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Note

Synthesis of *p*-aminophenyl 2-*O*-acetyl-3-*O*-α-L-rhamnopyranosyl-α-L-rhamnopyranoside*

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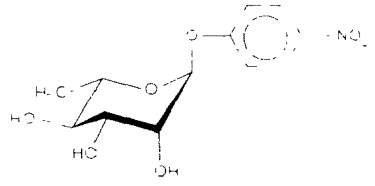
Artificial antigens prepared by linking di- and oligo-saccharides to suitable proteins are useful for making diagnostic reagents with high specificity[1]. We now describe an efficient synthesis of the title compound, which contains a structural element representing the *Shigella flexneri* O6 antigenic determinant[2]. The *O*-acetyl



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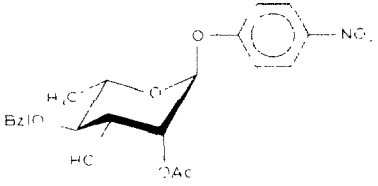

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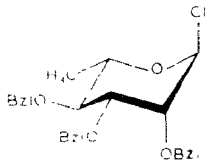

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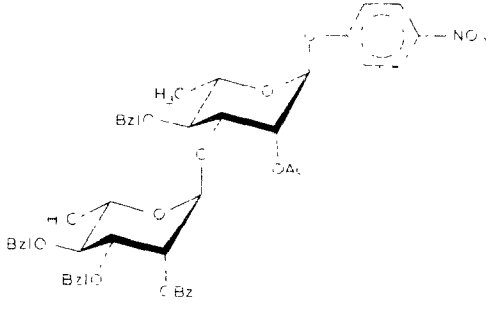

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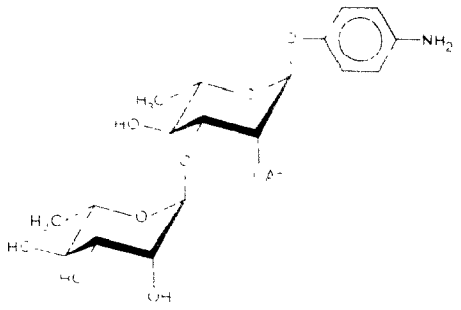

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*Disaccharides Related to *Shigella flexneri* O-Antigens. Part II. For Part I, see ref. 6.

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group was introduced at an early stage in the synthesis and only benzyl groups were used as protecting groups.

p-Nitrophenyl α-L-rhamnopyranoside (**1**) was treated first with triethyl orthoacetate under acid catalysis, and then with benzyl bromide and sodium hydride in *N,N*-dimethylformamide–toluene. On acidic opening of the resulting 2,3-orthoacetate, the 2-*O*-acetyl-4-*O*-benzyl derivative **2**, with an axially disposed *O*-acetyl group, was expected as the main product[4], and was formed in good yield (87%) and with high stereoselectivity. Silver triflate-promoted glycosidation of **2** with 2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl chloride (**3**) gave the disaccharide derivative **4** in 72% yield. Catalytic hydrogenation of **4** gave the title compound **5**. The immunological evaluation of **5** coupled to the amino group of a suitable protein carrier *via* its isothiocyanate derivative[5] will be reported elsewhere.

EXPERIMENTAL

The general methods were the same as those previously reported[6].

p-*Nitrophenyl 2-O-acetyl-4-O-benzyl-α-L-rhamnopyranoside* (**2**). — A mixture of *p*-nitrophenyl α-L-rhamnopyranoside[3] (**1**; 200 mg, 0.70 mmol), triethyl orthoacetate (1.0 mL), *p*-toluenesulfonic acid monohydrate (20 mg), and toluene (3 mL) was stirred at room temperature until a homogeneous solution was obtained (1 h). The mixture was diluted with dry *N,N*-dimethylformamide (3 mL), and sodium hydride (60 mg, 2.5 mmol) was added in portions. When hydrogen evolution had ceased, benzyl bromide (0.5 mL, 4.2 mmol) was added dropwise to the stirred mixture. After 3 h, methanol (1 mL) was added, and stirring was continued for 20 min. The mixture was then diluted with toluene, washed several times with water, and concentrated, to give a syrup containing mainly (^{13}C- and ^{1}H-n.m.r. spectroscopy) a diastereomeric mixture of 4-*O*-benzyl-2,3-orthoacetates. A solution of this mixture in 80% aqueous acetic acid was stored at room temperature for 1 h and then concentrated. The residue was purified by chromatography on silica gel with toluene–ethyl acetate (12:1), to give pure **2** (0.26 g, 87%) as a syrup, $[\alpha]_D$ −115° (*c* 1, chloroform). ^{13}C-N.m.r. ($CDCl_3$, 25°): δ 18.0 (C-6), 20.9 (acetyl CH_3), 68.9 (C-5), 69.7 (C-3), 71.9 (C-2), 75.3 (benzyl CH_2), 81.0 (C-4), 95.4 (C-1), 116.0, 125.5, 142.4, 160.4 (*p*-nitrophenyl C), and 170.4 (C=O). ^{1}H-N.m.r. ($CDCl_3$, 25°): δ 1.31 (d, $J_{5,6}$ 5.9 Hz, H-6), 2.20 (s, OAc), 2.48 (d, HO-3), 3.45 (t, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-4), 3.75 (m, H-5), 4.28 (m, H-3), 4.79 (q, benzyl CH_2), 5.27 (dd, $J_{1,2}$ 1.8, $J_{2,3}$ 3.7 Hz, H-2), 5.56 (d, $J_{1,2}$ 1.8 Hz, H-1), 7.08 (dt, *p*-nitrophenyl H), 7.33 (s, benzyl aromatic H), and 8.16 (dt, *p*-nitrophenyl H).

p-*Nitrophenyl 2-O-acetyl-4-O-benzyl-3-O-(2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside* (**4**). — Oxalyl chloride (0.4 mL) was added dropwise at −10° to a solution of 2,3,4-tri-*O*-benzyl-α-L-rhamnopyranose[7] (590 mg, 1.36 mmol) in dry *N,N*-dimethylformamide (5 mL). After stirring for 1 h at 0°, the mixture was diluted with dichloromethane, washed with water (0°), aqueous sodium hydrogencarbonate, and water, dried, and concentrated. The ^{1}H-n.m.r.

spectrum of the product (2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl chloride) indicated it to be at least 90% pure, and a solution in dichloromethane (2 mL) was added, with stirring and cooling to −40°, to a solution of **2** (410 mg, 0.98 mmol), silver triflate (440 mg), and 2,4,6-trimethylpyridine (0.15 mL) in dichloromethane (5 mL) containing 4Å molecular sieves. The mixture was allowed to attain room temperature, diluted with dichloromethane, and filtered. The filtrate was washed successively with aqueous sodium thiosulfate, water, 2M sulfuric acid, and aqueous sodium hydrogencarbonate, dried, and concentrated. Crystallisation of the syrupy residue from ethanol gave **4** (0.59 g, 72%), m.p. 110–111°, $[\alpha]_D$ −76° (*c* 1.1, chloroform). ^{13}C-N.m.r. ($CDCl_3$, 25°): δ 17.9 (C-6,6'), 21.0 (acetyl CH_3), 69.0 (C-5,5'), 71.5 (C-2), 71.9, 72.5, 75.0 (benzyl CH_2), 75.4 (C-2'), 77.4 (C-3), 79.3 (C-4,3'), 80.0 (C-4'), 94.8 (C-1), 100.4 (C-1'), 115.9, 125.4, 142.2, 160.2 (*p*-nitrophenyl C), and 170.0 (C=O). The $J_{C,H}$ spacings for C-1 and C-1' were 172 and 169 Hz, respectively, indicating[8] the α configuration at each anomeric center. ^{1}H-N.m.r. ($CDCl_3$, 25°): δ 1.24, 1.29 (2 d, H-6,6'), 2.17 (s, OAc), 5.12 (d, $J_{1,2}$ 1.0 Hz, H-1'), 5.30 (dd, $J_{1,2}$ 1.0, $J_{2,3}$ 3.2 Hz, H-2), and 5.56 (d, $J_{1,2}$ 1.0 Hz, H-1).

Anal. Calc. for $C_{48}H_{51}NO_{12}$: C, 69.1; H, 6.16; N, 1.68. Found: C, 69.3; H, 6.17; N, 1.63.

p-*Aminophenyl 2-O-acetyl-3-O-α-*L*-rhamnopyranosyl-α-*L*-rhamnopyranoside* (**5**). — A solution of compound **4** (250 mg) in 99% ethanol (10 mL) was hydrogenated at room temperature and atmospheric pressure over palladium-on-carbon (10%, 350 mg) for 18 h. Filtration and concentration then gave a syrup that was purified by chromatography on silica gel with ethyl acetate–methanol–water (80:15:5), to give pure **5** (108 mg, 81%), $[\alpha]_D$ −76° (*c* 1, water). ^{1}H-N.m.r. (D_2O, 25°): δ 1.68, 1.69 (2 overlapping doublets, $J_{5,6}$ 5.9 Hz, H-6,6'), 2.63 (s, OAc), 5.50 (d, $J_{1,2}$ 1.8 Hz, H-1'), 5.78 (dd, $J_{1,2}$ 1.8, $J_{2,3}$ 3.5 Hz, H-2), and 5.89 (d, $J_{1,2}$ 1.8 Hz, H-1). ^{13}C-N.m.r. (D_2O, 25°): δ 17.6 (C-6,6'), 21.2 (acetyl CH_3), 70.2, 70.4, 70.9, 71.0, 72.3, 72.5, 72.7, 77.2 (C-2–C-5 and C-2'–C-5'), 96.9 (C-1), 103.3 (C-1'), 119.3, 119.8, 143.0, 150.6 (aromatic C), and 173.6 (C=O). The $J_{C,H}$ spacings for C-1 and C-1' were 174 and 170 Hz, respectively, indicating[8] the α configurations at each anomeric center.

Compound **5** decomposed on storage and was therefore freshly prepared for use in coupling reactions *via* its isothiocyanate.

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REFERENCES

1 B. SVENUNGSSON AND A. A. LINDBERG, *Acta Pathol. Microbiol. Scand., Sect. B*, 87 (1979) 29–36.
2 L. KENNE, B. LINDBERG, K. PETERSSON, E. KATZENELLENBOGEN, AND E. ROMANOWSKA, *Eur. J. Biochem.*, 91 (1978) 279–284.
3 O. WESTPHAL AND H. FEIER, *Chem. Ber.*, 89 (1956) 582–588.
4 R. U. LEMIEUX AND H. DRIGUEZ, *J. Am. Chem. Soc.*, 97 (1975) 4069–4075.
5 D. H. BUSS AND I. J. GOLDSTEIN, *J. Chem. Soc., C*, (1968) 1457–1461.
6 M. FORSGREN AND T. NORBERG, *Carbohydr. Res.*, 116 (1983) 39–47.
7 J. M. J. FRÉCHET AND H. H. BAER, *Carbohydr. Res.*, 42 (1975) 369–372.
8 K. BOCK AND C. PEDERSEN, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–297.