

**Direct synthesis and cytotoxic activity of new macrodiolides
with 2,5-disubstituted tetrahydrofuran moieties**

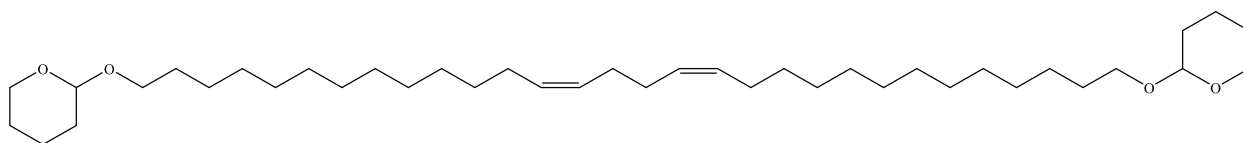
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Experimental section

IR spectra were recorded on a SILAB i-Red 7800u-L FTIR spectrometer using a QATR-S single reflection ATR accessory with diamond crystal, with 0.85 cm^{-1} resolution in the $400\text{--}4000\text{ cm}^{-1}$ range. NMR spectra were recorded in CDCl_3 on Bruker Ascend-500 ((500 MHz (^1H), 126 MHz (^{13}C)) instruments. The mass spectra were obtained on an UltraFlex III TOF/TOF (Bruker Daltonik GmbH, Bremen, Germany) operating in linear (TOF) and reflection (TOF/TOF) positive and negative ion modes. All solvents were dried and freshly distilled before use. All reactions were carried out under a dry argon atmosphere. Commercially available alkynols (TCI), Cp_2TiCl_2 , $\text{RuCl}_3\cdot\text{H}_2\text{O}$ and $\text{Hf}(\text{OTf})_4$ (Aldrich) were used.

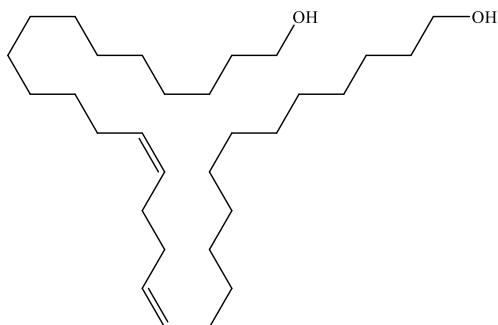
Compounds **3**, **4**, **6** synthesized according to procedures described in literature.^{S1}

(13Z,17Z)-1,30-Bis((tetrahydro-2H-pyran-2-yl)oxy)triaconta-13,17-diene (3)



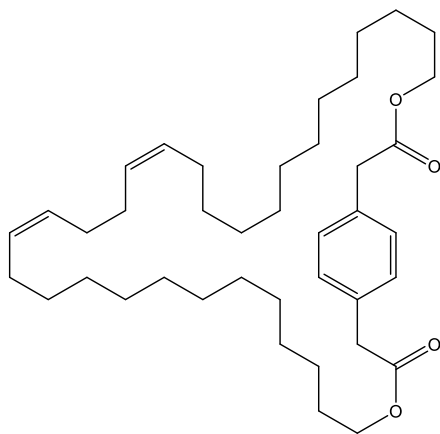
Yield: 86%, yellow oil. IR (ν / cm^{-1}): 647, 733, 908, 981, 1030, 1078, 1136, 1323, 1440, 2245, 2871, 2948. ^1H NMR (500 MHz, CDCl_3) δ : 5.44 – 5.31 (m, 4H), 4.59 – 4.56 (m, 2H), 3.89 – 3.71 (m, 4H), 3.51 – 3.36 (m, 4H), 2.08 – 1.82 (m, 8H), 1.73 – 1.53 (m, 16H), 1.32 – 1.19 (m, 36H). ^{13}C NMR (126 MHz, CDCl_3) δ : 130.34, 129.12, 98.79, 67.65, 62.26, 30.77, 29.75, 29.64, 29.60, 29.49, 29.31, 27.41, 27.25, 26.24, 25.51, 19.67. HRMS (ESI-TOF): found m/z 641.5521 [$\text{M} + \text{Na}$] $^+$; calcd. for $\text{C}_{40}\text{H}_{74}\text{O}_4\text{Na}^+$ 641.5479.

(13Z,17Z)-Triaconta-13,17-diene-1,30-diol (4).



Yield: 88%, yellow oil. IR (ν / cm^{-1}): 661, 719, 806, 981, 1051, 1168, 1369, 1457, 1706, 2854, 2925, 3004, 3317, 3374. ^1H NMR (500 MHz, CDCl_3) δ : 5.39 – 5.27 (m, 4H), 3.55 (t, J = 6.7 Hz, 4H), 2.08 – 1.89 (m, 8H), 1.55 – 1.45 (m, 8H), 1.32– 1.19 (m, 34H). ^{13}C NMR (126 MHz, CDCl_3) δ : 130.38, 129.16, 63.10, 32.81, 29.75, 29.65, 29.62, 29.57, 29.45, 29.33, 27.43, 27.27, 25.75. HRMS (ESI-TOF): found m/z 451.4509 $[\text{M} + \text{H}]^+$; calcd. for $\text{C}_{30}\text{H}_{59}\text{O}_2^+$ 451.4443.

(17Z,21Z)-4,35-Dioxa-1(1,4)-benzenacycloheptatriacontaphane-17,21-diene-3,36-dione (**6**).

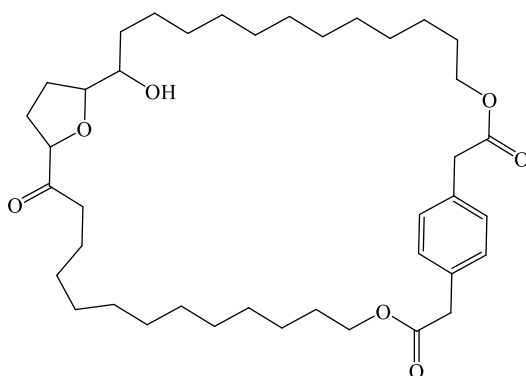


Yield: 76%, colorless oil. IR (ν / cm^{-1}): 664, 678, 711, 852, 995, 1132, 1172, 1324, 1408, 1454, 1709, 2944, 2979, 3015, 3028. ^1H NMR (500 MHz, CDCl_3) δ : 7.28 – 7.26 (m, 4H), 5.50 – 5.30 (m, 4H), 4.10 (t, J = 6.4 Hz, 4H), 3.61 (s, 4H), 2.34 – 1.88 (m, 8H), 1.66 – 1.59 (m, 4H), 1.36 – 1.28 (m, 36H). ^{13}C NMR (126 MHz, CDCl_3) δ : 171.53, 133.02, 130.37, 129.43, 129.35, 64.95, 41.36, 29.68, 29.57, 29.53, 29.46, 29.38, 29.18, 29.06, 28.62, 28.47, 28.21, 28.15, 27.56, 27.14, 25.77. HRMS (ESI-TOF): found m/z 609.4838 $[\text{M} + \text{H}]^+$; calcd. for $\text{C}_{40}\text{H}_{65}\text{O}_4^+$ 609.4877.

Oxidative cyclization of macrocyclic diene 6.

Water (0.55 mL) was poured into a glass reactor and heated to 70 °C, NaIO_4 (0.24 g, 1.1 mmol) was added, and the mixture was stirred for 5 min. Silica gel (0.92 g) was added to the mixture, and the reactor was gently shaken until a fine, homogeneous sodium periodate powder was formed. Then, THF (9 mL), CH_2Cl_2 (1 mL), and 1Z,5Z-diene **6** (0.5 mmol) were added to the reactor, and the mixture was cooled to 0 °C. A 0.1 M solution of $\text{RuCl}_3 \cdot \text{H}_2\text{O}$ in H_2O (0.01 mL, 1 μmol) was added dropwise to the suspension, and the reaction mixture was stirred at 0 °C until the reaction was completed (TLC monitoring, a 100% conversion of **6**). The reaction was stopped by adding an excess of i-PrOH (0.1 mL), the mixture was stirred for another 10 min, filtered off, and washed with EtOAc. The solvent was removed from the filtrate under reduced pressure (5–9 Torr). Product **7** was isolated by flash chromatography (15-20% EtOAc in hexanes).

34-Hydroxy-12,13,14,15-tetrahydro-15,21-dioxa-1(2,5)-furana-18(1,4)-benzenacyclotetratriacontaphane-2,16,20-trione (7).

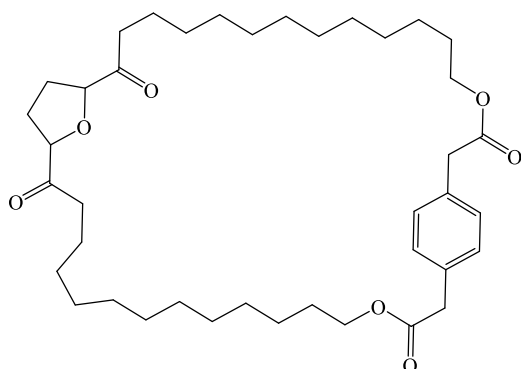


Yield: 67%, white waxy solid. IR (ν / cm^{-1}): 684, 709, 817, 875, 918, 956, 1008, 1025, 1064, 1122, 1172, 1278, 1311, 1398, 1469, 1710, 1782, 2360, 2848, 2915, 2956, 3432. ^1H NMR (500 MHz, CDCl_3) δ : 7.28 – 7.26 (m, 4H), 4.53 – 4.48 (m, 1H), 4.11 – 4.02 (m, 4H), 3.98 – 3.92 (m, 2H), 3.74 – 3.36 (m, 4H), 2.59 – 1.82 (m, 6H), 1.68 – 1.55 (m, 6H), 1.44 – 1.27 (m, 36H). ^{13}C NMR (126 MHz, CDCl_3) δ : 212.78, 171.63, 133.00, 129.41, 84.82, 82.59, 71.45, 65.06, 41.26, 41.15, 38.32, 32.76, 30.36, 29.69, 29.45, 29.36, 29.29, 29.22, 29.14, 29.07, 29.01, 28.94, 28.47, 25.73, 23.21, 23.13. HRMS (ESI-TOF): found m/z 657.4757 $[\text{M} + \text{H}]^+$; calcd. for $\text{C}_{40}\text{H}_{65}\text{O}_7^+$ 657.4725.

Oxidation of ketol 7 with the Dess—Martin periodinane.

Solid NaHCO_3 (0.204 g, 0.24 mmol) and the Dess—Martin periodinane (0.15 g, 0.36 mmol) were added to a solution of ketol 7 (0.24 mmol) in CH_2Cl_2 (2.4 mL) in a glass reactor at room temperature, and the mixture was stirred for 1 h. The mixture was diluted with CH_2Cl_2 (3.6 mL), neutralized with a 10% solution of $\text{Na}_2\text{S}_2\text{O}_3$ (3.6 mL) and a saturated NaHCO_3 solution (2.4 mL), and vigorously stirred for 30 min. Products were extracted with CH_2Cl_2 (5.2 mL), the organic phases were combined and washed with a 10% $\text{Na}_2\text{S}_2\text{O}_3$ solution (2 $\times$ 2.4 mL) and a saturated NaHCO_3 solution (2.4 mL). The organic phase was dried over anhydrous MgSO_4 , the solvent was evaporated. The residue was purified by flash chromatography (5-10% EtOAc in hexanes).

12,13,14,15-Tetrahydro-15,21-dioxo-1(2,5)-furana-18(1,4)-benzenacyclotetratetriacontaphane-2,16,20,34-tetraone (**8**).



Yield: 92%, light yellow waxy solid. IR (ν / cm^{-1}): 678, 709, 756, 800, 918, 954, 1024, 1068, 1106, 1178, 1203, 1272, 1311, 1405, 1429, 1470, 1712, 1766, 2850, 2917. ^1H NMR (500 MHz, CDCl_3) δ : 7.28 – 7.26 (m, 4H), 4.48 – 4.38 (m, 2H), 4.09 (s, 4H), 3.61 (s, 4H), 2.71 – 2.35 (m, 4H), 2.21 – 1.86 (m, 4H), 1.71 – 1.59 (m, 8H), 1.44 – 1.27 (m, 32H). ^{13}C NMR (126 MHz, CDCl_3) δ : 211.04, 171.54, 133.02, 129.33, 84.71, 64.94, 41.38, 38.68, 29.69, 29.65, 29.48, 29.43, 29.35, 29.25, 29.04, 28.54, 28.44, 28.40, 25.74, 23.06. HRMS (ESI-TOF): found m/z 677.4291 $[\text{M} + \text{Na}]^+$; calcd. for $\text{C}_{40}\text{H}_{62}\text{O}_7\text{Na}^+$ 677.4388.

Biological studies

Cell Culturing

Human cancer cell lines Jurkat, A549, and HEK293 were obtained from the European Authentic Cell Culture Collection and further cultured according to established standard protocols and using sterile methods. The cells were cultivated in RPMI 1640 (for Jurkat) and DMEM (for A549 and HEK293) media (Gibco), with the addition of 4 μM glutamine, 10% FBS (Sigma), and 100 U/mL penicillin-streptomycin (Sigma). All cell types were cultivated in a humidified atmosphere containing 5% CO_2 at a temperature of 37 $^\circ\text{C}$. Subculturing was performed at two- to three-day intervals. The cells were subsequently seeded into 24-well plates at a density of 5×10^4 cells per well and incubated overnight. Subsequent subculturing was then performed at two-day intervals, with a seeding density of 1×10^5 cells per 24-well plate, using RPMI medium with 10% FBS. Cytotoxicity analysis was performed using flow cytometry according to the procedure described in the literature.^{S2}

NMR data

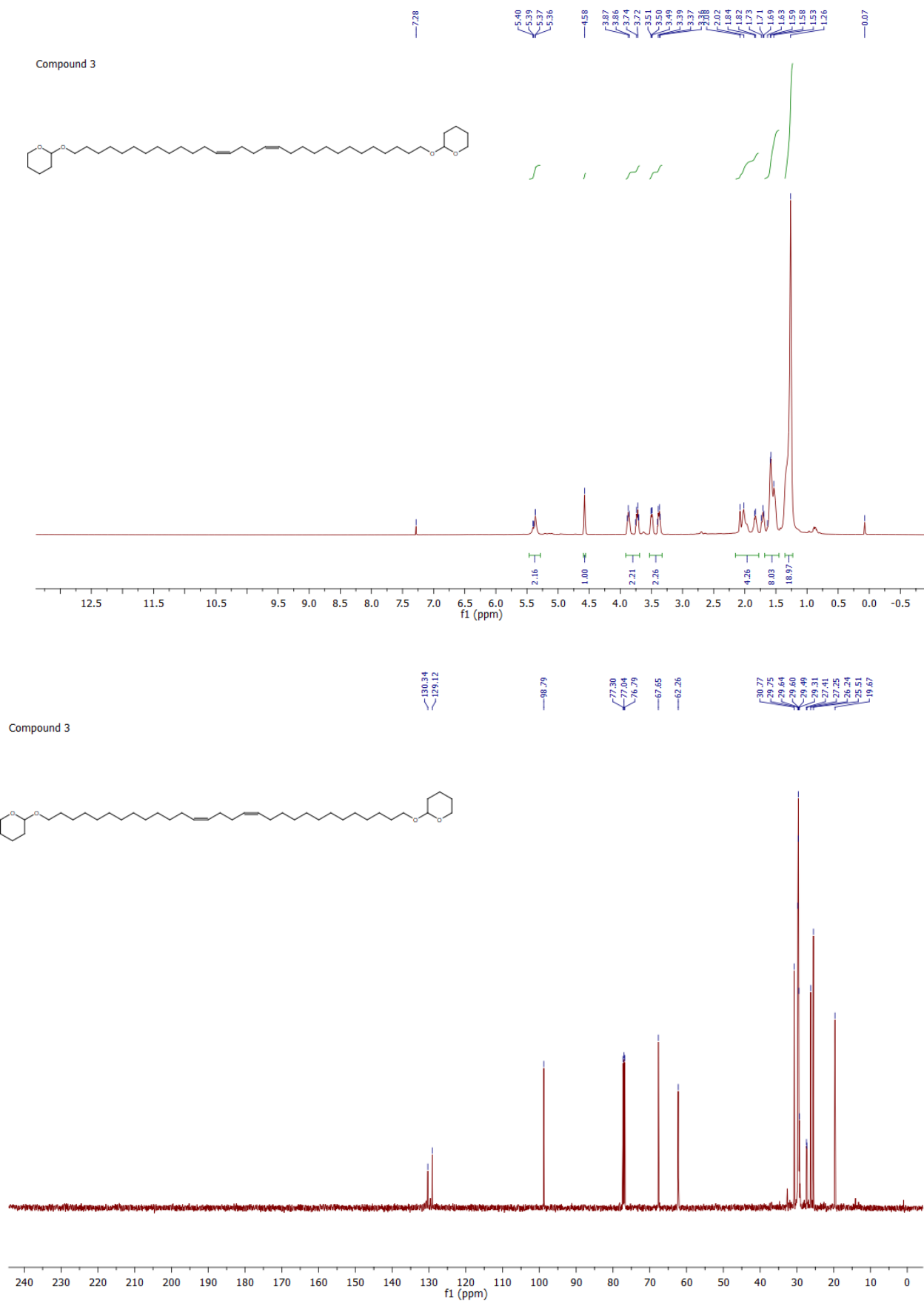


Figure S1. ^1H and ^{13}C NMR spectra of compound **3** in CDCl_3 .

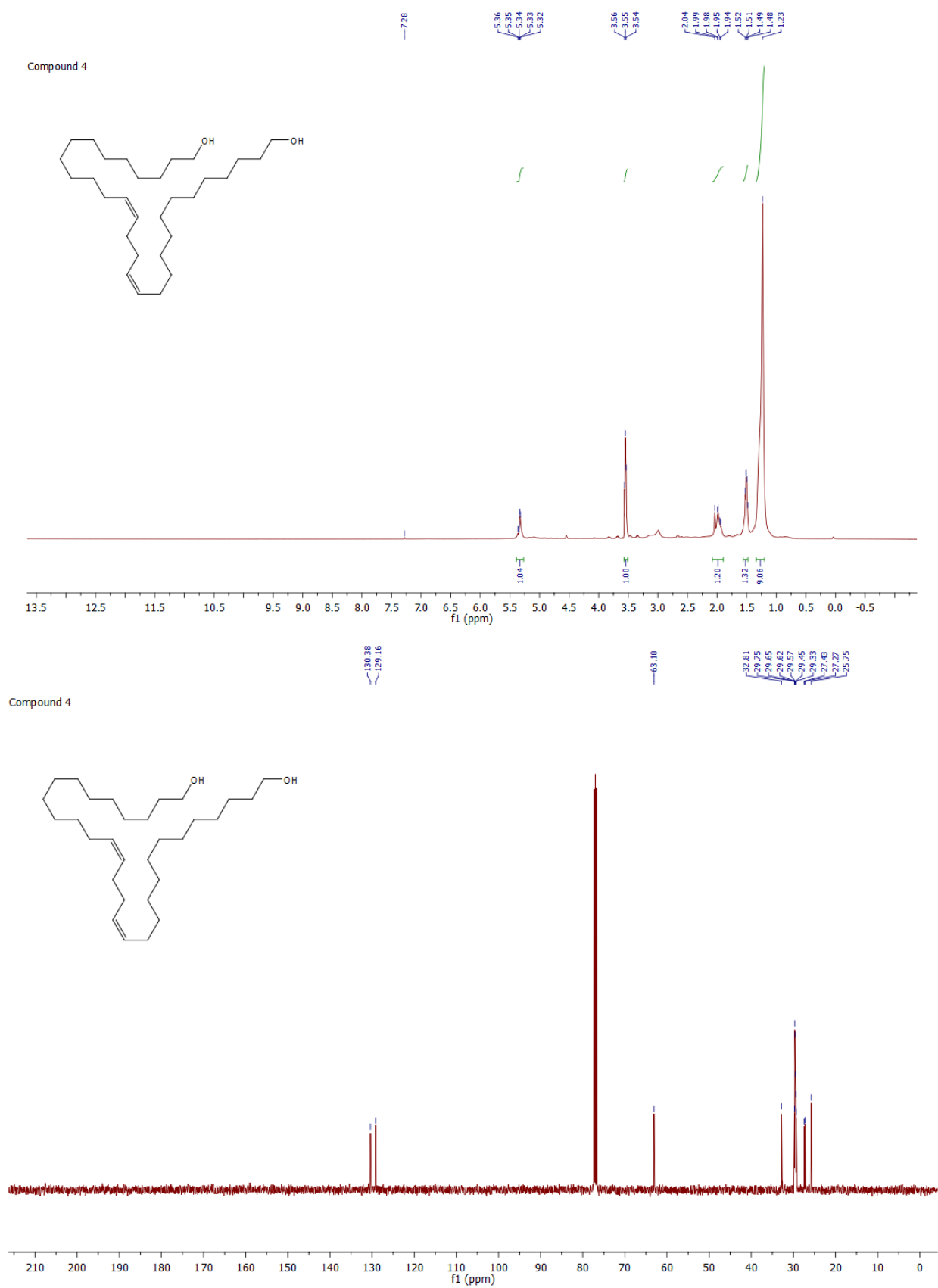


Figure S2. ^1H and ^{13}C NMR spectra of compound 4 in CDCl_3 .

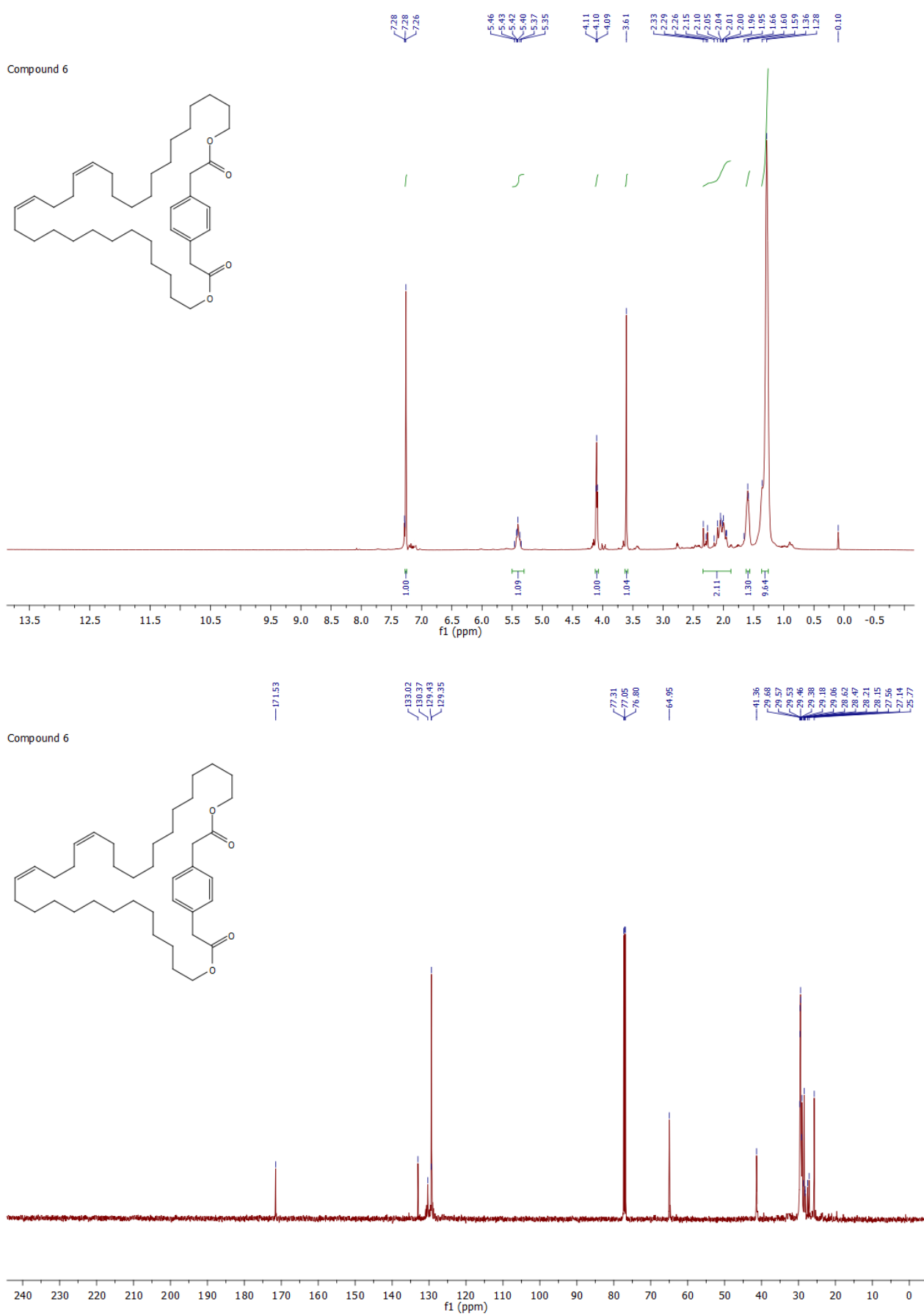


Figure S3. ^1H and ^{13}C NMR spectra of compound **6** in CDCl_3 .

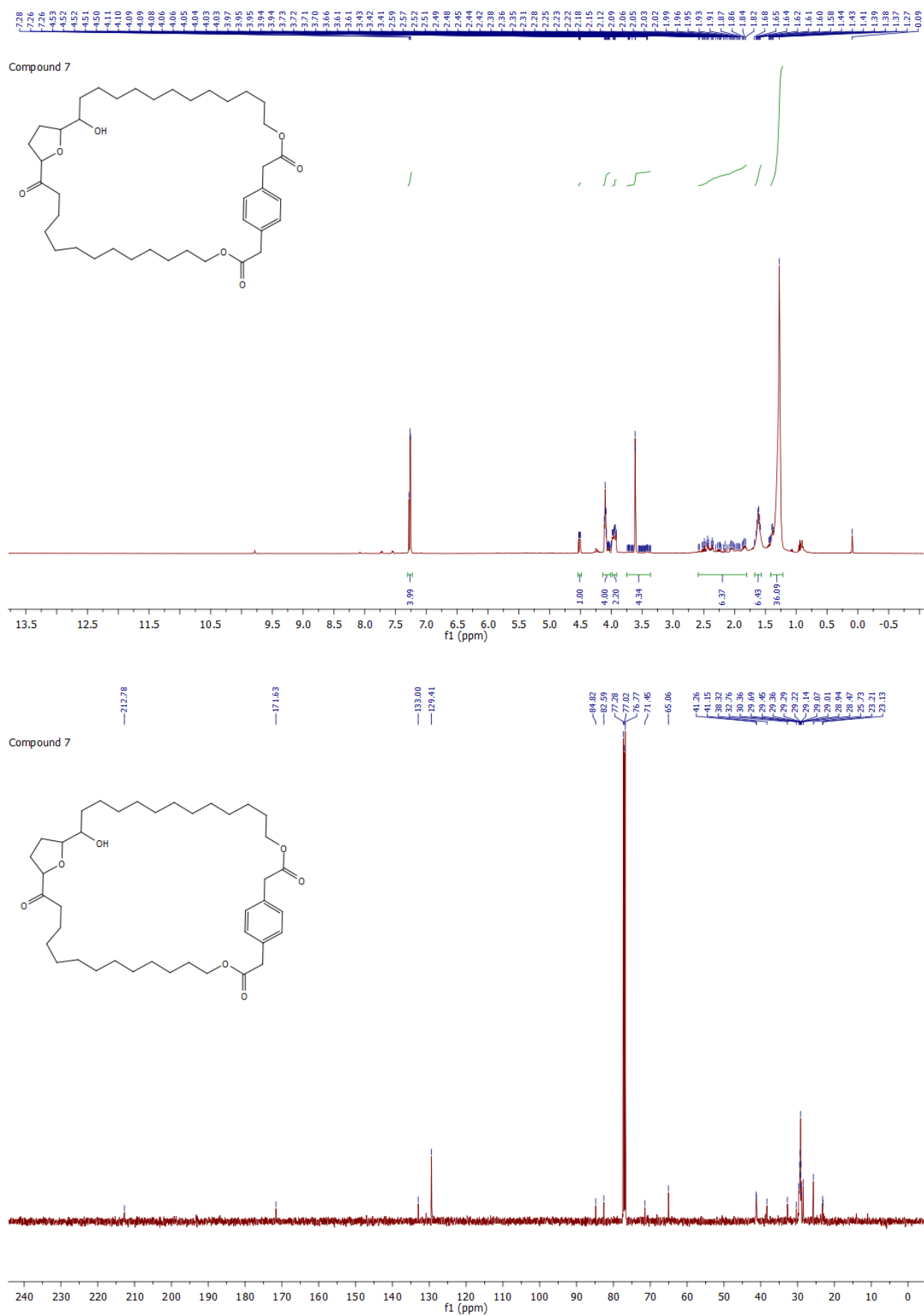


Figure S4. ¹H and ¹³C NMR spectra of compound 7 in CDCl₃.

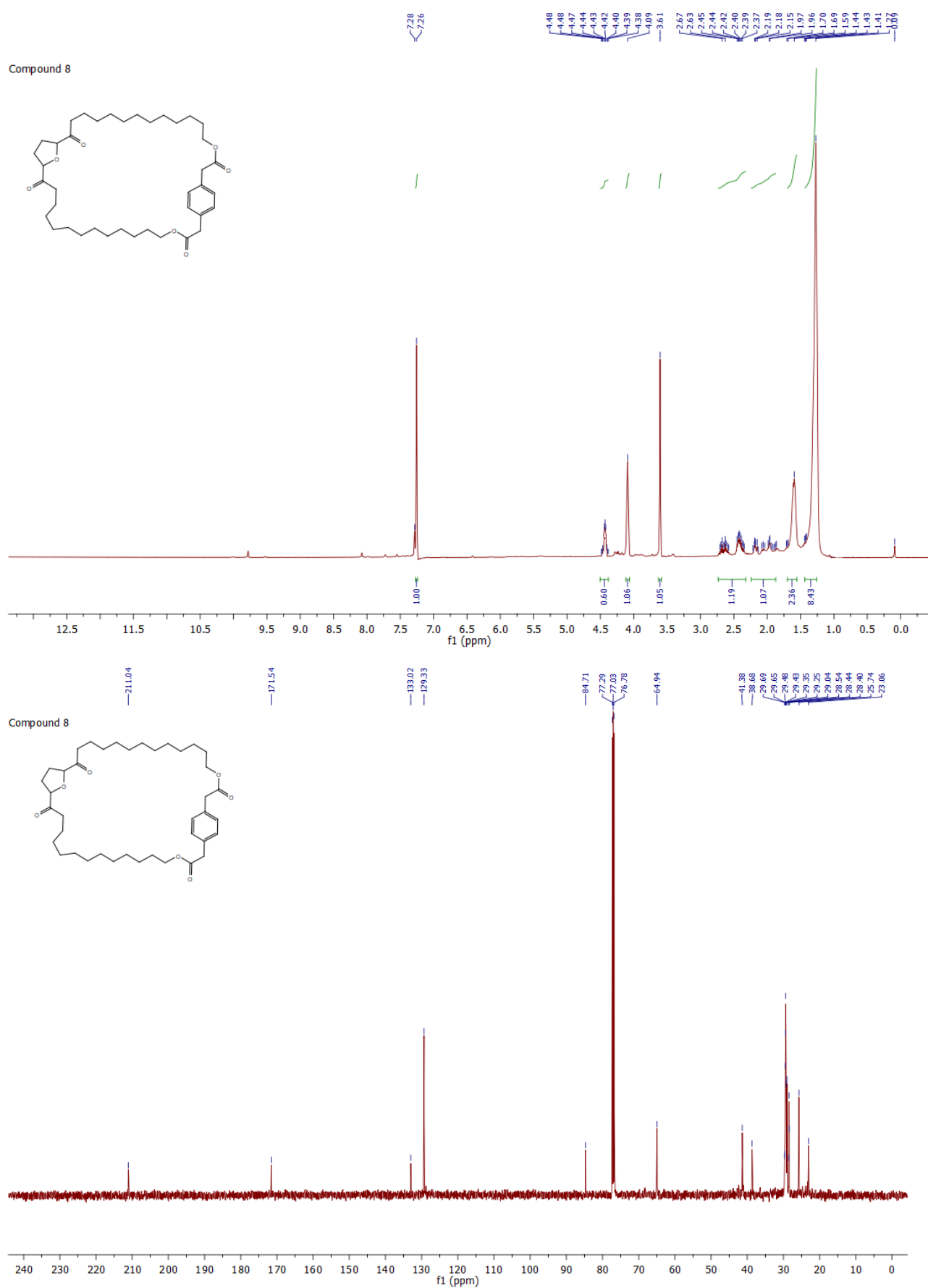


Figure S5. ^1H and ^{13}C NMR spectra of compound **8** in CDCl_3 .

References

- S1. V. A. D'yakonov, I. I. Islamov, L. U. Dzhemileva, E. M. Khusainova, M. M. Yunusbaeva and U. M. Dzhemilev, 2018, **74**, 4606; <https://doi.org/10.1016/j.tet.2018.07.031>.
- S2. N. S. Dyrkheeva, A. S. Filimonov, O. A. Luzina, K. A. Orlova, I. A. Chernyshova, T. E. Kornienko, A. A. Malakhova, S. P. Medvedev, A. L. Zakharenko, E.S. Ilina, R. O. Anarbaev, K. N. Naumenko, K. V. Klabenkova, E. A. Burakova, D. A. Stetsenko, S. M. Zakian, N. F. Salakhutdinov and O. I. Lavrik, *Int. J. Mol. Sci.*, 2021, **22**, 11336; <https://doi.org/10.3390/ijms222111336>.