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Synthesis and antibacterial studies of binaphthyl-based tripeptoids. Part 1

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ABSTRACT

An efficient synthesis of 29 new binaphthyl-based neutral, and mono- and di-cationic, peptoids is described. Some of these compounds had antibacterial activities with MIC values of 1.9–3.9 μg/mL against *Staphylococcus aureus*. One peptoid had a MIC value of 6 μg/mL against a methicillin-resistant strain of *S. aureus* (MRSA) and a MIC value of 2 μg/mL against vancomycin-resistant strains of enterococci (VRE).

1. Introduction

With the increasing spread of antibacterial resistance,[1–3] including resistance by Gram-positive pathogenic bacteria to vancomycin,[4,5] there is a strong imperative for the development of new antibacterials.[6,7] In this context, we initiated a program investigating the design and synthesis of cyclic cationic peptoids linked by a hydrophobic scaffold as potential antibacterial agents, and thus far, have shown the binaphthyl (**1**)[8] and carbazole (**2**) scaffolds[9,10] within these cyclic peptoids produce antibacterial agents, whilst the smaller indole[11] and phenyl[12] based cyclic peptoids had weak activities or were inactive (Fig. 1). More recently[13] we reported the preparation of further novel binaphthyl cyclic and acyclic cationic peptoids in which the peptoid moieties were attached to the 2 and 2′ positions of the binaphthyl system, rather than the 3 and 3′ positions as in peptoid **1**. Of these compounds the acyclic peptoid **3** showed the highest antibacterial activity against *Staphylococcus aureus* with a MIC value of 4 μg/mL.[13] This compound was more active than its cyclic counterpart (*S. aureus* MIC = 15–31 μg/mL) that was prepared from a ring-closing metathesis reaction of a protected version of **3**. The study provided us a platform for further development of acyclic peptoidal antimicrobial agents with improved activity against *S. aureus*. Recently we reported a new sub-class of synthetic acyclic di-cationic peptoids, compounds **4** and **5** (Fig. 1), 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.[14] These compounds, however, were generally weaker in activity against Gram-negative bacteria. These compounds were readily bactericidal in a concentration dependant manner with an extended post-antibacterial effect. In vivo potency of these compounds was also maintained. Resistance was very slow to develop in vitro and compound **4** is being developed for topical indications including wound and catheter-related infections.

In this paper we wish to disclose the synthesis and antibacterial activities against Gram-positive bacteria of other novel acyclic tripeptoid molecules based largely on compound **6** and which ultimately led, together with other changes as will be outlined in later papers, to the development of the lead compounds **4** and **5**. After our discovery of the acyclic peptoid **3**, with an embedded single cationic amino acid residue, our next target molecule was the peptoid **6** which contains cationic lysine and arginine residues and retains the hydrophobic 2,2′-disubstituted binaphthyl system and the two allylic residues of **3**. Initially the D-configured basic amino acids were chosen to minimise possible enzymatic cleavage of the peptoid side chain. Unlike **3**, however, we chose L-allylglycine benzyl ester as the terminal amino acid residue in **6** and in the majority of the other analogues reported here. This was a result of other ongoing studies in our laboratories on a different series of compounds which indicated that the more hydrophobic benzyl

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esters with the L-configuration showed better activities against *S. aureus* than the L or D-configured methyl esters. These studies will be the subject of a future paper.

2. Results and discussion

The synthesis of the peptoid **6** is shown in Scheme 1. The hydrochloride salt of benzyl L-allylglycine **7** underwent a peptide coupling reaction with Fmoc-D-Arg(Pmc)-OH under standard conditions using EDCI and HOBt in $MeCN$[12–15] to give the dipeptide **8** in 51% yield. Fmoc-deprotection of **8** upon exposure to piperidine in CH_3CN[12–15] at rt gave the primary amine **9** in 70% yield. This peptide coupling then deprotection sequence was repeated on amine **9** except using Fmoc-D-Lys(Boc)-OH in the first step to give the primary amine **11**[15] in 55% overall yield for the two steps. This compound was then coupled with (2′-allyloxy-[1,1′]-(*S*)-binaphthalen-2-yloxy)acetic acid **12**[13] to give the tripeptide **13** in 79% yield. *N*-Boc deprotection of **13** by exposure to TFA,[12–15] followed by anion exchange with HCl, provided the hydrochloride salt **6** in 62% yield (Scheme 1).

The hydrochloride salts of the peptoids **67–69** shown in Scheme 2 were also prepared using a similar sequence of reactions to that described in Scheme 1. Details of these syntheses including the respective intermediate compounds can be found in the Supplementary data. These compounds **67–79** were prepared in order to examine the effects on antibacterial potency of systematic changes in the constitution and configuration of the amino acid residues in the critically important peptoid chain. Compounds **67** and **68** were similar to **6** except **67** contained two D-Arg residues while **68** had two D-Lys residues. Peptoids **69–74** all had a non-basic amino acid side chain (Gly, D-Ala, D-allyl-Gly, D-Leu, D-Phe and D-norLeu) at C-8, while peptoid **75** had the opposite configuration at C-2 to **6**. Peptoids **76–79** all had non-basic amino acid side chains at C-2 (L-Leu, L-Ala and Gly), while **77** also had a non-basic residue at C-8 (D-Leu) in comparison to **6**. The peptoid compounds **80–82** (Fig. 2), having a C-2 allyl or phenyl substituent and a C-terminal 1-naphthylmethyl or methyl ester, were also prepared (via the general methodology outlined in Scheme 1) with a view to mapping hydrophobicity requirements in the C-terminal region.

In order to avoid possible later problems with the biological oxidation of the allyl residues in compounds **6** and **67–79** in vivo we looked for potentially less reactive alternatives. Based on the results of ongoing studies in our laboratories on a different series of compounds we chose to replace the allyl substituents with more hydrophobic groups. These were the *iso*-butyl group at the C-terminus and the *iso*-pentyl group on the binaphthyl moiety. Thus the compounds **86** and **89** were prepared via the acid **87**[14] and the respective amines **84** and **11** (Scheme 3). The peptoid salts **86** and **89** differed from **6** by replacement of the binaphthyl *O*-allyl group by an *iso*-pentyl group, while in **89** the C-2 allyl group in **6** was replaced by an *iso*-butyl group. Also synthesised were the peptoids **90–96** (Fig. 3) having the *O*-allyl group on the binaphthyl

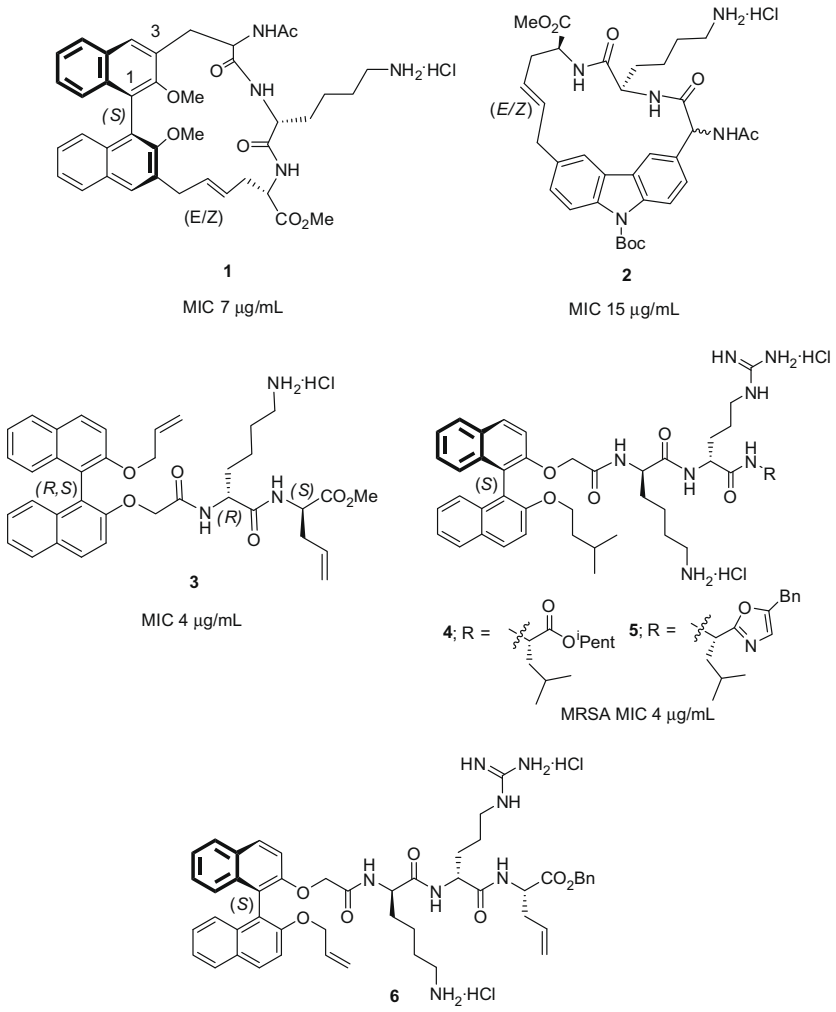
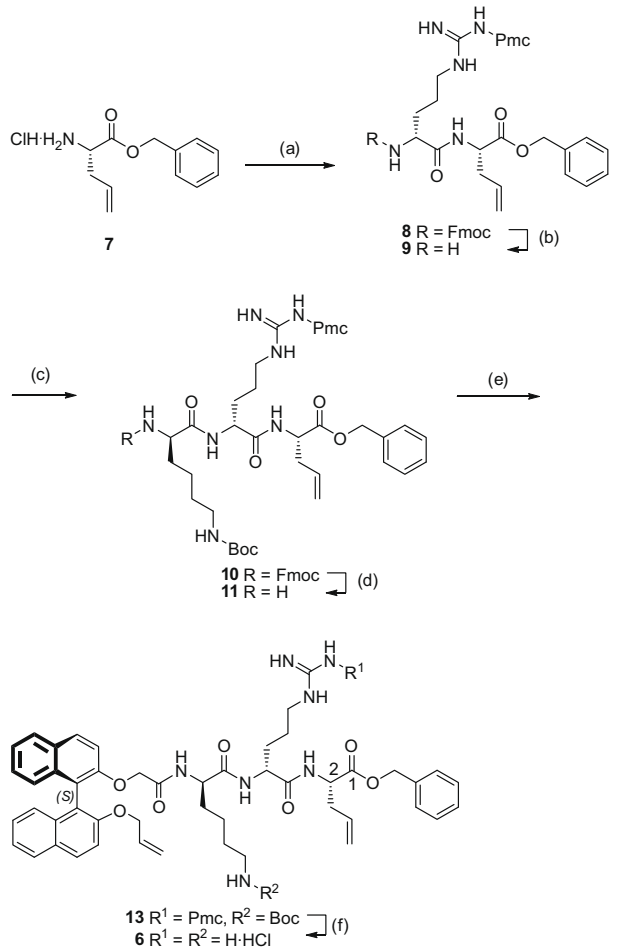


Figure 1. MIC values against *S. aureus* of cyclic peptoids **1–3**, MIC values against methicillin-resistant *S. aureus* (MRSA) of cyclic peptoids **4** and **5** and the structure of peptoid **6**.



Scheme 1. Synthesis of compound **6**. Reagents and conditions: (a) DIPEA, Fmoc-D-Arg(Pmc)-OH, EDCI, HOBt, CH_3CN, rt, 3 h, 51%; (b) piperidine, CH_3CN, rt, 3 h, 70%; (c) Fmoc-D-Lys(Boc)-OH, EDCI, HOBt, CH_3CN, rt, 3 h, 59%; (d) piperidine, CH_3CN, rt, 3 h, 93%; (e) (2′-allyloxy-[1,1′]-(*S*)-binaphthalen-2-yloxy)-acetic acid **12**, EDCI, HOBt, CH_3CN, rt, 3 h, 79%; (f) TFA/CH_2Cl_2 (1:1), rt, 16 h, then 1 M HCl–Et_2O, 62%.

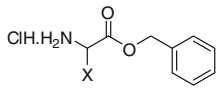
moiety replaced by an *iso*-pentyl group and the C-2 allyl group at the C-terminus replaced by an *iso*-butyl group, together with variations of the basic amino acid side chain at C-5.

In addition, to assist further with the structure–activity relationships studies, the tripeptoid **101** (Scheme 4), having no basic amino acid side chains, and the salts **106** and **107** (Scheme 5), having only one basic amino acid side chain residue each but with differing separation from the hydrophobic binaphthyl moiety, were also prepared. Details of the syntheses of these compounds can be found in the Supplementary data.

The aforementioned deprotected peptoid 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 1–3. The positive control, vancomycin, showed a MIC value of 1.95 μg/mL against *S. aureus* and had MIC values of 1.56, >25, >25 and 3.13 μg/mL against the vancomycin-sensitive and partially resistant enterococci strains, VRE_{243}, VRE_{449}, VRE_{820} and VRE_{987}, respectively (Table 1, last entry). Of the diallyl substituted peptoids shown in Table 1, peptoids **6** and **76** were equipotent and the most active against *S. aureus* (MIC values of 1.95 μg/mL) and against the four VRE strains, with MIC values of 31.3 μg/mL for all VRE strains. These compounds differed structurally only at the non-basic C-2 substituent (L-allyl for **6** and L-*iso*-butyl for **76**) and were more potent than our earlier compound **3** (albeit with a methyl ester in this case) which indicated that the additional cationic residue contributed positively to antibacterial activity. Compound **67** having two D-Arg residues was significantly less active than **6** against *S. aureus* (MIC = 31.3 μg/mL) and was relatively inactive against the four VRE strains (MIC values ⩾125 μg/mL). Peptoid **68**, with two D-Lys residues, maintained good activity against *S. aureus* (MIC = 3.9 μg/mL), however its activity against the VRE strains was significantly reduced (MIC = 62.5–125 μg/mL). Peptoids **69–74**, that have the D-Lys residue of **6** replaced with a non-basic residue at C-8 were all less active than **6**. However, peptoid **72** with a C-8 D-Leu residue was as active against *S. aureus* and equally, or in some cases more, active against the four VRE strains than **68**, having a basic D-Lys residue at C-8. The peptoids **78** and **79**, differing only from peptoids **6** and **76** by the nature of the C-2 substituent (Me (L-Ala) or H (Gly), respectively) showed significantly less activity than **6** and **76** suggesting that the more hydrophobic C-terminal L-allylglycinate or L-Leu residues in compounds **6** and **76** also contribute to their antibacterial activities. Our results highlight the importance of both hydrophobicity and cationic character within the peptoid for antibacterial activity. Clearly the nature and placement of the cationic residues is also important in determining antibacterial activity. Svendsen[16] has provided a pharmacophore model for peptoid antibacterial compounds which indicates that two hydrophobic and two cationic sites are important for antibacterial activity. Our results are generally consistent with this model. In our case the C-terminal allylglycinate or L-Leu residues of **6** and **76** would represent the second, albeit considerably smaller, hydrophobic group when compared to the binaphthyl residue.

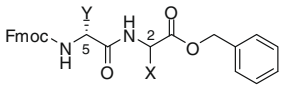
The antibacterial activities of compounds **90–96** are shown in Table 2. These peptoids have the allyl substituents found in the compounds **6** and **76** replaced with the more hydrophobic *iso*-butyl and *iso*-pentyl groups and have different basic residues at C-5. The di-cationic peptoids **90**, **92** and **94** had the highest activities against *S. aureus* (MIC = 1.95 μg/mL) and were equipotent to **6** and **76**. Of these three aforementioned peptoids, compound **90** showed an increased activity over that of **6** and **76**, against two of the VRE strains (VRE_{449} and VRE_{820}). Compounds **90**, **92** and **94** have a 2, 3 or 4 carbon linker, respectively, to a C-5 terminal amino residue rather than a guanidine residue as in peptoids **6** and **76**. Notably, the peptoids **91** and **93**, having a C-5 nor-Arg and a *N*-methyl-Arg residue, respectively, showed slightly reduced activities against *S. aureus* (MIC = 2.6 and 3.13 μg/mL, respectively) and the former was less active against two of the Enterococcal strains. Peptoid **96** showed a slightly reduced activity against *S. aureus* (MIC = 3.9 μg/mL) but was the most potent of all the compounds tested against the four VRE strains (MIC = 7.8–15.6 μg/mL). Peptoid **95**, the C-2 epimer of **96** and the C-2 *iso*-butyl and the C-2 *iso*-butyl and *O*-*iso*-pentyl analogues of **76** and **6**, respectively, was less active than **96** against the VRE strains and had the same activity as **96** against *S. aureus*. Compound **95** however, was more active than **6** and **76** (also having C-5 Arg/C-8-Lys moieties) against the VRE strains.

Peptoid **80**, the 1-naphthalenemethyl ester analogue of **6**, had poor activity against *S. aureus* (MIC = 15.6 μg/mL) and was inactive against the four VRE strains while the *S* and *R* binaphthyl, C-2 phenylalanine, methyl ester analogues **81** and **82**, respectively, showed slightly better activity against *S. aureus* (MIC = 3.9–7.8 μg/mL) (Table 3). The low activities for compound **80** may be due to adverse steric interactions between the large 1-napthalenemethyl moiety and the antibacterial target while that of the methyl esters may be due to the reduced hydrophobicity at the C-terminus or due to ester hydrolysis to the corresponding carboxylic acid. The carboxylic acid derivative of ester **4** has been shown to be produced in vivo and to be much less active against *S. aureus* (MIC = 25 μg/mL).[14] The di-cationic peptoids **86** and **89** demonstrated good activities against *S. aureus* and the four VRE strains



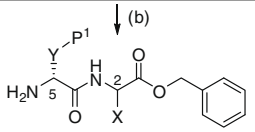
7 L-allyl-Gly
14 D-allyl-Gly
15 L-Leu
16 L-Ala
17 Gly

DIPEA, FmocNHCH(YP^1)CO$_2$H, (a)



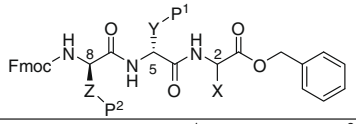
	C2	C5	P^1	Yield %
8	L-allyl-Gly	D-Arg	Pmc	51
18	L-allyl-Gly	D-Lys	Boc	72
19	D-allyl-Gly	D-Arg	Pmc	67
20	L-Leu	D-Arg	Pmc	73
21	L-Ala	D-Arg	Pmc	71
22	Gly	D-Arg	Pmc	82

(b)



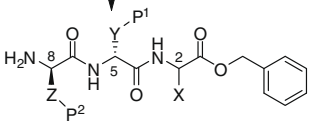
	C2	C5	P^1	Yield %
9	L-allyl-Gly	D-Arg	Pmc	70
23	L-allyl-Gly	D-Lys	Boc	90
24	D-allyl-Gly	D-Arg	Pmc	90
25	L-Leu	D-Arg	Pmc	73
26	L-Ala	D-Arg	Pmc	74
27	Gly	D-Arg	Pmc	95

FmocNHCH(ZP^2)CO$_2$H, (a)

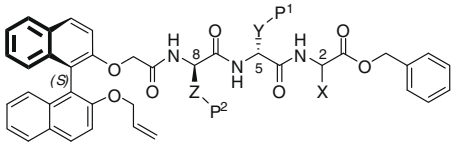


	C2	C5	P^1	C8	P^2	Yield %
10	L-allyl-Gly	D-Arg	Pmc	D-Lys	Boc	59
28	L-allyl-Gly	D-Arg	Pmc	D-Arg	Pmc	90
29	L-allyl-Gly	D-Lys	Boc	D-Lys	Boc	88
30	L-allyl-Gly	D-Arg	Pmc	Gly	-	51
31	L-allyl-Gly	D-Arg	Pmc	D-Ala	-	68
32	L-allyl-Gly	D-Arg	Pmc	D-allyl-Gly	-	70
33	L-allyl-Gly	D-Arg	Pmc	D-Leu	-	52
34	L-allyl-Gly	D-Arg	Pmc	D-Phe	-	70
35	L-allyl-Gly	D-Arg	Pmc	D-norLeu	-	84
36	D-allyl-Gly	D-Arg	Pmc	D-Lys	Boc	49
37	L-Leu	D-Arg	Pmc	D-Lys	Boc	82
38	L-Ala	D-Arg	Pmc	D-Leu	-	97
39	L-Ala	D-Arg	Pmc	D-Lys	Boc	93
40	Gly	D-Arg	Pmc	D-Lys	Boc	73

(b)



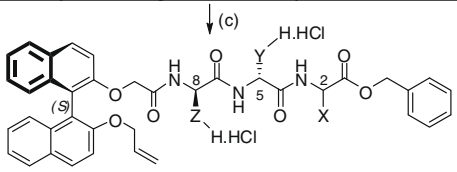
12, (a)



	C2	C5	P^1	C8	P^2	Yield %
11	L-allyl-Gly	D-Arg	Pmc	D-Lys	Boc	93
41	L-allyl-Gly	D-Arg	Pmc	D-Arg	Pmc	94
42	L-allyl-Gly	D-Lys	Boc	D-Lys	Boc	69
43	L-allyl-Gly	D-Arg	Pmc	Gly	-	75
44	L-allyl-Gly	D-Arg	Pmc	D-Ala	-	75
45	L-allyl-Gly	D-Arg	Pmc	D-allyl-Gly	-	91
46	L-allyl-Gly	D-Arg	Pmc	D-Leu	-	88
47	L-allyl-Gly	D-Arg	Pmc	D-Phe	-	76
48	L-allyl-Gly	D-Arg	Pmc	D-norLeu	-	97
49	D-allyl-Gly	D-Arg	Pmc	D-Lys	Boc	100
50	L-Leu	D-Arg	Pmc	D-Lys	Boc	91
51	L-Ala	D-Arg	Pmc	D-Leu	-	93
52	L-Ala	D-Arg	Pmc	D-Lys	Boc	86
53	Gly	D-Arg	Pmc	D-Lys	Boc	93

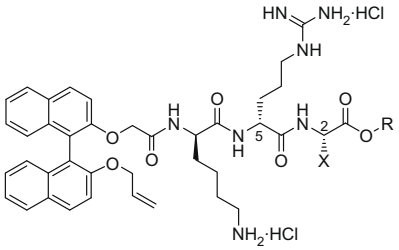
	C2	C5	P^1	C8	P^2	Yield %
13	L-allyl-Gly	D-Arg	Pmc	D-Lys	Boc	79
54	L-allyl-Gly	D-Arg	Pmc	D-Arg	Pmc	90
55	L-allyl-Gly	D-Lys	Boc	D-Lys	Boc	94
56	L-allyl-Gly	D-Arg	Pmc	Gly	-	82
57	L-allyl-Gly	D-Arg	Pmc	D-Ala	-	85
58	L-allyl-Gly	D-Arg	Pmc	D-allyl-Gly	-	95
59	L-allyl-Gly	D-Arg	Pmc	D-Leu	-	66
60	L-allyl-Gly	D-Arg	Pmc	D-Phe	-	87
61	L-allyl-Gly	D-Arg	Pmc	D-norLeu	-	70
62	D-allyl-Gly	D-Arg	Pmc	D-Lys	Boc	72
63	L-Leu	D-Arg	Pmc	D-Lys	Boc	84
64	L-Ala	D-Arg	Pmc	D-Leu	-	92
65	L-Ala	D-Arg	Pmc	D-Lys	Boc	88
66	Gly	D-Arg	Pmc	D-Lys	Boc	77

(c)



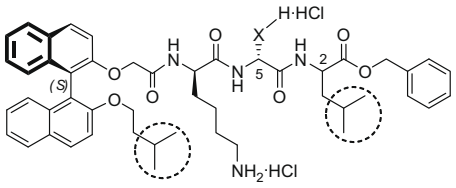
	C2	C5	C8	Yield %
6	L-allyl-Gly	D-Arg	D-Lys	62
67	L-allyl-Gly	D-Arg	D-Arg	85
68	L-allyl-Gly	D-Lys	D-Lys	98
69	L-allyl-Gly	D-Arg	Gly	77
70	L-allyl-Gly	D-Arg	D-Ala	71
71	L-allyl-Gly	D-Arg	D-allyl-Gly	95
72	L-allyl-Gly	D-Arg	D-Leu	63
73	L-allyl-Gly	D-Arg	D-Phe	90
74	L-allyl-Gly	D-Arg	D-norLeu	59
75	D-allyl-Gly	D-Arg	D-Lys	78
76	L-Leu	D-Arg	D-Lys	86
77	L-Ala	D-Arg	D-Leu	76
78	L-Ala	D-Arg	D-Lys	74
79	Gly	D-Arg	D-Lys	92

Scheme 2. Synthesis of compounds **6** and **67–79**. Reagents and conditions: (a) EDCI, HOBt, CH_3CN, rt, 1–3 h; (b) piperidine, CH_3CN, rt, 3–14 h; (c) TFA/CH_2Cl_2 (1:1), rt, 1 h (for Boc) or 14 h (for Pmc), then 1 M HCl–Et_2O.



	Binaph	C2	R
80	(S)	L-allyl-Gly	1-naphthalenemethyl
81	(*S*)	L-Phe	Me
82	(*R*)	L-Phe	Me

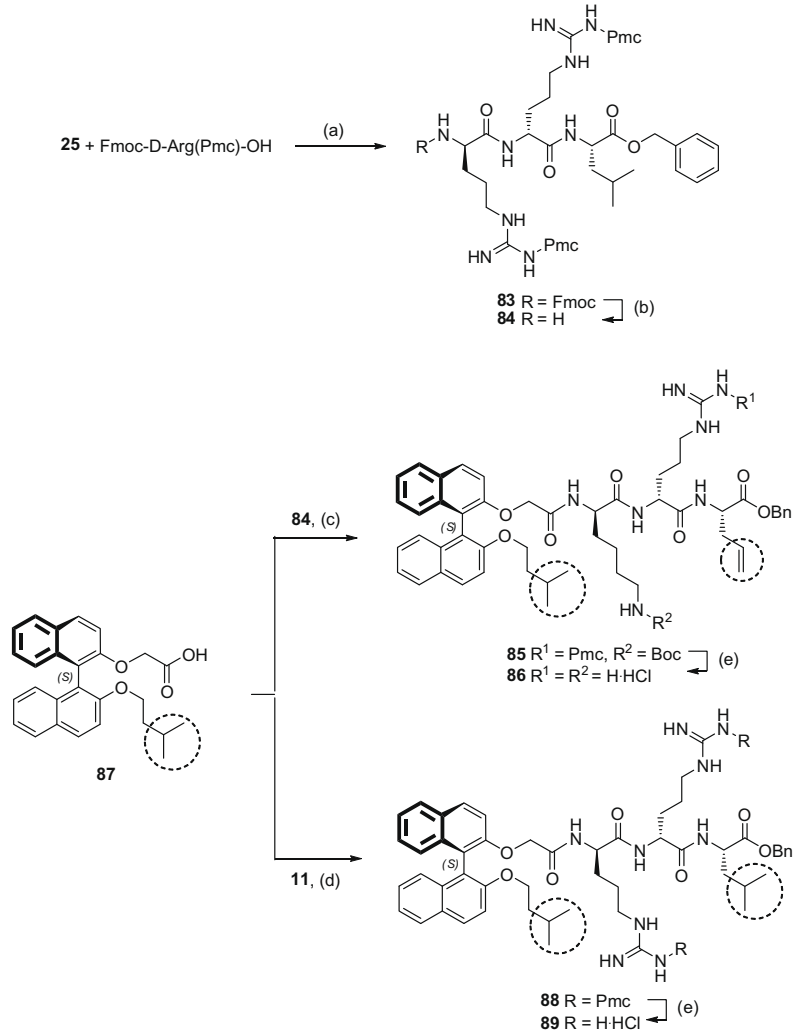
Figure 2.



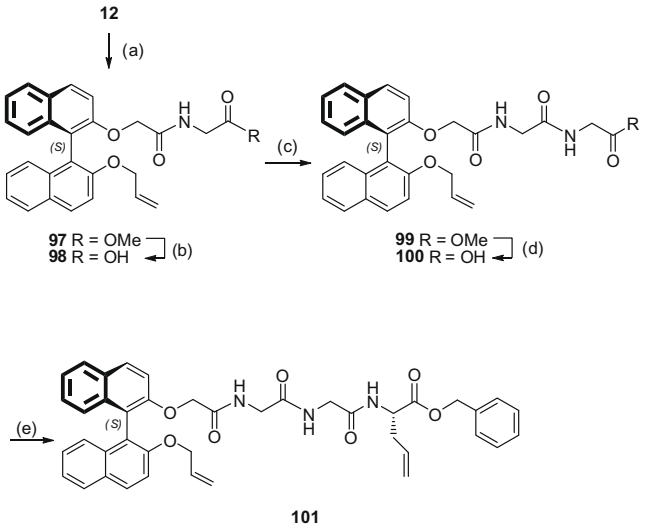
	C2	C5
90	L	Dab
91	L	norArg
92	L	Orn
93	L	N^{ω}-Me-Arg
94	L	Lys
95	D	Arg
96	L	Arg

Figure 3.

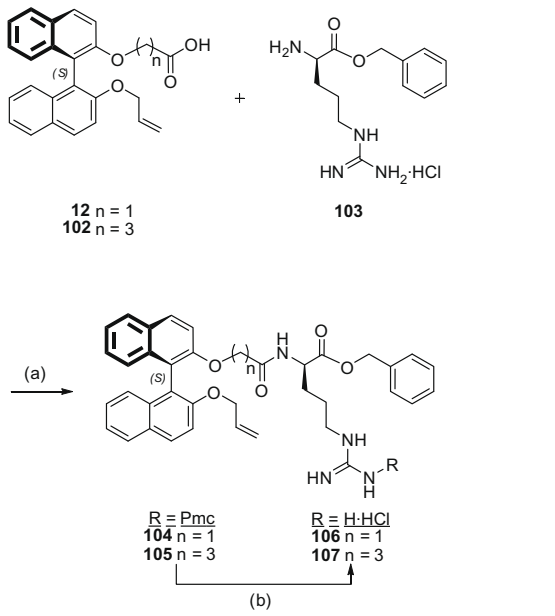
(Table 3). With the latter di-D-Arg peptoid showing a much improved activity over its relatively inactive diallyl analogue **67** (Table 1). Interestingly, the non-cationic peptoid **101** showed relatively good antibacterial activities against *S. aureus* (MIC = 3.9 μg/mL) and the four VRE strains (MIC values 15.6–62.5 μg/mL) when compared to a number of less active mono- and di-cationic peptoids in Tables 1 and 2. This result further demonstrates the importance of hydrophobic interactions and their contributions to antibacterial activity. The mono-cationic peptoids **106** and **107** having a single D-Arg group were generally less active than **101** and had similar activity to the mono-cationic D-Arg peptoid **72**, even though the distance between the binaphthyl system and the D-Arg group was different in each compound.



Scheme 3. Synthesis of compounds **86** and **89**. Reagents and conditions: (a) EDCI, HOBt, CH_3CN, rt, 3 h, 95%; (b) piperidine, CH_3CN, rt, 3 h, 95%; (c) EDCI, HOBt, CH_3CN, rt, 3 h, 56%; (d) EDCI, HOBt, CH_3CN, rt, 3 h, 84%; (e) TFA/CH_2Cl_2 (1:1), rt, 16 h, then 1 M HCl–Et_2O, 86% (**86**), 29% (**89**).



Scheme 4. Synthesis of compound **101**. Reagents and conditions: (a) Gly-OMe·HCl, EDCI, HOBt, CH_3CN, rt, 1 h, 77%; (b) $LiOH/H_2O$, THF, rt, 3 h, 98%; (c) Gly-OMe·HCl, EDCI, HOBt, CH_3CN, rt, 1 h, 72%; (d) $LiOH/H_2O$, THF, rt, 3 h, 90%; (e) **7**, EDCI, HOBt, CH_3CN, rt, 1 h, 21%.



Scheme 5. Synthesis of compounds **106** and **107**. Reagents and conditions: (a) EDCI, HOBt, CH_3CN; (b) TFA/CH_2Cl_2, then 1 M HCl–Et_2O.

Peptoid **96** was chosen for further testing based on its good activity against the four VRE strains. It was tested against methicillin-sensitive (MSSA), methicillin-resistant (MRSA) and vancomycin-intermediate (VISA) *S. aureus*, *Staphylococcus epidermis* and vancomycin-resistant (VRE) and vancomycin-sensitive (VRS) enterococci. The test compound showed good activity against five of these Gram-positive bacteria (MIC generally in the range 3–6 μg/mL) and showed only moderate activity against VSE (Table 4). This compound was slightly less active against MSSA, MRSA, VISA and VSE than the peptoids **4** and **5** that were subjected to a broader range of screening and against a larger variety of organisms.[14]

Although the mode of action of the tripeptoids has not been established, our earlier results on peptoid **4** suggested that more than one mode of action was involved. Our ESI-MS studies on **4** indicated that inhibition of cell wall cross linking through peptide complexation could be one possible mode of action. Our other results suggested that a more general Gram-positive bacterial cell membrane disruption activity component.[14] Uncertainty still surrounds the precise events responsible for bacterial death in vitro on exposure to other cationic peptides. Evidence for either cytoplasmic membrane damage inducing changes in permeability[17] or a combination of both membrane damage and interaction with other targets within the cell has been reported.[18] This type of dual action has been demonstrated for the vancomycin analogue telavancin which affects cell wall synthesis and the integrity of the cell membrane.[19]

In conclusion, a total of 29 novel acyclic tripeptoids have been prepared and tested for antibacterial activity against *S. aureus* and four clinical VRE strains. The di-cationic peptoids **6**, **76**, **90**, **95** and **96** showed the best overall activities. Peptoid **96** showed good activity against *S. epidermidis* and MSSA, MRSA, VISA and VRE organisms. Our results highlight the importance of both hydrophobic and cationic character within the peptoid and showed that the nature and placement of these cationic residues was important in determining antibacterial activity. Unfortunately we have not been able to perform any useful quantitative SAR studies on these peptoids because of their extreme flexibility which results in many computationally generated low energy conformations. We have however demonstrated that cationic residues at C-5 and C-8 are critical for good antibacterial activity. When the C-8 residue is D-Lys then for low activity against *S. aureus* (MIC = 1.95 μg/mL), we require C-5 to be D-Arg, Dab, Orn or Lys. However the combination of C-5 D-Lys and C-8 D-Arg seems to be the best for activity against the four clinical VRE strains. The study provided us a platform for further development of antimicrobial agents with improved activity against *S. aureus* and other pathogenic and drug resistant bacteria and these studies will be reported in future publications.

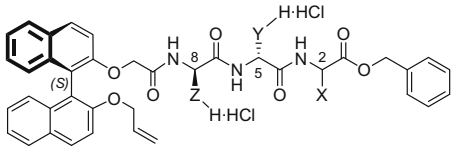
3. Experimental

3.1. General methods were as described previously[14]

3.1.1. Synthesis of compound 6

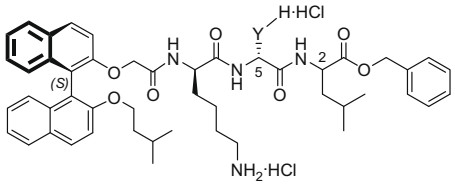
3.1.1.1. Benzyl (2*S*,5*R*,8*R*)-2-allyl-11-[2-((*S*)-2′-allyloxy-1,1′-binaphthalen-2-yloxy)]-3,6,9-triaza-8-(*tert*-butoxycarboxamidobutyl)-5-{3-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)guanidino]propyl}-4,7,10-trioxoundecanoate (13). To a solution of (2-allyloxy-[1,1′]-(*S*)-binaphthalen-2-yloxy)-acetic acid (28 mg, 0.073 mmol) in acetonitrile (10 mL) at rt was added **11**[15] (63 mg, 0.073 mmol), followed by HOBt (10.8 mg, 0.080 mmol) and EDCI (15.4 mg, 0.080 mmol). The mixture was stirred at rt for 16 h before the solvent being evaporated. Purification by column chromatography (5% $MeOH/CH_2Cl_2$) yielded **13** (71 mg, 0.058 mmol, 79%) as a white solid. Mp 72–74 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.71–0.86, m, 2H, H2‴′; 0.87–1.13, m, 2H, H2″; 1.18–1.27, m, 2H, H3‴″; 1.27, s, 6H, 2 × 2‴-CH_3; 1.30–1.42, m, 2H, H1‴″; 1.40, s, 9H, C$(CH_3)_3$; 1.44–1.62, m, 2H, H1″; 1.75, t, J = 6.3 Hz, H3‴; 2.08, s, 3H, 8‴-CH_3; 2.42–2.64, m, 4H, H1′ and H4‴); 2.53 (s, 3H, 5‴-CH_3); 2.55 (s, 3H, 7‴-CH_3); 2.82–2.96, m, 2H, H4‴″; 3.00–3.16, m, 2H, H3″; 3.99–4.13, m, 1H, H8; 4.37–4.62, m, 4H, H1‴′, H2 and H5; 4.76–5.10, m, 6H, H11, H3′ and H3‴′; 5.13, AB_q, J = 12.3 Hz, 2H, PhCH_2O; 5.56–5.72, m, 2H, H2′ and H2‴′; 6.20–6.22, m, 2H, NH; 7.08–7.44, m, 13H, ArH; 7.80–7.89, m, 2H, ArH; 7.90–7.98, m, 2H, ArH. ^{13}C NMR ($CDCl_3$, 75 MHz): δ 12.1, 8‴-CH_3; 17.5, 5‴-CH_3; 18.5, 7‴-CH_3; 21.3, C1″; 21.4, C3‴; 22.4, C3‴″; 25.3, C2‴″; 26.7, 2‴-CH_3; 28.4, C$(CH_3)_3$; 29.0, C1‴; 31.3, C4‴; 32.7, C2″; 35.8, C1′; 40.0, C4‴″; 40.4, C3″; 52.0, C5; 52.7, C2; 53.4, C8; 67.0, C1‴′; 67.8, ArCH_2; 70.6, C10; 73.5, C2‴; 78.9, C$(CH_3)_3$; 114.2, ArCH; 115.9, ArCH; 116.1, ArCH; 116.9, ArCH; 117.3, ArC4a‴; 117.8, C3′; 118.9, C3‴′; 119.3, ArCH;

Table 1
MIC values against *S. aureus* of the peptoids **6**, **67–79** as their hydrochloride salts



	C2	C5	C8	MIC (μg/mL)				
				S. aureus	VRE_{243}	VRE_{449}	VRE_{820}	VRE_{987}
6	L-Allyl-Gly	D-Arg	D-Lys	1.95	31.3	31.3	31.3	31.3
67	L-Allyl-Gly	D-Arg	D-Arg	31.3	>125	125	125	>125
68	L-Allyl-Gly	D-Lys	D-Lys	3.9	125	62.5	62.5	125
69	L-Allyl-Gly	D-Arg	Gly	15.6	>125	125	125	>125
70	L-Allyl-Gly	D-Arg	D-Ala	5.2	62.5	62.5	62.5	62.5
71	L-Allyl-Gly	D-Arg	D-allyl-Gly	15.6	125	125	62.5	125
72	L-Allyl-Gly	D-Arg	D-Leu	3.9	62.5	31.3	31.3	62.5
73	L-Allyl-Gly	D-Arg	D-Phe	26.1	>125	62.5	31.3	>125
74	L-Allyl-Gly	D-Arg	D-norLeu	52.1	125	83	62.5	125
75	D-Allyl-Gly	D-Arg	D-Lys	15.6	125	104.2	62.5	125
76	L-Leu	D-Arg	D-Lys	1.95	31.3	31.3	31.3	31.3
77	L-Ala	D-Arg	D-Leu	5.2	62.5	62.5	62.5	62.5
78	L-Ala	D-Arg	D-Lys	7.8	125	125	125	125
79	Gly	D-Arg	D-Lys	3.9	125	62.5	125	125
Vancomycin				1.56	1.56	>25	>25	3.13

Table 2
MIC values against *S. aureus* of the peptoids **90–96** as their hydrochloride salts



	C2	C5	MIC (μg/mL)					
			S. aureus	*S. epi.*	VRE_{243}	VRE_{449}	VRE_{820}	VRE_{987}
90	L	Dab	1.95	—	31.3	15.6	15.6	31.3
91	L	Nor-Arg	2.6	—	52.1	31.3	27.8	52.1
92	L	Orn	1.95	—	62.5	31.3	15.6	62.5
93	L	N^m-Me-Arg	3.13	3.13	25	25	25	25
94	L	Lys	1.95	1.95	62.5	31.3	26.1	62.5
95	D	Arg	3.9	—	31.3	15.6	15.6	31.3
96	L	Arg	3.9	—	15.6	15.6	7.8	15.6
Vancomycin			1.56	3.13	1.56	>25	>25	3.13

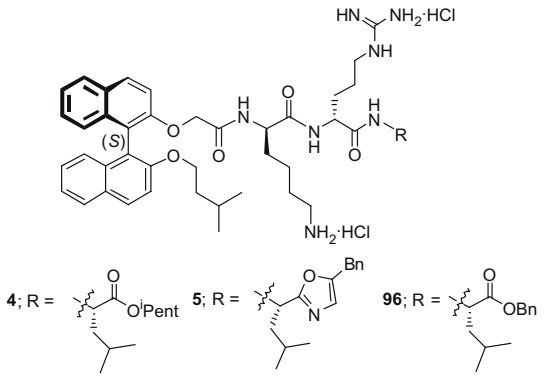
120.1, ArCH; 123.8, ArC8‴; 123.9, ArCH; 124.2, ArCH; 125.4, ArCH; 126.6, ArCH; 128.0, ArCH; 128.2, ArCH; 128.3, ArC; 128.4, ArC; 128.5, ArC; 129.1, ArCH; 129.2, ArCH; 129.7, ArCH; 129.9, ArC; 132.4, C2′; 133.3, C2⁗; 133.5, ArC; 133.7, ArC; 134.8, ArC; 135.3, ArC5‴; 135.4, ArC7‴; 152.1, ArC8‴; 153.5, ArC; 153.8, ArC; 156.0, NCO_2; 156.1, ArC6‴; 156.9, CN_3; 170.1, C10; 171.3, C7; 171.4, C4; 172.0, C1. MS (ESI, +ve) *m/z* 1222 (10%) $[M+H]^+$, 1172 (100%). HRMS (ESI, +ve) calcd for $C_{68}H_{84}N_7O_{12}S$ 1222.5899, found 1222.5889.

3.1.1.2. Benzyl (2*S*,5*R*,8*R*)-2-allyl-11-[2-((*S*)-2′-allyloxy-1,1′-binaphthalen-2-yloxy)]-3,6,9-triaza-8-(butylamino)-5-(3-guanidinopropyl)-4,7,10-trioxoundecanoate dihydrochloride (6). To a solution of **13** (65 mg, 0.055 mmol) in CH_2Cl_2 (5 mL) was added TFA (5 mL). The mixture was allowed to stir at rt for 3 h. The solvent was removed under reduce pressure, and the residue was suspended in a minimal amount of methanol. The solution was then treated with an excess of 1 M HCl/ether solution and the solvent evaporated. The crude product was purified by precipitation from

Table 3
MIC values against *S. aureus* of the peptoids **80–82**, **86**, **89**, **101**, **106** and **107** as their hydrochloride salts

	MIC (μg/mL)					
	S. aureus	*S. epi.*	VRE_{243}	VRE_{449}	VRE_{820}	VRE_{987}
80	15.6	—	>125	125	125	>125
81	3.9	—	125	125	125	125
82	7.8	—	125	125	125	125
86	3.9	—	31.3	15.6	15.6	31.3
89	1.95	19.5	62.5	31.3	15.6	31.3
101	3.9	3.9	62.5	31.3	15.6	31.3
106	3.3	—	62.5	62.5	62.5	62.5
107	3.9	—	62.5	62.5	62.5	125
Vancomycin	1.56	3.13	1.56	>25	>25	3.13

Table 4
MIC values of compounds **4**, **5** and **96** against Gram-positive isolates



Strain[a]	MIC_{50} (range) or MIC (μg/mL)			
	4[14]	**5**[14]	**96**	Van
S. aureus				
MSSA (8)	4	4	6	1–2
MRSA (7)	4 (2–4)	4	6	1–2
VISA (1)[b]	4	4	6	6–8
S. epidermidis (3)	4	4	3	2–3
E. faecium				
VRE (4)	4	4	2	>32
VSE (4)	4 (2–4)	4	16	2–4

[a] Compounds **4** and **5** were tested against a variety of strains (number in brackets) of *S. aureus*, *S. epidermidis* and *E. faecium* while compound **96** was tested against only one strain of each organism. Where the MIC was the same for each strain, no range is given.

[b] Where only one strain was tested, the value given is an MIC. *S. epidermidis* = *Staphylococcus epidermidis*; *E. faecium* = *Enterococcus faecium*; MSSA = methicillin-sensitive *S. aureus*; MRSA = *methicillin-resistant S. aureus*; VISA = vancomycin-intermediate *S. aureus*; VRSA = vancomycin-resistant *S. aureus*; VRE = vancomycin-resistant enterococci; VSE = vancomycin-sensitive enterococci. Minimum inhibitory concentration (μg/mL).

CH_2Cl_2 by additional of diethyl ether to yield **6** (29 mg, 62%) as a highly hydroscopic cream solid. ^{1}H NMR (CD_3OD, 300 MHz): δ 0.95–1.03, m, 2H, H2‴; 1.05–1.21, m, 2H, H2″; 1.40–1.88, m, 6H, H1″, H1‴ and H3‴; 2.40–2.60, m, 2H, H1′; 2.68–2.86, m, 2H, H4‴; 3.04–3.12, m, 2H, H3″; 4.06–4.18, m, 1H, H8; 4.32–4.68, m, 6H, H2, H5, H11 and H1⁗; 4.86–5.15, m, 4H, H3′ and H3⁗; 5.16, AB_q, J = 3.6 Hz, 2H, PhCH_2; 5.69–5.80, m, 2H, H2′ and H2⁗; 7.06, d, J = 7.8 Hz, 2H, ArH; 7.20–7.52, m, 2H, ArH; 7.32–7.37, m, 7H, ArH; 7.48 (d, J = 9.3 Hz, 1H, ArH); 7.55 (d, J = 9.3 Hz, 1H, ArH); 7.92, d, J = 7.8 Hz, 2H, ArH; 8.01 (d, J = 6.0 Hz, 1H, ArH); 8.04 (d, J = 6.0 Hz, 1H, ArH); 8.16–8.30, m, 1H, NH. ^{13}C NMR (CD_3OD, 75 MHz): δ 23.2, C3‴; 26.2, C2‴; 27.8, C2″; 30.3, C1‴; 32.2, C1″; 36.7, C1′; 40.4, C4‴; 41.9, C3″; 53.6, C8; 53.7, C2; 53.9, C5; 68.1, $ArCH_2$; 69.2, C1⁗; 70.9, C11; 116.0, ArCH; 116.9, C3⁗; 117.0, C3′; 119.1, ArC; 120.5, ArC; 21.6, ArCH; 124.9, ArCH; 125.3, ArCH; 126.0, ArCH; 126.4, ArCH; 127.6, ArCH; 129.2, ArCH; 129.3, ArCH; 129.3, ArCH; 129.4, ArC; 129.6, ArC; 130.8, ArCH; 130.8, ArCH; 131.0, ArCH; 131.4, ArCH; 134.2, ArC; 135.0, ArC; 135.1, C2⁗; 135.1, C2′; 137.1, ArC; 154.1, ArC; 155.4, ArC; 158.5, CN_3; 170.9, C10; 172.5, C7; 173.2, C2; 173.8, C4. MS (ESI, +ve) *m/z* 856 (100%) $[M+H]^+$. HRMS (ESI, +ve) calcd for $C_{49}H_{58}N_7O_7$ 856.4398, found 856.4367.

3.1.2. Synthesis of compound 96

3.1.2.1. Benzyl (2*S*,5*R*)-3-aza-5-(9*H*-9-fluorenylmethoxycarboxamido)-8-{3-[(3,4-dihydro-2,2,5,7,8-pentamethyl-2*H*-1-benzopyran-6-yl)sulfonyl]guanidino}-2-(3-methylpropyl)-4-oxooctanoate (20). To a solution of **15**[20] (160 mg, 0.723 mmol) in acetonitrile (10 mL) was added diisopropylethylamine (0.15 mL, 0.87 mmol). The reaction mixture was stirred for 1 min, and then Fmoc-(*R*)-Arg(Pmc)-OH (662.8 mg, 1.00 mmol) was added, followed by HOBt (117 mg, 0.87 mmol) and EDCI (167 mg, 0.87 mmol). The mixture was stirred at rt for 3 h before the solvent being evaporated to dryness. Purification by flash column chromatography (3% MeOH/CH_2Cl_2) afforded the desired product (460 mg, 73%) as an off white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.79–0.81 (m, 6H, $2CH_3$), 1.21 and 1.22 (2s, 6H, $2CH_3$ (Pmc)), 1.46–1.78 (m, 6H), 1.67 (t, J = 6.9 Hz, 2H, CH_2 (Pmc)), 1.80–1.98 (m, 1H), 2.04 (s, 3H, CH_3 (Pmc)), 2.45–2.58 (m, 2H, CH_2 (Pmc)), 2.54 (s, 3H, CH_3 (Pmc)), 2.58 (s, 3H, CH_2 (Pmc)), 3.10–3.30 (m, 2H, CH_2N), 4.01–4.06 (m, 1H), 4.14–4.34 (m, 3H), 4.46–4.59 (m, 1H), 5.02 and 5.08 (ABq, J = 12.3 Hz, 2H), 6.12–6.46 (br s, 3H, NH), 7.16–7.34 (m, 9H, ArH), 7.50 (d, J = 7.3 Hz, 2H, ArH), 7.69 (d, J = 7.3 Hz, 2H, ArH). MS (ESI +ve) *m/z* 888 ($[M+Na]^+$, 100%), 866 ($[M+H]^+$, 10%).

3.1.2.2. Benzyl (2*S*,5*R*)-5-amino-3-aza-8-{3-[(3,4-dihydro-2,2,5,7,8-pentamethyl-2*H*-1-benzopyran-6-yl)sulfonyl]guanidino}-2-(3-methylpropyl)-4-oxooctanoate (25). To a solution of **20** (450 mg, 0.520 mmol) in acetonitrile (10 mL) was added piperidine (57 μL, 0.57 mmol). The mixture was stirred overnight at rt, and then the solvent was evaporated to dryness. Purification by flash column chromatography afforded the desired product (244 mg, 73%) an off white solid. R_f = 0.07 (5% MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86–0.90 (m, 6H, $2CH_3$), 1.28 (s, 6H, $2CH_3$ (Pmc)), 1.53–1.62 (m, 5H), 1.75–1.79 (m, 3H), 1.86 (s, br, 2H, NH_2), 2.08 (s, 3H, CH_3 (Pmc)), 2.54 (s, 3H, CH_3 (Pmc)), 2.58 (s, 3H, CH_3 (Pmc)), 2.54–2.60 (m, 2H, CH_2 (Pmc)), 3.02–3.24 (m, 2H, CH_2N), 3.30–3.44 (m, 1H), 4.42–4.76 (m, 1H), 5.05 and 5.13 (ABq, J = 12.3 Hz, 2H), 6.22–6.46 (m, 3H, NH), 7.20–7.37 (m, 5H, ArH), 7.78 (d, J = 7.6 Hz, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.0, 17.3, 18.4, 21.3, 21.5, 22.7, 24.7, 25.2, 26.6, 31.7, 32.6, 40.5, 50.7, 54.1, 66.8, 73.5, 117.8, 123.9, 128.0, 128.2, 128.4, 133.3, 134.6, 135.2, 135.3, 153.4, 156.2, 172.7, 175.5. MS (ESI +ve) *m/z* 644 ($[M+H]^+$, 100%).

3.1.2.3. Benzyl (2*S*,5*R*,8*R*)-3,6-diaza-8-(9*H*-9-fluorenylmethoxycarboxamido)-5-{3-[(3,4-dihydro-2,2,5,7,8-pentamethyl-2*H*-1-benzopyran-6-yl)sulfonyl]guanidinopropyl}-12-(1,1-dimethylethoxycarbonylamino)-2-(3-methylpropyl)-4,7-dioxododecanoate (37). To a solution of **25** (240 mg, 0.373 mmol) in acetonitrile (10 mL) was added Fmoc-(*R*)-Lys(Boc)-OH (187 mg, 0.4 mmol), followed by HOBt (60 mg, 0.45 mmol) and EDCI (86 mg, 0.45 mmol). The mixture was stirred at rt for 3 h before the solvent being evaporated to dryness. Purification by flash column chromatography (3% MeOH/CH_2Cl_2) afforded the desired product (336 mg, 82%) as an off white solid. R_f = 0.28 (5% MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.81–0.85 (m, 6H, $2CH_3$), 1.06–1.23 (m, 2H), 1.06–1.88 (m, 15H), 1.18 (s, 6H, $2CH_3$ (Pmc)), 1.40 (s, 9H, $C(CH_3)_3$), 2.02 (s, 3H, CH_3 (Pmc)), 2.40–2.55 (m, 2H, CH_2 (Pmc)), 2.52 (s, 3H, CH_3 (Pmc)), 2.55 (s, 3H, CH_3 (Pmc)), 3.02 (s, br, 2H, CH_2N (Lys)), 3.18 (s, br, 2H (CH_2N (Arg)), 3.78–4.02 (m, 1H), 4.04–4.40 (m, 3H), 4.42–4.64 (m, 1H), 4.74–5.16 (m, 2H), 6.10–6.68 (m, 4H, NH), 7.17–7.36 (m, 9H, ArH), 7.43 (d, J = 7.6 Hz, NH), 7.42–7.54 (m, 2H, ArH), 7.60 (s, br, 1H, NH), 7.69 (d, J = 7.6 Hz, 2H, ArH)); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.0, 17.3, 17.5, 18.5, 21.2, 22.7, 26.5, 26.6, 28.4, 28.9, 29.5, 31.7, 32.5, 39.9, 46.7, 48.2, 53.1, 53.4, 66.8, 67.2, 73.5, 79.0, 117.8, 119.7, 123.9, 125.0, 125.2, 126.8, 127.5, 127.8, 128.1, 128.3, 133.0, 134.7, 135.2, 140.9, 141.0, 143.4, 144.0, 153.5, 156.2, 156.8, 171.9, 172.9, 173.0. MS (ESI +ve) *m/z* 1116 ($[M+Na]^+$, 80%), 1094 ($[M+H]^+$, 100%).

3.1.2.4. Benzyl (2*S*,5*R*,8*R*)-8-amino-3,6-diaza-5-{3-[(3,4-dihydro-2,2,5,7,8-pentamethyl-2*H*-1-benzopyran-6-yl)sulfonyl]guanidinopropyl}-12-(1,1-dimethylethoxycarbonylamino)-2-(3-methylpropyl)-4,7-dioxododecanoate (50). To a solution of **37** (330 mg, 0.302 mmol) in acetonitrile (10 mL) was added piperidine

(33 μL, 0.33 mmol). The mixture was stirred overnight at rt, and then the solvent was evaporated to dryness. Purification by flash column chromatography afforded the desired product (239 mg, 91%) an off white solid. R_f = baseline (5% MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82 (d, J = 5.8 Hz, 3H, CH_3), 0.84 (d, J = 5.8 Hz, 3H, CH_3), 1.24–2.02 (m, 13H), 1.27 (s, 6H, 2CH_3 (Pmc)), 1.38 (s, 9H, $C(CH_3)_3$), 1.76 (t, J = 6.0, 2H, CH_2 (Pmc)), 2.07 (s, 3H, CH_3 (Pmc)), 2.52 (s, 3H, CH_3 (Pmc)), 2.54 (s, 3H, CH_3 (Pmc)), 2.64–2.62 (m, 2H, CH_2 (Pmc)), 2.90–3.08 (m, 2H, CH_2N (Lys)), 3.09–3.24 (m, 1H, CH_2N (Arg, H_a)), 3.25–3.36 (m, 1H, (Arg, H_b)), 4.38–4.61 (m, 2H), 4.84–4.98 (m, NH), 5.03 and 5.09 (ABq, J = 12.3 Hz, 1H), 6.20–6.50 (m, 3H, NH), 7.24–7.29 (m, 5H, ArH), 7.58 (d, J = 7.9 Hz, NH), 7.95 (d, J = 7.3 Hz, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.0, 17.3, 18.4, 21.2, 21.4, 22.6, 24.7, 25.4, 26.6, 28.3, 29.6, 29.8, 32.6, 34.6, 40.1, 40.3, 50.8,52.1, 54.8, 66.7, 73.5, 78.8, 117.8, 123.8, 127.8, 128.1, 128.4, 133.3, 134.6, 135.2, 135.3, 153.4, 156.2, 171.8, 172.6, 175.8. MS (ESI +ve) m/z 872 ([M+H]$^+$, 100%).

3.1.2.5. Benzyl (2*S*,5*R*,8*R*)-3,6,9-triaza-5-{3-[(3,4-dihydro-2,2,5,7,8-pentamethyl-2*H*-1-benzopyran-6-yl)sulfonyl]guanidinopropyl}-11-[(*S*)-2′-(3-methylbutoxy)-1,1′-binaphthalen-2-yloxy]-8-[4-(1,1-dimethylethoxycarbonylamino)butyl]-2-(2-methylpropyl)-4,7,10-trioxoundecanoate (protected 96). To a solution of **87**[14] (50 mg, 0.121 mmol) and **50** (110 mg, 0.126 mmol) in acetonitrile (10 mL) was added HOBt (20 mg, 0.15 mmol) and EDCI (28 mg, 0.15 mmol). The mixture was stirred at rt for 3 h before the solvent being evaporated to dryness. Purification by flash column chromatography (3% MeOH/CH_2Cl_2) afforded the desired product (114 mg, 74%) as an off white solid. R_f = 0.16 (5% MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.46 (d, J = 6.2 Hz, 3H), 0.52 (d, J = 6.2 Hz, 3H), 0.70–1.00 (m, 9H), 1.20–1.35 (m, 5H), 1.28 (s, 6H, CH_3 (Pmc)), 1.38–1.54 (m, 2H), 1.41 (s, 9H, $C(CH_3)_3$), 1.57–1.87 (m, 7H), 2.09 (s, 3H, CH_3 (Pmc)), 2.40–2.48 (m, 2H, CH_2 (Pmc)), 2.55 (s, 3H, CH_3 (Pmc)), 2.57 (s, 3H, CH_3 (Pmc)), 2.80–2.98 (m, 2H, CH_2N (Lys)), 3.00–3.30 (m, 2H, CH_2N (Arg)), 3.78–3.92 (m, 1H), 3.99–4.15 (m, 2H), 4.34–4.60 (m, 4H), 4.78–4.90 (m, 1H, NH), 5.07 and 5.16 (ABq, J = 12.6 Hz, 1H), 6.08 (s, br, NH), 6.18 (d, J = 7.0 Hz, NH), 6.29 (br s, NH), 6.48 (br s, NH), 7.09–7.21 (m, 11H, ArH), 7.45 (d, J = 9.1 Hz, 2H, ArH), 7.69–7.90 (m, 2H, ArH), 7.91–8.01 (m, 2H, ArH), 8.06 (d, J = 8.8 Hz, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.1, 17.5, 18.6, 21.4, 21.6, 22.0, 22.3, 22.9, 24.5, 24.9, 25.2, 26.8, 28.5, 29.2, 32.8, 38.0, 39.9, 40.1, 40.4, 40.6, 51.1, 52.2, 53.3, 67.0, 68.0, 68.7, 73.6, 79.2, 114.1, 116.2, 117.9, 119.8, 120.2, 124.0, 124.1, 124.3, 125.1, 125.5, 126.7, 126.8, 128.0, 128.1, 128.3, 128.5, 128.6, 129.3, 129.6, 129.8, 130.4, 133.5, 133.6, 133.9, 134.9, 135.47, 135.54, 152.1, 153.5, 154.4, 156.2, 169.5, 171.4, 173.1. MS (ESI +ve) m/z 1291 ([M+Na]$^+$, 70%), 1268 ([M+H]$^+$, 100%). HRMS (ESI +ve) calcd for $C_{71}H_{94}N_7O_{12}S$ 1268.6681, found 1268.6687 ([M+H]$^+$, 100%).

3.1.2.6. Benzyl (2*S*,5*R*,8*R*)-3,6,9-triaza-8-butylamino-5-(3-guanidinopropyl)-11-[(*S*)-2′-(3-methylbutoxy)-1,1′-binaphthalen-2-yloxy]-8-(4-(1,1-dimethylethoxycarbonylamino)butyl)-2-(2-methylpropyl)-4,7,10-trioxoundecanoate dihydrochloride (96). To a solution of protected **96** (104 mg, 0.082 mmol) in CH_2Cl_2 (5 mL) was added TFA (5 mL). The mixture was stirred for 16 h at rt. The solvent was evaporated under reduced pressure. The crude residue was redissolved in CH_2Cl_2 (3 mL). 1 M HCl–Et_2O (excess) was added, and the mixture was stirred for a minute before the solvent being removed. This step was repeated twice more. The crude product was purified by precipitation from CH_2Cl_2 by additional of diethyl ether to yield the desired product (78 mg, 98%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 0.38 (d, J = 6.2 Hz, 3H), 0.43 (d, J = 6.2 Hz, 3H), 0.64–0.93 (m, 9H), 0.94–1.20 (m, 3H), 1.1.24–1.82 (m, 10H), 2.69 (s, br, 2H), 3.05 (s, br, 2H), 3.72–3.90 (m, 1H), 3.93–4.10 (m, 2H), 4.20–4.54 (m, 4H), 4.94–5.14 (m, 2H), 6.93–6.96 (m, 2H, ArH), 7.03–7.09 (m, 2H, ArH), 7.14–7.30 (m, 7H, ArH), 7.34 (d, J = 9.1 Hz, 1H, ArH), 7.42 (d, J = 9.1 Hz, 1H, ArH), 7.75–7.93 (m, 4H, ArH); ^{13}C NMR (75 MHz, CD_3OD) δ 21.7, 22.5, 22.8, 23.1, 23.3, 25.5, 25.9, 26.2, 27.7, 30.1, 32.1, 39.2, 40.4, 41.0, 41.9, 52.3, 53.6, 54.1, 67.8, 68.9, 69.2, 115.9, 116.9, 120.4, 121.7, 124.8, 125.1, 125.9, 126.3, 127.4, 127.5, 129.0, 129.1, 129.3, 129.5, 129.6, 130.6, 130.8, 130.9, 131.3, 135.0, 135.1, 137.1, 153.9, 155.8, 158.4, 170.9, 173.1, 173.6, 174.0. MS (ESI +ve) m/z 902 ([M+H]$^+$, 10%), 452 ([M+H]$^{2+}$, 100%). HRMS (ESI +ve) calcd for $C_{52}H_{68}N_7O_7$ 902.5180, found 902.5189 ([M+H]$^+$, 100%).

3.2. HPLC method

Gradient system comprised of H_2O containing 10% CH_3CN and 0.1% TFA (A), and CH_3CN containing 0.1% TFA (B). The gradient profile was 0–2 min, isocratic 15% B; 3–35 min, linear gradient 15–60% of B; 35–36 min, isocratic 60% B; 37–42 min, isocratic, 100% B. t_R = 28.7, 99% pure.

3.3. 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.

Compound **96** was tested at ImQuest BioSciences on *S. aureus* wild types (ATCC 6538P), Mu50 (ATCC 700699) and MRSA (ATCC 43300), *S. epidermidis* (ATCC 700562) and *E. faecium* (ATCC 700221). MICs of compounds were determined using micro-broth dilution analysis grown in Mueller Hinton II broth (cation adjusted) to a concentration of 1×10^6 colony forming units per mL. 100 mL was placed into triplicate wells containing 100 mL of test compound serially diluted twofold in Mueller Hinton II broth. The plates were incubated for 24 h at 37 °C and the MIC determined at the lowest compound dilution that completely inhibited microbial growth.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.02.033.

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