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A method for glycoconjugate synthesis

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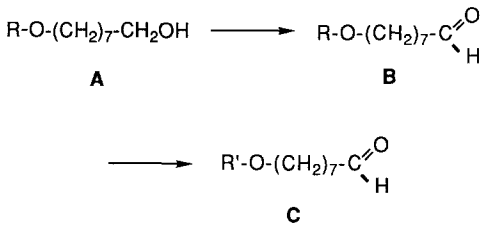
7-Formylheptyl glycosides of 2-acetamido-2-deoxy-β-D-glucopyranose and *O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-α-L-rhamnopyranose were synthesized and were coupled by reductive amination to bovine serum albumin and aminopropyl glass, respectively.

Keywords: glycoconjugate synthesis, reductive amination, ω-aldehydoalkyl aglycon, immunoadsorbent, disaccharide

Introduction

Reductive amination [1–3] is a frequently used technique for the preparation of carbohydrate-based, synthetic antigens, immunoadsorbents and other glycoconjugates [4–7]. It involves condensation of the carbonyl group of the carbohydrate with the amino group of the carrier to form an intermediate Schiff-base which is chemoselectively reduced with sodium cyanoborohydride at or near neutral pH. The product is a stable, secondary or tertiary amine [4]. Reductive amination conserves the original net charge on the proteins which is partly responsible for the good retention of their immunogenic activity [4]. The procedure is experimentally undemanding, needs no highly reactive, unstable intermediates and can be applied without difficulty to oligosaccharides containing carboxyl or acetamido groups. Reductive amination has been successfully used for the coupling of free (anomerically unsubstituted) oligosaccharides having D-glucose as the reducing end, terminal residue [4–8]. However, this approach could not be applied to oligosaccharides terminated at the reducing end by D-galactose [8] or by a ketose (e.g. fructose or KDO) [3]. In such cases, reduction of the oxo group was faster than the formation and/or reduction of the intermediate imine [3]. Another disadvantage is that reductive amination of free oligosaccharides converts the reducing end glycose residue into a polyhydroxyalkyl fragment. These barriers can be overcome by the use of O- or S-glycosides having an ω-aldehydo(oxa)alkyl aglycon [9–11]. Regrettably, synthesis of such glycosides is not without difficulty. For example, the aldehydo group of 6-hydroxyhexanal, an apparently ideal candidate as an aglycon, has to be protected to avoid formation of an internal, cyclic hemiacetal. Attempted glycosylation of the derivative 6,6-dimethoxyhexanol under Helferich conditions leads mostly to methyl instead of the target 6,6-dimethoxyhexyl glycoside (V. Pozsgay, unpublished results). Although ozonolysis of the allyl [12–14] and related glycosides [15, 16] is an established procedure for the preparation of ω-aldehydoalkyl glycosides, it needs special equipment which is not always available. This procedure was shown by NMR spectroscopy to lead to cyclic hemiacetals from allyl [13, 14] and butenyl hexopyranosides [13]. This was independent of the anomeric configuration. The use of such an approach for complex, synthetic oligosaccharides, which has yet to be reported, would put serious constraints on the array of the applicable protecting groups during the synthetic manipulations.

The first preparation of such glycosides is described here by an oxidative procedure other than ozonolysis, by way of examples of a mono- and a disaccharide. Briefly, the procedure (Scheme I) involves the synthesis of an 8-hydroxyoctyl glycoside (**A**) which is subjected to Swern-oxidation [17] of the C-8 carbon atom of the aglycon to generate the formyl group shown by the general formula **B**, followed by the removal of the protecting groups from the sugar moiety (→**C**). The C_8 aglycon is similar to Lemieux's [18] hydrophobic spacer. By virtue of the length of the aglycon, the carbon atom of the terminal, formyl group should not be involved to any significant extent in stable, cyclic hemiacetal formation with the sugar hydroxyl groups. Therefore, the proposed spacer moiety was expected to promote efficient and accelerated coupling reactions with matrices having amino groups.

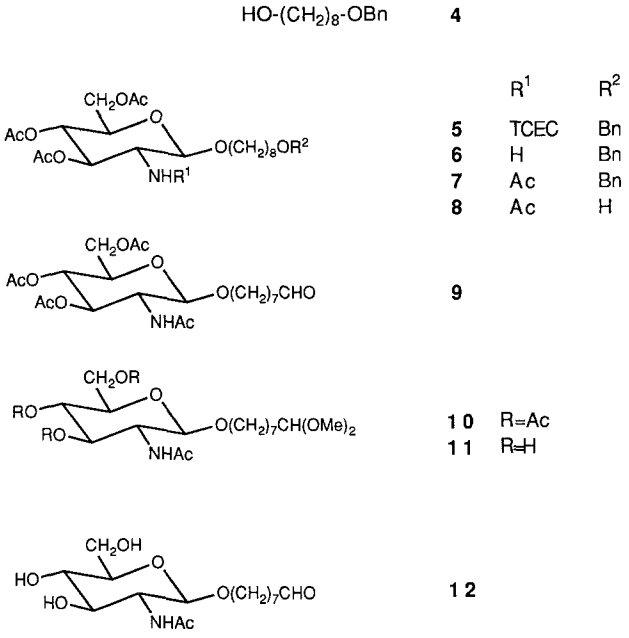


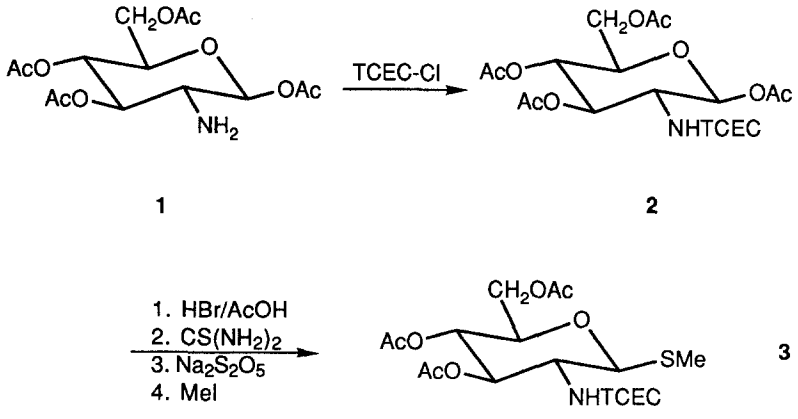
R = protected glycose
R' = unprotected glycose

Scheme 1

Results and discussion

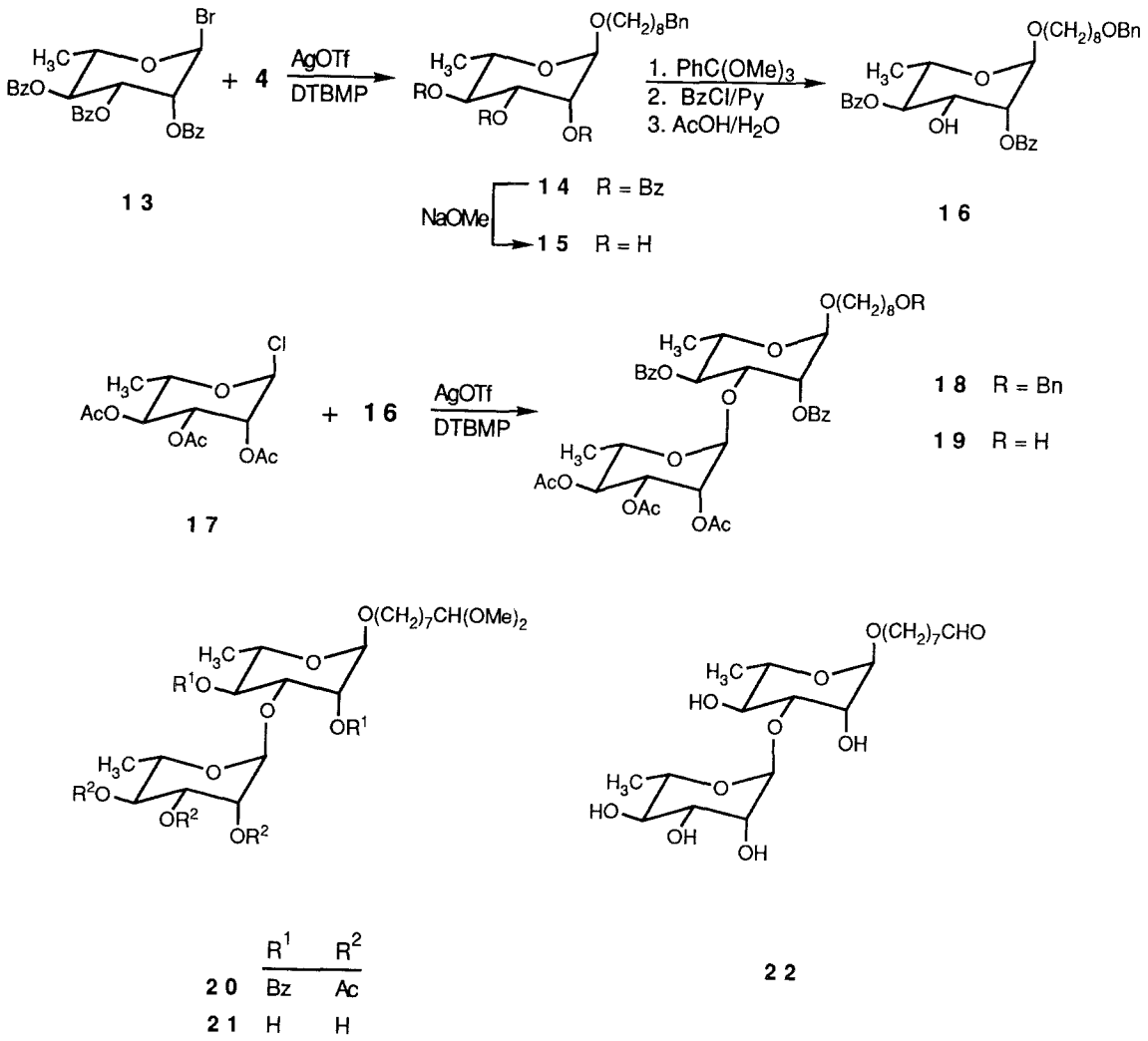
2-Acetamido-2-deoxy-D-glucose was selected as the monosaccharide example. The synthesis of the target 7-formylheptyl glycoside **12** was based on thioglucoside **3** which was readily obtained from the amine **1** [19] in five steps, through the intermediacy of the tetraacetate **2** (Scheme 2). The 2,2,2-trichloroethoxycarbonyl (TCEC) group as the temporary N-protecting group in **3** promotes 1,2-*trans* stereoselective glycosylation with nucleophiles [20] without the formation of 1,2-*cis* glycoside or sugar oxazolidinone, of which the latter was reportedly formed with another urethane-type N-protection [21]. A particularly pleasing characteristic of the TCEC group is that it can be removed under very mild conditions with zinc and acetic acid [22], leaving most other protecting groups including *O*-acetyl groups unaffected. Reaction of 8-benzyloxyoctanol (**4**) with compound **3** under promotion by methyl trifluoromethanesulfonate [23] gave glycoside **5** in 85% yield. Subsequently, the TCEC group was replaced by an acetyl group in two steps [(i) AcOH-Zn (→**6**); (ii) Ac_2O (→**7**)] and the benzyl protecting group was removed by hydrogenolysis (→**8**) to expose the chain-end hydroxyl group. Swern-oxidation of compound **8** gave aldehyde **9** which was converted *in situ* to dimethoxy derivative **10** through acid-catalyzed acetal-exchange with 2,2-dimethoxypropane. Sequential treatment of **10** first with NaOMe in MeOH (→**11**) then with dilute aqueous trifluoroacetic acid afforded the formylheptyl glycoside **12**. In deuterium oxide, **12** exists both as the free aldehyde and the hydrated form, as indicated in its ^1H NMR spectrum by a broad signal at 9.56 ppm (CHO) and a triplet at 5.01 ppm (J = 5 Hz) [CH(OH)$_2$], respectively. Diagnostic resonances in the ^{13}C NMR spectrum appear at 210.2 ppm (CHO) and at 91.9 ppm [CH(OH)$_2$]. The C-7′ carbon atom of the spacer moiety gives rise to two resonances (43.9 and 37.8 ppm) corresponding to the free aldehyde and the hydrated form, respectively. The hydrated form amounts to about 30% within 10 min after dissolution in deuterium oxide and to about 60% after





TCEC = C(O)OCH$_2$CCl$_3$

Scheme 2



Scheme 3

18 h. The observation that in the ^{13}C NMR spectrum all sugar carbons appear as sharp signals of comparable intensity without resonances attributable to diastereoisomers also supports the conclusion that **12** develops no cyclic hemiacetal structure in aqueous solution. Using a standard protocol [3] published for reductive amination, aldehyde **12** was covalently attached to bovine serum albumin (BSA). In the example described in the experimental part, the level of incorporation was estimated to be 50 molecules of hapten per one BSA molecule, using a protocol that was described by Lemieux and coworkers [18].

The α-(1 → 3)linked rhamnobiose [24] **22** was selected as the disaccharide example which occurs in a number of bacterial, cell-surface glycoconjugates, e.g., in the O-specific polysaccharide of *Shigella dysenteriae* type 1 [25]. The synthesis of compound **22** is outlined in Scheme 3. Rhamnosylation of the spacer **4** with the donor **13** [26] in the presence of silver trifluoromethanesulfonate and 2,6-di-t-butyl-4-methylpyridine gave **14** in 96% yield. Removal of the benzoyl groups by transesterification afforded the triol **15** which was regioselectively O-benzoylated in a three-step procedure [27] to give the acceptor **16** in 68% yield. The rhamnosyl block **17** was obtained by chlorinolysis of methyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside [26] in 79% yield. Condensation of the acceptor **16** with the chloride **17** afforded the disaccharide **18** (91%). Hydrogenolytic removal of the benzyl group (→**19**) followed by Swern-oxidation and acetalization as described for **8** gave compound **20** (76%) which was deacylated (→**21**) and deacetalated to give **22**. The NMR spectra of **22** exhibited characteristics similar to those of **12**. Hapten **22** was coupled to aminopropyl glass (Sigma) in an aqueous mixture at pH 8 in the presence of $NaCNBH_3$. The level of incorporation was estimated to be 20 μM of hapten **22** per one gram of the aminopropyl glass using the phenol–sulfuric acid assay [28]. Thus, every fourth reactive site is occupied by the ligand, based on the manufacturer's data for the NH_2 group equivalents (81 μM/g) in the aminopropyl glass.

In conclusion, a new protocol is described for the preparation of glycoconjugates. This approach exploits the

advantages of the reductive amination technique and conserves the integrity of the reducing-end sugar residue. The reactive, anchor-equipped intermediates are stable under the usual conditions of storage, and require no prior activation for coupling.

Materials and methods

General methods

All reagents and solvents were of commercial grade. Anhydrous solvents were purchased from Aldrich and were used as received. Aminopropyl glass was obtained from Sigma (Cat. No G-4643). Organic solutions were dried with anhydrous Na_2SO_4 before concentration. Column chromatography was performed on silica gel 60 (0.040–0.063 mm). Melting points were measured with a Thomas Hoover apparatus and are uncorrected. Optical rotations were measured at 22 °C with a Perkin-Elmer Type 241MC polarimeter, in chloroform, except where indicated otherwise. The NMR spectra were recorded on a Gemini-300 spectrometer (300 MHz for ^1H, 75 MHz for ^{13}C), or on a Bruker AM-250 spectrometer (250 MHz for ^1H, 62 MHz for ^{13}C), at 23–25 °C. Internal references: Me_4Si (0.000 ppm for ^1H for solutions in organic solvents), Me_2CO (2.225 ppm for ^1H for solutions in ^2H_2O), Me_2CO (31.05 ppm for ^{13}C for solutions in ^2H_2O), C^2HCl_3 (77.00 ppm for ^{13}C for solutions in C^2HCl_3), $C^2H_3O^2H$ (49.00 ppm for solutions in $C^2H_3O^2H$). For compounds **12** and **22** the assignments for the hydrated forms are shown in italics. Subscripts A, B for compounds **18**–**22** refer to the individual sugar residues, with A standing for the reducing-end unit. Aglyconic atoms are denoted by a prime. Protons linked to the same carbon atom are differentiated by an asterisk. Mass spectra were obtained by the chemical ionization technique (CIMS), using NH_3 as the ionizing gas. Positive ion, fast atom bombardment mass spectra were obtained in the low (FABMS) and high resolution mode (HRFAB), respectively, using 3-nitrobenzyl alcohol as the matrix. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA, USA, or by Oneida Research Services, Inc., Whitesboro, NY, USA.

*1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-*D*-glucopyranose* (**2**)

To a stirred mixture of **1** [19] (3.5 g) in acetonitrile (20 ml) and 5% aqueous sodium hydrogen carbonate (5 ml) was added at 0 °C 2,2,2-trichloroethyl chloroformate (1.37 ml) dropwise, then the mixture was stirred for 25 min. The volatiles were removed under vacuum and the residue was partitioned between chloroform and water. The organic layer was dried and concentrated. Crystallization of the residue from ether afforded **2** (4.2 g, 80%).

M.p. 129–130 °C, $[\alpha]_D$ +11° (*c* 0.9). NMR data (C^2HCl_3): ^{13}C, δ 20.6, 20.7, 20.8 (CH_3CO) 55.1 (C-2), 61.6 (C-6), 68.0 (C-4), 72.1, 72.8, 74.5 (C-3,5, CH_2CCl_3) 92.3 (C-1); ^1H, δ 2.05, 2.09, 2.11 (CH_3CO), 3.86 (ddd, H-5), 3.97 (ddd, $J_{2,3}$ 9.7 Hz, H-2), 4.13 (dd, $J_{5,6}$ 1.9 Hz, $J_{6,6*}$ 12.5 Hz, H-6), 4.30 (dd, $J_{5,6*}$ 4.6 Hz, H-6*), 4.732 (s, CH_2CCl_3), 5.12 (t, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4), 5.26 (t, H-3), 5.37 (bd, NH), 5.75 (d $J_{1,2}$ 8.8 Hz, H-1). CIMS: *m/z* 539 $[M + 18]^+$.

Analytical data calculated for $C_{17}H_{22}Cl_3NO_{11}$: C, 39.06; H, 4.24; N, 2.68; found: C, 39.35; H, 4.13, N, 2.56.

*Methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-1-thio-β-*D*-glycopyranoside* (**3**)

A solution of **2** (9 g) in a 3/2 mixture of acetic anhydride and dichloromethane (20 ml) was treated with 30% hydrogen bromide in acetic acid (50 ml) at room temperature for 3 h. The mixture was cooled to 0 °C, and partitioned between chloroform (100 ml) and ice-water (200 ml). The organic layer was successively extracted with ice-water (3 × 200 ml), 5% ice-cold, aqueous sodium hydrogen carbonate (50 ml) and ice-water (100 ml), was dried and concentrated. To a stirred solution of the residue in acetonitrile (60 ml) was added thiourea (1.4 g). The mixture was heated. Separation of a solid was observed within 2 min after dissolution of thiourea. Heating was continued for a further period of 5 min then the mixture was cooled in ice for 2 h. Filtration afforded a solid (8 g). A solution of this material (7.5 g) in water (50 ml) containing sodium metabisulfite (2.4 g) was stirred at 80 °C for 5 min. Chloroform (50 ml) was then added and the mixture was stirred under reflux for 20 min. The mixture was cooled to room temperature. The organic layer was washed with water, dried and concentrated to afford a syrup (5.8 g). A solution of this syrup (5.0 g) in a 5/1 mixture of acetone and 5% aqueous sodium hydrogen carbonate (60 ml) was treated with methyl iodide (2 ml). The mixture was stirred for 1 h. Most of the acetone was removed under vacuum, then the aqueous solution was treated with water (50 ml). Filtration of the precipitate followed by recrystallization from methanol afforded **3** (4.7 g).

M.p. 156–157 °C, $[\alpha]_D$ − 17° (*c* 1.3). NMR data (C^2HCl_3): ^{13}C, δ 11.8 (SMe) 20.6, 20.7 (CH_3CO), 54.6 (C-2), 61.2 (C-6), 68.4 (C-4), 73.4 (C-3), 74.5 (CH_2CCl_3), 76.0 (C-5), 84.3 (C-1), 154.1, 169.3, 170.7 (C=O); ^1H, δ 2.04, 2.09, 2.21 (SMe, CH_3CO), 3.67 (ddd, H-5), 3.83 (ddd, H-2), 4.16 (dd, $J_{5,6}$ 2 Hz, $J_{6,6*}$ 12.3 Hz, H-6), 4.28 (dd, $J_{5,6*}$ 3.2 Hz, H-6*), 4.50 (d, $J_{1,2}$ 10.2 Hz, H-1), 4.68 and 4.82 (2d *J* 12 Hz for each, CH_2CCl_3), 5.10 (t, $J_{3,4} = J_{4,5}$ = 8.3 Hz, H-4), 5.21 (t, $J_{2,3}$ H-3), 5.22 (d *J* 9 Hz, NH). CIMS: *m/z* 527 $[M + 18]^+$.

Analytical data calculated for $C_{16}H_{22}Cl_3NO_9S$: C, 37.62; H, 4.34; N, 2.74; found: C, 37.80; H, 4.27, N, 2.63.

8-Benzyloxyoctanol (**4**)

Sodium hydride (0.8 g) was added in small portions to a stirred solution of 1,8-octanediol (5.5 g) in *N*,*N*-dimethylformamide (20 ml) at 0 °C. The mixture was stirred for 30 min. Benzyl bromide (4 ml) was added in small portions

over a period of 30 min. Stirring was continued for 3 h. The solution was concentrated and the residue was partitioned between chloroform and water. Concentration of the organic layer afforded a liquid which was purified by column chromatography (hexane:ethyl acetate 10:1 → 4:1 by vol) to give first 1,8-di-*O*-benzyloctanol (3 g, 24%; CIMS: *m/z* 344 $[M + 18]^+$) followed by compound **4** as a liquid (4.2 g, 47%).

NMR data (C^2HCl_3): ^{13}C, δ 25.6, 26.1, 29.3, 29.35, 29.7, 32.7 (C-2,3,4,5,6,7), 62.8 (C-1), 70.4, 72.8 (C-8, CH_2Ph), 127.3–128.2, 138.5 (aromatic C); ^1H, δ 1.26–1.39, 1.47–1.66 [$(CH_2)_6$], 3.46 (t, *J* 6.6 Hz, CH_2), 3.6 (bt, CH_2), 4.50 (s, CH_2Ph), 5.27 (s, OH), 7.2–7.35 (aromatic H). CIMS: *m/z* 237 $[M + 1]^+$, 254 $[M + 18]^+$.

*8-Benzyloxyoctyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glycopyranoside* (**5**)

Methyl triflate [22] (400 µl) was added to a stirred mixture of **3** (1.54 g), **4** (1.0 g) and 4 Å molecular sieves (3 g) in dichloromethane (50 ml) at room temperature. After 4 days the mixture was filtered through a layer of Celite and concentrated. Column chromatography (hexane:ethyl acetate 2:1 by vol) of the residue afforded **5** as a syrup (1.8 g, 85%).

$[\alpha]_D$ − 12° (*c* 1). NMR data (C^2HCl_3): ^{13}C, δ 20.3, 20.4 (CH_3CO), 25.5, 25.8, 28.9, 29.0, 29.1, 29.4 [$(CH_2)_6$], 55.9 (C-2), 62.0 (C-6), 68.8 (C-4), 69.9, 70.1, 72.5, 74.1 (CH_2CCl_3, CH_2Ph, C-1′, C-8′), 71.3 (C-5), 100.4 (C-1), 127.2–128.0, 138.4 (aromatic C), 153.9, 169.2, 170.3, 170.4 (C=O); ^1H, δ 1.25–1.65 [$(CH_2)_6$], 2.00, 2.02, 2.07 (CH_3CO), 3.64 (ddd, $J_{1,2}$ 8.5 Hz, $J_{2,3}$ 10 Hz, H-2), 3.72 (ddd, H-5), 4.29 (dd, $J_{5,6}$ 4.7 Hz, $J_{6,6*}$ 12.1 Hz, H-6*), 4.49 (s, CH_2Ph), 4.65 (d, H-1), 4.65 and 4.77 (2d *J* 12 Hz for each, CH_2CCl_3), 5.05 (t, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.33 (t, H-3), 5.89 (bd, NH), 7.2–7.38 (aromatic H). CIMS: *m/z* 715 $[M + 18]^+$. HRFAB: Found *m/z* 700.1848, $C_{30}H_{43}{}^{35}Cl^{37}Cl_2NO_{11}$ requires 700.1879.

*8-Benzyloxyoctyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside* (**6**)

A mixture of **5** (1.6 g), zinc dust (2.3 g) and glacial acetic acid (20 ml) was stirred at room temperature for 90 min. The mixture was filtered and the solution concentrated. The residue was partitioned between chloroform and 5% aqueous sodium hydrogen carbonate. The organic layer was dried and concentrated. Trituration of the residue in ether:hexane afforded amorphous **6** (1.1 g, 94%).

$[\alpha]_D$ − 28° (*c* 0.8). NMR data (C^2HCl_3): ^{13}C, δ 20.5, 20.6, 20.7 (CH_3CO), 25.7, 26.0, 29.2 (2C), 29.3, 29.6 [$(CH_2)_6$], 55.0 (C-2), 62.2 (C-6), 68.8 (C-4), 70.3 (2 CH_2), 72.7 (CH_2), 71.7, 75.3 (C-3,5), 103.9 (C-1), 127.3–128.2, 138.6 (aromatic C), 169.6, 170.6(2C) (CH_3CO); ^1H, δ 1.24–1.74 [$(CH_2)_6$], 2.03, 2.08 (CH_3CO), 2.92 (m, H-2), 3.42–3.56 [m, CH_2 (aglycon)], 3.68 (ddd, H-5), 3.90 [m, CH_2 (aglycon)], 4.11 (dd, $J_{5,6}$ 2.3 Hz, $J_{6,6*}$ 12.2 Hz, H-6), 4.27 (d, $J_{1,2}$ 10 Hz, H-1), 4.30 (dd, $J_{5,6*}$ 4.7 Hz, H-6*), 4.51 (s, CH_2Ph), 4.94–5.5 (m, H-3,4), 7.23–7.45 (m, aromatic H). CIMS: *m/z* 524 $[M + 1]^+$, 541 $[M + 18]^+$.

Analytical data calculated for $C_{27}H_{41}NO_9$: C, 61.93; H, 7.89; N, 2.67; found: C, 61.92; H, 7.85, N, 2.51.

*8-Benzyloxyoctyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside* (**7**)

Acetic anhydride (0.5 ml) was added to a stirred solution of **6** (430 mg) in methanol (5 ml) at 0 °C. After 15 min the solution was concentrated. Trituration of the residue in ether:hexane afforded **7** (470 mg, 99%).

M.p. 82–84 °C, $[\alpha]_D$ − 12° (*c* 0.9). NMR data (C^2HCl_3): ^{13}C, δ 20.6, 20.65, 20.71 (CH_3COO), 23.3 (CH_3CON), 25.7, 26.1, 29.2, 29.3 (2C), 29.7 (C-2′,3′,4′,5′,6′,7′), 54.9 (C-2), 62.2 (C-6), 68.7 (C-4), 69.9, 70.4, 71.7, 72.3, 72.8 (C-3,5,1′,8′, CH_2Ph), 100.6 (C-1), 127.4, 127.6, 128.3 (CH_3CO); ^1H, δ 1.23–1.86 (m, $(CH_2)_6$), 1.93, 2.02, 2.03, 2.08 (CH_3CO), 3.41–3.52 (m, H-1′, H-8′, 8′*), 3.71 (ddd, H-5), 3.77–3.90 (m, H-2, H-$1'_B$), 4.12 (dd, $J_{5,6}$ 2.5 Hz, $J_{6,6*}$ 12.2 Hz, H-6), 4.27 (dd, $J_{5,6*}$ 4.7 Hz, H-6), 4.50 (s, CH_2Ph), 4.69 (d, $J_{1,2}$ 8.3 Hz, H-1), 5.06 (t, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.32 (dd, $J_{2,3}$ 9.3 Hz, H-3), 5.57 (bd, NH), 7.27–7.34 (aromatic H). CIMS: *m/z* 566 $[M + 1]^+$, 583 $[M + 18]^+$.

Analytical data calculated for $C_{29}H_{43}NO_{10}$: C, 61.57; H, 7.66; N, 2.48; found: C, 61.67; H, 7.76, N, 2.38.

*8-Hydroxyoctyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside* (**8**)

A solution of **7** (400 mg) in a mixture of ethanol:acetic acid (10 ml, 10:1 by vol) was hydrogenolyzed over Pd/C (10%, 100 mg) at atmospheric pressure for 24 h. Filtration followed by concentration afforded **8** (320 mg, 95%).

M.p. 107–110 °C, $[\alpha]_D$ + 30° (c 1). NMR data (C^2HCl_3): ^{13}C, δ 20.6, 20.68, 20.73 (CH_3COO), 23.3 (CH_3CON), 25.3, 25.4, 28.8, 29.0, 29.1, 32.5 (C-2′,3′,4′,5′,6′,7′), 54.8 (C-2), 62.2, 62.9 (C-6,8′), 68.7 (C-4), 69.7 (C-1′), 71.8, 72.4 (C-3,5), 100.7 (C-1), 169.4, 170.2, 170.8 (CH_3CO); ^1H, δ 1.32–1.75 [$(CH_2)_6$], 1.95, 2.02, 2.03, 2.09 (CH_3CO), 4.13 (dd, $J_{5,6}$ 2.5 Hz, $J_{6,6*}$ 12.1 Hz, H-6), 4.25 (dd, $J_{5,6*}$ 4.7 Hz, H-6*), 4.65 (d, $J_{1,2}$ 8.3 Hz, H-1), 5.07 (t, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 5.27 (dd, $J_{2,3}$ 9.4 Hz, H-3), 5.75 (bd, NH). CIMS: *m/z* 476 $[M + 1]^+$, 493 $[M + 18]^+$.

Analytical data calculated for $C_{22}H_{37}NO_{10}$: C, 55.57 H, 7.84; found: C, 55.21; H, 7.72.

*8,8-Dimethoxyoctyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside* (**10**)

To a stirred solution of oxalyl chloride (340 µl) in dichloromethane (20 ml) at −70 °C was added dimethyl sulfoxide (660µl) in 3 min. After 5 min a solution of **8** (1.6 g) in dichloromethane (40 ml) was added in 5 min. The solution was further stirred for 10 min, then treated with

triethylamine (3 ml). After 10 min the solution was concentrated. A solution of the residue in 2,2-dimethoxypropane (10 ml) was treated with *p*-toluenesulfonic acid monohydrate at 5 °C for 10 min. Triethylamine (2 ml) was added and the mixture was concentrated. The residue was equilibrated between chloroform and water. The organic layer was concentrated. Column chromatography of the residue (hexane:ethyl acetate, 1:3 by vol) afforded crystalline **10** (1.3 g, 74%).

M.p. 79–81 °C, $[\alpha]_D$ − 14° (*c* 0.6). NMR data (C^2HCl_3): ^{13}C, δ 20.63, 20.69, 20.7 (CH_3COO), 23.3 (CH_3CON), 24.4, 25.6, 29.1, 29.3 (2C), 32.4 (C-2',3',4',5',6',7'), 52.7 (C-2), 54.9 (CH_3O), 62.2 (C-6), 68.7 (C-4), 69.8, 71.7, 72.3 (C-1',3,5), 100.6 (C-1), 104.6 (C-8'), 169.4, 170.1, 170.7, 170.8 (CH_3CO); ^1H, δ 1.28–1.33 and 1.53–1.62 [$(CH_2)_6$], 1.94, 2.02, 2.03, 2.08 (CH_3CO), 3.31 (s, CH_3O), 3.70 (ddd, H-5), 3.78 (ddd, $J_{2,3}$ 10.2 Hz, H-2), 4.13 (dd, $J_{5,6}$ 2.4 Hz, $J_{6,6*}$ 12.1 Hz, H-6), 4.27 (dd, $J_{5,6*}$ 4.7 Hz, H-6*), 4.348 (t, *J* 5.7 Hz, H-8'), 4.70 (d, $J_{1,2}$ 8.3 Hz, H-1), 5.06 (t, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-4), 5.32 (dd, $J_{2,3}$ 10 Hz, H-3), 5.60 (d, *J* 8.5 Hz, NH). CIMS: *m/z* 518 $[M + H^+ - H_2]$, 537 $[M + 18]^+$.

Analytical data calculated for $C_{24}H_{41}NO_{11}$: C, 55.48; H, 7.95; N, 2.69; found: C, 55.92; H, 8.05; N, 2.62.

8,8-Dimethoxyoctyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**11**)

A solution of **10** (500 mg) in anhydrous methanol (10 ml) at 0 °C was treated with a catalytic amount of sodium methoxide. After 6 h the solution was concentrated to give **11** as an amorphous solid (370 mg, 98%).

$[\alpha]$ − 32° (*c* 0.6, *N,N*-dimethylformamide). NMR data ($C^2H_3O^2H$): ^{13}C, δ 23.0 (CH_3CON), 25.5, 27.0, 30.4, 30.6 (2C), 33.7 (C-2',3',4',5',6',7'), 53.3 (CH_3O), 57.4 (C-2), 62.8 (C-6), 70.5, 72.1, 76.0, 77.9 (C-1',3,4,5), 102.7 (C-1), 106.2 (C-8'); ^1H, δ 1.28–1.33 and 1.53–1.62 [$(CH_2)_6$], 2.03 (CH_3CO), 3.31 (s, CH_3O), 3.70 (ddd, H-5), 3.78 (ddd, $J_{2,3}$ 10.2 H-2), 4.13 (dd, $J_{5,6}$ 2.4 Hz, $J_{6,6*}$ 12.1 Hz, H-6), 4.27 (dd, $J_{5,6*}$ 4.7 Hz, H-6*), 4.348 (t, *J* 5.7 Hz, H-8', 8'*), 4.70 (d, $J_{1,2}$ 8.3 Hz, H-1), 5.06 (t, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-4), 5.32 (dd, $J_{2,3}$ 10 Hz, H-3), 5.60 (d, *J* 8.5 Hz, NH). FABMS: *m/z* 330 $[M + H^+ - 2CH_3OH]$, 362 $[M + H^+ - CH_3OH]$, 394 $[M + 1]^+$, 416 $[(M + 23]^+$.

Analytical data calculated for $C_{18}H_{35}NO_8$: C, 54.95; H, 8.97; N, 3.56; found: C, 54.11; H, 8.65; N, 3.82.

7-Formylheptyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**12**)

A solution of **11** (290 mg) in 20 μM trifluoroacetic acid (10 ml) was warmed to boiling in 2 min. After 30 s the solution was cooled to 0 °C and the volatiles were removed by freeze-drying to give **12** (250 mg, 97%) as an amorphous solid.

$[\alpha]_D$ − 33° (*c* 0.2, *N,N*-dimethylformamide). NMR data (^2H_2O): ^{13}C, δ 22.1 (C-5'), 23.0 (CH_3CON), 24.8 (*C-5'*), 25.69 (C-6'), 25.77 (*C-6'*), 28.9, 29.1, 29.3 (C-2',3',4'), 37.8 (*C-7'*), 43.9 (C-7), 56.4 (C-2), 61.6 (C-6), 71.2 (C-1'), 70.7, 74.6, 76.9 (C-3,4,5), 91.9 ($^1J_{C\text{-}8',H\text{-}8'}$ = 163 Hz, *C-8'*), 101.9 ($^1J_{C\text{-}1,H\text{-}1}$ = 161 Hz, C-1), 175.1 (CH_3CO), 210.2 (CHO); ^1H, δ 1.28–1.38 and 1.5–1.65 [$(CH_2)_5$], 2.03, (CH_3CO), 2.52 (dt, $J_{7',8'}$ 2.0 Hz, $J_{6',7'}$ 7.0 Hz, H-7'), 3.67 (H-2), 4.49 (d, $J_{1,2}$ 8.4 Hz, H-1), 5.01 (t, $J_{7',8'}$ 5 Hz, *H-8*), 9.67 (b, CHO). FABMS: *m/z* 204 $[C_8H_{14}NO_5]^+$, 320 $[M\text{-}CHO + 2]^+$, 348 $[M + 1]^+$.

Analytical data calculated for $C_{16}H_{29}NO_7$. 1/2 H_2O: C, 53.91; H, 8.48; N, 3.93; found: C, 53.83; H, 8.28; N, 3.99.

Coupling of **12** *to bovine serum albumin*

A solution of **12** (69.4 mg), bovine serum albumin (67 mg), and sodium cyanoborohydride (130 mg) in 0.1 ml phosphate buffer (pH 7.0, 6 ml) was kept at room temperature for 24 h then at 37 °C for 12 h. The solution was dialyzed against distilled water. Freeze-drying afforded an amorphous product (88 mg) which was estimated to contain 50 mol of the hapten per one mol of bovine serum albumin.

8-Benzyloxyoctyl 2,3,4-tri-O-benzyl-α-L rhamnopyranoside (**14**)

To a stirred solution of bromide [26] **13** (7.7 g), alcohol **4** (5 g) and 2,6-di-t-butyl-4-methylpyridine (2.5 g) in dichloromethane (50 ml) containing 4 Å molecular sieves (3 g) at −20 °C was added silver trifluoromethanesulfonate (5 g). Stirring was continued for 2 h during which the temperature of the mixture reached 23 °C. Ice-cold, 5% aqueous sodium hydrogen carbonate (20 ml) was added and the mixture was filtered. The organic layer was concentrated. Column chromatography of the residue (hexane:ethyl acetate, 6:1 by vol) afforded **14** as a syrup (9.15 g, 96%).

$[\alpha]_D$ + 118° (*c* 2). NMR data (C^2HCl_3): ^{13}C, δ 17.7 (C-6), 26.1, 26.2, 29.4 (3C), 29.8 (C-2',3',4',5',6',7'), 66.6 (C-5), 68.5, 70.5 (C-1',8'), 70.1 (C-3), 71.0 (C-2), 72.0 (C-4), 72.8 (CH_2Ph), 97.5 (C-1), 127.3–129.8, 132.9–133.3 (aromatic C), 165.4, 165.5, 165.7 (C=O); ^1H, δ 1.36 (d, H-6), 1.35–1.48 and 1.6–1.75 [$(CH_2)_6$], 3.48 (t, $J_{7',8'}$ 6.6 Hz, H-8',8'*), 3.53 and 3.78 (2m, H-1',1'*), 4.19 (dq, H-5), 4.51 (s, CH_2Ph), 4.99 (d, $J_{1,2}$ 1.7 Hz, H-1), 5.65 (dd, $J_{2,3}$ 3.5 Hz, H-2), 5.66 (t, $J_{3,4} = J_{4,5}$ = 9.9 Hz, H-4), 5.84 (dd, H-3). CIMS: *m/z* 695 $[M + 1]^+$, 712 $[M + 18]^+$.

Analytical data calculated for $C_{42}H_{46}O_9$: C, 72.59; H, 6.67; found: C, 72.55; H, 6.68.

8-Benzyloxyoctyl α-L-rhamnopyranoside (**15**)

A solution of **14** (8.8 g) in methanol (100 ml) was treated with a catalytic amount of sodium methoxide at room temperature. After 4 h, the solution was neutralized with Dowex 50W(H^+). The solution was concentrated. Column chromatography of the residue (hexane:ethyl acetate, 1:1 then 1:2 by vol) afforded syrupy **15** (4.0 g, 82%).

$[\alpha]_D$ − 47° (*c* 0.5). NMR data (C^2HCl_3): ^{13}C, δ 17.7 (C-6), 26.0, 26.1, 29.3, 29.4 (2C), 29.7 (C-2',3',4',5',6',7'), 67.8 (C-5), 67.7, 70.4 (C-1',8'), 71.1 (C-2), 71.8 (C-3), 72.8 (CH_2Ph), 73.0

(C-4), 99.6 (C-1), 127.4–128.2, 138.5 (aromatic C); ^{1}H, δ 1.28–1.38 and 1.5–1.66 (H-6, $(CH_2)_6$), 3.38 and 3.63 (2m, H-1′,1′*), 3.45 (H-4 and H-8′,8′*), 3.63 (dq, H-5), 3.74 (dd, $J_{2,3}$ 3.1 Hz, $J_{3,4}$ 9.4 Hz, H-3), 3.89 (dd H-2), 4.50 (s, CH_2Ph), 4.73 (d, $J_{1,2}$ 1.7 Hz, H-1), 7.18–7.35 (aromatic H). CIMS: *m/z* 383 $[M + 1]^+$, 400 $[M + 18]^+$.

Analytical data calculated for $C_{21}H_{34}O_6$: C, 65.94; H, 8.96; found: C, 65.81; H, 8.99.

*8-Benzyloxyoctyl 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside* (**16**)

To a stirred solution of **15** (3.8 g) in trimethyl orthobenzoate (3 ml) was added 10-camphorsulfonic acid at room temperature. The reaction flask was evacuated (1 Torr or less) for 10 min. The mixture was cooled to 0 °C. Pyridine (5 ml) and benzoyl chloride (4 ml) were added under cooling. The reaction mixture was allowed to reach room temperature. After 2 h the volatiles were removed under vacuum. The residue was dissolved in 80% aqueous acetic acid (20 ml) at 0 °C. After 10 min the solution was concentrated under vacuum. The residue was equilibrated between chloroform and water. The organic layer was concentrated. Column chromatography of the residue (hexane:ethyl acetate, 7:1 by vol) afforded **16** as a syrup (4.0 g, 68%).

$[\alpha]_D$ − 32° (*c* 1.4). NMR data (C^2HCl_3): ^{13}C, δ 17.9 (C-6), (26.0, 26.1, 29.3 (3C), 29.7 (C-2′,3′,4′,5′,6′,7′), 66.1 (C-5), 68.2, 70.4, (C-1′,8′), 68.9, 73.4, 75.6 (C-2,3,4), 72.8 (CH_2Ph), 97.3 (C-1), 127.4–129.8, 138.5 (aromatic C), 166.0, 167.0 (C=O); ^{1}H, δ 1.31 (d, $J_{5,6}$ 6.4 Hz, H-6), 1.3–1.5 and 1.58–1.7 $[(CH_2)_6]$, 3.47 (t, *J* 6.6 Hz, H-8′,8′*), 3.48 and 3.72 (2m, H-1′,1′*), 4.06 (dq, H-5), 4.33 (dd, H-3), 4.50 (s, CH_2Ph), 4.95 (d, $J_{1,2}$ 1.5 Hz, H-1), 5.28 (t, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4), 5.38 (dd, $J_{2,3}$ 3.3 Hz, H-2). CIMS: *m/z* 355 $[C_{20}H_{19}O_6]^+$, 591 $[M + 1]^+$, 608 $[M + 18]^+$.

Analytical data calculated for $C_{35}H_{42}O_8$: C, 71.16; H, 7.17; found: C, 71.19; H, 7.10.

*2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl chloride* (**17**)

A solution of methyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside (20 g) in dichloromethane (60 ml) was treated at 0 °C with a solution of chlorine in carbon tetrachloride until the yellow color persisted. After 10 min hex-1-ene was added until the solution became colorless. The solution was concentrated. Crystallization of the residue from hexane afforded **17** (15.3 g, 79%).

M.p. 72–74 °C, $[\alpha]_D$ − 129° (*c* 1); lit. m.p. 72–73 °C, $[\alpha]_D$ − 116° (*c* 0.64, chloroform) [29]. NMR data (C^2HCl_3): ^{1}H, δ 1.27 (d, $J_{5,6}$ 6.3 Hz, H-6), 2.01, 2.08, 2.17 (CH_3CO), 4.17 (dq, H-5), 5.14 (t, $J_{3,4} = J_{4,5}$ = 10 Hz, H-4), 5.39 (dd, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 3.4 Hz, H-2), 5.56 (dd, H-3), 5.94 (d, H-1). CIMS: *m/z* 273 $[M - 35]^+$, 326 $[M + 18]^+$.

Analytical data calculated for $C_{12}H_{17}ClO_7$: C, 46.68; H, 5.55; Cl, 11.48; found: C, 46.58; H, 5.60; Cl, 11.57.

*8-Benzyloxyoctyl 3-*O*-(2,3,4,-tri-*O*-acetyl-α-L-rhamnopyranosyl)-2,4-di-*O*-benzoyl-α-L-rhamnopyranoside* (**18**)

To a stirred solution of **16** (3.6 g), **17** (2.8 g), and 2,6-di-t-butyl-4-methylpyridine (1.5 g) in dichloromethane (40 ml) containing 4 Å molecular sieves (3 g) at −45 °C was added silver trifluoromethanesulfonate (2.5 g). The reaction mixture was allowed to reach room temperature in 3 h. Work-up as described for **14** followed by column chromatography (hexane:ethyl acetate, 6:1 by vol) afforded syrupy **18** (4.8 g, 91%).

$[\alpha]_D$ + 22° (*c* 1). NMR data (C^2HCl_3): ^{13}C, δ 17.2, 17.7 ($C\text{-}6_A,6_B$), 20.5, 20.7 (CH_3CO), 26.0, 26.2, 29.4 (3C), 29.7 (C-2′,3′.4′,5′,6′,7′), 66.6, 67.1 ($C\text{-}5_A,5_B$), 68.3 (C-1′), 68.4 ($C\text{-}3_B$), 69.7, 70.9 ($C\text{-}2_A,2_B$), 70.4, 72.8 (C-8′, CH_2Ph), 72.3, 73.3 ($C\text{-}4_A,4_B$), 75.7 ($C\text{-}3_A$), 97.1, 99. 1($C\text{-}1_A,1_B$), 127.4–133.4, (aromatic C), 165.4, 166.0 [C=O (Bz)], 169.0, 169.3, 169.9 [C=O (Ac)]; 1H, δ 1.04 (d, $J_{5,6}$ 6.2 Hz, $H\text{-}6_B$) 1.30 (d, $J_{5,6}$ 6.3 Hz, $H\text{-}6_A$), 1.83, 1.88, 1.89 (CH_3CO), 3.48 (2m, H-1′,8,8′*), 3.7 (m, H-1′*), 3.86 (dq, $H\text{-}5_B$), 4.01 (dq, $H\text{-}5_A$), 4.38 (dd, $J_{2,3}$ 3.5 Hz, $J_{3,4}$ 9.8 Hz, $H\text{-}3_A$), 4.5 (CH_2Ph), 4.85–4.95 ($H\text{-}1_A,1_B,2_B,4_B$), 5.07 (dd $J_{2,3}$ 3.4 Hz, $J_{3,4}$ 9.8 Hz, $H\text{-}3_B$), 5.41 (dd, $J_{1,2}$ 2.1 Hz, $J_{2,3}$ 3.3 Hz, $H\text{-}2_A$), 5.49 (t, $J_{3,4} = J_{4,5}$ = 9.8 Hz, $H\text{-}4_A$). CIMS: *m/z* 880 $[M + 18]^+$.

Analytical data calculated for $C_{47}H_{58}O_{15}$: C, 65.41; H, 6.77; found: C, 64.29; H, 6.67.

*8-Hydroxyoctyl 3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-2,4-di-*O*-benzoyl-α-L-rhamnopyranoside* (**19**)

A solution of **18** (2.6 g) in a mixture of ethanol:acetic acid (53 ml, 50:3 by vol) was hydrogenolyzed over 10% Pd/C (100 mg) at room temperature and atmospheric pressure. After 12 h the mixture was filtered and concentrated. Column chromatography (hexane:ethyl acetate, 1:1 by vol) of the residue afforded **19** as an amorphous solid (1.78 g, 76%).

$[\alpha]_D$ + 32° (*c* 0.8). NMR data (C^2HCl_3): ^{13}C, δ 17.2, 17.9 ($C\text{-}6_A,6_B$), 20.5, 20.9 (CH_3CO), 25.9, 26.0, 29.3 (2C), 32.7 (C-2′,3′,4′,5′,6′,7′), 62.9 (C-8′), 66.6, 67.1 ($C\text{-}5_A,5_B$), 68.2 (C-1′), 68.3 ($C\text{-}3_B$), 69.7, 70.9 ($C\text{-}2_A,2_B$), 72.3, 73.2 ($C\text{-}4_A,4_B$), 75.8 ($C\text{-}3_A$), 97.1, 99.0 ($C\text{-}1_A,1_B$), 128.3–133.4, (aromatic C), 164.5, 166.0 [C=O (Bz)], 169.0, 169.3, 169.9 [C=O (Ac)]; ^{1}H, δ 1.04 (d, $J_{5,6}$ 6.2 Hz, $H\text{-}6_B$) 1.31 (d, $J_{5,6}$ 6.3 Hz, $H\text{-}6_A$), 1.35–1.44 and 1.53–1.69 $[(CH_2)_6]$, 1.83, 1.90 (CH_3CO), 3.48 and 3.73 (2m, H-1′,1′*), 3.64 (t, H-8′,8′*), 3.86 (dq, $H\text{-}5_B$), 4.01 (dq, $H\text{-}5_A$), 4.38 (dd, $J_{2,3}$ 3.5 Hz, $J_{3,4}$ 9.7 Hz, $H\text{-}3_A$), 4.84–4.96 ($H\text{-}1_A,1_B,2_B,4_B$), 5.07 (dd $J_{2,3}$ 3.5 Hz, $J_{3,4}$ 9.8 Hz, $H\text{-}3_B$), 5.41 (dd, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.3 Hz, $H\text{-}2_A$), 5.49 (t, $J_{3,4} = J_{4,5}$ = 9.8 Hz, $H\text{-}4_A$), 7.4–7.65, 8.03–8.17 (aromatic H). CIMS: *m/z* 790 $[M + 18]^+$.

Analytical data calculated for $C_{40}H_{52}O_{15}$: C, 62.17; H, 6.78; found: C, 62.12; H, 6.83.

8′,8′-Dimethoxyoctyl 3-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-2,4-di-O-benzoyl-α-L-rhamnopyranoside (**20**)

Compound **19** was oxidized then acetalized as described for compound **8**. The crude product was purified by column chromatography (hexane:ethyl acetate, 1:2 by vol) to give compound **20** as a syrup (76% overall yield).

$[\alpha]_D$ + 27° (*c* 0.51). NMR data (C^2HCl_3): ^{13}C, δ 17.2, 17.7 (C-6_A,6_B), 20.55, 20.6 (CH_3CO), 25.0, 26.0, 29.3, 29.4 (2C), 32.5 (C-2′,3′,4′,5′,6′,7′), 52.6 (CH_3O), 66.6, 67.8 (C-5_A,5_B), 68.3 (C-1′), 68.4 (C-3_B), 69.7, 70.0 (C-2_A,2_B), 72.3, 73.3 (C-4_A,4_B), 75.7 (C-3_A), 97.2, 99.1 (C-1_A,1_B), 104.5 (C-8′), 123.7–135.8, (aromatic C); ^1H, δ 1.04 and 1.31 (2d, $J_{5,6}$ 6.3 Hz, H-6_A,6_B), 1.33–1.42 and 1.58–1.68 [$(CH_2)_6$], 1.82, 1.89, 1.90 (CH_3CO), 3.48 and 3.72 (2m, H-1′,1′*), 3.86 and 4.01 (2dq, H-5_A,5_B), 4.37 (t, H-8′) 4.38 (dd, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 9.6 Hz, H-3_A), 4.84–4.96 (H-1_A,1_B,2_B,4_B), 5.07 (dd $J_{2,3}$ 3.5 Hz, $J_{3,4}$ 9.8 Hz, H-3_B), 5.41 (dd, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.4 Hz, H-2_A), 5.49 (t, $J_{3,4} = J_{4,5}$ = 9.9 Hz, H-4_A), 7.26–7.32, 7.42–7.7, 8.04–8.17, 8.6–8.65 (aromatic H). CIMS: *m/z* 834 $[M + 18]^+$.

Analytical data calculated for $C_{42}H_{52}O_6$: C, 61.75; H, 6.91; found: C, 61.86; H, 6.92.

8,8′-Dimethoxyoctyl 3-O-(α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**21**)

To a solution of **20** (500 mg) in methanol (20 ml) was added a catalytic amount of sodium methoxide. After 2 days at room temperature the solution was carefully neutralized with Dowex 50W(H^+) then filtered immediately. The filtrate was treated with one drop of concentrated ammonium hydroxide then concentrated. A solution of the residue in water was extracted thrice with hexane. Freeze-drying of the aqueous layer afforded amorphous **21** (280 mg, 95%).

$[\alpha]_D$ − 58° (*c* 0.3). NMR data (^2H_2O): ^{13}C, δ 17.2, 17.7 (C-6_A,6_B), 20.55, 20.6 (CH_3CO), 25.0, 26.0, 29.3, 29.4 (2C), 32.5 (C-2′,3′,4′,5′,6′,7′), 52.6 (CH_3O), 66.6, 67.8 (C-5_A,5_B), 68.3 (C-1′), 68.4 (C-3_B), 69.7, 70.0 (C-2_A,2_B), 72.3, 73.3 (C-4_A,4_B), 75.7 (C-3_A), 97.2, 99.1 (C-1_A,1_B), 105.9 (C-8′), 123.7–135.8, (aromatic C); ^1H, δ 1.287 (d, $J_{5,6}$ 6.2 Hz, H-6_A), 1.293 (d, $J_{5,6}$ 6.3 Hz, H-6_B), 1.31–1.42 and 1.52–1.68 [$(CH_2)_6$], 3.36 (CH_3O), 3.45 (t, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4_B), 3.53 (t, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4_A), 3.78 (dd, $J_{2,3}$ 3.5 Hz, $J_{4,5}$ 9.8 Hz, H-3_A), 3.83 (dd, $J_{2,3}$ 4.5 Hz, $J_{3,4}$ 9.8 Hz, H-3_B), 3.99 (dd, $J_{1,2}$ 1.8 Hz, H-2_A), 4.06 (dd, $J_{1,2}$ 1.7 Hz, H-2_B), 4.51 (t, $J_{7',8'}$ 5.8 Hz, H-8′), 4.75 (d, H-1_A), 5.03 (d, H-1_B). CIMS: *m/z* 834 $[M + 18]^+$.

Analytical data calculated for $C_{22}H_{42}O_{11} \cdot H_2O$: C, 52.79; H, 8.86; found: C, 52.74; H, 8.30.

7-Formylheptyl 3-O-(α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**22**)

Compound **21** was hydrolyzed as described for **11**. Compound **22** was obtained as an amorphous solid (95%).

$[\alpha]_D$ − 66° (*c* 0.5, H_2O). NMR data (^2H_2O): ^{13}C, δ 17.4 (C-6_A,6_B), 22.1 (C-5′), 24.7 (*C-5′*), 25.9 (C-6′), 26.0 (*C-6′*), 28.9–29.2 (C-2′,3′,4′), 37.7 (*C-7′*), 43.9 (C-7), 68.7 (C-1′), 69.5, (C-5_A), 69.8 (C-5_B), 70.7, 70.9 (2C) (C-2_A,2_B,3_B), 72.2, 72.7 (C-4_A,4_B), 78.8 (C-3_A), 91.9 (*C-8′*), 100.3 (C-1_A), 103.0 (C-1_B); ^1H, δ 1.29 (2d, H-6_A,6_B), 1.3–1.4 and 1.55–1.68 [$(CH_2)_5$], 2.53 (dt, $J_{7',8'}$ 1.9 Hz, $J_{6',7'}$ 7.3 Hz, H-7′), 3.45 (t, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4_B), 3.53 (t, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4_A), 3.79 (dd, $J_{2,3}$ 3.6 Hz, H-3_A), 3.83 (dd, $J_{2,3}$ 3.5 Hz, H-3_B), 3.99 (dd, H-2_A), 4.06 (dd, H-2_B), 4.75 (d, $J_{1,2}$ 1.5 Hz, H-1_A), 5.02 (t, $J_{7',8'}$ 5.6 Hz, *H-8*), 5.03 (d, $J_{1,2}$ 1.5 Hz, H-1_B), 9.67 (t, CHO). FABMS: *m/z* 437 $[M + 1]^+$, 459 $[M + Na]^+$.

Coupling of **22** *to aminopropyl glass*

A solution of **22** (50 mg) and sodium cyanoborohydride (29 mg) in 0.2 M phosphate buffer (pH 8, 5 ml) containing aminopropyl glass (Sigma, amine content 81 μM g^{-1}) was slowly stirred at 37 °C for 4 h. More sodium cyanoborohydride (26 mg) was added and the mixture was further stirred for 4 h. The mixture was then cooled to 25 °C and was filtered. The solid was successively washed with water (200 ml), 0.1% sodium dodecyl sulfate (15 × 30 ml), 2% acetic acid (200 ml), and water (200 ml) then was dried under vacuum.

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