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SYNTHESIS OF *p*-ISOTHIOCYANATOPHENYL 3-*O*-(3,6-DIDEOXY-α-D-*ribo*-HEXOPYRANOSYL)-α-D-MANNOPYRANOSIDE

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ABSTRACT

The synthesis of the title disaccharide derivative (**10**), corresponding to the *Salmonella* O-factor 2[1], is described Treatment of 2-*O*-benzyl-4-*O*-*p*-nitrobenzoyl-α-paratosyl bromide (**5**) with *p*-nitrophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannoside in dichloromethane, in the presence of mercuric cyanide, gave the α- and β-linked disaccharide derivatives (**6a** and **6b**) in yields of 34 and 5%, respectively The disaccharide derivative **10** can react with free amino groups in proteins to produce artificial antigens useful in studies on *Salmonella* immunology

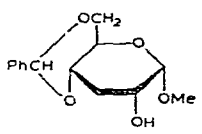
INTRODUCTION

This paper continues a series of investigations aimed at producing 3,6-dideoxy-α-D-hexopyranosyl-(1→3)-α-D-mannopyranosides, with the mannosyl unit α-linked to a *p*-isothiocyanatophenyl group These derivatives are suitable for covalent linkage, *via* a thiourea moiety, to amino groups in proteins The 3,6-dideoxyhexoses are tyvelose, abequose, and paratose, and the materials thus produced are artificial antigens that may be used for diagnosis, and possibly also prophylaxis, of *Salmonella* infections In the previous syntheses of an α-tyvelosyl (3,6-dideoxy-α-D-*arabino*-hexopyranosyl) and α-abequosyl (3,6-dideoxy-α-D-*xylo*-hexopyranosyl) disaccharides in which O-1 and O-2 of the dideoxyhexose unit were *trans* and *cis*, respectively, it was possible to use 3,6-dideoxy-α-D-hexosyl bromides with *p*-nitrobenzoyl groups in positions 2 and 4, and the Helferich modification of the Koenigs–Knorr reaction[2,3] Reasonable yields of the α-D-linked disaccharides were obtained However, in the synthesis of the α-paratosyl (3,6-dideoxy-α-D-*ribo*-hexopyranosyl) disaccharide **10**, the use of 2,4-di-*O*-*p*-nitrobenzoyl-α-paratosyl bromide produced disaccharides containing, almost exclusively, β-paratosyl units[4], a scheme involving a paratosyl bromide with a non-participating group at position 2 was therefore devised

RESULTS AND DISCUSSION

Methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*arabino*-hexopyranoside, available from previous work[2], was oxidized with ruthenium tetraoxide[5] to give methyl 4,6-*O*-

benzylidene-3-deoxy-α-D-*erythro*-hexopyranosid-2-ulose which, on reduction with sodium borohydride in ethanol, gave methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*ribo*-hexopyranoside (**1**, 55% overall yield) Treatment of **1** with *N*-bromosuccinimide[6] and hydrogenation of the resulting 6-bromohexoside produced the 3,6-dideoxyhexoside **2** (54% from **1**) Benzylation of **2** with benzyl trifluoromethanesulphonate[7–9] produced **3** (80%), the benzoyl group of which was removed with methanolic sodium methoxide and replaced with a *p*-nitrobenzoyl group to give **4** (80%) Treatment of **4** with hydrogen bromide in dichloromethane produced the 3,6-dideoxyhexosyl bromide **5**, which was treated with *p*-nitrophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside[2] in dichloromethane with mercury(II) cyanide as promoter[10] The resulting α- and β-linked disaccharides were separated after removal of the *p*-nitrobenzoyl group in the paratose residue The pure α-linked and β-linked disaccharides **6a** and **6b** were thus obtained in yields of 34 and 5%, respectively. Acetylation of **6a** gave **7**, and the synthesis then followed a route similar to that described for the



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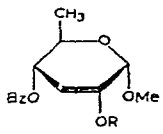
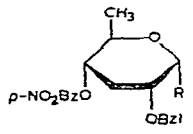
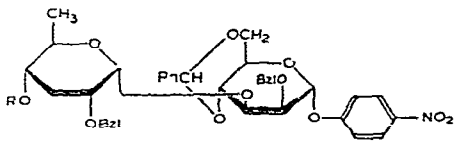


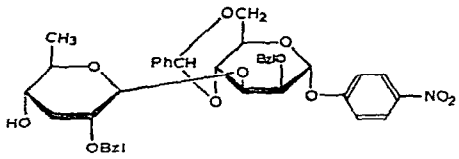
2 R = H
3 R = Bzl



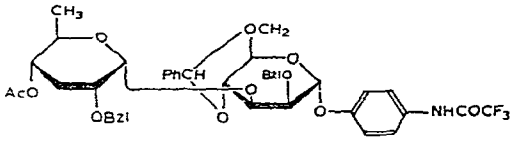
4 R = OMe
5 R = Br



6a R = H
7 R = Ac



6b



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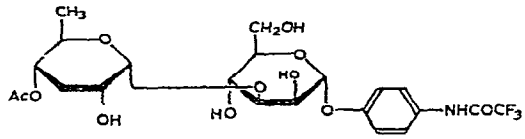
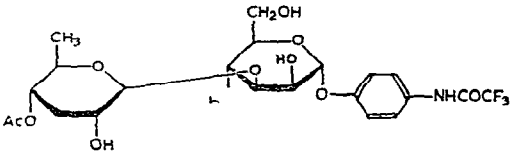
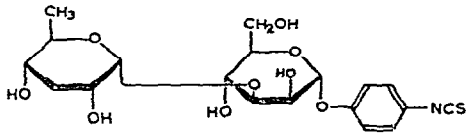


9c



9b



10

synthesis of disaccharides containing tyvelose and abequose[2,3] Hydrogenation of 7 over palladium to give the analogue of **9a** having a free amino group was expected to proceed in low yield due to catalyst poisoning[2], and hence the nitro group in **7** was first hydrogenated (Adams' catalyst) to an amino group which was then trifluoroacetylated to produce **8** Hydrogenation of **8** over palladium gave **9a** (69% from **6a**) For identification purposes, the same reaction sequence was performed with **6b** to give **9b** Deacylation of **9a**, followed by reaction with thiophosgene[11], gave **10** (76% from **9a**)

The constitution of the various intermediates and the final product followed from the methods of synthesis, elemental analyses, and n m r, g l c, and i r data The constitution of the disaccharide derivatives **9a** and **9b** was corroborated by sugar analyses[12,13] and methylation analyses[14,15], and their anomeric configurations followed from the optical rotations and $J_{1,2}$ values for the paratose residues

EXPERIMENTAL

General methods — Concentrations were performed under reduced pressure at below 40° (bath) Melting points are corrected Optical rotations were measured at 20–22° with a Perkin–Elmer 141 instrument I r and ^{1}H-n m r spectra were recorded with Perkin–Elmer 257 and with Varian A-60 A and XL-100 instruments, respectively Chemical shifts are recorded in p p m downfield from that of internal tetramethylsilane, and coupling constants were measured to the nearest 0 5 Hz ^{1}H-N m r spectra were recorded for all new compounds and were in agreement with postulated structures, hence, only selected data are presented below

T l c. was performed on silica gel F_{254} (Merck) with detection by charring with sulphuric acid Preparative separations were performed with Merck silica gel (0 040–0 063 mm), on prepacked silica gel columns, or on 0 25-mm layers of silica gel F_{254} G l c was performed with a Perkin–Elmer 900 instrument equipped with an ECNSS-M column (3% on Gas Chrom Q), and g l c–m s with Perkin–Elmer 270 or Varian MAT 311 instruments equipped with OV-225 columns (3% on Gas Chrom Q) Mass spectra were recorded at 70 eV

*Methyl 4,6-O-benzylidene-3-deoxy-α-*D*-ribo-hexopyranoside*[5] (**1**) — Methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*arabino*-hexopyranoside[16] (2 g) was stirred with a mixture of potassium metaperiodate (3 4 g), ruthenium dioxide (42 mg), and potassium carbonate in ethanol-free chloroform (10 ml) and water (9 ml) After 18 h, t l c (ether–chloroform, 1 1) showed the absence of starting material Propan-2-ol (1 ml) was added and stirring was continued for 15 min The mixture was filtered through Celite, and the aqueous layer was extracted with chloroform The extract was dried ($MgSO_4$), filtered, and concentrated to a syrup (1 67 g) which crystallized from ether–light petroleum to yield methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*erythro*-hexopyranosid-2-ulose (1 2 g), m p 107–108°, $[\alpha]_D$ +97° (*c* 0 5, chloroform), lit[17] m p 114–115°, $[\alpha]_D$ +109° (chloroform)

A solution of the 2-ulose (9 6 g) and sodium borohydride (1 3 g) in ethanol

(400 ml) was boiled under reflux for 1 h, and then neutralized with Dowex 50(H^+) resin, filtered, and concentrated Boric acid was removed from the residue by repeated evaporation of methanol therefrom G l c (ECNSS-M) revealed the *ribo* isomer **1** to be the only product (9 6 g), m p 187–189°, $[\alpha]_D$ +101° (*c* 0 2, chloroform), lit [18] m p 190–192°, $[\alpha]_D$ +116° (chloroform)

Methyl 4-O-benzoyl-3,6-dideoxy-α-D-ribo-hexopyranoside (**2**) — A mixture of **1** (9 6 g), *N*-bromosuccinimide (7 6 g), barium carbonate (26 g), and carbon tetrachloride (350 ml) was boiled under reflux with vigorous stirring for 3 h T l c (ether–chloroform, 1 1) then showed the absence of **1** The mixture was diluted with chloroform, filtered, washed with water, dried (Na_2SO_4), filtered, and concentrated The amorphous product (11 1 g), methyl 4-*O*-benzoyl-6-bromo-6-deoxy-α-D-*ribo*-hexopyranoside, was used without further purification A small amount of the material was purified by chromatography on silica gel (chloroform–methanol, 8 1) and then had $[\alpha]_D$ +127° (*c* 0 6, chloroform), lit [19] +169° (chloroform)

Anal Calc for $C_{14}H_{17}BrO_5$ C, 48 7, H, 4 96, Br, 23 1 Found C, 48 9, H, 4 78, Br 23 0

A solution of the 6-bromo compound (11 g) in methanol (150 ml) containing triethylamine (15 ml) was hydrogenated over 10% palladium-on-carbon (0 5 g) at 410 KPa and room temperature for 20 h The catalyst was removed, the filtrate was concentrated, and a solution of the residue in chloroform was washed successively with water, saturated, aqueous sodium hydrogen carbonate, and water, dried (Na_2SO_4), filtered, and concentrated The product was purified by chromatography on silica gel (chloroform–acetone, 8 1) to yield **2** (5 3 g), $[\alpha]_D$ +170° (*c* 0 67, chloroform) N m r data (60 MHz, $CDCl_3$) δ 1 22 (d, 3 H, H-6), 1 80 (q, 1 H, H-3*ax*), 2 12 (1 H, OH), 2 40 (dtr, 1 H, H-3*eq*), 3 50 (s, 3 H, OMe), 3 7–4 1 (m, 2 H, H-2,5), 4 67 (d, 1 H H-1), 4 75 (m 1 H, H-4), 7 3–7 6 (3 H, Ph), and 7 9–8 1 (2 H, Ph), $J_{1,2}$ 3 5, $J_{2,3eq}$ 5 0 $J_{2,3ax}$ 11 5, $J_{3eq,3ax}$ 11 5, $J_{3eq,4}$ 5 0, $J_{3ax,4}$ 11 5, $J_{4,5}$ 9, and $J_{5,6}$ 6 Hz

Anal Calc for $C_{14}H_{18}O_5$ C, 63 1, H, 6 81 Found C, 63 3 H, 6 72

Hydrolysis of **2** with 0 25M sulphuric acid at 100° for 12 h, followed by reduction with sodium borohydride and acetylation [12], gave 1,2,4,5-tetra-*O*-acetyl-3,6-dideoxy-D-*ribo*-nexitol indistinguishable from an authentic sample by g l c–m s [12 13]

Methyl 4-O-benzoyl-2-O-benzyl-3,6-dideoxy-α-D-ribo-hexopyranoside (**3**) — A solution of trifluoromethanesulphonic anhydride (1 35 ml) in anhydrous dichloromethane (35 ml) at −60° was added to a solution of benzyl alcohol (0 85 ml) and 2,6-di-*tert*-butyl-4-methylpyridine (1 7 g) in anhydrous dichloromethane (20 ml) After 30 min, a solution of **2** (970 mg) and 2,6-*tert*-butyl-4-methylpyridine (4 g) in anhydrous dichloromethane (25 ml) was added during 30 min at −60° The temperature was allowed to rise to −20° and the mixture was stirred thereat for 15 h [8 9] Pyridine was then added slowly, and the mixture was diluted with dichloromethane and shaken with water The organic layer was dried (Na_2SO_4), filtered, and concentrated The product was purified by chromatography on silica gel (toluene–ethyl acetate, 9 1) to yield **3** (1 10 g), $[\alpha]_D$ +50° (*c* 1 02, chloroform) N m r data (100 MHz,

$CDCl_3$) δ 1 19 (d, 3 H, H-6), 1 98 (q, 1 H, H-3*ax*), 2 43 (dtr, 1 H, H-3*eq*), 3 46 (s, 3 H, OMe) 3 64 (m, 1 H, H-2), 3 92 (m, 1 H, H-5), 4 50 and 4 61 (2 H, AB-spectrum, $PhCH_2$), 4 64 (d, 1 H, H-1), 4 74 (m, 1 H, H-4), 7 2–7 6 (m, 3 H, Bz), 7 32 (s, 5 H, Ph), and 7 9–8 1 (m, 2 H, Bz), $J_{1,2}$ 3 5, $J_{2,3eq}$ 5, $J_{2,3ax}$ 11 5, $J_{3eq,3ax}$ 11 5, $J_{3eq,4}$ 5, $J_{3ax,4}$ 11 5, $J_{4,5}$ 10, $J_{5,6}$ 6, and $J_{H,H(PhCH_2)}$ 12 5 Hz

Methyl 2-O-benzyl-3,6-dideoxy-4-O-p-nitrobenzoyl-α-D-ribo-hexopyranoside (**4**) — A solution of **3** (1 2 g) in methanol (50 ml) containing sodium (20 mg) was boiled under reflux for 30 min, and then neutralized with Dowex 50(H^+) resin, filtered, and concentrated To a solution of the residue in pyridine (20 ml) at 0°, a solution of *p*-nitrobenzoyl chloride (1 g) in pyridine (10 ml) was added with stirring The mixture was stored at room temperature for 4 h Ice–water was then added, the mixture was extracted with chloroform, and the extract was washed with water, dried (Na_2SO_4), and concentrated Purification of the product by chromatography on silica gel (toluene–ethyl acetate, 8 1) yielded **4** (1 2 g), m p 78–79° (from ether–light petroleum), $[\alpha]_D$ +35° (*c* 0 65, chloroform) N m r data (100 MHz, $CDCl_3$) δ 1 20 (d, 3 H, H-6), 2 02 (q, 1 H, H-3*ax*), 2 44 (m, 1 H, H-3*eq*), 3 45 (s, 3 H, OMe), 3 64 (m, 1 H, H-2), 3 92 (m, 1 H, H-5), 4 66 (d, 1 H, H-1), 4 74 (m, 1 H, H-4), 4 53, 4 63 (2 H, AB-spectrum, $PhCH_2$), 7 1–7 4 and 8 0–8 3 (m, 9 H, aromatic H), $J_{1,2}$ 3 5, $J_{2,3eq}$ 5, $J_{2,3ax}$ 11 5, $J_{3eq,3ax}$ 11 5, $J_{3eq,4}$ 5, $J_{3ax,4}$ 11 5, $J_{4,5}$ 10, $J_{5,6}$ 6, and $J_{H,H(PhCH_2)}$ 12 5 Hz

Anal Calc for $C_{21}H_{23}NO_7$ C, 62 8, H, 5 78, N, 3 48 Found C, 62 8, H, 5 75, N, 3 61

p-*Nitrophenyl 2-O-benzyl-3-O-(2-O-benzyl-3,6-dideoxy-α-D-ribo-hexopyranosyl)-4,6-O-benzylidene-α-D-mannopyranoside* (**6a**) — A solution of **4** (327 mg) in dichloromethane (50 ml) at −20° was saturated with hydrogen bromide After 3 h at −20°, t l c (toluene–ethyl acetate, 9 1) revealed the absence of **4**, and the solution was concentrated to give syrupy 2-*O*-benzyl-3,6-dideoxy-4-*O*-*p*-nitrobenzoyl-α-D-*ribo*-hexopyranosyl bromide (**5**), $[\alpha]_D$ +152° (*c* 0 2, chloroform)

A solution of **5** (prepared from 327 mg of **4**) in dichloromethane (3 ml) was added with stirring to a solution of *p*-nitrophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside[2] (422 mg) and mercury(II) cyanide (285 mg) in dichloromethane (6 ml) After stirring at room temperature under dry nitrogen for 18 h, the mixture was diluted with dichloromethane, washed successively with water, saturated, aqueous sodium hydrogen carbonate, and water, dried (Na_2SO_4), filtered, and concentrated to a syrup (682 mg) Column chromatography (toluene–ethyl acetate, 9 1) yielded a syrup (493 mg), R_F 0 54 (t l c), a solution of which in methanol (50 ml) containing sodium methoxide (from 30 mg of sodium) was boiled under reflux for 30 min, then neutralized with Dowex 50(H^+) resin, filtered, and concentrated Column chromatography (toluene–ethyl acetate, 2 1) of the product yielded **6a** (210 mg), R_F 0 52, $[\alpha]_D$ +143° (*c* 0 4, chloroform), and **6b** (33 mg), R_F 0 46, $[\alpha]_D$ +89° (*c* 0 7, chloroform) N m r data (100 MHz, $CDCl_3$) for **6a** δ 1 24 (d, 3 H, $J_{5,6}$ 6 Hz, H-6, paratose residue), 1 7–2 3 (m, 2 H, H-3*eq*,3*ax*, paratose residue), 4 84 and 5 05 (2 H, AB-spectrum, $J_{H,H}$ 12 Hz, $PhCH_2$), 5 37 (d, 1 H, $J_{1,2}$ 3 Hz, H-1, paratose residue), 5 46 (s, 1 H,

PhCH), 5 62 (d, 1 H, $J_{1,2}$ 1 5 Hz, H-1, mannose residue), 7 12 and 8 20 (2 d. 4 H, J 9 Hz, *p*-$NO_2C_6H_4O$), for **6b** δ 1 27 (d, 3 H, $J_{5,6}$ 6 Hz, H-6, paratose residue), 1 4–1 9 (m, 1 H, H-3*ax*, paratose residue), 2 3–2 6 (m, 1 H, H-3*eq*, paratose residue), 5 52 (d, 1 H, $J_{1,2}$ 1 5 Hz, H-1, mannose residue), 5 64 (s, 1 H, PhCH), 6 99 and 8 16 (2 d, 4 H, J 10 Hz, *p*-$NO_2C_6H_4O$)

Anal (for **6a**) Calc for $C_{39}H_{41}NO_{11}$ C, 66 9, H, 5 90, N, 2.00 Found C, 66 8, H, 5 93, N, 1 94

p-*Nitrophenyl 3*-O-(*4*-O-*acetyl-2*-O-*benzyl-3,6-dideoxy*-α-D-ribo-*hexopyranosyl*)-*2*-O-*benzyl-4,6*-O-*benzylidene*-α-D-*mannopyranoside* (**7**) — Treatment of **6a** (328 mg) with acetic anhydride (3 ml) in pyridine (3 ml) for 15 min at 100°, followed by concentration with codistillation with toluene, gave **7** as a syrup (332 mg), $[\alpha]_D$ +165° (*c* 0 8, chloroform) N m r data (100 MHz, $CDCl_3$) δ 1 17 (d, 3 H, $J_{5,6}$ 6 Hz, H-6, paratose residue), 1 7–2 3 (m, 2 H, H-3*eq*,3*ax*, paratose residue), 2 07 (s, 3 H, OAc), 4 86 and 5 05 (2 H, AB-spectrum, $J_{H,H}$ 11 Hz, $PhCH_2$), 5 38 (d, 1 H, $J_{1,2}$ 3 Hz, H-1, paratose residue), 5 46 (s, 1 H, PhCH), 5 63 (d, 1 H, $J_{1,2}$ 1 5 Hz, H-1, mannose residue), 7 10 and 8 20 (2 d, 4 H, J 9 Hz, *p*-$NO_2C_6H_4O$)

p-*Trifluoroacetamidophenyl 3*-O-(*4*-O-*acetyl-2*-O-*benzyl-3,6-dideoxy*-α-D-ribo-*hexopyranosyl*)-*2*-O-*benzyl-4,6*-O-*benzylidene*-α-D-*mannopyranoside* (**8**) — A solution of **7** (332 mg) in ethyl acetate was hydrogenated over Adams' catalyst (40 mg) at room temperature and atmospheric pressure When sufficient hydrogen ($NO_2 \rightarrow NH_2$) had been consumed, trifluoroacetic anhydride (0 4 ml) and pyridine (1 0 ml) were added, and the reaction mixture was kept at 60° for 30 min, and then filtered and concentrated A solution of the residue in toluene was washed with water, dried (Na_2SO_4), filtered, and concentrated to a syrup (367 mg), which was purified by column chromatography (toluene–ethyl acetate, 4 1) to yield **8** (270 mg), $[\alpha]_D$ +144° (*c* 0 5, chloroform) N m r data (100 MHz, $CDCl_3$) δ 1 16 (d, 3 H, $J_{5,6}$ 6 Hz, H-6, paratose residue), 2 06 (s, 3 H, OAc), 4 86 and 5 02 (2 H, AB-spectrum, $J_{H,H}$ 11 Hz, $PhCH_2$), 5 38 (d, 1 H, $J_{1,2}$ 3 Hz, H-1, paratose residue), 5 45 (s, 1 H, PhCH), 5 53 (d, 1 H, $J_{1,2}$ 1 5 Hz, H-1, mannose residue), 7 02 and 7 46 (2 d, 4 H, J 9 Hz, *p*-$CF_3CONHC_6H_4O$)

Anal Calc for $C_{43}H_{44}F_3NO_{12}$ C, 62 8, H, 5 27, F, 6 93, N, 1 70 Found C, 62 9, H, 5 46, F, 6 79, N, 1 81

p-*Trifluoroacetamidophenyl 3*-O-(*4*-O-*acetyl-3,6-dideoxy*-α-D-ribo-*hexopyranosyl*)-α-D-*mannopyranoside* (**9a**) — A solution of **8** (246 mg) in ethanol (25 ml) was hydrogenated at room temperature and atmospheric pressure over 10% palladium-on-charcoal (80 mg) When hydrogen consumption had ceased, the mixture was filtered and concentrated to give nearly pure **9a** as a syrup (160 mg) A portion, purified by t l c (chloroform–methanol, 9 1), had $[\alpha]_D$ +170° (*c* 0 7, methanol) N m r data (100 MHz, $CDCl_3$) δ 1 14 (d, 3 H, $J_{5,6}$ 6 Hz, H-6, paratose residue), 1 7–2 3 (m, 2 H, H-3*eq*,3*ax*, paratose residue), 2 05 (s, 3 H, OAc), 5 07 (d, 1 H, $J_{1,2}$ 3 Hz, H-1, paratose residue), 5 50 (d, 1 H, $J_{1,2}$ 1 5 Hz, H-1, mannose residue), 7 13 and 7 56 (2 d, 4 H, $J_{H,H}$ 9 Hz, *p*-$CF_3CONHC_6H_4O$)

Anal Calc for $C_{22}H_{28}F_3NO_{11}$ C, 49 0, H, 5 23, F, 10 5, N, 2 59 Found C, 49 0, H, 5 35, F, 10 5, N, 2 68

Hydrolysis of **9a** with 0 25M sulphuric acid for 12 h at 100°, followed by reduction with sodium borohydride and treatment with acetic anhydride in pyridine[12], gave paratitol tetra-acetate and mannitol hexa-acetate, which were indistinguishable from authentic samples in g l c –m s [12 13]

Methylation[14] of **9a**, followed by hydrolysis, reduction, and acetylation as described above, gave methylalditol acetates which were indistinguishable from authentic 1,5-di-*O*-acetyl-3,6-dideoxy-2,4-di-*O*-methyl-D-*ribo*-hexitol and 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-mannitol, respectively, on g l c –m s [15]

p-*Trifluoroacetamidophenyl* 3-O-(*4-O-acetyl-3,6-dideoxy-β*-D-ribo-*hexopyranosyl*)-α-D-*mannopyranoside* (**9b**) — The above sequence used for the conversion **6a**→**9a** was applied to **6b** (74 mg) to give **9b** (29 mg), $[\alpha]_D$ +77° (*c* 0 3, methanol) N m r data (100 MHz, $CDCl_3$) δ 1 23 (d, 1 H, $J_{5\,6}$ 6 Hz, H-6, paratose residue), 1 56 (q, 1 H, $J_{2,3ax} = J_{3ax,4} = J_{3eq,3ax} = 11\,5$ Hz, H-3*ax*, paratose residue), 2 06 (s, 3 H, OAc), 2 40 (dt, 1 H, $J_{2\,3eq} = J_{3\,4eq} = 5\,5$ Hz, $J_{3eq\,3ax}$ 11 5 Hz, H-3*eq*, paratose residue), 4 46 (d, 1 H, $J_{1,2}$ 7 Hz, H-1, paratose residue), 5 54 (d, 1 H, $J_{1,2}$ 2 Hz, H-1, mannose residue), 7 14 and 7 54 (2 d, 4 H, $J_{H\,H}$ 9 Hz, *p*-$CF_3CONHC_6H_4O$)

Sugar and methylation analyses, as described above for **9a**, gave the same results for **9b**

The ^{1}H-n m r spectrum of the tetrakis(trimethylsilyl) ether of **9b** contained signals for anomeric protons at δ 4 48 ($J_{1\,2}$ 7 Hz, paratose residue) and 5 34 ($J_{1\,2}$ 3 Hz, mannose residue) confirming a β-interglycosidic linkage

p-*Isothiocyanatophenyl* 3-O-(*3,6-dideoxy*-α-D-ribo-*hexopyranosyl*)-α-D-*mannopyranoside* (**10**) — A solution of **9a** (50 mg) in saturated, methanolic ammonia (10 ml) was kept at room temperature for 20 h, and then concentrated The syrupy residue, t l c (chloroform–methanol, 3 1) of which showed the presence of one compound only, was dissolved in 80% aqueous ethanol (10 ml) and the pH was adjusted to ~8 with barium carbonate and maintained thereat by adding barium carbonate whilst thiophosgene (0 1 ml) was added and the reaction mixture was stirred for 2 h[11] Filtration and concentration gave a syrup which was purified by t l c (chloroform–methanol, 5 1) to give **10** (31 mg), $[\alpha]_D$ +174° (*c* 0 3, water), ν_{max}^{KBr} 2130 cm^{-1} (broad)

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