Carbohydrate Research, 183 (1988) 183–199
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

SYNTHESIS OF A TRISACCHARIDE OF 3-DEOXY-D-*manno*-2-OCTULOPYRANOSYLONIC ACID (KDO) RESIDUES RELATED TO THE GENUS-SPECIFIC LIPOPOLYSACCHARIDE EPITOPE OF *Chlamydia**

PAUL KOSMA,
Institut für Chemie der Universität für Bodenkultur Wien, A-1180 Vienna (Austria)

GERHARD SCHULZ,
Sandoz-Forschungsinstitut Wien, A-1235 Vienna (Austria)

AND HELMUT BRADE
Forschungsinstitut Borstel, D-2061 Borstel (Federal Republic of Germany)

(Received October 31st, 1987; accepted for publication, February 18th, 1988)

ABSTRACT

The disaccharides, *O*-(sodium 3-deoxy-α- and -β-D-*manno*-2-octulopyranosylonate)-(2→8)-sodium (allyl 3-deoxy-α-D-*manno*-2-octulopyranosid)onate, were prepared *via* glycosylation of methyl (allyl 4,5,7-tri-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosid)onate with methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-D-*manno*-2-octulopyranosyl bromide)onate under Helferich and Koenigs–Knorr conditions, respectively. Based on g.l.c.–m.s. data of the α- and β-(2→8)-linked disaccharide derivatives, obtained after carbonyl- and carboxyl-group reduction, followed by methylation, the α-anomeric configuration was assigned to the terminal KDO-residue in the KDO-region of *Chlamydial* lipopolysaccharide. The trisaccharide *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→8)-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→4)-sodium (allyl 3-deoxy-α-D-*manno*-2-octulopyranosid)onate was obtained *via* block synthesis using an α-(2→8)-linked disaccharide bromide derivative as the glycosyl donor. Copolymerization of the allyl glycosides with acrylamide gave water-soluble macromolecular antigens, suitable for defining epitope specificities of monoclonal antibodies directed against *Chlamydial* LPS.

INTRODUCTION

One of the major surface antigens of *Chlamydiae* is a glycolipid[1,2] which has been shown[3,4] to contain the characteristic constituents of typical lipopolysaccharides

*Presented, in part, at the 4th European Carbohydrate Symposium, Darmstadt, F.R.G., July 12–17, 1987. Financial support of this work by Bundesministerium für Forschung und Technologie (Bonn) is gratefully acknowledged (grant: 0.1 ZR 8604).

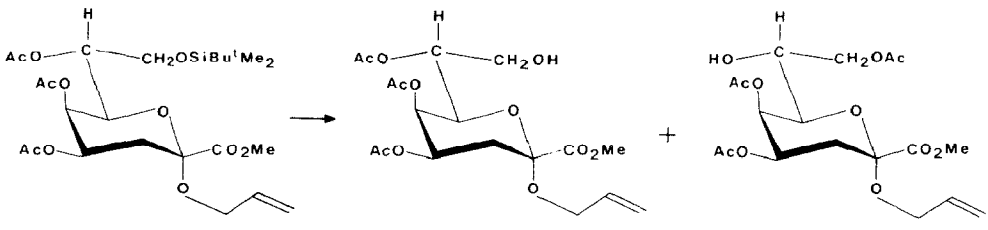
0008-6215/88/$ 03.50

known from gram-negative bacteria, *i.e.*, D-glucosamine, KDO, phosphoric ester, and long chain fatty acids among which 3-hydroxy fatty acids are always found. Thus, chlamydiae possess a lipopolysaccharide which, in addition, has been reported[4,5] to share antigenic determinants with the LPS from the deep rough mutant strain R595 [R*e* chemotype containing an α-(2→4)-linked KDO disaccharide as constituent of the saccharide portion]. In addition to this cross-reactive antigenic determinant, a chlamydia-specific epitope has been detected serologically with a monoclonal antibody[6]; however, its chemical structure was hitherto unknown. This epitope could be expressed by the tools of molecular genetics in Re-mutant recipients[7] being transformed with a plasmid containing a 6.5 kb insert[8] of chlamydial DNA. Structural investigations of the lipopolysaccharide of such recombinant bacteria revealed[9] the presence of an α-(2→4)-linked KDO disaccharide, a ?-(2→8)-linked KDO disaccharide, and a KDO trisaccharide having the sequence ?-KDO-(2→8)-KDO-(2→4)-KDO.

Previously, we have reported the synthesis of polyacrylamide copolymers[10,11] containing α-KDO*p*, β, KDO*p*, β-D-Rib*f*-(1→7)-β-KDO*p*, α-KDO*p*-(2→4)-α-KDO*p*, α-KDO*p*-(2→4)-β-KDO*p*, β-KDO*p*-(2→4)-α-KDO*p*, and β-KDO*p*-(2→4)-β-KDO*p* residues[12,13], which allowed the epitope specificities of two monoclonal antibodies to be determined[14]. One of them (clone 25) recognizes, in enterobacterial LPS, an α-(2→4)-linked KDO-disaccharide, whereas the other (clone 20) reacts specifically with a terminal pyranoside KDO group in the α-D anomeric configuration. The work described herein was performed to determine the anomeric configuration of the terminal KDO group residue in the trisaccharide aimed at the determination of the chemical structure of the clamydia-specific epitope, and reports the synthesis of allyl glycosides and polyacrylamide copolymers containing α- and β-KDO*p*-(2→8)-α-KDO*p*, and α-KDO*p*-(2→8)-α-KDO*p*-(2→4)-α-KDO*p* residues.

RESULTS AND DISCUSSION

For the synthesis of the disaccharide derivatives **7** and **13**, the previously described[12] methyl (allyl 4,5,7-tri-*O*-acetyl-8-*O*-*tert*-butyldimethylsilyl-3-deoxy-α-D-*manno*-2-octulopyranosid)onate (**1**) was employed as the starting material. Cleavage of Bu^tMe_2Si by the action of 2% hydrogen fluoride in acetonitrile[15] gave



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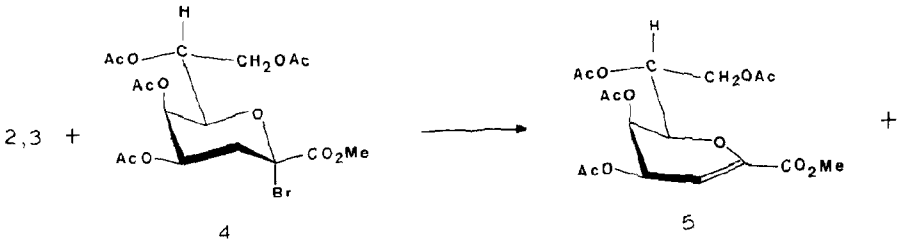

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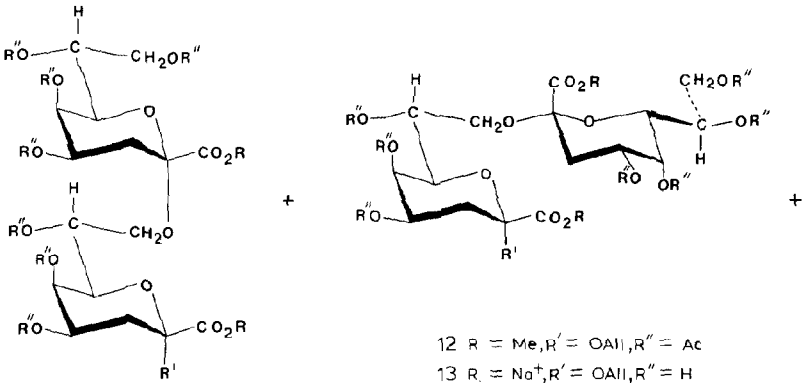
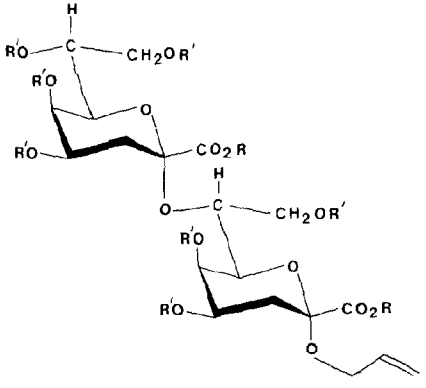


6 R = Me, R′ = OAll, R″ = Ac
7 R = Na^+, R′ = OAll, R″ = H
8 R = Me, R′ = OCH=CHCH$_3$, R″ = Ac
9 R = Me, R′ = OH, R″ = Ac
10 R = Me, R′ = OAc, R″ = Ac
11 R = Me, R′ = Br, R″ = Ac

12 R = Me, R′ = OAll, R″ = Ac
13 R = Na^+, R′ = OAll, R″ = H
14 R = Me, R′ = OCH=CHCH$_3$, R″ = Ac
15 R = Me, R′ = OH, R″ = Ac



16 R = Me, R′ = Ac

a 10:1 mixture of **2** and the 8-*O*-acetyl derivative **3** in 98% yield. Owing to extensive acetyl migration upon attempted separation of the isomers on silica gel, the crude mixture was immediately glycosylated with 2.7 molar equivalents of methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosyl bromide)onate[16] (**4**). Under modified Helferich conditions, *i.e.*, mercury(II) cyanide, molecular sieve 4Å, and nitromethane, a 4:1 mixture of the α- and β-(2→8)-linked disaccharide derivatives **6** and **12** was obtained in 90% yield. The mixture of the isomers **6** and **12** was isolated by column chromatography on silica gel after separation from the glycal ester derivative[17] **5** and a small amount (8%) of the α-(2→7)-linked disaccharide derivative **16**.

Crystallization of the mixture from ethyl acetate–hexane gave crystalline **6** in 59% yield. The structures of **6** and **16** were based on the 250-MHz, ^{1}H-n.m.r. data; the chemical shift values of the signals attributable to H-4′ (δ 5.33 for **6**, δ 5.37–5.22 for **16**) were indicative of the α-anomeric configuration[18,19]. The constitution of **6** and **16** was deduced from the chemical shift values of the signals attributable to H-8a, H-8b (δ 3.81 and 3.72) and H-7 (δ 4.10), respectively.

The β-(2→8)-linked disaccharide derivative **12** was obtained as the major isomer by performing the glycosylation reaction in dichloromethane with silver carbonate as promoter. Thus, the reaction of **2** and **3** with 2.3 molar equivalents of **4** gave a 58% yield of a 1:3 mixture of **6** and **12**. Four successive recrystallizations of the solid material obtained after chromatography afforded crystalline **12**. The purity of the crystalline fractions and the mother liquors were monitored by h.p.t.l.c. in 80:1 chloroform–methanol as eluent. The structural assignments of **12** were based on the chemical shift values of the 250-MHz, ^{1}H-n.m.r. signals attributable to H-4′ (δ 4.90) and H-8a, H-8b (δ 4.12 and 3.62).

The disaccharide derivatives **6** and **12** were deprotected by Zemplén deacetylation in methanolic sodium methoxide, and subsequent conversion of the methyl ester groups into the sodium salts by the action of aqueous NaOH gave *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→8)-sodium (allyl 3-deoxy-α-D-*manno*-2-octulopyranosid)onate (**7**, 96%), and *O*-(sodium 3-deoxy-β-D-*manno*-2-octulopyranosylonate)-(2→8)-sodium (allyl 3-deoxy-α-D-*manno*-2-octulopyranosid)onate (**13**, 96%), respectively.

The differences in the chemical shifts of the equatorial and axial protons at C-3′ (0.25 p.p.m. for **7** and 0.62 p.p.m. for **13**) confirmed the configurations assigned[19].

Assignment of the anomeric configuration of the terminal KDO residue in disaccharide **18**. — Prior to the g.l.c. and g.l.c.–m.s. comparison of a (2→8)-linked KDO-disaccharide derivative[9] isolated from *Chlamydia psittaci* and a recombinant of *Salmonella minnesota* R 595-207 with the synthetic disaccharides, the allyl group was removed as follows. A low yield (46%) of the reducing disaccharide **9** was obtained by use of selenium dioxide in acetonitrile in the presence of acetic acid[20] at 45° for 2 days. Better results were achieved *via* isomerization of the allyl glyco-

sides **6** and **12** into *trans*-1-propenyl glycosides with 1,5-cyclooctadienebis(methyldiphenylphosphine) iridium hexafluorophosphate[21,22], which gave **8** and **14** in 87% and 93% yield, respectively. Cleavage of the propenyl glycosides was effected by iodine in aqueous oxolane[23], which furnished **9** and **15**, in 79% and 78% yields, respectively. The 250-MHz, ^{1}H-n.m.r. signals of axial H-3 protons were observed at a field lower than those of equatorial H-3 protons, similarly to the reported data for KDO-derivatives having OH-2 free[24,25].

To obtain the same compound from the natural KDO*p*-(2→8)-KDO disaccharide and the synthetic counterparts, the following derivatization sequence was performed. The carbonyl group of the natural disaccharide was reduced and the resulting compound methylated by a modified[26] Hakomori procedure[27] to yield the pseudodisaccharide methyl (methyl 3-deoxy-8-*O*-(3-deoxy-4,5,7,8-tetra-*O*-methyl-D-*manno*-2-octulopyranosylonate)-2,4,5,6,7-penta-*O*-methyl-D-*glycero*-D-*galacto*/*talo*-octonate (**17**). The g.l.c. and g.l.c.–m.s. data for this compound have been published[9].

The carboxyl group of **17** was reduced with sodium borohydride in 1:1 methanol–water and the resulting compound methylated to give 3-deoxy-8-*O*-(3-deoxy-1,4,5,7,8-penta-*O*-methyl-D-*manno*-2-octulopyranosyl)-1,2,4,5,6,7-hexa-*O*-methyl-D-*glycero*-D-*galacto*/*talo*-octitol (**18**). The synthetic disaccharides **9** and **15**

TABLE I

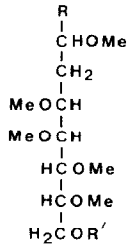
G.L.C. AND G.L.C.–M.S. DATA FOR COMPOUNDS **18–20**

Compound	*Mol. wt.*[a]	*Retention time (min)*[b]	*Characteristic e.i. fragments*[c] (m/z)
18	586	22.07–22.39	45 (21.8), 59 (17.4), 71 (16.5), 75 (26.8), 88 (26.8), 89 (40.1), 101 (100), 115 (55.8), 127 (18.3), 147 (16.2), 159 (6.5), 181 (9.4) 191 (8.8), 213 (20.9), 245 (23), 277 (6.8), 293 (7.4)
20	586	22.84–23.22	45 (18.2), 59 (16.6), 71 (16.4), 75 (24.9), 88 (21.5), 89 (45), 101 (100), 115 (53.2), 127 (17.7), 147 (9.3), 159 (3.7), 181 (11), 191 (3.6), 213 (29.3), 245 (27.8), 277 (3.6) 293 (14.2)
19	586	22.07–22.39	45 (21.2), 59 (16.4), 71 (15.9), 75 (26.4), 88 (26.1), 89 (41.1), 101 (100), 115 (54.4), 127 (18.9), 147 (15.7), 159 (7.2), 181 (9.1) 191 (8.8), 213 (20.9), 245 (23.8), 277 (6.2), 293 (6.9)

[a]Determined by c.i.m.s. (ammonia) (ref. 26) on the basis of peaks at *m/z* for $(M + 1)^+$ and $(M + 18)^+$. [b]Fused-silica capillary column (25 m × 0.32 mm, i.d.) with chemically bonded CP WAX 52 (0.16-μm film thickness), temperature program of 180° for 5 min and then 2°/min→230°, and H_2 as carrier gas (0.05 MPa). [c]Determined by e.i.m.s. (70 eV) (ref. 26). The fragment ions listed were obtained from the earlierst eluted isomer. [d]Percent of basepeak in parentheses.

were reduced with sodium borohydride in 1:1 methanol–water, followed by deacetylation in sodium methoxide and methylation to give 3-deoxy-8-*O*-(3-deoxy-1,4,5,7,8-penta-*O*-methyl-α-D-*manno*-2-octulopyranosyl)-1,2,4,5,6,7-hexa-*O*-methyl-D-*glycero*-D-*galacto*/*talo*-octitol (**19**) and 3-deoxy-8-*O*-(3-deoxy-1,4,5,7,8-penta-*O*-methyl-β-D-*manno*-2-octulopyranosyl)-1,2,4,5,6,7-hexa-*O*-methyl-D-*glycero*-D-*galacto*/*talo*-octitol (**20**), respectively. Since the reduction of the carbonyl group was not stereospecific, each compound was obtained as a mixture of the D-*glycero*-D-*galacto* and D-*glycero*-D-*talo* isomers, which were separated by g.l.c. (Table I). The retention times of **18** were identical to those of **19**, thus indicating that the anomeric configuration of the terminal KDO residue in the natural KDO*p*-(2→8)-KDO disaccharide was α.

C.i.m.s. (ammonia) yielded a pseudomolecular ion peak at *m/z* 604 $(M + 18)^+$, confirming the calculated mol. wt. of 586. The characteristic fragment ions obtained after e.i.m.s. are included in Table I. They were derived from cleavage of the glycosidic bond to yield the primary fragment ions at *m/z* 293 and 277, which comprise the alditol chain and the nonreducing terminus, respectively, and their corresponding daughter ions after loss of methanol. Fragment ions at *m/z* 147 and 115 (147 − 32) and at *m/z* 191 and 159 (191 − 32) were assigned to the C-1–C-4 and C-1–C-5 fragments of the alditol chain. Methylation analysis performed on **18**,



17 R = CO_2Me
R′ = methyl (3-deoxy-4,5,7,8-tetra-*O*-methyl-D-manno-2-octulopyranosyl)onate

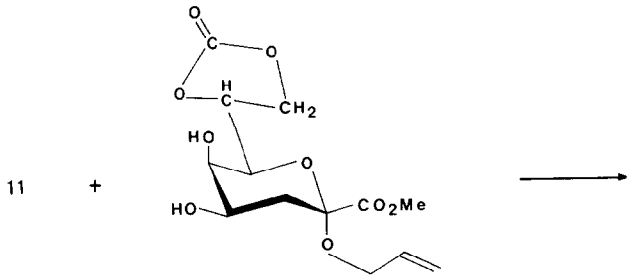
18 R = CH_2OMe
R′ = 3-deoxy-1,4,5,7,8-penta-*O*-methyl-D-manno-2-octulopyranosyl

19 R = CH_2OMe
R′ = 3-deoxy-1,4,5,7,8-penta-*O*-methyl-α-D-manno-2-octulopyranosyl

20 R = CH_2OMe
R′ = 3-deoxy-1,4,5,7,8-penta-*O*-methyl-β-D-manno-2-octulopyranosyl

19, and **20** yielded 2,6-di-*O*-acetyl-3-deoxy-1,4,5,7,8-penta-*O*-methyl-D-*glycero*-D-*galacto/talo*-octitol[26] and 8-*O*-acetyl-3-deoxy-1,2,4,5,6,7-hexa-*O*-methyl-D-*glycero*-D-*galacto/talo*-octitol[28] in equimolar amounts (data not shown). The mass spectral data of these 3-deoxyoctitol derivatives have been published[26,28].

The α-anomeric configuration of the terminal KDO-residue in the LPS from *Chlamydia* having thus been ascertained, the synthesis of the trisaccharide **24** was accomplished as follows. Acetylation of **9** (acetic anhydride–pyridine) gave a quantitative yield of the octa-*O*-acetyl derivative **10**. As in the case of **6** and **16**, the low-field ^{1}H-n.m.r. signal attributable to H-4 (δ 5.32) indicated the α-anomeric configuration. Reaction of **10** with titanium tetrabromide in dichloromethane gave a 98% yield of the disaccharide bromide derivative **11**, which was immediately employed as the glycosyl donor. Glycosylation of 4 molar equivalents of the previously described[12] 7,8-*O*-carbonyl derivative **21** with **11** in nitromethane with



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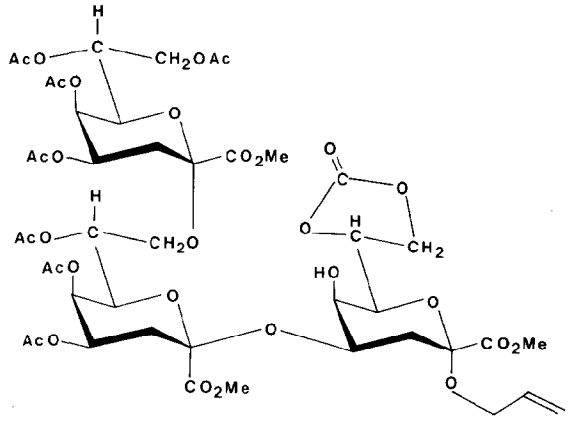


22

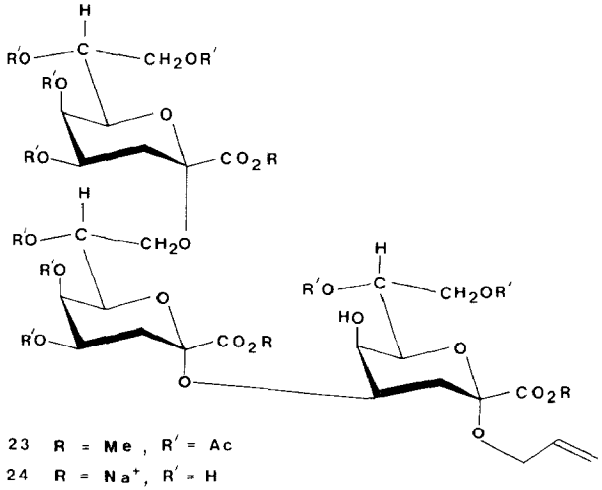
mercury(II) cyanide as promoter gave a 5% yield of the trisaccharide derivative **23**. In acetonitrile, however, a 2:5 mixture of the trisaccharide derivatives **22** and **23** was obtained in 30% yield; the mixture was separated by column chromatography

on silica gel. The 250-MHz, ^{1}H-n.m.r. spectra of **22** and **23** containing three signals for methyl ester groups and seven signals for acetoxy groups were in keeping with the overall structure of the trisaccharide derivatives. Furthermore, the signals attributable to H-8a and H-8b, which participate in the cyclic carbonate of the glycosyl acceptor, were well separated from the bulk of signals and easily assigned. The β-D-anomeric configuration at C-2′ in compound **22** was assigned on the basis of the chemical shift value of H-4′ (δ 4.82); for the isomer **23**, the corresponding signal attributable to H-4′ occurred in the bulk of signals between δ 5.34 and 5.14, thus being indicative of the α-D-anomeric configuration of the respective octulopyranosylonic residue.

Sequential deprotection of **23** by deacylation in methanolic sodium methoxide and alkaline hydrolysis of the methyl ester groups in aqueous NaOH afforded *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→8)-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→4)-sodium (allyl 3-deoxy-α-D-*manno*-2-octulopyranosid)onate (**24**) in 97% yield.

The Fourier-transform, proton-decoupled, ^{13}C-n.m.r. chemical shift data of **7, 13,** and **24** compared favorably with those of related allyl glycosides (Table II). The signals for C-8 of compounds **7** and **13** experienced a glycosidation shift of +1.6 and +1.7 p.p.m., respectively, the signals of C-7 being shifted −1.9 and −0.9 p.p.m., respectively. The chemical shift patterns of the ring carbon atoms of **7** and **24** were consistent with the assignment of the α-anomeric configuration of the respective KDO residues, whereas the corresponding signals of the terminal KDO-residue of **13** confirmed the β-anomeric configuration[31,32]. The assignment of the ^{13}C-n.m.r. resonances and a conformational analysis of the KDO-oligosaccharides by H.S.E.A. calculations will be published elsehwere[32].

Copolymerization with acrylamide[33]. — The allyl ketosides **7, 13,** and **24** were treated with 4 molar equivalents of acrylamide in the presence of *N,N,N′N′*-tetramethylethylene diamine and ammonium peroxosulfate as previously described[12].



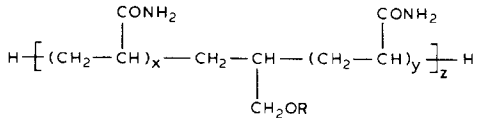
23 R = Me , R′ = Ac
24 R = Na$^+$, R′ = H

TABLE II

EMPIRICAL ASSIGNMENT[a] OF ^{13}C-N.M.R. CHEMICAL SHIFTS FOR COMPOUNDS **7**, **13**, AND **24**

Carbon atom	*Compound*					
	*α-KDO*p*OAll*[b]	*α-KDO*p*-(2→4)-α-KDO*p*OAll*[b]	**24**	**7**	**13**	*β-KDO*p*OAll*[b]
1	176.1	176.0	176.0	176.1	176.1	174.6
2	101.1	101.0	101.0	100.9	101.1	101.9
3	35.1	34.2	34.2	34.9	35.1	35.6
4	66.9	69.5	71.0[c]	66.8	66.9	68.3
5	67.2	65.2	64.6	67.1	67.2	66.2
6	72.5	72.4	72.3	72.2	72.1	74.3
7	70.4	70.5	70.5	68.5	69.0	69.9
8	64.1	64.0	64.0[d]	65.7	66.6	64.9
1′		176.8	176.9[e]	176.7	174.5	
2′		100.2	100.7	101.4	101.8	
3′		35.4	35.4	34.7	35.5	
4′		66.8	66.7[f]	66.7	68.5	
5′		67.2	67.8	67.0	66.3	
6′		73.3	73.0	72.5	74.2	
7′		70.9	70.3[c]	70.1	70.0	
8′		64.0	65.3	63.9	65.0	
1″			176.0[e]			
2″			100.7			
3″			35.1			
4″			66.8[f]			
5″			67.3			
6″			72.2			
7″			70.1			
8″			64.1[d]			
OCH_2	65.4	65.0	65.1	65.1	65.4	66.6
–CH=	134.8	135.0	135.2	134.6	134.9	134.8
CH_2=	119.0	118.8	117.7	118.3	118.7	119.0

[a]Ref. 32. [b]Ref. 12. [c,d,e,f]Assignments may be interchanged.



25 R = α-KDOp-(2→8)-α-KDOp-(2→

26 R = β-KDOp-(2→8)-α-KDOp-(2→

27 R = α-KDOp-(2→8)-α-KDOp-(2→4)-α-KDOp-(2→

The water-soluble linear copolymers **25–27** were isolated by passage through Sephadex G-25 and desalted on Bio-Gel P-2. Immunochemical results obtained with these artificial antigens will be published elsehwere.

EXPERIMENTAL

General methods. — Melting points were determined with a Kofler hot-stage and are uncorrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. ^{1}H-N.m.r.-spectra were recorded with a Bruker WM-250 instrument and tetramethylsilane was the internal standard. Coupling constants are first order. ^{13}C-N.m.r.-spectra were recorded at 62.9 MHz for solutions in deuterium oxide at 24° with 32K of memory and a spectral width of 12 kHz. The instrument was operated in the F.t. mode with complete proton-decoupling. Chemical shifts are given from the signal of tetramethylsilane (resonance frequency set at δ 67.40 upfield from an external signal of 1,4-dioxane in deuterium oxide). T.l.c. was performed on Merck precoated plates (5 × 10 cm, layer-thickness 0.25 mm, Silica Gel 60 F_{254}). Spots were detected by u.v. light and by spraying with an anisaldehyde–H_2SO_4 reagent[34]. Column chromatography was performed on Merck–Lichroprep columns (size A, 24 × 1; B, 31 × 2.5, and C, 44 × 3.7 cm; silica gel 40–63 μm) under pressure (0.2 MPa). Acrylamide was twice recrystallized from chloroform. Elemental analyses were performed by Dr. J. Zak, Mikroanalytisches Laboratorium am Institut für Physikalische Chemie, Universität Wien.

*Isolation of KDO*p*-(2→8)-KDO disaccharide from LPS of* Chlamydia psittaci *and* Salmonella minnesota *R595-207.* — LPS was extracted from purified elementary bodies of *C. psitacci*[3] and from the recombinant strain R595-207 of *S. minnesota*[7] which carries a pUC8 plasmid containing a 6.5 kbase insert of chlamydial DNA[8]. Both LPS have been shown[9] to release, upon hydrolysis in 20mM sodium acetate buffer at 70°, KDO, KDO*p*-(2→4)-KDO,KDO*p*-(2→8)-KDO, and KDO*p*-(2→8)-KDO*p*-(2→4)-KDO. The compounds were separated by g.l.c. using a fused-silica capillary column (25 m × 0.32 mm, i.d.) with a chemically-bonded separating phase (CP Wax 52; 0.16 μm film thickness), a temperature program (180° for 5 min with an increase of 2°/min to a final temperature of 230°), and H_2 as carrier gas (0.05 MPa). The experimental conditions for e.i.m.s. and c.i.m.s. (ammonia) have been already described[26].

*Methyl (allyl 4,5,7-tri-*O*-acetyl-3-deoxy-α-*D*-manno-2-octulopyranosid)onate* (**2**) *and methyl (allyl 4,5,8-tri-*O*-acetyl-3-deoxy-α-*D*-manno-2-octulopyranosid)onate* (**3**). — A solution of **1** (520 mg) in dry acetonitrile (10 mL) was stirred with 2% HF in acetonitrile (3 mL) for 4 h at room temperature. After addition of $NaHCO_3$, the mixture was evaporated. The residue was dissolved in dichloromethane (50 mL), and the solution was washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$) and evaporated to dryness to give a mixture of **2** and **3** as a colorless syrup (yield 400 mg, 98%); ^{1}H-n.m.r. ($CDCl_3$) (**2**): δ 5.90 (m, 1 H, =CH–), 5.38 (ddd, 1 H, $J_{4,5}$ ~3.0, $J_{4,3e}$ ~5.0, $J_{4,3a}$ ~12.5 Hz, H-4), 5.36 (unresolved signal, 1 H, H-5), 5.31 (dq, 1 H, $=CH_{2\,trans}$), 5.20 (dq, 1 H, $=CH_{2\,cis}$), 5.12 (dt, 1 H, $J_{7,6}$ ~9.5, $J_{7,8a}$ ~$J_{7,8b}$ ~3.0 Hz, H-7), 4.20 (dd, 1 H, $J_{6,5}$ ~1.0 Hz, H-6), 4.13 (m, 1 H) and 3.93 (m, 1H, OCH_2), 3.90–3.84 (m, 2 H, H-8a,b), 3.81 (s, 3 H, CO_2CH_3), 2.24 (dd, 1 H, $J_{3e,3a}$ ~12.5 Hz, H-3*e*), 2.08 (t, 1 H, H-3*a*), 2.10, (s, 3 H), 2.05 (s, 3 H), and 1.98 (s, 3 H, CH_3CO).

O-[*Methyl (4,5,7,8-tetra*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosyl)onate*]-*(2→8)-methyl (allyl 4,5,7-tri*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosid)onate* (**6**) *and* O-[*methyl (4,5,7,8-tetra*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosyl)onate*]-*(2→7)-methyl (allyl 4,5,8-tri*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosid)onate* (**16**). — A solution of **4** (580 mg, 1.2 mmol) in dry nitromethane (2 mL) was added dropwise during 6 h at room temperature to a suspension of **2** and **3** (180 mg, 0.43 mmol), $Hg(CN)_2$ (380 mg, 1.5 mmol), and molecular sieves 4A (1 g) in nitromethane (5 mL) under dry N_2. After being stirred for 15 h, the mixture was diluted with dichloromethane (50 mL), filtered over Celite, extracted with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated. Purification of the residue on a column of silica gel (size C, 5:2 toluene–ethyl acetate) gave 320 mg (90%) of **6** and **12** as a solid. Recrystallization from 2:1 hexane–ethyl acetate afforded 59% of **6**. The mother liquor contained **12** as the major component as evidenced by t.l.c. on h.p.t.l.c. plates (Merck, Kieselgel 60 F_{254}); by use of 80:1 chloroform–methanol as eluent, **6** emerged as the slightly faster moving isomer, colorless prisms, m.p. 189–190° (hexane–ethyl acetate), $[\alpha]_D^{20}$ +87° (*c* 1.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.92 (m, 1 H, =CH–), 5.37 (unresolved signal, 1 H, H-5′), 5.33 (m, 2 H, H-4′,5), 5.30 (dq, 1 H, $=CH_{2\ trans}$), 5.21 (m, 2 H, $-CH_{2\ cis}$, H-7′), 5.19 (ddd, 1 H, H-7), 5.15 (ddd, 1 H, $J_{4,5}$ ~3.0, $J_{4,3e}$ ~5.5, $J_{4,3a}$ ~12.5 Hz, H-4), 4.61 (dd, 1 H, $J_{8'a,7'}$ ~2.5, $J_{8'a,8'b}$ ~12.5 Hz, H-8′a), 4.15 (dd, 1 H, $J_{8'b,7'}$ ~4.0 Hz, H-8′b), 4.12 (dd, 1 H, $J_{6',5'}$ ~1.0, $J_{6',7'}$ ~9.0 Hz, H-6′), 4.09 (m, 1 H, OCH_2), 4.05 (dd, 1 H, OCH_2), 3.86 (dd, 1 H, $J_{8a,7}$ ~2.5, $J_{8a,8b}$ ~11.5 Hz, H-8a), 3.81 (s, 3 H) and 3.80 (s, 3 H, CO_2CH_3), 3.72 (dd, 1 H, $J_{8b,7}$ ~5.5 Hz, H-8b), 2.23 (dd, 1 H, $J_{3e,4}$ ~5.0, $J_{3e,3a}$ ~12.5 Hz, H-3*e*), 2.16 (dd, 1 H, $J_{3'e,4'}$ ~5.5, $J_{3'e,3'a}$ ~13.0 Hz, H-3′*e*), 2.15–1.95 (m, 2 H, including H-3*a*,3*a*′), 2.12 (s, 3 H), 2.08 (s, 6 H), 2.07 (s, 3 H), 2.00 (s, 3 H), 1.98 (s, 3 H), and 1.96 (s, 3 H, CH_3CO).

Anal. Calc. for $C_{35}H_{48}O_{22}$: C, 51.22; H, 5.89. Found: C, 51.24; H, 5.90.

Further elution of the column with toluene–ethyl acetate gave 30 mg (8%) of **16**, colorless syrup, $[\alpha]_D^{20}$ +64° (*c* 2.2, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.88 (m, 1 H, =CH–), 5.45 (unresolved signal, 1 H) and 5.37 (unresolved signal 1 H, H-5,5′), 5.33 (dq, 1 H, $=CH_{2\ trans}$), 5.37–5.22 (m, 3 H, H-4,4′,7′), 5.23 (dq, 1 H, $=CH_{2\ cis}$), 4.51 (dd, 1 H, $J_{8a,7}$ ~2.5, $J_{8a,8b}$ ~12.0 Hz, H-8a), 4.45 (dd, $J_{8'a,7'}$ ~3.5, $J_{8'a,8'b}$ ~13.0 Hz, H-8′a), 4.34 (dd, 1 H, $J_{8'b,7'}$ ~2.5 Hz, H-8′b), 4.30 (dd, 1 H, $J_{6',7'}$ ~9.0, $J_{6',5'}$ ~1.5 Hz, H-6′), 4.10 (dt, 1 H, $J_{7,6}$ ~7.5, $J_{7,8b}$ ~2.5 Hz, H-7), 3.98 (dd, 1 H, $J_{6,5}$ ~1.0 Hz, H-6), 3.97–3.85 (m, 3 H, H-8b, OCH_2), 3.84 (s, 3 H) and 3.79 (s, 3 H, CO_2CH_3), 2.25–1.95 (m, 4 H, including H-3*e*,3′*e*,3*a*,3′*a*), 2.16 (s, 3 H), 2.13 (s, 3 H), 2.08 (s, 3 H), 2.06 (s, 3 H), 2.03 (s, 3 H), and 1.97 (s, 6 H, 2 CH_3CO).

O-[*Methyl (4,5,7,8-tetra*-O-*acetyl-3-deoxy-β*-D-manno-*2-octulopyranosyl)onate*]-*(2→8)-methyl (allyl 4,5,7,8-tri*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosid)onate* (**12**). — A solution of **4** (280 mg, 0.58 mmol) in dry dichloromethane (2 mL) was added dropwise during 3 h to a suspension of Ag_2CO_3 (193 mg, 0.7 mmol), **2** and **3** (106 mg, 0.25 mmol), and molecular sieves 4Å (1 g) in dichloromethane (5 mL) under N_2 and with exclusion of light. After being stirred for 16 h at room

temperature, the mixture was diluted with dichloromethane (50 mL), filtered over Celite, washed sequentially with 5% aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and taken to dryness. The residue was purified on a column of silica gel (C, 5:2 toluene–ethyl acetate) to give **5** (139 mg, 35% based on **4**), and a mixture of **6** and **12** (122 mg, 58%), colorless crystals; four successive crystallizations from 1:1 hexane–ethyl acetate afforded **12** (67 mg, 32%), colorless needles, m.p. 156–158°, $[\alpha]_D^{20}$ +63° (*c* 0.99, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.89 (m, 1 H, =CH–), 5.41–5.35 (m, 2 H, H-4,5), 5.30 (dq, 1 H, $=CH_{2\ trans}$), 5.29 (unresolved signal, 1 H, H-5′), 5.19 (dq, 1 H, $=CH_{2\ cis}$), 5.12 (dt, 2 H, H-7,7′), 4.90 (ddd, 1 H, $J_{4',3'e}$ ~5.0, $J_{4',3'a}$ ~13.0, $J_{4',5'}$ ~3.0 Hz, H-4′), 4.35 (dd, 1 H, $J_{8'a,8'b}$ ~12.5, $J_{8'a,7'}$ ~2.5 Hz, H-8′a), 4.28 (dd, 1 H, $J_{8'b,7'}$ ~4.0 Hz, H-8′b), 4.17–4.03 (m, 4 H, including H-6,8a,6′, and OCH_2), 3.92 (m, 1 H, OCH_2), 3.81 (s, 6 H, 2 CO_2CH_3), 3.62 (dd, 1 H, $J_{8b,7}$ ~4.0, $J_{8b,8a}$ ~11.5 Hz, H-8b), 2.30 (dd, 1 H, $J_{3'e,3'a}$ ~12.5 Hz, H-3′*e*), 2.21 (dd, 1 H, $J_{3e,4}$ ~5.5, $J_{3e,3a}$ ~12.5 Hz, H-3*e*), 2.14 (t, 1 H, $J_{3'a,4'}$ ~13.0 Hz, H-3′*a*), 2.21–1.98 (m, 1 H, H-3*a*), 2.11 (s, 3 H), 2.08 (s, 6 H), 2.04 (s, 3 H), 2.00 (s, 3 H), 1.99 (s, 3 H), and 1.98 (s, 3 H, CH_3CO).

Anal. Calc. for $C_{35}H_{48}O_{22}$: C, 51.22; H, 5.89. Found: C, 51.21; H, 5.84.

O-[*Sodium (3-deoxy-α-*D-manno-*2-octulopyranosyl)onate*]-*(2→8)-sodium (allyl 3-deoxy-α-*D-manno-*2-octulopyranosid)onate* (**7**). — A solution of **6** (65 mg) in dry methanol (5 mL) was stirred with mM methanolic sodium methoxide for 70 min at room temperature. The solution was made neutral by addition of Dowex 50 (H^+) cation-exchange resin, filtered, and evaporated to dryness. The residue was dissolved in water (5 mL) and stirred with 0.2M aqueous NaOH (1.4 mL) for 90 min at room temperature. The pH of the solution was adjusted to 8.0 by addition of Dowex 50 (H^+) resin, the mixture was filtered, and the filtrate was lyophilized. Purification of the residue on a column of Bio-Gel P-2 (2.6 × 100 cm) gave **7** (yield 41 mg, 96%), amorphous powder, $[\alpha]_D^{20}$ +59° (*c* 0.47, water); ^{1}H-n.m.r. (D_2O): δ 5.98 (m, 1 H, =CH–), 5.36 (dq, 1 H, $=CH_{2\ trans}$), 5.26 (dq, 1 H, $=CH_{2\ cis}$), 4.17–3.83 (m, 10 H) and 3.70–3.57 (m, 4 H, including H-4,5,6,7,8a,8b,4′,5′,6′,7′,8′a,8′b, and OCH_2), 2.06 (2 dd, 2 H, H-3*e*,3′*e*), and 1.81 (2 t, 2 H, $J_{3e,3a}$ ~$J_{3'e,3'a}$ ~$J_{3a,4}$ ~12.5 Hz, H-3*a*,3′*a*).

O-[*Sodium (3-deoxy-β-*D-manno-*2-octulopyranosyl)onate*]-*(2→8)-sodium (allyl 3-deoxy-α-*D-manno-*2-octulopyranosid)onate* (**13**). — A solution of **12** (30 mg) in dry methanol (5 mL) was stirred with 0.01M methanolic sodium methoxide (5 mL) for 2 h at room temperature. The solution was made neutral by addition of Dowex 50 (H^+) resin, filtered, and taken to dryness. The residue was dissolved in water (10 mL) and treated with 0.2M aqueous NaOH (1 mL) for 60 min at room temperature. The solution was processed as described for **7** to give **13** (19 mg, 96%), amorphous powder, $[\alpha]_D^{20}$ +56° (*c* 0.46, water); ^{1}H-n.m.r. (D_2O): δ 5.98 (m, 1 H, =CH–), 5.37 (dq, 1 H, $=CH_{2\ trans}$), 5.25 (dq, 1 H, $=CH_{2\ cis}$), 4.13–3.50 (m, 14 H, including H-4,5,6,7,8a,8b,4′,5′,6′,7′,8′a,8′b and OCH_2), 2.45 (dd, 1 H, $J_{3'e,3'a}$ ~12.5, $J_{3'e,4}$ ~5.0 Hz, H-3′*e*), 2.05 (dd, 1 H, $J_{3e,3a}$ ~13.0, $J_{3e,4}$ ~5.0 Hz, H-3*e*), 1.83 (t, 1 H, $J_{3'a,4'}$ ~12.5 Hz, H-3′*a*), and 1.79 (dd, 1 H, $J_{3a,4}$ ~12.0 Hz, H-3*a*).

O-[*Methyl (4,5,7,8-tetra*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosyl)onate*]-*(2→8)-methyl* (trans-*1-propenyl 4,5,7-tri*-O-*acetyl-3-deoxy-α*-D-manno-*octulopyranosid)onate* (**8**). — A solution of **6** (62 mg) and 1,5-cyclooctadienebis(methyldiphenylphosphine)iridium hexafluorophosphate (0.5 mg) in dry oxolane (3 mL) was degassed, placed under O_2-free N_2, and degassed once more. After activation of the catalyst by H_2, the solution was degassed and stirred under N_2 for 90 min at room temperature. After removal of the solvent, the residue was dissolved in dichloromethane (25 mL), extracted with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated. Column chromatography of the residue on silica gel (C, 1:1 toluene–ethyl acetate) gave **8**, colorless syrup, (yield 54 mg, 87%), $[\alpha]_D^{20}$ +84° (*c* 2.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 6.16 (dd, 1 H, $J_{a,b}$ ~12.0, $J^4_{a,c}$ ~1.5 Hz, OC*H*), 5.39 and 5.35 (unresolved signals, 2 H, H-5,5′), 5.27 (dt, 1 H, $J_{b,c}$ ~7.0 Hz, =C*H*CH$_3$), 5.33–5.13 (m, 4 H, including H-4,4′,7,7′), 4.63 (dd, 1 H, $J_{8',a,7'}$ ~2.5, $J_{8'a,8'b}$ ~12.5 Hz, H-8′a), 4.16–4.05 (m, 3 H, including H-6,6′,8′b), 3.82 and 3.81 (s, 6 H, 2 CO_2CH_3), 3.78 (dd, 1 H, $J_{8a,8b}$ ~11.0, $J_{8a,7}$ ~2.5 Hz, H-8a), 3.61 (dd, 1 H, $J_{8b,7}$ ~6.5 Hz, H-8b), 2.23 (dd, 1 H, $J_{3e,3a}$ ~12.5, $J_{3e,4}$ ~5.0 Hz, H-3*e*), 2.15 (dd, 1 H, $J_{3'3e,3'a}$ ~13.0, $J_{3'e,4'}$ ~5.0 Hz, H-3′*e*), 2.15–2.01 (unresolved signals, 2 H, H-3*a*,3′*a*), 2.10 (s, 3 H), 2.09 (s, 3 H), 2.08 (s, 6 H), 1.99 (s, 3 H), 1.98 (s, 3 H) and 1.96 (s, 3 H, CH_3CO), and 1.58 (dd, 3 H, =CHC*H*$_3$).

Anal. Calc. for $C_{35}H_{48}O_{22}$: C, 51.22; H, 5.89. Found: C, 50.71; H, 5.83.

O-[*Methyl (4,5,7,8-tetra*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosyl)onate*]-*(2→8)-methyl 4,5,7-tri*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosonate* (**9**). — A solution of **8** (50 mg) in 4:1 oxolane-water (5 mL) was treated with I_2 (30 mg) for 30 min at room temperature. The solution was diluted with dichloromethane (50 mL), washed sequentially with 5% aqueous $NaHSO_3$, saturated aqueous $NaHCO_3$, and water, and dried ($MgSO_4$). The residue obtained upon evaporation was purified by column chromatography on silica gel (B, 3:2 toluene–ethyl acetate) to afford **9** (37 mg, 79%), colorless syrup, $[\alpha]_D^{20}$ +62° (*c* 1.3, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.68 (d, 1 H, $J_{H\text{-}3a,OH}$ ~1.8 Hz, OH), 5.46 (ddd, 1 H, $J_{4,3e}$ ~4.5, $J_{4,3a}$ ~12.5, $J_{4,5}$ ~3.0 Hz, H-4), 5.44 (unresolved signal, 1 H, H-5′), 5.39 (ddd, 1 H, $J_{4',3'e}$ ~5.0 $J_{4',3'a}$ ~12.5, $J_{4',5'}$ ~3.0 Hz, H-4′), 5.35 (unresolved signal, 1 H, H-5), 5.23 (ddd, 1 H, $J_{7',8'a}$ ~2.0, $J_{7',8'b}$ ~3.6, $J_{7',6'}$ ~10.0 Hz, H-7′), 5.02 (ddd, 1 H, $J_{7,6}$ ~10.0, $J_{7,8a}$ ~3.0, $J_{7,8b}$ ~1.5 Hz, H-7), 4.63 (dd, 1 H, $J_{6,5}$ ~1.5 Hz, H-6), 4.43 (dd, 1 H, $J_{8'a,8'b}$ ~12.3 Hz, H-8′a), 4.17 (dd, 1 H, H-8′b), 4.04 (dd, 1 H, $J_{6',5'}$ ~1.5 Hz, H-6′), 3.89 (s, 3 H) and 3.78 (s, 3 H, CO_2CH_3), 3.66 (dd, 1 H, $J_{8a,8b}$ ~11.0 Hz, H-8a), 3.58 (dd, 1 H, H-8b), 2.46 (dt, 1 H, $J_{3a,3e}$ ~12.5 Hz, H-3*a*), 2.20–1.87 (m, 3 H, including H-3*e*,3′*e*,3′*a*), 2.10 (s, 3 H), 2.09 (s, 3 H), 2.08 (s, 3 H), 2.06 (s, 3 H), 2.05 (s, 3 H), 2.01 (s, 3 H), and 1.97 (s, 3 H, CH_3CO).

Anal. Calc. for $C_{32}H_{44}O_{22}$: C, 49.23; H, 5.68. Found: C, 49.56; H, 5.80.

O-[*Methyl (4,5,7,8-tetra*-O-*acetyl-3-deoxy-β*-D-manno-*2-octulopyranosyl)onate*]-*(2→8)-methyl (1-propenyl 4,5,7-tri*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosid)onate* (**14**). — A solution of **12** (68 mg) in dry oxolane (3 mL) was treated with 1,5-cyclooctadienebis(methyldiphenylphosphine)iridium hexafluorophosphate

(0.3 mg) as described for **8**. The product was purified by column chromatography on silica gel (B, 2:1 toluene–ethyl acetate) to give **14** (63 mg, 93%), colorless syrup, $[\alpha]_D^{20}$ +72° (*c* 1.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 6.25 (dd, 1 H, $J_{a,b}$ ~12.5, $J^4_{a,e}$ ~1.5 Hz, –OCH=), 5.38 (unresolved signal, 1 H, H-5), 5.35 (ddd, 1 H, $J_{4,5}$ ~3.0, $J_{4,3e}$ ~5.0, $J_{4,3a}$ ~12.0 Hz, H-4), 5.29 (unresolved signal, 1 H, H-5′), 5.20 (dt, 1 H, $J_{b,c}$ ~7.0 Hz, =C*H*CH$_3$), 5.17–5.10 (m, 2 H, H-7,7′), 4.90 (ddd, 1 H, $J_{4',3'e}$ ~4.5, $J_{4',3'a}$ ~13.0, $J_{4',5'}$ ~3.0 Hz, H-4′), 4.37 (dd, 1 H, $J_{8'a,8'b}$ ~12.0, $J_{8'a,7'}$ ~2.5 Hz, H-8′a), 4.30 (dd, 1 H, $J_{8'b,7'}$ ~4.5 Hz, H-8′b), 4.14 (dd, 1 H, $J_{8a,7}$ ~2.5, $J_{8a,8b}$ ~11.0 Hz, H-8a), 4.10 and 4.09 (dd, 2 H, *J* ~9.0, *J* ~1.0 Hz, H-6,6′), 3.83 (s, 6 H, 2 CO_2CH_3), 3.50 (dd, 1 H, $J_{8b,7}$ ~5.0 Hz, H-8b), 2.33 (dd, 1 H, $J_{3'e,3'a}$ ~12.5 Hz, H-3′*e*), 2.21 (dd, 1 H, $J_{3e,3a}$ ~12.5 Hz, H-3*e*), 2.18–1.95 (m, 2 H, H-3*a*,3′*a*), 2.11 (s, 3 H), 2.10 (s, 6 H), 2.05 (s, 3 H), 2.03 (s, 3 H), 2.01 (s, 3 H) and 2.00 (s, 3 H, CH_3CO), and 1.55 (dd, 1 H, =CH–C*H*$_3$).

Anal. Calc. for $C_{35}H_{48}O_{22}$: C, 51.22; H, 5.89. Found: C, 50.87; H, 5.86.

O-[*Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-β-*D-manno-*octulopyranosyl)onate*]-*(2→8)-methyl 4,5,7-tri-*O*-acetyl-3-deoxy-α-*D-manno-*2-octulopyranosonate* (**15**). — A solution of **14** (30 mg) in 4:1 oxolane–water (2 mL) was treated with I_2 (10 mg) as described for **9**. Purification of the product on a column of silica gel (B, 2:1 toluene–ethyl acetate) gave **15** (20 mg, 70%), syrup, $[\alpha]_D^{20}$ +57° (*c* 0.9, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.36 (dd, 1 H, $J_{4,3e}$ ~4.5, $J_{4,3a}$ ~13.0 Hz, H-4), 5.36 (unresolved signal, 1 H, H-5), 5.28 (unresolved signal, 1 H, H-5′), 5.16 (ddd, 1 H, $J_{7,6}$ ~9.5, $J_{7,8a}$ ~4.5, $J_{7,8b}$ ~2.5 Hz, H-7), 5.10 (ddd, 1 H, $J_{7',6'}$ ~10.0, $J_{7',8'a}$ ~2.5, $J_{7',8'b}$ ~4.5 Hz, H-7′), 4.86 (ddd, 1 H, $J_{4',5'}$ ~2.5, $J_{4',3'e}$ ~4.5, $J_{4',3'a}$ ~13.0 Hz, H-4′), 4.53 (d, 1 H, $J^4_{OH,3a}$ ~2.5 Hz, OH), 4.43 (dd, 1 H, $J_{8'a,8'b}$ ~13.0 Hz, H-8′a), 4.39 (dd, 1 H, $J_{6',5'}$ ~1.0 Hz, H-6′), 4.26 (dd, 1 H, H-8′b), 4.10 (dd, 1 H, $J_{6,5}$ ~1.5 Hz, H-6), 3.99 (dd, 1 H, $J_{8a,8b}$ ~11.5 Hz, H-8a), 3.85 (s, 3 H) and 3.84 (s, 3 H, CO_2CH_3), 3.44 (dd, 1 H, H-8b), 2.39 (dd, 1 H, $J_{3'e,3'a}$ ~13.0 Hz, H-3′*e*), 2.36 (dt, 1 H, $J_{3e,3a}$ ~13.0 Hz, H-3*a*), 2.17–1.92 (m, 2 H, H-3*e*,3′*a*), 2.12 (s, 6 H), 2.10 (s, 3 H), 2.04 (s, 3 H), 2.01 (s, 3 H), and 1.99 (s, 6 H, 2 CH_3CO).

Anal. Calc. for $C_{32}H_{44}O_{22}$: C, 49.23; H, 5.68. Found: C, 50.07; H, 5.72.

O-[*Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-*D-manno-*2-octulopyranosyl)onate*]-*(2→8)-methyl 2,4,5,7-tetra-*O*-acetyl-3-deoxy-α-*D-manno-*2-octulopyranosonate* (**10**). — To a solution of **9** (60 mg) in dry pyridine (5 mL) was added acetic anhydride (0.5 mL) at 0°. After stirring of the solution for 15 h at room temperature, the solvent was removed *in vacuo*. The residue was dissolved in dichloromethane (50 mL), and the solution washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated. Purification of the residue on a column of silica gel (A, 1:1 toluene–ethyl acetate) afforded **10** (62 mg, 100%), colorless syrup, $[\alpha]_D^{20}$ +90° (*c* 0.86, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.45 (dd, 1 H, $J_{5,6}$ ~1.5, $J_{5,4}$ ~3.0 Hz, H-5), 5.35 (unresolved signal, 1 H, H-5′), 5.32 (ddd, 1 H, $J_{4,3e}$ ~5.5, $J_{4,3a}$ 12.5 Hz, H-4), 5.25 (ddd, 1 H, $J_{7',8'a}$ 2.5, $J_{7',8'b}$ ~5.0, $J_{7',6'}$ ~9.5 Hz, H-7′), 5.18 (ddd, 1 H, $J_{4',5'}$ ~3.0, $J_{4',3'e}$ ~5.0, $J_{4',3'a}$ ~12.0 Hz, H-4′), 5.02 (dt, 1 H, $J_{7,6}$ ~10.0, $J_{7,8a}$ ~$J_{7,8b}$ ~2.5 Hz, H-7), 4.56 (dd, 1 H, $J_{8'a,8'b}$ ~12.5 Hz, H-8′a), 4.34 (dd, 1 H, H-6), 4.14

(dd, 1 H, H-8'b), 4.07 (dd, 1 H, $J_{6',5'}$ ~1.5 Hz, H-6'), 3.79 (s, 3 H) and 3.77 (s, 3 H, CO_2CH_3), 3.70 (dd, 1 H, $J_{8a,8b}$ ~12.0 Hz, H-8a), 3.62 (dd, 1 H, H-8b), 2.33–1.96 (m, 4 H, H-3*e*,3'*e*,3*a*,3'*a*), 2.11 (s, 3 H), 2.10 (s, 3 H), 2.08 (s, 3 H), 2.06 (s, 3 H), 2.05 (s, 3 H), 2.03 (s, 3 H), 2.01 (s, 3 H), and 1.97 (s, 3 H, CH_3CO).

Anal. Calc. for $C_{34}H_{46}O_{23}$: C, 49.63; H, 5.64. Found: C, 49.64; H, 5.65.

O-[*Methyl (4,5,7,8-tetra*-O-*acetyl*-α-D-manno-*2-octulopyranosyl)onate*]-(2→8)-*methyl (allyl 4,5,7-tri*-O-*acetyl*-D-manno-*2-octulopyranosyl bromide)onate* (**11**). — To a solution of **10** (76.4 mg) in dry dichloromethane (25 mL) was added at −10° $TiBr_4$ (200 mg). The solution was kept for 15 h at 4°, diluted with chloroform (100 mL), and quickly extracted with ice-cold saturated aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$) and evaporated to give **11** (77 mg, 98%), as a slightly yellow syrup, which was immediately employed in the glycosylation reaction.

O-[*Methyl (4,5,7,8-tetra*-O-*acetyl-3-deoxy*-α-D-manno-*2-octulopyranosyl)onate*]-(2→8)-O-[*methyl (4,5,7-tri*-O-*acetyl-3-deoxy*-β-D-manno-*2-octulopyranosyl)onate*]-(2→4)-*methyl (allyl 7,8*-O-*carbonyl-3-deoxy*-α-D-manno-*2-octulopyranosid)onate* (**22**) *and* O-[*methyl (4,5,7,8-tetra*-O-*acetyl-3-deoxy*-α-D-manno-*2-octulopyranosyl)onate*]-(2→8)-O-[*methyl (4,5,7-tri*-O-*acetyl-3-deoxy*-α-D-manno-*2-octulopyranosyl)onate*]-(2→4)-*methyl (allyl 7,8*-O-*carbonyl-3-deoxy*-α-D-manno-*2-octulopyranosid)onate* (**23**). — A solution of **11** (77 mg, 0.09 mmol) in dry acetonitrile (3 mL) was added during 3 h at 0° to a suspension of **21** (122 mg, 0.38 mmol), mercury(II) cyanide (60 mg, 0.24 mmol), and molecular sieves 4A (0.5 g) in dry acetonitrile (5 mL) under dry N_2. The mixture was stirred for 72 h at room temperature, diluted with dichloromethane (50 mL), and filtered over Celite. The filtrate was washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated. The residue was subjected to column chromatography on silica gel (B, 3:2→1:2 toluene–ethyl acetate). Pooling and evaporation of the fractions containing the component having R_F 0.38 (1:2 toluene–ethyl acetate) gave **22** (8.9 mg, 9%), syrup, $[\alpha]_D^{20}$ +61° (*c* 1.3, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.88 (m, 1 H, =CH–), 5.35 (unresolved signal, 1 H) and 5.29 (unresolved signal, 1 H, H-5',5''), 5.26 (dq, 1 H, $=CH_{2\ trans}$), 5.17 (dq, 1 H, $=CH_{2\ cis}$), 5.30–5.11 (m, 4 H, including H-7,7',4'',7''), 4.87 (dd, 1 H, $J_{8a,8b}$ ~9.0, $J_{8a,7}$ ~7.0 Hz, H-8a), 4.82 (ddd, 1 H, $J_{4',5'}$ ~3.0, $J_{4',3'e}$ ~4.5, $J_{4',3'a}$ ~12.5 Hz, H-4'), 4.58 (t, 1 H, $J_{8b,7}$ ~9.0 Hz, H-8b), 4.54 (dd, 1 H, $J_{8''a,8''b}$ ~12.5, $J_{8''a,7''}$ 2.5 Hz, H-8''a), 4.18 (dd, 1 H, $J_{8''b,7''}$ ~4.5 Hz, H-8''b), 4.18–3.98 (m, 8 H, including H-4,5,6,6',8'a,6'', and OCH_2), 3.88 (s, 3 H) and 3.80 (s, 6 H, 2 CO_2CH_3), 3.53 (dd, 1 H, $J_{8'a,8'b}$ ~10.5, $J_{8'b,7'}$ ~8.5 Hz, H-8'b), 2.38 (dd, 1 H, $J_{3'e,3'a}$ ~13.0 Hz, H-3'*e*), 2.37 (d, 1 H, $J_{OH,5}$ ~2.5, OH), 2.22–1.95 (m, 5 H, including H-3'*e*,3*a*,3'*a*,3''*e*,3''*a*), 2.11 (s, 6 H), 2.09 (s, 3 H), 2.05 (s, 3 H), 2.03 (s, 3 H), 1.99 (s, 3 H), and 1.97 (s, 3 H, CH_3CO).

Further elution of the column with 1:2 toluene–ethyl acetate gave **23** (21 mg, 21%), colorless syrup, $[\alpha]_D^{20}$ +75° (*c* 0.34, chloroform); ^{1}H-n.mr. ($CDCl_3$): δ 5.88 (m, 1 H, =CH–), 5.38 (unresolved signal, 1 H, H-5'), 5.34–5.14 (m, 7 H, including H-4',7',4'',5'',7'', and $=CH_2$), 4.94 (ddd, 1 H, $J_{7,8a}$ ~7.0, $J_{7,8b}$ ~8.0, $J_{7,6}$ ~3.0 Hz, H-7), 4.80 (dd, 1 H, $J_{8a,8b}$ ~9.0 Hz, H-8a), 4.55 (t, 1 H, H-8b), 4.53 (dd, 1 H, $J_{8''a,7''}$

~2.5, $J_{8''a,8''b}$ ~12.0 Hz, H-8''a), 4.18 (dd, 1 H, $J_{8''b,7''}$ ~6.0 Hz, H-8''b), 4.17 (ddd, 1 H, $J_{4,5}$ ~3.0 Hz, H-4), 4.16 (dd, 1 H, $J_{6',5'}$ ~1.5 Hz, H-6'), 4.06 (dd, 1 H, $J_{6'',7''}$ ~10.0, $J_{6'',5''}$ ~1.5 Hz, H-6''), 4.01–3.97 (m, 2 H, OCH_2), 3.96–3.86 (m, 2 H, H-5,6), 3.83 (s, 3 H), 3.81 (s, 3 H) and 3.79 (s, 3 H, CO_2CH_3), 3.68–3.61 (m, 2 H, H-8'a,8'b), 2.83 (d, 1 H, $J_{OH,5}$ ~3.5 Hz, OH), 2.22 (dd, 1 H, $J_{3'e,3'a}$ ~13.0, $J_{3'e,4'}$ ~5.0 Hz, H-3'*e*), 2.18–1.96 (m, 5 H, including H-3*e*,3*a*,3'*a*,3''*e*,3''*a*), 2.11 (s, 6 H), 2.09 (s, 3 H), 2.06 (s, 3 H), 2.03 (3 H), 1.98 (s, 3 H), and 1.97 (s, 3 H, CH_3CO).

Anal. calc. for $C_{45}H_{60}O_{30}$: C, 50.00; H, 5.59. Found: C, 50.10; H, 5.78.

O-[*Sodium (3-deoxy-α-*D-manno-*2-octulopyranosyl)onate*]-*(2→8)*-O-[*sodium (3-deoxy-α-*D-manno-*2-octulopyranosyl)onate*]-*(2→4)-sodium (allyl 3-deoxy-α-*D-manno-*2-octulopyranosid)onate* (**24**). — A solution of **23** (27.5 mg) in dry methanol (5 mL) was stirred with 0.1M methanolic sodium methoxide (0.5 mL) for 2 h at room temperature. The solution was made neutral by addition of Dowex 50 (H^+) cation-exchange resin, filtered, and evaporated. A solution of the residue (19.3 mg) in water (5 mL) was stirred with 0.1M aqueous NaOH (1 mL) for 3 h at room temperature. The pH of the solution was adjusted to 8.5 by addition of Dowex 50 (H^+) resin. The solution was filtered and evaporated, and the residue was subjected to chromatography on Bio-Gel P-2 to give 18.8 mg (97%) of **24**, amorphous powder, $[\alpha]_D^{20}$ +62° (*c* 0.4, water); ^{1}H-n.m.r. (D_2O): δ 5.98 (m, 1 H, =CH–), 5.33 (dq, 1 H, $=CH_{2\ trans}$), 5.21 (dq, 1 H, $=CH_{2\ cis}$), 4.21–3.51 (m, 20 H, including H-4,5,6,7,8a,8b,4',5',6',7',8'a,8'b,4'',5'',6'',7'',8''a,8''b, and OCH_2), 2.12 (dd, 1 H, $J_{3'e,4'}$ ~4.5, $J_{3'e,3'a}$ ~12.5 Hz, H-3'*e*), 2.05 (dd, 1 H, $J_{3''e,3''a}$ ~12.5, $J_{3''e,4''}$ ~5.0 Hz, H-3''*e*), 2.02 (dd, 1 H, $J_{3e,3a}$ ~12.0, $J_{3a,4}$ ~5.0 Hz, H-3*e*), 1.90 (t, 1 H, $J_{3a,4}$ ~12.0 Hz, H-3*a*), and 1.80 (t, 2 H, $J_{3'a,4'} \sim J_{3''a,4''}$ ~12.5 Hz, H-3'*a*,3''*a*).

Copolymerization[33]. — A solution of **7** (28.1 mg), acrylamide (14.0 mg), and *N,N,N',N'*-tetramethylethylenediamine (2 μL) in water (1 mL) was degassed for 30 min. After addition of $(NH_4)_2S_2O_8$ (1 mg), the solution was kept for 18 h at 4°. The product was isolated by column chromatography on Sephadex G-50 (2.6 × 100 cm; eluent, 0.01M aqueous $NaHCO_3$) and desalted on Bio-Gel P-2 (2.6 × 100 cm), to yield **25** (14.7 mg), amorphous powder, $[\alpha]_D^{20}$ +19° (*c* 0.5, water). The copolymers **26** (21 mg of **13** and 12.0 mg of acrylamide) and **27** (8.7 mg of **24** and 4.0 mg of acrylamide) were prepared in a similar manner to yield 13.7 mg of **26**, $[\alpha]_D^{20}$ +18° (*c* 0.5, water), and 6.0 mg of **27**, $[\alpha]_D^{20}$ +3.8° (*c* 0.56, water).

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