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Synthesis of Heptaprenyl-Lipid IV to Analyze Peptidoglycan Glycosyltransferases

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Abstract

Bacteria are surrounded by a cell wall containing layers of peptidoglycan, the integrity of which is essential for bacterial survival. In the final stage of peptidoglycan biosynthesis, peptidoglycan glycosyltransferases (PGTs; also known as transglycosylases) catalyze the polymerization of Lipid II to form linear glycan chains. PGTs have tremendous potential as antibiotic targets, but the potential has not yet been realized. Mechanistic studies have been hampered by a lack of substrates to monitor enzymatic activity. We report here the total synthesis of heptaprenyl-Lipid IV and its use to study two different PGTs from *E. coli*. We show that one PGT can couple Lipid IV to itself whereas the other can only couple Lipid IV to Lipid II. These *in vitro* differences in enzymatic activity may reflect differences in the biological functions of the two major glycosyltransferases in *E. coli*.

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. In order 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

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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>.

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.^{3(a),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 multi-gram 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 tetrachlorophthalimido (TCP) group hindered reaction of the unprotected C3 hydroxyl of **6**, while favoring the β -anomeric product, enabling regio- and stereo-selective 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 9 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,^{3(a)} was coupled to the anomeric phosphate **16** using CDI. The addition of SnCl₂ 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 ¹⁴C-acetic anhydride to make *Lipid IV (**17**) in order 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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1a $m=7$, $n=3$
1b $m=4$, $n=2$

2a $m=7$, $n=3$
2b $m=4$, $n=2$

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) $m\text{CPBA}$, CH_2Cl_2 , -78 to -60 °C, 93%; (d) i. $(\text{Bu}_3\text{Sn})_2\text{O}$, MeOH , reflux; ii. Bu_4NI , BnBr , toluene, 91 °C, 75%; (e) $m\text{CPBA}$, CH_2Cl_2 , -78 to -60 °C, 91%; (f) Tf_2O , DTBMP , ADMB , $\text{MS } 4\text{\AA}$, CH_2Cl_2 , -60 to -30 °C, 75%; (g) $m\text{CPBA}$, CH_2Cl_2 , -78 to -60 °C, 86%; (h) Tf_2O , DTBMP , ADMB , $\text{MS } 4\text{\AA}$, CH_2Cl_2 , -60 to -40 °C, 58%; (i) Tf_2O , DTBMP , ADMB , $\text{MS } 4\text{\AA}$, CH_2Cl_2 , -40 °C, 77%.

Scheme 2.

Completion of the synthesis^a

^a Conditions: (a) (NH₂CH₂)₂, THF/CH₃CN/EtOH (1:2:1), 60 °C; (b) Ac₂O, MeOH/H₂O (5:1), rt, 75% two steps; (c) NaH, *S*-(−)-2-bromo-propionic acid, THF, 0 °C to rt; (d) TMSCHN₂, benzene/MeOH (3:1), 0 °C, 70% two steps; (e) NIS, CH₃CN/H₂O (5:1), rt, 75%; (f) 1*H*-tetrazole, (*i*-Pr)₂NP(OBn)₂, CH₂Cl₂, −40 to −20 °C; (g) *m*CPBA, CH₂Cl₂, −40 °C to rt, 84% two steps; (h) TBAF, THF, 0 °C to rt; (i) 1.3 M KOH, THF/H₂O (10:1), rt, 64% two steps; (j) HATU, DIEA, DMF, rt, 60%; (k) Pd(OH)₂/C, H₂, 44%; (l) ammonium heptaprenyl phosphate, 1,1'-carbonyl diimidazole, THF, rt, then **16**, SnCl₂, DMF, rt, 50%; (m) TBAF, DMF, rt, 69%; (n) (CH₃¹⁴CO)₂O, toluene/16 mM NaOH in MeOH (1:1), sonication, 37 °C, 50%.

Scheme 3.

Reactions catalyzed by PBP1b (top) and PBP1a (top and bottom)^a

^aLipid II is proposed to add to the reducing end of the growing glycan chain, as shown in the top depiction.¹³

Table 1Percentage of peptidoglycan formed from ^{14}C -Lipid IV (**17**)^a

enzyme (nM)	time (min)	^{12}C 1 (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**.