Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Mastering the Magnetic Susceptibility of Bicelles with Cholesterylamine and Complexed Lanthanide Ions

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1. Bicelle Preparation Protocol

The Bicelles were prepared following our previously reported protocols.[1] The phospholipids, cholesterol, and the cholestyramine dopants were dissolved in 10 mg/ml chloroform stock solutions. The lanthanide ions were dissolved in methanol to provide 10 mM stock solution of thulium chloride, dysprosium chloride, and ytterbium chloride. A dry lipid film was obtained from the solution of compounds by evaporating the organic solvents under reduced pressure at 40 °C. The dry lipid film was then hydrated with a 50 mM sodium phosphate buffer at a pH value of 7.5. The corresponding buffer in D_2O was employed for SANS experiments. The total lipid concentration was fixed to 15 mM with DMPC/dopant/DMPE-DTPA/Tm^{3+} (molar ratio 16:4:5:5). The dopant was either cholesterol, 3β-cholest-5-en-3-amine (Chol-NH_2) or the cholesterylamine conjugate (Chol-C_2OC_2-NH_2). The freshly hydrated samples were subject to five consecutive freeze-thaw cycles consisting of freezing in liquid nitrogen before heating to 50 °C. The samples were finally extruded (Lipex Biomembranes, Vancouver, Canada) 10 times through a polycarbonate membrane (Sterico, Dietikon, Switzerland) with a pore size of 200 nm and another 10 times through a polycarbonate membrane with a pore size of 100 nm. The bicelle samples were stored at room temperature and measured within a week of their preparation to guaranty their stability.[1a]

2. Radially Averaged SANS Curves and Fittings

The bicelle radius was calculated from radially averaged SANS curves measured at 5 °C and in the absence of any magnetic field. The curves were fitted with a Porod cylinder model with a thickness of 4.6 nm and a LogNorm distribution with $\sigma = 0.5$. The SASfit software package was employed for fittings of all the reported samples in Table 1 and Figure S1.[2] The measurements were conducted on the SANS-I beamline at PSI, Villigen, Switzerland with a neutron wavelength of 0.8 nm and a 2D ^{3}He detector at 2, 6 or 18 m to cover a q-range of 0.03 to 1.5 nm^{-1}. Data were corrected for the blank cell, transmission, and detector efficiency.

3. Cryo-TEM Micrographs of 3β-cholest-5-en-3-amine (Chol-NH_2) containing Bicelles

The Cryo-TEM micrograph of the DMPC/Chol-NH_2/DMPE-DTPA/Tm^{3+} (16:4:5:5) bicelle sample presented in Figure 1A of the manuscript is shown without artificial contrast enhancement in Figure S2A. The sample holder was tilted 65° in Figure S2B. The existence of planar bicelle structures is emphasized with a red arrow in Figure S2A and S2B as the round bicelle seen from top-on view at 0° becomes oval-shaped when viewed at a 65° angle. Moreover, the appearance of bicelles seen from side-on view at 65° is shown with blue arrows in Figure S2B. The presence of bicelles is confirmed with another sample of DMPC/Chol-NH_2/DMPE-DTPA/Tm^{3+} (16:4:5:5) in the micrograph of Figure S2C. It was not necessary to artificially enhance the contrast of the later when measuring at a 30° angle.

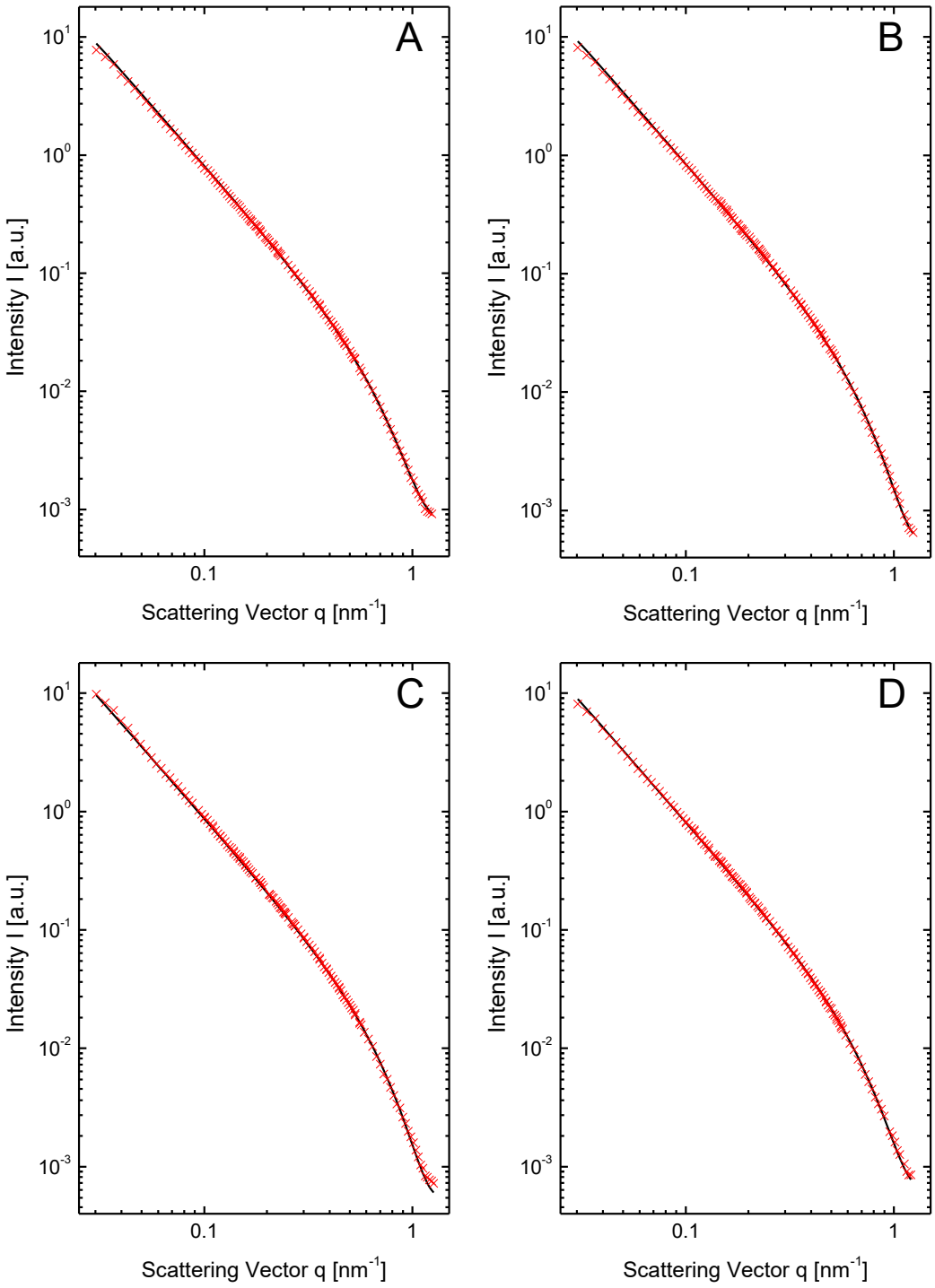


Figure S1. Radially averaged SANS curves (data points) and fittings (solid lines) of DMPC/Chol-NH_2/DMPE-DTPA/Ln^{3+} (16:4:5:5) bicelles at 5 °C with the lanthanide ion (Ln^{3+}) A) Tm^{3+}, B) Dy^{3+} or C) Yb^{3+}. D) radially averaged SANS curve and fitting of DMPC/Chol-C_2OC_2-NH_2/DMPE-DTPA/Tm^{3+} (16:4:5:5) bicelles at 5 °C. The SANS 2D scattering patterns were measured in the absence of any magnetic field.

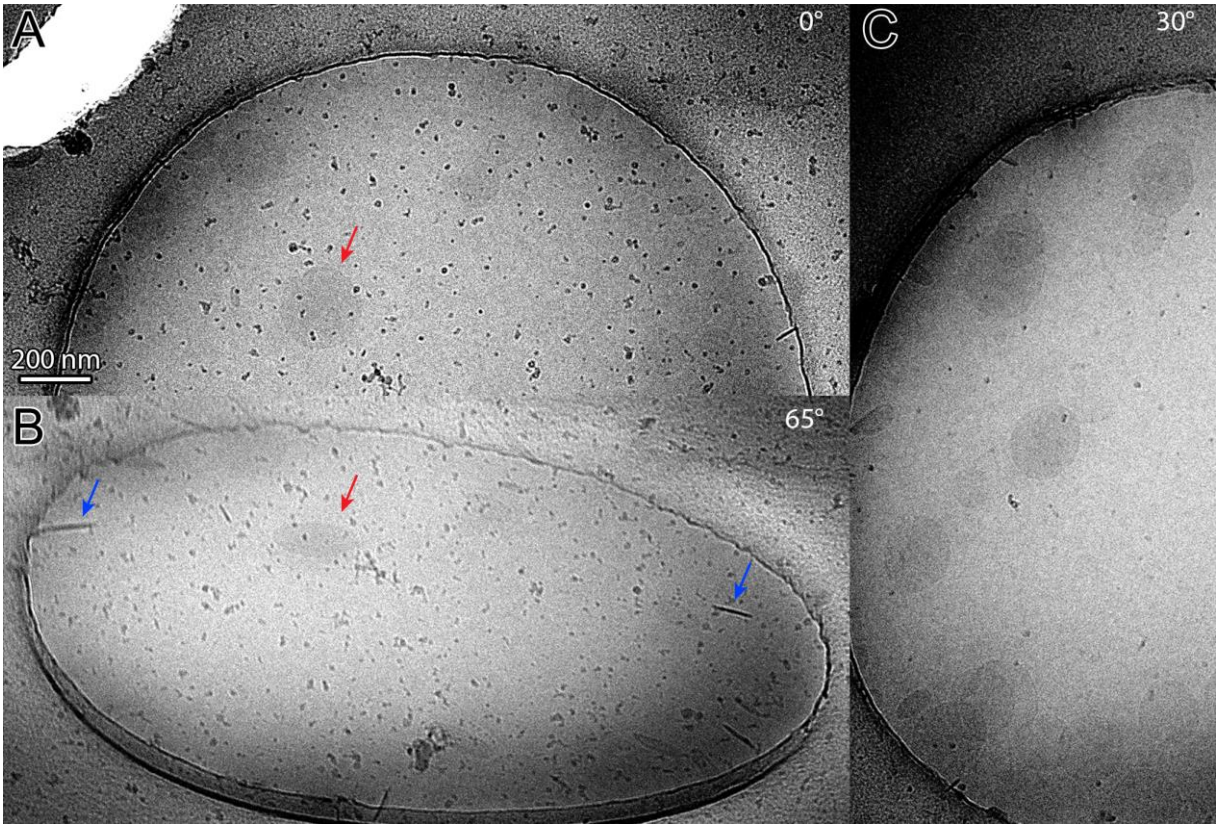


Figure S2. Cryo-TEM micrographs of DMPC/Chol-NH_2/DMPE-DTPA/Tm^{3+} (16:4:5:5) flash frozen at 5 °C with a sample holder tilt angle of A) 0°, B) 65°, and C) 30°. The same sample is shown in A and B. Bicelles from top-on view are labeled with red arrows and from side-on view with blue arrows. The micrograph in C is from another sample. The scale bar represents 200 nm and no artificial contrast enhancement was applied. Dark particles are ice crystals resulting from the flash freezing procedure.

4. Birefringence as a function of temperature of 3β-cholest-5-en-3-amine (Chol-NH_2) containing Bicelles

Monitoring the birefringence signal of DMPC/Chol-NH_2/DMPE-DTPA/Tm^{3+} (16:4:5:5) bicelles under a 5.5 T field was undertaken as described elsewhere.[1b,3] The birefringence signal of the sample was monitored as it underwent a heating and cooling cycle from 5 to 55 °C and back at a rate of 1 °C/min in Figure S3. The strength of the birefringence signal is correlated to the degree of alignment of the bicelles.[1c] Sterol-free DMPC/DMPE-DTPA/Ln^{3+} are known to undergo a thermo-reversible transition into magnetically non-alignable vesicles at the phase transition temperature of DMPC of 24 °C causing a zeroing of the birefringence signal.[1a] When cholesterol or cholesterol conjugates are present in the bicelle's bilayer, the clear phase transition temperature of DMPC disappears as a liquid-ordered phase is induced. The bicelles become more resistant to higher temperatures.[1b,1e,3] This phenomenon is readily monitored as the collapse of the birefringence signal occurs at higher temperatures and serves as evidence that the cholesterol or cholesterol conjugates are present in the bilayer. For the DMPC/Chol-NH_2/DMPE-DTPA/Tm^{3+} bicelle sample presented in Figure S3, the collapse of the birefringence signal occurs at 50 °C on heating and the regeneration at 40 °C on cooling. The existence of alignable species above 24 °C proves the incorporation of aminocholesterol in the bicelle's bilayer. Moreover, the thermo-reversible collapse occurs in a similar range to previously reported DMPC/Cholesterol /DMPE-DTPA/Tm^{3+} bicelle systems.[1b]

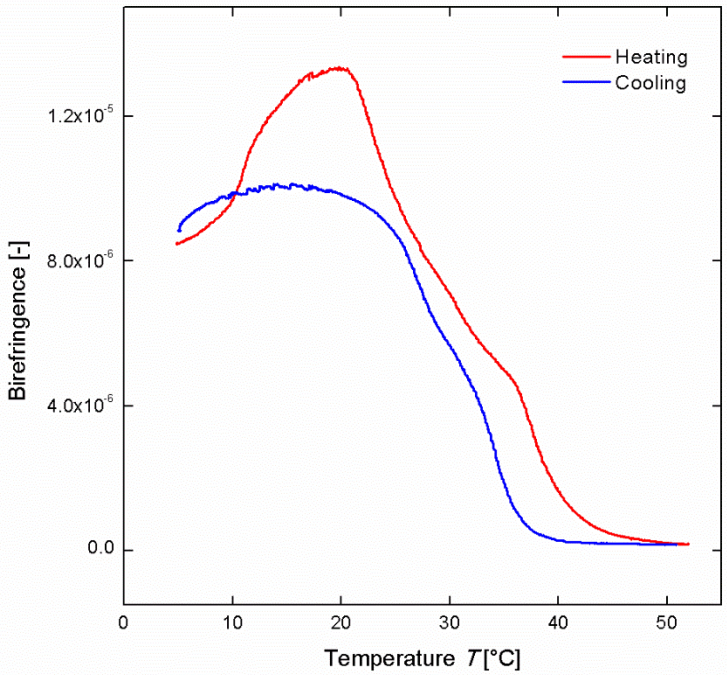


Figure S3. Birefringence signal as a function of temperature at 5.5 T of DMPC/Chol-NH_2/DMPE-DTPA/Tm3+ (16:4:5:5) bicelles. The sample was subject to a heating (red line) and cooling (blue line) cycle from 5 to 55 °C at 1 °C/min.

5. Synthesis Procedures

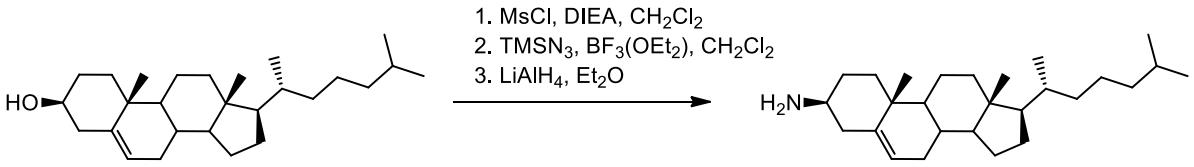
All reactions were conducted according to published protocols.[3-5] The concerned literature reference is explicitly stated in each synthesis description. Unless otherwise stated, all chemicals were purchased either from Sigma Aldrich, VWR, Merck or ABCR and were used without further purifications.

Reactions were conducted in three necked round bottom flasks under nitrogen atmosphere. They were monitored by analytical thin-layer chromatography (TLC) using silica gel baked on aluminum-foil with a fluorescent indicator 254 nm from Merck. The TLCs were visualized under UV-light at 254 nm and further developed with aqueous alkaline potassium permanganate solution. Flash chromatography was performed with a CombyFlash TeledyneISCO system from Companion using RediSep*TM* normal phase disposable columns of various sizes. Both isocratic flows and gradients of solvent mixtures were employed. Compounds were dried for 24 hours at room temperature (22 °C) and 10 mbar in a Salvislab vacuum oven. Yield was calculated based on the mass of the dried and purified compounds.

Nuclear magnetic resonance (NMR) spectra were recorded at room temperature in 5 mm broadband inverse probes on a Bruker spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Deuterated chloroform (ARMAR) was used as a solvent for ^{1}H NMR and a 4:1 deuterated chloroform/methanol mixture was used for ^{13}C NMR of the final products (spectra calibrated on the chloroform peak). Data is reported as follows: chemical shifts (δ) in parts per million (ppm), corresponding signal integral with multiplicity abbreviation (s: singlet, d: doublet, t: triplet, m: multiplet), and if possible identification according to the molecular structure.

Mass spectra were recorded on an ESI-HRMS from Thermo Scientific (exactive) with direct injection dissolved in an appropriate concentration of methanol. Chloroform was also used whenever necessary. Computed masses were based on single isotope masses for high-resolution spectra. Full calibration was conducted before measurements using the appropriate solutions for both positive and negative modes.

5.1. Synthesis of 3β-cholest-5-en-3-amine (Chol-NH_2)



The synthesis of Chol-NH_2 was conducted following the three-step reaction protocol proposed by B. R. Peterson et al.[4]

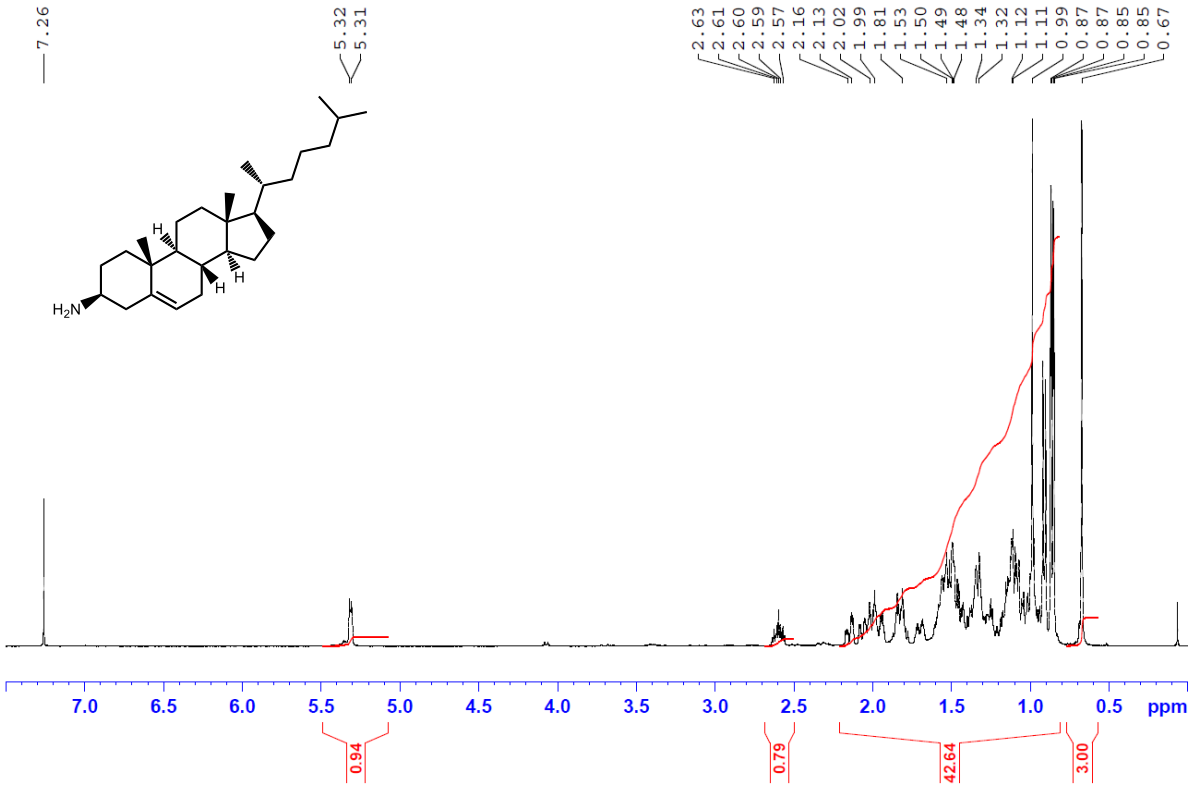
Step 1: 50 mmol of cholesterol (Amresco, USA) was dissolved in 250 ml of anhydrous dichloromethane and cooled to 4 °C. 100 mmol of triethylamine was added to the cholesterol solution followed by the dropwise addition of 55 mmol of methanesulfonyl chloride in 50 ml of anhydrous dichloromethane. The reaction ran for 3 hours at 4 °C and was monitored by TLC (heptane/ethyl acetate 4:1). The solvents were removed under vacuum and the residue dissolved in 15 ml of dicholormethane. The intermediate product (3β-cholest-5-en-3-ol methanesulfonate) was precipitated with 150 ml of methanol and recrystallized in methanol to achieve a pure compound with 94% yield.

Step 2: 43 mmol of trimethylsilyl azide and 77 mmol of boron trifluoride etherate were added to a solution of 38 mmol of 3β-cholest-5-en-3-ol methanesulfonate (product from step 1) in 200 ml of anhydrous dichloromethane. The reaction ran for 2 hours at 22 °C before it was quenched with 200 ml of a saturated sodium bicarbonate solution in water. The organic layer was recovered and the aqueous layer was extracted three times with 100 ml of methyl tert-butyl ether. The organic layers were combined and washed with 200 ml of deionized water, dried over sodium sulfate, and concentrated under vacuum to afford the crude intermediate product. The compound was purified by flash chromatography (heptane/ethyl acetate 9:1) to afford 3β-azido-5-cholestene with 78% yield.

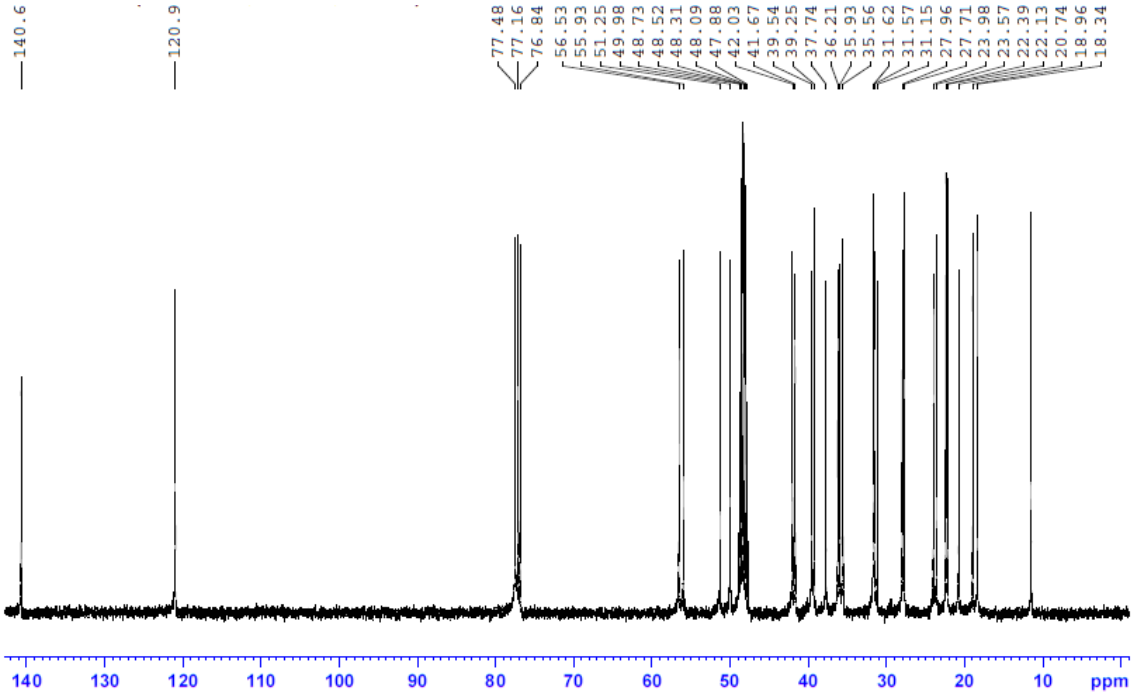
Step 3: 12 mmol of 3β-azido-5-cholestene (product from step 2) was dissolved in 80 ml of anhydrous diethyl ether at 4 °C. 18 mmol of $LiAlH_4$ were subsequently added in four equal portions over 30 min.

The reaction was gradually heated to 22 °C and stirred for an additional 2 hours. The reaction was monitored by TLC (heptane/ethyl acetate 2:1). The mixture was subsequently cooled to 4 °C and quenched by dropwise addition of 10 ml of cold deionized water. The resulting solution was pored over an ice bath and the organic layer was recovered. The aqueous layer was extracted twice with 40 ml of ethyl acetate. The organic layers were combined, washed with 100ml of a saturated NaCl solution and 100 ml of deionized water, dried over sodium sulfate, and concentrated under vacuum. The recovered solid was further recrystallized in acetonitrile to afford pure 3β-cholest-5-en-3-amine with 63% yield. **HR-MS(+):** calculated for $C_{27}H_{48}N$ $[M+H]^+$: 386.3787. Found: 386.3781. *Δm/z:* 1.5 ppm. **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 5.32 (d, 1H, H-6), 2.61 (m, 1H), 0.85-2.16 (m, 43H, cholesterol), 0.67 (s, 3H, cholesterol) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$/MeOD 4:1) δ: 140.69, 120.97, 56.53, 55.93, 51.25, 49.98, 42.03, 41.67, 39.54, 39.25 37.74, 36.21, 35.93, 35.56, 31.62, 31.57, 31.15, 27.96, 27.71, 23.98, 23.57, 22.39, 22.13, 20.73, 18.96, 18.34, 11.48.

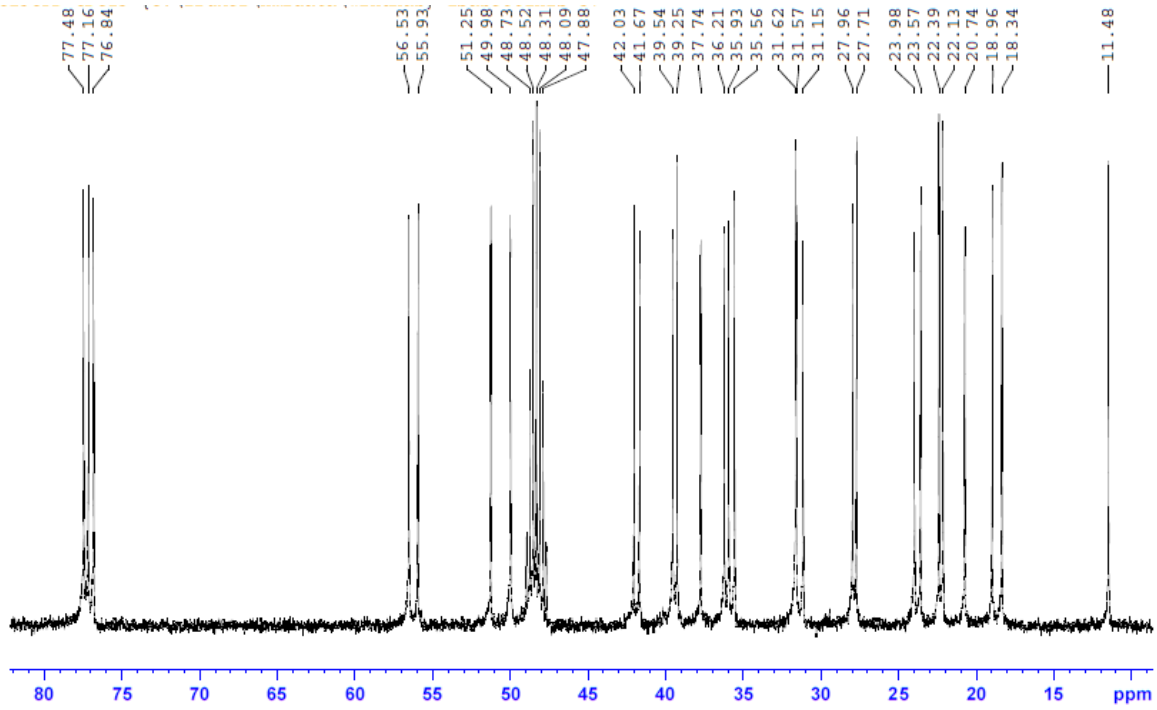
^{1}H NMR of Chol-NH_2



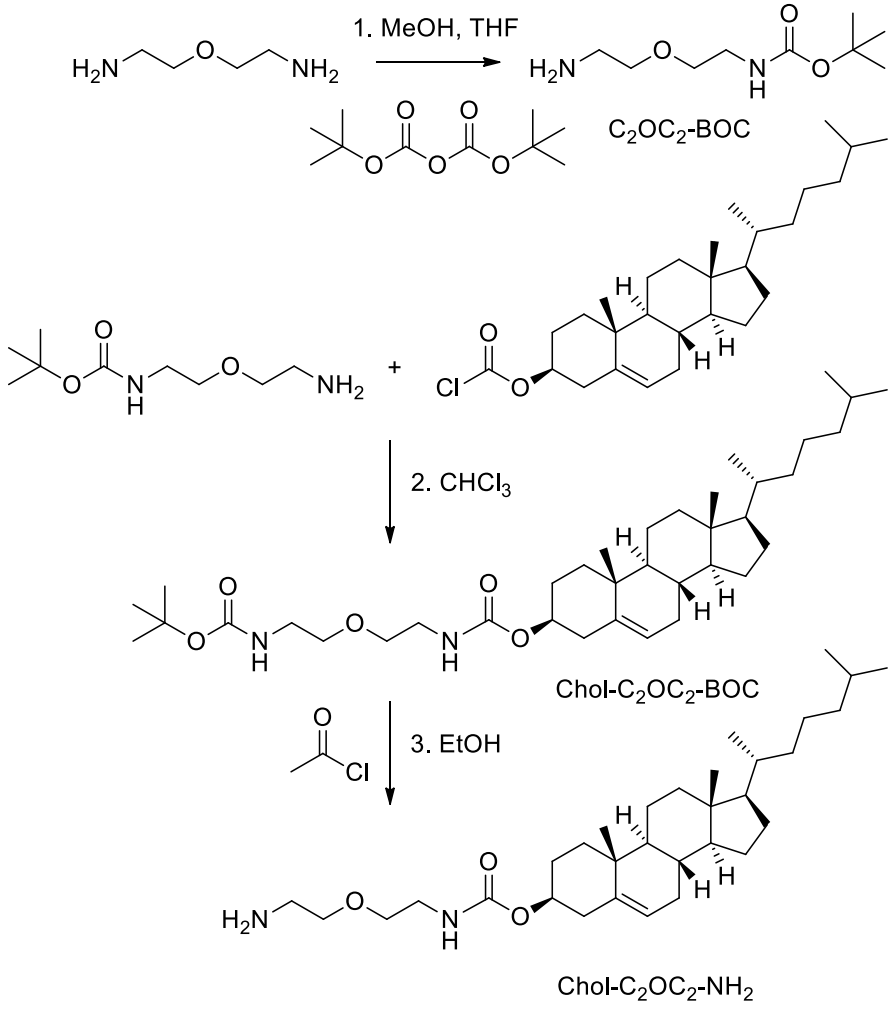
^{13}C NMR of Chol-NH_2



^{13}C NMR of Chol-NH_2 Zoom



5.2. Synthesis of the Chol-C_2OC_2-NH_2 cholesterylamine conjugate



The synthesis of Chol-C_2OC_2-NH_2 was conducted following the first three steps of our reported synthesis protocol for Chol-C_2OC_2-DTPA.[3] The third step is similar to the protocol described by Waterhouse *et al*.[5]

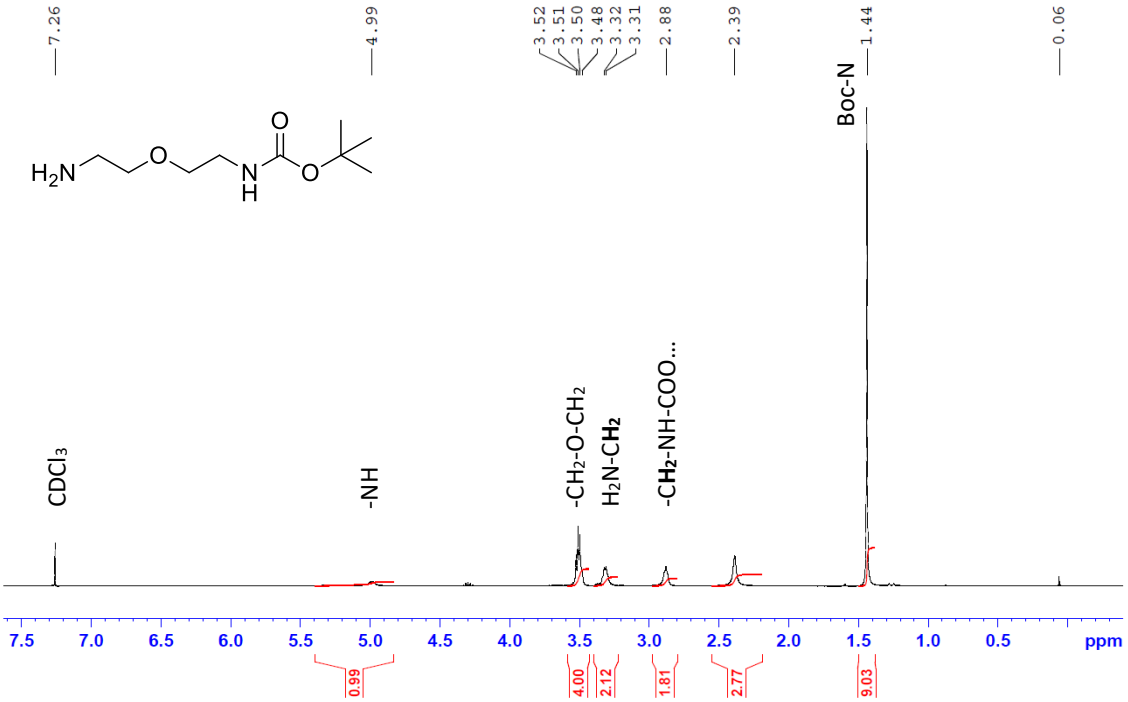
Step 1: 48 mmol of 2,2'-Oxybis(ethylamine) was dissolved in 500 ml of methanol (dried over 3 Å molecular sieves) at 0 °C. A 150 ml solution of THF (dried over 4 Å molecular sieves) containing 43 mmol of di-tert-butyl dicarbonate was subsequently added drop wise. The reaction mixture was heated to room temperature and stirred for 38 hours. The residue after solvent removal was purified by flash chromatography with isocratic $CHCl_3$/EtOH (1:1) and, subsequently, isocratic ammonia/EtOH/$CHCl_3$ (2:5:5). The product (C_2OC_2-BOC) yield was 57%. **HR-MS(+):** calculated for $C_9H_{21}N_2O_3$ $[M+H]^+$: 205.1547. Found: 205.1545. *Δm/z:* 1.0 ppm. **^{1}H-NMR** (400 MHz, $CDCl_3$) δ: 4.99 (s, 1H, NH), 3.48 – 3.52 (m, 4H, CH_2-O-CH_2), 3.31 (m, 2H, H_2N-**CH_2**), 2.88 (t, 2H, **CH_2**-NH-COO…), 2.39 (s, 2H, **H_2N**-CH_2), 1.44 (s, 9H, Boc-N) ppm.

Step 2: 10.4 mmol of C_2OC_2-BOC (product from step 1) was dissolved in 85 ml of chloroform and added dropwise to a 50 ml solution containing 11.7 mmol of cholesteryl chloroformate in anhydrous, amylene stabalized chloroform. The reaction was stirred 24 hours at room temperature and monitored by TLC (heptane/ethyl acetate 3:1). The reaction vessel was periodically flushed with nitrogen to remove the produced HCl gas. 70 ml of a 5% $NaHCO_3$ solution was used to quench the reaction. Two extractions with 140 ml of a saturated $NaHCO_3$ solution and a last extraction with 140 ml of deionized water. The recovered organic layer was dried over sodium sulfate before solvent removal under vacuum. The recovered crude product was further purified by flash chromatography using heptane/ethyl acetate 3:1. The product (Chol-C_2OC_2-BOC) yield was 87%. **HR-MS(+):** calculated for $C_{37}H_{64}N_2O_5Na$ $[M+Na]^+$: 639.4707. Found: 639.4701. *Δm/z:* 0.9 ppm. **^{1}H-NMR** (400 MHz, $CDCl_3$) δ: 5.37 (d, 1H, H-6), 4.79 - 5.10

(s, 2H, NH), 4.49 (dt, 1H, H-3), 3.49 – 3.52 (m, 4H, CH_2-O-CH_2), 3.30 – 3.36 (m, 4H, N-CH_2-…-CH_2-N), 1.45 (s, 9H, Boc-N), 0.67-2.35 (m, 44H, cholesterol) ppm.

Step 3: 6.2 mmol of Chol-C_2OC_2-BOC (product from step 2) was dissolved in 150 ml of anhydrous ethanol. 16.7 mmol of acetyl chloride was added dropwise to the ice-cooled mixture. The reaction was stirred for 24 hours and monitored by TLC (heptane/ethyl acetate 2:1). An additional 10% of acetyl chloride (1.53 mmol) was added and the reaction left for another 24 hours to react at room temperature and another 24 hours at 40 °C. The solvent was removed under vacuum and the crude product was purified by crystallization in ethanol and acetonitrile. The Chol-C_2OC_2-NH_2 product yield was 74%. **HR-MS(+):** calculated for $C_{32}H_{57}N_2O_3$ $[M+H]^+$: 517.4364. Found: 517.4360. *Δm/z:* 0.8 ppm. **^{1}H-NMR** (400 MHz, $CDCl_3$) δ: 8.39 (s, 3H, NH_3^+) 5.36 (d, 1H, H-6), 4.46 (dt, 1H, H-3), 3.55 – 3.76 (m, 4H, -CH_2-O-CH_2-), 3.24 – 3.35 (m, 4H, N-CH_2-…-CH_2-N), 0.67-2.33 (m, 44H, cholesterol) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$/MeOD 4:1) δ: 156.75, 139.32, 122.02, 74.07, 69.84, 65.86, 56.28, 55.78, 49.63, 41.86, 42.70, 40.03, 39.33, 39.20, 39.09, 38.11, 36.58, 36.09, 35.78, 35.40, 31.43, 27.81, 27.69, 27.53, 23.84, 23.46, 22.31, 22.06, 20.62, 18.80, 18.25, 11.38.

^{1}H NMR of C_2OC_2-BOC



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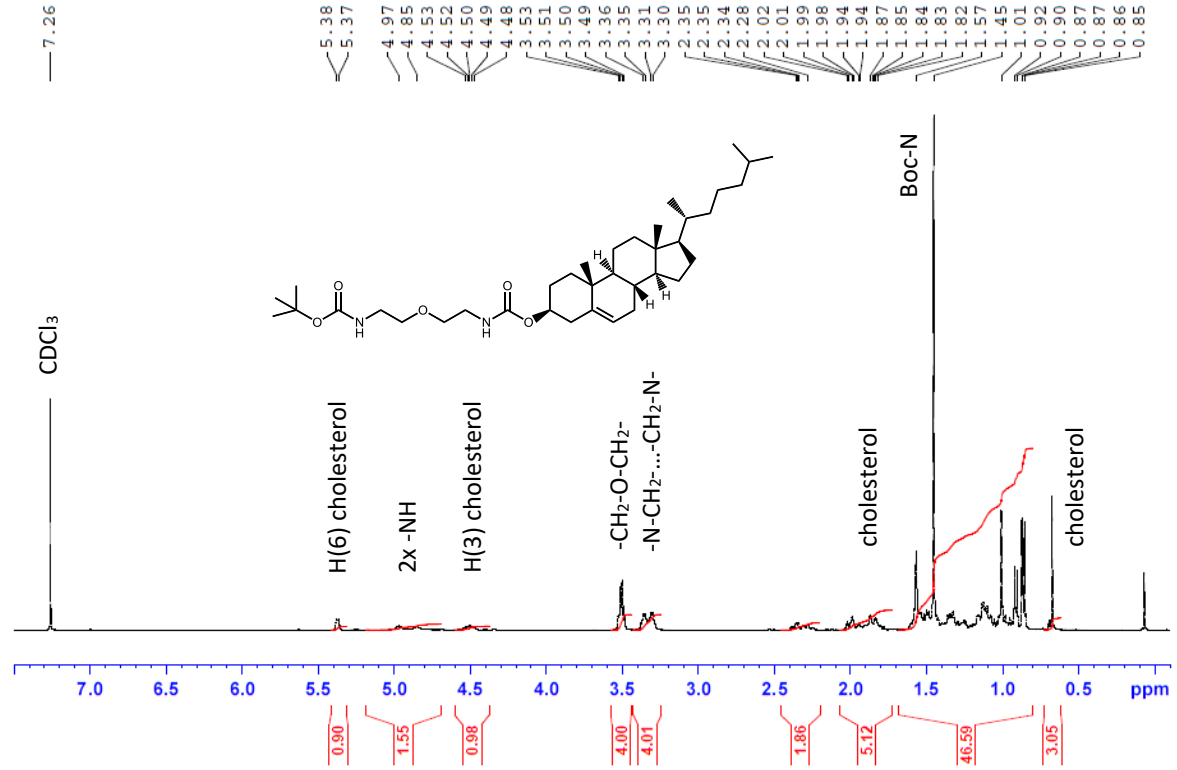
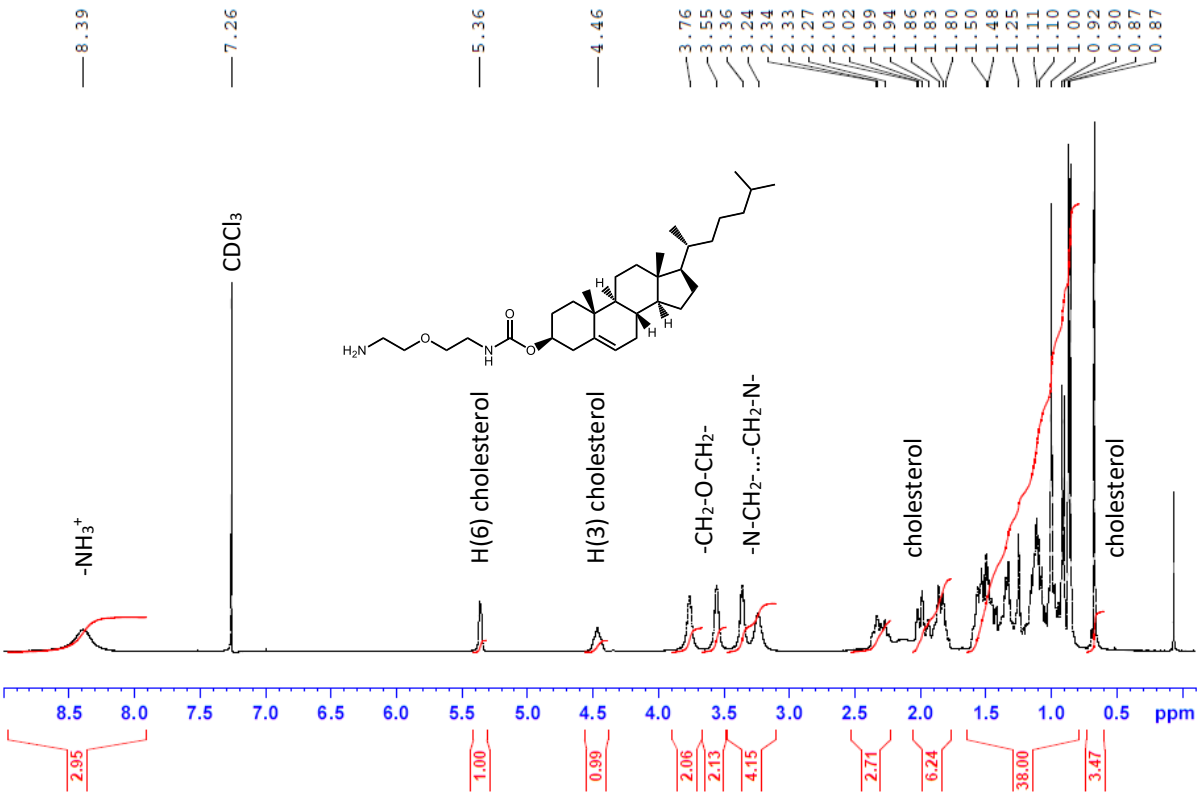


^{1}H



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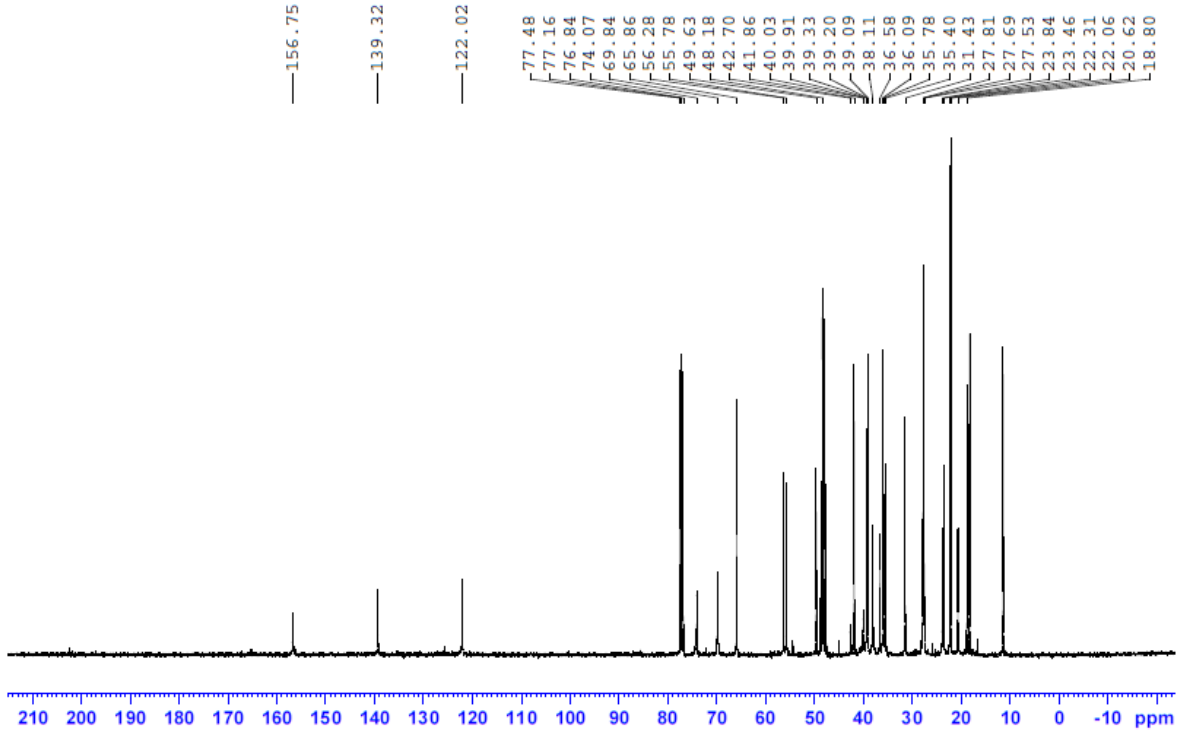
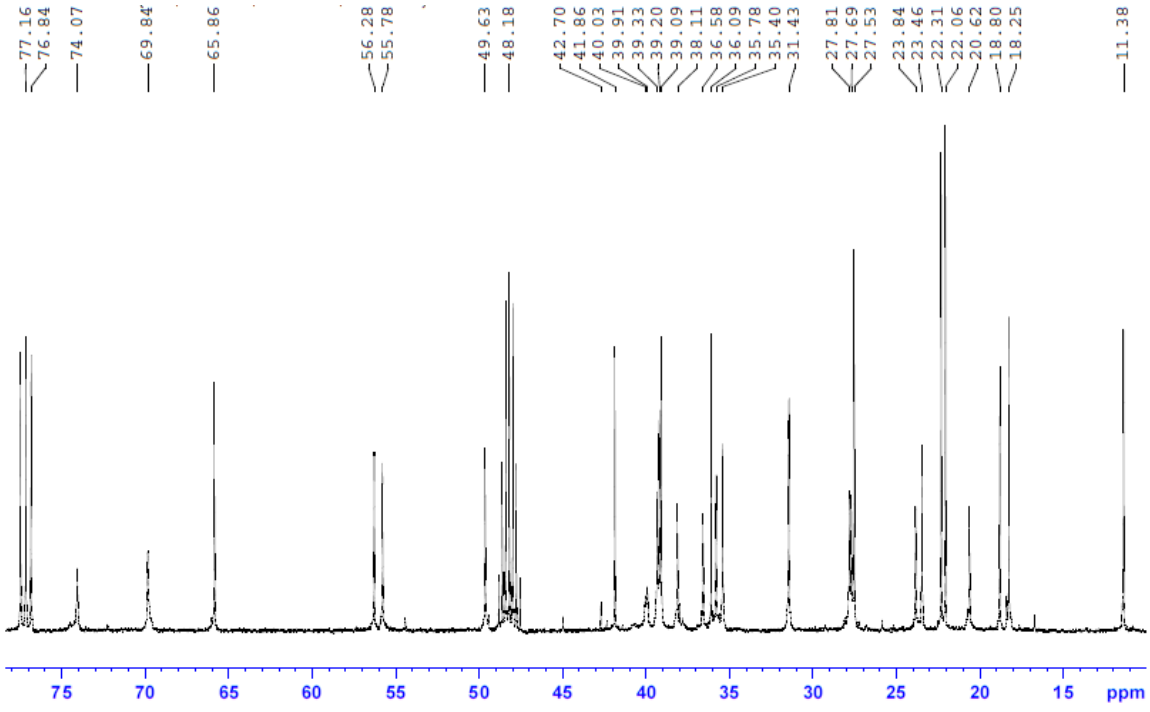


^{13}C NMR of Chol-C_2OC_2-NH_2 Zoom



6. References

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