A Route to Polyprenol Pyrophosphate-Based Probes of O-Polysaccharide Biosynthesis in *Klebsiella pneumoniae* O2a

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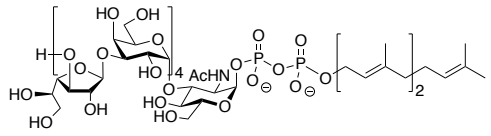
Supporting Information Placeholder



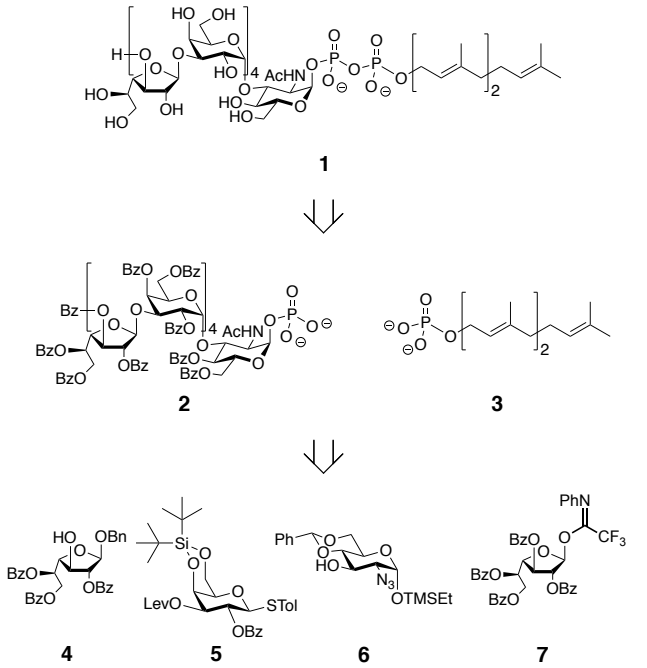
ABSTRACT: An approach for the assembly of polyprenol pyrophosphate-based probes of O-polysaccharide biosynthesis in *Klebsiella pneumoniae* serotype O2a is described. This convergent route features high yielding, diastereoselective glycosylations and the late stage installation of the polyprenol pyrophosphate moiety. Although applied to the synthesis of a nonasaccharide bearing a farnesyl group (**1**), the modular nature of the route makes it amenable to the synthesis of additional derivatives containing either larger glycans or different lipid domains.

Lipopolysaccharide (LPS) is a key structural component of the outer membrane in Gram-negative bacteria, contributing to their structural integrity and shielding the organisms from external threats.[1] It is also an important immunomodulatory molecule that elicits immune responses from infected hosts.[2] LPS consists of three components: lipid A, the core oligosaccharide, and the O-antigenic polysaccharide (O-PS).[3] The O-PS plays a major role in host–pathogen interactions as an essential virulence determinant,[4] accounting for the resistance to complement-mediated serum killing.[5,6] Understanding O-PS biosynthesis will potentially lead to novel approaches or therapeutics to suppress the virulence of Gram-negative bacteria.[7-9]

O-PS biosynthesis occurs via one of three pathways: the synthase-dependent pathway,[10] the wzy-dependent pathway,[11] or the ATP-binding cassette (ABC) transporter-dependent pathway.[12] In the ABC transporter-dependent pathway, which is relevant to the work described in this paper, the glycan is assembled by glycosyltransferases to its full length on a polyprenol pyrophosphate carrier embedded in the cytoplasmic face of the inner membrane. After its biosynthesis, the transporter exports the O-PS to the periplasm, where it is ligated to the already assembled lipid A–core oligosaccharide domain. O-PS chain length control and subsequent export across the inner membrane occurs by one of two mechanisms: 1). Termination of chain extension by a modification (cap)[13] or 2). A process where the O-PS does not involve the addition of a terminal capping motif.[14]

The O-PS in *K. pneumoniae* serotype O2a is a polymer consisting of a Gal*f*–Gal*p* disaccharide repeating unit. Known as D-galactan I,[15] this polysaccharide is synthesized via the ABC transporter dependent pathway where there is no terminal modification that caps the glycan.[14,16] This mechanism of O-PS biosynthesis is not well understood, in part due to the lack of suitable probe molecules for interrogating the process. Given that O-PS extension occurs on a polyprenol pyrophosphate-based species embedded in the membrane, we hypothesized that both of these domains would be key components of an efficient probe. In particular, a previous study with mycobacterial carbohydrate processing enzymes showed that acceptor substrates possessing both a pyrophosphate and a polyprenol moiety were 500–1000 times better substrates (i.e., k_{cat}/K_M) than those with a simple alkyl aglycone.[17] Previous work has reported the synthesis of *K. pneumoniae* serotype O2a O-PS fragments; however, none have incorporated the pyrophosphate or polyprenol motifs.[18-22] We describe here a modular route for the preparation such glycosylphospholipids and apply it to the synthesis of a nonasaccharide, **1** (Scheme 1). We consider **1** to be sufficiently complex to serve as an appropriate model compound to demonstrate the feasibility of an approach that could be applied to more elaborate compounds, i.e., those with longer glycan or lipid domains.

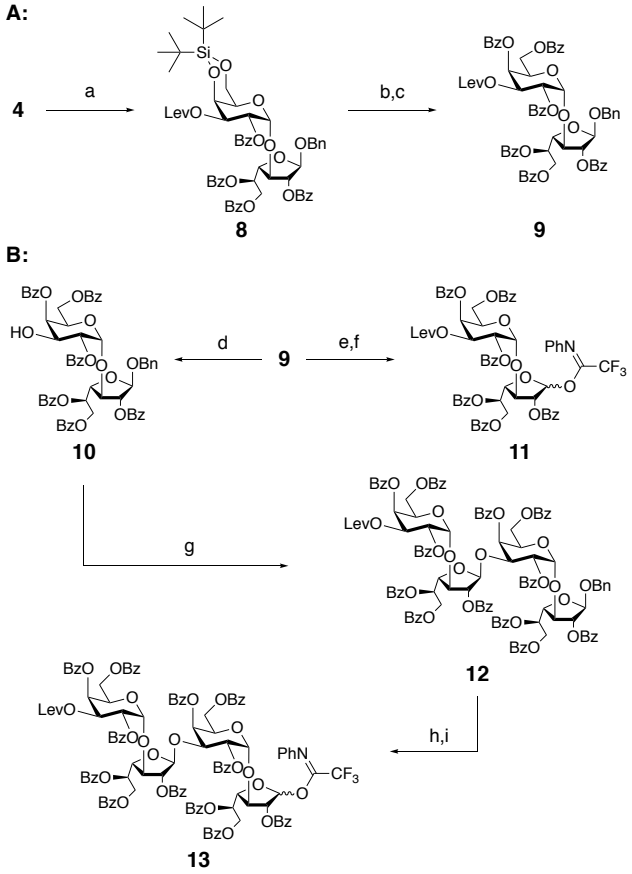
Scheme 1. Retrosynthesis of 1



To access the target, we envisioned (Scheme 1) doing a late stage pyrophosphate coupling between sugar phosphate **2** and farnesyl phosphate (**3**),[23] which, after removal of the benzoyl esters, would give **1**. Nonasaccharide **2** could be assembled from monosaccharides **4**–**7**, which were synthesized as described in the Supporting Information (**4**–**6**) or as reported (**7**).[24] The 2-*O*-benzoyl groups on galactofuranosides **4** and **7** would give 1,2-*trans*-β selectivity in the glycosylation reactions, while the 4,6-*O*-di-*tert*-butylsilylidene (DTBS) group on galactopyranoside **5** would provide α-selectivity.[25]

The synthesis began (Scheme 2) by preparing a building block that could be used to introduce the repeating unit structures in a dimeric form. To that end, activation of thioglycoside **5** with NIS/TfOH in the presence of alcohol **4** provided disaccharide **8** in 77% yield and in high α-selectivity; none of the β-anomer was detected. It was found that treatment of the crude reaction mixture with PPh_3 in CH_3OH–THF–H_2O was necessary to facilitate the purification of **8**. Doing this removed a by-product (believed to be *N*-thiotolulyl succinimide) that co-eluted with the disaccharide. Removal of the DTBS group, followed by benzoylation, gave a 94% yield of disaccharide **9**, which was split into two portions.

Scheme 2. Synthesis of Tetrasaccharide Donor 13



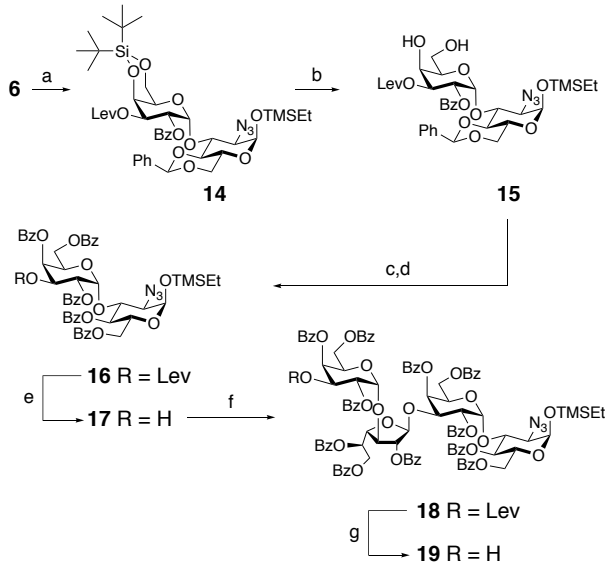
Legend: (a) **5**, NIS, TfOH, 4 Å M.S., CH_2Cl_2, 0 °C → rt, then PPh_3, THF–CH_3OH–H_2O, rt, 77%; (b) HF·pyridine, pyridine–THF, 0 °C → rt; (c) BzCl, pyridine–CH_2Cl_2, 0 °C → rt, 94% (over 2 steps); (d) H_2NNH_2·AcOH, CH_2Cl_2–CH_3OH, rt, 95%; (e) Raney-Ni, EtOH (200 proof), reflux, 80%; (f) $CF_3CN(Ph)Cl$, Cs_2CO_3, CH_2Cl_2, rt, 86%; (g) **11**, TfOH, 4 Å M.S., CH_2Cl_2, 0 °C → rt, 95%; h) Raney-Ni, EtOH (200 proof), reflux, 66%; (i) $CF_3CN(Ph)Cl$, Cs_2CO_3, CH_2Cl_2, rt, 90%.

One portion of **9** was treated with H_2NNH_2·AcOH to remove the levulinoyl ester giving disaccharide alcohol **10** in 95% yield. Conversion of the other portion to a disaccharide donor proved unexpectedly difficult. Use of standard hydrogenation conditions (H_2 and a range of palladium catalysts) did not lead to removal of the anomeric benzyl group efficiently. However, treatment of **9** with Raney-Ni[26] gave the desired lactol, which was then converted to imidate **11** in 69% yield over the two steps. Activation of **11** with TfOH in the presence of **10** gave tetrasaccharide **12** in 95% yield with complete β-selectivity. Again, reduction of the benzyl group with Raney-Ni gave the corresponding reducing sugar. Subsequent reaction with 2,2,2-trifluoro-*N*-phenylacetamidoyl chloride and cesium carbonate produced imidate **13** in 60% yield over two steps.

With a route to **13** in place, our attention turned to synthesizing the reducing end of the molecule (Scheme 3). Activation of thioglycoside **5** with NIS/TfOH, this time in the presence of **6**, yielded disaccharide **14** in 84% yield and in excellent α-selectivity. As was observed in the previous reaction with **5**, none of the β-linked product was detected. We next simplified the protecting groups on the disaccharide to facilitate the final deprotection. Removal of the DTBS group with HF·pyridine was facile, cleanly producing diol **15** in 94% yield. On the other hand, cleavage of the benzylidene acetal was problemat-

ic. Acid hydrolysis removed the acetal, but only in modest yields, presumably due to concomitant cleavage of the TMSEt glycoside. On the other hand, oxidative conditions, $KBrO_3/Na_2S_2O_4$,[27] transformed the acetal to an inseparable mixture of O-4 and O-6-benzoyl ester regioisomers. The formation of a mixture at this stage was not an issue as both compounds could be converted to the desired penta-*O*-benzoylated derivative **16** (90% over two steps) by treatment with BzCl/DMAP in pyridine at reflux. These forcing conditions were required as O-4 of the glucosamine residue was reluctant to undergo benzoylation at lower temperatures. Once **16** was in hand, cleavage of the levulinoyl ester with $H_2NNH_2{\cdot}AcOH$ gave disaccharide **17** in 94% yield. The combination of **11** and **17**, promoted by TfOH, furnished tetrasaccharide **18** in 95% yield. Finally, removal of the levulinoyl group in **18** under the usual conditions provided alcohol **19** in 81% yield.

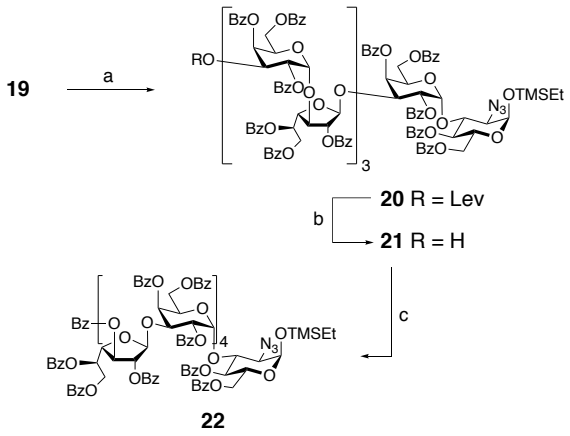
Scheme 3. Synthesis of Tetrasaccharide Acceptor 19



Legend: (a) **5**, NIS, TfOH, 4 Å M.S., CH_2Cl_2, 0 °C → rt, 84%; (b) HF·pyridine, pyridine–THF, 0 °C → rt; 94% (c) $KBrO_3$, $Na_2S_2O_4$, EtOAc–H_2O, rt; (d) BzCl, DMAP, pyridine, 0 °C → reflux, 90% (over 2 steps); (e) $H_2NNH_2{\cdot}AcOH$, CH_2Cl_2–CH_3OH, rt, 94%; (f) **11**, TfOH, 4 Å M.S., CH_2Cl_2, 0 °C → rt, 95%; (g) $H_2NNH_2{\cdot}AcOH$, CH_2Cl_2–CH_3OH, rt, 81%.

After tetrasaccharides **13** and **19** were in hand, they could be conveniently elaborated into a larger structure (Scheme 4). First, a TfOH-promoted 4 + 4 glycosylation between **13** and **19** yielded octasaccharide **20** in 97% yield. Treatment of **20** with $H_2NNH_2{\cdot}AcOH$ gave alcohol **21**, which was then combined with **7** in the presence of TBSOTf resulting in nonasaccharide **22** in excellent yield over the two steps. In this 8 + 1 glycosylation, it was found that a lower product yield was obtained using TfOH as the promotor.

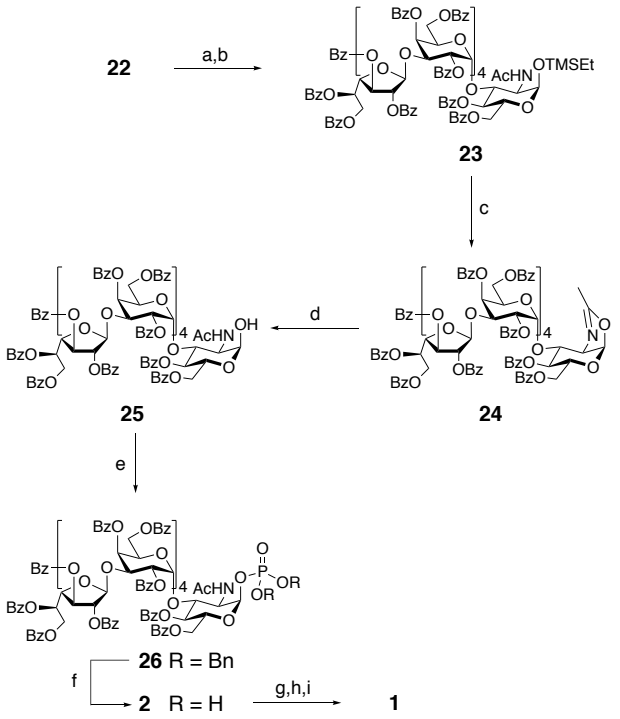
Scheme 4. Synthesis of Nonasaccharide 22



Legend: (a) **13**, TfOH, 4 Å M.S., CH_2Cl_2, 0 °C → rt, 97%; (b) $H_2NNH_2{\cdot}AcOH$, CH_2Cl_2–CH_3OH, rt, 97%; (c) **7**, TBSOTf, 4 Å M.S., CH_2Cl_2, 0 °C → rt, 97%.

Conversion of **22** into **1** required introduction of the polyprenol pyrophosphate motif at the reducing end and some functional group interconversions (Scheme 5). First, the azide in **22** was transformed to the corresponding acetamido derivative by Staudinger reduction and acetylation, giving **23** in 78% overall yield. Next, cleavage of the TMSEt glycoside was needed. Treatment of **23** with TFA–CH_2Cl_2–H_2O gave oxazoline **24**, which, when further treated with a 9:1 solution of TFA–H_2O, yielded lactol **25** in 74% yield over the two steps. Phosphitylation of **25** with $i\text{-}Pr_2NP(OBn)_2$ and tetrazole, followed by oxidation with *m*-CPBA resulted in a 73% overall yield of phosphate **26**. Hydrogenolysis of **26** under standard conditions gave, in quantitative yield, the free phosphate **2**. Finally, CDI-activation of **2**, followed by coupling with farnesyl phosphate (**3**) and removal of the benzoyl protecting groups with a $NaOCH_3$, yielded **1** in 30% yield over three steps.

Scheme 5. Synthesis of Target 1



Legend: (a) PMe_3, $NaOH_{(aq)}$, THF–H_2O, rt → 50 °C; (b) Ac_2O, pyridine, 0 °C → rt, 78% (over 2 steps); (c) TFA–CH_2Cl_2–H_2O (80:44:1, v/v/v), 0 °C → rt; (d) TFA–H_2O (9:1, v/v), rt, 74% (over 2 steps); (e) *i*-$Pr_2NP(OBn)_2$, tetrazole, CH_2Cl_2, 0 °C → rt, then *m*-CPBA, –78 °C → rt, 73%; (f) H_2, Pd/C, THF, rt, quant.; (g) CDI, CH_2Cl_2, rt; (h) **3**, DMF, rt; (i) $NaOCH_3$ (5 mM), CH_2Cl_2–CH_3OH, rt, 30% (over 4 steps)

In conclusion, we report here a convergent, modular approach to the synthesis of *K. pneumoniae* serotype O2a O-PS biosynthetic intermediates. The approach features highly diastereoselective glycosylations, for both 1,2-*trans*-β and 1,2-*cis*-α linkages, leading to an advanced intermediate that was further functionalized with a polyprenol pyrophosphate motif. We demonstrated the utility of the approach via the synthesis of a nonasaccharide target (**1**). However, the route is amenable to preparing larger derivatives, starting from **21**, via iterative glycosylations with tetrasaccharide **13** and levulinoyl ester removal. Similarly, replacing **3** with other polyprenol phosphates in the pyrophosphate-forming reaction would allow the synthesis of other derivatives, including the naturally-occurring undecaprenyl species. Compound **1** is currently being used in investigations of O-PS biosynthesis in *K. pneumoniae* O2a, with the goal of better understanding how chain-length is controlled. The synthesis of more complex derivatives of **1** is also underway, including those with longer glycan and polyprenol moeities .

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental procedures, characterization data, and ^1H and ^{13}C NMR spectra for all new compounds (PDF).

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Notes

The authors declare no competing financial interest.

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