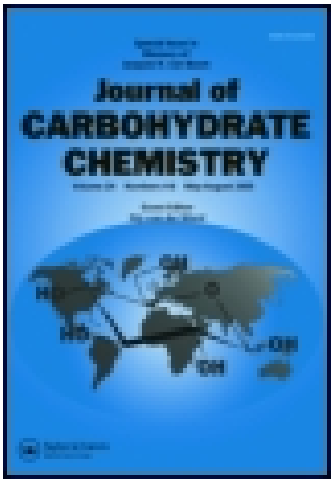


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lcar20

Synthesis and Semisynthesis of Some Structural Elements of Oligo-Mannose Type N-Glycoproteins

Zoltán Szurmai [a] , Lóránt Jánossy [a] , Zoltán Szilágyi [b] & Károly Vékey [b]

[a] Institute of Biochemistry , L. Kossuth University , P. O. Box 55, H-4010, Debrecen, Hungary
[b] Central Research Institute for Chemistry , Hungarian Academy of Sciences , P. O. Box 17, H-1525, Budapest, Hungary
Published online: 15 Aug 2006.

To cite this article: Zoltán Szurmai , Lóránt Jánossy , Zoltán Szilágyi & Károly Vékey (1998) Synthesis and Semisynthesis of Some Structural Elements of Oligo-Mannose Type N-Glycoproteins, Journal of Carbohydrate Chemistry, 17:3, 417-437, DOI: 10.1080/07328309808002903

To link to this article: http://dx.doi.org/10.1080/07328309808002903

J. CARBOHYDRATE CHEMISTRY, 17(3), 417-437 (1998)

SYNTHESIS AND SEMISYNTHESIS OF SOME STRUCTURAL ELEMENTS OF OLIGO-MANNOSE TYPE N-GLYCOPROTEINS

Zoltán Szurmai,[a]* Lóránt Jánossy,[a] Zoltán Szilágyi,[b] and Károly Vékey[b]

[a]Institute of Biochemistry, L. Kossuth University, P. O. Box 55, H-4010 Debrecen, Hungary
[b]Central Research Institute for Chemistry of the Hungarian Academy of Sciences, P. O. Box 17, H-1525 Budapest, Hungary

Received January 24, 1997 - Final Form December 15, 1997

ABSTRACT

For the construction of N-glycoprotein glycan chains, valuable potential glycosyl donors, *O*-α-D-mannopyranosyl-(1→2)-α-D-mannopyranose octaacetate (**19**) and *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→2)-α-D-mannopyranose undeca-acetate (**20**) were obtained in gram-scale by the acetylation and subsequent partial acetolysis of baker's yeast, without the isolation of mannan. The acetolytic products were investigated by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. Octaacetates of mannobiose (1→3) (**11**) and (1→6) (**17**) were chemically synthesized.

INTRODUCTION

Synthetic methods are very important in the field of glycobiology for the production of highly pure materials in large amounts for biological investigations. Over the past two decades, a number of excellent publications have appeared about N-glycoprotein glycan chains.[1-12] The preparation of structural elements of such materials

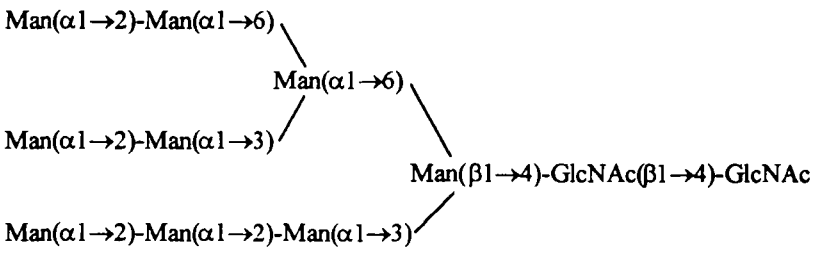


Figure 1

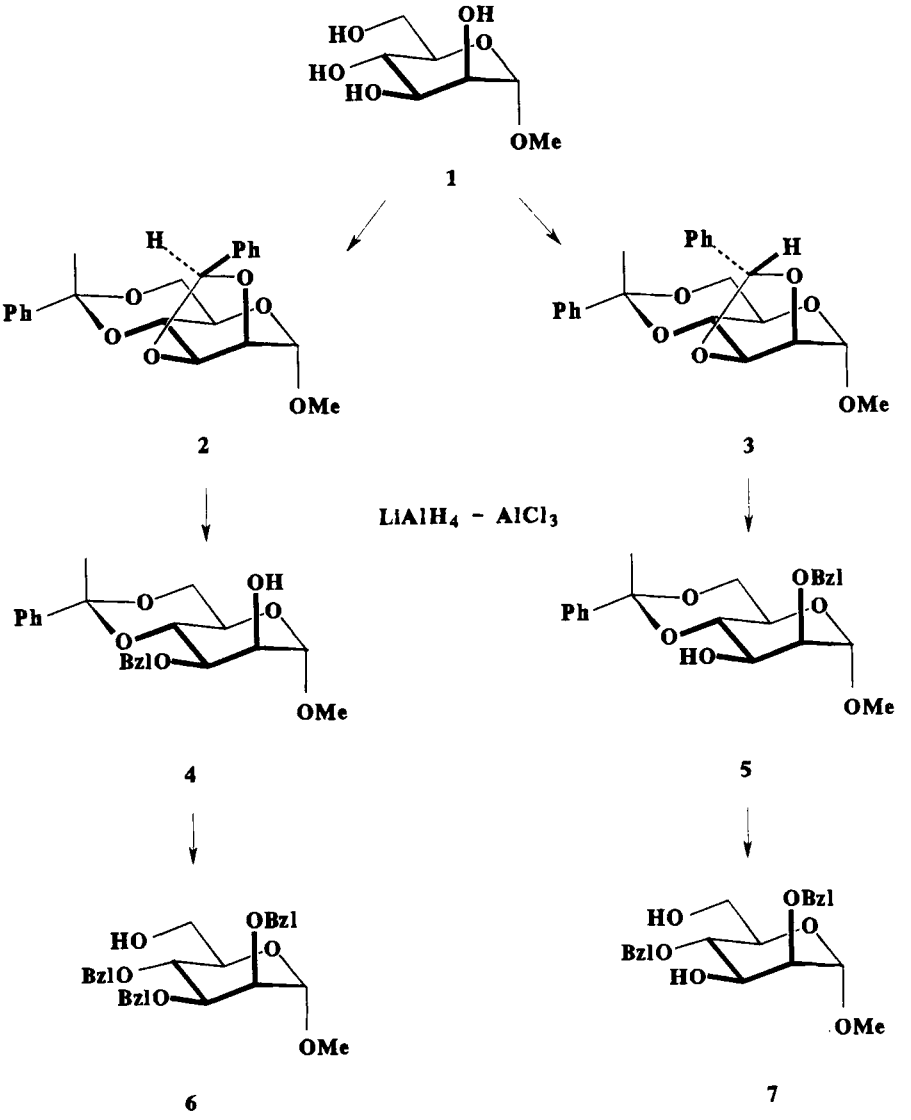
has been one of the ongoing research programmes in our laboratories.[13-18] Figure 1 represents the structure of a typical oligo-mannose type oligosaccharide. Recently a mg-scale preparation of this oligosaccharide was published by Ogawa *et al.*.[19]

In the past in order to synthesize suitable oligomannosides to serve as building blocks for the synthesis of high mannose type oligosaccharides, partially substituted methyl and phenyl-1-thio-α-D-mannopyranoside derivatives were prepared[15] and potential mannobiose-(1→2) glycosyl donors were synthesized by different chemical methods.[15,17] A novel semisynthetic route was recently proposed by us to prepare the mannobiose (1→2) octaacetate based on the direct acetylation and subsequent acetolysis of crude baker's yeast.[17] Now we report on the gram-scale preparation of mannobiose and mannotriose peracetates with (1→2) linkages, and also the chemical syntheses of mannobiose (1→3) and (1→6) derivatives. The efficiency of MALDI-TOF mass spectrometry for the investigation of the acetolytic products is reported in this article.

RESULTS AND DISCUSSION

Syntheses of mannobiose compounds

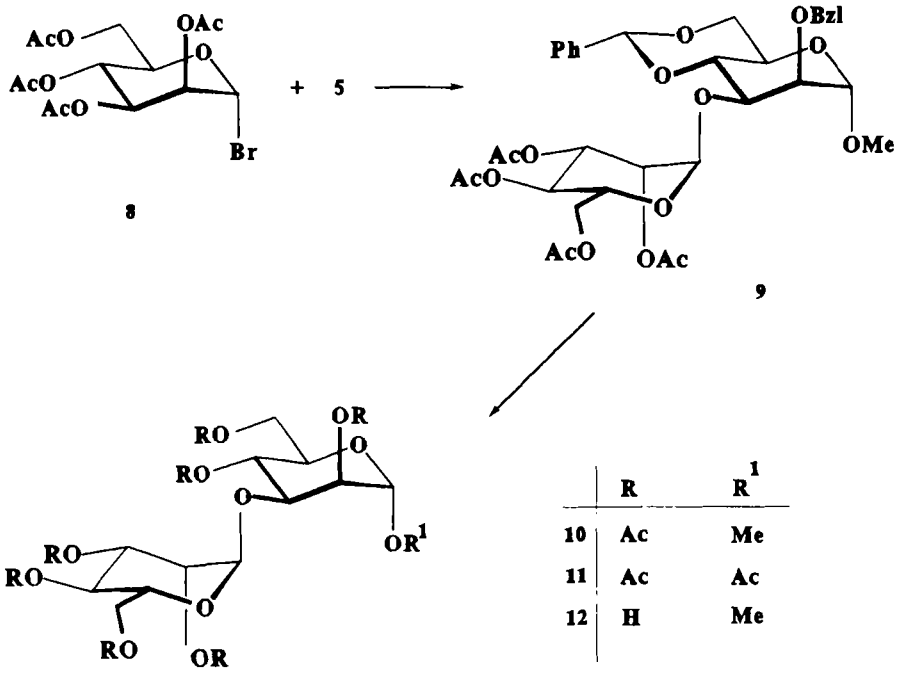
A series of partially substituted mannose derivatives was prepared earlier (such as **4**, **5**, **6** and **7**) *via* the *exo*-(**2**) and *endo*-(**3**) dibenzylidene acetals of methyl α-D-mannopyranoside (**1**) by chemo- stereo- and regioselective reactions with the $LiAlH_4$-$AlCl_3$ reagent[20,15] (Scheme 1). Derivatives of mannobiose-(1→2) have also been



Scheme 1

synthesized[15,17] and the interglycosidic linkage formed using silver triflate[21] or $HgBr_2$ as promoters[22] or *via* the imidate procedure.[23]

Bearing in mind that the HO-3 of mannose is considered to be a rather unreactive glycosyl acceptor, **5**[20] was reacted with **8** using the effective silver triflate promoter[21] to give the crystalline disaccharide **9** (81%). Despite the highly acidic medium, the

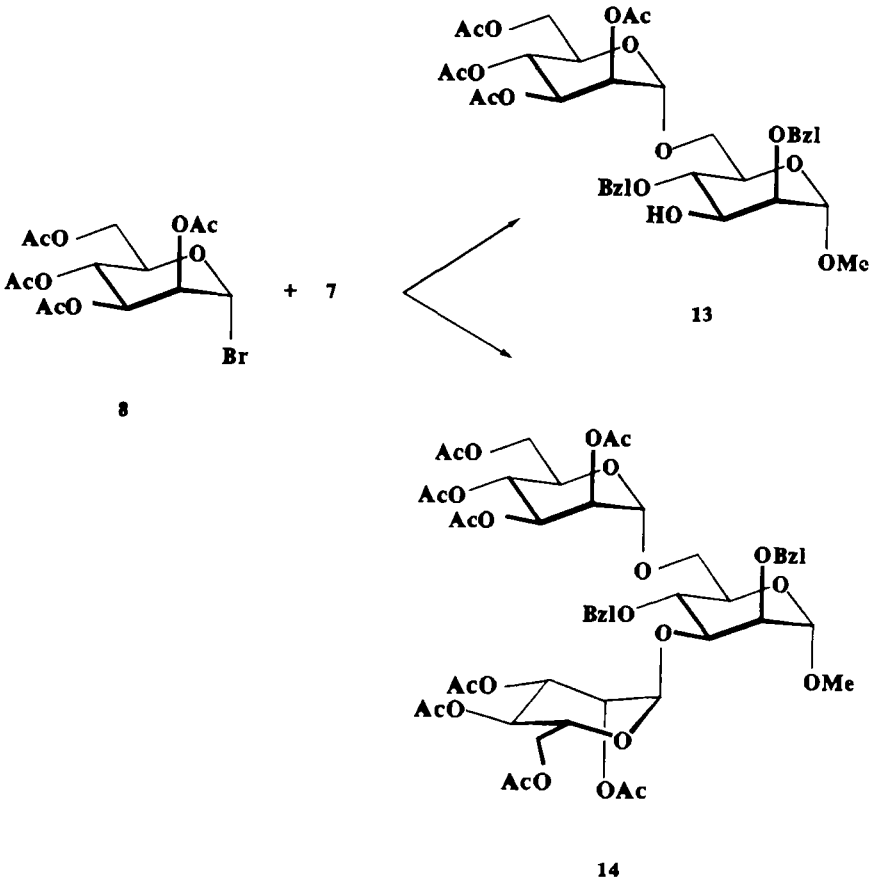


	R	R^1
10	Ac	Me
11	Ac	Ac
12	H	Me

Scheme 2

benzylidene acetal was stable at -40 °C, as was indicated in the ^{1}H NMR spectrum of **9** (δ 5.61 ppm for PhC*H*). Disaccharide **9** was converted into **10** by standard methods (Pd-C/H_2 and Ac_2O/Py) followed by the acetolysis of this latter compound (Ac_2O/H_2SO_4) at 0 °C to give **11**. The known methyl α-glycoside (**12**) was obtained on deprotection of **9** (Scheme 2).

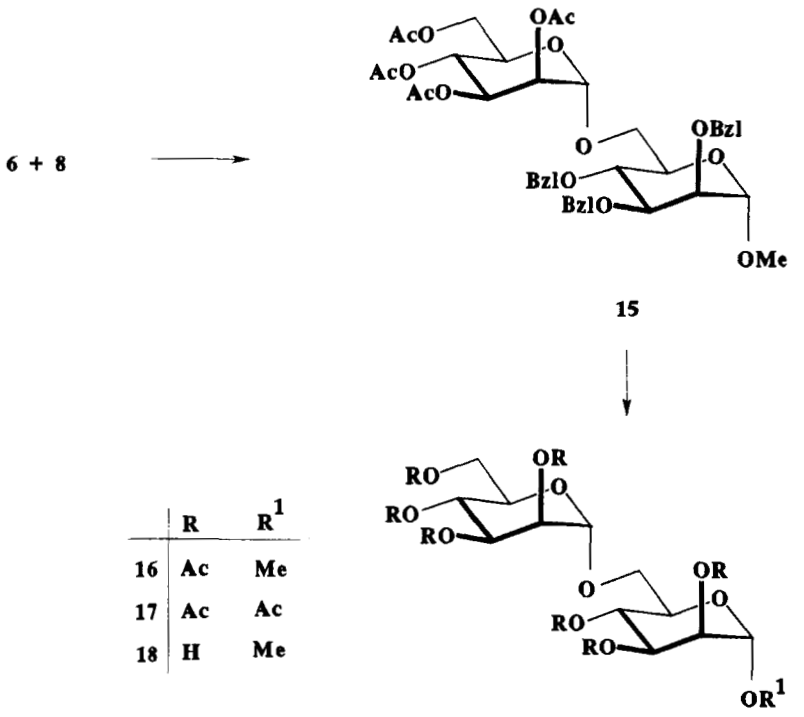
In order to synthesize the α(1→6)-linked mannobiose, as existing in N-glycoprotein antennae, methyl 2,4-di-*O*-benzyl-(**7**)- and methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranosides (**6**)[15] were selected as glycosyl acceptors. For construction of the (1→6) linkage, the $HgBr_2$ promoter[22] was used to minimize the possibility of orthoester formation.[24] However, selective mannosylation of diol **7** failed and the reaction with 1.2 equivalents of **8** (Scheme 3) resulted in a mixture of the corresponding (1→6) disaccharide **13** (26% isolated yield) and the protected trisaccharide **14** (18% isolated



Scheme 3

yield). This experiment showed that the HO-3 of D-mannose can be somewhat reactive to glycosylating reagents.

Similar conditions were applied for coupling **6** with 2 equivalents of **8**. The crude product was *O*-deacylated, the residue was partitioned between CH_2Cl_2 and water and then the organic phase was concentrated and reacetylated. Column chromatography of this product resulted in disaccharide **15** in an acceptable yield (67%). Disaccharide **15** was then converted into **16** by standard methods (Pd-C/H_2 and Ac_2O/Py). Acetolysis of this latter compound (Ac_2O/H_2SO_4) at 0 °C yielded **17** (Scheme 4). The (1→6)



	R	R^1
16	Ac	Me
17	Ac	Ac
18	H	Me

Scheme 4

disaccharide **16** was more sensitive to the reaction conditions than was **10**, so a little mannose peracetate was formed and detected on TLC. Zemplén *O*-deacetylation of compound **16** gave methyl 6-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (**18**).

The physical data for **10-12** and **16-18** were in good agreement with those reported in the literature.[25]

Acetylation and subsequent partial acetolysis of baker's yeast

All the chemical syntheses of manno-oligosaccharides are expensive and time consuming. As an alternative, we recently proposed a novel semisynthetic route for obtaining such materials from crude baker's yeast,[17] a rich source of manno-oligosaccharides. It has to be noted that partial acetolysis of isolated yeast mannan is a known procedure.[26] As described previously, powdered baker's yeast (7 g) was subjected

to acetylation and then partial acetolysis conditions to give 130 mg of *O*-α-D-mannopyranosyl-(1→2)-α-D-mannopyranose octaacetate **19**.[17] The second major product was a linear trisaccharide (200 mg) which is now shown to be *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→2)-α-D-mannopyranose undeca-acetate (**20**). For the second experiment 1 kg of baker's yeast was suspended in warm water, then filtered. Prior to treatment with 10% (v/v) H_2SO_4 in Ac_2O at 70 °C for 10 h, the substance was air-dried (225 g). After processing the solid product and the extract of the water phase showed different TLC patterns. Thus, these portions of the product of acetolysis were separately subjected to column chromatography. The "extract" (32 g) on 600 g of Kieselgel 60 gave several fractions which were collected as monosaccharide *(A)*, disaccharide *(B)*, trisaccharide *(C)*, and tetrasaccharide *(D)* regions. It is worth noting that Region *B* contained two components, one giving a "black spot" and the other "brown one" on TLC (charring with H_2SO_4). Column chromatography of the "solid" product (70 g) on 1 kg of Kieselgel 60 was more complicated and yielded not only regions *A*, *B*, *C*, and *D*, but also mixed fractions (*AB*, *BC*, *CD*), as well as fractions of higher oligosaccharides (*E* and *F*). The monosaccharide (*A*), disaccharide (*B*), trisaccharide (*C*), and tetrasaccharide (*D*) regions obtained by means of column chromatography from the "extract" and the "solid" were combined, respectively.

The acetolytic products (the combined Regions *B*, *C*, and *D*, respectively) were investigated by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. Region *B* contained exclusively per-*O*-acetylated disaccharides (*m/z* 701.6 [M+Na], 717.6 [M+K]), whilst per-*O*-acetylated trisaccharides (*m/z* 989.7 [M+Na], 1005.8 [M+K]) and tetrasaccharides (*m/z* 1278.1 [M+Na], 1294.2 [M+K]) were the main components of Region *C* and *D*, respectively (Figure 2).

In order to obtain valuable manno-oligosaccharides, some fractions were re-purified. Region *A* was a mixture of mannose and glucose peracetates from which penta-*O*-acetyl-α-D-glucopyranose crystallized readily. The main component of the mixed regions *AB* was octaacetyl-α,α-trehalose. Rechromatography of region *B* gave

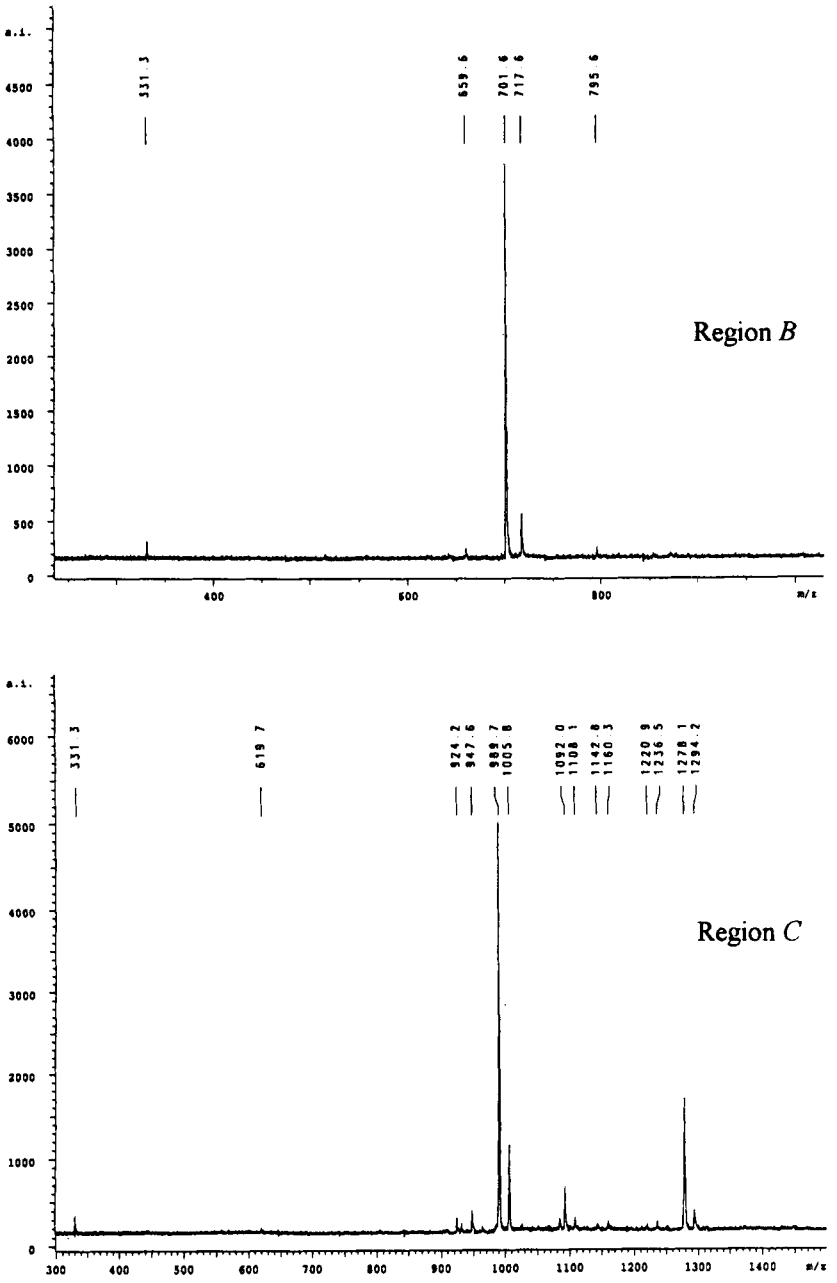


Figure 2

MALDI-TOF spectra of oligosaccharides (region *B* and region *C*)

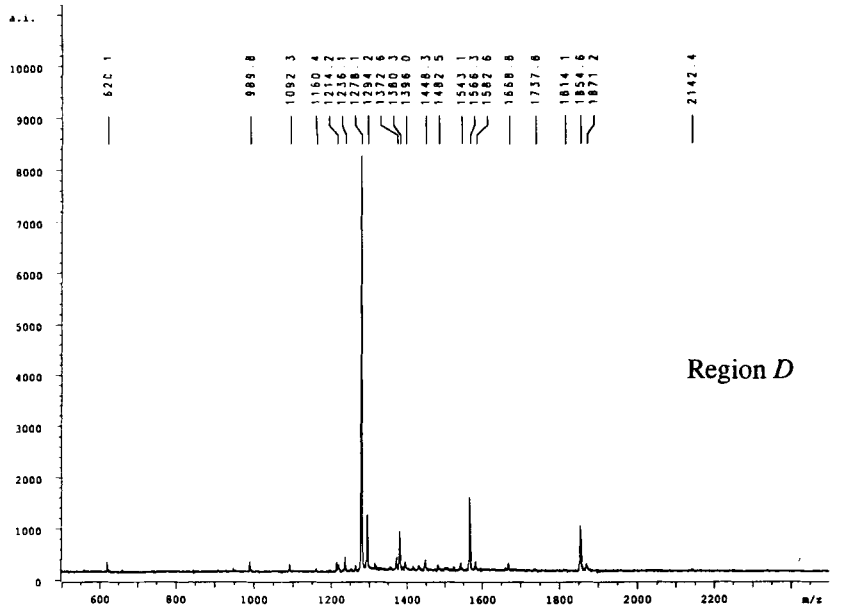


Figure 2 (continued)
MALDI-TOF spectra of oligosaccharides (region *D*)

octaacetyl-α,α-trehalose and *O*-α-D-mannopyranosyl-(1→2)-α-D-mannopyranose octaacetate (**19**). *O*-α-D-Mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→2)-α-D-mannopyranose undecaacetate (**20**) was obtained by means of column chromatography from region *C*. Unfortunately, region *D* was a very complicated mixture as indicated by TLC in different solvent systems. However, a linear tetrasaccharide could be obtained by repeated column chromatography. Mixed regions *BC* and *CD* were not further chromatographed.

As expected, compounds **19**, **20**, and the linear tetrasaccharide gave very simple MALDI-TOF spectra. Among the peaks, the $[M+Na]^+$ predominated. The MALDI-TOF spectrum of the tetrasaccharide derivative indicated that this substance was contaminated with a small amount of penta- and hexasaccharide acetates. Subsequent HPLC investigations confirmed this fact and showed only 92% purity of the sample. The exact

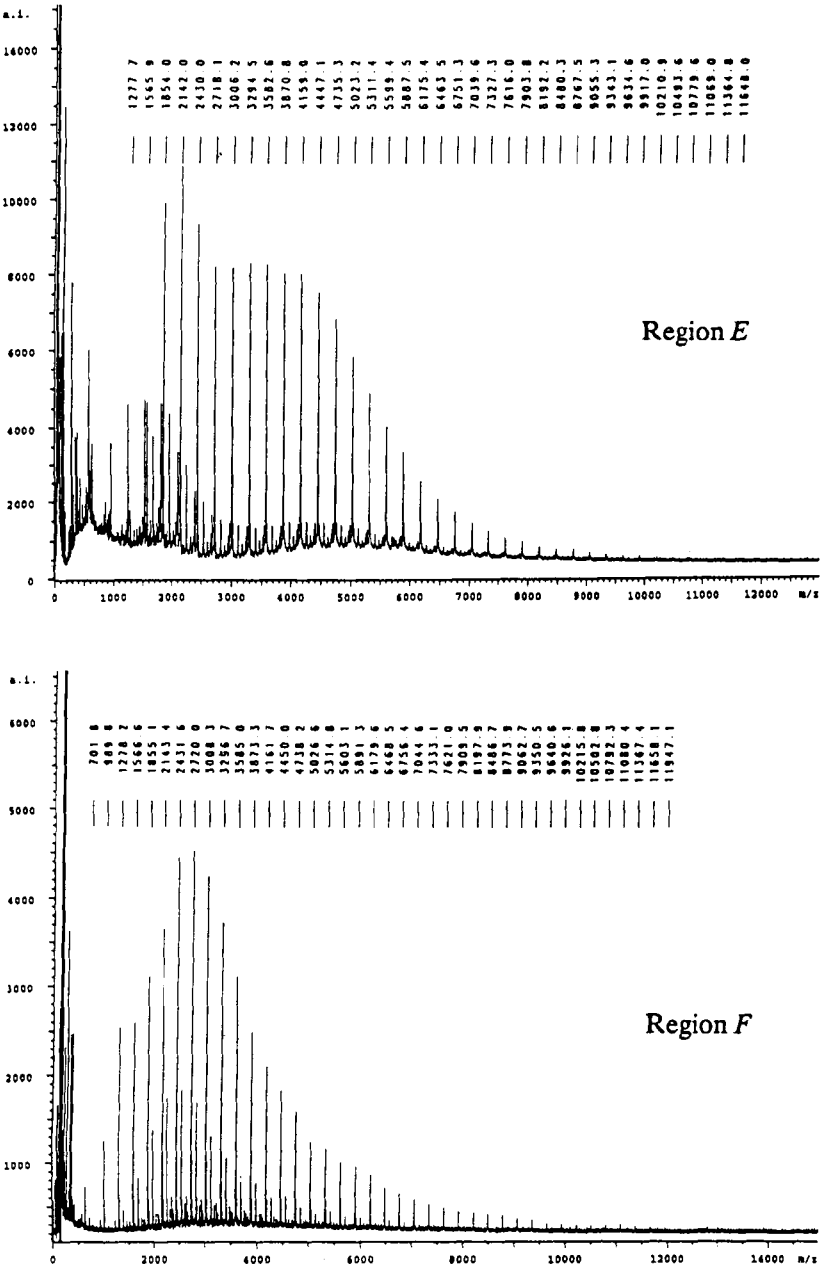
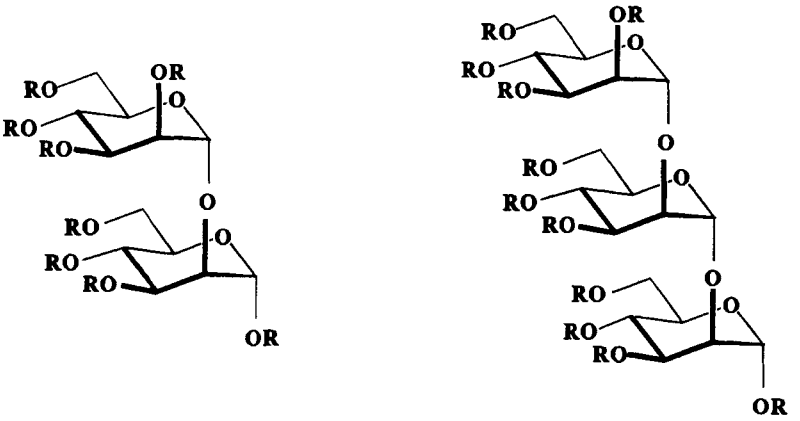


Figure 3

MALDI-TOF spectra for higher oligosaccharides (region *E* and region *F*)

Table 1
MALDI-TOF data for higher oligosaccharides (region *E*)

	m/z			
Sugar unit	[M+Na]	[M+Na-$C_{14}H_{19}O_9$]	[M-$C_2H_3O_2$]	[M-$C_4H_6O_4$]
4	1277.7	947.5	1195.1	1136.6
5	1565.9	1235.7	1482.5	1424.2
6	1854.0	1523.8	1770.3	
7	2142.0	1812.0	2058.2	
8	2430.0	2100.2	2346.6	
9	2718.1	2388.3		
10	3006.2	2676.7		
11	3294.5	2964.8		
12	3582.6	3253.1		
13	3870.8	3541.2		
14	4159.0	3829.2		
15	4447.1	4117.7		
16	4735.3	4405.7		
17	5023.2	4693.5		
18	5311.4	4981.4		
19	5599.4	5270.1		
20	5887.5	5558.5		
21	6175.4	5846.6		
22	6463.5	6133.0		
23	6751.3	6421.1		
24	7039.6	6708.9		
25	7327.3	6994.6		
26	7616.0	7282.1		
27	7903.8	7572.9		
28	8192.2	7861.2		
29	8480.3	8154.3		
30	8767.5			
35	10210.9			
40	11648.0			



	R
19	Ac
21	H

	R
20	Ac
22	H

Scheme 5

structure of this tetrasaccharide was not further investigated. The higher oligosaccharide region (*E*) had a very interesting spectrum. In the main series of the peaks from *m/z* 1277.7 (tetrasaccharide) to *m/z* 11648.0 (tetracontasaccharide), the peaks followed each other strictly by a 288 unit difference (Figure 3). The experimental data fitted in with the calculated ones within 0.1% accuracy. The covering curve of the peaks in this $[M+Na]^+$ series showed an almost ideal chromatographic elution profile. Comparing the intensity of the corresponding peaks it was shown that the heptasaccharide peracetate was the main component of region *E*. Some satellite series of peaks were observed with smaller intensities, such as $[M+Na\text{-}C_{14}H_{19}O_9]^+$ (for data, see Table 1). The other higher oligosaccharide region *F* showed the same pattern, but nonasaccharide peracetate being the main component.

Zemplén *O*-deacylation of **19** and **20** gave the well-known mannobiose **21**[25] and mannotriose **22**[27] derivatives, respectively (Scheme 5). Physical data of **21** and **22** were

in good accordance with those reported in the literature. The ^{13}C NMR spectrum of **22** clearly showed the (1→2) interglycosidic linkages.[28]

For the construction of glycan chains of oligo-mannose type N-glycoproteins, mannosyl peracetates (**11**, **17**, **19** and **20**) were prepared for conversion to the Koenigs-Knorr-donors or glycosyl imidates. The semisynthetic procedure using crude baker's yeast seems to be a simple and inexpensive route to some valuable manno-oligosaccharides, although the preparation of homogeneous tetra- or higher oligosaccharides is very complicated. It has been shown that MALDI-TOF mass spectrometry is an applicable method to investigate structures of the acetolytic products.

EXPERIMENTAL

General methods. Melting points (uncorrected) were determined on a Kofler hot-stage apparatus. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. NMR spectra were recorded with a Bruker WP-200 SY or Bruker DRX 500 spectrometer. Reactions were monitored by TLC on Kieselgel $60F_{254}$ (Merck) with detection by charring with H_2SO_4. Kieselgel 60 (Merck) was used for short-column chromatography. The MALDI measurements were carried out with a Bruker Biflex™ MALDI-TOF mass spectrometer, equipped with a 337-nm nitrogen laser. The instrument was used in both linear and reflector modes. The accelerating and the reflector voltages were 19.5 and 20.0 kV, respectively. 2,5-Dihydroxybenzoic acid was used as matrix and 100-200 laser shots were applied for each spectrum.

Methyl 2-*O*-Benzyl-4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (9). To a stirred mixture of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**5**, 745 mg, 2 mmol),[20] 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**8**, 987 mg, 2.4 mmol), 4A molecular sieves (powdered, 3 g), and dry CH_2Cl_2 (20 mL) was added a solution of silver triflate (617 mg, 2.4 mmol) in dry toluene (20 mL) for 10 min at -40 °C in an Ar atmosphere. Stirring was continued for another 30 min at -40 °C then pyridine (2 mL) was added. The mixture was diluted

with CH_2Cl_2 (50 mL), and filtered through Celite. The filtrate was washed with aq 10% $Na_2S_2O_3$ (2 x 20 mL) and water (2 x 20 mL), dried, and concentrated. The crystalline residue was recrystallized twice from EtOH to give **9** (640 mg; 45.5%): mp 166-168 °C; $[\alpha]_D$ +43° (*c* 1.53, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.50-7.23 (m, 10 H, aromatics), 5.61 (s, 1 H, PhC*H*), 5.48-5.16 (m, 4 H, H-1',2',3',4'), 4.80 (s, 2 H, PhCH_2), 4.76 (d, 1 H, H-1), 3.35 (s, 3 H, OMe), 2.08, 2.07, 2.06, and 2.00 (4 s, 12 H, 4 OAc); The combined mother liquors were concentrated and purified by means of column chromatography (9:1 CH_2Cl_2-EtOAc) to give an additional amount of **9** (498 mg; 35.4%). The overall yield was 1,138 mg (81%).

Anal. Calcd for $C_{35}H_{42}O_{15}$: C, 59.82; H, 6.03. Found: C, 59.99; H, 5.98.

Methyl 2,4,6-Tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (10). A mixture of **9** (703 mg, 1 mmol), EtOH (100 mL), HOAc (10 mL), and Pd-C (100 mg) was stirred under H_2 atmosphere overnight. The catalyst was filtered off and the filtrate was concentrated. The residue was dissolved in pyridine (8 mL) and Ac_2O (8 mL) was added. The mixture was kept overnight, at room temperature, then it was concentrated and co-evaporated with toluene (3 x 10 mL). Column chromatography of the residue (75:25 CH_2Cl_2-EtOAc) gave **10**: 618 mg (95%); $[\alpha]_D$ +35.6° (*c* 0.45, $CHCl_3$); Lit. $[\alpha]_D$ +34.9° ($CHCl_3$),[29] ^1H NMR ($CDCl_3$): δ 4.98 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.72 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 3.38 (s, 3 H, OMe), 2.21, 2.13, 2.12, 2.11, 2.06, 2.05, and 1.99 (7 s, 21 H, 7 OAc).

1,2,4,6-Tetra-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranose (11). To a solution of **10** (325 mg, 0.5 mmol) in Ac_2O (1 mL) was slowly added 4% (v/v) H_2SO_4 in Ac_2O (1 mL) at 0 °C. The mixture was stirred for 3 h at 0 °C then another 3 h at room temperature, poured into ice-water containing $NaHCO_3$, and then extracted with CH_2Cl_2 (3 x 30 mL). The combined extracts were washed with aq 5% $NaHCO_3$ (15 mL) and water (3 x 20 mL), dried, concentrated and co-evaporated with toluene (3 x 10 mL). The product was purified by column chromatography (6:4 hexane-EtOAc) to yield **11** (295 mg; 87%) as a foam; $[\alpha]_D$ +36.3°

(*c* 0.3, $CHCl_3$); Lit. $[\alpha]_D$ +35.9°($CHCl_3$);[30] mp 170-172 °C; $[\alpha]_D$ +37.6° ($CHCl_3$),[31] ^1H NMR ($CDCl_3$): δ 6.10 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), 5.03 (bs, 1 H, H-1'), 2.24, 2.15, 2.09, 2.06, and 2.00 (5 s, 24 H, 8 OAc).

Methyl 3-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (12). A mixture of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-manno-pyranosyl)-α-D-mannopyranoside (**9**; 246 mg, 0.35 mmol), EtOH (10 mL), HOAc (2 mL), and Pd-C (30 mg) was stirred in an H_2 atmosphere overnight. The catalyst was filtered off, the filtrate was concentrated and co-evaporated with toluene (3 x 10 mL). The residue was dissolved in dry MeOH (30 mL), NaOMe (catalytic amount) was added, and the solution was kept overnight at room temperature. The mixture was neutralized with Amberlite IR 120 (H^+) resin, filtered, and concentrated. The product was passed through a column of Kieselgel 60 with BuOH-MeOH-H_2O 2:1:1 as an eluent to give 99 mg (79%) of **12**; amorphous; $[\alpha]_D$ +94.2° (*c* 1, MeOH), +81.2° (*c* 0.16, H_2O); Lit. $[\alpha]_D$ +94.8° (H_2O);[32] ^{13}C NMR (D_2O): δ 103.04 (C-1'), 101.46 (C-1), 78.90 (C-3), 61.64 and 61.52 (C-6,6'), 55.44 (OMe); FABMS Calcd for $C_{13}H_{24}O_{11}$ (356.3). Found: *m/z* 357 $[M+H]^+$, 379 $[M+Na]^+$.

Methyl 2,4-Di-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (13) and Methyl 2,4-Di-*O*-benzyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (14). A mixture of methyl 2,4-di-*O*-benzyl-α-D-mannopyranoside[15] (**7**, 374 mg, 1 mmol), compound **8** (493 mg, 1.2 mmol), dry CH_2Cl_2 (8 mL), and 4A activated powdered molecular sieves (1.5 g) was stirred under Ar for 15 min. $HgBr_2$ (360 mg, 1 mmol) was added and stirring was continued for 24 h. The mixture was diluted with CH_2Cl_2 (40 mL) and filtered through Celite. The filtrate was washed with aq 5% KI (2 x 10 mL) and water (3 x 10 mL), dried, and concentrated. The products were separated by column chromatography (95:5 CH_2Cl_2-acetone). The compound that first eluted was **13** as a foam: 182 mg (25.8%); $[\alpha]_D$ +56.3° (*c* 3.9, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.40-7.25 (m, 10 H, aromatics), 5.39-5.24 (m, 3 H, H-2',3',4'), 5.00-4.53 (m, 4 H, 2 PhCH_2), 4.95 (d, 1 H, $J_{1',2'}$ 1.6 Hz, H-1'),

4.75 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 3.35 (s, 3 H, OMe), 2.36 (bs, 1 H, OH, deuterable), 2.15, 2.07, 2.03, and 1.98 (4 s, 12 H, 4 OAc);

Anal. Calcd for $C_{35}H_{44}O_{15}$: C, 59.65; H, 6.29. Found: C, 59.82; H, 6.30.

Second eluted was **14** as a foam: 190 mg (18.4%); $[\alpha]_D$ +70° (*c* 0.58, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.44-7.23 (m, 10 H, aromatics), 5.42-5.16 (m, 6 H, H-2',3',4' and H-2",3",4"), 5.13 (d, 1 H, $J_{1",2"}$ 1.0 Hz H-1") 4.90 (d, 1 H, $J_{1',2'}$ 1.4 Hz, H-1'), 4.85-4.57 (m, 4 H, 2 PhCH_2), 4.76 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 3.35 (s, 3 H, OMe), 2.14, 2.06, 2.05, 2.04, 2.03, 2.00, and 1.98 (7 s, 24 H, 8 OAc).

Anal. Calcd for $C_{49}H_{62}O_{24}$: C, 56.86; H, 6.04. Found: C, 56.99; H, 5.95.

Methyl 2,3,4-tri-*O*-Benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (15). Methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside[15] (**6**, 465 mg, 1 mmol) was reacted with **8** (822 mg, 2 mmol) in the presence of $HgBr_2$ (360 mg, 1 mmol) as described for the preparation of **13** and **14**. The crude product was dissolved in dry MeOH (20 mL), the pH was adjusted to 8 by the addition of NaOMe and the solution was kept overnight at room temperature. After neutralization (HOAc) and concentration the residue was partitioned between CH_2Cl_2 (40 mL) and water (5 mL). The organic phase was separated, dried, and concentrated. The product was dissolved in pyridine (2 mL), Ac_2O (2 mL) was added and the reaction mixture was kept at room temperature for 4 h. The solution was concentrated and co-evaporated with toluene (3 x 5 mL). The residue was purified by column chromatography (9:1 CH_2Cl_2-EtOAc) to give **15** as a glass: 533 mg (67%); $[\alpha]_D$ +59.2° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.43-7.23 (m, 15 H, aromatics), 5.41-5.20 (m, 3 H, H-2',3',4'), 3.32 (s, 3 H, OMe), 2.14, 2.05, 2.02, and 1.97 (4 s, 12 H, 4 OAc);

Anal. Calcd for $C_{42}H_{50}O_{15}$: C, 63.46; H, 6.34. Found: C, 63.30; H, 6.28.

Methyl 2,3,4-Tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (16). Compound **15** (397 mg, 0.5 mmol) was hydrogenated and then acetylated as described for the preparation of **10** to give **16** as a foam: 280 mg (86%); $[\alpha]_D$ +60.5° (*c* 1.01, $CHCl_3$); Lit. $[\alpha]_D$ +62.5° ($CHCl_3$),[33] ^1H NMR ($CDCl_3$): δ

5.40-5.18 (m, 6 H, H-2,3,4,2',3',4'), 4.87 (bs, 1 H, H-1'), 4.69 (bs, 1 H, H-1), 3.42 (s, 3 H, OMe), 2.16, 2.11, 2.06, 2.05, 2.00, and 1.99 (6 s, 21 H, 7 OAc).

1,2,3,4-Tetra-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranose (17). Compound **16** (325 mg, 0.5 mmol) was acetolyzed as described for the preparation of **11** until the starting material disappeared (1 h). TLC (6:4 hexane-EtOAc) showed the presence of a small amount of mannose-pentaacetate. Column chromatography of the crude product (6:4 hexane-EtOAc) gave **17** (245 mg; 72%); $[\alpha]_D$ +64.2° (*c* 0.52, $CHCl_3$); Lit. mp 162 °C; $[\alpha]_D$ +68° ($CHCl_3$),[34] ^1H NMR ($CDCl_3$): δ 6.05 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.83 (bs, 1 H, H-1'), 2.20, 2.19, 2.16, 2.11, 2.08, 2.06, 2.02, and 1.98 (8 s, each 3 H, 8 OAc).

Methyl 6-*O*-(α-D-Mannopyranosyl)-α-D-mannopyranoside (18). To a solution of **16** (200 mg, 307 μmol) in dry MeOH (20 mL) was added a catalytic amount of NaOMe. The reaction mixture was kept overnight at room temperature, then neutralized with Amberlite IR 120 (H^+) resin, filtered, and concentrated to give 105 mg (96%) of **18** as an amorphous foam: $[\alpha]_D$ +73.2° (*c* 0.23, H_2O); $[\alpha]_D$ +76.1° (*c* 0.76, MeOH); lit. $[\alpha]_D$ +88.3° (H_2O),[35] ^{13}C NMR (D_2O): δ 101.71 (C-1'), 100.16 (C-1), 66.30 (C-6), 61.65 (C-6'), 55.48 (OMe); FABMS Calcd for $C_{13}H_{24}O_{11}$ (356.3). Found: *m/z* 357 $[M+H]^+$, 379 $[M+Na]^+$.

Acetylation and Subsequent Partial Acetolysis of Baker's Yeast. Crude baker's yeast (1 kg) was suspended in warm (45 °C) water, then filtered and air-dried. This material (225 g) was added in portions to a vigorously stirred mixture of Ac_2O (600 mL) and concd H_2SO_4 (60 mL) for a period of 30 min, then stirring was continued for 10 h at 70 °C. After cooling, the mixture was poured into vigorously stirred water (6 L). The separated solid was filtered off, washed with water (2 x 300 mL) and then air-dried (134 g). The water phase was extracted with CH_2Cl_2 (3 x 400 mL), the organic solution was successively washed with aq 10% NaOH (2 x 120 mL) and water (3 x 120 mL), dried, and concentrated to give syrupy crude material (32 g). The dry material (134 g) was suspended in CH_2Cl_2 (1.5 L) and the mixture was filtered. The filtrate was washed

with 10% aq NaOH (2 x 200 mL), water (4 x 200 mL), dried over $MgSO_4$, and concentrated (70 g). The crude products were chromatographed separately. In the case of the "extract" (32 g), column chromatography on Kieselgel 60 (600 g) with CH_2Cl_2-acetone (9:1→85:15→8:2) as an eluent, gave several fractions which were collected as monosaccharide region *A* (5.5 g), disaccharide region *B* (5.4 g), trisaccharide region *C* (2.25 g), and tetrasaccharide region *D* (2.1 g). The crude product from "solid" (70 g) on 1 kg of Kieselgel, under the same conditions yielded regions *A* (0.6 g), *AB* (5.24 g), *B* (3.75 g), *BC* (6.6 g), C (5.75 g), *CD* (4.52 g), and *D* (9.18 g). Continued elution with 8:2 CH_2Cl_2-MeOH gave higher oligosaccharide regions *E* (5.26 g); $[\alpha]_D$ +65.9° (*c* 1.55 $CHCl_3$); and *F* (8.99 g); $[\alpha]_D$ +70.5° (*c* 0.96 $CHCl_3$). For MALDI-TOF data, see Table 1.

Recrystallization of the combined regions *A* (twice from EtOH) gave only penta-*O*-acetyl-α-D-glucopyranose. In the case of region *AB* the same procedure yielded octaacetyl-α,α-trehalose.

Compound **19** was isolated by rechromatography of the combined regions *B* (9.15 g) on a column of Kieselgel 60 (550 g) using 9:1→85:15 CH_2Cl_2-acetone as an eluent; 2.78 g; $[\alpha]_D$ +36.7° (*c* 1 $CHCl_3$); mp 138-140 °C (EtOH); ^1H NMR (500 MHz, $CDCl_3$): 6.24 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 5.43 (t, 1 H, $J_{4',5'}$ 10.0 Hz H-4') 5.41 (dd, 1 H, $J_{3',4'}$ 10.0 Hz, H-3'), 5.29 (dd, 1 H, $J_{3,4}$ 10.1 Hz H-3) 5.27 (dd, 1 H, $J_{2',3'}$ 3.4 Hz, H-2'), 5.26 (t, 1 H, $J_{4,5}$ 10.1 Hz, H-4), 4.95 (d, 1 H, $J_{1',2'}$ 1.6 Hz, H-1'), 4.25-4.11 (m, 5 H, H-5', H-6a,6b, H-6a'6b'), 4.05 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 4.01 (m, 1 H, H-5), 2.15, 2.14, 2.10, 2.08, 2.05, 2.04, 2.035, and 2.01 (8 s, each 3 H, 8 OAc); ^{13}C NMR ($CDCl_3$): δ 170.84, 170.71, 170.45, 169.88, 169.68, 169.41, 169.15, and 168.15 (C=O), 99.18 ($J_{C-1',H-1'}$ 176 Hz, C-1'), 91.36 ($J_{C-1,H-1}$ 179 Hz, C-1), 75.82 (C-2), 70.62, 69.76, 69.63, 69.54, 68.25, 66.13, and 65.39 (skeleton C-s), 62.35 and 61.65 (C-6 and C-6'), 20.81, 20.55, and 19.66 (C-Me); MALDI-TOF: Calcd for $C_{28}H_{38}O_{19}$ (678.60). Found: *m/z* 701.5 [M+Na], 717.6 [M+K].

Compound **20** was obtained by means of column chromatography of the combined regions *C* (8 g) on a column of Kieselgel 60 (500 g): 3.56 g; $[\alpha]_D$ +39.1° (*c* 0.89 $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): 6.25 (d, 1 H, $J_{1,2}$ 2.3 Hz, H-1), 5.14 (d, 1 H, $J_{1'',2''}$ 2.1 Hz, H-1''), 4.95 (d, 1 H, $J_{1',2'}$ 1.9 Hz, H-1'), 4.15 (t, 1 H, H-2), 4.08 (t, 1 H, H-2'), 2.16, 2.157, 2.14, 2.13, 2.10, 2.09, 2.06, 2.05, 2.048, 2.046, and 2.01 (11 s, each 3 H, 11 OAc) ; ^{13}C NMR ($CDCl_3$): δ 170.82, 170.41, 170.14, 169.98, 169.71, 169.41, 169.30, 169.20, and 168.20 (C=O), 99.81 ($J_{C\text{-}1'',H\text{-}1''}$ 175.4 Hz, C-1''), 99.31 ($J_{C\text{-}1',H\text{-}1'}$ 173.5 Hz, C-1'), 91.49 ($J_{C\text{-}1,H\text{-}1}$ 180 Hz, C-1), 77.36 (C-2), 75.38 (C-2'), 70.71, 69.92, 69.60, 69.47, 68.33, 66.20, 65.98, and 65.47 (skeleton C-s), 62.41, 62.12, and 61.60 (C-6, C-6', and C-6''), 20.77 and 20.55 (C-Me); MALDI-TOF: Calcd for $C_{40}H_{54}O_{27}$ (966.86). Found: *m/z* 989.4 [M+Na], 1005.4 [M+K].

Repeated column chromatography of a sample (0.5 g) of the combined regions *D* resulted in a tetrasaccharide: 70 mg; $[\alpha]_D$ +35.6° (*c* 0.64 $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 99.79, 99.38, 99.00, and 91.40 (anomeric region), 62.51, 62.38, 61.76, and 61.53 (for C-6,6',6'',6'''); MALDI-TOF: Calcd for $C_{52}H_{70}O_{35}$: (1255.12). Found: *m/z* 1278.0 [M+Na], 1294.2 [M+K]. The exact structure of this tetrasaccharide was not further investigated.

***O*-α-D-Mannopyranosyl-(1→2)-α-D-mannopyranose (21).** To a solution of compound **19** (100 mg, 147 μmol) in dry MeOH (25 mL) was added a catalytic amount of NaOMe. The mixture was kept overnight at room temperature, then neutralized with Amberlite IR 120 (H^+) resin, filtered and concentrated to give **21** (49 mg; 97%) as a white foam; $[\alpha]_D$ +51.8° (*c* 0.52 1:1 H_2O-MeOH); Lit. $[\alpha]_D$ +48° (*c* 0.08 H_2O),[26] +60° (*c* 0.1 H_2O),[36] ^{13}C NMR (D_2O): δ 102.86 (C-1'), 93.21 (C-1), 79.80 (C-2), 61.75 (C-6), 61.63 (C-6').

***O*-α-D-Mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→2)-α-D-mannopyranose (22).** Compound **20** (140 mg, 145 μmol) was deacetylated as described for **21** to yield **22** (70 mg, 96%) as a white foam; $[\alpha]_D$ +53.9° (*c* 1 H_2O); Lit. $[\alpha]_D$ +55° (H_2O)[37]; +55.3° (H_2O),[36] +48° (H_2O),[38] ^{13}C NMR (D_2O): δ 102.95 (C-1''), 101.27 (C-

1'), 93.20 (C-1), 80.06 (C-2), 79.27 (C-2'), 61.78 with roughly double int., and 61.66 (C-6,6',6").

ACKNOWLEDGMENTS

The authors thank Prof. András Lipták, Mr. Károly Ágoston, and Miss Mónika Szakos for their interest and help. Financial support from the National Science Foundation **(OTKA 1694** and **OTKA T015543)** is gratefully acknowledged.

REFERENCES

1. T. Ogawa, K. Katano, and M. Matsui, *Carbohydr. Res.*, **64**, C3 (1978).
2. J. Arnarp and J. Lönngren, *J. Chem. Soc., Chem. Commun.*, 1000 (1980).
3. T. Ogawa and K. Sasajima, *Tetrahedron*, **37**, 2787 (1981).
4. J. Arnarp, M. Haraldson, and J. Lönngren, *J. Chem. Soc., Perkin Trans. 1.*, 1841 (1982).
5. F. M. Winnik, J. P. Carver, and J. J. Krepinsky, *J. Org. Chem.*, **47**, 2701 (1982).
6. T. Ogawa, T. Kitajima, and T. Nukada, *Carbohydr. Res.*, **123**, C8 (1983).
7. H. Paulsen, M. Heume, Z. Györgydeák, and R. Lebuhn, *Carbohydr. Res.*, **144**, 57 (1985).
8. S. H. Tahir and O. Hindsgaul, *Can. J. Chem.*, **69**, 1771 (1986).
9. W. Günther and H. Kunz, *Carbohydr. Res.*, **228**, 217 (1992).
10. A. Dan, Y. Ito, and T. Ogawa, *Carbohydr. Lett.*, **1**, 469 (1996).
11. P. Grice, S. V. Ley, J. Pietruszka, and H. W. M. Priepke, *Angew. Chem. Int. Ed. Engl.*, **35**, 197 (1996).
12. C. Unverzagt, *Angew. Chem. Int. Ed. Engl.*, **35**, 2350 (1996).
13. J. Kerékgyártó, J. P. Kamerling, J. B. Bouwstra, J. F. G. Vliegenthart, and A. Lipták, *Carbohydr. Res.*, **186**, 51 (1989).
14. J. Kerékgyártó, J. G. M. van der Ven, J. P. Kamerling, A. Lipták, and J. F. G. Vliegenthart, *Carbohydr. Res.*, **238**, 135 (1993).
15. Z. Szurmai, L. Balatoni, and A. Lipták, *Carbohydr. Res.*, **254**, 301 (1994).
16. J. G. M. van der Ven, J. Kerékgyártó, J. P. Kamerling, A. Lipták, and J. F. G. Vliegenthart, *Carbohydr. Res.*, **264**, 45 (1994).
17. Z. Szurmai and L. Jánossy, *Carbohydr. Res.*, **296**, 279 (1996).
18. F. W. Lichtenthaler, U. Kläres, Z. Szurmai, and B. Werner, *Carbohydr. Res.*, submitted for publication
19. Yu. Nakahara, S. Shibayama, Yo. Nakahara, and T. Ogawa, *Carbohydr. Res.*, **280**, 67 (1996).
20. A. Lipták, I. Czégény, J. Harangi, and P. Nánási, *Carbohydr. Res.*, **73**, 327 (1979).
21. F. J. Kronzer and C. Schuerch, *Carbohydr. Res.*, **27**, 379 (1973); S. Hanessian and J. Banoub, *Carbohydr. Res.*, **53**, C13 (1977).

22. J.-C. Jacquinet, D. Duchet, M.-L. Milat, and P. Sinay, *J. Chem. Soc., Perkin Trans. 1*, 326 (1981).
23. R. R. Schmidt and J. Michel, *Angew. Chem.*, **92**, 763 (1980).
24. T. Ogawa, K. Katano, K. Sasajima, and M. Matsui, *Tetrahedron*, **37**, 2779 (1981).
25. A. Lipták, P. Fügedi, and Z. Szurmai, Handbook of Oligosaccharides, Vol. I. Disaccharides, CRC Press Inc., Boca Raton, FL (1990).
26. S. Peat, J. R. Turvey, and D. Doyle, *J. Chem. Soc.*, 3918 (1961).
27. A. Lipták, Z. Szurmai, J. Harangi, and P. Fügedi, Handbook of Oligosaccharides, Vol. II. Trisaccharides, CRC Press Inc., Boca Raton, FL (1990).
28. P. A. J. Gorin, *Can. J. Chem.*, **51**, 2375 (1973).
29. M. S. Chowdhary, R. K. Jain, S. S. Rana, and K. L. Matta, *Carbohydr. Res.*, **152**, 323 (1986).
30. M. M. Ponpipom, *Carbohydr. Res.*, **59**, 311 (1977).
31. Y. Itoh and S. Tejima, *Chem. Pharm. Bull.*, **32**, 957 (1984).
32. T. Ogawa and K. Sasajima, *Carbohydr. Res.*, **93**, 53 (1981).
33. L. V. Backinowsky, Y. E. Tsvetkov, N. F. Balan, N. E. Byramova, and N. K. Kochetkov, *Carbohydr. Res.*, **85**, 209 (1980).
34. C. M. Reichert, *Carbohydr. Res.*, **77**, 141 (1979).
35. R. Madiyalakan, M. S. Chowdhary, S. S. Rana, and K. L. Matta, *Carbohydr. Res.*, **152**, 183 (1986).
36. T. Ogawa and H. Yamamoto, *Carbohydr. Res.*, **104**, 271 (1982).
37. P. A. J. Gorin and A. S. Perlin, *Can. J. Chem.*, **35**, 262 (1957).
38. A. Lipták, J. Imre, and P. Nánási, *Carbohydr. Res.*, **114**, 35 (1983)