Tetrahedron Vol. 37, No. 16, pp. 2787 to 2792, 1981
Printed in Great Britain

0040-4020/81/162787-06$02.00/0
Pergamon Press Ltd

RECONSTRUCTION OF GLYCAN CHAINS OF GLYCOPROTEIN[a]

BRANCHING MANNOPENTAOSIDE AND MANNOHEXAOSIDE

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(*Received in Japan* 6 *September* 1980)

Abstract—Synthetic routes to the branching mannopentaoside **4** and mannohexaoside **5** are described employing properly protected mannobioside **13** as a key intermediate.

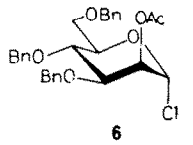
In 1978, Li *et al.*[2] proposed the structure **1** for the lipid-linked oligosaccharide precursor in glycoprotein biosynthesis. According to the proposed biosynthetic scheme,[3] the whole oligosaccharide unit in **1** is transfered from the lipid carrier to the newly synthesized protein and is further processed to give rise to the various high mannose type[4] of glycan chains linked to proteins. Typical examples of these glycan chains may be represented by glycopeptides **2** and **3** which have been reported to be involved in such glycoproteins as ovalbumin,[5] *Asperigillus oryzae* α-amylase,[6] Chinese hamster ovary cell membrane,[7] and human IgM[8].

As part of our project on the synthetic studies on glycan chains present at cell surfaces, we describe a regio- and stereo-controlled approach to the synthesis of branching mannopentaoside **4** and mannohexaoside **5**, the outer chains of glycopeptides **2** and **3**. Synthetic studies on **4** and **5** may be considered as part of the experiments directed toward the construction of the whole glycan unit present in lipid-linked oligosaccharide precursor **1**.

Structures of target molecules **4** and **5** could be retrosynthesized into three monosaccharide synthons, **6**, **7** and **8**. It is to be noted that synthons **6** and **7** are properly protected to have dual functions; first acting as a glycosyl donor toward OH groups and then upon selective removal of the protective groups being transformed into a glycosyl acceptor.

Synthesis of the key intermediate disaccharide

Acetylation of methyl 2,4 - di - O - benzyl - α - D - mannopyranoside **8**[9] gave diacetate **10** and subsequent acetolysis of **10** afforded a 72% yield of triacetate **11**. Configuration at C-1 of **11** was determined to be α according to ^{13}C NMR data which showed the signal for C-1 at δ 90.9 ($^1J_{CH}$ = 175.0 Hz) in good agreement with the empirical rule of Bock and Pedersen.[10] Of the two



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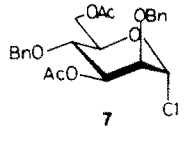

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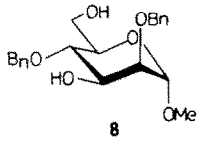

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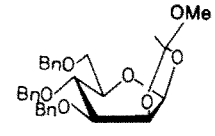

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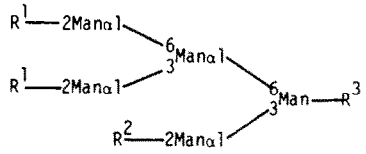

1 R^1=Manα1, R^2=Glcβ1—2Glcβ1—3Glcβ1—3Manα1—2Manα1
R^3=β1—4GlcNAcβ1—4GlcNAcα1—P—P—dolichol

2 R^1=H, R^2=Manα1, R^3=β1—4GlcNAcβ1—4GlcNAcβ1—Asn

3 R^1=R^2=H, R^3=β1—4GlcNAcβ1—4GlcNAcβ1—Asn

4 R^1=R^2=H, R^3=α1—OMe

5 R^1=H, R^2=Manα1, R^3=α1—OMe

[a]Synthetic studies on cell surface glycans Part 4. For Part 3, see Ref. 1.

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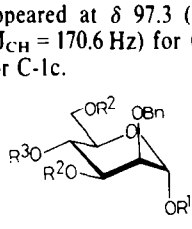
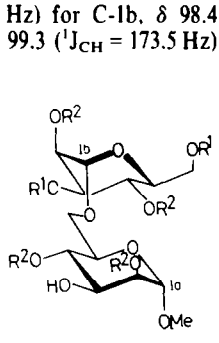
benzyl groups in **10**, one at O-4 was proved to be more susceptible to acetolysis; thus acetolysis of **10** under the same condition but at 25° gave rise the formation of tetraacetate **12**. Stereochemistry at C-1 of **12** was assigned to be α by comparing the proton chemical shift of the anomeric hydrogen of **12** (δ 6.14, d, J = 2 Hz) with that of **11** (δ 6.15, d, J = 2 Hz). And the signal for H-4 appeared at δ 5.46 as a triplet with $J_{34} = J_{45} = 10$ Hz, thus confirming the cleavage of the benzyl group at O-4. Similar susceptibility of the benzyl group at O-4 compared to that at O-3 was recently observed by Ponpipom.[11]

Treatment of **11** in CH_2Cl_2 saturated with HCl led to the chloride **7** in 99.4% yield, which showed the signal for C-1 at 90.0 with $^1J_{CH} = 180.9$ Hz indicating α anomeric stereochemistry.

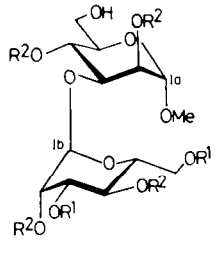
Having properly protected glycosyl donor **7** prepared, selective glycosidation at O-6 of the diol **8** with **7** was next studied. When **8** was glycosylated with 0.86 molar equivalents of glycosyl donor **7** under Hanessian–Banoub conditions,[12] protected mannobiosides **13** and **16** were isolated in 38.6 and 5.9% yield respectively, along with a 6.5% yield of the protected mannotrioside **19**, and starting diol **8** was recovered in 45% yield. However, by employing two molar equivalents of glycosyl donor **7** in this reaction, **13** was isolated in 56.4% yield as well as a 19.8% yield of **19**. Using excess of chloride **7** and minimizing both the formation of the isomer **16** and the recovery of **8**, overall conversion of **8** into **13** became practical, although the ratio of **13** vs **19** was lowered to 3:1 from 6:1. Newly introduced stereochemistry in **13**, **16** and **19** were determined by the following ^{13}C. NMR data. In the case of **13**, signals for two anomeric carbons appeared at δ 97.4 ($^1J_{CH} = 168.6$ Hz) for C-1b and δ 97.7 ($^1J_{CH} = 168.6$ Hz) for C-1a. In the case of **16**, signals for two anomeric carbons appeared at δ 98.8 ($^1J_{CH} = 169.1$ Hz) for C-1a and δ 99.3 ($^1J_{CH} = 172.1$ Hz) for C-1b. In the case of **19**, signals for three anomeric carbons appeared at δ 97.3 ($^1J_{CH} = 170.6$ Hz) for C-1b, δ 98.4 ($^1J_{CH} = 170.6$ Hz) for C-1a, and δ 99.3 ($^1J_{CH} = 173.5$ Hz) for C-1c.



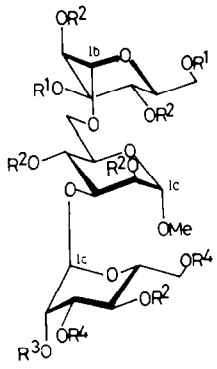
10: R¹ = Me, R² = Ac, R³ = Bn
11: R¹ = R² = Ac, R³ = Bn
12: R¹ = R² = R³ = Ac



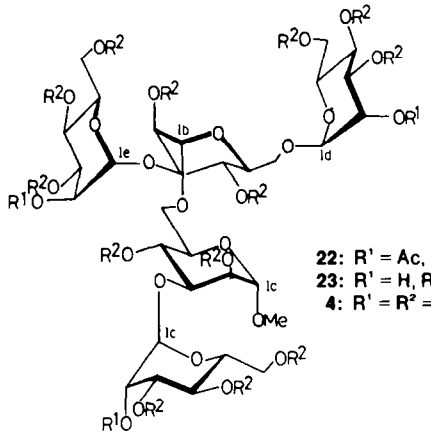
13: R¹ = Ac, R² = Bn
14: R¹ = H, R² = Bn
15: R¹ = R² = H



16: R¹ = Ac, R² = Bn
17: R¹ = H, R² = Bn
18: R¹ = R² = H



19: R¹ = R⁴ = Ac, R² = R³ = Bn
20: R¹ = R⁴ = H, R² = R³ = Bn
21: R¹ = R² = R³ = R⁴ = H
24: R¹ = R³ = Ac, R² = R⁴ = Bn
25: R¹ = R³ = H, R² = R⁴ = Bn



22: R¹ = Ac, R² = Bn
23: R¹ = H, R² = Bn
4: R¹ = R² = H

Structure of **13** was further confirmed by the transformation into free mannobioside **15**. Zémplen deacetylation of **13** into **14** and subsequent hydrogenolysis of **14** afforded **15** as an amorphous solid. ^1H NMR spectrum taken in D_2O at 60° showed the signals of two anomeric protons at δ 4.73 (d, J = 2 Hz, H-1a) and δ 4.91 (d, J = 2 Hz, H-1b). ^{13}C NMR spectrum in D_2O showed the signals for two anomeric carbons at δ 100.3 ($^1J_{CH} = 170.9$ Hz, C-1b) and δ 101.8 ($^1J_{CH} = 170.9$ Hz, C-1a). The formation of 1→6 interglycosidic linkage in mannobioside **15** was also proven by ^{13}C NMR data which showed the presence of a deshielded signal[13] for C-6a at δ 66.4 compared to the signal for C-6b which appeared at δ 61.6.

The structures of two minor products **16** and **19** were confirmed as follows. Compound **16** was saponified into the authentic triol **17** which had been prepared by a different and an unambiguous route[14] and had been converted into free mannobioside **18**.[15] Compound **19** was saponified into **20** which was hydrogenolysed giving mannotrioside **21** identical with the authentic sample.[1]

As the key intermediate **13** was prepared in 5 steps from dibenzylether **8** and the structure was determined unequivocally, further elongation of glycan chains of the glycosyl acceptors **13** and **14** was undertaken.

Synthesis of branching mannopentaoside **4** *and mannohexaoside* **5**

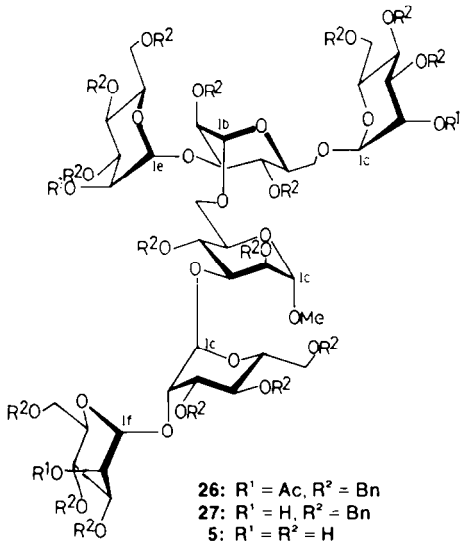
Triol **14** was glycosylated according to Hanessian–Banoub procedure[12] with the mannosyl donor **6**[15] prepared from 5.5 molar equivalents of orthoester **9**[17] to give a 73.3% yield of protected mannopentaoside **22** after column chromatography over silicagel. From less polar fractions of the chromatogram, 2 - O - acetyl - 3,4,6 - tri - O - benzyl - D - glucal was isolated as a side product in

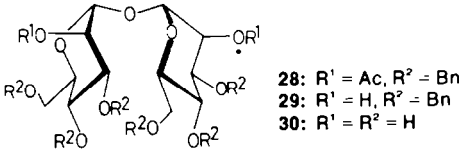
18.4% yield based on **9**. ^{1}H NMR spectrum of **22** showed three singlets for acetyl groups at δ 2.03, 2.06 and 2.10 which indicate the successful introduction of three mannosyl residues on the glycosyl acceptor **14** simultaneously. Anomeric configurations of newly introduced mannosyl residues were assigned to be α according to ^{13}C NMR data which showed signals at δ 96.9 ($^1J_{CH}$ = 169.1 Hz, C-1d), 98.2 ($^1J_{CH}$ = 170.6 Hz, C-1a and C-1b), and 99.6 ($^1J_{CH}$ = 173.5 Hz, C-1c and C-1e) corresponding to five anomeric carbons. Zémplen saponification of **22** in MeOH–THF–NaOMe afforded triol **23** in 87.6% yield which was subsequently hydrogenolysed over 10% Pd–C in aq EtOH giving the target mannopentaoside **4** as an amorphous solid. The strucure of **4** was deduced from the synthetic sequence and was confirmed by the following ^{1}H and ^{13}C NMR data. ^{1}H NMR spectrum taken in D_2O at 60° showed the signals for five anomeric protons as doublets at δ 4.72 (H-1a), 4.87 (H-1b), 4.89 (H-1d), 5.09 (H-1e) and 5.12 (H-1c). ^{13}C NMR spectrum (in D_2O) showed the signals for five anomeric carbons at δ 100.2 (C-1b and C-1d), 101.9 (C-1a), 103.1 (C-1e), and 103.3 (C-1c), and each signals showed showed $^1J_{CH} \doteqdot 170$ Hz confirming the α-configurations at all anomeric centers.

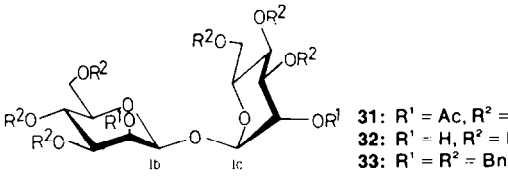
The protected mannobioside **13** could also be employed as a key intermediate for the synthesis of mannohexaoside **5** in a following way.

Glycosidation of **13** with mannosyl donor **6** under Hanessian–Banoub conditions gave a 90.7% yield (based on **13**) of protected mannotrioside **24** as well as a 22.0% yield (based on **9**) of 2 - O - acetyl - 3,4,6 - tri - O - benzyl - D - glucal as a by-product. ^{1}H NMR spectrum of **24** showed three singlets for acetyl groups at δ 1.96, 2.01 and 2.06 and ^{13}C NMR spectrum showed signals for three anomeric carbons with $^1J_{CH}$ = 172–174 Hz at δ 97.6 (C-1b), 98.5 (C-1a) and 99.6 (C-1c), confirming introduction of a new mannopyranosyl residue at O-3a of **13** with α anomeric configuration. Deacetylation of **24** gave an 83.2% yield of triol **25**, of which structure was further confirmed by the transformation into free mannotrioside **21** identical with the authentic sample.[1]

Glycosidation of the glycosylacceptor **25** with 6.5 molar equivalents of glycosyl donor **6** and subsequent chromatography of the product gave a 31.7% yield of the protected mannohexaoside **26**, R_f 0.40 in toluene–THF (10:1), along with four side products at R_f 0.67, 0.62, 0.44 and 0.30. A compound with R_f 0.62 was obtained as a 1:1 mixture with a compound with R_f 0.67 and was tentatively assigned as 2 - O - acetyl - 3,4,6 - tri - O - benzyl - D - glucal from its R_f value. The by-product at R_f 0.44 was isolated in a 6.8% yield based on **9** and was assigned to be protected mannobioside **28** from ^{1}H and ^{13}C NMR data which revealed a singlet (6H) for two acetyl groups at δ 2.10 and a signal for two anomeric carbons at δ 92.8 with $^1J_{CH}$ = 173.5 Hz. Saponification of **28** and subsequent hydrogenolysis led to free mannobioside **30** which showed a singlet for two anomeric carbons at δ 95.5 with $^1J_{CH}$ = 171.9 Hz in ^{13}C NMR in agreement with the reported data.[18] The product at R_f 0.30 was isolated in an 1.3% yield based on **9** and was determined to be **31** from ^{1}H and ^{13}C NMR data showing a singlet for two acetyl groups at δ2.12 and two signals for anomeric carbons at δ 98.0 with $^1J_{CH}$ = 160.3 Hz for C-1b and at δ 98.1 with $^1J_{CH}$ = 170.6 Hz for C-1a. The structure of **31** was further confirmed by its transformation into free mannobioside **33** which showed two doulbets with J = 2 Hz for two anomeric protons at δ 4.84 and 5.12 in ^{1}H NMR spectrum, and two signals for







two anomeric carbons at δ 100.0 ($^1J_{CH}$ = 159.2 Hz) for C-1b and 100.7 ($^1J_{CH}$ = 171.9 Hz) for C-1a.

Protected mannohexaoside **26** was saponified into **27** which in turn was hydrogenolysed in a usual way to give the target mannohexaoside **5**. ^{1}H and ^{13}C NMR data confirmed the structure as follows. Six doublets with J = 2 Hz for anomeric protons appeared at δ 4.70 (H-1a), 4.85 (H-1b), 4.88 (H-1d), 5.02 (H-1f) 5.12 (H-1e) and 5.28 (H-1c), and four signals with $^1J_{CH} \doteqdot 170$ Hz at δ 99.6 (C-1b and C-1d), 101.1 (C-1c), 101.3 (C-1a), 102.5 (C-1e and C-1f) confirmed α configuration at all anomeric centers.

In conclusion, a practical synthetic route toward branching mannopentaoside **4** and mannohexaoside **5** which constitute a part structure of high mannose type glycan chain of several glycoproteins such as ovalbumin, *Asperigillus oryzae* α-amylase, Chinese hamster ovary cell membranes and human IgM, has been developed using regioselectively protected mannobioside **13** as a key intermediate.

EXPERIMENTAL

All m.ps were determined with a Yanagimoto micro m.p. apparatus and were uncorrected. Optical rotations were determined with a Perkin–Elmer Model 141 polarimeter for solns in $CHCl_3$ at 25°, unless otherwise noted. IR spectra were recorded with an EPI-G2 Hitachi spectrophotometer, as KBr discs for the crystalline samples and as neat films for the liquid samples.

^{1}H-NMR spectra were recorded with a Varian HA-100 NMR spectrometer, using TMS as an internal standard. ^{13}C NMR spectra were recorded with a JNM-FX 100FT NMR spectrometer at 25.05 MHz. The values of δ_C and δ_H are expressed in ppm downward from the internal standard for the solns in $CDCl_3$ unless otherwise noted. Column chromatography was performed on columns of Silica Gel Merck (70–230 mesh; E. Merck, Darmstadt, West Germany). Tlc was performed on precoated plates (layer thickness, 0.25 mm; E. Merck, Darmstadt, West Germany) of Silica Gel 60 F_{254}.

Methyl 3,6 - *di* - O - *acetyl* - 2,4 - *di* - O - *benzyl* - *α* - D - *mannopyranoside* **10**

To a soln of **8** (2.05 g, 5.5 mmol) in pyridine (10 ml) was added Ac_2O (5 ml) at −5°–0° and the mixture was stirred for 3 days at room temp. Excess Ac_2O was destroyed by adding MeOH (22 ml) at 0°. Evaporation of the mixture *in vacuo* gave a residual oil which was co-evaporated 3 times with toluene to give a crude **10** which was used for the next step. An analytical sample was obtained by chromatography of the crude **10** over SiO_2 (toluene–EtOAc 5:1), R_f 0.53 in toluene–EtOac (3:1). $[\alpha]_D$ + 12.6° (c = 1.36). NMR δ_H: 1.94 (3H, s, OAc), 2.03 (3H, s, OAc), 3.32 (3H, s, OMe), 5.20 (1H, dd, J_{23} = 3 Hz, J_{34} = 9 Hz, H-3). δ_C: 20.9 ($COCH_3$), 55.0 (OMe), 63.4 (C-6), 69.6 (C-5), 72.9 (C_2O–CH_2Ph), 73.3 (C-3), 73.9 (C-4), 74.7 (C_4O–CH_2Ph), 75.7 (C-2), 98.7 (C-1, $^1J_{CH}$ = 169.1 Hz). (Found: C, 65.56; H, 6.63. $C_{25}H_{30}O_8$ requires: C, 65.49; H, 6.60%).

1,3,6 - *Tri* - O - *acetyl* - 2,4 - *di* - O - *benzyl* - *α* - D - *mannopyranose* **11**

To a soln of **10** (2.16 g, 4.6 mmol) in Ac_2O (30 ml) and AcOH (15 ml) was slowly added conc H_2SO_4 (0.45 ml) at −10°. The mixture was stirred for 1 hr at −10° and NaOAc (5 g) was added. Ac_2O and AcOH was evaporated *in vacuo* and the residue was partitioned between ice-water (50 ml) and EtOAc (70 ml × 3). Organic layer was washed with water, $NaHCO_3$aq, dried ($MgSO_4$) and evaporated to give an oily product. Chromatography over SiO_2 (150 g, toluene–EtOAc 5:1) afforded **11** as a viscous oil (1.645 g, 71.8%). Compound **11** gave the same R_f value with **10** in toluene–EtOAc (3:1) but could be separated from **10** in CCl_4–CH_3COCH_3 (10:1), R_f (**10**) 0.55 and R_f (**11**) 0.48. $[\alpha]_D$ + 31.1° (c = 0.09). NMR δ_H: 1.96 (3H, s, Ac), 2.04 (3H, s, Ac), 2.08 (3H, s, Ac), 5.20 (1H, dd, J_{23} = 3 Hz and J_{34} = 9.5 Hz, H-3), 6.15 (1H, d, J_{12} = 2 Hz, H-1). δ_C: 20.8 ($COCH_3$), 21.0 ($COCH_3$), 62.8 (C-6), 71.9 (C-5), 72.5 (C-3), 72.5 (C_2O–CH_2Ph), 73.0 (C-4), 74.2 (C-2), 74.8 (C_4O–CH_2Ph), 90.9 (C-1, $^1J_{CH}$ = 175.0 Hz). (Found: C, 63.97; H, 6.14. $C_{26}H_{30}O_9$ requires: C, 64.18; H, 6.22%).

When above acetolysis of **10** was performed under the same conditions but at 25°, a more polar product (R_f 0.34 in toluene–EtOAc 3:1) began to appear after 2–3 hr, which was isolated and purified by chromatography (SiO_2, toluene–EtOAc 3:1) to afford 1,3,4,6, - *tetra* - O - *acetyl* - 2 - O - *benzyl* - *α* - D - *mannopyranose* **12** as an oil. $[\alpha]_D$ + 11.9° (c = 1.35). NMR δ_H: 1.97 (3H, s, Ac), 2.01 (3H, s, Ac), 2.05 (3H, s, Ac), 2.10 (3H, s, Ac), 5.16 (1H, dd, J_{23} = 3 Hz, J_{34} = 10 Hz, H-3), 5.46 (1H, t, J_{34} = J_{45} = 10 Hz, H-4), 6.14 (1H, d, J_{12} = 2 Hz, H-1). (Found: C, 57.18; H, 5.95. $C_{21}H_{26}O_{10}$ requires: C, 57.53; H, 5.98%).

3,6 - *Di* - O - *acetyl*-2,4 - *di* - O - *benzyl*-*α* - D - *mannopyranosyl chloride* **7**

A soln of **11** (873 mg, 1.79 mmol) in CH_2Cl_2 (45 ml) was saturated with dry HCl at −5°–10° and stirred for 5 hr at 25°. The mixture was evaporated *in vacuo* below 30° (bath) to afford **7** as a syrup (824 mg, 99.4%) which was used directly for the next step, R_f 0.62 in toluene–EtOAc (3:1). $[\alpha]_D$ + 54.7° (c = 0.70). NMR δ_H: 1.96 (3H, s, Ac), 2.04 (3H, s, Ac), 5.46 (1H, dd, J_{23} = 3 Hz, J_{34} = 9 Hz, H-3), 6.00 (1H, d, J_{12} = 2 Hz, H-1). δ_C: 20.8 ($COCH_3$), 62.4 (C-6), 72.4 (C-3, C-4 and C-5), 73.1 (C_2O–CH_2Ph), 74.9 (C_4O–CH_2Ph), 78.4 (C-2), 90.0 (C-1, $^1J_{CH}$ = 180.9 Hz).

Selective mannosylation of methyl 2,4 - *di* - O - *benzyl* - *α* - D - *mannopyranoside* **8**

(A) a mixture of **8** (554 mg, 1.48 mmol) and $AgSO_3CF_3$ (540 mg, 2.10 mmol) in two-necked flask was dried for 3 hr at room temp in high vacuum. To this mixture was injected $Me_2NCONMe_2$ (0.6 ml) and CH_2Cl_2 (0.7 ml) under Ar. To the mixture was further added a soln of **7** (590.5 mg, 1.27 mmol) in CH_2Cl_2 (0.7 ml) at −5°. And the mixture was stirred for 1 hr at −5° and for 24 hr at 25° under Ar. Usual work-up and chromatography of the product over SiO_2 (700 g, CH_2Cl_2–Me_2CO 40:1) afforded **19** (119.0 mg, 6.5%), R_f 0.57 in CH_2Cl_2–Me_2CO (30:1). $[\alpha]_D$ + 36.6° (c = 0.145). NMR δ_H: 1.93 (3H, s, Ac), 1.94 (3H, s, Ac), 1.98 (3H, s, Ac), 2.00 (3H, s, Ac), 3.28 (3H, s, OMe). δ_C: 54.6 (OMe), 66.2 (C-6a), 77.1 (C-3a), 97.3 ($^1J_{CH}$ = 170.6 Hz, C-1b), 98.4 ($^1J_{CH}$ = 170.6 Hz, C-1a), 99.3 ($^1J_{CH}$ = 173.5 Hz, C-1c). (Found: C, 67.15; H, 6.49. $C_{69}H_{78}O_{20}$ requires: C, 67.52; H, 6.40%). Further elution with CH_2Cl_2–Me_2CO (30:1) afforded **13** (457 mg, 38.6%), R_f 0.49 in CH_2Cl_2–Me_2CO (30:1). $[\alpha]_D$ + 31.1° (c = 0.785). NMR δ_H: 1.96 (3H, s, Ac), 2.02 (3H, s, Ac), 3.30 (3H, s, OMe). δ_C: 54.6 (OMe), 66.2 (C-6a), 97.4 ($^1J_{CH}$ = 168.6 Hz, C-1b), 97.7 ($^1J_{CH}$ = 168.6 Hz, C-1a). (Found: C, 67.55; H, 6.49. $C_{45}H_{52}O_{13}$ requires: C, 67.48; H, 6.55%).

Further elution with CH_2Cl_2–Me_2CO (10:1) afforded **16** (70 mg, 5.9%), R_f 0.58 in CH_2Cl_2–Me_2CO (10:1). $[\alpha]_D$ + 25.7° (c = 0.925). NMR δ_H: 1.97 (3H, s, Ac), 2.00 (3H, s, Ac), 3.31 (3H, s, OMe). δ_C: 54.6 (OMe), 77.1 (C-3a), 98.8 ($^1J_{CH}$ = 169.1 Hz, C-1a), 99.3 ($^1J_{CH}$ = 172.1 Hz, C-1b). (Found: C, 65.77; H, 6.44. $C_{45}H_{52}O_{13}$ requires: C, 67.48; H, 6.55%). Further elution with the same solvent afforded **8** (249.5 mg, 45%) recovered.

(B) To a soln of **8** (375 mg, 1 mmol), $AgSO_3CF_3$ (900 mg, 3.5 mmol) and $Me_2NCONMe_2$ (1 ml, 8 mmol) in CH_2Cl_2 (0.5 ml) was injected 1/2 of a soln of **7** (928 mg, 2.0 mmol) in CH_2Cl_2 (1.6 ml) at −5° and the mixture was stirred for 5 hr at 25° under Ar. A remaining soln of **7** in CH_2Cl_2 was again injected at 25° and the mixture was further stirred for 16 hr at 25°. Usual work-up and chromatography of the product over SiO_2 (200 g, CH_2Cl_2–Me_2CO 30:1) afforded **19** (240 mg, 19.8%). Further elution with the same solvent gave **13** (454 mg, 54.4%).

Methyl 2,4 - *di* - O - *benzyl* - 6 - O - (2,4 - *di* - O - *benzyl* - *α* - D - *mannopyranosyl*) - *α* - D - *mannopyranoside* **14**

A soln of **13** (444 mg, 0.5 mmol) in MeOH (10 ml)–2NNaOMe–MeOH (1 ml) was stirred for 16 hr at 20°. Neutralization with Amberlist 15 (H^+) and usual work-up afforded an oily product which was chromatographed over SiO_2 (30 g, CH_2Cl_2–Me_2CO 25:1) to give **14** (300 mg, 83.1%), R_f 0.40 in CH_2Cl_2–Me_2CO (20:1). $[\alpha]_D$ + 43.2° (c = 0.25). NMR δ_H: 3.30 (3H, s, OMe), 5.06 (1H, d, J = 2 Hz, H-1b). δ_C: 54.8 (OMe), 66.1 (C-6a), 97.1 ($^1J_{CH}$ = 167.7 Hz) and 97.9 ($^1J_{CH}$ = 166.2 Hz) for C-1a and C-1b. (Found: C, 68.30; H, 6.74. $C_{41}H_{48}O_{11}$ requires: C, 68.70; H, 6.75%).

Methyl 6 - O - (*α* - D - *mannopyranosyl*) - *α* - D - *Mannopyranoside* **15**

A mixture of **14** (300 mg, 0.42 mmol) and 10% Pd–C (200 mg) in EtOH (20 ml) and H_2O (2 ml) was stirred under H_2 for 11 hr at room temp. Usual work-up afforded an amorphous powder **15** (153.8 mg, 98.0%), R_f 0.42 in nBuOH–EtOH–H_2O (2:1:1). $[\alpha]_D$ + 90.3° (c = 0.67, H_2O). NMR δ_H (D_2O at 60°): 3.40 (3H, s, OMe), 4.73 (1H, d, J = 2 Hz, H-1a), 4.91 (1H, d, J = 2 Hz, H-1b). δ_C (D_2O at 60°): 55.7 (OMe), 61.8 (C-6b), 66.4 (C-6a), 67.4 (C-4a), 67.7 (C-4b), 70.8 (C-2a and C-2b), 71.5 (C-3a and C-3b), 71.6 (C-5a), 73.6 (C-5b), 100.3 ($^1J_{CH}$ = 170.9 Hz, C-1b), 101.8 ($^1J_{CH}$ = 170.9 Hz, C-1a). (Found: C, 41.04; H, 6.37. $C_{13}H_{24}O_{11}\cdot H_2O$ requires: C, 41.71; H, 7.00%).

Methyl 3 - O - (2,4 - *di* - O - *benzyl* - *α* - D - *mannopyranosyl*) - 2,4 - *di* - O - *benzyl* - *α* - D - *mannopyranoside* **17**

A soln of **16** (110 mg) in MeOH (10 ml) and 2N–NaOMe in MeOH (0.1 ml) was stirred for 16 hr at 20°. Neutralization with Amberlist 15 (H^+) and the filtrate was concentrated *in vacuo* to give an oily residue which was chromatographed over SiO_2 (40 g, CH_2Cl_2–Me_2CO, 10:1) to give **17** (48 mg, 48.8%), R_f 0.22 in CH_2Cl_2–Me_2CO (10:1). $[\alpha]_D$ + 33.1° (c = 0.32). **17** was identified with the authentic sample prepared by an unambiguous route[14] through comparison of ^{13}C NMR data.

Methyl 3,6 - *di* - O - (2,4 - *di* - O - *benzyl* - α - D - *mannopyranosyl*) - 2,4 - *di* - O - *benzyl* - α - D - *mannopyranoside* **20**

A soln of **19** (439 mg, 0.36 mmol) in MeOH (15 ml) and 0.2 N NaOMe in MeOH (1.5 ml) was stirred for 16 hr at 20°. Usual work-up gave an oily residue (429 mg) which was chromatographed over SiO_2 (30 g, CH_2Cl_2–Me_2CO, 20:1) to afford **20** (298 mg, 78.6%), R_f 0.25 in CH_2Cl_2–Me_2CO (20:1). $[\alpha]_D$ + 49.4° (*c* = 0.235). NMR δ_H: 3.27 (3H, s, OMe), 5.10 (1H, d, J = 2 Hz), 5.21 (1H, d, J = 2 Hz). δ_C: 54.8 (OMe), 65.7 (C-6a), 97.2 ($^1J_{CH}$ = 170.6 Hz, C-1b), 98.4 ($^1J_{CH}$ = 167.7 Hz, C-1a), 99.0 ($^1J_{CH}$ = 169.1 Hz, C-1c). (Found: C, 69.16; H, 6.71. $C_{61}H_{70}O_{16}$ requires: C, 69.17; H, 6.66%).

Methyl 3,6 - *di* - O - (α - D - *mannopyranosyl*) - α - D - *mannopyranoside* **21**

The mixutre of **20** (253 mg, 0.24 mmol) and 10% Pd–C (200 mg) in EtOH (40 ml) and H_2O (4 ml) was stirred under H_2 for 16 hr at 20° and then for 1 hr at 50°. After addition of H_2O (2 ml), the mixture was further stirred under H_2 for 1 hr at 50°. Usual work-up gave **21** as an amorphous solid (117.3 mg, 94.6%), R_f 0.37 in nBuOH–EtOH–H_2O (2:1:1). $[\alpha]_D$ + 99.0° (*c* = 0.50, H_2O). NMR δ_H(D_2O, at 60°): 3.40 (s, OMe), 4.72 (1H, d, J = 2 Hz, H-1a), 4.91 (1H, d, J = 2 Hz, H-1b), 5.11 (1H, d, J = 2 Hz, H-1c). **21** was identified with the authentic sample prepared previously.[1,9]

Methyl 2,4 - *di* - O - *benzyl* - 3 - O - (2 - O - *acetyl* - 3,4,6 - *tri* - O - *benzyl* - α - D - *mannopyranosyl*) - 6 - O - [2,4 - *di* - O - *benzyl* - 3,6 - *di* - O - (2 - O - *acetyl* - 3,4,6 - *tri* - O - *benzyl* - α - D - *mannopyranosyl*) - α - D - *mannopyranosyl*] - α - D - *mannopyranoside* **22**

A mixture of **14** (590 mg, 0.82 mmol) and $AgSO_3CF_3$ (1.60 g, 6.2 mmol) was coevaporated with toluene (two times) *in vacuo* and further dried in high vacuum for 3 hr. To this mixture was injected $Me_2NCONMe_2$ (1.1 ml, 9.2 mmol) and CH_2Cl_2 (3 ml) under Ar, and then 1/2 volume of a soln of **6** (prepared from **9** 2.28 mg, 4.5 mmol) in CH_2Cl_2 (5 ml) successively with stirring at −10°. After stirring for 3 hr at 20°, the remaining soln of **6** in CH_2Cl_2 was injected and the mixture was stirred for 16 hr at 20°. The reaction mixture was diluted with CH_2Cl_2 and inorganic ppt was filtered off. Filtrate was washed with $NaCHO_3$aq, dried ($MgSO_4$) and evaporated to give an oil (3.53 g), which was chromatographed over SiO_2 (330 g, toluene–THF 10:1), affording **22** as a foam (1.290 g, 73.3%), R_f 0.27 in toluene–THF (10:1). $[\alpha]_D$ + 45.2° (*c* = 0.575). NMR δ_H: 2.03 (3H, s, Ac), 2.06 (3H, s, Ac), 2.10 (3H, s, Ac), 3.17 (3H, s, OMe). δ_C: 54.8 (OMe), 96.9 ($^1J_{CH}$ = 169.1 Hz, C-1d), 98.2 ($^1J_{CH}$ = 170.6 Hz, C-1b and C-1a), 99.6 ($^1J_{CH}$ = 173.5 Hz, C-1c and C-1e). (Found: C, 71.80; H, 6.52. $C_{128}H_{138}O_{29}$ requires: C, 71.82; H, 6.50%). From a less polar fraction, a minor product, 2 - O - *acetyl* - 3,4,6 - *tri* - O - *benzyl* - D - *glucal*, (392 mg, 18.4% from **9**) was isolated. R_f 0.61 in toluene–THF (10:1). $[\alpha]_D$ + 26.1° (*c* = 0.44). NMR δ_H: 2.03 (3H, s, Ac), 6.59 (1H, s, H-1). (Found: C, 73.27; H, 6.39. $C_{29}H_{30}O_6$ requires: C, 73.40; H, 6.37%).

Methyl 2,4 - *di* - O - *benzyl* - 3 - O - (3,4,6 - *tri* - O - *benzyl* - α - D - *mannopyranosyl*) - 6 - O - [2,4 - *di* - O - *benzyl* - 3,6 - *di* - O - (3,4,6 - *tri* - O - *benzyl* - α - D - *mannopyranosyl*) - α - D - *mannopyranosyl*] - α - D - *mannopyranoside* **23**

A soln of **22** (912 mg, 0.43 mmol) in MeOH (25 ml)–THF (10 ml)–2N NaOMe in MeOH (0.8 ml) was stirred for 2.5 hr at 20°. The mixture was diluted with MeOH (30 ml) and was neutralized with Amberlist 15 (H^+). Usual work-up and chromatography over SiO_2 (150 g, CH_2Cl_2–THF 20:1) gave **23** (759 mg, 87.6%) as a foam. R_f 0.24 in CH_2Cl_2–THF (30:1). $[\alpha]_D$ + 63.2° (*c* = 0.31). NMR δ_H: 3.24 (3H, s, OMe), 4.90, 4.99, 5.10, 5.21 and 5.23 (five 1H, bs, for five anomeric protons). δ_C: 54.6 (OMe), 96.8 ($^1J_{CH}$ = 170.6 Hz, C-1a), 98.2 ($^1J_{CH}$ = 169.1 Hz, C-1b), 99.5 ($^1J_{CH}$ = 170.6 Hz, C-1d), 101.1 ($^1J_{CH}$ = 167.7 Hz, C-1c and C-1e).

Methyl 3 - O - (α - D - *mannopyranosyl*) - 6 - O - [3,6 - *di* - O - (α - D - *mannopyranosyl*) - α - D - *mannopyranosyl*] - α - D - *mannopyranoside* **4**

A mixture of **23** (353 mg, 0.17 mmol) and 10% Pd–C (200 mg) in EtOH (20 ml) and H_2O (4 ml) was stirred under H_2 for 2 days at 25°. Usual work-up afforded **4** as an amorphous solid (137 mg, 87.8%), R_f 0.11 in nBuOH–EtOH–H_2O (2:1:1). $[\alpha]_D$ + 108.1° (*c* = 0.185, H_2O), + 115.0° (*c* = 0.30, MeOH). NMR δ_H (D_2O, 60°): 4.72 (1H, d, J = 2 Hz, H-1a), 4.87 (1H, d, J = 2 Hz, H-1b), 4.89 (1H, d, J = 2 Hz, H-1d), 5.09 (1H, d, J = 2 Hz, H-1e), 5.12 (1H, d, J = 2 Hz, H-1c). δ_C (D_2O, 60°): 55.7 (OMe), 79.1 and 79.3 from C-3a and C-3b, 100.2 ($^1J_{CH}$ = 170.6 Hz, C-1b and C-1d), 101.9 ($^1J_{CH}$ = 170.6 Hz, C-1a), 103.1 ($^1J_{CH}$ = 169.1 Hz, C-1e), 103.3 ($^1J_{CH}$ = 169.1 Hz, C-1c). (Found: C, 41.87; H, 6.31. $C_{31}H_{54}O_{26}\cdot 3H_2O$ requires: C, 41.52; H, 6.74%).

Methyl 3 - O - (2 - O - *acetyl* - 3,4,6 - *tri* - O - *benzyl* - α - D - *mannopyranosyl*) - 6 - O - (3,6 - *di* - O - *acetyl* - 2,4 - *di* - O - *benzyl* - α - D - *mannopyranosyl*) - α - D - *mannopyranoside* **24**

A mixture of **13** (1.966 g, 2.45 mmol) and $AgSO_3CF_3$ (1.95 g, 7.59 mmol) was coevaporated with toluene 2 times and was dried *in vacuo* for 2 hr. To this mixture was injected CH_2Cl_2 (5 ml) and $Me_2NCONMe_2$ (1.8 ml, 15 mmol) and then at −15° one half of the soln of **6** (prepared from **9** 2.54 g, 5 mmol) in CH_2Cl_2 (5 ml) with stirring under Ar. After stirring for 3 hr at 20°, remaining soln of **6** was injected and the mixture was further stirred for 16 hr at 20°. Usual work-up afforded an oily product (6.562 g) which was chromatographed over SiO_2 (500 g, CH_2Cl_2–Me_2CO 60:1) to give **24** (2.835 g, 90.7%). R_f 0.64 in CH_2Cl_2–Me_2CO (20:1). $[\alpha]_D$ + 41.0° (*c* = 0.385). NMR δ_H: 1.96 (3H, s, Ac), 2.01 (3H, s, Ac), 2.06 (3H, s, Ac), 3.24 (3H, s, OMe). δ_C: 54.8 (OMe), 97.6 ($^1J_{CH}$ = 172.1 Hz, C-1b), 98.5 ($^1J_{CH}$ = 172.1 Hz, C-1a), 99.6 ($^1J_{CH}$ = 173.5 Hz, C-1c). (Found: C, 69.63; H, 6.46. $C_{74}H_{82}O_{19}$ requires: C, 69.68; H, 6.48%). From less polar fraction, 2 - O - *acetyl* - 3,4,6 - *tri* - O - *benzyl* - D - *glucal* was isolated in 22.0% yield (523 mg) from **9**. It is to be noted that when above reaction was performed using 1.25 eq. of halide **6**, the yield of **24** was lowered to 54.4% and 26.1% of **13** was recovered.

Methyl 2,4 - *di* - O - *benzyl* - 6 - O - (2,4 - *di* - O - *benzyl* - α - D - *mannopyranosyl*) - 3 - O - (3,4,6 - *tri* - O - *benzyl* - α - D - *mannopyranosyl*) - α - D - *mannopyranoside* **25**

A soln of **24** (2.73 g, 2.14 mmol) in MeOH (75 ml)–THF (30 ml) and 2N–NaOMe in MeOH (0.5 ml) was stirred for 16 hr at 20°. Usual work-up gave an oily product (3.13 g) which was chromatographed over SiO_2 (220 g, CH_2Cl_2–Me_2CO 25:1) to afford **25** (2.046 g, 83.2%) as a foam, R_f 0.30 in CH_2Cl_2–Me_2CO (20:1). $[\alpha]_D$ + 46.5° (*c* = 0.55). NMR δ_H: 3.23 (3H, s, OMe), 5.11 (1H, bd, J ≒ 2 Hz) and 5.23 (1H, bd, J ≒ 2 Hz) for H-1b and H-1c. δ_C: 54.7 (OMe), 97.1 ($^1J_{CH}$ = 169.1 Hz, C-1b), 98.3 ($^1J_{CH}$ = 167.7 Hz, C-1a), 101.1 ($^1J_{CH}$ = 167.7 Hz, C-1c). (Found: C, 71.15; H, 6.83. $C_{68}H_{76}O_{16}$ requires: C, 71.06; H, 6.67%).

Hydrogenolysis of **25** under the same conditions as in the case of **20** afforded a high yield of the trimannoside which was identified with **21** through the comparison of ^{13}C and ^1H NMR data.

Methyl 3 - O - [2 - O - (2 - O - *acetyl* - 3,4,6 - *tri* - O - *benzyl* - α - D - *mannopyranosyl*) - 3,4,6 - *tri* - O - *benzyl* - α - D - *mannopyranosyl*] - 6 - O - [3,6 - *di* - O - (2 - O - *acetyl* - 3,4,6 - *tri* - O - *benzyl* - α - D - *mannopyranosyl*) - 2,4 - *di* - O - *benzyl* - α - D - *mannopyranosyl*] - 2,4 - *di* - O - *benzyl* - α - D - *mannopyranoside* **26**

A mixture of **25** (1.548 g, 1.35 mmol) and $AgSO_3CF_3$ (3.4 g, 13.2 mmol) was dried by co-evaporation with toluene for two times and then under high vacuum for 5 hr at 20°. To this mixture was injected $Me_2NCONMe_2$ (2.1 ml, 17.5 mmol), CH_2Cl_2 (20 ml), and 1/3 of a soln of **6** (prepared from **9**, 4.44 g, 8.76 mmol) in CH_2Cl_2 (20 ml) with stirring at −15° under Ar. Each of the one half of the remaining soln of **9** in CH_2Cl_2 was injected separately after stirring the mixture for 4 hr and 12 hr at 20° respectively. After further stirring for 24 hr at 20°, the mixture was processed as usual to give an oily product (6.97 g), which was chromatographed over SiO_2 (420 g). Elution with toluene–THF (15:1) afforded a fraction which contains an unidentified product of R_f 0.67 and 2 - O - acetyl - 3,4,6 - tri - O - benzyl - D - glucal, R_f 0.62 in a ratio of about 1:1 (1.53 g). Further elution with the same solvent afforded crude **26** (2.84 g) which contains two minor

spots at R_f 0.44 and 0.35 in addition to the major sport at R_f 0.40. Rechromatography over SiO_2 (500 g, toluene–THF, 18:1) afforded 2 - O - *acetyl* - 1 - O - (2 - O - *acetyl* - 3,4,6 - *tri* - O - *benzyl* - *α* - D - *mannopyranosyl*) - 3,4,6 - *tri* - O - *benzyl* - *α* - D - *mannopyranose* **28** (577 mg, 6.8% from **9**) R_f 0.44 in toluene–THF (10:1). NMR δ_H: 2.10 (6H, s, Ac×2), δ_C: 92.8 ($^1J_{CH}$ = 173.5 Hz, C-1a and C-1b). (Found: C, 72.59; H, 6.52. $C_{58}H_{62}O_{13}$ requires: C, 72.03; H, 6.46%). Further elution gave **26** (1.10 g, 31.7%) R_f 0.40 in toluene–THF (10:1). $[\alpha]_D$ + 45.8° (c = 0.275). NMR δ_H: 2.05 (3H, s, Ac), 2.09 (3H, s, Ac), 2.10 (3H, s, AC), 3.17 (3H, s, OMe). δ_C: 54.7 (OMe), 96.9 ($^1J_{CH}$ = 172.1 Hz, C-1a), 98.0 ($^1J_{CH}$ = 170.0 Hz, C-1b), 98.2 ($^1J_{CH}$ = 170.0 Hz, C-1d), 99.4 ($^1J_{CH}$ = 170.6 Hz, C-1f), 99.6 ($^1J_{CH}$ = 170.6 Hz, C-1e), 101.0 ($^1J_{CH}$ = 175.0, C-1c). (Found: C, 72.21; H, 6.52. $C_{155}H_{166}O_{34}$ requires: C, 72.35; H, 6.50%). Further elution with the same solvent afforded another minor product, 2 - O - *acetyl* - 1 - O - (2 - O - *acetyl* - 3,4,6 - *tri* - O - *benzyl* - *β* - D - *mannopyranosyl*) - 3,4,6 - *tri* - O - *benzyl* - *α* - D - *mannopyranose* **31** (107 mg, 1.3% yield from **9**). R_f 0.30. $[\alpha]_D$ + 18.4° (c = 0.62). NMR δ_H: 2.12 (6H, s, Ac×2). δ_C: 98.0 ($^1J_{CH}$ = 160.3 Hz, C-1b), 98.1 ($^1J_{CH}$ = 170.6 Hz, C-1a). (Found: C, 71.71; H, 6.62. $C_{58}H_{62}O_{13}$ requires: C, 72.03; H, 6.46%).

Methyl 2,4 - *di* - O - *benzyl* - 3 - O - [3,4,6 - *tri* - O - *benzyl* - 2 - O - (3,4,6 - *tri* - O - *benzyl* - *α* - D - *mannopyranosyl*) - *α* - D - *mannopyranosyl*] - 6 - O - [2,4 - *di* - O - *benzyl* - 3,6 - *di* - O - [3,4,6 - *tri* - O - *benzyl* - *α* - D - *mannopyranosyl*) - *α* - D - *mannopyranosyl*] - *α* - D - *mannopyranoside* **27**

A soln of **26** (744 mg, 0.29 mmol) in MeOH (30 ml)–THF (12 ml) and 2N NaOMe–MeOH (0.5 ml) was stirred for 7 hr at 20°. Usual work-up and chromatography over SiO_2 (300 g, CH_2Cl_2–THF, 50:1) afforded **27** (686 mg, 96.3%) as a syrap, R_f 0.30 in CH_2Cl_2–THF (30:1). $[\alpha]_D$ + 51.1° (c = 0.27). NMR δ_H: 3.18 (3H, s, OMe). δ_C: 54.7 (OMe), 96.9 ($^1J_{CH}$ ≒ 170 Hz, C-1a), 98.0 ($^1J_{CH}$ ≒ 170 Hz, C-1b), 99.6 ($^1J_{CH}$ ≒ 170 Hz, C-1d), 101.0 ($^1J_{CH}$ ≒ 170 Hz, C-1e and C-1c), 101.4 ($^1J_{CH}$ ≒ 170 Hz, C-1f). (Found: C, 72.63; H, 6.68. $C_{149}H_{160}O_{31}$ requires: C, 73.14; H, 6.59%).

Methyl 3 - O - [2 - O - (*α* - D - *mannopyranosyl*) - *α* - D - *mannopyranosyl*] - 6 - O - [3,6 - *di* - O - (*α* - D - *mannopyranosyl*) - *α* - D - *mannopyranosyl*] - *α* - D - *mannopyranoside* **5**

A soln of **27** (207 mg, 0.84 mmol) in EtOH (25 ml)–H_2O (5 ml) was stirred under H_2 in the presence of 10% Pd–C (250 mg) for 14 hr at 20°. Usual work-up afforded **5** (90.6 mg, 94.4%) as an amorphous powder, R_f 0.13 in nBuOH–EtOH–H_2O (2:1:1). $[\alpha]_D$ + 92.0° (c = 0.44, H_2O). NMR δ_H (D_2O, 60°): 4.70 (1H, d, J = 2 Hz, H-1a), 4.85 (1H, d, J = 2 Hz, H-1b), 4.88 (1H, d, J = 2 Hz, H-1d), 5.02 (1H, d, H = 2 Hz, H-1f), 5.12 (1H, d, J = 2 Hz, H-1e), 5.28 (1H, d, J = 2 Hz, H-1c). δ_C (D_2O, 60°): 55.2 (OMe), 99.6 ($^1J_{CH}$ = 169.9 Hz, C-1b and C-1d), 101.1 ($^1J_{CH}$ = 173.8 Hz, C-1c), 101.3 ($^1J_{CH}$ = 173.8 Hz, C-1a), 102.5 ($^1J_{CH}$ = 170.9 Hz, C-1e and C-1f). (Found: C, 35.59; H, 5.92. $C_{37}H_{64}O_{31}\cdot 7H_2O$ requires: C, 39.93; H, 6.88%).

1 - O - (*α* - D - *Mannopyranosyl*) - *α* - D - *mannopyranose* **30**

Deacetylation of **28** (270 mg, 0.28 mmol) was performed in a usual way. Chromatography of the product over SiO_2 (20 g, CH_2Cl_2–Me_2CO, 20:1) gave **29** as a syrup (180 mg, 72.9%). R_f 0.39 in CH_2Cl_2–Me_2CO (10:1). $[\alpha]_D$ + 49.2° (c = 0.61). NMR δ_C: 94.9 ($^1J_{CH}$ = 170.9 Hz, two anomeric carbons). (Found: C, 73.16; H, 6.59. $C_{54}H_{58}O_{11}$ requires: C, 73.45; H, 6.62%). Catalytic hydrogenolysis of **29** (106 mg, 0.12 mmol) in EtOH (10 ml)–H_2O (2 ml) in the presence of 10% Pd–C (100 mg) was performed in a usual way to afford **30** (42.8 mg) as an amorphous powder. R_f 0.27 in nBuOH–EtOH–H_2O (2:1:1). NMR δ_C (D_2O, 60°): 61.2 (C-6), 66.9 (C-4), 70.2 (C-3), 70.6 (C-2), 73.8 (C-5), 95.5 ($^1J_{CH}$ = 171.9 Hz, C-1).

1 - O - (*β* - D - *Mannopyranosyl*) - *α* - D - *mannopyranose* **33**

Deacetylation of **31** (50 mg) as in the case of **29** and chromatography over SiO_2 (10 g, CH_2Cl_2–MeCO, 15:1) gave **32** (31.4 mg, 68.7%). R_f 0.48 in CH_2Cl_2–Me_2CO (10:1). $[\alpha]_D$ + 55.6° (c = 0.18). NMR δ_C: 98.5 ($^1J_{CH}$ = 155.9 Hz, C-1b), 98.9 ($^1J_{CH}$ = 172.1 Hz, C-1a). (Found: C, 73.12; H, 6.66. $C_{54}H_{58}O_{11}$ requires: C, 73,45; H, 6.62%). Hydrogenolysis of **32** (198.5 mg, 0.22 mmol) in EtOH (20 ml)–H_2O (3 ml) in the presence of 10% Pd–C (100 mg) was performed in a usual way to give **33** (77 mg, 99.1%). R_f 0.30 in nBuOH–EtOH–H_2O (2:1:1). $[\alpha]_D$ + 34.7° (c = 0.285). NMR δ_H (D_2O, 60°): 4.84 (1H, d, J = 1 Hz, H-1b), 5.12 (1H, d, J = 2 Hz, H-1a). δ_C (D_2O, 60°): 100.0 ($^1J_{CH}$ = 159.2 Hz, C-1b), 100.7 ($^1J_{CH}$ = 171.9 Hz, C-1a). (Found: C, 40.12; H, 6.56. $C_{12}H_{22}O_{11}\cdot H_2O$ requires: C, 40.00; H, 6.71%).

Acknowledgements—We thank Dr. J. Uzawa and Mrs. T. Chijimatsu for recording and measuring the NMR spectra, and Dr. H. Homma and his staff for the elemental analysis. We also thank emeritus scientist Prof. M. Matsui for his encouragement and Miss A. Sone for the technical assistance.

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