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Carbohydrate Research 329 (2000) 549–560

CARBOHYDRATE RESEARCH

Synthesis of neoglycoproteins containing D-*glycero*-D-*talo*-oct-2-ulopyranosylonic acid (Ko) ligands corresponding to core units from *Burkholderia* and *Acinetobacter* lipopolysaccharide

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Received 5 June 2000; accepted 10 July 2000

Abstract

Glycal esters of Kdo derivatives were converted into 2,3-anhydro intermediates, which were transformed into D-*glycero*-D-*talo*-oct-2-ulopyranosylonic acid (Ko), as well as 3-*O*- and 4-*O*-*p*-nitrobenzoyl-Ko derivatives. The *exo*-allyl orthoester derivative, methyl {5,7,8-tri-*O*-acetyl-4-*O*-(4-nitrobenzoyl)-2,3-*O*-[(1-*exo*-allyloxy)-ethylidene]-D-*glycero*-β-D-*talo*-oct-2-ulopyranos}onate, prepared from the 4-*O*-*p*NBz-protected Ko derivative, was elaborated into the α-Ko allyl ketoside, the reducing disaccharide α-Kdo*p*-(2→4)-Ko and the disaccharide α-Kdo*p*-(2→4)-Ko*p*-(2→OAll). Conversely, methyl[4,5,7,8-tetra-*O*-acetyl-3-*O*-(4-nitrobenzoyl)-α-D-*glycero*-D-*talo*-2-octulopyranosyl bromide]onate [*Carbohydr. Res.*, 244 (1993) 69–84], was coupled with a Kdo acceptor to give the disaccharide α-Ko*p*-(2→4)-Kdop-(2→OAll) after orthoester rearrangement and deprotection. The allyl glycosides were treated with cysteamine and converted into neoglycoproteins. The ligands correspond to inner core units from *Acinetobacter haemolyticus* and *Burkholderia cepacia* lipopolysaccharides.

Keywords: Lipopolysaccharides; Kdo; Ko; Neoglycoproteins

1. Introduction

Lipopolysaccharides (LPS) are complex glycolipids located in the outer membrane of Gram-negative bacteria. Within the core region of bacterial LPS, 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) provides the linkage of the core to the lipid A domain [1]. In a few bacterial strains, however, Kdo is replaced by 3-deoxy-D-*threo*-hex-2-ulosonic acid [2], 3-deoxy-D-*arabino*-hept-2-ulosaric acid [3] or D-*glycero*-D-*talo*-oct-2-ulopyranosylonic acid (Ko). Ko has first been detected as a constituent of the main chain in the LPS core from *Acinetobacter calcoaceticus* NCTC 10305 and *Acinetobacter haemolyticus* [4–6]. Members of this genus are frequently involved in a wide spectrum of nosocomial infections, which are difficult to treat due to increasing antibiotic resistance of the strains [7,8]. Ko has also been found as a lateral substituent of a Kdo residue in the inner core of *Burkholderia cepacia* [9], a bacterium which is gaining increasing biomedical interest, since it may cause severe necrotizing pneumonias (cepacia-syndrome) in patients with cystic fibrosis [10].

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0008-6215/00/$ - see front matter
PII: S0008-6215(00)00213-5

In continuation of previous work on the synthesis of the disaccharide α-Ko*p*-(2→6)-β-D-Glc*p*NAc [11], we report herein the synthesis of disaccharide ligands containing α-(2→4)-interlinked Ko and Kdo units. Neoglycoproteins derived from the allyl glycosides will be used for the preparation and characterization of Ko-specific monoclonal antibodies, as well as for the further characterization of the epitope specificities of known Kdo-reactive monoclonal antibodies.

2. Results and discussion

The known glycal ester **1** [12] was used as starting material for the synthesis of the D-*glycero*-D-*talo*-oct-2-ulopyranosylonic acid (Ko, **4**). An alternative synthetic approach to obtain **4** has been published recently [13]. Epoxidation with *m*-chloroperbenzoic acid gave the α-configured oxirane **2** [14], which was hydrolyzed with wet silica gel. Subsequent O-acetylation afforded the crystalline D-*glycero*-D-*talo*-configured derivative **3** in 40% overall yield. Hydrogenolysis of the benzyl ester group followed by deacetylation and Bio-Gel P-2 chromatography furnished sodium D-*glycero*-D-*talo*-oct-2-ulopyranosylonate (**4**) in 95% yield. ^{1}H and ^{13}C NMR data obtained for **4** (Table 1) indicated the presence of α-pyranose (40%), β-furanose (40%), α-furanose (15%) and β-pyranose (5%). The ^{1}H NMR signals for H-3, H-4 and H-5 of the furanoses displayed a downfield shift (3.99–4.28 ppm) out of the bulk region and could be assigned to both anomeric forms considering the chemical shift of the ^{13}C NMR signal for C-3 (74.36 for the α anomer and 76.87 for the β anomer). Hydrolysis of the previously reported α-2,3-*anhydro*-derivative **6**, obtained from the glycal methyl ester **5**, gave a mixture of compounds [11]. NMR analysis of the hydrolysis products indicated that acetyl migration from O-4 to O-3 had occurred. Hence, this by-product was regarded as a suitable precursor for the synthesis of α-(2→4)-linked disaccharide derivatives **22** and **24**. Upon

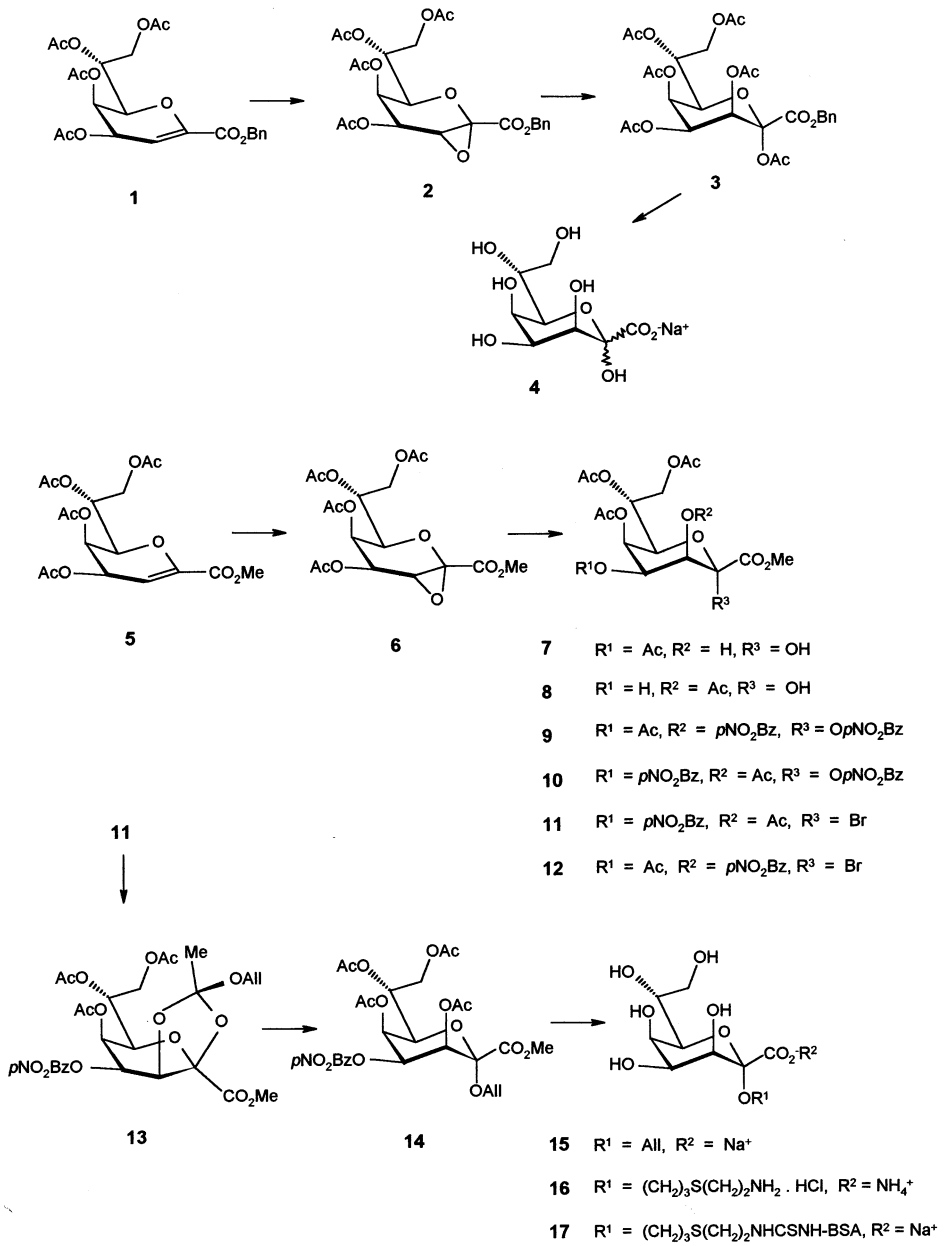
Table 1
^{13}C NMR data [a] for monosaccharide **4** and for the disaccharides **22**, **24** and **31**

Residue	Carbon	**4** α-pyr	**4** β-fur	**4** α-fur	**4** β-fur	**22** α-pyr	**24**	**31**
α-K(d)o-(2→	1	n.d. [b]	176.36	n.d.	n.d	176.16 [e]	176.51	174.34
	2	98.23	n.d.	n.d.	n.d.	100.77	100.60	101.82
	3	72.50	74.36	76.87	72.92	35.46	35.22	72.26
	4	67.12	71.32	71.32	68.19	n.d.	66.73	66.51
	5	69.14	83.46	82.97	68.52	67.03	67.08	68.70
	6	72.17	71.82 [d]	70.43	74.48	73.30	73.35	72.74
	7	70.22	71.96 [d]	n.d.	69.80	70.59	70.69	70.41
	8	63.87 [c]	63.87 [c]	63.80[c]	64.64	64.23	63.86	63.47
→4-α-K(d)o*p*	1					176.64 [e]	174.14	175.51
	2					98.51	102.65	100.45
	3					72.22	69.38	33.78
	4					67.37	72.71	69.56
	5					69.65	67.31	64.74
	6					n.d.	72.33	71.91
	7					70.49	70.28	69.84
	8					64.23	63.86	63.47
Allyl	1						65.15	64.57
	2						134.45	134.42
	3						118.27	117.69

[a] Spectra were recorded at 297 K and referenced to 1,4-dioxane (67.40 ppm).
[b] Not determined.
[c] Assignments may be reversed.
[d] Assignments may be reversed.
[e] Assignments may be reversed.

treatment of the material present in the hydrolysis mixture with 4-nitrobenzoyl chloride and 4-*N*,*N*-dimethylaminopyridine in pyridine, the 4-*O*-*p*-nitrobenzoyl derivative **10** was isolated as the major product, together with the previously described 3-*O*-*p*-nitrobenzoyl compound **9** [11]. Conversion of **10** into the bromide donor **11** (95%) was effected with $TiBr_4$. The reactivity of the glycosyl donor **11** was first investigated by its reaction with allyl alcohol under Helferich conditions, which furnished the *exo*-allyl orthoester derivative **13** (96%). The assignment of the *exo*-orientation of the allyloxy group was based on the ^1H NMR chemical shift of the *endo* methyl group observed at 1.82 ppm [15].

Orthoester rearrangement in the presence of substoichiometric amounts of trimethylsilyl trifluoromethanesulfonate [16] furnished exclusively the α-allyl glycoside **14** (74%). Deprotection of **14** by Zemplén de-O-acylation and subsequent alkaline hydrolysis of the methyl ester gave the previously known sodium allyl D-*glycero*-α-D-*talo*-oct-2-ulopyranosidonate (**15**, 93%) [17]. Radical addition of cysteamine hydrochloride to the allyl group

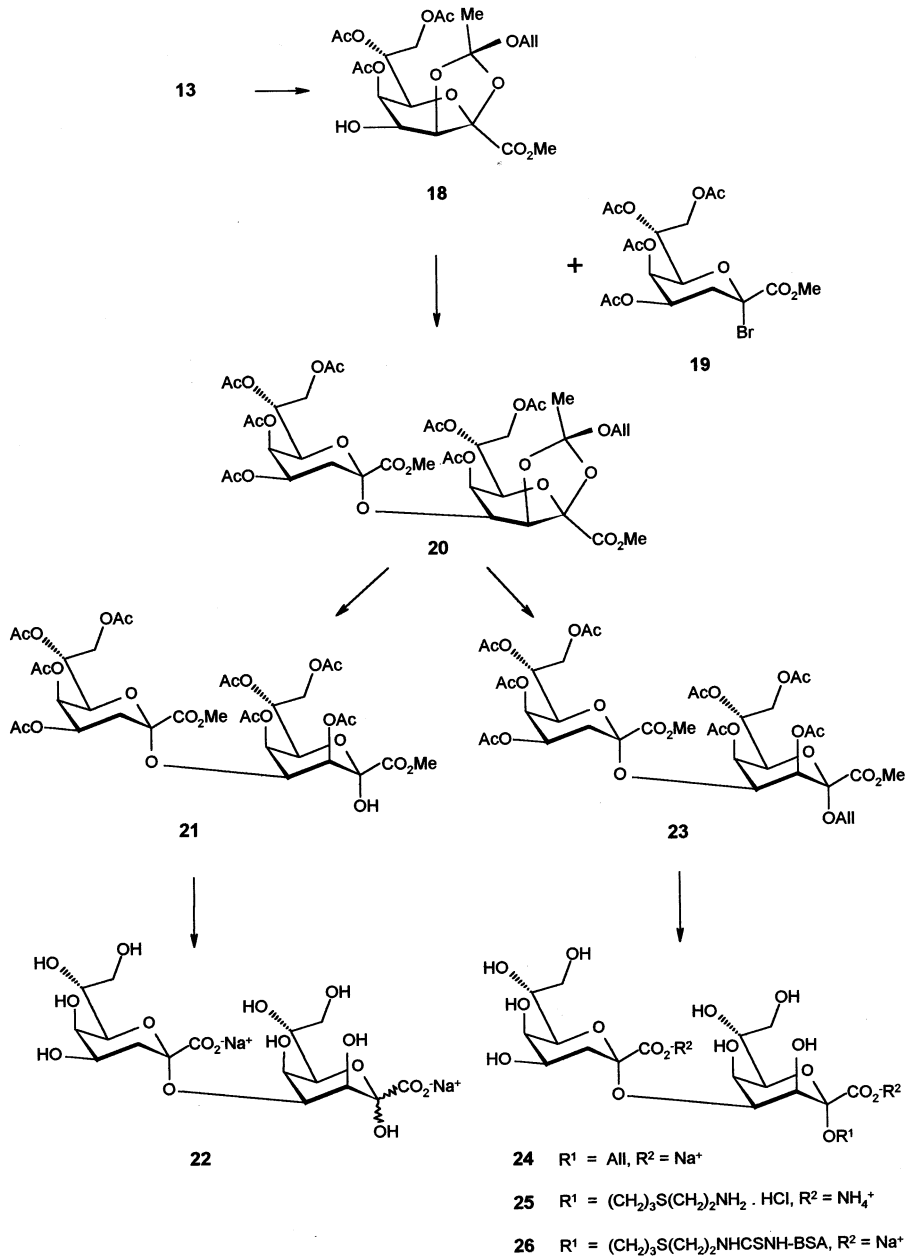


Scheme 1.

afforded the corresponding 3-(2-aminoethylthio)propyl glycoside **16** (65%). It was activated with thiophosgene and coupled to bovine serum albumin [18–20] (Scheme 1).

For the synthesis of the α-Kdo*p*-(2→4)-linked disaccharide derivatives, orthoester **13** was selectively de-O-acylated at O-4 using ammonium hydrogencarbonate/aqueous NH_3 at 0 °C, to afford the glycosyl acceptor **18** (78%). Glycosylation of **18** with 2 equivalents of the Kdo bromide donor **19** gave the (2→4)-linked disaccharide (87%) in a 5:1 α/β ratio. The disaccharide orthoester **20**, obtained from the foregoing anomeric mixture by column chromatography was hydrolyzed with dilute TFA to afford the reducing disaccharide **21** (96%). Similar to the reducing monosaccharide derivative **3**, the disaccharide remained stable under alkaline conditions used for the removal of the ester groups. Purification on Bio-Gel P-2 finally gave the α-Kdo*p*-(2→4)-Ko disaccharide **22** (95%).

Trimethylsilyl trifluoromethanesulfonate promoted orthoester rearrangement of **20** furnished the α-allyl disaccharide derivative **23**. The minor amount of the β-(2→4)-linked iso-

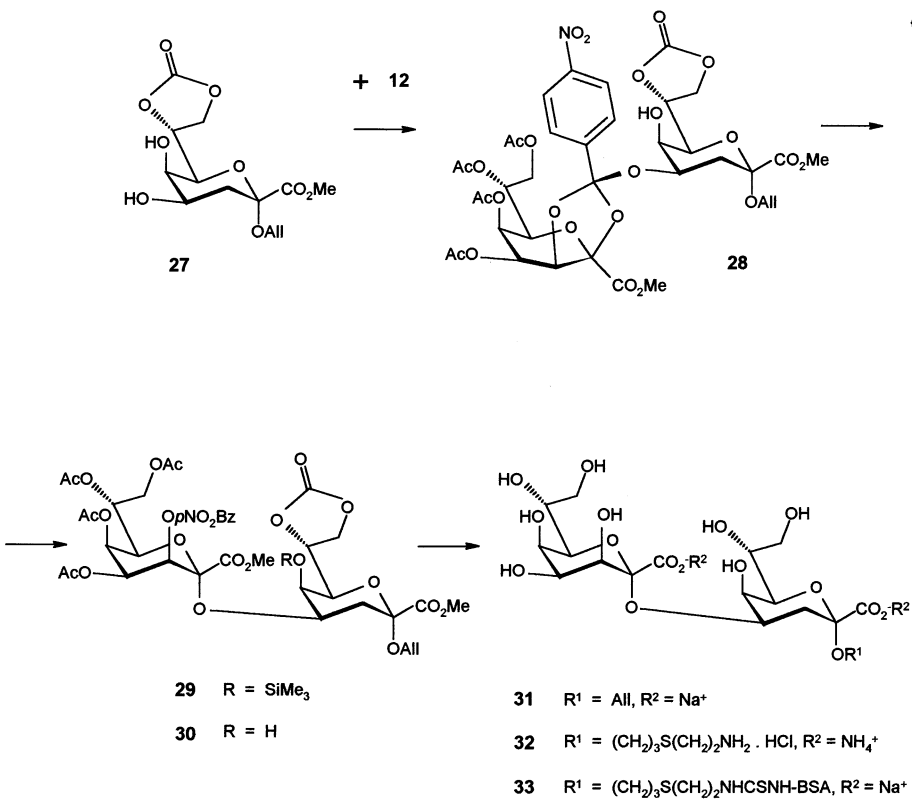


Scheme 2.

mer present could be separated at this stage. Deprotection of **23**, as described above, gave the disaccharide α-Kdo*p*-(2→4)-Ko*p*-(2→OAll) (**24**, 92%). The allyl glycoside was converted into the spacer compound **25** and coupled to BSA, to give the neoglycoconjugate **26** (Scheme 2).

For the synthesis of the disaccharide α-Ko*p*-(2→4)-Kdo*p*-(2→OAll), the previously reported [11] 3-*O*-*p*-nitrobenzoyl bromide donor **12** was used. Other disaccharide orthoester derivatives prepared from the Ko donor **11** or methyl (3,4,5,7,8-penta-*O*-acetyl-D-*glycero*-α-D-*talo*-oct-2-ulopyranosyl)onate bromide led to the formation of hydrolysis products, upon attempted rearrangement using trimethylsilyl trifluoromethanesulfonate or boron trifluoride etherate. Helferich glycosylation of the 7,8-*O*-carbonyl acceptor derivative **27** [21] furnished the disaccharide nitrobenzylidene orthoester **28** (85%). The presence of the orthoester structure was inferred from the upfield-shift of the ^{1}H NMR signal of H-3′ (4.86 ppm). Rearrangement of the orthoester into the α-configured glycoside was effected by treatment with equimolar amount of trimethylsilyl trifluoromethanesulfonate in CH_2Cl_2, affording the 5-*O*-Me_3Si–ether derivative **29** and the crystalline disaccharide compound **30** in yields of 31 and 42%, respectively (Scheme 3).

The NMR signal of H-3′ of both compounds **29** and **30** displayed a downfield shift (5.79 and 5.74 ppm, respectively), confirming the presence of an ester group at O-3. Removal of the Me_3Si group of **29** was accomplished by treatment with 2% HF in MeCN. Zemplén de-O-acylation and alkaline hydrolysis of the methyl ester groups of **30** afforded the disaccharide α-Ko*p*-(2→4)-Kdo*p*-(2→OAll) (**31**, 95%). The disaccharide is related to the inner core of *B. cepacia* LPS and the ^{13}C NMR data (Table 1) are in good agreement with the data of a similar methyl glycoside obtained from native LPS [9]. Conversion of **31** into the spacered ligand **32** was performed as described for similar conversions. The compound was attached to BSA via thiophosgene activation of the terminal amino group. Determination of the ligand/protein ratio 6.6, 2.4 and 1.9 mol/mol for **17**, **26** and **33** was based on MALDI TOF data, respectively. Immuno-



Scheme 3.

chemical results obtained with the neoglycoproteins will be reported elsewhere.

3. Experimental

General methods.—Melting points were determined with a hot stage and are uncorrected. Optical rotations were measured with a Perkin–Elmer 243 B polarimeter. ^{1}H NMR spectra were recorded at 297 K with Bruker AC 300F and DPX instruments operating at 300 MHz for ^{1}H using $CDCl_3$ as solvent and tetramethylsilane as internal standard, unless stated otherwise. Coupling constants are given in Hz (first order values). ^{13}C NMR spectra were measured at 75.47 MHz and referenced to 1,4-dioxane (δ 67.40). Homo- and heteronuclear 2D NMR spectroscopy was performed with Bruker standard software. TLC was performed on E. Merck precoated plates (5×10 cm, layer thickness 0.25 mm, Silica Gel $60F_{254}$); detection was effected by spraying with anisaldehyde–H_2SO_4 [22]. For column chromatography, silica gel (0.040–0.063 mm) was used. Concentration of solns was performed at reduced pressure and $<$ 40 °C. UV-irradation was performed at 254 nm with a 176 W UV-lamp. Elemental analyses were provided by Dr J. Theiner, Mikroanalytisches Laboratorium, Institut für Physikalische Chemie, University of Vienna. MALDI-TOF mass spectra were obtained on a Finnigan MAT instrument in the positive ion mode using 2% 2,5-dihydroxybenzoic acid as matrix, by Dr F. Altmann, Institut für Chemie, University of Agricultural Sciences, Vienna.

Benzyl (2,3,4,5,7,8-hexa-O-*acetyl*-D-glycero-α-D-talo-*oct-2-ulopyranos)onate* (**3**).—A soln of **1** (600 mg, 1.25 mmol) and 3-chloroperbenzoic acid (800 mg) in CH_2Cl_2 (50 mL) was stirred under reflux for 48 h. Ethyl acetate (50 mL) and Silica Gel 60 (0.043-0.060 mm, 3 g) were added and the suspension was heated at 40 °C for 48 h. After filtration, the solids were washed with EtOAc and the filtrate was concd. The residue was dissolved in dry pyridine (10 mL), 4-*N*,*N*-dimethylamino pyridine (10 mg) and Ac_2O (2.5 mL) were added. The soln was stirred for 15 h at rt and concd. The residue was taken up in CH_2Cl_2 (50 mL), washed with satd aq $NaHCO_3$ and dried (Na_2SO_4). Concentration gave a syrup which was chromatographed (1:1 toluene–EtOAc) to give **3** as colourless crystals, mp 178–179 °C (EtOAc–pentane). Yield: 300 mg (40%); $[\alpha]_D^{20}$ + 74° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.38–7.33 (m, 5 H, arom H), 5.43 (dd, 1 H, $J_{3,4}$ 3.7, $^3J_{3,5}$ 1.0 Hz, H-3), 5.39 (t, 1 H, $J_{4,5}$ 3.7 Hz, H-4), 5.35 (ddd, 1 H, H-5), 5.34 (ddd, 1 H, H-7), 5.21 and 5.10 (AB, 2 H, $J_{A,B}$ 11.9 Hz, CH_2), 4.53 (dd, 1 H, $J_{8a,7}$ 2.2, $J_{8a,8b}$ − 12.5 Hz, H-8a), 4.21 (dd, 1 H, $J_{6,7}$ 9.9, $J_{6,5}$ 1.6 Hz, H-6), 4.18 (dd, 1 H, $J_{8b,7}$ 3.3 Hz, H-8b), 2.15 (s, 3 H), 2.05 (s, 6 H), 1.99, 1.96 and 1.75 (3 s, each 3 H, total 6 Ac). Anal. Calcd for $C_{27}H_{32}O_{15}$: C, 54.40; H, 5.38. Found: C, 54.42; H, 5.28.

*Sodium (*D-glycero-D-talo-*oct-2-ulos)onate* (**4**).—A soln of **3** (40 mg, 0.067 mmol) in MeOH (10 mL) was stirred with 5% Pd–C (40 mg) under H_2 (atmospheric pressure) for 1 h at rt. The catalyst was filtered off and the filtrate was treated with 0.1 M methanolic NaOMe (2 mL) for 2.5 h at rt. Dowex 50 cation-exchange resin (H^+-form) was added until the soln reached pH 7. The resin was removed and the filtrate was concd. The residue was purified on a Bio-Gel P-2 column (2.5×100 cm, 95:1 water–EtOH), which afforded **4** as an amorphous powder. Yield: 17.6 mg (95%). $[\alpha]_D^{20}$ + 11 → + 9° after 16 h (*c* 0.5, H_2O); ^{1}H NMR (D_2O): δ 4.31 (dd, 1 H, H-4 α-furanose), 4.28 (dd, 1 H, $J_{4,5}$ 4.6, $J_{5,6}$ 1.2 Hz, H-5 β-furanose), 4.21 (t, 1 H, $J_{4,3}$ 5.9 Hz, H-4 β-furanose), 4.20 (dd, 1 H, H-5 α-furanose), 4.14 (d, 1 H, H-3 β-furanose), $J_{3,5}$ 1.0, $J_{4,3}$ 2.0 Hz, H-3 α-pyranose).

Methyl [4,5,7,8-tetra-O-*acetyl-2,3-di*-O-*(4-nitrobenzoyl)*-D-glycero-α-D-talo-*oct-2-ulopyranos]onate* (**9**) *and methyl [3,5,7,8-tetra*-O-*acetyl-2,4-di*-O-*(4-nitrobenzoyl)*-D-glycero-α-D-talo-*oct-2-ulopyranos]onate* (**10**).—A soln of **5** (3.24 g, 8.07 mmol) and 3-chloroperbenzoic acid (4.90 g, 28.4 mmol) in CH_2Cl_2 (22 mL) was stirred for 36 h under reflux. The soln was diluted with EtOAc (5 mL) and stirred with silica gel (5 g) under reflux for 12 h affording a heterogeneous mixture containing **7**, **8** and furanose diols. The mixture was dried with Na_2SO_4, filtered and concd.

The colorless syrup (1.30 g) was dissolved in pyridine (30 mL) and stirred with 4-nitrobenzoyl chloride (1.5 g, 8.1 mmol) and 4-*N*,*N*-dimethylaminopyridine (15 mg) for 24 h. The soln was diluted with toluene (200 mL) and stirred with K_2CO_3 (150 mg, 1.1 mmol) for 30 min. The suspension was filtered, coevaporated with toluene (150 mL) and concd. A soln of the residue in CH_2Cl_2 (250 mL) was washed with satd aq $NaHCO_3$, the organic phase was dried (Na_2SO_4) and evaporation of the solvent afforded a yellow syrup, which upon chromatography (2:1 *n*-hexane–EtOAc) and crystallization (hexane–EtOAc) gave **9** as slightly yellow crystals, mp 207 °C, lit. 206 °C [11]. Yield: 710 mg (12%); $[\alpha]_D^{20}$ +49° (*c* 1.2, $CHCl_3$), lit. +51° (*c* 0.75, $CHCl_3$) [11].

Further elution gave 1.78 g (30%) of **10** as yellow crystals; mp 192–195 °C (*n*-hexane–EtOAc); $[\alpha]_D^{20}$ +95° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.36–8.04 (m, 8 H, Ar H), 5.83 (dd, 1 H, H-3), 5.75 (t, 1 H, $J_{3,4} = J_{4,5}$ 3.7 Hz, H-4), 5.61 (ddd, 1 H, $J_{5,6}$ 1.2 Hz, H-5), 5.45 (d, 1 H, $J_{7,6}$ 9.8 Hz, H-7), 4.53 (dd, 1 H, $J_{8a,7}$ 2.2, $J_{8a,8b}$ −12.6 Hz, H-8a), 4.33 (dd, 1 H, H-6), 4.23 (dd, 1 H, $J_{8b,7}$ 3.2 Hz, H-8b), 3.85 (s, 3 H, CO_2Me), 2.12, 1.97, 1.68, and 1.55 (4 s, each 3 H, 4 Ac). Anal. Calcd for $C_{31}H_{29}N_2O_{19}$: C, 50.76; H, 3.99; N, 3.82. Found: C, 50.49; H, 3.88; N, 3.79.

Methyl [3,5,7,8-tetra-O-*acetyl-4*-O-*(4-nitrobenzoyl)*-D-glycero-α-D-talo-*oct-2-ulopyranosyl bromide]onate* (**11**).—A soln of **10** (405 mg, 0.552 mmol) and $TiBr_4$ (800 mg, 2.18 mmol) in CH_2Cl_2 (50 mL) was stirred for 12 h under reflux. The soln was diluted with $CHCl_3$ (150 mL) and washed with ice-cold satd aq $NaHCO_3$, the organic phase was dried (Na_2SO_4), and concd. Flash-chromatography (2:1 toluene–EtOAc) afforded **11** as a slightly yellow syrup. Yield: 377 mg (95%); $[\alpha]_D^{20}$ +125° (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.32–8.02 (m, 4 H, Ar H), 5.99 (dd, 1 H, $J_{3,4}$ 3.6, $J_{3,5}$ 0.9 Hz, H-3), 5.97 (t, 1 H, $J_{4,5}$ 3.6 Hz, H-4), 5.65 (ddd, 1 H, $J_{5,6}$ 1.8 Hz, H-5), 5.47 (d, 1 H, $J_{7,6}$ 9.8 Hz, H-7), 4.63 (dd, 1 H, H-6), 4.52 (dd, 1 H, $J_{8a,7}$ 2.2, $J_{8a,8b}$ −12.6 Hz, H-8a), 4.30 (dd, 1 H, $J_{8b,7}$ 3.8 Hz, H-8b), 3.92 (s, 3 H, CO_2Me), 2.13, 2.09, 2.08 and 2.04 (4 s, each 3 H, 4 Ac). Anal. Calcd for $C_{24}H_{26}NO_{15}Br$: C, 44.46; H, 4.04; N, 2.16. Found: C, 44.51; H, 4.02; N, 2.22.

Methyl {5,7,8-tri-O-*acetyl-4*-O-*(4-nitrobenzoyl)-2,3*-O-*[(1*-exo-*allyloxy)-ethylidene]*-D-glycero-β-D-talo-*oct-2-ulopyranos}onate* (**13**).—A suspension of **11** (125 mg, 0.193 mmol), allyl alcohol (200 μL, 2.9 mmol), $Hg(CN)_2$ (220 mg, 0.87 mmol), and 4 Å molecular sieves (100 mg) in dry $CHCl_3$ (3 mL) was stirred for 2 h at 40 °C. The suspension was diluted with $CHCl_3$ (150 mL), washed with aq KI (20%) and satd aq $NaHCO_3$ and dried (Na_2SO_4). Evaporation of the solvent and chromatography (2:3 toluene–EtOAc) gave **13** as a colourless syrup. Yield: 117 mg (95%); $[\alpha]_D^{20}$ +48° (*c* 2.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.32–8.16 (m, 4 H, arom H), 5.89 (m, 1 H, =CH–), 5.47 (ddd, 1 H, $J_{5,4}$ 3.7 Hz, H-5), 5.44 (t, 1 H, H-4), 5.27 (dq, 1 H, $=CH_{2trans}$), 5.22 (m, 1 H, $J_{7,6}$ 9.8 Hz, H-7), 5.15 (dq, 1 H, $=CH_{2cis}$), 4.82 (dd, 1 H, $J_{3,4}$ 4.0, $J_{3,5}$ 0.9 Hz, H-3), 4.48 (dd, 1 H, $J_{8a,7}$ 2.3, $J_{8a,8b}$ −12.3 Hz, H-8a), 4.34 (dd, 1 H, $J_{8b,7}$ 4.3 Hz, H-8b), 4.28 (dd, 1 H, H-6), 4.18 (m, 1 H, OCH_2), 4.08 (m, 1 H, OCH_2), 3.92 (s, 3 H, CO_2Me), 2.08 (s, 6 H) and 2.04 (s, 3 H, 3 Ac) and 1.82 (s, 3 H, *endo*-CH_3). Anal. Calcd for $C_{27}H_{31}NO_{16}$: C, 51.84; H, 5.00; N, 2.24. Found: C, 52.00; H, 4.86; N, 2.18.

Methyl [allyl 5,7,8-tri-O-*acetyl-4*-O-*(4-nitrobenzoyl)*-D-glycero-α-D-talo-*oct-2-ulopyranosid]onate* (**14**).—Trimethylsilyl trifluoromethanesulfonate (0.120 mL, 0.579 mmol) was added at 0 °C under Ar to a suspension of **13** (522 mg, 0.8345 mmol) and 4 Å molecular sieves (200 mg) in dry CH_2Cl_2 (5 mL). The suspension was stirred for 2.5 h at rt. Then triethylamine (0.2 mL) was added at 0 °C, the suspension was stirred for 10 min, diluted with CH_2Cl_2 (150 mL), filtered, and the filtrate was washed with satd aq $NaHCO_3$ (75 mL). The organic layer was dried (Na_2SO_4) and concd to dryness. The residue was chromatographed (2:1 toluene–EtOAc), which afforded **14** as a colourless syrup. Yield: 388 mg (74%); $[\alpha]_D^{20}$ +53° (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.30–8.01 (m, 4 H, arom H), 5.89 (m, 1 H, =CH–), 5.72 (dd, 1 H, $J_{3,4}$ 3.6, $J_{3,5}$ 0.9 Hz, H-3), 5.65 (t, 1 H, $J_{4,5}$ 3.7 Hz, H-4), 5.54 (ddd, 1 H, H-5), 5.43 (dt, 1 H, $J_{7,6}$ 10.0 Hz, H-7), 5.36 (dq, 1 H, $=CH_{2trans}$), 5.27 (dq, 1 H, $=CH_{2cis}$), 4.73 (dd, 1 H, $J_{8a,7}$ 2.3, $J_{8a,8b}$ −12.4 Hz, H-8a), 4.32 (dd, 1 H, H-6), 4.28 (dd, 1 H,

$J_{8b,7}$ 3.2 Hz, H-8b), 4.17 (m, 1 H, OCH_2), 3.92 (m, 1 H, OCH_2), 3.81 (s, 3 H, CO_2Me), 2.10 (s, 3 H), 2.08 (s, 3 H), 2.06 (s, 3 H) and 2.00 (s, 3 H, 4 Ac). Anal. Calcd for $C_{27}H_{31}NO_{16}$: C, 51.84; H, 5.00; N, 2.24. Found: C, 51.91; H, 5.03; N, 2.18.

Sodium (allyl D-glycero-α-D-talo-*oct-2-ulopyranosid)onate* (**15**).—A soln of **14** (35 mg, 0.056 mmol) in dry MeOH (8 mL) was stirred with 0.1 M methanolic NaOMe (0.2 mL) for 1 h at rt. After deionization with Dowex 50-AG WX8 (H^+) cation-exchange resin, a soln of the crude product in water (2 mL) was treated with 0.2 M NaOH (2 mL) for 2 h at rt. The pH of the soln was adjusted to 8.5 by addition of Dowex 50 resin. Filtration and lyophilization of the filtrate gave a residue, which was purified on Bio-Gel P-2 (2.6 × 100 cm, water) to afford **15** as an amorphous powder. Yield: 16.5 mg (93%). ^{1}H NMR data were identical to literature values [17].

Ammonium [3-(2-aminoethylthio)propyl D-glycero-α-D-talo-*oct-2-ulopyranosid]onate hydrochloride* (**16**).—A soln of **15** (12.5 mg, 0.0363 mmol) and cysteamine hydrochloride (16 mg, 0.143 mmol) in water (0.36 mL) was irradiated at 254 nm for 9 h at rt. The soln was diluted with water (0.5 mL) and passed through a column of Dowex AG-WX8 resin (NH_4^+ form) using a gradient water → 0.1 M aq NH_3 as eluant. Carbohydrate-containing fractions were pooled, lyophilized and further purified on Bio-Gel P-2 (2.6 × 100 cm, water) to afford **16** as a colorless solid. Yield: 11 mg (65%); $[\alpha]_D^{20}$ +48° (*c* 0.5, H_2O); ^{1}H NMR (D_2O): δ 4.07 (m, 1 H, H-5), 4.02–3.93 (m, 4 H, H-3, H-4, H-7, H-8a), 3.72 (dd, 1 H, $J_{8a,8b}$ −11.8, $J_{8b,7}$ 6.3 Hz, H-8b), 3.62 (d, 1 H, $J_{6,7}$ 8.7 Hz, H-6), 3.55 (m, 1 H, OCH_2), 3.33 (m, 1 H, OCH_2), 3.21 (t, 2 H, CH_2N), 2.85 (t, 2 H, SCH_2), 2.66 (t, 2 H, CH_2S) and 1.86 (m, 2 H, CH_2). ^{13}C NMR (D_2O): δ 174.64 (CO), 102.92 (C-2), 72.74 (C-6), 72.48 (C-3), 70.42 (C-7), 69.22 (C-5), 67.51 (C-4), 64.12 (C-8), 62.89 (OCH_2), 39.36 (CH_2N), 29.51 (SCH_2), 29.28 (CH_2) and 28.54 (CH_2S). MALDI-TOF MS: *m/z* 809.0 2[M + Na^+ − Cl^- + NH_4^+].

Synthesis of BSA-conjugate **17**.—A soln of thiophosgene (2.7 μL, 0.035 mmol) in $CHCl_3$ (1 mL) was added to a soln of **16** (5.4 mg, 0.0127 mmol) in 0.1 M $NaHCO_3$ (1.5 mL) and the mixture was vigorously stirred for 6 h at rt. The organic phase was separated and the aq phase was washed three times with $CHCl_3$ (1 mL portions), and finally purged with N_2 until a clear soln was obtained. The soln was transferred to a soln of BSA (6.3 mg) in 0.1 M aq $NaHCO_3$/0.3 M aq NaCl buffer (1 mL) and slowly stirred for 48 h at rt, then passed through a Sephadex G-25 column (1.6 × 50 cm, 0.01 M aq $NaHCO_3$). Ninhydrine-positive fractions were combined and dialyzed twice against water (4 L) for 48 h. Lyophilization gave the BSA-conjugate **17**. Yield: 6.6 mg. MALDI-TOF-MS: M^+ 69316; (determined for BSA: 66431); 6.6 mol ligand/mol BSA.

Methyl {5,7,8-tri-O-*acetyl-2,3*-O-*[(1*-exo-*allyloxy)-ethylidene]*-D-glycero-β-D-talo-*oct-2-ulopyranos}onate* (**18**).—A soln of **13** (50 mg, 0.082 mmol), NH_4HCO_3 (23 mg, 0.27 mmol) and aq NH_3 (25%, 0.1 mL) in MeOH (5 mL) was stirred for 5 h at 0 °C. The soln was passed through a column (10 × 3 cm) of silica gel (EtOAc), dried (Na_2SO_4), concd and chromatographed (1:1 toluene–EtOAc) which furnished **18** as a colourless syrup. Yield: 30 mg (78%); $[\alpha]_D^{20}$ +34° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.91 (m, 1 H, =CH–), 5.32 (ddd, 1 H, $J_{5,4}$ 4.0 Hz, H-5), 5.28 (dq, 1 H, $=CH_{2trans}$), 5.17 (dq, 1 H, $=CH_{2cis}$), 5.10 (dq, 1 H, $J_{7,6}$ 9.7 Hz, H-7), 4.66 (dd, 1 H, $J_{3,4}$ 4.0, $J_{3,5}$ 0.4 Hz, H-3), 4.47 (dd, 1 H, $J_{8a,7}$ 2.4, $J_{8a,8b}$ −12.3 Hz, H-8a), 4.30 (dd, 1 H, $J_{8b,7}$ 4.3 Hz, H-8b), 4.18 (m, 1 H, OCH_2), 4.10 (m, 1 H, OCH_2), 4.08 (dd, 1 H, $J_{4,OH}$ 9.2 Hz, H-4), 4.06 (dd, 1 H, $J_{5,6}$ 1.0 Hz, H-6), 3.87 (s, 3 H, CO_2Me), 2.63 (d, 1 H, OH), 2.12, 2.07 and 2.06 (3 s, each 3 H, 3 Ac) and 1.79 (s, 3 H, *endo*-CH_3). Anal. Calcd for $C_{20}H_{28}O_{13}$: C, 50.42; H, 5.92. Found: C, 50.24; H, 5.81.

Methyl (4,5,7,8-tetra-O-*acetyl-3-deoxy-α*-D-manno-*oct-2-ulopyranosyl)onate-(2→4)-methyl {5,7,8-tri*-O-*acetyl-2,3*-O-*[(1*-exo-*allyloxy)-ethylidene]*-D-glycero-β-D-talo-*oct-2-ulopyranos}onate* (**20**).—A suspension of **18** (95 mg, 0.2 mmol), **19** (167 mg, 0.346 mmol), $Hg(CN)_2$ (131 mg, 0.519 mmol) and 4 Å molecular sieves (80 mg) in dry CH_3CN (4 mL) was stirred for 4 h at rt under Ar. A second portion of **19** (40 mg, 0.083 mmol) and $Hg(CN)_2$ (35 mg, 0.138 mmol) was added, and

stirring was continued overnight at rt. The mixture was diluted with $CHCl_3$ (150 mL), washed with aq KI (10%) and satd aq $NaHCO_3$ (75 mL), dried (Na_2SO_4) and concd to dryness. The residue was chromatographed (3:2 → 1:1 toluene–EtOAc), which afforded **20** as a colourless syrup. Yield: 153 mg (87%, α:β = 5:1). A second column chromatography on silica gel (3:2 → 1:2 toluene–EtOAc) gave pure α anomer **20** as a colourless syrup; $[\alpha]_D^{20}$ + 77° (*c* 0.85, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.98 (m, 1 H, =CH–), 5.37 (dd, $J_{5',4'}$ 3.0 Hz, H-5′), 5.33 (dq, 1 H, $=CH_{2trans}$), 5.30 (m, 1 H, H-4′), 5.23 (dd, 1 H, H-5), 5.22 (dq, 1 H, $=CH_{2cis}$), 5.10 (dt, 1 H, H-7′), 5.04 (dq, 1 H, $J_{7,6}$ 9.5 Hz, H-7), 4.72 (dd, 1 H, $J_{8a',8b'}$ − 12.0, $J_{8a',7'}$ 3.2 Hz, H-8a′), 4.60 (dd, 1 H, $J_{3,4}$ 4.0 Hz, H-3), 4.54 (t, 1 H, H-4), 4.44 (dd, 1 H, $J_{8a,7}$ 2.3, $J_{8a,8b}$ − 12.5 Hz, H-8a), 4.26 (dd, 1 H, $J_{8b,7}$ 4.0 Hz, H-8b), 4.18–4.12 (m, 2 H, OCH_2), 4.13 (dd, 1 H, H-6′), 4.00 (dd, 1 H, H-8b′), 3.98 (dd, 1 H, H-6), 3.87 (s, 3 H, CO_2Me), 3.85 (s, 3 H, CO_2Me), 2.08 (s, 3 H), 2.07 (s, 6 H), 2.02, 2.01, 1.98 and 1.97 (4 s, each 3 H, total 7 Ac) and 1.78 (s, 3 H, *endo*-CH_3). Anal. Calcd for $C_{37}H_{50}O_{24}$: C, 50.57; H, 5.73. Found: C, 50.38; 5.57.

Methyl (4,5,7,8-tetra-O-*acetyl-3-deoxy*-α-D-manno-*oct*-2-*ulopyranosyl)onate*-(2 → 4)-*methyl (3,5,7,8-tetra*-O-*acetyl*-D-glycero-α-D-talo-*oct*-2-*ulopyranos)onate* (**21**).—A soln of **20** (10 mg, 0.011 mmol), trifluoroacetic acid (1 mL of a 2% soln in CH_3CN) and water (0.06 mL) was stirred for 2.5 h at 40 °C. The soln was neutralized with solid K_2CO_3 (5 mg, 0.036 mmol), filtered and concd. The residue was chromatographed (2:3 toluene–EtOAc) and crystallization (EtOAc-*n*-hexane) afforded **21**, mp 222–223 °C. Yield: 9.2 mg (96%) $[\alpha]_D^{20}$ + 60° (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.45 (dd, 1 H, $J_{3,4}$ 3.7 Hz, H-3), 5.37 (dd, 1 H, H-5), 5.22 (dt, 1 H, $J_{7',8a'} = J_{7',8b'}$ 3.0 Hz, H-7′), 5.15 (m, 1 H, H-4′), 5.15 (dd, $J_{5',4'}$ 4.0 Hz, H-5′), 5.08 (dt, 1 H, $J_{7,6}$ 9.8 Hz, H-7), 4.91 (t, 1 H, H-4), 4.68 (dd, 1 H, $J_{8a,7}$ 2.9, $J_{8a,8b}$ − 12.4 Hz, H-8a), 4.58 (dd, 1 H, $J_{6,5}$ 1.0 Hz, H-6), 4.32 (dd, 1 H, $J_{6',7'}$ 10.0, $J_{6',5'}$ 1.2 Hz, H-6′), 4.39 (dd, 1 H, H-8a′), 4.50 (s, 1 H, OH), 4.39 (dd, 1 H, H-8b′), 4.24 (m, 1 H, H-8b), 3.86 (s, 3 H, CO_2Me), 3.80 (s, 3 H, CO_2Me), 2.14 (s, 3 H), 2.13 (s, 3 H), 2.12 (s, 3 H), 2.06 (s, 6 H), 2.01 (s, 3 H), 2.00 (s, 3 H) and 1.98 (s, 3 H, total 8 Ac). Anal. Calcd for $C_{34}H_{46}O_{24}$: C, 48.69; H, 5.53. Found: C, 48.48; H, 5.80.

Sodium (3-deoxy-α-D-manno-*oct*-2-*ulopyranosyl)onate*-(2 → 4)-*sodium (*D-glycero-D-talo-*oct*-2-*ulopyranos)onate* (**22**).—A soln of **21** (24 mg, 0.029 mmol) in dry MeOH (5 mL) was stirred with 0.1 M methanolic NaOMe (0.2 mL) for 1 h at rt. The soln was neutralized by addition of Dowex 50 AG-WX8 (H^+) cation-exchange resin, filtered, and concd. A soln of the residue in water (1 mL) was treated with 0.2 M NaOH (2 mL) for 3 h at rt. The pH of the soln was adjusted to 8.0 by addition of Dowex cation-exchange resin. Filtration and lyophilization of the filtrate gave a residue, which was purified on Bio-Gel P-2 (2.6 × 100 cm, water) to afford **22** as a white amorphous powder yield. Yield: 14 mg (95%); $[\alpha]_D^{20}$ + 45 → 60° after 8 h (*c* 0.9, water); ^{1}H NMR (D_2O): selected signals: δ 4.16 (ddd, 1 H, $J_{4',3a'}$ 12.3 Hz, H-4′), 3.55 (dd, 1 H, $J_{6,5}$ 0.8, $J_{6,7}$ 8.5 Hz, H-6), 2.23 (dd, 1 H, $J_{3e',4'}$ 5.0, $J_{3a',3e'}$ − 13.2 Hz, H-3e′), 1.79 (dd, 1 H, $J_{3a',4'}$ 12.3 Hz, H-3a′). MALDI-TOF MS: *m*/*z* 499.1 [M − H_2O].

Methyl (4,5,7,8-tetra-O-*acetyl-3-deoxy*-α-D-manno-*oct*-2-*ulopyranosyl)onate*-(2 → 4)-*methyl (allyl 3,5,7,8-tetra*-O-*acetyl*-D-glycero-α-D-talo-*oct*-2-*ulopyranosid)onate* (**23**).—A suspension of **20** (80 mg, containing 0.076 mmol α anomer and 0.015 mmol β anomer), Me_3SiO–triflate (0.037 mL, 0.220 mmol) and 4 Å molecular sieves (80 mg) was stirred in dry CH_2Cl_2 (1 mL) for 1 h at rt under Ar. K_2CO_3 (30 mg, 0.22 mmol) was added at 0 °C, the suspension was filtered, diluted with CH_2Cl_2 (150 mL), washed with satd aq $NaHCO_3$ (50 mL) and the organic phase was dried (Na_2SO_4). The soln was concd and chromatographed (2:3 toluene–EtOAc), which afforded **23** as a colourless syrup. Yield: 51 mg (80%); $[\alpha]_D^{20}$ + 79° (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$): 5.84 (m, 1 H, =CH–), 5.62 (dd, 1 H, $J_{3,4}$ 4.0 Hz, $J_{3,5}$ 0.8 Hz, H-3), 5.39 (ddd, 1 H, H-5′), 5.26 (dt, 1 H, $J_{7',8a'}$ 2.8 Hz, H-7′), 5.25 (m, 1 H, $=CH_{2trans}$), 5.20 (dq, 1 H, $=CH_{2cis}$), 5.17 (m, 1 H, H-4′), 5.15 (ddd, 1 H, $J_{5,4}$ 4.0 Hz, H-5), 5.10 (dt, 1 H, $J_{7,6}$ 9.7 Hz, H-7), 4.89 (t, 1 H, H-4), 4.87 (dd, 1 H, $J_{8a,8b}$ − 12.8, $J_{8a,7}$ 2.5 Hz, H-8a), 4.72 (dd, 1 H, $J_{6,5}$ 1.8 Hz, H-6), 4.67 (dd, 1 H, H-8b), 4.22 (dd, 1 H, $J_{8a',8b'}$

−12.5, H-8a′), 4.11 (dd, 1 H, $J_{6',5'}$ 1.8 Hz, H-6′), 4.03 (dd, 1 H, $J_{8b',7'}$ 3.2 Hz, H-8b′), 3.86 (s, 3 H, CO_2Me), 3.76 (s, 3 H, CO_2Me), 2.17 (m, 1 H, $J_{3a',3e'}$ −12.5 Hz, H-3e′), 2.13 (s, 3 H), 2.09 (m, 1 H, H-3a′), 2.11 (s, 3 H), 2.09 (s, 3 H), 2.07 (s, 3 H), 2.05 (s, 3 H), 2.02 (s, 3 H) and 1.98 (s, 6 H, total 8 Ac). Anal. Calcd for $C_{37}H_{50}O_{24}$: C, 50.57; H, 5.73. Found: C, 50.48; H, 5.54.

*Sodium (3-deoxy-α-*D*-manno-oct-2-ulopyranosyl)onate-(2→4)-sodium (allyl* D*-glycero-α-*D*-talo-oct-2-ulopyranosidonate* (**24**).—A soln of **23** (20 mg, 0.023 mmol) in dry MeOH (4 mL) was stirred with 0.1 M methanolic NaOMe (0.2 mL) for 1 h at rt. The soln was neutralized by addition of Dowex 50AG-WX8 (H^+) cation-exchange resin, filtered, and concd. The residue was stirred with 0.2 M NaOH (1 mL) for 2 h at rt. The pH of the soln was adjusted to 8.5 by addition of Dowex cation-exchange resin. After filtration, the filtrate was purified on Bio-Gel P-2 (2.6 × 100 cm, water) to afford **24** as a colorless solid. Yield: 12 mg (92%); $[\alpha]_D^{20}$ +47° (*c* 0.3, water); ^{1}H NMR (D_2O): δ 5.94 (m, 1 H, =CH–), 5.32 (dq, 1 H, $=CH_{2trans}$), 5.21 (dq, 1 H, $=CH_{2cis}$), 4.17 (m, 1 H, H-4′), 4.14 (ddd, 1 H, H-5), 4.05 (dd, 1 H, $J_{5',4'}$ 2.8 Hz, H-5′), 4.04–3.75 (m, 5 H, H-3, H-7, H-8a, H-7′, H-8a′), 3.98 (m, 1 H, H-4), 3.90 (m, 1 H, OCH_2), 3.79 (m, 1 H, OCH_2), 3.73-3.62 (m, 2 H, H-8b, H-8b′), 3.67 (dd, 1 H, $J_{6',7'}$ 8.5 Hz, H-6′), 3.58 (dd, 1 H, $J_{6,7}$ 8.6, $J_{6,5}$ 1.2 Hz, H-6), 2.23 (dd, 1 H, $J_{3e',4'}$ 4.5 Hz, H-3*e*′), 1.79 (t, 1 H, $J_{3a',3e'}$ 13.0 Hz, $J_{3a',4'}$ 13.0 Hz, H-3*a*′). MALDI-TOF MS: *m*/*z* 535.6 [M + Na^+].

*Ammonium (3-deoxy-α-*D*-manno-oct-2-ulopyranosyl)onate]-(2→4)-ammonium [3-(2-aminoethylthio)propyl* D*-glycero-α-*D*-talo-oct-2-ulopyranosid]onate hydrochloride* (**25**).—A soln of **24** (3.5 mg, 0.0063 mmol) and cysteamine hydrochloride (4.2 mg, 0.038 mmol) in water (0.27 mL) was irradiated at 254 nm for 4 h at rt. The soln was diluted with water (0.5 mL) and passed through a column of Dowex AG-WX8 resin (NH_4^+ form) using a gradient water → 0.1 M aq NH_3. Carbohydrate-containing fractions were pooled, lyophilized and further purified on Bio-Gel P-2 (2.6 × 100 cm, water) to afford **25** as a white amorphous powder. Yield: 2.8 mg (68%); $[\alpha]_D^{20}$ +36° (*c* 0.2, water); ^{1}H NMR (D_2O): δ 4.18 (m, 1 H, H-4′), 4.17 (dd, 1 H, $J_{5,6}$ 1.2 Hz, H-5), 4.04 (dd, 1 H, $J_{5',4'}$ 3.2 Hz, H-5′), 4.04–3.92 (m, 5 H, H-3, H-7, H-8a, H-7′, H-8a′), 4.01 (t, 1 H, $J_{4,5}$ 3.1 Hz, H-4), 3.70 (m, 3 H, H-6, H-8b, H-8b′), 3.60 (dd, 1 H, $J_{6',7'}$ 8.9, $J_{6'.5'}$ 1.0 Hz, H-6′), 3.52 (m, 1 H, OCH_2), 3.31 (m, 1 H, OCH_2), 3.19 (t, 2 H, CH_2N), 2.86 (t, 2 H, SCH_2), 2.68 (t, 2 H, CH_2S), 2.24 (dd, 1 H, $J_{3e',4'}$ 5.0 Hz, H-3*e*′), 1.87 (m, 2 H, CH_2), 1.80 (t, 1 H, $J_{3a',3e'}$ −13.2, $J_{3a',4'}$ 13.2 Hz, H-3*a*′). MALDI-TOF MS: *m*/*z* 614.9 [M − HCl + Na^+].

Synthesis of BSA-conjugate **26**.—A soln of thiophosgene (1 μL, 0.013 mmol) in $CHCl_3$ (1 mL) was added to a soln of **25** (2.8 mg, 0.0043 mmol) in 0.1 M aq $NaHCO_3$ (1 mL) and the mixture was vigorously stirred for 4 h at rt. The soln was separated and processed as described for **17**, transferred to a soln of BSA (3.2 mg) in 0.1 M aq $NaHCO_3$/0.3 M aq NaCl buffer (1 mL) and slowly stirred for 48 h at rt. Work-up as described above gave the BSA-conjugate **26**, yield: 2.7 mg. MALDI-TOF MS: *m*/*z* 68,020 (*m*/*z* BSA: 66431) (2.4 mol ligand/mol BSA).

*Methyl {{4,5,7,8-tetra-*O*-acetyl-2,3-*O*-[1-exo-(allyl 7,8-*O*-carbonyl-3-deoxy-4-yl-α-*D*-manno-oct-2-ulopyranosid)onate]-4-nitrobenzylidene}-*D*-glycero-β-*D*-talo-oct-2-ulopyranos}}onate* (**28**).—A suspension of **27** (346 mg, 0.534 mmol), **12** (255 mg, 0.80 mmol), $Hg(CN)_2$ (244 mg, 0.966 mmol), $HgBr_2$ (530 mg, 1.47 mmol) and 4 Å molecular sieves (250 mg) in dry CH_3CN (7 mL) was stirred under Ar for 15 min at 0 °C and then for 10 h at rt. The mixture was diluted with $CHCl_3$ (200 mL), filtered, washed with 10% aq KI (250 mL) and satd aq $NaHCO_3$ (50 mL) and dried (Na_2SO_4). The soln was concd and chromatography (2:3 toluene–EtOAc → EtOAc) afforded **28** as a colourless syrup. Yield: 400 mg (85%); $[\alpha]_D^{20}$ +6° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.32–7.88 (m, 4 H, arom H), 5.84 (m, 1 H, =CH–), 5.29 (dd, 1 H, $J_{5',4'}$ 3.8 Hz, H-5′), 5.26 (t, 1 H, $J_{4',3'}$ 3.9 Hz, H-4′), 5.21 (dq, 1 H, $=CH_{2trans}$), 5.16 (dq, 1 H, $=CH_{2cis}$), 4.92 (m, 2 H, H-7, 7′), 4.86 (dd, 1 H, H-3′), 4.68 (dd, 1 H, $J_{8a,8b}$ −9.0 Hz, H-8a), 4.55 (t, 1 H, H-8b), 4.39 (dd, 1 H, $J_{8a',7'}$ 2.5,

$J_{8a',8b'}$ −12.5 Hz, H-8a′), 4.36 (ddd, 1 H, H-4), 4.19 (dd, 1 H, $J_{6',7'}$ 9.8 Hz, H-6′), 4.19 (dd, 1 H, $J_{8b',7'}$ 4.5 Hz, H-8b′), 3.96 (dd, 1 H, H-5), 3.94 (s, 3 H, CO_2Me), 3.88 (dd, 1 H, $J_{6,7}$ 5.0 Hz, H-6), 3.80 (s, 3 H, CO_2Me), 2.20 (m, 1 H, $J_{3a,3e}$ −12.5 Hz, H-3*e*), 2.18 (m, 1 H, H-3*a*), 2.15, 2.08, 1.97 and 1.77 (4 s, each 3 H, 4 Ac). Anal. Calcd for $C_{37}H_{43}NO_{24}$: C, 50.17; H, 4.89; N, 1.58. Found: C, 50.38; H, 5.02; N, 1.48.

Methyl [4,5,7,8-tetra-O-*acetyl-3*-O-*(4-nitrobenzoyl)*-D-glycero-α-D-talo-*oct-2-ulopyranosyl]onate-(2→4)-methyl (allyl 7,8*-O-*carbonyl-3-deoxy-5*-O-*trimethylsilyl*-α-D-manno-*oct-2-ulopyranosid)onate* (**29**) *and methyl [4,5,7,8-tetra*-O-*acetyl-3*-O-*(4-nitrobenzoyl)*-D-glycero-α-D-talo-*oct-2-ulopyranosyl]onate-(2→4)-methyl (allyl 7,8*-O-*carbonyl-3-deoxy*-α-D-manno-*oct-2-ulopyranosid)onate* (**30**).—A suspension of **28** (400 mg, 0.451 mmol) and 4 Å molecular sieves (200 mg) in dry CH_2Cl_2 (7 mL) was treated under Ar with Me_3SiO–triflate (0.081 mL, 0.452 mmol) for 5 min at 0 °C and for 45 min at rt. The suspension was then stirred with triethylamine (0.065 mL) at 0 °C for 10 min, diluted with CH_2Cl_2 (150 mL), filtered, the filtrate was washed with satd aq $NaHCO_3$ (50 mL) and the organic phase was dried (Na_2SO_4). The soln was concd and chromatographed (2:1→1:2 toluene–EtOAc), which afforded first the silylated product **29** as a colourless syrup. Yield: 136 mg (31%); $[\alpha]_D^{20}$ −2° (*c* 0.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.34–8.21 (m, 4 H, arom H), 5.83 (m, 1 H, =CH–), 5.79 (dd, 1 H, $J_{3',4'}$ 3.6 Hz, H-3′), 5.47 (t, 1 H, H-4′), 5.45 (m, 1 H, H-7′), 5.44 (dd, 1 H, H-5′), 5.23 (dq, 1 H, $=CH_{2trans}$), 5.17 (dq, 1 H, $=CH_{2cis}$), 4.87 (dd, 1 H, $J_{8a',8b'}$ −12.5 Hz, H-8a′), 4.76 (m, 1 H, H-7), 4.54 (m, 2 H, H-8a, H-8b), 4.37 (dd, 1 H, $J_{6',7'}$ 9.5, $J_{6',5'}$ 1.5 Hz, H-6′), 4.19 (ddd, 1 H, H-4), 4.12 (dd, 1 H, $J_{8b',7'}$ 4.4 Hz, H-8b′), 3.97 (dd, 2 H, OCH_2), 3.97 (dd, 1 H, H-5), 3.79 (s, 3 H, CO_2Me), 3.67 (m, 1 H, H-6), 3.66 (s, 3 H, CO_2Me), 2.34 (t, 1 H, $J_{3a,3e}$ −12.0 Hz, H-3*e*), 2.23 (dd, 1 H, $J_{3a,4}$ 4.5 Hz, H-3*a*), 2.09, 1.99, 1.94 and 1.87 (4 s, each 3 H, 4 Ac), 0.26 (s, 9 H, Me_3Si). Anal. Calcd for $C_{40}H_{51}NO_{24}$ Si: C, 50.15; H, 5.37; N, 1.46. Found: C, 50.18; H, 5.21; N, 1.40.

Further elution gave the main product **30** as colourless crystals; mp 213–216 °C (*n*-hexane–EtOAc). Yield: 166 mg (42%); $[\alpha]_D^{20}$ +53° (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.36–8.20 (m, 4 H, arom H), 5.84 (m, 1 H, =CH–), 5.74 (dd, 1 H, $J_{3',4'}$ 3.6 Hz, H-3′), 5.44 (m, 1 H, H-5′), 5.43 (m, 1 H, H-4′), 5.43 (m, 1 H, H-7′), 5.24 (dq, 1 H, $=CH_{2trans}$), 5.18 (dq, 1 H, $=CH_{2cis}$), 4.92 (dd, 1 H, $J_{8a',8b'}$ −12.8 Hz, H-8a′), 4.90 (m, 1 H, H-7), 4.72 (dd, 1 H, $J_{8a,8b}$ −9.0, $J_{8a,7}$ 6.8 Hz, H-8a), 4.54 (t, 1 H, H-8b), 4.35 (ddd, 1 H, H-4), 4.28 (dd, 1 H, $J_{6',7'}$ 9.8, $J_{6',5'}$ 1.7 Hz, H-6′), 4.09 (dd, 1 H, H-8b′), 4.00 (dd, 2 H, OCH_2), 3.92 (dd, 1 H, H-6), 3.81 (s, 3 H, CO_2Me), 3.66 (s, 3 H, CO_2Me), 3.65 (dd, 1 H, H-5), 2.21 (d, 1 H, OH), 2.22 (m, 2 H, $J_{3a,3e}$ 12.5 Hz, H-3*a*, H-3*e*), 2.14, 1.99, 1.93 and 1.88 (4 s, each 3 H, 4 Ac). Anal. Calcd for $C_{27}H_{31}NO_{16}$: C, 50.17; H, 4.89; N, 1.58. Found: C, 50.38; H, 4.95; N, 1.48.

Deprotection of **29**.—A soln of **29** (150 mg, 0.157 mmol) was desilylated by treatment with 2% HF–CH_3CN (3 mL) and H_2O (0.5 mL) for 2 h at rt. The pH of the soln was adjusted to 5 by addition of Dowex 50AG–1X8(OH^-) anion-exchange resin. The suspension was filtered, the filtrate was dried (Na_2SO_4) and concd to afford **30**. Yield: 135 mg (97%).

*Sodium (*D-glycero-α-D-talo-*oct-2-ulopyranosyl)onate-(2→4)-sodium (allyl 3-deoxy*-α-D-manno-*oct-2-ulopyranosid)onate* (**31**).—A soln of **30** (105 mg, 0.119 mmol) in dry MeOH (7 mL) was stirred with 0.1 M methanolic NaOMe (2 mL) for 2 h at rt. The soln was deionized with Dowex 50AG-WX8 (H^+) cation-exchange resin, filtered, and concd. The residue was treated with 0.2 M NaOH (5 mL) for 2 h at rt, the pH was adjusted to 9 with Dowex 50 resin. After filtration, the filtrate was purified on Bio-Gel P-2 (2.6 × 100 cm, water) to afford **31** as a white amorphous powder. Yield: 62 mg (95%) $[\alpha]_D^{20}$ +68° (*c* 0.5, H_2O); ^{1}H NMR (D_2O): δ 5.96 (m, 1 H, =CH–), 5.32 (dq, 1 H, $=CH_{2trans}$), 5.20 (dq, 1 H, $=CH_{2cis}$), 4.16 (ddd, 1 H, $J_{4,3a}$ 12.6 Hz, H-4), 4.08 (dd, 1 H, H-5), 4.08 (m,1 H, H-5′), 4.03 (m, 1 H, H-7′), 4.02 (dd, 1 H, $J_{3',5'}$ 1.0 Hz, H-3′), 3.97 (t, 1 H, $J_{4',3'}$ 3.2 Hz, H-4′), 3.91 (m, 1 H, H-7), 3.91 (dd, 1 H, $J_{8a,8b}$ −12.0, $J_{8a,7}$ 2.0 Hz, H-8a), 3.89 (dd, 1 H, $J_{8a',8b'}$ −12.2 Hz, H-8a′), 3.82 (m, 1 H, OCH_2), 3.78 (m, 1 H, OCH_2), 3.75 (dd, 1 H, $J_{8b',7'}$ 7.0 Hz, H-8b′), 3.63 (dd, 1 H, $J_{6',7'}$ 7.8, $J_{6',5'}$ 1.0 Hz, H-6′), 3.58 (dd, 1 H, $J_{8b,7}$ 5.0 Hz, H-8b), 3.54 (dd, 1 H, $J_{6,5}$ 1.0, $J_{6,7}$ 8.9 Hz, H-6), 2.03 (dd, 1 H, $J_{3e,4}$

5.0 Hz, H-3*e*), 1.90 (t, 1 H, $J_{3a,3e}$ −12.6 Hz, H-3*a*). MALDI-TOF-MS: *m/z* 537.9 $[M + Na + 2H]^+$.

*Ammonium (*D-glycero-α-D-talo-*oct-2-ulopyranosyl)onate-(2→4)-ammonium [3-(2-aminoethylthio)propyl 3-deoxy-*α-D-manno-*oct-2-ulopyranosid]onate* (**32**).—A soln of **31** (9.6 mg, 0.0172 mmol) and cysteamine hydrochloride (10.0 mg, 0.090 mmol) in water (0.86 mL) was irradiated at 254 nm for 3 h at rt. The soln was diluted with water (0.75 mL) and was passed through a column of Dowex 50AG-WX8 resin (NH_4^+ form) using a gradient water→0.1 M aq NH_3. Carbohydrate-containing fractions were pooled, lyophilized and further purified on Bio-Gel P-2 (2.6 × 100 cm, water) to afford **32** as amorphous solid. Yield: 10.4 mg (91%); $[\alpha]_D^{20}$ +57° (*c* 0.4, water); ^{1}H NMR (D_2O): $\delta = 4.10$ (ddd, 1 H, H-4), 4.03 (m, 1 H, H-5), 4.02 (m,1 H, H-5′), 4.00 (m, 1 H, H-7′), 3.97 (t, 1 H, $J_{4',3'}$ 3.2 Hz, H-4′), 3.95 (dd, 1 H, $J_{3',5'}$ 1.3 Hz, H-3′), 3.95 (m, 1 H, H-8a), 3.91–3.81 (m, 2 H, H-7, H-8a′), 3.66 (dd, 1 H, $J_{8a',8b'}$ 12.0, $J_{8b',7'}$ 7.0 Hz, H-8b′), 3.56 (m, 1 H, H-6′), 3.55 (m, 1 H, H-8b), 3.48 (dd, 1 H, $J_{6,7}$ 8.8 Hz, H-6), 3.37 (m, 1 H, OCH_2), 3.25 (m, 1 H, OCH_2), 3.16 (t, 2 H, CH_2N), 2.80 (t, 2 H, SCH_2), 2.63 (t, 2 H, CH_2S), 1.94 (dd, 1 H, $J_{3e,4}$ 5.0, $J_{3a,3e}$ −13.0 Hz, H-3*e*), 1.81 (m, 1 H, H-3*a*), 1.81 (m, 2 H, CH_2); ^{13}C NMR (D_2O): Kdo-carbons δ 175.62 (CO), 100.31(C-2), 33.89 (C-3), 69.87 (C-4), 64.92 (C-5), 71.88 (C-6), 69.76 (C-7), 63.68 (C-8), Ko-carbons: δ 174.13 (CO), 102.10 (C-2), 72.28 (C-3), 66.50 (C-4), 68.71 (C-5), 72.87 (C-6), 70.52 (C-7), 63.47 (C-8), 61.70 (OCH_2), 38.79 (CH_2N), 29.99 (CH_2), 28.79 (SCH_2), 28.36 (CH_2S); MALDI-TOF MS: *m/z* 614.5 $[M - HCl + Na]^+$.

Synthesis of BSA-conjugate **33**.—A soln of thiophosgene (2.0 μL, 0.026 mmol) in $CHCl_3$ (1 mL) was added to a soln of **32** (3.60 mg, 0.0054 mmol) in 0.1 M $NaHCO_3$ (1.3 mL) and the mixture was vigorously stirred for 6 h at rt. Processing as described for **17** gave a soln which was transferred to a soln of BSA (4.1 mg) in 0.1 M aq $NaHCO_3$/0.3 M aq NaCl buffer (1 mL). Work-up as described above afforded the BSA-conjugate **33**. Yield: 4.4 mg. The carbohydrate-BSA-ratio was determined via MALDI-TOF MS: *m/z* 67695 (1.9 mol ligand/mol BSA).

Acknowledgements

Technical assistance by Maria Hobel is gratefully acknowledged. The authors thank Dr Andreas Hofinger and Dr Michael Puchberger for recording NMR spectra, and Dr Fritz Altmann for providing the MALDI data. Financial assistance by Fonds zur Förderung der wissenschaftlichen Forschung (FWF-grant P 12573 MOB) is gratefully acknowledged.

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