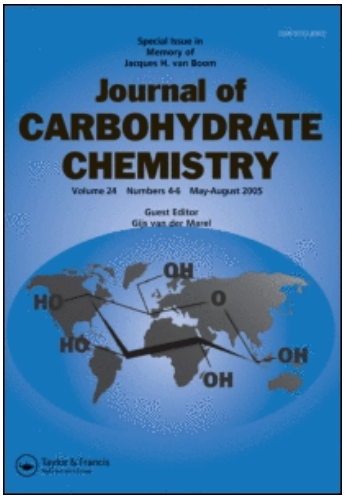


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

A Synthetic Approach to the Glycan Chain of High Mannose Type N-Glycoprotein

Slim Cherif; Jean-Marc Clavel; Claude Monneret

To cite this Article Cherif, Slim , Clavel, Jean-Marc and Monneret, Claude(1998) 'A Synthetic Approach to the Glycan Chain of High Mannose Type N-Glycoprotein', Journal of Carbohydrate Chemistry, 17: 8, 1203 — 1218
To link to this Article: DOI: 10.1080/07328309808001894
URL: http://dx.doi.org/10.1080/07328309808001894

J. CARBOHYDRATE CHEMISTRY, 17(8), 1203-1218 (1998)

A SYNTHETIC APPROACH TO THE GLYCAN CHAIN OF HIGH MANNOSE TYPE N-GLYCOPROTEIN

Slim Cherif, Jean-Marc Clavel, Claude Monneret*

UMR 176, CNRS-Institut Curie, Section Recherche, 26, rue d'Ulm,
F-75248 Paris Cedex 05, France

Received November 8, 1997 - Final Form May 26, 1998

ABSTRACT

The syntheses of α-D-glucopyranose-(1→3)-D-mannopyranose, methyl α-D-glucopyranose-(1→3)-α-D-mannopyranoside and methyl α-D-glucopyranose-(1→3)-α-D-mannopyranose-(1→2)-α-D-mannopyranoside are reported. High stereoselectivity was observed during the coupling of glucose and mannose residues by the use of glucosyl trichloroacetimidate as donor.

INTRODUCTION

N-Glycoproteins are widely distributed in eukaryotic cells, and their biosynthesis is well documented.[1] In particular, a common oligosaccharide precursor is transferred from a glycolipid to the nascent protein within the endoplasmic reticulum (E.R.)[2] where correct folding of secretory proteins is mediated by molecular chaperons. This step is essential for the transport of proteins to the connecting compartments of the biosynthetic pathway. Calnexin and calreticulin represent two E.R. proteins acting as chaperons for many secretory proteins.[3] These proteins possess lectin-like activity, and have been shown to bind with N-linked oligosaccharides. For calnexin, such binding is transient,[4] and depends on the presence of a single glucose in the N-linked oligosaccharide side-chain partially trimmed by glucosidases I and II. Once initial contact is established, calnexin is believed to associate in a more stable fashion with a hydrophobic peptide moiety. The substrate protein

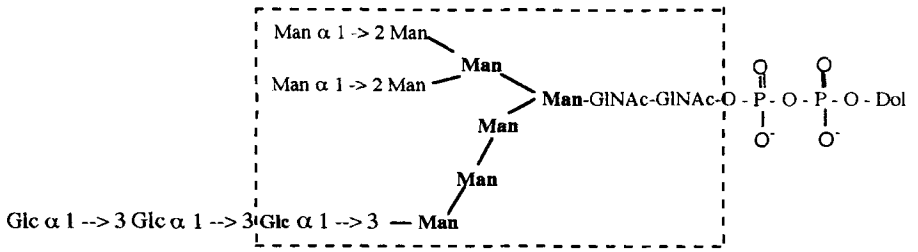


Figure 1. Schematic representation of the oligosaccharide-lipid precursor.

The $Glc_1Man_9GlcNAc_2$ dolichol-linked oligosaccharide that is bound by calnexin is enclosed by the box (cf. ref. 5). The Glc_1Man_5 which binds to calreticulin is shown in bold.

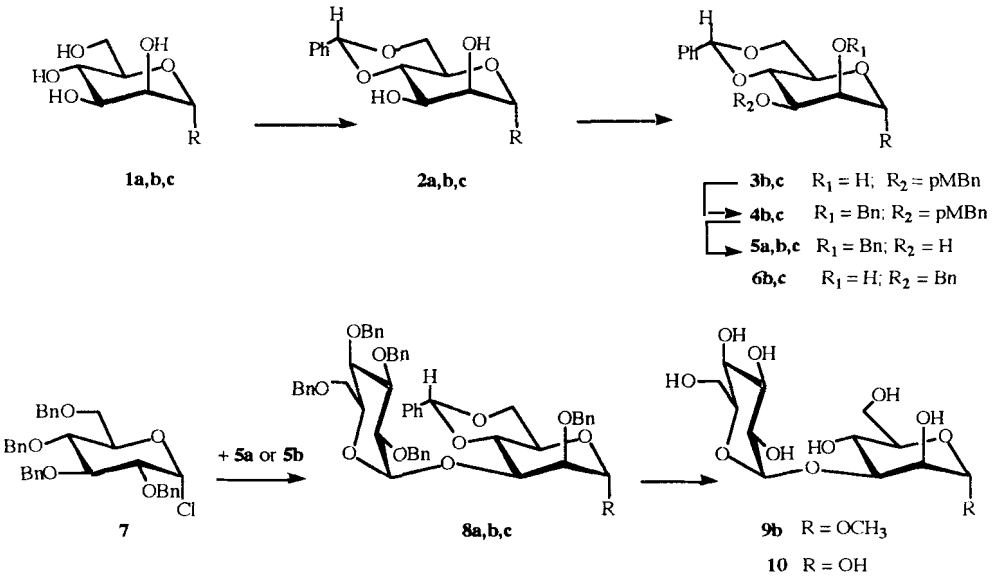
remains associated with the chaperon until it has folded and lost the conformation features responsible for the attachment.

Recent experiments have shown that soluble calnexin specifically binds[5] with $Glc_1Man_9GlcNAc_2$ species, whereas binding with $Glc_1Man_{5-7}GlcNAc_2$ oligosaccharides is relatively poor. A similar observation[6] has been reported with calreticulin, since truncation of $Glc_1Man_9GlcNAc_2$ to Glc_1Man_4 results in loss of binding. However, for $Glc_1Man_5GlcNAc$, approximately 65% of the initial binding capacity was maintained. This indicates that beyond the presence of the terminal mannose-linked glucose residue as determinant, at least a mannose after the 3,6-branching point is required for chaperon binding to occur.

RESULTS AND DISCUSSION

On the basis of these observations, it was interesting to see whether synthetically modified oligosides bearing the crucial portion α-D-Glc*p* -(1→3)-α-D-Man*p* interact with the chaperon and in this context we report the synthesis of this determinant, as well as the synthesis of the corresponding trisaccharide α-D-Glc*p* (1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-OMe.

Although approaches to asparagine-linked glycoprotein oligosaccharides have been the subject of many investigations,[7,8] most have been directed toward construction of the tri- or pentasaccharide core structure around the 3,6-branching point.[9] Recently, the synthesis of the undecasaccharide $Man_9GlcNAc_2$ was reported by Ogawa *et al.*[10] In

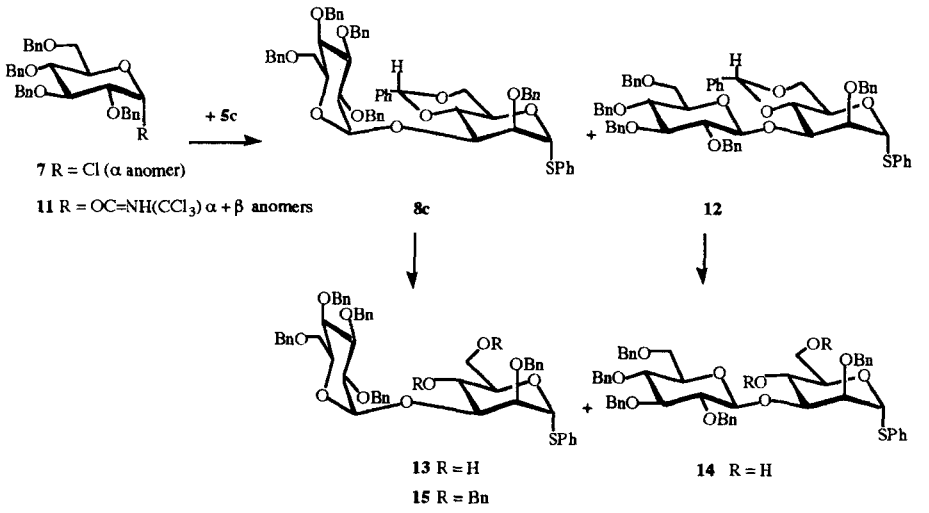


Scheme 1

contrast, the syntheses of oligosides containing the α-D-Glc*p* -(1 → 3)-Man*p* moiety as their terminal unit have received little attention.[11-13]

To obtain access to the disaccharide α-D-Glc*p* -(1 → 3)-Man*p* and its methyl glycoside, the benzyl and methyl glycosyl acceptors **5a** and **5b**, having a free OH group at the C-3 position, were initially prepared. As illustrated in Scheme 1, the benzyl **1a** and the methyl mannopyranoside **1b** were first converted into the corresponding 4,6-*O*-benzylidene derivatives **2a** and **2b** (α,α-dimethoxytoluene, TsOH).[14] Subsequent selective benzylation of the 2-OH was achieved under phase transfer conditions,[15] giving **5a** and **5b** in 51% and 36% yields, respectively. Alternatively, **5b** was also prepared regioselectively following a three-step sequence reported for the corresponding phenyl thioglycoside:[16] i) formation of a stannylene acetal, *p*-methoxybenzylation of the 3-OH (68% yield); ii) benzylation of **3b**; iii) removal of the *p*-methoxybenzyl group (CAN, CH_3CN-H_2O, 0 °C, 87%) present in **4b**.

After column chromatography (cyclohexane-EtOAc 9:1), condensation of **5a** and **5b** with tetra-*O*-benzylglucosyl chloride **7** (CF_3SO_3Ag, *sym*-coll., CH_2Cl_2, -78 °C)[17] afforded disaccharide **8a** (55%) and **8b** (34%). These intermediates were deprotected (H_2, Pd/C 10% in EtOH:EtOAc, 1:1, *v/v*) to give the expected disaccharide **9b** and the corresponding analog **10** (α + β anomers at the C-1 position) in almost quantitative yields.

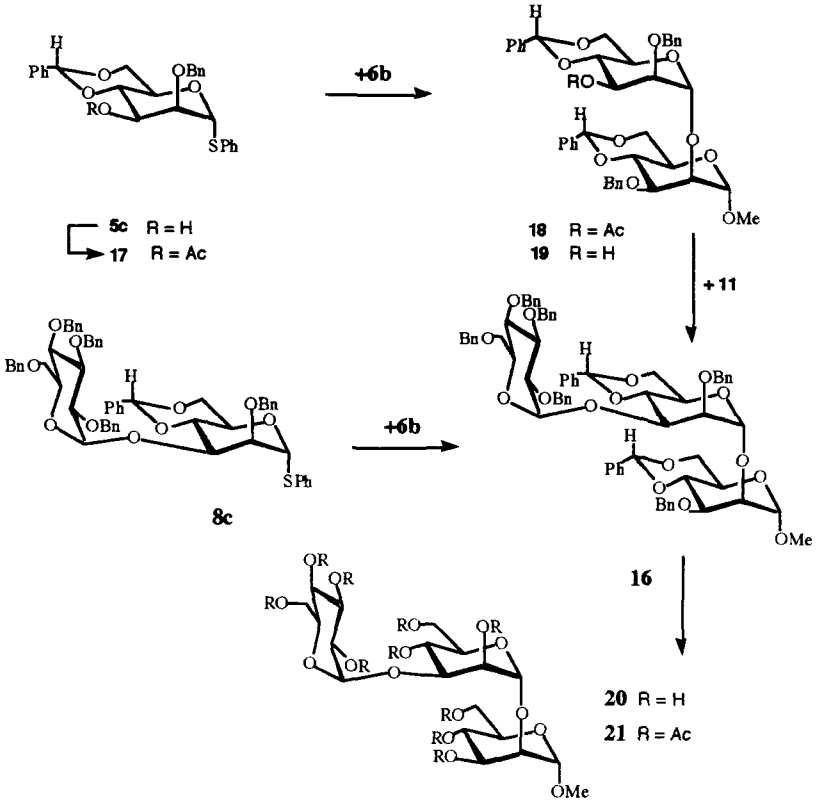


Scheme 2

In a subsequent approach to the formation of the trisaccharides α-D-Glc*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-OMe and α-D-Glc*p*-(1→3)-α-D-Man*p*(1→2)-Man, the phenyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thiomannopyranoside **5c** was used in place of the methyl or benzyl glycosides **5a** or **5b**. As for **5b**, compound **5c** was synthesized from the corresponding 4,6-*O*-benzylidene derivative **2c** via the formation of *p*-methoxybenzyl ether **3c** (81%), benzylation (90%), and treatment of **4c** with CAN (or, preferably, with DDQ) (86%). Condensation of **5c** with **7** under the above conditions (CF_3SO_3Ag, *sym*-coll, CH_2Cl_2, -78 °C) led to a mixture not easily separable (ratio α/β = 80:20, HPLC analysis) of α and β disaccharides **8c** and **12** (Scheme 2).

Anticipating that purification would be easier after chemical modification, reductive cleavage of the acetal group was subsequently attempted with $LiAlH_4$-$AlCl_3$[18] or with $NaBH_3CN$,[19] in order to obtain either the 6-*O*-benzyl or the 4-*O*-benzyl mannopyranoside moieties. In fact, separation was only effective after hydrolysis ($SnCl_2$)[20] of the benzylidene ring, affording **13** (which has been also characterized as its perbenzylated derivative **15**) and **14**.

As different attempts to achieve exclusive formation of **8c** from the glucosyl chloride **7** did not succeed ($HgCN_2$/$HgBr_2$[13] or $HgBr_2$) we turned our attention to the trichloroacetimidate derivative **11**.[21] Coupling of **11** with **5c** was stereoselective (ratio **8c**/**12** = 93/7 by HPLC analysis) providing disaccharide **8c** in high isolated yield (85%).



Scheme 3

Essentially pure compound **8c** was obtained after a second flash chromatography (> 97%, HPLC analysis).

In order to prepare the trisaccharide α-D-Glc*p*-(1→3)-α-D-Man*p* -(1→2)-α-D-Man*p*-OMe in the next step, two complementary routes were followed (Scheme 3). In the first, the disaccharide α-D-Glc*p*-(1→3)-α-D-Man*p*-SPh **8c** was employed as donor, and the mannose **6b** as the acceptor. Compound **6b** was easily obtained from methyl 4,6-*O*-benzylidene-α-D-mannopyranoside **2b** by selective 3-*O*-benzylation via the stannylene intermediate. Surprisingly, in spite of repeated experiments, condensation between **8c** and **6b** (NIS, CF_3SO_3Ag), to give the expected trisaccharide **16** proceeded in low yield (≈ 11%).

Therefore, we turned our attention to the other route, starting from the glucosyl trichloroacetimidate **11** as donor and suitably protected α-D-Man*p*-(1→2)-α-D-Man*p*-OMe

19 as the acceptor. Compound **19** was prepared in two steps involving condensation of phenyl thioglycoside **17** with **6b** to afford the protected α-D-man*p*-(1→2)-α-D-Man*p*-OMe disaccharide **18** (60% yield) and subsequent deprotection of **18** led to **19** (100% yield). Finally, trisaccharide **16** was obtained in satisfactory yield (≈ 50%) by coupling the trichloroacetimidate derivative **11** with **19**. Total debenzylation of **16** was achieved by hydrogenolysis to give **20**, and **21** after peracetylation.

In conclusion, we achieved the synthesis of the target disaccharides α-D-Glc*p*-(1→3)-D-Man*p* **9b** and its methyl glycoside **10**, and of the trisaccharide α-D-Glc*p*-(1→3)-α-D-Man*p* -(1→2)-α-D-Man*p*-OMe **20** in good yield and in a stereocontrolled manner. Studies are underway to construct glycoprotein mimics including this determinant, as well as terminal mannose residues as those present on the two other branches of natural glycoproteins.

EXPERIMENTAL

General methods. Melting points are reported uncorrected. IR spectra were recorded in chloroform solution using a Perkin-Elmer 1710 spectrophotometer, calibrated against a polystyrene film and are expressed in cm^{-1}. Optical rotations were determined with a Perkin-Elmer 241 polarimeter (589 nm), at 20 °C, with a concentration expressed in g/100 mL. ^1H NMR spectra were recorded using a Bruker AC300 (300 MHz) spectrophotometer. Chemical shifts are expressed in ppm downfield from internal Me_4Si with the notations indicating the multiplicity of the signal (s, singlet; bs, broad singlet; d, doublet; dd, doublet of doublet; t, triplet and m, multiplet). The coupling constants are expressed as J values in units of Hertz. For mass spectra, CI (NH_3) were recorded with a Nermag R10-10C and FAB (M+ Na^+) was recorded with a Jeol MS 700. TLC was performed on Silica gel $60F_{254}$ (Merck). Silica gel (Merck, particle size 0.040-0.063 nm) was used for flash chromatography.[22] Analytical HPLC were performed on a Nova Pak Silica Waters Part n° 36980 (3.9 mm diameter x 15 cm) or a Sigma-Aldrich Silica Waters, Spherisorb silica (4.6 mm diameter x 25 cm) with a mixture of heptane-EtOAc (94:6) as solvent. A flow rate of 1 mL/min was used with detection at 254 nm.

Benzyl 2-*O*-Benzyl 4,6-*O*-benzylidene-α-D-mannopyranoside (5a) was prepared in 51% yield according to Garegg *et al*[15] from benzyl 4,6-benzylidene-α-D-mannopyranoside **2a**.[24]

Methyl 4,6-*O*-Benzylidene-3-*O*-*p*-methoxybenzyl-α-D-mannopyranoside (3b). Dibutyltin oxide (3.5 g, 14 mmol) was added to a solution of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside **2b**[25] (3.6 g, 12.7 mmol) in anhydrous toluene

(400 mL). The resulting mixture was heated under Dean-Stark conditions for 4 h prior to the addition of tetrabutylammonium iodide (2.35 g, 6.35 mmol) and of 4-methoxybenzyl chloride (2.1 g, 14 mmol). After additional reflux for 5 h and concentration to ≈ 200 mL, the mixture was diluted with H_2O (200 mL) and extracted three times with EtOAc (3 x 100 mL). The combined organic layers were washed with H_2O and dried over $MgSO_4$. Concentration under reduced pressure, followed by flash chromatography (cyclohexane-EtOAc, 9:1 then 6:1), afforded **3b** (3.5 g, 68%) as a syrup; R_f = 0.3 (cyclohexane-EtOAc, 2:1); $[\alpha]_D$ + 43° (*c* 1.5, chloroform); IR ($CDCl_3$) 3587 cm^{-1} (OH); ^1H NMR ($CDCl_3$): δ 7.52-7.26 (m, 7H, Ar), 6.84-6.89 (m, 2H, Ar), 5.61 (s, 1H, H-7), 4.75 (s, 1H, H-1), 4.78 and 4.63 (2d, J = 11.5 Hz, CH_2Ph), 4.28 (m, 1H, H-6b), 4.07 (dd, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 4.01 (dd, 1H, H-2), 3.90 (dd, 1H, $J_{2,3}$ = 3, $J_{3,4}$ = 9.5 Hz, H-3), 3.37 (m, 2H, H-6a, H-5), 2.69 (d, 1H, J = 1.1 Hz, OH); MS (DCI/NH_3) *m/z* 420 $(M + NH_4)^+$, 403 $(M + H)^+$.

Anal. Calcd for $C_{22}H_{26}O_7$: C, 65.64; H, 6.52. Found: C, 65.75; H, 6.48.

Methyl 2-*O*-Benzyl-4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-α-D-mannopyranoside (4b). A solution of **3b** (3.5 g, 9 mmol) in anhydrous DMF (125 mL) was cooled to 0 °C prior to addition of NaH (382 mg, 9.5 mmol), Bu_4NI (3.5 g, 9.5 mmol), and benzyl bromide (1.1 mL, 9.5 mmol). At the end of addition, the mixture was allowed to reach room temperature and stirred for 48 h. Dilution with H_2O was followed by extraction with EtOAc, washing with H_2O and concentration under reduced pressure. Flash chromatography of the residue (4 g) with cyclohexane and cyclohexane-EtOAc (9:1) as eluent led to **4b** (3.51 g, 79%) isolated as a syrup; R_f 0.15 (cyclohexane-EtOAc, 9:1) ; $[\alpha]_D$ +18° (*c* 1, chloroform); ^1H NMR ($CDCl_3$): δ 5.65 (s; 1H, H-7), 4.78 (dd, 2H, $C\underline{H}_2Ph$), 4.69 (s, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 4.66 (dd, 2H, $C\underline{H}_2Ph$), 4.27 (dd, 1H, $J_{5,6b}$ = 4.5 Hz, H-6b), 4.23 (dd, 1H, $J_{3,4} = J_{4,5}$ = 10 Hz, H-4), 3.95 (dd, 1H, $J_{2,3}$ = 3.2, $J_{3,4}$ = 10 Hz, H-3), 3.90 (t, 1H, J = 10 Hz, H-6a), 3.82 (s, 3H, OCH_3), 3.80 (d, 1H, $J_{2,1}$ = 1.5 Hz, H-2), 3.76 (dd, 1H, $J_{5,6a}$ = 10, $J_{5,6b}$ = 4.5 Hz, H-5), 3.33 (s, 3H, OCH_3); MS (DCI/NH_3) *m/z* 493 $(M + H)^+$.

Anal. Calcd for $C_{29}H_{32}O_7$: C, 70.70; H, 6.55. Found: C, 70.75; H, 6.38.

Methyl 2-*O*-Benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (5b). 1) From **2b**: Following the protocol reported by Garegg *et al*,[15] **5b** was obtained in 36% yield from **2b** and isolated as a crystalline compound from EtOH.

2) From **4b**: To a cooled solution of **4b** (99 mg, 0.2 mmol) in a mixture of CH_3CN-H_2O (4:1, 5 mL), cerium ammonium nitrate (244 mg, 0.48 mmol) was added and the mixture was stirred for 2 h at the same temperature. After dilution with EtOAc (20 mL) and washings with H_2O, the organic solution was dried over $MgSO_4$ and concentrated under

reduced pressure. Flash chromatography (cyclohexane-EtOAc, 9:1, then 7:1) afforded **5b** (65 mg, 87%) as a crystalline compound (mp 41-42 °C, EtOH); $[\alpha]_D$ -6° (*c* 0.5, chloroform); Lit.:[26] mp 42-44 °C (EtOH), $[\alpha]_D$ +20° (*c* 1, chloroform); IR ($CDCl_3$) 3562 cm^{-1} (OH); ^1H NMR ($CDCl_3$): δ 7.52-7.26 (m, 10H, Ar), 5.58 (s, 1H, H-7), 4.77 (bs, 1H, H-1), 4.72 and 4.70 (2d, 2H, J = 12 Hz, CH_2Ph), 4.28 (dd, 1H, H-6a), 4.08 (m, 1H, H-5), 3.92 (dd, 1H, $J_{3,4} = J_{4,5} = 9$ Hz, H-4), 3.85-3.75 (m, 3H, H-2, H-3, H-6b), 3.37 (s, 3H, OCH_3), 2.40 (d, 1H, J = 8 Hz, OH); MS (DCI/NH_3) *m/z* 390 $(M + NH_4)^+$, 373 $(M + H)^+$.

Methyl 3-*O*-Benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (6b). Dibutyltin oxide (2.35 g, 9.43 mmol) was added to a solution of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside **2b** (2.42 g, 8.58 mmol) in anhydrous toluene (150 mL). The resulting mixture was heated under Dean-Stark conditions for 4 h prior to the addition of tetrabutylammonium iodide (2.45 g, 6.62 mmol) and of benzyl bromide (1.22 mL, 10.43 mmol). After additional reflux for 15 h, the mixture was diluted with EtOAc (150 mL), washed with H_2O and dried over $MgSO_4$. Concentration under reduced pressure, followed by flash chromatography (cyclohexane-EtOAc, 4:1) afforded **6b** (2.55 g, 80%) as a syrup; R_f = 0.3 (cyclohexane-EtOAc, 2:1); $[\alpha]_D$ +52° (*c* 1.2, chloroform); ^1H NMR ($CDCl_3$): δ 7.53-7.27 (m, 10H, Ar), 5.63 (s, 1H, H-7), 4.87 and 4.73 (2d, 2H, J = 12 Hz, CH_2Ph), 4.77 (d, 1H, $J_{1,2} = 1$ Hz, H-1), 4.30 (m, 1H, H-6a), 4.12 (t, 1H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 4.05 (dd, 1H, $J_{1,2} = 1$, $J_{2,3} = 3$ Hz, H-2), 3.92 (dd, 1H, $J_{3,4} = 9.5$, $J_{3,2} = 3$ Hz, H-3), 3.85 (m, 1H, H-6b), 3.81 (dd, 1H, $J_{5,6} = 4$ Hz, H-5), 3.39 (s, 3H, OCH_3), 2.75 (s, 1H, OH); ^{13}C NMR ($CDCl_3$): δ 128.86, 128.39, 128.13, 126.81, 126 (C-Ar), 101.52 (C-7), 101.19 (C-1), 78.82 (C-4), 75.64 (C-3), 72.98 ($\underline{C}H_2Ph$), 69.76 (C-2), 68.84 (C-6), 63.27 (C-5), 54.82 ($O\underline{C}H_3$).

Anal. Calcd for $C_{21}H_{24}O_6$: C,67.73; H, 6.50. Found: C, 66.92; H, 6.53.

Phenyl 4,6-*O*-Benzylidene-1-thio-α-D-mannopyranoside (2c). A mixture of phenyl α-D-thiomannopyranoside[27] (15 g, 55 mmol), benzaldehyde dimethylacetal (18.8 mL, 58.6 mmol) and tetrafluoroboric acid[28] (50% solution in ether, 7.6 mL, 55 mmol) in anhydrous DMF (400 mL) was stirred at room temperature for 3 h. After neutralization by addition of Et_3N, dimethylformamide was removed by distillation under vacuum (0.1 mm Hg) . The residue was dissolved in EtOH and concentrated under reduced pressure, giving **2c** (13.65 g, 69%) as a crystalline compound; R_f (cyclohexane-EtOAc, 2:1), mp 200 °C (EtOH), $[\alpha]_D$ +292° (*c* 0.5, MeOH); Lit.:[29] mp 182-184 °C $[\alpha]_D^{27}$ +294.6° (*c* 0.5, acetone).

Phenyl 4,6-*O*-Benzylidene-3-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (3c). It was prepared under the conditions as reported for **3b** and obtained

in 81% yield as a syrup; R_f 0.6 (cyclohexane-EtOAc, 4:1); $[\alpha]_D$ +232.5° (*c* 1, chloroform); IR ($CDCl_3$) 3567 cm^{-1} (OH); ^1H NMR ($CDCl_3$): δ 7.54-6.92 (m, 12H, Ar), 6.92-6.88 (m, 2H, Ar), 5.63 (s, 1H, H-7), 5.60 (s, 1H, H-1), 4.80 and 4.68 (2d, 2H, J = 11 Hz, CH_2PhOCH_3), 4.34 (m, 1H, H-5), 4.25 (m, 1H, H-2), 4.20 (m, 1H, H-6a), 4.17 (dd, 1H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 3.95 (dd, 1H, $J_{2,3} = 3.5$ Hz, $J_{3,4} = 9.5$, H-3), 3.86 (dd, 1H, J = J' = 10 Hz, H-6b), 3.82 (s, 3H, OCH_3), 2.88 (d, 1H, J = 1 Hz, OH); MS (DCI/NH_3) *m/z* 498 $(M + NH_4)^+$, 481 $(M + H)^+$.

Anal. Calcd for $C_{27}H_{28}O_6S$: C, 67.48; H, 5.87; S, 6.67. Found: C, 67.58; H, 5.86; S, 6.59.

Phenyl 2-*O*-Benzyl-4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (4c). Prepared under the same conditions as that described for **4b**, compound **4c** was obtained in 90% after flash chromatography (cyclohexane-EtOAc, 9:1); syrup, R_f = 0.72 (cyclohexane-EtOAc, 4:1); $[\alpha]_D$ +114° (*c* 1.2, chloroform); ^1H NMR ($CDCl_3$): δ 7.55-7.26 (m, 17H, H-Ar), 6.89-6.86 (m, 2H, Ar), 5.66 (s, 1H, H-1), 5.50 (d, 1H, $J_{1,2} = 1.3$ Hz, H-1), 4.72 (s, 2H, CH_2Ph), 4.75 and 4.60 (2d, 2H, J = 12 Hz, CH_2Ph), 4.31-4.20 (m, 3H, H-4, H-5, H-6a), 4.01 (d, 1H, J = 1.5 Hz, H-2), 3.90 (m, 2H, H-3, H-6b), 3.83 (s, 1H, OCH_3); MS (DCI/NH_3) *m/z* 588 $(M + NH_4)^+$.

Phenyl 2-*O*-Benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (5c).

1) From **2c**: Prepared under phase-catalyzed transfer, conditions as described for **5a**. Compound **5c** was obtained in 33% yield.

2) From **4c**: DDQ (151 mg, 0.66 mmol) was added to a solution of **4c** (316 mg, 5.54 mmol) in 10 mL of a mixture of dichloromethane-H_2O (20:1) and stirred at room temperature for 24 h. Dilution with dichloromethane (20 mL), followed by filtration, gave an organic solution which was washed with saturated aqueous solution of $NaHCO_3$, with brine and dried over $MgSO_4$. After concentration under reduced pressure, flash chromatography of the residue (cyclohexane-EtOAc, 9:1) afforded 216 mg (86%) of **5c**; syrup; R_f = 0.37 (cyclohexane-EtOAc, 4:1); mp 148-150 °C (Lit.[30] 147-149 °C); $[\alpha]_D$ +130° (*c* 1.02, chloroform)(Lit.[30] $[\alpha]_D$ +145° (*c* 0.8, chloroform); IR ($CDCl_3$) 3573 (OH); ^1H NMR ($CDCl_3$): δ 7.54-7.26 (m, 15H, H-Ar), 5.60 (s, 2H, H-1, H-7),4.76-4.65 (2d, 2H, J = 11.6 Hz, CH_2Ph), 4.38-4.30 (m, 2H, H-4, H-5), 4.23 (dd, 1H, $J_{5,6b} = 10$, $J_{6b,6a} = 5$ Hz, H-6b), 4.12 (bs, 1H, H-2), 4.02 (dd, 1H, $J_{3,4} = 10$, $J_{2,3} = 3.5$ Hz, H-3), 3.85 (t, 1H, J = 10 Hz, H-6a), 2.48 (d, 1H, J = 8 Hz, OH); MS (DCI/NH_3) *m/z* 468 $(M + NH_4)^+$, 451 $(M + H)^+$.

Anal. Calcd for $C_{26}H_{26}O_5S$: C, 69.31; H, 5.82; S, 7.12. Found: C, 69.39; H, 5.76; S, 7.11.

Benzyl (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (8a). To a solution of **5a** (76 mg, 0.17 mmol) in anhydrous dichloromethane (5 mL), CF_3SO_3Ag (66 mg, 0.25 mmol), 4 Å powdered molecular sieves (45 mg) and *sym*-collidine (12 μL, 0.09 mmol) were added. The stirred suspension was then cooled to -78 °C, before addition of a solution of chloride **7**[17] (125 mg, 0.22 mol) in dry dichloromethane (5 mL). The reaction mixture was stirred at -78 °C for 1.5 h and the temperature was then gradually raised to -10 °C and maintained for 1.5 h at this temperature before addition of *sym*-collidine (17 μL, 0.14 mmol). The crude mixture was filtered through Celite and the organic solution was washed with aqueous saturated $NaHCO_3$, with water, and concentrated, giving a crude residue (200 mg). Purification by flash chromatography on silica gel (cyclohexane, EtOAc, 9:1) gave the expected disaccharide **8a** (90 mg, 55%) as a syrup; $[\alpha]_D$ +66° (*c* 1.05, chloroform); IR ($CDCl_3$): 3562 cm^{-1} (OH); ^1H NMR ($CDCl_3$): δ 5.56 (d,1H,J =3.5Hz, H-1'), 5.30 (s, 1H, H-7); MS (DCI/NH_3) *m/z* 989 $(M + NH_4)^+$.

Methyl (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (8b). Prepared from **5b** and **7** under the same conditions as above and isolated after flash chromatography (cyclohexane-EtOAc, 9:1) in 34% yield; syrup; R_f = 0.42 (cyclohexane-EtOAc, 4:1); $[\alpha]_D$ +66° (*c* 1.05, chloroform); IR ($CDCl_3$): 3562 cm^{-1} (OH); ^1H NMR ($CDCl_3$): δ 7.48-6.97 (m, 30H, Ar), 5.53 (d,1H, J = 3.5 Hz, H-1'), 5.47 (s, 1H, H-7), 4.71 (s, 1H, H-1), 3.33 (s, 3H, OCH_3); MS (DCI/NH_3) *m/z* 913 $(M + NH_4)^+$.

Methyl (α-D-Glucopyranosyl)-(1→3)-α-D-mannopyranoside (9b). A solution of **8b** (348 mg, 0.39 mmol) in a mixture of EtOH (12 mL) and EtOAc (6 mL) was stirred for 3 h under a hydrogen atmosphere (1 atm) in the presence of 10% Palladium-on-charcoal (288 mg). The suspension was then filtered, and the filtrate was concentrated under reduced pressure to give a syrup (130 mg, 94%) which crystallized from ethanol. R_f = 0.17 (EtOAc-*i*PrOH-H_2O, 3:3:2); mp 104-107 °C; $[\alpha]_D$ +108° (*c* 1, MeOH); ^1H NMR (CD_3OD): δ 4.62 (d, 1H, J = 1.8 Hz, H-1'), 4.87 (d, H-1, OH), 5.07 (d, 1H, J = 4 Hz, H-1); MS (electrospray) *m/z* 379 $(M + Na)^+$.

Anal. Calcd for $C_{13}H_{24}O_{11}$, H_2O: C, 41.71; H, 7.00. Found: C, 41.83; H, 6.92.

α-D-Glucopyranosyl-(1→3)-D-mannopyranose (10). Applied to **8a**, the same procedure as described above led to **10** as crystals (97%). R_f (EtOAc-*i*PrOH-H_2O, 3:3:2) 0.51; mp 107-110 °C (EtOH); MS (electrospray) *m/z* 365 $(M + Na)^+$.

Anal. Calcd for $C_{12}H_{22}O_{11}$, H_2O: C, 40.00; H, 6.71. Found: C, 40.29; H, 6.68.

Phenyl (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-thiomannopyranoside (8c) and Phenyl

(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-thiomannopyranoside (12).

1) From **5c** and glucosyl chloride **7**. A mixture of **5c** and **7** (4.87 g, 1.1 eq) was treated under the same conditions as described for **8a** and **8b**, affording 10 g of a crude product. Flash chromatography (cyclohexane-EtOAc, 9:1) led to **8c** and **12** as a mixture (4.9 g, 76%; **8c/12** = 80/20 as determined by HPLC analysis). A sample of pure α-anomer **8c** could be obtained after two column chromatographies over H-60 silica gel (pentane-ether, 5:1 and cyclohexane-ether, 6:1); syrup, R_f = 0.4 (pentane-ether, 3:1); $[\alpha]_D$ +98° (*c* 1.1, chloroform); ^{1}H NMR ($CDCl_3$): δ 7.44-6.97 (m, 35H, Ar), 5.56 (s, 1H, H-7), 5.53 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H-1'), 5.50 (s, 1H, H-1), 4.93 and 4.73 (2d, 2H, J = 11 Hz, CH_2Ph), 4.86-4.40 (m, 6H, 3 CH_2Ph), 4.62 and 4.31 (2d, 2H, J = 12.5 Hz, CH_2Ph), 4.45 (m, 2H, H-4, H-3), 4.34 (s, 1H, H-5), 4.09 (s, 1H, H-2), 3.98 (dd, 1H, $J_{2',3'}$ = $J_{3',4'}$ = 9 Hz, H-3'), 3.87 (t, 1H, J = 9.5 Hz, H-6'a), 3.76 (m, 1H, H-5'), 3.66 (m, 2H, H-6a, H-6b), 3.60 (dd, 1H, $J_{4',3}$ = $J_{4',5'}$ = 9.2 Hz, H-4'), 3.50 (dd, 1H, $J_{2',3'}$ = 9, $J_{2',1'}$ = 3.5 Hz, H-2'); ^{13}C NMR ($CDCl_3$): δ 138-125 (C-Ar), 102.5 (C-7), 97 (C-1'), 87.30 (C-1), 81.40 (C-3'), 79.56 (C-4), 79.20 (C-2), 78.87 (C-2'), 77.44 (C-4'), 75.50 ($\underline{C}H_2Ph$), 74.97 ($\underline{C}H_2Ph$), 73.50 ($\underline{C}H_2Ph$), 73.34 ($\underline{C}H_2Ph$), 72.87 (C-3), 70.99 (C-5'), 70.68 ($\underline{C}H_2Ph$), 68.62 (C-6 and C-6'), 65.35 (C-5); MS (DCI/NH_3) *m/z* 990 (M + NH_4)$^+$.

Anal. Calcd for $C_{60}H_{60}O_5S$: C, 74.04; H, 6.22. Found: C, 73.50; H, 6.23.

2) From **5c** and glucopyranosyl trichloroacetamidate **11**. A solution of trichloroacetimidate **11**[21] (100 mg, 0.44 mmol) and of **5c** (148 mg, 0.33 mmol) in anhydrous ether (15 mL) and in the presence of 4 Å powdered molecular sieves (500 mg, freshly activated) was cooled to -55 °C. After adding trimethylsilyl triflate (158 μL, 0.87 mmol), the mixture was stirred at -55 °C for 18 h. Dilution with ether (50 mL) was followed by filtration over Celite and the filtrate was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. Subsequent flash chromatography (cyclohexane-EtOAc, 9:1) gave **8c** (271 mg, 85%) slightly contaminated with the β-interglycoside anomer **12** (ratio **8c/12** > 93/7 as determined by HPLC analysis). A second flash chromatography using the same mixture of solvents, but in 95:5 ratio, afforded a pure sample for description.

Compound 12: syrup; R_f = 0.4 (pentane-ether, 3:1) ; ^{1}H NMR ($CDCl_3$): δ 7.43-7.18 (m, 30H, Ar), 5.56 (s, 1H, H-7), 5.53 (d, 1H, $J_{1,2}$ = 1 Hz, H-1), 4.90 and 4.81 (2d, 2H, J = 11 Hz, CH_2Ph), 4.88 (d, 1H, $J_{1',2'}$ = 9 Hz, H-1'), 4.59-4.45 (m, H, H-2'), 4.02 (dd, 1H, $J_{1,2}$ = 1, $J_{2,3}$ = 3 Hz, H-2), 3.89 (dd, 1H, $J_{2,3}$ = 3, $J_{3,4}$ = 8.5 Hz, H-3), 3.71 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 10 Hz, H-4 or 4'), 3.68-3.51 (m, 4H, H-6a, H-6b, H-5', H-3'); MS (DCI/NH_3) *m/z* 990 (M + NH_4)$^+$.

Phenyl (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2-*O*-benzyl-α-D-thiomannopyranoside (13) and Phenyl (2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-2-*O*-benzyl-α-D-thiomannopyranoside (14). The crude mixture of **8c** and **12** (505 mg, 0.51 mmol, readily obtained from **5c** and **7**) was dissolved in dichloromethane (25 mL) and tin chloride dihydrate (234 mg, 1 mmol) was added. The resulting solution was stirred for 36 h at room temperature, washed with water, with brine, and the organic layer was concentrated under reduced pressure. Flash chromatography (cyclohexane-EtOAc, 6:1) led to the mixture of **13** and **14** (340 mg, 74%) and, in the following fractions to unreacted **8c** + **12** (100 mg). A second column chromatography with silica gel 60H (same eluent) successively afforded **14** (75 mg, 16%), **14** + **13** (15 mg) and **13** (250 mg, 54%).

Compound 14: syrup, R_f = 0.31 (cyclohexane-EtOAc, 2:1); $[\alpha]_D$ +66° (*c* 0.3, chloroform); ^1H NMR ($CDCl_3$): δ 7.43-7.18 (m, 30H, H-Ar), 5.50 (d, 1H, $J_{1,2}$ = 1 Hz, H-1), 4.95-4.70 (m, 6H, 3 CH_2Ph), 4.88 (d, 1H, $J_{1',2'}$ = 9 Hz, H-1'), 4.83 and 4.75 (2d, 2H, J = 11 Hz, CH_2Ph), 4.59-4.45 (m, 5H), 4.02 (dd, 1H, $J_{1,2}$= 1, $J_{2,3}$= 3 Hz, H-2), 3.89 (dd, 1H, $J_{2,3}$ = 3, $J_{3,4}$ = 8.5 Hz, H-3), 3.71 (dd, 1H, $J_{4,5}$ = 10 Hz, H-4 or H-4'), 3.68-3.51 (m, 4H, H-6a, H-6b, H-5', H-3'), 2.00 and 1.60 (2 bs, 2H, OH).

Anal. Calcd for $C_{53}H_{56}O_{10}S$: C, 71.92; H, 6.38. Found: C, 71.64; H, 6.28.

Compound 13: syrup, R_f = 0.25 (cyclohexane-EtOAc, 2:1); $[\alpha]_D$ +88° (*c* 0.7, chloroform); ^1H NMR ($CDCl_3$): δ 7.45-7.15 (m, 30, Ar), 5.48 (d, 1H, $J_{1,2}$ = 1 Hz, H-1), 4.96 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1'), 4.93 and 4.86 (2d, 2H, J = 11 Hz, CH_2Ph), 4.84 and 4.70 (2d, 2H, J = 11 Hz, CH_2Ph), 4.60 and 4.42 (2d, 2H, J = 11 Hz, CH_2Ph), 4.20-4.05 (m, 3H, including H-2), 4.0-3.55 (m, 8H, H-4, H-4', H-5, H-5', H-6a, H-6b, H-6'a, H-6b), 3.76 (dd, 1H, $J_{1',2'}$= 3.5, $J_{2',3'}$ = 9 Hz, H-2'), 2.00 and 1.60 (2 bs, 2 OH), MS (electrospray) *m/z* 907 $(M + Na)^+$.

Phenyl (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-α-D-thiomannopyranoside (15). A dispersion of NaH (60% in mineral oil, 10.7 mg, 0.27 mmol) was added to a solution of **13** (100 mg, 0.1 mmol) in anhydrous DMF containing Bu_4NI (99 mg,10 mL) and benzyl bromide (32 μL, 0.27 mmol). The resulting mixture was stirred for 16 h at room temperature and quenched by addition of H_2O (10 mL). The solution was extracted with EtOAc and washed with water, dried over $MgSO_4$ and concentrated under reduced pressure to give a crude product (150 mg). Flash chromatography (cyclohexane-EtOAc, 9:1) led to 95 mg (79%) of **15**; syrup; R_f = 0.70 (cyclohexane-EtOAc, 2:1); $[\alpha]_D$ +82.5° (*c* 1, chloroform); ^1H NMR ($CDCl_3$): δ 7.58-7.17 (m, 40H, arom H), 5.73 (bs, 1H, H-1); 5.19 (d, 1H, J = 3.39 Hz, H-1), 5.03 and 4.88 (2d, J = 11 Hz, OCH_2-Ph), 4.91 and 4.50 (2d, 2H, J = 10.9 Hz, OCH_2Ph), 4.43-4.77 (m, 12H), 4.29-3.60 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 139.01-138.05 (8C,

arom), 131.84-127.40 (m, 40C, arom), 99.63 (C-1'), 85.07 (C-1), 81.98, 81.33, 79.94, 79.32, 78.09, 74.69, 73.04, 71.33, 76.73 (C ring), 75.76, 73.71, 73.37, 73.21, 71.17, 69.46, 68.91 (9C, $O\underline{C}H_2$); MS (DCI/NH_3) *m/z* 1083 $(M + NH_4)^+$.

Anal. Calcd for $C_{67}H_{68}O_{10}S$: C, 74.28; H, 6.51; O, 2.98. Found: C,73.83; H, 6.41; O, 2.81.

Methyl (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (16).

From 8c and 6b: A solution of disaccharide **8c** (300 mg, 0.3 mmol) and of methyl mannoside derivative **6b** (88 mg, 0.2 mmol) in dry dichloromethane (10 mL) was cooled to -35 °C under argon atmosphere. After stirring for 15 min, in the presence of 4 Å powdered molecular sieves (330 mg), NIS (61 mg, 0.27 mmol) and CF_3SO_3Ag (67 mg, 0.26 mmol) were added with stirring and the mixture was maintained at the same temperature for 16 h. Dilution with CH_2Cl_2 (30 mL) was followed by filtration over Celite and the insoluble residue was washed twice with CH_2Cl_2. The combined organic layers were washed with an aqueous solution of sodium thiosulfate, with brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography (cyclohexane/EtOAc, 85:15) led to isolation of **16** as a syrup (33 mg, 11%).

From 11 and 19: A solution of glucoside donor **11** (136 mg, 0.19 mmol) and disaccharide **19** (98 mg, 0.15 mmol) in 5 mL of dry ether in the presence of 4 Å powdered molecular sieves (250 mg, freshly activated) was cooled to -50 °C before addition of *tert*-butyldimethylsilyl trifluoromethanesulfonate (4.5 μL, 0.19 mmol). After stirring for 1.15 h at -50 °C, the reaction mixture was diluted with ether (30 mL) and filtered over Celite. The organic solution was washed with water and dried over $MgSO_4$, affording 200 mg of product. Flash chromatography (cyclohexane-EtOAc, 9:1) gave 93 mg of **16** (50%); syrup. R_f = 0.63 (cyclohexane-EtOAc, 2:1); $[\alpha]_D$ + 23° (*c* 1, chloroform); ^1H NMR ($CDCl_3$): δ 7.47-7.01 (m, 40H, Ar), 5.57 (d, $J_{1'',2''}$ = 3.5 Hz, H-1''), 5.50 (s, 1H, H-7), 5.46 (s, 1H, H-7'), 5.26 (d, 1H, $J_{1',2'}$ = 1 Hz, H-1'), 5.00-3.50 (m, 29 H), 3.38 (s, 3H, OCH_3); MS (DCI/NH_3) *m/z* 1253 $(M + NH_4)^+$.

Anal. Calcd for $C_{75}H_{78}O_{16}$: C, 72.92; H, 6.36. Found: C, 72.85; H, 6.40.

Phenyl 3-*O*-Acetyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-thiomannopyranoside (17). A solution of **5c** (1 g, 2.22 mmol) in pyridine (20 mL) was stirred at room temperature for 5 h in the presence of acetic anhydride (3 mL). Extraction with EtOAc and washings with aqueous H_2SO_4 solution, with water and with saturated $NaHCO_3$ solution afforded, after drying over $MgSO_4$ and concentration under reduced pressure, 1.3 g of residue. This was purified by flash chromomatography affording 1 g (92%) of pure **17** as a syrup; R_f = 0.6 (cyclohexane-EtOAc, 4:1); $[\alpha]_D$ +78.5° (*c* 1,

chloroform); ^{1}H NMR ($CDCl_3$): δ 7.50-7.26 (m, 15H, Ar), 5.60 (s, 1H, H-7), 5.56 (s, 1H, H-1), 5.29 (dd, 1H, $J_{2,3}$ = 3, $J_{3,4}$ = 10 Hz, H-3), 4.70 and 4.54 (2d, 2H, J = 12 Hz, CH_2Ph), 4.40 (m, 1H, H-5), 4.30-4.20 (m, 3H, H-2, H-6a, H-6b), 3.89 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 10 Hz, H-4), 2.00 (s, 3H, OAc); MS (DCI/NH_3): *m/z* 510 $(M + NH_4)^+$, 493 $(M + H)^+$.

Anal. Calcd for $C_{28}H_{28}O_6S$: C, 68.27; H, 5.73. Found: C, 67.97; H, 5.81.

Methyl (3-*O*-Acetyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (18). To a cooled solution (-35 °C) of **17** (360 mg, 0.73 mmol) and of **6b** (227 mg, 0.6 mmol) in dry dichloromethane (10 mL) and in the presence of 4 Å powdered molecular sieves (300 mg), NIS (158 mg, 0.7 mmol) and CF_3SO_3Ag (172 mg, 0.67 mmol) were successively added. The reaction mixture was stirred at the same temperature for 72 h, diluted with dichloromethane (50 mL) and allowed to reached room temperature. Filtration over Celite was followed by washings with water and drying over $MgSO_4$. Flash chromatography (cyclohexane-EtOAc, 9:1) of the residue gave 280 mg (60%) of **18** as a pure compound and subsequently 50 mg (12%) of slightly impure **18**. For the pure compound: R_f = 0.37 (cyclohexane-EtOAc, 4:1); $[\alpha]_D$ -41° (*c* 0.9, chloroform); ^{1}H NMR: δ 7.57-7.12 (m, 20H, Ar), 5.72 (s, 1H, H-7), 5.58 (s, 1H, H-7'), 5.38 (dd, 1H, $J_{2',3'}$ = 3.5, $J_{3',4'}$ = 10 Hz, H-3'), 5.29 (bs, 1H, H-1'), 4.72 (s, 1H, H-1), 4.89 and 4.65 (2d, 2H, J = 11 Hz, CH_2Ph), 4.40 and 4.23 (2d, 2H, J = 12 Hz, CH_2Ph), 4.15 (2dd, 2H, J = 9.5 Hz, H-4 and H-4'), 4.13 (s, 1H, H-2), 4.07 (dd, $J_{1',2'}$ = 1, $J_{2',3'}$ = 3 Hz, H-2'), 4.04-3.77 (m, 5H, H-3, H-5, H-5', H-6, H-6'), 3.39 (s, 3H, OCH_3), 2.01 (s, 3H, OAc);^{13}C NMR ($CDCl_3$): δ 170 (CO), 138-137 (4C, quatern. arom.), 129-126.1 (20C, arom.), 101.6 (C-7'), 101.4 (C-7), 100.9 (C-1), 100.3 (C-1'), 79.3 (C-2'), 76.2 (C-2, C-3, C-4, C-4'), 73.8 and 72.9 (C-8 and C-8'), 70 (C-3'), 68.7 (C-6 and C-6'), 64.4 and 63.8 (C-5 and C-5'), 54.7 (OCH_3), 21 ($CO\underline{C}H_3$); MS (DCI/NH_3): *m/z* 755 $(M + H)^+$, 772 $(M + NH_4)^+$.

Anal. Calcd for $C_{43}H_{46}O_{12}$: C, 68.42; H, 6.14. Found: C, 68.38; H, 6.27.

Methyl (2-*O*-Benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (19). A solution of **18** (110 mg, 0.15 mmol) in a mixture of Et_3N/MeOH and H_2O (1/8/1; 20 mL) was stirred at room temperature for 48 h and concentrated to dryness. Repeated co-concentrations with toluene afforded 98 mg (94%) of **19** as a pure compound; syrup; R_f = 0.4 (cyclohexane-EtOAc, 2:1) 0.31; $[\alpha]_D$ -11° (*c* 0.85, chloroform); ^{1}H NMR ($CDCl_3$): δ 7.60-7.22 (m, 20H, Ar), 5.70 (s, 1H, H-7), 5.60 (s, 1H, H-7'), 5.36 (s, 1H, H-1'), 4.74 (bs,1H, H-1), 4.91 and 4.66 (2d, 2H, J = 11.4 Hz, CH_2Ph), 4.46 and 4.35 (2d, 2H, J = 12 Hz, CH_2Ph), 4.31 (m, 2H including H-3'), 4.18-4.11 (m, 3H including H-2),

4.05 (d, 1H), 4.00 (dd, 1H, $J_{1',2'} \approx 1$, $J_{2',3'} = 3.5$ Hz, H-2'), 3.95-3.84 (m, 6H, H-6a, H-6b, H-6'a, H-6'b, H-5, H-5'), 3.40 (s, 3H, OCH_3), 2.56 (bs, 1H, OH); ^{13}C NMR ($CDCl_3$): δ 102.17 (C-7'), 101.68 (C-7), 101.21 (C-1), 100.3 (C-1'), 9.97 (C-1'), 79.64, 78.62, 76.38, 76.22 (5C), 73.95 and 73.16 (C-8, C-8'), 68.98 and 68.86 (C-6, C-6'), 68.37 (C-2), 64.27, 63.89 (C-5, C-5'), 54.98 (OCH_3); MS (DCI/NH_3): *m/z* 730 $(M + NH_4)^+$.

Anal. Calcd for $C_{41}H_{44}O_{11}$: C, 69.07; H, 6.23. Found: C, 69.15; H, 6.28.

Methyl (2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (21). A solution of **16** (83 mg, 0.067 mmol) in EtOH-EtOAc (2:1, 18 mL) was stirred overnight under H_2 atmosphere (1 atm) in the presence of 10% Pd-on-charcoal. Filtration and concentration under reduced pressure afforded **20** as an amorphous powder; $R_f = 0.7$ (dichloromethane-MeOH, 1:1); $[\alpha]_D$ +43° (*c* 0.45, MeOH); MS (FAB) *m/z* 541 $(M+Na)^+$. A solution of this crude compound in pyridine (10 mL), and Ac_2O (2 mL) was stirred for 24 h at room temperature and then heated at 60 °C for 1 h. After reaching room temperature, water was added (10 mL) and the reaction mixture was extracted three times with EtOAc (3 x 50 mL). The separated organic solution was washed with 5% aqueous H_2SO_4 solution, with water and, finally, with aqueous solution of $NaHCO_3$. After drying, concentration under reduced pressure led to 57 mg of crude product. Flash chromatography (cyclohexane-EtOAc, 4:1) gave 50 mg (79%) of pure **21** which crystallized from MeOH; mp 156 °C; $R_f = 0.23$ (cyclohexane-EtOAc, 12:1); $[\alpha]_D$ +60° (*c* 1, chloroform); ^1H NMR ($CDCl_3$): δ 5.31 (dd, 1H, $J_{2'',3''} = J_{3'',4''} = 9.8$ Hz, H-3"), 5.31-5.19 (m, 5H), 5.06 (dd, $J_{3'',4''} = J_{4'',5''} = 9.7$ Hz, 1H, H-4"), 4.86 (bs, 1H, H-1'), 4.78 (bs, 1H, H-1), 4.78 (dd, 1H, J = 4 Hz, H-2"), 4.36 (dd, 1H, $J_{5''a,6''} = 2.5$, $J_{6''a,6''b} = 12.5$ Hz, H-6"a), 4.25-4.02 (m, 8H), 3.98 (d, 1H, J = 1.7 Hz, H-2), 3.88 (m, 1H, H-5 or H-5'), 3.37 (s, 3H, OCH_3), 2.19-1.84 (m, 30H, 10 $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 170.50-169.30 (10C, CO), 96.56 (C-1"), 99.17 and 99.04 (C-1 and C-1'), 72.34 (C-2 or C-2'), 70.63, 70.48, 70.14, 69.32, 69.22, 68.27, 68.07, 67.82 (8C), 67.52 (C-4"), 65.88 (C-3"), 62.44, 62.32 (C-6, C-6'), 60.92 (C-6"), 55.08 (OCH_3), 20.79, 20.67 and 20.55 (10C, $COCH_3$); MS (DCI/NH_3): *m/z* 956 $(M + NH_4)^+$.

Anal. Calcd for $C_{39}H_{54}O_{26}$: C, 49.89; H, 5.80. Found: C, 49.91; H, 5.89.

ACKNOWLEDGMENTS

We wish to thank Profs. D. Louvard and A. Helenius for helpful suggestions and discussions. Thanks are also due to CNRS and Institut Curie for financial support and to the MNESR for a fellowship (Slim Cherif).

REFERENCES

1. J. Montreuil, *Adv. Carbohydr. Chem. Biochem.*, **37**, 158 (1980).
2. A. Helenius, *J. Biol. Chem.* **270**, 26060 (1995); *Mol. Biol. Cell.*, **5**, 2531 (1994).
3. F. Parlati, R. Hemming, W.-J. Ou, J.J.M. Bergeron and D.Y. Thomas, in *Calreticulin*, edited by M. Michalak, R.G. Landes Company, Chapman Hall, New York, 1996, p. 43.
4. A.R. Rodan, J.F. Simons, E.S. Trombetta and A. Helenius, *The EMBO Journal*, **15**, 6921 (1996) and ref. cited therein.
5. F.E. Ware, A. Vassilakos, P.A. Peterson, M.R. Jackson, M.A. Lehrman and D.B. Williams, *J. Biol. Chem.*, **270**, 4697 (1995).
6. R.G. Spiro, Q. Zhu, V. Bhoyroo and H.D. Söling, *J. Biol. Chem.*, **271**, 11588 (1996).
7. H. Kunz, *Ang. Chem. Intl. Ed.*, **26**, 294 (1987).
8. H. Paulsen, *Ang. Chem. Intl. Ed.*, **29**, 823 (1990).
9. A. Dan, Y. Ito and T. Ogawa, *Tetrahedron. Lett.*, **36**, 7487 (1995); S.H. Khan, C.F. Piskorz and K.L. Matta, *J. Carbohydr. Chem.*, **13**, 1025 (1994).
10. Y. Nakahara, S. Shibayama, Y. Nakahara and T. Ogawa, *Carbohydr. Res.*, **280**, 67 (1996).
11. R.K. Jain, X.-G. Liu, S.R. Oruganti, E.V. Chandrasekaran and K.L. Matta, *Carbohydr. Res.*, **271**, 185 (1995).
12. T. Ogawa, T. Nukada and T. Kitajima, *Carbohydr. Res.*, **123**, C12 (1983).
13. T. Ogawa, H. Yamamoto, T. Nukada, T. Kitajima and M. Sugimoto, *Pure Appl. Chem.*, **56**, 779 (1984).
14. J. Defaye, A. Gadelle and C.C. Wong, *Carbohydr. Res.*, **95**, 131 (1989).
15. P.J. Garegg, T. Iversen and S. Oscarson, *Carbohydr. Res.*, **50**, C12 (1976).
16. T. Oshitari and S. Kobayashi, *Tetrahedron Lett.*, **36**, 1089 (1995); T. Oshitari, M. Shibasaki, T. Yoshizawa, M. Tomita, K.-i. Takao and S. Kobayashi, *Tetrahedron*, **53**, 10993 (1997).
17. H. Spohr, M. Bach and R.G. Spiro, *Can. J. Chem.*, **71**, 1928 (1993).
18. S.S. Bhattacharjee and P.A.J. Gorin, *Can. J. Chem.*, **47**, 1195 (1969).
19. P.J. Garegg, H. Hultberg and S. Wallin, *Carbohydr. Res.*, **108**, 97 (1982).
20. J. Xia and Y. Hui, *Synthetic Commun.*, **26**, 881 (1996).
21. R.R. Schmidt, J. Michel, and M. Roos, *Liebigs Ann. Chem.*, 1343 (1984).
22. W.C. Still, M. Kahn and A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).
23. F.M. Winnik, J.P. Carver and J.J. Krepinsky, *J. Org. Chem.*, **47**, 2701 (1982).
24. P.A.J. Gorin and A.S. Perlin, *Can. J. Chem.*, **39**, 2474 (1961).
25. C.W. Baker and R.L. Whistler, *Carbohydr. Res.*, **33**, 372 (1974); b) T. Bieg, W. Szeja, *Synthesis*, 317 (1986).
26. G. Alfredsson, H.B. Boren and P.J. Garegg, *Acta Chem.Scand.*, **26**, 3431 (1972).
27. K. Kobata and H. Meguro, *Agric. Biol. Chem.*, **46**, 919 (1982); S.K. Maity, S.K. Dutta, A.K. Banerjee, B. Achari and M. Singh, *Tetrahedron*, **50**, 6965 (1994).
28. R. Albert, K. Dax, R. Pleschko and A.E. Stutz,*Carbohydr. Res.*, **137**, 282 (1985).
29. H. Franzyk, M. Meldal, H. Paulsen and K. Bock, *J. Chem. Soc., Perkin Trans 1*, 2883 (1995).
30. Z. Szurmai, L. Balatoni and A. Lipták, *Carbohydr. Res.*, **254**, 301 (1994).