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Total Synthesis of Polyprenyl *N*-Glycolyl Lipid II as a Mycobacterial Transglycosylase Substrate

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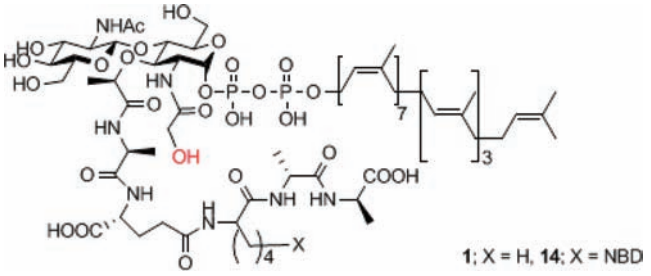
ABSTRACT



A feasible synthetic approach toward the *Mycobacterium tuberculosis* (*Mtb*) *N*-glycolyl lipid II-like molecule 1 is described. Compound 1 bears pendant undecaprenol and L-lysin moieties instead of the naturally occurring decaprenol and meso-diaminopimelic acid, which are not readily available. Functionalization of 1 with a fluorophore on the peptide side chain gave 14, which was found to be recognized as an *Mtb* TGase substrate. This result suggests it has tremendous utility for mechanistic studies, the characterization of mycobacterial enzymes, and mycobacterial TGase inhibitor evaluation.

Nearly two million people die annually from tuberculosis, an infectious disease caused by *Mycobacterium tuberculosis*.[1] Recently the rise of antibiotic-resistant infections caused by MDR- and XDR-TB (multidrug resistant and extensively drug-resistant *tuberculosis*) has been recognized as a serious public health threat,[2] and new antibiotics or new strategies to tackle this problem are urgently required. One class of possible antibiotic targets are the enzymes responsible for the assembly of mycobacterial cell walls. For example, penicillin-binding protein (PBP) PonA possesses two catalytic domains for transpeptidase and transglycosylase activities, and it plays an important role for mycobacterial cell wall biosynthesis.[3,4]

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(1) (a) Chopra, P.; Meena, L. S.; Singh, Y. *Indian J. Med. Res.* **2003**, *117*, 1–9. (b) Johnson, R.; Streicher, E. M.; Louw, G. E.; Warren, R. M.; Helden, P. D. v.; Victor, T. C. *Curr. Issues Mol. Biol.* **2006**, *8*, 97–112. (c) Cole, S. T.; Brosch, R.; Parkhill, J.; Garnier, T.; Churcher, C.; Harris, D.; Gordon, S. V.; Eiglmeier, K.; Gas, S.; Barry, C. E.; Tekaia, F.; Badcock, K.; Basham, D.; Brown, D.; Chillingworth, T.; Connor, R.; Davies, R.; Devlin, K.; Feltwell, T.; Gentles, S.; Hamlin, N.; Holroyd, S.; Hornsby, T.; Jagels, K.; Krogh, A.; McLean, J.; Moule, S.; Murphy, L.; Oliver, K.; Osborne, J.; Quail, M. A.; Rajandream, M. A.; Rogers, J.; Rutter, S.; Seeger, K.; Skelton, J.; Squares, R.; Squares, S.; Sulston, J. E.; Taylor, K.; Whitehead, S.; Barrell, B. G. *Nature* **1998**, *393*, 537–544.

(2) Patru, M.-M.; Pavelka, M. S., Jr. *J. Bacteriol.* **2010**, *192*, 3043–3054.

(3) (a) Hett, E. C.; Chao, M. C.; Rubin, E. J. *PLoS Pathog.* **2010**, *6*, e1001020. (b) Bhakta, S.; Basu, J. *Biochem. J.* **2002**, *361*, 635–639. (c) Lepage, S.; Dubois, P.; Ghosh, T.; Joris, B.; Mahapatra, S.; Kundu, M.; Basu, J.; Chakrabarti, P.; Cole, S.; Nguyen-Disteche, M.; Ghuysen, J. *J. Bacteriol.* **1997**, *179*, 4627–4630.

(4) Goffin, C.; Ghuysen, J.-M. *Microbiol. Mol. Biol. Rev.* **2002**, *66*, 702–738.

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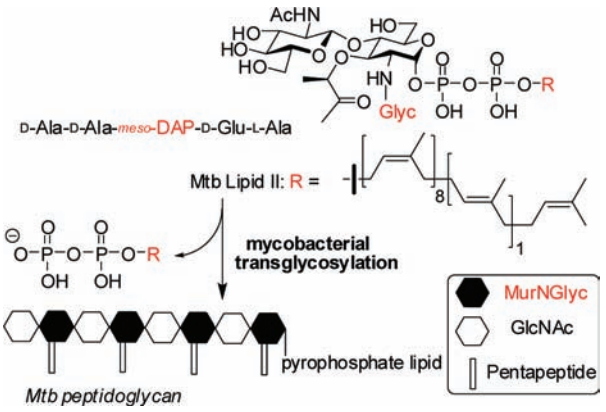
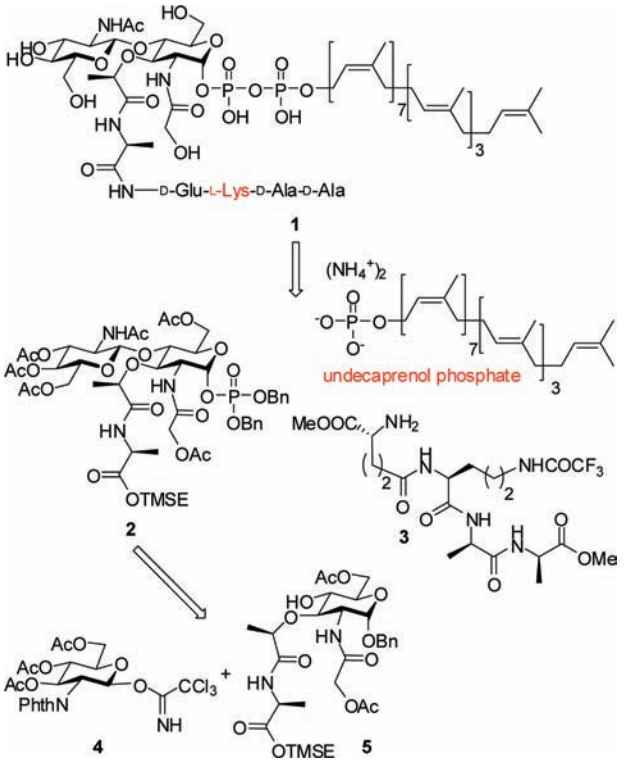


Figure 1. Mycobacterial Lipid II as the substrate in transglycosylation for mycobacterial peptidoglycan formation.

The transpeptidase responsible for the cross-linking of peptidoglycans is a target for existing antibiotics.[4,5] Although the transglycosylase (TGase) is also a potential target, no antibiotics have yet been developed.[6,7] Mycobacterial TGase catalyzes the polymerization of Lipid II to form peptidoglycan (Figure 1).[8,9] During transglycosylation, the sugar moiety from the activated polymeric peptidoglycan (a glycosyl donor) is linked to the specific hydroxyl group (4-OH) of Lipid II (a glycosyl acceptor) with associated release of a decaprenyl pyrophosphate (Figure 1). Since TGase is located on the external surface of bacterial membranes, it allows easy access to inhibitors and, because of the lack of a eukaryotic counterpart, thus eliminates the risk of serious side effects. As such, it is thought an attractive target for antibiotic discovery and development.[8] Unfortunately, the study of mycobacterial transglycosylation for drug discovery has been hampered by the difficulty in acquiring *N*-glycolyl Lipid II from natural sources.[10]

Structually, *Mtb* *N*-glycolyl Lipid II comprises the disaccharide of *N*-acetylglucosamine (GlcNAc) and *N*-glycolylmuramic acid (MurNGlyc), pyrophosphate, decaprenol lipid tail, and the pentapeptide moiety (L-Ala-D-Glu-*meso*-DAP-D-Ala-D-Ala (Figure 1).[9−11] Notably, the *N*-glycolyl muramic acid is a special component and only observed in

Scheme 1. Retrosynthetic Analysis of Mycobacterial *N*-Glycolyl Lipid II-like Molecule (**1**)



mycobacterial cell walls, and considered a potential biomarker. The *N*-glycolyl groups in peptidoglycan chains may play an impotant role in the resistance to lysozyme and in the innate immune response during a mycobacterial infection.[12]

Currently, only *Mtb* Park's nucleotide, a peptidoglycan precursor, has been synthesized by Kurosu and coworkers.[13] To the best of our knowledge, chemical syntheses of *N*-glycolyl Lipid II and its analogues have not been explored. Herein, we describe the first synthesis of a *N*-glycolyl Lipid II-like molecule, GlcNAcMurNglyc-(L-Ala-D-Glu-L-Lys-D-Ala-D-Ala)-undecaprenyl phosphate (**1**). The decaprenyl phosphate and *meso*-diaminopimelic acid (*meso*-DAP) moieties in the original *Mtb* *N*-glycolyl Lipid

(5) (a) Chambers, H.; Moreau, D.; Yajko, D.; Miick, C.; Wagner, C.; Hackbarth, C.; Kocagoz, S.; Rosenberg, E.; Hadley, W.; Nikaido, H. *Antimicrob. Agents Chemother.* **1995**, *39*, 2620–2624. (b) Anishetty, S.; Pulimi, M.; Pennathur, G. *Comput. Biol. Chem.* **2005**, *29*, 368–378.

(6) Barry, C. E.; Crick, D. C.; McNeil, M. R. *Infect. Disord.: Drug Targets* **2007**, *7*, 182–202.

(7) Vollmer, W.; Holtje, J.-V. *Antimicrob. Agents Chemother.* **2000**, *44*, 1181–1185.

(8) (a) Ye, X.-Y.; Lo, M.-C.; Brunner, L.; Walker, D.; Kahne, D.; Walker, S. *J. Am. Chem. Soc.* **2001**, *123*, 3155–3156. (b) Halliday, J.; McKeveney, D.; Muldoon, C.; Rajaratnam, P.; Meutermans, W. *Biochem. Pharmacol.* **2006**, *71*, 957–967.

(9) Crick, D. C.; Mahapatra, S.; Brennan, P. J. *Glycobiology*. **2001**, *11*, 107R–118R.

(10) Mahapatra, S.; Yagi, T.; Belisle, J. T.; Espinosa, B. J.; Hill, P. J.; McNeil, M. R.; Brennan, P. J.; Crick, D. C. *J. Bacteriol.* **2005**, *187*, 2747–2757.

(11) (a) Kaur, D.; Brennan, P. J.; Crick, D. C. *J. Bacteriol.* **2004**, *186*, 7564–7570. (b) Vollmer, W. *FEMS Microbiol. Rev.* **2008**, *32*, 287–306.

(12) (a) Raymond, J. B.; Mahapatra, S.; Crick, D. C.; Pavelka, M. S. *J. Biol. Chem.* **2005**, *280*, 326–333. (b) Daryaee, F.; Kobarfard, F.; Khalaj, A.; Farnia, P. *Eur. J. Med. Chem.* **2009**, *44*, 289–295.

(13) Li, K.; Kurosu, M. *Heterocycles*. **2008**, *76*, 455–469.

(14) (a) Khidyrova, N. K.; Shakhidoyatov, K. M. *Chem. Nat. Compd.* **2002**, *38*, 107–121. (b) Ruiz-Rodriguez, A.; Bronze, M.-R.; Ponte, M. N. d. *J. Supercrit. Fluids* **2008**, *45*, 171–176.

(15) Schwartz, B.; Markwalder, J. A.; Wang, Y. *J. Am. Chem. Soc.* **2001**, *123*, 11638–11643.

(16) (a) Liu, C.-Y.; Guo, C.-W.; Chang, Y.-F.; Wang, J.-T.; Shih, H.-W.; Hsu, Y.-F.; Chen, C.-W.; Chen, S.-K.; Wang, Y.-C.; Cheng, T.-J. R.; Ma, C.; Wong, C.-H.; Fang, J.-M.; Cheng, W.-C. *Org. Lett.* **2010**, *12*, 1608–1611. (b) Stachyra, T.; Dini, C.; Ferrari, P.; Bouhss, A.; van Heijenoort, J.; Mengin-Lecreulx, D.; Blanot, D.; Biton, J.; Le Beller, D. *Antimicrob. Agents Chemother.* **2004**, *48*, 897–902.

(17) VanNieuwenhze, M. S.; Mauldin, S. C.; Zia-Ebrahimi, M.; Winger, B. E.; Hornback, W. J.; Saha, S. L.; Aikins, J. A.; Blaszczak, L. C. *J. Am. Chem. Soc.* **2002**, *124*, 3656–3660.

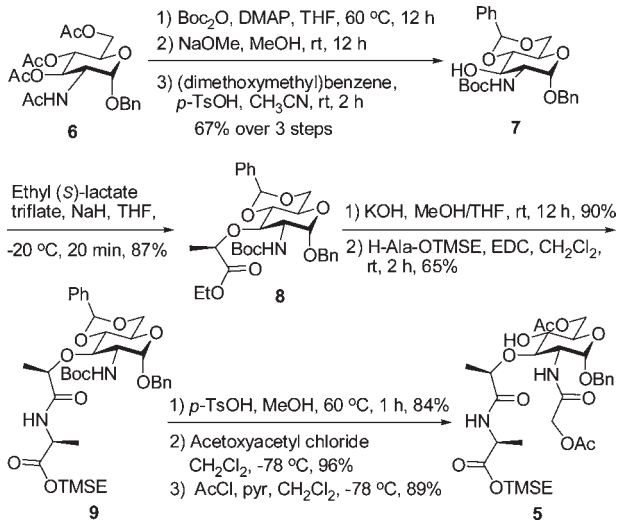
(18) Benakli, K.; Zha, C.; Kerns, R. J. *J. Am. Chem. Soc.* **2001**, *123*, 9461–9462.

II were substituted with two much more readily available alternatives, undecaprenyl phosphate and L-lysine, respectively.[14] The *N*-glycolyl Lipid II-like molecule (**1**) was subsequently converted to a fluorescent probe in order to study whether or not this structurally modified probe could be recognized as an *Mtb* TGase substrate.[15,16]

Our synthetic strategy toward **1** is illustrated in Scheme 1. We planned to assemble three proposed fragments, including an undecaprenol phosphate, disaccharide **2**, and tetrapeptide **3**, to obtain the target molecule. To circumvent the problems associated with handling an acid labile α-glycosyl diphosphate group, construction of the diphosphate moiety was proposed last, following conjugation of an undecaprenol phosphate and a phosphoryl disaccharide, in turn, prepared from tetrapeptide **3** and disaccharide **2**. Notably, the key intermediate **2** could be prepared from glycosyl donor **4** and acceptor **5**.[17]

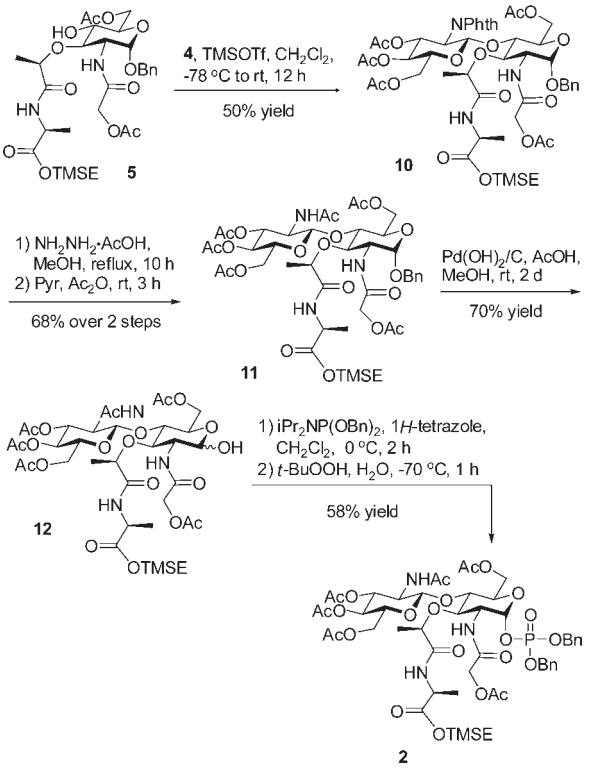
We started with preparation of the intermediate, *N*-glycolyl muramic acid **5**. *N*-Boc protection of **6**, prepared from D-glucosamine,[18] followed by the removal of the *N*-acetyl and *O*-acetyl moieties under Zemplen conditions[19] (cat. One M NaOMe solution in MeOH) and regioselective benzylidene acetalization resulted in **7** in a yield of 67% over three steps (Scheme 2).[18] Attempts to undergo *O*-alkylation at the C3-hydroxy position in **7** with *S*-(−)-2-chloropropionic acid and NaH at 60 °C were unsuccessful because the *N*-Boc moiety was unexpectedly deprotected and many unidentified side products were observed (see Supporting Information, Table S1). After examination of various reaction conditions, saccharide **8** was finally obtained in a yield of 87% by using methyl (*S*)-lactate triflate[13] as the electrophile. Hydrolysis of **8**, followed by

Scheme 2. Synthesis of Monosaccharide **5**



conjugation with H-Ala-OTMSE, deprotection of both benzylidene acetal and *N*-Boc moieties, *N*-acetylation with acetoxyacetyl chloride at the C2-amino group, and selective *O*-acetylation at the C6-hydroxy group, gave the key intermediate **5** in 42% overall yield over five steps (Scheme 2).

Scheme 3. Synthesis of Disaccharide **2**



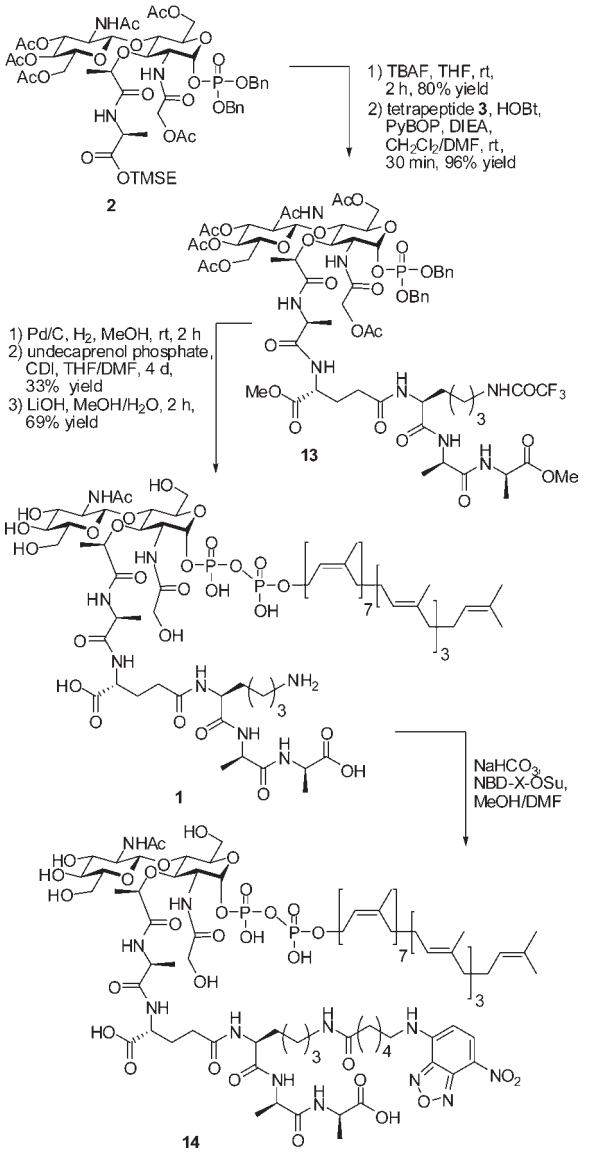
Glycosylation between donor **4** and acceptor **5** mediated by TMSOTf afforded the corresponding disaccharide **10** in 50% yield (Scheme 3). The β-linkage of the newly formed glycosidic bond was confirmed by ^{1}H NMR spectroscopy. Debenzylation of **11** by catalytic hydrogenation ($Pd(OH)_2/C$, H_2, 1 atm) was not successful, and only **11** was observed (see Supporting Information, Table S2). Fortunately, use of high pressure hydrogen (4.5 bar) in the presence of a catalytic amount of acetic acid gave lactol **12** in a reasonable yield (70%). Phosphorylation of **12** was carried out in a phosphitylation/oxidation sequence to deliver phosphoryldiester **2** as a single diastereomer. The α-configuration of **2** was confirmed by ^{1}H NMR spectroscopy (Scheme 3).

Selective deprotection of the OTMSE group in **2** by treatment with TBAF in THF, followed by coupling with the tetrapeptide **3** (D-Glu-L-Lys-D-Ala-D-Ala), gave disaccharyl pentapeptide **13** in 77% overall yield in two steps (Scheme 4). Finally, debenzylation of **13**, followed by conjugation with an undecprenyl phosphate, and global deprotection under basic conditions gave the desired target **1** in a yield of 21% over two steps. A novel fluorescent probe **14** was prepared from **1** by attaching a

(19) Chen, Q.; Cheng, Q. Y.; Zhao, Y. C.; Han, B.-H. *Macromol. Rapid Commun.* **2009**, *30*, 1651–1655.

(20) de Kruijff, B.; van Dam, V.; Breukink, E. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **2008**, *79*, 117–21.

Scheme 4. Synthesis of *Mtb N*-Glycolyl Lipid II-like Moelcule (**1**) and Its Fluorescent Probe (**14**)



nitrobenzoxadiazole (NBD) fluorophore at the terminal ε-NH_2 site of lysine on the peptide side chain.

The feasibility of **14** as a TGase substrate was examined in our HPLC-based functional enzyme assay system.[16a] To our delight, **14** (2.5 μM) was almost completely consumed (98%) in 4 h during the transglycosylation reaction catalyzed by *Mtb* PonA (10 nM) (Figure 2). This observation indicates that the undecaprenol and lysine moieties do not dramatically attenuate the substrate efficiency toward enzymes. Next, moenomycin A, a known bacterial TGase inhibitor,[20] was used to test the inhibitory activity of *Mtb* Pon A in this mycobacterial assay system. Around 87% of this enzyme reaction progress was blocked in the presence of 1 μM of moenomycin A (see Supporting Information, Figure S2).[7,21]

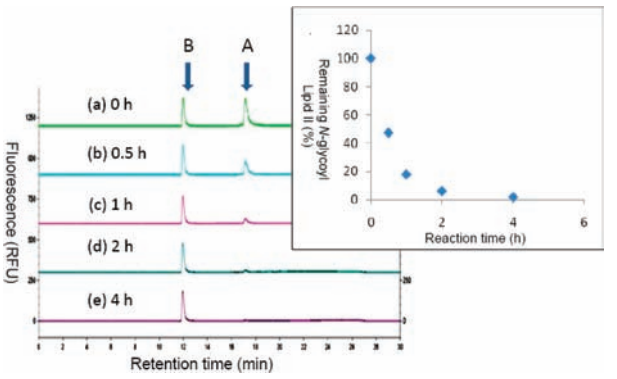


Figure 2. Measurement of transglycosylation progress by HPLC. The reactions (a–e) are at t = 0, 0.5, 1, 2, and 4 h. Peaks A and B represent **14** and an internal standard, respectively.

In summary, synthetic protocols for the preparation of an *Mtb N*-glycolyl lipid II-based analogue and its fluorescent probe have been successfully developed and several transformations, such as **7** to **8** and **11** to **12**, have been optimized.[22] This convergent methodology can be potentially scaled up, and the structure of target molecules can be modified easily. Preliminary studies show this probe to be an *Mtb* TGase substrate and, therefore, suggest it has tremendous utility for mechanistic studies, the characterization of mycobacterial enzymes, and mycobacterial TGase inhibitor evaluation.

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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.

(21) Shih, H.-W.; Chen, K.-T.; Chen, S.-K.; Huang, C.-Y.; Cheng, T.-J. R.; Ma, C.; Wong, C.-H.; Cheng, W.-C. *Org. Biomol. Chem.* **2010**, *8*, 2586–2593.

(22) The studies of transformations are in the Supporting Information.