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Synthesis of Structurally Defined Nitroxide Spin-Labeled Glycolipids as Useful Probes for Electron Paramagnetic Resonance (EPR) Spectroscopy Studies of Cell Surface Glycans

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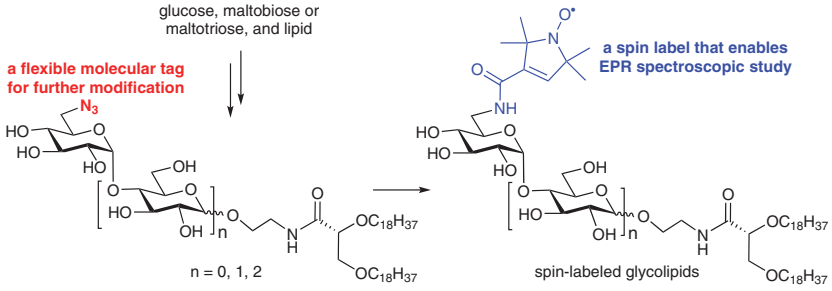

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Abstract Four glycolipids carrying different glycans and a nitroxide free radical spin at the glycan non-reducing end were designed and synthesized from free glucose and maltooligosaccharides by an efficient and streamlined synthetic strategy. The main features of this synthetic strategy include regioselective functionalization of the free carbohydrates and coupling of the radical spin label with functionalized free glycans as the last synthetic step. These glycolipids are useful probes for the study of cell surface glycans by electron paramagnetic resonance spectroscopy. Moreover, the key synthetic intermediates, free glycolipids carrying a flexible azido group at the glycan non-reducing end, are widely useful platforms for accessing glycolipids with other molecular labels.

Key words glycolipids, carbohydrates, glycans, nitroxides, radical spin label, electron paramagnetic resonance spectroscopy

The cell surface is covered by a layer of glycans, which is known as the cell glycocalyx. Cell surface glycans are anchored to the cell membrane in forms of glycoconjugates, such as glycolipids and glycoproteins, and play a pivotal role in various biological functions, such as cell recognition, proliferation, communication, etc.[1] To accomplish these functions, glycans need to be not only structurally complex and diverse, but also flexible to adopt certain organization and conformation on the cell surface.[2–4] For example, most tumor-associated carbohydrate antigens are glycans that are also expressed by some normal cells in much lower concentrations, but these antigens are still useful cancer biomarkers[5,6] recognized by our immune system due to their different concentrations and organizations on cancer and normal cells.[7,8]

To facilitate exploring the spatial organization of glycans on cells, we have recently established a metabolic glycoengineering-based method to label cell surface glycans with free radical spins to enable electron paramagnetic resonance (EPR) spectroscopy studies.[9] Line shape analyses and simulations of EPR spectra can provide information about the alignments, mobility, and dynamics of labeled molecules and their adjacent environments[10–18] to afford more insights into the structure–function relationships of glycans. However, spin labeling of cell surface glycans through metabolic glycoengineering is non-selective, and thus related EPR studies can only provide average data of diverse glycans in different locations and environments. It is difficult to draw definite conclusions from these data without a deep understanding of the EPR behaviors and properties of glycans in defined environments. To this end, spin-labeled glycoconjugates with well-defined structures are necessary to deliver the standard EPR spectra and the unique EPR parameters of glycans in various environments on the cell surface. To meet this demand, we have developed a series of structurally defined glycolipids carrying a nitroxide spin label; the chemical synthesis of these molecular probes is described here.

Figure 1 depicts the designed glycolipid probes **1**–**4**, which carry a nitroxide spin label. In these molecules, the lipid is attached to the reducing end anomeric position of a glycan, to mimic natural glycolipids, whilst the nitroxide spin is linked to the glycan non-reducing end C6-position through a metabolically stable amide linker. Glycolipids **1** and **2** are anomeric isomers, and **1**, **3**, and **4** are glycosyl homologs containing a mono-, di-, and trisaccharide, respectively. When **1**–**4** are anchored to cells or liposomes, their spins will have defined distances from the membrane surface. Moreover, we design the target molecules to contain unnatural lipids for several reasons. First, they should be more stable than natural glycolipids to lipidases and glycosidases, which is especially useful for their cellular studies. Second, we hope that they will not interact specifically with cell membrane components, so that their EPR results

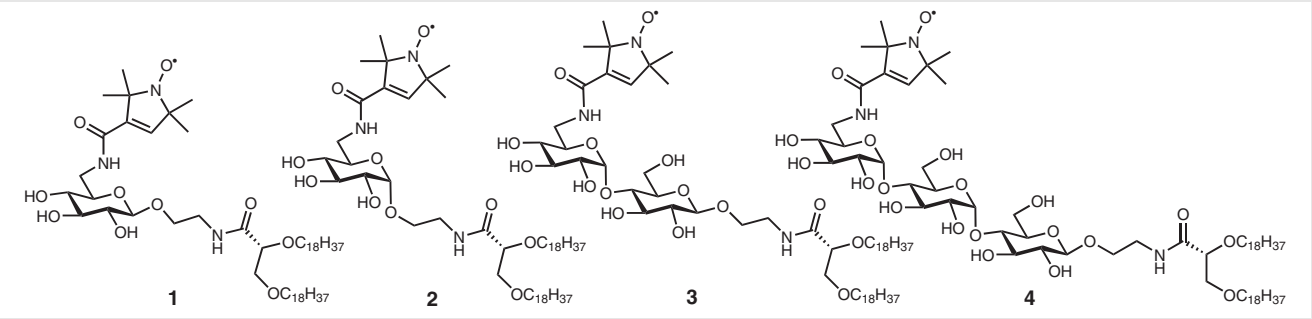
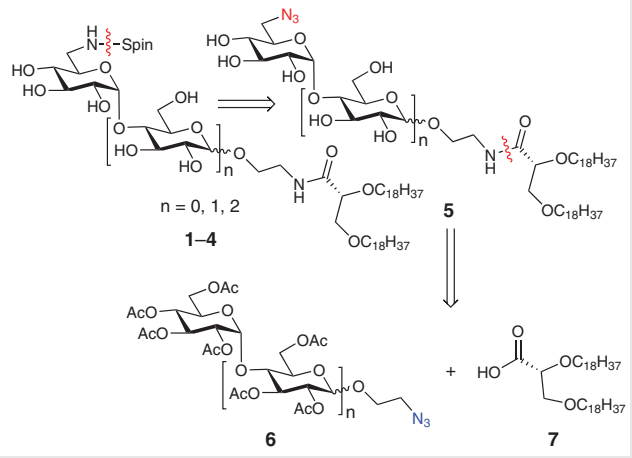


Figure 1 Structures of designed spin-labeled glycolipid probes **1–4**

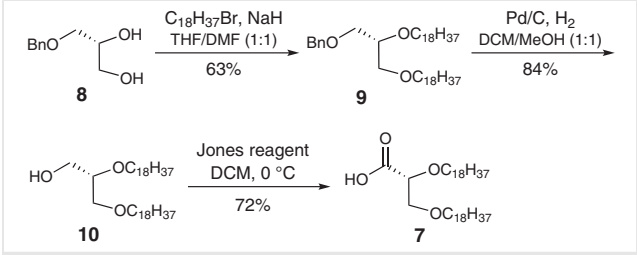
will be simple and relatively easily interpretable. Third, they should be more easily synthesized than most natural glycolipids. Nonetheless, due to the structural differences of these probes, their EPR studies can provide useful information about the influences of glycosidic linkage form and glycan length on the EPR spectra and line shapes of labeled glycans and the relationships between the cell surface environment and the mobility of glycans within the cell glycocalyx. The results can be used to analyze the environments, organizations, mobility, and other dynamic properties of glycans on the cell surface.

The synthesis of glycolipids **1–4** can be a challenge due to the presence of a reduction-sensitive nitroxide radical in their structure, as it limits the scope of selected protecting groups. Accordingly, we planned to employ the acetyl group for global protection of hydroxyl groups. In addition, the amphiphilic property of glycolipids makes the handling of our final synthetic targets and certain intermediates difficult. As shown in Scheme 1, compound **6** with an azidoethyl group as the linker would be derived from D-glucose and commercially available maltooligosaccharides, which could be linked to lipid **7** via a well-established procedure. Thereafter, the non-reducing end C6-position of the glycan would be regioselectively functionalized to facilitate the attachment of the spin label, which is the last step of the synthesis. In fact, glycolipids **5** with an azido group are also flexible platforms for accessing molecules with various other probes, such as fluorophores.



Scheme 1 Retrosynthetic plan for glycolipids **1–4**

Our synthesis commenced with the preparation of lipid **7** from (*R*)-3-(benzyloxy)propane-1,2-diol (**8**),[19] as outlined in Scheme 2 (detailed reaction conditions for all synthetic schemes are given in the experimental section). Alkylation of **8** using 1-bromooctadecane and NaH, followed by removal of the benzyl group in the resultant **9** by using Pd/C and H_2 gave alcohol **10**. Oxidation of **10** with the Jones reagent provided the desired lipid **7**.[20]



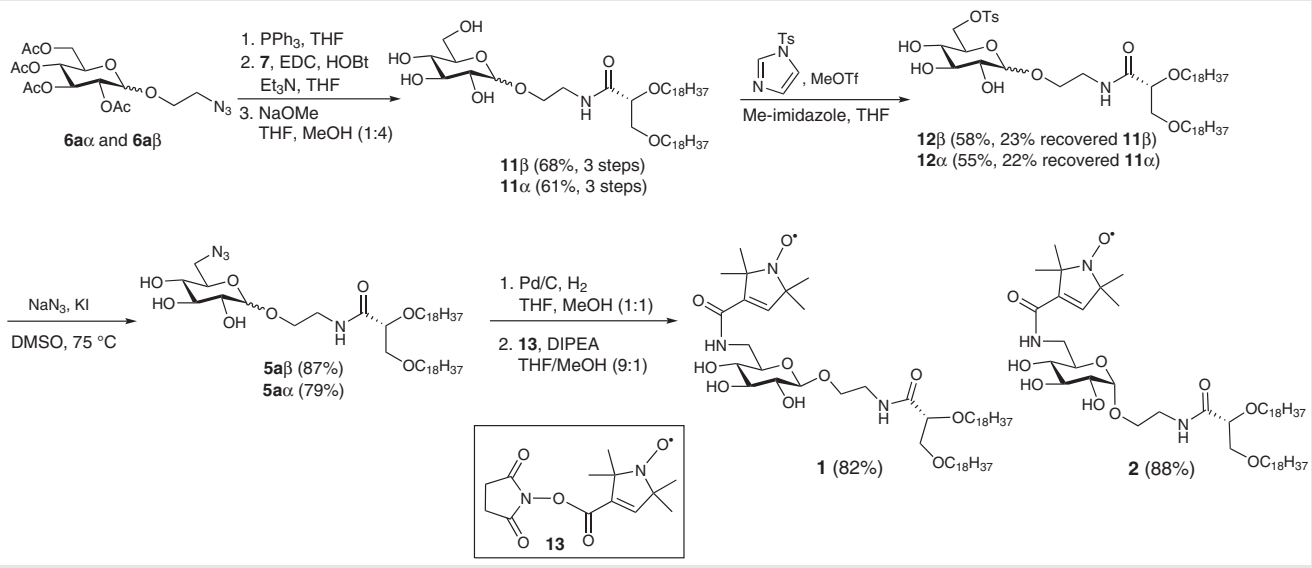
Scheme 2 Synthesis of lipid **7**

Spin-labeled glycolipids **1** and **2** were synthesized from 2-azidoethyl 2,3,4,6-tetra-*O*-acetyl-α- and β-D-glucopyranosides **6aβ** and **6aα**, respectively (Scheme 3). In turn, **6aβ** and **6aα** were derived from D-glucose according to reported procedures.[21,22] First, the azido group in **6aβ** and **6aα** was chemoselectively reduced with PPh_3; this was followed by coupling of the resultant free amines with lipid **7** in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and hydroxybenzotriazole (HOBt)[23] and then *O*-deacetylation with NaOMe to provide **11aβ** and **11aα** in 68% and 61% overall yields (for three steps), respectively. Subsequently, the 6-hydroxyl group in deprotected **11aβ** and **11aα** was regioselectively tosylated with 1-tosylimidazole by using methyl triflate and *N*-methylimidazole as the promoters[24] to provide **12aβ** and **12aα**. This reaction was carried out at a low temperature (0 °C) for a relatively short time (6 h) and was terminated before its completion to warrant a high regioselectivity, thereby affording moderate

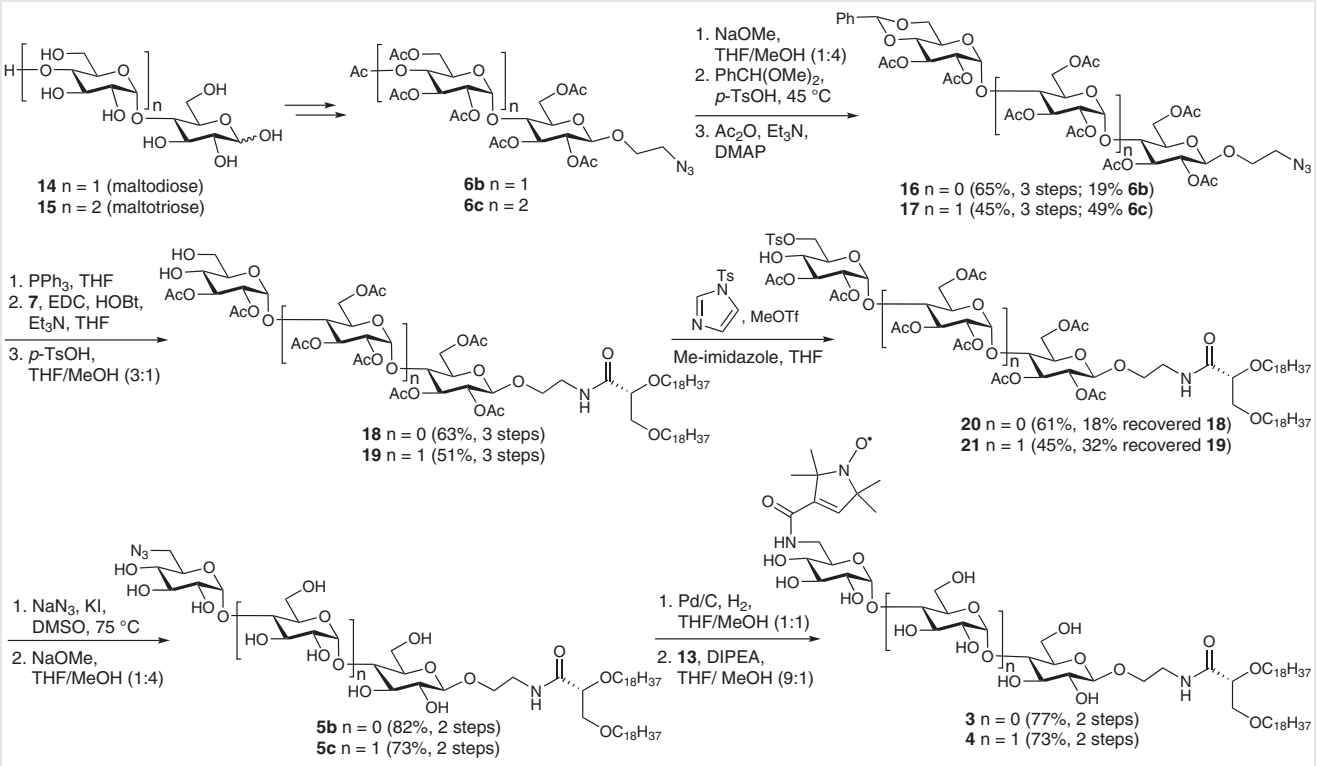
yields of the desired products (55–58%) with substantial amounts of the substrates recovered (22–23%). Thereafter, the *O*-tosylate in **12aβ** and **12aα** was smoothly substituted with an azido group by using NaN_3 and KI to afford the key intermediates, glycolipids **5aβ** and **5aα** having an azido group linked to the glycan non-reducing end C6-position. Finally, the azido group in **5aβ** and **5aα** was reduced with Pd/C and H_2, and the resultant amines were coupled with the spin label by using its activated ester **13** to produce synthetic targets **1** and **2**.[25] The final products and all synthetic intermediates were characterized by NMR and HRMS data. It is worth noting that the presence of a paramagnetic spin label in the structures of **1** and **2** significantly broadened their NMR signals. Thus, their NMR spectra can only provide basic structural information, such as broad signals of some key protons, but no details about their splitting patterns and coupling constants. On the other hand, the significantly broadened NMR signals of **1** and **2** proved the presence of a spin label in these molecules. The attachment of a spin label to **1** and **2** was also verified by their EPR spectra, which are under close investigation in our lab currently. In conclusion, our NMR, MS, IR, and EPR data validated unambiguously the structures of the final products. A useful feature about this synthetic strategy is that the spin label is installed as the last step via amine acylation by using an activated ester, which is well-established and proved to be highly selective. There is no stereochemical or any similar concerns for this step, and hence, typically, MS should be sufficient to validate the reaction and products.

Spin-labeled glycolipids **3** and **4** were synthesized from maltodiose **14** and maltotriose **15** (Scheme 4), respectively. First, **14** and **15** were converted into 2-azidoethyl glycosides **6b** and **6c** according to reported methods.[26,27] After all of the acetyl groups in **6b** and **6c** were removed by using NaOMe, the 4,6-*O*-positions were selectively protected with a benzylidene group by a reaction with benzaldehyde dimethyl acetal under the influence of *p*-toluenesulfonic acid (*p*-TsOH); this was followed by *O*-acetylation to give **16** and **17**.[28–30] Next, the azido groups in **16** and **17** were reduced and the resultant free amines were coupled with lipid **7** according to the above-described protocols; this was followed by removal of the benzylidene group in methanol by using *p*-TsOH as the catalyst to afford diols **18** and **19** in good overall yields. Then, the primary 6-hydroxyl group in **18** and **19** was selectively tosylated with 1-tosylimidazole to provide **20** and **21**. Subsequent transformations were similar to those used for the synthesis of **1** and **2**, including substitution of the tosylate with an azido group, global deprotection to remove all the acetyl groups, reduction of the azido group, and coupling with activated ester **13** to introduce the spin label, to finally yield the synthetic targets **3** and **4**. Again, the structures of all synthetic intermediates were characterized by NMR and HRMS data, and, for the final products, with IR and EPR as well.

In this study, we have designed spin-labeled glycolipids **1**–**4** and developed an efficient strategy for their chemical synthesis. The synthesis is highlighted with regioselective modifications (including tosylation and azido substitution) of the glycan non-reducing end sugar residue, early-stage global deprotection, and introduction of the radical spin to free glycans as the last synthetic step, which can minimize any potential complications caused by the spin label. The synthesis of glycolipids **1**–**4** carrying mono-, di-, and trisaccharides followed essentially the same protocol, thus the streamlined synthesis should be applicable to other glycolipids as well. The final products **1**–**4** should be useful mo-



Scheme 3 Synthesis of spin-labeled glycolipids **1** and **2**



Scheme 4 Synthesis of spin-labeled glycolipids **3** and **4**

lecular probes to study glycans on the cell surface or on other lipid membranes by EPR spectroscopy, which is currently pursued in our laboratories. Furthermore, free glycolipids **5a–c** carry a flexible azido group at their glycan nonreducing end, which makes them versatile and useful platforms for the access to other types of glycolipid probes. For example, fluorescent labels can be readily attached to **5a–c** either by an amide linker as described above or via a click reaction. Therefore, **1–4**, **5a–c**, and glycolipids with other labels can form a diverse and useful toolbox for investigating and better understanding the structures, presentation forms, and structure–activity relationships of glycans on the cell surface.

Chemicals and materials were purchased from commercial sources and were used as received without further purification unless otherwise noted. Analytical TLC was carried out on silica gel 60 Å F254 plates with detection by a UV detector and/or by charring with 10% (v/v) H_2SO_4 in EtOH. Flash column chromatography was performed on silica gel 60 (230–400 mesh). NMR spectra were acquired on a Bruker 400 or 600 MHz machine with chemical shifts δ, reported in ppm, referenced to $CHCl_3$ (^{1}H NMR: δ = 7.26) or $CDCl_3$ (^{13}C NMR: δ = 77.1). NMR peak assignments and coupling constants are made based on ^{1}H NMR, ^{1}H–^{1}H COSY, and ^{1}H–^{13}C HSQC experiments. 2-Azidoethyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**6aα**), 2-azidoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**6aβ**), 2-azidoethyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (**6b**), and 2-azidoethyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (**6c**) were synthesized according to reported procedures, and their ^{1}H and ^{13}C NMR data matched those described in the literature.[21,22,26,27]

(*R*)-[2,3-Bis(octadecyloxy)propoxy]methylbenzene (9)

(*R*)-3-Benzyloxy-1,2-propanediol (**8**; 8.0 g, 44.0 mmol) was co-evaporated with toluene twice and then dissolved in anhydrous THF/DMF (1:1, 180 mL). After the solution was cooled in an ice bath, NaH (60% in mineral oil, 4.9 g, 133 mmol) was added in portions. After the bubbling ceased, the suspension was stirred at 0 °C for an additional 20 min, followed by dropwise addition of 1-bromooctadecane (36.7 g, 110 mmol) dissolved in THF (20 mL). Thereafter, the reaction mixture was kept in an ice bath for 1 h and gradually warmed to rt. The mixture was stirred overnight and then cooled in an ice bath, followed by addition of sat. NH_4Cl aq solution (150 mL). The mixture was extracted with EtOAc, and the organic phases were combined, washed with brine, dried with anhydrous Na_2SO_4, filtrated, and condensed under vacuum. The crude product was purified by column chromatography (silica gel, hexane/EtOAc, 15:1); this gave **9**.

Yield: 19.0 g (27.6 mmol, 63%); white solid; R_f = 0.40 (hexane/EtOAc, 12:1).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.33 (d, *J* = 4.4 Hz, 4 H), 7.29–7.26 (m, 1 H), 4.55 (s, 2 H), 3.62–3.46 (m, 7 H), 3.42 (t, *J* = 6.7 Hz, 2 H), 1.60–1.50 (m, 4 H), 1.25 (br, 60 H), 0.88 (t, *J* = 7.0 Hz, 6 H).

^{13}C NMR (151 MHz, $CDCl_3$): δ = 128.3, 127.6, 127.5, 77.9, 77.2, 77.0, 76.8, 73.4, 71.7, 70.8, 70.6, 70.3, 31.9, 30.1, 29.7, 29.7, 29.5, 29.4, 26.1, 26.1, 22.7, 14.1.

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{46}H_{86}O_3Na$: 709.6469; found: 709.6485.

(*S*)-2,3-Bis(octadecyloxy)propan-1-ol (10)

Compound **9** (18.8 g, 27.3 mmol) and 10% Pd/C (1.8 g) were mixed in DCM/MeOH (1:1, 100 mL). After flushing with anhydrous N_2, the atmosphere in the reaction vessel was replaced with H_2, and a balloon filled with H_2 was attached. The reaction was monitored by TLC, and after the starting material **9** had disappeared, the reaction vessel was flushed with N_2. The reaction mixture was filtered and condensed under vacuum. The crude product was purified by column chromatography (silica gel, hexane/EtOAc, 9:1) to give **10**.

Yield: 13.7 g (24.0 mmol, 84%); white solid; R_f = 0.55 (hexane/EtOAc, 4:1).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.72 (ddd, *J* = 10.7, 6.4, 3.8 Hz, 1 H), 3.64–3.58 (m, 2 H), 3.56–3.40 (m, 6 H), 2.17 (t, *J* = 6.2 Hz, 1 H), 1.61–1.50 (m, 4 H), 1.25 (br, 60 H), 0.88 (t, *J* = 7.0 Hz, 6 H).

^{13}C NMR (151 MHz, $CDCl_3$): δ = 78.2, 77.2, 77.0, 76.8, 71.9, 70.9, 70.4, 63.2, 31.9, 30.1, 29.7, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 26.1, 22.7, 14.1.

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{39}H_{80}O_3Na$: 619.6000; found: 619.6028.

(*R*)-2,3-Bis(octadecyloxy)propanoic Acid (7)

Jones reagent, obtained by dropping concd H_2SO_4 (5.6 mL, 4.5 equiv) into an ice-cooled aq CrO_3 (6.8 g, 68.4 mmol) solution in deionized (DI) water (60 mL), was added dropwise to a solution of **10** (13.6 g, 122.8 mmol) in DCM (200 mL) in an ice bath. The suspension was slowly warmed to rt, and the completion of the reaction was confirmed by TLC. The suspension was diluted with *i*-PrOH (50 mL) and filtrated. The filtrate was washed with brine, dried with anhydrous Na_2SO_4, filtrated, and then condensed under vacuum. The resulting product was purified by column chromatography (silica gel, hexane/EtOAc, 9:1 to 4:1) to give **7**.

Yield: 10.0 g (16.4 mmol, 72%); off-white solid; R_f = 0.25 (hexane/EtOAc, 2:1).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 4.04 (dd, *J* = 5.1, 3.2 Hz, 1 H), 3.80 (dd, *J* = 10.5, 3.2 Hz, 1 H), 3.70 (dd, *J* = 10.5, 5.1 Hz, 1 H), 3.67–3.59 (m, 2 H), 3.53–3.43 (m, 2 H), 1.67–1.61 (m, 2 H), 1.59–1.53 (m, 2 H), 1.37–1.19 (br, 60 H), 0.88 (t, *J* = 7.0 Hz, 6 H).

^{13}C NMR (151 MHz, $CDCl_3$): δ = 171.5, 78.6, 77.2, 77.0, 76.8, 72.1, 71.7, 70.4, 31.9, 29.7, 29.7, 29.7, 29.6, 29.6, 29.6, 29.6, 29.4, 29.4, 26.0, 25.9, 22.7, 14.1.

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{39}H_{80}O_3Na$: 633.5792; found: 633.5770.

2-[(*R*)-2,3-Bis(octadecyloxy)propanamido]ethyl α-D-Glucopyranoside (11α)

After PPh_3 (409 mg, 1.56 mmol) was added to a solution of **6aα** (500 mg, 1.20 mmol) in THF (3 mL), the reaction mixture was stirred at rt and monitored by TLC until its completion. The mixture containing the resultant free amine was directly used in the following step. In another reaction vessel, **7** (1.47 g, 2.40 mmol) dissolved in THF (5 mL) was cooled to 0 °C; addition of Et_3N (0.84 mL, 6.00 mmol), HOBt (461 mg, 3.0 mmol, containing ~12% water), and EDC•HCl (559 mg, 3.6 mmol) followed. Then, the free amine solution was added slowly at 0 °C. The reaction mixture was gradually warmed to rt and stirred overnight. After the reaction was complete as determined by TLC, the mixture was quenched with sat. $NaHCO_3$ aq solution (10 mL) and extracted with EtOAc (3×). The combined organic phases were washed with brine, dried with anhydrous Na_2SO_4, filtrated, and condensed under vacuum. The resulting product was briefly purified by column chromatography (silica gel, hexane/EtOAc, 1:1) and then dissolved in THF/MeOH (1:4, 10 mL). NaOMe in MeOH (~5 M, 20 μL, 0.12 mmol) was added into the solution. After the completion of reaction as determined by TLC, the mixture was neutralized with Amberlyst 15 H$^+$ resin, filtrated, condensed under vacuum, and purified by column chromatography (silica gel, DCM/MeOH, 19:1 to 12:1) to give **11α**.

Yield: 595 mg (0.729 mmol, 61%); off-white solid; R_f = 0.40 (DCM/MeOH, 9:1).

^{1}H NMR (600 MHz, $CDCl_3$ with 1% MeOD-d_4): δ = 7.24 (t, *J* = 5.7 Hz, 1 H), 4.84 (d, *J* = 3.7 Hz, 1 H, α-anomeric H), 3.90 (dd, *J* = 5.3, 2.7 Hz, 1 H), 3.85–3.73 (m, 4 H), 3.71–3.52 (m, 7 H), 3.45 (m, 5 H), 1.61 (m, 2 H), 1.56 (m, 2 H), 1.25 (br, *J* = 2.6 Hz, 60 H), 0.88 (t, *J* = 6.9 Hz, 6 H).

^{13}C NMR (151 MHz, $CDCl_3$ with 1% MeOD-d_4): δ = 171.5, 99.1 (anomeric C), 80.2, 77.2, 77.0, 76.8, 74.2, 72.2, 71.9, 71.7, 71.6, 71.0, 70.6, 67.6, 62.3, 38.8, 31.9, 29.7, 29.6, 29.6, 29.5, 29.4, 29.4, 29.4, 26.0, 22.7, 14.1.

HRMS (ESI-TOF): *m*/*z* [M + H]$^+$ calcd for $C_{47}H_{94}NO_9$: 816.6923; found: 816.6933.

2-[(*R*)-2,3-Bis(octadecyloxy)propanamido]ethyl β-D-Glucopyranoside (11β)

Compound **11β** was synthesized from **6aβ** (500 mg, 1.20 mmol) by the same procedure and conditions used for the synthesis of **11α**. The compound was purified by column chromatography (silica gel, DCM/MeOH, 19:1 to 12:1).

Yield: 663 mg (0.812 mmol, 68% in 3 steps); white solid; R_f = 0.40 (DCM/MeOH, 9:1).

^{1}H NMR (600 MHz, $CDCl_3$ with 1% MeOD-d_4): δ = 7.34 (t, *J* = 5.7 Hz, 1 H), 4.32 (d, *J* = 7.7 Hz, 1 H, β-anomeric H), 3.89 (dt, *J* = 9.1, 3.8 Hz, 2 H), 3.86–3.82 (m, 2 H), 3.79–3.73 (m, 2 H), 3.64 (dd, *J* = 10.6, 4.9 Hz, 1 H), 3.55 (t, *J* = 6.9 Hz, 2 H), 3.49 (ddt, *J* = 13.6, 9.6, 7.3 Hz, 5 H), 3.44–3.39 (m, 1 H), 3.32 (dd, *J* = 10.7, 6.1 Hz, 2 H), 1.61 (p, *J* = 7.0 Hz, 2 H), 1.55 (p, *J* = 6.9 Hz, 2 H), 1.35–1.20 (br, 60 H), 0.88 (t, *J* = 6.9 Hz, 6 H).

^{13}C NMR (151 MHz, $CDCl_3$): δ = 171.2, 103.0 (anomeric C), 80.4, 77.3, 77.0, 76.9, 76.8, 76.3, 76.1, 73.5, 71.9, 71.7, 70.9, 69.8, 68.9, 61.6, 49.8, 49.7, 49.6, 49.4, 39.0, 31.9, 29.9, 29.7, 29.6, 29.6, 29.5, 29.4, 29.4, 29.3, 25.9, 25.9, 22.7, 14.7.

HRMS (ESI-TOF): *m*/*z* [M + H]$^+$ calcd for $C_{47}H_{94}NO_9$: 816.6923; found: 816.6936.

2-[(*R*)-2,3-Bis(octadecyloxy)propanamido]ethyl 6-*O*-Tosyl-α-D-glucopyranoside (12α)

Compound **11α** (106.0 mg, 0.13 mmol) and *N*-methylimidazole (20.7 μL, 0.26 mmol) were dissolved in anhydrous THF (2.0 mL). In a separate reaction vessel, methyl triflate (22.1 μL, 0.20 mmol) was added to a solution of 1-tosylimidazole (46.2 mg, 0.21 mmol) in THF (1.0 mL) at 0 °C, and the solution was stirred at 0 °C for 30 min. This suspension was added to the solution of **11α** at 0 °C, and the reaction mixture was stirred at 0 °C for 6 h, before being warmed to rt and condensed under vacuum. The product was purified by column chromatography (silica gel, DCM/MeOH, 49:1 to 24:1) to give **12α**.

Yield: 69.0 mg (71.1 μmol, 55%); white solid; recovery of some **11α** (23 mg, 28.2 μmol, 22%); R_f = 0.60 (DCM/MeOH, 19:1).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.80 (d, *J* = 8.1 Hz, 2 H), 7.34 (d, *J* = 8.0 Hz, 2 H), 7.06 (t, *J* = 6.4 Hz, 1 H), 4.81 (d, *J* = 3.8 Hz, 1 H, α-anomeric H), 4.27 (d, *J* = 3.5 Hz, 2 H), 3.77 (dq, *J* = 9.9, 3.2 Hz, 2 H), 3.70–3.57 (m, 5

H), 3.54–3.50 (m, 2 H), 3.47–3.35 (m, 8 H), 2.45 (s, 3 H), 1.61 (p, *J* = 6.8 Hz, 2 H), 1.54 (p, *J* = 6.8 Hz, 2 H), 1.35–1.17 (br, 60 H), 0.88 (t, *J* = 7.0 Hz, 6 H).

^{13}C NMR (151 MHz, $CDCl_3$): δ = 171.6, 144.9, 132.9, 129.9, 129.8, 128.0, 98.9 (anomeric C), 80.2, 77.2, 77.0, 76.8, 74.3, 72.0, 71.9, 71.5, 70.9, 70.6, 69.8, 69.7, 69.0, 38.8, 31.9, 29.7, 29.7, 29.7, 29.6, 29.5, 29.5, 29.4, 29.4, 26.5, 26.0, 26.0, 26.0, 22.7, 21.7, 14.1.

HRMS (ESI-TOF): *m*/*z* [M + H]$^+$ calcd for $C_{54}H_{100}NO_{11}S$: 970.7012; found: 970.7018.

2-[(*R*)-2,3-Bis(octadecyloxy)propanamido]ethyl 6-*O*-Tosyl-β-D-glucopyranoside (12β)

Compound **12β** was synthesized from **11β** (90.0 mg, 0.11 mmol) by the same procedure and conditions employed in the synthesis of **12α**. It was purified by column chromatography (silica gel, DCM/MeOH, 49:1 to 24:1).

Yield: 62.0 mg (63.9 μmol, 58%); white solid; 21.0 mg of **11β** (25.7 μmol, 23%) recovered; R_f = 0.55 (DCM/MeOH, 19:1).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.80 (d, *J* = 8.0 Hz, 2 H), 7.34 (d, *J* = 8.0 Hz, 2 H), 7.06 (t, *J* = 6.4 Hz, 1 H), 4.30–4.22 (m, 2 H, overlapped β-anomeric H), 3.91–3.84 (m, 2 H), 3.72–3.57 (m, 5 H), 3.57–3.36 (m, 13 H), 3.35–3.26 (m, 3 H), 2.45 (s, 3 H), 1.61 (p, *J* = 6.6 Hz, 2 H), 1.53 (p, *J* = 6.8 Hz, 2 H), 1.32–1.23 (br, 60 H), 0.88 (t, *J* = 6.9 Hz, 6 H).

^{13}C NMR (151 MHz, $CDCl_3$): δ = 171.6, 145.0, 132.8, 129.9, 128.0, 103.0 (anomeric C), 80.3, 77.2, 77.0, 76.9, 76.8, 76.3, 73.6, 73.2, 71.9, 71.4, 70.9, 70.6, 69.5, 69.3, 68.7, 39.0, 31.9, 29.7, 29.7, 29.6, 29.5, 29.5, 29.4, 26.5, 26.0, 26.0, 22.7, 21.7, 14.1.

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{54}H_{99}NO_{11}SNa$: 992.6831; found: 992.6833.

2-[(*R*)-2,3-Bis(octadecyloxy)propanamido]ethyl 6-Azido-6-deoxy-α-D-glucopyranoside (5aα)

Compound **12α** (69.0 mg, 71 μmol), NaN_3 (46.2 mg, 0.71 mmol), and KI (0.1 mg) were suspended in DMSO (2.0 mL). The mixture was heated to 75 °C and stirred at this temperature for 2 d. After the mixture was cooled to rt, diluted with EtOAc, and then cooled to 0 °C, water (2.0 mL) was added. The mixture was extracted with EtOAc (3×). The combined organic phase was washed with brine, dried with anhydrous Na_2SO_4, filtrated, and condensed under vacuum. The crude product was purified by column chromatography (silica gel, DCM/MeOH, 49:1 to 24:1) to give **5aα**.

Yield: 47.3 mg (56.2 μmol, 79%); white solid; R_f = 0.60 (DCM/MeOH, 19:1).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.10 (t, *J* = 6.4 Hz, 1 H), 4.88 (d, *J* = 3.7 Hz, 1 H, α-anomeric H), 3.90 (dd, *J* = 4.9, 2.7 Hz, 1 H), 3.85 (td, *J* = 7.0, 3.5 Hz, 1 H), 3.80–3.73 (m, 2 H), 3.72–3.57 (m, 5 H), 3.56–3.40 (m, 9 H), 1.61 (q, *J* = 7.0 Hz, 2 H) 1.54 (q, *J* = 7.0 Hz, 2 H), 1.25 (d, *J* = 2.4 Hz, 60 H), 0.88 (t, *J* = 7.0 Hz, 6 H).

^{13}C NMR (151 MHz, $CDCl_3$): δ = 171.6, 99.0 (anomeric C), 80.2, 77.2, 77.0, 76.8, 74.5, 72.2, 71.9, 71.5, 71.1, 70.9, 70.9, 68.1, 51.4, 38.9, 31.9, 29.7, 29.7, 29.6, 29.5, 29.4, 29.4, 26.0, 26.0, 22.7, 14.1.

HRMS (ESI-TOF): *m*/*z* [M + H]$^+$ calcd for $C_{47}H_{93}N_4O_8$: 841.6988; found: 841.6996.

2-[(*R*)-2,3-Bis(octadecyloxy)propanamido]ethyl 6-Azido-6-deoxy-β-D-glucopyranoside (5aβ)

Compound **5aβ** was synthesized from **12β** (62.0 mg, 64 μmol) by the same procedure and conditions employed in the synthesis of **5aα**. The product was purified by column chromatography (silica gel, DCM/MeOH, 49:1 to 24:1).

Yield: 47.0 mg (48.7 μmol, 87%); white solid; R_f = 0.55 (DCM/MeOH, 19:1).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.04 (t, *J* = 6.0, 1 H), 4.35 (d, *J* = 7.7 Hz, 1 H, β-anomeric H), 3.94 (ddd, *J* = 11.9, 8.2, 3.5 Hz, 1 H), 3.78–3.72 (m, 1 H), 3.72–3.57 (m, 4 H), 3.57–3.49 (m, 5 H), 3.45 (dtd, *J* = 16.3, 9.6, 4.7 Hz, 4 H), 3.38–3.31 (m, 2 H), 1.61 (q, *J* = 6.9 Hz, 2 H), 1.54 (q, *J* = 7.1 Hz, 2 H), 1.26 (br, 60 H), 0.88 (t, *J* = 7.0 Hz, 6 H).

^{13}C NMR (151 MHz, $CDCl_3$): δ = 171.6, 102.9 (anomeric C), 80.4, 77.2, 77.0, 76.8, 76.8, 76.6, 75.4, 73.4, 71.9, 71.4, 71.0, 70.9, 69.1, 51.5, 39.0, 31.9, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.3, 26.0, 26.0, 22.7, 14.1.

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{47}H_{92}N_4O_8Na$: 863.6813; found: 863.6818.

2-[(*R*)-2,3-Bis(octadecyloxy)propanamido]ethyl 6-Deoxy-6-[(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrole)-3-carboxamido]-β-D-glucopyranoside (1)

Compound **5aβ** (5.0 mg, 6 μmol) was dissolved in THF/MeOH (1:1, 1.0 mL); addition of 10% Pd/C (3.0 mg) followed. The reaction vessel was flushed with H_2 and attached to a balloon filled with H_2. After completion of the reaction as determined by TLC, the mixture was filtrated through a cotton pad (2×). The resulting solution was condensed under vacuum, and the crude amine intermediate was dissolved in THF/MeOH (9:1, 2.0 mL). Then, DIPEA (8.4 μL, 48 μmol) and the activated ester **13** (13.5 mg, 48 μmol) were added. After completion of the reaction as determined by TLC, the mixture was condensed under vacuum and the product was purified by column chromatography (silica gel, DCM/MeOH, 49:1 to 19:1) to give **1**.

Yield: 4.8 mg (4.89 μmol, 82%); pale-yellow syrup; R_f = 0.55 (DCM/MeOH, 19:1).

IR (neat, KBr): 3321.48, 2916.06, 2849.44, 1655.29, 1532.81, 1466.60, 1358.35, 1049.61, 720.00 cm^{-1}.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.22 (br, 1 H), 4.90 (br d, 1 H, α-anomeric H), 3.91–3.29 (m), 1.61 (br, 2 H) 1.54 (br, 2 H), 1.25 (br, 60 H), 0.88 (br, 6 H).

HRMS (ESI-TOF): *m*/*z* [M + H]$^+$ calcd for $C_{56}H_{107}N_3O_{10}$: 981.7956; found: 981.7947.

2-[(*R*)-2,3-Bis(octadecyloxy)propanamido]ethyl 6-Deoxy-6-[(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrole)-3-carboxamido]-α-D-glucopyranoside (2)

Compound **2** was synthesized from **5aα** (4.2 mg, 5.1 μmol) by the same procedure and conditions employed in the synthesis of **1**. It was purified by column chromatography (silica gel, DCM/MeOH, 49:1 to 19:1).

Yield: 4.2 mg (4.3 μmol, 88%); pale-yellow syrup; R_f = 0.55 (DCM/MeOH, 19:1).

IR (neat, KBr): 3387.10, 2916.85, 2849.76, 1783.40, 1698.25, 1652.35, 1530.06, 1466.00, 1427.17, 1305.24, 1213.50, 1163.64, 1076.90, 1049.18, 998.66, 893.02, 813.99, 717.92 cm^{-1}.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.49 (br, 1 H), 4.43 (br, 1 H, β-anomeric H), 4.01–3.36 (m), 1.61 (br, 2 H), 1.54 (br, 2 H), 1.26 (br, 60 H), 0.88 (br, 6 H).

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{56}H_{106}N_3O_{10}Na$: 1003.7776; found: 1003.7783.

2-Azidoethyl 2,3-Di-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (16)

NaOMe in MeOH (~5 M, 24 μL, 0.12 mmol) was added to a solution of **6b** (421 mg, 0.6 mmol) dissolved in THF/MeOH (1:4, 10 mL). After completion of the reaction as determined by TLC, the mixture was neutralized with Amberlyst 15 H$^+$ resin, filtrated, and condensed under vacuum. The residue was dissolved in DMF, and to the solution were then added benzaldehyde dimethyl acetal (0.45 mL, 3 mmol) and *p*-TsOH (11.4 mg, 60 μmol). The mixture was heated to 45 °C under reduced pressure (50 mbar), stirred overnight, quenched with Et_3N (0.1 mL), and condensed under vacuum. The residue was dissolved in THF (20.0 mL), and then Et_3N (1.3 mL, 9.0 mmol), DMAP (14.7 mg, 0.12 mmol), and Ac_2O (0.57 mL, 6.0 mmol) were added. The solution was stirred overnight, quenched with sat. aq $NaHCO_3$ solution (10.0 mL), and extracted with EtOAc. The combined organic phase was washed with brine, dried with anhydrous Na_2SO_4, filtrated, and condensed under vacuum. The product was purified by column chromatography (silica gel, hexane/EtOAc, 3:2 to 1:1); this gave **16**.

Yield: 276 mg (0.39 mmol, 65%); light-yellow syrup; with some **6b** (80 mg, 0.11 mmol, 19%) recovered; R_f = 0.60 (hexanes/EtOAc, 1:1).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.45–7.39 (m, 2 H), 7.38–7.32 (m, 3 H), 5.48 (s, 1 H), 5.46 (t, *J* = 10.0 Hz, 1 H), 5.37 (d, *J* = 4.2 Hz, 1 H, α-anomeric H), 5.26 (t, *J* = 9.1 Hz, 1 H), 4.91–4.82 (m, 2 H), 4.63–4.57 (m, 2 H, overlapped β-anomeric H), 4.28–4.21 (m, 2 H), 4.07–3.97 (m, 2 H), 3.86 (td, *J* = 9.9, 4.8 Hz, 1 H), 3.77–3.66 (m, 3 H), 3.63 (t, *J* = 9.7 Hz, 1 H), 3.48 (ddd, *J* = 13.4, 8.4, 3.3 Hz, 1 H), 3.25 (ddd, *J* = 13.4, 4.9, 3.2 Hz, 1 H), 2.12 (s, 3 H), 2.06 (s, 3 H), 2.05 (s, 3 H), 2.03 (s, 3 H), 2.01 (s, 3 H).

^{13}C NMR (151 MHz, CDCl3) δ = 170.8, 170.3, 170.2, 169.8, 169.7, 136.7, 129.1, 128.2, 126.2, 101.6, 100.2 (β-anomeric C), 96.5 (α-anomeric C), 78.8, 75.4, 72.6, 72.3, 72.0, 70.8, 68.7, 68.5, 63.7, 62.3, 50.5, 20.9, 20.8, 20.7, 20.7, 20.6. These NMR data matched that reported in the literature.[28–30]

2-Azidoethyl 2,3-Di-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (17)

Compound **17** was synthesized from **6c** (500 mg, 0.5 mmol) by the same procedure and conditions employed in the synthesis of **16**, with part of **6c** (245 mg, 0.245 mmol, 49%) recovered. Product **17** was purified by column chromatography (silica gel, hexane/EtOAc, 1:1 to 2:3).

Yield: 209 mg (0.209 mmol, 42%); light-yellow syrup; R_f = 0.50 (hexanes/EtOAc, 1:1).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.45 (dd, *J* = 6.9, 2.8 Hz, 2 H), 7.37 (dd, *J* = 5.2, 2.0 Hz, 3 H), 5.51–5.45 (m, 2 H), 5.43 (dd, *J* = 10.3, 8.7 Hz, 1 H), 5.38 (d, *J* = 4.2 Hz, 1 H, α-anomeric H), 5.34–5.26 (m, 2 H, overlapped α-anomeric H), 4.93–4.83 (m, 2 H), 4.77 (dd, *J* = 10.3, 4.0 Hz, 1 H), 4.64 (d, *J* = 7.8 Hz, 1 H, β-anomeric H), 4.57 (ddd, *J* = 20.5, 12.3, 2.7 Hz, 2 H), 4.34–4.22 (m, 3 H), 4.07–3.94 (m, 4 H), 3.86 (td, *J* = 9.8, 4.7 Hz, 1 H), 3.75 (s, 1 H), 3.78–3.69 (m, 2 H), 3.65 (t, *J* = 9.7 Hz, 1 H), 3.51 (ddd, *J* = 12.3, 8.4, 3.4 Hz, 1 H), 3.32–3.25 (m, 1 H), 2.20 (s, 3 H), 2.15 (s, 3 H), 2.09 (s, 3 H), 2.07 (s, 3 H), 2.05 (s, 6 H), 2.04 (s, 3 H), 2.01 (s, 3 H).

^{13}C NMR (151 MHz, $CDCl_3$): δ = 170.9, 170.7, 170.4, 170.2, 170.1, 169.77, 169.7, 169.7, 136.7, 129.2, 128.2, 126.2, 101.7, 100.2 (β-anomeric C), 96.5 (α-anomeric C), 95.8 (α-anomeric C), 78.8, 77.2, 77.0, 76.8, 75.3, 73.5, 72.4, 72.3, 72.0, 71.9, 70.9, 70.5, 69.0, 68.7, 68.5, 63.8, 62.6, 62.2, 50.5, 20.9, 20.9, 20.8, 20.7, 20.6, 20.6.

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{43}H_{55}N_3O_{24}$: 1020.3073; found: 1020.3060.

2-[(*R*)-2,3-Bis(octadecyloxy)propanamido]ethyl 2,3-Di-*O*-acetyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (18)

Compound **18** (149 mg, 0.125 mmol, 63% for three steps) as colorless syrup was synthesized from **16** (142 mg, 0.20 mmol) by a similar procedure used in the synthesis of **11α**. However, after the coupling product was briefly purified and dissolved in THF/MeOH (3:1, 4 mL), *p*-toluenesulfonic acid (3.8 mg, 20 μmol, 0.1 equiv), instead of NaOMe, was added. The reaction was stirred at rt for 6–8 h to allow for completion as indicated by TLC and then quenched with triethylamine (0.1 mL). The solvent was removed in vacuum, and the product **18** was purified by column chromatography (silica gel, hexane/EtOAc 1:1 to EtOAc).

Yield: 149 mg (0.125 mmol, 63% for three steps); colorless syrup; R_f = 0.35 (hexanes/EtOAc, 2:3).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 6.95 (t, *J* = 5.9 Hz, 1 H), 5.34 (d, *J* = 4.0 Hz, 1 H, α-anomeric H), 5.26–5.18 (m, 2 H), 4.84–4.75 (m, 2 H), 4.58–4.53 (m, 2 H, overlapped β-anomeric H), 4.18 (dd, *J* = 12.2, 4.5 Hz, 1 H), 3.97 (t, *J* = 9.2 Hz, 1 H), 3.89 (dd, *J* = 6.1, 2.5 Hz, 1 H), 3.83 (s, 3 H), 3.74 (dd, *J* = 10.7, 2.5 Hz, 1 H), 3.68 (p, *J* = 4.4, 3.7 Hz, 4 H), 3.61–3.38 (m, 7 H), 2.14 (s, 3 H), 2.09 (s, 3 H), 2.05 (s, 3 H), 2.02 (s, 3 H), 2.00 (s, 3 H), 1.60 (q, *J* = 7.1 Hz, 2 H), 1.53 (q, *J* = 6.9 Hz, 2 H), 1.36–1.12 (br, 60 H), 0.88 (t, *J* = 6.9 Hz, 6 H).

^{13}C NMR (151 MHz, $CDCl_3$): δ = 171.5, 171.0 170.84 170.7 170.1, 169.6, 100.3 (β-anomeric C), 95.8 (α-anomeric C), 80.4, 77.2, 77.0, 76.8, 75.5, 72.5, 72.4, 72.4, 72.3, 72.1, 71.7, 71.5, 70.0, 70.0, 68.6, 62.8, 62.3, 38.8, 31.9, 29.7, 29.7, 29.7, 29.5, 29.5, 29.5, 29.4, 26.1, 26.0, 22.7, 20.9, 20.9, 20.6, 14.1.

HRMS (ESI-TOF): *m*/*z* [M + H]$^+$ calcd for $C_{47}H_{94}NO_9$: 1188.7980; found: 1188.7990.

2-[(*R*)-2,3-Bis(octadecyloxy)propanamido]ethyl 2,3-Di-*O*-acetyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (19)

Compound **19** was synthesized from **17** (200 mg, 0.2 mmol) by the same procedure and conditions utilized in the synthesis of **18**. Product **19** was purified by column chromatography (silica gel, hexane/EtOAc, 1:1, to EtOAc).

Yield: 150 mg (0.102 mmol, 51% in three steps); colorless syrup; R_f = 0.30 (hexanes/EtOAc, 1:2).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 6.94 (t, *J* = 5.8 Hz, 1 H), 5.39 (dd, *J* = 10.3, 8.4 Hz, 1 H), 5.31 (d, *J* = 4.0 Hz, 1 H, α-anomeric H), 5.30–5.18 (m, 3 H, overlapped α-anomeric H), 4.82–4.71 (m, 3 H), 4.55 (d, *J* = 7.9 Hz, 1 H, β-anomeric H), 4.48 (ddd, *J* = 17.9, 12.4, 2.8 Hz, 2 H), 4.27–4.19 (m, 2 H), 3.98–3.37 (m, 20 H), 2.17 (s, 3 H), 2.14 (s, 3 H), 2.08 (s, 3 H), 2.06 (s, 3 H), 2.01 (s, 3 H), 2.01 (s, 2 H), 2.00 (s, 3 H), 1.98 (s, 3 H), 1.57 (dp, *J* = 37.8, 6.9 Hz, 4 H), 1.41–1.13 (br, 60 H), 0.87 (t, *J* = 6.9 Hz, 6 H).

^{13}C NMR (151 MHz, $CDCl_3$): δ = 171.3, 170.9, 170.8, 170.8, 170.6, 170.0, 169.7, 169.6, 100.3 (β-anomeric C), 96.0 (α-anomeric C), 95.6 (α-anomeric C), 80.4, 77.2, 77.0, 76.8, 75.3, 73.4, 72.7, 72.2, 72.2, 72.1, 71.8, 71.7, 71.6, 71.5, 71.5, 70.4, 70.2, 70.0, 69.3, 68.6, 63.1, 62.6, 62.3, 38.8, 31.9, 29.7, 29.7, 29.5, 29.5, 29.5, 29.4, 26.1, 26.0, 26.0, 22.7, 20.9, 20.9, 20.9, 20.7, 20.6, 20.6, 14.1.

HRMS (ESI-TOF): *m*/*z* [M + NH_4]$^+$ calcd for $C_{75}H_{133}N_2O_{27}$: 1493.9090; found: 1493.9099.

2-[(*R*)-2,3-Bis(octadecyloxy)propanamido]ethyl 2,3-Di-*O*-acetyl-6-*O*-tosyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (20)

Compound **20** was synthesized from **18** (45 mg, 37.9 μmol) by the same procedure and conditions used in the synthesis of **12α**, with part of **18** (8 mg, 6.7 μmol, 18%) recovered. Product **20** was purified by column chromatography (silica gel, hexane/EtOAc, 3:2 to 2:3).

Yield: 31 mg (23.1 μmol, 61%); colorless syrup; R_f = 0.60 (hexanes/EtOAc, 2:3).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.83 (d, *J* = 8.3 Hz, 2 H), 7.40 (d, *J* = 8.1 Hz, 2 H), 6.97 (t, *J* = 5.9 Hz, 1 H), 5.30 (d, *J* = 4.0 Hz, 1 H, α-anomeric H), 5.25–5.17 (m, 2 H), 4.84–4.74 (m, 2 H), 4.55 (d, *J* = 7.9 Hz, 1 H, β-anomeric H), 4.49 (dd, *J* = 12.1, 2.6 Hz, 1 H), 4.41 (dd, *J* = 11.3, 3.6 Hz, 1 H), 4.17 (dd, *J* = 11.3, 1.9 Hz, 1 H), 4.12 (dd, *J* = 12.2, 4.6 Hz, 1 H), 3.93–3.79 (m, 4 H), 3.77 (dd, *J* = 10.7, 2.6 Hz, 1 H), 3.73–3.40 (m, 12 H), 2.97 (d, *J* = 5.9 Hz, 1 H), 2.49 (s, 3 H), 2.11 (s, 3 H), 2.10 (s, 3 H), 2.06 (s, 3 H), 2.04 (s, 3 H), 2.01 (s, 3 H), 1.61 (p, *J* = 6.9 Hz, 2 H), 1.56 (p, *J* = 6.9 Hz, 2 H), 1.28 (d, *J* = 3.1 Hz, 60 H), 0.90 (t, *J* = 7.0 Hz, 6 H).

^{13}C NMR (151 MHz, $CDCl_3$): δ = 171.3, 170.8, 170.6, 170.6, 170.1, 169.6, 145.2, 132.7, 129.9, 128.0, 100.2 (β-anomeric C), 100.0, 95.8 (α-anomeric C), 80.4, 77.2, 77.0, 76.8, 75.4, 72.7, 72.4, 72.0, 71.8, 71.7, 71.5, 71.5, 70.9, 69.8, 68.6, 68.3, 67.9, 62.7, 38.8, 31.9, 29.7, 29.7, 29.7, 29.7, 29.5, 29.5, 29.5, 29.4, 26.5, 26.1, 26.0, 22.7, 21.7, 20.9, 20.8, 20.8, 20.6, 20.6, 14.1.

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{70}H_{119}NO_{21}SNa$: 1364.7893; found: 1364.7908.

2-[(*R*)-2,3-Bis(octadecyloxy)propanamido]ethyl 2,3-Di-*O*-acetyl-6-*O*-tosyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (21)

Compound **21** was prepared from **19** (42 mg, 28.4 μmol) by the same procedure and conditions utilized in the synthesis of **12α**, with part of **19** (13.4 mg, 9.1 μmol, 32%) recovered. Product **21** was purified by column chromatography (silica gel, hexane/EtOAc, 1:1 to 1:2).

Yield: 20.8 mg (12.8 μmol, 45%); colorless syrup; R_f = 0.45 (hexanes/EtOAc, 1:2).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.80 (d, *J* = 8.0 Hz, 2 H), 7.36 (d, *J* = 8.0 Hz, 2 H), 6.93 (t, *J* = 5.5 Hz, 1 H), 5.36 (t, *J* = 9.5 Hz, 1 H), 5.29–5.21 (m, 3 H, 2 overlapped α-anomeric H), 5.18 (t, *J* = 9.9 Hz, 1 H), 4.82–4.76 (m, 1 H), 4.72 (ddd, *J* = 14.3, 10.1, 4.2 Hz, 2 H), 4.54 (d, *J* = 7.8 Hz, 1 H, β-anomeric H), 4.48 (dd, *J* = 12.0, 3.0 Hz, 1 H), 4.41 (dd, *J* = 12.3, 2.3 Hz, 1 H), 4.36 (dd, *J* = 11.2, 3.6 Hz, 1 H), 4.27–4.20 (m, 1 H), 4.17 (d, *J* = 11.1 Hz, 1 H), 4.11 (dd, *J* = 12.3, 4.2 Hz, 1 H), 3.97–3.68 (m, 9 H), 3.68–3.62 (m, 2 H), 3.61–3.49 (m, 4 H), 3.49–3.36 (m, 6 H), 2.46 (s, 3 H), 2.15 (s, 3 H), 2.10 (s, 3 H), 2.08 (s, 3 H), 2.05 (s, 3 H), 2.01 (s, 6 H), 1.99 (s, 3 H), 1.98 (s, 3 H), 1.60 (q, *J* = 6.9 Hz, 2 H), 1.53 (q, *J* = 6.9 Hz, 2 H), 1.32–1.24 (m, 28 H), 0.88 (t, *J* = 6.9 Hz, 6 H).

^{13}C NMR (151 MHz, $CDCl_3$): δ = 171.2, 170.8, 170.7, 170.6, 170.6, 170.5, 170.1, 169.7, 169.6, 145.1, 132.7, 130.0, 129.9, 129.8, 128.0, 127.9, 100.3 (β-anomeric C), 100.3, 96.0 (α-anomeric C), 95.8 (α-anomeric C), 95.6, 80.4, 77.2, 77.1, 77.0, 76.8, 75.3, 73.4, 72.9, 72.6, 72.2, 72.1, 71.8, 71.7, 71.6, 71.5, 70.9, 70.8, 70.6, 70.4, 70.0, 69.3, 68.9, 68.6, 68.3, 68.0, 63.0, 62.5, 62.2, 58.5, 38.8, 31.9, 29.7, 29.7, 29.5, 29.5, 29.5, 29.4, 26.5, 26.3, 26.1, 26.0, 22.7, 21.7, 21.7, 20.9, 20.9, 20.8, 20.8, 20.6, 20.6, 20.5, 14.1.

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{82}H_{135}NO_{29}SNa$: 1652.8738; found: 1652.8719.

(*R*)-2,3-Bis(octadecyloxy)propanamidoethyl 6-Azido-6-deoxy-α-D-glucopyranosyl-(1→4)-β-D-glucocopyranoside (5b)

Compound **20** (31.0 mg, 23.1μmol), NaN_3 (30.0 mg, 0.46 mmol), and KI (0.1 mg) were suspended in DMSO (2.0 mL). The mixture was heated to 75 °C and stirred for 2 d. The mixture was cooled to rt and diluted with EtOAc, and then cooled to 0 °C; addition of water (4.0 mL) and extraction with EtOAc (3×) followed. The combined organic phase was washed with brine, dried with anhydrous Na_2SO_4, filtrated, and condensed under vacuum. The crude product was dissolved in THF/MeOH (1:4, 5 mL), followed by addition of NaOMe in MeOH (~5 M, 4 μL). After TLC showed the completion of the reaction, the solution was neutralized with Amberlyst 15 H^+ resin. The mixture was filtrated and condensed under vacuum. The product was purified by column chromatography (silica gel, DCM/MeOH, 24:1 to 15:1) to give **5b**.

Yield: 19 mg (18.9 μmol, 82%); white solid; R_f = 0.45 (DCM/MeOH, 15:1).

^{1}H NMR (600 MHz, $CDCl_3$ with 2% MeOD): δ = 7.10 (m, 1 H), 5.16 (d, *J* = 3.7 Hz, 1 H, α-anomeric H), 4.35 (d, *J* = 7.7 Hz, 1 H, β-anomeric H), 3.88–3.78 (m, 4 H), 3.78–3.68 (m, 4 H), 3.67–3.53 (m, 8 H), 3.50–3.33 (m, 7 H), 1.62 (m, 2 H), 1.55 (m, 2 H), 1.36–1.25 (m, 60 H), 0.90 (t, *J* = 6.9 Hz, 6 H).

^{13}C NMR (151 MHz, $CDCl_3$): δ = 171.5, 102.9 (β-anomeric C), 101.5 (α-anomeric C), 80.3, 80.1, 77.2, 77.0, 76.8, 75.9, 75.4, 73.6, 73.2, 72.5, 72.1, 71.9, 71.9, 71.6, 71.0, 70.7, 69.0, 63.8, 61.2, 51.7, 39.1, 39.0, 31.9, 29.7, 29.7, 29.7, 29.7, 29.6, 29.5, 29.4, 29.4, 29.4, 26.0, 26.0, 22.7, 14.1.

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{53}H_{102}N_4O_{13}Na$: 1025.7341; found: 1025.7352.

2-[(*R*)-2,3-Bis(octadecyloxy)propanamido]ethyl 6-Azido-6-deoxy-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (5c)

Compound **5c** was prepared from **21** (20 mg, 12.3 μmol) by the same procedure and conditions used in the synthesis of **5b**. Product **5c** was purified by column chromatography (silica gel, DCM/MeOH, 19:1 to 9:1).

Yield: 10.4 mg, 8.92 μmol, 73%); R_f = 0.55 (DCM/MeOH, 9:1).

^{1}H NMR (600 MHz, $CDCl_3$ with 2% MeOD): δ = 7.43–7.31 (m, 1 H), 5.12 (t, *J* = 3.4 Hz, 2 H, 2 overlapped α-anomeric H), 4.34 (d, *J* = 7.7 Hz, 1 H, β-anomeric H), 3.93–3.86 (m, 4 H), 3.86–3.72 (m, 7 H), 3.62–3.53 (m, 6 H), 3.51–3.38 (m, 7 H), 1.65–1.59 (m, 2 H), 1.55 (t, *J* = 7.7 Hz, 2 H), 1.33–1.24 (m, 60 H), 0.89 (t, *J* = 7.5, 6 H).

^{13}C NMR (151 MHz, $CDCl_3$ with 5% MeOD-d_4): δ = 171.7, 102.8 (β-anomeric C), 101.5 (2 α-anomeric C), 80.2, 77.2, 77.0, 76.8, 75.0, 73.5, 72.1, 71.8, 71.5, 71.3, 68.6, 61.0, 51.8, 39.0, 31.9, 29.8, 29.8, 29.7, 29.7, 29.7, 29.6, 29.5, 29.5, 29.4, 26.1, 26.0, 22.7, 14.1.

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{59}H_{112}N_4O_{18}Na$: 1187.7869; found: 1187.7875.

2-[(*R*)-2,3-Bis(octadecyloxy)propanamido]ethyl 6-Deoxy-6-[(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrole)-3-carboxamido]-α-D-glucopyranosyl-(1→4)-β-D-glucocopyranoside (3)

Compound **3** was synthesized from **5b** (4.8 mg, 4.78 μmol) by the same procedure and conditions employed in the synthesis of **1**. The product was purified by column chromatography (silica gel, DCM/MeOH, 24:1 to 12:1).

Yield: 4.2 mg (3.67 μmol, 77%); pale-yellow syrup; R_f = 0.40 (DCM/MeOH, 15:1).

IR (neat, KBr): 3341.57, 2916.64, 2849.70, 1780.23, 1707.52, 1648.24, 1535.75, 1466.26, 1378.11, 1304.37, 1214.40, 1074.04, 1039.95, 816.28, 717.99 cm^{-1}.

^1H NMR (600 MHz, $CDCl_3$ with 2% MeOD): δ = 7.36 (br, 1 H), 5.40–5.10 (br, α-anomeric H), 4.40–4.25 (br, β-anomeric H), 3.98–3.43 (m), 1.62 (br, 2 H), 1.55 (br, 2 H), 1.36–1.25 (br, 60 H), 0.90 (br, 6 H).

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{62}H_{116}N_3O_{15}Na$: 1165.8304; found: 1165.8322.

2-[(*R*)-2,3-Bis(octadecyloxy)propanamido]ethyl 6-Deoxy-6-[(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrole)-3-carboxamido]-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (4)

Compound **4** was prepared from **5c** (4.1 mg, 3.52 μmol) by the same procedure and conditions employed in the synthesis of **1**. The product was purified by column chromatography (silica gel, DCM/MeOH, 19:1 to 7:1).

Yield: 3.4 mg, 2.60 μmol, 73%); pale-yellow syrup; R_f = 0.45 (DCM/MeOH, 9:1).

IR (neat, KBr): 3328.96, 2916.72, 2849.82, 1710.34, 1653.09, 1535.44, 1465.93, 1377.64, 1212.11, 1072.22, 1031.38, 815.57, 719.36 cm^{-1}.

^1H NMR (600 MHz, $CDCl_3$ with 2% MeOD): δ = 5.50–5.00 (br, α-anomeric H), 4.30–3.97 (br, β-anomeric H), 3.97–3.30 (m), 1.56–1.50 (br), 1.33–1.24 (br, 60 H), 0.89 (br, 6 H).

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{68}H_{126}N_3O_{20}Na$: 1327.8832; found: 1327.8812.

Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/a-1768-2138.

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