The First Total Synthesis of Bacterial Cell Wall Precursor UDP−*N*-Acetylmuramyl-Pentapeptide (Park Nucleotide)

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During the past 50 years antimicrobial chemotherapy has revolutionized the treatment of infectious diseases. Several of the most widely used antibacterial agents, including the β-lactams and glycopeptides, possess a mode of action which involves inhibition of bacterial cell wall biosynthesis.[1] Recently however, an alarming increase in antimicrobial resistance has threatened the clinical efficacy of these and other classes of antibiotic agents.[2] To aid in understanding emerging antimicrobial resistance mechanisms at the molecular level and to identify new agents to counter this threat by rational, structure-based design and by devising new mechanism based screening techniques, access to bacterial cell wall precursors is invaluable. The importance of these compounds has been further heightened by recent advances in the determination of bacterial genome sequences, a development likely to unveil new targets in the cell wall pathway. Unfortunately the isolation of cell wall precursors from the bacteria themselves is a laborious process and is impractical for gram scale quantities.[3] For this reason we have embarked on a program to develop practical total syntheses of these biosynthetic intermediates for use in the discovery of new antibiotic agents.

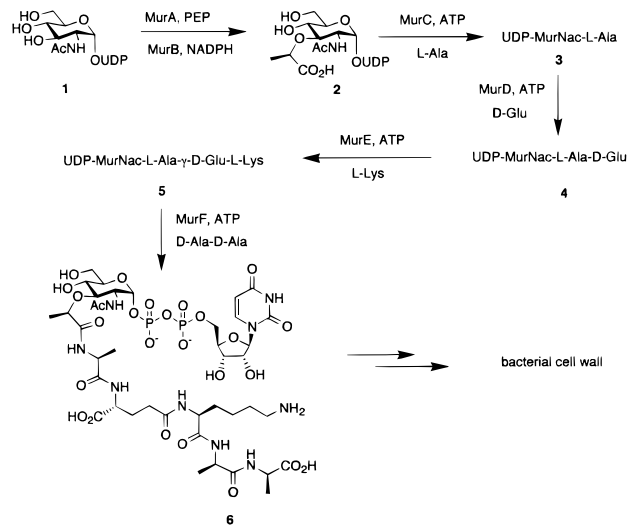
The bacterial cell wall is composed of a framework of alternating *N*-acetyl glucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) units cross-linked via peptide chains appended to the muramyl moiety. The first committed steps in bacterial peptidoglycan biosynthesis begin within the cytoplasm with the synthesis of UDP-MurNAc-L-Ala-γ-D-Glu-X-D-Ala-D-Ala, the so-called Park nucleotide,[4] **6** from UDP-GlcNAc **1** where X is usually meso-diaminopimelate in Gram-negative bacteria and L-Lys in Gram-positive bacteria (Scheme 1).[5]

The non-DNA-encoded peptide chain is established via sequential addition of L-Ala, D-Glu, L-Lys (or meso-DAP), and then D-Ala-D-Ala to UDP-MurNAc **2** by ATP-dependent amino acid ligases. An undecaprenyl carrier is then attached to **6**, and GlcNAc is added, followed by additional amino acid residues in some bacteria, before transport through the cell membrane and incorporation into the cell wall. Descibed herein is the first total synthesis of UDP-muramyl pentapeptide **6**. The convergent strategy employed should be adaptable to afford both rationally designed inhibitors of the steps outlined in Scheme 1[6] as well as the biosynthetic precursors to **6** (**2**−**5**).

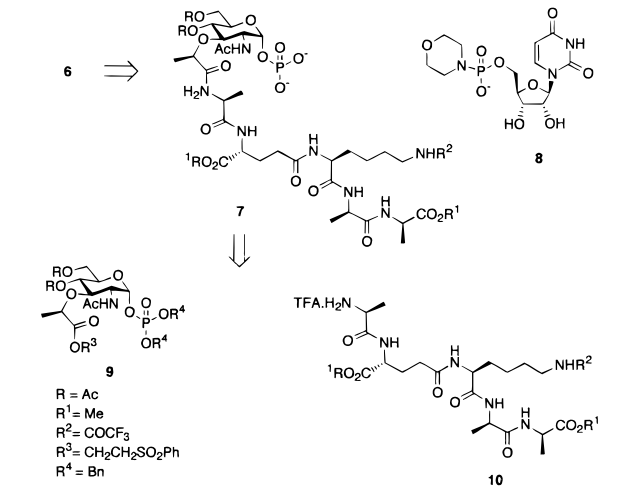
Recent advances in enzyme mediated oligosaccharide synthesis have served to fuel synthetic interest in glycosyl nucleoside diphosphates since they are used as activated substrates for glycosyl transferases.[7] As a result, both enzymatic[8] and chemical[9] routes have been explored. Synthetically, the most widely employed method for construction of these compounds has been late-stage formation of the diphosphate moiety via coupling of glycosyl phosphates with nucleoside 5′-morpholidophosphates (Khorana-Moffatt procedure).[10] Retrosynthetically, this first disconnection reveals monophosphate **7** (Scheme 2).

Traditionally, the Khorana-Moffatt coupling process is performed after the removal of all protecting groups as the final step in glycosyl nucleotide diphosphate syntheses. The reaction is

Scheme 1



Scheme 2



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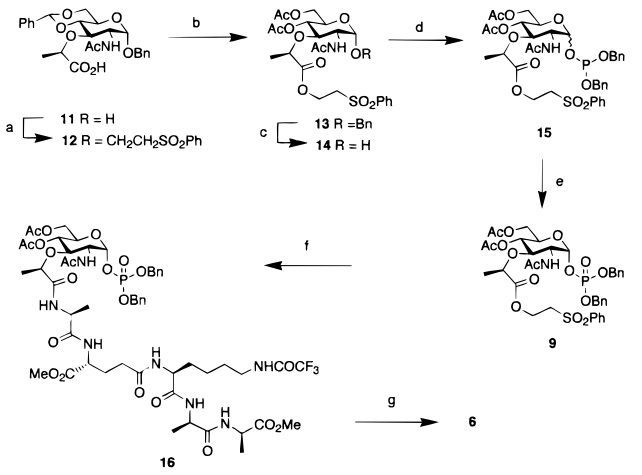
Scheme 3[a]



[a] Reagents: (a) DCC, NHS, 2-(phenylsulfonyl)ethanol, THF 96%; (b) (i) AcOH, H_2O, reflux; (ii) Ac_2O, pyridine, 85%; (c) H_2, Pd/C, AcOH, 95% (d) dibenzyl-*N*,*N*-diethylphosphoramidite, 1,2,4-triazole, CH_2Cl_2; (e) 30% H_2O_2, THF −78 °C to room temperature, 42% from **14**; (f) (i) DBU, CH_2Cl_2, (ii) DCC, NHS, **10**, 70%; (g) (i) H_2, Pd/C, cyclohexylamine, MeOH, (ii) **8**, DMF, 45 °C, 14 days; then NaOH, H_2O, 32% from **16**.

often limited therefore by the solubility of the unmasked glycosyl monophosphates in suitable organic solvents. Conscious that the pentapeptide chain present in our muramyl coupling partner was likely to exacerbate this problem, we proposed to perform the coupling reaction on a *protected* form of peptidomuramyl phosphate (**7**). The choice of protection for the peptidosugar was then dictated by the need to unmask the final molecule, maintaining the labile anomeric diphosphate moiety intact. To address this goal, acetate was chosen to block the hydroxyl groups, methyl ester to protect the carboxylic acid moieties, and trifluoroacetamide to mask the amino functionality. Thus, global deprotection could be accomplished by exposure to hydroxide as the final step.[11]

Approaches to glycosyl monophosphates[12] fall broadly into two categories wherein the sugar is either the electrophilic component (nucleophilic addition of phosphate anion) or the nucleophile (phosphorylation or phosphitylation/oxidation at the anomeric hydroxyl group). In the former case, carbohydrates bearing a neighboring participating group at C-2, favor the formation of 1,2-trans linked glycosyl phosphates (β-anomer in the case of GlcNAc). Although addition of phosphate diesters to carbohydrate derived oxazolines under thermodynamic control has been reported to deliver α-phosphates in some cases,[8b,13] decomposition has been reported in others.[14] The milder conditions offered by phosphitylation of carbohydrate lactols and subsequent oxidation[12b] suggested a more attractive alternative considering the complex substrate dictated by target **6**.

Thus, the synthesis commenced with the conversion of benzyl *N*-acetyl-4,6-benzylidenemuramic acid **11**,[15] available in three steps from *N*-acetyl glucosamine, to the corresponding phenylsulfonylethyl ester **12**. Selective acid mediated removal of the benzylidene group and acetylation next yielded diacetate **13**.

The anomeric hydroxyl group was then cleanly unmasked under hydrogenolytic conditions to deliver lactol **14** in readiness for introduction of the anomeric phosphate. Treatment of **14** with dibenzyl *N*,*N*-diethylphosphoramidite[12b] in the presence of 1,2,4-triazole afforded the corresponding labile anomeric phosphites **15** as an α/β mixture (2.5:1). Although chromatographically separable at this juncture, oxidation of the anomeric phosphite mixture followed by rapid chromatography of the resultant phosphate **9** proved more convenient due to the lability of the anomeric phosphites. Under these conditions **9** was obtained *solely as the desired* α-anomer in 42% overall yield from **14**. As has been noted for other 2-acetamido-2-deoxy glycosyl phosphates, the 1,2 trans isomers are typically not isolable due to the destabilizing effect of the neighboring participating group.[16] The carboxyl group was next unmasked via treatment of **9** with DBU in preparation for appendage of the pentapeptide fragment **10**. Pentapeptide **10** was, in turn, assembled from commercially available Cbz-D-Ala-D-Ala using standard Boc protection and EDC coupling procedures. Coupling of peptide **10** with the muramyl carboxyl fragment resulted in the corresponding amide **16** in 70% yield. Hydrogenolytic debenzylation of **16** in the presence of cyclohexylamine then yielded the corresponding cyclohexylammonium phosphate salt which, to our satisfaction, proved to be soluble in most organic solvents as anticipated. Although slow (14 days), the coupling of the cyclohexylammonium phosphate salt with uridine 5′-monophosphomorpholidate under anhydrous conditions in DMF cleanly afforded the corresponding protected UDP−*N*-acetylmuramyl pentapeptide. Rapid deprotection with aqueous sodium hydroxide[11] and chromatographic purification by reverse phase HPLC finally afforded pure UDP−*N*-acetylmuramyl pentapeptide **6** in 32% yield from **16** as a hygroscopic white powder. The structure of **6** was confirmed by analysis of spectral data (see Supporting Information). Further structural corroboration was obtained by HPLC and mass spectral comparison with an authentic sample of **6** obtained from *Staphylococcus aureus*[17] and from the observation that synthetic **6** was successfully converted into polymerized peptidoglycan using a bacterial enzyme preparation.[18]

The identification of new antibiotics that disrupt steps in bacterial cell wall biosynthesis not targeted by existing agents is an attractive counter-offensive strategy in the evolutionary battle against emerging antimicrobial drug resistance. The synthesis of UDP−*N*-acetylmuramyl pentapeptide outlined here should be amenable to other intermediates in the bacterial cell wall pathway which, in addition to **6**, are useful biochemical tools for the discovery of antibiotics with novel modes of action.

Supporting Information Available: Experimental details and spectral data for all compounds are provided (22 pages). See any current masthead page for ordering and Web access instructions.

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