1, 55.4, 47.1, 42.1, 40.1, 28.2, 28.2, 28.0, 25.2, 24.1, 18.4, 12.0; IR (ATR).vmax 3324, 2976, 2064, 1717, 1615, 1543, 1446, 1304, 1248, 1117, 840; HRMS (ESI): m/z calcd for $C_{44}H6_0N_8O_{10}S$ $[M + H]^+$: 893.4153; found: 893.4224.

4.3.15. ^{1}H NMR and ^{13}C NMR spectra of (9H-fluoren-9-yl)methyl tert-butyl (6-((2-((tert-butoxycarbonyl)amino)ethyl)amino)-6-oxohexane-1,5-diyl)(S)-dicarbamate (21a)

The title compound **21a** was prepared from compound **18a** (0.34 g, 2.13 mmol) and N_α-Fmoc-N_ε-Boc-*L*-lysine (1.0 g, 2.13 mmol) according to the protocol 2. Off-white solid (0.65 g, 50%); m.p.: 173.7–175.2 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.90-7.87 (m, 3H), 7.72 (t, J = 6.00 Hz, 2H), 7.42-7.38 (m, 3H), 7.32 (t, J = 6.00 Hz, 2H), 6.73 (d, J = 6.00 Hz, 2H), 4.28-4.27 (m, 1H),

4.24-4.20 (m, 2H), 3.88-3.87 (m, 1H), 3.12-3.00 (m, 2H), 2.99-2.96 (m, 2H), 2.90-2.86 (m, 2H), 1.74-1.46 (m, 3H), 1.36 (s, 18H), 1.28-1.19 (m, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.5, 156.4, 156.1, 156.0, 144.4, 144.2, 141.2, 128.1, 127.5, 125.7, 120.6, 79.6, 79.4, 79.2, 79.2, 78.2, 77.8, 66.1, 55.2, 47.1, 46.3, 46.3, 32.1, 29.7, 28.7, 28.6, 26.4, 26.3, 23.3; IR (ATR).νmax 3356, 2943, 2265, 2106, 1526, 1447, 1389, 1526, 1240, 1167, 1025, 836, 735; HRMS (ESI): m/z calcd for $C_{33}H_{46}N_4O_7Na$ [M + Na]$^+$: 633.3264; found: 633.3261.

4.3.16. ^{1}H NMR and ^{13}C NMR spectra of (9H-fluoren-9-yl)methyl tert-butyl (6-((2-(2,3-dibocguanidino)ethyl)amino)-6-oxohexane-1,5-diyl)(S)-dicarbamate (21b)

The title compound **21b** was prepared from compound **18b** (0.643 g, 2.13 mmol) and N_α-Fmoc-N_ε-Boc-*L*-lysine (1.0 g, 2.13 mmol) according to the protocol 2. Off-white solid (0.89 g, 55%); m.p.: 102.2–103.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.43 (s, 1H), 8.65 (br s, 1H), 8.07 (br s, 1H), 7.75 (d, *J* = 8.00 Hz, 2H), 7.59 (d, *J* = 8.00 Hz, 2H), 7.39 (t, *J* = 8.00 Hz, 2H), 7.33-7.26 (m, 2H), 5.65 (d, *J* = 4.00 Hz, 1H), 4.59 (br s, 1H), 4.38-4.36 (m, 1H), 4.22-4.17 (m, 1H), 3.57-3.53 (m, 2H), 3.45-3.42 (m, 2H), 3.10-3.08 (m, 2H), 1.87-1.80 (m, 1H), 1.70-1.66 (m, 1H), 1.50 (s, 9H), 1.48 (s, 9H), 1.42 (s, 9H), 1.48-1.32 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6, 162.8, 157.6, 156.0, 155.9, 153.0, 143.9, 141.3, 127.7, 127.0, 125.2, 120.0, 83.7, 80.0, 79.1, 66.9, 54.8, 47.2, 41.5, 40.3, 32.9, 29.8, 28.4, 28.3, 28.0, 22.3; IR (ATR).νmax 3316, 2973, 1713, 1612, 1516, 1448, 1410, 1325, 1244, 1129, 1046, 857; HRMS (ESI): m/z calcd for $C_{39}H_{56}N_6O_9Na$ [M + Na]$^+$: 775.4006; found: 775.4006.

4.3.17. ^{1}H NMR and ^{13}C NMR spectra of tert-butyl (S)-(1-((3'-((2-((tert-butoxycarbonyl)amino)ethyl)carbamoyl)-[1,1'-biphenyl]-3-yl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)carbamate (22a)

The title compound was prepared from compound **13** (100 mg, 0.20 mmol) and **18a** (32 mg, 0.20 mmol) following the general protocol 1 to afford **22a** as an off-white solid (83 mg, 65%).^{1}H NMR (400 MHz, DMSO-*d6*): δ 10.82 (br s, 1H), 10.20 (br s, 1H), 8.60 (t, *J* = 4.00 Hz, 1H), 8.10 (s, 1H), 7.90 (br s, 1H), 7.85 (d, *J* = 4.00 Hz, 1H), 7.75 (d, *J* = 8.00 Hz, 1H), 7.67 (d, *J* = 8.00 Hz, 2H), 7.57 (t, *J* = 8.00 Hz, 1H), 7.46-7.40 (m, 2H), 7.33 (d, *J* = 4.00 Hz, 1H), 7.20 (s, 1H), 7.08-6.92 (m, 4H), 4.44-4.40 (m, 1H), 3.18-2.99 (m, 6H), 1.37 (s, 18H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.7, 156.2, 140.6, 140.6, 136.5, 135.7, 129.8, 129.4, 127.8, 126.9, 125.9, 124.3, 122.3, 121.3, 119.2, 119.1, 118.7, 118.3, 111.8, 110.4, 79.6, 78.6, 78.2, 28.7, 28.6, 28.3; IR (ATR).νmax 3298, 2974, 1669, 1492, 1453, 1247, 1158, 1010, 853; HRMS (ESI): m/z calcd for $C_{36}H_{43}N_5O_6Na$ [M + Na]$^+$: 664.3111; found: 664.3102.

4.3.18. ^{1}H NMR and ^{13}C NMR spectra of tert-butyl (S)-(1-((3'-((2-(2,3-dibocguanidino)ethyl)carbamoyl)-[1,1'-biphenyl]-3-yl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)carbamate (22b)

The title compound was prepared from compound **13** (100 mg, 0.20 mmol) and **18b** (61 mg, 0.2 mmol) following the general protocol 1 to afford **22b** as an off-white solid (94 mg, 60%); m.p.: 127.2–127.6 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.49 (br s, 1H), 10.81 (br s, 1H), 10.15 (br s, 1H), 8.67 (t, *J* = 6.00 Hz, 1H), 8.47 (t, *J* = 6.00 Hz, 1H), 8.08 (br s, 1H), 7.91 (s, 1H), 7.82 (d, *J* = 12.00 Hz, 1H), 7.75 (d, *J* = 12.00 Hz, 1H), 7.67-7.66 (m, 2H), 7.56 (t, *J* = 6.00 Hz, 1H), 7.32 (d, *J* = 6.00 Hz, 1H), 7.19 (br s, 1H), 7.05 (t, *J* = 6.00 Hz, 1H), 6.99-6.95 (m, 2H), 4.40 (q, *J* = 6.00 Hz, 1H), 3.52 (q, *J* = 6.00 Hz, 2H), 3.46 (q, *J* = 6.00 Hz, 2H), 3.17-3.13 (m, 1H), 3.04-3.00 (m, 1H), 1.43 (s, 9H), 1.37 (s, 9H), 1.33 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.1, 166.9, 163.7, 163.5, 156.2, 155.7, 152.3, 140.6, 140.5, 140.1, 136.5, 135.6, 129.8, 129.7, 129.4, 129.4, 127.7, 126.7, 125.9, 124.2, 122.2, 121.3, 119.2, 119.0, 118.6, 118.2, 111.7, 110.4, 83.3, 78.6, 78.5, 67.4, 56.3, 40.5, 39.2, 31.7, 28.7, 28.6, 28.4, 28.2, 28; IR (ATR).νmax 2977, 1612, 1535, 1364, 1326, 1229, 1133, 875; HRMS (ESI): m/z calcd for $C_{42}H_{53}N_7O_8$ [M + H]$^+$: 784.3956; found: 784.4031.

4.3.19. ^{1}H NMR and ^{13}C NMR spectra of methyl N^6-(tert-butoxycarbonyl)-N^2-(3'-((S)-2-((tert-butoxycarbonyl)amino)-3-(1H-indol-3-yl)propanamido)-[1,1'-biphenyl]-3-carbonyl)-L-lysinate (22c)

The title compound was prepared from compound **13** (100 mg, 0.20 mmol) and **18c** (52 mg, 0.2 mmol) following the general protocol 1 to afford **22c** as an off-white solid (109 mg, 74%); m.p.: 235.5–236.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.82 (s, 1H), 10.17 (s, 1H), 8.84 (d, *J* = 8.00 Hz, 1H), 8.14 (s, 1H), 7.91-7.88 (m, 2H), 7.79 (d, *J* = 8.00 Hz, 1H), 7.68 (t, *J* = 4.00 Hz, 1H), 7.59 (t, *J* = 8.00 Hz, 1H), 7.47-7.41 (m, 2H), 7.32 (d, *J* = 8.00 Hz, 1H), 7.20 (s, 1H), 7.06 (t, *J* = 8.00 Hz, 1H), 7.00-6.97 (m, 2H), 6.78 (t, *J* = 8.00 Hz, 1H), 4.47-4.38 (m, 2H), 3.66 (s, 3H), 3.17-2.89 (m, 4H), 1.81 (t, *J* = 8.00 Hz, 1H), 1.41-1.37 (m, 2H), 1.34 (s, 18H), 1.24-1.18 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ173.2, 166.9, 156.0, 155.7, 140.7, 140.5, 140.1, 136.5, 134.8, 130.0, 129.8, 129.4, 127.7, 127.1, 126.1, 124.2, 122.3, 121.3, 119.0, 118.6, 118.2, 111.7, 110.4, 79.6, 78.5, 77.8, 53.2, 52.3, 30.7, 29.5, 28.7, 28.6, 28.2, 23.5; IR (ATR).νmax 2929, 2312, 1992, 1671, 1521, 1433, 1363, 1228, 1160, 1010, 860; HRMS (ESI): m/z calcd for $C_{41}H_{51}N_5O_8Na$ [M + Na]$^+$: 764.3635; found: 764.3630.

4.3.20. ^{1}H NMR and ^{13}C NMR spectra of Methyl N^2-(3'-((S)-2-((tert-butoxycarbonyl)amino)-3-(1H-indol-3-yl)propanamido)-[1,1'-biphenyl]-3-carbonyl)-N^w-((4-methoxy-2,3,6-trimethylphenyl)sulfonyl)-L-argininate (22d)

The title compound was prepared from compound **13** (100 mg, 0.20 mmol) and **18d** (80 mg, 0.2 mmol) following the general protocol 1 to afford **22d** as an off-white solid (104 mg, 59%); m.p.: 172.2–173.1 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.82 (s, 1H), 10.17 (s, 1H), 8.88 (d, *J* = 12.00 Hz, 1H), 8.13 (br s, 1H), 7.92-7.88 (m, 2H), 7.79 (d, *J* = 12.00 Hz, 1H), 7.68-7.66 (m, 2H), 7.60 (t, *J* = 6.00 Hz, 1H), 7.47-7.41 (m, 2H), 7.33 (d, *J* = 12.00 Hz, 1H), 7.19 (br s, 1H), 7.08-7.04 (m, 1H), 7.00-6.96 (m, 2H), 6.74-6.66 (m, 2H), 6.48-6.26 (m, 2H), 4.48-4.40 (m, 2H), 3.77 (s, 3H), 3.65 (s, 3H), 3.15-2.99 (m, 4H), 2.59 (s, 3H), 2.51 (s, 3H),

2.02 (s, 3H), 1.82-1.74 (m, 2H), 1.52-1.49 (m, 2H), 1.34 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.0, 171.8, 166.9, 157.9, 156.6, 155.7, 140.7, 140.5, 140.1, 138.1, 136.5, 134.8, 130.1, 129.8, 129.5, 127.7, 127.1, 126.1, 124.3, 122.3, 121.3, 119.2, 119.1, 118.6, 118.3, 112.2, 111.8, 110.4, 79.6, 78.6, 56.3, 55.9, 53.0, 52.3, 28.6, 28.4, 24.1, 18.5, 12.2; IR (ATR).νmax 3305, 2932, 1669, 1541, 1456, 1364, 1303, 1227, 1159, 1114, 1013; HRMS (ESI): m/z calcd for $C_{46}H_{55}N_7O_9S$ [M + H]$^+$: 882.3782; found: 882.3854.

4.3.21. ^{1}H NMR and ^{13}C NMR spectra of tert-butyl ((S)-5-(3'-((S)-2-((tert-butoxycarbonyl)amino)-3-(1H-indol-3-yl)propanamido)-[1,1'-biphenyl]-3-carboxamido)-6-((2-((tert-butoxycarbonyl)amino)ethyl)amino)-6-oxohexyl)carbamate (22e)

The title compound was prepared from compound **13** (100 mg, 0.20 mmol) and **18e** (77 mg, 0.20 mmol) following the general protocol 1 to afford **22e** as an off-white solid (139 mg, 80%); m.p.: 131.5–131.7 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.82 (s, 1H), 10.16 (s, 1H), 8.55 (d, *J* = 6.00 Hz, 1H), 8.16 (s, 1H), 8.01 (t, *J* = 6.00 Hz, 1H), 7.92-7.91 (m, 2H), 7.77-7.75 (m, 1H), 7.68-7.67 (m, 1H), 7.57 (t, *J* = 12.00 Hz, 1H), 7.45-7.43 (m, 2H), 7.32 (d, *J* = 6.00 Hz, 1H), 7.20 (s, 1H), 7.06 (t, *J* = 6.00 Hz, 1H), 6.99-6.96 (m, 2H), 6.77-6.74 (m, 2H), 4.43-4.37 (m, 2H), 3.18-2.86 (m, 8H), 1.77-1.68 (m, 2H), 1.37-1.36 (m, 2H), 1.36 (s, 9H), 1.34 (s, 18H), 1.24-1.15 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.4, 171.8, 166.7, 156.1, 156.0, 155.8, 140.6, 140.6, 136.5,135.3, 129.8, 129.3, 127.7, 127.2, 126.3, 124.3, 121.3, 119.2, 119.1, 118.7, 118.3, 111.7, 110.4, 78.6, 78.1, 77.7, 56.3, 54.1, 40.5, 39.3, 31.8, 31.4, 29.7, 23.7, 22.5; IR (ATR).νmax 2972, 2930, 1683, 1521, 1455, 1246, 1163, 1010, 861; HRMS (ESI): m/z calcd for $C_{47}H_{63}N_7O_9Na$ [M + Na]$^+$: 892.4585; found: 892.4575.

4.3.22. ^{1}H NMR and ^{13}C NMR spectra of (S)-3'-(2-amino-3-(1H-indol-3-yl)propanamido)-N-(2-aminoethyl)-[1,1'-biphenyl]-3-carboxamide (23a)

The title compound was prepared from compound **22a** (75 mg, 0.116 mmol) following the general protocol 4 to afford **23a** as a white solid (45 mg, 89%); m.p.: 164.5–164.7 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.06 (d, *J* = 6.00 Hz, 1H), 10.70 (s, 1H), 8.78 (t, *J* = 6.00 Hz, 1H), 8.30 (br s, 3H), 8.13 (t, *J* = 6.00 Hz, 1H), 7.90-7.87 (m, 5H), 7.79-7.77 (m, 1H), 7.68 (d, *J* = 12.00 Hz, 1H), 7.62-7.60 (m, 2H), 7.50-7.49 (m, 2H), 7.37 (d, *J* = 12.00 Hz, 1H), 7.26 (d, *J* = 6.00 Hz, 1H), 7.10-7.07 (m, 1H), 6.99-6.97 (m, 1H), 4.21-4.20 (m, 1H), 3.55 (q, *J* = 6.00 Hz, 2H), 3.39-3.35 (m, 2H), 3.28-3.25 (m, 2H), 3.03 (q, *J* = 6.00 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 167.9, 167.2, 140.6, 140.4, 139.1, 136.7, 135.3, 130.1, 129.9, 129.6, 127.5, 127.1, 126.1, 125.4, 123.1, 121.7, 119.6, 119.0, 118.9, 118.5, 111.9, 107.1, 54.1, 40.5, 39.1, 37.6, 27.8; IR (ATR).νmax 3041, 2890, 1774, 1664, 1525, 1429, 1318, 1179, 835, 795, 744; HRMS (ESI): m/z calcd for $C_{26}H_{27}N_5O_2$ [M + H]$^+$: 442.2165; found: 442.2234.

4.3.23. ^{1}H NMR and ^{13}C NMR spectra of (S)-3'-(2-amino-3-(1H-indol-3-yl)propanamido)-N-(2-guanidinoethyl)-[1,1'-biphenyl]-3-carboxamide (23b)

The title compound was prepared from compound **22b** (90 mg, 0.122 mmol) following the general protocol 4 to afford **23b** as a white solid (29 mg, 50%); m.p.: 236.7–238.0 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.05 (br s, 1H), 10.69 (s, 1H), 8.77 (t, *J* = 6.00 Hz, 1H), 8.28 (br s, 3H), 8.12 (t, *J* = 6.00 Hz, 1H), 7.89-7.87 (m, 2H), 7.78-7.77 (m, 1H), 7.70-7.67 (m, 2H), 7.62-7.59 (m, 2H), 7.49-7.48 (m, 3H), 7.38-7.36 (m, 2H), 7.26 (d, *J* = 6.00 Hz, 2H), 7.10-7.07 (m, 1H), 6.99-6.97 (m, 1H), 4.20 (t, *J* = 6.00 Hz, 1H), 3.45 (q, *J* = 6.00 Hz, 2H), 3.39-3.36 (m, 1H), 3.28-3.24 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 167.9, 167.1, 157.5, 140.6, 140.4, 139.1, 135.3, 130.1, 129.8, 129.6, 127.5, 126.9, 126.0, 125.4, 123.1, 121.7, 119.6, 119.0, 118.9, 118.5, 112.0, 107.1,54.1, 40.8, 39.1, 27.8; IR (ATR).νmax 3178, 3080, 2340, 2110, 1657, 1542, 1430, 1316, 1200, 1148, 835, 797, 743; HRMS (ESI): m/z calcd for $C_{27}H_{29}N_7O_2$ [M + H]$^+$: 484.2383; found: 484.2455.

4.3.24. ^{1}H NMR and ^{13}C NMR spectra of Methyl (3'-((S)-2-amino-3-(1H-indol-3-yl)propanamido)-[1,1'-biphenyl]-3-carbonyl)-L-lysinate (23c)

The title compound was prepared from compound **22c** (50 mg, 0.067 mmol) following the general protocol 4 to afford **23c** as a gummy solid (36 mg, 70%); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.00 (s, 1H), 10.70 (s, 1H), 8.89 (d, *J* = 6.00 Hz, 1H), 8.29 (br s, 3H), 8.14-8.13 (m, 1H), 7.93-7.91 (m, 1H), 7.87-7.86 (m, 1H), 7.80-7.75 (m, 4H), 7.68 (d, *J* = 12.00 Hz, 1H), 7.62-7.60 (m, 2H), 7.50-7.48 (m, 2H), 7.37-7.36 (m, 1H), 7.26 (d, *J* = 6.00 Hz, 1H), 7.10-7.07 (m, 1H), 6.99-6.97 (m, 1H), 4.50-4.46 (m, 1H), 4.20 (br s, 1H), 3.67 (s,3H), 3.35-3.25 (m, 2H), 2.80-2.79 (m, 2H), 1.86-1.39 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.1, 167.9, 166.9, 158.6, 158.4, 140.6, 140.4, 139.1, 136.7, 134.8, 130.2, 130.1, 129.6, 127.5, 127.2, 126.2, 125.4, 123.2, 121.6, 119.6, 118.95, 118.9, 118.6, 111.9, 107.1, 54.1, 53.0, 52.4, 40.5, 40.4, 39.0, 30.4, 27.8, 27.0, 23.1; IR (ATR).νmax 3251, 3044, 2932, 2098, 1666, 1524, 1432, 1338, 1178, 1118, 835, 796, 743, 719; HRMS (ESI): m/z calcd for $C_{31}H_{35}N_5O_4$ [M + H]$^+$: 542.2689; found: 542.2157.

4.3.25. ^{1}H NMR and ^{13}C NMR spectra of Methyl (3'-((S)-2-amino-3-(1H-indol-3-yl)propanamido)-[1,1'-biphenyl]-3-carbonyl)-L-argininate (23d)

The title compound was prepared from compound **22d** (90 mg, 0.10 mmol) following the general protocol 4 to afford **23d** as a gummy solid (50 mg, 54%).^{1}H NMR (600 MHz, DMSO-d_6): δ 11.04 (s, 1H), 10.66 (s, 1H), 8.93 (d, *J* = 6.00 Hz, 1H), 8.27 (br s, 3H), 8.13 (br s, 1H), 7.92 (d, *J* = 6.00 Hz, 2H), 7.79 (d, *J* = 12.00 Hz, 1H), 7.73-7.45 (m, 6H), 7.38-7.25 (m, 2H), 7.10-6.97 (m, 3H), 4.52-4.49 (m, 1H), 4.19-4.18 (m, 1H), 3.68 (s, 3H), 3.35-3.12 (m, 4H), 1.92-1.54 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.9, 167.9, 166.9, 162.2, 158.7, 158.5, 157.2, 140.6, 140.4, 139.1, 136.7, 134.8, 130.2, 130.1, 129.6, 127.5, 127.2, 126.2, 125.4, 123.2, 121.7, 119.6, 118.9, 118.9, 118.8, 118.5, 112.0, 107.1, 54.1, 52.8, 52.5, 40.7, 40.5, 28.1, 27.8, 25.8; IR (ATR).νmax

, 3192, 3065, 2118, 1660, 1539, 1434, 1315, 1227, 1159, 836, 797, 744; HRMS (ESI): m/z calcd for $C_{31}H_{35}N_7O_4$ [M + H]$^+$: 570.3114; found: 570.2820.

4.3.26. ^{1}H NMR and ^{13}C NMR spectra of N-((S)-6-amino-1-((2-aminoethyl)amino)-1-oxohexan-2-yl)-3'-((S)-2-amino-3-(1H-indol-3-yl)propanamido)-[1,1'-biphenyl]-3-carboxamide (23e)

The title compound was prepared from compound **22c** (100 mg, 0.114 mmol) following the general protocol 4 to afford **23e** as a white solid (45 mg, 70%); m.p.: 237.6–238.0 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.05 (d, *J* = 4.00 Hz, 1H), 10.71 (s, 1H), 8.68 (d, *J* = 4.00 Hz, 1H), 8.29-8.23 (m, 4H), 8.16 (s, 1H), 7.94 (d, *J* = 4.00 Hz, 1H), 7.85 (br s, 4H), 7.77-7.75 (m, 4H), 7.67 (d, *J* = 8.00 Hz, 1H), 7.60-7.58 (m, 2H), 7.51-7.47 (m, 2H), 7.36 (d, *J* = 8.00 Hz, 1H), 7.25 (d, *J* = 4.00 Hz, 1H), 7.08 (t, *J* = 4.00 Hz, 1H), 6.97 (t, *J* = 4.00 Hz, 1H), 4.44-4.41 (m, 1H), 4.21-4.20 (m, 1H), 3.37-3.24 (m, 4H), 2.88-2.77 (m, 4H), 1.86-1.70 (m, 2H), 1.60-1.52 (m, 2H), 1.44-1.31 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.8, 167.9, 166.8, 158.9, 158.7, 158.5, 140.7, 140.3, 139.1, 136.7, 135.2, 130.0, 129.4, 127.5, 127.3, 126.3, 125.4, 123.2, 121.7, 119.6, 118.9, 118.6, 116.7, 111.9, 107.1, 54.1, 53.8, 40.5, 39.1, 38.9, 36.9, 31.2, 27.8, 27.2, 23.2; IR (ATR).νmax 3257, 3052, 2930, 2099, 1664, 1524, 1431, 1317, 1170, 797, 744, 720; HRMS (ESI): m/z calcd for $C_{32}H_{39}N_7O_3$ [M + H]$^+$: 570.3114; found: 570.2820.

4.3.27. ^{1}H NMR and ^{13}C NMR spectra of tert-butyl ((S)-1-(((S)-1-((3'-((2-((tert-butoxycarbonyl)amino)ethyl)carbamoyl)-[1,1'-biphenyl]-3-yl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (24a)

The title compound was prepared from compound **17** (100 mg, 0.15 mmol) and **18a** (25 mg, 0.15 mmol) following the general protocol 1 to afford **24a** as an off-white solid (94 mg, 80%); m.p.: 126.7–128.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.82 (s, 1H), 10.15 (s, 1H), 8.57-8.56 (m, 1H), 8.43 (d, *J* = 8.00 Hz, 1H), 8.10 (s, 1H), 7.81-7.67 (m, 7H), 7.52 (t, *J* = 8.00 Hz, 1H), 7.30 (d, *J* = 8.00 Hz, 1H), 7.17-6.91 (m, 9H), 6.76 (d, *J* = 8.00 Hz, 1H), 4.75 (q, *J* = 8.00 Hz, 1H), 4.26-4.21 (m, 1H), 3.29-3.03 (m, 6H), 2.73-2.72 (m, 1H), 2.60-2.54 (m, 1H), 1.36 (s, 9H), 1.29 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.9, 166.7, 160.0, 156.2, 155.7, 155.1, 140.0, 139.8, 138.9, 138.3, 137.1, 136.5, 135.6, 134.9, 129.7, 129.3, 129.3, 128.3, 127.7, 127.5, 126.5, 125.3, 124.2, 121.4, 120.2, 119.0, 118.7, 111.7, 110.1, 78.5, 78.1, 56.0, 54.5, 37.8, 28.7, 28.5, 28.2; IR (ATR).νmax 3299, 2976, 1663, 1518, 1455, 1246, 1160, 1118, 1021, 806; HRMS (ESI): m/z calcd for $C_{45}H_{52}N_6O_7Na$ [M + Na]$^+$: 811.3795; found: 811.3789.

4.3.28. ^{1}H NMR and ^{13}C NMR spectra of tert-butyl ((S)-1-(((S)-1-((3'-((2-(2,3-dibocguanidino)ethyl)carbamoyl)-[1,1'-biphenyl]-3-yl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (24b)

The title compound was prepared from compound **17** (100 mg, 0.15 mmol) and **18b** (46 mg, 0.15 mmol) following the general protocol 1 to afford **24b** as an off-white solid (95 mg, 68%); m.p.: 206.3–206.5 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.50 (s, 1H), 10.80 (s, 1H), 10.20 (s, 1H), 8.41-8.45 (m, 1H), 8.14 (br s, 1H), 8.07 (br s, 1H), 7.88 (s, 1H), 7.82 (d, *J* = 4.00 Hz, 1H), 7.73 (d, *J* = 4.00 Hz, 1H), 7.62 (t, *J* = 8.00 Hz, 2H), 7.55 (t, *J* = 4.00 Hz, 1H), 7.42-7.40 (m, 2H), 7.32-7.31(m, 1H), 7.20-7.13 (m, 7H), 7.05 (t, *J* = 4.00 Hz, 1H), 6.97-6.92 (m, 2H), 4.75-4.74 (m, 1H), 4.18-4.17 (m, 1H), 3.50 (t, *J* = 4.00 Hz, 2H), 3.45 (q, *J* = 4.00 Hz, 2H), 3.25-3.21 (m, 1H), 3.13-3.09 (m, 1H), 2.93-2.90 (m, 1H), 2.73-2.69 (m, 1H), 1.42 (s, 9H), 1.37 (s, 9H), 1.28 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 171.9, 170.8, 166.9, 163.6, 156.2, 155.7, 152.3, 140.6, 140.5, 139.9, 138.4, 136.5, 135.7, 129.8, 129.6, 129.4, 128.4, 127.8, 126.7, 126.6, 125.9, 124.1, 122.4, 121.4, 119.2, 118.9, 118.7, 118.3, 111.7, 110.0, 83.3, 78.6, 56.4, 54.6, 40.5, 39.2, 37.9, 31.7, 28.7, 28.6, 28.0; IR (ATR).νmax 2974, 1641, 1539, 1364, 1326, 1250, 1133, 1049, 876; HRMS (ESI): m/z calcd for $C_{51}H_{62}N_8O_9Na$ [M + Na]$^+$: 953.4537; found: 953.4533.

4.3.29. ^{1}H NMR and ^{13}C NMR spectra of Methyl N^6-(tert-butoxycarbonyl)-N^2-(3'-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)-3-(1H-indol-3-yl)propanamido)-[1,1'-biphenyl]-3-carbonyl)-L-lysinate (24c)

The title compound was prepared from compound **17** (100 mg, 0.15 mmol) and **18c** (39 mg, 0.15 mmol) following the general protocol 1 to afford **24c** as an off-white solid (106 mg, 80%); m.p.: 132.5–133.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.85 (s, 1H), 10.20 (s, 1H), 8.83 (d, *J* = 8.00 Hz, 1H), 8.16-8.12 (m, 2H), 7.90-7.88 (m, 2H), 7.76 (d, *J* = 8.00 Hz, 1H), 7.65-7.56 (m, 3H), 7.46-7.43 (m, 2H), 7.32 (d, *J* = 8.00 Hz, 1H), 7.21-7.14 (m, 6H), 7.06 (t, *J* = 4.00 Hz, 1H), 6.99-6.93 (m, 2H), 6.78 (t, *J* = 4.00 Hz, 1H), 4.76 (q, *J* = 8.00 Hz, 1H), 4.44 (q, *J* = 8.00 Hz, 1H), 4.22-4.16 (m, 1H), 3.66 (s, 3H), 3.27-3.21 (m, 1H), 3.15-3.09 (m, 1H), 2.95-2.94 (m, 3H), 2.75-2.69 (m, 1H), 1.83-1.80 (m, 2H), 1.40-1.37 (m, 2H), 1.34 (s, 9H), 1.29 (s, 9H), 1.18-1.14 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.2, 171.9, 170.8, 166.9, 156.0, 155.7, 140.6, 140.5, 139.8, 138.4, 136.5, 134.8, 130.0, 129.8, 129.6, 129.5, 128.4, 127.8, 127.2, 126.6, 126.1, 124.1, 122.4, 121.4, 119.2, 118.9, 118.7, 118.3, 118.7, 118.3, 111.7, 110.0, 78.7, 77.7, 56.4, 54.6, 53.2, 37.9, 31.4, 30.7, 29.6, 28.7, 28.6; IR (ATR).νmax 3305, 3047, 2925, 1652, 1509, 1453, 1363, 1245, 1161, 1012, 855; HRMS (ESI): m/z calcd for $C_{50}H_{60}N_6O_9Na$[M + Na]$^+$: 911.4319; found: 911.4316.

4.3.30. ^{1}H NMR and ^{13}C NMR spectra of Methyl N^2-(3'-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)-3-(1H-indol-3-yl)propanamido)-[1,1'-biphenyl]-3-carbonyl)-N^w-((4-methoxy-2,3,6-trimethylphenyl)sulfonyl)-L-argininate (24d)

The title compound was prepared from compound **17** (100 mg, 0.15 mmol) and **18d** (60 mg, 0.15 mmol) following the general protocol 1 to afford **24d** as an off-white solid (115 mg, 75%); m.p.: 179.5–179.7 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.85 (s, 1H), 10.20 (s, 1H), 8.87 (d, *J* = 4.00 Hz, 1H), 8.15-8.12 (m, 2H), 7.89-7.88 (m, 2H), 7.77 (d, *J* = 4.00 Hz, 1H), 7.64-7.57 (m, 3H), 7.45-7.41 (m, 2H), 7.33 (d, *J* = 4.00 Hz, 1H), 7.21-7.13 (m, 6H), 7.07-7.04 (m, 1H), 6.98-6.93 (m, 2H), 6.66 (br s, 2H), 4.75 (q, *J* = 4.00 Hz, 1H), 4.46-4.42 (m, 1H), 4.21-4.17 (m, 1H), 3.77 (s, 3H), 3.65 (s, 3H), 3.25-3.22 (m, 1H), 3.14-3.06 (m, 3H), 2.94-2.91 (m, 1H), 2.74-2.70 (m, 1H), 2.59 (s, 3H), 2.02 (s, 3H), 1.84-1.59 (m, 2H), 1.52-1.47 (m, 2H), 1.29 (s, 9H); ^{13}C NMR (150 MHz,

DMSO-d_6): δ 172.9, 171.9, 166.9, 157.9, 155.7, 140.7, 140.5, 139.8, 138.4, 138.1, 136.5, 136.0, 134.8, 130.1, 129.8, 129.6, 129.5, 128.4, 127.8, 127.2, 126.6, 126.1, 124.1, 122.5, 121.4, 119.3, 118.9, 118.7, 118.3, 112.1, 111.7, 110.0, 79.6, 78.7, 56.4, 55.9, 52.9, 52.4, 40.5, 37.9, 28.6, 28.4, 24.1, 18.5, 12.1; IR (ATR).vmax 2931, 1649, 1541, 1454, 1363, 1304, 1227, 1158, 1114, 1013; HRMS (ESI): m/z calcd for $C_{55}H_{64}N_8O_{10}S$ [M + H]$^+$: 1029.4466; found: 1029.4540.

4.3.31. ^{1}H NMR and ^{13}C NMR spectra of tert-butyl ((S)-1-(((S)-3-(1H-indol-3-yl)-1-oxo-1-((3'-(((S)-2,2,18,18-tetramethyl-4,9,16-trioxo-3,17-dioxa-5,8,15-triazanonadecan-10-yl)carbamoyl)-[1,1'-biphenyl]-3-yl)amino)propan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (24e)

The title compound was prepared from compound **17** (100 mg, 0.15 mmol) and **18e** (58 mg, 0.15 mmol) following the general protocol 1 to afford **24e** as an off-white solid (106 mg, 70%); m.p.: 131.9–132.4 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.84 (s, 1H), 10.19 (s, 1H), 8.53 (d, *J* = 6.00 Hz, 1H), 8.13 (br s, 2H), 8.00 (t, *J* = 6.00 Hz, 1H), 7.91-7.88 (m, 2H), 7.73 (d, *J* = 12.00 Hz, 1H), 7.63-7.62 (m, 2H), 7.56 (t, *J* = 6.00 Hz, 1H), 7.44-7.42 (m, 2H), 7.31 (d, *J* = 12.00 Hz, 1H), 7.21-7.13 (m, 6H), 7.06-7.03 (m, 1H), 6.97-6.92 (m, 2H), 6.76-6.73 (m, 2H), 4.75 (q, *J* = 6.00 Hz, 1H), 4.39-4.37 (m, 1H), 4.20-4.16 (m, 1H), 3.25-3.05 (m, 4H), 3.00-2.86 (m, 5H), 2.73-2.69 (m, 1H), 1.75-1.67 (m, 2H), 1.34 (s, 9H), 1.33 (s, 9H), 1.28 (s, 9H), 1.16-1.13 (m, 2H), 0.96-0.94 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.4, 171.9, 170.8, 166.7, 156.1, 156.0, 155.7, 140.6, 140.5, 139.8, 138.4, 136.5, 135.3, 129.8, 129.6, 129.3, 128.4, 127.8, 127.2, 126.6, 126.2, 124.1, 122.5, 121.4, 118.9, 118.3, 111.7, 110.0, 78.7, 78.1, 77.7, 56.4, 54.6, 54.0, 56.4, 54.6, 54.0, 40.5, 39.3, 37.9, 31.8, 29.7, 28.7, 28.6, 28.5, 23.6; IR (ATR).vmax 3292, 2973, 1650, 1510, 1453, 1247, 1163, 1045, 856, 741; HRMS (ESI): m/z calcd for $C_{56}H_{72}N_8O_{10}Na$[M + Na]$^+$: 1039.5269; found: 1039.5261.

4.3.32. ^{1}H NMR and ^{13}C NMR spectra of tert-butyl ((S)-1-(((S)-1-((3'-(((S)-6-amino-1-((2-(2,3-dibocguanidino)ethyl)amino)-1-oxohexan-2-yl)carbamoyl)-[1,1'-biphenyl]-3-yl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (24f)

The title compound was prepared from compound **17** (100 mg, 0.15 mmol) and **18f** (80 mg, 0.15 mmol) following the general protocol 1 to afford **24f** as an off-white solid (128 mg, 74%); m.p.: 192.5–194.0 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.50 (s, 1H), 10.80 (s, 1H), 10.19 (s, 1H), 8.52 (d, *J* = 12.00 Hz, 1H), 8.35 (t, *J* = 6.00 Hz, 1H), 8.13 (br s, 3H), 7.90-7.74 (m, 2H), 7.73 (d, *J* = 12.00 Hz, 1H), 7.64-7.62 (m, 2H), 7.55 (t, *J* = 12.00 Hz, 1H), 7.44-7.41 (m, 2H), 7.31 (d, *J* = 12.00 Hz, 1H), 7.20-7.12 (m, 6H), 7.07-7.03 (m, 1H), 6.98-6.92 (m, 2H), 6.73 (t, *J* = 6.00 Hz, 1H), 4.75 (q, *J* = 6.00 Hz, 1H), 4.40 (q, *J* = 12.00 Hz, 1H), 4.20-4.15 (m, 1H), 3.34-3.36 (m, 2H), 3.30-3.10 (m, 4H), 2.94-2.86 (m, 3H), 2.74-2.71 (m, 1H), 1.75-1.68 (m, 2H), 1.43 (s, 9H), 1.38 (s, 9H), 1.37-1.34 (m, 2H), 1.28 (s, 9H), 1.32 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.6, 172.0, 170.8, 166.5, 163.5, 156.1, 155.7, 152.3, 151.8, 140.6, 140.5, 139.8, 136.5, 135.3, 129.8, 129.6, 129.3, 128.4, 127.8, 127.2, 126.6, 126.2, 124.1, 121.3, 119.3, 118.9, 118.7,118.4, 111.7, 110.0, 83.3, 79.6, 78.6, 77.7, 56.4, 54.6, 54.1, 38.3, 31.9, 29.7, 28.7, 28.5, 28.4, 28.0, 23.6; IR (ATR).vmax 3284, 3066, 2973, 1635, 1540, 1363, 1322, 1247, 1131, 1022, 856, 741; HRMS (ESI): m/z calcd for $C_{62}H_{82}N_{10}O_{12}Na$[M + Na]$^+$: 1181.6011; found: 1181.6010.

4.3.33. ^{1}H NMR and ^{13}C NMR spectra of tert-butyl ((S)-1-(((S)-1-((3'-(((S)-1-((2-(2,3-dibocguanidino)ethyl)amino)-5-(3-((4-methoxy-2,3,6-trimethylphenyl)sulfonyl)guanidino)-1-oxopentan-2-yl)carbamoyl)-[1,1'-biphenyl]-3-yl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (24g)

The title compound was prepared from compound **17** (50 mg, 0.075 mmol) and **18g** (50 mg, 0.15 mmol) following the general protocol 1 to afford **24g** as a gummy solid (50 mg, 49%).^{1}H NMR (600 MHz, DMSO-d_6): δ 11.40 (s, 1H), 10.84 (s, 1H), 10.19 (s, 1H), 8.56 (d, *J* = 12.00 Hz, 1H), 8.33 (t, *J* = 6.00 Hz, 1H), 8.18-8.14 (m, 3H), 7.91-7.89 (m, 2H), 7.75-7.89 (m, 2H), 7.74 (d, *J* = 12.00 Hz, 1H), 7.69-7.62 (m, 2H), 7.56 (t, *J* = 12.00 Hz, 1H), 7.43-7.40 (m, 2H), 7.32 (d, *J* = 12.00 Hz, 1H), 7.21-7.09 (m, 6H), 7.06 (t, *J* = 6.00 Hz, 1H), 6.95 (dd, *J* = 12.00, 24.00 Hz, 2H), 6.78-6.66 (m, 2H), 6.56-6.36 (m, 2H), 4.75 (q, *J* = 6.00 Hz, 1H), 4.43 (q, *J* = 12.00 Hz, 1H), 4.21-4.15 (m, 1H), 3.75 (s, 3H), 3.27-3.05 (m, 8H), 2.94-2.90 (m, 1H), 2.74-2.68 (m, 1H), 2.57 (s, 3H), 2.49 (s, 3H), 2.00 (s, 3H), 1.80-1.53 (m, 4H), 1.41 (s, 9H), 1.38 (s, 9H), 1.28 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.4, 170.8, 166.6, 163.5, 157.9, 156.6, 156.0, 152.3, 140.5, 140.53, 139.8, 138.4, 138.1, 136.5, 136.0, 135.1, 129.8, 129.6, 129.3, 128.4, 127.7, 126.6, 126.2, 124.1, 121.4, 118.9, 112.1, 111.7, 110.0, 83.3, 79.6, 78.7, 56.4, 55.9, 54.6, 53.6, 40.5, 38.3, 29.5, 28.5, 28.4, 28.0, 24.0, 18.4, 12; IR (ATR).vmax 3310, 2305, 2095, 1868, 1609, 1543, 1363, 1304, 1246, 1112, 740, 683; HRMS (ESI): m/z calcd for $C_{67}H_{86}N_{12}O_{13}S$ [M + H]$^+$: 1299.6158; found: 1299.6229.

4.3.34. ^{1}H NMR and ^{13}C NMR spectra of 3'-((S)-2-((S)-2-amino-3-phenylpropanamido)-3-(1H-indol-3-yl)propanamido)-N-(2-aminoethyl)-[1,1'-biphenyl]-3-carboxamide (25a)

The title compound was prepared from compound **24a** (80 mg, 0.101 mmol) following the general protocol 4 to afford **25a** as a white solid (38 mg, 65%); m.p.: 206.9–207.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.86 (d, *J* = 4.00 Hz, 1H), 10.40 (s, 1H), 8.94 (t, *J* = 4.00 Hz, 1H), 8.60 (d, *J* = 8.00 Hz, 1H), 8.27 (br s, 3H), 8.16 (br s, 1H), 7.84-7.83 (m, 2H), 7.76-7.67 (m, 5H), 7.55 (t, *J* = 16.00 Hz, 1H), 7.32 (d, *J* = 8.00 Hz, 1H), 7.20-7.05 (m, 6H), 7.02-6.98 (m, 3H), 4.77-4.76 (m, 2H), 3.70 (q, *J* = 4.00 Hz, 1H), 3.54 (q, *J* = 8.00 Hz, 1H), 3.20-3.17 (m, 1H), 3.07-2.99 (m, 3H), 2.89-2.83 (m, 1H), 2.63-2.57 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.3, 170.9, 167.2, 164.7, 140.1, 139.1, 137.6, 136.6, 135.2, 134.8, 129.8, 129.5, 129.4, 128.5, 127.7, 127.5, 126.8, 125.5, 124.4, 121.4, 120.3, 119.1, 118.7, 111.8, 109.9, 55.3, 54.6, 39.1, 37.9, 28.6; IR (ATR).vmax 2919, 2848, 2250, 1654, 1537, 1397, 1299, 1173, 1067, 743; HRMS (ESI): m/z calcd for $C_{35}H_{36}N_6O_3$ [M + H]$^+$: 589.2849; found: 589.2911.

4.3.35 ^{1}H NMR and ^{13}C NMR spectra of 3'-((S)-2-((S)-2-amino-3-phenylpropanamido)-3-(1H-indol-3-yl)propanamido)-N-(2-guanidinoethyl)-[1,1'-biphenyl]-3-carboxamide (25b)

The title compound was prepared from compound **24b** (80 mg, 0.08 mmol) following the general protocol 4 to afford **25b** as a white gummy solid (27 mg, 49%).^{1}H NMR (600 MHz, DMSO-d_6): δ 10.89 (br s, 1H), 10.35 (br s, 1H), 8.94 (d, *J* = 4.00 Hz, 1H), 8.75 (t, *J* = 4.00 Hz, 1H), 8.11-7.93 (m, 4H), 7.87-7.85 (m, 1H), 7.77-7.75 (m, 1H), 7.68 (d, *J* = 8.00 Hz, 1H), 7.63-7.58 (m, 4H), 7.46-7.42 (m, 4H), 7.26-7.15 (m, 6H), 7.08-6.97 (m, 4H), 4.82 (q, *J* = 4.00 Hz, 1H), 4.07 (t, *J* = 4.00 Hz, 1H), 3.43 (q, *J* = 4.00 Hz, 2H), 3.28-3.24 (m, 1H), 3.14-3.08 (m, 2H), 2.97-2.93 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 169.8, 166.6, 158.1, 157.9, 156.9, 140.2, 140.0, 139.3, 136.0, 134.8, 129.5, 129.4,129.41, 129.1, 128.4, 127.2, 127.0, 126.3, 125.5, 123.7, 122.1, 121.0, 118.9, 118.4, 118.3, 117.8, 111.4, 109.2, 87.8, 54.5, 53.3, 40.3, 38.6, 37.1, 28.0; IR (ATR).νmax 3276, 3189, 3064, 1651, 1538, 1433, 1318, 1201, 1150, 836, 798, 742 HRMS (ESI): m/z calcd for $C_{36}H_{38}N_8O_3$ [M + H]$^+$: 631.3067; found: 631.3141.

4.3.36.^{1}H NMR and ^{13}C NMR spectra of Methyl (3'-((S)-2-((S)-2-amino-3-phenylpropanamido)-3-(1H-indol-3-yl)propanamido)-[1,1'-biphenyl]-3-carbonyl)-L-lysinate (25c)

The title compound was prepared from compound **24c** (90 mg, 0.112 mmol) following the general protocol 4 to afford **25c** as a white gummy solid (46 mg, 60%).^{1}H NMR (600 MHz, DMSO-d_6): δ 10.89 (br s, 1H), 10.35 (s, 1H), 8.94 (d, *J* = 4.00 Hz, 1H), 8.87 (d, *J* = 8.00 Hz, 1H), 8.13-7.90 (m, 5H), 7.79-7.68 (m, 5H), 7.63-7.59 (m, 2H), 7.46-7.44 (m, 2H), 7.33 (d, *J* = 4.00 Hz, 1H), 7.26-7.15 (m, 6H), 7.07-7.05 (m, 1H), 6.99-6.97 (m, 1H), 4.81 (q, *J* = 4.00 Hz, 1H), 4.49-4.45 (m, 1H), 4.07 (t, *J* = 4.00 Hz, 1H), 3.66 (s, 3H), 3.28-3.24 (m, 1H), 3.14-3.09 (m, 2H), 2.99-2.94 (m, 1H), 2.79-2.77 (m, 2H), 1.85-1.80 (m, 2H), 1.60-1.53 (m, 2H), 1.47-1.38 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 173.1, 170.3, 168.6, 167.0, 158.5, 158.3, 140.6, 140.5, 139.9, 136.6, 135.2, 134.8, 130.0, 130.0, 129.5, 128.9, 127.7, 127.5, 127.1, 126.2, 124.2, 122.6, 121.5, 119.4, 118.9, 118.8, 118.3, 111.8, 109.7, 55.0, 53.7, 52.9, 52.4, 39.1, 37.6, 30.4, 28.5, 27.0, 23.1; IR (ATR).νmax 3276, 3195, 3081, 2109, 1540, 1433, 1318, 1198, 798, 743; HRMS (ESI): m/z calcd for $C_{40}H_{44}N_6O_5$ [M + H]$^+$: 689.3373; found: 689.3448.

4.3.37. ^{1}H NMR and ^{13}C NMR spectra of Methyl (3'-((S)-2-((S)-2-amino-3-phenylpropanamido)-3-(1H-indol-3-yl)propanamido)-[1,1'-biphenyl]-3-carbonyl)-L-argininate (25d)

The title compound was prepared from compound **24d** (90 mg, 0.087 mmol) following the general protocol 4 to afford **25d** as a brown gummy solid (25 mg, 40%).^{1}H NMR (600 MHz, DMSO-d_6): δ 10.89 (s, 1H), 10.35 (s, 1H), 8.94 (dd, *J* = 4.00, 12.00 Hz, 2H), 8.13-8.12 (m, 4H), 7.93-7.90 (m, 2H), 7.79-7.78 (m, 1H), 7.69 (d, *J* = 8.00 Hz, 1H), 7.64-7.60 (m, 3H), 7.48-7.33 (m, 4H), 7.27-7.16 (m, 7H), 7.08-7.06 (m, 1H), 7.00-6.98 (m, 1H), 4.82 (q, *J* = -4.00 Hz, 1H), 4.52-4.49 (m, 1H), 4.10 (br s, 1H), 3.67 (s, 3H), 3.29-3.25 (m, 1H), 3.15-3.09 (m, 4H), 2.99-2.95 (m, 1H), 1.91-1.54 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.9, 170.3, 168.4, 166.9, 158.2, 157.2, 140.6, 140.4, 139.8, 136.6, 135.1, 134.8, 130.1, 130.0, 129.9, 129.5, 128.9, 129.6, 128.9, 127.7, 126.1, 124.2, 122.6, 121.5, 119.4, 118.9, 118.9, 118.3, 111.8, 109.7, 55.0, 53.7, 52.8, 52.4, 40.7, 40.5, 37.5, 28.5, 28.1, 25.8; IR (ATR).νmax 3272, 3191, 3063, 2103, 1529, 1434, 1340, 1029, 835, 798, 744, 720; HRMS (ESI): m/z calcd for $C_{40}H_{44}N_8O_5$ [M + H]$^+$: 717.3435; found: 717.3501.

4.3.38. ^{1}H NMR and ^{13}C NMR spectra of N-((S)-6-amino-1-((2-aminoethyl)amino)-1-oxohexan-2-yl)-3'-((S)-2-((S)-2-amino-3-phenylpropanamido)-3-(1H-indol-3-yl)propanamido)-[1,1'-biphenyl]-3-carboxamide (25e)

The title compound was prepared from compound **24e** (90 mg, 0.088 mmol) following the general protocol 4 to afford **25e** as a gummy solid (45 mg, 49%).^{1}H NMR (600 MHz, DMSO-d_6): δ 10.91 (s, 1H), 10.36 (s, 1H), 8.97 (d, *J* = 6.00 Hz, 1H), 8.68 (d, *J* = 6.00 Hz, 1H), 8.24 (t, *J* = 6.00 Hz, 1H), 8.16-7.67 (m, 12H), 7.68 (d, *J* = 6.00 Hz, 1H), 7.62-7.58 (m, 2H), 7.46-7.45 (m, 2H), 7.33 (d, *J* = 12.00 Hz, 1H), 7.26-7.15 (m, 6H), 7.06 (t, *J* = 6.00 Hz, 1H), 6.98 (t, *J* = 6.00 Hz, 1H), 4.81 (q, *J* = 6.00 Hz, 1H), 4.44-4.41 (m, 1H), 4.09 (t, *J* = 6.00 Hz, 1H), 3.31-3.30 (m, 2H), 3.27 (dd, *J* = 6.00, 12.00 Hz, 1H), 3.15-3.09 (m, 2H), 2.97 (dd, *J* = 12.00, 15.00 Hz, 1H), 2.87 (t, *J* = 6.00 Hz, 2H), 2.77 (br s, 2H), 1.84-1.81 (m, 1H), 1.76-1.72 (m, 1H), 1.59-1.52 (m, 2H), 1.42-1.32 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.8, 170.3, 168.5, 166.9, 158.5, 158.3, 140.5, 139.8, 136.6, 135.1, 135.12, 130.0, 129.9, 129.4, 128.8, 127.7, 127.5, 127.2, 126.2, 124.2, 122.6, 121.5, 119.3, 118.9, 118.7, 118.3, 118.8, 109.7, 55.03, 53.8, 53.7, 40.5, 39.5, 39.2, 39.9, 37.4, 36.9, 31.3, 28.4, 27.1, 23.2; IR (ATR).νmax 3274, 3056, 2926, 2119, 1661, 1526, 1431, 1200, 1121, 836, 797, 743, 721; HRMS (ESI): m/z calcd for $C_{41}H_{48}N_8O_4$ [M + H]$^+$: 717.3435; found: 717.3501.

4.3.39. ^{1}H NMR and ^{13}C NMR spectra of N-((S)-6-amino-1-((2-guanidinoethyl)amino)-1-oxohexan-2-yl)-3'-((S)-2-((S)-2-amino-3-phenylpropanamido)-3-(1H-indol-3-yl)propanamido)-[1,1'-biphenyl]-3-carboxamide (25f)

The title compound was prepared from compound **24f** (100 mg, 0.086 mmol) following the general protocol 4 to afford **25f** as a gummy solid (41 mg, 40%).^{1}H NMR (600 MHz, DMSO-d_6): δ 10.90 (d, *J* = 6.00 Hz, 1H), 10.35 (s, 1H), 8.93 (br s, 1H), 8.65 (d, *J* = 6.00 Hz, 1H), 8.20-8.13 (m, 4H), 7.96-7.91 (m, 2H), 7.84-7.53 (m, 7H), 7.51-7.43 (m, 2H), 7.37-7.10 (m, 8H), 7.10-7.04 (m, 1H), 7.00-6.97 (m, 1H), 6.54 (br s, 1H), 4.82 (q, *J* = 6.00 Hz, 1H), 4.47-4.44 (m, 1H), 4.06 (br s, 1H), 3.46-3.39 (m, 2H), 3.27-2.77 (m, 8H), 1.91-1.24 (m, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.9, 170.3, 166.7, 158.5, 158.3, 157.4, 140.5, 139.7, 136.5, 135.1, 130.0, 129.9, 129.4, 128.9, 127.7, 127.5, 126.3, 124.2, 123.4, 122.63, 122.6, 121.5, 119.4, 118.9, 118.7, 118.4, 118.3, 116.7, 111.8, 109.7, 54.9, 54.0, 53.7, 40.8, 40.5, 39.2, 38.4, 31.4, 28.5, 27.2, 23.2; IR (ATR).νmax 3265, 3065, 1655, 1537, 1428, 1170, 1128, 1001,787; HRMS (ESI): m/z calcd for $C_{42}H_{50}N_{10}O_4$ [M + H]$^+$: 759.4017; found: 759.4084.

4.3.40.^{1}H NMR and ^{13}C NMR spectra of 3'-((S)-2-((S)-2-amino-3-phenylpropanamido)-3-(1H-indol-3-yl)propanamido)-N-((S)-5-guanidino-1-((2-guanidinoethyl)amino)-1-oxopentan-2-yl)-[1,1'-biphenyl]-3-carboxamide (25g)

The title compound was prepared from compound **24g** (48 mg, 0.036 mmol) following the general protocol 4 to afford **25g** as a gummy solid (20 mg, 40%).^{1}H NMR (600 MHz, DMSO-d_6): δ 10.84 (s, 1H), 10.27 (s, 1H), 8.84 (s, 1H), 8.62 (d, *J* = 6.00 Hz, 1H), 8.16-8.11 (m, 2H), 7.94 (d, *J* = 6.00 Hz, 1H), 7.76 (d, *J* = 12.00 Hz, 1H), 7.67 (d, *J* = 12.00 Hz, 1H), 7.62-7.56 (m, 3H), 7.46 (d, *J* = 6.00 Hz, 2H), 7.34 (d, *J* = 12.00 Hz, 1H), 7.28-7.14 (m, 8H), 7.08-7.06 (m, 2H), 7.01-6.97 (m, 1H), 4.82 (q, *J* = 6.00 Hz, 1H), 4.51-4.46 (m, 1H), 4.07 (br s, 1H), 3.25-3.19 (m, 6H), 3.15-3.12 (m, 3H), 2.96-2.94 (m, 1H), 1.89-1.84 (m, 1H), 1.76-1.72 (m, 1H), 1.60-1.50 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.6, 170.3, 166.8, 158.6, 158.4, 158.2, 157.5, 157.2, 140.6, 140.5, 139.8, 136.6, 135.1, 130.0, 130.0, 129.8, 129.4, 128.8, 128.8, 127.7, 127.4, 127.2, 126.2, 124.1, 122.6, 121.4, 119.4, 118.9, 118.7, 118.4, 116.8, 111.8, 109.8, 55.0, 53.5, 40.98, 40.9, 40.6, 38.4, 29.1, 28.4, 25.8; IR (ATR).vmax 3279, 3186, 3086, 1646, 1529, 1431, 1175, 1125, 798, 743; HRMS (ESI): m/z calcd for $C_{42}H_{50}N_{12}O_4$ [M + H]$^+$: 787.4078; found: 787.4152.

4.3.41. ^{1}H NMR and ^{13}C NMR spectra of 3'-nitro-[1,1'-biphenyl]-3-carboxylic acid (26)

To the solution of **9** (1.2 g, 3.88 mmol) in THF (10.0 mL) and MeOH (10.0 mL), was added a 1N $NaOH_{(aq)}$ (7.77 mL, 7.77 mmol) and stirred at room temperature for 16 h. Ethyl acetate was added and the layers were separated. The aqueous layer was then acidified with 1N HCl and then extracted with CH_2Cl_2 (2 X 100 mL) and then the solvent was removed under reduced pressure to yield **26** (0.84 g, 90%) as a pale-yellow solid; m.p.: 209.4–210.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.19 (br s, 1H), 8.44 (s, 1H), 8.25 (br s, 2H), 8.18 (d, *J* = 7.60 Hz, 1H), 8.02 (t, *J* = 8.00 Hz, 1H), 7.78 (t, *J* = 8.00 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.5, 148.9, 141.3, 138.6, 133.9, 132.2, 131.9, 131.1, 130.1, 129.8, 128.1, 123.1, 121.8; IR (ATR).vmax 2965, 2840, 2541, 2099, 1681, 1590, 1514, 1421, 1189, 1095, 928, 853; HRMS (ESI): m/z calcd for $C_{13}H_9NO_4Na$ [M + Na]$^+$: 266.0429; found: 266.0424.

4.3.42. ^{1}H NMR and ^{13}C NMR spectra of N-(2-(2,3-dibocguanidino)ethyl)-3'-nitro-[1,1'-biphenyl]-3-carboxamide (27)

The title compound **27** was prepared from compound **18b** (0.621 g, 2.00 mmol) and **33** (0.5 g, 2.00 mmol) according to the protocol 2. Off-white solid (0.47 g, 45%); m.p.: 117.6–118.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.51 (br s, 1H), 8.83-8.82 (m, 1H), 8.26-8.16 (m, 3H), 8.00-7.97 (m, 1H), 7.84-7.82 (m, 1H), 7.66-7.63 (m, 1H), 7.56 (t, *J* = 8.00 Hz, 1H), 3.80-3.77 (m, 2H), 3.71-3.67 (m, 2H), 1.51 (s, 9H), 1.49 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.1, 162.7, 157.9, 153.0, 148.8, 142.0, 139.1, 135.4, 133.2, 129.9, 129.8, 129.1, 126.9, 126.3, 122.4, 122.0, 83.8, 79.9, 79.7, 42.5, 39.7, 28.3, 28.2, 28.0; IR (ATR).vmax 3196, 2985, 2930, 1784, 1719, 1532, 1473, 1268, 1139, 869; HRMS (ESI): m/z calcd for $C_{41}H_{51}N_5O_8$ [M + Na]$^+$: 550.2278; found: 550.2273.

4.3.43. ^{1}H NMR and ^{13}C NMR spectra of 3'-amino-N-(2-guanidinoethyl)-[1,1'-biphenyl]-3-carboxamide (28)

The title compound **28** was prepared from **27** (0.25 g, 0.47 mmol) according to the protocol 4. Pale brown solid (139 mg, 90% yield); m.p.: 183.8–184.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.93 (t, *J* = 5.20 Hz, 1H), 8.54 (t, *J* = 2.00 Hz, 1H), 8.28-8.25 (m, 3H), 7.98 (t, *J* = 7.60 Hz, 2H), 7.83-7.75 (m, 2H), 7.65 (t, *J* = 8.00 Hz, 1H), 7.26 (br s, 3H), 3.48-3.43 (m, 2H), 3.39-3.35 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.8, 157.5, 148.9, 141.5, 138.3, 135.5, 133.9, 131.1, 130.3, 129.8, 128.0, 126.1, 123.0, 121.7, 40.7, 39.1; IR (ATR).vmax 3280, 3155, 2341, 2112, 1638, 1522, 1457, 1344, 1299, 1192, 1114, 996, 864, 798, 675, 730; HRMS (ESI): m/z calcd for $C_{16}H_{17}N_5O_3$ [M + H]$^+$: 327.1331; found: 328.1401.

4.3.44. ^{1}H NMR and ^{13}C NMR spectra of 3'-amino-N-(2-guanidinoethyl)-[1,1'-biphenyl]-3-carboxamide (29)

To a stirred solution of **28** (0.1 g, 0.305 mmol) in anhydrous THF (100 mL) under nitrogen atmosphere 10% palladium on activated charcoal (0.15 g) was added. The reaction was evacuated and placed under a hydrogen atmosphere and stirred overnight. The reaction mixture was filtered through celite, and the solvent was removed under reduced pressure to yield the desired product **29** as a brown solid (39 mg, 40% yield); m.p.: 233.0–233.6°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.76 (t, *J* = 5.20 Hz, 1H), 8.07 (br s, 1H), 7.83 (d, *J* = 7.60 Hz, 1H), 7.72 (d, *J* = 8.00 Hz, 1H), 7.66 (t, *J* = 5.60 Hz, 1H), 7.54 (t, *J* = 8.00 Hz, 1H), 7.14 (t, *J* = 7.60 Hz, 1H), 6.90-6.84 (m, 2H), 6.62-6.59 (m, 1H), 5.28 (br s, 2H), 3.44-3.37 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.2, 157.5, 149.5, 141.6, 140.6, 135.0, 129.9, 129.7, 129.3, 126.4, 125.7, 115.0, 114.0, 112.7, 40.8, 39.0; IR (ATR).vmax 3262, 3144, 2849, 2587, 2342, 1621, 1541, 1471, 1309, 1182, 1129, 807; HRMS (ESI): m/z calcd for $C_{16}H_{19}N_5O$ [M + H]$^+$: 298.1590; found: 298.1660.

4.3.45. ^{1}H NMR and ^{13}C NMR spectra of Methyl L-tryptophanate (31)

The title compound **31** was prepared by adding thionyl chloride (0.79 mL, 9.80 mmol) dropwise to *L*-Tryptophan (1.0 g, 4.90 mmol) in methanol (20.0 mL) at 0° C and then stirred at room temperature for 16 h. The solvents were removed and reduced pressure and the residue was diluted with ethylacetate (100.0 mL) and washed with saturated $NaHCO_3$(40 mL), saturated brine (40 mL), then dried (Na_2SO_4) and concentrated under reduced pressure to afford a white solid (1.00 g, 94%); m.p.: 89.4–89.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.87 (s, 1H), 7.48 (d, *J* = 8.00 Hz, 1H), 7.33 (d, *J* = 8.00 Hz, 1H), 7.12 (br s, 1H), 7.05 (t, *J* = 8.00 Hz, 1H), 6.97 (t, *J* = 8.00 Hz, 1H), 3.63 (t, *J* = 8.00 Hz, 1H), 3.55 (s, 1H), 3.05-2.91 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.1, 136.5, 127.8, 124.1, 121.3, 118.8, 118.7, 111.8, 110.3, 55.6, 51.8, 40.6, 31.1; IR (ATR).vmax 3356, 3292, 3096, 3051, 2916, 2871, 2338, 2095, 1727, 1567, 1448, 1351, 1290, 1222, 1104, 1012, 945, 890, 805, 737; HRMS (ESI): m/z calcd for $C_{12}H_{14}N_2O_2$ [M + H]$^+$: 219.1055; found: 219.1125.

4.3.46. ^{1}H NMR and ^{13}C NMR spectra of Methyl (tert-butoxycarbonyl)-L-phenylalanyl-L-tryptophanate (32)

The title compound was prepared *via* protocol 1, using *N*-(*tert*-Butoxycarbonyl)-*L*-phenylalanine (1.21 g, 4.6 mmol) and **31** (1.00 g, 4.6 mmol) to afford the coupled product **32** as an off-white solid (1.40 g, 65%); m.p.: 135.3–135.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.89 (s, 1H), 8.29 (d, *J* = 8.00 Hz, 1H), 7.49 (d, *J* = 8.00 Hz, 1H), 7.34 (d, *J* = 8.00 Hz, 1H), 7.23-7.18 (m, 6H), 7.07 (t, *J* = 8.00 Hz, 1H), 6.99 (t, *J* = 8.00 Hz, 1H), 6.87 (d, *J* = 12.00 Hz, 1H), 4.55 (q, *J* = 8.00 Hz, 1H), 4.23-4.18 (m, 1H), 3.55 (s, 3H), 3.20-3.07 (m, 2H), 2.95-2.90 (m, 1H), 2.72-2.66 (m, 1H), 1.28 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.6, 172.2, 155.6, 138.5, 136.5, 129.6, 128.4, 127.5, 126.6, 124.2, 121.4, 118.9, 118.4, 111.8, 109.6, 78.5, 56.0, 53.5, 52.2, 37.8, 28.5, 28.2, 27.5; IR (ATR).νmax 3295, 3001, 2952, 1694, 1648, 1525, 1433, 1288, 1164, 1112, 922, 846; HRMS (ESI): m/z calcd for $C_{26}H_{31}N_3O_5$ [M + H]$^+$: 465.23; found: 337.14340

4.3.47. ^{1}H NMR and ^{13}C NMR spectra of (tert-butoxycarbonyl)-L-phenylalanyl-L-tryptophan (33)

To a solution of **31** (1.4 g, 3.00 mmol) in THF (10.0 mL) and MeOH (10.0 mL), was added a 1N $NaOH_{(aq)}$ (6.0 mL, 6.00 mmol) and stirred at room temperature for 16 h. Ethyl acetate was added and the layers were separated. The aqueous layer was then acidified with 1N HCl and then extracted with CH_2Cl_2 (2 X 100 mL) and then the solvent was removed under reduced pressure to yield **33** (1.23 g, 91%) as an off-white solid; m.p.: 133.2–134.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.89 (s, 1H), 8.08 (d, *J* = 8.00 Hz, 1H), 7.54 (d, *J* = 8.00 Hz, 1H), 7.33 (d, *J* = 8.00 Hz, 1H), 7.24-7.17 (m, 6H), 7.08-7.04 (m, 1H), 7.00-6.96 (m, 1H), 6.87 (d, *J* = -8.00 Hz, 1H), 4.51 (q, *J* = 8.00 Hz, 1H), 4.21-4.15 (m, 1H), 3.25-3.17 (m, 1H), 3.11-3.06 (m, 1H), 2.96-2.92 (m, 1H), 2.71-2.65 (m, 1H), 1.28 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.6, 172.1, 155.6, 138.5, 136.5, 129.6, 128.4, 127.7, 126.5, 124.1, 121.3, 118.8, 118.6, 111.8, 110.3, 110.0, 78.5, 56.1, 53.3, 37.9, 31.4, 28.5, 27.5, 22.5, 14.4; IR (ATR).νmax 3310, 3046, 2926, 1650, 1509, 1436, 1364, 1247, 1158, 1011, 848; HRMS (ESI): m/z calcd for $C_{25}H_{29}N_3O_5Na$[M + H]$^+$: 474.2005; found: 474.1998.

4.3.48. ^{1}H NMR and ^{13}C NMR spectra of tert-butyl ((S)-1-(((S)-1-((2-(2,3-dibocguanidino)ethyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (34)

The title compound **34** was prepared from compound **18b** (0.333 g, 1.1 mmol) and **33** (0.5 g, 1.1 mmol) according to the protocol 2. Off-white solid (0.36 g, 45%); m.p.: 180.6–181.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.47 (br s, 1H), 10.80 (d, *J* = 4.00 Hz, 1H), 8.37 (t, *J* = 4.00 Hz, 1H), 8.13 (t, *J* = 4.00 Hz, 1H), 7.91 (d, *J* = 8.00 Hz, 1H), 7.57 (d, *J* = 8.00 Hz, 1H), 7.30 (d, *J* = 8.00 Hz, 1H), 7.20-7.06 (m, 6H), 7.07-7.02 (m, 1H), 6.98-6.94 (m, 1H), 6.88 (d, *J* = 8.00 Hz, 1H), 4.48 (q, *J* = 4.00 Hz, 1H), 4.15-4.09 (m, 1H), 3.27-2.95 (m, 6H), 2.90-2.85 (m, 1H), 2.69-2.63 (m, 1H), 1.44 (s, 9H), 1.38 (s, 9H), 1.28 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 171.6, 163.5, 156.0, 155.6, 152.3, 138.5, 136.5, 129.6, 128.4, 127.8, 126.6, 124.0, 121.3, 118.9, 118.6, 111.7, 110.3,83.3, 78.7, 78.6, 56.4, 53.8, 38.5, 37.9, 37.8, 28.6, 28.5, 28.4, 28.1; IR (ATR).νmax 2974, 1637, 1523, 1363, 1248, 1132, 1021; HRMS (ESI): m/z calcd for $C_{38}H_{53}N_7O_8$ [M + H]$^+$: 736.3956; found: 736.4023.

4.3.49. ^{1}H NMR and ^{13}C NMR spectra of (S)-2-amino-N-((S)-1-((2-guanidinoethyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)-3-phenylpropanamide (35 TFA Salt)

The title compound was prepared via protocol 4, using **34** (0.150 g, 0.203 mmol) to yield the desired product 35 as a brown gummy solid (40 mg, 47%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.87 (br s, 1H), 8.81 (d, *J* = 8.00 Hz, 1H), 8.30 (t, *J* = 4.00 Hz, 1H), 8.06 (br s, 3H), 7.64-7.62 (m, 2H), 7.34-7.24 (m, 9H), 7.16 (d, *J* = 4.00 Hz, 1H), 7.08-7.04 (m, 1H), 7.00-6.96 (m, 1H), 4.58-4.52 (m, 1H), 4.03 (br s, 1H), 3.26-2.88 (m, 8H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 168.3, 157.5, 136.6, 135.2, 130.0, 129.0, 127.64, 127.6, 124.3, 121.4, 118.9, 118.7, 111.8, 110.0, 54.2, 53.6, 38.5, 37.5, 28.6; IR (ATR).νmax 3060, 2919, 1670, 1542, 1421, 1199, 1128, 1002, 825; HRMS (ESI): m/z calcd for $C_{23}H_{29}N_7O_2$ [M + H]$^+$: 436.2383; found: 436.2451.

4.4 Antibacterial activity

The antimicrobial activity of compounds was evaluated through the broth micro dilution assay using the procedure described by CLSI.[64] Briefly, *Staphylococcus aureus* [SA38] were grown to mid-log phase in Muller-Hinton broth (MHB) with shaking at 120 rpm and incubated at 37 °C for 18-24 h. Following incubation, bacteria were washed three times using PBS pH 7.4 with centrifugation at 3500 g for 10 min after each wash. After washing, bacteria were diluted with fresh MHB. The turbidity of the bacterial suspensions were adjusted so that OD_{600nm} was 0.1, which gave 1×10^8 cfu/ml, and then further diluted to achieve $1\text{-}2\times10^5$ cfu/ml as a final bacterial concentration. Each compound was added at concentrations ranging from 250-3.9 µM through serial two-fold dilution. Wells in microtitre plates were loaded with 100 µl of inoculum containing $1\text{-}2\times10^5$ cfu/ml bacteria. Wells containing only bacteria and without any compound were used as a negative controls (i.e. no inhibition of growth). Wells without bacteria but containing compound acted as another control. The microtitre plate was wrapped with paraffin to prevent evaporation and incubated with shaking at 120 rpm and 37 °C for 18-24 h. After incubation, spectrophotometric reading of the wells was taken at 600nm. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of the biphenyl compounds that causes 100% inhibition of microbial growth. The same procedure was followed for the two Gram-negative bacteria, *Pseudomonas aeruginosa* [PA01], and *Escherichia coli* [K12]. Each experiment was performed in triplicate and was repeated in three independent experiments. The MICs of the compounds were compared to published MIC [15, 52, 53] of MSI-78. The method for determining the MIC of MSI-78 [52, 53] , that recommended by the CLSI (formerly named the National Committee for Clinical Laboratory Standards (NCCLS)) was exactly the same as described herein for the current compounds.

4.5 Toxicity assay

Normal human lung fibroblasts MRC-5 were cultured in minimal essential medium (MEM, Invitrogen) supplemented with 10% foetal calf serum (FCS), 1% L-glutamine–penicillin–streptomycin, 2% sodium bicarbonate, 1% non-essential amino acids (NEAA) and 1% sodium pyruvate. The cell line was maintained at 37 °C in 5% CO_2 as an adherent monolayer and was passaged upon reaching confluence by standard cell culture techniques. MRC-5 cells were seeded at 2×10^4 cells per well in 96-well plates to ensure full confluence (quiescence). Cells were treated 24 h after seeding with 0.1 to 1000 µM of compounds. After 72 h drug incubation, the treated media was replaced with fresh media containing 10% Alamar blue and the cells were incubated for another 6 h. The metabolic activity was detected by spectrophotometric analysis by assessing the absorbance of Alamar blue as previously described by Pasquier *et al*. [65] Cell proliferation was determined and expressed as a percentage of untreated control cells. The determination of IC_{50} values was performed using GraphPad Prism 6 (San Diego, CA, USA).]. Each experiment was performed in triplicate and was repeated in three independent experiments.

4.6 Tethered bilayer lipid membranes

The ability of the biphenyl derivatives to interact with lipid bilayers was tested using tethered bilayer lipid membranes (tBLMs) in association with AC electrical impedance spectroscopy techniques [55, 56]. Lipid bilayers were anchored to a gold substrate which were created using the solvent exchange technique described previously [56, 57]. In short, tethered benzyl-disulfide (tetra-ethyleneglycol) n=2 C20-phytanyl tethers: benzyl-disulfide-tetra-ethyleneglycol-OH spacers were pre-prepared in the ratio of 1:10 and were coated onto a gold patterned polycarbonate slide containing a large gold return electrode (SDx Tethered Membranes Pty Ltd, Australia). Attached to this slide was a specialised cartridge chamber which allows for easy addition of reagent between tethering and reference electrodes. A 3 mM solution of a standard mobile lipid phase [70% zwitterionic C20 Diphytanyl-Glycero-Phosphatidylcholine lipid: 30% C20 Diphytanyl-diglyceride-OH ether] was added to the tethering chemistries. All lipids were dissolved in 100% ethanol. Lipids were left for 2 minutes to associate with the tethering chemistries before being washed with 3 x 200 ml phosphate buffered saline (PBS). The membranes formed from these lipids are designated as the "zwitterionic membranes". Alternatively, negatively charged membranes, that resemble those present in many bacterial species, were produced using the same mobile lipid phase which was instead supplemented with 30% palmitoyl-oleoyl-phosphatidylglycerol (POPG) (Avanti Lipids, USA). The presence of a lipid bilayer was verified using AC electrical impedance spectrometry. These measures were done using a TethaPod™ operated with TethaQuick™ software (SDx Tethered Membranes Pty Ltd, Australia). The signal employed was 50 mV peak-to-peak AC excitation at 0.1–2000 Hz with four steps per decade. Impedance data were fitted to a constant phase element (CPE) in series with a resistor/capacitor. The CPE represents the capacitance of the gold electrode interface, whist the resistor and capacitor represent the resistance and capacitance of the tethered membrane respectively. Fitting was done using a proprietary adaptation of a Lev Mar fitting routine as described previously [56]. All tethered membrane assays were performed in triplicate. Individual results shown are of typical responses to the antimicrobial compounds.

4.7 Cytoplasmic membrane permeability assay

The method was adopted from Wu et al. [66] with slight modification. Bacterial cytoplasmic membrane permeability was determined using membrane potential sensitive dye diSC3-5 (3,3'-dipropylthiadicarbocyanine iodide) which penetrates inside bacterial cells depending on the membrane potential gradient of the cytoplasmic membrane. *Staphylococcus aureus* [SA38] and *Escherichia coli* [K12] were grown in MHB to mid-log phase by incubating with shaking at 37 °C for 18-24 h. Following incubation bacteria were washed with 5 mM HEPES containing 20 mM glucose pH (7.2) and resuspended in the same buffer to an OD_{600} 0.05-0.06 which gave 1×10^7 CFU/ml. diSC3-5 was added at 4 µM to the bacterial suspension. The suspensions were incubated at room temperature for 1 h in the dark for maximum dye take-up by bacterial cell. Then, 100 mM KCl was added to balance the K+ outside and inside the bacterial cell to prevent further uptake or outflow of the dye. For Gram-negative bacteria, 0.5 mM EDTA was used to remove the stabilizing divalent cations from the lipopolysaccharide (LPS) layer which help in dye penetration without affecting bacterial growth. 100 µl of bacterial suspension was added in a 96-well microtiter plate with equal volume of antimicrobial compounds. Dimethyl sulfoxide (DMSO) was used as a positive control to achieve maximum fluorescence while bacterial suspension containing only dye and HEPES buffer set as blank to subtract the background. Fluorescence was measured with a luminescence spectrophotometer at 3 min intervals at an excitation wavelength of 622 nm and an emission wavelength of 670 nm. The experiment was performed in triplicate.

4.8 Biofilm inhibition assay

Bacterial cultures (*S. aureus* and *E. coli*) were grown in LB_{10} media overnight at 37 °C with shaking at 150 rpm. Cultures were diluted (1:20) in LB medium and 200 µl aliquots were dispensed to flat bottom 96-well plate wells (Sarstedt Australia). Cultures were supplemented with varying concentrations of synthetic compounds dissolved in DMSO. The cultures were grown in 96-well plate wells overnight along with synthetic compounds. Control cultures were supplemented with an equal amount of DMSO. Plates were sealed with self-adhesive microplate sealers (TopSeal-A, PerkinElmer) to allow air diffusion and to prevent condensation. Cultures were incubated overnight at 37 °C with shaking at 150 rpm. Biofilms adhered on polystyrene substratum were quantified by crystal violet staining as described previously[67]. All cultures were prepared in triplicate.

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