Synthesis of Standardized Building Blocks as a β-D-Mannosyl Donors with a Temporary Protection to be 3,6-Di-O-glycosyl Acceptors, for Constructing the Inner Core of Glycoproteins and Artificial Antigens

El Sayed H. El Ashry*,† and Conrad Schuerch
Department of Chemistry, State University of New York,
College of Environmental Science and Forestry,
Syracuse, New York 13210, U.S.A.
(Received April 8, 1985)

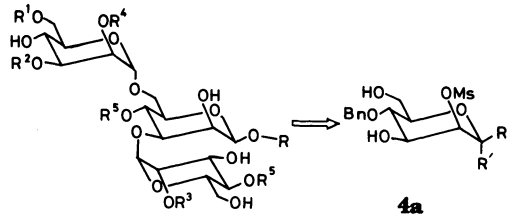
A synthetic scheme has been achieved for the core block of glycoproteins having D-mannose configuration, which is standardized to be a 3,6-di-O-glycosyl acceptor and react stereoselectively as a β-D-mannosyl donor. The synthesis was started by methyl 2,3-O-isopropylidene-α-D-mannopyranoside, which upon benzylation, deisopropylidenation, and selective acetolysis of the 6-O-benzyl group afforded the corresponding tetraacetate, which could be converted to 3,6-di-O-allyl-4-O-benzyl-1,2-O-(1-methoxyethylidene)-β-D-mannopyranose. The orthoester could be separated into the *exo* and *endo* isomers. Hydrolysis of the orthoester ring followed by reaction with mesyl chloride gave 3,6-di-O-allyl-4-O-benzyl-2-O-mesytl-α-D-mannopyranosyl chloride, that upon reaction via its 1-O-trifluoroethanesulfonate with 2-[4-(*p*-toluenesulfonamido)phenyl]-ethanol gave the corresponding glycosides in β:α ratio of 3:1. Deallylation of the β-glycosides gave the diol which are suitable for branching and could react selectively at the 6-position allowing its potential for glycosylation by different glycosyl donors.

Many glycoproteins existing at cell surfaces and in inter-cellular systems have a common feature[1-4] in which a 3,6-di-O-(α-D-mannopyranosyl)-D-mannopyranosyl moiety is linked via a 1,4-β-linkage to a chitobiose residue which is linked in turn to asparagine. Glycan chains in these glycoproteins can be classified[4-12] into three types according to their origin as well as the kind of the monosaccharide existing in the branched units of the glycan chains. The first type (**1**) is oligosaccharides having high mannose content which was isolated from calf thyroglobule, the second type (**2**) which is considered as complex one and was isolated from immunoglobulin, and the third type (**3**) which is a hybrid and was isolated from ovalbumin. They have various biological functions such as the recognition signals at cell surfaces.

The artificial antigen, prepared by coupling a carbohydrate residue to a protein support, produces antibodies specific for the carbohydrate moiety, when immunized to animals. This led to great efforts towards the estimation of the immunological determinants of various antigens, via selecting artificial antigen containing particular oligosaccharide which may provide homogeneous antibody of predefined specificity.[13-16] The availability of homogeneous antibody specific for the synthetic hapten may provide an approach for studying the antigen-antibody interaction. The success in the synthesis of artificial antigen is largely due to the recent advances[17-22] in the stereoselective synthesis of the requisite oligosaccharides in high yield as well as their involvement of functional groups capable of coupling to proteins. α-D-Manp-(1→6)-α-D-manp, and α-D-manp-(1→2)-α-D-manp have been described[16] as the antigenic backbone and side chain of the yeast mannan. α-D-Manp-(1→3)-α-D-manp has been also described[23] as the antigenic branching point of the glycoproteins.

The object of this synthetic project aimed to provide an efficient synthetic approach for the synthesis of the protein conjugate of the trisaccharide 3,6-di-O-(α-D-mannopyranosyl)-D-mannose (**4**) as artificial antigen which represents the core structure in the glycoproteins.[1-3] The prerequisite for that is the synthesis of a core block of the mannose moiety, standardised (such as **4a**) to act as a glycosyl acceptor in the 3 and 6 positions, and meantime could act stereoselectively as a β-D-mannosyl donor toward a hydroxyl group, which is not yet reported elsewhere because it is de facto the most problematic part, and therefore its synthesis is described in the present work.

The available methods[24-27] for the stereoselective synthesis of 1,2-*cis* glycosides of gluco and galacto



R=4GlcNAcβ1→4GlcNaAcβ1→Asn

1 $R^4=R^5=H$, $R^1=R^2=R^3=$Man α1→2 Manα1-
2 $R^1=R^2=R^5=H$, $R^3=R^4=$SA α2→6 Gal β1→4 GlcNAcβ_1-
3 $R^3=R^4=H$, $R^1=R^2=$Man α1-, $R^5=$GlcNAcβ1-
4 $R=R^1=R^2=R^3=R^4=R^5=H$

Scheme 1.

† On leave of absence from Chemistry Department, Faculty of Science, Alexandria University, Alexandira, Egypt.

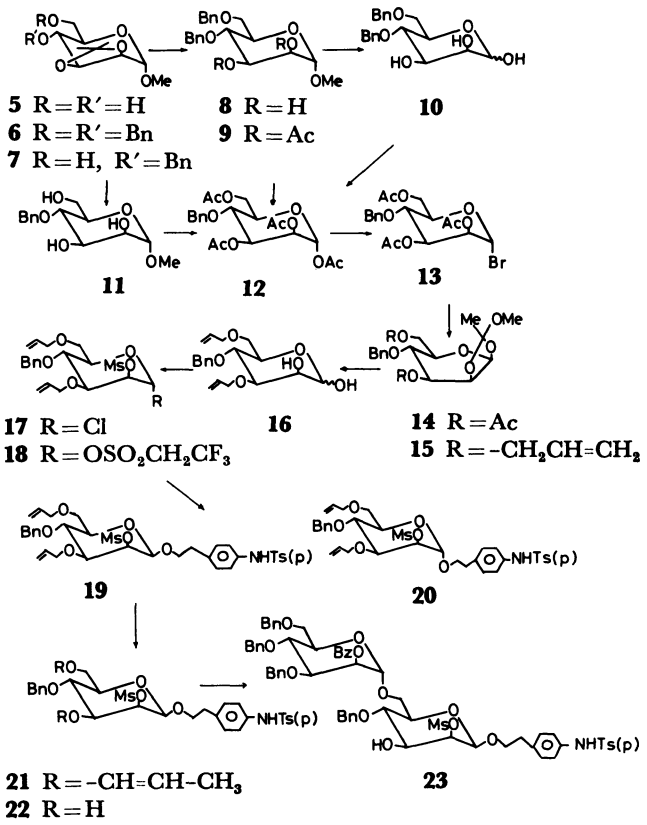
types are not of general utility for the synthesis of that of manno type. The use of glycosyl halides with *O*-2, *O*-3 bridging substituents that permit β-attack at *C*-1 has been reported.[28–31] This method does not allow the different temporary protection of *C*-2 and *C*-3 hydroxyl groups prior to the glycosidation of the mannosyl residue. The approach, which has found wide application in oligosaccharide synthesis,[32–35] utilises a suitably protected D-glucopyranosyl halide having a participating group at *C*-2 to favor β-linkage, followed by deacylation of *O*-2, oxidation and reduction of the resulting ketone to the manno epimer. A stereospecific synthesis of β-mannopyranosides with the use of insoluble silver catalysts has been also reported.[36] Enhancing the *C*-2-*O*-dipole by appropriate substitution has also been used to afford stereoselective synthesis[21,22] of β-D-mannosides. In a previous report,[22] a model study of the latter method with temporary blocking groups proved its successful application. Having this in mind, the design for the synthesis of 3,6-di-*O*-allyl-4-*O*-benzyl-2-*O*-mesyl-α-D-mannopyranosyl chloride (**17**) will provide a promising synthon to be used for synthesizing the target complex oligosaccharides by glycosidation to form the β-linkage followed by deallylation to generate hydroxyl groups at 3 and 6 positions, suitable for further construction.

Results and Discussion

Upon benzylation with benzyl chloride–powdered potassium hydroxide, methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside (**5**)[37] gave the dibenzyl derivative (**6**) that upon deisopropylidenation afforded methyl 4,6-di-*O*-benzyl-α-D-mannopyranoside (**8**) in 82% yield. This dibenzyl derivative (**8**) is a potential starting material,* since it can be prepared on a large scale in crystalline form. The presence of two benzyl groups in **8** was confirmed form its ^{1}H and ^{13}C NMR spectra.

Moreover, the structure was also confirmed by its acetylation to give the diacetate (**9**) whose ^{1}H NMR spectrum showed the presence of two acetyl singlets at δ 2.15 and 1.93 and a downfield shift of H-2 and H-3 (δ 5.27 and 5.35, $J_{2,3}$ 4 Hz and $J_{3,4}$ 8 Hz). Acid hydrolysis of **8** by hydrochloric acid in aqueous dioxane afforded 89% yield of 4,6-di-*O*-benzyl-D-mannopyranose (**10**) based on the recovery of the starting material which could be recycled. The acetylation of **10** gave a mixture of the corresponding α- and β-tri-*O*-acetyl derivatives[23] in 55 and 30% yield, respectively. Acetolysis of **8** under controlled temperature and reaction time afforded 1,2,3,6-tetra-*O*-acetyl-4-*O*-benzyl-α-D-mannopyranose (**12**) in 73% yield. Compound **12** could also be obtained in 78 and 86% yield by acetolysis of **11**[38] and **10**, respectively. For convenience, direct conversion of **8** into **12** was found to be satisfactory. The structure of **12** was confirmed from ^{1}H NMR spectrum which showed the presence of four acetyl singlets in addition to the downfield shift of H-1, H-2, H-3, and H-6 signals, and from ^{13}C NMR spectrum which showed, in addition to the presence of one benzyl group, an upfield shift of the *C*-6. The acetate **12** was assigned the α-configuration based on its optical rotation. The reaction of **12** with hydrogen bromide afforded the bromide (**13**).

The orthoester **14** was prepared from **13** by reaction with methanol in the presence of 2,6-lutidine. It exists as a mixture of diastereoisomers in a ratio 1:12 as found from its ^{1}H NMR spectrum, and the major isomer could be crystallized in a pure form. Deacetylative allylation of the mixture of isomers of **14** gave the diallyl derivative (**15**), also as a mixture of isomers that could be separated by LC to give the major isomer **15a** as a crystalline derivative in 82% yield [singlets at δ 1.72 (CMe) and 3.30 (OMe)] and the minor isomer **15b** as a syrup in 6% yield [singlets at δ 1.53 (CMe) and 3.48 (OMe)]. For assignment of the diastereoisomeric orthoacetates, the chemical shifts for protons of the orthester ring could be used,[39] which fall into one of two patterns. From the fact that H-1, H-2, and CMe are less shielded and OMe more shielded in **15b**, than in **15a**, and the comparison of chemical shifts of OMe with that of



Scheme 2.

* This compound was used by V. K. Handa, V. V. Barlow, and K. L. Matta, *Carbohydr. Res.*, **76**, Cl (1979); but no data was given for it.

methanol, the isomer **15a** should tentataively have its OMe *exo*, and the other isomer *endo*. Opening of the orthoester ring of **15** with aqueous acetic acid afforded 85% yield of 3,6-di-*O*-allyl-4-*O*-benzyl-D-mannopyranose (**16**). Its ^{13}C NMR spectrum showed a ratio of ≈2.3:1.0 of α:β anomers, as indicated by the two signals of the anomeric carbon atoms at δ 94.17 and 94.63, respectively. The corresponding 3,4,6-tri-*O*-allyl analog[22] of **16** showed almost a similar ratio (≈2.5:1.0) for their anomeric carbon atoms. Reaction of **16** with mesyl chloride in 2,6-lutidine gave 68% yield of 3,6-di-*O*-allyl-4-*O*-benzyl-2-*O*-mesyl-α-D-mannopyranosyl chloride (**17**). Its ^{13}C NMR spectrum showed signals at δ 90.58 and 38.92 due to the anomeric carbon atom and the mesyl group, respectively.

The stereoselectivity and yield in glycosidation reactions can be optimized if the reactivity of *C*-1 is carefully balanced by an appropriate choice of *C*-1 leaving group. A wide spectrum of reactivities is now available by selecting the leaving group with the proper electronegativity, form the series of sulfonates (tosyl), 2,2,2-trifluoroethanesulfonyl (tresyl), or trifluromethanesulfonyl (triflyl). In the present work, the tresylate was selected as the leaving group, as it provides a moderate rate of glycosidation which may favor the β-glycosidation. Thus, reaction of **17** with silver tresylate followed by reaction with 2-[4-(*p*-toluenesulfonamido)phenyl]ethanol afforded 60% yield of the corresponding β-D-mannopyranoside (**19**) and 25% yield of α-anomer (**20**), after separation by LC. The assigned anomeric configurations was based on the comparison of the specific rotations of the two anomers, as well as their ^{1}H NMR spectral data. The β-anomeric proton appeared at δ 4.45 and that of α at δ 4.8, within the anticipated range[22,40] for β (δ 4.4—4.7) and α (δ 4.8—5.0), respectively. The ^{13}C NMR spectra showed *C*-1 at δ 98.03 and 98.64 for **20** and **19**, respectively. Rearrangement of the allyl groups in compound **19** into the 1-propenyl groups was achieved by action of tris(triphenylphosphine)rhodium chloride. Partial depropenylation of **21** was observed during the reaction and it was completed by the action with a mixture of mercury(II) oxide and mercury(II) chloride to give 77% yield of **22**. Although the 3- and 6-positions of **22** are available for glycosidation, but, the 6-position could be selectively glycosylated as shown by allowing its reaction with the tresylate derived from 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride to give the disaccharide **23**.

In conclusion, the synthesis of the orthoesters **14** and **15** and the chloride **17** are of great value as precursors for complex oligosaccharides of biological significance, and actually the disaccharide **23** was successfully synthesized.

Experimental

Generals. ^{1}H NMR spectra were determined with a Varian A-60-A or XL-100-15 spectrometer for solutions in chloroform with tetramethylsilane as internal reference. ^{13}C NMR spectra were determined with Varian XL-100-15 or CFT-20 spectrometers in pulsed Fourier-transform proton-noise-decoupled mode on similar solutions. Optical rotations were determined with a Perkin-Elmer model 141 polarimeter in jacketed, 1-dm cells. Melting points were determined with a "Meltemp" apparatus with a 76-mm immersion thermometer. TLC was performed on "Baker flex" silica gel 1B-F (2.5×7.5 cm) plates; the solvents were ethyl acetate–hexane (1:1 solvent A; 2:1, solvent B; 1:3, solvent C). High pressure liquid chromatography (LC) was carried out using a Valvco septumless injector (1.0 ml), a Gleno pump, model HPLPS-1, and Waters differential refractometer R-401. A stainless-steel column (1×25 cm inside diameter) containing silica gel (Whatman, partisil M 9 10/25) was used. Preparative LC was performed on a Waters Prep-500 instrument, using a silica gel column.

Methyl 4,6-Di-*O*-benzyl-α-D-mannopyranoside (8). To a stirring mixture of methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside[37] (46.8 g, 0.2 mol) and powdered potassium hydroxide (50 g) in toluene (200 ml) was added benzyl chloride (150 ml) gradually under heating. After 1 h, potassium hydroxide (50 g) was again added and the reaction was continued till TLC showed complete conversion into **6** (5 h). The mixture was cooled and filtered, and the filtrate wa washed with water, dried, and evaporated. The remaining oil was dissolved in methanol (200 ml) and 0.5 M hydrochloric acid (50 ml), and solution was heated under reflux until TLC showed complete hydrolysis (30 min), neutralized with excess of sodium hydrogencarbonate, and then filtered. The filtrate was evaported, and the residue was extracted with dichloromethane. The usual work-up of the extract gave the product which was crystallized from hexane (61.7 g, 82% yield); R_f=0.39 (solvent A); mp 101—103 °C; $[\alpha]_D^{29}$ +75.3° (c 2.0, chloroform); ^{1}H NMR δ=7.3 (m, 10 H, 2×Ph), 4.78, 4.64, 4.52, 4.50 (each d, 5 H, 2×CH_2Ph and hidden H-1), 3.7 (m, 6 H, H-2,3,4,5,6, and 6′), 3.32 (s, 3 H, OMe), 3.1 (bs, 2 H, 2×OH).

Found: C, 67.59; H, 6.95%. Calcd for $C_{21}H_{26}O_6$: C, 67.36; H, 7.00%.

1,2,3,6-Tetra-*O*-acetyl-4-*O*-benzyl-α-D-mannopyranose (12). a) A suspension of compound **8** (20.0 g, 0.05 mol) in acetic anhydride (80 ml) was cooled and treated with a cold solution on 1.0% sulfuric acid in acetic anhydride (160 ml). The mixture was stirred till complete dissolution and then kept at room temperature for 2 h. The resulting solution was poured onto crushed ice and the mixture was extracted with dichloromethane. The usual processing of the extract gave an oil that was purified by preparative LC with ethyl acetate–hexane (1:3) to give the pure product (17.0 g, 73% yield); R_f=0.59 (solvent B).

(b) A cooled solution of compound **8** (3.0 g, 8.0 mmol) in pyridine (10 ml) and acetic anhydride (6 ml) was left overnight in the ice box, poured onto crushed ice, and extracted with dichloromethane. The usual work-up of the

extract gave **9** that was purified by LC with ethyl acetate: hexane (1:3) as a solvent to give a pure product (3.1 g. 84% yield); $[\alpha]_D^{27}$+46.2° (c 1.0, chloroform); ^{1}H NMR δ=7.2 (m, 10 H, 2×Ph), 5.35 (dd, 1 H, $J_{2,3}$=4, $J_{3,4}$=8 Hz, H-3), 5.27 (dd, 1 H, H-2), 4.78—4.42 (m, 5 H, 2×CH_2Ph and H-1), 4.1—3.6 (m, 4 H, H-4,5,6, and 6′), 3.36 (s, 3 H, OMe), 2.15 and 1.93 (each s, 6 H, 2×Ac). Acetolysis of compound **9** showed similar results as in (a).

(c) Compound **7** was partially hydrolyzed with 0.5 M hydrochloric acid and the resulting compound **11** (0.2 g, 0.44 mmol) was treated with 0.67% sulfuric acid in acetic anhydride (2.5 ml) and the mixture was processed as before to give **12** (0.15 g, 78% yield).

(d) A solution of compound **8** (14.0 g, 0.04 mmol) in dioxane (150 ml) and 1 M hydrochloric acid (60 ml) was heated on a steam bath for 20 h. TLC indicated that further heating did not cause a complete transformation. The reaction mixture was cooled, neutralized with sodium hydrogencarbonate, concentrated under vacuum, and the remaining solid was extracted with ethyl acetate. The usual work-up of the extract gave a syrup that was crystallized from dichloromethane–hexane to give **10** (8.5 g); mp 110—112 °C. The mother liquor was evaporated and subjected to preparative LC with dichloromethane–ethyl acetate (1:1) to give a further crop (1.3 g) of **10**, in addition to the starting material (2.5 g) which could be recycled (the total yield based on recovery of the starting material was 89%); $[\alpha]_D^{27}$+29.8° (*c* 0.7, chloroform); ^{1}H NMR δ=7.2 (m, 10 H, 2×Ph), 5.22 (d, 1 H, $J_{1,2}\leq$1 Hz, H-1), 4.88—4.35 (m, 4H, 2×CH_2Ph, H-1), 4.1—3.3 (m, 6 H, H-2,3,4,5,6, and 6′), 3.08, 2.74, and 1.94 (each bs, 3 H, 3×OH).

A mixed cooled solution of **10** (1.8 g, 5.0 mmol) in acetic anhydride (7.0 ml) and 1.0% sulfuric acid in acetic anhydride (14.0 ml) was kept at room temperature for 1.5 h, poured onto crushed ice, and the mixture was processed as before to give, after separation by preparative LC the product (1.9 g, 86% yield); $[\alpha]_D^{27}$+60.2° (*c* 2.8 chloroform); ^{1}H NMR δ=7.3 (m, 5 H, Ph), 6.04 (d, 1 H, $J_{1,2}$=2 Hz, H-1), 5.37 (dd, 1 H, $J_{2,3}$=3.5, $J_{3,4}$=8.0 Hz, H-3), 5.26 (dd, 1 H, H-2), 4.7 and 4.6 (ABq, 2 H, J=12 Hz, CH_2Ph), 4.3 (m, 2 H, H-6 and 6′), 3.9 (m, 2 H, H-4 and 5), 2.12, 2.08, 2.04, and 1.99 (each s, 12 H, 4×Ac).

Found: C, 57.29; H, 5.84%. Calcd for $C_{21}H_{26}O_{10}$: C, 57.52; H, 5.98%.

2,3,6-Tri-*O*-acetyl-4-*O*-benzyl-α-D-mannopyranosyl Bromide (13). A cold solution of **12** (10.0 g, 22.8 mmol) in the least volume of dichloroemthane was added to a saturated solution of hydrogen bromide in dichloromethane (100 ml) at room temperature. The resulting solution was kept for 2 h, whereby TLC showed the disappearance of the starting material. Nitrogen was passed into the solution, then the usual processing gave a syrup that purified immediately by preparative LC with ethyl acetate–hexane (1:2) to give a pure product (7.1 g, 68% yield); R_f=0.63 (solvent B); ^{1}H NMR δ=7.3 (m, 5 H, Ph), 6.28 (d, 1 H, $J_{1,2}$=2 Hz, H-1), 5.71 (dd, 1 H, $J_{2,3}$=3.5, $J_{3,4}$=9 Hz, H-3), 5.45 (dd, 1 H, H-2), 4.70, and 4.64 (ABq, 2 H, J=12 Hz, CH_2Ph), 4.34 (m, 2 H, H-6 and 6′), 4.1 (m, 1 H, H-5), 3.95 (dd, $J_{4,5}$=9 Hz, H-4), 2.14, 2.07, and 1.99 (each s, 9 H, 3×Ac).

3,6-Di-*O*-acetyl-4-*O*-benzyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose (14). A mixed solution of compound **13** (5.0 g, 10.9 mmol) in chloroform (15 ml), 2,6-lutidine (3 ml) and methanol (20 ml) was left at room temperature overnight, diluted with chloroform and water. The usual processing of the organic layer gave a crystallized product, that was recrystallized from dichloromethane–hexane to give colorless needles, (3.7 g, 83% yield); mp 150—151 °C; R_f=0.44 (solvent B); $[\alpha]_D^{23}$−4.3° (*c* 1.7, chloroform); ^{1}H NMR δ=7.3 (m, 5 H, Ph), 5.43 (d, 1 H, $J_{1,2}$=2.5 Hz, H-1), 5.14 (dd, 1 H, $J_{2,3}$=4, $J_{3,4}$=9 Hz, H-3), 4.7—4.6 (q, 2 H, CH_2Ph), 4.6 (m, 1 H, H-2), 4.36, and 4.21 (each q, $J_{5,6}$=3.0, $J_{5,6'}$=4.5, $J_{6,6'}$=12 Hz, H-6 and 6′), 3.89 (t, 1 H, H-4), 3.55 (m, 1 H, H-5), 3.27 (s, 3 H, OMe), 2.11, and 2.05 (each s, 6 H, 2×Ac), 1,72 (s, 3 H, CMe).

Found: C, 58.13; H, 6.29%. Calcd for $C_{20}H_{26}O_9$: C 58.53; H, 6.39%.

3,6-Di-*O*-allyl-4-*O*-benzyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose (15). A hot solution of **14** (1.6 g, 2.4 mmol) and allyl bromide (6 ml) in benzene (30 ml) was treated with powdered potassium hydroxide (1.0 g), and the mixture was heated with stirring under reflux for 2 h, and then additional amount of powdered potassium hydroxide (1.0 g) was added and heating was continued for more 2 h. After cooling, the mixture was filtered. The usual processing of the filtrate gave a syrup composed from two diastereoisomers in a ratio 12:1, that separated on LC with 1:4 ethyl acetate–hexane as a solvent. The fast moving isomer was the minor product (0.1 g, 6% yield) R_f=0.55 (solvent B); $[\alpha]_D^{23}$ +13.1° (*c* 0.3, chloroform); ^{1}H NMR δ=7.3 (m, 5 H, Ph), 5.9 (m, 2 H, 2×CH in allyl), 5.2 (m, 4 H, 2×CH_2 in allyl), 5.16 (d, 1 H, $J_{1,2}$=3 Hz, H-1), 4.9, and 4.65 (ABq, 2 H, J=11 Hz, CH_2Ph), 4.3 (m, 2 H, H-2), 4.0 (m, 4 H, 2×OCH_2 in allyl), 3.7 and 3.5 (each m, 5 H, H-3,4,5,6, and 6′), 3.48 (s, 3 H, OMe), 1.53 (s, 3 H, CMe).

Found: C, 64.67; H, 7.58%. Calcd for $C_{22}H_{30}O_7$: C, 65.01; H, 7.44%.

The slow moving isomer was the major product that was crystallized from hexane (1.3 g, 82% yield); R_f=0.53 (solvent B); mp 43—44 °C; $[\alpha]_D^{23}$+25.8° (*c* 0.2, chloroform); ^{1}H NMR δ=7.3 (m, 5 H, Ph), 5.9 (m, 2 H, 2×CH of allyl), 5.34 (d, 1 H, $J_{1,2}$=3 Hz, H-1), 5.2 (m, 4 H, 2×CH_2 in allyl), 4.88, and 4.63 (ABq, 2 H, J=11 Hz, CH_2Ph), 4.48 (t, 1 H, H-2), 4.22 and 4.01 (each bd, 4 H, 2×OCH_2 in allyl), 3.65, and 3.45 (2m, 5 H, H-3,4,5,6, and 6′), 3.3 (s, 3 H, OMe), 1.72 (s, 3 H, CMe).

Found: C, 64.77; H, 7.88%. Calcd for $C_{22}H_{30}O_7$: C, 65.01: H, 7.44%.

3,6-Di-*O*-allyl-4-*O*-benzyl-D-mannopyranose (16). A solution of **15** (1.0 g, 2.46 mmol) in a mixture of glacial acetic acid (12 ml) and water (8 ml) was heated for 1 h on a steam bath and then concentrated in vacuo. The resulting syrup was dissolved in dichloromethane which was washed with aqueous sodium hydrogencarbonate, dried, and evaporated. The resulting syrup in a solution of methanol was deacetylated by catalytic amount of sodium methoxide for overnight at 5 °C, neutralized with acetic acid and evaporated. The residue was purified by LC with 1:1 ethyl acetate–hexane to give a pure syrup (0.74 g, 85%); $[\alpha]_D^{23}$+39.6° (*c* 1.3, chloroform); ^{1}H NMR δ=7.3 (m, 5 H, Ph), 5.9 (m, 2 H, 2×CH in allyl), 5.2 (m, 4 H, 2×CH_2 in allyl and H-1), 4.87 and 4.57 (ABq, 2 H, J=12 Hz, CH_2Ph), 4.68 (s, <1 H, H-1), 4.2—3.3 (m, 10 H, H-2,3,4,5,6,6′, and 2×OCH_2 in allyl).

Found: C, 64.51; H, 7.76%. Calcd for $C_{19}H_{26}O_6$: C, 65.12;

H, 7.48%.

3,6-Di-*O*-allyl-4-*O*-benzyl-2-*O*-mesyl-α-D-mannopyranosyl Chloride (17). To an ice-cooled solution of **16** (2.0 g, 5.7 mmol) in a mixture of dichloromethane (30 ml) and 2,6-lutidine (10 ml) was added dropwise with stirring mesyl chloride (2 ml), and the mixture was kept at 0 °C for 0.5 h and then at room temperature till TLC showed complete conversion (ca. 3 h). The usual processing of the reaction mixture gave a syrup which was purified by LC with 1:2 ethyl acetate–hexane (1.7 g, 68% yield); $[\alpha]_D^{27}$+75.3° (*c* 0.9, chloroform).

Found: Cl, 7.56%. Calcd for $C_{20}H_{20}ClO_7S$: Cl, 7.93%.

2-[4-(*p*-Toluenesulfonamide)phenyl]ethyl 3,6-Di-*O*-allyl-4-*O*-benzyl-2-*O*-mesyl-β- and α-D-Mannopyranoside (19 and 20). A solution of **17** (0.20 g, 0.45 mmol) in acetonitrile (2 ml) was treated with a solution of silver 2,2,2-trifluoroethanesulfonate in acetonitrile to form the corresponding 1-trifluoroethanesulfonate (**18**), whereby silver chloride precipitated out. After 1 h, the solution was filtered on 2-[4-(*p*-toluenesulfonamide)phenyl]ethanol (0.15 g, 0.51 mmol). All operations were performed on a high vacuum rack as described before. The reaction mixture was kept in the dark overnight, diluted with dichloromethane, washed with a solution of sodium hydrogencarbonate, sodium thiosulfate and water, and dried ($MgSO_4$). The usual processing of the extract and purification of the product with LC with 1:1 ethyl acetate–hexane as a solvent gave the major product **19** (0.19 g, 60% yield), in addition to a minor product 20 (0.08 g, 25% yield).

20: R_f=0.38 (solvent A); $[\alpha]_D^{23}$+22.1° (*c* 0.5, chloroform); ^{1}H NMR δ=7.65 (d, 2 H, *p*-substituted Ph), 7.2 (m, 12 H, aromatic and NH), 5.9 (m, 2 H, 2×CH in allyl), 5.2 (m, 4 H, 2×CH_2 in allyl), 4.8 (m, 2 H, H-1 and 2), 4.85 and 4.55 (ABq, 2 H, *J*=12 Hz, CH_2Ph), 4.1 (m, 5 H, H-3 and 2×OCH_2 in allyl), 3.6 (m, 6 H, H-4,5,6,6′ and OCH_2 in ethyl), 3.10 (s, 3 H, Ms), 2.8 (q, 2 H, CH_2 in ethyl), 2.36 (s, 3 H, Me).

Found: C, 57.94; H, 6.13%. Calcd for $C_{35}H_{43}NO_{10}S_2 \cdot H_2O$: C, 58.39; H, 6.02%.

19: R_f=0.33 (solvent A); $[\alpha]_D^{27}$−22.3° (*c* 0.3, chloroform); ^{1}H NMR δ=7.68 (d, 2 H, *p*-substituted Ph), 7.2 (m, 12 H, aromatic and NH), 5.9 (m, 2 H, 2×CH in allyl), 5.2 (m, 4 H, 2×CH_2 in allyl), 4.95 (m, 1 H, H-2), 4.9 and 4.55 (ABq, 2 H, *J*=11 Hz, CH_2Ph), 4.45 (s, 1 H, H-1), 4.1 (m, 5 H, H-3 and 2×OCH_2 in allyl), 3.6 (m, 6 H, H-4,5,6,6′ and OCH_2 in ethyl), 2.82 (bq, 2 H, CH_2 in ethyl), 2.80 (s, 3 H, Ms), 2.35 (s, 3 H, Me).

Found: C, 60.46; H, 6.00%. Calcd for $C_{35}H_{43}NO_{10}S_2$: C, 59.89; H, 6.18%.

2-[4-(*p*-Toluenesulfonamido)phenyl]ethyl 4-*O*-Benzyl-2-*O*-mesyl-β-D-mannopyranoside (22). The mixture of **19** (0.7 g, 1.0 mmol) and tris(triphenylphosphine)rhodium-(I) chloride (0.13 g) in 9:1 (v/v) ethanol–water (20 ml) was heated under reflux till TLC showed complete disappearance of **19** (2 h) and then evaporated to a syrup. The syrup was extracted with dichloromethane, and the usual processing of the extract gave a mixture of 1-propenyl and depropenylated derivatives which was heated with mercury(II) oxide (0.9 g) and mercury(II) chloride (0.9 g) in 9:1 (v/v) acetone–water under reflux for 15 min, filtered, and the filtrate was evaporated. The remaining residue was extracted with dichloromethane and the organic phase was washed with aqueous potassium iodide and water, dried ($MgSO_4$), and evaporated to a syrup, which was purified by using HPLC with ethyl acetate–hexane (0.48 g, 77% yield); $[\alpha]_D^{23}$ −20.0° (*c* 0.1, chloroform); ^{1}H NMR δ=7.66 (d, 2 H, *p*-substituted Ph), 7.3 (m, 12 H, aromatic and NH), 4.8 (d, 1 H, H-2), 4.83 and 4.63 (ABq, 2 H, *J*=11 Hz, CH_2Ph), 4.48 (s, 1 H, H-1), 4.01 (m, 1 H, H-3), 3.7—3.3 (m, 6 H, H-4,5,6,6′ and OCH_2 in ethyl), 2.81 (t, 2 H, CH_2 in ethyl), 2.8 (s, 3 H, Ms), 2.4 (bs, 2 H, 2×OH), 2.34 (s, 3 H, Me).

Found: C, 55.12; H, 5.75%. Calcd for $C_{29}H_{35}NO_{10}S_2 \cdot 0.5H_2O$: C, 55.22; H, 5.75%.

2-[4-(*p*-Toluenesulfonamide)phenyl]ethyl 6-*O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-4-*O*-benzyl-2-*O*-mesyl-β-D-mannopyranoside (23). Under high vacuum, **22** (0.1 g, 0.16 mmol), 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride (1.5 equivalent) and silver tresylate were left in separate joined flask for 5 h at room temperature. Acetonitrile (2 ml) was distilled onto the chloride, and the resulting solution was mixed with silver tresylate. After 0.5 h, the solution of the tresylate was filtered over onto compound **22**. The resulting solution was kept for overnight at room temperature in the dark. The reaction mixture was diluted with dichloromethane, washed with saturated solution of sodium thiosulfate and sodium hydrogencarbonate, water and dried ($MgSO_4$). The resulting syrup after evaporation of the solvent, was separated on LC with ethyl acetate–hexane (2:3) to give the disaccharide as a syrup (0.12 g, 64% yield); $[\alpha]_D^{23}$ −4.3° (*c* 0.3, chloroform).

Found: C. 64.31; H, 5.75%. Calcd for $C_{63}H_{67}NO_{16}S_2 \cdot H_2O$: C, 64.32; H, 5.91%.

This work supported by Grant No. 5R01AI 12509 from the National Institute of Allergy and Infections Diseases, National of Health, U.S. Public Health Service. We thank Mr. R. Dushin for his skillfull techniques and efforts in preparing the starting material on large scale. We also thank Prof. J. Yoshimura and Dr. R. Eby for their helpfull discussions.

References

1) J. Montreuil, *Adv. Carbohydr. Chem. Biochem.*, **37**, 157 (1980).

2) R. Cornfeld and S. Kornfeld, *Annual Rev. Biochem.*, **45**, 217 (1976).

3) F. M. Winnik, J. P. Carver, and J. J. Krepinsky, Personnal communication.

4) T. Ogawa, K. Katano, K. Sasajima, and M. Matsui, *Tetrahedron*, **37**, 2779 (1981), and references cited in.

5) S. Ito, D. Yamashita, R. G. Spiro, and A. Kobata, *J. Biochem. (Tokyo)*, **81**, 1621 (1977).

6) J. Baenziger, S. Kornfeld, and S. Kochwa, *J. Biol. Chem.*, **249**, 1897 (1974).

7) J. Baenziger and S. Kornfeld, *J. Biol. Chem.*, **249**, 7260 (1974).

8) T. Tai, K. Yamashita, S. Ito, and A. Kobata, *J. Biol. Chem.*, **252**, 6687 (1977).

9) K. Yamashita, Y. Tachibana, and A. Kobata, *J. Biol. Chem.*, **253**, 3862 (1978).

10) J. Montreuil, *Pure Appl. Chem.*, **42**, 431 (1975).

11) D. Burke, L. Mendonca-previato, and C. E. Ballou,

Proc. Natl. Acad. Sci. U. S. A., **77**, 318 (1980).
12) R. C. Hughes and N. Sharon, *Nature*, **274**, 637 (1978).
13) W. F. Goebels and O. T. Avery, *J. Exp. Med.*, **50**, 521, 533 (1929).
14) W. F. Goebels, F. H. Babers, and O. T. Avery, *J. Exp. Med.*, **55**, 761 (1932).
15) W. F. Goebels, *J. Exp. Med.*, **64**, 29 (1936).
16) R. Eby and C. Schuerch, *Carbohydr. Res.*, **77**, 61 (1979).
17) R. U. Lemieux, K. B. Hendricks, R. V. Stock, and K. James, *J. Am. Chem. Soc.*, **97**, 4056 (1975).
18) S. Hannesian and J. Banoub, *Carbohydr. Res.*, **53**, c13 (1977).
19) R. Eby and C. Schuerch, *Carbohydr. Res.*, **34**, 79 (1974).
20) E. S. Rachaman, R. Eby, and C. Schuerch, *Carbohydr. Res.*, **67**, 147 (1978).
21) V. K. Srivastave and C. Schuerch, *J. Org. Chem.*, **46**, 1121 (1981).
22) E. S. H. El Ashry and C. Schuerch, *Carbohydr. Res.*, **105**, 33 (1982).
23) L. Awad, E. S. H. El Ashry, and C. Schuerch, *Carbohydr. Res.*, **122**, 69 (1983).
24) K. Igarashi, *Adv. Carbohydr. Chem. Biochem.*, **34**, 243 (1977).
25) G. Wulff and G. Röhle, *Angew. Chem., Int. Ed. Engl.*, **13**, 157 (1974).
26) C. Schuerch, "Anomeric Effect, Origin and Consequences," ed by W. A. Szarek and D. Horton, Am. Chem. Soc., Symp. Ser., **87**, 80 (1979).
27) J. R. Pougny and P. Sinay, *Tetrahedron Lett.*, **1976**, 4073.
28) S. Perlin, *Pure Appl. Chem.*, **50**, 1401 (1978).
29) R. J. Garegg and T. Iversen, *Carbohydr. Res.*, **70**, c13 (1979).
30) R. J. Garegg, T. Iversen, and R. Johansson, *Acta Chem. Scand., Ser. B.*, **34**, 505 (1980).
31) J. S. Brimacombe, *Specialist Periodical Repts. Chem. Soc. Carbohydrates*, **11**, 25 (1979).
32) O. Theander, *Acta Chem. Scand.*, **12**, 1883 (1958).
33) O. Theander, *Adv. Carbohydr. Chem.*, **17**, 223 (1962).
34) G. Ekborg, B. Lindberg, and J. Lönngren, *Acta Chem. Scand.*, **26**, 3287 (1972).
35) E. E. Lee, G. Keaveney, and P. S. O'Colla, *Carbohydr. Res.*, **59**, 268 (1977).
36) H. Paulsen and O. Lockhoff, *Chem. Ber.*, **114**, 3102 (1981).
37) M. E., Evans and F. W. Parrish, *Carbohydr. Res.*, **54**, 105 (1977).
38) R. Eby and C. Schuerch, *Carbohydr. Res.*, **100**, c41 (1982).
39) A. S. Perlin, *Can. J. Chem.*, **41**, 399 (1963).
40) R. Kasai, M. Okihara, J. Asakawa, K. Mizutani, and G. Tanaka, *Tetrahedron*, **35**, 1427 (1979).