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A highly convergent approach for the synthesis of disaccharide repeating units of peptidoglycan

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Abstract—A highly convergent strategy has been developed for the synthesis of the two possible saccharide-repeating units of peptidoglycan using TBDMS and PMB ethers as orthogonal protecting groups and a two-directional glycosylation strategy for the efficient assembly of the spacer containing disaccharides.

Septic shock resulting from gram-positive bacterial infections has increased progressively over the last two decades and currently causes one third of all cases of sepsis.[1,2] Gram-positive sepsis is often linked to a systemic inflammatory response to peptidoglycan (PGN) in the blood of infected patients. A major structural component of the cell wall of gram-positive bacteria is a large branched glycopeptide polymer (MW>125 000), PGN that consists of alternating β(1-4)-linked D-Glc-*N*Ac and *N*-acetylmuramic acid (Mur*N*Ac, the 3-*O*-D-lactyl ether of D-Glc*N*Ac) residues (Fig. 1) to which is appended peptide chains. This backbone is inter-linked by peptide bridges, thus forming an insoluble three-dimensional network. PGN exerts its clinical effects by initiating the production of multiple host-derived inflammatory mediators (e.g. tumor necrosis factor; TNFα), which in turn cause the deleterious effects of gram-positive sepsis. The first step in the production of these mediators is the binding of PGN to the cluster differentiation antigen CD14 on mononuclear phagocytes. The Toll-like receptor 2, a transmembrane receptor protein, is also involved in the transmission of the PGN signal to the interior of the cell.[3,4]

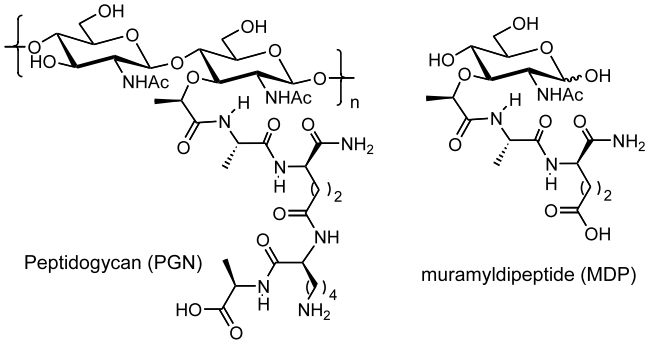

Figure 1. Structures of PGN and muramyldipeptide.

Data from our laboratory indicate that activation of CD14 and Toll-like receptor 2, leading to the production of TNFα, requires a multivalent presentation of a partial structure of PGN.[5] This finding was established with synthetic polymers functionalized with pendant muramyl dipeptide moieties (MDP, a partial-structure of the repeating unit of PGN, Fig. 1). This neoglycopeptide polymer was shown to induce dose-dependent production of TNFα by human monocytes, whereas monomeric MDP had no effect. The activity of the synthetic polyvalent MDP was, however, an order of a magnitude smaller than that of PGN and we conjectured that a more complete epitope might be required for its optimal binding of CD14 and Toll like receptor 2.

As part of a program designed to clarify which structural elements of PGN are required for binding to CD14 and Toll-like receptor 2 and thus the induction of endogenous proinflammatory mediators, we report here a highly convergent synthesis of the protected PGN-repeating disaccharides **9** and **11**. The lactate moiety of these disaccharides can be coupled to peptides and the artificial anomeric spacer allows conjugation of these units to an activated polymer to give polyvalent ligands.

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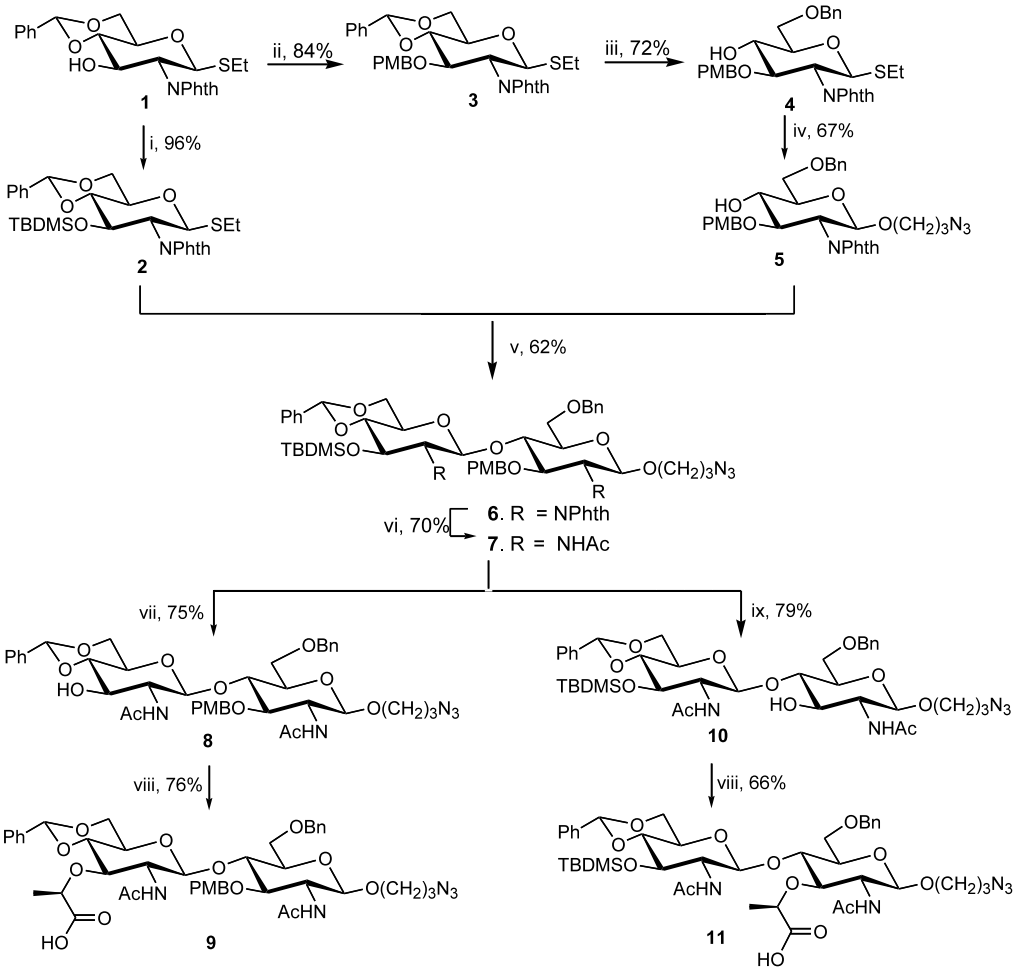
Contemporary oligosaccharide synthesis often makes use of orthogonally protected saccharide building blocks that can be assembled into complex structures using efficient glycosylation strategies.[6,7] It was expected that the disaccharide **6**, protected with orthogonally TBDMS and PMB ethers, would be a convenient intermediate for the synthesis of both **9** and **11** (Scheme 1). The key disaccharide **6** could easily be prepared from monosaccharide building blocks **2** and **5**, which could themselves be prepared from precursor **1**.[8] The spacer-containing saccharide **6** could be assembled by a two-directional glycosylation strategy[9,10] involving coupling of the partially protected glycosyl donor **4** with 3-azido propanol to give **5**, which in the next step could be used as an acceptor in glycosylation with **2**.

The C-3 hydroxyl of key intermediate **1** was converted into a TBDMS ether by reaction with TBDMSOTf in presence of 2,6-lutidine to give glycosyl donor **2** in a yield of 96%.[11] Conventional silylation conditions such as TBDMSCl and imidazole resulted only in recovery of starting material.[12] Compound **1** was also protected with a PMB group using NaH, PMBCl in THF to give **3** in a good yield of 84%. Treatment of **3** with $BH_3{\cdot}Me_3N$, and $AlCl_3$[13] led to regioselective opening of the benzylidene acetal function to give the glycosyl donor **4** in a yield of 72%. NIS/TMSOTf-mediated coupling[14] of partially protected glycosyl donor **4** with the highly reactive acceptor 3-azido-propanol gave **5** in good yield. Due to the low reactivity of the C-4 hydroxyl of **4**, no self-condensation was observed. Compound **5** could immediately be used as a glycosyl acceptor in a NIS/TMSOTf-mediated coupling with glycosyl donor **2** to give disaccharide **6** in a yield of 62%.

In an earlier attempt to obtain an orthogonally protected chitibiose derivative, we employed the combination of allyl- and *p*-methoxybenzyl ethers. However, NIS/TMSOTf mediated coupling of **5** with a derivative of **2** that had an allyl in lieu of the TBDMS ether gave substantial quantities of a disaccharide that had its allyl moiety iodinated. Several other promoters were investigated but each was incompatible with the allyl ether.

Removal of the phthalimido functions in disaccharide **6** using ethylene diamine in ethanol[15,16] followed by acetylation of the revealed amino groups with acetic anhydride in methanol gave derivative **7**. *n*-Tetrabutylammonium fluoride (TBAF) treatment of **7**



Scheme 1. *Reagents and conditions*. (i) TBDMSOTf, 2,6-lutidine, DCM; (ii) PMBCl, NaH, TBAI, THF, Δ; (iii) $Me_3N{\cdot}BH_3$, $AlCl_3$, THF; (iv) $HO(CH_2)_3N_3$, NIS, TMSOTf; (v) NIS, TMSOTf; (vi) $H_2N(CH_2)_2NH_2$, EtOH then Ac_2O, MeOH; (vii) Bu_4NF, THF; (viii) NaH, *S*-2-chloropropionic acid, dioxane; (ix) DDQ, DCM, H_2O.

resulted in clean removal of the TBDMS ether to give **8**. Several conditions were explored to install a D-lactate moiety at C-3′ of **8** but the best results were obtained with NaH and *S*-2-chloropropionic acid in 1,4-dioxan to give **9** in a yield of 76%.[17,18] Alternatively, disaccharide **7** could serve in the synthesis of the target compound **11** by removal of the PMB ether using DDQ in dichloromethane/water[19] to give **10** which was treated with NaH and *S*-2-chloropropionic acid.

Recently, considerable attention has been focussed on the development of efficient procedures for the synthesis of the repeating unit of PGN.[20–22] A major synthetic obstacle was lactonization of muramic acid derivatives that have a free C-4 hydroxyl. In one approach,[22] this problem was resolved by first installing L-alanine, the first amino acid residue of the muramyl peptide chain, followed by conversion into a glycosyl acceptor for the synthesis of a disaccharide. We addressed this problem by first synthesizing a disaccharide and thereafter incorporating the lactate arm. This approach has the advantage that one disaccharide can be used for the synthesis of both possible repeating units of PGN (Mur*N*Ac-Glc-*N*Ac and Glc*N*Ac-Mur*N*Ac). Alternative synthetic approaches have employed 2-amino-2-deoxyglucopyranosyl donors protected by a *N*-trichloroethoxycarbonyl (Troc) group. The rationale for the use of a *N*-Troc group is that the corresponding glycosyl donors are 40 times more reactive than a corresponding phthalimido protected derivative[23] and moreover that it directs a glycosylation to give exclusively a 1,2-*trans* linked product by neighboring group participation. However, we found that glycosyl donors that were protected with phthalimido groups led to high yielding glycosylations. More importantly, the phthalimido group is compatible with the rather strong basic conditions used for the incorporation of the lactate moiety whereas the Troc would not survive these conditions.

Acknowledgements

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18. All new compounds gave satisfactory NMR spectroscopic, mass spectroscopic data. Selected data for compound **9**: Colorless syrup; R_f 0.36 (MeOH: DCM, 8:92, v/v) MALDI-TOF $m/z=900.6$ $[M+Na]^+$, 916.4 $[M+K]^+$. Selected signals in ^1H NMR ($CDCl_3$, 500 MHz) δ 5.5 (s, 1H, PhC*H*), 4.2 (d, 3H, H-1, $J=7.79$ Hz), 2.1 (s, 3H, NHCOCH_3), 2.0 (s, 3H, NHCOCH_3), 1.8 (m, 2H, $OCH_2CH_2CH_2N_3$), 1.4 (d, 3H, $J=6.79$ Hz, (CH(CH_3)COOH). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 101.3 (Ph*C*H), 100.9, 100.7 (C-1, C-1′), 29.0 (OCH_2CH_2-CH_2N_3), 23.5 (NHCO*C*H$_3$), 22.7 (NHCO*C*H$_3$), 18.7 (CH(*C*H$_3$)COOH). Compound **11**: Colorless syrup; R_f 0.21 (MeOH: DCM, 8:92, v/v) MALDI-TOF $m/z=895.5$ $[M+Na]^+$. ^1H NMR (CD_3OD, 500 MHz) δ 5.3 (s, 1H, PhC*H*), 5.0 (dd, 2H, $J=11.7$ Hz, H-1, H-1′), 1.9 (s, 3H, NHCOCH_3), 1.9 (s, 3H, NHCOCH_3), 1.7 (m, 2H, $OCH_2CH_2CH_2N_3$), 1.1 (d, 3H, $J=6.83$ Hz, (CH(CH_3)-COOH). ^{13}C NMR (CD_3OD, 75 MHz) δ 101.1 (C-1, C-1′), 29.2 (CH(*C*H$_3$)COOH), 23.8 (NHCO*C*H$_3$), 22.0 ($OCH_2CH_2CH_2N_3$), 21.8 (NHCO*C*H$_3$).
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