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SYNTHESIS OF TWO 3-*O*-(3,6-DIDEOXY-α-D-*xylo*-HEXOPYRANOSYL)-α-D-MANNOPYRANOSIDES SUITABLE FOR COVALENT ATTACHMENT TO A PROTEIN CARRIER

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ABSTRACT

3-*O*-(3,6-Dideoxy-α-D-*xylo*-hexopyranosyl)-α-D-mannopyranosides of 8-methoxycarbonyloctan-1-ol and of *p*-trifluoroacetamidophenol were synthesised by the reaction of 2,4-di-*O*-benzyl-3,6-dideoxy-α-D-*xylo*-hexopyranosyl bromide with suitably protected mannosides, using halide-assisted glycosylation, and then deprotecting the products of glycosylation.

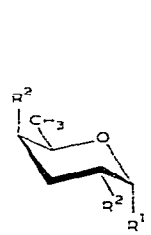
INTRODUCTION

During investigations of artificial *Salmonella* antigens for improved diagnostics, the need arose for further quantities of *p*-aminophenyl 3-*O*-(3,6-dideoxy-α-D-*xylo*-hexopyranosyl)-α-D-mannopyranoside, previously made[1] by condensing 2,4-di-*O*-*p*-nitrobenzoyl-α-D-*xylo*-hexopyranosyl bromide with *p*-nitrophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside, using mercuric cyanide as promotor in the key glycosylation step (Helferich procedure). In order to evaluate the importance of the nature of the linking arm between the disaccharide group and the protein, we also required 8-methoxycarbonyloct-1-yl 3-*O*-(3,6-dideoxy-α-D-*xylo*-hexopyranosyl)-α-D-mannopyranoside, not previously made. We now describe efficient syntheses of these substances, using the halide-assisted glycosylation conditions devised by Lemieux and his co-workers[2].

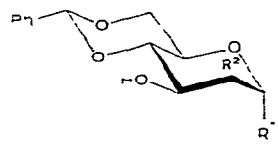
RESULTS AND DISCUSSION

Conventional benzylation of methyl 3,6-dideoxy-α-D-*xylo*-hexopyranoside[3] (**1**) gave the crystalline 2,4-dibenzyl ether **2** in 84% yield. Treatment of **2** with trimethylsilyl bromide[4] gave the abequosyl bromide **3**, which reacted under halide-assisted conditions, using molecular sieves as acid acceptor[2], with the mannoside **5**. The latter compound was obtained from *p*-nitrophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside[5] by hydrogenation over Adams' catalyst, followed by *N*-trifluoroacetylation of the amine **4**. The α-D-linked disaccharide **7** was isolated crystalline in

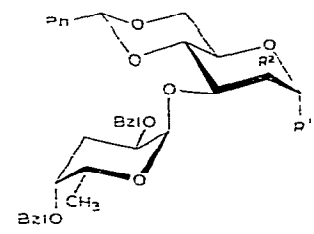
47% yield. Catalytic hydrogenolysis of **7** gave the desired product, identical to previously synthesised[1] **10**. The chief advantages of the present synthesis lie in the use of the *p*-trifluoroacetamidophenyl mannoside **5** rather than the corresponding *p*-nitrophenyl mannoside, and also in the use of the 2,4-di-*O*-benzylabequosyl bromide **3** rather than the corresponding 2,4-di-*O*-(*p*-nitrobenzoyl)abequosyl bromide. This made the deprotection route after glycosylation much shorter than that described before. Also, a higher yield. of a crystalline product, was obtained in the halide-assisted glycosylation step.



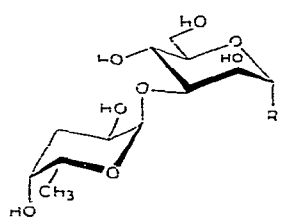
	R^1	R^2
1	OMe	OH
2	OMe	OBzl
3	Br	OBzl



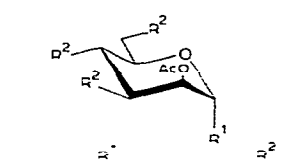
	R^1	R^2
4	p-$NH_2C_6H_4O$	OBzl
5	p-$(CF_3CONH)C_6H_4O$	OBzl
6	$MeO_2C(CH_2)_8O$	OAc



	R^1	R^2
7	p-$(CF_3CONH)C_6H_4O$	OBzl
8	$MeO_2C(CH_2)_8O$	OAc
9	$MeO_2C(CH_2)_8O$	OH



	R
10	p-$(CF_3CONH)C_6H_4O$
11	$MeO_2C(CH_2)_8O$



	R^1	R^2
12	Cl	OBzl
13	$MeO_2C(CH_2)_8O$	OBzl

2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride[6] (**12**) was treated with 8-methoxycarbonyloctan-1-ol[7], using silver triflate as promotor. The product **13**, obtained in 69% yield, was hydrogenolysed over palladium-on-carbon and then treated with α,α-dimethoxytoluene in acidic acetonitrile, to give **6** in 40% yield. This was treated under halide-assisted conditions, using molecular sieves as acid acceptor, with the abequosyl bromide **3** to give, after deacetylation, the disaccharide **9** in 58% yield. Deprotection of **9** gave the desired disaccharide **11**.

EXPERIMENTAL

General methods were the same as those reported[8]. Column chromatography was performed in the "flash" mode[9]. Non-aqueous solutions were dried, when

necessary, over magnesium sulfate. The molecular sieves used in the glycosylations were supplied by Union Carbide. Chemical shifts were measured from internal and external tetramethylsilane for solutions in $CDCl_3$ and D_2O, respectively.

*Methyl 2,4-di-*O*-benzyl-3,6-dideoxy-α-*D*-xylo-hexopyranoside* (**2**). — Methyl 3,6-dideoxy-α-D-*xylo*-hexopyranoside[3] (**1**; 9.1 g, 56.2 mmol) in *N*,*N*-dimethylformamide (110 mL) was treated with sodium hydride (55% in oil; 6.5 g, 149 mmol). After 30 min, benzyl bromide (14.8 mL, 124 mmol) was added dropwise. Stirring was continued at room temperature overnight. The mixture was treated with a few millilitres of methanol during 30 min and then partitioned between ether and water. The ethereal layer was washed with water, dried, and concentrated, to give a syrup that was purified by chromatography on silica gel (500 g), using toluene–ethyl acetate (85:15). Pure **2** (16.1 g, 84%) was obtained as a syrup that crystallised on standing. Recrystallisation from hexane gave material with m.p. 45–46°, $[\alpha]_D$ +18° (*c* 0.4, chloroform).

Anal. Calc. for $C_{21}H_{26}O_4$: C, 73.7; H, 7.65. Found: C, 73.6; H, 7.77.

*2,4-Di-*O*-benzyl-3,6-dideoxy-α-*D*-xylo-hexopyranosyl bromide* (**3**). — The methyl glycoside **2** (100 mg) was treated with trimethylsilyl bromide (0.5 mL). After 3 h at room temperature, the mixture was concentrated and dichloromethane was twice evaporated from the residue, which then showed a ^{13}C-n.m.r. spectrum ($CDCl_3$) containing major peaks corresponding to the desired bromide (*inter alia*: 16.0, 29.0, 70.7, 71.1, 71.3, 74.4, and 95.8 p.p.m.) and minor (<10%) peaks corresponding to **2**. The $J_{1,2}$ value (3 Hz) in the proton spectrum indicated the α configuration. The bromide was unstable and was therefore used immediately in the next step.

*p-Aminophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-*D*-mannopyranoside* (**4**). — A solution of *p*-nitrophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside[5] (7.5 g) in ethyl acetate (150 mL) containing prehydrogenated Adams' catalyst (0.5 g) was hydrogenated at room temperature and atmospheric pressure until hydrogen consumption had ceased. The solution was filtered and concentrated, and the residue was recrystallised from cold ethyl acetate–hexane to give **4** (6.8 g, 96%) in 2 crops; m.p. 130–131°, $[\alpha]_D$ +77° (*c* 0.5, chloroform).

Anal. Calc. for $C_{26}H_{27}NO_6$: C, 69.5; H, 6.05 Found: C, 69.3; H, 6.18.

*p-Trifluoroacetamidophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-*D*-mannopyranoside* (**5**). — Compound **4** (6.75 g, 15.0 mmol) in pyridine (50 mL) was treated with trifluoroacetic anhydride (4.18 mL, 30.0 mmol), with stirring and cooling in an ice-bath. After 10 min, ~2 mL of ice was added to the mixture, and stirring was continued for 30 min. The mixture was poured into cold water (1 L), and crude **5** (7.65 g) was collected and recrystallised from ethyl acetate–hexane (or dichloromethane in the cold), to give **5** (6.5 g, 79%), m.p. 210–212°, $[\alpha]_D$ +79° (*c* 0.5, chloroform).

Anal. Calc. for $C_{28}H_{26}F_3NO_7$: C, 61.6; H, 4.80. Found: C, 61.5; H, 4.72.

*p-Trifluoroacetamidophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2,4-di-*O*-benzyl-3,6-dideoxy-α-*D*-xylo-hexopyranosyl)-α-*D*-mannopyranoside* (**7**). — A solution of the bromide **3** [prepared from **2** (3.5 g, 10.0 mmol)] in dry dichloromethane (5 mL) was added to a solution of the alcohol **5** (3.30 g, 6.0 mmol) in dichloromethane (20

mL) containing *N*,*N*-dimethylformamide (1.5 mL), tetraethylammonium bromide (1.26 g, 6.0 mmol), and powdered 4 Å molecular sieve. The mixture was stirred at room temperature overnight and then filtered. The filtrate was washed with water and aqueous sodium hydrogencarbonate, dried, and concentrated. Chromatography on silica gel, using toluene–ethyl acetate (8:2), yielded fractions containing **7** and minor impurities. These fractions were combined and evaporated, and the residue was crystallised from chloroform–hexane to give pure **7** (2.4 g, 47%) in several crops; m.p. 84–88°, $[\alpha]_D$ +102° (*c* 0.6, chloroform). Several elemental analyses gave unsatisfactory results; the reason for this is unclear. However, the structure and purity of the material were evident from its n.m.r. spectra. 25-MHz, ^{13}C-N.m.r. data ($CDCl_3$): δ 16.5 (abequosyl C-6), 26.8 (abequosyl C-3), 96.6, 96.9, and 102.0 (anomeric and acetal carbons). 100-MHz, ^{1}H-N.m.r. data ($CDCl_3$): δ 1.19 (d, 3 H, $J_{5,6}$ 6.7 Hz, abequosyl H-6), 5.45 (s, 1 H, acetal H), 5.49 (d, 1 H, $J_{1,2}$ 3.3 Hz, abequosyl H-1), and 5.58 (d, 1 H, $J_{1,2}$ 1.4 Hz, mannosyl H-1).

p-*Trifluoroacetamidophenyl* 3-O-(*3,6-dideoxy*-α-D-xylo-*hexopyranosyl*)-α-D-*mannopyranoside*[1] (**10**). — A solution of **7** (1.1 g) in 95% ethanol (50 mL) and tetrahydrofuran (5 mL) was hydrogenated over palladium-on-carbon (10%, 0.5 g) at 400 kPa overnight and then filtered and concentrated. The glassy residue (650 mg) was chromatographically homogeneous **10**, $[\alpha]_D$ +115° (*c* 0.5, water). 25-MHz, ^{13}C-N.m.r. data (D_2O): δ 16.7 (abequosyl C-6), 34.2 (abequosyl C-3), 61.7 (mannosyl C-6), 64.8, 66.9, 68.0, 69.5, 71.2, 74.7, 79.4 (abequosyl C-2,4,5, mannosyl C-2,3,4,5), 99.3 (mannosyl C-1), 101.5 (abequosyl C-1), 117.0 (q, $J_{C,F}$ 287 Hz, CF_3), 118.5, 124.4, 130.8, and 154.7 (aromatic C). 100-Hz, ^{1}H-N.m.r. data (D_2O): δ 1.13 (d, 3 H, $J_{5,6}$ 6.6 Hz, abequosyl H-6), 1.9–2.1 (m, 2 H, H-3, abequosyl H-3,3'), 5.12 (d, 1 H, $J_{1,2}$ 3.7 Hz, abequosyl H-1), 5.56 (d, 1 H, $J_{1,2}$ 2.0 Hz, mannosyl H-1), 7.47, and 7.18 (dd, 4 H, aromatic H).

8-Methoxycarbonyloct-1-yl 2-O-*acetyl-3,4,6-tri*-O-*benzyl*-α-D-*mannopyranoside* (**13**). — Crystalline 3,4,6-tri-*O*-benzyl-1,2-*O*-methoxyethylidene-β-D-mannopyranose[6,10] (3.0 g, 5.9 mmol) was dissolved in trimethylsilyl chloride (6 mL). After 15 min at room temperature, toluene was added and the solution was concentrated. Toluene and dichloromethane were evaporated from the residue **12**, which was then taken up in 1:1 nitromethane–toluene (15 mL) containing 8-methoxycarbonyloctan-1-ol[7] (1.8 g, 9.5 mmol) and powdered 4 Å molecular sieve. After stirring for a few minutes to ensure dryness, the mixture was cooled to −20° and a solution of silver trifluoromethanesulfonate (2.1 g, 8.3 mmol) and 2,4,6-trimethylpyridine (0.59 mL, 4.5 mmol) in 1:1 nitromethane–toluene (6 mL) was added. After 15 min, more 2,4,6-trimethylpyridine (0.5 mL) was added and the mixture was allowed to attain room temperature. After dilution with ether (100 mL) and filtration, the mixture was washed successively with aqueous sodium thiosulfate, water, 2M sulfuric acid, and aqueous sodium hydrogencarbonate. Drying and concentration left a syrup that was purified by chromatography on silica gel (250 g) with toluene–ethyl acetate (4:1), to give chromatographically homogeneous **13** (2.7 g, 69%), $[\alpha]_D$ +15° (*c* 0.5, chloroform).

8-Methoxycarbonyloct-1-yl 2-O-*acetyl-4,6*-O-*benzylidene*-α-D-*mannopyranoside*

(**6**). — Hydrogenation at 400 kPa of a solution of **13** (2.5 g) in 95% ethanol (75 mL) containing palladium-on-carbon (10%, 0.5 g) gave, after filtration and concentration, a syrup which was directly taken up in acetonitrile (35 mL) containing α,α-dimethoxytoluene (2.3 mL). A catalytic amount of *p*-toluenesulfonic acid (25 mg) was added, and, after 30 min, the mixture was partitioned between dichloromethane and aqueous sodium hydrogencarbonate. The organic phase was washed with water, dried, and concentrated. Chromatography of the residue on silica gel with toluene–ethyl acetate (7:3) gave chromatographically homogeneous **6** (0.73 g, 40%) as a syrup, $[\alpha]_D$ +25° (*c* 0.5, chloroform). The ^{1}H-n.m r. spectrum showed that no acetyl migration had taken place during the acetalation. 25-MHz, ^{13}C-n.m.r. data ($CDCl_3$). δ 20.9 (CH_3CO), 51.3 (OCH_3), 63 3, 66.8, 68.0, 68.6, 72.3, 78.9 (ring carbons, OCH_2), 98.3, 101.9 (anomeric and acetal carbons), 170.4, and 174.1 (C=O). 100-MHz, ^{1}H-n.m.r. data ($CDCl_3$): δ 2.23 (s, 3 H, Ac), 3.69 (s, 3 H, OMe), 4.80 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 5.23 (dd, 1 H, $J_{1,2}$ 1.4 , $J_{2,3}$ 3.6 Hz, H-2), and 5.63 (s, 1 H, acetal H).

*8-Methoxycarbonyloct-1-yl 4,6-*O*-benzylidene-3-*O*-(2,4-di-*O*-benzyl-3,6-dideoxy-α-*D*-xylo-hexopyranosyl)-α-D-mannopyranoside* (**9**). — A solution of the bromide **3** [prepared from **2** (0.96 g, 2.7 mmol)] in dichloromethane (2 mL) was added to the alcohol **6** (0.70 g, 1.46 mmol) in dichloromethane (6 mL) containing *N*,*N*-dimethylformamide (0.5 mL), tetraethylammonium bromide (210 mg), and powdered 4 Å molecular sieve. After being stirred at room temperature overnight, the mixture was worked-up as in the preparation of **7**. Chromatography on silica gel with toluene–ethyl acetate (4:1) yielded fractions containing the disaccharide **8** together with minor impurities. The ^{1}H- and ^{13}C-n.m.r. spectra required the material to be at least 80% pure. After treatment with 0.2M sodium methoxide in methanol at room temperature overnight, subsequent neutralisation with Dowex-50 (H^+) resin, and concentration, the material was purified by chromatography on silica gel. Toluene–ethyl acetate (3:2) eluted syrupy **9** (0.60 g) as the main band; $[\alpha]_D$ +72° (*c* 0.5, chloroform). 25-MHz, ^{13}C-n.m.r. data ($CDCl_3$): δ 16.5 (abequosyl C-6), 51.4 (OCH_3), 96.4, 100.1, 102.0 (anomeric and acetal carbons), and 174.1 (C=O).

*8-Methoxycarbonyloct-1-yl 3-*O*-(3,6-dideoxy-α-D-xylo-hexopyranosyl)-α-D-mannopyranoside* (**11**). — A solution of **9** (0.55 g) in 95% aqueous ethanol (40 mL) was hydrogenated over palladium-on-carbon (10%, 0.3 g) at 400 kPa. Filtration and concentration gave chromatographically homogeneous **11** (0.35 g) as a glass; $[\alpha]_D$ +90° (*c* 0.4, water). 25-MHz, ^{13}C-n.m.r. data (D_2O): δ 17.0 (abequosyl C-6), 34.4 (abequosyl C-3), 52.9 (OCH_3), 61.9, 64.8, 67.0, 67.7, 68.9, 69.6, 71.8, 74.0, 79.5 (mannosyl C-2–C-6, abequosyl C-2,4,5, OCH_2), 100.9, 101.4 (anomeric carbons), and 176.5 (C=O). 100-MHz, ^{1}H-n.m.r. data (D_2O): δ 1.15 (d, 3 H, $J_{5,6}$ 7 Hz, abequosyl H-6), 1.9–2.1 (m, 2 H, abequosyl H-3,3′), 2 37 (t, 2 H, *J* 7 Hz (OCH_2), 3.67 (s, 3 H, OCH_3), 4.82 (d, 1 H, $J_{1,2}$ 2 Hz, mannosyl H-1), and 5.08 (d, 1 H, $J_{1,2}$ 4 Hz, abequosyl H-1).

Methylation analysis of **11**, comprising complete methylation, acidic hydrolysis, sodium borohydride reduction, and acetylation, gave results confirming the structure given.

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