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ARTIFICIAL CARBOHYDRATE ANTIGENS: THE SYNTHESIS OF A TETRASACCHARIDE HAPTEN, A *Shigella flexneri* O-ANTIGEN REPEATING UNIT*

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ABSTRACT

The 8-methoxycarbonyloctyl glycoside of the tetrasaccharide hapten, *O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside and the trisaccharide glycoside 8-methoxycarbonyloctyl *O*-α-L-rhamnopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside were synthesized by sequential Koenigs–Knorr reactions from monosaccharide units. The tetrasaccharide represents the complete skeletal repeating unit of *Shigella flexneri* serogroup Y lipopolysaccharide. Both oligosaccharide haptens are functionalized for covalent attachment to proteins, cell surfaces, and solid supports. ^{1}H-N.m.r. evidence for the conformations of these oligosaccharides in solution is presented and shown to be consistent with predictions based on the *exo*-anomeric effect.

INTRODUCTION

We have previously described[1–3] the synthesis of four di- and two tri-saccharides which represent portions of the O-antigen repeating unit of *Shigella flexneri*. Our particular immunochemical interest in a linear polysaccharide antigen has been discussed in detail[1]. Recent treatments of polysaccharide structure–conformation relationships[4–6] and of the orientation about glycosidic linkages[7] suggest that predictions as to the most likely conformations of polymeric antigens in solution may be undertaken. The structural analysis of the *S. flexneri* O-antigens has been completed in the Arrhenius laboratory and reveals a series of increasingly elaborate antigenic structures[8–11], based upon a linear tetrasaccharide repeating unit, that of the serogroup Y lipopolysaccharide (LPS)[10]. This antigen was chosen as the object of a synthetic project aimed at providing an artificial antigen representing the complete tetrasaccharide repeating unit and which, in conjunction with somatic cell-fusion techniques, may provide homogeneous antibody of predefined specificity[12,13]. The availability of homogeneous antibody specific for the synthetic tetrasaccharide hapten

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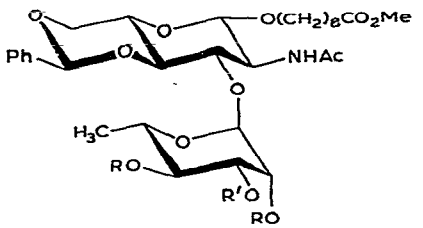
is particularly attractive for studies of antigen–antibody interaction. A detailed knowledge of the conformation of the synthetic antigen and, by inference, the polysaccharide, is possible, and information has already accumulated from earlier experiments[1-3]. These results suggest that the *exo*-anomeric effect[14] exerts a profound influence upon the conformation of *S. flexneri* LPS antigens in solution. Appreciation of these solution properties must be a prerequisite of studies of the chemical basis of antigen–antibody interaction. In this paper, we report the synthesis of an artificial tetrasaccharide hapten, 8-methoxycarbonyloctyl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (**12**), which constitutes the repeating unit of the *S. flexneri* serogroup Y LPS. We also report the synthesis of a trisaccharide hapten (**9**).

RESULTS AND DISCUSSION

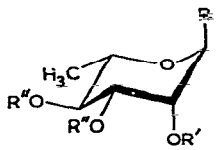
The oligosaccharides were synthesized as 8-methoxycarbonyloctyl glycosides in order to prepare artificial antigens by covalent attachment to protein, following deblocking of the hapten. This procedure follows the well established methodology of Lemieux *et al.*[15,16]. The immunogenicity of the artificial antigens obtained in this way has been verified both with respect to the level of the humoral response[16] and the carbohydrate specificity of these antibodies[16,17].

The partially deblocked disaccharide **1**, the synthesis of which is described elsewhere[1], was the starting point for the syntheses reported here. Conversion of **1** to the 2,4-dibenzoate **2** followed a procedure similar to that used to prepare 8-methoxycarbonyloctyl 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside[3]. Reaction of 8-methoxycarbonyloctyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (**1**) with trimethyl orthobenzoate gave a 2,3-orthobenzoate. Benzoylation of this intermediate, which was not isolated, by excess benzoyl chloride in pyridine was considered a hazard, due to the potential[18] for formation of 2,2-di-*N*-acyl derivatives of the 2-amino-2-deoxy-glucopyranoside residue. This complication was avoided by performing the benzoylation of the L-rhamnopyranose O-4 position in chloroform solution, at 0°, with a slight excess of benzoyl chloride and pyridine. The stereoselective opening of the 2,3-orthobenzoate to provide the 2,4-dibenzoate **2** was performed in aqueous acetic acid. Reaction of **2** with different L-rhamnopyranosyl halides **3**–**5** provided the trisaccharides **6** and **7**, the latter being the precursor of the tetrasaccharide **11**.

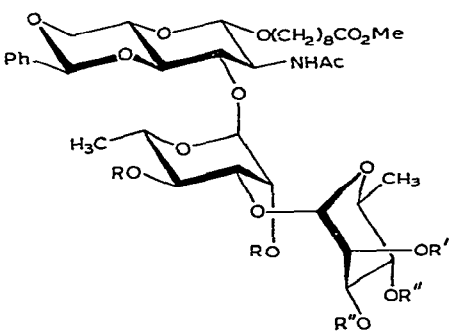
We have shown that 1,2-orthoacetates are intermediates in Koenigs–Knorr reactions promoted by silver trifluoromethanesulfonate (triflate)[19]. When the weak base, 1,1,3,3-tetramethylurea, is used as the proton acceptor[20,21], the acidity of the reaction is reduced so as to render protection with acetal groups compatible[21]. However, the conjugate acid of 1,1,3,3-tetramethylurea catalyzes the rearrangement of 1,2-orthoacetates to 1,2-*trans*-glycosides and other products[19]. In the case of 1,2-*trans*-glycosyl halides that are prone to orthoester formation[22-24], the use of silver triflate–1,1,3,3-tetramethylurea has, therefore, been particularly successful in providing α-L-rhamno-



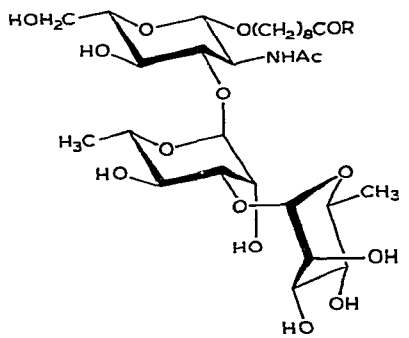
1 R = R′ = H
2 R = Bz, R′ = H



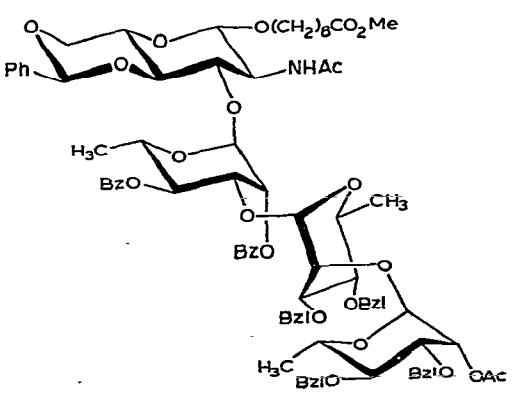
3 R = Br, R′ = R″ = Ac
4 R = Cl, R′ = R″ = Ac
5 R = Cl, R′ = Ac, R″ = Bzl



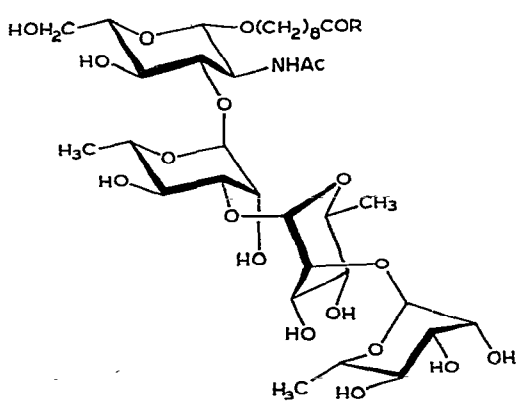
6 R = Bz, R′ = R″ = Ac
7 R = Bz, R′ = Ac, R″ = Bzl
8 R = Bz, R′ = H, R″ = Bzl



9 R = OMe
10 R = $NHNH_2$



11



12 R = OMe
13 R = $NHNH_2$

pyranosides in high yield[1-3,25], and we have used these conditions throughout these studies. Thus, reaction of 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide[26] (**3**) with the partially blocked disaccharide **2** gave a 61% yield of blocked trisaccharide **6**. In this instance, excess bromide **3** and promoter were added after 8 h, when unreacted **2** was observed by t.l.c. When 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl chloride (**4**) was treated with **2** in analogous fashion, a 45% yield of trisaccharide **6** occurred. The terminal 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl group is unsuitable for chain extension to the tetrasaccharide, and the approach to 2-*O*-substituted rhamnopyranosides used previously[1-3] was employed here. Direct reaction of the 1,2-orthoacetate, 3,4-di-*O*-benzyl-1,2-*O*-(methoxyethylidene)-β-L-rhamnopyranose with 8-methoxycarbonyloctanol under standard conditions of orthoester glycosylation has been shown to give 8-methoxycarbonyloctyl 3,4-di-*O*-benzyl-α-L-rhamnopyranoside in 63% yield[1]. By comparison, Koenigs–Knorr reaction between 8-methoxycarbonyloctanol and 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl chloride (**5**, prepared in essentially quantitative yield from the benzylated 1,2-orthoacetate[3]) provided 8-methoxycarbonyloctyl 3,4-di-*O*-benzyl-α-L-rhamnopyranoside in 75% yield[25]. This clearly demonstrates that the latter reaction is the most efficient route to α-L-rhamnopyranosides possessing persistent blocking groups at C-3 and C-4, and consequently this 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl chloride (**5**) has been used in both chain extension and chain termination steps. When the partially blocked disaccharide **2** was treated with **5** under Koenigs–Knorr conditions, the blocked trisaccharide **7** was obtained in 67% yield. Compared to the yields of 45% and 61% for trisaccharide **6**, this result indicates that glycosyl halides bearing ether groups at O-3 and O-4 are more efficient glycosylating derivatives than per-*O*-acetyl-rhamnopyranosyl halides. Similar observations have been reported by others[27]. Selective removal of the 2″-acetate group from trisaccharide **7** with magnesium methoxide[3] did not affect the 2′,4′-dibenzoate groups and yielded the partially blocked derivative **8** in 80% yield. Reaction of **5** with **8** gave the tetrasaccharide **11**. Removal of the protecting group from **11** was achieved by hydrogenolysis, hydrolysis of the benzylidene acetal, and finally transesterification of the ester groups. The intermediates were not isolated, and the yield of **12** from **11** was 63% after chromatography. The trisaccharide **6** was deblocked in a similar fashion. Both the trisaccharide **9** and tetrasaccharide **12** were converted to their respective hydrazide derivatives **10** and **13** in the usual manner. Purification of the impure hydrazides was accomplished most readily by chromatography on Sephadex LH-20 with methanol as the solvent. These hydrazides are the immediate precursors to artificial antigen synthesis, by which procedure[15] the hydrazide is converted to an acyl azide that is used without isolation to acylate amino groups, such as those of the L-lysine residues of a protein carrier.

Earlier work with the fully blocked derivative of the trisaccharide glycoside, 8-methoxycarbonyloctyl *O*-α-L-rhamnopyranoside-(1→2)-*O*-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranoside[3] and the disaccharide 8-methoxycarbonyloctyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside[1] showed that the exocyclic methyl protons of the L-rhamnose

residues had substantial upfield shifts in ^{1}H-n.m.r. spectra. These shifts were attributed to anisotropic shielding by the aromatic nuclei of adjacent blocking groups, benzoate esters[3] and benzylidene acetal[1,2]. Since the tetrasaccharide **11** incorporates all the blocking groups previously used[1-3], similar ^{1}H-n.m.r. shifts were observed for this fully blocked tetrasaccharide. The necessary juxtaposition of the H-6 protons in question, at C-6′ (adjacent to the phenyl group of the benzylidene acetal group[1,2]), and at C-6‴ (disposed toward the C-4′ benzoate group[3]) were most convincingly explained on the basis of preferred glycosidic orientations[1-3]. This conformational bias finds its origin in the *exo*-anomeric effect, and we have suggested[2,3] that the aforementioned ^{1}H-n.m.r. data provide supporting evidence for the validity of the effect in dictating the conformations of oligosaccharides in solution. The *exo*-anomeric effect[7] may be expressed in terms of the torsional angle ϕ about the bond between C-1 and O-1. When $\phi \simeq |60°|$, the energetically favorable antiperiplanar orientation of the O-5–C-1 bond with a *p*-orbital of O-1 is achieved. If the torsional angle ϕ is held in the range of $|50°|-|60°|$, then the second torsional angle ψ, which defines the torsional angle about the bond between O-1 and the carbon atom of the aglycon, approximates 0° (the eclipsed conformer), when nonbonded interactions are minimized[7]. When these criteria ($\phi \simeq |60°|$ and $\psi \simeq |0°|$) are applied to space-filling models of the tetrasaccharide **13**, the required juxtaposition of exocyclic methyl groups with the aromatic nuclei of the benzoate and benzylidene groups is fulfilled. Within the limitations of a two-dimensional presentation, these situations may be appreciated in structures **1**, **2**, **8**, and **11**. Although all the ^{1}H-n.m.r. evidence for conformational preference was obtained for blocked derivatives in organic solvents, it is anticipated that these glycosidic conformations will be maintained in aqueous solution. Indeed, preliminary evidence based upon T_1 measurements[3] has confirmed this, and further experiments are in progress to substantiate this interpretation. These observations agree with the hard-sphere calculations of Lemieux and Koto[7] based on the *exo*-anomeric effect, and accumulated X-ray data for disaccharides support[28] the concept of such a conformational bias. Furthermore, recent molecular orbital calculations[29,30] provide a theoretical basis for both the anomeric and *exo*-anomeric effects. Although the n.m.r. data accumulated in this and earlier studies[1-3] have provided good indications as to the glycosidic conformations adopted, we have used T_1 measurements[3] to provide indirect evidence for the values of the angles ϕ and ψ. This technique, together with the measurement of three-bond ^{13}C, ^{1}H-coupling constants[31] across the glycosidic bond, should provide additional information on these crucial questions of glycosidic orientation.

EXPERIMENTAL

General methods. — Thin-layer chromatography was performed with precoated silica gel plates (Merck, 60F-254), and the detection of compounds was achieved by charring after spraying with 5% sulfuric acid in ethanol. Column chromatography was performed on silica gel G-60 (Merck, 70–230 mesh) with redistilled solvents.

The loading on all columns was 1:100. Separations were also performed on a high-pressure liquid chromatograph, Prep 500 (Waters Associates), with solvent systems similar to those used for conventional chromatography. The hydrazide derivatives were purified on a Sephadex LH-20 column with methanol as solvent. Skellysolve B refers to hexane supplied by Getty Refining and Marketing Company, Tulsa, OK 74102. Palladium-on-charcoal (10%) was purchased from Engelhard Industries Division, Iselin, NJ 08830. Solvents were purified and dried according to standard procedures[32]. Processed solutions were dried with anhydrous sodium sulfate, and solvent removal was achieved with bath temperatures at 40° or lower, unless otherwise stated. All solvent mixtures are v/v. Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured at 589 nm in a 1-dm cell at room temperature (20–23°). Carbon-13 and ^{1}H-n.m.r. spectra were recorded at 20 and 79.9 MHz, respectively, in the pulsed, Fourier-transform mode with a Varian CFT-20 spectrometer. Proton chemical-shifts are expressed relative to 1% tetramethylsilane (Me_4Si) for chloroform-*d* and methanol-d_4, and for deuterium oxide solutions relative to sodium 3-trimethylsilylpropionate-*2,2,3,3-d*$_4$ (TSP). Carbon-13 shifts are expressed relative to internal Me_4Si for chloroform-*d* and methanol-d_4, and relative to external Me_4Si for deuterium oxide solutions. Carbon-13 assignments are tentative.

*8-Methoxycarbonyloctyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(α-*L*-rhamnopyranosyl)-β-*D*-glucopyranoside* (**1**). — 8-Methoxycarbonyloctyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside[1] (16.5 g, 22.0 mmol) was dissolved in methanol (500 mL) containing a catalytic amount of sodium. The syrup obtained after removal of sodium ions with Rexyn 101 (H^+) resin, filtration, and evaporation was purified on a silica gel column with 7:1 chloroform–methanol. This gave pure **1** as a foam (13.0 g, 95% yield), $[\alpha]_{589}^{20-23}$ −61.8° (*c* 1.1, methanol); t.l.c. (7:1 chloroform–methanol): R_F 0.28; ^{1}H-n.m.r. (CD_3OD): δ 0.74 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6′), 1.10–1.80 [m, 12 H, -$(CH_2)_6$-], 2.00 (s, 3 H, NHCOC*H*$_3$), 3.10–4.90 (ring H), 3.65 (s, 3 H, OCH_3), 5.56 (s, 1 H, C*H*Ph), and 7.10–7.50 (m, 5 H, aromatic); ^{13}C-n.m.r. (CD_3OD): 103.1 (C-1), 103.0 (2 C, *C*HPh and C-1′), 81.3 (C-3), 78.9 (C-4), 73.8 (C-4′), 72.4 (C-5), 72.1 (C-3′), 70.9 (C-2′), 69.9 (C-5′), 69.6 (OCH_2), 67.8 (C-6), 57.7 (C-2), and 17.6 (C-6′).

Anal. Calc. for $C_{31}H_{47}NO_{12}$: C, 59.51; H, 7.57; N, 2.24. Found: C, 59.34; H, 7.70; N, 2.31.

*8-Methoxycarbonyloctyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,4-di-*O*-benzoyl-α-*L*-rhamnopyranosyl)-β-*D*-glucopyranoside* (**2**). — The disaccharide **1** (13.0 g, 20.8 mmol), trimethyl orthobenzoate (5.0 g, 26.5 mmol), and *p*-toluenesulfonic acid (300 mg) in dry *N,N*-dimethylformamide (100 mL) was stirred for 20 h at room temperature. Triethylamine (2 mL) was added, and the mixture was evaporated. The residue was treated three times with Skellysolve B and then dissolved in chloroform (500 mL), which was extracted with a 5% water solution of sodium chloride (50 mL). The syrup obtained after drying and evaporation was dried under high vacuum overnight. This gave the 2,3-orthoester (14.5 g, yield 94%). To the 2,3-

orthoester (12.0 g, 16.1 mmol) in chloroform (200 mL) containing pyridine (10 mL) was added dropwise benzoyl chloride (2.3 mL, 20.0 mmol) at 0°. The reaction mixture was stirred overnight at 0°, and then processed by extraction with 0.1M hydrochloric acid and washing with sodium hydrogencarbonate solution and water. After drying and evaporation, the syrup was dissolved in aqueous 80% acetic acid and the solution stirred for 30 min. Evaporation and purification on a silica gel column with 1:2 Skellysolve B–ethyl acetate gave pure **2** (8.3 g, 62% yield), m.p. 204–205° (recryst. from carbon tetrachloride), $[\alpha]_{589}^{20-23}$ −17.3° (*c* 1.1, chloroform); t.l.c. (1:2 Skellysolve B–ethyl acetate): R_F 0.35; ^{1}H-n.m.r. ($CDCl_3$): δ 0.71 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6′), 0.90–1.80 [m, 12 H, -$(CH_2)_6$-], 2.05 (s, 3 H, $COCH_3$), 2.30 (t, 2 H, $COCH_2$), 3.20–5.30 (ring H), 3.65 (s, 3 H, OCH_3), 5.55 (s, 1 H, C*H*C_6H_5), and 7.10–8.20 (m, 15 H, aromatic); ^{13}C-n.m.r. ($CDCl_3$): 102.2 (*C*HPh), 100.3 (C-1), 97.7 (C-1′), 80.5 (C-3), 75.5 (2 C, C-4 and -2′), 73.8 (C-4′), 70.2 (C-5), 68.9 (C-3′), 68.5 (C-5′), 66.3 (2 C, OCH_2 and C-6), 58.8 (C-2), and 16.8 (C-6).

Anal. Calc. for $C_{45}H_{55}NO_{14}$: C, 64.81; H, 6.65; N, 1.84. Found: C, 64.92; H, 6.80; N, 1.68.

8-Methoxycarbonyloctyl O-(*2,3,4-tri*-O-*acetyl*-α-L-*rhamnopyranosyl*)-(1→3)-O-(*2,4-di*-O-*benzoyl*-α-L-*rhamnopyranosyl*)-(1→3)-*2-acetamido-4,6*-O-*benzylidene*-*2*-*deoxy*-β-D-*glycopyranoside* (**6**). — The partially protected glycoside **2** (0.84 g, 1.0 mmol) was dissolved in dichloromethane (20 mL) containing silver triflate (0.52 g, 2.0 mmol) and 1,1,3,3-tetramethylurea (2.4 mL, 20 mmol). This solution was cooled to −70° and 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide[26] (**3**, 0.7 g, 2.0 mmol) dissolved in dichloromethane (10 mL) was added dropwise with stirring. After 8 h, the reaction mixture was allowed to warm to 0°, then silver triflate (0.52 g, 2.0 mmol) was added, and the temperature was lowered again to −70°, and additional **3** (0.7 g, 2.0 mmol) in dichloromethane (10 mL) was added. The reaction mixture was allowed to warm overnight to room temperature, and the mixture was then filtered. Following extraction with saturated sodium hydrogencarbonate and water, the concentrated syrup was purified on a silica gel column (500 g) with 9:1 chloroform–methanol. This gave pure **6** (0.68 g, 61% yield), m.p. 108–110° (recryst. from ethyl acetate–Skellysolve B), $[\alpha]_{589}^{20-23}$ −9.6° (*c* 1.1, chloroform); t.l.c. (9:1 chloroform–methanol): R_F 0.40; ^{1}H-n.m.r. ($CDCl_3$): δ 0.70 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6″), 0.92 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6″), 1.00–1.85 [m, 12 H, -$(CH_2)_6$-], 1.82 (s, 3 H, $COCH_3$), 1.85 (s, 3 H, $COCH_3$), 1.91 (s, 3 H, $COCH_3$), 2.06 (s, 3 H, N*H*$COCH_3$), 2.30 (t, 2 H, -CH_2CO-), 3.10–5.50 (ring H), 3.66 (s, 3 H, OCH_3), 5.57 (s, 1 H, C*H*Ph), and 7.05–8.20 (m, 15 H, aromatic); ^{13}C-n.m.r. ($CDCl_3$): 102.1 (*C*HPh), 100.1 (C-1), 98.9 (C-1″), 97.3 (C-1′), 80.4 (C-3), 76.3 (C-3′), 74.9 (C-4), 72.8 (C-2′), 72.5 (C-4′), 71.0 (C-4′), 70.2 (C-5), 69.9 (C-3″), 68.9 (C-2″), 68.4 (C-5′), 67.2 (C-5″), 66.6 (OCH_2), 66.2 (C-6), 59.1 (C-2), 17.1 (C-6″), and 16.7 (C-6′).

Anal. Calc. for $C_{57}H_{71}NO_{21}$: C, 61.89; H, 6.47; N, 1.27. Found: C, 61.79; H, 6.61; N, 1.22.

8-Methoxycarbonyloctyl O-α-L-*rhamnopyranosyl*-(1→3)-O-α-L-*rhamnopyranosyl*-(*1→3*)-*2-acetamido-2-deoxy*-β-D-*glucopyranoside* (**9**). — The trisaccharide **6**

(700 mg, 0.63 mmol) was dissolved in methanol (50 mL) containing a catalytic amount of sodium. After 48 h, the solution was de-ionized and evaporated. The residue was dissolved in 90% aqueous trifluoroacetic acid (5 mL) at 0° and kept for 45 min. Evaporation and co-distillation with ethanol gave a syrup that was purified on a silica gel column with 7:2:1 ethyl acetate–methanol–water. This gave pure **9** (340 mg, 80% yield), $[\alpha]_{589}^{20-23}$ −55.0° (*c* 1.1, methanol); t.l.c. (7:2:1 ethyl acetate–methanol–water): R_F 0.46; ^{1}H-n.m.r. (D_2O, 85°): δ 1.10–1.90 [m, 18 H, H-6′, -6″, -$(CH_2)_6$-], 2.05 (s, 3 H, NHCOCH_3), 2.36 (t, 2 H, -CH_2CO), 3.71 (s, 3 H, OCH_3), 3.10–4.20 (ring H), 4.55 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.90 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1′), and 5.05 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1″); ^{13}C-n.m.r. (D_2O): 103.6 (C-1″), 102.5 (C-1′), 101.8 (C-1), 82.9 (C-3), 79.4 (C-3′), 77.2 (C-5), 73.2 (C-4), 72.5 (C-4′), 71.7 (C-4″), 71.4 (3C, C-2′, -2″, and -3″), 70.2 (2C, C-5′ and -5″), 69.8 (OCH_2), 62.1 (C-6), 56.5 (C-2), 17.9 (C-6″), and 17.7 (C-6′).

Anal. Calc. for $C_{30}H_{53}NO_{16}$: C, 52.70; H, 7.81; N, 2.05. Found: C, 52.85; H, 7.80; N, 2.01.

8-Hydrazinocarbonyloctyl O-α-L-*rhamnopyranosyl*-(*1*→*3*)-O-α-L-*rhamnopyranosyl*-(*1*→*3*)-*2-acetamido-2-deoxy*-β-D-*glucopyranoside* (**10**). — The deblocked trisaccharide **9** (52.0 mg, 0.08 mmol) was dissolved in ethanol (3 mL) to which an 85% solution of hydrazine hydrate (0.5 g) was added. The solution was stirred for 48 h, then evaporated and dried under high vacuum. Purification on a Sephadex LH-20 column with methanol gave pure hydrazide **10** (48.7 mg, 94% yield), $[\alpha]_{589}^{20-23}$ −55.1° (*c* 0.9, water); t.l.c. (6:3:1 ethyl acetate–methanol–water): R_F 0.30; ^{1}H-n.m.r. (D_2O, 85°): δ 1.10–1.70 [m, 18 H, H-6′, -6″, and -$(CH_2)_6$-], 2.03 (s, 3 H, NHCOCH_3), 2.20 (t, 2 H, -CH_2CO), 3.20–4.15 (ring H), 4.53 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.86 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1′), and 5.02 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1″); ^{13}C-n.m.r. (D_2O): 103.6 (C-1″), 102.5 (C-1′), 101.8 (C-1), 82.9 (C-3), 79.4 (C-3′), 77.2 (C-5), 73.2 (C-4), 72.5 (C-4′), 71.7 (2 C, C-4″ and -3″), 71.4 (2 C, C-2′ and -2″), 70.2 (2 C, C-5′ and -5″), 69.7 (OCH_2), 62.0 (C-6), 56.5 (C-2), 17.9 (C-6′), and 17.7 (C-6″).

Anal. Calc. for $C_{29}H_{53}N_3O_{15}$: C, 50.94; H, 7.81; N, 6.15. Found: C, 50.71; H, 7.95; N, 6.17.

8-Methoxycarbonyloctyl O-(*2*-O-*acetyl*-*3,4-di*-O-*benzyl*-α-L-*rhamnopyranosyl*)-(*1*→*3*)-O-(*2,4-di*-O-*benzoyl*-α-L-*rhamnopyranosyl*)-(*1*→*3*)-*2-acetamido-4,6*-O-*benzylidene-2-deoxy*-β-D-*glucopyranoside* (**7**). — The partially protected glycoside **2** (3.0 g, 3.6 mmol) was dissolved in dichloromethane (30 mL) containing silver triflate (1.3 g, 5.2 mmol) and 1,1,3,3-tetramethylurea (2.4 mL, 20 mmol). This solution was cooled to −70°, and 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl chloride[3] (**5**, 2.0 g, 5.0 mmol) dissolved in dichloromethane (15 mL) was added dropwise with stirring. The reaction mixture was allowed to warm overnight to room temperature, and was then filtered. Following extraction with saturated sodium hydrogencarbonate and water, the concentrated syrup was purified on a silica gel column with 1:1 Skellysolve B–ethyl acetate. This gave pure **7** (2.9 g, 67% yield), m.p. 153–155° (recryst. ethyl acetate–Skellysolve B), $[\alpha]_{589}^{20-23}$ +5.0° (*c* 1.0, chloroform); t.l.c. (1:1 Skellysolve B–ethyl acetate): R_F 0.36; ^{1}H-n.m.r. ($CDCl_3$): δ 0.72 (d, 3 H, $J_{5,6}$

6.0 Hz, H-6′), 0.98 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6″), 1.00–1.90 [m, 12 H, -$(CH_2)_6$-], 1.94 (s, 3 H, $COCH_3$), 2.06 (s, 3 H, $NHCOCH_3$), 2.31 (t, 2 H, -$COCH_2$-), 3.10–5.50 (ring H), 3.67 (s, 3 H, OCH_3), 5.58 (s, 1 H, C*H*Ph), 7.00–7.80 (m, 21 H, aromatic), and 7.90–8.15 (m, 4 H, aromatic); ^{13}C-n.m.r. ($CDCl_3$): 102.2 (*C*HPh), 101.2 (C-1), 100.2 (C-1″), 97.5 (C-1′), 80.6 (C-3), 79.5 (2 C, CH_2Ph), 76.1 (C-3′), 75.0 (C-4″), 74.4 (C-4), 73.4 (C-2′), 72.8 (C-4′), 71.7 (C-3″), 70.3 (C-5), 69.0 (C-2″), 68.6 (C-5″), 68.5 (C-5′), 66.5 (OCH_2), 66.3 (C-6), 59.1 (C-2), 17.7 (C-6″), and 16.8 (C-6′).

Anal. Calc. for $C_{67}H_{79}NO_{19}$: C, 66.93; H, 6.62; N, 1.16. Found: C, 66.82; H, 6.70; N, 1.16.

8-Methoxycarbonyloctyl O-(*3,4-di*-O-*benzyl*-α-L-*rhamnopyranosyl*)-(*1→3*)-O-(*2,4-di*-O-*benzoyl*-α-L-*rhamnopyranosyl*)-(*1→3*)-*2-acetamido-4,6*-O-*benzylidene*-*2-deoxy*-β-D-*glucopyranoside* (**8**). — Compound **7** (2.4 g, 2.0 mmol) in 2:1 methanol–tetrahydrofuran (30 mL) was cooled to 0°, and a freshly prepared solution of magnesium methoxide in methanol (12 mL of a 1% solution) was added. The reaction mixture was stirred for 48 h at 0°. After removal of magnesium ions with Rexyn 101 (H^+) resin, filtration, and evaporation, the syrup obtained was purified on a silica gel column with 3:5 Skellysolve B–ethyl acetate. This gave pure **8** (1.85 g, 80% yield), m.p. 137–139° (recryst. ethyl acetate–Skellysolve B), $[\alpha]_{589}^{20-23}$ +3.4° (*c* 1.0, chloroform); t.l.c. (3:5 Skellysolve B–ethyl acetate): R_F 0.40; ^{1}H-n.m.r. ($CDCl_3$): δ 0.71 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6′), 1.06 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6″), 1.00 –1.90 [m, 12 H, -$(CH_2)_6$-], 2.06 (s, 3 H, $NHCOCH_3$), 2.31 (t, 2 H, -$COCH_2$-), 3.10–5.50 (ring H), 3.67 (s, 3 H, OCH_3), 5.58 (s, 1 H, C*H*Ph), 6.90–7.80 (m, 21 H, aromatic), and 7.85–8.20 (m, 4 H, aromatic); ^{13}C-n.m.r. ($CDCl_3$): 102.2 (*C*HPh), 100.2 (C-1), 99.2 (C-1″), 97.4 (C-1′), 80.5 (C-3), 79.5 ($CHPh_2$), 77.5 (CH_2Ph), 77.3 (C-3′), 76.6 (C-4″) 75.0 (C-4), 73.0 (C-2′), 72.4 (C-4′), 71.4 (C-3″), 70.3 (C-5), 69.0 (2 C, C-2″ and -5″), 68.8 (C-5′), 66.5 (OCH_2), 66.3 (C-6), 59.0 (C-2), 17.7 (C-6″), and 16.7 (C-6′).

Anal. Calc. for $C_{65}H_{77}NO_{18}$: C, 67.28; H, 6.69; N, 1.21. Found: C, 67.48; H, 6.83; N, 1.16.

8-Methoxycarbonyloctyl O-(*2*-O-*acetyl*-*3,4-di*-O-*benzyl*-α-L-*rhamnopyranosyl*)-(*1→2*)-O-(*3,4-di*-O-*benzyl*-α-L-*rhamnopyranosyl*)-(*1→3*)-O-(*2,4-di*-O-*benzoyl*-α-L-*rhamnopyranosyl*)-(*1→3*)-*2-acetamido-4,6*-O-*benzylidene*-*2-deoxy*-β-D-*glucopyranoside* (**11**). — The partially blocked trisaccharide **8** (0.80 g, 0.7 mmol) was dissolved in dichloromethane (30 mL) containing silver triflate (0.4 g, 1.6 mmol) and 1,1,3,3-tetramethylurea (1.0 mL, 16 mmol). This solution was cooled to −70°, and 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl chloride[3] (**5**, 0.6 g, 1.5 mmol) dissolved in dichloromethane (10 mL) was added dropwise with stirring. The reaction mixture was allowed to warm to room temperature overnight, and then filtered. Following extraction with saturated sodium hydrogencarbonate and water, the concentrated syrup was purified on a silica gel column with 1:1 Skellysolve B–ethyl acetate. This gave pure **11** (0.73 g, 70% yield), $[\alpha]_{589}^{20-23}$ +8.1° (*c* 1.2, chloroform); t.l.c. (1:1 Skellysolve B–ethyl acetate): R_F 0.60; ^{1}H-n.m.r. ($CDCl_3$): δ 0.69 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6′), 1.02 (d, 6 H, H-6″ and -6‴), 1.00–1.90 [m, 12 H, -$(CH_2)_6$-], 2.07 (s, 3 H, $COCH_3$), 2.07 (s, 3 H, $NHCOCH_3$), 2.31 (t, 2 H, -$COCH_2$-), 3.00–5.50 (ring H),

3.68 (s, 3 H, OCH_3), 5.57 (s, 1 H, C*H*Ph), 6.80–7.70 (m, 31 H, aromatic), and 7.75–8.15 (m, 4 H, aromatic); ^{13}C-n.m.r. ($CDCl_3$): 102.1 (*C*HPh), 101.0 (C-1), 99.9 (C-1″), 98.7 (C-1‴), 97.4 (C-1′), 80.6 (C-3), 80.0 (*C*H_2Ph), 79.5 (*C*H_2Ph), 79.3 (*C*H_2Ph), 77.6 (*C*H_2Ph), 76.3 (C-2″), 75.5 (2 C, C-3′ and -4″), 74.3 (2 C, C-4 and -4‴), 73.7 (C-3″), 73.1 (C-2′), 72.7 (C-4′), 71.7 (2 C, C-3‴ and -5), 70.4 (C-2‴), 69.1 (C-5″), 68.7 (C-5‴), 68.2 (C-5′), 66.6 (OCH_2), 66.2 (C-6), 59.8 (C-2), 17.8 (2 C, C-6″ and -6‴), and 16.8 (C-6′).

Anal. Calc. for $C_{87}H_{101}NO_{23}$: C, 68.35; H, 6.66; N, 0.92. Found: C, 68.56; H, 6.70; N, 0.89.

8-Methoxycarbonyloctyl O-α-L-*rhamnopyranosyl-(1→2)*-O-α-L-*rhamnopyranosyl-(1→3)*-O-α-L-*rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-β*-D-*glucopyranoside* (**12**). — Compound **11** (1.00 g, 0.65 mmol) was dissolved in acetic acid (50 mL) and hydrogenated in the presence of 10% palladium-on-charcoal (0.5 g) at 505 kPa for 3 h. Filtration and co-evaporation with toluene (3 × 50 mL) gave a syrup, which was dissolved in 90% trifluoroacetic acid (10 mL) at 0°, and kept for 1 h. Evaporation and co-evaporation with toluene again gave a syrup, which was dissolved in methanol (50 mL) containing a catalytic amount of sodium. The solution was kept for 60 h at room temperature. The syrup, obtained after removal of sodium ions with Rexyn 101 (H^+) resin, filtration, and evaporation was purified on a silica gel column with 7:2:1 ethyl acetate–methanol–water to give pure **12** (0.34 g, 63% yield), $[\alpha]_{589}^{20-23}$ −58.1° (*c* 1.2, water); t.l.c. (7:2:1 ethyl acetate–methanol–water): R_F 0.35; ^{1}H-n.m.r. (D_2O, 85°): δ 1.20–1.75 [m, 21 H, H-6′, -6″, -6‴, and -$(CH_2)_6$-], 2.09 (s, 3 H, NHCOC*H*$_3$), 2.33 (t, 2 H, -$COCH_2$-), 3.40–4.20 (ring H), 3.75 (s, 3 H, OCH_3), 4.59 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.92 (d, 1 H, $J_{1,2}$ 0.7 Hz, H-1′), 5.03 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1‴), and 5.21 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1″); ^{13}C-n.m.r. (D_2O): 103.4 (C-1‴), 102.5 (C-1′), 102.0 (2 C, C-1 and -1″), 82.9 (C-3), 79.2 (C-2″), 78.5 (C-3′), 77.2 (C-5), 73.3 (3 C, C-4, -4′, and -4″), 72.9 (C-4‴), 71.3 (4 C, C-2′, -2‴, -3″, and -3‴), 70.3 (3 C, C-5′, -5″, and -5‴), 69.7 (OCH_2), 62.1 (C-6), 56.5 (C-2), 17.9 (2 C, C-6′ and -6″), and 17.6 (C-6‴).

Anal. Calc. for $C_{36}H_{63}NO_{20}$: C, 52.10; H, 7.65; N, 1.69. Found: C, 52.01; H, 7.72; N, 1.75.

8-Hydrazinocarbonyloctyl O-α-L-*rhamnopyranosyl-(1→2)*-O-α-L-*rhamnopyranosyl-(1→3)*-O-α-L-*rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β*-D-*glucopyranoside* (**13**). — The deblocked tetrasaccharide **12** (70.0 mg, 0.08 mmol) was dissolved in ethanol (3 mL), and an 85% solution of hydrazine hydrate (0.5 g) was added. The solution was stirred for 48 h, then evaporated and dried under high vacuum. Purification on a Sephadex LH-20-column with methanol gave pure hydrazide **13** (63.1 mg, 90% yield), $[\alpha]_{589}^{20-23}$ −56.2° (*c* 1.1, water); t.l.c. (6:3:1 ethyl acetate–methanol–water): R_F 0.25; ^{1}H-n.m.r. (D_2O, 85°): δ 1.10–1.80 [m, 21 H, H-6′, -6″, -6‴, and -$(CH_2)_6$-], 2.02 (s, 3 H, $COCH_3$), 2.19 (t, 2 H, -$COCH_2$-), 3.10–4.20 (ring H), 4.52 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.87 (bs, 1 H, H-1′), 4.97 (s, 1 H, $J_{1,2}$ 1.6 Hz, H-1‴), and 5.15 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1″); ^{13}C-n.m.r. (D_2O): 103.4 (C-1‴), 102.5 (C-1′), 102.0 (C-1″), 101.8 (C-1), 82.8 (C-3), 79.2 (C-2″), 78.5 (C-3′), 77.2 (C-5), 73.2 (2 C,

C-4′ and -4″), 72.8 (2 C, C-4 and -4‴), 71.7 (C-3″), 71.2 (3 C, C-2′, -2‴, and -3‴), 70.3 (2 C, C-5″ and -5‴), 70.1 (C-5′), 69.7 (OCH_2), 62.0 (C-6), 56.5 (C-2), 17.9 (2 C, C-6′ and -6″), and 17.6 (C-6‴).

Anal. Calc. for $C_{35}H_{63}N_3O_{19}$: C, 50.66; H, 7.65; N, 5.06. Found: C, 50.87; H, 7.59; N, 4.89.

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