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Biosynthesis

One-Pot Protection-Glycosylation Reactions for Synthesis of Lipid II Analogues

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Abstract: (2,6-Dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methyl trichloroacetimidate (**3**) and its polymer-supported reagent **4** can be successfully applied to a one-pot protection-glycosylation reaction to form the disaccharide derivative **7d** for the synthesis of lipid II analogues. The temporary protecting group or linker at the C-6 position and *N*-Troc protecting group of **7d** can be cleaved simultaneously through a reductive condition. Overall yields of syntheses of lipid II (**1**) and neryl-lipid II N^{ε}-dansylthiourea are significantly improved by using the described methods.

Lipid II (**1**) is a membrane-anchored cell-wall precursor that is essential for the growth and replication of both Gram-positive and Gram-negative bacteria. The effectiveness of targeting the enzymes associated with lipid II or lipid II itself as an antibacterial strategy is highlighted by the fact that it is the target for at least four different classes of antibiotics, including the clinically important glycopeptide antibiotics.[1] The reactions necessary for the biogenesis of peptidoglycan (PG) have been known for decades, and the biosynthesis of peptidoglycan of *E. coli* has been discussed extensively in reviews by van Heijenoort.[2] Lipid II is an important biochemical tool for studying MurG, flippase that translocates lipid II across the cytoplasmic membrane, penicillin binding proteins (PBPs), and the mode of action of glycopeptide antibiotics.[3] Lipid II displays very poor water solubility, and thus, it is not trivial to use the natural form of lipid II for biological investigations or to screen for inhibitor molecules. To date, several lipid II analogues have been synthesized and applied in biochemical studies (Figure 1).[4] We have recently identified that neryl-lipid II N^{ε}-dansyl analogue **2**-N^{ε}-dansylthiourea could be recognized by mycobacterial transglycosylase (TGase) to form polymerized products with significant enhancement of visible-light absorption at 400 nm. Because the dansyl group itself does not have absorption in the visible-light region, a bathochromic shift of the polymerized-**2**-N^{ε}-dansylthiourea is an unusual physicochemical observation. Thus, the polymerized-**2**-N^{ε}-dansylthiourea formed by reaction with *Mtb* TGase can be distinguished from unreacted **2**-N^{ε}-dansylthiourea in the reaction mixture with visible light without separation (see Supporting Information). The observed physicochemical property of polymerized-**2**-N^{ε}-dansylthiourea has been applied as a convenient assay for the discovery of new TGase inhibitor molecules in our laboratory. In order to perform high-throughput screening against TGase, it is indispensable to establish a convenient synthetic route to access neryl-lipid II (**2**). To date, the total chemical and biochemical syntheses of lipid II and its derivatives have been accomplished by a few research groups.[4] We have accomplished chemoenzymatic and total chemical synthesis of UDP-MurNAc-pentapeptide (Park's nucleotide) and prenyl-MurNAc-pentapeptide (lipid I).[5] However, to the best of our knowledge, total chemical synthesis is more feasible than other methods to generate enough lipid II analogue for high-throughput screening (HTS). Herein, we report an improved

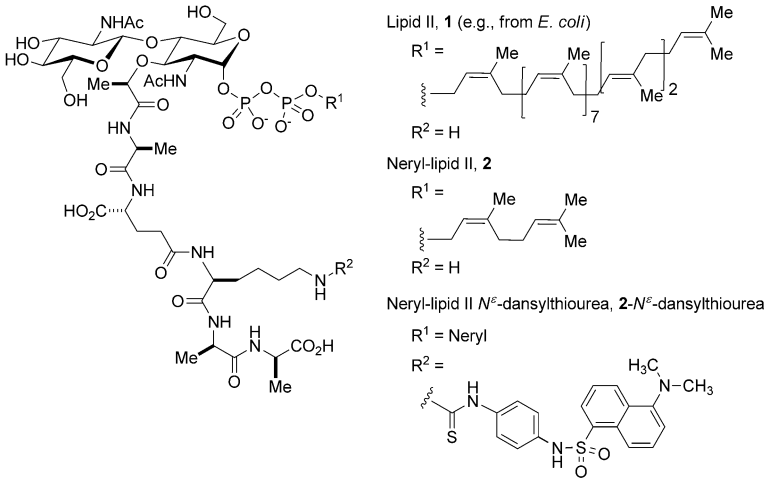

Figure 1. Structures of lipid II and neryl-lipid II analogues for studying transglycosylase (TGase).

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chemical synthesis of lipid II (**1**) and neryl-lipid II analogue **2**-N^{ε}-dansylthiourea through one-pot protection and glycosylation reactions.

Total chemical synthesis of lipid II reported previously revealed that limited combinations of protecting groups of glycosyl acceptors and donors (**i** and **ii** in Figure 2) can be used to successfully synthesize lipid II disaccharide (**iii** and **iv**); the glycosyl donors such as *N*-phthaloyl 3,4,6-*O*-triacetyl-2-deoxyl-2-amino-D-glucopyranosyl 1-bromide (P^2 = phthaloyl, L = Br in **i**), *N*-2,2,2-trichloroethoxycarbonyl 3,4,6-*O*-triacetyl-2-deoxyl-2-amino-D-glucopyranosyl 1-bromide (P^2 = Troc, L = Br in **i**), or *N*-phthaloyl 2-deoxy-2-amino-3,4,6-*O*-triacetate-D-glucopyranosyl 1-(2,2,2-trichloroacetoimidate) (P^2 = phthaloyl, L = *O*-imidate in **i**) have been utilized in the synthesis of the disaccharide **iv** with the C6-protected donor **ii** (R^3 = Ac or Bn).[4] In general, the glycosyl acceptors **ii**, whose C6-position was protected with acyl groups, showed slow reaction rate and low conversion.[4f]

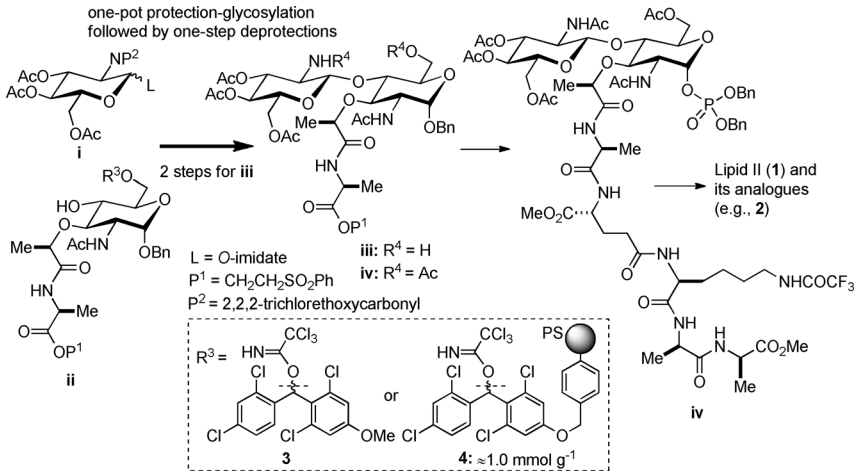
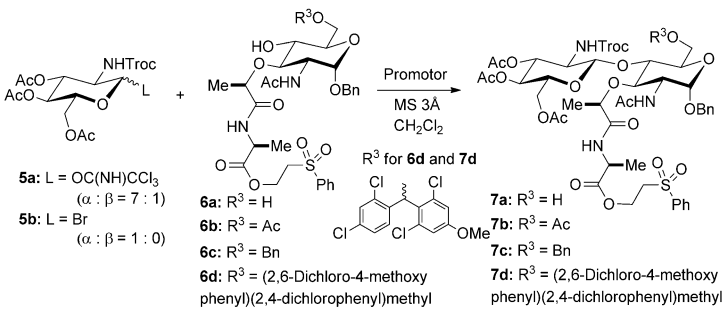


Figure 2. One-pot protection–glycosylation to synthesize GlcNAc-MurNAc-peptide **iv** for lipid II.

In our studies, glycosylation of the C6-acetylated MurNAc derivative **6a**[5a] with the glycosyl imidate **5a** did not yield the desired product **7a** (e.g., Entry 1 in Table 1). Because the *N*-Troc-protected imidate **5a** is one of the convenient GlcNAc sources in chemical glycosylations, we have sought an appropriate C6-ether-protected MurNAc to efficiently synthesize the lipid II disaccharide **iv** (Figure 2).[6] The C6-benzyl ether-protected MurNAc **6b** was introduced for Königs–Knorr type glycosylation of **5b** by Saha et al.[7] The AgOTf-catalysed glycosylation of **6c** with **5b** was performed in our laboratory; however, these reactions resulted in the formation of the desired β-linked disaccharide **7c** in moderate yield (Entry 5 in Table 1). The same glycosyl acceptor **6c** was applicable to the glycosylation with the imidate **5a**; TMSOTf-catalyzed glycosylation of **6c** with **5a** furnished **7c** in a moderate yield of 50% (Entry 2 in Table 1, TMS = trimethylsilyl).[8] Although synthesis of the C6-benzyl-protected glycosyl acceptor **6b** from the corresponding diol **ii** (R^3 = H in Figure 2) and selective debenzylation of the primary benzyl ether **7b** after the glycosylation were reported, in our hands, each step for these transformations provided the undesired diastereomers that require time-consuming chromatography, and/or the yield of each step was 45–65%.[7] We have previously reported that primary alcohols can be protected selectively with (2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methyl trichloroacetimidates [**3**, monomethoxydiphenylmethyl acetimidate (MDPM-imidate)] to afford the corresponding ethers in good to excellent yields at controlled temperatures.[9] The MDPM-ether protecting group of **6d** was stable under Schmidt glycosylation conditions for glycosyl trichloroacetimidate; TMSOTf-catalyzed glycosylation of **6d** with **5a** gave rise to the β-glycoside **7d** as a mixture of two inseparable diastereomers in 65% yield in 3 h (Entry 3 in Table 1). The isolation yield of the same glycosylation reaction of **6d** with **5a** was increased by 20% using $BF_3{\cdot}OEt_2$ (Entry 4 in Table 1).

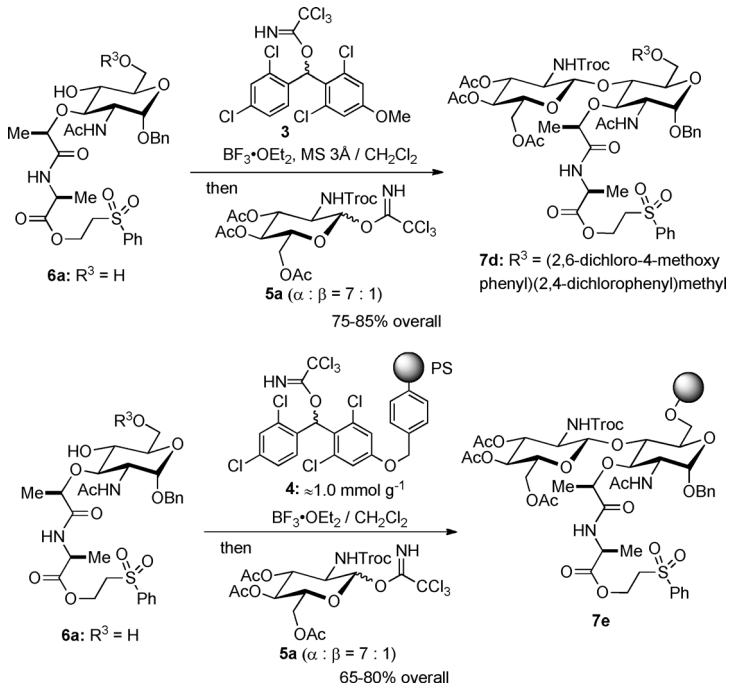
MDPM-imidate **3** reacted against only the primary alcohol of **6a** even at room temperatures with near quantitative yield, and the by-product, 2,2,2-trichloroacetamide, is an innocent species in Schmidt glycosylations. Thus, a one-pot protection–glycosylation protocol was envisioned for the synthesis of **7d** directly from the diol **6a** (Figure 2). As expected, the desired lipid II disaccharide derivative **7d** could be synthesized from

Table 1. Synthesis of lipid II disaccharide **7**.[a]



Entry	Donor	Acceptor	Promoter	Temp [°C]	Time [h]	Product	Yield [%]
1	**5a**	**6b**	TMSOTf	0	12	**7b**	0
2	**5a**	**6c**	TMSOTf	0	3	**7c**	50
3	**5a**	**6d**	TMSOTf	0	3	**7d**	65
4	**5a**	**6d**	$BF_3{\cdot}OEt_2$	0	3	**7d**	85
5	**5a**	**6c**	AgOTf	0	3	**7c**	45[b]

[a] 2.5 Equivalents of glycosyl donor were used. [b] The reported isolated yield is 74%.

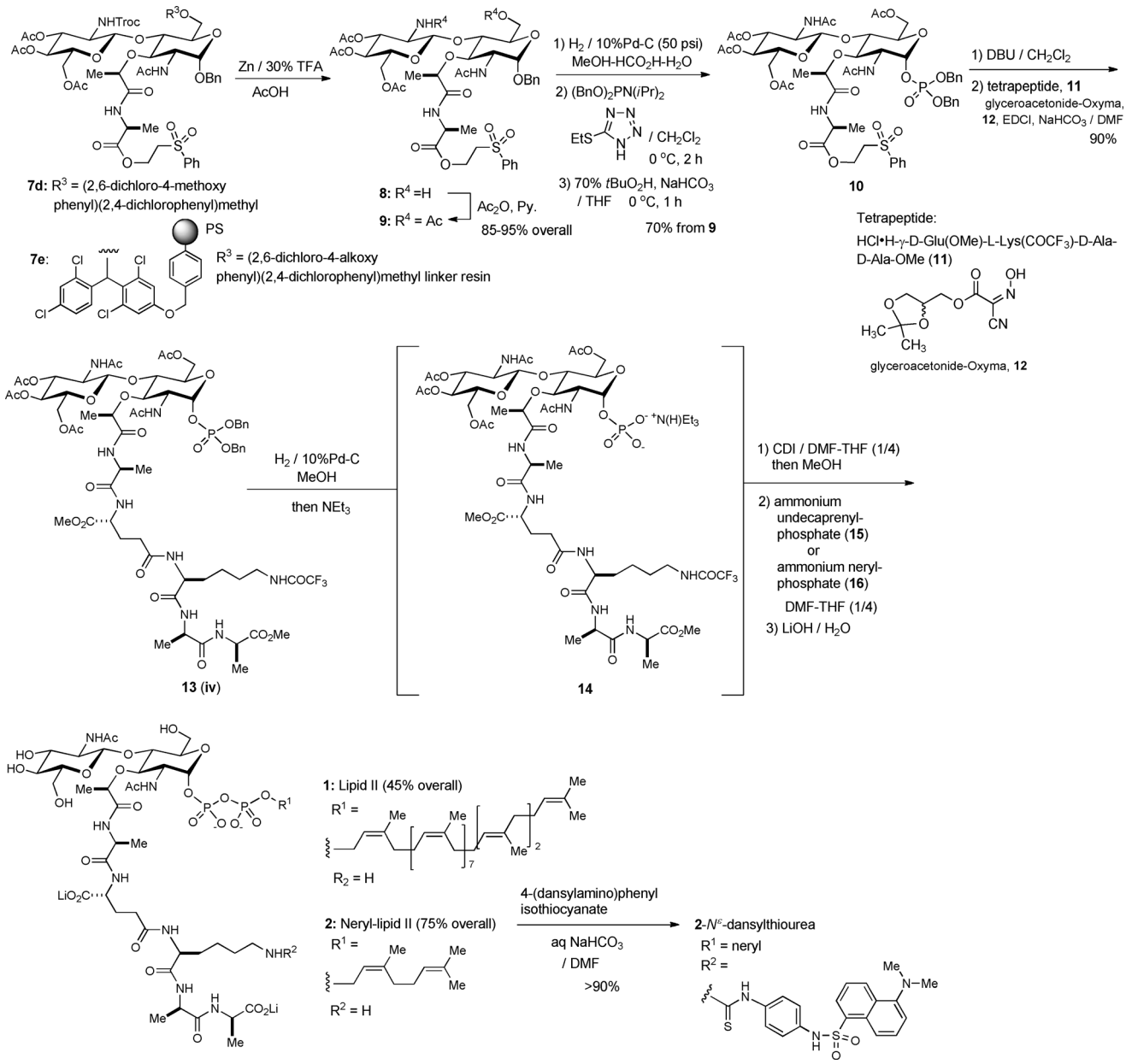


Scheme 1. One-pot protection–glycosylation to synthesize GlcNAc-MurNAc-peptide **7**. PS = polystyrene, MS = molecular sieves.

6a in 75–85% yield in a one-pot two-step strategy (Scheme 1). It is worth mentioning that the (2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methyl ether protecting group possesses a characteristic UV absorption, and isolation of the disaccharide **7d** from the crude reaction mixture with chromatography was relatively simple compared to **7c** (Entry 2 in Table 1).

In order to further facilitate the synthesis of the key intermediate **iv** in Figure 2, (2,6-dichloro-4-alkoxyphenyl)(2,4-dichlorophenyl)methyl trichloroacetimidate linker resin **4** was applied to a one-pot two-step strategy for the synthesis of **iii** (Figure 2).[10] Loading of the diol **6a** onto the linker resin **4** was completed with $BF_3{\cdot}OEt_2$ (5 equivalents) at room temperature in 1 h; in this step, progress of the reaction was monitored by measuring the consumption of **6a** with LC-MS. Once the loading step was completed, the imidate **5a** was added into the reaction mixture to afford the desired β-glycoside resin **7e** in 65–80% yield which was determined based on the isolated **7a** (Table 1) after the cleavage of **7e** with 30% trifluoroacetic acid (TFA) in CH_2Cl_2 for 1 h. Accordingly, convenient synthetic procedures for **7d** and **7e** for the syntheses of lipid II analogues were accomplished. The other convenient feature of (2,6-dichloro-4-alkoxyphenyl)(2,4-dichlorophenyl)methyl ether protecting group and linker is that they can be deprotected or cleaved simultaneously when the *N*-Troc group is removed under reductive conditions. The *N*-Troc and C6-ether protecting group or linker of **7d** or **7e** were deprotected with Zn in 30% TFA-AcOH to furnish the amino alcohol **8** (Scheme 2). Acetylations of the free amine and alcohol of **8** afforded **9** in 85–90% yield from **7d** or **7e**. α-Phosphorylation and diphosphate ester formation of **9** were carried out using established protocols with minor modifications. Deprotection of the anomeric Bn protecting group was performed by Pd-C catalyzed hydrogenation reaction to afford a mixture of α/β-anomers, which were subjected to α-selective phosphite formation using dibenzyl *N,N*-diisopropylphosphoramidite and 5-(ethylthio)-1*H*-tetrazole. The generated α-phosphite intermediate was oxidized with $tBuO_2H$ to afford the α-phosphate **10** in 70% overall yield for three steps. Deprotection of the 2-(phenylsulfonyl)ethanol protecting group of **10** was achieved by treatment with 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) to furnish the α-phosphoryl GlcNAc-MurNAc-monopeptide derivative. The tetrapeptide, HCl·H-L-Ala-γ-D-Glu(OMe)-L-Lys($COCF_3$)-D-Ala-D-Ala-OMe (**11**) was synthesized in water media with water-soluble reagents (glyceroacetonide-Oxyma (**12**), EDCI, and $NaHCO_3$; see Supporting Information).[11] Coupling of the free carboxylic acid α-phosphoryl GlcNAc-MurNAc-monopeptide with the tetrapeptide **11** under mild conditions (**12**, EDCI, and $NaHCO_3$) in H_2O yielded the α-phosphoryl GlcNAc-MurNAc-pentapeptide **13** in over 90% overall yield from **10**. Conveniently, all reagents and excess tetrapeptide used in this step could be removed with a basic water work-up. Hydrogenolytic debenzylations of **10** followed by the treatment with Et_3N resulted in the corresponding monotriethylammonium phosphate **14** in quantitative yield, whose structure was established by ^1H NMR analysis. Triethylammonium phosphate **14** was then applied to a carbonyldiimidazole (CDI) promoted diphosphate-formation reaction.[4e,f] Triethylammonium α-phosphoryl GlcNAc-MurNAc-pentapeptide **14** was first activated with CDI and the excess CDI was quenched with MeOH to afford 1*H*-imidazole-1-carboxylic (phosphoric) anhydride and methyl 1*H*-imidazole-1-carboxylate, which was not reactive against the phosphate nucleophiles. All volatiles were extensively removed and the resulting mixture was subjected to the cross-coupling reaction with the ammonium undecaprenyl phosphate (**15**) or neryl phosphate (**16**). Progress in the coupling reactions was monitored with reverse-phase HPLC (0.05 M NH_4HCO_3:MeOH to MeOH for lipid II). The reaction mixture was lyophilized and the fully protected product was subjected to global deprotection reactions with aq. LiOH. Lipid II (**1**) was synthesized in 45% overall yield from **13** after purification with reverse-phase HPLC. The structure of **1** was confirmed by ^1H NMR, negative ESI-TOF-MS spectroscopy, and retention time in HPLC analysis.[4e] Similarly, neryl-lipid II analogue (**2**) could be synthesized in 75% overall yield by using excess ammonium neryl phosphate (**16**). The dansyl group was conjugated to the N^{ε}-lysine moiety of **2** with 4-(dansylamino)-phenyl isothiocyanate to furnish neryl-lipid II N^{ε}-dansylthiourea, **2**-N^{ε}-dansylthiourea, in greater than 90% yield.

In conclusion, chemical syntheses of lipid II and neryl-lipid II analogues were accomplished with one-pot protection–glycosylation protocols. (2,6-Dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methyl trichloroacetimidate (**3**) and its linker resin **4** were demonstrated to be useful temporary protecting groups for the primary alcohol of the diol **6a** that could be compatible



Scheme 2. Synthesis of lipid II and neryl lipid II analogues.

with Schmidt glycosylation reactions. In addition, the (2,6-dichloro-4-alkoxyphenyl)(2,4-dichlorophenyl)methyl ether linkage could be deprotected simultaneously with the deprotection of *N*-Troc groups. Accordingly, the synthetic intermediate **10** for lipid II was efficiently synthesized from **6a** in 45–54% overall yield with a minimum number of chemical steps. Gram quantities of the tetrapeptide building block **11** can readily be synthesized in water, and the peptide-forming reagents used for the synthesis of **13** could be removed with simple water workups. Detailed experimental procedures for improved syntheses of lipid II and its neryl analogues are summarized in the Supporting Information. As mentioned in the introduction, neryl lipid II-N^{ε}-dansylthiourea, **2**-N^{ε}-dansylthiourea is a very useful biochemical tool for studying transglycosylase (TGase). Characterization of polymerized **2**-N^{ε}-dansylthiourea by TGase and development of high-throughput screening (HTS) against TGase will be reported elsewhere.

Experimental Section

One-pot protection and glycosylation reaction of 6a

To a stirred suspension of **6a** (0.20 g, 0.32 mmol), (2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methyl trichloroacetimidate (**3**, 0.29 g, 0.57 mmol), and MS 3 Å (0.5 g) in CH_2Cl_2 (2.5 mL) was added BF_3·OEt_2 (0.5 mmol) at 0 °C. After 1 h, the imidate **5a** (0.34 g, 0.54 mmol) in dichloromethane (1.0 mL) was added. The reaction mixture was stirred for 3 h at 0 °C, and quenched with sat. aq $NaHCO_3$ solution. The resulting suspension was filtered through celite. The aqueous layer was extracted with ethyl acetate and the combined organic layer was dried over Na_2SO_4, filtered, and evapo-

rated. Purification by silica gel column chromatography (hexanes/ethyl acetate = 20/80) afforded **7d** (0.39 g, 85%).

(2,6-Dichloro-4-alkoxyphenyl)(2,4-dichlorophenyl)methyl trichloroacetimidate linker resin 4

(2,6-Dichloro-4-alkoxyphenyl)(2,4-dichlorophenyl)methanol linker resin was prepared according to the procedure reported previously. The resin (5 g, active surface: $\approx$1.0 mmol g^{-1}) was suspended in CH_2Cl_2 (10 mL) and CCl_3CN (5 mmol) and DBU (1 mmol). The suspension was gently stirred for 3 h. The polymer resins were washed with THF/*i*PrOH (3/1), THF, and THF/CH_2Cl_2 (2/1), and dried under high vacuum to furnish **4** (5.72 g, quantitative yield).

One-pot loading and glycosylation of 6a

To a stirred suspension of **6a** (0.20 g, 0.32 mmol) and (2,6-dichloro-4-alkoxyphenyl)(2,4-dichlorophenyl)methyl trichloroacetimidate linker resin **4** (1.6 g, $\approx$1.0 mmol g^{-1}) in CH_2Cl_2 (5.0 mL) was added $BF_3{\cdot}OEt_2$ (0.64 mmol) at RT. After 1 h, the imidate **5a** (0.596 g, 0.96 mmol) in dichloromethane (2.0 mL) was added at 0 °C. The reaction mixture was gently stirred for 3 h at 0 °C, and the resins were washed with THF/*i*PrOH (3/1), THF, and THF/CH_2Cl_2 (2/1), and dried under high vacuum to furnish **7e** (0.25 g). In order to determine the overall yield, the disaccharide resin **7e** was cleaved with 30% TFA for 1 h and the generated **7a** was quantified with LC-MS (65–80% yield).

Deprotections of *N*-Troc and (2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methyl ether groups and acetylations

To a stirred solution of **7d** (0.35 g, 0.25 mmol) in 30% TFA/AcOH (3.5 mL) was added zinc powder (0.16 g, 2.5 mmol). After stirring the solution for 6 h at RT, the reaction mixture was filtered and all volatiles were evaporated to afford the crude **8**. To a solution of **8** in pyridine (Py, 2.0 mL) was added Ac_2O (2.0 mL). After 4 h at RT, all volatiles were removed to afford the crude product. Purified by silica gel column chromatography (ethyl acetate/methanol = 95/5) to afford **9** (0.24 g, 95% overall yield). Similarly, the disaccharide resin **7e** was converted to **9** (85% overall yield).

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Keywords: biosynthesis · lipid II · one-pot synthesis · peptidoglycans · transglycosylase (TGase)

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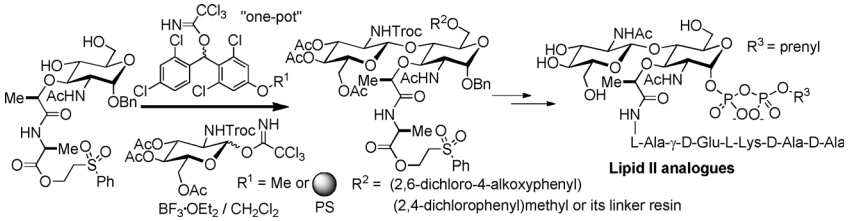

Sweet synthetic methods: A one-pot protection glycosylation reaction of the diol glycosyl acceptor is developed for synthesis of the lipid II disaccharide (see figure, Troc = 2,2,2-trichloroethoxycarbonyl). Improved syntheses of lipid II and neryl-lipid II analogues are summarized.