Electronic Supplementary Material (ESI) for RSC Advances.
This journal is © The Royal Society of Chemistry 2017

Supplementary Data

Fabrication of nanostructures through self-assembly of non-ionic amphiphiles for biomedical applications

Suchita Prasad,[a] Katharina Achazi,[b] Christoph Böttcher,[c]

Rainer Haag,[b,*] Sunil K. Sharma[a,*]

[a]*Department of Chemistry, University of Delhi, Delhi 110007, India*

[b]*Institut für Chemie und Biochemie, Freie Universität Berlin, Takustraße 3, 14195 Berlin, Germany*

[c]*Forschungszentrum für Elektronenmikroskopie, Institut für Chemie und Biochemie, Freie Universität Berlin, Fabeckstraße 36a, 14195 Berlin, Germany*

Table of Contents

1. Experimental Section

1.1. Materials

1.2. Methods and Instrumentation

1.3. Synthetic Procedures

2. Figures

Figure S1: ^{1}H & ^{13}C NMR spectra of 2,2-di(prop-2-yn-1-yl)propane-1,3-diyl bis(4-(decyloxy)benzoate) (**9**)

Figure S2: ^{1}H & ^{13}C NMR spectra of 2,2-di(prop-2-yn-1-yl)propane-1,3-diyl bis(4-(octadecyloxy)benzoate) (**10**)

Figure S3: ^{1}H & ^{13}C NMR spectra of methoxypolyethylene glycol carboxymethyl ether (**13**)

Figure S4: ^{1}H & ^{13}C NMR spectra of methoxypolyethylene glycol carboxymethyl ether (**14**)

Figure S5: ^{1}H & ^{13}C NMR spectra of (methoxypolyethylene glycol carboxymethyl ether)ethylester (**15**)

Figure S6: ^{1}H & ^{13}C NMR spectra of (methoxypolyethylene glycol carboxymethyl ether)ethylester (**16**)

Figure S7: ^{1}H & ^{13}C NMR spectra of 2-azidopropane-1,3-diyl bis(methoxypoly(oxyethylene)oate) (**18**)

Figure S8: ^{1}H & ^{13}C NMR spectra of 2-azidopropane-1,3-diyl bis(methoxypoly(oxyethylene)oate) (**19**)

Figure S9: ^{1}H & ^{13}C NMR spectra of amphiphile **20**

Figure S10: ^{1}H & ^{13}C NMR spectra of amphiphile **21**

Figure S11: ^{1}H & ^{13}C NMR spectra of amphiphile **22**

Figure S12: DEPT-135 NMR spectrum of amphiphile **21**

Figure S13: ^{2}D HETCOR and COSY NMR spectra of amphiphile **22**

Figure S14: Gel permeation chromatogram of amphiphiles **20**-**22**

Figures S15, **S16** and **S17**: Critical aggregation concentration (CAC) of amphiphiles **20** and **21** in aqueous solution by surface tension and fluorescence measurements at 25 ^{o}C

Figure S18: Size distribution profile (by intensity, volume and number) of **20**-**22** before encapsulation

Figure S19: Cryo-TEM micrographs of amphiphiles **20** and **21**

Figure S20: Size distribution profile (by volume) of **20**-**22** after encapsulation

Figure S21: Variation of the fluorescence intensity of Nile red with varying amounts of amphiphile **20** and Nile red in water

Figure S22: UV absorbance spectra of Nile red encapsulated samples in methanol

Figure S23: UV absorbance spectra of nimodipine with varying amounts of nimodipine in aqueous solution of amphiphile **20** at 5 mg mL^{-1} concentration

Figure S24: UV absorbance spectra of nimodipine encapsulated samples in ethanol

Figure S25: Calibration curve of curcumin in methanol

Figure S26: UV absorbance spectra of curcumin encapsulated samples in methanol using (a) 1 mg and (b) 2.5 mg of curcumin

Figure S27: HPLC chromatogram of dexamethasone encapsulated samples using 1 mg of dexamethasone

Figure S28: HPLC chromatogram of dexamethasone encapsulated samples using 2.5 mg of dexamethasone

Figure S29: Confocal laser scanning fluorescence microscopy images from A549 cells after 5 and 24 h incubation with Nile red encapsulated by amphiphiles **20** and **22** and non-treated control cells

3. References

1. Experimental Section

1.1. Materials

All the chemicals and solvents used were obtained from Spectrochem Pvt. Ltd., India and Sigma-Aldrich Chemicals, USA. Immobilized *Candida antarctica* lipase (Novozym 435) was procured from Julich Chiral Solutions GmbH (Jülich, Germany). All the dyes/drugs used for encapsulation studies were purchased from Fluka Chemie GmbH, (Buchs, Switzerland) and Sigma-Aldrich Chemicals, USA with maximum purity. The solvents used in the reactions were dried and distilled prior to use. Pre-coated TLC plate (Merck silica gel $60F_{254}$) was used to monitor the progress of the reactions with visualization of the spots on TLC using cerric solution. Silica gel (100-200 mesh) was used for column chromatography. Benzoylated dialysis tubing (molecular weight cut-off (MWCO) 2000 Da), procured from Sigma-Aldrich was used for the purification of amphiphiles. Millipore water used for preparing samples for physico-chemical characterization and transport analysis, was obtained from Merck Millipore Milli-Q Integral System. Cremophor® ELP (purified grade of Cremophor® EL (polyethoxylated castor oil)) was obtained from BASF, Ludwigshafen, Germany.

1.2. Methods and Instrumentation

Infrared spectra (IR) of the samples were recorded using a Perkin-Elmer FT-IR model 9 spectrometer. The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on JEOL 400 MHz, Bruker DRX 400, and Bruker AMX 500 MHz spectrometers with referencing done using the residual solvent peak. The chemical shift values are on a δ scale and the coupling constant values (*J*) are in Hertz. High-resolution mass spectrometry (HRMS) data were recorded on Q-TOF LCMS-Agilent Technology-6530 and HPLC/MS - Agilent 6210 (Agilent Technologies).

1.2.1. Gel permeation chromatography (GPC)

An Agilent GPC system equipped with Agilent 1100 pump, refractive index detector, and PLgel columns, was used to determine the molecular weight $\overline{M}_w$, $\overline{M}_n$ and $\overline{M}_z$ of amphiphiles using tetrahydrofuran (THF) as an eluent at a flow rate of 1.0 mL min^{-1} and molecular weight calibration carried using polystyrene standards.

1.2.2. Critical aggregation concentration (CAC) measurements

1.2.2.1. Surface tension method

Tensiometer OCA 20 from DataPhysics Instruments GmbH, Filderstadt, Germany, was used for calculating the CAC of amphiphiles by following the pendant drop method.[1] The samples at a concentration of 2 mM in Milli-Q water were stirred vigorously for 20 h followed by two-fold serial dilution to form solutions of different concentration. Subsequently, the surface tension values were recorded for these samples at 25 ± 5 °C until the deviation in the values stabilized. In order to calculate the CAC, the obtained surface tension values were plotted against log [amphiphile concentration].

1.2.2.2. Pyrene fluorescence method

CAC of the amphiphiles was also determined by fluorescence method using 'pyrene' as a model dye.[2] A stock solution of pyrene at a concentration of 1 mg mL^{-1} (4.9 x 10^{-3} M) was prepared in acetone, which was further diluted to form 4.9 x 10^{-4} M solution. The pyrene solution (10 µL) was taken into empty vials and acetone was allowed to evaporate completely. Stock solutions of amphiphiles (2 mM) were prepared using Milli-Q water, and allowed to stir for 1 h, which were then serially diluted and transferred to the vials having thin film of pyrene and allowed to stir overnight. The final concentration of pyrene was kept at 2.45 x 10^{-6} M. All the solutions were filtered using 0.45 µm polytetrafluoroethylene (PTFE) filter to remove non-encapsulated dye. The fluorescence spectra were recorded for the filtered clear solutions using Cary Eclipse fluorescence spectrophotometer and the ratio of the fluorescence intensity of I_3 (385 nm) and I_1 (374 nm) vibronic bands were plotted against the log of the concentration of each amphiphilic sample to obtain the CAC value.

1.2.2.3. Nile red fluorescence method

The CAC of the synthesized amphiphiles was determined by fluorescence technique using 'Nile red' as a model dye.[3] A stock solution of the dye at a concentration of 1 mg mL^{-1} (3.14 x 10^{-3} M) was prepared in THF, which was further diluted to form 3.14 x 10^{-4} M solution. 10 µL of 3.14 x 10^{-4} M Nile red solution was added in each empty vial followed by complete evaporation of THF. The amphiphilic stock solutions were prepared at a concentration of 1 mM using Milli-Q water, and allowed to stir for 1 h. Two-fold serial dilution of the stock solutions was done to achieve different concentrations of the amphiphiles, which were then transferred to the vials having thin

film of the dye followed by overnight stirring. The final concentration of Nile red was kept at 0.79 x 10^{-6} M. The non-encapsulated dye in all the solutions was removed by filtration through 0.45 μm polytetrafluoroethylene (PTFE) filter with subsequent fluorescence measurements using Cary Eclipse fluorescence spectrophotometer. The plot of fluorescence intensity maxima values against log [amphiphile concentration] for different samples afforded the CAC values.

1.2.3. Dynamic light scattering (DLS) measurements

Malvern Zetasizer Nano ZS analyzer equipped with a thermostated sample chamber and integrated with 4 mW He-Ne laser, λ = 633 nm based on back scattering detection (scattering angle θ = 173^{o}) using an avalanche photodiode as a detector was used for characterizing the nanostructures (micelles/micellar aggregates) formed in the aqueous solution (Milli-Q water) at a concentration of 5 mg mL^{-1}, well above their CACs. Disposable micro BRAND ultraviolet (UV)-cuvettes were used for the measurements. The sample was prepared by vigorous stirring for 20 h followed by filtration using 0.45 μm PTFE filter and equilibrated for 1 h at room temperature before the measurements.

1.2.4. Cryogenic transmission electron microscopy (cryo-TEM)

The size of the nanoparticles formed by the amphiphiles in the aqueous solution was also evaluated by cryo-TEM image using a Tecnai F20 transmission electron microscope (FEI Company, Oregon, USA) operated at 160 kV accelerating voltage at a concentration of 1 mg mL^{-1}. The cryo preparation was accomplished by applying droplets of the sample solution to 1 μm hole diameter perforated carbon film, covered with 200 mesh grids (R1/4 batch of the Quantifoil Micro Tools GmbH, Jena, Germany), which had been hydrophilized before use. An ultra-thin layer of the sample solution was prepared, so that the solution spanned the holes of the carbon film by removing the supernatant fluid with a filter paper. The samples were immediately vitrified by propelling the grids into liquid ethane at its freezing point (90 K) with a guillotine-like plunging device. The vitrified samples were then transferred to the microscope using a Gatan (Gatan, Inc., California) cryoholder and stage (model 626). The samples were kept at a temperature of 94 K. Imaging was performed using the low-dose protocol of the microscope at a calibrated primary magnification of 50 000x with the defocus set to 4.9 μm. Data were recorded by an Eagle 4k CCD-camera set to binning factor 2.

1.2.5. Drug/Dye encapsulation and quantification

Nile red, nimodipine, curcumin and dexamethasone encapsulation was studied by following the film method[4] for Nile red, nimodipine and dexamethasone and solid dispersion method[5] for curcumin, using ultraviolet-visible (UV-Vis), fluorescence spectra measurements and high performance liquid chromatography (HPLC) technique. The dye/drug was solubilized at a concentration of 5 mg mL^{-1} for all the amphiphiles using 0.12 mg of Nile red / 0.5 mg of nimodipine / 1 mg of either curcumin or dexamethasone. The required amount of Nile red, nimodipine and dexamethasone was dissolved in THF and acetone, respectively, and the solvent was allowed to evaporate so that a uniform layer of dye/drug was obtained at the bottom of the vial, followed by the addition of 1 mL of aqueous amphiphilic solution in 1X phosphate-buffered saline (PBS) buffer (pH 7.4). However, for curcumin encapsulation, 1 mg of it and 5 mg of amphiphile were dissolved in methanol, stirred for 30 min and methanol evaporated, followed by the addition of 1 mL of 1X PBS buffer (pH 7.4). After stirring for 20 h at room temperature, the non-encapsulated fraction of dye/drug was removed by filtering it (twice), slowly through 0.45 μm pore size PTFE filter. For the quantification of encapsulated dye/drug, the encapsulated samples were lyophilized and re-dissolved in anhydrous methanol in case of Nile red and curcumin and anhydrous ethanol for nimodipine. The absorbance (220–800 nm) spectra were recorded on a Perkin Elmer LAMBDA 950 UV/Vis/near-infrared (NIR) spectrophotometer using standard disposable poly(methyl methacrylate) (PMMA) UV/Vis cuvettes with a path length of 1 cm from PLASTIBRAND. Fluorescence measurements (450-800 nm) were performed on a Jasco FP-6500 spectro-fluorimeter using a variable slit system from 575-800 nm for Nile red, and 450-700 nm for curcumin. The fluorescence emission spectra were recorded by carrying out the excitation at 550 nm for Nile red, and at 420 nm for curcumin with excitation and emission slits set at 5 nm in case of Nile red and 5 and 3 nm, respectively, in case of curcumin. Following the similar protocol, curcumin encapsulated samples of amphiphiles and standard excipient, Cremophor® ELP were recorded for their absorbance using Cary-300 series UV-Vis spectrophotometer from Agilent Technologies. Knauer Smartline HPLC system equipped with refractive index (RI) detector 2300, UV detector 2550, 1050 pump, and the reversed-phase (RP) Gemini C18 column was used in order to quantify the encapsulated dexamethasone. Lyophilized samples dissolved in acetonitrile:water (40:60) were subjected to chromatography using the same mixture as the mobile phase at a flow rate of 1 mL min^{-1} under isocratic regime with UV detector

at 254 nm and 93 bar pressure. Microsoft Excel® and Origin 8 softwares were used for data analysis.

1.2.6. Cellular uptake study

Cellular uptake of Nile red (NR) encapsulated by the amphiphilic systems in A549 cells (DSMZ no.: ACC 107, Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures) was monitored by confocal laser scanning microscopy (cLSM). The cells were routinely propagated in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 2% glutamine, 100 U mL^{-1} penicillin, 100 µg mL^{-1} streptomycin (all from Gibco BRL, Eggenstein, Germany), and 10% fetal calf serum (Biochrom AG, Berlin, Germany) at 37 °C with 5% CO_2, and subcultured twice a week. For cLSM, cells were seeded in 8-well ibidi µ-slides (27.000 cells/well) in colourless cell culture medium. After 1 day, the compounds were added at a final test concentration of 0.5 mg mL^{-1} and incubated for either 5 h or 24 h. CellLight GFP (green fluorescent protein) reagents (Life Technologies GmbH, Darmstadt, Germany) were used according to the manufacturer's instructions to label either early endosomes or lysosomes in cells 5 h incubated with the encapsulated dye. Non-treated labeled and non-labeled cells served as controls to adjust the settings. Confocal images from the living cells were taken with an inverted confocal laser scanning microscope Leica DMI6000CSB SP8 (Leica, Wetzlar, Germany) with a 63x/1.4 HC PL APO CS2 oil immersion objective using the manufacture given Leica Application Suite X (LAS X) software. All images were taken using the same settings to ensure that the fluorescence intensity between the different images and times can be compared.

1.2.7. Cytotoxicity study

The cytotoxicity study of the amphiphiles was carried out in PBS buffer at a concentration of 2, 1, 0.5 and 0.1 mg mL^{-1} after 24 h using CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay from Promega (Mannheim, Germany) according to the manufacturer's instructions. In short, A549 cells (DSMZ no.: ACC 107, Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures) were seeded in a 96-well plate (4000 cells/well in 90 µL) and cultured overnight at 37 °C before adding the sample substances (10 µL) in serial dilutions (2, 1, 0.5, 0.1 mg mL^{-1}). The surfactant, sodium dodecyl sulphate (SDS) (0.01%) and non-treated cells served as controls. For background subtraction, wells containing no cells but only samples were used. Cells were incubated for 24 h at 37 °C before the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-

carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) solution (20 µL) was added. After an incubation period of 2 h and 30 min, absorbance was measured at a wavelength of 490 nm and a reference wavelength of 630 nm with a Tecan plate reader (Infinite pro200, TECAN-reader Tecan Group Ltd., Männedorf, Switzerland). Measurements were done in triplicates and repeated three times. The cell viability was calculated by setting the non-treated control to 100% and the non-cell control to 0% by subtracting the background using Microsoft Excel 2013. GraphPad Prism (5.01) served for data visualization.

1.2.8. Enzyme-triggered release study

For the time-dependent enzymatic release study, Nile red was used as a model dye and encapsulated in the amphiphilic solution in 1X PBS buffer (pH 7.4, 2 mL) following the same protocol used for quantification (Section 2.2.5.). After removing the non-encapsulated dye through 0.45 µm PTFE filter, a few drops of *n*-butanol and 200 wt % of the enzyme were added. The final solutions having pH 7.4 were incubated at 37 ^{o}C and 200 rpm for 12 days under dark conditions. The time-dependent release was studied using fluorescence spectroscopy (Cary Eclipse fluorescence spectrophotometer, Agilent Technologies) by measuring the emission maxima.

1.3. Synthetic Procedures

Non-ionic mPEG based amphiphiles were synthesized *via* Cu (I) catalysed Huisgen 1,3-dipolar cycloaddition reaction (**Scheme 3**). The hydrophobic and hydrophilic units, in turn, were synthesized following **Schemes 1** and **2**.

*1.3.1. Synthesis of 2,2-di(prop-2-yn-1-yl)propane-1,3-diyl bis(4-(n-alkyloxy)benzoate) (**9**/**10**).*

*1.3.1.1. Synthesis of diethyl 2,2-di(prop-2-yn-1-yl)malonate (**2**).*

Diethyl 2,2-di(prop-2-yn-1-yl)malonate (**2**) was synthesized by following the literature procedure.[6] In a round-bottom flask, sodium hydride (NaH) (60% dispersion in mineral oil) (1.87 g, 2.5 equiv.) in THF (100 mL) was stirred for about 15 min at 0 ^{o}C. Diethyl malonate (**1**) (5 g, 1 equiv.) was added slowly under N_2 atmosphere at 0 ^{o}C. After stirring for about an hour, propargyl bromide (5.91 mL, 2.5 equiv.) was added dropwise at 0 ^{o}C and the reaction mixture was stirred overnight at 10-15 ^{o}C. After completion of the reaction, THF was evaporated under reduced pressure and the reaction mixture was quenched with water followed by extraction with

ethyl acetate. The organic layer was washed with brine (2 x 100 mL), dried over anhydrous sodium sulphate and evaporated *in vacuo* to obtain the desired crude product (**2**) which was further purified by column chromatography using silica gel (Ethyl acetate:Pet. ether :: 1:99) (Yield: 80%).

*1.3.1.2. Synthesis of 2,2-di(prop-2-yn-1-yl)propane-1,3-diol (**3**).*

Reduction of diethyl 2,2-di(prop-2-yn-1-yl)malonate (**2**) using lithium aluminium hydride ($LiAlH_4$) with some modifications afforded 2,2-di(prop-2-yn-1-yl)propane-1,3-diol (**3**).[7] Diethyldipropargyl malonate (**2**) (5 g, 1 equiv.) in THF (25 mL) was added slowly to a stirred suspension of $LiAlH_4$ (8.03 g, 10 equiv.) in THF (300 mL) at 0 ^oC. After stirring for 12 h at room temperature, water was added carefully until no H_2 was produced and the suspension turned white followed by filtration through celite. After removal of THF by rotary evaporation, the residue was extracted with diethyl ether (3 x 100 mL) and then dried over anhydrous sodium sulphate. Evaporation of the solvent under reduced pressure gave the diol (**3**) in 90% yield, which was used without further purification.

*1.3.1.3. Synthesis of 4-(n-alkyloxy)benzoic acid (**7**/**8**).*

Modified literature procedure[8] was used in order to synthesize 4-(*n*-alkyloxy)benzoic acid (**7**/**8**). Potassium carbonate (K_2CO_3) (4.98 g, 3 equiv.) and ethyl 4-hydroxybenzoate (**4**) (2 g, 1 equiv.) dissolved in dimethylformamide (DMF) (50 mL) were stirred at room temperature for 30 min followed by the addition of 1-bromoalkane (1.1 equiv.). The reaction mixture was stirred at 40 ^oC and on completion of the reaction (6 h), it was poured over ice. The solid so obtained was filtered, washed with water and dried. Further, a solution of ethyl 4-(*n*-alkyloxy)benzoate (**5**/**6**) (1 equiv.) in ethanol (50 mL), refluxed along with aqueous potassium hydroxide (KOH) solution (4 equiv., 3 mL) for 4 h followed by acidification with hydrochloric acid (HCl) solution (1N) led to the formation of the hydrolyzed product, 4-(*n*-alkyloxy)benzoic acid (**7**/**8**) in almost quantitative yield.

*1.3.1.4. Synthesis of 2,2-di(prop-2-yn-1-yl)propane-1,3-diyl bis(4-(n-alkyloxy)benzoate) (**9**/**10**).*

To a stirred and clear solution of 2,2-di(prop-2-yn-1-yl)propane-1,3-diol (**3**) (0.5 g, 1 equiv.) and 4-(*n*-alkyloxy)benzoic acid (**7**/**8**) (2.5 equiv.) in anhydrous dichloromethane (DCM):DMF (4:1, 100 mL) was added *N*-(3-dimethylaminopropyl)-*N*-ethyl-carbodiimide hydrochloride (EDC) (3

equiv.) followed by 4-dimethylaminopyridine (DMAP) (1.2 equiv.) at 0 ^{o}C. The reaction mixture was stirred at 30 ^{o}C for 48 h with subsequent removal of the solvent under reduced pressure. The resulting product was extracted with chloroform (3 x 100 mL) and dried over anhydrous sodium sulphate. Removal of the solvent and subsequent purification by column chromatography using silica gel (Ethyl acetate:Pet. ether :: 1:49) afforded 2,2-di(prop-2-yn-1-yl)propane-1,3-diyl bis(4-(*n*-alkyloxy) benzoate) (**9**/**10**) in 70-72% yield.

*1.3.1.4.1. 2,2-Di(prop-2-yn-1-yl)propane-1,3-diyl bis(4-(decyloxy)benzoate) (**9**)*

The reaction of 2,2-di(prop-2-yn-1-yl)propane-1,3-diol (**3**) (0.5 g, 3.29 mmol) and 4-(decyloxy)benzoic acid (**7**) (2.28 g, 8.21 mmol) gave the title compound **9** as a viscous oil (1.55 g, 70%) by following the general procedure; IR (KBr) ν_{max}: 3290, 2920, 2853, 1714, 1608, 1511, 1470, 1252, 1168, 1101, 1030, 844, 768, 650 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 6H, *J* = 6.9 Hz, H-10'), 1.24-1.35 (m, 24H, H-4' - H-9'), 1.40-1.52 (m, 4H, H-3'), 1.75-1.83 (m, 4H, H-2'), 2.07 (t, 2H, *J* = 2.3 Hz, H-5''), 2.61 (d, 4H, *J* = 2.7 Hz, H-3''), 4.00 (t, 4H, *J* = 6.9 Hz, H-1'), 4.45 (s, 4H, H-1''), 6.89 (d, 4H, *J* = 8.4 Hz, H-4 & H-6), 7.95 (d, 4H, *J* = 8.4 Hz, H-3 & H-7); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 14.3 (C-10'), 22.8, 22.9, 26.1, 29.3, 29.5, 29.5, 29.7, 32.1 & 40.9 (C-2' - C-9', C-2'' & C-3''), 65.7 (C-1''), 68.4 (C-1'), 72.0 (C-5''), 79.1 (C-4''), 114.3 (C-4 & C-6), 122.0 (C-2), 131.8 (C-3 & C-7), 163.4 (C-5), 166.0 (C-1); HRMS: *m/z* [*M* + Na]$^{+}$ Calculated for $C_{43}H_{60}O_6$: 695.4288; found: 695.4340.

*1.3.1.4.2. 2,2-Di(prop-2-yn-1-yl)propane-1,3-diyl bis(4-(octadecyloxy)benzoate) (**10**)*

The reaction of 2,2-di(prop-2-yn-1-yl)propane-1,3-diol (**3**) (0.5 g, 3.29 mmol) and 4-(octadecyloxy)benzoic acid (**8**) (3.20 g, 8.21 mmol) gave the title compound **10** as a white solid (4.24 g, 72%) by following the general procedure; IR (KBr) ν_{max}: 3287, 2918, 2850, 1713, 1610, 1510, 1472, 1294, 1265, 1252, 1180, 1100, 1038, 840, 766, 657 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 6H, *J* = 6.8 Hz, H-18'), 1.23-1.35 (m, 56H, H-4' - H-17'), 1.41-1.50 (m, 4H, H-3'), 1.73-1.85 (m, 4H, H-2'), 2.07 (t, 2H, *J* = 2.8 Hz, H-5''), 2.61 (d, 4H, *J* = 2.7 Hz, H-3''), 3.99 (t, 4H, *J* = 6.9 Hz, H-1'), 4.45 (s, 4H, H-1''), 6.88 (d, 4H, *J* = 8.7 Hz, H-4 & H-6), 7.95 (d, 4H, *J* = 8.7 Hz, H-3 & H-7); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 14.3 (C-18'), 22.8, 22.9, 26.2, 29.3, 29.6, 29.8, 29.8, 29.9, 29.9, 32.1 & 40.9 (C-2' - C-17', C-2'' & C-3''), 65.7 (C-1''), 68.4 (C-1'), 72.0 (C-5''), 79.1 (C-4''), 114.3 (C-4 & C-6), 122.0 (C-2), 131.8 (C-3 & C-7), 163.4 (C-5), 166.1 (C-1); HRMS: *m/z* [*M* + Na]$^{+}$ Calculated for $C_{59}H_{92}O_6$: 919.6792; found: 919.6804.

*1.3.2. Synthesis of 2-azidopropane-1,3-diyl bis(methoxypoly(oxyethylene)oate) (**18**/**19**).*

*1.3.2.1. Synthesis of methoxypolyethylene glycol carboxymethyl ether (**13**/**14**).*

Methoxypolyethylene glycol (**11**/**12**) (M_n: 550/1000 g mol^{-1}) (10 g, 1 equiv.) was dissolved in distilled water (150 mL) in a 500 mL round-bottom flask. The reaction mixture was stirred at room temperature and sodium hydroxide (4 equiv.) was added. After cooling the reaction mixture to 0 ^oC, potassium permanganate ($KMnO_4$) (6 equiv.) was added in small amounts in approx. 2-3 h. Subsequently, the reaction mixture was allowed to attain room temperature and then heated to 80 ^oC for 24 h. Upon completion of the reaction as monitored by TLC, the reaction mixture was filtered while still a little warm. The filtrate was acidified using 2N HCl solution to pH 2 and then extracted 4-5 times using chloroform. The organic layer was washed with cold water (2 x 200 mL), treated with anhydrous sodium sulphate, and concentrated under vacuum to get the desired mPEG acid (**13**/**14**) in 90% yield.

*1.3.2.1.1. Methoxypolyethylene glycol carboxymethyl ether (**13**)*

The oxidation reaction of methoxypolyethylene glycol (**11**) (M_n: 550 g mol^{-1}) (10 g, 18.18 mmol) with potassium permanganate (17.24 g, 109.09 mmol) gave the title compound **13** as a colourless liquid (9.22 g, 90%) by following the general procedure; IR ($CHCl_3$) ν_{max}: 3492, 2876, 1741, 1644, 1458, 1353, 1216, 1100, 947, 852, 751, 668 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.36 (s, 3H, $-OCH_3$), 3.52-3.74 (m, 44H, $-(OCH_2CH_2)_{n\sim11}$), 4.14 (s, 2H, H-2); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 59.0 ($-OCH_3$), 68.8 (C-2), 70.4, 70.6, 70.6, 71.1, 71.9 ($-(OCH_2CH_2)_{n\sim11}$), 172.4 (C-1).

*1.3.2.1.2. Methoxypolyethylene glycol carboxymethyl ether (**14**)*

The oxidation reaction of methoxypolyethylene glycol (**12**) (M_n: 1000 g mol^{-1}) (10 g, 10 mmol) with potassium permanganate (9.48 g, 60 mmol) gave the title compound **14** as a colourless liquid (9.12 g, 90%) by following the general procedure; IR ($CHCl_3$) ν_{max}: 3488, 2884, 1744, 1649, 1462, 1355, 1249, 1112, 949, 851, 757, 668 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.36 (s, 3H, $-OCH_3$), 3.52-3.78 (m, 84H, $-(OCH_2CH_2)_{n\sim21}$), 4.14 (s, 2H, H-2); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 58.9 ($-OCH_3$), 68.6 (C-2), 69.9, 70.2, 70.2, 70.3, 70.5, 71.7, 72.6 ($-(OCH_2CH_2)_{n\sim21}$), 171.9 (C-1).

*1.3.2.2. Synthesis of (methoxypolyethylene glycol carboxymethyl ether)ethyl ester (**15**/**16**).*

Methoxypolyethylene glycol carboxymethyl ether (**13**/**14**), (9 g) was dissolved in absolute ethanol (200 mL) followed by addition of a catalytic amount of concentrated sulphuric acid (H_2SO_4). The reaction mixture was refluxed for 12 h and the progress of the reaction was monitored by TLC. Upon completion, the solvent was evaporated under vacuum and the product so obtained was suspended in cold water and extracted using chloroform (2 x 100 mL). The organic layer was dried with anhydrous sodium sulphate followed by removal of the solvent *in vacuo* to get the desired mPEG ethyl ester (**15**/**16**) in 75-78% yield.

*1.3.2.2.1. (Methoxypolyethylene glycol carboxymethyl ether)ethyl ester (**15**)*

The esterification reaction of methoxypolyethylene glycol carboxymethyl ether (**13**) (M_n: 564 g mol^{-1}) (9 g, 15.96 mmol) using ethanol gave the title compound **15** as a colourless liquid (7.37 g, 78%) by following the general procedure; IR ($CHCl_3$) ν_{max}: 3564, 2877, 1749, 1454, 1358, 1288, 1244, 1212, 1098, 945, 852 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.24 (t, 3H, J = 6.9 Hz, H-2'), 3.34 (s, 3H, -OCH_3), 3.50-3.69 (m, 44H, -$(OCH_2CH_2)_{n\sim11}$), 4.11 (s, 2H, H-2), 4.17 (q, 2H, J = 6.9 Hz, H-1'); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 14.1 (C-2'), 58.9 (-OCH_3), 60.7 (C-1'), 68.6 (C-2), 70.4, 70.5, 70.5, 70.8, 71.8 (-$(OCH_2CH_2)_{n\sim11}$), 170.4 (C-1).

*1.3.2.2.2. (Methoxypolyethylene glycol carboxymethyl ether)ethyl ester (**16**)*

The esterification reaction of methoxypolyethylene glycol carboxymethyl ether (**14**) (M_n: 1014 g mol^{-1}) (9 g, 8.88 mmol) using ethanol gave the title compound **16** as a colourless liquid (6.94 g, 75%) by following the general procedure; IR ($CHCl_3$) ν_{max}: 3565, 2870, 1749, 1645, 1458, 1352, 1290, 1249, 1205, 1094, 946, 850 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.22 (t, 3H, J = 6.8 Hz, H-2'), 3.31 (s, 3H, -OCH_3), 3.47-3.68 (m, 84H, -$(OCH_2CH_2)_{n\sim21}$), 4.08 (s, 2H, H-2), 4.15 (q, 2H, J = 6.8 Hz, H-1'); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 13.4 (C-2'), 58.0 (-OCH_3), 59.8 (C-1'), 67.7 (C-2), 69.7, 70.0, 71.1, 72.0 (-$(OCH_2CH_2)_{n\sim21}$), 169.5 (C-1).

*1.3.2.3. Synthesis of 2-azidopropane-1,3-diol (**17**).*

2-Azidopropane-1,3-diol (azido glycerol) (**17**) was synthesized from glycerol in four steps by following a previously reported procedure.[9]

*1.3.2.4. Synthesis of 2-azidopropane-1,3-diyl bis(methoxypoly(oxyethylene)oate) (**18**/**19**).*

2-Azidopropane-1,3-diol (**17**) (0.3 g, 1 equiv.) and mPEG ethyl ester (**15**/**16**) (2 equiv.) were taken in a round-bottom flask and stirred for 10 min. to make the reaction mixture homogeneous.

Novozym 435, 10% by weight (the sum of both reactants), was then added and further stirred at 70 ^{o}C under vacuum (1-2 mbar) for 60 h. Addition of chloroform (50 mL) led to the quenching of the reaction mixture with subsequent filtration of the enzyme. The filtrate was concentrated and then purified by column chromatography to get the product (**18**/**19**) in 62-70% yield.

*1.3.2.4.1. 2-Azidopropane-1,3-diyl bis(methoxypoly(oxyethylene)oate) (**18**)*

The reaction of 2-azidopropane-1,3-diol (**17**) (0.3 g, 2.56 mmol) and mPEG ethyl ester (**15**) (3.04 g, 5.12 mmol) gave the title compound **18** as a colourless oily product (2.2 g, 70%) by following the general procedure; IR ($CHCl_3$) ν_{max}: 2871, 2102, 1755, 1457, 1349, 1285, 1248, 1199, 1093, 947, 850, 749 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.33 (s, 6H, 2 x -OCH_3), 3.48-3.77 (m, 88H, 2 x -$(OCH_2CH_2)_{n\sim11}$), 3.86-3.92 (m, 1H, H-2), 4.13-4.38 (m, 8H, H-1, H-3 & 2 x H-2'); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 58.3 (C-2), 58.9 (-OCH_3), 63.1 (C-1 & C-3), 68.2 (C-2'), 70.4, 70.4, 70.8, 70.9, 71.8 (-$(OCH_2CH_2)_{n\sim11}$), 169.9 (C-1').

*1.3.2.4.2. 2-Azidopropane-1,3-diyl bis(methoxypoly(oxyethylene)oate) (**19**)*

The reaction of 2-azidopropane-1,3-diol (**17**) (0.3 g, 2.56 mmol) and mPEG ethyl ester (**16**) (5.57 g, 5.12 mmol) gave the title compound **19** as a colourless oily product (3.35 g, 62%) by following the general procedure; IR ($CHCl_3$) ν_{max}: 2869, 2103, 1754, 1647, 1457, 1349, 1248, 1200, 1092, 947, 848, 749 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.36 (s, 6H, 2 x -OCH_3), 3.46-3.81 (m, 168H, 2 x -$(OCH_2CH_2)_{n\sim21}$), 3.88-3.94 (m, 1H, H-2), 4.13-4.40 (m, 8H, H-1, H-3 & 2 x H-2'); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 58.5 (C-2), 59.1 (-OCH_3), 63.3 (C-1 & C-3), 68.4 (C-2'), 70.4, 70.6, 71.0, 71.1, 72.0 (-$(OCH_2CH_2)_{n\sim21}$), 170.0 (C-1').

*1.3.3. Synthesis of amphiphiles (**20-22**).*

2,2-Di(prop-2-yn-1-yl)propane-1,3-diyl bis(4-(*n*-alkyloxy)benzoate) (**9**/**10**) (0.5 g, 1 equiv.) and 2-azidopropane-1,3-diyl bis(methoxypoly(oxyethylene)oate) (**18**/**19**) (2.2 equiv.) were taken in a 100 mL flask and dissolved in anhydrous dichloromethane (60 mL) under a nitrogen atmosphere. Then tris(triphenylphosphine)copper(I) bromide (0.05 equiv.) and *N*,*N*-diisopropylethylamine (DIPEA) (12.5 equiv.) were added and the reaction mixture was stirred for 72 h at 30 ^{o}C. The solvent was removed and the product was washed with hexane (by sonication) to remove non-polar reactants and residual copper catalyst, tris(triphenylphosphine)copper(I) bromide. The crude product was subjected to purification using column chromatography / dialysis (2000

MWCO dialysis tubing; chloroform; 48 h) to yield the purified amphiphiles (**20**-**22**) in 55-60% yield.

*1.3.3.1. Amphiphile **20***

The reaction of 2,2-di(prop-2-yn-1-yl)propane-1,3-diyl bis(4-(decyloxy)benzoate) (**9**) (0.5 g, 0.74 mmol) and 2-azidopropane-1,3-diyl bis(methoxypoly(oxyethylene)oate) (**18**) (2.0 g, 1.63 mmol) gave the title compound **20** as a viscous oil (1.38 g, 60%) by following the general procedure; IR (KBr) ν_{max}: 2921, 2866, 1758, 1606, 1461, 1352, 1253, 1107, 947, 851 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.81 (t, 6H, *J* = 7.6 Hz, H-10'), 1.16-1.42 (m, 28H, H-3' - H-9'), 1.68-1.77 (m, 4H, H-2'), 2.79-2.92 (m, 4H, H-3''), 3.31 (s, 12H, 4 x $-OCH_3$), 3.43-3.78 (m, 170H, 4 x $-(OCH_2CH_2)_{n\sim11}$), 3.93 (t, 4H, *J* = 6.1 Hz, H-1'), 4.04-4.60 (m, 20H, 2 x H-1'', 2 x H-b & H-c, 4 x H-e), 5.37-5.43 (m, 2H, 2 x H-a), 6.82 (d, 4H, *J* = 8.4 Hz, H-4 & H-6), 7.61-7.93 (m, 6H, H-5'', H-3 & H-7); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 13.9 (C-10'), 22.4, 25.8, 28.9, 29.1, 29.2, 29.3, 31.7 & 41.4 (C-2' - C-9', C-2'' & C-3''), 58.2, 58.8 ($-OCH_3$), 63.0, 68.0, 68.3, 70.3, 70.7, 70.7, 71.7, 114.0 (C-4 & C-6), 121.7 (C-2), 124.6 (C-5''), 131.4 (C-3 & C-7), 142.6 (C-4''), 162.9 (C-5), 165.7 (C-1), 170.0 (C-d); GPC (THF, 1 mL min^{-1}): $\overline{M}_w$ = 2082.4 g mol^{-1}, $\overline{M}_n$ = 1944.4 g mol^{-1}, $\overline{M}_z$ = 2203.6 g mol^{-1}, polydispersity index (PDI) = 1.07.

*1.3.3.2. Amphiphile **21***

The reaction of 2,2-di(prop-2-yn-1-yl)propane-1,3-diyl bis(4-(decyloxy)benzoate) (**9**) (0.5 g, 0.74 mmol) and 2-azidopropane-1,3-diyl bis(methoxypoly(oxyethylene)oate) (**19**) (3.44 g, 1.63 mmol) gave the title compound **21** as a low melting solid (2.0 g, 55%) by following the general procedure; IR ($CHCl_3$) ν_{max}: 2922, 2867, 1753, 1647, 1607, 1461, 1351, 1251, 1200, 1101, 948, 851 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83 (t, 6H, *J* = 6.9 Hz, H-10'), 1.19-1.45 (m, 28H, H-3' - H-9'), 1.70-1.79 (m, 4H, H-2'), 2.83-2.93 (m, 4H, H-3''), 3.33 (s, 12H, 4 x $-OCH_3$), 3.48-3.79 (m, 336H, 4 x $-(OCH_2CH_2)_{n\sim21}$), 3.95 (t, 4H, *J* = 6.5 Hz, H-1'), 4.08-4.61 (m, 20H, 2 x H-1'', 2 x H-b & H-c, 4 x H-e), 5.39-5.45 (m, 2H, 2 x H-a), 6.84 (d, 4H, *J* = 8.4 Hz, H-4 & H-6), 7.60-7.95 (m, 6H, H-5'', H-3 & H-7); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 14.1 (C-10'), 22.6, 25.9, 29.1, 29.3, 29.3, 29.5, 31.8 & 41.7 (C-2' - C-9', C-2'' & C-3''), 59.0 ($-OCH_3$), 60.8, 63.7, 68.2, 68.5, 68.9, 70.5, 70.8, 71.9, 114.2 (C-4 & C-6), 121.8 (C-2), 124.8 (C-5''), 131.6 (C-3 & C-7), 142.9 (C-4''), 163.1 (C-5), 165.9 (C-1), 170.4 (C-d); GPC (THF, 1 mL min^{-1}): $\overline{M}_w$ = 2420.0 g mol^{-1}, $\overline{M}_n$ = 2022.0 g mol^{-1}, $\overline{M}_z$ = 2771.4 g mol^{-1}, PDI = 1.19.

*1.3.3.3. Amphiphile **22***

The reaction of 2,2-di(prop-2-yn-1-yl)propane-1,3-diyl bis(4-(octadecyloxy)benzoate) (**10**) (0.5 g, 0.56 mmol) and 2-azidopropane-1,3-diyl bis(methoxypoly(oxyethylene)oate) (**19**) (2.59 g, 1.23 mmol) gave the title compound **22** as an off-white sticky solid (1.65 g, 58%) by following the general procedure; IR (KBr) ν_{max}: 2918, 2860, 1756, 1608, 1511, 1461, 1352, 1285, 1253, 1107, 948, 850 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, 6H, *J* = 7.6 Hz, H-18'), 1.11-1.48 (m, 60H, H-3' - H-17'), 1.72-1.81 (m, 4H, H-2'), 2.84-2.94 (m, 4H, H-3''), 3.35 (s, 12H, 4 x -OCH_3), 3.41-3.82 (m, 315H, 4 x -$(OCH_2CH_2)_{n\sim21}$), 3.97 (t, 4H, *J* = 6.1 Hz, H-1'), 4.09-4.62 (m, 20H, 2 x H-1'', 2 x H-b & H-c, 4 x H-e), 5.41-5.48 (m, 2H, 2 x H-a), 6.86 (d, 4H, *J* = 8.4 Hz, H-4 & H-6), 7.63-7.97 (m, 6H, H-5'', H-3 & H-7); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 14.1 (C-18'), 22.6, 29.3, 29.4, 29.5, 29.6, 29.6, 31.9 & 41.7 (C-2' - C-17', C-2'' & C-3''), 59.0 (-OCH_3), 63.7, 68.2, 68.9, 69.9, 70.0, 70.5, 70.8, 71.8, 114.2 (C-4 & C-6), 121.8 (C-2), 124.7 (C-5''), 131.6 (C-3 & C-7), 142.8 (C-4''), 163.1 (C-5), 165.9 (C-1), 170.4 (C-d); GPC (THF, 1 mL min^{-1}): $\overline{M}_w$ = 3070.7 g mol^{-1}, $\overline{M}_n$ = 2475.8 g mol^{-1}, $\overline{M}_z$ = 3575.2 g mol^{-1}, PDI = 1.24.

2. Figures

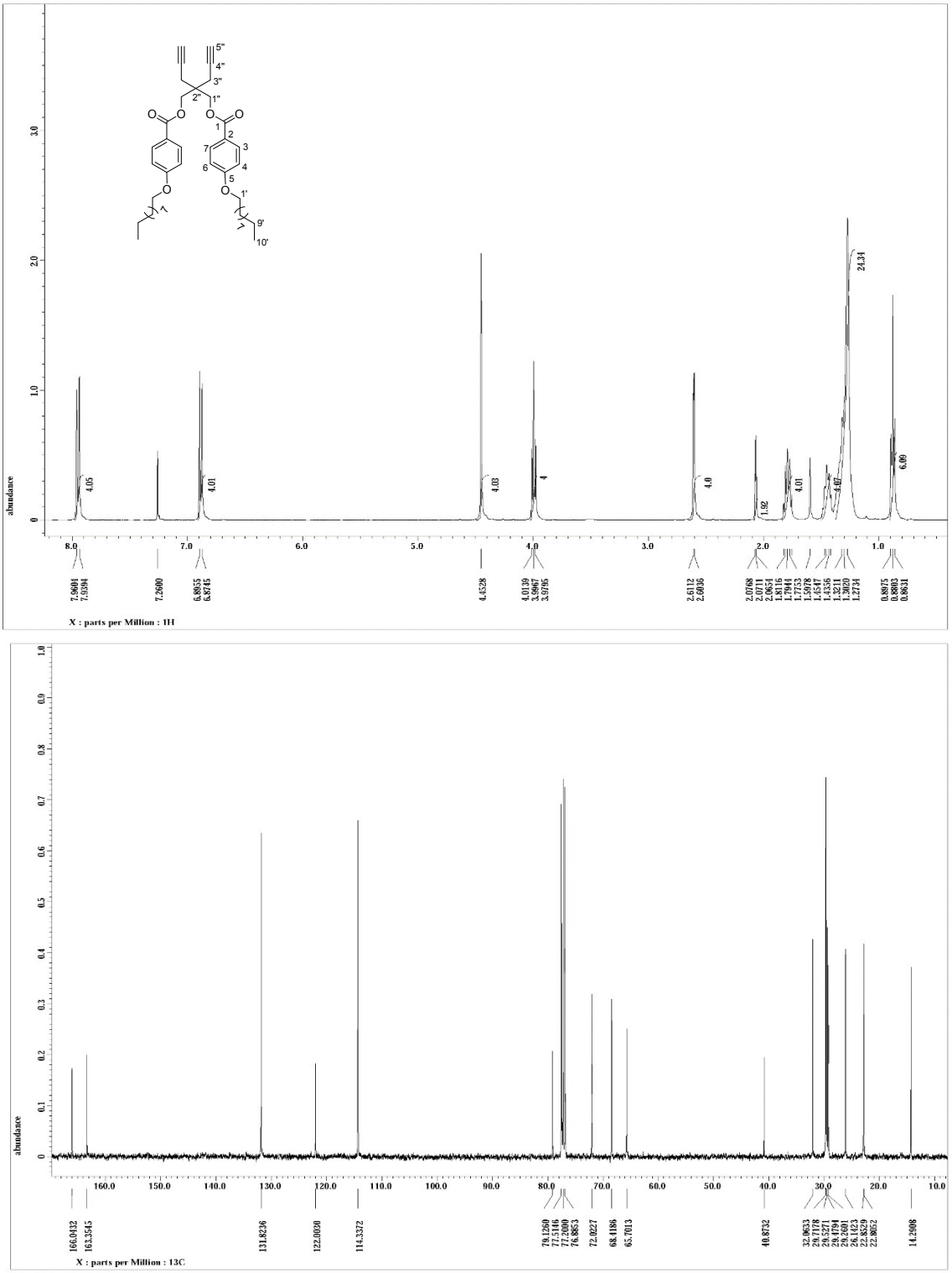

Figure S1. ^{1}H & ^{13}C NMR spectra of 2,2-di(prop-2-yn-1-yl)propane-1,3-diyl bis(4-(decyloxy)benzoate) (**9**)

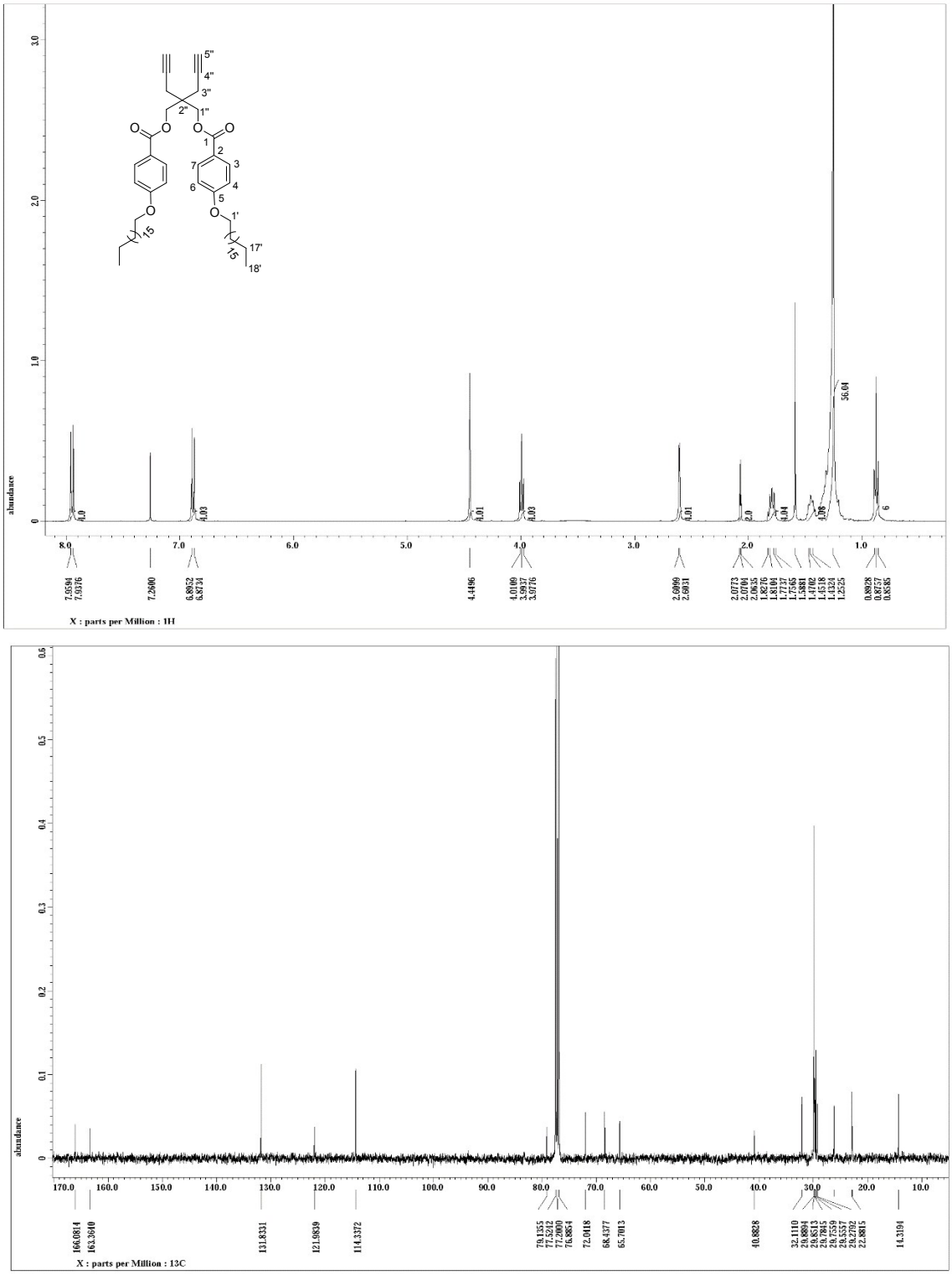

Figure S2. ^{1}H & ^{13}C NMR spectra of 2,2-di(prop-2-yn-1-yl)propane-1,3-diyl bis(4-(octadecyloxy)benzoate) (**10**)

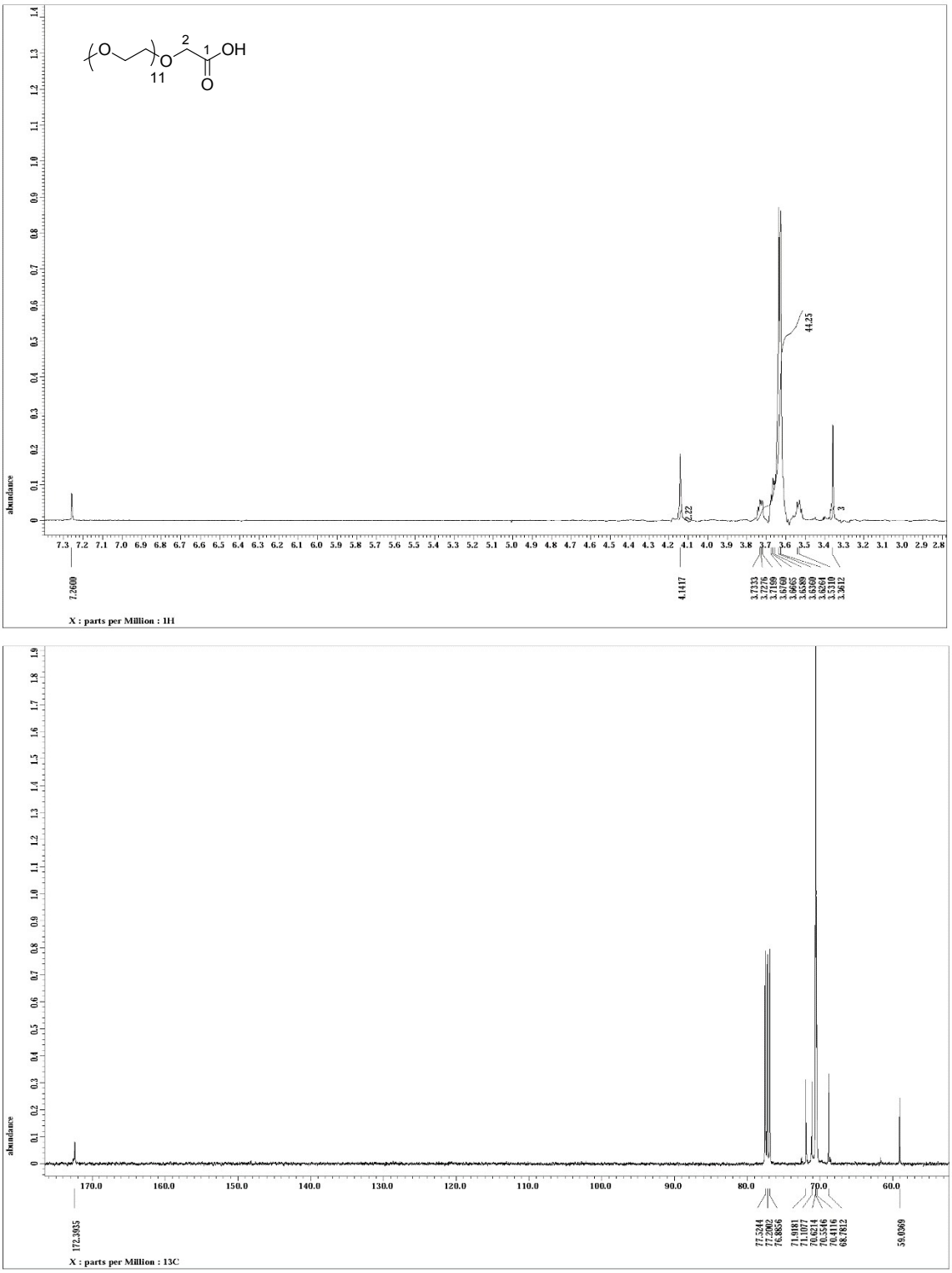

Figure S3. ^{1}H & ^{13}C NMR spectra of methoxypolyethylene glycol carboxymethyl ether (**13**)

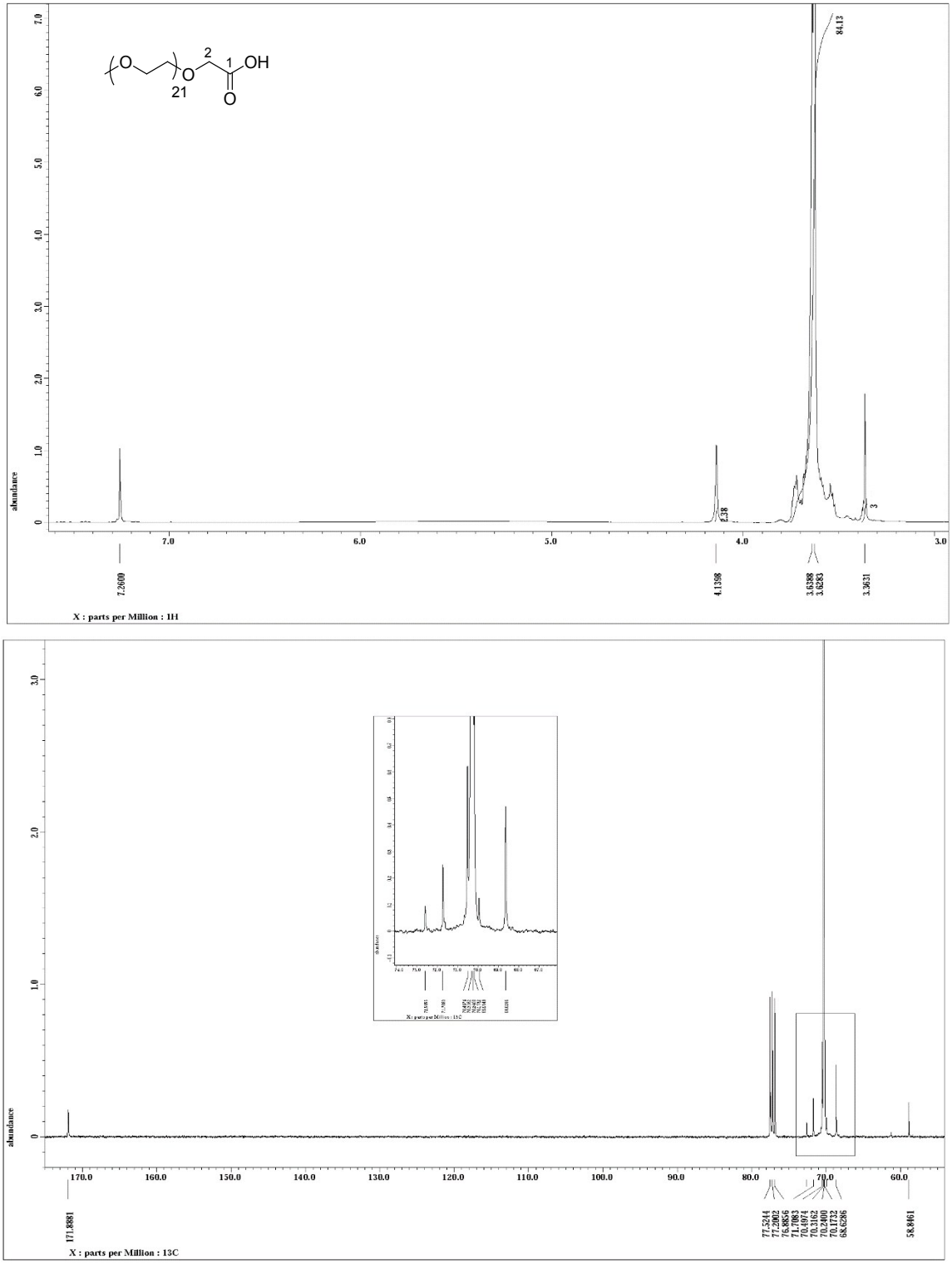

Figure S4. ^{1}H & ^{13}C NMR spectra of methoxypolyethylene glycol carboxymethyl ether (**14**)

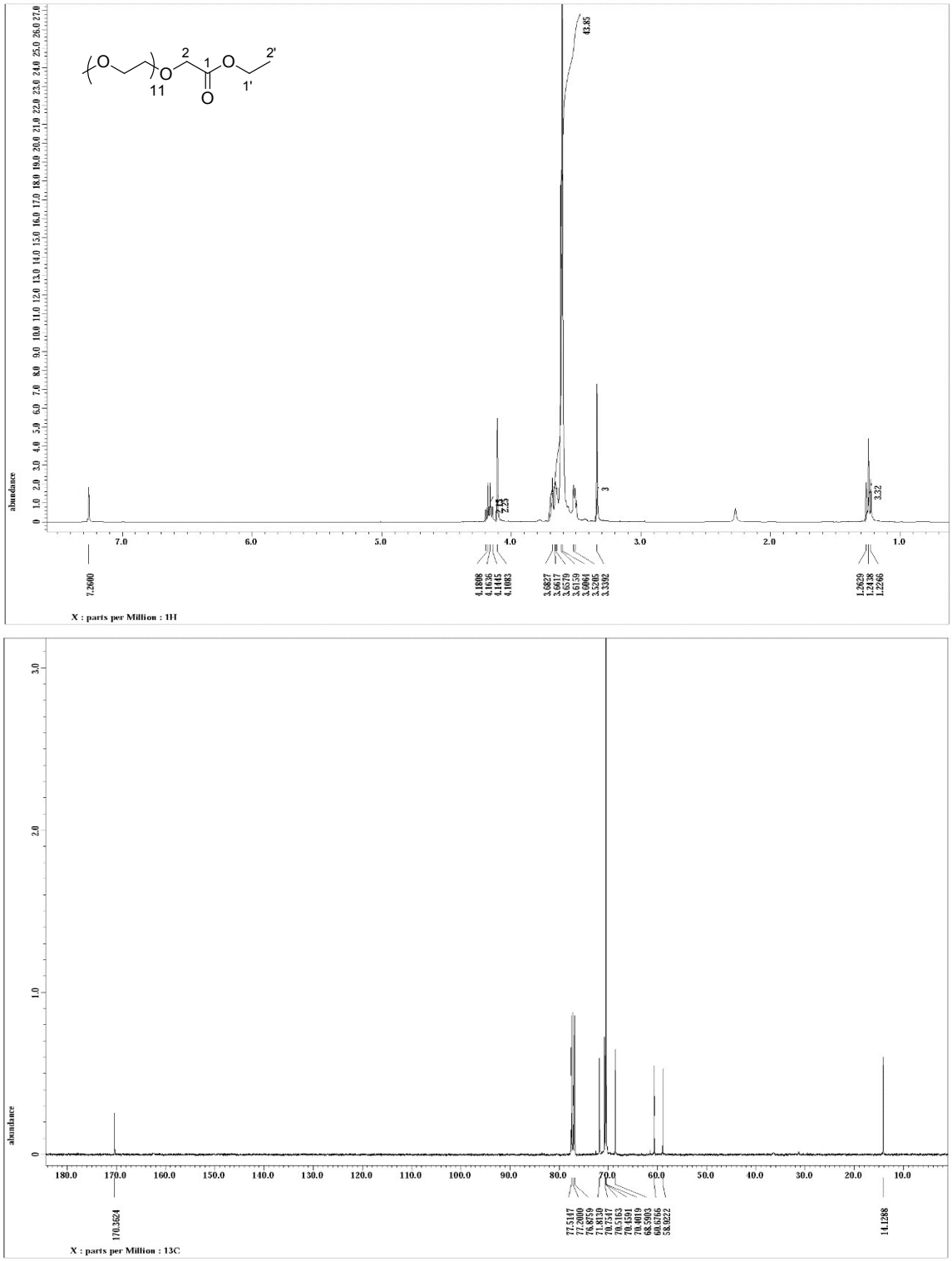

Figure S5. ^{1}H & ^{13}C NMR spectra of (methoxypolyethylene glycol carboxymethyl ether)ethylester (**15**)

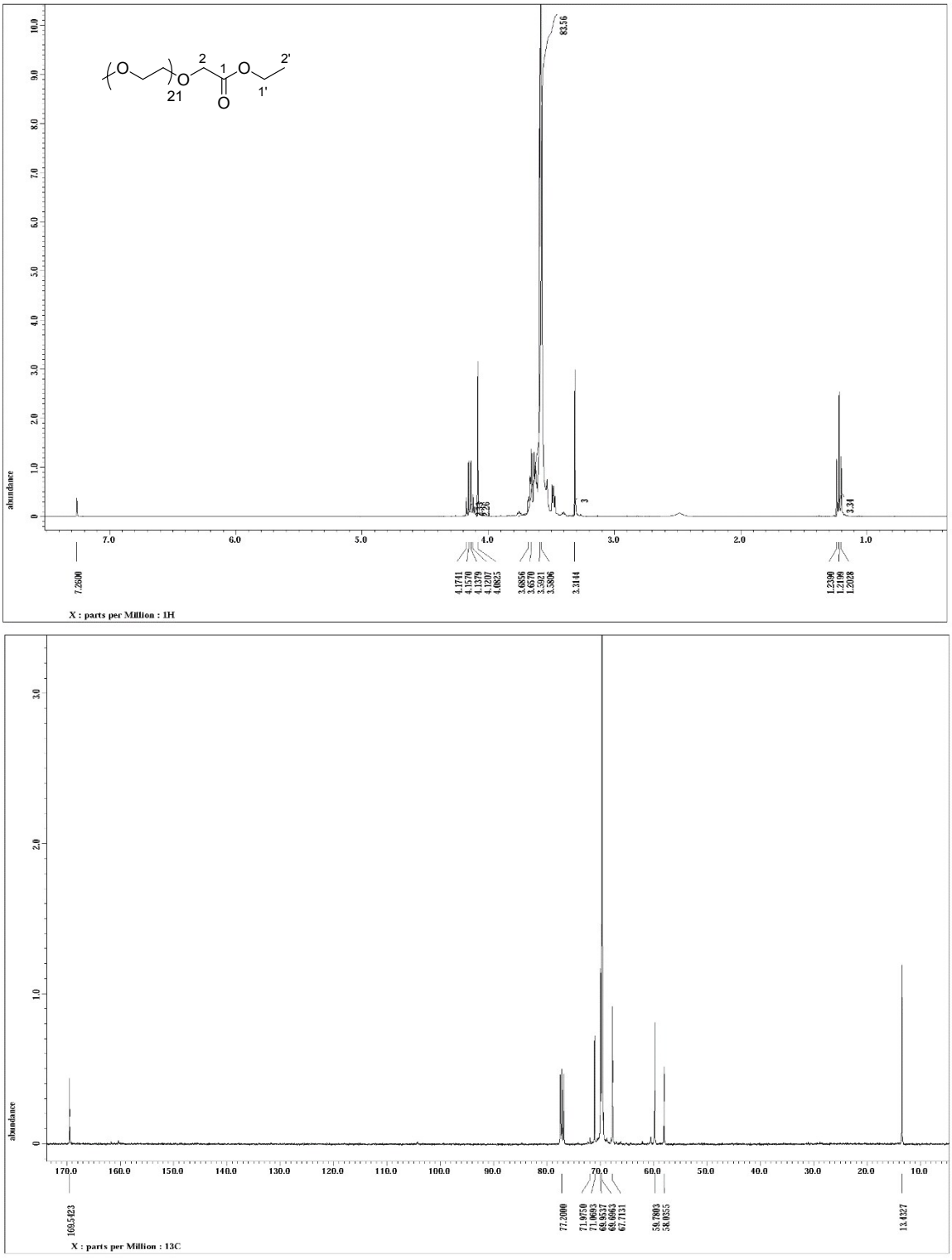

Figure S6. ^{1}H & ^{13}C NMR spectra of (methoxypolyethylene glycol carboxymethyl ether)ethylester (**16**)

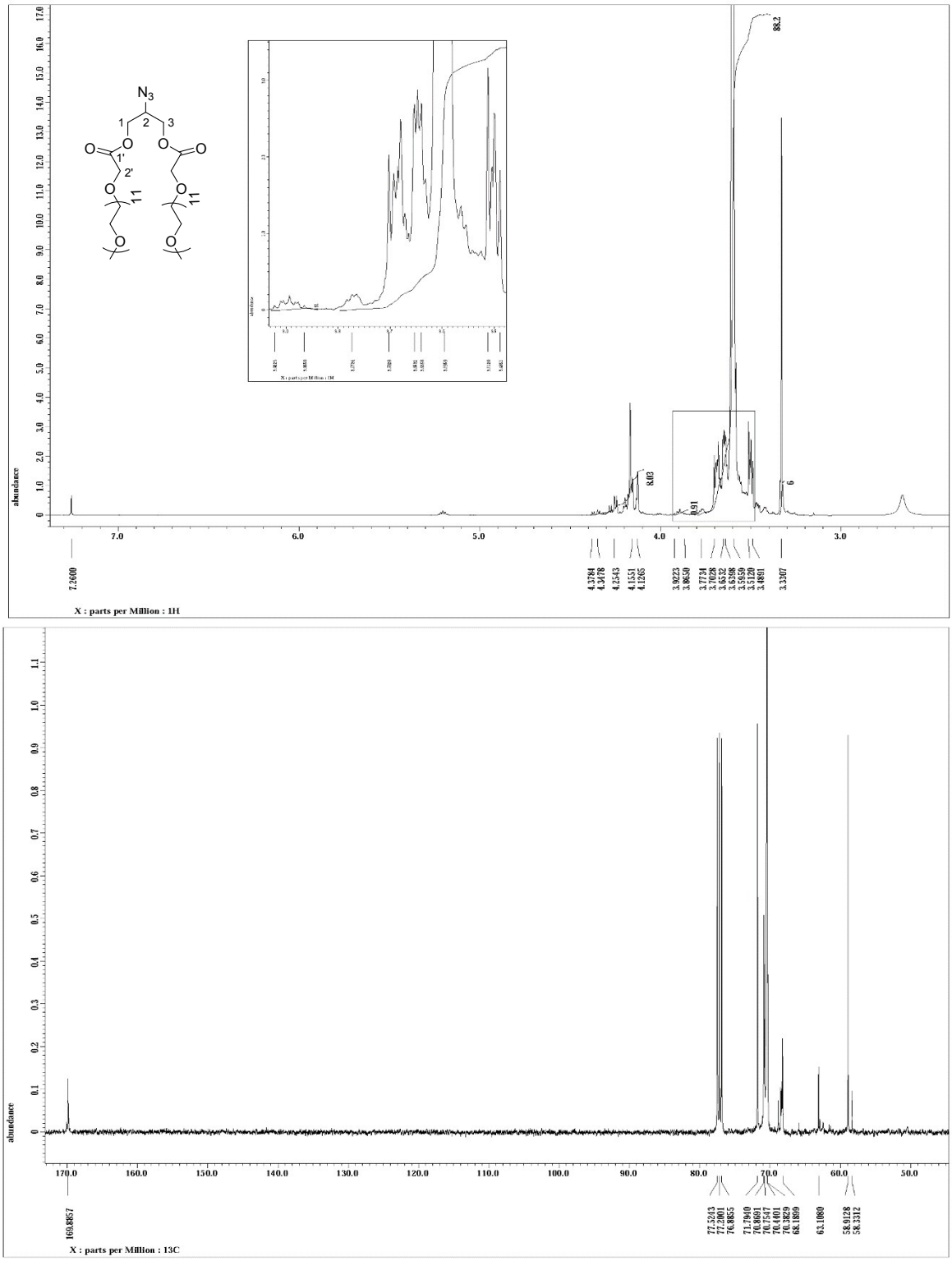

Figure S7. ^{1}H & ^{13}C NMR spectra of 2-azidopropane-1,3-diyl bis(methoxypoly(oxyethylene)oate) (**18**)

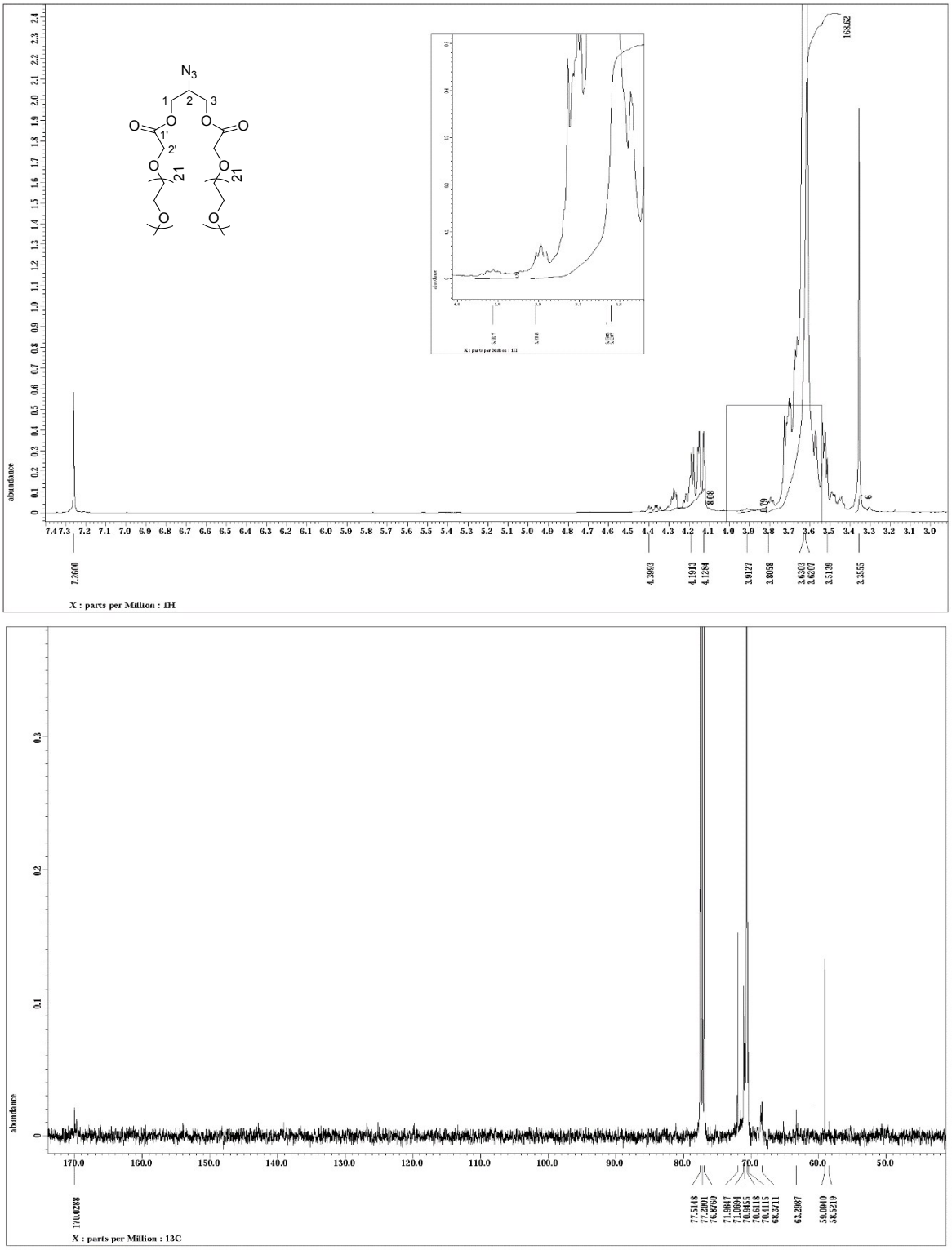

Figure S8. ^{1}H & ^{13}C NMR spectra of 2-azidopropane-1,3-diyl bis(methoxypoly(oxyethylene)oate) (**19**)

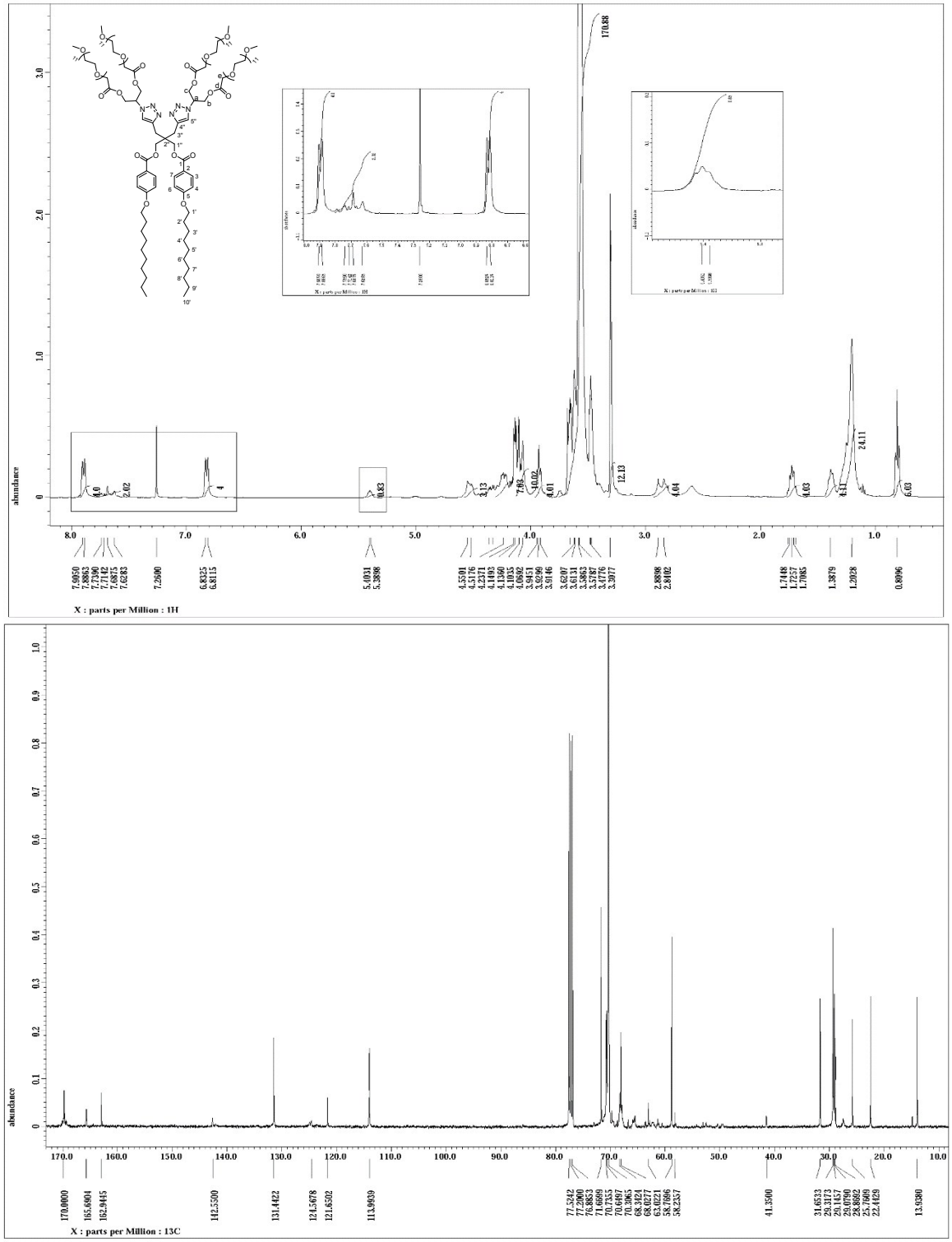

Figure S9. ^{1}H & ^{13}C NMR spectra of amphiphile **20**

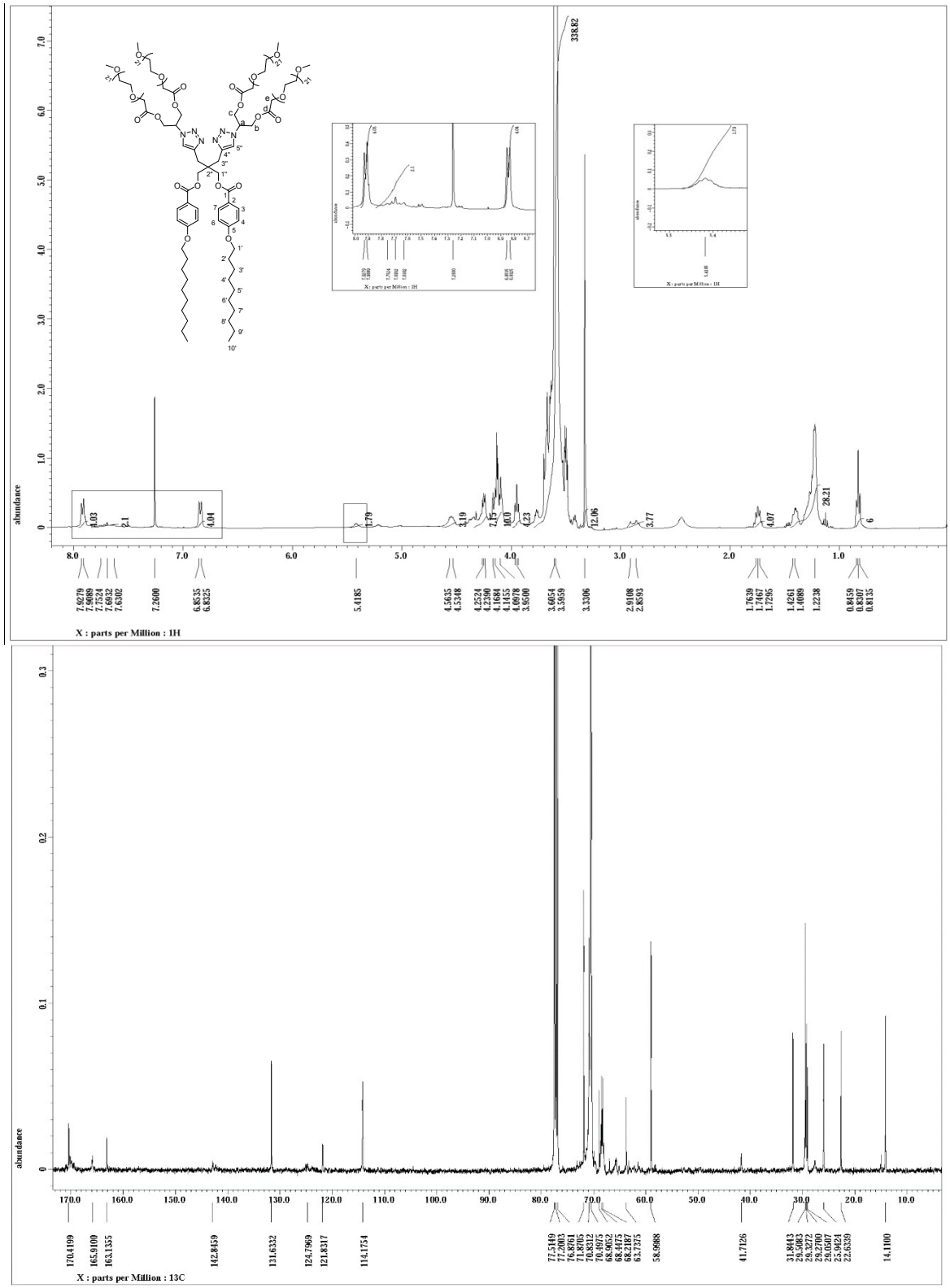

Figure S10. ^{1}H & ^{13}C NMR spectra of amphiphile **21**

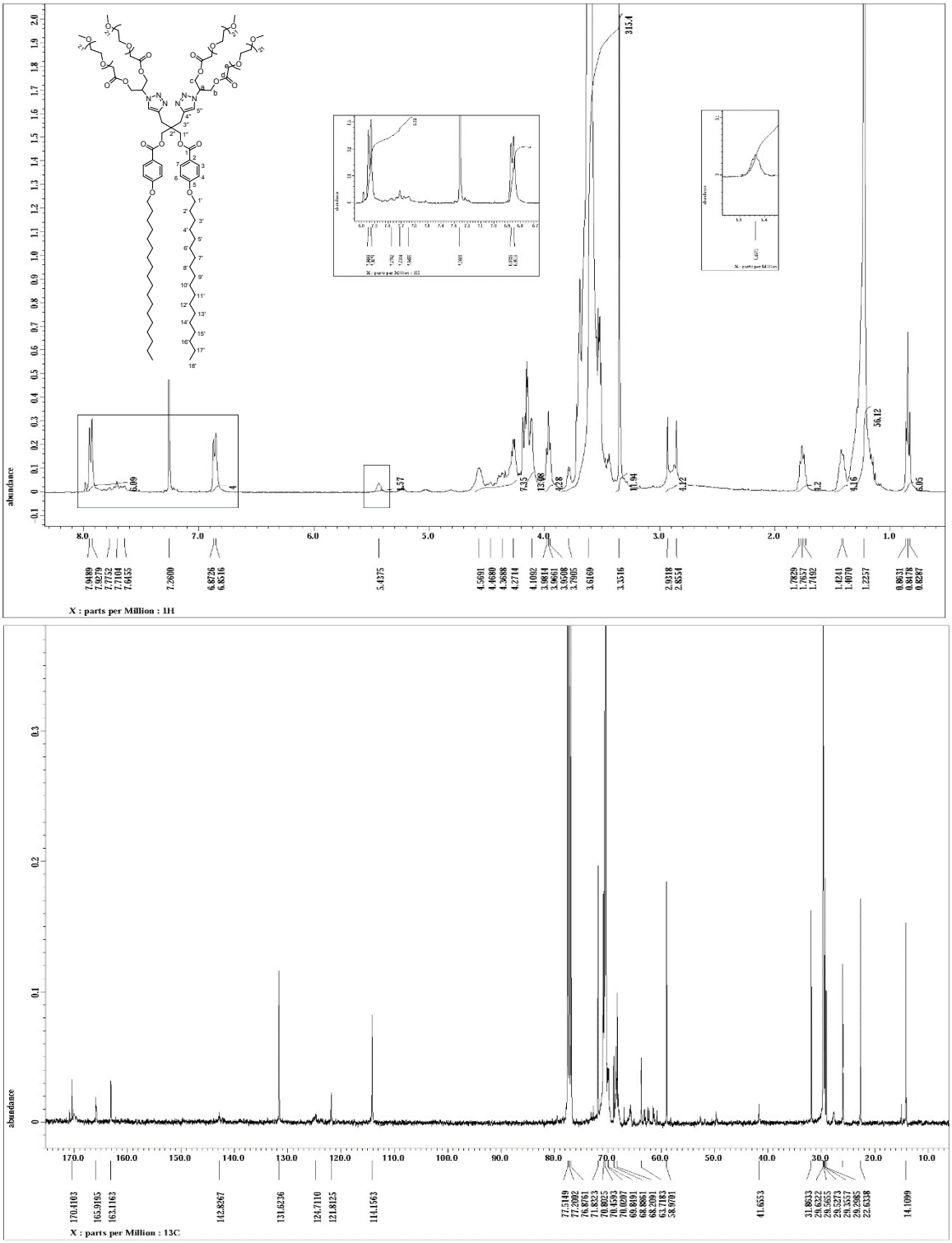

Figure S11. ^{1}H & ^{13}C NMR spectra of amphiphile **22**

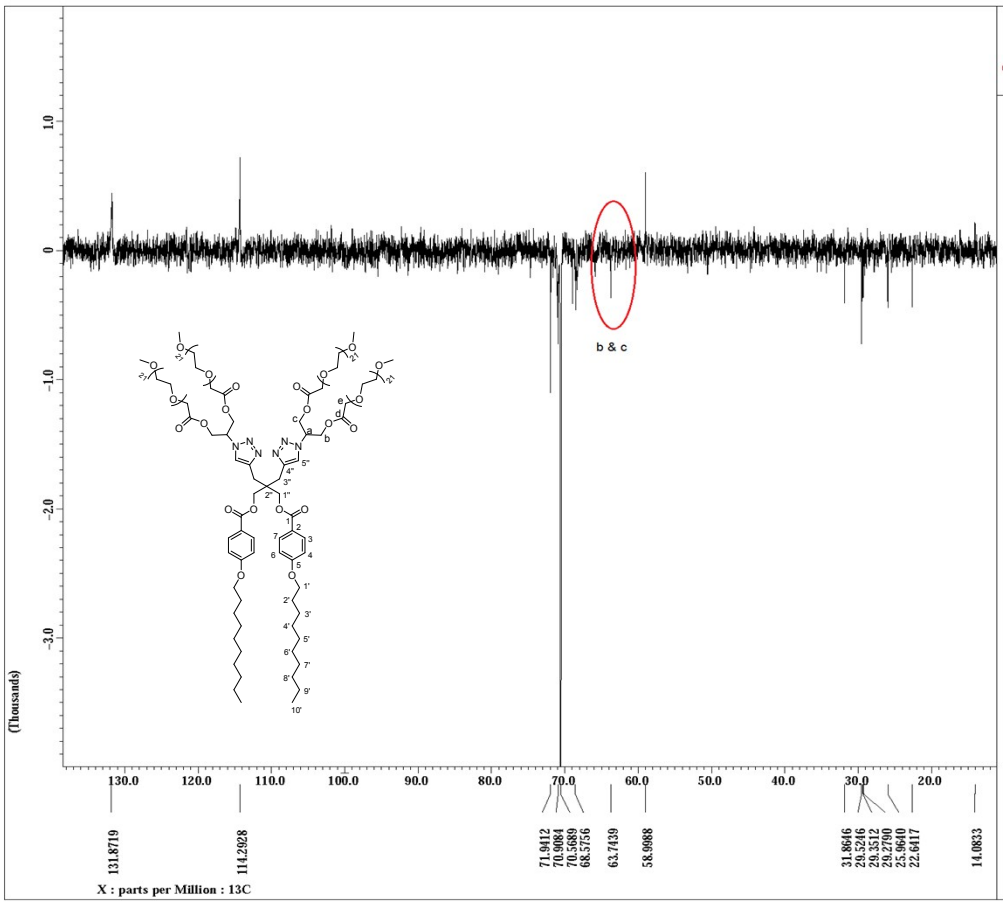

Figure S12. DEPT-135 NMR spectrum of amphiphile **21**

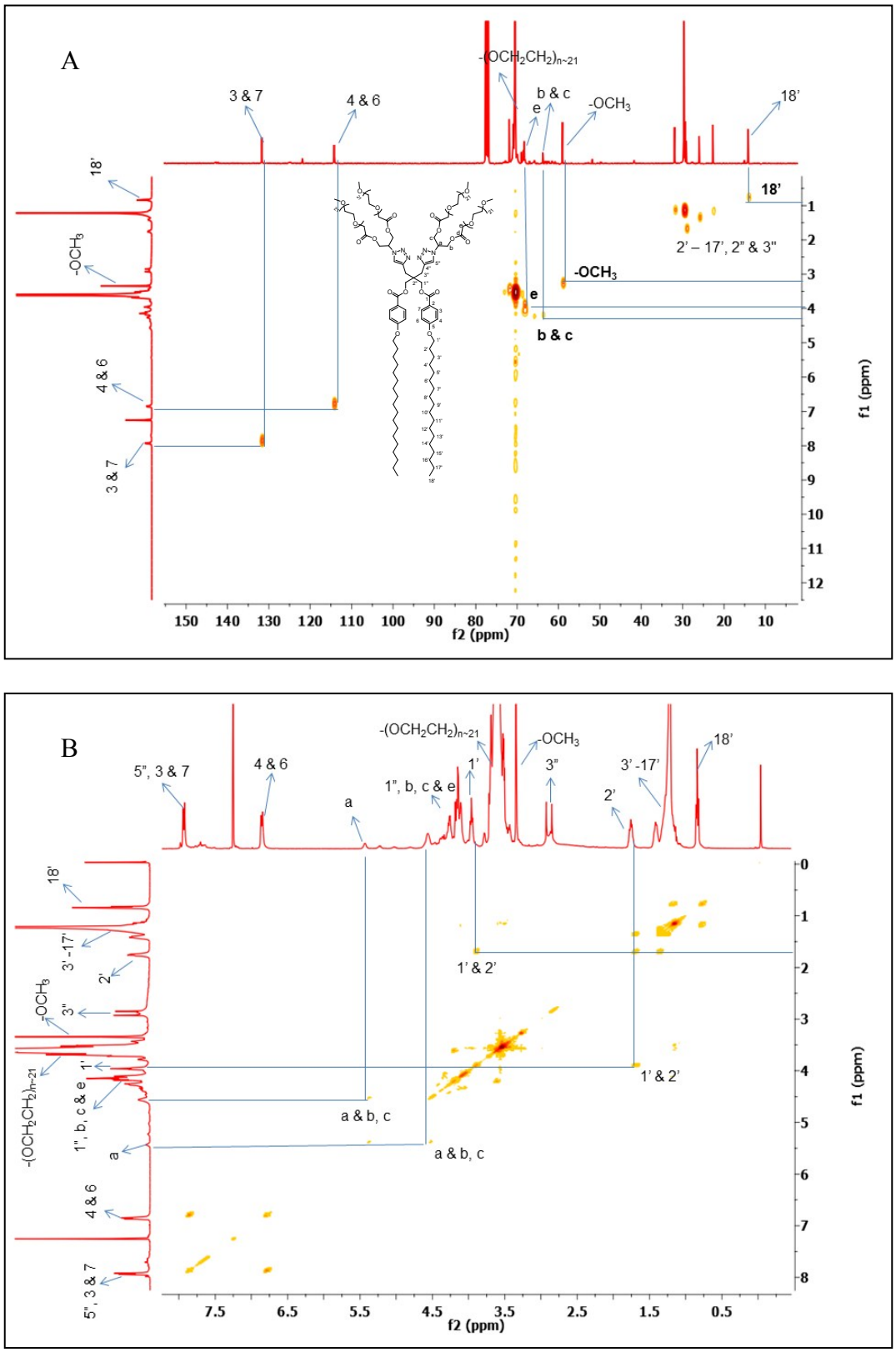

Figure S13. [2]D (A) HETCOR (B) COSY NMR spectra of amphiphile **22**

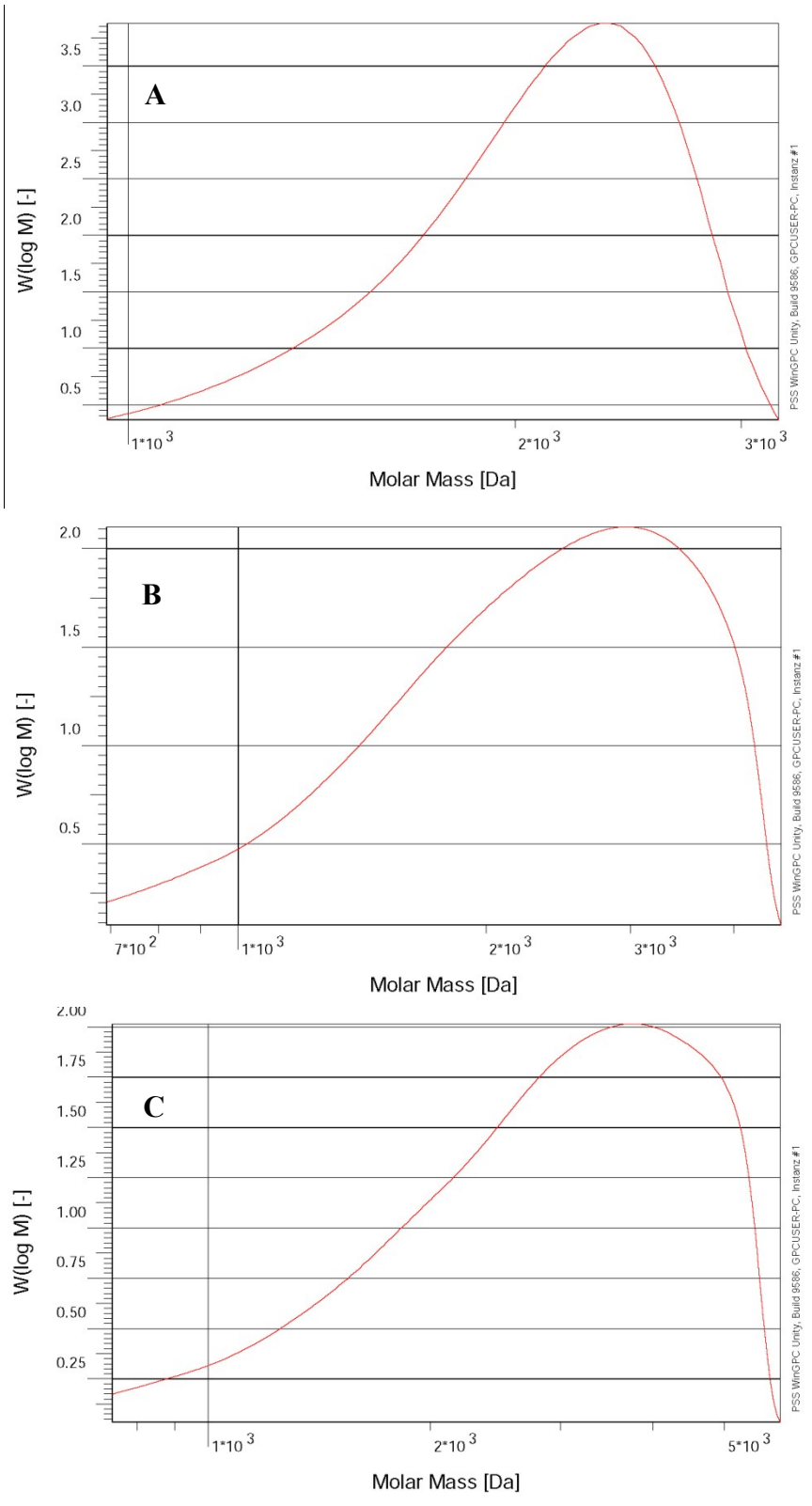

Figure S14. Gel permeation chromatogram of amphiphiles (A) **20** (B) **21** (C) **22**.

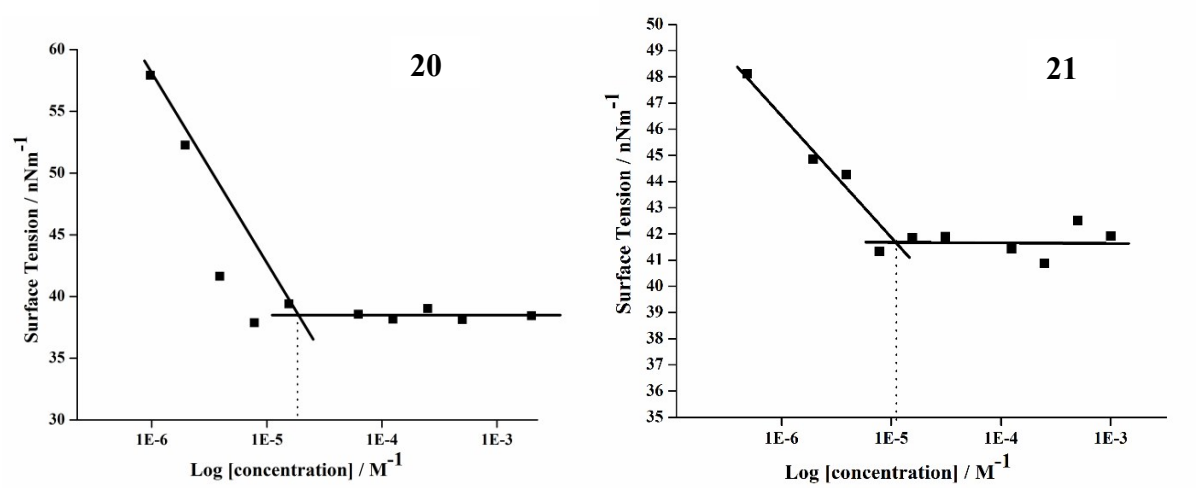

Figure S15. Critical aggregation concentration (CAC) of amphiphiles **20** and **21** in aqueous solution by surface tension measurements at 25 °C.

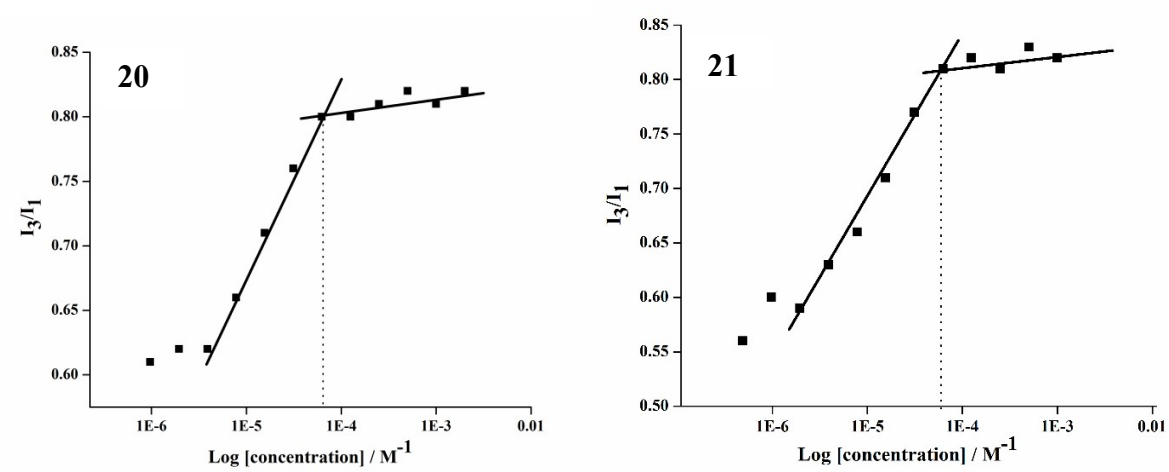

Figure S16. Critical aggregation concentration (CAC) of amphiphiles **20** and **21** in aqueous solution by pyrene fluorescence measurements at 25 °C.

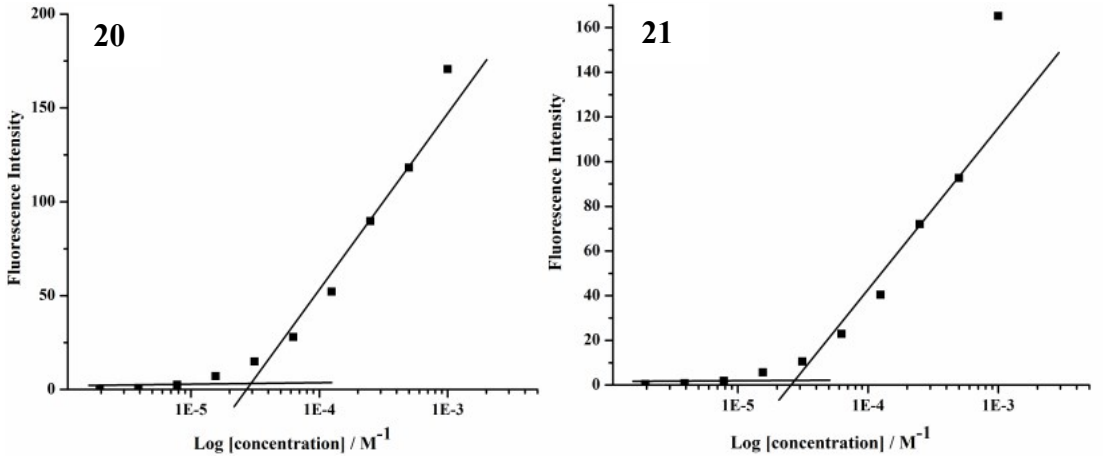

Figure S17. Critical aggregation concentration (CAC) of amphiphiles **20** and **21** in aqueous solution by Nile red fluorescence measurements at 25 °C.

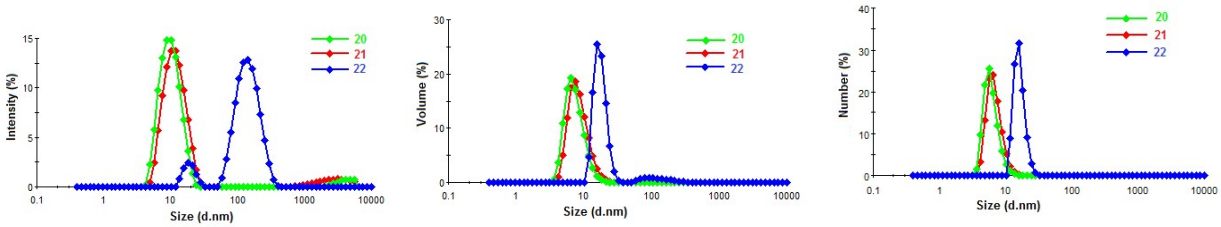

Figure S18. Size distribution profile (by intensity, volume and number) of **20**-**22** before encapsulation.

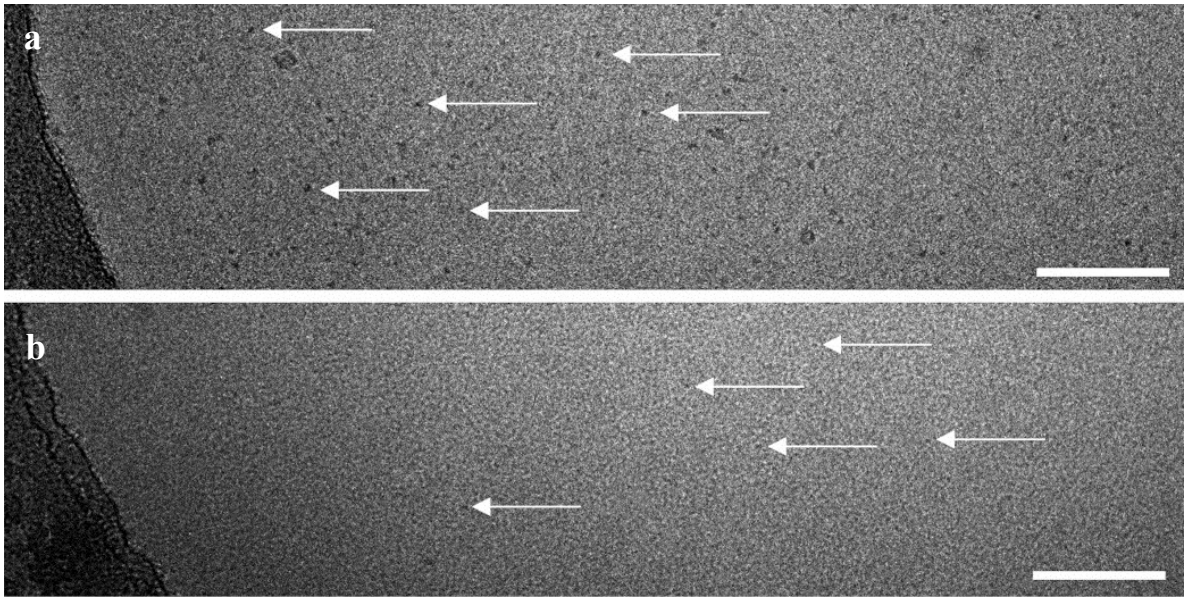

Figure S19. Cryo-TEM micrographs of amphiphiles (a) **20** and (b) **21** showing spherical micellar particles in the < 5 nm range. Scale bar is 100 nm.

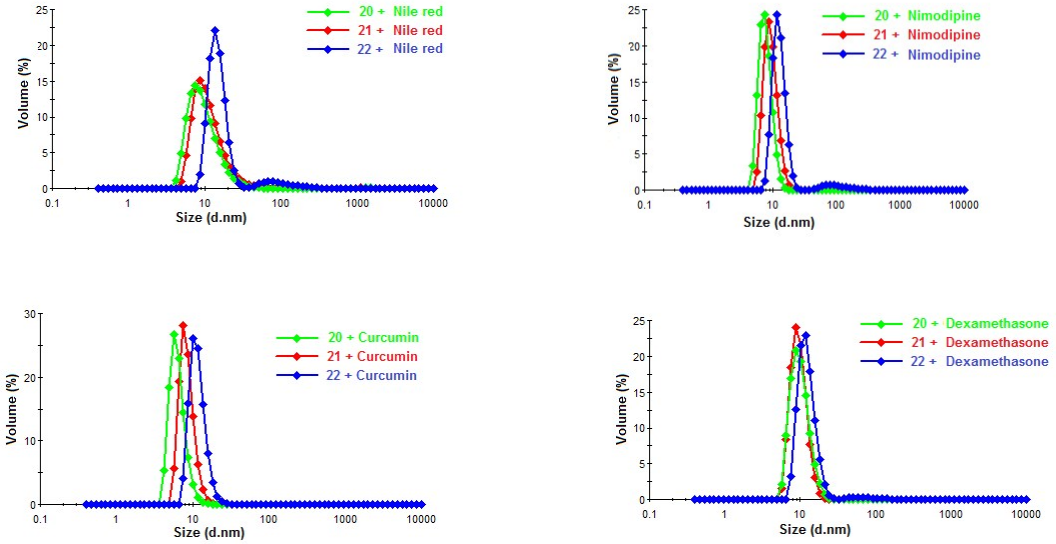

Figure S20. Size distribution profile (by volume) of **20**-**22** after encapsulation.

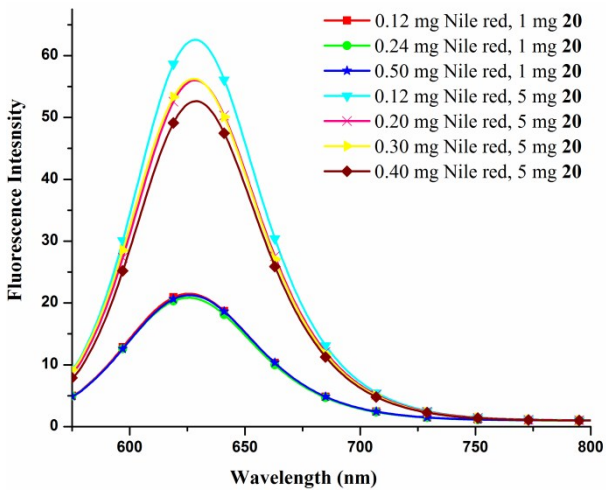

Figure S21. Variation of the fluorescence intensity of Nile red with varying amounts of amphiphile **20** and Nile red in water.

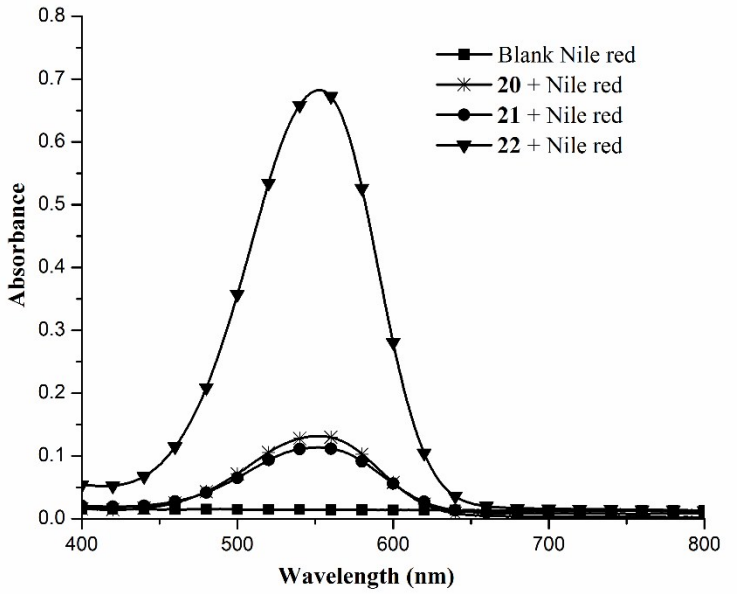

Figure S22. UV absorbance spectra of Nile red encapsulated samples in methanol.

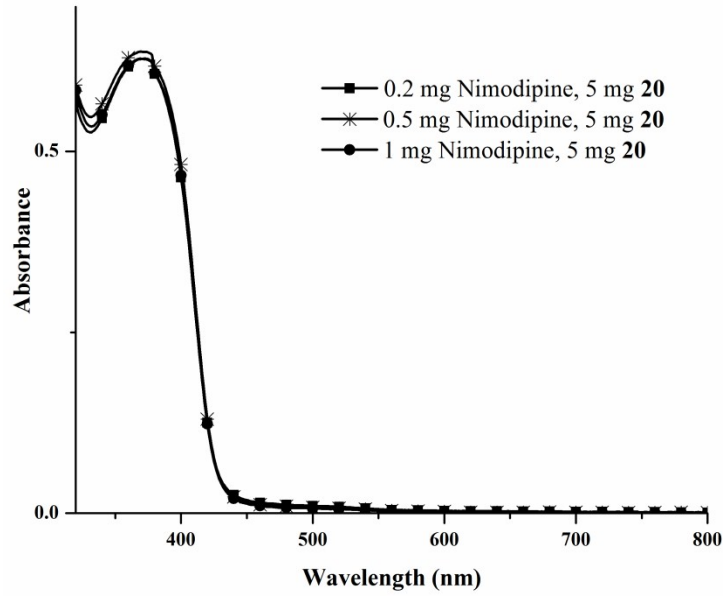

Figure S23. UV absorbance spectra of nimodipine with varying amounts of nimodipine in aqueous solution of amphiphile **20** at 5 mg mL^{-1} concentration.

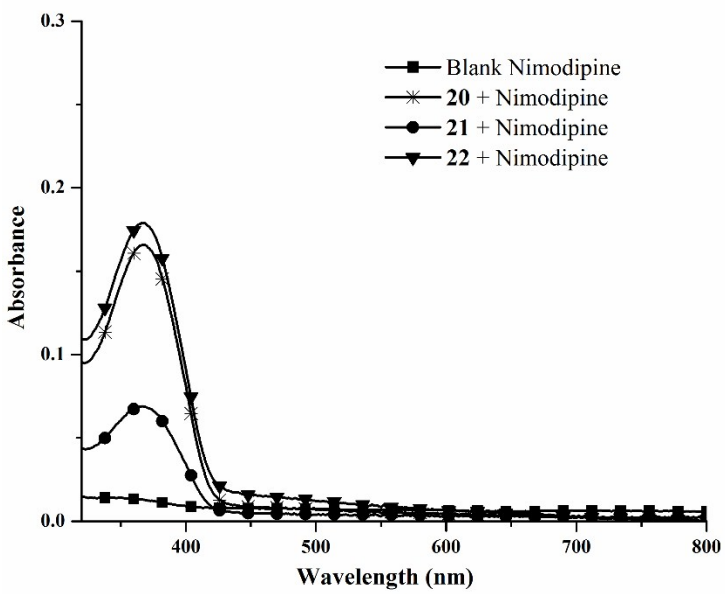

Figure S24. UV absorbance spectra of nimodipine encapsulated samples in ethanol.

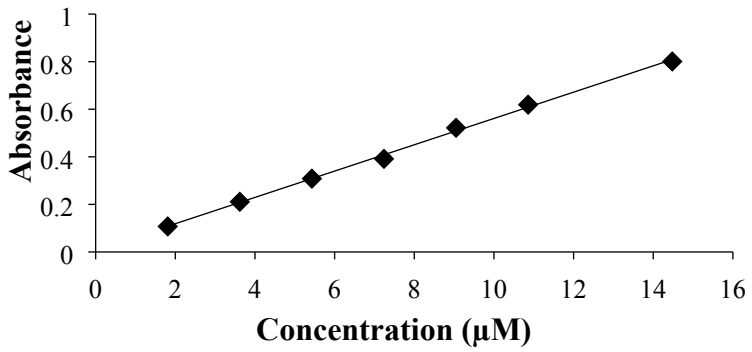

Figure S25. Calibration curve of curcumin in methanol.

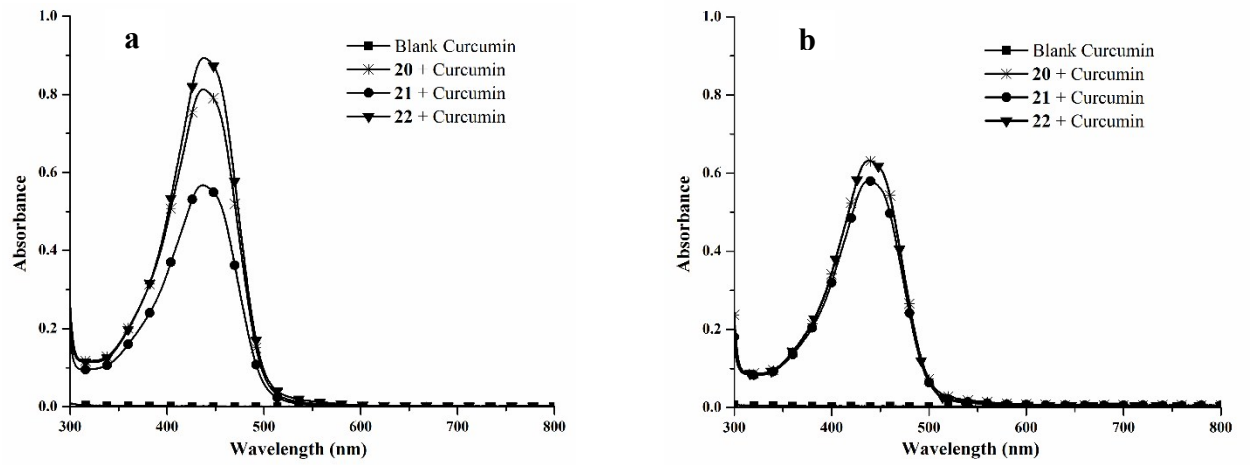

Figure S26. UV absorbance spectra of curcumin encapsulated samples in methanol using (a) 1 mg and (b) 2.5 mg of curcumin.

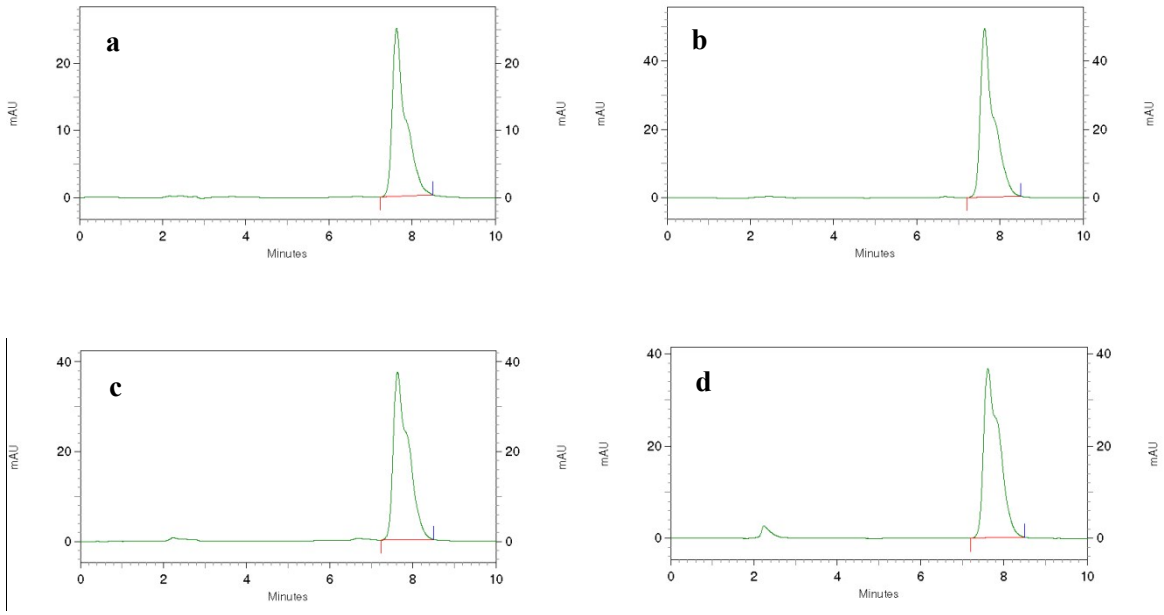

Figure S27. HPLC chromatogram of (a) blank dexamethasone (1 mg) (b) dexamethasone (1 mg) encapsulated in 5 mg mL^{-1} of **20** (c) dexamethasone (1 mg) encapsulated in 5 mg mL^{-1} of **21** (d) dexamethasone (1 mg) encapsulated in 5 mg mL^{-1} of **22**.

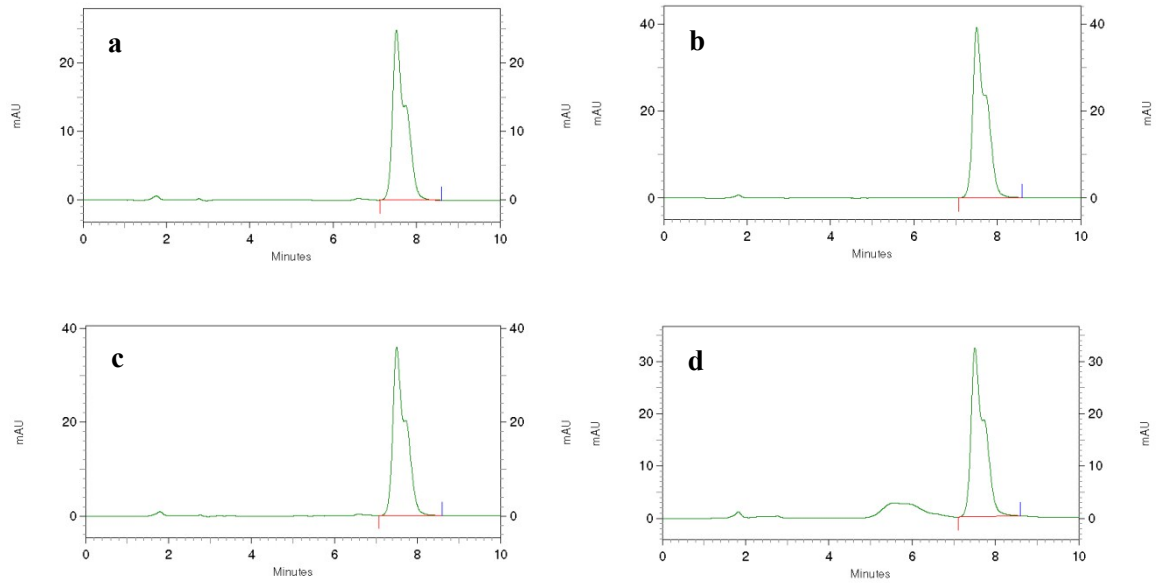

Figure S28. HPLC chromatogram of (a) blank dexamethasone (2.5 mg) (b) dexamethasone (2.5 mg) encapsulated in 5 mg mL^{-1} of **20** (c) dexamethasone (2.5 mg) encapsulated in 5 mg mL^{-1} of **21** (d) dexamethasone (2.5 mg) encapsulated in 5 mg mL^{-1} of **22**.

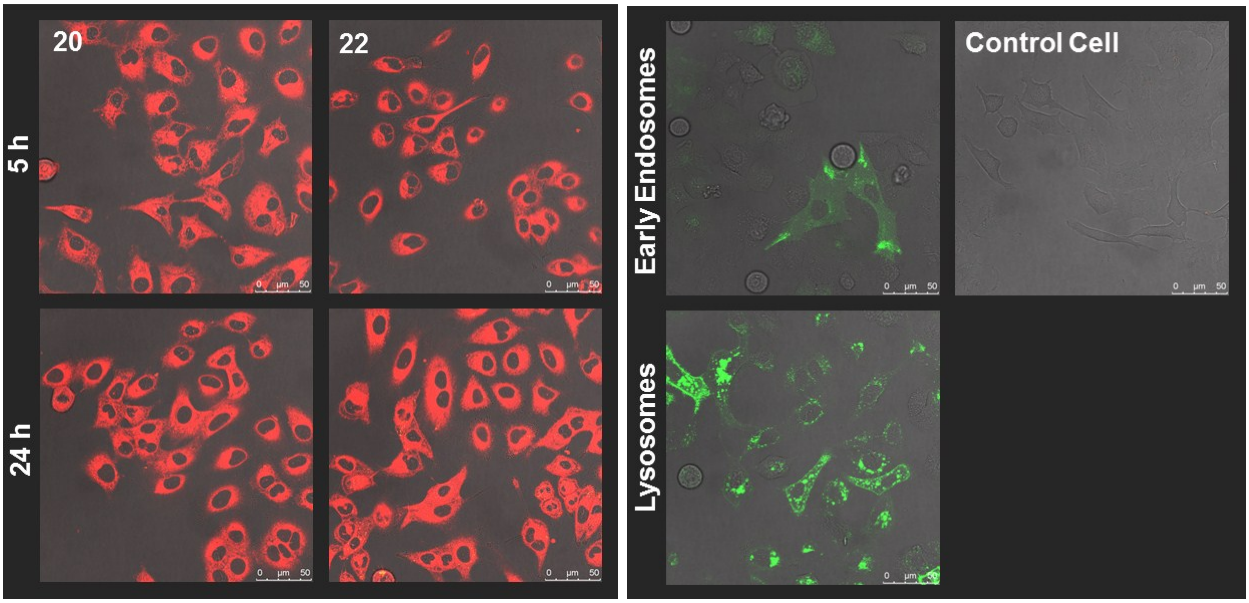

Figure S29. Confocal laser scanning fluorescence microscopy images from A549 cells after 5 and 24 h incubation with Nile red encapsulated by amphiphiles **20** and **22** and non-treated control cells. Nile red is shown in red color and labeled early endosomes and lysosomes in green. The bright field channel is shown in grey scale.

3. References

1. B. N. S. Thota, H. V. Berlepsch, C. Böttcher and R. Haag, *Chem. Commun.*, 2015, **51**, 8648-8651.
2. A. C. Greene, J. Zhu, D. J. Pochan, X. Jia and K. L. Kiick, *Macromolecules*, 2011, **44**, 1942-1951.
3. A. C. Rodrigo, A. Barnard, J. Cooper and D. K. Smith, *Angew. Chem. Int. Ed*., 2011, **50**, 4675-4679.
4. E. Fleige, B. Ziem, M. Grabolle, R. Haag and U. Resch-Genger, *Macromolecules*, 2012, **45**, 9452-9459.
5. V. Kumar, B. Gupta, G. Kumar, M. K. Pandey, E. Aiazian, V. S. Parmar, J. Kumar and A. C. Watterson, *J. Macromol. Sci., Pure Appl. Chem*., 2010, **47**, 1154-1160.
6. S. Das, A. Bedi, G. R. Krishna, C. M. Reddy and S. S. Zade, *Org. Biomol. Chem*., 2011, **9**, 6963-6972.
7. L. Q. Xu, F. Yao and G. D. Fu, *Macromolecules*, 2009, **42**, 6385-6392.
8. X. Y. Hu, K. Jia, Y. Cao, Y. Li, S. Qin, F. Zhou, C. Lin, D. Zhang and L. Wang, *Chem. Eur. J.*, 2015, **21**, 1208-1220.
9. S. Gupta, B. Schade, S. Kumar, C. Böttcher, S. K. Sharma and R. Haag, *Small*, 2013, **9**, 894-904.