European Journal of Medicinal Chemistry 46 (2011) 4201–4211

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and antibacterial activity of C_2-symmetric binaphthyl scaffolded amino acid derivatives

Mark Robertson [a], John B. Bremner [a,*], Jonathan Coates [b,1], John Deadman [b,1], Paul A. Keller [a,*], Stephen G. Pyne [a,*], Kittiya Somphol [a], David I. Rhodes [b,1]

[a] School of Chemistry, University of Wollongong, Wollongong, NSW 2522, Australia
[b] Avexa Ltd, 576 Swan St, Richmond, Vic 3121, Australia

ARTICLE INFO

Article history:
Received 26 October 2010
Received in revised form
7 May 2011
Accepted 18 June 2011
Available online 25 June 2011

Keywords:
Antibacterial
Peptides
Binaphthyl scaffolds
VRE
S. aureus
Resistant bacteria

ABSTRACT

The synthesis of eleven novel antibacterial agents is reported. The structures are based on a C_2-symmetric binaphthyl scaffold which holds two identical chains consisting of a short linker, a basic amino acid and a small hydrophobic side chain. Antibacterial activity is revealed for a number of derivatives down to an MIC of 2 μg/mL (2 μM) against *Staphylococcus aureus* – comparable to vancomycin, and an MIC of 31 μg/mL (31 μM) against some vancomycin-resistant enterococcal strains.

1. Introduction

The emergence of resistance to vancomycin in Gram-positive staphylococci and enterococci presents a serious health care challenge [1–5]. This challenge is heightened by cross-resistance to other antibacterials including linezolid [6]. In an attempt to overcome this resistance, high molecular weight cyclic peptides like oritavancin [7] or large vancomycin dimers linked *via* a rigid actinocin group [8] have been developed. Another strategy is to reduce molecular size with a view to the design of simpler cationic amino acids that possess additional features related to vancomycin. Such analogues could act in a similar fashion to vancomycin while concurrently maintaining the capability to bind strongly with the altered peptido-glycan cell wall moiety in vancomycin-resistant strains [3]. The same structural entity in sensitive strains would also be susceptible and thus inhibition by the same molecule would broaden the antibacterial spectrum. A similar structural minimisation strategy has been effectively pursued by Svendsen and co-workers with respect to other cationic peptidomimetics based on dipeptides which contain two cationic charges and two sterically bulky hydrophobic units [9–11]. Subsequently cationic tripeptide analogues were also developed [12,13]. The compounds have good activity against both Gram-positive (including MRSA) and Gram-negative bacteria, but activity against vancomycin-resistant strains was not assessed [12,13].

In our research the design principles we adopted [14] for the scaffolded amino acids included: 1) the presence of a basic amino acid residue to engage in an ionic interaction with the terminal carboxylate group of D-Ala or D-Lac in the cell wall peptide-glycan unit; 2) amino acid moieties for H-bond interactions with either of these two terminal groups; and 3) a 1,1′-binaphthyl system (or heteroaryl [15,16] or phenyl [17] systems) to enable hydrophobic interactions with the D-Ala methyl group or with other hydrophobic regions; such a system also offered the potential, through the atropisomers, for further stereochemical refinements if required. The initial exploration of these design principles involved amino acids contained within a scaffolded cyclic structure on the 2,2′-dimethoxy-1,1′-binaphthyl system with the macrocyclic ring connected *via* the 3,3′-positions of the binaphthyl core and a D-lysine as the basic amino acid residue. This produced a derivative with good antibacterial activity (Minimum Inhibitory Concentration; MIC) of 15 μg/mL (16 μM) against *Staphylococcus aureus* [14].

* Corresponding authors. Tel.: +61 2 4221 4692; fax: +61 2 4221 4287.
E-mail addresses: jbremner@uow.edu.au (J.B. Bremner), keller@uow.edu.au (P.A. Keller), spyne@uow.edu.au (S.G. Pyne).
[1] Tel.: +61 3 9208 4094; fax: +61 3 9208 4100.

0223-5234/$ – see front matter
doi:10.1016/j.ejmech.2011.06.024

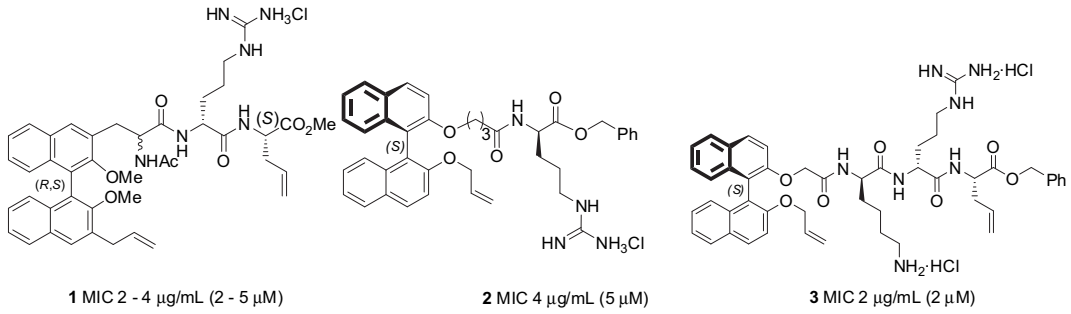
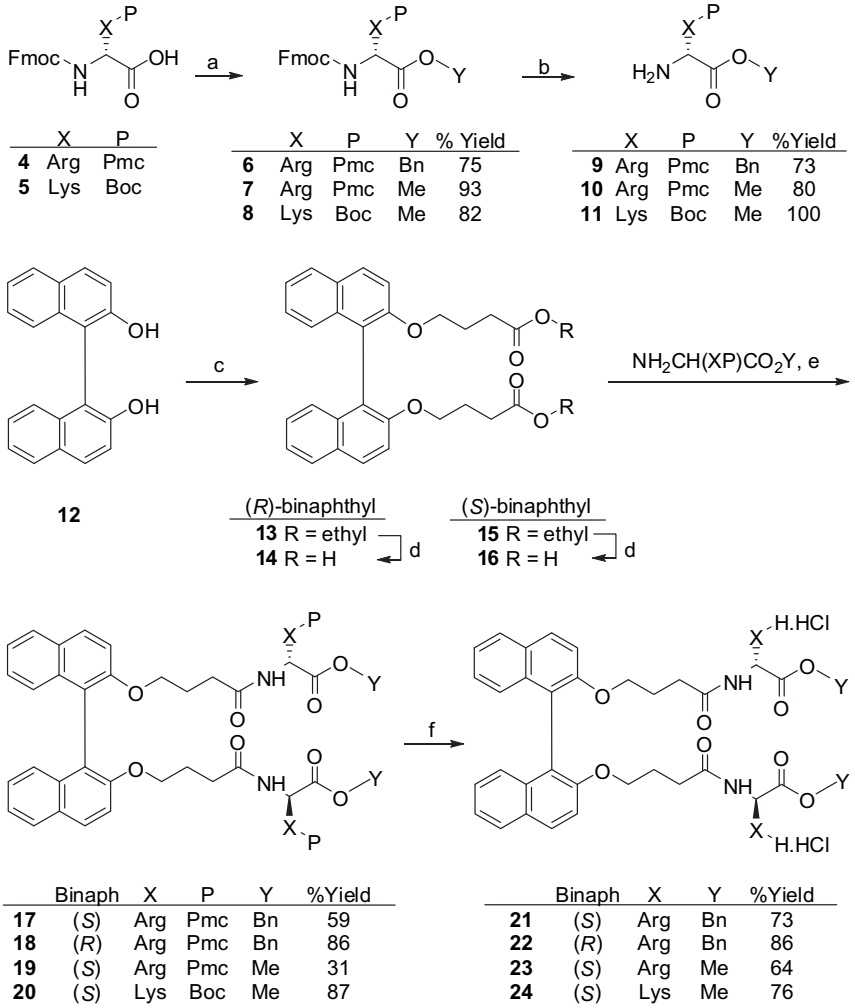


Fig. 1. Cyclic and acyclic hydrophobic scaffolded antibacterial agents containing amino acid moieties with MIC values against *S. aureus* [23].

Testing of the deprotected acyclic precursors of these macrocycles, *e.g.* **1** (Fig. 1), revealed significant activities (*e.g.* **1**, MIC = 2–4 μg/mL, 2–5 μM, against *S. aureus*) [18]. Further development of this class of compounds revealed that moving the aryl substituents from the binaphthyl 3,3′-positions to the 2,2′-aryloxy positions maintained activity (*e.g.* **2** (MIC = 4 μg/mL, 5 μM) and **3** (MIC = 2 μg/mL, 2 μM) while allowing for a significantly easier synthetic access [19,20]. Our structure activity relationship (SAR) studies have already revealed the importance of the basic side chains within the molecule [21]. However, the relative position of these amino acids has yet to be confirmed. The presence of the allyloxy substituent in **2** and **3** is a remnant of its requirement for use in a metathesis reaction to produce cyclic structures [14], and hence it could be replaced by additional basic amino acid units. The necessity of the binaphthyl aromatic unit in our acyclic antibacterial agents has been previously established – if the binaphthyl scaffold is replaced with an indole [15], carbazole [16], or a simple benzene unit [17], no significant antibacterial activity was found. We recently reported



	X	P
4	Arg	Pmc
5	Lys	Boc

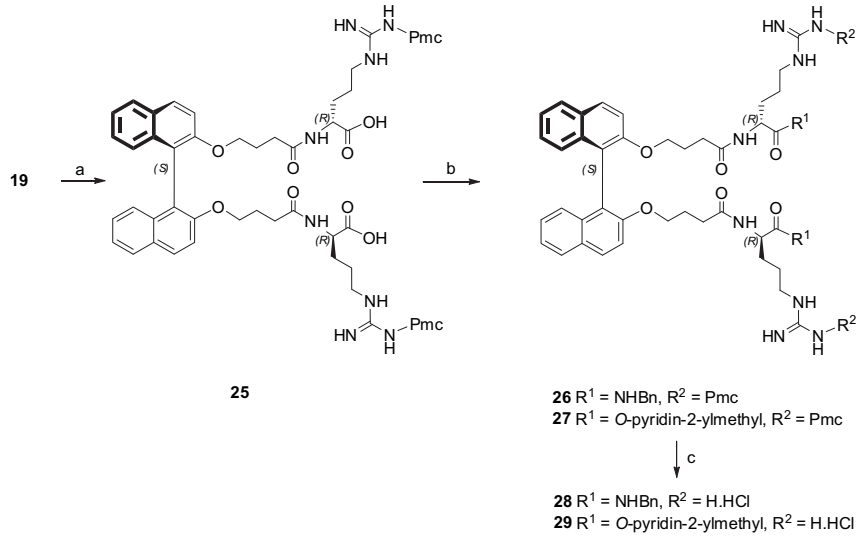
	X	P	Y	% Yield
6	Arg	Pmc	Bn	75
7	Arg	Pmc	Me	93
8	Lys	Boc	Me	82

	X	P	Y	%Yield
9	Arg	Pmc	Bn	73
10	Arg	Pmc	Me	80
11	Lys	Boc	Me	100

	Binaph	X	P	Y	%Yield
17	(*S*)	Arg	Pmc	Bn	59
18	(*R*)	Arg	Pmc	Bn	86
19	(*S*)	Arg	Pmc	Me	31
20	(*S*)	Lys	Boc	Me	87

	Binaph	X	Y	%Yield
21	(*S*)	Arg	Bn	73
22	(*R*)	Arg	Bn	86
23	(*S*)	Arg	Me	64
24	(*S*)	Lys	Me	76

Scheme 1. Synthesis of compounds **21**–**24**. Reagents and conditions: a) for **6** and **7**: BnOH or MeOH, $SOCl_2$, 0–70 °C, overnight, for **8**: MeOH, EDCI, HOBt, CH_3CN, RT, 3 h; b) piperidine, CH_3CN, RT, 3 h; c) ethyl 4-bromobutyrate, K_2CO_3, acetone, reflux, 24 h; d) LiOH, H_2O, THF, RT, 4 h; e) EDCI, HOBt, CH_3CN, RT, 3 h; f) TFA, CH_2Cl_2, RT, 3 h to overnight, then 1 M HCl–Et_2O.



Scheme 2. Synthesis of compounds **28** and **29**. Reagents and conditions: a) LiOH, H_2O, THF, RT, 90 min, 74%; b) $BnNH_2$ or 2-pyridinecarbinol, EDCI, HOBt, CH_3CN, RT, 3 h; 27% (**26**), 64% (**27**); c) TFA, CH_2Cl_2, RT, overnight, then 1 M HCl–Et_2O, 75% (**28**), 90% (**29**).

the importance of the presence of two basic amino acid side chains for activity across a broad spectrum of bacterial strains [20,22], however these reported analogues positioned these amino acids sequentially pendant from one of the naphthyl units of the hydrophobic scaffold. We describe here the synthesis and antibacterial results for significantly different derivatives which maintain the binaphthyl hydrophobic scaffold but have incorporated the required basic amino acid moieties in chains attached to both binaphthyl 2,2′-oxy positions.

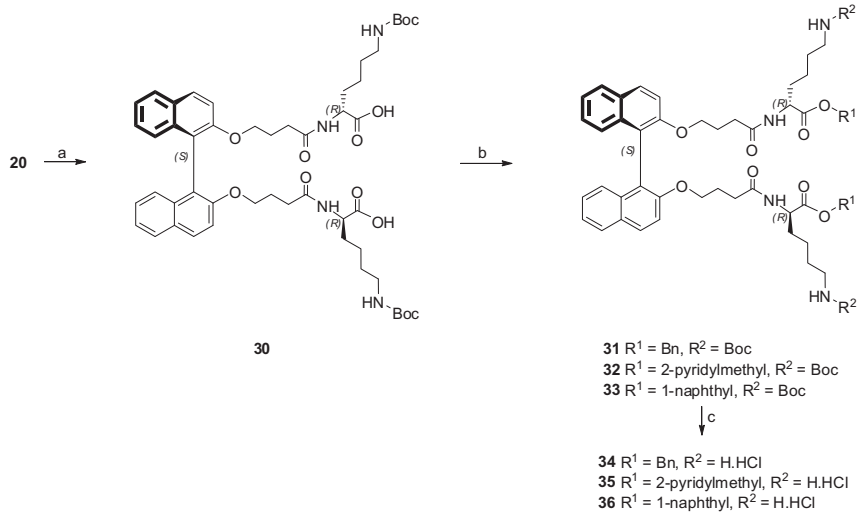
2. Results and discussion

2.1. Chemistry

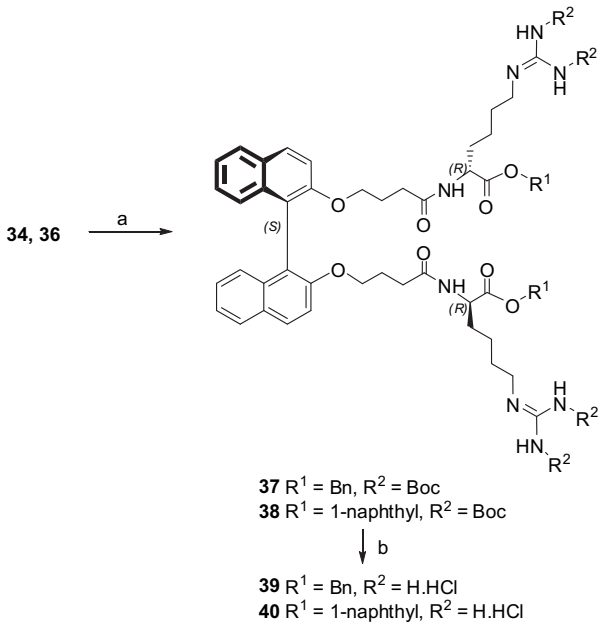
Our target compounds are of the type exemplified by compounds **21**–**24** (Scheme 1). We incorporated a linker between the naphthyl and amino acid units as previous studies have shown that the presence of three contiguous methylene units can be tolerated within the structure (*e.g.* **2**, MIC 5 μM, *S. aureus*) – this further ensured there was no steric interference from the binaphthyl units for any potential binding with the peptide side chain to its targets. Both Arg and Lys side chains were used together with either the *R*- or *S*-binaphthyl anchors and a butanoic acid-based linking group. The amino acid chain was initially terminated by a benzyl ester as in one of our previous examples (**3**, Fig. 1). However, we also further investigated a variety of ester and amide units to cap the chain.

The facile synthesis of the first target structures **21**–**24** is outlined in Scheme 1. The protected Lys and Arg derivatives were esterified with either a methyl or benzyl moiety (**6**–**8**) before deprotection of the α-amino group in these esters to give the amines (**9**–**11**). These amino esters were then added to the (*R*)- [24] or (*S*)-binaphthyl scaffold, which had been chain extended at the 2,2′-positions with an oxybutanoic acid moiety (**14** and **16**), to yield derivatives **17**–**20**. Deprotection of **17**–**20** gave the binaphthyl amino acid derivatives **21**–**24** respectively, after conversion to their hydrochloride salts.

The arginine derivative **19** was also used to vary the ester terminus in the corresponding *N*-benzyl amide **28** and 2-pyridylmethyl ester **29** (Scheme 2). Similarly, the lysine derivative



Scheme 3. Synthesis of compounds **34**–**36**. Reagents and conditions: a) LiOH, H_2O, THF, RT, 90 min, 92%; b) PPh_3, DIAD, THF, benzyl alcohol (for **31**), 2-pyridinecarbinol (for **32**), 1-naphthalene methanol (for **33**), 0 °C to RT, overnight; c) TFA, CH_2Cl_2, RT, 3 h, then 1 M HCl–Et_2O, 80% (**34**, 2 steps), 54% (**35**, 2 steps), 83% (**36**, 2 steps).



Scheme 4. Synthesis of compounds **39** and **40**. Reagents and conditions: a) Et_3N, *N,N′*-bis(*tert*-butoxycarbonyl)-*N″*-triflyl guanidine, CH_2Cl_2, RT, overnight, 79% (**37**), 61% (**38**); b) TFA, CH_2Cl_2, RT, overnight, then 1 M HCl–Et_2O, 86% (**39**), 61% (**40**).

20 was used to generate the analogues **34**, **35** and **36** where the terminal methyl ester was replaced with a benzyl, 2-pyridylmethyl and 1-naphthyl ester respectively (Scheme 3). Finally, a guanylation reaction of the lysine benzyl ester **34** and the lysine 1-naphthyl ester **36** provided the corresponding homoarginyl derivatives, **39** and **40**, respectively (Scheme 4).

In all the reactions to produce the derivatives, there was no evidence for racemisation of the binaphthyl unit occurring. This is consistent with our previous results [14,18–22] and experience with this moiety during the synthesis of such antibacterial agents.

2.2. Evaluation of antibacterial activities

The results from the antibacterial testing of these compounds against *S. aureus* (ATCC6538P; vancomycin sensitive) and vancomycin sensitive and resistant strains of *Enterococcus faecium* (#243, 449, 820, 987; clinical isolates) are summarised in Table 1. The most active compound against *S. aureus* was the (*S*)-diarginine-based structure **21** with an MIC of 2 μg/mL, equipotent with vancomycin. In addition, this activity was not sensitive to the binaphthyl stereochemistry with similar activity being observed for the corresponding (*R*)-binaphthyl isomer **22**. This result is not inconsistent with the binaphthyl scaffold having a general hydrophobic interaction role. The terminal ester appears to require a reasonable degree of hydrophobic steric bulk with the smaller methyl diester **23** showing notably less activity than **21** or **22**. However, the introduction of a heteroatom into the terminal aryl ester, *e.g.* the corresponding 2-pyridylmethyl diester **29** with the potential for hydrogen-bonding interaction in this region, was detrimental with the compound showing very poor activity. This trend is emulated with the dilysine series with **34** (dibenzyl ester) showing the best activity, with both the larger dinaphthyl diester **36** and the 2-pyridylmethyl diester **35** showing poorer activity. By extending the length of the basic amino acid side chains, we produced the homoarginine derivatives **39** and **40** with terminal benzyl and naphthyl esters respectively. The extra length in the side chains showed no advantage, with respect to antibacterial activity.

With *E. faecium*, compounds **21** and **22** showed the best activity, a highlight being the moderate activity (MICs 31 μM) shown against the vancomycin-resistant strains VRE_{449} and VRE_{820} in comparison to vancomycin which was completely ineffective (MIC >86 μM, VRE_{820}). Interestingly, a direct comparison between **21** and **28**, where the only difference is the replacement of the terminal dibenzyl ester with a *N,N*-dibenzyl amide showed a similar trend for the vancomycin sensitive enterococcal strains, however the amide **28** was significantly less potent against the resistant strains compared to the corresponding ester derivative. A comparison between the activities shown for *S. aureus* and VRE_{987}, which were both vancomycin sensitive strains, showed our derivatives to be essentially ineffective against the latter. Indeed, generally the trends in activities against *E. faecium* were poor, with decreasing activity observed when the terminal ester was reduced in size (*e.g.* to a methyl ester), and when an aryl heteroatom was incorporated into the terminal ester.

In general, the use of C_2-symmetric systems of the type illustrated in Table 1 showed no particular advantage or insight into the antibacterial activities of our scaffolded amino acid molecules. Although the structures **21** and **22** showed equivalent activity to our previous acyclic lead compounds **2** and **3**, this study was unable to improve antibacterial activities against *E. faecium*. We have since further developed our acyclic binaphthyl scaffolded amino acids and have shown that they possess consistently good activities, including against *S. aureus* and resistant strains of bacteria [20]. This current study has highlighted that the repositioning of the

Table 1
Antibacterial activities of our synthesised compounds.

MIC Values										
	S. aureus		VRE_{243}		VRE_{449}		VRE_{820}		VRE_{987}	
	μg/mL	μM	μg/mL	μM	μg/mL	μM	μg/mL	μM	μg/mL	μM
21	2	2	63	61	31	31	31	31	33	31
22	4	4	31	31	31	31	31	31	31	31
23	16	18	–	–	>125	>143	>125	>143	–	–
24	>125	>153	–	–	–	–	–	–	–	–
28	2	2	63	61	63	61	125	122	125	122
29	16	15	>125	>122	>125	>122	>125	>122	>125	>122
34	4	4	125	129	83	65	63	65	125	129
35	125	129	>125	>129	>125	>129	>125	>129	>125	>129
36	8	7	>125	>117	125	117	63	59	125	117
39	4	4	83	59	63	59	63	59	104	119
40	8	7	>125	>109	125	109	63	54	>125	>108
Vancomycin	2	1	2	1	63	43	>125	>86	<1	<1

S. aureus (ATCC 6538P), *E. faecium* #243, *E. faecium* #449, *E. faecium* #820, *E. faecium* #987. Note: MIC values reported are medium have been rounded.

amino acid basic side chains relative to the scaffold and the terminal esters was particularly detrimental to the activity against the resistant strains tested and these results have assisted with the SAR picture developing for this class of inhibitors.

3. Conclusion

In conclusion, some of the C_2-symmetric scaffolded cationic amino acid derivatives synthesised here showed good activity against *S. aureus*, and in selected cases equalled the activity shown by our lead compounds and vancomycin. However, further development of this series was curtailed in the light of better activities shown by simpler compounds with just one side chain attached to the binaphthyl scaffold.

4. Experimental protocols

4.1. General notes

Melting point determinations were carried out on a Gallenkamp melting point apparatus and are uncorrected. Chemical ionization (CI) and electron impact (EI) mass spectra were obtained on a Shimadzu QP-5000 mass spectrometer by a direct insertion technique with an electron beam energy of 70 eV. Electrospray ionization (ESI) mass spectra were obtained on a VG Autospec spectrometer. High-resolution mass spectra (HRMS) were determined on a micromass QTof2 spectrometer using polyethylene glycol or polypropylene glycol as the internal standard. The *m/z* values are stated with their peak intensity as a percentage in parentheses. Proton and carbon nuclear magnetic resonance (NMR) spectra were obtained as specified on a Varian Mercury 300 MHz or Varian Inova 500 MHz spectrometer. Spectra were recorded in the specified deuterated solvent, and referenced to the residual non-deuterated solvent signal. Chemical shifts (δ) in ppm were measured relative to the internal standard. Multiplet (*m*) signals are reported as ranges; 'app' refers to apparent when describing some multiplets. Analytical thin layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} pre-coated aluminium plates with a thickness of 0.2 mm. All column chromatography was performed under 'flash' conditions on Merck silica gel 60 (230–400 mesh). Chromatography solvent mixtures were measured by volume. All compounds were judged to be of greater than 95% purity based upon ^{1}H NMR and TLC analysis. Starting materials and reagents were purchased from Sigma–Aldrich Pty Ltd or Auspep Pty Ltd and were used as received. Solvent extracts were dried over magnesium sulfate.

All final tested compounds are fully characterized with the exception where LRMS revealed only an *m/2* peak due to instrumentation limitations, the HRMS measurements on *m/2* ions was not possible.

4.2. General protocols

4.2.1. Protocol 1: peptide coupling

To a stirred solution of an acid (1 equiv.) and an amine (1 equiv.) in dry acetonitrile or DMF (5–10 mL) was added EDCI (1.2 equiv.) and HOBt (1.2 equiv.). If the amine was an HCl salt then 1 equiv. of DIPEA was also added. The reaction mixture was stirred overnight before the solvent was removed under reduced pressure and the resultant residue subjected to flash silica gel column chromatography (normally using 2% MeOH/CH_2Cl_2 as the eluant) to afford the desired compound.

4.2.2. Protocol 2: N-fmoc deprotection

To a stirred solution of the Fmoc-protected peptide in dry acetonitrile (5–10 mL) was added piperidine (0.1 mL). The resultant solution was then stirred at room temperature for 3 h. The solvent was then removed under reduced pressure and the resultant residue subjected to flash silica gel chromatography using a short column with 2% MeOH/CH_2Cl_2 then 5% MeOH/CH_2Cl_2) upon removal of Fmoc by-products to afford the desired compound.

4.2.3. Protocol 3: N-boc and Pmc/Pbf-deprotection

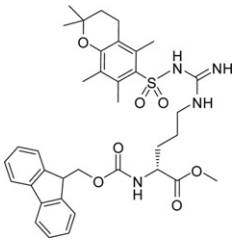
To a stirred solution of the protected peptide in CH_2Cl_2 (2 mL) was added TFA (2 mL). The reaction mixture was stirred at room temperature for 3 h before the solvent was removed under reduced pressure. After triturating twice more with CH_2Cl_2 (2 mL), the residue was taken up in CH_2Cl_2 (2 mL) and treated with a 1 M HCl/diethyl ether solution (2 mL), stirred for 1 min and then evaporated to dryness. This treatment with HCl was repeated twice more. For Boc-deprotection this is the final step, for Pmc/Pbf-deprotection the following is completed. The residue was taken up in the minimum required CH_2Cl_2 (or dry MeOH if insoluble in CH_2Cl_2), precipitated by the addition of diethyl ether and the solid collected by centrifugation. This step is repeated once more to remove the protecting group by product. The resultant solid was then dried to yield the desired compound as its hydrochloride salt.

4.2.4. Protocol 4: esterification

To an ice cold solution of an acid (1 equiv.) in dry THF (30 mL) was added DIAD (2.1 equiv.) and PPh_3 (2.1 equiv.) under a nitrogen atmosphere. The alcohol (2.1 equiv.) was then added and the solution was allowed to warm to room temperature and was then stirred overnight. The solvent was then evaporated under reduced pressure and the crude product was purified by flash column chromatography with 0–5% MeOH/CH_2Cl_2 to yield an ester product.

4.3. Preparation of derivatives

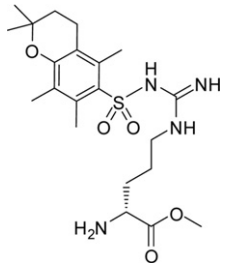
4.3.1. Methyl (2R)-2-(9H-9-fluorenylmethoxycarboxamido)-5-{3-[(2,3-dihydro-2,2,5,7,8-pentamethyl-2H-1-benzopyran-6-yl)sulfonyl]guanidino}pentanoate (**7**)



To Fmoc-(*R*)-Arg(Pmc)-OH (1.00 g, 1.51 mmol) dissolved in dry MeOH (5 mL) was added thionyl chloride (0.2 mL). The solution was then heated at reflux overnight before being evaporated to dryness. Water (20 mL) was then added and the solution was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were dried and evaporated to dryness to yield the desired product (952 mg, 93%) as a foamy white solid which was of sufficient purity to carry onto the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (s, 6H), 1.51–1.63 (m, 2H), 1.72–1.76 (m, 2H), 1.80 (t, J = 6.9 Hz, 2H), 2.10 (s, 3H), 2.54–2.64 (m, 2H), 2.56 (s, 3H), 2.60 (s, 3H), 3.63 (s, 3H, OCH_3), 4.12–4.16 (m, 1H), 4.27–4.35 (m, 3H), 5.95 (d, J = 8.4 Hz, 1H, NH), 6.30 (br s, 1H, NH), 6.35 (br s, 2H, NH), 7.26 (t, J = 7.4 Hz, 2H), 7.36 (t, J = 7.4 Hz, 2H), 7.57 (d, J = 7.5 Hz, 2H), 7.73 (d, J = 7.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.0, 17.4, 18.5, 21.3, 24.9, 26.6, 29.3, 32.6,

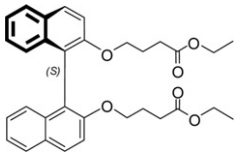
36.4, 40.5, 46.9, 52.2, 66.9, 73.4, 117.7, 119.6, 123.7, 124.9, 126.8, 127.4, 133.1, 134.5, 135.1, 140.9, 143.4, 143.5, 153.2, 156.0, 157.2, 162.3, 172.4. MS (ESI + ve) *m/z:* 677 (100%) $[M + H]^+$.

*4.3.2. Methyl (2R)-2-amino-5-{3-[(2,3-dihydro-2,2,5,7,8-pentamethyl-2H-1-benzopyran-6-yl)sulfonyl]guanidino}pentanoate (**10**)*



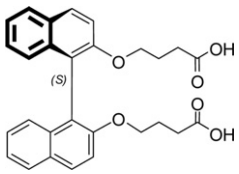
The title compound was prepared *via* protocol **2**, using Fmoc-(*R*)-Arg(Pmc)-OMe (**7**) (356 mg, 0.53 mmol) to yield the desired product (186 mg, 80%) as a white foam [25]. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (s, 6H), 1.46–1.62 (m, 3H), 1.66–1.76 (m, 1H), 1.74 (br s, 2H, NH_2), 1.80 (t, J = 6.8 Hz, 2H), 2.10 (s, 3H), 2.54 (s, 3H), 2.55 (s, 3H), 2.62 (t, J = 6.8 Hz, 2H), 3.12–3.21 (m, 2H), 3.38–3.45 (m, 1H), 3.67 (s, 3H, OCH_3), 6.41 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.0, 17.4, 18.4, 21.3, 25.5, 26.7, 32.7, 40.6, 61.9, 53.8, 73.5, 117.8, 123.9, 133.3, 134.7, 135.3, 153.4, 156.2, 176.1.

*4.3.3. Diethyl (S)-4,4′-(1,1′-binaphthyl-2,2′-bisoxy)dibutanoate (**15**)*



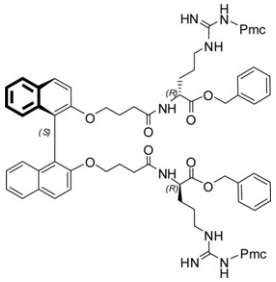
To a suspension of (*S*)-1,1′-binaphthalene-2,2′-diol (5.0 g, 17.5 mmol) and anhydrous potassium carbonate (12.0 g, 87.6 mmol) dissolved in acetone (200 mL), was added ethyl bromobutyrate (6.0 mL, 2.2 equiv.) under a nitrogen atmosphere. The mixture was then heated at reflux for 24 h before being evaporated to dryness and the residue partitioned between ethyl acetate (100 mL) and water (100 mL). The organic layer was then washed with water (2 × 50 mL) before being dried and evaporated to yield an oil (7.56 g, 85%) which was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17 (t, J = 7.2 Hz, 6H), 1.73–1.79 (m, 4H), 1.87–1.94 (m, 4H), 3.97–4.08 (m, 8H), 7.18–7.26 (m, 4H), 7.31–7.36 (m, 2H), 7.39 (d, J = 8.8 Hz, 2H), 7.84 (d, J = 8.0 Hz, 2H), 7.92 (d, J = 8.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 24.5, 29.9, 59.9, 68.3, 115.5, 120.4, 123.5, 125.2, 126.1, 127.7, 129.15, 129.20, 133.9, 153.9, 173.1. MS (EI, +ve) *m/z* 514 (100%) [M]. HRMS (ESI, +ve) calcd for $C_{32}H_{35}O_6$ 515.2434, found 515.2434.

*4.3.4. (S)-4,4′-(1,1′-Binaphthyl-2,2′-bisoxy)dibutanoic acid (**16**)*



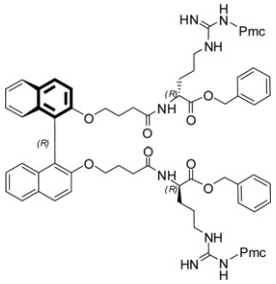
To **15** (598 mg, 1.52 mmol) dissolved in THF (30 mL) was added a solution of LiOH (250 mg, 10.50 mmol) in water (20 mL). After stirring at RT for 4 h, diethyl ether was added and the layers were separated. The aqueous layer was then acidified with diluted HCl solution. This was then extracted with diethyl ether (3 × 20 mL). The combined extracts were dried and the solvent was evaporated under reduced pressure to yield **16** (342 mg, 64%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.52–2.00 (m, 8H), 3.81–4.05 (m, 4H), 7.14–7.20 (m, 4H), 7.25–7.30 (m, 2H), 7.35 (d, J = 9.1 Hz, 2H), 7.81 (d, J = 8.2 Hz, 2H), 7.90 (d, J = 8.8 Hz, 2H), 11.84 (br s, 2H, 2 × OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.1, 29.6, 68.1, 115.6, 120.4, 123.7, 125.2, 126.3, 127.9, 129.30, 129.32, 133.9, 153.9, 179.8. MS (ESI + ve) *m/z* 458 $[M + H]^+$, 372, 286 (100%).

*4.3.5. Dibenzyl (2R)-7,7′-((S)-1,1′-binaphthyl-2,2′-bisoxy)-2,2′-di{3-[(2,3-dihydro-2,2,4,6,7-pentamethyl-2H-1-benzofuran-5-yl)sulfonyl]guanidinopropyl}-3,3′-diaza-4,4′-dioxodiheptanoate (**17**)*



The title compound was prepared *via* protocol 1, using **9** [21] (159 mg, 0.30 mmol; prepared *via* **4** and **6**) and **16** (68.7 mg, 0.15 mmol) to yield **17** as a white solid (124 mg, 59%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (s, 12H), 1.32–1.39 (m, 4H), 1.54–1.81 (m, 16H), 2.06 (s, 6H), 2.42–2.60 (m, 4H), 2.50 (s, 6H), 2.53 (s, 6H), 2.94–3.15 (m, 4H), 3.74–3.78 (m, 2H), 3.94–3.97 (m, 2H), 4.29–4.40 (m, 2H), 5.04 (s, 4H) 6.12 (br s, 2H, NH), 6.23 (br s, 4H, NH), 6.43 (d, J = 7.2 Hz, 2H, NH), 7.08 (d, J = 8.4 Hz, 2H), 7.16 (dist t, 2H), 7.18–7.28 (m, 12H), 7.32 (d, J = 9.0 Hz, 2H), 7.80 (d, J = 8.4 Hz, 2H), 7.86 (d, J = 9.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.0, 17.4, 18.5, 21.3, 25.1, 25.3, 26.7, 29.1, 31.7, 32.6, 40.5, 51.9, 67.0, 68.2, 73.6, 115.7, 117.9, 120.3, 123.7, 124.0, 125.2, 126.3, 127.9, 128.1, 128.3, 128.5, 129.2, 129.4, 133.2, 134.0, 134.7, 135.2, 135.3, 153.5, 153.8, 156.1171.8, 173.2. MS (ESI +ve) *m/z* 742.7 (100%) $[M + H]^+$.

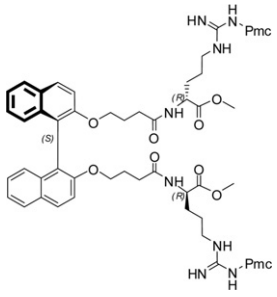
*4.3.6. Dibenzyl (2R)-7,7′-((R)-1,1′-binaphthyl-2,2′-bisoxy)-2,2′-di{3-[(2,3-dihydro-2,2,4,6,7-pentamethyl-2H-1-benzofuran-5-yl)sulfonyl]guanidinopropyl}-3,3′-diaza-4,4′-dioxodiheptanoate (**18**)*



The title compound was prepared *via* protocol 1, using **9** [21] (125 mg, 0.236 mmol) and **14** [24] (45 mg, 0.098 mmol) to yield **18** as a white solid (123 mg, 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (s, 12H), 1.28–1.35 (m, 4H), 1.43–1.75 (m, 16H), 2.07 (s, 6H), 2.52 (s, 6H), 2.53–2.57 (m, 4H), 2.54 (s, 6H), 2.95–3.14 (m, 4H), 3.77–3.82 (m, 2H), 3.94–3.99 (m, 2H), 4.34–4.41 (m, 2H), 5.04 (s, 4H), 6.05 br

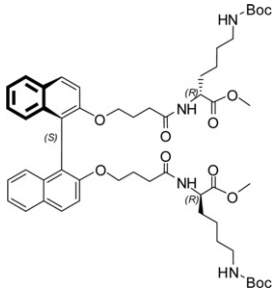
s, 2H, NH), 6.19 (br s, 2H, NH), 6. 21 (s, 4H, NH), 7.10, d, $J = 8.5$ Hz, 2H), 7.15–7.20 (m, 2H), 7.22–7.31 (m, 12H), 7.33 (d, $J = 9.1$ Hz, 2H), 7.80 (d, $J = 7.9$ Hz, 2H), 7.87 (d, $J = 9.1$ Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.0, 17.4, 18.5, 21.3, 24.9, 25.2, 26.7, 29.2, 31.5, 32.6, 40.5, 51.7, 67.0, 73.5, 76.6, 115.4, 117.9, 120.2, 123.7, 124.0, 125.3, 126.3, 127.9, 128.1, 128.4, 128.5, 129.1, 129.4, 133.3, 134.0, 134.7, 135.2, 135.3, 153.5, 153.7, 156.1171.7, 173.1. MS (ESI +ve) *m/z* 1483.4 (10%) $[M + H]^+$; 742.4 (20) $[M + H]^{2+}$.

*4.3.7. Dimethyl (2R)-7,7′-((S)-1,1′-binaphthyl-2,2′-bisoxy)-2,2′-di {3-[(2,3-dihydro-2,2,4,6,7-pentamethyl-2H-1-benzofuran-5-yl) sulfonyl]guanidinopropyl}-3,3′-diaza-4,4′-dioxodiheptanoate (**19**)*



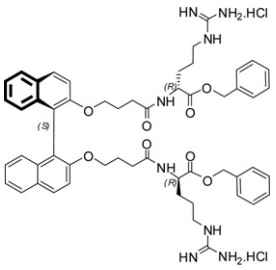
The title compound was prepared *via* protocol 1, using (*R*)-Arg(Pmc)-OMe **(10)** (40 mg, 0.088 mmol) and **16** (20 mg, 0.044 mmol) to yield **19** as a white solid (18 mg, 31%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (s, 12H), 1.35–1.40 (m, 4H), 1.48–1.55 (m, 2H), 1.60–1.78 (m, 14H), 2.09 (s, 6H), 2.53 (s, 6H), 2.54–3.61 (m, 4H), 2.56 (s, 6H), 3.03–3.18 (m, 4H), 3.64 (s, 6H), 3.83–3.89 (m, 2H), 4.00–4.05 (m, 2H), 4.32–4.39 (m, 2H), 6.06 (br s, 2H, NH), 6.18–6.29 (m, 6H, NH), 7.13 (d, $J = 8.7$ Hz, 2H), 7.21 (app t, 2H), 7.32 (app t, 2H), 7.39 (d, $J = 9.0$ Hz, 2H), 7.84 (d, $J = 8.1$ Hz, 2H), 7.92 (d, $J = 9.3$ Hz, 2H).

*4.3.8. Dimethyl (2R)-7,7′-((S)-1,1′-binaphthyl-2,2′-bisoxy)-2,2′-di [(1,1′-dimethylethoxycarbonylamino)butyl]-3,3′-diaza-4,4′-dioxodiheptanoate (**20**)*



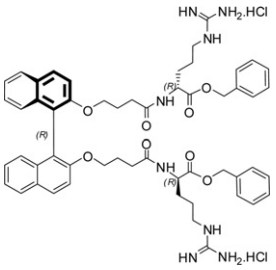
The title compound was prepared *via* protocol 1, using **16** (50 mg, 0.11 mmol) and (*R*)-Lys(BOC)-OMe **(11)** (68 mg, 0.23 mmol) to yield the desired product **20** as an off white solid (90 mg, 87%). $R_f = 0.44$ (5% MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15–1.26 (m, 4H), 1.30–1.55 (m, 6H), 1.43 (s, 18H), 1.59–1.76 (m, 10H), 2.97–3.02 (m, 4H), 3.73 (s, 6H), 3.84–3.91 (m, 2H), 4.11–4.18 (m, 2H), 4.42–4.49 (m, 2H), 4.53 (br s, 2H, 2 × NHBoc), 5.62 (d, $J = 8.2$ Hz, NH), 7.16–7.19 (m, 2H), 7.23–7.58 (m, 2H), 7.33–7.38 (m, 2H), 7.45 (d, $J = 9.1$ Hz, 2H), 7.91 (d, $J = 8.2$ Hz, 2H), 7.99 (d, $J = 8.8$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.4, 24.9, 28.4, 29.4, 31.7, 32.0, 33.9, 40.1, 51.6, 52.2, 68.1, 79.0, 115.6, 120.4, 123.6, 125.2, 126.2, 127.8, 127.9, 129.2, 129.3, 134.0, 153.7, 155.8, 172.1, 172.6. MS (ESI +ve) *m/z* 981.5 (30%) $[M + K]^+$; 968.6 (100) $[M + Na]^+$; 943.6 (10) $[M + H]^+$.

*4.3.9. Dibenzyl (2R)-7,7′-((S)-1,1′-binaphthyl-2,2′-bisoxy)-2,2′-di(3-guanidinopropyl)-3,3′-diaza-4,4′-dioxodiheptanoate dihydrochloride (**21**)*



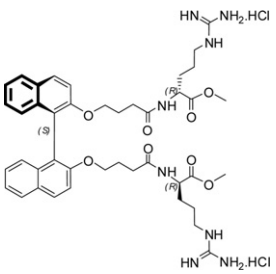
The title compound was prepared *via* protocol 3, using **17** (124 mg, 0.089 mmol) to yield **21** as a white solid (65.9 mg, 73%). ^{1}H NMR (300 MHz, CD_3OD) δ 1.42–2.00 (m, 16H), 3.00–3.17 (m, 4H), 3.90–3.97 (m, 4H), 4.23–4.35 (m, 2H), 5.02–5.12 (m, 4H), 6.96–6.99 (m, 2H), 7.04–7.15 (m, 2H), 7.16–7.34 (m, 12H), 7.37–7.49 (m, 2H), 7.80 (d, $J = 7.8$ Hz, 2H), 7.89 (d, $J = 8.7$ Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 26.3, 26.7, 29.4, 31.7, 32.8, 41.8, 53.4, 67.9, 69.6, 116.7, 121.4, 124.6, 126.2, 127.2, 129.1, 129.3, 129.4, 129.6, 130.5, 130.8, 135.3, 137.0, 155.4, 158.4173.0, 173.2. MS (ESI +ve) *m/z* 951 (5%) $[M + H]^+$; 476 (100) $[M + H]^{2+}$. HRMS (ESI, +ve) calcd for $C_{54}H_{63}N_8O_8$ 951.4769, found 951.4805.

*4.3.10. Dibenzyl (2R)-7,7′-((R)-1,1′-binaphthyl-2,2′-bisoxy)-2,2′-di(3-guanidinopropyl)-3,3′-diaza-4,4′-dioxodiheptanoate dihydrochloride (**22**)*



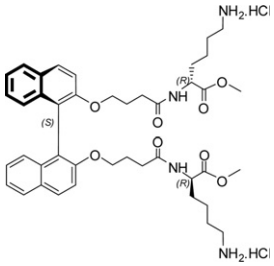
The title compound was prepared *via* protocol 3, using **18** (120 mg, 0.081 mmol) to yield **22** as a white solid (71 mg, 86%). ^{1}H NMR (300 MHz, CD_3OD) δ 1.54–1.61 (m, 4H), 1.63–1.70 (m, 6H), 1.78–1.87 (m, 2H), 1.90–1.95 (m, 4H), 3.10–3.15 (m, 4H), 3.90–4.04 (m, 4H), 4.31–4.36 (m, 2H), 5.10 (ABq, $J = 12.3$ Hz, 4H), 7.00–7.03 (m, 2H), 7.10–7.15 (m, 2H), 7.23–7.32 (m, 12H), 7.45 (d, $J = 9.0$ Hz, 2H), 7.83 (d, $J = 7.9$ Hz, 2H), 7.93 (d, $J = 9.1$ Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 26.3, 26.7, 29.4, 31.7, 32.8, 41.8, 53.4, 67.9, 69.6, 116.7, 121.4, 124.6, 126.2, 127.2, 129.1, 129.3, 129.4, 129.6, 130.5, 130.8, 135.3, 137.0, 155.4, 158.4, 173.0, 173.2. MS (ESI +ve) *m/z* 476 (100%) $[M + H]^{2+}$.

*4.3.11. Dimethyl (2R)-7,7′-((S)-1,1′-binaphthyl-2,2′-bisoxy)-2,2′-di(3-guanidinopropyl)-3,3′-diaza-4,4′-dioxodiheptanoate dihydrochloride (**23**)*



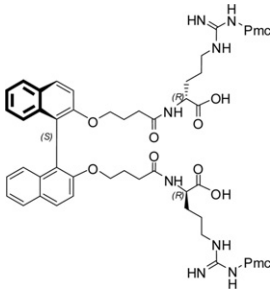
The title compound was prepared *via* protocol 3, using **19** (20 mg, 0.015 mmol) to yield **23** as a white solid (9 mg, 64%). ^{1}H NMR (300 MHz, CD_3OD) δ 1.54–1.63 (m, 10H), 1.73–1.95 (m, 6H), 3.07–3.23 (m, 4H), 3.69 (s, 6H), 3.78–3.91 (m, 2H), 3.92–4.06 (m, 2H), 4.25–4.29 (m, 2H), 6.69–6.82 (m, 2H), 6.93–7.00 (m, 4H), 7.38 (d, J = 8.8 Hz, 2H), 7.64 (d, J = 7.7 Hz, 2H), 7.81 (d, J = 8.3 Hz, 2H). ^{13}C NMR (75 MHz, CD_3OD) δ 26.3, 26.7, 29.8, 32.9, 41.9, 52.9, 55.1, 69.7, 116.8, 121.7, 124.7, 126.3, 127.3, 129.1, 130.5, 130.9, 135.4, 155.6, 158.6, 174.9, 175.7. MS (ESI +ve) *m/z:* 401 (100%) $[M + 2H]^{2+}$. HRMS (ESI +ve) calcd for $C_{42}H_{55}N_8O_8$ 799.4143, found 799.4133.

*4.3.12. Dimethyl (2R)-7,7′-((S)-1,1′-binaphthyl-2,2′-bisoxy)-2,2′-dibutylamino-3,3′-diaza-4,4′-dioxodiheptanoate dihydrochloride (**24**)*



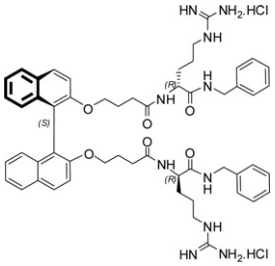
The title compound was prepared *via* protocol 3, using **20** (88 mg, 0.093 mmol) to yield the desired product **24** as an off white solid (50 mg, 76%). ^{1}H NMR (300 MHz, CD_3OD) δ 1.22–1.52 (m, 4H), 1.53–1.89 (m, 12H), 1.90–2.14 (m, 4H), 2.64–3.00 (m, 4H), 3.69 (s, 6H), 3.90–4.19 (m, 4H), 4.22–4.40 (m, 2H), 7.04 (dist d, J = 8.0 Hz, 2H), 7.16–7.21 (m, 2H), 7.23–7.39 (m, 2H), 7.48–7.61 (m, 2H), 7.88–7.90 (m, 2H), 7.94–8.08 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 23.8, 26.7, 27.9, 31.8, 32.8, 40.5, 52.8, 53.3, 69.7, 116.8, 124.5, 124.7, 126.2, 127.2, 129.1, 130.5, 130.8, 135.4, 155.5, 173.8, 175.6. MS (ESI +ve) *m/z* 743.3 (10%) $[M + H]^+$; 372.6 (100) $[M + 2H]^{2+}$. HRMS (ESI +ve) calcd for $C_{42}H_{55}N_4O_8$ 743.4020, found 743.4033.

*4.3.13. (2R)-7,7′-((S)-1,1′-binaphthyl-2,2′-bisoxy)-2,2′-di{3-[(2,3-dihydro-2,2,4,6,7-pentamethyl-2H-1-benzofuran-5-yl)sulfonyl] guanidinopropyl}-3,3′-diaza-4,4′-dioxodiheptanoic acid (**25**)*



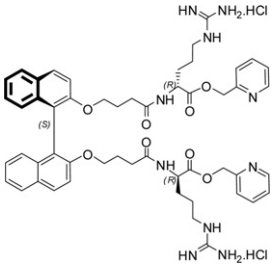
To a soln of **19** (200 mg) in THF (20 mL), was added a solution of LiOH (75 mg, 3.1 mmol) in water (10 mL). After stirring at room temperature for 90 min, ethyl acetate was added and the layers were separated. The aqueous layer was then acidified with a diluted potassium bisulphate solution. This was then extracted with CH_2Cl_2 (3 × 20 mL) and then the solvent was removed under reduced pressure to yield **25** (145 mg, 74%) as a white foam. ^{1}H NMR (300 MHz, CD_3OD) δ 1.23 (s, 12H), 1.35–1.97 (m, 20H), 2.03 (s, 6H), 2.30–2.63 (m, 10H), 2.94–3.22 (m, 4H), 3.66–3.86 (m, 2H), 3.89–4.09 (m, 2H), 4.20–4.42 (m, 2H), 6.15–6.62 (m, 6H), 7.01–7.11 (m, 4H), 7.22–7.32 (m, 4H), 7.68–7.81 (m, 4H), 8.51 (br s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.1, 17.4, 18.5, 21.3, 25.2, 26.7, 28.7, 31.6, 40.5, 52.5, 67.9, 68.1, 73.7, 112.0, 115.6, 118.1, 120.3, 123.6, 124.1, 125.2, 126.4, 127.9, 128.4, 129.2, 133.9, 134.9, 135.4, 138.1, 153.8, 156.2, 174.3, 175.2. MS (ESI +ve) *m/z* 1326 (80%) $[M + Na]^+$; 1304 (100) $[M + H]^+$. HRMS (ESI, +ve) calcd for $C_{68}H_{87}N_8O_{14}S_2$ 1303.5789, found 1303.5783.

*4.3.14. (2R)-7,7′-((S)-1,1′-Binaphthyl-2,2′-bisoxy)-N,N′-dibenzyl-2,2′-di(3-guanidinopropyl)-3,3′-diaza-4,4′-dioxodiheptanamide (**28**)*



The title compound was prepared *via* protocol 1 using **25** (70 mg, 0.054 mmol) and benzylamine (0.1 mL, 0.92 mmol) to yield the coupled product **26** (21.4 mg, 27%). This was then deprotected directly *via* protocol 3 to yield **28** (10 mg, 75%). ^{1}H NMR (300 MHz, CD_3OD) δ 1.51–1.85 (m, 12H), 1.95–2.02 (m, 4H), 3.09–3.22 (m, 4H, 2 × CH_2N), 3.92–4.08 (m, 4H, 2 × CH_2O), 4.30–4.35 (m, 6H, 2 × (CH (Arg) and CH_2Ph)), 7.03 (d, J = 8.4 Hz, 2H, ArH), 7.16–7.33 (m, 14H, ArH), 7.49 (d, J = 9.0 Hz, 2H, ArH), 7.88 (d, J = 8.1 Hz, 2H, ArH), 7.97 (d, J = 9.0 Hz, 2H, ArH), 8.47 (app t, 2H, NH); ^{13}C NMR (75 MHz, CD_3OD) δ 26.3, 30.3, 32.9, 41.9, 44.0, 54.3, 69.7, 116.8, 121.6, 124.7, 126.3, 127.2, 128.2, 128.4, 129.1, 129.6, 130.5, 130.9, 135.4, 139.7, 135.5, 158.5, 174.0, 175.7. MS (ESI +ve) *m/z* 949 ($[M + H]^+$, 10%), 475 ($[M + 2H]^{2+}$, 100). HRMS (ESI +ve) calcd for $C_{54}H_{65}N_{10}O_6$ 949.5089, found 949.5071.

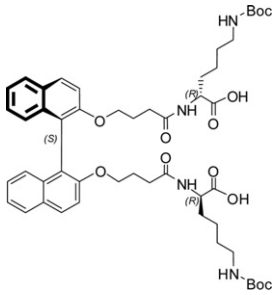
*4.3.15. Di(1-methylpyridyl) (2R)-7,7′-((S)-1,1′-binaphthyl-2,2′-bisoxy)-2,2′-di(3-guanidinopropyl)-3,3′-diaza-4,4′-dioxodiheptanoate dihydrochloride (**29**)*



The protected precursor was prepared *via* protocol 1 using **25** (60 mg, 0.046 mmol) and 2-pyridinemethanol (0.02 mL) to yield **27** as an impure light brown solid (44 mg, 64%, MS (ESI +ve) *m/z* 1485.5 (10%) $[M + H]^+$; 743.3 (20) $[M + 2H]^{2+}$). This was then deprotected directly *via* protocol 3 to yield **29** as an off white solid (28 mg, 90%). ^{1}H NMR (300 MHz, CD_3OD) δ 1.47–1.84 (m, 10H), 1.85–2.04 (m, 6H), 3.05–3.23 (m, 4H), 3.85–4.09 (m, 4H), 4.30–4.42 (m, 2H), 5.44 (ABq, J = 14.9 Hz, 4H), 6.94–6.96 (m, 2H), 7.09–7.14 (m, 2H), 7.21–7.26 (m, 2H), 7.43–7.46 (m, 2H), 7.80–7.98 (m, 8H), 8.41–8.46 (m, 2H), 8.68–8.80 (m, 2H); ^{13}C NMR (75 MHz,

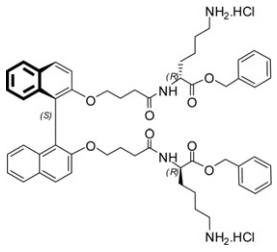
CD_3OD) δ 26.3, 26.7, 29.0, 32.7, 41.9, 53.5, 63.6, 69.9, 116.7, 121.4, 124.7, 126.2, 127.2, 127.2, 127.5, 129.0, 130.5, 130.8, 135.3, 143.7, 147.5, 152.2, 155.5, 158.5, 172.5, 176.0. MS (ESI +ve) *m/z* 477.5 (100%) $[M + 2H]^{2+}$.

*4.3.16. (2R)-7,7′-((S)-1,1′-binaphthyl-2,2′-bisoxy)-2,2′-di[(1,1′-dimethylethoxycarbonylamino)butyl]-3,3′-diaza-4,4′-dioxodiheptanoic acid (**30**)*



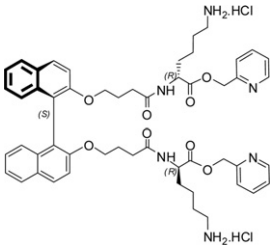
To a soln of **20** (200 mg, 0.212 mmol) in THF (20 mL), was added a solution of LiOH (75 mg, 3.14 mmol) in water (10 mL). After stirring at room temperature for 90 min, ethyl acetate was added and the layers were separated. The aqueous layer was then acidified with a dilute potassium bisulfate solution. This was then extracted with CH_2Cl_2 (3 × 20 mL) and then the solvent was removed under reduced pressure to yield **30** (178 mg, 92%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21–1.26 (m, 4H), 1.30–1.62 (m, 4H), 1.40 (s, 18H), 1.62–1.86 (m, 12H), 2.90–3.11 (m, 4H), 3.89–3.91 (m, 2H), 4.01–4.15 (m, 2H), 4.38–4.54 (m, 2H), 4.79–4.81 (m, 2H, 2 × NHBoc), 6.20–6.40 (m, 2H, 2 × NH), 7.12 (d, J = 8.1 Hz, 2H), 7.19 (app t, 2H), 7.30 (t, J = 7.2 Hz, 2H), 7.41 (d, J = 9.0 Hz, 2H), 7.84 (d, J = 7.8 Hz, 2H), 7.92 (d, J = 8.7 Hz, 2H), 10.51 (br s, 2H, 2 × OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.7, 22.4, 25.0, 29.2, 31.3, 31.5, 40.0, 51.7, 68.1, 79.2, 115.8, 120.5, 123.7, 125.2, 126.3, 127.8, 129.2, 129.3, 134.0, 153.7, 156.2, 158.0, 173.5, 175.5. MS (ESI +ve) *m/z* 937 (80%) $[M + Na]^+$; 915 (100) $[M + H]^+$.

*4.3.17. Dibenzyl (2R)-7,7′-((S)-1,1′-binaphthyl-2,2′-bisoxy)-2,2′-dibutylamino-3,3′-diaza-4,4′-dioxodiheptanoate dihydrochloride (**34**)*



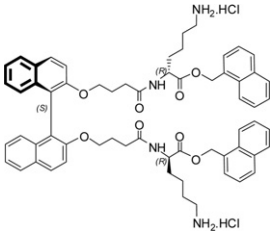
The title compound was prepared *via* protocol 4, using **30** (120 mg, 0.131 mmol), triphenylphosphine (73 mg, 0.278 mmol), DIAD (0.055 mL, 0.275 mmol) and BnOH (0.05 mL, 0.275 mmol). The Boc-protected intermediate **31** eluted at the same time as a reaction by product, and so this material was then deprotected *via* protocol 3 to yield the desired product **34** as a pale yellow hydrochloride salt (101 mg, 80%). ^{1}H NMR (300 MHz, CD_3OD) δ 1.20–1.40 (m, 4H), 1.49–1.72 (m, 10H), 1.67–1.84 (m, 2H), 1.85–2.01 (m, 4H), 2.70–2.87 (m, 4H), 3.82–4.06 (m, 4H), 4.27–4.32 (m, 2H), 5.08 (ABq, J = 12.3 Hz, 4H), 6.98 (dist d, J = 8.5 Hz, 2H), 7.11 (app t, 2H), 7.21–7.36 (m, 10H), 7.41–7.50 (m, 2H), 7.55–7.61 (m, 2H), 7.81 (d, J = 7.9 Hz, 2H), 7.91 (d, J = 8.8 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 21.2, 22.6, 25.6, 26.8, 30.6, 31.7, 39.3, 52.4, 66.8, 68.5, 115.6, 120.4, 123.6, 125.1, 126.1, 128.0, 128.2, 128.3, 218.5, 128.8, 129.0, 129.4, 129.7, 131.8, 132.0, 132.7, 134.2, 136.1, 154.3, 172.0, 174.6. MS (ESI +ve) *m/z* 895.5 (10%) $[M + H]^+$; 825.4 (40) $[M - lys]^+$; 448.7 (100) $[M + 2H]^{2+}$. HRMS (ESI +ve) calcd for $C_{54}H_{63}N_4O_8$ 895.4646, found 895.4670.

*4.3.18. Di(2-methylpyridyl) (2R)-7,7′-((S)-1,1′-binaphthyl-2,2-bisoxy)-2,2′-dibutyl-3,3′-diaza-4,4′-dioxodiheptanoate dihydrochloride (**35**)*



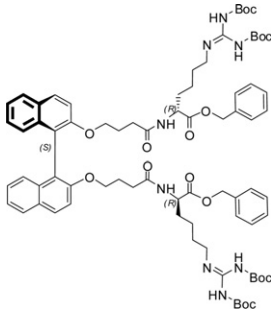
The crude Pmc protected precursor **32** was prepared *via* protocol 4, using **30** (120 mg, 0.131 mmol) and 2-pyridinemethanol (0.026 mL) to yield an impure light brown solid. This was then deprotected *via* protocol 3 to yield **35** as an off white solid (68 mg, 54%). ^{1}H NMR (300 MHz, CD_3OD) δ 1.28–1.51 (m, 4H), 1.52–1.79 (m, 8H), 1.80–2.03 (m, 4H), 2.78–2.94 (m, 4H), 3.94–3.98 (m, 4H), 4.25–4.38 (m, 2H), 5.45 (ABq, J = 14.4 Hz, 4H), 6.94–6.97 (m, 2H), 7.11–7.16 (m, 2H), 7.25 (t, 2H), 7.44–7.47 (m, 2H), 7.82 (d, J = 7.9 Hz, 2H), 7.86–7.96 (m, 4H), 7.99–8.01 (m, 2H), 8.41–8.52 (m 2H), 8.74–8.75 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.3, 26.7, 29.4, 31.7, 32.8, 41.8, 53.4, 67.9, 69.6, 116.7, 121.4, 124.6, 126.2, 127.2, 129.1, 129.3, 129.4, 129.6, 130.5, 130.8, 135.3, 137.0, 155.4, 158.4173.0, 173.2. MS (ESI +ve) *m/z* 449.4 (100%) $[M + 2H]^{2+}$.

*4.3.19. Di(1-naphthalenylmethyl) (2R)-7,7′-((S)-1,1′-binaphthyl-2,2′-bisoxy)-2,2′-dibutylamino-3,3′-diaza-4,4′-dioxodiheptanoate dihydrochloride (**36**)*



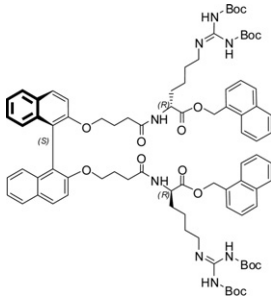
The title compound was prepared *via* protocol 4, using **30** (60 mg, 0.066 mmol), triphenylphosphine (73 mg, 278 mmol), DIAD (0.055 mL, 0.275 mmol) and 1-naphthalene methanol (45 mg, 0.028 mmol). The Boc-protected intermediate **33** eluted at the same time as a reaction by product, and so this material was then deprotected *via* protocol 3 to yield the desired product **36** as a pale yellow hydrochloride salt (58 mg, 83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.19–1.25 (m, 4H), 1.48–1.72 (m, 12H), 1.78–1.94 (m, 4H), 2.64–2.68 (m, 4H), 3.80–3.93 (m, 4H), 4.22–4.45 (m, 2H), 5.54 (ABq, J = 12.3 Hz, 4H), 6.96–7.00 (m, 2H), 7.11 (app t, 2H), 7.23 (app t, 2H), 7.32–7.50 (m, 10H), 7.73–7.90 (m, 8H), 7.95 (d J = 8.4 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 23.6, 26.5, 27.8, 31.6, 32.7, 40.3, 53.6, 66.3, 69.5, 116.7, 121.4, 124.6, 126.2, 126.3, 127.1, 127.2, 127.7128.9, 129.0, 129.7, 130.5, 130.7, 132.4, 132.8, 132.9, 135.1, 135.3, 142.9, 155.3, 173.1, 175.6. MS (ESI +ve) *m/z* 995.5 (10%) $[M + H]^+$; 825.4 (40) $[M - lys]^+$; 448.7 (100) $[M + 2H]^{2+}$. HRMS (ESI +ve) calcd for $C_{62}H_{67}N_4O_8$ 995.4959, found 995.4990.

*4.3.20. Dibenzyl (2R)-7,7′-((S)-1,1′-binaphthyl-2,2-bisoxy)-2,2′-di[4-(1,1′-dimethylcarbonylbutyl)guanidinobutyl]-3,3′-diaza-4,4′-dioxodiheptanoate (**37**)*



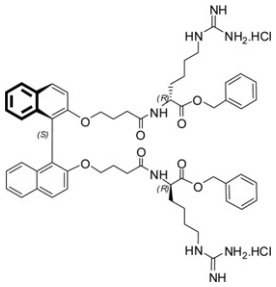
To a soln of **34** (20 mg, 0.021 mmol) in CH_2Cl_2 (2 mL) was added triethylamine (0.09 mL) and *N,N′*-bis(*tert*-butoxycarbonyl)-*N″*-triflylguanidine (25 mg, 0.062 mmol) under nitrogen. The solution was allowed to stir overnight before being evaporated to dryness. The resultant residue was then subjected to flash column chromatography using 2% MeOH/CH_2Cl_2 as the eluant to yield the desired compound **37** as a pale yellow oil (23 mg, 79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.11–1.23 (m, 4H), 1.38–1.61 (m, 10H), 1.48 (s, 18H), 1.49 (s, 18H), 1.65–1.80 (m, 6H), 3.20–3.32 (m, 4H), 3.79–3.86 (m, 2H), 4.06–4.11 (m, 2H), 4.41–4.48 (m, 2H), 5.16 (ABq, J = 12.3 Hz, 4H), 5.54 (d, J = 8.2 Hz, 2H, 2 × NH), 7.16 (dist d, J = 8.2 Hz, 2H), 7.23–7.27 (m, 2H), 7.31–7.40 (m, 15H), 7.44–7.52 (m, 1H), 7.53–7.78 (m, 2H), 7.87 (d, J = 7.9 Hz, 2H), 7.94 (d, J = 9.1 Hz, 2H), 8.28 (br s, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.5, 24.8, 28.0, 28.2, 28.4, 31.6, 31.8, 40.5, 51.7, 67.1, 68.0, 79.4, 83.2, 107.8, 115.6, 120.4, 121.5, 123.8, 125.4, 126.4, 128.0, 128.3, 128.5, 128.6, 129.2, 129.4, 132.0, 132.1, 134.1, 135.2, 153.2, 153.8, 156.1, 163.4, 172.0, 172.7. MS (ESI +ve) *m/z* 1401.7 (40%) $[M + Na]^+$; 1379 (100) $[M + H]^+$.

*4.3.21. Di(1-naphthalenemethyl) (2R)-7,7′-((S)-1,1′-binaphthyl-2,2-bisoxy)-2,2′-di[4-(1,1′-dimethylcarbonylbutyl)guanidinobutyl]-3,3′-diaza-4,4′-dioxodiheptanoate (**38**)*



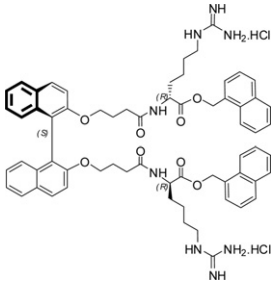
To a soln of **36** (47 mg, 0.044 mmol) in CH_2Cl_2 (2 mL) was added triethylamine (0.19 mL) and *N,N′*-bis(tert-butoxycarbonyl)-*N″*-triflylguanidine (53 mg, 0.13 mmol) under nitrogen. The solution was allowed to stir overnight before being evaporated to dryness. The resultant residue was then subjected to flash column chromatography eluting with 2% MeOH/CH_2Cl_2 to yield the desired compound **38** as an off white solid (40 mg, 61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99–1.57 (m, 14H), 1.47 (s, 18H), 1.48 (s, 18H), 1.63–1.71 (m, 6H), 3.11–3.18 (m, 4H), 3.70–3.77 (m, 2H), 3.97–4.03 (m, 2H), 4.38–4.45 (m, 2H), 5.51 (d, J = 7.9 Hz, 2H, NH), 5.61 (ABq, J = 12.3 Hz, 4H), 7.12 (dist d, J = 7.9 Hz, 2H), 7.18–7.32 (m, 6H), 7.44–7.60 (m, 8H), 7.78–7.82 (m, 4H), 7.87–7.92 (m, 4H), 7.97 (dist d, J = 7.9 Hz, 2H), 8.24 (br s, 2H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.4, 24.8, 28.0, 28.2, 28.3, 31.6, 40.5, 51.8, 53.4, 65.6, 67.9, 79.5, 83.2, 115.5, 120.4, 121.5, 123.4, 123.8, 125.2, 126.1, 126.4, 126.7, 128.0, 128.8, 129.2, 129.4, 129.7, 130.7, 131.5, 133.7, 134.0, 153.2, 153.6, 156.1, 163.3, 172.0, 172.9. MS (ESI +ve) *m/z* 1501.8 (10%) [M + Na]+; 1479.7 (10) $[M + H]^+$; 740.5 (20) $[M + 2H]^{2+}$.

*4.3.22. Dibenzyl (2R)-7,7′-((S)-1,1′-binaphthyl-2,2′-bisoxy)-2,2′-di(3-guanidinobutyl)-3,3′-diaza-4,4′-dioxodiheptanoate dihydrochloride (**39**)*



The title compound was prepared *via* protocol 3, using **37** (20 mg, 0.014 mmol) to yield the desired product **39** as a light brown solid (15 mg, 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16–1.31 (m, 4H), 1.41–1.32 (m, 12H), 1.36–1.49 (m, 4H), 3.06–3.09 (m, 4H), 3.96–4.04 (m, 4H), 4.24–4.36 (m, 2H), 5.06–5.16 (m, 4H), 7.01 (dist d, J = 8.1 Hz, 2H), 7.17–7.20 (m, 2H), 7.30–7.31 (m, 9H), 7.44–7.55 (m, 3H), 7.58–7.65 (m, 2H), 7.85 (t, J = 6.9 Hz, 2H), 7.93–7.99 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.9, 26.7, 29.3, 32.0, 32.1, 32.8, 42.2, 67.9, 69.7 (CH_2), 53.4 (CH), 116.8, 124.7, 126.3, 127.3, 129.1, 129.3, 129.4, 129.6, 130.5 (ArCH), 121.6, 130.9, 135.4, 137.2, 155.5 (ArC), 158.6 (CN_3), 173.3, 175.7 (CO). MS (ESI +ve) *m/z* 490.5 (60%) $[M + 2H]^{2+}$; 452.4 (100) $[M + H - Ph]^{2+}$; 414.5 (80) $[M + 2H - 2Ph]^{2+}$. HRMS (ESI +ve) calcd for $C_{56}H_{67}N_8O_8$ 979.5082, found 979.5044.

*4.3.23. Dibenzyl (2R)-7,7′-((S)-1,1′-binaphthyl-2,2-bis(oxy)-2,2′-di(4-guanidinobutyl)-3,3′-diaza-4,4′-dioxodiheptanoate dihydrochloride (**40**)*



The title compound was prepared *via* protocol 3, using **38** (38 mg, 0.026 mmol) to yield the desired product **40** as a light brown solid (18 mg, 61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.09–1.45 (m, 6H), 1.46–1.80 (m, 10H), 1.81–1.98 (m, 4H), 2.85–3.00 (m, 2H), 3.02–3.15 (m, 2H), 3.83–4.04 (m, 4H), 4.20–4.35 (m, 2H), 5.49–5.37 (m, 4H), 6.98–7.02 (m, 2H), 7.13–7.18 (m, 2H), 7.22–7.30 (m, 2H), 7.33–7.51 (m, 10H), 7.79–7.98 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.7, 22.8, 25.5, 25.55, 28.0, 28.1, 30.7, 30.8, 31.55, 31.6, 40.95, 41.0, 52.2, 52.5, 65.2, 68.3, 68.5, 115,5, 115.6, 120.35, 120.4, 123.5, 125.1, 125.2, 125.5, 126.0, 126.1, 126.5, 127.8, 127.9, 128.6, 129.3, 129.4, 129.65, 129.7, 131.4, 131.8, 134.1, 134.2, 154.25, 154.3, 157.4, 172.1, 172.8, 174.5. MS (ESI +ve) *m/z* 540.4 (20%) $[M + 2H]^{2+}$; 477.3 (95) [M + H-naphthyl]$^{2+}$; 414.4 (100) $[M + H - 2 \times \text{naphthyl}]^{2+}$. HRMS (ESI +ve) calcd for $C_{64}H_{71}N_8O_8$ 1079.5395, found 1079.5409.

4.4. Determination of Minimum Inhibitory Concentration (MIC)

MIC studies were performed on *S. aureus* wild type (ATCC 6538P) in Luria Broth. MIC determinations for wild type and clinical isolates of *E. faecium* were conducted by growth in Enterococcosal broth

(Becton Dickinson Microbiology Systems). Briefly, overnight stationary phase cultures were diluted 1:1000 into fresh media and then incubated with two fold dilutions of compound in media, typically with a highest concentration of 125 μg/mL, in a 96 well plate. Plates were incubated overnight at 37 °C and the MIC recorded as the highest concentration at which bacterial growth was observed. Dilution factors give rise to concentrations of 125, 62.5, 31.3, 15.6, 7.8, 3.9 and 1.95 μg/mL which are subsequently rounded off.

Acknowledgements

We thank Avexa Limited, the University of Wollongong and the National Health and Medical Research Council (Development Grants 303415 and 404528) for their support and Dr Susan Cox for her support in the initial development of this project.

References

[1] A. Srinivasan, J.D. Dick, T.M. Perl, Clin. Microbiol. Rev. 15 (2002) 430–438.
[2] B. Bagga, J.L. Shenep, Pediatr. Infect. Dis. J. 29 (2010) 662–664.
[3] D. Kahne, C. Leimkuhler, W. Lu, C. Walsh, Chem. Rev. 105 (2005) 425–448.
[4] D.P. Levine, South. Med. J. 101 (2008) 284–291.
[5] D.M. Sievert, J.T. Rudrik, J.B. Patel, L.C. McDonald, M.J. Wilkins, J.C. Hageman, Clin. Infect. Dis. 46 (2008) 668–674.
[6] R.D. Gonzales, P.C. Schreckenberger, M.B. Graham, S. Kelkar, K. DenBesten, J.P. Quinn, Lancet 357 (2001) 1179–1183.
[7] S.J. Projan, P.A. Bradford, Curr. Opin. Microbiol. 10 (2007) 441–446.
[8] J. Lu, O. Yoshida, S. Hayashi, H. Arimoto, Chem. Commun. (2007) 251–253.
[9] M.B. Strom, B.E. Haug, M.L. Skar, W. Stensen, T. Stiberg, J.S. Svendsen, J. Med. Chem. 46 (2003) 1567–1570.
[10] B.E. Haug, W. Stensen, W. Stiberg, J.S. Svendsen, J. Med. Chem. 47 (2004) 4159–4162.
[11] B.E. Haug, W. Stensen, J.S. Svendsen, Bioorg. Med. Chem. Lett. 17 (2007) 2361–2364.
[12] B.E. Haug, W. Stensen, M. Kalaaji, O. Rekdal, J.S. Svendsen, J. Med. Chem. 51 (2008) 4306–4314.
[13] K. Flemming, C. Klingenberg, J.P. Cavanagh, M. Sletteng, W. Stensen, J.S. Svendsen, T. Flaegstad, J. Antimicrob. Chemother. 63 (2009) 136–145.
[14] J.B. Bremner, J.A. Coates, D.R. Coghlan, D.M. David, P.A. Keller, S.G. Pyne, New J. Chem. 26 (2002) 1549–1552.
[15] V.S. Au, J.B. Bremner, J.A. Coates, P.A. Keller, S.G. Pyne, Tetrahedron 62 (2006) 9373–9382.
[16] J.B. Bremner, J.A. Coates, P.A. Keller, S.G. Pyne, H.M. Witchard, Tetrahedron 59 (2003) 8741–8755.
[17] T.P. Boyle, J.B. Bremner, J.A. Coates, J. Deadman, P.A. Keller, S.G. Pyne, D. Rhodes, Tetrahedron 64 (2008) 11270–11290.
[18] J.B. Bremner, S.G. Pyne, P.A. Keller, D.R. Coghlan, A. Garas, H.M. Witchard, T.P. Boyle, J.A. Coates, WO 03/002545 A1, Peptoid Compounds, AU2002/00850 (2003).
[19] A. Garas, J.B. Bremner, J. Coates, J. Deadman, P.A. Keller, S.G. Pyne, D.I. Rhodes, Bioorg. Med. Chem. Lett. 19 (2009) 3010–3013.
[20] J.B. Bremner, P.A. Keller, S.G. Pyne, T.P. Boyle, Z. Brkic, D.M. David, J. Morgan, M. Robertson, K. Somphol, P. Ambrose, S. Bhavnani, T.R. Fritsche, D.J. Biedenbach, R.N. Jones, M.H. Miller, R.W. Buckheit Jr., K.M. Watson, D. Baylis, J.A. Coates, J. Deadman, D. Jeevarajah, A. McCracken, D.I. Rhodes, Angew. Chem. Int. Ed. 49 (2010) 537–540.
[21] J.B. Bremner, P.A. Keller, S.G. Pyne, T.P. Boyle, Z. Brkic, D.M. David, M. Robertson, K. Somphol, D. Baylis, J.A. Coates, J. Deadman, Da. Jeevarajah, D.I. Rhodes, Bioorg. Med. Chem. 18 (2010) 2611–2620.
[22] J.B. Bremner, P.A. Keller, S.G. Pyne, T.P. Boyle, Z. Brikic, J. Morgan, K. Somphol, J.A. Coates, J. Deadman, D.I. Rhodes, Bioorg. Med. Chem. 18 (2010) 4793–4800.
[23] Our previous publications reported antibacterial activities as μg/mL not rounded off – we have reported the values here rounded off and in μM.
[24] B.S. Rasmussen, U. Elezcano, T. Skrydstrup, J. Chem.Soc. Perkin Trans. 1 14 (2002) 1723–1733.
[25] R.J. Biediger, H. Bui, K.M. Henry, T. Thrash, PCT Int. Appl. WO2008079371 (2008).