Synthesis of an experimental glycolipoprotein vaccine against Lyme disease

Vince Pozsgay and **Joanna Kubler-Kielb**

Laboratory of Developmental and Molecular Immunity, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892-2423

Abstract

A novel glycolipid was synthesized that corresponds to cholesteryl palmitoyl-galactopyranoside **1** found in the spirochete *Borrelia burgdorferi*, the causative agent of Lyme disease. In order to fashion **1** in a conjugatable form, the palmitoyl residue was modified to include a terminal aldehydo moiety that anchored the glycolipid to aminooxypropylated serum albumin using oxime chemistry. The glycolipoprotein so obtained incorporates an average of 18 glycolipid moieties per albumin molecule. The novel glycolipoprotein constructs are soluble in water and are candidates towards developing a semisynthetic vaccine against Lyme disease.

Keywords

Aminooxy; *Borrelia burgdorferi*; Conjugation; Glycolipid; Glycolipoprotein; Lyme disease; Oxime; Vaccine


Lyme disease (LD) is caused by the spiral-shaped Gram-negative bacterium *Borrelia burgdorferi* which belongs to the Spirochaetaceae family.[1] LD was first reported in the United States in 1977 when a large number of children in and around the townships Lyme and Old Lyme in Connecticut were diagnosed as having juvenile rheumatoid arthritis. The disease can be manifested as a multi-system disorder affecting the skin, eyes, the joints, internal organs and the nervous system. LD has been recognized in Europe since the 1880's under various names such as erythema migrans (EM) and in 1948 EM biopsies revealed the presence of spirochetes.[2] That LD is caused by *B. burgdorferi* was firmly established in 1982. While the progression of the symptoms of LD can be successfully treated with antibiotics, there is no vaccine available after the withdrawal from medical use of a vaccine (LYMErix, Smith Kline Beecham) in 2002 that was based on the outer surface lipoprotein of *B. burgdorferi* which is a single polypeptide chain consisting of 257 amino acids having covalently bound lipids at its N-terminus.

Borrelia are lacking lipooligo- and lipopolysaccharides characteristic of the cell-surface of most Gram-negative bacteria. A recently published work identified cholesteryl 6-*O*-palmitoyl-β-ᴅ-galactopyranoside **1** as a surface component of *B. burgdorferi*.[3] Compound **1** was shown to elicit specific immune response when administered in Freund's adjuvant or in PBS.[3] Because of this propensity, glycolipid **1** became a target for vaccine development. We reported chemical

---

Correspondence to: Vince Pozsgay.

synthesis of **1** and that of a congener in which the palmitoyl group is replaced by an oleoyl group.[4] The availability of the synthetic glycolipids dispelled any uncertainty that arose from the fact that the structural studies were performed on the inseparable mixture of **1** and its oleoyl congener, and paved the way towards the synthesis of a functionalized derivative suitable for covalent attachment to proteins for the purpose of enhancing its immunogenicity without the need for an adjuvant.
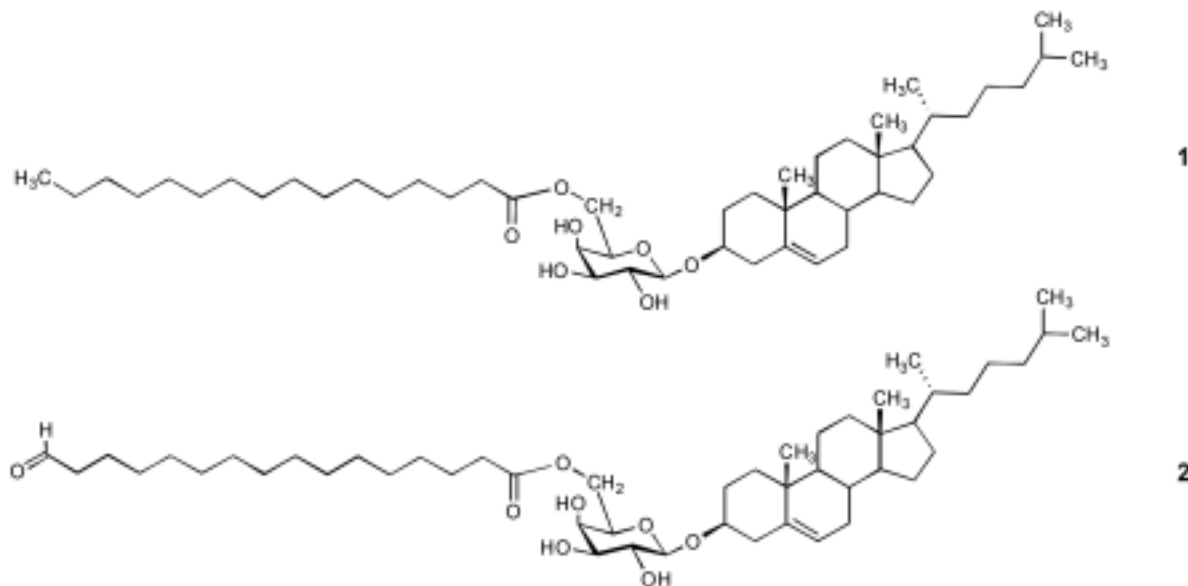
Here, we describe the synthesis of a bioconjugatable derivative (**2**) of glycolipid **1** and show that it can be covalently linked to aminooxypropylated bovine serum albumin (BSA)[5] through oxime linkages, to yield a four-domain, water-soluble glycolipoprotein.

Our approach to the oxo derivative **2** was modeled after the reported synthesis of compound **1**, relying on the triol[4] **3** as the starting material that was converted to the key intermediate **6** in a series of protection-deprotection steps. We reasoned that the use of a *p*-methoxybenzylidene acetal protection on HO-3 and 4 would optimize the overall yield because of the ease and selectivity for the construction and the cleavage of dioxolane-type benzylidene acetals.[6] Thus, the triol[4] **3** was treated with *p*-methoxybenzaldehyde dimethyl acetal in the presence of camphorsulfonic acid to afford a diastereomeric mixture of the benzylidene acetals *R,S*-**4** in 82% yield. (Scheme 1) That the dioxolane ring was, indeed, formed on O-3 and O-4 of the galactose moiety was shown by the appearance of the H-2 protons as two triplets of approximately equal intensity at 5.08 and 5.01 ppm ($J \sim 7.8$ Hz for each) upon acetylation (Ac$_2$O/C$_5$H$_5$N) of *R,S*-**4**. (Not described in the Experimental part.) Next, sodium hydride-mediated *p*-methoxybenzylaton using freshly prepared *p*-methoxybenzyl bromide afforded the fully protected intermediate *R,S*-**5** in 91 % yield. We note that although the individual diastereomers were well-separated on TLC, no attempt was made to their isolation in stereochemically homogeneous form. Treatment of the silyl ether *R,S*-**5** with tetrabutylammonium fluoride generated the alcohol *R,S*-**6** as a diastereomeric mixture in 96 % yield, featuring the linkage site to the aldehydo palmitic acid moiety unprotected. Aldehydo palmitic acid derivative **7** was prepared from the commercially available 16-hydroxypalmitic acid as described for 6,6-dimethoxyhexanoic acid.[7] Condensation of alcohol **6** with the acid **7** under promotion by dicyclohexylcarbodiimide and 4-dimethylaminopyridine afforded the fully protected key intermediate *R,S*-**8**. Following the assembly of the target, our attention turned to the removal of the protecting groups from the galactosyl and the palmitoyl moieties,

respectively. Treatment of *R,S*-**8** with trifluoroacetic acid selectively removed the anisylidene protecting group to afford compound **9** in 91 % yield. Next, the *p*-methoxybenzyl group was oxidatively removed by treatment with DDQ to generate the triol **10**. Finally, acetic acid-mediated hydrolysis of the acetal moiety provided the targeted glycolipid derivative **2**.

Having accomplished the synthesis of the modified glycolipid derivative **2**, our attention turned to its covalent attachment to proteins. We have shown that synthetic oligosaccharides containing an aldehydo or keto group in their aglyconic part can be efficiently linked to aminooxypropylated serum albumin through oxime linkages and that the conjugates so prepared induce anti-hapten antibodies in mice.[5,8-10] Our initial attempts to link **2** with aminooxypropylated bovine serum albumin[5] were hampered by the low solubility of **2** in most organic solvents and by its insolubility in aqueous media. Eventually, we envisaged the use of the "surfactant reversed micelle" technique in organic solvents.[11,12] The method creates a microheterogeneous environment for the hapten and the albumin counterparts thus bringing the partners in close proximity for reaction. In applying this technique, a buffered aqueous solution of aminooxypropylated BSA was added to a solution of the anionic detergent sodium bis(2-ethylhexyl)sulfosuccinate in octane under stirring. A transparent, clear solution indicated the formation of reversed micelles. Next, aminooxypropyl-BSA was treated with the aldehydo-derivatized hapten **2** in pyridine to form the conjugate which was isolated by precipitation with alcohol followed by purification by size-exclusion chromatography. The solution of the conjugate so obtained was slightly opalescent but did not precipitate from the aqueous solution. In order to prove that hapten **2** was, indeed, covalently linked to BSA, the conjugate was subjected to SDS polyacrylamide gel eletrophoresis (SDS-PAGE) that confirmed the increase of the molecular mass of aminooxypropylated BSA upon treatment with the hapten **2**. (Figure 1) Under our conditions, described in detail in the Experimental section, the average loading density was 18 glycolipid moieties per BSA molecule as determined by MALDI-TOF mass spectrometry. In a control experiment carried out under identical conditions, the parent glycolipid **1** failed to react with aminooxypropylated BSA as evidenced by SDS-PAGE and by mass spectrometry.

In summary, we described a simple route to a conjugatable derivative **2** of the natural glycolipid **1** which was covalently linked to bovine serum albumin under mild conditions using oxime chemistry. The glycolipoprotein so obtained is currently being evaluated for its immunogenic properties.

4. Experimental

4.1. General Methods

All chemicals of were of commercial grade and were used without purification. Anhydrous solvents were obtained from Aldrich (St. Louis, MO). Column chromatography was performed on silica gel 60 (0.040–0.063 mm) and on dimethyloctadecylsilyl-bonded amorphous silica (C-18 adsorbent, 125 Å, Waters, MA. NMR spectra were obtained using either a Varian Mercury 300 spectrometer or a Bruker DRX-500 spectrometer at 300 K. Internal references: TMS (0.000 ppm for ^{1}H for solutions in CDCl$_3$), acetone (2.225 ppm for ^{1}H and 31.07 ppm for ^{13}C for solutions in D$_2$O), methanol (3.358 ppm for ^{1}H and 49.68 ppm for ^{13}C for solution in CD$_3$OD), and CDCl$_3$ (77.00 ppm for ^{13}C for solutions in CDCl$_3$). Coupling constants are given in Hertz. The high resolution electrospray ionisation mass spectra (HRESIMS) were recorded at the Laboratory of Bioorganic Chemistry, NIDDK, NIH, Bethesda, MD. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. For SDS-PAGE 14%, gels were used according to the manufacturer's instructions (BioRad, Hercules, CA).

4.2. Cholesteryl 6-*O*-(*tert*-butyldiphenylsilyl)-3,4-*O*-*p*-methoxybenzylidene-β-ᴅ-galactopyranoside (*R,S*-4)

To a stirred solution of compound[4] **3** (4.0 g, 5.1 mmol) in CH_2Cl_2 (40 mL) were added at 23 °C *p*-methoxybenzaldehyde dimethyl acetal (6 mL, 33.4 mmol) and a catalytic amount of camphorsulfonic acid. After 24 h, the solution was treated with Et_3N followed by extraction with aq $NaHCO_3$ and H_2O. The organic phase was concentrated. Column chromatography of the residue using 6:1 → 4:1 hexanes–EtOAc gradient containing 0.05 % of Et_3N afforded *R,S*-**4** (3.75 g, 82%) as an amorphous solid: ^{1}H NMR, partial ($CDCl_3$): δ 5.36 (m, 1H), 4.40 (dd, 0.6H, *J* 5.4 Hz, *J* 7.6 Hz), 4.33 (dd, 1H, *J* 6.9 Hz, *J* 8.0 Hz), 4.24 (dt, 1H, *J* 2.6 Hz, *J* 6.1 Hz), 4.19 (dd, 0.4H, *J* 6.0 Hz, *J* 7.0 Hz), 3.83 (0.6H, *J* 1.8 Hz, *J* 6.9 Hz), 3.81 (s), 3.79 (s), 3.69 (dd, 0.4H, *J* 2.0 Hz, *J* 8.0 Hz); ^{13}C NMR ($CDCl_3$): δ 160.3, 160.1, 140.3, 135.6, 133.3, 133.2, 130.7, 129.6-127.7, 122.1, 113.63, 113.61, 104.6, 103.3, 100.6, 100.5, 79.7, 79.1, 78.9, 78.1, 75.9, 74.3, 74.0, 73.6, 73.2, 71.1, 62.9, 62.8, 56.7, 56.1, 55.3, 55.2, 50.1, 42.3, 39.7, 39.5, 38.82, 38.79, 37.2, 36.7, 36.2, 35.8, 31.9, 31.8, 29.70, 29.68, 28.2, 28.0, 26.72, 26.68, 24.3, 23.8, 22.8, 22.6, 21.0, 19.3, 19.21, 19.17, 18.7, 11.8; HRESIMS: Calcd for $(C_{57}H_{80}O_7Si + Li)^+$, *m/z* 911.5795, found, *m/z* 911.5833. Anal. Calcd for $C_{57}H_{80}O_7Si$ + 1/3 H_2O: C, 75.25; H, 8.92. Found: C, 75.22; H, 8.88.

4.3. Cholesteryl 6-*O*-(*tert*-butyldiphenylsilyl)-2-*O*-*p*-methoxybenzyl-3,4-*O*-*p*-methoxybenzylidene-β-ᴅ-galactopyranoside (*R,S*-5)

To a stirred solution of *R,S*-**4** (3.60 g, 4.0 mmol) in and DMF (30 mL) was added at 0 °C NaH (0.7 g of a 60% suspension in oil). After 20 min, freshly prepared *p*-methoxybenzyl bromide (2.7 g, 16 mmol) was added at 0 °C. The mixture was allowed to reach 23 °C. After 40 min, the mixture was re-cooled to 0 °C and was treated with MeOH (1 mL) followed by acetic acid until the pH of the mixture reached 5-6 as determined by a wet indicator paper. The mixture was concentrated at 25 °C under vacuum. Extractive work-up ($CHCl_3/H_2O$) followed by column chromatographic purification of the residue using 10:1 → 6:1 hexanes–EtOAc gradient containing 0.05 % of Et_3N afforded *R,S*-**5** (3.7 g, 91%) as a syrup: ^{1}H NMR, partial ($CDCl_3$): δ 5.36 (m, 1H), 4.85 (d, *J* 11 Hz), 4.76 (d, *J* 11 Hz), 4.74 (d, *J* 11 Hz), 4.79 (d, *J* 11 Hz), 4.24 (t, *J* 6.3 Hz), 4.22 (dd, *J* 1.9 Hz, *J* 5.5 Hz), 4.19 (dd, *J* 2.1 Hz, *J* 6.1 Hz), 3.81 (s, 1.2H), 3.79 (s, 3H), 3.78 (s, 1.8H), 3.48 (dd, *J* 7.2 Hz, *J* 7.7 Hz), 3.43 (dd, *J* 6.5 Hz, *J* 8.0 Hz; ^{13}C NMR ($CDCl_3$): δ 160.24, 160.21, 159.2, 159.1, 140.6, 135.6, 133.4-129.9, 113.64, 113.60, 113.5, 104.4, 103.2, 101.7, 101.4, 80.3, 79.8, 79.4, 79.3, 78.7, 75.8, 73.8, 73.6, 73.5, 73.3, 73.1, 63.1, 62.9, 56.7, 56.1, 55.31, 55.26, 55.24, 55.2, 50.1, 42.3, 39.7, 39.5, 39.1, 39.0, 37.2, 36.7, 36.2, 35.8, 32.0, 31.9, 29.91, 29.88, 28.22, 28.0, 26.72, 26.68, 24.3, 23.8, 22.8, 22.6, 21.0, 19.4, 19.2, 19.1, 15.8; HRESIMS: Calcd for $(C_{65}H_{88}O_8Si + Li)^+$, *m/z* 1031.6390, found, *m/z* 1031.6409. Anal. Calcd for $C_{65}H_{88}O_8Si$ + 1/3 H_2O: C, 75.69; H, 8.66. Found: C, 75.66; H, 8.60.

4.4. Cholesteryl 2-*O*-*p*-methoxybenzyl-3,4-*O*-*p*-methoxybenzylidene-β-ᴅ-galactopyranoside (*R,S*-6)

To a solution of *R,S*-**5** (3.65 g, 3.6 mmol) in THF (30 mL) was added a solution of tetrabutylammonium fluoride (5 mL of a 1 M solution in THF) at 23 °C. After 2 h, the solution was concentrated. Purification of the residue by column chromatography using 6:1 → 1:1 hexanes–EtOAc gradient containing 0.05 % of Et_3N afforded *R,S*-**6** (2.70 g, 96%) as an amorphous solid: ^{1}H NMR, partial ($CDCl_3$): δ 5.37 (m, 1H), 4.85 (d, *J* 11 Hz), 4.76 (d, *J* 11 Hz), 4.74 (d, *J* 11 Hz), 4.71 (d, *J* 11 Hz), 4.52-4.48 (m), 3.65-3.55 (m), 3.49 (t, 1H, *J* 7.8 Hz), 3.46 (dd, 1H, *J* 6.6 Hz, *J* 8.0 Hz); ^{13}C NMR ($CDCl_3$): δ 160.42, 160.37, 159.2, 159.1, 140.39, 140.38, 130.24, 130.2, 129.9, 128.9, 128.3, 127.8, 122.1, 122.0 113.8, 113.7, 113.6, 104.7, 103.3, 101.5, 101.3, 80.5, 79.6, 79.5, 78.8, 76.4, 76.0, 73.9, 73.6, 73.1, 73.0, 62.6, 62.3, 56.7, 56.1, 55.3, 55.2, 55.1, 50.1, 42.3, 39.7, 39.5, 39.0, 37.2, 36.7, 36.2, 35.8, 31.9, 31.8, 29.94, 29.91, 28.2, 28.0, 24.3, 23.8, 22.8, 22.5, 21.0, 19.4, 18.7, 11.8; HRESIMS: Calcd for

$(C_{49}H_{70}O_8 + Li)^+$, *m/z* 793.5209, found, *m/z* 793.5231. Anal. Calcd for $C_{49}H_{70}O_8$ + 1/3 H_2O: C, 74.21; H, 8.98. Found: C, 74.36; H, 8.92.

4.5. (16,16-Dimethoxy)palmitic acid (7)

To a solution of $(COCl)_2$ (2.74 mL, 31.3 mmol) in anh. CH_2Cl_2 (20 mL) was added at -62 °C a solution of DMSO (4.95 mL, 62.5 mmol) in anh. CH_2Cl_2 (10 mL) under stirring, in a period of 5 min. The solution was stirred for 2 min after the addition was complete. To this solution was added dropwise a solution of methyl 16-hydroxyhexadecaneoate in and CH_2Cl_2 [prepared by treatment of a solution of 16-hydroxydecanoic acid (7.4 g, 27 mmol) in anh CH_2Cl_2 with CH_2N_2 until a yellow color persisted]. The solution was stirred at -50 °C for 1 h and then was allowed to reach -10 °C. After 15 min, the solution was treated with Hünig's base (15 mL) and then was allowed to reach 23 °C. The solution was washed with H_2O (3 × 25 mL), dried (Na_2SO_4), and concentrated to afford a semisolid. To a solution of the material in 2,2-dimethoxypropane (30 mL) was added a catalytic amount of 4-toluenesulfonic acid. After 30 min, approx. half of the volatiles were removed by distillation. The solution so obtained was treated with Et_3N (3 mL) and then concentrated. The residue was equilibrated between $CHCl_3$ and H_2O. The organic layer was dried (Na_2SO_4) and concentrated to give a syrup. To a solution of this material in a dioxane (50 mL) were added H_2O (5 mL) and solid KOH (1.7 g) at 23 °C. After 24 h, thin-layer chromatography (9:1 hexanes-EtOAc) indicated that most of the starting material disappeared. Water (5 mL) was added. After an additional 3 h, the solution was treated with H_2O (50 mL) followed by removal of most of the organic solvent by distillation under reduced pressure. More H_2O (100 mL) was added. The solution was acidified with citric acid until pH 3.5-4 was reached as detected by an indicator stripe. The mixture containing a precipitate was extracted with $CHCl_3$ (3 × 50 mL). The combined organic layer was washed with H_2O (2 × 30 mL), dried (Na_2SO_4) and concentrated to afford **7** (5.2 g, 64%) as an amorphous solid: ^1H NMR ($CDCl_3$): δ 4.35 (m, 1H), 3.32 (s, 3H), 3.31 (s, 3H), 2.38-2.31 (m, 2H), 1.68-1.54 (m, 4H), 1.37-1.17 (m, 22H); ^{13}C NMR ($CDCl_3$): δ 179.6, 104.5, 52.5, 34.0, 32.4, 29.6, 29.5, 29.44, 29.39, 29.3, 29.2, 29.0, 24.7, 24.6. Anal. Calcd for $C_{18}H_{36}O_4$: C, 68.31; H, 11.47. Found: C, 68.51; H, 11.52.

4.6. Cholesteryl 6-*O*-(16,16-dimethoxy)palmitoyl-2-*O*-p-methoxybenzyl-3,4-*O*-p-methoxybenzylidene-β-ᴅ-galactopyranoside (8)

A solution of **6** (2.65 g, 3.4 mmol), compound **7** (1.8 g, 5.7 mmol), dicyclohexylcarbodiimide (1.8 g, 8.7 mmol), and 4-dimethylaminopyridine (0.9 g, 7.3 mmol) in EtOAc (50 mL) was stirred at 23 °C for 12 h. Water (0.5 mL) was added and stirring was continued for an other 30 min. The solids were removed by filtration and were washed with ethyl acetate. The combined filtrate and washings was concentrated. The residue was purified by chromatography using 5:1 → 3:1 hexanes–EtOAc gradient containing 0.05 % of Et_3N to afford compound **8** (3.4 g, 93%) as an amorphous material: ^1H NMR ($CDCl_3$): δ 7.35-7.24 (m, 4H), 6.90-6.80 (m, 4H), 5.85 (s, 0.5H), 5.43 (s, 0.5H), 5.37 (m, 1H), 8.84, 4.75, 4.73, 4.68 (4d, *J* ~ 11.3 Hz for each, 2H), 4.50-4.43 (m, 3H), 4.31 (dd, 1H, *J* 4.4 Hz, *J* 11.6 Hz), 4.27 (t, 1H, *J* 6.4 Hz), 4.16 (dd, 1H, *J* 2.4 Hz, *J* 6.1 Hz), 4.13 (dd, 1H, *J* 2.0 Hz, *J* 5.4 Hz), 3.82, 3.81, 3.79, 3.78 (4 s, 6H), 3.31 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 173.52, 173.49, 160.4, 160.3, 159.2, 159.1, 154.1, 140.57, 140.55, 130.3-129.1, 121.9, 113.8-113.6, 104.7, 104.5(2C), 103.3, 101.8, 101.5, 79.3, 78.7, 77.9, 77.8, 77.6, 76.4, 75.7, 73.5, 73.1, 71.2, 70.5, 63.4, 63.1, 56.7, 56.1, 55.31, 55.26, 55.2, 52.63, 52.55, 50.2, 49.6, 42.3, 39.7, 39.5, 39.1, 37.3, 36.7, 36.1, 36.0, 35.8, 34.3, 32.8, 32.5, 31.9, 31.8, 29.9, 29.5-29.3, 28.2, 28.0, 26.4, 25.5, 25.4, 25.3, 25.0, 24.7, 24.6, 24.3, 23.8, 22.8, 22.5, 21.1, 19.4, 18.7, 11.8; HRESIMS: Calcd for $(C_{67}H_{104}O_{11} + Li)^+$, *m/z* 1091.7771, found, *m/z* 1091.77389. Anal. Calcd for $C_{67}H_{104}O_{11}$ + 1/2 H_2O: C, 73.52; H, 9.67. Found: C, 73.29; H, 10.01.

4.7. Cholesteryl 6-*O*-(16,16-dimethoxy)palmitoyl-2-*O*-*p*-methoxybenzyl-β-ᴅ-galactopyranoside (9)

To a solution of **8** (3.4 g, 3.1 mmol) in CH_2Cl_2 (100 mL) and MeOH (25 mL) was added trifluoroacetic acid (1 mL) at 23 °C. After 2 h, the solution was treated with Et_3N (3 mL). Extractive workup ($CHCl_3$/aq $NaHCO_3$) followed by column purification of the residue using 3:1 → 1:1 hexanes–EtOAc gradient containing 0.05 % of Et_3N afforded **9** (2.75 g, 91%) as a syrup: ^{1}H NMR, partial ($CDCl_3$): δ 7.30-7.26 (m, 3H), 6.90-6.87 (m, 2H), 5.36 (m, 1H), 4.90 (d, 1H, *J* 11 Hz), 4.45 (d, 1H, *J* 7.7 Hz), 4.36 (t, 1H, *J* 5.7 Hz), 4.30 (d, 1H, *J* 6.5 Hz), 3.81 (s, 3H), 3.63 (t, 1H, *J* 7.2 Hz), 3.45 (dd, 1H, *J* 7.7 Hz, *J* 9.3 Hz), 3.31 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 173.7, 159.4, 140.5, 130.4, 129.9, 122.0, 114.0, 104.5, 102.2, 79.8, 78.3, 74.1, 72.9, 71.8, 68.1, 62.5, 56.7, 56.1, 55.2, 52.5, 50.1, 42.3, 39.7, 39.5, 39.1, 36.7, 36.1, 35.8, 34.2, 32.5, 31.9, 31.8, 29.9, 29.66, 29.65, 29.56, 29.52, 29.48, 29.3, 29.2, 28.2, 28.0, 24.9, 24.6, 24.3, 23.8, 22.8, 22.5, 21.0, 19.4, 18.7, 11.8; HRESIMS: Calcd for $(C_{59}H_{98}O_{10} + Li)^+$, *m/z* 973.7328, found, *m/z* 973.7320. Anal. Calcd for $C_{59}H_{98}O_{10} + 1/3 H_2O$: C, 72.80; H, 10.22. Found: C, 72.85; H, 10.20.

4.8. Cholesteryl 6-*O*-(16,16-dimethoxy)palmitoyl-β-ᴅ-galactopyranoside (10)

To a solution of **8** (1.40 g, 1.4 mmol) in moist CH_2Cl_2 (30 mL) was added at 23 °C 4,5-dichloro-5,6-dicyano-1,4-benzoquinone (1.5 g, 6.6 mmol). After 30 min, aq $NaHCO_3$ was added. Extractive work-up ($CHCl_3$/H_2O) followed by concentration of the organic layer afforded a syrup that was purified by column chromatography using 1:2 hexanes–EtOAc → EtOAc gradient containing 0.05 % of Et_3N afforded **10** (1.0 g, 82%) as a syrup: ^{1}H NMR ($CDCl_3$): δ 5.35 (m, 1H), 4.36 (t, 1H, *J* 5.8 Hz), 4.37-4.27 (m, 3H), 3.31 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 173.7, 140.3, 122.0, 104.5, 101.5, 79.5, 73.3, 72.3, 71.4, 68.5, 62.7, 56.7, 56.1, 52.5, 50.1, 42.3, 39.7, 39.5, 38.8, 37.2, 36.7, 36.1, 35.8, 34.2, 32.4, 31.9, 31.8, 29.73, 29.70, 29.68, 29.5, 29.43, 29.36, 29.3, 28.2, 28.0, 24.9, 24.6, 24.3, 23.8, 22.8, 22.5, 21.0, 19.3, 18.7, 11.8; HRESIMS: Calcd for $(C_{51}H_{90}O_9 + Li)^+$, *m/z* 853.6750, found, *m/z* 853.6745. Anal. Calcd for $C_{51}H_{9}O_9 + 1/2 H_2O$: C, 71.54; H, 10.71. Found: C, 71.60; H, 10.66.

4.9. Cholesteryl 6-*O*-(16-oxo)palmitoyl-β-ᴅ-galactopyranoside (2)

To a stirred solution of **9** (1.9 g, 2.2 mmol) in *i*-PrOH were added AcOH (50 mL) and H_2O (15 mL). The solution was kept at 85-90 °C for 3 h. The volatiles were removed by distillation under reduced pressure. Column chromatography of the residue using 100:4 CH_2Cl_2-MeOH afforded **2** (1.25 g, 84%) as an amorphous substance: ^{1}H NMR ($CDCl_3$): δ 9.76 (t, 1H, *J* 1.9 Hz), 5.34 (m, 1H), 4.45-4.17 (m, 4H), 0.99 (s, 3H), 0.91 (d, 3H, *J* 6.4 Hz), 0.87 (d, 3H, *J* 6.7 Hz), 0.86 (d, 3H. *J* 6.6 Hz), 0.67 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 203.0, 173.6, 140.3, 122.0, 101.7, 79.6, 73.4, 72.4, 71.1, 68.7, 62.9, 56.7, 56.2, 50.1, 43.9, 42.3, 39.7, 39.5, 38.8, 37.3, 36.6, 36.2, 35.8, 34.2, 31.9, 31.8, 29.44, 29.41, 29.37, 29.3, 29.2, 28.2, 28.0, 24.9, 24.2, 23.8, 22.8, 22.5, 22.0, 21.0, 19.4, 18.7, 11.0; HRESIMS: Calcd for $(C_{49}H_{84}O_8 + Na)^+$, *m/z* 823.6064, found, *m/z* 823.6077. Anal. Calcd for $C_{49}H_{84}O_8 + H_2O$: C, 71.84; H, 10.58. Found: C, 72.27; H, 10.52.

4.10. Conjugation of 2 with aminooxypropylated bovine serum albumin

To a solution of aminooxypropylated BSA[5] (15 mg, 0.21 μmol, having an average of 24 aminooxypropyl moieties per BSA) in PBS (3 mL, containing 0.1% glycerol, 0.005 M EDTA, and 1% octyl glucoside, at pH 7.4) was added a 0.5 M solution of sodium bis(2-ethylhexyl) sulfosuccinate in octane (18 mL) under vigorous stirring. After the formation of a transparent, clear solution, a solution of the aldehydo-derivatized hapten **2** (15 mg, 19 μmol) in pyridine (200 μL) was added. The solution was kept at 37 °C for 20 h followed by addition of 80% aqueous EtOH (80 mL). The mixture was centrifuged and the supernatant discarded. The precipitate was resuspended in 0.2 M NaCl (4 mL, containing 0.3% octyl glucoside) followed

by the addition of 80% aqueous EtOH (16 mL). The precipitate obtained was isolated by centrifugation as before and was resuspended in 0.2 M NaCl (2 mL, containing 0.3% octyl glucoside). The insoluble material was removed by centrifugation and the supernatant was passed through a Sephadex G-50 column (1 × 50 cm) made in 0.2 M NaCl., using the same solution as the eluant. The void volume fractions were combined and the solution so obtained was concentrated by ultrafiltration. The average molecular mass of the conjugates was 86.4 kDa as determined by MALDI-TOF mass spectrometry. The precipitate insoluble in 0.2 M NaCl containing 0.3% octyl glucoside was solubilized in 0.5% sodium dodecylsulfate (1 mL). The solution so obtained was applied to a Sephadex G-50 column and the conjugate was isolated as described earlier. The molecular weight (MALDI-TOF) of the product eluted in the void volume was identical to that obtained above.

Acknowledgments

Reference List

1. MMWR 2004;53:365–369. [PubMed: 15129194]

2. European Union Concerted Action on Lyme Borreliosis. 2005. http://www.oeghmp.at/eucalb/disease-overview_1.html

3. Ben-Menachem G, Kubler-Kielb J, Coxon B, Yergey A, Schneerson R. Proc Natl Acad Sci USA 2003;100:7913–7918. [PubMed: 12799465]

4. Pozsgay V, Kubler-Kielb J, Coxon B, Ekborg G. Tetrahedron 2005;61:10470–10481.

5. Kubler-Kielb J, Pozsgay V. J Org Chem 2005;70:6987–6990. [PubMed: 16095333]2006;71:5422–5422.

6. Greene, TW.; Wuts, PGM. Protecting groups in organic synthesis. John Wiley & Sons; New York: 1999. p. 217

7. Pozsgay V. J Org Chem 1998;63:5983–5999. [PubMed: 11672203]

8. Pozsgay V, Kubler-Kielb J. ACS Symp Ser 2006;960:238–252.

9. Pozsgay V, Kubler-Kielb J. ACS Symp Ser. 2007In press

10. Fekete A, Hoogerhout P, Zomer G, Kubler-Kielb J, Schneerson R, Robbins JB, Pozsgay V. Carbohydr Res 2006;341:2037–2048. [PubMed: 16458277]

11. Yatsimirskaya EA, Gavrilova EM, Egorov AM, Levashov AV. Steroids 1993;58:547–550. [PubMed: 7505959]

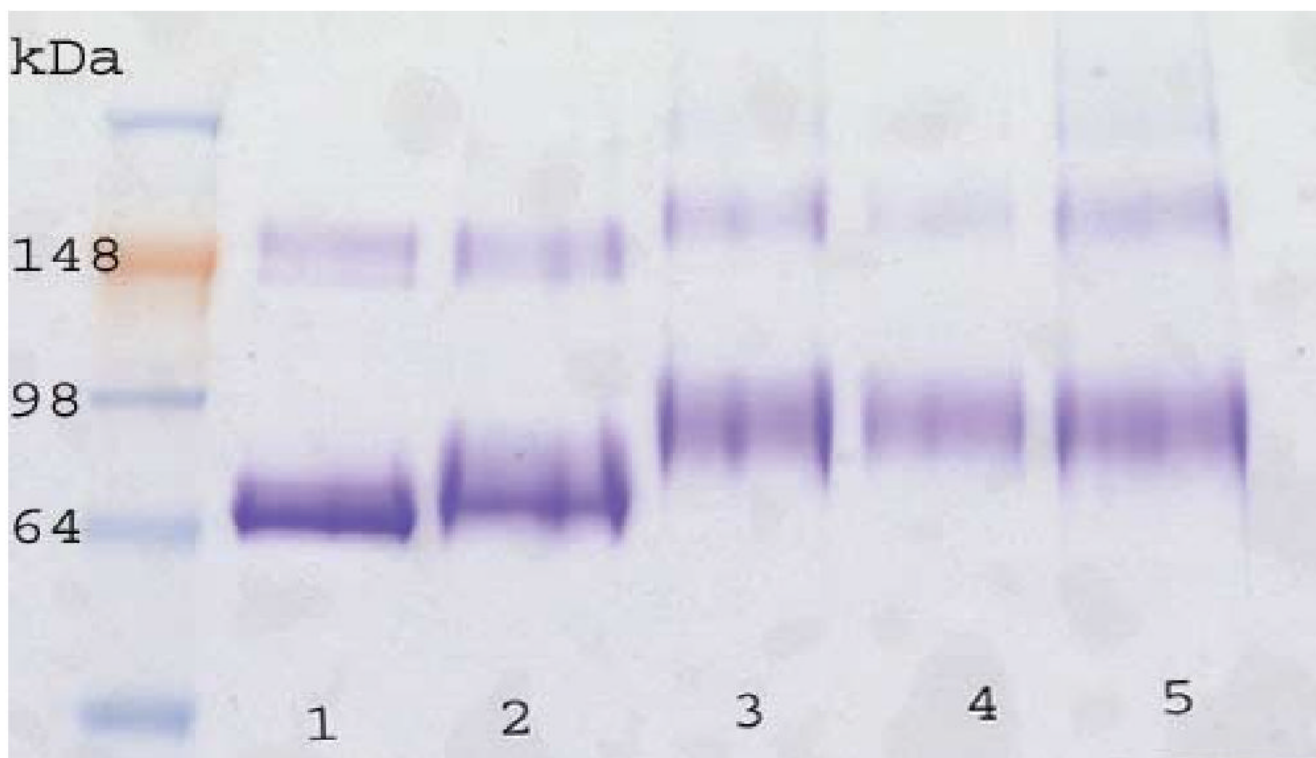12. Pauillac S, Naar J, Mouratou B, Guesdon JL. J Immunological Methods 2002;263:75–83.

Figure 1.
Sodium dodecylsulfate-polyacrylamide gel electrophoresis of the conjugates. Left lane:
molecular weight markers; Lane 1: BSA; Line 2: aminooxypropylated BSA, MW 71.7 kDa
indicating an average of 24 aminooxypropyl moieties per BSA; Lanes 3, 4, 5: BSA conjugates
of glycolipid **2**, average MW: 86.4 kDa indicating an average of 18 glycolipid moieties per
BSA. The bands at about 150 kDa correspond to dimers. MW's were determined by MALDI-
TOF.
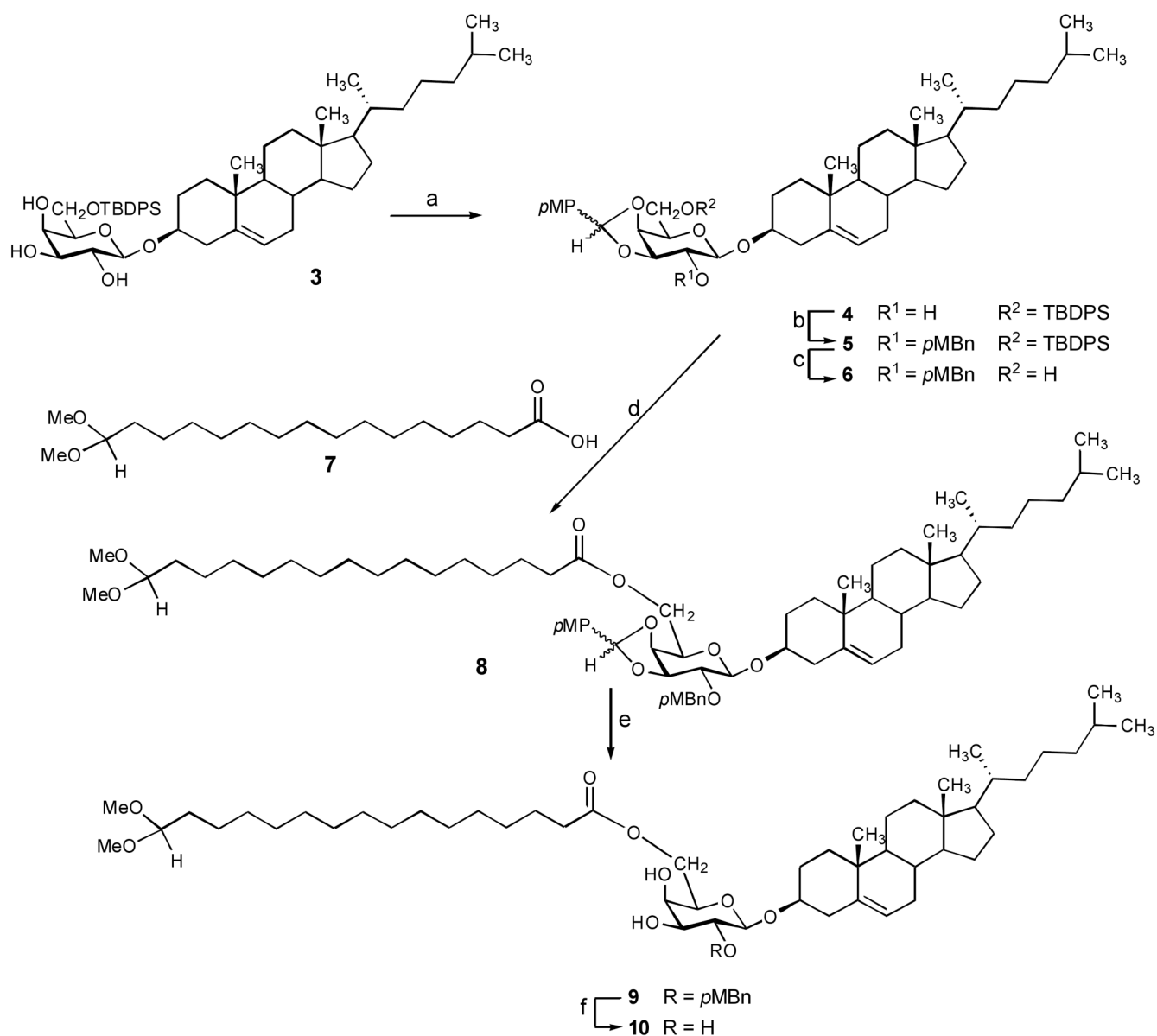
Scheme 1.
Reagents and conditions: (a) *p*-methoxybenzaldehyde dimethylacetal (6.5 eq), CSA (cat), CH$_2$Cl$_2$, 23 °C, 24 h, 82%; (b) *p*-methoxybenzyl bromide (4 eq), NaH, DMF, 0 → 23 °C, 40 min, 91%; (c) Bu$_4$F, 23 °C, 2 h, THF, 96%; (d) **7** (1.7 eq), DCC (2.6 eq), DMAP (2.1 eq), 23 °C, 30 min, EtOAc, 93%; (e) TFA, 23 °C, 2 h, CH$_2$Cl$_2$, MeOH, 91%; (f) DCC (4.7 eq), 23 °C, 30 min, CH$_2$Cl$_2$, H$_2$O, 82%.