

Stereoselective synthesis and antitumor activity of macrodiolides containing 1Z,5Z-diene and 1,3-diyne moieties

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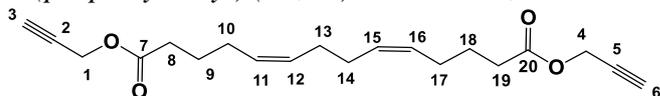
Experimental section

General methods

All reactions were carried out in an inert atmosphere. The ethereal and aromatic solvents were distilled from sodium metal. Triethylamine, pyridine were dried over NaOH. Commercial 2-propyn-1-ol, 3-butyne-1-ol, 4-pentyn-1-ol, 5-hexyn-1-ol, PEG 400, CuCl₂, Hf(OTf)₄ and Cp₂TiCl₂ (Aldrich) were used as purchased. (5Z,9Z)-Tetradeca-5,9-dienedioic acid **1** was prepared from hex-5-yn-1-ol by a reported procedure [V. A. D'yakonov *et al*, *Steroids*, 2015, **102**, 110]. Alkadiyne- α,ω -diols was prepared from 2-propyn-1-ol, 3-butyne-1-ol, 4-pentyn-1-ol, 5-hexyn-1-ol as described [L. Brandsma, *Synthesis of Acetylenes, Allenes, Cumulenes: Methods and Techniques*, Elsevier Academic Press: Bilthoven, the Netherlands, 2004, 470 pp]. One- (¹H, ¹³C) and two-dimensional heteronuclear (HSQC, HMBC) NMR spectra were recorded in CDCl₃ on Bruker Avance-400 [(400.13 MHz (¹H), 100.62 MHz (¹³C))] and Bruker Ascend-500 [(500 MHz (¹H), 125 MHz (¹³C))] instruments. IR spectra were recorded on Bruker VERTEX 70V using KBr discs over the range of 400–4000 cm⁻¹. Elemental analyses were measured on a 1106 Carlo Erba apparatus. Mass spectra were obtained on MALDI TOF/TOF spectrometer in a sinapic acid matrix.

Reaction of alkynols with (5Z,9Z)-tetradeca-5,9-dienedioic acid 1. To a mixture of alkynol (3.5 mmol), (5Z,9Z)-tetradeca-5,9-dienedioic acid **1** (1.0 mmol) and 4-dimethylaminopyridine (0.28 mmol) in dichloromethane (10 ml) was added a solution of dicyclohexylcarbodiimide (2.8 mmol) in dichloromethane (5 ml) under argon. The mixture was stirred at room temperature for 8 h. After removal of the precipitate by filtration, the solution was concentrated under vacuum. The crude product was purified by column chromatography (silica gel) using hexane/ethyl acetate as the elution solvent to afford α,ω -diynes **3**.

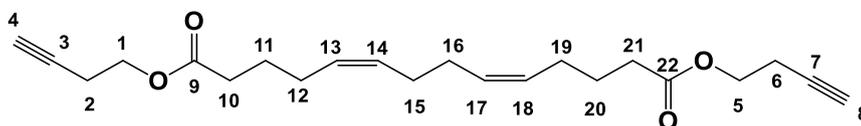
Di(prop-2-yn-1-yl) (5Z,9Z)-tetradeca-5,9-dienedioate (3a)



Yield: 80%, colourless oil. MS (MALDI-TOF), m/z : 330[M]⁺. Found (%): C, 72.57; H, 7.98. Calc. for C₂₀H₂₆O₄: (%): C, 72.70; H, 7.93. IR (ν / cm⁻¹): 3294, 2929(C≡H), 1735 (C=O), 1243, 1180 (C–O). ¹H NMR (400 MHz, CDCl₃) δ : 5.50 – 5.28 (4H, m, =CH, H¹¹, H¹², H¹⁵, H¹⁶),

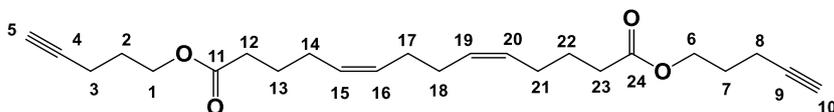
4.70 (4H, d, $J = 2.4$ Hz, O-CH₂, H¹, H⁴), 2.49 (2H, t, $J = 2.4$ Hz, ≡CH, H³, H⁶), 2.40 – 2.35 (4H, m, CH₂, H⁸, H¹⁹), 2.15 – 1.96 (8H, m, CH₂, H¹⁰, H¹³, H¹⁴, H¹⁷), 1.75 – 1.69 (4H, m, CH₂, H⁹, H¹⁸). ¹³C NMR (101 MHz, CDCl₃) δ: 172.8 (C=O, C⁷, C²⁰), 130.4 (CH, C¹¹, C¹⁶), 128.8 (CH, C¹², C¹⁵), 77.8 (C, C², C⁵), 74.8 (C, C³, C⁶), 51.8 (CH₂O, C¹, C⁴), 33.3 (CH₂, C⁸, C¹⁹), 27.2 (CH₂, C¹⁰, C¹⁷), 26.5 (CH₂, C¹³, C¹⁴), 24.7 (CH₂, C⁹, C¹⁸).

Di(but-3-yn-1-yl) (5Z,9Z)-tetradeca-5,9-dienedioate (3b)



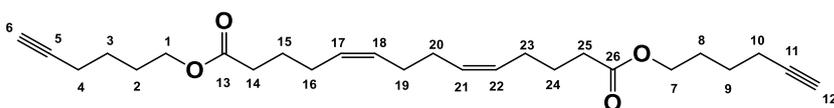
Yield: 81%, colourless oil. MS (MALDI-TOF), m/z : 358[M]⁺. Found (%): C, 73.66; H, 8.48. Calc. for C₂₂H₃₀O₄: (%): C, 73.71; H, 8.44. IR (ν / cm^{-1}): 3306, 2971 (C≡H), 1738 (C=O), 1237, 1168 (C–O). ¹H NMR (400 MHz, CDCl₃) δ: 5.45 – 5.35 (4H, m, =CH, H¹³, H¹⁴, H¹⁷, H¹⁸), 4.20 (4H, t, $J = 6.8$ Hz, O-CH₂, H¹, H⁵), 2.57 – 2.53 (4H, m, CH₂, H², H⁶), 2.39 – 2.32 (4H, m, CH₂, H¹⁰, H²¹), 2.16 – 1.96 (10H, m, CH₂, ≡CH, H⁴, H⁸, H¹², H¹⁵, H¹⁶, H¹⁹), 1.81 – 1.65 (4H, m, CH₂, H¹¹, H²⁰). ¹³C NMR (101 MHz, CDCl₃) δ: 173.4 (C=O, C⁹, C²²), 130.3 (CH, C¹³, C¹⁸), 128.9 (CH, C¹⁴, C¹⁷), 80.1 (C, C³, C⁷), 69.9 (C, C⁴, C⁸), 61.9 (CH₂O, C¹, C⁵), 33.5 (CH₂, C¹⁰, C²¹), 27.3 (CH₂, C¹², C¹⁹), 26.6 (CH₂, C¹⁵, C¹⁶), 24.8 (CH₂, C¹¹, C²⁰), 18.9 (CH₂, C², C⁶).

Di(pent-4-yn-1-yl) (5Z,9Z)-tetradeca-5,9-dienedioate (3c)



Yield: 80%, colourless oil. MS (MALDI-TOF), m/z : 386[M]⁺. Found (%): C, 74.49; H, 8.78. Calc. for C₂₄H₃₄O₄: (%): C, 74.58; H, 8.87. IR (ν / cm^{-1}): 3299, 2937 (C≡H), 1734 (C=O), 1233, 1167 (C–O). ¹H NMR (400 MHz, CDCl₃) δ: 5.48 – 5.31 (4H, m, =CH, H¹⁵, H¹⁶, H¹⁹, H²⁰), 4.19 (4H, t, $J = 6.4$ Hz, O-CH₂, H¹, H⁶), 2.40 – 2.27 (8H, m, CH₂, H³, H⁸, H¹², H²³), 2.16 – 1.96 (10H, m, CH₂, ≡CH, H⁵, H¹⁰, H¹⁴, H¹⁷, H¹⁸, H²¹), 1.92 – 1.84 (4H, m, CH₂, H², H⁷), 1.75 – 1.66 (4H, m, CH₂, H¹³, H²²). ¹³C NMR (101 MHz, CDCl₃) δ: 173.6 (C=O, C¹¹, C²⁴), 130.2 (CH, C¹⁵, C²⁰), 128.9 (CH, C¹⁶, C¹⁹), 83.0 (C, C⁴, C⁹), 69.0 (C, C⁵, C¹⁰), 62.8 (CH₂O, C¹, C⁶), 33.7 (CH₂, C¹², C²³), 27.6 (CH₂, C², C⁷), 27.3 (CH₂, C¹⁴, C²¹), 26.6 (CH₂, C¹⁷, C¹⁸), 24.8 (CH₂, C¹³, C²²), 15.2 (CH₂, C³, C⁸).

Di(hex-5-yn-1-yl) (5Z,9Z)-tetradeca-5,9-dienedioate (3d)



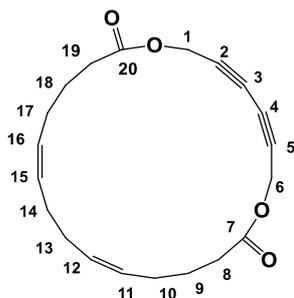
Yield: 82%, colourless oil. MS (MALDI-TOF), m/z : 414 [M]⁺. Found (%): C, 75.24; H, 9.29. Calc. for C₂₆H₃₈O₄: (%): C, 75.32; H, 9.24. IR (ν / cm^{-1}): 3310, 2951 (C≡H), 1730 (C=O), 1231, 1174 (C–O). ¹H NMR (400 MHz, CDCl₃) δ: 5.48 – 5.32 (4H, m, =CH, H¹⁷, H¹⁸, H²¹, H²²), 4.11 (4H, t, $J = 6.4$ Hz, O-CH₂, H¹, H⁷), 2.40 – 2.21 (8H, m,

$\underline{\text{C}}\underline{\text{H}}_2$, H^4 , H^{10} , H^{14} , H^{25}), 2.16 – 1.95 (10H, m, $\underline{\text{C}}\underline{\text{H}}_2$, $\equiv\text{CH}$, H^6 , H^{12} , H^{16} , H^{19} , H^{20} , H^{23}), 1.79 – 1.59 (12H, m, $\underline{\text{C}}\underline{\text{H}}_2$, H^2 , H^3 , H^8 , H^9 , H^{15} , H^{24}). ^{13}C NMR (101 MHz, CDCl_3) δ : 173.7 ($\underline{\text{C}}\underline{\text{O}}\underline{\text{O}}$, C^{13} , C^{26}), 130.2 (CH , C^{17} , C^{22}), 128.9 (CH , C^{18} , C^{21}), 83.8 (C , C^5 , C^{11}), 68.7 (C , C^6 , C^{12}), 63.7 ($\underline{\text{C}}\underline{\text{H}}_2\text{O}$, C^1 , C^7), 33.7 (CH_2 , C^{14} , C^{25}), 27.7 (CH_2 , C^2 , C^8), 27.3 (CH_2 , C^{16} , C^{23}), 26.6 (CH_2 , C^{19} , C^{20}), 25.0 (CH_2 , C^3 , C^9), 24.9 (CH_2 , C^{15} , C^{24}), 18.1 (CH_2 , C^4 , C^{10}).

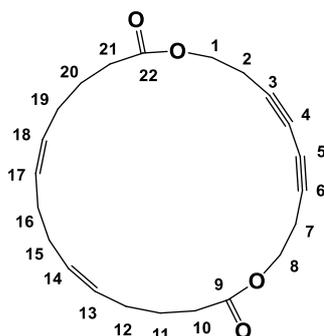
General procedure synthesis of macrodiolides. Method 1. (5Z,9Z)-Tetradeca-5,9-dienedioic acid **1** (51 mg, 0.2 mmol, 1.0 equiv.) and the corresponding diynediol (0.2 mmol, 1.0 equiv.) were dissolved in toluene (40 ml, 5 mM). Then $\text{Hf}(\text{OTf})_4$ (15 mg, 0.02 mmol, 0.1 equiv.) was added to the solution, and the mixture was heated to 110 °C. The mixture was stirred at this temperature for 16-18 h. After cooling to room temperature, silica gel (~ 1 ml) was added, and the slurry was concentrated under reduced pressure and purified by column chromatography (elution with light petroleum/EtOAc, 15:1) to afford the desired products as colourless oils.

Method 2. To a vial equipped with a stirring bar was added CuCl_2 (5.0 mg, 0.44 mmol, 25 mol.%) and $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (8.5 mg, 0.44 mmol, 25 mol.%). Polyethylene glycol 400 (3.05 ml), triethylamine (0.046 ml, 0.33 mmol, 3 equiv.) and pyridine (0.046 ml, 0.55 mmol, 5 equiv.) were added, and the mixture was stirred at room temperature for 15 min or until the salts were solubilized. A solution of diyne diester **3** (0.11 mmol) in MeOH (1.5 ml) was added to the homogenous mixture in one portion. Oxygen was bubbled through the solution for 5 min, and the vial was then closed with a screw cap. The vial was warmed to 60 °C, the consumption of the starting material was monitored by TLC (oxygen was bubbled again through the solution every 12 h). When the starting material was completely consumed (TLC), the vial was cooled to room temperature and the crude mixture was loaded directly on a silica column. Final elution with light petroleum/EtOAc (15:1) afforded the desired products as a colourless oils.

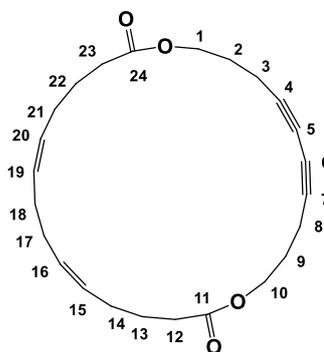
(13Z,17Z)-1,8-Dioxacyclodocosa-13,17-diene-3,5-diyne-9,22-dione (**2a**).



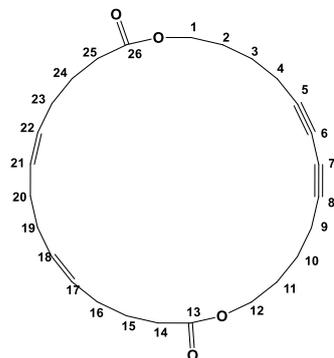
Yield (method 1/ method 2): 55% / 58%, colourless oil. MS (MALDI-TOF), m/z : 328 $[\text{M}]^+$. Found (%): C, 73.09; H, 7.29. Calc. for $\text{C}_{20}\text{H}_{24}\text{O}_4$: (%): C, 73.15; H, 7.37. IR (ν / cm^{-1}): 1737 ($\text{C}=\text{O}$), 1239, 1156 ($\text{C}-\text{O}$). ^1H NMR (500 MHz, CDCl_3) δ : 5.59 – 5.26 (4H, m, $=\underline{\text{C}}\underline{\text{H}}$, H^{11} , H^{12} , H^{15} , H^{16}), 4.76 (4H, s, $\text{O}-\underline{\text{C}}\underline{\text{H}}_2$, H^1 , H^6), 2.42 – 2.32 (4H, m, $\underline{\text{C}}\underline{\text{H}}_2$, H^8 , H^{19}), 2.21 – 1.96 (8H, m, H^{10} , H^{13} , H^{14} , H^{17}), 1.75 (4H, m, $\underline{\text{C}}\underline{\text{H}}_2$, H^9 , H^{18}). ^{13}C NMR (125 MHz, CDCl_3) δ : 172.6 ($\underline{\text{C}}\underline{\text{O}}\underline{\text{O}}$, C^7 , C^{20}), 130.8 (CH , C^{11} , C^{16}), 128.6 (CH , C^{12} , C^{15}), 76.8 (C , C^2 , C^5), 73.7 (C , C^3 , C^4), 51.6 ($\underline{\text{C}}\underline{\text{H}}_2\text{O}$, C^1 , C^6), 32.8 (CH_2 , C^8 , C^{19}), 27.1 (CH_2 , C^{10} , C^{17}), 26.0 (CH_2 , C^{13} , C^{14}), 24.5 (CH_2 , C^9 , C^{18}).

(15Z,19Z)-1,10-Dioxacyclotetracos-15,19-diene-4,6-diyne-11,24-dione (2b)

Yield (method 1/ method 2): 59% / 61%, colourless oil. MS (MALDI-TOF), m/z : 356 $[M]^+$. Found (%): C, 74.08; H, 7.98. Calc. for $C_{22}H_{28}O_4$: (%): C, 74.13; H, 7.92. IR (ν / cm^{-1}): 1740 (C=O), 1244, 1167 (C–O). ^1H NMR (400 MHz, CDCl_3) δ : 5.58 – 5.25 (4H, m, =CH, H^{13} , H^{14} , H^{17} , H^{18}), 4.19 (4H, t, $J = 5.4$ Hz, O-CH₂, H^1 , H^8), 2.62 (4H, t, $J = 5.4$ Hz, CH₂, H^2 , H^7), 2.37 (4H, t, $J = 7.3$ Hz, CH₂, H^{10} , H^{21}), 2.21 – 1.95 (8H, m, CH₂, H^{12} , H^{15} , H^{16} , H^{19}), 1.82 – 1.68 (4H, m, CH₂, H^{11} , H^{20}). ^{13}C NMR (101 MHz, CDCl_3) δ : 170.3 (COO, C^9 , C^{22}), 130.5 (CH, C^{13} , C^{18}), 128.9 (CH, C^{14} , C^{17}), 74.07 (C, C^3 , C^6), 66.4 (CH₂O, C^1 , C^8), 61.6 (C, C^4 , C^5), 33.5 (CH₂, C^{10} , C^{21}), 27.3 (CH₂, C^{12} , C^{19}), 26.4 (CH₂, C^{15} , C^{16}), 24.7 (CH₂, C^{11} , C^{20}), 19.9 (CH₂, C^2 , C^7).

(17Z,21Z)-1,12-Dioxacyclohexacos-17,21-diene-5,7-diyne-13,26-dione (2c)

Yield (method 1/ method 2): 64% / 74%, as a colorless oil. MS (MALDI-TOF), m/z : 384 $[M]^+$. Found (%): C, 74.84; H, 8.34. Calc. for $C_{24}H_{32}O_4$: (%): C, 74.97; H, 8.39. IR (ν / cm^{-1}): 1731 (C=O), 1239, 1177 (C–O). ^1H NMR (400 MHz, CDCl_3) δ : 5.54 – 5.26 (4H, m, =CH, H^{15} , H^{16} , H^{19} , H^{20}), 4.21 (4H, t, $J = 5.9$ Hz, O-CH₂, H^1 , H^{10}), 2.44 – 2.33 (8H, m, CH₂, H^3 , H^8 , H^{12} , H^{23}), 2.18 – 1.98 (8H, CH₂, H^{14} , H^{17} , H^{18} , H^{21}), 1.93 – 1.84 (4H, m, CH₂, H^2 , H^9), 1.78 – 1.68 (4H, m, CH₂, H^{13} , H^{22}). ^{13}C NMR (101 MHz, CDCl_3) δ : 173.7 (COO, C^{11} , C^{24}), 130.3 (CH, C^{15} , C^{20}), 128.9 (CH, C^{16} , C^{19}), 76.2 (C, C^4 , C^7), 65.9 (CH₂O, C^1 , C^{10}), 62.9 (C, C^5 , C^6), 33.5 (CH₂, C^{12} , C^{23}), 27.3 (CH₂, C^{14} , C^{21}), 27.1 (CH₂, C^2 , C^9), 26.5 (CH₂, C^{17} , C^{18}), 24.7 (CH₂, C^{13} , C^{22}), 16.4 (CH₂, C^3 , C^8).

(19Z,23Z)-1,14-Dioxacyclooctacos-19,23-diene-6,8-diyne-15,28-dione (2d)

Yield (method 1/ method 2): 62% / 79%, colourless oil. MS (MALDI-TOF), m/z : 412 $[M]^+$. Found (%): C, 75.60; H, 8.88. Calc. for $C_{26}H_{36}O_4$: (%): C, 75.69; H, 8.80. IR (ν / cm^{-1}): 1729 (C=O), 1236, 1178 (C–O). ^1H NMR (400 MHz, CDCl_3) δ : 5.55 – 5.30 (4H, m, =CH, H^{17} , H^{18} , H^{21} , H^{22}), 4.11 (4H, t, $J = 6.4$ Hz, O-CH₂, H^1 , H^{12}), 2.36 – 2.30 (8H, m, CH₂, H^4 , H^9 , H^{14} , H^{25}), 2.19 – 2.01 (8 H, CH₂, H^{16} , H^{19} , H^{20} , H^{23}), 1.82 – 1.54 (12H, m, CH₂, H^2 , H^3 , H^{10} , H^{11} , H^{15} , H^{24}). ^{13}C NMR (101 MHz, CDCl_3) δ : 173.7 (COO, C^{13} , C^{26}), 130.3 (CH, C^{17} , C^{22}), 129.0 (CH, C^{18} , C^{21}), 76.9 (C, C^5 , C^8), 65.8 (CH₂O, C^1 , C^{12}), 63.8 (C, C^6 , C^7), 33.8 (CH₂, C^{14} , C^{25}), 27.8 (CH₂, C^2 , C^{11}), 27.3 (CH₂, C^{16} , C^{23}), 26.6 (CH₂, C^{19} , C^{20}), 25.0 (CH₂, C^3 , C^{10}), 24.8 (CH₂, C^{15} , C^{24}), 19.0 (CH₂, C^4 , C^9).

Biology studies

Cell culturing. Cells (Jurkat, K562, U937, HL-60) were purchased from Russian Cell Culture Collection (Institute of Cytology of the Russian Academy of Sciences) and cultured according to standard mammalian tissue culture protocols and sterile technique. All cell lines used in the study were tested and shown to be free of mycoplasma and viral contamination. Cells were maintained in RPMI 1640 (Jurkat, K562, U937) (Gibco) supplemented with 4 mM glutamine, 10% FBS (Sigma) and 100 units per ml penicillin-streptomycin (Sigma). All types of cells were grown in an atmosphere of 5% CO_2 at 37 °C. The cells were subcultured at 2-3 days intervals. Cells were then seeded in 24 well plates at 5×10^4 cells per well and incubated overnight. Jurkat, K562, U937, HL-60 cells were subcultured at 2 day intervals with a seeding density of 1×10^5 cells per 24 well plates in RPMI with 10% FBS.

Cytotoxicity assay. Viability (live/dead) assessment was performed by staining cells with 7-AAD (7-Aminoactinomycin D) (Biolegend). After treatment the cells were harvested, washed 1-2 times with phosphate-buffered saline (PBS) and centrifuged at 400 g for 5 min. The cell pellets were resuspended in 200 μl of flow cytometry staining buffer (PBS without Ca^{2+} and Mg^{2+} , 2.5% FBS) and stained with 5 μl of 7-AAD staining solution for 15 min at room temperature in the dark. Samples were acquired on NovoCyte™ 2000 FlowCytometry System (ACEA) equipped

with 488 nm argon laser. Detection of 7-AAD emission was collected through a 675/30 nm filter in FL4 channel.

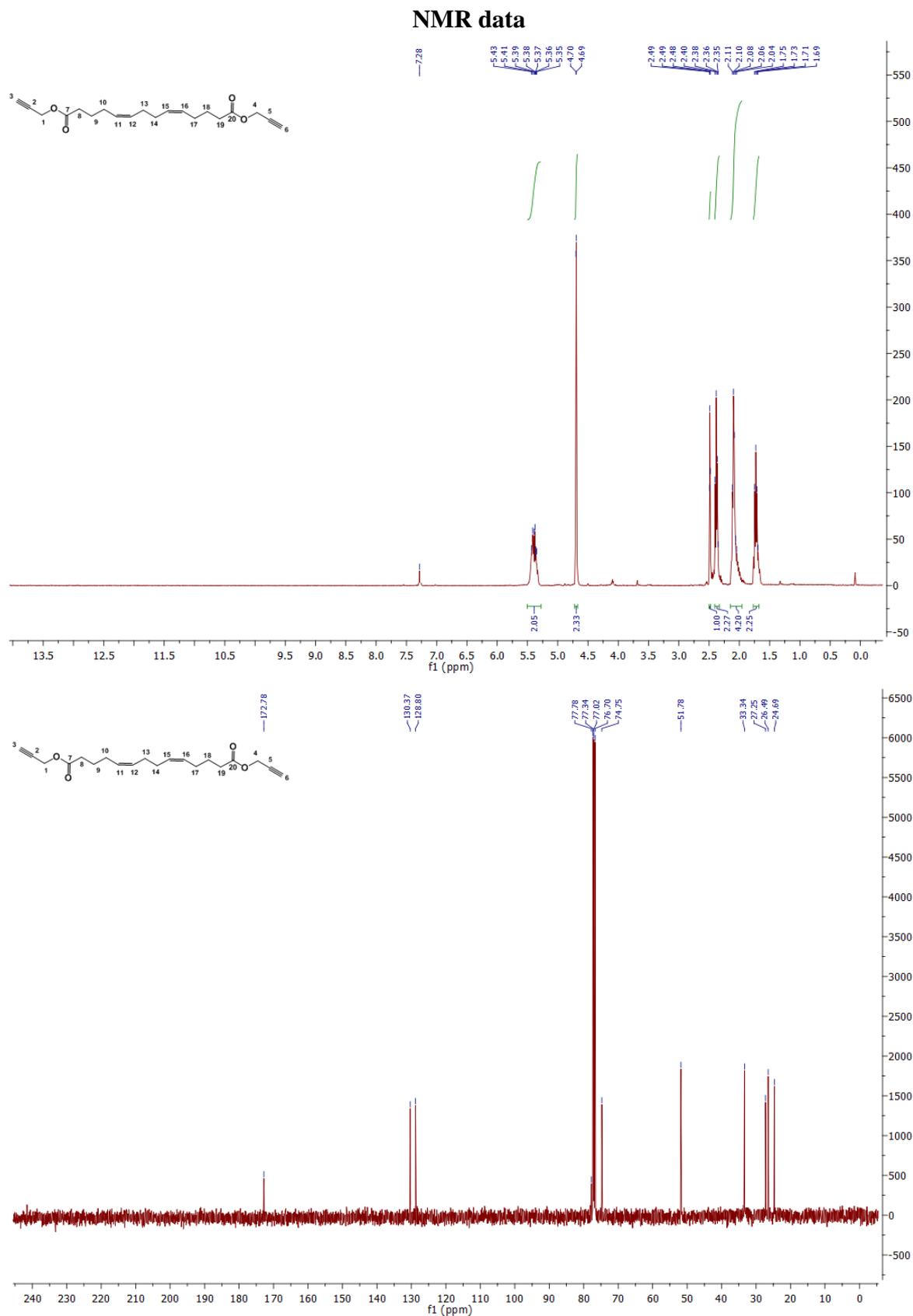


Figure S1. ¹H and ¹³C NMR spectra of compound **3a** in CDCl₃.

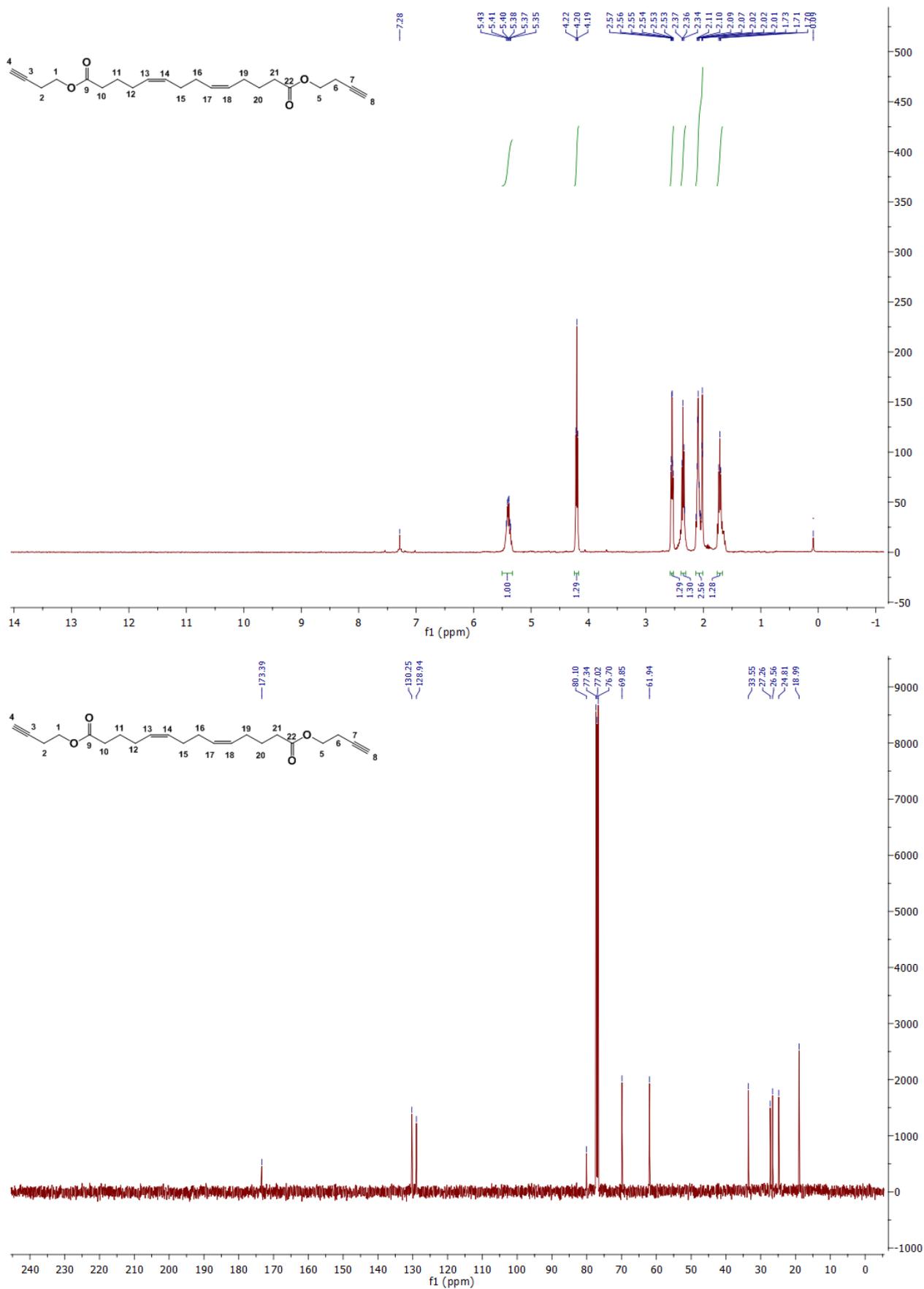


Figure S2. ^1H and ^{13}C NMR spectra of compound **3b** in CDCl_3 .

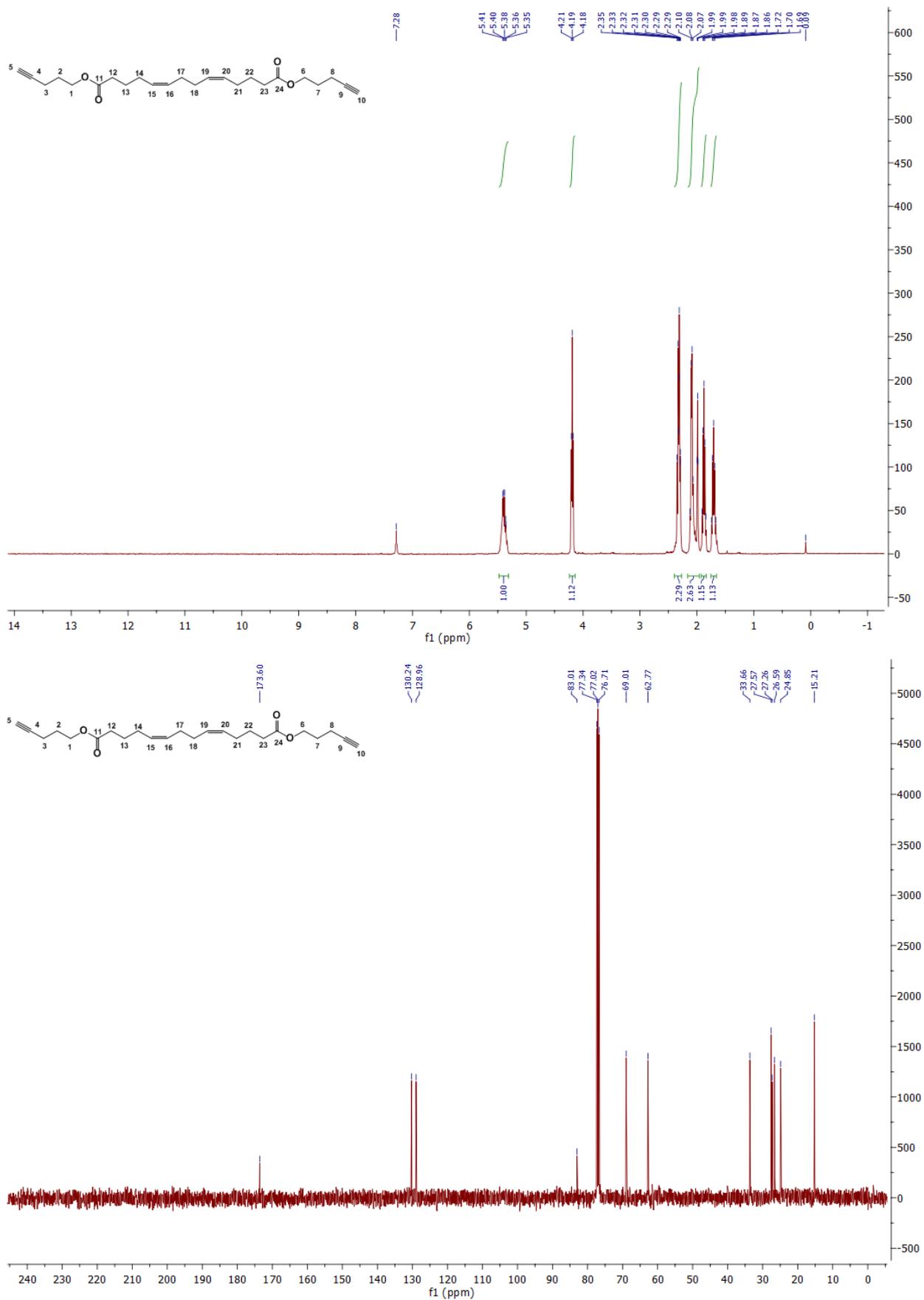


Figure S3. ^1H and ^{13}C NMR spectra of compound **3c** in CDCl_3 .

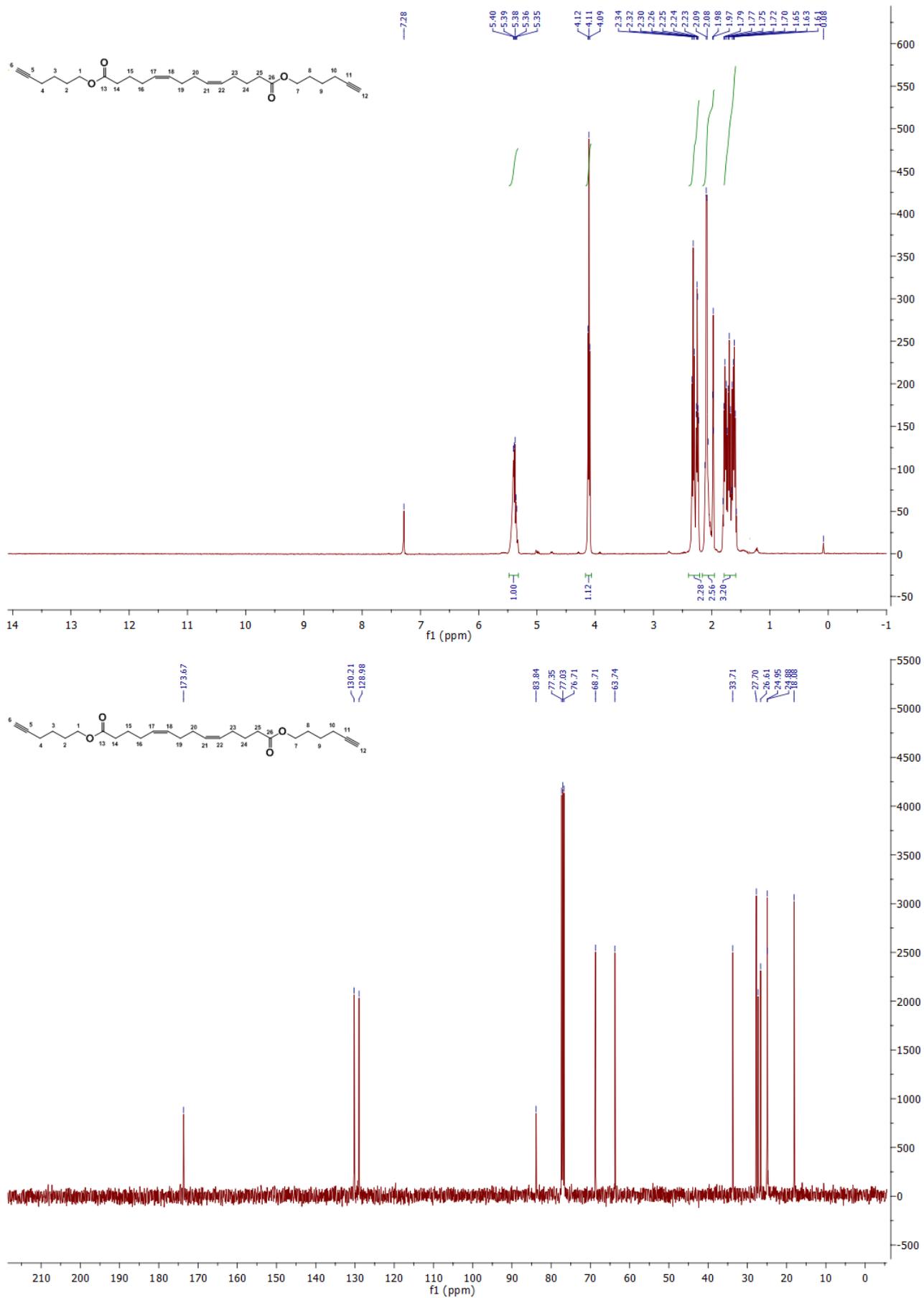


Figure S4. ^1H and ^{13}C NMR spectra of compound **3d** in CDCl_3 .

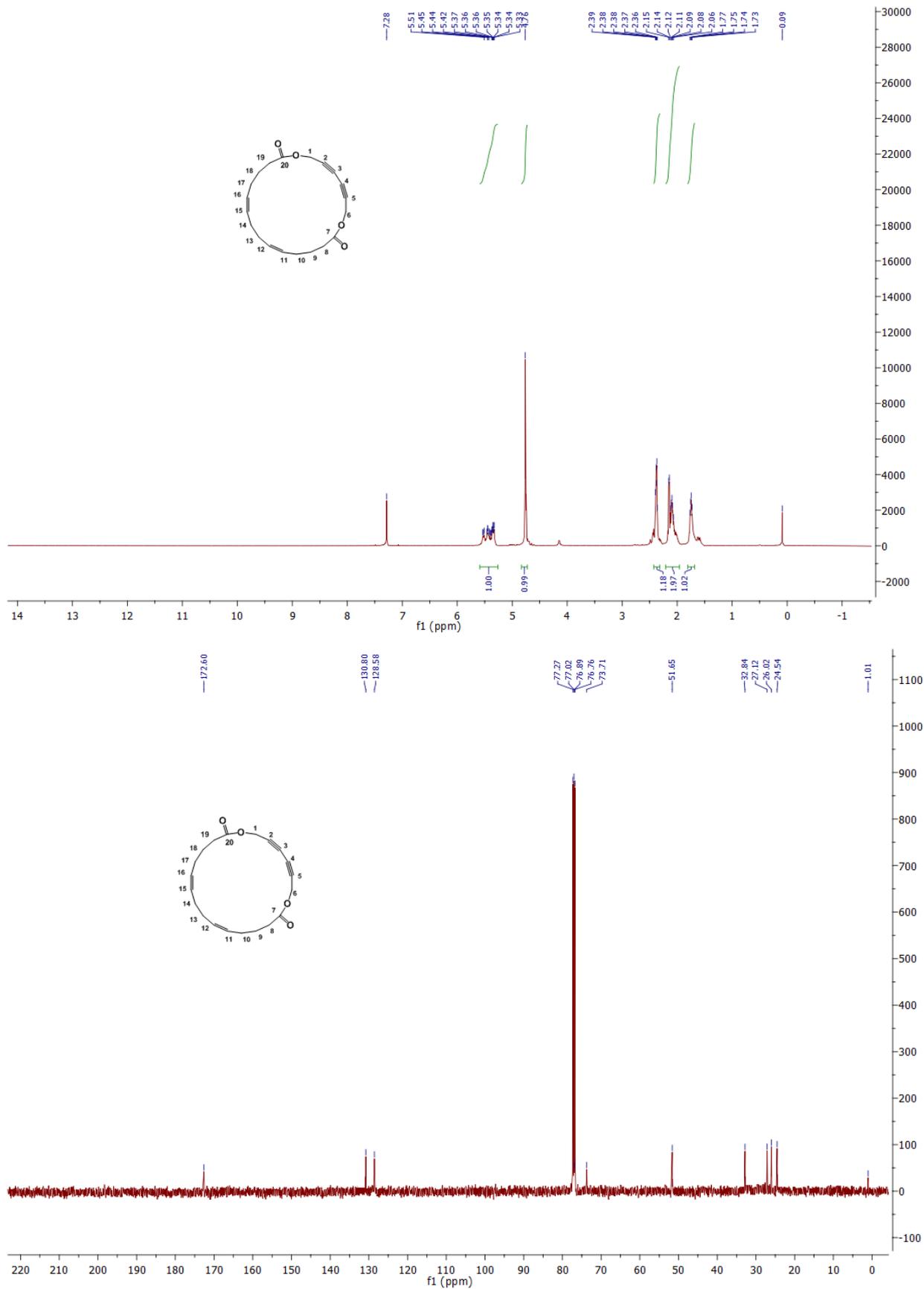


Figure S5. ¹H and ¹³C NMR spectra of compound 2a in CDCl₃.

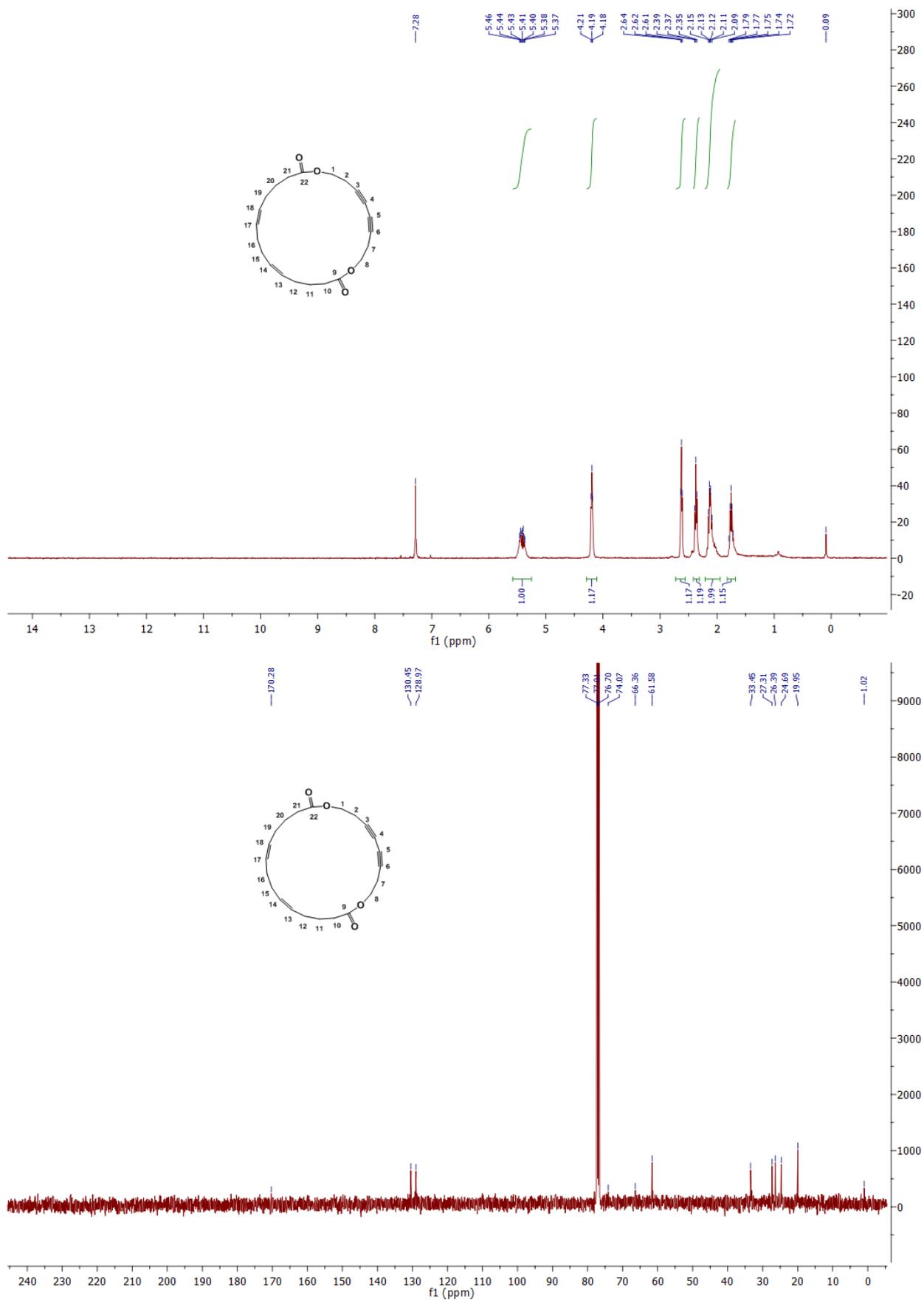


Figure S6. ¹H and ¹³C NMR spectra of compound **2b** in CDCl₃.

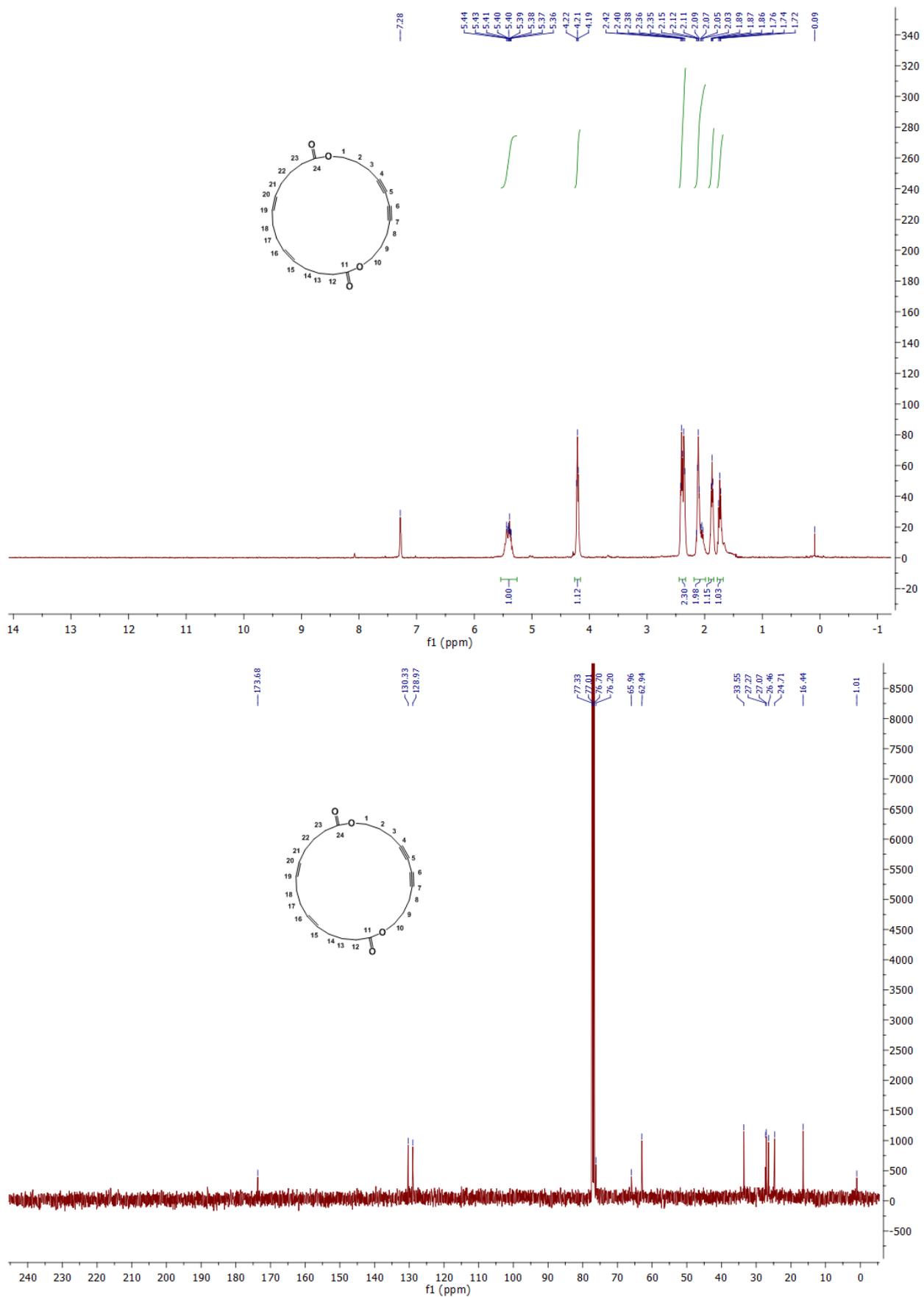


Figure S7. ¹H and ¹³C NMR spectra of compound **2c** in CDCl₃.

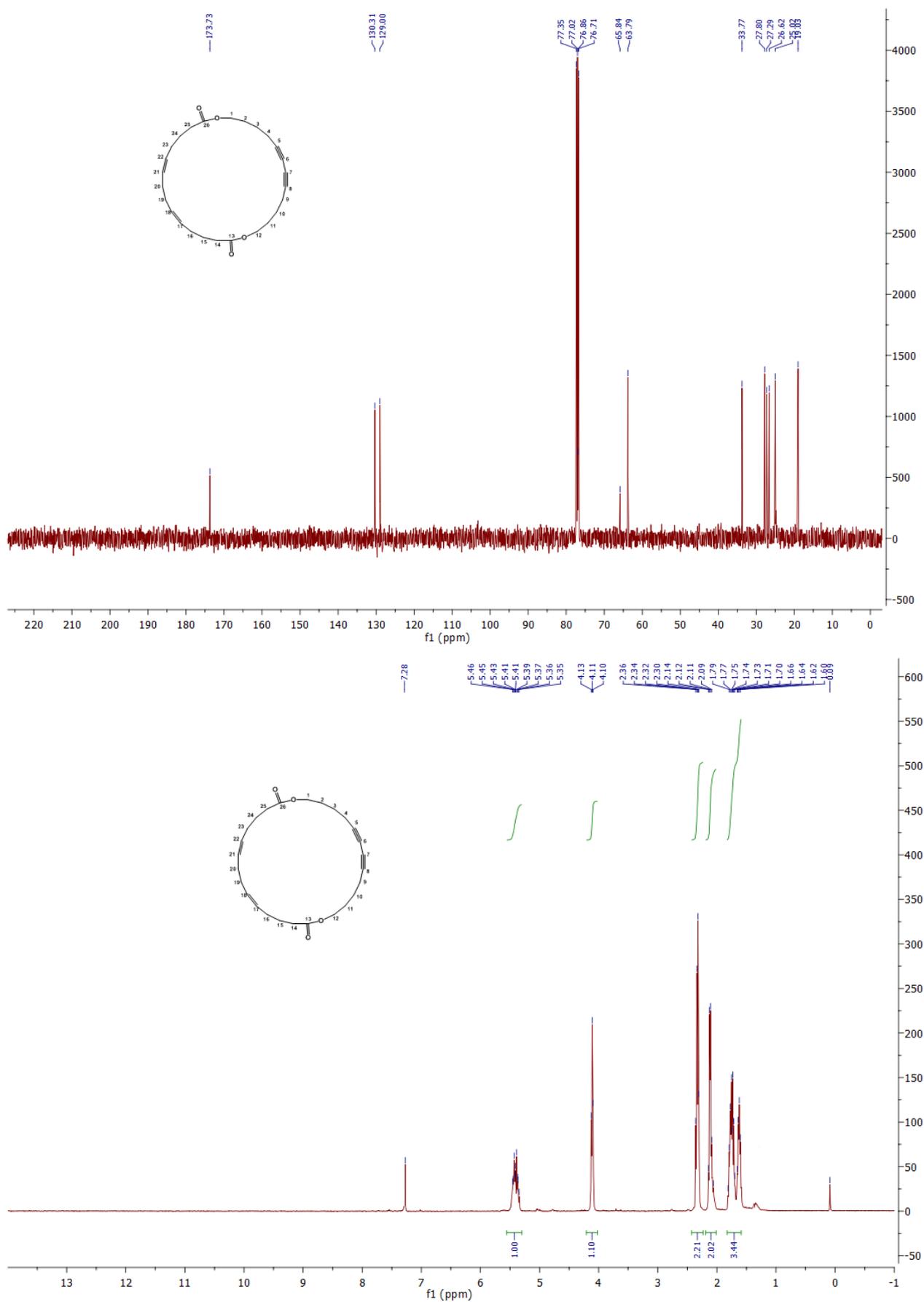


Figure S8. ¹H and ¹³C NMR spectra of compound **2d** in CDCl₃.