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

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A synthesis of the disaccharide derivative *p*-isothiocyanatophenyl 3-*O*-(3,6-dideoxy-α-D-*xylo*-hexopyranosyl)-α-D-mannopyranoside, corresponding to the Salmonella O-factor 4, is described. The *p*-isothiocyanatophenyl group is useful for attaching the abequosylmannosyl disaccharide unit to free amino groups in a protein. The glycosylation step in the synthetic sequence was performed using the Helferich procedure and 2,4-di-*O*-*p*-nitrobenzoyl-α-abequosyl bromide as the glycosylating reagent.

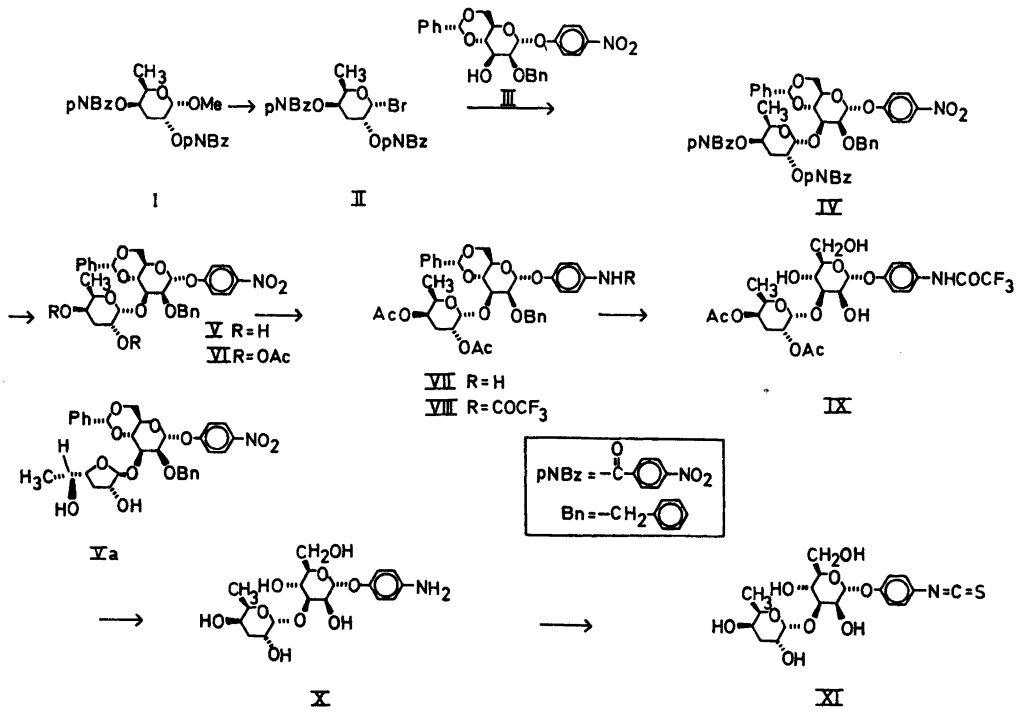
Access to α-D-3,6-dideoxyhexosyl-(1→3)-α-D-mannosides in which the dideoxyhexose residue is abequose, paratose or tyvelose and with the mannose residue linked to an aglycone containing a functional group suitable for attachment to proteins is of considerable immunological interest.[1] These three antigens include the majority of the more severe types of Salmonellosis from a diagnostic point of view and are of potential value for prophylaxis.

We have previously synthesized *p*-isothiocyanatophenyl 3-*O*-α-tyvelosyl-α-D-mannopyranoside [2] and have demonstrated its immunological potential.[1] We now describe the synthesis of the analogous abequosyl disaccharide XI, corresponding to Salmonella O-factor 4.[3]

In Ref. 4 we describe the synthesis of an α-abequosyl disaccharide, using two different abequosyl bromides, one with a non-participating benzyl group in the 2-position and a *p*-nitrobenzoyl group in the 4-position, and the other with *p*-nitrobenzoyl groups in both positions. These exploratory studies showed that a non-participating group at C-2 was unneccessary for obtaining a good yield of the desired α-abequosyl disaccharide and the more readily accessible 2,4-di-*O*-*p*-nitrobenzoyl-α-abequosyl bromide (II) was therefore used in the present work. A complication, however, arose in the use of II in abequoside synthesis. Treatment of methyl 2,4-di-*O*-*p*-nitrobenzoyl-α-abequoside (I) with hydrogen bromide in dichloromethane to obtain II and the subsequent use of the crude product in glycosylation also produced a β-furanoside as a minor component.[4] Bock and Pedersen have shown that treatment of acetylated methyl glycopyranosides with hydrogen bromide in acetic acid gives glycofuranosyl bromides in addition to the expected, glycopyranosyl bromides.[5] In the present work a minor component of glycosylation yields NMR data, suggesting, by analogy [4,5] the presence of an abequosyl unit in the β-furanose ring form.

The major product of treatment of methyl 2,4-di-*O*-*p*-nitrobenzoyl-α-abequoside [6] (I) with hydrogen bromide in dichloromethane is the α-bromo sugar II. The α-configuration is indicated by the optical rotation ($[\alpha]_D + 181°$) and by NMR ($J_{1,2}$ 4 Hz).

Mercury cyanide in benzene-nitromethane was used to promote the condensation of bromo sugar II with the mannoside III.[7] All hydroxyl groups on the latter were protected except for the one at C-3.[2] After chromatography the disaccharide derivative IV was obtained in a 42 % yield. The assignment of the anomeric configuration is based on optical rotation and NMR. The anomeric signals of the abequosyl and mannosyl residues were identified and differentiated by comparison with NMR results,



including spin decoupling experiments, obtained for the analogous derivative of methyl 3-*O*-α-abequosyl-α-D-mannopyranoside.[4]

The following steps, from IV to XI, were analogous to those previously described for the corresponding tyvelosyl disaccharide:[2] The *p*-nitrobenzoyl groups in the abequose residue in IV were removed (V) and replaced by acetyl groups (VI). The nitro group in VI was reduced by hydrogenation with Adams catalyst (VII) and the resulting amino group was protected with a trifluoroacetyl group (VIII). Benzyl and benzylidene groups were removed by catalytic hydrogenation (IX). Treatment of IX with methanolic ammonia afforded the product X, which was converted into the isothiocyanatophenyl [8] compound XI. The conversion of V to XI proceeded in yields exceeding 85 % in the individual steps.

EXPERIMENTAL

General methods were the same as those recently reported.[2] NMR spectra were recorded using a Varian XL-100 instrument, in deuteriochloroform unless otherwise stated. Chemical shifts were recorded in ppm downfield from tetramethylsilane as internal standard. NMR spectra were recorded for all new compounds and were in agreement with postulated structures. Only selected NMR data are therefore presented below.

3,6-Dideoxy-2,4-di-O-(p-*nitrobenzoyl*)-α-D-xylo-*hexopyranosyl bromide (II).* A solution of methyl 2,4-di-*O*-*p*-nitrobenzoyl-α-abequoside (I) [6] (0.5 g) in dichloromethane (25 ml) was cooled to −20 °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 −20 °C when no starting material remained, the solution was concentrated to a syrup which was used directly in the next step. The optical rotation of the syrup was $[\alpha]_D$ +181°. NMR: δ 1.30 (3 H, d, $J_{5,6}$ 7 Hz, H-6), 2.2–2.8 (2 H, m, H-3, H-3′), 4.44 (1 H, q, $J_{5,6}$ 7 Hz, H-5), 5.16-5.48 (2 H, m, H-2, H-4), 6.84 (1 H, d, $J_{1,2}$ 4 Hz, H-1).

p-*Nitrophenyl* 2-O-*benzyl-4,6*-O-*benzylidene-3*-O-*(3,6-dideoxy-2,4-di*-O-p-*nitrophenyl*-α-D-xylo-*hexopyranosyl)*-α-D-*mannopyranoside (IV).* A solution of *p*-nitrophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (III) [2] (505 mg) in nitromethane-benzene 1:1 (20 ml) was boiled until 6 ml solvent mixture had distilled off and then it was cooled to room temperature. Mercury(II) cyanide (353 mg) and the glycosyl halide II (prepared from 500 mg of the glycoside

I and used immediately) dissolved in dry dichlormethane (2 ml) were added and the solution was stirred at room temperature under nitrogen for 24 h. The mixture was diluted with toluene and washed with water, saturated aqueous sodium hydrogen carbonate and finally water. The solution was dried over anhydrous sodium sulfate, filtered and concentrated to a syrup (970 mg). Column chromatography on silica gel (toluene – ethyl acetate 16:1) yielded chromatographically homogeneous IV, R_F 0.45 (TLC, same solvent system) (406 mg). The material crystallized from ethanol, m.p. 127 – 129 °C, $[\alpha]_D$ +123° (chloroform). (Found: C 60.9; H 4.66; N 4.84. $C_{46}H_{41}N_3O_{17}$ requires: C 60.9; H 4.53; N 4.63). NMR: δ 1.18 (3 H, d, $J_{5,6}$ 7 Hz, H-6), 1.6 – 2.7 (2 H, m, H-3, H-3'), 4.83, 4.93 (2 H, AB spectrum, $J_{H,H'}$ 12 Hz, benzylic protons), 5.20 (1 H, $J_{1,2}$ 4 Hz, H-1, abequose residue), 5.52 (1 H, s, benzylidene proton), 5.75 (1 H, d, $J_{1,2}$ 1.5 Hz, H-1, mannose residue), 7.0 – 8.4 (22 H, aromatic protons).

p-Nitrophenyl 2-O-benzyl-4,6-O-benzylidene-3-O-(3,6-dideoxy-α-D-xylo-hexopyranosyl)-α-D-mannopyranoside (V). The above disaccharide derivative IV (406 mg) in methanol (100 ml) containing barium oxide (200 mg) was refluxed for 1 h. The mixture was neutralized with solid carbon dioxide and concentrated. The residue was taken up in ethyl acetate (100 ml) and filtered. The filtrate was concentrated to a syrup which was purified on a silica gel column (toluene-ethyl acetate 2:3) to yield chromatographically pure V (195 mg), $[\alpha]_D$ +114° (chloroform). NMR: δ 1.17 (3 H, d, $J_{5,6}$ 7 Hz, H-6 abequose residue), 1.84 (2 H, broad s, OH), 1.9 – 2.5 (2 H, m, H-3 and H-3' abequose residue), 4.79 (2 H, s, benzylic protons), 5.13 (1 H, d, $J_{1,2}$ 4 Hz, H-1, abequose residue), 5.61 (2 H, nearly s, benzylidene and H-1, mannose residue), 7.08 and 8.19 (2 H each, both d, both $J_{H,H}$ 9.5 Hz, p-$NO_2C_6H_4O$-protons), 7.2 – 7.5 (10 H, other aromatic protons).

In a separate experiment the above major component IV was isolated together with a minor one. Debenzoylation of this mixture as described above for IV afforded a product containing V and the presumed furanoside Va in a ratio of 92:8. Chromatographically homogeneous Va (TLC, toluene – ethyl acetate 1:1) had $[\alpha]_D$ +40° (chloroform). NMR for Va: δ 1.17 (3 H, d, $J_{5,6}$ 7 Hz, H-6, abequose residue), 1.6 – 2.6 (2 H, m, H-3 and H-3', abequose residue), 4.70, 4.84 (2 H, AB spectrum, $J_{H,H}$ 12 Hz, benzylic protons), 5.00 [1 H, $J_{1,2}$ 0 Hz, H-1, abequose (furanosidic residue)], 5.55 (1 H, s, benzylidene proton), 5.58 (1 H, d, $J_{1,2}$ 1.5 Hz, H-1, mannose residue), 5.07 and 8.18 (2 H each, d, each $J_{1,2}$ 9 Hz, p-$NO_2C_6H_4O$-protons).

p-*Nitrophenyl-2-O-benzyl-4,6-O-benzylidene-3-O-(2,4-di-O-acetyl-3,6-dideoxy-α-D-xylo-hexopyranosyl)-α-D-mannopyranoside* (VI). V (771 mg) was acetylated with acetic anhydride (5 ml) in pyridine (7 ml) at room temperature overnight. The solution was concentrated yielding the diacetate VI (815 mg) which crystallized from diethyl ether-light petroleum, m.p. 95 – 98 °C, $[\alpha]_D$ +107° (chloroform). (Found: C 62.0; H 5.67; N 1.97. $C_{36}H_{39}O_{13}$ requires: C 62.3; H 5.67; N 2.02). NMR: δ 1.07 (3 H, d, $J_{5,6}$ 7 Hz, H-6 abequose residue), 1.62 (3 H, s, 2-OAc), 2.12 (3 H, s, 4-OAc), 1.8 – 2.4 (2 H, m, H-3 and H-3' abequose residue), 4.75, 4.85 (2 H, AB spectrum $J_{H,H}$ 13 Hz, benzylic protons), 5.32 (1 H, d, $J_{1,2}$ 4 Hz, H-1, abequose residue), 5.58 (1 H, s, benzylidene proton), 5.70 (1 H, d, $J_{1,2}$ 1.5 Hz, H-1, mannose residue, 7.13 and 8.21 (1 H each, both d, both $J_{H,H'}$ 9 Hz), p-$NO_2C_6H_4O$-protons), 7.3 – 7.5 (10 H, other aromatic protons).

p-*Trifluoroacetamidophenyl 3-O-(2,4-di-O-acetyl-3,6-dideoxy-α-D-xylo-hexopyranosyl)-2-O-benzyl-4,6-O-benzylidene-α-D-mannopyranoside (VIII).* The foregoing compound VI (850 mg) was hydrogenated at room temperature and atmospheric pressure in ethyl acetate (50 ml) using Adams catalyst (80 mg). When sufficient hydrogen to account for the conversion of a nitro to an amino group had been consumed, trifluoroacetic anhydride (0.8 ml) and pyridine (1.9 ml) were added and the mixture was kept at 60 °C for 30 min. The catalyst was removed by filtration and the filtrate was concentrated. The residue was dissolved in toluene and shaken with water. The organic layer was dried over sodium sulfate, filtered and concentrated to a syrup (848 mg) which crystallized from methanol, m.p. 105 – 107 °C, $[\alpha]_D$ +74° (chloroform). A satisfactory elemental analysis could not be obtained for the compound. NMR: δ 1.07 (3 H, d, $J_{5,6}$ 7 Hz, H-6 abequose residue), 1.8 – 2.3 (2 H, m, H-3 and H-3', abequose residue), 1.62 (3 H, s, 2-OAc), 2.12 (3 H, s, 4-OAc), 4.70, 4.84 (2 H, AB spectrum, $J_{H,H'}$ 13 Hz, benzylic protons), 5.32 (1 H, d, $J_{1,2}$ 4 Hz, H-1, abequose residue), 5.57 (1 H, s, benzylidene proton), 5.60 (1 H, d, $J_{1,2}$ 1.5 Hz, H-1, mannose residue), 7.05 and 7.50 (2 H each, both d, both $J_{H,H'}$, 9 Hz, p-$CF_3CONHC_6H_4O$-), 7.2 – 7.6 (10 H, other aromatic protons).

p-*Trifluoroacetamidophenyl 3-O-(2,4-di-O-acetyl-3,6-dideoxy-α-D-xylo-hexopyranosyl)-α-D-mannopyranoside (IX).* The syrupy product VIII (780 mg) from the above reaction was hydrogenated at atmospheric pressure in ethanol (30 ml) using 10 % palladium on charcoal (300 mg) as catalyst. When hydrogen consumption had ceased, the catalyst was removed by filtration and the filtrate was concentrated to yield IX as a syrup (611 mg). This material was chromatographically pure on TLC (chloroform-methanol 3:1). A small amount was purified by column chromatography on silica gel and crystalline IX was obtained from ethyl acetate-light petroleum, m.p. 191 – 194 °C, $[\alpha]_D$ +145° (methanol). (Found: C 50.2; H 5.39; N 2.37; F 9.70. $C_{24}H_{30}NO_{12}F_3$ requires: C 49.6; H 5.20; N 2.41; F 9.80. NMR: δ 1.12 (3 H, d, $J_{5,6}$ 7 Hz, H-6 abequose residue), 1.8 – 2.6 (2 H, m, H-3, H-3' abequose residue), 2.08

(3 H, s, 2-OAc), 2.14 (3 H, s, 4-OAc), 5.29 (1 H, d, $J_{1,2}$ 4 Hz, H-1 abequose residue), 5.49 (1 H, d, $J_{1,2}$ 1.5 Hz, H-1, mannose residue), 7.15 and 7.56 (2 H each, both d, both $J_{H,H'}$ 9 Hz, *p*-$CF_3CONHC_6H_4O-$).

p-*Isothiocyanatophenyl 3-O-(3,6-dideoxy-α-D-xylo-hexopyranosyl)-α-D-mannopyranoside (XI).* The above pure disaccharide derivative IX (98 mg) was dissolved in saturated methanolic ammonia (10 ml) in a capped serum bottle and kept at room temperature for 48 h. Concentration afforded a syrup which on TLC (chloroform – methanol 3:1) showed the presence of one component only (X). The syrup was dissolved in 80 % aqueous ethanol (20 ml) and the pH was adjusted to about 8 by adding barium carbonate and maintained at this pH level throughout the reaction by adding more barium carbonate as required. Thiophosgene (0.1 ml) was added and the reaction was stirred for 30 min. Filtration and concentration gave a syrup which was purified by TLC (chloroform – methanol 3:1) to give pure XI (67 mg), $[\alpha]_D$ +158°. IR (KBr) showed a broad absorption band centred at 2120 cm^{-1}. The material crystallized to a low-melting hygroscopic material with m.p. too diffuse for useful characterization. NMR (CD_3OD): δ 1.16 (3 H, d, $J_{5,6}$ 7 Hz, H-6 abequose residue), 1.80 – 2.30 (2 H, m, H-3, H-3' abequose residue, 5.06 (1 H, d, $J_{1,2}$ 4 Hz, H-1, abequose residue), 5.49 (1 H, d, $J_{1,2}$ 1.5 Hz, H-1, mannose residue), 7.00 – 7.30 (4 H, aromatic protons).

An aliquot of XI was hydrolyzed with 0.25 M aqueous sulfuric acid for 12 h at 100 °C. The product was reduced with sodium borohydride and acetylated.[9] The abequitol triacetate and mannitol pentaacetate thus obtained were indistinguishable from authentic materials on GLC [9] and MS.[10]

Another aliquot of IX was methylated,[11] hydrolyzed, reduced with sodium borohydride and acetylated.[9] The two methylalditol acetates thus obtained were indistinguishable from authentic 1,5-di-*O*-acetyl-3,6-dideoxy-2,4-di-*O*-methyl-D-*xylo*-hexitol and 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-mannitol, respectively, on GLC and MS.[12]

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