Synthesis of neoglycoproteins containing L-*glycero*-α-D-*manno*-heptopyranosyl-(1→4)- and -(1→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→4)-Kdo and Hep-(1→5)-Kdo 4-phosphate-(2→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→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-ulosonic 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 group[11] and subsequent activation of the terminal amino group with $CSCl_2$, 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, known[14] 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→4)-linked crystalline disaccharide derivative **3** ($J_{\mathbf{1}',2'}$ 1.5 Hz at δ 5.06, $J_{\mathbf{C\text{-}1}',\mathrm{H\text{-}1}'}$ 172 Hz) in 61% isolated yield. The structure of compound **3** was assigned on the basis of the ^{1}H 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. ^{13}C 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 ^{1}H and ^{13}C 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*-diisopropylphosphoramidite[16] 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 described[21] L-*glycero*-α-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 tetraisopropyldisiloxane-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 ^{1}H, ^{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 $J_{C,P}$ 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 previously[13] noted for the spacer derivative of α-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 described[13] 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 ^{1}H NMR spectrum. Owing to the propensity of the acetamido group to compete in the *O*-glycosidation reaction, the sequence of glycosylation steps was therefore reversed by first preparing the disaccharide donor Hep-(1→5)-Kdo **27**.

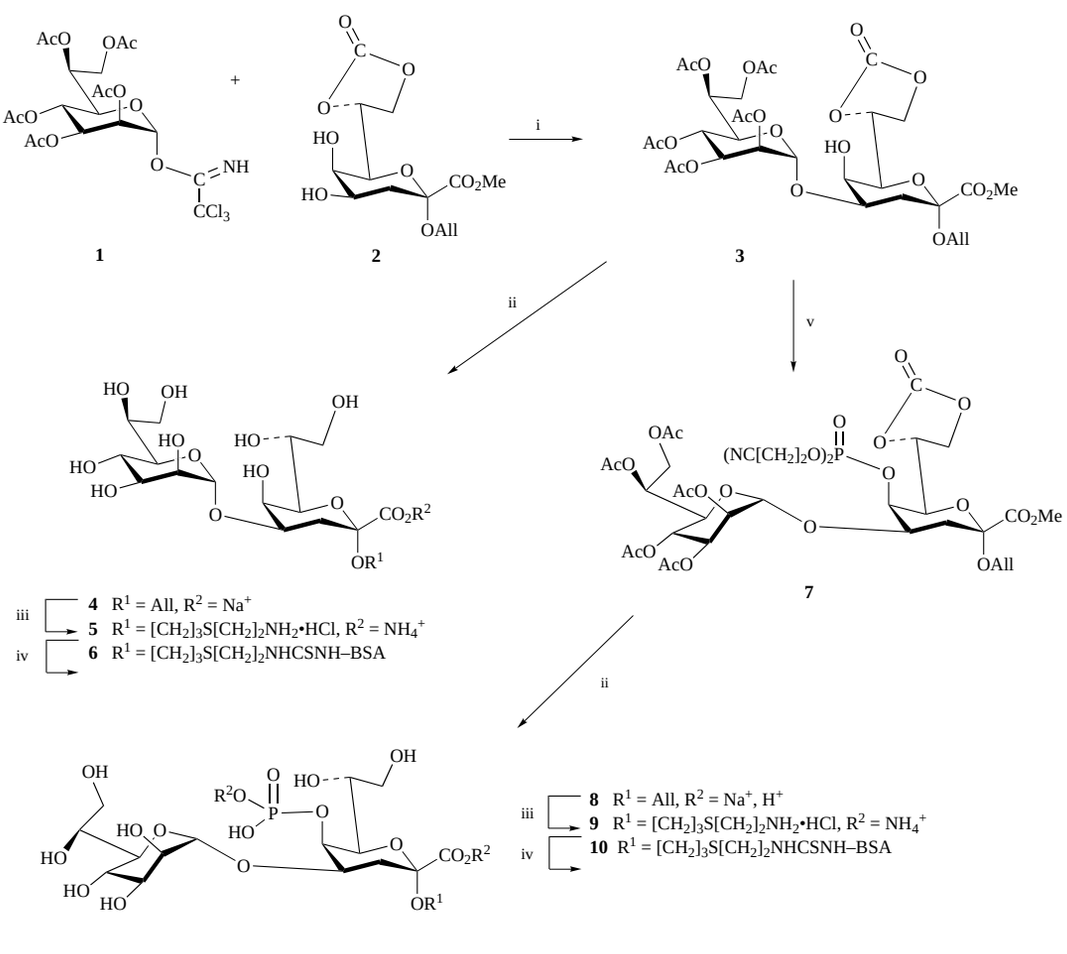
The 4-*O*-(*p*-methoxybenzyl) ether derivative **20** was prepared in 64% yield—together with a small amount (9%) of the methyl ester **21**—by treatment of compound **19**[23] with dibutyltin oxide (DBTO)–*p*-methoxybenzyl chloride in toluene (Scheme 4). After separation of the products by silica gel 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 $J_{C\text{-}1',H\text{-}1'}$ (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 glycosidation reaction with the previously reported[24] glucosamine acceptor derivative **28**. Promotion of the reaction by a 1:3 mixture of $HgBr_2$–$Hg(CN)_2$ 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 ^{1}H 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 dithiocarbonate[25] 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

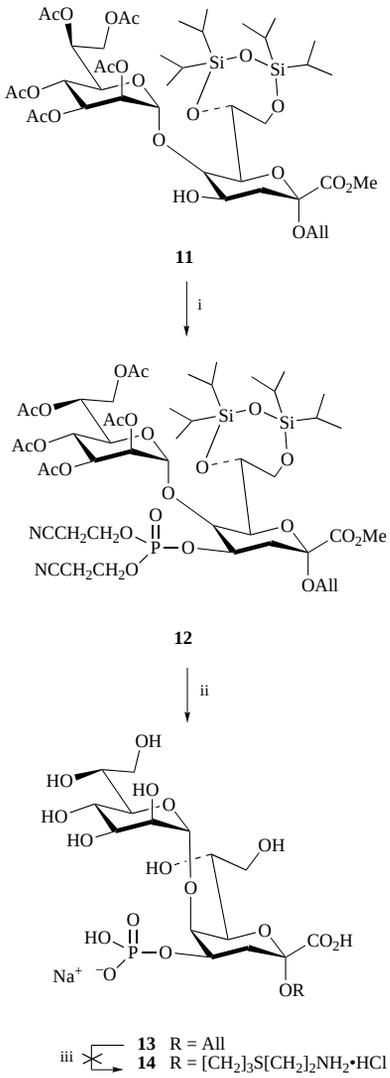
Table 2 ^{1}H NMR chemical shifts (δ) and coupling constants (J/Hz) for compounds **4**, **8**, **13** and **33**

	Chemical shifts (δ)			
	4	**8**	**13**	**33**
Hep-(1→				
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.
→Kdo-(2→				
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)
→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. $HSCH_2CH_2NH_2$·HCl, *hv*; 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. $HSCH_2CH_2NH_2$•HCl, *hν*

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 phosphate[26] or of Kdo by the thiobarbiturate assay after hydrolysis in 0.1 M HCl as described[27] 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; $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H NMR spectra were recorded at 297 K with a Bruker AC 300F instrument operating at 300 MHz for ^{1}H using $CDCl_3$ as solvent and tetramethysilane 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 $60F_{254}$); 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 °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

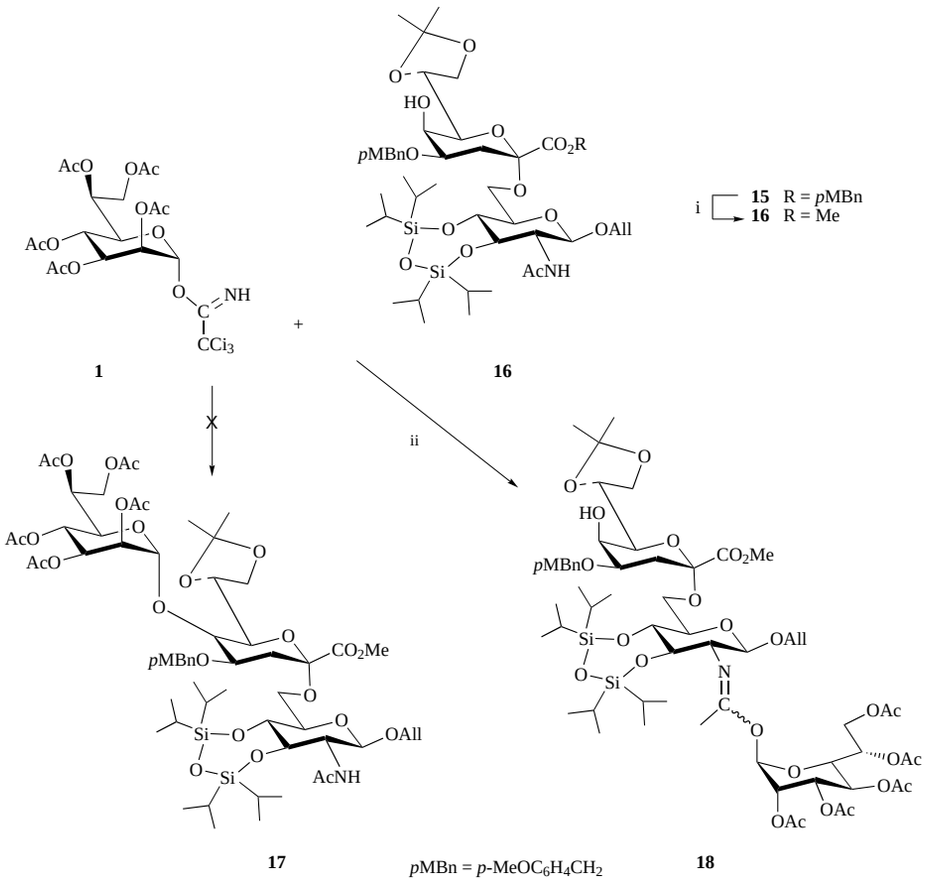
A suspension of compound **1** (150 mg, 0.27 mmol), compound **2** (75 mg, 0.24 mmol) and molecular sieves 4 Å (300 mg) in dry CH_2Cl_2 (3 cm^3) was stirred at ambient temperature for 2.5 h under N_2. After cooling of the mixture to 0 °C, a solution of TMSOTf in CH_2Cl_2 (0.4 M; 205 mm^3, 0.083 mmol) 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 °C (from hexane–EtOAc); $[\alpha]_D^{20}$ +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, $=CH_{2trans}$), 5.22–5.20 (2 H, m, 2′- and 6′-H), 5.18 (1 H, m, $=CH_{2cis}$), 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, $J_{7'a,7'b}$ 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, $J_{7'b,6'}$ 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 × Ac) (Found: C, 49.9; H, 5.6. $C_{30}H_{40}O_{20}$ 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, $[\alpha]_D^{20}$ +80 (*c* 0.8, water); $\delta_H(D_2O)$ (*inter alia*) 5.99 (1 H, m, =CH), 5.35 (1 H, dq, *J* 17.2, 1.6, $=CH_{2trans}$), 5.23 (1 H, dq, *J* 10.3, 1.3, $=CH_{2cis}$) and 3.98–3.92 and 3.86–3.86 (2 H, m, OCH_2). Additional ^{1}H NMR and ^{13}C NMR data are presented in Tables 1 and 2 (Found: C, 41.0; H, 6.4. $C_{18}H_{29}NaO_{14}$•$2H_2O$ 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

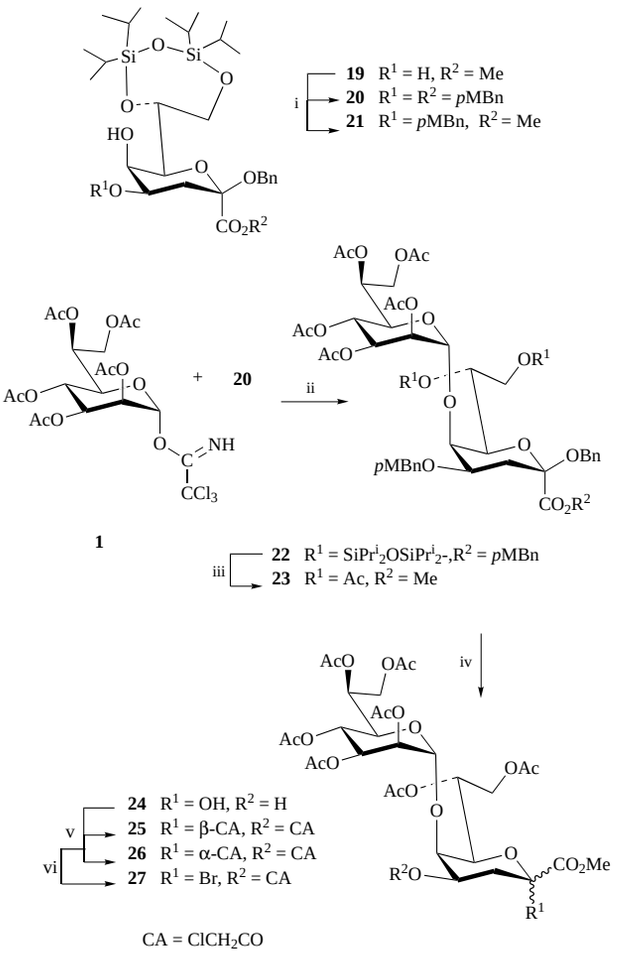
Table 3 Chemical analysis of the neoglycoconjugates **6**, **10** and **35**

Compound	Structure	Phosphate (nmol/ml)	BSA (μg/ml)	Ligand/protein quotient (nmol/mg)
6	Hep-(1→4)-Kdo		860	115
10	Hep-(1→4)-Kdo 5*P*	102	781	131
35	Hep-(1→5)-Kdo 4*P* (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 cm^3) 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, $[\alpha]_D^{20}$ +71 (*c* 0.3, water); $\delta_H(D_2O)$ 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′-H_2), 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. $C_{20}H_{40}ClN_2O_{14}S\cdot H_2O$ 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 cm^3) 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 cm^3) was added. The reaction was quenched after one hour by addition of 10% aq. $Na_2S_2O_5$ (0.2 cm^3). 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%), $[\alpha]_D^{20}$ +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, $=CH_{2trans}$), 5.19 (2 H, m, $=CH_{2cis}$, 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 × OCH_2CH_2CN), 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-H_2) 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. $C_{36}H_{47}N_2O_{23}P$ requires C, 47.6; H, 5.2; N, 3.1%).



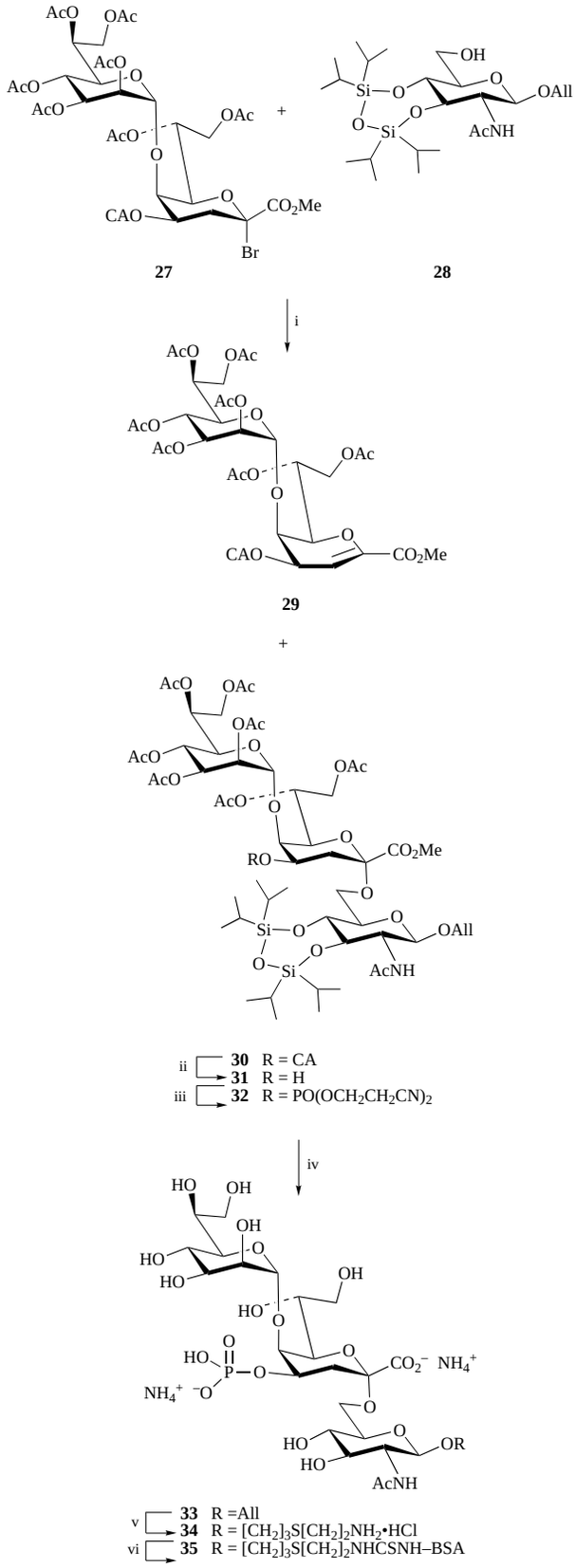
Scheme 4 *Reagents:* i, (*a*) DBTO, toluene; (*b*) TBAB, *p*-$MeOC_6H_4CH_2Cl$; ii, TMSOTf, CH_2Cl_2; iii, (*a*) TBAF, THF; (*b*) MeONa–MeOH; (*c*) Ac_2O, pyridine; iv, H_2, Pd–carbon, MeOH; v, CA_2O, pyridine; vi, $TiBr_4$, CH_2Cl_2

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 cm^3) in dry MeOH (5 cm^3) 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 cm^3) 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%); $[\alpha]_D^{20}$ +83 (*c* 0.5, water); $\delta_H(D_2O)$ (*inter alia*) 5.96 (1 H, m, CH=), 5.34 (1 H, dq, *J* 17.3, 1.6, $=CH_{2trans}$), 5.21 (1 H, dq, *J* 10.3, 1.2, $=CH_{2cis}$), 3.97–3.89 and 3.84–3.79 (2 H, m, OCH_2). Additional ^1H NMR and ^{13}C NMR data are given in Tables 1 and 2 (Found: C, 35.9; H, 5.6. $C_{18}H_{30}NaO_{17}P\cdot 2H_2O$ 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-ulopyranosidonate 5-(ammonium hydrogen phosphate) hydrochloride 9

A solution of compound **8** (5 mg, 8.1 μmol) and cysteamine hydrochloride (8.8 mg, 78 μmol) in water (0.2 cm^3) 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, $[\alpha]_D^{20}$ +53 (*c* 0.27, water); $\delta_H(D_2O)$ 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), 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′-H_2), 3.68–3.30 (5 H, m, 4′-, 6- and 8-H[b], OCH_2), 3.21 (2 H, t, NCH_2), 2.86 (2 H, t, SCH_2), 2.70 (2 H, t, SCH_2) and 2.01–1.85 (4 H, m, 3-H_2, CH_2); δ_C 176.18 (C-1),



Scheme 5 *Reagents and conditions:* i, $HgBr_2$, $Hg(CN)_2$, $MeNO_2$; ii, HDTC, 2,6-lutidine, HOAc; iii, bis(2-cyanoethyl) *N*,*N*-diisopropyl-phosphoramidite, 1*H*-tetrazole, CH_2Cl_2; then MCPBA; iv, (*a*) TBAF, THF; (*b*) MeONa–MeOH; (*c*) aq. NaOH; then NH_4OH; v, aq. $HSCH_2CH_2NH_2\cdot HCl$, *hv*; vi (*a*) $CSCl_2$, aq. $NaHCO_3$; (*b*) BSA, aq. $NaHCO_3$–NaCl

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 (OCH_2), 39.18 (NCH_2), 34.03 (C-3) and 29.39, 29.13 and 28.62 (2 × SCH_2,CH_2) (Found: C, 33.2; H, 6.5; N, 6.1; S, 4.5. $C_{20}H_{45}ClN_3O_{17}PS·H_2O$ 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-tetraisopropyldisiloxane-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 CH_2Cl_2 (5 cm^3) was treated with five portions of bis-(2-cyanoethyl) *N*,*N*-diisopropyl-phosphoramidite (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 CH_2Cl_2 (1 cm^3) was added. After 90 min, 10% aq. $Na_2S_2O_5$ (0.25 cm^3) was added and the reaction mixture was processed as described for compound **7** to give *compound* **12** (32 mg, 70%) as a syrup, $[\alpha]_D^{20}$ +28 (*c* 0.5, $CHCl_3$); 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 × OCH_2CH_2CN), 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, OCH_2), 4.08 (1 H, t, $J_{8a,8b}$ 9.5, $J_{8a,7}$ 9.5, 8-H[a]), 3.92 (1 H, ddt, OCH_2), 3.83 (1 H, d, 8-H[b]), 3.80 (3 H, s, CO_2Me), 2.83 (4 H, t, 2 × CH_2CN), 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), 2.11 (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 × Pr^iSi) (Found: C, 50.8; H, 6.6; N, 2.5. $C_{47}H_{75}N_2O_{23}PSi_2$ 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 cm^3) was stirrred with a 1 M solution of TBAF in THF (36 mm^3) for 30 min at room temperature. Methanol (0.3 cm^3) was added and the solution was concentrated and treated with 0.1 M methanolic NaOMe (0.5 cm^3) in MeOH (5 cm^3) 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 cm^3) and the solution was stirred with 0.2 M aq. NaOH (1 cm^3) for 22 h. Work-up of the solution as described for compound **8** afforded *compound* **13** as a syrup (12.3 mg, 83%), $[\alpha]_D^{20}$ +67 (*c* 0.3, water); $\delta_H(D_2O)$ 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, OCH_2). Additional ^1H and ^{13}C NMR data are presented in Tables 1 and 2 (Found: C, 36.4; H, 5.6. $C_{18}H_{30}NaO_{17}P·H_2O$ requires C, 36.6; H, 5.5%).

Methyl [2-acetamido-1-*O*-allyl-2,6-dideoxy-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-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 cm^3) was stirred with 0.1 M methanolic NaOMe (1.5 cm^3) 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%), $[\alpha]_D^{20}$ +33 (*c* 1.4, $CHCl_3$); 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, CH_2Ar), 4.44 (1 H, m, $J_{7',8a'}$ 6.3, $J_{7',8b'}$ 5.6, 7′-H), 4.19 (1 H, m, OC*H*H 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], OC*H*H allyl), 3.81 (1 H, m, 6-H[a]), 3.79 (3 H, s, ArO*Me*), 3.74 (1 H, dd, $J_{6,7'}$ 7.5, 6′-H), 3.74 (3 H, s, CO_2Me), 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, NH*Ac*), 1.40 and 1.39 (each 3 H, together s, 2 × Me) and 1.05–0.97 (28 H, m, 4 × Pr^iSi) (Found: C, 58.1; H, 8.0; N, 1.5. $C_{43}H_{71}NO_{15}Si_2$ 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-tetraisopropyldisiloxane-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 CH_2Cl_2 (4 cm^3) was stirred under N_2 for 4 h. A solution of 0.02 M TMSOTf in CH_2Cl_2 (0.15 cm^3) was added in two portions during 2 h at room temperature. CH_2Cl_2 (2 cm^3) and triethylamine (10 mm^3) 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, $[\alpha]_D^{20}$ +20 (*c* 0.15, $CHCl_3$); 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, $J_{3'',4''}$ 10.1, $J_{3'',2''}$ 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, CH_2Ar), 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′-H_2 and OC*H*H allyl), 3.97–3.87 (3 H, m, 4′-H, 8′-H[b], OCH*H* allyl), 3.82–3.79 (4 H, m, 6-H[a], ArO*Me*), 3.74–3.70 (4 H, m, 6′-H, CO_2Me), 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, CMe_2) and 1.06–0.98 (28 H, m, 4 × Pr^iSi) (Found: C, 55.2; H, 7.2; N, 1.0. $C_{60}H_{93}NO_{26}Si_2$ 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-tetraisopropyldisiloxane-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-tetraisopropyldisiloxane-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 cm^3) 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 cm^3, 20.4 mmol) were added and the reaction was kept at 90 °C for 4.5 h. MeOH (1.2 cm^3) 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 (Na_2SO_4) 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, $[\alpha]_D^{20}$ +0.6 (*c* 1.6, $CHCl_3$); 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, CH_2Ar), 4.78 and 4.40 (2 H, AB system, $J_{A,B}$ 11.5, CH_2Ar), 4.51 and 4.40 (2 H, AB system, $J_{A,B}$ 11.5, CH_2Ar), 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^{i}Si) (Found: C, 63.1; H, 7.8. $C_{43}H_{62}O_{11}Si_2$ requires C, 63.7; H, 7.7%).

Yield for *compound* **21**: 0.26 g (9%), syrup $[\alpha]_D^{20}$ −0.6 (*c* 0.9, $CHCl_3$); 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, $J_{A,B}$ 11.6, CH_2Ar), 4.57 and 4.49 (2 H, AB system, $J_{A,B}$ 11.6, CH_2Ar), 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, ArO*Me*), 3.75 (1 H, dd, 8-H^b), 3.66 (3 H, s, CO_2Me), 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^{i}Si) (Found: C, 61.3; H, 8.0. $C_{36}H_{56}O_{10}Si_2$ 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-tetraisopropyldisiloxane-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 CH_2Cl_2 (20 cm^3) was stirred with molecular sieves 4 Å (1 g) for 90 min at room temperature. A solution of TMSOTf (0.16 cm^3, 0.895 mmol) in CH_2Cl_2 (1 cm^3) was added under N_2. After 20 min, the reaction was quenched by addition of triethylamine (0.1 cm^3). The suspension was filtered over Celite®, and washed successively with EtOAc and water. The organic phase was dried (Na_2SO_4), concentrated and purified by silica gel chromatography [hexane–EtOAc (5:1)] which furnished *compound* **22** (1.39 g, 64%) as a syrup, $[\alpha]_D^{20}$ +11 (*c* 2.7, $CHCl_3$); 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, $J_{A,B}$ 11.7, OCH_2Ar), 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, $J_{A,B}$ 11.8, CH_2Ar), 4.60 (1 H, dd, 5′-H), 4.55 and 4.16 (2 H, AB system, $J_{A,B}$ 10.9, OCH_2Ar), 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^{i}Si) (Found: C, 59.6; H, 7.0. $C_{60}H_{84}O_{22}Si_2$ 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 cm^3) was treated with a 1.1 M solution of TBAF in THF (1.2 cm^3) for 1 h at room temperature. MeOH (85 cm^3) was added and the solution was evaporated to dryness. A solution of the residue in dry MeOH (70 cm^3) was stirred with 0.1 M methanolic NaOMe (28 cm^3) 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 cm^3), and the solution was cooled to 0 °C and stirred with acetic anhydride (1 cm^3) for 4 h. MeOH (5 cm^3) was added and the solution was stirred for 15 min and concentrated. Co-evaporation with toluene (3 × 20 cm^3) afforded a syrup, which was subjected to silica gel chromatography [toluene–EtOAc (1:1)] to give *compound* **23** as a syrup (760 mg, 73%), $[\alpha]_D^{20}$ +11 (*c* 0.8, $CHCl_3$); 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, $J_{6',7'a}$ 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, $J_{A,B}$ 11.8, CH_2Ar), 4.63 and 4.40 (2 H, AB system, $J_{A,B}$ 11.9, CH_2Ar), 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′-H_2), 3.91 (1 H, d, 6-H), 3.82 (1 H, br s, 5-H), 3.81 (3 H, s, ArO*Me*), 3.61 (3 H, s, CO_2Me), 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. $C_{45}H_{56}O_{22}$ 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 cm^3) was stirred with 5% Pd/C (150 mg) under H_2 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%), $[\alpha]_D^{20}$ +14 (*c* 1.5, $CHCl_3$); 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, $J_{6',7'a}$ 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, $J_{7'a,7'b}$ 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, CO_2Me), 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. $C_{30}H_{42}O_{21}$·1/2 H_2O 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 cm^3) was cooled to 0 °C and stirred with chloroacetic anhydride (1.8 g, 10.5 mmol) for 35 min. MeOH (4 cm^3) was added, and the solution was concentrated, and co-evaporated three times with toluene (50 cm^3). Purification of the residue on silica gel [toluene–EtOAc (1:1)] furnished compound **25** as the faster migrating isomer (124 mg, 25%), syrup, $[\alpha]_D^{20}$ +24 (*c* 0.6, $CHCl_3$); 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, ClCH_2CO), 4.19 (2 H, s, ClCH_2CO), 4.15 (1 H, m, 7′-H^b), 4.09 (1 H, br s, 5-H), 3.78 (3 H, s, CO_2Me), 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. $C_{34}H_{44}Cl_2O_{23}$ 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%), $[\alpha]_D^{20}$ +46 (*c* 0.3, $CHCl_3$); 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′-H_2), 4.23 (2 H, s, ClCH_2CO), 4.20 (2H, s, ClCH_2-CO), 3.83 (3 H, s, CO_2Me), 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-ulopyranosyl)onate 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 $CHCl_3$ (100 cm^3) and extracted with saturated aq. $NaHCO_3$. The organic layer

was dried (Na_2SO_4), 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, $CHCl_3$); 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′-H_2 and $ClCH_2CO$), 3.94 (3 H, s, CO_2Me), 2.72 (1 H, dd, $J_{3a,3e}$ 13.6, 3-H^e), 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-tetraisopropyldisiloxane-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 $MeNO_2$ (2 cm^3) 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 $MeNO_2$ (2 cm^3) at room temperature under N_2. After 60 h, the suspension was diluted with EtOAc (50 cm^3), filtered over Celite® and washed with MeOH–EtOAc (1:1). Filtrate and washings were combined, concentrated, then diluted with CH_2Cl_2 (100 cm^3). The organic layer was extracted in turn with saturated aq. $NaHCO_3$, then with 10% aq. KI, and dried (Na_2SO_4). Purification of the residue obtained upon concentration on silica gel [toluene–EtOAc (1:1)] gave, first the *glycal ester* **29** as a syrup (120 mg, 50%), $[\alpha]_D^{20}$ −135 (*c* 0.3, $CHCl_3$); 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, $ClCH_2CO$), 3.82 (3 H, s, CO_2Me) 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. $C_{25}H_{41}ClO_{21}$ 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, $CHCl_3$); 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, $J_{8a',7'}$ 2.2, 8′-H^a), 4.80 (1 H, d, 1″-H), 4.32 (1 H, ddt, OCH_2), 4.27–4.07 (8 H, m, 5′- and 6′-H, 8′-H^b, 5″-H, 7″-H_2 and $ClCH_2CO$), 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, CO_2Me), 3.66 (1 H, t, $J_{6b,5}$ 8.0, 6-H^b), 3.54 (1 H, m, $J_{5,4}$ 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^e), 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, NH*Ac*) and 1.10–0.99 (28 H, m, 4 × Pr^{i}Si) (Found: C, 50.8; H, 6.5; N, 1.0. $C_{55}H_{86}ClNO_{28}Si_2$ 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-tetraisopropyldisiloxane-1,3-diyl)-β-D-glucopyranos-6-yl *O*-(2″,3″,4″,6″,7″-penta-*O*-acetyl-L-*glycero*-α-D-*manno*-hepto-pyranosyl)-(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 cm^3) was cooled to 0 °C and treated with hydrazine dithiocarbonate (HDTC) (0.37 cm^3, 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 cm^3), 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, $CHCl_3$); 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, $J_{8a',7'}$ 2.3, 8′-H^a), 4.32 (1 H, dd, $J_{5'',6''}$ 3.1, 5″-H), 4.32 (1 H, dd, $J_{7''a,7b''}$ 11.7, $J_{7a'',6''}$ 5.3, 7″-H^a), 4.28 (1 H, dt, OCH*H*), 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, OC*H*H), 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, CO_2Me), 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, NH*Ac*), 1.91 (1 H, t, 3′-H^a) and 1.07–0.99 (28 H, m, 4 × Pr^{i}Si) (Found: C, 51.9; H, 6.7; N, 1.2. $C_{53}H_{85}NO_{27}Si_2$ 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-tetraisopropyldisiloxane-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 CH_2Cl_2 (6 cm^3) was stirred for 23 h at room temperature under N_2. The reaction vessel was cooled to −20 °C and a solution of MCPBA (67 mg) in CH_2Cl_2 (2 cm^3) was added. After 30 min, 10% aq. $Na_2S_2O_5$ (0.5 cm^3) 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 $=CH_2$), 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, $J_{8a',8b'}$ 12.8, 1- and 4′-H, 8′-H^a), 4.37–4.23 (7 H, m, 5″-H, 7′-H^a, OC*H*H, 2 × OCH_2CH_2CN), 4.19–4.07 (5 H, m, 5′- and 6′-H, 8′- and 7″-H^b, $OCHH_2$), 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, CO_2Me), 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 × CH_2CN), 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, NH*Ac*) and 1.06–0.99 (28 H, m, 4 × Pr^{i}Si) (Found: C, 50.1; H, 6.3; N, 2.9. $C_{59}H_{92}N_3O_{30}PSi_2$ 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 cm^3) was stirred with 1 M TBAF in THF (23 mm^3, 0.023 mmol) for 2 h at ambient temperature. MeOH (1 cm^3) was added and the solution was concentrated, and treated with 0.1 M methanolic NaOMe (0.5 cm^3) and MeOH (3 cm^3) 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 cm^3) and the solution was stirred with 0.2 M aq. NaOH (1 cm^3) 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%), $[\alpha]_D^{20}$ +33 (*c* 0.6, water); $\delta_H(D_2O)$ 5.90 (1 H, m, =CH), 5.31 (1 H, dq, *J* 17.3, 1.6, $=CH_{2trans}$), 5.25 (1 H, dq, *J* 10.6, 1.3, $=CH_{2cis}$), 4.34 and 4.15 (2 H, ddt, OCH_2) and 2.03 (3 H, s, NH*Ac*). Additional ^1H and ^{13}C NMR data are presented in Tables 1 and 2 (Found: C, 37.9; H, 6.7; N, 4.9. $C_{26}H_{50}N_3O_{22}P{\cdot}2H_2O$ requires C, 37.9; H, 6.6; N, 5.1%).

Ammonium {2-acetamido-1-*O*-[3-(2-aminoethylthio)propyl]-2,6-dideoxy-β-D-glucopyranos-6-yl *O*-(L-*glycero*-α-D-*manno*-heptopyranosyl)-(1″→5′)-3′-deoxy-α-D-*mannno*-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%), $[\alpha]_D^{20}$ +32 (*c* 0.3, water); $\delta_H(D_2O)$ (*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 OC*H*H), 3.82–3.65 (5 H, m, 2-, 4″- and 5″-H, 7″-H^b, OCH*H*), 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-H_2), 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, NH*Ac*), 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. $C_{28}H_{58}ClN_4O_{22}PS{\cdot}H_2O$ 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 × 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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