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ω-Mercapto Analogs of Naturally Occurring Lipids

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Abstract: Analogs of natural lipids, where one of the alkyl chains carries a terminal thiol functionality, were prepared by *N*- or *O*-acylation of sphingosine or monoacylglycerol derivatives, respectively, thus creating lipid mimics suitable for anchoring to e.g. gold surfaces.

Glycolipids are ubiquitous components of cell membranes, where they function as receptors for proteins, antibodies, and other biomolecules, but are also used for adhesion by opportunistic pathogens such as bacteria and viruses during the initial phase of an infection.[1] The study of these phenomena has traditionally been performed with natural or synthetic glycolipids presented to the binding protein or microbe on TLC plates, microtiter plates, glass or plastic particles, or as liposomes. However, these procedures are not always reliable, as exemplified by the binding of Verotoxin to different glycolipid analogs; the mode of presentation of the glycolipids (i.e. on TLC or microtiter plates) greatly influenced the binding.[2] Furthermore, the mode of presentation is important for the amount of non-specific binding of proteins and microbes, and therefore it has an influence on the signal-to-noise ratio in adhesion measurements.

In an attempt to create natural mimics of cell surfaces, we now introduce a series of lipid analogs (Fig. 1), where one of the alkyl chains carries a terminal thiol group. Anchoring of mixtures of these lipids to gold surfaces should result in mimics of cell membranes that closely resemble natural surfaces, thereby avoiding non-specific protein binding. The material thus obtained would be suitable for adhesion studies based on e.g. surface plasmon resonance. The anchoring of thiols to gold is a well-known phenomenon,[3] and glycosides carrying various single-chain alkyl thiol linker arms have been used in amperometric biosensor[4] and surface plasmon resonance[5] measurements.

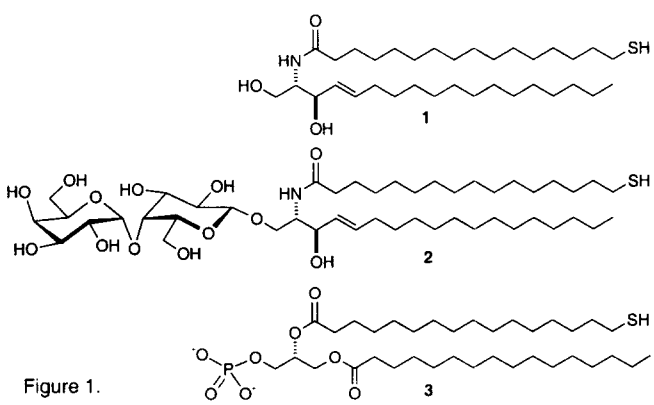

Figure 1.

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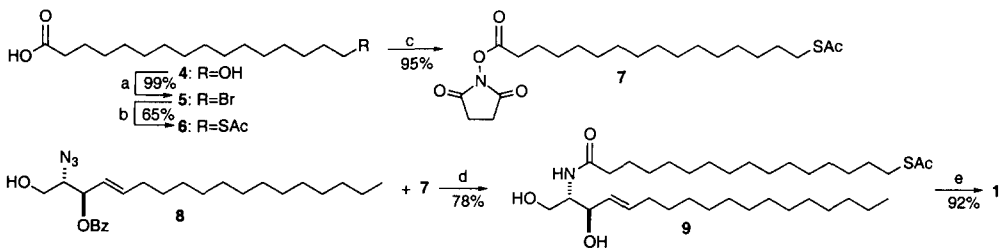
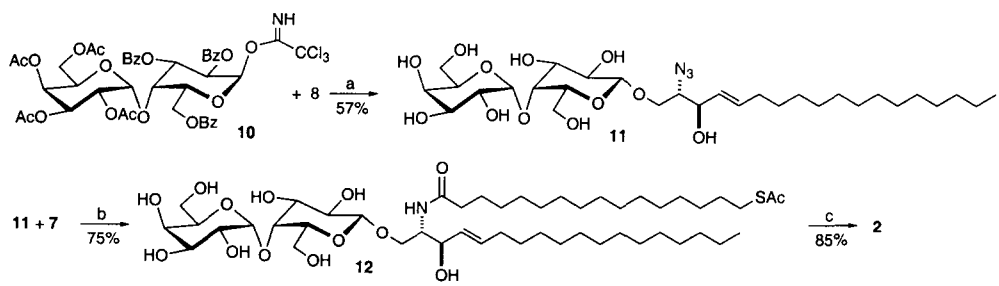
Synthesis of the lipids **1-3** was performed by standard methods, although variations and improvements had to be employed in order to obtain good yields of intermediates and products. As depicted in Scheme 1, the known[6] azidosphingosine derivative **8** was de-*O*-benzoylated with methanolic MeONa, the product was dissolved in a mixture of pyridine, Et_3N, and MeOH and reduced with H_2S, and the resulting amine was acylated overnight with the N-hydroxysuccinimide-activated carboxylic acid derivative **7** in CH_2Cl_2-solution to give the protected thiol **9** (78%). Removal of the acetyl protecting group of **9** by treatment with methanolic sodium methoxide then gave the ceramide mimic **1** (92%). Compound **7** was prepared from 16-hydroxyhexadecanoic acid (**4**) by treatment with concentrated aqueous HBr in AcOH under reflux for 3 days (→**5**[7], 99%), followed by replacement of bromide ion by treatment with AcSK in DMF at 60 °C for 8 h (→**6**, 65%). Treatment of **6** with N-hydroxysuccinimide and ethyl-3-(3-dimethylaminopropyl)carbodiimide in CH_2Cl_2 overnight gave **7** (95%).



Scheme 1. a) HBr, AcOH, reflux, 3 d. b) AcSK, DMF, 60 °C, 8 h. c) N-hydroxysuccinimide, EDC, CH_2Cl_2, 12 h. d) MeONa, MeOH, 12 h, then pyridine, Et_3N, MeOH, H_2S, 22 °C, 16 h, then CH_2Cl_2, **7**, Et_3N, 22 °C,12 h. e) MeONa, MeOH, 22 °C, 12 h.

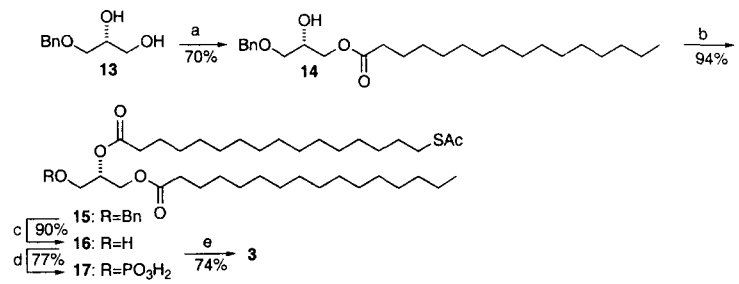
The galabiosyl ceramide mimic **2** was prepared as depicted in Scheme 2. A mixture of the galabiosyl trichloroacetimidate **10** (prepared by well-known methods[8]) and the azidosphingosine derivative **8** in CH_2Cl_2 was treated for 3 h with BF_3OEt_2 in the presence of molecular sieves (MS 300 AW) to give the corresponding fully acylated derivative (66%). Removal of the acetyl and benzoyl protecting groups by treatment with methanolic sodium methoxide, followed by chromatography, furnished the azidosphingosinyl galabioside **11** in 57% overall yield. Essentially as in the synthesis of **9** above, the azido functionality of **11** was reduced with H_2S and the resulting amine was acylated by addition of compound **7** to yield the protected thiol **12** (75%). Removal of the acetyl protecting group of **12** by treatment with methanolic sodium methoxide then gave the galabiosylceramide mimic **2** (85%).



Scheme 2. a) CH_2Cl_2, MS 300 AW, BF_3OEt_2, 22 °C, 3 h, then MeONa, MeOH, 22 °C, 12 h. b) pyridine, Et_3N, MeOH, H_2S, 22 °C, 16 h, then CH_2Cl_2, **7**, Et_3N, 22 °C,12 h. c) MeONa, MeOH, 22 °C, 3 h.

In order to demonstrate that the procedures presented here are generally useful for the preparation of lipid analogs, we also synthesized a compound (**3**) belonging to the glycerol ester lipids (Scheme 3). Acylation of the benzyl-protected glycerol derivative **13** with palmitic acid[9] was promoted by

diisopropylcarbodiimide (DIC) in CH_2Cl_2/DMAP-solution at -10 °C (1 h)→0 °C (4 h), to give the palmitoyl derivative **14** in 70% yield after chromatographic removal of the corresponding regioisomer and diacylated material. A second acylation with the thioacetylpalmitic acid **6** was performed under similar conditions (DIC, CH_2Cl_2/DMAP, 0→22 °C for 0.5+4.5 h), which furnished the bis-acylglycerol derivative **15** (94%). The benzyl protecting group of **15** was removed by treatment[10] with BCl_3 in CH_2Cl_2 at -75 °C for 1 h (attempted hydrogenolytic de-*O*-benzylation was unsuccessful), which yielded the bis-acylglycerol **16** (90%). Phosphorylation of **16** was performed by treatment[11] with $POCl_3$ in a mixture of toluene, Et_3N, and hexane at 0 °C for 0.5 h, then at room temperature overnight, to furnish the phosphatidic acid derivative **17** in 77% yield. Removal of the acetyl protecting group of **17** by treatment with methanolic sodium methoxide then gave the phosphatidic acid mimic **3** (74%), together with a small amount (14%) of the corresponding disulfide.



Scheme 3. a) CH_2Cl_2, $CH_3(CH_2)_{14}COOH$, DMAP, DIC, -10°C, 1 h, then 0 °C, 4 h. b) CH_2Cl_2, **6**, DMAP, DIC, 0 °C, 0.5 h, then 22 °C, 4.5 h. c) CH_2Cl_2, BCl_3/hexane, -75 °C, 1 h. d) toluene, $POCl_3$, Et_3N, hexane, 0 °C, 0.5 h, then 22 °C, 12 h. e) MeONa, MeOH, 22 °C, 12 h.

The use of lipid mimics such as compounds **1-3** for anchoring to gold surfaces in connection with surface plasmon resonanse-based investigations of protein and microbe binding, will be reported in due course.

EXPERIMENTAL

The experimental conditions were as described in Schemes 1-3. Selected ^{1}H-NMR data were as follows. **1** ($CDCl_3$): δ 5.54 (dd, 1 H, *J* 6.4, 9.1 Hz, =C*H*CHOH), 2.68 (t, 2 H, *J* 7.3 Hz, CH_2S). **2** (CD_3OD) δ 4.97 (d, 1 H, *J* 3.3 Hz, H-1'), 4.28 (d, 1 H, *J* 6.3 Hz, H-1), 2.68 (t, 2 H, *J* 7.2 Hz, CH_2S). **3** ($CDCl_3$/CD_3OD 1:1): δ 4.11 (dd, 1 H, *J* 3.5, 8.4 Hz, H-*sn*1), 2.39 (t, 2 H, *J* 7.2 Hz, CH_2S). **6** ($CDCl_3$): δ 2.87 (t, 2 H, *J* 7.3 Hz, CH_2SAc), 2.36 (t, 2 H, *J* 7.4 Hz, CH_2COO). **7** ($CDCl_3$): δ 2.87 (m, 6 H, CH_2SAc, $COCH_2CH_2CO$), 2.61 (t, 2 H, *J* 7.4 Hz, CH_2COON). **9** ($CDCl_3$): δ 5.54 (dd, 1 H, *J* 6.4, 9.1 Hz, =C*H*CHOH), 2.87 (t, 2 H, *J* 7.3 Hz, CH_2SAc), 2.24 (t, 2 H, *J* 7.5 Hz, CH2CON). **11** (CD_3OD): δ 5.55-5.48 (m, 1 H, =C*H*CHOH), 4.97 (d, 1 H, *J* 2.1 Hz, H-1'), 4.31 (d, 1 H, *J* 7.0 Hz, H-1). **12** ($CDCl_3$): δ 5.45 (m, 1 H, =C*H*CHOH), 4.97 (d, 1 H, *J* 3.3 Hz, H-1'), 4.28 (d, 1 H, *J* 6.3 Hz, H-1), 2.86 (t, 2 H, *J* 7.2 Hz, CH_2SAc). **15** ($CDCl_3$): δ 4.20 (dd, 1 H, *J* 3.9, 8.1 Hz, H-*sn*1), 2.87 (t, 2 H, *J* 7.3 Hz, CH_2SAc). **16** ($CDCl_3$): δ 4.24 (dd, 1 H, *J* 6.3, 5.5 Hz, H-*sn*1), 2.87 (t, 2 H, *J* 7.3 Hz, CH_2SAc). **17** ($CDCl_3$/CD_3OD 1:1): δ 4.11 (dd, 1 H, *J* 3.5, 8.4 Hz, H-*sn*1), 2.55 (t, 2 H, *J* 8.0 Hz, CH_2SAc).

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