Synthesis of *p*-Nitrophenyl 3-*O*-(2-Acetamido-2-deoxy-β-D-gluco-pyranosyl)-α-L-Rhamnopyranoside Corresponding to a Fragment of the *Streptococcus* Group A Cell Wall Polysaccharide

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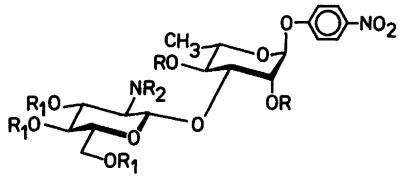
The cell-wall of *Streptococcus* Group A bacteria has been reported[1] to contain the polysaccharide depicted below:

β-D-GlcNAc*p*
1
↓
3
⟨→3)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→⟩$_n$

The disaccharide β-D-GlcNAc*p*-(1→3)-α-L-Rha*p* has now been synthesized in the form of the *p*-nitrophenyl glycoside *3*, suitable for coupling to proteins. Studies on the antigenic properties of the disaccharide coupled to a protein will be performed.

The free disaccharide has been prepared before[2] and also, recently, the 8-methoxycarbonyloctyl-α-glycoside.[3]

The synthesis of *3* is based on silver triflate-assisted glycosidation[4] of *p*-nitrophenyl 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside[5] with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phtalimido-β-D-glucopyranosyl bromide.[4] The protected disaccharide *1*, obtained in 64% yield after chromatography, was subsequently deprotected with hydrazine hydrate[4] and acetylated to give the crystalline acetate *2* in an 80% yield. De-*O*-acetylation gave the title disaccharide *3* which, after conversion[6] to the corresponding isothiocyanate derivative, can be used for coupling to proteins.



1: R = Bz, R_1 = Ac, R_2 =

2: R = R_1 = Ac, R_2 = 

3: R = R_1 = H, R_2 = 

Experimental. General methods were the same as those reported before.[7]

p-*Nitrophenyl 3-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phtalimido-β-D-glucopyranosyl)-2,4-di-O-benzoyl-α-L-rhamnopyranoside (1).* A mixture of *p*-nitrophenyl 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside[5] (1.20 g, 2.24 mmol), silver triflate (0.82 g, 3.2 mmol) and 2,4,6-trimethylpyridine (0.33 ml, 2.5 mmol) in 1:1 nitromethane – toluene (20 ml) containing powdered 4 Å molecular sieve was stirred and cooled to −20 °C. A solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phtalimido-β-D-glucopyranosyl bromide[4] (1.49 g, 3.0 mmol) in 1:1 nitromethane – toluene (10 ml) was added. After 15 min, more bromide (1.0 g) was added, followed by silver triflate (1.0 g) and 2,4,6-trimethylpyridine (0.4 ml). After 4 h at −20 °C, the mixture was diluted with 1:1 diethyl ether – ethyl acetate and filtered. The filtrate was washed successively with aqueous sodium thiosulfate, water, 2 M aqueous sulfuric acid and aqueous sodium hydrogencarbonate. The residue after drying ($MgSO_4$) and concentration was purified by column chromatography on silica gel (250 g). Dichloromethane – ethyl acetate – acetone (40:5:1) eluted pure *1* which upon concentration was obtained as a colorless foam (1.35 g, 64%), $[\alpha]_D$ −14° (*c* 0.5, $CHCl_3$). Further elution gave a fraction containing the dehydrohalogenation product of the bromo sugar[4] (0.82 g).

p-*Nitrophenyl 3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4-di-O-acetyl-α-L-rhamnopyranoside (2).* Disaccharide *1* (1.1 g) was treated with a mixture of 95 % ethanol and 98 % hydrazine hydrate (2:1, 30 ml) at 70 °C for 10 min. After concentration the residue was acetylated (90 °C, 15 min) with pyridine (40 ml) and acetic anhydride (15 ml) and then again concentrated. Purification by column chromatography on silica gel (150 g) using ethyl acetate as eluant gave a solid material which was recrystallized from ethanol. Pure *2* (0.59 g, 80 %) was obtained, m.p. 209 – 213 °C, $[\alpha]_D$ −58° (*c* 0.5, $CHCl_3$). Anal.: $C_{30}H_{38}N_2O_{17}$: C,H,N.

p-*Nitrophenyl 3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-L-rhamnopyranoside (3).* Compound *2* (0.56 g) was treated with 0.1 M methanolic sodium methoxide (20 ml) at room temperature. After 1 h the mixture was neutralized with Dowex 50 (H^+) and concentrated. The residue, after partitioning between diethyl ether – water and lyophilization of the aqueous phase, was pure *3* (0.36

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g, 92 %) as an amorphous solid, $[\alpha]_D$ −104° (*c* 0.4, H_2O). The 1→3-linkage was demonstrated by methylation analysis – GLC-MS.[8] ^1H NMR (D_2O, 85 °C, external TMS): δ 5.64 (d, $J_{1,2}$ 2.0 Hz, Rha H-1), 4.89 (d, $J_{1,2}$ 7.9 Hz, GlcNAc H-1), 2.03 (acetyl CH_3), 1.20 (d, $J_{5,6}$ 5.4 Hz, Rha CH_3). ^{13}C NMR (D_2O, 25 °C, external TMS), δ 176.0 (acetyl C=O), 161.9, 142.9, 126.9, 117.5 (aromatic C), 104.1 (GlcNAc C-1), 98.5 (Rha C-1), 81.1 – 70.7 (ring carbons), 61.6 (GlcNAc C-6), 56.9 (GlcNAc C-2), 23.4 (acetyl CH_3) 18.0 (Rha CH_3).

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