Electronic Supplementary Information

Liposomes containing mannose-6-phosphate-cholesteryl conjugates for lysosome-specific delivery

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Table of Contents

Materials and Methods

I. General Information

1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and cholesterol used in the liposomes preparation were purchased from Avanti Polar Lipids Inc. (Alabaster, AL). All materials and reagents used in the synthesis and in the samples preparation were purchased from Sigma Aldrich Co. (Stenheim, Germany) unless otherwise stated and used without purification. All solvents were analytically pure and dried before use. TLC was carried out on plastic sheets pre-coated with silica gel 60 F254 (Merck), visualized under UV light and developed with immersion in a permanganate solution followed by charring or immersion in a phosphomolybdic solution (for phosphorus containing compounds). Column chromatography was performed using silica gel 60 (70-230 mesh ASTM). Optical rotations were measured at the sodium D-line with a Perkin-Elmer-241 polarimeter (path length cell 1 dm) and concentrations expressed as g/100 mL. Melting point were measured on a Electrothermal apparatus IA 9000 and are uncorrected. ^{1}H NMR Spectra were recorded on a Varian Mercury 400 (400 MHz) at 25°C. Chemical shifts (δ) are given in ppm and referenced using residual solvent signals (7.26 ppm for $CHCl_3$). Coupling constants *(J)* are given in Hertz. ^{13}C NMR Spectra and ^{31}P NMR Spectra were recorded on a Varian Mercury 400 (100 MHz and 162 MHz, respectively). Chemical shifts (δ) are given in ppm and referenced using residual solvent signals (77.0 ppm for $CHCl_3$) for ^{13}C and external aqueous 85% H_3PO_4 for ^{31}P. Electron Spray Ionization mass spectrometry (ESI-MS) was performed on an Agilent Series 1100 MSD Mass Spectrometer.

II. Synthesis of 4-(8-(Cholest-5-en-3β-yloxy)-carbonylamino-acetamido)-phenyl-α-D-mannopyranoside-6-phosphate, disodium salt [Chol-M6P]

4-nitrophenyl-2,3,4-tri-O-benzoyl-6-O-trityl-α-D-mannopyranoside,[1] 2

To a solution of 4-nitrophenyl-α-D-mannopyranoside **1** (150 mg, 0.5 mmol) in pyridine (5 mL) were added DMAP (12 mg, 0.1 mmol) and trityl chloride (279 mg, 1 mmol). The solution was heated to 70°C for 8 h then it was allowed to come to room temperature and BzCl (2 mmol, 0.232 mL) was added before stirring for 12 h. Then pyridine was removed *in vacuo* and the crude mixture was diluted with DCM (20 mL), washed with saturated aqueous $NaHCO_3$ (5 mL), H_2O (5 mL) followed by brine (5 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to obtain a residue, which was purified by silica gel column chromatography (petroleum ether-EtOAc, 8 : 2) to afford **2** (368 mg, 86%) as a white solid. $[\alpha]_D$ -4.2 (*c* 2.4, $CHCl_3$); mp 111-113°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.34 (d, $J_{6a,5}$ = 3.6, 2H, H6a, H6b), 4.13-4.18 (m, 1H, H5), 5.90 (dd, $J_{2,1}$ = 1.6, $J_{2,3}$ = 3.2, 1H, H2), 5.94 (d, $J_{1,2}$ = 1.6, 1H, H1), 5.96 (dd, $J_{3,2}$ = 3.2, 1H, H3), 6.10 (dd, $J_{4,3}$ = $J_{4,5}$ = 10.4, 1H, H4), 7.10-7.68 (m, 24H, Ar-H), 7.74 (d, J = 7.2, 2H, Ar-H), 7.87 (d, J = 7.2, 2H, Ar-H), 8.12 (d, J = 7.2, 2H, $PhNO_2$), 8.17 (d, J = 7.2, 2H, Ar-H), 8.27 (d, J = 8.8, 2H, $PhNO_2$). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 61.8, 66.3, 69.9, 70.1, 71.6, 86.8, 95.9, 116.7, 125.9, 127.0, 127.7, 128.2, 128.4, 128.5, 128.8, 129.7, 129.8, 130.0, 133.3, 133.3, 133.8, 143.0, 143.5, 160.5, 165.0, 165.5, 165.6. ESI-MS: m/z 877.8 $[M+Na]^+$.

Methyl 2-(8-(Cholest-5-en-3β-yloxy)-carbonylamino)-acetate, 4

A solution of Boc-Gly-OCH_3 (416 mg, 2.2 mmol) and TFA (0.5 mL/100 mg) in DCM (5 mL) was stirred at room temperature and the reaction was monitored by TLC. After completion of reaction, the solvent was evaporated and the crude product was washed with Et_2O, to give the trifluoroacetate salt as a foamy solid. This product was suspended in a mixture of DMAP (27 mg, 0.22 mmol), TEA (0.65 mL, 4.6 mmol), cholesterylchloroformate (1.08 g, 2.4 mmol) in dry DCM (4 mL) under nitrogen atmosphere. The reaction was stirred for 2 h, then the mixture was extracted with HCl 1M (5 mL) and EtOAc (3 x 5mL). The organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$:CH_3OH, 99:1) to afford **4** (0.85 g, 77%) as a white solid. mp 162-164°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.67 (s, 3H, Chol 18-H3), 0.86 (d, *J* = 6.8, 3H, Chol 26-H3), 0.86 (d, *J* = 6.8, 3H, Chol 27-H3), 0.91 (d, *J* = 6.4, 3H, Chol 21-H3), 1.01 (s, 3H, Chol 19-H3), 0.86-2.03 (m, 26H, Chol 1-H2, 2-H2, 4-H2, 8-H1, 9-H1, 11-H2, 12-H2, 14-H1, 15-H2, 16-H2, 17-H1, 20-H1, 22-H2, 23-H2, 24-H2 and 25-H1), 2.26-2.39 (m, 2H, Chol 7-H2), 3.76 (s, 3H, OCH_3), 3.97 (d, *J* = 4.0, 2H), 4.47-4.56 (m, 1H, Chol 3-H1), 5.09 (br s, 1H, NH), 5.37 (d, *J* = 5.2, 1H, Chol 6-H1); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 11.8, 18.7, 19.3, 21.0, 22.6, 22.8, 23.8, 24.3, 28.0, 28.0, 28.2, 31.8, 31.9, 35.8, 36.2, 36.5, 36.9, 38.4, 39.5, 39.7, 42.3, 42.5, 50.0, 52.3, 56.1, 56.7, 74.9, 122.6, 139.7, 155.9, 170.6; ESI-MS: m/z 1026.7 $[2M+Na]^+$.

2-(8-(Cholest-5-en-3β-yloxy)-carbonylamino)-acetic acid, 5

To a solution of compound **4** (502 mg, 1 mmol) in THF (5 mL) was added NaOH 1M (5 mL, 5 mmol) at 0°C and the reaction was stirred for 2 h. Then the mixture was poured into HCl 1M and extracted with EtOAc. The organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give **5** (466 mg, 95%) as a white solid, which was used without further purification. mp 205-207°C (dec); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.67 (s, 3H, Chol 18-H3), 0.86 (d, *J* = 6.8, 3H, Chol 26-H3), 0.86 (d, *J* = 6.4, 3H, Chol 27-H3), 0.91 (d, *J* = 6.8, 3H, Chol 21-H3), 1.01 (s, 3H, Chol 19-H3), 0.86-2.03 (m, 26H, Chol 1-H2, 2-H2, 4-H2, 8-H1, 9-H1, 11-H2, 12-H2, 14-H1, 15-H2, 16-H2, 17-H1, 20-H1, 22-H2, 23-H2, 24-H2 and 25-H1), 2.26-2.39 (m, 2H, Chol 7-H2), 4.01 (d, *J* = 5.2, 2H), 4.48-4.56 (m, 1H, Chol 3-H1), 5.12 (br t, *J* = 5.2, 1H, NH), 5.37 (d, *J* = 5.2, 1H, Chol 6-H1); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 11.9, 18.7, 19.3, 21.0, 22.6, 22.8, 23.8, 24.3, 28.0, 28.2, 29.7, 31.8, 31.9, 35.8, 36.2, 36.5, 36.9, 38.4, 39.5, 39.7, 42.3, 42.4, 50.0, 56.1, 56.7, 75.2, 122.7, 139.6, 156.2, 173.3; ESI-MS: m/z 998.6 $[2M+Na]^+$.

4-(8-(Cholest-5-en-3β-yloxy)-carbonylamino-acetamido)-phenyl-2,3,4-tri-O-benzoyl-6-O-trityl-α-D-mannopyranoside, 6

A solution of compound **2** (346 mg, 0.4 mmol) and Pd/C (10%, 346 mg) in dry methanol (5 mL) and dry DCM (2 mL) was refluxed under hydrogen atmosphere for 1 h. The reaction mixture was then filtered through celite, washed with methanol and the filtrate concentrated under reduced

pressure affording **3** (240 mg, 73%), which was used without further purification. ESI-MS: m/z 848.2 $[M+Na]^+$. Then to a solution of compound **3** (240 mg, 0.3 mmol) and compound **5** (146 mg, 0.3 mmol) in DCM (2 mL) was added DMAP (37 mg, 0.3 mmol) and DCC (62 mg, 0.3 mmol). The reaction was stirred at room temperature over night. Then the mixture was filtered and extracted with EtOAc (2 x 5 ml) and water (2 mL). The organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether-EtOAc, 7 : 3) to afford **6** (244 mg, 63%) as a foamy solid. $[\alpha]_D$ -15.2 (*c* 2.3, $CHCl_3$); mp 123-125°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.67 (s, 3H, Chol 18-H3), 0.86 (d, J = 6.4, 3H, Chol 26-H3), 0.86 (d, J = 6.8, 3H, Chol 27-H3), 0.91 (d, J = 6.4, 3H, Chol 21-H3), 1.01 (s, 3H, Chol 19-H3), 0.86-2.06 (m, 26H, Chol 1-H2, 2-H2, 4-H2, 8-H1, 9-H1, 11-H2, 12-H2, 14-H1, 15-H2, 16-H2, 17-H1, 20-H1, 22-H2, 23-H2, 24-H2 and 25-H1), 2.29-2.42 (m, 2H, Chol 7-H2), 3.27 (dd, $J_{6a,5}$ = 4.4, $J_{6a,6b}$ = 10.8, 1H, H6a), 3.36 (dd, $J_{6b,5}$ = 2.0, $J_{6b,6a}$ = 10.8,1H, H6b), 3.99 (d, J = 5.6, 2H), 4.24-4.28 (m, 1H, H5), 4.53-4.60 (m, 1H, Chol 3-H1), 5.35 (br s, 1H, NH), 5.38 (d, J = 4.8, 1H, Chol 6-H1), 5.78 (s, 1H, H1), 5.87-5.88 (m, 1H, H2), 5.96 (dd, $J_{3,2}$ = 2.8, $J_{3,4}$ = 10.0, 1H, H3), 6.12 (dd, $J_{4,3}$ = $J_{4,5}$ = 10.0, 1H, H4), 7.07-7.52 (m, 27H, Ar-H), 7.62-7.65 (m, 1H, Ar-H), 7.73 (d, J = 8.4, 2H, Ar-H), 7.87 (d, J = 8.4, 2H, Ar-H), 8.05 (br s, 1H, NH), 8.18 (d, J = 8.4, 2H, Ar-H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 11.8, 18.7, 19.3, 21.0, 22.6, 22.8, 23.8, 24.3, 28.0, 28.1, 28.2, 31.8, 31.9, 35.8, 36.2, 36.5, 36.9, 38.4, 39.5, 39.7, 42.3, 50.0, 56.1, 56.7, 61.9, 66.6, 70.3, 70.5, 70.9, 75.5, 86.6, 96.3, 117.3, 121.5, 122.8, 126.8, 127.7, 128.2, 128.3, 128.6, 128.7, 129.4, 129.7, 129.8, 130.0, 133.1, 133.1, 133.6, 139.5, 143.6, 152.8, 165.0, 165.6, 167.2.

4-(8-(Cholest-5-en-3β-yloxy)-carbonylamino-acetamido)-phenyl-2,3,4-tri-O-benzoyl-α-D-mannopyranoside,[2] 7

To a solution of compound **6** (233 mg, 0.18 mmol) in methanol (2 mL) and chloroform (2 mL) was added *p*-toluensulfonic acid (7 mg, 0.036 mmol) and the reaction was stirred at room temperature over night. Then the reaction was neutralized with TEA (20 µl) and the solvents were removed under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether-EtOAc, 6 : 4) to afford **7** (126 mg, 67%) as a white solid. $[\alpha]_D$ -28.9 (*c* 1.9, $CHCl_3$); mp 108-110°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.67 (s, 3H, Chol 18-H3), 0.86 (d, J = 6.4, 3H, Chol 26-H3), 0.86 (d, J = 6.4, 3H, Chol 27-H3), 0.91 (d, J = 6.4, 3H, Chol 21-H3), 1.01 (s, 3H, Chol 19-H3), 0.86-2.06 (m, 26H, Chol 1-H2, 2-H2, 4-H2, 8-H1, 9-H1, 11-H2, 12-H2, 14-H1, 15-H2, 16-H2, 17-H1, 20-H1, 22-H2, 23-H2, 24-H2 and 25-H1), 2.29-2.42 (m, 2H, Chol 7-H2), 3.73 (dd, $J_{6a,5}$ = 2.8, $J_{6a,6b}$ = 13.2, 1H, H6a), 3.81 (dd, $J_{6b,5}$ = 2.0, $J_{6b,6a}$ = 13.2, 1H, H6b), 3.98 (d, J = 6.0, 2H), 4.13-4.17 (m, 1H, H5), 4.51-4.60 (m, 1H, Chol 3H-1), 5.35-5.43 (m, 2H, Chol 6H-1 and NH), 5.79 (br s, 1H, H1), 5.86-5.87 (m, 1H, H2), 5.94 (dd, $J_{4,3}$ = $J_{4,5}$ = 10.0, 1H, H4), 6.19 (dd, $J_{3,2}$ = 3.6, $J_{3,4}$ = 10.4, 1H, H3), 7.15 (d, J = 8.8, 2H, Ar-H), 7.26-7.65 (m, 11H, Ar-H), 7.85 (d, J = 8.0, 2H, Ar-H), 7.99 (d, J = 7.2, 2H, Ar-H), 8.09-8.14 (m, 3H, 2Ar-H and NH). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 11.8, 18.7, 19.3, 21.0, 22.6, 22.8, 23.8, 24.3, 28.0, 28.1, 28.2, 31.8, 31.9, 35.8, 36.2, 36.5, 36.9, 38.4, 39.5, 39.7, 42.3, 50.0, 56.1, 56.7, 61.0, 67.0, 69.4, 70.3, 71.6, 75.5, 96.2, 117.0, 121.7, 122.8, 128.4, 128.5, 128.7, 129.7, 130.0, 130.0, 133.3, 133.7, 133.8, 139.5, 152.5, 163.9, 165.5, 165.5, 166.6.

4-(8-(Cholest-5-en-3β-yloxy)-carbonylamino-acetamido)-phenyl-α-D-mannopyranoside-6-phosphate, disodium salt [Chol-M6P] 9

To a solution of compound **7** (126 mg, 0.12 mmol) and TEA (55 µl, 0.4 mmol) in dry DCM (2 mL), $POCl_3$ (24 µl, 0.26 mmol) was added dropwise at 0°C under nitrogen atmosphere[3]. The mixture was allowed to reach room temperature and it was stirred over night. Then the reaction was quenched with ice water (1 mL), stirred vigorously for another 4 h and extracted with DCM (2 x 5mL). The organic layers were dried over Na_2SO_4, filtered and concentrated to give a residue, that was triturated and washed with Et_2O to furnish compound **8** (0.086 mmol, 72%) as a brown solid. ^{31}P-NMR (162 MHz, $CDCl_3$): δ 1.32 (s). Then the acid **8** (0.086 mmol, 97 mg) was dissolved in dry methanol (1 mL) and dry DCM (1 mL) and $NaOCH_3$ (0.129 mmol, 184 µl) was added at room temperature. After 24 h, the reaction was neutralized with Amberlyst H15, filtered and the filtrate concentrated *in vacuo*. The residue was triturated with Et_2O and dried to afford a solid (0.06 mmol, 50 mg). ^{1}H-NMR (400 MHz, $CDCl_3$:CD_3OD): δ 0.52 (s, 3H, Chol 18-H3), 0.70 (d, *J* = 6.4, 3H, Chol 26-H3), 0.70 (d, *J* = 6.8, 3H, Chol 27-H3), 0.76 (d, *J* = 6.4, 3H, Chol 21-H3), 0.86 (s, 3H, Chol 19-H3), 0.70-2.22 (m, 28H, Chol 1-H2, 2-H2, 4-H2, 8-H1, 9-H1, 11-H2, 12-H2, 14-H1, 15-H2, 16-H2, 17-H1, 20-H1, 22-H2, 23-H2, 24-H2, 25-H1 and 7-H2), 3.52-4.37 (m, 10H, Chol 3H-1, 2H, H1, H2, H3, H4, H5, H6a and H6b), 5.18-5.33 (m, 2H, Chol 6H-1 and NH), 6.86 (d, *J* = 8.8, 2H, Ar-H), 7.27 (d, *J* = 8.0, 2H, Ar-H). ^{31}P-NMR (162 MHz, $CDCl_3$:CD_3OD): δ 1.64 (s). This product was suspended in $CHCl_3$ (2 mL) and CH_3OH (2 mL) and TEA (0.12 mmol, 17 µl) was added. After removal of the solvents, the residue was suspended in H_2O (3mL) and treated with Dowex Na cation exchange resin. The aqueous layer was lyophilized, to afford compound **9** as a white solid in quantitative yield. ESI-MS: m/z 819.5 $[M-2H]^-$.

III. Preparation of liposomes

Liposomes were prepared by the "thin film hydration" method[4]. Appropriate amounts of chloroform solutions of DOPC and Cholesterol or Cholesterol-M6P were mixed to obtain 95:5, 90:10, 85:15 and 80:20 mol/mol ratio. The solvent was removed slowly by evaporation under reduced pressure and the thin film obtained was dried under vacuum overnight. This dried film was then resuspended under stirring for 30 min in the required amount of PBS 20 mM (for Size and ζ Potential measurements) or alternatively of 58 mM calcein solution (pH 7.49, 558 mOsM) (for confocal and fluorescent microscopy experiments) at a final concentration of DOPC 1.00 mM and guest lipid 0.25 mM. The resulting multilamellar vesicle dispersions were sonicated (8 min, 40%) at 0°C with a vibra cell sonicator (Sonics Vibra Cell Mod. VCx130) equipped with a tapered micro tip, until the liposome dispersion was completely clear. Separation of liposomes from non-encapsulated calcein was achieved by size exclusion chromatography through a Sephadex G-50 columns pre-hydrated with PBS-K buffer (155 mM containing 112 mM NaCl, pH 7.49, 558 mOsM). After purification of liposomes from non-entrapped dye, the percent calcein latency is measured in order to be sure that separation of free calcein from liposome-entrapped is complete. Liposomes were re-purified until the calcein latency was higher than 95%; for calculation of latency[5] a sample from the liposome dispersion (10 µL) is diluted with 1 mL of the isosmotic PBS-K buffer, pH 7.49, and the

fluorescence intensity (FI) of the samples was measured on a BioTek Synergy HT MicroPlate Reader Spectrophotometer (EX 485 nm, EM 528 nm), before and after vesicle disruption, by addition of Triton X-100 to a final concentration of 1% (v/v). The percent of calcein latency (% latency) is calculated from the following equation,

% Latency = [(Fa-Fb) / Fa] x 100

where Fb and Fa are the calcein FIs of the sample before and after addition of Triton X-100, respectively.

IV. Particle size and ζ Potential

Size distribution (mean diameter and polydispersity index [PDI]) and zeta-potential of the liposomes were measured by Dynamic Light Scattering (DLS) and Electrophoretic Light Scattering using a Nanosizer (Nano-ZS, Nanoseries, Malvern). An aliquot of the liposomes suspensions was diluted at a final concentration of 2.5 x 10^{-2} mM with the appropriate buffer and filtered with 0.20 μm pore size cellulose acetate filters (Sartorius Stedim Biotech). Measurements were performed at 25°C with a fixed angle of 173°. Sizing measurements are the intensity-based mean diameters and the polydispersity index (PdI) was directly calculated by the software of the apparatus. Zeta potentials are determined from the electrophoretic mobility applying the Henry equation. For all samples investigated the data represent the average of at least three different autocorrelations carried out for each sample.

V. Cells and biological assays

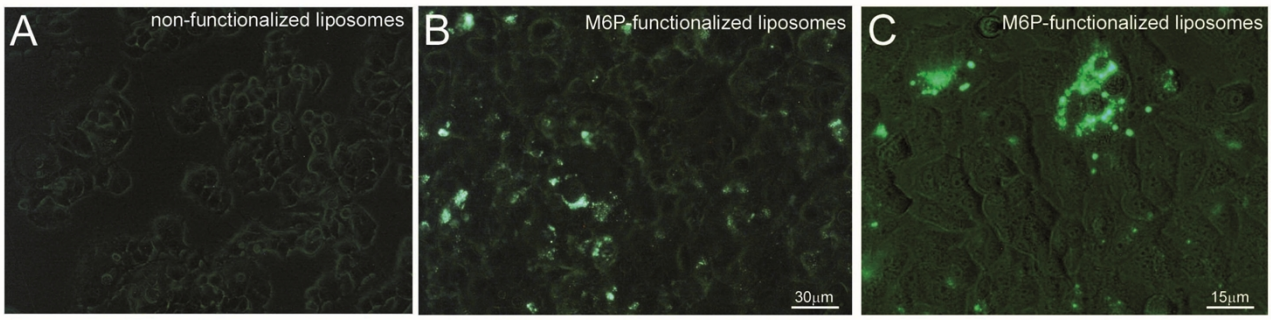
The mouse embryonic fibroblast cell line NIH-3T3 (kindly provided by Dr Mauro Provinciali), expressing the cation-independent mannose-6-phosphate receptor, was used to assess and trace the internalization of Chol-M6P functionalized liposomes containing calcein. NIH-3T3 cells were maintained at 37°C and 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium, DMEM, (Lonza) containing 4,5 mg/L glucose, 1% penicillin/streptomycin and 10% Foetal Calf Serum, FCS (Lonza) until the day of the experiments. Two days before the performance of the assay, cells were plated on a 12 wells plate ($2x10^5$ cells/well) in order to achieve 70-80% confluence at the time of assay. The day of the experiment, serum containing medium was replaced with DMEM w/o antibiotics and w/o serum, then, 25 μl of vesicles containing calcein, prepared as described above, were diluted in DMEM and added to each cell sample in order to reach a final total volume of 200 μl/well; cells were thus incubated at 37°C and 5% CO_2 atmosphere with liposomes to allow the interaction with cell surfaces and, after 2 hrs incubation, unbound vesicles were washed away and 10% FCS and 1% p/s was added to the medium. Evidence of liposomes uptake was assessed 24 or 48 hrs later after washing cells twice with 1X PBS (Lonza), by fluorescence microscopy or confocal microscopy depending on the type of assay.

VI. Confocal microscopy analysis

Confocal microscopy analysis was carried out to trace calcein loaded liposomes trafficking in NIH-3T3 cells during internalization experiments. The assays were performed with DOPC/Cholesterol-M6P liposomes, and Rhodamine-labeled liposomes or Lysotracker labeled lysosomes (red) carrying calcein (endogenous green). Confocal images were obtained through a motorized Leica DM6000 microscope at different magnifications. Fluorescence signal was detected with a Leica TCS-SL confocal scanning system equipped with Argon and He/Ne mixed gas lasers. Excitation wavelength used were respectively 488 nm for calcein and 546 nm for liposomes and lysosomes. Finally, nuclei were stained with TO-PRO-3 (Molecular Probes) applied on cells preparation for 10 min at the end of the procedures and excited with 646 nm visualized in blue. Images were obtained sequentially from two channels using a pinhole of 1.1200 and stored as TIFF files. Brightness and contrast of individual images as well as the merge were obtained and adjusted using the Leica software.

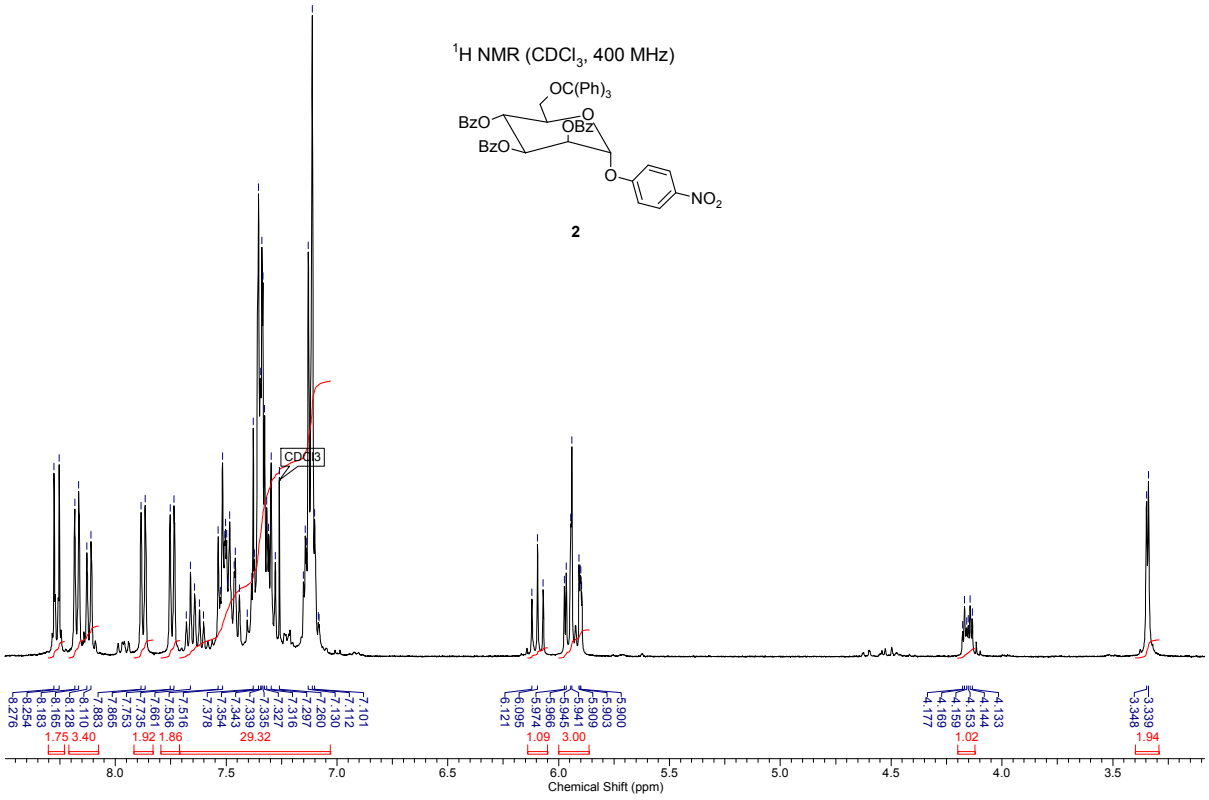
VII. Fluorescence microscopy analysis

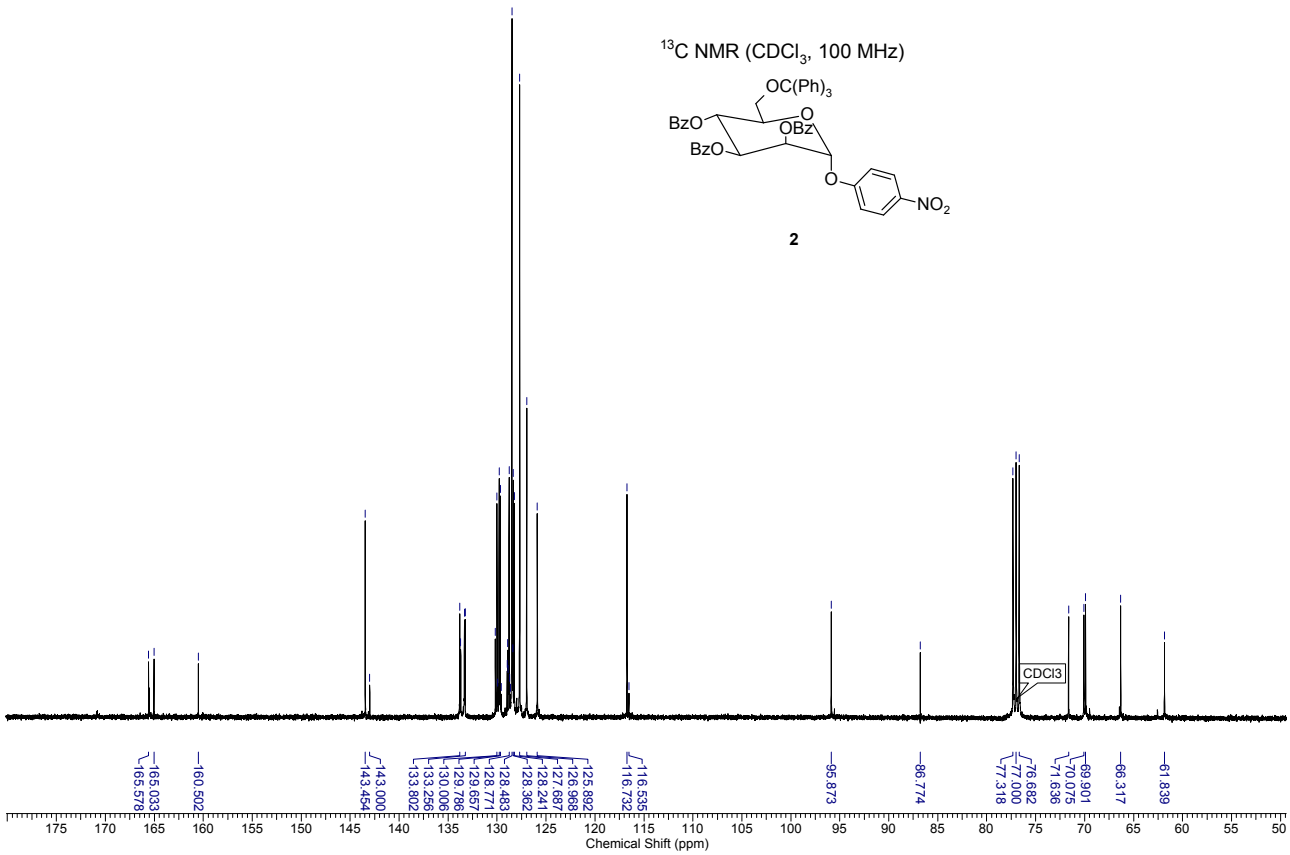
Figure S1: Controls performed with epifluorescent microscope. A) Cells were treated with non-functionalized liposomes. After 48 hrs the preparation was visualized under fluorescent light and resulted completely negative for calcein staining. B) Cells treated with DOPC/M6P-Cholesterol liposomes after 48 hrs display a clear green staining (calcein) within several cells scattered in the culture chamber. C) At higher magnification the morphological details of cells are more visible and calcein appeared in the cytoplasmic space both as clusters or diffuse.



VIII. NMR and Mass Spectra

Figure S2. ^{1}H-, ^{13}C-NMR and Mass Spectra of compound **2**.





ESI-MS (ES$^+$): $[C_{52}H_{41}O_{11}N + Na]^+$ 877.8

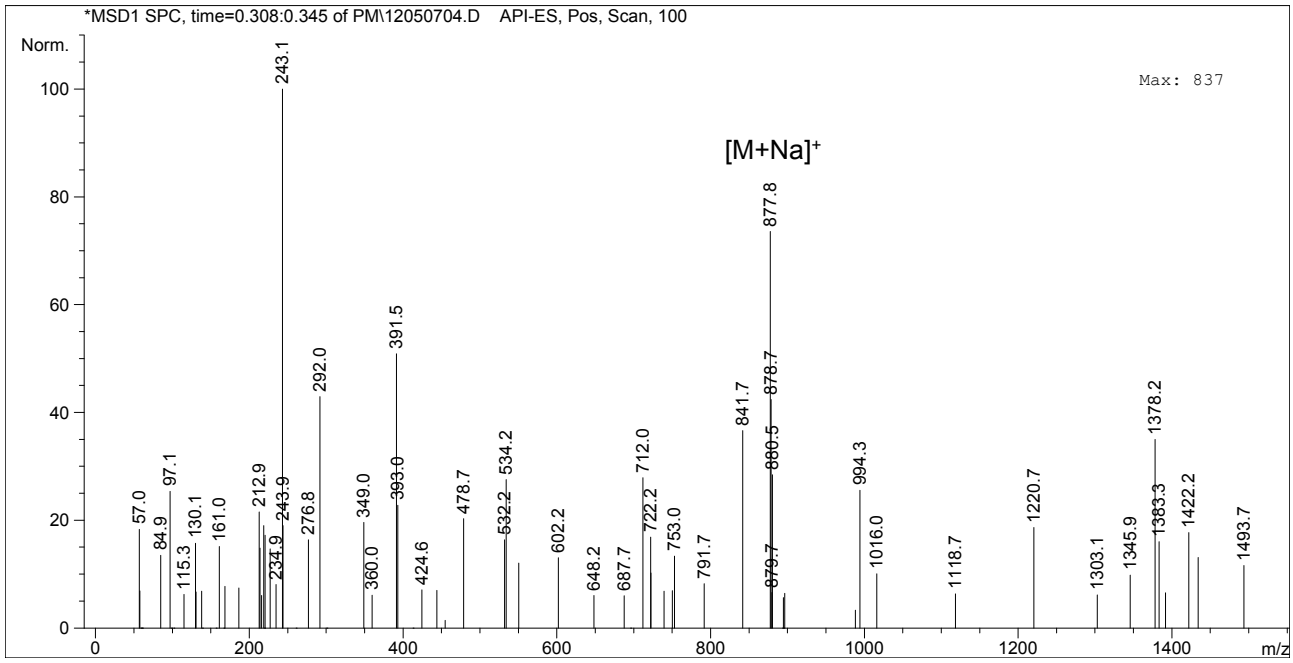
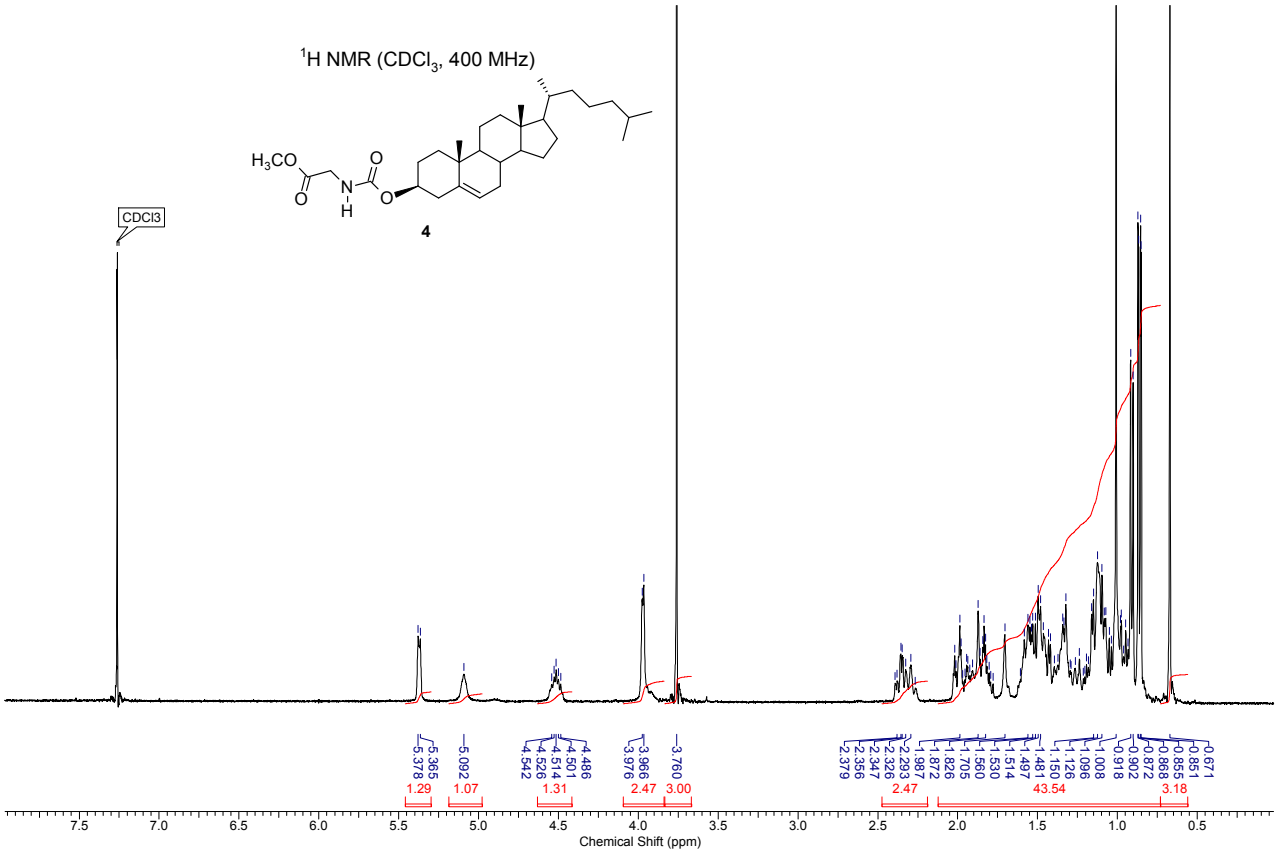
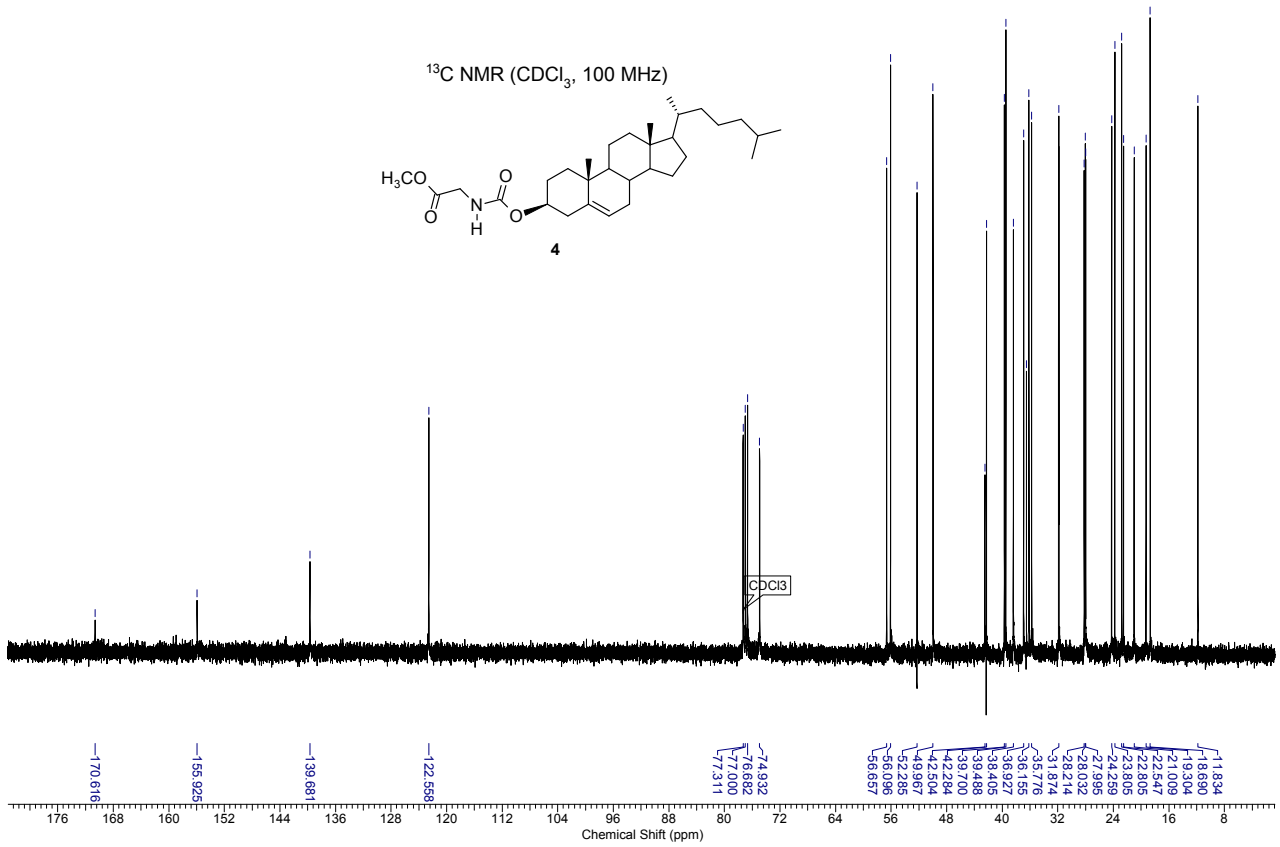


Figure S3. ^{1}H-, ^{13}C-NMR and Mass Spectra of compound **4**.





ESI-MS (ES^+): $[(C_{31}H_{51}O_4N)_2 + Na]^+$ 1026.7

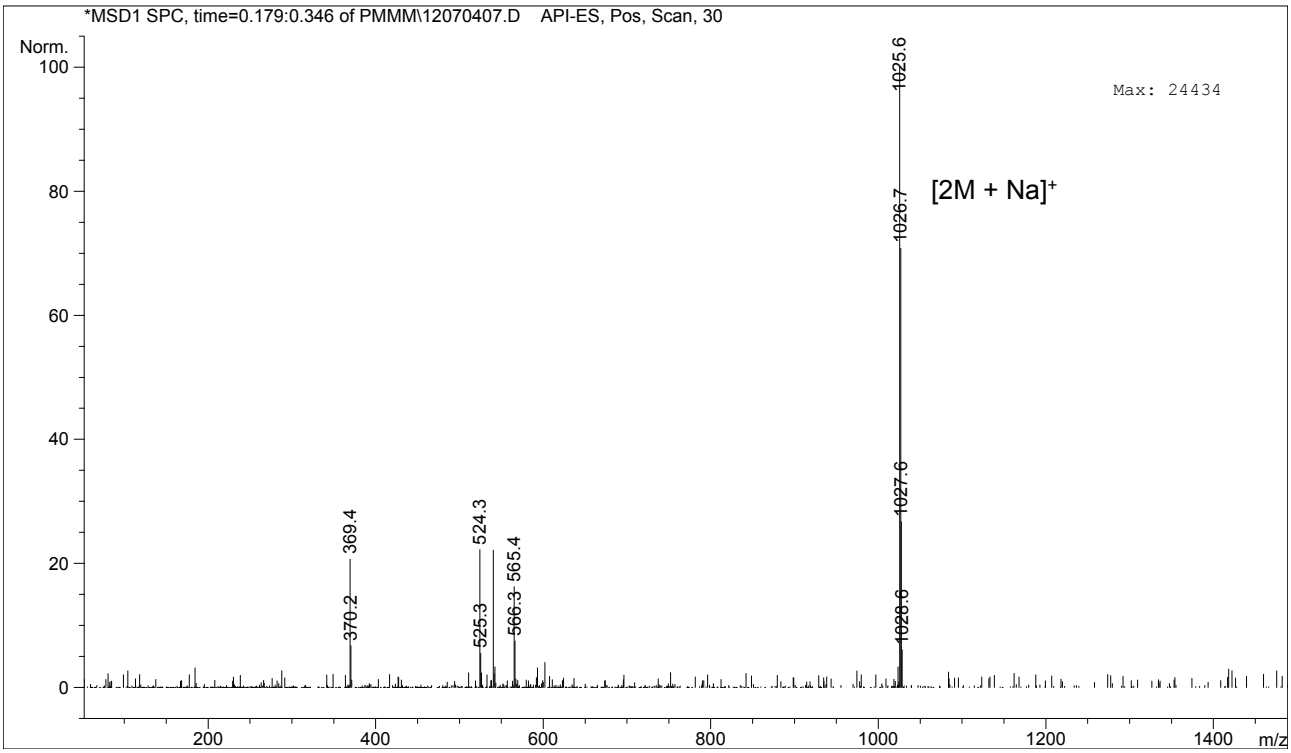
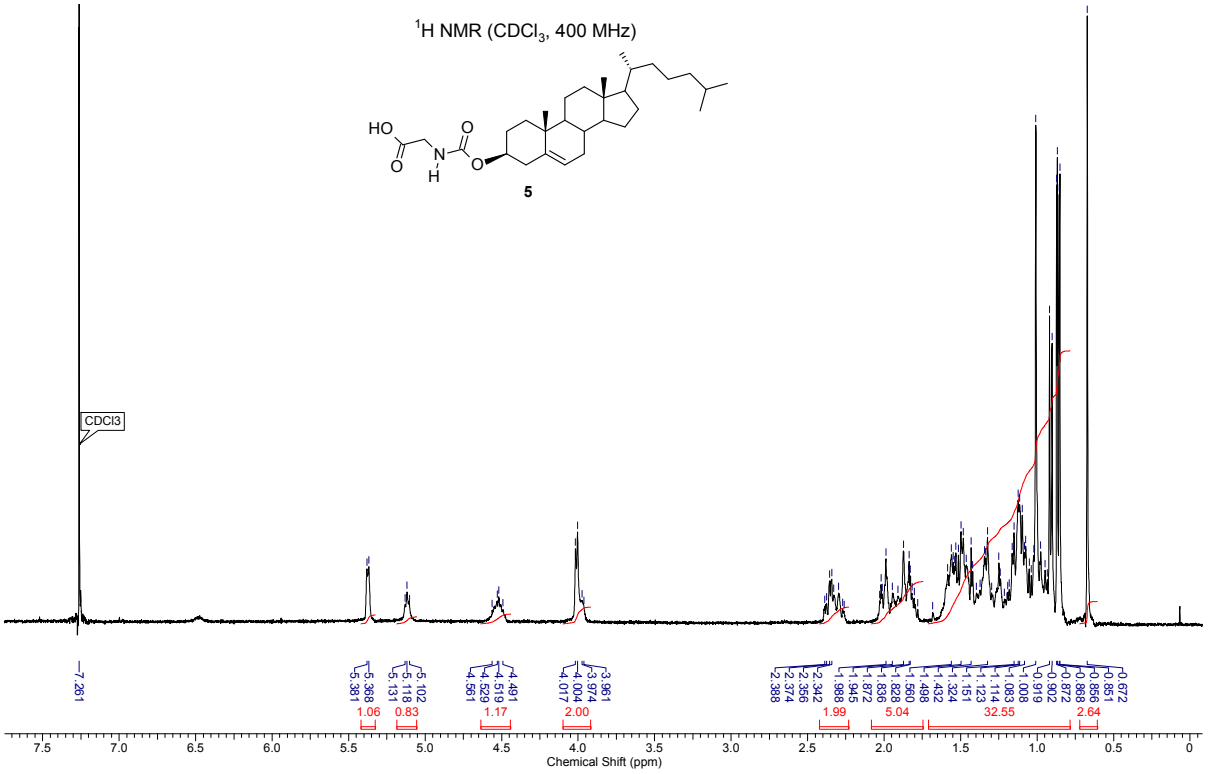
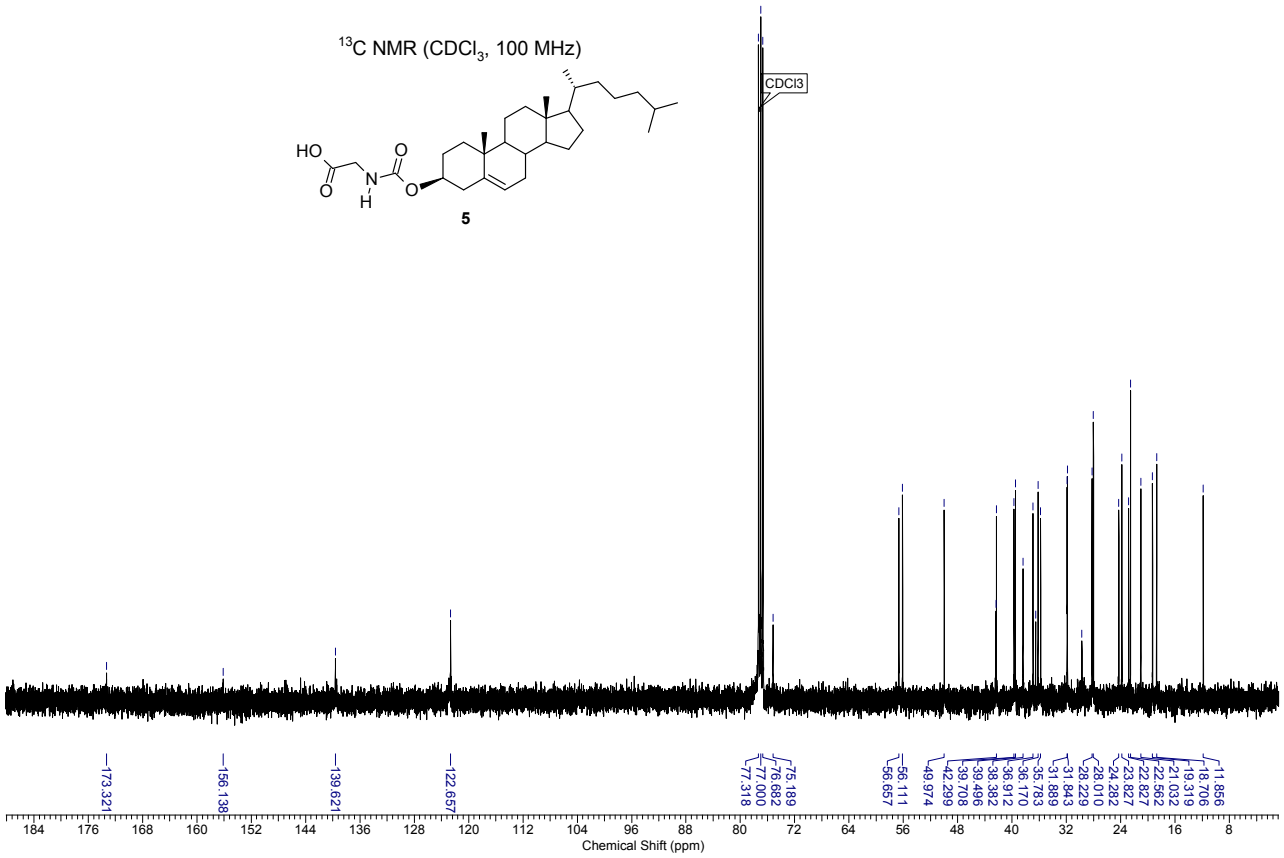


Figure S4. ^{1}H-, ^{13}C-NMR and Mass Spectra of compound **5**.





ESI-MS (ES^+): $[(C_{30}H_{49}O_4N)_2 + Na]^+$ 998.6

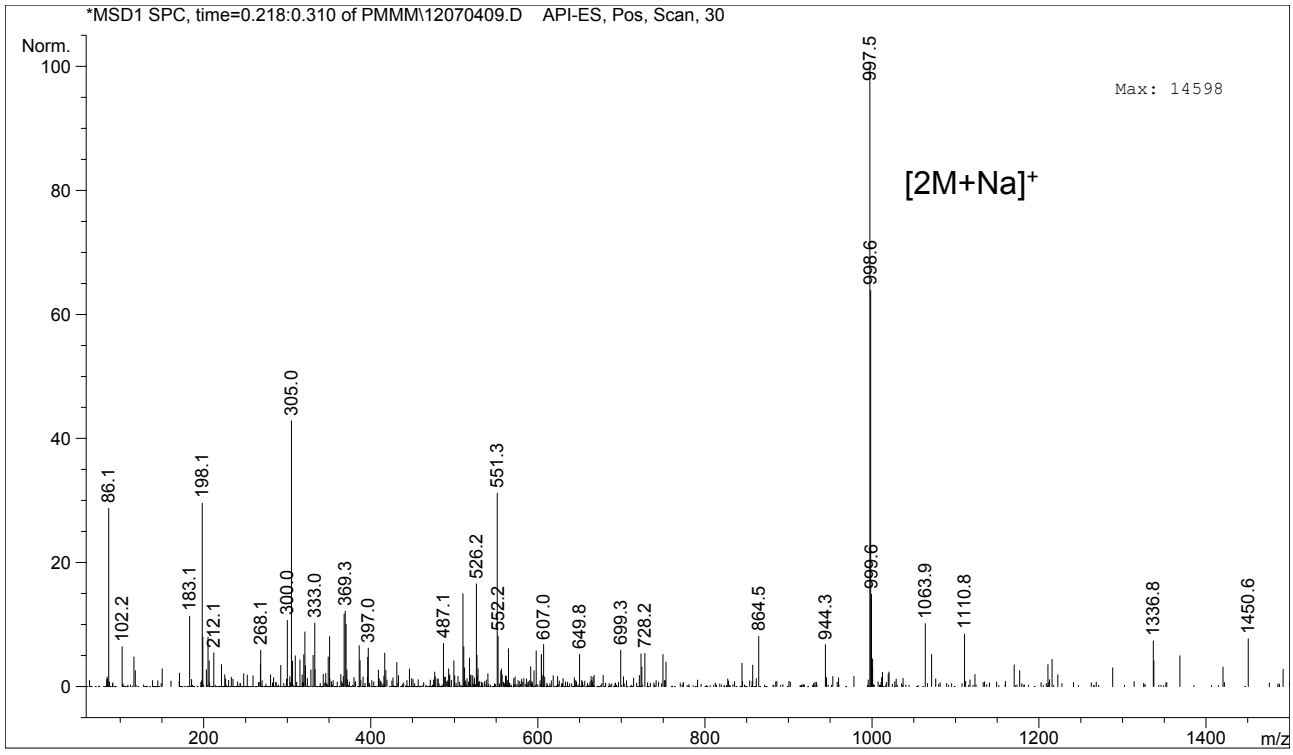
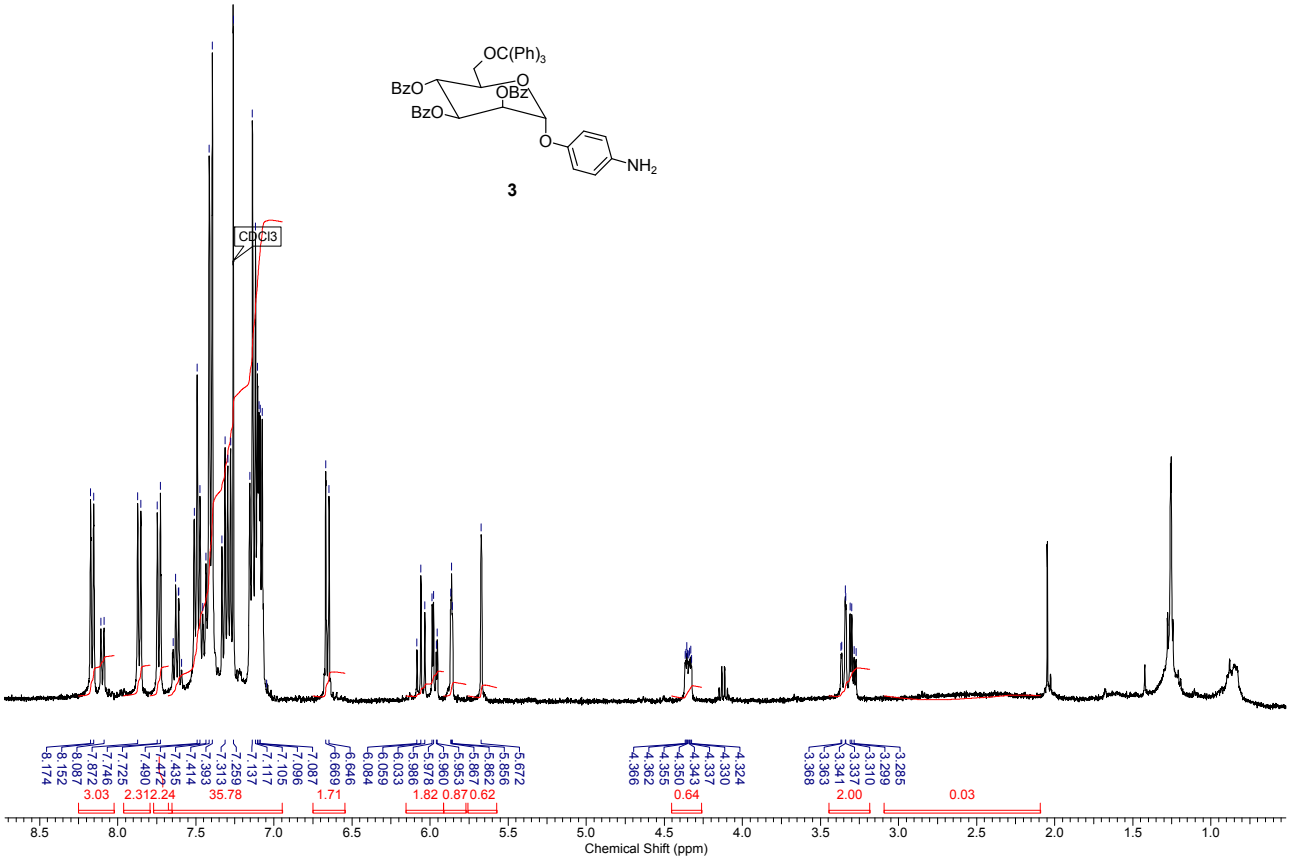
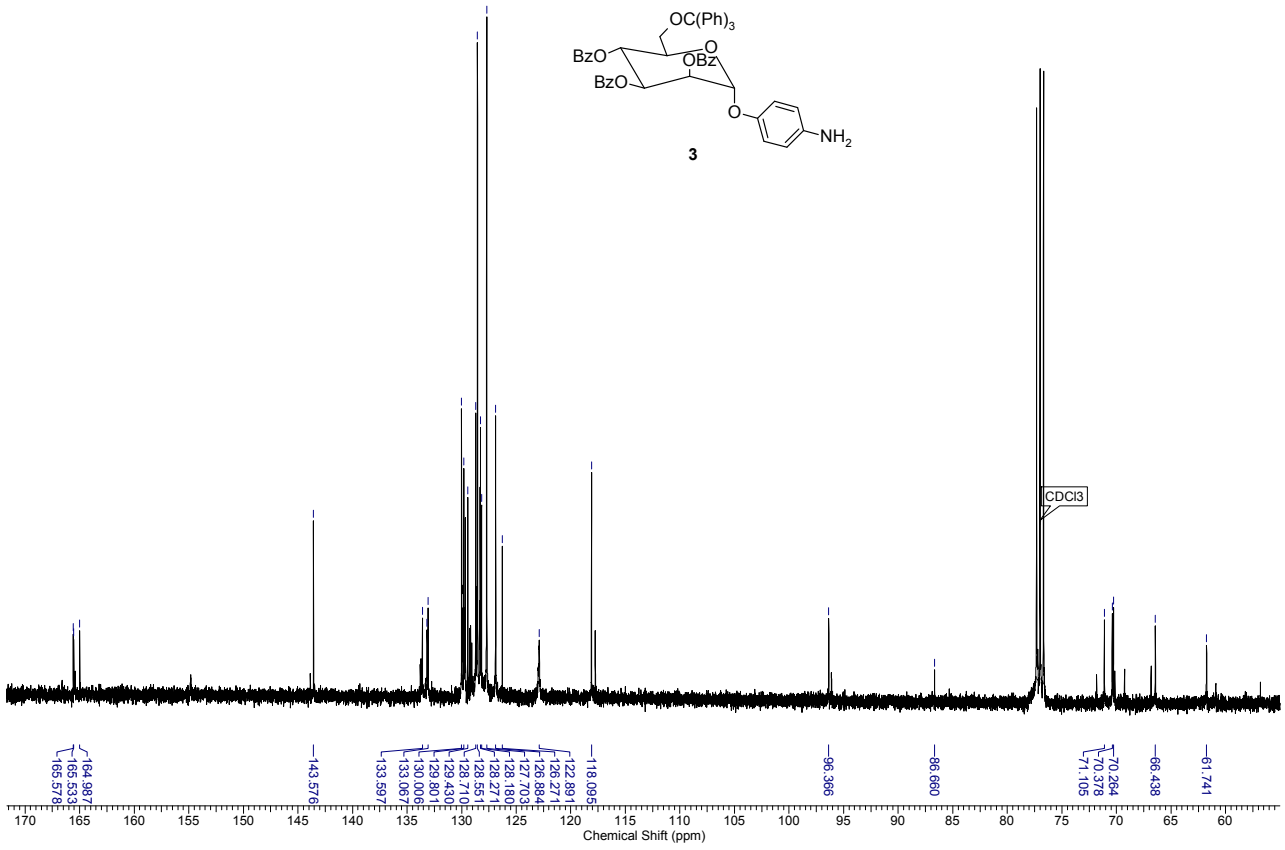


Figure S5. ^{1}H-, ^{13}C-NMR and Mass Spectra of compound **3**.





ESI-MS (ES$^+$): $[C_{52}H_{43}O_9N + Na]^+$ 848.2

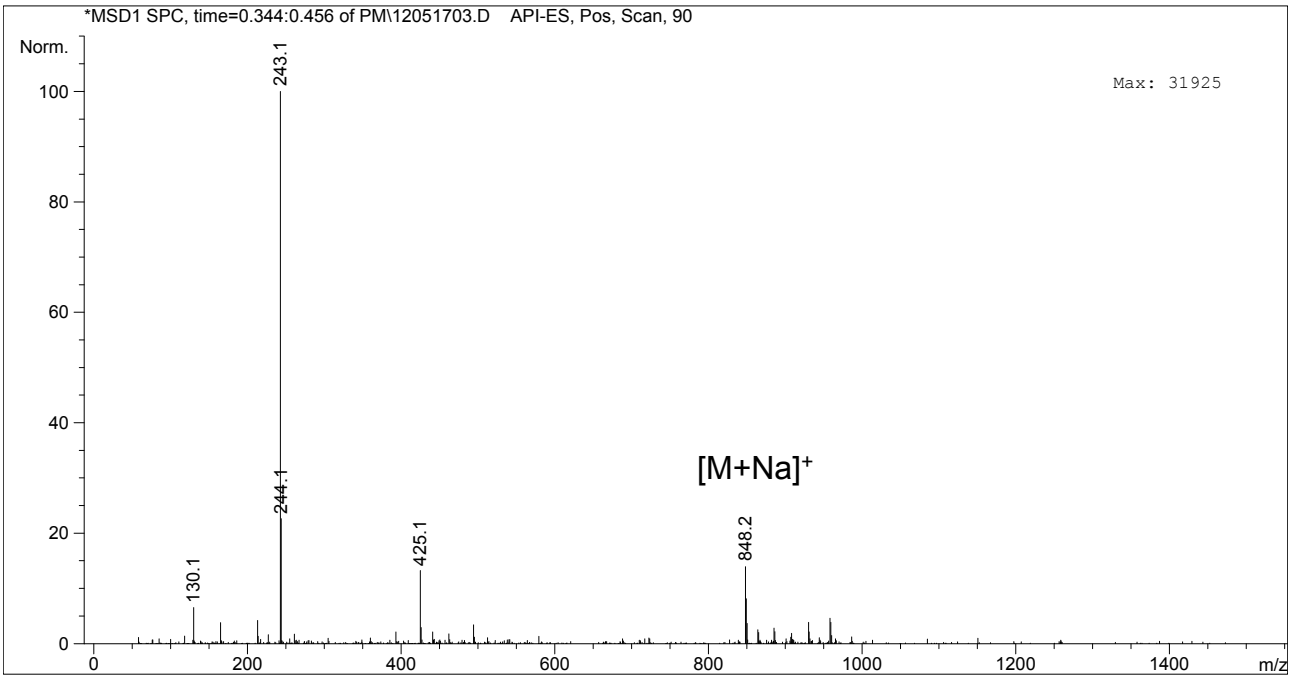
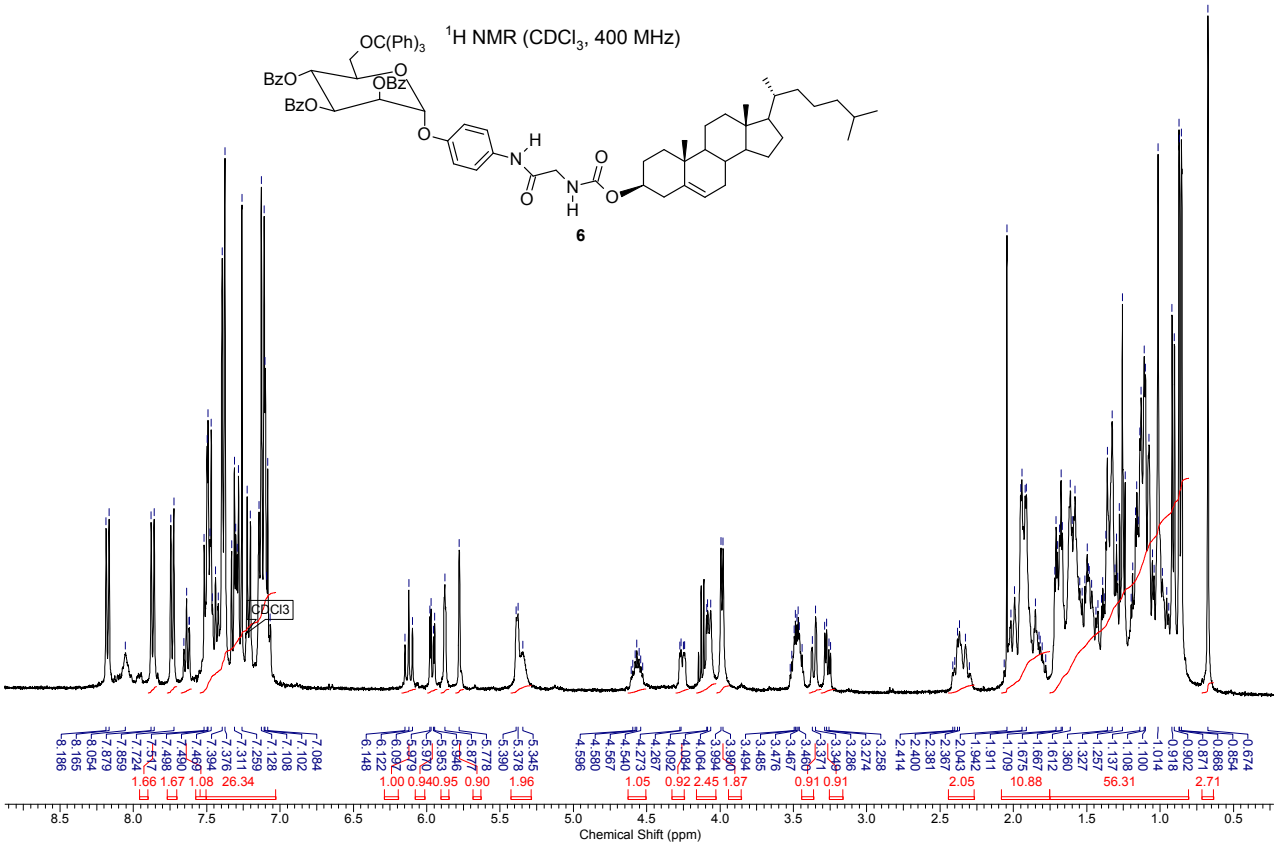


Figure S6. ^{1}H- and ^{13}C-NMR Spectra of compound **6**.



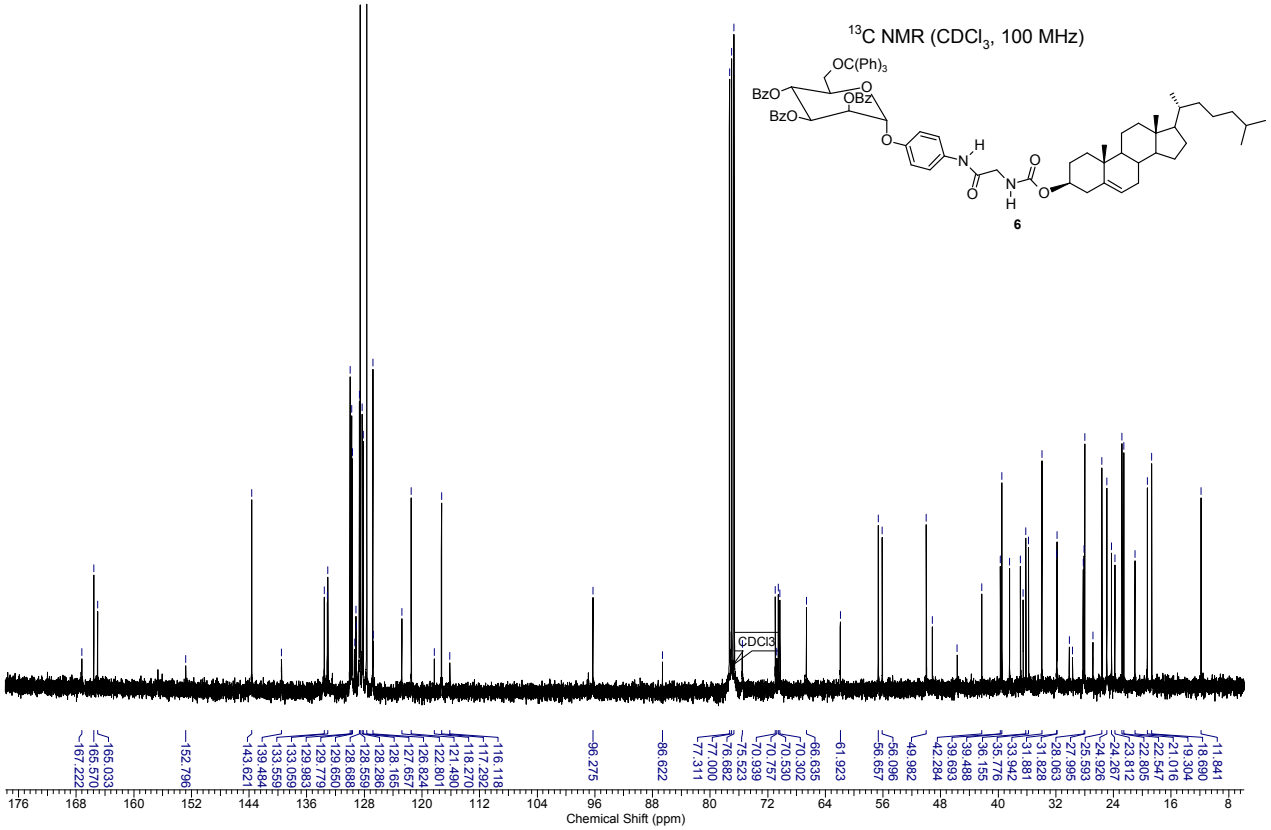
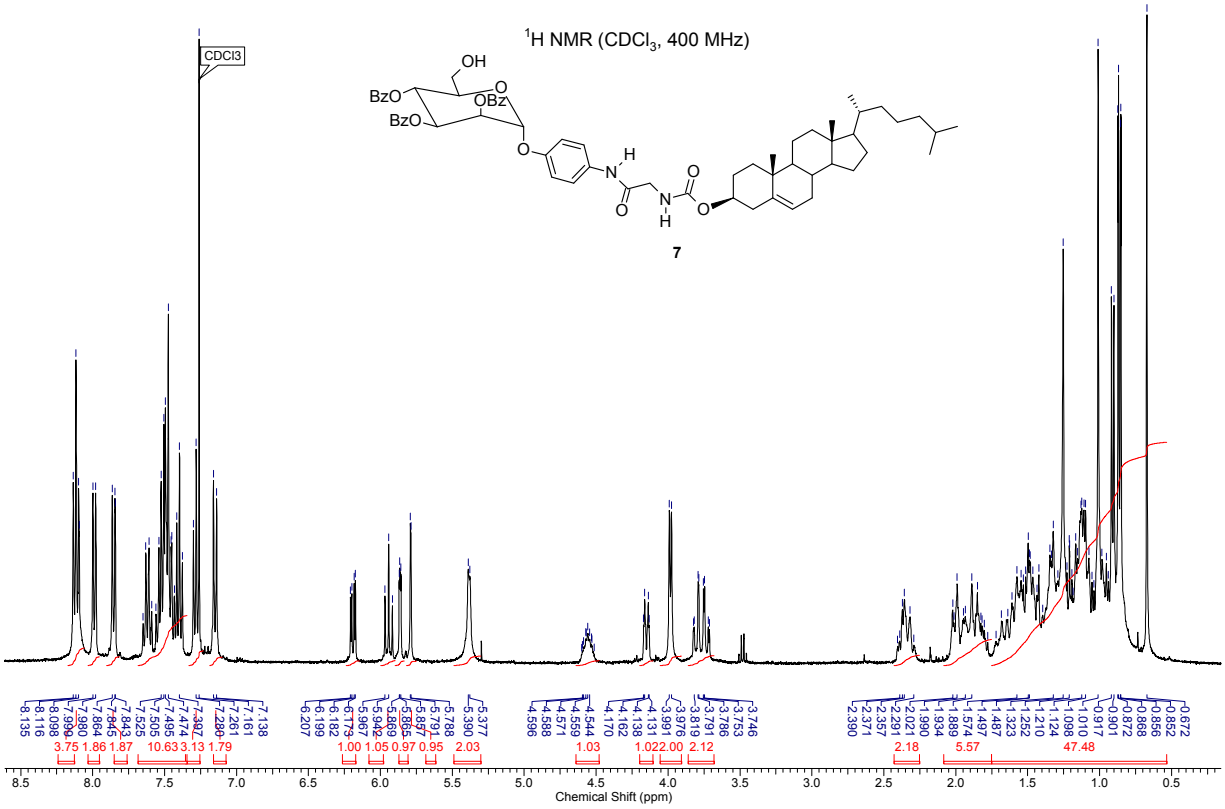

^{13}C NMR ($CDCl_3$, 100 MHz)
OC(Ph)3
BzO
OBz
BzO
O
N
H
O
N
H
O
O
6
CDCl3
167.222
165.033
165.570
152.796
143.621
139.484
133.559
133.059
129.983
129.779
129.650
128.688
128.559
128.286
128.165
127.657
126.824
122.801
121.490
118.270
117.292
116.118
96.275
86.622
77.311
77.000
76.682
75.523
70.939
70.757
70.530
70.302
66.635
61.923
56.657
56.096
49.982
42.284
39.693
39.488
36.155
35.776
33.942
31.881
31.828
28.063
27.995
25.593
24.926
24.267
23.812
22.805
22.547
21.016
19.304
18.690
11.841
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
Chemical Shift (ppm)

Figure S7. ^{1}H- and ^{13}C-NMR Spectra of compound **7**.



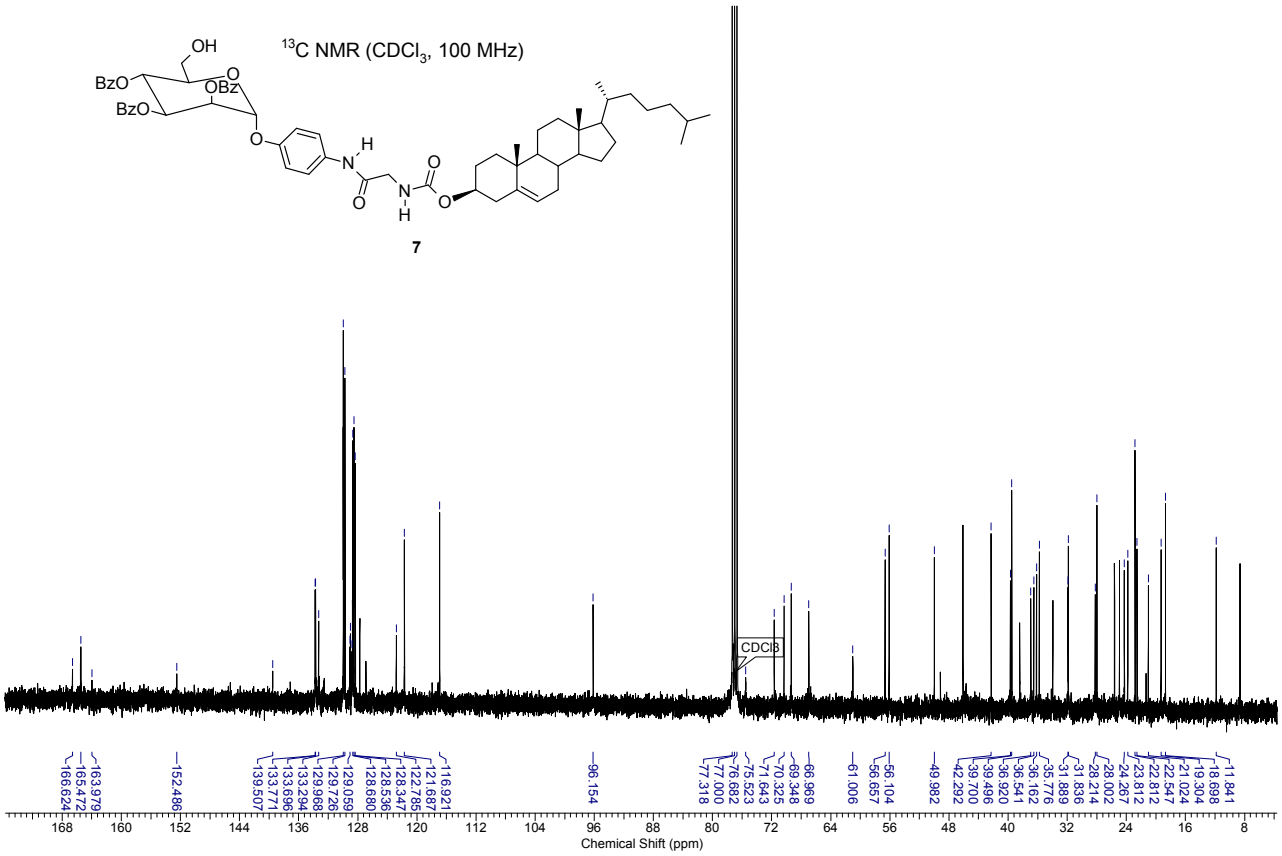


Figure S8. ^{31}P-NMR Spectrum of compound **8**.

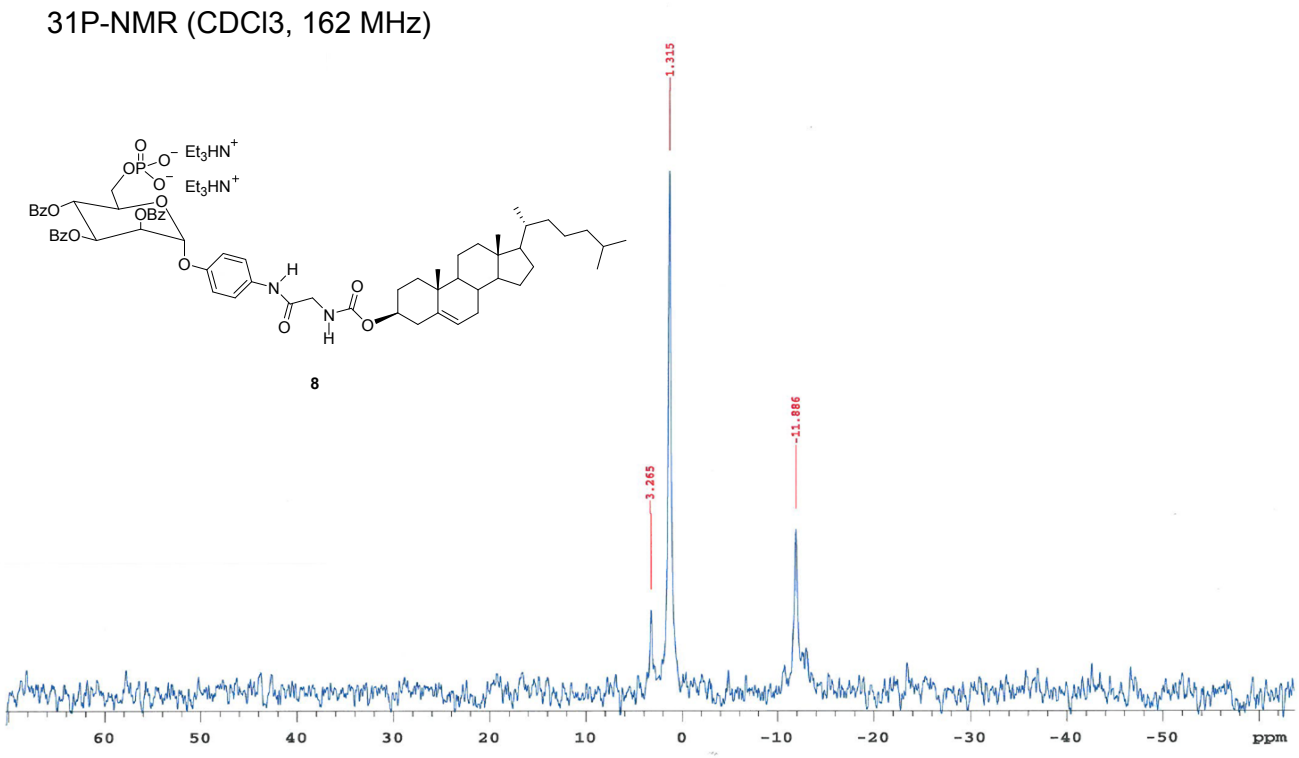
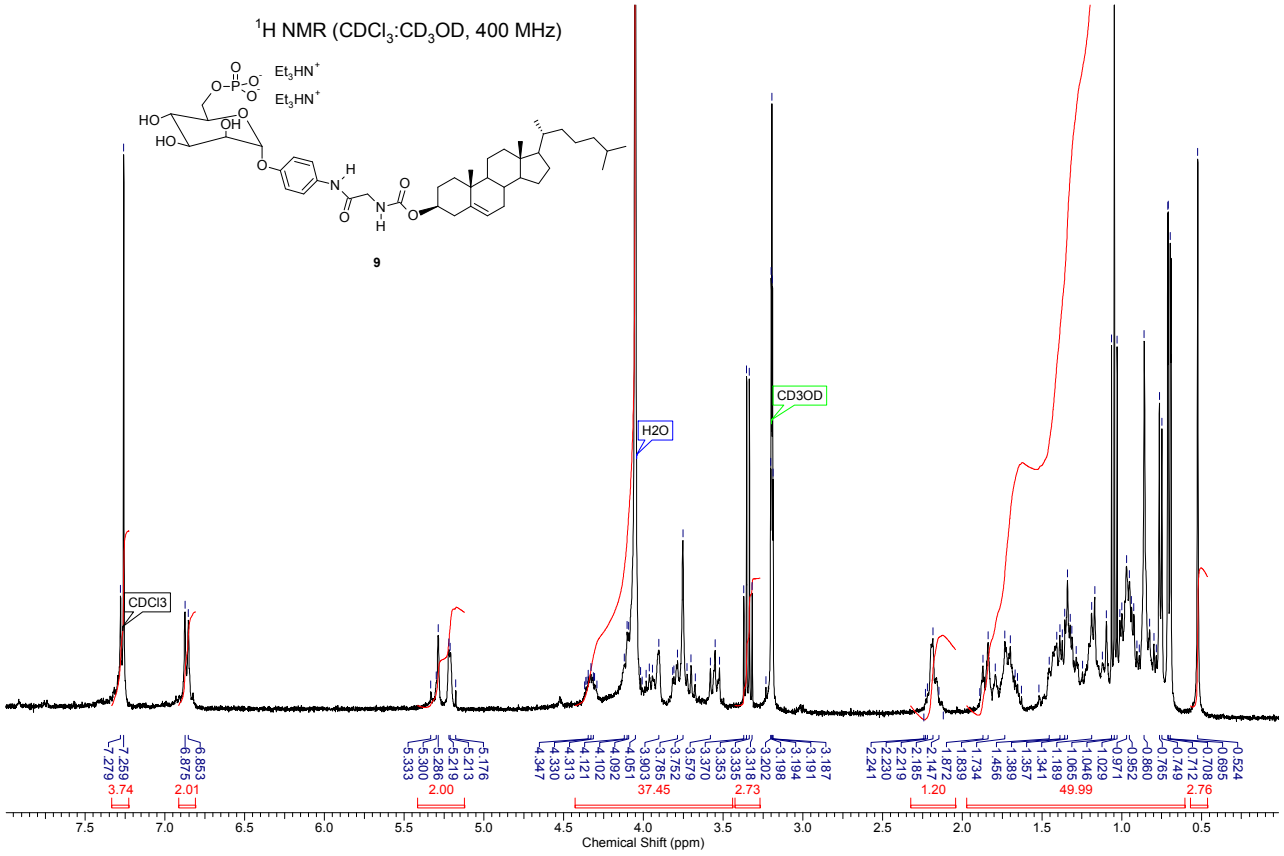
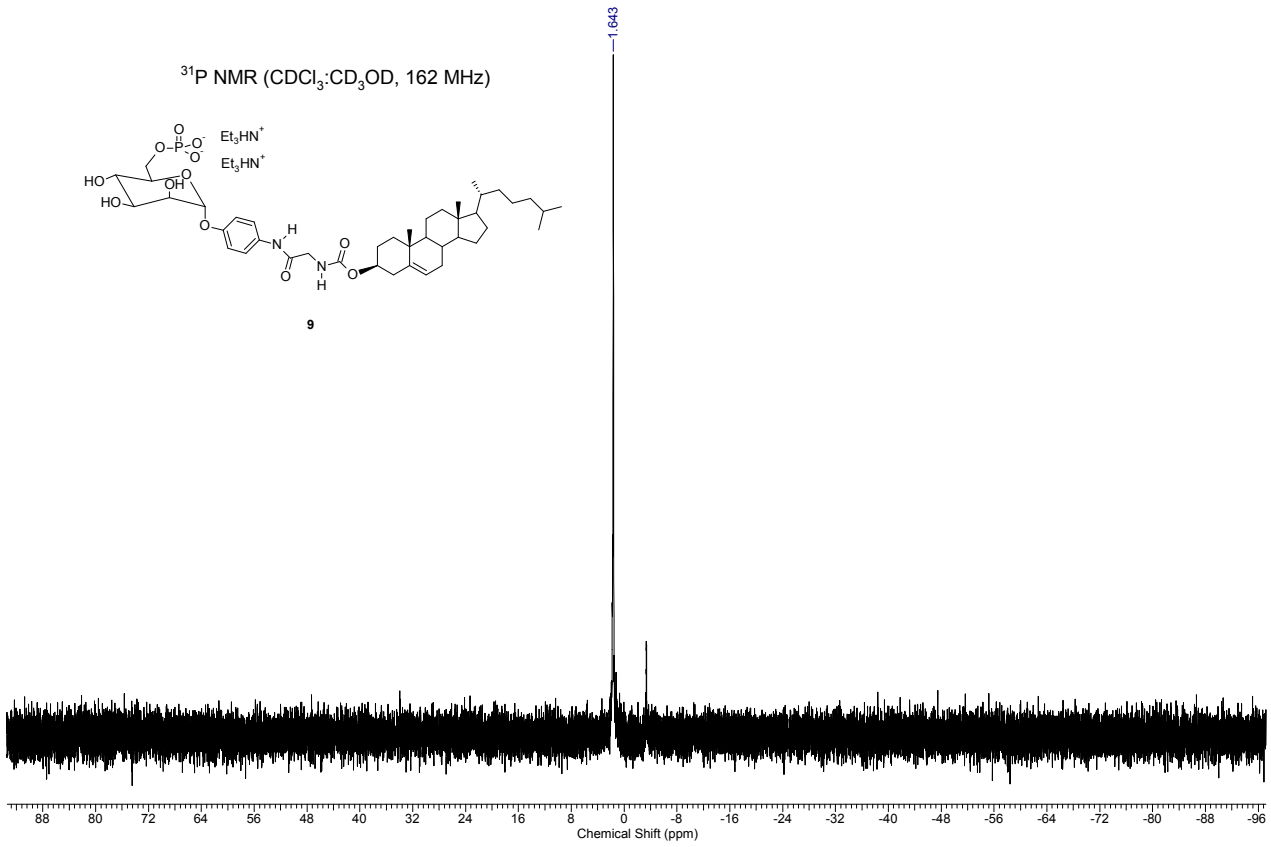


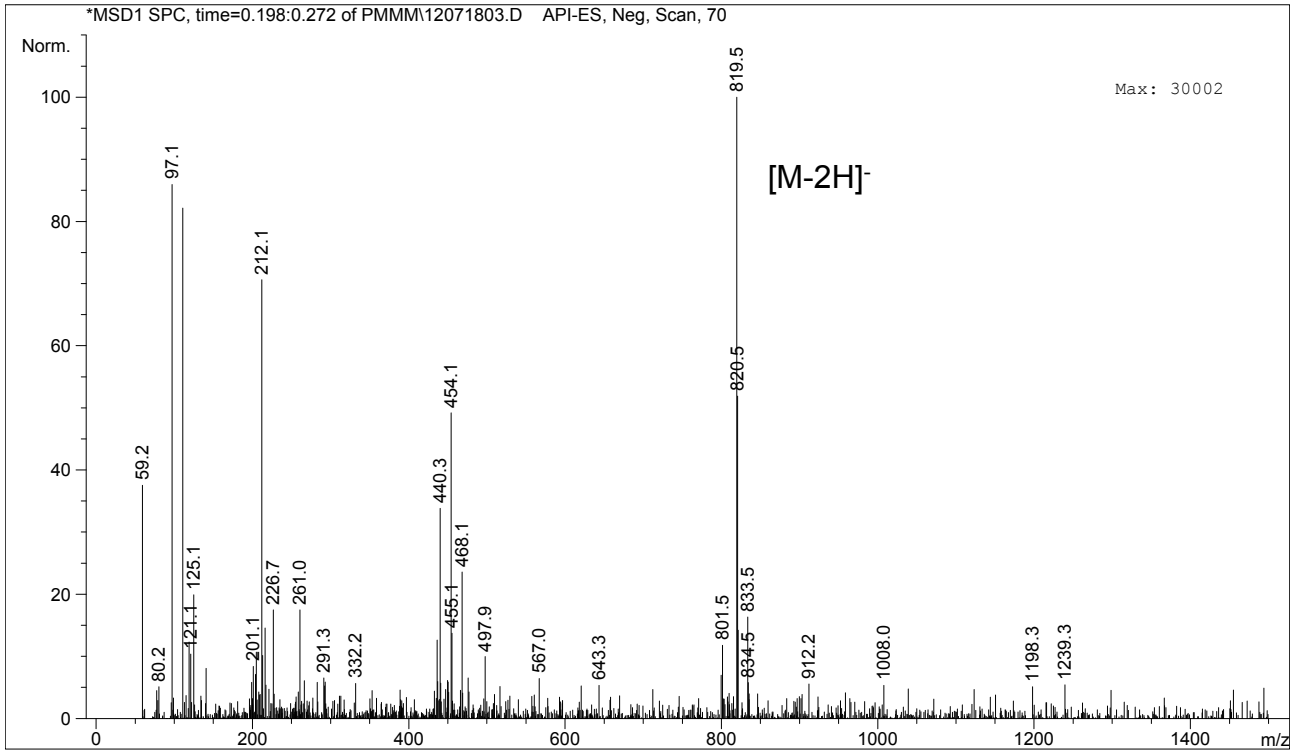
Figure S9. ^{1}H-, ^{31}P-NMR and Mass Spectra of compound **9**.[a]

[a] ^{1}H- and ^{31}P-NMR Spectra before treatment with Dowex cation exchange resin.





ESI-MS (ES$^+$): $[C_{42}H_{63}O_{12}N_2P\text{-}2H]^-$ 819.5



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