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Synthesis of a spacer-containing disaccharide fragment of *Bordetella pertussis* lipopolysaccharide

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Abstract

The disaccharide 2-(*p*-aminophenyl)ethyl 4-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-2,3-diacetamido-2,3-dideoxy-α-D-mannopyranoside uronate, which is assumed to be a partial structure of the *Bordetella pertussis* polysaccharide, was synthesized starting from D-glucose and D-glucosamine, respectively. The major synthetic transformations were conversion of D-glucosamine into the donor ethyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-β-D-glucopyranoside and conversion of glucose, by a sequence involving 2,3-epoxide formation/opening, nucleophilic triflate displacement in the 3-position, and necessary protecting group manipulations, into the acceptor 2-(*p*-trifluoroacetamidophenyl)ethyl 6-*O*-benzyl-2,3-diazido-2,3-dideoxy-α-D-mannopyranoside. Coupling of the donor and acceptor units promoted by dimethyl(methylthio)sulfonium triflate followed by selective oxidation of the 6′-position and deprotection gave the target disaccharide.

Keywords: *Bordetella pertussis*; Glycoconjugate; Vaccine

1. Introduction

Bacterial lipopolysaccharides are important bacterial surface components, that have been studied for nearly a century by immunologists and chemists. Chemical structures are now known for lipopolysaccharides of a great number of bacterial species [1]. Immunologically, the carbohydrate part of bacterial lipopolysaccharides give rise to highly specific immune responses. Because of this, oligosaccharides derived from bacterial lipopolysaccharides should be efficient components of glycoconjugate vaccines, in analogy with what has been shown [2] with bacterial capsular polysaccharides. Here, the efficacy of polysaccharide–protein conjugate vaccines is a proven fact, and commercial vaccines already exist, e.g., against childhood meningitis caused by *Haemophilus influenzae* type B bacteria. Even short synthetic fragments [3] of the natural polysaccharide have been shown to be highly immunogenic when conjugated to a protein.

To aid in the development of conjugate vaccines, we have previously synthesized penta- and decameric fragments [4,5] as well as a pentameric analog [6] of *H. influenzae* type B capsular polysaccharide, and a nonasaccharide fragment [7] of the capsular polysaccharide from *Streptococcus pneumoniae* 19F. The present article describes synthesis work on a *Bordetella pertussis* lipopolysaccharide fragment. The entire lipopolysaccharide structure of *B. pertussis* has not yet been published, but the partial structure shown

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below has been proposed [8,9]. Our own preliminary NMR investigations [10] of a pentasaccharide obtained from partial degradation of *B. pertussis* LPS confirmed the GlcNAc-(1→4)-Man*p*A2,3-$(NAc)_2$- structural element, and indicated that both the glycosidic linkages were alpha. The absolute configurations of the component monosaccharides are, to our knowledge, unknown, but our working hypothesis is that the GlcNAc and Man-$A(NAc)_2$ units are in the D form. Based on this tentative structure and indications that this part of the lipopolysaccharide is highly immunogenic [8–10], we undertook the synthesis of the disaccharide **16**, the details of which is hereby reported. The synthesized disaccharide carries an amino group for later simple and specific conjugation to proteins. The proposed partial structure [8,9] of *B. pertussis* lipopolysaccharide is shown in (1):

GlcNAc*p*-(1→4)-Man*p*A2,3-$(NAc)_2$-(1→3)-

Fuc*p*N(Ac)Me-(1 – – – → (1)

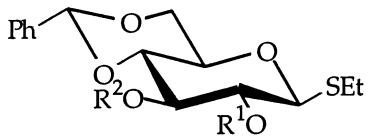
2. Results and discussion

The major challenge in the synthesis of disaccharide **16** is the construction of the 2,3-diacetamidomannuronate unit, a monosaccharide, which to our knowledge, has not been synthesized before. It has been detected as a constituent of both *B. pertussis* [8,9] and *Pseudomonas aureginosa* [11] lipopolysaccharides, in a glycoprotein from *Bacillus stearothermophilus* [12,13], and in teichuronic acids [14]. It was decided to synthesize a 2,3-diacetamido-D-mannuronic acid derivative from D-glucose, and to introduce first the acetamido functions via azide opening of a 2,3-epoxide (to give a 2-azido function) followed by azide displacement of a 3-triflate (to give a 3-azido function), and then to introduce the carboxylate function at the disaccharide stage of the synthesis. The following steps were carried out to synthesize disaccharide **16** (Scheme 1).

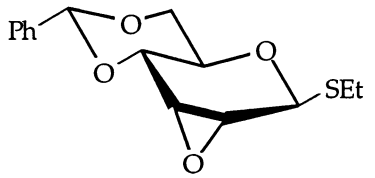
Compound **1** [15] was monotosylated in the 3-position with *p*-toluenesulfonyl chloride in pyridine to give crystalline **2** in 50% yield. The position of tosylation was evident from the ^{1}H NMR data of **2**. Treatment of **2** with sodium hydroxide in toluene–dimethyl sulfoxide–butanol in the presence of tetrabutylammonium hydrogensulfate and potassium carbonate gave the *allo*-epoxide **3**, which was directly reacted with sodium azide and ammonium chloride in dimethyl sulfoxide. The expected 2-azide **4** (diaxial epoxide opening) was isolated in a total yield of 82% (from **2**). In the literature, similar preparations of 2-azido-*altro* compounds carrying other glycosidic substituents have been reported [16]. To introduce a second azido function, compound **4** was treated with triflic anhydride–pyridine to give an intermediate 3-triflate **5**, which was directly reacted with sodium azide in dimethyl sulfoxide. The resulting S_N2 displacement product, crystalline 2,3-diazido compound **6** (manno configuration) was isolated in 52% yield. Next, the temporary thioglycoside function in **6** was replaced by a 2-(*p*-trifluoroacetamidophenyl)-ethyl glycosidic function. Reaction of the ethyl thioglycoside **6** with 2-(*p*-trifluoroacetamidophenyl)ethanol and dimethyl(methylthio)-sulfonium triflate (DMTST) [17,18] resulted in the formation of the two possible anomeric glycosides **7** and **8**, which were separated by chromatography. The desired α glycoside **7** was isolated in 44% yield. The anomeric configuration of **7** was ascertained by the $^3J_{C\text{-}1,H\text{-}1}$ value (171 Hz for **7**, as compared with 158 Hz for **8**), as well as by observed strong nOe contacts between H-1 and H-3/H-5 in **8** but not in **7**. Variations of the glycosylation conditions (including use of glycosyl bromide and silver triflate or tetraethylammonium bromide) in order to obtain a more favorable α/β ratio were unsuccessful.

Opening of the benzylidene ring in **7** with sodium cyanoborohydride–fluoroboric acid yielded compound **9** (73% yield from **7**), which was used as an acceptor in a DMTST-promoted disaccharide coupling with donor **10** (prepared as described [19] from D-glucosamine). As expected, the α-disaccharide derivative **11** was formed as a major product together with minor amounts of the corresponding β isomer. However, the α/β mixture (86% total glycosylation yield, α/β ratio 83:17) proved difficult to resolve by chromatography at this stage, so the crude mixture was instead

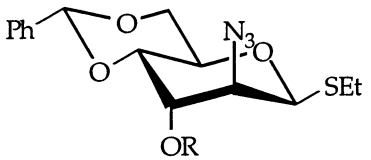
subjected to treatment with hydrogen sulfide in pyridine–triethylamine–water (to reduce the azido functions), followed by acetylation of the formed triamino products with acetic anhydride in pyridine. The resulting mixture of triacetamido derivatives could now be



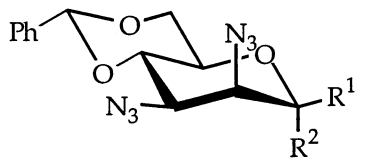
1 $R^1 = R^2 = H$
2 $R^1 = H$, $R^2 = Ts$



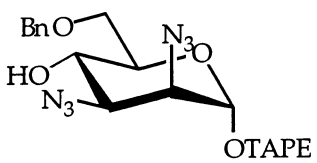
3



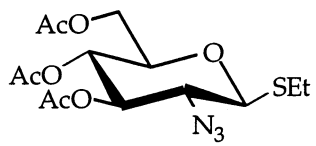
4 R = H
5 R = Tf



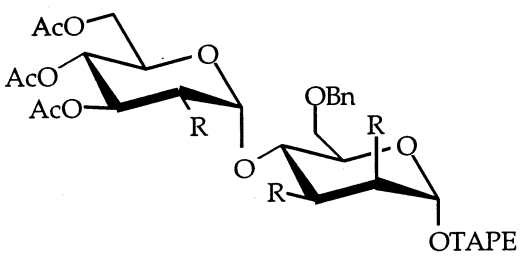
6 R^1 = SEt, $R^2 = H$
7 $R^1 = H$, R^2 = OTAPE
8 R^1 = OTAPE, $R^2 = H$



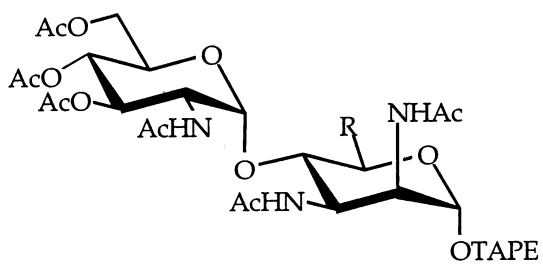
9



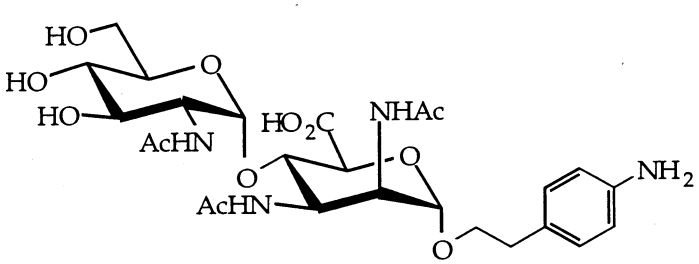
10



11 R=N_3
12 R=NH_2
13 R=NHAc

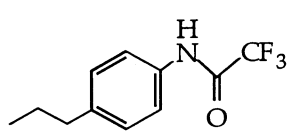


14 R=CH_2OH
15 R=CHO



16

TAPE =



Scheme 1.

resolved by column chromatography to give the pure α anomer **13** in 40% yield (calculated from **10**). Removal of the benzyl group of **13** by catalytic hydrogenation gave the 6′-OH compound **14**, which was then treated with dimethyl sulfoxide–acetic anhydride. The resulting oxidation product (aldehyde **15**) was stable to further oxidation by the reagent, and was therefore isolated (58% yield) and treated with iodine in dioxane–water to complete the oxidation to a carboxyl group (several other attempts to carry out the hydroxyl to carboxyl oxidation, i.e., with 2,2,6,6-tetramethylpiperidinyloxy radical or pyridinium dichromate, were made but gave inferior results). The crude carboxylic acid was treated in situ with aqueous potassium hydroxide to remove *O*-acetyl groups. The conditions required for removal of the acetate groups resulted in loss also of the *N*-trifluoroacetyl group, so the final product disaccharide **16** was isolated as a 2-(*p*-aminophenyl)ethyl glycoside in 67% yield (from **15**). Purification by gel filtration and analysis by NMR and mass spectroscopy verified the structure of **16** in all respects. Conjugation of **16** to proteins and immunological studies with the obtained neoglycoconjugate will be reported elsewhere.

3. Experimental

General methods.—Concentrations were performed at reduced pressure (bath temperature < 40 °C, unless otherwise stated). Weighed yields are calculated from residues dried in vacuum overnight. Optical rotations were measured at 27 °C (*c* 1.0, chloroform, unless otherwise stated), using a Perkin–Elmer 341 polarimeter. NMR spectra were recorded for solutions in $CDCl_3$ (internal Me_4Si, $\delta = 0.00$) or D_2O (internal acetone, ^1H $\delta = 2.225$, ^{13}C $\delta = 30.7$) at 303 K unless otherwise stated with Bruker DRX 400 or DRX 600 spectrometers. Only selected NMR data are reported. Assignments were corroborated by appropriate 2D experiments. Coupling constants (in Hz) are given within brackets. The FABMS spectra were recorded with a Jeol JMS-SX/SX-102A instrument. Ions were produced by a beam of Xe-atoms (6 keV), using a matrix of glycerol or *m*-nitrobenzyl alcohol. For HRMS, PEG was used as an internal standard. TLC was performed on Silica Gel F_{254} (E. Merck, Darmstadt, Germany) with detection by UV light and by staining with 5% sulfuric acid in ethanol or 0.5% ninhydrin in butanol. Column chromatography was performed on Matrex silica gel 60 Å (35–70 μm, Amicon). Molecular sieves (powdered 3 Å and 4 Å) were dried at 280 °C in vacuo overnight. Dichloromethane was distilled from P_2O_5 when necessary. Dimethyl(thiomethyl)sulfonium triflate (DMTST) was prepared by mixing equimolar amounts of methyl triflate and dimethyl disulfide in dry dichloromethane at 0 °C, precipitating with dry ether, and filtering off (the hygroscopic) solid, essentially as previously described [17,18]. The material was stored under dry nitrogen at −20 °C.

*Ethyl 4,6-*O*-benzylidene-3-*O*-p-toluenesulfonyl-1-thio-β-*D*-glucopyranoside* (**2**).—To a stirred and cooled (0 °C) solution of ethyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**1**) [15] (5.0 g, 16.0 mmol) in pyridine was added *p*-toluenesulfonyl chloride (9.2 g, 48.2 mmol) and the mixture was slowly allowed to reach rt. After stirring for 70 h the mixture was cooled (0 °C) and water (0.9 mL) was added. The mixture was stirred for another 40 min, then diluted with toluene, washed with water, aq 2 M sulfuric acid, aq. 1 M sodium hydrogen carbonate, dried (Na_2SO_4), filtered and concentrated to give a liquid residue ($\approx$ 20 mL). This residue was applied to a short, wide column (100 g silica, packed and eluted with 6:1 toluene–ethyl acetate), pure fractions were concentrated and crystallized from ethanol. More crystals were obtained by repeating the chromatographic and crystallization procedure with the contaminated chromatographic fractions and the concentrated mother liquor, giving a total yield of compound **2** of 3.76 g (50%) as needles, mp 115–116 °C, $[\alpha]_D$ −83°; NMR data ($CDCl_3$): ^{13}C, δ 15.2 ($SCH_2\mathit{C}H_3$), 21.7 (CH_3Ar), 24.9 ($S\mathit{C}H_2CH_3$) 68.5 (C-6), 70.7 (C-5), 71.9 (C-2), 77.3 (C-4), 83.1 (C-3), 86.8 (C-1), 101.7 (CHPh), ^1H, δ 1.32 (t, 3H, $SCH_2C\mathit{H}_3$), 2.31 (s, 3H, CH_3Ar), 2.76 (m, 2H, $SC\mathit{H}_2CH_3$), 3.03 (d, 1 H, OH, $J_{OH,2} = 2.4$), 3.48 (m, 1 H, H-5), 3.63

(t, 1 H, H-4, $J_{4,5} = 9.5$), 3.67–3.74 (m, 2 H, H-2/H-6a), 4.34 (dd, 1 H, H-6b, $J_{6b,5} = 5.0$, $J_{6b,6a} = 10.6$), 4.51 (d, 1 H, H-1, $J_{1,2} = 9.7$), 4.70 (dd, 1 H, H-3, $J_{3,2} = 8.6$, $J_{3,4} = 9.5$), 5.37 (s, 1 H, CHPh). (HRMS: Calc. for $C_{22}H_{27}O_7S$: 467.1198. Found: 467.1219 $[M + H^+]$.

*Ethyl 2-azido-4,6-*O*-benzylidene-2-deoxy-1-thio-β-*D*-altropyranoside* (**4**).—To a stirred solution of **2** (4.38 g, 9.38 mmol) in toluene–dimethyl sulfoxide–*n*-butanol (80/4/1.6 mL) was first added tetrabutylammonium hydrogensulfate (0.64 g, 1.89 mmol), then a powdered mixture of sodium hydroxide (0.98 g, 24.5 mmol) and potassium carbonate (3.0 g, 21.7 mmol). The mixture was stirred for 2 h at rt, then diluted with toluene, washed with water, dried (Na_2SO_4), filtered and concentrated. A mixture of the residue (containing crude **3**), sodium azide (2.45 g, 37.7 mmol) and ammonium chloride (2.52 g, 47.1 mmol) in dimethyl sulfoxide (40 mL) was stirred at 80 °C for 20 h. The reaction mixture was then diluted with ethyl acetate, washed with water and aq 1 M sodium hydrogen carbonate, dried (Na_2SO_4), filtered and concentrated. The residue was crystallized from ethanol giving needles of **4** (1.36 g, 43%). Column chromatography of the mother liquor with ethyl acetate in petroleum ether (stepwise gradient elution, 20–25%) gave more **4** (1.24 g, 39%), mp 105–106 °C, $[\alpha]_D$ −28°; NMR data ($CDCl_3$): ^{13}C, δ 15.1 (SCH_2CH_3), 26.1 (SCH_2CH_3), 65.6 (C-2), 67.1 (C-5), 68.6 (C-3), 69.1 (C-6), 76.4 (C-4), 82.1 (C-1), 102.3 (CHPh), ^1H, δ 1.32 (t, 3 H, SCH_2CH_3), 2.77 (q, 2 H, SCH_2CH_3), 3.82 (t, 1 H, H-6a, $J_{6a,6b} = 10.2$), 3.86 (dd, 1 H, H-2, $J_{2,3} = 3.5$), 3.91 (dd, 1 H, H-4, $J_{4,5} = 9.6$), 3.97 (m, 1 H, H-5), 4.27 (t, 1 H, H-3, $J_{3,4} = 3.0$), 4.33 (dd, 1 H, H-6b, $J_{6b,5} = 4.8$), 5.13 (d, 1 H, H-1, $J_{1,2} = 1.7$), 5.63 (CHPh). HRMS: Calc. for $C_{15}H_{20}N_3O_4S$: 338.1174. Found: 338.1183 $[M + H^+]$.

*Ethyl 2,3-diazido-4,6-*O*-benzylidene-2,3-dideoxy-1-thio-β-*D*-mannopyranoside* (**6**).—A solution of **4** (2.82 g, 8.36 mmol) in 2:1 dichloromethane–pyridine (30 mL) was stirred and cooled (0 °C) while triflic anhydride (3.5 mL, 20.9 mmol) was added dropwise during 15 min. After 1 h, cooling was removed and the reaction mixture was stirred another 1.5 h, then diluted with dichloromethane, washed with aq 1 M sulfuric acid, aq 1 M sodium hydrogen carbonate, water, dried (Na_2SO_4), filtered and concentrated. The residue (containing crude **5**) was dissolved in dimethyl sulfoxide (20 mL) and added to a mixture of sodium azide (2.72 g, 41.7 mmol) and dimethyl sulfoxide (50 mL). After 30 min at rt the reaction mixture was partitioned between toluene and water, and the toluene layer was washed with water, dried (Na_2SO_4), filtered and concentrated. Column chromatography of the residue with ethyl acetate in petroleum ether (stepwise gradient elution, 17–50%) gave **6** (1.57 g, 52%) as a solid which could be crystallized from ethanol giving needles, mp 144–145 °C; $[\alpha]_D$ −5°; NMR data ($CDCl_3$): ^{13}C, δ 15.1 (SCH_2CH_3), 26.0 (SCH_2CH_3), 63.1 (C-3), 65.1 (C-2), 68.4 (C-6), 72.1 (C-5), 77.4 (C-4), 84.5 (C-1), 101.7 (CHPh), ^1H, δ 1.31 (t, 3 H, SCH_2CH_3), 2.75 (q, 2 H, SCH_2CH_3), 3.46 (m, 1 H, H-5, $J_{5,6b} = 5.0$), 3.86 (t, 1 H, H-6a, $J_{6a,6b} = 10.4$), 3.92 (dd, 1 H, H-3, $J_{3,4} = 10.1$), 3.97 (dd, 1 H, H-2, $J_{2,3} = 3.7$), 4.07 (t, 1 H, H-4, $J_{4,5} = 9.3$), 4.32 (dd, 1 H, H-6b), 4.73 (d, 1 H, H-1, $J_{1,2} = 1.5$), 5.64 (CHPh). HRMS: Calc. for $C_{15}H_{19}N_6O_3S$: 363.1239, Found: 363.1284 $[M + H^+]$.

*2-(*p*-Trifluoroacetamidophenyl)ethyl 2,3-diazido-6-*O*-benzyl-2,3-dideoxy-α-*D*-mannopyranoside* (**9**).—To a stirred mixture of **6** (0.65 g, 1.79 mmol), 2-(trifluoroacetamidophenyl)-ethanol (0.50 g, 2.14 mmol) and 4 Å molecular sieves in dichloromethane (30 mL) was added DMTST (1.38 g, 5.36 mmol), and the reaction mixture was stirred at rt for 70 h, after which pyridine (1.5 mL) was added, and stirring was continued for 30 min. The mixture was then filtered through Celite, diluted with dichloromethane, washed with aq 2 M sulfuric acid, aq 1 M sodium hydrogen carbonate, water, dried (Na_2SO_4), filtered and concentrated. Column chromatography of the residue with ethyl acetate in petroleum ether (stepwise gradient elution, 25–33%) gave first **7** (0.42 g, 44%, NMR data ($CDCl_3$): 4.74 (1.6)/98.2 (171) (H-1/C-1) and then **8** (0.34 g, 36%, NMR data ($CDCl_3$): 4.62 (nd)/100.8 (158) (H-1/C-1)). To a stirred mixture of the α-glycoside **7** (0.15 g, 0.28 mmol), sodium cyanoborohydride (71 mg, 1.12 mmol) and 3

Å molecular sieves in THF (4 mL) at 0 °C, was added dropwise a solution of fluoroboric acid (54% in diethyl ether; 0.15 mL, 1.12 mmol) in THF (1 mL). Additional fluoroboric acid (0.05 mL, 0.37 mmol) was added after 90 min, and after 4 h the reaction mixture was filtered through Celite, diluted with ethyl acetate, washed with aq 1 M sodium hydrogen carbonate, brine, dried (Na_2SO_4), filtered and concentrated. Column chromatography of the residue with ethyl acetate in toluene (stepwise gradient elution, 17–25%) gave the title compound **9** as a solid (0.11 g, 73%), $[\alpha]_D$ + 62°. NMR data ($CDCl_3$): ^{13}C, δ 35.4 (CH_2Ar), 61.9 (C-2), 62.1 (C-3), 68.4 (OCH_2CH_2Ar), 69.2 (C-4), 70.2 (C-5), 70.6 (C-6), 73.8 (CH_2Ph), 97.4 ($J_{H,C} = 171$, C-1), ^1H, δ 2.88 (t, 2 H, CH_2Ar), 3.40 (m, 1 H, H-5), 3.59 (dd, 1 H, H-6a, $J_{6a,6b} = 9.9$, $J_{6a,5} = 5.8$), 3.64–3.68 (m, 2H, H-6b, OCH*H*CH_2Ar), 3.74 (dd, 1 H, H-2, $J_{2,3} = 3.6$), 3.79 (dd, 1 H, H-3, $J_{3,4} = 9.7$), 3.83–3.92 (m, 2 H, H-4, OC*H*HCH_2Ar), 4.50 and 4.59 (2 d, 2 H, CH_2Ph), 4.74 (d, 1 H, H-1, $J_{1,2} = 1.3$). HRMS: Calc. for $C_{23}H_{25}F_3N_7O_5$: 536.1869, Found: 536.1851 [M + H^+].

*2-(*p-*Trifluoroacetamidophenyl)ethyl 4*-O-*(2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-α*-D-*glucopyranosyl)-2,3-diacetamido-6*-O-*benzyl-2,3-dideoxy-α*-D-*mannopyranoside* (**13**).—A mixture of ethyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-α-D-glucopyranoside (**10** [19], 0.169 g, 0.451 mmol), **9** (0.254 g, 0.47 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (0.049 g, 0.237 mmol) and 4 Å molecular sieves in dichloromethane (8 mL), was stirred at rt for 45 min. DMTST (0.349 g, 1.35 mmol) was added and the reaction mixture was stirred at rt for 14 h. Then pyridine was added, the mixture was filtered through Celite, diluted with dichloromethane, washed with aq. 2 M sulfuric acid, aq 1 M sodium hydrogen carbonate, dried (Na_2SO_4), filtered and concentrated. Column chromatography of the residue with ethyl acetate in toluene (20%) gave the α-disaccharide **11** in admixture with its β isomer (0.328 g, 86%, α/β ratio: 83:17). To a solution of this syrupy α/β-mixture (0.32 g, 0.38 mmol) in 2:1.5:1 pyridine–triethylamine–water (19.5 mL) was slowly bubbled hydrogen sulfide for 55 min. The flask was then sealed and stirring was continued for 1 h. The resulting mixture was concentrated and co-concentrated twice with pyridine. The residue, containing crude tri-amine **12**, was stirred with 2:1 pyridine–acetic anhydride (9 mL) for 90 min, then concentrated and co-concentrated twice with toluene. Flash column chromatography of the residue with methanol in ethyl acetate (stepwise gradient elution, 10–20%) gave the title compound **13** as a syrup (0.154 g, 40%, calculated from **10**), $[\alpha]_D$ + 70°. NMR data ($CDCl_3$) are given in the table below.

	1	2	3	4	5	6a	6b
Glc	5.07 (3.5) 97.8 (175)	4.28 (10.7) 51.4	5.02 (10.6) 71.7	5.17 (9.9) 67.4	3.64 69.2	3.86 (12.6) 61.4	4.01
Man	4.60 (1.9) 97.2 (170)	4.15 (2.9) 53.6	4.73 (9.7) 50.5	3.82 (nd) 71.4	2.89 (nd) 71.2	3.31 (11.1) 69.2	3.43

Further $^1H/^{13}C$ chemical shifts are 1.91/23.7 (s, 6 H, 2 CH_3CO), 2.00/20.7, 2.04/20.9, 2.08/20.9, 2.10/23.3 (4 s, 12 H, 4 CH_3CO), 2.85, 2.98/35.8 (CH_2Ar), 3.86, 4.01/61.4 (CH_2CH_2-Ar), 5.87, 5.94, 6.30 (3 d, 3H, 3N*H*Ac). HRMS: Calc. for $C_{41}H_{52}F_3N_4O_{15}$: 897.3381. Found: 897.3428 [M + H^+].

*2-(*p-*Aminophenyl)ethyl 4*-O-*(2-acetamido-2-deoxy-α*-D-*glucopyranosyl)-2,3-diacetamido-2,3-dideoxy-α*-D-*mannopyranoside uronate* (**16**).—A mixture of **13** (50 mg, 0.056 mmol) and Pd/C (25 mg; 10%) in 1:1 ethanol–ethyl acetate (6 mL) was hydrogenated at atmospheric pressure for 2 h, then additional Pd/C (50 mg; 10%) was added and the mixture was hydrogenated for another 3 h. The mixture was filtered through Celite, diluted with ethanol, concentrated and dried in vacuum for 12 h. The residue (containing crude **14**) was dissolved in a mixture of 2:1 dimethyl sulfoxide–acetic anhydride (3 mL) and stirred at rt. for 6 h, then diluted with ethyl acetate, washed with water, dried (Na_2SO_4), filtered and concentrated. Column chromatography of the residue with methanol in 9:1 ethyl acetate gave the aldehyde **15** as a syrup (26.2 mg, 58%). To a stirred solution of **15** (22 mg, 0.015 mmol) and iodine (8 mg, 0.0185 mmol) in 6:4

dioxane–water (1.5 mL) was added dropwise aq 4% (w/v) potassium hydroxide. The pH was kept between 8–9 for 1 h until **15** was consumed, then an additional aq 4% (w/v) potassium hydroxide was added until the pH was 11. The reaction mixture was heated to 60 °C and stirred for another 36 h. Then dry ice was added until neutral pH, and the mixture was concentrated. Column chromatography of the residue (12:3:3:2–4:3:3:2 ethyl acetate–methanol–acetic acid–water) followed by gel filtration (Sephadex G-15 column) using 95:5 water–butan-1-ol as eluant gave the title compound **16** as a white powder (11 mg, 67% from **15**), $[\alpha]_D$ +19° (*c* 0.5, water). NMR data ($CDCl_3$) are given in the table below.

	1	2	3	4	5	6a	6b
Glc	5.07	3.82	3.69	3.47	3.72	3.81	3.81
	(3.8)						
	96.6	53.9	70.7	69.8	72.1	60.3	
Man	4.69	4.17	4.37	3.95	3.96		
		(3.2)	(9.1)				
	98.1	51.1	50.7	70.8	73.9	176.1	

Further $^1H/^{13}C$ chemical shifts are 1.88/22.1, 2.01/22.2, 2.03/22.1 (3 s, 9 H, 3 CH_3CO), 2.95/34.6 (CH_2Ar), 3.76, 3.96/68.5 (CH_2-CH_2Ar), 7.10/120.1, 7.34/130.4 (Ar), 174.5, 174.8, 174.9 (3 C=O). HRMS: Calc. for $C_{26}H_{39}N_4O_{12}$: 599.2564. Found: 599.2581 $[M + H^+]$.

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