Synthesis of extended bacterial cell-wall precursor analogues for ligand binding studies with glycopeptide antibiotics

Thomas Staroske, Jochen Görlitzer, Richard M. H. Entress, Matthew A. Cooper and Dudley H. Williams *

Cambridge Centre for Molecular Recognition, University Chemical Laboratory, Lensfield Road, Cambridge, UK CB2 1EW. E-mail: dhw1@cus.cam.ac.uk

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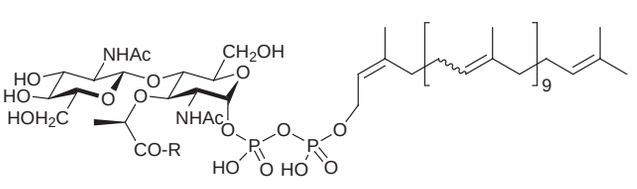
The synthesis of bacterial cell-wall precursor analogues, which resemble the naturally occurring precursor (lipid II) more closely than those hitherto used in NMR binding studies with glycopeptide antibiotics, is reported.

Introduction

The cell walls of Gram-positive bacteria are assembled from disaccharide precursors consisting of *N*-acetylglucosamine and *N*-acetylmuramate with an attached pentapeptide, and a C55 hydrocarbon chain attached *via* a diphosphate linkage (Fig. 1). The hydrocarbon chain serves to anchor the precursor in the bacterial cell membrane. During the biosynthesis of the cell wall, the disaccharide units of two precursors are coupled by a transglycosylase. Subsequently, the peptide sidechains from two linear peptidoglycan chains are crosslinked by a transpeptidase to achieve the relatively rigid structure of the bacterial cell wall.[1]

Glycopeptide antibiotics bind to the C-terminal sequence -Lys-D-Ala-D-Ala of the bacterial cell-wall precursors, thereby inhibiting transglycosylation and transpeptidation, and ultimately leading to cell death.[2,3] Two glycopeptides, vancomycin and teicoplanin, are currently used clinically to treat infections with multiply-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA).[4,5] Recently, bacteria resistant to glycopeptide antibiotics have been observed.[6] These bacteria produce cell-wall precursors terminating in -D-lactate (-D-Lac) rather than in -D-Ala.[7] This change means that an NH group of the cell-wall precursor (which forms a hydrogen bond to the antibiotics) is replaced with an oxygen, thereby transforming an attractive hydrogen bond into a repulsive interaction, thus leading to a dramatic decrease in the affinity of the antibiotics to such precursors.[8,9]

NMR studies have shown that glycopeptide antibiotics form back-to-back homo-dimers and it has been postulated that dimerisation may be important in the mode of action of these antibiotics.[10] After one half of the dimer has bound to a cell-wall precursor on the surface of a bacterium, the binding of the second half of the dimer to another precursor is effectively intramolecular, and thus entropically favoured.[10] Furthermore, dimerisation and ligand binding are cooperative (*i.e.* dimerisation increases ligand binding and *vice versa*).[11] In previous studies, we have shown that using phosphatidylcholine



R = –NH-Ala-D-γ-Glu-Lys(*N*-ε-Gly_5-NH_2)-D-Ala-D-Ala-OH

Fig. 1 Structure of a bacterial cell-wall precursor (lipid II) of *Staphylococcus aureus*.

R^1-NH-Gly-Ala-D-γ-Glu-Lys(*N*-ε-Ac)-D-Ala-R^2

R^1 = $C_9H_{19}CO$ **R^2** = D-Ala-OH

R^2 = D-Lac-OH

R^1 = $C_{21}H_{43}CO$ **R^2** = D-Lac-OH

Fig. 2 Structures of some of the cell-wall precursor analogues used in previous NMR studies.

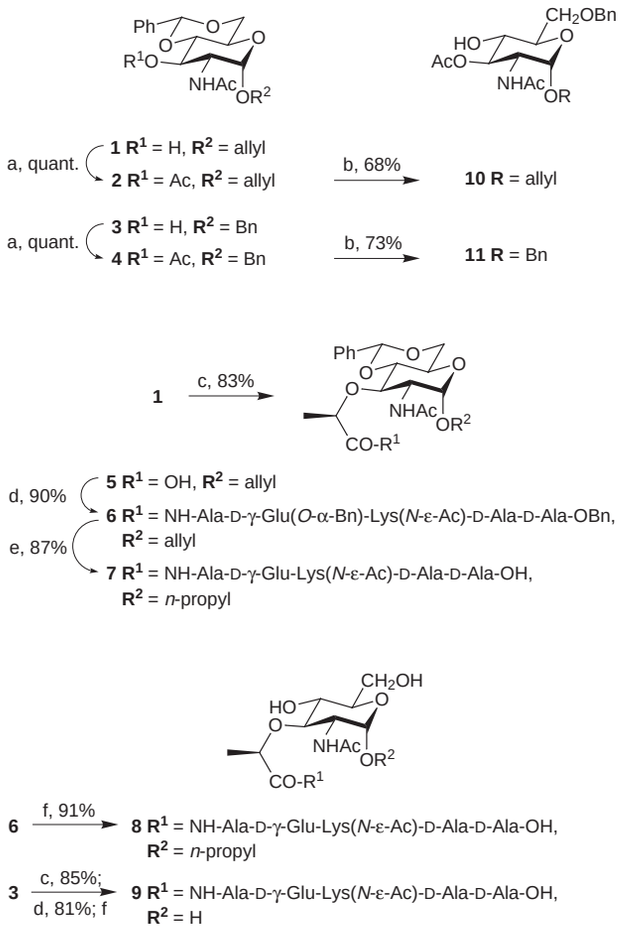
vesicles and docosanoylated peptide cell-wall precursor analogues as a model system for the surface of a bacterium, the affinity of the antibiotics increases *ca.* 100-fold to ligands terminating in -D-Ala and *ca.* 10,000-fold to ligands terminating in -D-Lac.[12,13] This enhancement is the result of cooperativity expressed through the binding of a dimeric molecule to two ligands on the vesicle surface.

The interactions between peptide ligands and glycopeptide antibiotics have been studied in detail, particularly through the use of intermolecular NOEs in proton NMR spectroscopy.[8,14] However, these precursor analogues did not contain the sugar moiety of the natural cell-wall precursors and little is known about the interactions between these disaccharides and the glycopeptide antibiotics. To further improve our surface binding model system, and to study the interactions between the sugar moieties of the cell-wall precursors and the antibiotics, we now report the synthesis of bacterial cell-wall precursor analogues which resemble naturally occurring precursors more closely than those used in previous NMR studies.

Results and discussion

The structures of some of the precursor analogues used in previous NMR studies[12,13] are shown in Fig. 2. Glycine (which is not found in this position in the naturally occurring precursors) was introduced into the peptide chain in between the alkanoyl tail and the N-terminus of the pentapeptide as a spacer to fill the space normally occupied in the natural precursors by the sugar residues. The synthesis of the extended precursors[15] described here is illustrated in Scheme 1. The pentapeptide chain was synthesised by standard solution phase peptide chemistry using a Boc/benzyl ester strategy described previously.[12] The protected sugar intermediate **1** was prepared from *N*-acetylglucosamine according to literature procedures.[16] The allyl group in **1** serves as a protecting group for the anomeric centre (only the α-configuration is found in natural precursors).

S_N2 reaction of **1** with (*S*)-2-chloropropionic acid[17] afforded the muramic acid derivative **5**. HBTU-mediated coupling of the pentapeptide to **5** yielded the fully protected cell-wall precursor



Scheme 1 *Reagents and conditions:* a) Ac_2O, pyridine, cat. DMAP, RT, 3 h; b) $NaBH_3CN$, 4 M HCl in dioxane, THF, RT; c) NaH (3.5 equiv.), THF, DMF, (*S*)-chloropropionic acid, 70 °C, 18 h; d) HBTU, pentapeptide, Pr^i_2NEt, CH_2Cl_2, RT, 4 h; e) 3% AcOH in EtOH–DMF (1 : 1), Pd/C, H_2 (3×10^5 Pa), RT, 5 h; f) EtOH–AcOH (3 : 1), Pd/C, H_2 (3×10^5 Pa), RT, 18 h.

analogue **6**. Deprotection[18] of **6** and saturation of the allyl group with Pd/C and H_2 at 3×10^5 Pa in 3% acetic acid in ethanol–DMF (1 : 1) gave **7** selectively, with the benzylidene acetal still present. Hydrogenation of **6** in 25% acetic acid in ethanol afforded the desired compound **8**.

The protected *N*-acetylglucosamine **3** was prepared from *N*-acetylglucosamine in a two-step reaction according to literature procedures.[19] Conversion of **3** into the cell-wall precursor analogue **9** (with an unprotected anomeric centre) was achieved using a similar reaction sequence as in the preparation of **8**. Due to problems during the purification of **9**, this compound was only characterised by high resolution mass spectrometry and the stereochemistry at the anomeric centre is yet not known.

As natural bacterial cell-wall precursors contain a *N*-acetylglucosamine moiety attached to the 4 position of the muramate (Fig. 1), suitable compounds for the synthesis of disaccharide cell-wall precursor analogues were prepared. Acetylation of **1** and **3** with acetic anhydride in pyridine afforded **2** and **4**, respectively, in excellent yields. Both compounds were selectively deprotected at the 4 position with $NaBH_3CN/HCl$.[20] The products of this deprotection, **10** and **11**, are thus suitable for attachment of a protected *N*-acetylglucosamine at the 4 position. Cleavage of the *O*-acetyl group should then lead to compounds which may be converted to cell-wall precursor analogues similar to **8** and **9** but bearing a disaccharide as found in the natural precursor.

The allyl group at the anomeric centre of the muramate moieties of the cell-wall precursor analogues synthesised in this study does not only serve as a protecting group. It should also be possible to convert this group into a lipophilic sidechain *via* an olefin metathesis reaction[21,22] with an appropriate terminal alkene. This sidechain could then serve as a membrane anchor in binding studies with model cell membrane systems. Another approach for the attachment of a lipophilic sidechain could be *via* a Heck reaction using halo-arenes.

Conclusion

The compounds prepared in this study will be used in ligand binding studies with a variety of glycopeptide antibiotics to gain a deeper insight into the interactions of the sugar moieties of bacterial cell-wall precursors with glycopeptide antibiotics. Furthermore, attachment of a membrane anchor to the compounds should improve our model system for studying binding of glycopeptides at the surface of a bacterium. It may also be interesting to investigate the potential of these compounds as transglycosylase inhibitors.[23,24]

Experimental

General

^{1}H NMR spectra were recorded on a Bruker DRX-500 (proton) and a Bruker AM-400 (carbon) spectrometer at 300 K; *J* values are given in Hz. High resolution mass spectra were obtained on a Fourier transform ion cyclotron resonance mass spectrometer (Bruker Daltronics, Billerica, USA) equipped with a 4.7 T superconducting magnet and an external electrospray ionisation source (Analytica of Branford, Branford, USA).

2-*N*-Acetyl-1-α-*O*-*n*-propyl-4,6-dihydroxy-3-muramyl-alanyl-D-γ-glutamyl-lysyl(*N*-ε-acetyl)-D-alanyl-D-alanine 8

Compound **6** (100 mg, 90 μmol) was dissolved in EtOH–AcOH 3 : 1 (10 ml). 10% Pd/C (85 mg) was added and the suspension was shaken in a Parr hydrogenator under an atmosphere of hydrogen (3×10^5 Pa, ambient temperature). After 18 h the mixture was filtered and the solvent was evaporated from the filtrate to give a white crystalline compound (70 mg, 82 μmol, 91%). ^{1}H NMR spectroscopy and mass spectrometry showed the formation of **8** as the sole product. δ_H(500 MHz; DMSO-d_6) 0.89 (3H, t, *J* 7.3, propyl-CH_3), 1.21 (3H, d, *J* 6.8, CH_3), 1.23 (3H, d, *J* 6.8, CH_3), 1.26 (3H, d, *J* 6.8, CH_3), 1.29 (3H, d, *J* 7.8, CH_3), 1.79 (3H, s, NAc), 1.81 (3H, s, NAc), 3.00 (2H, dt, *J* 6.8, 5.8, Lys-CH_2N), 3.74 (1H, ddd, *J* 10.6, 7.8, 3.4, 2-H), 4.72 (1H, d, *J* 3.4, H-1), 7.66 (1H, d, *J* 7.8, NH), 7.79 (1H, t, *J* 5.4, N*H*Ac), 8.02 (1H, d, *J* 6.8, NH), 8.04 (1H, d, *J* 8.3, NH), 8.05 (1H, d, *J* 7.8, NH), 8.14 (1H, d, *J* 7.8, NH), 8.24 (1H, d, br, *J* 6.3, NH); δ_C(100 MHz; DMSO-d_6) 10.40, 12.43, 17.42, 17.90, 18.89, 19.02, 20.07, 22.03, 22.49, 22.54, 22.69, 28.72, 31.30, 31.51, 35.69, 38.25, 41.49, 47.66, 52.81, 53.13, 54.78, 60.54, 68.44, 69.67, 72.79, 76.18, 78.77, 96.34 (C-1), 169.0 (CO), 169.4 (CO), 171.4 (2 × CO), 171.6 (2 × CO), 172.5 (CO), 174.0 (CO); *m*/*z* (ES) 443.6949 ($C_{36}H_{62}N_7O_{16}K$ $[M + H + K]^{2+}$ requires 443.6943).

2-*N*-Acetyl-3-muramyl-alanyl-D-γ-glutamyl-lysyl(*N*-ε-acetyl)-D-alanyl-D-alanine 9

The precursor of **9** (40 mg, 35 μmol) was reacted under the same conditions as described above to give an unseparable mixture of two products. Compound **9** was characterised by high resolution mass spectrometry. *m*/*z* (ESI) 806.3797 ($C_{33}H_{56}N_7O_{16}$ $[M + H]^+$ requires 806.3783).

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