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Tandem Glycosyl Iodide Glycosylation and Regioselective Enzymatic Acylation Affords 6-*O*-Tetradecanoyl-α-D-cholesterylglycosides

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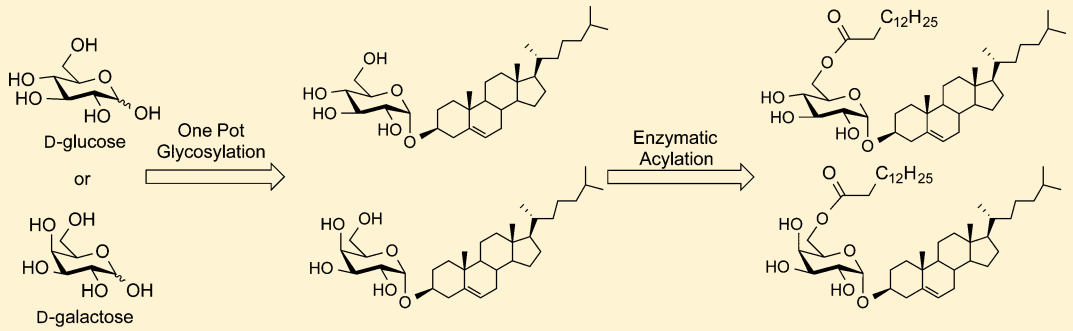
Supporting Information



ABSTRACT: A generalized synthesis of α-D-cholesterylglycosides has been achieved using one-pot per-*O*-trimethylsilyl glycosyl iodide glycosidation. Both cholesteryl α-D-glucopyranoside (αCG) and cholesteryl α-D-galactopyranoside were prepared in high yield. These compounds were further esterified using regioselective enzymatic acylation with tetradecanoyl vinyl ester to afford 6-*O*-tetradecanoyl-α-D-cholesteryl glucopyranoside (αCAG) of *Helicobacter pylori* and the corresponding galactose analogue in 66−78% overall yields from free sugars. The tandem step-economy sequence provides novel analogues to facilitate glycolipidomic profiling.

Recently, three cholesterylglucosides (Figure 1) have been isolated from *Helicobacter pylori*, a bacteria that is believed

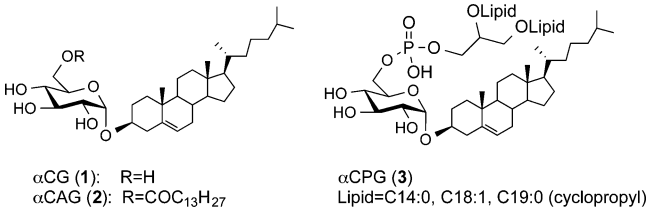


Figure 1. Structures of *H. pylori* cholesterylglucosides.

to infect 50% of the world population. While in many cases, *H. pylori* infection goes undetected, chronic infection can lead to gastric ulcers or even cancer.[1−8] The core glucoside structure includes glucose α-linked to cholesterol (αCG (**1**)). One analogue is acylated at the sugar C6-hydroxyl with tetradecanoic acid and is referred to as αCAG (**2**). A third analogue contains a phosphatidyl group instead of the fatty acid chain and is abbreviated αCPG (**3**). The lipid portion on the phosphatidyl glycerol may vary in its composition of three different lipids.[9] Together, these glucosides make up approximately 25% of the lipid content of *H. pylori* and are a distinguishing feature of these bacteria.[5,6] Most notably, these compounds have been shown to stimulate the human immune system and, since *H. pylori* must obtain cholesterol from the host, a symbiotic relationship between microbe and man has been suggested.

To date, biological studies involving these cholesterylglycosides have mostly relied upon natural sources, which furnish mixtures of all three glycolipids, making it difficult to determine the independent roles of each constituent.[7,8] We have initiated a synthetic campaign directed toward developing step-economy syntheses of cholesterylglycosides. As part of that effort, we are interested in making both glucosyl and galactosyl analogues of αCAG available to the biological community for structure activity relationship and glycolipidomic profiling studies. Access to these standards may also facilitate the identification of naturally occurring analogues that have yet to be discovered.

We recently reported a five-step synthesis of αCAG (**2**),[10] which relied upon enzymatic acylation of free glucose to afford 6-*O*-tetradecanoyl glucose (Scheme 1). The remaining hydroxyls were protected as trimethylsilyl ethers, and the resulting compound was treated with trimethylsilyl iodide to generate the α-iodide. The C-6 acyl functionality had a remarkable effect on the reaction rate, requiring 14 h to quantitatively produce the iodide as compared to the same reaction employing per-*O*-silyl glucose, which took only 30 min.[10] Moreover, subsequent glycosidation proceeded in only 45% yield, whereas glycosidations with per-*O*-silyl glycosyl iodides are typically much higher. This synthetic protocol was

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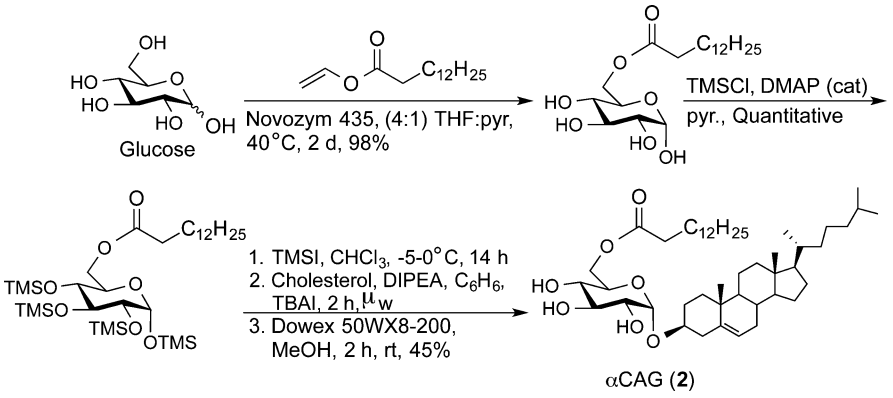
Scheme 1. First-Generation Synthesis of αCAG



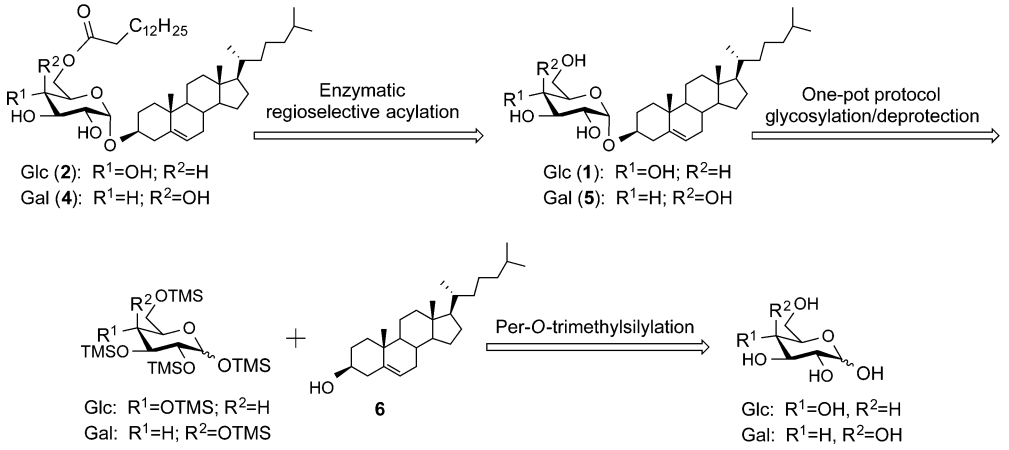


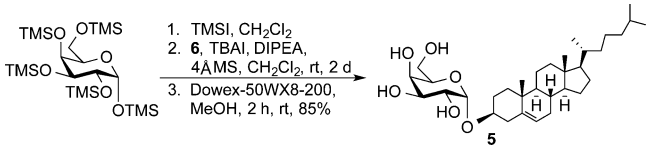
Figure 2. Retrosynthesis of a generalized strategy for making acylated α-cholesterylglycosides.

further limited by the fact that enzymatic acylation failed when attempted with free galactose.

To address these shortcomings, we have implemented an alternative strategy that relies upon the one-pot glycosidation of cholesterol to give the corresponding α-cholesterylglycoside, followed by enzymatic acylation (Figure 2). The synthesis of αCG (**1**) had been previously reported to require eight steps from free D-glucose with the key step requiring a metallic Lewis acid promoted glycosylation, which afforded a 1:1 α:β ratio of CG in 75% yield.[11] Recent developments in our lab led us to believe that the yield and α-selectivity of the glycosylation reaction could be improved using the per-*O*-silyl glycosyl iodide methodology, which had proven successful in the synthesis of α-linked glycolipids.[12−14] We were hopeful that the lipase-catalyzed regioselective acylation of the resulting cholesterylglycosides would uneventfully produce the acylated cholesterylglycosides for both glucosyl and galactosyl substrates, addressing the inability to esterify free α-D-galactose. This approach could also circumvent the low yields we previously encountered with chemical acylation of cholesterylglycosides.[14]

The new strategy began with glycosylation of the monosaccahrides. Work previously performed in our laboratory had already accomplished the glycosidation of cholesterol (**6**) with per-*O*-TMS galactosyl iodide to afford the α-cholesterylgalactoside **5** in 85% yield (Scheme 2).[14] This protocol relies upon using excess per-*O*-TMS glycosyl iodide, which can be made quantitatively from relatively inexpensive D-galactose.[15] After glycosidation, the TMS-protecting groups can be removed with ease using Dowex-50WX8-200 acidic resin in methanol. To assess the stereochemical integrity of the reaction, the crude reaction material is typically acetylated, which allows facile separation of anomers. However, the β-linked product was not observed when using the per-*O*-TMS galactosyl iodide donor.

Scheme 2. Synthesis of α-Cholesteryl Galactose



Following this same strategy, attention turned to the synthesis of αCG (**1**). First, per-*O*-TMS glucoside was reacted with iodotrimethylsilane (TMSI) to generate the glucosyl iodide, which was directly cannulated into a solution of cholesterol (**6**), TBAI, and DIPEA, and the mixture was stirred for 2 days at ambient temperature. The solvent was then removed, and TBAI precipitated upon adding a 1:1 mixture of ethyl acetate and hexanes with cooling. The resulting solid TBAI was removed by suction filtration, and the filtrate was concentrated to give a white solid, which was treated with methanol and Dowex-50WX8-200 acidic resin to remove the silyl protecting groups. Acetylation of the crude products afforded a mixture of CG anomers in a 39:1 α:β ratio. The anomers were separated by HPLC chromatography, and each anomer was then deactylated using sodium methoxide in a

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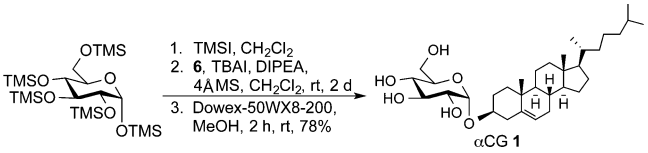
methanol:dichloromethane (10:1) solution to yield αCG (**1**) in 78% along with 2% of the β-anomer (Scheme 3).

Scheme 3. Glycosidation of Glucosyl Iodide with Cholesterol



The glycosylation/deprotection protocol provided a step-economy approach to α-linked cholesteryl monosaccharides, and the acetylated analogues turned out to be crystalline. X-ray crystallography revealed distinguishing structural features of the two cholesterylglucoside anomers. The carbohydrate headgroup of αCG orients perpendicular to the cholesterol backbone, whereas, in the β-anomer, the pyranose is parallel and the carbohydrate group is in an extended oritentation (Figure 3). Understanding the biological relevance of different presentation geometries will require further investigation.

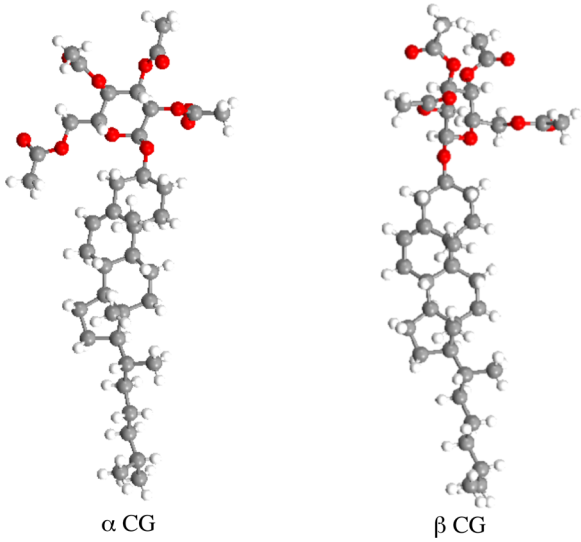
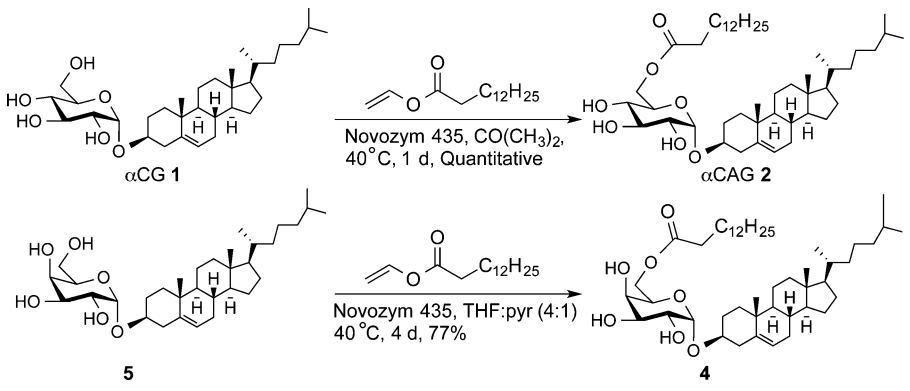


Figure 3. X-ray crystal structures of per-*O*-acetylated αCG (left) and per-*O*-acetylated βCG (right) displaying different carbohydrate orientations.

With the cholesterylglycosides **1** and **5** in hand, the regioselective enzymatic acylation of the C6-hydroxyl group was attempted using the protocol we published in 2012.[10] We were delighted to find that the enzymatic acylation allowed smooth transformation of αCG to αCAG in a quantitative manner using 3 equiv of myristic acid vinyl ester (Scheme 4). The esterification proceeded in both a tetrahydrofuran:pyridine (4:1 THF:pyr.) solvent system[10] and in acetone, which had proven effective in the regioselective acylation of amygdalin by Vemula et al.[16] However, the galactosyl analogue (**5**) failed to give any acylated product in a variety of solvents, including acetone, THF:pyridine (4:1), dioxane:pyridine (4:1), dioxane:-pyridine (1:1), dioxane:pyridine (3:7), THF:pyridine (1:1), and THF:pyridine (3:7). Aqueous solvent was not investigated because the cholesterylglycosides were not water-soluble and the lipase enzyme required anhydrous conditions.[17,18] We initially thought that the enzyme was specific for glucose until we learned of one example that reported C-6 acetylation of α-D-methyl galactoside using 22 equiv of vinyl acetate in 74% yield.[19] Believing the reaction to be independent of solvent, we again subjected α-cholesterylgalactoside to enzymatic acylation using increasing amounts of myristic acid vinyl ester in the THF:pyr (4:1) solvent system (Scheme 4). No reaction was observed using 6 equiv of fatty acid vinyl ester, even after 4 days. However, we were encouraged to find that doubling the amount of vinyl ester to 12 equiv yielded 51% of **4**. The reaction was further optimized using 18 equiv of fatty acid vinyl ester to afford **4** in 77% isolated yield after 4 days. Although the reaction was considerably slower than the glucose analogue, and required a large excess of vinyl ester, the yield compares favorably with previous chemical acylation processes[14,20] and, as far as we are aware, represents the first example of lipase catalyzed regioselective fatty acid acylation of galactose.

A step-economy synthetic protocol involving the glycosidation of cholesterol prior to enzymatic regioselective acylation has allowed expansion of the acylated α-cholesterylglycoside inventory to include galactose analogues. The glycosidation of per-*O*-silylated glucose provides better α-selectivity (39:1) than past syntheses (8:1 α-selectivity)[10,11] and higher glycosylation yields due to the armed nature of per-*O*-silyl donors. Moreover, unlike free galactose, which failed to undergo regioselective esterification, α-cholesteryl galactose was a substrate for lipase, giving a 77% yield of the C-6 ester. The lipase catalyzed process was highly regioselective and gave increased yields compared to previous chemical esterification (43% yield) methods.[14] The new methodolgy has allowed for the preparation of αCG (**1**) and αCAG (**2**) in 78% overall yields from D-glucose (compared

Scheme 4. Enzymatic Regioselective Acylation Using Novozym 435



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to 45% previously)[10] as well as the synthesis of 6-*O*-tetradecanoyl-α-D-cholesterylgalactoside in an overall yield of 66% from D-galactose. Little is known about the biological roles of cholesterylglycosides in general. While the galactose analogues reported herein have not yet been isolated from natural sources, αCG and αCAG are known to inhibit T-cell activation and thus aid in immune evasion of *H. pylori*.[7]

EXPERIMENTAL SECTION

Cholesteryl-α-D-glucopyranose (1). In a flame-dried argon-purged round-bottom flask containing 4 Å molecular sieves (300 mg) were added TBAI (140 mg, 0.38 mmol, 4.5 equiv), cholesterol (32 mg, 0.08 mmol, 1.0 equiv), and anhydrous dichloromethane (2.5 mL). Hünig's base (90 μL, 0.51 mmol, 6.0 equiv) was then added to the solution, and the reaction was stirred for 30 min. In a separate flame-dried argon-purged round-bottom flask was placed per-*O*-trimethylsilylated glucose (140 mg, 0.25 mmol),[15] which was azeotroped with anhydrous benzene (2 × 3 mL), and then anhydrous chloroform was added and removed under reduced pressure (2 × 3 mL). The per-*O*-TMS sugar[15] was placed under vacuum for at least 2 h prior to use, but typically was dried on vacuum overnight. Once dried according to the above protocol, the reaction was diluted with anhydrous dichloromethane (2.5 mL), and TMSI (40 μL, 0.28 mmol, 3.3 equiv) was added and allowed to react for 10 min at rt. The in situ generated glucosyl iodide was then transferred via cannula into the acceptor flask and allowed to stir for 2 days at rt. The solvent was then filtered to remove the molecular sieves and concentrated under reduced pressure. A 1:1 ratio of ethyl acetate and hexanes (20 mL) was then added to the round-bottom flask, and the flask was cooled in a dry ice/acetone bath, causing the excess TBAI to precipitate. The solid was filtered, and the solution was again concentrated to give an oil. Methanol (5 mL) and Dowex 50WX8-200 acidic resin (300 mg) were then added, and the reaction was stirred for 2 h. Initially, the crude reaction mixture was acetylated to separate the anomers and to obtain an accurate α:β ratio.

Cholesteryl-2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose. The acetylated mixture was purified by HPLC using a silica microsorb normal phase 250 × 10 mm column and a gradient mobile phase starting with 20% ethyl acetate in hexanes with gradual polarity increasing to 40% ethyl acetate in hexanes over a 23 min time period and a flow rate of 4 mL per min. The α-anomer had an HPLC retention time of 14.4 min and was obtained as a white powder. The purified compound was then recrystallized from diethyl ether and methanol to afford glasslike, rod-shaped crystals. mp = 196.4−196.8 °C. R_f = 0.72 (hexanes:acetone 6:4). $[\alpha]_D^{20}$ +70.2 (*c* 0.95, CH_2Cl_2). ^{1}H NMR (800 MH_Z, $CDCl_3$): δ 0.67 (s, 3H), 0.85 (d, *J* = 6.6 Hz, 3H), 0.86 (d, *J* = 6.6 Hz, 3H), 0.91 (d, *J* = 6.6 Hz, 3H), 1.02 (s, 3H), 1.03−1.17 (m, 9H), 1.23−1.29 (m, 2H), 1.32−1.39 (m, 2H), 1.41−1.58 (m, 9H), 1.75−1.85 (m, 2H), 1.95−2.00 (m, 2H), 2.01 (s, 3H), 2.03 (s, 3H), 2.05 (s, 3H), 2.08 (s, 3H), 2.30 (ddd, *J* = 2.2, 4.9, 13.2 Hz, 1H), 3.36−2.38 (m, 1H), 3.40−3.44 (m, 1H), 4.11 (dd, *J* = 2.2, 12.2 Hz, H6, 1H), 4.14 (ddd, *J* = 2.2, 4.8 10.2 Hz, H5, 1H), 4.23 (dd, *J* = 4.8, 12.2 Hz, H6′, 1H), 4.80 (dd, *J* = 3.8, 10.2 Hz, H2, 1H), 5.03 (app. t, *J* = 10.2 Hz, H4, 1H), 5.22 (d, *J* = 3.8 Hz, H1, 1H), 5.33−5.34 (m, 1H), 5.48 (app. t, *J* = 10.0 Hz, H3, 1H). ^{13}C NMR (200 MHz, $CDCl_3$): δ 170.9, 170.4, 170.3, 169.9, 140.4, 122.3, 94.3 (C1), 78.9, 71.2 (C2), 70.3 (C3), 68.8 (C4), 67.3 (C5), 62.2 (C6), 56.8, 56.2, 50.2, 42.4, 40.1, 39.8, 39.6, 37.1, 36.8, 36.3, 35.9, 32.1, 32.0, 28.4, 28.2, 28.0, 24.4, 24.0, 23.0, 22.7, 21.2, 20.94, 20.91, 20.9, 20.8, 19.5, 18.8, 12.0. HRMS (ESI-Ion Trap) *m*/*z*: $[M + Na]^+$ calcd for $C_{41}H_{64}O_{10}Na$ 739.4397; found 739.4404.

Cholesteryl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose. The β-anomer had an HPLC retention time of 16.8 min and was obtained as a white powder and was then recrystallized from diethyl ether and methanol to afford glasslike, rod-shaped crystals. mp = 149.7−152.3 °C. R_f = 0.70 (hexanes:acetone 6:4). $[\alpha]_D^{20}$ −10.0 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (800 MH_Z, $CDCl_3$): δ 0.66 (s, 3H), 0.85 (d, *J* = 6.6 Hz, 3H), 0.86 (d, *J* = 6.0 Hz, 3H), 0.91 (d, *J* = 6.0 Hz, 3H), 0.98 (s, 3H), 1.00−1.17 (m, 9H), 1.22−1.28 (m, 2H), 1.32−1.37 (m, 2H), 1.41−1.54 (m, 5H), 1.56−1.62 (m, 3H), 1.79−1.90 (m, 3H), 1.96−2.06 (m, 2H), 2.00 (s, 3H), 2.01 (s, 3H), 2.04 (s, 3H), 2.07 (s, 3H), 2.16−2.20 (m, 1H), 2.25 (ddd, *J* = 2.4, 4.8, 13.3 Hz, 1H), 3.48 (dp, *J* = 0.6, 4.8, 9.6 Hz, 1H), 3.67 (ddd, *J* = 2.4, 4.8, 9.6 Hz, H5, 1H), 4.11 (dd, *J* = 2.4, 12.6 Hz, H6, 1H), 4.25 (dd, *J* = 4.8, 12.6 Hz, H6′, 1H), 4.59 (d, *J* = 7.8 Hz, H1, 1H), 4.95 (dd, *J* = 8.0, 9.6 Hz, H2, 1H), 5.07 (app. t, *J* = 9.6 Hz, H4, 1H), 5.19 (app. t, *J* = 9.6, Hz, H3, 1H), 5.35−5.36 (m, 1H). ^{13}C NMR (200 MHz, $CDCl_3$): δ 170.8, 170.5, 169.5, 169.4, 140.5, 122.3, 99.8 (C1), 80.2, 73.0 (C3), 71.8 (C5), 71.6 (C2), 68.7 (C4), 62.2 (C6), 56.9, 56.3, 50.3, 42.5, 39.9, 39.7, 39.1, 37.3, 36.9, 36.3, 35.9, 32.1, 32.0, 29.6, 28.4, 28.2, 24.4, 24.0, 23.0, 22.7, 21.2, 20.9, 20.87, 20.8, 20.76, 19.5, 18.9, 12.0. HRMS (ESI-Ion Trap) *m*/*z*: $[M + NH_4]^+$ calcd for $C_{41}H_{68}NO_{10}$ 734.4843; found 734.4843.

Alternatively, the crude mixture could be purified by flash column chromatography using a 9:1 dichloromethane:methanol solvent system to afford compound (**1**) (35 mg, 0.06 mmol, 78% yield).

$[\alpha]_D^{23}$ +30.2 (*c* 0.54, $CHCl_3$:MeOH). R_f = 0.26 (dichloromethane:-methanol 9:1). mp = 220.1−221.8 °C. ^{1}H NMR NOESY presaturation 1D to suppress water (4.23 ppm) and the spectrum was locked to CD_3OD (800 MH_Z, $CDCl_3$: CD_3OD (3:1)): δ 0.49 (s, 3H), 0.67 (d, *J* = 6.6 Hz, 3H), 0.68 (d, *J* = 6.6 Hz, 3H), 0.74 (d, *J* = 6.6 Hz, 3H), 0.78−0.81 (m, 1H), 0.82 (s, 3H), 0.83−0.99 (m, 8H), 1.05−1.13 (m, 1H), 1.14−1.18 (m, 3H), 1.24−1.39 (m, 8H), 1.64−1.66 (m, 1H), 1.67−1.69 (m, 1H), 1.72−1.74 (m, 1H), 1.76−1.79 (m, 1H), 1.82−1.83 (m, 1H), 2.14−2.17 (m, 2H), 3.22 (dd, *J* = 3.8, 10.2 Hz, H2, 1H), 3.23 (app. t, *J* = 9.6 Hz, H4, 1H), 3.28−3.32 (m, 1H), 3.47 (app. t, *J* = 9.6 Hz, H3, 1H), 3.46−3.49 (m, H5, 1H), 3.58−3.60 (m, H6, H6′, 2H), 4.79 (d, *J* = 3.8 Hz, H1, 1H), 5.15−5.20 (m, 1H). ^{13}C NMR (200 MHz, $CDCl_3$: CD_3OD (3:1)): δ 140.4, 121.8, 96.7 (C1), 77.5, 73.8 (C3), 71.8 (C2), 71.5 (C5), 70.0 (C4), 61.3 (C6), 56.6, 55.9, 49.9, 42.1, 39.8, 39.6, 39.3, 36.8, 36.5, 36.0, 35.6, 31.7, 31.6, 28.0, 27.8, 27.4, 24.0, 23.6, 22.5, 22.2, 20.8, 19.0, 18.4, 11.6. HRMS (MALDI-TOF) *m*/*z*: $[M + Na]^+$ calcd for $C_{33}H_{56}O_6Na$ 571.3969; found 571.3937.

Cholesteryl-6-*O*-tetradecanoyl-α-D-glucopyranoside (2). Compound **1** (20.0 mg, 0.036 mmol) was placed into an oven-dried screw cap vial. Then, anhydrous acetone (0.9 mL) and commercial lipase Novozym 435 (36 mg on solid support) were added to the vial, followed by myristic vinyl ester (6.0 equiv, 60 μL). The vial was then placed on a thermoplate shaker at 40 °C for 18 h. Upon completion of the reaction, the solution was decanted into a round-bottom flask and the enzyme was rinsed twice with methanol, followed by chloroform. The organic solvents were combined and concentrated to afford a white solid. This solid was purified by flash chromatography using 100% hexanes, and the characterization data matched that of what was previously reported.[14]

Cholesteryl-6-*O*-tetradecanoyl-α-D-galactopyranoside (4). Compound **5** (10.0 mg, 0.018 mmol) was placed into an oven-dried screw cap vial. Then, anhydrous pyridine (60 μL), followed by THF (240 μL), was added to give a M = 0.1 concentration of **13**. Then, commercial lipase Novozym 435 (10 mg on solid support) was added to the vial, followed by myristic vinyl ester (18.0 equiv, 95 μL, 0.32 mmol). The vial was then placed on a thermoplate shaker at 40 °C for 4 days, after which the solution was diluted with chloroform and decanted into a round-bottom flask. The enzyme was rinsed twice with methanol, followed by chloroform. The organic solvents were combined and concentrated to afford a white solid, which was purified by flash chromatography on a 3.6 cm diameter glass column that was 25.5 cm long plugged with cotton and filled with 150 mL of silica. A gradient solvent system of 100% hexanes (100 mL) with increasing polarity to 1:1 hexanes:ethyl acetate (100 mL), followed by 100% ethyl acetate (100 mL) to remove the excess fatty acid, which does not stain on the TLC plate, was used. After using 100 mL of each solvent ratio, a 9:1 ethyl acetate:methanol solvent system was used to elute compound **4** (10 mg, 0.014 mmol) in 77% yield. R_f = 0.73 (ethyl acetate:methanol 9:1). $[\alpha]_D^{24}$ +41.1 (*c* 0.48, CH_2Cl_2). ^{1}H NMR (800 MH_Z, $CDCl_3$): δ 0.67 (s, 3H), 0.86 (d, *J* = 6.6 Hz, 3H), 0.87 (d, *J* = 6.6 Hz, 3H), 0.89 (t, *J* = 7.2 Hz, 3H), 0.92 (d, *J* = 6.6 Hz, 3H), 0.96−0.99 (m, 1H), 1.00 (s, 3H), 1.02−1.17 (m, 7H), 1.25−1.27 (m, 22H), 1.27−1.36 (m, 2H), 1.42−1.62 (m, 13H), 1.81−1.89 (m, 4H), 1.95−2.02 (m, 2H), 2.31−2.39 (m, 4H), 3.48−3.50 (m, 1H), 3.74−3.76 (m, H2, H3, 2H), 3.95−

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3.96 (m, H4, 1H), 4.07 (app. t, J = 4.8 Hz, H5, 1H), 4.21 (dd, J = 8.0, 12.0 Hz, H6, 1H), 4.41 (dd, J = 4.8, 12.0 Hz, H6′, 1H), 5.06 (d, J = 4.0 Hz, H1, 1H), 5.35−5.36 (m, 1 H). ^{13}C NMR (200 MHz, $CDCl_3$): δ 174.1, 140.4, 122.4, 97.3 (C1), 78.6, 71.3 (C3), 69.6 (C2), 69.0 (C4), 68.3 (C5), 63.5(C6), 56.8, 56.3, 50.2, 42.4, 40.2, 39.8, 39.6, 37.1, 36.8, 36.3, 35.9, 34.4, 32.1, 32.0, 31.9, 29.9, 29.89, 29.85, 29.84, 29.8, 29.53, 29.5, 29.4, 28.4, 28.2, 28.1, 25.1, 24.4, 24.0, 23.0, 22.9, 22.7, 21.6, 19.5, 18.8, 14.3, 12.0. HRMS (ESI-Ion Trap) m/z: $[M + Na]^+$ calcd for $C_{47}H_{82}O_7Na$ 781.5958; found 781.6014.

Cholesteryl-α-D-galactopyranose (5). In a flame-dried argon-purged round-bottom flask containing 4 Å molecular sieves (300 mg) were added TBAI (140 mg, 0.38 mmol, 1.5 equiv), cholesterol (32 mg, 0.083 mmol, 0.3 equiv), and anhydrous dichloromethane (2.5 mL). Hünig's base (DIPEA) (90 μL, 0.51 mmol, 2.0 equiv) was then added to this solution, which was stirred for 30 min. In a separate flame-dried argon-purged round-bottom flask was placed per-*O*-trimethylsilylated galactose made from the synthetic protocol of Bhat and Gervay-Hague[15] (140 mg, 0.25 mmol) and azeotroped with anhydrous chloroform (2 × 3 mL). After azeotroping, the sugar was placed under vacuum for at least 2 h prior to use. The resulting residue was then diluted with anhydrous dichloromethane (2.5 mL), and TMSI (40 μL, 0.28 mmol, 1.1 equiv) was added and allowed to react for 10 min. The in situ generated galactosyl iodide was then transferred via cannula into the acceptor flask and allowed to stir for 2 days at rt. The solvent was then filtered to remove the molecular sieves, and the solvent was removed under reduced pressure. A 1:1 ratio of ethyl acetate and hexanes (20 mL) was then added to the round-bottom flask, and the flask was cooled using dry ice. The cooling helped solidify the TBAI, which was then filtered off, and the solution was again concentrated. Methanol (5 mL) and Dowex 50WX8-200 acidic resin (300 mg) were then added, and the mixture was stirred for 2 h. The solution was then filtered to remove the acidic resin and concentrated under reduced pressure. The crude reaction mixture was then acetylated to identify the α:β-ratio of the glycosylation. However, no β-anomer was observed or isolated.

Cholesteryl-2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose. The α-anomer of the cholesteryl galactoside was further purified by HPLC using a silica microsorb normal phase 250 × 10 mm column and a gradient mobile phase starting with 20% ethyl acetate in hexanes with gradual polarity increasing to 50% ethyl acetate in hexanes over a 20 min time period and a flow rate of 4 mL per min. It had an HPLC retention time of 9.6 min and was obtained as a white foam. The white foam was also recrystallized from diethyl ether and methanol. R_f = 0.72 (hexanes:acetone 6:4). $[\alpha]_D^{20}$ +8.6 (c 0.40, CH_2Cl_2). mp = 153.6−155.9 °C. ^{1}H NMR (800 MH_Z, $CDCl_3$): δ 0.68 (s, 3H), 0.85 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.91 (d, J = 6.6 Hz, 3H), 0.96−1.01 (m, 1H), 1.02 (s, 3H), 1.03−1.14 (m, 8H), 1.23−1.28 (m, 2H), 1.31−1.38 (m, 2H), 1.44−1.52 (m, 7H), 1.53−1.56 (m, 2H), 1.74−1.77 (m, 1H), 1.81−1.85 (m, 2H), 1.96−2.01 (m, 1H), 1.99 (s, 3H), 2.04 (s, 3H), 2.07 (s, 3H), 2.13 (s, 3H), 2.30 (ddd, J = 2.2, 4.9, 13.2 Hz, 1H), 3.34−2.38 (m, 1H), 3.40−3.44 (m, 1H), 4.07 (dd, J = 7.2, 11.2 Hz, H6, 1H), 4.11 (dd, J = 5.6, 11.2 Hz, H6′, 1H), 4.34 (app t, J = 7.2 Hz, H5, 1H), 5.06 (dd, J = 4.0, 11.2 Hz, H2, 1H), 5.25 (d, J = 4.0 Hz, H1, 1H), 5.35 (dd, J = 3.2, 11.2 Hz, H3, 1H), 5.35−5.36 (m, 1H), 5.45 (dd, J = 1.6, 3.2 Hz, H4, 1H). ^{13}C NMR (200 MHz, $CDCl_3$): δ 170.6, 170.5, 170.4, 170.2, 140.5, 122.3, 95.0 (C1), 78.9, 68.5 (C2), 68.4 (C4), 67.8 (C3), 66.4 (C5), 62.1 (C6), 56.8, 56.3, 50.3, 42.5, 40.1, 39.9, 39.7, 37.1, 36.8, 36.3, 35.9, 32.1, 32.0, 29.8, 28.4, 28.2, 24.4, 24.0, 23.0, 22.7, 21.2, 21.0, 20.9, 20.8, 20.8, 19.5, 18.9, 12.0. HRMS (ESI-Ion Trap) m/z: $[M + Na]^+$ calcd for $C_{41}H_{64}O_{10}Na$ 739.4397; found 739.4404.

Alternatively, the crude solid after removing the trimethylsilyl protecting groups could be purified with a 9:1 dichloromethane:methanol solvent system, and the white powder was collected to afford the target compound (**5**) (39 mg, 0.071 mmol, 85%). The ^{1}H NMR utilized NOESY presaturation 1D to suppress water (4.23 ppm) and the spectrum was locked to CD_3OD. R_f = 0.25 (dichloromethane:-methanol 9:1). $[\alpha]_D^{25}$ +55.6 (c 0.52, $CHCl_3$:MeOH). mp = 223.6−224.3 °C. ^{1}H NMR (800 MH_Z, $CDCl_3$: CD_3OD (3:1)): δ 0.53 (s, 3H), 0.70 (d, J = 6.6 Hz, 3H), 0.71 (d, J = 6.6 Hz, 3H), 0.77 (d, J = 6.6 Hz, 3H), 0.76−0.79 (m, 1H), 0.85 (s, 3H), 0.87−1.03 (m, 8H), 1.08−1.13 (m, 5H), 1.16−1.23 (m, 3H), 1.27−1.44 (m, 8H), 1.66−1.68 (m, 1H), 1.69−1.72 (m, 1H), 1.72−1.73 (m, 1H), 1.76−1.79 (m, 1H), 1.80−1.87 (m, 1H), 2.19−2.20 (m, 2H), 3.31−3.35 (m, 1H), 3.57−3.70 (m, H3, H6, H6′, H2, 4H), 3.75 (app. t, J = 6.0 Hz, H5, 1H), 3.83−3.84 (m, H4, 1H), 4.87 (d, J = 3.5 Hz, H1, 1H), 5.18−5.19 (m, 1H). ^{13}C NMR (200 MHz, $CDCl_3$: CD_3OD (3:1)): δ 140.4, 121.9, 97.1 (C1), 77.6, 70.3 (C3), 69.9 (C5), 69.7 (C4), 68.9 (C2), 61.6 (C6), 56.6, 56.0, 50.0, 42.2, 39.9, 39.6, 39.4, 36.9, 36.5, 36.0, 35.7, 31.7, 31.6, 28.1, 27.9, 27.6, 24.1, 23.6, 22.6, 22.3, 20.9, 19.1, 18.5, 11.6. HRMS (MALDI-TOF) m/z: $[M + Na]^+$ calcd for $C_{33}H_{56}O_6Na$ 571.3969; found 571.3968.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR, MS, and X-ray diffraction data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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