ELSEVIER

Carbohydrate Research 281 (1996) 47–60

CARBOHYDRATE RESEARCH

Synthesis of the methyl α-glycosides of a di-, tri-, and a tetra-saccharide fragment mimicking the terminus of the O-polysaccharide of *Vibrio cholerae* O:1, serotype Ogawa [1]

Ping-sheng Lei, Yuji Ogawa [2], Pavol Kováč *

NIDDK, National Institutes of Health, 8 Center Drive, Bethesda, MD 20892-0815, USA

Received 24 June 1995; accepted 30 August 1995

Abstract

Methyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside was acetylated, and the fully protected methyl glycoside was treated with dichloromethyl methyl ether–$ZnCl_2$ (DCMME–$ZnCl_2$) reagent to give 3-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl chloride (**3**). Condensation of **3** with methyl 3-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (**4**) gave the fully acetylated disaccharide **5**, which was deacetylated yielding the methyl α-glycoside of title disaccharide. The disaccharide glycosyl donor required for the blockwise synthesis of the title tri- and the tetra-saccharide, 3-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-(1 → 2)-3-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl chloride (**12**), was obtained by condensation of **3** with the 1-*O*-acetyl analog of **4**, followed by treatment of the disaccharide formed with DCMME–$ZnCl_2$. The synthesis of the methyl α-glycoside of the title trisaccharide involved a condensation of **12** with **4**, followed by deacetylation. Similarly, the condensation of **12** with **15**, the latter being the analog of **5** having a free HO-2, followed by deacetylation, gave the methyl α-glycoside of the title tetrasaccharide. All glycosylation reactions were mediated by silver trifluoromethanesulfonate in the presence of 2,4,6-trimethylpyridine. 4-(3-Deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α,β-D-mannopyranose was prepared for the first time. It was characterized by NMR spectroscopy, and via its crystalline per-*O*-acetyl

* Corresponding author.

[1] Synthesis of ligands related to the *Vibrio cholerae* O-specific antigen. Part 8. For Part 7, see ref. [1].

[2] On leave from Fuji Chemical Industries, Ltd., 530 Chokeiji, Takaoka, Toyama 933, Japan.

Elsevier Science Ltd.
SSDI 0008-6215(95)00331-2

derivative. It is the saccharide whose α-form constitutes the terminal, non-reducing end-group of the O-PS of *V. cholerea* O:1, serotype Ogawa.

Keywords: Polysaccharide; *Vibrio cholerae*; Glycoside, α-linked; Synthesis; Oligosaccharides

1. Introduction

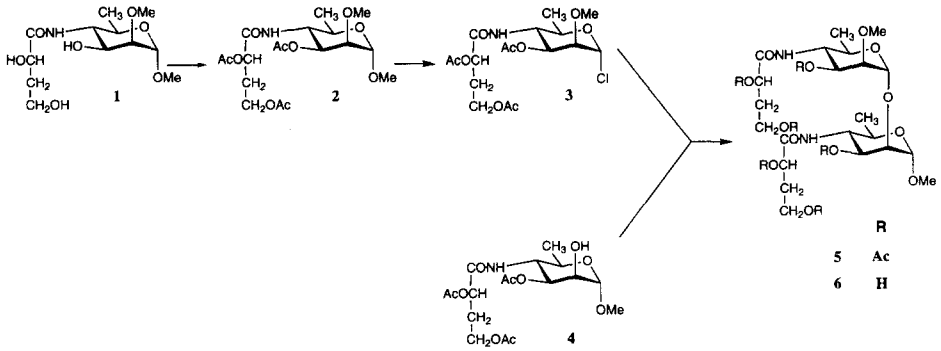
In connection with our studies on the binding of antigens and antibodies involving *Vibrio cholerae* O:1, a substantial part of our effort is directed towards the synthesis of ligands related to the O-specific polysaccharides (O-PS) of the two main strains of the species, Inaba and Ogawa. The structure of the two O-PSs, whose internal part consists of a chain of (1 → 2)-linked, *N*-3-deoxy-L-*glycero*-tetronylated 4-amino-4,6-dideoxy-α-D-mannopyranose (α-D-perosamine), differs in the presence of a methyl group at O-2 in the nonreducing (upstream [2,3]) α-D-perosaminyl group in the O-SP of the Ogawa strain [4,5]. Among the ligands we have synthesized [1,3,6–9], only one, the crystalline methyl 4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-2-*O*-methyl-α-D-mannopyranoside (**1**) [7], carried the 2-methoxyl group characteristic of the Ogawa O-PS. Here, we describe syntheses of three oligosaccharides which imitate the upstream [2,3] part of the O-PS of the strain Ogawa. Formation of the intersaccharidic linkages was achieved using silver trifluoromethanesulfonate (triflate) as a promotor of the glycosyation reactions. Under these conditions, the α-mannosides are formed from mannosyl halides, with or without neighboring group participation [10]. While the analogous trisaccharide related to the O-PS of the serotype Inaba was synthesized [1] in a stepwise manner, the tri- and the tetra-saccharide described here were constructed in the blockwise fashion.

2. Results and discussion

The synthetic strategy applied here is similar to that developed during our preparation of the trisaccharide fragment of the O-PS of *V. cholerae*, serotype Inaba, and which is described in the preceding part in this series [1].

The starting material for the synthesis (Scheme 1) of the key glycosyl donor **3** was the monosaccharide glycoside **1** [7] that mimicks the upstream terminal unit of the O-PS of *V. cholerae* O:1, serotype Ogawa. It was acetylated to give **2**, which was treated with dichloromethyl methyl ether (DCMME)–$ZnCl_2$ reagent [11]. With a large excess of DCMME, which are the conditions routinely applied to convert acetylated methyl glycosides to the corresponding glycosyl chlorides [12], a number of side products were formed. When only two molar equivalents of the reagent were used, the glycosyl donor **3** was obtained in virtually theoretical yield. The amount of $ZnCl_2$ only affected the reaction rate, but not the eventual outcome of the reaction.

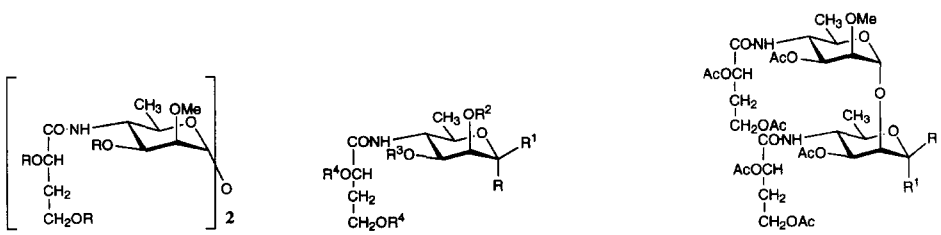
The product resulting from the silver trifluoromethanesulfonate (triflate, AgOTf)-mediated condensation of **3** with **4** [1] contained one major and several minor products. During the isolation of the major product, later shown to be the desired disaccharide **5**, a portion of it was eluted from the chromatography column together with a byproduct. The latter was eventually found to be the nonreducing disaccharide **8**. We have previously isolated [13–15] such oligosaccharides from oligosaccharide-forming reactions, where



Scheme 1.

compounds of this class were formed by reactions of glycosyl donors used with products of their own hydrolysis. When the mixture of **5** and **8** was deacetylated (Zemplén), the resulting mixture of **6** and **7** was readily resolved by chromatography. In this way, the overall yield of the disaccharide **6** was ~ 70%. While the NMR spectra of **5** and **6** were consistent with the expected structures, the NMR spectra of **7** and **8** did not immediately reveal that these compounds were disaccharides. Because of the symmetry of the molecules, the number of signals appearing in the NMR spectra of these compounds corresponded to that expected of monosaccharides. The structure of **8** was finally deduced from the NMR spectra of **7**, aided by the FABMS spectrum showing a peak at m/z 541 $[M + 1]^+$, 563 $[M + Na]^+$.

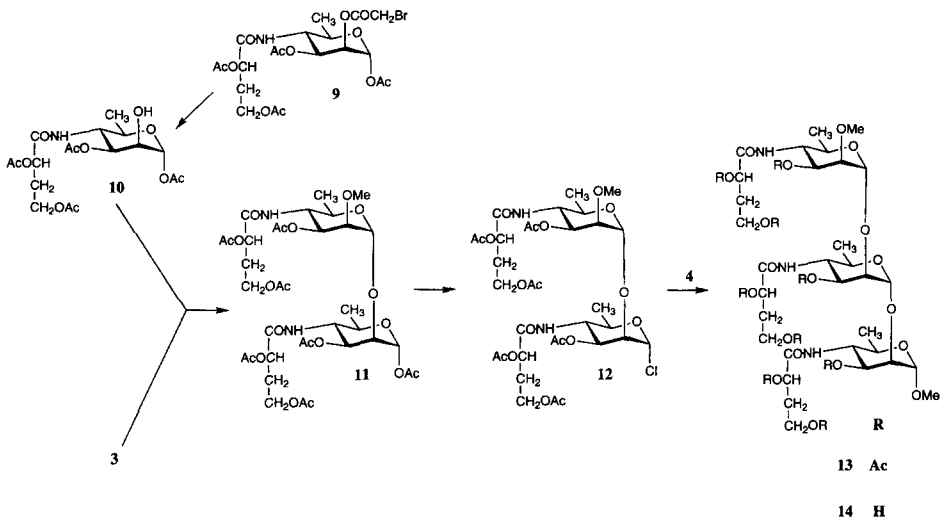
To prepare the trisaccharide **14** (Scheme 2), compound **9** [1] was *O*-debromoacetylated with thiourea. In addition to the desired product **10** (major), preparative chromatography also yielded **19** and **20**. Compound **20** was formed from **10** by a simple acetyl group migration. On the other hand, because of the *trans*-diaxial arrangement of acetyl groups at positions 1 and 2 in **10**, the apparent 1-*O*-acetyl → 2-*O*-acetyl group



	R
7	H
8	Ac

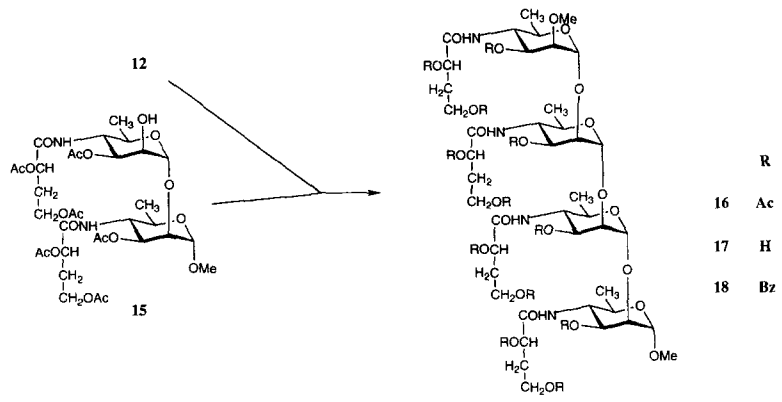
	R	R^1	R^2	R^3	R^4
19	HOH		Ac	Ac	Ac
20	OAc	H	Ac	H	Ac
21	HOH		Me	Ac	Ac
22	OAc	H	Me	Ac	Ac
23	H	OAc	Me	Ac	Ac
24	HOH		Me	H	H

	R	R^1
25	HOH	
26	OAc	H



Scheme 2.

migration, to effect the conversion **10** → **19**, must have been preceded by anomerization. The structure of these substances was deduced from the analysis of their NMR spectra. This was readily accomplished as the ^{1}HNMR chemical shifts of H-2 and H-3, as well as the ^{13}CNMR chemical shift of C-1 in these substances, is strongly affected by the absence or presence of the *O*-acetyl group at positions 2 and 3. Also, compared with 3-*O*-acetylated substances, the ^{13}CNMR resonance of C-4 in the 3-hydroxy compound **20** is shifted downfield, because of the absence of the negative shift effect of the 3-*O*-acetyl group. While the signal for H-1 in 1-*O*-acetyl derivatives **10** and **20** appeared downfield (δ 6.04 and 6.06), the signal for H-1 in the 1-hydroxy compound **19** was shifted upfield and appeared as a part of a multiplet at δ 5.20–5.17. Finally, a mutual comparison of the spectra of the 2-*O*-acetyl, 3-*O*-acetyl, and 2,3-di-*O*-acetyl derivatives, **20**, **10**, and **19**, respectively, showed that the chemical shifts for H-2,3,2′, and H-4′ were consistent with the presence of acetyl groups at the anticipated positions. The extent of the acetyl group migration during the conversion **9** → **10** could be minimized by buffering the *O*-debromoacetylation medium with a weak, nonnucleophilic organic base [16,17]. In this way, the AcO-1 → AcO-2 migration could be eliminated completely (TLC), and the extent of the AcO-3 → AcO-2 migration was minimal. Eventually, the desired glycosyl acceptor **10** was obtained in 75–80% yield, following chromatography. Compound **10** was used as a nucleophile in the condensation with **3**, the product of which, **11**, when treated with DCMME gave the disaccharide glycosyl donor **12**. For economic reasons, an excess of the synthetically more valuable chloride **12** was avoided in the condensation with **4**. The reaction of equimolar proportions of synthons **4** and **12** furnished the intermediate trisaccharide **13** (70%), which was deacylated (Zemplén) to give the desired trisaccharide **14**. The substance mimicks the terminal, upstream [2,3] trisaccharide segment of the O-PS of *V. cholerae*, serotype Ogawa.



Scheme 3.

The preparation of the tetrasaccharide **17** involved the condensation of the building blocks **12** and **15** [1], to give the fully protected tetrasaccharide **16**, followed by deacetylation. In a search for a crystalline derivative of **17** we prepared the per-*O*-benzoyl derivative **18**, but the compound could not be induced to crystallize.

Although the presence of 4-*N*-tetronylated 2-*O*-methyl-perosamine as a significant constituent in the O-PS of *V. cholerae*, serotype Ogawa has been convincingly established [4,5], the substance itself has never been isolated. During their structural investigation, Ito et al. [5] isolated and purified only the free amine, 2-*O*-methyl-perosamine and described its NMR and mass spectral characteristics. During this work, the byproduct of the condensation of **3** with **10**, the 1-hydroxy derivative **21**, offered a way to obtain 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α,β-D-mannopyranose (**24**). Conventional acetylation of **21** gave material that was chromatographed to afford the crystalline 1,3-di-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranose (**22**) in high yield. Deacetylation of **22** gave the amorphous perosamine derivative **24**, which forms the upstream [2,3] end-group of the O-PS of *V. cholerea* O:1, serotype Ogawa. NMR characteristics observed for **24** were consistent with the anticipated structure.

3. Experimental

General methods.—Unless stated otherwise, optical rotations were measured at ambient temperature for solutions in $CHCl_3$ with a Perkin–Elmer automatic polarimeter, model 241MC. Thin-layer chromatography (TLC) was performed with *A*, 2:1 hexane–ethyl acetate, *B*, 5:1 toluene–acetone, *C*, 20:1 $CHCl_3$–MeOH; *D*, 3:1:0.2 $CHCl_3$–MeOH–25% NH_4OH; *E*, 5:1 $CHCl_3$–acetone; *F*, 1:4 hexane–EtOAc; *G*, 3:2:0.4 $CHCl_3$–MeOH–25% NH_4OH; *H*, 3:2 toluene–acetone, and *I*, 10:1 $CHCl_3$–acetone. The detection during TLC, preparative chromatography, NMR and MS spectroscopy, purification of solvents, drying of solutions, and other laboratory techniques was done as described previously [1]. When reporting NMR data, sugar residues in oligosaccharides are serially numbered, beginning with the one bearing the aglycon, and are identified by

a superscript in listings of signal assignments. Nuclei without a superscript notation have not been individually assigned. Thus, for example, in a spectrum of an oligosaccharide, a resonance denoted H-3 can be that of H-3 of any sugar residue.

*Methyl 3-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-*D*-mannopyranoside* (**2**).—Methyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside (**1**, [7], 4.9 g) was acetylated conventionally with excess of acetic anhydride in pyridine. The crude product was eluted from a short column of silica gel (solvent *A*), to give pure **2**, (6.2 g, 94%): mp 69.5–70 °C, $[\alpha]_D$ +61° (*c* 0.8); ^{1}H NMR ($CDCl_3$): δ 5.95 (d, 1 H, $J_{4,NH}$ 9.2 Hz, NH), 5.12 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 11.2 Hz, H-3), 5.03 (dd, $J_{2',3'a}$ 4.6, $J_{2',3'b}$ 7.7 Hz, H-2'), 4.67 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.18 (m, 1 H, H-4), 4.12–3.98 (m, 2 H, H-4'a,b), 3.59–3.48 (m, 1 H, H-5), 3.44 (s, partially overlapped, OCH_3-2), 3.42 (dd, partially overlapped, H-2), 3.30 (s, 3 H, OCH_3-1), 2.10, 2.06, 1.98 (3 s, overlapping H-3'a,b resonances, 3 $COCH_3$), and 1.15 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 171.84, 170.80, 169.75, 169.33 (4 CO), 98.58 (C-1), 77.85 (C-2), 71.02 (C-3), 70.93 (C-2'), 68.37 (C-5), 59.85 (C-4'), 59.56 (OCH_3-2), 54.89 (OCH_3-1), 51.58 (C-4), 30.56 (C-3'), 20.96, 20.77, 20.71 (3 $COCH_3$), and 17.83 (C-6); CIMS: *m/z* 420 $[M + 1]^+$, 437 $[M + 18]^+$. Anal. Calcd for $C_{18}H_{29}NO_{10}$: C, 51.55; H, 6.97; N, 3.33. Found: C, 51.61; H, 6.95; N, 3.35.

*Methyl 3-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamino)-4,6-dideoxy-2-*O*-methyl-α-*D*-mannopyranosyl-(1 → 2)-3-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-α-*D*-mannopyranoside* (**5**) .—A mixture of the foregoing per-*O*-acetyl derivative **2** (1 g, 2.4 mmol) and DCMME (0.42 mL, 4.8 mmol) in alcohol-free $CHCl_3$ (10 mL) was treated with freshly fused $ZnCl_2$ (~ 50 mg) for 1 h at 50 °C. TLC (solvent *B*) showed complete conversion of the starting material, and that one major product was formed. After filtration, the filtrate was concentrated with coevaporation of toluene to remove the excess of DCMME to give after chromatography the amorphous 3-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl chloride (**3**, 0.95 g, ~ 95%): ^{1}H NMR ($CDCl_3$): δ 6.12 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 6.08 (d, 1 H, $J_{4,NH}$ 10.2 Hz, NH), 5.49 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 11.1 Hz, H-3), 5.10 (dd, 1 H, $J_{2',3'a}$ 4.9, $J_{2',3'b}$ 7.6 Hz, H-2'), 4.38–4.29 (m, 1 H, H-4), 4.20–4.05 (m, 2 H, H-4'a,b), 3.99–3.88 (m, 1 H, H-5), 3.69 (dd, 1 H, H-2), 3.53 (s, 1 H, OCH_3), 2.18, 2.16, 2.06 (3 s, overlapping H-3'a,b resonances, 3 $COCH_3$), and 1.25 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}CNMR ($CDCl_3$): δ 90.37 (C-1), 80.48 (C-2), 71.52 (C-5), 70.92 (C-2'), 69.23 (C-3), 59.80 (C-4'), 59.58 (OCH_3), 51.17 (C-4), 30.57 (C-3'), and 17.48 (C-6), CIMS: *m/z* 424 $[M + 1]^+$ and 441 $[M + 18]^+$.

A solution of **3** (783 mg, 1.9 mmol), **4** (ref. [1], 0.5 g, 1.2 mmol) and 2,4,6-trimethylpyridine (0.24 mL, 1.9 mmol) in CH_2Cl_2 (10 mL) was added at 0 °C to a stirred mixture of AgOTf (0.76 g, 1.9 mmol) in CH_2Cl_2 (5 mL). The cooling bath was removed, and stirring was continued for 30 min when TLC (solvent *C*) showed that the donor **3** was no longer present. The mixture was filtered, the filtrate washed with a mixture of aqueous $NaHCO_3$ and $Na_2S_2O_3$, the organic phase was dried and concentrated, and the residue was chromatographed to give first **5** (706 mg, 0.89 mmol): $[\alpha]_D$ +36° (*c* 0.8); ^{1}H NMR ($CDCl_3$): δ 6.22, 6.15 (2 d, 1 H each, $J_{4,NH}$ 9.1 and 9.3 Hz, $NH^{1,2}$), 5.23 (dd, partially overlapped, $J_{2,3}$ 3.2, $J_{3,4}$ 11.5 Hz, H-3^1), 5.20 (dd, partially overlapped, $J_{2,3}$ 3.0, $J_{3,4}$ 11.3 Hz, H-3^2), 5.12–5.04 (m, 2 H, H-$2'^{1,2}$), 5.02 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1^2),

4.64 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1^1), 4.32–4.22 (m, partially overlapped, H-4^2), 4.22–4.04 (5 H, H-4^1,4$'^{1,2}_{a,b}$), 3.96 (dd, 1 H, H-2^1), 3.72–3.60 (m, 3 H, H-2^2,5^{1,2}), 3.53 (OCH_3-2), 3.38 (OCH_3-1), 2.22, 2.17, 2.15, 2.09, 2.06, 2.05 (6 s, 6 $COCH_3$ overlapping multiplets of H-3$'^{1,2}$), 1.24, and 1.18 (2 d, 3 H each, $J_{5,6}$ 6.3 and 6.2 Hz, respectively, H-6^{1,2}); ^{13}C NMR ($CDCl_3$): δ 99.71 (C-1^1), 99.46 (C-1^2), 77.77 (C-2^2), 74.61 (C-2^1), 70.87 (2 C, C-2$'^{1,2}$), 70.72 (C-3^2), 70.51 (C-3^1), 69.55 (C-5^2), 68.27 (C-5^1), 59.82 (2 C, C-4$'^{1,2}$), 59.69 (OCH_3-2), 54.96 (OCH_3-1), 51.68 (2 C, C-4^{1,2}), 30.60, 30.51 (C-3$'^{1,2}$), and 17.82 (2 C, C-6^{1,2}); CIMS: m/z 793 $[M+1]^+$ and 810 $[M+18]^+$. Anal. Calcd for $C_{34}H_{52}N_2O_{13}$: C, 51.51; H, 6.61; N, 3.53. Found: C, 51.44; H, 6.64; N, 3.44.

The material eluted next was a mixture of two substances, one of which was **5** (NMR). Deacetylation (Zemplén) of this mixture, followed by chromatography (solvent *D*), gave material identical with the compound **6** described below (32 mg, 0.06 mmol, total yield of the desired product, 0.95 mmol, 79%).

Eluted later was the trehalose-type, nonreducing disaccharide, 4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-2-*O*-methyl-α-D-mannopyranosyl-(1 → 1)-4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-2-*O*-methyl-α-D-mannopyranoside (**7**), the product of deacetylation of 3-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-(1 → 1)-3-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-*O*-methyl-α-D-mannopyranoside (**8**) present in the mixture. Compound **8** was formed during the condensation just described by a reaction of the glycosyl chloride **3** with the product of its hydrolysis **21**. ^{1}H NMR (D_2O): δ 5.23 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.28 (dd, 1 H, $J_{2',3'a}$ 3.9, $J_{2',3'b}$ 8.6 Hz, H-2'), 4.05 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 10.3 Hz, H-3), 3.92–3.82 (m, partially overlapped, H-5), 3.81 (t, partially overlapped, *J* 10.1 Hz, H-4), 3.73 (m, 2 H, H-4'a,b), 3.62 (dd, 1 H, H-2), 3.50 (s, 3 H, OCH_3), 2.09–1.99, 1.89–1.79 (2 m, 1 H each, H-3'a,b), and 1.17 (d, 3 H, $J_{5,6}$ 5.9 Hz, H-6); ^{13}C NMR (D_2O): δ 92.76 ($J_{C,H}$ 170.2 Hz, C-1), 79.19 (C-2), 69.10 (C-2'), 68.19 (C-5), 67.48 (C-3), 59.02 (OCH_3), 57.94 (C-4'), 53.41 (C-4), 36.03 (C-3'), and 16.98 (C-6); FABMS: m/z 541 $[M+1]^+$ and 563 $[M+Na]^+$.

Acetylation of pure **7** gave **8**: $[\alpha]_D$ +15° (*c* 0.8); ^{1}H NMR ($CDCl_3$): δ 6.10 (d, 1 H, $J_{4,NH}$ 9.1 Hz, NH), 5.18 (dd, partially overlapped, $J_{2,3}$ 3.0, $J_{3,4}$ ~ 11.5 Hz, H-3), 5.16 (d, partially overlapped, $J_{1,2}$ ~ 1.9 Hz, H-1), 5.09 (dd, 1 H, $J_{2',3'a}$ 4.7, $J_{2',3'b}$ 7.7 Hz, H-2'), 4.35–4.25 (m, 1 H, H-4), 4.22–4.06 (m, 2 H, H-4'a,b), 3.69–3.60 (m, 1 H, H-5), 3.53 (bs, 4 H, H-2, OCH_3), 2.19. 2.15, 2.06 (3 s, overlapping signals of H-3', 3 $COCH_3$), and 1.22 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 93.76 (C-1), 77.69 (C-2), 70.89 (C-2'), 70.73 (C-3), 69.46 (C-5), 59.81 (C-4'), 59.66 (OCH_3), 51.35 (C-4), 30.54 (C-3'), and 17.86 (C-6); CIMS: m/z 810 $[M+18]^+$. Anal. Calcd for $C_{34}H_{52}N_2O_{19}$: C, 51.51; H, 6.61; N, 3.53. Found: C, 51.22; H, 6.80; N, 3.36.

Eluted last was the product of hydrolysis of the chloride **3**, 3-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α,β-D-mannopyranose (**21**, α:β ~ 2:1, 0.28 g), as shown by NMR spectroscopy and mass spectrometry. ^{1}H NMR ($CDCl_3$): δ 6.11 (d, $J_{4,NH}$ 9.3 Hz, NHα), 6.01 (d, $J_{4,NH}$ 9.0 Hz, NHβ), 5.24 (d, $J_{1,2}$ 2.9 Hz, H-1β), 5.20 (d, $J_{1,2}$ 2.1 Hz, H-1α), 5.04–4.97 (m, partially overlapped, H-3α,2'α,β), 4.95 (dd, partially overlapped, $J_{2,3}$ 2.6, $J_{3,4}$ 9.2 Hz, H-3β), 4.25–3.98 (m, H-4α,4β,4'α,4'β), 3.88–3.77 (m, H-5α), 3.57 (s, OCH_3β), 3.51 (bd, H-2β), 3.48 (bt, H-2α), 3.45 (OCH_3α), 3.40–3.30 (m, H-5β), 2.10–1.99 (m, $COCH_3$α,β), 1.17

(d, $J_{5,6}$ 5.7 Hz, H-6β), and 1.12 (d, $J_{5,6}$ 6.3 Hz, H-6α); ^{13}C NMR ($CDCl_3$): δ 93.23 (C-1β, $J_{C,H}$ 160.8 Hz), 92.19 (C-1α, $J_{C,H}$ 170.2 Hz), 79.09 (C-2β), 78.19 (C-2α), 73.36 (C-3β), 71.68 (C-5β), 70.96, 70.72 (C-2′α,β,3α), 68.29 (C-5α), 61.88 (OCH_3 β), 59.93 (C-4′α), 59.85 (C-4′β), 59.61 (OCH_3 α), 51.63 (C-4α), 51.19 (C-4β), 30.57 (C-3′α,β), 17.89 (C-6α), and 17.80 (C-6β); CIMS: m/z 423 $[M + 18]^+$.

Methyl 4,6-dideoxy-4-(3-deoxy-L-glycero-*tetronamido)-2*-O-*methyl-α*-D-*mannopyranosyl-(1 → 2)-4,6-dideoxy-4-(3-deoxy*-L-glycero-*tetronamido)-α*-D-*mannopyranoside* (**6**).—The fully acetylated disaccharide **5** (0.65 g) was deacetylated conventionally (Zemplén). After processing, the product was eluted from a small column of silica gel (solvent *D*), and freeze-dried, to give **6** as a white, hygroscopic solid that was pure according to TLC and NMR spectroscopy: $[\alpha]_D$ +2.7° (*c* 0.9, H_2O); ^{1}H NMR (D_2O): δ 5.18 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1^2), 4.82 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1^1), 4.27, 4.28 (2 dd, 2 H, $J_{2',3'a}$ 3.8, $J_{2',3'b}$ 8.6 Hz, H-2′^{1,2}), 4.09 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 10.4 Hz, H-3^2), 4.03 (dd, 1 H, $J_{2,3}$ 3.8, $J_{3,4}$ 10.1 Hz, H-3^1), 3.96 (dd, partially overlapped, H-2^1), 3.95–3.77 (m, partially overlapped, H-4′^{1,2},5^{1,2}), 3.76 (dd, partially overlapped, H-2^2), 3.76–3.71 (m, partially overlapped, H-4′^{1,2}), 2.10–1.97, 1.90–1.78 (2 m, 4 H, H-3′^{1,2}), 1.19, and 1.16 (2 d, 3 H each, $J_{5,6}$ 5.9 and 6.1, respectively, H-6^{1,2}); ^{13}C NMR ($CDCl_3$): δ 99.65 (C-1^1), 99.09 (C-1^2), 79.05 (C-2^2), 78.04 (C-2^1), 69.08 (2 C, C-2′^{1,2}), 68.04 (C-5), 67.60 (C-3), 67.55 (C-3,5), 58.84 (OCH_3-2), 57.93 (2 C, C-4′^{1,2}), 55.05 (OCH_3-1), 53.28, 53.10 (C-4^{1,2}), 36.05 (2 C, C-3′^{1,2}), and 16.93 (2 C, C-6^{1,2}); CIMS: m/z 541 $[M + 1]^+$ and 558 $[M + 18]^+$.

1,3-Di-O-*acetyl-4-(2,4-di*-O-*acetyl-3-deoxy*-L-glycero-*tetronamido)-4,6-dideoxy-2*-O-*methyl-α*- (**22**) *and β*-D-*mannopyranose* (**23**), *and 4,6-dideoxy-4-(3-deoxy*-L-glycero-*tetronamido)-2*-O-*methyl-α,β*-D-*mannopyranose* (**24**).—The foregoing reducing sugar **21** (0.18 g) was treated for 2 h at room temperature with excess of 1:1 acetic anhydride–pyridine. After processing, elution from a small column of silica gel (solvent *E*) gave first 1,3-di-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranose (**22**, 0.17 g, 86%): mp 115–117 °C (from CH_2Cl_2–ether); $[\alpha]_D$ +41° (*c* 1.2); ^{1}H NMR ($CDCl_3$): δ 6.16 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 6.08 (d, 1 H, $J_{4,NH}$ 9.4 Hz, NH), 5.22 (dd, $J_{2,3}$ 3.1, $J_{3,4}$ 11.1 Hz, H-3), 5.08 (dd, 1 H, $J_{2',3'a}$ 4.9, $J_{2',3'b}$ 7.7 Hz, H-2′), 4.35–4.05 (m, 3 H, H-4,4′a,b), 3.81–3.72 (m, 1 H, H-5), 3.54 (s, 3 H, OCH_3), 3.52 (dd, 1 H, H-2), 2.16, 2.18, 2.14, 2.06 (4 s overlapping signals of H-3′ protons, 4 $COCH_3$), and 1.22 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 91.07 ($J_{C,H}$ 175.4 Hz, C-1), 76.74 (C-2), 70.83 (C-2′), 70.79 (C-5), 70.16 (C-3), 59.73 (C-4′), 59.44 (OCH_3), 51.18 (C-4), 30.45 (C-3′), and 17.79 (C-6); CIMS: m/z 465 $[M + 18]^+$. Anal. Calcd for $C_{19}H_{29}NO_{11}$: C, 51.00; H, 6.53; N, 3.13. Found: C, 50.89; H, 6.48; N, 3.06.

Eluted next was 1,3-di-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-β-D-mannopyranose (**23**, 0.02 g, 10%): ^{1}H NMR ($CDCl_3$): δ 5.98 (d, 1 H, $J_{4,NH}$ 9.6 Hz, NH), 5.68 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 5.07 (dd, 1 H, $J_{2',3'a}$ 5.0, $J_{2',3'b}$ 7.7 Hz, H-2′), 5.01 (dd, 1 H, $J_{2,3}$ 2.7, $J_{3,4}$ 11.0 Hz, H-3), 4.25–4.06 (m, 3 H, H-4,4′a,b), 3.68 (bdd, 1 H, H-2), 3.61 (s, 3 H, OCH_3), 3.57–3.47 (m, 1 H, H-5), 2.17, 2.16, 2.14, 2.06 (4 s overlapping signals of H-3′a,b protons, 4 $COCH_3$), and 1.27 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 92.43 ($J_{C,H}$ 161.1 Hz, C-1), 77.40 (C-2), 72.86 (C-5), 72.41 (C-3), 70.97 (C-2′), 61.68 (OCH_3), 59.80 (C-4′), 51.19 (C-4), 30.56 (C-3′), and 17.73 (C-6); CIMS: m/z 441 $[M + 1]^+$ and 465 $[M + 18]^+$.

An intermediate, mixed fraction was also obtained.

Deacetylation of **22** (Zemplén) gave **24** in virtually theoretical yield as a mixture of α and β anomers ($\alpha:\beta \sim 3:1$); CIMS: m/z 297 $[M + 18]^+$ and 280 $[M + 1]^+$.

Spectral data for **24** (α): ^{1}H NMR (D_2O): δ 5.30 (d, $J_{1,2}$ 1.8 Hz, H-1), 4.27 (dd, $J_{2',3'a}$ 3.9, $J_{2',3'b}$ 8.7 Hz, H-2'), 4.04–3.94 (m, H-3,5), 3.83–3.68 (m, H-4,4'a,b), 3.55 (dd, $J_{2,3}$ 3.3 Hz, H-2), 3.47 (s, OCH_3), 2.08–1.98 (m, H-3'a), 1.89–1.79 (m, H-3'b), and 1.13 (d, partially overlapped, $J_{5,6} \sim 6.2$ Hz, H-6); ^{13}C NMR (D_2O): δ 90.90 ($J_{C,H}$ 169.4 Hz, C-1), 79.97 (C-2), 69.10 (C-2'), 67.54, 67.06 (C-3,5), 58.89 (OCH_3), 57.95 (C-4'), 53.54 (C-4), 36.06 (C-3'), and 17.07 (C-6).

Spectral data for **24** (β): ^{1}H NMR (D_2O): δ 4.84 (d, $J_{1,2}$ 0.9 Hz, H-1), 4.27 (dd, $J_{2',3'a}$ 3.9, $J_{2',3'b}$ 8.7 Hz, H-2'), 3.76–3.69 (m, partially overlapped, H-4,4'a,b), 3.66 (dd, $J_{2,3}$ 3.2 Hz, H-2), 3.60 (s, OCH_3), 2.08–1.98 (m, H-3'a), 1.89–1.79 (m, H-3'b), 1.16 (d, partially overlapped, $J_{5,6} \sim 6.2$ Hz, H-6); ^{13}C NMR (D_2O): δ 94.02 ($J_{C,H}$ 159.7 Hz, C-1), 81.00 (C-2), 71.07 (3,5), 69.10 (C-2'), 62.03 (OCH_3), 57.95 (C-4'), 53.11 (C-4), 36.06 (C-3'), 17.07 (C-6).

1,2-di-O- (**20**) *and 1,3-di-O-acetyl-4-(2,4-di*-O-*acetyl-3-deoxy*-L-glycero-*tetronamido)-4,6-dideoxy-α*-D-*mannopyranose* (**10**).—A solution of thiourea (0.9 g, 12 mmol) in MeOH (15 mL) was added at 0 °C to a stirred solution of **9** (2.2 g, 4 mmol) and 2,4,6-trimethylpyridine (0.8 mL, 6 mmol) in CH_2Cl_2 (30 mL), and stirring was continued at 0 °C for 1 h, when TLC (solvent *F*) showed that all starting material was consumed. The mixture was partitioned between water and CH_2Cl_2, the organic phase was dried, concentrated, and the residue was chromatographed to give first the 3-*O*-acetyl derivative **10** (1.4 g, 79%): $[\alpha]_D$ +57° (*c* 1.2); ^{1}H NMR ($CDCl_3$): δ 6.13 (δ, 1 H, $J_{4,NH}$ 9.3 Hz, NH), 6.03 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 5.14 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 11.1 Hz, H-3), 4.98 (dd, 1 H, $J_{2',3'a}$ 4.8, $J_{2',3'b}$ 7.7 Hz, H-2'), 4.3–4.21 (m, 1 H, H-4), 4.12–4.01 (m, 2 H, H-4'a,b), 3.90 (bt, 1 H, H-2), 3.81–3.71 (m, 1 H, H-5), 2.10, 2.06, 1.99 (3 s, 12 H, 3 $COCH_3$, overlapping H-3' resonances), 1.15 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 93.11 ($J_{C,H}$ 172.9 Hz, C-1), 70.86 (C-2'), 70.41 (C-5), 70.35 (C-3), 67.75 (C-2), 59.80 (C-4'), 50.75 (C-4), 30.47 (C-3'), 20.94, 20.75, 20.64 (CO), 17.73 (C-6); CIMS: m/z 451 $[M + 18]^+$, 434 $[M + 1]^+$. Anal. Calcd for $C_{18}H_{27}NO_{11}$: C, 49.88; H, 6.28; N, 3.23. Found: C, 49.61; H, 6.26; N, 3.15.

Eluted next was the 2-*O*-acetyl derivative **20** (30 mg, 17%) resulting from acetyl group migration: ^{1}H NMR ($CDCl_3$): δ 6.14 (d, 1 H, $J_{4,NH}$ 8.6 Hz, NH), 6.06 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 5.18 (dd, $J_{2',3'a}$ 5.2, $J_{2',3'b}$ 6.8 Hz, H-2'), 5.09 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 4.35–3.75 (m, 5 H, H-3,4,5,4'a,b), 2.17, 2.16, 2.12, 2.06 (4 s, 3 H each, 4 $COCH_3$, overlapping H-3' resonances), 1.24 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 90.75 ($J_{C,H}$ 176.7 Hz, C-1), 71.54 (C-2'), 70.37 (C-2), 69.28 (C-5), 67.91 (C-3), 59.86 (C-4'), 54.13 (C-4), 30.77 (C-3'), 17.87 (C-6); CIMS: m/z 451 $[M + 18]^+$, 434 $[M + 1]^+$.

When the debromoacetylation described above was carried out in the absence of 2,4,6-trimethylpyridine, compounds **10** and **20** were isolated in a ratio of 2:1. Also, a small amount of material was eluted just before the 1,3-di-*O*-acetyl derivative **10**. Its NMR spectra indicated that it was 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose: ^{1}H NMR ($CDCl_3$): δ 6.10 (δ, 1 H, $J_{4,NH}$ 8.8 Hz, NH), 5.37 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 11.1 Hz, H-3), 5.20–5.17 (m, 2 H, H-1,2), 5.11 (dd, 1 H, $J_{2',3'a}$ 4.8, $J_{2',3'b}$ 5.9 Hz, H-2'),

4.28–4.07 (m, 3 H, H-4,4'a,b), 4.00–3.92 (m, 1 H, H-5), 2.18, 2.17, 2.07, 2.06 (4 s, 3 H each, 3 $COCH_3$, overlapping H-3' resonances), 1.23 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 92.03 ($J_{C,H}$ 170.9 Hz, C-1), 70.98 (C-2'), 69.74 (C-5), 68.32, 68.08 (C-2,3), 59.85 (C-4'), 51.66 (C-4), 30.60 (C-3'), 17.88 (C-6); CIMS: m/z 451 $[M + 18]^+$, 434 $[M + 1]^+$.

*3-O-Acetyl-4-(2,4-di-O-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2-O-methyl-α-*D*-mannopyranosyl-(1 → 2)-1,3-di-O-acetyl-4-(2,4-di-O-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-α-*D*-mannopyranose* (**11**).—A solution of the glycosyl donor **3** (2.5 g, 4.9 mmol), the glycosyl acceptor **10** (1.4 g, 3.2 mmol) and 2,4,6-trimethylpyridine (0.7 mL, 4.9 mmol) in CH_2Cl_2 (70 mL) was added at 0 °C to a stirred suspension of AgOTf (1.25 g, 4.9 mmol) in CH_2Cl_2 (70 mL). After the mixture had been stirred at room temperature for 1 h, when TLC (solvent *C*) showed that the chloride **3** was all consumed, the mixture was worked up as described for the preparation of **5**. Chromatography (twice, solvent *F*) gave pure **11** (1.8 g, 67%): $[\alpha]_D$ +40° (*c* 0.5). ^1H NMR ($CDCl_3$): δ 6.19, 6.06 (2 d, 1 H each, $J_{4,NH}$ 9.1 Hz, 2 NH), 6.03 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1^1), 5.26 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 11.1 Hz, H-3^1), 5.19 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 11.2 Hz, H-3^2), 5.13–5.05 (m, 3 H, incl. d for H-1^2 at 5.06, H-$2'^{1,2}$), 4.36–4.04 (m, 6 H, H-$4^{1,2}$,$4'^{1,2}$a,b), 4.01 (dd, 1 H, H-2^1), 3.80–3.68 (m, partially overlapped, H-$5^{1,2}$), 3.69 (dd, partially overlapped, H-2^2), 3.54 (s, 3 H, OCH_3), 2.22, 2.18, 2.16, 2.15, 2.12, 2.06 (6 s, the highest-field s being of double intensity, overlapping H-$3'^{1,2}$ resonances, 7 $COCH_3$), 1.25, and 1.20 (2 d, 3 H each, $J_{5,6}$ 6.2 Hz, H-$6^{1,2}$); ^{13}C NMR ($CDCl_3$): δ 99.28 ($J_{C,H}$ 171.5 Hz, C-1^2), 92.33 ($J_{C,H}$ 175.8 Hz, C-1^1), 77.64 (C-2^2), 72.53 (C-2^1), 70.89 (C-5^1), 70.85 (2 C, C-$2'^{1,2}$), 70.67 (C-3^2), 70.11 (C-3^1), 69.85 (C-5^2), 59.85 (OCH_3), 59.77 (2 C, C-$4'^{1,2}$), 51.48 (C-4^2), 51.38 (C-4^1), 30.64, 30.50 (C-$3'^{1,2}$), 17.84 (C-6^1), and 17.65 (C-6^2); CIMS: m/z 838 $[M + 18]^+$. Anal. Calcd for $C_{35}H_{52}N_2O_{20}$: C, 51.22; H, 6.39; N, 3.41. Found: C, 51.17; H, 6.59; N, 3.45.

*Methyl 3-O-acetyl-4-(2,4-di-O-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2-O-methyl-α-*D*-mannopyranosyl-(1 → 2)-3-O-acetyl-4-(2,4-di-O-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-α-*D*-mannopyranosyl-(1 → 2)-3-O-acetyl-4-(2,4-di-O-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-α-*D*-mannopyranoside* (**13**).—A solution of the 1-*O*-acetyl derivative **11** (0.8 g, 0.975 mmol), DCMME (0.172 mL, 1.95 mmol), and freshly fused $ZnCl_2$ (~ 20 mg) in alcohol-free $CHCl_3$ (10 mL) was stirred at 40 °C until almost all starting material was consumed (~ 1 h), as shown by TLC (solvent *B*). One major and one very minor product, both showing faster chromatographic mobility than **11**, were formed, the slower of which largely predominated. After processing as described above for the preparation of **3**, chromatography gave the desired 3-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-(1 → 2)-3-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl chloride (**12**, 640 mg, 82%): ^1H NMR ($CDCl_3$): δ 6.26, 6.23 (2 d, partially overlapped, 2 H, $J_{4,NH}$ ~ 9.9 Hz, $NH^{1,2}$), 6.03 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1^1), 5.54 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 11.1 Hz, H-3^1), 5.17 (dd, 1 H, $J_{2,3}$ 2.9, $J_{3,4}$ 11.1 Hz, H-3^2), 5.10–5.04 (m, 2 H, H-$2'^{1,2}$), 5.02 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1^2), 4.35–4.06 (m, 7 H, H-2^1,$4^{1,2}$,$4'^{1,2}$a,b), 4.03–3.94 (m, 1 H, H-5), 3.72–3.63 (m, 2 H, H-2^2,5), 3.53 (s, 3 H, OCH_3), 2.24–2.02 (m, 22 H, 6 OCH_3,$3'^{1,2}$a,b), 1.28, and 1.21 (2 d, 3 H each, $J_{5,6}$ 6.2 and 6.3 Hz, respectively, H-$6^{1,2}$); ^{13}C NMR ($CDCl_3$): δ 99.72

(C-1^2), 91.11 (C-1^1), 77.54 (C-2^2), 77.09 (C-2^1), 71.27 (C-5), 70.86, 70.82 (C-$2'^{1,2}$), 70.47 (C-3^2), 69.85 (C-5), 68.76 (C-3^1), 59.79 (2 C, C-$4'^{1,2}$), 59.68 (OCH_3), 51.45, 51.14 (C-$4^{1,2}$), 30.50, 30.47 (C-$3'^{1,2}$), 17.73, and 17.40 (C-$6^{1,2}$); CIMS: m/z 814 $[M + 18]^+$.

A solution of the glycosyl donor **12** (528 mg, 0.66 mmol), the glycosyl acceptor **4** (270 mg, 0.66 mmol) and 2,4,6-trimethylpyridine (96 μL, 0.73 mmol) in CH_2Cl_2 (5 mL) was added at room temperature to a stirred suspension of AgOTf (187 mg, 0.73 mmol) in CH_2Cl_2 (5 mL). After 1 h, when TLC (solvent *C*) showed that the chloride **12** was all consumed, the mixture was worked up as described for the preparation of **5**. Chromatography removed some unchanged **4** and most of byproducts and gave material enriched in the desired trisaccharide **13**, as shown by ^{1}H NMR spectroscopy. Re-chromatography (solvent *F*) gave pure **13** (0.54 g, 70%): $[\alpha]_D$ +53° (*c* 0.5); ^{1}H NMR ($CDCl_3$): δ 6.39, 6.33, 6.27 (3 d, 1 H each, $J_{4,NH}$ 9.4, 9.2, 9.3 Hz, respectively, 3 NH), 5.23 (dd, partially overlapped, $J_{2,3}$ 3.2, $J_{3,4}$ ~ 10.7 Hz, H-3^2), 5.19 (dd, partially overlapped, $J_{2,3}$ ~ 3.1, $J_{3,4}$ ~ 8.2 Hz, H-3^1), 5.17 (dd, partially overlapped, $J_{2,3}$ ~ 3.3, $J_{3,4}$ ~ 8.1 Hz, H-3^3), 5.11–5.03 (m, 7 H, incl. bs of H-1^3 at 5.05, H-$2'^{1-3}$), 4.94 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1^2), 4.63 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1^1), 4.33–4.05 (m, 10 H, H-2^2,4^{1-3},$4'^{1-3}$a,b), 3.93 (bdd, 1 H, H-2^1), 3.80–3.63 (m, 4 H, incl. bdd of H-2^3 at 3.64, H-5^{1-3}), 3.53 (s, 3 H, OCH_3-2), 3.38 (s, 3 H, OCH_3-1), 2.22, 2.20, 2.16, 2.15, 2.13, 2.09, 2.07, 2.06, 2.05 (9 s, overlapping signals of H-$3'^{1-3}$, 9 $COCH_3$), 1.24, 1.21, and 1.15 (3 d, $J_{5,6}$ 6.3 Hz, H-6^{1-3}); ^{13}C NMR ($CDCl_3$): δ 100.56 (C-1^2), 99.53 (C-1^1), 99.37 (C-1^3), 77.58 (C-2^3), 75.28 (C-2^1), 75.16 (C-2^2), 70.75, 70.65 (C, 2 C, C-$2'^{1-3}$), 70.45 (C-3^3), 69.91 (C-3^1), 69.71 (C-3^2), 69.25, 68.85 (C-$5^{2,3}$), 67.79 (C-5^1), 59.69, 59.66 (2 C, C, C-$4'^{1-3}$), 59.46 (OCH_3), 54.78 (OCH_3), 51.69, 51.25 (C, 2 C, C-4^{1-3}), 30.35 (3 C, C-$3'^{1-3}$), and 17.58 (3 C, C-6^{1-3}); CIMS: m/z 1183 $[M + 18]^+$. Anal. Calcd for $C_{50}H_{75}N_3O_{28}$: C, 51.50; H, 6.48; N, 3.60. Found: C, 51.41; H, 6.53; N, 3.51.

Eluted next was a mixture of the unchanged nucleophile **4** and the product of hydrolysis **25** of the glycosyl chloride **12**.

Eluted last was pure **25**: ^{1}H NMR ($CDCl_3$) for the α anomer (largely predominating): δ 6.36, 6.32 (2 d, 2 H, partially overlapped, $J_{4,NH}$ 9.0 and 8.6 Hz, respectively, $NH^{1,2}$), 5.34 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 10.9 Hz, H-3^1), 5.20 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 11.1 Hz, H-3^2), 5.17 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1^1), 5.11–5.04 (m, 2 H, H-$2'^{1,2}$), 5.03 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1^2), 4.32–4.04 (m, 6 H, H-$4^{1,2}$,$4'^{1,2}$a,b), 4.02–3.92 (m, 2 H, incl. dd at 4.00, H-2^1,5), 3.74–3.67 (m, 2 H, incl. dd at 3.68, H-2^2,5), 3.52 (s, 3 H, OCH_3), 2.20, 2.17, 2.16, 2.10, 2.06 (5 s, the one at 2.06 appearing to be of double intensity, 6 $COCH_3$, overlapping signals of H-$3'^{1,2}$), 1.21, and 1.17 (2 d, 3 H each, $J_{5,6}$ 6.3 and 6.4 Hz, respectively, H-$6^{1,2}$); ^{13}C NMR ($CDCl_3$): δ 99.28 ($J_{C,H}$ 171.2 Hz, C-1^2), 93.18 ($J_{C,H}$ 170.2 Hz, C-1^1), 77.72 (C-2^2), 74.95 (C-2^1), 70.92, 70.84 (C-$2'^{1,2}$), 70.68 (C-3^2), 70.24 (C-3^1), 69.27, 68.00 (C-$5^{1,2}$), 59.91 (2 C, C-$4'^{1,2}$), 59.63 (OCH_3), 51.60 (C-$4^{1,2}$), 30.55 (C-$3'^{1,2}$), 17.93, and 17.73 (C-$6^{1,2}$); CIMS: m/z 796 $[M + 18]^+$.

The foregoing product of hydrolysis **25** (140 mg) was treated overnight at room temperature with excess of 1:1 pyridine–acetic anhydride (1 mL). TLC (solvent *E*) showed that two products were formed, the less polar one largely predominating. After concentration, chromatography gave material identical (TLC, NMR) with the α anomer **11** described above.

A small amount of the pure 3-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-(1 → 2)-1,3-di-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-β-D-mannopyranose (**26**, ~ 5 mg) was obtained after elution of the intermediate, mixed, fraction. ^{1}H NMR ($CDCl_3$): δ 6.23, 5.98 (2 d, 1 H each, $J_{4,NH}$ 8.7 and 9.3 Hz, respectively, 2 NH), 5.71 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1^1), 5.26 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 11.2 Hz, H-3^2), 5.13–5.03 (m, 4 H, H-1^2,3^1,2′^{1,2}), 4.34–4.02 (m, 7 H, 2^1,4^{1,2},4′^{1,2}a,b), 3.74 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 2.9 Hz, H-2^2), 3.55 (s, 3 H, OCH_3), 2.21, 2.17, 2.16, 2.13, 2.11, 2.06, 2.05 (7 s, overlapping H-3′^{1,2} resonances, 7 $COCH_3$), 1.29, and 1.19 (2 d, 3 H each, $J_{5,6}$ 6.2 and 6.3 Hz, respectively, H-6^{1,2}); ^{13}C NMR ($CDCl_3$): δ 99.09 (C-1^2), 91.43 (C-1^1), 77.84 (C-2^2), 73.40, 73.24, 72.47 (C-2^1,3^1,5^1), 70.95 (2 C, C-2′^{1,2}), 70.76 (C-3^2), 69.30 (C-5^2), 59.80 (C-4′^{1,2}), 59.73 (OCH_3), 51.76, 51.32 (C-4^{1,2}), 30.54, 29.69 (C-3′^{1,2}), 17.96, and 17.81 (C-6^{1,2}); CIMS: *m*/*z* 838 $[M + 18]^+$ and 821 $[M + 1]^+$.

Methyl 4,6-dideoxy-4-(3-deoxy-L-glycero-*tetronamido)-2*-O-*methyl*-α-D-*mannopyranosyl-(1 → 2)-4,6-dideoxy-4-(3-deoxy*-L-glycero-*tetronamido)*-α-D-*mannopyranosyl-(1 → 2)-4,6-dideoxy-4-(3-deoxy*-L-glycero-*tetronamido)*-α-D-*mannopyranoside* (**14**).—A solution of the fully acetylated trisaccharide (450 mg) in MeOH (15 mL) was treated, at room remperature overnight with M sodium methoxide in MeOH (1 mL). TLC (solvent *E* and *G*) showed that the reaction was complete. After processing, as described for the preparation of **6**, chromatography (solvent *G*) gave **14** (270 mg, 89%): $[\alpha]_D$ +1.6° (*c* 0.9, H_2O); ^{1}H NMR (D_2O): δ 5.23 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1^3), 5.21 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1^2), 4.82 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1^1), 4.36–4.28 (m, 3 H, H-2′$^{1-3}$), 3.50 (s, 3 H, OCH_3-2), 3,41 (s, 3 H, OCH_3-1), 2.12–2.0, 1.94–1.80 (2 m, 3 H each, H-3′$^{1-3}$a,b), 1.22, 1.21, and 1.15 (3d, partially overlapped, 9 H, $J_{5,6}$ ~ 5.9 Hz, H-6$^{1-3}$); ^{13}C NMR (D_2O): δ 100.90 (C-1^2), 99.78 (C-1^1), 99.06 (C-1^3), 79.09 (C-2^3), 77.71 (C-2^2), 77.62 (C-2^1), 69.14 (3 C, C-2′$^{1-3}$), 68.39, 68.05, 67.85, 67.68, 67.60, 67.48 (C-3$^{1-3}$, 5$^{1-3}$), 58.84 (OCH_3-2), 57.98 (3 C, C-4′$^{1-3}$), 55.01 (OCH_3-1), 53.33, 53.18, 53.10 (C-4$^{1-3}$), 36.18 (3 C, C-3′$^{1-3}$), and 16.99 (3 C, C-6$^{1-3}$); FABMS: *m*/*z* 810 $[M + Na]^+$ and 788 $[M + 1]^+$.

Methyl 3-O-*acetyl-4-(2,4-di*-O-*acetyl-3-deoxy*-L-glycero-*tetronamido)-4,6-dideoxy-2*-O-*methyl*-α-D-*mannopyranosyl-(1 → 2)-3*-O-*acetyl-4-(2,4-di*-O-*acetyl-3-deoxy*-L-glycero-*tetronamido)-4,6-dideoxy*-α-D-*mannopyranosyl-(1 → 2)-3*-O-*acetyl-4-(2,4-di*-O-*acetyl-3-deoxy*-L-glycero-*tetronamido)-4,6-dideoxy*-α-D-*mannopyranosyl-(1 → 2)-3*-O-*acetyl-4-(2,4-di*-O-*acetyl-3-deoxy*-L-glycero-*tetronamido)-4,6-dideoxy*-α-D-*mannopyranoside* (**16**).—A solution of the glycosyl chloride **12** (384 mg, 0.48 mmol) in CH_2Cl_2 (5 mL) was added dropwise at room temperature to a stirred mixture of the glycosyl acceptor **15** [1] (250 mg, 0.32 mmol), 2,4,6-trimethylpyridine (64 μL) and AgOTf (124 mg, 0.48 mmol). After 30 min, when TLC (solvent *C*) showed that all of **12** was consumed, the mixture was worked up as described for the preparation of **5**. Chromatography (solvent *C*) gave material highly enriched in the desired tetrasaccharide **16**. Rechromatography (solvent *H*) gave **16** (240 mg, 49%): $[\alpha]_D$ +31° (*c* 0.9); ^{1}H NMR ($CDCl_3$): δ 6.44, 6.33, 6.27, 6.08 (4 d, 1 H each, $J_{4,NH}$ ~ 9.5 Hz, 4 NH), 5.25–5.17 (m, 4 H, H-3$^{1-4}$), 5.12–5.02 (m, 5 H, H-1^4,2′$^{1-4}$), 5.01 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1^3), 4.92 (d, 1 H, $J_{1,2}$ 2.1 Hz, H-1^2), 4.65 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1^1), 4.35–4.04 (m, 14 H, H-2^{2,3},4$^{1-4}$,4′$^{1-4}$a,b), 3.93 (dd, $J_{2,3}$ 3.1 Hz, H-2^1), 3.77–3.61 (m, 5 H, H-5$^{1-4}$, incl. dd,

$J_{1,2}$ 1.7, $J_{2,3}$ 3.1 Hz, H-2^4), 3.53 (s, 3 H, OCH_3-2), 3.39 (s, 3 H, OCH_3-1), 2.21, 2.19, 2.17, 2.16, 2.15, 2.12 (triple int.), 2.06, 2.05 (double int.), and 2.04 (9 s, overlapping H-3$'^{1-4}$ signals, 12 $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 100.54 (C-1^2), 100.34 (C-1^3), 99.63 (C-1^1), 99.44 (C-1^4), 77.73 (C-2^4), 75.41, 75.35, 74.92 (C-2$^{1-3}$), 70.91, 70.85, 70.80 (C, C, 2C, C-2$'^{1-4}$), 70.67, 70.03, 69.97, 69.54 (C-3$^{1-4}$), 69.47, 69.24, 68.89 (C-5$^{2-4}$), 68.08 (C-5^1), 59.84, 59.81 (2 C, 2C, C-4$'^{1-4}$), 59.63 (OCH_3-2), 55.02 (OCH_3-1), 52.10, 51.60, 51.45 (C, 2 C, C, C-4$^{1-4}$), 30.55, 30.51 (2 C, 2C, C-3$'^{1-4}$), 17.92, and 17.76 (C, 3C, C-6$^{1-4}$); CIMS: *m/z* 1557 $[M + 18]^+$. Anal. Calcd for $C_{66}H_{98}N_4O_{37}$: C, 51.49; H, 6.42; N, 3.64. Found: C, 51.22; H, 6.48; N, 3.56.

Methyl 4,6-dideoxy-4-(3-deoxy-L-glycero-*tetronamido)-2*-O-*methyl*-α-D-*manno*-*pyranosyl-(1 → 2)-4,6-dideoxy-4-(3-deoxy*-L-glycero-*tetronamido)*-α-D-*mannopyranosyl-(1 → 2)-4,6-dideoxy-4-(3-deoxy*-L-glycero-*tetronamido)*-α-D-*mannopyranosyl-(1 → 2)-4,6-dideoxy-4-(3-deoxy*-L-glycero-*tetronamido)*-α-D-*mannopyranoside* (**17**).—The foregoing compound **16** was deacetylated (Zemplén) as described for the preparation of **6**. Chromatography (solvent *G*), followed by freeze-drying, gave **17** as a white, hygroscopic solid in virtually theoretical yield, $[\alpha]_D$ +1.5° (*c* 1, H_2O); ^{1}H NMR ($CDCl_3$): δ 5.21 (bd, 1 H, $J_{1,2}$ ~ 1.8 Hz, H-1^4), 5.19, 5.16 (2 bd, 1 H each, $J_{1,2}$ ~ 1.1 and ~ 1.2 Hz, H-1^{2,3}), 4.80 (bd, 1 H, $J_{1,2}$ ~ 1.7 Hz, H-1^1), 4.33–4.26 (m, 4 H, H-2$'^{1-4}$), 4.20–4.15 (m, 4 H, H-2^{2,3},3,3), 4.08 (dd, partially overlapped, $J_{2,3}$ 3.5, $J_{3,4}$ ~ 10.3 Hz, H-3), 4.03 (dd, partially overlapped, $J_{2,3}$ ~ 3.0, $J_{3,4}$ 10.6 Hz, H-3), 3.99–3.82 (m, 9 H, H-2^1,4$^{1-4}$,5$^{1-4}$), 3.79–3.71 (m, 9 H, H-2^4,4$'^{1-4}$a,b), 3.49 (s, 3 H, OCH_3-4), 3.40 (s, 3 H, OCH_3-1), 2.12–1.99, 1.91–1.79 (2 m, 4 H each, H-3$'^{1-4}$a,b), 1.20, 1.18, and 1.15 (3 d, partially overlapped, 12 H, H-6$^{1-4}$); ^{13}C NMR ($CDCl_3$): δ 100.84, 100.72 (C-1^{2,3}), 99.69 (C-1^1), 99.69 (C-1^4), 79.01 (C-2^4), 77.59, 77.53, 77.28 (C-2$^{1-3}$), 69.11 (C-2$'^{1-4}$), 68.35, 68.31, 68.00, 67.78, 67.64, 67.54 (2 C), 67.47 (C-3$^{1-4}$,5$^{1-4}$), 58.81 (OCH_3-4), 57.95 (C-4$'^{1-4}$), 54.94 (OCH_3-1), 53.24, 53.14, 53.05 (2 C) (C-4$^{1-4}$), 36.09 (4 C, C-3$'^{1-4}$), 16.95, and 16.89 (2 C, 2 C, C-6$^{1-4}$); FABMS: *m/z* 1035 $[M + 1]^+$.

A solution of a small amount of **17** in pyridine was treated overnight at room temperature with excess of benzoyl chloride. After conventional processing, the crude material was eluted from a column of silica gel (solvent *I*) to give methyl 3-*O*-benzoyl-4-(2,4-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-(1 → 2)-3-*O*-benzoyl-4-(2,4-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)-3-*O*-benzoyl-4-(2,4-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)-3-*O*-benzoyl-4-(2,4-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (**18**), which could not be induced to crystallize. ^{1}H NMR ($CDCl_3$): δ 6.82 (bs, 1 H, NH), 6.62, 6.59 (2 d, partially overlapped, 2 H, 2 NH), 6.20 (d, $J_{4,NH}$ 9.5 Hz, NH), 5.55–5.32 (m, 8 H, H-2$'^{1-4}$,3$^{1-4}$), 5.02, 5.01 (2 bd, 2 H, 2 H-1), 4.97 (bd, 1 H, H-1), 4.57 (bd, 1 H, H-1), 3.38 (s, 3 H, OCH_3-4), 3.23 (s, 3 H, OCH_3-1), 2.25–1.80 (m, 8 H, H-3$'^{1-4}$), 1.07, 1.02, 0.94, and 0.84 (4 d, 3 H each, $J_{5,6}$ ~ 6.2 Hz, H-6$^{1-4}$); ^{13}C NMR ($CDCl_3$): δ 100.36, 99.76, 99.60 (C, 2 C, C, C-1$^{1-4}$), 78.06 (C-2^4), 75.66, 75.50, 74.78 (C-2$^{1-3}$), 71.90, 71.86, 71.74, 71.65, 70.95, 70.50, 69.90, 69.55, 69.11, 68.75 (C, C,C, 2 C, C, C, C, C, C, C (C-2$'^{1-4}$,3$^{1-4}$,5$^{2-4}$), 68.33 (C-5^1), 60.75, 60.60 (3 C, C, C-4$'^{1-4}$), 59.91 (OCH_3-4), 55.08 (OCH_3-1), 52.65, 51.86, 51.56, 51.35 (C-4$^{1-4}$), 31.14, 31.05, 30.85 (C, 2 C, C, C-3$'^{1-4}$), 17.90, 17.77, and 17.49 (C, 2C, C, C-6$^{1-4}$); FABMS: *m/z* 2284 $[M]^+$.

References

[1] P.-s. Lei, Y. Ogawa, and P. Kováč, *Carbohydr. Res.*, 279 (1995) 117–131.
[2] V. Pavliak, V. Pozsgay, P. Kováč, A. Karpas, C. Chu, R. Schneerson, J. Robbins, and C.P.J. Glaudemans, *J. Biol. Chem.*, 268 (1993) 25797–25802.
[3] M. Gotoh and P. Kováč, *J. Carbohydr. Chem.*, 13 (1994) 1193–1213.
[4] K. Hisatsune, S. Kondo, Y. Isshiki, T. Iguchi, and Y. Haishima, *Biochem. Biophys. Res. Commun.*, 190 (1993) 302–307.
[5] T. Ito, T. Higuchi, M. Hirobe, K. Hiramatsu, and T. Yokota, *Carbohydr. Res.*, 256 (1994) 113–128.
[6] Y. Ogawa, P.-s. Lei, and P. Kováč, *Carbohydr. Res.*, 277 (1995) 327–331.
[7] P.-s. Lei, Y. Ogawa, J.L. Flippen-Anderson, and P. Kováč, *Carbohydr. Res.*, 275 (1995) 117–129.
[8] M. Gotoh and P. Kováč, *Carbohydr. Res.*, 268 (1995) 73–84.
[9] M. Gotoh, C.L. Barnes, and P. Kováč, *Carbohydr. Res.*, 260 (1994) 203–218.
[10] H. Paulsen, *Angew. Chem., Int. Ed. Engl.*, 29 (1990) 823–938.
[11] H. Gross, I. Farkas, and R. Bognár, *Z. Chem.*, 18 (1978) 201–210.
[12] R. Bognár, I. Farkas-Szabó, I. Farkas, and H. Gross, *Carbohydr. Res.*, 5 (1967) 241–243.
[13] P. Kováč, and R.B. Taylor, *Carbohydr. Res.*, 167 (1987) 153–173.
[14] P. Kováč, J. Hirsch, and V. Kováčik, *Carbohydr. Res.*, 75 (1979) 109–116.
[15] P. Kováč, *Collect. Czech. Chem. Commun.*, 45 (1980) 892–900.
[16] P. Kováč, H.C.J. Yeh, and C.P.J. Glaudemans, *Carbohydr. Res.*, 140 (1985) 277–288.
[17] P. Kováč, C.P.J. Glaudemans, W. Guo, and T.C. Wong, *Carbohydr. Res.*, 140 (1985) 299–311.