SUPPLEMENTARY INFORMATION

Candida albicans Steryl 6-*O*-Acyl-α-D-Mannosides Agonize Signalling through Mincle

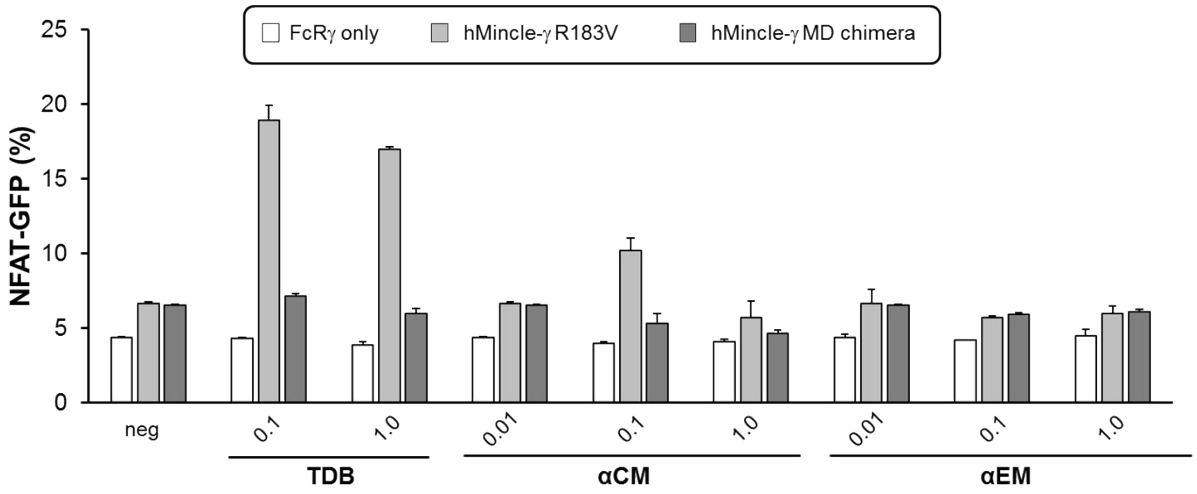

Figure S1. Signalling of αCM and αEM through mutants of hMincle.

Signalling of synthetic αCM and αEM through human Mincle R183V and hMincle MD chimera (residues 195–202 in hMincle is replaced by the corresponding region of Dectin-2 (residues 192–199)).[1] NFAT-GFP 2B4 reporter cells expressing either human Mincle (R183V)/FcRγ, human Mincle-MD chimera/FcRγ or FcRγ alone were tested for their reactivity to plate-bound trehalose dibehenate (TDB) and steryl mannosides. Quantities denote the amount in nmol; assays were performed in duplicate; the mean values and standard deviations are shown.

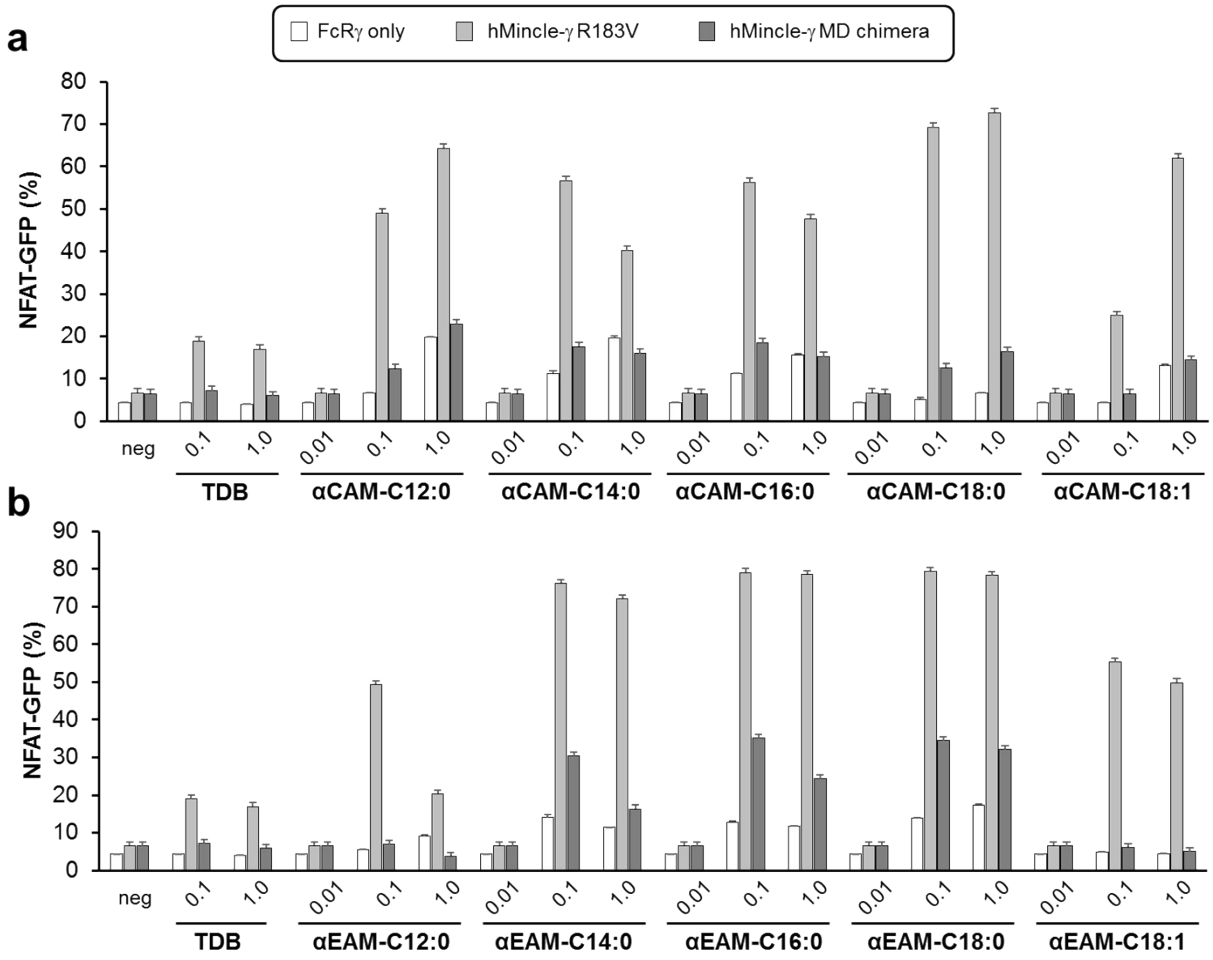

Figure S2. Signalling of αCAMs and αEAMs through mutants of hMincle.

Signalling of synthetic αCAMs and αEAMs through human Mincle R183V and hMincle MD chimera (residues 195–202 in hMincle is replaced by the corresponding region of Dectin-2 (residues 192–199)).[1] NFAT-GFP 2B4 reporter cells expressing either human Mincle (R183V)/FcRγ, human Mincle-MD chimera/FcRγ or FcRγ alone were tested for their reactivity to plate-bound trehalose dibehenate (TDB) and steryl mannosides. Quantities denote the amount in nmol; assays were performed in duplicate; the mean values and standard deviations are shown.

Experimental

General

Pyridine was distilled over KOH before use. Dichloromethane and THF were dried over alumina according to the method of Pangborn *et al*.[2] Reactions were monitored using thin layer chromatography, performed with silica gel 60 F254. Detection was effected by charring in a mixture of 5% sulfuric acid in methanol, 10% phosphomolybdic acid in EtOH, and/or visualizing with UV light. Flash chromatography was performed using silica gel 60according to the method of Still *et al*.[3] NMR experiments were conducted on 400 MHz instruments, with chemical shifts referenced relative to residual protiated solvent, and are in ppm. $^{1}H-^{1}H$ COSY spectra were used to confirm proton assignments. Mass spectra were acquired using an ESI quadrupole orbitrap.

Cholesteryl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside (1)

A solution of cholesterol (0.516 g, 1.34 mmol) and 2,3,4,6-tetra-*O*-benzoyl-mannopyranosyl trichloroacetimidate[4] (0.90 g, 1.21 mmol) in dry dioxane (134 mL) was stirred with activated 3 Å molecular sieves at r.t for 30 min. The mixture was then cooled to 0 ºC and TfOH (0.036 g, 21 μL, 0.242 mmol) was added. The reaction mixture was stirred at 0 ºC for 30 min. The reaction was quenched with NEt_3 (0.245 g, 2.42 mmol, 0.337 mL) at 0 ºC, then was diluted with diethyl ether and the solid was removed by filtration through a pad of Celite and washed with diethyl ether. The combined filtrates were washed with aq. $NaHCO_3$, aq. brine and dried ($MgSO_4$). The solvent was removed under reduced pressure to give a crude residue that was purified by flash chromatography (Et_2O:pet. spirit, 5:95) to afford a white solid that was recrystallised from ethanol to afford the pure α-anomer **1** as a white solid (0.514 g, 44%). ^{1}H NMR (400 Hz, $CDCl_3$) δ 8.06-8.08 (4 H, t, *J* = 7.5 Hz), 7.97-7.99 (2 H, d, *J* = 8.3 Hz), 7.83-7.85 (2 H, d, *J* = 8.2 Hz), 7.49-7.62 (3 H, m), 7.35-7.45 (7 H, m), 7.25-7.29 (2 H, t, *J* = 7.7 Hz), 6.02-6.07 (1 H, t, *J* = 9.9 Hz, H4), 5.93-5.96 (1 H, dd, *J* = 10.1, 3.2, H3), 5.64-5.65 (1 H, m, H2), 5.27 (1 H, m, CH=), 5.25 (1 H, d, *J* = 1.5 Hz, H1), 4.65-4.68 (1 H, dd, *J* = 11.6, 1.5 Hz, H6a), 4.53-4.57 (1 H, m, H5), 4.47-4.51 (1 H, dd, *J* = 11.7, 5.4 Hz, H6b), 3.59-3.66 (1 H, m, cholesteryl-H3), 2.46-2.48 (2 H, m), 1.82-2.04 (5 H, m), 0.83-1.60 (21 H, m, cholesteryl-CH), 1.03 (3 H, s), 0.92-0.93 (3 H, d, *J* = 6.4 Hz), 0.87 (3 H, d, *J* = 6.6 Hz), 0.86 (3 H, d, *J* = 6.6 Hz), 0.69 (3 H, s). ^{13}C NMR (100 Hz, $CDCl_3$) δ 166.0, 165.4, 165.3, 140.0, 133.3, 133.0, 132.8, 129.7, 129.6, 129.6, 129.3, 129.0, 128.8, 128.4, 128.3, 128.2, 128.1, 122.1, 95.8, 78.4, 71.0, 70.0, 68.8, 67.1, 63.1, 56.6, 56.0, 49.9, 42.2, 39.9, 39.6, 39.4, 36.8, 36.5, 36.0, 35.6, 31.8, 31.7, 28.1, 27.9, 27.7, 24.2, 23.7, 22.7, 22.4, 20.9, 19.3, 19.2, 18.6, 11.7. HRMS (ESI^+) *m/z* calcd. for $[C_{61}H_{72}O_{10}+H]^+$: 965.5198, obsd: 965.5199.

Cholesteryl α-D-mannopyranoside (3)

NaOMe (25% w/w in MeOH) was added dropwise into a solution of protected cholesteryl α-D-mannopyranoside **1** (0.514 g, 0.532 mmol) in dry MeOH:THF (3 mL: 2 mL) to achieve pH = 10. The solution was stirred at r.t for 1 h then was quenched with Dowex resin (H^+ form), filtered and concentrated to afford a white solid. This solid was recrystallised from EtOAc containing a few drops of methanol to afford cholesteryl mannoside **3** (0.178 g, 61%). ^{1}H NMR (400 Hz, DMSO-d_6) δ 5.29-5.30 (1 H, d, *J* = 4.5 Hz, CH=), 4.75 (1 H, d, *J* = 1.5 Hz, H1), 4.67-4.68 (1 H, d, *J* = 4.8 Hz, OH-4), 4.63-4.65 (1 H, d, *J* = 4.4 Hz, OH-2), 4.51-4.52 (1 H, d, *J* = 5.9 Hz, OH-3), 4.39-4.42 (1 H, t, *J* = 5.8 Hz, OH-6), 3.61-3.66 (1 H, dd, *J* = 10.6, 6.1 Hz, H5), 3.54 (1 H, m, H2), 3.29-3.45 (5 H, m, H3,4,6a,6b,cholesteryl-H3), 2.31-2.35 (1 H, dd, *J* = 12.7, 4.3 Hz), 2.16-2.22 (1 H, t, *J* = 11.7 Hz), 1.76-1.97 (5 H, m), 0.99-1.55 (21 H, m, cholesteryl-CH), 0.95 (3 H, s), 0.89-0.90 (3 H, d, *J* = 6.4 Hz), 0.85-0.86 (3 H, d, *J* = 6.6 Hz), 0.83-0.84 (3 H, d, *J* = 6.6 Hz), 0.65 (3 H, s). ^{13}C NMR (100 Hz, DMSO-d_6) δ 140.6, 121.1, 97.9, 75.2, 74.1, 70.9, 70.8, 67.1, 61.4, 56.2, 55.6, 49.5, 41.9, 36.6, 36.2, 35.7, 35.2, 31.4, 31.3, 27.8, 27.4, 23.9, 23.2, 22.7, 22.4, 20.6, 19.1, 18.6, 11.7. HRMS (ESI^+) *m/z* calcd. for $[C_{33}H_{56}O_6+H]^+$: 549.4150, obsd: 549.4150.

Cholesteryl 2,3,4-tri-*O*-trimethylsilyl-α-D-mannopyranoside (5)

Trimethylsilyl chloride (0.176 g, 1.62 mmol, 0.206 mL) and NEt_3 (0.170 mg, 1.68 mmol, 0.235 mL) were added to a stirred mixture of cholesteryl α-D-mannopyranoside **3** (0.178 g, 0.324 mmol) in CH_2Cl_2 (3 mL). The reaction was stirred overnight and then diluted with CH_2Cl_2 and aq. $NaHCO_3$. The combined organic layers were separated and washed with sat aq. $NaHCO_3$, water, aq. Brine, dried (Na_2SO_4) and concentrated. The residue was dissolved in $CHCl_3$ (3 mL) and NH_4OAc (55 mg, 0.71 mmol) was added. The reaction was allowed to stir for 48 h then concentrated and purified by flash chromatography (pre-treated with 4% NEt_3/pet. spirits, EtOAc:pet spirits 5:95) to afford **5** as a clear glass (248 mg, 76%). ^{1}H NMR (400 Hz, $CDCl_3$) δ 5.32-5.33 (1 H, dd, *J* = 3.4, 1.8 Hz, CH=), 4.73 (1 H, d, *J* = 1.9 Hz, H1), 3.84-3.88 (1 H, t, *J* = 9.1 Hz, H4), 3.76-3.78 (1 H, dd, *J* = 9.0, 2.5 Hz, H3), 3.73 -3.76 (1 H, dd, *J* = 11.4, 2.7 Hz, H6a), 3.69-3.70 (1 H, m, H2), 3.66-3.68 (1 H, m, H6b), 3.61-3.64 (1 H, m, H5), 2.28-2.31 (2 H, m), 1.94-2.01 (3 H, m), 1.83-1.85 (3 H, m), 0.86-1.60 (22 H, m, cholesteryl-CH), 1.00 (3 H, s), 0.90-0.91 (3 H, d, *J* = 6.2 Hz), 0.86-0.87 (3 H, d, *J* = 6.6 Hz), 0.85 (3 H, d, *J* = 6.6 Hz), 0.67 (3 H, s), 0.16 (9 H, s, 0.14 (9 H, s), 0.12 (9H, s). ^{13}C NMR (100 Hz, $CDCl_3$) δ 140.8, 122.0, 99.1, 77.5, 74.4, 74.1, 72.6, 68.4, 62.4, 56.9, 56.3, 50.3, 42.5, 40.2, 39.9, 39.7, 37.2, 36.9, 36.3, 35.9, 32.1, 28.4, 28.2, 27.9, 24.4, 24.0, 23.0, 22.7, 21.2, 19.5, 18.9, 12.0, 0.83, 0.79, 0.52.

Ergosteryl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside (2)

A solution of ergosterol (0.456 g, 1.15 mmol) and 2,3,4,6-tetra-*O*-benzoyl-mannopyranosyl-trichloroacetimidate[4] (0.741 g, 1.00 mmol) in dry CH_2Cl_2 (100 mL) was stirred with activated 3 Å molecular sieves at r.t for 30 min. The mixture was cooled to 0 ºC then TBDMSOTf (0.053 g, 0.200 mmol, 46 µL) was added. The mixture was stirred at 0 ºC for 30 min then was quenched with NEt_3 (0.202 g, 2.00 mmol, 0.278 mL) at 0 ºC. The mixture was diluted with diethyl ether and the solids were removed by filtration through a pad of Celite and washed with diethyl ether. The combined filtrates were washed with aq. $NaHCO_3$, aq. brine and dried ($MgSO_4$). The solvent was removed under reduced pressure to give a crude residue, which was purified by flash chromatography (ether:pet. spirit, 5:95) to afford a white solid. The solid was recrystallised from ethanol to afford compound **2** as a white solid (0.537 g, 61%). ^{1}H NMR (400 Hz, $CDCl_3$) δ 8.06-8.08 (4 H, d, *J* = 7.1 Hz), 7.97-7.99 (2 H, d, *J* = 8.3 Hz), 7.83-7.85 (2 H, d, *J* = 8.3 Hz), 7.29-2.62 (12 H, m), 6.03-6.07 (1 H, t, *J* = 9.8 Hz, H4), 5.93-5.96 (1 H, dd, *J* = 10.0, 2.6 Hz, H3), 5.66 (1 H, m, H2), 5.49-5.50 (1 H, dd, *J* = 5.3, 2.0 Hz, ring-B CH=), 5.36-5.37 (1 H, m, ring-B CH=), 5.38 (1 H, d, *J* = 1.7 Hz, H1), 5.19-5.35 (2 H, t, *J* = 7.1 Hz, C22/23 CH=CH), 4.64-4.66 (1 H, d, *J* = 10.9 Hz, H6a), 4.49-4.57 (2 H, m, H5,6b), 3.71-3.78 (1 H, ddd, *J* = 15.4, 11.0, 4.4 Hz, ergosteryl-H3), 1.20-2.10 (20 H, m, ergosteryl-CH), 1.04-1.05 (3 H, d, *J* = 6.6 Hz), 0.97 (3 H, s), 0.92-0.93 (3 H, d, *J* = 6.8 Hz), 0.83-0.85 (6 H, t, *J* = 7.1 Hz), 0.65 (3 H, s). ^{13}C NMR (100 Hz, $CDCl_3$) δ 166.4, 165.7, 165.7, 141.6, 139.2, 135.7, 133.6, 133.3, 133.2, 133.1, 130.0, 130.0, 129.9, 128.7, 128.6, 128.5, 120.2, 116.5, 96.0, 71.3, 70.3, 69.2, 67.3, 63.4, 55.9, 54.8, 46.3, 43.0, 40.6, 39.2, 38.7, 38.2, 37.4, 33.3, 28.5, 28.3, 23.2, 21.3, 21.2, 20.1, 19.8, 17.8, 16.4, 12.2. HRMS (ESI$^+$) *m/z* calcd. for $[C_{62}H_{70}O_{10}+Na]^+$: 997.4861, obsd: 997.4856.

Ergosteryl α-D-mannopyranoside (4)

NaOMe (25% w/w in MeOH) was added dropwise into a solution of protected ergosteryl α-D-mannopyranoside **2** (0.537 g, 0.550 mmol) in dried MeOH:THF (33 mL: 22 mL) to achieve pH = 10. The reaction was stirred at r.t for 1 h then was quenched with Dowex resin (H^+ form), filtered and concentrated to afford a white solid. This solid was recrystallised from EtOAc containing a few drops of methanol to afford compound **4** (0.200 g, 65%). ^{1}H NMR (400 Hz, DMSO-d_6) δ 5.50-5.51 (1 H, d, *J* = 4.2 Hz, ring-B CH=), 5.34 (1 H, m, ring-B CH=), 5.15-5.27 (2 H, qd, *J* = 15.4, 7.4 Hz, C22/23 CH=CH), 4.79 (1 H, s, H1), 4.67-4.69 (1 H, d, *J* = 4.9 Hz, OH-H4), 4.65-4.66 (1 H, d, *J* = 4.4 Hz, OH-H2), 4.52-4.53 (1 H, d, *J* = 5.9 Hz, OH-H3), 4.41-4.44 (1 H, t, *J* = 5.8 Hz, OH-H6), 3.62-3.67 (1 H, dd, *J* = 10.4, 6.0 Hz, H5), 3.55 (1 H, s, H2), 3.37-3.52 (5 H, m, H3,4,6a,6b,ergosteryl-H3), 2.21-2.33 (1 H, t, *J* = 12.9 Hz), 1.21-2.02 (22 H, m, ergosteryl-CH), 1.00-1.02 (3 H, d, *J* = 6.6 Hz), 0.87-0.90 (3 H, d, *J* = 6.8 Hz), 0.87 (3 H, s), 0.79-0.82 (3 H, t, *J* = 6.1 Hz), 0.59 (3 H, s). ^{13}C NMR (100 Hz, DMSO-d_6) δ 140.9, 140.2, 135.8, 131.9, 119.6, 116.5,

98.3, 74.6, 74.2, 71.3, 71.2, 67.6, 61.8, 55.5, 54.3, 46.0, 42.8, 42.5, 38.8, 38.5, 37.9, 37.1, 32.9, 28.4, 28.1, 23.0, 21.4, 21.0, 20.2, 19.9, 17.8, 16.4, 12.3. HRMS (ESI^+) *m/z* calcd. for $[C_{34}H_{54}O_6+H]^+$: 581.3813, obsd: 581.3810.

Ergosteryl 2,3,4-tri-*O*-trimethylsilyl-α-D-mannopyranoside (6)

Trimethylsilyl chloride (0.176 g, 1.62 mmol, 0.206 mL) and NEt_3 (0.170 mg, 1.68 mmol, 0.235 mL) were added to a stirred mixture of ergosteryl α-D-mannopyranoside **4** (0.181 g, 0.324 mmol) in CH_2Cl_2 (6 mL). The reaction was stirred overnight and then diluted with CH_2Cl_2 and aq. $NaHCO_3$. The combined organic layers were separated and washed with sat aq. $NaHCO_3$, water, aq. brine and dried (Na_2SO_4) and concentrated. The residue was dissolved in $CHCl_3$ (6 mL) and NH_4OAc (55 mg, 0.71 mmol) was added. The reaction was allowed to stir for 48 h then concentrated and purified by flash chromatography (pre-treated with 4% NEt_3/pet. spirits, EtOAc:pet spirits 5:95) to afford **6** as a clear glass (130 mg, 52%). ^1H NMR (400 Hz, $CDCl_3$) δ 5.53-5.54 (1 H, d, *J* = 4.4 Hz, ring-B CH=), 5.37 (1 H, s, ring-B CH=), 5.13-5.25 (2 H, m, C22/23 CH=CH), 4.76 (1 H, s, H1), 3.52-3.89 (9 H, m), 2.46-2.51 (1 H, dd, *J* = 14.0, 3.1 Hz), 2.30-2.37 (1 H, m), 1.25-1.89 (17 H, m, ergosteryl-CH), 1.02-1.04 (3 H, d, *J* = 6.7 Hz), 0.94 (3 H, s), 0.91-0.92 (3 H, d, *J* = 6.7 Hz), 0.81-0.84 (6 H, t, *J* = 6.3 Hz), 0.62 (3 H, s), 0.16 (9 H, s), 0.15 (9 H, s), 0.13 (9 H, s). ^{13}C NMR (100 Hz, $CDCl_3$) δ 141.0, 139.6, 135.4, 131.8, 119.5, 116.2, 98.7, 75.1, 74.0, 73.9, 72.3, 68.0, 62.0, 58.6, 55.6, 54.5, 46.0, 45.5, 42.7, 40.3, 39.0, 38.4, 38.0, 37.1, 33.0, 28.1, 28.2, 27.9, 22.9, 21.0, 19.8, 19.5, 17.5, 16.2, 11.9, 10.4, 0.55, 0.52, 0.24.

General Procedure for Acylation of cholesteryl and ergosteryl 2,3,4-tri-*O*-trimethylsilyl-α-D-mannosides

Acyl chloride (2 equiv) was added a solution of DMAP (1 equiv) and steryl 2,3,4-tri-*O*-trimethylsilyl-mannoside **5** or **6** (1 equiv) in pyridine/CH_2Cl_2 (3:97, 10 mL/mmol) at 0 ºC. The solution was stirred at r.t for 2 h then MeOH (1 mL/mmol) was added and the mixture was stirred for 0.5 h. The solvent was evaporated and the residue was redissolved in $CHCl_3$ (10 mL/mmol) and Dowex 50WX8-200 (H^+ form, approx. 100 mg) was added. After stirring for 1 h, the mixture was filtered and the filtrate concentrated. Flash chromatography (EtOAc + 1% AcOH) afforded the product as a white solid.

Cholesteryl 6-*O*-dodecanoyl-α-D-mannopyranoside (7)

The General Protocol for Acylation between **5** (30 mg, 0.039 mmol) and lauroyl chloride (17.1 mg, 0.078 mmol, 18 μL) after flash chromatography afforded **7** as white solid (20.2 mg, 71%). ^1H NMR (400 Hz, $CDCl_3$) δ 5.33-5.35 (1 H, d, *J* = 4.3 Hz, CH=), 5.01 (1 H, s, H1), 4.55-4.59 (1 H, dd, *J* =

12.3, 3.9 Hz, H6a), 4.20-4.23 (1 H, d, *J* = 11.9 Hz, H6b), 3.91 (1 H, s, H2), 3.81-3.86 (2 H, dd, *J* = 19.4, 7.9 Hz, H3,5), 3.53-3.58 (1 H, t, *J* = 9.6 Hz, H4), 3.48-3.50 (1 H, m, cholesteryl-H3), 2.36-2.40 (2 H, t, *J* = 7.6 Hz), 2.29-2.31 (2 H, d, *J* = 7.8 Hz), 1.80 - 2.02 (6 H, m), 1.43-2.15 (12 H, m, cholesteryl-CH), 1.26 (18 H, m, lipid), 1.10 (8 H, m), 1.00 (3 H, s), 0.90-0.92 (3 H, d, *J* = 6.5 Hz), 0.87-0.89 (3 H, t, *J* = 6.7 Hz, ω-CH_3), 0.85-0.87 (3 H, d, 6.6 Hz), 0.86-0.87 (3 H, d, *J* = 6.6 Hz), 0.67 (3 H, s). ^{13}C NMR (100 Hz, $CDCl_3$) δ 175.0, 140.4, 121.8, 97.6, 77.0, 71.2, 70.8, 70.3, 67.8, 63.2, 56.6, 56.0, 50.0, 42.2, 39.8, 39.6, 39.4, 36.8, 36.5, 36.0, 35.6, 34.10, 31.8, 31.8, 29.5, 29.4, 29.2, 29.1, 29.1, 28.1, 27.9, 27.5, 24.8, 24.2, 23.7, 22.7, 22.5, 22.4, 20.9, 19.2, 18.6, 14.0, 11.7. HRMS (ESI$^+$) *m/z* calcd. for $[C_{45}H_{78}O_7+Na]^+$: 753.5640, obsd: 753.5645.

Cholesteryl 6-*O*-tetradecanoyl-α-D-mannopyranoside (8)

The General Protocol for Acylation between **5** (30 mg, 0.039 mmol) and myristoyl chloride (19.3 mg, 0.078 mmol, 21.2 μL) after flash chromatography afforded **8** as white solid (22.4 mg, 76%). ^{1}H NMR (400 Hz, $CDCl_3$) δ 5.33-5.34 (1 H, d, *J* = 4.3 Hz, CH=), 4.99 (1 H, s, H1), 4.50-4.53 (1 H, dd, *J* = 11.8, 4.5 Hz, H6a), 4.25-4.27 (1 H, d, *J* = 11.7 Hz, H6b), 3.91 (1 H, s, H2), 3.82-3.85 (1 H, t, *J* = 8.4 Hz, H3), 3.47-3.49 (1 H, m, cholesteryl-H3), 3.33 (1 H, s, OH), 3.05 (1 H, s, OH), 2.70 (1 H, s, OH), 2.35-2.37 (2 H, t, *J* = 7.6 Hz), 2.30-2.31 (2 H, d, *J* = 7.0 Hz), 1.95-2.02 (2 H, m), 1.84-1.86 (2 H, m), 1.30-1.70 (18 H, m), 1.25 (22 H, m, lipid), 1.09-1.15 (6 H, m), 1.00 (3 H, s), 0.91-0.02 (3 H, d, *J* = 6.4 Hz), 0.87-0.89 (3 H, t, *J* = 6.4 Hz, ω-CH_3), 0.85-0.87 (6 H, *J* = 5.9 Hz), 0.67 (3 H, s). ^{13}C NMR (100 Hz, $CDCl_3$) δ 174.8, 140.4, 121.8, 97.6, 77.0, 71.2, 70.9, 70.3, 67.8, 63.3, 56.6, 56.0, 50.0, 42.2, 39.8, 39.6, 39.4, 36.9, 36.5, 36.9, 36.5, 36.0, 35.7, 34.1, 31.8, 31.7, 29.6, 29.5, 29.4, 29.23, 29.19, 29.12, 28.1, 27.9, 27.5, 24.8, 24.1, 23.7, 22.7, 22.5, 22.4, 20.9, 19.2, 18.6, 14.0 11.7. HRMS (ESI$^+$) *m/z* calcd. for $[C_{47}H_{82}O_7+Na]^+$: 781.5953, obsd: 781.5950. The ^{1}H NMR spectrum was consistent with that reported.[5]

Cholesteryl 6-*O*-hexadecanoyl-α-D-mannopyranoside (9)

The General Protocol for Acylation between **5** (30 mg, 0.039 mmol) and palmitoyl chloride (21.4 mg, 0.078 mmol, 23.7 μL) after flash chromatography afforded **9** as white solid (23.9 mg, 78%). ^{1}H NMR (400 Hz, $CDCl_3$) δ 5.33-5.34 (1 H, d, *J* = 3.2 Hz, CH=), 5.00 (1 H, s, H1), 4.54-4.55 (1 H, dd, *J* = 11.8, 4.3 Hz, H6a), 4.21-4.24 (1 H, d, *J* = 11.8 Hz, H6b), 3.91 (1 H, s, H2), 3.81-4.84 (2 H, m, H3,5), 3.55 (1 H, t, *J* = 9.6 Hz, H4), 3.47-3.49 (1 H, m, cholesteryl-H3), 2.35-2.39 (2 H, t, *J* = 7.4 Hz), 2.29-2.31 (2 H, d, *J* = 7.8 Hz), 1.84-1.89 (6 H, m), 1.04-1.62 (12 H, m, cholesteryl-CH), 1.25 (26 H, s, lipid), 1.11 (8 H, m), 0.99 (3 H, s), 0.90-0.92 (3 H, d, *J* = 5.6 Hz), 0.85-0.87 (6 H, d, *J* = 6.6 Hz), 0.87-0.89 (3 H, t, *J* = 6.7 Hz, ω-CH_3), 0.67 (3 H, s). ^{13}C NMR (100 Hz, $CDCl_3$) δ 175.2,

140.7, 122.1, 97.9, 77.2, 71.5, 71.2, 70.6, 68.1, 63.6, 56.9, 56.3, 50.3, 42.5, 40.1, 39.9, 39.7, 37.2, 36.9, 36.4, 36.0, 34.4, 32.1, 32.0, 29.9, 29.8, 29.7, 29.6, 29.4, 29.4, 28.4, 28.2, 27.8, 25.2, 24.4, 24.0, 23.0, 22.9, 22.7, 21.2, 19.5, 18.9, 14.3, 12.0. HRMS (ESI^+) *m/z* calcd. for $[C_{49}H_{86}O_7+Na]^+$: 809.6266, obsd: 809.6258.

Cholesteryl 6-*O*-octadecanoyl-α-D-mannopyranoside (10)

The General Protocol for Acylation between **5** (30 mg, 0.039 mmol) and stearoyl chloride (23.6 mg, 0.078 mmol, 26.3 μL) after flash chromatography afforded **10** as white solid (24.8 mg, 78%). ^1H NMR (400 Hz, $CDCl_3$) δ 5.33-5.34 (1 H, d, *J* = 4.1 Hz, CH=), 5.01 (1 H, s, H1), 4.54-4.58 (1 H, dd, *J* = 12.2, 4,4 Hz, H6a), 4.22-4.24 (1 H, d, *J* = 11.4 Hz, H6b), 3.91 (1 H, s, H2), 3.81-3.88 (2 H, m, H3,5), 3.53-3.58 (1 H, t, *J* = 9.6 Hz, H4), 3.47-3.51 (1 H, m, cholesteryl-H3), 2.35-2.39 (2 H, t, *J* = 7.6 Hz), 2.29-2.31 (2 H, d, *J* = 7.7 Hz), 1.95-2.02 (2 H, m), 1.80-1.89 (3 H, m), 1.05-1.64 (13 H, m, cholesteryl-CH), 1.25 (30 H, lipid), 1.10 (8 H, m), 1.00 (3 H, s), 0.90-0.92 (3 H, d, *J* = 6.4 Hz), 0.87-0.89 (3 H, t, *J* = 6.8 Hz, ω-CH_3), 0.87 (3 H, d, *J* = 1.2 Hz), 0.85 (3 H, d, *J* = 1.2 Hz), 0.67 (3 H, s). ^{13}C NMR (100 Hz, $CDCl_3$) δ 175.1, 140.5, 122.0, 97.7, 71.3, 71.0, 70.5, 67.9, 63.3, 56.73, 56.2, 50.1, 42.3, 39.6, 39.7, 39.5, 37.0, 36.7, 36.2, 35.8, 34.3, 31.9, 31.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.7, 28.2, 28.0, 27.6, 25.0, 24.3, 23.8, 22.8, 22.7, 22.5, 21.0, 19.3, 18.7, 14.1, 11.8. HRMS (ESI^+) *m/z* calcd. for $[C_{51}H_{90}O_7+Na]^+$: 837.6579, obsd: 837.6580.

Cholesteryl 6-*O*-oleyl-α-D-mannopyranoside (11)

The General Protocol for Acylation between **5** (30 mg, 0.039 mmol) and oleoyl chloride (23.5 mg, 0.078 mmol, 25.8 μL) after flash chromatography afforded **11** as white solid (27.2 mg, 86%). ^1H NMR (400 Hz, $CDCl_3$) δ 5.32-5.35 (3 H, m), 4.99 (1 H, s, H1), 4.51-4.55 (1 H, dd, *J* = 11.8, 4.1 Hz, H6a), 4.23-4.25 (1 H, d, *J* = 11.6 Hz, H6b), 3.91 (1 H, s, H2), 3.81-3.87 (2 H, m, H3,5), 3.56-3.59 (1 H, m), 3.46-3.49 (1 H, m, cholesteryl-H3), 2.34-2.38 (2 H, t, *J* = 7.6 Hz), 2.29-2.31 (2 H, d, *J* = 7.5 Hz), 2.01-2.00 (6 H, m), 1.29 (14 H, m, lipid), 1.26 (12 H, m, lipid), 1.11-1.86 (12 H, m, cholesteryl-CH), 0.99 (3 H, s), 1.11 (6 H, m), 0.90-0.92 (3 H, d, *J* = 6.5 Hz), 0.87-0.89 (3 H, t, *J* = 6.9 Hz, ω-CH_3), 0.85-0.89 (6 H, d, *J* = 6.6 Hz), 0.67 (3 H, s). ^{13}C NMR (100 Hz, $CDCl_3$) δ 174.9, 140.4, 129.9, 129.5, 121,8, 97.6, 77.0, 71.2, 70.8, 70.3, 67.8, 63.2, 56.6, 56.0, 50.0, 42.2, 39.8, 39.6, 39.4, 36.8, 36.5, 36.0, 35.6, 34.1, 31.8, 31.7, 29.6, 29.6, 29.4, 29.2, 29.0, 28.1, 27.9, 27.5, 27.1, 27.0, 24.8, 24.1, 23.7, 22.4, 20.9, 19.2, 18.6, 14.0, 11.7. HRMS (ESI^+) *m/z* calcd. for $[C_{51}H_{88}O_7+Na]^+$: 812.6530, obsd: 812.6538.

Ergosteryl 6-*O*-dodecanoyl-α-D-mannopyranoside (12)

The General Protocol for Acylation between **6** (23.5 mg, 0.030 mmol) and lauroyl chloride (13.1 mg, 0.060 mmol, 13.8 μL) after flash chromatography afforded **12** as white solid (16.2 mg, 73%). ^{1}H NMR (400 Hz, $CDCl_3$) δ 5.54-5.55 (1 H, d, *J* = 4.0 Hz, ring-B CH=), 5.36-5.37 (1 H, m, ring-B CH=), 5.13-5.25 (2 H, qd, *J* = 15.3, 7.3 Hz, C22/C23 CH=CH), 5.03 (1 H, s, H1), 4.52-4.56 (1 H, dd, *J* = 12.2, 4.6 Hz, H6a), 4.22-4.25 (1 H, d, *J* = 11.4 Hz, H6b), 3.92 (1 H, s, H2), 3.81-3.84 (2 H, m, H3,5), 3.57-3.59 (2 H, m, H4, ergosteryl-H3), 2.45-2.49 (1 H, dd, *J* = 14.5, 2.8 Hz), 2.34-2.38 (3 H, t, *J* = 7.7 Hz), 1.25 (18 H, m, lipid), 1.25-2.04 (18 H, m, ergsoteryl-CH), 1.02-1.04 (3 H, d, *J* = 6.6 Hz), 0.93 (3 H, s), 0.90-0.93 (3 H, d, *J* = 6.9 Hz), 0.86-0.89 (3 H, t, *J* = 6.8 Hz, ω-CH_3), 0.81-0.84 (6 H, t, *J* = 6.4 Hz), 0.62 (3 H, s). ^{13}C NMR (100 Hz, $CDCl_3$) δ 175.2, 141.5, 139.6, 135.7, 132.1, 120.0, 116.4, 97.8, 75.7, 71.6, 71.2, 70.7, 68.0, 63.7, 55.9, 54.7, 46.3, 43.0, 42.9, 40.6, 39.2, 38.6, 38.2, 37.4, 34.5, 33.2, 32.1, 29.9, 29.8, 29.6, 29.5, 29.5, 28.5, 28.1, 25.2, 23.2, 22.9, 21.3, 21.2, 20.1, 19.8, 17.8, 16.3, 14.3, 12.2. HRMS (ESI$^+$) *m/z* calcd. for $[C_{46}H_{76}O_7+Na]^+$: 763.5483, obsd: 763.5480.

Ergosteryl 6-*O*-tetradecanoyl-α-D-mannopyranoside (13)

The General Protocol for Acylation between **6** (23.5 mg, 0.030 mmol) and myristoyl chloride (14.8 mg, 0.060 mmol, 16.3 μL) after flash chromatography afforded **13** as white solid (16.8 mg, 73%). ^{1}H NMR (400 Hz, $CDCl_3$) δ 5.55-5.56 (1 H, d, *J* = 3.8 Hz, ring B CH=), 5.37-5.38 (1 H, m, ring B CH=), 5.15-5.25 (2 H, m, C22/23 CH=CH), 5.04 (1 H, s, H1), 4.54-4.56 (1 H, d, *J* = 11.6 Hz, H6a), 4.23-4.25 (1 H, d, *J* = 11.9 Hz, H6b), 3.92 (1 H, s, H2), 3.81-3.87 (2 H, m, H3,5), 3.54-3.63 (2 H, m, H4, cholesteryl-H3), 3.56 (2 H, m), 2.46-2.50 (1 H, dd, *J* = 14.8, 2.4 Hz), 2.36-2.39 (3 H, m), 1.34-2.05 (16 H, m, ergosteryl-CH), 1.25 (22 H, m, lipid), 1.03-1.04 (3 H, d, *J* = 6.6 Hz), 0.94 (3 H, s), 0.91-0.92 (3 H, d, *J* = 6.8 Hz), 0.87-0.88 (3 H, t, *J* = 6.9 Hz, ω-CH_3), 0.82-0.85 (6 H, t, *J* = 7.2 Hz), 0.63 (3 H, s). ^{13}C NMR (100 Hz, $CDCl_3$) δ 175.2, 141.5, 139.6, 135.7, 132.1, 120.0, 116.4, 97.8, 75.7, 71.5, 71.1, 70.7, 68.1, 63.5, 58.3, 55.9, 54.7, 46.3, 43.0, 40.6, 39.2, 38.6, 38.2, 37.4, 34.4, 33.2, 32.1, 29.9, 29.8, 29.7, 29.5, 29.4, 29.4, 28.4, 28.1, 25.2, 23.2, 22.9, 21.3, 21.2, 20.2, 20.1, 19.8, 17.8, 16.4, 14.3, 12.2, 8.3. HRMS (ESI$^+$) *m/z* calcd. for $[C_{48}H_{80}O_7+Na]^+$: 791.5796, obsd: 791.5794.

Ergosteryl 6-*O*-hexadecanoyl-α-D-mannopyranoside (14)

The General Protocol for Acylation between **6** (23.5 mg, 0.030 mmol) and palmitoyl chloride (16.5 mg, 0.060 mmol, 18.2 μL) after flash chromatography afforded **14** as white solid (21.5 mg, 90%). ^{1}H NMR (400 Hz, $CDCl_3$) δ 5.56 (1 H, d, *J* = 3.8 Hz, ring B CH=), 5.38-5.39 (1 H, m, ring B CH=), 5.21-5.26 (2 H, qd, *J* = 15.3, 7.5 Hz, C22/23 CH=CH), 5.05 (1 H, s, H1), 4.52-4.55 (1 H, dd, *J* = 12.0, 4.6 Hz, H6a), 4.26-4.28 (1 H, d, *J* = 11.2 Hz, H6b), 3.93 (1 H, s, H2), 3.83-3.85 (2 H, m,

H3,5), 3.56-3.62 (4 H, m), 2.48-2.51 (1 H, dd, *J* = 14.9, 2.3 Hz), 2.36-2.38 (4 H, m), 1.42-2.06 (15 H, m, ergosteryl- CH), 1.26 (26 H, m, lipid), 1.04-1.05 (3 H, d, *J* = 6.6 Hz), 0.95 (3 H, s), 0.92-0.93 (3 H, d, *J* = 6.8 Hz), 0.88-0.90 (3 H, t, *J* = 6.9 Hz, ω-CH_3), 0.83-0.86 (6 H, t, *J* = 7.2 Hz), 0.64 (3 H, s). ^{13}C NMR (100 Hz, $CDCl_3$) δ 175.1, 141.5, 139.6, 135.7, 132.7, 119.9, 116.4, 97.8, 75.7, 71.6, 71.1, 70.7, 68.1, 63.6, 58.6, 55.9, 54.7, 46.3, 43.0, 40.6, 39.2, 38.6, 38.2, 37.4, 34.4, 33.2, 32.1, 30.0, 29.8, 29.7, 29.5, 29.4, 29.3, 28.4, 28.1, 25.2, 23.2, 23.0, 21.2, 21.2, 20.1, 19.8, 17.8, 16.4, 14.3, 12.2, 8.4. HRMS (ESI$^+$) *m/z* calcd. for $[C_{50}H_{84}O_7+Na]^+$: 819.6109, obsd: 819.6113.

Ergosteryl 6-*O*-octadecanoyl-α-D-mannopyranoside (15)

The General Protocol for Acylation between **6** (23.5 mg, 0.030 mmol) and stearoyl chloride (18.2 mg, 0.060 mmol, 20.3 μL) after flash chromatography afforded **15** as white solid (18.5 mg, 75%). ^{1}H NMR (400 Hz, $CDCl_3$) δ 5.54-5.55 (1 H, d, *J* = 3.8 Hz, ring B CH=), 5.36-5.37 (1 H, m, ring B CH=), 5.14-5.25 (2 H, qd, *J* = 15.3, 7.5 Hz, C22/23 CH=CH), 5.02 (1 H, s, H1), 4.47-4.50 (1 H, dd, *J* = 11.9, 5.1 Hz, H6a), 4.27-4.30 (1 H, d, *J* = 11.4 Hz, H6b), 3.91 (1 H, m, H2), 3.84 (2 H, m, H3,5), 3.57-3.60 (2 H, m), 2.46-2.50 (1 H, dd, *J* = 14.7, 2.3 Hz), 2.34-2.37 (3 H, t, *J* = 7.7 Hz), 1.25-2.07 (18 H, m, ergosteryl-CH), 1.25 (30 H, m, lipid) 1.03-1.04 (3 H, d, *J* = 6.6 Hz), 0.93 (3 H, s), 0.91-0.92 (3 H, d, *J* = 7.0 Hz), 0.86-0.89 (3 H, t, *J* = 6.9 Hz, ω-CH_3), 0.82-0.85 (6 H, t, *J* = 7.1 Hz), 0.62 (3 H, s). ^{13}C NMR (100 Hz, $CDCl_3$) δ 175.0, 141.4, 139.5, 135.7, 132.1, 120.0, 116.4, 97.9, 75.8, 71.6, 71.2, 70.7, 68.1, 63.7, 55.9, 54.7, 46.3, 43.0, 42.9, 40.6, 39.2, 38.6, 38.2, 37.4, 34.5, 33.2, 21.1, 29.88, 29.82, 29.79, 29.51, 29.49, 29.40, 28.4, 28.1, 23.1, 22.9, 21.3, 21.2, 20.1, 19.8, 17.8, 16.3, 14.3, 12.2. HRMS (ESI$^+$) *m/z* calcd. for $[C_{52}H_{88}O_7+Na]^+$: 847.6422, obsd: 847.6424.

Ergosteryl 6-*O*-oleyl-α-D-mannopyranoside (16)

The General Protocol for Acylation between **6** (23.5 mg, 0.030 mmol) and oleoyl chloride (18.1 mg, 0.060 mmol, 19.8 μL) after flash chromatography afforded **16** as white solid (20.5 mg, 82%). ^{1}H NMR (400 Hz, $CDCl_3$) δ 5.54-5.56 (1 H, dd, *J* = 5.2, 1.7 Hz, ring B CH=), 5.37-5.38 (1 H, m, ring B CH=), 5.33-5.35 (dt, 2 H, *J* = 5.6, 4.3 Hz), 5.15-5.25 (dq, 2 H, *J* = 15.3, 7.5 Hz, C22/23 CH=CH), 5.03 (1 H, s, H1), 4.53-4.56 (1 H, dd, *J* = 12.2, 4.5 Hz, H6a), 4.23-4.25 (1 H, d, *J* = 10.9 Hz, H6b), 3.92 (1 H, m, H2), 3.81-3.87 (2 H, m, H3,5), 3.55-3.62 (2 H, m), 2.46-2.50 (1 H, dd, *J* = 14.7, 2.4 Hz), 2.35-2.38 (3 H, m), 1.61-2.08 (18 H, m, ergosteryl-CH), 1.31 (14 H, m, lipid), 1.27 (12 H, m, lipid), 1.03-1.04 (3 H, d, *J* = 6.6 Hz), 0.93 (3 H, s), 0.91-0.92 (3 H, d, *J* = 6.8 Hz), 0.86-0.89 (3 H, t, *J* = 6.9 Hz), 0.82-0.85 (6 H, t, *J* = 7.2 Hz), 0.63 (3 H, s). ^{13}C NMR (100 Hz, $CDCl_3$) δ 175.2, 141.5, 139.6, 135.7, 132.2, 130.2, 129.9, 120.0, 116.4, 97.8, 75.8, 71.6, 71.1, 70.7, 68.1, 63.6, 55.9, 54.7, 46.3, 43.0, 40.6, 39.2, 38.6, 38.2, 37.4, 34.4, 33.2, 32.1, 29.93, 29.88, 29.69, 29.49,

29.48, 29.35, 29.31, 28.4, 28.1, 27.39, 27.37, 25.1, 23.2, 22.8, 21.3, 21.2, 20.1, 19.8, 17.8, 16.4, 14.3, 12.2. HRMS (ESI$^+$) *m/z* calcd. for $[C_{52}H_{86}O_7+Na]^+$: 845.6266, obsd: 845.6270.

Mincle reporter cell signalling assays

2B4-NFAT-GFP reporter cells expressing mouse or human Mincle (or the R135L mutant;[6] or the Mincle R183V and hMincle MD chimera (residues 195–202 in hMincle is replaced by the corresponding region of Dectin-2 (residues 192–199)[1]), together with FcRγ, as well as an FcRγ only control, were prepared as described previously.[7, 8] Each glycolipid was dissolved in chloroform methanol (2:1) at 5 or 10 mg mL^{-1}, then diluted in isopropanol, was added to 96-well plates at 20 μL per well, followed by the evaporation of the solvent. Reporter cells were stimulated for 16−20 h, and activation of NFAT-GFP was monitored by flow cytometry.

Bone marrow-derived dendritic cell (BMDC) preparation

Mincle-deficient mice[9] were backcrossed for at least fifteen generations with C57BL/6 mice. All mice were maintained in a filtered-air laminar-flow enclosure and given standard laboratory food and water ad libitum. All animal experiments were performed in accordance with Institute guidelines. Preparation of BMDCs followed previously reported approach.[10] For preparing BMDCs, bone marrow cells were suspended in RPMI 1640 medium supplemented with 10% fetal calf serum and β-mercaptoethanol, plated in the presence of 20ng mL^{-1} recombinant mouse granulocyte macrophage colony stimulating factor (BioLegend) and cultured for 7 days at 37 °C.

BMDC stimulation assay

To stimulate BMDCs, each glycolipid was dissolved in chloroform methanol (2:1) at 5 or 10 mg mL^{-1}, then diluted in isopropanol, was added to 96-well plates at 20 μL per well, followed by the evaporation of the solvent. LPS was added at final concentration of 10 ng mL^{-1}. BMDCs were stimulated for 1 day, and the supernatant was harvested. Tumour necrosis factor (TNF) was measured by ELISA (BD Biosciences).

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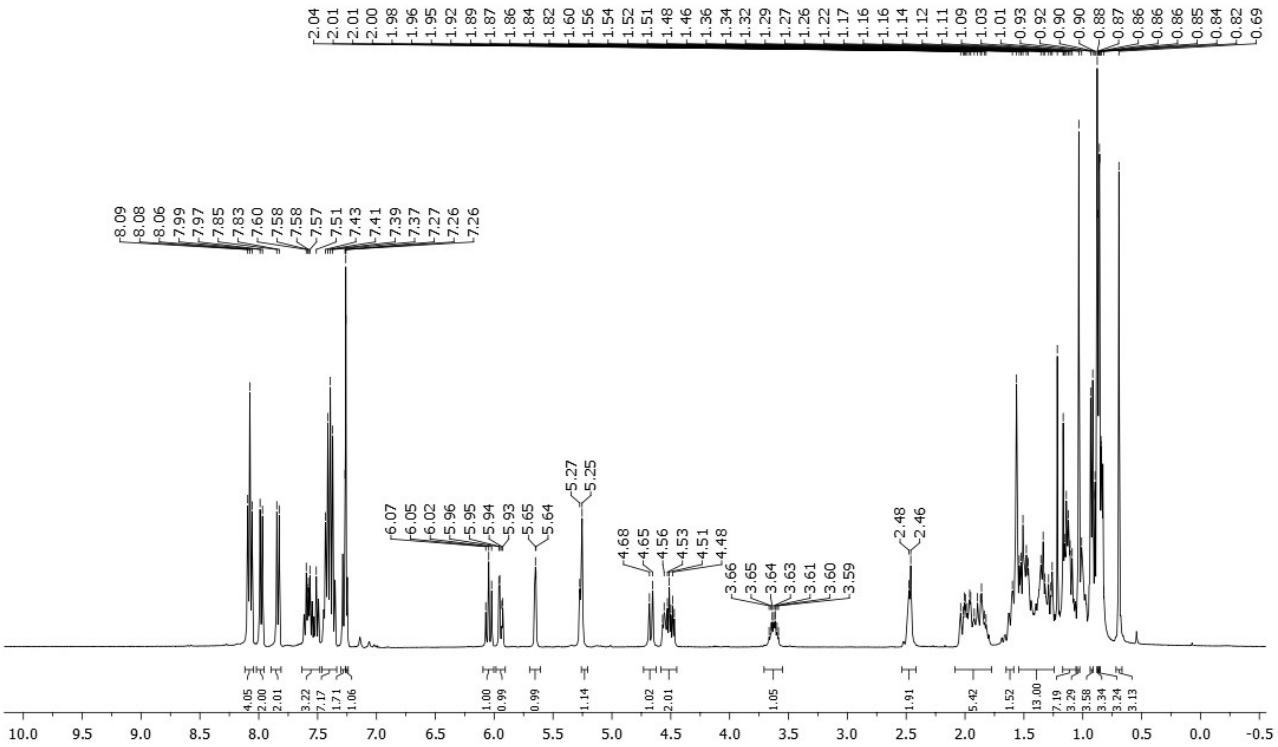
NMR Spectra

Cholesteryl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside (1)

^{1}H NMR



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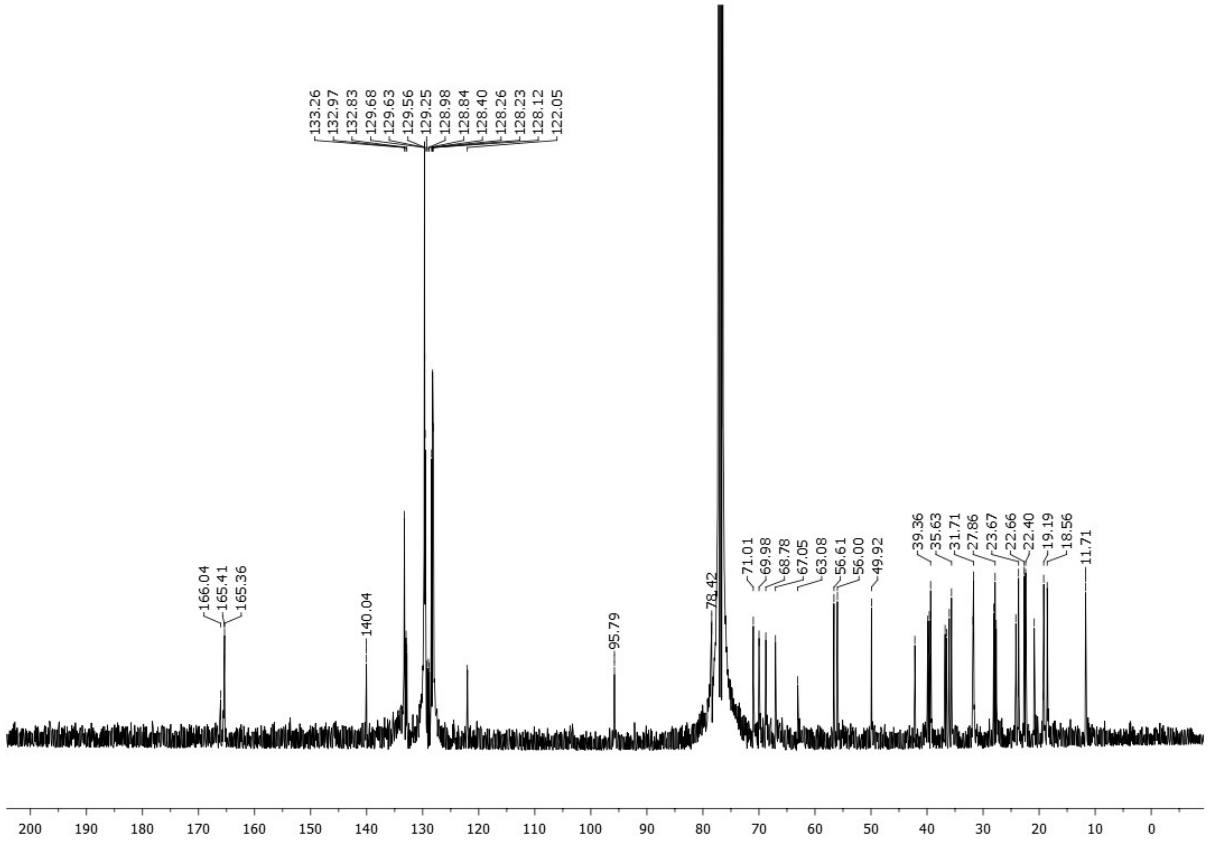
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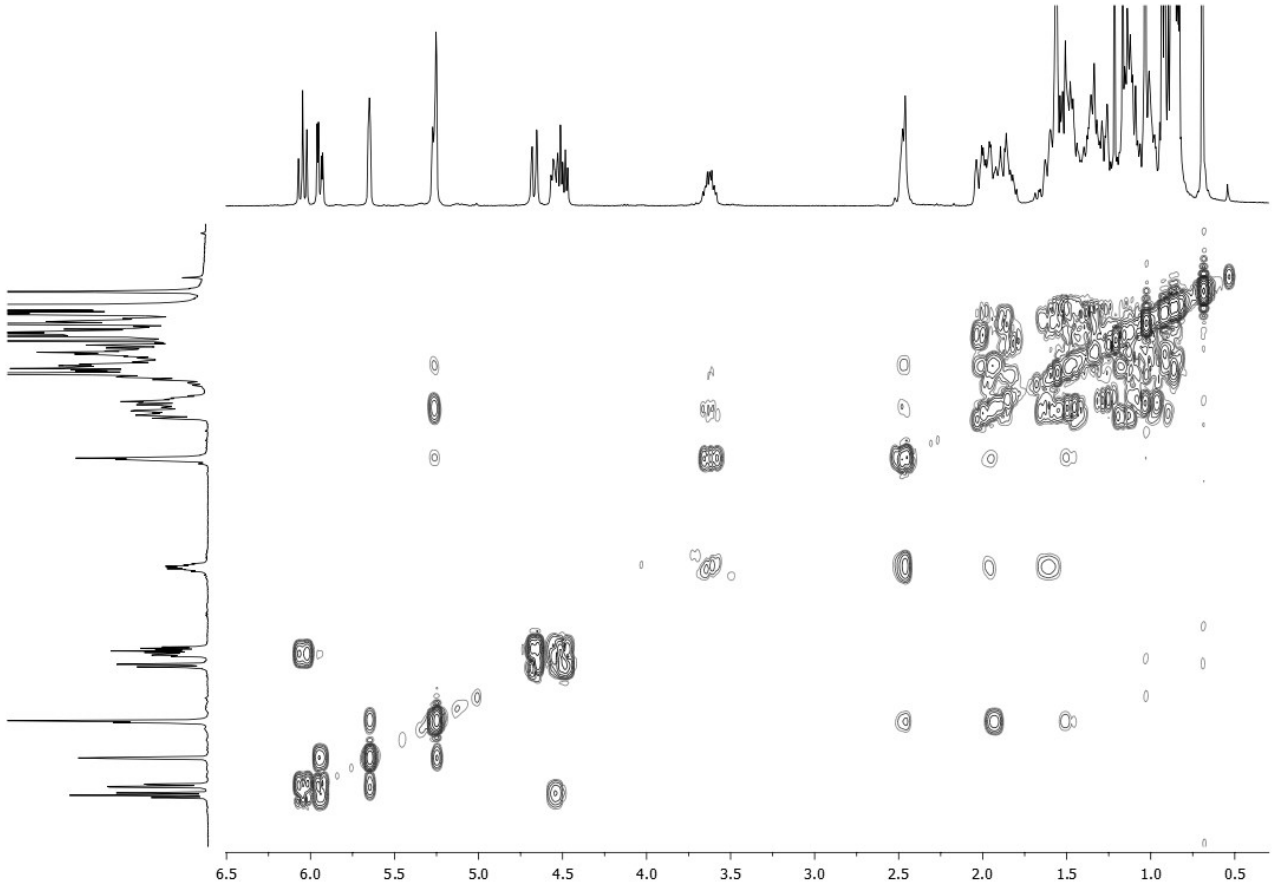
^{1}H- ^{1}H COSY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Cholesteryl α-D-mannopyranoside (3)

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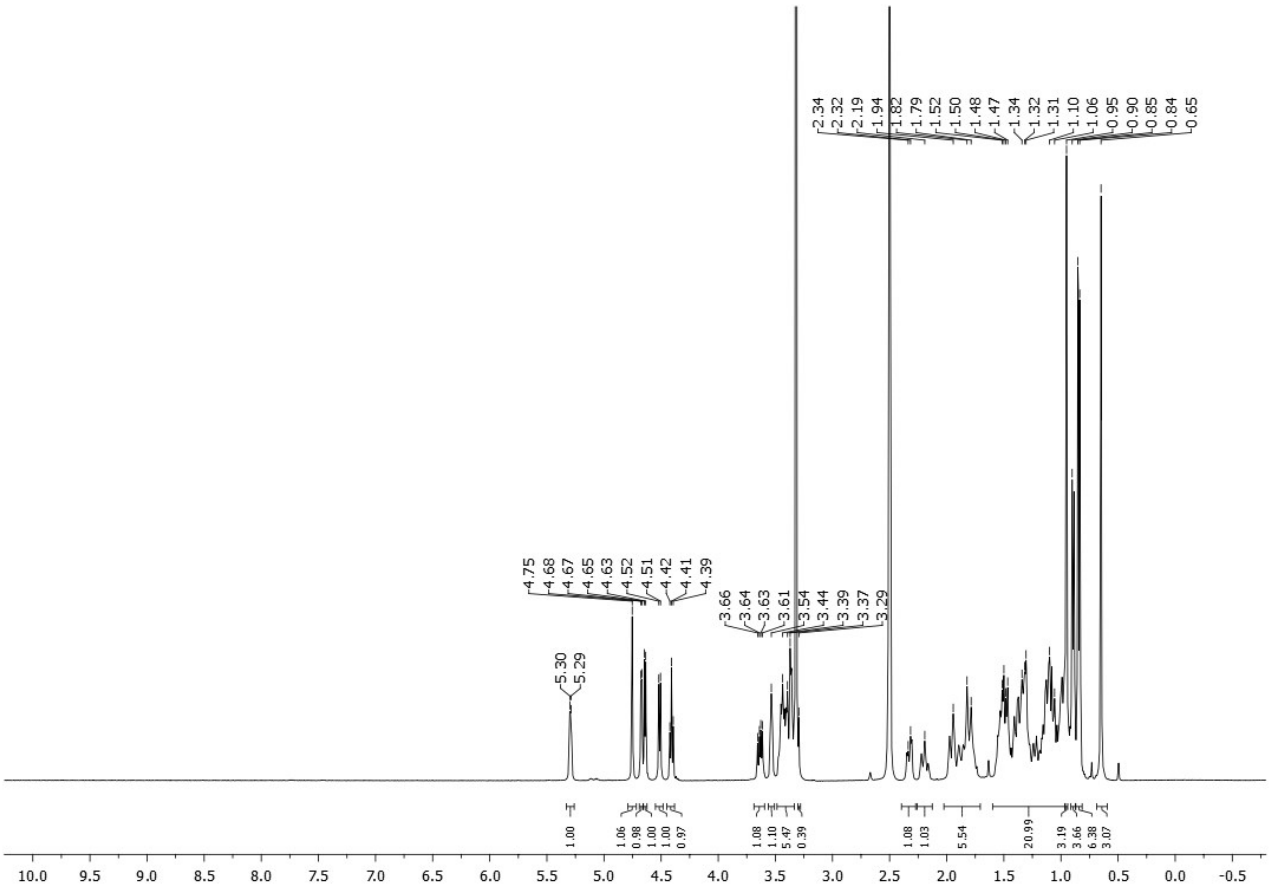
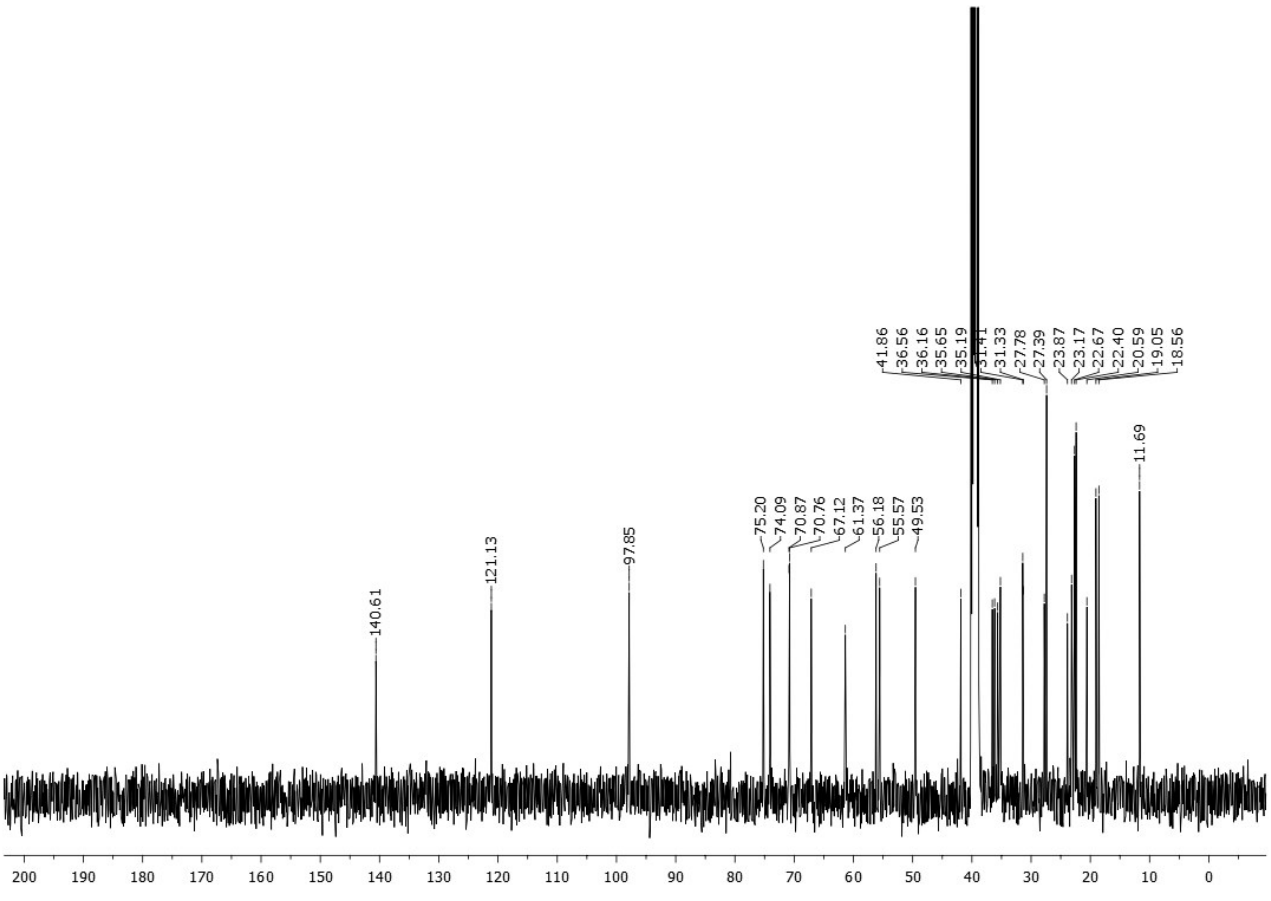
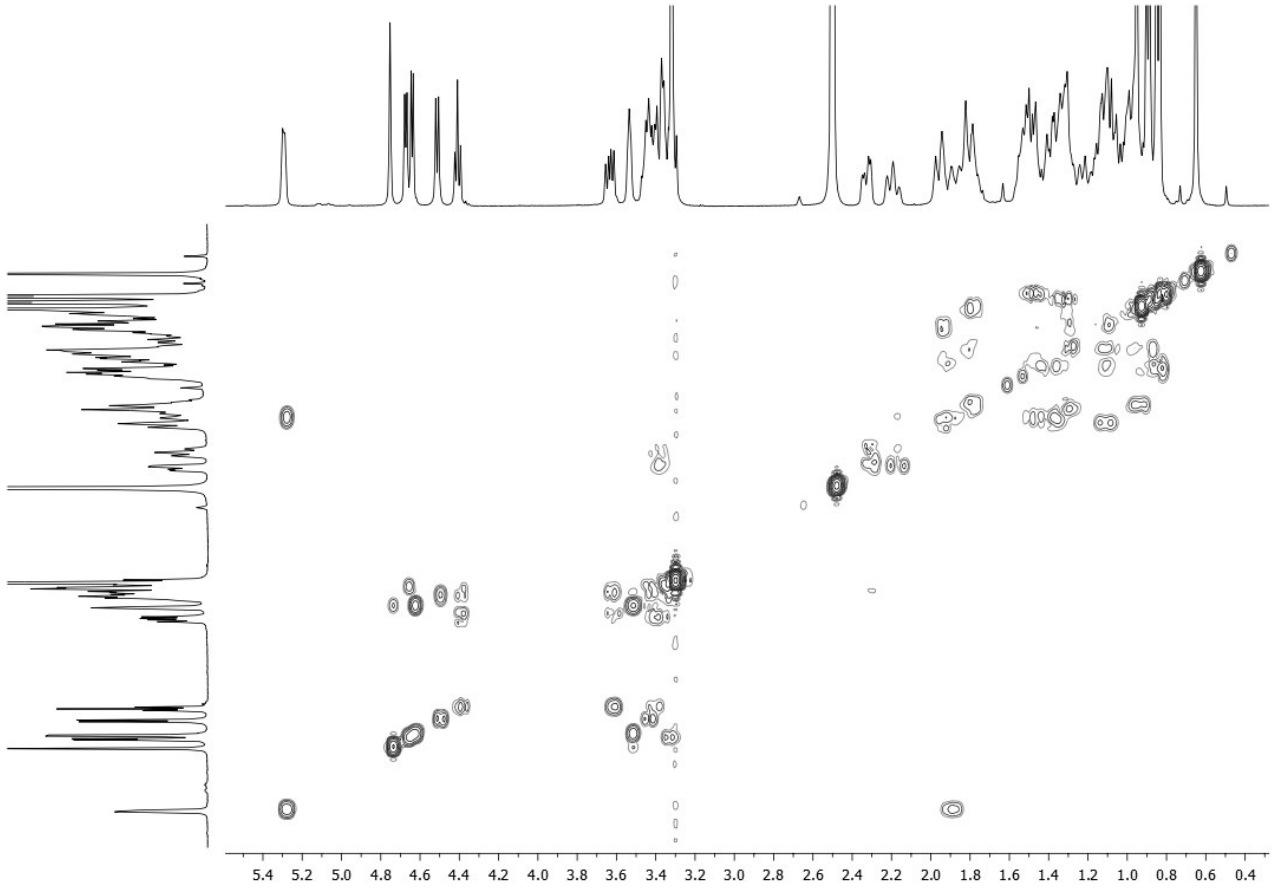


^{13}C NMR

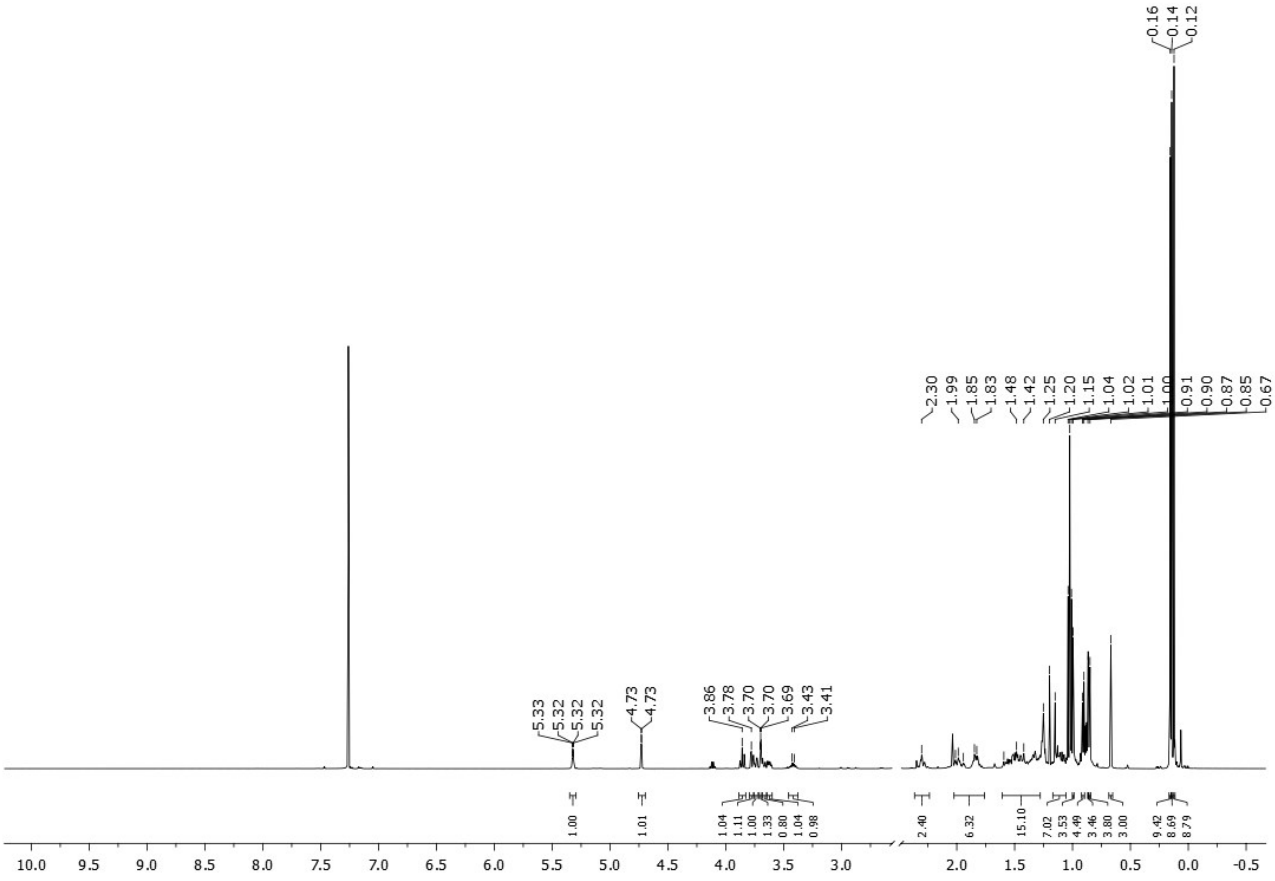


^{1}H- ^{1}H COSY

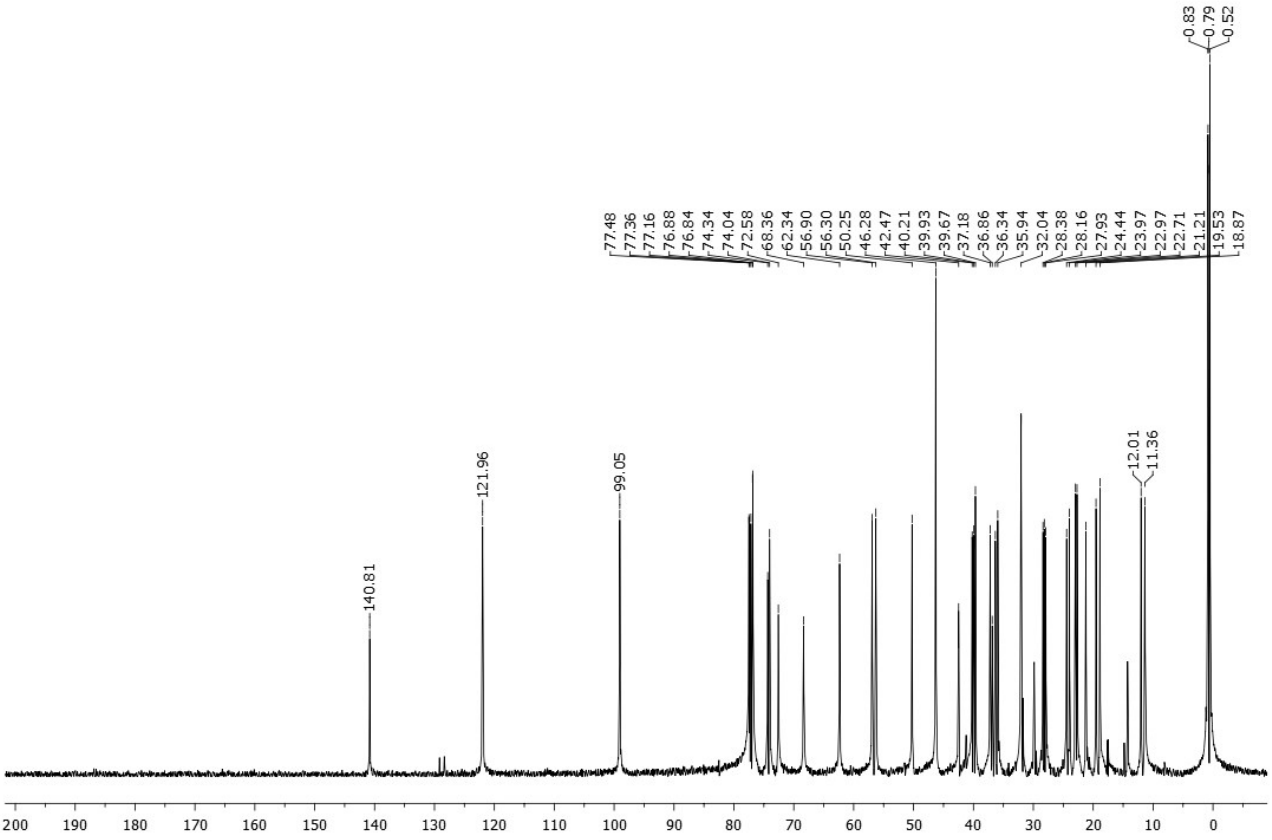

5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4

Cholesteryl 2,3,4-tri-*O*-trimethylsilyl-α-D-mannopyranoside (5)

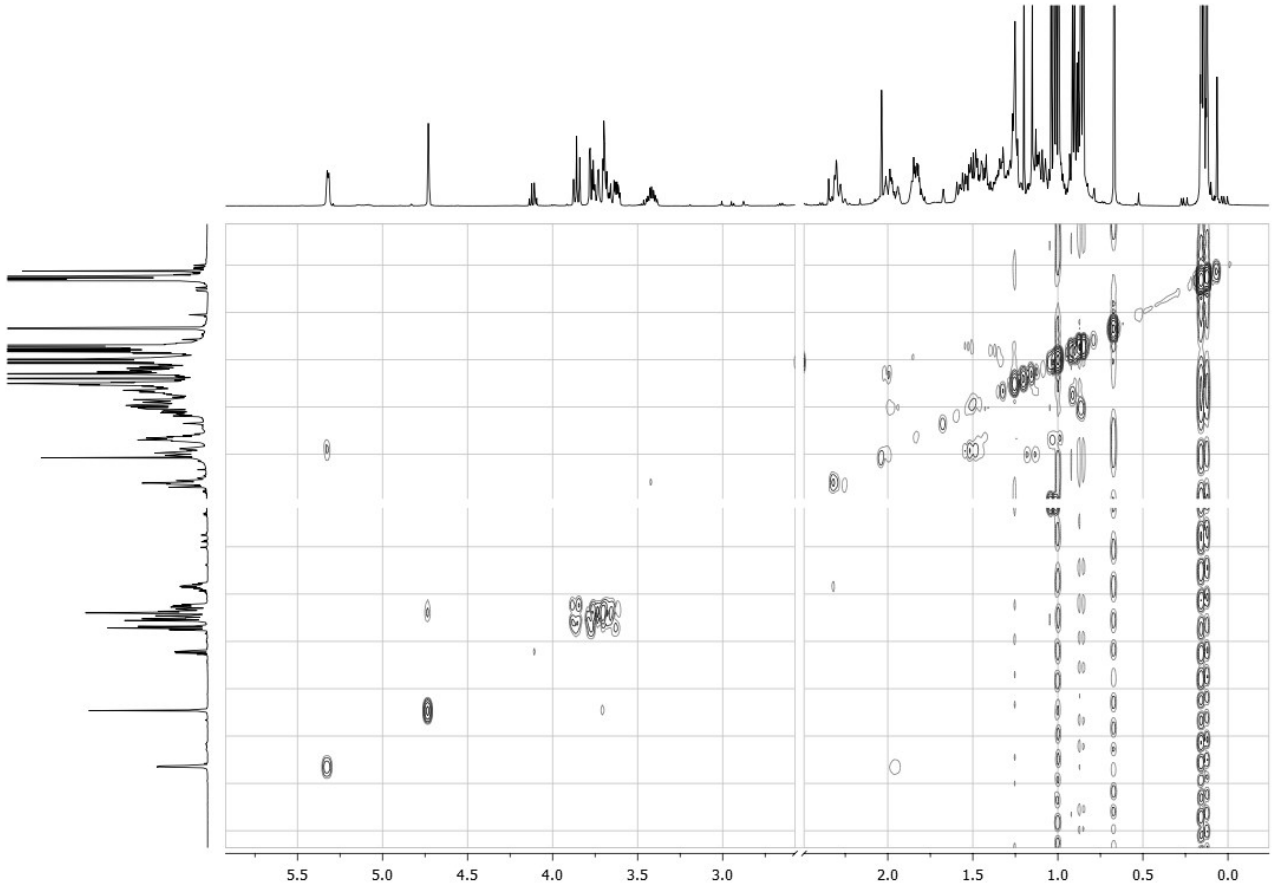
^{1}H NMR



^{13}C NMR

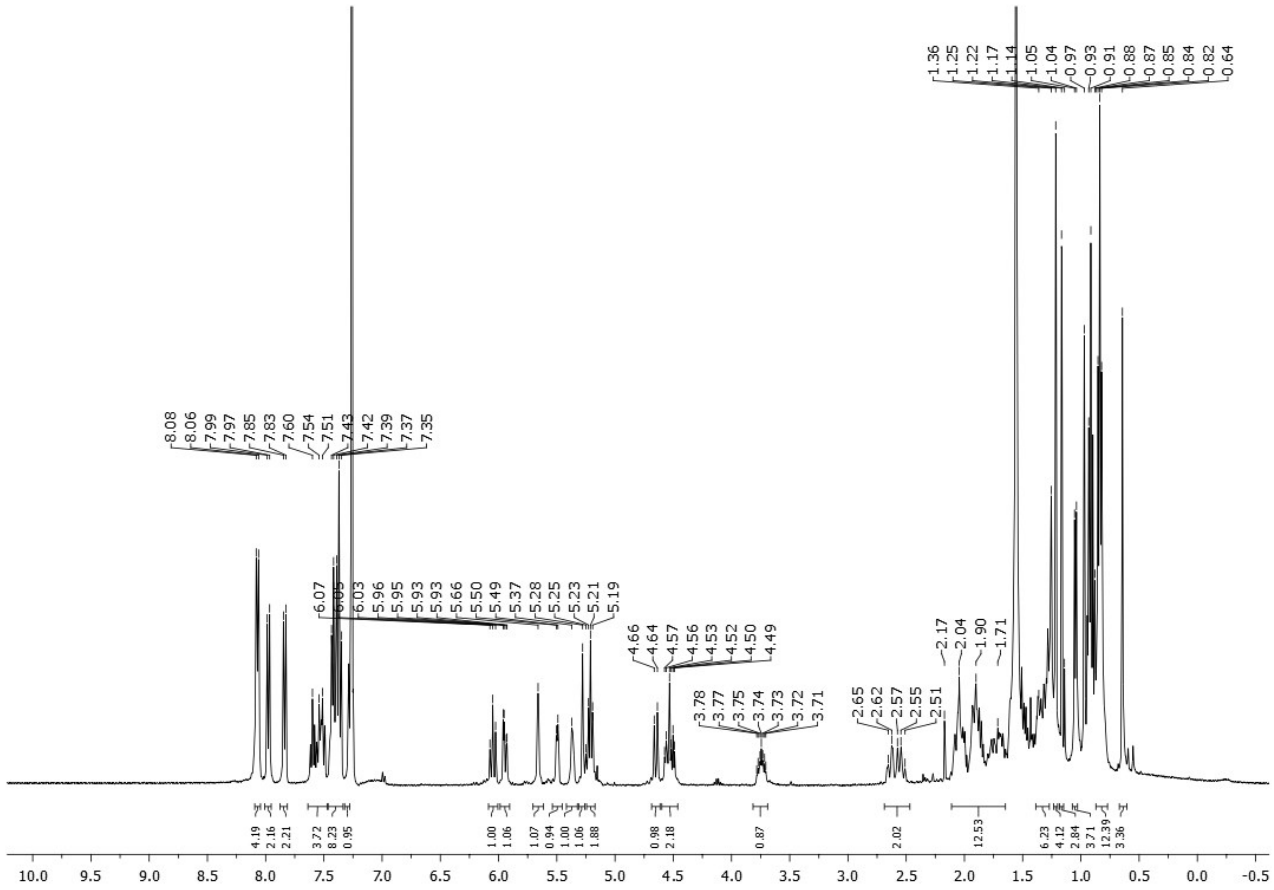


^{1}H- ^{1}H COSY

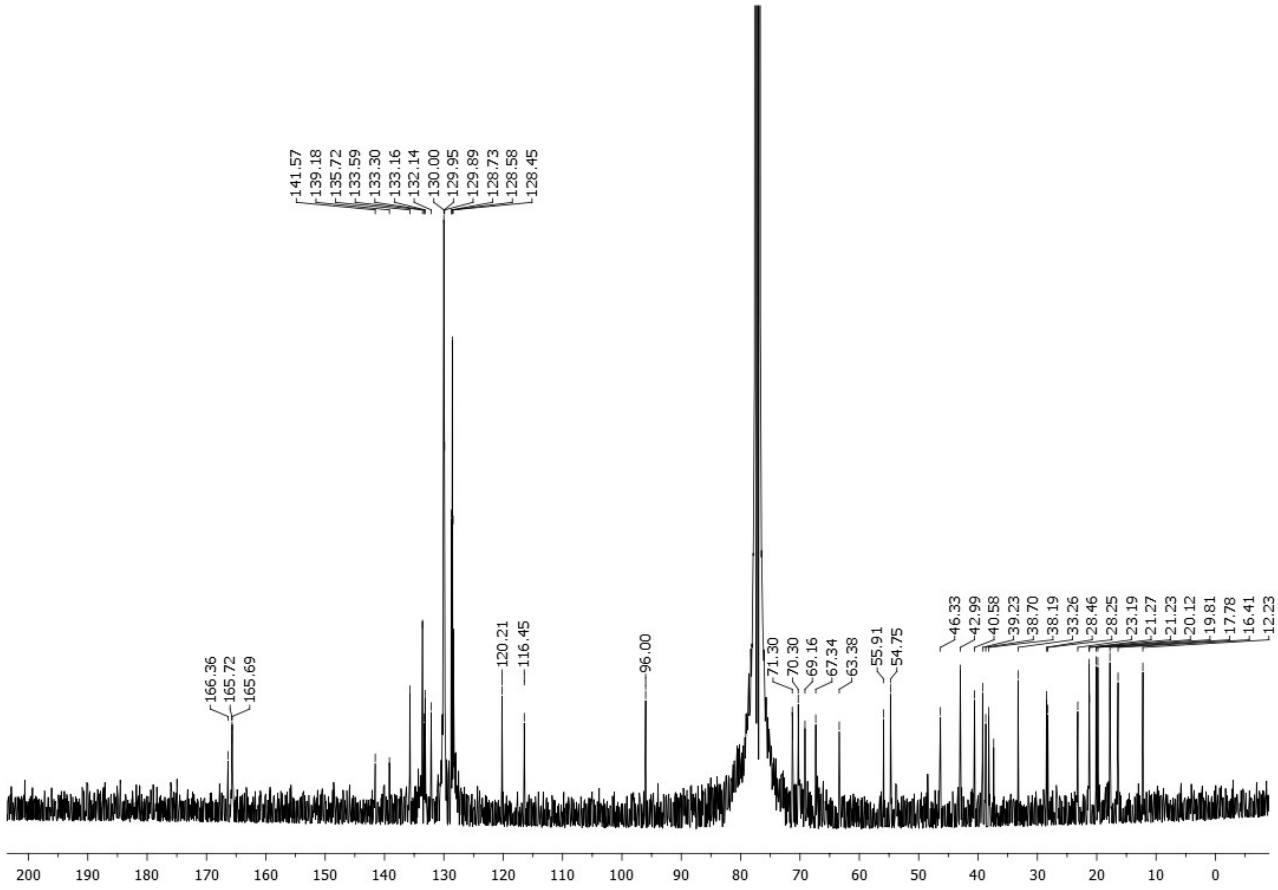


Ergosteryl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside (2)

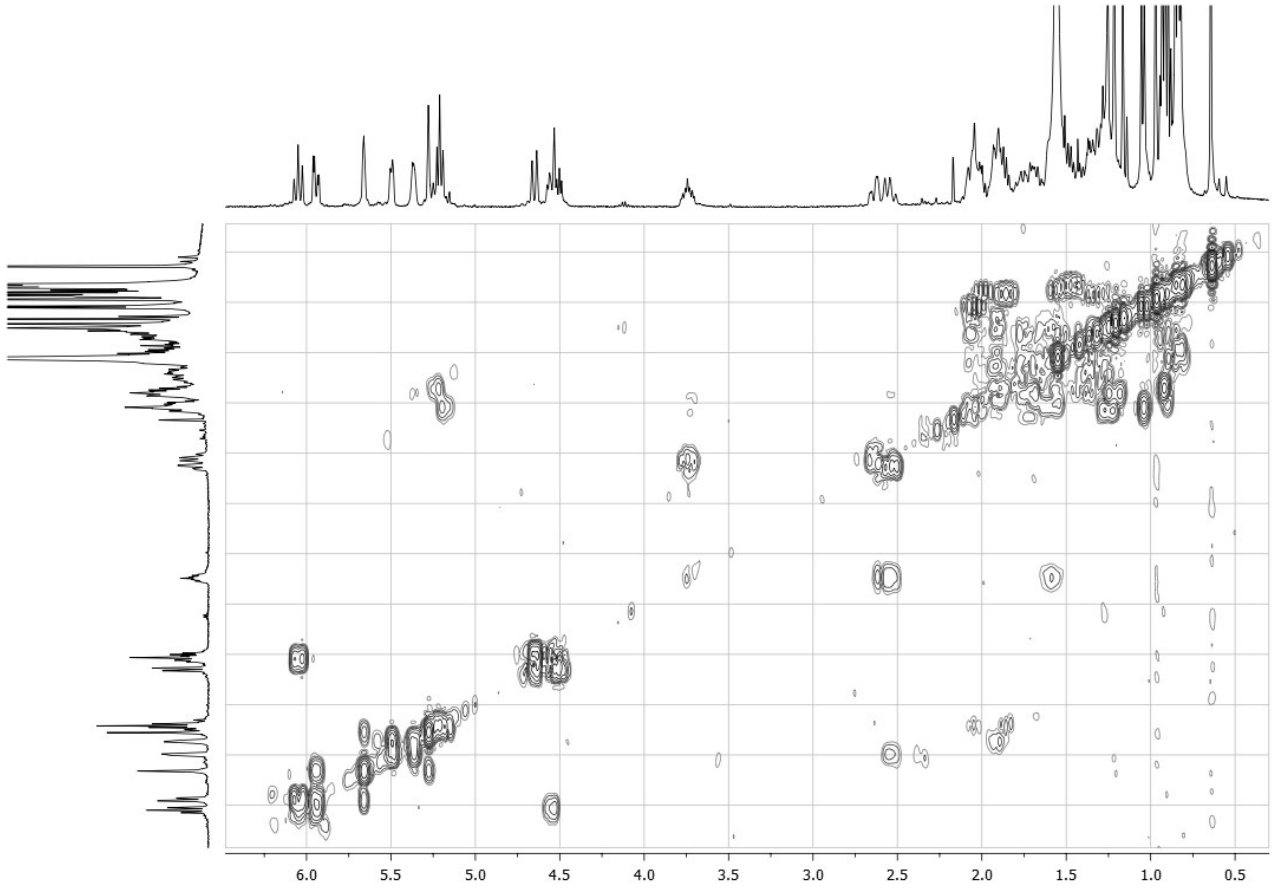
^{1}H NMR



^{13}C NMR

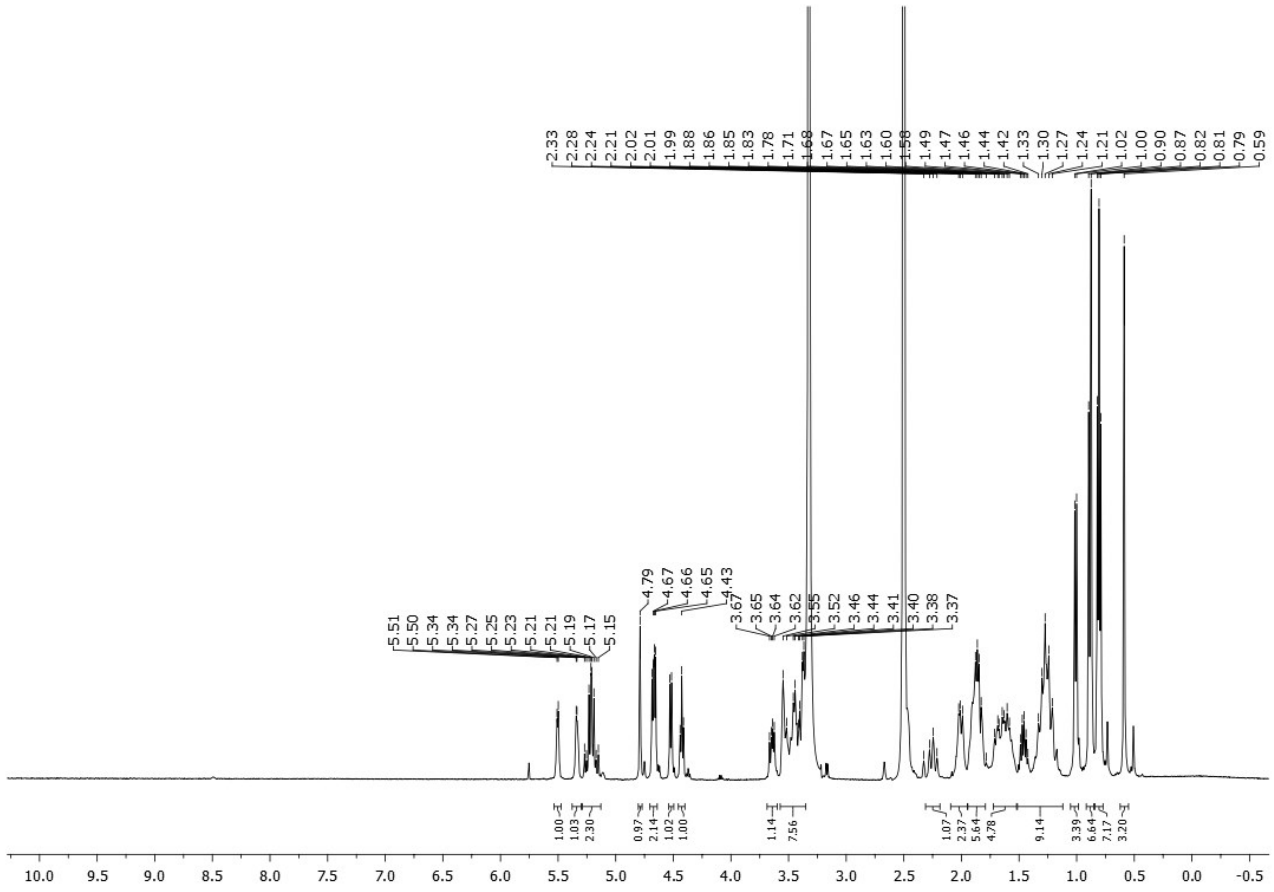


^{1}H- ^{1}H COSY

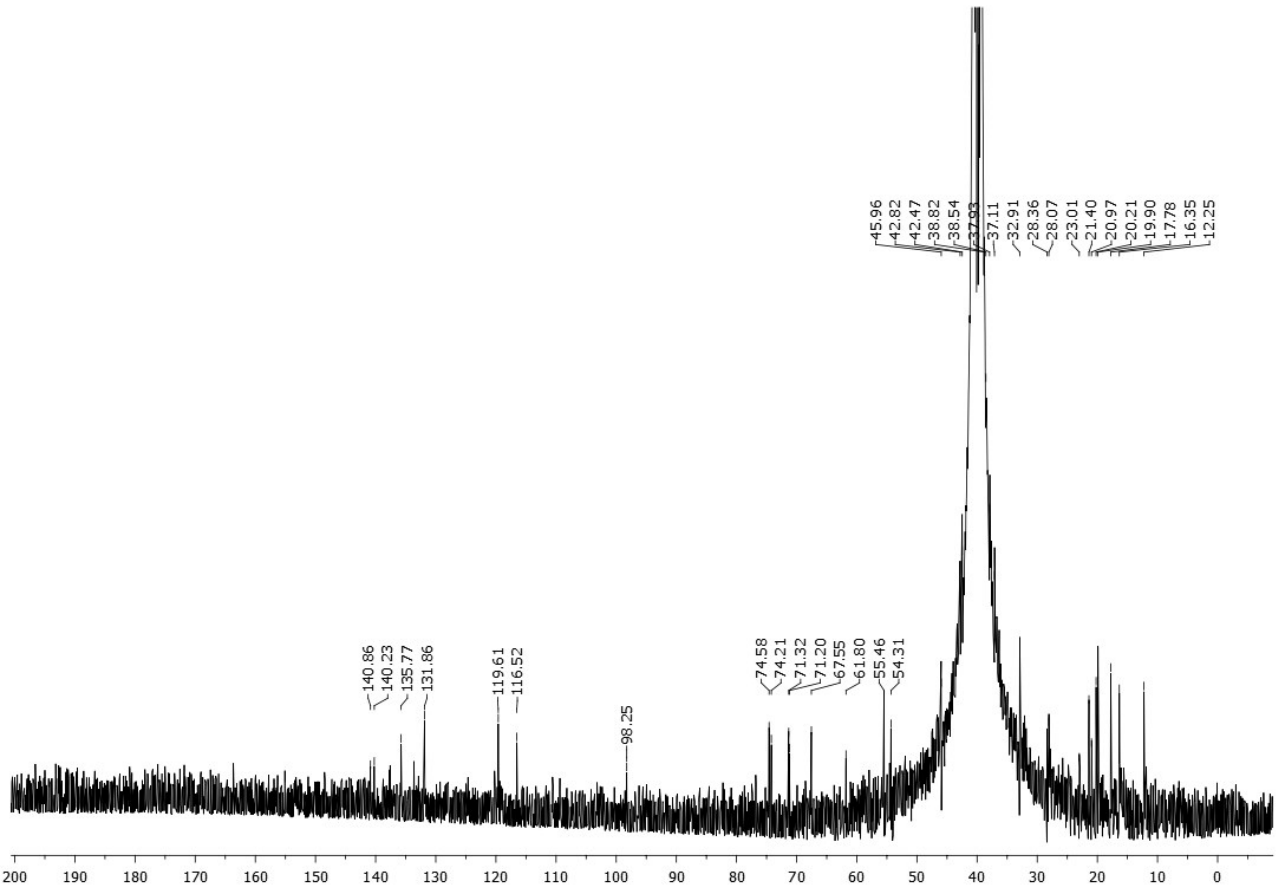


Ergosteryl α-D-mannopyranoside (4)

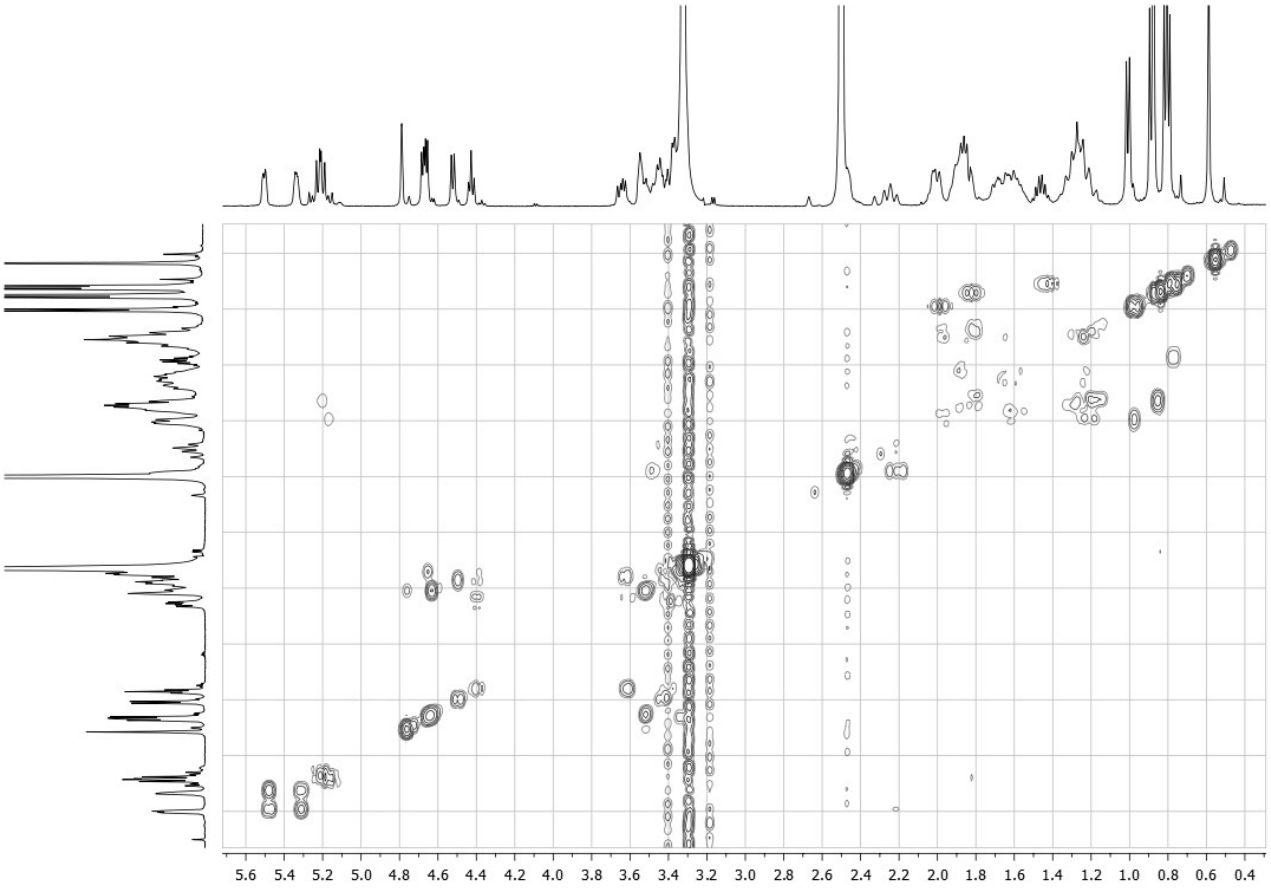
^{1}H NMR



^{13}C NMR

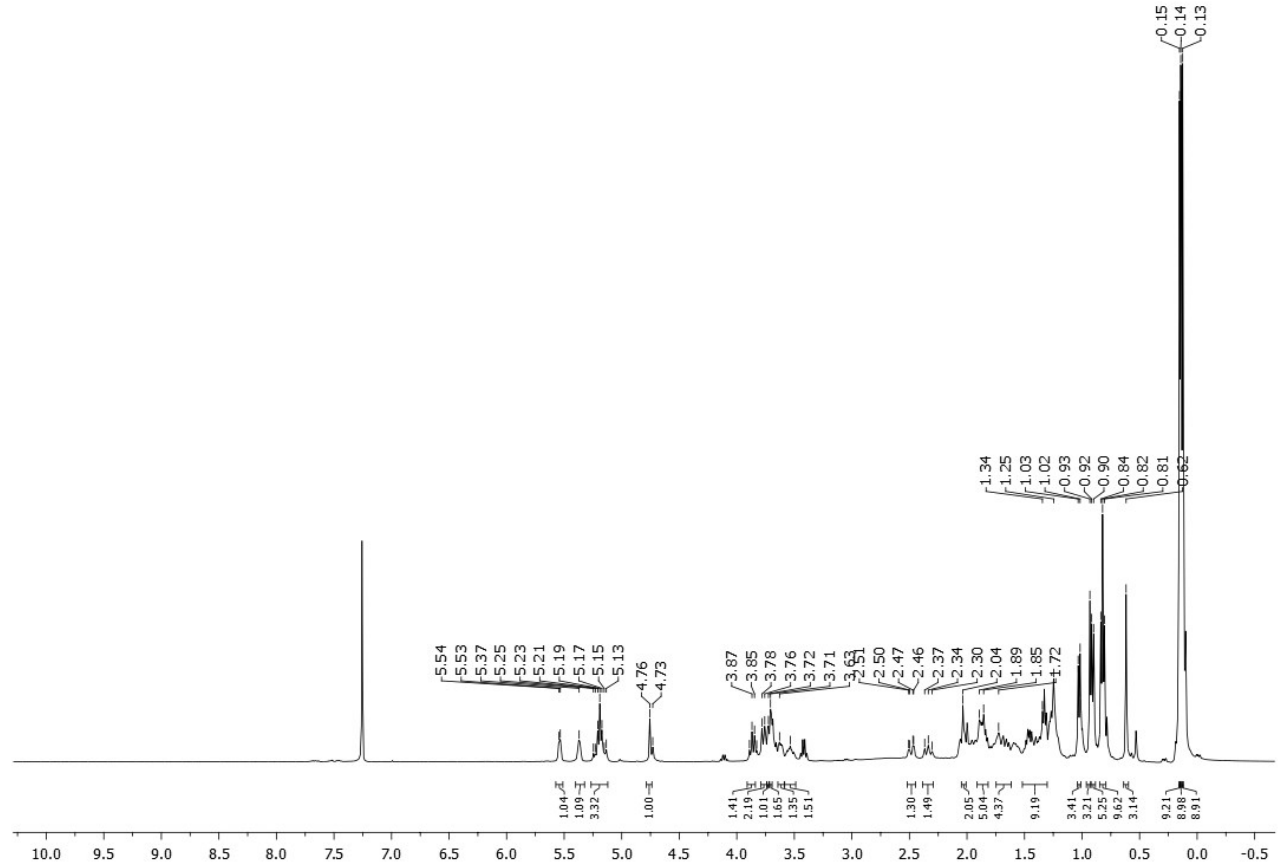


^{1}H- ^{1}H COSY

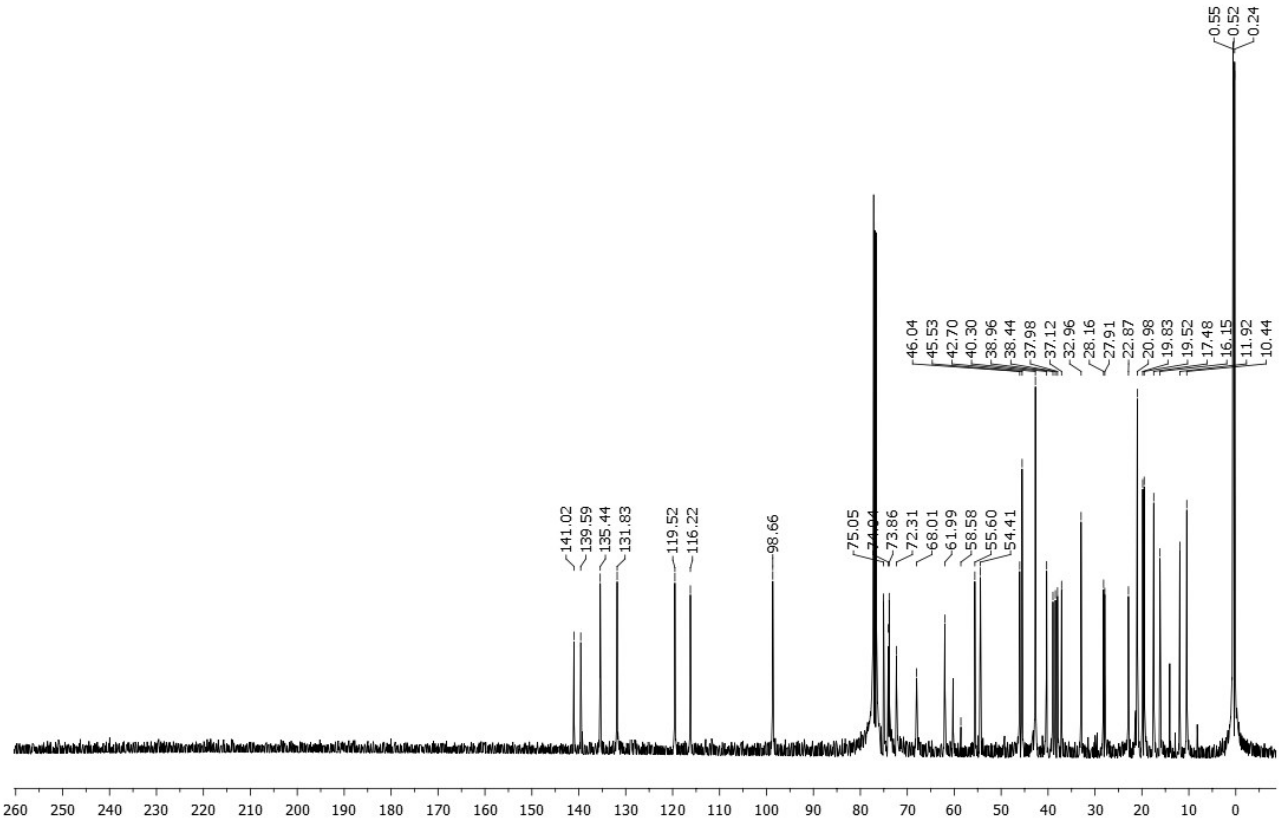

5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4

Ergosteryl 2,3,4-tri-*O*-trimethylsilyl-α-D-mannopyranoside (6)

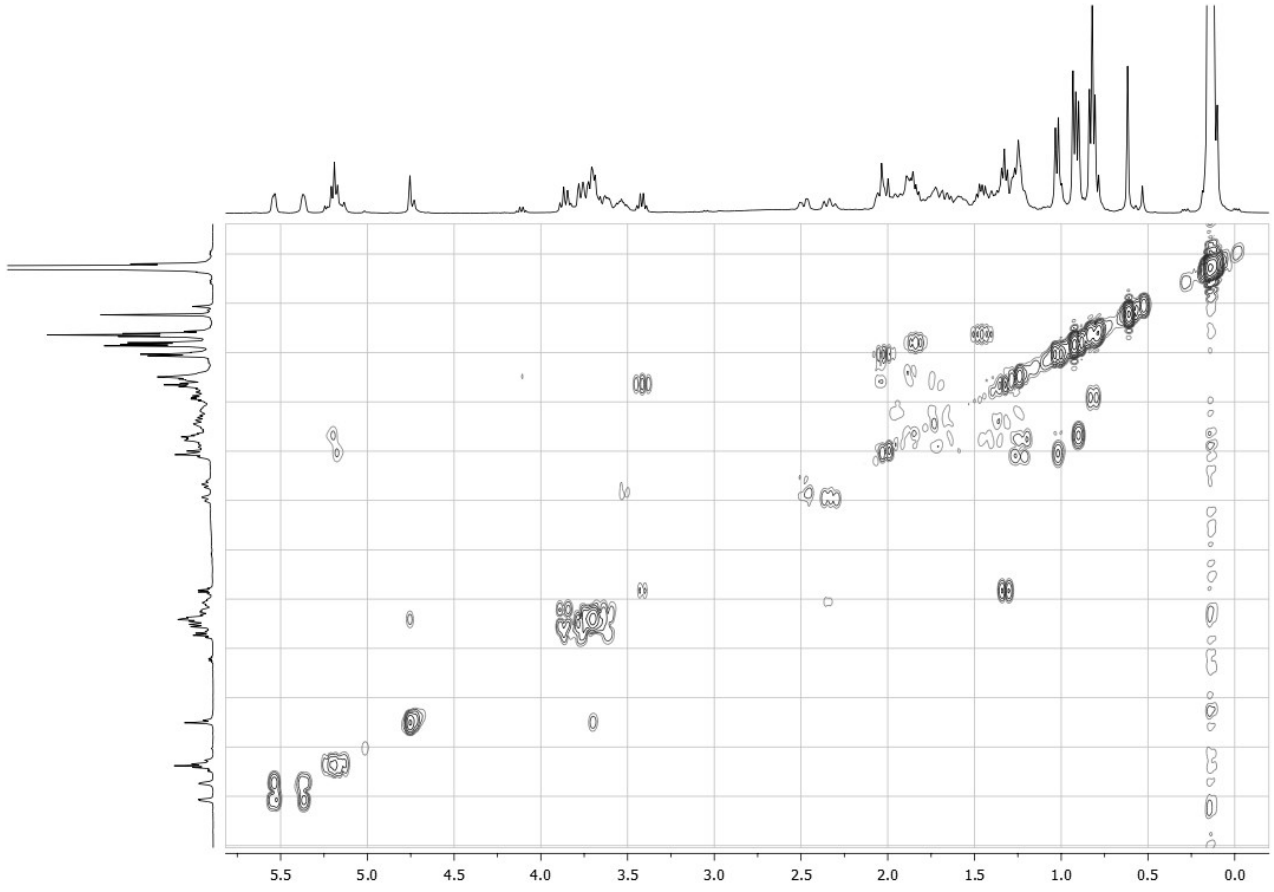
^{1}H NMR



^{13}C NMR

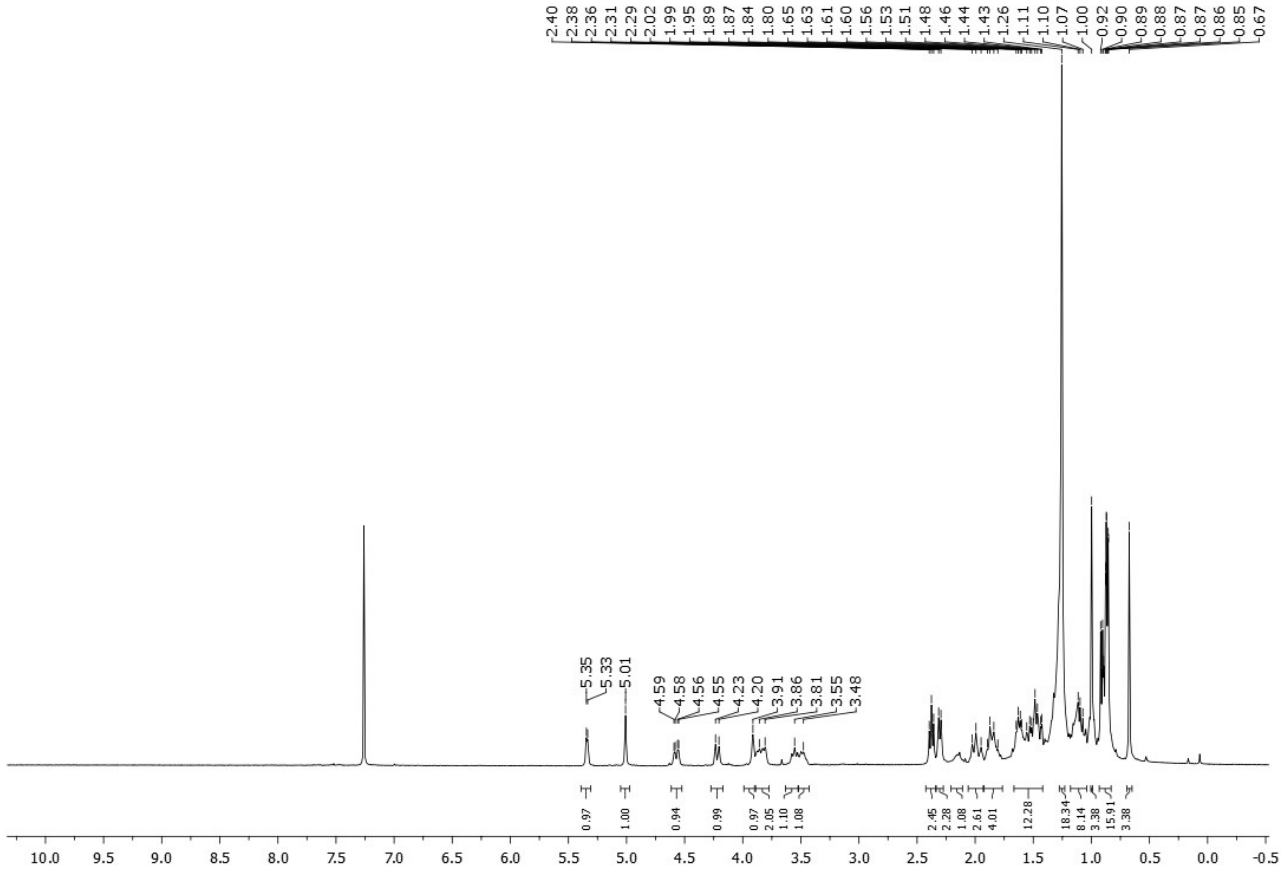


^{1}H- ^{1}H COSY

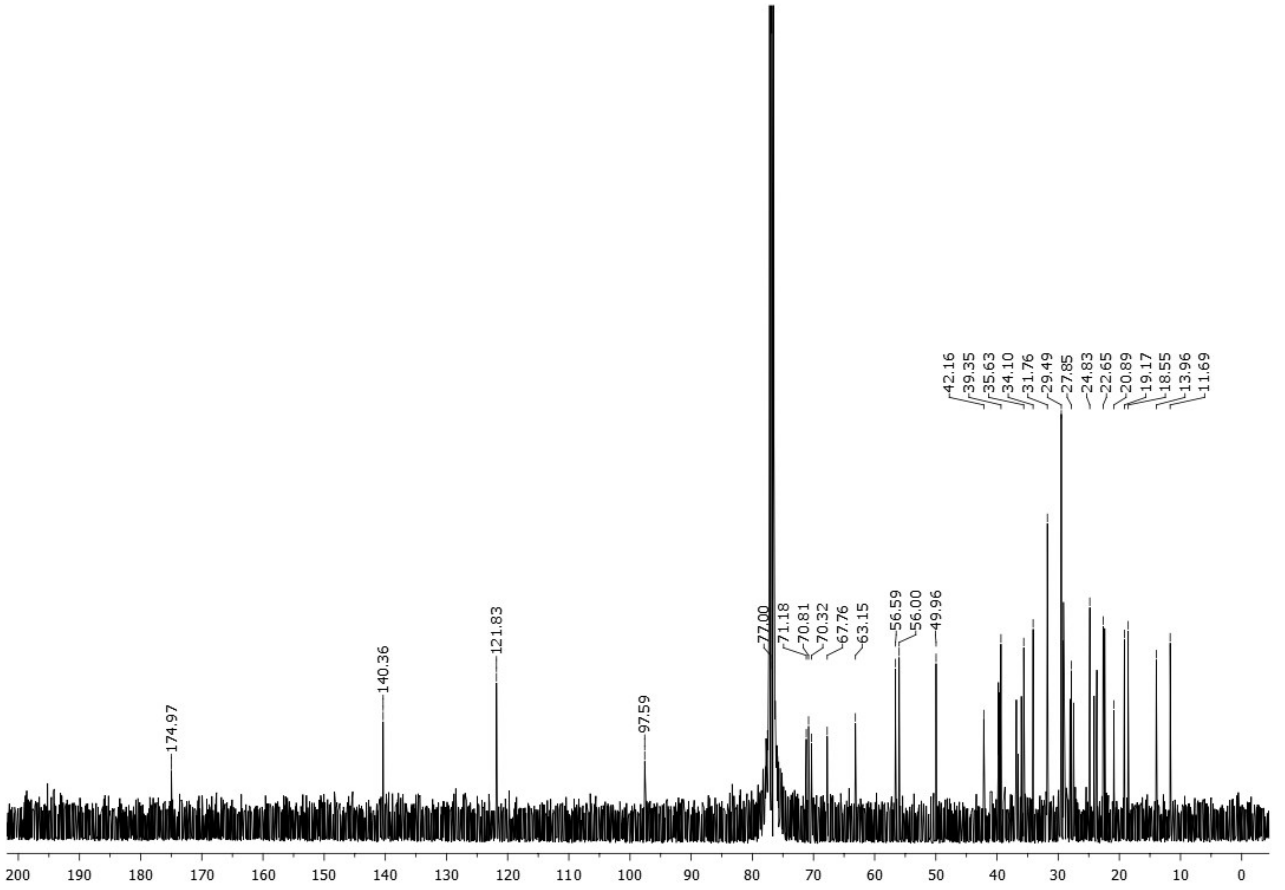


Cholesteryl 6-*O*-dodecanoyl-α-D-mannopyranoside (7)

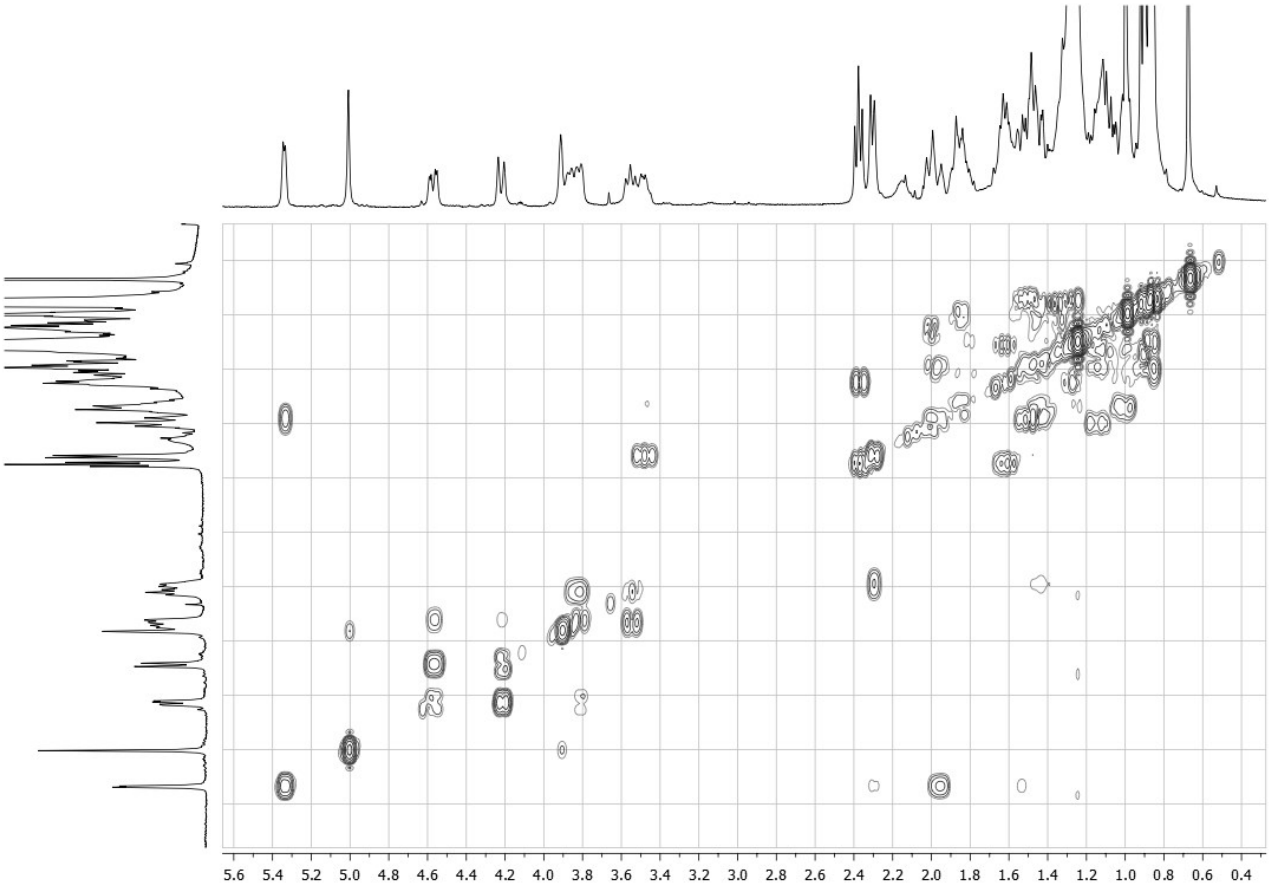
^{1}H NMR



^{13}C NMR

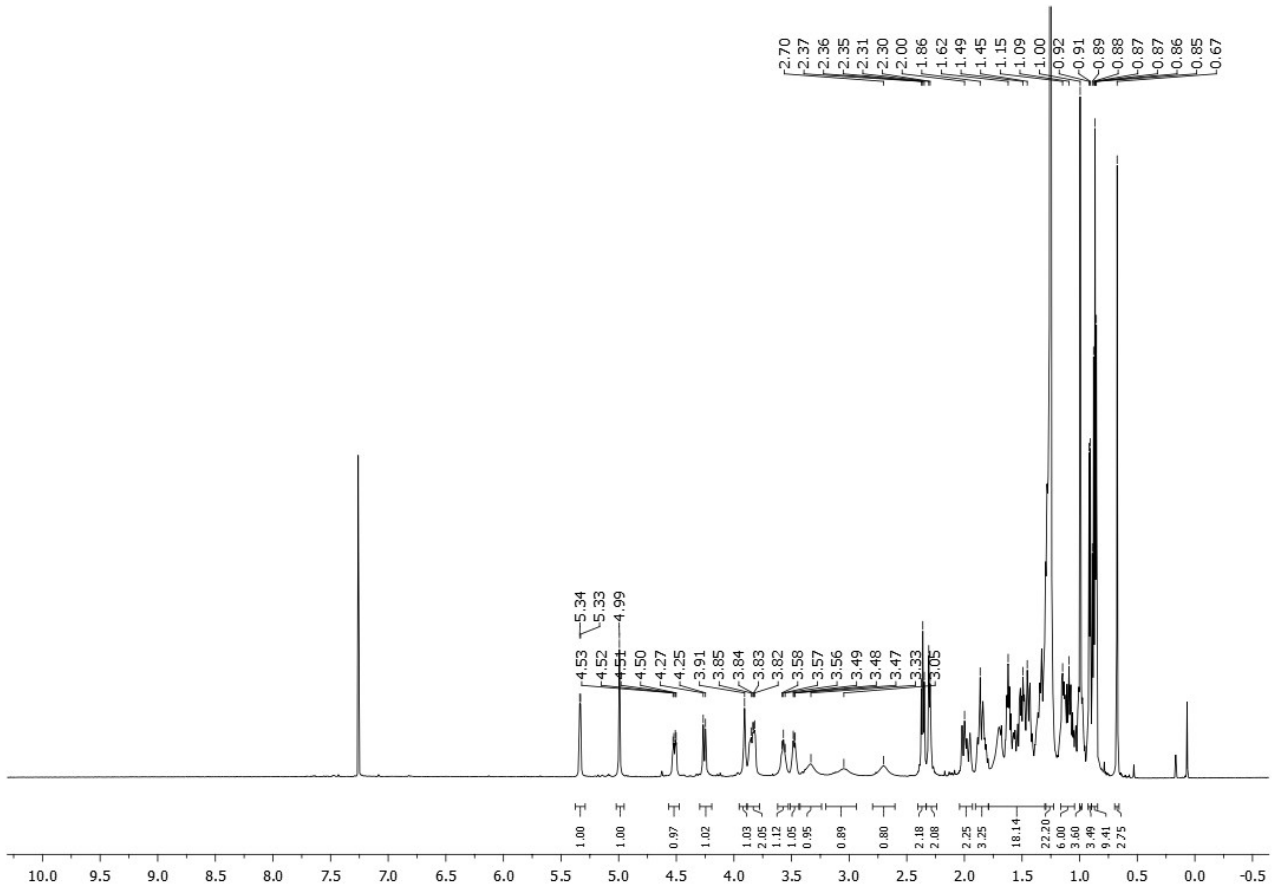


^{1}H- ^{1}H COSY

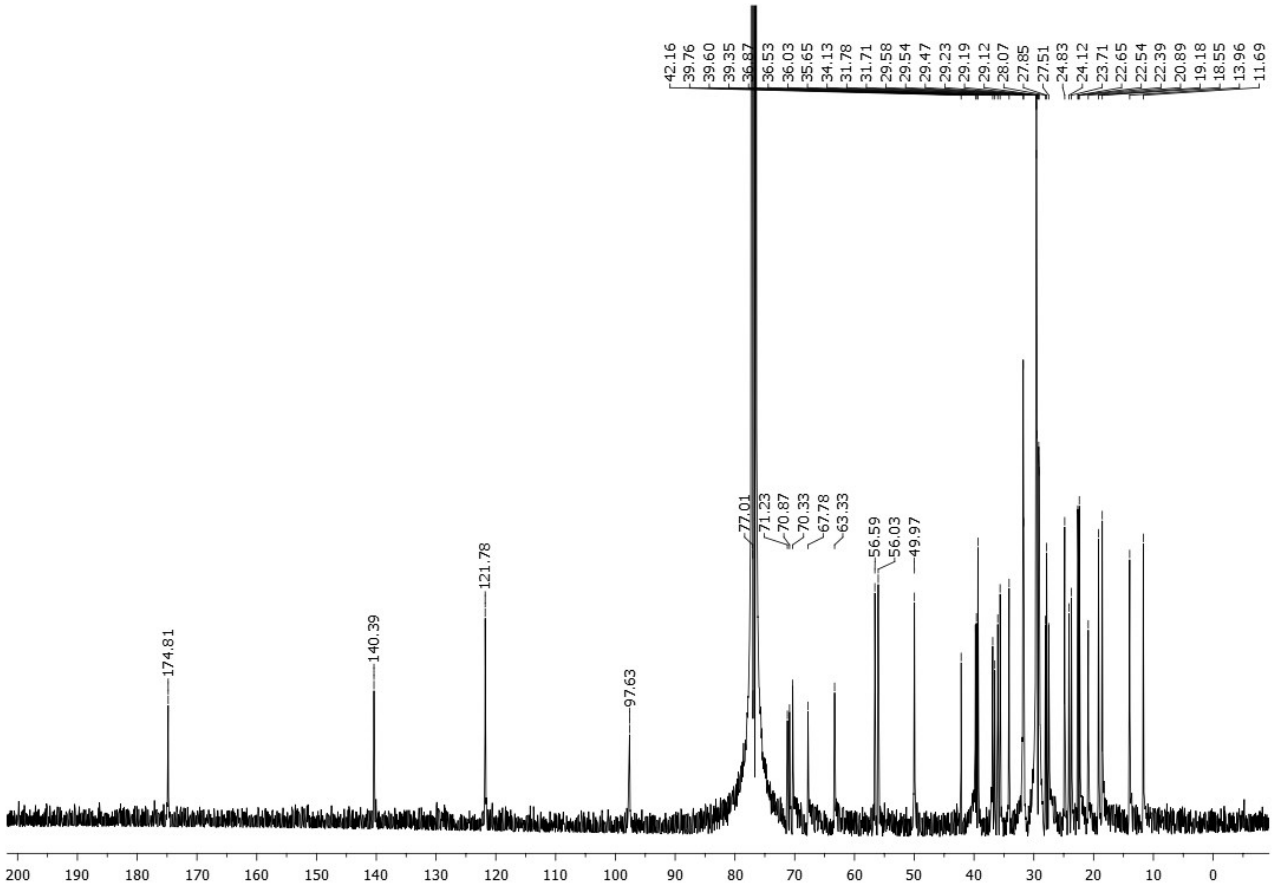

5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4

Cholesteryl 6-*O*-tetradecanoyl-α-D-mannopyranoside (8)

^{1}H NMR

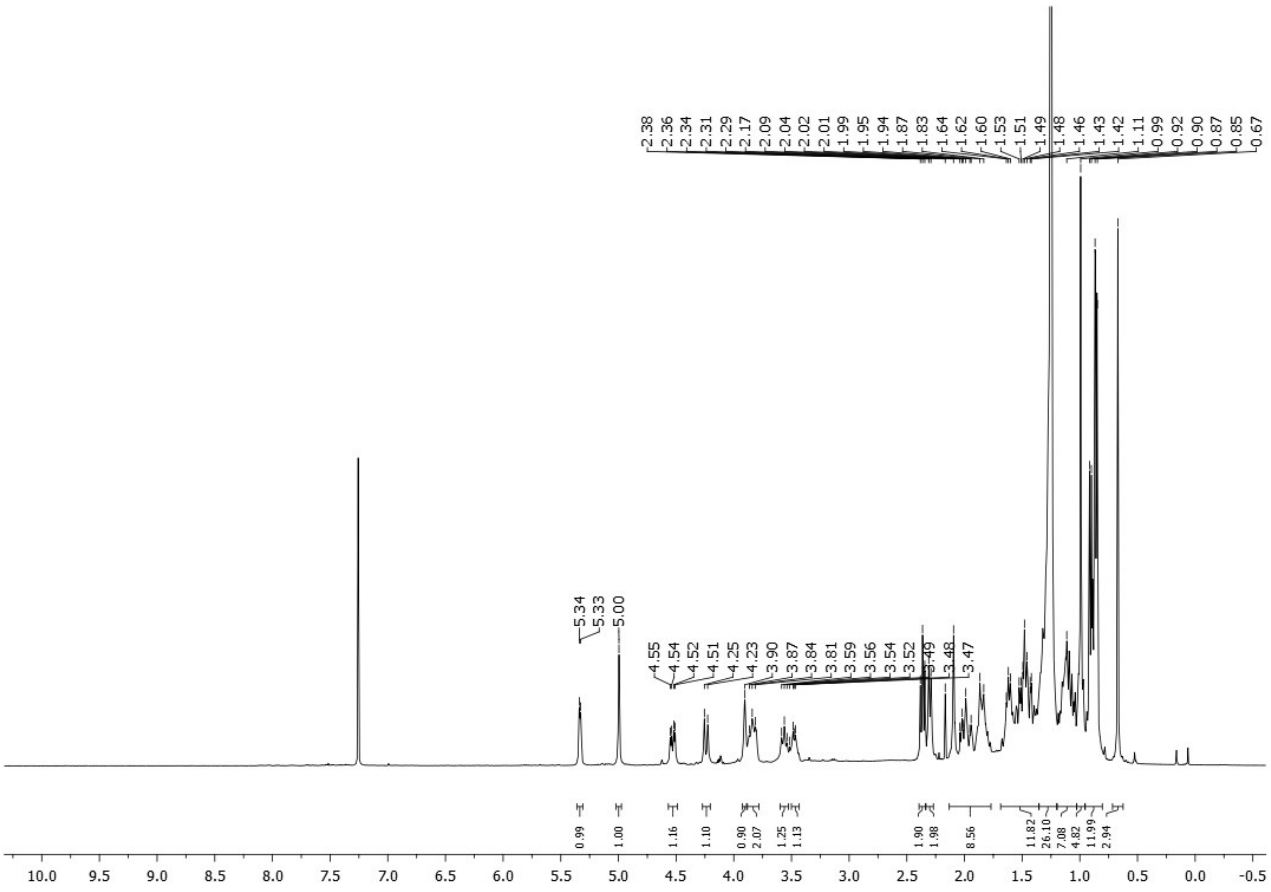


^{13}C NMR

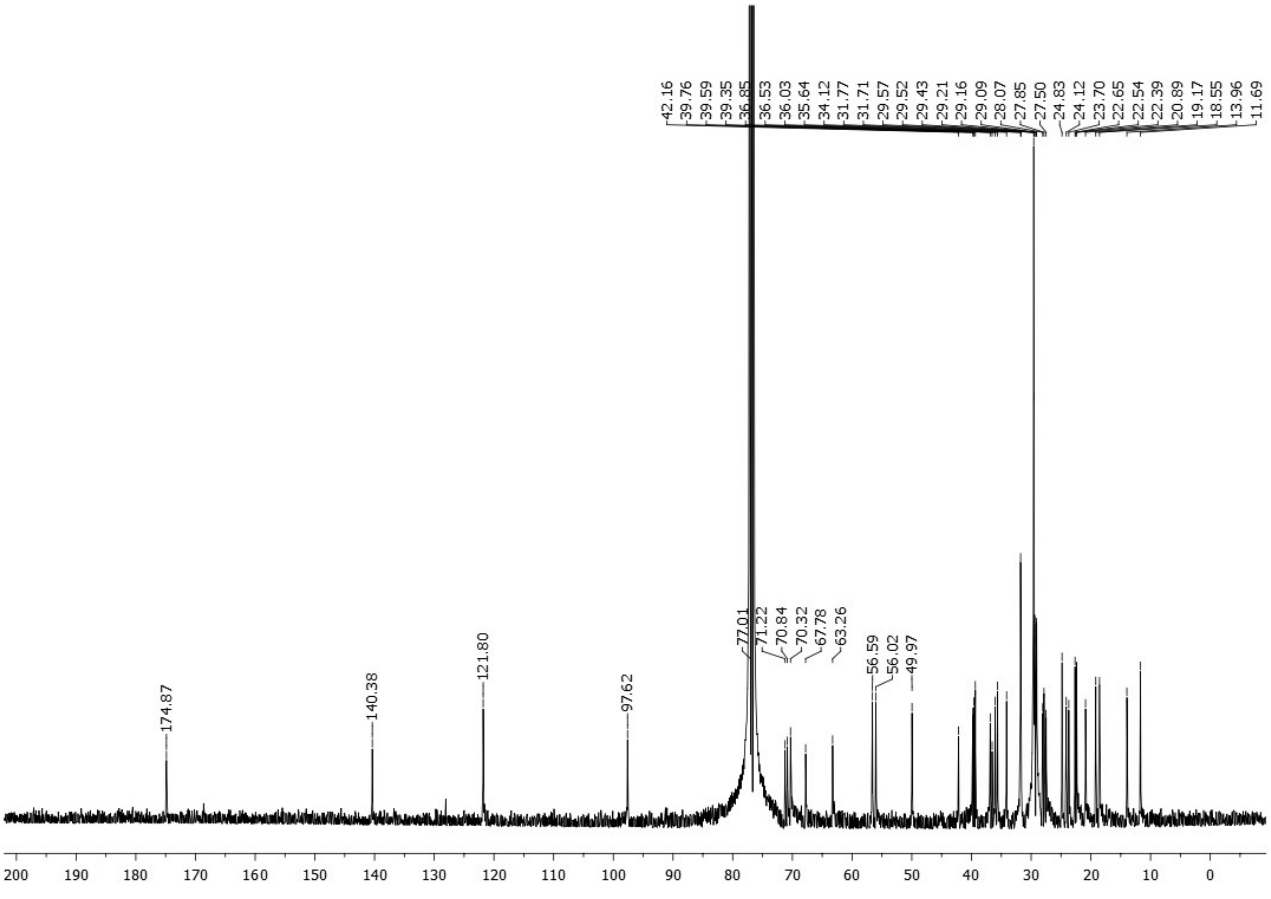


Cholesteryl 6-*O*-hexadecanoyl-α-D-mannopyranoside (9)

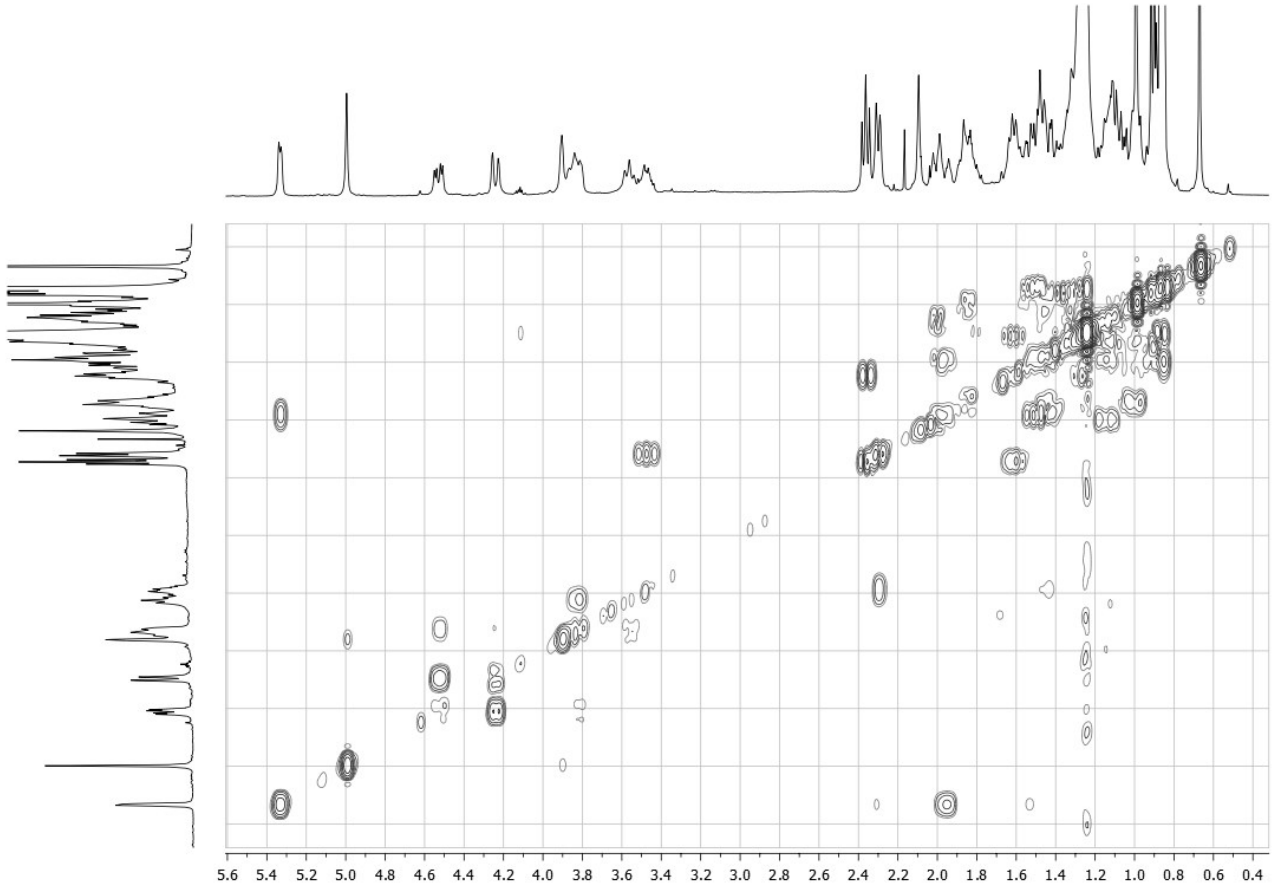
^{1}H NMR



^{13}C NMR

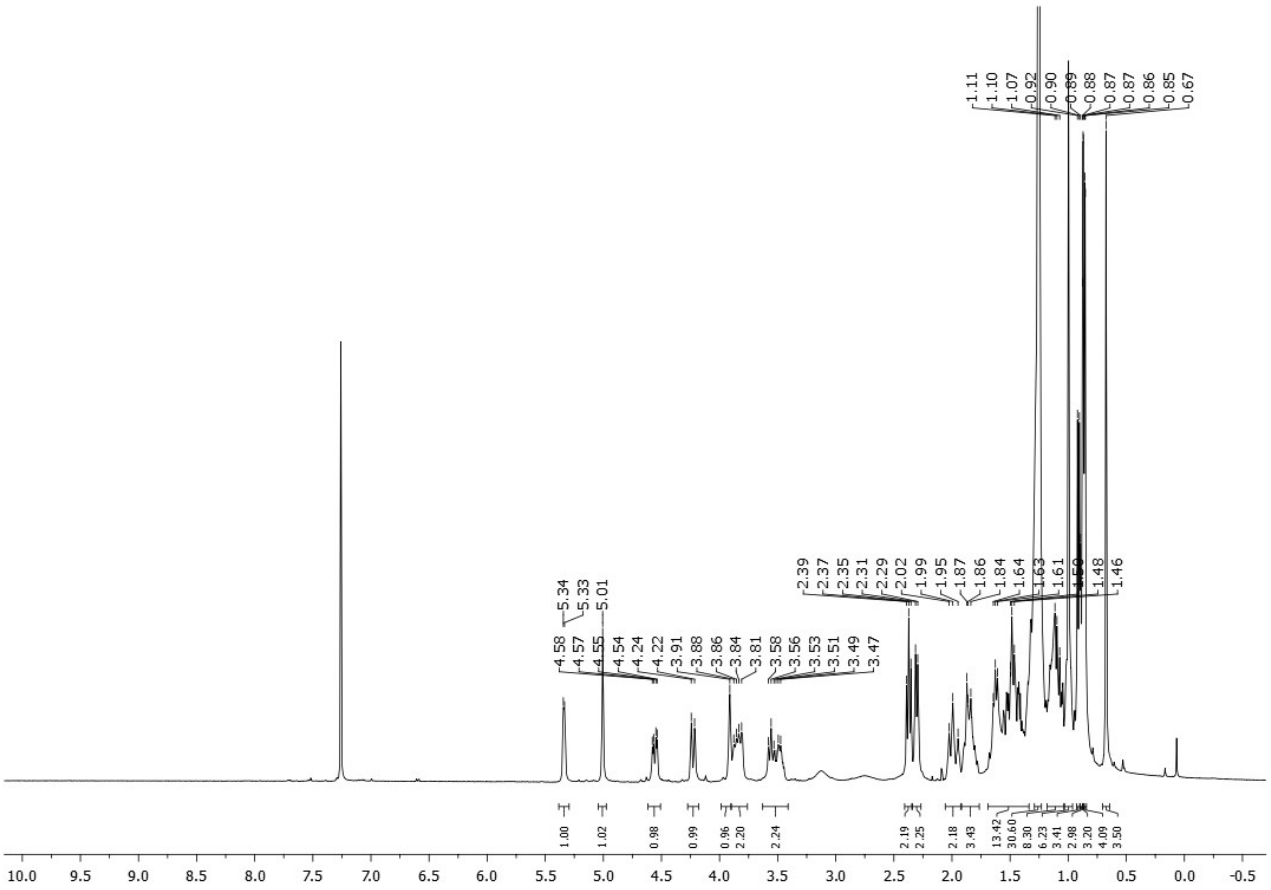


^{1}H- ^{1}H COSY

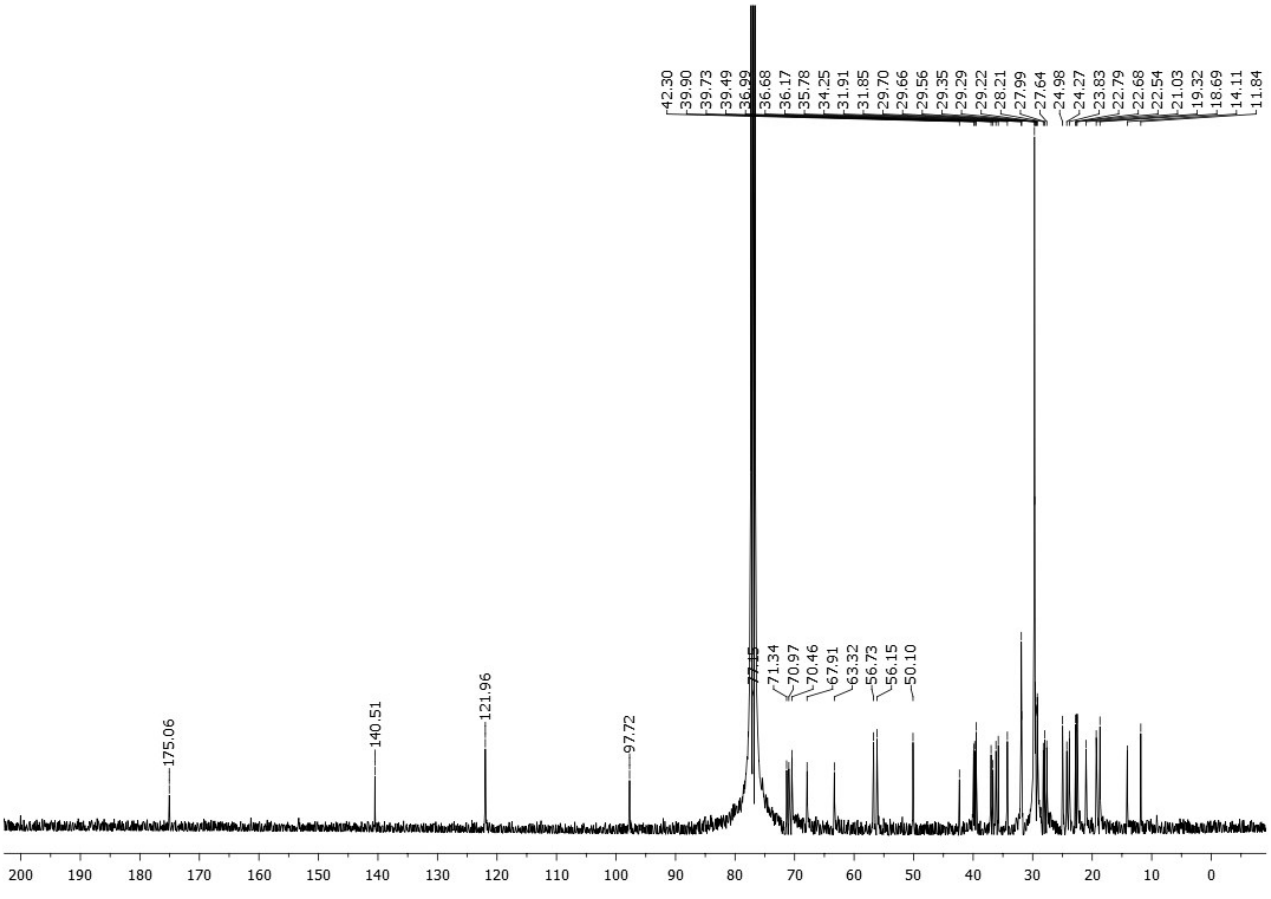

5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4

Cholesteryl 6-*O*-octadecanoyl-α-D-mannopyranoside (10)

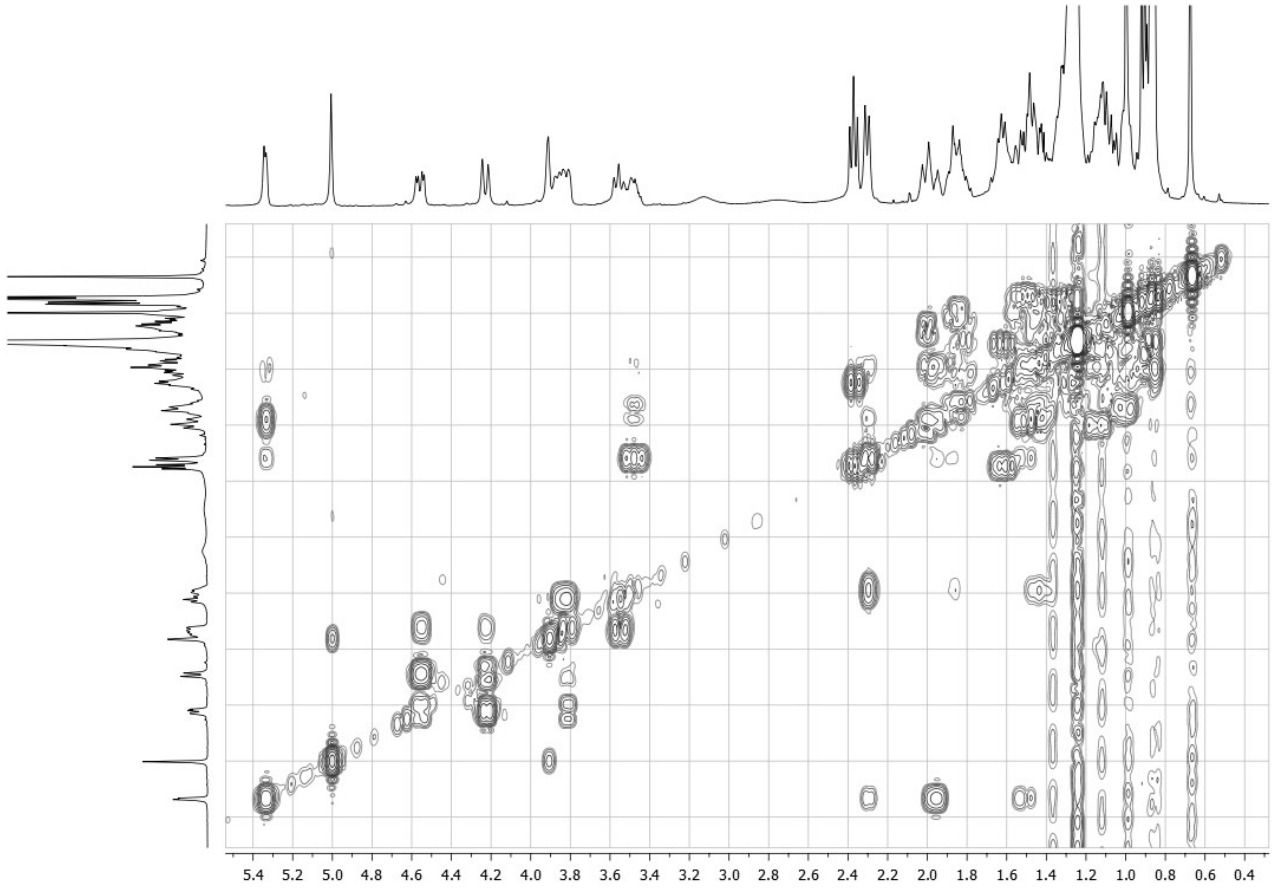
^{1}H NMR



^{13}C NMR

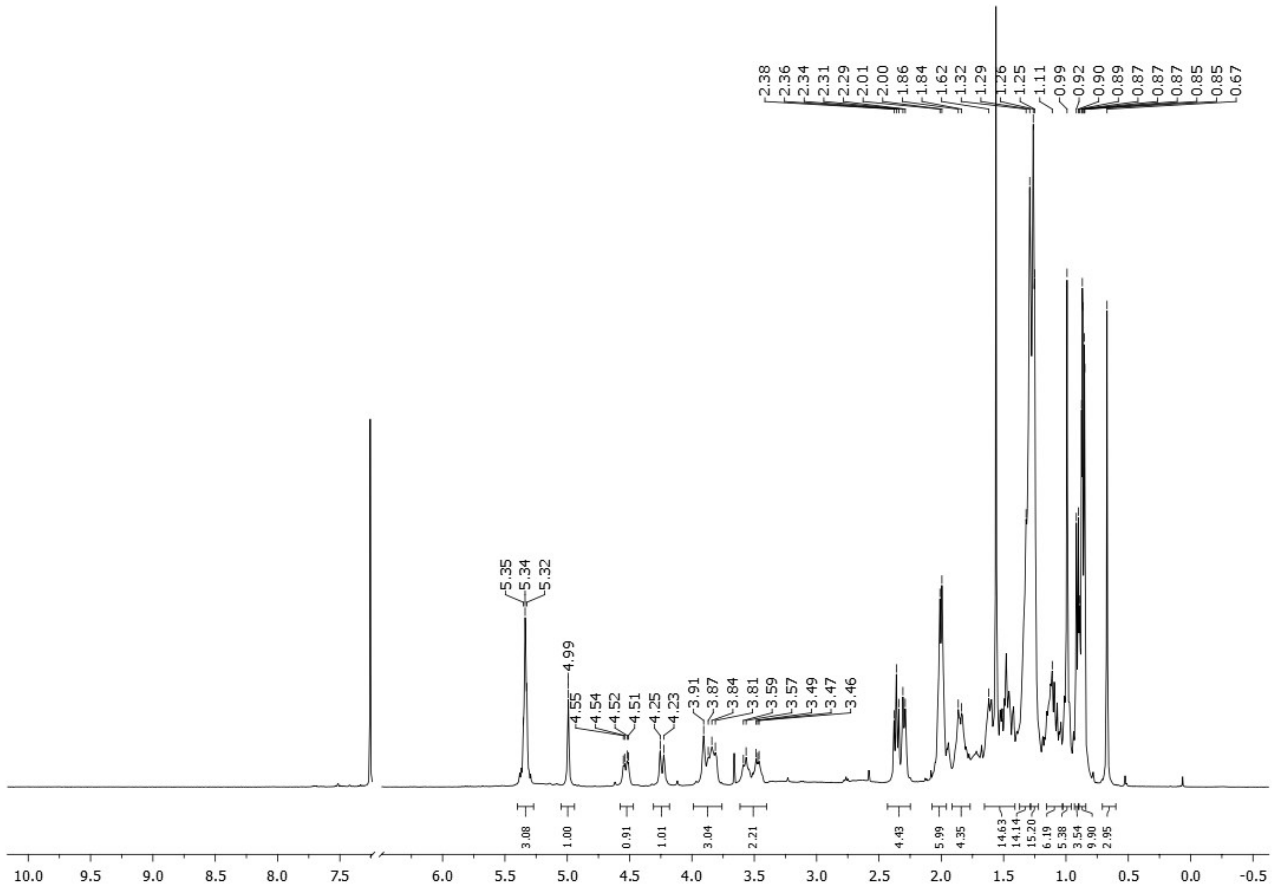


^{1}H- ^{1}H COSY

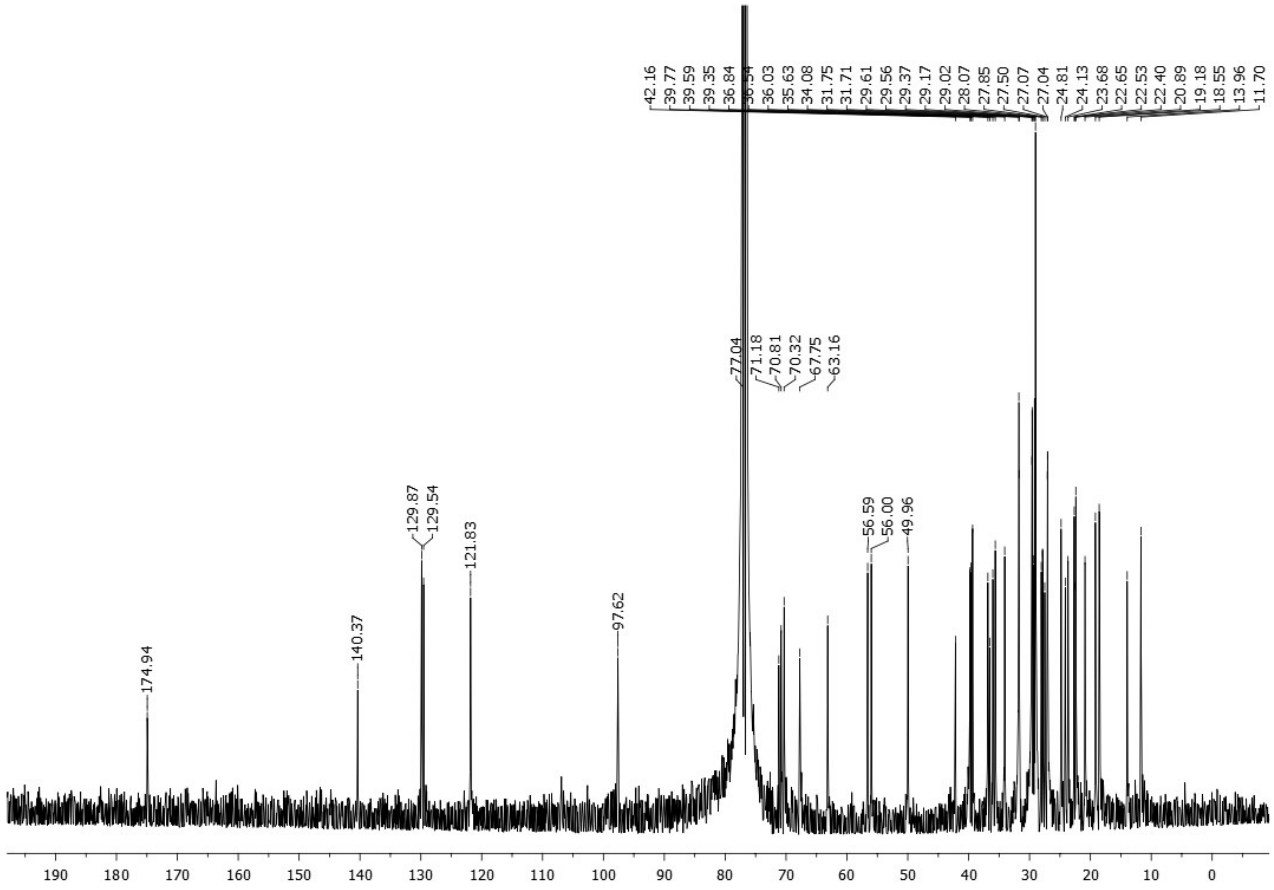

5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4

Cholesteryl 6-*O*-oleyl-α-D-mannopyranoside (11)

^{1}H NMR

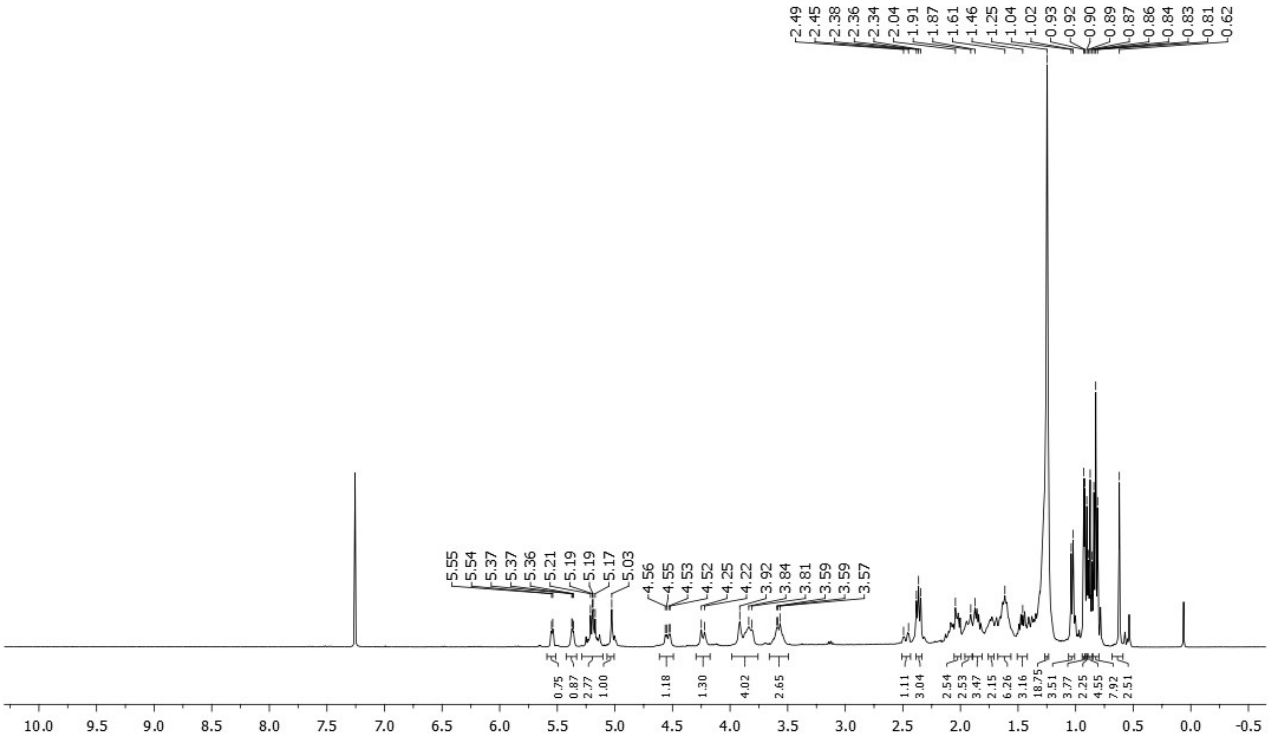


^{13}C NMR

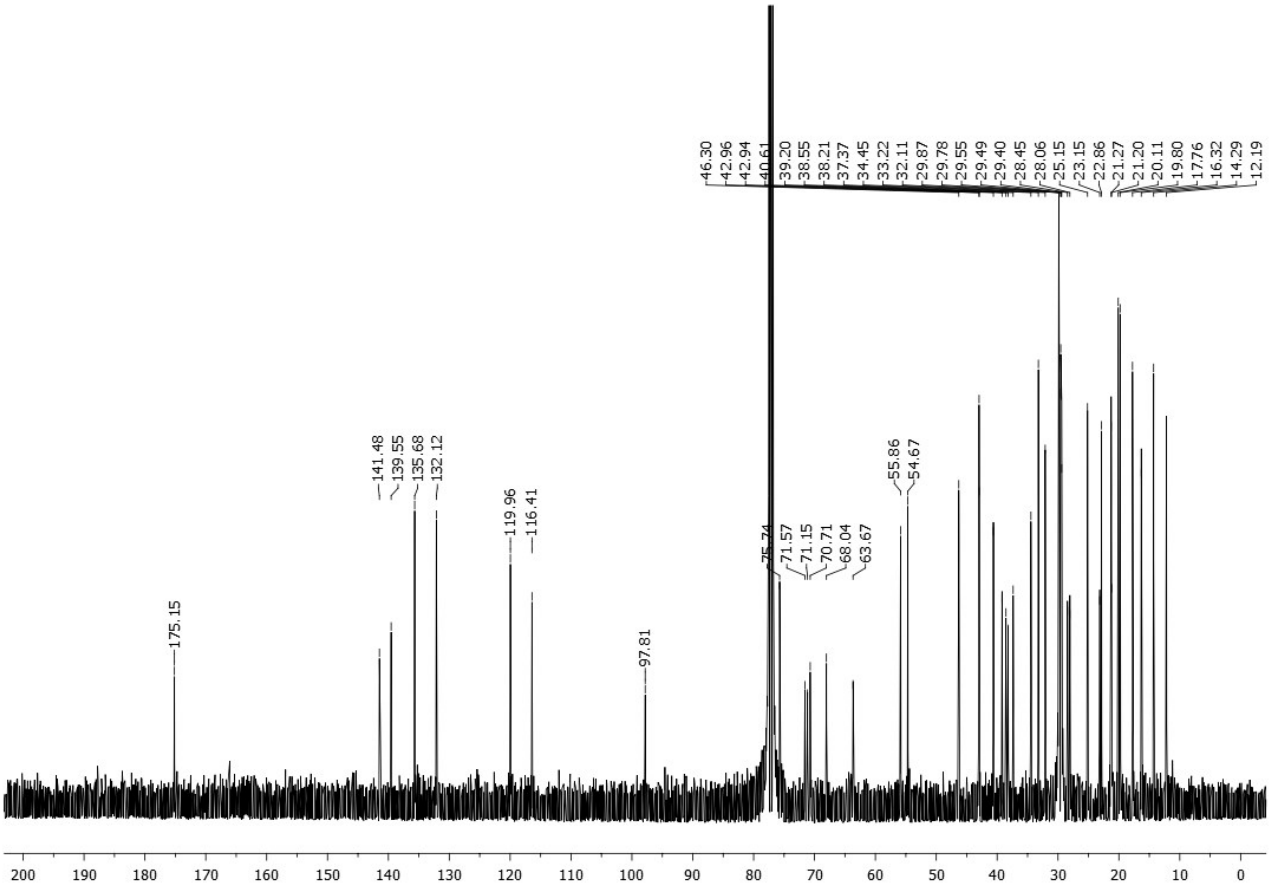


Ergosteryl 6-*O*-dodecanoyl-α-D-mannopyranoside (12)

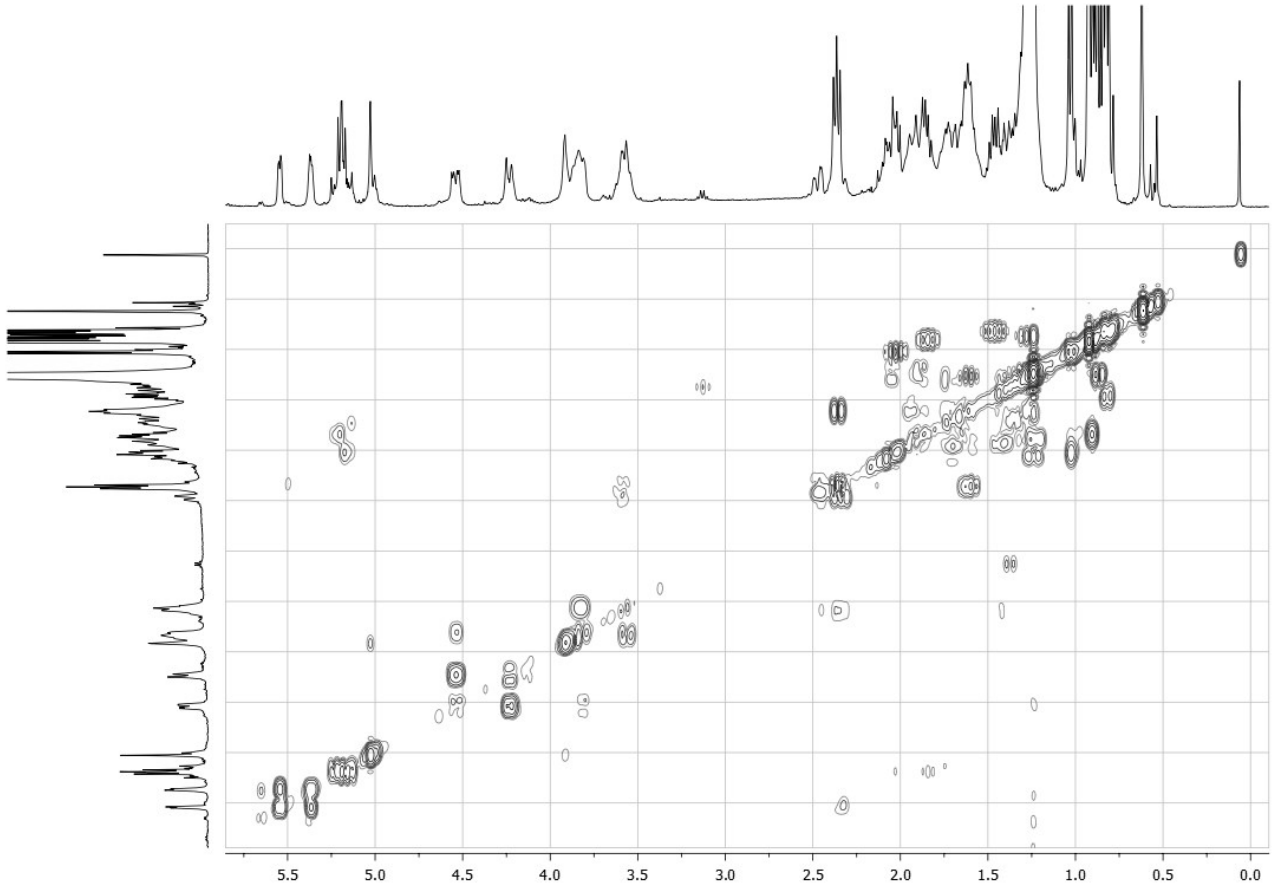
^{1}H NMR



^{13}C NMR

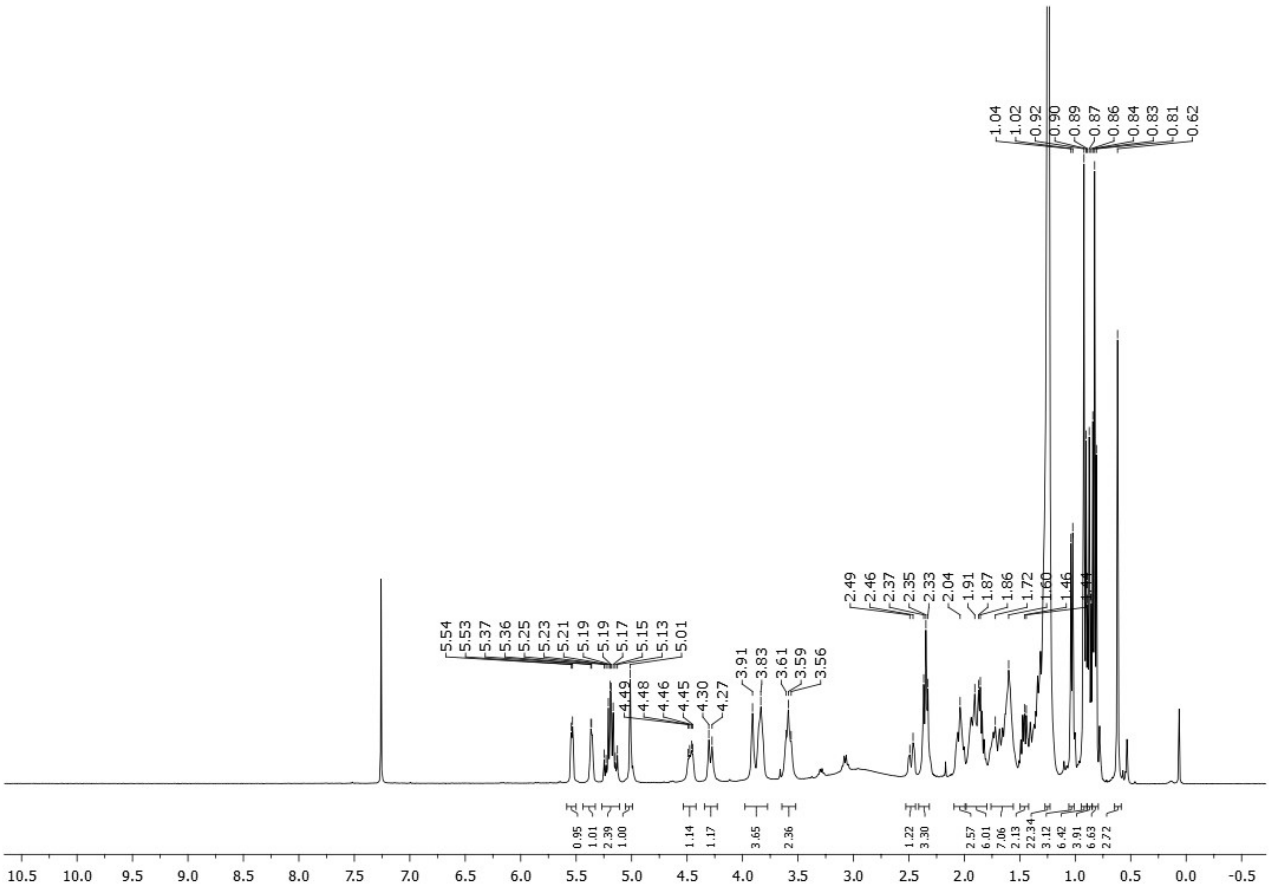


^{1}H- ^{1}H COSY

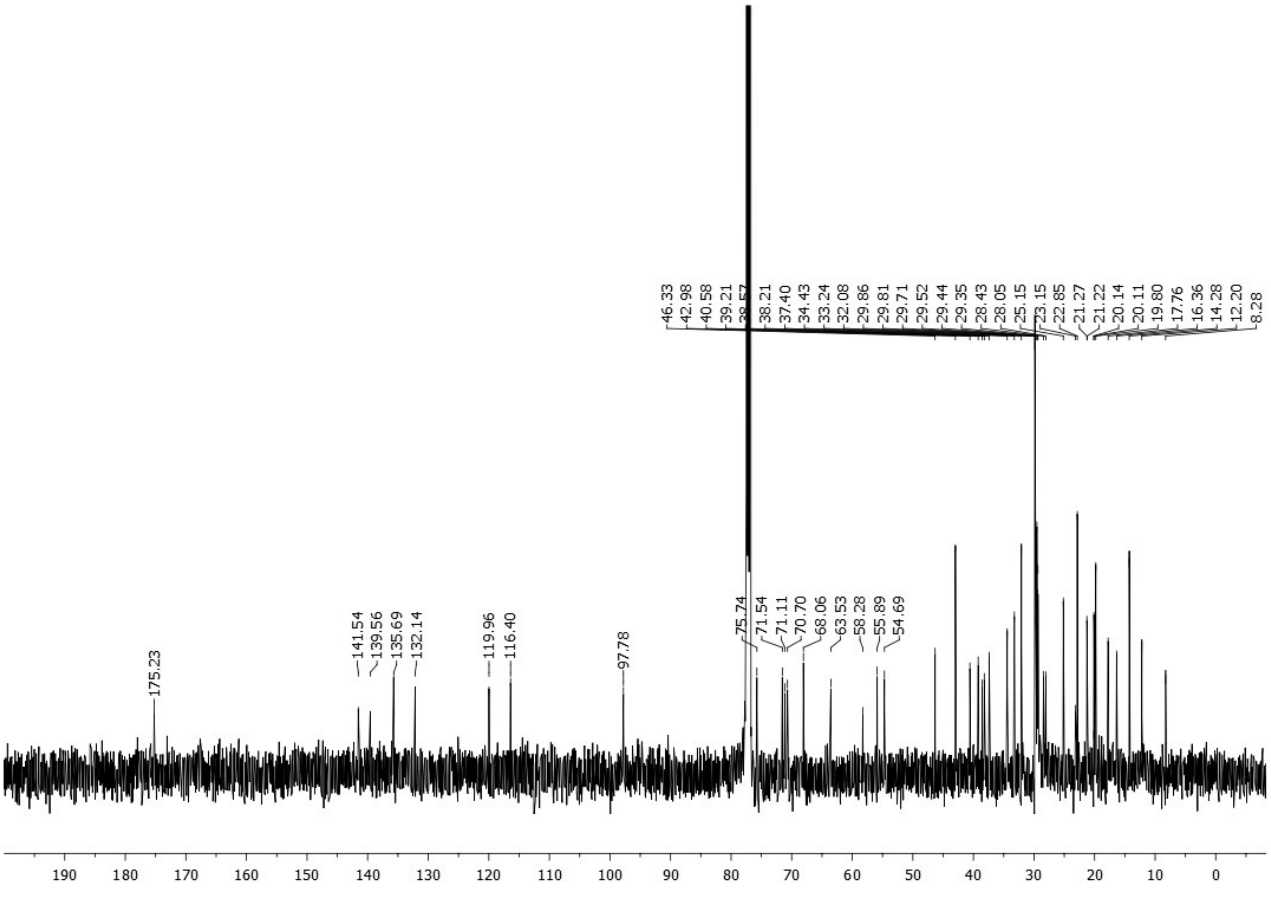


Ergosteryl 6-*O*-tetradecanoyl-α-D-mannopyranoside (13)

^{1}H NMR

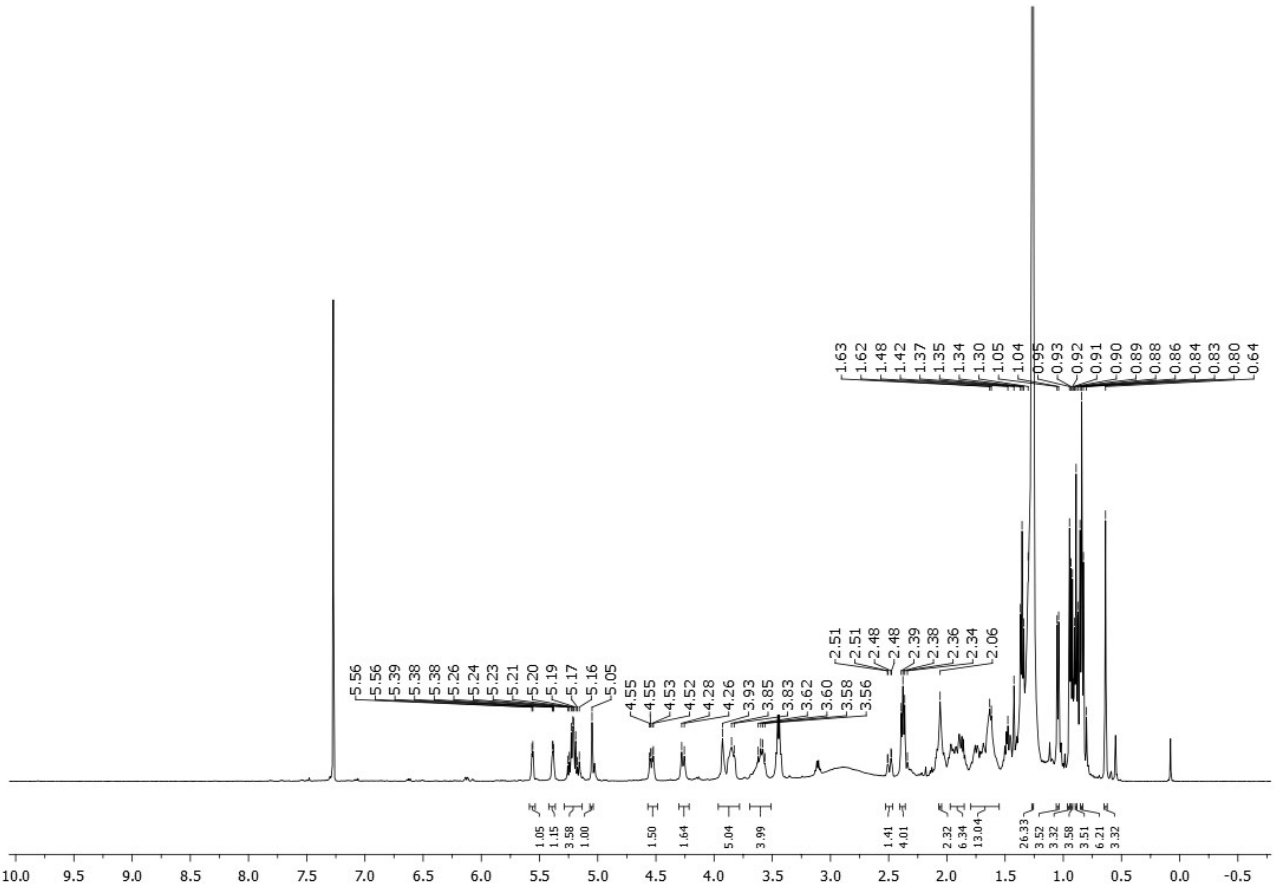


^{13}C NMR

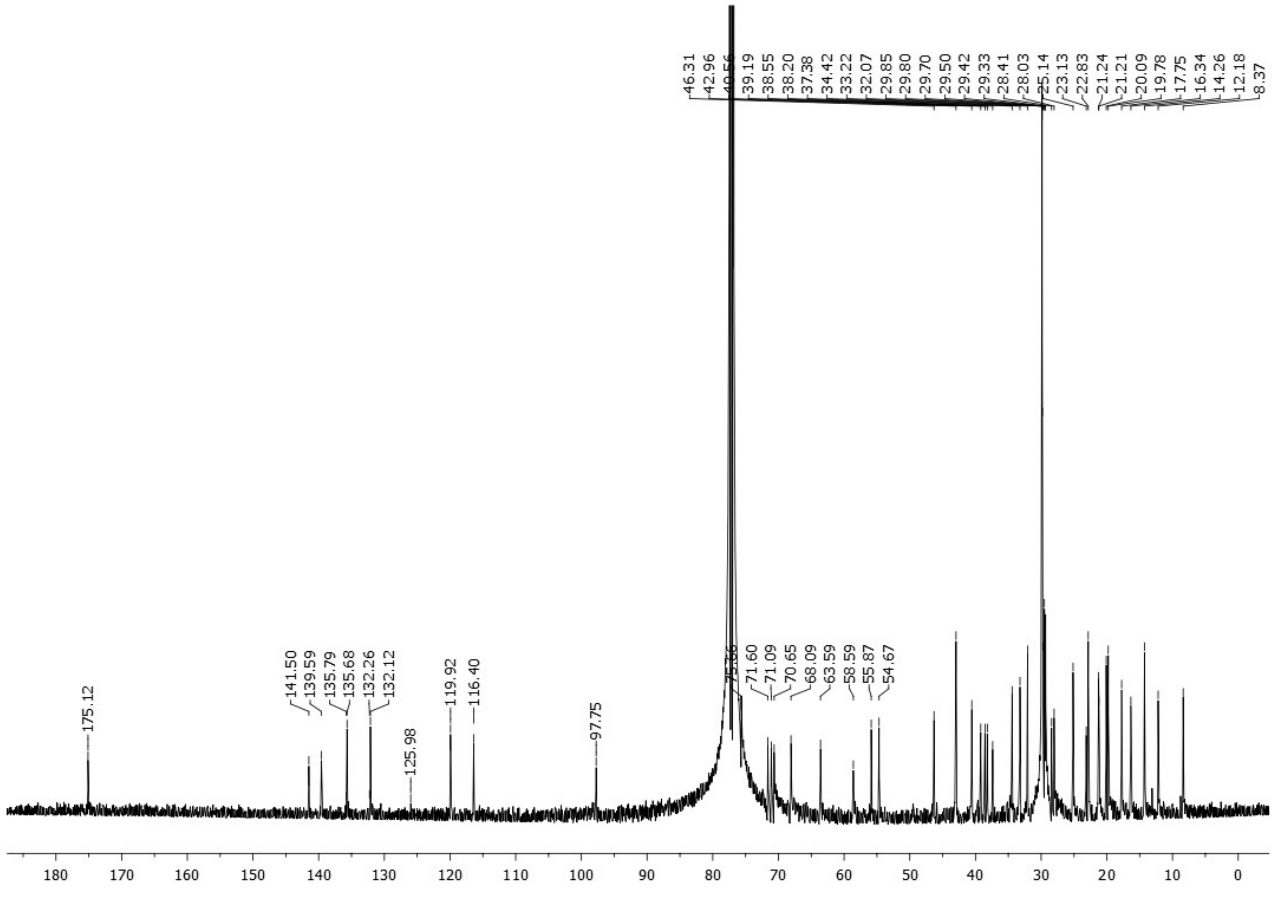


Ergosteryl 6-*O*-hexadecanoyl-α-D-mannopyranoside (14)

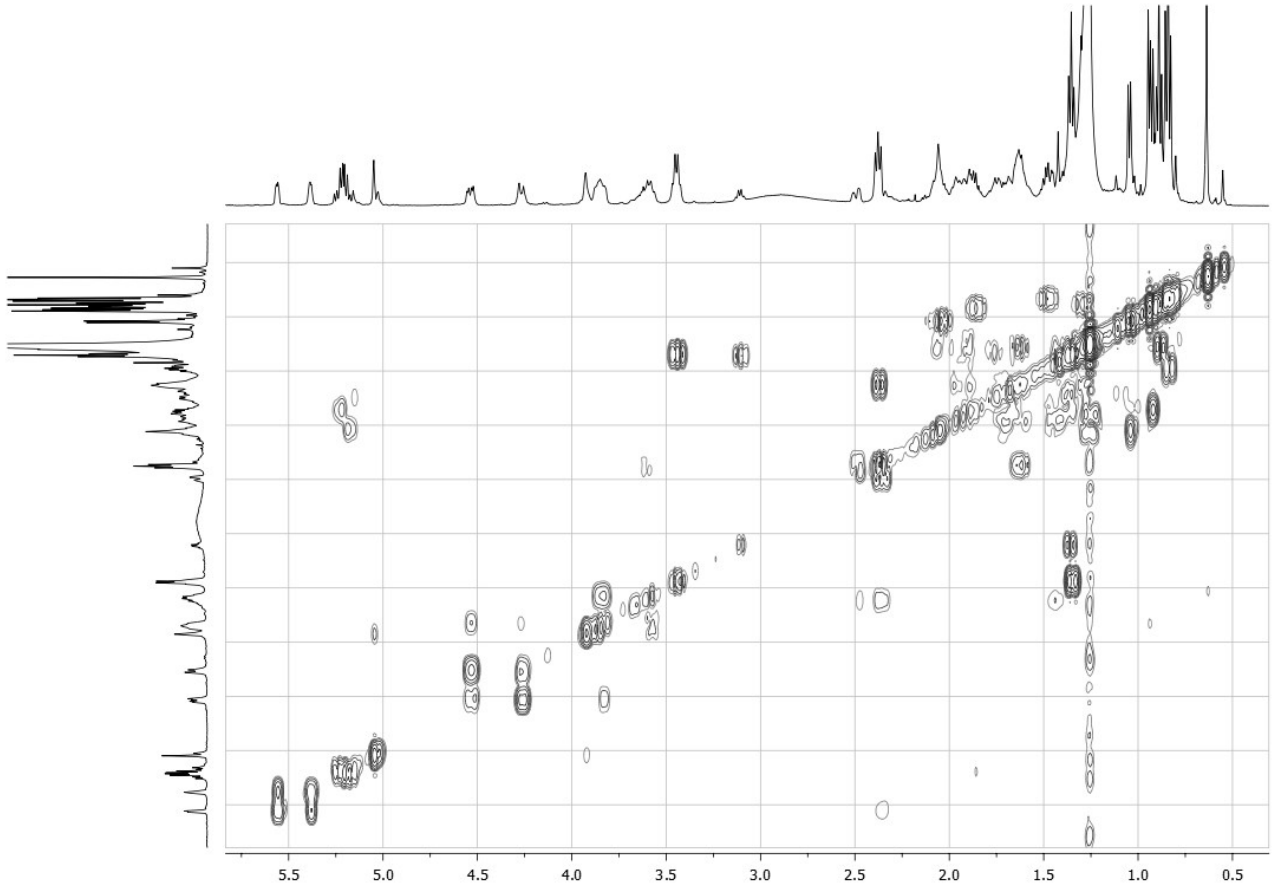
^{1}H NMR



^{13}C NMR

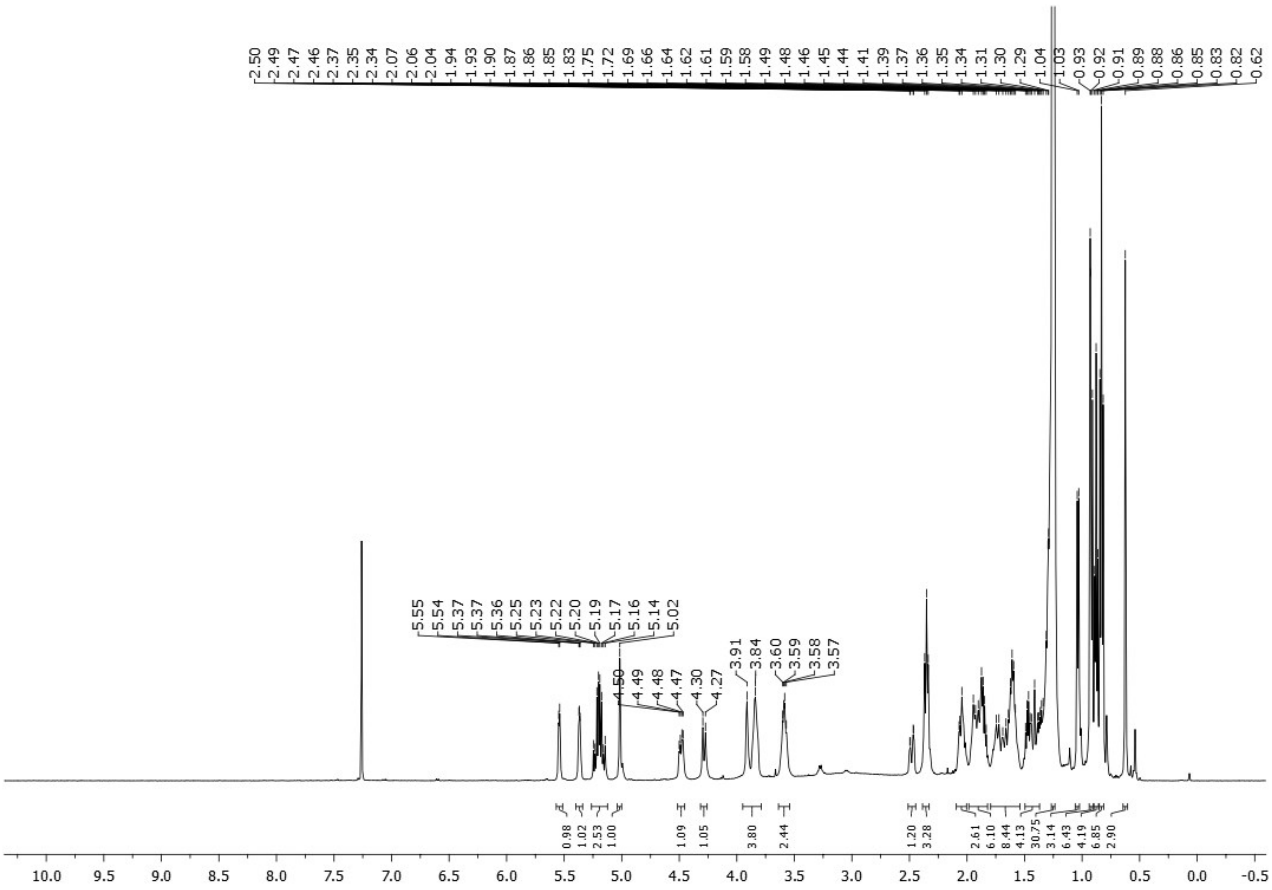


^{1}H- ^{1}H COSY

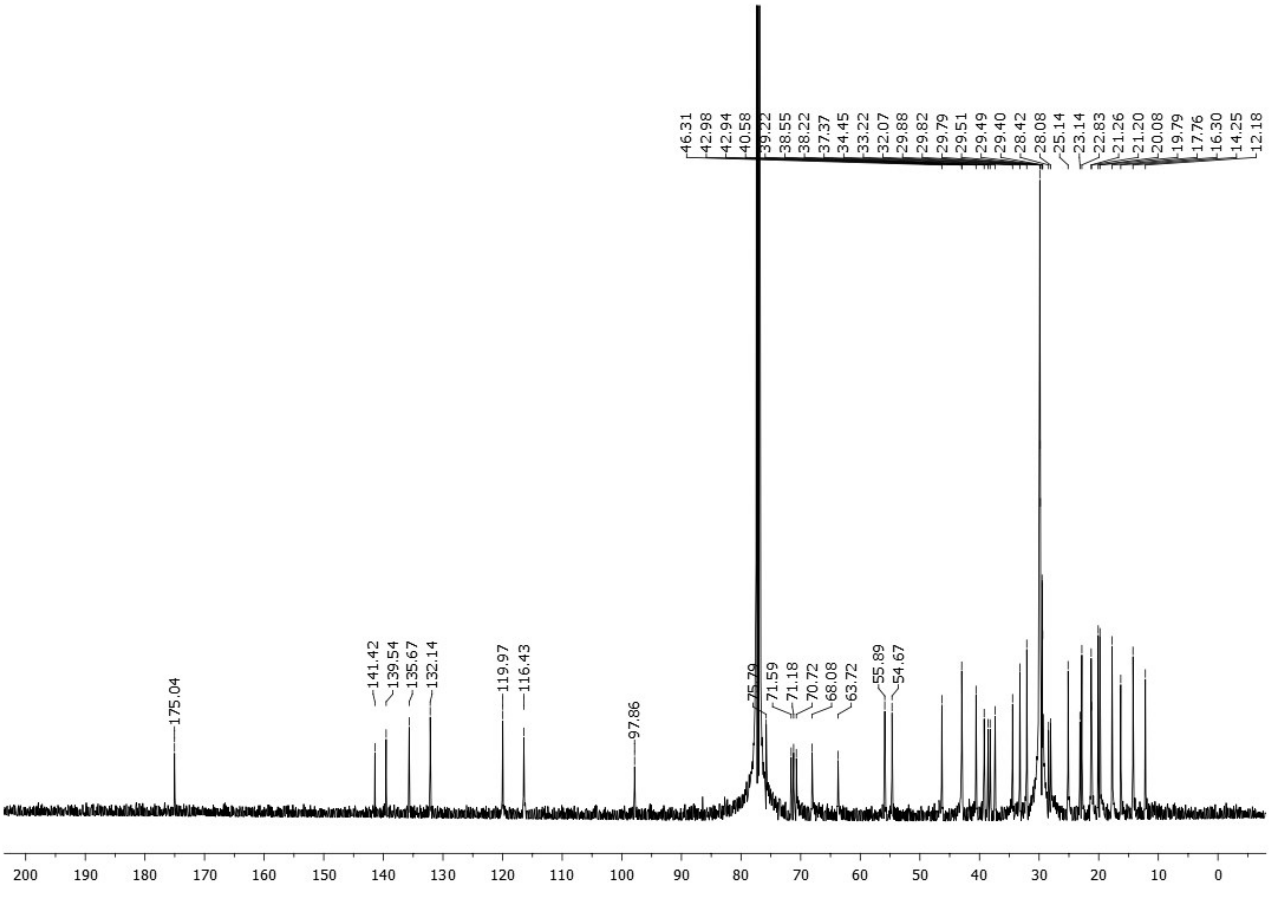


Ergosteryl 6-*O*-octadecanoyl-α-D-mannopyranoside (15)

^{1}H NMR



^{13}C NMR



Ergosteryl 6-*O*-oleyl-α-D-mannopyranoside (16)

^{1}H NMR

^{13}C NMR