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Note

Synthesis of 6-thio pseudo glycolipids and their orientation on a gold slide studied by IRRAS

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ABSTRACT

We have synthesized four 6-thio pseudo glycolipid analogues and assessed how two of them self-assembled on a gold surface. These structures were designed as candidate tethers molecules to anchor bilayer lipid membranes on gold. 6-Deoxy-6-thiogalactose was chosen to anchor the macromolecule to the gold and define an aqueous zone at the gold surface. A long alkane chain (C-12 or C-18) linked to the anomeric position of the sugar residue was chosen to anchor a bilayer lipid membrane. The linkage between the carbohydrate and the hydrophobic chains is either a glycosidic bond or a 1,4-disubstituted triazole formed by copper(I)-catalysed alkyne-azide cycloaddition (CuAAC) of the propargyl glycoside with azido-dodecane and azido-octadecane. We are expecting that the hydrocarbon chains will orient themselves perpendicular to the gold surface and be incorporated into the first leaflet of the bilayer membrane. We have studied self assembled monolayers of the C-12 aglycone analogues on gold using infrared reflection absorption spectroscopy (IRRAS). We compared the results given by the IRRAS experiments to the IR spectra recorded by attenuated total reflection (ATR) spectroscopy on films of the randomly oriented analogues. Our results demonstrate that the C-12 analogues did bind to gold and did orient themselves perpendicular to the gold slide.

The complexity of biological membranes makes their direct study very challenging and the design and development of artificial model membranes constitutes an area of intensive research.[1–3] Amongst these artificial model membranes, artificial bilayer lipid membranes (BLMs) supported on glass, quartz, silicon or gold are of considerable interest since they can be characterized easily using various analytical techniques: SPR, EIS, AFM etc.[4–14] These artificial bilayer membranes are either freely supported on the solid surface[4,5] or tethered to the surface.[6–14] While the freely supported membranes maintain better fluidity than the tethered BLMs, they are often unstable and present structural defects.[1–5] In contrast, tethered artificial bilayer membranes exhibit good stability and thus many such models have been developed in the past two decades.[1–3,6–14] Whether tethered or freely supported, the supported BLMs designed for the study of transmembrane proteins and events must be separated from the solid support by an hydrophilic layer[1–3] that allows the incorporation of the transmembrane proteins[9,11,13,14] or the transport of ions and molecules.[15] For this purpose many tethers have been developed and they are usually made up of three domains: (1) a lipid chain that inserts itself in the first leaflet of the BLM; (2) an hydrophilic spacer molecule such as an amphiphilic copolymer,[6] a polyethyleneglycol chain[7–10] or a peptide chain[11–14] that provides the necessary hydrophilic region between the BLM and the solid support and (3) an anchoring functional group such as a disulfide[6–8,10–12,16] or thiol[8,12–14,17] for gold support or a silane for glass immobilization.[9] In this study, we are proposing the development of a new type of tether molecules in which the hydrophilic spacer molecule is based on a thiocarbohydrate that is self adsorbed on gold. Self-assembled monolayers (SAM) of 1-β-D-thioglucose on gold have been described by Revell[18] and used by Quirk[19] to study the enzymatic digestion of freely supported cellulose fibres by atomic force microscopy. However, it is only very recently[20] that SAMs of 1-β-D-thioglucose on gold were studied at the molecular level by electrochemical scanning tunnelling microscopy (EC-STM) and polarization modulation infrared reflection adsorption spectroscopy (PM-IRRAS). These studies[20] demonstrated that, upon self-assembly on gold, 1-β-D-thioglucose oriented itself perpendicular to the gold surface, thus providing an organised hydrophilic layer on the gold slide. Thus, one may suggest that an alkane chain at O-4 of 1-β-D-thioglucose would extend perpendicular to the gold surface and be able to insert itself in the first leaflet of a BLM while the glucose moiety provides a hydrophilic zone between the solid substrate and the BLM. However, it has been shown that 1-β-D-thioglucose adsorbed on a solid silver electrode had the potential to anomerize to a mixture of α and β anomers.[21] Thus, since the configuration of the anomeric thiol linked to gold will determine the orientation of the sugar and, therefore, the orientation of the O-4 linked lipid chains,

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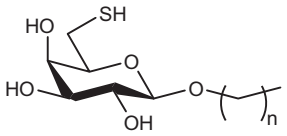
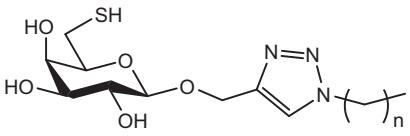
the ability of 1-thioglucose to anomerize precludes its use as such a carbohydrate template. We envisioned that the introduction of a thiol at C-4 or C-6 of a sugar unit together with that of a long alkane chain at the anomeric position would give novel interesting tether molecules that would not be able to undergo anomerization on the gold surface. We postulated that a 6-thio hexose would be able to self-assemble perpendicularly to the gold surface and that a lipid chain positioned equatorial (i.e., β) at the anomeric position would extend perpendicular to the gold surface and thus be able to anchor a BLM as represented schematically on Figure 1. Based on its ease of synthesis, we chose to use 6-deoxy-6-thiogalactose to anchor the macromolecule to the gold surface and define the aqueous zone between the gold surface and the bilayer while C-12 or C-18 alkane chains linked at the anomeric position were chosen with the expectation that they would be able to insert themselves in the first leaflet of a BLM (Fig. 1). The linkage between the carbohydrate and the hydrophobic chains is either a β glycosidic bond as in compounds **1** and **2**, or a 1,4-disubstituted triazole formed by copper(I)-catalysed alkyne-azide cycloaddition (CuAAC) as in analogues **3** and **4**.[22,23] Thus, we report here the synthesis of four pseudo glycolipid analogues **1**–**4** as a first generation of carbohydrate-based tether molecules to anchor bilayer lipid membranes to gold. The construction and study of tethered BLMs using these novel 6-thio pseudo glycolipids are beyond the scope of this paper. However, in order to demonstrate that our analogues did bind and orient themselves on a gold slide, self-assembled monolayers of **1** and **3** were studied by infrared reflection absorption spectroscopy (IRRAS).[24] Analogues **1** and **3** did indeed orient themselves on the gold slide in such a way that the lipid chains extended perpendicular to the gold surface and should therefore be able to insert themselves in the first leaflet of a BLM as proposed on Figure 1.

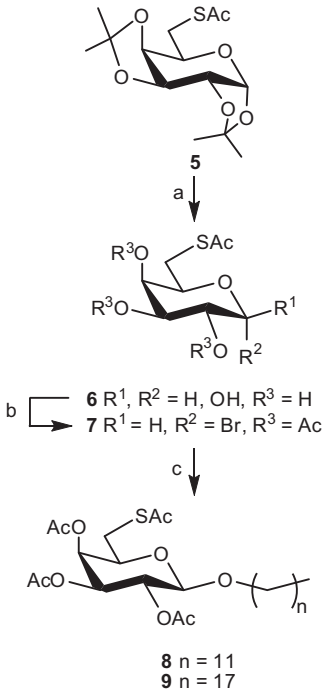


1 n = 11
2 n = 17



3 n = 11
4 n = 17

The synthesis of compounds **1** and **2** was easily carried out in four steps from the known[25] 6-deoxy-6-thioacetate diisopropylidene galactose derivative **5** (Scheme 1). We first attempted to remove the isopropylidene groups and in situ acetylate the intermediate polyol **6** as described previously by Elhalabi and Rice using a mixture of acetic anhydride–acetic acid–sulfuric acid.[26] However, in our hands the desired peracetate was obtained in only 5% yield. Thus, diisopropylidene **5** was treated with a mixture of TFA–AcOH–H_2O and the resulting polyol **6** was isolated and purified by column chromatography (Scheme 1). Acetylation followed by treatment of the peracetate intermediate with HBr in acetic acid gave the known[27] glycosyl bromide **7** which was, in turn, glycosidated with either dodecanol or octadecanol under Helfrich conditions. To allow an efficient purification of the desired glycosides, the excess alcohol was acetylated in situ by treating the reaction mixtures with acetic anhydride–pyridine and the desired glycosides **8** and **9** were isolated in good yield upon column chromatography. Finally, Zemplèn deacetylations gave the glycolipid analogues **1** and **2** in excellent yields.



5

6 R^1, R^2 = H, OH, R^3 = H
7 R^1 = H, R^2 = Br, R^3 = Ac

8 n = 11
9 n = 17

Scheme 1. Reagents and conditions: (a) TFA–AcOH–H_2O (5:3:2), rt (95%); (b) (i) Ac_2O–pyridine, 50 °C, (ii) 33% HBr–AcOH, rt (50%); (c) dodecanol or octadodecanol, $Hg(CN)_2$, toluene, MS 4 Å, 40 °C (76%).

Cu^I-catalysed azide-alkyne 1,3-dipolar cycloaddition or the so-called 'click' chemistry is an attractive approach to efficiently couple two entities[28] and since its development[22,23] it has been used to make a large number of new compounds including glycoclusters, glycodendrimers and glycopolymers.[29–34] In this report, we applied this method to link the propargyl glycoside **10** to the known azides **11**[35] and **12**[36] (Scheme 2). Thus, propargyl glycoside **10** was prepared

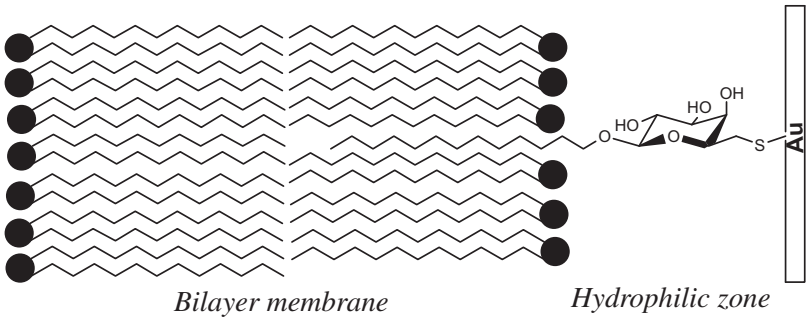
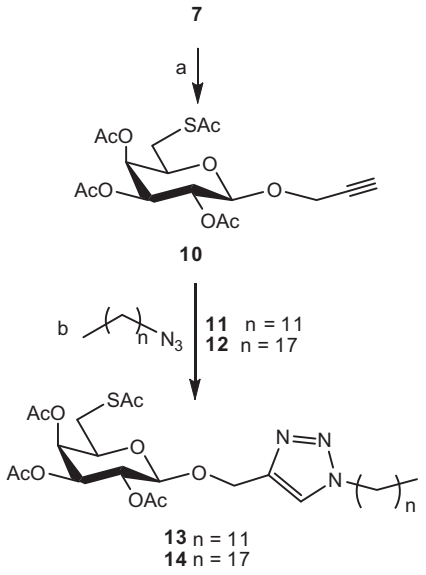
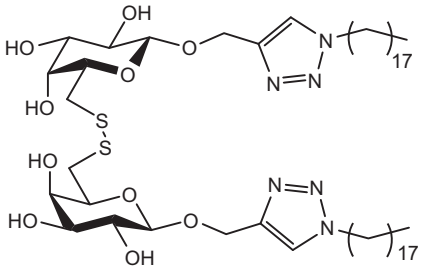


Figure 1. Proposed model of a tethered bilayer lipid membrane using carbohydrate-based tether **1** as the anchoring molecule.



Scheme 2. Reagents and conditions: (a) AgOTf, TMU, propargyl alcohol, CH_2Cl_2, −40 °C to rt (56%); (b) **11** or **12**, THF–water 11:3, Na ascorbate, $CuSO_4$, $5H_2O$, rt (55% for **13**, 59% for **14**).

from glycosyl bromide **7** through glycosidation with propargyl alcohol. We first attempted this glycosidation under Helfrich conditions as described above for the synthesis of glycosides **8** and **9**. However, under these conditions the desired glycoside was obtained contaminated with the corresponding orthoester. Best results were obtained when the reaction was carried out under AgOTf–TMU activation and even though propargyl glycoside **10** was isolated in a mediocre 56% yield it could be easily purified by column chromatography. The preparation of the known[35,36] azides **11** and **12** was accomplished in two steps from the corresponding alcohols. Dodecanol and octadecanol were first converted into the corresponding iodide analogues (PPh_3, imidazole, I_2) which were, in turn, submitted to nucleophilic displacement with sodium azide in DMF. The desired azido-dodecane **11** and azido-octadecane **12** were obtained in 70% and 78% yields, respectively, over the two steps. The cycloaddition between propargyl glycoside **10** and the azido-alkane **11** and **12** was carried out in a mixture of THF and water using a slight excess of azide (1.1 equiv). Copper(I) was generated in situ through the reduction of the copper(II) sulfate by sodium ascorbate and the 'click' adducts **13** and **14** were obtained in 55% and 59% yields, respectively. These yields are comparable to that reported recently for the cycloaddition between peracetylated propargyl β-glucopyranoside and 11-azidoundecanoic acid.[37] Attempts to further improve the yield of these reactions by using copper(I) iodide in THF were unsuccessful. The structure of analogue **13** and **14** was confirmed by ^1H and ^{13}C NMR as well as HR-ESIMS. Indeed, the 1,4 disubstituted triazole ring gave a typical singlet at 7.48 ppm and typical signals at ~145 and ~125 ppm in the ^1H and ^{13}C NMR spectra, respectively. The large difference in chemical shift measured for the triazole carbon atoms [$\Delta(\delta_{C4} - \delta_{C5})$ = ~20 ppm] was characteristic in both cases of the expected formation of the 1,4 regioisomers.[38] Zemplèn deacetylation of analogue **13** gave the desired pseudo glycolipid analogue **3** in quantitative yield. Interestingly, Zemplèn deacetylation of analogue **14** under the same conditions than used to prepare the thiols **1**–**3** gave the thiol **4** contaminated with 10% of the corresponding disulfide **15**. The deacetylation of analogue **14** was carried out on multiple samples and it was observed that the amount of disulfide formation varied from one deacetylation to the next. We also observed that dissolving the product of this reaction in DMSO to acquire NMR data favoured the oxidation of thiol **4** to the disulfide **15**. Even though both thiols[8,12–14,17] and disulfides[6–8,10–16] can be immobilized on gold and , therefore, do not need to be separated, column chromatography easily allowed separation of the thiol **4** and disulfide **15**. It is important to note that the C-18 aglycone analogues **2** and **4** were poorly soluble in any given pure solvent other than DMSO. However, they could be solubilized into a 9:1 mixture of $CDCl_3$–CD_3OD and this constituted the best solvent to acquire NMR data without favouring the oxidation of the thiol to the disulfide. We were able, in this solvent mixture, to identify key NMR features that are characteristic to the thiol and disulfide, respectively. Indeed, H-6a and H-6b in thiol **4** gave signals in the ^1H NMR spectrum at 2.82 and 2.67 ppm, respectively, while the same signals in disulfide **15** were found at a lower field: 3.05 and 2.96 ppm, respectively. An even more obvious difference in chemical shift of C-6 in the thiol and disulfide was also found in the ^{13}C NMR spectra. Indeed, C-6 in thiol **4** gave a signal at 24.4 ppm while the same carbon in disulfide **15** gave a signal at 39.4 ppm. The NMR features identified in thiol **4** were also found in analogues **1**–**3** confirming that these analogues did not contain any disulfide.



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Compounds **1** and **3** were self-assembled on polycrystalline gold slides and studied using infrared reflection absorption spectroscopy (IRRAS), an analytical technique which allows the recording of IR data on gold-adsorbed monolayer films. For comparative purposes, films of compounds **1** and **3** were deposited on a ZnSe internal reflection element and studied using attenuated total reflection (ATR) spectroscopy, an analytical technique that allows the recording of IR data for a film (~1 μm thick) of randomly oriented molecules. Comparing the two sets of IR data gave us information on the adsorption and orientation of the pseudo-glycolipids **1** and **3** on the gold slide.[24] Figures 2 and 3 show two regions of the ATR and IRRAS spectra obtained for **1** and **3**. Figure 2 shows the asymmetric $\nu_{as}(CH_2)$, $\nu_{as}(CH_3)$, and symmetric $\nu_s(CH_2)$, $\nu_s(CH_3)$ stretching vibrations observed in the ATR and IRRAS spectra of compounds **1** and **3**. As can be seen in the IRRAS spectra these bands [$\nu_{as}(CH_3)$, $\nu_{as}(CH_2)$, $\nu_s(CH_3)$, $\nu_s(CH_2)$] are centred at 2964, 2926, 2877 and 2854 cm^{-1}, respectively, and are in total agreement with those recorded in the ATR spectra (centred at 2954, 2920, 2872 and 2852 cm^{-1}, respectively) clearly indicating a successful adsorption of **1** and **3** on the gold slide. In addition, when compared to the ATR spectra obtained for the randomly oriented glycolipids **1** and **3** on ZnSe, the IRRAS spectra of the SAM of **1** and **3** on gold show a clear decrease in band intensity for the $\nu_{as}(CH_2)$ relative to $\nu_{as}(CH_3)$. This decrease in $\nu_{as}(CH_2)$ intensity suggests that the lipid chains in glycolipids **1** and **3** are extended and oriented perpendicular to the surface of the gold slide.[24] This orientation of the lipid chains in **1** and **3** is ideal for their insertion into the fist leaflet of a bilayer. Finally, the clear differences observed in Figure 3 that shows the IRRAS and ATR spectra in the ~1500–1000 cm^{-1} region also suggest orientation at the gold surface.[24]

Thus as postulated the 6-thio galactose analogues **1** and **3** did self-assemble perpendicularly to the gold surface allowing the lipid chains positioned equatorial (i.e., β) at the anomeric position to extend perpendicular to the gold surface for insertion into the first leaflet of a BLM. Further studies are on going to assess the ability of the hydrocarbon chains to tether an artificial bilayer membrane

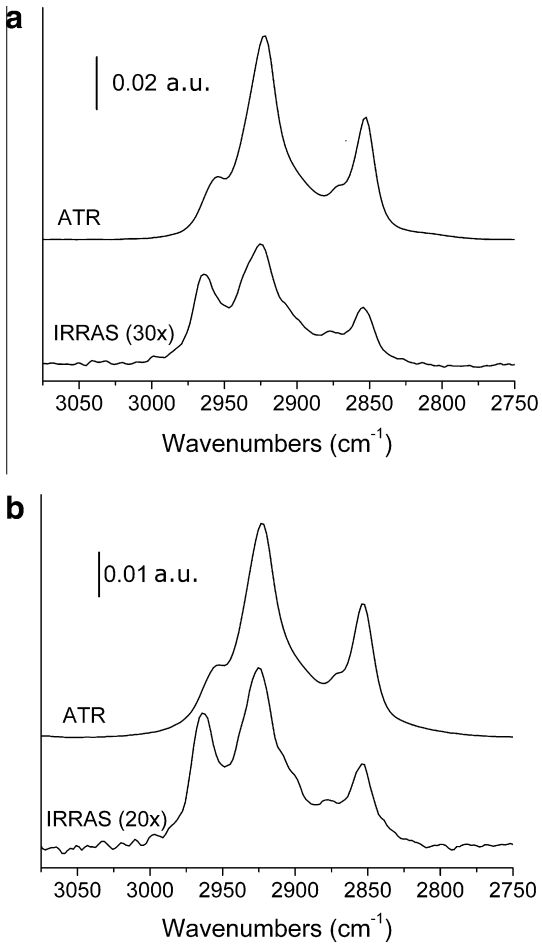

Figure 2. ATR (top trace) and IRRAS (bottom trace, collected at a 70° incident angle) spectra of (a) compound **1** and (b) compound **3** for the asymmetric $\nu_{as}(CH_2)$, $\nu_{as}(CH_3)$, and symmetric $\nu_s(CH_2)$, $\nu_s(CH_3)$ stretching vibrations (a.u. denotes absorbance units).

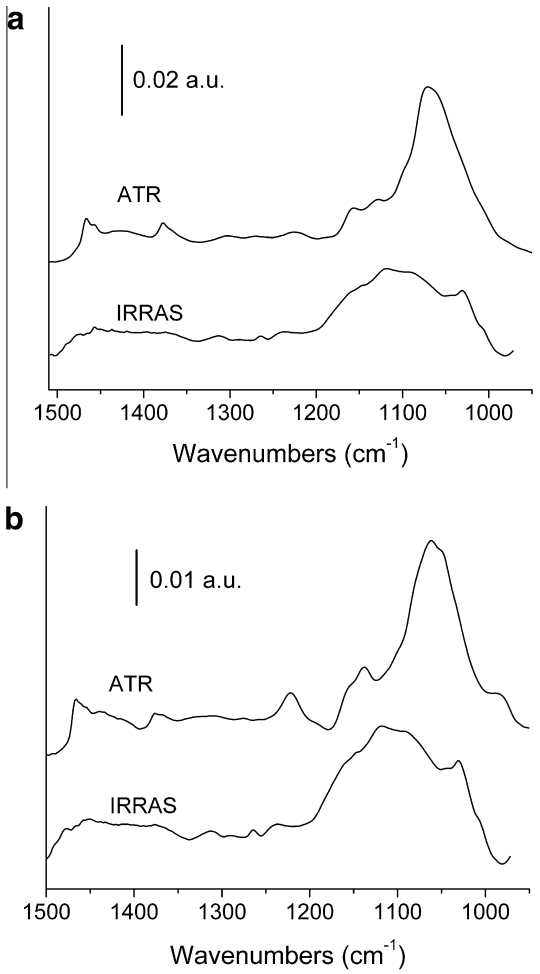

Figure 3. ATR (top trace) and IRRAS (bottom trace, collected at a 80° incident angle) spectra of (a) compound **1** and (b) compound **3** for the ~1500–1000 cm^{-1} region (a.u. denotes absorbance units).

while the galactose residue provides the required hydrophilic region between the first leaflet of the bilayer and the gold support. However, when tethering BLMs to gold using analogues **1**–**4**, it is clear that one sugar residue will not provide a hydrophilic zone thick enough to allow the insertion of transmembrane proteins into the model membrane. Indeed, these first generation tether candidates (**1**–**4**) were synthesized to test our hypothesis that if the thiol was placed at C-6 and the lipid chain equatorial at the anomeric position, the orientation of the lipid chain could allow anchoring of a BLM. We are now preparing a second generation of carbohydrate-based tether molecules using di- and tri- saccharides as carbohydrate templates. The disaccharide-based pseudo glycolipids will have a thiol at the 6-position of the non-reducing end sugar that will be β-linked to the 4-position of a D-glucoside residue carrying a β-linked lipid chain at the anomeric position. Based on the results presented herein, we expect that the use of such disaccharides (6-thio lactose or cellobiose) will maximize an extended orientation of the disaccharide perpendicular to the gold surface and that the β-linked lipid chains will also extend perpendicular to the gold surface for tethering of a BLM.

1. Experimental

1.1. General methods

^1H NMR (600.13 or 400.13 MHz) and ^{13}C NMR (150.9 or 100.6 MHz) spectra were recorded with Bruker Avances spectrometers in $CDCl_3$ (internal standard, for ^1H residual $CHCl_3$ δ 7.24; for ^{13}C $CDCl_3$ δ 77.0), CD_3OD (internal standard, for ^1H residual CD_2HOD δ 3.30; for ^{13}C CD_3OD δ 49.0) or DMSO-d_6 (internal standard, for ^1H residual CD_2HSOCD_3 δ 2.49; for ^{13}C DMSO-d_6 δ 39.0). Chemical shifts (ppm) and coupling constants (J, Hz) were obtained from a first-order analysis of one-dimensional spectra and assignments of proton and carbon resonances were based on two dimensional ^1H–^1H and ^{13}C–^1H correlation experiments. ^1H NMR data are reported using standard abbreviations: singlet (s), doublet (d), triplet (t), doublet of doublet (dd), triplet of doublet (td) and multiplet (m). TLC was performed on aluminium plates precoated with Silica Gel 60 (250 μm) containing a fluorescent indicator. The plates were visualized under UV and charred with a 10% solution of H_2SO_4 in EtOH. Compounds were purified by flash chromatography with Silica Gel 60 (230–400 mesh) unless otherwise stated. Solvents were distilled and dried according to standard procedures[39] and organic solutions were dried over Na_2SO_4 and concentrated under reduced pressure below 40 °C. Optical rotations were measured at 23 °C on a Rudolph Research Autopol III polarimeter and reported as follows: $[\alpha]_D$ (c in grams per 100 mL, solvent). High resolution electrospray ionization mass spectra (HRESI MS) were recorded by the analytical services of the McMaster Regional Center for Mass Spectrometry, Hamilton, Ontario.

1.2. Dodecyl 6-thio-β-D-galactopyranoside (1)

Sodium (20 mg, 0.866 mmol, 4 equiv) was added to a solution of peracetate **8** (115 mg, 0.217 mmol, 1 equiv) in anhyd MeOH

(8.5 mL), the reaction mixture was stirred at rt for 1 h and deionized with Dowex 50 (H^+) resin. The resin was filtered off, washed with MeOH (25 mL) and the combined filtrate and washings were concentrated yielding pure glycolipid analogue **1** (74 mg, 94%) as a white amorphous powder. $[\alpha]_D$ +8.0 (*c* 1.0, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 4.18 (d, 1H, *J* = 7.2 Hz, H-1), 3.91 (d, 1H, *J* = 2.1 Hz, H-4), 3.85 (m, 1H, $OCH_aH_b(CH_2)_{10}CH_3$), 3.55 (m, 1H, $OCH_aH_b(CH_2)_{10}CH_3$), 3.48–3.41 (m, 3H, H-2, H-3, H-5), 2.79 (dd, 1H, *J* = 7.3, 13.6 Hz, H-6a), 2.66 (dd, 1H, *J* = 6.6, 13.6 Hz, H-6b), 1.62 (2H, m, $OCH_2CH_2(CH_2)_9CH_3$), 1.28 (m, 18H, $OCH_2CH_2(CH_2)_9CH_3$), 0.89 (t, 3H, *J* = 6.6 Hz, $O(CH_2)_{11}CH_3$). ^{13}C NMR (100 MHz, CD_3OD): δ 105 (C-1), 78.0 (C-5), 75.0 (C-3), 72.4 (C-2), 71.0 ($OCH_2(CH_2)_{10}CH_3$), 70.3 (C-4), 33.1, 30.9, 30.8, 30.6, 30.5, 27.1, 23.7 ($OCH_2(CH_2)_{10}CH_3$), 25.2 (C-6), 14.5 ($O(CH_2)_{11}CH_3$). HRESIMS calcd for $C_{18}H_{40}NO_5S$ $[M+NH_4]^+$: 382.2627, found: 382.2637.

1.3. Octadecyl 6-thio-β-D-galactopyranoside (2)

Sodium (11.2 mg, 0.486 mmol, 2 equiv) was added to a solution of peracetate **9** (150 mg, 0.243 mmol, 1 equiv) in anhyd MeOH (2.5 mL) and the reaction was left to proceed (2 h) and worked up as described Section 1.2 for the preparation of compound **1**. Glycolipid analogue **2** (80 mg, 73%) was obtained pure as a white amorphous powder. $[\alpha]_D$ −9.5 (*c* 1.0, 9:1 $CHCl_3$–MeOH). ^{1}H NMR (400 MHz, 9:1 $CDCl_3$–CD_3OD): δ 4.14 (m, 1H, H-1), 3.91 (s, 1H, H-4), 3.83 (m, 2H, $OCH_aH_b(CH_2)_{16}CH_3$), 3.54–3.43 (m, 3H, H-2, H-3, $OCH_aH_b(CH_2)_{16}CH_3$), 3.39 (t, 1H, *J* = 7.0 Hz, H-5), 2.81 (m, 1H, H-6a), 2.65 (dd, 1H, *J* = 6.7, 14.3 Hz, H-6b), 1.56 (m, 2H, $OCH_2CH_2(CH_2)_{15}CH_3$), 1.33–1.10 (m, 30H, $OCH_2CH_2(CH_2)_{15}CH_3$), 0.80 (t, 3H, *J* = 6.4 Hz, $O(CH_2)_{17}CH_3$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.81 (d, 1H, *J* = 4.2 Hz, OH-2), 4.71, (d, 1H, *J* = 5.0 Hz, OH-3), 4.51 (d, 1H, *J* = 4.8 Hz, OH-4), 4.04 (d, 1H, *J* = 7.1 Hz, H-1), 3.74–3.65 (m, 2H, H-4, $OCH_aH_b(CH_2)_{16}CH_3$), 3.40 (m, 1H, $OCH_aH_b(CH_2)_{16}CH_3$), 3.34–3.29 (m, 1H, H-5), 3.27–3.19 (m, 2H, H-2, H-3), 2.67–2.54 (m, 2H, H-6a, H-6b), 2.18 (t, 1H, *J* = 8.9 Hz, SH), 1.50 (m, 2H, $OCH_2CH_2(CH_2)_{15}CH_3$), 1.33–1.18 (m, 30H, $OCH_2CH_2(CH_2)_{15}CH_3$), 0.84 (t, 3H, *J* = 6.5 Hz, $O(CH_2)_{17}CH_3$). ^{13}C NMR (100 MHz, 9:1 $CDCl_3$–CD_3OD): δ 103.0 (C-1), 76.4 (C-5), 73.4 (C-3), 71.3 (C-2), 70.2 ($OCH_2(CH_2)_{16}CH_3$), 68.7 (C-4), 31.8, 29.6, 29.5, 29.3, 29.2, 25.8, 24.5, 22.6 ($OCH_2(CH_2)_{16}CH_3$), 24.3 (C-6), 14.0 ($O(CH_2)_{17}CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 103.4 (C-1), 76.0 (C-5), 73.3 (C-3), 70.3 (C-2), 68.6 ($OCH_2(CH_2)_{16}CH_3$), 68.3 (C-4), 31.3, 29.3, 29.0, 28.9, 28.7, 25.5, 22.1 ($OCH_2(CH_2)_{16}CH_3$), 24.3 (C-6), 13.9 ($O(CH_2)_{17}CH_3$). HRESIMS calcd for $C_{24}H_{52}NO_5S$ $[M+NH_4]^+$: 466.3566, found: 466.3596.

1.4. 1-Dodecane-4-(6-thio-β-D-galactopyranosyloxymethyl)-[1,2,3]-triazole (3)

Sodium (11 mg, 0.497 mmol, 4 equiv) was added to a solution of peracetate **13** (76 mg, 0.124 mmol, 1 equiv) in anhyd MeOH (12 mL) and the reaction was left to proceed (1 h) and worked up as described Section 1.2 for the preparation of compound **1**. Glycolipid analogue **3** (55 mg, quant.) was obtained pure as a slightly yellowish foam. $[\alpha]_D$ −4.5 (*c* 1.0, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 8.01 (s, 1H, =CH), 4.95 (d, 1H, *J* = 12.5 Hz, OCH_aH_b), 4.77 (d, 1H, *J* = 12.5 Hz, OCH_aH_b), 4.42–4.32 (m, 3H, H-1, $NCH_2(CH_2)_{10}CH_3$, 3.92 (d, 1H, *J* = 2.6 Hz, H-4), 3.57–3.43 (m, 3H, H-2, H-3, H-5), 2.81 (dd, 1H, *J* = 7.2, 13.6 Hz, H-6a), 2.67 (dd, 1H, *J* = 6.6, 13.6 Hz, H-6b), 1.88 (t, 2H, *J* = 7.2 Hz, $NCH_2CH_2(CH_2)_9CH_3$), 1.38–1.22 (m, 18H, $NCH_2CH_2(CH_2)_9CH_3$), 0.88 (t, 3H, *J* = 6.6 Hz, $N(CH_2)_{11}CH_3$). ^{13}C NMR (100 MHz, CD_3OD): δ 145.7 (C=CH), 125.2 (C=CH), 104.2 (C-1), 78.1 (C-5), 74.8 (C-3), 72.2 (C-2), 70.2 (C-4), 63.2 (OCH_2), 51.4 ($NCH_2(CH_2)_{10}CH_3$), 33.0, 31.3, 30.7, 30.6, 30.4, 30.1, 27.5, 23.7 ($NCH_2(CH_2)_{10}CH_3$), 25.2 (C-6), 14.5 ($N(CH_2)_{11}CH_3$). HRESIMS calcd for $C_{21}H_{39}O_5N_3SNa$ $[M+Na]^+$: 468.2508, found: 498.2505.

1.5. 1-Octadecane-4-(6-thio-β-D-galactopyranosyloxymethyl)-[1,2,3]-triazole (4) and 1-octadecane-4-(6-thio-β-D-galacto-pyranosyloxymethyl)-[1,2,3]-triazole disulfide (15)

Sodium (7 mg, 0.304 mmol, 4 equiv) was added to a solution of peracetate **14** (53 mg, 76 μmol, 1 equiv) in anhyd MeOH (7.6 mL) and the reaction was left to proceed (1.5 h) and worked up as described Section 1.2 for the preparation of compound **1**. After evaporation of the solvent, the mixture (35 mg, 88%, **4**/**15** = 9:1 estimated by ^{1}H NMR) of glycolipid analogue **4** and disulfide **15** was obtained as an amorphous powder. For analytical purpose, these two products were separated by column chromatography (MeOH–CH_2Cl_2, 8:92).

1.5.1. Analytical data for thiol 4

$[\alpha]_D$ −10.0 (*c* 1.0, 9:1.0 $CHCl_3$–MeOH). ^{1}H NMR (400 MHz, 9:1 $CDCl_3$–CD_3OD): δ 7.57 (s, 1H, =CH), 4.92 (d, 1H, *J* = 12.2 Hz, OCH_aH_b), 4.67 (d, 1H, *J* = 12.2 Hz, OCH_aH_b), 4.30–4.22 (m, 3H, H-1, $NCH_2(CH_2)_{16}CH_3$), 3.90 (d, 1H, *J* = 2.6 Hz, H-4), 3.56–3.44 (m, 2H, H-2, H-3), 3.40 (t, 1H, J = 7.0 Hz, H-5), 2.82 (dd, 1H, *J* = 7.3, 13.8 Hz, H-6a), 2.67 (dd, 1H, *J* = 6.4, 13.7 Hz, H-6b), 1.81 (t, 2H, *J* = 6.8 Hz, $NCH_2CH_2(CH_2)_{15}CH_3$), 1.28–1.10 (m, 30H, $NCH_2CH_2(CH_2)_{15}CH_3$), 0.79 (t, 3H, *J* = 6.6 Hz, $N(CH_2)_{17}CH_3$). ^{13}C NMR (100 MHz, 9:1 $CDCl_3$–CD_3OD): δ 144.0 (C=CH), 122.7 (C=CH), 102.6 (C-1), 76.6 (C-5), 73.4 (C-3), 71.1 (C-2), 68.6 (C-4), 62.1 (OCH_2), 50.5 ($NCH_2(CH_2)_{16}CH_3$), 31.9, 30.2, 29.6, 29.5, 29.3, 28.9, 26.4, 22.6 ($NCH_2(CH_2)_{16}CH_3$), 24.4 (C-6), 14.0 ($N(CH_2)_{17}CH_3$). HRESIMS calcd for $C_{27}H_{51}O_5SN_3Na$ $[M+Na]^+$: 552.3447, found: 552.3461.

1.5.2. Analytical data for disulfide 15

$[\alpha]_D$ −12.0 (*c* 0.3, 9:1 $CHCl_3$–MeOH). ^{1}H NMR (400 MHz, 9:1 $CDCl_3$–CD_3OD): δ 7.59 (s, 2H, =CH), 4.94 (d, 2H, *J* = 12.3 Hz, OCH_aH_b), 4.67 (d, 2H, *J* = 12.2 Hz, OCH_aH_b), 4.30 (d, 2H, *J* = 7.1 Hz, H-1), 4.25 (t, 2H, *J* = 7.2 Hz, $NCH_2(CH_2)_{16}CH_3$), 3.87 (s, 2H, H-4), 3.67 (t, 2H, J = 6.7 Hz, H-5), 3.58–3.49 (m, 4H, H-2, H-3), 3.05 (dd, 2H, *J* = 7.5, 14.0 Hz, H-6a), 2.96 (dd, 2H, *J* = 5.8, 13.8 Hz, H-6b), 1.81 (t, 4H, *J* = 6.8 Hz, $NCH_2CH_2(CH_2)_{15}CH_3$), 1.30–1.13 (m, 60H, $NCH_2CH_2(CH_2)_{15}CH_3$), 0.81 (t, 6H, *J* = 6.6 Hz, $N(CH_2)_{17}CH_3$). ^{13}C NMR (100 MHz, 9:1 $CDCl_3$–CD_3OD): δ 143.9 (C=CH), 122.8 (C=CH), 102.5 (C-1), 73.5 (C-5), 73.3 (C-3), 71.0 (C-2), 69.1 (C-4), 62.0 (OCH_2), 50.4 ($NCH_2(CH_2)_{16}CH_3$), 39.4 (C-6), 31.8, 30.1, 29.6, 29.5, 29.3, 29.2, 28.9, 26.4, 22.6 ($NCH_2(CH_2)_{16}CH_3$), 14.0 ($N(CH_2)_{17}CH_3$). HRESIMS calcd for $C_{54}H_{101}O_{10}S_2N_6$ $[M+H]^+$: 1057.7021, found: 1057.7053.

1.6. 6-*S*-Acetyl-6-thio-α,β-D-galactopyranose (6)

The known[25] 1:2,3:4-di-*O*-isopropylidene-6-*S*-acetyl-6-thio-α,β-D-galactopyranose **5** (1.01 g, 3.18 mmol) was dissolved in a mixture of 2:3:5 H_2O–AcOH–TFA (30 mL) and left at rt for 1 h. The mixture was diluted with toluene (30 mL), solvents were evaporated and the residue was co-concentrated with toluene (2 × 30 mL). Chromatography (EtOAc–MeOH–H_2O, 90:6:4) of the residue gave hemiacetal **6** (718 mg, 95%, α/β ratio 1:1) as a white foam. ^{1}H NMR (400 MHz, CD_3OD): δ 5.09 (d, 1H, *J* = 3.2 Hz, H-1α), 4.38 (dd, 1H, *J* = 3.7, 3.8 Hz, H-1β), 3.98 (t, 1H, *J* = 6.7 Hz, H-5α), 3.83 (d, 1H, *J* = 1.7 Hz, H-4α), 3.78 (s, 1H, H-4β), 3.75–3.67 (m, 2H, H-2α, H-3α), 3.49 (td, 1H, *J* = 7.3, 0.9 Hz, H-5β), 3.45–3.39 (m, 2H, H-2β, H-3β), 3.19–3.02 (m, 4H, H-6α,β), 2.31 (s, 6H, $SCOCH_3$-α,β). ^{13}C NMR (100 MHz, CD_3OD): δ 197.2 (C=O), 98.7 (C-1β), 94.2 (C-

1α), 75.2 (C-5β), 74.9, 73.5 (C-2β, C-3β), 71.9 (C-4α), 71.2 (C-4β), 71.2, 70.2 (C-2α, C-3α), 70.4 (C-5α), 30.7, 30.6 (C-6α,β), 30.4 (SCOCH_3-α,β). HRESIMS calcd for $C_8H_{14}O_6SNa$ $[M+Na]^+$: 261.0409, found: 261.0407.

1.7. 2,3,4-Tri-*O*-acetyl-6-*S*-acetyl-6-thio-α-D-galactopyranosyl bromide (7)

Hemiacetal **6** (148 mg, 0.624 mmol) was dissolved in a mixture Ac_2O–pyridine (1:1, 2 mL) and the solution was stirred at 50 °C for 1.5 h. The solvents were co-evaporated with toluene (2 × 3 mL) and a solution of the residue in CH_2Cl_2 (15 mL) was washed successively with 2 M HCl (3 × 15 mL) and satd aq $NaHCO_3$ (3 × 15 mL). The aq layers were re-extracted with CH_2Cl_2 (2 × 15 mL) and the combined organic layers were dried and concentrated. The crude peracetylated intermediate was obtained as a yellowish foam (204 mg, 80%), dried overnight and dissolved in anhyd CH_2Cl_2 (2.5 mL) under N_2. A solution of HBr in AcOH (33%, 1 mL) was added dropwise to the reaction mixture which was stirred for 30 min at rt, diluted with CH_2Cl_2 (15 mL) and quickly washed with satd aq $NaHCO_3$ (40 mL). The aq layer was re-extracted with CH_2Cl_2 (2 × 20 mL) and the combined organic layers were dried and concentrated. Chromatography (EtOAc–hexanes, 4:6) of the residue gave the pure bromide **7** (168 mg, 63%) as a brownish syrup. $[\alpha]_D$ +213 (*c* 1.0, CH_2Cl_2), lit.[27] $[\alpha]_D^{25}$ +197 (*c* 1.1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 6.64 (d, 1H, *J* = 3.7 Hz, H-1), 5.48 (dd, 1H, *J* = 1.1, 3.2 Hz, H-4), 5.34 (dd, 1H, *J* = 3.2, 10.6 Hz, H-3), 4.97 (dd, 1H, *J* = 3.9, 10.6 Hz, H-2), 4.23 (t, 1H, *J* = 7.1 Hz, H-5), 3.15 (dd, 1H, *J* = 7.2, 13.9 Hz, H-6a), 2.95 (dd, 1H, *J* = 7.0, 13.9 Hz, H-6b), 2.31 (s, 3H, SCOCH_3), 2.14, 2.07, 1.97 (3s, 9H, OCOCH_3 × 3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 194.0, 170.0, 170.0, 169.7 (C=O), 88.2 (C-1), 72.4 (C-5), 68.2 (C-3), 67.7 (C-4), 67.6 (C-2), 30.4 (SCOCH_3), 27.8 (C-6), 20.7, 20.6, 20.5 (OCOCH_3 × 3).

1.8. Dodecyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-galactopyranoside (8)

A solution of dodecanol (327 mg, 1.76 mol, 5 equiv) and bromide **7** (150 mg, 0.351 mmol, 1 equiv) in anhyd toluene (3.5 mL) containing freshly activated MS 4 Å (175 mg) was stirred under N_2 for 30 min at rt. $Hg(CN)_2$ (133 mg, 0.527 mmol, 1.5 equiv) was added to the reaction mixture that was then stirred at 40 °C for 22 h. A mixture pyridine–Ac_2O (1:1, 2 mL) was added to the mixture which was then heated to 50 °C for 3 h. The reaction mixture was allowed to cool down to rt, diluted with EtOAc (30 mL) and filtered over a pad of Celite®. The solids were washed with EtOAc (10 mL) and the combined filtrate and washings were washed with 2 M HCl (3 × 30 mL) and satd aq $NaHCO_3$ (3 × 30 mL). The aq phases were re-extracted with EtOAc (2 × 15 mL) and the combined organic layers were dried and concentrated. Chromatography (EtOAc–hexanes, 2:8) of the residue gave the dodecyl glycoside **8** pure as a white amorphous powder (142 mg, 76%). $[\alpha]_D$ +23.2 (*c* 1.0, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$): δ 5.38 (dd, 1H, *J* = 0.6, 3.1 Hz, H-4), 5.15 (dd, 1H, *J* = 8.0, 10.5 Hz, H-2), 4.95 (dd, 1H, *J* = 3.4, 10.5 Hz, H-3), 4.39 (d, 1H, *J* = 8 Hz, H-1), 3.86 (m, 1H, OCH_aH$_b$$(CH_2)_{10}CH_3$), 3.63 (td, 1H, *J* = 0.8, 7.4 Hz, H-5), 3.45 (m, 1H, OCH$_a$$H_b$$(CH_2)_{10}CH_3$), 3.08 (dd, 1H, *J* = 7.0, 13.8 Hz, H-6a), 3.02 (dd, 1H, *J* = 7.1, 13.9 Hz, H-6b), 2.31 (s, 3H, SCOCH_3), 2.14, 2.02, 1.94 (3s, 9H, OCOCH_3), 1.55 (m, 2H, $OCH_2CH_2(CH_2)_9CH_3$), 1.25 (m, 18H, $OCH_2CH_2(CH_2)_9CH_3$), 0.85 (t, 3H, *J* = 6.8 Hz, $O(CH_2)_{11}CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 194.7, 170.4, 170.1, 169.4 (C=O), 101.2 (C-1), 72.0 (C-5), 71.1 (C-3), 70.3 ($OCH_2(CH_2)_{10}CH_3$), 68.9 (C-2), 68.0 (C-4), 31.9, 29.6, 29.4, 29.3, 28.6, 22.7 ($OCH_2(CH_2)_{10}CH_3$), 30.4 (SCOCH_3), 25.8 (C-6), 20.7, 20.6 (OCOCH_3), 14.1 ($O(CH_2)_{11}CH_3$). HRESIMS calcd for $C_{26}H_{44}O_9SNa$ $[M+Na]^+$: 555.2604, found: 555.2580.

1.9. Octadecyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-galactopyranoside (9)

Octadecanol (475 mg, 1.76 mol, 5 equiv) was glycosylated with bromide **7** (150 mg, 0.351 mmol, 1 equiv) under $Hg(CN)_2$ (133 mg, 0.527 mmol, 1.5 equiv) activation as described above Section 1.8 for the synthesis of glycoside **8**. After work up (as described Section 1.8), chromatography (EtOAc–hexanes, 2:8) of the residue gave the octadecyl glycoside **9** pure (165 mg, 76%) as a white amorphous powder. $[\alpha]_D$ +19.5 (*c* 1.0, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$): δ 5.38 (d, 1H, *J* = 2.7 Hz, H-4), 5.15 (dd, 1H, *J* = 8.0, 10.4 Hz, H-2), 4.95 (dd, 1H, *J* = 3.4, 10.5 Hz, H-3), 4.39 (d, 1H, *J* = 8.0 Hz, H-1), 3.83 (m, 1H, OCH$_a$H$_b$$(CH_2)_{16}CH_3$), 3.63 (td, 1H, *J* = 0.8, 7.0 Hz, H-5), 3.42 (m, 1H, OCH$_a$$H_b$$(CH_2)_{16}CH_3$), 3.09 (dd, 1H, *J* = 7.0, 13.9 Hz, H-6a), 3.02 (dd, 1H, *J* = 7.1, 13.8 Hz, H-6b), 2.32 (s, 3H, SCOCH_3), 2.14, 2.02, 1.95 (3s, 9H, OCOCH_3), 1.55 (m, 2H, $OCH_2CH_2(CH_2)_{15}CH_3$), 1.33–1.20 (m, 30H, $OCH_2CH_2(CH_2)_{15}CH_3$), 0.85 (t, 3H, *J* = 6.6 Hz, $O(CH_2)_{17}CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 194.7, 170.3, 170.1, 169.4 (C=O), 101.2 (C-1), 72.0 (C-5), 71.1 (C-3), 70.3 ($OCH_2(CH_2)_{16}$–CH_3), 68.9 (C-2), 68.0 (C-4), 31.9, 29.7, 29.6, 29.4, 29.3, 25.8, 22.7 ($OCH_2(CH_2)_{16}CH_3$), 30.4 (SCOCH_3), 28.6 (C-6), 20.7, 20.6 (OCOCH_3), 14.1 ($O(CH_2)_{17}CH_3$). HRESIMS calcd for $C_{32}H_{60}NO_9S$ $[M+NH_4]^+$: 634.3989, found: 466.4033.

1.10. 2-Propynyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-galactopyranoside (10)

A mixture of propargyl alcohol (96.7 μL, 1.66 mmol, 3 equiv) and *N*,*N*-tetramethylurea (99.3 μL, 0.830 mmol, 1.5 equiv) in anhyd CH_2Cl_2 (2 mL) containing silver trifluoromethanesulfonate (156.5 mg, 0.609 mmol, 1.1 equiv) was cooled to −40 °C under N_2. A solution of bromide **7** (238 mg, 0.554 mmol, 1 equiv) in anhyd CH_2Cl_2 (3.5 mL) was added dropwise to the mixture and after 30 min at −40 °C the reaction mixture was allowed to warm up to rt slowly and the reaction was left under stirring for a further 3 h at rt. The solids were filtered off on Celite®, washed with CH_2Cl_2 (15 mL) and the combined filtrate and washings were washed with satd aq $NaHCO_3$ (20 mL). The aq phase was re-extracted with CH_2Cl_2 (2 × 10 mL) and the combined organic layers were dried and concentrated. Chromatography (EtOAc–hexanes, 3:7) of the residue gave propagyl glycoside **10** pure as a yellowish oil (125 mg, 56%). $[\alpha]_D$ −2.0 (*c* 1.0, CH_2Cl_2). ^1H NMR (600 MHz, $CDCl_3$): δ 5.39 (d, 1H, *J* = 3.3 Hz, H-4), 5.17 (dd, 1H, *J* = 8.1, 10.3 Hz, H-2), 5.0 (dd, 1H, *J* = 3.3, 10.3 Hz, H-3), 4.68 (d, 1H, *J* = 8.0 Hz, H-1), 4.38 (t, 2H, *J* = 2 Hz, OCH_2C≡CH), 3.69 (t, 1H, *J* = 7.1 Hz, H-5), 3.1 (dd, 1H, *J* = 6.9, 13.9 Hz, H-6a), 3.02 (dd, 1H, *J* = 7.1, 13.9 Hz, H-6b), 2.44 (t, 1H, *J* = 2.1 Hz, C≡CH), 2.32 (s, 3H, SCOCH_3), 2.14, 2.04, 1.95 (3s, 9H, OCOCH_3 × 3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 194.6, 170.3, 170.1, 169.6 (C=O), 98.6 (C-1), 78.3 (*C*≡CH), 75.3 (C≡*C*H), 72.3 (C-4), 71 (C-2), 68.5 (C-3), 67.9 (C-5), 55.9 (OCH_2C≡CH), 30.5 (SCOCH_3), 28.6 (C-6), 20.8, 20.7, 20.6 (OCOCH_3). HRESIMS calcd for $C_{17}H_{22}O_9SNa$ $[M+Na]^+$: 425.0882, found: 425.0898.

1.11. 1-Azido-dodecane (11)

PPh_3 (2.89 g, 11 mmol, 4.1 equiv), I_2 (2.12 g, 8.34 mmol, 3.1 equiv) and imidazole (751 mg, 11 mmol, 4.1 equiv) were added at rt to a solution of dodecanol (501 mg, 2.7 mmol, 1 equiv) in toluene (70 mL). The reaction was heated to reflux for 2 h, then allowed to cool down to rt and satd aq $NaHCO_3$ (70 mL) was added. The biphasic mixture was stirred for 5 min and I_2 was added until the organic phase remained brown. A 20% aq $Na_2S_2O_3$ solution was then added to the reaction mixture until it became colourless and the biphasic mixture was transferred to a separatory funnel. The aq layer was drained and the organic phase was washed with water (3 × 70 mL), dried and concentrated.

Chromatography (EtOAc–hexanes, 1:9) of the residue gave the pure 1-iodo-dodecane (606 mg, 76%) intermediate as a yellowish oil. The iodide (527 mg, 1.8 mmol, 1 equiv) was dissolved in anhyd DMF (25 mL) and NaN_3 (694 mg, 11 mmol, 6 equiv) was added to the solution. The reaction mixture was heated to 60 °C for 20 h, allowed to cool down to rt and diluted with water (25 mL). The mixture was extracted with Et_2O (3 × 50 mL) and the organic phases were washed with water (3 × 50 mL), dried and concentrated. Chromatography (EtOAc–hexanes, 2:98) of the residue gave the azido **11** (347 mg, 92%) pure as a colourless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.23 (t, 2H, J = 7.0 Hz, CH_2N_3), 1.58 (m, 2H, J = 7.0 Hz, $CH_2CH_2N_3$), 1.35 (2H, m, $CH_2CH_2CH_2N_3$), 1.25 (16H, m, $CH_3(CH_2)_8CH_2CH_2CH_2N_3$), 0.86 (t, 3H, J = 6.8 Hz, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 51.5 (CH_2N_3), 31.9, 29.6, 29.5, 29.3, 29.2, 28.8, 26.7, 22.6 ($CH_3(CH_2)_{10}CH_2N_3$), 14.1 (CH_3). The NMR data is in accordance to the literature.[35]

1.12. 1-Azido-octadecane (12)

Octadecanol (504 mg, 1.86 mmol, 1 equiv) in toluene (50 mL) was converted into the corresponding iodide using PPh_3 (2.0 g, 7.6 mmol, 4 equiv), I_2 (1.47 g, 5.8 mmol, 3 equiv) and imidazole (520 mg, 7.6 mmol, 4 equiv) as described above Section 1.11 for the synthesis of 1-iodo-dodecane. The reaction was worked up as described Section 1.11 and chromatography (EtOAc–hexanes, 2:98) gave the pure 1-iodo-octadecane (579 mg, 82%) as a yellowish oil. It was converted (553 mg, 1.45 mmol, 1 equiv) to the corresponding 1-azido octadecane as described above Section 1.11 for the synthesis of 1-azido-dodecane and after work up and chromatography (EtOAc–hexanes, 2:98) the 1-azido-octadecane **12** (409 mg, 95%) was isolated pure as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.23 (t, 2H, J = 7.0 Hz, CH_2N_3), 1.58 (m, 2H, J = 6.8 Hz, $CH_2CH_2N_3$), 1.37–1.20 (30H, m, $CH_3(CH_2)_{15}CH_2CH_2N_3$), 0.86 (t, 3H, J = 6.7 Hz, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 51.5 (CH_2N_3), 31.9, 29.7, 29.6, 29.5, 29.4, 29.2, 28.8, 26.7, 22.7 ($CH_3(CH_2)_{16}CH_2N_3$), 14.1 (CH_3). The NMR data is in accordance to the literature.[36]

1.13. 1-Docecane-4-(2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-galactopyranosyloxy-methyl)-[1,2,3]-triazole (13)

Propargyl glycoside **10** (114 mg, 0.284 mmol, 1 equiv) and 1-azido-dodecane **11** (66 mg, 0.312 mmol, 1.1 equiv) were dissolved in a mixture THF-water (11:3, 14 mL) and a 1 M aq solution of sodium ascorbate (567 μL, 0.567 mmol, 2 equiv) followed by a 0.1 M aq solution of copper(II) sulfate pentahydrate (284 μL, 0.284 mmol, 0.1 equiv) were added. The reaction mixture was stirred at rt for 23 h and the solvents were evaporated. The residue was dissolved in EtOAc (20 mL), washed with satd aq $NaHCO_3$ (3 × 20 mL) and the aq phases were re-extracted with EtOAc (2 × 10 mL). The combined organic layers were dried, concentrated and chromatography (EtOAc–hexanes, 4:6) of the residue gave the cyclo-adduct **13** (97 mg, 55%) pure as a colourless amorphous glass. $[\alpha]_D$ +1.6 (c 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48 (s, 1H, =CH), 5.38 (d, 1H, J = 2.9 Hz, H-4), 5.18 (dd, 1H, J = 8.0, 10.4 Hz, H-2), 4.96 (m, 2H, H-3, OCH_aH_b), 4.78 (d, 1H, J = 12.5 Hz, OCH_aH_b), 4.58 (d, 1H, J = 8.0 Hz, H-1), 4.30 (t, 2H, J = 7.0 Hz, $NCH_2(CH_2)_{10}CH_3$), 3.69 (t, 1H, J = 6.9 Hz, H-5), 3.10 (dd, 1H, J = 6.8, 13.9 Hz, H-6a), 3.01 (dd, 1H, J = 7.2, 13.9 Hz, H-6b), 2.32 (s, 3H, $SCOCH_3$), 2.14, 1.95, 1.94 (3s, 9H, $OCOCH_3$), 1.86 (t, 2H, J = 7.0 Hz, $NCH_2CH_2(CH_2)_9CH_3$), 1.31–1.19 (m, 18H, $NCH_2CH_2(CH_2)_9CH_3$), 0.84 (t, 3H, J = 6.6 Hz, $N(CH_2)_{11}CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 194.5, 170.2, 170.0, 169.5 (C=O), 144.0 (C=CH), 122.4 (C=CH), 100.2 (C-1), 72.2 (C-5), 70.9 (C-3), 68.7 (C-2), 67.9 (C-4), 63.0 (OCH_2), 50.3 ($NCH_2(CH_2)_{10}CH_3$), 31.8, 30.3, 29.5, 29.3, 28.9, 26.4, 22.6 ($NCH_2(CH_2)_{10}CH_3$), 30.4 ($SCOCH_3$), 28.5 (C-6), 20.8, 20.7, 20.5 ($OCOCH_3$), 14.0 ($N(CH_2)_{11}CH_3$). HRESIMS calcd for $C_{29}H_{48}O_9SN_3$ $[M+H]^+$: 614.3131, found: 614.3104.

1.14. 1-Octadecane-4-(2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-galactopyranosyloxy-methyl)-[1,2,3]-triazole (14)

Propargyl glycoside **10** (57 mg, 0.142 mmol, 1 equiv) and 1-azido-octadecane **12** (46 mg, 0.156 mmol, 1.1 equiv) were reacted as described Section 1.13 for the synthesis of cyclo-adduct **13**. After work up as described above Section 1.13, chromatography (EtOAc–hexanes, 4:6) of the residue gave cyclo-adduct **14** (58 mg, 59%) pure as a colourless amorphous glass. $[\alpha]_D$ +6.2 (c 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48 (s, 1H, =CH), 5.38 (dd, 1H, J = 0.5, 2.9 Hz, H-4), 5.17 (dd, 1H, J = 8.0, 10.4 Hz, H-2), 4.96 (m, 2H, H-3, OCH_aH_b), 4.78 (d, 1H, J = 12.5 Hz, OCH_aH_b), 4.58 (d, 1H, J = 8.0 Hz, H-1), 4.30 (t, 2H, J = 6.9 Hz, $NCH_2(CH_2)_{16}CH_3$), 3.69 (t, 1H, J = 7.0 Hz, H-5), 3.10 (dd, 1H, J = 6.8, 13.9 Hz, H-6a), 3.01 (dd, 1H, J = 7.2, 13.9 Hz, H-6b), 2.32 (s, 3H, $SCOCH_3$), 2.13, 1.95, 1.93 (3s, 9H, $OCOCH_3$), 1.86 (t, 2H, J = 7.0 Hz, $NCH_2CH_2(CH_2)_{15}CH_3$), 1.32–1.17 (m, 30H, $NCH_2CH_2(CH_2)_{15}CH_3$), 0.83 (t, 3H, J = 6.6 Hz, $N(CH_2)_{17}CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 194.5, 170.3, 170.0, 169.5 (C=O), 144.1 (C=CH), 122.4 (C=CH), 100.3 (C-1), 72.2 (C-5), 71.0 (C-3), 68.7 (C-2), 67.9 (C-4), 63.0 (OCH_2), 50.4 ($NCH_2(CH_2)_{16}CH_3$), 31.9, 30.3, 29.6, 29.5, 29.4, 29.3, 29.0, 26.5, 22.6 ($NCH_2(CH_2)_{16}CH_3$), 30.5 ($SCOCH_3$), 28.6 (C-6), 20.7, 20.5 ($OCOCH_3$), 14.1 ($N(CH_2)_{17}CH_3$). HRESIMS calcd for $C_{35}H_{60}O_9SN_3$ $[M+H]^+$: 698.4050, found: 698.4057.

1.15. Attenuated total reflection (ATR) spectroscopy on films of randomly oriented compounds 1 and 3

Sample films were deposited onto the internal reflection element by evaporation of a 2 mg mL^{-1} solution of either compound **1** or **3** in MeOH. ATR spectra were collected at room temperature on a Nicolet Nexus 870 spectrometer (Madison, WI) equipped with an MCT-A detector (Nicolet, Madison, WI) and a VeeMax II variable angle specular reflectance accessory (Pike Technologies, Madison, WI). The instrumental resolution was 4 cm^{-1}. Spectra were collected at a 45° incident angle with a 45° face angle ZnSe internal reflection element (Pike Technologies, Madison, WI).

1.16. Infrared reflection absorption spectroscopy (IRRAS) on self-assembled mono layers of 1 and 3 on gold

Self-assembled monolayers were obtained by incubating a gold slide in a 2 mg mL^{-1} solution of either compound **1** or **3** in MeOH for 24 h. The gold slide was then rinsed, first with methanol and then with water. IRRAS spectra were collected at room temperature on a Nicolet Nexus 870 spectrometer (Madison, WI) equipped with an MCT-A detector (Nicolet, Madison, WI), a VeeMax II variable angle specular reflectance accessory (Pike Technologies, Madison, WI), and a ZnSe wire-grid polarizer (Pike Technologies, Madison, WI). The instrumental resolution was 4 cm^{-1}. For the CH stretching region, the incident angle was 70°. For the 1500–1000 cm^{-1} region, the incident angle was 80°.

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Supplementary data

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