Synthesis of C_3-symmetric star shaped amphiphiles for drug delivery applications

Supplementary information

Ayushi Mittal[a], Aarti[a], Sudhanshu Vats[a], Fatemeh Zabihi[b,c], Katharina Achazi[c], Fiorenza Rancan[b] , Anika Vogt[b], Rainer Haag[c] and Sunil K. Sharma[a,*]

[a]*Department of Chemistry, University of Delhi, Delhi 110 007, India*

[b]*Clinical Research Center for Hair and Skin Science, Department of Dermatology and Allergy, Charité-Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany*

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

***Email**: sk.sharma90@gmail.com; **Phone number**: +91 -11-27666646*

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3 References

1. Experimental section

1.1 Materials

Chemicals and solvents were procured from Sigma-Aldrich Chemicals Private Limited (India), Spectrochem Pvt. Ltd., Sisco Research Laboratories (SRL) Pvt. Ltd. India. Nile red and nimodipine were purchased from Fluka Chemie GmbH (Switzerland) and Sigma Aldrich Chemicals, USA. Immobilized *Candida antarctica* lipase was purchased from Julich, Germany. Pre-coated TLC plate was used to monitor the reaction and silica gel of mesh size 100-200 was used in column chromatography for purification. Milli-Q water was used for physicochemical characterization and transport analysis.

1.2 Methods and Instrumentation

1.2.1 Compounds synthesized were characterized by IR (Infra-red), ^{1}H & ^{13}C NMR (Nuclear magnetic resonance), GPC (Gel permeation chromatography) and high resolution mass spectroscopy techniques. IR analysis of the samples was performed using Perkin-Elmer FT-IR model Nicolet iS50. ^{1}H and ^{13}C NMR spectra of amphiphiles were recorded on JEOL 400 MHz spectrometer. The chemical shift values were measured on ppm scale and coupling constant in Hertz (Hz). Mass of the intermediates was analysed by Q-TOF LCMS-Agilent Technology 6530 instrument. The Waters GPC system was used to record molecular weight M_w, M_n, and M_z of the amphiphiles. It consists of PLgel based columns and Agilent 1100 pump and its flow rate set at 1.2 mL min^{-1}. Polystyrene standards were used for the calibration and THF used as an eluent.

1.2.2 Cytotoxicity study

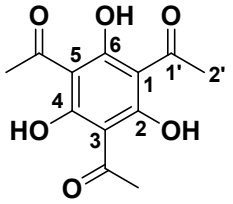
Cytotoxicity of amphiphiles was analysed at a concentration of 0.5, 0.1 and 0.05 mg mL^{-1} in PBS buffer using the CCK-8 kit according to manufacturer's instructions. Before adding samples of all concentrations, HeLa and MCF7 cells were seeded in a 96-well plate and were cultured overnight at 37 ^{o}C. For control measurements, sodium dodecyl sulphate (SDS, 1 %) and non-treated cells were taken, whereas background subtraction was done by only samples. Cells were incubated for 24 h at 37 ^{o}C and then 10 µL of the CCK-8 solution was added, after 2 h and 30 min of incubation period absorbance was recorded at a wavelength of 490 nm and reference was done with 630 nm of Tecan plate reader (Infinite Pro200, TECAN-reader Tecan Group Ltd., Männedorf, Switzerland). Measurements were carried out 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. GraphPad Prism was used for data visualization.

1.3 Synthetic details

1.3.1 Synthesis of 1,3,5-triacetyl-2,4,6-trihydroxybenzene (1)

Anhydrous phloroglucinol (1 g, 1 equiv.) was taken in 50 mL round bottom flask and dissolved in acetic anhydride (3.75 mL, 5 equiv.). Then a few drops of sulphuric acid (4-5) were added in the above solution and the reaction mixture refluxed at 120 ^{o}C for 24 h.[1] Reaction was monitored by the thin layer chromatography (TLC) in the mixture of ethyl acetate and hexane. After completion, the reaction mixture was poured into the ice-cold water (100 mL) and was continuously stirred. The yellow colour solid so formed was filtered out by using a vacuum pump. The crude product was then purified by column chromatography in the mixture of hexane and ethyl acetate. After purification a white colour shining compound (**1**) was obtained in 70 % yield.

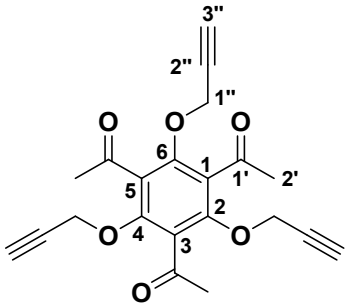


IR (KBr) ν_{max}: 3200, 2910, 2850, 1650, 948, 850 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$): δ** 2.71 (s, 9H, H-2'); **^{13}C NMR (100.5 MHz, $CDCl_3$): δ** 32.89 (C-2'), 103.19 (C-1), 175.81 (C-2), 205.05 (C-1').

1.3.2 Synthesis of 1, 3, 5-triacetyl-2, 4, 6-tripropargyloxy benzene (2)

Compound **1** (1 g, 1 equiv.) was taken in a 50 mL round bottom flask and was dissolved in 20 mL of acetonitrile. Potassium carbonate (5.27 g, 9 equiv.) was added to the above solution and stirred for 30 min at room temperature, after that propargyl bromide (1.69 mL, 6 equiv.) was added dropwise to the above solution and the reaction mixture was stirred for 48 h at 50 ^{o}C. After completion of the reaction unreacted potassium carbonate was filtered off and the solvent was evaporated using a rotary evaporator. The crude product so obtained was extracted with ethyl acetate (2 x 50 mL), the combined organic layer was dried over anhydrous sodium sulphate and the solvent was evaporated under on a rotary evaporator.

Crude product so obtained was purified by column chromatography using silica gel in the mixture of hexane and ethyl acetate. The target compound (**2**) was obtained in 60 % yield.

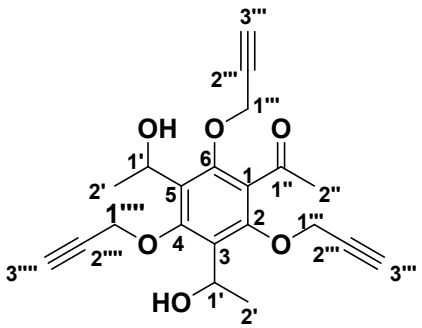


IR (KBr) ν_{max}: 3324, 2918, 2860, 2126, 1650 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$): δ** 2.56 (t, *J* = 2 Hz, 3H, H-3"), 2.60 (s, 9H, H-2'), 4.57 (d, *J* = 2.4 Hz, 6H, H-1"); **^{13}C NMR (100.5 MHz, $CDCl_3$): δ** 32.69 (C-2'), 64.89 (C-1"), 77.41 (C-3"), 129.52 (C-1), 153.18 (C-2), 200.11 (C-1'); HRMS: *m/z* $[M + H]^+$ Calculated for $C_{21}H_{18}O_6$: 366.1103; found: 367.1201.

1.3.3 1-(3,5-bis(1-hydroxyethyl)-2,4,6-tris(propargyloxy)phenyl)ethan-1-one (3) & 1,3,5-tri-hydroxyethyl-2,4,6-tripropargyloxybenzene (4)

In a 25 mL round bottom flask 50 mg (18 equiv.) of sodium borohydride was weighed and dissolved in 5 mL of anhydrous methanol under a nitrogen atmosphere and the solution was kept stirring for 15 min at 0 °C. Then compound **2** (25.5 mg, 1 equiv.) was added to the solution of sodium borohydride and the reaction was initially stirred at 0 °C for 1 h and then at 20 °C for 24 h. Reaction was monitored by thin layer chromatography in the ethyl acetate and hexane mixture and after completion of reaction methanol was evaporated and the reaction mixture was extracted by chloroform (2 x 25 mL) followed by purification of crude product by column chromatography in the ethyl acetate and hexane mixture to give partially reduced (di-reduced) compound **3** in 55 % yield. Compound **3** was further subjected to reduction in the presence of lithium aluminium hydride to give compound **4**. Lithium aluminium hydride ($LiAlH_4$) (50 mg, 5 equiv.) was dissolved in anhydrous THF in nitrogen flushed round bottom flask and stirred for 30 min at 0 °C. Then compound **3** (100 mg, 1 equiv.) was dissolved in a minimum amount of THF (0.5-1 mL) and added dropwise in the $LiAlH_4$ solution under a nitrogen atmosphere at 0 °C. Reaction mixture was stirred for 72 h at 0 °C. After completion of the reaction water was slowly added to the reaction mixture to deactivate the unreacted lithium aluminium hydride in ice conditions followed by evaporation of solvent under vacuum. Reaction mixture was extracted with chloroform (2 x 25 mL) to give fully reduced compound **4**.

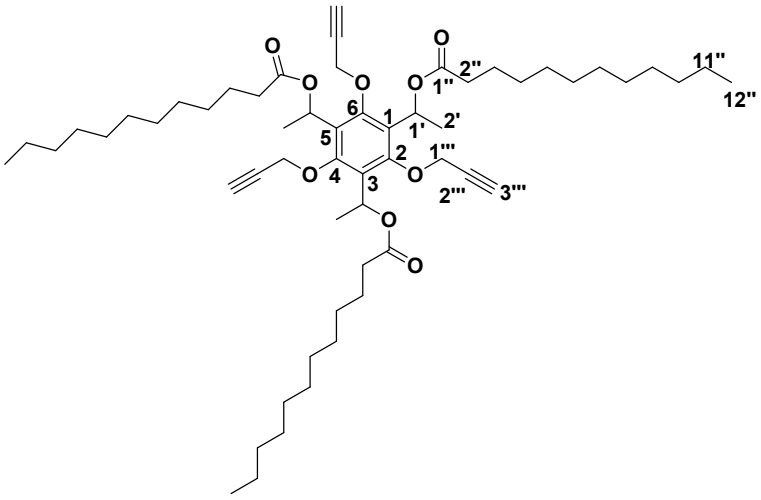
1.3.3.1 Compound **2** reacts with sodium borohydride gives di-reduced compound **3** in 55 % yield as white solid.



IR (KBr) ν_{max}: 3200, 2950, 2870, 2150, 1608, 1352, 1285 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$): δ** 1.63 (d, *J* = 2.8 Hz, 6H, H-2'), 2.54 (s, 3H, H-2''), 2.60 (t, *J* = 2.8 Hz, 2H, H-3'''), 2.64 (t, *J* = 2.4 Hz, 1H, H-3''''), 2.82 (brs, 2H, -OH), 4.50-4.69 (m, 6H, H-1''' & 1''''), 5.26-5.27 (m, 2H, H-1'); **^{13}C NMR (100.5 MHz, $CDCl_3$): δ** 23.55, 32.87, 63.85, 64.26, 78.28, 78.55, 129.11, 129.98, 130.05, 153.30, 156.34, 202.74; HRMS: *m/z* $[M + H]^+$ Calculated for $C_{21}H_{22}O_6$: 370.1416; found: 370.1501.

1.3.4 Synthesis of (2,4,6-tris(prop-2-yn-1-yloxy)benzene-1,3,5-triyl)tris(ethane-1,1-diyl) tridodecanoate (5)

Compound **4** (100 mg, 1 equiv.) was dissolved in anhydrous dichloromethane (DCM) in 25 mL round bottom flask and then dodecanoic acid (172 mg, 3.2 equiv.) was added to the above stirred solution at 0 °C. *N*-(3-dimethylaminopropyl)-*N*-ethyl-carbodiimide hydrochloride (EDC.HCl) (257 mg, 5 equiv.) was added followed by 4-dimethylaminopyridine (72.2 mg, 2.2 equiv.) maintaining the temperature of the reaction mixture at 0 °C. The reaction mixture was stirred at 25 °C for 12 h, the reaction was monitored by thin layer chromatography. After completion, the reaction mixture was poured into a saturated solution of sodium bicarbonate followed by extraction with chloroform (3 x 25 mL), the combined organic layer was dried over anhydrous sodium sulphate. The solvent was evaporated using rotary evaporator, and the crude product was purified by column chromatography in the mixture of ethyl acetate and hexane to yield compound **5** in 50 % yield.



IR (KBr) ν_{max}: 3312, 2922, 2853, 1734, 1573, 1460, 1233, 1142, 1108, 721, 667 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$): δ** 0.87 (t, *J* = 6.8 Hz, 9H, H-12"), 1.24-1.28 (m, 48H, H-4" - H-11"), 1.59-1.70 (m, 15H, H-3" & H-2'), 2.28-2.38 (m, 6H, H-2"), 2.56 (t, *J* = 2.4 Hz, 3H, H-3'"), 4.57-4.69 (m, 6H, H-1'"), 6.37-6.43 (m, 3H, H-1'); **^{13}C NMR (100.5 MHz, $CDCl_3$): δ** 14.18, 19.65, 19.77, 20.02, 22.75, 25.00, 29.67, 31.97, 34.81, 63.08, 63.16, 63.63, 65.94, 66.20, 75.62, 75.97, 78.64, 78.89, 156.33, 156.49, 157.33, 173.06; HRMS: *m/z* $[M + H]^+$ Calculated for $C_{57}H_{90}O_9$: 918.6567; found: 919.6640.

1.3.5 Synthesis of mPEG 550/1000 azide (**8** / **9**)

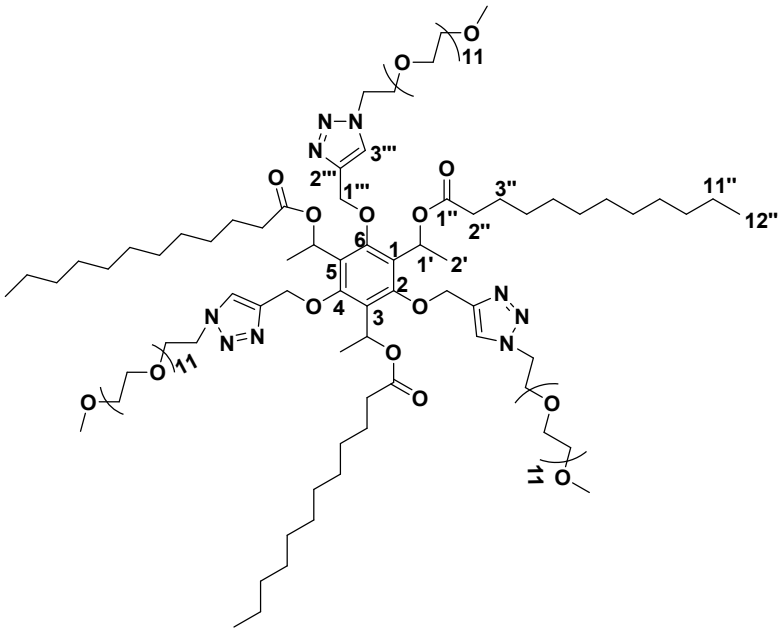
The mesylation of monomethoxypolyethylene glycol (**6/7**) (M_n: 550/1000 g mol^{-1}) (1 g, 1 equiv.) was carried out by dissolving reactant in DCM (20 mL) in 100 mL round bottom flask followed by addition of triethyl amine (0.3041/0.167 mL, 1.2 equiv.) and the solution stirred for 30 min under nitrogen atmosphere. Then methanesulfonyl chloride (0.211/0.116 mL, 1.5 equiv.) was added to the reaction mixture at 0 °C and was stirred for 2 h. After completion of reaction solvent was evaporated and the crude was extracted using chloroform (3 x 100 mL). The combined organic layer was dried over anhydrous sodium sulphate followed by the evaporation of solvent under reduced pressure using a rotary evaporator. The crude mesylated mPEG (1.41/1.08 g, 1 equiv.) was dissolved in DMF (10 mL) followed by the addition of sodium azide (0.583/0.26 g, 3.5 equiv.) and the solution was kept stirring for 12 h at 80 °C. After completion of the reaction, DMF was evaporated on rotary evaporator and water was added to the crude product which was extracted with chloroform (4 x 100 mL) and then organic layer was dried over anhydrous sodium sulphate. The crude product so obtained was

purified by column chromatography in chloroform and methanol mixture to give the compound **8**/**9** in 70 % yield.

1.3.6 Synthesis of compound **10** & **11**

Compound **5** (50 mg, 1 equiv.) was dissolved in *N*,*N*-dimethyl formamide (DMF) in 10 mL round bottom flask, and hydrophilic unit, *i.e.* mPEG 550/1000 azide (**8**/**9**) (0.125 g/0.223 g, 4 equiv.) was added to the above solution. Then bromotris(triphenylphosphine) copper(I) catalyst (5 mg) and DIPEA were added to the reaction mixture, and the reaction was allowed to stir at 80 ^{o}C for 24 h. After completion, DMF was evaporated followed by the addition of water to the reaction mixture and the solution was extracted with chloroform (3 x 20 mL). The organic layer was dried using anhydrous sodium sulphate and then the solvent was evaporated. The crude compound was purified using column chromatography using silica gel of mesh size 100-200 in the mixture of chloroform and methanol to give the compound **10**/**11**.

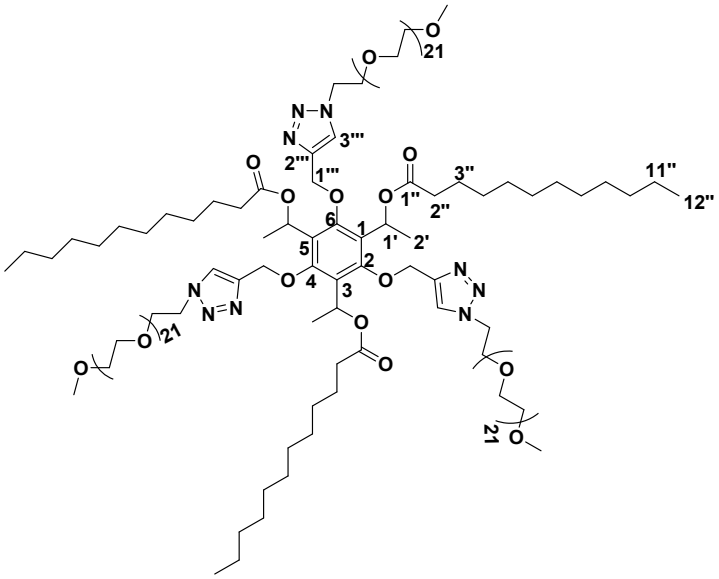
1.3.6.1 Compound **5** was reacted with hydrophilic mPEG 550 azide unit **8** *via* click reaction giving compound **10** as yellow viscous oil in 40 % yield by following the mentioned procedure.



IR (KBr) ν_{max}: 2930, 2860, 1608, 1511, 1285, 1253, 1107 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$): δ** 0.86 (t, *J* = 6.8 Hz, 9H, H-12"), 1.20-1.23 (m, H-4" - H-11"), 1.61-1.62 (m, 9H, H-2'), 1.70-1.79 (m, 6H, H-3"), 2.26-2.45 (m, 6H, H-2"), 3.37 (s, 9H, -OCH_3), 3.53-3.64 (m, -OCH_2CH_2 of mPEG region), 3.92-4.59 (m, 6H, H-1'"), 5.10-5.32 (m, 3H, H-1'), 6.43-6.59 (m,

2H, H-3'''), 7.94-8.05 (m, 1H, H-3'''); **^{13}C NMR (100.5 MHz, $CDCl_3$): δ** 14.19 (C-12''), 19.82, 22.72, 24.97, 29.38, 29.65, 31.94, 34.67, 50.70, 59.08, 61.66, 69.57, 70.07, 70.58, 70.72, 71.95, 72.78, 114.14, 124.25, 124.82, 126.46, 143.71, 144.31, 173.14, 173.38; **GPC (THF, 1.2 mL min^{-1})**: M_w: 2419.1 g mol^{-1}, M_n: 2305.9 g mol^{-1}, M_z: 2543.7 g mol^{-1}, polydispersity index (PDI): 1.049.

1.3.6.2 Compound **5** was reacted with compound **9** *via* click reaction giving compound **11** as a yellow viscous oil in 50 % yield.



IR (KBr) ν_{max}: 2919, 2862, 2105, 1732, 1458, 1352, 1246, 1098, 946, 848 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$): δ** 0.85 (t, *J* = 6.8 Hz, 9H, H-12''), 1.23-1.36 (m, H-4'' - H-11''), 1.60-1.65 (m, 9H, H-2'), 1.89-1.96 (m, 6H, H-3''), 2.01-2.30 (m, 6H, H-2''), 3.35 (s, 9H, -OCH_3), 3.62-3.85 (m, -OCH_2CH_2 of mPEG region), 4.20-4.58 (m, 6H, H-1'''), 5.09-5.32 (m, 3H, H-1'), 7.50-8.02 (m, 3H, H-3'''); **^{13}C NMR (100.5 MHz, $CDCl_3$): δ** 14.11 (C-12''), 19.80, 22.67, 24.93, 29.32, 29.60, 31.89, 34.70, 50.69, 59.033, 61.71, 69.54, 70.03, 70.29, 70.57, 70.64, 71.94, 72.58, 124.17, 124.69, 124.79, 126.43, 126.62, 126.77, 143.86; **GPC (THF, 1.2 mL min^{-1})**: M_w: 3594.2 g mol^{-1}, M_n: 3759.9 g mol^{-1}, M_z: 3916.3 g mol^{-1}, polydispersity index (PDI): 1.046.

2 Figures

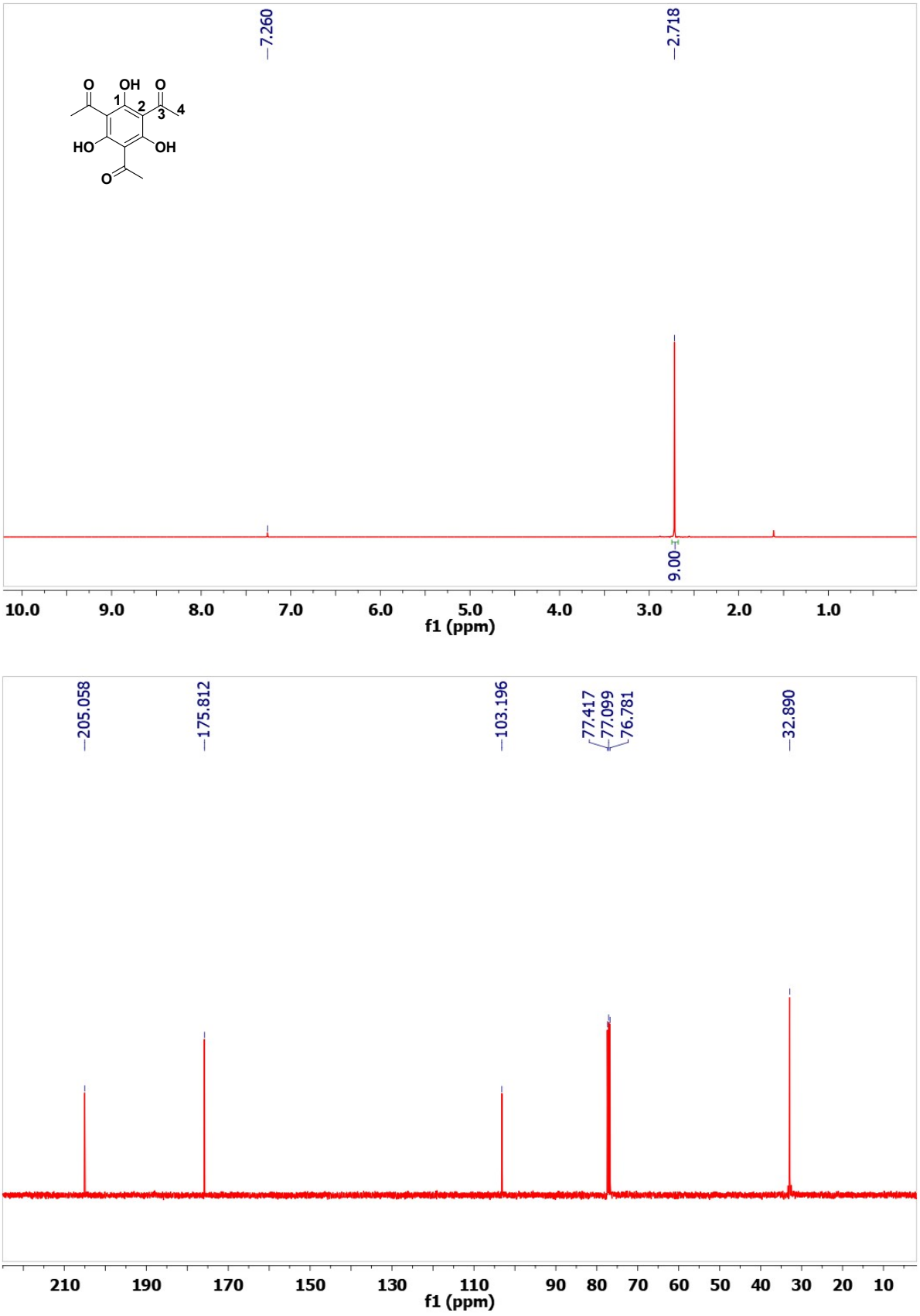


Fig. S1 ^{1}H & ^{13}C NMR spectra of 1,1',1''-(2,4,6-trihydroxybenzene-1,3,5-triyl)tris(ethan-1-one) (**1**).

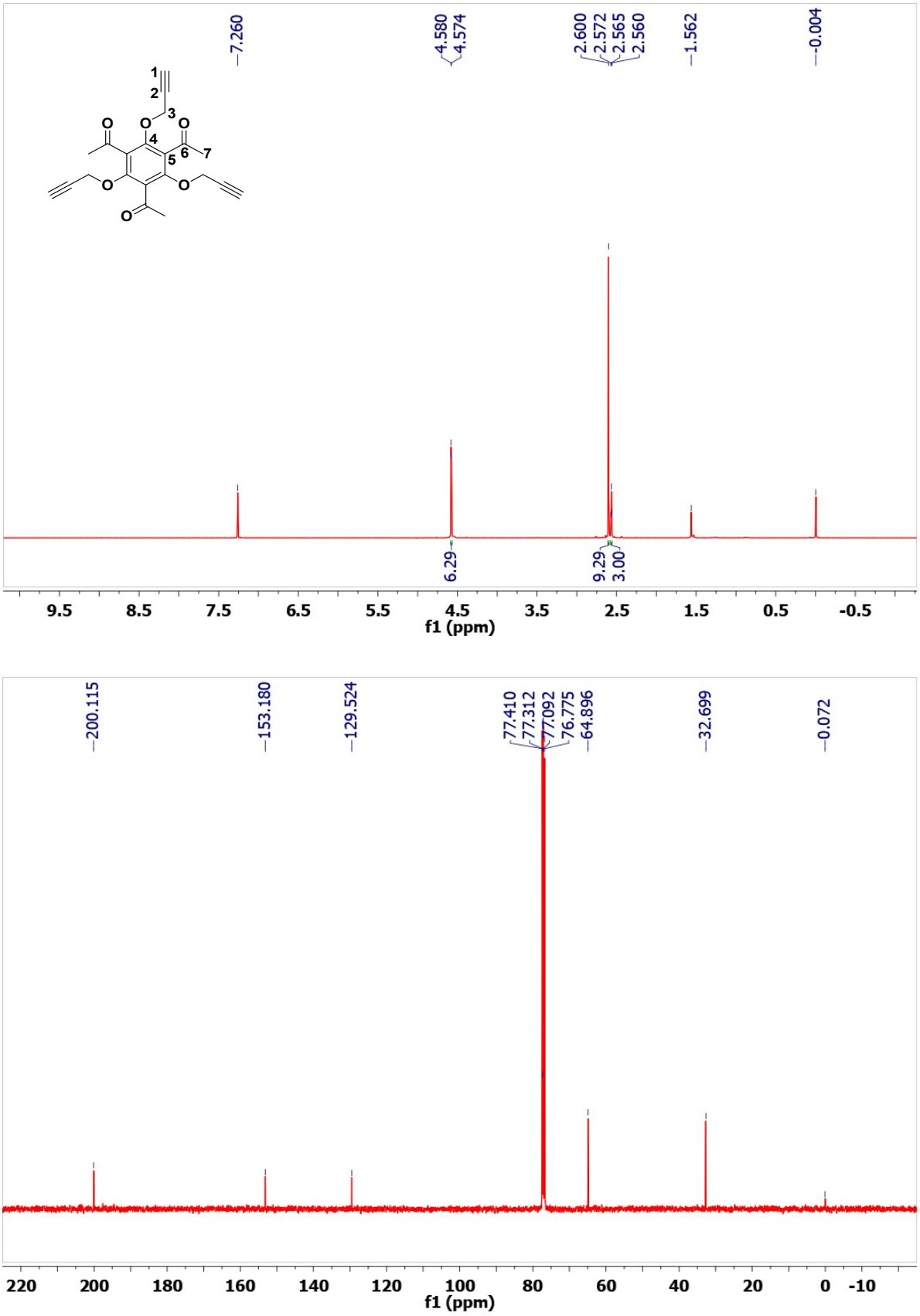


Fig. S2 ^{1}H & ^{13}C NMR spectra of 1,1',1''-(2,4,6-tris(prop-2-yn-1-yloxy)benzene-1,3,5-triyl)tris(ethan-1-one) (**2**).

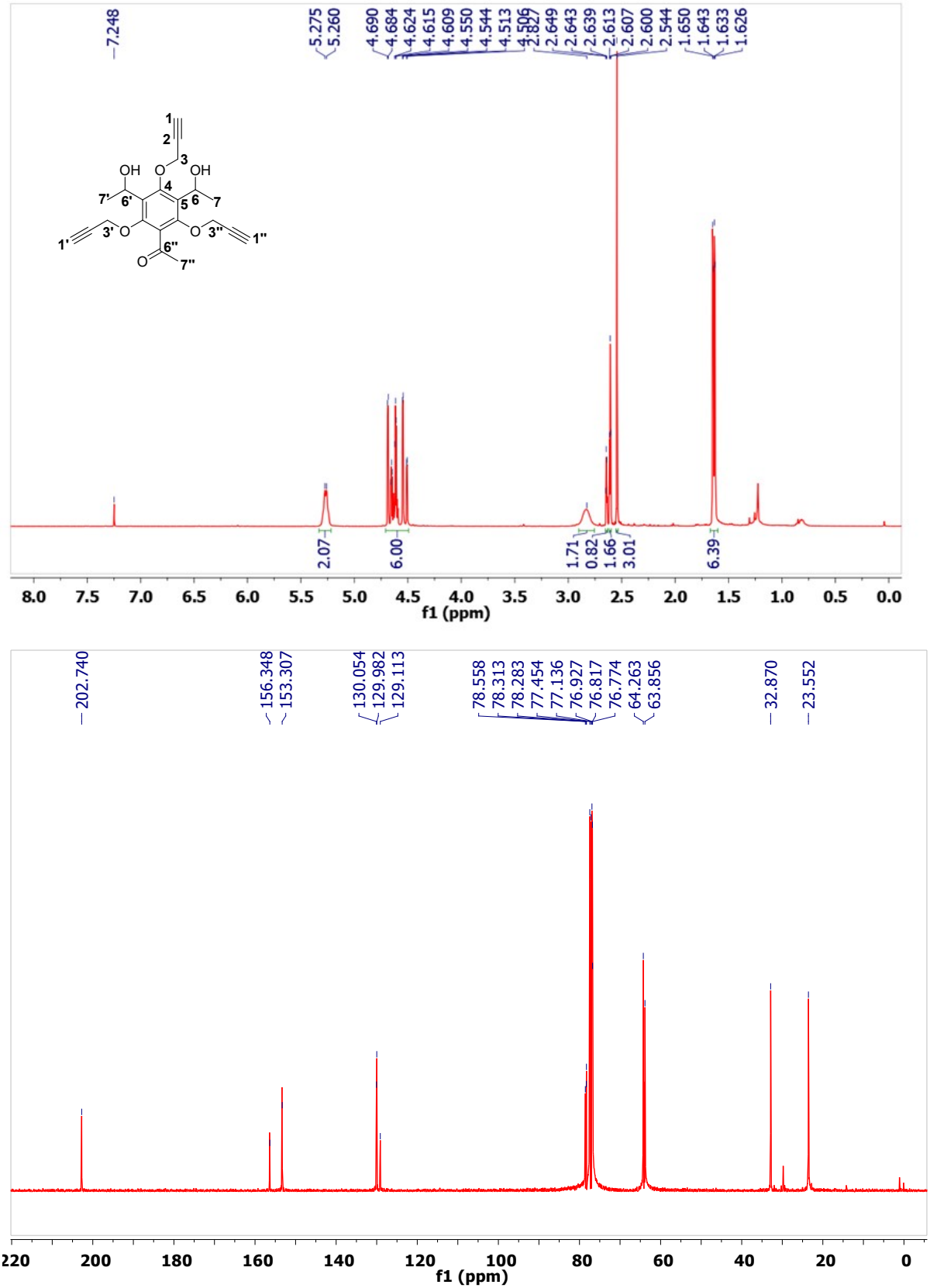


Fig. S3 ^{1}H & ^{13}C NMR spectra of 1-(3,5-bis(1-hydroxyethyl)-2,4,6-tris(prop-2-yn-1-yloxy)phenyl)ethan-1-one (**3**).

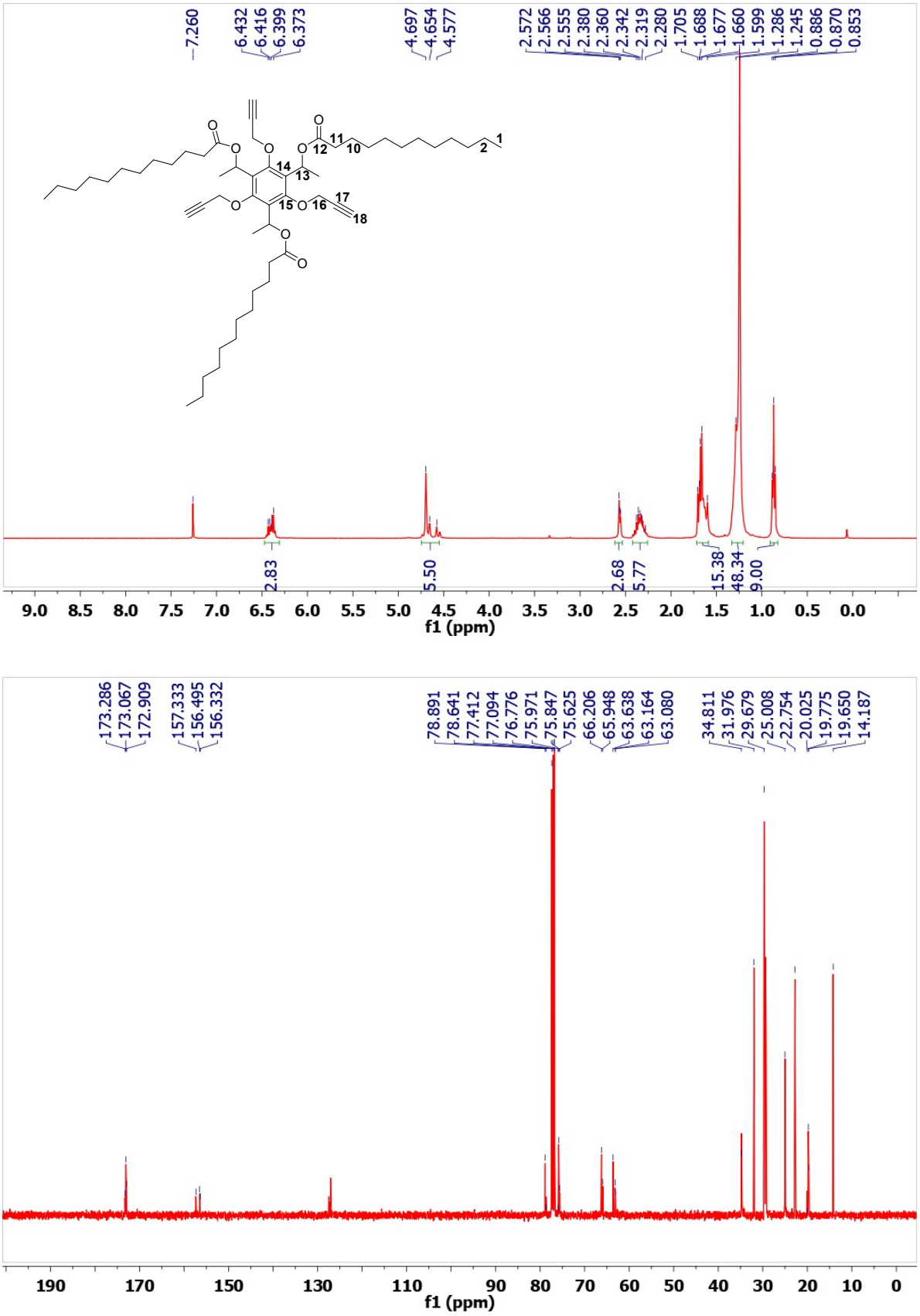


Fig. S4 ^{1}H & ^{13}C NMR spectra of (2,4,6-tris(prop-2-yn-1-yloxy)benzene-1,3,5-triyl)tris(ethane-1,1-diyl)tridodecanoate (**5**).

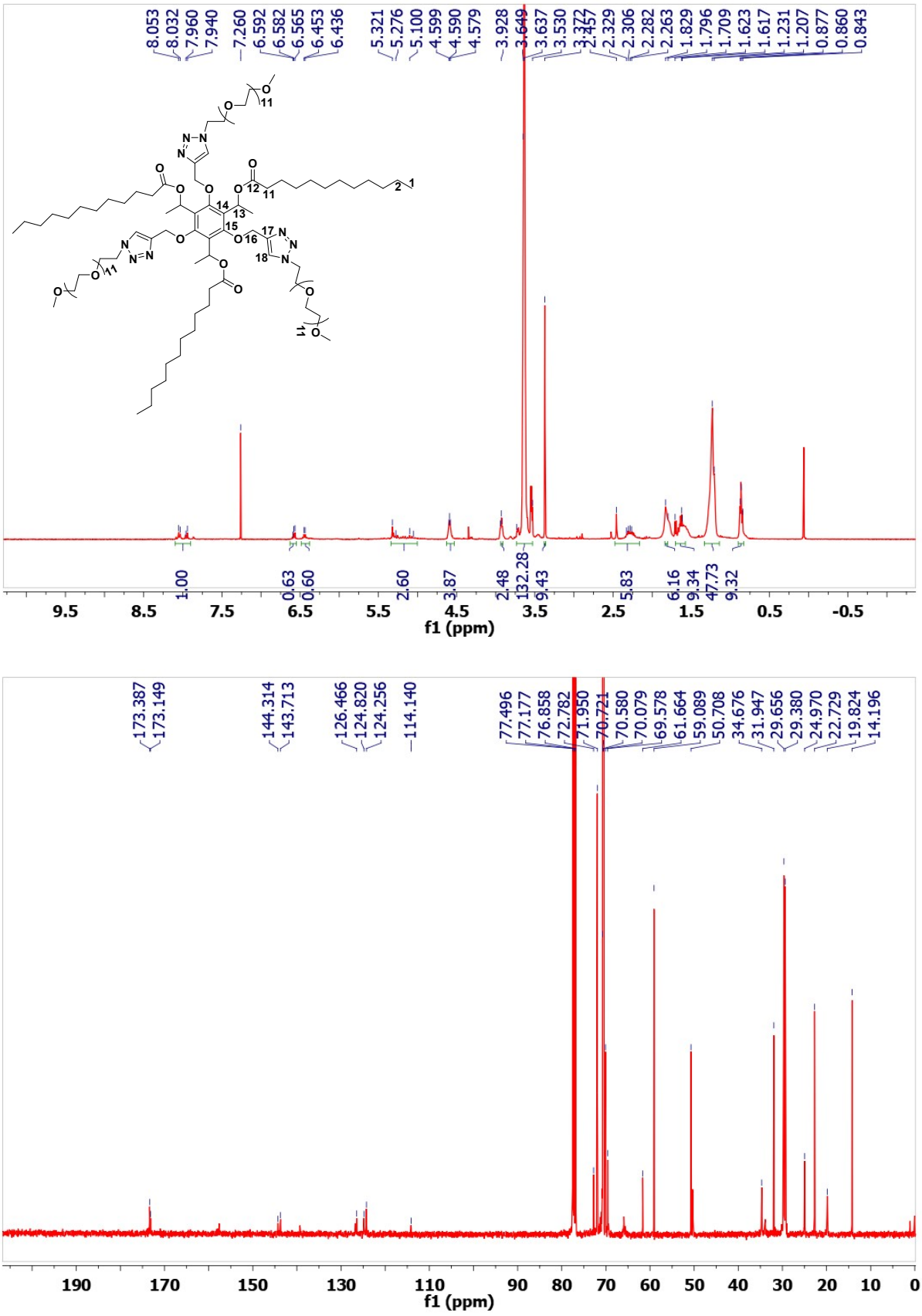


Fig. S5 ^{1}H & ^{13}C NMR spectra of compound (**10**).

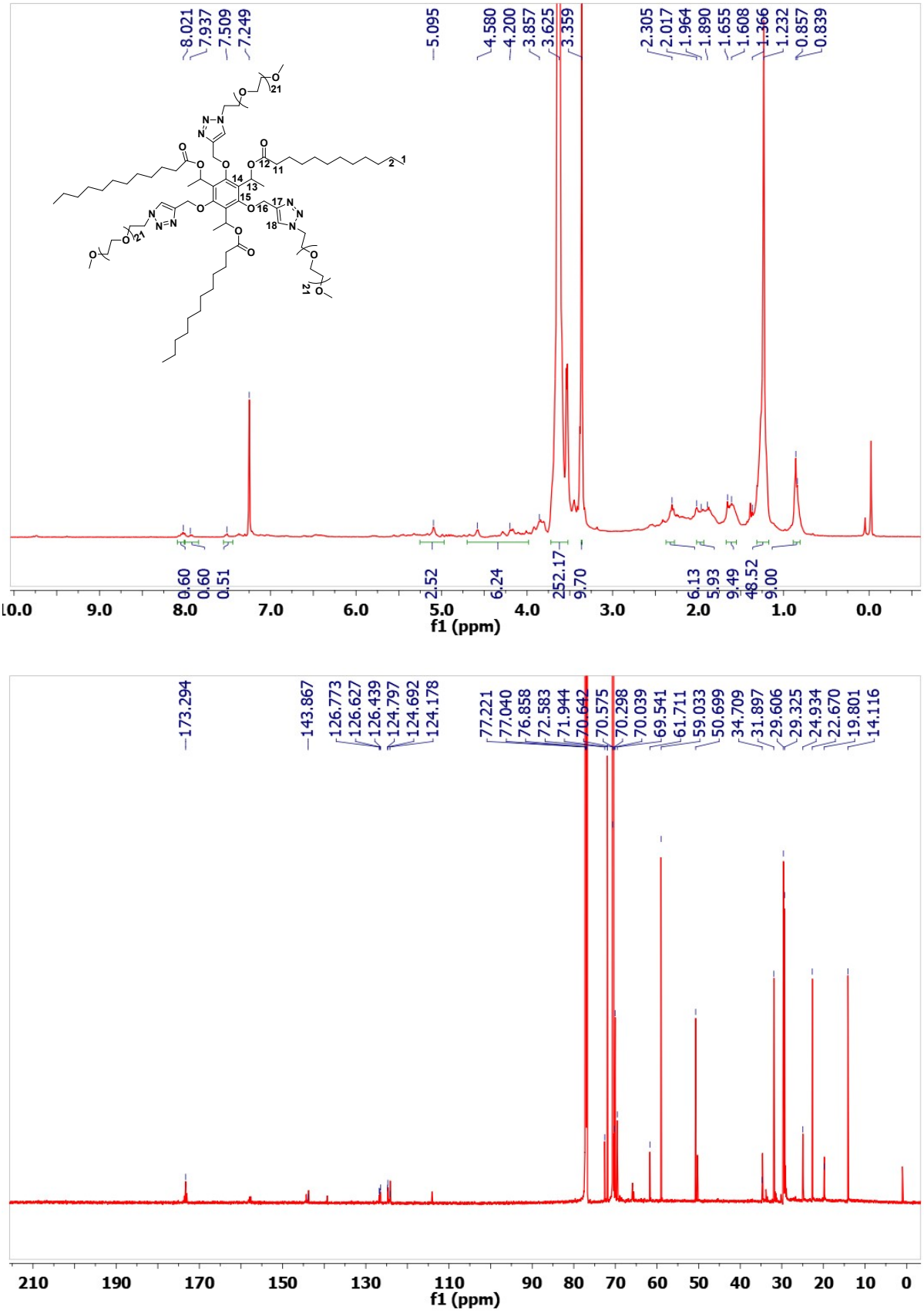


Fig. S6 ^{1}H & ^{13}C NMR spectra of compound (**11**).

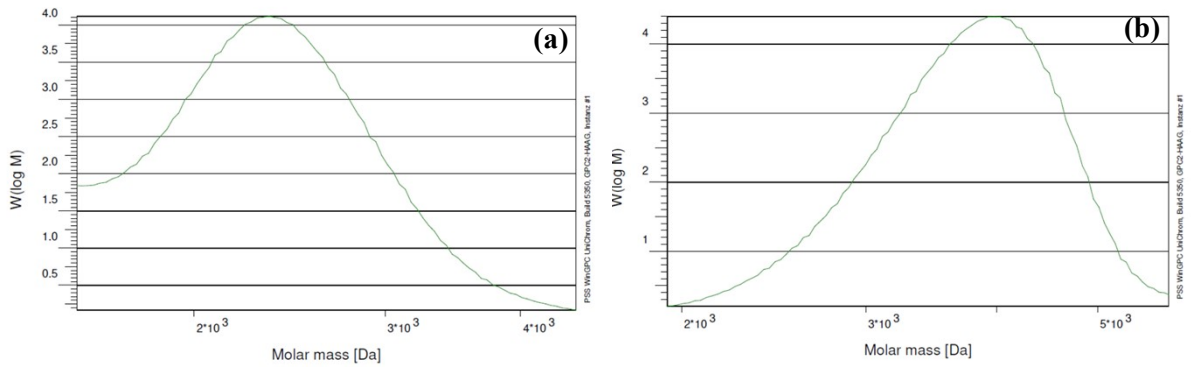


Fig. S7 Gel permeation chromatogram of molecules (a) **10**; (b) **11**.

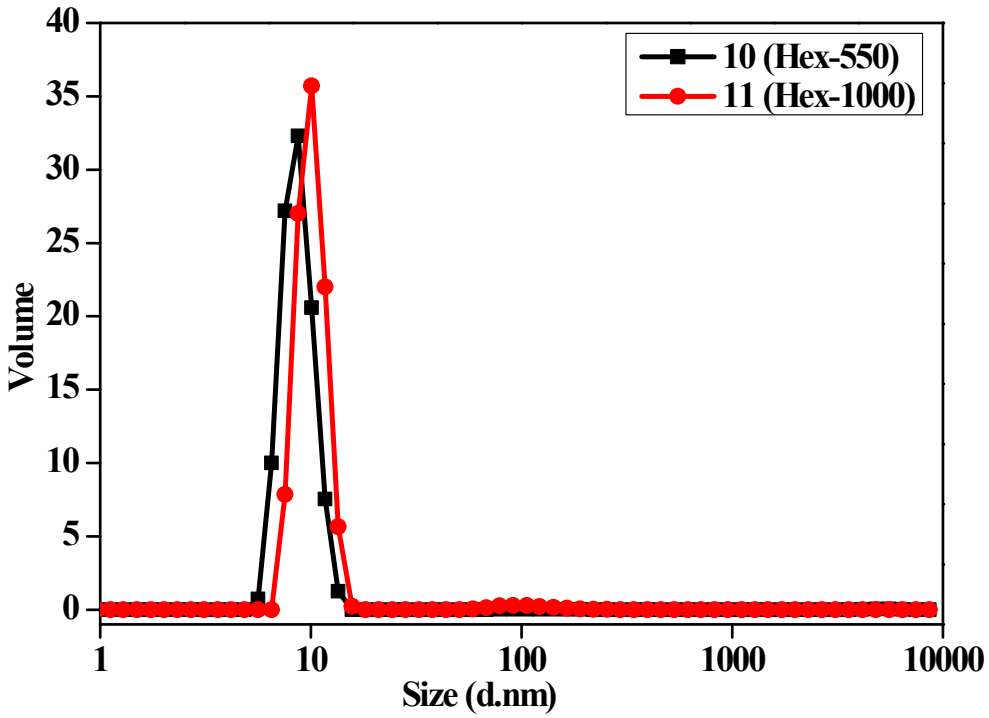


Fig. S8 DLS volume profile of the amphiphiles **10** and **11**.

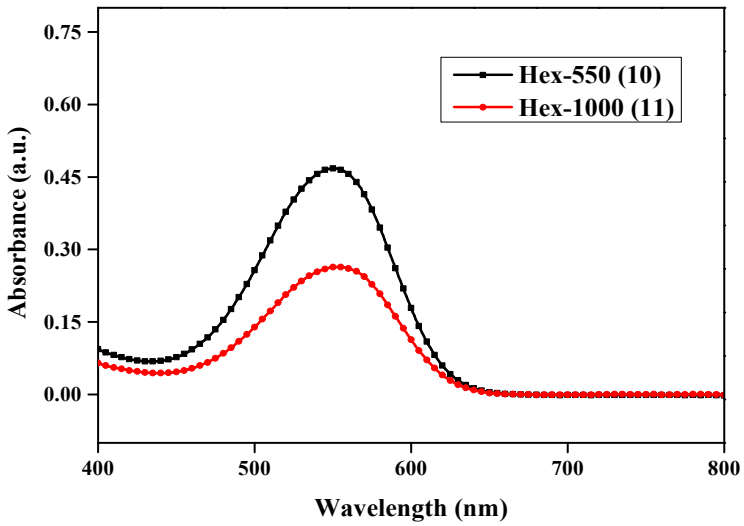


Fig. S9 UV absorbance quantification spectrum of Nile red loaded samples in methanol.

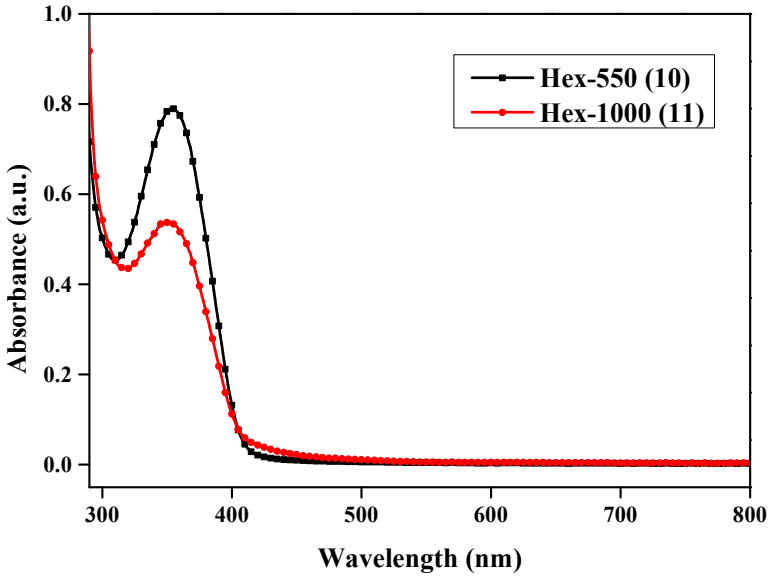


Fig. S10 UV absorbance quantification spectrum of nimodipine loaded samples in methanol.

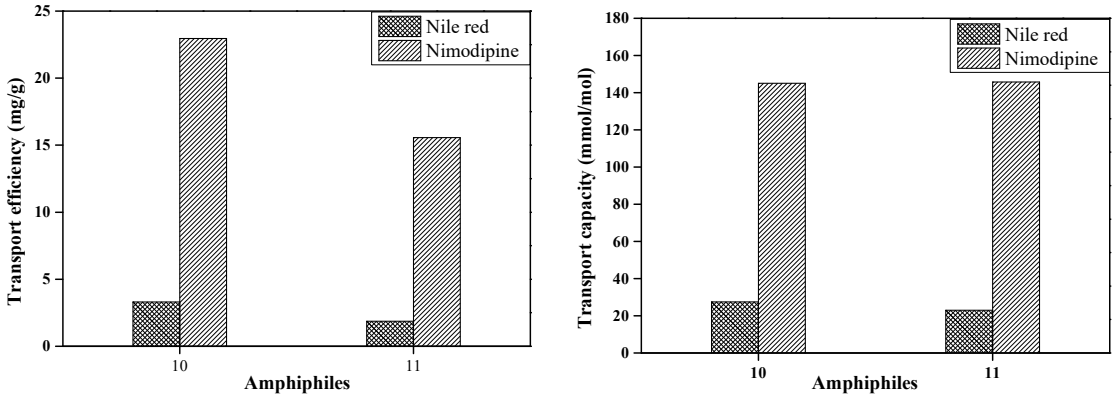


Fig. S11 (a) Transport efficiency and; (b) transport capacity of amphiphiles **10** and **11** for Nile red and nimodipine.

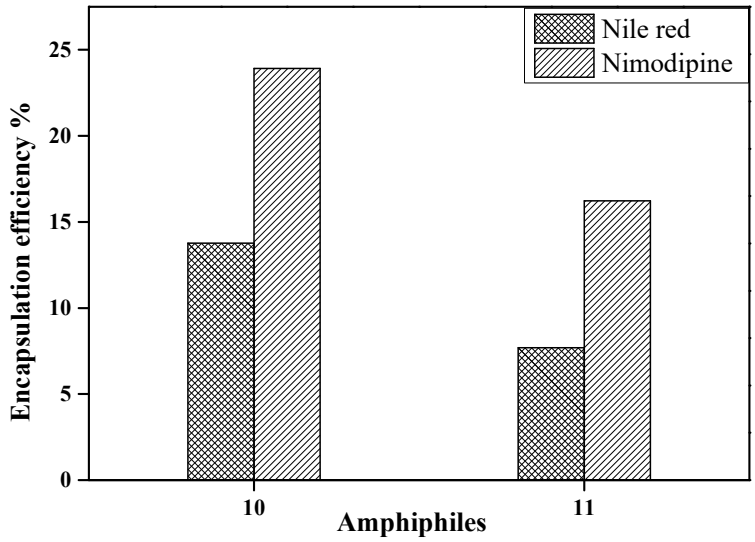


Fig. S12 Encapsulation efficiency of Nile red and nimodipine loaded amphiphile **10** and **11**.

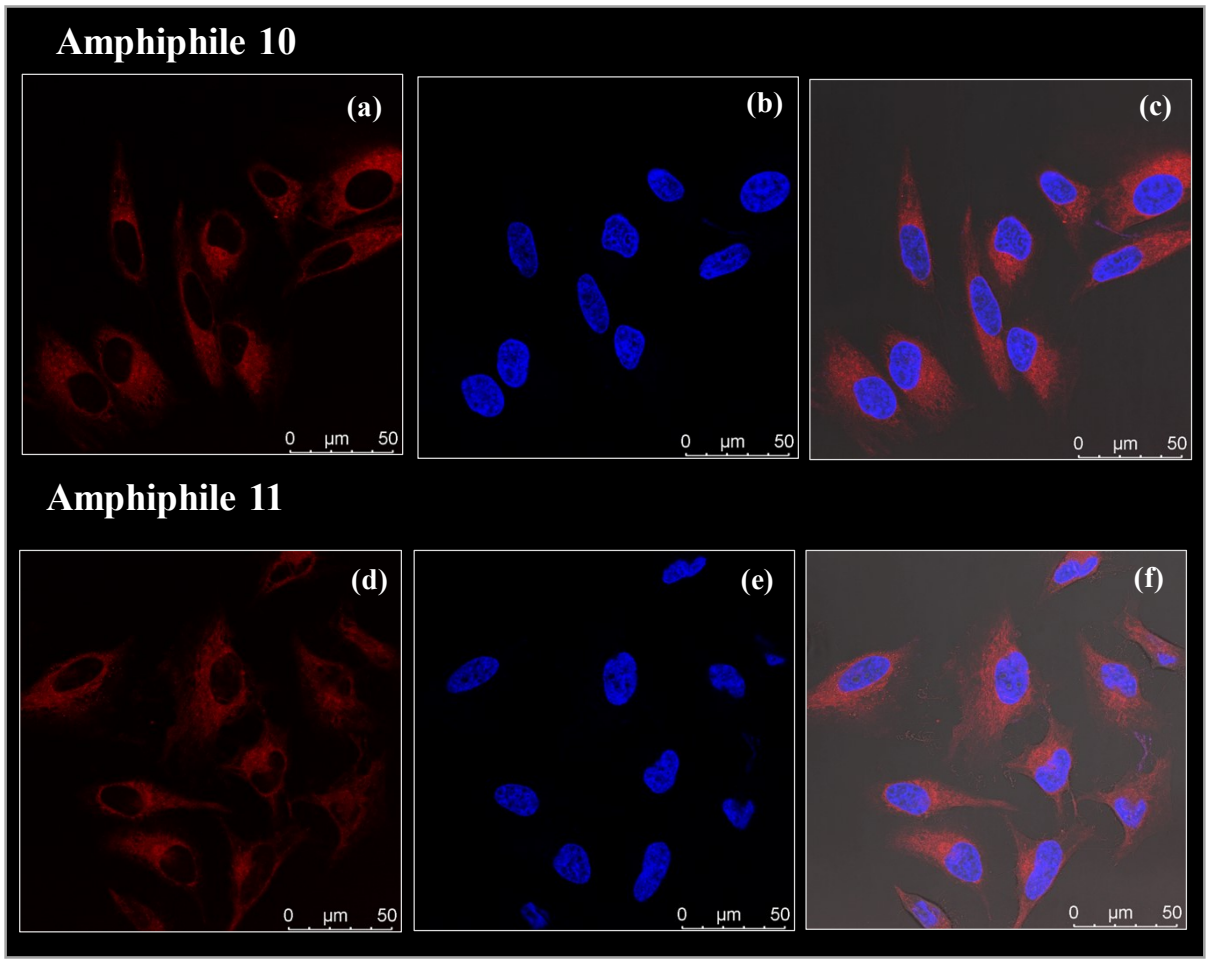


Fig. S13 cLSM images from HeLa cells after 24 h incubation of Nile red encapsulated amphiphiles **10** (a-c) and **11** (d-f). Nile red is shown in red colour and nucleus stained with Hoechst 33342 in blue colour. The scale is 50 µm.

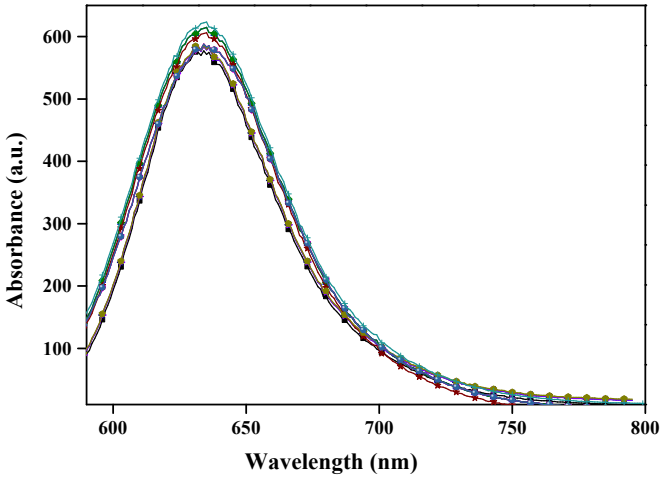


Fig. S14 Release profile of Nile red encapsulated amphiphile **11** under physiological conditions in the absence of enzyme.

3 References

1 T. Kusumaningsih, M. Firdaus, A. N. Artanti and W. E. Prasetyo, *In IOP Conference Series: Materials Science and Engineering*, 2019, **578**, 012057.