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Syntheses of deuterium-labelled cholesteryl neoglycolipids

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Four deuterium-labelled neoglycolipids derived from cholesterol were synthesized for embedment into liposomes. Deuterium atoms were either incorporated by CH_2 replacement with a CD_2 group in the triethylene glycol spacer arm between the cholesteryl residue and the sugar moiety (products 2–4) or incorporated directly on the acetamido function in the sugar head (compound 5).

Keywords: neoglycolipids; deuterium; N-acetyl-D-glucosamine; glycosylation; hydrophobic anchor; isotopically labelled synthesis; deuterium

Introduction

Deuterium-labelled glycolipids have been synthesized for more than 25 years to study their behaviour in multicomponent model membranes.[1–5] Two different strategies have been developed for the replacement of selected hydrogen atoms by deuterium in labelled glycolipids. In the first strategy, deuterium was incorporated into the lipidic moiety according to known methods, which have been reviewed 15 years ago.[6] Then, the labelled lipidic part (most often an acid or an acyl chloride) was condensed to a sugar derivative generally via an amide[7,8] or an ester[9–11] bond; deuterated alcohols have also been employed for the preparation of ether bond.[11] For example, an efficient and convenient synthesis of deuterium-labelled seminolipid isotopomers was recently described by epoxide ring opening of (R)-glycidyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside: either the opening was performed with labelled [16,16,16-D_3]-hexadecanol under acidic conditions (and the resulting hydroxyl group acylated with hexadecanoic acid) or the epoxide was opened with hexadecanol (and then acylated with labelled [16,16,16-D_3]-hexadecanoic acid).[11] In the second strategy, the deuterium was incorporated in the glycolipids on the saccharidic moieties on the exocyclic hydroxymethyl group of terminal galactose, galactosamine[2,12] or glucosamine[13] residues either by enzymatical or chemical methods or by replacement of the *N*-acetyl group by an *N*-trideuteroacetyl group.[2,12]

The present paper deals with the syntheses of deuterium-labelled analogues of *N*-acetyl-D-glucosamine glycosides of cholesteryl oligoethylene glycol, which were used as anchors into phosphatidylcholine bilayers.[14–16]

Results and discussion

Previous work in the laboratory has demonstrated that *N*-acetyl-β-D-glucosaminyl glycosides of cholesteryl oligoethylene glycol could be used as anchors into phosphatidylcholine liposomes, the distance between the ligand (*N*-acetyl-D-glucosamine) and the membrane surface being a major factor in the lectin accessibility, during recognition phenomenon with Wheat Germ Agglutinin (WGA). The most interesting results were obtained with triethylene glycol and tetraethylene glycol spacer arms.[14,15] The replacement of a methylene group of the spacer by a dideuteriomethylene group at different places would give more information about the glycolipid incorporation into the membrane. The present paper describes the syntheses of analogues **2–5** of [8-(cholest-5-en-3β-yloxy)-3,6-dioxaoctyl] 2-acetamido-2-deoxy-β-D-glucopyranoside (**1**)[14] bearing deuterium atoms on the triethylene glycol spacer respectively at positions C-1 (compound **2**), C-4 (compound **3**) and C-7 (compound **4**) or on the carbohydrate residue on the acetamido function (compound **5**; Figure 1).

Syntheses of glycosylation acceptors **9**, **13** and **17** are described in Scheme 1. Compound **9** was obtained in three steps from cholesterol tosylate **6**.[17] Compound **6** was reacted with diethylene glycol in refluxing dioxane, affording product **7** in 81% yield. Several methods were checked to realize the diethylene chain elongation on derivative **7**: the reaction with chloroacetic acid in THF in the presence of sodium hydride as described by Abe

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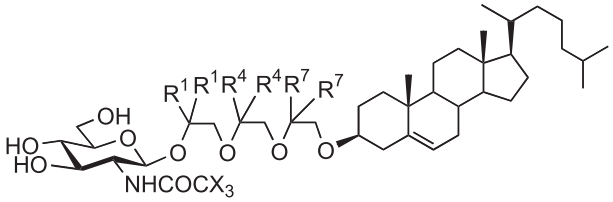


1 X = R^1 = R^4 = R^7 = H
2 X = R^4 = R^7 = H, R^1 = D
3 X = R^1 = R^7 = H, R^4 = D
4 X = R^1 = R^4= H, R^7 = D
5 X = D, R^1 = R^4= R^7 = H

Figure 1. Structure of glycolipids **1–5**.

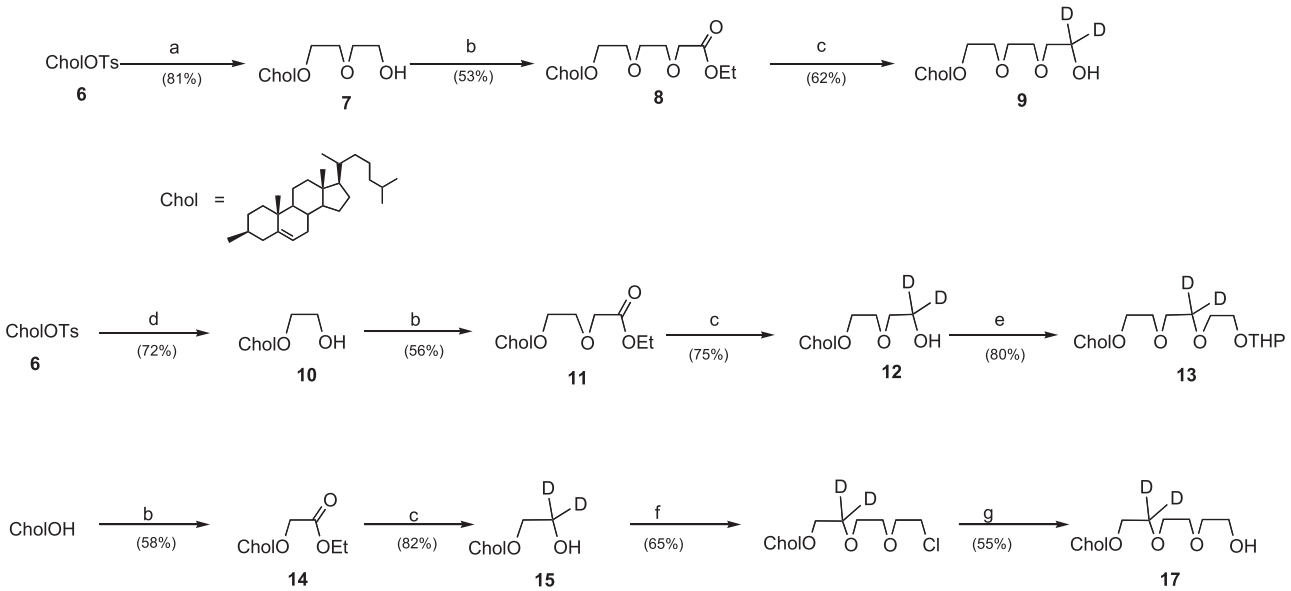
et al.[18] gave the expected acid in very low yields (30% approximatively). However, under catalytic acidic conditions (boron trifluoride etherate) at −20 °C in dichloromethane, alcohol **7** was reacted with ethyl diazoacetate, leading to ester **8** in 53% yield after purification. Increasing the temperature gave more by-products and decreased the yield. Alcohol **9** was obtained by reduction of ester **8** with lithium aluminium deuteride in THF. Similar reactions were applied for the preparation of alcohol **10** (72%), ester **11** (56%) and alcohol **12** (75%). Condensation of alcohol **12** with pyranylated 2-chloroethylene glycol under phase transfer conditions[19] in the presence of powder sodium hydroxide and tetrabutylammonium hydrogen sulfate afforded tetrahydropyranylated alcohol **13** in 80% yield. We did not try to deprotect the tetrahydropyranyl ether before the glycosylation step, because cleavage must occur under the glycosylation conditions. Reaction of cholesterol with ethyl diazoacetate as described previously for **7** and **10** gave ester **14** (58% yield). This compound has formerly been synthesized in the literature by using ethyl chloroacetate and benzene in the presence of potassium.[20,21] Ester **14** was reduced by lithium aluminium deuteride (80% yield), and the chain elongation on alcohol **15** was realized using di(chloroethyl)ether under phase transfer conditions.[19] Chloride **16** was recovered in 65% yield. Two steps were necessary for the conversion of chloride **16** into alcohol **17** by the intermediate of a formate[22], which was saponified under basic conditions.

Compounds **9**, **13** and **17** were glycosylated with 1,2,3,4-tetra-*O*-acetyl-2-allyloxycarbonylamino-2-deoxy-β-D-glucopyranose (**18**)[14,23,24] as donor (1.05 equiv.) in dichloromethane at −20 °C, the reaction being promoted by trimethylsilyl trifluoromethanesulfonate; glycosides **19** (from **9**), **20** (from **13**) and **21** (from **17**) were obtained in 75–79% yields (Scheme 2). As expected, the cleavage of the tetrahydropyranyl group occurred under the acidic conditions of the glycosylation reaction. *N*-allyloxycarbonyl group cleavage for compounds **19–21** and for non-deuterated analogue **22**[14] was catalysed by tetrakis(triplenylphosphine)palladium in the presence of diethyl malonate in THF. The amino-free derivatives were *N*-acetylated either with acetic anhydride for the deuterium-labelled compounds or with hexadeuterated acetic anhydride for the non-labelled derivative. Compounds **23–26** were finally de-*O*-acetylated according to Zemplén method by using catalytic sodium methylate in methanol, giving products **2–5** in good yields.

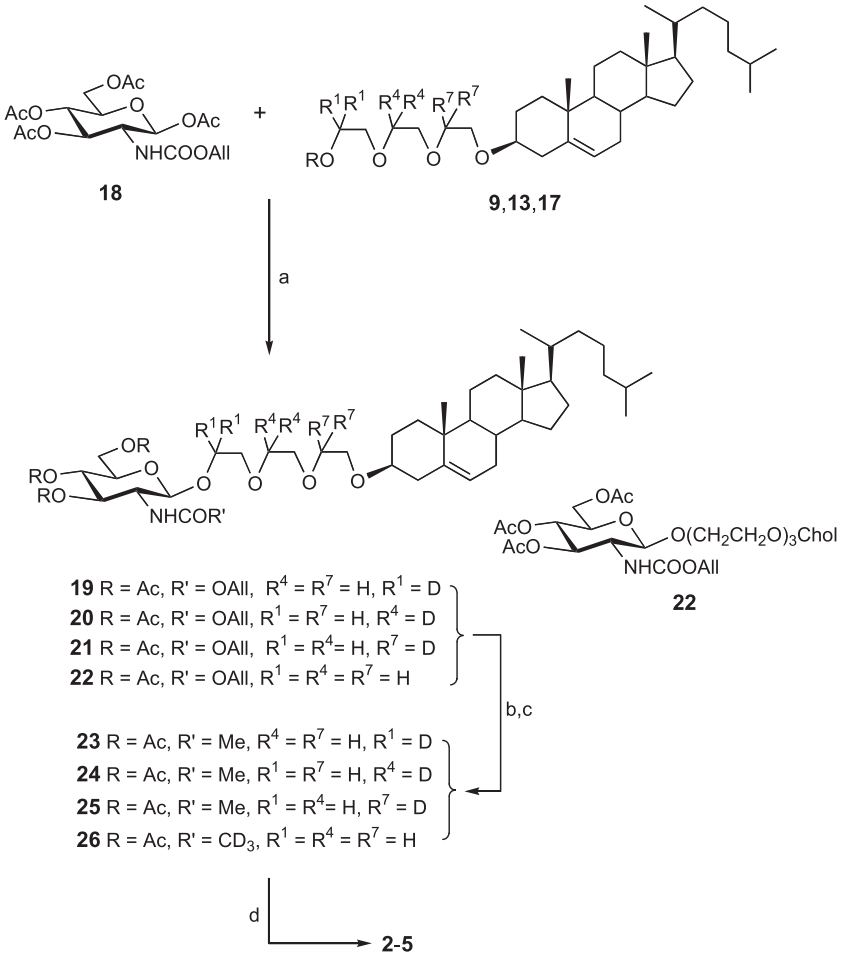
Experimental

General methods

Dichloromethane was washed twice with water, dried with $CaCl_2$ and distilled from CaH_2. THF and dioxane were distilled from sodium–benzophenone. Methanol was distilled from magnesium. Dioxane, THF and CH_2Cl_2 were stored over 0.4 nm molecular sieves, and MeOH was stored over 0.3 nm molecular sieves. Melting points were determined on a Büchi apparatus and were uncorrected. Thin layer chromatography was performed on aluminium sheets coated with silica gel 60 F_{254} (Merck Darmstadt, Germany). Compounds were visualized by spraying the thin layer



Scheme 1. Reagents and conditions: (a) $HO(CH_2CH_2O)_2H$, dioxane, reflux; (b) N_2CH_2COOEt, CH_2Cl_2, −20 °C; (c) $LiAlD_4$, THF; (d) $HOCH_2CH_2OH$, dioxane, reflux; (e):$ClCH_2CH_2OTHP$, Bu_4NHSO_4, powder NaOH, 65 °C; (f): $(ClCH_2CH_2O)_2O$, Bu_4NHSO_4 powder NaOH, 65 °C; (g) NaOCHO, Bu_4NBr, DMSO, 115 °C and then 12.5 N NaOH, rt.



Scheme 2. Syntheses of glycosides **2–5**, reagents and conditions: (a) TMSOTf, CH_2Cl_2, −20 °C; (b) $Pd(PPh_3)_4$, $CH_2(COOMe)_2$, THF; (c) Ac_2O, MeOH (for **19–21**) or $(CD_3CO)_2O$ (for **22**), MeOH; (d) MeONa (cat), MeOH.

chromatography plates with dilute 15% aq H_2SO_4, followed by charring at 150 °C for a few minutes. Column chromatography was performed on silica gel Geduran Si 60 (Merck). Optical rotations were recorded on a Perkin Elmer (Waltham, Massachussets, USA) 241 polarimeter in a 1 dm cell at 21 °C. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker (Wissembourg, France) AC-200 spectrometer working at 200 MHz and 50 MHz, respectively, with Me_4Si as the internal standard. Elemental analyses were performed by the 'Laboratoire Central d'Analyses du CNRS' (Vernaison, France). ESI high resolution mass spectrometry measurements were performed with a MAT 95 XL (Thermoquest Finnigan, Bremen, Germany) electromagnetic mass spectrometer by the 'Centre Commun de Spectrometry de Masse' (UMR 5246, Lyon, France).

*5-(Cholest-5-en-3β-yloxy)-3-oxapentanol (**7**)*

Product **7** was prepared as already described from cholesteryl tosylate **6** (4.00 g, 7.40 mmol) and diethylene glycol (25 mL, 0.263 mol) in dry dioxane (45 mL).[14] Product **7** was obtained in 81% yield as a white solid: mp 52 °C (lit.[14] mp 51–52 °C), R_f 0.40 (1:1 ethyl acetate–petroleum ether), $[\alpha]_D$ −29.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.34 (bd, 1H, H-6$_{chol}$), 3.71–3.60 (m, 8H, 4CH_2O), 3.20 (m, 1H, H-3$_{chol}$), 2.79 (bs, 1H, O*H*), 2.40–0.67 (m, 43H, H cholesterol); ^{13}C NMR ($CDCl_3$): δ 140.78 (C-5$_{chol}$), 121.77 (C-6$_{chol}$), 79.66 (C-3$_{chol}$), 72.69, 70.82 ($CH_2OCH_2CH_2OH$), 67.48 (CH_2OChol), 61.83 (CH_2OH), 56.94 (C-14$_{chol}$), 56.25 (C-17$_{chol}$), 50.24 (C-9$_{chol}$), 42.38 (C-13$_{chol}$), 39.86 (C-12$_{chol}$), 39.60 (C-24$_{chol}$), 39.05 (C-4$_{chol}$), 37.28 (C-1$_{chol}$), 36.91 (C-10$_{chol}$), 36.28 (C-22$_{chol}$), 35.87 (C-20$_{chol}$), 31.95 (C-7$_{chol}$), 31.90 (C-8$_{chol}$), 28.38 (C-2$_{chol}$), 28.32 (C-16$_{chol}$), 28.07 (C-25$_{chol}$), 24.37 (C-15$_{chol}$), 23.94 (C-23$_{chol}$), 22.91 (C-27$_{chol}$), 22.66 (C-26$_{chol}$), 21.15 (C-11$_{chol}$), 19.45 (C-19$_{chol}$), 18.81 (C-21$_{chol}$), 11.94 (C-18$_{chol}$).

*Ethyl 8-(cholest-5-en-3β-yloxy)-3,6-dioxaoctanoate (**8**)*

Boron trifluoride etherate (15 μL, 0.012 mmol) was added to a cold solution (−20 °C) of alcohol **7** (0.687 g, 1.45 mmol) and ethyl diazoacetate (0.18 mL, 1.71 mmol, 1.18 equiv.) in dry CH_2Cl_2 (10 mL). The solution was stirred overnight at −20 °C and then poured into a saturated aqueous $NaHCO_3$ solution (30 mL), and the product was extracted with CH_2Cl_2 (2 × 15 mL). The organic phase was dried (Na_2SO_4) and concentrated under diminished pressure. The crude product was purified by column chromatography (1:5 ethyl acetate–petroleum ether). Compound **8** was recovered as an oily material in 53% yield: 0.428 g, R_f 0.23 (1:5 ethyl acetate–petroleum ether), $[\alpha]_D$ −10.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.35 (bd, 1H, H-6$_{chol}$), 4.23 (q, 2H, *J* 7.1 Hz, CH_3CH_2OCO), 4.15 (s, 2H, OCH_2COOEt), 3.75–3.63 (m, 8H, 4OCH_2), 3.18 (m, 1H, H-3$_{chol}$), 1.28 (t, 3H, CH_3CH_2O), 2.40–0.67 (m, 43H, H cholesterol); ^{13}C NMR ($CDCl_3$): δ 170.50 (COOEt), 140.97 (C-5$_{chol}$), 121.57 (C-6$_{chol}$), 79.55 (C-3$_{chol}$), 70.95, 70.92, 70.74, 68.79 ($CH_2OCH_2CH_2OCH_2COOEt$), 67.35 (CH_2OChol), 60.78

($COOCH_2CH_3$), 56.84 (C-14_{chol}), 56.23 (C-17_{chol}), 50.25 (C-9_{chol}), 42.37 (C-13_{chol}), 39.85 (C-12_{chol}), 39.57 (C-24_{chol}), 39.12 (C-4_{chol}), 37.30 (C-1_{chol}), 36.91 (C-10_{chol}), 36.25 (C-22_{chol}), 35.84 (C-20_{chol}), 31.97 (C-7_{chol}), 31.92 (C-8_{chol}), 28.41 (C-2_{chol}), 28.28 (C-16_{chol}), 28.05 (C-25_{chol}), 24.34 (C-15_{chol}), 23.89 (C-23_{chol}), 22.86 (C-27_{chol}), 22.61 (C-26_{chol}), 21.12 (C-11_{chol}), 19.42 (C-19_{chol}), 18.78 (C-21_{chol}), 14.26 ($COOCH_2CH_3$), 11.91 (C-18_{chol}).

Anal. calc. for $C_{35}H_{60}O_5$ (560.83): C, 74.95; H, 10.78. Found: C, 74.80; H, 10.92.

*8-(Cholest-5-en-3β-yloxy)-1-[$^2H_2$]-3,6-dioxaoctan-1-ol (**9**)*

A solution of ester **8** (0.400 g, 0.71 mmol) in THF (2 mL) was slowly added during 15 min. under argon to a suspension of lithium aluminium deuteride (0.070 g, 1.67 mmol) in THF (3 mL). Stirring was maintained overnight, and the excess of lithium aluminium deuteride was destroyed by careful addition of a 15% NaOH aqueous solution. Then, the mixture was acidified to pH 5–6, and THF was evaporated *in vaccuo*. The product was extracted with diethyl ether (4 × 30 mL), and the organic phase was washed with water (20 mL) and dried. After concentration, the residue was purified by column chromatography by using pure ethyl acetate as eluent. Pure compound **9** was recovered in 62% yield as an amorphous solid: 0.235 g, R_f 0.45 (ethyl acetate), $[\alpha]_D$ −23.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.35 (bd, 1H, H-6_{chol}), 3.67–3.61 (m, 10H, 5CH_2O), 3.19 (m, 1H, H-3_{chol}), 2.40–0.67 (m, 43H, H cholesterol); ^{13}C NMR ($CDCl_3$): δ 140.88 (C-5_{chol}), 121.58 (C-6_{chol}), 79.54 (C-3_{chol}), 72.54, 70.88, 70.67, 70.39 ($CH_2OCH_2CH_2OCH_2$), 67.28 (CH_2OChol), 60.95 (q, *J* 21.2 Hz, CD_2), 56.81 (C-14_{chol}), 56.24 (C-17_{chol}), 50.22 (C-9_{chol}), 42.35 (C-13_{chol}), 39.84 (C-12_{chol}), 39.56 (C-24_{chol}), 39.06 (C-4_{chol}), 37.28 (C-1_{chol}), 36.87 (C-10_{chol}), 36.26 (C-22_{chol}), 35.85 (C-20_{chol}), 31.97 (C-7_{chol}), 31.93 (C-8_{chol}), 28.35 (C-2_{chol}), 28.29 (C-16_{chol}), 28.03 (C-25_{chol}), 24.35 (C-15_{chol}), 23.92 (C-23_{chol}), 22.88 (C-27_{chol}), 22.63 (C-26_{chol}), 21.02 (C-11_{chol}), 19.41 (C-19_{chol}), 18.79 (C-21_{chol}), 11.91 (C-18_{chol}).

Anal. calc. for $C_{33}H_{56}D_2O_4$,H_2O (538.46): C, 73.55; H, 11.58. Found: C, 73.27; H, 11.18.

*2-(Cholest-5-en-3β-yloxy)-ethanol (**10**)*

Product **10** was prepared from cholesteryl tosylate **6** (5.90 g, 10.9 mmol) and ethylene glycol (40 mL, 0.717 mol) in dry dioxane (60 mL) as already described.[14] Product **10** was obtained in 72% yield as a white solid: mp 96–97 °C (lit.[14] mp 96–97 °C), R_f 0.62 (1:1 ethyl acetate–petroleum ether), $[\alpha]_D$ −30.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.35 (bs, 1H, H-6_{chol}), 3.75–3.71 (m, 2H, CH_2OChol), 3.60–3.56 (m, 2H, CH_2OH), 3.20 (3, 1H, H-3_{chol}), 2.40–0.67 (m, 43H, H cholesterol); ^{13}C NMR ($CDCl_3$): δ 140.38 (C-5_{chol}), 121.84 (C-6_{chol}), 80.55 (C-3_{chol}), 69.13 (CH_2OChol), 62.14 (CH_2OH), 56.87 (C-14_{chol}), 56.28 (C-17_{chol}), 50.27 (C-9_{chol}), 42.41 (C-13_{chol}), 39.88 (C-12_{chol}), 39.62 (C-24_{chol}), 39.20 (C-4_{chol}), 37.28 (C-1_{chol}), 39.95 (C-10_{chol}), 36.30 (C-22_{chol}), 35.90 (C-20_{chol}), 32.07 (C-7_{chol}), 31.95 (C-8_{chol}), 28.50 (C-2_{chol}), 28.34 (C-16_{chol}), 28.11 (C-25_{chol}), 24.39 (C-15_{chol}), 23.51 (C-23_{chol}), 22.92 (C-27_{chol}), 22.67 (C-26_{chol}), 21.18 (C-11_{chol}), 19.47 (C-19_{chol}), 18.82 (C-21_{chol}), 11.96 (C-18_{chol}).

*Ethyl 5-(cholest-5-en-3β-yloxy)-3-oxapentanoate (**11**)*

Boron trifluoride etherate (12 µL, 0.010 mmol) was added to a cold solution (−20 °C) of alcohol **10** (0.430 g, 1.00 mmol) and ethyl diazoacetate (0.11 mL, 1.10 mmol, 1.10 equiv.) in dry CH_2Cl_2 (10 mL). The reaction was treated as described previously, and pure compound **11** was recovered as an oily material in 56% yield: 0.289 g, R_f 0.48 (1:5 ethyl acetate–petroleum ether), $[\alpha]_D$ −24.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.33 (bd, 1H, H-6_{chol}), 4.23 (q, 2H, *J* 7.1 Hz, $COOCH_2CH_3$), 4.17 (s, 2H, OCH_2COOEt), 3.74–3.66 (m, 4H, OCH_2CH_2O), 3.19 (m, 1H, H-3_{chol}), 1.29 (t, 3H, $COOCH_2CH_3$), 2.40–0.67 (m, 43H, H cholesterol); ^{13}C NMR ($CDCl_3$): δ 170.43 (*C*OOEt), 140.80 (C-5_{chol}), 121.52 (C-6_{chol}), 79.48 (C-3_{chol}), 71.17, 68.72 (CH_2OCH_2COOEt), 67.32 (CH_2OChol), 60.66 ($COOCH_2CH_3$), 56.73 (C-14_{chol}), 56.14 (C-17_{chol}), 50.14 (C-9_{chol}), 42.27 (C-13_{chol}), 39.75 (C-12_{chol}), 39.48 (C-24_{chol}), 38.99 (C-4_{chol}), 37.18 (C-1_{chol}), 36.80 (C-10_{chol}), 36.15 (C-22_{chol}), 35.75 (C-20_{chol}), 31.89 (C-7_{chol}), 31.85 (C-8_{chol}), 28.27 (C-2_{chol}), 28.19 (C-16_{chol}), 27.95 (C-25_{chol}), 24.24 (C-15_{chol}), 23.80 (C-23_{chol}), 22.77 (C-27_{chol}), 22.52 (C-26_{chol}), 21.03 (C-11_{chol}), 19.32 (C-19_{chol}), 18.68 (C-21_{chol}), 14.17 ($COOCH_2CH_3$), 11.81 (C-18_{chol}).

Anal. calc. for $C_{33}H_{56}O_4$ (516.78): C, 76.69; H, 10.92. Found: C, 76.37; H, 10.78.

*5-(Cholest-5-en-3β-yloxy)-1-[$^2H_2$]-3-oxapentan-1-ol (**12**)*

Compound **12** was obtained as described previously from compound **9**, starting from ester **11** (0.600 g, 1.56 mmol) and lithium aluminium deuteride (0.130 g, 3.12 mmol). Pure compound **12** was recovered as an oily material in 75% yield after purification by column chromatography (3:2 ethyl acetate–petroleum ether): 0.422 g, R_f 0.52 (1:1 ethyl acetate–petroleum ether), $[\alpha]_D$ −28.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.36 (bd, 1H, H-6_{chol}), 3.68–3.62 (m, 6H, 3CH_2O), 3.18 (m, 1H, H-3_{chol}), 2.40–0.67 (m, 43H, H cholesterol); ^{13}C NMR ($CDCl_3$): δ 140.80 (C-5_{chol}), 121.71 (C-6_{chol}), 79.46 (C-3_{chol}), 72.48, 70.84 (CH_2OCH_2), 67.45 (CH_2OChol), 61.18 (q, *J* 21.0 Hz, CD_2), 56.83 (C-14_{chol}), 56.23 (C-17_{chol}), 50.23 (C-9_{chol}), 42.36 (C-13_{chol}), 39.83 (C-12_{chol}), 39.56 (C-24_{chol}), 39.03 (C-4_{chol}), 37.25 (C-1_{chol}), 36.97 (C-10_{chol}), 36.24 (C-22_{chol}), 35.82 (C-20_{chol}), 31.98 (C-7_{chol}), 31.93 (C-8_{chol}), 28.375 (C-2_{chol}), 28.26 (C-16_{chol}), 28.03 (C-25_{chol}), 24.32 (C-15_{chol}), 23.68 (C-23_{chol}), 22.85 (C-27_{chol}), 22.60 (C-26_{chol}), 21.11 (C-11_{chol}), 19.41 (C-19_{chol}), 18.76 (C-21_{chol}), 11.89 (C-18_{chol}).

Anal. calc. for $C_{31}H_{52}D_2O_3$,0.5H_2O (485.77): C, 76.64; H, 11.00. Found: C, 76.94; H, 11.19.

*8-(Cholest-5-en-3β-yloxy)-4-[$^2H_2$]-1-tetrahydropyranyl-3,6-dioxaoctan-1-ol (**13**)*

A mixture of compound **12** (0.360 g, 0.756 mmol), tetrahydropyranylated 2-chloroethanol (0.520 g, 3.50 mmol, 4.63 equiv.), tetrabutylammonium hydrogen sulfate (0.167 g, 0.49 mmol) and powder sodium hydroxide (0.152 g, 3.80 mmol) was stirred at 65 °C for 2 h. After cooling to room temperature, the mixture was poured into water, and the product was extracted with dichloromethane (2 × 50 mL). The organic phase was washed with water (20 mL), dried (Na_2SO_4) and concentrated to afford a crude product, which was purified by column chromatography by using 1:2 ethyl acetate–petroleum ether as eluent. Product **13** was recovered in a pure form as an oily material in 80% yield: 0.357 g, R_f 0.53 (1:2 ethyl acetate–petroleum ether), $[\alpha]_D$ −20.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.34 (bd, 1H, H-6_{chol}), 4.63 (dd, 1H, OCH_{THP}), 3.95–3.45 (m, 12H, 5CH_2O, OCH_{2THP}), 3.18 (m, 1H, H-3_{chol}), 2.40–0.67 (m, 49H, 3CH_{2THP}, H cholesterol); ^{13}C NMR ($CDCl_3$): δ 140.97 (C-5_{chol}), 121.50 (C-6_{chol}), 79.48 (C-3_{chol}), 70.92, 70.53, 70.46 ($CH_2OCH_2CD_2OCH_2$), 69.86 (q, *J* 21.4 Hz, CD_2), 67.32 (CH_2OChol), 66.65 (CH_2OTHP), 62.04 (OCH_{2THP}), 56.78 (C-14_{chol}), 56.20 (C-17_{chol}), 50.20 (C-9_{chol}), 42.31 (C-13_{chol}), 39.80 (C-12_{chol}), 39.52 (C-24_{chol}), 39.08 (C-4_{chol}), 37.26 (C-1_{chol}), 36.83 (C-10_{chol}), 36.21 (C-22_{chol}), 35.79 (C-20_{chol}), 31.94 (C-7_{chol}), 31.89 (C-8_{chol}), 30.55 (CH_{2THP}), 28.36 (C-2_{chol}), 28.22

(C-16$_{chol}$), 27.98 (C-25$_{chol}$), 25.47 (CH_{2THP}), 24.29 (C-15$_{chol}$), 23.86 (C-23$_{chol}$), 22.83 (C-27$_{chol}$), 22.58 (C-26$_{chol}$), 21.08 (C-11$_{chol}$), 19.44 (C-19$_{chol}$), 19.39 (CH_{2THP}), 18.74 (C-21$_{chol}$), 11.86 (C-18$_{chol}$).

Anal. calc. for $C_{38}H_{64}D_2O_5$ (604.94): C, 75.45; H, 11.33. Found: C, 75.28; H, 11.27.

*Ethyl 2-(cholest-5-en-3β-yloxy)-ethanoate (**14**)*

Boron trifluoride etherate (64 μL, 0.52 mmol) was added to a cold solution (−20 °C) of cholesterol (2.00 g, 5.17 mmol) and ethyl diazoacetate (0.60 mL, 5.70 mmol, 1.10 equiv.) in dry CH_2Cl_2 (10 mL). The solution was stirred overnight at −20 °C and then poured into a saturated aqueous $NaHCO_3$ solution (30 mL), and the product was extracted with CH_2Cl_2 (2 × 25 mL). The organic phase was dried (Na_2SO_4) and concentrated under diminished pressure. The crude product was purified by column chromatography (1:10 ethyl acetate–petroleum ether). Compound **14** was recovered as an amorphous solid in 58% yield: 1.12 g, R_f 0.60 (1:10 ethyl acetate–petroleum ether), $[\alpha]_D$ −30.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.34 (bd, 1H, H-6$_{chol}$), 4.16 (q, 2H, *J* 7.1 Hz, CH_3CH_2OCO), 4.11 (s, 2H, OCH_2COOEt), 3.19 (m, 1H, H-3$_{chol}$), 2.40–0.67 (m, 43H, H cholesterol), 1.28 (t, 3H, CH_3CH_2O); ^{13}C NMR ($CDCl_3$): δ 170.86 (*C*OOEt), 140.53 (C-5$_{chol}$), 121.94 (C-6$_{chol}$), 80.035 (C-3$_{chol}$), 65.76 (CH_2OChol), 60.78 ($COOCH_2CH_3$), 56.80 (C-14$_{chol}$), 56.22 (C-17$_{chol}$), 50.21 (C-9$_{chol}$), 42.35 (C-13$_{chol}$), 39.80 (C-12$_{chol}$), 39.567 (C-24$_{chol}$), 38.75 (C-4$_{chol}$), 37.19 (C-1$_{chol}$), 36.84 (C-10$_{chol}$), 36.24 (C-22$_{chol}$), 35.82 (C-20$_{chol}$), 31.92 (C-7$_{chol}$), 31.92 (C-8$_{chol}$), 28.26 (C-2$_{chol}$), 28.12 (C-16$_{chol}$), 28.03 (C-25$_{chol}$), 24.32 (C-15$_{chol}$), 23.88 (C-23$_{chol}$), 22.85 (C-27$_{chol}$), 22.60 (C-26$_{chol}$), 21.11 (C-11$_{chol}$), 19.38 (C-19$_{chol}$), 18.76 (C-21$_{chol}$), 14.25 ($COOCH_2CH_3$), 11.89 (C-18$_{chol}$).

Anal. calc. for $C_{31}H_{52}O_3$ (472.73): C, 78.76; H, 11.09. Found: C, 78.43; H, 11.25.

*2-(Cholest-5-en-3β-yloxy)-1-[$^2H_2$]-ethano1 (**15**)*

Compound **15** was prepared as described previously for **8** from ester **14** (1.70 g, 3.60 mmol) and lithium aluminium deuteride (0.300 g, 7.20 mmol). The crude reaction product was purified by column chromatography by using 1:1 ethyl acetate–petroleum ether as eluent. Pure compound **15** was recovered in 82% yield as a solid: 1.28 g, R_f 0.60 (1:1 ethyl acetate–petroleum ether), mp 98 °C, $[\alpha]_D$ −29.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.33 (bd, 1H, H-6$_{chol}$), 3.57 (s, 2H, CH_2OChol), 3.18 (m, 1H, H-3$_{chol}$), 2.40−0.67 (m, 43H, H cholesterol); ^{13}C NMR ($CDCl_3$): δ 140.64 (C-5$_{chol}$), 121.70 (C-6$_{chol}$), 79.44 (C-3$_{chol}$), 68.95 (CH_2OChol), 61.28 (q, *J* 21.1 Hz, CD_2), 56.76 (C-14$_{chol}$), 56.19 (C-17$_{chol}$), 50.18 (C-9$_{chol}$), 42.30 (C-13$_{chol}$), 39.79 (C-12$_{chol}$), 39.52 (C-24$_{chol}$), 39.08 (C-4$_{chol}$), 37.19 (C-1$_{chol}$), 36.83 (C-10$_{chol}$), 36.20 (C-22$_{chol}$), 35.79 (C-20$_{chol}$), 31.91 (C-7$_{chol}$), 31.88 (C-8$_{chol}$), 28.38 (C-2$_{chol}$), 28.32 (C-16$_{chol}$), 27.99 (C-25$_{chol}$), 24.28 (C-15$_{chol}$), 23.86 (C-23$_{chol}$), 22.80 (C-27$_{chol}$), 22.55 (C-26$_{chol}$), 21.07 (C-11$_{chol}$), 19.35 (C-19$_{chol}$), 18.79 (C-21$_{chol}$), 11.85 (C-18$_{chol}$).

Anal. calc. for $C_{29}H_{48}D_2O_2$,0.5H_2O (441.73): C, 78.85; H, 12.09. Found: C, 78.91; H, 11.84.

*1-Chloro-8-(cholest-5-en-3β-yloxy)-7-[$^2H_2$]-3,6-dioxaoctane (**16**)*

A mixture of compound **15** (1.00 g, 2.26 mmol), 2-chloroethyl ether (1.60 mL, 13.65 mmol), powder sodium hydroxide (0.550 g, 13.75 mmol) and tetrabutylammonium hydrogen sulfate (0.550 g, 1.62 mmol) was heated for 1 h at 65 °C with vigorous stirring. After cooling to room temperature, the mixture was diluted with dichloromethane (80 mL), and the organic phase was washed with water (2 × 20 mL) and dried (Na_2SO_4). The crude residue obtained after concentration under diminished pressure was purified by column chromatography (1:3 ethyl acetate–petroleum ether). Pure product **16** was recovered as an oily material in 65% yield: 0.817 g, R_f 0.55 (1:3 ethyl acetate–petroleum ether), $[\alpha]_D$ −21.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.35 (bd, 1H, H-6$_{chol}$), 3.79 (t, 2H, CH_2Cl), 3.64–3.54 (m, 8H, $OCH_2CH_2OCH_2CH_2$OChol), 3.18 (m, 1H, H-3$_{chol}$), 2.40–0.67 (m, 43H, H cholesterol); ^{13}C NMR ($CDCl_3$): δ 140.93 (C-5$_{chol}$), 121.53 (C-6$_{chol}$), 79.48 (C-3$_{chol}$), 71.37, 70.75, 70.70.71 (4 CH_2O), 70.23 (q, *J* 21.0 Hz, CD_2), 67.16 (CH_2OChol), 56.77 (C-14$_{chol}$), 56.16 (C-17$_{chol}$), 50.18 (C-9$_{chol}$), 42.67 (CH_2Cl), 42.31 (C-13$_{chol}$), 39.78 (C-12$_{chol}$), 39.51 (C-24$_{chol}$), 39.07 (C-4$_{chol}$), 37.24 (C-1$_{chol}$), 36.86 (C-10$_{chol}$), 36.19 (C-22$_{chol}$), 35.78 (C-20$_{chol}$), 31.94 (C-7$_{chol}$), 31.89 (C-8$_{chol}$), 28.35 (C-2$_{chol}$), 28.23 (C-16$_{chol}$), 28.00 (C-25$_{chol}$), 24.29 (C-15$_{chol}$), 23.83 (C-23$_{chol}$), 22.82 (C-27$_{chol}$), 22.57 (C-26$_{chol}$), 21.07 (C-11$_{chol}$), 19.37 (C-19$_{chol}$), 18.72 (C-21$_{chol}$), 11.99 (C-18$_{chol}$).

Anal. calc. for $C_{33}H_{55}ClD_2O_3$ (539.25): C, 73.49; H, 11.02. Found: C, 72.91; H, 10.44.

*8-(Cholest-5-en-3β-yloxy)-7-[$^2H_2$]-3,6-dioxaoctan-1-ol (**17**)*

A mixture of compound **16** (0.800 g, 1.48 mmol), sodium formate (0.250 g, 3.68 mmol) and tetrabutylammonium bromide (0.030 g, 0.09 mmol) in DMSO was stirred at 115 °C for 24 h. After cooling to room temperature, 12.5 M sodium hydroxide (0.23 mL, 2.95 mmol) was added, and stirring was maintained overnight at room temperature. The crude product was obtained after addition of water (10 mL), extraction with dichloromethane (2 × 30 mL), drying (Na_2SO_4) and concentration of the organic phase. Purification was realized by column chromatography (2:1 ethyl acetate–petroleum ether). Compound **17** was obtained as an oily material in 55% yield: 0.425 g, R_f 0.53 (2:1 ethyl acetate–petroleum ether), $[\alpha]_D$ −21.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.34 (bd, 1H, H-6$_{chol}$), 3.70–3.63 (m, 10H, $5CH_2O$), 3.18 (m, 1H, H-3$_{chol}$), 2.40–0.67 (m, 43H, H cholesterol); ^{13}C NMR ($CDCl_3$): δ 140.85 (C-5$_{chol}$), 121.54 (C-6$_{chol}$), 79.51 (C-3$_{chol}$), 72.56, 70.53, 70.34 ($CH_2OCH_2CH_2O$), 70.00 (q, *J* 21.2 Hz, CD_2), 67.07 (CH_2OChol), 61.68 (CH_2OH), 56.74 (C-14$_{chol}$), 56.15 (C-17$_{chol}$), 50.17 (C-9$_{chol}$), 42.33 (C-13$_{chol}$), 39.77 (C-12$_{chol}$), 39.50 (C-24$_{chol}$), 38.98 (C-4$_{chol}$), 37.20 (C-1$_{chol}$), 36.83 (C-10$_{chol}$), 36.18 (C-22$_{chol}$), 35.76 (C-20$_{chol}$), 31.91 (C-7$_{chol}$), 31.87 (C-8$_{chol}$), 28.28 (C-2$_{chol}$), 28.20 (C-16$_{chol}$), 27.97 (C-25$_{chol}$), 24.26 (C-15$_{chol}$), 23.82 (C-23$_{chol}$), 22.79 (C-27$_{chol}$), 22.54 (C-26$_{chol}$), 21.05 (C-11$_{chol}$), 19.35 (C-19$_{chol}$), 18.70 (C-21$_{chol}$), 11.84 (C-18$_{chol}$).

Anal. calc. for $C_{33}H_{56}D_2O_4$,0.75H_2O (534.61): C, 74.14; H, 11.59. Found: C, 74.14; H, 11.21.

General method for the glycosylation of deuterated cholesteryl derivatives

Trimethylsilyl trifluoromethanesulfonate (1.05 equiv.) was added at −20 °C under argon to a mixture of 1,3,4,6-tetra-*O*-acetyl-2-allyloxycarbonylamino-2-deoxy-β-D-glucopyranose (**18**)[23,24] (1–1.05 equiv) and alcohol **9** (or **17**) or tetrahydropyranylated alcohol **13** (1 equiv.) in dry alcohol-free CH_2Cl_2 (15 mL/mmol). The mixture was stirred for 16 h at −20 °C and then poured into a saturated aqueous $NaHCO_3$ solution (30 mL); the aqueous phase was extracted with CH_2Cl_2 (2 × 20 mL), and the organic phase was dried and concentrated under diminished pressure. The crude product was purified by column chromatography (2:1 EtOAc–petroleum ether) to afford the pure glycosides.

[8-(Cholest-5-en-3β-yloxy)-1-[$^2H_2$]-3,6-dioxaoctyl] 3,4,6-tri-O-acetyl-2-allyloxy-carbonylamino-2-deoxy-β-D-glucopyranoside (**19**)

Compound **19** was obtained in 79% yield as described previously from alcohol **9** (0.520 g, 1.00 mmol) and donor **18** (0.452 mmol, 1.05 mmol): 0.705 g, R_f 0.70 (2:1 ethyl acetate–petroleum ether), $[\alpha]_D$ −17.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.89 (m, 1H, C*H*), 5.78 (m, 1H, N*H*), 5.34 (bd, 1H, H-6_{chol}), 5.30–5.13 (m, 3H, CH_2=, H-3), 5.10 (dd, 1H, $J_{3,4}$ 9.3 Hz, $J_{4,5}$ 9.9 Hz, H-4), 4.80 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 4.57 (m, 2H, allyl CH_2), 4.27 (dd, 1H, $J_{5,6a}$ 4.6 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.12 (dd, 1H, $J_{5,6b}$ 2.2 Hz, H-6b), 3.95–3.55 (m, 12H, 5CH_2O, H-2, H-5), 3.18 (m, 1H, H-3_{chol}), 2.16, 2.08, 2.01 (3s, 9H, 3CH_3COO), 2.40–0.67 (m, 43H, H cholesterol); ^{13}C NMR ($CDCl_3$): δ 170.74, 170.49, 169.46 (CH_3COO), 156.23 (NHCOO), 140.90 (C-5_{chol}), 132.93 (CH_2=*C*H), 121.58 (C-6_{chol}), 117.25 (CH_2=), 101.87 (C-1), 79.43 (C-3_{chol}), 73.01 (C-3), 71.68 (C-5), 70.66, 70.57 ($CH_2OCH_2CH_2OCH_2$), 70.03 (q, *J* 21.2 Hz, CD_2), 68.82 (C-4), 67.33 (CH_2OChol), 65.49 (allyl CH_2), 62.21 (C-6), 56.78 (C-14_{chol}), 56.16 (C-17_{chol}), 55.96 (C-2), 50.21 (C-9_{chol}), 42.32 (C-13_{chol}), 39.75 (C-12_{chol}), 39.52 (C-24_{chol}), 39.08 (C-4_{chol}), 37.24 (C-1_{chol}), 36.87 (C-10_{chol}), 36.19 (C-22_{chol}), 35.78 (C-20_{chol}), 31.95 (C-7_{chol}), 31.90 (C-8_{chol}), 28.37 (C-2_{chol}), 28.22 (C-16_{chol}), 28.01 (C-25_{chol}), 24.29 (C-15_{chol}), 23.82 (C-23_{chol}), 22.83 (C-27_{chol}), 22.58 (C-26_{chol}), 21.08 (C-11_{chol}), 20.78, 20.72, 20.65 (CH_3COO), 19.40 (C-19_{chol}), 18.73 (C-21_{chol}), 11.87 (C-18_{chol}).

Anal. calc. for $C_{49}H_{77}D_2NO_{13}$ (892.145): C, 65.96; H, 9.15; N 1.57. Found: C, 65.71; H, 9.05; N: 1.78.

[8-(Cholest-5-en-3β-yloxy)-4-[$^2H_2$]-3,6-dioxaoctyl] 3,4,6-tri-O-acetyl-2-allyloxy-carbonylamino-2-deoxy-β-D-glucopyranoside (**20**)

Compound **20** was obtained in 75% yield as described previously from tetrahydropyranylated derivative **13** (0.362 g, 0.60 mmol) and donor **18** (0.265 g, 0.62 mmol): 0.401 g, mp 110 °C (petroleum ether), R_f 0.70 (2:1 ethyl acetate–petroleum ether), $[\alpha]_D$ −19.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.89 (m, 1H, C*H*), 5.78 (m, 1H, N*H*), 5.34 (bd, 1H, H-6_{chol}), 5.24–5.09 (m, 3H, CH_2=, H-3), 5.05 (dd, 1H, $J_{3,4}$ 9.3 Hz, $J_{4,5}$ 9.8 Hz, H-4), 4.79 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 4.57 (m, 2H, allyl CH_2), 4.27 (dd, 1H, $J_{5,6a}$ 4.7 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.11 (dd, 1H, $J_{5,6b}$ 2.0 Hz, H-6b), 3.95–3.60 (m, 12H, 5CH_2O, H-2, H-5), 3.19 (m, 1H, H-3_{chol}), 2.16, 2.08, 2.01 (3s, 9H, 3CH_3COO), 2.40–0.67 (m, 43H, H cholesterol); ^{13}C NMR ($CDCl_3$): δ 170.55, 170.28, 169.35 (CH_3COO), 156.16 (NHCOO), 140.73 (C-5_{chol}), 132.93 (CH_2=*C*H), 121.46 (C-6_{chol}), 117.06 (CH_2=), 101.62 (C-1), 79.32 (C-3_{chol}), 72.92 (C-3), 71.52 (C-5), 70.96, 70.67, 70.42 ($CH_2CH_2OCD_2CH_2OCH_2$), 69.83 (q, *J* 21.2 Hz, CD_2), 68.82 (C-4), 67.32 (CH_2OChol), 65.30 (allyl CH_2), 62.14 (C-6), 56.68 (C-14_{chol}), 56.09 (C-17_{chol}), 55.81 (C-2), 50.10 (C-9_{chol}), 42.23 (C-13_{chol}), 39.71 (C-12_{chol}), 39.44 (C-24_{chol}), 39.00 (C-4_{chol}), 37.16 (C-1_{chol}), 36.75 (C-10_{chol}), 36.12 (C-22_{chol}), 35.72 (C-20_{chol}), 31.85 (C-7_{chol}), 31.81 (C-8_{chol}), 28.27 (C-2_{chol}), 28.17 (C-16_{chol}), 27.90 (C-25_{chol}), 24.22 (C-15_{chol}), 23.77 (C-23_{chol}), 22.78 (C-27_{chol}), 22.53 (C-26_{chol}), 21.01 (C-11_{chol}), 20.69, 20.60, 20.53 (CH_3COO), 19.31 (C-19_{chol}), 18.68 (C-21_{chol}), 11.81 (C-18_{chol}).

Anal. calc. for $C_{49}H_{77}D_2NO_{13}$ (892.145): C, 65.96; H, 9.15; N 1.57. Found: C, 65.68; H, 9.00; N: 1.69.

[8-(Cholest-5-en-3β-yloxy)-7-[$^2H_2$]-3,6-dioxaoctyl] 3,4,6-tri-O-acetyl-2-allyloxy-carbonylamino-2-deoxy-β-D-glucopyranoside (**21**)

Compound **21** was obtained in 75% yield as described previously from alcohol **17** (0.362 g, 0.60 mmol) and donor **18** (0.265 g, 0.62 mmol): 0.401 g, mp 109 °C (petroleum ether), R_f 0.70 (2:1 ethyl acetate–petroleum ether), $[\alpha]_D$ −17.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.89 (m, 1H, C*H*), 5.75 (m, 1H, N*H*), 5.34 (bd, 1H, H-6_{chol}), 5.24–5.09 (m, 3H, CH_2=, H-3), 5.05 (dd, 1H, $J_{3,4}$ 9.3 Hz, $J_{4,5}$ 9.7 Hz, H-4), 4.79 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), 4.56 (m, 2H, allyl CH_2), 4.27 (dd, 1H, $J_{5,6a}$ 4.7 Hz, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.12 (dd, 1H, $J_{5,6b}$ 2.0 Hz, H-6b), 3.95–3.60 (m, 12H, 5CH_2O, H-2, H-5), 3.18 (m, 1H, H-3_{chol}), 2.17, 2.08, 2.01 (3s, 9H, 3CH_3COO), 2.40–0.67 (m, 43H, H cholesterol); ^{13}C NMR ($CDCl_3$): δ 170.76, 170.51, 169.48 (CH_3COO), 156.25 (NHCOO), 140.91 (C-5_{chol}), 132.88 (CH_2=*C*H), 121.60 (C-6_{chol}), 117.26 (CH_2=), 101.90 (C-1), 79.48 (C-3_{chol}), 73.01 (C-3), 71.68 (C-5), 70.66, 70.57, 70.42 ($CH_2CH_2OCH_2CH_2OCH_2$), 70.03 (q, *J* 21.2 Hz, CD_2), 68.81 (C-4), 67.32 (CH_2OChol), 65.50 (allyl CH_2), 62.21 (C-6), 56.78 (C-14_{chol}), 56.16 (C-17_{chol}), 55.97 (C-2), 50.20 (C-9_{chol}), 42.32 (C-13_{chol}), 39.72 (C-12_{chol}), 39.52 (C-24_{chol}), 39.08 (C-4_{chol}), 37.23 (C-1_{chol}), 36.8775 (C-10_{chol}), 36.20 (C-22_{chol}), 35.78 (C-20_{chol}), 31.95 (C-7_{chol}), 31.90 (C-8_{chol}), 28.36 (C-2_{chol}), 28.24 (C-16_{chol}), 28.00 (C-25_{chol}), 24.29 (C-15_{chol}), 23.83 (C-23_{chol}), 22.83 (C-27_{chol}), 22.57 (C-26_{chol}), 21.08 (C-11_{chol}), 20.78, 20.72, 20.65 (CH_3COO), 19.40 (C-19_{chol}), 18.73 (C-21_{chol}), 11.87 (C-18_{chol}).

Anal. calc. for $C_{49}H_{77}D_2NO_{13}$ (892.145): C, 65.96; H, 9.15; N 1.57. Found: C, 65.82; H, 9.07; N: 1.53.

General procedure for the preparation of compounds 23–26

Tris(dibenzylideneacetone)dipalladium (0.016 g, 0.0168 mmol) and PPh_3 (0.050 g, 0.190 mmol) were reacted for 10 min in dry oxygen-free THF (2 mL) under argon. The solution was added to a solution of the *N*-allyloxycarbonyl derivatives **19–22** (0.40 mmol) and diethyl malonate (0.80 mL) in dry THF (2–3 mL), and the mixture was stirred for 16 h under argon. After concentration, the residue was eluted on a short column of silica gel to separate the free amino derivative, which was acetylated with Ac_2O (1.5 equiv.) in dry MeOH (4 mL) (from **19** to **21**) or with $(CD_3CO)_2O$ in MeOH (from **22**). The pure peracetylated derivatives **23–26** were obtained after concentration and purification of the residue by column chromatography by using 5:1 ethyl acetate–acetone mixture as eluent.

[8-(Cholest-5-en-3β-yloxy)-1-[$^2H_2$]-3,6-dioxaoctyl] 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (**23**)

Compound **23** was obtained in 80% yield as described previously from glycoside **19** (0.357 g, 0.40 mmol): 0.271 g, R_f 0.55 (5:1 ethyl acetate–acetone), mp 126 °C, $[\alpha]_D$ −26.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.70 (d, 1H, $J_{2,NH}$ 9.3 Hz, N*H*), 5.31 (bd, 1H, H-6_{chol}), 5.11–5.02 (m, 2H, H-3, H-4), 4.79 (d, 1H, $J_{1,2}$ 8.6 Hz, H-1), 4.25 (dd, 1H, $J_{5,6a}$ 4.6 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.10 (dd, 1H, $J_{5,6b}$ 2.2 Hz, H-6b), 4.09–4.04 (m, 1H, H-2), 3.80–5.55 (m, 11H, 5CH_2O, H-5), 3.15 (m, 1H, H-3_{chol}), 2.07, 1.99, 1.99, 1.97 (4s, 12H, 4CH_3CO), 2.34–0.66 (m, 43H, H cholesterol); ^{13}C NMR ($CDCl_3$): δ 170.92, 170.84, 170.82, 169.47 (CH_3CO), 140.87 (C-5_{chol}), 121.88 (C-6_{chol}), 102.13 (C-1), 79.60 (C-3_{chol}), 73.56 (C-3), 71.84 (C-5), 71.76, 71.04, 70.87, 70.83 (CH_2OCH_2-CH_2OCH_2), 68.85 (C-4), 67.45 (CH_2OChol), 62.34 (C-6), 56.89 (C-14_{chol}), 56.28 (C-17_{chol}), 53.96 (C-2), 50.33 (C-9_{chol}), 42.45 (C-13_{chol}), 39.90 (C-12_{chol}), 39.63 (C-24_{chol}), 39.29 (C-4_{chol}), 37.33 (C-1_{chol}), 36.99 (C-10_{chol}), 36.27 (C-22_{chol}), 35.91 (C-20_{chol}), 32.06 (C-7_{chol}), 32.02 (C-8_{chol}), 28.43 (C-2_{chol}), 28.36 (C-16_{chol}), 28.14 (C-25_{chol}), 24.41 (C-15_{chol}), 23.93 (C-23_{chol}), 23.23 (NHCOCH_3), 22.96 (C-27_{chol}), 22.70 (C-26_{chol}), 21.18 (C-11_{chol}), 20.91, 20.87, 20.78 (CH_3COO), 19.52 (C-19_{chol}), 18.85 (C-21_{chol}), 12.00 (C-18_{chol}).

Anal. calc. for $C_{47}H_{75}D_2NO_{12}$ (850.105): C, 66.40; H, 9.36; N 1.65. Found: C, 66.35; H, 9.17; N: 1.64.

*[8-(Cholest-5-en-3β-yloxy)-4-[$^2H_2$]-3,6-dioxaoctyl] 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (**24**)*

Compound **24** was obtained in 75% yield as described previously from glycoside **20** (0.350 g, 0.39 mmol): 0.250 g, R_f 0.55 (5:1 ethyl acetate–acetone), mp 127 °C, $[\alpha]_D$ −28.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.73 (d, 1H, $J_{2,NH}$ 9.2 Hz, N*H*), 5.33 (bd, 1H, H-6_{chol}), 5.12–5.03 (m, 2H, H-3, H-4), 4.80 (d, 1H, $J_{1,2}$ 8.6 Hz, H-1), 4.25 (dd, 1H, $J_{5,6a}$ 4.6 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.11 (dd, 1H, $J_{5,6b}$ 2.2 Hz, H-6b), 4.10–4.07 (m, 1H, H-2), 3.91–3.58 (m, 11H, 5CH_2O, H-5), 3.17 (m, 1H, H-3$_{chol}$), 2.08, 2.00, 2.00, 1.97 (4s, 12H, 4CH_3CO), 2.40–0.67 (m, 43H, H cholesterol); ^{13}C NMR ($CDCl_3$): δ 170.99, 170.99, 170.89, 169.52 (*C*H_3CO), 140.68 (C-5_{chol}), 121.93 (C-6_{chol}), 102.22 (C-1), 79.66 (C-3_{chol}), 73.60 (C-3), 71.89 (C-5), 71.84, 70.89, 70.79, 68.88 (O*C*H_2*C*H_2*C*H_2O*C*D_2*C*H_2O*C*H_2), 68.88 (C-4), 67.49 (*C*H_2OChol), 62.37 (C-6), 56.93 (C-14_{chol}), 56.32 (C-17_{chol}), 53.91 (C-2), 50.37 (C-9_{chol}), 42.49 (C-13_{chol}), 39.94 (C-12_{chol}), 39.69 (C-24_{chol}), 39.32 (C-4_{chol}), 37.37 (C-1_{chol}), 37.03 (C-10_{chol}), 36.36 (C-22_{chol}), 35.95 (C-20_{chol}), 32.10 (C-7_{chol}), 32.05 (C-8_{chol}), 28.46 (C-2_{chol}), 28.40 (C-16_{chol}), 28.19 (C-25_{chol}), 24.46 (C-15_{chol}), 23.99 (C-23_{chol}), 23.28 (NHCO*C*H_3), 23.00 (C-27_{chol}), 22.74 (C-26_{chol}), 21.25 (C-11_{chol}), 20.97, 20.92, 20.83 (*C*H_3COO), 19.57 (C-19_{chol}), 18.90 (C-21_{chol}), 12.04 (C-18_{chol}).

Anal. calc. for $C_{47}H_{75}D_2NO_{12}$ (850.105): C, 66.40; H, 9.36; N 1.65. Found: C, 66.17; H, 9.21; N: 1.66.

*[8-(Cholest-5-en-3β-yloxy)-7-[$^2H_2$]-3,6-dioxaoctyl] 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (**25**)*

Compound **25** was obtained in 78% yield as described previously from glycoside **21** (0.350 g, 0.39 mmol): 0.260 g, R_f 0.55 (5:1 ethyl acetate–acetone), mp 126 °C, $[\alpha]_D$ −28.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.74 (d, 1H, $J_{2,NH}$ 9.2 Hz, N*H*), 5.32 (bd, 1H, H-6_{chol}), 5.12–5.03 (m, 2H, H-3, H-4), 4.79 (d, 1H, $J_{1,2}$ 8.6 Hz, H-1), 4.25 (dd, 1H, $J_{5,6a}$ 4.5 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.10 (dd, 1H, $J_{5,6b}$ 2.1 Hz, H-6b), 4.10–4.07 (m, 1H, H-2), 3.92–3.58 (m, 11H, 5CH_2O, H-5), 3.16 (m, 1H, H-3$_{chol}$), 2.07, 1.99, 1.99, 1.97 (4s, 12H, 4CH_3CO), 2.36–0.66 (m, 43H, H cholesterol); ^{13}C NMR ($CDCl_3$): δ 170.98, 170.90, 170.87, 169.51 (*C*H_3CO), 140.89 (C-5_{chol}), 121.93 (C-6_{chol}), 101.18 (C-1), 79.66 (C-3_{chol}), 73.60 (C-3), 71.88 (C-5), 71.88, 70.84, 70.80 (*C*H_2OCH_2*C*H_2), 68.86 (C-4), 68.86 (O*C*H_2), 67.34 (*C*H_2OChol), 62.36 (C-6), 56.92 (C-14_{chol}), 56.31 (C-17_{chol}), 54.01 (C-2), 50.35 (C-9_{chol}), 42.48 (C-13_{chol}), 39.93 (C-12_{chol}), 39.67 (C-24_{chol}), 39.29 (C-4_{chol}), 37.36 (C-1_{chol}), 37.02 (C-10_{chol}), 36.34 (C-22_{chol}), 35.94 (C-20_{chol}), 32.09 (C-7_{chol}), 32.04 (C-8_{chol}), 28.45 (C-2_{chol}), 28.29 (C-16_{chol}), 28.17 (C-25_{chol}), 24.45 (C-15_{chol}), 23.98 (C-23_{chol}), 23.27 (NHCO*C*H_3), 22.99 (C-27_{chol}), 22.73 (C-26_{chol}), 21.24 (C-11_{chol}), 20.95, 20.91, 20.78 (*C*H_3COO), 19.56 (C-19_{chol}), 18.88 (C-21_{chol}), 12.03 (C-18_{chol}).

Anal. calc. for $C_{47}H_{75}D_2NO_{12}$ (850.105): C, 66.40; H, 9.36; N 1.65. Found: C, 66.64; H, 9.20; N: 1.68.

*[8-(Cholest-5-en-3β-yloxy)-3,6-dioxaoctyl] 3,4,6-tri-O-acetyl-2-deoxy-2-trideuterioacetamido-β-D-glucopyranoside (**26**)*

Compound **26** was obtained in 81% yield as described previously from glycoside **22** (0.350 g, 0.39 mmol): 0.270 g, R_f 0.39 (6:1 ethyl acetate–acetone), mp 127 °C, $[\alpha]_D$ −29.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.78 (d, 1H, $J_{2,NH}$ 9.3 Hz, N*H*), 5.31 (bd, 1H, H-6_{chol}), 5.11–5.01 (m, 2H, H-3, H-4), 4.79 (d, 1H, $J_{1,2}$ 8.6 Hz, H-1), 4.24 (dd, 1H, $J_{5,6a}$ 4.5 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.08 (dd, 1H, $J_{5,6b}$ 2.0 Hz, H-6b), 4.07–4.03 (m, 1H, H-2), 3.85–3.55 (m, 13H, 6CH_2O, H-5), 3.15 (m, 1H, H-3$_{chol}$), 2.05, 1.97, 1.97 (3s, 9H, 3CH_3CO), 2.35–0.65 (m, 43H, H cholesterol); ^{13}C NMR ($CDCl_3$): δ 170.82, 170.82, 170.69, 169.40 (*C*H_3CO), 140.78 (C-5_{chol}), 121.79 (C-6_{chol}), 102.07 (C-1), 79.52 (C-3_{chol}), 73.48 (C-3), 71.75 (C-5), 71.75, 70.95, 70.77, 70.72, 68.72 (*C*H_2O*C*H_2*C*H_2O*C*H_2*C*H_2), 68.81 (C-4), 67.35 (*C*H_2OChol), 62.28 (C-6), 56.81 (C-14_{chol}), 56.20 (C-17_{chol}), 53.86 (C-2), 50.25 (C-9_{chol}), 42.37 (C-13_{chol}), 39.82 (C-12_{chol}), 39.57 (C-24_{chol}), 39.21 (C-4_{chol}), 37.26 (C-1_{chol}), 36.91 (C-10_{chol}), 36.25 (C-22_{chol}), 35.84 (C-20_{chol}), 31.98 (C-7_{chol}), 31.94 (C-8_{chol}), 28.35 (C-2_{chol}), 28.29 (C-16_{chol}), 28.06 (C-25_{chol}), 24.34 (C-15_{chol}), 23.88 (C-23_{chol}), 22.90 (C-27_{chol}), 22.64 (C-26_{chol}), 21.14 (C-11_{chol}), 20.83, 20.79, 20.70 (*C*H_3COO), 19.45 (C-19_{chol}), 18.79 (C-21_{chol}), 11.93 (C-18_{chol}).

Anal. calc. for $C_{47}H_{74}D_3NO_{12}$ (851.13): C, 66.32; H, 9.47; N 1.65. Found: C, 66.05; H, 9.29; N: 1.68.

General procedure for the cleavage of the *O*-deacetylation of compounds 23–26

A solution of compounds **23–26** (0.200 g, 0.23 mmol) in MeOH (25 mL) containing a chip of sodium was stirred overnight at room temperature. After neutralization with Amberlyst IR 120 [H^+] and filtration, the solvent was evaporated under diminished pressure to afford products **2–5**.

*[8-(Cholest-5-en-3β-yloxy)-1-[$^2H_2$]-3,6-dioxaoctyl] 2-acetamido-2-deoxy-β-D-glucopyranoside (**2**)*

Product **2** was obtained in 92% yield as described previously from glycoside **23** (0.200 g, 0.23 mmol): 0.156 g, R_f 0.62 (67:25:8 ethyl acetate–ethanol–water), amorphous solid, $[\alpha]_D$ −45.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (C_5D_5N): δ 8.82 (d, 1H, $J_{2,NH}$ 8.5 Hz, N*H*), 5.43 (bd, 1H, H-6_{chol}), 5.10 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), 4.55 (ddd, 1H, $J_{2,3}$ 10.1 Hz, H-2), 4.54 (dd, 1H, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 12.1 Hz, H-6a), 4.39–4.33 (m, 2H, H-3, H-6b), 4.21 (dd, 1H, $J_{3,4}$ 8.6 Hz, $J_{4,5}$ 9.5 Hz, H-4), 3.91 (ddd, 1H, $J_{5,6b}$ 5.6 Hz H-5), 3.72–3.70 (m, 10H, 5OCH_2), 3.28 (m, 1H, H-3$_{chol}$), 2.15 (s, 3H, CH_3CO), 2.58–0.67 (m, 43H, H cholesterol).

HRMS (ESI) calc. for MNa: $C_{41}H_{69}D_2NNaO_9$, 746.5152. Found: 746.5154.

*[8-(Cholest-5-en-3β-yloxy)-4-[$^2H_2$]-3,6-dioxaoctyl] 2-acetamido-2-deoxy-β-D-glucopyranoside (**3**)*

Compound **3** was obtained in 91% yield as described previously from glycoside **24** (0.200 g, 0.23 mmol): 0.152 g, R_f 0.62 (67:25:8 ethyl acetate–ethanol–water), amorphous solid, $[\alpha]_D$ −45.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (C_5D_5N): δ 8.81 (d, 1H, $J_{2,NH}$ 8.2 Hz, N*H*), 5.52 (bd, 1H, H-6_{chol}), 5.08 (d, 1H, $J_{1,2}$ 8.3 Hz, H-1), 4.54–4.50 (m, 2H, H-2, H-6a), 4.37–4.32 (m, 2H, H-3, H-6b), 4.20 (dd, 1H, $J_{3,4}$ 8.8 Hz, $J_{4,5}$ 9.2 Hz, H-4), 4.14 (m, 1H, $CH_2CH_AH_BOC$-1), 3.92–3.84 (m, 2H, H-5, $CH_2CH_AH_BOC$-1), 3.73–3.70 (m, 8H, 4OCH_2), 3.28 (m, 1H, H-3$_{chol}$), 2.15 (s, 3H, CH_3CO), 2.56–0.68 (m, 43H, H cholesterol).

HRMS (ESI) calc. for MNa: $C_{41}H_{69}D_2NNaO_9$, 746.5152. Found: 746.5149.

*[8-(Cholest-5-en-3β-yloxy)-7-[$^2H_2$]-3,6-dioxaoctyl] 2-acetamido-deoxy-β-D-glucopyranoside (**4**)*

Compound **4** was obtained in 90% yield as described previously from glycoside **25** (0.200 g, 0.23 mmol): 0.150 g, R_f 0.62 (67:25:8 ethyl acetate–ethanol–water), amorphous solid, $[\alpha]_D$ −45.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (C_5D_5N): δ 8.82 (d, 1H, $J_{2,NH}$ 8.4 Hz, N*H*),

5.42 (bd, 1H, H-6_{chol}), 5.09 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), 4.55 (ddd, 1H, $J_{2,3}$ 9.9 Hz, H-2), 4.54 (m, 1H, H-6a), 4.40–4.33 (m, 2H, H-3, H-6b), 4.21 (dd, 1H, $J_{3,4}$ 8.8 Hz, $J_{4,5}$ 9.2 Hz, H-4), 4.15 (m, 1H, $CH_2CH_AH_BOC$-1), 3.88 (ddd, 1H, $J_{5,6a}$ 2.4 Hz, $J_{5,6b}$ 5.4 Hz, H-5), 3.86 (m, 1H, $CH_2CH_AH_BOC$-1), 3.75–3.67 (m, 8H, $4OCH_2$), 3.28 (m, 1H, H-3_{chol}), 2.15 (s, 3H, CH_3CO), 2.58–0.67 (m, 43H, H cholesterol).

HRMS (ESI) calc. for MNa: $C_{41}H_{69}D_2NNaO_9$, 746.5152. Found: 746.5155.

*[8-(Cholest-5-en-3β-yloxy)-3,6-dioxaoctyl] 2-deoxy-2-trideuteroacetamido-β-D-glucopyranoside (**5**)*

Compound **5** was obtained in 90% yield as described previously from glycoside **26** (0.200 g, 0.23 mmol): 0.150 g, R_f 0.62 (67:25:8 ethyl acetate–ethanol–water), amorphous solid, $[\alpha]_D$ −45.2 (*c* 1.0, $CHCl_3$); 8.82 (d, 1H, $J_{2,NH}$ 8.4 Hz, N*H*), 5.42 (bd, 1H, H-6_{chol}), 5.10 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), 4.59–4.53 (m, 2H, H-2, H-6a), 4.40–4.33 (m, 2H, H-3, H-6b), 4.21 (dd, 1H, $J_{3,4}$ 8.9 Hz, $J_{4,5}$ 9.3 Hz, H-4), 4.15 (m, 1H, $CH_2CH_AH_BOC$-1), 3.90 (m, 1H, H-5), 3.88 (m, 1H, $CH_2CH_AH_BOC$-1), 3.77–3.67 (m, 10H, $5OCH_2$), 3.28 (m, 1H, H-3_{chol}), 2.58–0.67 (m, 43H, H cholesterol).

HRMS (ESI) calc. for MNa: $C_{41}H_{68}D_3NNaO_9$, 747.5215. Found: 747.5217.

Conflict of Interest

The authors did not report any conflict of interest.

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