Carbohydrate Research, 194 (1989) 145–154
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

SYNTHESIS OF POLY(ACRYLAMIDE) COPOLYMERS CONTAINING 3,5-DIDEOXY-D-*arabino*-2-OCTULOPYRANOSYLONIC ACID (5-DEOXY-KDO) RESIDUES

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(Received November 29th, 1988; accepted for publication, January 11th, 1989)

ABSTRACT

The monosaccharides, sodium (allyl 3,5-dideoxy-α- and -β-D-*arabino*-2-octulopyranosid)onate, and the disaccharide, *O*-[sodium (3-deoxy-α-D-*manno*-2-octulopyranosyl)onate]-(2→4)-sodium (allyl 3,5-dideoxy-α-D-*arabino*-2-octulopyranosid)onate, corresponding to the 5-deoxy derivatives of Kdo-mono- and -di-saccharides, were synthesized *via* mercury(II) cyanide-promoted glycosylation of allyl alcohol or methyl (allyl 7,8-*O*-carbonyl-3,5-dideoxy-α-D-*arabino*-2-octulopyranosid)onate with Kdo or 5-deoxy-Kdo bromide derivatives, respectively. Removal of the protecting groups and subsequent copolymerization of the allyl glycosides with acrylamide gave artificial antigens suitable for the determination of epitope specificities displayed by monoclonal antibodies directed against the Kdo-region of enterobacterial lipopolysaccharides.

INTRODUCTION

3-Deoxy-D-*manno*-2-octulosonic acid (Kdo) has been originally discovered[1] as a characteristic component of enterobacterial lipopolysaccharides (LPS), constituting the link between "Lipid A" and the heptose-containing, inner-core oligosaccharides[2,3]. Recent structural investigations of a number of Rough-mutant lipopolysaccharides showed the presence of an α-(2→4)-linked Kdo-disaccharide[4–11]. Previously, we reported the synthesis of poly(acrylamide) copolymers[12,13] substituted by α- and β-Kdo*p*, α- and β-Kdo*p*-(2→4)-α-Kdo*p*, α- and β-Kdo*p*-(2→4)-β-Kdo*p*, α- and β-Kdo*p*-(2→8)-α-Kdo*p*, α-Kdo*p*-(2→8)-α-Kdo*p*-(2→4)-α-Kdo*p*, and α-Kdo*p*-(2→4)-α- and -β-Kdo*p*-(2→4)-α-Kdo*p* residues[14–18].

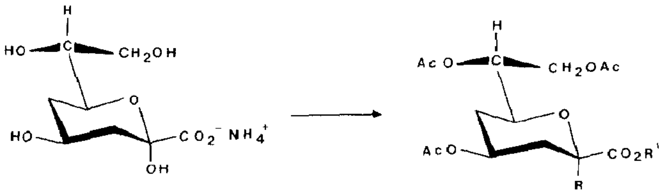
By use of these antigens, the epitope specificities of two monoclonal antibodies directed against the Kdo-region of enterobacterial LPS could be determined[18,19]. One of them (clone 25) recognizes, in enterobacterial LPS, an α-(2→4)-linked Kdo-disaccharide, whereas the other (clone 20) reacts with a terminal Kdo*p*-

0008-6215/89/$ 03.50

residue in the α-D-anomeric configuration. With the intention to investigate the involvement of the OH group attached to C-5 of the Kdo-residues in the polar binding-sites of these antibodies, we report herein the synthesis of poly(acrylamide) copolymers substituted by α-5-deoxy-Kdo*p*, β-5-deoxy-Kdo*p*, and α-Kdo*p*-(2→4)-α-5-deoxy-Kdo*p*-residues.

RESULTS AND DISCUSSION

Ammonium 3,5-dideoxy-D-*arabino*-2-octulosonate (**1**) was prepared according to the procedure of Cherniak *et al.*[20], *i.e.*, condensation of oxaloacetic acid with 2-deoxy-D-*erythro*-pentose. Acetylation of **1** (acetic anhydride–pyridine–4-dimethylaminopyridine) gave **2**, which was converted into the syrupy methyl ester derivative **3** by reaction with diazomethane (47% yield), similar to the procedures employed for the preparation of Kdo-derivatives[21].



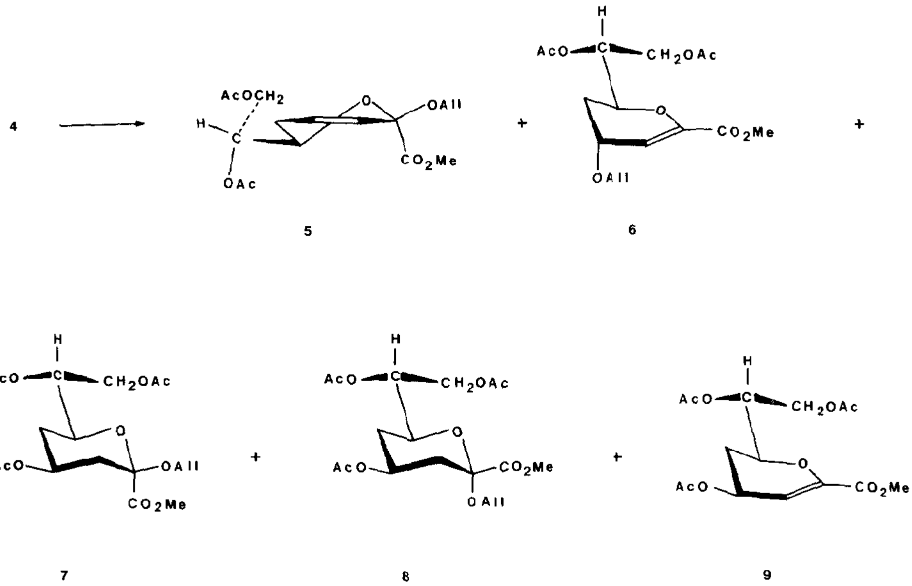
1

2 R = OAc , R' = H
3 R = OAc , R' = Me
4 R = Br , R' = Me

Treatment of **3** with titanium tetrabromide in dichloromethane[22] gave a 96% yield of the unstable bromide derivative **4** which was immediately glycosylated with allyl alcohol in the presence of mercury(II) cyanide in nitromethane. Thus, a 1:2 mixture of the allyl β- (**7**) and α-glycoside (**8**) (homogeneous by t.l.c., R_F 0.46, 2:1 toluene–ethyl acetate) was obtained in 57% yield. The mixture was separated from the glycal ester derivatives **6** (19%) and **9** (20%), and the 3,4-dehydro derivative **5** (2%) by column chromatography on silica gel. The D-*ribo* configuration of **6** and the D-*arabino* configuration of **9** was assigned on the basis of the values of the vicinal coupling constants $J_{4,5a}$ ($J_{4,5a}$ ~4.0 Hz for **6** and $J_{4,5a}$ ~7.5 Hz for **9**), similar to the values observed for acetylated 1,5-anhydro-2-deoxy-hex-1-enitols[23,24].

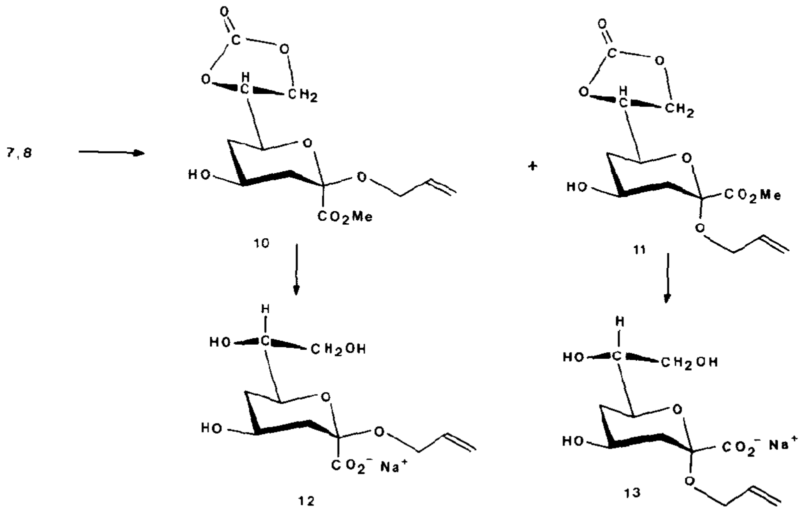
Separation of the mixture of anomers of **7** and **8** was achieved *via* Zemplén deacylation and subsequent conversion of the *O*-deacetylated compounds into the 7,8-*O*-carbonyl derivatives **10** and **11**, respectively, by the action of 4-nitrophenyl chloroformate in pyridine[25] in 50% yield.

The assignment of the anomeric configuration of the isomers was based on the ^{1}H-n.m.r. chemical-shift values of the signals attributable to H-4. Thus, similar



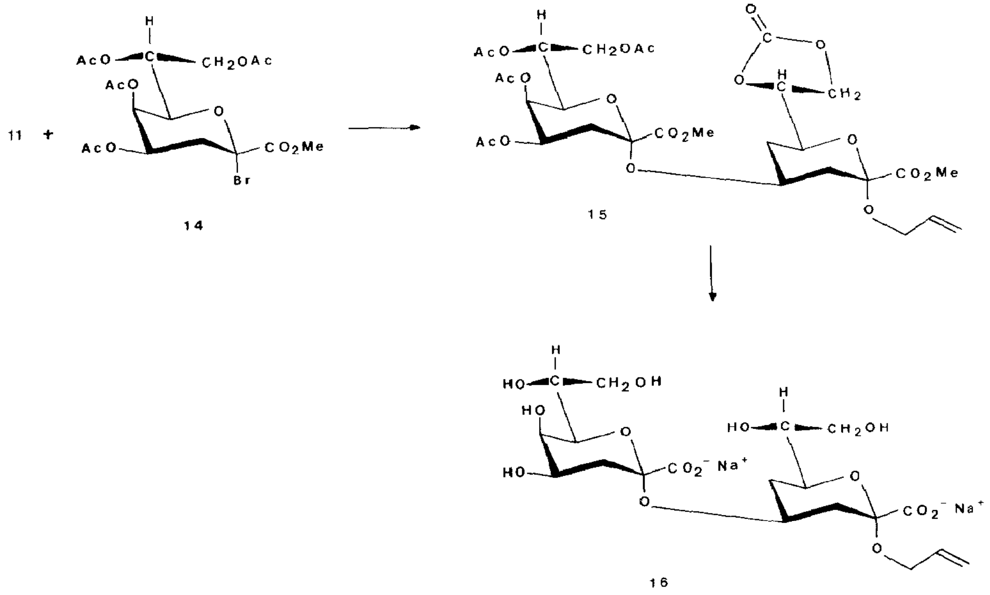
to the per-*O*-acetylated Kdo-derivatives[26], the signals for H-4 of the α anomer was shifted downfield relative to the corresponding signal of the β anomer (δ 4.15 for **8** *vs.* δ 3.80 for **7**). The assignment of the α–anomeric configuration of **11** was also substantiated by the smaller chemical-shift difference between the equatorial and axial protons at C-3 ($\delta_{H\text{-}3e} - \delta_{H\text{-}3a}$ ~0.85 for **11**, $\delta_{H\text{-}3e} - \delta_{H\text{-}3a}$ ~1.02 for **10**) (ref. 26).

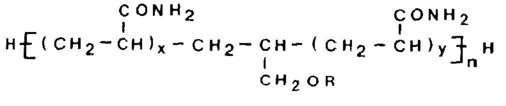
The products were deacylated with methanolic sodium methoxide and deesterified with aqueous sodium hydroxide to afford sodium (allyl 3,5-dideoxy-β-D-*arabino*-2-octulopyranosid)onate (**12**) and sodium (allyl 3,5-dideoxy-α-D-*arabino*-2-octulopyranosid)onate (**13**) in quantitative yield.



For the synthesis of **16**, the glycosyl acceptor **11** was glycosylated with 1.85 molar equivs. of the bromide derivative **14** (ref. 22) in nitromethane and mercury(II) cyanide as promoter. The α-(2→4)-linked disaccharide derivative **15** was obtained in 95% yield. The α-anomeric configuration of the Kdo-residue was assigned on the basis of the chemical-shift value of H-4′ (δ 5.25) which is indicative of the α-anomeric configuration of per-*O*-acetylated Kdo-derivatives[27]. Removal of the protecting group *via* Zemplén deacylation and saponification of the methyl ester groups gave a quantitative yield of *O*-[sodium (3-deoxy-α-D-*manno*-2-octulopyranosyl)onate]-(2→4)-sodium (allyl 3,5-dideoxy-α-D-*arabino*-2-octulopyranosid)onate (**16**) in quantitative yield.

The ^{13}C-n.m.r. chemical-shift data for **12**, **13**, and **16** compare favorably with values reported by Cherniak *et al.*[20] for **1** (α-pyranose), as well as the allyl glycoside of the α-(2→4)-α-linked Kdo-disaccharide[28].





$$H\!\left[(CH_2-\underset{}{\overset{CONH_2}{CH}})_x-CH_2-\underset{CH_2OR}{CH}-(CH_2-\overset{CONH_2}{CH})_y\right]_n H$$

17 R = 5-deoxy-β-KDO*p*-(2→

18 R = 5-deoxy-α-KDO*p*-(2→

19 R = α-KDO*p*-(2→4)-5-deoxy-α-KDO*p*-(2→

The allyl glycosides **12**, **13**, and **16** were copolymerized with acrylamide in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine and ammonium peroxosulfate[29].

The water-soluble, linear copolymers **17**, **18**, and **19** were isolated (with recovery of unreacted allyl glycoside) and desalted by passage through Sephadex G-50 and Bio-Gel P-2, respectively. The immunochemical results obtained with these artificial antigens will be published separately[19].

EXPERIMENTAL

General methods. —These were as described previously[14]. ^{13}C-N.m.r. spectra were recorded with a Bruker WM 250 instrument, at 62.9 MHz, for solutions in D_2O at 24°, by use of a 32 K data set of memory and a spectral width of 12 kHz. Chemical shifts are given from the signal of tetramethylsilane (shift set at δ 67.40 relative to the signal of 1,4-dioxane in D_2O). 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).

*Methyl (2,4,7,8-tetra-O-acetyl-3,5-dideoxy-α-*D-arabino-*2-octulopyranos)onate* (**3**). — Acetic anhydride (5 mL) was added dropwise at 4° to a solution of **1** (2.3 g) and 4-dimethylaminopyridine (100 mg) in pyridine (50 mL). The mixture was stirred for 18 h, methanol (8 mL) was added dropwise at 4°, and the solution was stirred for 1 h at room temperature and concentrated. The residue was dissolved in dichloromethane (150 mL), and extracted with saturated aqueous $NaHSO_4$ and water. The organic layer was dried ($MgSO_4$) and taken to dryness. A solution of the residue (1.9 g of **2**) in ether was treated with freshly prepared diazomethane. The residue obtained upon evaporation was subjected to column chromatography on silica gel (C, 4:1→2:1 toluene–ethyl acetate) to give **3** (1.9 g, 47%), syrup, $[\alpha]_D^{20}$ +90° (*c* 1.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.19 (ddt, 1 H, $J_{4,3e}$ ~5.0, $J_{4,3a}$ ~11.5, $J_{4,5a}$ ~12.0, $J_{4,5e}$ ~5.0 Hz, H-4), 5.09 (ddd, 1 H, $J_{7,6}$ ~7.5, $J_{7,8a}$ ~3.0, $J_{7,8b}$ ~5.0 Hz, H-7), 4.45 (dd, 1 H, $J_{8a,8b}$ ~12.0 Hz, H-8a), 4.16 (dd, 1 H, H-8b), 4.04 (ddd, 1 H, $J_{6,5e}$ ~2.0, $J_{6,5a}$ ~12.0 Hz, H-6), 3.78 (s, 3 H, CH_3OCO), 2.46 (ddd, 1 H, $J_{3e,5e}$ ~2.0, $J_{3a,3e}$ ~ −13.0 Hz, H-3*e*), 2.19 (dddd, 1 H, $J_{5e,5a}$ ~ −12.0 Hz, H-5*e*), 2.13 (s, 3 H), 2.08 (s, 3 H), 2.06 (s, 3 H) and 2.05 (s, 3 H, 4 CH_3CO), 1.78 (dd, 1 H, H-3*a*), and 1.50 (dt, 1 H, H-5*a*).

Anal. Calc. for $C_{17}H_{24}O_{11}$: C, 50.49; H, 5.98. Found: C, 50.53; H, 6.07.

*Methyl (4,7,8-tri-O-acetyl-3,5-dideoxy-*D-arabino-*2-octulopyranosyl bromide)-onate* (**4**). — Titanium tetrabromide (10 g) was added at −10° to a solution of **3** (7.5 g) in dichloromethane (30 mL). The solution was kept for 15 h at 4°, diluted with chloroform (50 mL), and quickly extracted with ice-cold, saturated aqueous $NaHCO_3$ solution. The organic layer was dried ($MgSO_4$) and evaporated to give **4** (7.6 g, 96%), a yellow syrup, which was immediately used for glycosylation.

*Methyl (allyl 7,8-di-O-acetyl-3,4,5-trideoxy-β-*D-erythro-*2-octulo-3-enopyranosid)onate* (**5**), *methyl (7,8-di-O-acetyl-4-O-allyl-2,6-anhydro-2,3,5-trideoxy-*D-ribo-*2-octen)onate* (**6**), *methyl [4,7,8-tri-O-acetyl-3,5-dideoxy-β-* (**7**) *and -α-*D-arabino-*2-octulopyranosid]onate* (**8**) *and methyl (4,7,8-tri-O-acetyl-2,6-anhydro-2,5-dideoxy-*D-arabino-*2-octen)onate* (**9**). — A solution of **4** (7.6 g, 1.78 mmol) in dry nitromethane (5 mL) was added dropwise to a suspension of allyl alcohol (5 mL, 7.5 mmol), mercury(II) cyanide (5.05 g, 20 mmol), and molecular sieve 3A in nitromethane (5 mL) during 30 min at −10°. The mixture was stirred for 15 h at room temperature, then diluted with dichloromethane (100 mL), filtered through Celite, and washed successively with saturated aqueous $NaHCO_3$ solution, 10% aqueous KI solution, and water. The organic layer was dried (Na_2SO_4) and evaporated. Purification of the residue on silica gel (800 × 25 mm, 3:1 toluene–ethyl acetate) gave **5** (122 mg, 2%), syrup, $[\alpha]_D^{20}$ −21° (*c* 1.2, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 6.21 (dddd, 1 H, $J_{4,5e}$ ~5.7, $J_{4,5a}$ ~2.3, $J_{4,3}$ ~10.1, $J_{4,6}$ ~0.5 Hz, H-4), 5.90 (m, 1 H, =CH–), 5.82 (ddd, 1 H, $J_{3,5e}$ ~1.4, $J_{3,5a}$ ~2.8 Hz, H-3), 5.28 (dq, 1 H, =CH_2-*trans*), 5.16 (dq, 1 H, CH_2-*cis*), 5.14 (ddd, 1 H, $J_{7,8a}$ ~2.8, $J_{7,8b}$ ~6.3, $J_{7,6}$ ~ 7.1 Hz, H-7), 4.51 (dd, 1 H, $J_{8a,8b}$ ~ −12.2 Hz, H-8a), 4.33 (dd, 1 H, H-8b), 4.21 (dddd, 1 H, $J_{6,5e}$ ~3.8, $J_{6,5a}$ ~10.0 Hz, H-6), 4.17–3.96 (m, 2 H, OCH_2), 3.78 (s, 3 H, CH_3OCO), 2.20 (ddt, 1 H, $J_{5a,5e}$ ~ −17.3 Hz, H-5*a*), 2.10 (s, 3 H) and 2.08 (s, 3 H, 2 CH_3CO), and 2.07 (dddd, 1 H, H-5*e*).

Anal. Calc. for $C_{16}H_{22}O_8$: C, 56.14; H, 6.48. Found: C, 55.90; H, 6.51.

Further elution of the column gave **6** (1.16 g, 19%), syrup, $[\alpha]_D^{20}$ +82° (*c* 1.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 6.22 (dd, 1 H, $J_{3,4}$ ~5.5, $J_{3,5e}$ ~2.0 Hz, H-3), 5.93 (m, 1 H, =CH–), 5.31 (dq, 1 H, =CH_2-*trans*), 5.29–5.22 (m, 1 H, H-7), 5.21 (dq, 1 H, =CH_2-*cis*), 4.51 (dd, 1 H, $J_{8a,7}$ ~3.5, $J_{8a,8b}$ ~ −12.0 Hz, H-8a), 4.26 (dd, 1 H, $J_{8b,7}$ ~6.0 Hz, H-8b), 4.17 (ddd, 1 H, $J_{6,7}$ ~6.0, $J_{6,5a}$ ~12.0, $J_{6,5e}$ ~2.5 Hz, H-6), 4.10–4.06 (m, 2 H, OCH_2), 4.00 (ddd, 1 H, $J_{4,5a}$ ~4.0, $J_{4,5e}$ ~5.5 Hz, H-4), 3.80 (s, 3 H, CH_3OCO), 2.11 (s, 3 H) and 2.07 (s, 3 H, 2 CH_3CO), 2.08 (dddd, 1 H, $J_{5e,5a}$ ~ −14.0 Hz, H-5*e*), and 1.65 (dddd, 1 H, H-5*a*).

Anal. Calc. for $C_{16}H_{22}O_8$: C, 5.14; H, 6.48. Found: C, 55.77; H, 6.28.

Further elution of the column afforded a mixture of **7** and **8** (4.1 g, 57%), syrup, ^{1}H-n.m.r. ($CDCl_3$) for **8**: δ 5.89 (m, 1 H, =CH–), 5.30 (dq, 1 H, =CH_2-*trans*), 5.23 (dddd, 1 H, $J_{4,3a}$ ~12.0, $J_{4,3e}$ ~4.5, $J_{4,5a}$ ~12.0, $J_{4,5e}$ ~4.5 Hz, H-4), 5.19 (dq, 1 H, =CH_2-*cis*), 5.10 (ddd, 1 H, $J_{7,6}$ ~8.0, $J_{7,8a}$ ~3.0, $J_{7,8b}$ ~5.0 Hz, H-7), 4.55 (dd, 1 H, $J_{8a,8b}$ ~ −12.5 Hz, H-8a), 4.21 (dd, 1 H, H-8b), 4.08–4.00 (m, 1 H, OCH_2), 3.97 (ddd, 1 H, $J_{6,5e}$ ~4.5, $J_{6,5a}$ ~12.0 Hz, H-6), 3.96–3.88 (m, 1 H, OCH_2), 3.78 (s, 3 H, CH_3OCO), 2.40 (ddd, 1 H, $J_{3e,3a}$ ~ −12.5, $J_{3e,5e}$ ~1.5 Hz, H-3*e*), 2.12 (dddd, 1 H, $J_{5e,5a}$ ~ −12.0 Hz, H-5*e*), 2.08 (s, 3 H), 2.07 (s, 3 H) and 2.04 (s, 3 H, 3 CH_3CO), 1.68 (dd, 1 H, H-3*a*), and 1.39 (dt, 1 H, H-5*a*).

Anal. Calc. for $C_{18}H_{25}O_{10}$: C, 53.86; H, 6.28. Found: C, 53.78; H, 6.50.

Final elution of the column with 2:1 toluene–ethyl acetate gave **9** (1.22 g, 20%), syrup, $[\alpha]_D^{20}$ −1.9° (*c* 1.4, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 6.06 (dd, 1 H, $J_{3,4}$ ~3.0, $J_{3,5e}$ ~0.7 Hz, H-3), 5.46 (ddd, 1 H, $J_{4,5a}$ ~7.5, $J_{4,5e}$ ~6.5 Hz, H-4), 5.25 (ddd, 1 H, $J_{7,6}$ ~7.8, $J_{7,8a}$ ~3.0, $J_{7,8b}$ ~5.0 Hz, H-7), 4.55 (dd, 1 H, $J_{8a,8b}$ ~ −12.3 Hz, H-8a), 4.38 (ddd, 1 H, $J_{6,5a}$ ~9.0, $J_{6,5e}$ ~3.0, H-6), 4.21 (dddd, 1 H, $J_{5e,5a}$ ~ −13.8 Hz, H-5*e*), 2.09 (s, 3 H), 2.075 (s, 3 H), and 2.05 (s, 3 H, 3 CH_3CO), and 1.90 (ddd, 1 H, H-5*a*).

Anal. Calc. for $C_{15}H_{19}O_9$: C, 52.48; H, 5.58. Found: C, 52.29; H, 5.47.

*Methyl (allyl 7,8-O-carbonyl-3,5-dideoxy-β-*D-arabino*-2-octulopyranosid)-*

onate (**10**) *and methyl (allyl 7,8-*O*-carbonyl-3,5-dideoxy-α-*D-arabino*-2-octulopyranosid)onate* (**11**). — A solution of **7** and **8** (4.1 g) in dry methanol (50 mL) was treated with a solution of sodium (160 mg) in methanol (20 mL) for 3 h at room temperature. The solution was made neutral by addition of Dowex 50 (H^+) cation-exchange resin, filtered, and concentrated to dryness. A solution of the residue in dry pyridine (15 mL) was stirred with *p*-nitrophenyl chloroformate (6.6 g) for 48 h at room temperature. The mixture was taken to dryness, and the residue dissolved in ethyl acetate (100 mL) and extracted three times with saturated aqueous $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4) and the residue obtained upon evaporation was subjected to column chromatography on silica gel (C, ethyl acetate) which furnished **10** (0.62 g, 19%), syrup, $[\alpha]_D^{20}$ +23° (*c* 1.4, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.85 (m, 1 H, =CH–), 5.26 (dq, 1 H, =CH_2-*trans*), 5.17 (dq, 1 H, =CH_2-*cis*), 4.67–4.55 (m, 3 H, H-7,8a,8b), 4.20 (ddt, 1 H, OCH_2), 3.97 (ddd, 1 H, $J_{7,6}$ ~6.5, $J_{6,5e}$ ~2.0, $J_{6,5a}$ ~11.5 Hz, H-6), 3.92 (ddt, 1 H, OCH_2), 3.81 (s, 3 H, CH_3OCO), 3.81 (br. m, 1 H, H-4), 2.66 (ddd, 1 H, $^4J_{3e,5e}$ ~2.0, $J_{3e,4}$ ~4.5, $J_{3e,3a}$ ~12.5 Hz, H-3*e*), 2.15 (dddd, 1 H, $J_{5e,5a}$ ~ −12.0, $J_{5e,4}$ ~4.5 Hz, H-5*e*), 2.13 (br. s, 1 H, OH), 1.84 (dd, 1 H, $J_{3a,4}$ ~11.5 Hz, H-3*a*), and 1.31 (dt, 1 H, $J_{5a,4}$ ~11.0 Hz, H-5*a*).

Anal. Calc. for $C_{13}H_{18}O_8$: C, 51.66; H, 6.00. Found: C, 51.79; H, 6.18.

Further elution of the column gave **11** (1.23 g, 38%), syrup, $[\alpha]_D^{20}$ +54° (*c* 1.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.88 (m, 1 H, =CH–), 5.30 (m, 1 H, =CH_2-*trans*), 5.20 (dq, 1 H, =CH_2-*cis*), 4.68 (ddd, 1 H, $J_{7,6}$ ~5.0, $J_{7,8a}$ ~6.5, $J_{7,8b}$ ~7.5 Hz, H-7), 4.56 (dd, 1 H, $J_{8a,8b}$ ~ −8.5 Hz, H-8a), 4.52 (dd, 1 H, H-8b), 4.15 (br. m, 1 H, H-4), 3.99–3.95 (m, 2 H, OCH_2), 3.94 (ddd, 1 H, $J_{6,5e}$ ~2.0, $J_{6,5a}$ ~12.0 Hz, H-6), 3.80 (s, 3 H, CH_3OCO), 2.41 (ddd, 1 H, $J_{3e,3a}$ ~ −13.0, $J_{3e,5e}$ ~2.0, $J_{3e,4}$ ~5.0 Hz, H-3*e*), 2.13 (ddd, 1 H, $J_{5e,5a}$ ~ −12.5, $J_{5e,4}$ ~4.5 Hz, H-5*e*), 2.03 (br. s, 1 H, OH), 1.56 (dd, 1 H, $J_{3a,4}$ ~11.0 Hz, H-3*a*), and 1.27 (dt, 1 H, $J_{5a,4}$ ~11.0 Hz, H-5*a*).

Anal. Calc. for $C_{13}H_{18}O_8$: C, 51.66; H, 6.00. Found: C, 51.12; H, 6.10.

*Sodium (allyl 3,5-dideoxy-β-*D-arabino*-2-octulopyranosid)onate* (**12**). — A solution of **10** (57 mg) in dry methanol (5 mL) was stirred with 0.1M methanolic sodium methoxide (2 mL) for 3 h at room temperature. The solution was made neutral by addition of Dowex 50 (H^+) cation-exchange resin, filtered, and concentrated. A solution of the residue in water (5 mL) was treated with 0.2M aqueous NaOH (2 mL) for 2 h at room temperature. The pH of the solution was adjusted to 8.5 by addition of Dowex 50 (H^+) resin. The mixture was filtered and the filtrate concentrated. The residue was purified by column chromatography on Bio-Gel P-2 to afford **12** (53 mg, 98%), amorphous powder, $[\alpha]_D^{20}$ +19° (*c* 1.0, water); ^{1}H-n.m.r. (D_2O): δ 5.94 (m, 1 H, =CH–), 5.34 (dq, 1 H, =CH_2-*trans*), 5.23 (ddt, 1 H, =CH_2-*cis*), 4.22 and 3.97 (m, 2 H, OCH_2), 3.85–3.70 (m, 5 H, H-4,6,7,8a,8b), 2.65 (ddd, 1 H, $J_{3e,3a}$ ~ −12.0, $J_{3e,5e}$ ~2.0, $J_{3e,4}$ ~4.5 Hz, H-3*e*), 2.07 (dddd, 1 H, H-5*e*), 1.37 (t, 1 H, $J_{3a,4}$ ~12.0 Hz, H-3*a*), and 1.30 (ddt, 1 H, $J_{5e,5a}$ ~12.0, $J_{5a,4}$ ~11.0, $J_{5a,6}$ ~12.0 Hz, H-5*a*); ^{13}C-n.m.r.: δ 174.77 (C-1), 134.87 (=CH–), 119.14 (=CH_2), 102.32 (C-2), 74.43 (C-7), 72.85 (C-6), 66.60 (OCH_2), 66.07 (C-4), 64.06 (C-8), 42.46 (C-3), and 35.36 (C-5).

Anal. Calc. for $C_{11}H_{17}NaO_7$: C, 46.49; H, 6.03. Found: C, 46.13; H, 6.25.

*Sodium (allyl 3,5-dideoxy-α-*D-arabino*-2-octulopyranosid)onate* (**13**). — A solution of **11** (16 mg) in dry methanol (2 mL) was stirred with 0.1M methanolic sodium methoxide (0.5 mL) for 2 h at room temperature. The solution was deionized by addition of Dowex 50 (H^+) cation-exchange resin, filtered, and taken to dryness. The residue was treated with 0.2M aqueous NaOH (1 mL) for 2 h at room temperature. The product was isolated, as described for **12**, to give **13** (15 mg, ~100%), amorphous powder, $[\alpha]_D^{20}$ +41° (*c* 0.6, water); ^{1}H-n.m.r. (D_2O): δ 5.98 (m, 1 H, =CH–), 5.35 (dq, 1 H, =CH_2-*trans*), 5.23 (m, 1 H, =CH_2-*cis*), 4.17 (ddt, $J_{4,3a}$ ~$J_{4,5a}$ ~11.2, $J_{4,5e}$ ~4.7 Hz, H-4), 3.96 (ddt, 1 H, OCH_2), 3.89 (dt, 1 H, $J_{7,6}$ ~$J_{7,8b}$ ~7.3, $J_{7,8a}$ ~3.7 Hz, H-7), 3.83 (ddt, 1 H, OCH_2), 3.81–3.73 (m, 1 H, H-6), 3.77 (dd, 1 H, $J_{8a,8b}$ ~ −11.5 Hz, H-8a), 3.63 (dd, 1 H, H-8b), 2.28 (ddd, 1 H, $J_{3e,3a}$ ~ −12.5, $^4J_{3e,5e}$ ~2.0, $J_{3e,4}$ ~4.7 Hz, H-3*e*), 2.01 (dddd, 1 H, H-5*e*), 1.42 (dd, 1 H, H-3*a*), and 1.39 (dt, 1 H, $J_{5a,6}$ ~11.0, $J_{5a,5e}$ ~ −12.0 Hz, H-5*a*); ^{13}C-n.m.r.: δ 176.55 (C-1), 134.97 (=CH–), 118.76 (=CH_2), 101.60 (C-2), 74.17 (C-7), 71.42 (C-6), 65.23 (OCH_2), 64.91 (C-4), 63.11 (C-8), 41.40 (C-3), and 34.19 (C-5).

O-[*Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-*D-manno*-2-octulopyranosyl)onate*]*-(2→4)-methyl (allyl 7,8-*O*-carbonyl-3,5-dideoxy-α-*D-arabino*-2-octulopyranosid)onate* (**15**). — A solution of **14** (1.7 g, 3.5 mmol) in dry nitromethane (3 mL) was added dropwise under N_2 to a suspension of **11** (0.58 g, 1.9 mmol) and molecular sieve 3A in nitromethane (5 mL) for 2 h at room temperature. After being stirred for 15 h, the mixture was diluted with dichloromethane (100 mL), filtered through Celite, and extracted successively with saturated aqueous $NaHCO_3$ solution, 10% aqueous KI solution, and water. The organic layer was dried (Na_2SO_4) and evaporated to give a syrup which was purified on a column of silica gel (C, 2:1 toluene–ethyl acetate), yielding **15** (1.24 g, 92%), colorless syrup, $[\alpha]_D^{20}$ +90° (*c* 2.2, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.85 (m, 1 H, =CH–), 5.37 (br. s, 1 H, H-5′), 5.25 (ddd, 1 H, $J_{4',5'}$ ~3.0, $J_{4',3'e}$ ~5.0, $J_{4',3'a}$ ~12.5 Hz, H-4′), 5.24 (dq, 1 H, =CH_2-*trans*), 5.19 (ddd, 1 H, $J_{7',8'a}$ ~3.0, $J_{7',8'b}$ ~3.5, $J_{7',6'}$ ~9.5 Hz, H-7′), 5.18 (dq, 1 H, =CH_2-*cis*), 4.78 (dd, 1 H, $J_{8'a,8'b}$ ~ −12.5 Hz, H-8′a), 4.60 (ddd, 1 H, $J_{7,8a}$ ~8.0, $J_{7,8b}$ ~5.0, $J_{7,6}$ ~11.0 Hz, H-7), 4.54 (t, 1 H, $J_{8a,8b}$ ~ −8.0 Hz, H-8a), 4.45 (dd, 1 H, H-8b), 4.32 (ddt, 1 H, $J_{4,5e}$ ~5.0, $J_{4,5a}$ ~11.0, $J_{4,3e}$ ~5.0, $J_{4,3a}$ ~11.0 Hz, H-4), 4.16 (dd, 1 H, $J_{6',5'}$ ~1.5 Hz, H-6′), 4.04 (dd, 1 H, H-8′b), 3.95 (m, 2 H, OCH_2), 3.85 (ddd, 1 H, $J_{6,5e}$ ~5.0, $J_{6,5a}$ ~12.5 Hz, H-6), 3.83 (s, 3 H) and 3.79 (s, 3 H) (2 CH_3OCO), 2.40 (ddd, 1 H, $J_{3e,5e}$ ~2.0, $J_{3e,3a}$ ~12.5 Hz, H-3*e*), 2.21 (ddd, 1 H, $J_{3'e,3'a}$ ~13.0 Hz, H-3′*e*), 2.06 (t, 1 H, H-3′*a*), 2.11 (s, 3 H), 2.07 (s, 3 H), and 1.98 (s, 6 H) (4 CH_3CO), 1.93 (dddd, 1 H, $J_{5e,5a}$ ~ −12.5 Hz, H-5*e*), 1.69 (dd, 1 H, H-3*a*), and 1.31 (dt, 1 H, H-5*a*).

Anal. Calc. for $C_{30}H_{40}O_{19}$: C, 51.13; H, 5.72. Found: C, 51.39; H, 5.68.

O-[*Sodium (3-deoxy-α-*D-manno*-2-octulopyranosyl)onate*]*-(2→4)-sodium-(allyl 3,5-dideoxy-α-*D-arabino*-2-octulopyranosid)onate* (**16**). — A solution of **15** (80 mg) in dry methanol (5 mL) was stirred with 0.01M methanolic sodium methoxide (6 mL) for 7 h at room temperature. The solution was made neutral by addition of Dowex 50 (H^+) cation-exchange resin, filtered, and concentrated. A solution of the residue in water (5 mL) was stirred with 0.2M aqueous NaOH (2 mL) for 1 h at room temperature. The product was isolated, as described for **12**, to give **16** (57 mg, 95%), amorphous powder, $[\alpha]_D^{20}$ +47° (*c* 0.5, water); ^{1}H-n.m.r. (D_2O): δ 5.87 (m, 1 H, =CH–), 5.34 (dq, 1 H, $=CH_2$-*trans*), 5.22 (dq, 1 H, $=CH_2$-*cis*), 4.18 (dddd, 1 H, $J_{4,5a}$ ~12.0, $J_{4,5e}$ ~5.0, $J_{4,3e}$ ~5.0, $J_{4,3a}$ ~12.0 Hz, H-4), 4.09–3.54 (m, 12 H, H-6,7,8a,8b,4′,5′,6′,7′,8′a,8′b, OCH_2), 2.12 (ddd, 1 H, $J_{3e,3a}$ ~ −12.5, $^4J_{3e,5e}$ ~2.0 Hz, H-3*e*), 2.10 (ddd, 1 H, $J_{3'e,4'}$ ~5.0, $^4J_{3'e,5'}$ ~2.0, $J_{3'e,3'a}$ ~ −13.0 Hz, H-3′*e*), 2.04 (dddd, 1 H, $J_{5e,5a}$ ~12.5 Hz, H-5*e*), 1.73 (t, 1 H, $J_{3'a,4'}$ ~12.0 Hz, H-3′*a*), 1.50 (t, 1 H, H-3*a*), and 1.40 (dt, 1 H, H-5*a*); ^{13}C-n.m.r.: δ 176.74, 176.45 (C-1,1′), 135.12 (=CH–), 118.09 ($=CH_2$), 101.49 (C-2), 100.26 (C-2′), 74.17 (C-7), 73.04 (C-6′), 71.50 (C-6), 70.96 (C-7′), 67.68 (C-4), 67.25 (C-5′), 66.85 (C-4′), 64.82 (OCH_2), 64.03 (C-8′), 62.98 (C-8), 40.39 (C-3), 35.60 (C-3′), and 32.40 (C-5).

Anal. Calc. for $C_{19}H_{28}Na_2O_{14}\cdot 3H_2O$: C, 39.32; H, 5.90. Found: C, 39.46; H, 6.06.

Copolymerization. — A solution of **12** (37 mg), acrylamide (37 mg), and *N,N,N′N′*-tetramethylethylenediamine (5 μL) in water (2 mL) was degassed at aspirator pressure for 25 min. After addition of $(NH_4)_2S_2O_8$ (1.5 mg), the mixture was kept for 20 h at 4°. The solution was purified on a column (2.6 × 100 cm) of Sephadex G-50 with 0.01M aqueous $NaHCO_3$ as eluent, at a flow rate of 55 mL h^{-1}, and 3.5-mL fractions were collected. The product-containing fractions 38–45 were pooled and lyophilized, and the residue was desalted on a column (2.6 × 100 cm) of Bio-Gel P-2 to give **17** (59 mg), amorphous powder, $[\alpha]_D^{20}$ +4.3° (*c* 1.8, water). The copolymers **18** (16 mg of **13** and 16 mg of acrylamide) and **19** (23 mg of **16** and 12.7 mg of acrylamide) were prepared in a similar manner to yield **18** (20.9 mg), $[\alpha]_D^{20}$ +2.6° (*c* 1.2, water) and **19** (21.4 mg), $[\alpha]_D^{20}$ +6.1° (*c* 0.7, water), respectively.

ACKNOWLEDGMENT

Financial support of this work by a grant from the Bundesministerium für Wissenschaft und Forschung is gratefully acknowledged.

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