SUPPORTING INFORMATION

Cholesteryl glucosides signal through the Carbohydrate Recognition Domain of the Macrophage inducible C-type lectin (Mincle)

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Contents

Experimental and Procedures

Chemistry

General: Prior to use, CH_2Cl_2 was distilled from P_2O_5, MeOH was distilled, Dowex-H^+ was washed with water, methanol, ethanol, and 6 M HCl, NEt_3 was distilled over KOH, toluene was dried and stored over Na wire, and the following solvents were distilled from drum (Solvent Supplies): acetone, ethyl acetate, and petroleum ether. The following chemicals were used as received: $CDCl_3$ (Aldrich), CD_3OD (Roth), EtOH (Fisher), dried $MgSO_4$ (Pure Science), seasand purified by acid and calcined (Chem Solute), H_2SO_4 (Pancreac), Et_2O (LabServ), DMF extra dry over molecular sieves (Acros Organics), TBAI (Riedel-de Haën), (Riedel-de Haën), $NaHCO_3$ (Pure Science), HCl (Chem Solute), sephadex LH-20 (Sigma), Dowex-H^+ (Serva), cholesterol (chem impex), β-sitosterol (carbosynth), stigmasterol (carbosynth), iodine (vickers), hexamethyldisiloxane (fluka), glucose (sigma), myristic acid (BDH Biochem), stearic acid (BDH Biochem), behenic acid (BDH Biochem), TMSCl (Acros Organics), novozyme 435 (sigma). Vinyl myristate,[1] vinyl stearate,[1] and trehalose dibehenate (TDB)[2] were synthesised according to previously published procedures. Reactions were monitored by TLC analysis on Macherey-Nagel silica gel coated plastic sheets (0.20 mm with fluorescent indicator UV254) via detection by UV-absorption (254 nm) where relevant and dipping in 10% H_2SO_4 in EtOH or a solution of $KMnO_4$ (0.05 M), K_2CO_3 (0.4 M), and NaOH (0.06%) in water, followed by charring. Column chromatography was performed using Pure Science silica gel (40 – 63 microns). High resolution mass spectrometry (HRMS) was performed on a Waters Q-TOF PremierTM Tandem Mass Spectrometer using electro-spray ionisation (ESI) in positive mode. Nuclear magnetic resonance spectra were recorded in $CDCl_3$ or $CDCl_3$: CD_3OD (3:1) using a Varian INOVA operating at 500 MHz. Chemical shifts are given in ppm (δ) relative to the solvent residue peak. NMR peak assignments were made using COSY, heteronuclear single quantum coherence spectroscopy (HSQC), and HMBC 2D experiments. Optical rotations were recorded on an Autopol II (Rudolph Research Analytical) at 589 nm (sodium D-line).

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1. Swern, D.; Jordan, E.F.; Vinyl laurate and other vinyl esters. *Org. Synth.* **1950**, *30*, 106-107.
2. AA Khan, SH Chee, RJ McLaughlin, JL Harper, F Kamena, MSM Timmer, BL Stocker. 'Long chain lipids are required for innate immune recognition of trehalose diesters by macrophages.' *ChemBioChem* **2011**, *17,* 2572-2576.

1,2,3,4,6-Penta-*O*-trimethylsilyl-α-D-glucopyranose (9). In an oven-dried and argon-purged round-bottom flask, D-glucose (**8**, 1.0 g, 5.6 mmol, 1 equiv) was dissolved in dry DMF (25 mL) by heating the solvent to 50 °C. After cooling the flask to 0 °C, triethylamine (4.4 mL, 5.5 equiv) and chlorotrimethylsilane (4.0 mL, 5.5 equiv) were added sequentially and the resulting mixture turned white. The reaction mixture was left to stir for 4 hours at room temperature, after which time the reaction was deemed to have gone to completion, as determined by TLC analysis (10:90 EtOAc:PE, *v/v*). To the reaction mixture was added hexanes (100 mL) and crushed ice (50 mL) followed by 10 mL of saturated $NaHCO_3$ solution. The mixture was poured into a separating funnel and the aqueous layer was extracted with hexanes (3 x 25 mL). The organic fractions were combined, washed with water (2 x 25 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residual colorless oil was further purified via silica gel flash column chromatography, eluting with toluene, to give **9** (2.1 g, 3.8 mmol, 70%). R_f = 0.62 (1:20 EtOAc:PE, *v/v*); IR (film): 2956, 2916, 1388, 1153, 833 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.00 (d, 1H, *J* = 3.0 Hz), 3.77 (t, 1H, *J* = 8.9 Hz), 3.74-3.63 (m, 3H), 3.39 (t, 1H, *J* = 8.9 Hz), 3.35 (dd, 1H, *J* = 3.1, *J* = 9.1 Hz), 0.18-0.12 (m, 45H); ^{13}C NMR ($CDCl_3$): δ 93.9, 74.2, 74.0, 72.4, 72.2, 62.3, 1.3, 0.9, 0.4, 0.2, 0.2; HRMS calcd. for $[C_{21}H_{56}NO_6Si_5]^+$: 558.2948; obsd. 558.2940. Spectral data matched that previously reported.[3]

General procedure for the preparation of steroidal α-D-glucosides: The synthesis of the steryl α□-D-glucosides was undertaken according to the general procedures developed by Gervey-Hague.[4] To an oven-dried and argon-purged round-bottom flask containing 4 Å activated molecular sieves (300 mg), was added TBAI (140 mg, 0.38 mmol, 1.5 equiv), steroid (**11a-c**, 0.3 equiv) and anhydrous CH_2Cl_2 (2.5 mL). To this solution was added DIPEA (90 μL, 0.51 mmol, 2.0 equiv) and the resulting mixture was stirred for 30 min at room temperature. In a separate flame-dried and argon-purged round-bottom flask was added TMS-protected D-glucose (**9**, 140 mg, 0.25 mmol), which was azeotroped with anhydrous toluene (2 × 3 mL). To the resulting residue in anhydrous CH_2Cl_2 (2.5 mL) was added freshly prepared TMSI (40 μL, 0.28 mmol, 1.1 equiv) and the resulting solution was stirred for 20 min to give the corresponding glucosyl iodide (**10**). The *in situ* generated glucosyl iodide **10** was then transferred to the steroid-containing flask and the resulting solution allowed to stir for two days

3. Bhat, A.S.; Gervay-Hague, J. Efficient syntheses of β-cyanosugars using glycosyl iodides derived from per-*O*-silylated mono- and disaccharides. *Org. Lett.* **2001**, *3*, 2081-2084.
4. Davis, R. A.; Fettinger, J. C.; Gervay-Hague, J. 'Tandem Glycosyl Iodide Glycosylation and Regioselective Enzymatic Acylation Affords 6-*O*-Tetradecanoyl-α-D-cholesterylglycosides.' *J. Org. Chem.* **2014**, *79*, 8447−8452.

at room temperature. The reaction mixture was then filtered to remove the molecular sieves, with care taken to rinse the reaction vessel with MeOH:CH_2Cl_2 (1:1), and concentrated to *ca.* 5 mL under reduced pressure to yield the TMS-protected sterol-α-glucoside. To the reaction mixture was added Dowex 50WX8-200 acidic resin until a pH of 6 was obtained. After two hours, as no TMS-protected sugar was observed by TLC (20:80 EtOAc:PE, *v/v*), the solution was filtered and concentrated under reduced pressure to give a yellow solid. The product was purified via silica gel flash column chromatography (5:95–10:90 MeOH:CH_2Cl_2, *v/v*) and Sephadex LH-20 size exclusion column chromatography (1:1 MeOH:CH_2Cl_2, *v/v*) to give the corresponding steroidal α-D-glucoside.

Cholesteryl α-D-glucopyranoside (1). The title compound **1** was obtained as a white solid according to the general procedure for the preparation of steroidal α-D-glucosides (36.4 mg, 0.07 mmol, 80% yield). R_f = 0.26 (1:9 MeOH:CH_2Cl_2, *v/v*). $[\alpha]_D^{20}$ = +10 (*c* = 0.15, $CHCl_3$:MeOH, 1:1); IR (film): 3152, 3045, 2932, 1627, 1524, 1404, 1022 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$: CD_3OD [3:1]): δ 5.34−5.29 (m, 1H, H-5), 4.93 (d, 1H, $J_{1',2'}$ = 3.8 Hz, H-1α′), 3.78−3.72 (m, 2H, H-6a′, H-6b′), 3.66−3.59 (m, 2H, H-5′, H-3′), 3.50−3.42 (m, 1H, H-3), 3.40−3.33 (m, 2H, H-4′, H-2′), 2.37−2.29 (m, 2H, cholesterol), 2.01−1.75 (m, 5H, cholesterol), 1.59−0.81 (m, 33H, cholesterol), 0.65 (s, 3H, cholesterol); ^{13}C NMR (125 MHz, $CDCl_3$: CD_3OD [3:1]): δ 140.5 (C-6), 121.8 (C-5), 96.8 (C-1′), 77.5 (C-3), 73.8 (C-3′), 71.9 (C-2′), 71.8 (C-5′), 70.2 (C-4′), 61.4 (C-6′) 56.7, 55.9, 50.1, 42.2, 39.9, 39.7, 39.4, 36.8, 36.5, 36.0, 35.6, 31.7, 31.6, 28.0, 27.8, 27.4, 24.1, 23.6, 22.5, 22.2, 20.8, 19.1, 18.4, 11.6 (cholesterol); HRMS calcd. for $[C_{33}H_{60}NO_6]^+$: 566.4415; obsd. 566.4487. Spectral data matched that previously reported.[4,5]

Sitosteryl α-D-glucopyranoside (5a). The title compound **5a** was obtained as a white solid according to the general procedure for the preparation of steroidal α-D-glucosides (33.3 mg, 0.06 mmol, 78% yield). R_f= 0.26 (1:9 MeOH:CH_2Cl_2, *v/v*). $[\alpha]_D^{20}$ = +4.9 (*c* = 0.9, $CHCl_3$:MeOH, 1:1); IR (film): 3133, 3042, 2957, 2929, 1724, 1626, 1403, 1270, 1039 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$: CD_3OD [3:1]): δ 5.34−5.29 (m, 1H, H-5), 4.93 (d, 1H, $J_{1',2'}$ = 4.1 Hz, H-1α′), 3.76−3.72 (m, 2H, H-6a′, H-6b′), 3.66−3.59 (m, 2H, H-3′, H-5′), 3.47−3.42 (m, 1H, H-3), 3.37−3.32 (m, 2H, H-2′, H-4′), 2.01−1.77 (m, 2H, sitosterol), 2.01−1.75 (m, 5H, sitosterol),

5. Davis, R. A.; Lin, C.-H.; Gervay-Hague, J. 'Chemoenzymatic synthesis of cholesteryl-6-*O*-tetradecanoyl-α-D- glucopyranoside: a product of host cholesterol efflux promoted by *Helicobacter pylori*.' *Chem. Commun.,* **2012**, *48*, 9083-9085.

1.71−0.72 (m, 37H, sitosterol), 0.65 (s, 3H, sitosterol); ^{13}C NMR (125 MHz, $CDCl_3$: CD_3OD [3:1]): δ 140.9 (C-6), 122.0 (C-5), 96.8 (C-1′), 77.4 (C-3), 73.7 (C-3′), 71.9 (C-2′), 71.7 (C-5′), 70.2 (C-4′), 61.4 (C-6′) 55.9, 50.1, 45.7, 42.2, 39.9, 39.7, 36.9, 36.5, 36.0, 35.6, 31.7, 33.9, 31.7, 29.0, 28.1, 27.5, 26.0, 24.1, 23.0, 21.0, 19.5, 19.1, 18.7, 18.5, 11.6 (sitosterol); HRMS calcd. for $[C_{35}H_{64}NO_6]^+$: 594.4728; obsd. 594.4721.

Stigmasteryl α-D-glucoside (6a). The title compound **6a** was obtained as a white solid according to the general procedure for the preparation of steroidal α-D-glucosides (33.2 mg, 0.06 mmol, 78% yield). R_f= 0.26 (1:9 MeOH:CH_2Cl_2, *v*/*v*). $[\alpha]_D^{20}$ = +23 (*c* = 0.1, $CHCl_3$:MeOH, 1:1); IR (film): 3132, 3042, 1626, 1403, 1020 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$: CD_3OD [3:1]): δ 5.33−5.29 (m, 1H, H-5), 5.12 (m, 1H, H-22), 4.99 (m, 1H, H-23), 4.93 (d, 1H, $J_{1',2'}$ = 4.1 Hz, H-1α′), 3.79−3.70 (m, 2H, H-6a, H-6b′), 3.66−3.59 (m, 2H, H-3′, H-5′), 3.50−3.41 (m, 1H, H-3), 3.39−3.32 (m, 2H, H-2′, H-4′), 2.37−2.30 (m, 2H, stigmasterol), 2.05−1.81 (m, 5H, stigmasterol), 1.72−0.72 (m, 33H, stigmasterol), 0.65 (s, 3H, stigmasterol); ^{13}C NMR [125 MHz, $CDCl_3$: CD_3OD (3:1)]: δ 140.5 (C-6), 138.5, (C-22), 129.2 (C-23), 122.0 (C-5), 96.8 (C-1), 77.4 (C-3), 73.7 (C-3′), 71.9 (C-2′), 71.7 (C-5′), 70.2 (C-4′), 61.4 (C-6′) 56.9, 55.9, 50.1, 50.0, 42.2, 40.4, 39.9, 39.5, 36.9, 36.5, 31.7, 28.1, 27.5, 26.0, 25.3, 24.2, 21.0, 20.7, 19.1, 11.9, 11.6 (stigmasterol); HRMS calcd. for $[C_{35}H_{62}NO_6]^+$: 592.4572; obsd. 592.4566.

*General procedure for the enzyme catalyzed formation of steryl 6-*O*-acyl-glucosides.* The indicated amount of steryl glycoside (ca. 0.01 mmol) was added to an oven-dried screw cap vial containing anhydrous acetone (0.5 mL) and novozym 435 (1 mg / 0.001 mmol glycoside). Vinyl myristate (6.0 equiv.) was then added to the vial, and the reaction mixture was left to stir on a rotatory evaporator at 50 °C for 3 hours. Following completion of the reaction, as determined by TLC analysis (R_f = 0.35, CH_2Cl_2:MeOH, 90:10, *v*/*v*), the solution was decanted into a round-bottom flask and the vial was rinsed with CH_2Cl_2:MeOH (1:1, *v*/*v* , 3 x 0.5 mL). The combined extracts were concentrated under reduced pressure to give an off-white solid, which was purified by silica gel flash column chromatography (CH_2Cl_2 → CH_2Cl_2:MeOH, 95:5, *v*/*v*) and size exclusion column chromatography on Sephadex LH-20 (CH_2Cl_2:MeOH, 1:1, *v*/*v*).

Cholesteryl 6-*O*-tetradecanoyl-α-D-glucopyranoside (2a). Compound **1** (5.0 mg, 0.010 mmol) was subjected to the general procedure for the enzyme catalyzed formation of steryl 6-

O-acyl-glucosides to give ester **2a** (6.8 mg, 0.009 mmol, 90%) as an off-white solid. R_f = 0.35 (CH_2Cl_2:MeOH, 90:10, *v/v*); $[\alpha]_D^{20}$ = +5 (*c* = 0.14, $CHCl_3$); IR (film): 3312, 3149, 2954, 2919, 2850, 1626, 1521, 1048 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 5.38−5.33 (m, 1H, H-5), 5.02 (d, 1H, $J_{1',2'}$ = 4.1 Hz, H-1α′), 4.47 (dd, 1H, $J_{5',6a'}$ = 4.9, $J_{6a',6b'}$ = 11.9 Hz, H-6a′), 4.25 (d, 1H, $J_{6a',6b'}$ = 11.8 Hz, H-6b′), 3.88−3.83 (m, 1H, H-5′), 3.72 (t, 1H, $J_{2',3'} = J_{3',4'}$ = 9.5 Hz, H-3′), 3.53−3.43 (m, 2H, H-2′, H-3), 3.36−3.29 (m. 1H, H-4′), 2.06−1.77 (m, 5H, lipid, cholesterol), 1.71−0.65 (m, 61H, lipid, cholesterol); ^{13}C NMR (125 MHz, $CDCl_3$): δ 175.0 (C-1″), 140.4 (C-6), 122.4 (C-5), 96.7 (C-1′), 78.7 (C-3), 74.6 (C-3′), 72.2 (C-2′), 70.0 (C-5′), 69.7 (C-4′), 63.1 (C-6′), 56.7, 56.2, 50.1, 42.4, 40.2, 39.7, 39.6, 37.0, 36.6, 36.2, 35.8, 34.2, 32.0, 29.68, 29.62, 29.4, 29.3, 29.3, 28.2, 28.0, 25.0, 24.6, 23.4, 22.9, 22.7, 21.1, 19.4, 18.7, 14.1, 11.9 (lipid, cholesterol); HRMS calcd. for $[C_{47}H_{86}NO_7]^+$: 776.6399; obsd. 776.6408. Spectral data matched that previously reported.[18,19]

Sitosteryl 6-*O*-tetradecanoyl-α-D-glucopyranoside (5b). Compound **5a** (10 mg, 0.017 mmol) was subjected to the general procedure for the enzyme catalyzed formation of steryl 6-*O*-acyl-glucosides to give ester **5b** (11.3 mg, 0.015 mmol, 86%) as an off-white solid. R_f = 0.35 (CH_2Cl_2:MeOH, 90:10, *v/v*); $[\alpha]_D^{20}$ = +19 (*c* = 0.55, $CHCl_3$); IR (film): 3361, 2955, 2923, 2852, 1628, 1380, 1107 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 5.36−5.34 (m, 1H, H-5), 5.01 (d, 1H, $J_{1',2'}$ = 4.1 Hz, H-1α′), 4.49 (dd, 1H, $J_{5',6a'}$ = 5.2, $J_{6a',6b'}$ = 11.4 Hz, H-6a′), 4.25 (d, 1H, $J_{6a',6b'}$ = 11.4 Hz, H-6b′), 3.85−3.78 (m, 1H, H-5′), 3.43 (t, 1H, $J_{2',3'} = J_{3',4'}$ = 9.5 Hz, H-3′), 3.51−3.41 (m, 2H, H-2′, H-3), 3.32−3.25 (m, 1H, H-4′), 2.12−1.80 (m, 5H, lipid, sitosterol), 1.71−0.65 (m, 65H, lipid, sitosterol); ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.8 (C=O), 140.4 (C-6), 122.4 (C-5), 96.9 (C-1′), 78.3 (C-3), 74.6 (C-3′), 72.2 (C-2′), 70.2 (C-5′), 69.7 (C-4′), 63.1 (C-6′), 56.7, 56.2, 50.2, 45.8, 42.3, 40.2, 39.7, 37.0, 36.6, 36.2, 34.2, 33.9, 32.0, 29.68, 29.62, 29.4, 29.36, 29.33, 28.2, 28.0, 25.0, 24.3, 23.1, 22.99, 20.92, 19.8, 19.4, 18.7, 14.1, 11.9 (lipid, sitosterol); HRMS calcd. for $[C_{49}H_{90}NO_7]^+$: 804.6712; obsd. 804.6721.

Stigmasteryl 6-*O*-tetradecanoyl-α-D-glucopyranoside (6b). Compound **6a** (8 mg, 0.014 mmol) was subjected to the general procedure for the enzyme catalyzed formation of steryl 6-*O*-acyl-glucosides to give ester **6b** (10.0 mg, 0.013 mmol, 91%) as an off-white solid. R_f = 0.35 (CH_2Cl_2:MeOH, 90:10, *v/v*); $[\alpha]_D^{20}$ = +10 (*c* = 0.15, $CHCl_3$); IR (film): 3357, 3149, 2955, 2923, 2852, 1632, 1523, 1023 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 5.37−5.34 (m, 1H, H-5), 5.15 (dd, 1H, $J_{21,22}$ = 8.9, $J_{22,23}$ = 15.2 Hz, H-22), 5.05−4.99 (m, 2H, H-1α′, H-23), 4.47 (dd, 1H,

$J_{5',6a'}$ = 4.6, $J_{6a',6b'}$ = 12.1 Hz, H-6a′), 4.25 (dd, 1H, $J_{5',6b'}$ = 1.4, $J_{6a',6b'}$ = 12.1 Hz, H-6b′), 3.89−3.84 (m, 1H, H-5′), 3.72 (t, 1H, $J_{2',3'}$= $J_{3',4'}$ = 9.5 Hz, H-3′), 3.53−3.43 (m, 2H, H-2′, H-3), 3.36−3.29 (m, 1H, H-4′), 2.39−2.31 (m, 4H, stigmasterol), 2.08−0.66 (m, 61H, lipid, stigmasterol); ^{13}C NMR (125 MHZ, $CDCl_3$): δ 176.1 (C=O), 140.4 (C-6), 138.3 (C-22), 129.3 (C-23), 122.3 (C-5), 96.7 (C-1′), 78.4 (C-3), 74.6 (C-3′), 72.2 (C-2′), 70.0 (C-5′), 69.7 (C-4′), 63.2 (C-6′), 56.7, 56.2, 51.2, 50.1, 42.2, 40.6, 40.0, 39.7, 39.6, 37.0, 36.6, 35.8, 34.3, 32.0, 29.67, 29.64, 29.4, 29.38, 29.35, 28.2, 28.0, 25.3, 24.8, 24.2, 22.9, 22.7, 22.6, 21.1, 19.4, 18.7, 14.1, 12.2, 11.9 (lipid, stigmasterol); HRMS calcd. for $[C_{49}H_{88}NO_7]^+$: 802.6555; obsd. 802.6544.

Cholesteryl 6-*O*-octadecanol-α-D-glucopyranoside (2c). To a solution of **1a** (6.0 mg, 0.011 mmol) in anhydrous acetone (0.5 mL) in an oven-dried screw cap vial was added novozym 435 (11 mg on solid support) and vinyl stearate (6.0 equiv). The reaction mixture was left to stir on a rotary evaporator at 50 °C for 3 hours. Following reaction completion (as determined by TLC analysis R_f = 0.41 [15:85 MeOH:CH_2Cl_2, *v/v*]), the solution was then decanted into a round-bottom flask, taking care to rinse the vial with MeOH:CH_2Cl_2 (1:1, 3 x 0.5 mL), and concentrated under reduced pressure to give an off-white solid. The residue obtained was purified by silica gel flash column chromatography (0:100 - 5:95 MeOH:CH_2Cl_2, *v/v*) followed by Sephadex LH-20 size exclusion column chromatography (1:1 MeOH:CH_2Cl_2, *v/v*) to give the title compound as an off-white solid (8.9 mg, 0.009 mmol, 80%). R_f = 0.41 (10:90 MeOH:CH_2Cl_2, *v/v*). $[\alpha]_D^{20}$ = +3 (*c* = 0.33, $CHCl_3$); IR (film): 3362, 2954, 2916, 2870, 2849, 1627, 1524, 1027 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.38−5.33 (m, 1H, H-5), 5.02 (d, 1H, $J_{1',2'}$ = 4.1 Hz, H-1α′), 4.47 (dd, 1H, $J_{5',6a'}$ = 4.9, $J_{6a',6b'}$ = 11.8 Hz, H-6a′), 4.25 (dd, 1H, $J_{5',6b'}$ = 1.4, *J* = 11.8 Hz, H-6b′), 3.88−3.83 (m, 1H, H-5′), 3.72 (t, 1H, $J_{2',3'}$ = $J_{3',4'}$ = 9.5 Hz, H-3′), 3.53−3.43 (m, 2H, H-2′, H-3), 3.36−3.29 (m, 1H, H-4′), 2.06−1.77 (m, 5H, lipid, cholesterol), 1.71−0.65 (m, 69H, lipid, cholesterol); ^{13}C NMR (125 MHZ, $CDCl_3$): δ 174.7 (C=O), 140.2 (C-6), 122.3 (C-5), 96.8 (C-1′), 78.5 (C-3), 74.6 (C-3′), 72.1 (C-2′), 70.0 (C-5′), 69.9 (C-4′), 63.1 (C-6′), 56.7, 56.2, 50.1, 42.3, 40.2, 39.7, 39.5, 37.0, 36.6, 36.2, 35.7, 34.2, 32.0, 29.7, 29.6, 29.4, 29.3, 29.2, 28.2, 28.0, 25.0, 24.3, 23.40, 22.9, 22.7, 22.0, 21.1, 19.3, 18.7, 14.2, 11.9 (lipid, cholesterol); HRMS calcd. for $[C_{51}H_{94}NO_7]^+$: 832.7025; obsd. 832.6990.

Cholesteryl β-D-glucopyranoside (7). To a round-bottom flask containing anhydrous dichloromethane (10 mL) was added trichloroacetimidate **13** (200 mg, 0.27 mmol, 1 equiv),

cholesterol (**11a**, 135 mg, 0.35 mmol, 1.3 equiv) and 4 Å molecular sieves. The mixture was stirred under an atmosphere of argon for 1 hour after which time it was cooled to 0 °C and TMSOTf (17 μL, 0.1 mmol) was added. The reaction was stirred for 30 minutes at 0 °C, warmed to room temperature, and left to stir overnight. The reaction was quenched with triethylamine, extracted with CH_2Cl_2, and the crude product was deprotected using NaOMe in MeOH (4 mL, 0.5 M). Following neutralization with Dowex-H^+ resin, the reaction mixture was concentrated under reduced pressure and the resulting residue was purified via silica-gel flash column chromatography (5:95–10:90 MeOH:CH_2Cl_2, *v/v*) to give **7a** as a white solid (45.8 mg, 0.08 mmol, 31% yield over two steps). R_f = 0.26 (1:9 MeOH:CH_2Cl_2, *v/v*). $[\alpha]_D^{20}$ = -35 (*c* = 0.20, $CHCl_3$:MeOH, 1:1); IR (film): 3144, 3047, 2931, 2867, 1631, 1405, 1016 cm^{-1}; ^1H NMR (500 MHZ, $CDCl_3$: CD_3OD [3:1]): δ 5.35−5.31 (m, 1H, H-5), 4.37 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1β′), 3.81 (dd, 1H, $J_{5',6a'}$ = 3.1 Hz, $J_{6a',6b'}$ = 12.0 Hz, H6-a′), 3.71 (dd, 1H, $J_{5',6b'}$ = 4.4 Hz, $J_{6a',6b'}$ = 12.0 Hz, H6-b′), 3.59−3.51 (m, 1H, H-3), 3.42−3.35 (m, 2H, H-4′, H-3′), 3.28−3.22 (m, 1H, H-5′) 3.36 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 8.3 Hz, H-2′), 2.40−2.33 (m, 1H, cholesterol), 2.23 (t, 1H, *J* = 12.4 Hz, cholesterol), 2.03−1.75 (m, 5H, cholesterol), 1.60−0.81 (m, 33H, cholesterol), 0.64 (s, 3H, cholesterol); ^{13}C NMR (125 MHz, $CDCl_3$: CD_3OD [3:1]): δ 140.2 (C-6), 121.7 (C-5), 101.0 (C-1′), 78.9 (C-3), 76.3 (C-3′), 75.7 (C-2′), 73.4 (C-5′), 70.1 (C-4′), 61.8 (C-6′), 56.7, 56.0, 50.1, 42.2, 39.7, 39.4, 38.7, 37.2, 36.6, 36.1, 35.7, 31.8, 29.5, 28.2, 27.9, 24.1, 23.6, 22.5, 22.2, 20.9, 19.1, 18.4, 11.7 (cholesterol); HRMS calcd. for $[C_{33}H_{60}NO_6]^+$: 566.4415; obsd. 566.4495.

Immunology Materials and Methods

Human Monocytes

The use of human leukocyte from healthy donors with written informed consent was approved by New Zealand Northern A Health and Disability Ethics Committee (approval number 15/NTA/178), and all experiments were performed in compliance with Victoria University of Wellington's policy on animal use and ethics. Human monocytes were negatively selected using RosettaSep Human Monocyte Enrichment Cocktail (StemCell) from whole blood according to manufacturer's protocol. Cell concentration was adjusted to 1 x 10^6 cell/mL in complete RMPI (10% FCS, 1% PenStrep) and 200 μL of cells were added per well in a 96-well plate. Monocytes were stimulated with 40 or 80 μM of ligand, TDB was used as a positive control. At 24h, supernatants were collected and IL-1β, TNFα, and IL-8 production was measured using ELISA (BD and R&D Systems).

2B4-NFAT-GFP reporter cells

2B4-NFAT-GFP reporter cells expressing mMincle + FcRγ, hMincle + FcRγ, h-Mincle (R135L) + FcRγ (CRAC – mutant), h-Mincle (EPN-QPD) + FcRγ (CRD – mutant) or FcRγ only have been previously described.[1, 2] Ligands (0.1 or 1 nmol/well) were coated on 96-well plates and 0.4 x 10^6 cells/mL NFAT-GFP 2B4 reporter cells were added and incubated at 37 °C, 5% CO_2. After 20 hours the cells were harvested, stained with DAPI, and analysed for NFAT-GFP expression using flow cytometry (FACS Calibur).

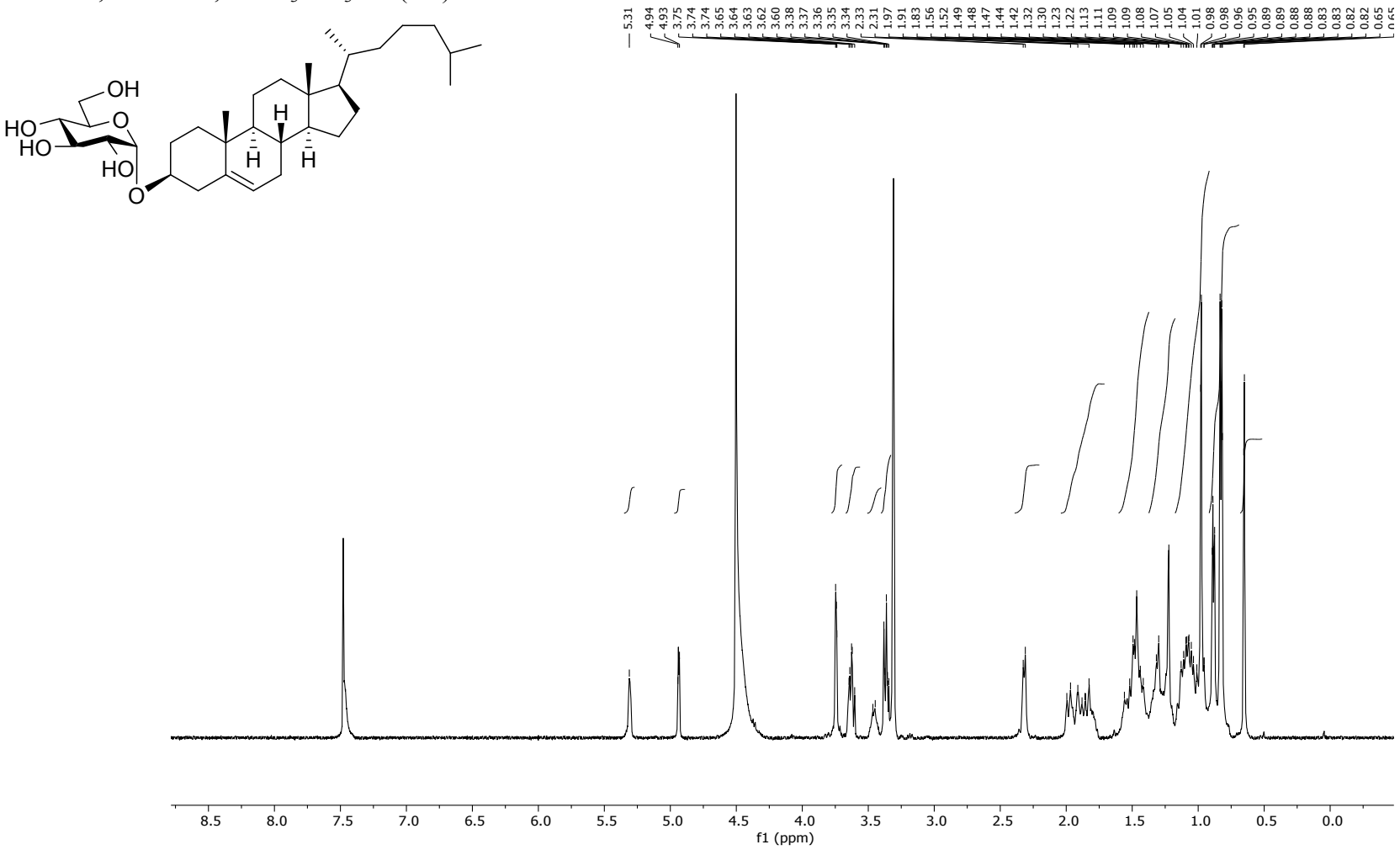
Statistics

Statistical significance of differences was assessed using 2-way ANOVA with Sidak's multiple comparisons test using Prism v7 software (GraphPad). *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001.

NMR Spectra

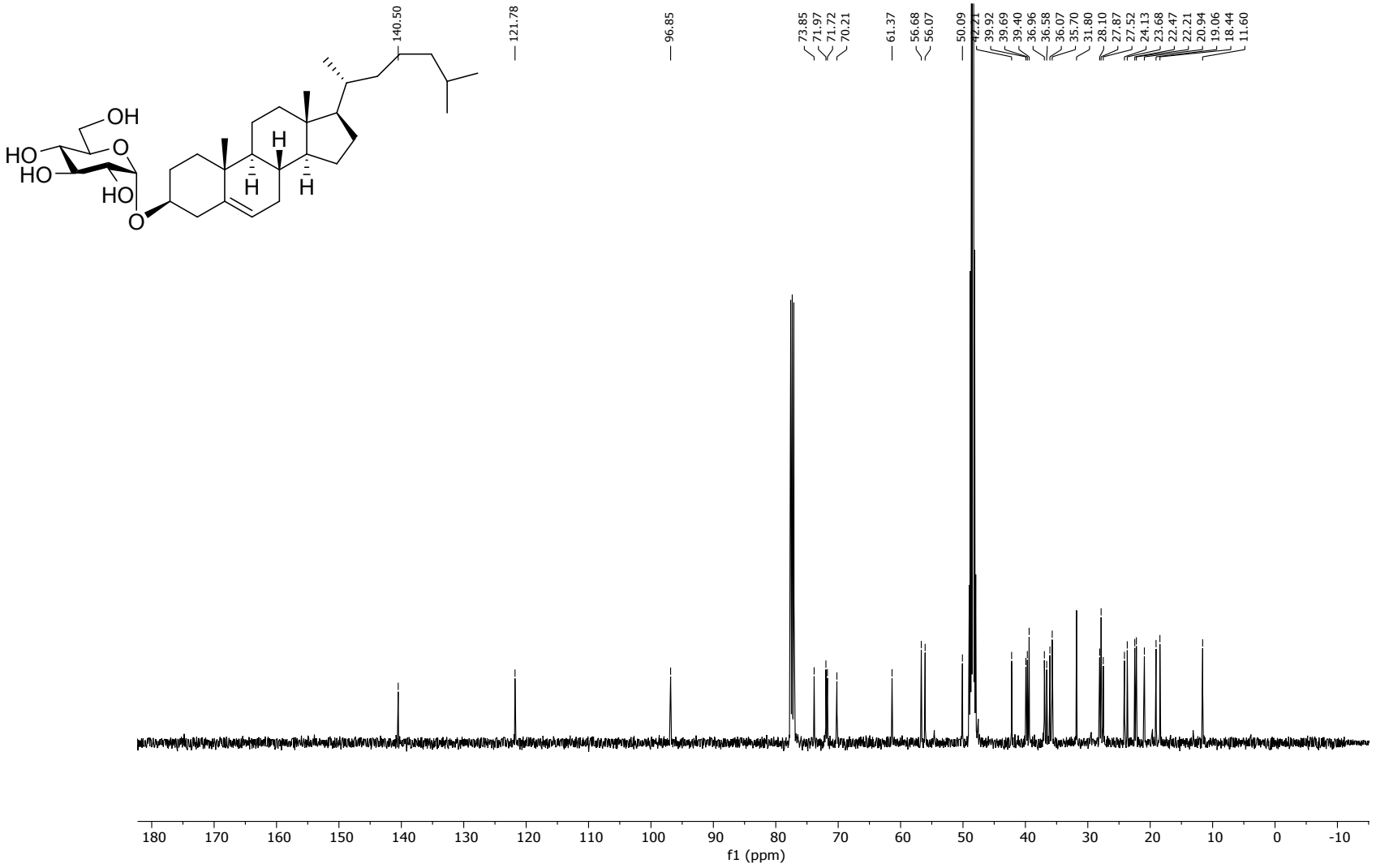
Cholesteryl α-D-glucopyranoside (1)

^{1}H-NMR, 500 MHz, $CDCl_3$:CD_3OD (1:3)



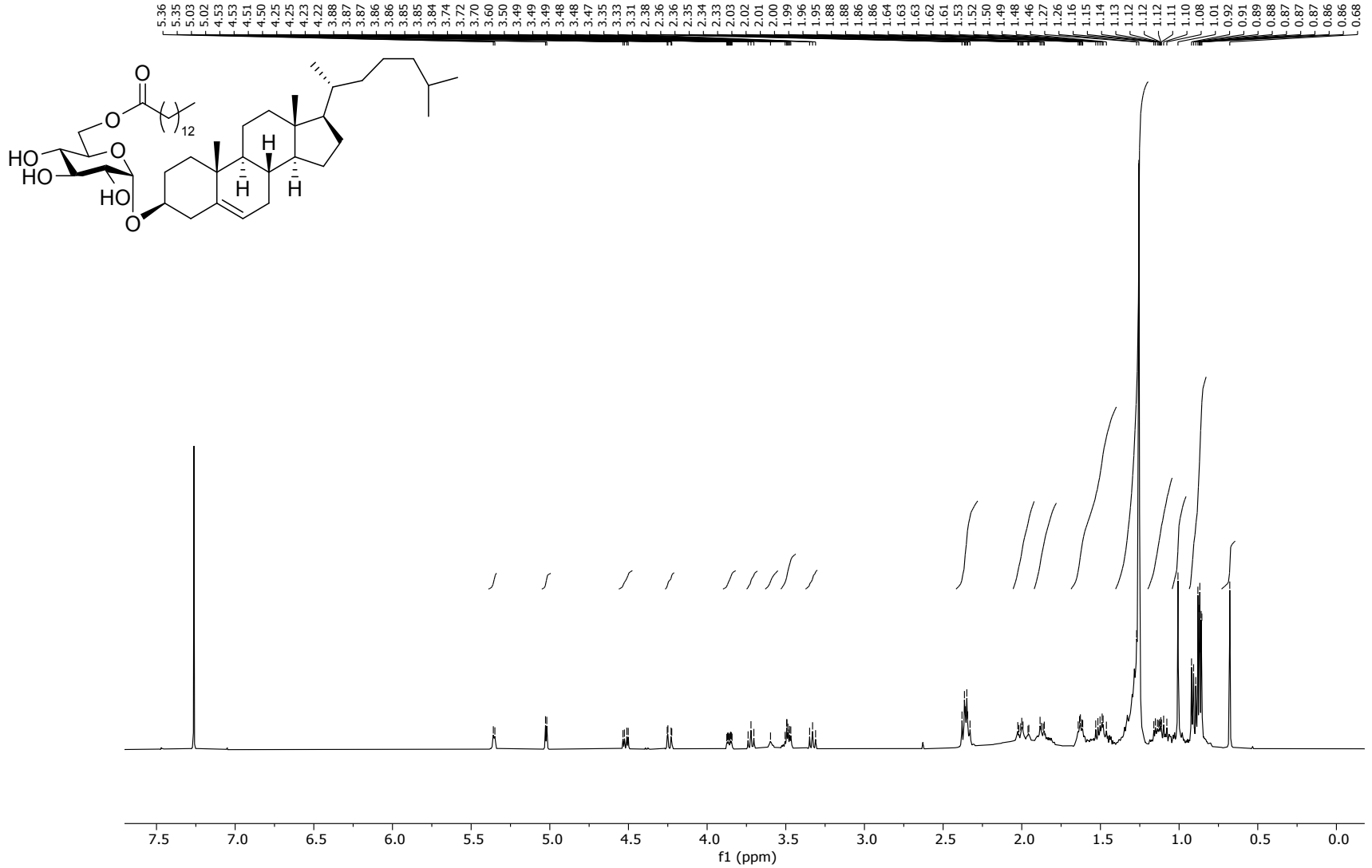
Cholesteryl α-D-glucopyranoside (1)

^{13}C-NMR, 125 MHz, $CDCl_3$:CD_3OD (1:3)



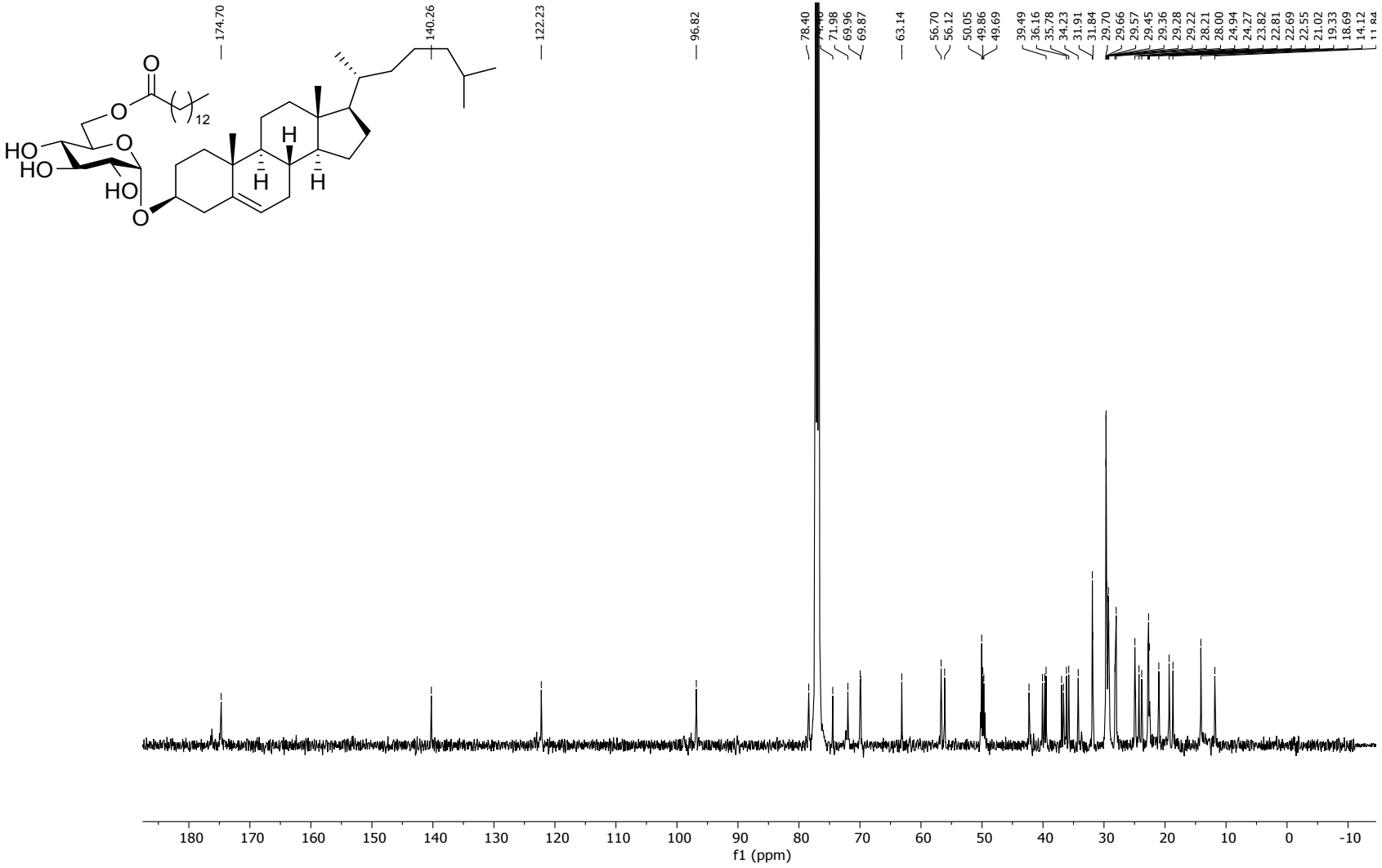
Cholesteryl 6-O-tetradecanoyl-α-D-glucopyranoside (2a)

^{1}H-NMR, 500 MHz, $CDCl_3$



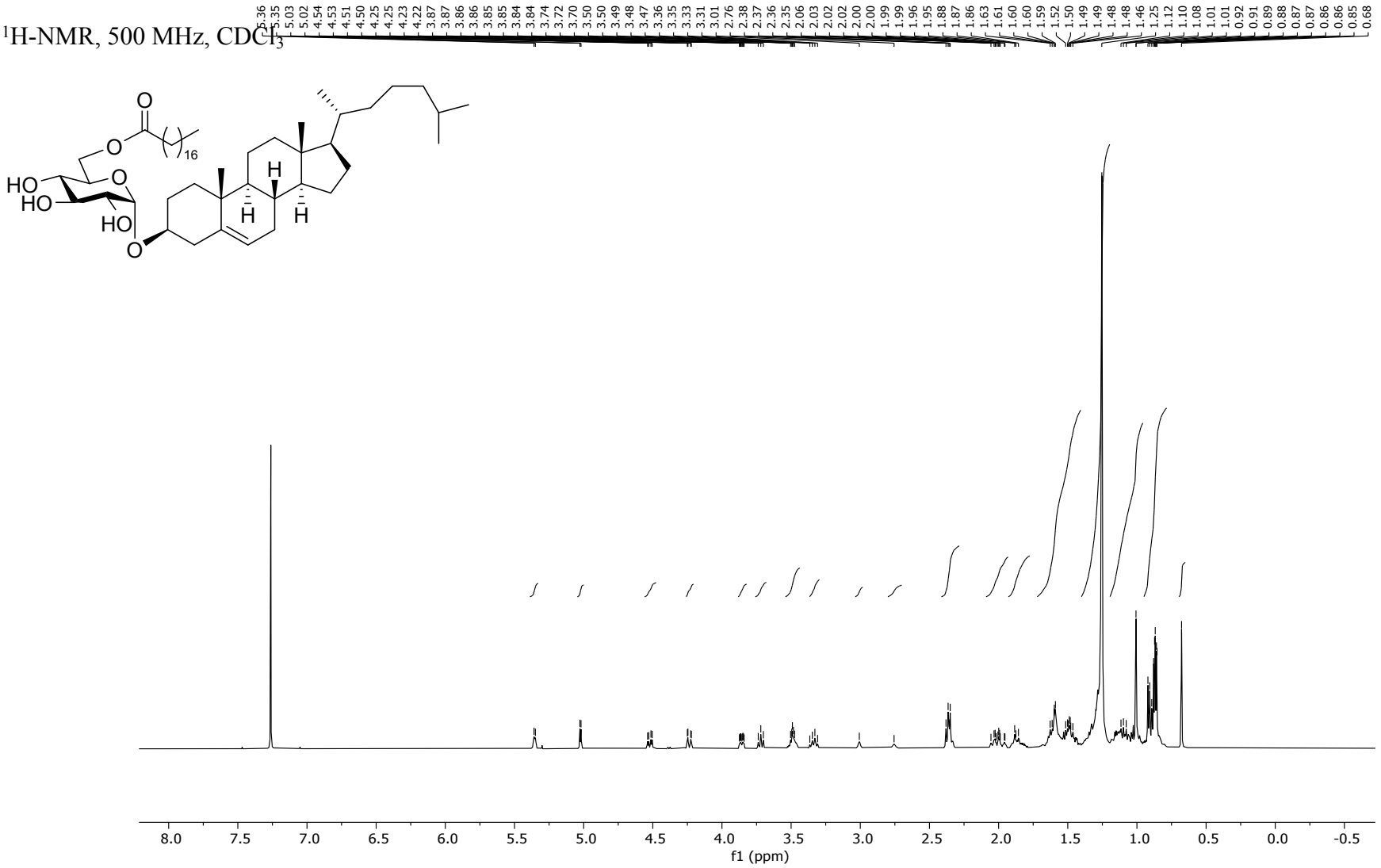
Cholesteryl 6-*O*-tetradecanoyl-α-D-glucopyranoside (2a)

^{13}C-NMR, 125 MHz, $CDCl_3$



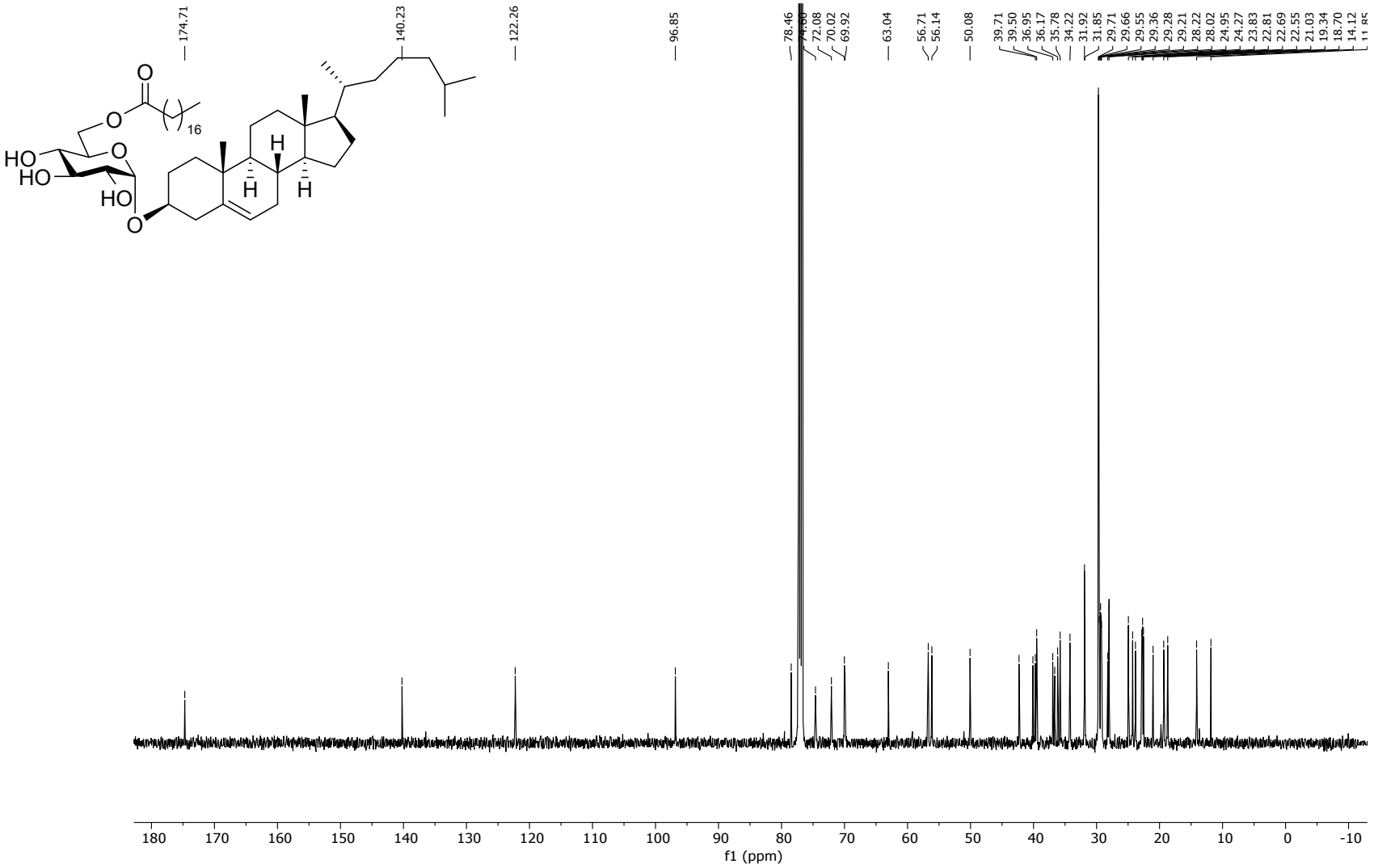
Cholesteryl 6-*O*-octadecanoyl-α-D-glucopyranoside (2c)

^{1}H-NMR, 500 MHz, $CDCl_3$



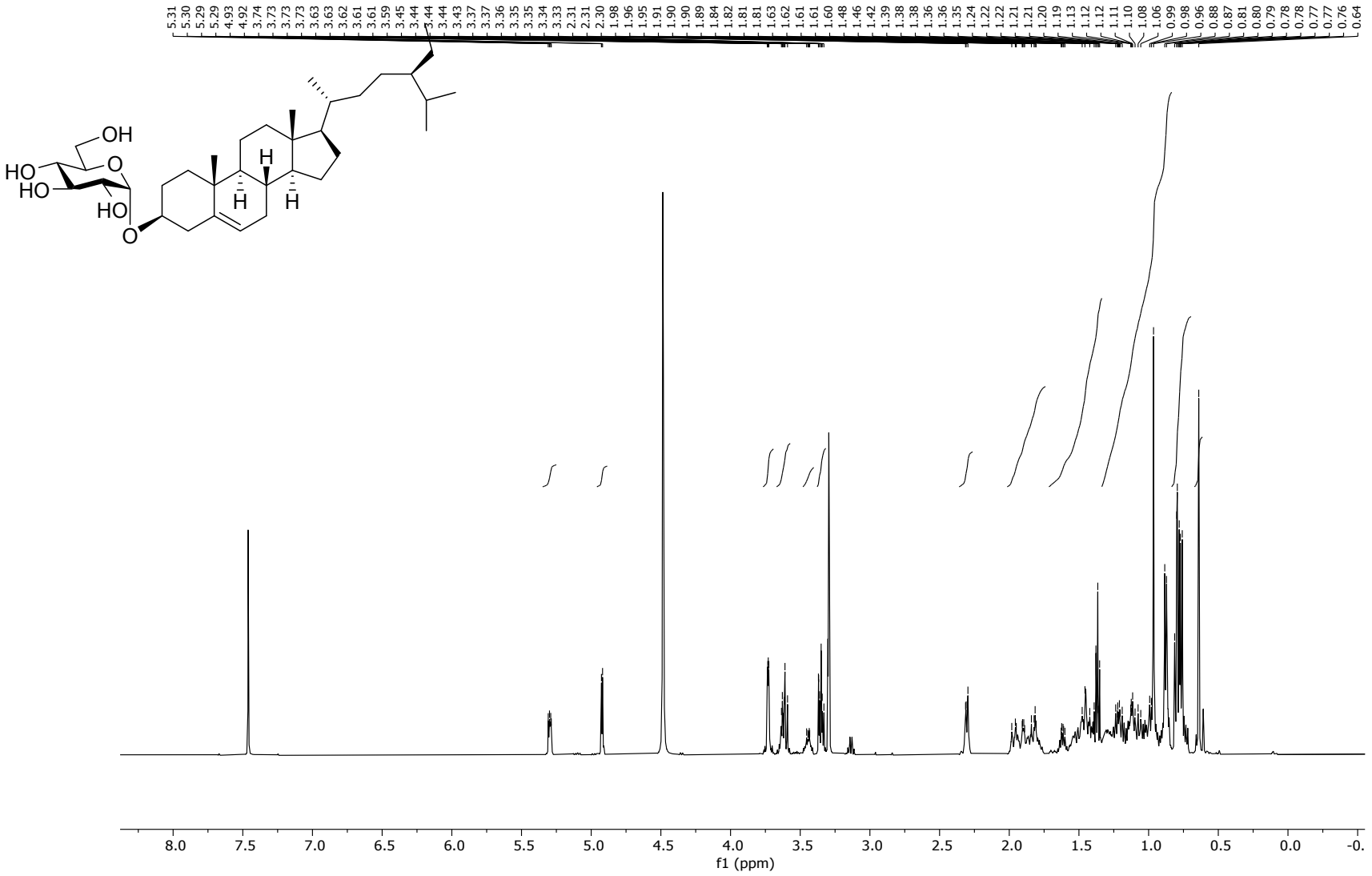
Cholesteryl 6-*O*-octadecanoyl-α-D-glucopyranoside (2c)

^{13}C-NMR, 125 MHz, $CDCl_3$



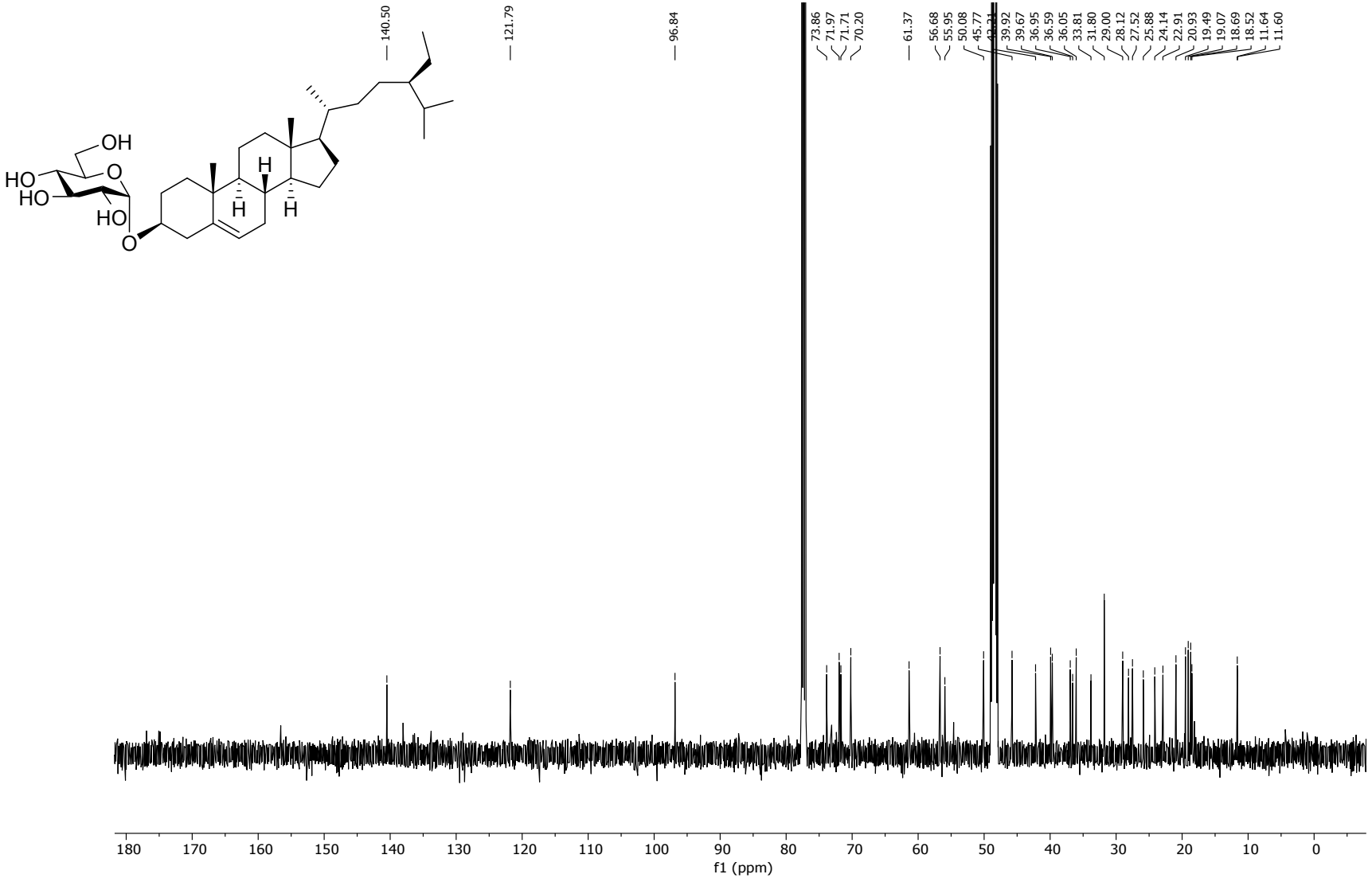
Sitosteryl α-D-glucopyranoside (5a)

^{1}H-NMR, 500 MHz, $CDCl_3$:CD_3OD (1:3)



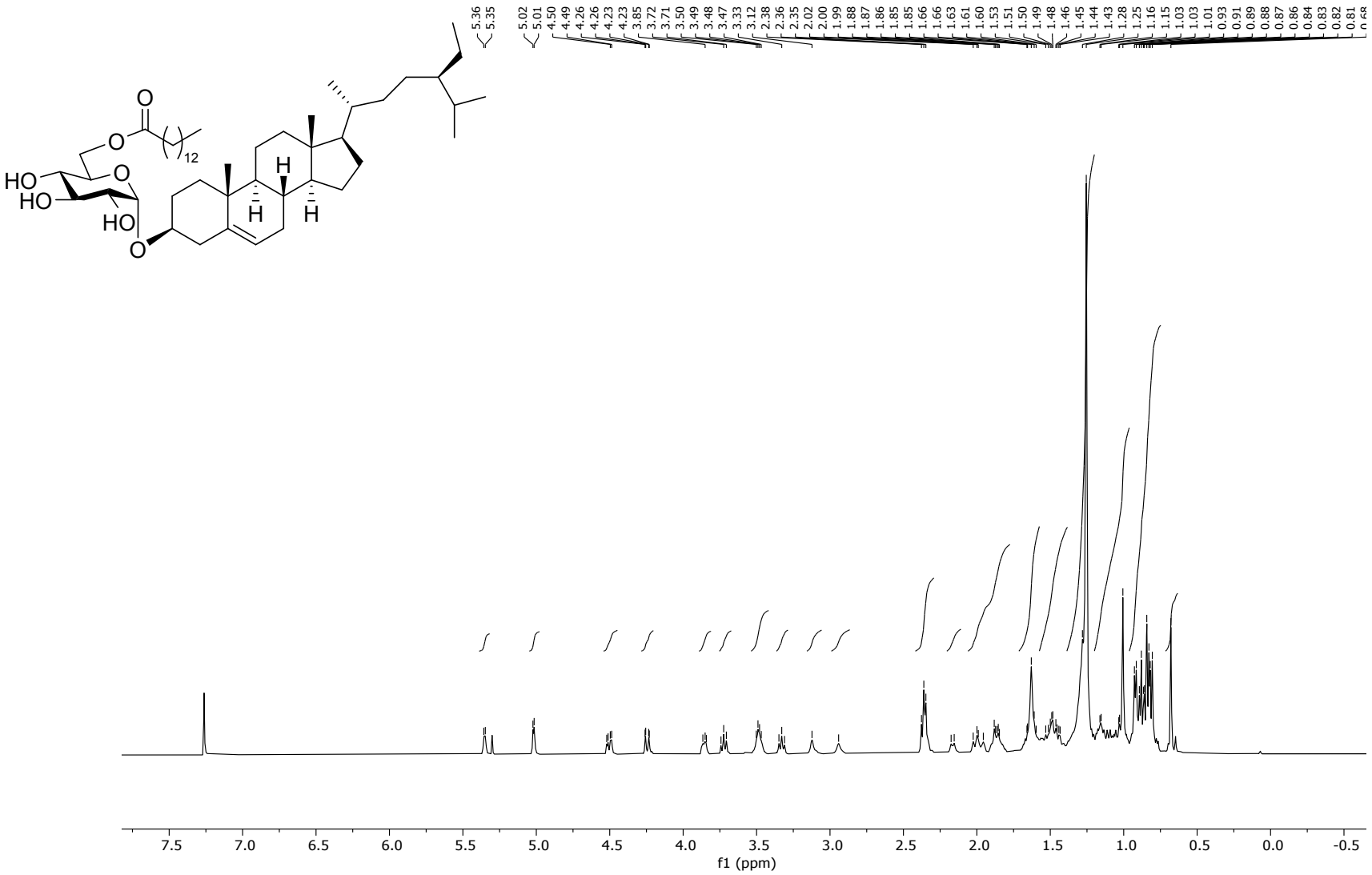
Sitosteryl α-D-glucopyranoside (5a)

^{13}C-NMR, 125 MHz, $CDCl_3$:CD_3OD (1:3)



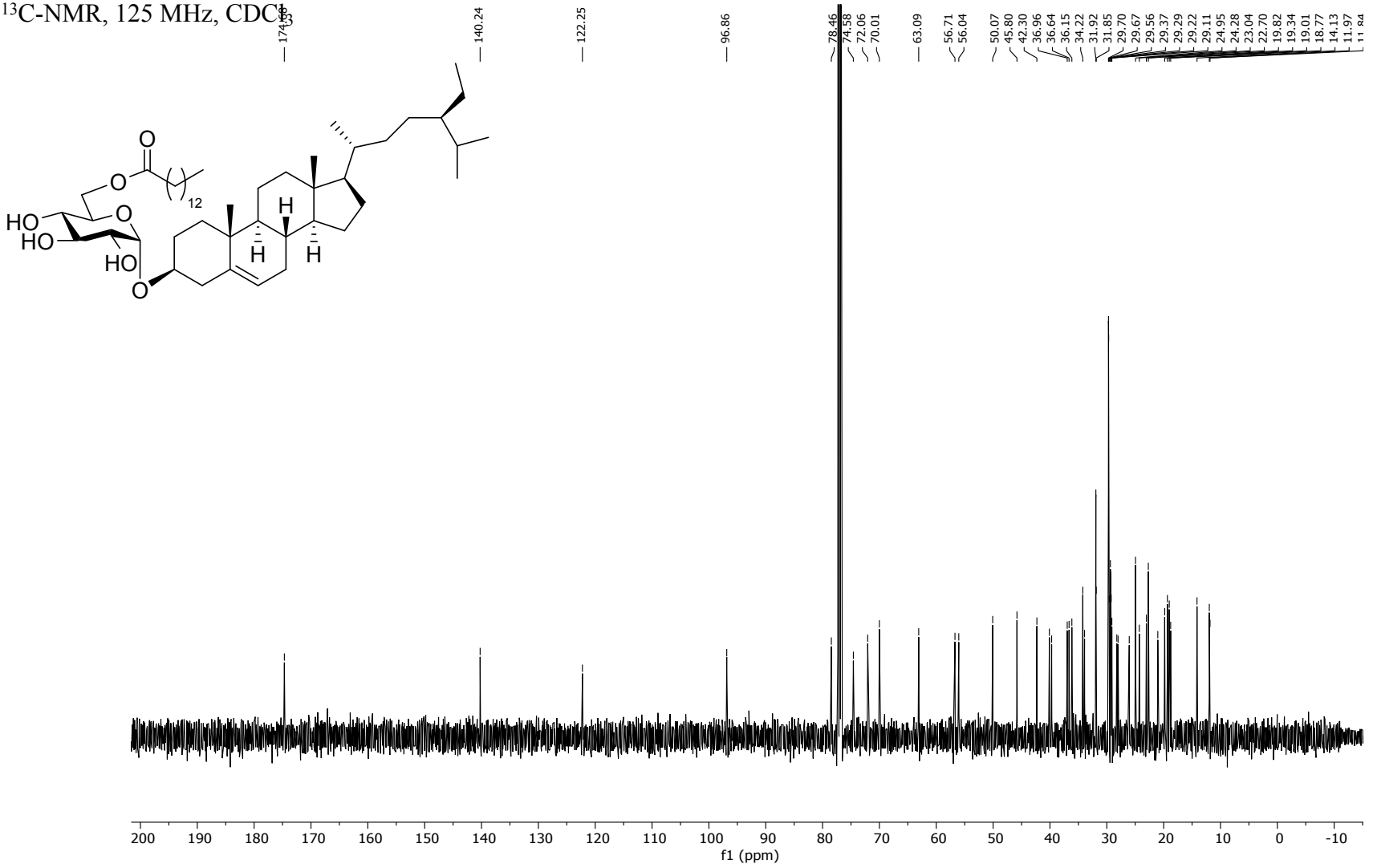
Sitosteryl 6-*O*-tetradecanoyl-α-D-glucopyranoside (5b)

^{1}H-NMR, 500 MHz, $CDCl_3$



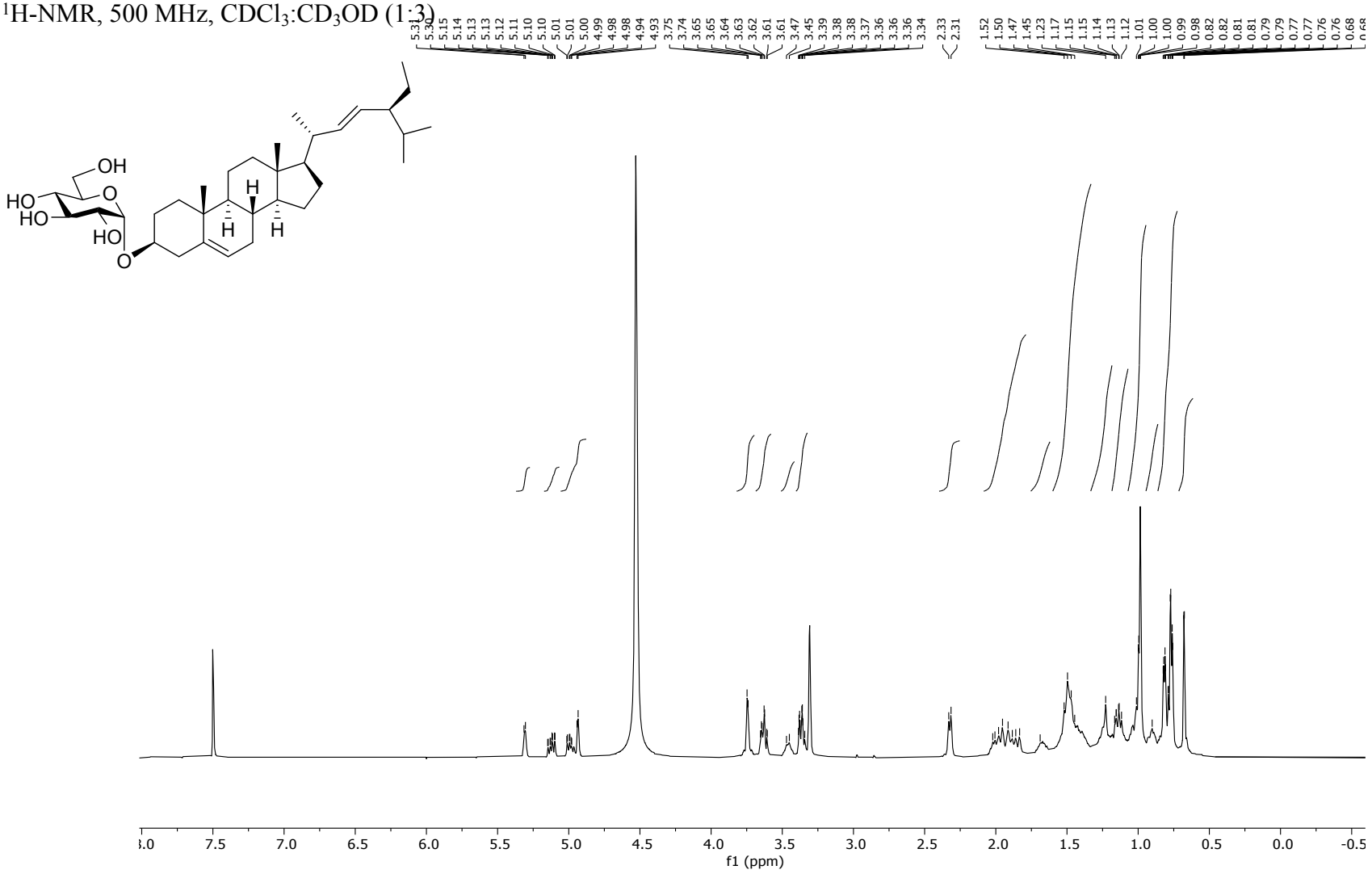
Sitosteryl 6-*O*-tetradecanoyl-α-D-glucopyranoside (5b)

^{13}C-NMR, 125 MHz, CDCl$_3$



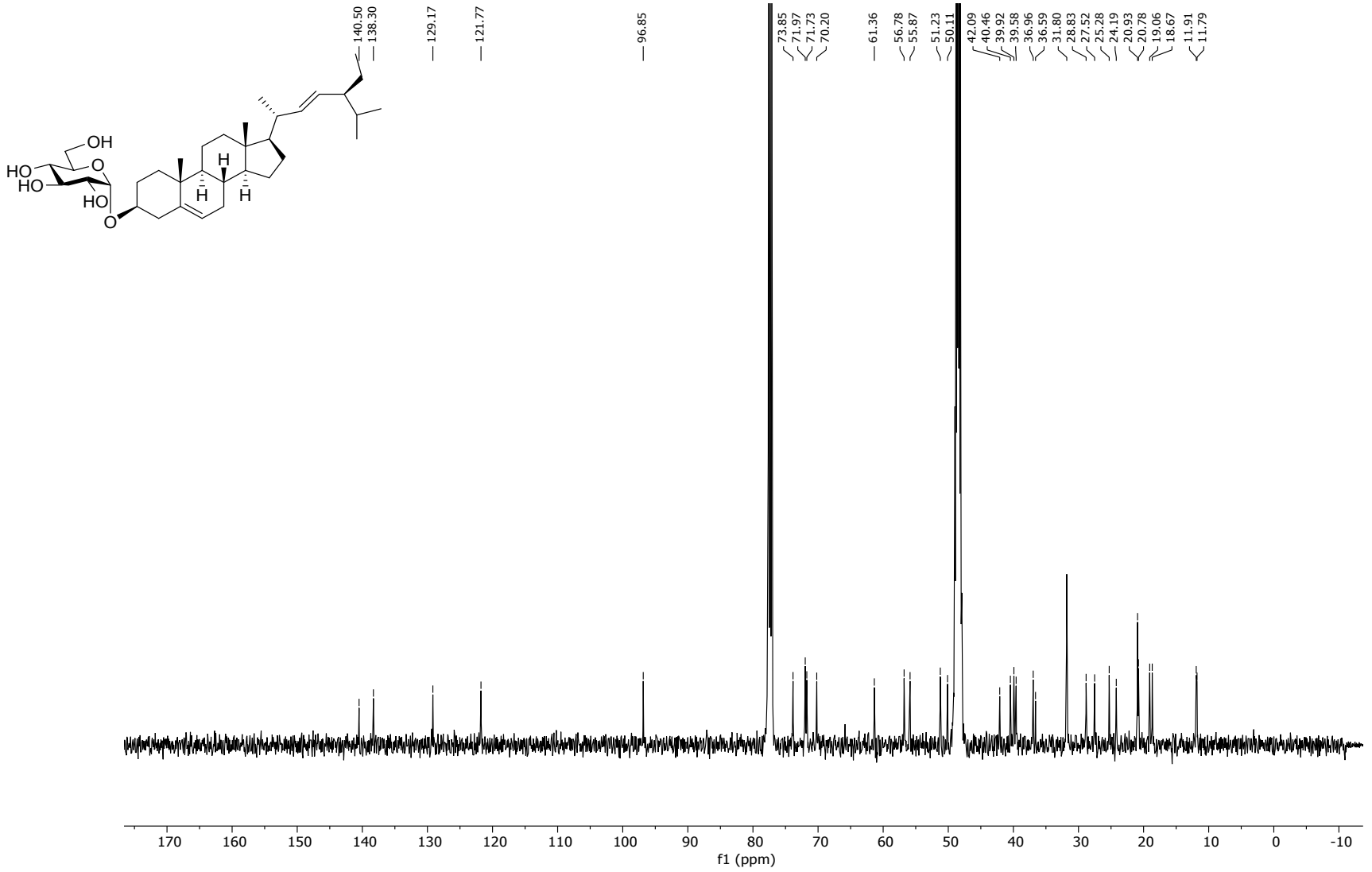
Stigmasteryl α-D-glucoside (6a)

^{1}H-NMR, 500 MHz, $CDCl_3$:CD_3OD (1:3)



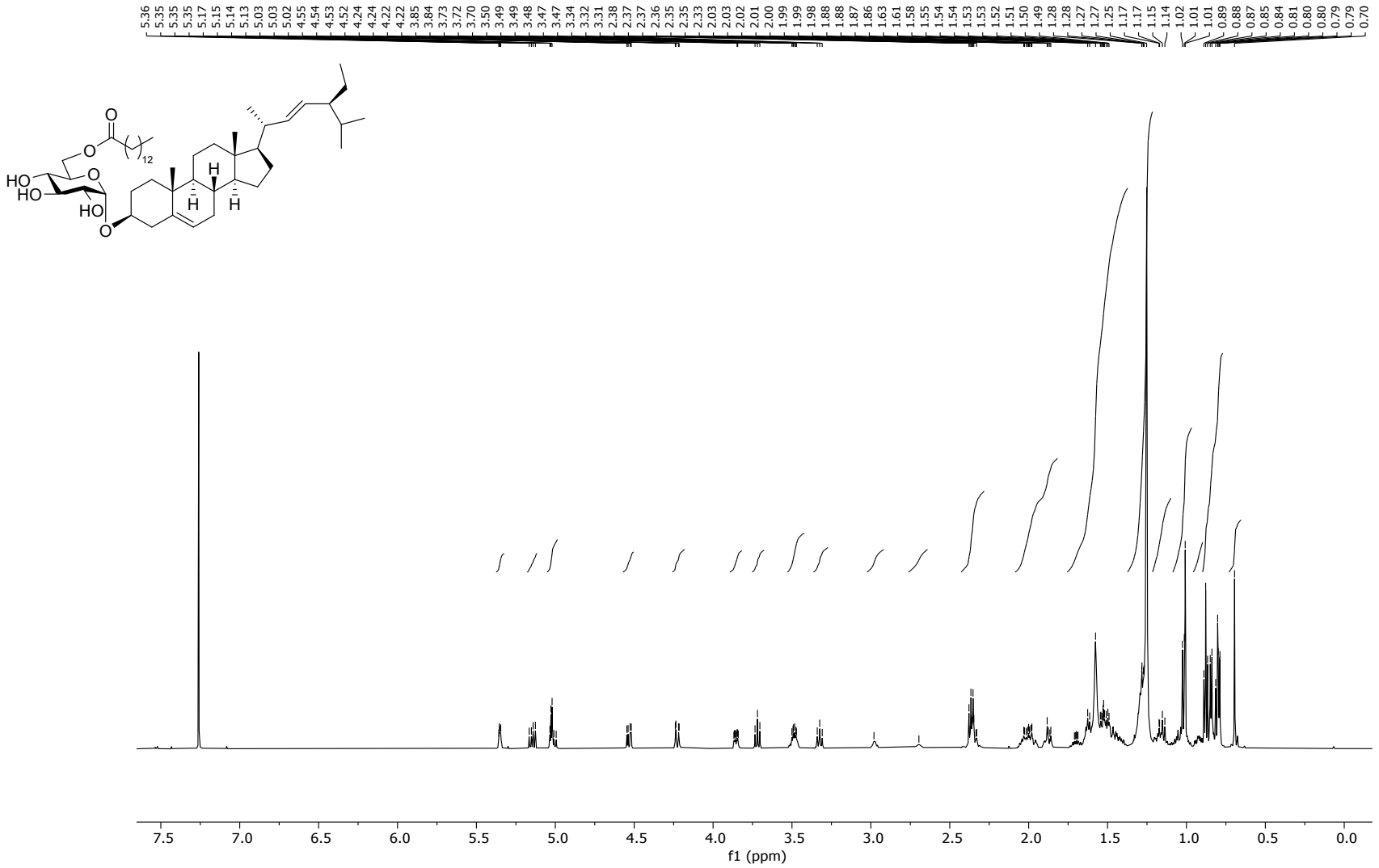
Stigmasteryl α-D-glucoside (6a)

^{13}C-NMR, 125 MHz, $CDCl_3$:CD_3OD (1:3)



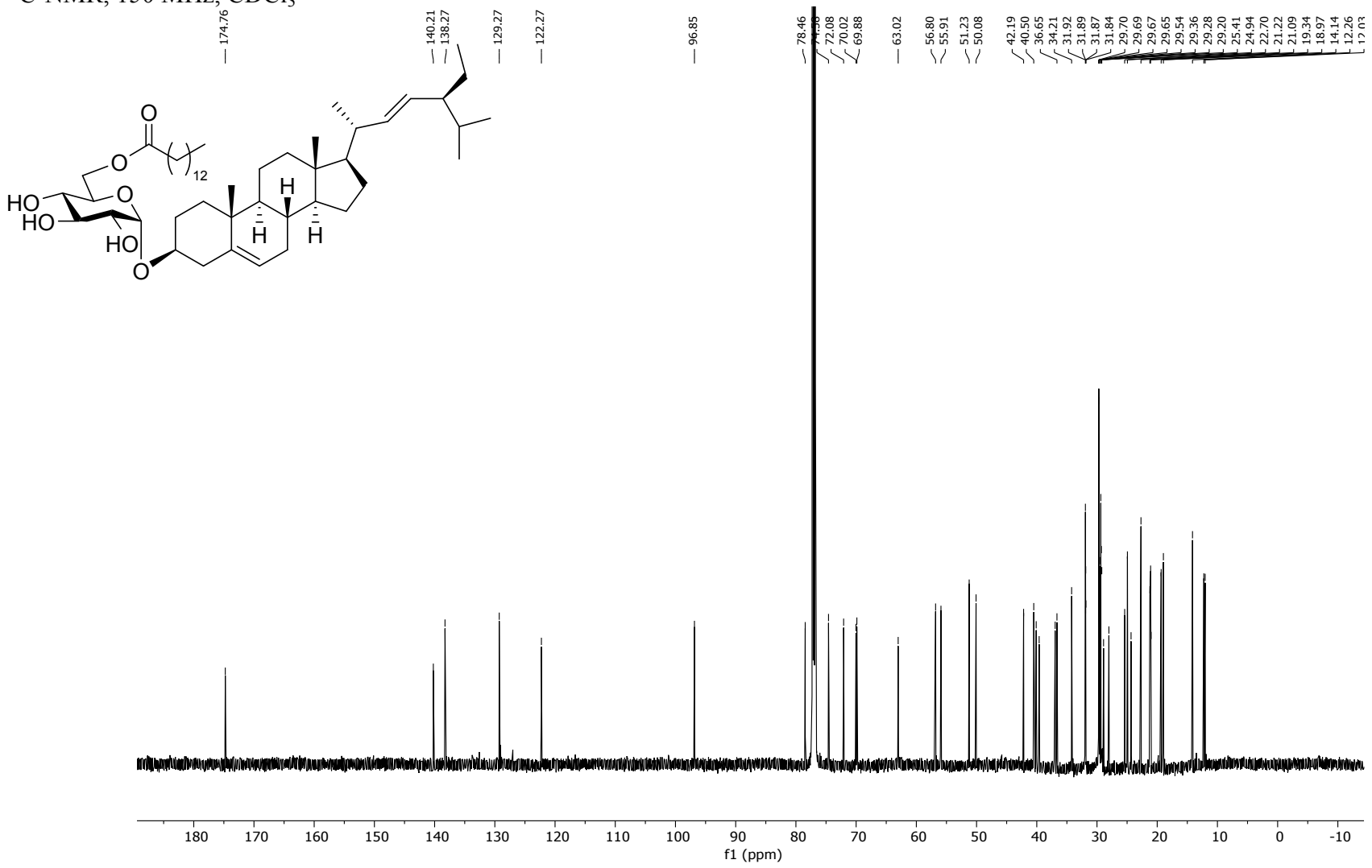
Stigmasteryl 6-*O*-tetradecanoyl-α-D-glucopyranoside (6b)

^{1}H-NMR, 600 MHz, $CDCl_3$



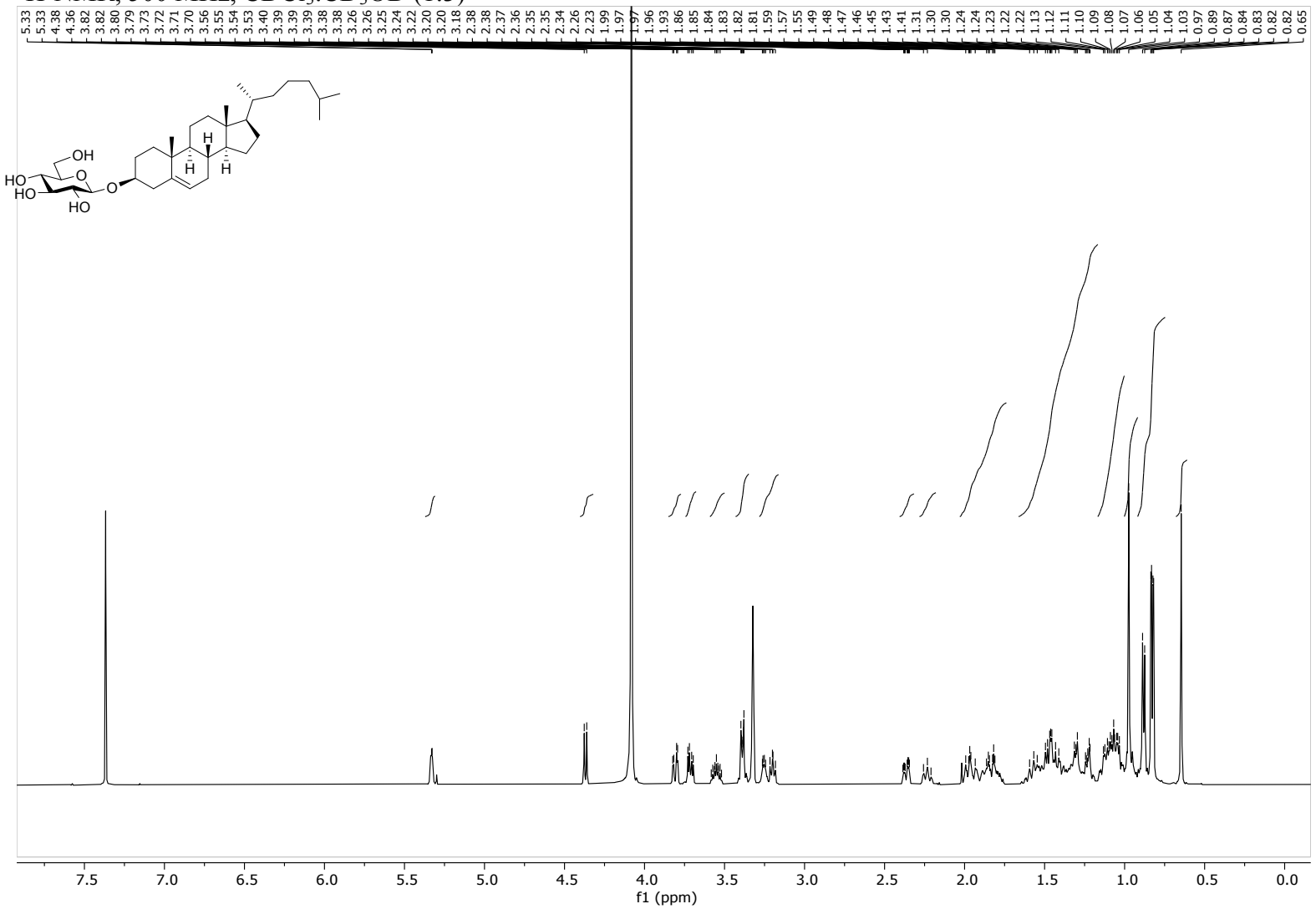
Stigmasteryl 6-*O*-tetradecanoyl-α-D-glucopyranoside (6b)

^{13}C-NMR, 150 MHz, $CDCl_3$



Cholesteryl β-D-glucopyranoside (7)

^{1}H-NMR, 500 MHz, $CDCl_3$:CD_3OD (1:3)



Cholesteryl β-D-glucopyranoside (7a)

^{13}C-NMR, 125 MHz, $CDCl_3$:CD_3OD (1:3)