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A SYNTHESIS OF 3-*O*-(α-D-MANNOPYRANOSYL)-D-MANNOSE AND ITS PROTEIN CONJUGATE

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ABSTRACT

Methods for the synthesis of 3-*O*-(α-D-mannopyranosyl)-D-mannose and 2-(4-aminophenyl)ethyl 3-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside have been investigated by a number of sequences. Glycosidations with 2,3-di-*O*-acetyl-4,6-di-*O*-benzyl-D-mannopyranosyl and 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-D-mannopyranosyl *p*-toluenesulfonates were found to give better yields than the Helferich modification, the use of a peracylated D-mannopyranosyl halide, or the use of triflyl leaving group. Only the α anomer was obtained. Factors influencing glycosidation reactions are discussed. A mercury(II) complex was used for selective 2-*O*-acylation of 4,6-di-*O*-benzyl-α-D-mannopyranosides. A disaccharide–protein conjugate was prepared by the isothiocyanate method.

INTRODUCTION

Studies by Haworth *et al.*[1] and many others[2-5] have established that the structure of bakers' yeast D-mannan consists of an α-(1→6)-linked backbone with (1→2)- and (1→3)-linked side-chains. The mannans were shown to be linked to protein by direct attachment to L-serine and L-threonine residues and indirectly by a β-linkage to O-4′ of a di-*N*-acetylchitobiose residue[6,7] linked in turn to asparagine, forming a mannan–protein conjugate. This conjugate is one of three main components of the cell wall of yeast[2].

Immunochemical studies of yeast cell-wall showed that the antigenic properties of yeast are largely determined by the mannan side-chains[2]. As Suzuki, Sunayama, and Saito have shown that 3-*O*-(α-D-mannopyranosyl)-D-mannose is a good inhibitor for rabbit antisera[8]. Ballou and Raschke[2] have suggested that this α-(1→3) linkage is the immunodominant structure in baker's yeast mannan.

Because of the importance of this linkage in biological studies, and because of the limited data in the literature concerning 3-*O*-(α-D-mannopyranosyl)--D-man-

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nose, we have concentrated on its synthesis and the synthesis of its α-linked methyl glycoside. Moreover, we have synthesized a protein conjugate of 2-(4-aminophenyl)ethyl 3-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside for use as an artificial antigen. The synthesis of a similar antigen of 2-*O*-(α-D-mannopyranosyl)-α-D-mannopyranose and its use in immunological investigations has been reported[9,10].

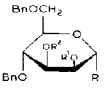
The synthetic methods tested in this research reflect the observation made over a period of years that stereoselectivity and yield in glycosidation reactions may be optimized if the reactivity of C-1 is carefully balanced by an appropriate choice of leaving group at C-1 and alcoholic substituents. If the reaction is performed in homogeneous solution, a series of sulfonate leaving-groups, *p*-toluenesulfonate, trifluoroethylsulfonyl (tresyl), and triflyl provide a wide range of reactivities. The better leaving-groups are more appropriate when there are a number of ester substituents on the alcoholic oxygen atoms, whereas the tosyl group is more useful with etherified glycosyl derivatives that are intrinsically more reactive. As ester groups are more susceptible to side reactions than ether functions, partial etherification is often advantageous. Steric control in these reactions is, of course provided by a 2-*O*-acyl group, and the (1,2-*trans*) α-glycoside is usually formed almost exclusively by neighboring-group participation. Many of these relationships are confirmed in the following experiments.

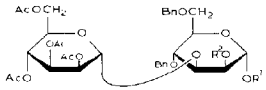
RESULTS AND DISCUSSION

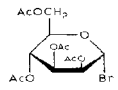
4,6-Di-*O*-benzyl-D-mannopyranose[11] (**1**) was used as starting material in the synthesis of both benzyl and 2-(4-nitrophenyl)ethyl α-D-mannopyranoside derivatives. Benzylation of **1** with benzyl alcohol and dry hydrogen chloride gave benzyl 4,6-di-*O*-benzyl-α-D-mannopyranoside (**2**) in 80% yield, whereas acetylation of **1** with acetic anhydride in pyridine afforded 1,2,3-tri-*O*-acetyl-4,6-di-*O*-benzyl-D-mannopyranose (**11**) as 55% of the α and 30% of the β anomer. Chlorination of **11** in a solution of ether saturated with hydrogen chloride gave 2,3-di-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl chloride in 86% yield. The structures of both of these compounds were confirmed by both ^{1}H- and ^{13}C-n.m.r. (Table I and Experimental section).

Garegg[14] has used phase-transfer catalysis for the selective monobenzylation of diols. Under the same conditions, we attempted to monobenzylate the diol 2-(4-nitrophenyl)ethyl 4,6-di-*O*-benzyl-α-D-mannopyranoside (**14**) selectively, but a mixture of decomposition products was obtained. The decomposition was probably caused by the effect of alkali on the nitro group.

Ayela and Melander[15] have used copper(II) and mercury(II) chelates for selective acylation. Acetylation of **14** was attempted by treatment of the disodium salt of **14** with mercury(II) chloride followed by acetic anhydride. The reaction resulted in rapid conversion of 59% of **14** to give 2-(4-nitrophenyl)ethyl 2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (**15**) in 79% yield and the 3-*O*-acetyl de-







1 R = OH, R^1 = R^2 = H
2 R = OBn, R^1 = R^2 = H
3 R = OBn, R^1 = Ac, R^2 = H
9 R = OMe, R^1 = Bn, R^2 = OH
11 R = OAc, R^1 = R^2 = OAc
12 R = Cl, R^1 = R^2 = OAc

13 R = OH_2CH_2C–C6H4–NO_2, R^1 = R^2 = OAc

14 R = OH_2CH_2C C6H4 NO_2, R^1 = R^2 = OH

15 R = OH_2CH_2C–C6H4–NO_2, R^1 = OAc, R^2 = OH

20 R = Cl, R^1 = Bz, R^2 = Bn

4 R^1 = Bn, R^2 = Ac
5 R^1 = Me, R^2 = Bn

8

rivative in 20% yield. The structure of **15** was confirmed by ^{1}H- and ^{13}C-n.m.r. spectroscopy (Table I and Experimental). Benzyl 2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (**3**) was similarly obtained in 82% yield, and the 3-*O*-acetyl derivative in 18% yield, from conversion of 70% of the starting compound **2**.

Tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**8**) has traditionally been used for the synthesis of 1,2-*trans* glycosides. Herein we tried to use it for the synthesis of α-(1→3)-linked mannose disaccharides by conventional methods. The condensation product of **8** and silver triflate (prepared previously in the presence of tetramethylurea in dichloromethane) was coupled with methyl 2,4,6-tri-*O*-benzyl-α-D-mannopyranoside[16] (**9**) in a high-vacuum rack to give methyl 2,4,6-tri-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**10**) in only 36% yield (based on starting material) because of a low degree of conversion. Under the same conditions, **15** gave decomposition products when it was treated with the condensation product of **8** and silver triflate. In a related coupling, the reaction even with a primary alcohol gave a low yield; when 2,3-di-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl chloride (**11**) was condensed with silver triflate under the previous conditions and the product then coupled with 2-(4-nitrophenyl)ethanol, only 33% of 2-(4-nitrophenyl)ethyl 2,3-di-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (**13**) was obtained. The same compound was synthesized under the conditions of Hanessian and Banoub[17] but, in contrast to their results, a still-lower yield (20%) was obtained. These low yields were probably due to the sensitivity of the acyl substituents to the glycosidation conditions, because deacetylated products were separated with **13**, as shown from ^{1}H-n.m.r. studies. Similarly, when the disaccharide **10** was synthesized according to Krepinsky *et al.*[18] by the Helferich modification of the Koenigs–Knorr reaction, only a 22% yield was obtained. When silver tresylate, instead of silver triflate, was condensed with **8** in acetonitrile and the 2,3,4,6-tetra-*O*-acetyl-1-*O*-tresyl-D-mannopyranose (not iso-

TABLE I

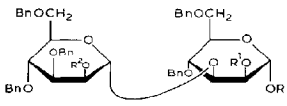
TENTATIVE ^{13}C-N.M.R. CHEMICAL SHIFTS OF MONOSACCHARIDES[a]

Resonance	*Compound*						
	2	**3**	**11**	**12**	**13**	**14**	**15**
C-1	99	97.3	91.1	90.8	97.6	99.6	97.5
C-2	71.2	71.5[b]	71.6	69.0[b]	71.8	71.1[b]	71.5
C-3	71.2	70.6	69.1[b]	72.6	70.2	71.3	70.7
C-4	76.1	76.1	74.0	74.9	73.1	75.9	76.0
C-5	72.0	72.8	72.4	76.1	71.8	71.9	72.6
C-6	69.1	69.0	68.5	68.5	68.8	69.1	68.9
Bn-CH_2-O-1	69.3	69.5	73.8	73.7	73.7	74.7	74.9
Bn-CH_2	74.8	75.0	75.0	73.5	74.8	73.7	73.7
	73.7	73.4					
Ac		21.0	20.7	20.7	20.8		21
C=O		171.0	168.6	168.7	170.0		171
			170.0	169.9	170.3		
			170.1	170.4			
p-$O_2NC_6H_4CH_2$-CH_2O					35.9	35.9	35.8
p-$O_2NC_6H_4CH_2$-CH_2O					67.7	67.4	67.5

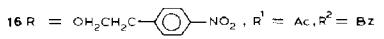
[a]These assignments are based on the ^{13}C-n.m.r. spectra of benzyl α-D-mannopyranoside[17], and the work of Rachaman, Eby, and Schuerch[13]. [b,c]These assignments may be interchanged in each vertical column. In general, however, peak heights of ring carbon atoms are somewhat higher than benzyl methylene carbon atoms, and are so selected here.

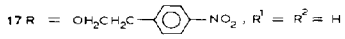
lated) was condensed directly with **3**, only 50% of benzyl 2-*O*-acetyl-4,6-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**4**) was obtained. Only one isomer was separated, as shown from ^{13}C-n.m.r. spectroscopy.

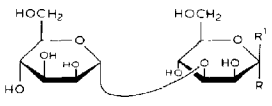
In addition to these highly electronegative leaving-groups, we also used *p*-toluenesulfonate, which is less electronegative. In this case, compound **13** was obtained in 82% yield when the condensation product of **12** and silver *p*-toluenesulfonate was coupled with 2-(4-nitrophenyl)ethanol on a high-vacuum rack. Only the α anomer was obtained, as established by ^{13}C- and ^{1}H-n.m.r. spectra, and the optical rotation (Table I and Experimental). 2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride[19] (**20**) was also condensed with silver *p*-toluenesulfonate in acetonitrile on a high-vacuum rack. The *p*-tolylsulfonyl derivative formed was not separated, but was coupled immediately with **3** or **15** under vacuum at room temperature to give the disaccharide **5** or **16** in 87 and 70% yields, respectively. Only the α anomer was separated as shown from ^{13}C- and ^{1}H-n.m.r. spectra, and the optical rotations (Experimental). These results are similar to those of Shaban and Jeanloz[20], who indicated that a D-glucopyranosyl halide having an acyl group at O-2 and ether groups at O-3, O-4, and O-6 gave higher stereoselectivity in the synthesis of 1,2-*trans*-glycosides and a higher reaction-rate than the corresponding peracylated derivatives. Transesterification of both **5** and **16** with sodium methoxide in methanol afforded the diols **6** and **17**.



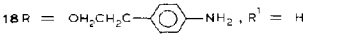
5 R = Bn, R^1 = Ac, R^2 = Bz
6 R = Bn, R^1 = R^2 = H

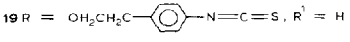






7 R, R^1 = OH, H





Catalytic reduction of the nitro group and removal of the benzyl groups from **17** was accomplished with palladium-on-carbon in aqueous ethanol. The deprotected disaccharide, 2-(4-aminophenyl)ethyl 3-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (**18**), was immediately treated with an excess of thiophosgene[21] to give the thioisocyanate derivative **19**, which was then coupled to bovine serum albumin (BSA) at pH 9 without isolation[21]. The carbohydrate content of the synthetic protein conjugate was determined by the quantitative anthrone method[22], with mannose as the standard. The product was found to be rich enough in carbohydrate to use as an artificial antigen, as it contained 17.4 mol of disaccharide per mol of protein.

Few data on 3-*O*-(α-D-mannopyranosyl)-D-mannose (**7**) have been reported. It was first obtained by acid-catalyzed reversion of mannose[23]. It was also obtained by partial hydrolysis of mannan[24]. Jeanes and co-workers[25] isolated this disaccharide from phosphomannan Y-2648. Recently, Ponpipom[26] reported a total synthesis. In our present work, disaccharide **7** was obtained in quantitative yield by catalytic hydrogenation of **6**. The ^{13}C-n.m.r. spectrum proved the presence of both α and β anomers. In the anomeric region, three signals were assigned at δ 102.79, 94.52, and 94.03 to C-1′, C-1α, and C-1β[27,28], respectively. Because of the interglycosidic linkage, the C-3α and C-3β signals were shifted downfield to δ 78.41 and 80.86, whereas removal of the benzyl group from C-3′ caused an upfield shift to δ 70.5. Overlap of the C-2α and C-2′ signals was observed, a two-carbon signal was assigned at δ 70.85, whereas C-2β resonated at δ 71.39. Signals at δ 67.28, 66.99, and 66.51 were assigned to C-4′, C-4′, and C-4β respectively, whereas C-5β, C-5′, and C-5α resonated downfield δ 76.44, 73.77, and 73.06, respectively. A 2-carbon signal at δ 61.42 was assigned to C-6 and C-6′.

EXPERIMENTAL

General methods. — ^{1}H- and ^{13}C-n.m.r. spectra, optical rotations, melting points, t.l.c., high-pressure liquid chromatography (l.c.), and microanalysis were performed as described previously[29]. Acetonitrile, oxolane, and dichloromethane were dried over calcium hydride. *p*-Nitrophenethyl alcohol and silver tri-

fluoromethanesulfonate were purchased from Aldrich Chemical Company, Inc. and silver *p*-toluenesulfonate from Eastman Organic Chemicals (Rochester, NY 14650). Silver 2,2,2-trifluoroethanesulfonate was prepared from 2,2,2-trifluoroethanesulfonyl chloride by hydrolysis and reaction with silver carbonate. Compounds **4**, **5**, **9**, and **13** were prepared in an inert atmosphere with the aid of a glass manifold that was connected *via* a stopcock to a vacuum line and *via* three ground-glass joints and a sintered-glass filter to three 10–20 mL flasks. The flask containing the alcohol undergoing glycosidation was attached to one outlet separated from the others by the glass filter. Solutions were dried with magnesium sulfate.

*Benzyl 4,6-di-*O*-benzyl-α-*D-mannopyranoside (**2**). — Dry hydrogen chloride was bubbled into a solution of 4,6-di-*O*-benzyl-D-mannopyranose[11] (**1**, 2 g) in dry benzyl alcohol (20 mL). The mixture was heated for 3–4 h with stirring. The excess of benzyl alcohol was removed by steam distillation. Compound **2** was extracted with dichloromethane from water and the extract was washed with sodium hydrogencarbonate and water, and dried. The syrup obtained crystallized from dichloromethane–hexane to give **2** (2 g, 80%); m.p. 90–92°, $[\alpha]_D^{22}$ +67.5° (*c* 1.22, chloroform); ^{1}H-n.m.r.: δ 7.26–7.38 (m, 15 H, aromatic), 4.94 (d, 1 H, H-1), 3.7–4 (m, 6 H, H-2,3,4,5,6,6′), and 2.2–2.3 (bs, 2 H, 2 OH).

Anal. Calc. for $C_{27}H_{30}O_6$: C, 71.97; H, 6.71. Found: C, 71.64; H, 6.71.

*Benzyl 2-*O*-acetyl-4,6-di-*O*-benzyl-α-*D-*mannopyranoside* (**3**). — To a well-stirred solution of **2** (0.3 g, 0.66 mmol) in dry oxolane (THF, 15 mL), sodium hydride was added (53 mg, 60% in mineral oil, 2.2 mmol) under anhydrous conditions. After evolution of hydrogen gas had ceased, anhydrous mercury(II) chloride (0.18 g, 0.66 mmol) was added. The mixture was stirred for 15 min and acetic anhydride (0.136 g, 1.3 mmol) was added. The mixture was stirred for 30 min, and then diluted with water and evaporated. The residue was extracted with dichloromethane, the extract washed with water, and dried. The resulting syrup was then separated by l.c. using 1:1 ethyl acetate–hexane. Starting material **2** (0.09 g, 30%) was recovered, and **3** was obtained in 82% yield (0.188 g) and the 3-*O*-acetyl derivative in 18% yield (0.041 g). Compound **3** crystallized from ether–hexane; m.p. 79°, $[\alpha]_D^{25}$ +54.7° (*c* 1, chloroform); ^{1}H-n.m.r. δ 7.22–7.39 (m, 15 H, aromatic), 5.12–5.18 (dd, 1 H, $J_{1,2}$ 2, $J_{2,3}$ 4 Hz, H-2), 4.96 (d, 1 H, H-1), 4.1–4.26 (bm, 1 H, H-3), 2.24–2.34 (bs, 1 H, OH), and 2.12 (s, 3 H, Ac).

Anal. Calc. for $C_{29}H_{32}O_7$: C, 70.71; H, 6.54. Found: C, 70.88; H, 6.11.

*Benzyl 2-*O*-acetyl-3-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-*D-*mannopyranosyl)-4,6-di-*O*-benzyl-α-*D-*mannopyranoside* (**5**). — Under high vacuum, compound **3** (0.17 g, 0.34 mmol), 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride (**20**, 0.237 g, 0.41 mmol) and silver *p*-toluenesulfonate (0.115 g, 0.41 mmol) were placed in separate, joined flasks for 3 h at room temperature. Acetonitrile (2 mL) was distilled onto **20**, and the resulting solution was mixed with silver *p*-toluenesulfonate. The solution of the sulfonate derivative was filtered over onto compound **3**. The homogeneous solution was kept for 40 h at room tempera-

ture in the dark. The mixture was diluted with dichloromethane, filtered, washed successively with saturated sodium thiosulfate, sodium hydrogencarbonate, and water, dried, and evaporated. The resulting syrup was separated by l.c. with 1:2 ethyl acetate–hexane to give the disaccharide **5** as a syrup (yield 0.30 g, 87%), $[\alpha]_D^{24}$ +22.2° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 8–8.1 (dd, 2 H, H-2,6 of aryl), 7.2–7.4 (m, 33 H, aromatic), 5.66–5.74 (t, 1 H, H-2′), 5.24–5.32 (d, 2 H, H-1′ and H-2), 4.96 (d, 1 H, H-1), and 2.08 (s, 3 H, Ac); ^{13}C-n.m.r.: δ 100.2 (C-1′), 96.8 (C-1), 78.1 (C-3,3′), 74.3, 74.8 (C-4,4′), 72.8 (C-5,5′), 71.6, 71.9 (C-2,2′), 68.9 (C-6,6′), 75.1, 73.6, 71.4 (Bn-CH_2), 69.3 (Bn-CH_2-O-1), 21 (Ac), 170.5, and 165.7 (C=O).

Anal. Calc. for $C_{63}H_{64}O_{13}$: C, 73.52; H, 6.26. Found*: C, 72.92; H, 6.53.

*Benzyl 4,6-di-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-α-*D*-mannopyranoside* (**6**). — A solution of **5** (30 mg) in methanol (10 mL) and THF (3 mL) was treated with a catalytic amount of sodium methoxide at room temperature for 48 h with stirring. The mixture was made neutral with acetic acid, evaporated, and extracted with dichloromethane. The extract was washed with sodium hydrogencarbonate and water, dried, and evaporated to a syrup that was separated by l.c. with 1:2 ethyl acetate–hexane. The diol **6**; in yield 24 mg (96%) was obtained as a syrup, $[\alpha]_D^{21}$ +41.3° (*c* 1.82, chloroform); ^{1}H-n.m.r.: δ 7.15–7.3 (m, 30 H, aromatic), 5.03–5.1 (d, 1 H, H-1′), 3.4–4.4 (m, 12 H, H-2,2′,3,3′,4,4′, H-5,5′,6,6′,6,6′) the signal H-1 overlapped those of the benzyl methylene protons; ^{13}C-n.m.r.: δ 100.6 (C-1′), 99.7 (C-1), 80.4, 82.9 (C-3,3′), 71.4 (C-2,2′), 69.5 (C-6,6′), and 69.2 (Bn-CH_2-O-1).

Anal. Calc. for $C_{54}H_{58}O_{11}$: C, 73.44; H, 6.62. Found: C, 72.64; H, 7.30.

*3-*O*-(α-*D*-Mannopyranosyl)-*D*-mannose* (**7**). — To a solution of **6** (0.09 g) in methanol (20 mL) and water (5 mL), was added palladium-on-carbon (0.04 g, 5%). The mixture was hydrogenated for 48 h with stirring under diminished pressure. It was filtered and freeze-dried to give a glassy solid in quantitative yield; $[\alpha]_D^{27}$ +48.4° (*c* 0.72, water) after 20 h in solution at room temperature, lit.[23–26] $[\alpha]_D^{25}$ +57.6° (*c* 0.434, water), $[\alpha]_D^{20}$ +46° (*c* 1.18, water), $[\alpha]_D^{20}$ +50° (*c* 0.86 water), and $[\alpha]_D^{27}$ +40° (*c* 1.55, methanol).

Anal. Calc. for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.47. Found: C, 42.55; H, 6.58.

*1,2,3-Tri-*O*-acetyl-4,6-di-*O*-benzyl-*D*-mannopyranose* (**11**). — A cold solution of **1** (0.5 g, 1.38 mmol) in pyridine (2 mL) was treated with acetic anhydride (0.7 g, 0.64 mL, 6.86 mmol). The mixture was kept overnight at room temperature with stirring. It was poured onto crushed ice, extracted with dichloromethane, washed with dilute hydrochloric acid followed by sodium hydrogencarbonate and water, dried, and separated by l.c. with 1:2 ethyl acetate–hexane. The α anomer of **11** was obtained as a syrup (0.371 g, 55%)$[\alpha]_D^{25}$ +61.3° (*c* 0.75, chloroform), whereas the β anomer was obtained in 30% yield (0.202 g), m.p. 97–99° from ether–hexane, $[\alpha]_D^{25}$ −6.5° (*c* 1.1, chloroform); ^{1}H-n.m.r.: δ 7–7.5 (m, 10 H,

*Certain syrupy products gave analytical data somewhat outside conventional limits, but n.m.r. and other data left no doubt concerning identity.

aromatic), 5.82, 6.08–6.12 (2s, 2 H, H-1β and H-1α), 5–5.3 (2d, 2 H, H-2,3), 3.4–4.2 (m, 4 H, H-4,5,6,6'), 1.92, 2.08, and 2.2 (3s, 9H, 3Ac).

Anal. Calc. for $C_{26}H_{30}O_9$: C, 64.18; H, 6.21. Found: C, 64.28; H, 6.23

2-(4-Nitrophenyl)ethyl 2,3-di-O-acetyl-4,6-di-O-benzyl-α-D-mannopyranoside (**13**). — A solution of **11** (0.3 g, 0.6 mmol) in dry ether (15 mL) was saturated with hydrogen chloride at 0°. The mixture was kept for 4–6 h (or until t.l.c. showed only one spot) at room temperature. Nitrogen was then bubbled into the solution. The resulting solution was diluted with ether, washed with cold dilute, sodium hydrogencarbonate and water, dried, and then separated by l.c. with 1:2 ethyl acetate–hexane. Syrupy 2,3-di-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl chloride (**12**) was obtained in 86% yield (0.245 g). The chloride **12** (0.12 g, 0.25 mmol), 2-(4-nitrophenyl)ethanol (0.052 g, 0.31 mmol), and silver *p*-toluenesulfonate (0.072 g, 0.25 mmol) were kept separately under vacuum on a high-vacuum rack for 6 h at room temperature. The product was then processed as described for **5**. The resulting syrup was separated by l.c. with 1:1 ethyl acetate–hexane. Compound **13** was obtained in 82% yield (0.126 g) as a syrup; $[\alpha]_D^{25}$ +41.4° (*c* 1, chloroform). The same compound (**13**) was obtained by using silver triflate instead of silver *p*-toluenesulfonate. 2-(p-Nitrophenyl)ethanol (0.05 g, 0.299 mmol), the chloride **12** (0.1 g, 0.23 mmol), and silver triflate (0.066 g, 0.25 mmol) were kept separately under vacuum for 3 h on a high-vacuum rack. Tetramethylurea (0.08 g, 0.082 mL, 0.68 mmol) was injected into the chloride (**12**) under nitrogen, and the mixture frozen. Under vacuum, dichloromethane (2 mL) was distilled onto the chloride **12** and tetramethylurea. The resulting solution was then mixed with silver triflate and this mixture was filtered onto 2-(4-nitrophenyl)ethanol. The mixture was kept for 24 h at 4°, and then processed as in the previous example. After separation, **13** was obtained in 33% yield (42 mg); ^{1}H-n.m.r.: 8.04–8.2 (2d, 2 H, H-3,5 of aryl), 7.06–7.5 (m, 12 H, aromatic), 4.8–4.82 (d, 1 H, H-1), 3.5–4.04 (m, 6 H, H-4,5,6,6', OH_2C-H_2C-$C_6H_4NO_2$-*p*), 2.9–3.1 (bt, 2 H, OH_2C-H_2C-C_6H_4-NO_2-*p*), 1.96, and 2.22 (2 s, 6 H, 2Ac).

Anal. Calc. for $C_{32}H_{35}NO_{15}$: C, 64.74; H, 5.94; N, 2.35. Found: C, 65.26; H, 6.40; N, 2.43.

2-(4-Nitrophenyl)ethyl 4,6-di-O-benzyl-α-D-mannopyranoside (**14**). — A solution of **13** (0.1 g, 0.16 mmol) in methanol (10 mL) was treated with a catalytic amount of sodium methoxide. The mixture was stirred for 30 min at room temperature, and then made neutral with acetic acid, evaporated, and extracted with dichloromethane, washed with sodium hydrogencarbonate and water, and dried. The diol **14** crystallized from dichloromethane–hexane; yield 77 mg (90%), m.p. 94–95° $[\alpha]_D^{27}$ +39.3° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 8.06–8.18 (2 d, 2 H, H-3,5 of aryl), 7.2–7.4 (m, 11 H, aromatic), 4.8–4.82 (d, 1 H, H-1), 3.42–4.0 (m, 8 H, H-2,3,4,5,6,6', OH_2C-H_2C-C_6H_4-NO_2-*p*), 2.86–3.04 (t, 2 H, OH_2C-H_2C-C_6H_4-NO_2-*p*), and 2.36–2.46 (bs, 2 H, 2OH).

Anal. Calc. for $C_{28}H_{31}NO_8$: C, 65.99; H, 6.13; N, 2.74. Found: C, 65.63; H, 6.09; N, 2.31.

2-(4-Nitrophenyl)ethyl 2-O-acetyl-4,6-di-O-benzyl-α-D-mannopyranoside (**15**). — To a stirred solution of **14** (0.1 g, 0.19 mmol) in THF (5 mL), sodium hydride (5 mg, 60%, 0.31 mmol) was added. When hydrogen gas ceased to be evolved, anhydrous mercury(II) chloride (0.053 g, 0.19 mmol) was added. The mixture was kept for 20 min and acetic anhydride (0.04 g, 0.035 mL, 0.39 mmol) was added. The mixture was then processed as described for **3**. Compound **15** was separated as a syrup; yield 85 mg (78.7%); $[\alpha]_D^{19}$ +29.1° (*c* 1.96, chloroform), whereas the 3-acetate was obtained in 20.3% yield (22 mg), calculated based on conversion of 59% of **14**; ^{1}H-n.m.r.: δ 8.06–8.18 (2 d, 2 H, H-3,5 of aryl), 7.2–7.4 (m, 12 H, aromatic), 5–5.08 (dd, 1 H, $J_{1,2}$ 1.4, $J_{2,3}$ 3.2 Hz, H-2), 4.82–4.86 (d, 1 H, 1.4 Hz, H-1), 3.49–4.02 (2 d, $J_{2,3}$ 3.2, $J_{3,4}$ 9.4 Hz, H-3), 3.4–3.9 (m, 6 H, H-4,5,6,6′, O*H*$_2$-C-H$_2$C-C$_6$H$_4$-NO$_2$-*p*), 2.88–3.04 (t, 2 H, OH$_2$C-*H*$_2$C-C$_6$H$_4$-NO$_2$-*p*), and 2.12 (s, 4 H, OH and Ac).

Anal. Calc. for $C_{30}H_{33}NO_9$: C, 65.32; H, 6.03; N, 2.53. Found: C, 64.71; H, 5.82; N, 2.14.

2-(4-Nitrophenyl)ethyl 4,6-di-O-benzyl-3-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**17**). — 2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride (**20**, 0.15 g, 0.39 mmol), **15** (0.12 g, 0.217 mmol), and silver *p*-toluenesulfonate (76 mg, 0.27 mmol) were condensed as described for **5**. After separation, the disaccharide **16** was obtained as a syrup in 70% yield (0.137 g), $[\alpha]_D^{25}$ +12.6° (*c* 0.7, chloroform); ^{1}H-n.m.r.: δ 5.24–5.29 (d, 1 H, H-1′), 5.12–5.2 (dd, 1 H, H-2), 5.64–5.7 (t, 1 H, H-2′), 4.04–4.26 (2 d, 2 H, H-3,3′), 3.4–4 (m, 8 H, H-4,4′,5,5′,6,6,6,6′), 2.8–3 (t, 2 H, OH$_2$C-*H*$_2$C-C$_6$H$_4$-NO$_2$-*p*), H-1 and O*H*$_C$-H$_2$C-C$_6$H$_4$-NO$_2$-*p*) overlapped benzyl methylene protons; ^{13}C-n.m.r.: 102.6 (C-1′), 99.5 (C-1), 80.4, 80.1 (C-3,3′), 71.4 (C-6,6′), 69.9 (OH$_2$C-H$_2$C-C$_6$H$_2$-NO$_2$-*p*), 38.4 (OH$_2$C-H$_2$C-C$_6$H$_4$-NO$_2$-*p*), 23.4 (Ac), 172.9, and 168.2 (C=O).

To a well stirred solution of **16** (0.1 g) in methanol (20 mL) and THF (5 mL), a catalytic amount of sodium methoxide was added at room temperature. The mixture was processed as described for **6**; separation by l.c. gave compound **17** as a syrup (82 mg, 95%), $[\alpha]_D^{18}$ +35.5° (*c* 1.68, chloroform); ^{1}H-n.m.r.: δ 5.24–5.28 (d, 1 H, H-1), 2.8–3 (t, 2 H, OH$_2$C-*H*$_2$C-C$_6$H$_4$-NO$_2$-*p*), and 2.46–2.58 (bs, 2 H, 2 OH); ^{13}C-n.m.r.: δ 100.7 (C-1′), 100.3 (C-1), 80.7, 80.2 (C-3,3′), 69.2 (C-6,6′), 67.2 (OH$_2$C-H$_2$C-C$_6$H$_4$-NO$_2$-*p*), and 35.9 (OH$_2$C-H$_2$C-C$_6$H$_4$-NO$_2$-*p*).

Anal. Calc. for $C_{55}H_{59}NO_{13}$: C, 70.12; H, 6.31; N, 1.49. Found: C, 69.57; H, 6.75; N, 1.99.

2-(4-Aminophenyl)ethyl 3-O-(α-D-mannopyranosyl)-α-D-mannopyranoside protein conjugate (**20**). — A solution of **17** (0.13 g) in ethanol (10 mL) and water (10 mL) was treated with palladium-on-carbon (40 mg, 5%). The mixture was processed as described for **7**. The disaccharide **18** was obtained in quantitative yield. The absence of benzyl groups was proved by ^{1}H-n.m.r. spectroscopy. Thiophosgene (0.037 g, 0.32 mmol, 0.025 mL) was added to a stirred solution of **18** (50 mg, 0.1 mmol) in 80% ethanol (20 mL). The mixture was stirred for 1.5 h at room temperature. The solution was brought to pH 6 and evaporated. The iso-

thiocyanate derivative **19** was dissolved with water and the solution concentrated to 5 mL. The aqueous solution of **19** was then added dropwise to a stirred solution of bovine serum albumin (BSA) (100 mg) in 0.15M sodium chloride (3 mL). The pH of the solution was maintained at 9 by addition of 0.1M sodium hydroxide. The solution was kept for 6 h at room temperature, and then overnight in a refrigerator. The pH was adjusted to **7** and the solution then subjected to ultrafiltration five times through a PM-10 membrane (Amicon). The protein–conjugate solution was freeze-dried and analyzed for carbohydrate content by the quantitative anthrone method[18]. It was found that every mol of protein contained 17.41 mol of disaccharide, and each mg of protein contained 81.06 mg of disaccharide.

Methyl 2,4,6-tri-O-benzyl-3-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**10**). — Methyl 2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**9**, 0.447 g, 0.96 mmol), 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**8**, 0.19 g, 0.046 mmol), and silver triflate (0.268 g, 3.89 mmol) were kept on a high-vacuum rack for 6 h at room temperature. Tetramethylurea (0.14 g, 0.15 mL, 1.28 mmol) was injected onto **8** under nitrogen. The mixture was processed as described for **13**. The resulting syrup was separated by l.c. with 1:1 ethyl acetate–hexane. The starting compound **9** was recovered (0.2 g, 45%) whereas **10** was obtained in 36% yield (0.27 g) as a syrup $[\alpha]_D^{25}$ +37.7° (*c* 1, chloroform). The disaccharide **10** was also synthesized by the Helferich modification of the Koenigs–Knorr reaction. Compound **9** (100 mg) in acetonitrile (2 mL) was treated with mercury(II) bromide (84.5 mg), followed by mercury(II) cyanide (0.0672 g) in the presence of 4A molecular sieves. A solution of **8** (88.8 mg) in acetonitrile (1 mL) was then added. The mixture was stirred for 48 h at room temperature, although t.l.c. showed no change after 30 min. The product was evaporated and isolated conventionally. The resulting syrup was separated by l.c. with 1:1 ethyl acetate–hexane; yield 37 mg (22%); ^{1}H-n.m.r.: δ 7.2–7.4 (m, 15 H, aromatic), 5.38–5.42 (d, 1 H, H-1′), 4.88–4.91 (d, 1 H, H-1), 4.1–4.26 (bt, 2 H, H-2,3), 5.12–5.24 (bt, 2 H, H-2′,3′), 3.3 (s, 3 H, OMe), 1.82, 1.98, 2.03, and 2.06 (4s, 12 H, 4 Ac). ^{13}C-n.m.r.: δ 99.2 (C-1′), 97.6 (C-1), 69.6 (C-2), 69.3 (C-2′), 54.5 (OMe), 25.2, 20.6 (Ac), 170.7, 170.2, and 169.7 (C=O).

Anal. Calc. for $C_{42}H_{50}O_{15}$: C, 63.46; H, 6.34. Found: C, 63.41; H, 6.32.

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REFERENCES

1 W. N. HAWORTH, R. L. HEATH, AND S. PEAT, *J. Chem. Soc.*, (1941) 833–842
2 C. E. BALLOU AND W. C. RASCHKE, *Science*, 184 (1974) 127–134.
3 P. A. J. GORIN AND A. S. PERLIN, *Can. J. Chem.*, 34 (1956) 1796–1803

4 J. C. LEE AND C. E. BALLOU, *Biochemistry*, 4 (1965) 257–264.
5 G. H. JONES AND C. E. BALLOU, *J. Biol. Chem.*, 243 (1968) 2443–2446; *ibid*, 244 (1969) 1043–1051; 1052–1059.
6 R. SENTANDREU AND D. H. NORTHCOTE, *Biochem. J.*, 109 (1968) 419–432; *Carbohydr. Res.*, 10 (1969) 584–585.
7 T. N. CAWLEY AND R. LETTERS, *Biochem. J.*, 115 (1969) 9P.
8 S. SUZUKI, H. SUNAYAMA, AND T. SAITO, *Jpn. J. Microbiol.*, 12 (1968) 19.
9 C. M. REICHERT, *Carbohydr. Res.*, 77 (1979) 141–147.
10 C. M. REICHERT AND I. J. GOLDSTEIN, *J. Immunol.*, 122 (1979) 1138–1145.
11 E. S. EL ASHRY AND C. SCHUERCH, unpublished results.
12 A. LIPTÁK, P. FUGEDI, P. NÁNÁSI, AND A. NESJEMBI, *Tetrahedron Lett.*, (1979) 1111–1119.
13 E. S. RACHAMAN, R. EBY, AND C. SCHUERCH, *Carbohydr. Res.*, 67 (1978) 147–161.
14 P. J. GAREGG, T. INVERSEN, AND S. OSCARSEN, *Carbohydr. Res.*, 50 (1976) c12–c14.
15 E. AVELA AND B. MELANDER, *Abstr. Pap. VI Int. Symp. Carbohydr. Chem.*, August 17 (1972).
16 V. K. HANDA, J. BARLOW, AND K. L. MATTA, *Carbohydr. Res.*, 76 (1979) c1–c3.
17 S. HANESSIAN AND J. BANOUB, *Carbohydr. Res.*, 53 (1977) c13–c16.
18 F. M. WINNIK, J. R. BRISSON, J. P. CARVER, AND J. J. KREPINSKY, *Carbohydr. Res.*, 103 (1982) 15–28.
19 R. EBY AND C. SCHUERCH, *Carbohydr. Res.*, 77 (1979) 61–70.
20 M. A. E. SHABAN AND R. W. JEANLOZ, *Carbohydr. Res.*, 52 (1976) 103–114, 115–127.
21 C. R. MCBROOM, C. H. SAMANEN, AND I. J. GOLDSTEIN, *Methods Enzymol.*, 288 (1972) 212–219.
22 R. J. DIMLER, W. C. SCHAEFER, C. S. WISE, AND C. E. RIST, *Anal. Chem.*, (1952) 1411–1414.
23 J. K. N. JONES AND W. H. NICHOLSON, *J. Chem. Soc.*, (1958) 27–33.
24 H. O. BOUVENG, I. BREMNER, AND B. LINDBERG, *Acta Chem. Scand.*, 19 (1965) 967–973.
25 A. R. JEANES, J. E. PITTSLEY, P. R. WATSON, AND J. H. SLONEKER, *Can. J. Chem.*, 40 (1962) 2256–2259.
26 M. M. PONPIPOM, *Carbohydr. Res.*, 59 (1977) 311–317.
27 T. OGAWA AND K. SASAJIMA, *Carbohydr. Res.*, 97 (1981) 205–227.
28 J. B. STOTHERS, *Carbon-13 NMR Spectroscopy*, Academic Press, New York, 1972, p. 461.
29 E. S. H. EL ASHRY AND C. SCHUERCH, *Carbohydr. Res.*, 105 (1980) 33–43.