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SYNTHESES OF TRISACCHARIDES RELATED TO *Salmonella* SEROGROUP E O-ANTIGENIC POLYSACCHARIDES

PER J GAREGG, CHRISTINA HENRICHSON, THOMAS NORBERG, AND PETRA OSSOWSKI
Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm (Sweden)

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ABSTRACT

Syntheses of *p*-trifluoroacetamidophenyl *O*-β-D-mannopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→3)-α-D-galactopyranoside (**3**) and *p*-trifluoroacetamidophenyl *O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-mannopyranosyl-(1→4)-α-L-rhamnopyranoside (**11**) are described. Silver zeolite was used as promoter for constructing the β-D-mannosyl linkages from 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl bromide and the appropriate monohydroxy compound.

INTRODUCTION

The structures of the O-antigenic polysaccharides attached to the cell wall of Salmonella bacteria belonging to serogroups E_1 and E_2 are depicted below.

$$[\rightarrow 6)\text{-}\beta\text{-D-Man}p\text{-}(1\rightarrow 4)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{-}(1\text{-}]_n \qquad (E_1)$$

$$[\rightarrow 6)\text{-}\beta\text{-D-Man}p\text{-}(1\rightarrow 4)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{-}(1\text{-}]_n \qquad (E_2)$$

Several syntheses of carbohydrate structures related to these polysaccharides have been reported. Tri-[1], hexa-[2], and nona-saccharides[2] have been synthesised, as well as regular polymers[3] of high molecular weight. Our involvement in the synthesis of oligosaccharide fragments related to *Salmonella* serogroups A, B, and D_1 polysaccharides led to the development[4] of a fast and reliable synthesis of the crystalline rhamnosylgalactosyl synthon **1**, which was then α-glycosylated with a suitably protected mannose derivative and further transformed to give tri- and tetra-saccharide fragments needed for immunological studies.

We now report β-D-mannosylation of **1** to give, after deprotection, the trisaccharide **3**. In order to make possible comparisons of immunological properties, we have also synthesised the trisaccharide **11**. Silver zeolite[7] was used for creating the β-D-mannosidic linkages.

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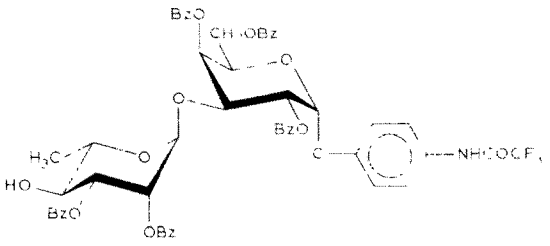

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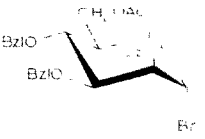

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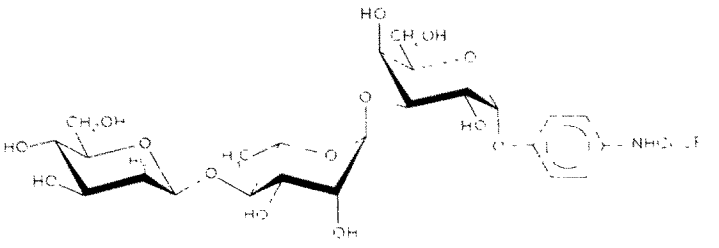
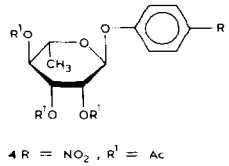


3

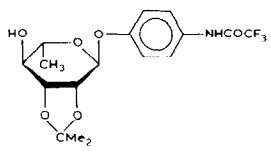
RESULTS

Silver triflate-assisted glycosidation of **1** with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl bromide gave[4], as expected, mainly the α-D-mannosyl trisaccharide. Treatment of **1** with the mannosyl bromide[5] **2**, using an insoluble promoter[6], should give a higher proportion of β-D-mannosyl trisaccharide derivative. Indeed, when silver silicate[5] was used as promoter, a 1:1 mixture of α- and β-D-mannosyl derivatives was obtained; the total yield was 82%. An improved α,β ratio (1:3) and a comparable yield (77%) were obtained by using silver zeolite[7] as promoter. The mixture of anomers from the glycosidation was deprotected by catalytic hydrogenation over palladium-on-carbon followed by treatment with methanolic sodium methoxide. The desired β-D-mannosyl derivative **3** crystallised from the mixture of trisaccharides in 31% yield (from **1**).

p-Nitrophenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside[8] (**4**) was used as starting material for the preparation of trisaccharide **11**. Catalytic hydrogenation of **4** over platinum and subsequent trifluoroacetylation gave **5** in 83% yield. Treatment of **5** with methanolic sodium methoxide gave **6**, which was directly treated with 2,2-dimethoxypropane and *p*-toluenesulfonic acid to give **7** (74% yield). Mannosylation of **7** with the mannosyl bromide **2**, using silver zeolite as promoter, gave the β-D-mannosyl derivative **8** (61%), together with some α-D-mannosyl anomer[7]. Compound **8** was treated with methanolic sodium methoxide to give the monohydroxy compound **9** in 95% yield. Silver triflate-promoted glycosidation of **9** with 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide[9] gave the trisaccharide de-



4 R = NO_2, R^1 = Ac
5 R = $NHCOCF_3$, R^1 = Ac
6 R = $NHCOCF_3$, R^1 = H



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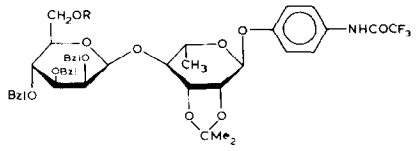
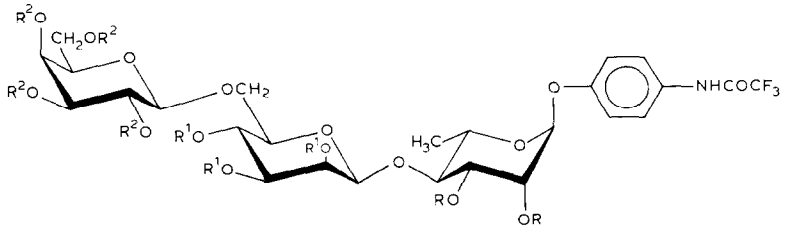


8 R = Ac
9 R = H



10 R, R = CMe_2, R^1 = Bzl, R^2 = Bz
11 R = R^1 = R^2 = H

rivative **10** (84% yield). Deprotection of **10** by catalytic hydrogenation over palladium, followed by treatment, successively, with methanolic sodium methoxide and 90% aqueous trifluoroacetic acid, gave 58% of the desired derivative **11**.

EXPERIMENTAL

General methods were the same as those previously reported[10].

Glycosidation of **1** *with the mannosyl bromide* **2**. — (*a*) *Using silver silicate.* A mixture of **1**[4] (70 mg, 0.068 mmol), 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl bromide[5] (**2**; 70 mg, 0.126 mmol), and powdered 4Å molecular sieve in dichloromethane (5 mL) was stirred at room temperature while silver silicate[5] (280 mg) was added. After 1 h, the mixture was filtered and concentrated. Purification by preparative t.l.c. (6:1 toluene–acetone) gave a syrupy mixture (1:1, estimated from ^{13}C-n.m.r. data) of the α-D-*manno* and β-D-*manno* trisaccharide derivatives (84 mg, 82%).

(*b*) *Using silver zeolite.* A mixture of **1** (1.02 g, 0.99 mmol) and **2** (1.40 g, 2.52 mmol) in dichloromethane (15 mL) was stirred at room temperature while silver zeolite[7] was added. After 4 h, the mixture was filtered and concentrated. Purification by column chromatography on silica gel (6:1 toluene–ethyl acetate) gave a syrupy mixture (1:3, estimated from ^{13}C-n.m.r. data) of the α-D-*manno* and β-D-*manno* trisaccharide derivatives (1.14 g, 77%).

p-*Trifluoroacetamidophenyl* O-β-D-*mannopyranosyl-(1→4)*-O-α-L-*rhamnopyranosyl-(1→3)*-α-D-*galactopyranoside* (**3**). — A solution of the mixture (1.10 g) obtained in (*b*) above in 1:20 ethyl acetate–ethanol (100 mL) was hydrogenated over Pd/C (10%, 0.50 g) at 400 kPa for 48 h. The mixture was filtered, concen-

trated, and passed through a short column of silica gel (elution with 20:20:1 ethyl acetate–dichloromethane–methanol). The fractions containing debenzylated trisaccharide derivatives were combined, concentrated, and treated with 0.1M methanolic sodium methoxide (10 mL) until t.l.c. revealed no further reaction (2 h). Neutralisation with Dowex 50 (H^+) resin and concentration gave a residue that was applied to a short column of silica gel. Elution with dichloromethane removed minor impurities. Further elution with methanol gave a product that crystallised from ethanol, to afford **3** (200 mg, 31% from **1**), m.p. 260° (dec.). The optical rotation and n.m.r. data were the same as those reported[4]. The material failed to give an acceptable elemental analysis (the reason for this is unclear), but the purity and identity of the material were evident from the n.m.r. spectra.

p-*Trifluoroacetamidophenyl 2,3,4-tri-O-acetyl-α-*L*-rhamnopyranoside* (**5**). — A solution of *p*-nitrophenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside[8] (19.6 g, 47.7 mmol) in ethyl acetate was hydrogenated at atmospheric pressure and room temperature over Pd/C (10%, 1.40 g), filtered, and concentrated. The residue (18.0 g) was taken up in pyridine (250 mL), and trifluoroacetic anhydride (13.0 mL, 93.5 mmol) was added dropwise with stirring and cooling in an ice-bath. After 1 h, ice was added, and stirring was continued for 30 min. The mixture was partitioned between dichloromethane and water, and the organic layer was washed with water, 2M sulfuric acid, and aqueous sodium hydrogencarbonate, dried, and concentrated. Crystallisation of the residue from methanol gave **5** (18.6 g, 83%), m.p. 165–166°, $[\alpha]_D$ −74° (*c* 0.5, chloroform).

Anal. Calc. for $C_{20}H_{22}F_3NO_9$: C, 50.3; H, 4.64; F, 12.0; N, 2.93. Found: C, 50.3; H, 4.73; F, 12.0; N, 2.91.

p-*Trifluoroacetamidophenyl 2,3-O-isopropylidene-α-*L*-rhamnopyranoside* (**7**). — Compound **5** (18.6 g) was taken up in 0.05M methanolic sodium methoxide, and the mixture was kept at room temperature for 3 h, neutralised with Dowex 50 (H^+) resin, and concentrated. The residue was suspended in acetone (250 mL) containing 2,2-dimethoxypropane (50 mL), *p*-toluenesulfonic acid monohydrate (100 mg) was added, and the mixture was stirred at room temperature for 6 h, neutralised by addition of pyridine, and concentrated. Crystallisation of the residue from methanol–water gave **7** (11.5 g, 74%), m.p. 192–193°, $[\alpha]_D$ −70° (*c* 0.5, ethanol). ^{13}C-N.m.r. data [$(CD_3)_2SO$, 25°]: δ 17.4 (C-6), 26.3, 28.0 (acetal Me), 66.9, 73.3, 75.3, 78.1 (C-2,3,4,5), 95.3 (C-1), 108.6 (acetal O-C-O), 117.2, 122.6, 130.7, and 153.2 (aromatic C). The CO and CF_3 carbons gave rise to quartet signals centered at 154.3 and 116.0, with $J_{C,F}$ spacings of 37.8 and 288.1 Hz, respectively.

Anal. Calc. for $C_{17}H_{20}F_3NO_6$: C, 52.2; H, 5.15; F, 14.6; N, 3.58. Found: C, 52.0; H, 5.17; F, 14.7; N, 3.56.

p-*Trifluoroacetamidophenyl 4-O-(6-O-acetyl-2,3,4-tri-O-benzyl-β-*D*-mannopyranosyl)-2,3-O-isopropylidene-α-*L*-rhamnopyranoside*[7] (**8**). — Silver zeolite[7] (5.0 g) was added with stirring at room temperature to a solution of 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl bromide[5] [prepared from 2.11 g (3.9 mmol) of 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranose[5], and used dire-

ctly] and **7** (1.21 g, 3.1 mmol) in dry dichloromethane (22 mL). After 20 h, the mixture was filtered, the filtrate concentrated to dryness, and the residue subjected to chromatography on silica gel. Elution with 4:1 toluene–ethyl acetate gave **8**[7] (1.65 g, 61%), $[\alpha]_D$ −85° (*c* 0.5, chloroform). Further elution afforded the corresponding α-linked disaccharide[7] in 9% yield.

p-*Trifluoroacetamidophenyl 2,3-O-isopropylidene-4-O-(2,3,4-tri-O-benzyl-β-*D-*mannopyranosyl)-α-*L-*rhamnopyranoside* (**9**). — Compound **8** (0.84 g) was treated with 0.1M methanolic sodium methoxide (7 mL) for 30 min, the base was then neutralised with Dowex 50 (H^+) resin, and the solution was concentrated, to give pure **9** (0.76 g, 95%) as a syrup, $[\alpha]_D$ −81° (*c* 0.5, chloroform). ^{13}C-N.m.r. data ($CDCl_3$, 25°): δ 17.7 (Rha C-6), 26.4, 27.9 (acetal Me), 62.4 (Man C-6), 65.4–82.4 (Rha and Man, C-2,3,4,5, benzyl CH_2), 95.6 (Rha C-1), 99.8 (Man C-1), 109.8 (acetal O-C-O), 117.0, 122.3, 129.7, and 154.1 (*p*-trifluoroacetamidophenyl aromatic C).

p-*Trifluoroacetamidophenyl* O-*(2,3,4,6-tetra-O-benzoyl-β-*D-*galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-benzyl-β-*D-*mannopyranosyl)-(1→4)-2,3-O-isopropylidene-α-*L-*rhamnopyranoside* (**10**). — A solution of silver triflate (0.39 g, 0.85 mmol) and 2,4,6-trimethylpyridine (0.11 mL, 0.81 mmol) in 1:1 nitromethane–toluene (7 mL) was added to a stirred and cooled (−30°) solution of **9** (0.70 g, 0.85 mmol) and 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide[9] (0.84 g, 1.27 mmol) in 1:1 nitromethane–toluene (10 mL) containing molecular sieves. After 10 min, more 2,4,6-trimethylpyridine (0.05 mL) was added, and the mixture was diluted with dichloromethane, filtered, washed successively with aqueous sodium thiosulfate, water, 2M sulfuric acid, and aqueous sodium hydrogencarbonate, dried, and concentrated. The syrupy residue was subjected to chromatography on silica gel (9:1 toluene–ethyl acetate), to give pure **10** (1.0 g, 84%) as a syrup, $[\alpha]_D$ −16° (*c* 0.5, chloroform). ^{13}C-N.m.r. data ($CDCl_3$, 25°): δ 17.9 (Rha C-6), 26.8, 28.2 (CMe_2), 62.4–82.8 (Rha C-2,3,4,5, Man C-2,3,4,5, Gal C-2,3,4,5,6, benzyl CH_2), 95.9 (Rha C-1), 100.4 (Man C-1), 101.5 (Gal C-1), 110.0 (CMe_2), 117.3, 122.6, 129.6, 154.5 (*p*-trifluoroacetamidophenyl aromatic C), and 165.4–166.3 (C=O).

p-*Trifluoroacetamidophenyl* O-β-D-*galactopyranosyl-(1→6)-O-β-*D-*mannopyranosyl-(1→4)-α-*L-*rhamnopyranoside* (**11**). — A solution of **10** (1.04 g) in 1:5 ethyl acetate–ethanol (24 mL) was hydrogenated at 400 kPa for 20 h over Pd/C (10%, 400 mg), filtered, and concentrated. The residue was taken up in 0.1M methanolic sodium methoxide (7 mL), and, after 1 h, the base was neutralised (Dowex 50, H^+) and the solution concentrated. The residue was taken up in 90% aqueous trifluoroacetic acid (2.5 mL). After 10 min, the mixture was concentrated, and the residue was subjected to chromatography on silica gel (12:3:3:2 ethyl acetate–acetic acid–methanol–water). The fractions containing **11** (320 mg) were combined and concentrated, and the residue was applied to a column of Bio-Gel P-2. Elution with water gave pure **11** (292 mg, 58%) as an amorphous solid, $[\alpha]_D$ −68° (*c* 0.5, water). N.m.r. data (D_2O, 85°): ^{1}H, δ 1.97 (d, *J* 5.9 Hz, Rha H-6), 5.13 (d,

J 7.8 Hz, Gal H-1), 5.57 (d, *J* 1.0 Hz, Man H-1), 6.18 (d, *J* 1.5 Hz, Rha H-1), 7.80, 7.89, 8.12, and 8.22 (aromatic H); ^{13}C, δ 18.3 (Rha C-6), 62.2 (Gal C-6), 67.8, 69.4, 69.8, 70.1, 71.4, 71.7, 71.9, 73.9, 74.2, 76.3, 76.6, 80.5 (Rha C-2,3,4,5, Man C-2,3,4,5,6, Gal C-2,3,4,5), 99.4 (Rha C-1), 101.7 (Man C-1), 104.5 (Gal C-1), 118.9, 125.2, 130.8, and 154.9 (aromatic C). The undecoupled ^{13}C-n.m.r. spectrum showed $J_{C,H}$ spacings of 160, 162, and 172 Hz for the anomeric carbons of Gal, Man, and Rha, respectively, indicating the β configuration for Gal and Man, and the α configuration for Rha. Sugar[11] and methylation[12] analyses were in agreement with the structure **11**.

ACKNOWLEDGMENTS

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