Carbohydrate Research, 254 (1994) 81–90
Elsevier Science B.V., Amsterdam

Synthesis of a hexasaccharide corresponding to part of the heptose–hexose region of the *Salmonella* Ra core, and a penta- and a tetra-saccharide that compose parts of this structure

Stefan Oscarson and Helena Ritzén

Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm (Sweden)

(Received May 14th, 1993; accepted August 13th, 1993)

ABSTRACT

The synthesis of the hexasaccharide 2-(4-trifluoroacetamidophenyl)ethyl *O*-α-D-galactopyranosyl-(1 → 3)-[*O*-α-D-galactopyranosyl-(1 → 6)]-*O*-α-D-glucopyranosyl-(1 → 3)-[*O*-L-*glycero*-α-D-*manno*-heptopyranosyl-(1 → 7)]-*O*-L-*glycero*-α-D-*manno*-heptopyranosyl-(1 → 3)-L-*glycero*-α-D-*manno*-heptopyranoside, corresponding to the heptose and part of the hexose region in the *Salmonella* Ra core, is described. Syntheses of the pentasaccharide 2-(4-trifluoroacetamidophenyl)ethyl *O*-α-D-galactopyranosyl-(1 → 3)-*O*-α-D-glucopyranosyl-(1 → 3)-[*O*-L-*glycero*-α-D-*manno*-heptopyranosyl-(1 → 7)]-*O*-L-*glycero*-α-D-*manno*-heptopyranosyl-(1 → 3)-L-*glycero*-α-D-*manno*-heptopyranoside and the tetrasaccharide 2-(4-trifluoroacetamidophenyl)ethyl *O*-α-D-glucopyranosyl-(1 → 3)-[*O*-L-*glycero*-α-D-*manno*-heptopyranosyl-(1 → 7)]-*O*-L-*glycero*-α-D-*manno*-heptopyranosyl-(1 → 3)-L-*glycero*-α-D-*manno*-heptopyranoside are also described. Coupling of methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside and methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-1-thio-β-D-glucopyranoside to a triheptoside derivative with a free HO-3′, using dimethyl(methylthio)sulfonium triflate and *N*-iodosuccinimide–silver triflate as promoters, gave the protected tetra- and penta-saccharide, respectively. Removal of the benzylidene group from the pentasaccharide followed by a regio- and stereo-selective coupling using halide-assisted conditions and 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide as donor gave the protected hexasaccharide. Deprotection then gave the target structures.

INTRODUCTION

The structure[1] of the dephosphorylated *Salmonella* Ra core is

α-D-Glc*p*NAc	α-D-Gal*p*	α-Hep*p*	α-Kdo*p*-(2 → 4)-α-Kdo*p*
1	1	1	2
↓	↓	↓	↓
2	6	7	4

→ 4)-α-D-Glc*p*-(1 → 2)-α-D-Gal*p*-(1 → 3)-α-D-Glc*p*-(1 → 3)-α-Hep*p*-(1 → 3)-α-Hep*p*-(1 → 5)-α-Kdo*p*-(2 →

Hep*p* = L-*glycero*-D-*manno*-heptopyranosyl

In our laboratory, we have earlier synthesized a number of oligosaccharide structures corresponding to the hexose and heptose part of this core[2–6], *inter alia*,

SSDI 0008-6215(93)E0315-R

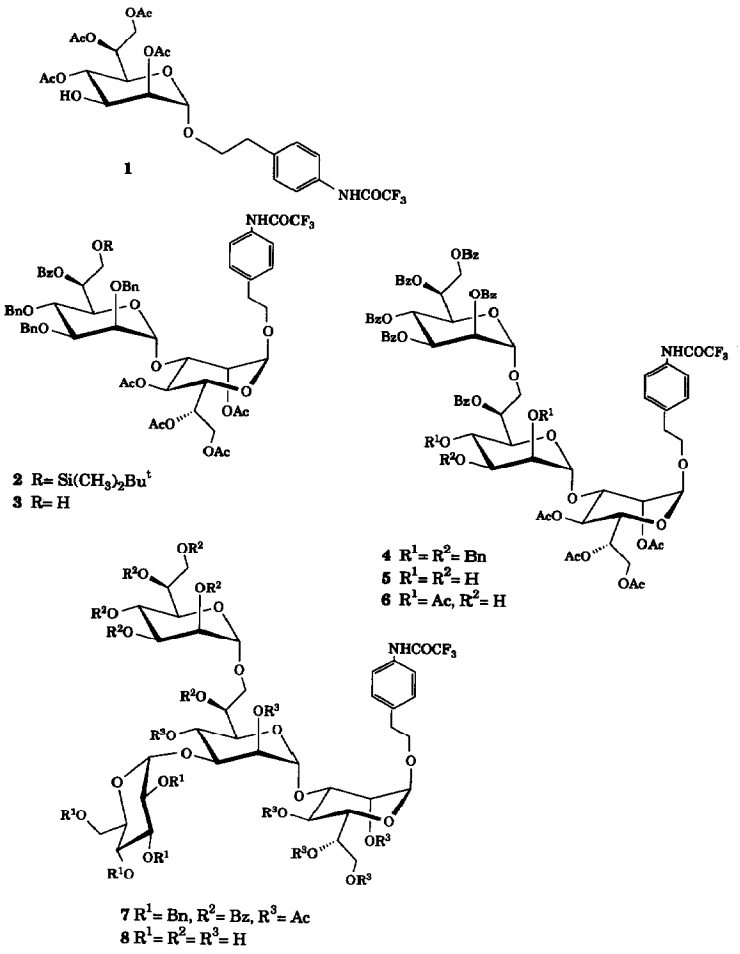
in order to investigate the specificity of monoclonal antibodies raised against rough mutants of *Salmonella* bacteria and to investigate the receptor structure of the phage G13, which is known to bind to the Ra core[7]. When these synthetic oligosaccharides were tested as inhibitors, they were found to be poor inhibitors especially towards the antibody–bacteria interaction[8,9]. Larger epitopes than the oligosaccharides used (di- to tetra-saccharides) are probably involved in these interactions. Therefore, to investigate further the specificity of the antibodies, larger synthetic oligosaccharides were needed. We describe here the synthesis of a hexasaccharide, found in the Ra core, that includes the heptose part, α-Hep*p*-(1 → 7)-α-Hep*p*-(1 → 3)-α-Hep*p*, and a trisaccharide part from the hexose region, α-D-Gal*p*-(1 → 3)-[α-D-Gal*p*-(1 → 6)]-α-D-Glc*p*. The synthesis of a pentasaccharide, including a disaccharide part from the hexose region [α-D-Gal*p*-(1 → 3)-α-D-Glc*p*], and a tetrasaccharide is also described. All the oligosaccharides were synthesized as their 2-(4-trifluoroacetamidophenyl)ethyl glycosides to allow their coupling to, e.g., proteins and their use as antigens.

RESULTS AND DISCUSSION

The synthesis of the triheptoside derivative **5** parallels that earlier described[2], the only difference is that an acetyl group instead of a chloroacetyl group was introduced in the 4-position. Using the same procedure as earlier[2,3] to convert a mannose 2,3,4-triol derivative into a 2,4-di-*O*-acetyl derivative, i.e., formation of the 2,3-orthoacetate followed by acetylation and regioselective opening of the ortho ester to the axial acetate, **5** was transformed into **6** (84%) with a free HO-3′. The structure of **6** was proved by COSY-NMR, from which it was shown that H-2′ and H-4′ were shifted downfield due to acetylation.

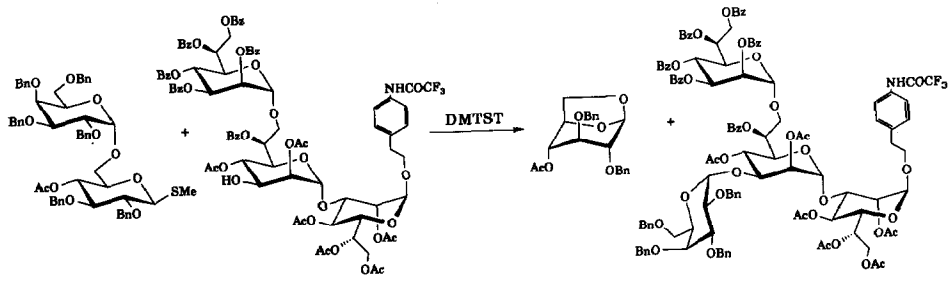
Dimethyl(methylthio)sulfonium triflate(DMTST)-promoted[10] coupling of **6** and methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside[5] in diethyl ether gave the α-(1 → 3)-linked tetrasaccharide **7** (66%). No β isomer could be detected by TLC, in contrast to a similar coupling performed earlier[3], with the same donor but with a mono-heptose acceptor, using methyl triflate as promoter, in which a 10% yield of the β anomer also could be isolated.

When a DMTST-promoted coupling between **6** and the known[4] trisaccharide methyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1 → 3)-[*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1 → 6)]-4-*O*-acetyl-2-*O*-benzyl-1-thio-β-D-glucopyranoside was attempted, the expected hexasaccharide was not formed, but instead a tetrasaccharide together with a disaccharide derivative. If the disaccharide methyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1 → 6)-4-*O*-acetyl-2,3-di-*O*-benzyl-1-thio-β-D-glucopyranoside was used instead as donor, the same tetrasaccharide was isolated. In the last coupling, a monosaccharide derivative was also isolated and was found to be a 1,6-anhydroglucose derivative (Scheme 1). Evidently in these reactions, the 6-oxygen had interacted with the anomeric center of the activated donor to give a 1,6-anhydro derivative together with an activated galacto-



syl donor, which then coupled to the aglycon. This loss of glycosyl moieties through interaction of an internal oxygen and decomposition of the activated donor has happened a number of times in our laboratory. Normally this problem can be circumvented by choosing a less active promoter, but when these couplings were performed using glycosyl bromides as donors and halide-assisted conditions[11], the aglycon was found to be too unreactive and no product was formed. Therefore, an alternative route had to be found to the hexasaccharide.

Methyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1 → 3)-2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside[4] was therefore tried as donor in a DMTST-promoted coupling with **6**, and this time the pentasaccharide **9** was formed, but a substantial proportion of the aglycon was not consumed. *N*-Iodosuccinimide(NIS)/silver triflate[12,13] as promoter finally gave a good yield of the pentasaccharide **9** (72%, 95% based on consumed aglycon). Removal of the



Scheme 1.

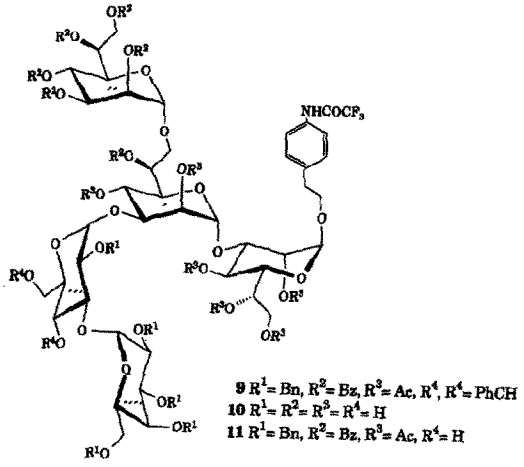
benzylidene group by treatment with aqueous acetic acid gave the 4,6-diol **11** (67%), which was used as the acceptor in a regio- and stereo-selective coupling, using halide-assisted conditions[11] and 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl bromide as donor, to give the α-(1 → 6)-linked hexasaccharide **12** in 90% yield. The structure of **12** is proved by the disappearance of a primary carbon signal (δ 63.2 ppm) in the ^{13}C NMR spectrum as compared to **11**, in combination with the presence of only two signals for primary hexose-carbons (δ 61.7 and 61.9 ppm) in the ^{13}C NMR spectrum of the deprotected hexasaccharide **13**. The $J_{C\text{-}1,H\text{-}1}$ coupling constants of the anomeric carbons in **12** show them all to be in the α configuration, which is further confirmed by the chemical shifts and the $J_{H\text{-}1,H\text{-}2}$ couplings of the anomeric protons in the deprotected hexasaccharide **13**.

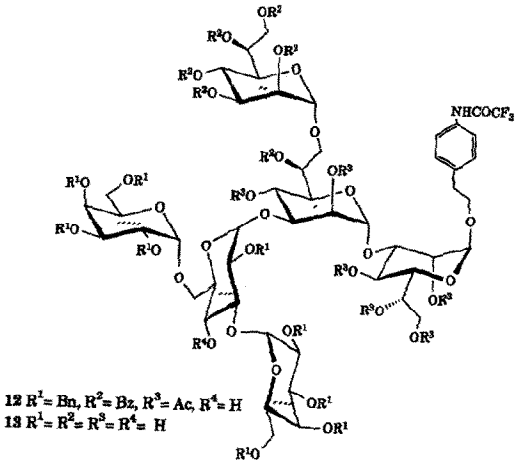
Deprotection of **7**, **9**, and **12** using standard conditions, i.e., Zemplén deacylation and catalytic hydrogenolyis, then gave the target structures **8**, **10**, and **13**.

EXPERIMENTAL

General methods.—These were as described[3]. Only selected NMR data are given. NMR spectra for solutions in D_2O were recorded at 30°C.

*2-(4-Trifluoroacetamidophenyl)ethyl 2,4,6,7-tetra-O-acetyl-*L-glycero-α-D-manno-*heptopyranoside* (**1**).—Trimethyl orthoacetate (0.45 mL) was added to a solution of 2-(4-trifluoroacetamidophenyl)ethyl 6,7-di-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranoside[2] (515 mg) and 4-toluenesulfonic acid (0.1 mL, 5% in MeCN) in dry MeCN (50 mL), and the mixture was stirred at room temperature for 30 min. Pyridine (4 mL), Ac_2O (4 mL), and 4-dimethylaminopyridine (a few crystals) were added and the stirring was continued for another 1 h. The solution was diluted with toluene, concentrated, and coevaporated twice with dry toluene. Aqueous CF_3CO_2H (90%, 0.1 mL) was added to a solution of the residue in MeCN (40 mL). After 30 min, the solution was concentrated and purified on a silica gel column (2:1 toluene–EtOAc) to give **1** (506 mg, 84%); $[\alpha]_D$ −23° (*c* 1.2, $CHCl_3$). NMR data ($CDCl_3$): ^{13}C, δ 20.7, 20.9 (CH_3CO), 35.3 (CH_2CH_2Ph), 62.8 (C-7), 67.2, 67.9, 68.4, 68.6, 68.7, 72.3 (C-2–6, OCH_2CH_2), 97.3 (C-1), 113.8, 118.0 (CF_3), 121.2–136.8 (aromatic C), 154.8, 155.4 (CF_3CO), 170.5, 170.8, 171.0, and 171.3 (CH_3CO); ^1H, δ 3.41 (H-5,





dd), 3.94–4.12 (H-3 and H-7), 4.85 (H-1, s), 4.94 (H-4, t), 5.03 (H-2, dd), and 5.17 (H-6, ddd).

2-(4-Trifluoroacetamidophenyl)ethyl O-(*6-O-benzoyl-2,3,4-tri-O-benzyl-7-O-tert-butyldimethylsilyl-*L-glycero-*α*-D-manno-*heptopyranosyl)-(1 → 3)-2,4,6,7-tetra-O-acetyl-*L-glycero-*α*-D-manno-*heptopyranoside* (**2**).—DMTST (375 mg) was added at 0°C to a solution of **1** (280 mg) and ethyl 6-*O*-benzoyl-2,3,4-tri-*O*-benzyl-7-*O*-*tert*-butyldimethylsilyl-1-thio-L-*glycero*-*α*-D-*manno*-heptopyranoside[2] (385 mg) in dry Et_2O (25 mL) containing 4A molecular sieves. The mixture was stirred for 2 h at room temperature, Et_3N (1 mL) was added, and stirring was continued for 30 min. The mixture was concentrated and purified on a silica gel column (9:1 toluene–EtOAc) to give **2** (507 mg, 84%); $[\alpha]_D + 17°$ (*c* 0.8, $CHCl_3$). ^{13}C NMR data ($CDCl_3$): δ −5.3, −5.2 [$Si(CH_3)_2$], 18.2 [$C(CH_3)_3$], 20.5, 20.7, 21.0 (CH_3CO), 25.8

[C(CH_3)$_3$], 35.4 (CH_2CH_2Ph), 61.8, 63.1, 66.8, 68.7, 68.8, 71.0, 71.6, 2 × 72.1, 73.0, 73.1, 74.0, 74.8, 75.3, 79.6 (C-2–7, C-2′–7′, CH_2Ph, OCH_2CH_2), 97.1 (C-1, $J_{C\text{-}1,H\text{-}1}$ 172 Hz), 100.2 (C-1′, $J_{C\text{-}1',H\text{-}1'}$ 174 Hz), 121.5–138.3 (aromatic C), 166.1 (benzoyl CO), 169.5, 169.9, 170.5, and 171.2 (acetyl CO).

2-(4-Trifluoroacetamidophenyl)ethyl O-*(6-O-benzoyl-2,3,4-tri-O-benzyl-*L-glycero-α-D-manno-*heptopyranosyl)-(1 → 3)-2,4,6,7-tetra-*O-*acetyl-*L-glycero-α-D-manno-*heptopyranoside* (**3**).—Compound **2** (507 mg) in aq 70% AcOH (25 mL) was stirred overnight at room temperature, then concentrated and coevaporated twice with toluene. The residue was purified by silica gel chromatography (1:1 toluene–EtOAc) to afford **3** (443 mg, 96%); $[\alpha]_D$ + 28° (*c* 1.1, $CHCl_3$). ^{13}C NMR data ($CDCl_3$): δ 20.6, 20.7, 20.9 (CH_3CO), 35.1 (CH_2CH_2Ph), 2 × 62.6, 66.8, 66.9, 68.5, 68.8, 71.4, 72.0, 72.2, 73.0, 73.1, 73.5, 74.0, 74.8, 75.1, 79.5 (C-2–7, C-2′–7′, CH_2Ph, OCH_2CH_2), 97.2 (C-1), 100.5 (C-1′), 121.5–138.2 (aromatic C), 167.0 (benzoyl CO), 169.4, 170.3, 170.4, and 170.8 (acetyl CO).

2-(4-Trifluoroacetamidophenyl)ethyl O-*(2,3,4,6,7-penta-*O-*benzoyl-*L-glycero-α-D-manno-*heptopyranosyl)-(1 → 7)-*O-*(6-*O-*benzoyl-2,3,4-tri-*O-*benzyl-*L-glycero-α-D-manno-*heptopyranosyl)-(1 → 3)-2,4,6,7-tetra-*O-*acetyl-*L-glycero-α-D-manno-*heptopyranoside* (**4**).—Silver trifluoromethanesulfonate (150 mg) was added to a mixture of **3** (460 mg), 2,3,4,6,7-penta-*O*-benzoyl-L-*glycero*-α-D-*manno*-heptopyranosyl bromide (made from 1 g of 1,2,3,4,6,7-hexa-*O*-benzoyl-L-*glycero*-α-D-*manno*-heptopyranose[2]) and 4A molecular sieves in CH_2Cl_2 (5 mL). The mixture was stirred at room temperature for 2 h, concentrated, and purified on a silica gel column (6:1 toluene–EtOAc) to give **4** (525 mg, 71%); $[\alpha]_D$ −17° (*c* 1.3, $CHCl_3$). ^{13}C NMR data ($CDCl_3$): δ 20.6, 20.7, 20.8, 20.9 (CH_3CO), 35.5 (CH_2CH_2Ph), 62.8, 63.9, 65.8, 66.0, 66.9, 68.2, 68.4, 68.5, 68.7, 69.2, 69.9, 70.6, 71.2, 71.5, 72.2, 72.9, 73.6, 74.6, 74.7, 75.0, 79.7 (C-2–7, C-2′–7′, C-2″–7″, CH_2Ph, OCH_2CH_2Ph), 97.3 ($J_{C\text{-}1,H\text{-}1}$ 174 Hz), 97.6 ($J_{C\text{-}1,H\text{-}1}$ 174 Hz), 100.7 ($J_{C\text{-}1,H\text{-}1}$ 168 Hz) (C-1–1″), 113.7, 117.9 (CF_3), 121.3–138.2 (aromatic C), 154.7, 155.2 (CF_3CO), 2 × 165.2, 165.3, 165.6, 2 × 166.1 (benzoyl CO), 169.5, 170.1, 170.5, and 171.0 (acetyl CO).

2-(4-Trifluoroacetamidophenyl)ethyl O-*(2,3,4,6,7-penta-*O-*benzoyl-*L-glycero-α-D-manno-*heptopyranosyl)-(1 → 7)-*O-*(6-*O-*benzoyl-*L-glycero-α-D-manno-*heptopyranosyl)-(1 → 3)-2,4,6,7-tetra-*O-*acetyl-*L-glycero-α-manno-*heptopyranoside* (**5**).—A solution of **4** (1.00 g) in EtOAc (50 mL) was hydrogenolyzed over Pd–C (100 mg) at 400 kPa for 16 h. The solution was filtered, evaporated, applied to a silica gel column, and eluted (1:3 toluene–EtOAc) to give **5** (754 mg, 88%); $[\alpha]_D$ −9° (*c* 1.2, $CHCl_3$). NMR data ($CDCl_3$): ^{13}C, δ 20.6, 2 × 20.7, 21.0 (CH_3CO), 35.6 (CH_2CH_2Ph), 62.6, 63.9, 65.6, 66.9, 67.0, 67.5, 68.3, 68.4, 68.9, 69.9, 70.0, 70.5, 70.5, 70.9, 71.1, 71.6, 74.4 (C-2–7, C-2′–7′, C-2″–7″, OCH_2CH_2), 97.9, 98.1, 102.0 (C-1–1″), 113.7, 117.9 (CF_3), 121.2–136.7 (aromatic C), 154.6, 155.2 (CF_3CO), 165.1, 165.4, 165.4, 165.5, 166.2 (benzoyl CO), 169.6, 170.4, 170.8, and 170.9 (acetyl CO); ^1H, δ 3.84 (H-2′), 3.73 (H-3′), and 3.65 (H-4′). Anal. Calcd. for $C_{81}H_{78}F_3NO_{30}$: C, 60.7; H, 4.91. Found: C, 60.0; H. 4.90.

2-(4-Trifluoroacetamidophenyl)ethyl O-*(2,3,4,6,7-penta-*O-*benzoyl-*L-glycero-α-D-

manno-*heptopyranosyl*)-(1 → 7)-O-(2,4-*di*-O-*acetyl*-6-O-*benzoyl*-L-glycero-α-D-manno-*heptopyranosyl*)-(1 → 3)-2,4,6,7-*tetra*-O-*acetyl*-L-glycero-α-D-manno-*heptopyranoside* (**6**).—Trimethyl orthoacetate (0.25 mL) was added to a solution of **5** (345 mg) and 4-toluenesulfonic acid (0.1 mL, 5% in MeCN) in dry MeCN (10 mL), and the mixture was stirred at room temperature for 30 min. Pyridine (2 mL), Ac_2O (1 mL), and 4-dimethylaminopyridine (a few crystals) were added and the stirring was continued for another 1 h. The solution was diluted with toluene, concentrated, and coevaporated twice with dry toluene. Aqueous CF_3CO_2H (90%, 0.1 mL) was added to a solution of the residue in MeCN (20 mL). After 30 min, the solution was concentrated and purified on a silica gel column (2:1 toluene–EtOAc) to give **6** (306 mg, 84%); $[\alpha]_D$ −26° (*c* 0.8, $CHCl_3$). NMR data ($CDCl_3$): ^{13}C δ 20.6, 20.7, 20.8, 2 × 21.0, 21.4 (CH_3CO), 35.7 (OCH_2CH_2), 62.7, 63.8, 65.0, 65.8, 66.4, 66.8, 2 × 67.9, 68.1, 68.2, 68.5, 68.6, 69.1, 69.6, 70.0, 70.8, 70.8, 72.5, 74.5 (C-2–7, C-2′–7′, C-2″–7″, OCH_2CH_2), 97.1, 97.9, 99.0 (C-1–1″), 113.7, 117.9 (CF_3), 121.3–136.6 (aromatic C), 154.7, 155.3 (CF_3CO), 2 × 165.3, 165.4, 165.6, 165.8, 166.2 (benzoyl CO), 170.2, 170.3, 170.6, 171.0, 171.9 (acetyl CO); ^1H, δ 3.92 (H-3′, dd), 4.87 (H-2′, dd), 5.17 (H-4′, t). Anal. Calcd. for $C_{85}H_{82}F_3NO_{32}$: C, 60.5; H, 4.90. Found: C, 60.2; H, 5.00.

2-(4-Trifluoroacetamidophenyl)ethyl O-(2,3,4,6-*tetra*-O-*benzyl*-α-D-*glucopyranosyl*)-(1 → 3)-[O-(2,3,4,6,7-*penta*-O-*benzoyl*-L-glycero-α-D-manno-*heptopyranosyl*)-(1 → 7)]-O-(2,4-*di*-O-*acetyl*-6-O-*benzoyl*-L-glycero-α-D-manno-*heptopyranosyl*)-(1 → 3)-2,4,6,7-*tetra*-O-*acetyl*-L-glycero-α-D-manno-*heptopyranoside* (**7**).—DMTST (100 mg) was added at 0°C to a solution of **6** (115 mg) and methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside[5] (50 mg) in dry Et_2O (2 mL) containing 4A molecular sieves. The mixture was stirred for 2 h at room temperature, Et_3N (0.5 mL) was added, and stirring was continued for 30 min. The mixture was concentrated and purified on a silica gel column (9:1 toluene–EtOAc) to give **7** (85 mg, 66%; 18 mg of **6** recovered); $[\alpha]_D$ −2° (*c* 0.4, $CHCl_3$). ^{13}C NMR data ($CDCl_3$): δ 20.6–21.5 (CH_3CO), 35.7 (OCH_2CH_2), 62.7–81.5 (C-2–7, C-2′–7′, C-2″–7″, C-2‴–6‴, 4 CH_2Ph, OCH_2CH_2), 97.0 ($J_{C\text{-}1,H\text{-}1}$ 174 Hz), 97.9 ($J_{C\text{-}1,H\text{-}1}$ 174 Hz), 98.7 ($J_{C\text{-}1,H\text{-}1}$ 174 Hz), 99.9 ($J_{C\text{-}1,H\text{-}1}$ 167 Hz) (C-1–1‴), 121.4–138.7 (aromatic C), 3 × 165.2, 165.6, 165.8, 166.2 (benzoyl CO), 169.8, 2 × 170.1, 170.3, 170.5, 170.9 (acetyl CO). Anal. Calcd. for $C_{119}H_{116}F_3NO_{36}$: C, 65.2; H, 5.33. Found: C, 65.1; H, 5.32.

2-(4-Trifluoroacetamidophenyl)ethyl O-α-D-*glucopyranosyl*-(1 → 3)-[O-L-glycero-α-D-manno-*heptopyranosyl*-(1 → 7)]-O-L-glycero-α-D-manno-*heptopyranosyl*-(1 → 3)-L-glycero-α-D-manno-*heptopyranoside* (**8**).—10% Pd–C (50 mg) was added to a solution of **7** in EtOH (10 mL) and the mixture was hydrogenolyzed in a Parr apparatus (400 kPa) overnight. The mixture was then filtered and concentrated, and the residue was dissolved in MeOH (5 mL) to which a catalytic amount of methanolic NaOMe was added. The reaction was stirred for 24 h at room temperature, then neutralized with Dowex (H^+) resin, filtered, and concentrated. A solution of the residue in water was washed with EtOAc, concentrated, purified on a Bio-Gel P2 column, and freeze-dried to give **8** (14.3 mg, 56%); $[\alpha]_D$ + 93° (*c*

0.49, H_2O). NMR data (D_2O): ^{13}C, δ 35.7 (OCH_2CH_2), 61.5, 63.7, 63.9, 65.9, 66.3, 66.7, 67.6, 68.3, 69.2, 69.3, 69.6, 70.4, 70.6, 70.8, 71.0, 71.6, 72.0, 72.4, 72.6, 73.0, 73.2, 73.6, 78.4, 79.6 (C-2–7, C-2′–7′, C-2″–7″, C-2‴–6‴, OCH_2CH_2), 99.7, 100.9, 101.3, 103.1 (C-1–1‴), 114.6, 118.8 (CF_3), 122.9–139.3 (aromatic C), 157.3, 157.9 (CF_3CO); ^1H, δ 5.25 (H-1‴, J 3.8 Hz), 5.09 (H-1′), 4.83, 4.82 (H-1,1″). FAB-mass spectrum: m/z 971.9 (M + 1). Calculated for $C_{37}H_{57}F_3NO_{25}$: m/z 972.3.

2-(4-Trifluoroacetamidophenyl)ethyl O-*(2,3,4,6-tetra*-O-*benzyl-α*-D-*galactopyranosyl)-(1 → 3)*-O-*(2*-O-*benzyl-4,6*-O-*benzylidene-α*-D-*glucopyranosyl)-(1 → 3)-[*O-*(2,3,4,6,7-penta*-O-*benzoyl*-L-glycero-*α*-D-manno-*heptopyranosyl)-(1 → 7)]*-O-*(2,4-di*-O-*acetyl-6*-O-*benzoyl*-L-glycero-*α*-D-manno-*heptopyranosyl)-(1 → 3)-2,4,6,7-tetra*-O-*acetyl*-L-glycero-*α*-manno-*heptopyranoside* (**9**).—A catalytic amount of silver trifluoromethanesulfonate was added to a stirred solution of **6** (240 mg), methyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1 → 3)-2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside[4] (235 mg), and *N*-iodosuccinimide (55 mg) in CH_2Cl_2 (2 mL) containing 4A molecular sieves. After 30 min, the mixture was filtered, concentrated, and subjected to column chromatography (6:1 toluene–EtOAc) to give **9** (270 mg, 72%; 54 mg of **6** recovered); $[\alpha]_D$ +3° (*c* 1.1, $CHCl_3$). ^{13}C NMR data ($CDCl_3$): δ 20.4, 20.6, 20.7, 21.1, 21.4 (CH_3CO), 35.6 (OCH_2CH_2), 62.7, 63.2, 63.9, 64.7, 64.9, 65.8, 66.3, 66.9, 67.5, 68.2, 68.5, 69.0, 69.1, 69.2, 69.7, 70.0, 70.7, 71.0, 71.3, 71.6, 72.8, 73.1, 73.7, 74.6, 75.0, 75.1, 75.7, 77.3, 78.0, 78.2, 83.1 (C-2–7, C-2′–7′, C-2″–7″, C-2‴–6‴, C-2⁗–6⁗, 5 CH_2Ph, OCH_2CH_2), 96.4 ($J_{C\text{-}1,H\text{-}1}$ 174 Hz), 97.1 ($J_{C\text{-}1,H\text{-}1}$ 172 Hz), 97.9 ($J_{C\text{-}1,H\text{-}1}$ 176 Hz), 99.0 ($J_{C\text{-}1,H\text{-}1}$ 176 Hz), 100.6 ($J_{C\text{-}1,H\text{-}1}$ 167 Hz) (C-1–1⁗), 101.9 (PhCH), 121.3–139.0 (aromatic C), 2 × 165.2, 165.3, 165.6, 165.9, 166.2 (benzoyl CO), 169.7, 170.0, 170.1, 170.3, 170.6, 170.8 (acetyl CO). Anal. Calcd. for $C_{139}H_{137}F_3NO_{42}$: C, 65.5; H, 5.41. Found: C, 65.6; H, 5.43.

2-(4-Trifluoroacetamidophenyl)ethyl O-*α*-D-*galactopyranosyl-(1 → 3)*-O-*α*-D-*glucopyranosyl-(1 → 3)*-O-*[*L-glycero-*α*-D-manno-*heptopyranosyl-(1 → 7)]*-O-L-glycero-*α*-D-manno-*heptopyranosyl-(1 → 3)*-L-glycero-*α*-D-manno-*heptopyranoside* (**10**).—Compound **9** (45 mg) in 3:1 EtOAc–EtOH (4 mL) was hydrogenolyzed over Pd–C (10%) in a Parr apparatus (400 kPa) overnight, and the mixture was then filtered and concentrated. The residue (30 mg) was dissolved in MeOH (2 mL) and methanolic NaOMe (0.2 mL, 1 M) was added. After stirring overnight the mixture was neutralized with Dowex (H^+) resin, filtered, and concentrated. Purification on a Bio-Gel P2 column followed by freeze-drying gave **10** (13.5 mg, 70%); $[\alpha]_D$ +134° (*c* 0.55, H_2O). NMR data (D_2O): ^{13}C, δ 35.6 (OCH_2CH_2), 61.3, 61.7, 63.7, 63.7, 66.0, 66.3, 66.8, 67.6, 68.3, 69.2, 69.3, 69.4, 69.6, 69.9, 70.1, 70.5, 70.8, 70.9, 71.0, 71.2, 71.5, 71.6, 72.0, 72.4, 72.9, 78.4, 79.5, 80.3 (C-2–7, C-2′–7′, C-2″–7″, C-2‴–6‴, C-2⁗–6⁗, OCH_2CH_2), 99.7, 100.0, 100.9, 101.4, 103.1 (C-1–1⁗), 114.6, 118.8 (CF_3), 123.0–139.4 (aromatic C), 157.3, and 157.9 (CF_3CO); ^1H, δ 5.41 (J 3.5 Hz), 5.29 (J 3.7 Hz) (H-1‴,1⁗), 5.11 (H-1′), 4.83, and 4.82 (H-1,1″). FAB-mass spectrum: m/z 1133.9 (M + 1). Calculated for $C_{43}H_{67}F_3NO_{30}$: m/z 1134.4.

2-(4-Trifluoroacetamidophenyl)ethyl O-*(2,3,4,6-tetra*-O-*benzyl-α*-D-*galactopyranosyl)-(1 → 3)*-O-*(2*-O-*benzyl-α*-D-*glucopyranosyl)-(1 → 3)-[*O-*(2,3,4,6,7-penta*-O-*benzoyl*-L-glycero-*α*-D-manno-*heptopyranosyl)-(1 → 7)]*-O-*(2,4-di*-O-*acetyl-6*-O-*benzoyl*-L-glycero-*α*-D-manno-*heptopyranosyl)-(1 → 3)-2,4,6,7-tetra*-O-*acetyl*-L-glycero-*α*-D-manno-*heptopyranoside* (**11**).—A solution of **9** (180 mg) in aq 80% AcOH (4 mL) was stirred at 60°C for 2 h, whereafter the mixture was concentrated and applied to a silica gel column (2:1 toluene–EtOAc) to give **11** (116 mg, 67%); $[\alpha]_D$ +9° (*c* 0.9, $CHCl_3$). ^{13}C NMR data ($CDCl_3$): δ 20.5–21.4 (CH_3CO), 35.6 (OCH_2CH_2), 62.6, 63.2, 63.9, 64.6, 65.1, 65.8, 66.3, 66.9, 68.2, 68.9, 69.1, 69.7, 70.0, 70.7, 70.9, 71.1, 71.9, 72.3, 72.6, 73.2, 73.3, 74.4, 74.5, 74.8, 76.9, 77.2, 78.6, 79.6, 82.9 (C-2–7, C-2′–7′, C-2″–7″, C-2‴–6‴, C-2⁗–6⁗, 5 CH_2Ph, OCH_2CH_2), 97.1, 97.9, 2 × 99.0, 101.0 (C-1–1⁗), 113.6, 117.9 (CF_3), 121.3–138.6 (aromatic C), 154.6, 155.2 (CF_3CO), 3 × 165.2, 165.6, 165.7, 166.2 (benzoyl CO), 2 × 170.0, 2 × 170.3, 170.7, and 170.8 (acetyl CO). Anal. Calcd. for $C_{131}H_{131}F_3NO_{42}$: C, 64.3; H, 5.39. Found: C, 64.3; H, 5.37.

2-(4-Trifluoroacetamidophenyl)ethyl O-*(2,3,4,6-tetra*-O-*benzyl-α*-D-*galactopynosyl)-(1 → 3)-[*O-*(2,3,4,6-tetra*-O-*benzyl-α*-D-*galactopyranosyl)-(1 → 6)]*-O-*(2*-O-*benzyl-α*-D-*glucopyranosyl)-(1 → 3)-[*O-*(2,3,4,6,7-penta*-O-*benzoyl*-L-glycero-*α*-D-manno-*heptopyranosyl)-(1 → 7)]*-O-*(2,4-di*-O-*acetyl-6*-O-*benzoyl*-L-glycero-*α*-D-manno-*heptopyranosyl)-(1 → 3)-2,4,6,7-tetra*-O-*acetyl*-L-glycero-*α*-D-manno-*heptopyranoside* (**12**).—Bromine (15 μL) was added at 0°C to a stirred solution of methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-*β*-D-galactopyranoside[14] (120 mg) in CH_2Cl_2 (2 mL) containing 4A molecular sieves. After 45 min, the mixture was filtered, concentrated, and co-concentrated with dry toluene. The residue in CH_2Cl_2 (0.5 mL) was added to a solution of **11** (116 mg) and tetraethylammonium bromide (100 mg) in CH_2Cl_2 (1 mL) containing 4A molecular sieves. The mixture was stirred for 40 h at room temperature, then applied to a silica gel column, and eluted (6:1 toluene–EtOAc) to give **12** (155 mg, 90%); $[\alpha]_D$ +14° (*c* 1.5, $CHCl_3$). ^{13}C NMR data ($CDCl_3$): δ 20.6, 20.8, 21.0, 21.4 (CH_3CO), 35.7 (OCH_2CH_2), 62.6, 63.8, 64.5, 65.0, 65.8, 66.3, 66.7, 66.8, 67.0, 68.1, 68.3, 68.8, 69.0, 69.2, 69.5, 69.6, 70.0, 70.7, 70.9, 71.1, 71.8, 72.4, 72.5, 72.9, 73.3, 74.2, 74.7, 74.8, 74.9, 76.3, 77.2, 78.6, 78.7, 79.2, 80.6 (C-2–7, C-2′–7′, C-2″–7″, C-2‴–6‴, C-2⁗–6⁗, C-2⁗′–6⁗′, C-2⁗″–6⁗″, 9 CH_2Ph, OCH_2CH_2), 97.0 ($J_{C\text{-}1,H\text{-}1}$ 172 Hz), 2 × 98.0 ($J_{C\text{-}1,H\text{-}1}$ 172 Hz), 98.9 ($J_{C\text{-}1,H\text{-}1}$ 174 Hz), 99.2 ($J_{C\text{-}1,H\text{-}1}$ 168 Hz), 99.7 ($J_{C\text{-}1,H\text{-}1}$ 170 Hz) (C-1–1⁗), 113.6, 117.9 (CF_3), 121.4–139.0 (aromatic C), 154.6, 155.2 (CF_3CO), 165.1, 165.2 (2 C), 165.6, 165.8, 166.2 (benzoyl CO), 169.8, 170.0 (2 C), 170.2, 170.4, and 170.8 (acetyl CO). Anal. Calcd. for $C_{166}H_{166}F_3NO_{47}$: C, 66.8; H, 5.61. Found: C, 66.8; H, 5.57.

2-(4-Trifluoroacetamidophenyl)ethyl O-*α*-D-*galactopyranosyl-(1 → 3)-[*O-*α*-D-*galactopyranosyl-(1 → 6)]*-O-*α*-D-*glucopyranosyl-(1 → 3)-[*O-L-glycero-*α*-D-manno-*heptopyranosyl-(1 → 7)]*-O-L-glycero-*α*-D-manno-*heptopyranosyl-(1 → 3)*-L-glycero-*α*-D-manno-*heptopyranoside* (**13**).—Compound **12** (60 mg) in 3:1 EtOAc–EtOH (4 mL) was hydrogenolyzed over Pd–C (10%) in a Parr apparatus (400 kPa) overnight, and the mixture was then filtered and concentrated. The residue (40 mg) was

dissolved in MeOH (2 mL) and methanolic NaOMe (0.2 mL, 1 M) was added. After stirring overnight, the mixture was neutralized with Dowex (H^+) resin, filtered, and concentrated. Purification on a Bio-Gel P2 column followed by freeze-drying gave **13** (15 mg, 58%); $[\alpha]_D$ +145° (*c* 0.52, H_2O). NMR data (D_2O): ^{13}C, δ 35.7 (OCH_2CH_2), 61.7, 61.9, 63.8, 63.9, 65.7, 66.5, 66.8, 67.7, 68.4, 69.1, 69.2, 69.3, 69.4, 69.7, 69.9, 70.0, 70.1, 70.4, 70.5, 70.8, 71.0, 71.3, 71.5, 71.6, 71.7, 71.7, 72.0, 72.5, 72.9, 77.7, 80.6, 80.8 (C-2–7, C-2′–7′, C-2″–7″, C-2‴–6‴, C-2⁗–6⁗, C-2⁗′–6⁗′, OCH_2CH_2), 99.1, 99.8, 100.2, 100.9, 101.8, 103.0 (C-1–1⁗′), 123.0, 130.8, 133.9, and 139.4 (aromatic C); ^1H, δ 5.38 (*J* 3.1 Hz), 5.27 (*J* 3.8 Hz), 5.14 (*J* 1.5 Hz), 5.0 (not resolved), 4.86 (*J* 1.6 Hz), and 4.84 (*J* 1.6 Hz) (H-1–1⁗′). FAB-mass spectrum: *m/z* 1296.6 (M + 1). Calculated for $C_{49}H_{77}F_3NO_{35}$: *m/z* 1296.4.

ACKNOWLEDGMENTS

We thank Professor Per J. Garegg for his interest in this work, and the Swedish Research Council for Engineering Studies for financial support.

REFERENCES

1 C.R.H. Raetz, *Annu. Rev. Biochem.*, 59 (1990) 129–170.
2 P.J. Garegg, S. Oscarson, H. Ritzén, and M. Szönyi, *Carbohydr. Res.*, 228 (1992) 121–128.
3 P.J. Garegg, S. Oscarson, and M. Szönyi, *Carbohydr. Res.*, 205 (1990) 125–132.
4 T. Norberg, M. Walding, and E. Westman, *J. Carbohydr. Chem.*, 7 (1988) 283–292.
5 T. Norberg and H. Ritzén, *Glycoconjugate J.*, 3 (1986) 135–142.
6 A.-C. Helland, *Chem. Commun., Univ. Stockholm*, No. 5 (1991).
7 G.W. Bruse, Doctoral Dissertation Thesis, Karolinska Institute, 1991.
8 J.M.C. Luk, Doctoral Dissertation Thesis, Karolinska Institute, 1991.
9 S. Lind, Doctoral Dissertation Thesis, Karolinska Institute, 1992.
10 P. Fügedi and P.J. Garegg, *Carbohydr. Res.*, 149 (1986) c9–c12.
11 R.U. Lemieux, K.B. Hendriks, R.V. Stick, and K. James, *J. Am. Chem. Soc.*, 97 (1975) 4056–4062.
12 P. Konradson, U.D. Udodong, and B. Fraser-Reid, *Tetrahedron Lett.*, 31 (1990) 4313–4316.
13 G.H. Veeneman, S.H. van Leeuwen, and J.H. van Boom, *Tetrahedron Lett.*, 31 (1990) 1331–1334.
14 S. Oscarson, *Chem. Commun., Univ. Stockholm*, No. 5 (1985).