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Synthesis of an Orthogonally Protected Precursor to the Glycan Repeating Unit of the Bacterial Cell Wall

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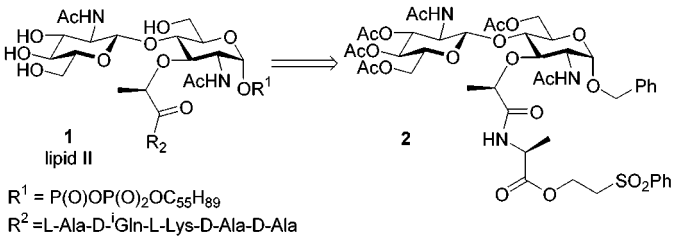
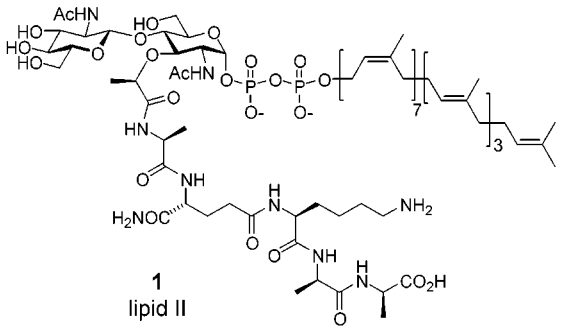
ABSTRACT



A synthesis of stereochemically pure and orthogonally protected *N*-acetyl-(2-deoxy-2-aminoglucopyranosyl)-*β*-[1,4]-*N*-acetylmuramyl (NAG-NAM) monopeptide (2), the glycopeptide core of lipid II and of the bacterial cell wall repeating unit, is reported.

Lipid II[1] (**1**) is the ultimate monomeric intermediate of bacterial cell wall (peptidoglycan) biosynthesis.[2] To fuel our synthetic efforts toward lipid II, we required an efficient and dependable synthetic route to orthogonally protected disaccharide **2**. Having successfully addressed this central issue, we report here a practical synthesis of an orthogonally protected version of the core NAG-NAM disaccharide **2**; the key starting material for our projected total synthesis of lipid II.

Formation of the desired *β*-[1,4]-glycosidic bond requires a 2-deoxy-2-aminoglucopyranose glycosyl donor with a nitrogen substituent that will favor equatorial approach[3] of the very modestly nucleophilic C(4) hydroxyl group of a muramic acid derivative.[4] Several approaches to this formidable glycosidation problem have been recorded,[5] but none is sufficiently effective in all regards for our purpose: preparation of **2** in quantities to enable the first total synthesis of lipid II.



The overall synthetic challenge of lipid II required protection and activation chemistries for utilization in several nested applications: (1) the functional group activation

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(1) This version of lipid II is common to most Gram-positive bacteria. In Gram-negative bacteria, the lysine residue is replaced by *meso*-diaminopimelic acid (see ref 2).

(2) For a comprehensive review of bacterial cell wall biosynthesis, see: Ghuysen, J.-M.; Hackenbeck, R. *Bacterial Cell Wall*; Elsevier Biomedical: Amsterdam, 1994.

(3) Jeanloz, R. W. *The Amino Sugars: The Chemistry and Biology of Compounds Containing Amino Sugars*; Academic Press: New York, 1969.

(4) The C(4) hydroxyl group is reported to be the least nucleophilic hydroxyl group in glucopyranose-based acceptors. See, for example: Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503.

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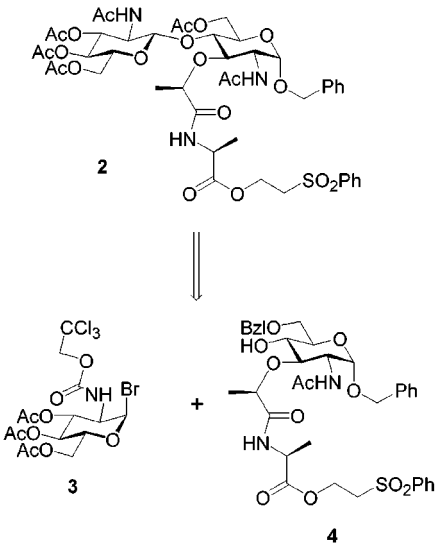
scheme required for the glycosidation reaction needed to be changed to a functional group protection scheme at the disaccharide stage, (2) triple orthogonality of disaccharide protecting groups (anomeric OH, peripheral OH, and carboxyl OH) was required to ensure staging flexibility in exploratory transformations for the lipid II endgame, (3) a protection scheme to preserve global deprotection by hydroxide ion as the final step to lipid II, and (4) minimizing the number of functional group interchange reactions for overall synthetic efficiency.

Our synthetic strategy (Scheme 1) dictated the use of benzyl protection for the anomeric hydroxyl of glycosyl

Scheme 1. Retrosynthetic Analysis of the Lipid II Glycopeptide Core

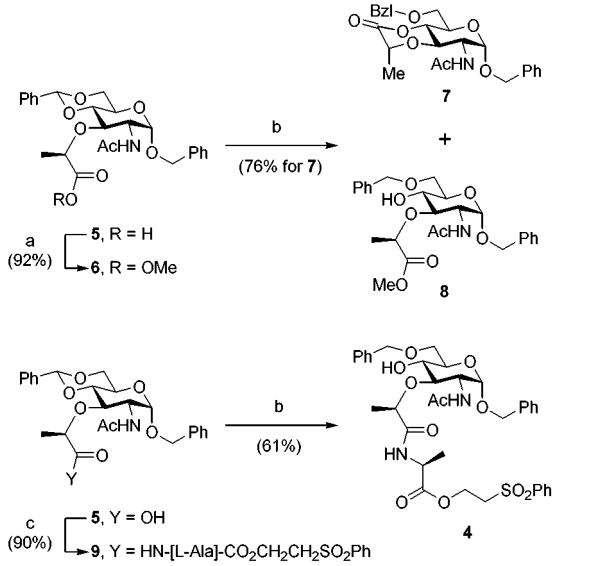


acceptor **4**. Literature precedent[5] reinforced by our own experience suggested that the acceptor would also require an electron-donating protective group at C(6) for activation in the glycosidation reaction; benzyl was selected for stability and ease of selective introduction. Of the several options available for orthogonal protection of the C(3) lactate ester, we selected phenylsulfonylethyl on the basis of its successful performance in the synthesis of Park nucleotide.[6]

With respect to design of glycosyl donor **3**, Wong and co-workers[7] recently measured relative rates of glycosidic bond formation using various 2-amino-2-deoxyglucopyranose donors (inter alia). Since they noted a 40-fold relative reactivity increase of trichloroethoxycarbonyl[8] (Troc) over phthaloyl, we chose to employ the former for protection of the 2-amino substituent of our glycosyl donor.

Our synthesis began with a study to assess the reductive opening of benzyl *N*-acetyl-4,6-benzylidinemuramic ester **6** as an efficient means for regioselective introduction of benzyl protection/activation at the C(6) hydroxyl of the muramic acid starting material (Scheme 2).

Scheme 2. Reductive Opening of the 4,6-*O*-Benzylidene Acetal[a]



[a] Reagents and conditions: (a) MeI, Cs_2CO_3, MeCN; (b) Et_3SiH (3 equiv), TFA (6 equiv), CH_2Cl_2, 0 °C, 5 h; (c) *N*-methylmorpholine, 2-chloro-4,6-dimethoxy-1,3,5-triazine, CH_2Cl_2, 2 h.

When **6**[9] was treated with trifluoroacetic acid and triethylsilane under the reported[10] conditions, 76% of lactone **7**[5i] was isolated along with a small amount of the desired **8**. The simple expedient of installing L-alanine, the first amino acid residue along the muramyl peptide chain, completely suppressed acid-catalyzed cyclization of the reduction product. Thus, subjecting **9** (Y = HN-[L-Ala]-$CO_2CH_2CH_2SO_2$-Ph) to the same reductive ring opening conditions afforded the muramylmonopeptide ester **4** in 61% yield.

When glycosyl donor **3**[11] and acceptor **4** were combined under rigorously anhydrous Königs−Knorr conditions (silver triflate/dichloromethane), the desired β-linked disaccharide **10** was obtained in 74% yield after chromatographic purification (Scheme 3).

Finally, the activation scheme of the glycosidation stage was exchanged for the protection scheme of the lipid II endgame. Four protecting group interchange reactions were accomplished in a single operation. Glycosidation product

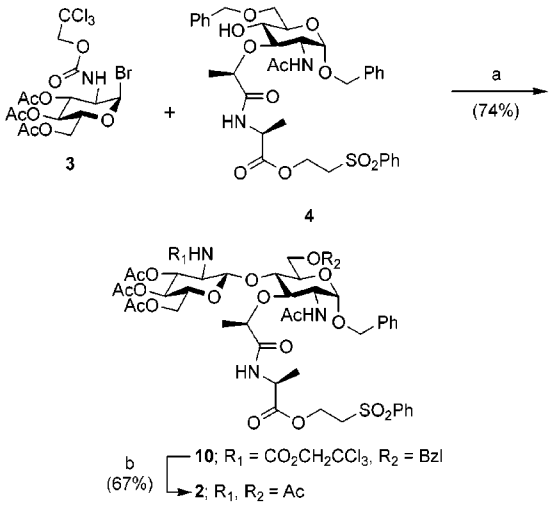
(5) (a) Merser, C.; Sinaÿ, P. *Tetrahedron Lett.* **1973**, *13*, 1029. (b) Durette, P. L.; Meitzner, E. P.; Shen, T. Y. *Carbohydr. Res.* **1979**, *77*, C1. (c) Kiso, M.; Kaneda, Y.; Shimizu, R.; Hasegawa, A. *Carbohydr. Res.* **1980**, *83*, C8. (d) Kiso, M.; Kaneda, Y.; Shimizu, R.; Hasegawa, A. *Carbohydr. Res.* **1982**, *104*, 253. (e) Kusumoto, S.; Yamamoto, K.; Imoto, M.; Inage, M.; Tsujimoto, M.; Kotani, S.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 1411. (f) Kusimoto, S.; Imoto, M.; Ogiku, T.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 1419. (g) Farkas, J.; Ledvina, M.; Brokes, J.; Jezek, J.; Zajicek, J.; Zaoral, M. *Carbohydr. Res.* **1987**, *163*, 63. (h) Kinzy, W.; Schmidt, R. R. *Liebigs Ann. Chem.* **1987**, 407. (i) Kantoci, D.; Keglevic, D.; Derome, A. *Carbohydr. Res.* **1987**, *162*, 227. (j) Termin, A.; Schmidt, R. R. *Liebigs Ann. Chem.* **1989**, 789. (k) Ledvina, M.; Farkas, J.; Zajicek, J.; Jezek, J.; Zaoral, M. *Collect. Czech. Chem. Commun.* **1989**, *54*, 2784. (l) Termin, A.; Schmidt, R. R. *Liebigs Ann. Chem.* **1992**, 527.

(6) Hitchcock, S. A.; Eid, C. N.; Aikins, J. A.; Zia-Ebrahimi, M.; Blaszczak, L. C. *J. Am. Chem. Soc.* **1998**, *120*, 1916.

(7) Zhang, Z.; Ollmann, I.; Ye, X.-S. Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734.

(8) Windholz, T. B.; Johnston, D. B. R. *Tetrahedron Lett.* **1967**, *7*, 2555.

Scheme 3. Glycosidation and Protective Group Exchange[a]



[a] Reagents and conditions: (a) AgOTf (3 equiv), 4Å molecular sieves (5.5 wt equiv), CH_2Cl_2, 25 °C, 20 h; (b) (i) anhydrous $ZnCl_2$, Ac_2O/AcOH (2:1), 25 °C, 20 h, (ii) Zn^0 dust (20 reducing equiv), 25 °C, 4 h.

10 was dissolved in acetic anhydride/acetic acid. Anhydrous zinc chloride was added to remove the muramic C(6) benzyl ether,[12] revealing the alcohol, which acetylated in situ. Zinc dust was then added to the same reaction mixture to remove the Troc group from the glucosamine nitrogen, which in turn acetylated in situ. Removal of solvent, extractive workup, flash chromatography, and crystallization provided the target compound, benzyl *N*-acetyl-(2-deoxy-2-aminoglucopyranosyl)-β-[1,4]-*N*-acetyl-muramylmonopeptide phenylsulfonylethyl ester **2**, in 67% yield.[13]

The concise synthesis of the glycopeptide **2** outlined here offers efficient access to multiple gram quantities of starting material for our projected synthesis of lipid II. The convergent approach, protecting group economy, and crystalline nature of the intermediates permit the process to be scaled to substantial volume. Moreover, the route provides a flexibility that is well suited to support syntheses of *N*-acetyl-(2-deoxy-2-aminoglucopyranosyl)-β-[1,4]-*N*-acetylmuramylpeptide analogues that are unavailable from nature.

Acknowledgment. The authors thank Dr. David A. Peake and his colleagues for application of their expertise in mass spectrometry to analysis of complex reaction mixtures. In addition, we are indebted to the Physical Chemistry Department for characterization of the carbohydrate intermediates.

Supporting Information Available: Experimental procedures and spectral data for compounds **2**, **4**, **9**, and **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) For a preparation of **5**, see: Jeanloz, R. W.; Walker, E.; Sinaÿ, P. *Carbohydr. Res.* **1968**, *6*, 184.

(10) DeNinno, M. P.; Etienne, J. B.; Duplantier, K. C. *Tetrahedron Lett.* **1995**, *36*, 669.

(11) Donor **3** may be prepared in three steps from commercially available glucosamine. See: Imoto, M.; Yoshimura, H.; Shimoto, T.; Sakaguchi, N.; Kusumoto, S.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2205.

(12) Yang, G.; Ding, X.; Kong, F. *Tetrahedron Lett.* **1997**, *38*, 6725.

(13) All compounds gave satisfactory combustion analysis (C, H, N). All spectral data (HRMS, ^{1}H NMR, ^{13}C NMR, and IR) was consistent with the assigned structures.