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Letter

The synthesis of a novel binaphthyl-based cyclic peptoid with anti-bacterial activity†

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The novel cyclic peptoid 1, based upon a 1,1′-binaphthyl scaffold and a bridging tripeptide moiety, was synthesised utilising a ring-closing metathesis reaction, and was shown to possess anti-bacterial properties.

There is currently great interest in trying to develop new anti-bacterial agents to address the pressing problem of drug-resistant, pathogenic bacteria.[1,2] Vancomycin, which inhibits bacterial cell wall synthesis, is used as an anti-bacterial agent of last resort for the treatment of infections caused by multi-drug resistant Gram-positive bacteria. However, the recent development of vancomycin resistance in multidrug-resistant enterococci, and less commonly in staphylococci, has raised serious concerns about the future efficacy of this last line of defence, and has flagged the urgent need for the development of new anti-bacterial agents.[3,4] At the molecular level, it is known that vancomycin binds to the terminal D-Ala–D-Ala moiety of bacterial cell wall peptidoglycan precursors, thus preventing their cross-linking and ultimately leading to cell lysis. One key structural element of vancomycin for binding is the cyclic peptoid unit that incorporates several H-bond donor NH groups and one H-bond acceptor amide carbonyl group for binding interactions with this terminus. The cationic, terminal *N*-methylamino group in vancomycin also stabilises the bound complex through a favourable electrostatic interaction with the carboxy group of the terminal D-Ala residue of the growing cell wall. These binding interactions are further enhanced by the hydrophobic aryl rings and side chains in vancomycin. In vancomycin-resistant bacteria, the cell wall precursor is terminated by L-Lys–D-Ala–D-Lac, resulting in a 1000-fold reduction in binding affinity due to a repulsive oxygen-oxygen electrostatic interaction in place of a H-bond.[4–6]

Vancomycin,[7] along with a number of its analogues[8] and related systems,[9] have been synthesised. However, we have undertaken a program[10] for the synthesis of scaffold-based cyclic peptoids in an attempt to mimic and potentiate these interactions that give rise to anti-bacterial activity. We report here the synthesis of the novel cyclic peptoid **1**, which, although much simpler than vancomycin, incorporates the necessary structural features for binding to both the L-Lys–D-Ala–D-Ala and the L-Lys–D-Ala–D-Lac termini. These structural features include: (1) a basic amino acid residue for electrostatic interaction with the carboxy group of terminal D-Ala or D-Lac, (2) a tripeptoid moiety for H-bonding interactions with both D-Ala and D-Lac, and (3) a hydrophobic 1,1′-binaphthyl system that should enhance the above binding interaction by the exclusion of some water molecules in an aqueous environment. Furthermore, the 1,1′-binaphthyl system potentially allows control of the conformation of the peptoid unit by employing either *R*- or *S*-enantiomers.

The retrosynthetic analysis shown in Fig. 1 illustrates the modular approach to the synthesis of our target cyclic peptoid. This convergent strategy allows for the production of a number of analogues suitable for modulation and optimisation of anti-bacterial activity. The addition of the initial amino acid residue is achieved *via* a nucleophilic displacement reaction. The substituted biaryl is further elaborated by the addition of an allyl unit using simple Stille reaction conditions. The addition of a range of allylated dipeptides using standard peptide-coupling conditions will produce the required diallyl intermediates. A key step in the synthesis of our target molecule is the ring-closing metathesis reaction between an

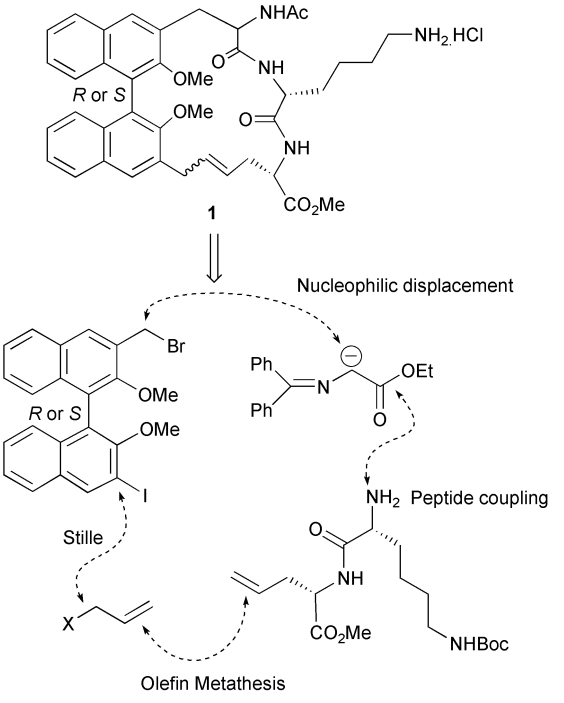
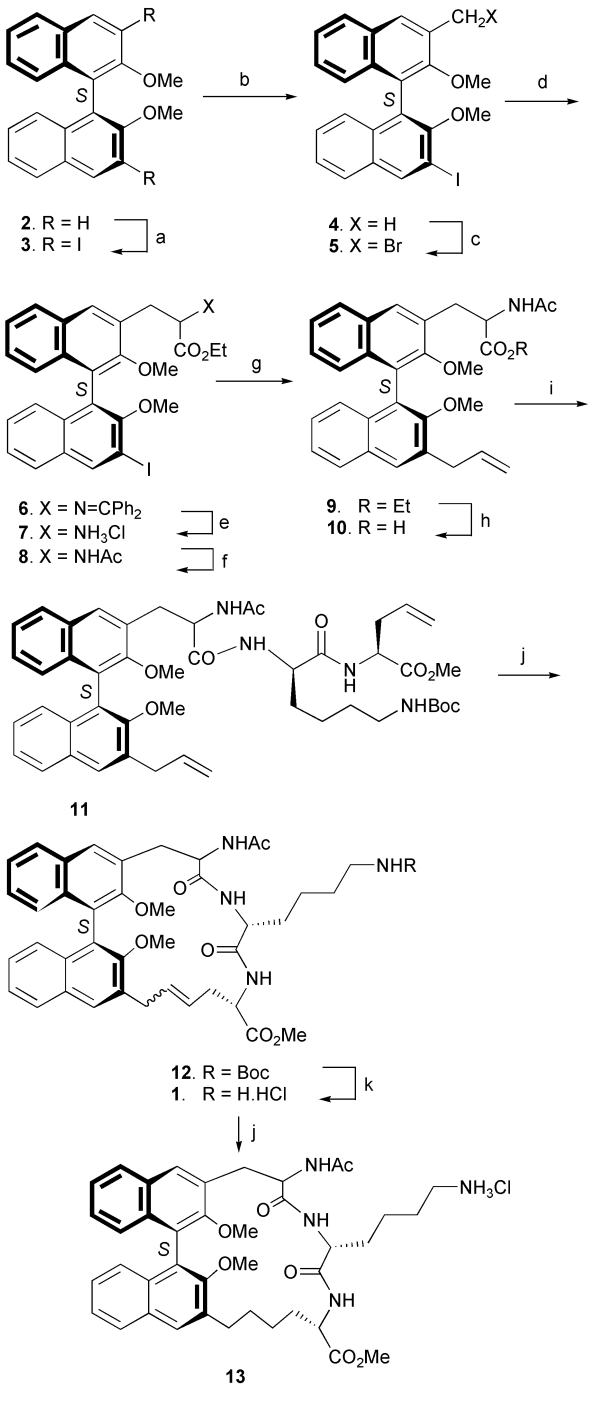


Fig. 1 Modular retrosynthetic analysis for the target cyclic peptoid illustrating the convergent synthesis.

† Electronic supplementary information (ESI) available: ESMS data. See http://www.rsc.org/suppdata/nj/b2/b205894b/

allyl glycine residue and the aryl-allyl group, providing efficient access to a protected version of **1**.

Therefore, the synthesis of **1** (Scheme 1) started with commercially available (*S*)-2,2′-dihydroxy-1,1′-binaphthyl, which was converted to the known 2,2′-dimethoxy derivative **2** using a published procedure.[11] Compound **2** was transformed to the known 3,3′-diiodo derivative **3**,[11] which was converted to the 3-iodo-3′-methyl derivative **4** by *trans*-metallation of **3** with *t*-BuLi, followed by quenching the resulting lithiated species with iodomethane. This reaction consistently produced a mixture (*ca* 5:3:2, respectively) of **4** and the known compounds 3-iodo-2,2′-dimethoxy-1,1′-binaphthyl[12] and 2,2′-dimethoxy-3,3′-dimethyl-1,1′-binaphthyl[13] as their *S*-enantiomers. The yield of **4** after purification by column chromatography was typically 38–42%. All attempts to improve the yield of **4** resulted in lower yields, including one or more of the following modifications: the use of *n*-BuLi as base, using TMEDA–diethyl ether as solvent or employing methyl triflate as methylating agent. Benzylic bromination of **4** gave the bromomethyl derivative **5**, which was converted to the α-imino ester **6** upon alkylation with the lithium salt of ethyl *N*-(diphenylmethylene)glycinate[14,15] as a mixture of diastereomers. A biphasic acid-catalysed hydrolysis of the imino group of **6** with 3% aqueous HCl–diethyl ether[15] gave the hydrochloride salt **7**, which was converted to the α-acetamido ester **8** upon base-catalysed acetylation. The overall yield for the conversion of **5** to **8** was typically 71%. A Stille-type allylation[16] using the 3-iodo group in **8** and allyltributylstannane gave the allylated product **9** in 77% yield, which showed a diastereomeric ratio of 1:2 by ^{1}H NMR analysis of the methylene protons of the ester. This was then converted to the carboxylic acid derivative **10** in good yield (75%; diastereomeric ratio 1:2 by ^{1}H NMR analysis of the amino acid α-proton) upon mild basic hydrolysis (LiOH–THF–H_2O) followed by acidification.[17] DCC-mediated coupling of the carboxylic acid group of **10** with the dipeptide, N_ε-Boc-D-lysine-L-allylglycine methyl ester gave the key tripeptide derivative **11** in moderate yield (49%). A ring-closing metathesis reaction[18,19] of **11** at high dilution in dichloromethane heated at reflux, using commercially available benzylidene bis(tricyclohexylphoshine) dichlororuthenium (Grubbs' catalyst), provided the fully protected cyclic peptoid **12** in good yield (72%). Silica gel column chromatography afforded **12** as two diastereomers isolated in yields of 27% and 45%, respectively, reflecting the approximate diastereomeric ratio seen by ^{1}H NMR spectroscopy analysis of the precursors **9** and **10**. These two components of **12** each showed satisfactory MS analysis (ES, +ve, m/z 795, $M+H^+$); however, their ^{1}H and ^{13}C NMR spectra were consistent with a mixture of *E* and *Z* isomers and amide rotomers, with the exact *E*/*Z* ratio in these unable to be determined from the ^{1}H NMR data. Deprotection of each component by removal of the N_ε-Boc group using TFA, followed by evaporation of excess TFA and anion exchange with HCl, gave the target molecules **1** as their hydrochloride salts in 84% and 67% yield, respectively (HRMS, ES, +ve, found m/z 695.3455 and 695.3427, respectively. $C_{40}H_{46}N_4O_7+H^+$ requires m/z 695.3445). Additionally, the major component of **1**, isolated from a separate experiment, was hydrogenated using standard conditions to yield **13** (ES, +ve, m/z 697, $M+H^+$), the saturated compound in which the stereoisomers have been deconvoluted down to a single diastereomer.



Scheme 1 Synthesis of the 1,1′-binaphthyl cyclic tripeptide. a.(i) *n*-BuLi, RT, diethyl ether, TMEDA; (ii) I_2, $-78\,^\circ C \rightarrow RT$, 12 h; b. (i) *t*-BuLi (2 equiv.), THF, $-78\,^\circ C \rightarrow RT$; (ii) MeI, $-78\,^\circ C$; c. NBS, CCl_4, *hv* (500 W Hg lamp); d. (i) LDA, THF, HMPA, $-78\,^\circ C$; (ii) ethyl *N*-(diphenylmethylene)glycinate, $-78\,^\circ C \rightarrow RT$; e. 3% aq. HCl–ether; f. acetic anhydride, Et_3N, DMAP; g. $PdCl_2$ (10 mol %) Ph_3P (40 mol %), allyltributylstannane, dioxane, Δ, 5 h; h. LiOH, H_2O–THF (1:2), $0\,^\circ C$; i. N_ε-Boc-D-lysine-L-allylglycine methyl ester, DCC, DMAP, DCM, $0\,^\circ C$; j. benzylidene[bis(tricyclohexylphosphine)] dichlororuthenium (Grubbs' catalyst, 5 mol %), DCM, Δ; k. (i) TFA; (ii) HCl in ether, $0\,^\circ C$; j. H_2, Pd/C.

Thus, we have developed an efficient synthetic route to the novel peptoid **1** that is sufficiently flexible to allow the synthesis of a range of analogues through the introduction of various D- and L-amino acids. Although some stereocontrol has been achieved, the complete stereoselective synthesis of these cyclic peptidomimetics should be realised by the use of chiral auxiliaries in the nucleophilic displacement reaction (see Fig. 1). Furthermore, these experiments should allow for complete assignment of stereochemistry to the individual diastereomers of **1**. These reactions are currently being investigated. In some preliminary tests, the individual major and minor diastereomers of compound **1** were shown to have promising anti-bacterial activity against *Staphylococcus aureus* with inhibition of microbial growth at 17 μg mL^{-1} (MIC) and 31 μg ml^{-1}, respectively; in this assay, vancomycin had an MIC of <1.5 μg ml^{-1}. Initial mass spectrometric binding studies

using a L-Lys-*R*/*S*-binaphthyl analogue of **1** indicated a greater affinity for Gly–Ala–Ala–D-Ala–D-Lac, a model 'resistant' bacterial cell wall precursor peptide, than for Gly–Ala–Ala–D-Ala–D-Ala, a model 'normal' bacterial cell wall precursor peptide, with unbound to bound ratios of 3.5:1 and 29:1, respectively. Additionally, compound **13** was tested in a similar assay and was found to inhibit microbial growth in the same strain at a minimum concentration of 15 μg mL^{-1}, thus indicating that the presence of either the *E* or the *Z* olefin in **1** was unlikely to be significant in the anti-bacterial properties of this class of compounds. These anti-bacterial results, including those of other analogues using this synthetic route, and the results from the fully stereoselective syntheses, will be reported in a full paper.

Experimental

In a typical metathesis reaction, the binaphthyl derivative **11** (0.120 g, 0.14 mmol) was dissolved in dichloromethane (20 mL). The solution was deoxygenated by bubbling argon gas through for 10 min before the addition of benzylidene[bis(tricyclohexylphosphine)] dichlororuthenium (0.011 g, 0.013 mmol). The reaction mixture was heated at reflux for 22 h. The cooled reaction mixture was evaporated to dryness and the resulting residue was purified by flash column chromatography to give **12** in two fractions, (total yield = 0.083 g, 72%). Fraction 1. Pale yellow glass-like solid (0.031 g), δ 7.81, d, $J = 8.1$ Hz, 2H, ArH; 7.75, s, 1H, ArH; 7.69, s, 1H, ArH; 7.38–7.31, m, 2H, ArH; 7.22–7.06, m, 2H, ArH; 7.00, d, $J = 8.4$ Hz, 1H, ArH; 6.92, d, $J = 8.4$ Hz, 1H, ArH; 6.85, d, $J = 6$ Hz, 1H, NH; 6.67, d, $J = 7.5$ Hz, 1H, NH; 6.42, d, $J = 6.9$ Hz, 1H, NH; 6.20, dd, $J = 5.2$, 15.5 Hz, 1H, =CH; 5.08, br t, $J = 13$ Hz, 1H; 4.9–4.85, m, 1H; 4.4–4.3, m, 2H, $2 \times \alpha$-CH; 3.71, dd, $J = 6.9$, 15.9 Hz, 1H; 3.64, s, 1H; 3.50, dd, $J = 4.1$, 13.3 Hz, 1H, 3.50, s, 3H, OCH_3; 3.38, br dd, $J = 2.5$, 16.0 Hz, 1H; 3.32, s, 3H, OCH_3; 3.22, dd, $J = 3.2$, 13.6 Hz, 1H; 3.14, s, 3H, OCH_3; 3.1–3.0, m, 2H; 2.7, br d, $J = 14$ Hz, 1H; 2.43, ddd, $J = 7$, 10.5, 14 Hz, 1H; 2.17, s, 3H, $COCH_3$; 1.94–1.74, m, 2H; 1.42, s, 9H, $C(CH_3)_3$; 1.32–1.20, m, 4H, $2 \times CH_2$. *m*/*z* (ES, +ve) 817 ($M + Na^+$, 4%), 795 ($M + H^+$, 3), 593 (20) and 297 [$O{=}P(C_6H_{11})_3 + H^+$, 100]. Fraction 2. Light brown solid (0.052 g), *m*/*z* (ES, +ve) 817, ($M + Na^+$, 12%), 795 ($M + H^+$, 33), 593 (5), and 297 [$O{=}P(C_6H_{11})_3 + H^+$, 100). $C_{45}H_{54}N_4O_9 + H^+$ requires 795. Note, these are unoptimised yields and subsequent ring-closing metathesis reactions on similar compounds reliably gave >90% yields. Further, all traces of tricyclohexylphosphine oxide in the metathesis products were eliminated in the subsequent Boc deprotection reactions (from ESMS, − and +, on **1**).

Acknowledgements

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