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Note

Synthesis of *p*-nitrophenyl 3-*O*-(3,6-dideoxy-α-D-*xylo*-hexopyranosyl)-α-L-rhamnopyranoside for making artificial antigens corresponding to the *Salmonella* O-factor 8

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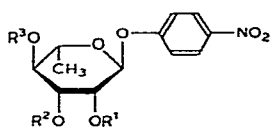
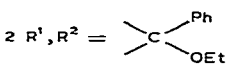
Artificial antigens containing 3,6-dideoxyhexosylmannose residues joined through *p*-aminophenyl units to proteins by means of a thiourea linkage are useful for the diagnosis of *Salmonella* infections brought about by bacteria that have cell-wall lipopolysaccharides containing these disaccharide moieties as immunological determinants. We have previously made artificial antigens corresponding to the *Salmonella* O-factors[1] 2, 4, and 9, and have evaluated their use in diagnosis[2].

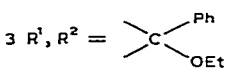
We now report the synthesis of the title abequosylrhamnoside **7**, corresponding to the *Salmonella* O-factor 8 (serogroups C_2 and C_3). After reduction of **7** to the corresponding *p*-aminophenyl compound, treatment with thiophosgene gave the corresponding isothiocyanate, which reacts with free amino groups in bovine serum albumin, thereby joining the disaccharide residue of **7** to the protein *via* a phenylthiourea grouping[3]. Tests now in progress show that the resulting material gives rise to anti-O-8 antibodies that are useful in the diagnosis of *Salmonella* infections.



1 $R^1 = R^2 = R^3 = H$

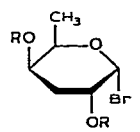


2 $R^1, R^2 =$; $R^3 = H$

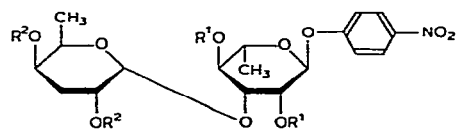


3 $R^1, R^2 =$; $R^3 = Bz$

4 $R^1 = R^3 = Bz$; $R^2 = H$



5 R = *p*-NO_2-Bz



6 $R^1 = Bz, R^2 = p$-NO_2-Bz
7 $R^1 = R^2 = H$
8 $R^1 = R^2 =$ Ac

p-Nitrophenyl α-L-rhamnopyranoside[4,5] (**1**) was converted into the 2,3-(ethyl orthobenzoate) **2**, which was benzoylated in the 4-position to give **3**. Opening of the orthoester grouping of **3** with weak acid occurred with the expected cleavage of the

equatorial *O*-3-orthoester carbon-bond[6], and yielded the 2,4-dibenzoate **4**. The sequence **1**→**4** was carried out without the purification of intermediates. Each reaction step gave only one main product and, after chromatographic purification, syrupy **4** was obtained in 57% yield from **1**. The structure of the 2,4-dibenzoate **4** was demonstrated by means of n.m.r. spectroscopy and by methylation analysis[7]. The 3-acetate of **4** was crystalline. The reaction of **4** with the abequosyl bromide[8] **5** in dichloromethane, with mercury(II) cyanide as promoter[9] and chromatographic purification of the product, gave the disaccharide derivative **6** in 54% yield. Debenzoylation of **6** with methanolic sodium methoxide then gave the title compound **7**, which was characterized by ^{1}H- and ^{13}C-n.m.r. data, optical rotation, hydrolysis and analysis of the monosaccharides obtained[10,11], and methylation analysis[7]. The tetra-aceate (**8**) of **7** was crystalline.

EXPERIMENTAL

General. — General methods were the same as those previously reported[2,12]. The purity of syrupy new compounds, for which elemental analyses were not performed, was carefully ascertained in solvent systems that gave R_F values of ~0.5, and the substances were rechromatographed until they were pure. N.m.r. data were recorded for all new compounds and were invariably in agreement with the postulated structures. Whenever necessary, the n.m.r. assignments were confirmed by spin-decoupling experiments. Only selected n.m.r. data are recorded below. Assignments of anomeric configurations are further based upon the optical rotations.

p-*Nitrophenyl 2,4-di*-O-*benzoyl*-α-L-*rhamnopyranoside* (**4**). — A solution of *p*-nitrophenyl α-L-rhamnopyranoside[4,5] (**1**, 3.0 g), triethyl orthobenzoate (30 ml), and *p*-toluenesulfonic acid (12 mg) in dry *N*,*N*-dimethylformamide (35 ml) was stirred at room temperature for 48 h. The solution was diluted with dichloromethane, washed with saturated aqueous sodium hydrogencarbonate and water, dried (Na_2SO_4), filtered, and concentrated to yield syrupy **2**, which was used directly in the next step. Benzoyl chloride (1.4 ml) was added slowly at room temperature to a solution of **2** (3.7 g) in dry pyridine (30 ml), and the solution was kept at room temperature for 3 h. Ice–water was added and the mixture was extracted with chloroform. The chloroform layer was washed with water, dried (Na_2SO_4), filtered, and concentrated to yield syrupy **3**, which was used directly in the next step. Aqueous 80% acetic acid (50 ml) was added to **3**, the mixture was stirred for 10–15 min, and the resulting solution was concentrated. The syrupy residue was purified on a prepacked column of silica gel (toluene–ethyl acetate, 6:1), to yield **4** (3.0 g), $[\alpha]_D$ −85° (*c* 1.1, chloroform). 100-MHz n.m.r. data ($CDCl_3$): δ 1.32 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6), 4.04–4.19 (m, 1 H, H-5), 4.37–4.63 (m, 1 H, H-3), 5.38 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.9 Hz), 5.59 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3.5 Hz, H-2), and 5.82 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1).

Conventional treatment of **4** with acetic anhydride in pyridine gave *p*-nitrophenyl 3-*O*-acetyl-2,4-di-*O*-benzoyl-α-L-rhamnopyranoside, m.p. 159–160°, $[\alpha]_D$ −50° (*c* 0.9, chloroform).

Anal. Calc. for $C_{28}H_{25}NO_{10}$: C, 62.8; H, 4.71; N, 2.62. Found: C, 62.7; H, 4.76; N, 2.60.

An aliquot of **4** was methylated with methyl trifluoromethanesulfonate[13,14]. The product was debenzoylated with methanolic sodium methoxide, hydrolyzed, and then reduced with sodium borohydride. Acetylation of this product gave a methylated alditol acetate that was indistinguishable from 1,2,4,5-tetra-*O*-acetyl-3-*O*-methylrhamnitol in g.l.c.–m.s.[7].

p-*Nitrophenyl 3*-O-(*3,6-dideoxy*-α-D-xylo-*hexopyranosyl*)-α-L-*rhamnopyranoside* (**7**). — A solution of 3,6-dideoxy-2,4-di-*O*-*p*-nitrobenzoyl-α-D-*xylo*-hexopyranosyl bromide[8] (**5**, 2.2 g) in dichloromethane (6 ml) was added under nitrogen to a stirred mixture of **4** (1.0 g) and mercury(II) cyanide (0.44 g) in dichloromethane (10 ml). After being stirred under nitrogen for 18 h, the mixture was diluted with dichloromethane, washed with water, saturated, aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), filtered, and concentrated. Syrupy **6** (1.0 g) was obtained as a foam following purification on a prepacked column of silica gel (toluene–ethyl acetate, 6:1); $[\alpha]_D$ +98° (*c* 1.0, chloroform). 100-MHz n.m.r. data ($CDCl_3$): δ 0.98 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6, abequose residue), 1.33 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6, rhamnose residue), and 2.18 (m, 2 H, H-3, abequose residue).

A catalytic amount of sodium was added to a solution of **6** (0.50 g) in methanol (20 ml). The solution was kept at room temperature for 16 h, neutralized with Dowex-50(H^+) resin, filtered, and concentrated. The product was purified on a prepacked column of silica gel (chloroform–methanol, 9:1), to yield **7** (0.20 g), $[\alpha]_D$ −30° (*c* 1.0, water). 100-MHz n.m.r. data (D_2O): δ 1.10 and 1.19 (2 d, each 3 H, $J_{5,6}$ 6.4, $J_{5,6}$ 6.9 Hz, H-6, abequose and rhamnose residues), 1.91–2.05 (m, 2 H, H-3, abequose residue), 4.96 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1, abequose residue), 5.64 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1, rhamnose residue), 7.16 and 8.13 (2 d, each 2 H, $J_{H,H}$ 9.3 Hz, aromatic H). 25-MHz ^{13}C-n.m.r. data (D_2O) for **7** (δ values in p.p.m. downfield from tetramethylsilane): δ 16.6 (C-6, abequose residue), 18.0 (C-6, rhamnose residue), 34.1 (C-3, abequose residue), 76.6 (C-3, rhamnose residue), 96.7 (C-1, rhamnose residue), 98.6 (C-1, abequose residue), 117.9, 127.3, 143.5, and 162.2 (aromatic C). No peaks other than those at δ 96.7 and 98.6 were observed in the anomeric region. Hydrolysis of **7**, followed by reduction and acetylation, gave alditol acetates that were indistinguishable from 1,2,4,5-tetra-*O*-acetylabequitol and 1,2,3,4,5-penta-*O*-acetylrhamnitol in g.l.c.–m.s.[10,11]. Methylation analysis of **7** yielded two compounds that were indistinguishable from 1,5-di-*O*-acetyl-2,4-di-*O*-methylabequitol and 1,3,5-tri-*O*-acetyl-2,4-di-*O*-methylrhamnitol in g.l.c.–m.s.[7].

Acetylation of **7** with acetic anhydride in pyridine yielded **8**, m.p. 180–181°, $[\alpha]_D$ −6° (*c* 1.2, chloroform).

Anal. Calc. for $C_{26}H_{33}NO_{14}$: C, 53.5; H, 5.70; N, 2.40. Found: C, 53.5; H, 5.69; N, 2.40.

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