

Synthesis of neoglycoproteins containing L-glycero- α -D-manno-heptopyranosyl-(1 \rightarrow 4)- and -(1 \rightarrow 5)-linked 3-deoxy- α -D-manno-oct-2-ulopyranosylonic acid (Kdo) phosphate determinants

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The disaccharide allyl glycosides **4**, **8**, **13** and the trisaccharide **33** have been prepared using heptopyranosyl trichloroacetimidate **1** or the disaccharide bromide **27** as glycosyl donors followed by efficient *O*-phosphorylation *via* the amidite procedure. The allyl glycosides **4**, **8** and **33** are converted into 3-(2-aminoethylthio)propyl glycosides and are coupled to bovine serum albumin. The resulting neoglycoconjugates **6** and **35** containing spacer-linked Hep-(1 \rightarrow 4)-Kdo and Hep-(1 \rightarrow 5)-Kdo 4-phosphate-(2 \rightarrow 6)- β -GlcNAc residues correspond to part structures of the inner core region in bacterial lipopolysaccharide, whereas compound **10** contains the artificial analogue Hep-(1 \rightarrow 4)-Kdo 5-phosphate. The compounds may be used in immunochemical characterisation of monoclonal antibodies.

Introduction

Surface structures of bacterial cell walls play a prominent role in the pathogenesis and virulence of bacterial infections. Lipopolysaccharides (LPS), as essential constituents of the outer membranes of Gram-negative bacteria, may be regarded as target structures for the development of novel antibiotics by inhibition of biosynthetic pathways or induction of protective poly- and mono-clonal antibodies.¹ LPS is composed of an *O*-specific heteropolysaccharide chain, a core region and lipid A, the latter part being responsible for endotoxic properties of LPS such as fever, inflammation, hypotension and toxicity. In LPS of enteric and most other Gram-negative bacteria, Kdo (3-deoxy-D-manno-oct-2-ulonic acid) provides the linkage of the core oligosaccharide to *O*-6 of the distal glucosamine residue of lipid A. Furthermore, Kdo is frequently substituted by either one or two additional Kdo unit(s) or by phosphate residues, respectively.² The presence of at least two acidic functions in the inner core region seems to be of general importance to maintain the functions and integrity of the outer membrane.

Kdo phosphates have been reported to occur in LPS from *Haemophilus*,³ *Bordetella pertussis*,⁴ *Vibrionaceae*,⁵ and *Bacteroides*⁶ species. Furthermore, whereas the inner core structure of the deep rough mutant of *Haemophilus influenzae* I-69 Rd⁻/b⁺—being composed of Kdo 4- and Kdo 5-phosphate linked to lipid A—constitutes the smallest functional LPS core found thus far, substitution by an L-glycero-D-manno-heptopyranosyl residue in position 5 of Kdo 4-phosphate is quite common. By contrast, substitution at *O*-4 with a heptosyl moiety has been found in the inner core region of *Hafnia alvei* strains.⁷ The potential role of enzymes responsible for the phosphorylation within the biosynthetic assembly of the core remains to be studied. Given the frequent presence of heptose-Kdo phosphate entities present in different bacterial genera, the development and characterisation of antibodies recognising phosphorylated core structures^{8,9} is desirable. For immunisation procedures with structurally well defined immunogens, the oligosaccharide derivatives have to be linked to protein carriers, since polyacrylamide derivatives,¹⁰ which are multivalent haptens useful for enzyme linked immunoassay (EIA)-inhibition studies, are only weakly immunogenic. Previously, the use of allyl glycosides for

the acid-labile glycosides of Kdo has found application both in the preparation of glycopolymers as well as neoglycoproteins following introduction of a spacer moiety. Addition of cysteamine to the allyl group¹¹ and subsequent activation of the terminal amino group with CSCl₂, and *in situ* coupling to ϵ -amino groups of lysine residues in bovine serum albumin (BSA) affords neoglycoconjugates for immunochemical applications.^{12,13}

Results and discussion

For the synthesis of disaccharide **4** corresponding to a part structure found in the core region of *Hafnia alvei* strains 32 and 1192, known¹⁴ diol glycosyl acceptor derivative **2** was treated with a small excess of the trichloroacetimidate donor **1**¹⁵ and 0.35 mol. equiv. of trimethylsilyl trifluoromethanesulfonate (triflate) (TMSOTf) which afforded regioselectively the α -(1 \rightarrow 4)-linked crystalline disaccharide derivative **3** ($J_{1,2}$: 1.5 Hz at δ 5.06, $J_{C-1',H-1'}$: 172 Hz) in 61% isolated yield. The structure of compound **3** was assigned on the basis of the ¹H NMR data, which showed the presence of a hydroxy group coupled to H-5 of the Kdo-residue, whereas H-4 displayed only spin-couplings to H-5 and the geminal protons at C-3. Removal of the ester groups by reaction with sodium methoxide and aq. NaOH furnished the deprotected allyl glycoside derivative **4** in 93% yield. ¹³C NMR data for compound **4** (Table 1) were in full agreement with the structure assigned. C-4 of the Kdo unit experienced a downfield shift to δ_C 71.90, whereas C-5 and C-3 were shifted slightly upfield (δ_C 63.85 and 33.89, respectively) compared with unsubstituted Kdo. Addition of cysteamine hydrochloride under radical conditions (UV irradiation at 254 nm) proceeded in an Anti-Markownikow fashion to give the 3-(2-aminoethylthio)propyl disaccharide compound **5** in 77% yield. In the ¹H and ¹³C NMR spectra of compound **5**, complete absence of the allyl signals was observed, whereas the respective signals of the spacer group were readily seen at δ 39.16 (C-N), 29.31, 29.30 and 28.40.

For comparison purposes the unnatural phosphorylated disaccharide derivative **8** was synthesized. Reaction of compound **3** with bis(2-cyanoethyl) *N,N*-diisopropylphosphoramidite¹⁶ and 1*H*-tetrazole followed by *in situ* oxidation with

Table 1 ^{13}C NMR chemical shifts (δ_{C}) and carbon–phosphorus coupling constants (J/Hz) for compounds **4**, **8**, **13** and **33**, measured at pD 7.5–8.5

	Chemical shifts (δ_{C})			
	4	8	13	33
Hep-(1→				
C-1	98.31	97.70	101.25 ^b	101.16 ^b
C-2	70.17	70.59	71.07	70.95
C-3	71.45	71.44	71.32	71.35
C-4	66.97	67.08	67.51	67.69
C-5	72.73 ^a	72.71	73.10	72.91
C-6	69.80	69.89	70.05	70.91
C-7	64.13	64.25	64.18	63.82 ^c
→Kdo-(2→				
C-1	176.03	175.96	175.82	175.71
C-2	100.79	100.77	101.02 ^b	101.12 ^b
C-3	33.89	34.01	35.17	35.27
C-4	71.90	69.89	70.63 (4.3)	69.48 (3.8)
C-5	63.85	67.11 (5.3)	73.32 (4.7)	73.54 (4.5)
C-6	72.31 ^a	72.57	72.72	72.98
C-7	71.06	69.89	70.77	70.17
C-8	63.85	63.48	64.18	64.44 ^c
→GlcNAc				
C-1				101.12 ^b
C-2				56.33
C-3				74.88
C-4				71.35
C-5				75.02
C-6				63.17
Allyl group				
C-1	65.05	64.98	65.34	71.35
C-2	134.78	134.82	134.67	134.31
C-3	118.10	117.95	118.81	118.80
			CH_3	22.98
			CO	175.38

^{a,b,c} Assignments may be reversed within a column.

m-chloroperbenzoic acid (MCPBA) furnished the phosphotriester derivative **7** in 67% yield. Removal of the blocking groups under alkaline conditions went smoothly to give the 5-*O*-phosphorylated disaccharide derivative **8** in near quantitative yield (96%). The presence of the 5-*O*-phosphomonoester in compound **8** was evident from the downfield shift of C-5 (δ_{C} 67.11) of the Kdo residue compared with that of compound **4** (δ_{C} 63.85), and the carbon–phosphorus coupling (J 5.3 Hz).

The disaccharide derivative **13**, which corresponds to the inner core structure of bacterial strains such as *Haemophilus influenzae*,¹⁷ *H. ducreyi*,¹⁸ *Vibrio cholerae*,⁷ *V. salmonicida*¹⁹ and *Bordetella pertussis*²⁰ was prepared from the previously described²¹ *L*-glycero-*a*-*D*-manno-heptopyranosyl-(1→5)-Kdo derivative **11**. *O*-Phosphorylation of the equatorial 4-OH group in compound **11** afforded the phosphotriester derivative **12** in 70% yield. Deprotection of compound **12** was accomplished by sequential removal of the tetraisopropylidisiloxane-1,3-diyl group using tetrabutylammonium fluoride (TBAF)²² followed by treatment with sodium methoxide and aq. NaOH which furnished the target allyl glycoside Hep-(1→5)-Kdo 4-phosphate **13** in 83% yield. The purity and structure of compound **13** were confirmed by ^1H , ^{13}C and ^{31}P NMR spectroscopy. The C-5 and C-4 signals of Kdo were shifted upfield and displayed a heteronuclear coupling constant $J_{\text{C,P}}$ of 4.7 and 4.3 Hz, respectively (Table 1), whereas the phosphate residue was observed at δ_{P} 1.32. Conversion of compound **13** into the spacer derivative **14**, however, met with difficulties since the 3-(2-aminoethylthio)propyl glycoside **14** was dephosphorylated upon chromatography on Bio-Gel P2 (Scheme 2). A similar

degradation due to an intramolecular reaction of the terminal amino group with the 4-*O*-phosphomonoester group was previously¹³ noted for the spacer derivative of *a*-Kdo 4-phosphate. In order to avoid hydrolysis of the 4-phosphate, the synthesis of a trisaccharide derivative with the spacer moiety attached to a β -configured 2-acetamido-2-deoxyglucopyranosyl residue was performed.

Within the first approach, the coupling reaction of the trichloroacetimidate donor **1** with the Kdo-(2→6)-GlcNAc derivative **16**—readily accessible from the previously described¹³ disaccharide **15**—in the presence of TMSOTf, Sn triflate or Ag triflate, respectively, did not furnish the expected *O*-glycoside **17** but gave rise to the formation of the unstable imidate compound **18**, which was isolated in low yield (Scheme 3). The structural assignment of compound **18** was based on the presence of an OH-group coupled to H-5 of the Kdo unit and the absence of an NH-signal in the ^1H NMR spectrum. Owing to the propensity of the acetamido group to compete in the *O*-glycosylation reaction, the sequence of glycosylation steps was therefore reversed by first preparing the disaccharide donor Hep-(1→5)-Kdo **27**.

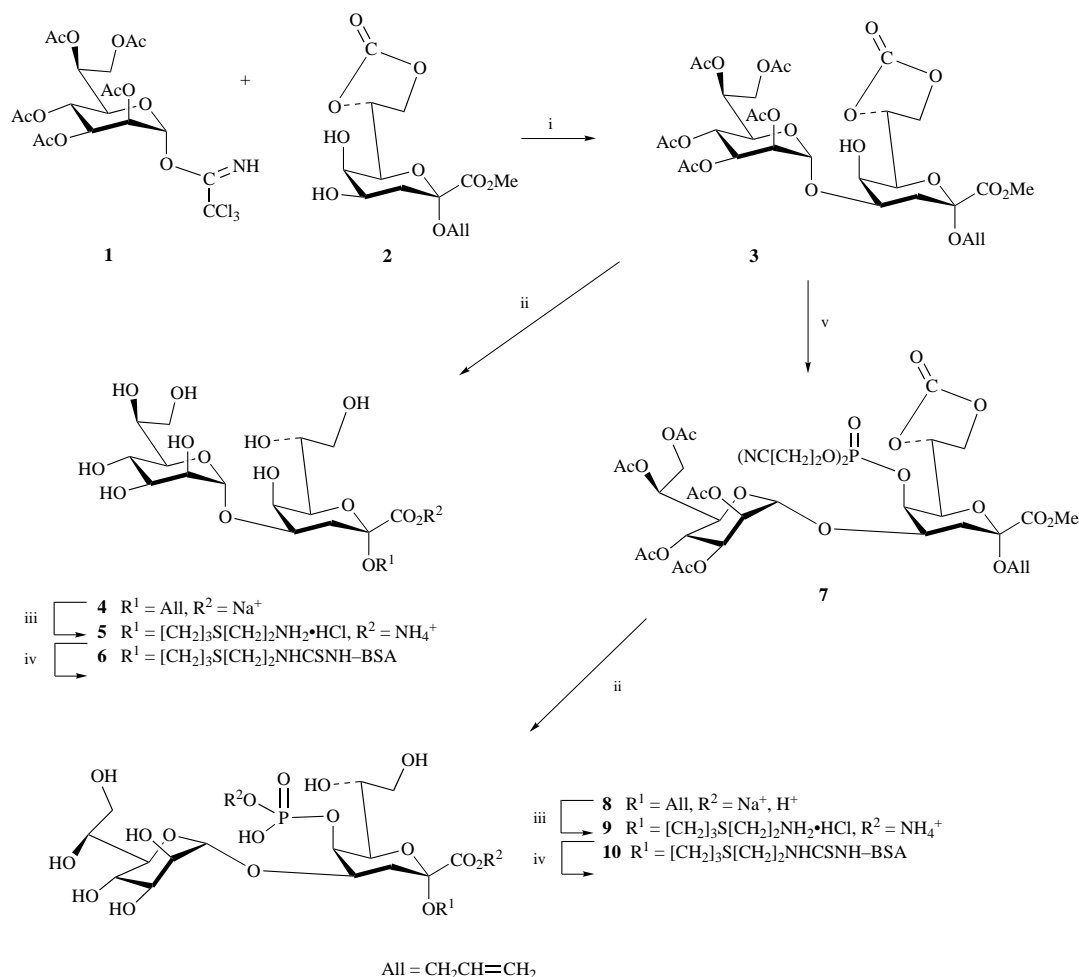
The 4-*O*-(*p*-methoxybenzyl) ether derivative **20** was prepared in 64% yield—together with a small amount (9%) of the methyl ester **21**—by treatment of compound **19**²³ with dibutyltin oxide (DBTO)-*p*-methoxybenzyl chloride in toluene (Scheme 4). After separation of the products by silica gel chromatography, compound **21** was subjected to glycosylation with acetimidate **1** in the presence of TMSOTf. Since separation of an orthoester derivative formed as a by-product was difficult, the *p*-methoxybenzyl ester derivative **20** was used. TMSOTf-catalysed coupling of compound **20** with the trichloroacetimidate donor **1** afforded the α -(1→5)-linked disaccharide derivative **22** in 64% yield. The presence of the α -anomeric configuration was evident from the value of the heteronuclear coupling constant $J_{\text{C-1',H-1'}}$ (173.1 Hz).

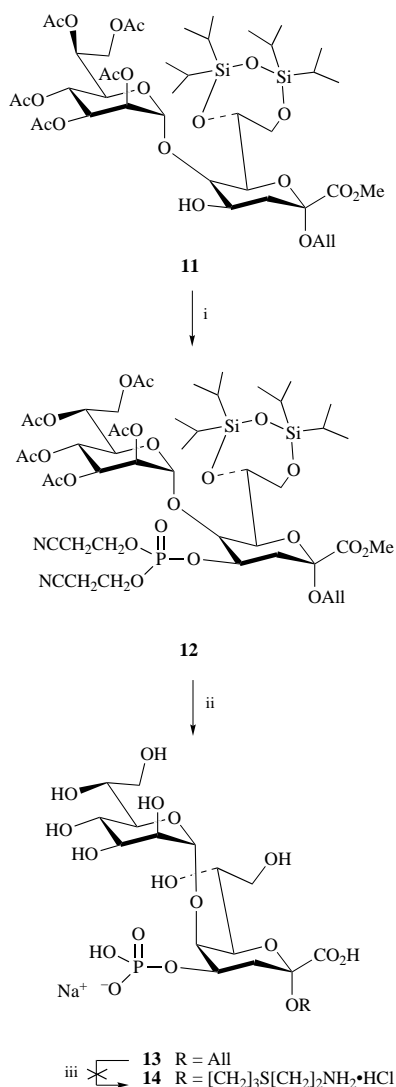
In order to provide a suitable disaccharide donor allowing for the introduction of the 4-*O*-phosphate group at a later stage of the synthesis, the cyclic silyl ether blocking group was cleaved by treatment with TBAF. Subsequent deacetylation under Zemplén conditions and *O*-acetylation with Ac_2O -pyridine afforded the methyl ester derivative **23** in 73% overall yield. Removal of the benzyl ether groups *via* hydrogenolysis with Pd-carbon (5%) in MeOH proceeded quantitatively to give the reduced oligosaccharide derivative **24** as an α,β -mixture. Chloroacetylation of diol **24** was attempted under various conditions. Whereas reactions with 2,6-dimethylpyridine (lutidine)-triethylamine-chloroacetic anhydride or 2,6-dimethylpyridine-chloroacetyl chloride in dichloromethane did not proceed in good yield, treatment with chloroacetic anhydride in pyridine at 0 °C for 35 min afforded a 71% yield of **25** and **26**. The anomeric mixture could be resolved by silica gel chromatography. The anomeric chloroacetates were then converted—by treatment with TiBr_4 —into the bromide derivative **27**, which was immediately used in the glycosylation reaction with the previously reported²⁴ glucosamine acceptor derivative **28**. Promotion of the reaction by a 1:3 mixture of HgBr_2 - $\text{Hg}(\text{CN})_2$ in nitromethane gave the α -(2→6)-linked trisaccharide derivative **30** (28%), traces of the β -linked isomer and a substantial amount (50% based on substrate **26**) of the glycal ester derivative **29**, which were separated by silica gel chromatography (Scheme 5). Assignment of the α -anomeric configuration of the Kdo unit in compound **30** was based on the ^1H NMR chemical shift value of H-4' and the small chemical shift difference between the equatorial and axial protons at C-3'. The 4'-*O*-chloroacetyl group was then removed with hydrazine dithiocarbonate²⁵ to afford the alcohol **31** in 94% yield. Subsequent phosphorylation similar to the preparation of compound **7** proceeded smoothly, and furnished the protected phosphotriester trisaccharide derivative **32** in high yield (99%). Removal of the blocking groups was accomplished by successive treatment

Table 2 ^1H NMR chemical shifts (δ) and coupling constants (J/Hz) for compounds **4**, **8**, **13** and **33**

	Chemical shifts (δ)			
	4	8	13	33
Hep-(1 \rightarrow)				
H-1	5.11 (1.7)	5.14 (1.6)	5.18 (1.7)	5.20 (1.7)
H-2	3.97	3.99 (3.0)	4.11 (3.3)	4.11 (3.4)
H-3	3.86	3.85	3.97 (9.6)	3.96 (9.6)
H-4	3.86	3.85	3.82 (9.8)	3.77 (9.8)
H-5	3.61 (1.5)	3.59 (1.6)	4.05 (1.9)	4.10 (2.7)
H-6	4.05 (6.6)	4.03 (6.7)	3.85	3.94
H-7a	3.73	3.72	n.d.	3.88 (4.8, 11.1)
H-7b	3.75	3.74	3.65 (5.6, 11.6)	n.d.
\rightarrow Kdo-(2 \rightarrow)				
H-3a	1.90 (12.2)	1.97 (12.7)	2.00 (12.8)	1.95 (12.8)
H-3e	2.11 (13.1)	2.06 (13.0)	2.24 (13.1)	2.23
H-4	4.16 (5.0)	4.25 (5.1)	4.53 (4.8)	4.45 (4.3)
H-5	4.25 (2.9)	4.68 (2.7)	4.28 (2.1)	4.27
H-6	3.61 (1.0)	3.58 (10.0)	3.71 (10.1)	3.72
H-7	3.96 (9.0)	n.d.	3.99	3.83
H-8a	3.93 (2.8)	n.d.	n.d.	3.92 (2.9)
H-8b	3.65 (6.3, 11.6)	3.64 (6.4, 11.7)	3.73 (7.6, 11.6)	3.63 (6.5, 11.5)
\rightarrow GlcNAc				
H-1				4.55
H-2				3.73
H-3				~3.50
H-4				n.d.
H-5				n.d.
H-6a				n.d.
H-6b				n.d.

n.d., Not determined.

**Scheme 1** Reagents and conditions: i, TMSOTf, CH_2Cl_2 ; ii, (a) MeONa–MeOH; (b) aq. NaOH; iii, aq. $\text{HSCH}_2\text{CH}_2\text{NH}_2\cdot\text{HCl}$, *lv*; iv, (a) CSCl_2 , aq. NaHCO_3 , (b) BSA, aq. NaHCO_3 – NaCl ; v, bis(2-cyanoethyl) *N,N*-diisopropylphosphoramidite, 1*H*-tetrazole, CH_2Cl_2 ; then MCPBA



Scheme 2 Reagents and conditions: i, bis(2-cyanoethyl) *N,N*-diisopropylphosphoramidite, 1*H*-tetrazole, CH_2Cl_2 ; then MCPBA; ii, (a) TBAF, THF; (b) MeONa–MeOH; (c) aq. NaOH; iii, aq. $\text{HSCH}_2\text{CH}_2\text{NH}_2 \cdot \text{HCl}$, *hv*

of compound **32** with TBAF in tetrahydrofuran (THF) followed by reaction with NaOMe and aq. NaOH to give the target trisaccharide derivative **33** in 68% yield. The ^{31}P NMR spectrum of compound **33** displayed a singlet at δ_{p} 2.16.

Introduction of the spacer function by addition of cysteamine hydrochloride provided the stable 3-(2-aminoethylthio)propyl derivative **34** (68%).

The neoglycoproteins **6**, **10** and **35** were prepared *via in situ* activation of the spacer compounds **5**, **9** and **34** with thiophosgene and coupling of the resulting isothiocyanate to bovine serum albumin (BSA). The glycoconjugates were isolated by gel chromatography on Sephadex G-25 and subsequent dialysis against water. The ratio of ligand to protein was determined by measuring the amount of phosphate²⁶ or of Kdo by the thio-barbiturate assay after hydrolysis in 0.1 M HCl as described²⁷ and a protein assay, respectively. Immobilised ligands were in the range 115–154 nmol/mg BSA which corresponds to 8–11 mol/mol (Table 3).

Experimental

General methods

Mps were determined with a Kofler hot stage and are uncorrected. Optical rotations were measured with a Perkin-Elmer 243 B polarimeter; $[\alpha]_{\text{D}}$ -values are given in units of $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$. ^1H NMR spectra were recorded at 297 K with a Bruker AC

300F instrument operating at 300 MHz for ^1H using CDCl_3 as solvent and tetramethylsilane as the internal standard, if not 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_{C} 67.40). ^{31}P NMR spectra were recorded at 121.49 MHz using orthophosphoric acid as external standard (δ_{p} 0.00). Homo- and hetero-nuclear 2D NMR spectroscopy were performed with Bruker standard software. TLC was performed on Merck precoated plates (5×10 cm; layer thickness 0.25 mm, Silica Gel 60F₂₅₄); spots were detected by spraying with anisaldehyde– H_2SO_4 . For column chromatography, silica gel (0.040–0.063 mm) was used. Concentration of solutions was performed at reduced pressure at temperatures $<40^\circ\text{C}$. Elemental analyses were provided by Dr J. Theiner, Mikroanalytisches Laboratorium, Institut für Physikalische Chemie, Universität Wien. Centrifugation was performed on a Heraeus Labofuge model 200.

Methyl [allyl *O*-(2',3',4',6',7'-penta-*O*-acetyl-*L*-glycero- α -*D*-manno-heptopyranosyl)-(1'→4)-methyl-7,8-*O*-carbonyl-3-deoxy- α -*D*-manno-oct-2-ulopyranosid]onate **3**

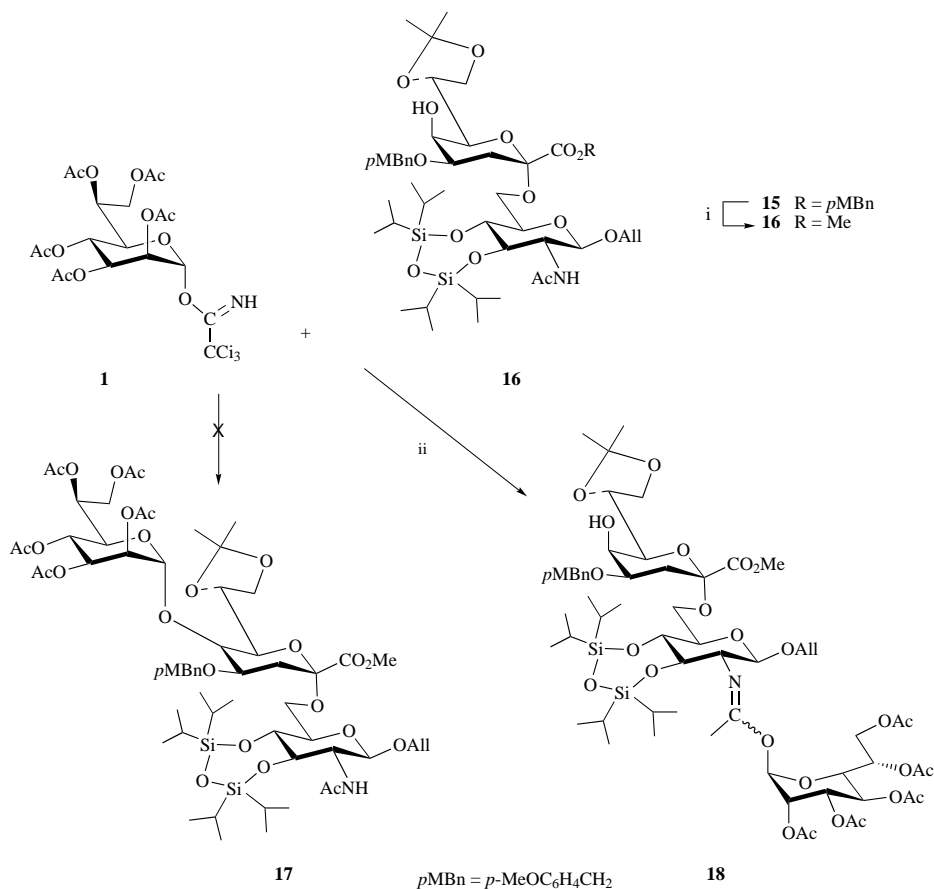
A suspension of compound **1** (150 mg, 0.27 mmol), compound **2** (75 mg, 0.24 mmol) and molecular sieves 4 Å (300 mg) in dry CH_2Cl_2 (3 cm^3) was stirred at ambient temperature for 2.5 h under N_2 . After cooling of the mixture to 0°C , a solution of TMSOTf in CH_2Cl_2 (0.4 M; 205 mm^3 , 0.083 mmol) was added. Stirring was continued for 2 h and triethylamine (0.2 cm^3) was added. The suspension was diluted with EtOAc (30 cm^3) and filtered over Celite. The filtrate was washed with saturated aq. NaHCO_3 and dried (Na_2SO_4). Removal of solvents followed by column chromatography on silica gel [toluene–EtOAc (1:1)] afforded compound **3** (104 mg, 61%) as crystals, mp 206–208 $^\circ\text{C}$ (from hexane–EtOAc); $[\alpha]_{\text{D}}^{20} +56$ (*c* 0.9, CHCl_3); δ_{H} 5.84 (1 H, m, =CH), 5.32 (1 H, t, $J_{9,9}$, 4'-H), 5.26 (1 H, dd, $J_{3,0}$, 3'-H), 5.25 (1 H, m, = $\text{CH}_{2\text{trans}}$), 5.22–5.20 (2 H, m, 2'- and 6'-H), 5.18 (1 H, m, = $\text{CH}_{2\text{cis}}$), 5.06 (1 H, d, $J_{1,5}$, 1'-H), 4.97 (1 H, ddd, $J_{7,6}$ 3.8, $J_{7,8a}$ 6.8, $J_{7,8b}$ 8.6, 7-H), 4.76 (1 H, dd, $J_{8,9}$, 8-H^a), 4.56 (1 H, t, 8-H^b), 4.27 (1 H, dd, $J_{7,6'}$ 6.7, $J_{7a,7b}$ 11.0, 7'-H^a), 4.22 (1 H, ddd, $J_{4,5}$ 3.0, $J_{4,3e}$ 5.6, $J_{4,3a}$ 11.1, 4-H), 4.17 (1 H, dd, $J_{7b,6'}$ 4.5, 7'-H^b), 4.10 (1 H, dd, $J_{5,6'}$ 2.3, 5'-H), 4.03 (1 H, br s, 5-H), 3.99–3.96 (3 H, m, 6-H, OCH_2), 3.80 (3 H, s, CO_2Me), 2.49 (1 H, br s, OH), 2.21–2.10 (2 H, m, 3- H_2) and 2.19, 2.14, 2.09, 2.03 and 2.00 (each 3 H together s, $5 \times \text{Ac}$) (Found: C, 49.9; H, 5.6. $\text{C}_{30}\text{H}_{40}\text{O}_{20}$ requires C, 50.0; H, 5.6%).

Sodium [allyl *O*-(*L*-glycero- α -*D*-manno-heptopyranosyl)-(1'→4)-(3-deoxy- α -*D*-manno-oct-2-ulopyranosid]onate **4**

A solution of disaccharide **3** (24 mg, 0.033 mmol) in 0.1 M methanolic NaOMe (2 cm^3) was stirred for 1.5 h at room temperature. Dowex[®] AG50 W-X8 resin (H^+ -form) was added until pH 6 was attained. The resin was removed and the filtrate was taken to dryness and then treated with 0.1 M aq. NaOH (2 cm^3) for 2 h. The pH of the solution was adjusted to 8.5 by adding Dowex resin, the suspension was filtered, and the residue obtained after lyophilisation of the filtrate was purified on a Bio-Gel P2 column [2.5×100 cm; EtOH–water (95:5)] to afford compound **4** (15.3 mg, 93%) as a syrup, $[\alpha]_{\text{D}}^{20} +80$ (*c* 0.8, water); δ_{H} (D_2O) (*inter alia*) 5.99 (1 H, m, =CH), 5.35 (1 H, dq, $J_{17,2}$ 1.6, = $\text{CH}_{2\text{trans}}$), 5.23 (1 H, dq, $J_{10,3}$ 1.3, = $\text{CH}_{2\text{cis}}$) and 3.98–3.92 and 3.86–3.86 (2 H, m, OCH_2). Additional ^1H NMR and ^{13}C NMR data are presented in Tables 1 and 2 (Found: C, 41.0; H, 6.4. $\text{C}_{18}\text{H}_{29}\text{NaO}_{14} \cdot 2\text{H}_2\text{O}$ requires C, 40.9; H, 6.3%).

Ammonium [3-(2-aminoethylthio)propyl *O*-(*L*-glycero- α -*D*-manno-heptopyranosyl)-(1'→4)-3-deoxy- α -*D*-manno-oct-2-ulopyranosid]onate hydrochloride **5**

A solution of compound **4** (5.6 mg, 0.011 mmol) and cysteamine hydrochloride (10.3 mg, 0.081 mmol) in water (180 mm^3) was placed in a quartz vial and irradiated at 254 nm for 15 h at room temperature. The reaction mixture was applied onto a column



Scheme 3 Reagents: i, MeONa–MeOH; ii, TMSOTf, CH_2Cl_2

Table 3 Chemical analysis of the neoglycoconjugates **6**, **10** and **35**

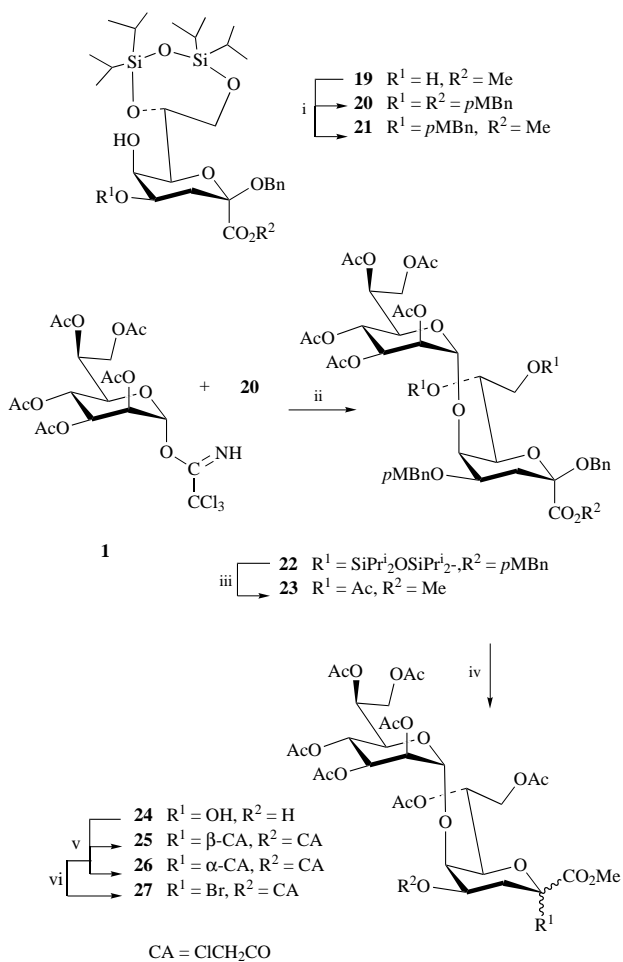
Compound	Structure	Phosphate (nmol/ml)	BSA ($\mu\text{g/ml}$)	Ligand/protein quotient (nmol/mg)
6	Hep-(1→4)-Kdo		860	115
10	Hep-(1→4)-Kdo 5P	102	781	131
35	Hep-(1→5)-Kdo 4P (2→6)- βGlcNAc	124	806	154

(0.5 × 10 cm) of Dowex AG50 W-X8 resin (NH_4^+ -form) and eluted with water → 0.1 M aq. NH_3 . Fractions (2.5 cm³) were collected and immediately neutralised by addition of 0.1 M aq. NH_3 . Ninhydrin-positive fractions (10–15) were pooled, lyophilised and purified on Bio-Gel P2 as described for compound **4** which furnished *compound 5* (5.3 mg, 77%) as an amorphous solid, $[\alpha]_{\text{D}}^{20} + 71$ (c 0.3, water); $\delta_{\text{H}}(\text{D}_2\text{O})$ 5.10 (1 H, d, $J_{1,5}$ 1'-H), 4.25 (1 H, d, 5-H), 4.14 (1 H, ddd, $J_{4,5}$ 3.0, $J_{4,3e}$ 5.2, $J_{4,3a}$ 12.0, 4-H), 4.06 (1 H, dt, $J_{6,2}$ 6'-H), 3.99–3.90 (3 H, m, 2'- and 7-H, 8-H^a), 3.87–3.84 (2 H, m, 3'- and 4'-H), 3.75–3.73 (2 H, m, 7'-H₂), 3.67 (1 H, dd, $J_{8b,7}$ 7.3, $J_{8b,8a}$ 12.5, 8-H^b), 3.62–3.58 (2 H, m, 6- and 5'-H), 3.49 and 3.55 (2 H, dt, OCH_2), 3.22 (2 H, t, NCH_2), 2.88 and 2.72 (4 H, t, SCH_2), 2.09 (1 H, dd, $J_{3e,3a}$ 12.5, 3-H^e), 1.89 (1 H, t, 3-H^a) and 1.89 (2 H, m, CH_2); δ_{C} 176.14 (C-1), 100.66 (C-2), 98.00 (C-1'), 72.75 (C-5'), 72.20 (C-6), 71.50 and 71.44 (C-4, -3'), 70.96 (C-2'), 70.10 (C-7), 69.85 (C-6'), 66.91 (C-4'), 64.29, 63.86, and 63.63 (C-5, -8, -7'), 62.29 (OCH_2), 33.81 (C-3), 39.16 (NCH_2) and 29.31, 29.30 and 28.40 (2 × SCH_2 , CH_2) (Found: C, 38.6; H, 7.0; N, 4.6; S, 5.1. $\text{C}_{20}\text{H}_{40}\text{ClN}_2\text{O}_{14}\text{S} \cdot \text{H}_2\text{O}$ requires C, 38.9; H, 6.85; N, 4.5; S, 5.2%).

Methyl {allyl O-(2',3',4',6',7'-penta-O-acetyl-L-glycero- α -D-manno-heptopyranosyl)-(1'→4)-7,8-O-carbonyl-5-O-[bis(2-cyanoethyl)phosphoryl]-3-deoxy- α -D-manno-oct-2-ulopyranosid}onate **7**

A suspension of compound **3** (20.0 mg, 0.028 mmol) and

molecular sieves 4 Å (200 mg) in dry CH_2Cl_2 (5 cm³) was stirred for 30 min at room temperature under N_2 . 1*H*-Tetrazole (15 mg, 0.21 mmol) and bis(2-cyanoethyl) *N,N*-diisopropylphosphoramidite (48 mg, 0.18 mmol) were added in four portions over a period of 64 h. The suspension was then cooled to -20°C and a solution of MCPBA (13 mg, 0.075 mmol) in CH_2Cl_2 (1 cm³) was added. The reaction was quenched after one hour by addition of 10% aq. $\text{Na}_2\text{S}_2\text{O}_5$ (0.2 cm³). The suspension was centrifuged at 5000 rpm and solids were washed thoroughly with EtOAc–MeOH (1:1). The supernatant and washings were combined, concentrated, and purified by silica gel chromatography (EtOAc) which gave *phosphotriester 7* as a syrup (17 mg, 67%), $[\alpha]_{\text{D}}^{20} + 42$ (c 0.3, CHCl_3); δ_{H} 5.83 (1 H, m, =CH), 5.50 (1 H, dd, $J_{2',3'}$ 3.3, $J_{1',2'}$ 1.6, 2'-H), 5.35 (1 H, t, $J_{4',3'} = J_{4',5'} = 10.3$, 4'-H), 5.22 (1 H, dq, J 17.2, 1.6, = $\text{CH}_{2\text{trans}}$), 5.19 (2 H, m, = $\text{CH}_{2\text{cis}}$, 6'-H), 5.14 (1 H, d, 1'-H), 5.11 (1 H, dd, 3'-H), 5.07 (1 H, m, 7-H), 4.89 (1 H, dd, $J_{5,6}$ 2.3, $J_{5,4}$ 8.8, 5-H), 4.69 (1 H, t, $J_{8a,8b} = J_{8a,7} = 8.9$, 8-H^a), 4.50 (1 H, dd, $J_{8b,7}$ 5.3, 8-H^b), 4.42–4.26 (6 H, m, 7'-H^a, 4-H, 2 × $\text{OCH}_2\text{CH}_2\text{CN}$), 4.14 (1 H, dd, $J_{7b',7a'}$ 9.7, $J_{7b',6'}$ 7.0, 7'-H^b), 4.08 (1 H, dd, $J_{5',6'}$ 2.0, 5'-H), 4.00–3.95 (2 H, m, OCH_2), 3.89 (1 H, ddd, $J_{6,7}$ 7.0, 6-H), 3.81 (3 H, s, CO_2Me), 2.91 (2 H, t, CH_2CN), 2.81 (2 H, m, CH_2CN), 2.23–2.13 (2 H, m, 3-H₂) and 2.17, 2.15, 2.09, 2.03 and 1.94 (each 3 H, s, total 5 × Ac) (Found: C, 47.3; H, 5.2; N, 3.15. $\text{C}_{36}\text{H}_{47}\text{N}_2\text{O}_{23}\text{P}$ requires C, 47.6; H, 5.2; N, 3.1%).



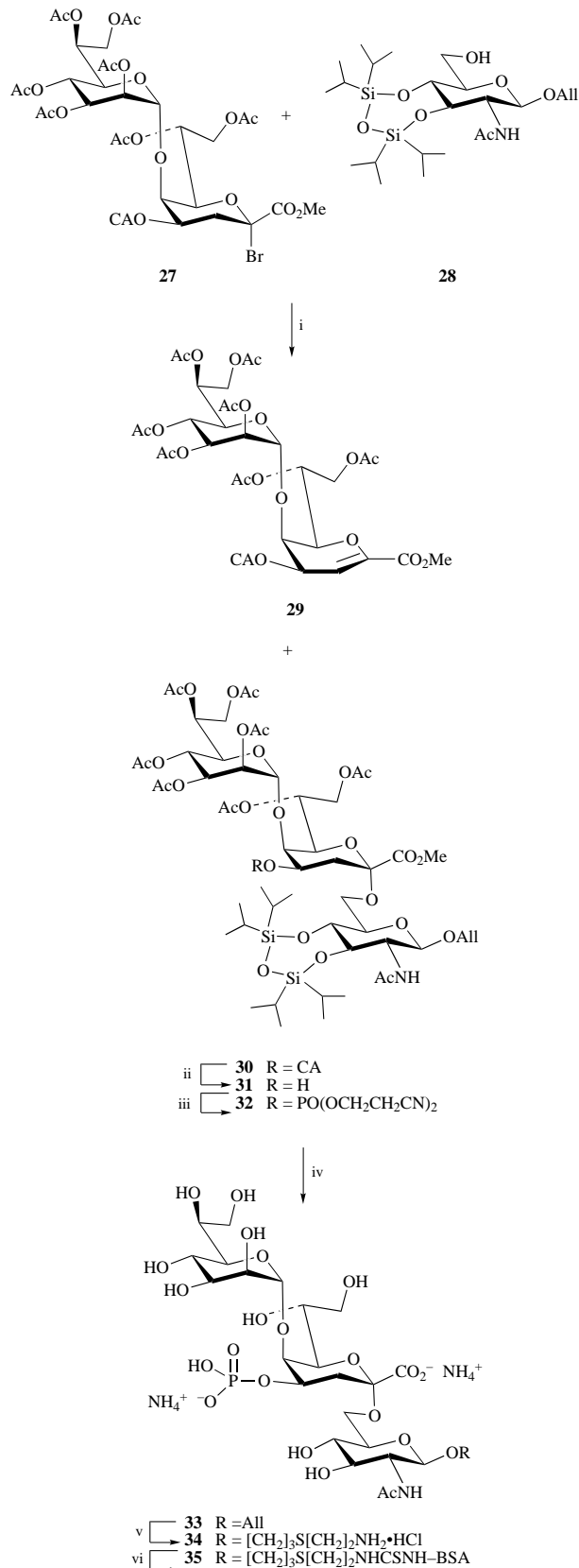
Scheme 4 Reagents: i, (a) DBTO, toluene; (b) TBAB, *p*-MeOC₆H₄CH₂Cl; ii, TMSOTf, CH₂Cl₂; iii, (a) TBAF, THF; (b) MeONa–MeOH; (c) Ac₂O, pyridine; iv, H₂, Pd–carbon, MeOH; v, CA₂O, pyridine; vi, TiBr₄, CH₂Cl₂

Allyl O-(L-glycero- α -D-manno-heptopyranosyl)-(1'→4)-3-deoxy- α -D-manno-oct-2-ulopyranosidonic acid 5-(sodium hydrogen phosphate) **8**

A solution of compound **7** (13 mg, 0.014 mmol) and 0.1 M methanolic NaOMe (0.3 cm³) in dry MeOH (5 cm³) was stirred for 20 h at ambient temperature. The pH of the solution was adjusted to 6.0 by adding Dowex® AG50W-X8 resin (H⁺-form). The resin was filtered off, and the filtrate was concentrated, and treated with 0.2 M aq. NaOH (0.6 cm³) for 4 h at room temperature. The pH of the solution was adjusted to 7.5 by addition of Dowex resin (H⁺-form), the suspension was filtered, and the filtrate was concentrated. The residue was purified on Bio-Gel P2 as described for compound **4**, which afforded compound **8** as a syrup (8.5 mg, 96%); [α]_D²⁰ +83 (*c* 0.5, water); δ_{H} (D₂O) (*inter alia*) 5.96 (1 H, m, CH=), 5.34 (1 H, dq, *J* 17.3, 1.6, =CH₂^{*trans*}), 5.21 (1 H, dq, *J* 10.3, 1.2, =CH₂^{*cis*}), 3.97–3.89 and 3.84–3.79 (2 H, m, OCH₂). Additional ¹H NMR and ¹³C NMR data are given in Tables 1 and 2 (Found: C, 35.9; H, 5.6. C₁₈H₃₀NaO₁₇P·2H₂O requires C, 36.1; H, 5.55%).

3-(2-Aminoethylthio)propyl O-(L-glycero- α -D-manno-heptopyranosyl)-(1'→4)-3-deoxy- α -D-manno-oct-2-ulopyranosidonic acid 5-(ammonium hydrogen phosphate) hydrochloride **9**

A solution of compound **8** (5 mg, 8.1 μ mol) and cysteamine hydrochloride (8.8 mg, 78 μ mol) in water (0.2 cm³) was irradiated at 254 nm for 20 h. The solution was processed as described for compound **5** to afford spacer compound **9** (2.7 mg, 65%) as a syrup, [α]_D²⁰ +53 (*c* 0.27, water); δ_{H} (D₂O) 5.20 (1 H, d, *J*_{2,1'} 1.6, 1'-H), 4.59 (1 H, dd, *J*_{5,4} 9.8, *J*_{5,6} 2.0, 5-H), 4.17 (1 H, m, 4-H), 4.07 (1 H, dd, *J*_{2,3'} 3.0, 2'-H), 4.04 (1 H, m, *J*_{6,7'} 6.0, 6'-H),



Scheme 5 Reagents and conditions: i, HgBr₂, Hg(CN)₂, MeNO₂; ii, HDTC, 2,6-lutidine, HOAc; iii, bis(2-cyanoethyl) *N,N*-diisopropylphosphoramidite, 1*H*-tetrazole, CH₂Cl₂; then MCPBA; iv, (a) TBAF, THF; (b) MeONa–MeOH; (c) aq. NaOH; then NH₄OH; v, aq. HSCH₂CH₂NH₂·HCl, *hv*; vi (a) CCl₄, aq. NaHCO₃; (b) BSA, aq. NaHCO₃–NaCl

3.99–3.93 (2 H, m, 7-H, 8-H^a), 3.85–3.82 (2 H, m, 3'- and 5'-H), 3.75 (2 H, d, 7'-H₂), 3.68–3.30 (5 H, m, 4'-, 6- and 8-H^b, OCH₂), 3.21 (2 H, t, NCH₂), 2.86 (2 H, t, SCH₂), 2.70 (2 H, t, SCH₂) and 2.01–1.85 (4 H, m, 3-H₂, CH₂); δ_{C} 176.18 (C-1),

100.67 (C-2), 97.44 (C-1'), 72.80 (C-5'), 72.80 (C-6), 71.47 (C-3'), 70.47 (C-2'), 70.01 and 69.87 (C-7, -6'), 69.69 (C-4), 67.13 (C-4'), 66.15 (C-5), 64.50 (C-8), 63.70 (C-7'), 62.20 (OCH₂), 39.18 (NCH₂), 34.03 (C-3) and 29.39, 29.13 and 28.62 (2 × SCH₂CH₂) (Found: C, 33.2; H, 6.5; N, 6.1; S, 4.5. C₂₀H₄₅ClN₃O₁₇PS·H₂O requires C, 33.55; H, 6.6; N, 5.9; S, 4.5%).

Methyl {allyl *O*-(2',3',4',6',7'-penta-*O*-acetyl-*L*-glycero- α -*D*-manno-heptopyranosyl)-(1'→5)-4-*O*-[bis(2-cyanoethyl)-phosphoryl]-3-deoxy-7,8-*O*-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)- α -*D*-manno-oct-2-ulopyranosid]onate 12

A suspension of compound **11** (40 mg, 0.042 mmol) and molecular sieves 4 Å (100 mg) in CH₂Cl₂ (5 cm³) was treated with five portions of bis-(2-cyanoethyl) *N,N*-diisopropylphosphoramidite (total amount 63 mg, 0.23 mmol) and 1*H*-tetrazole (17 mg, 0.24 mmol) for 96 h at room temperature. The solution was cooled to -20 °C and a solution of MCPBA (31 mg) in CH₂Cl₂ (1 cm³) was added. After 90 min, 10% aq. Na₂S₂O₅ (0.25 cm³) was added and the reaction mixture was processed as described for compound **7** to give *compound 12* (32 mg, 70%) as a syrup, [α]_D²⁰ +28 (*c* 0.5, CHCl₃); δ _H 5.86 (1 H, m, CH=), 5.37 (1 H, dd, *J*_{3',2'} 3.1, *J*_{3',4'} 10.3, 3'-H), 5.37–5.32 (1 H, m, 6'-H), 5.30 (1 H, dd, *J*_{2',1'} 1.8, 2'-H), 5.29 (1 H, t, *J*_{5',4'} 10.3, 4'-H), 5.28 (1 H, dq, *J* 1.6, =CH_{2trans}), 5.21 (1 H, d, 1'-H), 5.13 (1 H, dq, *J* 10.4, 1.3, =CH_{2cis}), 4.90 (1 H, m, 4-H), 4.44–4.29 (8 H, m, 5- and 7-H, 5'-H, 7'-H^a, 2 × OCH₂CH₂CN), 4.22 (1 H, dd, *J*_{7a',7b'} 11.7, *J*_{7b',6'} 9.0, 7'-H^b), 4.18 (1 H, dd, *J*_{6,5} 1.0, *J*_{6,7} 5.0, 6-H), 4.16 (1 H, ddt, OCH₂), 4.08 (1 H, t, *J*_{8a,8b} 9.5, *J*_{8a,7} 9.5, 8-H^a), 3.92 (1 H, ddt, OCH₂), 3.83 (1 H, d, 8-H^b), 3.80 (3 H, s, CO₂Me), 2.83 (4 H, t, 2 × CH₂CN), 2.46 (1 H, dd, *J*_{3e,3a} 12.3, *J*_{3e,4} 4.5, 3-H^e), 2.22 (1 H, t, *J*_{3a,4} 12.3, 3-H^a), 2.13 (3 H, s), 2.01 (3 H, s), 2.01 (6 H, s) and 1.97 (3 H, s) (total 5 × Ac) and 0.97–1.05 (28 H, m, 4 × PrⁱSi) (Found: C, 50.8; H, 6.6; N, 2.5. C₄₇H₇₅N₂O₂₃PSi₂ requires C, 50.3; H, 6.7; N, 2.5%).

Allyl *O*-(*L*-glycero- α -*D*-manno-heptopyranosyl)-(1'→5)-3-deoxy- α -*D*-manno-oct-2-ulopyranosidonic acid 4 (sodium hydrogen phosphate) 13

A solution of compound **12** (27 mg, 0.024 mmol) in THF (5 cm³) was stirred with a 1 M solution of TBAF in THF (36 mm³) for 30 min at room temperature. Methanol (0.3 cm³) was added and the solution was concentrated and treated with 0.1 M methanolic NaOMe (0.5 cm³) in MeOH (5 cm³) for 48 h at 4 °C. The pH of the solution was adjusted to 7.0 by addition of Dowex 50 resin (H⁺-form) and the filtrate obtained upon removal of the resin was evaporated to dryness. The residue was dissolved in water (2 cm³) and the solution was stirred with 0.2 M aq. NaOH (1 cm³) for 22 h. Work-up of the solution as described for compound **8** afforded *compound 13* as a syrup (12.3 mg, 83%), [α]_D²⁰ +67 (*c* 0.3, water); δ _H(D₂O) 5.69 (1 H, m, =CH), 5.37 (1 H, dq, =CH_{2trans}), 5.24 (1 H, dq, =CH_{2cis}) and 4.01–3.90 (m, OCH₂). Additional ¹H and ¹³C NMR data are presented in Tables 1 and 2 (Found: C, 36.4; H, 5.6. C₁₈H₃₀NaO₁₇P·H₂O requires C, 36.6; H, 5.5%).

Methyl [2-acetamido-1-*O*-allyl-2,6-dideoxy-3,4-*O*-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)- β -*D*-glucopyranos-6-yl 3'-deoxy-7',8'-*O*-isopropylidene-4'-*O*-(*p*-methoxybenzyl)- α -*D*-manno-oct-2'-ulopyranosid]onate 16

A solution of compound **15** (122 mg, 0.12 mmol) in MeOH (30 cm³) was stirred with 0.1 M methanolic NaOMe (1.5 cm³) for 5 h at room temperature. The pH of the solution was adjusted to 7.0 by addition of Dowex (H⁺)-resin. The resin was removed and the filtrate was concentrated, and purified on silica gel [toluene–EtOAc (1:1)] to give *compound 16* as a syrup (100 mg, 93%), [α]_D²⁰ +33 (*c* 1.4, CHCl₃); δ _H 7.23 (2 H, m) and 6.86 (2 H, m) (together ArH), 5.84 (1 H, m, CH=), 5.50 (1 H, d, *J*_{NH,2} 7.8, NH), 5.21 (1 H, dq, *J* 17.1, 1.6, =CH_{2trans}), 5.16 (1 H, dq, *J* 10.4, 1.2, =CH_{2cis}), 4.96 (1 H, d, *J*_{1,2} 8.4, 1-H), 4.52 (2 H, d, *J* 2.6,

CH₂Ar), 4.44 (1 H, m, *J*_{7,8a'} 6.3, *J*_{7,8b'} 5.6, 7'-H), 4.19 (1 H, m, OCHH allyl), 4.19 (1 H, dd, *J*_{8'a,8b'} 8.5, 8'-H^a), 4.17 (1 H, m, 3-H), 4.12 (1 H, br s, 5'-H), 4.00–3.86 (3 H, m, 4'-H, 8'-H^b, OCHH allyl), 3.81 (1 H, m, 6-H^a), 3.79 (3 H, s, ArOMe), 3.74 (1 H, dd, *J*_{6,7} 7.5, 6'-H), 3.74 (3 H, s, CO₂Me), 3.53–3.46 (2 H, m, 5- and 6-H^b), 3.40 (1 H, m, 4-H), 3.20 (1 H, m, *J*_{2,3} 9.1, 2-H), 2.26 (1 H, br s, OH), 2.24 (1 H, dd, *J*_{3e',3a'} 12.8, *J*_{3e',4'} 4.6, 3'-H^e), 2.01 (1 H, t, 3'-H^a), 1.95 (3 H, s, NHAc), 1.40 and 1.39 (each 3 H, together s, 2 × Me) and 1.05–0.97 (28 H, m, 4 × PrⁱSi) (Found: C, 58.1; H, 8.0; N, 1.5. C₄₃H₇₁NO₁₅Si₂ requires C, 57.5; H, 8.0; N, 1.6%).

Methyl {1-*O*-allyl-2,6-dideoxy-2-[2-(2',3',4',6',7'-penta-*O*-acetyl-*L*-glycero- α -*D*-manno-heptopyranosyl)ethylimino]-3,4-*O*-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)- β -*D*-glucopyranos-6-yl 3'-deoxy-7',8'-*O*-isopropylidene-4'-*O*-(*p*-methoxybenzyl)- α -*D*-manno-oct-2'-ulopyranosid]onate 18

A suspension of compounds **16** (20 mg, 0.022 mmol) and **1** (25 mg, 0.044 mmol) and molecular sieves 4 Å (0.2 g) in CH₂Cl₂ (4 cm³) was stirred under N₂ for 4 h. A solution of 0.02 M TMSOTf in CH₂Cl₂ (0.15 cm³) was added in two portions during 2 h at room temperature. CH₂Cl₂ (2 cm³) and triethylamine (10 mm³) were added, and the suspension was filtered, and washed with EtOAc. The filtrate was concentrated, and chromatographed on silica gel [toluene–EtOAc (3:2)] which gave *compound 18* (3.7 mg, 13%) as a syrup, [α]_D²⁰ +20 (*c* 0.15, CHCl₃); δ _H 7.22 (2 H, m) and 6.86 (2 H, m) (together ArH), 6.37 (1 H, d, *J*_{2',1'} 1.7, 1'-H), 5.76 (1 H, m, CH=), 5.38 (1 H, dd, *J*_{3',2'} 3.3, 3'-H), 5.30 (1 H, t, *J*_{4',5'} 10.1, 4'-H), 5.30–5.26 (1 H, m, 6''-H), 5.22 (1 H, dd, 2''-H), 5.14 (1 H, dq, *J* 17.3, 1.6, =CH_{2trans}), 5.07 (1 H, dq, *J* 10.5, 1.6, =CH_{2cis}), 4.53 and 4.49 (2 H, AB system, *J*_{A,B} 11.2, CH₂Ar), 4.46 (1 H, m, 7'-H), 4.37 (1 H, d, *J*_{1,2} 7.7, 1-H), 4.23–4.11 (6 H, m, 5'-H, 8'-H^a, 5''-H, 7'-H₂ and OCHH allyl), 3.97–3.87 (3 H, m, 4'-H, 8'-H^b, OCHH allyl), 3.82–3.79 (4 H, m, 6-H^a, ArOMe), 3.74–3.70 (4 H, m, 6'-H, CO₂Me), 3.60 (1 H, t, *J*_{3,2} 7.9, 3-H), 3.57–3.45 (3 H, m, 4- and 5-H, 6-H^b), 3.21 (1 H, t, 2-H), 2.27 (1 H, s, OH), 2.24 (1 H, dd, *J*_{3'a,3'e} 13.0, *J*_{3'e,4'} 4.7, 3'-H^e), 2.18 (3 H, s) and 2.13 (3 H, s) (together 2 × Ac), 2.01 (4 H, m, 3'-H^a, Ac), 1.99 (3 H, s) and 1.98 (6 H, s) (together CMe and 2 × Ac), 1.40 (6 H, s, CMe₂) and 1.06–0.98 (28 H, m, 4 × PrⁱSi) (Found: C, 55.2; H, 7.2; N, 1.0. C₆₀H₉₃NO₂₆Si₂ requires C, 55.4; H, 7.2; N, 1.1%).

***p*-Methoxybenzyl [benzyl 3-deoxy-4-*O*-(*p*-methoxybenzyl)-7,8-*O*-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)- β -*D*-manno-oct-2-ulopyranosid]onate 20 and methyl [benzyl 3-deoxy-4-*O*-(*p*-methoxybenzyl)-7,8-*O*-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)- β -*D*-manno-oct-2-ulopyranosid]onate 21**

A solution of compound **19** (2 g, 4.24 mmol) and DBTO (2.6 g, 10.4 mmol) in toluene (120 cm³) was heated under reflux with continuous separation of water for 24 h. After cooling of the mixture to 90 °C, molecular sieves 4 Å (1 g) were added. Tetrabutylammonium bromide (TBAB) (2.72 g, 8.4 mmol) and *p*-methoxybenzyl chloride (2.76 cm³, 20.4 mmol) were added and the reaction was kept at 90 °C for 4.5 h. MeOH (1.2 cm³) was added dropwise and stirring was continued for 30 min. The suspension was filtered over Celite[®], and washed successively with EtOAc and water. The organic layer was dried (Na₂SO₄) and evaporated. Purification of the residue on silica gel [toluene–EtOAc (20:1)] afforded a mixture of products **20** and **21** which were separated by further chromatography [hexane–EtOAc (15:1 → 5:1)]. Yield for *compound 20*: 2.23 g (65%), syrup, [α]_D²⁰ +0.6 (*c* 1.6, CHCl₃); δ _H 7.28–7.18 (9 H, m, ArH), 6.87–6.83 (4 H, m, ArH), 5.09 and 4.99 (2 H, AB system, *J*_{A,B} 11.5, CH₂Ar), 4.78 and 4.40 (2 H, AB system, *J*_{A,B} 11.5, CH₂Ar), 4.51 and 4.40 (2 H, AB system, *J*_{A,B} 11.5, CH₂Ar), 4.30 (1 H, m, *J*_{7,8a} 1.5, *J*_{7,8b} 8.6, *J*_{7,6} 8.6, 7-H), 4.28 (1 H, dd, *J*_{8a,8b} 12.0, 8-H^a), 4.00 (1 H, br s, 5-H), 3.79 (3 H, s, OMe), 3.78 (3 H, s, OMe), 3.62 (1 H, dd, 8-H^b), 3.38 (1 H, ddd, *J*_{4,5} 2.6, *J*_{4,3a} 12.2, *J*_{4,3e} 4.4, 4-H), 3.28 (1 H, d, 6-H), 2.59 (1 H, dd, *J*_{3a,3e} 12.5, 3-H^e),

2.24 (1 H, d, OH), 2.09 (1 H, t, 3-H^a) and 1.09–0.99 (28 H, m, 4 × Pr^dSi) (Found: C, 63.1; H, 7.8. C₄₃H₆₂O₁₁Si₂ requires C, 63.7; H, 7.7%).

Yield for **compound 21**: 0.26 g (9%), syrup [α]_D²⁰ –0.6 (c 0.9, CHCl₃); δ_{H} 7.31–7.22 (7 H, m, ArH), 6.89–6.86 (2 H, m, ArH), 4.82 and 4.48 (2 H, AB system, $J_{\text{A,B}}$ 11.6, CH₂Ar), 4.57 and 4.49 (2 H, AB system, $J_{\text{A,B}}$ 11.6, CH₂Ar), 4.34 (1 H, m, $J_{7,8a}$ 1.6, $J_{7,8b}$ 9.0, $J_{7,6}$ 8.4, 7-H), 4.33 (1 H, dd, $J_{8a,8b}$ 12.3, 8-H^a), 4.06 (1 H, br s, 5-H), 3.80 (3 H, s, ArOMe), 3.75 (1 H, dd, 8-H^b), 3.66 (3 H, s, CO₂Me), 3.43 (1 H, ddd, $J_{4,3a}$ 12.3, $J_{4,3e}$ 4.6, $J_{4,5}$ 2.7, 4-H), 3.34 (1 H, d, $J_{6,5}$ 1.1, 6-H), 2.55 (1 H, dd, $J_{3e,3a}$ 12.5, 3-H^e), 2.30 (1 H, dd, OH), 2.13 (1 H, t, 3-H^a) and 1.10–1.0 (28 H, m, 4 × Pr^dSi) (Found: C, 61.3; H, 8.0. C₃₆H₅₆O₁₀Si₂ requires C, 61.1; H, 7.95%).

p*-Methoxybenzyl [benzyl *O*-(2',3',4',6',7'-penta-*O*-acetyl-L-glycero- α -D-manno-heptopyranosyl)-(1'→5)-3-deoxy-4-*O*-(*p*-methoxybenzyl)-7,8-*O*-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)- β -D-manno-oct-2-ulopyranosid]onate **22*

A suspension of compounds **20** (1.45 g, 1.79 mmol) and **1** (2.02 g, 3.58 mmol) in CH₂Cl₂ (20 cm³) was stirred with molecular sieves 4 Å (1 g) for 90 min at room temperature. A solution of TMSOTf (0.16 cm³, 0.895 mmol) in CH₂Cl₂ (1 cm³) was added under N₂. After 20 min, the reaction was quenched by addition of triethylamine (0.1 cm³). The suspension was filtered over Celite[®], and washed successively with EtOAc and water. The organic phase was dried (Na₂SO₄), concentrated and purified by silica gel chromatography [hexane–EtOAc (5:1)] which furnished **compound 22** (1.39 g, 64%) as a syrup, [α]_D²⁰ +11 (c 2.7, CHCl₃); δ_{H} 7.31–7.18 (9 H, m, ArH), 6.87–6.82 (4 H, m, ArH), 5.37 (1 H, dd, $J_{2,1'}$ 1.6, $J_{2,3'}$ 3.4, 2'-H), 5.32 (1 H, dd, $J_{3,4'}$ 10.0, 3'-H), 5.18 and 5.01 (2 H, AB system, $J_{\text{A,B}}$ 11.7, OCH₂Ar), 5.16 (1 H, t, $J_{4,5'}$ 10.0, 4'-H), 5.15 (1 H, d, 1'-H), 4.91 (1 H, dt, $J_{6,5'}$ 2.6, $J_{6,7b}$ 2.1, 6'-H), 4.81 and 4.51 (2 H, AB system, $J_{\text{A,B}}$ 11.8, CH₂Ar), 4.60 (1 H, dd, 5'-H), 4.55 and 4.16 (2 H, AB system, $J_{\text{A,B}}$ 10.9, OCH₂Ar), 4.16–4.05 (2 H, m, 8-H^a, 7'-H^a), 4.07 (1 H, d, $J_{4,5}$ 2.3, 5-H), 3.96 (1 H, dd, $J_{8a,8b}$ 13.2, $J_{8b,7}$ 3.9, 8-H^b), 3.91 (1 H, m, 7-H), 3.88 (1 H, dd, $J_{7a,7b}$ 11.9, 7'-H^b), 3.81 (3 H, s, OMe), 3.80 (3 H, s, OMe), 3.57 (1 H, d, $J_{6,7}$ 9.3, 6-H), 3.43 (1 H, dd, $J_{4,3a}$ 12.4, $J_{4,3e}$ 4.1, 4-H), 2.68 (1 H, dd, $J_{3e,3a}$ 12.4, 3-H^e), 2.14 (1 H, t, 3-H^a), 2.11, 2.05 and 2.01 (each 3 H, s, total 3 × Ac), 1.95 (6 H, s, 2 × Ac) and 1.06–0.97 (28 H, m, 4 × Pr^dSi) (Found: C, 59.6; H, 7.0. C₆₀H₈₄O₂₂Si₂ requires C, 59.4; H, 7.0%).

Methyl [benzyl *O*-(2',3',4',6',7'-penta-*O*-acetyl-L-glycero- α -D-manno-heptopyranosyl)-(1'→5)-7,8-di-*O*-acetyl-3-deoxy-4-*O*-(*p*-methoxybenzyl)- β -D-manno-oct-2-ulopyranosid]onate **23**

A solution of compound **22** (800 mg, 0.66 mmol) in THF (60 cm³) was treated with a 1.1 M solution of TBAF in THF (1.2 cm³) for 1 h at room temperature. MeOH (85 cm³) was added and the solution was evaporated to dryness. A solution of the residue in dry MeOH (70 cm³) was stirred with 0.1 M methanolic NaOMe (28 cm³) for 4.5 h. The pH of the solution was adjusted to 7.5 by addition of Dowex resin (H⁺-form) and the suspension was filtered. The filtrate was evaporated and dried. The residue was dissolved in pyridine (30 cm³), and the solution was cooled to 0 °C and stirred with acetic anhydride (1 cm³) for 4 h. MeOH (5 cm³) was added and the solution was stirred for 15 min and concentrated. Co-evaporation with toluene (3 × 20 cm³) afforded a syrup, which was subjected to silica gel chromatography [toluene–EtOAc (1:1)] to give **compound 23** as a syrup (760 mg, 73%), [α]_D²⁰ +11 (c 0.8, CHCl₃); δ_{H} 7.34–7.21 (7 H, m, ArH), 6.88–6.85 (2 H, m, ArH), 5.39 (1 H, dd, $J_{3,2'}$ 3.4, $J_{4,3'}$ 10.1, 3'-H), 5.24 (1 H, t, $J_{4,5'}$ 10.1, 4'-H), 5.21 (1 H, dd, $J_{2,1'}$ 1.7, 2'-H), 5.16 (1 H, m, $J_{6,5'}$ 2.2, $J_{6,7b}$ 4.7, $J_{6,7a}$ 7.2, 6'-H), 5.08 (1 H, m, $J_{7,8a}$ 3.4, $J_{7,8b}$ 2.4, $J_{7,6}$ 9.4, 7-H), 4.85 (1 H, d, 1'-H), 4.80 and 4.46 (2 H, AB system, $J_{\text{A,B}}$ 11.8, CH₂Ar), 4.63 and 4.40 (2 H, AB system, $J_{\text{A,B}}$ 11.9, CH₂Ar), 4.47 (1 H, dd, $J_{8a,8b}$ 12.2, 8-H^a), 4.45 (1 H, br d, 5'-H), 4.38 (1 H, dd, 8-H^b), 4.19–4.16 (2 H, m, 7'-H₂), 3.91 (1 H, d, 6-H), 3.82 (1 H, br s, 5-H), 3.81 (3 H, s,

ArOMe), 3.61 (3 H, s, CO₂Me), 3.38 (1 H, ddd, $J_{4,3e}$ 3.5, $J_{4,3a}$ 12.8, $J_{4,5}$ 2.5, 4-H), 2.61 (1 H, dd, $J_{3a,3e}$ 12.5, 3-H^e), 2.16 (3 H, s, Ac), 2.12–2.08 (10 H, m, 3-H^a and 3 × Ac) and 2.02, 2.00 and 1.97 (each 3 H, s, total 3 × Ac) (Found: C, 56.5; H, 5.7. C₄₅H₅₆O₂₂ requires C, 56.9; H, 5.9%).

Methyl *O*-(2',3',4',6',7'-penta-*O*-acetyl-L-glycero- α -D-manno-heptopyranosyl)-(1'→5)-7,8-di-*O*-acetyl-3-deoxy-D-manno-oct-2-ulopyranosylonate **24**

A solution of compound **23** (580 mg) in dry MeOH (30 cm³) was stirred with 5% Pd/C (150 mg) under H₂ at atmospheric pressure for 90 min. The catalyst was removed by filtration over Celite[®] and the filtrate was taken to dryness to afford compound **24** as a syrup (450 mg, ~100%), [α]_D²⁰ +14 (c 1.5, CHCl₃); δ_{H} 5.43 (1 H, dd, $J_{3,4'}$ 9.8, $J_{3,2'}$ 3.3, 3'-H), 5.29 (1 H, t, $J_{4,5'}$ 9.7, 4'-H), 5.28 (1 H, m, $J_{6,7a}$ 4.7, $J_{6,7b}$ 7.1, $J_{6,5'}$ 3.3, 6'-H), 5.16 (1 H, dd, $J_{2,1'}$ 2.3, 2'-H), 5.10 (1 H, m, $J_{7,6}$ 9.5, $J_{7,8a}$ 2.6, $J_{7,8b}$ 3.6, 7-H), 4.94 (1 H, d, 1'-H), 4.47 (1 H, dd, $J_{8a,8b}$ 12.3, 8-H^a), 4.40 (1 H, dd, $J_{7a,7b}$ 11.8, 7'-H^a), 4.38 (1 H, dd, 5'-H), 4.21 (1 H, dd, 8-H^b), 4.18 (1 H, dd, 7'-H^b), 4.15 (1 H, dd, $J_{6,5}$ 0.8, 6-H), 4.12 (1 H, m, 4-H), 3.94 (1 H, br s, 5-H), 3.89 (3 H, s, CO₂Me), 3.87 (1 H, d, $J_{3e,OH}$ 2.1, 2-OH), 2.74 (1 H, d, $J_{4,OH}$ 8.7, 4-OH), 2.30 (1 H, m, 3-H^e), 2.16, 2.13 and 2.10 (each 3 H, s), 2.06 (6 H, s), 2.03 and 2.02 (each 3 H, s) (together 7 × Ac), 1.97 (1 H, m, 3-H^a) (Found: C, 48.1; H, 5.7. C₃₀H₄₂O₂₁·1/2 H₂O requires C, 48.2; H, 5.8%).

Methyl [chloroacetyl *O*-(2',3',4',6',7'-penta-*O*-acetyl-L-glycero- α -D-manno-heptopyranosyl)-(1'→5)-7,8-di-*O*-acetyl-4-*O*-chloroacetyl-3-deoxy- β -D-manno-oct-2-ulopyranosid]onate **25 and methyl [chloroacetyl *O*-(2',3',4',6',7'-penta-*O*-acetyl-L-glycero- α -D-manno-heptopyranosyl)-(1'→5)-7,8-di-*O*-acetyl-4-*O*-chloroacetyl-3-deoxy- α -D-manno-oct-2-ulopyranosid]onate **26****

A solution of diol **24** (420 mg, 0.57 mmol) in pyridine (15 cm³) was cooled to 0 °C and stirred with chloroacetic anhydride (1.8 g, 10.5 mmol) for 35 min. MeOH (4 cm³) was added, and the solution was concentrated, and co-evaporated three times with toluene (50 cm³). Purification of the residue on silica gel [toluene–EtOAc (1:1)] furnished compound **25** as the faster migrating isomer (124 mg, 25%), syrup, [α]_D²⁰ +24 (c 0.6, CHCl₃); δ_{H} 5.45 (1 H, dd, $J_{3,2'}$ 3.4, $J_{3,4'}$ 10.0, 3'-H), 5.35–5.30 (1 H, m, 6'-H), 5.29 (1 H, t, $J_{4,5'}$ 10.0, 4'-H), 5.17 (1 H, ddd, $J_{4,3e}$ 4.5, $J_{4,3a}$ 12.6, $J_{4,5}$ 2.5, 4-H), 5.08 (1 H, m, $J_{7,8a}$ 2.2, $J_{7,8b}$ 3.3, $J_{7,6}$ 9.8, 7-H), 5.06 (1 H, dd, $J_{2,1'}$ 2.0, 2'-H), 4.82 (1 H, d, 1'-H), 4.61 (1 H, d, 6-H), 4.54 (1 H, dd, $J_{8a,8b}$ 12.5, 8-H^a), 4.30–4.23 (3 H, m, 8-H^b, 7'-H^a, 5'-H), 4.22 (2 H, s, ClCH₂CO), 4.19 (2 H, s, ClCH₂CO), 4.15 (1 H, m, 7'-H^b), 4.09 (1 H, br s, 5-H), 3.78 (3 H, s, CO₂Me), 2.47 (1 H, dd, $J_{3e,3a}$ 12.6, 3-H^e), 2.26 (1 H, t, 3-H^a) and 2.17, 2.13, 2.10, 2.08, 2.05, 2.04 and 2.03 (each 3 H, s, total 7 × Ac) (Found: C, 45.5; H, 4.6; Cl, 7.9. C₃₄H₄₄Cl₂O₂₃ requires C, 45.8; H, 5.0; Cl, 7.95%).

Further elution of the column afforded *stereoisomeric compound 26* as a syrup (224 mg, 46%), [α]_D²⁰ +46 (c 0.3, CHCl₃); δ_{H} 5.39 (1 H, dd, $J_{3,2'}$ 3.4, $J_{3,4'}$ 9.9, 3'-H), 5.33–5.29 (2 H, m, 4- and 6'-H), 5.28 (1 H, t, $J_{4,5'}$ 9.8, 4'-H), 5.14 (1 H, m, $J_{7,6}$ 9.7, $J_{7,8a}$ 2.4, 7-H), 5.09 (1 H, dd, $J_{2,1'}$ 2.2, 2'-H), 4.84 (1 H, d, 1'-H), 4.67 (1 H, dd, $J_{8a,8b}$ 12.3, 8-H^a), 4.28–4.09 (6 H, m, 5-, 5'- and 6-H, 8-H^b and 7'-H₂), 4.23 (2 H, s, ClCH₂CO), 4.20 (2 H, s, ClCH₂CO), 3.83 (3 H, s, CO₂Me), 2.35 (1 H, dd, $J_{3a,3e}$ 12.6, $J_{3e,4}$ 5.0, 3-H^e), 2.27 (1 H, t, $J_{3a,4}$ 12.2, 3-H^a), 2.16, 2.14, 2.09 and 2.05 (each 3 H, s), 2.04 (6 H, s) and 2.03 (3 H, s) (together 7 × Ac) (Found: C, 45.8; H, 4.7; Cl, 7.5%).

Methyl *O*-(2',3',4',6',7'-penta-*O*-acetyl-L-glycero- α -D-manno-heptopyranosyl)-(1'→5)-7,8-di-*O*-acetyl-4-*O*-chloroacetyl-3-deoxy- α -D-manno-oct-2-ulopyranosylonate bromide **27**

A solution of stereoisomers **25** and **26** (285 mg, 0.32 mmol) and titanium tetrabromide (733 mg, 2 mmol) was kept at 4 °C for 42 h. The solution was diluted with ice-cold CHCl₃ (100 cm³) and extracted with saturated aq. NaHCO₃. The organic layer

was dried (Na₂SO₄), evaporated to dryness and immediately used for the glycosylation step. Yield for compound **27**: 278 mg (99%), syrup, $[\alpha]_D^{20} +66$ (c 0.6, CHCl₃); δ_H 5.51 (1 H, ddd, $J_{4,5}$ 2.8, $J_{4,3e}$ 4.4, $J_{4,3a}$ 11.8, 4-H), 5.37 (1 H, dd, $J_{2',3'}$ 3.2, $J_{4',3'}$ 9.9, 3'-H), 5.28 (1 H, t, $J_{4',5'}$ 9.5, 4'-H), 5.30–5.25 (1 H, m, 6'-H), 5.17 (1 H, dt, $J_{7,6}$ 9.8, $J_{7,8a}$ 2.0, 7-H), 5.02 (1 H, dd, $J_{2',1'}$ 2.0, 2'-H), 4.82 (1 H, d, 1'-H), 4.53 (1 H, dd, $J_{8a,8b}$ 12.6, 8-H^a), 4.41 (1 H, br d, 6-H), 4.32–4.10 (7 H, m, 5-, 8-H^b, 5'-H, 7'-H₂ and ClCH₂CO), 3.94 (3 H, s, CO₂Me), 2.72 (1 H, dd, $J_{3a,3e}$ 13.6, 3-H^a), 2.40 (1 H, t, 3-H^a) and 2.17, 2.14, 2.10 and 2.09 (3 H each, s) and 2.04 (9 H, s) (together 7 × Ac).

Methyl *O*-(2',3',4',6',7'-penta-*O*-acetyl-*L*-glycero- α -D-manno-heptopyranosyl)-(1'→5)-7,8-di-*O*-acetyl-2,6-anhydro-4-*O*-chloroacetyl-3-deoxy-D-manno-oct-2-enoate **29 and methyl [2-acetamido-1-*O*-allyl-2,6-dideoxy-3,4-*O*-(1,1,3,3-tetraisopropyl-disiloxane-1,3-diyl)- β -D-glucopyranos-6-yl *O*-(2'',3'',4'',6'',7''-penta-*O*-acetyl-*L*-glycero- α -D-manno-heptopyranosyl)-(1''→5')-7',8'-di-*O*-acetyl-4'-*O*-chloroacetyl-3'-deoxy- α -D-manno-oct-2'-ulopyranosid]onate **30****

A solution of compound **27** (267 mg, 0.3 mmol) in MeNO₂ (2 cm³) was added to a suspension of compound **28** (290 mg, 0.57 mmol), mercury(II) cyanide (68 mg, 0.27 mmol), mercury(II) bromide (32 mg, 0.09 mmol) and molecular sieves 4 Å (0.5 g) in dry MeNO₂ (2 cm³) at room temperature under N₂. After 60 h, the suspension was diluted with EtOAc (50 cm³), filtered over Celite® and washed with MeOH–EtOAc (1:1). Filtrate and washings were combined, concentrated, then diluted with CH₂Cl₂ (100 cm³). The organic layer was extracted in turn with saturated aq. NaHCO₃, then with 10% aq. KI, and dried (Na₂SO₄). Purification of the residue obtained upon concentration on silica gel [toluene–EtOAc (1:1)] gave, first the *glycol ester* **29** as a syrup (120 mg, 50%), $[\alpha]_D^{20} -135$ (c 0.3, CHCl₃); δ_H 5.95 (1 H, dd, $J_{3,4}$ 3.2, $J_{3,5}$ 1.0, 3-H), 5.71 (1 H, m, $J_{4,5}$ 4.3, $J_{4,6}$ 1.3, 4-H), 5.46 (1 H, m, $J_{7,6}$ 9.6, 7-H), 5.33–5.26 (3 H, m, 3', 4'- and 6'-H), 5.15 (1 H, t, $J_{2',1'}$ 2.2, $J_{2',3'}$ 2.2, 2'-H), 4.94 (1 H, d, 1'-H), 4.68 (1 H, dd, $J_{8a,8b}$ 12.5, $J_{8a,7}$ 2.6, 8-H^a), 4.40 (1 H, br d, $J_{7a,7b}$ 9.6, 7'-H^a), 4.30 (1 H, br s, 5-H), 4.25–4.12 (5 H, m, 6-H, 8-H^b, 7'-H^b, ClCH₂CO), 3.82 (3 H, s, CO₂Me) and 2.17, 2.13, 2.12, 2.08, 2.05, 2.04 and 2.00 (each 3 H, s, together 7 × Ac) (Found: C, 42.5; H, 5.8. C₂₅H₄₁ClO₂₁ requires C, 42.1; H, 5.8%).

Pooling and evaporation of the following fractions afforded starting material **28** (140 mg, 48% recovery); finally *trisaccharide* **30** was obtained as a syrup (110 mg, 28%), $[\alpha]_D^{20} +33$ (c 0.3, CHCl₃); δ_H 5.90 (1 H, m, CH=), 5.45 (1 H, d, $J_{NH,2}$ 7.6, NH), 5.41 (1 H, dd, $J_{3',2'}$ 3.4, $J_{3',4'}$ 10.0, 3'-H), 5.37–5.28 (3 H, m, 4'- and 6''-H, =CH_{2trans}), 5.28 (1 H, t, $J_{4',5'}$ 10.1, 4''-H), 5.18 (1 H, dq, J 10.4, 1.6, =CH_{2cis}), 5.11 (1 H, m, $J_{7',6'}$ 9.5, 7'-H), 5.01 (1 H, dd, $J_{2',1'}$ 2.0, 2''-H), 4.88 (1 H, d, $J_{1,2}$ 8.4, 1-H), 4.86 (1 H, dd, $J_{8'a,8'b}$ 12.1, $J_{8'a,7}$ 2.2, 8'-H^a), 4.80 (1 H, d, 1''-H), 4.32 (1 H, ddt, OCH₂), 4.27–4.07 (8 H, m, 5'- and 6'-H, 8'-H^b, 5''-H, 7''-H₂ and ClCH₂CO), 4.06 (1 H, t, $J_{3,4}$ 7.7, 3-H), 3.84 (1 H, dd, $J_{6a,6b}$ 8.0, $J_{6a,5}$ 1.8, 6-H^a), 3.79 (3 H, s, CO₂Me), 3.66 (1 H, t, $J_{6b,5}$ 8.0, 6-H^b), 3.54 (1 H, m, $J_{3,4}$ 9.0, 5-H), 3.39 (1 H, dd, 4-H), 3.31 (1 H, ddd, $J_{2,3}$ 10.2, 2-H), 2.27 (1 H, dd, $J_{3'e,3'a}$ 12.5, $J_{3'e,4'}$ 4.7, 3'-H^a), 2.17–2.12 (7 H, m, 3'-H^a and 2 × Ac), 2.10, 2.08, 2.04, 2.03 and 2.02 (each 3 H, s, total 5 × Ac), 1.96 (3 H, s, NHAc) and 1.10–0.99 (28 H, m, 4 × Pr^dSi) (Found: C, 50.8; H, 6.5; N, 1.0. C₅₅H₈₆ClNO₂₈Si₂ requires C, 50.8; H, 6.7; N, 1.1%).

Methyl [2-acetamido-1-*O*-allyl-2,6-dideoxy-3,4-*O*-(1,1,3,3-tetraisopropyl-disiloxane-1,3-diyl)- β -D-glucopyranos-6-yl *O*-(2'',3'',4'',6'',7''-penta-*O*-acetyl-*L*-glycero- α -D-manno-heptopyranosyl)-(1''→5')-7',8'-di-*O*-acetyl-3'-deoxy- α -D-manno-oct-2'-ulopyranosid]onate **31**

A solution of chloroacetate **30** (60 mg, 0.046 mmol) in 2:1 lutidine–HOAc (5 cm³) was cooled to 0 °C and treated with hydrazine dithiocarbonate (HDTC) (0.37 cm³, 0.138 mmol) for 30 min at 0 °C, then was stirred at room temperature for an additional 60 min. The solution was co-evaporated six times

with toluene (50 cm³), concentrated and chromatographed on silica gel [EtOAc–toluene (2.5:1)] which afforded *compound 31* as a syrup (53 mg, 94%), $[\alpha]_D^{20} +12$ (c 0.4, CHCl₃); δ_H 5.88 (1 H, m, CH=), 5.47 (1 H, d, $J_{NH,2}$ 8.1, NH), 5.40 (1 H, dd, $J_{3',2'}$ 3.3, $J_{3',4'}$ 9.6, 3'-H), 5.28 (1 H, dq, J 17.2, 1.6, =CH_{2trans}), 5.27 (1 H, t, $J_{4',5'}$ 9.6, 4''-H), 5.24–5.16 (3 H, m, 7'- and 6''-H, =CH_{2cis}), 5.13 (1 H, t, 2''-H), 4.92 (1 H, d, $J_{1',2'}$ 2.2, 1''-H), 4.90 (1 H, d, $J_{1,2}$ 8.3, 1-H), 4.80 (1 H, dd, $J_{8'a,8'b}$ 12.3, $J_{8'a,7}$ 2.3, 8'-H^a), 4.32 (1 H, dd, $J_{5',6'}$ 3.1, 5''-H), 4.32 (1 H, dd, $J_{7'a,7'b}$ 11.7, $J_{7'a,6'}$ 5.3, 7''-H^a), 4.28 (1 H, dt, OCHH), 4.18 (1 H, dd, $J_{7b',6'}$ 7.0, 7''-H^b), 4.17–4.03 (5 H, m, 3-, 4'- and 6'-H, 8'-H^b, OCHH), 3.90 (1 H, br s, 5'-H), 3.81 (1 H, d, $J_{6a,6b}$ 9.0, $J_{6a,5} <1.5$, 6-H^a), 3.78 (3 H, s, CO₂Me), 3.60 (1 H, t, $J_{6b,5}$ 8.5, 6-H^b), 3.53 (1 H, t, $J_{5,4}$ 8.3, 5-H), 3.37 (1 H, t, 4-H), 3.26 (1 H, m, 2-H), 2.80 (1 H, d, $J_{OH,4'}$ 9.1, OH), 2.24 (1 H, dd, $J_{3'a,3'e}$ 12.5, $J_{3'e,4'}$ 4.5, 3'-H^e), 2.15, 2.12, 2.08, 2.07, 2.05, 2.03 and 2.02 (3 H each, s, together 7 × Ac), 1.96 (3 H, s, NHAc), 1.91 (1 H, t, 3'-H^a) and 1.07–0.99 (28 H, m, 4 × Pr^dSi) (Found: C, 51.9; H, 6.7; N, 1.2. C₅₃H₈₅NO₂₇Si₂ requires C, 52.0; H, 7.0; N, 1.1%).

Methyl [2-acetamido-1-*O*-allyl-2,6-dideoxy-3,4-*O*-(1,1,3,3-tetraisopropyl-disiloxane-1,3-diyl)- β -D-glucopyranos-6-yl *O*-(2'',3'',4'',6'',7''-penta-*O*-acetyl-*L*-glycero- α -D-manno-heptopyranosyl)-(1''→5')-7',8'-di-*O*-acetyl-4'-*O*-[bis(2-cyanoethyl)phosphoryl]-3'-deoxy- α -D-manno-oct-2'-ulopyranosid]onate **32**

A suspension of compound **31** (48 mg, 0.039 mmol), bis(2-cyanoethyl) *N,N*-diisopropylphosphoramidite (63 mg, 0.234 mmol), 1*H*-tetrazole (27 mg, 0.39 mmol) and molecular sieves 4 Å (0.3 g) in CH₂Cl₂ (6 cm³) was stirred for 23 h at room temperature under N₂. The reaction vessel was cooled to –20 °C and a solution of MCPBA (67 mg) in CH₂Cl₂ (2 cm³) was added. After 30 min, 10% aq. Na₂S₂O₅ (0.5 cm³) was added and the suspension was centrifuged. Solids were repeatedly washed with EtOAc–MeOH (1:1). Washings and supernatant were combined, concentrated and purified by two successive column chromatographic separations on silica gel [EtOAc–MeOH (40:1); then EtOAc–MeOH–hexane (10:1:1)] to give *phosphotriester 32* as a syrup (55 mg, 99%); δ_H 5.92 (1 H, m, CH=), 5.46 (1 H, d, $J_{NH,2}$ 8.4, NH), 5.40 (1 H, dd, $J_{3',2'}$ 3.2, $J_{3',4'}$ 10.0, 3'-H), 5.31 (1 H, t, $J_{4',5'}$ 10.0, 4''-H), 5.31–5.17 (3 H, m, 6''-H and =CH₂), 5.12 (1 H, m, $J_{7',6'}$ 9.3, 7'-H), 5.03 (1 H, dd, $J_{2',1'}$ 1.9, 2''-H), 4.98 (1 H, d, 1''-H), 4.89–4.82 (3 H, m, $J_{1,2}$ 8.6, $J_{8'a,8'b}$ 12.8, 1- and 4'-H, 8'-H^a), 4.37–4.23 (7 H, m, 5''-H, 7'-H^a, OCHH, 2 × OCH₂CH₂CN), 4.19–4.07 (5 H, m, 5'- and 6'-H, 8'- and 7''-H^b, OCHH₂), 4.02 (1 H, t, $J_{3,4}$ 9.0, 3-H), 3.85 (1 H, d, $J_{6a,6b}$ 8.7, 6-H^a), 3.80 (3 H, s, CO₂Me), 3.65–3.54 (2 H, m, 5-H and 6-H^b), 3.40–3.30 (2 H, m, 2- and 4-H), 2.83–2.79 (4 H, m, 2 × CH₂CN), 2.38 (1 H, dd, $J_{3e,3'a}$ 12.3, $J_{3e,4'}$ 4.1, 3'-H^e), 2.29 (1 H, t, $J_{3'a,4'}$ 12.2, 3'-H^a), 2.16, 2.10, 2.09, 2.08, 2.02, 2.01 and 2.00 (each 3 H, s, together 7 × Ac), 1.96 (3 H, s, NHAc) and 1.06–0.99 (28 H, m, 4 × Pr^dSi) (Found: C, 50.1; H, 6.3; N, 2.9. C₅₉H₉₂N₃O₃₀PSi₂ requires C, 50.4; H, 6.6; N, 2.9%).

Ammonium [2-acetamido-1-*O*-allyl-2,6-dideoxy- β -D-glucopyranos-6-yl *O*-(*L*-glycero- α -D-manno-heptopyranosyl)-(1''→5')-3-deoxy- α -D-manno-oct-2'-ulopyranosid]onate 4'- (ammonium hydrogen phosphate) **33**

A solution of compound **32** (22 mg, 0.015 mmol) in THF (5 cm³) was stirred with 1 M TBAF in THF (23 mm³, 0.023 mmol) for 2 h at ambient temperature. MeOH (1 cm³) was added and the solution was concentrated, and treated with 0.1 M methanolic NaOMe (0.5 cm³) and MeOH (3 cm³) for 2 h. The pH of the solution was adjusted to 6.5 by addition of Dowex 50 resin (H⁺-form), the resin was filtered off, and the filtrate was taken to dryness which afforded the de-*O*-acetylated compound (18.9 mg) as a syrup. The residue was dissolved in water (2 cm³) and the solution was stirred with 0.2 M aq. NaOH (1 cm³) for 15 h. The solution was cooled to 0 °C and quickly passed through a column (5 × 0.5 cm) of Dowex resin (H⁺-form); the

carbohydrate-containing effluents were immediately neutralised by addition of 0.1 M aq. NH_3 and lyophilised. Final purification of the material on Bio-Gel P2 afforded the target *trisaccharide* **33** as a hygroscopic solid (8.9 mg, 68%), $[\alpha]_{\text{D}}^{20} + 33$ (c 0.6, water); $\delta_{\text{H}}(\text{D}_2\text{O})$ 5.90 (1 H, m, =CH), 5.31 (1 H, dq, J 17.3, 1.6, = $\text{CH}_{2\text{trans}}$), 5.25 (1 H, dq, J 10.6, 1.3, = $\text{CH}_{2\text{cis}}$), 4.34 and 4.15 (2 H, ddt, OCH_2) and 2.03 (3 H, s, NHAc). Additional ^1H and ^{13}C NMR data are presented in Tables 1 and 2 (Found: C, 37.9; H, 6.7; N, 4.9. $\text{C}_{26}\text{H}_{50}\text{N}_3\text{O}_{22}\text{P}\cdot 2\text{H}_2\text{O}$ requires C, 37.9; H, 6.6; N, 5.1%).

Ammonium {2-acetamido-1-*O*-[3-(2-aminoethylthio)propyl]-2,6-dideoxy- β -D-glucopyranosyl-6-yl *O*-(L-glycero- α -D-mannoheptopyranosyl)-(1'' \rightarrow 5')-3'-deoxy- α -D-manno-oct-2'-ulopyranosid}onate 4'-(ammonium hydrogen phosphate) hydrochloride **34**

An aq. solution of compound **33** (6 mg, 0.007 mmol) and cysteamine hydrochloride (2.5 mg, 0.022 mmol) (0.2 cm^3) was irradiated at 254 nm for 15 h at room temperature. The solution was processed as described for compound **9**, which afforded *compound 34* as a syrup (4.3 mg, 68%), $[\alpha]_{\text{D}}^{20} + 32$ (c 0.3, water); $\delta_{\text{H}}(\text{D}_2\text{O})$ (*inter alia*) (1 H, br s, 1''-H), 4.50 (1 H, d, $J_{1,2}$ 8.5, 1-H), 4.48 (1 H, m, 4'-H), 4.26 (1 H, br s, 5'-H), 4.10 (1 H, m, 2''-H), 4.08 (1 H, dd, $J_{6,7}$ 8.0, 6'-H), 4.01–3.82 (6 H, m, 7'-H, 8'-H^a, 3''- and 6''-H, 7''-H^a and OCHH), 3.82–3.65 (5 H, m, 2-, 4''- and 5''-H, 7''-H^b, OCHH), 3.64 (1 H, dd, $J_{8b,7}$ 5.6, $J_{8b,8a}$ 10.7, 8'-H^b), 3.60–3.48 (5 H, m, 3-, 4-, 5-H and 6-H₂), 3.22 (2 H, t, NCH_2), 2.86 (2 H, t, SCH_2), 2.64 (2 H, t, SCH_2), 2.24 (1 H, dd, $J_{3e',3a'}$ 13.0, $J_{3e',4'}$ 4.4, 3'-H^e), 2.03 (3 H, s, NHAc), 1.97 (1 H, t, $J_{3a',4'}$ 12.7, 3'-H^a) and 1.84 (2 H, m, CH_2); δ_{C} 175.60 and 175.32 (NHCO , C-1'), 102.24 (C-1), 101.16 (C-1''), 100.93 (C-2'), 75.13 (C-5), 74.91 (C-3), 73.46 (C-5'), 72.96 and 72.91 (C-6', -5''), 71.34 (C-3''), 70.99 (C-4), 70.91 (C-7', -2''), 70.08 (C-6''), 69.85 (C-4'), 69.37 (OCH_2), 67.62 (C-4''), 64.40 and 63.94 (C-8', -7''), 62.87 (C-6), 56.39 (C-2), 38.59 (CN), 35.21 (C-3'), 29.25, 29.02 and 27.70 (CSC, CH_2) and 23.00 (CH_3) (Found: C, 36.3; H, 6.8; N, 5.9; S, 3.3. $\text{C}_{28}\text{H}_{58}\text{ClN}_4\text{O}_{22}\text{PS}\cdot\text{H}_2\text{O}$ requires C, 36.6; H, 6.6; N, 6.1; S, 3.5%).

Synthesis of BSA-conjugates **6, **10** and **35****

A solution of thiophosgene (2 mm^3 , 0.026 mmol) in CHCl_3 (2 cm^3) was added to a solution of compound **5** (5.3 mg, 0.009 mmol) in 0.1 M aq. NaHCO_3 (1.5 cm^3) and the mixture was vigorously stirred for 4 h at room temperature. The organic phase was separated, and the aqueous layer was washed three times with CHCl_3 (2 cm^3 portions) and purged with N_2 until a clear solution was obtained. The solution was transferred to a solution of BSA (SIGMA[®]; 5 mg) in 0.1 M aq. NaHCO_3 –0.3 M aq. NaCl (1 cm^3). The solutions were stirred slowly at room temperature for 72 h, then passed through a Sephadex G-25 column (1.6 \times 50 cm; 0.01 M aq. NaHCO_3). Ninhydrin-positive fractions were pooled, and dialysed twice against distilled water (2000 cm^3) for 24 h. Lyophilisation gave the BSA-conjugate **6** (6.0 mg) as a fluffy powder. Yield for conjugate **10** (4.5 mg of **9**, 2 mm^3 of CSCl_2 , 5.0 mg of BSA): 4.0 mg. Yield for conjugate **35** (3.3 mg of **34**, 2 mm^3 of CSCl_2 , 4.0 mg of BSA): 5.0 mg.

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