CHEMMEDCHEM
Chemistry & Drug Discovery

Supporting Information

Development of Cyclobutene- and Cyclobutane-Functionalized Fatty Acids with Inhibitory Activity against *Mycobacterium tuberculosis*

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Benzyl 9-decenoate was prepared by a known procedure.[1] Thionyl chloride (36.9 mL, 51 mmol) was added to a stirred solution of 9-decenoic acid **DA** (8.67 g, 51 mmol) and catalytic dimethylformamide (DMF, 0.12 mL, 1.5 mmol) in toluene (400 mL). The reaction was stirred at rt until the starting material was consumed (TLC, ~ 24 h). The reaction was concentrated and the residue redissolved in toluene (400 mL) and triethylamine (8.5 mL). The solution was stirred for 24 h and washed with water. The aqueous layer was then extracted with ether. The combined organic solution were combined, dried with Na_2SO_4, and concentrated under vacuum. The residue was purified by flash column chromatography on silica gel (5-10% EtOAc in Hex) to give benzyl 9-decenoate (13.1 g, 99%) as a colorless oil: R_f = 0.40 (10% EtOAc/ Hex); IR (ZnSe, cm^{-1}): 3072, 2927, 2855, 1734, 1161, 908, 730 cm^{-1}; ^{1}H NMR: δ 7.38-7.32 (m, 5H), 5.83 (ddt, J = 17, 10.2, 6.6 Hz, 1H), 5.14 (s, 2H), 5.02 (ddd, J = 7.6 Hz, 2H), 5.00 (dq, , J = 17.0, 1.7 Hz, 1H), 4.96 (d of quintet, J = 10.2, 1.2 Hz, 1H), 2.37 (t, J = 7.3 Hz, 2H), 2.10-2.02 (br q, J = 7.3 Hz, 2H), 1.70-1.63 (br m, 2H), 1.41-1.48 (m, 10H); ^{13}C NMR: δ 173.5, 138.9, 136.1, 128.4, 128.1, 128.0, 114.1, 65.9, 34.2, 33.7, 28.99, 28.97, 28.8, 28.7, 24.8, 22.6, 14.0; HRESI-MS: calcd. for $C_{17}H_{24}O_2Na$: $[M+Na]^+$ 283.1674; Found: 283.1671.

(Z)-9-Octadecenoate, benzyl ester (benzyl oleate) was prepared by a variation of a known procedure.[2] Benzyl alcohol (3.83 g, 35.4 mmol) was added to a stirred solution of oleic acid (5.136 g, 18.1 mmol) and DMAP (43.7 mg, 3.57 mmol) in CH_2Cl_2 (40 mL). The solution was cooled to 0 $^\circ$C and DCC (4.211 g, 20.4 mmol) was added. The mixture was allowed to warm to rt and stirred for 2.5 h. The resulting suspension was filtered through a cotton plug and the precipitate was washed with CH_2Cl_2. The CH_2Cl_2 solution was washed with water and dried over Na_2SO_4. Evaporation of the organic solvent and flash chromatography of the residue over silica gel, using 5% EtOAc/Hex, gave benzyl oleate (5.73 g, 85 %) as a colorless oil: R_f = 0.83 (10%

EtOAc/Hex); IR (ZnSe, cm^{-1}) 2924, 2853, 1738, 1456, 1162, 696 cm^{-1}; ^1H NMR: δ 7.38-7.31 (5H), 5.41-5.31 (2H), 5.13 (s, 2H), 2.37 (t, J = 7.6 Hz, 2H), 2.05-2.00 (4H), 1.70-1.62 (m, 2H), 1.40-1.29 (20H), 0.90 (t, J = 6.8 Hz, 3H); ^{13}C NMR δ 173.6, 136.12, 129.9, 129.7, 128.5 (two overlapping signals), 128.11, 128.09, 66.0, 34.3, 31.9, 29.7, 29.6, 29.50, 29.3, 29.1, 29.1, 27.2, 27.1, 24.9, 22.7, 14.1.

(*E*)-9-octadecenoic acid, benzyl ester (benzyl elaidate)[3] was prepared from the reaction of elaidic acid (0.9836, 3.5 mmol), DCC (0.79 g, 3.8 mmol), DMAP (0.087 g, 0.7 mmol), and benzyl alcohol (0.73 mL, 7 mmol) in CH_2Cl_2 (10 mL) by a similar procedure as employed for benzyl oleate. The product was purified by flash column chromatography on silica gel (2.5% / 5% EtOAc/Hex) to afford 1.2280 g (95%) of benzyl elaidate as a colorless oil: *Rf* = 0.57 (10% EtOAc/Hex). IR (ZnSe, cm^{-1}) 2922, 2852, 2737, 1160, 966, 696 cm^{-1}; ^1H NMR: δ 7.38-7.34 (m, 5H), 5.41-5.38 (m, 2H), 5.13 (s, 2H), 2.37 (t, J = 7.2 Hz, 2H), 1.98 (br s, 4H), 1.68-1.63 (m, 2H), 1.38-1.18 (br m, 20H), 0.90 (t, J = 7.2 Hz, 3H); ^{13}C NMR δ 173.6, 136.1, 130.4, 130.2, 128.5, 128.1, 66.0, 34.3, 32.57, 32.52, 31.9, 29.6, 29.52, 29.46, 29.3, 29.2, 29.1, 29.0, 28.9, 24.9, 22.7, 14.1.

Data from cytotoxicity studies

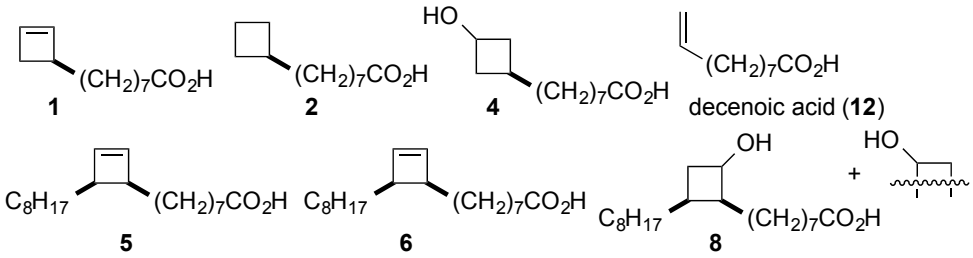


Figure S1. Analogs investigated

Table S1. Toxicity of Fatty acid analogs in RAW 2647 macrophages

Compound	Average % viability at indicated concentration (μM)					
	0	25	50	100	250	Control[a]
1 (DA-CB)	100	67.8	58.5	48.1	0.9	0.2
2 (DA-satCB)	100	72.9	49.2	7.5	0.6	0.2
4 (DA-alcCB)	100	86.5	78.7	77.5	58.8	0.2
5 (OA-CB)	100	93.8	91.8	89.3	73.4	0.2
6 (OA-satCB)	100	87.8	84.6	82.5	78.9	0.2
8 (OA-alcCB)	100	5.5	4.4	4.4	3.7	0.2
DA	100	83.5	77.3	73	38.3	0.2
D-cycloserine	100	81.8	77.5	72.2	69.4	0.2
Isoniazid	100	76.5	75.1	71.7	64	0.2

a. Sodium dodecyl sulfate (SDS)

Thermal Stability (Figure S1-S11)

Differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA) were acquired simultaneously on 4-10 mg samples of the indicated molecules, using a Perkin Elmer STA 6000 analyzer operated at a temperature ramp of 2 °C/minute except for compound **5** at a 5 °C/minute

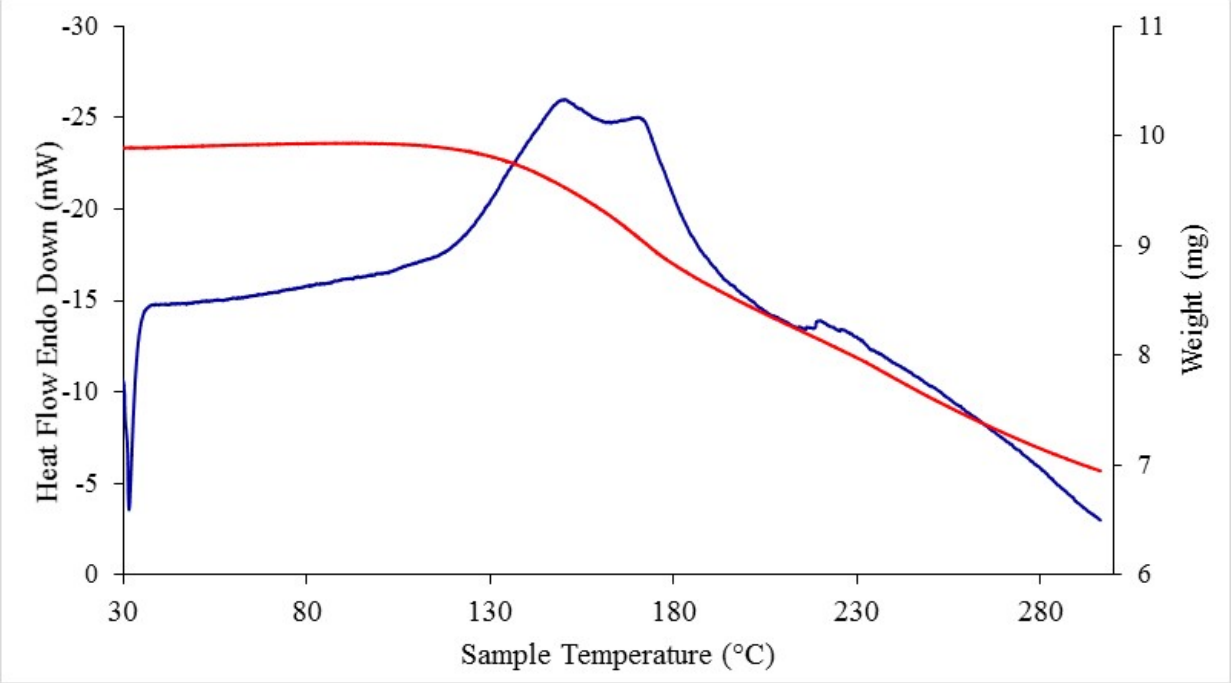


Figure S1. Thermal stability of **1**. TGA-measured mass (red) and DSC-measured heat flow (blue).

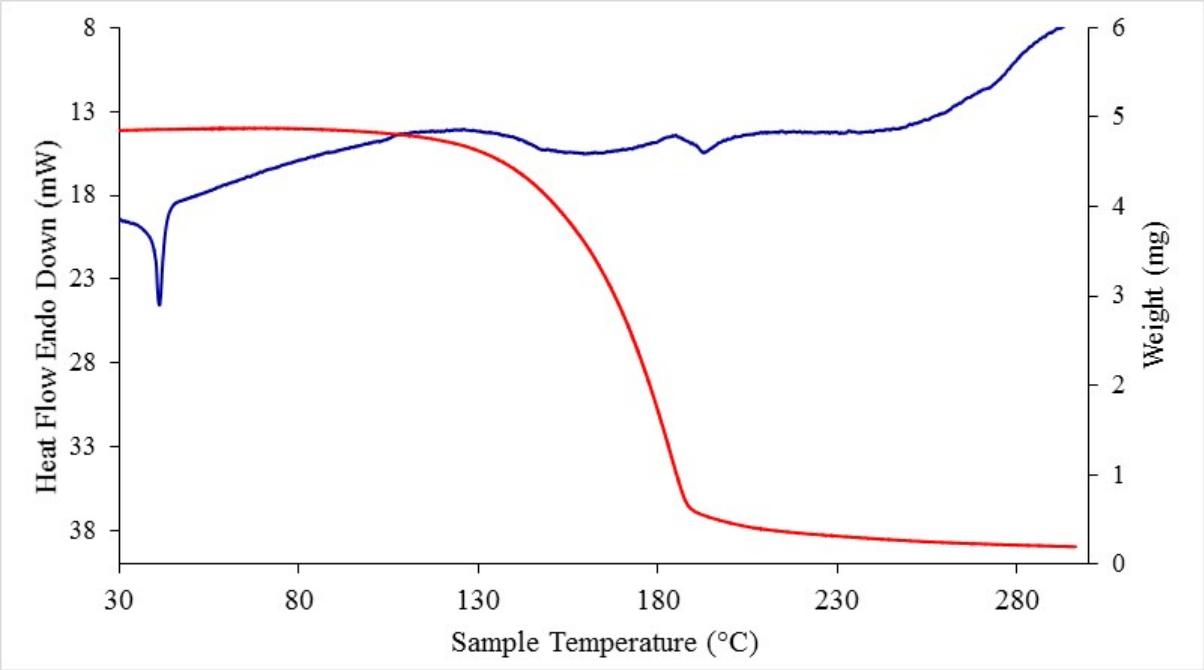


Figure S2. Thermal stability of **2**. TGA-measured mass (red) and DSC-measured heat flow (blue).

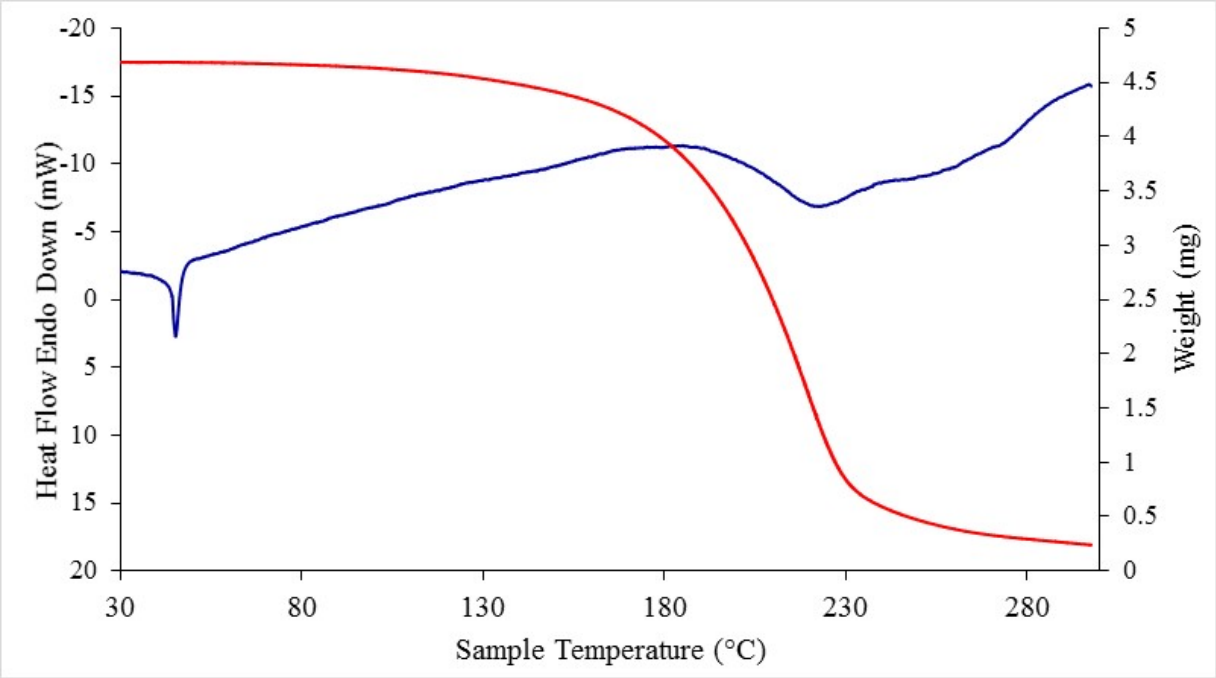


Figure S3. Thermal stability of **3**. TGA-measured mass (red) and DSC-measured heat flow (blue).

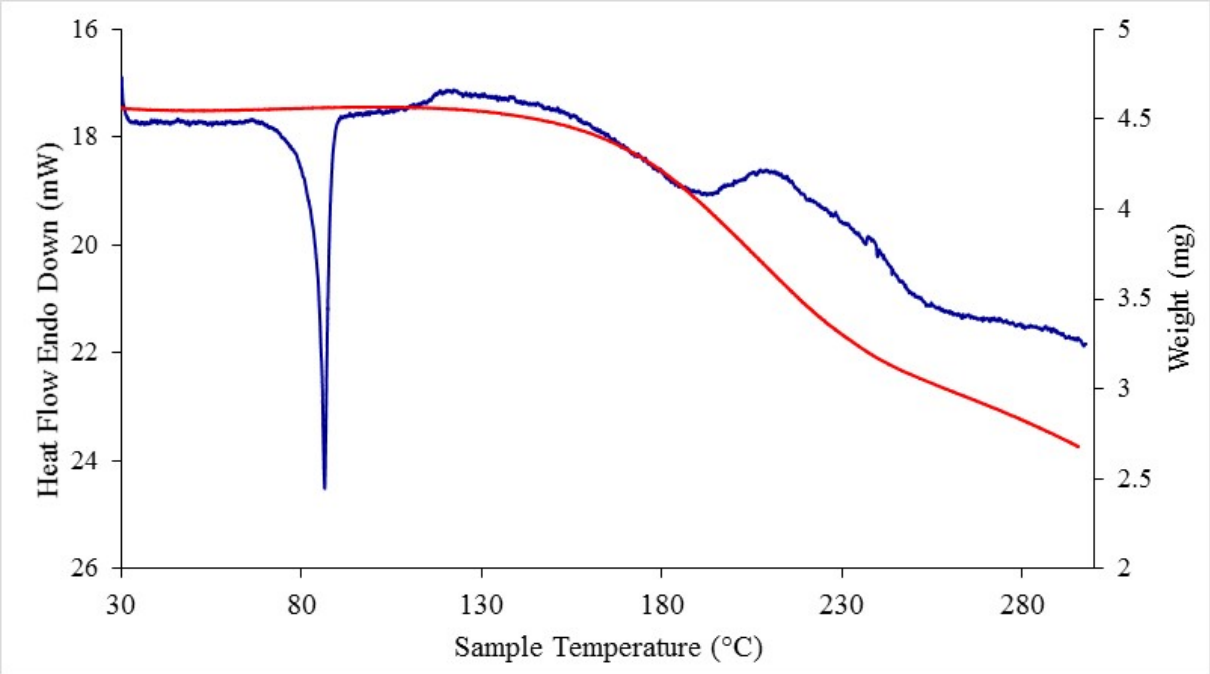


Figure S4. Thermal stability of **4**. TGA-measured mass (red) and DSC-measured heat flow (blue).

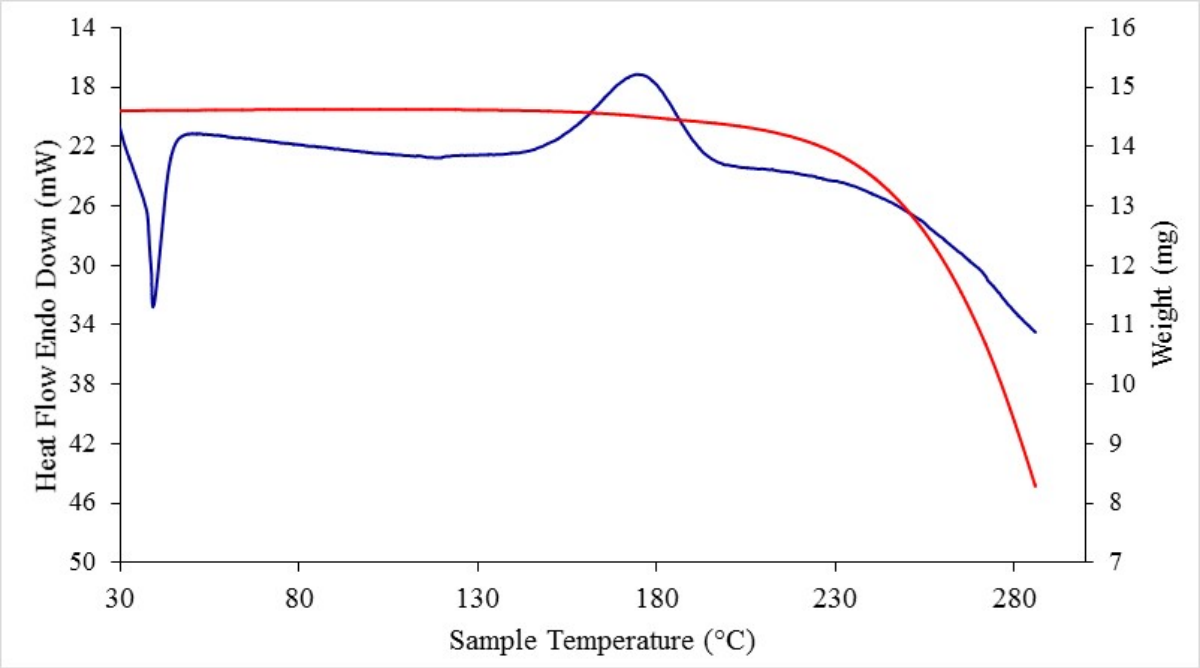


Figure S5. Thermal stability of **5**. TGA-measured mass (red) and DSC-measured heat flow (blue).

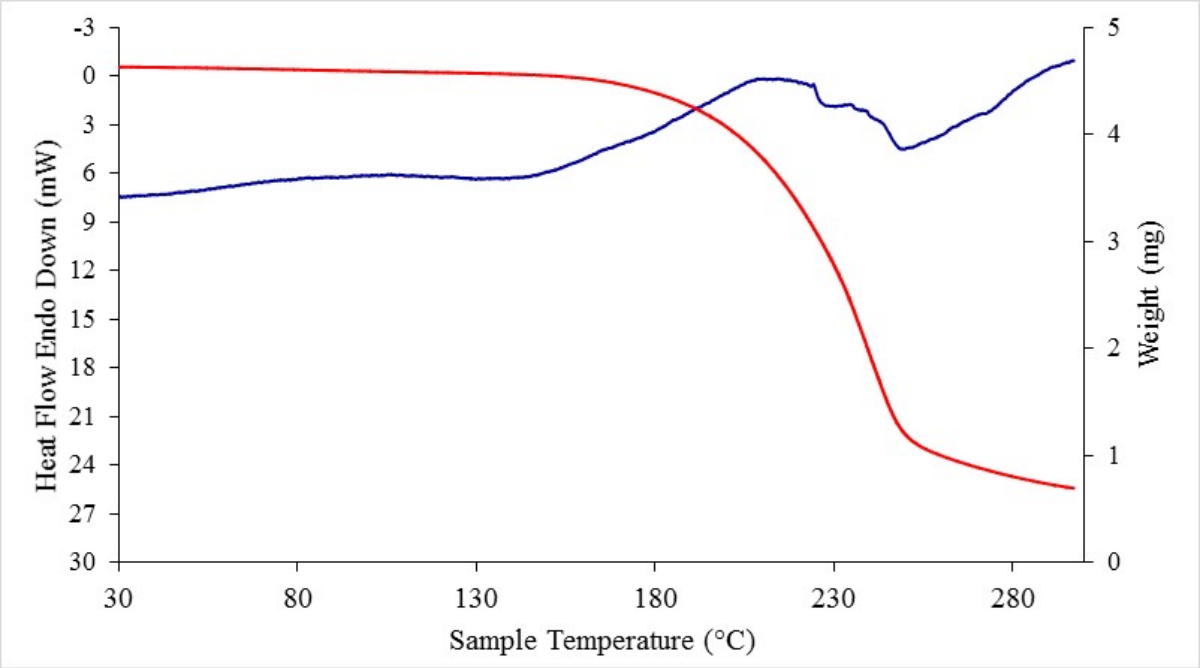


Figure S6. Thermal stability of **6**. TGA-measured mass (red) and DSC-measured heat flow (blue).

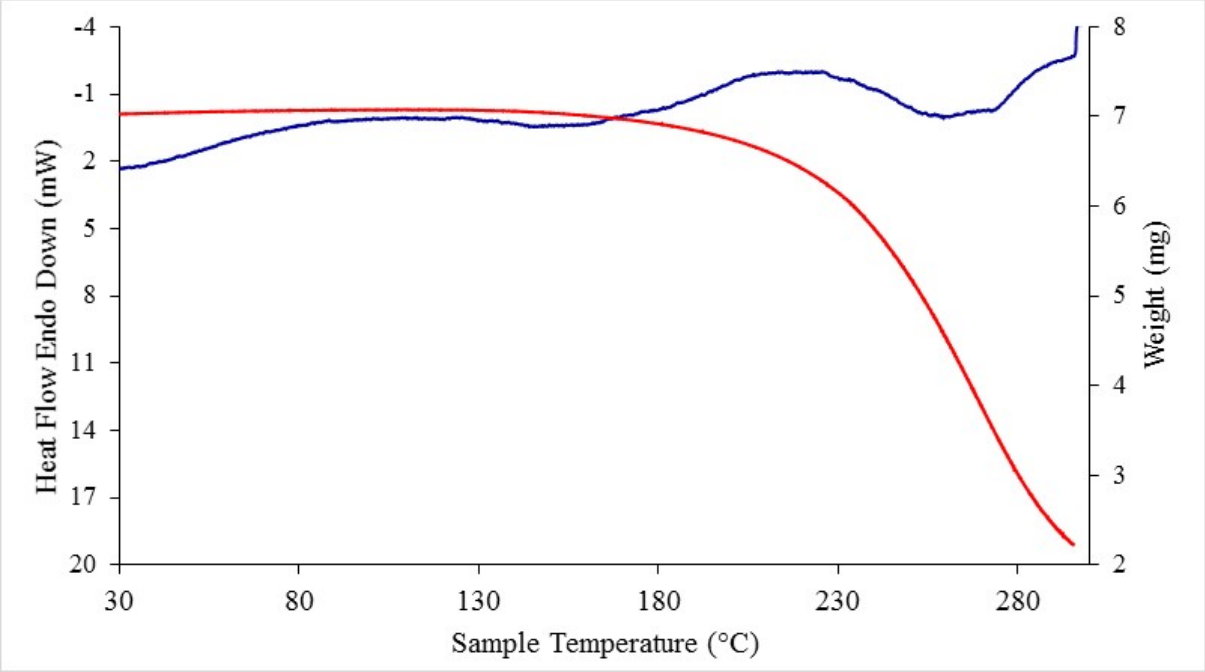


Figure S7. Thermal stability of **7**. TGA-measured mass (red) and DSC-measured heat flow (blue).

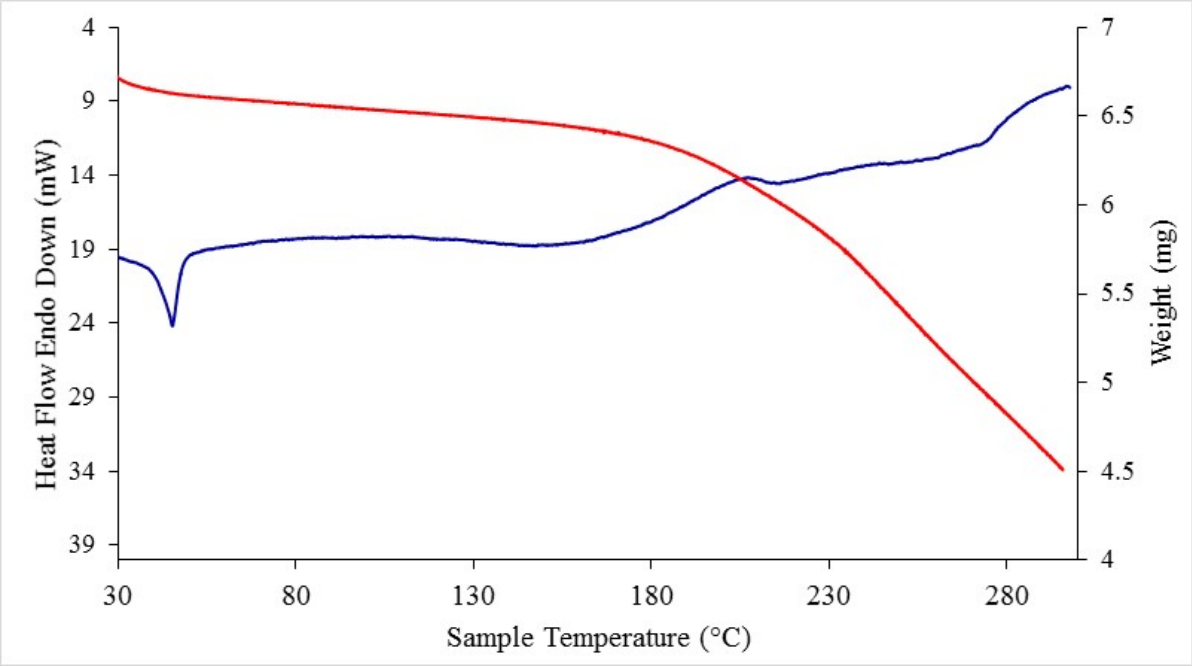


Figure S8. Thermal stability of **8**. TGA-measured mass (red) and DSC-measured heat flow (blue).

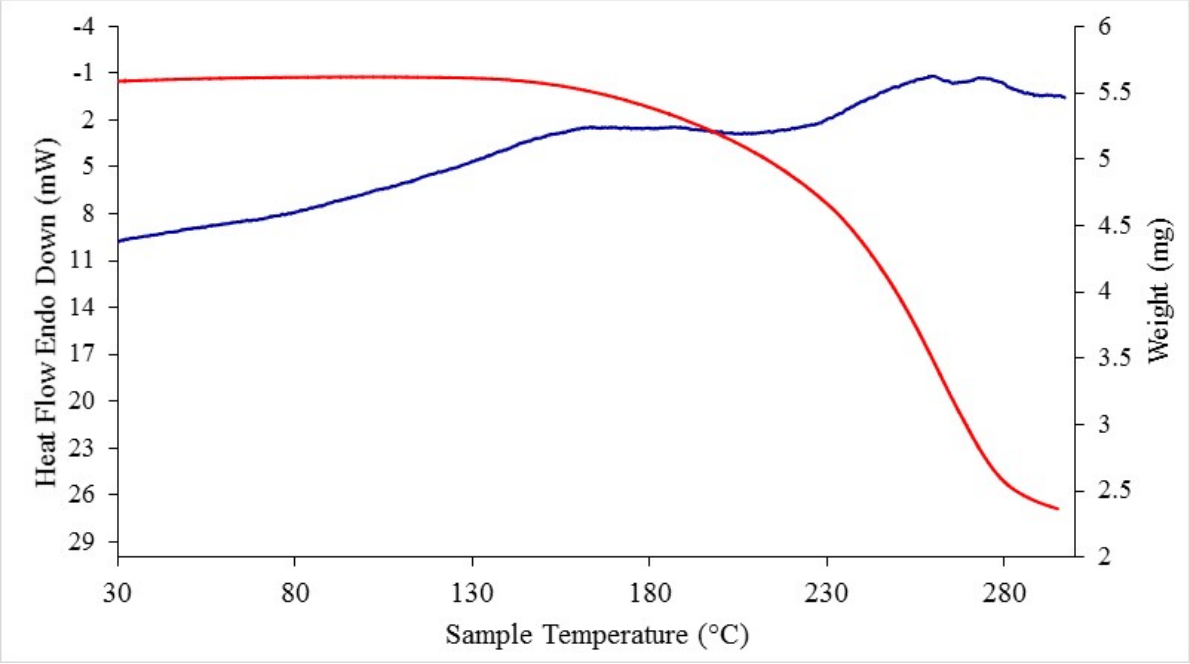


Figure S9. Thermal stability of **9.** TGA-measured mass (red) and DSC-measured heat flow (blue).

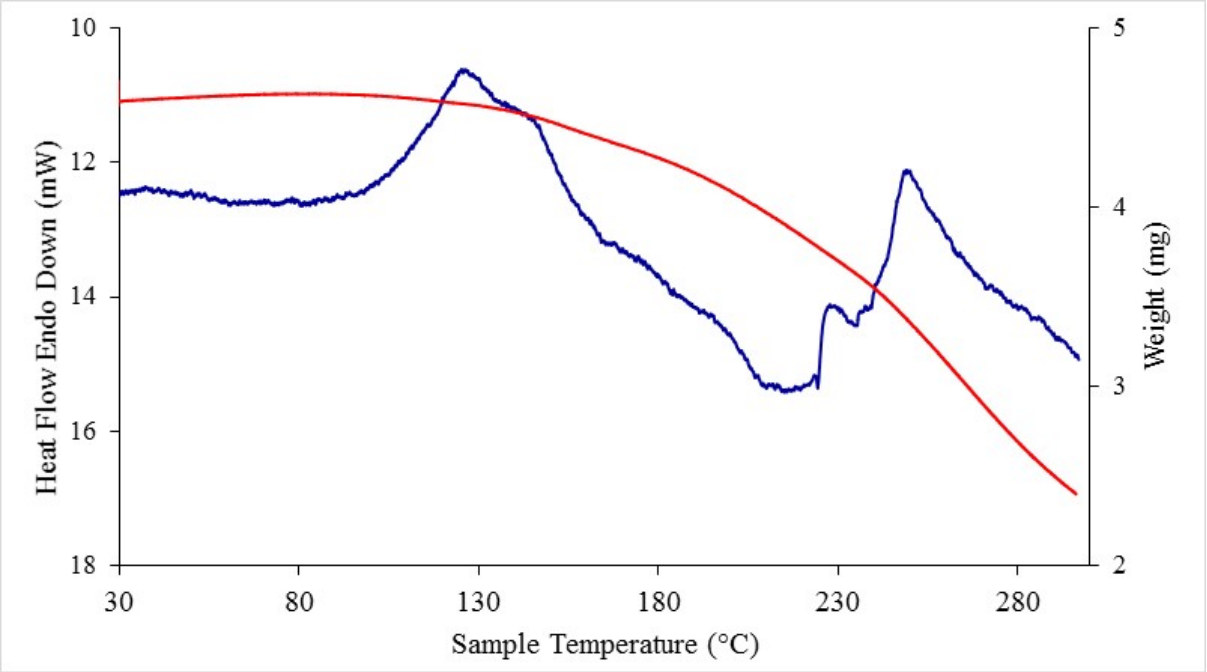


Figure S10. Thermal stability of **10**. TGA-measured mass (red) and DSC-measured heat flow (blue).

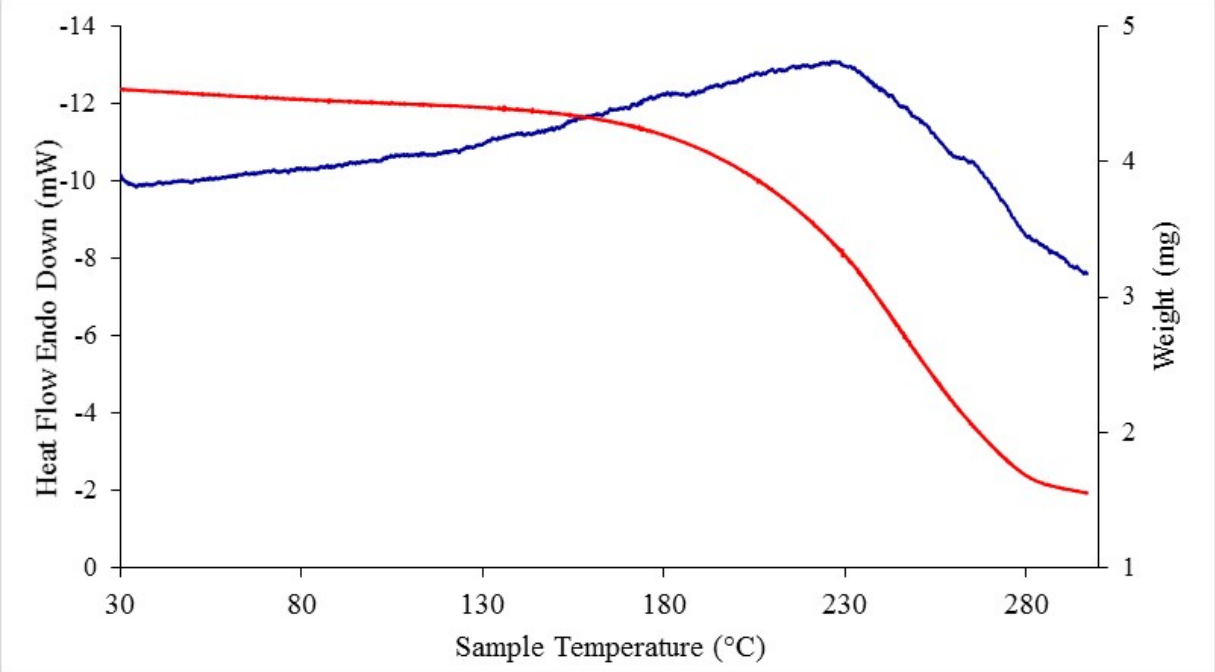
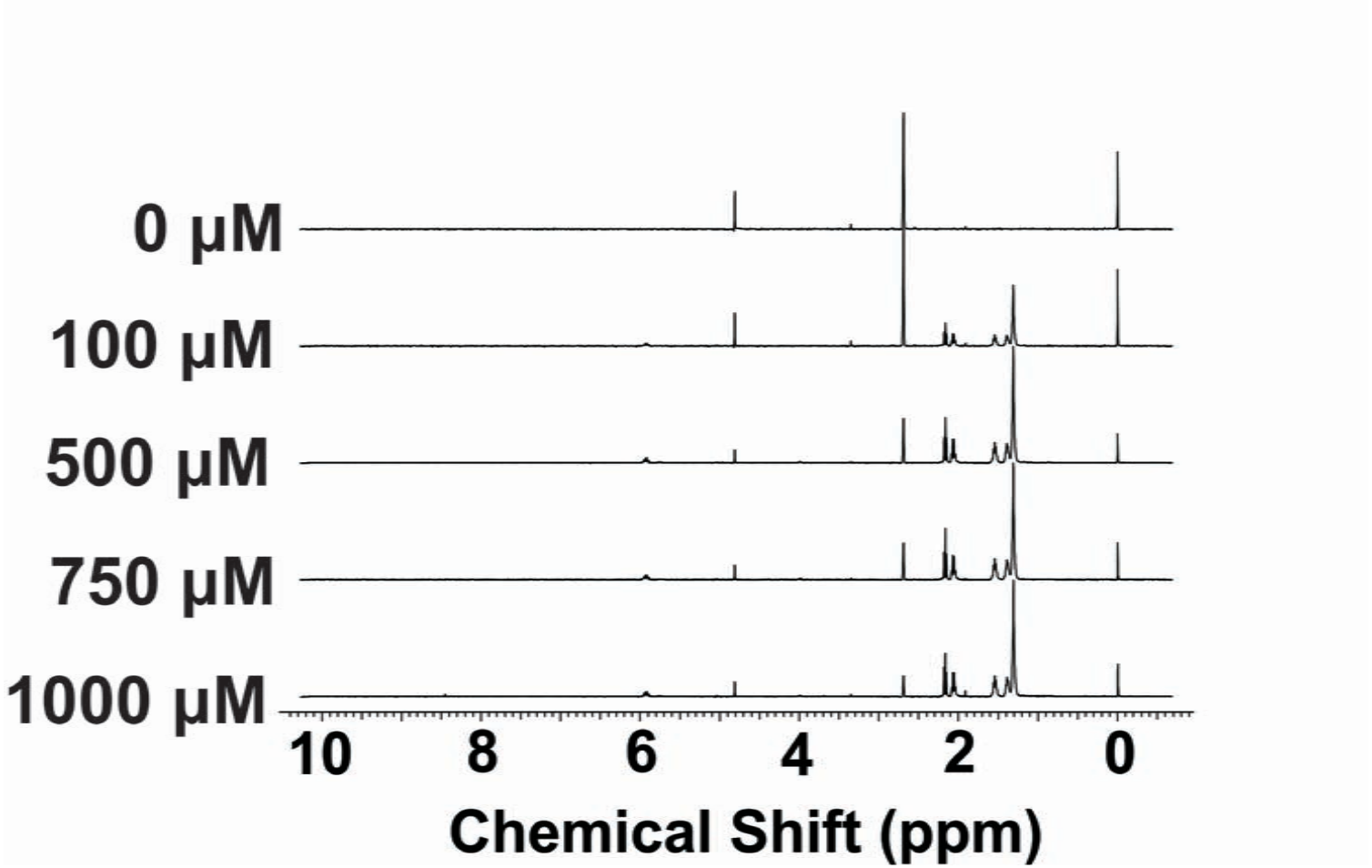
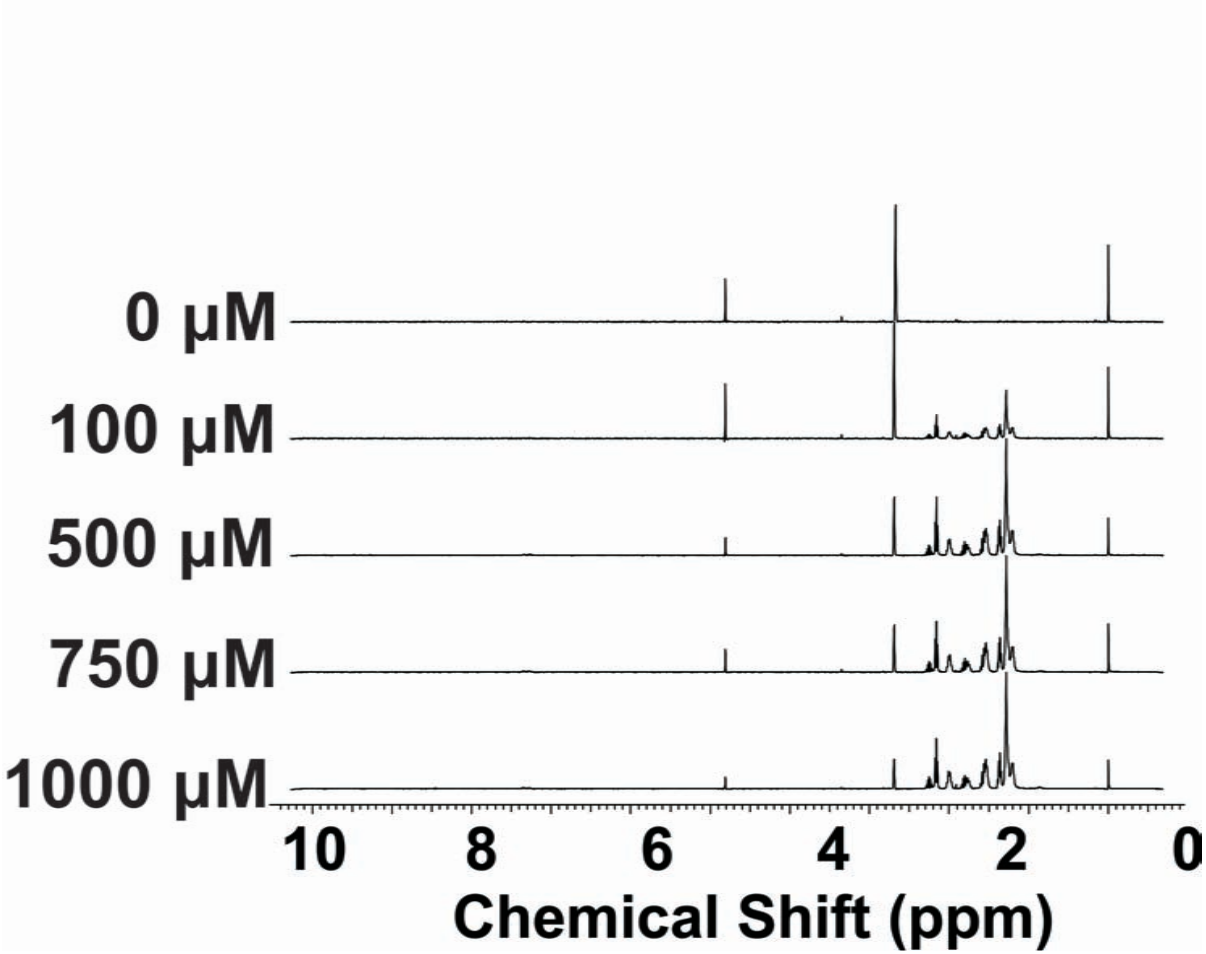


Figure S11. Thermal stability of **11**. TGA-measured mass (red) and DSC-measured heat flow (blue).

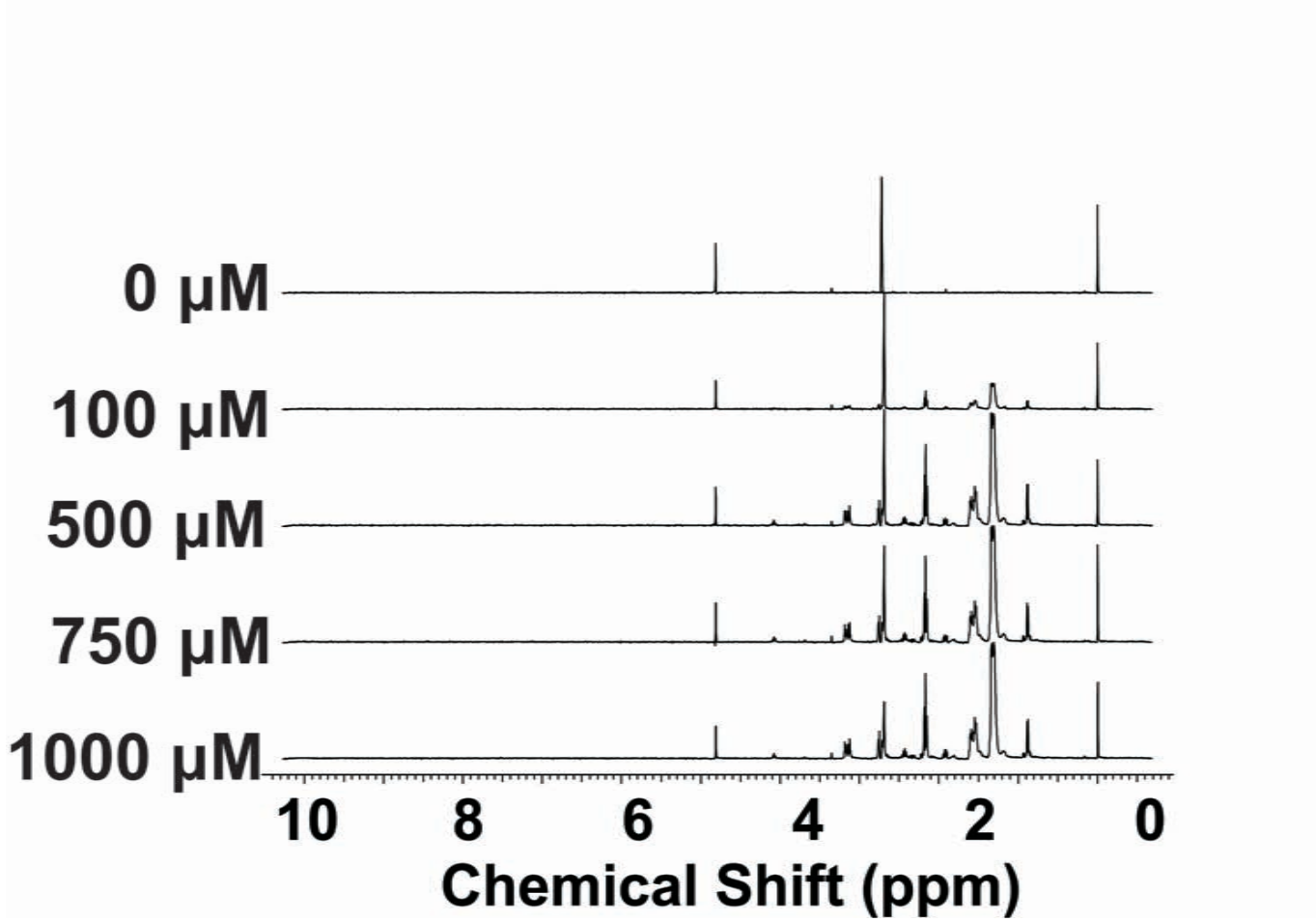
Decenoic acid: aggregation assay in phosphate buffer



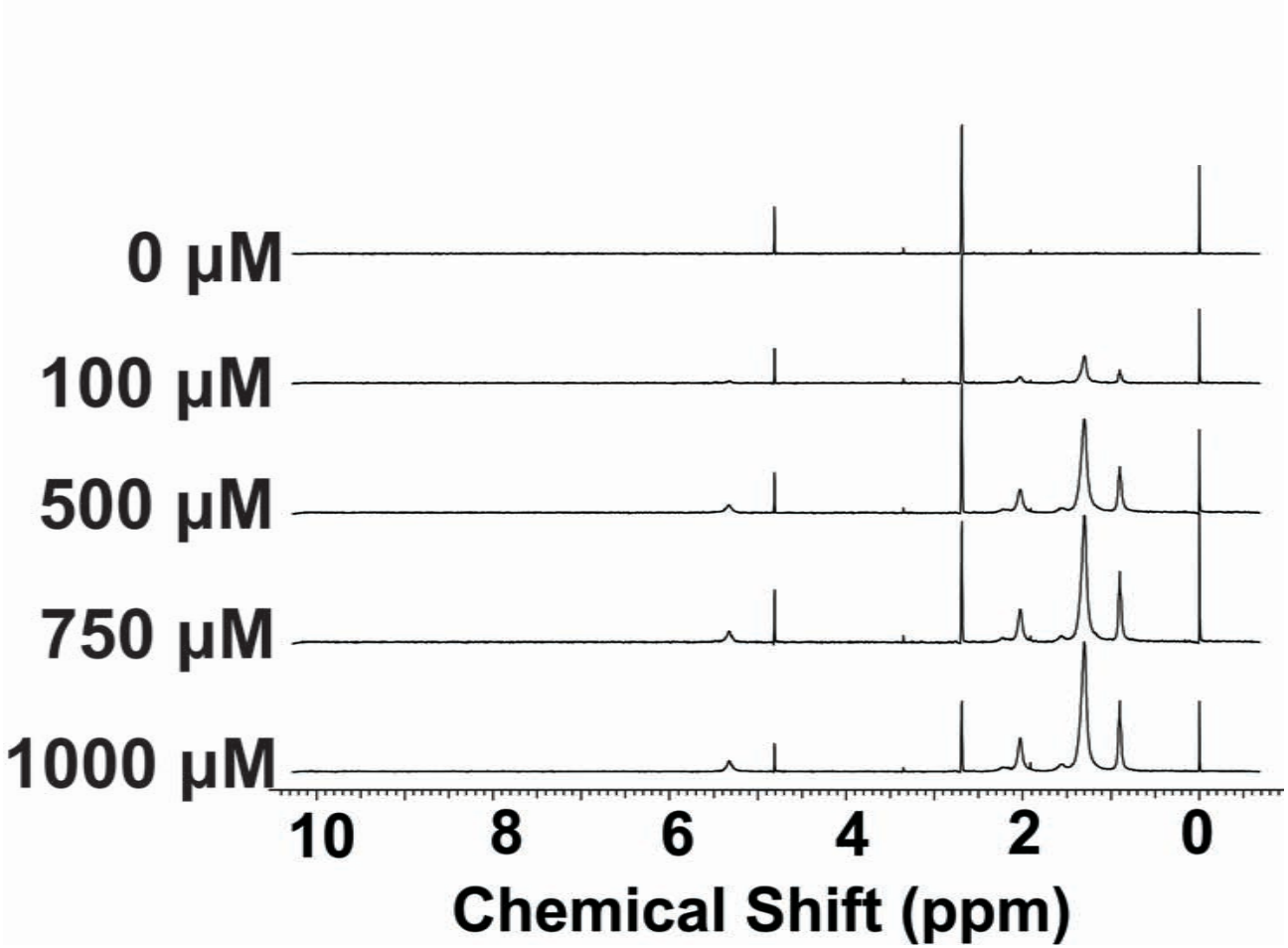
Analog **2** (C_{10} cyclobutane); aggregation assay in phosphate buffer



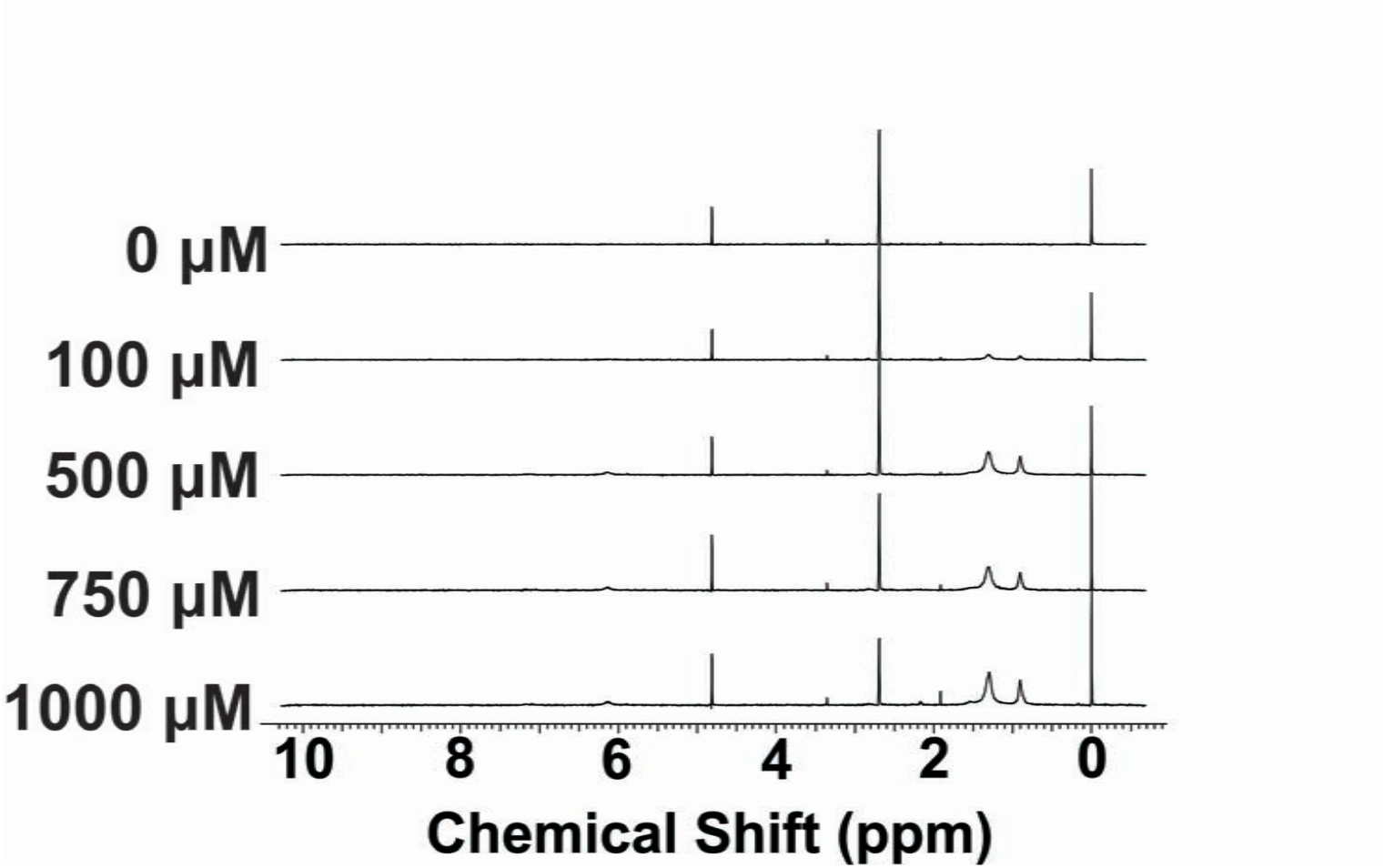
Analog **4** (C_{10} cyclobutanol); aggregation assay in phosphate buffer



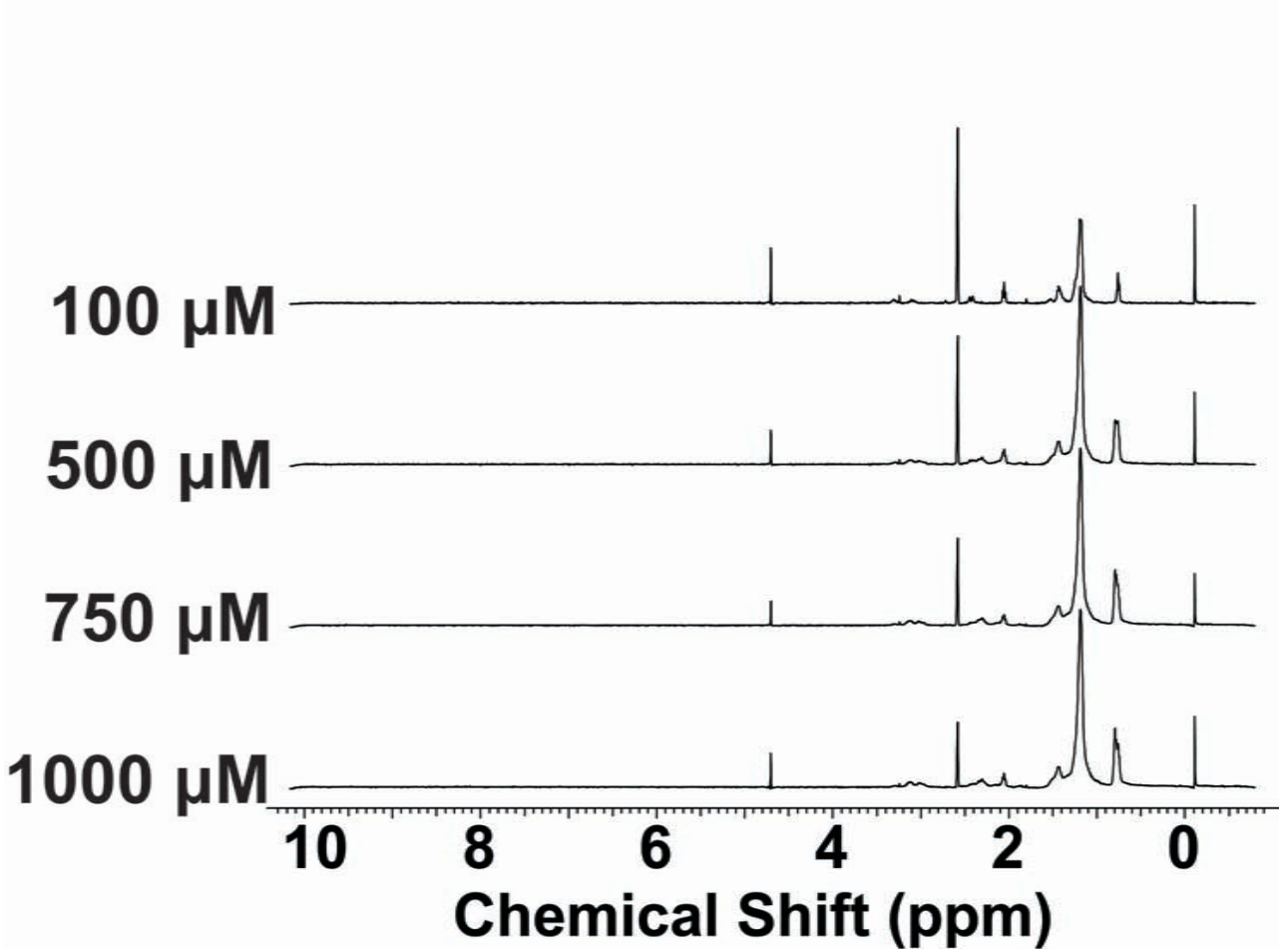
Oleic acid: aggregation assay in phosphate buffer



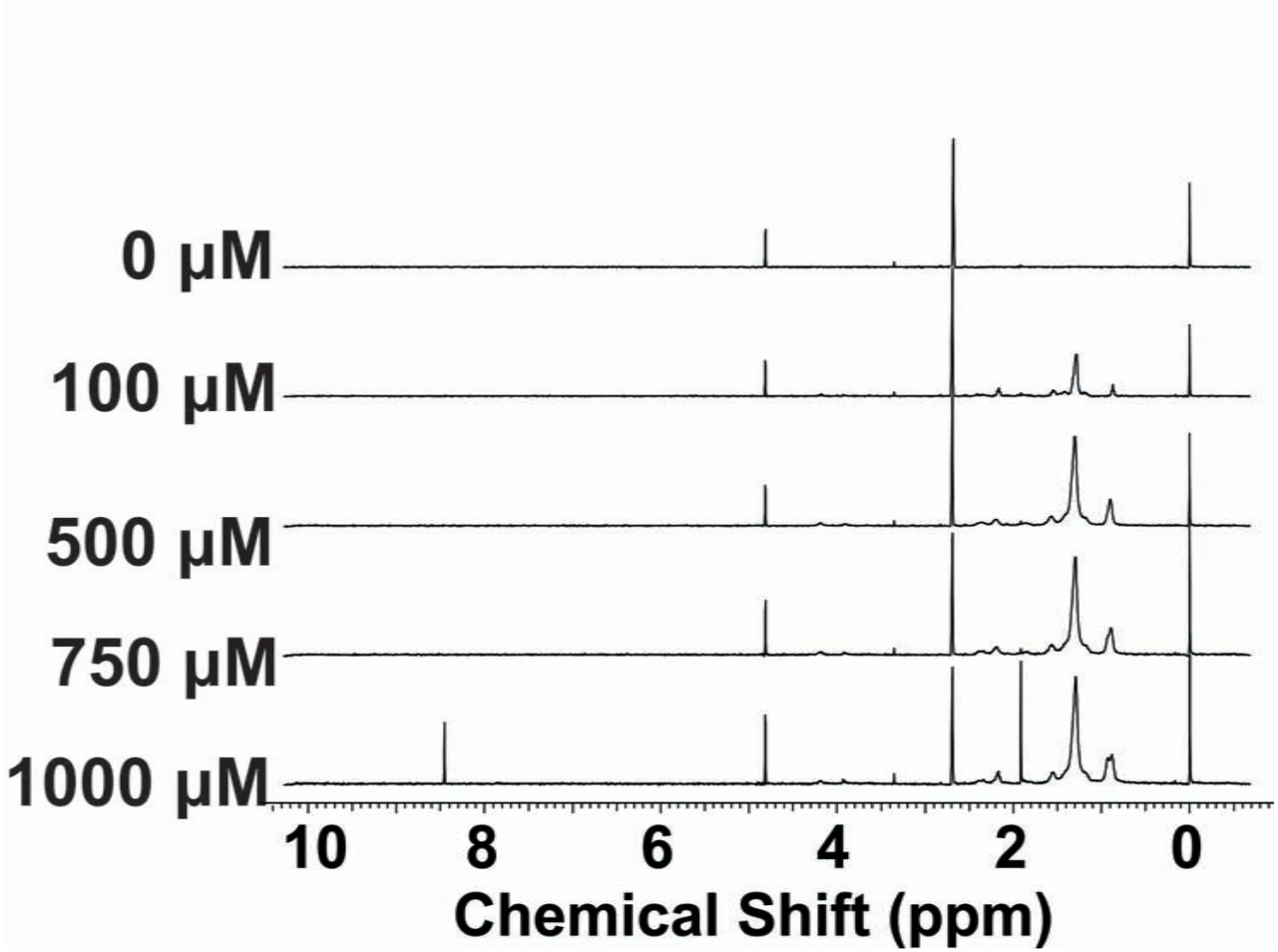
Analog **5** (C_{18} *cis*-cyclobutene); aggregation assay in phosphate buffer



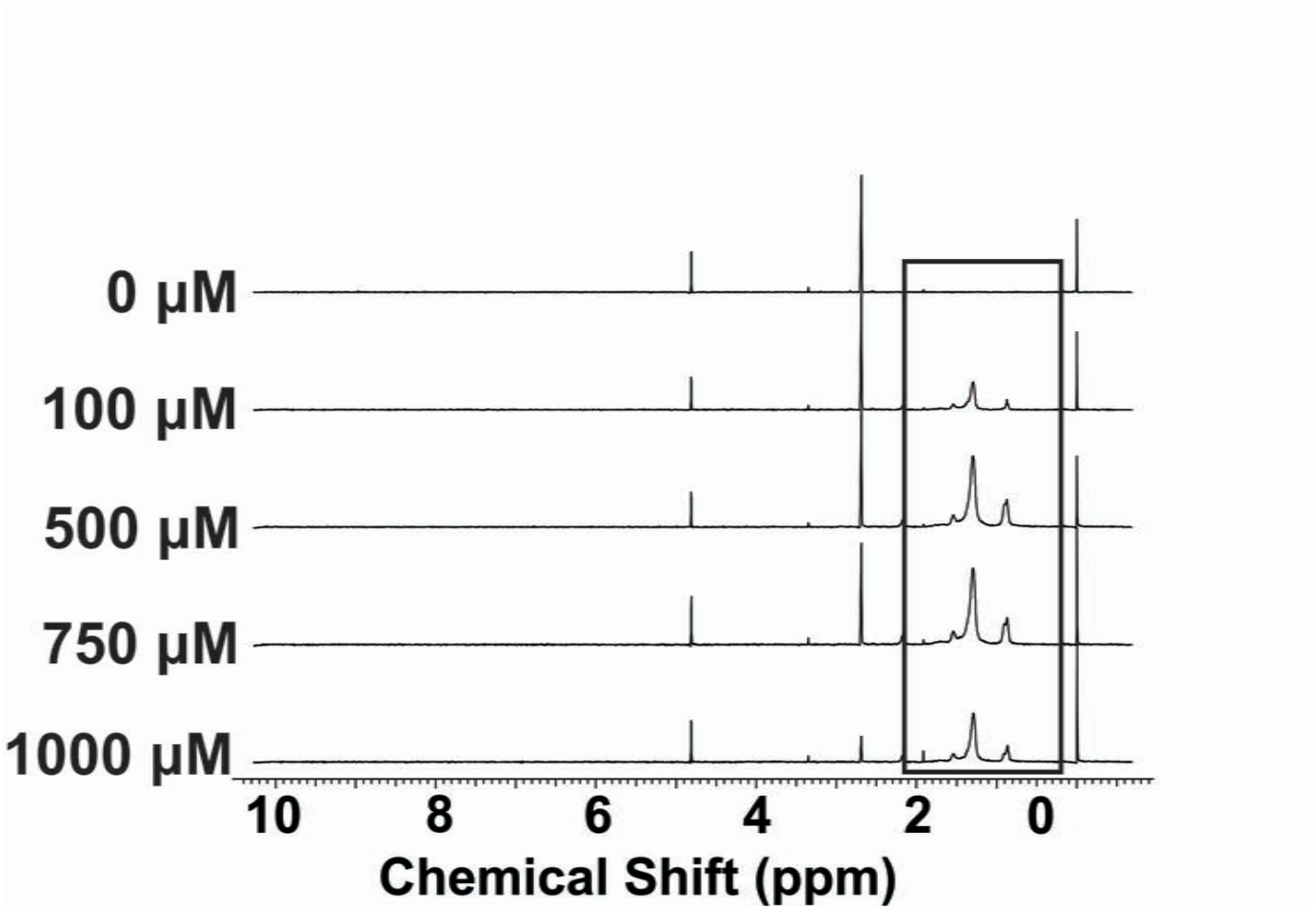
Analog **7** (C_{18} *cis*-cyclobutanone); aggregation assay in phosphate buffer



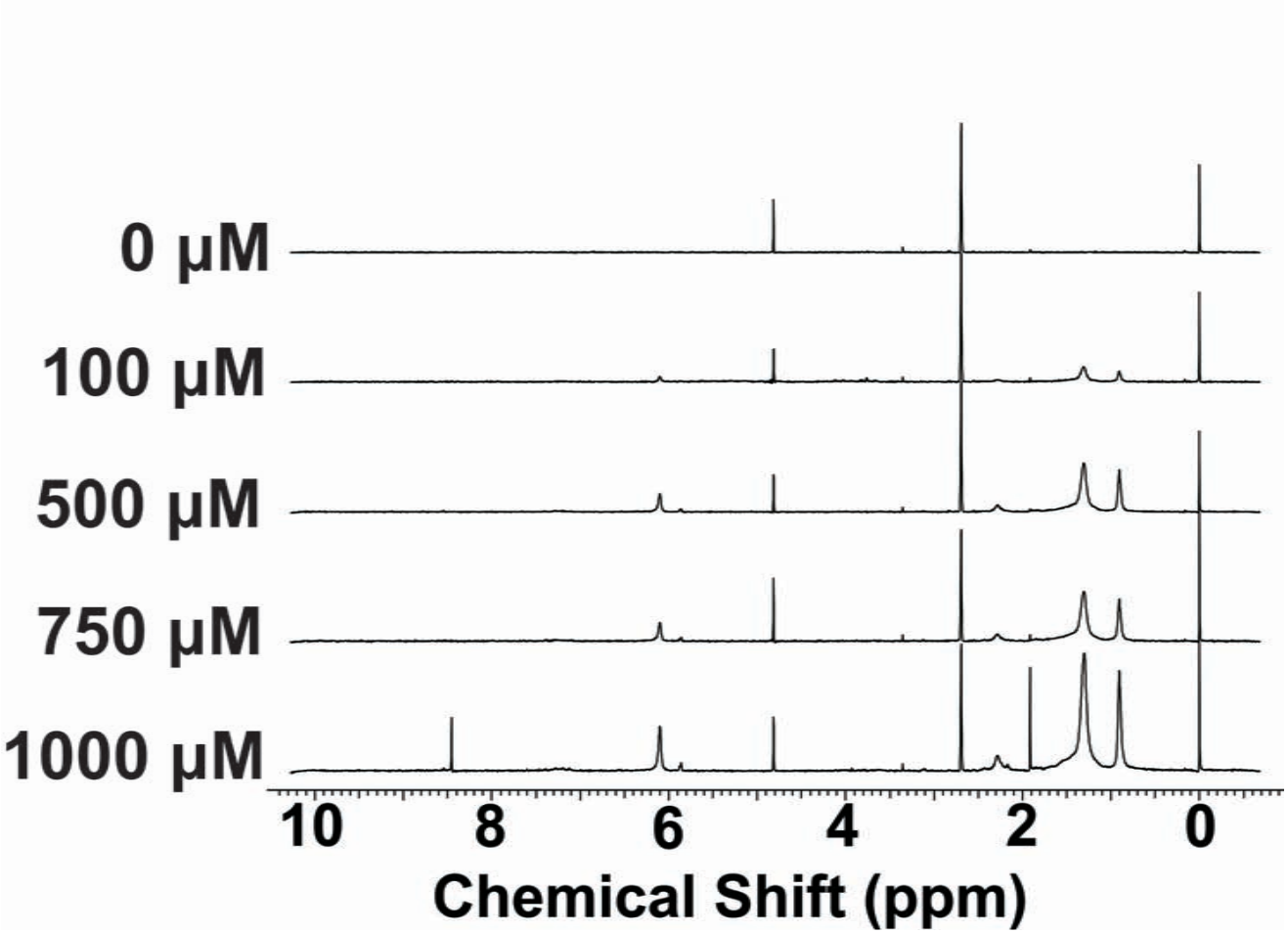
Analog **8** (C_{18} cyclobutanol); aggregation assay in phosphate buffer



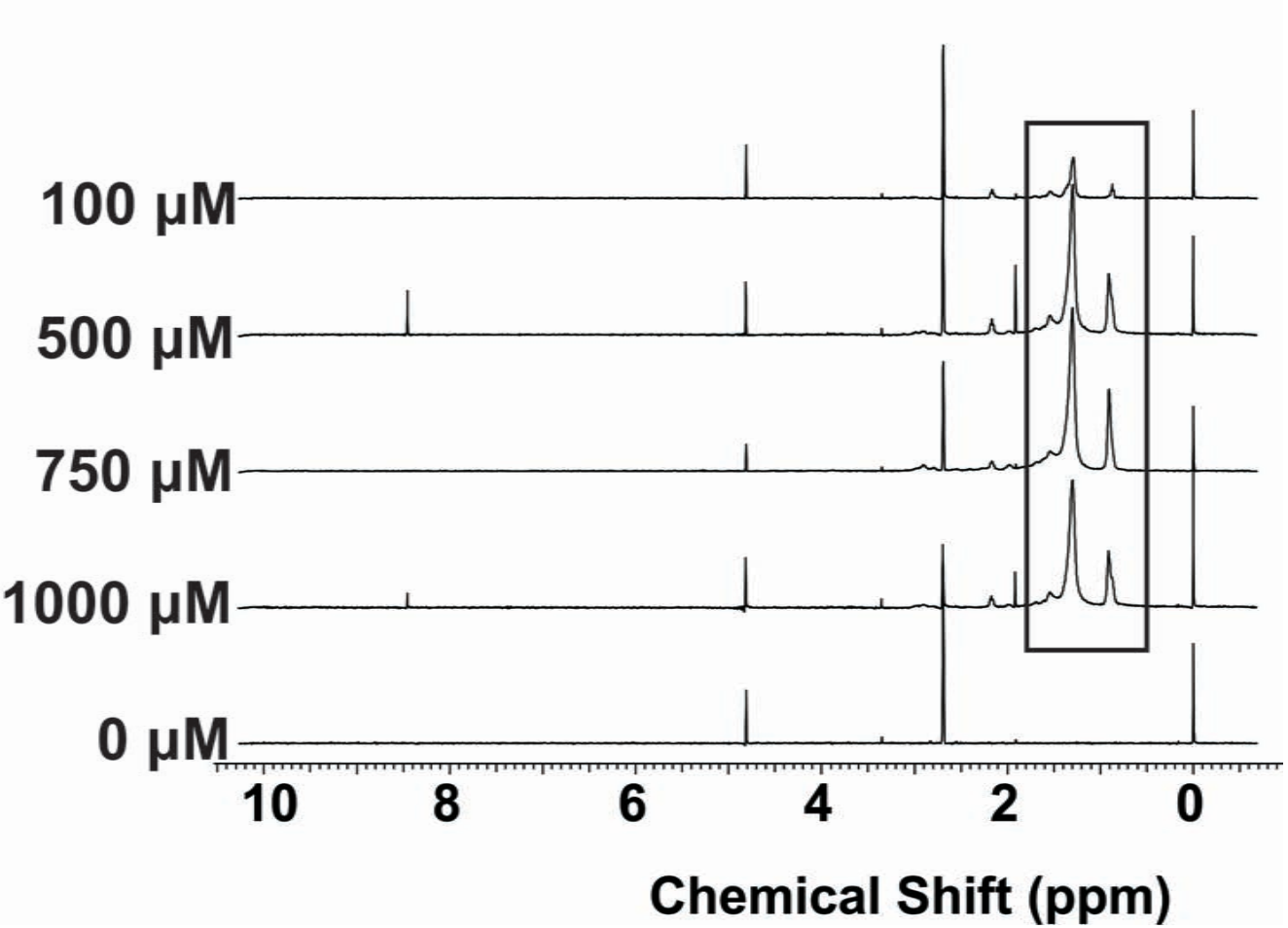
Analog **9** (C_{18} *cis*-chlorocyclobutanone); aggregation assay in phosphate buffer



Analog **10** (C_{18} *trans*-cyclobutene); aggregation assay in phosphate buffer



Analog **11** (C_{18} *trans*-chloroketone); aggregation assay in phosphate buffer



References

[1] J, J. Li, C. Limberakis, D. A. Pflum, *Modern Organic Synthesis in the Laboratory: A Collection of Standard Experimental Procedures*, Oxford University Press, New York, **2007**, p 45.

[2] B. Neises, W. Steglich, *Angew. Chem. Int. Ed.* **1978**, *17*, 522–524. Previous report: E. G. Maleeva, *Zhurnal Obshchei Khimii* **1953**, *23*, 1662–1664; K. Murai, G. J. Akazome, *Jpn Oil Chem. Soc.* **1955**, *4*, 125–127. (no detail for preparation)

[3] E. G. Maleeva, *Zhurnal Obshchei Khimii*. **1953**, *23*, 1662–1664 [*Chem. Abstr.* **1954**, *48*, 77506].

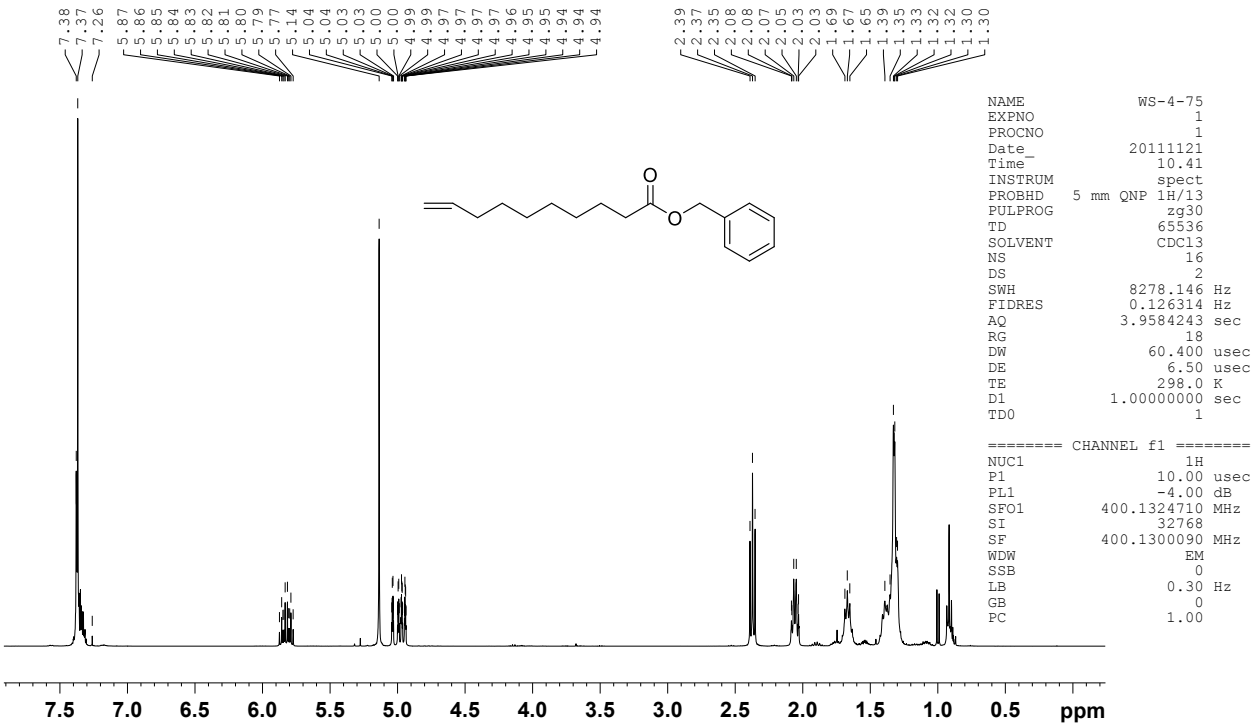


Figure S1.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum.

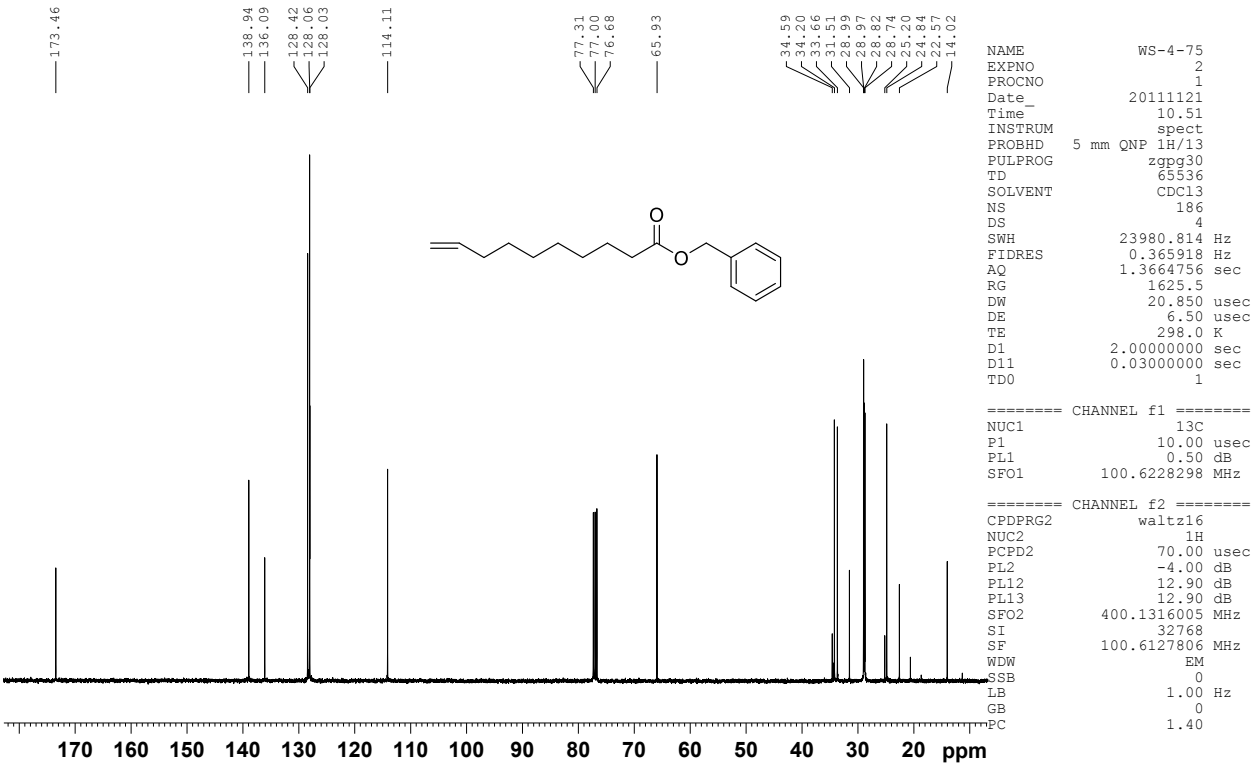


Figure S1.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum.

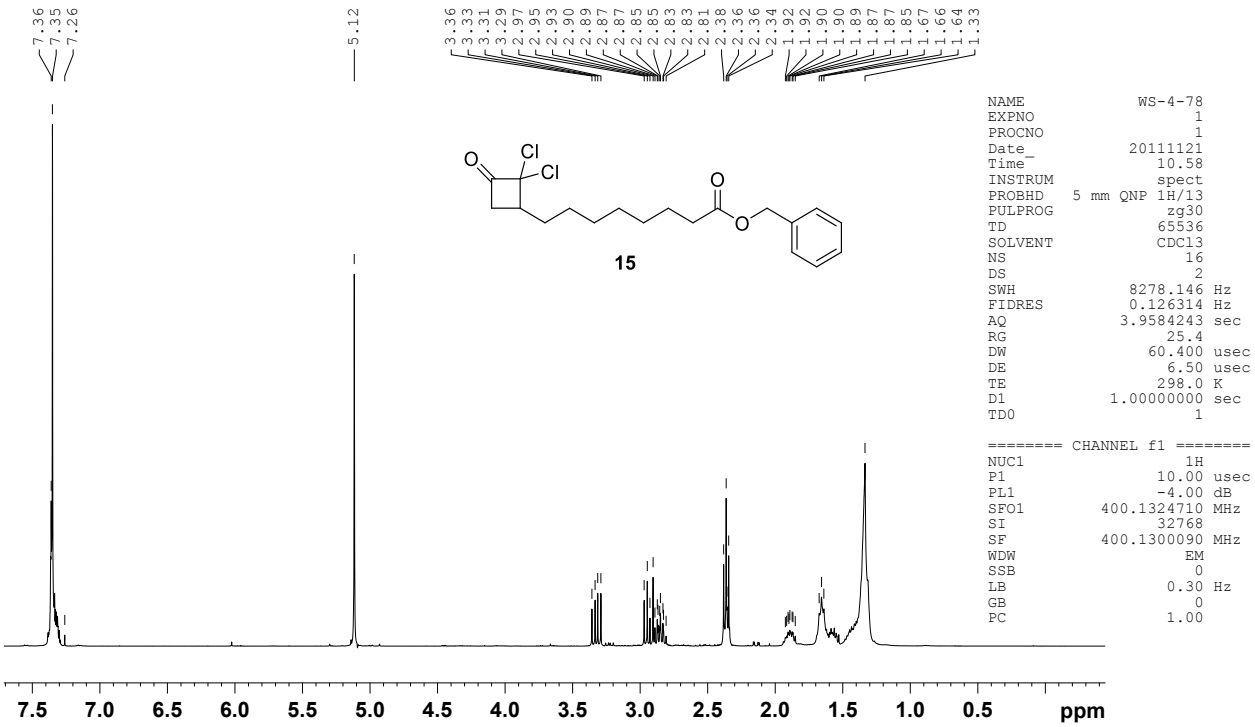


Figure S2.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of **15**.

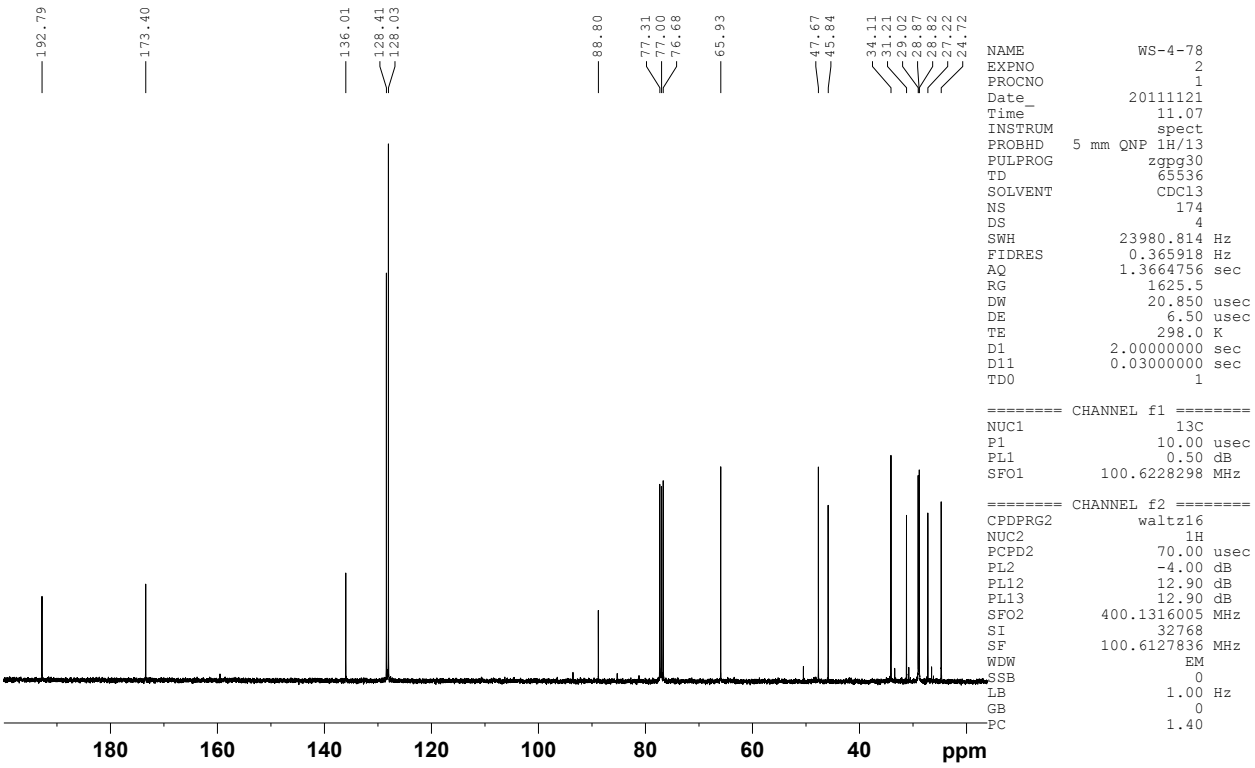


Figure S2.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of **15**.

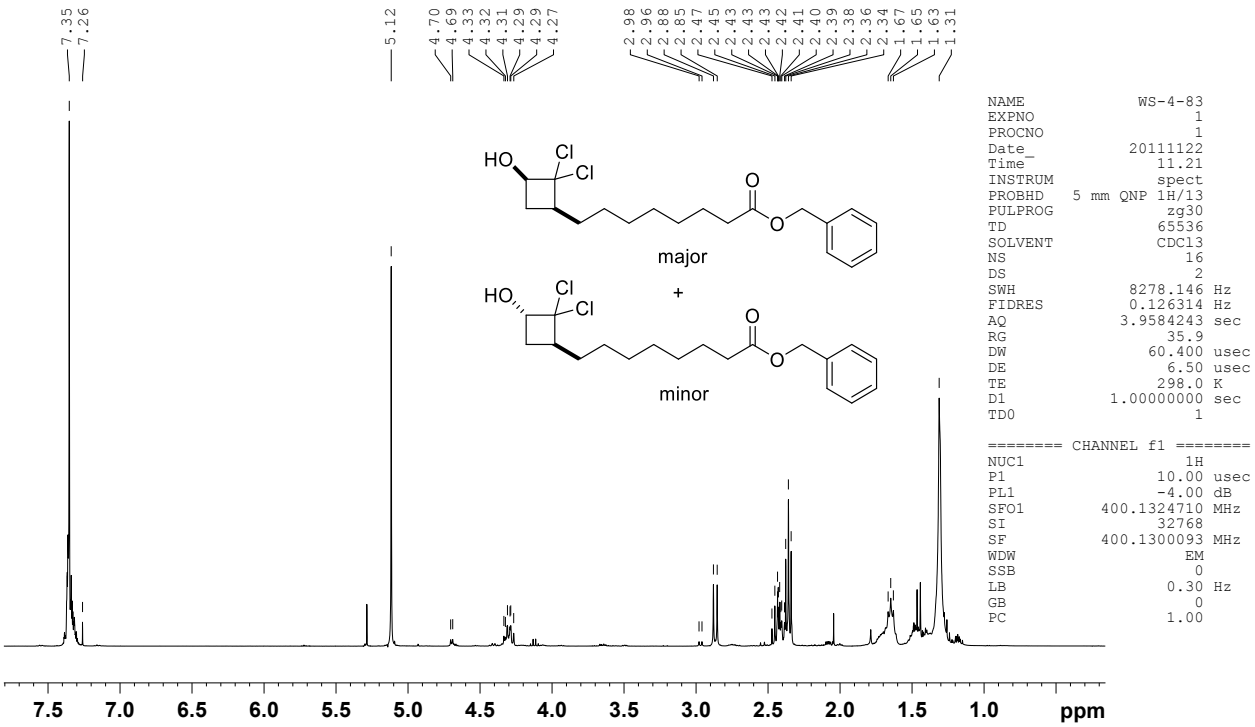


Figure S3.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum.

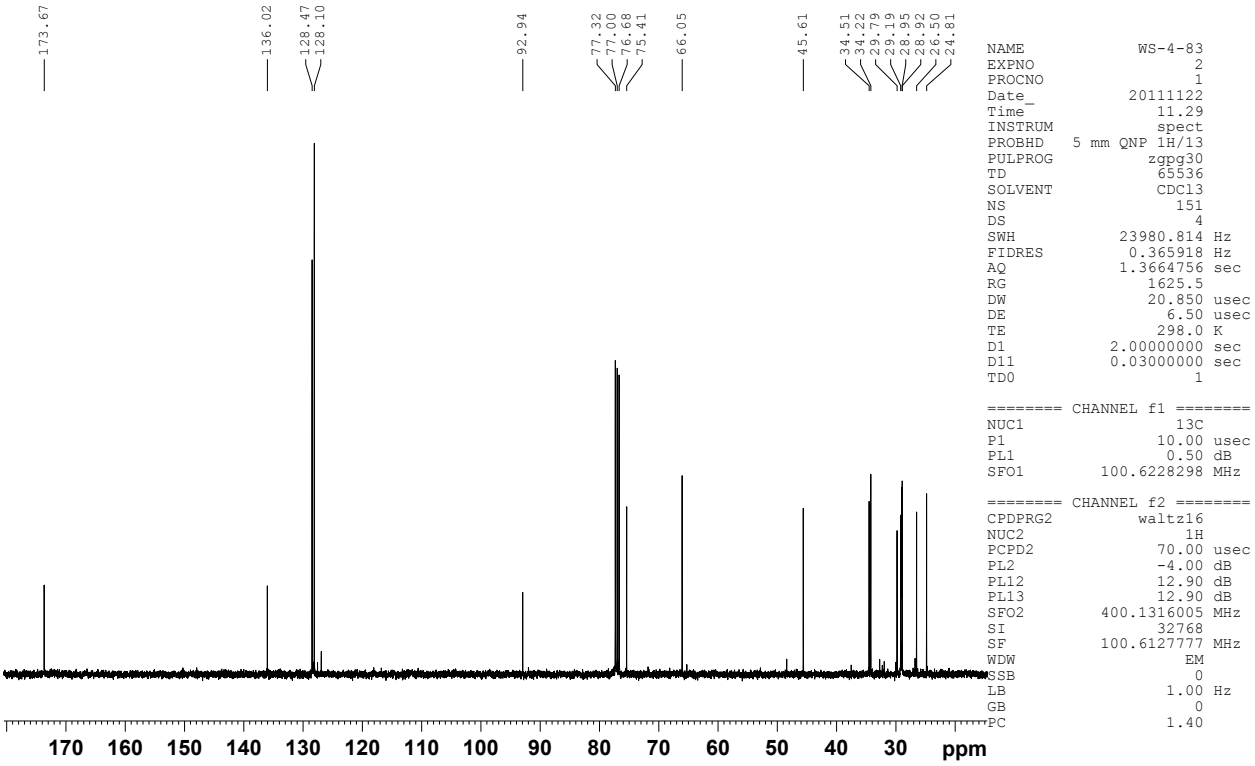


Figure S3.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum.

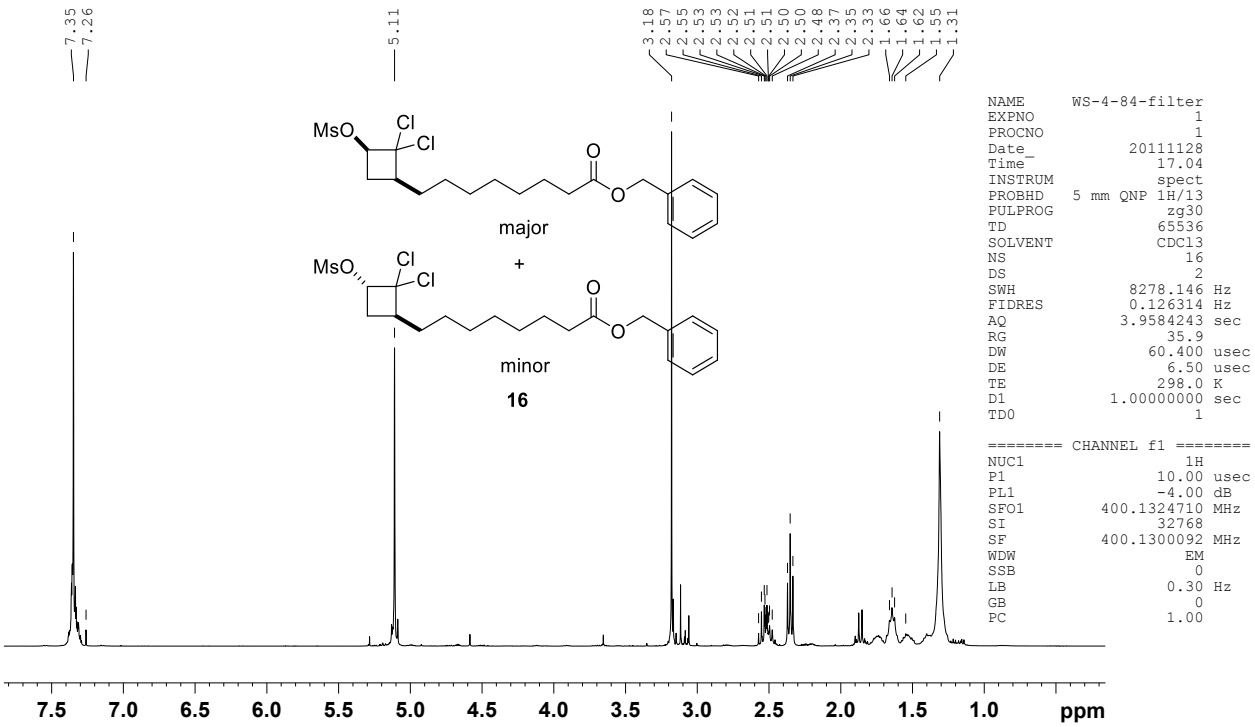


Figure S4.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of **16**.

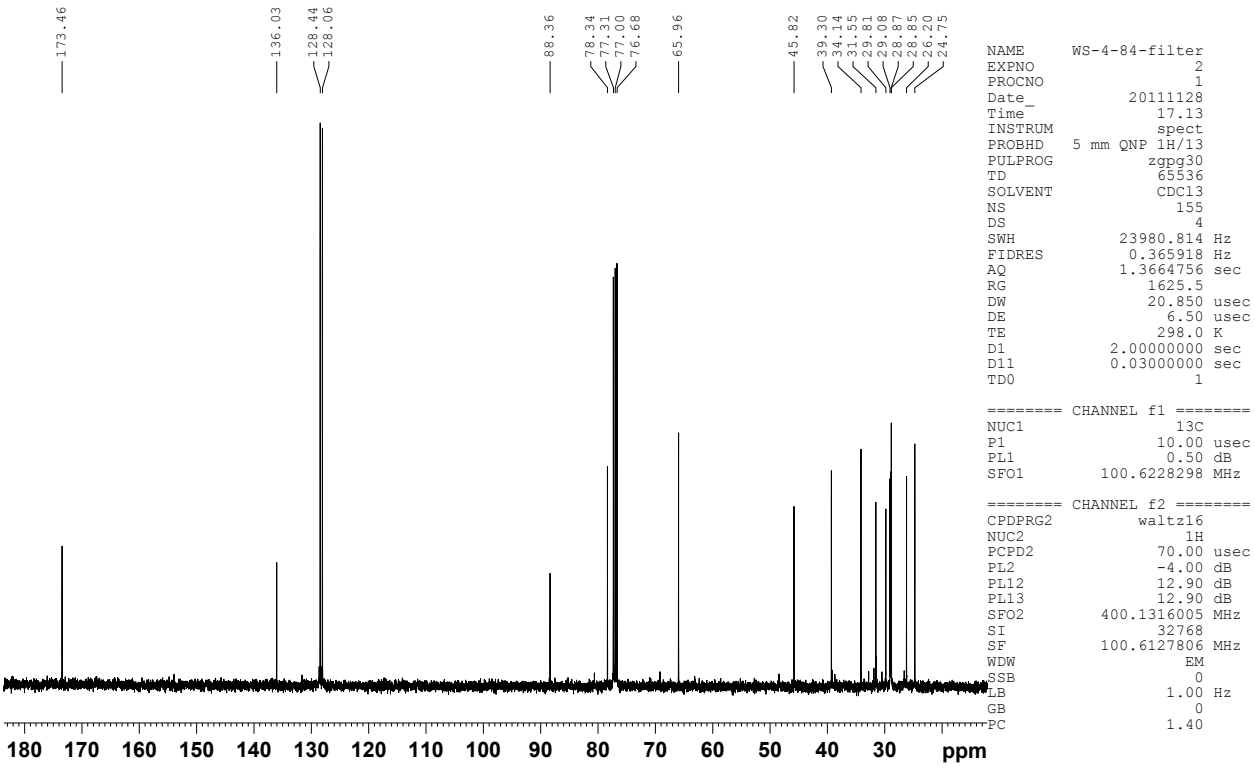


Figure S4.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of **16**.

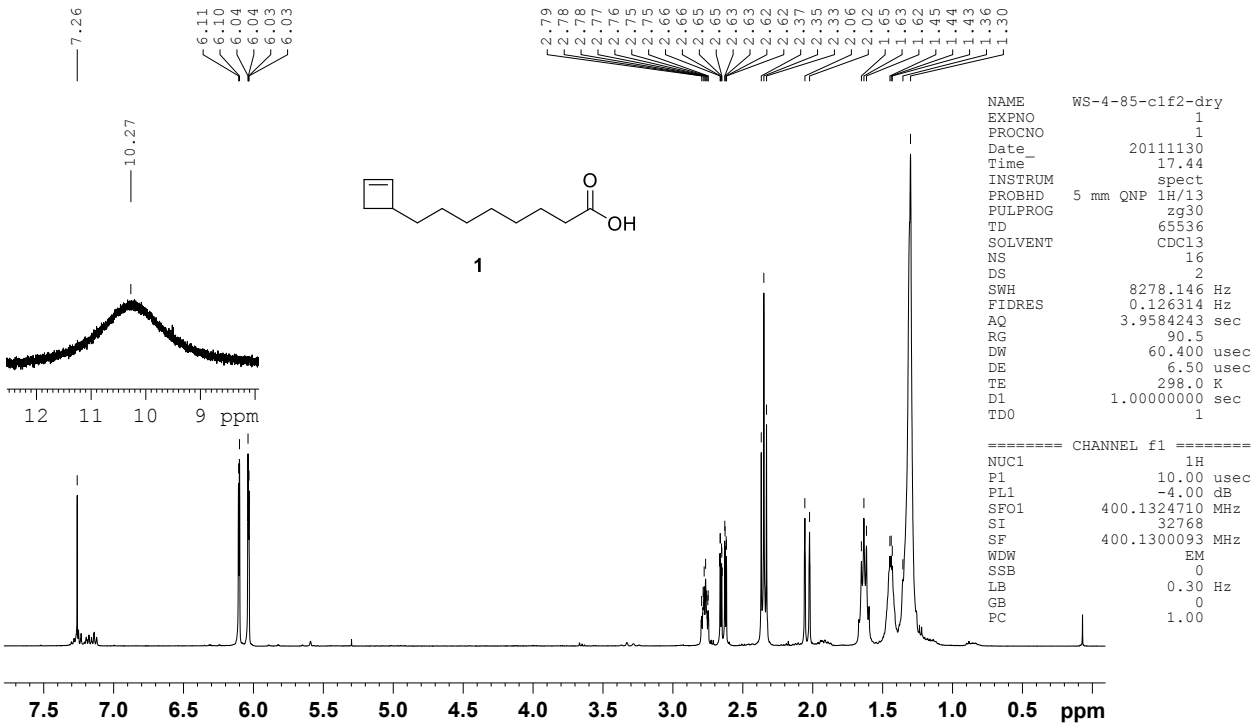


Figure S5.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of **1**.

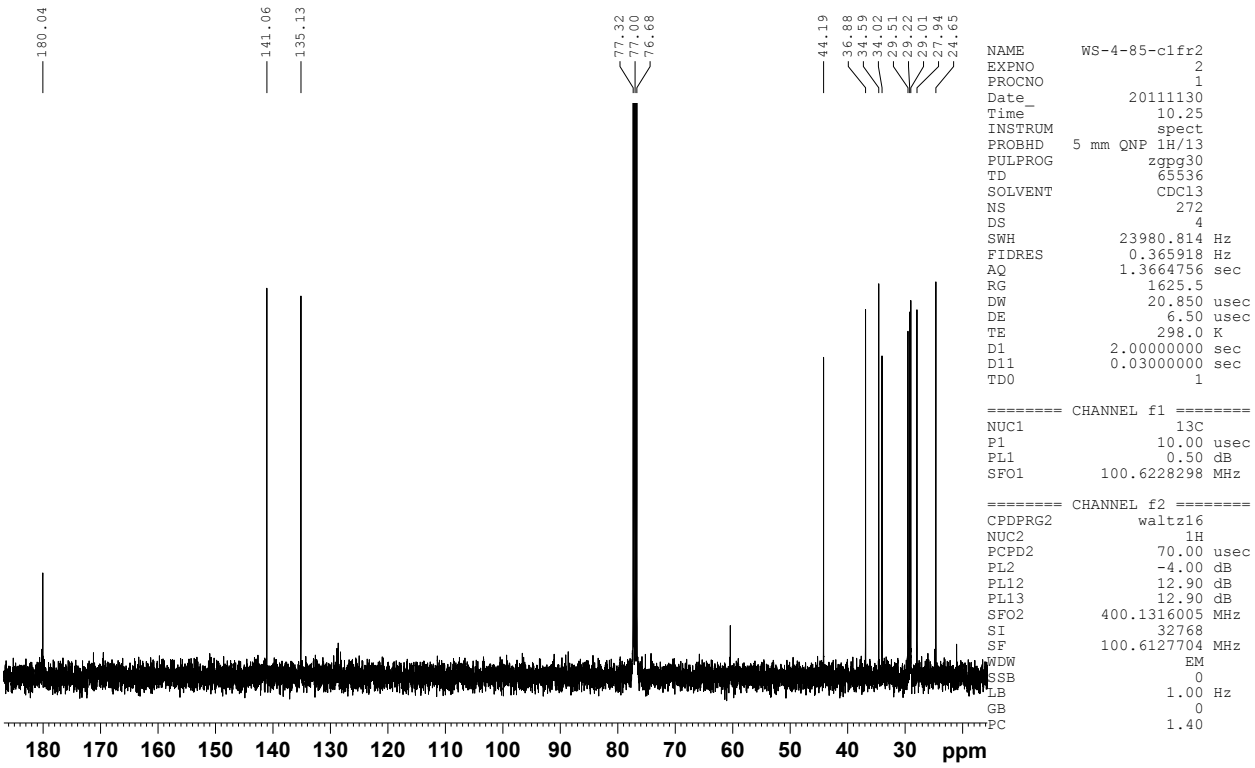


Figure S5.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of **1**.

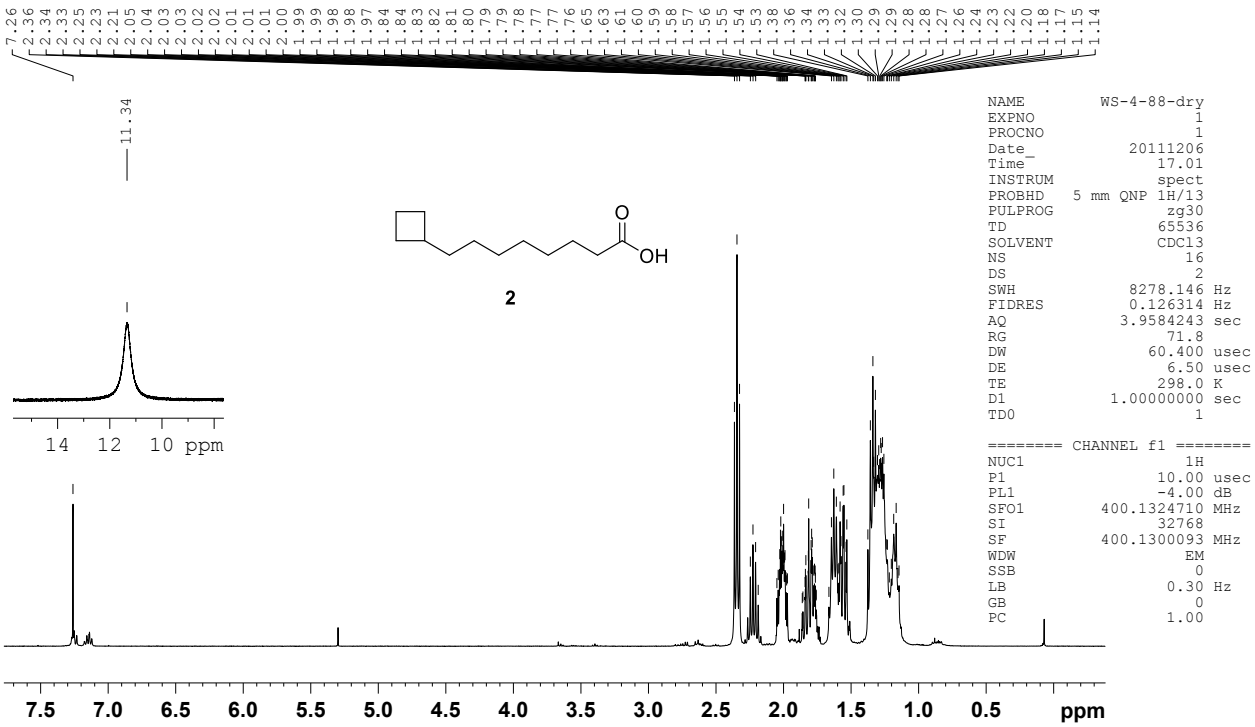


Figure S6.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of **2**.

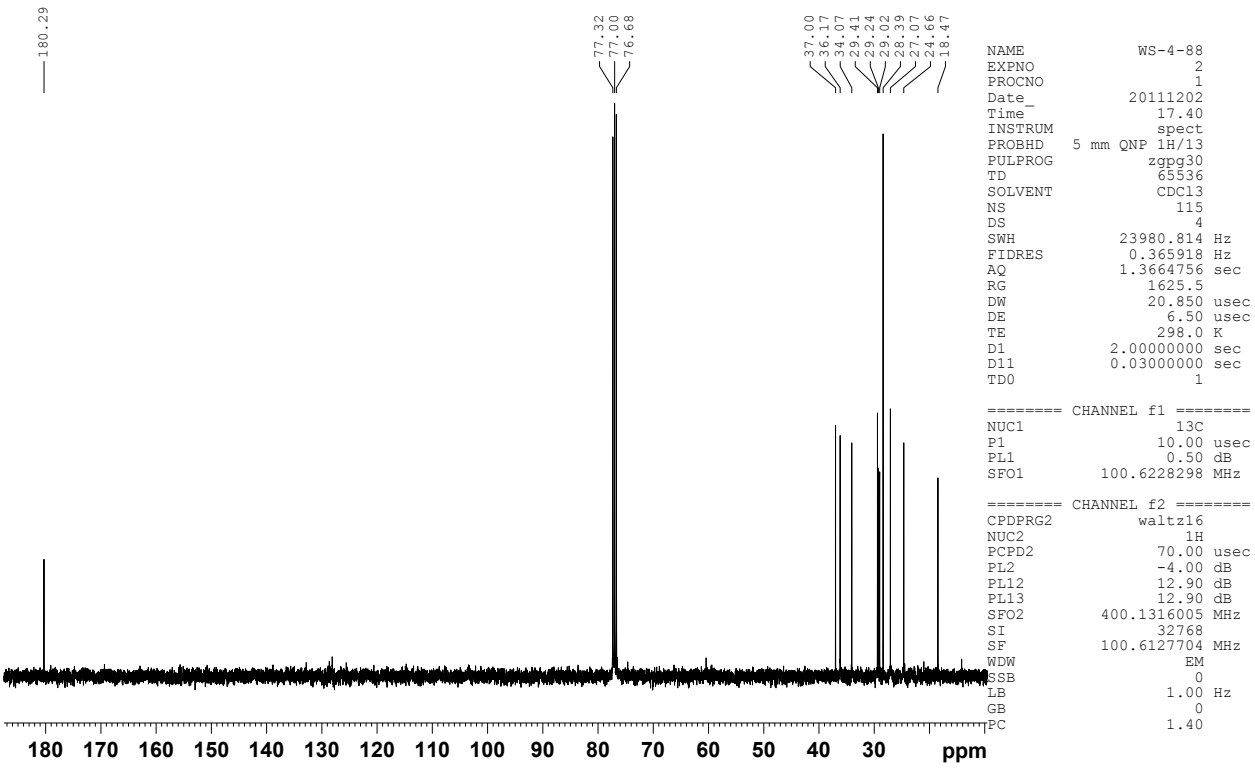


Figure S6.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of **2**.

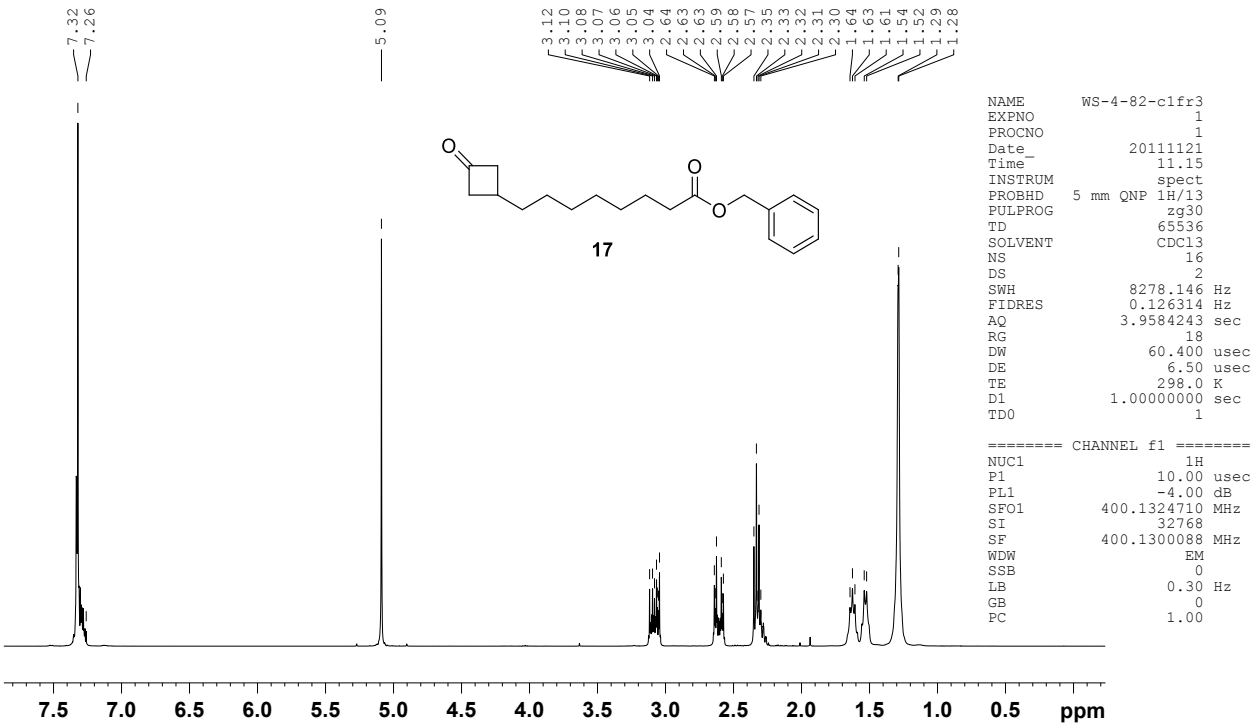


Figure S7.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of **17**.

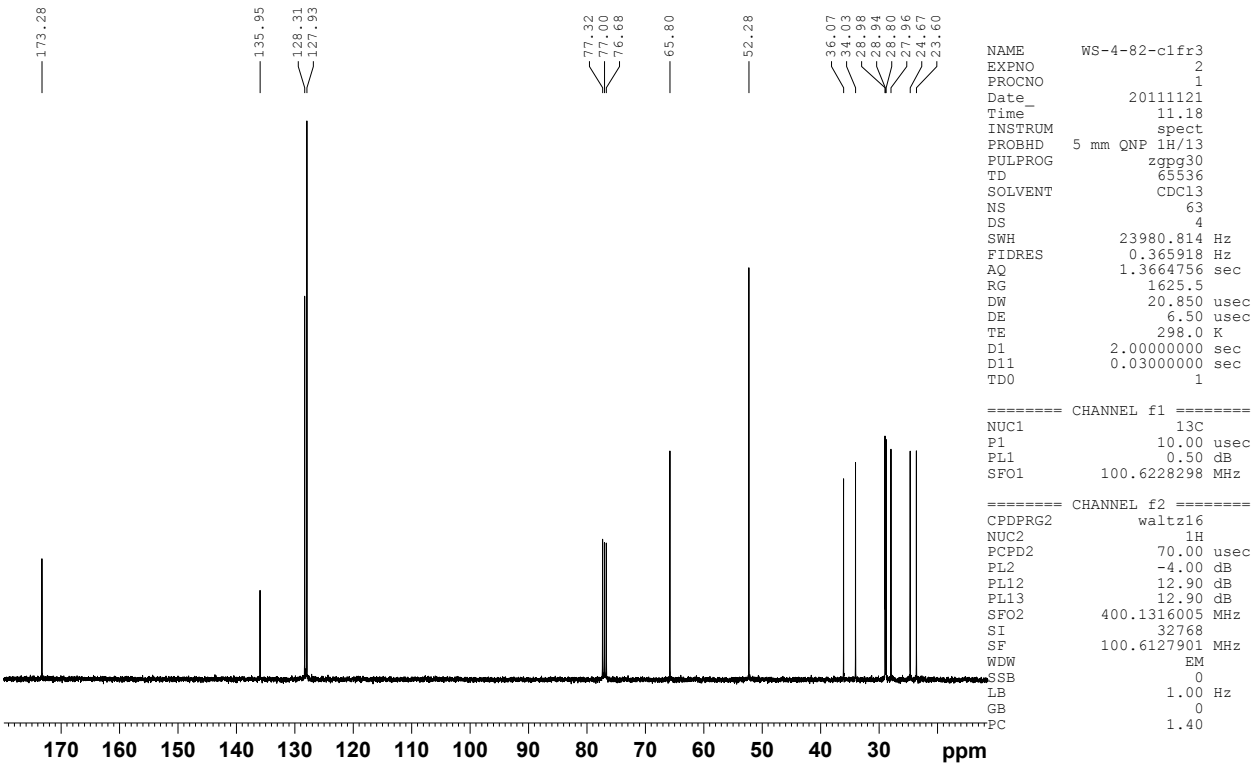


Figure S7.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of **17**.

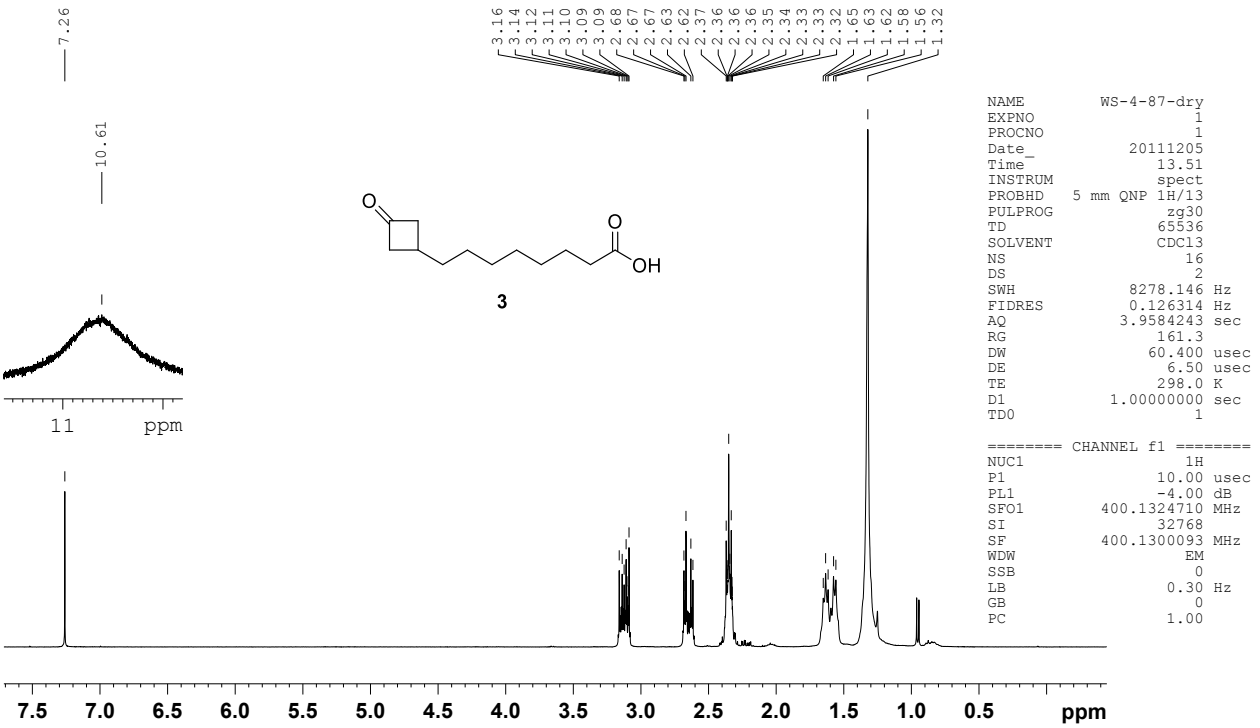


Figure S8.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of **3**.

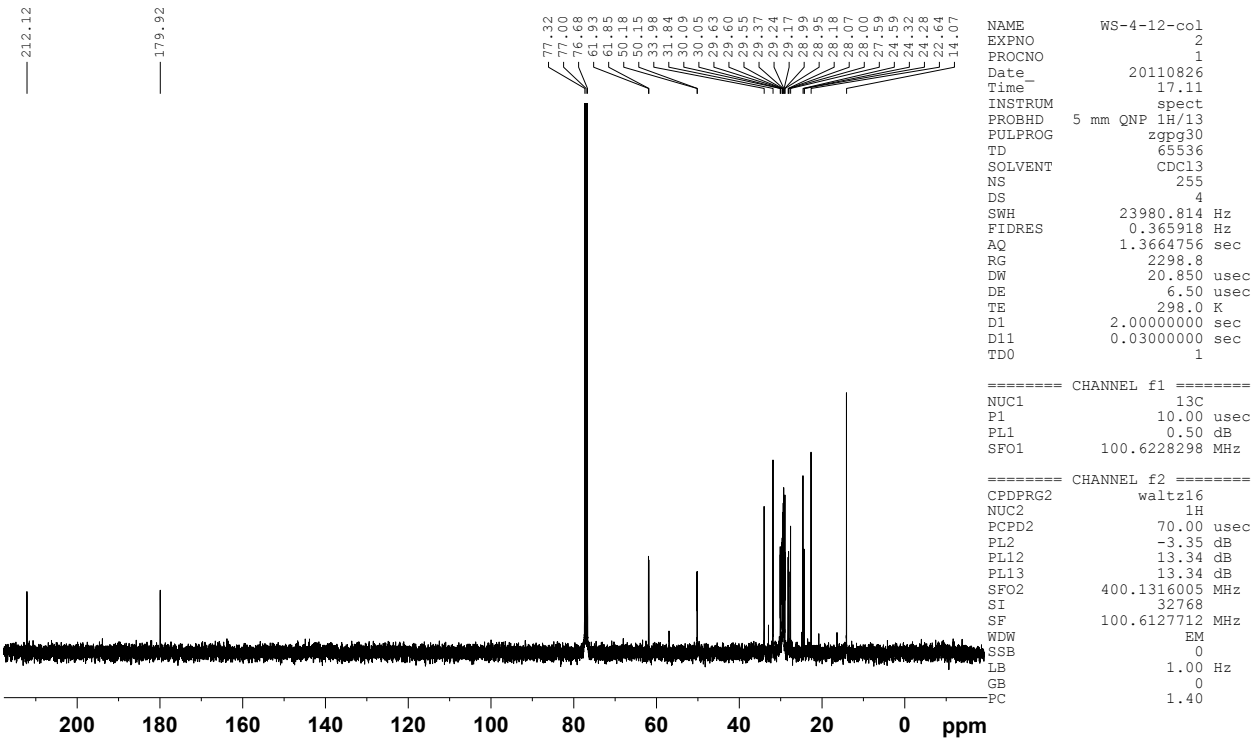


Figure S8.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of **3**.

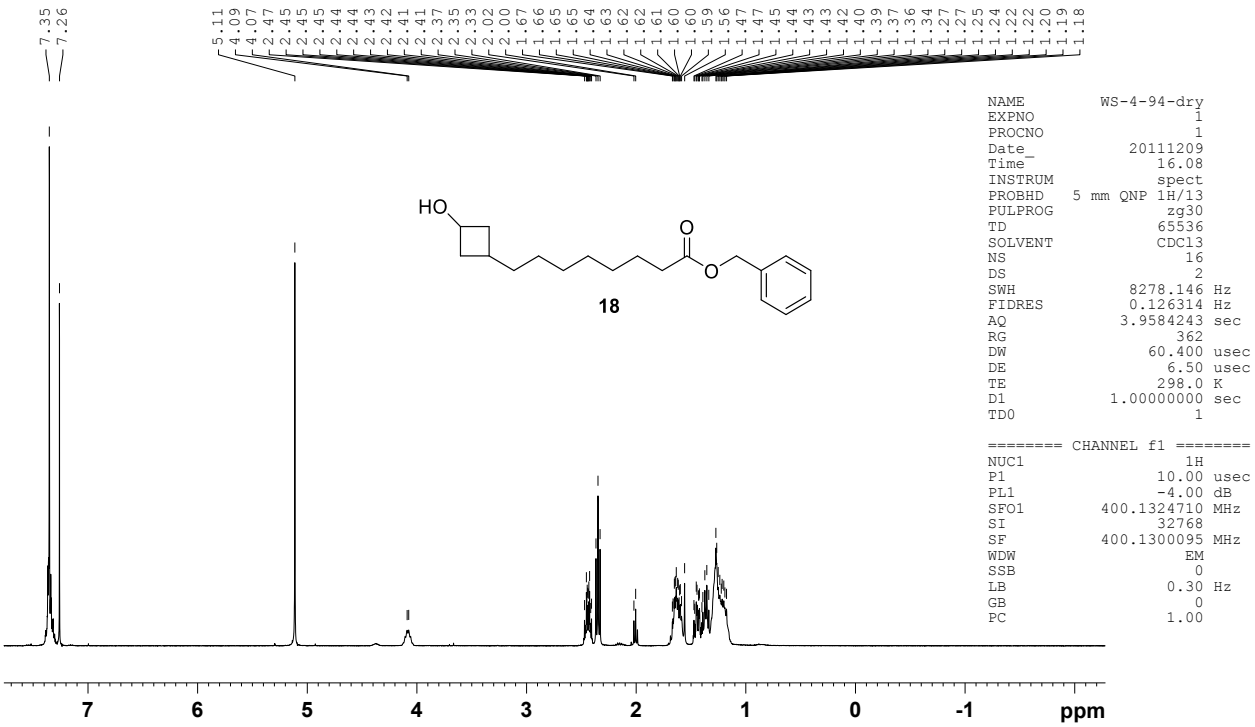


Figure S9.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of **18**.

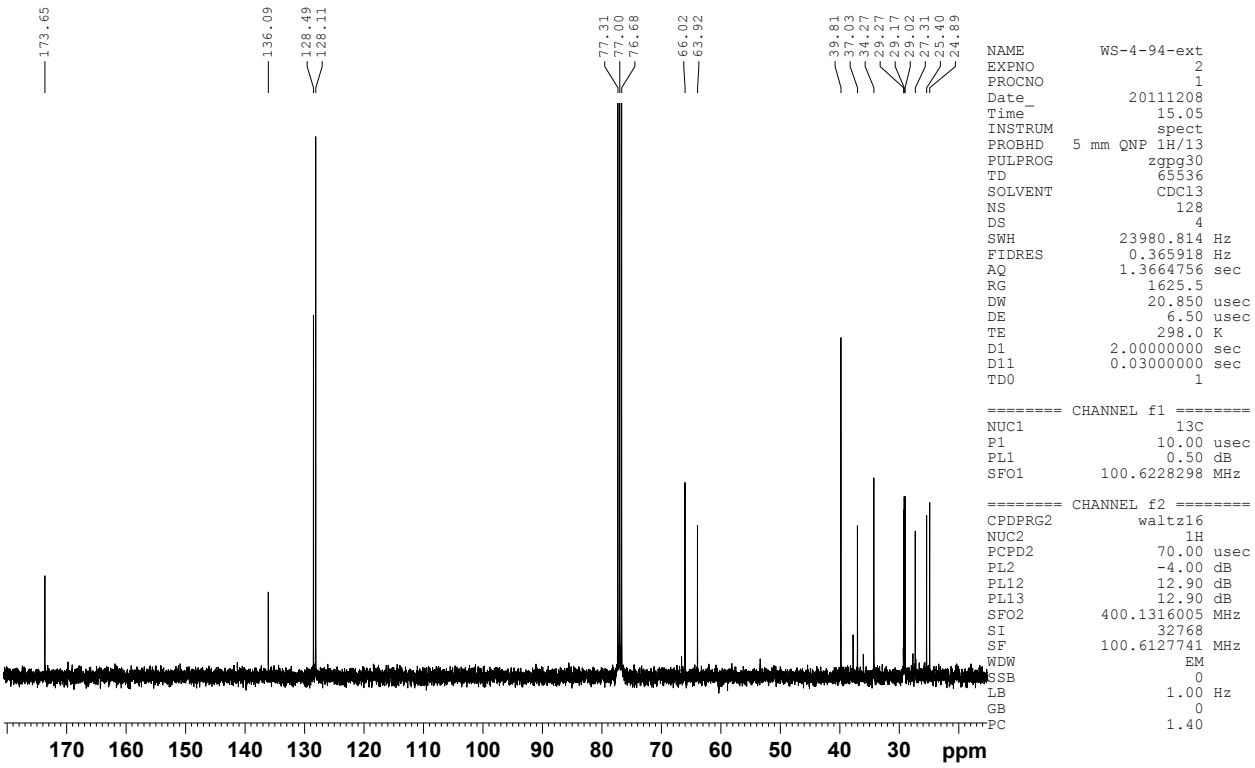


Figure S9.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of **18**.

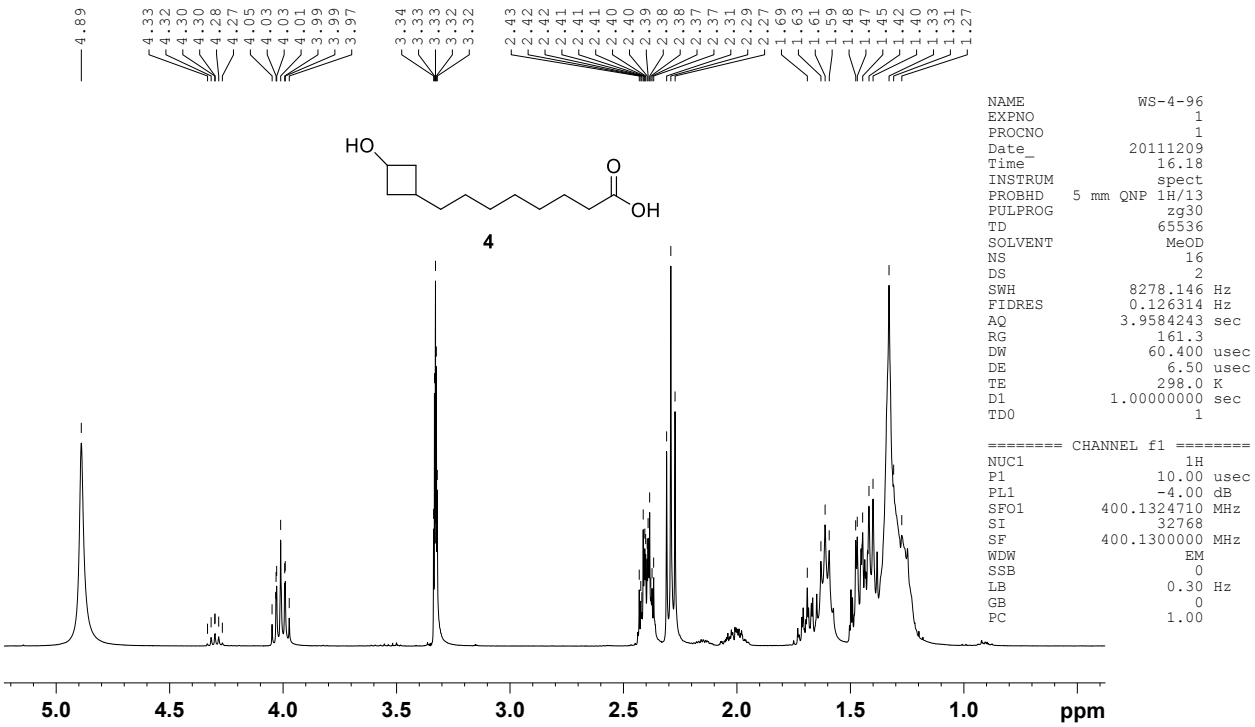


Figure S10.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of **4**.

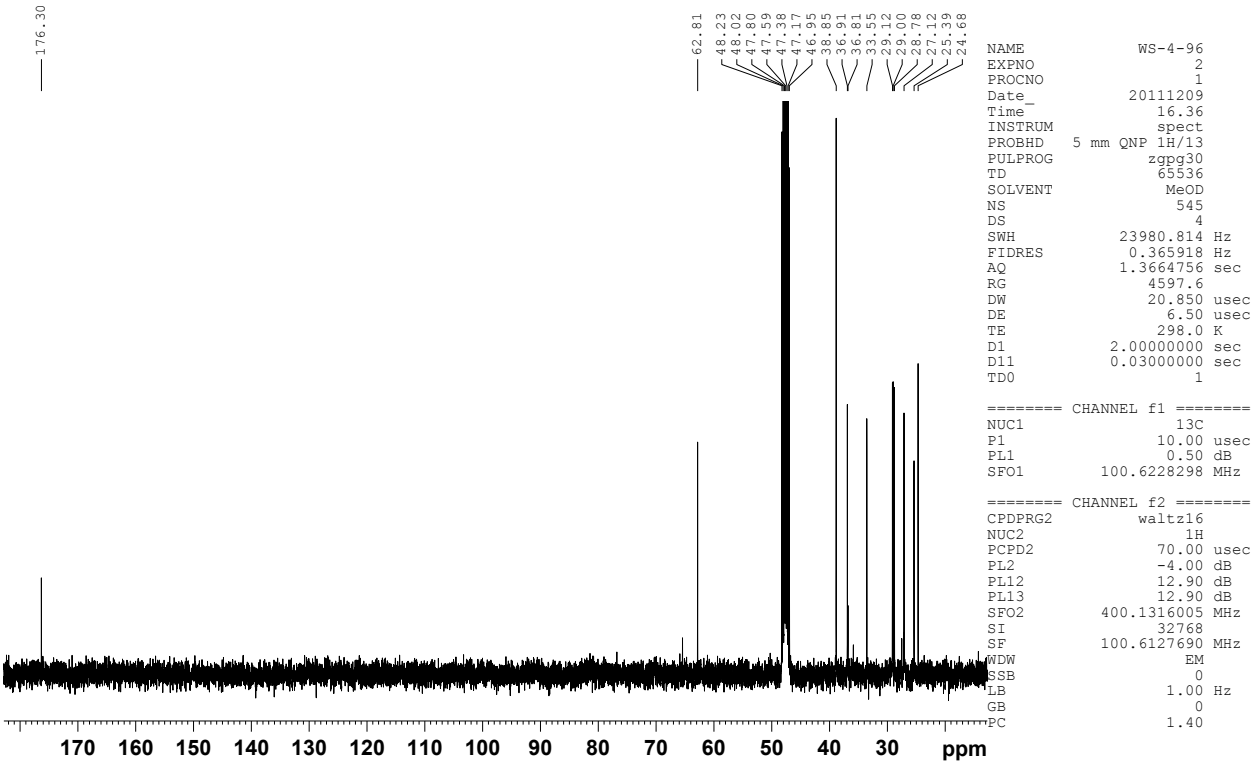


Figure S10.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of **4**.

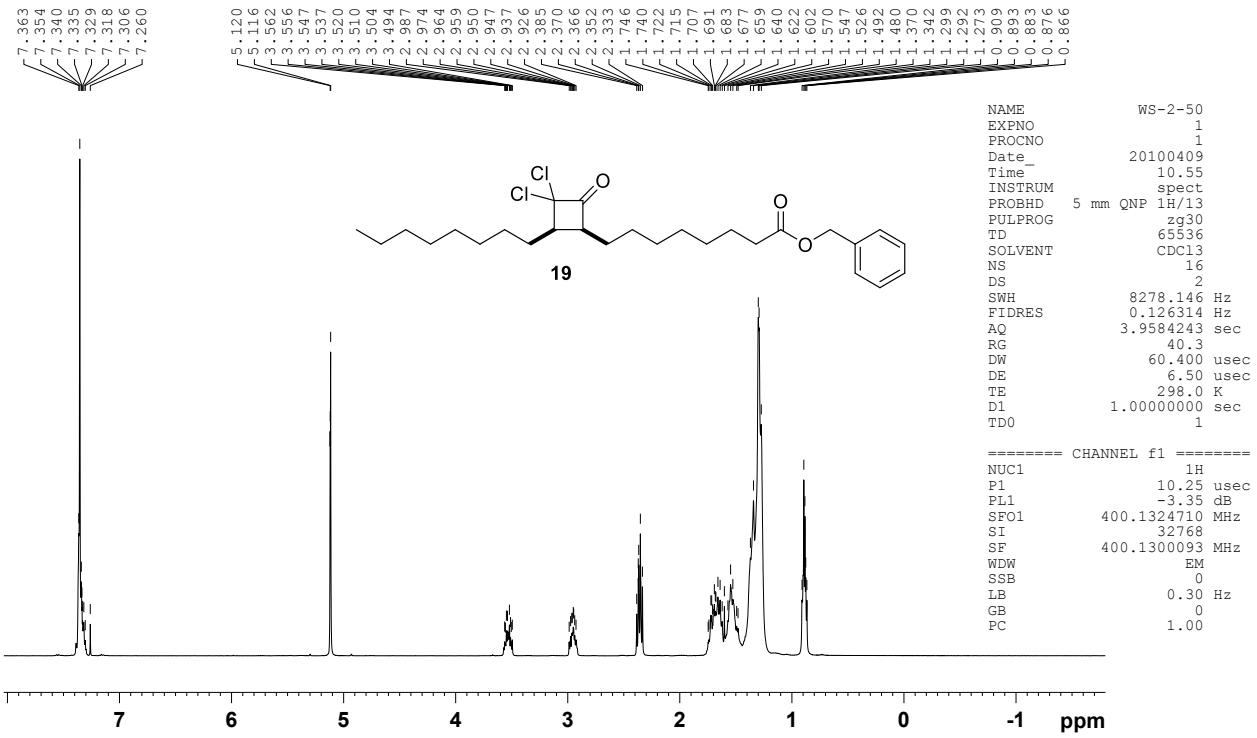


Figure S11.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of **19**.

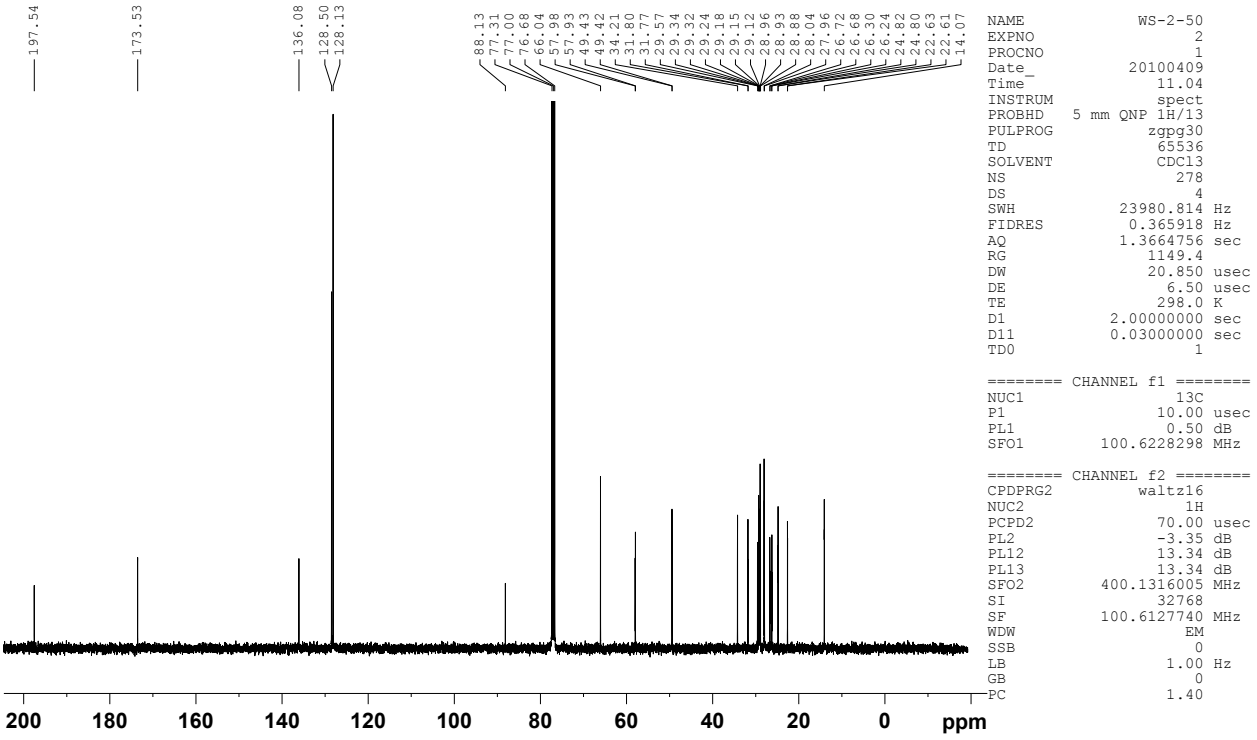


Figure S11.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of **19**.

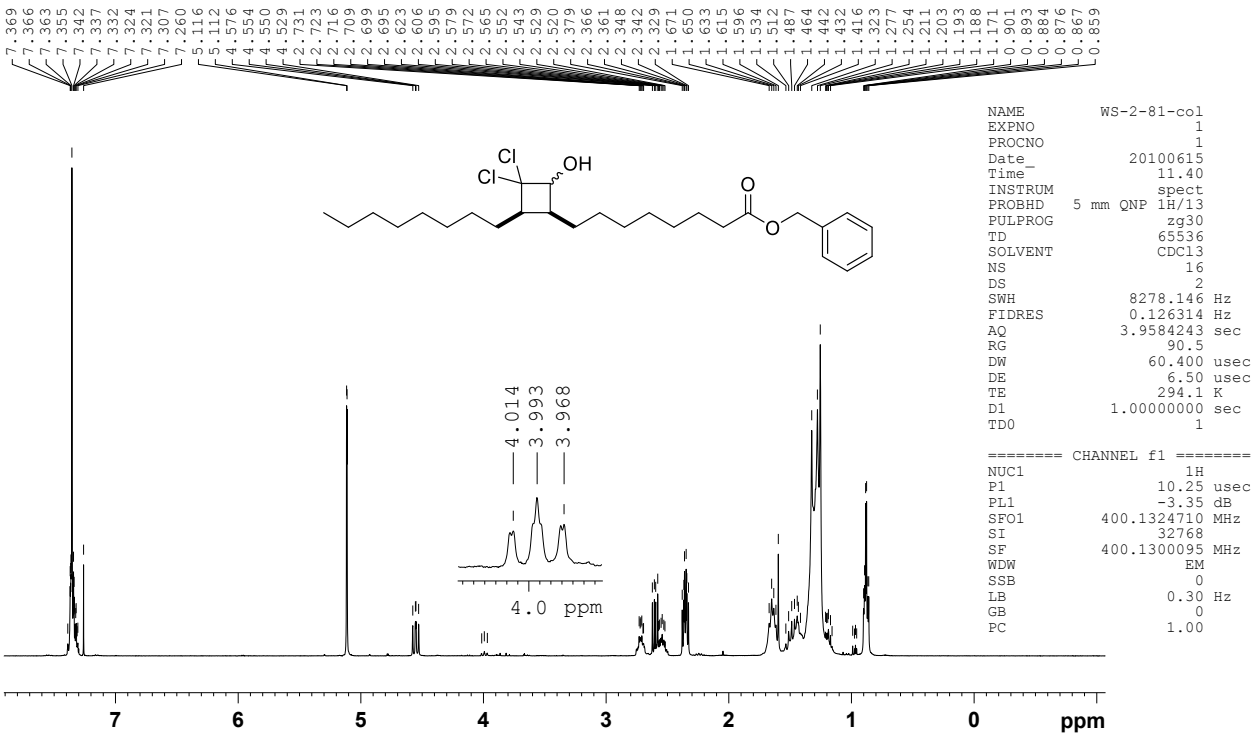


Figure S12.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of the crude.

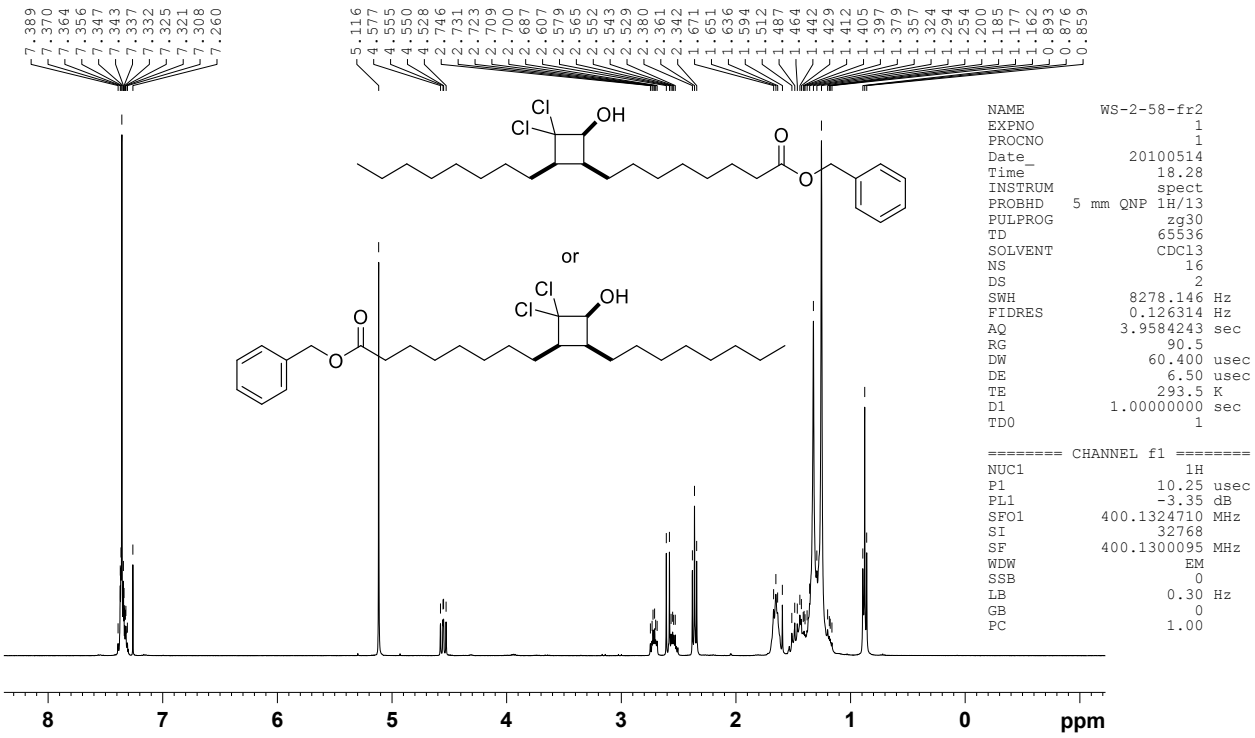


Figure S12.2. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of first eluting *cis* isomer.

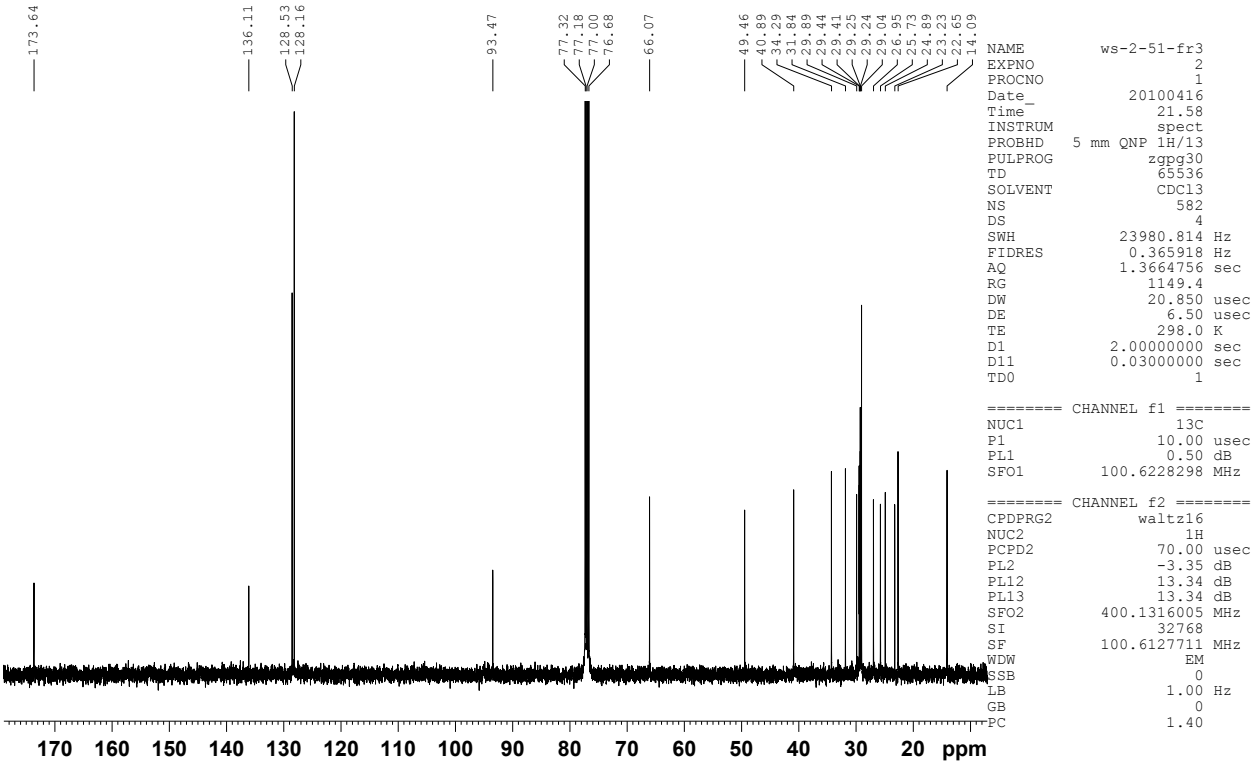


Figure S12.3. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of first eluting *cis* isomer.

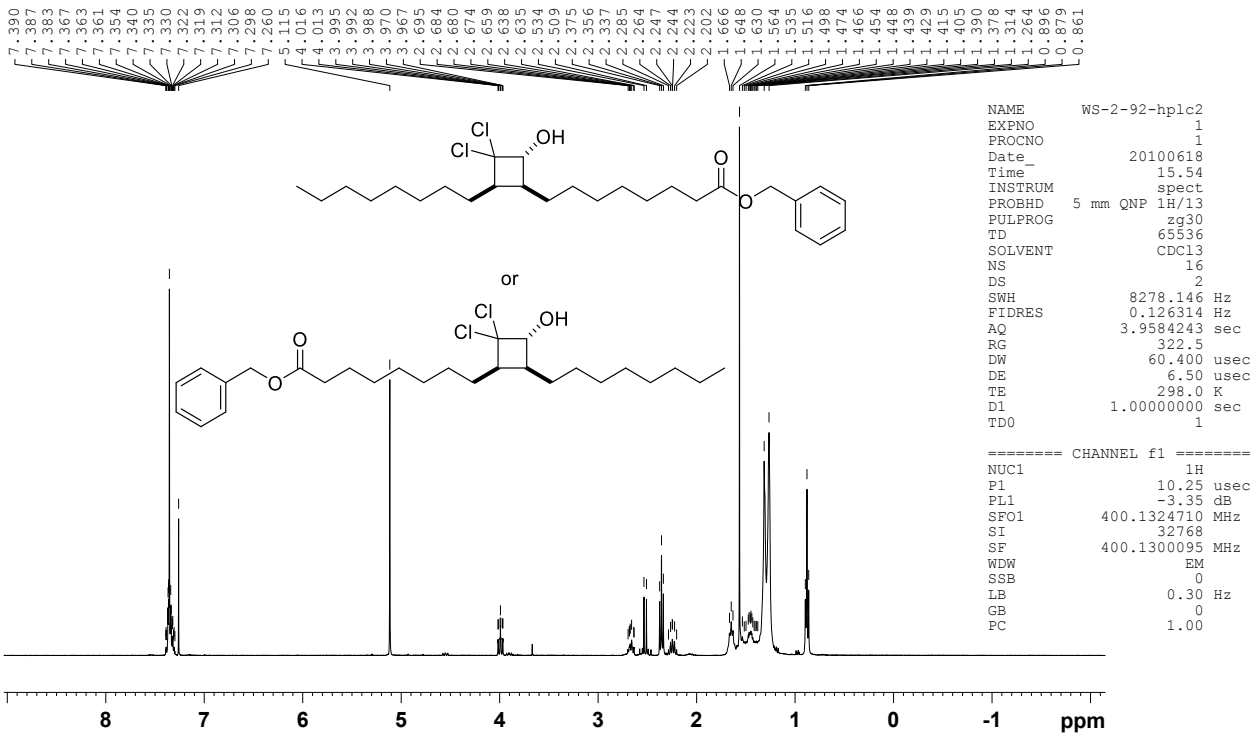


Figure S12.4. [1]H-NMR (400 MHz, chloroform-*d*) spectrum of first eluting *trans* isomer.

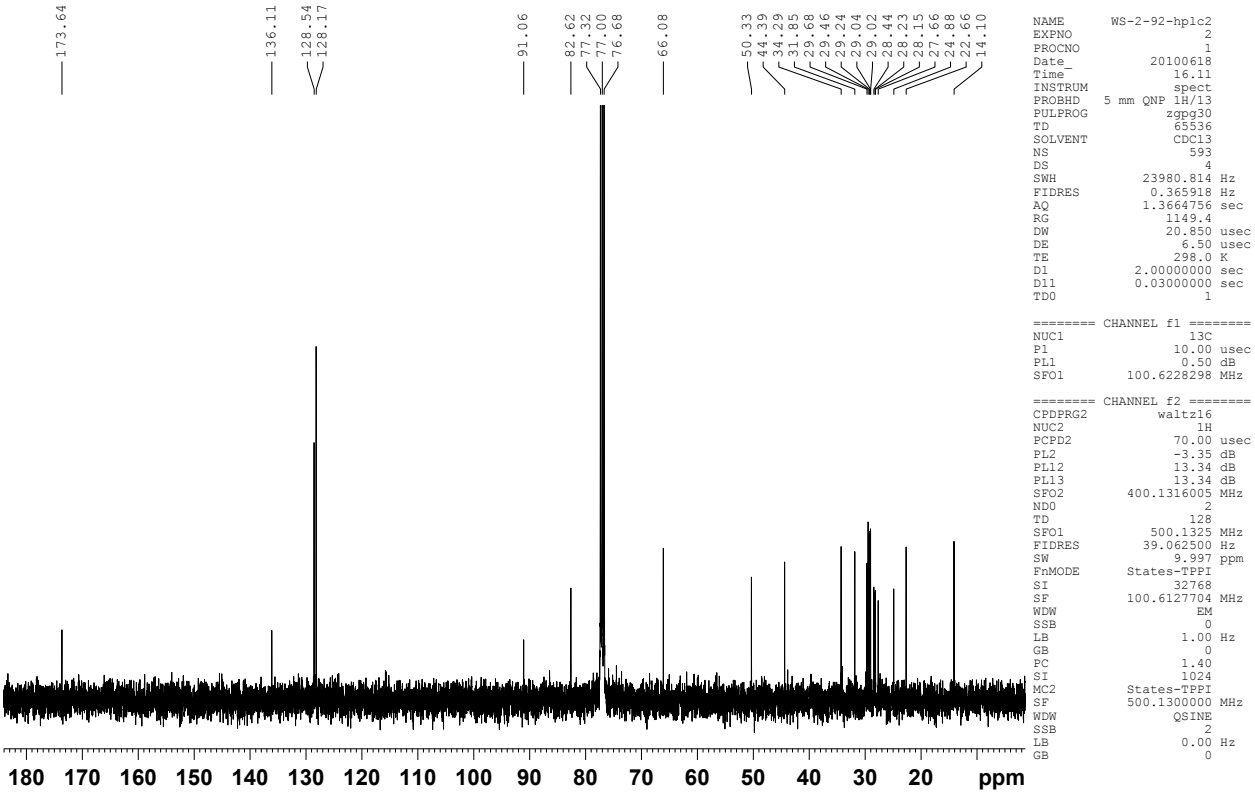


Figure S12.5. [13]C-NMR (100 MHz, chloroform-*d*) spectrum of first eluting *tran* isomer.

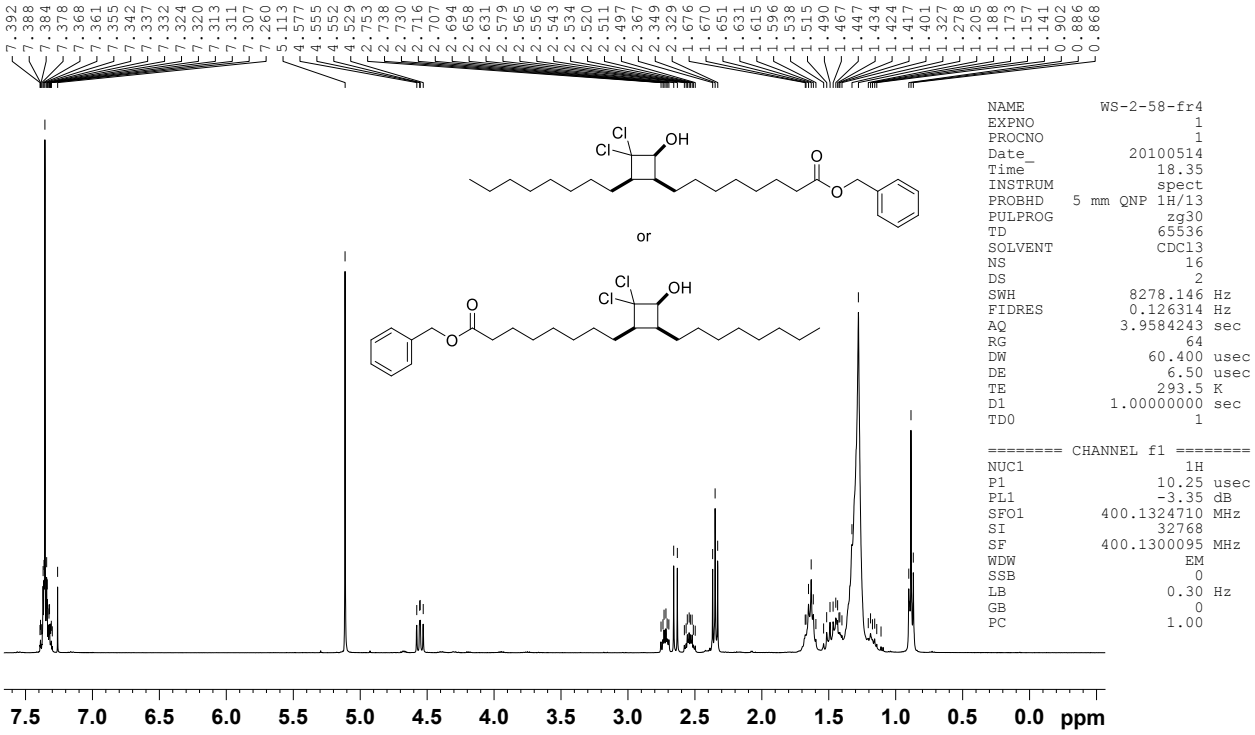


Figure S12.6. ^{1}H-NMR(400 MHz, chloroform-*d*) spectrum of second eluting *cis* isomer.

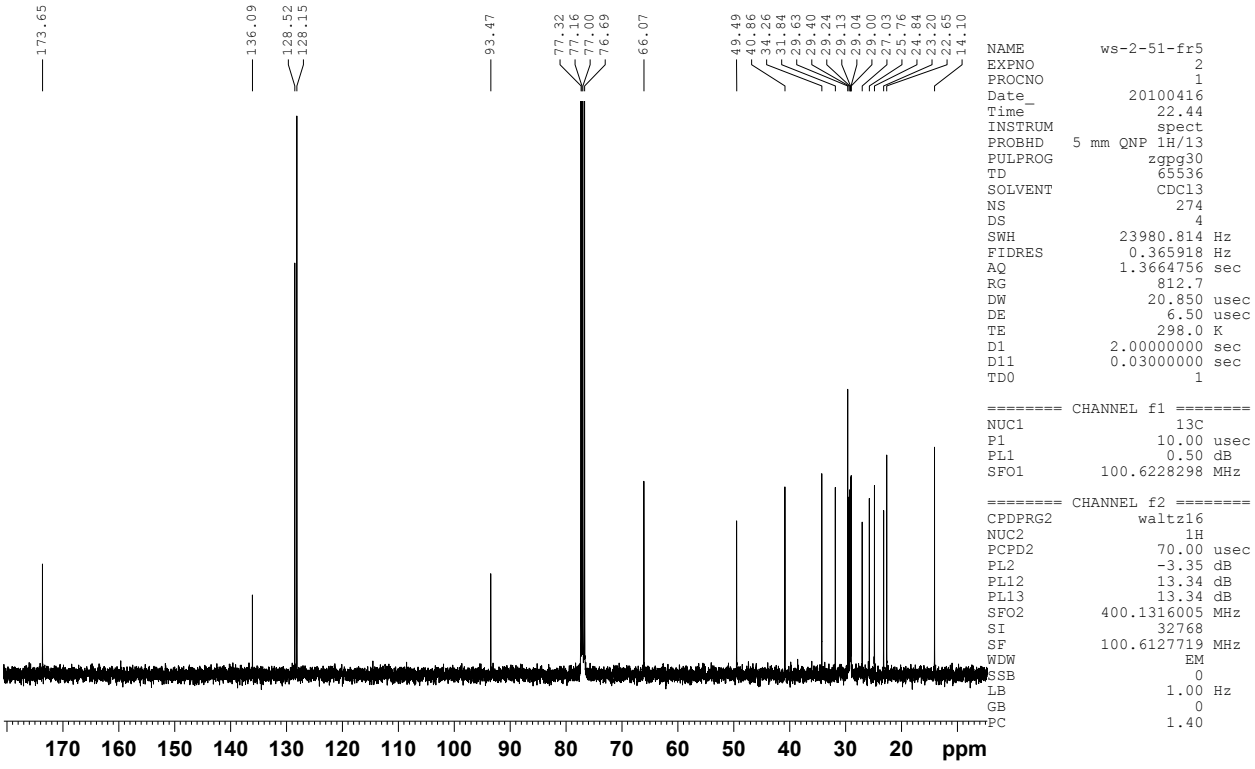


Figure S12.7. ^{13}C-NMR(100 MHz, chloroform-*d*)spectrum of second eluting *cis* isomer.

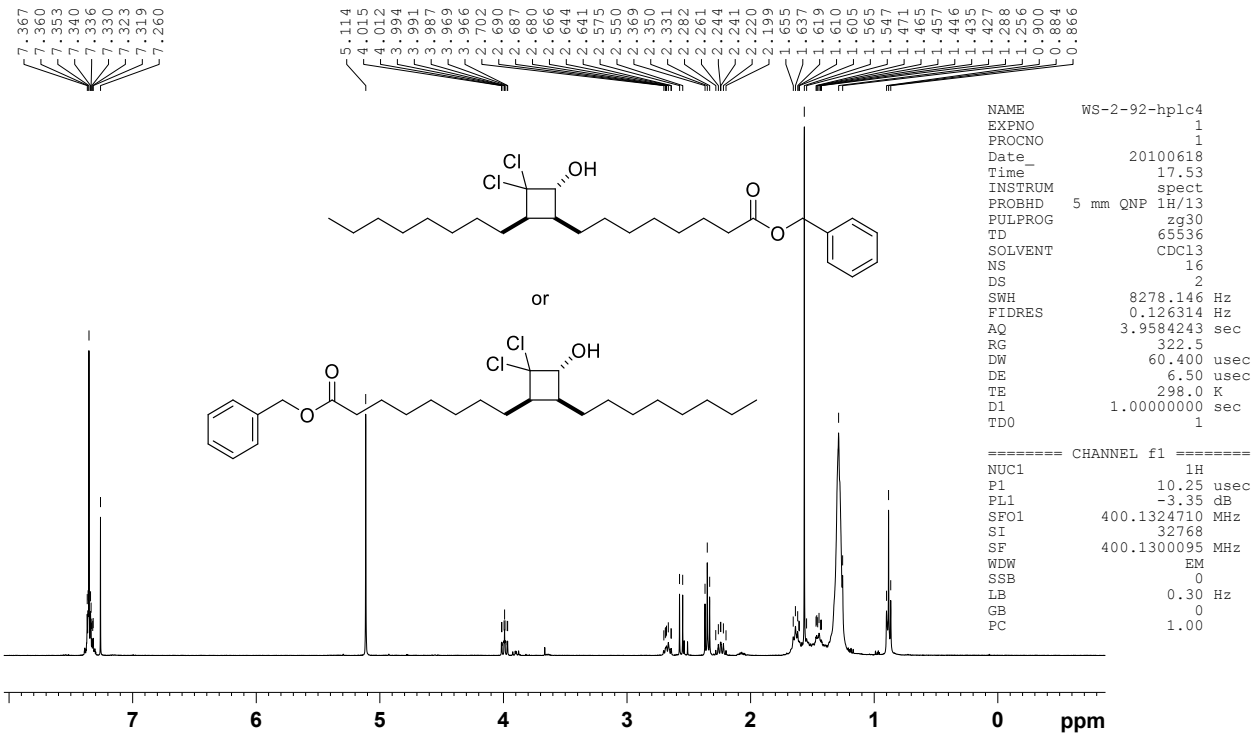


Figure S12.8. ^{1}H-NMR(400MHz, chloroform-*d*)spectrum of second eluting *trans* isomer.

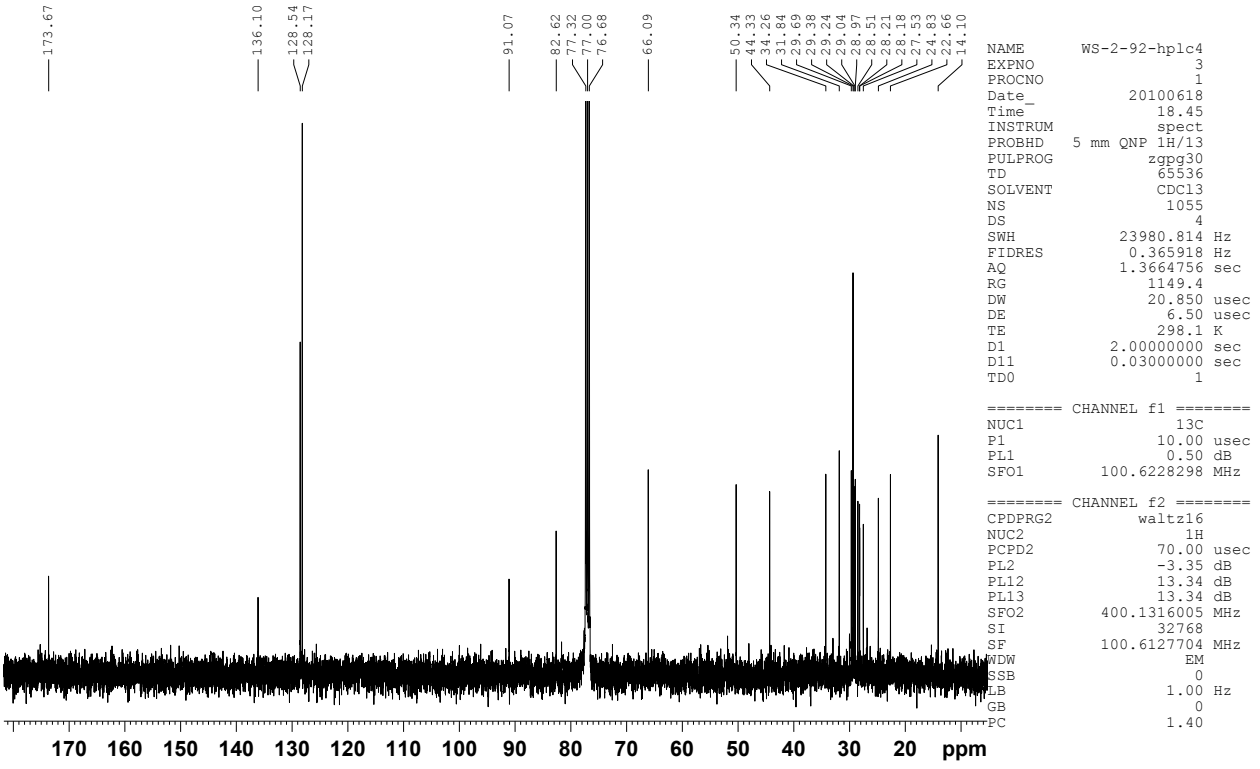


Figure S12.9. ^{13}C-NMR(100MHz,chloroform-*d*) spectrum of second eluting *trans* isomer.

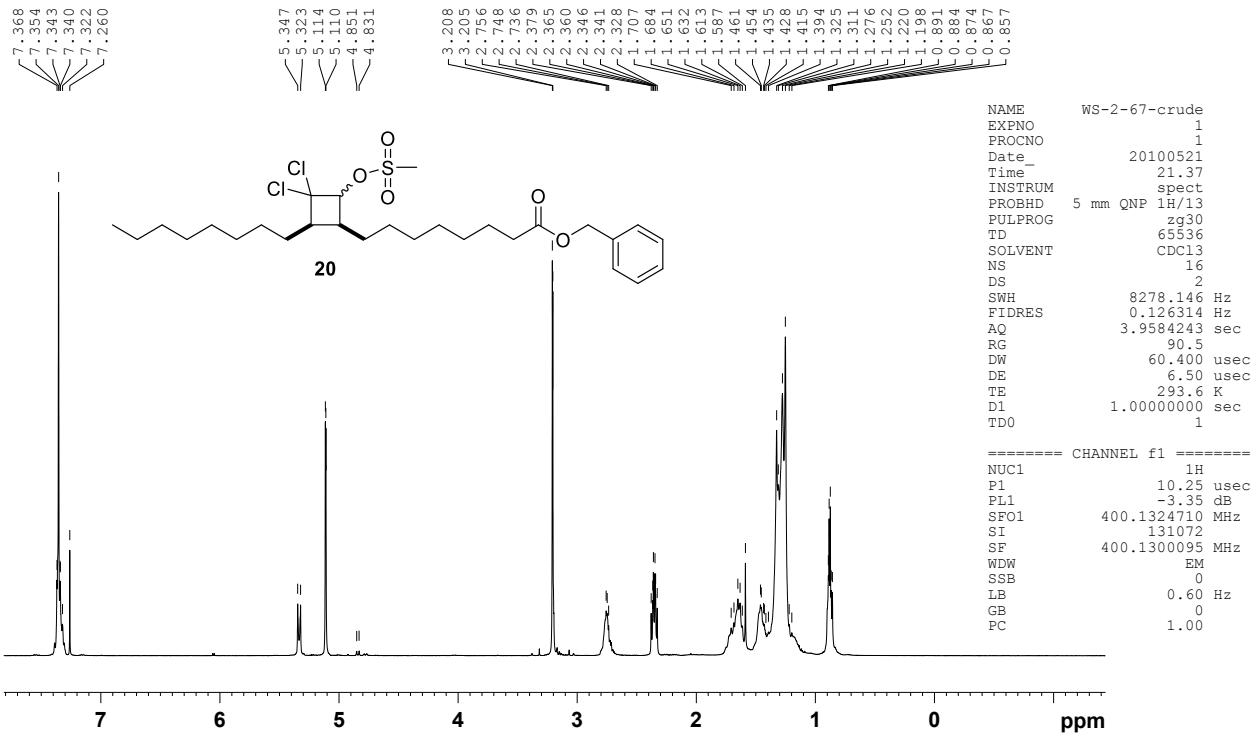


Figure S13.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of crude **20**.

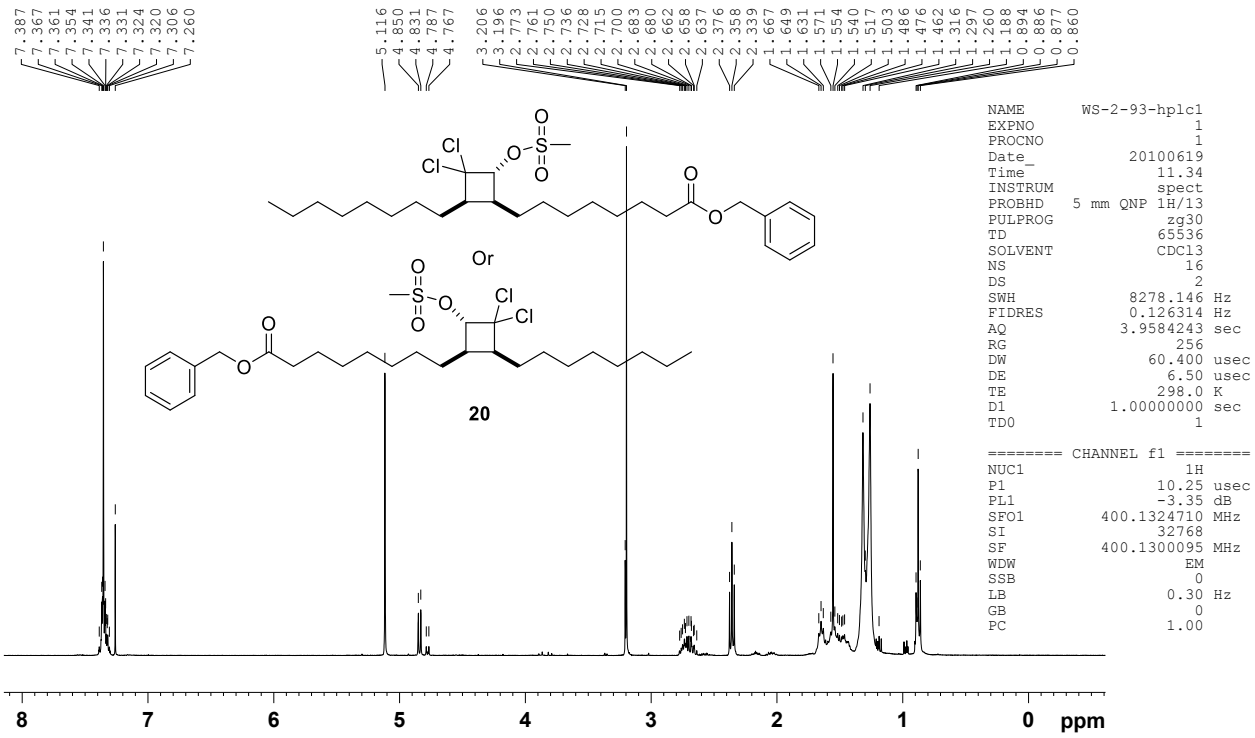


Figure S13.2. [1]H-NMR (400 MHz, chloroform-*d*) spectrum of first eluting *trans* isomer of **20**.

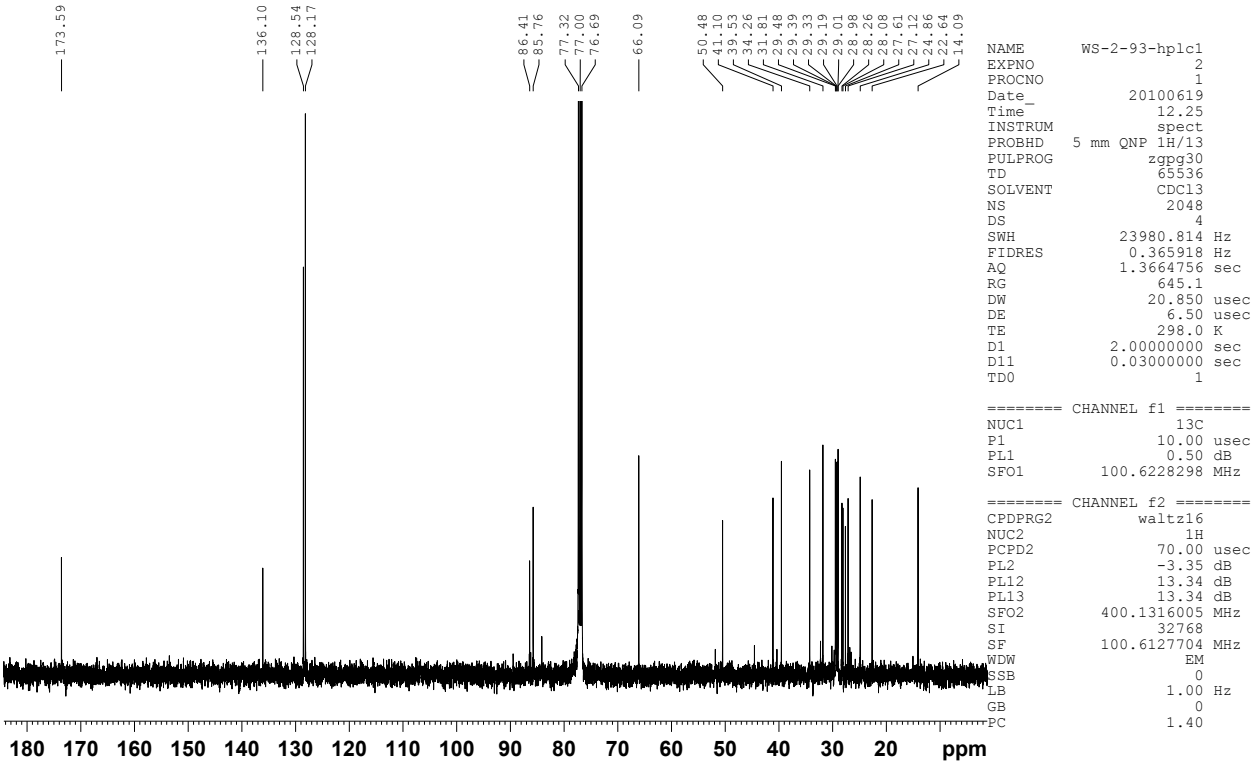
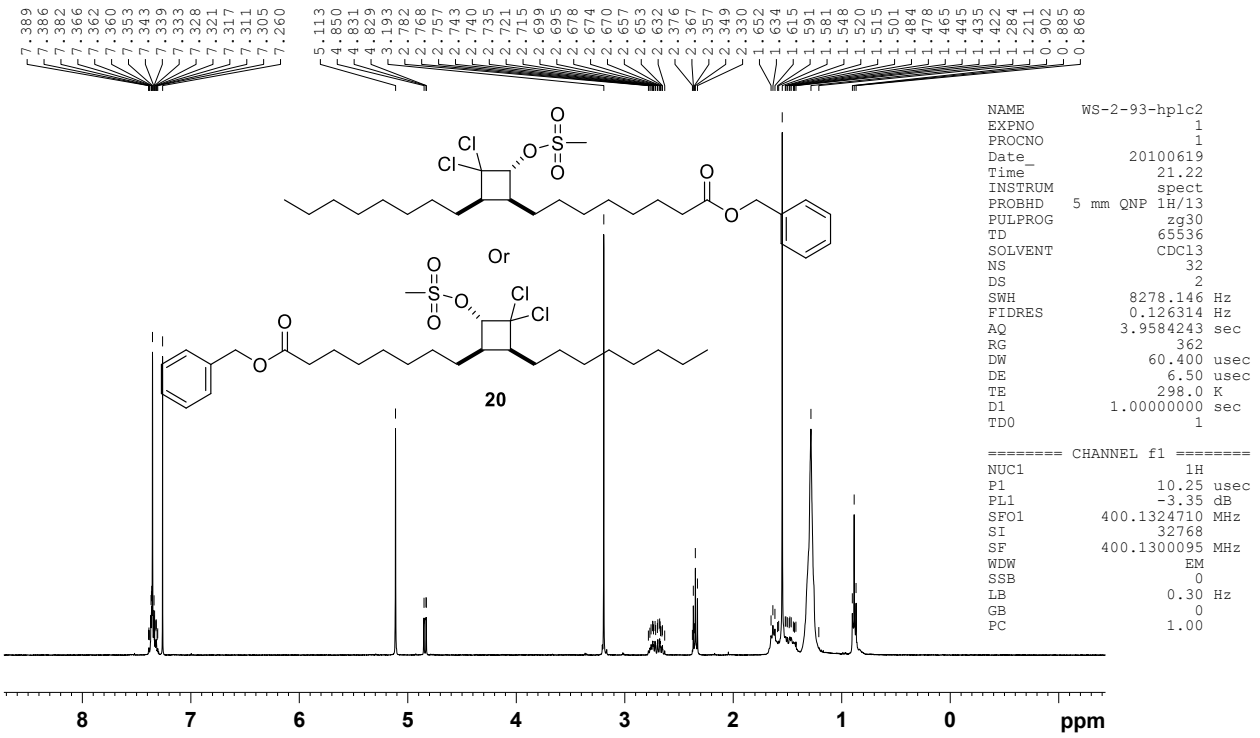
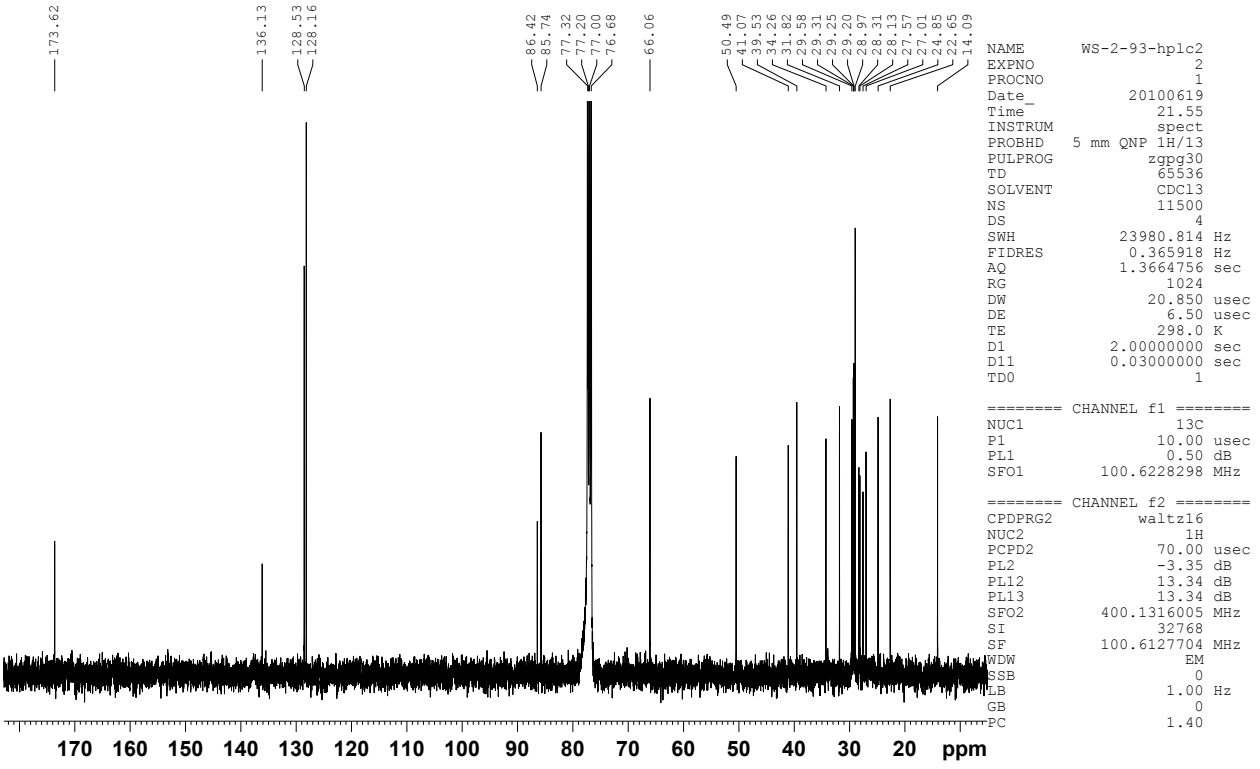


Figure S13.3. [13]C-NMR(100 MHz, chloroform-*d*) spectrum of first eluting *trans* isomer of **20**.



FigureS13.4. [1]H-NMR(400MHz,chloroform-*d*)spectrum of second eluting *trans* isomer of **20**.



FigureS13.5.[13]C-NMR(100MHz,chloroform-*d*)spectrum of second eluting *trans* isomer of **20**.

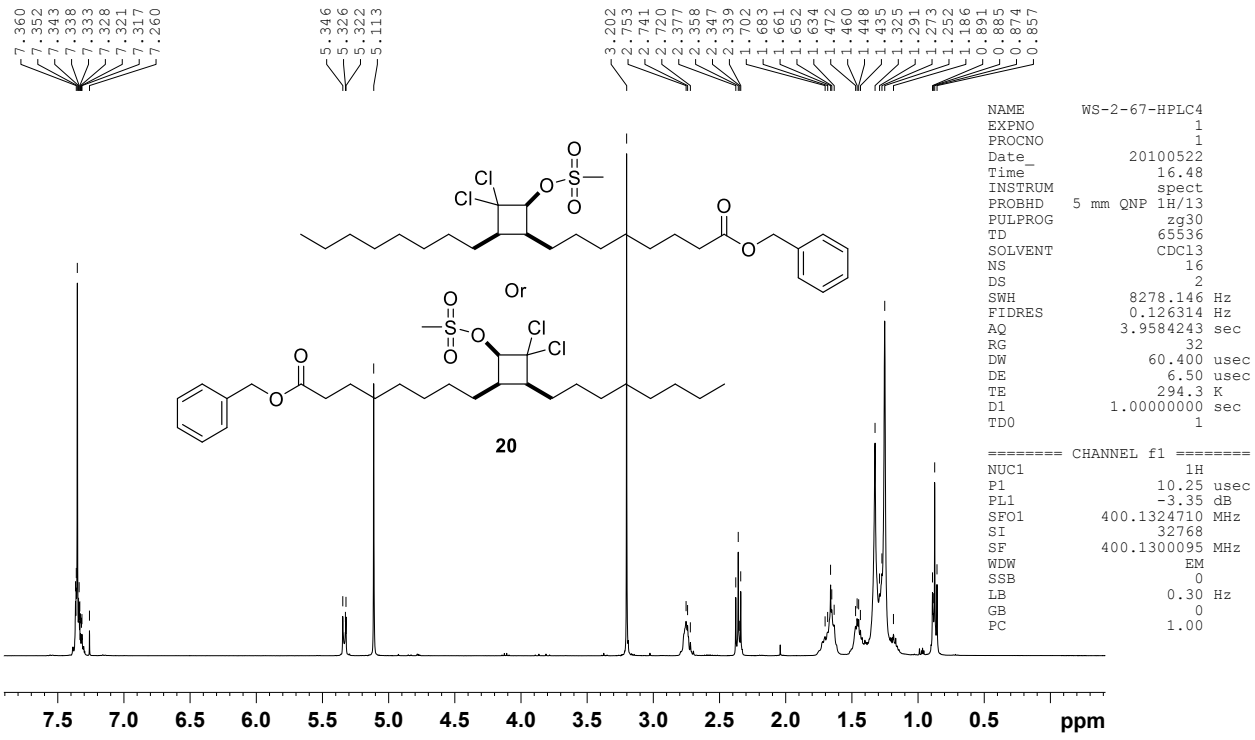


Figure S13.6. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of first eluting *cis* isomer of **20**.

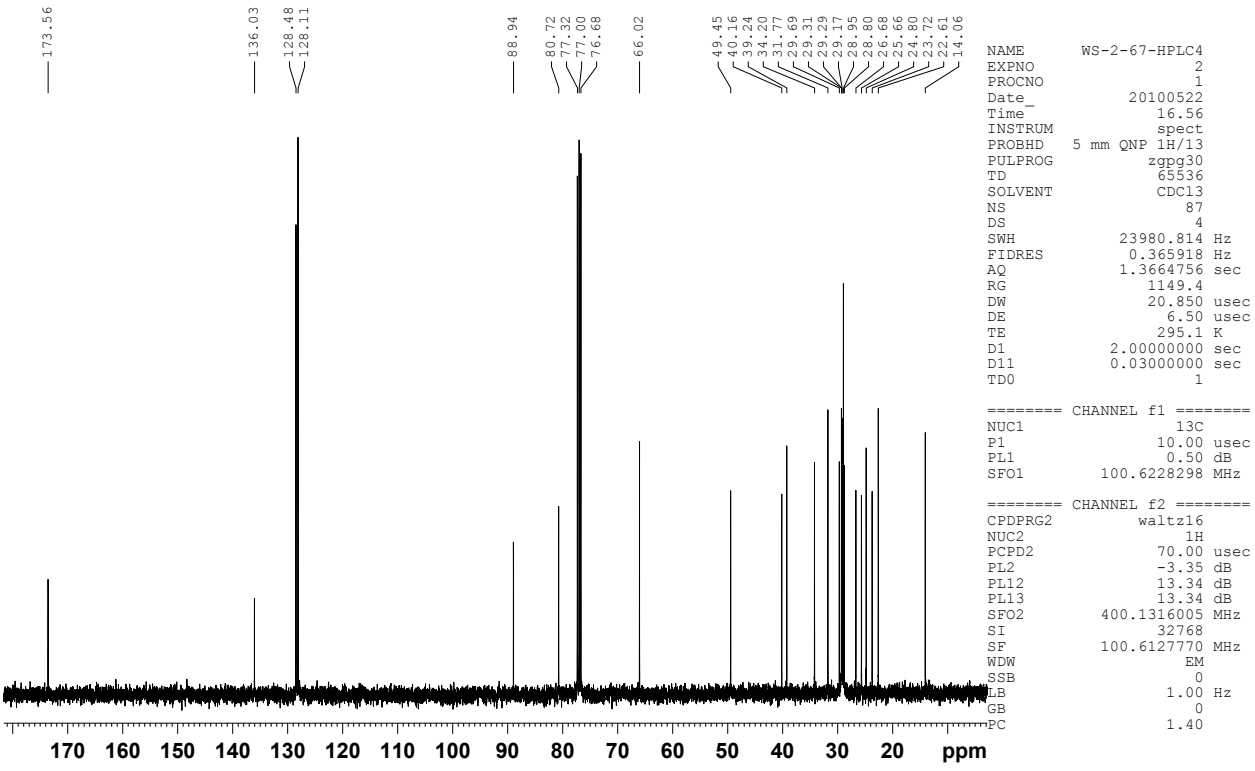


Figure S13.7. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of first eluting *cis* isomer of **20**.

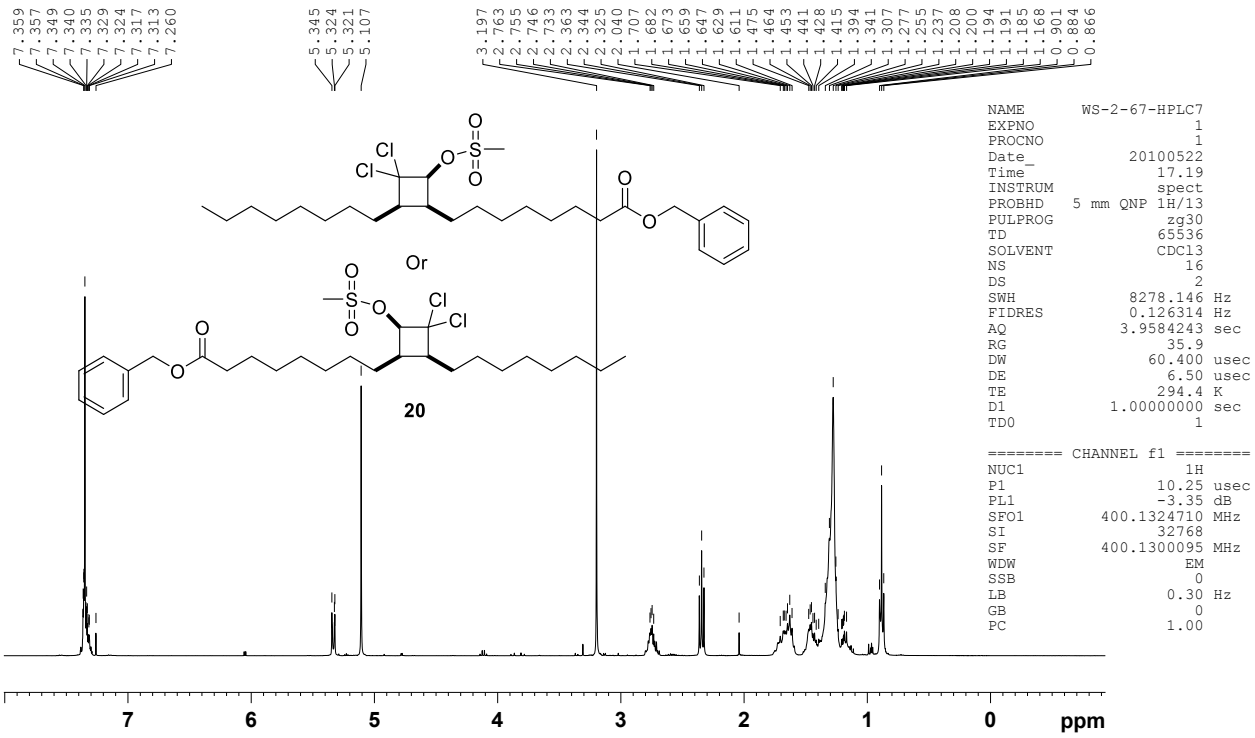


Figure S13.8. [1]H-NMR(400 MHz, chloroform-*d*) spectrum of second eluting *cis* isomer of **20**.

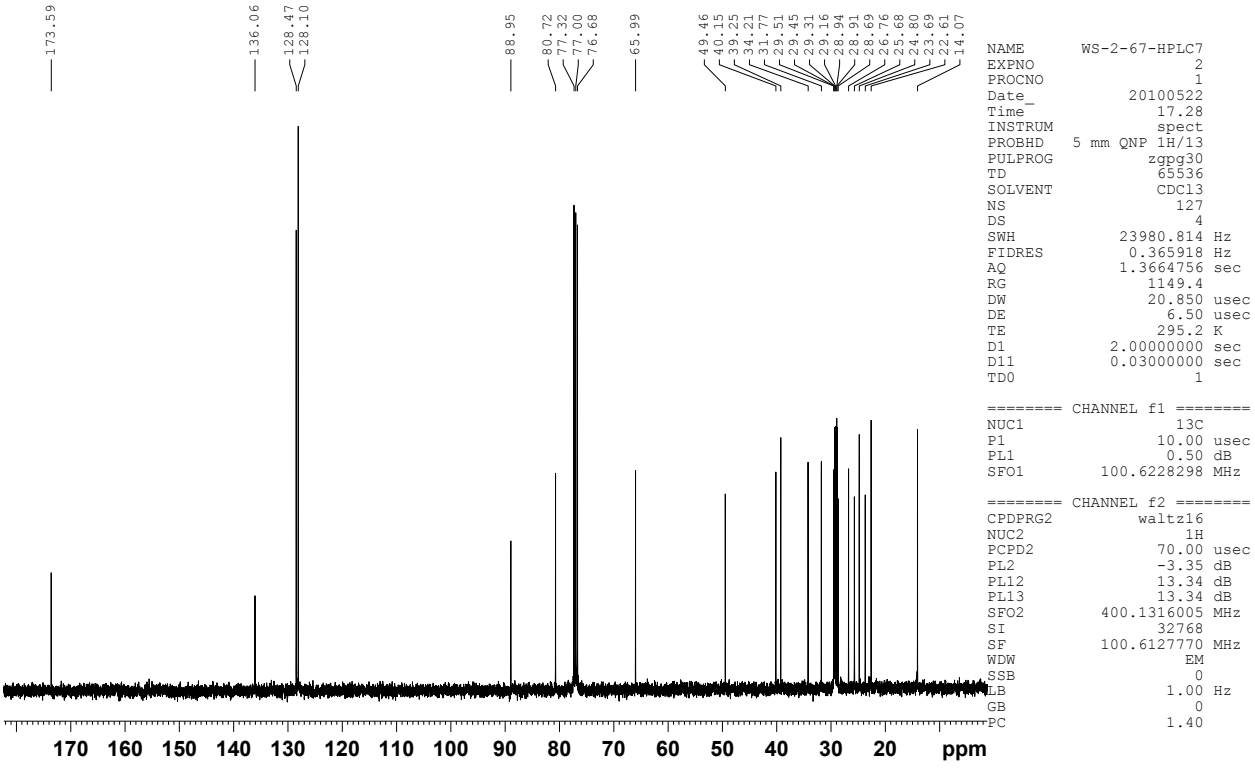


Figure S13.9. [13]C-NMR(100 MHz, chloroform-*d*)spectrum of second eluting *cis* isomer of **20**.

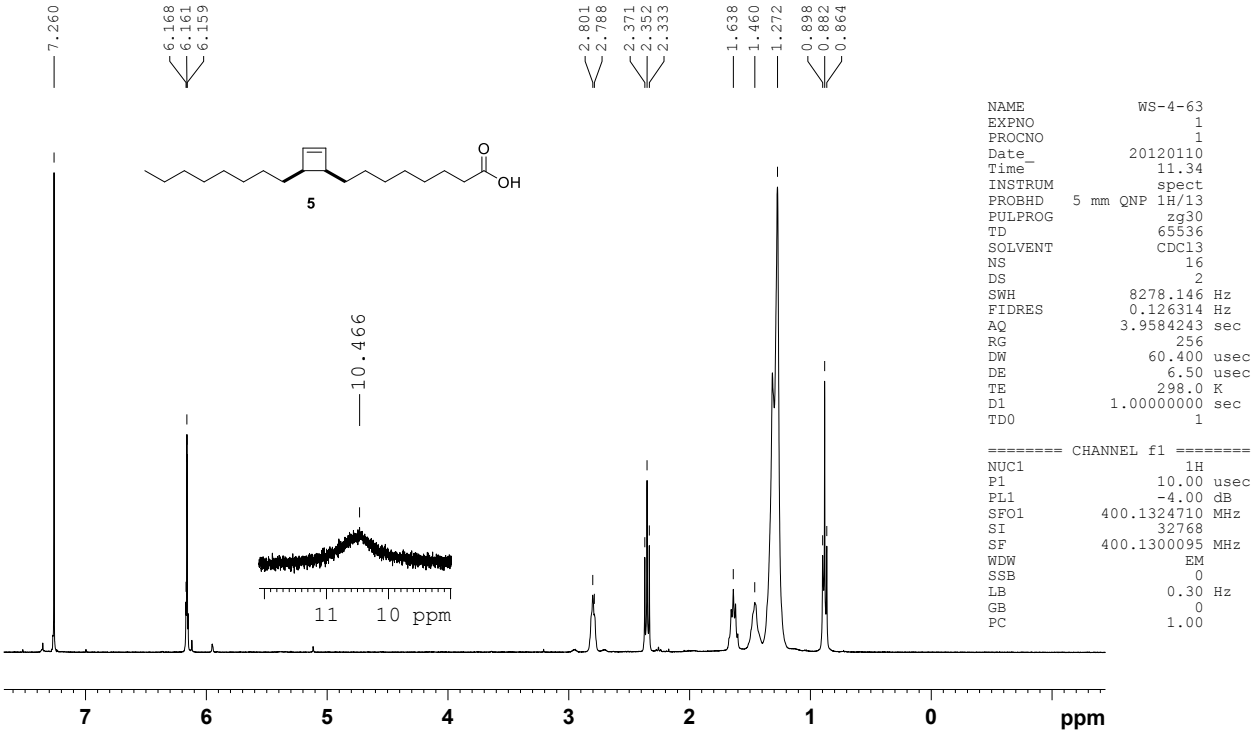


Figure S20.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of compound **5**.

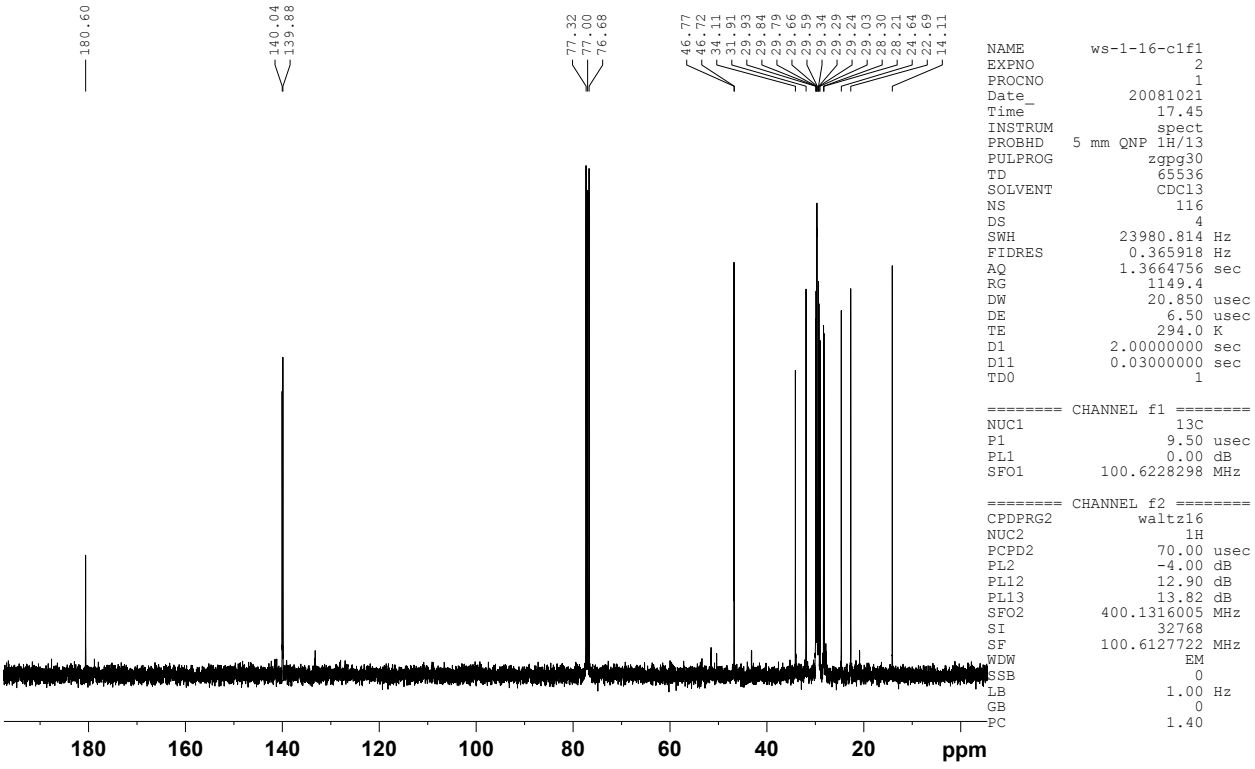


Figure S20.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of compound **5**.

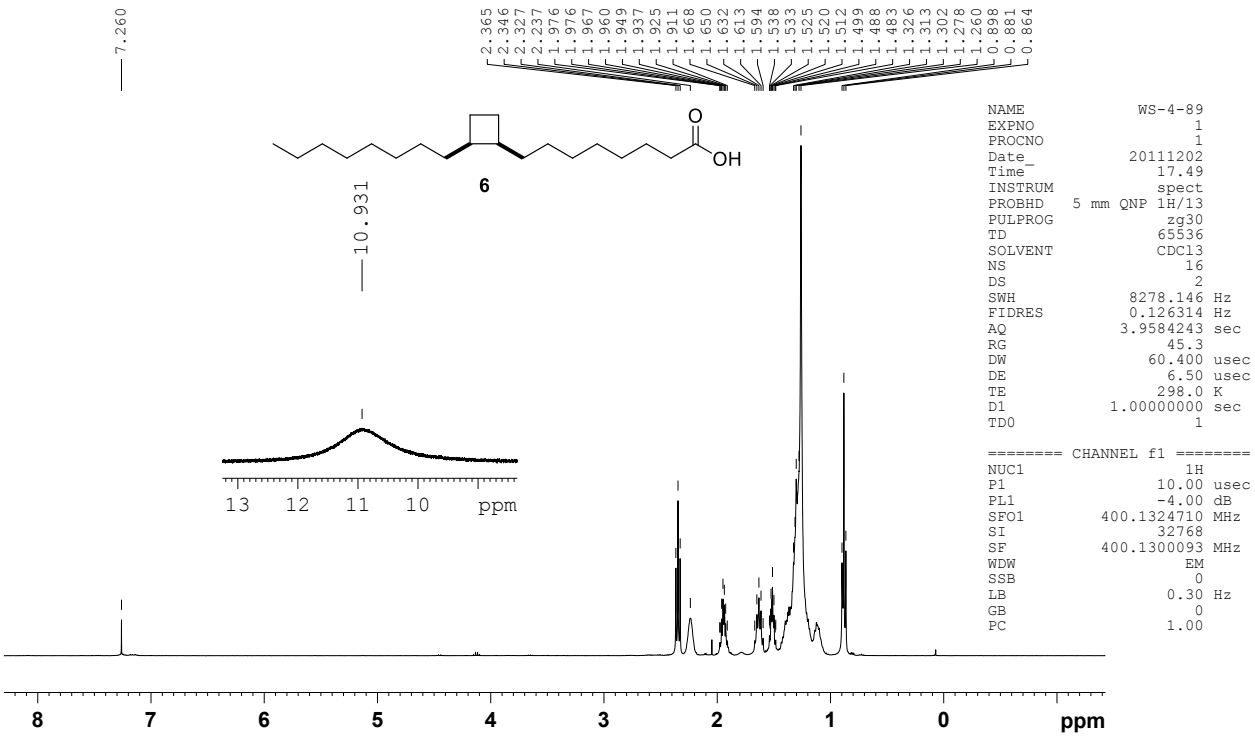


Figure S21.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of compound **6**.

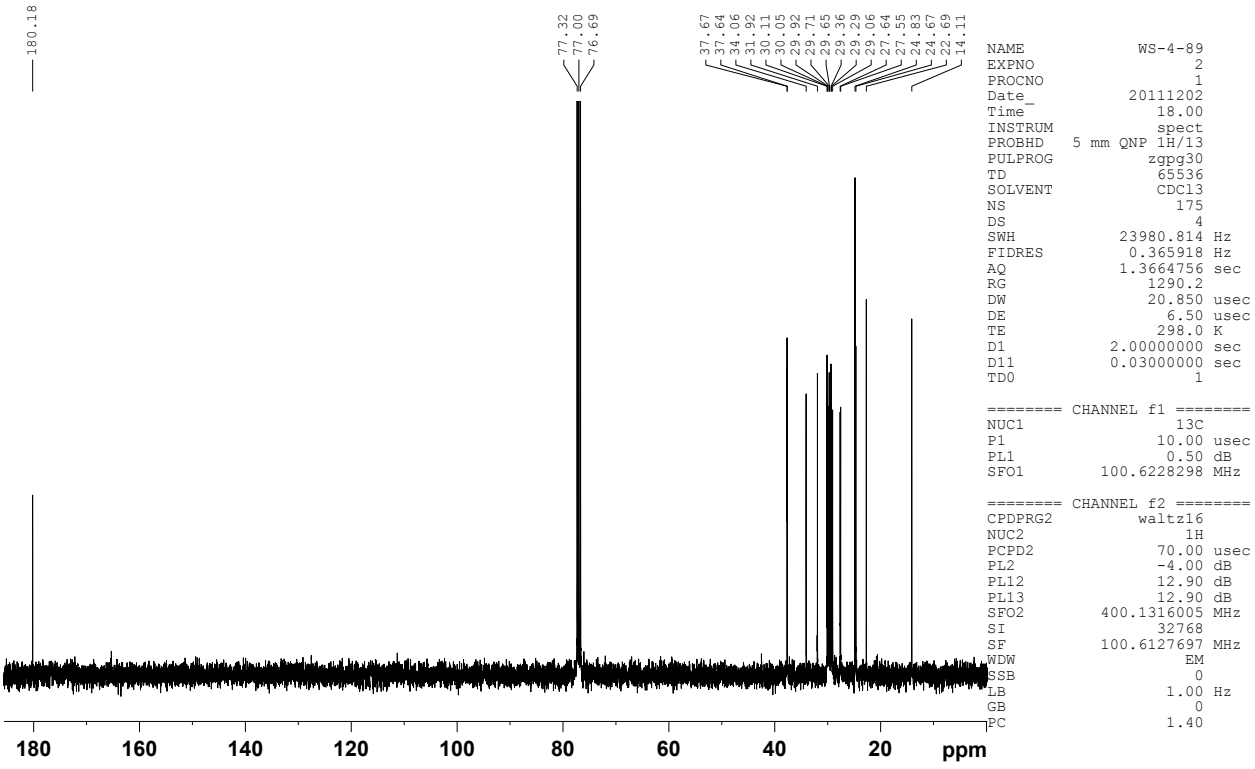


Figure S21.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of compound **6**.

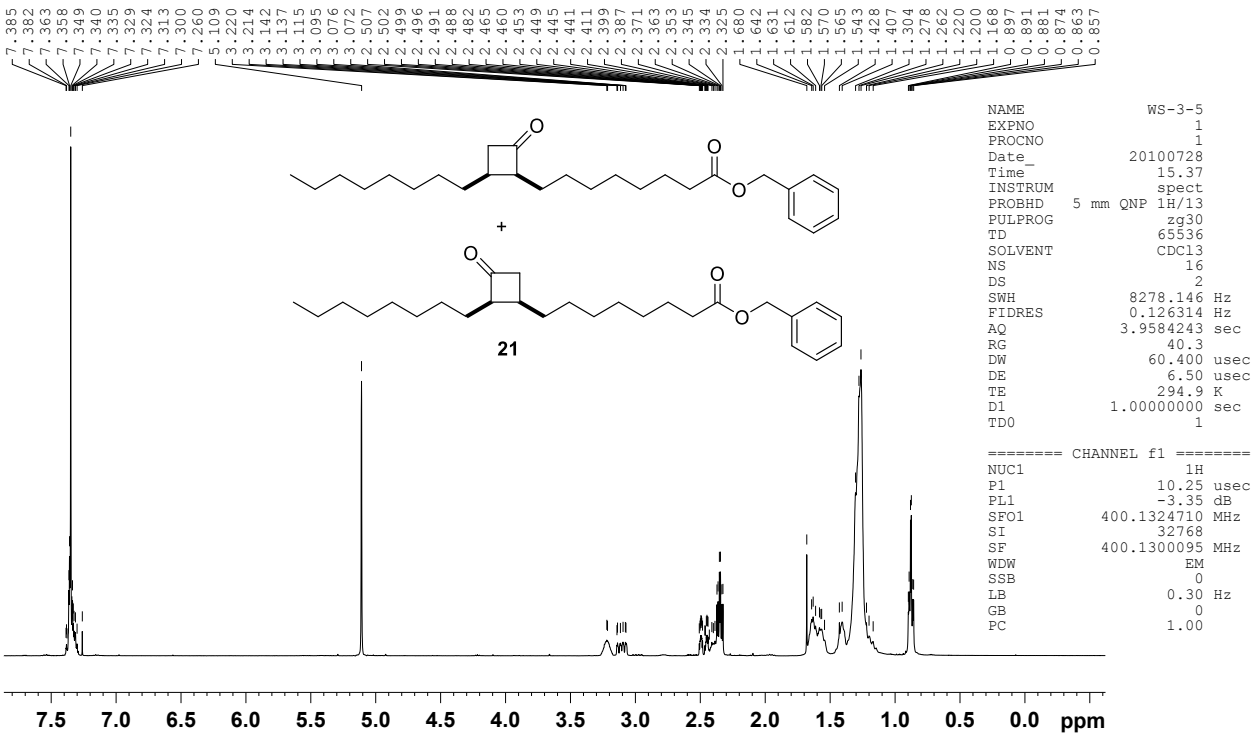


Figure S22.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of compound **21**.

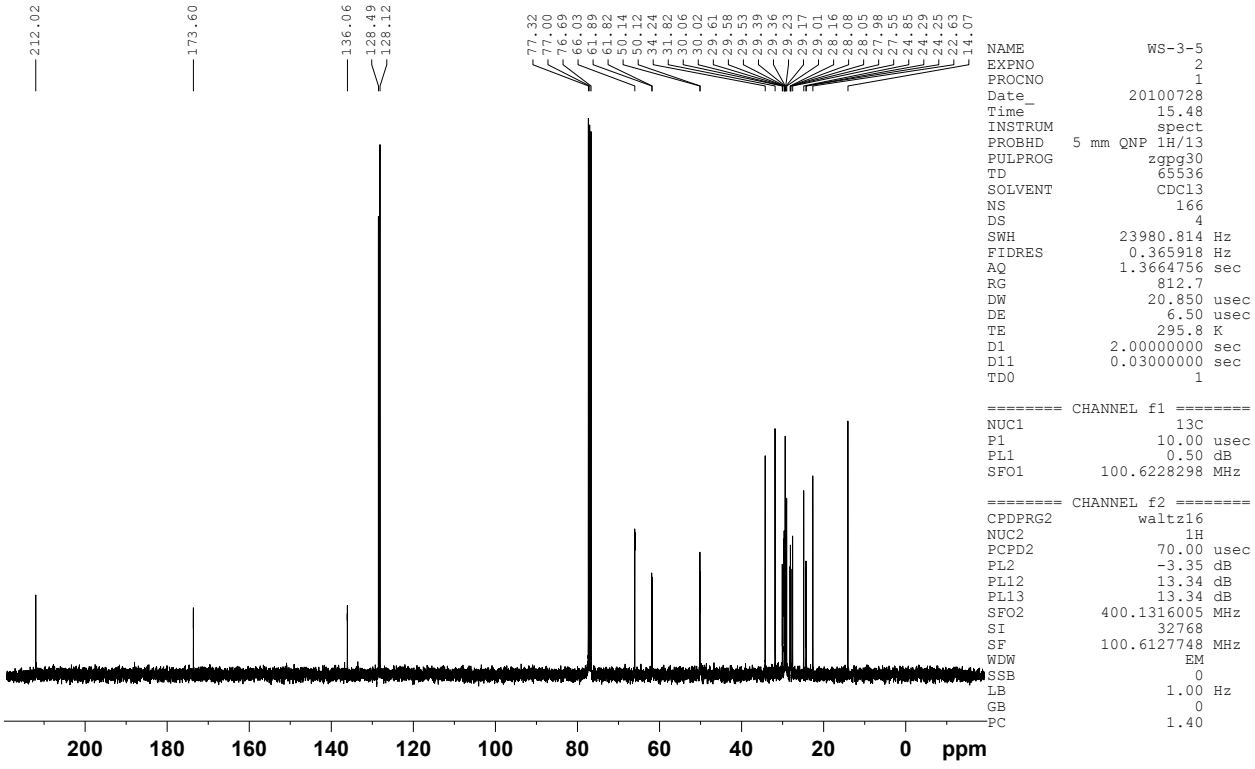


Figure S22.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of compound **21**.

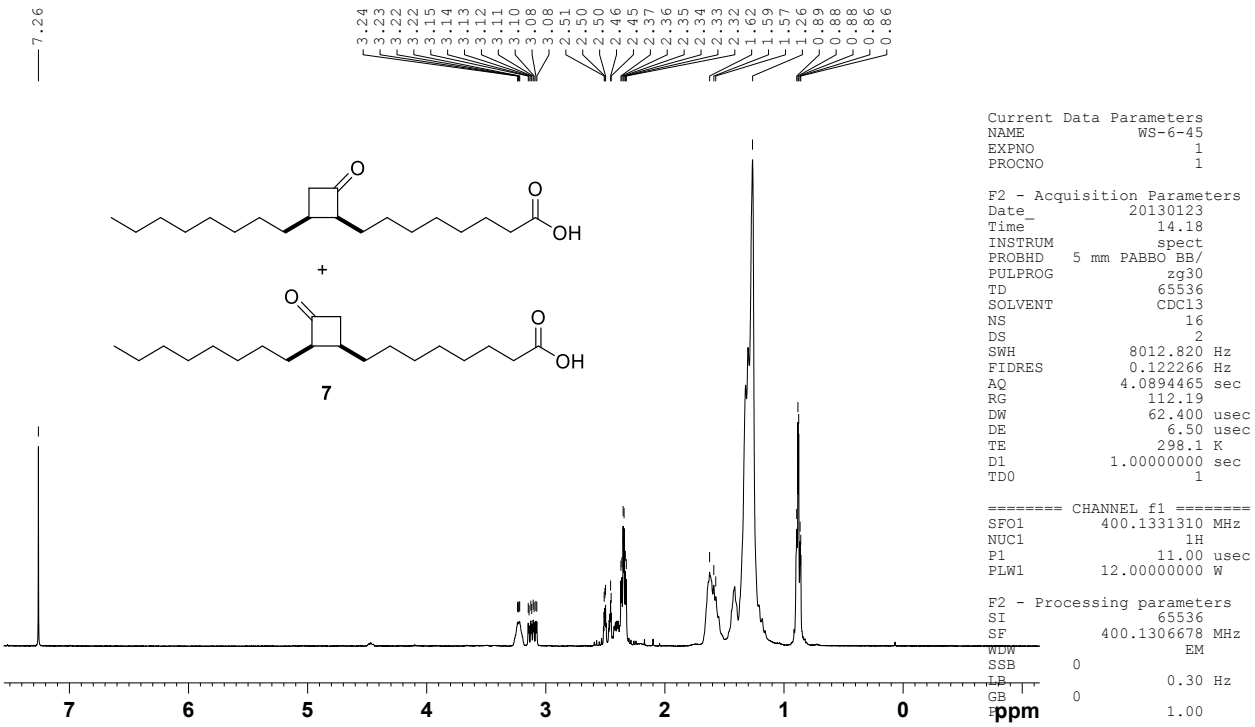


Figure S23.1. [1]H-NMR (400 MHz, chloroform-*d*) spectrum of compound **7**.

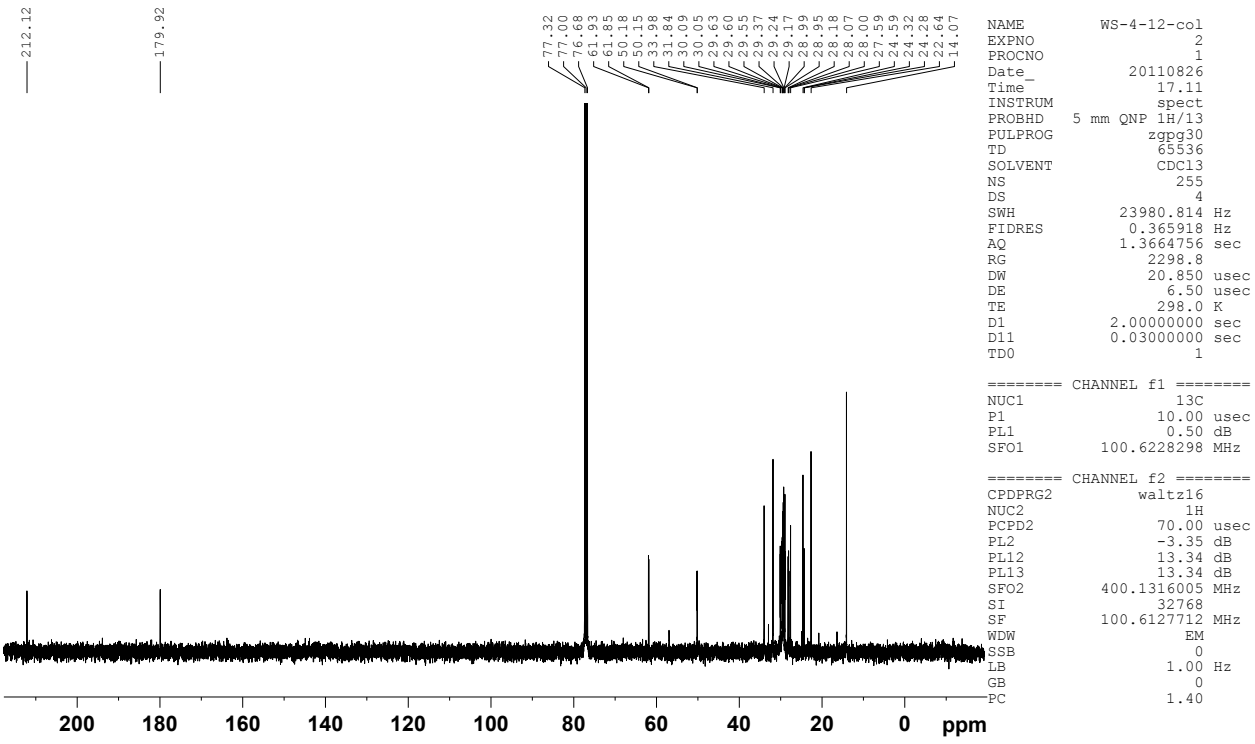


Figure S23.2. [13]C-NMR (100 MHz, chloroform-*d*) spectrum of compound **7**.

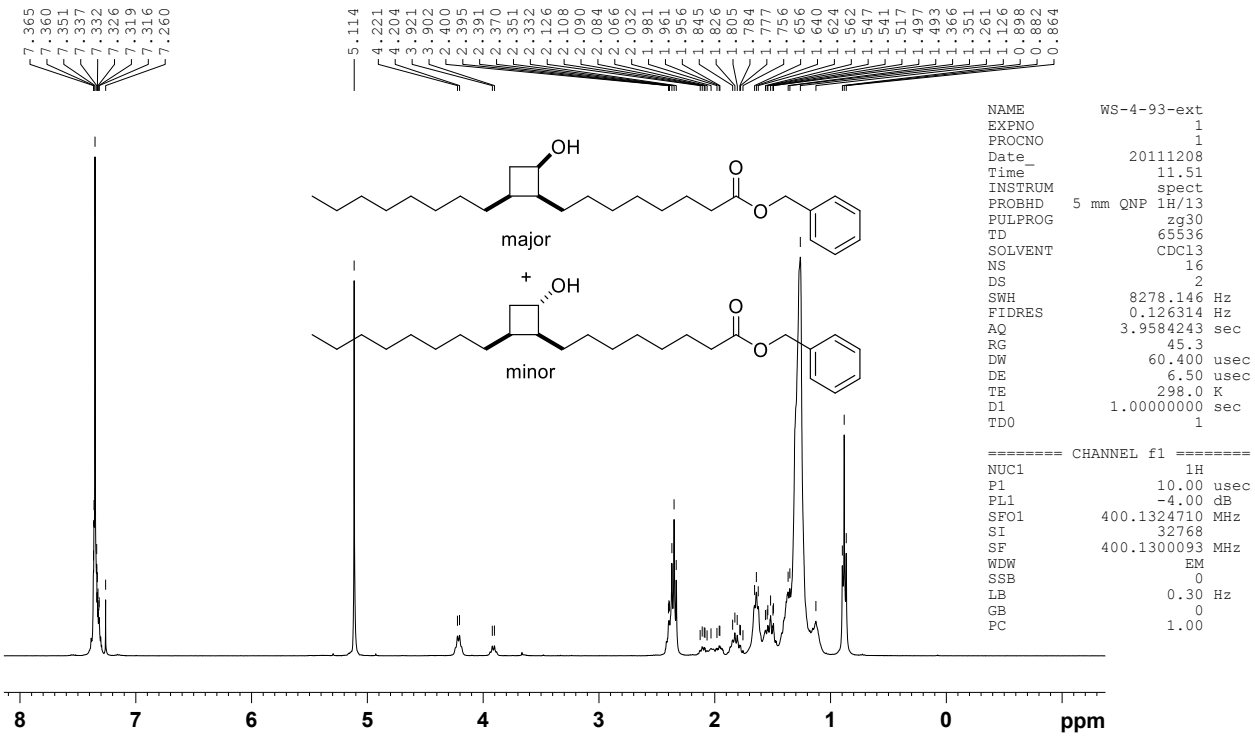


Figure S24.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum.

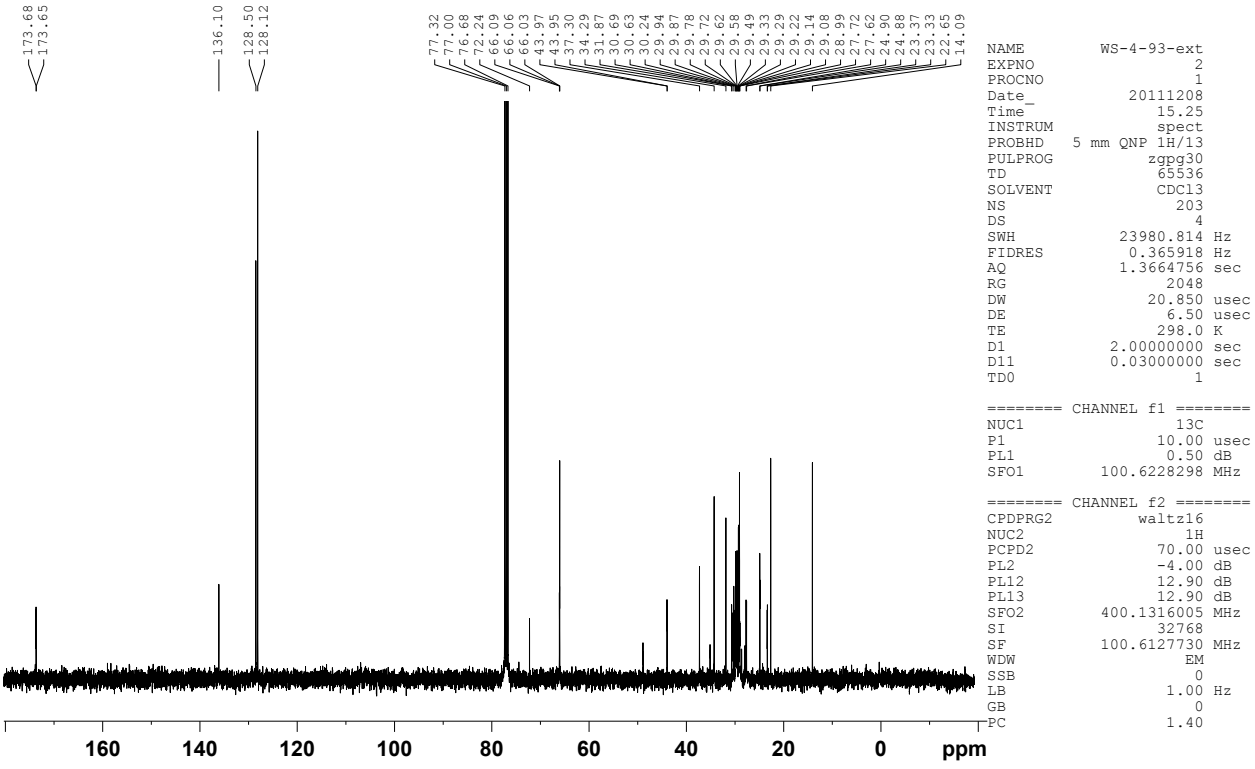


Figure S24.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum.

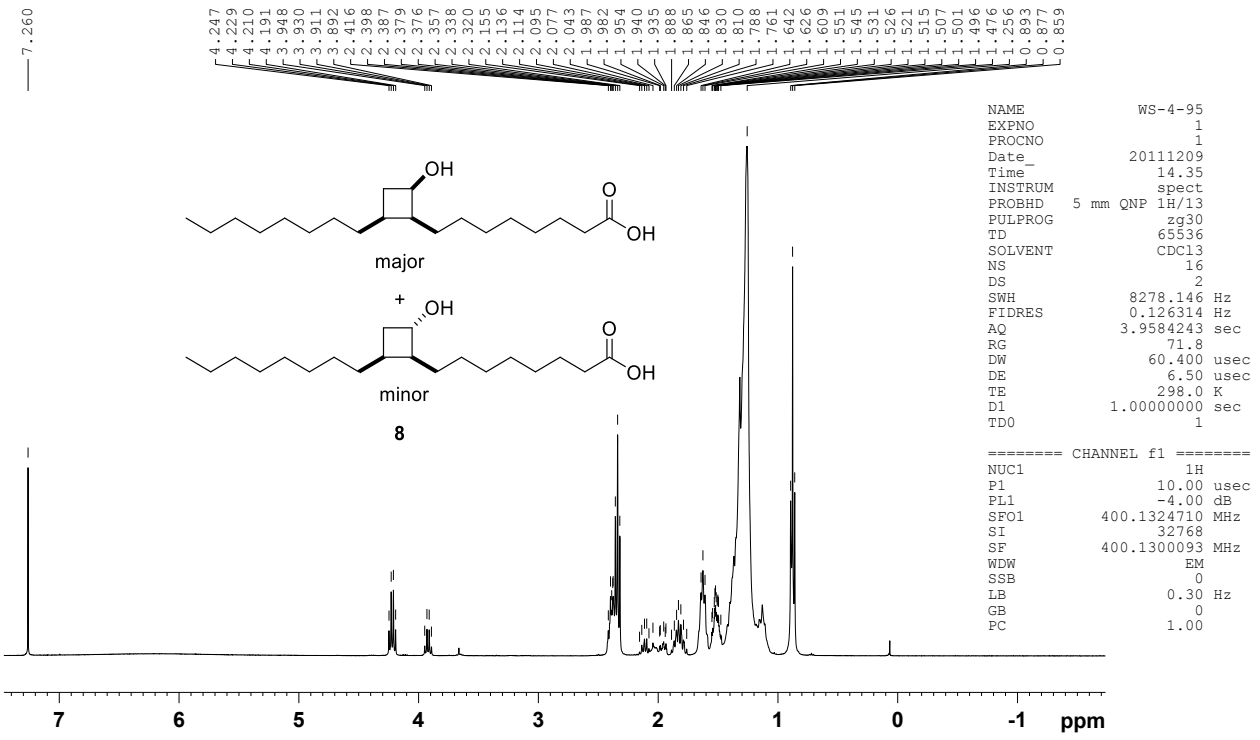


Figure S25.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of compound **8**.

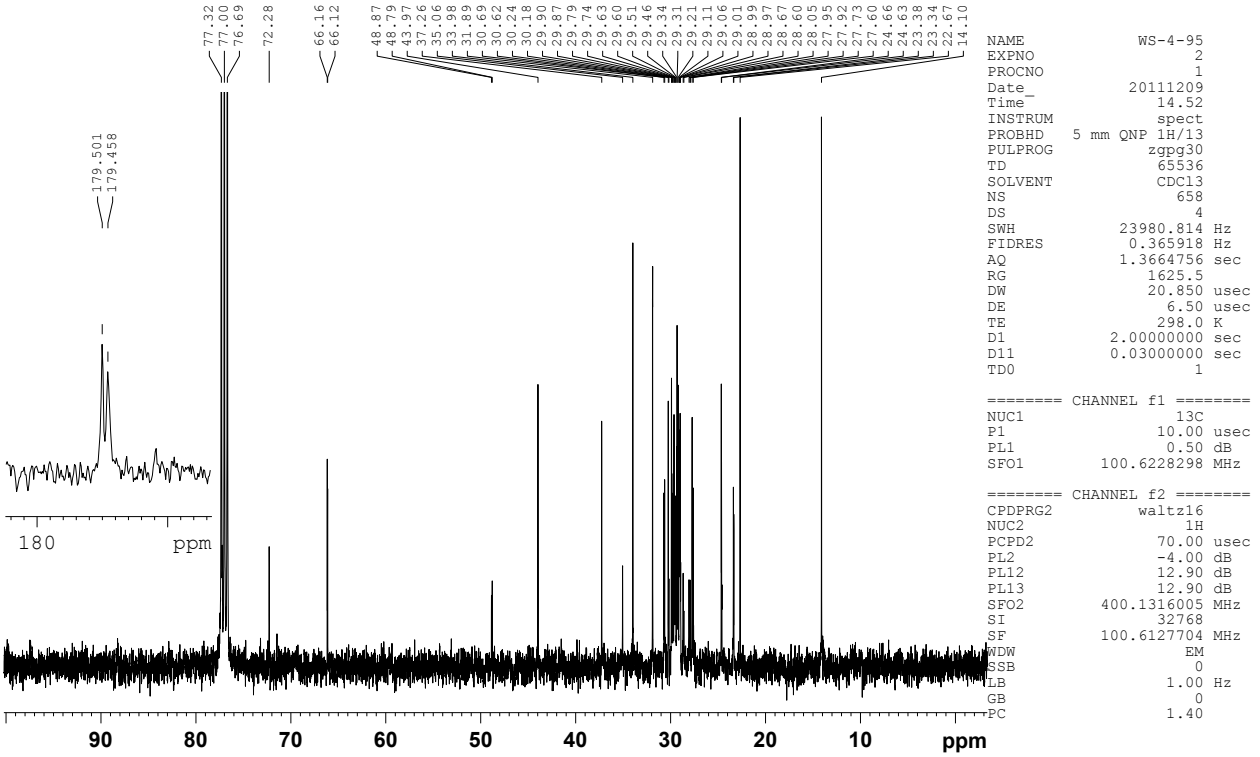


Figure S25.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of compound **8**.

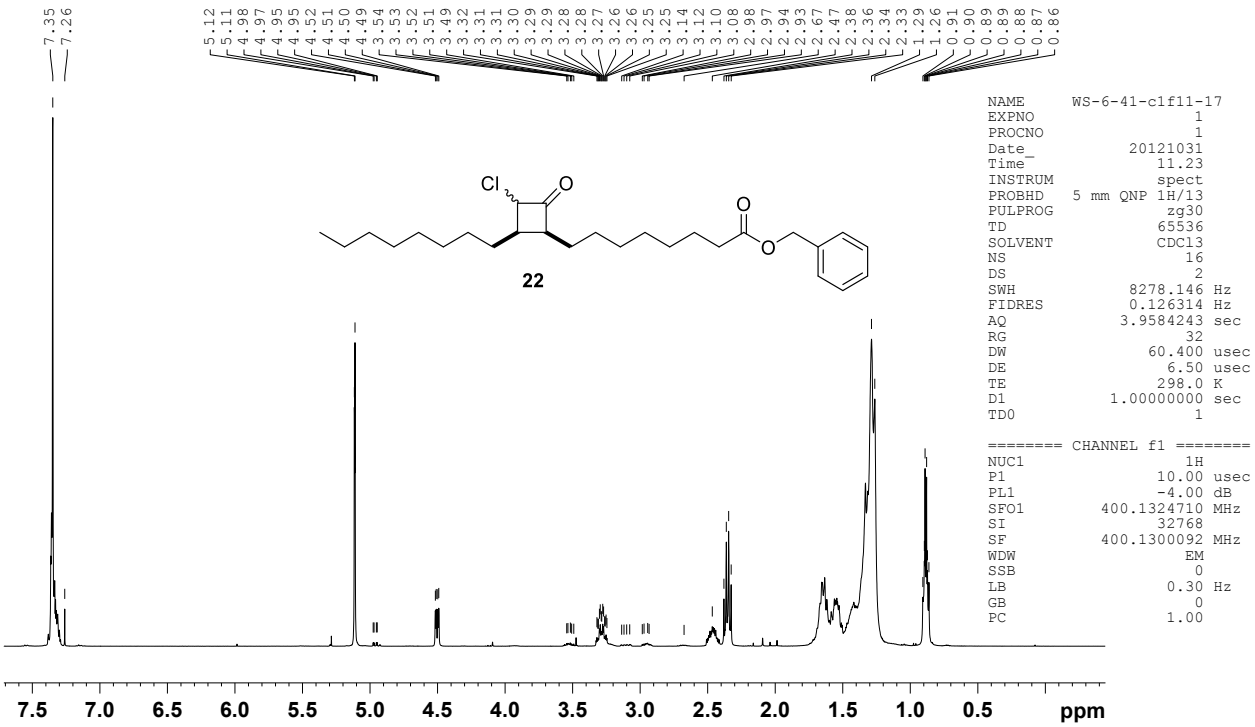


Figure S26.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of compound **22**.

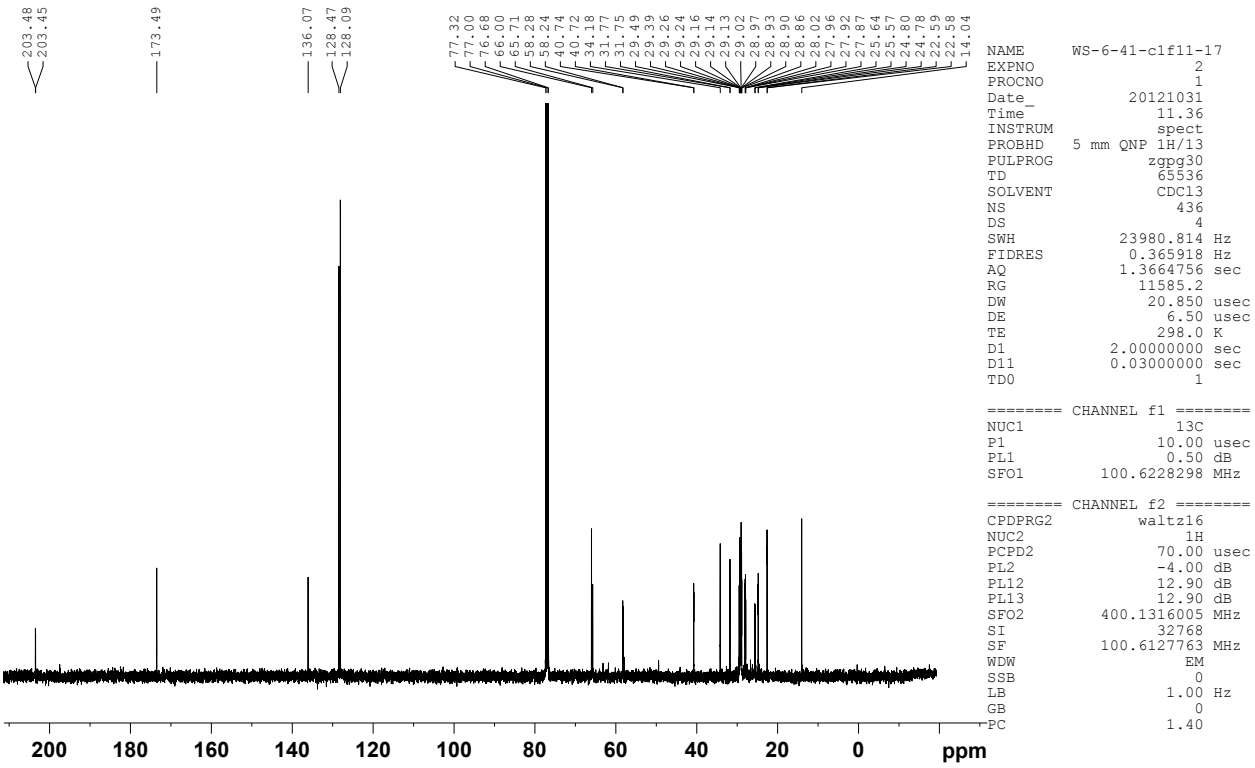


Figure S26.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of compound **22**.

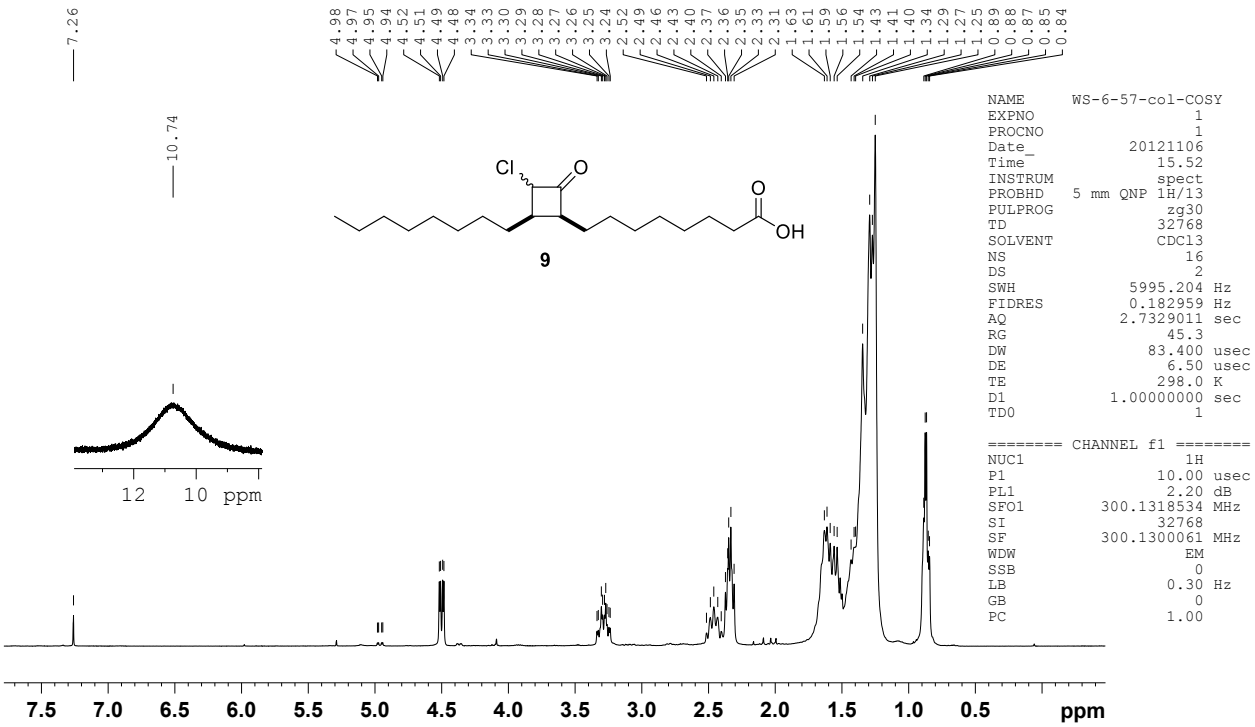


Figure S27.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of compound **9**.

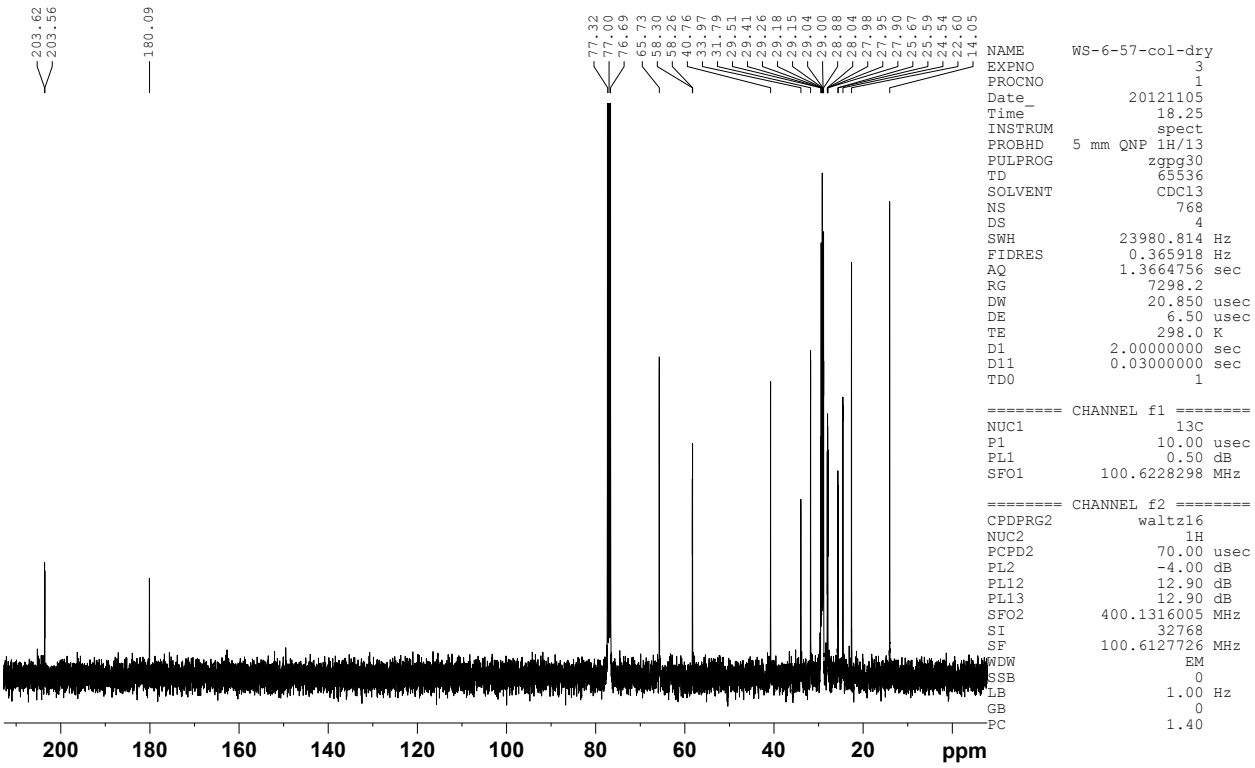


Figure S27.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of compound **9**.

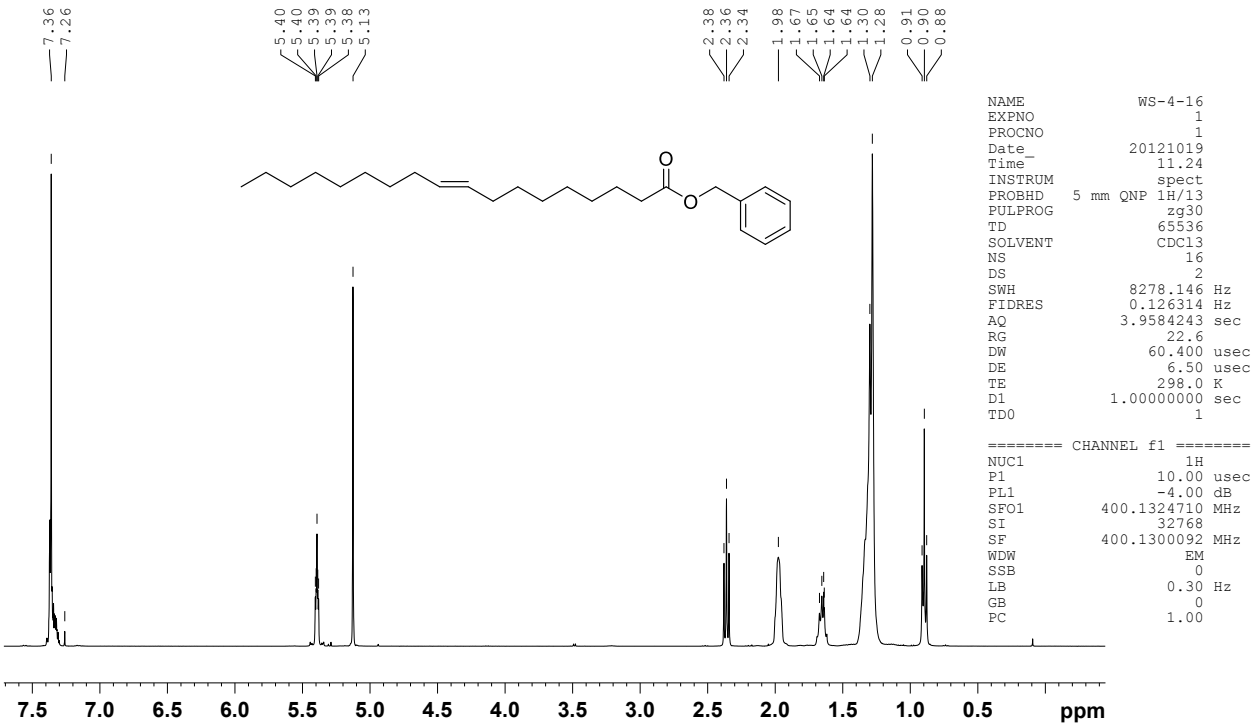


Figure S28.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum.

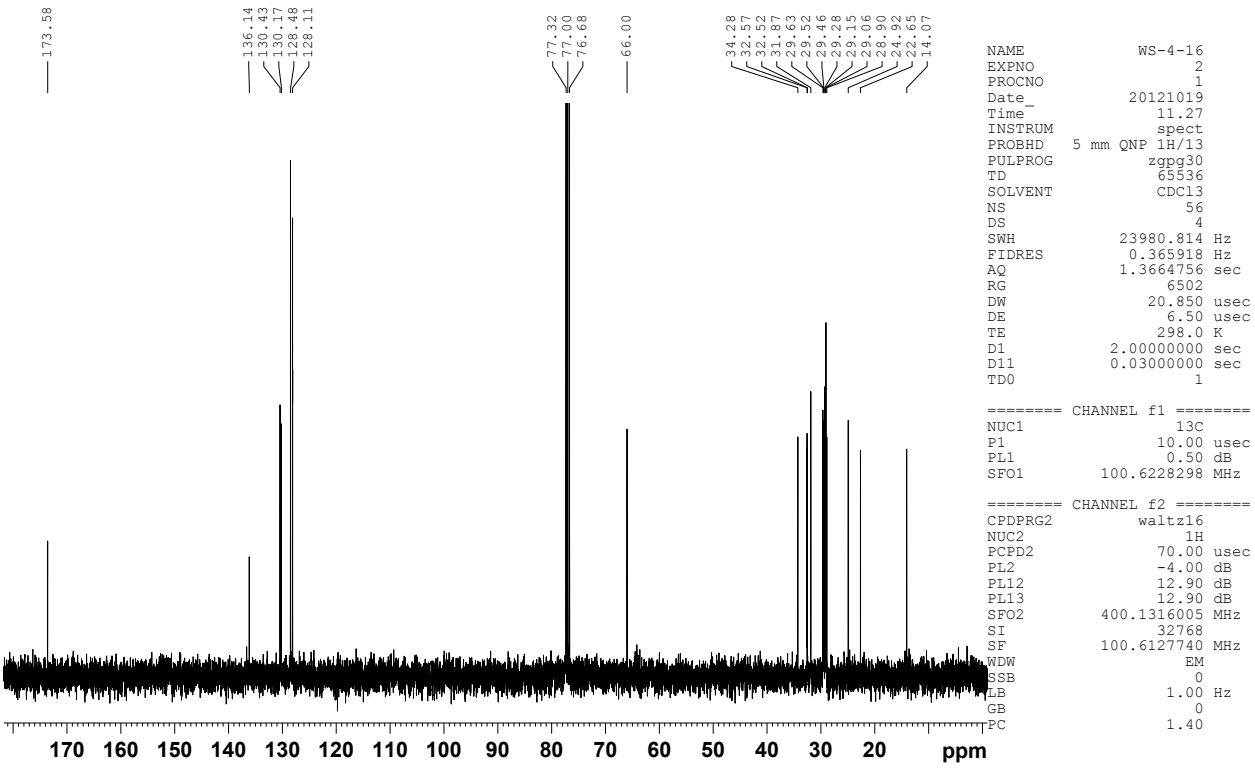


Figure S28.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum.

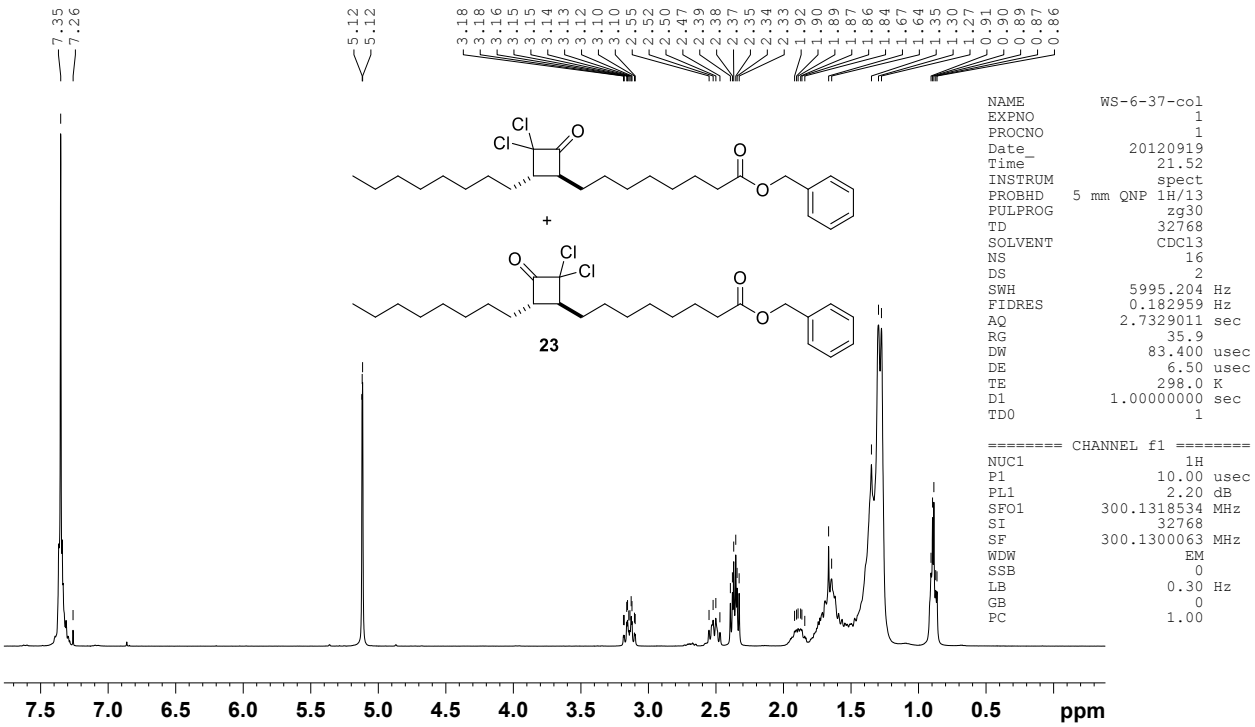


Figure S29.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of **23**.

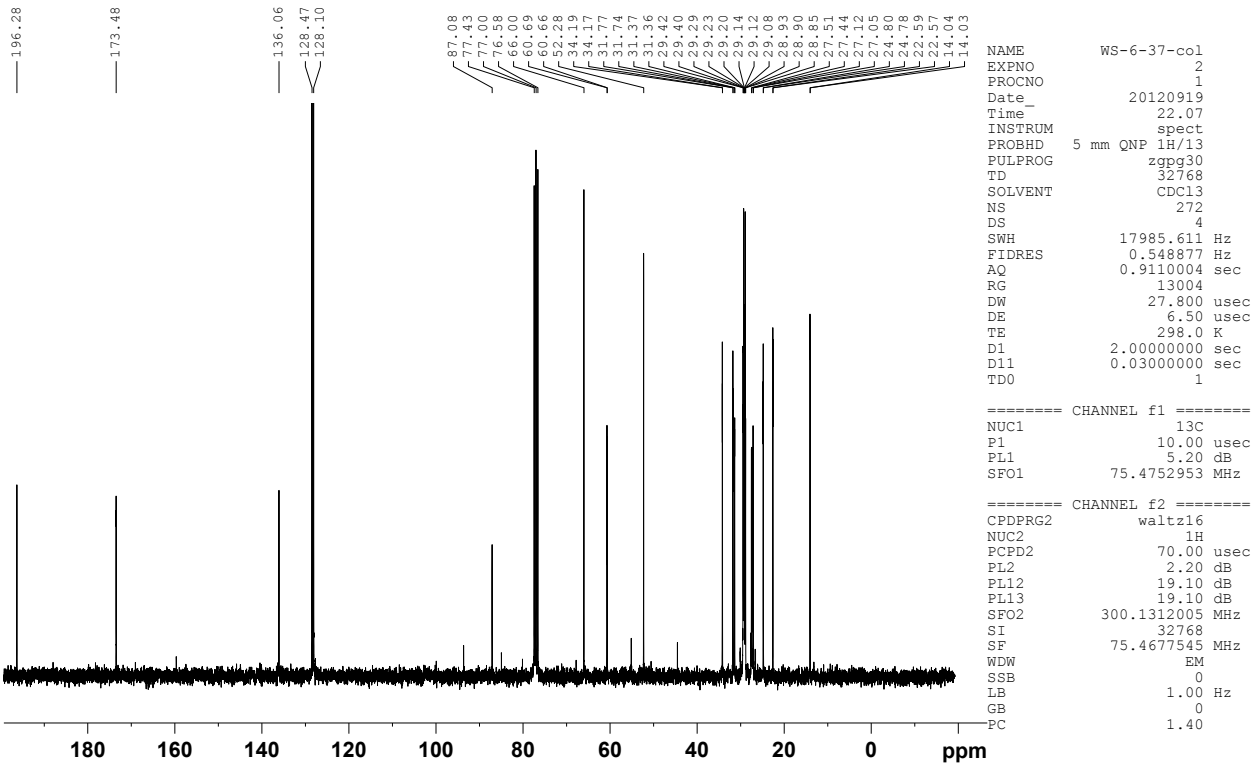


Figure S29.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of **23**.

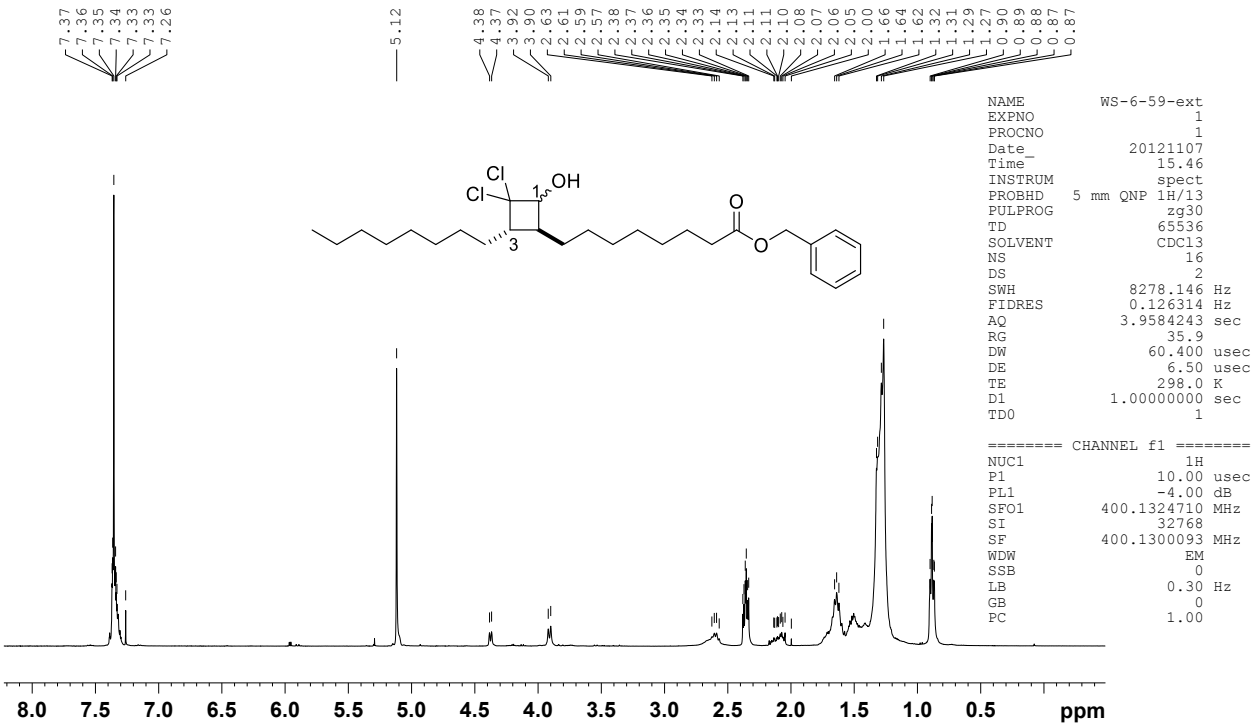


Figure S30.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum.

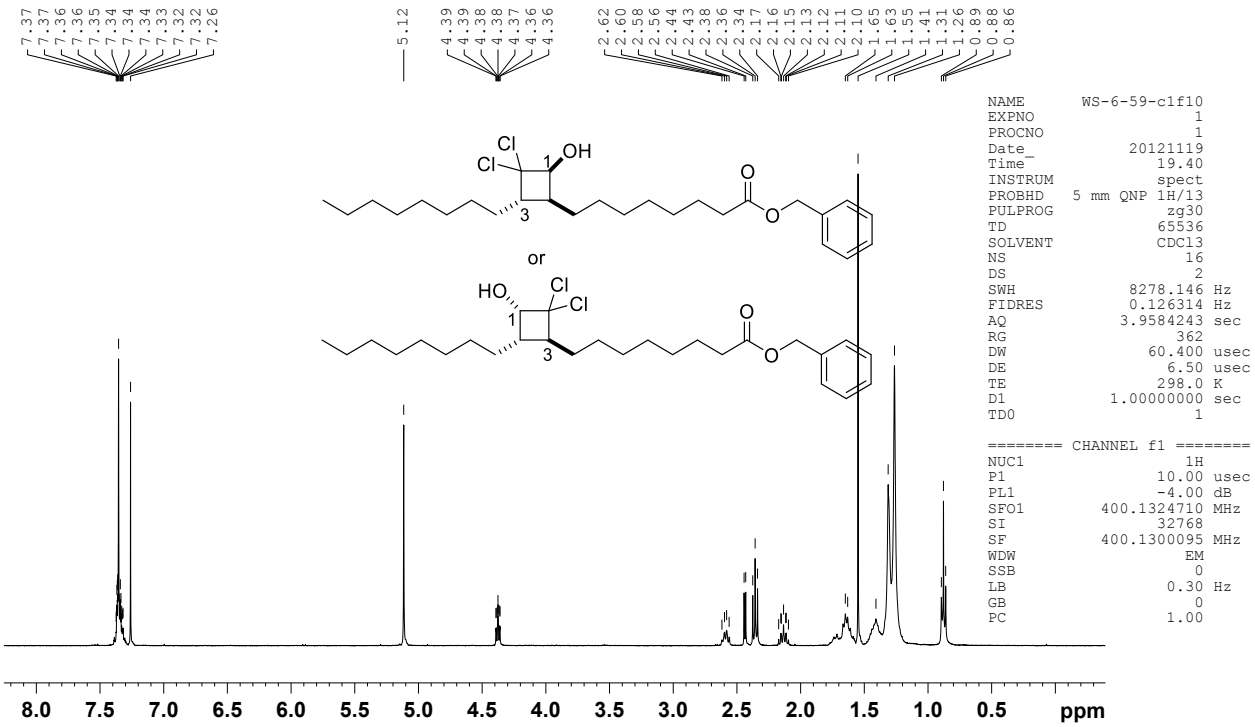


Figure S30.2. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of first eluting *trans* isomer.

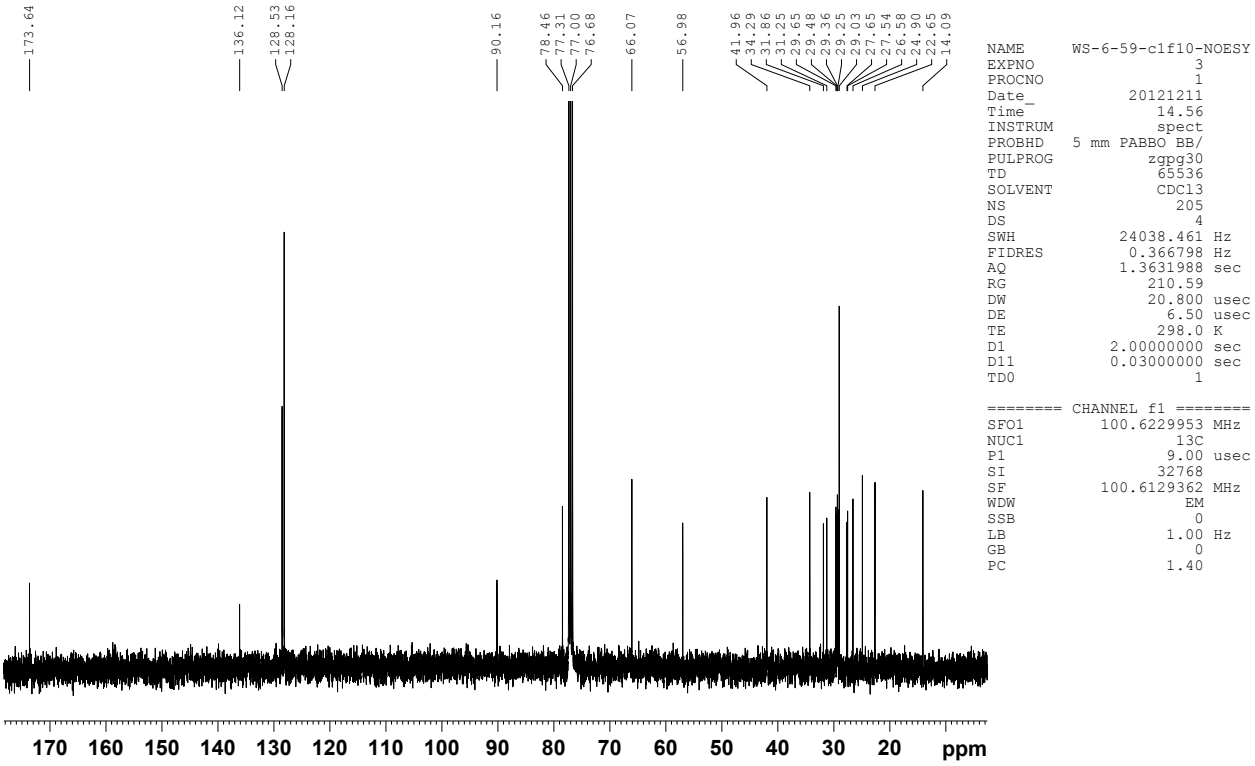


Figure S30.3. ^{13}C-NMR(100 MHz, chloroform-*d*) spectrum of first eluting *trans* isomer.

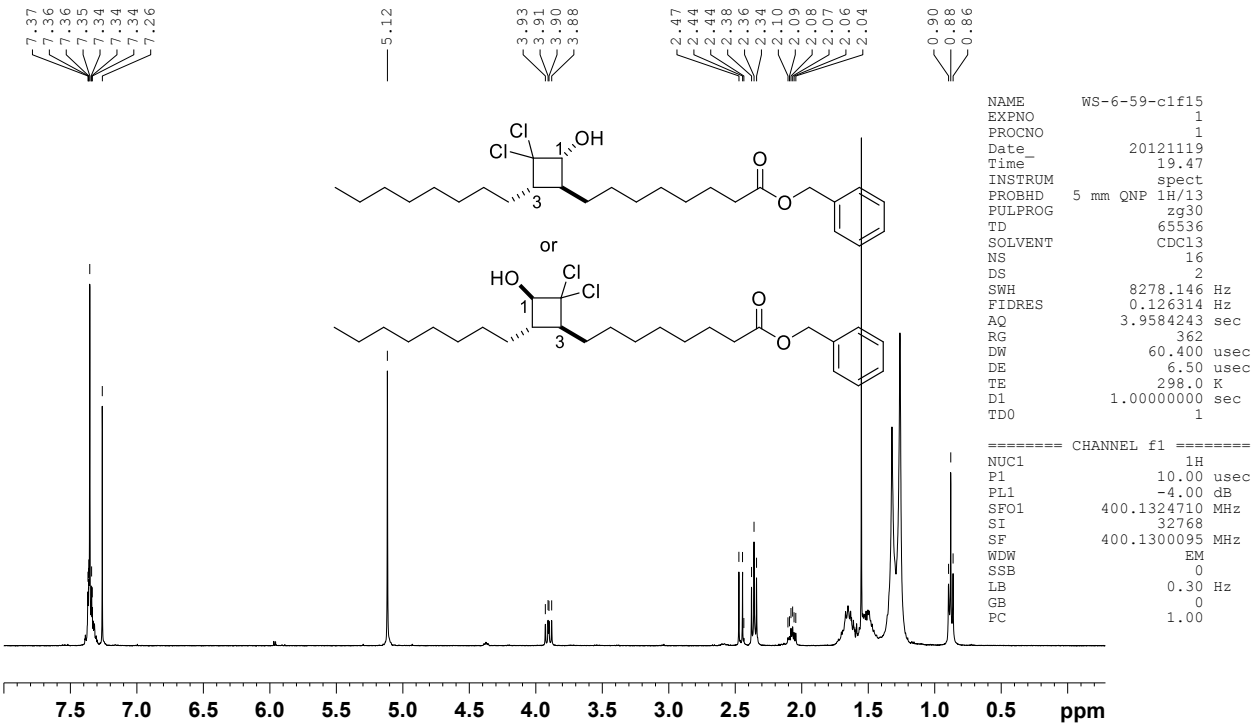


Figure S30.4. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of first eluting *cis* isomer.

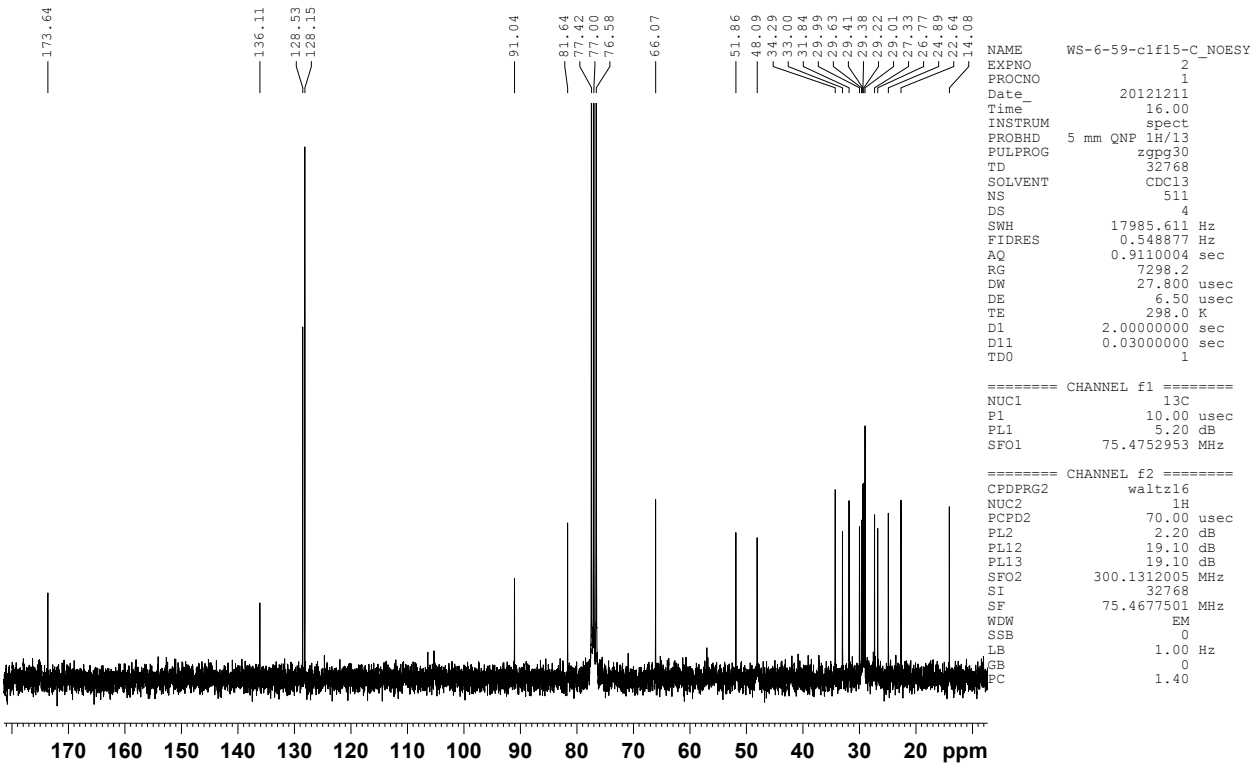


Figure S30.5. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of first eluting *cis* isomer.

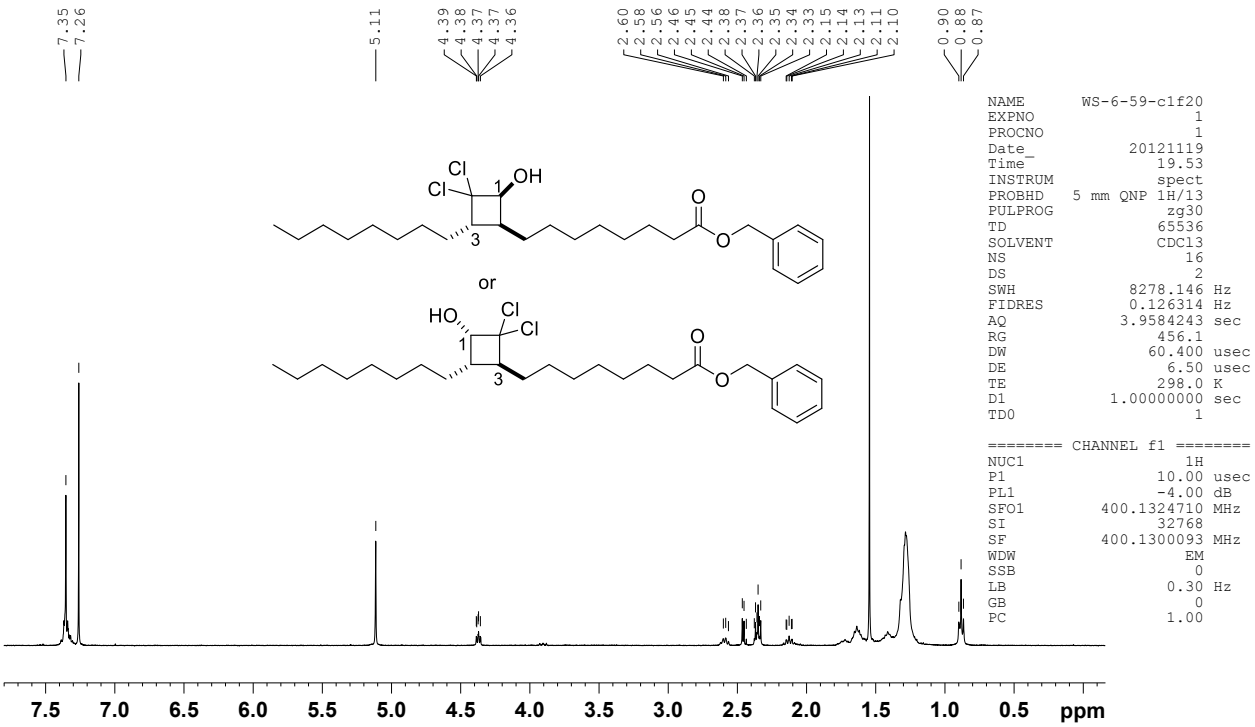


Figure S30.6. [1]H-NMR (400 MHz, chloroform-*d*) spectrum of second eluting *trans* isomer.

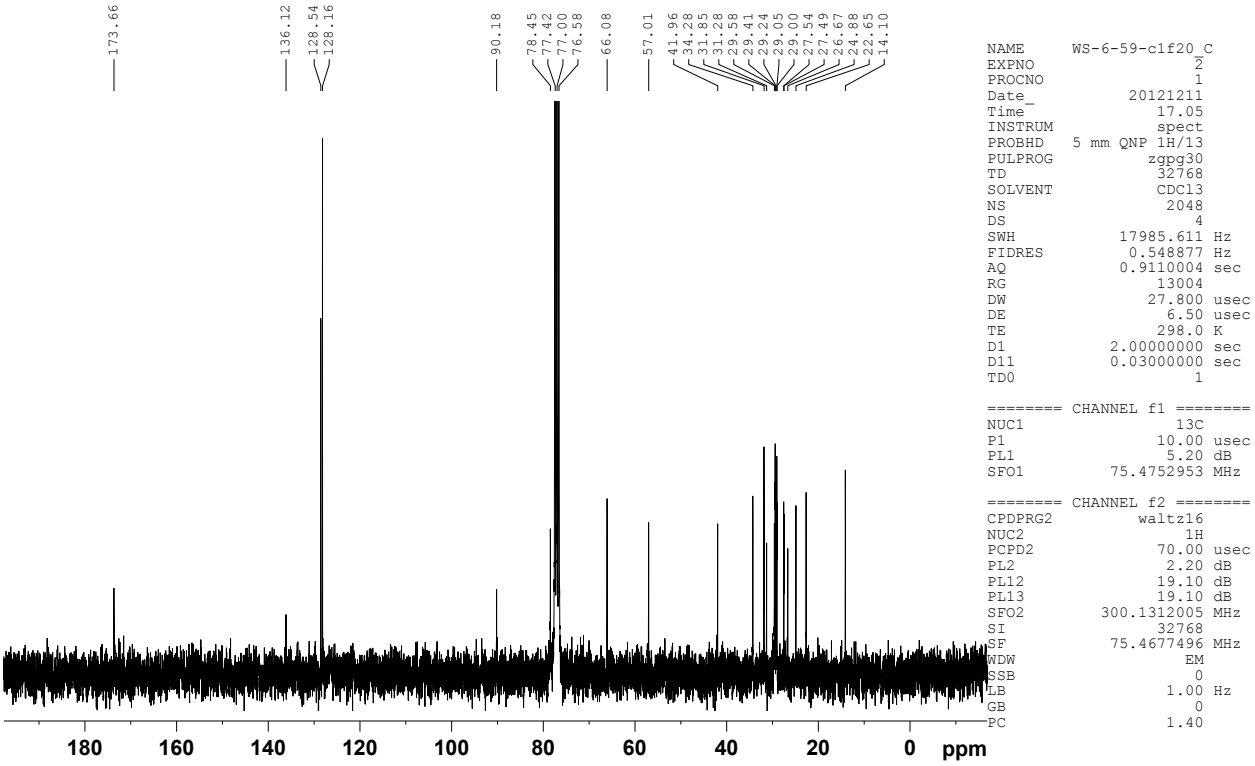


Figure S30.7.[13]C-NMR (100MHz, chloroform-*d*) spectrum of second eluting *trans* isomer.

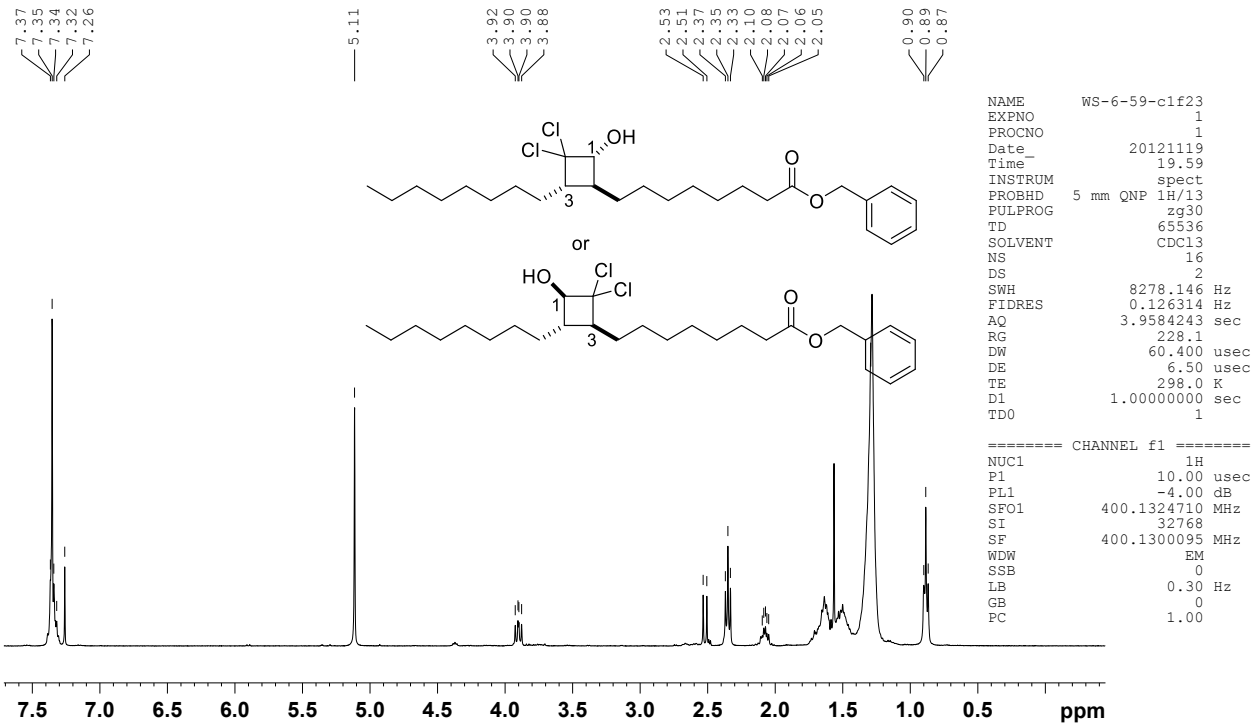


Figure S30.8. [1]H-NMR (400 MHz, chloroform-*d*) spectrum of second eluting *cis* isomer.

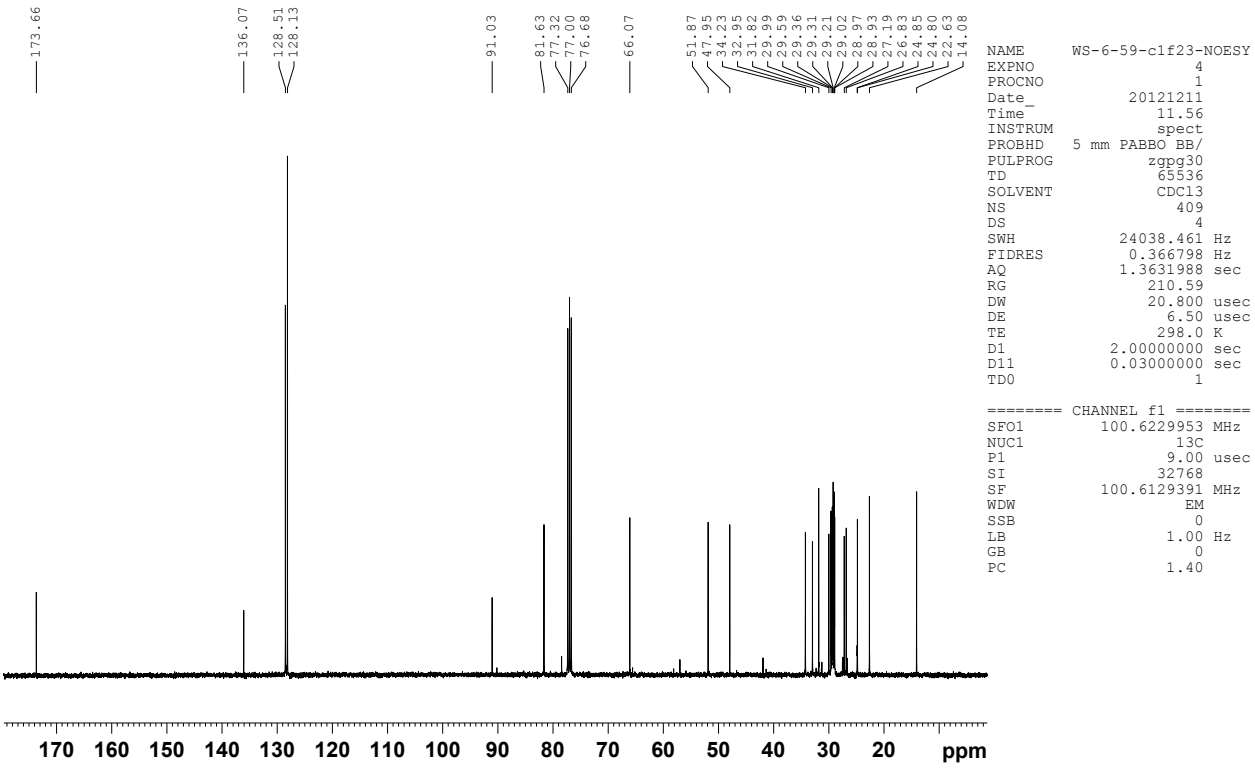


Figure S30.9. [13]C-NMR (100 MHz, chloroform-*d*) spectrum of second eluting *cis* isomer.

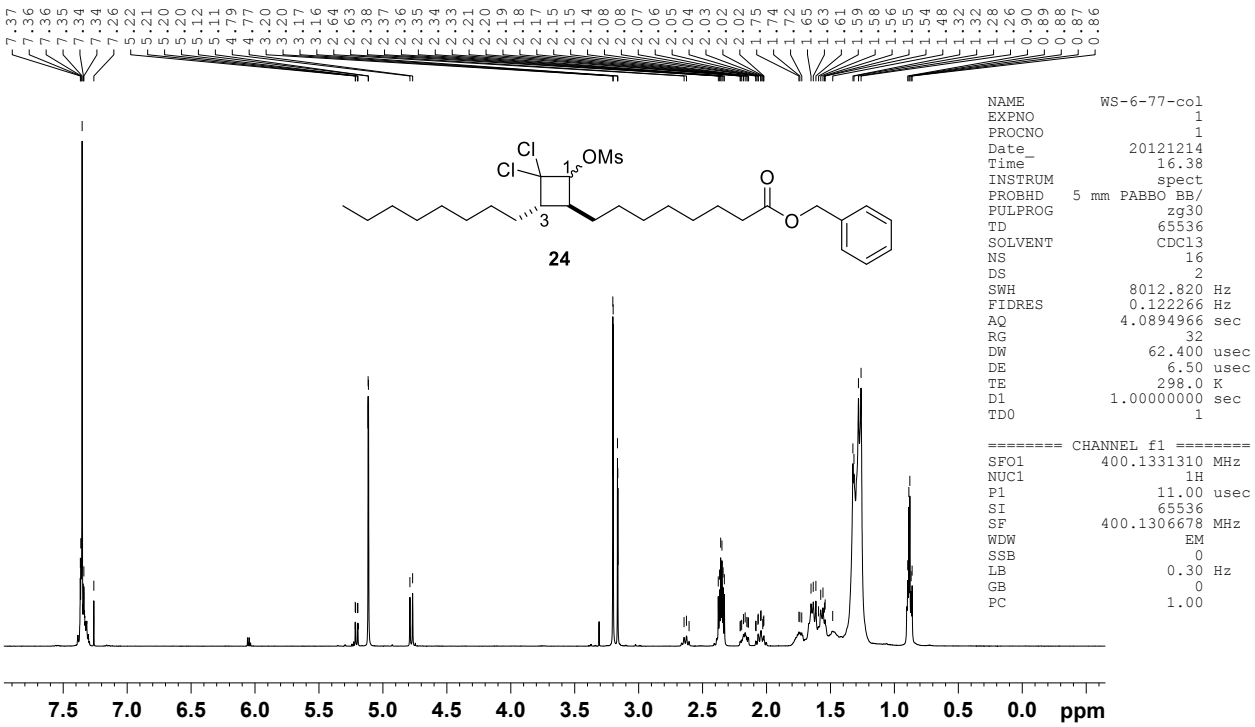


Figure S31.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of **24**.

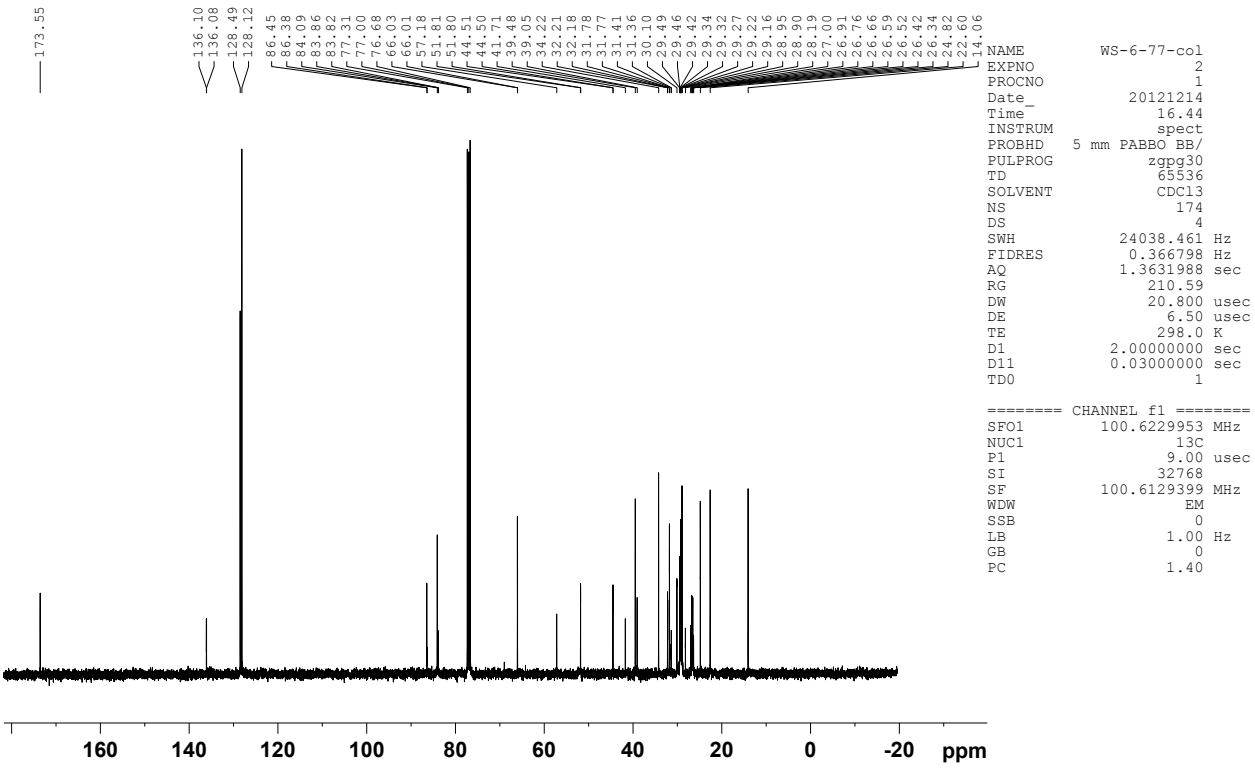


Figure S31.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of **24**.

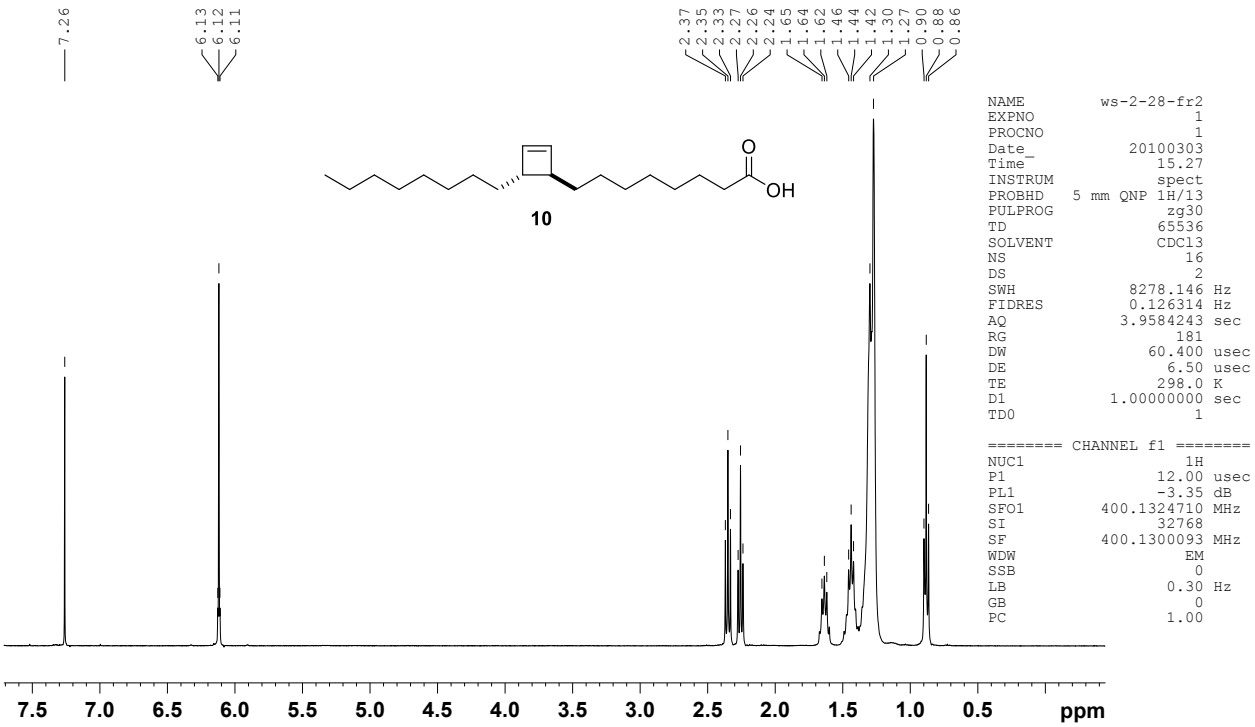


Figure S32.1. [1]H-NMR (400 MHz, chloroform-*d*) spectrum of **10**.

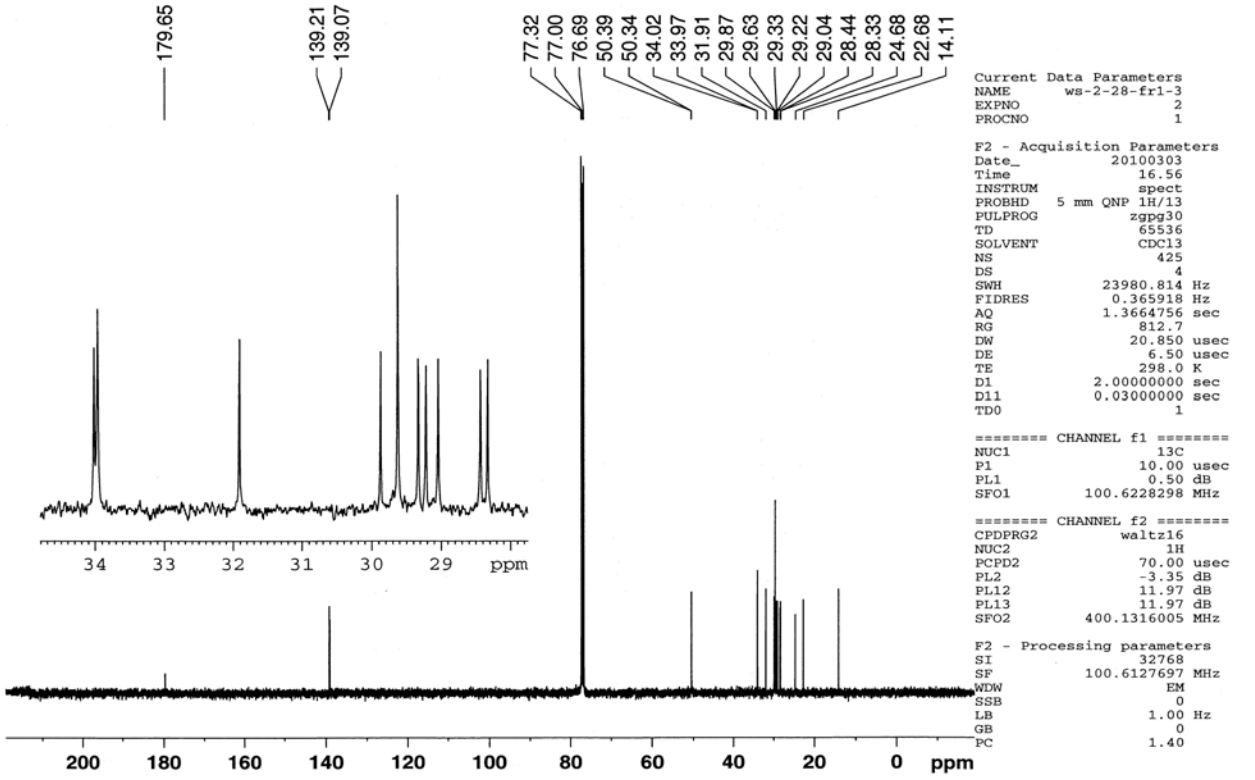


Figure S32.2. [13]C-NMR (100 MHz, chloroform-*d*) spectrum of **10**.

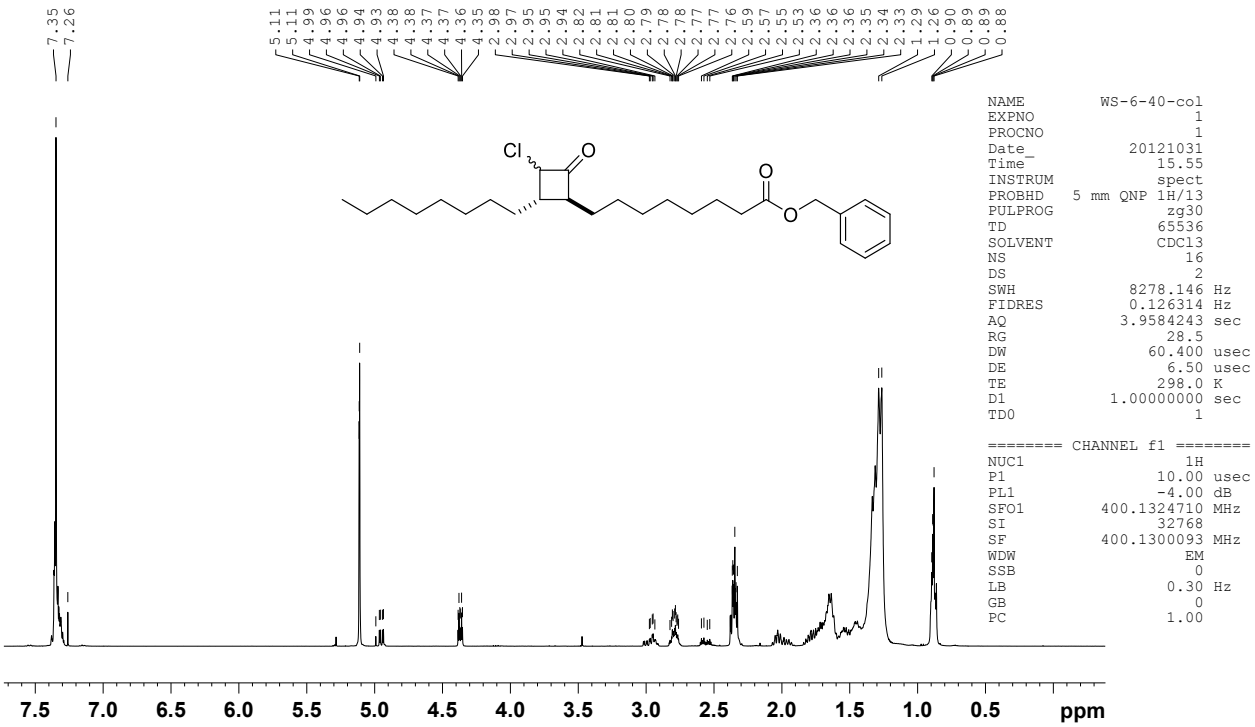


Figure S33.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum.

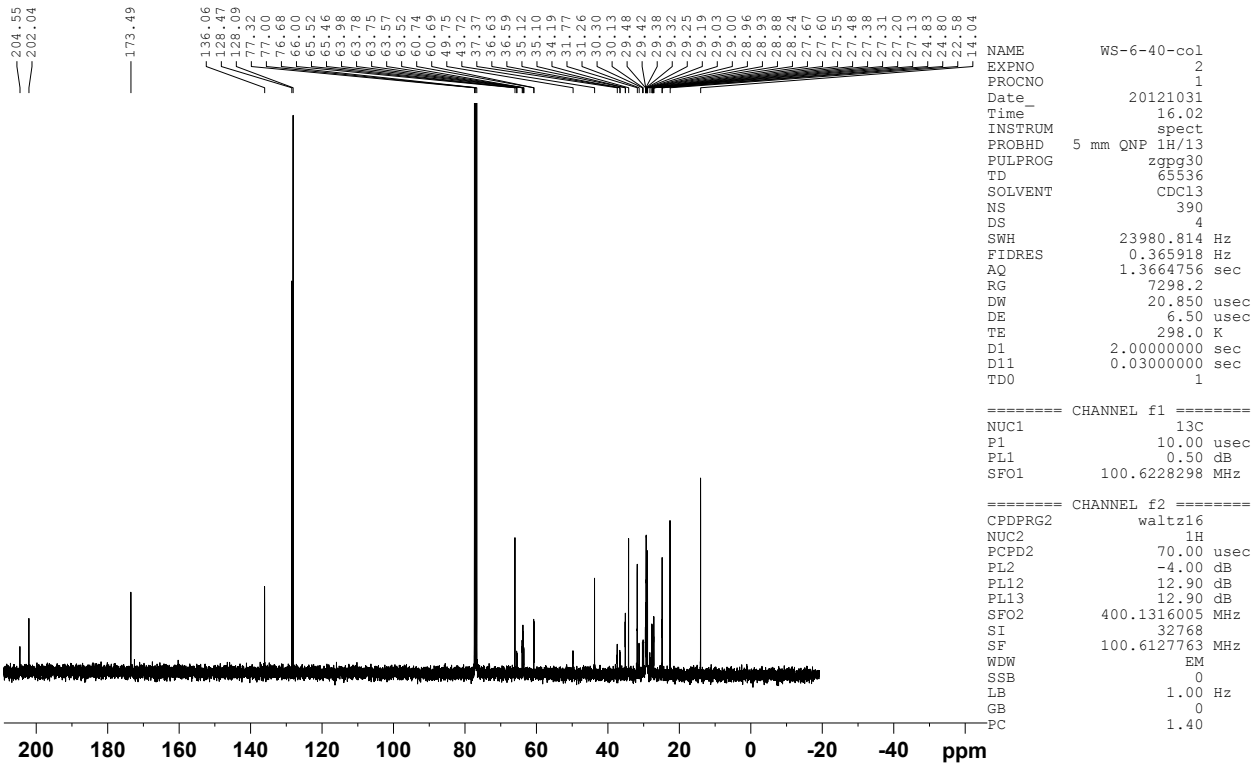


Figure S33.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum.

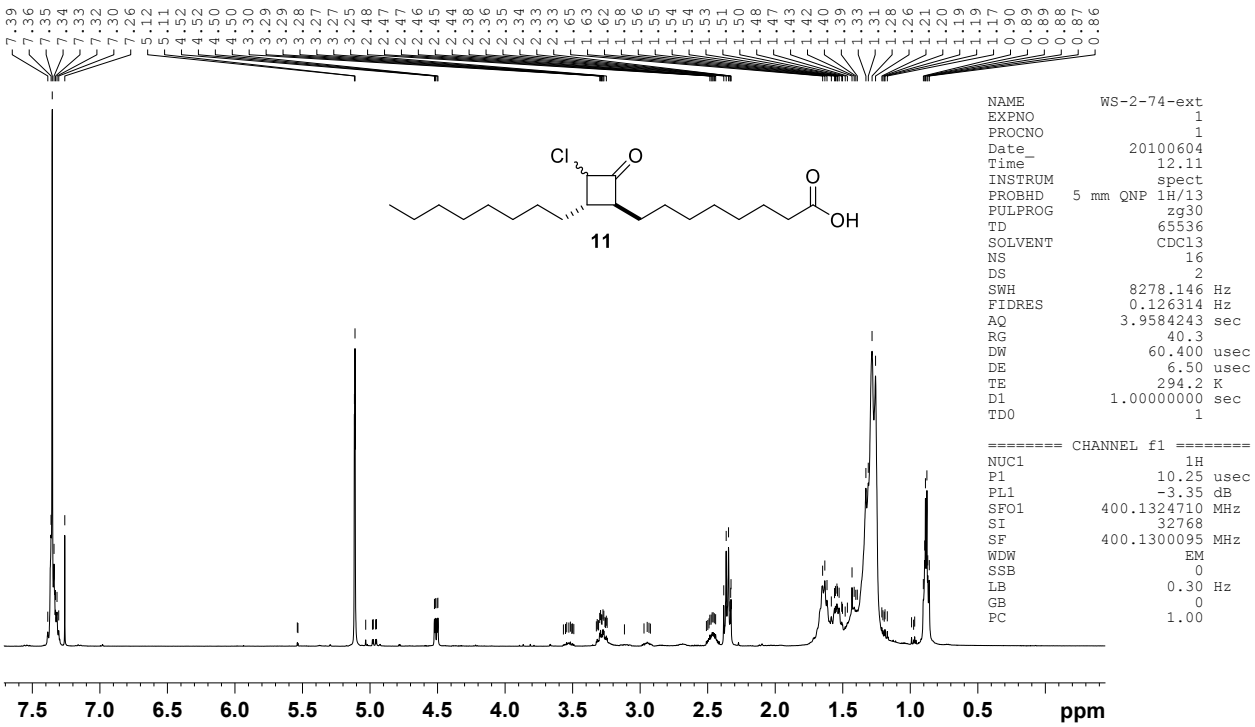


Figure S34.1. ^{1}H-NMR (400 MHz, chloroform-*d*) spectrum of **11**.

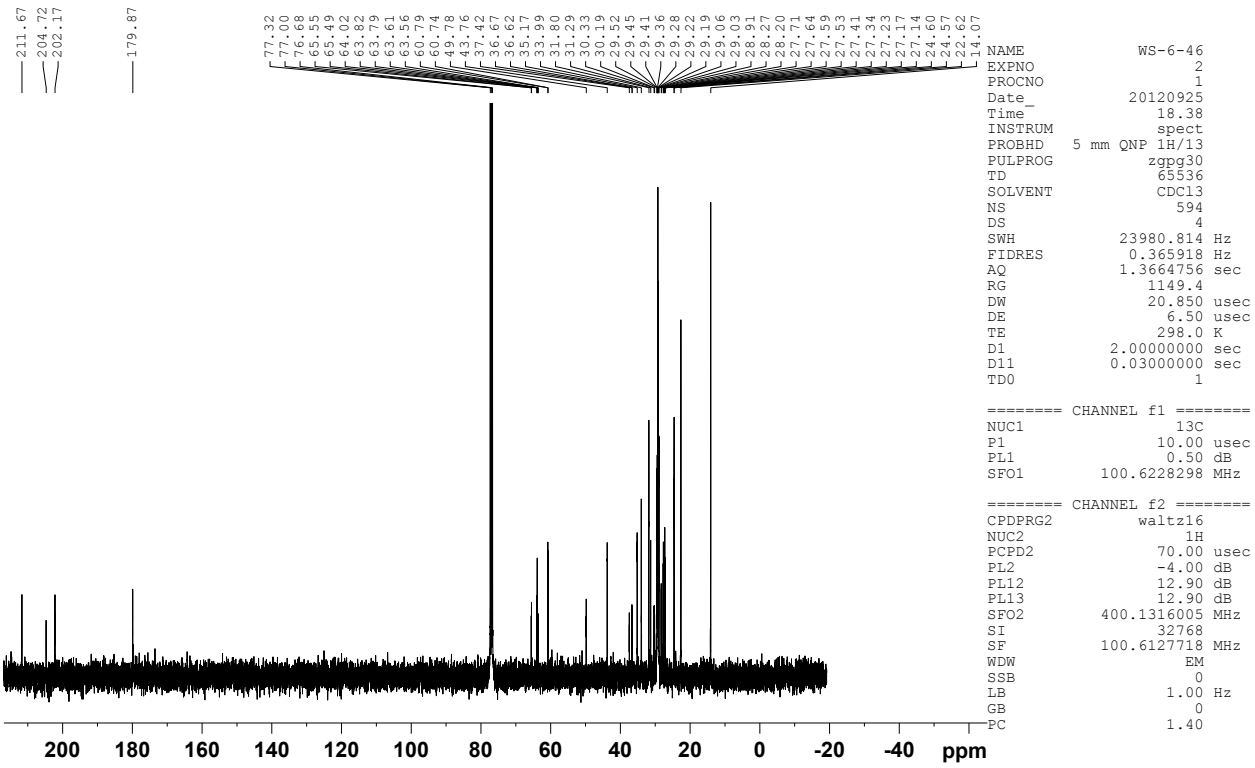


Figure S34.2. ^{13}C-NMR (100 MHz, chloroform-*d*) spectrum of **11**.