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Synthesis of Heptaprenyl−Lipid IV to Analyze Peptidoglycan Glycosyltransferases

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Peptidoglycan (PG) is a crosslinked carbohydrate polymer that surrounds and protects bacterial cell membranes, enabling bacteria to withstand large fluctuations in internal osmotic pressure. Because peptidoglycan is essential for bacterial cell survival, the enzymes involved in the biosynthesis of PG are targets for antibiotics.[1] Over the past fifteen years, great progress has been made toward understanding the different steps of PG synthesis,[1] but there is still one family of enzymes that remains poorly characterized: the peptidoglycan glycosyltransferases (PGTs) that catalyze formation of the carbohydrate chains of peptidoglycan from a disaccharide precursor called Lipid II (**1**, Figure 1).[2] Bacteria typically contain several different PGTs whose biological roles are poorly understood. Biochemical studies of PGTs have been hampered by difficulties in obtaining substrates to dissect the polymerization mechanism. The first coupling catalyzed by PGTs involves the condensation of two Lipid II substrates to form a tetrasaccharide, Lipid IV (**2**, Figure 1). Subsequent coupling cycles involve the elongation of the growing polymer by addition of Lipid II subunits. Therefore, after the first coupling cycle, the substrates used by PGTs are different. To probe the mechanism of glycosyltransfer and to characterize enzyme inhibitors, it is essential to have Lipid II substrates (**1**) as well as a longer substrate representing the growing polymer (such as Lipid IV, **2**). We and others have previously developed approaches to obtain Lipid II,[3] but longer substrates have not been reported.[4] Here we describe the total synthesis of heptaprenyl−Lipid IV (**2b**) and we show that both major *E. coli* PGTs, PBP1a and PBP1b, couple this substrate to heptaprenyl−Lipid II (**1b**).[5] Unexpectedly, PBP1a also couples Lipid IV subunits to one another, suggesting that some PGTs may be able to ligate longer glycan polymers in addition to building glycan chains from Lipid II.

Our route to Lipid II involves the chemical synthesis of Lipid I followed by the enzymatic construction of the β-1,4-glycosidic linkage using *E. coli* MurG.[3a,6] While this chemoenzymatic approach using a purified enzyme as the final step is efficient, enzymes to make longer glycan polymers with control over product length are not yet available. Therefore, all the glycosidic linkages in Lipid IV must be constructed chemically prior to installation of the sensitive diphospholipid moiety at the reducing end of the molecule. β-1,4 glycosidic linkages between 2-amino-2-deoxy sugars occur frequently in nature, but remain challenging to synthesize, in part because of the low reactivity of the C4 hydroxyl. We chose tetrasaccharide **11** (Scheme 1) as a key intermediate and established the goal of constructing all the glycosidic linkages using the sulfoxide glycosylation method. The monomer building blocks **5**, **6**, and **7** were readily synthesized in multigram quantities from a common intermediate **3** (Scheme 1). **5** and **6** were coupled to obtain the desired β-1,4-linked disaccharide **8** in 75% yield. The bulky

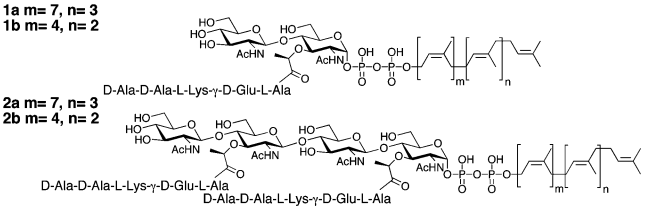
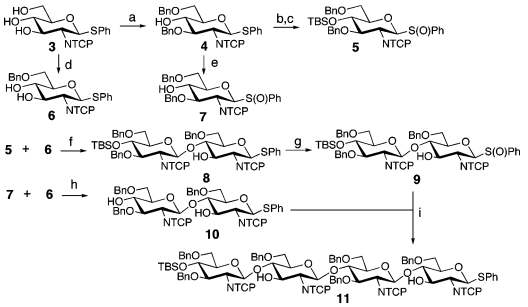


Figure 1. Undecaprenyl−Lipid II (**1a**) and Lipid IV (**2a**); heptaprenyl−Lipid II (**1b**) and Lipid IV (**2b**).

Scheme 1. Construction of Lipid IV Tetrasaccharide Backbone[a]



[a] Conditions: (a) Bu_2SnO, toluene, reflux, then Bu_4NI, BnBr, reflux, 51%; (b) TBSOTf, 2,6-lutidine, CH_2Cl_2, 91%; (c) *m*CPBA, CH_2Cl_2, −78 to −60 °C, 93%; (d) (i) $(Bu_3Sn)_2O$, MeOH, reflux; (ii) Bu_4NI, BnBr, toluene, 91 °C, 75%; (e) *m*CPBA, CH_2Cl_2, −78 to −60 °C, 91%; (f) Tf_2O, DTBMP, ADMB, MS 4 Å, CH_2Cl_2, −60 to −30 °C, 75%; (g) *m*CPBA, CH_2Cl_2, −78 to −60 °C, 86%; (h) Tf_2O, DTBMP, ADMB, MS 4 Å, CH_2Cl_2, −60 to −40 °C, 58%; (i) Tf_2O, DTBMP, ADMB, MS 4 Å, CH_2Cl_2, −40 °C, 77%.

tetrachlorophthalimido (TCP) group hindered reaction of the unprotected C3 hydroxyl of **6**, while favoring the β-anomeric product, enabling regio- and stereoselective glycosylation.[7] Disaccharide **10** was produced in 58% yield by inverse addition of the partially protected glycosyl donor **7** to the acceptor **6**.[8] Reaction of the C4 hydroxyl of **6** with activated donor **7** was favored over self-condensation of **7**, which also contains an unprotected C4 hydroxyl.[9] In the next step, however, this unprotected C4 hydroxyl reacted preferentially over the C3 hydroxyls of **9** and **10** to produce tetrasaccharide **11**. Thus, the use of partially protected glycosyl donors and acceptors at several different steps enabled a convergent tetrasaccharide synthesis with a minimum of protecting group manipulations.

The N−TCP amides of **11** were converted to the *N*-acetyl amides as shown, and the two D-lactate and 1-α-phosphate moieties were then installed to afford the desired intermediate **14** in nine steps with a 21% overall yield (Scheme 2). The silyl protected pentapeptide **15** was synthesized using Fmoc chemistry and coupled to **14** using standard peptide coupling conditions. Global hydrogenolysis provided **16**. Heptaprenyl phosphate, synthesized as described,[3a] was coupled to the anomeric phosphate **16** using CDI. The addition

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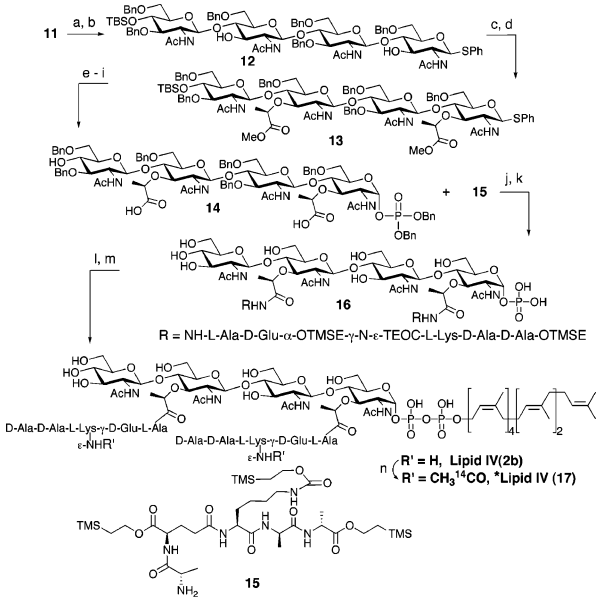
Scheme 2. Completion of the Synthesis[a]



[a] Conditions: (a) $(NH_2CH_2)_2$, THF/CH_3CN/EtOH (1:2:1), 60 °C; (b) Ac_2O, MeOH/H_2O (5:1), room temp, 75% two steps; (c) NaH, *S*-(−)-2-bromo-propionic acid, THF, 0 °C to room temp; (d) $TMSCHN_2$, benzene/MeOH (3:1), 0 °C, 70% two steps; (e) NIS, CH_3CN/H_2O (5:1), room temp, 75%; (f) 1*H*-tetrazole, $(i\text{-}Pr)_2NP(OBn)_2$, CH_2Cl_2, −40 to −20 °C; (g) *m*CPBA, CH_2Cl_2, −40 °C to room temp, 84% two steps; (h) TBAF, THF, 0 °C to room temp; (i) 1.3 M KOH, THF/H_2O (10:1), room temp, 64% two steps; (j) HATU, DIEA, DMF, room temp, 60%; (k) $Pd(OH)_2/C$, H_2, 44%; (l) ammonium heptaprenyl phosphate, 1,1′-carbonyl diimidazole, THF, room temp, then **16**, $SnCl_2$, DMF, room temp, 50%; (m) TBAF, DMF, room temp, 69%; (n) $(CH_3{}^{14}CO)_2O$, toluene/16 mM NaOH in MeOH (1:1), sonication, 37 °C, 50%.

Table 1. Percentage of Peptidoglyan Formed from ^{14}C-Lipid IV (**17**)[a]

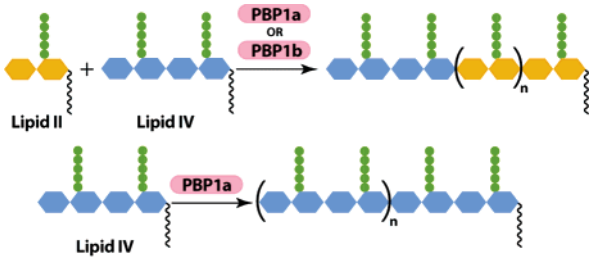
enzyme (nM)	time (min)	^{12}C **1b** (μM)	^{14}C **17** (μM)	% conversion to peptidoglycan[b]
84 (PBP1b)	90	0	0.4	undetectable
84 (PBP1b)	360	0	0.4	undetectable
84 (PBP1b)	10	4	0.32	40.8
86 (PBP1a)	90	0	0.4	27.9
86 (PBP1a)	360	0	0.4	46.0
86 (PBP1a)	10	4	0.32	51.1

[a] All the experiments were carried out in the presence of penicillin G to prevent peptide crosslinking. For experimental procedures, see Supporting Information. [b] Conversion is based on utilization of ^{14}C-labeled **17**.

of $SnCl_2$ as a Lewis acid accelerated the coupling reaction and improved the yield significantly.[10] Finally, global deprotection of the silyl groups led to the desired target, heptaprenyl−Lipid IV (**2b**). This compound was treated with ^{14}C-acetic anhydride to make *Lipid IV (**17**) to assay enzymatic activity.

*Lipid IV was incubated with either *E. coli* PBP1a or PBP1b (Table 1), and reactions were analyzed as described previously.[11] PBP1b did not utilize Lipid IV as a substrate unless Lipid II was also included in the reaction mixture (Table 1).[12] This result is consistent with the accepted mechanism for transglycosylation, in which Lipid II subunits are added sequentially to a growing polymer chain (Scheme 3, top). Surprisingly, however, PBP1a was able to convert Lipid IV to peptidoglycan polymer in the *absence* of Lipid II (Scheme 3, bottom), showing that Lipid II is *not* an obligatory substrate for all PGTs. This result suggests that the biological functions of some PGTs may include coupling peptidoglycan oligomers. The work reported here demonstrates the utility of Lipid II and Lipid IV substrates to probe the mechanisms of PGTs, and more detailed studies are underway.

Scheme 3. Reactions Catalyzed by PBP1b (Top) and PBP1a (Top and Bottom)[a]



[a] Lipid II is proposed to add to the reducing end of the growing glycan chain, as shown in the top depiction.[13]

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Supporting Information Available: Experimental procedures and spectral data for all compounds; enzyme expression, purification, and reaction conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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