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Synthesis of *p*-Isothiocyanatophenyl 3-*O*-(3,6-Dideoxy-α-D-*arabino*-hexopyranosyl)-α-D-mannopyranoside

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The synthesis of the disaccharide derivative Tyv*p* $\xrightarrow[\alpha]{1\to3}$ D-Man*p* $\xrightarrow[\alpha]{1}$ *p*-C_6H_4–N=C=S (XVI, title compound) is described. The key step is reaction of di-*p*-nitrobenzoyl 3,6-dideoxyhexosyl bromide VI with protected mannoside VIII using the Helferich glycosylation procedure. The disaccharide derivative XVI reacts with free amino groups in proteins to give disaccharide-protein antigens with the same specificity as *Salmonella* serogroup D_1 O-factor 9 (*e.g. S. typhi*). These disaccharide-protein conjugates have previously been found to be promising for specific diagnosis and possibly for immunization.

As part of our efforts [1–4] directed towards the preparation of disaccharide-protein conjugates having disaccharide or oligosaccharide moieties with serospecific *Salmonella* O-factors,[5] we now report the synthesis of a 3-*O*-tyvelosyl-α-D-mannopyranoside having a functional group in the mannoside aglycon useful for attachment of the disaccharide to proteins. Such synthetic antigens are of potential use for diagnosis and prophylactic procedures.[4]

In the present work 2,4-di-*O*-*p*-nitrobenzoyl α-tyvelosyl bromide (VI) was prepared and condensed with *p*-nitrophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (VIII) in acetonitrile using mercuric cyanide as a promotor.[6]

Methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*arabino*-hexopyranoside (I) [7] was treated with *N*-bromosuccinimide [8,9] to give the 6-bromo-4-*O*-benzoyl derivative II which upon catalytic

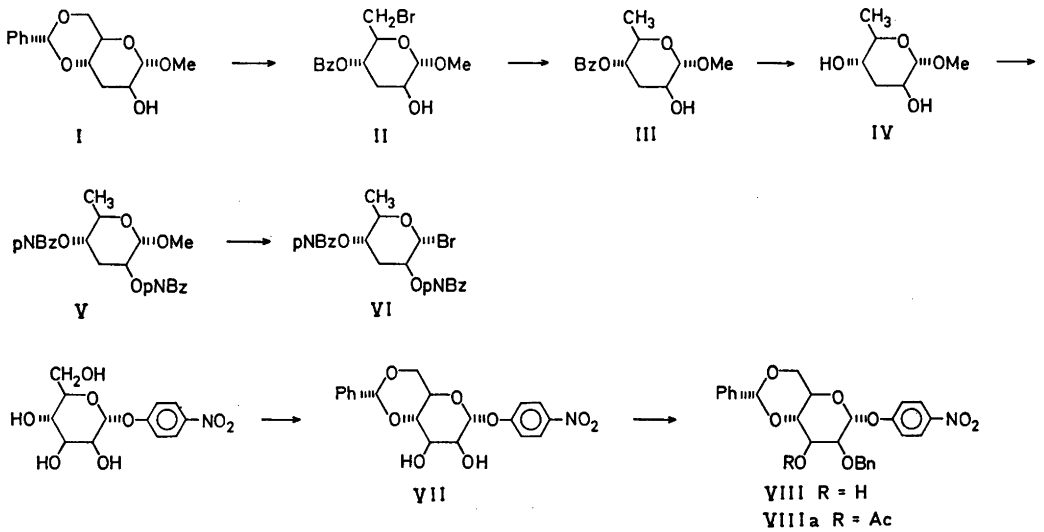


Table 1. Selected NMR shifts (in ppm downfield from internal TMS) and coupling constants

Substance	H-1	H-3	H-4	H-5	H-6 in C-CH_3	Other H-6
II	4.70 $J_{1,2}$<1	1.8–2.4 (2 H)m	5.30 sext $J_{4,3e}$5.5 $J_{4,3a}$ 10 $J_{4,5}$ 10	–	–	3.55d $J_{5,6}$5
III	4.59 $J_{1,2}$<1	1.9–2.35 (2 H)m	5.15 sext $J_{4,3e}$ 5.5 $J_{4,3e}$ 10 $J_{4,5}$ 10	–	1.28d $J_{5,6}$6.5	–
V	4.79 $J_{1,2}$<1	2.15–2.6 (2 H)m	–	4.17 oct $J_{4,5}$ 10 $J_{5,6}$ 6.5	1.36d $J_{5,6}$ 6.5	–
VI	6.55 $J_{1,2}$<1	2.35–2.85 (2 H)m	–	4.34 oct $J_{4,5}$ 10 $J_{5,6}$ 6	1.40d $J_{5,6}$ 6	–
VIII	5.60 $J_{1,2}$ 1.5	–	–	–	–	–
VIIIa	5.63 $J_{1,2}$ 1.5	5.52 $J_{3,2}$ 3 $J_{3,4}$ 9.5	–	–	–	–
VIIIa [a]	5.76 $J_{1,2}$ 1.5	6.28 $J_{3,2}$ 3 $J_{3,4}$ 9.5	–	–	–	–
IX	5.72 [b] $J_{1,2}$ 1.5	2.2–2.6 [c] (2H)m	–	–	1.38d $J_{5,6}$ 6.5	–
X	5.61 $J_{1,2}$ 1.5 [b] 5.00 $J_{1,2}$ 1.5 [c]	1.7–2.3 [c] (2 H)m	–	–	1.28d $J_{5,6}$ 5.5	–
XI	5.69 [b] $J_{1,2}$ 2	1.85–2.25 [c] (2 H)m	–	–	1.23d $J_{5,6}$ 6	–
XIII [b]	5.56 [b] $J_{1,2}$ 1.5	1.85–2.25 [c] (2 H)m	–	–	1.23d $J_{5,6}$ 6.5	–
XIV	5.51 [b] $J_{1,2}$ 1.5	1.85–2.3 [c] (2 H)m	–	–	1.21d $J_{5,6}$ 6	–
XVI	5.61 [b] $J_{1,2}$ 2	1.7–2.2 [c] (2 H)m	–	–	1.28d $J_{5,6}$ 5.5	–

[a] VIIIa With 0.2 mol $Eu(DPM)_3$ per mol sugar added. [b] Mannosyl residue. [c] Tyvelosyl residue.

(in Hz). Solvent: $CDCl_3$ throughout, except for XIV and XVI for which CD_3OD was used.

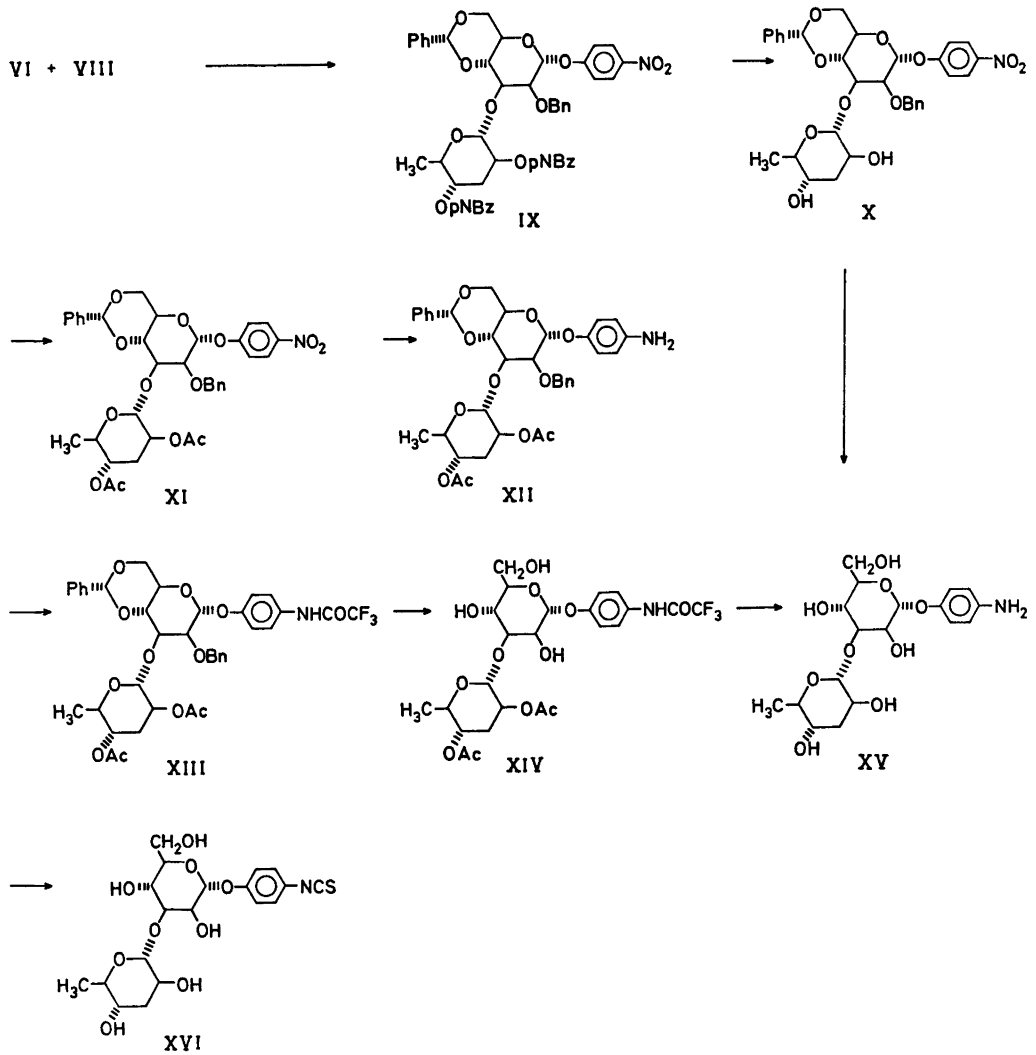
$OCOCH_3$	OCH_3	$PhCH_2$	PhCH	$pNO_2C_6H_4CO$	$pNO_2C_6H_4O$	pCF_3CONH C_6H_4	Other aromatic
–	3.50s	–	–	–	–	–	7.3–8.2 (5 H)
–	3.44s	–	–	–	–	–	7.3–8.2 (5 H)
–	3.52s	–	–	8.0–8.4 (8 H)	–	–	–
–	–	–	–	–	–	–	–
–	–	4.81s	5.57s		7.06d 8.16d $J_{H,H}$ 9	–	7.2–7.7 (10 H)
2.06s	–	4.73s	5.59s	–	7.10d 8.18d $J_{H,H}$ 9.5	–	7.25–7.6 (10 H)
2.50s	–	4.90s	5.72s	–	7.07d 8.18d $J_{H,H}$ 9.5	–	7.25–7.6 (10 H)
–	–	4.97s	5.67s	8.05–8.4 (8 H)	7.0–7.7 (2 H) 8.05–8.4 (2 H)	–	7.0–7.7 (10 H)
–		4.83s 4.86s	5.56s	–	7.06d 8.17d $J_{H,H}$ 9	–	7.2–7.6 (10 H)
2.03s 2.07s	–	4.91s	5.64s	–	7.09d 8.20d $J_{H,H}$ 9.5	–	7.25–7.6 (10 H)
2.03s 2.08s	–	4.87s	5.62s	–	–	7.04d 7.52d $J_{H,H}$ 9	7.2–7.5 (10 H)
2.06s 2.13s	–	–	–	–	–	7.19d 7.58d $J_{H,H}$ 9	–
–	–	–	–	–	–	Signals centered at 7.21 (2 H) m and 7.42 (2 H) m	–

[d] N–H 8.48 ppm (s).

hydrogenation (to give III), followed by debenzoylation (to give IV) and treatment with *p*-nitrobenzoyl chloride in pyridine afforded V in 35 % yield from I. Treatment of the glycoside V with hydrogen bromide in dichloromethane gave 2,4-di-*O*-*p*-nitrobenzoyl-α-D-*arabino*-hexopyranosyl bromide (VI) which was used directly in the glycosylation step described below.

p-Nitrophenyl α-D-mannopyranoside was treated with benzaldehyde in formic acid as described by Buchanan and Schwarz for the corresponding methyl mannoside.[10] The 4,6-*O*-benzylidene derivative VII, obtained in 36 % yield, was monobenzylated with benzyl bromide and silver oxide in dimethyl formamide [11,12] giving, after chromatography using silica gel, *p*-nitrophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (VIII) in 56 % yield. The structure of VIII was shown by acetylation of the remaining free hydroxyl group and examination of the product (VIIIa) by NMR. The large (9.5 Hz) and small (3 Hz) coupling observed for the hydrogen attached to the acetoxylated carbon demonstrates that this hydrogen is axial (*a*/*e* coupling to H-2, *a*/*a* coupling to H-4). This was confirmed by NMR in the presence of tris(dipivaloylmethanato)-europium (Table 1).

Condensation of VIII with the 3,6-dideoxy-glycosyl bromide VI in acetonitrile in the



presence of mercuric cyanide,[6] afforded the protected disaccharide IX in 54 – 70 % yield. *p*-Nitrobenzoyl groups were removed from IX by treatment with barium oxide in methanol. Further deprotection of the disaccharide and conversion to the *p*-aminophenyl glycoside XV by catalytic hydrogenation proved unsatisfactory because the nitro group was converted to an amino group and thereafter hydrogenolysis proceeded very slowly, with a maximum yield of XV from IX of only 10 %. We therefore proceeded by a more circuitous route. The disaccharide derivative X was acetylated (XI), the nitro group of XI was converted to an amino group by brief catalytic hydrogenation, then the amino group of XII was tri-fluoroacetylated giving XIII. Catalytic hydrogenation of XIII proceeded in good yield to give XIV which upon treatment with methanolic ammonia afforded XV. This more indirect route has the added attraction of crystalline intermediates (XI, XIII, and XIV). Treatment of the *p*-aminophenyl glycoside XV with thiophosgene [14] afforded the title compound XVI in a 44 % yield from IX.

Although the above synthetic scheme may appear lengthy, the good yields in each step and the relative ease of purification of the intermediates have permitted the synthesis of the disaccharide derivative IX in decagram quantities. The compound IX is stable and suitable for storage. Further conversions into the title compound XVI, in particular the conversion of XIV into XVI, have therefore only been carried out on a small scale, producing small quantities of XVI required for disaccharide-protein conjugates as directed by the needs of the immunological part of our studies.

EXPERIMENTAL

General methods. Melting points are corrected. Concentrations were performed at reduced pressure and a bath temperature below 40 °C. Optical rotations (*c* 0.5 – 1.0) were recorded at room temperature using a Perkin-Elmer 141 instrument. IR spectra were recorded in KBr discs using a Perkin-Elmer 257 instrument. NMR spectra were recorded with a Varian A-60 A instrument. Analytical TLC was performed on precoated silica gel F_{254} plates (Merck). Sulfuric acid was used as spray reagent. Separations on a scale less than 5 g were performed using Merck's prepacked silica gel columns; for larger-scale separations silica gel (Merck) of particle size 0.040 – 0.063 mm was used. GLC-MS was performed using Perkin-Elmer 900 and 270 instruments and an ECNSS-M column (3 % on silanized Gas-Chrom Q) for separation of components prior to recording mass spectra (manifold temperature 200 °C, ionization potential 70 eV, ionization current 60 μA, temperature at the ion source chamber 120 °C).

NMR spectra recorded for all new compounds were in agreement with the postulated structures. Pertinent NMR parameters are shown in Table 1.

Methyl 4-O-benzoyl-6-bromo-3,6-dideoxy-α-D-arabino-hexopyranoside (II). *N*-Bromosuccinimide (0.88 g) was added to a suspension of methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*arabino*-hexopyranoside (I, 1.12 g) and barium carbonate (3 g) in carbon tetrachloride (80 ml).[8,9] The mixture was refluxed with stirring for 2 h, diluted with chloroform and filtered. The filtrate was shaken with water and the organic phase dried over sodium sulfate, filtered and concentrated to a colourless amorphous powder (1.5 g). TLC (chloroform-methanol 20:1) showed the presence of major component, which was isolated by preparative TLC using the same solvent (or by column chromatography on silica gel in other preparations) to yield pure I (1.03 g). Material thus purified had $[\alpha]_D$ +96° (chloroform) (Found: C 48.9; H 5.01; O 23.0; Br 23.3. $C_{14}H_{17}O_5Br$ requires: C 48.7; H 4.96; O 23.2; Br 23.2).

Methyl 4-O-benzoyl-3,6-dideoxy-α-D-arabino-hexopyranoside (III). A solution of the 6-bromo compound II (3.7 g) in methanol (100 ml) containing 10 % palladium on carbon (0.5 g) and excess triethylamine (5 ml) was hydrogenated at 410 kPa and room temperature until no more hydrogen was consumed. Filtration and concentration gave a residue which was dissolved in dichloromethane. The dichloromethane solution was extracted with saturated aqueous sodium hydrogen carbonate and then water. After drying over sodium sulfate and filtering, the solution was concentrated to a syrup (2.61 g) which was used without further purification in the next step. An aliquot was purified by TLC (chloroform-methanol 100:5) to give a syrup, $[\alpha]_D$ +111° (chloroform) (Found C 63.1; H 6.94; $C_{14}H_{18}O_5$ requires: C 63.1; H 6.81).

Methyl 3,6-dideoxy-α-D-arabino-hexopyranoside (IV). The above 4-benzoate (47 g) was dissolved in methanol (500 ml). Sodium (0.95 g) was added and the solution refluxed for 1 h. TLC (chloroform-methanol 20:1) showed that no more starting material remained. The reaction mixture was neutralized with solid carbon dioxide, concentrated, suspended in water and the aqueous phase shaken twice with hexane (150 ml) and concentrated to dryness. The product was extracted with ethyl acetate; the combined ethyl acetate extracts were filtered and concentrated to a syrup (26.6 g), which

was chromatographically pure (above solvent) and indistinguishable from an authentic sample of IV by NMR and by GLC-MS on the derived alditol acetate.

*Methyl 3,6-dideoxy-2,4-di-*O*-(p-nitrobenzoyl)-*α*-D-arabino-hexopyranoside (V).* Methyl tyveloside (IV, 2.41 g) was dissolved in pyridine (150 ml) and cooled to 0 °C. *p*-Nitrobenzoyl chloride (8.28 g) was added with stirring. The reaction mixture was kept at 0 °C for 1 h and then at room temperature for 7 h. The solution was poured with stirring into ice-water (200 ml). After stirring for 15 min the aqueous mixture was extracted with chloroform. The combined chloroform extracts were shaken with, in turn, ice-cold 0.25 M aqueous sulfuric acid, saturated aqueous sodium hydrogen carbonate and water, dried over sodium sulfate and concentrated to a syrup (5.13 g) which crystallized from methanol, m.p. 139 – 141 °C; $[\alpha]_{578}$ – 59° (chloroform). (Found: C 55.0; H 4.56; N 6.20. $C_{21}H_{20}N_2O_{10}$ requires C 54.8; H 4.38; N 6.09).

*3,6-Dideoxy-2,4-di-*O*-(p-nitrobenzoyl)-*α*-D-arabino-hexopyranosyl bromide (VI).* A solution of the above glycoside V (2.0 g) in dichloromethane (60 ml) was cooled to 0 °C and then saturated at this temperature with hydrogen bromide. The reaction was monitored by TLC (toluene-ethyl acetate 4:1).
After 2 h at 0 °C, when no starting material remained, the solution was concentrated to a syrup (2.2 g) which was used directly in the next step. The NMR spectrum was compatible with the presumed structure (Table 1). *

p-*Nitrophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (VIII).* *p*-Nitrophenyl α-D-mannopyranoside was converted into the 4,6-benzylidene derivative as described by Buchanan and Schwarz for the corresponding methyl mannoside.[10] The benzylidene compound VII had m.p. 116 – 117 °C, $[\alpha]_D$ +196° (chloroform) and a satisfactory elemental analysis. It was converted into the monobenzyl derivative VIII by treatment with approximately 1 equivalent of benzyl bromide and silver oxide in dimethyl formamide.[11,12] The product VIII was obtained in a 56 % yield and had m.p. 118 – 120 °C, $[\alpha]_D$ +118° (chloroform) and a satisfactory elemental analysis. Details of the preparation of the two above compounds will be communicated in a separate paper.[13] An aliquot of VIII was converted into the 3-*O*-acetyl derivative VIIIa by acetylation at room temperature with acetic anhydride and pyridine. The usual work-up afforded VIIIa, the NMR spectrum of which is described in Table 1.

p-*Nitrophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(3,6-dideoxy-2,4-di-*O*-p-nitrobenzoyl-α-D-arabino-hexopyranosyl)-α-D-mannopyranoside (IX).* The hexosyl bromide VI (prepared from 25 g of V and used immediately) was dissolved in acetonitrile (62.5 ml) and the solution added over a period of 1 h to a mixture of VIII (25 g) and mercuric cyanide (9.25 g) in acetonitrile (62.5 ml) while the mixture was stirred at room temperature in an atmosphere of dry nitrogen. After the addition was completed, stirring was continued for 3 h. The reaction mixture was diluted with chloroform (600 ml). The chloroform solution was shaken with saturated aqueous sodium hydrogen carbonate and then water, dried over magnesium sulfate, filtered and concentrated to a syrup (51 g). Column chromatography on silica gel (toluene-ethyl acetate 8:1) yielded chromatographically homogeneous IX, R_F 0.65 (TLC, same solvent) (27 g) $[\alpha]_{578}$ +42° (chloroform). In another experiment on the 2 g scale, and otherwise as described above, a 70 % yield of pure IX was obtained.

*p-Nitrophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2,4-di-*O*-acetyl-3,6-dideoxy-α-D-arabino-hexopyranosyl)-α-D-mannopyranoside (XI).* The above disaccharide derivative IX (4 g) in methanol (1000 ml) containing barium oxide (2 g) was refluxed for 1 h. The mixture was concentrated to a syrup which was taken up in ethyl acetate (300 ml) and filtered. The filtrate was concentrated and purified by silica gel column chromatography (toluene-ethyl acetate 1:2) to yield chromatographically pure X, R_F 0.56 (TLC in the same solvent system) (2.45 g) $[\alpha]_{578}$ +125° (chloroform). X (2.25 g) was acetylated with acetic anhydride (15 ml) in pyridine (20 ml) overnight at room temperature. The product was concentrated and excess reagents removed by repeated codistillation with toluene. The diacetate XI was obtained as crystals from ethanol, m.p. 100 – 105 °C $[\alpha]_{578}$ +146° (chloroform). (Found C 62.2; H 5.65; N 1.97. $C_{36}H_{39}O_{13}N$ requires: C 62.3; H 5.67; N 2.02.

p-*Trifluoroacetamidophenyl-3-*O*-(2,4-di-*O*-acetyl-3,6-dideoxy-α-D-arabino-hexopyranosyl)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (XIII).* The above disaccharide derivative XI (2.40 g) was hydrogenated at room temperature and atmospheric pressure in ethyl acetate (150 ml) using Adam's catalyst (0.25 g). When the hydrogen comsumption had ceased, trifluoroacetic anhydride (2.5 ml) and pyridine (6 ml) were added. The solution was kept at 60 °C for 30 min, filtered and concentrated. The residue was dissolved in toluene (200 ml) and shaken with water, in order to remove pyridinium salts. The organic layer was dried over magnesium sulfate, filtered and concentrated to yield XIII (2.63 g) which crystallized from ethanol, m.p. 166 – 167 °C $[\alpha]_{578}$ +113° (chloroform) (Found: C 60.0; H 5.26; N 1.95; F 7.39. $C_{38}H_{40}NO_{12}F_3$ requires C 60.1; H 5.31; N 1.84; F 7.50).

p-*Trifluoroacetamidophenyl 3-*O*-(2,4-di-*O*-acetyl-α-D-arabino-hexopyranosyl)-α-D-mannopyranoside (XIV).* The above disaccharide derivative XIII (1.64 g) in 95 % ethanol

* *Added in proof.* A crude sample had $[\alpha]_{578}$ +23° (chloroform).

(60 ml) and tetrahydrofuran (40 ml) was hydrogenated at room temperature and atmospheric pressure using 10 % palladium on charcoal (1.5 g). When hydrogen consumption ceased, the catalyst was removed by filtration and the filtrate concentrated to dryness. The crude XIV (1.20 g) thus obtained crystallized and was recrystallized from ethyl acetate-diethyl ether to give the pure substance m.p. 228–230 °C (dec.). $[\alpha]_{578}$ +127° (methanol). (Found: C 49.8; H 5.31; N 2.55; F 9.94. $C_{24}H_{30}NO_{12}F_3$ requires: C 49.6; H 5.20; N 2.41; F 9.80).

p-*Isothiocyanatophenyl* 3-O-*(3,6-dideoxy-α-*D-arabino-*hexopyranosyl)-α-*D-*mannopyranoside (XVI)*. The above disaccharide derivative XIV (56 mg) was dissolved in saturated ammoniacal methanol (10 ml) in a capped serum bottle under nitrogen and allowed to stand at room temperature for 12 h. The solution was concentrated to a syrup which contained one component (XV) only (TLC, chloroform-methanol 3:1). The syrup was dissolved in 80 % aqueous ethanol (10 ml). The pH was adjusted to about 8 by adding barium carbonate and kept at this level by adding more barium carbonate as required throughout the reaction. After addition of thiophosgene (0.05 ml) the mixture was stirred for 1.5 h. Filtration and concentration gave a syrupy compound which was purified by TLC (chloroform-methanol 3:1) to give pure XVI (25 mg), $[\alpha]_D$ +180°. IR showed a broad absorption band centered at 2130 cm^{-1}.

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