Synthesis of *O*-α-D-Rha*p*-(1 → 3)-*O*-α-D-Rha*p*-(1 → 2)-*O*-α-D-Rha*p*-(1 → 12)-oxydodecanoyl-bovine serum albumin

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The title compound (**19**), a conjugate of a trisaccharide based upon the repeating trisaccharide of α-D-rhamnose, which comprises the polysaccharide portion of "A-band" lipopolysaccharide from a mutant (AK1401) of *Pseudomonas aeruginosa*, strain PAO1, with bovine serum albumin (BSA) was synthesized starting with methyl α-D-mannopyranoside. Suitably protected D-rhamnose derivatives, namely, ethyl 3,4-di-*O*-benzyl-1-thio-α-D-rhamnopyranoside (**11**) and 3-*O*-acetyl-2,4-di-*O*-benzyl-α-D-rhamnopyranosyl chloride (**12**), were used as the glycosyl acceptor and donor, respectively, in the synthesis of disaccharide **13**. *O*-Deacetylation of **13** gave **14**, a glycosyl acceptor that reacted with **12** to yield the trisaccharide **15**. *N*-Iodosuccinimide – trifluoromethanesulfonic acid was used as an activator of the thioglycoside in the synthesis of **16** and **17** from **15**. Compound **16** was converted into the hydrazide **18** by treatment with hydrazine. The conjugation was achieved by coupling of the intermediate acyl azide derivative of **18** with BSA.

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Utilisant l'α-D-mannopyranoside de méthyle comme produit de départ, on a synthétisé le composé **19** mentionné dans le titre, un conjugué d'un trisaccharide basé sur l'homoséquence du trisaccharide de l'α-D-rhamnose qui comprend la portion polysaccharidique de la «bande-A» du lipopolysaccharide d'un mutant (AK1401) du *Pseudomonas aeruginosa*, souche PAO1, d'une albumine de sérum bovin (ASB). On a utilisé des dérivés D-rhamnose protégés d'une façon appropriée, soit les 3,4-di-*O*-benzyl-1-thio-α-D-rhamnopyranoside d'éthyle (**11**) et le chlorure de 3-*O*-acétyl-2,4-di-*O*-benzyl-α-D-rhamnopyranosyle (**12**) comme respectivement accepteur et donneur de glycosyle dans la synthèse du disaccharide **13**. La *O*-déacétylation de **13** conduit à **14**, un accepteur de glycosyle, qui par réaction avec **12**, fournit le trisaccharide **15**. On a utilisé la combinaison *N*-iodosuccinimide – acide trifluorométhanesulfonique comme activant du thioglycoside dans la synthèse des composés **16** et **17** à partir du composé **15**. Par traitement avec de l'hydrazine, on a transformé le composé **16** en son hydrazide **18**. On a réalisé la conjugaison en procédant au couplage du dérivé intermédiaire **18** avec le BSA.

[Traduit par la rédaction]

Introduction

During the past decade an increasing number of studies have demonstrated that carbohydrate–protein conjugates possess important seroreactivity (1–6); some of the conjugates were developed with the intention of preparing synthetic antigens and vaccines (1–6). *Pseudomonas aeruginosa* is an opportunistic pathogen that infects persons having compromised immune systems, such as cystic fibrosis patients (7). Vaccines currently used against pathogenic Gram-negative bacteria commonly consist of a mixture of O-antigens from several serotypes of the same species. "A-band" lipopolysaccharide (LPS) is often expressed in established colonies of *Pseudomonas aeruginosa* that do not express O-antigen; since the "A-band" LPS is conserved upon infection, and since it is a common antigen (8), vaccines based on this type of LPS might be more successful in preventing pulmonary infections of *P. aeruginosa* than the current vaccines based on O-antigens.

Recently we (9) reported the results of structural studies on the polysaccharide portion of "A-band" LPS from a mutant (AK1401) of *Pseudomonas aeruginosa* strain PAO1. The main structural feature is a repeating trisaccharide of α-D-rhamnose having the following structure: [→ 2)-*O*-α-D-Rha*p*-(1 → 3)-*O*-α-D-Rha*p*-(1 → 3)-*O*-α-D-Rha*p*-(1 →]$_n$; this structural assignment has been confirmed by further studies involving methylation analysis and Smith-degradation studies (10). As part of our program concerned with the synthesis of glycoconjugates for the investigation of their serological activity, with the intention of preparing synthetic vaccines based upon subunits of the structure that could be used for protection against *Pseudomonas* infections, it was planned to synthesize a trisaccharide based upon this trisaccharide repeating unit by a strategy that would permit the trisaccharide units to be joined readily to the extent of a nonasaccharide. It was also planned to make neoglycoproteins of the tri-, hexa-, and nona-saccharides.

Methods for the synthesis of glycoconjugates include diazo coupling, formation of isothiocyanates, amidation, amination, guanidination, and amidination (see ref. 6). Conjugates containing several types of spacer arms, such as a nine-carbon chain (11–14), and chains containing dioxa (15–17), amide (18), thioether (19–23), phenylthiourea (24), or *p*-*N*-acryloylphenyl (25) functional groups, have been prepared. Four-, seven-, twelve-, and fifteen-carbon spacer arms have also been employed (26). Here we describe the synthesis of a conjugate of the trisaccharide and bovine serum albumin (BSA) by using a 12-carbon spacer arm.

Results and discussion

Methyl 2,3-*O*-isopropylidene-α-D-rhamnopyranoside (**3**) was prepared from methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside (**1**) in two steps (27) by treatment of **1** with

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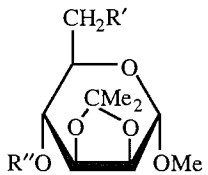
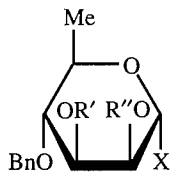
iodine–triphenylphosphine to give **2**, followed by catalytic hydrogenation of **2**. Benzylation of **3** with benzyl chloride – potassium hydroxide in dimethyl sulfoxide at room temperature gave **4** in good yield; the product, without further purification, was converted into **5** (28, 29) in 79% yield (from **3**) by treatment with 60% aqueous acetic acid at boiling temperature.

Benzylation of **5** to form methyl 2,4-di-*O*-benzyl-α-D-rhamnopyranoside (**6**) in 82% yield was performed in toluene at room temperature, in the presence of the phase-transfer catalyst, tetra-*n*-butylammonium iodide, and powdered potassium hydroxide. A selective benzylation of **5** to give **6** has been achieved also by a two-phase reaction in chloroform – aqueous sodium hydroxide using tetra-*n*-butylammonium bromide (28). In the present study, in addition to compound **6**, small amounts of methyl 3,4-di-*O*-benzyl-α-D-rhamnopyranoside (**7**) (ref. 30, for *L*-enantiomer, see refs. 31, 32) and another component having a higher R_f value, presumably methyl 2,3,4-tri-*O*-benzyl-α-D-rhamnopyranoside, were isolated also. Compound **7** could be prepared from **5** in large scale in 69% yield by reaction with (di-*n*-butyl) tin oxide, by way of its stannylene derivative (see refs. 33, 34), in toluene–benzene. Acetolysis of **6** or **7** with a 1.5% solution of sulfuric acid in acetic anhydride – acetic acid (5:2, v/v) at room temperature did not afford simply the corresponding acetate **8** (for L-enantiomer, see ref. 14) or **9** (32, 35), but resulted also in debenzylation. 1,2-Di-*O*-acetyl-3,4-di-*O*-benzyl-α-D-rhamnopyranose (**9**) could be obtained in 84% yield by hydrolysis of **7** in a 3% solution of sulfuric acid in water – acetic acid – acetone (1:1:1, v/v) at reflux temperature for 20 h, followed by acetylation with acetic anhydride – pyridine at room temperature for 4 h.



1 R′ = OH, R″ = H
2 R′ = I, R″ = H
3 R′ = H, R″ = H
4 R′ = H, R″ = Bn



5 R′ = R″ = H, X = OMe
6 R′ = H, R″ = Bn, X = OMe
7 R′ = Bn, R″ = H, X = OMe
8 R′ = Ac, R″ = Bn, X = OAc
9 R′ = Bn, R″ = Ac, X = OAc
10 R′ = Bn, R″ = Ac, X = Cl
11 R′ = Bn, R″ = H, X = SEt
12 R′ = Ac, R″ = Bn, X = Cl

Ethyl thioglycosides have usually been synthesized by the reaction of a glycosyl acetate with thioethanol in the presence of a Lewis acid; however, in many cases the protecting groups are labile under these conditions. Although boron trifluoride etherate has been used (35) in the preparation of thioglycoside **11**, debenzylation has been observed (36) under such conditions. Consequently, in the present work glycosyl chloride **10**, which was prepared quantitatively by treatment of **9** with hydrogen chloride in acetic acid – dichloromethane at room temperature for 2 h, was treated with potassium thioethoxide in acetone at room temperature for 10–15 min to give **11** in 91% overall yield; no β isomer was isolated. Gated ^{13}C NMR measurements revealed $J_{H\text{-}1,C\text{-}1}$ = 166.48 Hz, a value in the range reported (37) for the α anomer of some *O*-glycosides of mannose derivatives. The other suitably protected, monosaccharide unit **12** was obtained from **6** in good yield by the same methods as those used in the preparation of **10** from **7**.

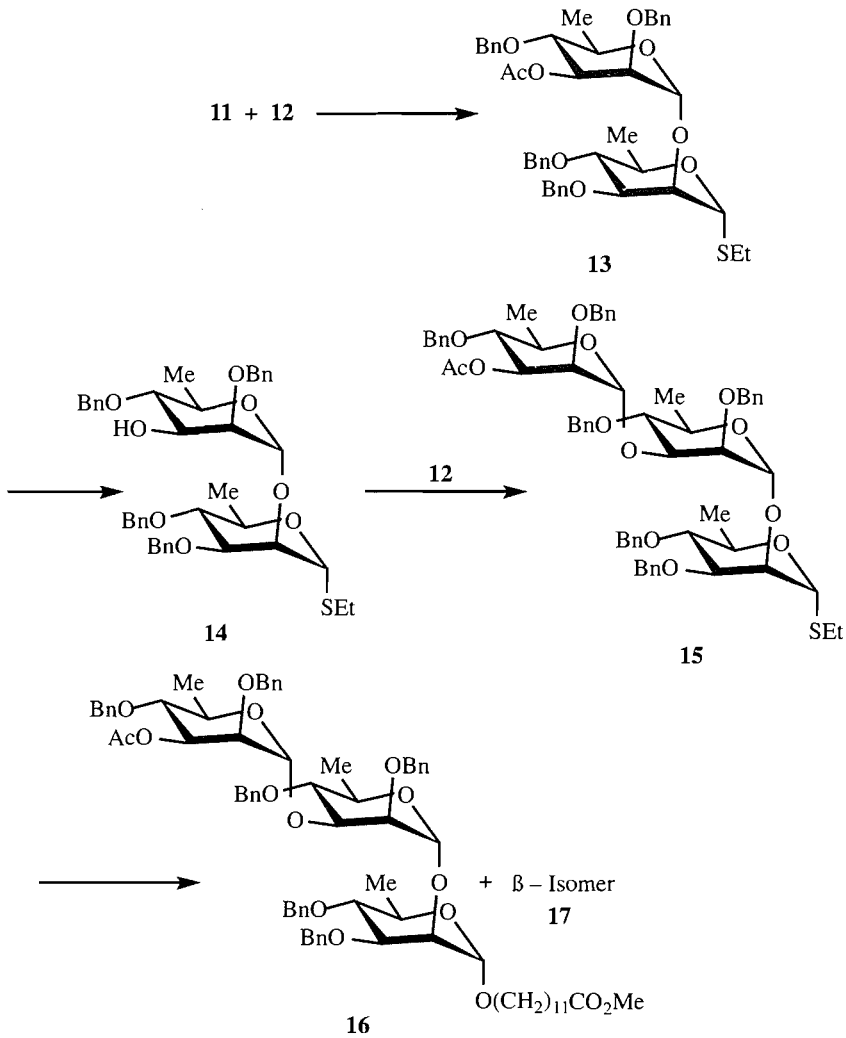
The reaction of the glycosyl acceptor **11** with the glycosyl donor **12** was performed in the presence of silver triflate and powdered 4 Å molecular sieves at −5 to −10°C to give the protected disaccharide **13** in 76% yield. The assignment of the configuration of the inter-glycosidic linkage was based on precedents in the literature (see, for example, refs. 37*a*, 37*b*, 38) for the synthesis of mannose-containing oligosaccharides under the same conditions. ^1H and ^{13}C NMR spectroscopy indicated the stereochemical integrity of **13**. *O*-Deacetylation of **13** with 0.5% sodium methoxide in methanol at room temperature for 4 h afforded quantitatively the glycosyl acceptor **14**, which was, in turn, glycosylated with the glycosyl donor **12**, again by use of silver triflate and powdered 4 Å molecular sieves, to give protected trisaccharide **15** as a syrup in 80% yield. ^1H NMR spectroscopy showed the presence of signals for three anomeric protons at δ 5.09 (H-1″), 5.19 (H-1′), and 5.21 (H-1); in the spectrum of **13** the signals for the two anomeric protons were observed at δ 5.14 (H-1′) and 5.25 (H-1).

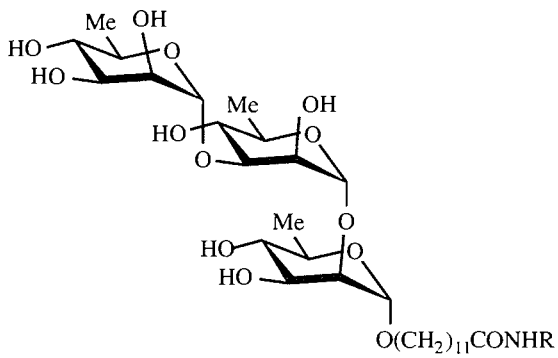
The thioglycoside **15** was treated with methyl 12-hydroxydodecanoate using *N*-iodosuccinimide – trifluoromethanesulfonic acid as an activator (39, 40), to give the two diastereomers **16** and **17** (~1:1) in 68% yield. The ratio of **16** to **17** and the yield were not changed significantly by changing the reaction temperature from 0°C to room temperature. The trisaccharides **16** and **17** were separated by chromatography on silica gel. The ^1H NMR resonances of **16** and **17** were assigned on the basis of 2D ^1H-homonuclear chemical-shift correlated (^1H-COSY) experiments.

Attempted *O*-debenzylation of **16** by catalytic hydrogenolysis over 10% Pd–C in methanol was unsuccessful possibly because of the presence of a trace of sulfide derived from the thioglycoside precursor; however, the *O*-debenzylation was achieved in a binary solvent of 40% acetic acid in methanol at room temperature. The product was then converted into the hydrazide **18** in 62% yield by treatment overnight with hydrazine hydrate in ethanol; during the conversion process the 3″-Ac group was also removed. The ^1H and ^{13}C NMR spectral data, including data from ^1H-COSY experiments, and the MS(FAB) spectrum were consistent with the structure assigned for **18**. In the ^1H NMR spectrum of **18** in CD_3OD at 25°C the signal of the anomeric proton (H-1) at δ 4.71 was obscured by a protiated-solvent signal; signals for only two anomeric protons were observed (δ 4.85 and 4.99). When the temperature was raised to 30°C the signals of three anomeric protons were clearly observed at δ 4.77 (H-1), 4.91 (H-1′), and 5.04 (H-1″) (see Fig. 1).

The hydrazide **18** was converted into its acyl azide, which was conjugated to the carrier protein, bovine serum albumin, by the method described by Chatterjee et al. (13), to give the glycoconjugate **19**. The conjugate **19** could be purified by column chromatography on Bio-Gel P-30. The fractions were monitored for carbohydrate by the phenol – sulfuric acid colorimetric method (41); protein was assayed using bicinchoninic acid (Pierce BCA Protein Assay Reagent). The results in Fig. 2 indicated a successful conjugation. The glycoconjugate material was dialyzed, lyophilized, and stored at 4°C prior to serological analyses.

Preliminary tests have shown that both **18** and **19** possess binding reactivity to the "A-band"-specific monoclonal antibody, MAb N1F10 (see refs. 8, 42). The syntheses of





18 R = NH_2
19 R = BSA

hexa- and nona-saccharides and their conjugates should be achievable from **15** and **16** by the reactions described in this article.

Experimental

Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer model 241 automatic polarimeter for solutions in a 0.1-dm cell at room temperature. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded on a Bruker AM-400 or Bruker AC-F200 spectrometer; chemical shifts (δ) are given in ppm downfield from the signal of Me_4Si. CI mass spectra were recorded on a VG Analytical ZAB-E mass spectrometer; the FAB mass spectrum was recorded on a VG Quattro mass spectrometer using *m*-nitrobenzyl alcohol as the matrix.

Thin-layer chromatography (TLC) was performed using glass precoated with E. Merck Silica Gel 60 F-254. The developed plates were dried and sprayed with a solution of cerium (IV) sulfate (1%) and molybdic acid (1.5%) in 10% aqueous sulfuric acid, and heated at 150°C. Column chromatography was performed using E. Merck 7734 Silica Gel 60 (70–230 mesh) as the solid phase. Solvents were evaporated under reduced pressure at <40°C.

Methyl 4-O-benzyl-α-D-rhamnopyranoside (5)

To a solution of **3** (27) (4.4 g, 20.1 mmol) in dimethyl sulfoxide (25 mL) were added powdered potassium hydroxide (4.0 g) and benzyl chloride (3.0 mL, 25.8 mmol) with stirring at room temperature, and the mixture was stirred for a further 4 h. Ice-water (50 mL) was then added and the solution was extracted with chloroform (3 × 50 mL). The combined organic solution was then washed with water (2 × 50 mL), dried over anhydrous sodium sulfate, and concentrated to give methyl 4-*O*-benzyl-2,3-*O*-isopropylidene-α-D-rhamnopyranoside (**4**) as an oil.

To the oil obtained above was added 60% aqueous acetic acid (50 mL) and the mixture was then stirred at 100°C for 1 h. Evaporation under reduced pressure gave a residue that crystallized in a short time after the addition of hexane (100 mL) to give a sample of **5** (4.31 g, 79.5% from **3**). The sample was recrystallized from ethyl acetate – hexane; mp 108–109°C, $[\alpha]_D$ +72.4 (*c* 1.56, $CHCl_3$) (lit. (28) mp 105°C, $[\alpha]_D$ +66.3 (*c* 5.3, $CHCl_3$); lit. (29) mp 105–107°C, $[\alpha]_D$ +72 (*c* 4, $CHCl_3$)); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.35 (3H, d, $J_{5,6}$ = 6.2 Hz, 6-CH_3), 2.32 (2H, br, 2-OH and 3-OH),

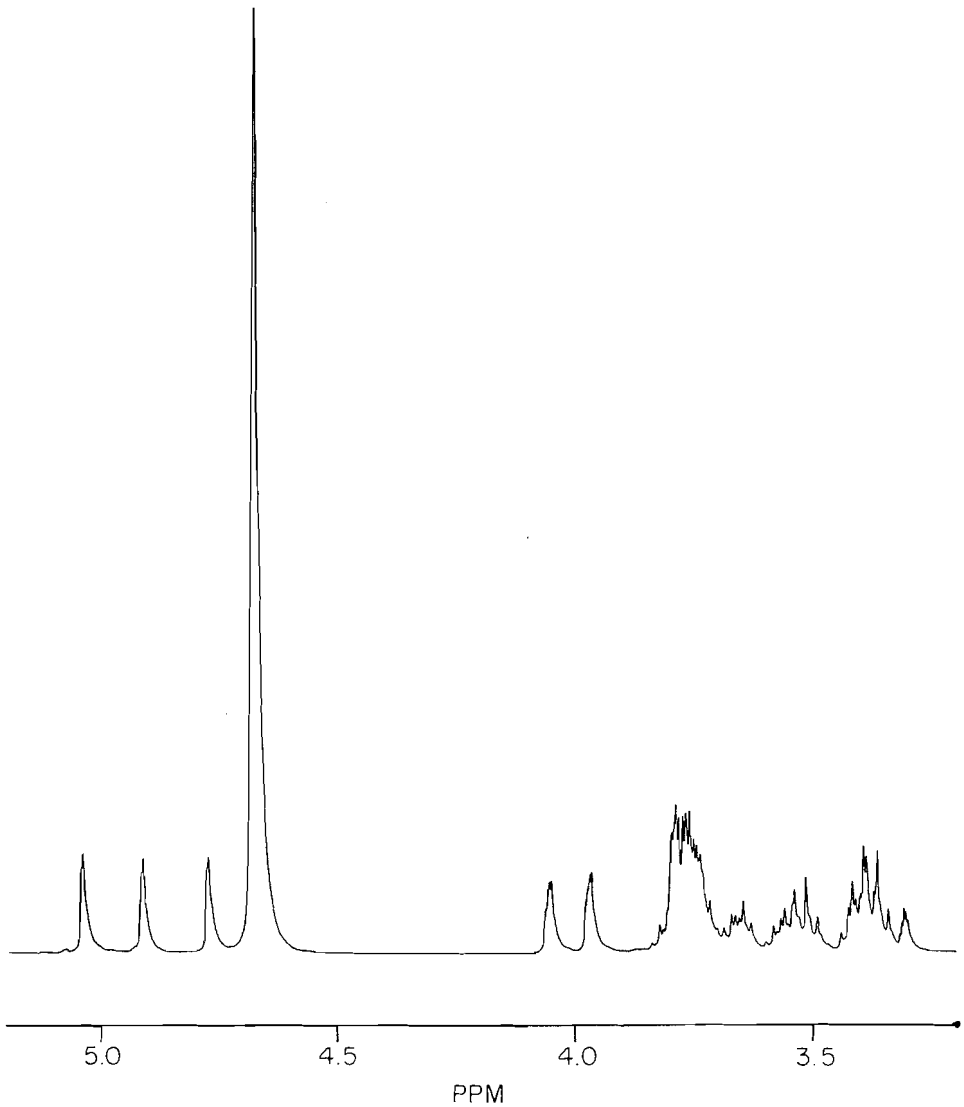


FIG. 1. Partial ^{1}H NMR spectrum of **18** at 400 MHz in CD_3OD at 30°C.

3.32 (1H, t, $J_{3,4} = J_{4,5} = 9.3$ Hz, H-4), 3.34 (3H, s, OMe), 3.71 (1H, dq, $J_{5,6} = 6.2$ Hz, $J_{4,5} = 9.3$ Hz, H-5), 3.84–3.91 (2H, m, H-2 and H-3), 4.65 (1H, br, H-1), 4.73 (2H, s, CH_2Ph), and 7.38 (5H, m, Ph). Anal. calcd. for $C_{14}H_{20}O_5$: C 62.67, H 7.52; found: C 62.46, H 7.34.

*Methyl 2,4-di-*O*-benzyl-α-*D*-rhamnopyranoside (6)*

To a solution of **5** (2.31 g, 8.62 mmol) in toluene (50 mL) were added tetra-*n*-butylammonium iodide (1.6 g, 4.3 mmol) and powdered potassium hydroxide (2.3 g), followed by benzyl bromide (1.47 g, 8.62 mmol). The mixture was stirred at room temperature for 4 h. Ice-cold water (50 mL) and ethyl acetate (50 mL) were added and the mixture was stirred for 10 h. The organic layer was then washed with water (2 × 50 mL), dried over anhydrous sodium sulfate, and evaporated to give a residue that was fractionated by column chromatography on silica gel (hexane – ethyl acetate (3:1, v/v)) to give compound **6** as an oil (2.52 g, 82%) and a small amount of **7**; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.34 (3H, d, $J_{5,6} = 6.2$ Hz, 6-CH_3), 2.24 (1H, br, 3-OH), 3.33 (3H, s, OMe), 3.35 (1H, t, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4), 3.64 (1H, dq, $J_{4,5} = 9.4$ Hz, $J_{5,6} = 6.2$ Hz, H-5), 3.73 (1H, dd, $J_{1,2} = 1.4$ Hz, $J_{2,3} = 3.7$ Hz, H-2), 3.94 (1H, dd, $J_{2,3} = 3.7$ Hz, $J_{3,4} = 9.4$ Hz, H-3), 4.59 and 4.76 (2H, d and d, $J_{gem} = 11.9$ Hz, CH_2Ph), 4.72 (1H, br, H-1), 4.66 and 4.91 (2H, d and d, $J_{gem} = 11.1$ Hz, CH_2Ph), and 7.26–7.50 (10H, m, 2 Ph).

*Methyl 3,4-di-*O*-benzyl-α-*D*-rhamnopyranoside (7)*

To a solution of **5** (4.0 g, 14.93 mmol) in benzene–toluene (1:1, v/v, 200 mL) was added (di-*n*-butyl)tin oxide (4.1 g), and the mixture was heated at reflux temperature with azeotropic removal of water for 5 h. Benzene was removed by distillation, and tetra-*n*-butylammonium iodide (5.6 g, 15.16 mmol) and benzyl bromide (2.65 mL, 20.6 mmol) were added; the mixture was heated at reflux temperature for 3 h and then cooled to room temperature. Water (100 mL) and ethyl acetate (100 mL) were added and the organic layer was washed sequentially with an aqueous solution of sodium hydrogen carbonate and water. The organic solution was dried over anhydrous sodium sulfate and concentrated to a residue that was fractionated by column chromatography on silica gel (hexane – ethyl acetate (3:1, v/v)) to give compound **7** as an oil (3.67 g, 69%); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.37 (3H, d, $J_{5,6} = 6.2$ Hz, 6-CH_3), 2.79 (1H, s, 2-OH), 3.37 (3H, s, OMe), 3.51 (1H, t, $J_{3,4} = J_{4,5} = 9.2$ Hz, H-4), 3.72 (1H, dq, $J_{5,6} = 6.2$ Hz, $J_{4,5} = 9.2$ Hz, H-5), 3.87 (1H, dd, $J_{3,4} = 9.2$ Hz, $J_{2,3} = 3.5$ Hz, H-3), 4.05 (1H, dd, $J_{1,2} = 1.5$ Hz, $J_{2,3} = 3.5$ Hz, H-2), 4.69 and 4.86 (2H, d and d, $J_{gem} = 11$ Hz, CH_2Ph), 4.72 (2H, s, CH_2Ph), 4.75 (1H, d, $J_{1,2} = 1.5$ Hz, H-1), and 7.25–7.45 (10H, m, 2 Ph).

*1,3-Di-*O*-acetyl-2,4-di-*O*-benzyl-α-*D*-rhamnopyranose (8)*

A solution of **6** (6.2 g, 17.32 mmol) in a 3% solution of sulfuric acid (80 mL) in acetic acid – water–acetone (1:1:1, v/v) was

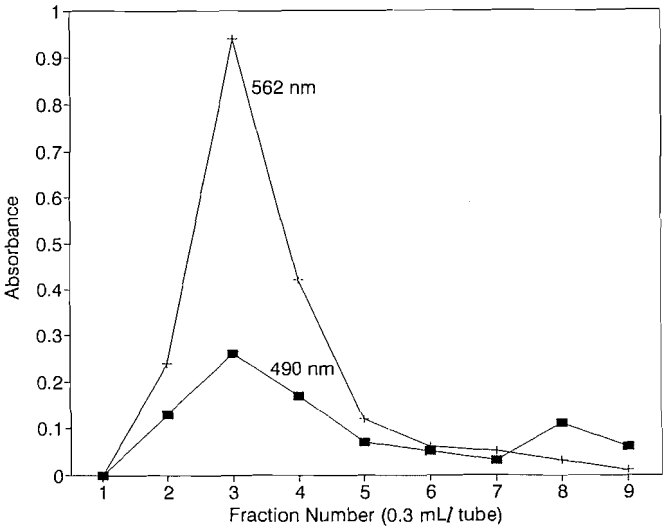


FIG. 2. Gel filtration chromatography of conjugate **19** on Bio-Gel P-30 (10 × 0.4 cm, water as eluant). Carbohydrate was assayed by the phenol – sulfuric acid colorimetric method at 490 nm (■); protein was assayed using bicinchoninic acid (Pierce BCA Protein Assay Reagent) at 562 nm (+).

heated at reflux temperature for 20 h. The solution was cooled to room temperature and extracted with chloroform (3 × 50 mL). The organic solution was washed sequentially with water (20 mL), an aqueous solution of sodium hydrogen carbonate (20 mL), and water (20 mL), dried over anhydrous sodium sulfate, and evaporated; the residue was treated with acetic anhydride – pyridine (1:1, v/v, 30 mL) at room temperature for 4 h. The mixture was poured into ice-water and extracted with ethyl acetate (4 × 25 mL). The solution was concentrated to a residue that was purified by column chromatography on silica gel (hexane – ethyl acetate (3:1, v/v)) to give compound **8** as a syrup (4.4 g, 60%), and another component (0.8 g) that appeared to be a mixture of α and β isomers; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.37 (3H, d, $J_{5,6}$ = 6.2 Hz, 6-CH_3), 1.98 and 2.09 (3H each, s and s, 2 Ac), 3.72 (1H, t, $J_{3,4} = J_{4,5}$ = 9.2 Hz, H-4), 3.95–3.98 (2H, m, H-2 and H-5), 4.69–4.80 (4H, m, 2 CH_2Ph), 5.13 (1H, dd, $J_{2,3}$ = 2.9 Hz, $J_{3,4}$ = 9.2 Hz, H-3), 6.09 (1H, d, $J_{1,2}$ = 1.4 Hz, H-1), and 7.20–7.40 (10H, m, 2 Ph). Anal. calcd. for $C_{24}H_{28}O_7$: C 67.27, H 6.59; found: C 67.66, H, 6.70.

*1,2-Di-*O*-acetyl-3,4-di-*O*-benzyl-α-*D*-rhamnopyranose (**9**)*

Compound **7** (2.8 g, 7.82 mmol) was treated as described for the preparation of **8** from **6** to give **9** in crystalline form from hexane and a mixture of α and β isomers (total 2.85 g, 84%); mp 107°C, $[\alpha]_D$ +22.8 (*c* 1.36, $CHCl_3$) (lit. (35) mp 106–108°C, $[\alpha]_D$ +22.3 (*c* 0.73, $CHCl_3$); for *L*-enantiomer, lit. (32) mp 107–109°C, $[\alpha]_D$ −20 (*c* 1.1, CH_2Cl_2)); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.33 (3H, d, $J_{5,6}$ = 6.2 Hz, 6-CH_3), 2.07 and 2.17 (3H each, s and s, 2 Ac), 3.47 (1H, t, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 3.81 (1H, dq, $J_{5,4}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.92 (1H, dd, $J_{3,4}$ = 9.5 Hz, $J_{2,3}$ = 3.4 Hz, H-3), 4.54 and 4.72 (2H, d and d, J_{gem} = 11.1 Hz, CH_2Ph), 4.62 and 4.92 (2H, d and d, J_{gem} = 10.7 Hz, CH_2Ph), 5.35 (1H, dd, $J_{1,2}$ = 2.0 Hz, $J_{2,3}$ = 3.4 Hz, H-2), 6.00 (1H, d, $J_{1,2}$ = 2.0 Hz, H-1), and 7.28–7.35 (10H, m, 2 Ph); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ: 18.02 (C-6), 20.91 and 20.98 (2 CH_3C=O), 67.83 (C-5), 70.05, 71.94 (CH_2Ph), 75.63 (CH_2Ph), 77.63, 79.53, 91.17 (C-1), 127.88, 128.04, 128.12, 128.46, 137.76, and 138.24 (2 Ph), 168.53 and 170.04 (2 C=O). Anal. calcd. for $C_{24}H_{28}O_7$: C 67.27, H 6.59; found: C 67.41, H 6.56.

*2-O-Acetyl-3,4-di-O-benzyl-α-D-rhamnopyranosyl chloride (**10**)*

A solution of **9** (1.92 g, 4.5 mmol) in dichloromethane – acetic acid (1:1, v/v, 50 mL) was saturated with HCl(g) at 0°C for 4 h; TLC (hexane – ethyl acetate (3:1, v/v)) showed that the starting material had been completely converted into a product having a higher R_f value. Dichloromethane (50 mL) was added and the solution was washed sequentially with ice-water (twice), an aqueous solution of sodium hydrogen carbonate, and ice-water. The organic solution was dried over anhydrous sodium sulfate and concentrated to give compound **10** in quantitative yield.

*Ethyl 3,4-di-*O*-benzyl-1-thio-α-*D*-rhamnopyranoside (**11**)*

To a solution of potassium thioethoxide (0.5 g, 5 mmol) in acetone (10 mL) was added a solution of **10** (1.8 g, 4.4 mmol) in acetone (15 mL). The mixture was stirred at room temperature for 10 min; TLC (hexane – ethyl acetate (3:1, v/v)) showed that the reaction was complete. The mixture was diluted with chloroform (100 mL) and washed with water several times. The organic solution was then concentrated to a residue that was purified by chromatography on silica gel (hexane – ethyl acetate (3:1, v/v)) to give compound **11** as a syrup (1.6 g, 92%); $[\alpha]_D$ +150.6 (*c* 1.6, $CHCl_3$) (lit. (35) $[\alpha]_D$ +127 (*c* 0.56, $CHCl_3$)); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.29 (3H, t, *J* = 7.4 Hz, SCH_2CH_3), 1.36 (3H, d, $J_{5,6}$ = 6.3 Hz, 6-CH_3), 2.58 (2H, m, SCH_2CH_3), 3.00 (1H, br, 2-OH), 3.55 (1H, t, $J_{3,4} = J_{4,5}$ = 9.2 Hz, H-4), 3.83 (1H, dd, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.2 Hz, H-3), 4.06–4.20 (2H, m, H-2 and H-5), 4.68 (2H, s, CH_2Ph), 4.61 and 4.92 (2H, d and d, J_{gem} = 11.0 Hz, CH_2Ph), 5.33 (1H, d, $J_{1,2}$ = 1.1 Hz, H-1), and 7.25–7.36 (10H, m, 2 Ph); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ: 14.92 (SCH_2CH_3), 17.83 (C-6), 24.97 (SCH_2CH_3), 67.90 (C-5), 70.15, 72.10 (CH_2Ph), 75.34 (CH_2Ph), 80.30 (2 C's), 83.20 (C-1, $J_{C1,H1}$ = 166.48 Hz), 127.71, 127.93, 128.03, 128.38, 128.58, 138.07, and 138.46 (2 Ph). Anal. calcd. for $C_{22}H_{28}O_4S$: C 68.01, H 7.26, S 8.25; found: C 67.65, H 7.23, S 8.59.

*3-O-Acetyl-2,4-di-O-benzyl-α-D-rhamnopyranosyl chloride (**12**)*

Compound **12** was prepared from **8** by the procedure described for the preparation of **10**.

*Ethyl 2-O-(3'-O-acetyl-2',4'-di-O-benzyl-α-D-rhamnopyranosyl)-3,4-di-O-benzyl-1-thio-α-D-rhamnopyranoside (**13**)*

To a solution of **11** (1.0 g, 2.58 mmol) in dichloromethane (15 mL) were added powdered 4 Å molecular sieves (2 g) and silver triflate (1.3 g, 5.06 mmol). The mixture was stirred at −10°C under N_2. A solution of compound **12** (0.9 g, 2.22 mmol) in dichloromethane (12 mL) was slowly added and the mixture was stirred at −5 to −10°C for 30 min and then at room temperature for 5 h. The reaction mixture was diluted with dichloromethane (20 mL) and filtered through Celite. The filtrate was washed with an aqueous solution of sodium hydrogen carbonate and water, dried over anhydrous sodium sulfate, and concentrated to a residue. The residue was purified by column chromatography on silica gel (hexane – ethyl acetate (4:1, v/v)) to give compound **13** as a syrup (1.27 g, 76%); $[\alpha]_D$ +44 (*c* 1.67, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.34 (3H, t, *J* = 7.4 Hz, SCH_2CH_3), 1.36 (3H, d, $J_{5',6'}$ = 6.3 Hz, 6'-CH_3), 1.41 (3H, d, $J_{5,6}$ = 6.2 Hz, 6-CH_3), 2.03 (3H, s, 3'-Ac), 2.66 (2H, m, SCH_2CH_3), 3.59 (1H, t, $J_{3',4'} = J_{4',5'}$ = 9.4 Hz, H-4'), 3.69 (1H, t, $J_{3,4} = J_{4,5}$ = 9.4 Hz, H-4), 3.87 (1H, dd, $J_{3,4}$ = 9.4 Hz, $J_{2,3}$ = 2.9 Hz, H-3), 3.95–4.10 (3H, m, H-2', H-5, and H-5'), 4.19 (1H, m, H-2), 4.32–4.99 (8H, m, 8H, 4 CH_2Ph), 5.14 (1H, d, $J_{1',2'}$ = 1.8 Hz, H-1'), 5.25 (1H, d, $J_{1,2}$ = 1.3 Hz, H-1), 5.34 (1H, dd, $J_{2',3'}$ = 3.4 Hz, $J_{3',4'}$ = 9.4 Hz, H-3'), and 7.26–7.38 (20H, m, 4 Ph); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ: 15.09 (SCH_2CH_3), 17.80 and 18.12 (C-6 and C-6'), 21.14 (CH_3C=O), 25.42 (SCH_2CH_3), 68.24 and 68.65 (C-5 and C-5'), 72.55 and 72.74 (2 CH_2Ph), 73.45, 74.94 and 75.44 (2 CH_2Ph), 76.03, 76.80, 79.33, 80.55, 80.68, 83.61 (C-1), 99.57 (C-1'), 127.75, 127.86, 128.00, 128.28, 128.44, 128.51, 138.53, and 138.72 (4 Ph), and 169.50 (C=O); MS (CI, NH_3): 774 $(M + NH_4)^+$, 684, 494. Anal. calcd. for $C_{44}H_{52}O_9S$: C 69.81, H 6.93, S 4.24; found: C 69.68, H 6.75, S 3.99.

*Ethyl 3,4-di-O-benzyl-2-O-(2',4'-di-O-benzyl-α-D-rhamnopyranosyl)-1-thio-α-D-rhamnopyranoside (**14**)*

A solution of compound **13** (0.49 g, 0.648 mmol) in 0.5% sodium methoxide – methanol (20 mL) was stirred at room temper-

ature for 4 h; TLC (hexane – ethyl acetate (4:1, v/v)) showed that the reaction was complete. Amberlite IR-120 (H^+ form) ion-exchange resin (5 mL) was added to the solution. The mixture was stirred for 5 min, filtered, and the filtrate was concentrated to give compound **14** in quantitative yield; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.28 (3H, t, J = 7.4 Hz, SCH_2CH_3), 1.31 (3H, d, $J_{5',6'}$ = 6.2 Hz, 6′-CH_3), 1.36 (3H, d, $J_{5,6}$ = 6.2 Hz, 6-CH_3), 2.40 (1H, d, J = 8.0 Hz, 3′-OH), 2.60 (2H, m, SCH_2CH_3), 3.40 (1H, t, $J_{3',4'} = J_{4',5'}$ = 9.3 Hz, H-4′), 3.52 (1H, t, $J_{3,5} = J_{4,5}$ = 9.4 Hz, H-4), 3.85–3.94 (3H, m, H-3′, H-5, and H-5′), 4.04–4.14 (2H, m, H-2′ and H-3), 4.19 (1H, dd, $J_{1,2}$ = 1.1 Hz, $J_{2,3}$ = 2.8 Hz, H-2), 4.35 and 4.50 (2H, d and d, J_{gem} = 11.9 Hz, CH_2Ph), 4.61 and 4.75 (2H, d and d, J_{gem} = 10.9 Hz, CH_2Ph), 4.70 and 4.97 (4H, d and d, J_{gem} = 11.0 Hz, 2 CH_2Ph), 5.19 (1H, d, $J_{1',2'}$ = 1.2 Hz, H-1′), 5.25 (1H, d, $J_{1,2}$ = 1.1 Hz, H-1), and 7.25–7.35 (20H, m, 4 Ph).

Ethyl 2-O-{3′-O-(3″-O-acetyl-2″,4″-di-O-benzyl-α-D-rhamnopyranosyl)-2′,4′-di-O-benzyl-α-D-rhamnopyranosyl}-3,4-di-O-benzyl-1-thio-α-D-rhamnopyranoside (15)

To a solution of **14** (0.47 g, 0.648 mmol) in dichloromethane (8 mL) were added powdered 4 Å molecular sieves (2 g) and silver triflate (0.3 g). The mixture was cooled to −10°C under N_2, and a solution of compound **12** (0.28 g, 0.692 mmol) in dichloromethane (3 mL) was added slowly; the mixture was stirred at −5 to −10°C for 1 h and then at room temperature overnight. The reaction mixture was processed as described for the preparation of **13**. Compound **15** was obtained as a syrup (0.56 g, 80%); $[\alpha]_D$ +52.5 (*c* 2.12, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.22 (3H, d, $J_{5,6}$ = 6.2 Hz, 6-CH_3), 1.26 (3H, t, J = 7.4 Hz, SCH_2CH_3), 1.27 (3H, d, $J_{5',6'}$ = 6.2 Hz, 6′-CH_3), 1.29 (3H, d, $J_{5',6''}$ = 6.2 Hz, 6″-CH_3), 1.95 (3H, s, 3″-Ac), 2.60 (2H, m, SCH_2CH_3), 3.44 (1H, t, $J_{3',4'} = J_{4',5'}$ = 9.4 Hz, H-4′), 3.62 (1H, t, $J_{3'',4''} = J_{4'',5''}$ = 9.4 Hz, H-4″), 3.66 (1H, t, $J_{3,4} = J_{4,5}$ = 9.4 Hz, H-4), 3.78–3.83 (3H, m, H-2′, H-3, and H-5), 3.87–3.89 (2H, m, H-2″ and 5″), 4.02 (1H, dq, $J_{5',6'}$ = 6.2 Hz, $J_{4',5'}$ = 9.4 Hz, H-5′), 4.12 (1H, dd, $J_{1,2}$ = 1.4 Hz, $J_{2,3}$ = 2.5 Hz, H-2), 4.19 (1H, d, J_{gem} = 12.0 Hz, one of CH_2Ph), 4.21 (1H, dd, $J_{2',3'}$ = 3.0 Hz, $J_{3',4'}$ = 9.4 Hz, H-3′), 4.39–4.88 (11H, m, 5.5 CH_2Ph), 5.09 (1H, d, $J_{1'',2''}$ = 1.4 Hz, H-1″), 5.19 (1H, d, $J_{1',2'}$ = 1.3 Hz, H-1′), 5.21 (1H, d, $J_{1,2}$ = 1.2 Hz, H-1), 5.32 (1H, dd, $J_{2'',3''}$ = 3.1 Hz, $J_{3'',4''}$ = 9.4 Hz, H-3″), and 7.2–7.4 (30H, m, 6 Ph); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ: 15.07 (SCH_2CH_3), 18.02 (C-6, C-6′, and C-6″), 21.09 (CH_3C=O), 25.49 (SCH_2CH_3), 68.26, 68.50, and 69.03 (C-5, C-5′, and C-5″), 72.38, 72.76, 74.73, and 75.38 (6 CH_2Ph), 73.65, 76.59, 76.92, 77.06, 77.70, 79.12, 80.39, 80.70, 80.86; 83.72 (C-1), 99.38 (C-1′ and C-1″), 127.10, 127.52, 127.59, 127.67, 127.86, 127.99, 128.26, 128.35, 128.40, 128.48, 138.09, and 138.52 (6 Ph), and 170.00 (C=O); MS (CI, NH_3): 1100 $(M + NH_4)^+$, 1010, 820, 774, 712, 638. Anal. calcd. for $C_{64}H_{74}O_{13}S$: C 70.95, H 6.89, S 2.95; found: C 70.90, H 6.81, S 2.79.

11-(Methoxycarbonyl)undecyl 2-O-{3′-O-(3″-O-acetyl-2″,4″-di-O-benzyl-α-D-rhamnopyranosyl)-2′,4′-di-O-benzyl-α-D-rhamnopyranosyl}-3,4-di-O-benzyl-α-D-rhamnopyranoside (16)

To a solution of **15** (0.31 g, 0.287 mmol) and methyl 12-hydroxydodecanoate (0.1 g, 0.435 mmol) in dichloromethane (8 mL) were added powdered 4 Å molecular sieves (0.3 g) and *N*-iodosuccinimide (0.15 g, 0.667 mmol). Trifluoromethanesulfonic acid (20 μL) was added at 0°C under N_2 and the mixture was stirred at room temperature for 4 h. Triethylamine (0.2 mL) in dichloromethane (5 mL) was added to neutralize the solution. The reaction mixture was filtered and the filtrate was concentrated to a residue that was fractionated by column chromatography on silica gel (hexane – ethyl acetate (3:1, v/v)) to give compound **16** (66 mg), its β form **17** (73 mg), and their mixture (184 mg) (68% combined yield).

Compound 16: $[\alpha]_D$ +10 (*c* 1.54, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.22 (3H, d, $J_{5,6}$ = 6.2 Hz, 6-CH_3), 1.27–1.34 (20H, m, 2 6-CH_3 and $(CH_2)_7$), 1.54 (2H, m, CH_2CH_2C=O), 1.64 (2H, m, OCH_2CH_2), 1.95 (3H, s, 3″-Ac), 2.31 (2H, t, J = 7.6 Hz, CH_2C=O), 3.35 (1H, m, one of $OCH_2(CH_2)_7$), 3.40 (1H, t, $J_{3,4} = J_{4,5}$ = 9.4 Hz, H-4), 3.61 (1H, t, $J_{3'',4''} = J_{4'',5''}$ = 9.5 Hz, H-4″), 3.63 (1H, t, $J_{3',4'} = J_{4',5'}$ = 9.6 Hz, H-4′), 3.65 (1H, m, one of $OCH_2(CH_2)_7$), 3.66 (1H, m, H-5), 3.67 (3H, s, OMe), 3.75–3.82 (3H, m, H-2′, H-5′, and H-5″), 3.83–3.88 (2H, m, H-2″ and H-3), 4.05 (1H, dd, $J_{1,2}$ = 1.5 Hz, $J_{2,3}$ = 2.5 Hz, H-2), 4.19 (1H, dd, $J_{2',3'}$ = 2.9 Hz, $J_{3',4'}$ = 9.6 Hz, H-3′), 4.38 and 4.20 (2H, d and d, J_{gem} = 12.2 Hz, CH_2Ph), 4.50–4.90 (10H, m, 5 CH_2Ph), 4.71 (1H, d, $J_{1,2}$ = 1.5 Hz, H-1), 5.13 (1H, d, $J_{1',2'}$ = 1.4 Hz, H-1′), 5.18 (1H, d, $J_{1'',2''}$ = 1.3 Hz, H-1″), 5.30 (1H, dd, $J_{2'',3''}$ = 3.3 Hz, $J_{3'',4''}$ = 9.5 Hz, H-3″), and 7.15–7.40 (30H, m, 6 Ph); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ: 18.01 (C-6, C-6′, and C-6″), 21.07 (CH_3C=O), 24.98, 26.17, 29.17, 29.26, 29.45, 29.56, and 34.13 ($(CH_2)_{10}$), 51.40 (OMe), 67.56 ($OCH_2(CH_2)_{10}$), 67.90, 68.21, and 68.83 (C-5, C-5′, and C-5″), 72.30, 72.42, 72.73, 74.70, and 75.42 (6 CH_2Ph), 73.63, 76.92, 77.39, 77.88, 79.11, 80.14, 80.61, 80.85; 98.94, 99.12, and 99.38 (C-1, C-1′,and C-1″), 127.03, 127.51, 127.58, 127.64, 127.74, 128.07, 128.24, 128.31, 128.40, 138.07, 138.11, 138.39, 138.57, and 138.72 (6 Ph), and 170.09 (C=O); MS (CI, NH_3): 1268 $(M + NH_4)^+$, 1178, 942, 820, 728, 638. Anal. calcd. for $C_{75}H_{94}O_{16}$: C 71.97, H 7.57; found: C 71.58, H 7.50.

Compound 17: $[\alpha]_D$ −16 (*c* 0.55, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.21 (3H, d, $J_{5'',6''}$ = 6.2 Hz, 6″-CH_3), 1.26 (17H, m, 6′-CH_3 and $(CH_2)_7$), 1.33 (3H, d, $J_{5,6}$ = 6.1 Hz, 6-CH_3), 1.61 (4H, m, OCH_2CH_2 and CH_2CH_2C=O), 1.92 (3H, s, 3″-Ac), 2.29 (2H, t, J = 7.5 Hz, CH_2C=O), 3.31 (1H, m, $J_{5,6}$ = 6.1 Hz, $J_{4,5}$ = 9.3 Hz, H-5), 3.40 (1H, m, one of OCH_2CH_2), 3.42 (1H, t, $J_{4,5} = J_{3,4}$ = 9.3 Hz, H-4), 3.51 (1H, dd, $J_{2,3}$ = 2.3 Hz, $J_{3,4}$ = 9.3 Hz, H-3), 3.59 (2H, t, $J_{3',4'} = J_{3'',4''} = J_{4',5'} = J_{4'',5''}$ = 9.5 Hz, H-4′ and H-4″), 3.66 (3H, s, OMe), 3.80–3.84 (3H, m, H-2′, H-2″, and H-5′), 3.91 (1H, m, one of OCH_2CH_2), 4.10 (1H, d, J_{gem} = 12.2 Hz, one of CH_2Ph), 4.19 (1H, d, $J_{2,3}$ = 2.3 Hz, H-2), 4.25 (1H, dd, $J_{2',3'}$ = 3.1 Hz, $J_{3',4'}$ = 9.5 Hz, H-3′), 4.31–4.36 (3H, m, H-1, H-5″, and one of CH_2Ph), 4.42–4.91 (10H, m, 5 CH_2Ph), 5.18 (1H, d, $J_{1'',2''}$ = 1.3 Hz, H-1″), 5.19 (1H, d, $J_{1',2'}$ = 1.3 Hz, H-1′), 5.29 (1H, dd, $J_{3'',4''}$ = 9.5 Hz, $J_{2'',3''}$ = 3.2 Hz, H-3″), and 7.18–7.35 (30H, m, 6 Ph); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ: 17.85 (C-6), 18.02 (C-6′ and C-6″), 21.09 (CH_3 C=O), 25.00, 26.18, 29.17, 29.29, 29.44, 29.57, 29.79, and 34.14 ($(CH_2)_{10}$), 51.44 (OMe), 68.04, 69.98 ($OCH_2(CH_2)_{10}$), 71.76; 72.10, 72.60, 73.64, 74.37, and 74.65 (6 CH_2Ph), 72.68, 73.41, 77.04, 77.15, 77.79, 78.16, 79.14, 80.44, 80.87, 82.90; 98.23, 99.30, and 100.04 (C-1, C-1′, and C-1″), 126.69, 127.14, 127.33, 127.50, 127.61, 127.85, 127.96, 128.11, 128.23, 128.32, 128.48, 128.56, 137.91, 138.27, 138.63, and 139.23 (6 Ph), and 170.07 (C=O); MS (CI, NH_3): 1268 $(M + NH_4)^+$, 1178, 1038, 900, 712. Anal. calcd. for $C_{75}H_{94}O_{16}$: C 71.97, H 7.57; found: C 71.60, H 7.42.

11-Carbazoylundecyl O-α-D-rhamnopyranosyl-(1″ → 3′)-O-α-D-rhamnopyranosyl-(1′ → 2)-α-D-rhamnopyranoside (18)

A solution of **16** (0.24 g, 0.19 mmol) in 3:2 (v/v) methanol – acetic acid (10 mL) was subjected to a hydrogen pressure of 50 psig (1 psi = 6.9 kPa) in the presence of 10% Pd–C (0.2 g) at room temperature for 20 h; TLC (ethyl acetate) showed that the debenzylation was complete. The catalyst was removed by filtration and the filtrate was concentrated to a residue under vacuum. The residue was dissolved in 95% ethanol (5 mL) and the solution was treated with hydrazine (0.2 mL) for 16 h at room temperature. The solvent was evaporated and traces of hydrazine were removed by codistillation with ethanol; the residue was dried under vacuum. Purification on a column of Sephadex LH-20 (40 × 1 cm, methanol) gave **18** (80 mg, 62%); $[\alpha]_D$ +77.3 (*c* 0.44, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.18 (3H, d, $J_{5,6}$ = 6.1 Hz, 6-CH_3), 1.19 (3H, d, $J_{5',6'}$ = 6.2 Hz, 6′-CH_3), 1.22 (3H, d, $J_{5'',6''}$ = 6.2 Hz, 6″-CH_3), 1.26 (14H, br, $(CH_2)_7$), 1.53 (4H, m, OCH_2CH_2 and CH_2CH_2C=O), 2.09 (2H, t, J = 7.5 Hz, CH_2C=O), 3.28–3.38

(3H, m, H-4″, H-5, and one of OCH_2CH_2), 3.43–3.53 (2H, m, H-3 and H-4′), 3.60 (1H, m, H-5″), 3.68 (1H, m, one of OCH_2CH_2), 3.65–3.72 (3H, m, H-3″, H-4, and H-5′), 3.72–3.75 (2H, m, H-2 and H-3′), 3.92 (1H, dd, $J_{1'',2''}$ = 1.6 Hz, $J_{2'',3''}$ = 3.3 Hz, H-2″), 4.00 (1H, dd, $J_{1',2'}$ = 1.6 Hz, $J_{2',3'}$ = 2.9 Hz, H-2′), 4.77 (1H, d, $J_{1,2}$ = 1.6 Hz, H-1), 4.91 (1H, d, $J_{1',2'}$ = 1.6 Hz, H-1′), and 5.04 (1H, d, $J_{1'',2''}$ = 1.6 Hz, H-1″); ^{13}C NMR (100.61 MHz, CD_3OD) δ: 17.94, 17.99, and 18.15 (C-6, C-6′, and C-6″), 26.84, 27.30, 30.24, 30.40, 30.46, 30.55, 30.58, 30.65, and 35.00 ($(CH_2)_{10}$), 68.55 (OCH_2), 69.86, 70.13, and 70.51 (C-5, C-5′, and C-5″), 71.80, 72.14, 72.17, 73.23, 74.04, 74.29, 79.22, 80.16; 100.26, 103.88, and 103.95 (C-1, C-1′, and C-1″), and 175.37 ($CONHNH_2$); MS (FAB): 691 $(M + Na)^+$, 669 $(M + H)^+$, 523, 539, 377, 231. Anal. calcd. for $C_{30}H_{56}N_2O_{14}$: C 53.88, H 8.44, N 4.19; found: C 53.73, H 8.58, N 3.87.

*O-α-D-Rhamnopyranosyl-(1″ → 3′)-O-α-D-rhamnopyranosyl-(1′ → 2)-O-α-D-rhamnopyranosyl-(1 → 12)-oxydodecanoyl-BSA (**19**)*

To a stirred solution of **18** (8 mg) in dry *N,N*-dimethylformamide (0.2 mL) at −30°C was added 1.3 M HCl–1,4-dioxane (60 μL). *tert*-Butyl nitrite in *N,N*-dimethylformamide (1:10, w/v, 50 μL) was added, and the solution was stirred for 10 min at −30°C. The solution of the acyl azide (−30°C) was added dropwise to a solution of BSA (15 mg) in 0.1 M $Na_2B_4O_7$ at 0°C. The solution was stirred at 0°C for 4 h and dialyzed for 20 h against five changes of deionized water using a membrane that retains proteins of molecular weight 12 000 or greater. The water was removed by lyophilization to give **19** (18 mg).

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