Stereoselective Synthesis of α- and β-L-Ara4N Glycosyl H-Phosphonates and a Neoglycoconjugate Comprising Glycosyl Phosphodiester Linked β-L-Ara4N

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Supporting Information

ABSTRACT: Stereoselective synthesis of variably protected α- and β-L-Ara4N glycosyl H-phosphonates as key intermediates in the syntheses of β-L-Ara4N-modified LPS structures and α-L-Ara4N-containing biosynthetic precursors is reported. A facile one-pot approach toward β-L-Ara4N glycosyl H-phosphonates includes anomeric deallylation of protected 4-azido β-L-Ara4N via terminal olefin isomerization followed by ozonolysis and methanolysis of formyl groups to furnish exclusively β-configured lactols that are phosphitylated with retention of configuration. The carbohydrate epitope of β-L-Ara4N-modified Lipid A, βGlcN(1→6)αGlcN(1→P=1)β-L-Ara4N, was stereoselectively synthesized and linked to maleimide-activated bovine serum albumin.

Modification of the terminal portion of lipopolysaccharide (LPS), a glycosphospholipid Lipid A, with 4-amino-4-deoxy-β-L-arabinose (L-Ara4N) is an adaptive mechanism that enables Gram-negative bacteria to resist recognition by the components of the host immune system.1,2 Inducible addition of L-Ara4N to at least one of the phosphate groups of Lipid A (at the 1 and/or 4’ positions) in Escherichia coli, Salmonella enterica serovar Typhimurium, Yersinia pestis, Pseudomonas aeruginosa, and Burkholderia cepacia complex is required for bacterial viability and largely contributes to antibiotic resistance and bacterial virulence (Figure 1).3–9 Inducible addition of cationic sugar L-Ara4N reduces the net negative charge of the bacterial membrane, which protects it from recognition by the cationic antimicrobial peptides (CAMPs) that comprise the foremost component of the innate immune response at epithelial surfaces. Occurrence of profound resistance to exogenous CAMP polymyxin B, a “last line of defense” antibiotic for the treatment of multi-drug-resistant Gram-negative infections, is also attributed to incorporation of L-Ara4N into the Lipid A moiety of LPS.10 L-Ara4N biosynthesis occurs in the cytoplasm, whereas in the final step, L-Ara4N is delivered by the long-chain isoprene lipid carrier undecaprenyl phosphate (UndP) to the periplasmic face of the inner bacterial membrane.11 There, a membrane lipid-to-lipid glycosyltransferase ArnT catalyzes transfer of L-Ara4N from undecaprenylphosphate-α-L-Ara4N to the phosphate groups of Lipid A.11,12 ArnT family is the last enzyme in the Ara4N biosynthesis pathway in Gram-negative bacteria and is thus an attractive target for development of antibacterial agents affecting LPS biosynthesis, which necessitates a synthetic access to α-L-Ara4N-containing UndP derivatives.13

Ara4N-modified LPS structures can hardly be obtained in pure form by isolation from bacterial sources, due to the inherent lability of the anomeric phosphodiester functionality. To clarify immuno-modulating and immunogenic potential of the Ara4N modification, a reliable synthetic approach toward β-L-Ara4N glycolipid phosphate-containing LPS partial structures is highly desirable. The Lipid A-based neoglycoconjugate, containing conserved epitope βGlcN(1→6)αGlcN(1→P=1)β-L-Ara4N of highly virulent Gram-negative human pathogens, is an important antigen that could be applied to help generate specific monoclonal antibodies. Such mAbs could be used in diagnostic immunoaffinity assays for rapid antigen determination in clinical samples and applied to screen not-yet-identified Ara4N-producing mutants in, for example, Y. pestis flea infection models.6

In contrast to the abundantly prevailing phosphodiester bonds connecting one anomeric and one non-anomeric sugar hydroxyl...
group, the L-Ara4N-modified Lipid A involves a glycosyl phosphodiester linkage connecting anomeric centers of aminosugars Ara4N and GlcN (Figure 1). Assembly of such a binary glycosyl phosphodiester requires both rigorous anomeric stereocontrol and very mild reaction conditions that allow for preservation of the labile glycosyl phosphites intermediates. We have recently shown that application of the H-phosphonate synthetic challenge in the synthesis of Ara4N-containing phosphitylation methodologies. Preparation of anomerically pure α- and β- H-phosphate monoesters of orthogonally protected L-Ara4N in a stereoselective manner comprises a major synthetic challenge in the synthesis of Ara4N-containing phosphodiesters (Figure 2).

At the anomeric center generally relies on the ultimate anomeric ratio in the lactol precursors and demands the preparation of anomerically enriched hemiacetals which should be straightforwardly converted into the H-phosphonates. Anomeric dealylation of 2 and 3 was carried out by sequential double bond isomerization with [Ir(1,5-Cod)(PMePh)2]PF6 followed by I2-assisted prop-1-enyl cleavage, to furnish anomeric mixtures 12 and 13, respectively (α/β = 1:1). Lactols 12 and 13 could be enriched with the β-anomer (α/β = 1:3) by treatment with diluted AcOH. Subsequent phosphorylation by reaction with 2-chloro-1,3,2-benzodioxaphosphorin-4-one (SalPCl) in pyridine yielded anomeric H-phosphonates 17 and 18 (α/β = 1:3). 2,3-O-TIPDS-protected 17-β was smoothly isolated in pure form, though in a moderate 35% yield (Scheme 2). In contrast, separation of the 2,3-di-O-TDMS-protected α/β mixture 18 was challenging and ineffective.

A predominant formation of the β-configured H-phosphonate was achieved by application of fairly reactive SalPCl, which quickly trapped the excess of axial β-lactol in 12, such that the initial α/β ratio was preserved. To guide in situ anomerization in favor of the α-lactol, a less reactive phosphorylation agent that would primarily react with the more nucleophilic equatorial 1- OH group, to shift the α/β ratio in favor of α-anomer, could be of use. Indeed, slow addition of diphenylphosphite to a solution of 12 (α/β = 1:1) in pyridine resulted in preponderant formation of the kinetic product, an equatorial glycosyl H-phosphonate (α/β = 2:1) 17α readily isolated in 62% yield (Scheme 2, Table 1).

Monitoring the progress of I2-mediated hydrolysis of the prop-1-enyl group in 7 by 1H NMR indicated that exclusively the β-lactol was formed when the reaction was performed at 0 °C (α/β ratio varied from 0:10 to 1:10), while the proportion of the α-configured lactol increased at a higher reaction temperature (25 °C, α/β = 1:1). Insight into the mechanistic pathway of I2-assisted prop-1-enyl glycoside cleavage in 7 suggested formation of the intermediate halohydrin (at 0 °C), which is cleaved without affecting the anomeric configuration, to form exclusively the β-configured lactol (Figures S1 and S2). At 25 °C, and upon chromatography on silica gel, a rapid anomerization toward α-lactol sets in. Attempts to avoid the purification step and to conduct the phosphorylation of the crude reaction mixture 12 enriched with the β-lactol (α/β = 1:10) failed due to formation of numerous byproducts.

To elaborate an efficient approach toward anomerically pure β-1-Ara4N H-phosphonates, we scrutinized the options for traceless hydrolysis of β-allyl glycosides without affecting the axial anomeric configuration at C-1. To this end, after allyl group isomerization, the anomeric prop-1-enyl ether could be oxidized by ozonolysis to give a stable formyl intermediate under mild conditions (Scheme 3). The formate group could be selectively hydrolyzed to give a β-lactol and volatile methyl formate, so that the crude β-lactol could be directly subjected to phosphorylation without needing chromatographic purification. Accordingly, prop-1-enyl intermediates 7 and 9–11 were treated with ozone at −78 °C followed by addition of thiourea. Formyl glycosides 19–22 were isolated in pure form by chromatography on deactivated silica gel. Finally, methanolysis (NEt3/MeOH) of the formate esters at −40 °C furnished solely β-configured lactols 12–16, which were used without further purification. Subsequent phosphorylation with SalPCl provided anomerically pure β-glycosyl H-phosphonates 17-β, 23-β, 24-β.
Table 1. Stereoselective Synthesis of α- and β-Glycosyl H-Phosphonates of L-Ara4N

| Method for removal of anomeric prop-1-enyl ether and phosphitylation: | a/β ratio yield isolated yieldβ |
|---|---|---|
| (A) (1) I₂ (2 equiv), THF/H₂O (2:1, v/v), rt; (2) slow addition (4 h) of (PhO)₂P(O)H (4 equiv), Py; (3) NEt₃/H₂O, rt, 30 min; | A: a/β = 2:1 pure α: 62% | |
| (B) (1) I₂ (2 equiv), THF/H₂O (2:1, v/v), 0 °C; (2) in situ anomerization with AcOH; (3) SalPCL (2 equiv), Py; (4) NEt₃/H₂O, rt, 30 min; | B: a/β = 1:3 (84%) pure β: 35% | |
| (C) (1) O₃, −78 °C, 5 min, thiourea (1.3 equiv); (2) MeOH, NEt₃, −40 °C; (3) SalPCL (2 equiv), Py; (4) NEt₃/H₂O, rt, 30 min. | C: a/β = 1:6 (90%) pure β: 78% | |

Yield of glycosyl β-L- or α-L-Ara4N H-phosphonate after conventional column chromatography on silica gel (not HPLC).

Scheme 3. Stereoselective Synthesis of β-L-Ara4N Glycosyl H-Phosphonates

and 25-β in 62–78% yield (Table 1). Exchange of the 4-azido group in 2 for the Fmoc-protected amino group, followed by anomic deallylation and phosphitylation with SalPCL, furnished an inseparable mixture of α- and β-L-Ara4N glycosyl H-phosphonates in 84% yield, in which the α-configured product prevailed (α/β = 2:1, Scheme S1).

Glycosyl H-phosphonate 17-β was coupled to the β(1→6)-linked diglucosamine lactol 26 (α/β = 2:1) in pyridine using pivaloyl chloride (PivCl) as activating agent to furnish H-phosphonate glycosyl phosphodiester 27 as an anomic mixture at GlcN moiety (α/β = 2:1, according to 1H and 31P NMR data) (Scheme 4). Oxidation of 27 by treatment withaq I₂ at −40 °C followed by chromatographic purification afforded anomerically pure binary glycosyl phosphodiester 29 in 58% yield, whereas an undesired β-anomeric product was expectedly destroyed upon aqueous I₂-mediated oxidation and isolation by chromatography on silica gel. Despite the apparent simplicity, this procedure requires strict control of reaction conditions since minor alterations can result in hydrolysis of the binary glycosyl phosphodiester or the appearance of byproducts. PivCl-mediated coupling can instigate formation of P-acyl byproducts resulting from an over-reaction of 17 or 27 with PivCl and the formation of GlcNAc-derived oxazolines. Oxidation of 27 into the P(V) counterpart 29 by treatment withaq I₂ could result in rapid hydrolysis, with the loss of Ara4N, if the optimized reaction conditions were not rigorously followed. To circumvent these possible drawbacks, we exchanged the activating agent to 3-nitro-1,2,4-triazol-1-yl-tris(pyrrolidin-1-yl)-phosphonium hexafluorophosphate (PyNTP), which selectively reacted with the electrophilic phosphorus atom of 17 to form a P-N-activated intermediate. Formation of the binary glycosyl H-phospho-
nate diester 27 was confirmed by appearance of the P–H-coupled signals (31P NMR, δ: 7.3 ppm, J_{PH} = 730 Hz and 5.6 ppm, J_{PH} = 754 Hz) corresponding to the β/β- and α/β-linked H-phosphonate diesters (Figure S3).

Anhydrous oxidation of tetra-coordinated H-phosphonate 27 was performed in two steps: by treatment with N,O-bis-(trimethylsilyl)acetamide (BTSA) in the presence of DBU which transformed 27 into the three-coordinated silylphosphate 28, and by oxidation of 28 with 2-(phenylsulfonyl)-3-(3-nitrophenyl)-oxaziridine (PNO) to furnish 1,1-di-(2-quinolinecarboxylato)

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Additional figures, experimental procedures, and NMR data (PDF)

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Notes

The authors declare no competing financial interest.

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