Synthesis of Neoglycoconjugates Containing 4-Amino-4-deoxy-L-arabinose Epitopes Corresponding to the Inner Core of Burkholderia and Proteus Lipopolysaccharides

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**Keywords:** Biomimetic synthesis / Glycoconjugates / Glycosylation / Glycolipids / Lipopolysaccharide / Carbohydrates

Disaccharides that contain 3-deoxy-d-manno-oct-2-ulosonic acid (Kdo) and d-glycero-d-talo-oct-2-ulosonic acid (Ko) substituted at the 8-position by 4-amino-4-deoxy-β-L-arabinopyranosyl (Ara4N) residues have been prepared. Coupling an N-phényltrifluoroacetimidate-4-azido-4-deoxy-L-arabinosyl-glycosyl donor to acetyl-protected allyl glycosides of Kdo and Ko afforded anomeric mixtures of disaccharide products in 74 and 90% yield, respectively, which were separated by chromatography. Further extension of an intermediate Ara4N-(1→8)-Kdo disaccharide acceptor, which capitalized on a regioselective glycosylation with a Kdo bromide donor under Helferich conditions, afforded the branched trisaccharide α-Kdo-(2→4)[β-L-Ara4N-(1→8)]-α-Kdo derivative. Deprotection of the protected di- and trisaccharide allyl glycosides was accomplished by TiCl4-promoted benzyl ether cleavage followed by the removal of ester groups and reduction of the azido group with thiol or Staudinger reagents, respectively. The reaction of the anomic allyl group with 1,3-propanedithiol under radical conditions afforded the thioether-bridged spacer glycosides, which were efficiently coupled to maleimide-activated bovine serum albumin. The neoglycoconjugates serve as immunoreagents with specificity for inner core epitopes of *Burkholderia* and *Proteus* lipopolysaccharides.

**Introduction**

4-Amino-4-deoxy-L-arabinose (Ara4N) constitutes an important sugar component within the outer membrane of Gram-negative bacteria. Thus, Ara4N residues occur in substoichiometric to stoichiometric amounts ester-linked to the 1- and 4-phosphate groups of the glucosamine disaccharide backbone of lipid A, which anchors the lipopolysaccharide (LPS) chain to the outer membrane.[1-3] In addition, Ara4N residues have also been detected glycosidically-linked to the LPS core sugar 3-deoxy-d-manno-oct-2-ulosonic acid (Kdo), which provides the linkage unit to the lipid A domain. In particular, Ara4N has been found in a β-(1→8) linkage to Kdo in the inner core region of many *Proteus* strains and in a *Providencia* strain.[14-6] Bacteria of the genus *Proteus* are important pathogens that cause nosocomial wound infections and urinary tract infections. Similarly, Ara4N has been detected linked to the 8-position of the Kdo-isosteric sugar d-glycero-d-talo-oct-2-ulosonic acid (Ko) in the core region of *Burkholderia*.[7-10] and in a *Serratia marcescens* strain.[11] *Burkholderia* species are responsible for severe and fatal infections in cystic fibrosis sufferers. The incorporation of Ara4N in the lipid A and core regions has been implicated in the development of antibiotic resistance in bacteria by masking the anionic charges of the phosphate and carboxylic acid groups, which counteracts the effects of cationic antimicrobial peptides.[12] Previously, antibodies raised against neoglycoconjugates containing the Ko-(2→4)-Kdo disaccharide did not bind to the LPS of *Burkholderia*, which could be because of steric hindrance by the terminal Ara4N residue, which masks Ko and Kdo epitopes.[13] In order to study the antigenic properties of Ara4N-substituted inner core LPS units and develop polyclonal and monoclonal antibodies for diagnostic applications, we have set out to synthesize the central Ara4N-Kdo/Ko units that correspond to part of the structures of the *Burkholderia* and *Proteus* LPS. Herein we present results pertaining to the development of new Ara4N glycosyl donors, assembly of the di- and trisaccharide units, and conversion into the corresponding neoglycoconjugates. An additional feature relates to the orthogonal utilization of allyl and benzyl protecting groups, which considerably adds to the repertoire of protecting group strategies in oligosaccharide syntheses.

**Results and Discussion**

Multigram amounts of methyl 4-azido-4-deoxy-α-L-arabinopyranoside (I) have been generated from commercial methyl β-d-xylopyranoside.[14] Benzyl groups were selected as nonparticipating protecting groups at the 2- and 3-positions...
tions, and 1 was converted into the 2,3-di-O-benzyl glycoside 2 by reaction with benzyl bromide/NaH in N,N-di-methylformamide (DMF) in 93% yield (Scheme 1). In order to generate hemiacetal 3, which was the precursor for the armed glycosyl donors 4–7, hydrolysis of 2 was accomplished by treatment with 2 M HCl in acetic acid at 65 °C, and lactol 3 was isolated after flash chromatography in 61% yield. As fluoride glycosyl donors have served as efficient donors in numerous glycoside syntheses,\textsuperscript{[15]} including coupling to 8-O-silylated Kdo derivatives,\textsuperscript{[16]} donor 4 was prepared by the reaction of 3 with diethylenosulfur trifluoride (DAST) in CH$_2$Cl$_2$ at –20 °C to afford an anomeric mixture of 4-azido-4-deoxy-L-arabinopyranosyl fluoride (4) in ca. 99% yield with preferential formation of the equatorial isomer (α/β ratio ca. 2:1). In addition, the tri-chloroacetimidate donor 5 (α/β ratio ca. 3:1) was prepared from the reaction of 3 with trichloroacetone/K$_2$CO$_3$ at room temperature in 78% yield.\textsuperscript{[17]} Similarly, conversion of 3 into the N-phenyltrifluoroacetimidate derivative 6 (α/β ratio ca. 7:1) was effected by treatment of 3 with N-phenyltrifluoroacetimidoyl chloride/K$_2$CO$_3$ in acetone in 84% overall yield.\textsuperscript{[18]} In order to exploit the stabilization of a putative oxacarbenium ion intermediate in the glycosylation step by the intramolecular participation of a 4-acylamino moiety, the 4-deoxy-4-trifluoroacetamido derivative 7 was also prepared.\textsuperscript{[19,20]} The azido group of 4 was subjected to a StaudINGER reaction with triphenylphosphine and trifluoroacetic anhydride in CH$_2$Cl$_2$, which gave a moderate yield (47%) of the corresponding 4-deoxy-4-trifluoroacetamidoarabino-syl fluoride derivative 7 together with the byproducts 8 and 9 (35% combined yield), which were removed by column chromatography. The anemic mixtures of 5, 6, and 7 were resolved by silica gel chromatography.

For the straightforward assembly of the (1–8)-linked disaccharide units, the peracetylated 8-O-tert-butylidimethylsilyl allyl glycoside derivatives of Kdo (10) and Ko (14) were chosen as glycosyl acceptors that allow direct coupling with fluoride donors and provide access to the respective alcohol used for the subsequent glycosylation step.

Gratifyingly, the reaction of 12 with tert-butylidimethylsilyl chloride (tBDMSiCl) in dry acetonitrile in the presence of diazabicyclo[2.2.2]octane (DABCO) afforded the 8-O-silyl derivative 13, which was acetylated with acetic anhydride/4-(dimethylamino)pyridine (DMAP) in pyridine to furnish the 3,4,5,7-tetra-O-acetyl Ko glycosyl acceptor 14 in 62% overall yield. The structural assignment of 14 was based on the low-field chemical shifts observed for protons H-3, H-4, H-5, and H-7 in the $^1$H NMR spectrum. Removal of the 8-O-acetyl group is prone to migration to the 8-position upon contact with silica gel, the crude products of 11 and 15 were used for the subsequent glycosylation step.

The glycosylation conditions were elaborated with the Kdo acceptor derivatives 10 and 11. Coupling reactions of 10 and 4 in the presence of 1.1 equiv. of BF$_3$·Et$_2$O as the promoter in CH$_2$Cl$_2$ and acetone, respectively, afforded low yields of glycosides (Table 1, Entries 1 and 2). Employing a large excess of promoter provided good yields of the glycosides with a very moderate stereoselectivity in favor of the cis-configured β-L-Ara4N glycosides (Entries 3 and 4). The trimethylsilyl triflate (TMSOTf)–promoted coupling reaction of 7 with 15 did not improve the stereochemical outcome of the reaction and gave a 55% yield of disaccharide (Entry 5). Coupling of 5 with 11 also gave a modest yield of products (Entry 6), whereas less reactive, armed 6 led to good yields of Ara4N-Kdo and Ara4N-Ko glycosides albeit with no improvement in anomeric selectivity (Entry 7).\textsuperscript{[24]} The disaccharide mixture generated from the reaction of 11 with 6 was partially resolved by chromatography, which allowed the separation of the equitorially-linked α-
l-glycoside 18 (Scheme 3). The assignment of the anomeric configuration of the Ara4N residue in 18 was based on the value of the coupling constant $J_{1,2}$ of ca. 6.2 Hz, which indicated a 1,2-trans-glycosidic linkage. The attachment site was identified by the low-field-shifted C-8 Kdo signal at 67.35 ppm in the $^{13}$C NMR spectrum.

ORDER TO PREVENT FORMATION OF A 4'-acetamido product. Thus, 21 was subjected to Zemplén deacetylation followed by hydrolysis of the Kdo methyl ester group to furnish the 4'-azido-4'-deoxy-disaccharide derivative 22 in 95% yield, which was purified by chromatography on BioGel P2. The reduction of the azido group of 22 was accomplished by treatment with dithiothreitol (DTT) in aqueous pyridine/diisopropylamine, which left the allyl group intact and provided the 4'-amino-4'-deoxy-l-arabinopyranosyl derivative 23. Purification was performed by ion-exchange chromatography on Dowex WX50 (H+ form) eluted with 1 M aqueous NH₃ followed by desalting on BioGel P2 to give the β-1-Ara4N-(1→8)-Kdo allyl glycoside as the ammonium salt 23 in 60% yield.

In order to produce neoglycoconjugates with covalently-linked Ara4N-K(d)h ligands, suitable spacer groups were introduced by using the remaining allylic function. The conjugation chemistry has to be compatible with the presence of both amino and carboxylic acid groups in order to retain the antigenic properties of these LPS inner core sugars. Hence, frequently-employed chain-elongation methods by addition of cysteamine or ω-mercaptoalkanoic acid derivatives to the allyl aglycon were not feasible.[27,28] Conjugation conditions also have to take into account the acid-labile glycosidic linkages of the Kdo residues. Hence, thiol-based conjugation chemistry was envisaged to provide chemoselective ligation to the protein matrix.[29] The introduction of the thiol-based spacer moiety was effected by UV-mediated addition of 1,3-propanedithiol to the allylic aglycon to give the thioether-bridged thiol 24 in 63% yield.[14] The resulting material contained a small fraction of the corresponding dimer and was immediately used for the preparation of the glycoconjugate.

The corresponding Ara4N-Ko glycoside was prepared in a similar way using the conditions established for the Ara4N-Kdo derivatives (Scheme 4). Thus, glycosylation of 15 with 6 proved to be highly efficient and gave a 90% yield of disaccharide products (Table 1, Entry 8). Again, a minor amount of the α-linked product 27 was formed, which was separated from the mixture of 25 and 28 by chromatography. Final purification of the target disaccharide 25 was achieved by deacetylation with NaOMe, which produced a separable mixture of the triol derivatives 26 (68%) and 29 (30%). The overall yield for the preparation of 26 from 15 was 38%. Subsequent reacetylation of the β-(1→8)-linked disaccharide 26 afforded 25 in 94% yield. Similar to the Ara4N-Kdo intermediate 16, the benzyl groups of 25 were cleaved by the action of TiCl₄ followed by acetylation to furnish the hexa-O-acetyl disaccharide 30 in 89% yield. Zemplén deacetylation and hydrolysis of the Ko methyl ester group gave 31 in 97% yield. The 4'-azido group of 31 was reduced with dithiothreitol to produce the 4'-amino glycoside 32 in 69% yield, which was transformed into the thio-spacer derivative 33 in 80% yield.

Proceeding towards the branched inner core unit related to the Proteus mirabilis LPS, the Ara-(1→8)-Kdo intermediate 17 was subjected to a regioselective glycosylation with the known Kdo bromide donor 34.[30] The outcome of the
Scheme 4. Reagents and conditions for the synthesis of Ara4N-(1→8)-Kdo disaccharide derivatives. (a) see Table 1; (b) 0.1 m NaOMe, MeOH, room temp., 4 h 68% for 26, 30% for 29; (c) Ac₂O, DMAP, pyridine, room temp., 24 h, 94%; (d) TiCl₄, CHCl₃, 0°C, 6 h, then Ac₂O, DMAP, pyridine, room temp., 24 h, 89%; (e) 0.1 m NaOMe, MeOH, room temp., 4 h, then 0.2 m NaOH, 3 h, 97%; (f) dithiothreitol, iPr₂N/H₂O, room temp., 1 h, then BioGel P-2, 80%.  

Helferich glycosylation procedure was dependant on the solvent system. With CH₂Cl₂, the reaction gave a low yield and prevailing formation of the (1→7)-linked product, whereas nitromethane afforded a better yield, regio-, and anomeric selectivity. The excess amount of 34 was limited to avoid formation of the (2→7)-linked trisaccharide and tetrascarachide byproducts. Separation of 38 and the glycal ester byproduct 37 was achieved by column chromatography followed by HPLC (Scheme 5). Acetylation of 35 afforded the hexa-O-acetyl derivative 36 in 75% yield. A small amount of the β-(2→4)-linked trisaccharide isomer was visible in the ¹H NMR spectrum of 36, which was removed in the next step. Deprotection was performed by TiCl₄-promoted hydrolysis of the benzyl protecting groups followed by acetylation to afford the branched octa-O-acetyl trisaccharide 39 in 68% yield. Remarkably, both of the acid-labile Kdo units were stable during the Lewis acid treatment, and only minor amounts of the hydrolysis products were observed. The ester groups were unblocked by transesterification with NaOMe followed by saponification with aqueous NaOH to give the branched trisaccharide 40, which was isolated as the disodium salt in 96% yield. The assignment of the structure of 40 was based on the ¹³C NMR spectrum, which showed low-field-shifted signals for C-4 (69.23 ppm) and C-8 (70.51 ppm) accompanied by high-field-shifted signals of the adjacent carbon atoms (C-7 68.52, C-3 33.94, C-5 64.92 ppm). Reduction of the azido group by treatment with dithiothreitol finally produced the 4′′-amino derivative 41 albeit with the formation of a minor byproduct, which was not fully removed by chromatography. Hence, reduction of the azido group was accomplished by treatment of 40 with trimethylphosphane to afford the 4′′-amino derivative 41 in 99% yield.

NMR spectroscopic data for the allyl glycosides 23, 32, and 41 are in full agreement with data measured for LPS–core oligosaccharides.[⁸]

Conjugation  
Chain elongation of trisaccharide 41 with 1,3-propanedithiol produced the spacer derivative 42 in excellent yield.

Scheme 5. Reagents and conditions for the synthesis of Ara4N-(1→8)-Kdo disaccharide derivatives. (a) Hg(CN)₂/HgBr₂, MeNO₂, room temp., 14 h, 19% for 35, 5.5% for 38; (b) Ac₂O, DMAP, pyridine, room temp., 12 h, 75%; c) TiCl₄, CHCl₃, room temp., 13 h, then Ac₂O, DMAP, pyridine, room temp., 24 h, 68%; d) 0.1 m NaOMe, MeOH, room temp., 4 h, then 0.1 m NaOH, 3 h, 96%; (e) Me₃P, THF/0.1 m NaOH, room temp., 4 h, 99%.

Scheme 6. Reagents and conditions for the synthesis of neoglycoconjugates 43–45. (a) HS(CH₂)₃SH, H₂O, hv, NH₄⁺, room temp., 1 h 99%; (b) room temp., 2 h.
Synthesis of Neoglycoconjugates

Conclusions

The synthesis of neoglycoconjugates that contain inner core LPS epitopes composed of 4-amino-4-deoxy-L-arabinose residues glycosidically-linked to Kdo and Ko has been accomplished by a straightforward strategy that used a benzylated N-phenyl-trifluoroacetimide Ara4N donor and acetylated allyl glycoside acceptors. Selective cleavage of the benzyl protecting groups in the presence of the allicy aglycon with TiCl4 allows for the subsequent deprotection of the anomic center or transformation of the remaining terminal alkene for spacer elongation by Michael addition of the thiol moieties. The spacer ligands are useful probes for the preparation of glycanoparticles and glycoarrays, and the neoglycoconjugates serve as specific immunoreagents.

Experimental Section

General: Maleimide-activated BSA was purchased from Sigma Aldrich. Known compounds were identified by comparison with reported melting points as well as 1H and 13C NMR spectroscopic data. Melting points were determined with a Kofler hot stage microscope. Optical rotations were measured with a Perkin–Elmer 243 B Polarimeter. [α]D values are given in units of 10°deg·cm2·g–1. 1H NMR spectra were recorded at 297 K with a Bruker DPX instrument operating at 300, 400, or 600 MHz for 1H with CDCl3 as the solvent and Me4Si as the standard, unless stated otherwise. 13C NMR spectra were measured at 75.47, 100.62, or 150.9 MHz and referenced to Me4Si ppm. Data analysis was performed with Eur. J. Org. Chem. 2012, 119–131

Methyl 4-Azido-2,3-di-O-benzyl-4-deoxy-3-L-arabinopyranoside (2): Sodium hydride (60% in oil, 1.06 g, 26.5 mmol) was added to a solution of I[14] (2.01 g, 10.6 mmol) in dry DMF (40 mL) at 0 °C and stirred for 10 min. Benzyl bromide (3.04 mL, 28.6 mmol) in DMF (10 mL) was added over 5 min, and the solution was stirred at room temp. for 1 h. The reaction was quenched by addition of methanol (5 mL). The solution was diluted with CH2Cl2 (50 mL) and washed with water and brine. The organic phase was dried (MgSO4) and concentrated. The residue was dissolved in vacuo to give crude 2 (3.65 g, 93%) as a yellowish syrup. [α]D20 = +29.4 (c = 0.24, CHCl3). 1H NMR (300 MHz, CDCl3): δ = 7.41–7.25 (m, 10 H, Ph), 4.85–4.66 (m, 4 H, CH2Ph), 4.22 (d, J1,2 = 6.75 Hz, 1 H, 1- H), 3.95 (dd, J3,4a = 3.3, J3,5b = 12.4 Hz, 1 H; 5a-H), 3.79–3.74 (m, 1 H, 4-H), 3.70–3.61 (m, 2 H, 2-H, 3-H), 3.51 (s, 3 H, OCH3), 3.43 (dd, J3,5b = 1.8 Hz, 1 H, 5b-H) ppm. 13C NMR (CDCl3, 100 MHz): δ = 138.33, 137.71, 128.42, 128.31, 127.96, 127.85, 127.81 and 126.76 (C-Ar), 104.39 (C-1), 79.36 (C-3), 78.57 (C-2), 74.92 (CH2Ph), 72.82 (CH3Ph), 62.98 (C-5), 58.57 (C-4), 56.77 (OCH3) ppm.

4-Azido-2,3-di-O-benzyl-4-deoxy-L-arabinopyranosyl Fluoride (4): A solution of 2 (29.5 g, 79 mmol) in acetic acid (290 mL) and HCl (73 mL, 2.3 m) was kept at 65 °C for 15 h. The brownish solution was diluted with toluene (450 mL) and cooled to 0 °C. NaHCO3 (125 g) was slowly added at 0 °C until CO2 formation had ceased. The organic layer was then washed with saturated aqueous NaHCO3 and NaCl solutions, dried (MgSO4), and concentrated. The residue was dissolved in n-hexane/EtOAc, silica gel was added, and the solvent was removed in vacuo. The remaining solid was subjected to flash chromatography on silica gel (toluene/EtOAc, 3:1) to afford 4 (17.21 g, 61%) as a colorless solid. M.p. 62–63 °C, [α]D20 = +49.6 (c = 0.28, CHCl3). 1H NMR for β-anomer (400 MHz, CDCl3): δ = 7.38–7.27 (m, 10 H, Ph), 5.13 (d, J1,2 = 3.9 Hz, 1 H, 1-H), 4.77–4.66 (m, 4 H, CH2Ph), 3.96 (dd, J3,5a = 2.4, J3,5b = 11.8 Hz, 1 H, 5a-H), 3.95 (dd, J3,4a = 3.7, J3,5b = 7.8 Hz, 1 H, 3-H), 3.82–3.78 (m, 1 H, 4-H), 3.73 (dd, 1 H, 2-H), 3.69 (dd, J3,5b = 4.8 Hz, 1 H, 5b-H) ppm. 13C NMR (CDCl3, 100 MHz): δ = 137.64–136.70 (m, Cq, Ar), 128.89–128.68 (m, C-Ar), 92.00 (C-1), 76.11 (C-3), 76.02 (C-2), 73.75 (CH3Ph), 73.04 (CH3Ph), 69.00 (C-5), 59.38 (C-4) ppm. 1H NMR for α-anomer (400 MHz, CDCl3): δ = 7.38–7.27 (m, 10 H, Ph), 4.81 (d, J1,2 = 3.9 Hz, 1 H, 1-H), 4.77–4.76 (m, 4 H, CH2Ph), 4.02 (dd, J5a,5b = 7.4, J5a,5b = 11.8 Hz, 1 H, 5a-H), 3.84 (dd, J3,4a = 3.2, J3,5b = 5.8 Hz, 1 H, 3-H), 3.80–3.75 (m, 1 H, 4-H), 3.71 (dd, J3,5b = 3.4 Hz, 1 H, 5b-H), 3.57 (dd, 1 H, 2-H) ppm. 13C NMR (CDCl3, 100 MHz): δ = 137.64–136.70 (m, Cq, Ph), 128.89–128.68 (m, CH, Ph), 94.40 (C-1), 77.50 (C-3), 75.79 (C-2), 73.87 (CH3Ph), 73.42 (CH3Ph), 59.04 (C-5), 56.05 (C-4) ppm. C19H33N3O5 (355.40): calcd. C 64.21, H 5.96, N 11.82; found C 64.22, H 5.79, N 11.66.

4-Azido-2,3-di-O-benzyl-4-deoxy-L-arabinopyranosyl Fluoride (4): Diethylaminosulfur trifluoride (111 μL, 0.84 mmol) was slowly added to a solution of 3 (250 mg, 0.70 mmol) in dry CH2Cl2 (5 mL) at −20 °C. After stirring for 2 h at −20 °C, MeOH (0.5 mL) was added and stirring was continued for a further 10 min. The solution was diluted with CH2Cl2 (50 mL), washed with saturated aqueous NaHCO3, dried (MgSO4), and concentrated. The crude yellow oil was purified by column chromatography on silica gel (hexane/EtOAc, 10:1) to give an amionic mixture of 4 (250 mg, 99%) as a colorless liquid. 1H NMR (600 MHz, CDCl3): δ = 7.43–7.27 (m, 20 H, 4 × Ph), 5.55 (dd, J1,2,F = ca. 53.1, J1,2,F = ca. 2.6 Hz, 1 H, 1b-H), 5.35 (dd, J1,2,F = ca. 54.0, J1,2,F = ca. 2.6 Hz, 1 H, 1a-H), 4.89–4.56 (m, 8 H, 4 × OCH2), 4.23–4.18 (m, 1 H, 1a-H), 4.06 (dd, J1,2,F = 9.7, J3,4a,F = 3.7, 1 H, 3b-H), 3.96–3.92 (m, 2 H, 2b-H, 5b-H), 3.89 (dd, J1,2 = 25.0 Hz, 2b-H), 3.85–3.82 (m, 1 H, 3a-H), 3.97–3.67 (m, 4 H, 2a-H, 4a-H, 5b-H, 5b-H) ppm. 13C NMR (150 MHz, CDCl3): δ = 137.78, 137.65, 137.30, 137.07 (4 × C-Ar),
The suspension was filtered, the filtrate was concentrated, and the acetimidate (5a) and 4-Azido-2,3-di
H), 3.97 (dd, 3.4 Hz, 5b-H) and 4.16 (m, 1 H, 3-H), 3.81 (m, 1 H, 4-H), 3.67 (dd, 3.8 ppm. 13C NMR (75 MHz, CDCl3): 8 = 161.09 (C=NH), 138.05, 137.74, 128.45, 128.35, 127.87, 126.77, 124.77, 123.71 (C-Ar), 95.09 (C-5), 76.13 (C-2), 75.51 (C-3), 73.22, 73.16 (2 × CH2Ph), 63.05 (C-5), 59.77 (C-4) ppm.

Further elution of the column gave 5a as a colorless amorphous solid (166 mg, 59%). 1H NMR (400 MHz, CDCl3): δ = 8.63 (br. s, 1 H, NH), 7.38–7.28 (m, 10 H, 2 × Ph), 5.88 (d, J = 4.8 Hz, 1 H, H-1), 4.83–4.62 (m, 4 H, 2 × CH2Ph), 4.14 (dd, J = 9.5 Hz, 1 H, 2-H), 4.06 (dd, J = 2.5 Hz, 1 H, 3-H), 3.93 (dd, J = 6.1 Hz, 1 H, 2-H), 3.83 (dd, J = 3.2 Hz, 1 H, 3-H), 3.81 (m, 1 H, 4-H), 3.67 (dd, J = 3.2 Hz, 1 H, 5b-H). 13C NMR (75 MHz, CDCl3): δ = 161.27 (C=NH), 137.48, 137.33, 128.59–127.85 (C-Ar), 96.76 (C-1), 77.39 (C-3), 75.10 (C-2), 74.77, 72.77 (2 × CH2Ph), 62.08 (C-5), 56.76 (C-4) ppm.

4-Azido-2,3-di-O-benzyl-4-deoxy-L-arabinopyranosyl N-Phenyltrifluoracetimidochloride (6a) and 4-Azido-2,3-di-O-benzyl-4-deoxy-L-arabinopyranosyl N-Phenyltrifluoracetimidochloride (6b): A suspension of 3 (1 g, 2.81 mmol), K2CO3 (780 mg, 5.63 mmol), and N-phenyl-2,2′-trifluoracetimidoyl chloride (780 mg, 5.63 mmol) prepared according to ref[16] in acetone (10 mL) was stirred for 12 h at room temperature. The suspension was filtered. The filtrate was concentrated and the residue was chromatographed on silica gel (hexane/EtOAc: 9:1). Pooling of the fractions containing the least polar compound gave 2,3-di-O-benzyl-4,5-di-deoxy-L-threo-pent-4-enopyranosyl fluoride (8) as a colorless syrup (28 mg, 9%). 1H NMR (400 MHz, CDCl3): δ = 7.38–7.26 (m, 10 H, Ph-Phe), 6.18 (dd, J = 5.9 Hz, 1 H, 1-H), 6.03 (d, J = 9.5 Hz, 1 H, 2-H), 4.14 (dd, J = 9.5 Hz, 1 H, 3-H), 3.90 (dd, J = 3.2 Hz, 1 H, 4-H), 3.67 (dd, J = 3.2 Hz, 1 H, 5b-H) ppm. 13C NMR (100 MHz, CDCl3): δ = 148.94 (C-3), 138.05, 136.53 and 128.58–123.79 (C-Ar), 105.13 (J = 2F, J = 226.3 Hz, C-1), 94.55 (C-4), 73.66 (OCH3), 70.73 (dd, J = 23.5 Hz, C-2), 69.21 (CH2Ph) and 61.20 (J = 4.7 Hz, C-5). Continued elution furnished 7a (87 mg, 29%) as a colorless syrup. 1H NMR (400 MHz, CDCl3): δ = 7.42–7.19 (m, 10 H, Ph-Phe), 6.49 (d, J = 4.7 Hz, 1 H, NH), 5.48 (m, 1 H, 4-H), 4.48 (m, 1 H, 5b-H). 1H NMR (400 MHz, CDCl3): δ = 7.38–7.26 (m, 10 H, Ph-Phe), 6.18 (dd, J = 5.9 Hz, 1 H, 1-H), 6.03 (d, J = 9.5 Hz, 1 H, 2-H), 4.14 (dd, J = 9.5 Hz, 1 H, 3-H), 3.90 (dd, J = 3.2 Hz, 1 H, 4-H), 3.67 (dd, J = 3.2 Hz, 1 H, 5b-H) ppm. 13C NMR (100 MHz, CDCl3): δ = 148.94 (C-3), 138.05, 136.53 and 128.58–123.79 (C-Ar), 105.13 (J = 2F, J = 226.3 Hz, C-1), 94.55 (C-4), 73.66 (OCH3), 70.73 (dd, J = 23.5 Hz, C-2), 69.21 (CH2Ph) and 61.20 (J = 4.7 Hz, C-5).

Finally, elution of the column gave 7b (55 mg, 18%) as a syrup. 1H NMR (400 MHz, CDCl3): δ = 7.40–7.22 (m, 12 H, Ph-Ph), 7.08 (t, J = 7.6 Hz, 1 H, N-Phe (H-p)), 6.73 (d, 2 H, N-Phe (2 × α-CH)): 6.40 (br. s, 1 H, 1-H), 4.86–4.67 (m, 4 H, 2 × CH2Ph), 4.10–3.97 (m, 2 H, 2-H, 3-H), 3.97–3.69 (m, 3 H, 4-H, 5a-H, 5b-H) ppm. 13C NMR (100 MHz, CDCl3): δ = 143.48 (N-Phe-CH), 137.87, 137.71, 128.70, 128.48, 128.42, 127.91, 127.81, 127.77, 125.22 (C-Ar), 124.25 (N-Phe-Ph), 119.41 (N-Phe-C-0), 74.64 (C-2), 75.31 (C-3), 73.63 and 73.24 (CH2Ph), 62.95 (C-5) and 59.62 (C-4) ppm.

Further elution of the column gave 6b (752 mg, 50%) as a colorless syrup. 1H NMR (400 MHz, CDCl3): δ = 8.15–7.22 (m, 12 H, Ph-Ph), 7.09 (t, J = 7.6 Hz, 1 H, N-Phe (H-p)), 6.79 (d, 2 H, N-Phe (2 × α-CH)): 6.50 (br. s, 1 H, 1-H), 4.75–4.65 (m, 4 H, 2 × CH2Ph), 4.06 (br. s, 1 H, 5a-H), 3.88 (br. s, 1 H, 2-H), 3.78 (br. s, 2 H, 3-H, 4-H), 3.54 (br. s, 1 H, 5b-H) ppm. 13C NMR (100 MHz, CDCl3): δ = 143.49 (N-Phe-CH), 137.39, 137.32, 128.70, 128.51, 128.49, 128.07, 128.06 and 127.91 (C-Ar), 124.28 (N-Phe Cp), 119.38 (N-Phe Cp), 78.04 (C-3), 75.33 (C-2), 74.51 and 72.95 (2 × OCH2), 62.50 (C-5) and 59.91 (C-4) ppm.
pyranoside)onate (250 mg, 0.48 mmol) in dry MeOH (5 mL) was treated with methanolic NaOMe (0.25 mL, 0.1 m) for 3 h at room temp. The solution was deionized with Dowex 50 (H+)-cation-exchange resin, filtered, and the filtrate was concentrated. The residue was dried and dissolved in dry acetonitrile (5 mL). DABCO (65 mg, 0.58 mmol) and ( tert-butyl)chlorodimethylsilane (87 mg, 0.58 mmol) were added. The mixture was stirred at room temp. for 15 h. The suspension was concentrated and the residue was purified by flash chromatography (EtOAc). The product was taken up in dry pyridine (5 mL) and cooled to −10 °C. A catalytic amount of DMAP was added followed by a mixture of pyridine/ Ac2O (1:1, 4 mL). The solution was stirred for 12 h at room temp., cooled to 0 °C, and dry MeOH (3 mL) was added. Stirring was continued at 0 °C for a further 15 min. The solution was coevaporated several times with toluene, and the residue was taken up in chloroform (50 mL). The organic layer was washed with saturated aqueous NaHCO3 solution and dried (MgSO4). Concentration left a syrup which was purified on a column of silica gel (toluene/EtOAc, 5:1 + 1% triethylamine) to afford 14 (179 mg, 62%) as a colorless syrup. [α]D20 +46 (c = 1.8, CHCl3).

1H NMR (300 MHz, CDCl3): δ = 5.89–5.75 (m, 1 H, –CH2–), 5.52 (dd, Jα,β = 3.7, Jβ,γ = 0.9 Hz, 1 H, 1-3), 5.43 (t, Jα,β = 3.7 Hz, 1 H, 1-H), 5.35 (dd, Jα,β = 1.7 Hz, 1 H, 5-H), 5.27 (dq, 1 H, =CH2trans), 5.21–5.15 (m, 1 H, =CH2cis, 7-H), 4.32 (dd, Jα,β = 9.9 Hz, 1 H, 6-H), 4.23 (dq, 1 H, OCH2), 4.06 (dd, Jα,β = 11.9, Jβ,γ = 2.2 Hz, 1 H, 8a-H), 3.89–3.80 (m, 2 H, OCH2, 8b-H), 3.75 (s, 3 H, CO2CH3), 2.05 (s 3 H), 2.04 (s, 3 H), 1.99 (s, 3 H) and 1.96 (s, 3 H, 4× COCH3). 0.87 [s, 9 H, (CH3)3], 0.04 (s, 3 H) and 0.01 [s, 3 H, Si(CH3)3] ppm. 13C NMR (75 MHz, CDCl3): δ = 170.34, 169.79, 169.36, 168.45 (4× COCH3), 166.01 (C-1), 132.59 (–CH=), 117.54 (=CH2), 99.08 (C-2), 70.15 (C-7), 67.66 (C-3), 67.02 (C-6), 66.16 (C-4), 65.08 (OCH2), 64.20 (C-5), 61.12 (C-8), 52.61 (CO2CH3), 25.84 (C(CH3)3), 20.80, 20.65, 20.52, 20.49 (4× COCH3) and 18.43 (C(CH3)3) ppm. HRMS (ESI-TOF): calcld. for C29H44O12Si [M + Na]+ 863.2287; found 863.2280.

Desilylation of 10: A solution of 10 (736 mg, 1.38 mmol) was treated with a solution of 2% HF in MeCN (0.6 mL) in dry acetonitrile (5 mL) at room temp. for 2 h. Solid NaHCO3 was added to the solution and stirring was continued for 20 min. The suspension was filtered, and the filtrate was concentrated, coevaporated with toluene three times, and thoroughly dried to give 11 (578 mg, 99%) as a syrup. The material was immediately used for the subsequent glycosylation step.

Desilylation of 14: A solution of 14 (550 mg, 0.93 mmol) was treated with a solution of 2% HF in MeCN (0.6 mL) in dry acetonitrile (10 mL) at room temp. for 2 h. Solid NaHCO3 was added to the solution and stirring was continued for 20 min. The suspension was filtered, and the filtrate was concentrated, coevaporated with toluene three times, and thoroughly dried to give 15 (443 mg, 99%) as a syrup. The material was immediately used for the subsequent glycosylation step.

Methyl 4-Azido-2,3-di-O-benzyl-4-deoxy-β-D-arabinofuranosyl-(1→8)-(allyl 4,5,7-tri-O-acetyl-3-deoxy-a-D-manno-2-ulopyranoside)onate (16) and Methyl 4-Azido-2,3-di-O-benzyl-4-deoxy-a-D-arabinofuranosyl-(1→8)-(allyl 4,5,7-tri-O-acetyl-3-deoxy-a-D-manno-2-ulopyranoside) onate (18): A solution of 11 (578 mg, obtained by desilylation of 10) in dry CH2Cl2 (5 mL) was added to a suspension of 6 (1.45 g, 2.76 mmol) in dry CH2Cl2 (20 mL) with 4 Å molecular sieves. The suspension was stirred for 2 h at −15 °C under Ar. A solution of TMSOTf (12.5 µL, 69 µmol) in dry CH2Cl2 (2 mL) was slowly added and stirring was continued for 1 h with gradual warming to room temp. The reaction was quenched by adding triethylamine (50 µL). The suspension was diluted with CH2Cl2 (20 mL), filtered through a short plug of Celite®, and was washed with CH2Cl2. The filtrate was washed with saturated aqueous NaHCO3, dried (MgSO4), and concentrated. The crude material (2 g) was directly applied to column chromatography (toluene/EtOAc, 3:1), which gave a mixture of 16/19 (550 mg, 52%) and 18 (230 mg, 22%) as a colorless syrup. [α]D20 +45 (c = 0.5, CHCl3).

1H NMR (400 MHz, CDCl3): δ = 7.36–7.27 (m, 10 H, Ph-H), 5.75 (m, 1 H, –CH=), 5.33 (dd, Jα,β = 3.0, Jβ,γ = 1.4 Hz, 1 H, 5-H), 5.29 (dd, Jα,β = 5.0, Jα,γ = 12.0 Hz, 1 H, 4-H), 5.24–5.18 (m, 2 H, 2-H, 7-H, =CH2trans), 5.12 (dq, 1 H, =CH2cis), 4.74–4.63 (m, 4 H, 2× CH2Ph), 4.32 (dq, Jα,β = 6.2 Hz, 1 H, 1’-H), 4.10 (dd, Jα,β = 2.4, Jβ,γ = 11.2 Hz, 1 H, 8a-H), 4.06 (dd, Jα,β = 9.6 Hz, 1 H, 6-H), 4.00 (m, 1 H, OCH3), 3.94 (dd, Jα,β = 12.1, Jβ,γ = 4.1 Hz, 1 H, 5’a-H), 3.87 (m, 1 H, OCH2), 2.73 (m, 3 H, CO2CH3), 3.77–3.72 (m, 2 H, 2’-H, 4’-H), 3.68–3.62 (m, 2 H, 2’-H, 3’-H), 3.42 (dd, Jα,β = 2.2 Hz, 1 H, 5’b-H), 2.18 (dd, Jα,β = 12.7 Hz, 1 H, 3e-H), 2.09–2.04 (m, 4 H, 3e-H, OCH2), 1.97 (s, 3 H) and 1.90 (s, 3 H, 2× COCH3) ppm. 13C NMR (100 MHz, CDCl3): δ = 170.46–169.79 (3× OCH3), 167.77 (C-1), 138.07, 137.61 (2× C-Ar), 133.28 (–CH=), 128.44, 128.37, 127.90, 127.80, 127.78 and 127.71 (6× C-Ar), 117.26 (=CH2), 102.85 (C-1’), 98.53 (C-2’), 78.99 (C-3’), 77.71 (C-2’), 74.63 and 73.71 (2× OCH2Ph), 68.83 (C-7), 68.73 (C-6), 67.34 (C-8), 66.41 (C-4), 64.77 (OCH2), 64.49 (C-5), 62.38 (C-5’), 57.94 (C-4’), 52.62 (OCH2), 31.93 (C-3), 20.84–20.67 (3× OCH3) ppm. HRMS (ESI-TOF): calcld. for C34H46O12N2 [M + HCOO + Na]+ 880.2884; found 880.2890.
Acetylation of 17: A solution of 17 (230 mg, 0.35 mmol) in dry pyridine (3 mL) was stirred with acetic anhydride (2 mL) in pyridine (1 mL) with a catalytic amount of DMAP for 24 h at room temp. The solution was cooled to 0 °C, methanol (3 mL) was added, and stirring was continued for 30 min. The solution was coevaporated with toluene. The residue was directly applied to column chromatography (toluene/EtOAc, 5:1) to give 16 (241 mg, 80 %) as a colorless syrup. \[\text{yield} = \frac{0.35}{0.23} \times 100\% = 80\%\]

Sodium 4-Azido-4-deoxy-β-L-arabinopyranosyl-(1→8)-(allyl 3-deoxy-α-manno-oct-2-ulopyranoside)onate (22): A solution of 21 (33 mg, 0.045 mmol) in dry methanol (2 mL) was stirred with methanolic NaOMe (0.1 mL, 0.1 mmol) for 4 h at room temp. The solution was neutralized with Dowex 50 (H⁺) cation-exchange resin. The suspension was filtered, and the filtrate was concentrated. A solution of the residue in water (2 mL) was then adjusted to pH 12 with aqueous NaOH (ca. 2 mL, 0.2 M) and stirred at room temp. for 3 h. The solution was neutralized with Dowex 50 (H⁺) cation-exchange resin. The resin was removed by filtration and the filtrate was lyophilized to give 22 (20 mg, 98 %) as a colorless syrup. \[\text{yield} = \frac{0.20}{0.23} \times 100\% = 98\%\]

Methyl 2,3-Di-O-acetyl-4-azido-4-deoxy-β-D-arabinopyranosyl-(1→8)-(allyl 4,5,7-tri-O-acetyl-3-deoxy-α-manno-oct-2-ulopyranoside)onate (21): A solution of 16 (56 mg, 0.074 mmol) in dry chloroform (5 mL) was stripped with argon. A solution of TiCl₄ (172 µL, 0.156 mmol) in chloroform (1 mL) was added slowly at 0 °C. After 1 h, additional TiCl₄ (17.2 µL) was added until the TLC showed the complete consumption of starting material and the appearance of a lower migrating compound (Rf 0.18, EtOAc/toluene, 1:1). Ethyl ether (10 mL) and saturated aqueous NaHCO₃ solution (5 mL) were added with caution and the solution was stirred for 30 min. The organic layer was dried (MgSO₄) and concentrated. The residue was purified by flash chromatography over silica gel (toluene/EtOAc, 1:1) to give the crude debenzylated product. The residue of this product in pyridine (2 mL) and a catalytic amount of DMAP was cooled to 0 °C. A solution of acetic anhydride in pyridine (2 mL; 2:1) was added slowly and the reaction was stirred for 24 h at room temp. Dry MeOH (2 mL) was added at 0 °C and stirring was continued for 30 min. The solution was coevaporated with toluene five times and concentrated. The residue was purified by column chromatography (toluene/EtOAc, 4:1) to furnish 21 (33 mg, 68 %) as a colorless syrup. \[\text{yield} = \frac{0.074}{0.035} \times 100\% = 69\%\]
100.80 (C-2), 99.92 (C-1’), 72.33 (C-6), 70.84 (C-8), 68.90, 68.53 (C-2’), 67.32 (C-7), 67.03 (C-4), 66.79 (C-5), 65.19 (OCH3), 60.07 (C-5’), 52.30 (C-4’) and 34.92 (C-3’) ppm. HRMS (ESI-TOF): calcd. for C16H33NO15 [M + H]+ 410.1657; found 410.1659.

Ammonium 4-Amino-deoxy-β-D-arabinofuranosyl(1→8)-(3-mercaptopyrrolidinyl)propyl 3-deoxy-a-D-manno-2-octulopyranoside (24): Propane-1,3-dithiol (0.1 mL) was added to a solution of 23 (2.6 mg, 6.3 μmol) in MeOH (2 mL) and stripped with argon in a quartz flask. The reaction mixture was stirred under UV radiation (254 nm) for 1 h at room temp. to give full conversion. The solution was diluted with H2O (2 mL) and washed with chloroform (2 mL) five times. The organic phases were reextracted with water, and the combined aqueous phases were stripped with argon until the solution became clear. The solution was lyophilized, and the residue was purified on BioGel P-2 (5%aq. EtOH) to give 24 (2.1 mg, 63%) as a white solid. [α]D 20 = +76.5 (c = 0.2, H2O). 1H NMR (400 MHz, D2O): δ = 5.04 (d, J1,2 = 3.7 Hz, 1 H, 1-1H), 4.19-4.05 (m, 5 H, 3’-H, 4-H, 5-H, 5’-α-H, 7-βH), 3.93 (dd, Jαβ,α = 10.6, Jβγ,α = 2.7 Hz, 1 H, 8a-H), 3.86 (dd, Jβγ,β = 7.5 Hz, 1 H, 8b-H), 3.80 (dd, Jγδ,γ = 9.8 Hz, 1 H, 2-H), 3.72 (dd, Jδε,γ = 12.9, Jεζ,ε = 2.2 Hz, 1 H, 3-H), 3.61 (dd, Jζη,ζ = 9.2 Hz, 1 H, 5-H), 3.63–3.49 (m, 2 H, 4’,H-OC2H3), 3.38–3.36 (m, 1 H, OC2H3), 2.73–2.64 (m, 6 H, 3’-CH2), 2.06 (sd, Jαβ,α = 13.1, Jαα,α = 5.0 Hz, 1 H, 0–H), 1.95–1.87 (m, 4 H, 2×CH2), 1.80 (t, Jαα,α = 13.1 Hz, 1 H, 3H-α-H) ppm. 13C NMR (100 MHz, D2O): δ = 176.28 (C-1), 101.05 (C-2), 99.83 (C-1’), 72.77 (C-6), 71.29 (C-8), 69.12 (C-2’), 68.73 (C-3’), 67.87 (C-7), 67.23 (C-4), 67.70 (C-5), 65.26 (OCH2CH3), 60.65 (C-5’), 52.38 (C-4’), 35.22 (C-3’), 33.58 (OCH2CH2CH3), 30.50 (OCH2CH2CH2CH3), 29.57 (SCH2CH2CH2SH), 26.22 (SCH2CH2CH3) ppm. HRMS (ESI-TOF): calcd. for C16H33NO15 [M – H]+ 516.1579; found 516.1576.

Methyl 4-Azido-2,3-di-O-benzyl-deoxy-β-D-arabinofuranosyl(1→8)-(allyl)3,4,5,7-tetra-O-acetyl-D-glycero-a-o-talo-oct-2-ulopyranoside (25) and Methyl 4-Azido-2,3-di-O-benzyl-deoxy-a-L-arabinofuranosyl(1→8)-(allyl)3,4,5,7-tetra-O-acetyl-D-glycero-a-o-talo-oct-2-ulopyranoside (27): A solution of 15 (443 mg, 0.93 mmol) was taken up in dry CH2Cl2 (5 mL) and added to a suspension of 6 (970 mg, 1.86 mmol) in dry CH2Cl2 (5 mL) containing molecular sieves 4 Å. The suspension was stirred under argon at –15 °C for 2 h. A solution of TMSOTf (8.4 μL, 0.04 mmol) dissolved in dry CH2Cl2 (5 mL) was slowly added. After 1 h, triethylamine (30 μL) was added slowly. The suspension was filtered through a short plug of Celite, and the Celite bed was washed with CH2Cl2. The filtrate was washed with saturated aqueous NaHCO3 solution, dried (MgSO4), filtered, and concentrated. The crude mixture was directly applied to column chromatography (toluene/EtOAc, 3:1) to give a mixture of 25/28 (430 mg, 57%) and 27 (284 mg, 37%) as a colorless syrup. [α]D 20 = +24 (c = 0.8, CHCl3).

1H NMR (600 MHz, CDCl3): δ = 7.39–7.27 (m, 10 H, Ph-H), 5.79 (m, 1 H, –CH2=), 5.22 (dd, Jαα,α = 13.1 Hz, 1 H, CH2=CH2), 5.11 (dd, Jαα,α = 13.1 Hz, 1 H, 3-H), 3.22–3.37 (m, 5 H, 3’-H, 4-H, 5-H, 5’-α-H, 7-βH), 3.20–3.04 (m, 2 H, 4’,H-OC2H3), 2.87–2.69 (m, 6 H, 3’-CH2), 1.79–1.62 (m, 4 H, 2×CH2), 1.19 (t, Jαα,α = 7.2 Hz, 3 H, 3H-α-H) ppm. 13C NMR (150 MHz, CDCl3): δ = 173.24 (C-1), 150.87 (C-2), 146.80 (C-3), 129.85 (C-4), 128.64, 128.57, 128.50 (C-Ar), 127.49 (C-2’), 126.80 (C-5), 126.68 (C-6), 126.52 (C-7), 126.37 (C-3’), 125.10 (C-4’), 113.27 (C-5’), 51.30 (C-2), 41.00 (C-3), 38.60 (C-4), 38.38 (C-5), 21.63 (C-6), 18.71 (C-8), 16.07 (C-9) ppm.
showed the complete consumption of starting material to a lower solution was neutralized with Dowex 50 (H +) cation-exchange C118.09 (=Sodium 4-Azido-4-deoxy-\(\text{H11032}\)) NMR (100 MHz, CDCl 3): 13C NMR (100 MHz, CDCl3): \(\delta\) = 174.04 (C-1), 134.25 (–CH=), 118.29 (\(\text{=CH}=\)), 102.66 (C-2), 101.33 (C-1’), 72.41 (C-3), 72.14 (C-6), 70.58 (C-8), 69.54 (C-2’), 69.40 (C-3’), 68.85 (C-5), 68.50 (C-7), 67.09 (C-4), 65.03 (OCH3), 63.08 (C-4’) and 61.21 (C-5’) ppm. HRMS (ESI-TOF): calcd. for C14H12N3O16 [M – H] – 450.1366; found 450.1366.

Methyl 4-Azido-2,3-di-O-acetyl-4-deoxy-\(\beta\)-1-arabinopyranosyl-(1-\(\beta\)-3)4,5,7-tetra-O-acetyl-4-glycero-a-talo-5-talo-2-olopyranoside)onate (30): A solution of 25 (169 mg, 0.207 mmol) in dry MeOH (10 mL) was diluted with H2O (2 mL) and washed with chloroform (2 mL) five times. The organic phases were reextracted with water and the combined aqueous phases were stripped with argon until the solution became clear again. The material obtained upon lyophilization was dissolved in H2O (1 mL) and transferred to a packed column of Dowex 50 (H +) cation-exchange resin. The column was washed with water (20 mL) under TLC control. The product was then eluted with ammonia (20 mL, 1 m) and lyophilized to give 32 (12 mg, 60%) as a white amorphous solid. HRMS (ESI-TOF): calcd. for \(\text{H11032}\) 348.2232; found 348.2224.

Methyl 4-Azido-2,3-di-O-acetyl-4-deoxy-\(\beta\)-1-arabinopyranosyl-(1-\(\beta\)-3)4,5,7-tetra-O-acetyl-4-glycero-a-talo-5-talo-2-olopyranoside)onate (30): A solution of 25 (169 mg, 0.207 mmol) in dry MeOH (10 mL) was diluted with H2O (2 mL) and washed with chloroform (2 mL) five times. The organic phases were reextracted with water and the combined aqueous phases were stripped with argon until the solution became clear again. The material obtained upon lyophilization was dissolved in H2O (1 mL) and transferred to a packed column of Dowex 50 (H +) cation-exchange resin. The column was washed with water (20 mL) under TLC control. The product was then eluted with ammonia (20 mL, 1 m) and lyophilized to give 32 (12 mg, 60%) as a white amorphous solid. HRMS (ESI-TOF): calcd. for \(\text{H11032}\) 348.2232; found 348.2224.

Ammonium 4-Amino-4-deoxy-\(\beta\)-1-arabinopyranosyl-(1-\(\beta\)-3)4,5,7-tetra-O-acetyl-4-glycero-a-talo-5-talo-2-olopyranoside)onate (32): A solution of 31 (20 mg, 0.045 mmol) in H2O/diisopropylamine (3:1, 2 mL) was stripped with argon in a light protected flask. Dithiothreitol (29.8 mg, 0.183 mmol) was added, and the solution was stirred for 2 h at room temp. under argon. Another portion of dithiothreitol (29.8 mg) was added to complete the conversion. The solution was diluted with H2O (2 mL) and washed with chloroform (2 mL) five times. The organic phases were reextracted with water and the combined aqueous phases were stripped with argon until the solution became clear again. The material obtained upon lyophilization was dissolved in H2O (1 mL) and transferred to a packed column of Dowex 50 (H +) cation-exchange resin. The column was washed with water (20 mL) under TLC control. The product was then eluted with ammonia (20 mL, 1 m) and lyophilized to give 32 (12 mg, 60%) as a white amorphous solid. HRMS (ESI-TOF): calcd. for \(\text{H11032}\) 348.2232; found 348.2224.
Synthesis of Neoglycoconjugates

(–8), 69.09 (C-2′, C-5), 68.72 (C-3′), 68.24 (C-7′), 67.34 (C-4′), 62.69 (OCH 2 ), 61.26 (C-5′), 51.23 (C-4′), 33.54 (OCH 2 CH 2 CH 2 SH), 30.42 (OCH 2 CH 2 CH 2 SH), 29.46 (SCH 2 CH 2 CH 2 SH), 28.83 (SCH 2 CH 2 CH 2 SH) and 23.59 (SCH 2 CH 2 CH 2 SH) ppm. HRMS (ESI-TOF): calcd. for C 46 H 62 NO 12 [M + Na] + 703.4526; found 703.4526.

Dimethyl 4-Azido-2,3-di-O-benzyl-4-deoxy-β-L-arabinopyranosyl-(1→8)-[(4,5,7,8-O-tetra-O-acetyl-3-deoxy-a-D-manno-2-0ct-o-2-ribofuranosylo)onate-(2→4)]-(allyl 3-deoxy-a-D-manno-2-0ct-o-2-ribofuranosylo)onate (35) and Dimethyl 4-Azido-2,3-di-O-benzyl-4-deoxy-β-L-arabinopyranosyl-(1→8)-[(4,5,7,8-O-tetra-O-acetyl-3-deoxy-a-D-manno-2-0ct-o-2-ribofuranosylo)onate-(2→7)]-(allyl 3-deoxy-a-D-manno-2-0ct-o-2-ribofuranosylo)onate (38): A suspension of 17 (88 mg, 0.139 mmol), Hg(CN) 2 (23.3 mg, 0.092 mmol), HgBr 2 (16.6 mg, 0.046 mmol), and 4 Å molecular sieves in dry nitromethane (10 mL) was stirred at room temp for 2 h. A solution of 34 (67.5 mg, 0.14 mmol) in MeNO 2 (5 mL) was added over 2 h using a syringe pump. After 6 h, additional portions of 34 (34 mg, 0.007 mmol), Hg(CN) 2 (12.0 mg, 0.046 mmol), and HgBr 2 (8.3 mg, 0.023 mmol) were added and stirring was continued for 8 h. The suspension was diluted with EtOAc (50 mL) and filtered through a short plug of Celite®. The Celite bed was washed with EtOAc, and the combined filtrates were washed with aqueous KI solution (15%), saturated aqueous NaHCO 3 solution, and dried (MgSO 4 ). The organic phase was concentrated and the residue was purified by column chromatography on silica gel (toluene/EtOAc, 1:1) to afford 36 (29 mg, 75%) as a colorless syrup.

Dimethyl 4-Azido-2,3-di-O-benzyl-4-deoxy-β-L-arabinopyranosyl-(1→8)-[(4,5,7,8-O-tetra-O-acetyl-3-deoxy-a-D-manno-2-0ct-o-2-ribofuranosylo)onate-(2→4)]-(allyl 5,7-di-O-acetyl-3-deoxy-a-D-manno-2-0ct-o-2-ribofuranosylo)onate (36): Acetic anhydride (0.2 mL) was added to a solution of 35 (36 mg, 0.034 mmol) in dry pyridine (4 mL) and a catalytic amount of DMAP at 0 °C. The solution was stirred for 12 h at room temp and the reaction was quenched by the addition of dry MeOH (1 mL) at 0 °C. The solution was concentrated and coevaporated several times with toluene. The residue was dissolved in CHCl 3 , washed with saturated aqueous NaHCO 3 solution and dried (MgSO 4 ). The solution was concentrated and the residue was purified by column chromatography on silica gel (toluene/etoAc, 1:1) to afford 36 (29 mg, 75%) as a colorless syrup.
at room temp. and processed as described for 36. The residue was afforded 39 (17 mg, 68%) as a colorless syrup. [α]D = +96° (c = 0.4, CHCl3). 1H NMR (600 MHz, CDCl3): δ = 5.88 (m, 1H, −CH=), 5.35–5.33 (br, 1H, 5′′-H), 5.25 [dq, 1H, =CH2(trans)], 5.03–5.16 (m, 2H, 3′′, 4′′-H), 5.15–5.10 [m, 5H, 1′′, 4′′-H, 5′-, 7′-, =CH2(cis)], 5.01 (dt, Jα=β = 9.8, Jα,β = 2.3 Hz, 1H, 7-H), 4.80 (dd, Jα,β = 2.4, Jα,γ = 12.2 Hz, 1H, 8′-a), 4.72 (dd, Jα,β = 3.0, Jα,γ = 4.8, Jγ,β = 11.9 Hz, 1H, 4-H), 4.15 (d, 1H, OCH3), 4.13 (d, Jα,β = 1.1, Jα,γ = 9.4 Hz, 1H, 6′-H), 4.11–4.08 (br, 1H, 1′-, 8′-H). 13C NMR (150 MHz, CDCl3): δ = 170.69, 170.44, 170.42, 170.31, 170.13, 170.11, 169.64 and 169.49, (8×COCH3), 167.81 (C-1′), 167.04 (C-1), 133.29 (−CH=), 116.84 (=CH2), 98.61, 97.31 (C-2′, C-2′′), 97.15 (C-1′′-), 69.39 (C-6′), 69.36 (C-7′), 69.12 (C-3′), 68.12 (C-2′), 68.06 (C-6′), 67.85 (C-8′), 66.34 (C-4′), 66.24 (C-24), 65.14 (C-8′), 64.95 (C-5′), 64.61 (OCH3), 64.28 (C-5′′), 61.33 (C-8′′), 59.97 (C-5′′′), 59.53 (C-4′′′), 52.66 and 52.63 (2×CO2CH3), 34.11 (C-3), 31.39 (C-3′′), 20.82–20.54 (8×CH3) ppm. HRMS (ESI-TOF): calcld. for C41H65N3O18 [M + H] + 866.4316; found: 866.4317.

**Disodium 4-Azido-4-deoxy-β-L-arabinopyranosyl-(1→8)-(3-deoxy-a-D-manno-oct-2-ulosylphosphonate)onate-(2→4)-(allyl 3-deoxy-a-D-manno-oct-2-ulosylphosphonate)onate** (33): A solution of 40 (3 mg, 4.5 μmol) in THF/0.1 M NaOH (1.2 mL) was stirred with trimethylphosphine (1.3 mL, 18.3 μmol) for 4 h at room temp. The solution was diluted with H2O (2 mL) and washed with chloroform (2 mL) five times. The organic phases were extracted with water. The combined aqueous phases were stripped with argon until the solution became clear again. The solution was lyophilized, and the residue was purified by gel chromatography (BioGel P-2) and lyophilized to give 41 (2.8 mg, 99% as a colorless amorphous solid. [α]D = +46.5° (c = 0.4, H2O). 1H NMR (600 MHz, D2O): δ = 5.93 (m, 1H, −CH=), 5.32 [dq, 1H, =CH2(trans)], 5.22 [dd, 1H, =CH2(cis)], 4.98 (d, Jα,β = 3.7 Hz, 1H, 1′-H), 4.22 (dd, Jα,β = 2.6, Jα,γ = 5.2, Jγ,β = 12.0 Hz, 1H, 4-H), 4.10 (dd, Jα,γ = 2.9, Jγ,β = 4.7 Hz, 1H, 3′-H), 4.07–4.02 (m, 3H, 4′-, 5′-, 7′-H), 4.00 (br. s, 1H, 5′-H), 3.94–3.90 (m, 3H, 7′-, 8′-a, OCH3), 3.83 (d, 1H, OCH3), 3.78 (dd, Jα,β = 10.9, Jα,γ = 6.0 Hz, 1H, 8a-H), 3.77 (dd, Jα,γ = 3.0, 1H, 1′-H, 8b-H), 3.72 (dd, Jα,β = 12.9, Jα,γ = 10.0 Hz, 1H, 5′′-b-H), 3.71 (dd, 1H, 2′-H), 3.68 (dd, Jα,γ = 13.8, 1H, 1′′-H, 8b-H), 3.62 (br. s, 1H, 4′-H), 3.59 (dd, Jα,γ = 9.8 Hz, 1H, 1′-H), 3.57 (dd, Jα,β = 9.5 Hz, 1H, 6′-H), 3.57 (dd, Jα,β = 13.1, Jα,γ = 5.1 Hz, 1H, 3′-H), 3.20 (dd, Jα,β = 13.6 Hz, 1H, 3′-H), 1.95 (dd, Jα,β = 12.4 Hz, 1H, 3′-H), 1.78 (1H, 3′-a′-H) ppm. 13C NMR (150 MHz, D2O): δ = 175.97 and 175.06 (C-1, C-1′), 134.07 (−CH=), 113.17 (=CH3), 100.20 and 99.32 (C-2′, C-2′′), 99.15 (C-1′′), 72.40 (C-6′), 71.41 (C-6), 70.00 (C-8), 70.63 (C-7′), 68.50 (C-4′), 68.17 (C-7′′), 67.86 (C-5′), 66.63 (C-5′′), 65.92 (C-3′′), 64.20 (C-5′′), 64.03 (OCH3), 63.18 (C-8′), 57.97 (C-5′′′), 51.66 (C-4′′′), 34.51 (C-3′′) and 32.31 (C-3′) ppm. HRMS (ESI-TOF): calcld. for C23H32NO18 [M + H] + 630.2240; found 630.2233.

Synthesis of Neoglycoconjugates 43–45: Thiol 24 (2.1 mg) was dissolved in a freshly prepared conjugation buffer [0.5 mL, 20 mM sodium phosphate buffer, 100 mM ethylenediaminetetraacetic acid (EDTA), 80 mM sucrose, pH 6.6] and immediately added to a solution of 5 mg/mL maleimide-activated BSA (20 mM sodium phosphate buffer, 230 mM NaCl, 2 mM EDTA, 80 mM sucrose, pH 6.6). The reaction mixture was purged with argon for 2 min and stirred at room temp for 2 h. The reaction mixture was purified over a Sephadex G-25M column (0.01 mL phosphate-buffered saline in double distilled H2O as eluent). The protein-containing fractions were pooled, lyophilized, and purified again over a P-2 gel column (30×1 cm, water) to afford 44 (18.4 mg) as a colorless solid. Neoglycoconjugate 45 was prepared from 33 (2.0 mg) by this procedure. Yield of 45: 20 mg. Neoglycoconjugate 43 was synthesized from 42.
Synthesis of Neoglycoconjugates

(2.0 mg) accordingly to give 43 (6.4 mg) as a colorless powder. MS (MALDI-TOF) measurements of the BSA-conjugates gave maximum peak intensities at 72733 for 43, 77264 for 44, and 75165 Da for 45, which correspond to average ligand-to-protein ratios of 5.4 for 43, 16.5 for 44, and 13.6 mol/mol for 45.

Supporting Information (see footnote on the first page of this article): 1H and 13C NMR spectra of deprotected oligosaccharides.

Acknowledgments

Financial support of this work by the Austrian Science Fund (FWF grant P 19295) is gratefully acknowledged. Technical assistance by Maria Holbel is acknowledged. The authors thank Martin Pabst and Ralph Hollaus for ESI-TOF MS data and Buko Lindner (Research Center Borstel) for providing MALDI-TOF MS data for the glycoconjugates.

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