Chemistry of Bacterial Endotoxins. Part 6.[1] Synthesis of Allyl 5-*O*-(α-D-mannopyranosyl)-(3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onic Acid and of Allyl 5-*O*-(α-D-mannopyranosyl)-(3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onic Acid 4-Phosphate and Their Copolymers with Acrylamide

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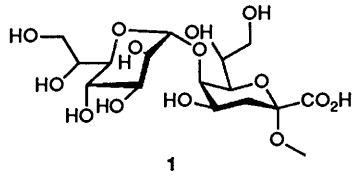
A mixture of methyl (allyl-7,8-*O*-cyclohexylidene-3-deoxy-α-, and -β-D-*manno*-oct-2-ulopyranosid)onates (3:1) was prepared from methyl 2,4,5,7,8-penta-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosonate in 3 steps and the anomers were separated by chromatography. Sequential treatment of the α-glycoside with dibutyltin oxide and *tert*-butyldimethylsilyl chloride gave the corresponding 4-*O*-*tert*-butyldimethylsilyl 1,5-lactone. Transformation of the lactone with MeONa to the corresponding ester, was accompanied by partial migration of the silyl group and yielded a 2:1 mixture of the 4-*O*-, and 5-*O*-*tert*-butyldimethylsilyl derivatives which were separated by chromatography. Condensation of the former with 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide in 1,2-dichloroethane in the presence of *N,N,N',N'*-tetramethylurea and under silver trifluoromethanesulphonate catalysis afforded a 71% yield of the protected α-linked disaccharide. The silyl group was removed selectively with tetrabutylammonium fluoride, whereafter deprotection by conventional methods produced the unprotected title disaccharide. The alcohol obtained after the removal of the silyl group was treated with bis(trichloroethyl) phosphorochloridate to afford the corresponding phosphotriester which, after deprotection, gave the phosphorylated title disaccharide. Both were copolymerized with acrylamide. ^{1}H NMR revealed that the phosphorylated disaccharide formed a complex with Ca^{2+} involving Man 4-O, 6-O, and oxygen atoms of the phosphate group.

Endotoxic lipopolysaccharides are obligate,[2] major elements of the outer membrane of gram negative bacteria. Their hydrophilic polysaccharide domain consists of two regions. One is the highly variable, species-specific *O*-chain (which may be absent), usually made up of repeating oligosaccharide units. The other, called core, is a non-repetitive sequence of glycoses, different from the repeating unit. The last constituent of the core, always[3] a 3-deoxy-D-*manno*-oct-2-ulosonic acid (KDO, dOclA), is attached through its glycosidic oxygen to C-6 of a glucosamine that is part of the hydrophobic region.[4] More than one KDO unit may be present and one of them is very often substituted by L-*glycero*-D-*manno*-heptopyranose to form the sequence: heptose-(1,5)-KDO-(KDO)-(2,6)-glucosamine. In many animals, including Man, endotoxic lipopolysaccharides elicit not only the main, humoral immune response which is directed against the species-specific elements of the *O*-chain, but also the production of antibodies which have serological specificities different from those directed against the *O*-chain. These are not species specific and appear to be identical for many lipopolysaccharides, derived from taxonomically unrelated microorganisms.[5] The existence of these cross-reacting antibodies indicates that endotoxic lipopolysaccharides share common, immunodeterminant molecular structures. Sera that are rich with respect to these antibodies provide protection against endotoxic shock.[6] Such antibodies, that can be raised in animals,[7] and in Man[8] by R-type endotoxin preparations (lipopolysaccharides of these endotoxins are devoid of the immunodominant *O*-chains), are directed against constituents of the core region.[9] Although only very few complete core-structures are known for wild-type (*i.e.* not obtained by induced mutagenesis) microorganisms, at least one KDO unit is always present, and the sequence L-*glycero*-D-*manno*-heptopyranosyl-(1,5)-3-deoxy-D-*manno*-oct-2-ulopyranosonic acid **1** appears frequently. Because of their potential interest as synthetic



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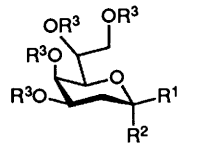
vaccines, hopefully providing protection against several gram negative pathogenic organisms, a considerable number of oligosaccharides containing one or more KDO units[10,11] or KDO substituted by L-*glycero*-D-*manno*-heptose have been synthesized.[12] In some cases[10,11] their allyl glycosides have been copolymerised with acrylamide to give polymers which had antigenic properties used in immunoassays to characterize the epitope-specificity of monoclonal antibodies directed to the KDO-region of *Salmonella minnesota* rough mutants.[13]

Endotoxin preparations of *Bordetella pertussis* cells, the etiologic agent for whooping cough, contain two types of lipopolysaccharides:[14] in one, the KDO unit that is 5-*O*-substituted by L-*glycero*-D-*manno*-heptose is phosphorylated in position 4, in the other it is not.[15] As we have observed previously (unpublished) that transformation—by oxidation with limiting amounts of periodate, followed by treatment with sodium borohydride—of the L-*glycero*-D-*manno*-heptose units present in this endotoxin into D-mannose units, did not change the examined biological properties significantly, the disaccharides named in the title and their acrylamide copolymers have been synthesized to be used in immunological work.

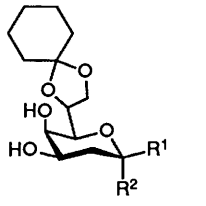
Results and Discussion

The starting material for the synthesis of the alcohol **8** was methyl 2,4,5,7,8-penta-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-

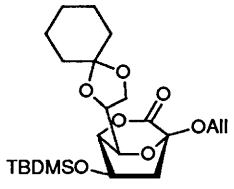
ulopyranosonate[16] **2** which was first transformed into the bromide[17] **3**. The crude product was immediately treated with allyl alcohol in the absence of added catalyst: progressive release of hydrogen bromide catalysed the solvolysis and favoured the formation of the thermodynamically more stable α-allyl glycoside. The crude, partially deacetylated glycosides thus formed were then fully deacetylated by Zemplén's method to afford, after column chromatography, the anomeric glycosides **4** and **5** as an inseparable mixture. The overall yield was less (47%) than obtainable by condensation in nitromethane with mercuric cyanide as the catalyst and 4 Å molecular sieves (78%),[10,11] but the α–β ratio was somewhat higher (8:2 *vs* 3:1). By treatment with methoxycyclohexene[18] under kinetically controlled conditions[19] the allyl glycosides **4** and **5** were transformed into the 7,8-*O*-cyclohexylidene derivatives **6** and **7**.



2 $R^1 = CO_2Me$, $R^2 = OAc$, $R^3 = Ac$
3 $R^1 = CO_2Me$, $R^2 = Br$, $R^3 = Ac$
4 $R^1 = CO_2Me$, $R^2 = OAll$, $R^3 = H$
5 $R^1 = OAll$, $R^2 = CO_2Me$, $R^3 = H$



6 $R^1 = CO_2Me$, $R^2 = OAll$
7 $R^1 = OAll$, $R^2 = CO_2Me$



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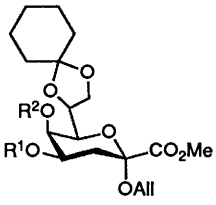
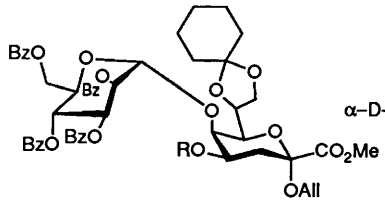


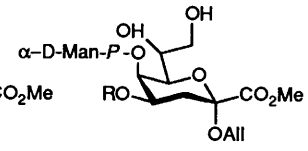
8 $R^1 = TBDMS$, $R^2 = H$
10 $R^1 = H$, $R^2 = TBDMS$
11 $R^1 = TBDMS$, $R^2 = Ac$
12 $R^1 = Ac$, $R^2 = TBDMS$

The anomers were then separated by column chromatography and their identity was assessed by ^1H NMR spectroscopy (α: H-3_{ax}, δ 1.90; H-3_{eq}, 2.16; H-4, 3.93–4.12. β: H-3_{ax}, δ 1.92; H-3_{eq}, 2.41; H-4, 3.61).[10] The cyclohexylidene acetal was preferred to the more usual isopropylidene acetal[20] because of its greater stability[21] in weakly acidic media; indeed, loss of the isopropylidene acetal has been observed during attempted glycosylation of an analogue of **6** catalysed by mercuric salts (unpublished observation). Selective protection of the equatorial 4-OH function[22] of the diol **6** by a *tert*-butyldimethylsilyl (TBDMS) group was then attempted. To that end, the diol **6** was treated first with dibutyltin oxide to afford the 4,5-stannylene derivative which was then made to react with TBDMSCl under tetrabutylammonium bromide catalysis. However, instead of the expected product **8**, the silylated 1,5-lactone **9** was obtained. We have observed previously[1] that 1,5-lactones are easily formed from analogous KDO-derivatives under similar conditions. Transesterification of the silylated lactone **9** was readily accomplished by treatment with sodium methoxide (0.1 mol dm^{-3}), but was accompanied by partial migration[23] (4⟶5) of the silyl group in the anhydrous alkaline medium. The resulting mixed silylated methyl esters **8** (65%) and **10** (26%) were separated by chromatography (overall yield 91%). The isomers were identified by the ^1H NMR spectra of their acetates, 5-H of **8** and 4-H of **10** being shifted to lower fields in the acetylated derivatives **11** (Δδ of 5-H = 1.51 ppm) and **12** (Δδ of 4-H = 1.16 ppm).

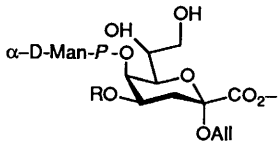
Condensation of the alcohol **8** with 2,3,4,6-tetra-*O*-benzoyl-α-D-mannosyl bromide in benzene–nitromethane (1:1) in the presence of mercuric cyanide and 4 Å molecular sieves[24] gave the protected disaccharide **13** in only 30% yield. However, a 70% yield was isolated when the reaction was carried out in rigorously anhydrous 1,2-dichloroethane[25] with silver triflate as the catalyst and tetramethylurea as the proton acceptor.[26] Loss of the silyl group previously observed in analogous cases[25,27] did not occur, nor was the formation of mannose orthobenzoate—which often accompanies glycosylation when α-acylated *trans* glycosyl halides are used as glycosyl donors[28]—detected. Selective removal of the silyl group by



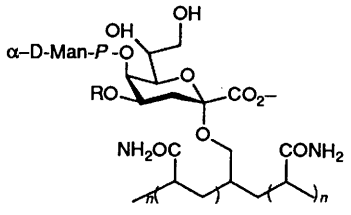
13 R = TBDMS
14 R = H
17 R = $PO(OCH_2CCl_3)_2$
18 R = PO_3^{2-}



15 R = H
19 R = PO_3^{2-}



16 R = H
20 R = PO_3^{2-}



21 R = H
22 R = PO_3^{2-}

acid hydrolysis could not be accomplished because of the lability of the exocyclic acetal to acid, but this was easily achieved by treatment of the silyl ether **13** with tetrabutylammonium fluoride in anhydrous tetrahydrofuran (THF) at room temperature[29] to afford the crystalline alcohol **14**. To obtain the unprotected disaccharide **16**, benzoate groups were removed by transesterification with sodium methoxide, and the acetal group by hydrolysis with HCl (0.2 mol dm^{-3}) in methanol, both at room temperature. The methyl ester **15** of the disaccharide thus formed was isolated, characterized by its ^1H NMR spectrum and then saponified to yield the allyl glycoside **16** which was isolated as its Ca-salt.

Phosphorylation of the alcohol **14** was accomplished with bis(trichloroethyl) phosphochloridate and *N*-methylimidazole[30] in anhydrous THF at 40 °C and afforded the phosphotriester **17** in 90% yield. This was treated with Zn–Ag in pyridine containing 10% acetic acid and purified by gel chromatography on Sephadex LH20 to remove Zn derivatives soluble in organic solvents, to give the phosphomonoester **18**. The benzoate groups were saponified by treatment with sodium methoxide, the methanolic solution was decationized, and methanol was replaced by allyl alcohol to avoid transglycosidation during the acid-catalysed removal of the cyclohexylidene acetal. After removal of the solvents, the resulting phosphomonoester **19** (74% yield from **17**) was isolated as its ammonium salt. Finally the carboxymethyl group was saponified with NaOH (0.5 mol dm^{-3}), the cooled (0 °C) solution was decationized and filtered while still cold, and neutralized (pH 7.5) with lime water. The disaccharide **20** was then isolated as its Ca-salt.

Analysis of the conformation of the Ca salts of the disaccharides **16** and **20** in aqueous solution was carried out by

Table 1

		Allyl 5-*O*-(α-D-mannopyranosyl)-(3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onic acid, Ca salt **16**			Allyl 5-*O*-(α-D-mannopyranosyl)-(3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onic acid 4-phosphate, Ca salt **20**		
		δ/ppm	J/Hz		δ/ppm	J/Hz	
Man*p*	1-H	5.04	(1,2)	2.0	5.15	(1,2)	1.5
	2-H	4.07	(2,3)	3.2	4.07	(2,3)	3.0
	3-H	3.88	(3,4)	9.2	3.88	(3,4)	10.0
	4-H	3.68	(4,5)	9.9	3.53	(4,5)	10.0
	5-H	3.95	(5,6)	5.1	4.30	(5,6)	5.5
	6-H	3.75	(5,6′)	2.6	3.62	(5,6′)	2.8
	6′-H	3.79	(6,6′)	12.3	3.95	(6,6′)	12.4
KDO*p*	3e-H	2.05	(3e,3a)	13.0	2.20	(3e,3a)	13.0
	3a-H	1.81	(3a,4)	12.8	1.92	(3a,4)	13.0
	4-H	4.12	(3e,4)	4.8	4.43	(3e,4)	4.8
	5-H	4.10	(4,5)	2.8	4.27	(4,5)	3.1
	6-H	3.63	(5,6)	1.0	3.66	(5,6)	<1.0
	7-H	3.84	(6,7)	10.0	3.79	(6,7)	9.5
	8-H	3.62	(7,8)	6.2	3.60	(7,8)	6.0
	8′-H	3.90	(7,8′)	2.9	3.90	(7,8′)	2.7
			(8,8′)	11.6		(8,8′)	11.5
						(4,P)	9.2
Allyl	=CH	5.92			5.94		
	$=CH_2$	5.30, 5.19			5.33, 5.20		
	OCH_2	3.89, 3.82			3.90, 3.82		

^{1}H NMR (500 MHz) and gave the following results. Assignments (Table 1) were deduced from double-quantum-filtered pure-phase COSY (DOCOSY) spectra and spin decoupling experiments. The chemical shifts of KDO 3e-H, KDO 3a-H[3], and that of Man 1-H unequivocally established α anomeric configurations for the KDO and mannose units, respectively, in both derivatives. The large values of $J_{3a,4}$ for the KDO units and $J_{3,4}$ and $J_{4,5}$ for the mannose units clearly show that the ring conformations are $^4C_1$ for mannose and $^5C_2$ for the KDO units in both disaccharides. The three-bond coupling $^3J_{4,P}$, and the downfield shift (Δδ 0.3 ppm) of KDO 4-H in **20** as compared to KDO 4-H in **16** confirmed the position assigned to the phosphate group in **20** which is corroborated by the expected smaller (Δδ < 0.2 ppm) downfield shifts of KDO 3e-H, KDO 3a-H and KDO 5-H due to phosphorylation of KDO 4-OH in **20**. Unexpectedly, in the phosphorylated disaccharide **20**, Man 5-H is shifted downfield, and Man 4-H and 6-H are shifted upfield (Table 1) with respect to their position in the non-phosphorylated disaccharide **16**. Glycoses are well known to form complexes with metal ions[31] including Ca^{2+}, and disaccharides have been shown to be able to form pentadentate complexes with Ca^{2+}.[31] Accordingly, the chemical shifts mentioned above may well indicate that in the phosphorylated disaccharide **20** Man 4-O and 6-O are ligated with Ca^{2+}, the other ligands being provided by oxygen atoms of the phosphate group (Fig. 1). Molecular models indicated that the formation of such a complex required the two pyranose rings of the phosphorylated disaccharide **20** to be locked in a unique conformation characterized by the quasi parallel position of the planes defined by Man C-2, -3, -5 and 5-O and KDO C-3, -4, -6 and 6-O, respectively. The existence of such a conformation is strongly suggested by the unique inter-annular NOE observed between Man 2-H and KDO 7-H of the phosphorylated disaccharide; this effect is absent from the non-phosphorylated disaccharide **16**. According to molecular models, the only inter-annular H-H distance short enough to be able to produce a nuclear Overhauser effect in the conformation indicated above, is that between Man 2-H and KDO 7-H. The value of $^3J_{4,P}$ indicates predominance of the *anti* conformer

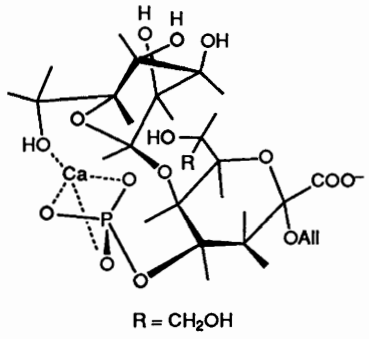


Fig. 1 Proposed structure for allyl 5-*O*-(α-D-mannopyranosyl)-(3-deoxy-α-D-*manno*-2-octulopyranosid)onic acid 4-(Ca-phosphate) in water

around the C(5)–O bond of KDO[32] in the equilibrium mixture of the rotamers; in the phosphorylated disaccharide **20** this conformation favours the co-ordination of Ca^{2+} as proposed.

It is well known that the antigenicity of haptenes can be considerably enhanced if they are attached to 'carriers' (*e.g.* proteins) or polymers (*e.g.* acrylamide). Accordingly, the disaccharides **16** and **20** were copolymerized with acrylamide according to the method of Horejsi *et al.*[33] Five molar equiv. of acrylamide were used per mol of disaccharide. The ratio of the number of molecules of disaccharide incorporated per polymer molecule was found to be 1 : 18 ± 2 as determined by integration of the NMR signals of the H atoms of the disaccharide moiety and those due to acrylamide (CH and CH_2). The average molecular weight of the acrylamide copolymers is dependent upon the concentrations of *N,N,N′,N′*-tetramethylethylenediamine (TMED) and ammonium persulphate in the reaction medium;[33] estimated values for TMED (3 mm^3 cm^{-3}) and persulphate[34] (1.4 mg cm^{-3}), and TMED (1 mm^3 cm^{-3}) and persulphate[9] (1 mg cm^{-3}) were 100–300 kDa and 60–100 kDa, respectively. Although not actually determined, on this basis the polymeric material obtained with TMED (4 mm^3 cm^{-3}) and persulphate (2 mg cm^{-3}) would be expected to be superior to 100 kDa.

Experimental

General Methods.—Evaporations were carried out under water-pump pressure at 40 °C bath temp. M.p.s were determined on a Kofler hot plate and are uncorrected. Optical rotations were measured with a Perkin-Elmer model 141 polarimeter at 19–22 °C. Me_4Si was used as internal reference for ^1H NMR spectra. All *J* values are in Hz. TLC was performed on silica gel (60 F_{254} on aluminium foil, Merck); compounds were located by spraying with sulphuric acid (10%) in ethanol and heating on a hot plate. Column chromatography was performed on silica gel Merck 60 (70–230 mesh); column dimension are given in cm as height by diameter.

Methyl (Allyl 3-Deoxy-α-, and -β-D-manno-oct-2-ulopyranosid)onate **4** *and* **5**.—Hydrobromic acid (4.5 mol equiv.) in acetic acid (32%, w/v) was added at room temperature to a stirred solution of methyl (2,4,5,7,8-penta-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (20 g, 43.3 mmol) dissolved in a mixture of anhydrous benzene (200 cm^3) and freshly distilled chloroform (20 cm^3). After 2.5 h, solvents were removed and anhydrous toluene (2 × 200 cm^3) was evaporated from the residue. Anhydrous $CaSO_4$ (10 g) and anhydrous allyl alcohol (20 cm^3) in a mixture of anhydrous benzene (100 cm^3) and distilled chloroform (40 cm^3) were added. The stirred mixture was set aside at room temperature for 24 h and then at 50 °C for 1 h. Allyl alcohol (5 cm^3) was added and stirring was continued at 50 °C for 2 h and then at room temperature overnight. Chloroform (5 cm^3) was added and the mixture neutralized with solid $NaHCO_3$. Solids were filtered off, and washed with a mixture of chloroform (147 cm^3) and allyl alcohol (3 cm^3). Solvents were removed from the combined filtrate and washings, and anhydrous benzene (2 × 100 cm^3) was added to and evaporated off the residual oil which was taken up in anhydrous methanol (150 cm^3). Sodium methoxide in methanol (1.5 cm^3; 1 mol dm^{-3}) was added, the mixture was stirred at room temperature for 2 h, decationized [Dowex AG50 WX8 (H^+) resin] and concentrated. Column (36 × 8) chromatography ($CHCl_3$–MeOH, 85:15) of the residue afforded the anomeric mixture of the allyl glycosides **4** and **5** (6 g, 47%) [TLC, single spot, solvent as above, R_F 0.40, α–β (8:2) by ^1H NMR) (Found: C, 49.2; H, 6.8. $C_{12}H_{20}O_8$ requires C, 49.3; H, 6.8%); δ_H(250 MHz; $CDCl_3$–CD_3OD, 9:1) 1.84–2.08 (2 H_α, 1 H_β, m, $3_{a,\alpha}$-H, $3_{e,\alpha}$-H, $3_{a,\beta}$-H), 2.32 (1 H_β, dd, $J_{3e,3a}$ 12.5, $J_{3e,4}$ 4.5, $3_{e\beta}$-H), 3.4–4.3 (11 H_α and 11 H_β, m, 4α-H, 5α-H, 6α-H, 7α-H, 8α-H, 8′α-H, $CO_2Me\alpha$, $OCH_2C=$ α, 4β-H, 5β-H, 6β-H, 7β-H, 8β-H, 8′β-H, CO_2Me β, $OCH_2C=$ β), 5.05–5.44 (2 H_α, 2 H_β, m, $CH_2=$ α, $CH_2=$ β) and 5.8–5.9 (1 H_α, 1 H_β, m, CH= α, CH=β).

Methyl (Allyl 7,8-O-Cyclohexylidene-3-deoxy-α-, and -β-D-manno-oct-2-ulopyranosid)onate **6** *and* **7**.—1-Methoxycyclohexene (3.1 cm^3, 0.9 mol equiv.) were added to a cooled (4 °C) and stirred solution of the mixed anomers described above (7.23 g, 25 mmol) in anhydrous dioxane (500 cm^3) containing anhydrous $CuSO_4$ (7 g) and dry toluene-*p*-sulphonic acid (300 mg). The mixture was allowed to reach ambient temperature and stirring was continued for a further 2 h. As formation of the acetal appeared to be incomplete (TLC, ethyl acetate–cyclohexane, 6:4), the mixture was cooled, methoxycyclohexene (1 cm^3, 0.3 mol equiv.) was added and stirring continued at room temperature for 16 h. The mixture was neutralized with $NaHCO_3$, diluted with diethyl ether (300 cm^3), and passed through a layer (4 × 9.5) of silica gel which was then washed with diethyl ether (500 cm^3). Solvents were removed. Column (18 × 6) chromatography (ethyl acetate–cyclohexane, 6:4) of the residue afforded first the β-*allyl glycoside* **7** (oil, 1.9 g, 13%; R_F 0.46, solvent as above); (Found: C, 57.9; H, 7.5. $C_{18}H_{28}O_8$ requires C, 58.1; H, 7.5%); $[\alpha]_D$ +38.6° (*c* 1.3, chloroform); δ_H (250 MHz; $CDCl_3$) 1.22–1.63 (10 H, m, $[CH_2]_5$), 1.92 (1 H, dd, $J_{3a,3e}$ 12.5, $J_{3a,4}$ 12, 3a-H), 2.41 (1 H, dd, $J_{3e,4}$ 5, 3e-H), 3.44 (1 H, dd, $J_{6,5}$ 1, $J_{6,7}$ 8.5, 6-H), 3.61 (1 H, m, $J_{4,5}$ 3, 4-H), 3.73 (3 H, s, CO_2Me), 3.83–4.25 (5 H, m, 5-H, 8-H, 8′-H and $OCH_2C=$), 4.28 (1 H, m, $J_{7,8\text{ or }7,8'}$ 6, $J_{7,8'\text{ or }7,8}$ 5, 6-H), 5.03–5.24 (2 H, m, $CH_2=$) and 5.80 (1 H, m, CH=).

Secondly, the *α-allyl glycoside* **6** was eluted (oil, 5.6 g, 61%; R_F 0.30, solvent as above), (Found: C, 57.9; H, 7.4. $C_{18}H_{28}O_8$ requires C, 58.1; H, 7.5%); $[\alpha]_D$ +55.7° (*c* 1.3, chloroform); δ_H(250 MHz; $CDCl_3$) 1.3–1.70 (10 H, m, $[CH_2]_5$), 1.90 (1 H, dd, $J_{3a,3e}$ 13.7, $J_{3a,4}$ 12, 3a-H), 2.16 (1 H, dd, $J_{3e,4}$ 4.7, 3e-H), 3.55 (1 H, dd, $J_{6,7}$ 8.2, $J_{6,5}$ *ca.* 0.5, 6-H), 3.79 (3 H, s, CO_2Me), 3.93–4.12 (5 H, m, 4-H, 5-H, 8-H and $OCH_2C=$), 4.15 (1 H, dd, $J_{8,8'}$ 9, $J_{8',7}$ 6, 8′-H), 4.41 (1 H, m, $J_{7,8}$ 4.5, 7-H), 5.15–5.37 (2 H, m, $CH_2=$) and 5.88 (1 H, m, CH=).

(Allyl 4-O-tert-Butyldimethylsilyl-7,8-O-cyclohexylidene-3-deoxy-α-D-manno-oct-2-ulopyranosid)ono-1,5-lactone **9**.—A solution of the diol **6** (3.1 g, 8.33 mmol) in anhydrous toluene (500 cm^3) containing dibutyltin oxide (2.65 g, 1.3 mol equiv.) was heated at reflux for 2 h in a flask equipped with a Dean–Stark water separator. Tetrabutylammonium bromide (2.8 g, 1 mol equiv.) was added and reflux continued during 1 h. *tert*-Butyldimethylchlorosilane (2.6 g, 2.1 mol equiv.) was added and reflux continued for 17 h. The reaction mixture was cooled and deposited on a bed (5 × 13) of silica gel containing solid $NaHCO_3$ (2 g) mixed into the top layers, and eluted with a mixture of ethyl acetate–cyclohexane (3:7, 1 dm^3). Fractions (125 cm^3) containing the lactone (R_F 0.74, solvent as above) were pooled; the residue (2.45 g, 65%) remaining after removal of the solvents crystallized, m.p. 119–121 °C (diethyl ether–cyclohexane); (Found: C, 60.7; H, 8.4. $C_{23}H_{38}O_7Si$ requires C, 60.8; H, 8.4%); $[\alpha]_D$ −24.5° (*c* 1, chloroform); δ_H(250 MHz; $CDCl_3$) 0.08, 0.11 [2 × 3 H, 2 s, $(Me)_2Si$], 0.89 [9 H, s, $(Me)_3CSi$], 1.33–1.68 (10 H, m, $[CH_2]_5$), 1.92 (1 H, *ca.* d, $J_{3,3'}$ 15, $J_{3,4}$ *ca.* 0, 3-H), 2.61 (1 H, dd, $J_{3',4}$ 9, 3′-H), 3.71 (1 H, *ca.* d, $J_{6,5}$ *ca.* 0, $J_{6,7}$ 5, 6-H), 3.92–4.15 (3 H, m, 7-H, 8-H and 8′-H), 4.19 (1 H, *ca.* d, $J_{4,5}$ 1.5, 4-H), 4.21–4.46 (2 H, m, $OCH_2C=$), 4.69 (1 H, d, H-5), 5.13–5.38 (2 H, m, $CH_2=$) and 5.98 (1 H, m, CH).

Methyl (Allyl 4-O-tert-Butyldimethylsilyl-7,8-O-cyclohexylidene-3-deoxy-α-D-manno-oct-2-ulopyranosid)onate **8** *and Methyl (2-O-Allyl-5-O-tert-butyldimethylsilyl-7,8-O-cyclohexylidene-3-deoxy-α-D-manno-oct-2-ulopyranosid)onate* **10**.—Methanolic sodium methoxide (1 mol dm^{-3}, 1 cm^3) was added at room temperature to a solution of the lactone **9** (2.25 g, 2.96 mmol) in a mixture of chloroform–methanol (1:1) and the mixture was stirred for 2 h. The mixture was deposited on a layer (6.5 × 2.5) of silica gel and eluted with a mixture of ethyl acetate–toluene (1:5; 100 cm^3). Solvents were removed from the eluent and the isomers present in the dry residue were separated by column (17.5 × 2.5) chromatography (ethyl acetate–toluene, 1:5). Eluted first, the 5-O-*silyl ether* **10** (636 mg, 26%, R_F 0.53, solvent as above) a crystalline solid; m.p. 84–86 °C (from diethyl ether–hexane) (Found: C, 59.4; H, 8.8. $C_{24}H_{42}O_8Si$ requires C, 59.3; H, 8.6%); $[\alpha]_D$ +55.0° (*c* 1.4, chloroform); δ_H(250 MHz; $CDCl_3$) 0.17, 0.24 [2 × 3 H, 2 s, $(Me)_2Si$], 0.95 [9 H, s, $(Me)_3CSi$], 1.30–1.75 (10 H, m, $[CH_2]_5$), 1.97 (1 H, dd, $J_{3a,3e}$ 12, $J_{3a,4}$ 11, 3a-H), 2.15 (1 H, dd, $J_{3e,4}$ 5, 3e-H), 3.41 (1 H, dd, $J_{6,5}$ 1, $J_{6,7}$ 9, 6-H), 3.78 (3 H, s, CO_2Me), 3.93 (1 H, dd, $J_{8,8'}$ 8.5, $J_{8,7}$ 4, 8-H), 3.97 (2 H, m, $OCH_2C=$), 4.05 (1 H, m, 4-H), 4.10 (1 H, m, 5-H), 4.18 (1 H, dd, $J_{8',7}$ 5.5, 8′-H), 4.27 (1 H, m, 7-H), 5.15–5.8 (2 H, m, $CH_2=$) and 5.90 (1 H, m, CH=).

Secondly, the 4-O-silyl ether **8** was eluted (1.56 g, 65%, R_F 0.37, solvent as for the isomer) a colourless oil $[\alpha]_D$ +43.2° (*c* 1, chloroform); (Found: C, 59.2; H, 8.8%); δ_H(250 MHz; $CDCl_3$) 0.07 [2 × 3 H, 2 s, $(Me)_2Si$], 0.87 [9 H, s, $(Me)_3CSi$], 1.23–1.58 (10 H, m, $[CH_2]_5$), 1.93 (1 H, dd, $J_{3a,3e}$ 12, $J_{3a,4}$ 11, 3a-H), 2.01

(1 H, dd, $J_{3e,4}$ 5, 3e-H), 3.53 (1 H, dd, $J_{6,5}$ 1.5, $J_{6,7}$ 8, 6-H), 3.73 (3 H, s, CO_2Me), 3.82 (1 H, m, $J_{5,4}$ *ca.* 3, 5-H), 3.89–3.98 (2 H, m, OCH_2C=), 3.98 (1 H, dd, $J_{8,8'}$ 8.5, $J_{8,7}$ 5, 8-H), 4.12 (1 H, m, H-4) and (1 H, dd, $J_{8',7}$ 6, 8'-H), 4.43 (1 H, m, 7-H), 5.09–5.35 (2 H, m, CH_2=) and 5.88 (1 H, m, CH=).

Methyl (*Allyl* 5-O-*Acetyl*-4-O-tert-*butyldimethylsilyl*-7,8-O-*cyclohexylidene*-3-*deoxy*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* **11**.—The alcohol **8** (41 mg, 0.08 mmol) was treated with a mixture of acetic anhydride–pyridine (1:1, 800 mm^3) for 12 h at room temperature. Anhydrous toluene was evaporated from the residue remaining after removal of the solvents. Column (2.5 × 1) chromatography (ethyl acetate–toluene, 1:5) of the residual material gave the acetate **11**, a colourless oil (37 mg, 84%) (Found: C, 59.5; H, 8.6. $C_{26}H_{44}O_9Si$ requires C, 59.1; H, 8.3%), $[\alpha]_D$ +37.5° (*c* 1.8, chloroform); δ_H(250 MHz; $CDCl_3$) 0.08, 0.09 [2 × 3 H, 2 s, $(Me)_2Si$], 0.85 [9 H, s, $(Me)_3CSi$], 1.31–1.70 (10 H, m, $[CH_2]_5$), 1.94 (1 H, dd, $J_{3a,3e}$ 12, $J_{3a,4}$ 11, 3a-H), 2.07 (1 H, dd, $J_{3e,4}$ 5, 3e-H), 2.10 (3 H, s, MeCO), 3.74 (1 H, dd, $J_{6,5}$ 1.5, $J_{6,7}$ 7.5, 6-H), 3.80 (3 H, s, CO_2Me), 3.92–4.09 (4 H, m, 7-H, 8-H, OCHC=, and 8'-H or OCHC=), 4.15–4.26 (2 H, m, 4-H and 8'-H or OCHC=), 5.14–5.32 (2 H, m, CH_2=), 5.33 (1 H, d, $J_{4,5}$ 3.1, 5-H) and 5.90 (m, 1 H, CH=).

Methyl (*Allyl* 4-O-*Acetyl*-5-O-tert-*butyldimethylsilyl*-7,8-O-*cyclohexylidene*-3-*deoxy*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* **12**.—Acetylation of the alcohol **10** (46.4 mg, 0.09 mmol) and purification of the product formed in conditions identical to those described for **11** gave the acetate **12** (42 mg, 83%) as a colourless oil (Found: C, 59.2; H, 8.4; $C_{26}H_{44}O_9Si$ requires C, 59.1; H, 8.3%); $[\alpha]_D$ +53.5° (*c* 1, chloroform); δ_H(250 MHz; $CDCl_3$) 0.08, 0.16 [2 × 3 H, 2 s, $(Me)_2Si$], 0.54 [9 H, s, $(Me)_3CSi$], 1.30–1.70 (10 H, m, $[CH_2]_5$), 2.07 (3 H, s, MeCO), 2.11 (1 H, dd, $J_{3e,3a}$ 12, $J_{3e,4}$ 5, 3e-H), 2.24 (1 H, t, $J_{3a,4}$ 12, 3a-H), 3.48 (1 H, dd, $J_{6,5}$ 0.8, $J_{6,7}$ 9, 6-H), 3.77 (3 H, s, CO_2Me), 3.90 (1 H, dd, $J_{8,8'}$ 8.5, $J_{8,7}$ 4.5, 8-H), 3.98 (2 H, m, OCH_2C=), 4.18 (1 H, dd, $J_{8',7}$ 6, 8'-H) and (1 H, m, 5-H), 4.24 (1 H, m, H-7), 5.14–5.38 (2 H, m, CH_2=), 5.21 (1 H, m, $J_{4,5}$ 2.5, 4-H) and 5.90 (1 H, m, CH=).

Methyl [*Allyl* 4-O-tert-*Butyldimethylsilyl*-7,8-O-*cyclohexylidene*-5-O-(2,3,4,6-*tetra*-O-*benzoyl*-α-D-*mannopyranosyl*)-α-D-manno-*oct*-2-*ulopyranosid*]*onate* **13**.—A solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide[35] (3.12 g, 2 mol equiv.) in anhydrous 1,2-dichloroethane (15 cm^3) was added dropwise to a stirred solution of the alcohol **10** (1.10 g, 2.37 mmol) in the same solvent (10 cm^3), kept in darkness at 40 °C under Ar and containing *N*,*N*,*N'*,*N'*-tetramethylurea (850 mm^3, 3 mol equiv.) and silver trifluoromethylsulphonate (triflate) (1.2 g, 2 mol equiv.). Stirring was maintained overnight at 40 °C. More tetramethylurea (250 mm^3, 0.75 mol equiv.), silver triflate (300 mg, 0.5 mol equiv.) and benzobromomannose (800 mg, 0.5 mol. equiv.) were then added in 1,2-dichloroethane (2 cm^3) and stirring was continued at 40 °C for 3 h. A mixture of anhydrous pyridine (2 cm^3) and dichloroethane (30 cm^3) was added to the cooled mixture which was then deposited on a layer (4.5 × 9.5) of silica gel and eluted with a mixture of toluene–diethyl ether (900 cm^3, 5:1). Solvents were removed from pooled fractions (100 cm^3) containing the product (R_F 0.22 in toluene–diethyl ether, 10:1) which was purified by column (17 × 5) chromatography (solvent as above). Removal of the solvent from the appropriate pooled fractions gave the crystalline, protected disaccharide **13** (1.7 g, 70.5%), m.p. 193–195 °C (diethyl ether–cyclohexane) (Found: C, 65.2; H, 6.2. $C_{58}H_{68}O_{17}Si$ requires C, 65.4; H, 6.4%); $[\alpha]_D$ −6.6° (*c* 1.6, chloroform); δ_H(250 MHz; $CDCl_3$) 0.15, 0.17 [2 × 3 H, 2 s, $(Me)_2Si$], 0.91 [9 H, s, $(Me)_3CSi$], 1.25–1.75 (10 H, m, $[CH_2]_5$), 2.11 (1 H, dd, $J_{3e,3a}$ 12.5, $J_{3e,4}$ 4, 3e-H), 2.31 (1 H, dd, $J_{3a,4}$ 11.5, 3a-H), 3.52 (1 H, d, $J_{6,5}$ *ca.* 1, $J_{6,7}$ 9.5, 6-H), 3.83 (3 H, s, CO_2Me), 3.86 (1 H, dd, $J_{8a,8b}$ 8.5, $J_{8a,7}$ 5, 8a-H), 4.00 (2 H, m, OCH_2C=), 4.18 (1 H, m, $J_{5,4}$ *ca.* 3, 5-H), 4.29 (1 H, m, 4-H), 4.31 (1 H, dd, $J_{8b,7}$ 6, 8b-H), 4.40 (1 H, dd, $J_{6'a,6'b}$ 12, $J_{6'a,5'}$ 2.6, 6'a-H), 4.47 (1 H, m, 7-H), 4.68 (1 H, dd, $J_{6'b,5'}$ 2.2, 6'b-H), 4.87 (1 H, m, $J_{5',4'}$ 10, 5'-H), 5.14–5.35 (2 H, m, CH_2=), 5.53 (1 H, d, $J_{1',2'}$ 1.7, 1'-H), 5.84 (1 H, dd, $J_{2',3'}$ 3.5, 2'-H), 5.87 (1 H, m, CH=), 6.01 (1 H, dd, $J_{3',4'}$ 10.1, 3'-H), 6.24 (1 H, t, 4'-H) and 7.2–8.2 (20 H, m, ArH).

Methyl [*Allyl* 7,8-O-*Cyclohexylidene*-3-*deoxy*-5-O-(2,3,4,6-*tetra*-O-*benzoyl*-α-D-*mannopyranosyl*)-α-D-manno-*oct*-2-*ulopyranosid*]*onate* **14**.—A solution of tetrabutylammonium fluoride (1 mol dm^{-3}, 5 cm^3; 3 mol equiv.) in anhydrous THF was added at room temperature to a stirred solution of the protected disaccharide **13** (1.7 g, 1.6 mmol) in the same solvent (53 cm^3) and stirring was continued for 1 h. The mixture was then deposited on a column (13 × 4.5) wetted with cyclohexane and then eluted with a mixture of cyclohexane–ethyl acetate (7:3). Solvents were removed from the pooled fractions containing the alcohol **14** (TLC, same solvent, R_F 0.36) which crystallized from ethyl acetate–cyclohexane (1.4 g, 94%), m.p. 107–109 °C (Found: C, 65.8; H, 5.8. $C_{52}H_{54}O_{17}$ requires C, 65.7; H, 5.7%); δ_H(250 MHz; $CDCl_3$) 1.25–1.76 (10 H, m, $[CH_2]_5$), 2.05 (1 H, dd, $J_{3a,3e}$ 13, $J_{3a,4}$ 11, 3a-H), 2.27 (1 H, dd, $J_{3e,4}$ 6, 3e-H), 3.51 (1 H, dd, $J_{6,7}$ 9, $J_{6,5}$ *ca.* 0.1, 6-H), 3.85 (3 H, s, CO_2Me), 3.94 (1 H, dd, $J_{8a,8b}$ 8.5, $J_{8a,7}$ 4.5, 8a-H), 4.03 (2 H, m, OCH_2C=), 4.23 (2 H, m, 4-H, 5-H), 4.34 (1 H, dd, $J_{8b,7}$ 6, 8b-H), 4.50 (1 H, dd, $J_{6'a,6'b}$ 12, $J_{6'a,5}$ 5.5, 6'a-H), 4.51 (1 H, m, 7-H), 4.70 (1 H, dd, $J_{6'b,5}$ 2.5, 6'b-H), 4.82 (1 H, m, $J_{5',4'}$ 10, 5'-H), 5.18–5.37 (2 H, m, CH_2=), 5.40 (1 H, d, $J_{1',2'}$ 1.5, 1'-H), 5.89 (1 H, dd, $J_{2',3'}$ 3, 2'-H), 5.90 (1 H, m, CH=), 5.96 (1 H, dd, $J_{3',4'}$ 10, 3'-H), 6.07 (1 H, t, 4-H) and 7.25–8.15 (20 H, m, ArH).

[*Allyl* 3-*Deoxy*-5-O-(α-D-*mannopyranosyl*)-α-D-manno-*oct*-2-*ulopyranosid*]*onate* **16**.—Sodium methoxide in methanol (1 mol dm^{-3}, 1 cm^3) was added to a solution of the protected disaccharide **14** (290 mg, 0.3 mmol) in methanol (9 cm^3) and the mixture stirred for 3 h at room temperature. Dowex AG50WX8 (H^+) resin was added to remove cations, the solid was filtered off and washed with methanol (5 cm^3), and the solvent was evaporated from the pooled filtrate and washings. The residue was dissolved in methanolic HCl (0.2 mol dm^3, 10 cm^3) and the progress of the removal of the cyclohexylidene group followed by TLC (chloroform–methanol–water, 8:4:0.5; R_F 0.43). When complete (36 h at room temperature), hydrochloric acid was removed with Amberlite IR 45 (OH^-) resin. The solid was filtered off and washed with methanol. From the pooled filtrate and washings the solvent was evaporated to leave as the residue practically pure *methyl* [*allyl* 3-*deoxy*-5-O-(α-D-*mannopyranosyl*)-α-D-manno-*oct*-2-*ulopyranosid*]*onate* (90 mg, 66%) characterized by its ^1H NMR spectrum; δ_H(250 MHz; CD_3OD) 1.96 (1 H, dd, $J_{3a,3e}$ 13, $J_{3a,4}$ 11, 3a-H), 2.12 (1 H, dd, $J_{3e,4}$ 5, 3e-H), 3.65–4.18 (14 H, m, OCH_2C=, 4-H, 5-H, 6-H, 7-H, 8a-H, 8b-H, 2'-H, 3'-H, 4'-H, 5'-H, 6'a-H and 6'b-H), 3.82 (3 H, s, CO_2Me), 5.12 (1 H, d, $J_{1',2'}$ 1.5, 1'-H), 5.15–5.36 (2 H, m, CH_2=) and 5.92 (1 H, m, CH=). The ester was dissolved in aqueous NaOH (0.5 mol dm^{-3}, 5 cm^3) and kept at room temperature for 2 h. Na ions were removed from the cooled (ice–water) mixture with Dowex AGWX8 (H^+) resin, solids were filtered off, and the pH of the solution was brought to 7.5 with $Ca(OH)_2$ (0.02 mol dm^{-3}). The syrup remaining after removal of the solvent was diluted with acetone (2 cm^3) whereupon the Ca-salt of the *title compound* precipitated. It was collected by centrifugation, washed with acetone (2 × 2 cm^2) and dried (90 mg, 59% from **14**) (Found: C, 40.4; H, 6.3. $C_{17}H_{27}Ca_{0.5}O_{13}\cdot 2.5H_2O$ requires: C, 40.5; H, 6.3%); $[\alpha]_D$ +87.4° (*c* 1.4, water); ^1H NMR data in Table 1.

Methyl [*Allyl* 7,8-O-*Cyclohexylidene*-3-*deoxy*-5-O-(2,3,4,6-*tetra*-O-*benzoyl*-α-D-*mannopyranosyl*)-α-D-manno-*oct*-2-

ulopyranosid]onate 4-[*Bis(trichloroethyl)*]*phosphate* **17**.—*N*-Methylimidazole (215 mm^3, 4 mol equiv.) and bis(trichloroethyl)phosphorochloridate (482 mg, 2 mol equiv.) were added to a solution of the disaccharide **14** (600 mg, 0.63 mmol) in anhydrous THF (6 cm^3), and the mixture, kept at 40 °C, was stirred for 1 h. The cooled mixture was soaked into a layer (3 × 5) of silica gel and eluted with a mixture of toluene–ethyl acetate (5:1, 500 cm^3). Fractions (100 cm^3) containing the phosphotriester (TLC, ethyl acetate–cyclohexane, 3:7; R_F 0.46) were pooled and the solvent was removed. The residual oil afforded after column (13.5 × 3.5) chromatography (ethyl acetate–cyclohexane, 2:8; 200 ml; then 3:7) the *title compound* (746 mg, 91%) as a colourless oil (Found: C, 51.8; H, 4.5. $C_{56}H_{57}Cl_6O_{20}P$ requires C, 52.0; H, 4.4%); $[\alpha]_D$ −1.3° (*c* 1, chloroform); δ_H(250 MHz; $CDCl_3$) 1.24–1.67 (10 H, m, $[CH_2]_5$), 2.48 (1 H, dd, $J_{3a,3e}$ 12, $J_{3a,4}$ 11.5, 3a-H), 2.59 (1 H, dd, $J_{3e,4}$ 5, 3e-H), 3.52 (1 H, *ca.* d, $J_{6,5}$, $J_{6,7}$ 9, 6-H), 3.88 (3 H, s, CO_2Me), 3.94 (1 H, dd, $J_{8a,8b}$ 9, $J_{8a,7}$ 4.5, 8a-H), 4.02 (2 H, m, OCH_2C=), 4.32 (1 H, dd, $J_{8b,7}$ 6.2, 8b-H), 4.45 (1 H, dd, $J_{6'a,6'b}$ 12.5, $J_{6'a,5'}$ 2.5, 6'a-H), 4.49 (1 H, *ca.* d, $J_{5,4}$ 3, 5-H), 4.51 (1 H, m, H-7), 4.70–4.81 (5 H, m, 5'-H and 2 × CH_2CCl_3), 4.94 (1 H, dd, $J_{6'b,5'}$ 2.1, 6'b-H), 5.14 (1 H, m, $J_{4,P}$ 9, 4-H), 5.20–5.35 (2 H, m, CH_2=), 5.47 (1 H, d, $J_{1',2'}$ 1.5, 1'-H), 5.86 (1 H, m, CH=), 5.90 (1 H, dd, $J_{2',3'}$ 3, 2'-H), 5.96 (1 H, dd, $J_{3',4'}$ 10, 3'-H), 6.28 (1 H, t, $J_{4',5'}$ 10, 4'-H) and 7.25–8.20 (20 H, m, ArH).

Methyl [*Allyl* 3-*Deoxy*-5-O-(α-D-*mannopyranosyl*)-α-D-manno-*oct*-2-*ulopyranosid*]*onate* 4-*Phosphate* **19**.—Activated Zn (400 mg) and silver carbonate (30 mg) were added to a solution of the phosphotriester **17** (700 mg, 0.54 mmol) in pyridine–acetic acid (8 cm^3, 10:1) and the mixture was stirred for 18 h at room temperature. Solids were filtered off and the filtrate was evaporated. Column (100 × 2.5) chromatography [Sephadex LH20; dichloromethane–methanol–ethyl acetate–water–conc. ammonia (*d* 0.91), 65:30:20:1:0.5] of the residue gave the fully protected, oily phosphomonoester **18** (514 mg) (TLC, chloroform–methanol–conc. ammonia, 65:24:4; R_F 0.47) which was recovered from the appropriate pooled fractions following removal of the solvents. It was taken up in methanolic sodium methoxide (0.1 mol dm^{-3}, 17 cm^3) and the mixture stirred for 7 h at room temperature. The solution was passed through a column (7 × 1.5) of Dowex AG50WX8 (H^+) resin, the column was washed with methanol (20 cm^3). Allyl alcohol (15 cm^3) was added to the pooled eluents, the volume was reduced to 25–30 cm^3, allyl alcohol (10 cm^3) was added and the volume again reduced to about 20 cm^3. Dowex AG50WX8 (H^+) resin was added, and the stirred mixture was kept at 56 °C for 10 h. Solids were filtered off and washed, the filtrate was neutralised with dilute (2%) ammonia, and the solvents were removed. The *monoammonium salt of the title compound* precipitated when the residue was diluted with methanol and acetone. It was recovered by centrifugation, washed with acetone and dried over phosphoric pentoxide (221 mg, 74%) (Found: C, 39.1; H, 6.2; N, 2.5. $C_{18}H_{34}NO_{16}P$ requires C, 39.2; H, 6.2; N, 2.5%); $[\alpha]_D$ +74.5° (*c* 1, water); δ_H(250 MHz; D_2O) 2.11 (1 H, dd, $J_{3a,3e}$ 13, $J_{3a,4}$ 11.5, 3a-H), 2.24 (1 H, dd, $J_{3e,4}$ 5.25, 3e-H), 3.59–4.20 (12 H, m, OCH_2C=, 6-H, 7-H, 8a-H, 8b-H, 2'-H, 3'-H, 4'-H, 5'-H, 6'a-H, 6'b-H), 3.80 (3 H, s, CO_2Me), 4.29 (1 H, m, 5-H), 4.54 (1 H, m, 4-H), 5.10 (1 H, d, $J_{1',2'}$ 2.5, 1'-H), 5.20–5.40 (2 H, m, CH_2=) and 5.92 (1 H, m, CH=).

[*Allyl* 3-*Deoxy*-5-O-(α-D-*mannopyranosyl*)-α-D-manno-*oct*-2-*ulopyranosid*]*onate* 4-*Phosphate* **20**.—A solution of the phosphorylated carboxymethyl ester **19** (188 mg, 0.34 mmol) in NaOH (5 cm^3, 0.5 mol dm^{-3}) was kept at room temperature for 2 h, diluted with water (5 cm^3), cooled (ice–water) and treated with Dowex AGWX8 (H^+) resin to remove cations. Solids were filtered off in the cold, washed with water and the pH of the filtrate was adjusted to 7.5 with aqueous $Ca(OH)_2$ (0.02 mol dm^{-3}) and its volume was reduced to about 0.5 cm^3. Addition of ethanol and acetone (final volume about 5 cm^3) caused the *Ca salt of the title compound* to precipitate; it was dried over phosphoric pentoxide (194 mg, 88%) (Found: C, 31.5; H, 5.6. $C_{17}H_{27}CaO_{16}P{\cdot}5H_2O$ requires C, 31.5; H, 5.7%); $[\alpha]_D$ +63.7° (*c* 1, water); ^{1}H NMR data: see Table 1.

Copolymerizations.—A solution of the allyl glycoside **16** (20 mg, 0.04 mmol), acrylamide (15 mg, 5 mol equiv.) and *N*,*N*,*N'*,*N'*-tetramethylethylenediamine (2 mm^3) in water (500 mm^3) was degassed by keeping it under water-pump pressure for 30 min. Ammonium persulphate (1 mg) was added and the mixture was kept at room temperature for 1 h and then at + 4 °C overnight. The solution was passed through a column (47 × 1.6) of Sephadex G-50 equilibrated with sodium hydrogen carbonate (0.1 mol dm^{-3}) and eluted with the same solvent. The volume of pooled fractions (50 drops) containing the copolymer (No. 19–28) was reduced to 1 cm^3, and the solution was desalted by passing it through a column (70 × 1.6) of Biogel P-2 in water. Lyophilization of the pooled fractions (50 drops; No. 22–31) gave the polymer **21** (20.5 mg) as an amorphous solid. Polymerization of the phosphorylated allyl glycoside **20** (20 mg), and purification of the product afforded the copolymer **22** (16.3 mg) as an amorphous solid.

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