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Attempts to prepare tethered bilayer lipid membranes using synthetic thioglycolipid anchors: synthesis of 6”-thiotrisaccharide glycolipid analogues and applications.

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Abstract

The synthesis of the three 6”-deoxy-6”-thio glycolipid analogues β-D-Gal-(1→6)-β-D-Gal-(1→4)-β-D-Glu-(1→OCH_2)-[1,2,3]-triazole-1-dodecane, β-D-Gal-(1→4)-β-D-Glu-(1→4)- β-D-Glu-(1→OCH_2)-[1,2,3]-triazole-1-dodecane and β-D-Gal-(1→4)-β-D-Glu-(1→4)-β-D-Glu-(1→OCH_2)-[1,2,3]-triazole-1-octadecane is presented. Glycosylation at position O-4’ of a

propargyl cellobioside glycosyl acceptor and position O-6' of a propargyl lactoside glycosyl acceptor with a 6-deoxy-6-thio galactosyl donor gave rise to two unique trisaccharides that in turn underwent copper-catalyzed azide-alkyne cycloadditions with either 1-azidododecane or 1-azidooctadecane. The potential for each of these analogues to function as tethers of lipid bilayers to Au(111) surface was assessed by differential capacitance experiments. A monolayer of the previously described monosaccharide 1-octadecane-4-(6-thio-β-D-galacto-pyranosyloxymethyl)-[1,2,3]-triazole either self-assembled or prepared by Langmuir-Blodgett (LB) transfer was found to support an outer leaflet monolayer (DMPC/cholesterol, 70:30) deposited by Langmuir-Schaefer (LS) touch. The bilayers obtained with this monosaccharide analogue had minimum differential capacitances of 1.0 and 0.9 μF/cm^2 when the inner monolayer was prepared by self-assembly and LS touch, respectively. Attempts to produce bilayers using the trisaccharides synthesized here were unsuccessful; we are attributing these unsuccessful results mostly to the high water solubility of trisaccharides combined with the relatively short length of the hydrocarbon chains used in this study.

1. Introduction

Since the study of the cell membrane is challenging, research has been directed towards designing simplified models of this system that accurately reflect its structure and function *in vivo*.[1] The leading design in membrane bilayer models is the tethered bilayer lipid membrane (tBLM), in which the bilayer is anchored to a solid support by a tether that supports hydrophilic zones on both sides of the membrane.[1-2] These models are simple to construct and possess long term stability. The challenge is to design a tether that maintains the fluidity (lipid lateral diffusion of 10^{-8} cm^2/s)[3] and insulative properties (a resistance of 10 MΩ/cm^2 and a capacitance below 1 μF/cm^{-2})[4] of a natural membrane without inhibiting the incorporation of membrane

proteins. Tethers are usually made up of three domains: 1. a lipid chain that inserts itself in the inner leaflet of the BLM; 2. an hydrophilic spacer molecule such as an amphiphilic copolymer,[2a] a polyethyleneglycol chain,[2b-e] or a peptide chain[2f-i] that provides the necessary hydrophilic region; 3. an anchoring functional group. Anchoring systems include thiols, sulfides or disulfides on silver or gold and trichloro- or trimethoxysilanes on oxide surfaces such as SiO_2 or TiO_2.[5] Lipoglycopolymeric tethers have been designed[6] but the concept of a purely carbohydrate based tether system is novel. Such a tethering system would mimic the properties of the glycocalyx by providing stabilization through hydrogen bonding networks and maintaining osmotic pressure within the compartment.[6] We have reported a first generation of carbohydrate based[7] tether in which a galactose residue was bound to a single-crystalline Au(111) surface by a sulfur atom at position C-6. Glycolipid analogues **1** and **2** bearing alkyl chains of 12 and 18 carbon units connected at the anomeric position *via* a 1,4-disubstituted triazole linkage were described.[7] Comparison of the attenuated total reflectance infrared spectrum (ATR-IR) of the randomly oriented analogue **1** to the infrared reflection absorption spectrum (IRRAS) of self-assembled **1** on gold suggested that the self-assembled molecules were oriented perpendicular to the gold surface and could potentially be useful to anchor a BLM.[7] However, monosaccharide-based tethers such as **1**, can only provide a water layer at the gold surface that is ~5 Å thick and as such are not and ideal tether molecules to anchor BLMs and study transmembrane proteins. To increase the size of the water interface, we describe here the chemical synthesis of trisaccharide glycolipid analogues **3**–**5**. As schematically proposed on Figure 1, the potential for each of these analogues as well as previously synthesized analogues **1** and **2** to function as tethers of lipid bilayers to Au(111) was assessed by differential capacitance experiments.

2. Results and discussion

2.1. Synthesis of analogues 3–5

The 6-thioacetate bromide glycosyl donor **6** was prepared as described previously[7] and converted in two steps to the trichloroacetimidate **8** (Scheme 1): hydrolysis of bromide **6** in the presence of silver carbonate gave hemiacetal **7** which was treated with trichloroacetonitrile and DBU to give donor **8**. Propargyl lactoside **11** free at O-4' and O-6' as well as propargyl cellobioside glycosyl acceptor **14** free at O-4' were both prepared from the known[8] propargyl lactoside **9** (Scheme 1). Lactoside **9** was protected with a benzylidene acetal at O-4' and O-6' ($PhCH(OMe)_2$/CSA), acetylated to give pentaacetate **10** and the benzylidene acetal was hydrolyzed (80% aq AcOH) to give the desired diol acceptor **11**. A portion of diol **11** was selectively acetylated at O-6' with acetyl chloride to give **12** which was treated with triflic anhydride to yield triflate **13**. Lactoside **13** was then converted to cellobioside **14** using a Lattrell-Dax mediated inversion[9]: treatment with sodium nitrite in DMF followed by an aqueous work up. The desired cellobioside glycosyl acceptor **14** was isolated in acceptable yield (57%). Selective glycosylation at O-6' of diol acceptor **11** to yield trisaccharide **15** was first attempted using the more reactive trichloroacetimidate donor **8** under catalysis with TMSOTf or $BF_3{\cdot}Et_2O$. Unfortunately we observed by TLC that these conditions led to the fast degradation of donor **8** and no trisaccharide could be isolated. Thus, we investigated the coupling of less reactive bromide donor **6** with diol **11**. While under Helfrich [$Hg(CN)_2$] activation at 40 °C, only a low yield of the desired trisaccharide **15** was obtained (22%), under Koenigs-Knorr (AgOTf) activation carefully raising the temperature from 5 to 10 °C in 45 min, the desired trisaccharide **15** was isolated in acceptable yield (55%). It is important to note that these glycosylation reactions were carried out with an excess amount of acceptor. Not surprisingly glycosylation at the less reactive O-4' of acceptor **14** with bromide glycosyl donor **6** under Helferich conditions

[$Hg(CN)_2$, 50 °C] failed to provide the desired trisaccharide. Thus, we carried out the coupling of trichloroacetimidate **8** with acceptor **14**, using conditions that we have established[10] to glycosylate O-4 of *N*-acetylglucosamine acceptors (excess $BF_3{\cdot}Et_2O$, 40 °C, 1.5 h) and obtained trisaccharide **16** in an acceptable 67% yield.

"Click chemistry" or Cu^I-catalyzed azide-alkyne 1,3-dipolar cycloaddition chemistry[11] (CuAAC) was then applied to couple propargyl trisaccharides **15** and **16** with 1-azidododecane[12] and/or 1-azidooctadecane[13]. We first attempted cycloaddition between trisaccharide **15** and 1-azidododecane using the conditions that were successful in our previous study[7] and that involved *in situ* reduction of copper (II) (from copper sulfate) to copper (I) by sodium ascorbate in a mixture of THF and water (11:3) at 25 °C. However, under these conditions the desired analogue **17** was only isolated in a low 21% yield. This yield was increased to 59% by using copper iodide as a source of copper (I), DIPEA as a base, and an excess of azidododecane (3.2 equiv) in anhydrous THF and allowing the reaction to proceed overnight at 25 °C. These same conditions were successful to prepare analogues **18** and **19** which were isolated in 71% and 70% yield, respectively. The three cycloadducts were characterized by HRMS and NMR. The typical signals expected[7, 14] for the 1,4-disubstituted triazole rings were found at 7.46–7.47 ppm in the ^1H spectra and at ~144 and ~122 ppm in the ^{13}C spectra.

Zemplén deacetylation of trisaccharide **17** followed by deionization of the reaction mixture with Dowex® 50WX8-100 resin gave the desired trisaccharide disulfide **3** in a mediocre 45% yield while a UV active impurity was also isolated. In the ^1H NMR spectrum, this impurity showed signals characteristic of divinylbenzene, a constituent of the resin, as well as signals characteristic of disulfide **3**. While this impurity could not be fully characterized we hypothesized that its formation originated from hydrophobic interactions between the long chain

of the analogue and the resin. Since the formation of this side product decreased the yield of desired product we investigated alternate work up procedure and attempted neutralizing the reaction mixture with a 20% solution of methanolic acetic acid. While disulfide **3** was seen as the major product on TLC, a more polar product was also observed and assumed to be the corresponding thiol. Thus, the solvent was evaporated and the residue was dissolved in methanol and stirred at 40 °C for 20 h which led to the air-promoted oxidation of the remaining thiol to disulfide **3** that was obtained pure in 75% yield. The chemical structure of disulfide **3** was confirmed by HRMS and NMR. ^{1}H NMR spectroscopy of disulfide **3** showed signals corresponding to H-6a" and H-6b" between 3.00 to 3.03 ppm which in the HSQC correlated to a ^{13}C found at 41.2 ppm. These signals are in agreement with those found for the disulfide of thiol **1** that we have reported.[7] Deacetylation of trisaccharides **18** and **19** was then carried out following the same procedure and work up than established to prepare disulfide **3**. The glycolipid **4** was purified by column chromatography and isolated in 70% yield. Interestingly, sodium acetate could be removed from the crude hydrophobic disulfide **5** by dissolving it in chloroform and washing with water. In turn the chloroform was evaporated and column chromatography gave the C-18 analogue **5** in 75% yield. The assembly of these analogues on gold and their potential as tethering molecules in the formation of artificial bilayer lipid membrane was then studied.

2.2. Monolayers and bilayers obtained using analogues 1–5 as inner leaflets.

2.2.1. Construction of bilayers by self-assembled (inner leaflet) followed by LS touch (outer leaflet). Monolayers of analogues **1**–**5** on Au(111) surface were prepared by self-assembly and the minimum capacitance of these monolayers was measured (Table 1). All the self-assembled monolayers (SAM) had minimum capacitances that were lower than that of bare gold (25 μF/cm^2

at 0.0 V) but, except for analogue **2**, these values were still much greater than the desired 1 μF/cm^2. We proceeded depositing a second monolayer of DMPC/cholesterol (70:30) by LS touch and measured the minimum capacitance of these lipid bilayers (Table 1). As can be seen in table 1, the minimum capacitance did not vary significantly for analogues **1**, **3** and **4** suggesting that the DMPC/cholesterol monolayers did not transfer or were defective. While the bilayer obtained when using the SAM of analogue **5** as inner leaflet showed a significant decrease in the minimum capacitance, this value (7.1 μF/cm^2) remained much greater than that desired of about 1 μF/cm^2. In contrast, analogue **2** produced a tethered bilayer that had the desired minimum differential capacitance of 1 μF/cm^2.

2.2.2. Construction of bilayers by LB transfer (inner leaflet) followed by LS touch (outer leaflet). We thus investigated if the preparation of the inner layers by LB transfer using analogues **2** and **5** that were most promising, would lead to a better supported bilayer. We have shown by IRRAS that analogue **1** was able to organize itself perpendicular to the gold surface.[7] Thus, despite the poor results obtained (see above) when monosaccharide **1** was used as the self-assembled inner layer, we included this analogue in the following study. A surface pressure of 35 mN/m was determined to be ideal for the Langmuir-Blodgett transfer of analogues **1**, **2** and **5** onto the gold surface. For analogue **1**, LB transfer produced a monolayer with a minimum capacitance of 9.8 μF/cm^2, which decreased to 5.6 μF/cm^2 upon assembly of the outer layer by LS touch (Table 2). This decrease in capacitance is evidence for successful assembly of a bilayer although of poor quality since the desired minimum capacitance of 1 μF/cm^2 was still not achieved. While the monolayer obtained by LB transfer for the more hydrophobic glycolipid monosaccharide **2** had a higher minimum capacitance than that obtained through self-assembly of the same analogue (compare Table 1 and Table 2, entries 2), the bilayer obtained by LS touch

was of excellent quality with a minimum capacitance of 0.9 μF/cm^2. The differential capacitance curves for the two bilayers obtained with analogue **2** (Figure 2) displayed a flat area in the target potential window (-0.40 V to +0.45 V), which is evidence of a compact monolayer with excellent surface coverage.[2j, k] In contrast, the results obtained for analogue **5** were disappointing. While the monolayer produced from the LB transfer of analogue of **5** showed a lower minimum capacitance than the self-assembled monolayer for this analogue (compare Table 1, entry 6 and Table 2, entry 3), the insignificant decrease in minimum capacitance observed upon LS touch to add the outer layer indicated that a bilayer did not form.

2.2.3 IRRAS studies on analogues 3 and 4. To attempt explaining our lack of success in producing bilayer lipid membranes using trisaccharides **3**–**5**, we assessed by IRRAS and ATR-IR spectroscopy the orientation that analogues **3** and **4** adopt when allowed to self-assemble on gold. The IRRAS spectrum of **3** self-assembled on gold was acquired and compared to a spectrum of the randomly oriented analogue acquired by ATR-IR (Figure 3). As can be seen on Figure 3, the relative intensity of the $\nu_{as}(CH_2)$ *vs.* $\nu_{as}(CH_3)$ bands (located at ~2926 cm^{-1} and ~2961 cm^{-1}, respectively) in the IRRAS spectrum of the self-assembled monolayer of **3** was the same as that measured by ATR for a random film. This indicated that trisaccharide **3** did not orient itself perpendicular to the gold but rather was adsorbed in a random fashion.[15] The increased flexibility around the non-reducing end β-D-Gal-(1→6)-D-Gal glycosidic bond may explain the lack of orientation of this trisaccharide on gold. IRRAS of a self-assembled monolayer of **4** on gold did not lead to any detectable IR bands indicating that the self-assembled analogue provided poor surface coverage. This result may reflect mixed orientations of the analogue on the gold surface combined with steric effects. This finding may explain why no tBLMs were obtained when the inner leaflets were prepared *via* self-assembly of analogues **3**, **4**

or **5** (Table 1 entries 3 and 4). It is interesting to point out however that the successful perpendicular orientation of analogue **1** which we have demonstrated[7] using the same combination of IR-based experiments did not warrant success in forming a tBLM using a SAM of analogue **1** as the inner leaflet (Table 1, entry 1).

3. Conclusion

Monosaccharide **2** produced the best quality monolayers by both self-assembly and LB transfer and may thus be considered as a promising tethering molecule. Although bilayers were produced by LS touch of outer layers on inner layers of self-assembled trisaccharide **5** and LB-transferred monosaccharide **1**, these bilayers were of poor quality. The experiments demonstrated that the monolayer of trisaccharide **5** deposited by LB transfer was better for bilayer assembly than the monolayer prepared by self-assembly. However, the high water solubility of the trisaccharide was a major barrier to producing monolayers of high quality *via* this method. Analogues **1** and **2** only differ by the length of the glycosidic hydrocarbon chain, C-12 and C-18, respectively. Thus the successful formation of tBLMs when using analogue **2** compared to the poor results obtained with analogue **1** indicate that the hydrocarbon chain must be at least 18 carbons long. However, the poor results that we obtained when using the C-18 trisaccharide **5** as the inner leaflet, supports the need for a structure that has balanced properties between the hydrophilicity of the sugar and the hydrophobicity of the hydrocarbon chain. Further synthetic efforts are on-going in our laboratory to prepare oligosaccharides connected to a C-20 phytanyl or a C-40 glycerol diphytanyl architecture.[2k, 16] These analogues will have the thiol positioned at C-4 instead of C-6 so that their orientation with respect to the gold surface is similar to that of 1-β-D-thioglucose, which is known to orient perpendicular to the substrate.[17]

4. Experimental Section

4.1. General Methods

^{1}H NMR (300.13, 400.13 or 600.13 MHz) and ^{13}C NMR (75.5, 100.6 or 150.9 MHz) spectra were recorded in $CDCl_3$ (internal standard, for ^{1}H residual $CHCl_3$ δ 7.24; for ^{13}C $CDCl_3$ δ 77.0) or CD_3OD (internal standard, for ^{1}H residual CD_2HOD δ 3.30; for ^{13}C CD_3OD δ 49.0). Chemical shifts (ppm) and coupling constants (*J*, Hz) were obtained from a first-order analysis of one-dimensional spectra and assignments of protons and carbon resonances were based on two dimensional ^{1}H-^{1}H and ^{13}C-^{1}H correlation experiments as well as 1D TOCSY experiments. ^{1}H NMR data are reported using standard abbreviations: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broadened (b). TLC was performed on aluminum plates precoated with Silica Gel 60 (250 μm) containing a fluorescent indicator. The plates were visualized under UV and/or 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,[18] and organic solutions were dried over Na_2SO_4 and concentrated under reduced pressure below 40 °C. Reversed-phase HPLC purifications were carried out on a Prep Nova Pak® HR C18, 6 μm 60 Å (25 x 100 mm) column using mixtures of MeCN and water as eluant. Optical rotations were measured at 23 °C and are 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. Au(111) electrodes were rinsed with Milli-Q water, flame annealed, allowed to cool then rinsed again and air dried. The area of the Au(111) surface was 0.22 cm^2. Glassware and electrochemical cells were rinsed with Milli-Q ultra-pure water then submerged in a solution of potassium permanganate (4 g $KMnO_4$ per 1 L of

Milli-Q water) overnight. Glassware was then rinsed with a hydrogen peroxide solution (20 mL H_2O_2 in 250 mL Milli-Q water) followed by Milli-Q water and dried in an oven at 80 °C. 1,2-Dimyristoyl-sn-glycerol-3-phosphocholine (DMPC) and cholesterol (chol) were purchased from *Sigma-Aldrich* (99%).

4.2. 2,3,4-Tri-*O*-acetyl-6-*S*-acetyl-6-deoxy-6-thio-α,β-D-galactopyranose (7)

Known[7] bromide **6** (1.72 g, 4.03 mmol) was dissolved in a mixture of acetone (8.8 mL) and water (8.8 mL), silver carbonate (0.830 g, 3.01 mmol, 0.75 equiv) was added and the reaction mixture was stirred at rt for 30 min. The solids were filtered off over a pad of Celite® and washed with acetone (20 mL). The filtrate was diluted with CH_2Cl_2 (100 mL) and washed successively with H_2O (100 mL), satd aq $NaHCO_3$ (100 mL) and 2 M aq HCl (100 mL). The aq layers were re-extracted with CH_2Cl_2 (2 × 100 mL) and the combined organic layers were dried and concentrated. Chromatography of the crude product (1:1, EtOAc-hexanes) gave hemiacetal **7** (1.13 g, 77%, α/β ratio 3:2) pure as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, 297 K): δ 5.52–5.50 (m, 2H, H-1α, H-4α), 5.45–5.44 (m, 1H, H-4β), 5.40 (dd, 1H, *J* = 3.3, 10.9 Hz, H-2α), 5.16 (dd, 1H, *J* = 3.4, 10.8 Hz, H-3α), 5.08–5.06 (m, 2H, H-2β, H-3β), 4.68 (dd, 1H, *J* = 8.6, 15.2 Hz, H-1β), 4.28 (m, 1H, H-5α), 3.78–3.74 (m, 1H, H-5β), 3.67 (d, 1H, *J* = 8.8 Hz, OH-1β), 3.19 (d, 1H, *J* = 1.1 Hz, OH-1α), 3.16–2.97 (m, 4H, H-6α, H-6β), 2.36 (s, 6H, α,β-SCOC*H*$_3$), 2.20, 2.12, 2.01 (3s, 18H, α,β-OCOC*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$, 297 K): δ 194.7, 194.6, 171.3, 170.4, 170.3, 170.3, 170.0, 170.0 (α,β-C=O × 8), 95.9 (C-1β), 90.7 (C-1α), 72.3 (C-5β), 71.0 (C-3β), 70.5 (C-2β), 68.9 (C-4α), 68.2 (C-2α or C-3α), 67.9 (C-4β), 67.6 (C-5α), 67.4 (C-2α or C-3α), 30.5, 30.4 (α,β-SCO*C*H$_3$), 28.4 (C-6α, C-6β), 20.9, 20.7 (α,β-OCO*C*H$_3$ × 6). HRESIMS calcd for $C_{14}H_{20}O_9S$ $[M+Na]^+$: 387.0726, found 387.0717.

4.3. 2,3,4-Tri-*O*-acetyl-6-*S*-acetyl-6-thio-α-D-galactopyranosyl trichloroacetimidate (8)

Hemiacetal **7** (1.07 g, 2.94 mmol) was dissolved in anhyd CH_2Cl_2 (15 mL) under N_2, trichloroacetonitrile (0.880 mL, 8.81 mmol, 3 equiv) followed by DBU (110 μL, 0.73 mmol, 0.25 equiv) were added and the reaction mixture was stirred for 3 h at rt. The solvent was evaporated and the residue was purified by chromatography (3:7, EtOAc:hexanes containing 0.1% triethylamine) to give trichloroacetimidate **8** (1.19 g, 79%) pure as a white foam. $[\alpha]_D$ 92° (*c* 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, 297 K): δ 8.64 (s, 1H, N*H*), 6.55 (d, 1H, *J* = 3.4 Hz, H-1), 5.55 (dd, 1H, *J* = 1.08, 3.0 Hz, H-4), 5.39–5.29 (m, 2H, H-2, H-3), 4.21–4.20 (m, 1H, H-5), 3.09–2.92 (m, 2H, H-6), 2.28 (s, 3H, SCOC*H*$_3$), 2.16, 2.00, 1.99 (3s, 9H, OCOC*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ 194.3, 170.2, 170.1, 169.9, 160.9 (C=O × 4), 93.4 (C-1), 70.3 (C-4), 68.2 (C-3), 67.8 (C-2), 66.8 (C-5), 30.4 (SCO*C*H$_3$), 28.4 (C-6), 20.5 (OCO*C*H$_3$ × 3). HRESIMS calcd for $C_{16}H_{20}O_9NSCl_3$ $[M+Na]^+$: 529.9822, found 529.9819.

4.4. 2-Propyn-1-yl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (10)

Known[8] propargyl lactoside **9** (2.81 g, 7.39 mmol) was suspended in anhyd acetonitrile (281 mL) under N_2. Benzaldehyde dimethyl acetal (5.01 mL, 33.2 mmol, 4.5 equiv) was added followed by CSA (1.12 g, 4.82 mmol, 0.65 equiv) and the reaction mixture was stirred at 70 °C for 2 h. The reaction was quenched with Et_3N (0.720 mL, 5.17 mmol, 0.7 equiv) and the solvent was evaporated. Chromatography (5:95 MeOH:CH_2Cl_2) gave the benzylidene acetal (2.14 g) intermediate which was dissolved in a 1:1 mixture of acetic anhydride and pyridine (33 mL). The reaction mixture was stirred at 50 °C for 45 min and concentrated, residual pyridine was co-evaporated with toluene (20 mL) and the oily residue was dissolved in CH_2Cl_2 (50 mL) and

washed successively with 2 M aq HCl (50 mL) and satd aq $NaHCO_3$ (50 mL). The aq layers were re-extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic layers were dried and concentrated. Chromatography (1:1, EtOAc-hexanes) gave disaccharide **10** (2.86 g, 57%) pure as a colourless glass. $[\alpha]_D$ 22° (*c* 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, 295 K): δ 7.45–7.42 (m, 2H, Ar), 7.36–7.35 (m, 3H, Ar), 5.45 (s, 1H, C*H* acetal), 5.26–5.20 (m, 2H, H-2', H-3), 4.93 (dd, 1H, *J* = 7.9, 9.7 Hz, H-2), 4.85 (dd, 1H, *J* = 3.6, 10.3 Hz, H-3'), 4.72 (d, 1H, *J* = 8.0 Hz, H-1), 4.51 (dd, 1H, *J* = 2.0, 12.0 Hz, H-6a), 4.44 (d, 1H, *J* = 7.9 Hz, H-1'), 4.32–4.30 (m, 4H, OC*H*$_2$C≡CH, H-4', H-6a'), 4.10 (dd, 1H, *J* = 5.0, 12.1 Hz, H-6b), 4.02 (dd, 1H, *J* = 1.5, 12.4 Hz, H-6b'), 3.80 (t, 1H, *J* = 9.7 Hz, H-4), 3.63–3.59 (m, 1H, H-5), 3.44 (s, 1H, H-5'), 2.43 (t, 1H, *J* = 2.4 Hz, C≡C*H*), 2.10, 2.02, 2.02, 2.01 (5s, 15H, OCOC*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$, 296 K): δ 170.8, 170.4, 170.2, 169.8, 168.9 (C=O × 5), 137.4 (Ar quaternary), 129.2, 128.3, 126.5 (Ar), 101.4 (CH acetal), 101.1 (C-1'), 98.1 (C-1), 78.1 (*C*≡CH), 75.9 (C-4), 75.5 (C≡*C*H), 73.1 (C-4'), 73.0 (C-5), 72.3 (C-3), 72.1 (C-3'), 71.1 (C-2), 69.0 (C-2'), 68.4 (C-6'), 66.5 (C-5'), 61.8 (C-6), 55.9 (O*C*H$_2$C≡CH), 20.9, 20.9, 20.8, 20.7, 20.7 (OCO*C*H$_3$ × 5). HRESIMS calcd for $C_{32}H_{38}O_{16}$ $[M+NH_4]^+$: 696.2504, found 696.2527.

4.5. 2-Propyn-1-yl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3-di-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (11)

Benzylidene actetal **10** (910 mg, 1.34 mmol) was stirred in 80% aq AcOH (50 mL) at 100 °C for 1 h and the mixture was then co-concentrated with toluene (2 × 100 mL). The residue was dissolved in CH_2Cl_2 (200 mL) and washed with satd aq $NaHCO_3$ (200 mL). The aq layers were re-extracted with CH_2Cl_2 (3 × 100 mL). The organic layers were combined, dried and concentrated. Chromatography (5:95, MeOH-CH_2Cl_2) gave diol **11** (595 mg, 75%) pure as a colourless glass. $[\alpha]_D$ –21° (*c* 0.5, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, 297 K): δ 5.22–5.14 (m,

2H, H-3, H-2'), 4.91–4.83 (m, 2H, H-2, H-3'), 4.70 (d, 1H, *J* = 7.8 Hz, H-1), 4.50–4.46 (m, 2H, H-1', H-6a), 4.30 (t, 2H, *J* = 2.3 Hz, OC*H*$_2$C≡CH), 4.10–4.04 (m, 2H, H-6b, H-4'), 3.89–3.78 (m, 3H, H-4, H-6a', H-6b'), 3.66–3.61 (m, 1H, H-5), 3.55–3.52 (m, 1H, H-5'), 3.25, 2.75 (bs, 1H, OH-4', OH-6'), 2.43 (t, 1H, *J* = 2.4 Hz, C≡C*H*), 2.08, 2.08, 2.05, 2.05, 2.04 (5s, 15H, OCOC*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$, 297 K): δ 170.5,170.5, 170.2, 169.8, 169.4 (C=O × 5), 101.0 (C-1'), 97.8 (C-1), 78.0 (*C*≡CH), 76.1 (C-4), 75.4 (C≡*C*H), 74.4 (C-5'), 73.4 (C-3'), 73.1 (C-3), 72.7 (C-5), 71.2 (C-2), 69.6 (C-2'), 67.8 (C-4'), 62.1 (C-6, C-6'), 55.8 (O*C*H$_2$C≡CH), 20.9, 20.8, 20.7, 20.7, 20.6 (OCO*C*H$_3$ × 5). HRESIMS calcd for $C_{25}H_{34}O_{16}$ $[M+NH_4]^+$: 608.2191, found 608.2175.

4.6. 2-Propyn-1-yl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (12)

Diol **11** (776 mg, 1.32 mmol) and collidine (3.5 mL, 2.68 mmol, 20 equiv) were stirred in anhyd CH_2Cl_2 (26 mL) under N_2 at –35 °C for 20 min. AcCl (234 μL, 3.28 mmol, 2.5 equiv) was added and the reaction mixture was allowed to warm up slowly to –10 °C in one h. More AcCl (47 μL, 0.5 equiv) was added and the reaction was kept at –10 °C for one hour. MeOH (5 mL) was added and the solvents were co-evaporated with toluene (2 × 100 mL). The residue was dissolved in CH_2Cl_2 (200 mL) and washed successively with 2 M aq HCl (100 mL) and satd aq $NaHCO_3$ (100 mL). The aq layers were re-extracted with CH_2Cl_2 (2 × 100 mL) and the combined organic layers were dried and concentrated. Chromatography (6:4, EtOAc-hexanes) gave disaccharide **12** (546 mg, 66%) pure as a colourless glass. $[\alpha]_D$ –22° (*c* 0.5, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, 295 K): δ 5.19–5.11 (m, 2H, H-3, H-2'), 4.90–4.81 (m, 2H, H-2, H-3'), 4.69 (d, 1H, *J* = 7.8 Hz, H-1), 4.46 (dd, 1H, *J* = 1.8, 12.0 Hz, H-6a), 4.39 (d, 1H, *J* = 1.8, 7.9 Hz, H-1'), 4.29 (t, 2H, *J* = 2.4 Hz, OC*H*$_2$C≡CH), 4.26–4.18 (m, 1H, H-6a'), 4.10–4.05 (m, 2H, H-6b, H-6b'), 3.97 (s, 1H,

H-4'), 3.75 (t, 1H, *J* = 9.6 Hz, H-4), 3.69–3.66 (m, 1H, H-5'), 3.63–3.59 (m, 1H, H-5), 2.82 (s, 1H, OH-4'), 2.42 (t, 1H, *J* = 2.4 Hz, C≡C*H*), 2.08, 2.06, 2.03, 2.01, 2.00 (6s, 18H, $OCOCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$, 297 K): δ 170.7, 169.1 (C=O × 6), 100.8 (C-1'), 97.8 (C-1), 78.0 (*C*≡CH), 76.0 (C-4), 75.3 (C≡*C*H), 73.1 (C-3'), 72.7 (C-5), 72.4 (C-3), 71.9 (C-5'), 71.1 (C-2), 69.3 (C-2'), 66.6 (C-4'), 61.8 (C-6, C-6'), 55.7 (OCH_2C≡CH), 20.7, 20.7, 20.6, 20.5 ($OCOCH_3$ × 6). HRESIMS calcd for $C_{27}H_{36}O_{17}$ $[M+NH_4]^+$: 650.2296, found 650.2308.

4.7. 2-Propyn-1-yl 2,3,6-tri-*O*-acetyl-4-O-{2,3,6-tri-O-acetyl-4-*O*-[(trifluoromethyl)sulfonyl]-β-D-galactopyranosyl}-β-D-glucopyranoside (13)

Disaccharide **12** (215 mg, 0.340 mmol) was dissolved in anhyd CH_2Cl_2 (1.6 mL) and anhyd pyridine (192 μL) under N_2 and the mixture was cooled to –20 °C. Triflic anhydride (114 μL, 0.680 mmol, 2 equiv) was added dropwise, the reaction mixture was stirred at rt for 30 min diluted with CH_2Cl_2 (50 mL) and washed successively with 2 M aq HCl (50 mL) and satd aq $NaHCO_3$ (50 mL). The aq layers were re-extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic layers were dried and concentrated to give triflate **13** (221 mg, 85%) pure as a white foam. $[\alpha]_D$ –27° (*c* 1.0, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$, 295 K): δ 5.21–5.16 (m, 2H, H-3, H-4'), 5.11 (dd, 1H, *J* = 7.7, 10.4 Hz, H-2'), 5.05 (dd, 1H, *J* = 3.0, 10.4 Hz, H-3'), 4.89 (dd, 1H, *J* = 7.9, 9.4 Hz, H-2), 4.71 (d, 1H, *J* = 7.9 Hz, H-1), 4.51 (d, 1H, 7.6 Hz, H-1'), 4.46 (dd, 1H, *J* = 2.04, 12.0 Hz, H-6a), 4.35 (dd, 1H, *J* = 3.5, 8.8 Hz, H-6a'), 4.30 (t, 2H, *J* = 2.4 Hz, OCH_2C≡CH), 4.06 (dd, 1H, *J* = 4.8, 12.0 Hz, H-6b), 3.98–3.91 (m, 2H, H-5', H-6b'), 3.78 (t, 1H, *J* = 9.6 Hz, H-4), 3.62–3.59 (m, H-5), 2.43 (t, 1H, *J* = 2.4 Hz, C≡C*H*), 2.10, 2.08, 2.07, 2.03, 2.03, 2.03, 2.00 (6s, 18H, $OCOCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$, 296 K): δ 170.1, 169.8, 169.5, 169.4, 168.4 (C=O × 6), 100.7 (C-1'), 97.7 (C-1), 79.8 (C-4'), 77.8 (*C*≡CH), 75.9 (C-4), 75.3 (C≡*C*H), 72.5 (C-5), 72.2 (C-3), 71.0 (C-2), 69.8 (C-5'), 69.6 (C-3'), 68.2 (C-2'), 61.5 (C-

6), 60.0 (C-6'), 55.7 (O*C*H$_2$C≡CH), 20.6, 20.5, 20.4, 20.3, 20.2 (OCO*C*H$_3$ × 6). HRESIMS calcd for $C_{28}H_{35}O_{19}SF_3$ $[M+NH_4]^+$: 782.1789, found 782.1824.

4.8. 2-Propyn-1-yl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside (14)

Triflate **13** (221 mg, 0.290 mmol) and $NaNO_3$ (242 mg, 3.50 mmol, 12 equiv) were stirred in anhyd DMF (17 mL) at 50 °C for 20 h under N_2. The solvent was evaporated and the residue, dissolved in CH_2Cl_2 (50 mL), was washed with brine (50 mL). The aq layer was re-extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic layers were dried and concentrated. Chromatography (6:4, EtOAc-hexanes) gave propargyl cellobioside **14** (105 mg, 57%) pure as a white foam. $[\alpha]_D$ –53° (*c* 0.5, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, 295 K): δ 5.17 (t, 1H, *J* = 9.4 Hz, H-3), 4.99–4.80 (m, 3H, H-2, H-2', H-3'), 4.70 (d, 1H, *J* = 7.9 Hz, H-1), 4.58–4.49 (m, 2H, H-6a, H-6a'), 4.44 (d, 1H, *J* = 7.9 Hz, H-1'), 4.30 (t, 2H, *J* = 2.4 Hz, OC*H*$_2$C≡CH), 4.18 (dd, 1H, *J* = 1.9, 12.5 Hz, H-6b'), 4.12–4.05 (m, 1H, H-6b), 3.76 (t, 1H, *J* = 9.6 Hz, H-4), 3.60–3.56 (m, 1H, H-5), 3.50–3.41 (m, 2H, H-4', H-5'), 3.17 (bs, 1H, OH-4'), 2.42 (t, 1H, *J* = 2.4 Hz, C≡C*H*), 2.11, 2.10, 2.03, 2.02, 2.01 (5s, 18H, OCOC*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$, 296 K): δ 171.9, 171.2, 170.3, 169.9, 169.8, 169.3 (C=O × 6), 100.9 (C-1'), 97.9 (C-1), 78.1 (*C*≡CH), 76.4 (C-4), 75.5 (C≡*C*H), 75.1 (C-3'), 74.3 (C-5'), 72.9 (C-5), 72.5 (C-3), 71.5 (C-2'), 71.2 (C-2), 68.2 (C-4'), 62.6 (C-6'), 61.7 (C-6), 55.9 (O*C*H$_2$C≡CH), 20.9, 20.8, 20.7, 20.6, 20.6 (OCO*C*H$_3$ × 6). HRESIMS calcd for $C_{27}H_{36}O_{17}$ $[M+NH_4]^+$: 650.2296, found 650.2292.

4.9. 2-Propyn-1-yl 2,3,6-tri-*O*-acetyl-4-*O*-[2,3-di-*O*-acetyl-6-*O*-(2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (15)

Bromide **6** (81 mg, 0.189 mmol), diol **11** (144 mg, 0.244 mmol, 1.2 equiv) and activated powdered MS 4 Å (127 mg) were stirred in anhyd CH_2Cl_2 (8 mL) under N_2 for 15 min at 5 °C. AgOTf (37 mg, 0.143 mmol, 0.8 equiv) was added and the reaction mixture was allowed to warm to 10 °C over 45 min. The mixture was diluted with CH_2Cl_2 (~10 mL), the solids filtered off on Celite® and washed with CH_2Cl_2 (~20 mL). The combined filtrate and washings were washed with satd aq $NaHCO_3$ (30 mL), the aq layer was re-extracted with CH_2Cl_2 (3 × 30 mL) and the organic layers were combined, dried and concentrated. Fash chromatography (6:4 EtOAc-hexanes) followed by reverse phase HPLC (7:3 to 3:7, H_2O-CH_3CN) gave trisaccharide **15** (97 mg, 55%) pure as a white foam. $[\alpha]_D$ 45° (*c* 0.5, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, 296 K): δ 5.48 (d, 1H, *J* = 3.2 Hz, H-4”), 5.20–5.09 (m, 3H, H3, H2’, H2”), 4.97 (d, 1H, *J* = 10.4 Hz, H3”), 4.90–4.83 (m, 2H, H-2, H-3’), 4.70 (d, 1H, *J* = 7.9 Hz, H-1), 4.49–4.45 (m, 2H, H-1’, H-6a), 4.40 (d, 1H, *J* = 7.8 Hz, H-1”), 4.30 (t, 2H, *J* = 2.3 Hz, OC*H*$_2$C≡CH), 4.10–4.05 (m, 2H, H-4’, H-6b), 4.00 (dd, 1H, *J* = 6.7, 10.3 Hz, H-6a’), 3.79–3.74 (m, 2H, H-6b’, H-4), 3.70–3.66 (m, 1H, H-5”), 3.61–3.58 (m, 2H, H-5’, H-5), 3.07–2.96 (m, 2H, H-6a”, H-6b”), 2.52 (bs, 1H, OH-4”), 2.43 (t, 1H, *J* = 2.4 Hz, C≡C*H*), 2.32 (s, 3H, SCOC*H*$_3$), 2.09, 2.05, 2.05, 2.03, 2.01, 2.01, 2.01, 2.00 (8s, 24H, OCOC*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$, 297 K): δ 194.6, 170.5, 170.3, 170.1, 170.0, 169.8, 169.7, 169.6, 169.3 (C=O × 9), 101.0 (C-1’), 100.9 (C-1”), 97.9 (C-1), 78.1 (*C*≡CH), 75.9 (C-4), 75.4 (C≡*C*H), 73.2 (C-3’, C-5, C-5’), 72.8 (C-3), 72.4 (C-5”), 71.2 (C-2), 70.9 (C-3”), 69.6 (C-2”), 68.5 (C-2”), 68.0 (C-4”), 67.2 (C-6’), 66.6 (C-4’), 61.9 (C-6), 55.9 (O*C*H$_2$C≡CH), 30.5 (SCO*C*H$_3$), 28.6 (C-6”), 20.9, 20.9, 20.8, 20.7, 20.6, 20.5 (OCO*C*H$_3$ × 8). HRESIMS calcd for $C_{39}H_{51}O_{24}S$ $[M+NH_4]^+$: 954.2913, found 954.2902.

4.10. 2-Propyn-1-yl 2,3,6-tri-*O*-acetyl-4-*O*-[2,3,6-tri-O-acetyl-4-*O*-(2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-glucopyranoside (16)

Trichloroacetimidate **8** (305 mg, 0.60 mmol, 4 equiv) and propargyl cellobioside **14** (95 mg, 0.12 mmol, 1 equiv) were dissolved in anhyd CH_2Cl_2 (3.7 mL) under N_2 and stirred at 40 °C. $BF_3{\cdot}Et_2O$ (0.029 mL, 0.18 mmol, 1.5 equiv) was added and the reaction mixture was stirred at 40 °C for 1.5 h. The reaction was quenched with Et_3N (45 μL) and the mixture was diluted with CH_2Cl_2 (10 mL) then washed with satd aq $NaHCO_3$ (10 mL). The aq layers were re-extracted with CH_2Cl_2 (3 × 10 mL) and the combined organic layers were dried and concentrated. Flash chromatography (1:1 to 7:3, EtOAc-hexanes) gave trisaccharide **16** (97 mg, 67%) pure as a colourless glass. $[\alpha]_D$ –10° (*c* 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, 295 K): δ 5.34 (d, 1H, *J* = 3.60 Hz, H-4"), 5.20–5.11 (m, 2H, H-3, H-3'), 5.03 (dd, 1H, *J* = 7.8, 10.4 Hz, H-2"), 4.91–4.82 (m, 3H, H-2, H-2', H-3"), 4.69 (d, 1H, *J* = 7.92 Hz, H-1), 4.52 (dd, 1H, *J* = 1.8, 12.0 Hz, H-6a), 4.47 (d, 1H, *J* = 7.9 Hz, H-1'), 4.33 (d, 1H, *J* = 7.8 Hz, H-1"), 4.32–4.30 (m, 3H, H-6a', OC*H*$_2$C≡CH), 4.10–4.05 (m, 2H, H-6b, H-6b'), 3.80–3.73 (m, 2H, H-4, H-4'), 3.61–3.56 (m, 3H, H-5, H-5', H-5"), 3.00–2.98 (m, 2H, H-6a", H-6b"), 2.42 (t, 1H, *J* = 2.3 Hz, C≡C*H*), 2.33 (s, 3H, SCOC*H*$_3$), 2.13, 2.11, 2.11, 2.04, 2.02, 2.01, 1.96, 1.92 (8s, 27H, OCOC*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$, 297 K): δ 194.3, 170.1, 170.0, 169.8, 169.6, 169.6, 169.1 (C=O × 10), 100.5 (C-1"), 100.3 (C-1'), 97.7 (C-1), 77.9 (*C*≡CH), 76.1 (C-4, C-4'), 75.2 (C≡*C*H), 72.6, 72.5 (C-3, C-3'), 72.7, 72.1, 72.1 (C-5, C-5', C-5"), 71.7 (C-2'), 71.0 (C-2), 70.9 (C-3"), 68.8 (C-2"), 67.1 (C-4"), 62.0 (C-6'), 61.4 (C-6), 55.6 (O*C*H$_2$C≡CH), 30.3 (SCO*C*H$_3$), 28.0 (C-6"), 20.7, 20.6, 20.5, 20.4, 20.3, 20.3 (OCO*C*H$_3$ × 9). HRESIMS calcd for $C_{41}H_{53}O_{25}S$ $[M+NH_4]^+$: 996.3019, found 996.2994.

4.11. 1-Dodecane-4-{2,3,6-tri-*O*-acetyl-4-*O*-[2,3-di-*O*-acetyl-6-*O*-(2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranosyloxymethyl}-[1,2,3]-triazole (17)

Trisaccharide **15** (21 mg, 0.022 mmol) and 1-azidododecane[12] (16 mg, 0.076 mmol, 3.4 equiv) were dissolved in anhyd THF (1.5 mL) under N_2 then CuI (1.6 mg, 0.008 mmol, 0.38 equiv) was introduced followed by DIPEA (10 μL, 0.057 mmol, 2.6 equiv). The reaction mixture was stirred at 25 °C for 20 h then the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (10 mL) and washed successively with satd aq NH_4Cl (2 × 10 mL) and satd aq $NaHCO_3$ (2 × 10 mL). The aq layer was re-extracted with CH_2Cl_2 (4 × 10 mL) and the organic layers were combined, dried and concentrated. Chromatography (7:3, EtOAc-hexanes) gave triazole **17** (15 mg, 59%) pure as a white foam. $[\alpha]_D$ 113° (*c* 0.5, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, 295 K): δ 7.47 (s, 1H, =CH), 5.38 (d, 1H, *J* = 2.7 Hz, H-4"), 5.17–5.10 (m, 3H, H-3, H-2', H-2"), 4.98 (d, 1H, *J* = 10.4 Hz, H-3"), 4.91–4.84 (m, 3H, H-2, H-3', OC*H*$_a$H$_b$), 4.77 (d, 1H, *J* = 12.6 Hz, OCH$_a$*H*$_b$), 4.60 (d, 1H, *J* = 7.9 Hz, H-1), 4.50–4.47 (m, 2H, H-1', H-6a), 4.42 (d, 1H, *J* = 7.9 Hz, H-1"), 4.31 (t, 2H, *J* = 7.2 Hz, NC*H*$_2$(CH$_2$)$_{10}$CH$_3$), 4.11–4.07 (m, 2H, H-6b, H-4'), 4.00 (dd, 1H, *J* = 6.8, 10.3 Hz, H-6a'), 3.79–3.74 (m, 2H, H-4, H-6b'), 3.71–3.67 (m, 1H, H-5"), 3.62–3.59 (m, 2H, H-5, H-5'), 3.08–2.97 (m, 2H, H-6a", H-6b"), 2.38 (bs, 1H, OH-4"), 2.32 (s, 3H, SCOC*H*$_3$), 2.16, 2.10, 2.06, 2.05, 2.03, 2.02, 1.96, 1.95 (8s, 24H, OCOC*H*$_3$), 1.86 (t, 2H, *J* = 7.3 Hz, NCH$_2$C*H*$_2$(CH$_2$)$_9$CH$_3$), 1.29–1.23 (m, 18H, NCH$_2$CH$_2$(C*H*$_2$)$_9$CH$_3$), 0.85 (t, 3H, *J* = 6.7 Hz, N(CH$_2$)$_{11}$C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$, 297 K): δ 194.7, 170.1, 169.9, 169.2 (C=O × 9), 144.1(*C*=CH), 122.3 (C=*C*H), 101.0 (C-1'), 100.8 (C-1"), 99.7 (C-1), 75.8 (C-4), 73.2 (C-3'), 73.1 (C-5, C-5'), 72.8 (C-3), 72.3 (C-5"), 71.5 (C-2), 70.8 (C-3"), 69.6 (C-2'), 68.6 (C-2"), 68.0 (C-4"), 67.2 (C-6'), 66.6 (C-4'), 63.1 (O*C*H$_2$), 61.9 (C-6), 50.4 (N*C*H$_2$(CH$_2$)$_{10}$CH$_3$), 31.9 (NCH$_2$CH$_2$(*C*H$_2$)$_9$CH$_3$), 30.5 (SCO*C*H$_3$), 30.3 (NCH$_2$*C*H$_2$(CH$_2$)$_9$CH$_3$), 29.6, 29.5, 29.3, 29.3, 29.0 (NCH$_2$CH$_2$(*C*H$_2$)$_9$CH$_3$), 28.6 (C-6"), 26.5, 22.6 (NCH$_2$CH$_2$(*C*H$_2$)$_9$CH$_3$), 20.9, 20.8, 20.7, 20.6

(OCO*C*H$_3$ × 8), 14.1 (N(CH$_2$)$_{11}$*C*H$_3$). HRESIMS calcd for $C_{51}H_{76}O_{24}SN_3$ $[M+H]^+$: 1148.47, found 1148.466.

4.12. 1-Dodecane-4-{2,3,6-tri-O-acetyl-4-*O*-[2,3,6-tri-O-acetyl-4-*O*-(2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-glucopyranosyloxymethyl}-[1,2,3]-triazole (18)

Trisaccharide **16** (26 mg, 0.027 mmol) and 1-azidododecane[12] (19 mg, 0.090 mmol, 3.4 equiv) were dissolved in anhyd THF (1.3 mL) under N_2 then CuI (2.3 mg, 0.012 mmol, 0.45 equiv) was introduced followed by DIPEA (12 µL, 0.069 mmol, 2.6 equiv). The reaction mixture was stirred at 26 °C for 18 h then the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (10 mL) and washed successively with satd aq NH_4Cl (2 × 10 mL) and satd aq $NaHCO_3$ (2 × 10 mL). The aq layer was re-extracted with CH_2Cl_2 (4 × 10 mL) and the organic layers were combined, dried and concentrated. Chromatography (7:3, EtOAc-hexanes) gave triazole **18** (21.7 mg, 71%) pure as a white foam. $[\alpha]_D$ 7° (*c* 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, 295 K): δ 7.46 (s, 1H, =CH), 5.34 (d, 1H, *J* = 3.3 Hz, H-4”), 5.15–5.11 (m, 2H, H-3, H-3’), 5.03 (dd, 1H, *J* = 7.9, 10.4 Hz, H-2”), 4.91–4.84 (m, 4H, H-2, H-2’, H-3”, OC*H$_a$*H$_b$), 4.78 (d, 1H, *J* = 12.8 Hz, OCH$_a$*H$_b$*), 4.59 (d, 1H, *J* = 7.9 Hz, H-1), 4.53 (dd, 1H, *J* = 0.1, 10.4 Hz, H-6a), 4.47 (d, 1H, *J* = 7.8 Hz, H-1’), 4.36 (d, 1H, *J* = 7.9 Hz, H-1”), 4.32–4.29 (m, 2H, H-6a’, NC*H$_2$*(CH$_2$)$_{10}$CH$_3$), 4.10–4.05 (m, 2H, H-6b, H-6b’), 3.80–3.72 (m, 2H, H-4, H-4’), 3.61–3.57 (m, 3H, H-5, H-5’, H-5”), 3.00–2.98 (m, 2H, H-6a”, H-6b”), 2.33 (s, 3H, SCOC*H$_3$*), 2.11, 2.09, 2.08, 2.01, 1.99, 1.93, 1.90 (9s, 27H, OCOC*H$_3$*), 1.87 (t, 2H, *J* = 7.0 Hz, NCH$_2$C*H$_2$*(CH$_2$)$_9$CH$_3$), 1.27–1.20 (m, 18H, NCH$_2$CH$_2$(C*H$_2$*)$_9$CH$_3$), 0.83 (t, 3H, *J* = 6.5 Hz, N(CH$_2$)$_{11}$C*H$_3$*). ^{13}C NMR (100 MHz, $CDCl_3$, 296 K): δ 194.4, 170.3, 170.3, 170.2, 170.1, 169.9, 169.7, 169.6, 169.3, 169.1 (C=O × 9), 144.0 (*C*=CH), 122.5 (C=*C*H), 100.7 (C-1”), 100.5 (C-1’), 99.8 (C-1), 76.3, 75.4 (C-4, C-4’), 72.8,

72.7 (C-3, C-3'), 72.9, 72.4, 72.3 (C-5, C-5', C-5"), 71.9, 71.5, 71.1 (C-2, C-2', C-3"), 69.1 (C-2"), 67.3 (C-4"), 63.1 (O*C*H_2), 62.2 (C-6'), 61.6 (C-6), 50.4 (N*C*$H_2(CH_2)_{10}CH_3$), 31.9 ($NCH_2CH_2(CH_2)_9CH_3$), 30.5 (SCO*C*H_3), 30.3 ($NCH_2CH_2(CH_2)_9CH_3$), 29.6, 29.5, 29.4, 29.3, 29.0 ($NCH_2CH_2(CH_2)_9CH_3$), 28.2 (C-6"), 26.5, 22.7 ($NCH_2CH_2(CH_2)_9CH_3$), 20.9, 20.8, 20.7, 20.7 (OCO*C*H_3 × 8), 14.1 ($N(CH_2)_{11}CH_3$). HRESIMS calcd for $C_{53}H_{78}O_{25}SN_3$ $[M+H]^+$: 1190.48, found 1190.477.

4.13. 1-Octadecane-4-{2,3,6-tri-*O*-acetyl-4-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-glucopyranosyloxymethyl}-[1,2,3]-triazole (19)

Trisaccharide **16** (40 mg, 0.041 mmol) and 1-azidooctadecane[13] (41 mg, 0.139 mmol, 3.4 equiv) were dissolved in anhyd THF (2 mL) under N_2 then CuI (3.0 mg, 0.016 mmol, 0.38 equiv) was introduced followed by DIPEA (19 μL, 0.106 mmol, 2.6 equiv). The reaction was allowed to proceed and the mixture worked up as described above for the preparation of analogue **18**.. Chromatography (6:4 to 9:1, EtOAc-hexanes) gave triazole **19** (36 mg, 70%) pure as a yellow oil. $[\alpha]_D$ –6° (*c* 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, 295 K): δ 7.46 (s, 1H, =CH), 5.34 (d, 1H, *J* = 3.3 Hz, H-4"), 5.15–5.11 (m, 2H, H-3, H-3'), 5.03 (dd, 1H, *J* = 7.9, 10.4 Hz, H-2"), 4.91–4.84 (m, 4H, H-2, H-2', H-3", OC*H*$_a$H$_b$), 4.76 (d, 1H, *J* = 12.6 Hz, OCH$_a$*H*$_b$), 4.59 (d, 1H, *J* = 7.9 Hz, H-1), 4.53 (dd, 1H, *J* = 1.83, 12.0 Hz, H-6a), 4.47 (d, 1H, *J* = 7.8 Hz, H-1'), 4.36 (d, 1H, *J* = 7.9 Hz, H-1"), 4.32–4.29 (m, 2H, H-6a', N*CH*$_2$(CH$_2$)$_{10}$CH$_3$), 4.10–4.05 (m, 2H, H-6b, H-6b'), 3.80–3.72 (m, 2H, H-4, H-4'), 3.61–3.57 (m, 3H, H-5, H-5', H-5"), 3.00–2.98 (m, 2H, H-6a", H-6b"), 2.33 (s, 3H, SCOC*H*$_3$), 2.11, 2.09, 2.08, 2.05, 2.03, 2.01, 1.99, 1.93, 1.90 (9s, 27H, OCOC*H*$_3$), 1.87 (t, 2H, *J* = 7.0 Hz, NCH$_2$C*H*$_2$(CH$_2$)$_9$CH$_3$), 1.27–1.20 (m, 30H, NCH$_2$CH$_2$(C*H*$_2$)$_{15}$CH$_3$), 0.83 (t, 3H, *J* = 6.5 Hz, N(CH$_2$)$_{11}$C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$,

296 K): δ 194.4, 170.3, 170.3, 170.2, 170.0, 169.9, 169.7, 169.6, 169.3, 169.1 (C=O × 9), 144.0 (*C*=CH), 122.5 (C=*C*H), 100.7 (C-1"), 100.5 (C-1'), 99.7 (C-1), 76.3, 75.4 (C-4, C-4'), 72.8, 72.7 (C-3, C-3'), 72.9, 72.4, 72.3 (C-5, C-5', C-5"), 71.9, 71.5, 71.1 (C-2, C-2', C-3"), 69.1 (C-2"), 67.3 (C-4"), 63.1 (O*C*H_2), 62.2 (C-6'), 61.6 (C-6), 50.4 (N*C*H_2(CH_2)$_{10}$$CH_3$), 31.9 (NC$H_2CH_2$(*C*$H_2$)$_9$$CH_3$), 30.5 (SCO*C*$H_3$), 30.3 (NC$H_2$*C*$H_2$($CH_2$)$_9$$CH_3$), 29.7, 29.7, 29.6, 29.5, 29.4, 29.4, 29.0 (NCH_2CH_2(*C*H_2)$_9$$CH_3$), 28.2 (C-6"), 26.5, 22.7 (NCH_2CH_2(*C*H_2)$_9$$CH_3$), 20.9, 20.8, 20.7, 20.7 (OCO*C*H_3 × 8), 14.1 (N(CH_2)$_{11}$*C*H_3). HRESIMS calcd for $C_{59}H_{91}O_{25}SN_3$ $[M+H]^+$: 1274.5741, found 1274.5706.

4.14. 1-Dodecane-4-{4-*O*-[6-*O*-(6-thio-β-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranosyloxymethyl}-[1,2,3]-triazole disulfide (3)

Sodium (3.3 mg, 0.14 mmol) was added to a solution of trisaccharide **17** (64 mg, 0.055 mmol) in anhyd MeOH (1.7 mL) and the reaction mixture was stirred at rt under N_2. After 40 min, the reaction mixture was quenched with 20% methanolic AcOH until pH strip indicated neutral. The solvent was evaporated and the residue was dissolved in methanol and stirred at 40 °C for 20 h. The solvent was again evaporated and chromatography of the residue (5:95 to 4:6 MeOH-CH_2Cl_2) gave disulfide **3** (32 mg, 75%) pure as a white foam. $[\alpha]_D$ 14° (*c* 0.5, MeOH). ^{1}H NMR (400 MHz, CD_3OD, 296 K): δ 8.00 (s, 2H, =CH), 4.95 (d, 2H, *J* = 12.4 Hz, OC$H_a$$H_b$), 4.77 (d, 2H, *J* = 12.4 Hz, OC$H_a$$H_b$), 4.43–4.35 (m, 8H, NC$H_2$($CH_2$)$_{10}$$CH_3$, H-1, H-1'), 4.31 (d, 2H, *J* = 7.4 Hz, H-1"), 4.03–3.80 (m, 14H, H-4", H-5, H-5', H-6a, H-6b, H-6a', H-6b'), 3.77–3.74 (m, 2H, H-5"), 3.59–3.47 (m, 14H, H-2 or H-2', H-2", H-3, H-3', H-3", H-4, H-4'), 3.29–3.28 (m, 2H, H-2 or H-2'), 3.03–3.00 (m, 4H, H-6a", H-6b"), 1.89 (t, 4H, *J* = 7.0 Hz, NCH_2CH_2(*C*H_2)$_9$$CH_3$), 1.32–1.28 (m, 36H, NC$H_2CH_2$(C$H_2$)$_9$$CH_3$), 0.88 (t, 6H, *J* = 6.6 Hz, N(CH_2)$_{11}$CH_3). ^{13}C NMR (150 MHz, CD_3OD, 295 K): δ 145.7 (*C*=CH), 125.3 (C=*C*H), 105.5,

105.1 (C-1 or C-1', C-1") 103.3 (C-1 or C-1'), 82.5, 76.6, 76.3, 75.8, 74.8, 74.6, 74.6, 74.5, 72.6, 72.4, 72.4, 71.4, 70.4, 70.2 (C-2, C-2', C-2", C-3, C-3', C-3", C-4, C-4', C-4", C-5, C-5', C-5"), 63.1 (OCH_2), 62.2 (C-6, C-6'), 51.4 ($NCH_2(CH_2)_{10}CH_3$), 41.2 (C-6"), 31.4 ($NCH_2CH_2(CH_2)_9CH_3$), 33.1, 30.8, 30.7, 30.6, 30.5, 30.2, 27.5, 23.8 ($NCH_2CH_2(CH_2)_9CH_3$), 14.5 ($N(CH_2)_{11}CH_3$). HRESIMS calcd for $C_{66}H_{116}O_{30}S_2N_6$ $[M+Na]^+$: 1559.7075, found 1559.7059.

4.15. 1-Dodecane-4-{4-*O*-[4-*O*-(6-thio-β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-glucopyranosyloxymethyl}-[1,2,3]-triazole disulfide (4)

Trisaccharide **18** (21 mg, 0.017 mmol) was dissolved in anhyd MeOH (3.8 mL) and stirred at 40 °C under N_2. A 1 M solution of NaOMe in MeOH (945 μL) was added and the reaction mixture was stirred at 40 °C for 15 h. The reaction was quenched with 20% methanolic AcOH until pH strip indicated neutral. The solvent was evaporated and the residue was dissolved in methanol and stirred at 40 °C for 20 h. The solvent was again evaporated and two successive column chromatographies of the residue (5:95 to 6:4, MeOH-CH_2Cl_2) gave disulfide **4** (7.3 mg, 70%) pure as a white foam. $[\alpha]_D$ 2° (*c* 1.0, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6, 295 K): δ 8.10 (s, 2H, =CH), 5.45 (d, 2H, *J* = 4.9 Hz, OH-2 or OH-2'), 5.19–5.18 (m, 4H, OH-2 or OH-2', OH), 4.90–4.78 (m, 6H, OCH_aH_b, OH × 2), 4.68–4.60 (m, 8H, OCH_aH_b, OH × 3), 4.46 (bs, 2H, OH), 4.34–4.29 (m, 8H, $NCH_2(CH_2)_{10}CH_3$, H-1, H-1'), 4.27 (d, 2H, *J* = 7.4 Hz, H-1"), 3.80–3.77 (m, 4H, H-6a, H-6a') 3.73–3.69 (m, 2H, H-5"), 3.65–3.56 (m, 6H, H-4", H-6b, H-6b'), 3.33–3.32 (m, 16H, H-2", H-3, H-3', H-3", H-4, H-4', H-5, H-5'), 3.10–3.04 (m, 4H, H-2, H-2'), 2.98–2.91 (m, 4H, H-6a", H-6b"), 1.78 (t, 4H, *J* = 7.1 Hz, $NCH_2CH_2(CH_2)_9CH_3$), 1.22–1.21 (m, 36H, $NCH_2CH_2(CH_2)_9CH_3$), 0.84 (t, 6H, *J* = 6.6 Hz, $N(CH_2)_{11}CH_3$). ^{13}C NMR (150 MHz, DMSO-d_6, 295 K): δ 143.6 (*C*=CH), 124.0 (C=*C*H), 103.7 (C-1"), 102.7, 101.8 (C-1, C-1'),

80.4, 79.8, 74.9, 74.8, 74.7, 73.1, 72.9, 70.1, (C-2, C-2', C-2", C-3, C-3', C-3", C-4, C-4', C-5, C-5', C-5"), 69.1 (C-4"), 61.7 (O*C*H_2), 60.3, 60.2 (C-6, C-6'), 49.3 (N*C*$H_2(CH_2)_{10}CH_3$), 39.4 (C-6"), 29.7 (NCH_2*C*$H_2(CH_2)_9CH_3$), 31.3, 29.0, 29.0, 28.9, 28.7, 28.4, 25.9, 22.1 (NCH_2CH_2(*C*$H_2)_9CH_3$), 14.0 ($N(CH_2)_{11}$*C*H_3). HRESIMS calcd for $C_{66}H_{116}O_{30}S_2N_6$ $[M+NH_4]^+$: 1554.7521, found 1554.7538.

4.16. 1-Octadecane-4-{4-*O*-[4-*O*-(6-thio-β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-glucopyranosyloxymethyl}-[1,2,3]-triazole disulfide (5)

Trisaccharide **19** (45 mg, 0.035 mmol) was dissolved in anhyd MeOH (890 μL) and stirred at 40 °C under N_2. A 1 M solution of NaOMe in MeOH (223 μL) was added and the reaction mixture was stirred at 40 °C for 15 h. The reaction was then quenched with 20% methanolic AcOH until pH strip indicated neutral. The solvent was evaporated and the residue was dissolved in methanol and stirred at 40 °C for 20 h. The solvent was again evaporated and the residue was dissolved in $CHCl_3$ (10 mL) and washed with H_2O (10 mL). The aq phase was re-extracted with $CHCl_3$ (5 × 10 mL). The organic layer was concentrated and chromatography of the residue (1:9 to 9:1 MeOH:CH_2Cl_2) gave disulfide **5** (23 mg, 75%) pure as a white foam. $[\alpha]_D$ 6° (*c* 1.0, 1:1 MeOH-CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO-d_6, 295 K): δ 8.11 (s, 2H, =CH), 5.48 (bs, 2H, OH-2 or OH-2'), 5.20–5.19 (m, 4H, OH-2 or OH-2', OH), 4.86–4.83 (m, 4H, OC$H_a$$H_b$, OH-5"), 4.67–4.61 (m, 8H, OC$H_a$$H_b$, OH-6a or OH-6b, OH-6a' or OH-6b', OH-6a or OH-6b or OH-6a' or OH-6b'), 4.49 (bs, 2H, OH), 4.35–4.30 (m, 8H, N*C*$H_2(CH_2)_{16}CH_3$, H-1, H-1'), 4.26 (d, 2H, *J* = 7.2 Hz, H-1"), 3.80–3.78 (m, 4H, H-6a, H-6a') 3.74–3.71 (m, 2H, H-5"), 3.66–3.57 (m, 6H, H-4", H-6b, H-6b'), 3.34–3.33 (m, 16H, H-2", H-3, H-3', H-3", H-4, H-4', H-5, H-5'), 3.10–3.05 (m, 4H, H-2, H-2'), 2.98–2.91 (m, 4H, H-6a", H-6b"), 1.79 (t, 4H, *J* = 6.8 Hz, NCH_2C*H*$_2(CH_2)_{15}CH_3$), 1.23–1.22 (m, 60H, NCH_2CH_2(C*H*$_2)_{15}CH_3$), 0.85 (t, 6H, *J* = 6.6 Hz,

$N(CH_2)_{17}CH_3$). ^{13}C NMR (150 MHz, DMSO-d_6, 295 K): δ 143.6 (*C*=CH), 124.1 (C=*C*H), 103.7 (C-1"), 102.8, 101.8 (C-1, C-1'), 80.4, 79.9, 75.0, 74.8, 74.7, 73.1, 72.9, 70.1 (C-2, C-2', C-2", C-3, C-3', C-3", C-4, C-4', C-5, C-5', C-5"), 69.2 (C-4"), 61.7 (OCH_2), 60.4, 60.2 (C-6, C-6'), 49.3 ($NCH_2(CH_2)_{16}CH_3$), 39.2 (C-6"), 29.8 ($NCH_2CH_2(CH_2)_{15}CH_3$), 31.3, 29.1, 29.0, 28.9, 28.7, 28.4, 25.9, 22.1 ($NCH_2CH_2(CH_2)_{15}CH_3$), 14.0 ($N(CH_2)_{17}CH_3$). HRESIMS calcd for $C_{78}H_{140}O_{30}S_2N_6$ $[M+H]^+$: 1727.8977, found 1727.9021.

4.17. Formation of lipid bilayers using analogues 1–5.

4.17.1. Passive self-assembly of inner leaflets on Au(111) surface. Prior to each experiment, the Au(111) electrode was flame-annealed, cooled in air, and rinsed with pure water. Self-assembly was carried out by immersing the surface of the Au(111) electrode surface in the methanol solution of the analogue (1mg / mL) overnight (18 to 20 h). The electrode covered with SAM was then washed with methanol followed by Milli-Q water and argon dried.

4.17.2. LB transfer of inner leaflets. Pure water was used as the subphase in both the LB and LS experiment. The subphase was heated to ~30 °C in order to improve the homogeneity and fluidity of the compressed film. A 1 mg/mL solution of each analogue in chloroform was prepared and ~30 μL of the solution was added to the subphase surface until the surface pressure reached ~10 mN/m. The analogues were compressed to a surface pressure of 35 mN/m prior to deposition. The transfer ratio of the LB deposition was 1.0 ± 0.1.

4.17.3. LS touch of outer leaflets. A mixture of DMPC/cholesterol (70:30) was dissolved in chloroform to give a solution of concentration ~1.0 mg/mL. Lipids were compressed to a surface pressure of 35 mN/m prior to LS touch.

4.17.4. Differential capacitance measurements. Differential capacitance measurements were carried out in an all-glass three-electrode cell using a hanging meniscus configuration. The cell was de-aerated by purging with argon for 30 minutes. To prevent the influx of oxygen, an argon blanket was maintained over the solution throughout the experiments. A saturated calomel (SCE) reference electrode located in a separate compartment was connected to the cell via a Luggin capillary and a flame annealed gold coiled wire was used as the counter electrode. All potentials reported in this paper are quoted versus the Ag/AgCl (sat.KCl) reference electrode. Electrochemical experiments were performed using a computer-controlled system, consisting of a potentiostat/galvanostat (HEKA PG590) and a lock-in amplifier (EG&G Instruments 7265 DSP). The differential capacitance curves were determined using a scan rate of 5 mV s^{-1} and an ac perturbation with a 25 Hz frequency and 5 mV *r.m.s.* amplitude. The differential capacitances were calculated from the in-phase and out-of-phase components of the ac signal assuming a simple series of RC equivalent circuit. Custom software was used to collect the data. This software was generously supplied by Prof. Dan Bizzotto, University of British Columbia, with modifications made by Prof. Ian Burgess, University of Saskatchewan.

4.18. Attenuated Total Reflection (ATR) spectroscopy on compounds 3 and 4.

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). Sample films were deposited onto the internal reflection element by evaporation of a 2 $mg.mL^{-1}$ solution of compounds **3** or **4** in methanol.

4.19. Infrared Reflection Absorption Spectroscopy (IRRAS) on self-assembled mono layer of 3 and 4 on gold.

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°. Self-assembled monolayers were obtained by incubating a gold slide in a 2 $mg.mL^{-1}$ solution of either compound **2** or **4** in methanol for 20 h. The gold slide was then rinsed, first with methanol and then water.

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Supplementary Data

Supplementary data associated with this article can be found in the online version at doi:

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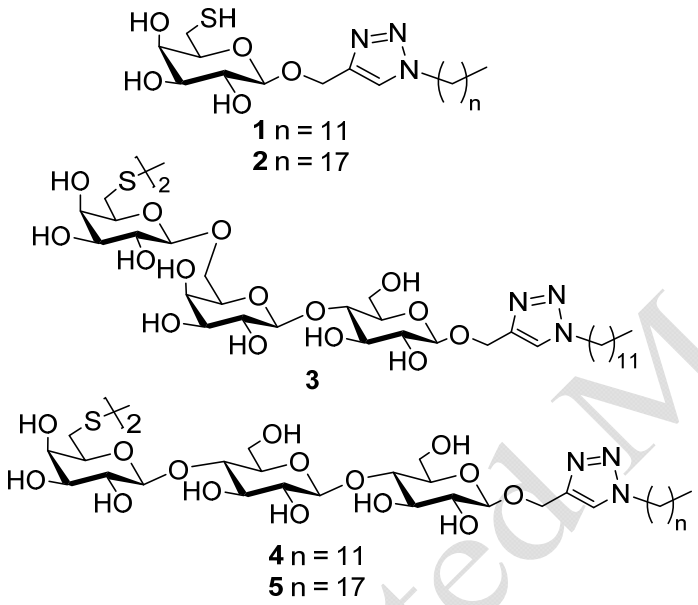
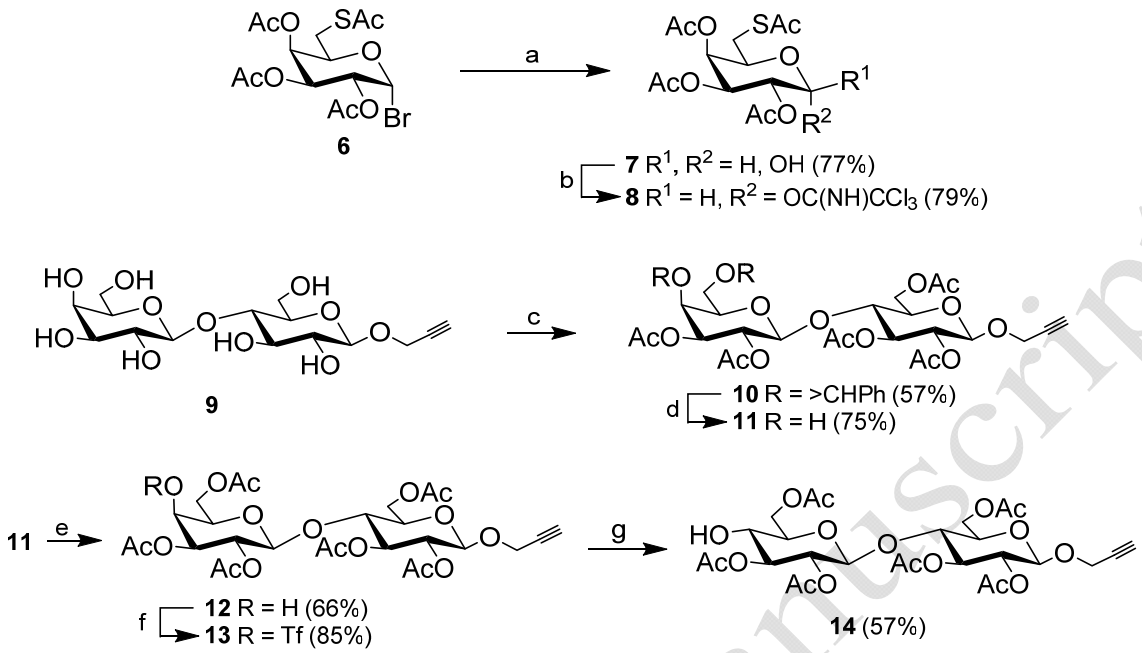
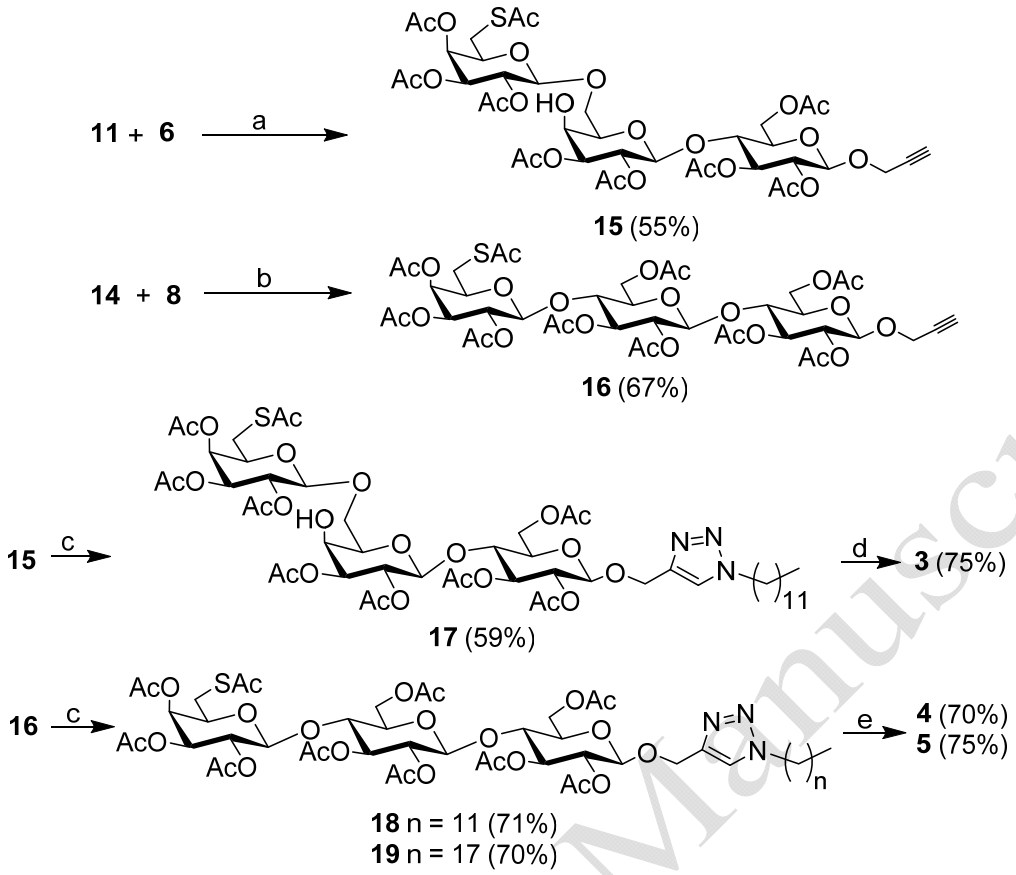


Chart 1



Scheme 1. *Reagents and conditions*: (a) Ag_2CO_3, 50% aq acetone, RT, 30 min. (b) Cl_3CCN, DBU, CH_2Cl_2, RT, 3 h. (c) i. $PhCH(OMe)_2$, CSA, MeCN, 70 °C, 2 h; ii. Ac_2O, pyr., 50 °C, 1 h. (d) 80% aq AcOH, 100 °C, 45 min. (e) AcCl, collidine, CH_2Cl_2, –35 °C to –5 °C, 2 h; (f) Tf_2O, pyr., CH_2Cl_2, –20 °C to RT, 30 min. (g) $NaNO_3$, DMF, 50 °C, 20 h.



Scheme 2. *Reagents and conditions:* (a) AgOTf 0.8 equiv, CH_2Cl_2, 5 °C to 10 °C, 45 min. (b) $BF_3{\cdot}Et_2O$ 1.5 equiv, CH_2Cl_2, 40 °C, 1.5 h. (c) CuI, DIPEA, THF, 25 °C, 18–20 h. (d) NaOMe/MeOH, RT, 40 min. (e) NaOMe/MeOH, 40 °C, 15 h.

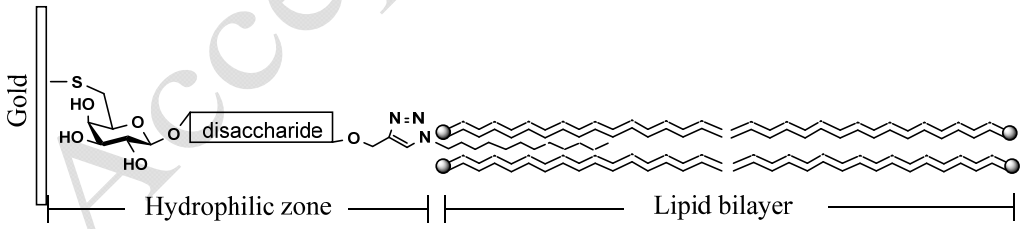


Figure 1. Proposed model of a tethered bilayer lipid membrane using a carbohydrate-based tether as the anchoring molecule.

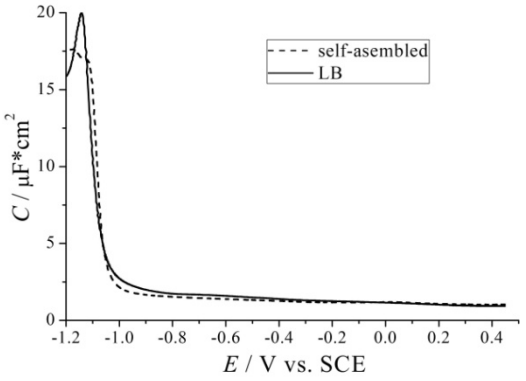


Figure 2. Comparison of differential capacitance curves of a bilayer on Au(111) in 0.1 M NaF. Inner leaflet: monosaccharide **2** prepared by LB transfer (solid line) and self-assembled (dash line); outer leaflet: DMPC/cholesterol (70:30) prepared by LS touch.

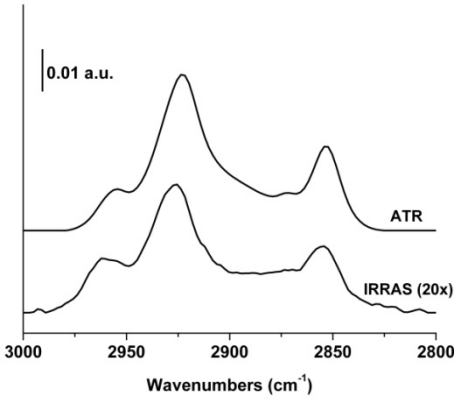


Figure 3. IR spectra (hydrocarbon chain region) of analogue **3** randomly oriented (ATR) and bound (IRRAS).

Table 1. Minimum capacitances for self-assembled monolayers (SAM) of analogues **1–5** and subsequent tethered bilayers (tBLM) prepared by LS touch.

Entry	Analogue	Minimum capacitances ($\mu F/cm^2$)	
		SAM	tBLM
1	**1**	8.8	9.2
2	**2**	1.1	1.0
3	**3**	8.6	8.9
4	**4**	8.8	8.6
5	**5**	15.2	7.1

Table 2. Minimum capacitances for LB transferred monolayers (LBM) of analogues **1**, **2** and **5** and subsequent tethered bilayers (tBLM) prepared by LS touch.

Entry	Analogue	Minimum capacitances ($\mu F/cm^2$)	
		LBM	tBLM
1	**1**	9.8	5.6
2	**2**	3.5	0.9
3	**5**	13.0	12.6