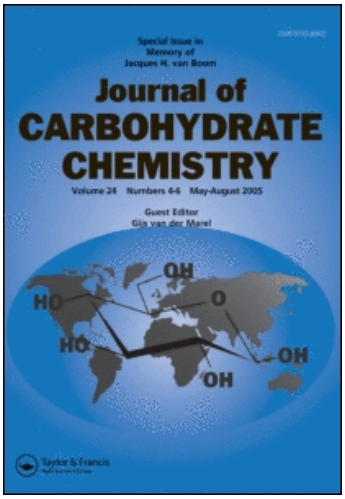

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Synthetic Antigens: Synthesis of 4-Aminophenyl *O*-α-D-Mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→6)-*O*-α-D-Mannopyranoside and A Related Di- and A Trisaccharide

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SYNTHETIC ANTIGENS: SYNTHESIS OF 4-AMINOPHENYL *O*-α-D-MANNOPYRANOSYL-(1→2)-*O*-α-D-MANNOPYRANOSYL-(1→6)-*O*-α-D-MANNOPYRANOSIDE AND A RELATED DI- AND A TRISACCHARIDE[1]

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ABSTRACT

4-Nitrophenyl 2,3-*O*-isopropylidine-α-D-mannopyranoside **2** was condensed with *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl bromide **1** and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide **11** in the presence of mercuric cyanide. Products were deprotected to yield, respectively, 4-nitrophenyl *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside **6** and 4-nitrophenyl *O*-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside **14**. The 4-nitrophenyl group of **6** was reduced to give title trisaccharide. Bromide **1** was also condensed with methyl 2,3,4-tri-*O*-benzyl-α-D-manopyranoside **3** in the presence of silver trifluoromethanesulfonate and tetramethylurea to give protected trisaccharide derivative which was deprotected to furnish, methyl *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside **10**. The identities of all protected and deprotected compounds were supported by ^{1}H and ^{13}C NMR spectral data.

INTRODUCTION

Pioneering work of Goebel and Avery[3,4] showed that simple oligosaccharides, normally nonimmunogenic could invoke immune response when conjugated to protein carriers and consequently can be used for the formation of carbohydrate specific antibodies. In the past oligosaccharides from natural sources constituted the major source for such

antigens. However, the difficult task[5] of obtaining these oligosaccharides in pure form for analyses, and subsequent production and screening of antibodies made it apparent that synthesis could provide an alternative source. It may also be pointed out that oligosaccharides isolated from natural sources are obtained as reducing sugars, whereas a synthetic approach can provide the total hapten,[6] thereby preserving the appropriate anomeric configuration of the sugar moiety at the reducing end. For these reasons our laboratory has been involved in the chemical synthesis of oligosaccharide determinants that occur on glycoconjugates. These oligosaccharide haptens are being synthesized in a form suitable for coupling to a carrier protein, so as to produce immunogens.[7-12] The title trisaccharide **7**, described here was coupled via a diazotization reaction[13,14] to bovine serum albumin, and the resulting glycoconjugate was employed to immunize rabbits.[12] Trisaccharide **10** and disaccharide **14** were used in enzyme immunoassays (EIA) to examine the specificity of the antibody.[12]

RESULTS AND DISCUSSION

Regioselective glycosylation of 4-nitrophenyl 2,3-*O*-isopropylidine-α-D-mannopyranoside **2**[15] with *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl bromide **1**[16] in 1:1 benzene-nitromethane at room temperature, in the presence of mercuric cyanide gave a crude product mixture (containing **4**) which was treated with aqueous trifluoroacetic acid in chloroform to afford, after chromatographic purification, trisaccharide derivative **5**. The ^{1}H NMR of **5** showed three doublets ($J \sim$ 1.5 Hz) at δ 5.69, 4.95, and 4.79, that were assigned to anomeric protons H-1, H-1', and H-1", respectively. *O*-Deacetylation of **5** (Zemplén) furnished **6** (74%). Reduction of the nitro group of **6** in the presence of PtO_2 gave trisaccharide derivative **7** which was coupled to BSA in 9:1 molar ratio.[12]

Condensation of methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside **3**[17] with bromide **1** in dichloromethane in the presence of silver trifluoromethanesulfonate and tetramethylurea gave a crude product mixture (containing **8**) which was *O*-deacetylated to furnish a partially protected trisaccharide **9**. The ^{1}H and ^{13}C NMR spectra of **9** contained signals supporting its overall structure (see Table 1). Catalytic hydrogenolysis of **9** in the presence of 10% Pd-C then gave the trisaccharide **10** (80%).

Reaction of diol **2** with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide **11**[18,19] in acetonitrile (promoted by mercuric cyanide) at room temperature, followed by chromatography, gave **12** (68%). Deacetonation of **12** with aqueous trifluoroacetic acid gave **13** (77%) which was subjected to Zemplén transesterification to give **14** (72%).

TABLE 1.

SELECTED ^{1}H AND ^{13}C NMR DATA FOR PROTECTED DI- AND TRISACCHARIDES[a]

Nucleus	5	9	12	13
H-1 ($J_{1,2}$)	5.69 (1.0)	5.16 (1.5)	5.92 (<1)	5.70 (1.0)
H-1' ($J_{1',2'}$)	4.95 (1.5)	4.95 (1.5)	4.72 (1.5)	4.87 (1.5)
H-1" ($J_{1",2"}$)	4.79 (1.5)	4.76 (1.5)	-	-
OCH_3	-	3.33	-	-
OAc	2.15 (6H), 2.08 2.07, 2.04, 2.01	-	2.22, 2.12 2.10, 1.91	2.17, 2.13 2.12, 1.96
$(CH_3)2$	-	-	1.55, 1.42	-
C_6H_4-NO_2 (^{3}J)	8.24 (9.0) 7.18 (9.0)	- -	8.26 (9.0) 7.20 (9.0)	8.22 (9.0) 7.17 (9.0)
C-1	97.60[b]	100.55[d]	94.88	97.57[f]
C-1'	97.88[b]	100.18[d]	97.06	97.18[f]
C-1"	99.09	104.14	-	-
C-6	66.34	67.79	66.48	66.27
C-6'	62.39[c]	62.92[e]	62.72	62.63
C-6"	62.64[c]	62.82[e]	-	-
OCH_3	-	55.33	-	-
$\underline{C}OCH_3$	171.34, 171.02, 170.77, 169.99 169.74, 169.69	-	170.99, 170.00 169.89, 169.53	171.19, 170.48 170.34, 169.86
$CO\underline{C}H_3$	20.85, 20.78, 20.75 20.67, 20.59	-	20.85, 20.78 20.33	20.88, 20.83 20.74, 20.59
$C(\underline{C}H_3)2$	-	-	28.05, 26.35	-
$\underline{C}(CH_3)2$	-	-	110.37	-
CNO2	160.46	-	160.26	160.44
CO (phenolic)	142.79	-	142.89	142.83

a. Spectra were recorded at 300 MHz (^{1}H in $CDCl_3$ for compounds **5** and **13** and in CD_3OD for **9**) or 400 MHz (^{1}H in $CDCl_3$, compound **12**) and 75.5 MHz (^{13}C in $CDCl_3$ for compounds **5** and **12** and **13** and in CD_3OD for **9**); n.d., Not determined.

b-f. Values with the same superscripts may be interchanged.

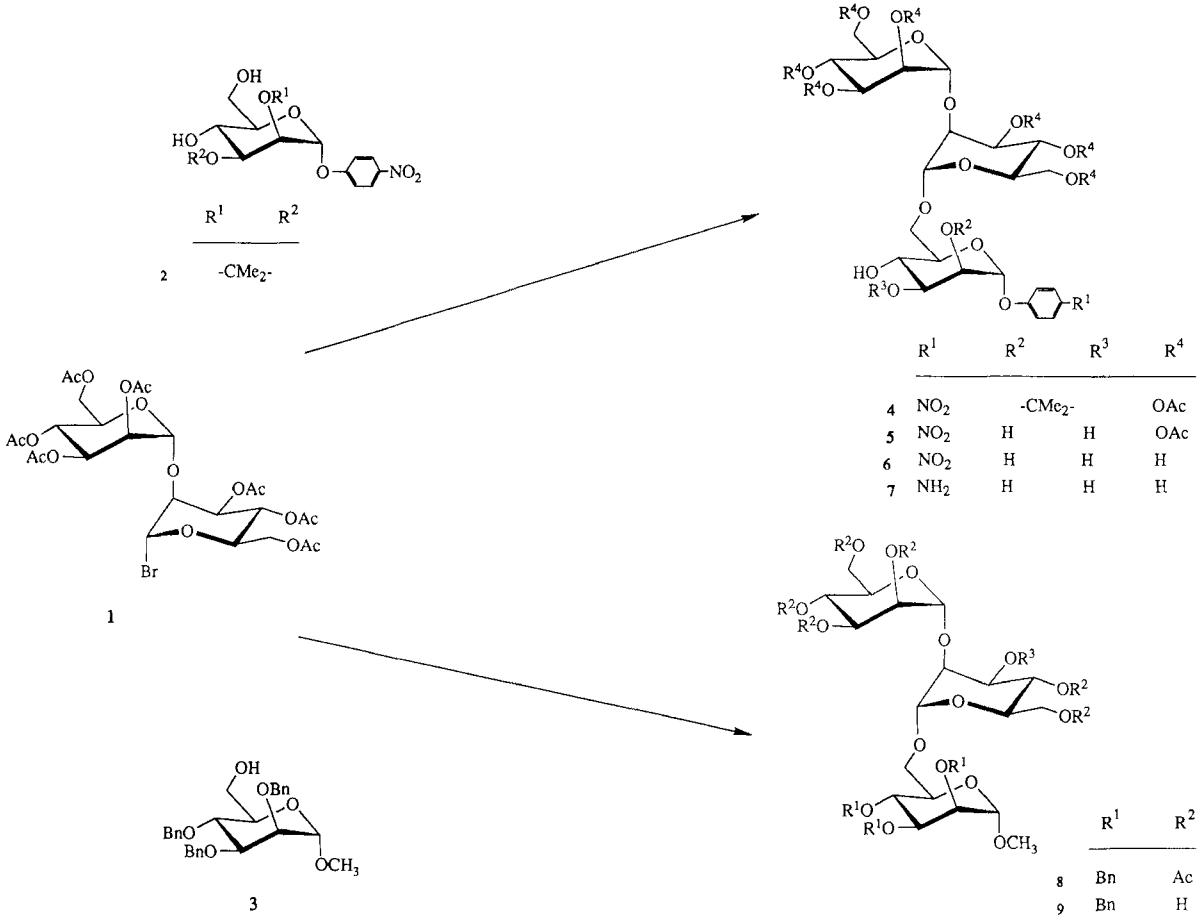
TABLE 2 .

SELECTED ^{1}H AND ^{13}C NMR DATA FOR UNPROTECTED DI- AND TRISACCHARIDES.[a]

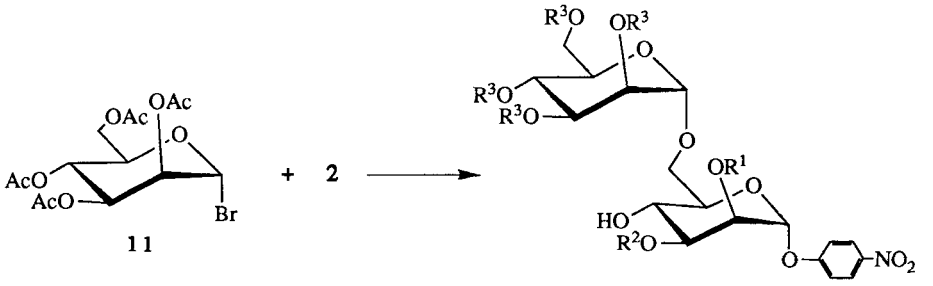
Nucleus	6	10	14
H-1 ($J_{1,2}$)	5.79 (<1)	5.14 (1.5)	5.77 (1.5)
H-1' ($J_{1',2'}$)	5.03 (<1)	5.02 (1.75)	4.73 (<1)
H-1" ($J_{1",2"}$)	4.76 (<1)	4.73 (1.5)	-
H-2' ($J_{2',3'}$)	4.01 (3.5)	4.01 (3.5)	n.d.
OCH_3	-	3.40	-
C_6H_4-NO_2 (^{3}J)	8.29 (9.0) 7.29 (9.0)	- -	8.25 (9.0) 7.26 (9.0)
C-1	98.35	101.86	98.40
C-1'	98.35	98.92	99.69
C-1"	103.13	103.15	-
C-2'	79.64	79.49	n.d.
C-6	66.72	66.62	66.20
C-6'	61.95[b]	61.95[c]	61.78
C-6"	61.77[b]	61.77[c]	-
OCH_3	-	55.62	-
CNO_2	161.49	-	161.45
CO (phenolic)	143.17	-	143.13
Aromatic	126.91, 117.63	-	126.84, 117.61

a. Spectra were recorded at 400 MHz (^{1}H in D_2O, compounds **6** and **14**), and 300 MHz (^{1}H in D_2O, compound **10**) and 75.5 MHz (^{13}C in D_2O); n.d., Not determined.

b,c. Values with the same superscripts may be interchanged.



Compound **14** had been previously[20] obtained by fusion of *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranose with 4-nitrophenyl in the presence of zinc chloride, followed by *O*-deacetylation, but no spectral and analytical data were reported.



	R^1	R^2	R^3
12	-CMe_2-		Ac
13	H	H	Ac
14	H	H	H

The site of glycosylation of partially protected diol acceptor **2** was expected to be at O-6, because of the inherent higher reactivity of the primary hydroxyl group as compared to the secondary hydroxyl group at C-4.[21] In the ^{13}C NMR spectra of all di- and trisaccharides, the resonances for C-6 of the reducing end residue showed a downfield shift (δ 66.20-67.79) confirming the site of glycosylation.

EXPERIMENTAL

General methods. Optical rotations were measured at 22±2 °C with a Perkin-Elmer 241 polarimeter. TLC was conducted on aluminum sheets, precoated with 0.2-mm layers of silica Gel 60F-254 (Merck); the compounds were located by quenching of fluorescence and/or by charring with 5% sulfuric acid. Column chromatography was performed on silica gel (Baker Analyzed, 60-200 mesh). ^1H NMR spectra were recorded at 300 (Bruker AM-300) or 400 MHz (Bruker AM-400). The chemical shift reference in organic solvents was internal Me_4Si (δ 0) and in D_2O was internal acetone (δ 2.225). ^{13}C NMR spectra were recorded either at 75.5 MHz (Bruker AM-300) on solutions in $CDCl_3$, CD_3OD (internal Me_4Si, δ 0) or D_2O (external 1% 1,4 dioxane in D_2O, δ 67.4). The assignments of ^{13}C NMR chemical shifts are tentative. FAB mass spectra were obtained

using an AEI MS-9 instrument with xenon as the bombarding gas with 1,4-dithiothreitol:1,4-dithioerythritol (5:1) as the matrix. Unless otherwise indicated, all reactions were carried out at ambient temperature, and in the work-up, solutions in organic solvents were washed with equal volumes of aqueous solutions. Organic solutions were generally dried (anhydrous Na_2SO_4) prior to concentration (at a bath temperature of 40-50 °C) on a rotary evaporator under the reduced pressure obtained from a water aspirator. Elemental analyses were performed by Robertson Laboratory, 29 Samson Ave., Madison, New Jersey 08940 (U.S.A.). The following solvent systems (v/v) were used for chromatography: **A**, chloroform-acetone (5:1); **B**, chloroform-ethanol (12:1); **C**, chloroform-methanol-water (5.5:4.5:1); **D**, ether-hexane (25:1); **E**, chloroform-methanol-water (32.5:7.5:1); **F**, chloroform-methanol-water (13:6:1); **G**, hexane-chloroform (1:1); **H**, chloroform-acetone (4:1); I, chloroform-acetone (3:1); **J**, chloroform-acetone (3:2).

4-Nitrophenyl *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-α-D-mannopyranoside (5). A stirred mixture of diol **2**[15] (1.7 g, 4.98 mmol), powdered $Hg(CN)_2$ (1.3 g, 5.15 mmol) in 1:1 (v/v) benzene-nitromethane (60 mL) was boiled until 20 mL of the solvent had distilled off. After cooling to room temperature, disaccharide bromide **1**[16] (3 g, 4.29 mmol in 15 mL 1:1 benzene-nitromethane) was added and stirring was continued at room temperature for 3 h under N_2. TLC (solvent A) of the mixture showed the disappearance of **1** and the presence of a major product, faster migrating than **2**; some slower migrating contaminants (presumably resulting from the decomposition of **1**), as well as some unchanged **2**, were also present. The mixture was filtered through a bed of Celite, the solids thoroughly washed with benzene, and the filtrate and washings were combined and diluted with benzene. The organic solution was successively washed with water, M KI solution, an aqueous $NaHCO_3$ solution, and water, dried, and concentrated to give a solid residue. The crude product mixture (3.3 g, containing **4**) was taken up in chloroform (120 mL) containing trifluoroacetic acid (15 mL) and water (0.2 mL) and stirred for 1 h at room temperature. After conncentration with coevaporation of toluene, the residue was chromatographed (solvent A→B) to afford **5** (1.8 g, 39%, based on **2**): syrup; $[\alpha]_D$ +66° (*c* 1, chloroform).

Anal. Calcd for $C_{38}H_{49}NO_{25}$: C, 49.62; H, 5.37; N, 1.52. Found: C, 49.61; H, 5.32; N, 1.51.

4-Nitrophenyl *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (6). Compound **5** (0.4 g, 0.43 mmol) in 25 mM methanolic sodium methoxide (20 mL) was stirred overnight at room temperature. The base was neutralized with Amberlite IR-120 (H^+) cation exchange resin. The resin was

removed by filtration through a bed of Celite and washed with methanol. The combined filtrate was concentrated, and a solution of the residue in water was lyophilized to give **6** (0.2 g, 73.5%): $[\alpha]_D$ +107° (*c* 0.5, water); FAB-MS *m/z* 626 $[M+1]^+$ and 648 $[M+Na]^+$.

Anal. Calcd for $C_{24}H_{35}NO_{18}.H_2O$: C, 44.79; H, 5.80; N, 2.18. Found: C, 44.98; H, 5.91; N, 2.14.

4-Aminophenyl *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (7). A mixture of compound **6** (0.1 g, 0.16 mmol) and Adams' catalyst (PtO_2, 0.06 g) in ethanol (5 mL) was stirred under hydrogen at atmospheric pressure for 1 h at room temperature. TLC (solvent C) of the mixture showed the disappearance of **6** and the presence of a major product, slower migrating than **6**. The mixture was filtered (celite) and the filtrate was concentrated *in vacuo* to give **7** (0.08 g, 84%). Because of the instability of the 4-aminophenyl trisaccharide, the subsequent conjugation was performed on the concentrate without further characterization.

Methyl *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (9). To a cold (0 °C, bath) stirred mixture of **3**[17] (1.4 g, 3 mmol), silver trifluoromethanesulfonate (silver triflate, 1.73 g, 6.73 mmol), tetramethylurea (2.7 mL, 23.9 mmol), and 4Å molecular sieves (2.5 g) in dichloromethane (15 mL), protected from light and moisture, was added dropwise, disaccharide bromide **1**[16] (2.6 g, 3.72 mmol) in dichloromethane (10 mL) and stirring was continued for 3 h at room temperature in an atmosphere of nitrogen. After 3 h and 24 h, additional amounts of silver triflate (0.87 g, 3.39 mmol) and bromide **1** (1.3 g, 1.86 mmol) were added and the stirring was continued for a total of 48 h. The mixture was diluted with dichloromethane (200 mL), the solids were filtered off (celite) and washed with dichloromethane. The filtrate and washings were combined and concentrated. Chromatography (solvent D) gave **8** as a syrup (1.85 g), contaminated with some slower migrating impurities (TLC, solvent D). This crude product was taken up in 50 mM methanolic sodium methoxide (20 mL) and stirred overnight at room temperature. After neutralizing with acetic acid the crude product was chromatographed (solvent E→F) to furnish **9** (0.9 g, 38%, based on **3**), syrup; $[\alpha]_D$ +49° (*c* 2.7, methanol).

Anal. Calcd for $C_{40}H_{52}O_{16}$: C, 60.90; H, 6.64. Found: C, 60.75; H, 6.66.

Methyl *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (10). A mixture of **9** (0.8 g, 1 mmol) and 10% Pd-C (1 g) in 1:1 (v/v) ethanol-glacial acetic acid (20 mL) was shaken under H_2 at 345 kPa for 12 h at room temperature. The suspension was filtered through a bed of Celite, the solid washed with methanol, and the filtrate and washings were combined and concentrated. The residue was chromatographed (solvent C), to give, after freeze-drying, **10** (0.42 g, 79%): $[\alpha]_D$ +77° (*c* 1, water); FAB-MS *m/z* 519 $[M+1]^+$ and 541 $[M+Na]^+$.

Anal. Calcd for $C_{19}H_{34}O_{16}.0.5\ H_2O$: C, 43.26; H, 6.69. Found: C, 43.43; H, 6.88.

4-Nitrophenyl *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-2,3-*O*-isopropylidine-α-D-mannopyranoside (12). To a mixture of diol **2**[15] (1.0 g, 2.93 mmol), powdered $Hg(CN)_2$ (0.74 g, 2.93 mmol), 4Å molecular sieves (1.3 g) in acetonitrile (5 mL) was added a solution of bromide **11**[19,20] (1.57 g, 3.82 mmol) in acetonitrile (7.5 mL), and stirring was continued for 4 h at room temperature. The mixture was filtered (Celite) and the solids were washed with chloroform. The filtrate and washings were combined and concentrated, to give a crude product which was taken up in chloroform (150 mL) and processed as described for the conversion **4**→**5**. After concentration, the residue was chromatographed (solvent G→chloroform) to give a solid which was dissolved in a small volume of dichloromethane. Addition of ether-hexane caused the precipatation of amorphous **12** (1.2 g, 68%, based on reacted **2**): $[\alpha]_D$ +79° (*c* 0.6, chloroform); TLC (solvent H), R_F 0.44.

Anal. Calcd for $C_{29}H_{37}NO_{17}$: C, 51.86; H, 5.55; N, 2.09. Found: C, 51.64; H, 5.38; N, 2.03.

Continued elution of the column gave unchanged **2** (0.1 g)

4-Nitrophenyl *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-α-D-mannopyranoside (13). A mixture of **12** (1.1 g, 1.64 mmol) in trifluoroacetic acid (2.7 mL) and water (0.2 mL) was stirred for 2 h at room temperature. After concentration with evaporation of toluene, the crude product was chromatographed (chloroform→solvent I) to give a solid residue which was dissolved in a small volume of chloroform. Addition of ether-hexane caused precipitation of amorphous **13** (0.8 g, 77%): $[\alpha]_D$ +95° (*c* 1.9, chloroform); TLC (solvent J), R_F 0.16.

Anal. Calcd for $C_{26}H_{33}NO_{17}$: C, 49.45; H, 5.27; N, 2.22. Found: C, 49.44; H, 5.14; N, 2.24.

4-Nitrophenyl *O*-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (14). Compound **13** (0.7 g, 1.11 mmol) was O-deacetylated with 30 mM methanolic sodium methoxide (52 mL) as described for the formation of **6** to give a material which was dissolved in a small amount of methanol. Addition of ether-hexane caused the precipitation of **14** (0.37 g, 72%), $[\alpha]_D$ +96° (*c* 1.1, water); lit.[20] $[\alpha]_D$ +114° (*c* 0.29, water); TLC (solvent F), R_F 0.3; FAB-MS *m/z* 464 $[M+1]^+$ and 486 $[M+Na]^+$.

Anal. Calcd for $C_{18}H_{25}NO_{13}.0.5\ H_2O$: C, 45.77; H, 5.55; N, 2.96. Found: C, 45.92; H, 5.71; N, 2.83.

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