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Binaphthyl-Based Dicationic Peptoids with Therapeutic Potential**

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While the cationic glycopeptide vancomycin has long been regarded as the gold standard for the treatment of recalcitrant Gram-positive bacterial infection, this position has been compromised by the emergence of resistant strains.[1–3] The first report[4] of such resistance emerged in 1988, and has subsequently widened amongst the enterococci[5] and staphylococci, including methicillin-resistant *Staphylococcus aureus* (MRSA);[1,2] cross-resistance to linezolid[6] is also a concern. Some recent chemical strategies for overcoming this resistance[7] have centered on other high molecular weight cyclic peptides,[8–10] elegantly crafted vancomycin[11] or vancomycin aglycone[12,13] analogues, potent dual-action vancomycin/β-lactam hybrid antibiotics,[14] or large vancomycin dimers.[15,16] An alternative strategy is to design smaller, simpler cationic peptoids with some related design features to vancomycin which could still interact with the altered peptide-glycan cell-wall moiety in both vancomycin-resistant[3] and -sensitive strains and thus broaden the antibacterial spectrum. Svendsen et al. designed minimal cationic peptidomimetics, and a pharmacophore has been developed for dipeptides which includes the presence of two cationic charges and two hydrophobic units of steric bulk.[17–19] Subsequently, cationic tripeptide analogues were developed[20,21] that demonstrated good activity against both Gram-positive (including MRSA) and Gram-negative bacteria, but were not evaluated with respect to vancomycin-resistant strains.[20,21]

In our minimization strategy we explored cyclic peptoids containing a hydrophobic scaffold[22,23] that specifically contained 1) a basic amino acid residue to potentially engage in an ionic interaction with the terminal carboxylate group of (*R*)-Ala or (*R*)-Lac in the bacterial cell-wall peptide-glycan unit, 2) a tripeptide moiety for H-bonding interactions with either of these two terminal groups, and 3) a 1,1′-binaphthyl system (or related systems[24]) to enable hydrophobic interactions with the (*R*)-Ala methyl group or with other hydrophobic regions. Subsequently, through selective manipulations of the C terminus and addition of a further basic amino acid component, the Lys-Arg-containing acyclic peptoid **1** (Scheme 1; unpublished work) was found with a minimum inhibitory concentration (MIC) of 2 $\mu g\,mL^{-1}$ against *S. aureus*, but 31 $\mu g\,mL^{-1}$ against vancomycin-resistant enterococci (VRE). Modification of the 2-naphthyl ether group and the C-terminal region then resulted in ester **2a** and the ester-bioisosteric oxazole **2b** (Scheme 1), which are the subject of this paper. These two dicationic peptoids represent a promising new antibacterial subclass of the cationic peptides.

A modular approach amenable to scaleup was adopted for the synthesis of **2a** and **2b** (Scheme 2). The key precursor for the scaffold was commercially available (*S*)-1,1′-binaphthalene-2,2′-diol (**3**). Base-induced mono-*O*-alkylation of **3** with 1-bromo-3-methylbutane, followed by *O*-alkylation of the remaining phenolic group with bromoacetic acid together with K_2CO_3, then provided access to key acid **4**. Compound **4** was then coupled with H-(*R*)-Lys(Boc)-OMe to give protected peptoid **5**, and subsequently acid **6** by base hydrolysis followed by acidification. *N*-Protected precursors of peptide amine salts **2a** and **2b** were accessed by diimide-mediated coupling of the appropriate starting amines **7** with an amine/amidine-group-protected (*R*)-arginine to give **8a,b**, followed by Fmoc deprotection to afford the amine intermediates **9a,b** in good yield. The EDCI/HOBT-promoted coupling of amines **9a,b** with acid **6** then gave the respective penultimate amide-coupled products (84% yield for the isopentyl ester

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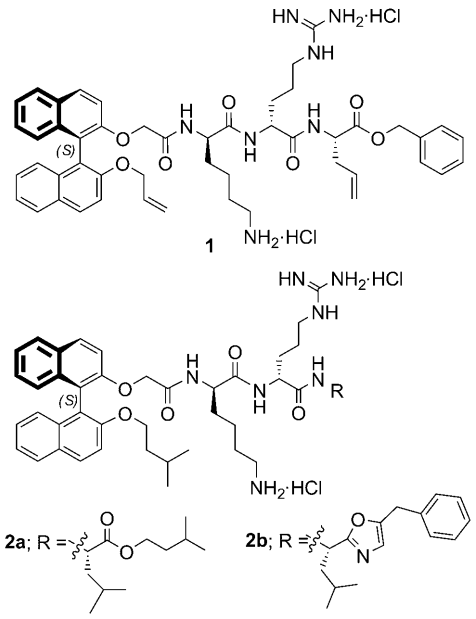
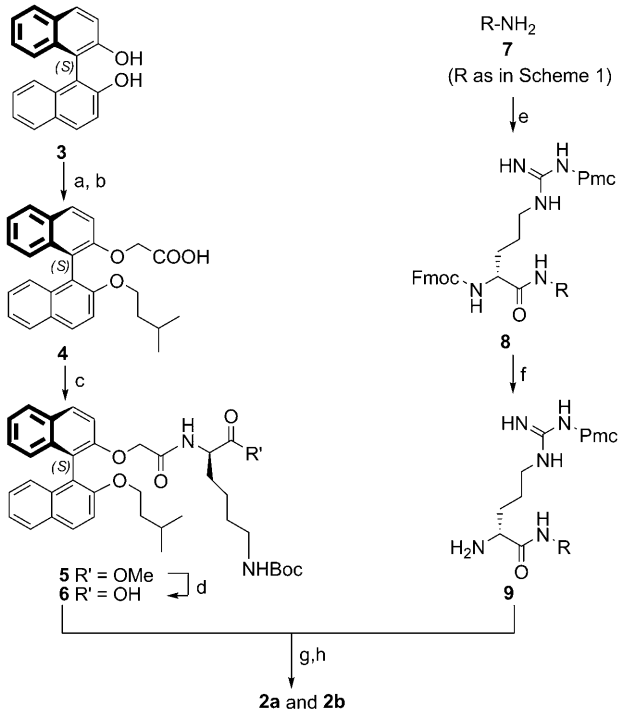


Scheme 1. Structures of dicationic peptoids **1** and **2a**/**b**.



Scheme 2. General synthetic route to **2a** and **2b**. (Note, in the compound numbers below, "**a**" corresponds to the R group in **2a** and "**b**" to the R group in **2b**). a) 1-Bromo-3-methylbutane, K_2CO_3, CH_3COCH_3, reflux, 16 h; b) $BrCH_2COOH$, K_2CO_3, MeOH, reflux, 3 h, 83% (2 steps); c) H-(*R*)-Lys(Boc)-OMe·HCl, EDCI, HOBt, MeCN, RT, 3 h, 90%; d) 4% LiOH/H_2O, THF, RT, 16 h, 89%; e) Fmoc-(*R*)-Arg(PG)-OH, EDCI, HOBt, MeCN, RT, 3 h, **8a** (97%), **8b** (94%); f) piperidine, MeCN, RT, 3 h, **9a** (91%), **9b** (97%); g) EDCI, HOBt, MeCN, RT, 3 h; h) TFA, CH_2Cl_2, RT, 16 h; then 1 M HCl/Et_2O, **2a** (92%), **2b** (62%). Boc = *tert*-butoxycarbonyl, EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, Fmoc = 9-fluorenylmethoxycarbonyl, HOBt = *N*-hydroxybenzotriazole, Pmc = 2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-1-benzopyran-6-sulfonyl, TFA = trifluoroacetic acid.

and 64% yield for the oxazole analogue). Deprotection of these amide products with TFA, addition of hydrogen chloride in diethyl ether, and freeze-drying gave the required salts **2a** and **2b** (Scheme 2). This general synthetic methodology was also used to access all diastereomers of peptoid **2a** for structure–activity relationship studies (see the Supporting Information). The structural and stereochemical integrity and purity (>95%) of **2a**, its diastereomers, and **2b** were established by HPLC and NMR analysis.

Compounds **2a** and **2b** are active against a variety of organisms, including *S. aureus*, coagulase-negative staphylococci, enterococci, and streptococci (Table 1 and the Supporting Information). The test compounds had a broad spectrum of activity against the Gram-positive bacteria, with MIC_{50} (or MIC) generally in the range 2–8 μg mL^{-1}, but reduced activity against streptococci with an MIC_{50} range of 4–16 μg mL^{-1} and poor activity against Gram-negative bacteria (8 to >32 μg mL^{-1}). The test compounds were also active against organisms resistant to vancomycin, methicillin, and linezolid, as well as community-acquired MRSA.

Table 1: In vitro antibacterial activity of **2a**, **2b**, and vancomycin (Van) against Gram-positive and Gram-negative isolates.

Strain (number of strains)	MIC_{50} (range) or MIC [μg mL^{-1}]		
	2a	**2b**	Van
S. aureus			
MSSA (8)	4 (2–4)	4	1 (1–2)
MRSA (7)[a]	4	4	2 (1–2)
VISA (1)[b]	4	4	8
VRSA (1)[b]	2	4	>32
PVL [CA-MRSA] (1)[b]	2	4	1
Linezolid R (1)[b]	4	4	1
S. epidermidis (3)[a]	4	4	2
E. faecium			
VRE (4)[a]	4	4	>32
VSE (4)	2 (2–4)	4	4 (2–8)
Streptococci (13)	8 (4–16)	8 (4–16)	1 (0.5–1)
Gram-negative bacilli			
E. coli (1)[b]	16	32	>32
K. pneumoniae (3)[a]	>32	>32	n.t.
A. baumannii (4)	4 (4 to >32)	8 (2 to >32)	n.t.
S. maltophilia (3)	8 (4–8)	16 (4–16)	n.t.

[a] 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*; *E. coli* = *Escherichia coli*; *K. pneumoniae* = *Klebsiella pneumoniae*; *A. baumannii* = *Acinetobacter baumannii*; *S. maltophilia* = *Stenotrophomonas maltophilia*. MSSA = methicillin-sensitive *S. aureus*; MRSA = methicillin-resistant *S.aureus*; VISA = vancomycin-intermediate *S. aureus*; VRSA = vancomycin-resistant *S. aureus*; PVL = Panton–Valentine Leucocidin-positive *S. aureus* (CA-MRSA = community-acquired MRSA); Linezolid R = linezolid-resistant; VRE = vancomycin-resistant enterococci; VSE = vancomycin-sensitive enterococci; n.t. = not tested.

In further biological testing, **2a** and **2b** showed rapid killing of MRSA within the first hour at concentrations of ≥2×MIC (Figure 1a and b).[25] The rate of killing was dose-dependent and faster and greater in magnitude than that

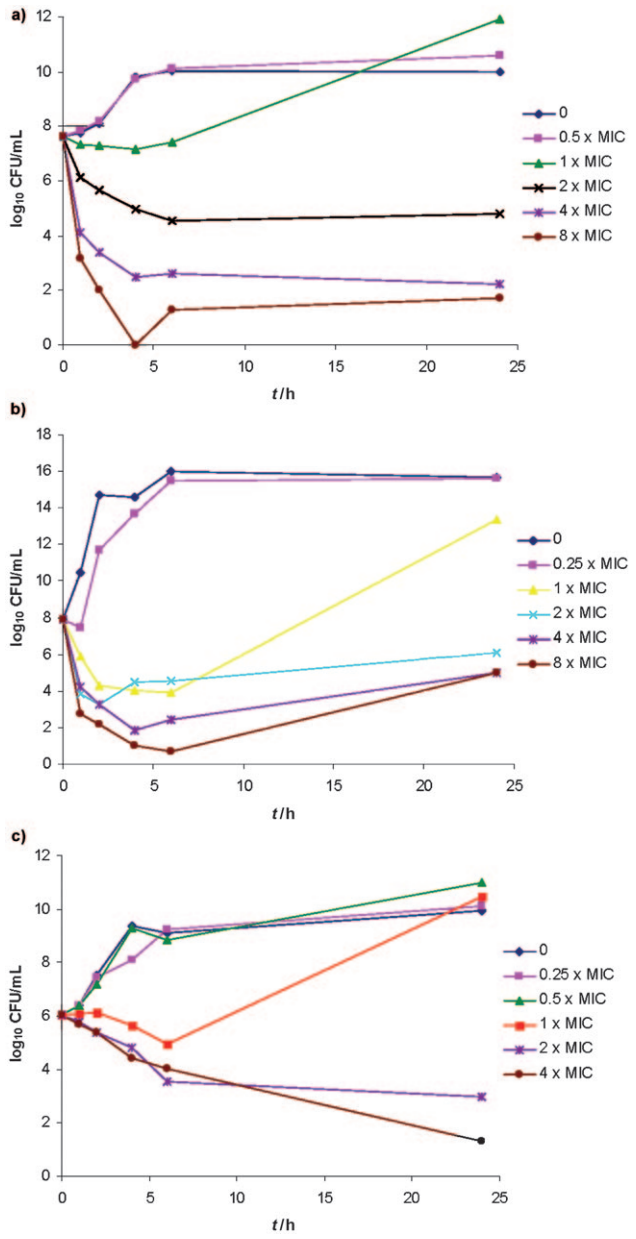


Figure 1. Mean time–kill values against MRSA for a) **2a**, b) **2b**, and c) vancomycin.

observed with vancomycin (Figure 1 a–c). Peptoids **2a** and **2b** were rapidly bactericidal (at 2 × MIC) with an associated, but not simultaneous, reduction in optical density suggesting the occurrence of bacterial cell lysis. The degree of bacteriolysis was concentration-dependent (not shown). The results indicate that bacteriolysis follows loss of cell viability, and possibly suggests a complex mode of bacterial killing by these compounds.

The observed in vitro activity was translated into in vivo activity both systemically and topically. Since oxazole **2b** is more resistant to enzymatic hydrolysis than the terminal ester analogue, it was tested systemically in an animal model which measured the bacterial growth of MRSA (vancomycin-sensitive strain BAA-41) in the spleens of infected mice. Compound **2b** dosed systemically (5 mg kg^{-1} intraperitoneal (i.p.) twice a day for 4 d; initial oral infection[26] with 5×10^{10} colony-forming units (cfu) per mouse, $n = 10$) effectively reduced the number of viable bacteria remaining in the spleens (11 cfu per spleen); this compared favorably with the result for vancomycin (6 cfu per spleen), while with the control (DMSO vehicle), 128 cfu per spleen remained. Similar results were obtained for **2b**, vancomycin, and the control for the other bacterial inocula tested (5×10^9 and 1.5×10^{10} cfu per mouse).

Activity was established topically by using the mouse nasal decolonization model[27–28] with MRSA. Twice-daily administration for 5 d of **2a** or **2b** resulted in a significant dose-dependent reduction in MRSA nasal carriage. A single administration of neat **2a** (5 wt %, approximately equimolar to 2% mupirocin) was as active as 2% mupirocin, the currently used clinical antibiotic,[29] administered twice daily for 5 d. Ester **2a** may act as a prodrug that is hydrolyzed to the corresponding carboxylic acid; separate synthesis (by LiOH-mediated hydrolysis of **2a** and acidification with HCl) and assessment of this acid showed it was considerably less active than **2a** (*S. aureus* (ATCC6538P) MIC 25 μg mL^{-1}). Ester **2a** was stable for up to 4 h in human and mouse plasma, and 19 h in minipig plasma (unpublished results). In vitro resistance selectivity studies showed that resistance to **2a** develops slowly in *S. aureus*. Some 50 generations were required for a tenfold increase in MIC to be observed. In comparison, vancomycin took 18 generations. These strains resistant to **2a** remained susceptible to vancomycin, and this suggests that **2a**, at least in part, has a different mode (or modes) of action.

The peptoid salt **2a**, with its ease of synthesis compared to the oxazole **2b**, coupled with reasonable plasma stability and amenability to stable nonaqueous formulation, is suitable for topical application and has no irritancy potential in rabbit skin irritation studies (data not shown). It is being developed for indications including nasal decolonization and/or wound infection/catheter-related infections.

Our results suggest that more than one mode of action is exhibited by compounds **2a** and **2b**. From ESI-MS studies, it was clear that **2a** could form complexes with the cell-wall model peptide sequences Ala-Lys-Ala-(*R*)-Ala-(*R*)-Ala (mimicking the sequence in vancomycin-sensitive *S. aureus*) and Ala-Lys-Ala-(*R*)-Ala-(*R*)-Lac (as an analogue of the sequence in vancomycin-resistant *S. aureus*) with signals for the doubly charged 1:1 complex evident at *m*/*z* 642 and 643, respectively, together with separate peaks for the individual components in each case. In contrast, vancomycin only complexed with the terminal (*R*)-Ala sequence (*m*/*z* 927). This indicates that inhibition of cell-wall cross-linking through peptide complexation could contribute to the mode of action. All diastereomers of **2a** were equipotent against *S. aureus* (vancomycin-sensitive and -resistant strains) and, together with activity of **2a** against MRSA and linezolid-resistant *S. aureus*, this suggests a more general activity component for disruption of Gram-positive bacterial cell membranes. The slow development of resistance and the rapid bactericidal action of **2a** and **2b** is also consistent with such an action. Controversy 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[30] or a combination of both membrane damage and interaction with other targets within the cell has been reported.[31,32] This type of dual action has been established for the vancomycin analogue telavancin, which compromises cell-wall synthesis and cell-membrane coherence.[10]

In conclusion, two novel dicationic peptoids have been designed and synthesized. These compounds show promising in vitro antibacterial potency against a range of Gram-positive pathogens including strains of *S. aureus* resistant to vancomycin, methicillin, and linezolid. Peptoids **2a** and **2b** are rapidly bactericidal with a concentration-dependent profile, and induced resistance has been shown to develop exceptionally slowly in vitro. These compounds may act by more than one mode of action including inhibition of cell-wall cross-linking and cell-membrane disruption. Systemic and topical in vivo potency was maintained in mouse models of infection, and **2a** is being developed for topical indications.

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