The Total Synthesis of Lipid I

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Abstract: A total synthesis of lipid I (**4**), a membrane-associated intermediate in the bacterial cell wall (peptidoglycan) biosynthesis pathway, is reported. This highly convergent synthesis will enable further studies on bacterial resistance mechanisms and may provide insight toward the development of new chemotherapeutic agents with novel modes of action.

Background and Introduction

Bacterial peptidoglycan consists of an intricate network of β-1,4-linked carbohydrate polymers, which are cross-linked by pendant peptide chains.[1] This rigid macromolecular structure precisely defines the shape and size of the bacterial cell in both Gram-positive and Gram-negative bacteria and enables the cell to resist lysis resulting from high internal osmotic pressure.[2] Many of the more widely used antibiotics, β-lactams and glycopeptides (e.g., vancomycin), function via mechanisms involving inhibition of key steps in the cell wall biosynthesis cascade.[3] The rapid emergence of antimicrobial resistance has now begun to erode the once dependable clinical efficacy of these chemotherapeutic agents.[4] In response to this rapid increase in resistance, a renewed emphasis has been placed on the study of the bacterial enzymes involved in peptidoglycan biosynthesis. Mechanistic and structural information gained from such studies could facilitate rational design of new antibacterial agents with novel mechanisms of action. While efficient access to many of the biosynthetic enzymes has been gained, the isolation of their respective precursors from natural sources has proven to be difficult.[5] For study of resistance mechanisms at the molecular level, access to isolable quantities of the biosynthetic precursors is required. To address this difficulty, we have established a program to develop practical and efficient synthetic routes to the biosynthetic precursors for use in the discovery and design of novel antibacterial agents. Previous efforts[6] by workers at Eli Lilly and Company culminated in a total synthesis of UDP-*N*-acetylmuramyl pentapeptide (Park Nucleotide[7]), the immediate biosynthetic precursor to lipid I (Scheme 1). As part of our continuing studies in bacterial cell wall biosynthesis, we report herein a total synthesis of lipid I, the penultimate intermediate utilized by Gram-positive bacteria in peptidoglycan biosynthesis.[8]

Peptidoglycan biosynthesis is believed to take place in three distinct stages. The first of these (Scheme 1) occurs in the cytoplasm of the bacterial cell and begins with the elaboration of UDP-*N*-acetylglucosamine (UDP-GlcNAc, **1**) into UDP-MurNAc-pentapeptide (Park Nucleotide, **3**).[1,2,7] The initial step in this sequence is mediated by the MurA enzyme and involves the transfer of an enolpyruvyl group onto the 3-hydroxyl group of UDP-GlcNAc. Conversion of the enol group into the lactyl moiety is accomplished via an NADPH-dependent reduction mediated by the reductase MurB providing UDP-*N*-acetylmuramic acid (UDP-MurNAc, **2**). An ATP-dependent ligase (MurC) introduces the first amide bond (L-Ala) onto the C(3)-lactyl moiety. Additional ATP-dependent ligases (MurD-F) introduce, in succession, D-Glu, L-Lys,[8] and D-Ala-D-Ala to provide the fully elaborated UDP-MurNAc-pentapeptide **3** (Park Nucleotide).[1,2,7]

The second stage of peptidoglycan biosynthesis takes place on the cytoplasmic surface of the bacterial membrane. First, MraY catalyzes a pyrophosphate exchange reaction in which UDP-MurNAc-pentapeptide **3** is coupled to a membrane-anchored lipid (C_{55}) carrier, with concomitant ejection of UMP, to provide undecaprenylpyrophosphoryl-MurNAc-pentapeptide **4** (lipid I). Second, MurG catalyzes the transfer of GlcNAc from UDP-GlcNAc to the C(4)-hydroxyl group of the lipid-linked MurNAc-pentapeptide. The product of this biochemical transformation, lipid II **5**, is the final monomeric intermediate in the cell wall biosynthesis cascade.[1,2,8]

In some Gram-positive bacteria 1−5 amino acid residues may be successively attached to the ϵ-amino group of the lysine residue. Typically, these additional residues are glycine, although organisms incorporating L-serine, L-threonine, and other amino acids are known.[2,9]

‡ Chemical Process Research and Development.

(1) Rogers, H. J.; Perkins, H. R.; Ward, J. B. *Biosynthesis of Peptidoglycan*; Chapman and Hall, Ltd.: London, 1980.

(2) (a) Hölt, J.-V. *Microbiol. Mol. Biol. Rev.* **1998**, *62*, 181. (b) Bugg, T. D. H.; Walsh, C. T. *Nat. Prod. Rep.* **1992**, 199.

(3) Gale, E. F.; Cundliffe, E.; Reynolds, P. E.; Richmond, M. H.; Waring, M. J. *The Molecular Basis of Antibiotic Action*, 2nd ed.; Wiley-Interscience: New York, 1981.

(4) For reviews, see: (a) Chu, D. T. W.; Plattner, J. J.; Katz, L. *J. Med. Chem.* **1996**, *39*, 3853. (b) Morris, A.; Kellner, J. D.; Low, D. E. *Curr Opin. Microbiol.* **1998**, *1*, 524.

(5) See, for example: (a) Anderson, J. S.; Matsuhashi, M.; Haskin, M. A.; Strominger, J. L. *J. Biol. Chem.* **1967**, *242*, 3180. (b) Kohlrausch, U.; Hoeltje, J.-V. *FEMS Microbiol. Lett.* **1991**, 78, 253. (c) Van Heijenoort, Y.; Gomez, M.; Derrien, M.; Ayala, J.; van Heijenoort, J. *J. Bacteriol.* **1992**, *174*, 3549.

(6) For the first total synthesis of the Park nucleotide, the biosynthetic precursor to lipid I, see: Hitchcock, S. A.; Eid, C. N.; Aikins, J. A.; Zia-Ebrahimi, M.; Blaszczak, L. C. *J. Am. Chem. Soc.* **1998**, *120*, 1916.

(7) Park, J. T. *J. Biol. Chem.* **1952**, *194*, 877.

(8) The biochemical pathway illustrated here is that utilized by Gram-positive bacteria. In the biosynthetic pathway utilized by Gram-negative bacteria *meso*-diaminopimelic (*meso*-DAP) is incorporated into the pentapeptide side chain in place of L-lysine.

(9) Schleifer, K. H.; Kandler, O. *Bacteriol. Rev.* **1972**, *36*, 407.

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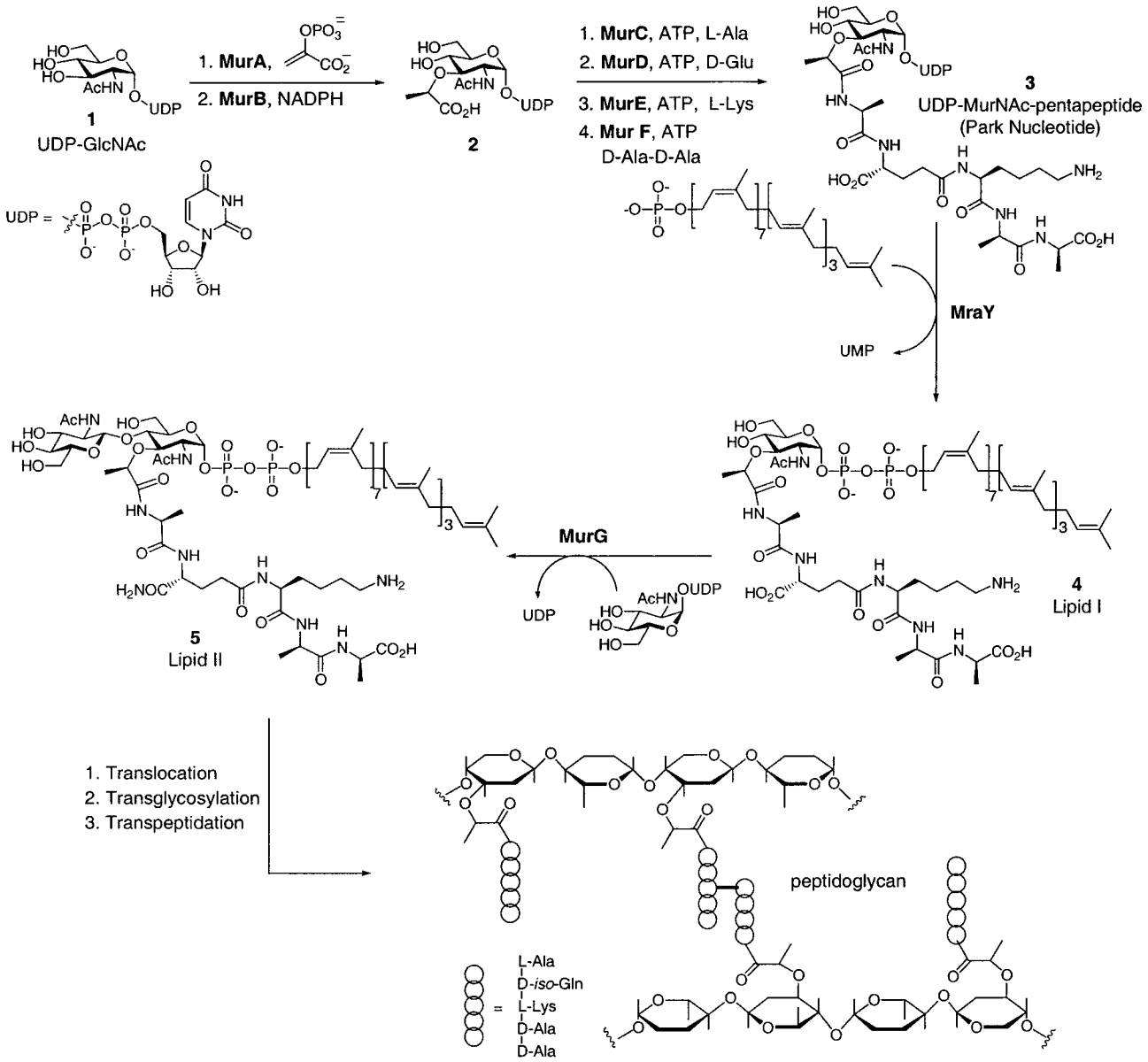
Scheme 1. Biochemical Pathway Utilized in Bacterial Cell Wall Biosynthesis



In the final stage of peptidoglycan biosynthesis, lipid II is translocated to the extracellular face of the bacterial cell membrane where it is subsequently polymerized into glycan strands through the action of transglycosylases. After polymerization of the carbohydrate backbone, the undecaprenyl lipid carrier is translocated back across the cytoplasmic membrane to be recycled.[1,2] The glycan polymers are subsequently cross-linked via amide bond formation between the terminal amino group of the lysine residue, or the α-amino terminus of an attached peptide chain (vide supra), and the penultimate D-Ala residue of a neighboring chain. The amide bond is established with concomitant loss of the terminal D-Ala residue. Mature peptidoglycan is a single macromolecule of muliple layers of cross-linked glycan strands that precisely defines the size and shape of the bacterial cell.[1,2]

To gain a greater understanding of the mechanisms by which bacteria acquire resistance to cell wall active agents, increasing effort has been directed toward the isolation and study of the individual biosynthetic enzymes and their natural substrates. Modern biochemical and genetics techniques have facilitated isolation and purification of the biosynthetic enzymes. With respect to the stage I enzymes, all are soluble and crystal structures of at least four of these enzymes have been published.[10−13] Although reports of the chemical synthesis of their respective substrates have yet to appear, efforts toward the rational design of inhibitors of the stage I enzymes have been reported.[14] The stage II and stage III enzymes have proven much more difficult to study. These are membrane-associated proteins that can make purification difficult and complicate mechanistic and structural analysis. In addition, the structural complexity

(10) MurA: (a) Schonbrunn, E.; Sack, S.; Eschenburg, S.; Perrakis, A.; Krekel, F.; Amrhein, N.; Mandelkow, E. *Structure* **1996**, *4*, 1065. (b) Skarzynski, T.; Mistry, A.; Wonacott, A.; Hutchinson, S. E.; Kelly, V. A.; Duncan, K. *Structure* **1996**, *4*, 1465. (c) Skarzynski, T.; Kim, D. H.; Lees, W. J.; Walsh, C. T.; Duncan, K. *Biochemistry* **1998**, *37*, 2572.

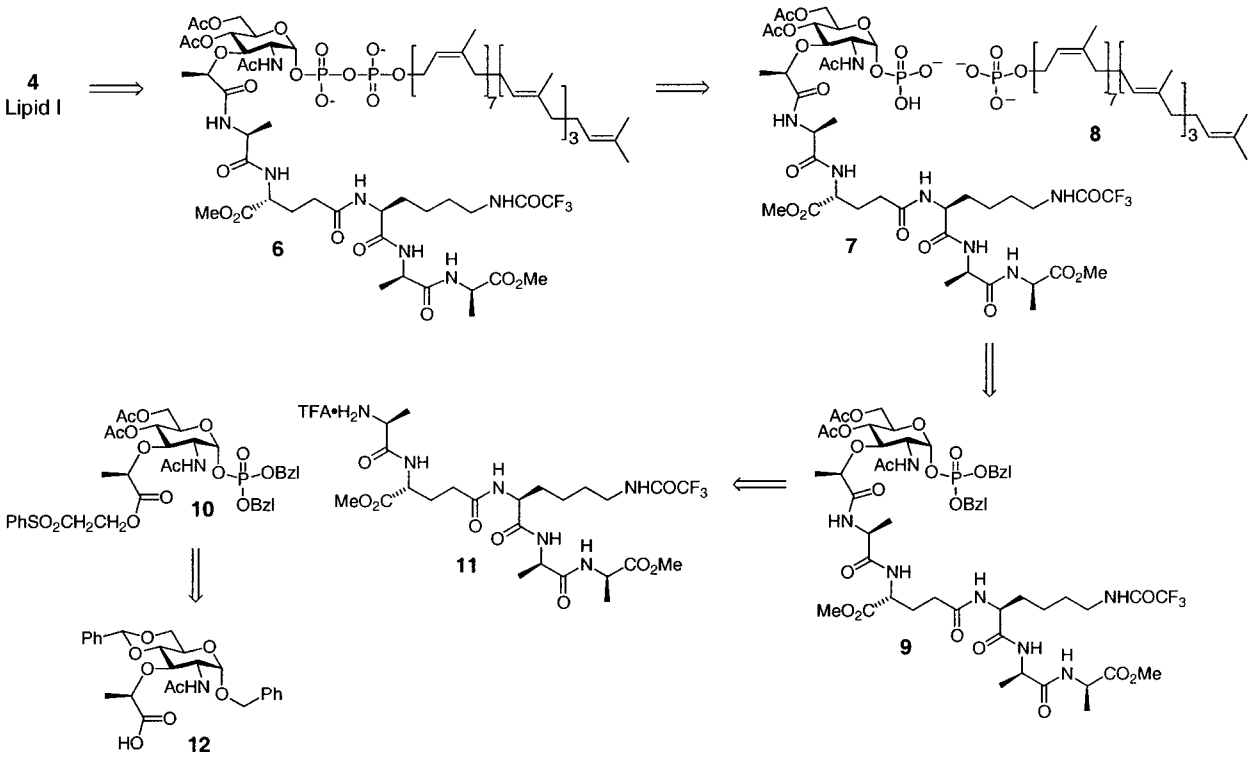
(11) MurB: (a) Benson, T. E.; Filman, D. J.; Walsh, C. T.; Hogle, J. M. *Nat. Struct. Biol.* **1995**, *2*, 644. (b) Benson, T. E.; Walsh, C. T.; Hogle, J. M. *Structure* **1996**, *4*, 47.

(12) Mur C: Emanuele, J. J., Jr.; Jin, H.; Jacobson, B. L.; Chang, C. Y.; Einspahr, H. M.; Villafranca, J. J. *Protein Sci.* **1996**, *5*, 2566.

(13) MurD: Bertrand, J. A.; Auger, G.; Fanchon, E.; Martin, L.; Blanot, D.; van Heijenoort, J.; Dideburg, O. *EMBO J.* **1997**, *16*, 3416.

(14) (a) Gegnas, L. D.; Waddell, S. T.; Chabin, R. M.; Reddy, S.; Wong, K. K. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1643. (b) Tanner, M. E.; Vaganay, S.; van Heijenoort, J.; Blanot, D. *J. Org. Chem.* **1996**, *61*, 1756. (c) Zeng, B.; Wong K. K.; Pompliano, D. L.; Reddy, S.; Tanner, M. E. *J. Org. Chem.* **1998**, *63*, 10081.

Scheme 2. Retrosynthetic Analysis for Lipid I



of the natural substrates for these enzymes is significantly greater than that of the stage I substrates. Moreover, the substrates themselves are difficult to obtain due to low natural abundances and difficult isolation procedures.[5] For example, experiments in *Escherichia coli* have determined lipid I to be present at cell copy numbers no higher than 700.[5c,15]

As part of our ongoing effort in the study of peptidoglycan biosynthesis, we required a supply of lipid I. The inherent structural complexity of lipid I, coupled with its low natural abundance, have hampered attempts at its isolation from cell culture. Recently, in efforts directed toward the purification and characterization of the MurG enzyme, Walker has reported a synthesis of lipid I,[16] as well as related substrate analogues.[17] These results have prompted us to report our total synthesis of lipid I **4**, the penultimate monomeric intermediate utilized by Gram-positive bacteria in peptidoglycan biosynthesis.

Retrosynthetic Analysis

Managing the interplay of structural complexity, chemical stability, and protective group strategy required a very carefully constructed synthetic plan that addressed several key issues. A major consideration during the development of our synthetic strategy involved the timing for introduction of the undecaprenyl side chain and the requisite diphosphate linkage. We were mindful of the technical obstacles (e.g., solubility, micelle formation, etc.) that could interfere with chemical transformations carried out after installation of the lipid side chain. In addition, introduction of the lipid side chain establishes a diphosphate moiety that is both glycosidic and allylic. We anticipated this linkage to be extremely acid-sensitive thus not only placing restrictions on the means for introducing the lipid side chain itself, but also precluding the use of protective groups requiring acidic conditions for any late stage global deprotection.

Our retrosynthetic analysis is presented in Scheme 2. Our "protected" version of lipid I (**6**), employed acetate protective groups for the peripheral hydroxyls, methyl esters for each carboxyl group in the pentapeptide side chain, and a trifluoroacetate group for the terminal amino group of the lysine residue. These base-cleavable protective groups offered a potential solution to our concern regarding the acid-lability of the anomeric diphosphate, and could be removed in a single operation as the final step in the synthesis.

Our first disconnection revealed glycosyl monophosphate **7** and undecaprenyl monophosphate **8**. A mild method for introduction of the chemically sensitive lipid diphosphate moiety would be required.[18] We also preferred a late-stage coupling of these partners to minimize the number of subsequent synthetic transformations that the diphosphate linkage must withstand.

Another consideration in the design of coupling partner **7** was the choice of protective group for the anomeric phosphate. This group must be orthogonal to the protective groups on the periphery of the carbohydrate, as well as those utilized in the peptide backbone to ensure selective unmasking of the anomeric phosphate prior to activation for coupling with lipid monophosphate **8**. For this purpose, we chose benzyl protection thus revealing monosaccharyl pentapeptide **9** as our next target in the retrosynthetic direction.

The pentapeptide side chain **11** would be prepared using standard peptide chemistry methods and would be introduced via coupling with an activated ester intermediate deriving from a muramic acid derivative **10**. Phenylsulfonylethyl protection of the lactyl ester in **10** would ensure complete protective group

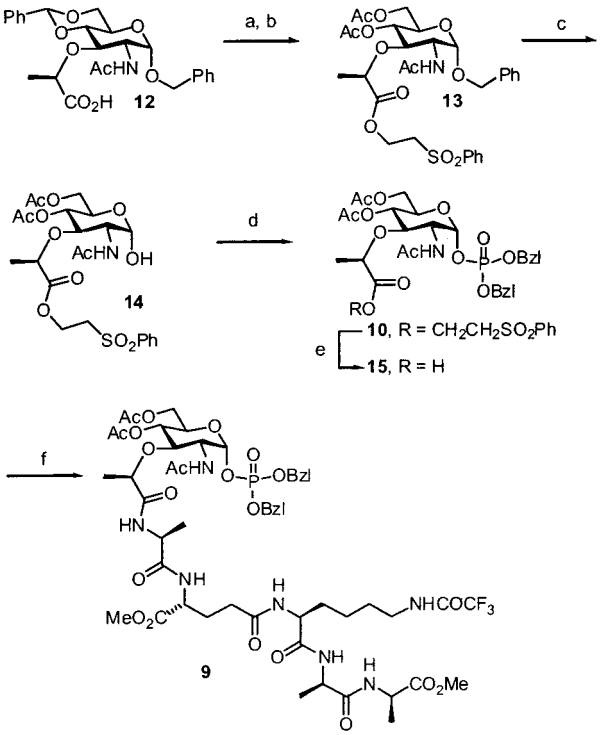
(15) Although the data cited are from a Gram-negative bacterium (*E. coli*), it serves to illustrate the difficulty inherent in obtaining lipid I from cell culture.

(16) Ye, X.-Y.; Lo, M.-C.; Brunner, L.; Walker, D.; Kahne, D.; Walker, S. *J. Am. Chem. Soc.* **2001**, *123*, 3155.

(17) Substrate analogues of Lipid I have been prepared in efforts to characterize MurG. (a) Ha, S.; Chang, E.; Lo, M.-C.; Men, H.; Park, P.; Ge, M.; Walker, S. *J. Am. Chem. Soc*. **1999**, *121*, 8415. (b) Men, H.; Park, P. Ge, M.; Walker, S. *J. Am. Chem. Soc*. **1998**, *120*, 2484.

(18) Undecaprenyl monophosphate bis-ammonium salt may be purchased from the Institute of Biochemistry and Biophysics, Polish Academy of Sciences, ul. Pawinskiego 5A, 02-106 Warszawa, Poland.

Scheme 3. Synthesis of Phosphomuramyl Pentapeptide **9**[a]



[a] Reagents and conditions: (a) EDCI, 2-phenylsulfonylethanol, DMAP, CH_2Cl_2, >95% yield; (b) (*i*) HOAc, H_2O, reflux and (*ii*) Ac_2O, pyridine, 81% yield; (c) H_2 (15 psi), Pd/C, HOAc, 53% yield; (d) (*i*) dibenzyl-*N*,*N*-diethylphosphoramidite, 1*H*-tetrazole, CH_2Cl_2 and (*ii*) 30% H_2O_2, THF, −78 °C to room temperature, 86% yield; (e) DBU, CH_2Cl_2, >95%yield; (f) EDCI, NHS, DMF, then **11**, $^iPr_2NEt$, 75% yield.

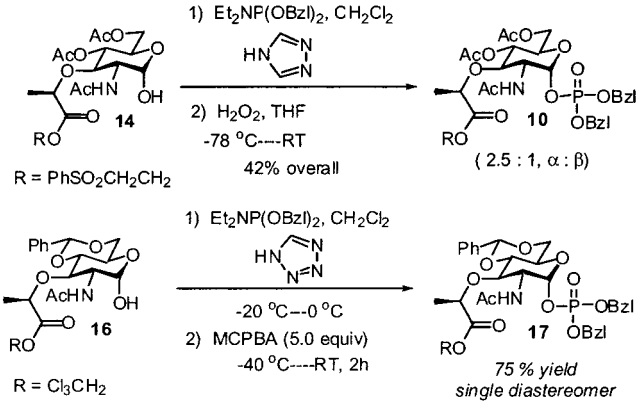
orthogonality and would allow mild conditions for unmasking of the carboxyl group for peptide coupling. The phenylsulfonylethyl ester **10** would in turn be prepared from a differentially protected muramic acid derivative **12**, the starting material for our total synthesis of lipid I.[19]

Synthesis of Phosphomuramyl Pentapeptide 9

Our synthesis began (Scheme 3) with conversion of muramic acid derivative **12**[19] to a phenylsulfonylethyl ester (> 95% yield). Subsequent acid-mediated cleavage of the 4,6-*O*-benzylidene acetal followed by acetylation of the liberated hydroxyl groups provided diacetate **13** in 81% yield. Hydrogenolytic cleavage of the anomeric benzyl protective group was achieved through exposure of **13** to H_2 (15 psi) and Pd/C in acetic acid solvent. This provided lactol **14** (predominantly α) in 53% yield and set the stage for introduction of the anomeric phosphate.

Methods for preparation of glycosyl monophosphates can be divided into two classes, wherein the carbohydrate component may function either as the nucleophilic or as the electrophilic component.[20] In the latter case, carbohydrate substrates bearing a functional group capable of neighboring group participation at C(2) generally favor formation of 1,2-trans linked glycosyl phosphates. This would not provide a glycosyl monophosphate with the proper anomeric stereochemistry for completion of the lipid I total synthesis. Addition of phosphate diesters

Chart 1. Comparison of Phosphitylation/Oxidation Protocols



to carbohydrate-derived oxazolines has been reported to provide α-phosphates in some cases,[21] although decomposition has been reported in others.[22] Of the available methods utilizing a nucleophilic carbohydrate component, we chose a phosphitylation/oxidation sequence for introduction of the anomeric phosphate.[17b,20a]

Literature precedent from the Park nucleotide synthesis[6] established the phosphitylation/oxidation of lactol **14**, existing predominantly as the α-anomer, displayed a modest preference (2.5:1) for the desired α-phosphate **10** (Chart 1). Walker subsequently reported a similar reaction sequence on a related substrate **16** (Chart 1) that provided an α-phosphate **17** as the exclusive product in good chemical yield.[17b]

Although different protection schemes were used in each of these cases, we felt the enhanced preference for the α-anomer observed in the latter example may be due to the choice of acid catalyst (tetrazole vs triazole). Since tetrazole ($pK_a = 4.9$) is a much better proton donor than triazole ($pK_a = 10.0$), the enhanced α-selectivity in the reaction employing tetrazole may be the result of a greater equilibrium concentration of the activated phosphoramidite reagent such that capture by the lactol α-anomer now occurs at a much faster rate than anomerization and capture by the more nucleophilic β-anomer.[23]

Thus, as depicted in Scheme 3, exposure of lactol **14** to dibenzyl-*N*,*N*-diethylphosphoramidite and 1*H*-tetrazole in dichloromethane followed by oxidation of the phosphite intermediate with 30% hydrogen peroxide provided the desired α-phosphate **10** in 86% yield ($^3J_{H1H2} = 3.4$ Hz).[24] The lactyl carboxyl group, the anchor for introduction of the pentapeptide side chain, was liberated through treatment of the phenylsufonylethyl ester with DBU (>95% yield). The stage was now set for introduction of the pentapeptide side chain. The pentapeptide fragment was conveniently prepared utilizing standard peptide synthesis protocols (Scheme 4). Carboxyl group activation of muramic acid derivative **15** was achieved through its conversion to the corresponding NHS-ester. Addition of a DMF solution of pentapeptide **11** to a solution of the NHS-ester deriving from muramic acid derivative **15** and $^iPr_2NEt$ in DMF provided the protected muramyl pentapeptide **9** in excellent yield (75% from **15**).

Thus we achieved efficient access to a differentially protected phosphomuramyl pentapeptide derivative **9** which set the stage for comple-

(19) Jeanloz, R. W.; Walker, E.; Sinay, P. *Carbohydr. Res.* **1968**, *6*, 184.

(20) For leading references, see: (a) Sim, M. M.; Kondo, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 2261. (b) Boons, G.-J.; Burton, A.; Wyatt, P. *Synlett* **1996**, 310. (c) Schmidt, R. R.; Braun, H.; Jung, K.-H. *Tetrahedron Lett.* **1992**, *33*, 1585. (d) Sabesan, S.; Neira, S. *Carbohydr. Res.* **1992**, *223*, 169. (e) Schmidt, R. R.; Wegmann, B.; Jung, K.-H. *Liebigs Ann. Chem.* **1991**, 121. (f) P. Pale, P.; Whitesides, G. M. *J. Org. Chem.* **1991**, *56*, 4547. (g) Veeneman, G. H.; Broxterman, H. J. G.; Van der Marel, G. A.; Van Boom, J. H. *Tetrahedron Lett.* **1991**, *32*, 6175. (h) Bannwarth, W.; Trzeciak, A. *Helv. Chim. Acta* **1987**, *70*, 175.

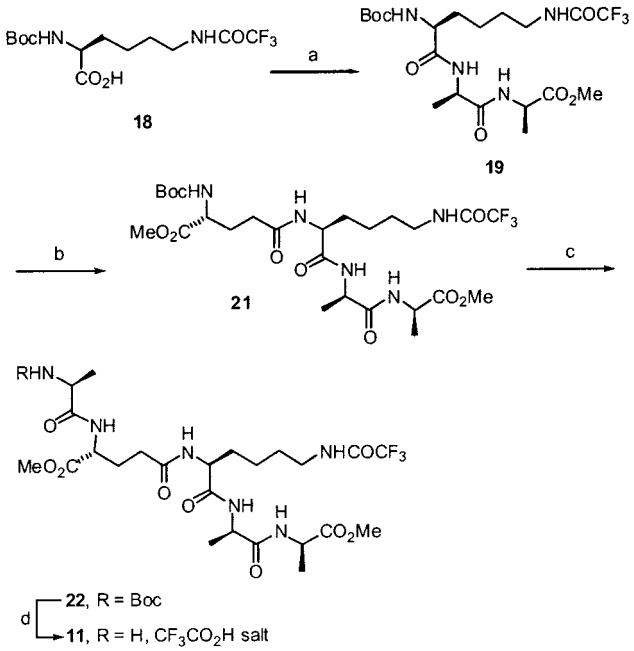
(21) (a) Khorlin, A. Y.; Zurabyan, S. E.; Antonenko, T. S. *Tetrahedron Lett.* **1970**, 4803. (b) Warren, C. D.; Herscovics, A.; Jeanloz, R. W. *Carbohydr. Res.* **1978**, *61*, 181. (c) Inage, M.; Chaki, H.; Kusumoto, S.; Shiba, T. *Tetrahedron Lett.* **1981**, *22*, 2281.

(22) Srivastava, G.; Alton, G.; Hindsgaul, O. *Carbohydr. Res.* **1990**, *207*, 259.

(23) While we have attributed the enhanced α-selectivity observed in 1*H*-tetrazole mediated phosphitylation/oxidation of **16** to the enhanced acidity of tetrazole compared to triazole, we could not discount the additional possibility that a torsional effect, exerted by the 4,6-*O*-benzylidene acetal, could also influence the observed preference for the α-anomer. See, for example: Fraser-Reid, B.; Wu, Z.; Andrews, C. W.; Skowronski, E.; Bowen, J. P. *J. Am. Chem. Soc.* **1991**, *113*, 1434.

(24) For mechanistic studies on the role of 1*H*-tetrazole in phosphoramidite coupling reactions, see: (a) Dahl, B. H.; Nielsen, J.; Dahl, O. *Nucleic Acids Res.* **1987**, *15*, 1729. (b) Berner, S.; Muehlegger, K.; Seliger, H. *Nucleic Acids Res.* **1989**, *17*, 853

Scheme 4. Synthesis of Pentapeptide **11**[a]



[a] Reagents and conditions: (a) (*i*) DCC, NHS, THF, 2 h and (*ii*) D-Ala-D-Ala-OMe (TFA salt), $^{i}Pr_2NEt$, THF, 72% yield; (b) CH_2Cl_2:TFA (1:1), then add to a solution of Boc-D-*iso*-Glu-γ-NHS-OMe **20**, $^{i}Pr_2NEt$, 56% yield; (c) CH_2Cl_2:TFA (1:1), then add to a solution of Boc-L-Ala-OBT, $^{i}Pr_2NEt$, 85% yield; (d) CH_2Cl_2:TFA (1:1), quantitative yield.

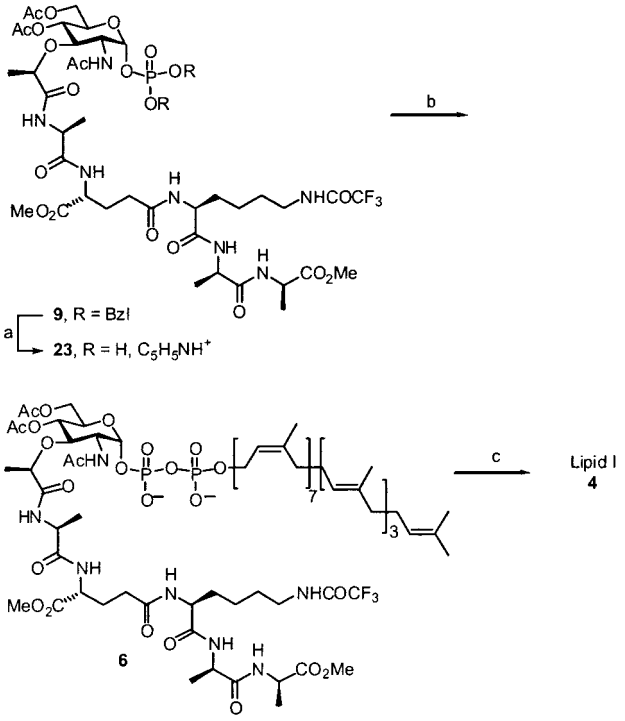
tion of the lipid I total synthesis. The remaining technical hurdles involved introduction of the lipid diphosphate linkage followed by global deprotection and purification. Our solutions to these issues are discussed in the following section.

Introduction of the Lipid Diphosphate: Lipid I Endgame

Numerous methods, both chemical[25] and enzymatic,[26] have been exploited for the preparation of glycosyl diphosphates. Perhaps the most widely used method for construction of anomeric diphosphates is the Khorana−Moffatt[27] protocol, the coupling of glycosyl monophosphates with phosphoromorpholidates (usually nucleoside 5′-phosphoromorpholidates). Although this reaction has enjoyed great utility in the construction of glycosyl nucleoside diphosphates, to the best of our knowledge, it has not been used for the construction of prenyl-linked glycosyl diphosphates. Moreover, application of this method would require independent synthesis of a phosphoromorpholidate deriving from either the carbohydrate or lipid fragment. Thus, in recognition of the potential instability of such activated glycosidic or allylic phosphate intermediates, and for synthetic ease and efficiency, we chose to investigate in situ activation protocols.

Several procedures have been developed for the construction of lipid-linked glycosyl diphosphates. For example, phosphoric anhydrides,[28] phosphoryl dichlorides,[29] and phosphoroimidazolidates[30] have been used as electrophiles for the addition of nucleophilic phosphate salts. The phosphoric anhydride protocol has been used to establish a carbohydrate−lipid diphosphate linkage that is also allylic. Of these procedures, however, only the phosphoroimidazolidate protocol proceeds via initial activation of the carbohydrate component. This was particularly attractive to us in that it would allow use of undecaprenyl monophosphate directly in a coupling reaction with an activated carbohydrate component, the end result being installation of the chemically sensitive diphosphate linkage under relatively mild reaction conditions.

Scheme 5. Lipid I Total Synthesis: The Final Stages[a]



[a] Reagents and conditions: (a) H_2, 10% Pd/C, MeOH, then pyridine; (b) (*i*) 1,1′-carbonyldiimidazole, THF/DMF (4:1), (*ii*) MeOH, and (*iii*) THF/DMF (4:1), undecaprenyl monophosphate bis-ammonium salt **8**, 1*H*-tetrazole, 4 d; (c) 1 N NaOH, dioxane/H_2O (1:1); 40% yield overall from **9**.

Encouraged by promising results with model systems,[31] our attention turned toward application of the phosphoroimidazolidate method to complete the lipid I total synthesis. Cleavage of the benzyl groups from the anomeric phosphotriester was readily achieved via hydrogenolysis (Scheme 5). The anomeric phosphate was isolated as the monopyridyl salt **23** and used directly without purification.[30a] Addition of 1,1′-carbonyldiimidazole to a solution of **23** in THF/DMF (4:1) provided the intermediate phosphoroimidazolidate. After quenching of excess carbonyldiimidazole via careful addition of methanol, a solution of undecaprenyl monophosphate bis-ammonium salt **8** and tetrazole in THF/DMF (4:1) was added carefully via syringe. Reaction progress was monitored by mass spectrometry until complete disappearance of the phosphoroimidazolidate intermediate was observed. The crude reaction solution was concentrated in vacuo, dissolved in a dioxane−water solvent mixture (1:1), and treated with 1 N NaOH. After being stirred for 2 h at room temperature, the mixture was filtered and purified by reverse-phase HPLC.[32] Lyophilization of the pure fractions provided pure lipid I in 40% overall yield from muramyl pentapeptide **9**.

(25) (a) Ichikawa, Y.; Sim, M. M.; Wong, C.-H. *J. Org. Chem.* **1992**, *57*, 2943. (b) Khan, S.; Hindsgaul, O. In *Molecular Glycobiology: Frontiers in Molecular Biology Series*; Fukuda, M., Hindsgaul, O., Eds.; Oxford University Press: Oxford, U.K., 1994; p 206. (c) Klaffke, W. *Carbohydr. Res.* **1995**, *266*, 285. (d) Muller, T.; Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1328. (e) Arlt, M.; Hindsgaul, O. *J. Org. Chem.* **1995**, *60*, 14.

(26) (a) Korf, U.; Thimm, J.; Thiem, J. *Synlett* **1991**, 313. (b) Heidlas, J. E.; Lees, W. J.; Pale, P.; Whitesides, G. M. *J. Org. Chem.* **1992**, *57*, 146. (c) Wong, C.-H.; Whitesides, G. M. Enzymes in Synthetic Organic Chemistry. In *Tetrahedron Organic Chemistry Series*; Baldwin, J. E., Magnus, P. D., Eds.; Elsevier Science: Oxford, U.K., 1994; Vol. 12, p 252. (d) Zervosen, A.; Elling, L. *J. Am. Chem. Soc.* **1996**, *118*, 1836.

(27) Roseman, S.; Distler, J. J.; Moffatt, J. G.; Khorana, H. G. *J. Am. Chem. Soc.* **1961**, *83*, 659.

(28) Warren, C. D.; Jeanloz, R. W. *Biochemistry* **1972**, *11*, 2565.

(29) Imperiali, B.; Zimmerman, J. W. *Tetrahedron Lett.* **1990**, *31*, 6485.

(30) (a) Fang, X.; Gibbs, B. S.; Coward, J. K. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2701. (b) Danilov, L. L.; Maltsev, S. D.; Shibaev, V. N.; Kochetkov, N. K. *Carbohydr. Res.* **1981**, *88*, 203.

(31) For example, coupling reactions utilizing the phosphoroimidazolidate deriving from the anomeric phosphate (α-configuration) of *N*-acetylglucosamine-3,4,6-triacetate and farnesyl monophosphate proceeded cleanly to the desired product as judged by mass spectrometry.

(32) Please refer to the Supporting Information for details regarding the HPLC purification of lipid I.

Conclusions

The emergence of bacterial resistance to antimicrobial agents that inhibit bacterial cell wall biosynthesis has hastened the search for novel, yet to be exploited, targets for chemotherapeutic intervention. Detailed study of the bacterial cell wall biosynthesis enzymes, as well as the resistance mechanisms themselves, has been slow to develop due to the limited availability of the natural substrates. Organic synthesis can provide a powerful solution to this problem as has already been demonstrated with the synthesis of Park Nucleotide, the immediate biosynthetic precursor to lipid I.[6] It is our anticipation that this total synthesis of lipid I will provide another valuable biochemical tool that may advance the understanding of key events in bacterial cell wall biosynthesis and, ultimately, lead to the identification of new chemotherapeutic agents with novel modes of action.

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Supporting Information Available: Complete experimental procedures and spectral data are available for all compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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