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# Synthesis of two glycolipid antigens of the causative agent of Lyme disease

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**Abstract**—As a prelude to development of a human vaccine against Lyme disease, the first chemical synthesis of glycolipid antigens of *Borrelia burkholderi* is reported. First, cholesteryl β-D-galactopyranoside was synthesized and was converted to partially protected congeners having the HO-6 group of the galactose moiety unprotected. Treatment of these intermediates with palmitic and oleic acid, respectively, under dehydrative conditions followed by removal of the protecting groups afforded cholesteryl 6-*O*-palmitoyl/oleoyl-β-D-galactopyranosides that were identical to the glycolipids isolated from *B. burkholderi*.
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## 1. Introduction

Lyme disease, a vector-borne infection caused by the tick-transmitted spirochaete *Borrelia burgdorferi*, frequently occurs in the United States, Europe, and North Asia.[1,2] This disease was identified in 1977 when an unusually large number of children with arthritis-like symptoms in and around the township of Lyme, Connecticut was identified.[3] Since then, the Centers for Disease Control and Prevention has reported a steadily increasing number of cases, reaching more than 23,000 in 2002.[4] Most cases occur in the age-groups 5–14 and 50–59 years[4] in northeastern, mid-Atlantic, and north central states. Borrelia organisms enter the circulatory system of the host via the bite of an infected Ixodes tick and infect various organs, including the brain and joints.[5] Some patients develop chronic Lyme disease that can be disabling[6] when the infection persists for years.

*B. burgdorferi* belongs to the bacterial order *Spirochaetales*, which is divided into two families, each divided into genera. The first family, *Spirochaetaceae* includes four genera: *Spirochaeta*, *Cristaspira*, *Treponema*, and *Borrelia*, whereas the second family, *Leptospiraceae* encompasses two genera: *Leptospira* and *Leptonema*.[7] The pathogen's helically shaped, motile cells are surrounded by an outer cell membrane, which together with flagella are assumed to play a role in the host–parasite interaction.[8] The bacterium's surface-exposed proteins are immunogenic[9] and a recombinant lipidated outer surface protein, referred to as OspA, was the major component of a licensed vaccine[10] LYMErix (SmithKline Beecham) that was withdrawn from medical use in 2002. Currently, there is no licensed vaccine to prevent Lyme disease.

*Borrelia* are considered Gram-negative bacteria but there is no evidence for the presence of a characteristic lipopoly- or lipooligosaccharide on their surface. Work in our laboratory[11] has identified two major glycolipids (**1**, **2**) in *B. burgdorferi* that contain cholesteryl β-galactoside in which O-6 of the galactose residue is substituted either by a palmitoyl or an oleoyl group, respectively. Cholesteryl glycosides occur in many plants[12] and fungi[13] but they are rare in animals and bacteria. The bacteria *Acholeplasma* spp.,[14] *Mycoplasma gallinarum*,[15] *Spiroplasma citri*,[16] *Borrelia hermsi*,[17] and *Helicobacter pylori*[18] have been reported to express cholesteryl glucoside but not galactoside. The presence of galactose in cholesteryl glycosides is, therefore, a unique feature of *B. burgdorferi*. The native glycolipids **1** and **2** differing in the fatty acid residue at O-6 of the galactose moiety could not be separated.[11] Therefore, the structural elucidation was performed on their mixture estimated to contain **1** and **2** in an approx. 1:1 ratio. Because of the difficulties in growing *B. burgdorferi*, glycolipids **1** and **2** could be obtained only in small quantities. This fact combined with the difficulty to separate them, prompted us to synthesize **1** and **2** in quantities and purity suitable for immunization experiments. Availability of well-defined compounds was expected to remove uncertainties that

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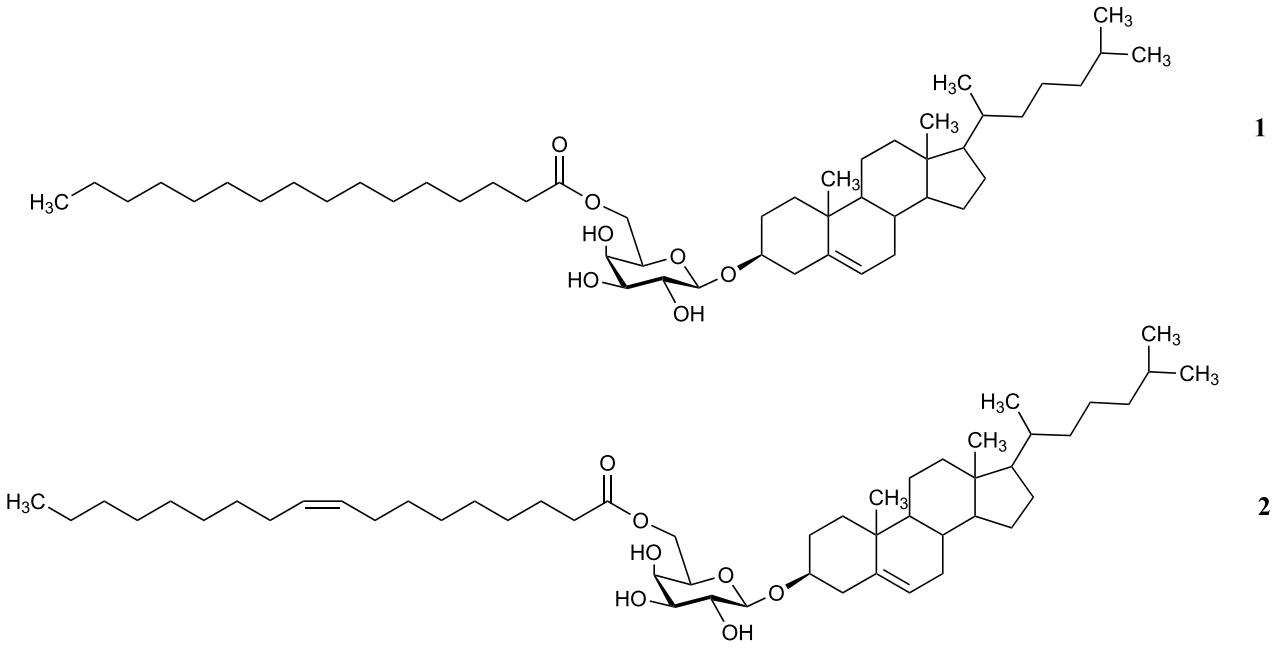
arose from the fact that the structural studies were carried out on a mixture. Here, we report chemical synthetic approaches to glycolipids **1** and **2**, and provide confirmatory evidence for the native structures. We also describe a simple method to eliminate the virtual couplings in the chloroform-*d* $^1$H NMR spectra of **1** and **2** and their synthetic intermediates.

## 2. Results and discussion

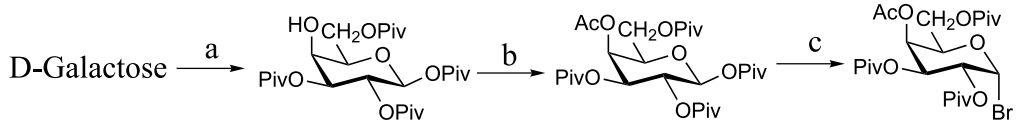
Two approaches were designed to the synthetic targets **1** and **2**. The first involved the preparation of a 6-*O*-palmitoyl/oleoyl-galactopyranosyl donor that was coupled to HO-3 of cholesterol. Because of the limitations imposed upon the synthetic scheme by the cholesterol's own double bond, as



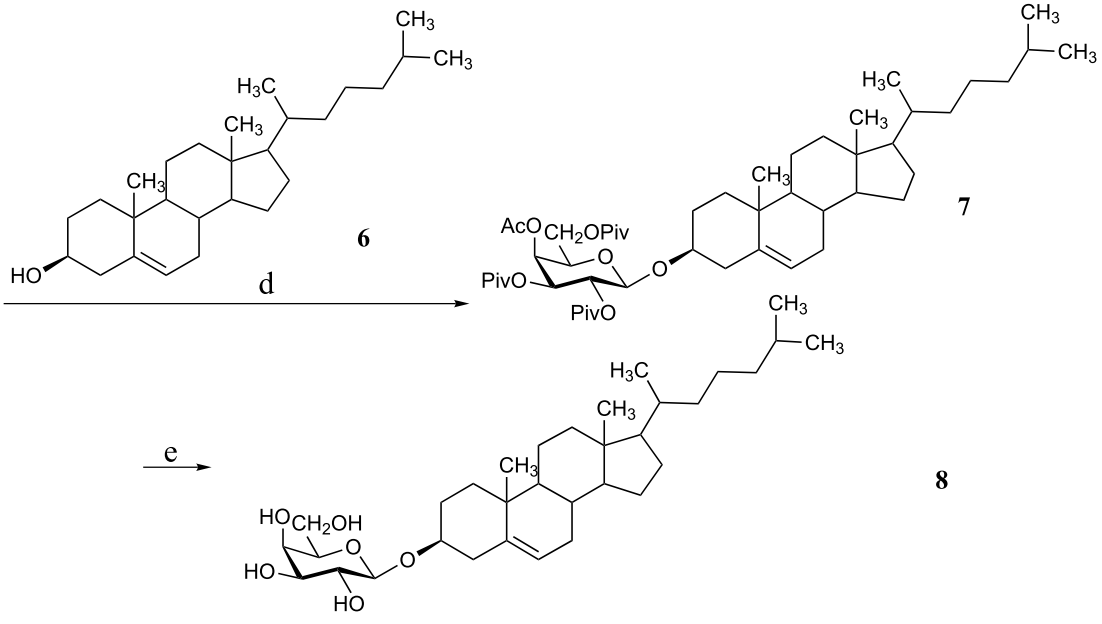
**1**



**2**



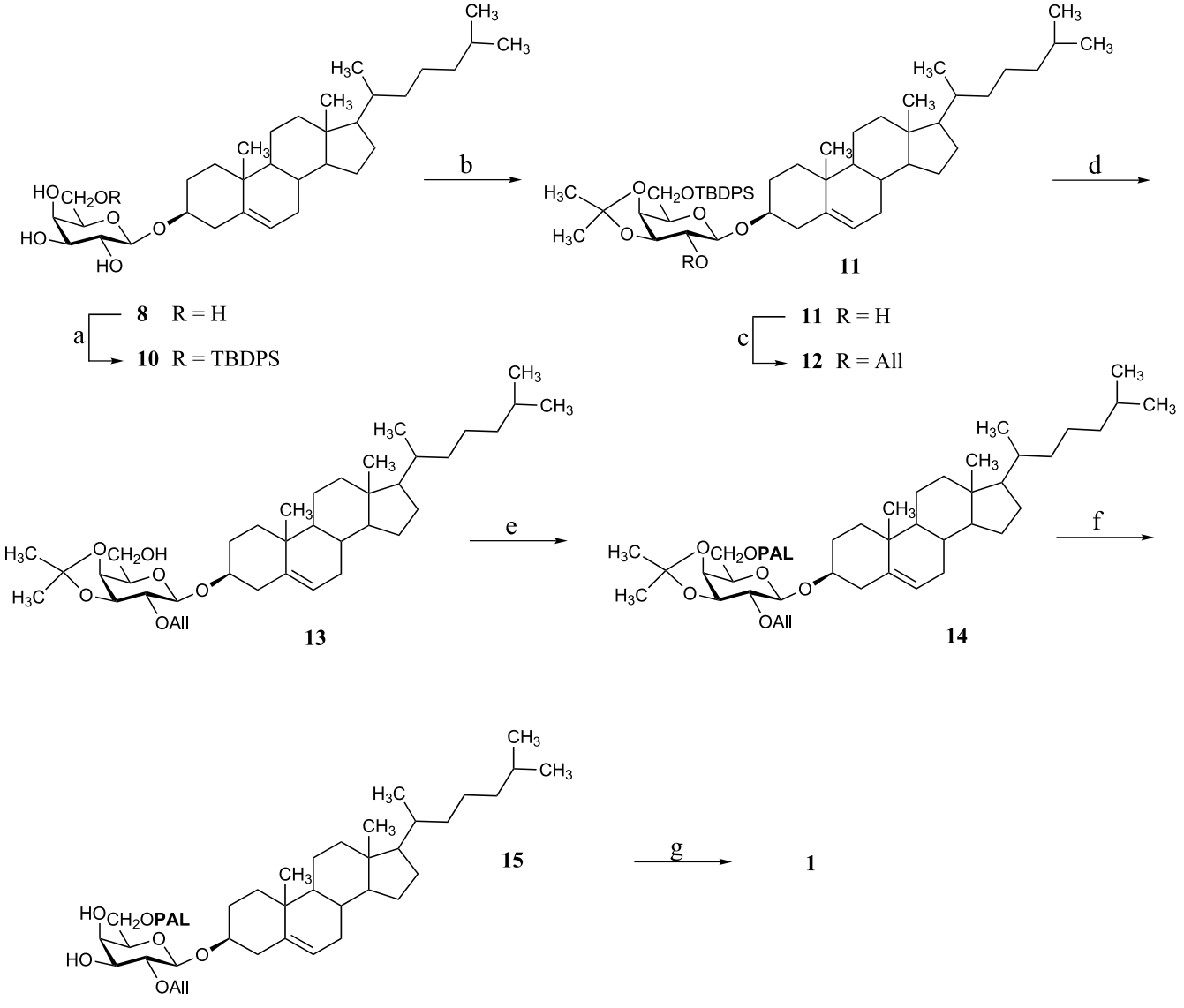
**3** **4** **5**



**6** **7** **8**

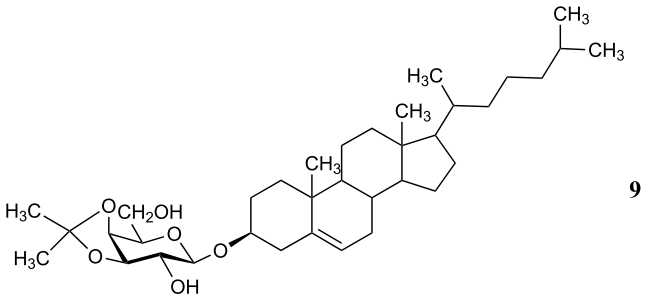
**Scheme 1.** Reagents and conditions: (a) 1.32 equiv of $(CH_3)_3COCl$, $C_5H_5N$, $CHCl_3$, 23 °C, 24 h, 30%; (b) $Ac_2O$, $C_5H_5N$, 23 °C, 4 h, quant.; (c) HBr/AcOH, $CH_2Cl_2$, 0→23 °C, (d) 1.44 equiv of **5**, 1.88 equiv of 2,6-di-*tert*-butyl-4-methylpyridine, 1.5 equiv of AgOTf, −40→0 °C, 1 h, 85%; (e) $CH_3ONa$ (excess), $CH_3OH$, $CH_2Cl_2$, 23 °C, 24 h, 94%.

well as the need for a 'participating' protecting group at O-2 to lead to the β anomeric stereochemistry, this scheme required a per-*O*-acylated donor where the *O*-acyl groups at HO-2, 3, and 4 were to be chemoselectively cleavable, leaving the palmitoyl/oleoyl group at O-6 intact during deprotection. For this purpose the levulinoyl and the chloroacetyl groups were tested because they can be selectively cleaved in the presence of alkanoyl and aroyl groups. Unfortunately, this approach proved to be abortive because attempted, selective cleavage of the protecting groups at O-2, 3, and 4 caused extensive acyl migrations leading to inseparable product mixtures. In the alternative approach, cholesteryl galactopyranoside **8** was prepared first as shown in Scheme 1, in which the 6-*O*-acyl moieties were installed at a later stage. Thus, galactose was first treated with pivaloyl chloride in pyridine containing 4-dimethylaminopyridine, to afford a mixture of fully and partially pivaloylated galactose derivatives. This protecting group was chosen because it suppresses the formation of unwanted orthoesters.[19] Complete pivaloylation could not be achieved even after extended reaction times at an elevated temperature. Previously, Kunz et al.[20] reported that pivaloylation of galactose with pivaloyl chloride in pyridine for 6 days afforded penta-pivaloyl galactopyranose in 63% yield.[20] Serendipitously, we found that 1,2,3,6-tetra-*O*-pivaloyl-β-D-galactopyranose **3** could be isolated from the pivaloylation reaction mixture in pure form after only 1 day by crystallization, in 30% yield. (Scheme 1) Because of the inexpensive nature of the reagents, no attempt was made for optimization. Compound **3** was converted to the fully acylated derivative **4** by treatment with $Ac_2O$ in pyridine, in a quantitative yield. Next, **4** was transformed into the



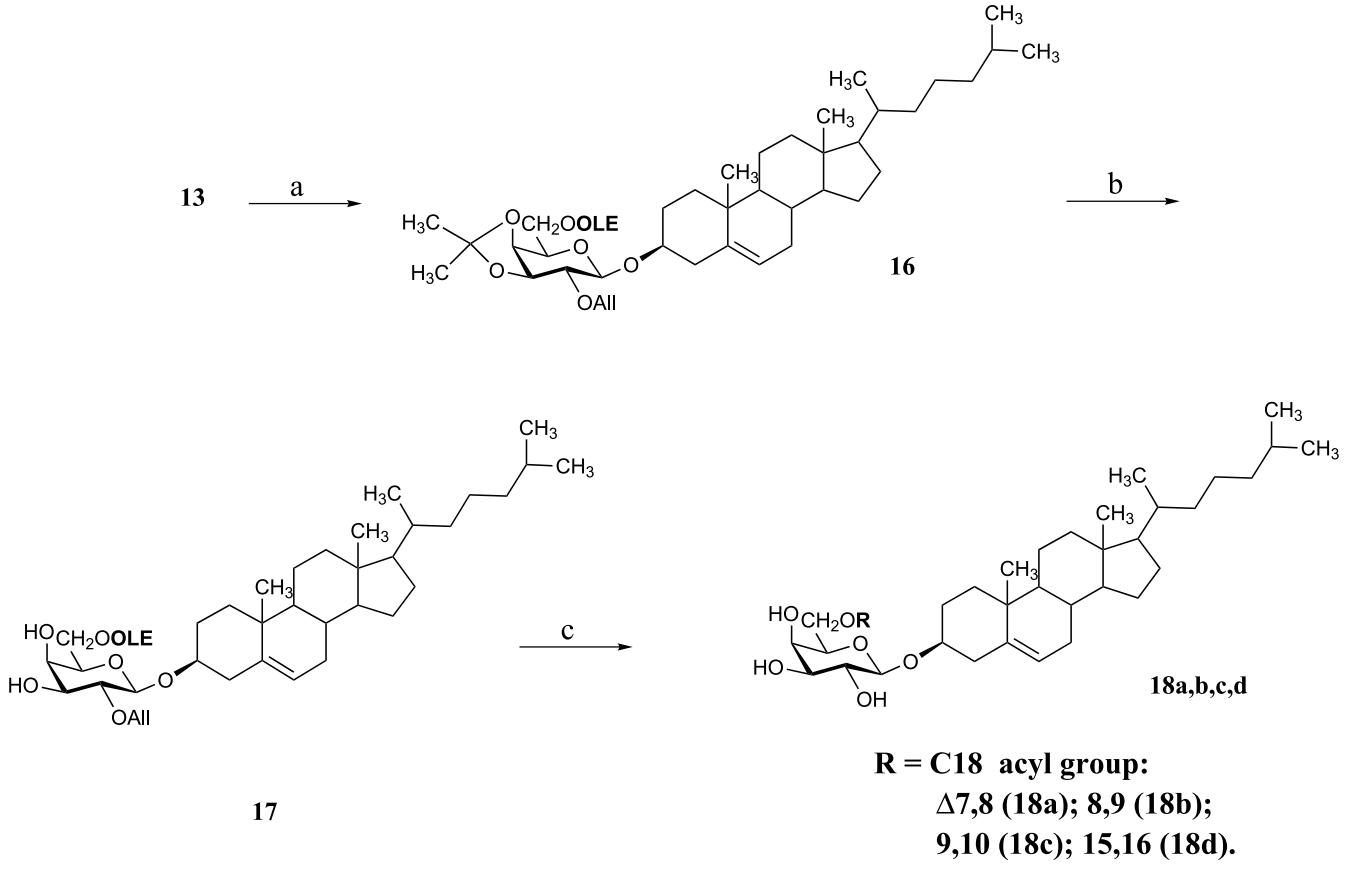

**Scheme 2.** Reagents and conditions: (a) 1.3 equiv of *tert*-butyl-diphenylsilyl chloride, $C_5H_5N$, 23 °C, 5 h, 96%; (b) $(CH_3O)_2C(CH_3)_2$ (excess), CSA (cat), 23 °C, 20 min, 91%; (c) 2.4 equiv of NaH, DMF, 1.4 equiv of All-Br, 0→23 °C, 92%; (d) $Bu_4NF$ (excess), THF, 23 °C, 2.5 h, 97%; (e) 3 equiv of palmitic acid, 6.4 equiv of DCC, MDAP, 23 °C, 6 h, 99%; (f) AcOH–MeOH–$H_2O$, 70 °C, 6 h, 70%; (g) $C_{34}H_{38}F_6IrP_3$, THF, 23 °C, 24 h then AcOH/TFA, 23 °C, 24 h, 88%.

galactosyl donor **5** by treatment with hydrogen bromide. Stereoselective glycosylation of cholesterol (**6**) with **5** under promotion by silver triflate afforded the glycoside **7** in 85% yield, without the formation of an orthoester.

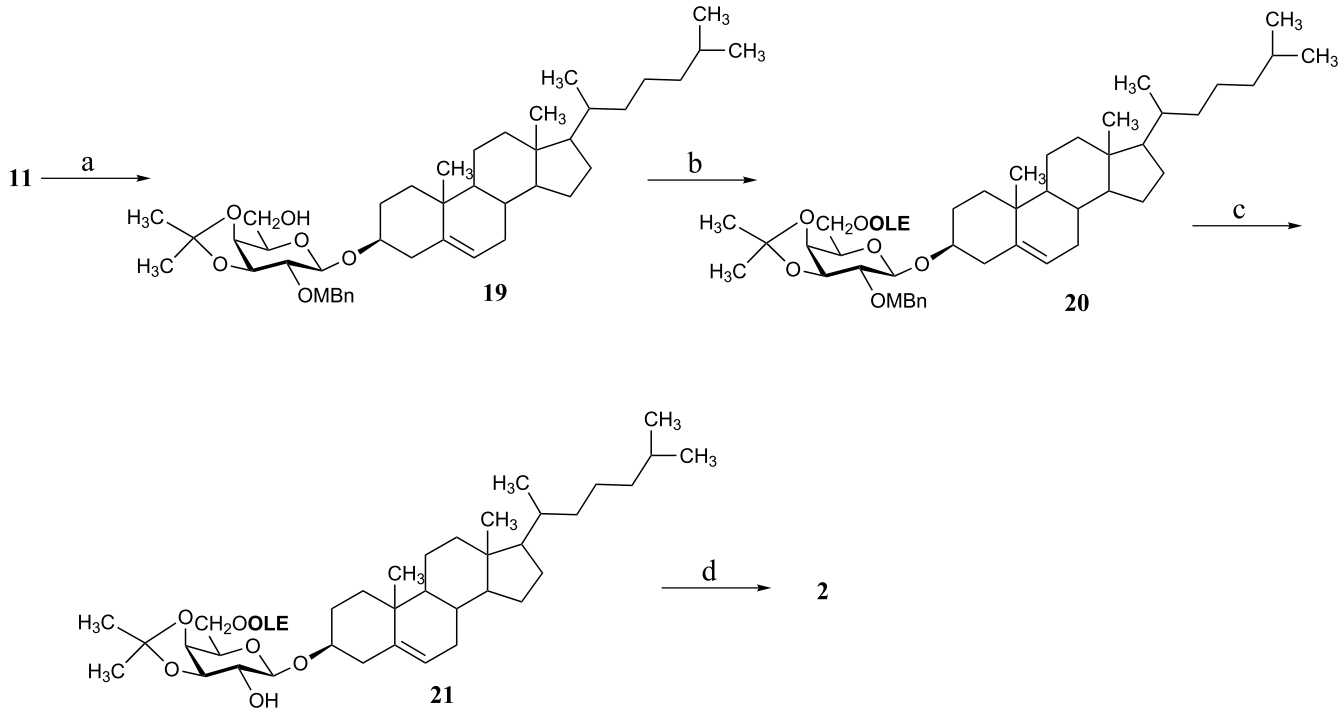


**9**

Treatment of the glycoside **7** with sodium methoxide afforded the tetraol **8** in 94% yield. The isolation of **8** in a pure form was facilitated by the fact that **8**, a crystalline material, is insoluble in most organic solvents, whereas all of the impurities are soluble. Next, we envisioned protection schemes for the introduction of the acyl moieties at O-6 of the galactose unit. Initially, we prepared the isopropylidene derivative **9** by treating the tetraol **8** with 2,2-dimethoxypropane in the presence of CSA (→**9**, 34%) in the hope that subsequent *O*-acylations would take place preferentially or exclusively at O-6. Unexpectedly, condensation of **9** with palmitic acid in the presence of DCC afforded at least two products that could not be separated and that are conceivably the 2-*O* and the 6-*O*-acyl derivatives. This led us to synthesize the highly protected intermediate **13** in a series of protective-deprotective steps, as shown in Scheme 2. Thus, compound **8** was first converted to the 6-*O*-silyl derivative **10** using *tert*-butyl-diphenylsilyl chloride in pyridine in 96% yield, followed by acid catalyzed transacetalization with 2,2-dimethoxypropane to afford the alcohol **11** (91%). Treatment of **11** with allyl bromide and NaH afforded the fully protected intermediate **12** (92%) as expected. Next, reaction of compound **12** with tetrabutylammonium fluoride provided the alcohol **13** (97%) from which acylation with palmitic acid/DCC provided the fully protected derivative **14** (99%). Compound **14** was deprotected in a three-step sequence involving acid-catalyzed hydrolysis of the isopropylidene acetal in AcOH–MeOH–$H_2O$ (→**15**, 70%), and allyl isomerization[21,22] with (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate in THF, followed by TFA hydrolysis of the 2-*O*-(1-propylene) derivative (not isolated) affording[23] **1** in 88% yield.




**Scheme 3.** Reagents and conditions: (a) 2 equiv of oleic acid, 5.9 equiv of DCC, DMAP, EtOAc, 23 °C, 2 h, 75%; (b) AcOH–MeOH–$CH_2Cl_2$, reflux, 6 h, 70%; (c) $C_{34}H_{38}F_6IrP_3$, THF, 23 °C, 24 h then AcOH/TFA, 23 °C, 24 h.




**Scheme 4.** Reagents and conditions: (a) 6.6 equiv of NaH, DMF, 0 °C, 10 min, then 6 equiv of MBn–Br, 0 °C, 6 h, then $Bu_4NF$ (excess), THF, 23 °C, 6 h, 78%; (b) 2.2 equiv of oleic acid, 6.8 equiv of DCC, DMAP, EtOAc, 23 °C, 6 h, 81%; (c) 1.3 equiv of CAN, $CH_2Cl_2$, $H_2O$, 23 °C, 6 h, 93%; (d) AcOH–$H_2O$, 65 °C, 4 h, 92%.

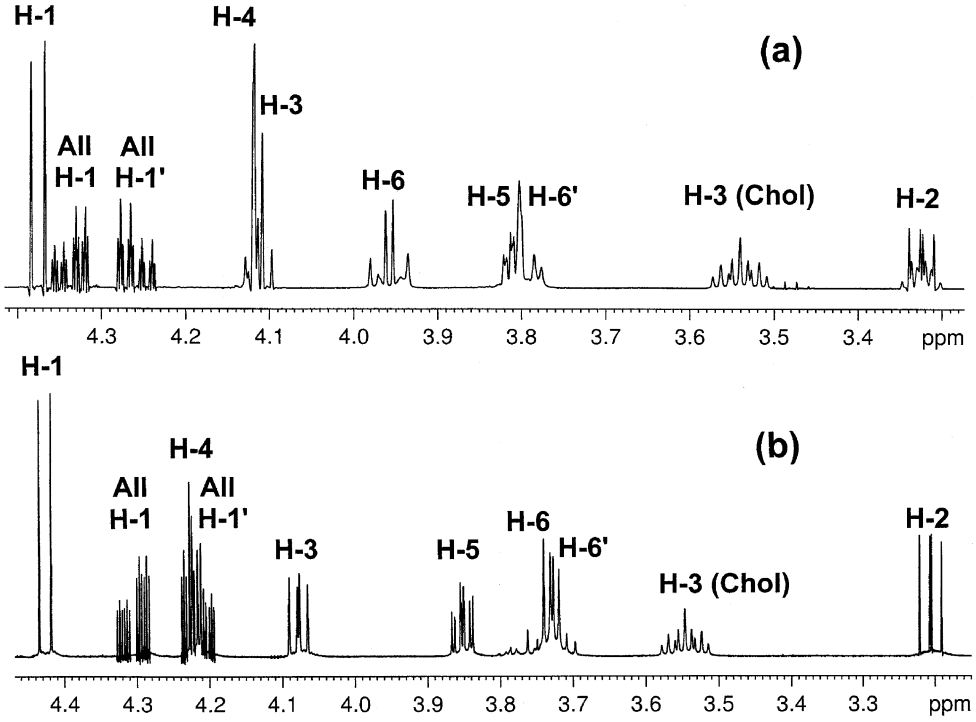



**Figure 1.** Partial $^1H$ NMR spectra of cholesteryl 2-*O*-allyl-3,4-di-*O*-isopropylidene-β-D-galactopyranoside **13** at 500 MHz; (a) the spectrum of a solution in $CDCl_3$ shows 'virtual coupling' in the H-2 multiplet because of strong coupling of H-3 and H-4, and in the H-6 multiplet because of strong coupling of H-5 and H-6′; (b) no virtual coupling is observed in the spectrum of a solution of **13** in acetone-$d_6$.

**Table 1**. Selected $^1$H chemical shifts[a] of synthetic glycolipid antigens and their precursors

| Compound | Galactose | | | | | | | Allyl | | | | | Cholesterol | | Others |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H-1 | H-2 | H-3 | H-4 | H-5 | H-6 | H-6′ | H-1 | H-1′ | H-2 | H-3 | H-3′ | H-3 | H-6 | |
| **1**[b] (at 300 K) | 4.335 | 3.474 | 3.535 | 3.827 | 3.739 | 4.388 | 4.153 | — | — | — | — | — | 3.500 | 5.357 | HO-2 4.051, HO-3 4.022, HO-4 3.674 |
| **2**[b] | 4.336 | 3.476 | 3.536 | 3.828 | 3.738 | 4.382 | 4.157 | — | — | — | — | — | 3.502 | 5.365 | Oleoyl H-9,10 5.371, 5.359 HO-2 4.059, HO-3 4.032, HO-4 3.678 |
| **3** | 5.666 | 5.466 | 5.014 | 4.033 | 3.899 | 4.336 | 4.292 | — | — | — | — | — | — | — | Piv Me 1.205, 1.194, 1.182, 1.128 |
| **4**[c] | 5.673 | 5.585 | 5.059 | 5.359 | 3.451 | 4.077 | 3.979 | — | — | — | — | — | — | — | Piv Me 1.143, 1.126, 1.110, 1.110 Ac Me 1.658 |
| **7** | 4.604 | 5.240 | 5.060 | 5.421 | 3.932 | 4.176 | 4.074 | — | — | — | — | — | 3.478 | 5.326 | Piv Me 1.180, 1.176, 1.112 Ac Me 2.130 |
| **8**[d] | 4.744 | 4.036 | 3.948 | 4.287 | 3.898 | 4.145 | 4.107 | — | — | — | — | — | 3.810 | 5.401 | |
| **9**[b,e] | 4.337 | 3.378 | 4.030 | 4.214 | 3.863 | 3.758 | 3.733 | — | — | — | — | — | 3.553 | 5.345 | Ip Me 1.416, 1.275 |
| **10** | 4.312 | 3.610 | ~3.577 | 4.049 | 3.519 | 3.916 | 3.876 | — | — | — | — | — | 3.556 | 5.344 | *t*-Bu Me 1.050 |
| **11** | 4.256 | 3.529 | 4.065 | 4.237 | 3.851 | 3.956 | 3.916 | — | — | — | — | — | 3.553 | 5.352 | Ip Me 1.507, 1.336, *t*-Bu Me 1.048 |
| **12** | 4.318 | 3.307 | 4.068 | 4.204 | 3.783 | 3.928 | 3.899 | 4.337 | 4.249 | 5.940 | 5.303 | 5.160 | 3.512 | 5.343 | Ip Me 1.491, 1.324, *t*-Bu Me 1.043 |
| **13**[b] | 4.435 | 3.207 | 4.078 | 4.220 | 3.853 | 3.745 | 3.714 | 4.306 | 4.216 | 5.909 | 5.307 | 5.100 | 3.546 | 5.361 | Ip Me 1.441, 1.278 |
| **14**[b] | 4.440 | 3.228 | 4.124 | 4.221 | 4.078 | 4.360 | 4.201 | 4.304 | 4.213 | 5.905 | 5.307 | 5.106 | 3.508 | 5.373 | Ip Me 1.458, 1.296 |
| **15** | 4.395 | 3.393 | 3.575 | 3.895 | 3.635 | 4.328 | 4.295 | 4.454 | 4.176 | 5.935 | 5.295 | 5.201 | 3.524 | 5.355 | |
| **16**[b] | 4.439 | 3.228 | 4.123 | 4.221 | 4.076 | 4.358 | 4.203 | 4.304 | 4.214 | 5.906 | 5.307 | 5.107 | 3.510 | 5.366 | Oleoyl H-9,10 5.366 Ip Me 1.459, 1.297 |
| **19**[b] | 4.479 | 3.268 | 4.117 | 4.210 | 3.856 | 3.740 | 3.709 | — | — | — | — | — | 3.578 | 5.364 | Ip Me 1.332, 1.268, MBn C$H_2$ 4.772, 4.694, H-2,6 7.322, H-3,5 6.894, OMe 3.784 |
| **20**[b] | 4.490 | 3.283 | 4.158 | 4.197 | 4.073 | 4.351 | 4.192 | — | — | — | — | — | 3.539 | 5.367 | Ip Me 1.344, 1.283, MBn C$H_2$ 4.769, 4.692, H-2,6 7.319, H-3,5 6.896, OMe 3.785, Oleoyl H-9, 10 5.367 |
| **21**[b] | 4.351 | 3.386 | 4.075 | 4.216 | 4.094 | 4.367 | 4.210 | — | — | — | — | — | 3.511 | 5.366 | Ip Me 1.433, 1.291, Oleoyl H-9, 10 5.366 |

[a] In ppm from internal $Me_4Si$, in $CDCl_3$ solution unless stated otherwise.
[b] In fresh acetone-$d_6$.
[c] In 4:1 v/v benzene-$d_6$/$CDCl_3$.
[d] In 1:1 v/v methylsulfoxide-$d_6$/pyridine-$d_5$.
[e] At 318 K.

**Table 2**. Selected $^{13}C$ chemical shifts[a] of synthetic glycolipid antigens and their precursors

| Compound | Galactose | | | | | | Allyl | | | Cholesterol | | | Others |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C-1 | C-2 | C-3 | C-4 | C-5 | C-6 | C-1 | C-2 | C-3 | C-3 | C-5 | C-6 | |
| **1**[b] | 102.89 | 72.16 | 74.42 | 69.74 | 73.40 | 64.38 | — | — | — | 79.39 | 141.82 | 122.16 | C═O 173.49 |
| **2**[b] | 102.85 | 72.10 | 74.36 | 69.69 | 73.33 | 64.37 | — | — | — | 79.33 | 141.75 | 122.17 | C═O 173.48, Oleoyl C-9,10 130.65, 130.53 |
| **3** | 92.26 | 67.54 | 72.94 | 66.89 | 72.99 | 61.71 | — | — | — | — | — | — | Piv Me 27.11, 27.11, 27.08, 26.89 |
| **4**[c] | 92.35 | 68.20 | 71.02 | 66.73 | 71.62 | 60.69 | — | — | — | — | — | — | Piv Me 27.20, 27.09, 27.07, 26.92, Ac Me 20.00<br>Piv C═O 177.20, 176.77, 176.36, 176.27<br>Ac C═O 169.68 |
| **7** | 100.19 | 68.88 | 70.97 | 67.08 | 70.69 | 61.47 | — | — | — | 80.13 | 140.29 | 122.10 | Piv Me 27.20, 27.06, 26.96, Ac Me 20.68<br>Piv C═O 177.95, 177.27, 176.51, Ac C═O 169.97 |
| **8**[d] | 102.29 | 71.76 | 74.43 | 69.25 | 75.92 | 61.50 | — | — | — | 77.92 | 140.76 | 121.69 | |
| **9**[b,e] | 102.07 | 74.27b | 80.65 | 74.71 | 74.77 | 62.38b | — | — | — | 78.89 | 141.77 | 122.24 | Ip Me 28.53, 26.67, Ip OCO 109.87 |
| **10** | 101.25 | 72.22 | 73.68 | 68.83 | 74.44 | 62.97 | — | — | — | 78.82 | 140.35 | 122.06 | TBDPS Ar 135.63, 135.55, 133.10, 132.94, 129.86, 129.85, 127.80, 127.79, *t*-Bu Me 26.77, *t*-Bu C-1 19.18 |
| **11** | 100.63 | 73.75 | 78.62 | 73.24 | 73.67 | 62.71 | — | — | — | 78.98 | 140.36 | 122.06 | TBDPS Ar 135.63, 135.60, 133.42, 133.35, 129.69, 127.70, 127.63, Ip OCO 110.04, Ip Me 28.21, 26.31, *t*-Bu Me, 26.72, *t*-Bu C-1 19.20 |
| **12** | 101.64 | 79.84 | 79.11 | 73.38 | 73.22 | 62.79 | 72.88 | 135.14 | 117.03 | 79.47 | 140.68 | 121.79 | TBDPS Ar 135.64, 135.60, 133.46, 133.38, 129.67, 127.69, 127.62, Ip OCO 109.75, Ip Me 28.03, 26.33, *t*-Bu Me 26.72, *t*-Bu C-1 19.20 |
| **13**[b] | 101.99 | 81.11 | 80.09 | 74.74 | 74.44 | 62.17 | 72.93 | 136.85 | 115.93 | 79.14 | 141.56 | 122.35 | Ip OCO 109.92, Ip Me 28.42, 26.64 |
| **14**[b] | 102.08 | 80.84 | 80.12 | 74.69 | 71.50 | 64.00 | 72.92 | 136.74 | 116.06 | 79.79 | 141.57 | 122.35 | C═O 173.39, Ip OCO 110.32, Ip Me 28.32, 26.60 |
| **15** | 102.29 | 78.67 | 72.95 | 68.19 | 71.89 | 62.46 | 73.52 | 134.70 | 117.66 | 79.95 | 140.55 | 121.95 | C═O 173.77 |
| **16**[b] | 102.06 | 80.83 | 80.10 | 74.68 | 71.48 | 63.99 | 72.91 | 136.73 | 116.06 | 79.78 | 141.56 | 122.35 | C═O 173.38, Oleoyl C-9,10 130.65, 130.50 Ip OCO, 110.31, Ip Me 28.31, 26.60 |
| **19**[b] | 101.99 | 80.78 | 80.13 | 74.72 | 74.43 | 62.18 | — | — | — | 79.09 | 141.57 | 122.37 | Ip Me 28.35, 26.66, Ip OCO 109.92<br>MBn $CH_2$ 73.64, C-1 132.04, C-2,6 130.21, C-3,5 113.23, C-4 160.07, OMe 55.48 |
| **20**[b] | 102.11 | 80.48 | 80.13 | 74.66 | 71.48 | 64.00 | — | — | — | 79.77 | 141.59 | 122.38 | Ip Me 28.24, 26.63, Ip OCO 110.31, MBn $CH_2$ 73.63, C-1 131.92, C-2,6 130.26, C-3,5 114.25, C-4 160.11, OMe 55.49<br>Oleoyl C-9,10 130.66, 130.51, C═O 173.39 |
| **21**[b] | 102.02 | 73.97 | 80.60 | 74.54 | 71.66 | 64.07 | — | — | — | 79.40 | 141.66 | 122.25 | Ip Me 28.44, 26.64, Ip OCO 110.19, Oleoyl C-9,10, C═O 173.41 |

[a] In ppm from internal $Me_4Si$, in $CDCl_3$ solution unless stated otherwise.
[b] In acetone-$d_6$.
[c] In 4:1 v/v benzene-$d_6$/$CDCl_3$.
[d] In 1:1 v/v methylsulfoxide-$d_6$/pyridine-$d_5$.
[e] At 318 K.

An initial approach to the synthesis of the oleoyl glycolipid **2** was based on the method just described for the preparation of **1**. (Scheme 3.) Thus, the alcohol **13** was acylated with oleic acid/DCC to afford compound **16** in a near quantitative yield, from which acid-catalyzed removal of the isopropylidene group provided the diol **17** in an uneventful transformation. Removal of the allyl group by isomerization with the iridium reagent, followed by hydrolysis as described for **1** gave a chromatographically homogeneous material (**18**) that showed the expected C-5 and C-6 $^{13}C$ NMR resonances for the double bond of the cholesterol unit (140 and 122 ppm, respectively) but lacked the characteristic olefinic carbon resonances of the oleoyl residue (18:1) at ~130 ppm. Instead, a number of low intensity signals appeared at ~130 ppm, hinting at extensive migration of the double bond. In order to gain insight into the extent of isomerization, compound **18** was subjected to transesterification with methanol followed by reaction with 2-amino-2-methylpropanol as described.[24] GC–MS of the resulting oxazoline derivatives revealed unsaturations mainly at C-7, C-8, and C-15. Thus, while the iridium reagent preserved the location of the cholesterol double bond, it caused extensive double bond migration in the oleoyl residue, necessitating an alternative approach depicted in Scheme 4. Alcohol **11** was first treated with 4-methoxybenzyl bromide/NaH followed by removal of the silyl protecting group with tetrabutylammonium fluoride in THF to afford compound **19** in 78% combined yield. Next, treatment of the alcohol **19** with oleic acid/DCC led to the desired oleoyl ester **20**, from which removal of the 4-methoxybenzyl (→**21**) and isopropylidene groups using standard procedures successfully afforded the oleoyl glycolipid **2** in 86% combined yield.

For solutions of members of this series of glycolipids in $CDCl_3$, virtual coupling[25] in their $^1H$ NMR spectra was extremely common. This was often due to strong coupling (large $J/\delta$) of H-3 and H-4 of the Gal residue, which led to second order effects in the H-2 and H-5 multiplets in the form of additional lines in the multiplets that complicated the first order analysis of the spectra. (Fig. 1) Rather than resorting to iterative spectral simulations, the virtual coupling problem was overcome by the use of solvent shifts in acetone-$d_6$ to increase the dispersion of the spectra. Selected $^1H$ and $^{13}C$ chemical shifts of the synthetic glycolipids **1** and **2** and their precursors are shown in Tables 1 and 2, respectively, and selected $^1H$ coupling constants in Table 3.

The measured values of the $^1H$–$^1H$ coupling constant $^3J_{1,2}$ = 7.5–8.4 Hz and $^1J_{C\text{-}1,H\text{-}1}$ = 155.2–166.3 Hz indicated that except for **5**, all of the galactopyranose precursors, glycolipid intermediates, and target glycolipids had the β anomeric configuration, as required for duplicative synthesis of the natural glycolipids. For the pivaloyl esters **3** and **4**, the observed values $^1J_{C\text{-}1,H\text{-}1}$ = 165.7–166.3 Hz (Table 3) are 5–10 Hz greater than the range for many β-D-galactopyranosides, due to the electronegativity of the acyloxy group at C-1.[26]

As usual, TOCSY experiments did not work well for compounds containing undistorted galactopyranose residues, because of the small $J_{4,5}$ values, which inhibited transfer of magnetization from H-4 through to H-5, H-6, and H-6′. However, in compounds **9**, **11**–**14**, **16**, and **19**–**21** the attachment of the 3,4-*O*-isopropylidene ring flattened the chair form of the galactopyranose ring somewhat, causing $J_{4,5}$ to be larger (~2 Hz, Table 3), with a resulting improvement in TOCSY transfer. Nevertheless, in the end, most of the $^1H$ assignments could be made by simple COSY experiments, and the $^{13}C$ assignments by HSQC based on the $^1H$ assignments. The positions of the various substituents on the Gal ring were indicated by HMBC connectivities.

**Table 3**. Selected $^1H$ coupling constants (Hz)[a] of synthetic glycolipid antigens and their precursors

| Compound | Galactose | | | | | | | | Allyl | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $J_{1,2}$ | $J_{2,3}$ | $J_{3,4}$ | $J_{4,5}$ | $J_{5,6}$ | $J_{5,6'}$ | $J_{6,6'}$ | $J_{C\text{-}1,H\text{-}1}$ | $J_{1,1'}$ | $J_{1,2}$ | $J_{1',2}$ | $J_{1,3}$ | $J_{1,3'}$ | $J_{1',3}$ | $J_{1',3'}$ | $J_{2,3}$ | $J_{2,3'}$ | $J_{3,3'}$ |
| **1**[b] (at 300 K) | 7.5 | 9.5 | 3.5 | 1.1 | 8.3 | 4.1 | 11.4 | 57.4 | | | | | | | | | | |
| **2**[b] | 7.5 | 9.5 | 3.5 | 1.1 | 8.2 | 4.2 | 11.3 | 56.1 | | | | | | | | | | |
| **3** | 8.3 | 10.3 | 3.4 | 1.1 | 6.2 | 6.7 | 11.5 | 165.7 | | | | | | | | | | |
| **4**[c] | 8.4 | 10.2 | 3.6 | 1.2 | 6.9 | 7.0 | 11.1 | 166.3 | | | | | | | | | | |
| **7** | 8.0 | 10.4 | 3.6 | 1.1 | 7.0 | 6.6 | 11.2 | 158.5 | | | | | | | | | | |
| **8**[d] | 7.6 | 9.6 | 3.4 | 1.1[e] | 6.1 | 6.3 | 11.0 | 156.3[e] | | | | | | | | | | |
| **9**[b,f] | 8.1 | 7.1 | 5.6 | 2.2 | 6.5 | 5.8 | 11.1 | 156.1 | | | | | | | | | | |
| **10** | 7.5 | 9.4 | ~2.0 | ~0.9 | 6.2 | 5.5 | 10.5 | 160.8 | | | | | | | | | | |
| **11** | 8.3 | 7.5 | 5.4 | 2.2 | 7.0 | 6.3 | 10.0 | 155.9 | | | | | | | | | | |
| **12** | 8.2 | 7.1 | 5.5 | 2.1 | 6.9 | 6.4 | 10.0 | 155.2 | 12.7 | 5.5 | 6.0 | 1.6 | 1.4 | 1.5 | 1.3 | 17.2 | 10.4 | 1.9 |
| **13**[b] | 8.1 | 7.1 | 5.6 | 2.1 | 6.5 | 5.9 | 11.1 | 156.8 | 13.4 | 5.9 | 5.5 | 1.7 | 1.6 | 1.6 | 1.5 | 17.3 | 10.5 | 2.0 |
| **14**[b] | 8.1 | 7.0 | 5.6 | 2.1 | 8.7 | 3.5 | 11.6 | 157.2 | 13.4 | 5.0 | 5.5 | 1.8 | 1.6 | 1.6 | 1.5 | 17.3 | 10.5 | 2.0 |
| **15** | 7.7 | 9.5 | 3.3 | 1.2 | 6.3 | 6.9 | 11.4 | 157.8 | 12.6 | 5.5 | 6.3 | 1.5 | 1.3 | 1.4 | 1.2 | 17.2 | 10.3 | 1.4 |
| **16**[b] | 8.1 | 7.0 | 5.6 | 2.1 | 8.6 | 3.6 | 11.5 | 157.0 | 13.4 | 5.1 | 5.5 | 1.8 | 1.5 | 1.7 | 1.4 | 17.3 | 10.5 | 2.1 |
| **19**[b,g] | 8.1 | 7.1 | 5.6 | 2.1 | 6.6 | 5.8 | 11.1 | 156.8 | | | | | | | | | | |
| **20**[b,g] | 8.1 | 6.9 | 5.6 | 2.1 | 8.7 | 3.6 | 11.5 | 157.0 | | | | | | | | | | |
| **21**[b] | 8.1 | 7.1 | 5.6 | 2.2 | 8.6 | 3.7 | 11.5 | 156.8 | | | | | | | | | | |

[a] Measured at 500 MHz, for $CDCl_3$ solutions unless stated otherwise.
[b] In fresh acetone-$d_6$, $J_{2,HO\text{-}2}$ = 3.5, $J_{3,HO\text{-}3}$ = 5.0, $J_{4,HO\text{-}4}$ = 4.0, $J_{5,HO\text{-}4}$ = 0.9.
[c] In 4:1 v/v benzene-$d_6$/$CDCl_3$.
[d] In 1:1 v/v methylsulfoxide-$d_6$/pyridine-$d_5$.
[e] In pyridine-$d_5$.
[f] At 318 K.
[g] MBn $J(CH_2)$ = 11.5 Hz.

The chemical synthesis of the palmitoyl (**1**) and oleoyl (**2**) glycolipids as separate entities afforded us the opportunity to test a previous interpretation[11] of the $^{13}C$ NMR spectrum of a mixture of two major and several minor glycolipids termed 'Glycolipid I', isolated from *B. burgdorferi*. It was proposed[11] that the doubling of the C-4, C-5, and C-6 signals of Gal and the C-3 resonance of the cholesteryl moiety that was observed for glycolipid I was due to its isolation as a chromatographically inseparable mixture of mainly **1** and **2**, even though the points of difference in the fatty acid residues of these molecules are quite remote from the nuclei showing the doubling of signals. When, in the current work we recorded the $^{13}C$ spectrum of a 1:1 mixture of synthetic **1** and **2**, this spectrum was almost identical with that of glycolipid I, thus confirming our previous interpretation.[11] This was true as long as care was taken with concentration, because **1** and **2** showed an unexpected sensitivity to concentration of the galactose C-2 resonance, possibly due to molecular aggregation, amounting to changes in the $^{13}C$ chemical shifts as large as 1 ppm. Molecular aggregation may produce this effect. Hydroxyl proton coupled $^{1}H$ NMR spectra were obtained for **1** and **2** by use of freshly opened vials of acetone-$d_6$ solvent (see OH chemical shifts and coupling constants in Tables 1 and 3, respectively). Otherwise, the use of acetone-$d_6$ that had been exposed to the atmosphere a number of times yielded $^{1}H$ NMR spectra in which a major proportion of the hydroxyl proton signals was absent due to decoupling of the OH protons by chemical exchange. In these spectra, the OH proton coupled species appeared only as a minor component amounting to 14% for **1** at 310 K, and 15% for **2** at 300 K.

## 3. Conclusion

In summary, we have presented successful syntheses of *O*-lipidated galactosyl cholesterols **1** and **2** that can now be used individually for detailed biological studies. Current work is directed towards synthesizing an experimental vaccine against Lyme disease. This vaccine will consist of glycolipid **1** carrying a reactive end group at the alkyl chain through which it will be covalently attached to protein carriers[27] to form immunogenic lipoglycoproteins.

## 4. Experimental

### 4.1. General methods

All chemicals were commercial grade and were used without purification. Solvents for chromatography were distilled prior to use. Anhydrous solvents were obtained from Aldrich. Column chromatography was performed on silica gel 60 (0.040–0.063 mm). Melting points were taken on a Meltemp capillary melting point apparatus and are uncorrected. Optical rotations were measured at 23 °C with a Perkin-Elmer Type 341 polarimeter.

*NMR spectroscopy*. NMR spectra were acquired at 300 K by use of a Bruker DRX-500 NMR spectrometer equipped with a 5 mm TXI HCN cryoprobe with *z*-gradient coil, a Silicon Graphics O2 workstation, and Bruker XWINNMR software, version 3.5, patch level 6. $^{1}H$ NMR spectra were acquired at 500 MHz by using 32,768 point data sets, a 30° pulse (2.2–2.4 μs), and a pulse recycle time of 6 s. An optimum concentration for obtaining good resolution and sensitivity was 5–6 mg of glycolipid intermediate in 0.5 mL of solvent. The resolution of the spectra was enhanced by Gaussian multiplication of the free induction decay, using a line-broadening of −1 to −3 Hz and a Gaussian truncation fraction of 0.3, together with linear prediction to 65,536 points, or linear prediction to 131,072 points with zero-filling to 262,144 points. $^{13}C$ NMR spectra were acquired at 126 MHz by using 65,536 point data sets, a 90° pulse (14 μs), and a pulse recycle time of 1.5 s. Resolution was enhanced by exponential multiplication with a line-broadening of −0.2 Hz, together with forward, complex linear prediction to 131,072 points. $^{1}H$ and $^{13}C$ chemical shifts were referenced to internal tetramethylsilane (0 ppm). $^{1}H$-coupled $^{13}C$ NMR spectra were acquired with the nuclear Overhauser effect (NOE) by use of gated, WALTZ-16 irradiation at the $^{1}H$ frequency. These spectra were used to measure $^{1}J_{C\text{-}1,H\text{-}1}$. All 2D NMR spectra were acquired by field gradient-selected methods. Forward linear prediction to larger data sizes was used to improve the resolution of 2D spectra, where necessary. 2D COSY and HMBC NMR spectra were obtained by using 2048 ($t_2$)×512 ($t_1$) point data sets, zero-filled to 2048 ($F_2$)×2048 ($F_1$) points. For 2D COSY and TOCSY, the spectral width was 5.48 kHz in each dimension, and for COSY, the read pulse was 30° (2.2–2.4 μs). For 2D HSQC and HMBC, the $^{1}H$ and $^{13}C$ spectral widths were 5.48 kHz ($F_2$) and 25.1 kHz ($F_1$), respectively. 2D TOCSY NMR spectra were acquired by use of 16,384 ($t_2$)×512 ($t_1$) point data sets, zero-filled to 32,768 ($F_2$)×2048 ($F_1$) points. High-resolution 2D HSQC spectra were acquired in 2048×512 point data sets, linear predicted to 4096 points in both dimensions.

The mass spectra were recorded at the Laboratory of Bioorganic Chemistry, NIDDK, NIH, Bethesda, MD. The fast atom bombardment (FAB) mass spectra were obtained using 6 keV Xe atoms to ionize samples from dithiothreitol/dithioerythritol, 3-nitrobenzyl alcohol, or glycerol as the matrix. The purity of all new compounds was determined to be >95% by $^{1}H$ NMR spectroscopy. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

**4.1.1. 1,2,3,6-Tetra-*O*-trimethylacetyl-β-D-galacto-pyranose (3).** To a stirred mixture of anhydrous $CHCl_3$ (80 mL), $C_5H_5N$ (45 mL), and trimethylacetyl chloride (45 mL, 365 mmol) was added D-galactose (10 g, 55 mmol, 0.75 equiv) in portions. After 24 h at room temperature, TLC (3:1 hexane/EtOAc) indicated the formation of several products. Their ratios did not change significantly after the addition of more trimethylacetyl chloride or heating for another 24 h. The mixture was concentrated under reduced pressure and the residue so obtained was triturated with diethyl ether. The solids were isolated by filtration and were discarded. To the filtrate was added isopropyl ether and the mixture was left standing at room temperature for 3 h. Filtration followed by washing with isopropyl ether afforded **3** (8.5 g, 30%) as a crystalline material: $[\alpha]_D$ +15 (*c* 0.8, $CHCl_3$). For NMR data, see Tables 1–3. HRMS *m/z* Calcd for $C_{26}H_{44}O_{10}Li$ 523.3105, found 523.3095.

**4.1.2. 4-*O*-Acetyl-1,2,3,6-tetra-*O*-(trimethylacetyl)-β-D-galactopyranose (4).** Compound **3** was treated with $Ac_2O$ and $C_5H_5N$ at room temperature followed by removal of the volatiles to afford **4** as a crystalline material in a quantitative yield: $[\alpha]_D$ +9 (*c* 1.2, $CHCl_3$). For NMR data, see Tables 1–3. HRMS *m/z* Calcd for $C_{28}H_{46}O_{11}Li$ 565.3230, found 565.3200.

**4.1.3. 4-*O*-Acetyl-2,3,6-tri-*O*-(trimethylacetyl)-α-D-galactopyranosyl bromide (5).** To a solution of compound **4** (8.5 g, 15 mmol) in anhydrous $CH_2Cl_2$ (50 mL) at 0 °C was added a 30% solution of hydrogen bromide in acetic acid (15 mL). The solution was allowed to reach room temperature. After 1 h, the solution was extracted with ice-water (5×100 mL) followed by drying ($Na_2SO_4$), filtration, and concentration to afford a colorless syrup that was used in the next step without further purification.

**4.1.4. Cholesteryl 4-*O*-acetyl-2,3,6-tri-*O*-(trimethylacetyl)-β-D-galactopyranoside (7).** To a stirred solution of compound **5** (2.0 g, 3.73 mmol), cholesterol (**6**, 1.0 g, 2.58 mmol), and di-*tert*-butyl-4-methylpyridine (1.0 g, 4.87 mmol) in anhydrous $CH_2Cl_2$ (10 mL) was added at −40 °C $CF_3SO_3Ag$ (2.0 g, 3.89 mmol). The mixture was allowed to reach 0 °C then was treated with $Bu_4NBr$ followed by aq $NaHCO_3$. The solids were removed by filtration and the organic layer was concentrated under vacuum. The residue was stirred in MeOH overnight. The solids were collected by filtration and were washed with cold MeOH to afford pure **7** (1.85 g, 85%) as a crystalline material: $[\alpha]_D$ −18 (*c* 0.5, $CHCl_3$). For NMR data, see Tables 1–3. HRMS *m/z* Calcd for $C_{50}H_{82}O_{10}Li$ 849.6071, found 849.6068.

**4.1.5. Cholesteryl β-D-galactopyranoside (8).** A stirred mixture of compound **7** (1.8 g, 2.1 mmol), $CH_2Cl_2$ (5 mL), and $CH_3OH$ (5 mL) was treated with an excess of $CH_3ONa$. After 24 h, the solids were isolated by filtration and were washed with $CH_3OH$ to afford **8** (1.10 g, 94%) as a crystalline material: $[\alpha]_D$ −58 (c 0.4, $C_5H_5N$). For NMR data, see Tables 1–3. Anal. Calcd for $C_{33}H_{56}O_7$: C, 72.22; H, 10.29. Found: C, 71.83; H, 10.29.

**4.1.6. Cholesteryl 3,4-*O*-isopropylidene-β-D-galactopyranoside (9).** To a solution of **8** (1.1 g, 2.0 mmol) in DMF (5 mL) was added 2,2-dimethoxypropane (5 mL) and camphorsulfonic acid (100 mg). Thin-layer chromatography (5:1 hexanes/EtOAc) revealed the initial formation of two products. After 48 h, the faster-moving component had almost completely disappeared. Neutralization ($NaHCO_3$) followed by extractive work-up ($CHCl_3/H_2O$) afforded an amorphous residue that was purified by silica gel column chromatography using 8:1 hexanes/EtOAc as the eluant to afford **9** (365 mg, 34%): $[\alpha]_D$ −24 (c 0.8, $CHCl_3$). For NMR data, see Tables 1–3. MS *m/z* Calcd for $C_{36}H_{60}O_6Li$ 593.4533, found 595.4550.

**4.1.7. Cholesteryl 6-*O*-(*tert*-butyl-diphenylsilyl)-β-D-galactopyranoside (10).** To a stirred solution of tetraol **9** (8.2 g, 14.9 mmol) in $C_5H_5N$ (50 mL) were added at room temperature *tert*-butyl-diphenylsilyl chloride (8.2 mL, 19.2 mmol) and a catalytic amount of 4-dimethylaminopyridine. After 5 h, MeOH was added (2 mL) followed by concentration under reduced pressure. Column chromatographic purification of the residue afforded **10** (11.93 g, 96%) as a syrup: $[\alpha]_D$ −44 (*c* 0.4, $CHCl_3$). For NMR data, see Tables 1–3. HRMS *m/z* Calcd for $C_{49}H_{74}O_6LiSi$ 793.5420, found 793.5415.

**4.1.8. Cholesteryl 6-*O*-(*tert*-butyl-diphenylsilyl)-3,4-*O*-isopropylidene-β-D-galactopyranoside (11).** To a stirred solution of triol **10** (10.4 g (18.9 mmol) in 2,2-dimethoxypropane (100 mL) was added at room temperature camphorsulfonic acid (0.5 g). After 20 min TLC (4:1 hexane/EtOAc) indicated that no starting material remained. To the solution was added triethylamine (2 mL). The solution was concentrated and the residue purified by column chromatography using a gradient of hexane/EtOAc 10:1–5:1 to obtain **11** (10.0 g, 91%) as a syrup: $[\alpha]_D$ −26 (*c* 0.6, $CHCl_3$). For NMR data, see Tables 1–3. HRMS *m/z* Calcd for $C_{48}H_{86}O_{11}LiSi$ 873.6071, found 873.6099.

**4.1.9. Cholesteryl 2-*O*-allyl-6-*O*-(*tert*-butyl-diphenylsilyl)-3,4-*O*-isopropylidene-β-D-galactopyranoside (12).** To a stirred solution of **11** (384 mg, 0.46 mmol) in DMF (2 mL) was added at 0 °C NaH (45 mg of a 60% suspension in oil). After 20 min, allyl bromide (56 μL, 0.65 mmol) was added. The solution was allowed to reach room temperature and was stirred for a further period of 45 min. The mixture was cooled to 0 °C and was treated with $CH_3OH$ (1 mL) followed by $CH_3CO_2H$ (0.5 mL).The mixture was concentrated and the residue was equilibrated between $CHCl_3$ and $H_2O$. Concentration of the organic layer followed by column chromatographic purification of the residue using 10:1 hexane/EtOAc afforded **12** (370 mg, 92%) as a syrup: $[\alpha]_D$ −18 (*c* 0.4, $CHCl_3$). For NMR data, see Tables 1–3. HRMS *m/z* Calcd for $C_{48}H_{86}O_{11}LiSi$ 873.6071, found 873.6099.

**4.1.10. Cholesteryl 2-*O*-allyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (13).** To a solution of compound **12** (2.9 g, 3.6 mmol) in anhydrous THF (20 mL) was added at room temperature a 1 M solution of tetrabutylammonium fluoride in tetrahydrofuran (1.2 mL) under argon. After 2 ½ h, the solution was concentrated. Silica gel column chromatographic purification of the residue using a gradient of 10:1–3:1 hexane/EtOAc afforded **13** (2.05 g, 97%) as a syrup: $[\alpha]_D$ −21 (*c* 1.0, $CHCl_3$). For NMR data, see Tables 1–3. HRMS *m/z* Calcd for $C_{39}H_{64}O_6Li$ 635.4866, found 635.4863.

**4.1.11. Cholesteryl 2-*O*-allyl-3,4-*O*-isopropylidene-6-*O*-palmitoyl-β-D-galactopyranoside (14).** A stirred solution of **13** (300 mg, 0.38 mmol), palmitic acid (300 mg, 1.17 mmol), dicyclohexyl carbodiimide (0.5 g, 2.42 mmol), and 4-dimethylaminopyridine (100 mg) in EtOAc (6 mL) was kept at room temperature for 6 h. MeOH (2 mL) was added and the mixture was filtered. The solids were discarded and the filtrated concentrated. Silica gel column chromatographic purification of the residue (100:2 $CHCl_3$/MeOH) afforded **14** (410 mg, 99%) as a syrup: $[\alpha]_D$ −20 (*c* 0.7, $CHCl_3$). For NMR data, see Tables 1–3. Anal. Calcd for $C_{55}H_{94}O_7$: C, 76.16; H, 10.92. Found: C, 75.95; H, 10.49.

**4.1.12. Cholesteryl 2-*O*-allyl-6-*O*-palmitoyl-β-D-galactopyranoside (15).** To a solution of **14** (400 mg, 0.46 mmol) in $CH_2Cl_2$ (2 mL) was added a solution of AcOH in MeOH (80%, 25 mL) followed by $H_2O$ (2 mL). The solution was stirred at 70 °C for 6 h. Removal of the volatiles under reduced pressure afforded a syrup, which was purified by silica gel column chromatography using 10:1 $CHCl_3$/MeOH as the eluant to afford **15** (270 mg, 70%) as a syrup: $[\alpha]_D$ −12 (*c* 0.1, $CHCl_3$). For NMR data, see Tables 1–3. MS *m/z* Calcd for $C_{52}H_{90}O_7Na$ 849.66, found 849.60. Anal. Calcd for $C_{52}H_{90}O_7$: C, 75.50; H, 10.97. Found: C, 74.47; H, 10.91.

**4.1.13. Cholesteryl 6-*O*-palmitoyl-β-D-galactopyranoside (1).** To a solution of **15** (240 mg, 0.29 mmol) in THF (5 mL) at room temperature was added a solution of (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate[21] (40 mg, 0.28 mmol) in THF (5 mL) through which $H_2$ was bubbled for 15 min before addition. After 24 h, the solution was concentrated and the residue was dissolved in AcOH (5 mL) followed by addition of TFA (1 mL) at room temperature. After 6 h, the solution was concentrated and the residue was purified by silica gel column chromatography using 20:1 $CHCl_3$/MeOH as the eluant to afford **1** (200 mg, 88%) as an amorphous substance: $[\alpha]_D$ −29 (*c* 0.5, $CHCl_3$). For NMR data, see Tables 1–3. MS *m/z* Calcd for $C_{49}H_{86}O_7Na$ 809.63, found 809.68. Anal. Calcd for $C_{49}H_{86}O_7 \cdot$MeOH: C, 73.30; H, 11.07. Found: C, 73.49; H, 11.00.

**4.1.14. Cholesteryl 2-*O*-allyl-3,4-*O*-isopropylidene-6-*O*-oleoyl-β-D-galactopyranoside (16).** A stirred solution of **13** (205 mg, 0.33 mmol), oleic acid (200 μL, 180 mg, 0.63 mmol), dicyclohexyl carbodiimide (0.4 g, 1.94 mmol), and 4-dimethylaminopyridine (100 mg) in EtOAc (5 mL) was kept at room temperature for 2 h. MeOH (1 mL) was added and the mixture was filtered. The solids were discarded and the filtrated concentrated. Silica gel column chromatographic purification of the residue (8:1 hexanes/EtOAc) afforded **16** (220 mg, 75%) as a syrup: $[\alpha]_D$ −23 (*c* 0.2, $CHCl_3$). For NMR data, see Tables 1–3. HRMS *m/z* Calcd for $C_{57}H_{96}O_7Li$ 899.7297, found 899.7361.

**4.1.15. Cholesteryl 2-*O*-allyl-6-*O*-oleoyl-β-D-galactopyranoside (17).** To a solution of **16** (200 mg, 0.22 mmol) in $CH_2Cl_2$ (2 mL) was added a solution of AcOH in MeOH (80%, 25 mL) followed by $H_2O$ (2 mL). The solution was stirred under reflux for 6 h. Removal of the volatiles under reduced pressure afforded a syrup, which was purified by silica gel column chromatography using 10:1 $CHCl_3$/MeOH as the eluant to afford **17** (133 mg, 70%) as a syrup: $[\alpha]_D$ −10 (*c* 0.3, $CHCl_3$). For NMR data, see Tables 1–3. Anal. Calcd for $C_{54}H_{92}O_7$: C, 76.01; H, 10.87. Found: C, 75.86; H, 11.06.

**4.1.16. Cholesteryl 6-*O*-octadecenoyl-β-D-galactopyranosides (18a,b,c,d).** Compound **17** was treated as described for the preparation of **1**. The product was purified by silica gel column chromatography using 20:1 EtOAc/MeOH as the eluant to afford **18a–d**, as determined by GC–MS.

**4.1.17. Cholesteryl 3,4-*O*-isopropylidene-2-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (19).** To a stirred solution of **11** (10.0 g, 12.1 mmol) in dry DMF (50 mL) at 0 °C was added NaH (2.2 g of a 60% suspension in oil). After 10 min, freshly prepared 4-methoxybenzyl bromide (15 mL) was added dropwise and the mixture was stirred for 6 h followed by the usual work-up and silica gel column chromatography using 4:1 hexanes/EtOAc as the eluant to afford a syrup. This was dissolved in dry THF (100 mL) and the solution so obtained was treated with a solution of tetrabutylammonium fluoride (25 mL of a 1 M solution in THF) at 23 °C. After 6 h, the solution was concentrated and the residue purified by silica gel column chromatography using a 4:1→1:1 hexanes/EtOAc gradient as the eluant to afford **19** (6.7 g, 78%) as a solid: $[\alpha]_D$ +22 (*c* 0.6, $CHCl_3$). For NMR data, see Tables 1–3. MS *m/z* Calcd for $C_{44}H_{68}O_7Na$ 731.49, found 732.08.

**4.1.18. Cholesteryl 3,4-*O*-isopropylidene-2-*O*-(4-methoxybenzyl)-6-*O*-oleoyl-β-D-galactopyranoside (20).** A stirred solution of **19** (1.1 g, 1.41 mmol), oleic acid (890 mg, 3.1 mmol), dicyclohexyl carbodiimide (2.0 g, 9.68 mmol), and 4-dimethylaminopyridine (100 mg) in EtOAc (6 mL) was kept at room temperature for 6 h. MeOH (4 mL) was added and the mixture was filtered. The solids were discarded and the filtrate was concentrated. Silica gel column chromatographic purification of the residue (100:2 $CHCl_3$/MeOH) afforded **20** (1.2 g, 81%) as a waxy solid: $[\alpha]_D$ +17 (*c* 1.0, $CHCl_3$). For NMR data, see Tables 1–3. Anal. Calcd for $C_{49}H_{86}O_7$: C, 76.50; H, 10.35. Found: C, 76.53; H, 10.51.

**4.1.19. Cholesteryl 3,4-*O*-isopropylidene-6-*O*-oleoyl-β-D-galactopyranoside (21).** To a stirred mixture of **20** (1.1 g, 1.13 mmol), $CH_2Cl_2$ (10 mL), and $H_2O$ (0.4 mL) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (333 mg, 1.47 mmol). After 6 h, the reaction mixture treated with aq $NaHCO_3$ followed by extractive work-up ($CHCl_3/H_2O$). Silica gel column chromatographic purification of the residue, using 6:1 hexanes/EtOAc as the eluant afforded **21** (1.0 g, 93%) as an amorphous solid: $[\alpha]_D$ −17 (*c* 0.6, $CHCl_3$). For NMR data, see Tables 1–3. HRMS *m/z* Calcd for $C_{54}H_{92}O_7Li$ 859.7032, found 859.7003.

**4.1.20. Cholesteryl 6-*O*-oleoyl-β-D-galactopyranoside (2).** A solution of **21** (250 mg, 0.29 mmol) in AcOH (15 mL) and $H_2O$ (1.3 mL) was stirred at 65 °C until TLC (1:1 hexanes/EtOAc) indicated disappearance of **21** (approx. 4 h). Removal of the volatiles under reduced pressure afforded a semisolid that was purified by silica gel column chromatography using a 1:1→1:3 hexanes/EtOAc gradient as the eluant to afford glycolipids **2** (220 mg, 92%) as a waxy solid: $[\alpha]_D$ −29 (*c* 0.5, $CHCl_3$). For NMR data, see Tables 1–3. MS *m/z* Calcd for $C_{51}H_{88}O_7Na$ 835.64, found 836.41. Anal. Calcd for $C_{51}H_{88}O_7 \cdot ½$MeOH: C, 74.59; H, 10.91. Found: C, 74.36; H, 10.99.

## References and notes

1. Steere, A. C.; Grodzicki, R. L.; Kornblatt, A. N.; Craft, J. E.; Barbour, A. G.; Burgdorfer, W.; Schmid, G. P.; Johnson, E.; Malawista, S. E. *N. Engl. J. Med.* **1983**, *308*, 733–740.

2. Steere, A. C. *N. Engl. J. Med.* **2001**, *345*, 115–125.
3. Steere, A. C.; Malawista, S. E.; Snydman, D. R.; Shope, R. E.; Andiman, W. A.; Ross, M. R.; Steele, F. M. *Arthritis Rheum.* **1977**, *20*, 7–17.
4. *Ann. Pharmacother.* **2004**, *38*, 1347.
5. Barbour, A. G.; Burgdorfer, W.; Grunwaldt, E.; Steere, A. C. *J. Clin. Invest.* **1983**, *72*, 504–515.
6. Steere, A. C.; Gibofsky, A.; Patarroyo, M. E.; Wichester, R. J.; Hardin, J. A.; Malawista, S. E. *Ann. Intern. Med.* **1979**, *90*, 896–901.
7. Canale-Parola, E. Order spirochaetales. In *Bergey's Manual of Systemic Bacteriology*; Kieg, N. R., Holt, J. G., Eds.; Williams and Williams: Baltimore, 1984; pp 38–39.
8. Gutierrez Fernandez, J.; Rodriguez Fernandez, M.; Nunez Murillo, F.; Maroto Vela, M. C. *Microbios.* **1997**, *91*, 165–174.
9. Wormser, G. P. *Infection* **1996**, *24*, 203–207.
10. Steere, A. C.; Sikand, V. K.; Meurice, F.; Parenti, D. L.; Fikrig, E.; Schoen, R. T.; Nowakowski, J.; Schmid, C. H.; Laukamp, S.; Buscarino, C.; Krause, D. S. *N. Engl. J. Med.* **1998**, *339*, 209–215.
11. Ben-Menachem, G.; Kubler-Kielb, J.; Coxon, B.; Yergey, A.; Schneerson, R. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 7913–7918.
12. Bolt, A. J. N.; Clarke, R. E. *Phytochemistry* **1970**, *9*, 819–822.
13. Kasteric-Suhadolc, T. *Biochim. Biophys. Acta* **1980**, *620*, 322–325.
14. Mayberry, W. R.; Smith, P. F. *Biochim. Biophys. Acta* **1983**, *752*, 434–443.
15. Smith, P. F. *J. Bacteriol.* **1971**, *108*, 986–991.
16. Patel, K. R.; Smith, P. F.; Mayberry, W. R. *J. Bacteriol.* **1978**, *136*, 829–831.
17. Livermore, B. P.; Bey, R. F.; Johnson, R. C. *Infect. Immun.* **1978**, *20*, 215–220.
18. Hirai, Y.; Haque, M.; Yoshida, T.; Yokota, K.; Yasuda, T.; Oguma, K. *J. Bacteriol.* **1995**, *177*, 5327–5333.
19. Kunz, H.; Harreus, A. *Justus Liebigs Ann. Chem.* **1982**, 41–48.
20. Kunz, H.; Sager, W.; Schanzenbach, D.; Decker, M. *Justus Liebigs Ann. Chem.* **1991**, 649–654.
21. Oltvoort, J. J.; van Boeckel, C. A. A.; de Koning, J. H.; van Boom, J. H. *Synthesis* **1981**, 305.
22. Baudry, D.; Ephritikhine, M.; Felkin, H. *J. Chem. Soc., Chem. Commun.* **1978**, 694–695.
23. Wu, D.; Xing, G. W.; Poles, M. A.; Horowitz, A.; Kinjo, Y.; Sullivan, B.; Bodmer-Narkevitch, V.; Plettenburg, O.; Kronenberg, M.; Tsuji, M.; Ho, D. D.; Wong, C. H. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 1351–1356.
24. Fay, L.; Richli, U. *J. Chromatogr.* **1991**, *541*, 89–98.
25. Musher, J. I.; Corey, E. J. *Tetrahedron* **1962**, *18*, 791–809.
26. Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 1* **1974**, *2*, 293–297.
27. Kubler-Kielb, J.; Pozsgay, V. *J. Org. Chem.* **2005**, *70*, 6987–6990.