## A New Synthetic Approach toward Bacterial Transglycosylase Substrates, Lipid II and Lipid IV

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A new synthetic approach toward the bacterial transglycosylase substrates, Lipid II (1) and Lipid IV (2), is described. The key disaccharide was synthesized using the concept of relative reactivity value (RRV) and elaborated to Lipid II and Lipid IV by conjugation with the appropriate oligopeptides and pyrophosphate lipids. Interestingly, the results from our HPLC-based functional TGase assay suggested Lipid IV has a higher affinity for the enzyme than Lipid II.

The increasing prevalence of drug-resistant infections has stimulated the pharmaceutical industry and scientific community to identify novel drug targets and develop new antibiotics against such resistant strains of bacteria as vancomycin-resistant *Enterococci* (VRE), methicillinresistant *Staphylococcus aureus* (MRSA), and even the newly found bacterium with the NDM-1 resistant gene.<sup>1</sup> In bacterial cell wall biosynthesis, the enzyme transglycosylases

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Figure 1. The bacterial transglycosylase (TGase) catalyzes its substrates, Lipid II, Lipid IV, and others, to form peptidoglycan.

(TGases) use Lipid II (1) to elongate the sugar backbone with another substrate such as Lipid II, Lipid IV (2), or a longer TGase substrate and construct the polymerized peptidoglycan chain (Figure 1).<sup>2</sup> Importantly, these bacterial TGases have similar binding motifs and conserved catalytic residues in most Gram-positive and Gram-negative bacteria, including drug-resistant bacteria, even though the peptide moieties of the peptidoglycan chain have mutated.<sup>3</sup>

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Accordingly, TGase is considered a potential drug target for antibiotic discovery and development.<sup>2,3</sup>

Unfortunately, the study of TGase is hampered by an acute shortage of substrates.<sup>3</sup> Isolation from natural sources is limited by the low natural abundance,<sup>3</sup> and the assembly of their complex structures is a very challenging task for synthetic chemists. Currently, a few studies related to the synthesis of Lipid II and its analogues,<sup>4</sup> and only one for Lipid IV, have been reported.<sup>5</sup> Novel synthetic strategies toward TGase substrates therefore are of great interest.

Our laboratory has already described and exemplified the utility of the RRV (relative reactivity value) concept for the synthesis of natural oligosaccharides and antigens.<sup>6</sup> Herein, we describe a new synthetic method to prepare Lipid II and Lipid IV via key oligosaccharides, the design of which is based on our RRV concept.

Our retrosynthetic analysis is illustrated in Figure 2. Importantly the correct choice of protecting groups or substituents for the key disaccharide would first be investigated. For example, the group at the C3 position in the first sugar plays two pivotal roles: to further conjugate with peptides, and to modulate the reactivity of the pendant thioglycosides via an inductive effect. To avoid an annoying aglycone-transfer side reaction, which occurred by the mismatch of the relative energies of thioglycosides,<sup>7</sup> six possible monothioglycoside-based building blocks 3-8 were prepared based on our design and existing RRV database.<sup>6</sup>

After quantitative determination of their RRVs (Figure 2), we discovered that a TBS group on the C4oxygen of **5** dramatically enhanced the glycosyl reactivity (RRV = 134.1) and that thioglycosides **3** and **4** had much lower RRVs. The building block **8** (RRV = 9), bearing an electron-withdrawing chloroacetyl group (ClAc) on O3, had a lower RRV compared with the lactylated **6** (18.9) and the unprotected **7** (21.6). Through our evaluation, building blocks **5** and **8** were chosen for our oligosaccharide synthesis.

Treatment of **5** and **8** with NIS-TMSOTf gave disaccharide **9** in almost quantitative yield (>90%) (Scheme 1). The  $\beta$ -linkage for this newly formed glycosidic bond was confirmed by the <sup>1</sup>H NMR spectrum ( $\delta$  5.17, J = 8.4 Hz).

Scheme 1. Synthesis of Disaccharide 9 and Tetrasaccharide 12

BnO

BnO



NIS (1.1 equiv)



Disaccharide 9 was an intermediate in our Lipid II synthesis, while the two requisite disaccharides 10 and 11 were intermediates for our Lipid IV synthesis. Deprotection of 9 either with NaOMe in MeOH (to give 10) or with TBAF (to give 11)

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Figure 2. Our synthetic strategy for Lipid II and Lipid IV. The RRVs of new desired monothioglycosides were determined as previously described.<sup>6</sup>

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took place in very good yield (Scehem 1). As expected, **10**, without the ClAc group (RRV = 31.3), was found to be more reactive than **9**(4.2) and **11** (5.3). A sufficient reactivity difference between **10** and **11** allowed glycosylation to proceed to deliver tetrasaccharide **12** in good yield (71%), without any side products being observed.<sup>8</sup> The configuration of the newly fomed glycosidic bond in **12** was identified as the  $\beta$ -anomer by NMR, as described above.

With the key oligosaccharides **9** and **12** in hand, attention turned to the synthesis of the target TGase substrates Lipid II (**1**) and Lipid IV (**2**). Unfortunately, initial attempts to deprotect the *N*-phthalimide moiety of **9** under several conditions (Table 1) such as  $NH_2NH_2$  (entry 1) and base-mediated conditions (entries 2 and 3) were unsuccessful.<sup>4,9</sup> A three-step, one-pot procedure (NaBH<sub>4</sub>, AcOH, and Ac<sub>2</sub>O) was eventually developed, in which the *N*-phthaloyl group of **9** was directly converted to the requisite *N*-acetyl group via reduction, intramolecular lactonization, and acetylation, with concomitant removal of the ClAc moiety (entries 5 and 6).<sup>10</sup> It is notable that use of a mixed solvent system (THF/*i*-BuOH/H<sub>2</sub>O) gave the best results (86%, Table 1, entry 6).

Table 1. Conditions of N-Phthalimide Deprotection

Bn TBSC Bn(	0 BnO BnO BnO BnO BnO TBSO O O O O O O O O O O O O O O O O O O	O STol NHAc
entry	conditions	yield (%)
1	NH <sub>2</sub> NH <sub>2</sub> H <sub>2</sub> O (20 equiv), EtOH, reflux, 48 h	_
2	6 N KOH, EtOH, reflux, 24 h	_
$3^a$	6 N KOH, EtOH, μ.w., 1 h	trace
$4^b$	(NH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> (20 equiv), <i>t</i> -BuOH, 90 °C, 24 h	3
5	NaBH <sub>4</sub> (10 equiv), MeOH, rt, 24 h; AcOH,	71
	90 °C, 3 h; then Ac <sub>2</sub> O, rt, 0.5 h	
$6^c$	NaBH <sub>4</sub> (10 equiv), THF/i-BuOH/H <sub>2</sub> O (2:6:1), rt,	86
	24 h; AcOH, 90 °C, 3 h; then Ac <sub>2</sub> O, rt, 0.5 h	

<sup>*a*</sup> The reaction was performed in a sealed tube at 120 W. <sup>*b*</sup> TBS group was also removed. <sup>*c*</sup> 20 equiv of NaBH<sub>4</sub> were used for **12**. <sup>*d*</sup> Isolated yield.

As shown in Scheme 2, treatment of **13** with methyl (*S*)lactate triflate under basic conditions gave **14** in 72% yield. Thiocresol deprotection (NIS, acetone/H<sub>2</sub>O) of **14**, followed by phosphorylation,<sup>4</sup> provided phosphoryldiester **15** as a single diastereomer. The  $\alpha$ -configuration of **15** was confirmed by NMR spectroscopy (<sup>3</sup>J<sub>H1,H2</sub> = 3.2 Hz). After successive fluoride-mediated desilylation, hydrolysis, conjugation with tetrapeptide **16**, and debenzylation, the pentapeptidyl phosphodisaccharide **17** was generated in 42% overall yield from **15**. Finally, the undecaprenyl Scheme 2. Preparation of Lipid II (1)





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 $(NH_4^+)_2$ 

1 h, 37% over 2 steps

phosphoroimidazolidate (C55PIm) was coupled to 17 in the presence of 1H-tetrazole. After global deprotection of the peptidyl protecting groups, Lipid II (1) was obtained in 37% yield.

Similarly, Lipid IV (2) was synthesized from 12 in an overall yield of approximately 5%. A new Lipid IV based fluorescent probe 19 was also prepared by reacting the  $\varepsilon$ -NH<sub>2</sub> of the lysine side chain with NBD-X-OSu (succinimidyl 6-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)hexanoate) fluorophore and applied to our TGase study (Scheme 3).<sup>4,11</sup>

In an HPLC-based functional enzyme assay,<sup>11</sup> *E. coli* PBP1b TGase was used as the enzyme model and the reaction was monitored by the consumption of **19** (see details in the Supporting Information).

When the assay contained **19** alone, no transglycosylation occurred (Figure 3). In contrast, when another substrate Lipid II (**1**) was added, **19** was consumed. These observations suggest the disaccharide building block provided by Lipid II is required for chain elongation in *E. coli* PBP1b TGase and are consistent with Kahne's report, using a radiolabeled Lipid IV.<sup>5</sup> Surprisingly, **19** (1 equiv) was completely consumed even in the presence of an excess (1.5 equiv) of **1** (Figure 3e), suggesting that Lipid IV may

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Scheme 3. Preparation of Lipid IV (2) and Its Fluorescent Analogue (19)



bind to the TGase enzyme with a higher affinity than Lipid II. It was also found that the rate of consumption of **19** showed a positive dependence with the amount of **1**. In Figure 3, all reactions were terminated by a well-known TGase inhibitor, Moenomycin  $A^{3,12}$ 

In conclusion, an RRV-based thioglycosylation strategy has been successfully used to design and prepare the key disaccharide and tetrasaccharide intermediates of Lipid II and Lipid IV. These intermediates were subsequently elaborated



**Figure 3.** Measurement of the transglycosylation reaction by HPLC. *E. coli* PBP1b was incubated with **19** (1 equiv) and various amounts of **1** (a–e: 0, 0.3, 0.6, 0.9, 1.5 equiv) in 2 h and then stopped by adding Moenomycin A (100  $\mu$ M). In trace f, Moenomycin A (100  $\mu$ M) was added first to stop the reaction (see details in the Supporting Information).

to the final targets in a practical and convergent manner. Lipid IV was conjugated with a fluorotag to generate a versatile fluorescent TGase probe. Preliminary biological studies suggest that Lipid IV may bind to *E. coli* PBP1b with a higher affinity than Lipid II. Syntheses of longer substrates or substrate-based inhibitors using this approach are currently in progress.

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**Supporting Information Available.** Experimental procedures, characterization of the synthetic compounds, and HPLC analysis of transglycosylation. This material is available free of charge via the Internet at http://pubs.acs.org.

<sup>(12)</sup> In this biological system with probe **19** as a substrate, Moenomycin A exhibited an  $IC_{50}$  value of 23 nM, and the detailed experiment was shown in the Supporting Information.