Bioorganic & Medicinal Chemistry 18 (2010) 4793–4800

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and antibacterial studies of binaphthyl-based tripeptoids. Part 2 ☆

John B. Bremner [a,*], Paul A. Keller [a,*], Stephen G. Pyne [a,*], Timothy P. Boyle [a], Zinka Brkic [a], Jody Morgan [a], Kittiya Somphol [a], Jonathan A. Coates [b,†], John Deadman [b,†], David I. Rhodes [b,†]

[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 23 March 2010
Revised 30 April 2010
Accepted 1 May 2010
Available online 2 June 2010

Keywords:
Antibacterial
Cationic peptides
Tripetoid
Binaphthyl

ABSTRACT

A compact synthesis of 15 new binaphthyl-based dicationic tripeptoids and one biphenyl based dicationic tripeptoid is described. Fourteen of these tripeptoids resulted from variation of the C-2′ ether substituent of the binaphthyl unit. An *O-iso*-butyl ether binaphthyl derivative was found to be the most active against *Staphylococcus aureus* (MIC 1.95 μg/mL). The biphenyl analogue also showed good activity against *S. aureus* (MIC 1.95 μg/mL). These compounds, however, were less active against four vancomycin-resistant strains of enterococci (VRE) than some of our previously developed compounds that had an *O-iso*-pentyl ether substituent on the binaphthyl unit and a C-2 L-Leu moiety.

1. Introduction

The development of new antibacterial agents, especially those active against the increasing number of drug resistant pathogenic strains,[2–4] including those resistant to vancomycin,[5,6] is of growing importance.[7,8] From our previous antibacterial studies on acyclic 1,1′-binaphthyl-based dicationic peptides we identified the peptoid **1** as a promising lead compound with good activity against the problematic human pathogenic bacterium *Staphylococcus aureus* (MIC 1.95 μg/mL).[1] From this platform the compounds **2**,[9] **3**,[9] and **4**[1] were developed that showed very promising in vitro antibacterial activity against a range of Gram-positive pathogens, including organisms resistant to vancomycin, methicillin (MRSA, MIC 4 μg/mL) and linezolid.[9] However the compounds were generally less active against Gram-negative bacteria (Fig. 1).

In part 1 of this series we showed the importance of cationic character within the tripeptoid and indicated that the nature and placement of these cationic residues was important in determining antibacterial activity.[1] We clearly demonstrated that cationic residues at C-5 and C-8 (especially C-5 D-Arg and C-8 D-Lys) were critical for good antibacterial activity against vancomycin-resistant enterococci (VRE) strains.[1] The importance of the hydrophobic groups at both termini of these dicationic tripeptoids was also shown but only in a limited manner. In this paper we wish to disclose our studies to optimise the hydrophobic N-terminus of these tripeptoids with systematic changes being made at the C-2′ ether substituent of the 1,1′-binaphthyl unit while maintaining a benzyl ester moiety at the C-terminus. Having optimised this ether substituent, an analogous dicationic tripeptoid having a 1,1′-biphenyl N-terminus was prepared and a comparison made of its antibacterial activities with the corresponding 1,1′-binaphthyl compound **4**. These studies ultimately led, together with other changes as will be outlined in later papers, to the development of the lead compounds **2** and **3**.

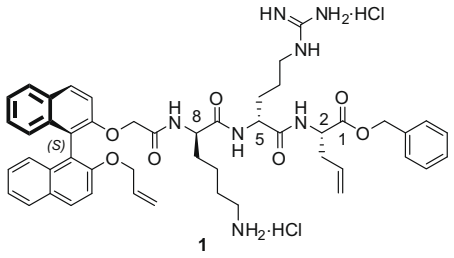
2. Results and discussion

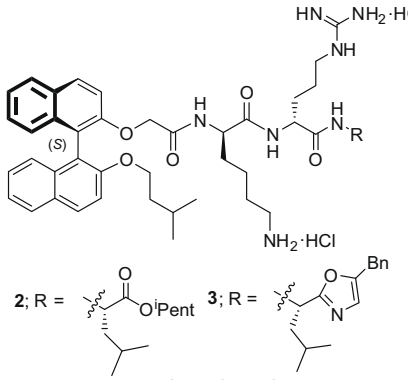
The synthesis of the target peptoids **51**–**64** (Scheme 3), in which systematic changes were made to the C-2′ ether substituent of the binaphthyl unit, began from (*S*)-1,1′-binaphthalene-2,2′-diol **5** (Schemes 1 and 2). From earlier work we had a supply of the ester **6**[1,9] that was prepared from **5** as shown in Scheme 1. This compound readily underwent de-O-allylation upon exposure to $Pd(PPh_3)_4$ in the presence of morpholine and the resulting phenolic ester **7** was O-alkylated with 2-cyclopropylethanol or 2-methoxymethanol under Mitsunobu reaction conditions using and PPh_3 and DIAD in THF to give the corresponding ethers **8** and **9**, in yields of 57% and 87%, respectively. The cyanomethyl ether **10** was efficiently prepared by a base catalysed alkylation of **7** with α-chloroacetonitrile in the presence of lithium iodide in 79% yield. The direct alkylation of **5** using this procedure was much less successful and produced the mono-cyanomethylated product in only 33% yield. Saponification of esters **8**–**10** with $LiOH/H_2O$/THF followed

☆ See Ref. 1.

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

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.05.005





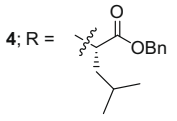
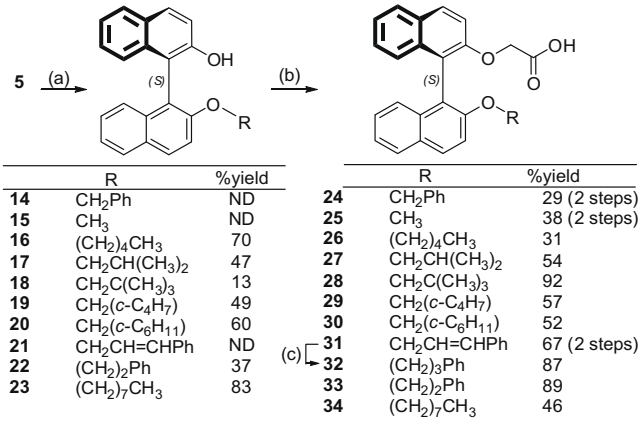


Figure 1. MIC value against *S. aureus* of peptoids **1**, MIC values against methicillin resistant *S. aureus* (MRSA) of peptoids **2** and **3** and the range of MIC values against vancomycin-resistant enterococci strain (VRE) of peptoid **4**.



	R	%yield		R	%yield
14	CH_2Ph	ND	**24**	CH_2Ph	29 (2 steps)
15	CH_3	ND	**25**	CH_3	38 (2 steps)
16	$(CH_2)_4CH_3$	70	**26**	$(CH_2)_4CH_3$	31
17	$CH_2CH(CH_3)_2$	47	**27**	$CH_2CH(CH_3)_2$	54
18	$CH_2C(CH_3)_3$	13	**28**	$CH_2C(CH_3)_3$	92
19	$CH_2(c\text{-}C_4H_7)$	49	**29**	$CH_2(c\text{-}C_4H_7)$	57
20	$CH_2(c\text{-}C_6H_{11})$	60	**30**	$CH_2(c\text{-}C_6H_{11})$	52
21	$CH_2CH{=}CHPh$	ND	(c) **31**	$CH_2CH{=}CHPh$	67 (2 steps)
22	$(CH_2)_2Ph$	37	**32**	$(CH_2)_3Ph$	87
23	$(CH_2)_7CH_3$	83	**33**	$(CH_2)_2Ph$	89
			34	$(CH_2)_7CH_3$	46

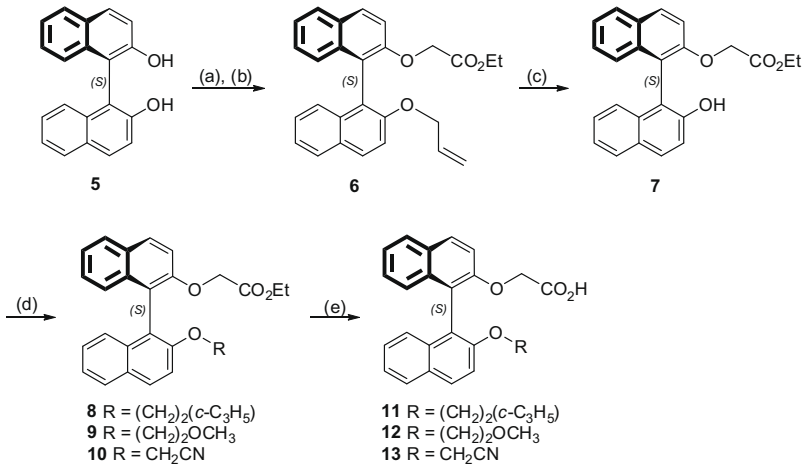
Scheme 2. Synthesis of compounds **24**–**34**. Reagents and conditions: (a) RX, K_2CO_3, acetone, reflux, 16 h to 3 days, (for **23**: $Cl(CH_2)_7CH_3$, K_2CO_3, KI, acetone, reflux, 3 days, for **18**: $BrCH_2C(CH_3)_3$, K_2CO_3, DMF, 80 °C, 7 days); (b) $BrCH_2COOH$, K_2CO_3, MeOH, 3 h to 4 days; (c) H_2, 5% Pd–C, rt, 16 h. The yields for **14**, **15** and **21** were not determined, the overall yields for the two steps is given for their corresponding acids **24**, **25** and **67**, respectively.

by acidification gave the corresponding carboxylic acids **11**–**13**, respectively (Scheme 1). In an alternative and more direct approach, the C-2′ ether substituent of the binaphthyl unit was introduced first by direct base catalysed mono-alkylation of **5** with an alkyl halide followed by introduction of the carboxylic acid linker in a subsequent base catalysed alkylation reaction of the initial products **14**–**23** with α-bromoacetic acid (Scheme 2). The alkylation reactions of **5** proceeded in moderate to good yields, except for the alkylation with the sterically hindered *neo*-pentylbromide that gave **18** in only 13% yield. In the case of the acids **24**, **25** and **31** their precursors, **14**, **15** and **21**, respectively, were not isolated but converted directly to their corresponding acids.

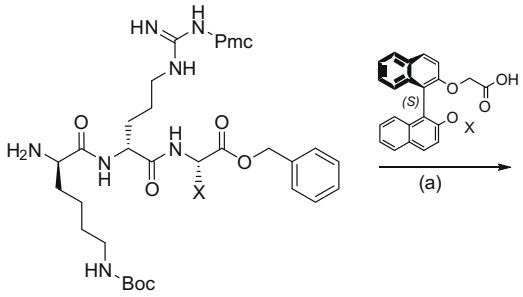
The acids **11**–**13** and **24**–**34** were then coupled to the protected dipeptides **35**[1] or **36**[1] (X = D-allyl-Gly or D-Leu) under the standard conditions using EDCI and HOBt in MeCN that we have described earlier (Scheme 3).[1,9] Deprotection of the resulting tripeptides **37**–**50** with TFA and then anion exchange with HCl in diethyl ether gave the target tripeptoids **51**–**64** as their hydrochloride salts. Deprotection of **44** gave the C-2′ hydroxy substituted binaphthyl tripeptoid derivative **58** from acid catalysed cleavage of the cinnamyl ether group.

In order to avoid possible later problems with the biological oxidation of the allyl residues in compound **1**, both allyl groups were converted to propyl groups by hydrogenation over Pd/C to give, after deprotection, the dicationic tripeptoid **66** as its hydrochloride salt (Scheme 4).

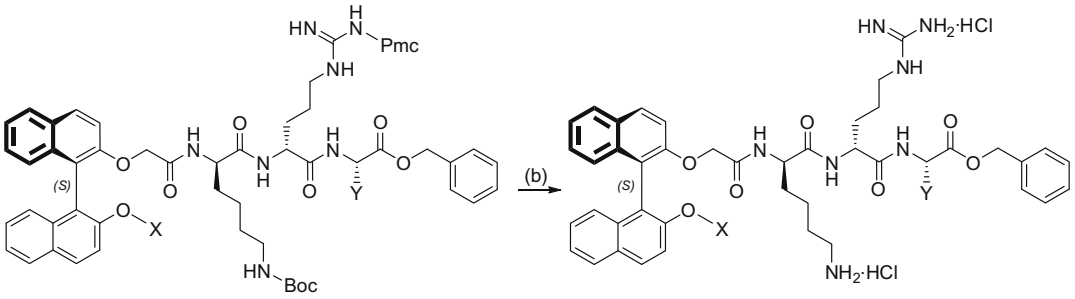
The biphenyl analogue **73** of **4** was prepared from 1,1′-biphenyl-2,2′-diol **67** in an analogous way to **51**–**64** (Scheme 5). The



Scheme 1. Synthesis of compound **11**–**13**. Reagents and conditions: (a) allyl bromide, K_2CO_3, CH_3COCH_3, reflux, overnight, 86%; (b) $BrCH_2CO_2Et$, K_2CO_3, CH_3COCH_3, reflux, overnight, 95%; (c) $Pd(PPh_3)_4$, morpholine, THF, rt, 24 h, 71%; (d) for **8** and **9**: R-OH, PPh_3, DIAD, THF, 0 °C to rt, overnight, 57% (**8**), 87% (**9**). For **10**: $ClCH_2CN$, K_2CO_3, KI, CH_3COCH_3, reflux, 18 h, 79%; (e) LiOH, H_2O, THF, 0 °C to rt, 3 h, 89% (**11**), 96% (**12**), 83% (**13**).



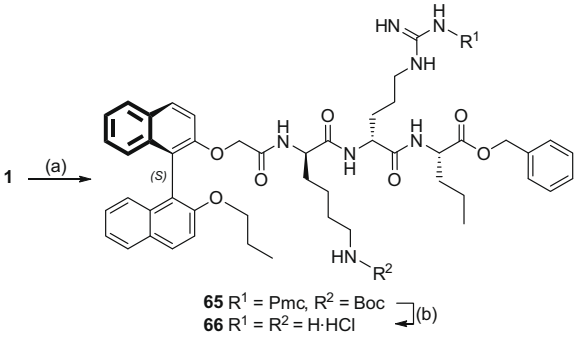
35, X = allyl-Gly—
36, X = Leu



	X	Y	%Yield
37	CH_2Ph	allyl-Gly	71
38	CH_3	allyl-Gly	66
39	$(CH_2)_4CH_3$	allyl-Gly	95
40	$CH_2CH(CH_3)_2$	allyl-Gly	70
41	$CH_2C(CH_3)_3$	allyl-Gly	61
42	$CH_2(c\text{-}C_4H_7)$	allyl-Gly	55
43	$CH_2(c\text{-}C_6H_{11})$	allyl-Gly	79
44	$CH_2CH{=}CHPh$	allyl-Gly	67
45	$(CH_2)_3Ph$	allyl-Gly	80
46	$(CH_2)_2Ph$	Leu	56
47	$(CH_2)_7CH_3$	Leu	46
48	$(CH_2)_2(c\text{-}C_3H_5)$	Leu	84
49	$(CH_2)_2OCH_3$	Leu	68
50	CH_2CN	Leu	70

	X	Y	%Yield
51	CH_2Ph	allyl-Gly	76
52	CH_3	allyl-Gly	72
53	$(CH_2)_4CH_3$	allyl-Gly	65
54	$CH_2CH(CH_3)_2$	allyl-Gly	56
55	$CH_2C(CH_3)_3$	allyl-Gly	65
56	$CH_2(c\text{-}C_4H_7)$	allyl-Gly	65
57	$CH_2(c\text{-}C_6H_{11})$	allyl-Gly	65
58	H	allyl-Gly	96
59	$(CH_2)_3Ph$	allyl-Gly	82
60	$(CH_2)_2Ph$	*i*Bu	65
61	$(CH_2)_7CH_3$	*i*Bu	65
62	$(CH_2)_2(c\text{-}C_3H_5)$	*i*Bu	65
63	$(CH_2)_2OCH_3$	*i*Bu	63
64	CH_2CN	*i*Bu	66

Scheme 3. Synthesis of tripeptoids **51**–**64**. Reagents and conditions: (a) binaphthyloxyacetic acid (**11**–**13** and **24**–**34**) EDCI, HOBt, CH_3CN, rt, 3 h; (b) TFA, CH_2Cl_2, rt, overnight, then 1 M HCl–Et_2O.

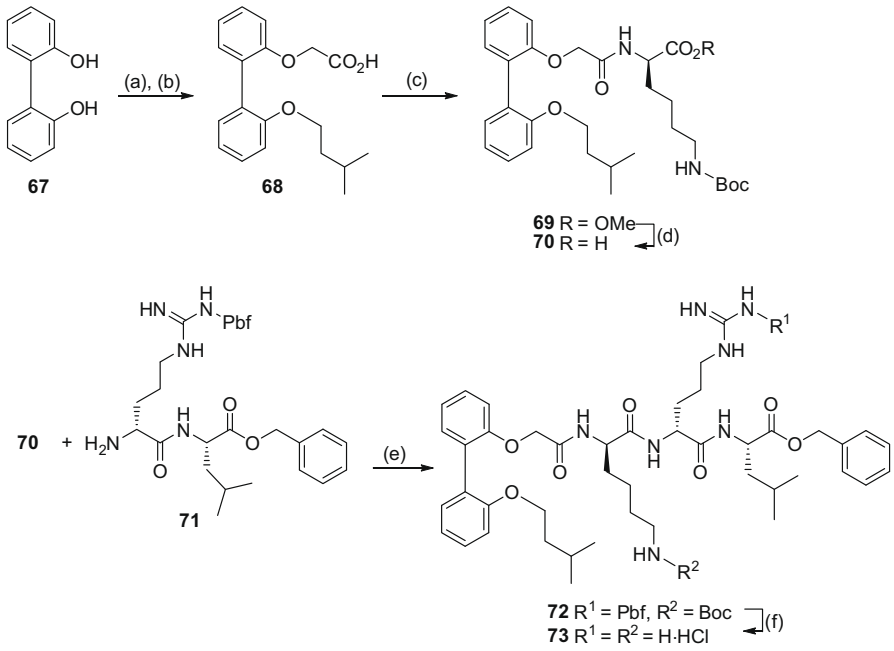


Scheme 4. Synthesis of compound **66**. Reagents and conditions: (a) H_2, Pd–C, THF, rt, 13 h, 71%; (b) TFA, CH_2Cl_2, rt, overnight, then 1 M HCl–Et_2O, 85%.

iso-pentyl substituent was first introduced by a base catalysed O-alkylation with 1-bromo-3-methylbutane in almost quantitative yield (99%), however introduction of the acetic acid residue by alkylation with α-bromoacetic acid was much less efficient (30% yield, Scheme 5). A peptide coupling reaction between the acid **68** and H-D-Lys(Boc)-OMe provided the amide **69** in 93% yield which was subsequently converted to the acid **70** by saponification with LiOH/THF/H_2O and then acidification. Coupling of this acid with the dipeptide **71**[1] using EDCI and HOBt in MeCN gave, after deprotection with TFA and anion exchange, the tripeptide **73** as its hydrochloride salt (Scheme 5).

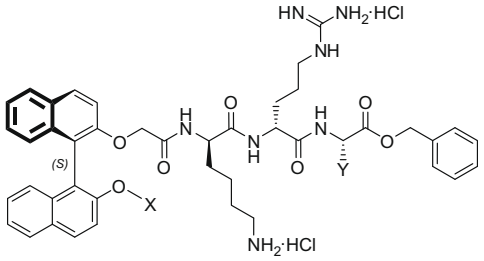
The tripeptoid hydrochloride salts were tested against the Gram-positive bacterium *S. aureus* (ATCC6538) and four clinical isolates of vancomycin-resistant enterococci (VRE) and the results are shown in Tables 1 and 2. The positive control, vancomycin, showed a MIC value of 1.95 μg/mL against *S. aureus* and had MIC values of 1.95, <0.98, 62.5 and >125 μg/mL against the vancomycin sensitive VRE_{243} and VRE_{987}, partially resistant VRE_{449} and resistant VRE_{820} enterococcal strains, respectively (Table 1, last entry).

Of the C-2 L-allyl-Gly (Y = allyl) substituted tripeptoids (**51**–**64**), the *O-iso*-butyl ether **54** was the most active against *S. aureus* (MIC 1.95 μg/mL, Table 1), slightly more active than analogue **4** (MIC 3.9 μg/mL, Table 2). The latter peptoid, however, showed a much higher activity against the four VRE strains, with MIC values ranging from 7.8–15.6 μg/mL (Table 2). The C-2 L-allyl-Gly compounds having the weakest activity against *S. aureus* were the *O*-methyl ether **52** and the free phenolic compound **58**, both with MIC values of 7.8 μg/mL (Table 1). Clearly, the reduction of hydrophobicity at the binaphthyl C-2′ site in these compounds had an adverse effect on their antibacterial activities, although the cyanomethyl ether (**64**) maintained activity against *S. aureus* possibly due to the accessibility of new counterbalancing interactions of the cyano group with the bacterial target site(s). The *O*-pentyl (**53**), *O-neo*-pentyl



Scheme 5. Synthesis of compound **73**. Reagents and conditions: (a) 1-bromo-3-methylbutane, K_2CO_3, CH_3COCH_3, reflux, 18 h, 99%; (b) bromoacetic acid, MeOH, 16 h, 30%; (c) H-D-Lys(Boc)-OMe·HCl, EDCI, HOBt, CH_3CN, rt, 3 h, 93%; (d) LiOH, H_2O, THF, 20 °C, 16 h, 62%; (e) EDCI, HOBt, CH_3CN, rt, 3 h, 71%; (f) TFA/TIPS/H_2O (95:2.5:2.5), overnight, then 1 M HCl–Et_2O, 93%.

Table 1



	X	Y	MIC$_{50}$ (μg/mL)				
			S. aureus	VRE$_{243}$	VRE$_{449}$	VRE$_{820}$	VRE$_{987}$
51	CH_2Ph	Allyl-Gly	3.9	62.5	62.5	62.5	125
52	CH_3	Allyl-Gly	7.8	125	62.5	62.5	125
53	$(CH_2)_4CH_3$	Allyl-Gly	3.9	62.5	62.5	31.3	62.5
54	$CH_2CH(CH_3)_2$	Allyl-Gly	1.95	62.5	52.1	31.3	62.5
55	$CH_2C(CH_3)_3$	Allyl-Gly	3.9	62.5	62.5	31.3	83.3
56	$CH_2(c\text{-}C_4H_7)$	Allyl-Gly	3.9	62.5	52.1	31.3	62.5
57	$CH_2(c\text{-}C_6H_{11})$	Allyl-Gly	3.9	62.5	62.5	31.3	62.5
58	H	Allyl-Gly	7.8	125	52.1	62.5	125
59	$(CH_2)_3Ph$	Allyl-Gly	3.9	62.5	31.3	31.3	62.5
60	$(CH_2)_2Ph$	*i*Bu	5.2	31.3	31.3	15.6	31.3
61	$(CH_2)_7CH_3$	*i*Bu	13	125	125	62.5	125
62	$(CH_2)_2(c\text{-}C_3H_5)$	*i*Bu	3.9	125	31.3	62.5	62.5
63	$(CH_2)_2OCH_3$	*i*Bu	3.9	125	31.3	62.5	62.5
64	CH_2CN	*i*Bu	3.9	62.5	31.3	31.3	62.5
Vancomycin			1.95	1.95	62.5	>125	<0.98

(**55**), *O*-cyclobutylmethyl (**56**), *O*-cyclohexylmethyl (**57**) and *O*-phenylpropyl (**59**) ether analogues all had MIC values of 3.9 μg/mL (Table 1). These compounds however showed much weaker activity compared to **4** and **54** against the four VRE strains (Table 1). Of the C-2 L-Leu (Y = ^{i}Bu) substituted tripeptoids (**60**–**64**), none showed superior activity to **4** (Table 2) while the *O*-octyl derivative **61** had the weakest activity of all compounds tested with a MIC of 13 μg/mL against *S. aureus* and essentially no activity against the four VRE strains (Table 1). We had anticipated that the octyl chain may help anchor our molecule to the bacterial cell membrane and enhance antibacterial activity. Clearly, however, the extra length of this hydrophobic substituent in **61** was deleterious to its antibacterial activities.

The saturated tripeptoid **66** (Table 2) showed a reduced activity against *S. aureus* (MIC value of 3.9 μg/mL) compared to its unreduced analogue **1** (MIC 1.95 μg/mL). Its activities were the same

Table 2

	MIC_{50} (μg/mL)				
	S. aureus	VRE_{243}	VRE_{449}	VRE_{820}	VRE_{987}
1	1.96	31.3	31.3	31.3	31.3
4	3.9	15.6	15.6	7.8	15.6
6	3.9	31.3	31.3	31.3	62.5
73	1.95	31.3	31.3	31.3	31.3
Vancomycin	1.95	1.95	62.5	>125	<0.98

as those of **1** against three of the VRE strains (MIC 31.3 μg/mL) but was different for the VRE_{987} strain (MIC 62.5 μg/mL for **66** and 31.3 μg/mL for **1**) (Table 2).

The peptoid **73**, the biphenyl tripeptoid analogue of **4**, had better activity against *S. aureus* (MIC 1.95 μg/mL) than **4** however it was not as active as **4** against the four VRE strains (Table 2).

Although the mode of action of the tripeptoids has not been established, our earlier results on peptoid **2** suggested that more than one mode of action was involved.[9,10] Unfortunately quantitative SAR studies on these peptoids were not productive because of the high flexibility of the structures which afforded many computationally generated low energy conformations. It is clear from this study, however, that the nature of hydrophobic C-2′ ether substituent of the 1,1′-binaphthyl unit is important for antibacterial activity. Chain branching and the length of the chain are important for activity against *S. aureus* and the four VRE strains.

In conclusion, a total of sixteen novel dicationic tripeptoids have been prepared and tested for antibacterial activity against *S. aureus* and four clinical VRE strains. Fifteen of these tripeptoids resulted from variation of the C-2′ ether substituent of the binaphthyl unit. The *O-iso*-butyl ether **54** was found to be the most active against *S. aureus* (MIC 1.95 μg/mL), more active than the previously reported compound **4**, having a *O-iso*-pentyl ether substituent on the binaphthyl unit and a C-2 L-Leu moiety. However **54** was much less active than **4** against the four VRE strains. The biphenyl analogue of **4**, the tripeptoid **73**, had a better activity against *S. aureus* (MIC 1.95 μg/mL) than **4** however it was also not as active against the four VRE strains. This study has thus provided us a platform for further development of C-2′ *O-iso*-pentyl-binaphthyl, C-2 Leu based dicationic peptoids as antimicrobial agents with improved activity against *S. aureus* and other pathogenic and drug resistant bacteria. These studies will be reported in future publications.

3. Experimental

General methods were as described previously[1] (see Supplementary data for details). See Supplementary data for compound numbering.

3.1. General synthetic procedures

3.1.1. Protocol 1: peptide coupling

To a solution of the acid in acetonitrile (10 mL/0.10 mmol) at rt was added EDCI (1.2 equiv), HOBt (1.2 equiv) and the amine (1 equiv). After stirring for 1–3 h, the solvent was removed under reduced pressure, and the resulting residue was subjected to silica gel column chromatography (1–4% MeOH/CH_2Cl_2 as the eluent) to afford a coupled product.

3.1.2. Protocol 2: *N*-Boc, Pmc and Pbf deprotection

The *N*-Boc/Pmc/Pbf protected amine was stirred for 1 h (for Boc) or overnight (for Pmc or Pbf) in a solution of 1:1 CH_2Cl_2/TFA (6 mL/0.10 mmol) at rt. The solvent was removed under reduced pressure, and the residue was re-suspended in a minimal volume of methanol or CH_2Cl_2. The solution was then treated with an excess of 2 M HCl/diethyl ether (2 mL/0.01 mmol) solution and the solvent evaporated. The crude product was purified by precipitation from CH_2Cl_2 or MeOH and diethyl ether.

3.2. Typical reaction for Scheme 2

3.2.1. (2′-Benzyloxy-1,1′-(*S*)-binaphthalen-2-yloxy)acetic acid 24

To a solution of 1,1′-(*S*)-binaphthol **5** (500 mg, 1.75 mmol) and K_2CO_3 (300 mg, 2.18 mmol) in acetone (6 mL) was added dropwise, benzyl bromide (0.21 mL, 1.75 mmol). The resulting mixture was heated at reflux with stirring for 16 h before being filtered, concentrated and dissolved in anhydrous MeOH (5 mL). To this solution was added K_2CO_3 (2.4 g, 17.4 mmol) and bromoacetic acid (740 mg, 5.25 mmol). This mixture was heated at reflux for a further 3 h before evaporation to dryness and dissolution in water (50 mL). The aqueous layer was then washed with diethyl ether (3 × 30 mL) before acidification with 3 M HCl. The acidified solution was extracted with CH_2Cl_2, dried ($MgSO_4$) before being evaporated to dryness to yield **24** (218 mg, 0.50 mmol, 29%) as a viscous yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.48 (AB_q, J = 17.1 Hz, 2H, H1″); 4.99 (AB_q, J = 12.6 Hz, 2H, CH_2-COOH); 6.86–6.89 (m, 2H, ArH); 7.00–7.07 (m, 3H, ArH); 7.10–7.21 (m, 4H, ArH); 7.25–7.39 (m, 4H, ArH); 7.78–7.92 (m, 4H, ArH); 10.30 (br s, 1H, COOH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 66.2, $ArCH_2$; 71.6, CH_2–COOH; 114.6, ArCH; 116.2, ArCH; 120.1, ArC; 120.6, ArC; 124.0, ArCH; 124.2, ArCH; 125.3, ArCH; 125.5, ArCH; 126.5, ArCH; 126.6, ArCH; 126.9, ArCH; 127.5, ArCH; 127.9, ArCH; 128.0, ArCH; 129.7 (×2) ArC; 129.8, ArC; 133.7, ArC; 133.8, ArC; 136.7, ArC; 152.7, ArC; 153.4, ArC; 172.9, COOH. MS (CI, +ve) *m/z* 435 (100%) [MH^+]. HRMS (ESI, +ve) calcd for $C_{29}H_{23}O_4$ 435.1596, found 435.1582.

3.2.2. (*S*)-2′-(Cyclohexylmethyloxy)-1,1′-binaphthalene-2-ol 20

Bromomethylcyclohexane (240 μL, 1.75 mmol) was added to a mixture of 1,1′-(*S*)-binaphthalene-2,2′-diol **5** (500 mg, 1.75 mmol), potassium carbonate (1.00 g, 7.27 mmol) and acetone (10 mL). The mixture was stirred at reflux for 3 days, and then cooled and concentrated in vacuo. The crude product was purified by flash chromatography with 1–10% ethyl acetate–petrol as eluent to give 400 mg (1.05 mmol, 60%) of the product **20** as a viscous oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.39–1.73 (m, 10H); 2.43–2.45 (m, 1H); 3.89–3.97 (m, 2H, OCH_2); 7.06 (d, J = 8.5 Hz, 1H, ArH); 7.19–7.38 (m, 8H); 7.44 (d, J = 9.2 Hz, 1H, ArH); 7.84 (d, J = 8.1 Hz, 1H, ArH); 7.89 (dd, J_1 = 6.2, J_2 = 6.2, 2H, ArH); 8.02 (d, J = 9.2 Hz, 1H, ArH). ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.5, 25.6, 26.3, 29.3 (×2), 37.5, 75.3, 109.8, 115.3, 115.7, 116.29, 117.4, 123.0, 124.1, 125.0, 126.2, 127.2, 128.0, 128.1, 129.1, 129.6, 130.8, 133.9, 134.1, 151.3, 155.7.

3.2.3. [2′-(Cyclohexylmethyl)-1,1′-(*S*)-binaphthalen-2-yloxy]-acetic acid 30

The above phenol **20** (400 mg, 1.05 mmol) was dissolved in methanol (10 mL) and then potassium carbonate (1.50 g, 10.9 mmol) and bromoacetic acid (900 mg, 6.48 mmol) were added, and the mixture was stirred at reflux for 2 days. The reaction mixture was cooled, methanol was removed in vacuo and the crude residue redissolved in water and acidified with 1 M HCl. Extraction with ether, drying (Na_2SO_4) and concentration gave the crude product that was purified by flash chromatography. Elution with chloroform gave 100 mg of unreacted **20** (25%). Further elution with 2% methanol–chloroform gave 240 mg of the acid **30** (0.55 mmol, 52%) as a viscous oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.46–0.57 (m, 2H); 0.72–0.94 (m, 3H); 1.14–1.20 (m, 2H); 1.26–1.41 (m, 4H); 3.69–3.72 (m, 1H, $OCH_2C_6H_{11}$ (H_a)); 3.77–3.81, m, $OCH_2C_6H_{11}$ (H_b)); 4.49 (AB_q, J = 16.6 Hz, 1H, OCH_2CO); 4.61 (AB_q, J = 16.6 Hz, 1H, OCH_2CO); 7.13–7.21 (m, 4H, ArH); 7.28–7.33 (m,

3H, ArH); 7.40 (d, J = 9.3 Hz, 1H, ArH); 7.82 (d, J = 7.8 Hz, 1H, ArH); 7.83 (d, J = 7.8 Hz, 1H, ArH); 7.91 (d, J = 9.3 Hz, 1H, ArH); 7.92 (d, J = 9.3 Hz, 1H, ArH); 9.25 (br s, 1H, COOH). ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.2, CH_2; 25.4, CH; 26.1, CH_2; 29.0, CH_2; 29.3, CH_2; 37.2, CH; 66.2, OCH_2; 76.5, OCH_2CO; 113.9, C; 116.6, CH; 120.1, CH; 120.6, C; 124.1, CH; 124.2, CH; 125.3, CH; 125.6, CH; 126.6, CH; 126.6, CH; 127.9, CH; 128.0, CH; 129.6, C; 129.8, CH; 129.9 (×2), CH; 133.6, C; 133.9, C; 152.4, C; 153.9, C; 171.3, CO. HRMS (ESI, +ve) for $C_{29}H_{29}O_4$, $[M+H]^+$, calculated 441.2066, found 441.2087.

3.3. Typical reaction for Scheme 3

3.3.1. Benzyl (2*S*,5*R*,8*R*)-2-allyl-3,6,9-triaza-11-[2-(2′-benzyloxy-1,1′-(*S*)-binaphthalen-2-yloxy)]-8-(*tert*-butoxycarboxamidobutyl)-5-{3-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenyl sulfonyl)guanidino]propyl}-4,7,10-trioxoundecanoate 37

The title compound was synthesised using the general peptide coupling procedure (protocol 1), from **35** (58 mg, 0.067 mmol) and **24** (29 mg, 0.067 mmol) to afford **37** (61 mg, 0.048 mmol, 71%) as a white solid. Mp 114–119 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.10–1.42 (m, 6H, H2″, H1‴, H2‴and H3‴); 1.27 (s, 6H, 2 × 2‴-CH_3); 1.41 (s, 9H, C(CH_3)$_3$); 1.47–1.56 (m, 2H, H1″); 1.75 (t, J = 6.6 Hz, H3‴); 2.08 (s, 3H, 8‴-CH_3); 2.47–2.59 (m, 4H, H1′ and H4‴); 2.54 (s, 3H, 5‴-CH_3); 2.57 (s, 3H, 7‴-CH_3); 2.81–2.95 (m, 2H, H4‴); 2.96–3.10 (m, 2H, H3″); 4.08 (br s, 1H, H8); 4.31–4.53 (m, 2H, H11); 4.56–4.63 (m, 1H, H5); 4.74–4.86 (m, 1H, H2); 4.93–5.20 (m, 6H, H11, PhCH_2O-ester and H3′); 5.59–5.72 (m, 1H, H2′); 6.12–6.34 (m, 3H, NH); 6.80 (d, J = 6.9 Hz, 1H, NH); 6.98–7.40 (m, 17H, ArH); 7.49 (d, J = 9.0 Hz, 1H, ArH); 7.82–7.97 (m, 4H, ArH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 12.1, 8‴-CH_3; 14.1, C1″; 17.5, 5‴-CH_3; 18.5, 7‴-CH_3; 21.4, C3‴; 22.6, C3‴; 25.2, C2‴; 26.7, 2‴-CH_3; 28.4, C(CH_3)$_3$; 29.0, C1‴; 29.3, C4‴; 32.7, C2″; 35.9, C1′; 40.0, C4‴; 40.5, C3″; 52.1, C5; 53.1, C2; 53.4, C8; 67.0, ArCH_2OAr; 67.9, CH_2-ester; 71.2, C10; 73.5, C2‴; 77.5, C(CH_3)$_3$; 114.2, ArCH; 116.2, ArC4a‴; 116.6, ArCH; 117.9, ArCH; 118.9, C3′; 119.1, ArC; 119.2, ArC; 119.7, ArCH; 120.1, ArCH; 123.9, ArC8‴; 124.0, ArCH; 124.2, ArCH; 125.0, ArCH; 125.3, ArCH; 125.5, ArCH; 126.7, ArCH; 126.8, ArCH; 127.4, ArCH; 127.8, ArCH; 128.1, ArC; 128.2, ArC; 128.3, ArCH; 128.5, ArCH; 129.3, ArCH; 129.9, ArCH; 132.5, ArC; 133.4, ArC; 133.6, C2′; 133.8, ArC; 134.8, ArC; 135.3, ArC; 135.4, ArC5‴; 137.0, ArC7‴; 152.1, ArC8‴; 153.5, ArC; 156.0, NCO; 153.9, CN_3; 156.1, ArC6‴; 169.1, C7; 171.3, C10; 171.4, C4; 171.6, C1. MS (ESI, +ve) *m/z* 1272 (100%) $[MH^+]$. HRMS (ESI, +ve) calcd for $C_{72}H_{86}N_7O_{12}S$ 1272.6055, found 1272.6061.

3.3.2. Benzyl (2*S*,5*R*,8*R*)-2-allyl-3,6,9-triaza-11-[2-(2′-benzyloxy-1,1′-(*S*)-binaphthalen-2-yloxy)]-8-(butylamino)-5-(3-guanidinopropyl)-4,7,10-trioxoundecanoate dihydrochloride 51

The title compound was synthesized using the general deprotection procedure (protocol 2), from **37** (50 mg, 0.039 mmol) to yield **51** (29 mg, 0.030 mmol, 76%) as a cream solid. Mp 116–118 °C. ^{1}H NMR (CD_3OD, 300 MHz): δ 0.70–1.20 (m, 4H, H2″ and H2‴); 1.21–1.78 (m, 6H, H1″, H1‴ and H3‴); 2.32–3.50 (m, 6H, H1′, H3″ and H4‴); 4.00–4.10 (m, 1H, H8); 4.14–4.36 (m, 3H, H5 and H11); 4.38–4.56 (m, 1H, H2); 4.78–5.02 (m, 6H, PhCH_2O, H1‴ and H3′); 5.46–5.60 (m, 1H, H2′); 6.62–7.23 (m, 18H, ArH); 7.54–7.94 (m, 4H, ArH). ^{13}C NMR (CD_3OD, 75 MHz): δ 23.2, C3‴; 26.1, C2‴; 27.6, C2″; 30.0, C1‴; 31.9, C1″; 36.5, C1′; 40.4, C4‴; 41.8, C3″; 53.6, C8; 53.9, C2; 54.0, C5; 68.0, ArCH_2; 68.7, C1‴ and C11; 116.6, C3′; 119.2, ArC; 120.2, ArCH; 120.6, ArCH; 125.1, ArCH; 125.5, ArCH; 126.3, ArCH; 126.7, ArCH; 126.9, ArCH; 127.4, ArCH; 128.8, ArCH; 129.1, ArCH; 129.2, ArCH; 129.3, ArCH; 129.5, ArCH; 129.6, ArC; 129.7, ArC; 130.1, ArCH; 130.6, ArCH; 131.1, ArCH; 132.6, ArC; 135.2, C2′; 135.9, ArCH; 136.8, ArCH; 140.8, ArC; 141.8, ArC; 142.1, ArC; 153.4, ArC; 154.1, ArC; 154.3, ArC; 158.2, CN_3; 171.1, C10; 172.4, C7; 173.4, C2; 173.6, C4. MS (ESI, +ve) *m/z* 906 (100%) $[M^{2+}]$. HRMS (ESI, +ve) calcd for $C_{53}H_{60}N_7O_7$ 906.4554, found 906.4544.

3.4. Procedures for Scheme 4

3.4.1. Benzyl (2*S*,5*R*,8*R*)-3,6,9-triaza-8-(*tert*-butoxycarboxamidobutyl)-5-{3-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)guanidino]propyl}-4,7,10-trioxo-2-propyl-11-{2-[2′-3-(propyloxy)- 1,1′-(*S*)-binaphthalen-2-yloxy]}undecanoate 65

To a solution of **1** (170 mg, 0.145 mmol) in THF (5 mL) was added 10% palladium on activated carbon (17 mg). The reaction vessel was degassed under vacuum and charged with hydrogen before being allowed to stir for 13 h. The solution was filtered, evaporated to dryness and dissolved in acetone (5 mL). To this solution was added K_2CO_3 (39 mg, 0.28 mmol) and benzyl bromide (24 mg, 0.14 mmol). After a further 13 h the reaction was concentrated by vacuum and the product isolated by flash column chromatography (5:95, MeOH/CH_2Cl_2) to yield **65** (127 mg, 0.10 mmol, 71%) as a white solid. Mp 118–123 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.43 (t, J = 7.2 Hz, 3H, H3′); 0.87 (t, J = 6.9 Hz, 3H, H3‴); 1.18–1.37 (m, 8H, H2′, H1″, H2″ and H1‴); 1.27 (s, 6H, 2 × 2‴-CH_3); 1.41 (s, 9H, C(CH_3)$_3$); 1.46–1.64 (m, 2H, H2‴); 1.70–1.92 (m, 6H, H1′, H3‴ and H2‴); 2.08 (s, 3H, 8‴-CH_3); 2.54 (s, 3H, 5‴-CH_3); 2.56 (s, 3H, 7‴-CH_3); 2.50–2.62 (m, 2H, H4‴); 2.84–3.00 (m, 2H, H4‴); 3.02–3.22 (m, 2H, H3″); 3.64–4.89 (m, 2H, H1‴); 3.95–4.06 (m, 1H, H8); 4.23–4.35 (m, 2H, H11); 4.38–4.56 (m, 2H, H2 and H5); 4.84–4.94 (m, 1H, N*H*Boc); 5.13 (AB_q, J = 12.6 Hz, 2H, PhCH_2O); 6.20 (d, J = 7.2 Hz, 1H, NH); 6.26 (br s, 2H, NH); 7.09–7.37 (m, 12H, ArH); 7.45, (d, J = 9.3 Hz, 1H); 7.85 (t, J = 7.8 Hz, 2H, ArH); 7.94 (dd, J = 2.4, 9.3 Hz, 2H, ArH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 9.0 C3‴; 12.0, 8‴-CH_3; 14.8, C3′; 17.4, 5‴-CH_3; 18.5, 7‴-CH_3; 18.8, C2′; 22.4, C3‴; 23.4, C2‴; 25.4, C3‴; 26.6, 2‴-CH_3; 27.7, C2″; 28.4, C(CH_3)$_3$; 29.0, C2‴; 31.0, C1‴; 31.3, C1″; 32.7, C4‴; 33.5, C1′; 39.9, C3″ and C4‴; 40.4, C3″; 52.2, C2; 52.6, C8; 52.8, C5; 67.8, ArCH_2; 68.4, C11; 71.2, C1‴; 73.5, C2‴; 78.8, C(CH_3)$_3$; 114.1, ArC; 115.8, ArC; 117.8, ArC4a‴; 119.3, C3′; 120.2, ArC8‴; 123.8, 2x ArCH; 124.1, ArCH; 124.9, ArCH; 125.4, ArCH; 126.5, ArCH; 126.6, ArCH; 126.9, ArCH; 127.4, ArCH; 127.9, ArCH; 128.0, ArCH; 128.2, ArCH; 128.4, ArCH; 128.5, ArCH; 129.1, ArCH; 129.6, ArC; 129.7, ArC; 133.4, ArC; 133.5, C2′; 133.8, ArC5‴; 134.7, ArC7‴; 135.4, 2 x ArC; 152.0, ArC8a‴; 153.4, ArC; 154.2, ArC; 156.0, NCO_2; 156.1, ArC6‴; 161.2, CN_3; 169.2, C10; 171.4, C7; 171.5, C1; 172.3, C4. MS (ESI, +ve) *m/z* 1226 (100%) $[MH^+]$. HRMS (ESI, +ve) calcd for $C_{68}H_{88}N_7O_{12}S$ 1226.6212, found 1226.6240.

3.4.2. Benzyl (2*S*,5*R*,8*R*)-3,6,9-triaza-8-(4-aminobutyl)-5-(3-guanidinopropyl)-4,7,10-trioxo-2-propyl-11-{2-[2′-3-(propyloxy)- 1,1′-(*S*)-binaphthalen-2-yloxy]}undecanoate dihydrochloride 66

The title compound was synthesized using the general deprotection procedure (protocol 2), from **73** (115 mg, 0.094 mmol) to yield **66** (75 mg, 0.080 mmol, 85%) as a highly hydroscopic white solid. ^{1}H NMR (CD_3OD, 500 MHz) δ 0.44–0.55 (m, 3H, H3′); 0.87–0.97 (m, 3H, H3‴); 1.06–2.11 (m, 16H, H1′, H2′, H1″, H2″, H1‴, H2‴, H2‴ and H3‴); 2.72–2.88 (m, 2H, H4‴); 3.15–3.30 (m, 2H, H3″); 3.86–3.89 (m, 2H, H1‴); 4.02–4.06 (m, 1H, H8); 4.13–4.15 (m, 1H, H2); 4.36–4.58 (m, 3H, H5 and H11); 5.01–5.17 (m, 2H, PhCH_2O); 7.03–7.06 (m, 1H, ArH); 7.14–7.18 (m, 2H, ArH); 7.27–7.33 (m, 8H, ArH); 7.44 (d, J = 9.0 Hz, 1H, ArH); 7.52 (d, J = 9.0 Hz, 1H, ArH); 7.86 (d, J = 8.5 Hz, 1H, ArH); 7.90 (d, J = 7.5 Hz, 1H, ArH); 8.00 (dd, J = 9.0, 16.0 Hz, 2H, ArH). ^{13}C NMR (CD_3OD, 125 MHz) δ 10.8, C3‴; 13.9, C3′; 20.0, C2′; 23.1, C3‴; 23.7, C2‴; 26.2, C2‴; 27.7, C2″; 30.1, C1‴; 32.2, C1″; 34.3, C1′; 40.4, C4‴;

41.9, C3″; 53.6, C8; 53.7, C2; 54.1, C5; 67.9, $ArCH_2$; 69.2, C11; 72.1, C1‴″; 116.0, ArCH; 116.8, ArCH; 120.4, ArCH; 121.7, ArCH; 124.8, ArCH; 125.2, ArCH; 125.9, ArCH; 126.3, ArCH; 127.4, ArCH; 127.6, ArCH; 128.2, ArC; 129.1, ArC; 129.3, 2× ArCH; 129.6, ArCH; 130.6, ArC; 130.9, ArC; 131.3, ArC; 135.1, ArCH; 135.1, ArCH; 137.1, ArC; 142.6, ArC; 153.9, ArC; 155.8, ArC; 158.4, CN_3; 170.8, C10; 173.1, C7; 173.3, C2; 173.9, C4. MS (ESI, +ve) *m/z* 860 (30%) [M^{2+}], 431 (100%). HRMS (ESI, +ve) calcd for $C_{49}H_{62}N_7O_7$ 860.4711, found 860.4730.

3.5. Procedures for Scheme 5

3.5.1. 2′-(Isopentyloxy)biphenyl-2-ol

To a solution of 1,1′-biphen-2,2′-diol **67** (0.21 g, 1.15 mmol) in dry acetone (25 mL) was added potassium carbonate (1.62 g, 12.0 mmol). To the resulting suspension a solution of 1-bromo-3-methylbutane (0.18 mL, 1.50 mmol) in dry acetone (10 mL) was added portion wise over 90 min. The reaction mixture was then heated at reflux for 18 h. The cooled mixture was filtered and the solid residue was washed with acetone (2 × 20 mL). The combined filtrate and washes were concentrated in vacuo to yield the title compound as a colourless oil (292 mg, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (d, J = 6.2 Hz, 6H); 1.56–1.83 (m, 3H); 4.13 (t, J = 6.4 Hz, 2H); 7.00–7.20 (m, 4H); 7.33–7.44 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.4, 24.8, 37.6, 68.2, 113.2, 117.5, 120.8, 122.2, 126.5, 127.8, 129.0, 131.2, 132.4, 153.8, 154.8. MS (EI) *m/z* 256 (30%) [M]$^+$; 186 (100) [M−($CH_2CH_2CH(CH_3)_2$)]$^+$. HRMS (EI) calcd for $C_{17}H_{20}O_2$ 256.1463, found 256.1462.

3.5.2. 2-[2′-(Isopentyloxy)biphenyl-2-yloxy]acetic acid 68

To a solution of 2′-(isopentyloxy)biphenyl-2-ol (0.29 g, 1.13 mmol) in methanol (20 mL) was added potassium carbonate (1.83 g, 13.2 mmol) and bromoacetic acid (0.56 g, 4.03 mmol) and the resulting suspension was heated at reflux for 16 h. The cooled reaction mixture was concentrated in vacuo and the residue was dissolved in distilled water (100 mL) then washed with ether (3 × 20 mL). The aqueous solution was acidified (10% HCl) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo to yield **68** as a thick colourless oil (111 mg, 30%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.77 (d, J = 4.4 Hz, 6H); 1.43–1.46 (m, 3H); 3.99 (t, J = 6.5 Hz, 2H); 4.62 (s, 2H); 6.87 (dd, J = 8.2, 0.9 Hz, 1H); 7.06–7.13 (m, 3H); 7.25–7.39 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.3, 24.8, 37.6, 69.4, 69.4, 111.1, 115.0, 122.1, 122.2, 128.3, 128.7, 128.9, 129.1, 131.3, 131.6, 154.1, 155.8, 170.8. MS (ESI, +ve) *m/z* 353 (12%) [M+K]$^+$; 337 (39) [M+Na]$^+$; 332 (96) [M+NH_4]$^+$; 315 (100) [M+H]$^+$; 245 (38). HRMS (ESI, +ve) calcd for $C_{19}H_{23}O_4$ 315.1596, found 315.1598.

3.5.3. (*R*)-Methyl 6-(*tert*-butoxycarbonylamino)-2-{2-[2′-(isopentyloxy)biphenyl-2-yloxy]acetamido}hexanoate 69

This compound was prepared via protocol 1, using (*R*)-Lysine (Boc)-methyl ester (0.10 g, 0.38 mmol) and **68** (0.11 g, 0.34 mmol) in anhydrous acetonitrile (10 mL) with EDCI (0.09 g, 0.45 mmol) and HOBt (0.08 g, 0.58 mmol). Purification with column chromatography using 1:99–2:98 MeOH/CH_2Cl_2 gave product **69** as a pale yellow oil (179 mg, 93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.76 (dd, J = 6.4 Hz, 6H); 1.01–1.15 (m, 2H); 1.26–1.59 (m, 3H); 1.41 (s, 9H, *t*Bu); 1.61–1.77 (m, 2H); 2.93–3.08 (m, 2H); 3.66 (s, 3H); 3.82–4.04 (m, 3H); 4.43 (AB_q, J = 14.7 Hz, 2H); 6.74 (d, J = 8.5 Hz, 1H); 6.86 (d, J = 7.9 Hz, 1H); 6.98–7.07 (m, 3H); 7.24–7.34 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.3, 22.3, 22.4, 24.9, 28.3, 29.3, 31.7, 32.1, 37.8, 40.1, 51.2, 52.1, 67.2, 67.4, 77.8, 111.8, 113.0, 120.6, 121.6, 128.0, 128.0, 128.6, 128.7, 131.1, 131.4, 154.4, 156.3, 168.2, 169.4, 171.8. MS (ESI, +ve) *m/z* 579 (11%) [M+Na]$^+$; 557 (100) [M+H]$^+$; 457 (29) [M-Boc+H]$^+$; 233 (88). HRMS (ESI, +ve) calcd for $C_{31}H_{45}N_2O_7$ 557.3227, found 557.3217.

3.5.4. (*R*)-6-(*tert*-Butoxycarbonylamino)-2-{2-[2′-(isopentyloxy)biphenyl-2-yloxy]acetamido}hexanoic acid 70

To a solution of **69** (0.18 g, 0.32 mmol) in THF/water (4:1) (15 mL) was added lithium hydroxide (0.11 g, 2.60 mmol) and the resulting solution was stirred at 20 °C for 16 h. The reaction mixture was diluted with water (5 mL), acidified with 10% HCl and extracted with CH_2Cl_2 (4 × 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo to give **70** as a pale yellow oil (108 mg, 62%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.77 (dd, J = 6.3, 4.4 Hz, 6H); 1.06–1.20 (m, 2H); 1.39–1.53 (m, 5H); 1.43 (s, 9H, *t*Bu); 1.75 (br s, 1H); 2.95–3.10 (m, 2H); 3.89–3.98 (m, 2H); 4.49 (AB_q, J = 14.6 Hz, 2H); 4.58–4.68 (m, 1H); 6.88 (d, J = 7.5 Hz, 2H); 6.98–7.02 (m, 2H); 7.05 (t, J = 7.8 Hz, 1H); 7.25–7.31 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.3, 22.4, 24.9, 25.4, 28.3, 29.4, 31.4, 37.8, 51.5, 67.1, 67.4, 67.8, 78.6, 111.9, 113.1, 120.7, 121.7, 128.1, 128.3, 128.7, 128.8, 131.1, 131.5, 151.1, 154.4, 156.3, 169.7, 174.8. MS (ESI, +ve) *m/z* 543, (100%) [M+H]$^+$; 487 (46) [M+H-56]$^+$; 443 (46) [M+H-Boc]$^+$. HRMS (ESI, +ve) calcd for $C_{30}H_{43}N_2O_7$ 543.3070, found 543.3066.

3.5.5. Benzyl (2*S*,5*R*,8*R*)-2-(3-methylbutyloxy)-3,6,9-triaza-8-(*tert*-butoxycarboxamidobutyl)-11-{2-[2′-(2-methylpropyloxy)-1,1′-biphenyl-2-yloxy]}-5-{3-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)guanidino]propyl}-4,7,10-trioxoundecanoate 72

This compound was prepared via protocol 1, using **70** (70 mg, 0.13 mmol) and **71** (77 mg, 0.12 mmol) in anhydrous acetonitrile with EDCI (40 mg, 0.21 mmol) and HOBt (39 mg, 0.29 mmol). Purification with column chromatography using 1:99–2:98 methanol/CH_2Cl_2 gave **72** as a clear colourless oil (103 mg, 71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.77 (dd, J = 6.4, 1.8 Hz, 6H); 0.88 (dd, J = 7.3, 7.3 Hz, 6H); 1.00–1.14 (m, 2H); 1.28–1.58 (m, 18H); 1.44 (s, 6H, 2 × CH_3 (Pbf)); 1.41 (s, 9H, *t*Bu); 1.58–1.76 (m, 5H); 1.81–1.98 (m, 1H); 2.05 (s, 3H); 2.47 (s, 3H); 2.54 (s, 3H); 2.91 (s, 2H); 2.94–3.07 (m, 2H); 3.08–3.26 (m, 2H); 3.84–4.02 (m, 2H); 4.18–4.60 (m, 5H); 4.80–4.94 (m, 1H); 5.14 (AB_q, J = 12.3 Hz, 2H); 6.15 (br s, 2H); 6.73–6.83 (m, 2H); 6.96–7.08 (m, 3H); 7.21–7.33 (m, 10H); 7.61 (d, J = 9.0 Hz, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.4, 17.9, 19.3, 21.5, 22.4, 22.7, 22.8, 24.8, 24.9, 25.7, 28.4, 58.4, 29.3, 31.4, 32.8, 37.8, 40.1, 40.3, 40.4, 43.1, 51.0, 53.2, 53.4, 66.8, 67.1, 67.5, 80.1, 86.3, 111.9, 113.2, 117.4, 120.7, 121.5, 124.5, 127.9, 128.0, 128.1, 128.2, 128.5, 128.6, 128.9, 131.3, 131.5, 132.1, 132.9, 135.6, 138.3, 154.2, 156.0, 156.3 (×2), 158.6, 169.6, 171.8, 172.6. MS (ESI, +ve) *m/z* 1153 (100%) [M+H]$^+$; 527 (68); 288 (98). HRMS (ESI, +ve) calcd for $C_{62}H_{88}N_7O_{12}S$ 1154.6212, found 1154.6227.

3.6. Determination of minimum inhibitory concentration (MIC)

MIC studies were performed on *S. aureus* wild type (ATCC 6538P), Mu50 (ATCC 700699) and MRSA (ATCC 43300) in Luria Broth. MIC determinations for wild type and clinical isolates of *Enterococcus 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 128 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.

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.

Supplementary data

Supplementary data (full experimental details and spectroscopic data for all compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.05.005.

References and notes

1. Bremner, J. B.; Keller, P. A.; Pyne, S. G.; Boyle, T. P.; Brkic, Z.; David, D. M.; Robertson, M.; Somphol, K.; Baylis, D.; Coates, J. A.; Deadman, J.; Jeevarajah, D.; Rhodes, D. I. *Bioorg. Med. Chem.* **2010**, in press (doi:10.1016/j.bmc.2010.02.033).
2. Nordmann, P.; Naas, T.; Fortineau, N.; Poirel, L. *Curr. Opin. Microbiol.* **2007**, *10*, 436.
3. Nicolaou, K. C.; Boddy, C. N. C. *Sci. Am.* **2001**, *284*, 54.
4. Noble, W. C.; Virani, Z.; Cree, R. G. A. *FEMS Microbiol. Lett.* **1992**, *93*, 195.
5. Kahne, D.; Leimkuhler, C.; Lu, W.; Walsh, C. *Chem. Rev.* **2005**, *105*, 425.
6. Appelbaum, P. C. *Clin. Microbiol. Infect.* **2006**, *12*, 16.
7. Rice, L. B. *Am. J. Med.* **2006**, *119*, S11.
8. Wright, G. D.; Sutherland, A. D. *Trends Mol. Med.* **2007**, *13*, 260.
9. Bremner, J. B.; Keller, P. A.; Pyne, S. G.; Boyle, T. P.; Brkic, Z.; David, D. M.; Morgan, J.; Robertson, M.; Somphol, K.; Miller, M. H.; Howe, A. H.; Ambrose, P.; Bhavnani, S.; Fritsche, T. R.; Biedenbach, D. J.; Jones, R. N.; Buckheit, R. W., Jr.; Watson, K. M.; Baylis, D.; Coates, J. A.; Deadman, J.; Jeevarajah, D.; McCracken, A.; Rhodes, D. I. *Angew. Chem., Int. Ed.* **2010**, *49*, 537.
10. For work on other cationic peptides and a pharmacophore model see: (a) Strom, M. B.; Haug, B. E.; Skar, M. L.; Stensen, W.; Stiberg, T.; Svendsen, J. S. *J. Med. Chem.* **2003**, *46*, 1567; (b) Haug, B. E.; Stensen, W.; Stiberg, T.; Svendsen, J. S. *J. Med. Chem.* **2004**, *47*, 4159; (c) Haug, B. E.; Stensen, W.; Svendsen, J. S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2361; (d) Haug, B. E.; Stensen, W.; Kalaaji, M.; Rekdal, O.; Svendsen, J. S. *J. Med. Chem.* **2008**, *51*, 4306; (e) Flemming, K.; Klingenberg, C.; Cavanagh, J. P.; Sletteng, M.; Stensen, W.; Svendsen, J. S.; Flaegstad, T. *J. Antimicrob. Chemother.* **2009**, *63*, 136.