[Chem. Pharm. Bull. 27(7)1589—1595(1979)] UDC 547.458.02.05 : 581.192

Synthesis of Methyl O-β-D-Galactopyranosyl-(1→6)-O-α-D-galactopyranosyl-(1→6)-O-α-D-galactopyranosyl-(1→6)-O-β-D-glucopyranoside

Tai Gi Chung, Hideko Ishihara, and Setsuzo Tejima

Faculty of Pharmaceutical Sciences, Nagoya City University[1]

(Received December 26, 1978)

The desired compound (**10**) was synthesized from crude stachyose (**1**). Compound **1**, separated from tubers of *Stachys sieboldi* Miq., was heated with aqueous acetic acid to yield manninotriose, which was isolated as a crystalline β-undecaacetate (**2**). Treatment of **2** with saturated hydrogen bromide in acetic acid afforded a crystalline acetobromomanninotriose (**3**). Methyl glycosidation of **3** followed by deacetylation gave methyl β-manninotrioside (**4**) as a white powder. Selective tritylation and subsequent acetylation of **4** afforded a crystalline methyl nona-O-acetyl-6″-O-trityl-β-manninotrioside (**6**). Stirring of **6** with acetobromogalactose in nitromethane in the presence of silver perchlorate and Drierite yielded the acetate (**9**) in 42% yield. Deacetylation of **9** gave **10** as a white powder. Methylation of **10** by Hakomori's method gave a crystalline fully methyl ether (**11**). The structure of **10** was confirmed by GC and GC–MS analyses of acid hydrolysates of **11**. β-Galactosidase from jack bean meal hydrolyzed **10** to D-galactose and methyl β-manninotrioside.

Keywords——linear oligosaccharide synthesis; *Stachys sieboldi* Miq.; stachyose; manninotriose undecaacetate; acetobromomanninotriose; methyl manninotrioside; GC–MS analysis; substrate of glycosidase

Oligosaccharides having well-defined structures are indispensable for studies of the action of glycosidases and elucidation of the role of carbohydrates in complex saccharides. However, the synthesis of an oligosaccharide having more than three monosaccharide units is a laborious task. We have been concerned with the syntheses of galactose-containing oligosaccharides[2] with a view to studying the substrate specificities of β-galactosidase.[3] We have sought to synthesize materials possessing biological activity.[4] We now report an extension of this work to provide a synthetic approach to linear oligosaccharides having more than four monosaccharide units. The starting material is manninotriose β-undecaacetate, which is easily prepared from crude stachyose. The occurrence of manninotriose, O-α-D-galactopyranosyl-(1→6)-O-α-D-galactopyranosyl-(1→6)-D-glucose, was known as early as 1902; it occurs in a free form in ash manna.[5] The sugar is also known as a product of partial acid hydrolysis, or invertase hydrolysis, of stachyose. The sequence of the monosaccharide linkages from the reducing terminus is similar to that of sphingolipids separated from animal tissues.[6] Although chemical synthesis of manninotriose β-undecaacetate has recently been reported by Adachi and Suami,[7] some derivatives of it are already known. We selected manninotriose derivatives as useful starting materials for higher oligosaccharide synthesis.

Crude stachyose (**1**) was prepared from fresh tubers of *Stachys sieboldi* Miq. The flow sheet of the preparation is shown in Chart 1. Thin-layer chromatography (TLC) and gas chromatographic (GC) analysis of the trimethylsilylated (TMS) derivative of the product

1) Location: *Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan.*
2) T.G. Chung and S. Tejima, *Chem. Pharm. Bull.* (Tokyo), **25**, 464 (1977); *idem, ibid.*, **26**, 3562 (1978).
3) S. Arakawa, T. Chiba, and S. Tejima, *Seikagaku*, **46**, 795 (1974).
4) T.G. Chung, H. Ishihara, and S. Tejima, *Chem. Pharm. Bull.* (Tokyo), **26**, 2147 (1978); T. Takamura, T. Chiba, and S. Tejima, *ibid.*, **27**, 721 (1979).
5) D. French, *Adv. Carbohyd. Chem.*, **9**, 170 (1954).
6) H. Wiegandt, *Adv. Lipid Res.*, **9**, 249 (1971).
7) R. Adachi and T. Suami, *Bull. Chem. Soc. Jpn.*, **50**, 1901 (1977).

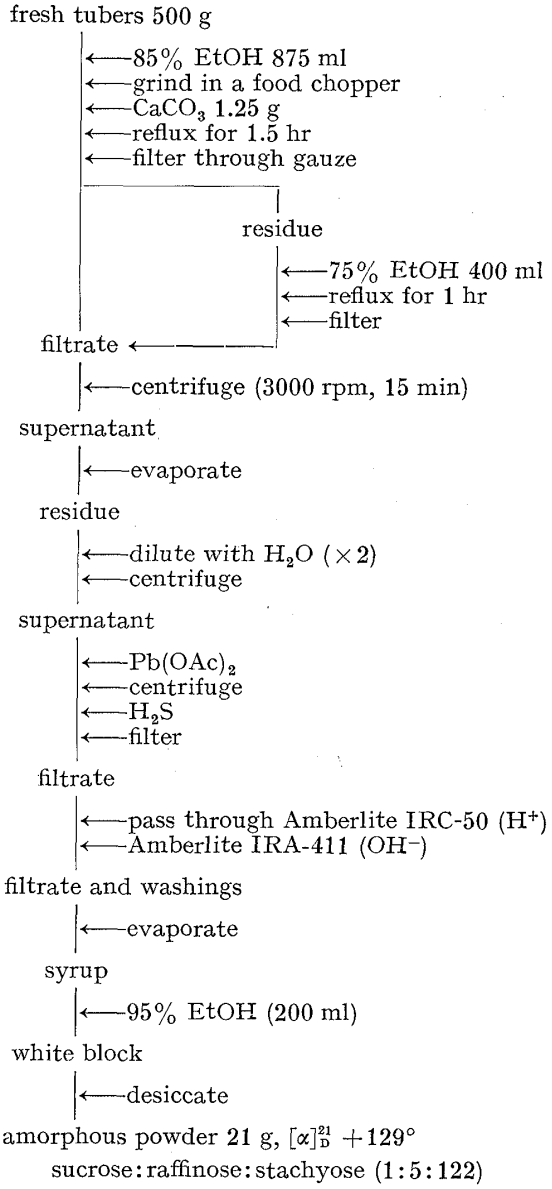


Chart 1. Preparation of Crude Stachyose from *Stachys sieboldi* MIQ.

indicated that it consisted of sucrose, raffinose, and stachyose in the ratio 1:5:122, but it was used for further studies without purification.

Manninotriose β-undecaacetate (**2**), mp 106—107°, $[\alpha]_D^{23}$ +138.2°, was synthesized in 49% yield by heating **1** in aqueous acetic acid, subsequent acetylation of the hydrolysate, and purification of the acetate by silica gel column chromatography. The values of melting point and specific rotation were in good agreement with the reported values,[7] mp 106—107.5°, $[\alpha]_D^{21}$ +137°.

Compound **2** was treated with acetic acid saturated with hydrogen bromide, and the mixture was processed in the usual way to afford acetobromomannnotriose (**3**) in 60% yield. The product was crystallized from ether, $[\alpha]_D^{27}$ +211.7°. The nuclear magnetic resonance (NMR) spectrum showed seven separate singlets due to ten acetoxyl groups. The signal due to the C-1 proton of the reducing terminus (α-Glc) was observed as a one-proton doublet with a spacing of 4 Hz. The large dextrorotatory value and the small coupling constant of the terminal anomeric proton were indicative of an α-D-configuration of **3**.

A mixture of **3** in methanol was stirred at room temperature in the presence of silver carbonate. Methyl glycosidation proceeded smoothly to yield methyl decaacetyl-β-manninotrioside (**4**). The product was isolated in 95% yield after chromatography. The NMR spectrum showed seven separate singlets due to ten acetoxyl groups and a three-proton singlet due to a methoxyl group. Deacetylation of **4** gave methyl β-manninotrioside (**5**) as a white powder in 93% yield. The NMR spectrum showed a three-proton singlet due to a methoxyl group. The signal due to the C-1 proton of the reducing terminus (β-Glc) was observed at 4.42 ppm as a one-proton doublet with a spacing of 7.5 Hz. The two anomeric protons due to the inner α-D-galactosidic linkages, H-1′, and H-1″, overlapped and they were observed as a two-proton doublet at 5.02 ppm with a spacing of 2 Hz.

Tritylation of **5** was performed by treatment of **5** with 1.5 molar equivalents of trityl chloride in pyridine at room temperature. The reaction proceeded very slowly, and was monitored by TLC. After 24 and 48 hours, further portions of trityl chloride were added, and the reaction was continued for 72 hours. TLC then indicated disappearance of **5**. In total 4.5 molar equivalents of trityl chloride were required. Without separation of the monotrityl ether, the reaction mixture was acetylated to yield methyl nonaacetyl-6″-O-trityl-β-manninotrioside (**6**) in 56% yield after chromatography. The NMR spectrum showed seven separate singlets due to nine acetoxyl groups, a three-proton singlet due to a methoxyl group, and fifteen-proton multiplet due to aromatic protons.

Detritylation of **6** with 80% aqueous acetic acid at 50° for three hours yielded two products, *Rf* 0.43 (major) and 0.41 (minor) on TLC. The mixture was chromatographed through a column of silica gel. Methyl nonaacetyl-β-manninotrioside (**7**) having an unprotected hydroxyl group at C-6″ was eluted first and isolated as needles in 61% yield. No migration of acetyl groups took place during the detritylation step, as retritylation of **7** gave **6**. The minor product was isolated as an amorphous powder (**8**) in 28% yield. The NMR spectrum of **8** showed five separate singlets due to nine acetoxyl groups and a three-proton singlet due to a methoxyl group. Retritylation of **8** did not give trityl ether, but **8** was recovered. Thus, **8** was assigned as an isomer of **7** formed by migration of the acetyl group at C-4″ to C-6″. Further studies on **8** were not carried out because of the low yield.

A mixture of **6** and 1.5 molar equivalents of acetobromogalactose in dry nitromethane was stirred at 0° in the presence of silver perchlorate and Drierite to synthesize acetylated tetrasaccharide (**9**). After thirty minutes, the mixture was freed from inorganic salts, acetylated, and processed in the usual way to afford an acetate, which was purified by chromatography. A small amount of methyl decaacetyl-β-manninotrioside (**4**) was isolated from the faster moving effluent. Further elution with the same solvent eluted **9** from the column. Removal of the solvent from the fraction having *Rf* 0.59 on TLC gave **9** as an amorphous powder in 42% yield. The synthetic route[8] suggested a β-configuration of the new galactosidic linkage in **9**. Further evidence for a β-configuration was obtained from the value of the molecular rotation of **9** (+111, 810), which was in good agreement with the value (+114290) calculated from those of the parent monosaccharide and trisaccharide derivative. The molecular rotation of **9** differed from the value (+167780) calculated for the α-1,6-linked tetrasaccharide (see Table I).

Table I. Molecular Rotation of Compound **9** Compared to the Sum of the Molecular Rotations of Constituents

Compound	$[\alpha]_D$[a]	Mol. wt.	$[M]_D$ (degree) $\times 10^{-2}$
Methyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside[b]	+133.3°	362	+482.5
Methyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside[b]	−14.5°	362	−52.4
Compound **9**[c]	+91.2°	1226	+1118.1
Compound **7**[d]	+133.4°	896	+1195.3
7+methyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside			+1677.8
7+methyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside			+1142.9

a) Optical rotations determined in chloroform.
b) J. Świderski and A. Temerius, *Carbohyd. Res.*, **3**, 225 (1966).
c) Methyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-α-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-α-D-galactopyranosyl)-(1→6)-O-2,3,4-tri-O-acetyl-β-D-glucopyranoside.
d) Methyl 2,2′,2″,3,3′,3″,4,4′,4″-nona-O-acetyl-β-manninotrioside.

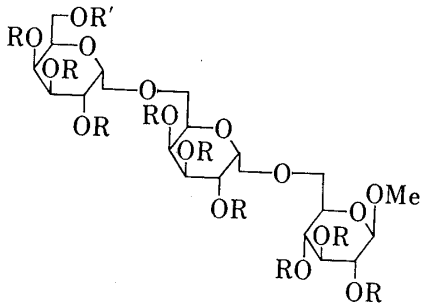
Deacetylation of **9** afforded the desired tetrasaccharide as a white powder. The NMR spectrum showed a one-proton doublet at 4.42 ppm with a spacing of 8 Hz due to the C-1 proton of the reducing terminus (β-Glc). The signal due to the C-1 proton of the non-reducing terminus (β-Gal) was observed at 4.46 ppm as a one-proton doublet with a spacing of 7.5 Hz. The two anomeric protons due to the inner α-D-galactosidic linkages, H-1′ and H-1″, overlapped and were observed as a two-proton doublet at 5.00 ppm with a spacing of 2 Hz.

8) H. Brederek, A. Wagner, G. Faber, H. Ott, and J. Rauther, *Chem. Ber.*, **92**, 1135 (1959).

Compound **9** was dissolved in dimethylsulfoxide (DMSO) and methylated by Hakomori's procedure.[9] The permethylated tetrasaccharide (**11**) was isolated as white needles in 86% yield after chromatography. The product was hydrolyzed in 10% sulfuric acid at 100° for ten hours. The hydrolysate was neutralized and evaporated to dryness, and the partially methylated alditol acetates were prepared by borohydride reduction followed by acetylation. The product was dissolved in chloroform and subjected to GC analysis and gas chromatography–mass spectrometry (GC–MS).

The gas chromatogram of the methylated alditol acetates obtained from the hydrolysate of **11** showed three peaks, (a), (b), and (c), having a 1: 1: 2 peak area ratio. They were assigned as 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylgalactitol (**12**), 1,5,6-tri-O-acetyl-2,3,4-tri-O-methylglucitol (**13**), and 1,5,6-tri-O-acetyl-2,3,4-tri-O-methylgalactitol (**14**), respectively, by comparing their retention times and mass spectra with those of authentic samples.

β-Galactosidase from jack bean hydrolyzed the tetrasaccharide to galactose and methyl β-manninotrioside. Thus, this tetrasaccharide may be a useful substrate for exo-β-galactosidases. In addition, compound **3**, **6**, and **7** should be useful intermediates for linear tetra-, penta-, and hexasaccharide synthesis. The results of further studies will be reported separately.



4: R=R′=Ac
5: R=R′=H
6: R=Ac, R′=Tr
7: R=Ac, R′=H
9: R=Ac, R′=2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl
10: R=H, R′=β-D-galactopyranosyl
11: R=CH_3, R′=2,3,4,6-tetra-O-methyl-β-D-galactopyranosyl

Ac=acetyl, Tr=trityl

Chart 2

Experimental

Solutions were evaporated down in a rotary evaporator below 40° under a vacuum. Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a Union Giken PM-201 automatic digital polarimeter in a 0.5 dm tube. Infrared (IR) spectra were measured with a Jasco IRA-2 spectrometer. NMR spectra were recorded at 100 MHz with a Jeol JNM-MH-100 spectrometer. TMS (in $CDCl_3$) and 2,2-dimethyl-2-silapentane-5-sulfonate (in D_2O) were used as internal standards. Chemical shifts are given on the δ scale. A Shimadzu gas chromatography, Model GC-4BPTF, equipped with a hydrogen flame ionization detector was employed for GC analysis. Retention times (t_R) are given in min. Mass spectra of partially methylated alditol acetates were measured with a Hitachi M-52 GC-MS spectrometer. TLC on Kieselgel GF_{254} (5×20 cm) (E. Merck, Darmstadt, Germany) was performed with the following solvent combinations (v/v): (A), CH_2Cl_2–acetone (5: 2), (B), 70% 2-PrOH–AcOEt (2: 1). Detection was effected with H_2SO_4 or by UV irradiation (short wavelength). Column chromatography was performed on Wakogel C-200 (Wako Pure Chemical Industries, Ltd., Osaka) with 1 g of a sample to be separated per 20 g of adsorbent. Solvent combinations of eluent are shown as v/v.

Preparation of Crude Stachyose (1)——Tubers of *Stachys sieboldi* MIQ. were obtained from the Herb Garden of this Faculty or purchased from the Kaminogo Branch, Mitaka Agricultural Co-operative Associa-

9) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

tion, Gifu Prefecture. Fresh tubers (500 g) were ground in a mincer and refluxed with 85% (v/v) EtOH (850 ml) containing precipitated $CaCO_3$ (1.25 g) for 1.5 hr. The liquid was removed by filtration and extraction was repeated with 75% EtOH (400 ml) for 1 hr. The extract was filtered and combined with the first filtrate to produce a clear brown solution which was concentrated to a syrup; yield 130 g. To a solution of the syrup in H_2O (260 ml), basic lead acetate (10 g) was added, and the precipitate was separated by filtration. Excess lead was removed from the filtrate as sulfides by saturation with H_2S. After removal of H_2S from the filtrate by aeration, the solution was passed through columns (5×30 cm) of Amberlite IRC-50 (H^+) cation-exchange resin (200 ml) and Amberlite IRA-400 (OH^-) anion-exchange resin (200 ml). The filtrate and washings were concentrated to a thick syrup which was triturated with 95% EtOH (200 ml). The insoluble part was collected and the solvent was removed under a vacuum to afford a white amorphous powder (21 g), $[\alpha]_D^{21}$ +129° (c=1, H_2O). TLC on a pre-coated Silica Gel 60 F_{254} plate (20×5 cm) (E. Merck) with 2-PrOH–acetone–0.1 M lactic acid (4: 4: 2, v/v) showed three spots corresponding to sucrose, raffinose, and stachyose. Detection was effected with diphenylamine reagent.[10] The product (10 mg) was converted into TMS ethers[11] about 30 min before injection and subjected to GC analysis. A stainless steel column (2 m×3 mm) packed with 3% JXR on Gas-Chrom Q (100—120 mesh) was used at 280° with a flow of 28 ml/min of N_2 gas passing through the column. GC analysis indicated that the ratio sucrose: raffinose: stachyose in **1** was 1: 5: 122. t_R: sucrose=2.00; raffinose=10.00; stachyose=67.00.

1,2,2′,2″,3,3′,3″,4,4′,4″,6″-Undeca-O-acetyl-β-manninotriose (2)——A solution of **1** (40 g, 60 mmol) in 20% (v/v) aq. AcOH (400 ml) was heated at 95—100° for 4 hr in a water bath. After cooling, the mixture was evaporated to dryness by repeated co-distillation with EtOH. The residue was acetylated with Ac_2O (200 ml) and AcONa (20 g) at 95—100° for 2 hr, then poured into ice-H_2O (2 l), and the mixture was extracted with CH_2Cl_2. The organic layer was washed with H_2O, sat. $NaHCO_3$, and H_2O. After desiccation over $CaCl_2$, the solvent was removed to afford a syrup which was chromatographed through a column of silica gel, eluting with CH_2Cl_2–acetone (20: 1). Fractions having *Rf* 0.63 (solvent A) were combined and the solvent was removed to afford an amorphous powder which was crystallized from EtOH. Recrystallization from EtOH gave white needles (28.4 g, 49%), mp 106—107°, $[\alpha]_D^{23}$ +138.2° (c=1.76, $CHCl_3$) [lit.[7] mp 106—107.5°, $[\alpha]_D^{21}$ +137° (c=1.27, $CHCl_3$)].

2,2′,2″,3,3′,3″,4,4′,4″,6″-Deca-O-acetyl-α-manninotriosyl Bromide (3)——Ice-cold AcOH (30 ml) saturated with HBr was added to a chilled solution of **2** (10 g, 10.35 mmol) in $CHCl_3$ (100 ml) at 0°. After standing at 0° for 3 hr, the mixture was poured into ice-H_2O (300 ml), and the organic layer was separated. The aqueous layer was extracted with $CHCl_3$. The combined $CHCl_3$–layer was washed with ice-H_2O, ice-cold aq. $NaHCO_3$, and ice-H_2O, then dried over $CaCl_2$. Removal of the solvent afforded an amorphous powder which was crystallied from ether. Recrystallization from ether gave **3** (6.1 g, 60%) as short needles, mp 125—127°, $[\alpha]_D^{27}$ +211.7° (c=1.02, $CHCl_3$). NMR $\delta_{ppm}^{CDCl_3}$: 1.98, 2.04, 2.09, 2.13, 2.15 (30H, all s, 10×OAc), 6.61 (1H, d, $J_{1,2}$=4 Hz, H-1, α-Glc). TLC: *Rf* 0.67 (solvent A). *Anal.* Calcd. for $C_{38}H_{51}BrO_{25}$: C, 46.21; H, 5.21. Found: C, 45.91; H, 5.21.

Methyl 2,2′,2″,3,3′,3″,4,4′,4″,6″-Deca-O-acetyl-β-manninotrioside (4)——A mixture of **3** (2.8 g, 2.83 mmol) and Ag_2CO_3 (5.6 g, 20.3 mmol) in dry MeOH (30 ml) was stirred at room temperature for 20 hr with exclusion of light and moisture. The reaction was monitored by TLC (solvent A), and the stirring was stopped when **3** had disappeared on the plate. The mixture was filtered, and the solvent was removed to afford an amorphous powder. The residue was dissolved in CH_2Cl_2 (2 ml) and chromatographed on a column of silica gel, eluting with CH_2Cl_2–acetone (10: 1). Fractions having *Rf* 0.62 (solvent A) were combined, and the solvent was removed to afford **4** (2.54 g, 95%), $[\alpha]_D^{19}$ +128.2° (c=1.70, $CHCl_3$), as an amorphous powder. NMR $\delta_{ppm}^{CDCl_3}$: 1.98, 2.00, 2.06, 2.07, 2.09, 2.11, 2.14 (30H, all s, 10×OAc), 3.53 (3H, s, OMe), 4.44 (1H, d, $J_{1,2}$= 8.5 Hz, H-1, β-Glc). TLC: *Rf* 0.62 (solvent A). *Anal.* Calcd. for $C_{39}H_{54}O_{26}$: C, 49.89; H, 5.79. Found: C, 49.65; H, 5.72.

Methyl β-Manninotrioside (5)——Methanolic NaOMe (0.3 N, 0.1 ml) was added to a solution of **4** (1.60 g, 1.70 mmol) in dry MeOH (20 ml) at room temperature with stirring, and the mixture was stirred for 4 hr. Deacetylation was monitored by TLC (solvent B). Dry Amberlite IR-120 (H^+) resin was added, and the mixture was stirred for 30 min, then filtered. Removal of the solvent gave an amorphous powder which was dissolved in MeOH. Addition of AcOEt to the solution precipitated **5** (820 mg, 93%), $[\alpha]_D^{24}$ +121.3° (c= 1.17, H_2O), as a white powder. NMR $\delta_{ppm}^{D_2O}$: 3.59 (3H, s, OMe), 4.42 (1H, d, $J_{1,2}$=7.5 Hz, H-1, β-Glc), 5.02 (2H, overlapping d, $J_{1',2'}$ and $J_{1'',2''}$=2 Hz, α-Gal). TLC: *Rf* 0.18 (solvent B). *Anal.* Calcd. for $C_{19}H_{34}O_{16}$: C, 44.02; H, 6.61. Found: C, 43.78; H, 6.73.

Methyl 2,2′,2″,3,3′,3″,4,4′,4″-Nona-O-acetyl-6″-O-trityl-β-manninotrioside (6)——Trityl chloride (1 g, 3.62 mmol) was added to a solution of **5** (1.25 g, 2.41 mmol) in pyridine (15 ml). The mixture was stirred for 24 hr at room temperature. After 24 and 48 hr further portions of trityl chloride (each 1 g) were added, and the stirring was continued for 72 hr; TLC (solvent B) then indicated disappearance of **5**. Ac_2O (15 ml) was added to the mixture at 0°. The mixture was left at room temperature overnight, poured into ice-H_2O

10) S.A. Hansen, *J. Chromatogr.*, **107**, 224 (1975).
11) C.C. Sweeley, R. Bentley, M. Makita, and W.W. Wells, *J. Am. Chem. Soc.*, **85**, 2497 (1963).

(300 ml), then extracted with CH_2Cl_2. The extract was washed with H_2O, dil. H_2SO_4, H_2O, satd. $NaHCO_3$, and H_2O, dried over Na_2SO_4, and evaporated to dryness. A solution of the residue in CH_2Cl_2 (2 ml) was applied to a column of silica gel, which was eluted with CH_2Cl_2 until tritanol emerged, and then with CH_2Cl_2–acetone (20: 1) to remove the trityl ether. The fractions having *Rf* 0.70 (solvent A) were concentrated to dryness and the resulting amorphous powder was crystallized from EtOH. Recrystallization from EtOH gave **6** (1.53 g, 56%) as fine needles, mp 242—243°, $[\alpha]_D^{21}$ +89.6° (c=0.92, $CHCl_3$). NMR $\delta_{ppm}^{CDCl_3}$: 1.89, 1.99, 2.01, 2.05, 2.06, 2.08, 2.16 (27H, all s, 9×OAc), 3.40 (3H, s, OMe), 4.41 (1H, d, $J_{1,2}$=7.5 Hz, H-1, β-Glc), 7.20—7.52 (15H, m, aromatic protons). TLC: *Rf* 0.70 (solvent A). *Anal.* Calcd. for $C_{56}H_{66}O_{25}$: C, 59.04; H, 5.84. Found: C, 59.00; H, 5.84.

Methyl 2,2′,2″,3,3′,3″,4,4′,4″-Nona-O-acetyl-β-manninotrioside (7) and Methyl 2,2′,2″,3,3′,3″,4,4′,6″-Nona-O-acetyl-β-manninotrioside (8)——A solution of **6** (1.55 g, 1.36 mmol) in 80% (v/v) aq. AcOH (60 ml) was warmed at 50°. After 3 hr at this temperature, H_2O (135 ml) was added, and the mixture was left at 5° for 18 hr. The resulting precipitate was filtered and washed with 25% AcOH (15 ml). The combined filtrate and washings were concentrated to dryness by repeated co-distillation with EtOH. A solution of the residue in CH_2Cl_2 showed two spots, *Rf* 0.43 (major) and 0.41 (minor), on TLC (solvent A). It was chromatographed on a column of silica gel, eluting with CH_2Cl_2–acetone (10: 1). Fractions having *Rf* 0.43 were concentrated, and the residue was crystallized from 2-PrOH as needles (**7**, 740 mg, 61%), mp 172—173°, $[\alpha]_D^{22}$ +133.4° (c=1.04, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3480 (OH). NMR $\delta_{ppm}^{CDCl_3}$: 2.00, 2.05, 2.07, 2.12, 2.13 (27H, all s, 9×OAc), 3.53 (3H, s, OMe). TLC: *Rf* 0.43 (solvent A). *Anal.* Calcd. for $C_{37}H_{52}O_{25}$: C, 49.55; H, 5.84. Found: C, 49.30; H, 5.80.

A solution of **7** (100 mg, 0.11 mmol) in dry pyridine (2 ml) was treated with trityl chloride (100 mg, 0.36 mmol) for 24 hr at room temperature; TLC (solvent A) then indicated the formation of a single product having an *Rf* value identical with that of **6**. The mixture was poured into ice-H_2O, and the resulting solid was collected and purified on a column of silica gel, eluting with CH_2Cl_2–acetone (20: 1). Removal of the solvent and crystallization of the residue from EtOH gave fine needles (57 mg, 45%) which were indistinguishable from **6** by mp, mixed mp, IR and NMR spectra.

The minor fraction (*Rf* 0.41) from column chromatography of the hydrolysate of **6** was evaporated down to give an amorphous powder (**8**, 340 mg, 28%), $[\alpha]_D^{20}$ +131.7° (c=0.53, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3480 (OH). NMR $\delta_{ppm}^{CDCl_3}$: 1.97, 1.99, 2.05, 2.08, 2.12 (27H, all s, 9×OAc), 3.53 (3H, s, OMe). Tritylation of **8** (100 mg) as described for **7** did not give trityl ether, but the starting **8** was recovered.

Methyl O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-α-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-α-D-galactopyranosyl)-(1→6)-O-2,3,4-tri-O-acetyl-β-D-glucopyranoside (9)——Drierite (300 mg) and **7** (600 mg, 0.53 mmol) were added to a solution of silver perchlorate (320 mg, 1.54 mmol) in dry nitromethane (15 ml) with stirring. The mixture was stirred for 30 min and then cooled at 0°. 2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl bromide[12] (320 mg, 0.78 mmol) was added at 0°, and the mixture was stirred vigorously for 30 min with exclusion of moisture; the solution turned orange and then AgBr and trityl perchlorate began to precipitate. The mixture was filtered, and the solid was washed with CH_2Cl_2. The combined filtrate and washings were evaporated to dryness. The residue was acetylated with Ac_2O (5 ml) and pyridine (5 ml). After standing overnight at room temperature, the mixture was poured into ice-H_2O and extracted with CH_2Cl_2. The extract was washed with H_2O, dil. H_2SO_4, satd. $NaHCO_3$, and H_2O, dried over $CaCl_2$, and then evaporated down to afford a syrup. The residue was dissolved in CH_2Cl_2 (2 ml), and chromatographed on a column of silica gel, eluting with CH_2Cl_2–acetone (10: 1). Removal of the solvent from the fractions having *Rf* 0.59 (solvent A) gave **9** (275 mg, 42%) as an amorphous powder, $[\alpha]_D^{22}$ +91.2° (c=1.16, $CHCl_3$). NMR $\delta_{ppm}^{CDCl_3}$ 1.98, 2.01, 2.06, 2.14 (39H, all s, 13×OAc), 3.52 (3H, s, OMe). TLC: *Rf* 0.59 (solvent A). *Anal.* Calcd. for $C_{51}H_{70}O_{34}$: C, 49.91; H, 5.75. Found: C, 49.67; H, 5.75.

Fractions having *Rf* 0.62 were evaporated down to afford an amorphous powder (200 mg). The product was indistinguishable from **4** by IR and NMR spectra.

Methyl O-β-D-Galactopyranosyl-(1→6)-O-α-D-galactopyranosyl-(1→6)-O-α-D-galactopyranosyl-(1→6)-O-β-D-glucopyranoside (10)——Methanolic NaOMe (0.5 N, 0.1 ml) was added to a solution of **9** (250 mg, 0.82 mmol) in dry MeOH (5 ml) at room temperature with stirring, and the mixture was stirred for 5 hr. Deacetylation was monitored by TLC (solvent B). Treatment of the mixture as described for **5** afforded **10** (120 mg, 86%), $[\alpha]_D^{25}$ +89.5° (c=1.05, H_2O), as a white powder. NMR $\delta_{ppm}^{D_2O}$: 3.58 (3H, s, OMe), 4.42 (1H, d, $J_{1,2}$=8 Hz, H-1, β-Glc), 4.44 (1H, d, $J_{1''',2'''}$=7.5 Hz, H-1‴, β-Gal), 5.00 (2H, overlapping d, $J_{1',2'}$ and $J_{1'',2''}$=2 Hz, H-1′ and H-1″, α-Gal). *Anal.* Calcd. for $C_{25}H_{44}O_{21}\cdot 1/2H_2O$: C, 43.54; H, 6.57. Found: C, 43.44; H, 6.49.

Methyl O-(2,3,4,6-Tetra-O-methyl-β-D-galactopyranosyl)-(1→6)-(2,3,4-tri-O-methyl-α-D-galactopyranosyl)-(1→6)-(2,3,4-tri-O-methyl-α-D-galactopyranosyl)-O-(1→6)-2,3,4-tri-O-methyl-β-D-glucopyranoside (11)——A suspension of NaH (500 mg, defatted with hexane beforehand) in dry DMSO (5 ml) was stirred at 50° for 2 hr under N_2 to furnish a solution of dimsyl carbanion. The prepared carbanion was added to a solution of **10** (250 mg, 0.36 mmol) in dry DMSO (5 ml). The mixture was stirred under N_2 for 2 hr, treated with CH_3I (5 ml), stirred for a further 15 hr in the dark, and then poured into ice-H_2O. It was extracted

12) H. Ohle, W. Marecek, and W. Bourjau, *Chem. Ber.*, **62**, 833 (1929).

with CH_2Cl_2, and the organic layer was washed with H_2O then dried over Na_2SO_4. After removal of the solvent, the resulting syrup was dissolved in CH_2Cl_2 and chromatographed on a column of silica gel, eluting with CH_2Cl_2–acetone (1: 1). Fractions having *Rf* 0.61 (solvent B) were pooled, and removal of the solvent gave a syrup which was crystallized from EtOH. Recrystallization from EtOH gave **11** (270 mg, 86%), mp 183—184°, $[\alpha]_D^{24}$ +77.2° (c=1.05, $CHCl_3$), as white needles. TLC: *Rf* 0.61 (solvent B). *Anal.* Calcd. for $C_{38}H_{70}O_{21}$: C, 52.89; H, 8.17. Found: C, 53.03; H, 8.19.

GC Analyses of Acid Hydrolysates of 11——A glass column (2 m×4 mm) packed with Gas-Chrom Q (100—120 mesh), coated with 3% ECNSS-M was used. The instrument temperature was 180° with a flow of 30 ml/min of N_2 passing through the column.

A mixture of **11** (6 mg) and 10% (w/v) H_2SO_4 (5 ml) was heated at 100° for 10 hr with stirring. The solution was neutralized with $BaCO_3$, filtered through Celite, and evaporated to dryness. The residue in H_2O (2 ml) was hydrogenated with $NaBH_4$ (15 mg) in H_2O (2 ml) for 3 hr at room temperature with stirring. Excess $NaBH_4$ was destroyed with Amberlite IR-120 (H^+) resin and, after removal of the resin by filtration, boric acid was removed by repeated evaporation with MeOH. The residue was acetylated with Ac_2O (2 ml) and pyridine (2 ml), left overnight, and then evaporated down by repeated co-distillation with EtOH and toluene to afford a syrup. The syrup was dissolved in $CHCl_3$, and subjected to GC analysis. The product gave three peaks of methylated alditol acetates (a), (b), and (c), having a 1: 1: 2 peak area ratio. They were assigned as 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylgalactitol (**12**), 1,5,6-tri-O-acetyl-2,3,4-tri-O-methylglucitol (**13**), and 1,5,6-tri-O-acetyl-2,3,4-tri-O-methylgalactitol (**14**), respectively, by comparing their retention times and mass spectra with those of authentic samples. The authentic **12**, **13**, and **14** were synthesized from methyl 2,3,4,6-tetra-O-methyl-α-D-galactopyranoside,[13] methyl 2,3,4-tri-O-methyl-6-O-trityl-α-D-glucopyranoside,[14] and 1,6-anhydro-2,3,4-tri-O-methyl-β-D-galactopyranose,[15] respectively, *via* three steps: acid hydrolysis, borohydride reduction, and acetylation. t_R: (a)=2.18; (b)=4.00; (c)=5.30; **12**=2.18; **13**=4.00; **14**=5.30.

GC-MS of Acid Hydrolysates of 11——A glass column (1.5 m×3 mm) packed with Gas-Chrom Q (80—100 mesh), coated with 2% OV-17 was used. Conditions for recording mass spectra were as follows: ionization potential, 20 eV; ion source temp., 170°; separation temp., 150°. MS *m/e* values of methylated alditol acetates were as follows; (a): 43 (55), 45 (13.3), 71 (16.6), 87 (20), 101 (83.3), 117 (100), 129 (65), 145 (86.6), 161 (75), 205 (41.7); (b): 43 (46.5), 87 (30.2), 99 (62.8), 101 (100), 117 (93), 129 (67.4), 161 (48.8), 189 (32.5); (c): 43 (44.3), 87 (24.5), 99 (61.3), 101 (67), 117 (100), 129 (53.8), 161 (24.5), 189 (24.5).

Hydrolysis of 10 with β-Galactosidase from Jack Bean Meal——β-Galactosidase was purified from jack bean meal (Sigma Chemical Co.) by the method of Arakawa *et al.*[16] The enzyme solution (0.05 ml, 0.08 unit[17]) was added to a solution of **10** (0.7 mg) in 0.15 M sodium citrate buffer (0.2 ml), pH 3.5. The mixture was incubated at 37° with a drop of toluene and the hydrolysis was monitored on a TLC plate (5×20 cm). TLC was performed by the ascending method with 2-PrOH–acetone–1 M lactic acid (4: 4: 2, v/v), and detection was effected with diphenylamine reagent.[10] After incubation for 2 hr, **10** (*Rf* 0.33) was completely hydrolyzed to D-galactose (*Rf* 0.61) and methyl β-manninotrioside (*Rf* 0.46).

Acknowledgements We thank Misses T. Kamimura, E. Masuda, and T. Warabino for their technical assistance. Thanks are also due to Miss T. Ito for the NMR spectral measurements, Misses S. Iwauchi and T. Naito for the microanalyses, and Mr S. Kato for the cultivation of *Stachys sieboldi* MIQ.

13) D.J. Bell, *J. Chem. Soc.*, **1940**, 1543.
14) A. Robertson and R.B. Waters, *J. Chem. Soc.*, **1931**, 1709.
15) D. McCreath, F. Smith, E.G. Cox, and A.I. Wagstaff, *J. Chem. Soc.*, **1939**, 387.
16) M. Arakawa, S. Ogata, and T. Muramatsu, *J. Biochem.* (Tokyo), **75**, 707 (1974).
17) One enzyme unit is the amount of enzyme required to release 1 μmol of *p*-nitrophenol from *p*-nitrophenyl β-D-galactopyranoside per min.