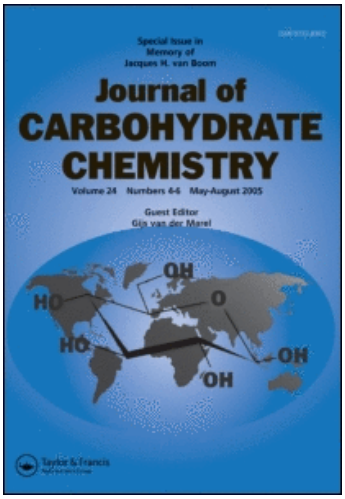


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

SYNTHESIS OF THE TETRASACCHARIDE Glc α (1⟶3) Man α (1⟶2) Man α (1⟶2) Man α (OMe) AS INHIBITOR OF CALNEXIN BINDING TO $GlcMan_9GlcNAc_2$ [a]

Slim Cherif[a]; Jean-Marc Clavel[a]; Claude Monneret[a]
[a] Section de Recherche, UMR 176 CNRS-Institut Curie, Paris, France

Online publication date: 20 May 2002

To cite this Article Cherif, Slim , Clavel, Jean-Marc and Monneret, Claude(2002) 'SYNTHESIS OF THE TETRASACCHARIDE Glc α (1⟶3) Man α (1⟶2) Man α (1⟶2) Man α (OMe) AS INHIBITOR OF CALNEXIN BINDING TO $GlcMan_9GlcNAc_2$ [a]', Journal of Carbohydrate Chemistry, 21: 1, 123 — 130
To link to this Article: DOI: 10.1081/CAR-120003743
URL: http://dx.doi.org/10.1081/CAR-120003743

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

J. CARBOHYDRATE CHEMISTRY, 21(1&2), 123–130 (2002)

SYNTHESIS OF THE TETRASACCHARIDE Glc α (1→3) Man α (1→2) Man α (1→2) Man α (OMe) AS INHIBITOR OF CALNEXIN BINDING TO $GlcMan_9GlcNAc_2$

Slim Cherif, Jean-Marc Clavel, and Claude Monneret*

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

Dedicated to Prof. Joachim Thiem on the occasion of his 60th birthday.

ABSTRACT

Tetrasaccharide $GlcMan_3$ is an inhibitor of $GlcMan_9GlcNAc_2$ binding to calnexin, a chaperone protein involved in CFTR-ΔF 508 retention. A convergent route to its methyl glycoside, the title tetrasaccharide, was developed. The key building block Glc α (1→3) Man **6** was stereoselectively obtained by condensation of a trichloroacetimidate glucosyl donor with an ethyl thiomannopyranoside acceptor. Di-mannose moiety **10** and final compound **12** resulted from thioglycoside activations.

INTRODUCTION

Cystic fibrosis [1] or mucoviscidosis is a severe autosomal recessive disease of fatal consequence. Over eight hundred mutations in the gene encoding CFTR (cystic fibrosis transmembrane conductance regulator) have been associated with the disease, but the most frequent mutation in patients (70%) with cystic fibrosis is deletion of phenylalanine

*Corresponding author. Fax: +33-1-42-34-66-31; E-mail: claude.monneret@curie.fr

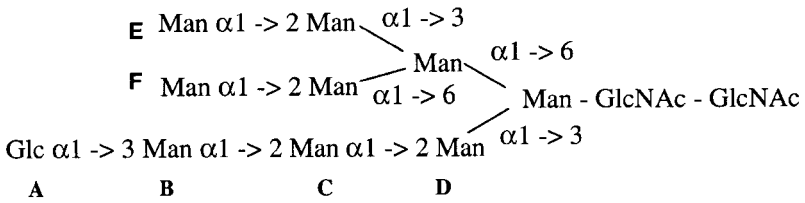
at position 508 (ΔF 508) of the protein. As a consequence, the newly synthesized ΔF 508 CFTR protein fails to fold properly, and this prevents the trafficking of CFTR from the rough endoplasmic reticulum (ER) to the plasma membrane.[2] This misfolding and retention abolish the normal function of CFTR which usually provides a pathway for the movement of Cl^- ions across the apical membrane and regulate water transport. Any attempt to overcome the biosynthetic arrest of ΔF 508 CFTR protein requires the knowledge of the mechanism that causes this retention.[3]

Newly synthesized glycoproteins bind to certain molecular chaperones only subsequent to the processing of the carbohydrate units (a triglucosyl sequence) to the monoglucosylated state.[4] Most of the evidence for this lectin-like activity has been obtained through studies on calnexin, but also on a luminal chaperone, calreticulin. Calnexin and calreticulin are components of the quality control system that retains misfolded or incompletely folded assembled proteins in the ER. Among the novel pharmacologic therapies for cystic fibrosis, one approach may be to restore normal CFTR traffic through the Golgi to the cell surface by modulating chaperone associations. This design is strongly supported by the fact that the presence of CFTR in the apical membrane can be restored by decreasing the temperature[5] (presumably through a reduction in misfolding) and, moreover, that the correctly localized ΔF 508 CFTR is still functional.[6] Binding studies to calnexin[7] and to calreticulin[8] of a monoglucosylated oligosaccharide containing progressively fewer mannose residues have led to the concluding remark that a minimum structure like $Glc_1Man_5GlcNAc$ with its α 1→6 branch point is required for a relevant chaperone binding.

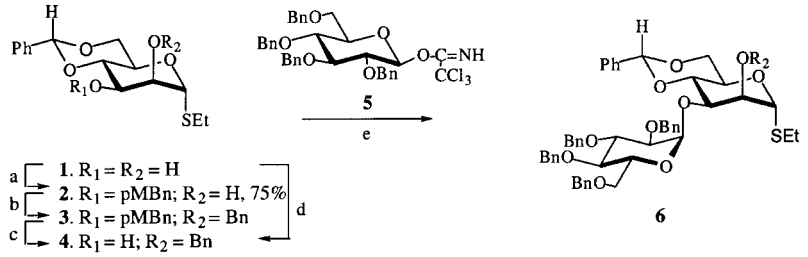
En route towards this oligosaccharide, we already reported[9] the synthesis of disaccharide **A-B** and trisaccharide moieties **A-B-C**, namely α−D-Glc-(1→3)-Man and α−D-Glc-(1→3)-Man-(1→2)-Man. While this work was in progress, it was reported that the tetrasaccharide $GlcMan_3$ **A-B-C-D** is an inhibitor of $GlcMan_9GlcNAc_2$ binding to calnexin with an IC_{50} of 4 μM (Scheme 1).[10]

The present paper relies on the report of the synthesis of the corresponding methyl glycoside, $GlcMan_3$ (OMe). Instead of using a linear strategy as already reported by Jain et al.,[11] to build up this tetrasaccharide, we considered a convergent synthesis by coupling an α−D-Glc-(1→3)-Man donor to an α−D-Man-(1→2)-Man acceptor.

The synthesis of the disaccharide donor **6** was achieved following two synthetic pathways. In the first, ethyl 4,6-*O*-benzylidene-α-thiomannopyranoside **1** was converted into ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside **4** according to Garegg et al.[12] by phase transfer catalysis (BnBr, Bu_4NHSO_4, NaOH, 50%) and isolated along with the corresponding 3-*O*-benzyl derivatives (20% yield). A second, less straightforward route[13] was next developed for the conversion of **1** into **4** (Scheme 2).



Scheme 1. CFTR oligosaccharide core.

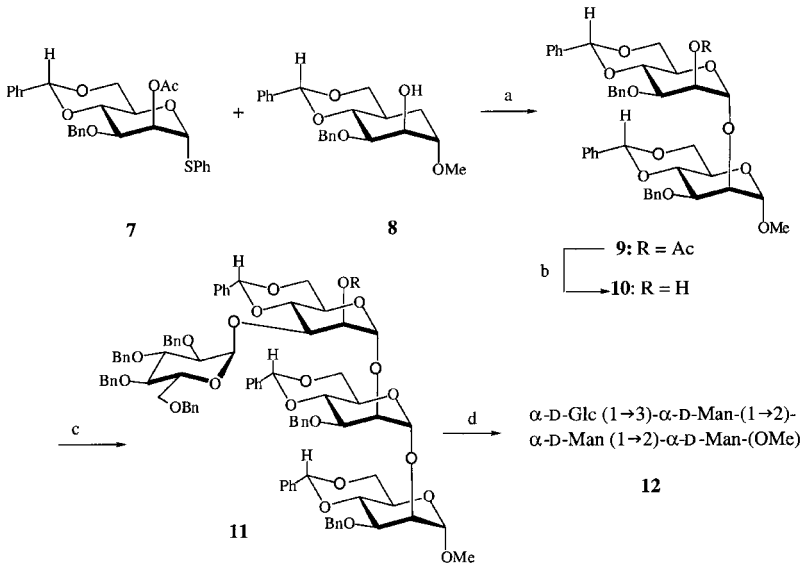


Scheme 2. a) Bu_2SnO, toluene, reflux, 4 h then Bu_4NI, *p*MBnCl, rt, 5 h, 75%; b) HNa, Bu_4NI, BnBr, 0 °C → rt 18 h, 71% c) CAN, CH_3CN-H_2O (4:1), 0 °C, 80%; d) BnBr, CH_2Cl_2, nBu_4NHSO_4, NaOH aq, reflux 18 h, 50%; e) 4 Å molecular sieves, Et_2O, - 30 °C then TMSOTf (1 eq) 16 h,49%.

This involved the transitory and regioselective *p*-methoxybenzylation of the 3-OH of **1** which was achieved via the formation of the 2,3-stannylene derivative. Next, benzylation of **2** was carried out and subsequent removal of the *p*-methoxybenzyl group, as present in **3** (Scheme 2), afforded **4**.

Condensation of **4** with the tetra-*O*-benzylglucosyl trichloroacetimidate **5** (TMSOTf, ether, 4 Å molecular sieves, −30 °C) afforded the disaccharide **6** in 49% yield.

On the other hand, the di-mannose derivative **10** was prepared in 50% overall yield by coupling **7**, readily obtained by acetylation of a known phenyl thiomannopyranoside precursor,[9] with the methyl mannopyranoside acceptor **8**[9] in the presence of dimethyl(thiomethyl)sulfonium triflate or DMTST, [15] and 2,6-di-*tert*-butyl-4-methylpyridine, followed by Zemplén deacetylation (NaOMe, MeOH, rt, 2 h) of **9**.



Scheme 3. a) **7** (1.3 eq), **8** (1 eq), 4 Å molecular sieves, 2,6-DTBMP (1 eq), CH_2Cl_2, 20 min then DTMST (4 eq), 2.5 h, 48%; b) NaOMe-MeOH, 2h, 98%; c) **6** (1 eq), 4 Å molecular sieves, NIS (2 eq), CF_3SO_3Ag (3 eq), Et_2O, -10 °C, 12 h, 47%; d) H_2, Pd/C MeOH, 2 h, 93%.

The title compound **12** [11] was finally isolated in 44% overall yield by coupling the two disaccharide units **6** and **10** in the presence of NIS/CF_3SO_3Ag in dry ether at −10 °C and treating the tetrasaccharide **11** by hydrogenolysis (Pd/C 10%, MeOH). Acetylation of **12** with an excess of Ac_2O afforded the corresponding per-*O*-acetylated derivative **13** (Scheme 3).

In spite of its higher convergent character, this new route to the title tetrasaccharide is very similar to that already reported by Jain et al. [11] in terms of number of steps and overall yield. The use of a trichloroacetimidate donor instead of thiomethyl ether to obtain the α-linkage between the glucose and mannose units with high stereoselectivity, resulted, as expected, in a slight improvement of the yield (49% vs. 39%). Unfortunately, the limiting step was the glycosylation of the two building blocks which never exceeded 50% yield in our hands.

EXPERIMENTAL

General Methods. Melting points were determined with a Kofler-block apparatus and are uncorrected. Optical rotations were measured at 20 °C using a Perkin–Elmer 241 polarimeter (589 nm) (concentration expressed in g/100 mL). Infrared spectra were measured with a F.T. Perkin–Elmer 1710 spectrometer. NMR spectra were recorded at 300 MHz on a Bruker apparatus. Chemical shifts are expressed in parts per million downfield from tetramethylsilane. Mass spectra were recordered on a Nermag Rl0-l0C mass spectrometer by chemical ionization (DCI/NH_3) or on a Jeol MS-700 for FAB. Analyses were performed by the Laboratoire Central de Microanalyses du CNRS. Thin-layer chromatography was effected on Merck Kieselgel 60 F254 plates using sulfuric acid (5%) in ethanol as revealer. Flash column chromatography was conducted on Kieselgel Merck 60 (230–400 Mesh) silica gel column. Commercial solvents were distilled before use.

Ethyl 4,6-*O*-Benzylidene-3-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (2). To a solution of **1** [12] (2.85 g, 9.1 mmol) in anhydrous toluene (200 mL) was added tin dibutyloxide (2.73 g, 10.1 mmol), and the suspension was heated under reflux for 4 h with continuous removal of water (Dean–Stark) and concentrated to half-volume (100 mL). Then, tetrabutylammonium iodide (4.05 g, 10.1 mmol) and *p*-methoxybenzyl chloride (1.48 g, 10.1 mmol) were added, and the reflux was maintained for 5 h, after which the reaction mixture was diluted with water (100 mL) and extracted with ethyl acetate. The organic layer was separated, washed with water, with brine, and dried over $MgSO_4$. Purification by flash chromatography on silica gel (cyclohexane-EtOAc, 9:1 then 8:1) gave 2 (2.95 g, 75%). R_f (cyclohexane-EtOAc, 9:1) 0.11; $[\alpha]_D^{20} + 124.5°$ (*c* 0.83, MeOH); IR ($CDCl_3$) ν_{max} (cm^{-1}): 3564 (OH); ^1H NMR ($CDCl_3$): δ 5.62 (s, 1H, H-7), 5.37 (s, 1H, H-1), 4.71 (q, 2H, J = 11.3 Hz, CH_2-Ph-O-CH_3), 4.27–4.20 (m, 2H, H-4, H-5), 4.13 (t, 1H, H-6b), 4.08 (d, 1H, H-2), 3.92–3.84 (m, 2H, H-3, H-6a), 3.81 (s, 3H, Ph-OCH_3), 2.70–2.54 (m, 1H, S-CH_2-CH_3), 1.30 (t, 1H, S-CH_2-CH_3); ^{13}C NMR ($CDCl_3$): δ 126, 128.14, 128.86, 129.59, 129.85 (arom), 101.50 (C-7), 84.40 (C-1), 79.08 (C-4), 75.59 (C-2), 72.70 (CH_2-Ph), 71.26 (C-3), 68.60 (C-6), 63.95 (C-5), 55.16 (O-CH_3), 24.90 (S-CH_2), 14.86 (S-CH_2-CH_3).

Anal. Calcd for $C_{23}H_{28}O_6S$ (432.53): C, 63.87; H, 6.52. Found: C, 63.75; H, 6.75.

Ethyl 2-*O*-Benzyl-4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (3). To a cooled solution (0 °C) of **2** (2.8 g, 6.4 mmol) in DMF (125 mL), sodium hydride (272 mg, 6.8 mmol), tetrabutylammonium iodide (2.51 g, 6.8 mmol) and benzyl bromide (0.8 mL, 6.7 mmol) were added. The reaction mixture was allowed to reach room temperature and stirred for 18 h. It was then diluted with water (50 mL) and extracted with ethyl acetate (200 mL). The organic layer was separated and washed several times with water before drying over $MgSO_4$ and concentration in vacuo. Flash chromatography on silica gel (cyclohexane-EtOAc, 7:1) led to compound 3 (2.4 g, yield: 71%) as a syrup. R_f (cyclohexane-EtOAc, 2:1) 0.7; $[\alpha]_D^{20} + 94°$ (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.66 (s, 1H, H-7), 5.31 (d, 1H, H-1), 4.76 (m, 2H, CH_2-Ph-OCH_3), 4.78−4.58 (2d, 2H, J = 11.7 Hz, CH_2-Ph), 4.28−4.18 (m, 3H, H-4, H-5, H-6b), 3.95−3.90 (m, 3H, H-3, H-2, H-6a), 3.81 (s, 3H, OCH_3), 2.60 (m, 2H, S-CH_2), 1.26 (t, 3H, S-CH_2-CH_3); ^{13}C NMR ($CDCl_3$) δ 101.52 (C-7), 83.60 (C-1), 79.20 (C-4), 78.40 (C-2), 76.21 (C-3), 73.14 (*C*H_2-Ph), 72.67 (O-*C*H_2-Ph), 68.60 (C-6), 64.80 (C-5), 55.10 (O-CH_3), 25.30 (S-CH_2), 15.00 (S-CH_2-*C*H_3).

Anal. Calcd for $C_{30}H_{34}O_6S$: C, 68.94; H, 6.56. Found: C, 68.69; H, 6.47.

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

1) *From 1*: A solution of **1** (0.62 g, 2 mmol), benzylbromide (0.23 mL, 2.2 mmol) and *n*-Bu_4HSO_4 (0.6 g, 2.2 mmol) in CH_2Cl_2 (60 mL) was stirred in the presence of an aqueous NaOH solution (5 mL, 30%) and heated at reflux overnight. After cooling to rt, the organic layer was separated, washed with water, with a saturated aqueous $NaHCO_3$ solution and with brine. This afforded, after concentration in vacuo and chromatography as below, 0.5 g (50%) of crude **4**.

2) *From 3*: A solution of **3** (1.68 g, 3.21 mmol) in a mixture of acetonitrile−water (4:1, 5 mL) was cooled to 0 °C prior to addition of cerium ammonium nitrate (3.5 g, 6.4 mmol). After stirring for 2 h, the reaction mixture was diluted with ethyl acetate (100 mL) and the organic layer was washed with water and dried over $MgSO_4$. After concentration in vacuo, flash chromatography using cyclohexane/EtOAc afforded 1.03 g (80%) of **4** as a syrup. R_f (cyclohexane-EtOAc, 2:1) 0.66; $[\alpha]_D^{20} + 96°$ (*c* 1.14, $CHCl_3$) [Lit.:(11) mp 66−68 °C (ether/petroleum ether), $[\alpha]_D^{20} + 102.8°$ (*c* 1.30, $CHCl_3$)]; IR ($CDCl_3$) ν_{max} (cm^{-1}) 3566 (OH); ^{1}H NMR ($CDCl_3$) δ 7.52−7.26 (m, 10H, H-Ar), 5.58 (s, 1H, H-7), 5.39 (s, 1H, H-1), 4.77 and 4.66 (2d, 2H, J = 11.6 Hz, CH_2-Ph), 4.26−4.17 (m, 2H, H-5, H-6), 4.07−4.04 (dd, 1H, J = 3.5, J = 8 Hz, H-3), 3.99−3.92 (m, 2H, H-2, H-4), 3.85 (m, 1H, H-6′), 2.61 (m, 2H, S-CH_2), 2.43 (d, 1H, J = 8 Hz, OH), 1.27 (t, 1H, J = 7.38 Hz, S-CH_2-CH_3); ^{13}C NMR ($CDCl_3$): δ 101.98 (C-7), 82.77 (C-1), 80.23 and 79.62 (C-2, C-4), 73.19 (O*C*H_2-Ph), 69.01 (C-3), 68.46 (C-6), 64.05 (C-5), 25.24 (S-*C*H_2-CH_3), 14.96 (S-CH_2-*C*H_3); MS (DCI/NH_3) *m/z*: 420 $[M+NH_4]^+$, 403 $[M+H]^+$.

Ethyl *O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (6). Trichloroacetimidate donor **5** (0.7 g, 1 mmol) and acceptor **4** (0.31 g, 0.77 mmol) were dissolved in ether (15 mL) containing 4 Å powdered molecular sieves. The mixture was cooled to −30 °C prior to the addition of trimethylsilyl triflate (139 μL, 0.77 mmol) and stirred at the same temperature for 16 h.

Dilution with ethyl acetate (50 mL) was followed by filtration through a celite pad. The filtrate was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Flash chromatography of the residue on a silica gel column eluting with 9:1 cyclohexane/EtOAc afforded 0.35 g (49%) of **6** as a syrup; R_f (cyclohexane-EtOAc, 2:1) 0.85; $[\alpha]_D^{20} + 84°$ (*c* 0.85, $CDCl_3$); ^1H NMR ($CDCl_3$) δ 7.32−6.97 (m, 25 H, H Ar), 5.50 (d, 1H, J = 3.6 Hz, H-1′), 5.47 (s, 1H, H-7), 5.34 (d, 1H, J = 0.7 Hz, H-1), 4.97 and 4.43 (2d, 2H, J = 10.9 Hz, CH_2Ph), 4.86 and 4.78 (2d, 2H, J = 12 Hz, CH_2Ph), 4.82 and 4.43 (2d, 2H, J = 12.3 Hz, CH_2Ph), 4.37 (m, 3H, H), 4.18 (dd, 1H, J = 4.8 Hz, J = 10.18 Hz, H-6′b), 3.97 (t, 1H, J = 9.2 Hz, H-3′), 3.92 (dd, 1H, J = 2.3 Hz, J = 1 Hz, H-2), 3.88 (t, 1H, J = 10.1 Hz, H-6′a), 3.73 (m, 1H, H-5 or H-5′), 3.63 (dd, 1H, J = 3.6 Hz, J = 8 Hz, H), 3.57 (t, 1H, J = 9.1 Hz, H), 2.59 (m, 2H, S-CH_2-CH_3), 1.26 (t, 3H, S-CH_2-CH_3).

Phenyl 2-*O*-Acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-thiomannopyranoside (7). A solution of phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-thiomannopyranoside[9] (1 g, 2.22 mol) in pyridine (20 mL) was stirred at room temperature for 5 h in the presence of Ac_2O (3 mL). Subsequent dilution with ethyl acetate, followed by washings with aqueous solution of H_2SO_4, with water and with a saturated aqueous solution of sodium hydrogencarbonate led to isolation of 1.3 g of crude product. Flash chromatography of the residue on a silica gel column eluting with 9:1 cyclohexane/EtOAc afforded 1 g (92%) of **7** as a syrup; R_f (cyclohexane-EtOAc, 4:1) 0.76; $[\alpha]_D^{20} + 113°$ (*c* 1, $CDCl_3$); ^1H NMR ($CDCl_3$) δ 7.56−7.24 (m, 15H, Ar), 5.65 (s, 1H, H-7), 5.62 (dd, 1H, $J_{2,3}$ = 3.4 Hz, $J_{2,1}$ = 1.4 Hz, H-2), 5.46 (d, 1H, J = 1 Hz, H-1), 4.71 (s, 2H, CH_2Ph), 4.44−3.90 (m, 4H, H-4, H-5, H-6a, H-6b), 3.86 (dd, 1H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9,5 Hz, H-3), 2.16 (s, 3H, OAc); MS (DCI/NH_3) *m/z* 510 $[M + NH_4]^+$, 493 $[M + H]^+$.

Anal. Calcd for $C_{28}H_{28}O_6S$ (492.59): C, 68.27; H, 5.73. Found: C, 68.12; H, 5.89.

Methyl *O*-(2-*O*-Acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (9). A solution of **7** (0.530 g, 1.07 mmol) and **8**[9] (0.308 g, 0.82 mol) in anhydrous CH_2Cl_2 containing 4 Å molecular sieves (0.4 g) and 2,6-di-*tert*-butyl-4-methylpyridine (170 mg, 0.8 mmol) was stirred for 20 min at rt. Then, dimethyl(methylthio)sulfonium triflate (DMTST) [prepared extemporaneously by dropwise addition of methyl trifluoromethanesulfonate (375 μL, 3.31mmol) to methyl disulfide (293 μL, 3.31 mmol)] was added and stirring was maintained for 2.5 h before filtration over a celite pad. The solution was washed with brine and dried over $MgSO_4$. Concentration in vacuo, followed by flash chromatography using cyclohexane/EtOAc (9:1) as eluent, gave **9** (0.3 g, 48%) as a syrup; R_f (cyclohexane-EtOAc, 2:1) 0.66; $[\alpha]_D^{20}$+4.5° (*c* 1, $CDCl_3$); ^1H NMR ($CDCl_3$) δ 7.41− 7.21 (m, 20H, Ar), 5.67 and 5.66 (2s, 2H, H-7 and H-7′), 5.16 (d, 1H, J = 1.43 Hz, H-1), 4.88 and 4.72 (2d, 2H, J = 11.5 Hz, OCH_2-Ph), 4.78 and 4.66 (2d, J = 12 Hz, OCH_2-Ph), 4.69 (s, 1H, H-1′), 4.29 (m, 2H, H-6, H-6′), 4.10−3.77 (m, 8H, H-2, H-2′, H-3, H-3′, H-4, H-4, H-5, H-5′), 3.36 (s, 3H, OCH_3), 2.15 (s, 3H, Ac); MS (DCI/NH_3) *m/z* 772 $[M + NH_4]^+$.

Methyl *O*-(3-*O*-Benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (10). A methanolic solution (20 mL) of **9** (0.25 g, 0.33 mmol) was stirred for 2 h in the presence of sodium methoxide (100 mg). The solution was neutralized by addition of IRC_{50} H^+ Amberlite and filtered. Flash chromatography using cyclohexane-EtOAc (85:15) led to **10** (0.23 g, 98%). R_f

(cyclohexane-EtOAc, 2:1) 0.45; $[\alpha]_D^{20}$+38° (*c* 0,45, $CDCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.53−7.25 (arom), 5.64 and 5.63 (2s, 2H, H-7, H-7′), 5.20 (d, 1H, J = 1 Hz, H-1), 4.91 and 4.77 (2d, 2H, J = 11.5 Hz, CH_2-Ph), 4.70 (s, 1H, J = 1.7 Hz, H-1′), 4.83 and 4.69 (2d, 2H, J = 12 Hz, CH_2-Ph), 4.28−3.76 (m, 12H, CH), 2.63 (s, 1H, OCH_3); MS (DCI/NH_3) *m/z* 730.2 $[M + NH_4]^+$.

Anal. Calcd for $C_{41}H_{44}O_{11}$ (712.78): C, 69.09; H 6.22. Found: C, 68.55; H 6.39.

Methyl *O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (11). To a solution of **6** (130 mg, 0.14 mmol) and of **10** (100 mg, 0.14 mmol) in dry ether cooled to −10 °C were added *N*-iodosuccinimide (65 mg, 0.28 mmol) and silver trifluoromethanesulfonate (100 mg, 0.42 mmol). The reaction mixture was stirred until complete disappearance of **6** (TLC: cyclohexane/EtOAc, 2:1), then diluted with ether (30 mL) and filtered through a celite pad. The organic layer was washed with water, with brine and concentrated in vacuo. Flash chromatography on silica gel using cyclohexane/EtOAc (85:15) yielded **11** (104 mg, 47%) as a clear syrup; $[\alpha]_D^{20}$ + 27° (*c* 0.45, $CDCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.38−7.02 (m, 50H, arom.), 5.63, 5.42, 5.24 (3s, 3H, H-7, H-7′, H-7), 5.54 (d, 1H, J = 3.45 Hz, H-1‴), 5.25 (s, 1H, H-1), 5.22 (s, 1H, H-1′), 4.91−3.59 (m, 39H, H ring and OCH_2), 3.34 (s, 3H, OCH_3); R_f (cyclohexane-EtOAc, 2:1) 0.56; MS (FAB) *m/z*: 1598.5 $[M + Na]^+$.

Methyl *O*-(α-D-Glucopyranosyl)-(1→3)-*O*-(α-D-mannopyranosyl)-(1→2)-*O*-(α-D-mannopyranosyl)-(1→2)-α-D-mannopyranose (12). A methanolic solution (10 mL) of **11** (50 mg, 0.31mmol) was stirred for 2 h under hydrogen atmosphere (1 atm) in the presence of palladium-on-charcoal (20 mg). The suspension was filtered off, and the solids were washed with methanol. Filtrate and washings were combined and concentrated in vacuo. This afforded **12** as an amorphous compound (20 mg, 93%); $[\alpha]_D^{20}$ + 178° (*c* 0.5, MeOH). [Lit: (12) $[\alpha]_D^{20}$ + 164° (*c* 1.1, H_2O)]; MS (FAB) *m/z* 703 $[M + Na]^+$. ^{1}H NMR (D_2O) was in full agreement with that reported in the literature.[11]

Methyl *O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (13). A solution of **12** (10 mg, 0.14 mmol) in pyridine (5 mL) was stirred at rt for 2 h in the presence of Ac_2O (500 mL). The mixture was then diluted with EtOAc (25 mL) and the organic layer was separated, washed with a 2N H_2SO_4 aqueous solution, with water, and concentrated in vacuo. This afforded a crude residue (15 mg, 87%). ^{1}H NMR ($CDCl_3$) δ 5.37 (bs, 1H, H-1‴), 5.31 (dd, 1H, J = J′ = 9 Hz, H-3), 4.92 (bs, 1H, H-1), 4.86 (bs, 1H, H-1′), 4.84 (bs, 1H, H-1), 3.40 (s, 3H, OMe), 2.22−1.80 (13s, 39H, 13 $COCH_3$). HR-MS (FAB+LiCl) *m/z* calculated for 1233,3949 $[M + Li]^+$. Found 1233,3908.

REFERENCES

1. Welsh, M.J.; Smith, A.E. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell **1993**, *73*, 1251−1254.

2. Kopito, R.R. Biosynthesis and degradation of CFTR. Physiol. Rev. **1999**, *79*, S167–S173.
3. Riordan, J.R. Cystic fibrosis as a disease of misprocessing of the cystic fibrosis transmembrane conductance regulator glycoprotein. Am. J. Hum. Genet. **1999**, *64*, 1499–1504.
4. Helenius, A. How N-linked oligosaccharide affect glycoprotein folding in the endoplasmic reticulum. Mol. Biol. Cell **1994**, *5*, 253–265.
5. Denning, G.M.; Anderson, M.P.; Amara, J.F.; Marshall, J.; Smith, A.E.; Welsh, M.J. Processing of mutant cystic fibrosis transmembrane conductance regulator is temperature-sensitive. Nature **1992**, *358*, 761–764.
6. Li, C.; Ramjeesingh, M.; Reyes, E.; Jensen, T.; Chang, X.; Rommens, J.M.; Bear, C.E. The cystic fibrosis mutation (ΔF508) does not influence the chloride channel activity of CFTR. Nat. Genet. **1993**, *3*, 312–316.
7. Ware, F.E.; Vassilakos, A.; Peterson, P.A.; Jackson, M.R.; Lehrman, M.A.; Williams, D.B. The molecular chaperone calnexin binds $Glc_1Man_9GlcNAc_2$ oligosaccharide as an initial step in recognizing unfolded glycoproteins. J. Biol. Chem. **1995**, *270*, 4697–4704.
8. Spiro, R.G.; Zhu, Q.; Bhoyroo, V.; Söling, H.-D. Definition of the lectin-like properties of the molecular chaperone, calreticulin and demonstration of its copurification with endomannosidase from rat liver golgi. J. Biol. Chem. **1996**, *271*, 11588–11594.
9. Cherif, S.; Clavel, J.-M.; Monneret, C. A synthetic approach to the glycan chain of high mannose type *N*-glycoprotein. J. Carbohydr. Chem. **1998**, *17*, 1203–1218.
10. Vassilakos, A.; Michalak, M.; Lehrman, M.A.; Williams, D.B. Oligosaccharide binding characteristics of the molecular chaperones calnexin and calreticulin. Biochemistry **1998**, *37*, 3480–3490.
11. Jain, R.K.; Liu, X.-G.; Oruganti, S.R.; Chandrasekaran, E.V.; Matta, K.L. Synthesis of oligosaccharide substrates for *N*-linked glycoprotein processing enzymes. Carbohydr. Res. **1995**, *271*, 185–196.
12. Garegg, P.J.; Kvarnström, I.; Niklasson, A.; Niklasson, G.; Svensson, S.C.T. Partial substitution of thioglycosides by phase transfer catalyzed benzoylation and benzylation. J. Carbohydr. Chem. **1993**, *12*, 933–953.
13. Oshitari, T.; Kobayashi, S. Preparation of 2-*O*-(3-*O*-Carbamoyl-α-D-mannopyranosyl)-L-gulopyranose: synthetic study on the sugar moiety of antitumor antibiotic bleomycin. Tetrahedron Lett. **1995**, *36*, 1089–1092.
14. Fügedi, P.; Garegg, P.J. A novel promoter for the efficient construction of 1,2-*trans* linkages in glycoside synthesis, using thioglycosides as glycosyl donors. Carbohydr. Res. **1986**, *149*, C9–C12.

Received June 15, 2001
Accepted December 31, 2001