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ARTIFICIAL CARBOHYDRATE ANTIGENS: A BLOCK SYNTHESIS OF A LINEAR, TETRASACCHARIDE REPEATING-UNIT OF THE *Shigella flexneri* VARIANT Y POLYSACCHARIDE†

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ABSTRACT

Glycosylation of methyl 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside with 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide gave methyl 2,4-di-*O*-benzoyl-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**4**) in 93% yield. Conversion of **4** into the corresponding glycosyl bromide was accomplished with dibromomethyl methyl ether. Under Koenigs–Knorr conditions, this bromide reacted with 8-(methoxycarbonyl)octyl 2-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glycopyranosyl)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside, to provide the protected tetrasaccharide in 91% yield. Removal of blocking groups gave 8-(methoxycarbonyl)octyl *O*-α-L-rhamnopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside. Together with previously synthesized tetrasaccharides of the *Shigella flexneri* Y O-antigen, this oligosaccharide has been used to study the conformation of O-antigens and to assist in the selection of *S. flexneri*, variant Y, specific monoclonal antibodies.

INTRODUCTION

Two of the four possible tetrasaccharides of the *Shigella flexneri*, Y-variant, O-antigen have been synthesized[1,2], together with four trisaccharides[1–4]. Serological testing of these oligosaccharides revealed the trisaccharide sequence α-L-Rha-(1→3)-β-D-GlcNAc-(1→2)-α-L-Rha to be one of the most active inhibitors of rabbit anti-serum. On conformational grounds, we argued that the tetrasaccharide α-L-Rha-(1→3)-α-L-Rha-(1→3)-β-D-GlcNAc-(1→2)-α-L-Rha permits presentation of *C*-methyl groups from the terminal L-rhamnosyl group and L-rhamnose residue, with the polar acetamido residue intervening[5]. The surface topography resulting is a potential, antibody-binding surface associated with serological factors 3 and 4. To

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test this hypothesis, and to assist in the selection of hybrid myeloma antibodies from somatic cell-fusion experiments[6], this tetrasaccharide sequence was synthesized.

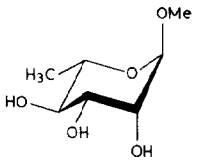
RESULTS

We had described the synthesis of various *S. flexneri* oligosaccharides by sequential, chain extension[1,4]. The synthesis of the frame-shifted tetrasaccharide α-L-Rha-(1→3)-α-L-Rha-(1→3)-β-D-GlcNAc-(1→2)-α-L-Rha was envisaged as a block synthesis starting from a suitably protected 3-*O*-α-L-rhamnopyranosyl-α-L-rhamnopyranose derivative and a 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-L-rhamnopyranoside.

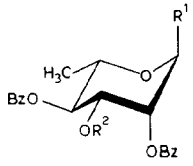
The first of these disaccharides was synthesized from methyl α-L-rhamnopyranoside (**1**), which was converted into its 2,3-orthobenzoate in acetonitrile solution. Removal of solvent, followed by benzoylation in pyridine gave a fully substituted intermediate which was not isolated, but was converted into the 2,4-dibenzoate **2** in aqueous acetic acid[7]. Reaction of **2** with two mol of 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide (**7**) in dichloromethane, with mercuric cyanide as a promoter, gave the disaccharide glycoside **9** in 93% yield after chromatography. When the reaction was conducted with 1.4 mol of **7**, the yield of **9** was 80%. Catalysis of a similar reaction with silver triflate–1,1,3,3-tetramethylurea gave yields of 62–67%. So-called "open" syntheses of disaccharides closely related to **9** have been reported[8,9] in yields that range from 11 to 80%. Reaction of 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl chloride[4] (**8**) with **2** in the presence of mercuric cyanide and mercuric bromide gave disaccharide **14** in 88% yield. In this instance, the reaction conditions were not optimized, and purification is hampered by the similar chromatographic mobilities of **2** and **14**. This difficulty was overcome by selective *O*-deacetylation of impure fractions to give **15**, which is readily purified and reacetylated, to give more pure compound **14**.

A variety of conditions for the direct conversion of disaccharide glycosides **9** and **14** into glycosyl halides was investigated. On reaction with bromotrimethylsilane[10], **9** gave only starting material, even under forcing conditions employing the Lewis acid $ZnBr_2$, an eight-molar excess of reagent, and reaction for 18 h in toluene at 80°. Iodotrimethylsilane, prepared *in situ*[11], gave a similar result. Acetyl bromide in glacial acetic acid–acetic anhydride[12] gave the desired glycosyl bromide **10** together with monosaccharide bromides. The most convenient preparation of **10** was the reaction of **9** with dibromomethyl methyl ether in dichloromethane containing a catalytic amount of zinc bromide[13]. Product **10** was obtained spectroscopically pure in 50% yield following preparative, 4-MPa l.c. to remove monosaccharide components. During this reaction, partial cleavage of the interglycosidic linkage occurred, and the glycosyl bromides **3** and **10** were trapped as their isopropyl glycosides **4** and **11**, respectively.

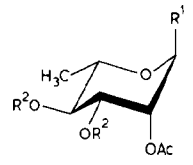
The disaccharide glycoside **14** could not be converted into a glycosyl halide in



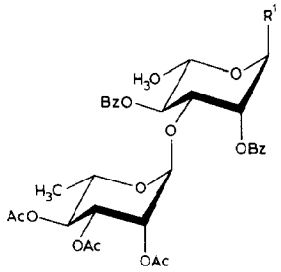
1



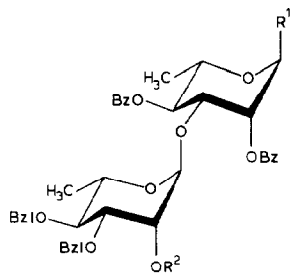
2 R^1 = OMe, R^2 = H
3 R^1 = Br, R^2 = H
4 R^1 = $OCHMe_2$, R^2 = H
5 R^1 = OMe, R^2 = Ac
6 R^1 = OMe, R^2 = $PhCH_2$



7 R^1 = Br, R^2 = Ac
8 R^1 = Cl, R^2 = $PhCH_2$



9 R^1 = OMe
10 R^1 = Br
11 R^1 = $OCHMe_2$
12 R^1 = OH
13 R^1 = Ac

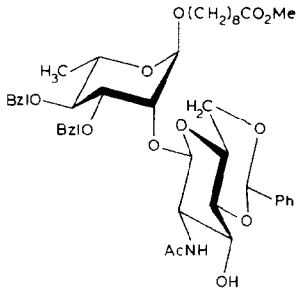


14 R^1 = OMe, R^2 = Ac
15 R^1 = OMe, R^2 = H
16 R^1 = OAc, R^2 = Ac
Bzl = $PhCH_2$

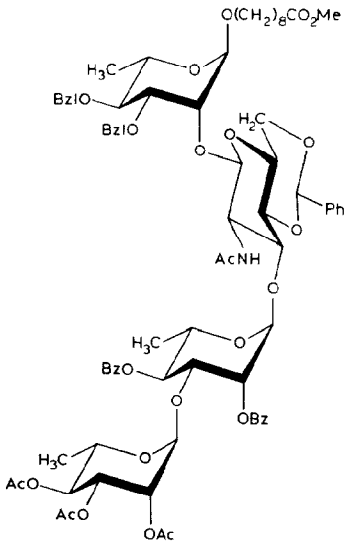
a fashion analogous to the conversion of **9** into **10**, as the benzyl ether groups were labile to dibromomethyl methyl ether. Acetolysis of **14** gave the acetate **16**, from which a glycosyl halide could be prepared. Although not elaborated here, such a derivative would provide a tetrasaccharide from which higher saccharides could be readily prepared after selective *O*-deacetylation, as shown for **14**.

The selectively protected disaccharide glycoside **17**, prepared according to published procedures[3], was treated with a 1-molar excess of the glycosyl bromide **10** in dichloromethane in the presence of mercuric cyanide and molecular sieve. After chromatography, the tetrasaccharide **18** was obtained in 90% yield, along with the reducing disaccharide **12**. After acetylation of this by-product to give **13**, the latter may be used to prepare more bromide **10**. The blocked tetrasaccharide **18** in acetic acid–methanol was hydrogenolyzed in the presence of palladium-on-charcoal, to give the acylated intermediate **19**, which, after transesterification, provided the deblocked tetrasaccharide **20**. Reaction with hydrazine in ethanol converted the 8-(methoxycarbonyl)octyl group of **20** into the corresponding 8-(hydrazidocarbonyl)octyl glycoside **21**, the precursor of the acyl azide used to prepare synthetic glycoconjugates[14].

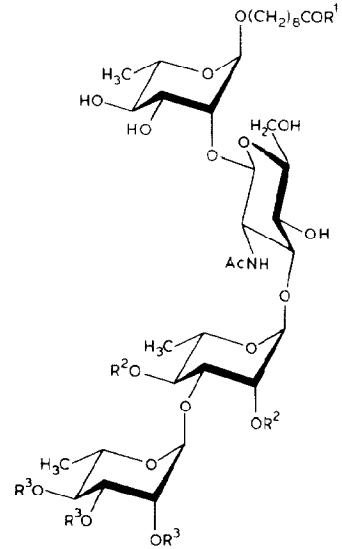
The structures assigned were in accordance with their ^{13}C- and ^{1}H-n.m.r.



17



18



19 R^1 = OMe, R^2 = Bz, R^3 = Ac
20 R^1 = OMe, R^2 = R^3 = H
21 R^1 = $NHNH_2$, R^2 = R^3 = H

data. Although, for the most part, ^{1}H-n.m.r. data were unexceptional, the ^{13}C data, especially for blocked and partially protected derivatives, are worthy of note and allow assignments of the tetrasaccharide spectra. Rhamnose and many of its derivatives have been characterized by ^{13}C-n.m.r. spectroscopy[15,16], which provided comparative data for the compounds reported here. Thus, carbon shifts of the 2,4-dibenzoate **2** compare well with those for the analogous 2,4-diacetate[15]. Assignments of disaccharide **9** were possible by comparison with the fully blocked rhamnopyranosides **5** and **6**. Generally, the known shift-rules[15–17] could be satisfactorily applied. Resonances of the benzylic methylene groups were confirmed by recording the coupled, ^{13}C-n.m.r. spectra. The chemical shifts of the mono- and di-rhamnopyranosides investigated are listed in Table I.

TABLE I

^{13}C-CHEMICAL SHIFTS OF MONO- AND DI-RHAMNOPYRANOSIDES IN $CDCl_3$

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1'*	*C-2'*	*C-3'*	*C-4'*	*C-5'*	*C-6'*	*OCH_3*	*Others*[a]
2	98.5	73.3	68.9	75.6	66.2	17.7							55.3	
9	98.4	72.2	75.5	73.4	66.6	17.7	99.3	69.8	68.5	71.1	67.4	17.2	55.3	20.5, 20.7 (OAc)
8							98.8	69.0	78.1	80.1	67.7	18.1	54.8	21.1 (OAc), 71.8 (Bzl 3'), 75.4 (Bzl 4)
14	98.4	72.0	75.9	73.4	66.5	17.7	99.4	68.9	77.4	79.4	68.7	17.7	55.3	20.8 (OAc), 71.4 (Bzl 3'), 74.4 (Bzl 4')
15	98.5	72.4	75.5	73.7	66.6	17.7	101.2	68.7	79.5	79.5	68.4	17.7	55.3	71.9 (Bzl 3'), 74.4 (Bzl 4')
16	90.5	70.9	75.7	72.7	68.8	17.7	99.5	68.8	77.2	79.3	68.8	17.7		20.6, 20.8 (OAc), 71.3 (Bzl 3'), 74.3 (Bzl 4')
12	92.0	72.6	75.1	73.4	66.9	17.8	99.2	69.8	68.5	71.1	67.2			20.6, 20.7 (OAc)
13	90.7	70.4	75.3	72.8	69.1	17.8	99.3	69.7	68.4	72.8	67.4	17.3		20.6, 20.7, 21.0 (OAc)
5	98.7	70.6	69.3	71.9	66.6	17.8							55.3	20.7 (OAc)
6	99.0	69.0	74.3	73.3	66.6	17.8							55.2	70.9 (Bzl 3)

[a]Bzl = benzyl.

High-resolution, ^{1}H-n.m.r. data for the tetrasaccharide **20** were in agreement with chemical-shift data previously published for *S. flexneri* oligosaccharides[5]. This indicates that the conformation of **20** in solution is well approximated by the model proposed for related, frame-shifted oligosaccharides[5]. Thus, the scheme of synthesis reported here provides potentially useful antigens and inhibitors for studies of the interaction of the *Shigella flexneri* Y antigen with monoclonal antibodies.

EXPERIMENTAL

General methods. — The methods employed were similar to those reported[2]. Optical rotations were measured at 589 nm in a 1-dm cell at 20–23°. Carbon-13 and ^{1}H-n.m.r. spectra were recorded at 20.0 and 79.9, and 400 MHz, respectively. Proton chemical-shifts are expressed relative to 1% of tetramethylsilane (Me_4Si) for solutions in chloroform-*d* and benzene-d_6, and, for solutions in deuterium oxide, relative to internal acetone (δ 2.12) at 400 MHz, and to sodium 4,4-dimethyl-4-silapentanoate-2,2,3,3-d_4. Carbon-13 chemical-shifts are expressed relative to internal Me_4Si for solutions in chloroform-*d*, and relative to external Me_4Si for solutions in deuterium oxide.

Methyl 2,4-di-O-benzoyl-α-L-rhamnopyranoside (**2**). — Methyl α-L-rhamnopyranoside[18] (**1**) (18.0 g, 100 mmol) was dissolved in dry acetonitrile (250 mL) by heating and stirring. This solution was cooled, and trimethyl orthobenzoate (23 g, 126 mmol), *p*-toluenesulfonic acid (50 mg), and 3 Å molecular sieve (2.0 g) were added, the solution stirred for 5 h, and evaporated. A solution of the resulting syrup in dry pyridine (150 mL) was cooled to 0°, benzoyl chloride (15 mL, 130 mmol) was added dropwise, and after 18 h, the solution was diluted with dichloromethane (500 mL), the resultant suspension filtered, and the filtrate successively washed with M hydrochloric acid, saturated aqueous sodium hydrogencarbonate, and water (100 mL each), and evaporated. The residue was dissolved in 80% aqueous acetic acid (100 mL), and, after 18 h, evaporation yielded crude product, which was purified by preparative, 4-MPa l.c. using 3:1 (v/v) Skellysolve B–ethyl acetate. Compound **2** was obtained as a homogeneous syrup (22.7 g, 59%); $[\alpha]_D^{24}$ +57.3° (*c* 2.4, dichloromethane); ^{1}H-n.m.r. (C_6D_6): δ 1.30 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 3.07 (s, 3 H, OMe), 4.00 (dq, 1 H, H-5), 4.45 (dd, 1 H, $J_{3,4}$ 9.8 Hz, H-3), 4.86 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.68 (dd, 1 H, $J_{2,3}$ 3.6 Hz, H-2), 5.72 (dd, ~t, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 6.96–7.25 (m, 6 H, aromatic), and 8.05–8.35 (m, 4 H, aromatic).

Anal. Calc. for $C_{21}H_{22}O_7$: C, 65.28; H, 5.74. Found: C, 65.04; H, 5.79.

Methyl 2,4-di-O-benzoyl-3-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**9**). — A solution of 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide[19] (**7**; 12 g, 310 mmol) in absolute dichloromethane (100 mL) was added dropwise during 2 h to a stirred solution of **2** (6.1 g, 15.8 mmol) in absolute dichloromethane (at −80°) containing mercuric cyanide (4.0 g, 16 mmol) and 4A molecular sieve (3 g). The mixture was allowed to warm overnight to room temper-

ature, and filtered. The filtrate was successively washed with saturated aqueous sodium hydrogencarbonate and water, and evaporated to a syrup which was purified on a column of silica gel (600 g) with 2:1 (v/v) Skellysolve B–ethyl acetate. Analytically pure **9** (9.7 g, 93%) was obtained as a syrup; $[\alpha]_D^{24}$ +46.0° (*c* 1.0, dichloromethane); ^{1}H-n.m.r. (C_6D_6): δ 1.23 (d, 3 H, $J_{5',6'}$ 6.4 Hz, H-6'), 1.32 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6), 1.49 (s, $OCOCH_3$), 1.53 (s, $OCOCH_3$), 1.56 (s, $OCOCH_3$), 3.07 (s, 3 H, OMe), 4.02 (dq, H-5), 4.28 (dq, H-5'), 4.58 (dd, 1 H, $J_{3,4}$ 9.8 Hz, H-3), 4.87 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 5.09 (d, 1 H, $J_{1',2'}$ 1.9 Hz, H-1'), 5.27 (dd, 1 H, $J_{2',3'}$ 3.0 Hz, H-2'), 5.35 (dd, ~t, 1 H, $J_{4',5'}$ 8.9 Hz, H-4'), 5.55 (dd, 1 H, $J_{3',4'}$ 9.8 Hz, H-3'), 5.83 (dd, 1 H, $J_{2,3}$ 3.5 Hz, H-2), 5.95 (dd, ~t, 1 H, $J_{4,5}$ 10.1 Hz, H-4), 6.96–7.25 (m, 6 H, aromatic), and 8.05–8.35 (m, 4 H, aromatic).

Anal. Calc. for $C_{33}H_{38}O_{14}$: C, 60.18; H, 5.82. Found: C, 59.90; H, 5.92.

*Methyl 3-O-(2-O-acetyl-3,4-di-O-benzyl-α-*L*-rhamnopyranosyl)-2,4-di-O-benzoyl-α-*L*-rhamnopyranoside* (**14**). — A solution of 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl chloride[4] (**8**) (1.9 g, 5.0 mmol) in absolute dichloromethane (25 mL) was added dropwise during 5 h to a stirred solution of **2** (1.1 g, 2.85 mmol) in absolute dichloromethane (20 mL) containing mercuric cyanide (1.5 g, 6 mmol), mercuric bromide (1.0 g, 2.77 mmol), and 3A molecular sieve (3 g). After being stirred for 18 h at room temperature, the mixture was boiled under reflux for 20 h, cooled, filtered, and the filtrate successively washed with saturated aqueous sodium hydrogencarbonate and water, and evaporated, to give crude **14**, which was chromatographed on silica gel (300 g) with 3:1 (v/v) Skellysolve B–ethyl acetate. Homogeneous **14** (1.12 g) was obtained as an analytically pure syrup; $[\alpha]_D^{24}$ +63.7° (*c* 1.4, dichloromethane). Rechromatography of impure fractions gave **14** (1.57 g; total yield, 75.4%). An additional 156 mg of **14** could be obtained by *O*-deacetylation ation to give **15**, chromatography of **15**, and acetylation thereof, to afford an overall yield of 1.83 g (88%) of **14**; ^{1}H-n.m.r. (C_6D_6): δ 1.35 (d, $J_{5',6'}$ 6.2 Hz, H-6'), 1.38 (d, $J_{5,6}$ 6.2 Hz, H-6), 1.61 (s, 3 H, $OCOCH_3$), 3.08 (s, 3 H, OMe), 3.54 (dd, ~t, 1 H, $J_{4',5'}$ 9.3 Hz, H-4'), 3.99 (d, $J_{A,B}$ −11.0 Hz, PhCHa', *H*b'), 4.04 (dd, $J_{3',4'}$ 9.3 Hz, H-3'), 4.04 (dq, H-5), 4.20 (dq, H-5'), 4.22 (d, PhC*H*a', Hb'), 4.45 (d, $J_{A,B}$ −11.6 Hz, PhCHa, *H*b), 4.56 (dd, 1 H, $J_{3,4}$ 9.9 Hz, H-3), 4.86 (d, PhC*H*a, Hb), 4.92 (d, $J_{1,2}$ 1.7 Hz, H-1), 5.10 (d, $J_{1',2'}$ 1.8 Hz, H-1'), 5.37 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-2'), 5.95 (dd, $J_{2,3}$ 3.5 Hz, H-2), 6.03 (dd, ~t, $J_{3,4}$ 9.9 Hz, H-4), 6.89–7.21 (m, 6 H, aromatic), and 8.11–8.35 (m, 4 H, aromatic).

Anal. Calc. for $C_{41}H_{46}O_{12}$: C, 68.42; H, 6.34. Found: C, 68.25; H, 6.05.

*Methyl 2,4-di-O-benzoyl-3-O-(3,4-di-O-benzyl-α-*L*-rhamnopyranosyl)-α-*L*-rhamnopyranoside* (**15**). — A 1:1 mixture of **14** and unreacted **2** (500 mg, from the previous experiment) was dissolved in methanol (10 mL), and a freshly prepared 1% solution of magnesium methoxide in methanol (2 mL) was kept for 48 h at 5°. Neutralization of the base with Rexyn-101 (H^+) resin, filtration, and evaporation gave a syrup that, after chromatography with 3:2 Skellysolve B–ethyl acetate as the eluant, gave 156 mg of **15**; $[\alpha]_D^{24}$ +66.4° (*c* 1.9, dichloromethane).

Anal. Calc. for $C_{39}H_{44}O_{11}$: C, 69.09; H, 6.22. Found: C, 68.93; H, 6.11.

2,4-Di-O-benzoyl-3-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl bromide (**10**). — A solution of **9** (7.0 g, 10.6 mmol) in absolute dichloromethane containing a catalytic amount of zinc bromide (100 mg) and dibromethyl methyl ether (1.1 mL, 11.7 mmol) was stirred under an atmosphere of dry nitrogen for 15 h at −15° and then for 5 h at 0°, freeze-dried, and the product purified by preparative, 4-MPa l.c. with 3:1 (v/v) Skellysolve B–ethyl acetate. Compound **10** was obtained as a homogeneous (t.l.c.) syrup (3.8 g, 50%); ^{1}H-n.m.r. (C_6D_6): δ 1.20 (d, 6 H, $J_{5,6}$ 6.3 Hz, H-6,6′), 1.44 (s, 3 H, $OCOCH_3$), 1.48 (s, 3 H, $OCOCH_3$), 1.52 (s, 3 H, $OCOCH_3$), 4.10 (dq, $J_{4,5}$ 9.9 Hz, H-5), 4.29 (dq, $J_{4',5'}$ 8.9 Hz, H-5′), 4.98 (dd, 1 H, H-3), 5.09 (d, 1 H, $J_{1',2'}$ 2.0 Hz, H-1′), 5.23 (dd, $J_{2',3'}$ 3.5 Hz, H-2′), 5.31 (dd, ~t, $J_{3',4'}$ 9.7 Hz, H-4′), 5.50 (dd, 1 H, H-3′), 5.95 (dd, ~t, $J_{3,4}$ 10.0 Hz, H-4), 5.98 (dd, $J_{2,3}$ 3.5 Hz, H-2), 6.40 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 6.89–7.06 (m, 6 H, aromatic), and 7.83–8.15 (m, 4 H, aromatic).

Isopropyl 2,4-di-O-benzoyl-3-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**11**) *and isopropyl 2,4-di-O-benzoyl-α-L-rhamnopyranoside* (**4**). — The reaction of **9** (77 mg, 0.12 mmol) with dibromomethyl methyl ether was conducted as described for the synthesis of **10**. The crude reaction products were not separated and after the freeze-drying step, the syrup was dissolved in dry 2-propanol (1 mL). 1,1,3,3-Tetramethylurea (150 μL) was added to this solution and, after 18 h, dilution with dichloromethane (30 mL) and extraction with water (2 × 15 mL) afforded two products that fluoresced under u.v. light. These products were isolated by preparative t.l.c. using 1-mm plates and 3:2 (v/v) Skellysolve B–ethyl acetate. The major component of the mixture was glycoside **11** (27 mg, 34%); $[\alpha]_D^{24}$ +32.2° (*c* 0.63, dichloromethane); ^{1}H-n.m.r. (C_6D_6): δ 1.03 (d, 3 H, J_{CH,CH_3} 6.1 Hz, $CHCH_3$), 1.15 (d, 3 H, J_{CH,CH_3} 6.1 Hz, $CHCH_3$), 1.23 (d, 3 H, $J_{5',6'}$ 6.3 Hz, H-6′), 1.40 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6), 1.48 (s, $OCOCH_3$), 1.49 (s, $OCOCH_3$), 1.55 (s, $OCOCH_3$), 3.78 (qq, ~sept, 1 H, $CHCH_3$), 4.26 (dq, $J_{4,5}$ 9.9 Hz, H-5), 4.32 (dq, $J_{4',5'}$ 8.9 Hz, H-5′), 4.74 (dd, 1 H, $J_{3,4}$ 9.8 Hz, H-3), 5.12 (d, $J_{1,2}$ 1.8 Hz, H-1), 5.28 (d, $J_{1',2'}$ 2.0 Hz, H-1′), 5.33 (dd, $J_{2',3'}$ 3.0 Hz, H-2′), 5.39 (dd, H-4′), 5.63 (dd, $J_{3',4'}$ 9.8 Hz, H-3′), 5.90 (dd, $J_{2,3}$ 3.4 Hz, H-2), 6.10 (dd, ~t, H-4), 6.83–7.23 (m, 6 H, aromatic), and 8.06–8.40 (m, 4 H, aromatic).

Anal. Calc. for $C_{35}H_{42}O_{14}$: C, 61.22; H, 6.17. Found: C, 61.38; H, 6.30.

The monosaccharide glycoside **4** (15 mg, 30%) was also isolated; $[\alpha]_D^{24}$ +38.6° (*c* 0.37, dichloromethane); ^{1}H-n.m.r. (C_6D_6): δ 1.00 [d, 3 H, J_{CH,CH_3} 6.1 Hz, $CH(CH_3)_2$], 1.12 [d, 3 H, J_{CH,CH_3} 6.1 Hz, $CH(CH_3)_2$], 1.35 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 3.73 [qq, ~sept, 1 H, $CH(CH_3)_2$], 4.25 (dq, 1 H, $J_{4,5}$ 9.7 Hz, H-5), 4.56 (dd, 1 H, H-3), 5.21 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 5.63 (dd, 1 H, $J_{2,3}$ 3.6 Hz, H-2), 5.77 (dd, ~t, 1 H, $J_{3,4}$ 9.9 Hz, H-4), 6.82–7.16 (m, 6 H, aromatic), and 7.93–8.25 (m, 4 H, aromatic).

Anal. Calc. for $C_{23}H_{26}O_7$: C, 66.65; H, 6.37. Found: C, 66.43; H, 6.22.

1-O-Acetyl-3-O-(2-O-acetyl-3,6-di-O-benzyl-α-L-rhamnopyranosyl)-2,4-di-O-benzoyl-α-L-rhamnopyranose (**16**). — A solution of **14** (600 mg, 0.82 mmol) in acetic anhydride (10 mL), glacial acetic acid (4 mL), and sulfuric acid (0.1 mL) was

kept for 15 min at 20°. Sodium acetate was added, and the mixture was stirred for 10 min and poured into cold, aqueous sodium hydrogencarbonate solution. Extraction with ethyl acetate (2 × 50 mL), concentration, and chromatography with 2:1 (v/v) Skellysolve B–ethyl acetate as the eluant gave **16** as a homogeneous syrup (324 mg, 52%); $[\alpha]_D^{24}$ +47.3° (*c* 2.86, dichloromethane).

Anal. Calc. for $C_{42}H_{46}O_{13}$: C, 67.51; H, 5.92. Found: C, 67.97; H, 5.71.

8-(Methoxycarbonyl)octyl O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-(1→3)-O-(2,4-di-O-benzoyl-α-L-rhamnopyranosyl)-(1→3)-O-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (**18**). — A solution of the disaccharide bromide **10** (3.8 g, 5.25 mmol) in absolute dichloromethane (20 mL) was slowly added to a stirred solution (at −80°) of the selectively protected disaccharide[3] **17** (1.68 g, 2.08 mmol) in absolute dichloromethane (20 mL) containing mercuric cyanide (1.01 g, 4 mmol) and 4A molecular sieve (2 g). The mixture was stirred overnight, and allowed to warm to room temperature. Filtration, successive washing of the filtrate with aqueous sodium hydrogencarbonate solution and water, and evaporation gave a syrup that was chromatographed on silica gel (400 g) with 3:2 (v/v) Skellysolve B–ethyl acetate as the eluant. The pure tetrasaccharide **18** (2.7 g, 90.6%) was obtained as a homogeneous syrup; $[\alpha]_D^{24}$ −8.7° (*c* 0.52, dichloromethane). Tetrasaccharide **18** was best characterized by its ^{13}C-n.m.r. spectrum ($CDCl_3$): δ 102.9 (C-1′), 102.1 ($PhCH{<}^{O}_{O}$), 98.9 (2 C, C-1,1‴), 97.6 (C-1″), 81.2 (C-4), 79.9 (2 C, C-3,3′), 78.1 (C-2), 77.1 (C-4′), 75.6 (2 C, C-3″, CH_2Ph), 73.3 (C-5′), 71.1 (C-4‴), 69.9 (C-2‴), 68.6 (2 C, C-3‴, CH_2Ph), 67.8 (C-6′), 67.5 [$OCH_2(CH_2)_7$-], 67.2 (C-5‴), 66.6 (C-5″), 57.2 (C-2′), 51.5 (OCH_3), 34.1 (-CH_2COCH_3), 29.5–25.0 [$OCH_2(CH_2)_6CH$-], 23.5 ($NHCOCH_3$), 20.6 (3 C, $OCOCH_3$), 18.0 (C-6), 17.1 (C-6‴), and 16.9 (C-6″).

Anal. Calc. for $C_{77}H_{93}NO_{25}$: C, 64.56; H, 6.54; N, 0.98. Found: C, 64.13; H, 6.60; N, 0.89.

The disaccharide **12** (2.0 g, 3.1 mmol) was obtained as a by-product, $[\alpha]_D^{24}$ +40.0° (*c* 0.85, dichloromethane).

Anal. Calc. for $C_{32}H_{36}O_{14}$: C, 59.62; H, 5.63. Found: C, 59.49; H, 5.50.

1-O-Acetyl-2,4-di-O-benzoyl-3-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranose (**13**). — A solution of **12** (170 mg, 263 μmol) in absolute pyridine (5 mL) and acetic anhydride (3 mL) was kept overnight at 5°. Evaporation, co-distillation with toluene, and purification on a column of silica gel using 3:2 (v/v) Skellysolve B–ethyl acetate as the eluant gave **13** as a syrup (150 mg, 83%); $[\alpha]_D^{24}$ +10.9° (*c* 0.57, dichloromethane).

Anal. Calc. for $C_{34}H_{38}O_{15}$: C, 59.74; H, 5.58. Found: C, 59.65; H, 5.70.

8-(Methoxycarbonyl)octyl O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-(1→3)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-α-L-rhamnopyranoside (**19**). — A solution of the fully protected tetrasaccharide **18** (418 mg, 0.29 mmol) in glacial acetic acid (20 mL) and absolute methanol (20 mL) was hydrogenolyzed overnight with hydrogen at 505 kPa in the presence of 10% palladium-

on-charcoal. Filtration, and repeated co-evaporation with toluene, gave **19** (327 mg, 96%) as a homogeneous syrup; $[\alpha]_D^{24}$ −20° (*c* 0.66, methanol); ^{13}C-n.m.r. ($CDCl_3$): δ 102.0 (C-1′), 99.1 (3 C, C-1,1″,1‴), 85.1 (C-3′), 79.1 (C-2), 75.9 (C-5′), 75.5 (C-3″), 73.6 (C-4″), 72.5 (2 C, C-4,2″), 71.5 (C-4′), 71.0 (C-4‴), 70.4 (C-3), 69.6 (C-2‴), 68.5 (C-3‴), 68.3 (C-5), 67.9 (C-5″), 67.6 [$OCH_2(CH_2)_7$-], 67.3 (C-5‴), 62.5 (C-6′), 56.1 (C-2′), 51.5 (OMe), 34.1 (CH_2CO_2Me), 29.4–25.0 [$OCH_2(CH_2)_6CH_2$-], 23.5 ($NHCOCH_3$), 20.6 (3 C, $OCOCH_3$), 17.6 (2 C, C-6,6″), and 17.1 (C-6‴).

Anal. Calc. for $C_{56}H_{77}NO_{25}$: C, 57.77; H, 6.67; N, 1.20. Found: C, 57.49; H, 6.64; N, 1.14.

8-(Methoxycarbonyl)octyl O-α-L-*rhamnopyranosyl-(1→3)*-O-α-L-*rhamnopyranosyl-(1→3)*-O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1→2)*-α-L-*rhamnopyranoside* (**20**). — A solution of the partially deprotected tetrasaccharide **19** in absolute methanol and methanolic sodium methoxide solution [0.4 mL of a 0.25% (w/v) solution of sodium in methanol] was kept for 48 h; more sodium methoxide solution (0.2 mL) was then added. After 16 h, sodium ions were removed with Rexyn 101 (H^+) ion-exchange resin. Filtration, evaporation, and chromatography of the resulting syrup on silica gel with 9:3:1 (v/v) ethyl acetate–methanol–water as the eluant, gave pure tetrasaccharide **20** (443 mg, 85%); $[\alpha]_D^{24}$ −55.3° (*c* 0.87, methanol); ^{1}H-n.m.r. (D_2O, 400 MHz): δ 1.13 (d, H-6″), 1.15 (d, H-6), 1.19 (d, H-6‴), 1.96 (s, $NHCOCH_3$), 3.59 (s, 3 OMe), 3.21 (dd, ~t, H-4), 3.34 (ddd, H-5′), 3.37 (dd, ~t, H-4‴), 3.42 (dd, ~t, H-4′), 3.44 (dd, ~t, H-4″), 3.52 (dd, ~t, H-3′), 3.57 (dq, H-5), 3.65 (dd, H-6b′), 3.67 (dq, H-5‴), 3.71 (dd, H-3), 3.71 (dd, H-2′), 3.73 (dd, H-3″), 3.75 (dd, H-3‴), 3.77 (dd, H-2″), 3.82 (dd, H-6a′), 3.89 (dd, H-2), 3.93 (dq, H-5″), 3.96 (dd, H-2‴), 4.63 (d, H-1′), 4.72 (d, $J_{1'',2''}$ 1.6 Hz, H-1″), 4.84 (d, $J_{1,2}$ 1.6 Hz, H-1), and 4.91 (d, $J_{1''',2'''}$ 1.5 Hz, H-1‴); ^{13}C-n.m.r. (CD_3OD): δ 103.7 ($_{C1'',H1''}$ 170.0 Hz, C-1″), 103.7 ($J_{C1',H1'}$ 156.7 Hz, C-1′), 102.7 ($J_{C1''',H1'''}$ 169.4 Hz, C-1‴), 100.3 ($J_{C1,H1}$ 170.3 Hz, C-1), 83.1 (C-3′), 80.6 (C-2), 79.2 (C-3″), 77.7 (C-5′), 74.0 (2 C, C-4,4′), 72.9 (C-4‴), 72.0 (4 C, C-3,4″,3‴), 70.4 (2 C, C-2″,5″), 69.9 (C-5‴), 69.7 [$OCH_2(CH_2)_6$], 68.4 (C-5), 62.4 (C-6′), 56.8 (C-2′), 51.9 (OCH_3), 34.6 ($CH_2CO_2CH_3$), 30.4–25.8 [$OCH_2(CH_2)_6CH_2$], 23.2 ($NHCOCH_3$), and 17.9 (3 C, C-6,6″,6‴).

Anal. Calc. for $C_{36}H_{62}NO_{20}$: C, 52.12; H, 7.56; N, 1.69. Found: C, 51.96; H, 7.34; N, 1.60.

8-(Hydrazidocarbonyl)octyl O-α-L-*rhamnopyranosyl-(1→3)*-O-α-L-*rhamnopyranosyl-(1→3)*-O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1→2)*-α-L-*rhamnopyranoside* (**21**). — The tetrasaccharide **20** (75 mg, 90.5 mmol) was dissolved in ethanol (0.5 mL), and 85% hydrazine hydrate solution (0.1 mL) was added. After 16 h, the solution was evaporated, and the product dried under high vacuum. Two chromatographic separations on LH20 with 1:1 (v/v) methanol–water as the eluant gave pure hydrazide **21** (52 mg, 66%); $[\alpha]_D^{24}$ −53.1° (*c* 0.94, methanol).

Anal. Calc. for $C_{35}H_{62}N_3O_{19}$: C, 50.72; H, 7.54; N, 5.07. Found: C, 50.89; H, 7.60; N, 4.93.

Methyl 3-O-acetyl-2,4-di-O-benzoyl-α-L-rhamnopyranoside (**5**). — A solution of **2** (300 mg, 0.78 mmol) in absolute pyridine (15 mL) and acetic anhydride (8 mL) was kept overnight at 0°. Evaporation, co-distillation with toluene, and filtration through a micro-column of silica gel gave **5** in quantitative yield (331 mg). Crystallization from ethyl acetate–Skellysolve B furnished **5**, m.p. 91°, $[\alpha]_D^{24}$ +88.5° (*c* 2.2, dichloromethane); ^{1}H-n.m.r. (C_6D_6): δ 1.37 (d, 1 H, $J_{5,6}$ 6.3 Hz, H-6), 1.49 (s, 3 H, $OCOCH_3$), 3.06 (2, 3 H, OCH_3), 4.11 (m, 1 H, H-5), 4.70 (d, 1 H, $J_{1,2}$ ~1 Hz, H-1), 5.84–6.08 (m, 3 H, H-2,3,4), 6.91–7.15 (m, 6 H, aromatic), and 7.98–8.29 (m, 4 H, aromatic).

Anal. Calc. for $C_{23}H_{24}O_8$: C, 64.48; H, 5.65. Found: C, 64.65; H, 5.57.

Methyl 2,4-di-O-benzoyl-3-O-benzyl-α-L-rhamnopyranoside (**6**). — Compound **6** was prepared analogously to the corresponding 4-*O*-acetyl-2-*O*-benzoyl-L-rhamnopyranoside[20]. Precise details have been reported[21]. The ^{13}C-data for **6** are given in Table I.

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