Supporting Information For:

Single-event spectroscopy and unravelling kinetics of covalent domains based on cyclobutane mechanophores

Brandon H. Bowser[1,2‡], Shu Wang[1,2‡], Tatiana B. Kouznetsova[1,2], Haley K. Beech[1,3], Bradley D. Olsen[1,3,*], Michael Rubinstein[1,2,4,5*] and Stephen L. Craig[1,2,*]

[1]NSF Center for the Chemistry of Molecularly Optimized Networks; [2]Department of Chemistry, Duke University, Durham, North Carolina 27708, United States; [3]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States; [4]Departments of Physics, Mechanical Engineering and Materials Science, and Biomedical Engineering, Duke University, Durham, North Carolina 27708, United States; [5]World Premier Institute for Chemical Reaction Design and Discovery (WPI ICReDD), Hokkaido University, Sapporo, JAPAN.

*To whom correspondence should be addressed. Phone (919) 660-1538. Fax: (919) 660-1605. Email: stephen.craig@duke.edu

Table of Contents

Materials and Methods

General Information.

All reagents were purchased from Sigma-Aldrich, Alfa Aesar, or Tokyo Chemical Industry Co., LTD and used without further purification. Glassware was dried in an oven (160˚C) overnight and cooled under an inert gas (N_2 or Ar). All reactions were performed under nitrogen atmosphere. Flash chromatography was performed using Silicycle SiliaFlash® F60 gel (40-63 μm particle size, 230-400 mesh) and medium pressure liquid chromatography (MPLC) was performed on a Teledyne ISCO CombiFlash Rf 200. All gel permeation chromatography (GPC) was performed on in-line two columns (Agilent PLgel 10^5 Å, 7.5 x 300 mm, 5μm, part number PL1110-6550) at room temperature using inhibitor free THF at a flow rate of 1.0 mL/min. The flow rate was set using an Agilent 1260 Infinity Isocratic pump, molecular weights were calculated using in line Wyatt Optilab T-rEX refractive index detector and Wyatt miniDAWN TREOS multi-angle light scattering detector, and UV absorbance was measured with an in-line Agilent 1260 Infinity UV detector. The UV detector monitored 190 to 800 nm with step of 2.0 nm and slit width of 4.0 nm. The refractive index increment (dn/dc) values were determined by using on-line 100% mass recovery assumption calculations built into Wyatt Astra software using injections of known concentration and mass. Before GPC analysis, 1-2 mg/mL in THF solutions were filtered through a 0.2 μm pore size PTFE syringe filters.

Small Molecule Characterization.

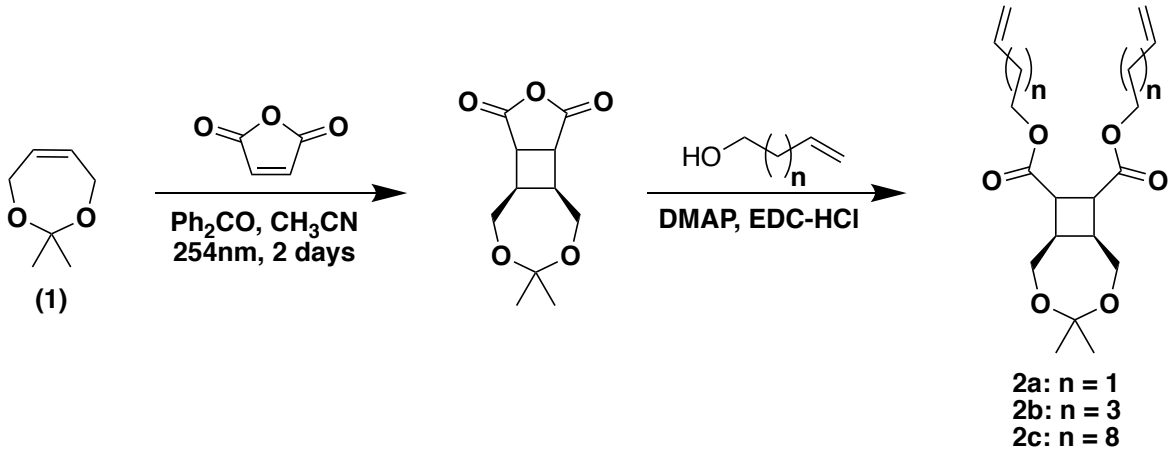
^{1}H-NMR and ^{13}C-NMR spectra were obtained on either a 500 MHz Varian spectrophotometer and the residual solvent peaks ($CDCl_3$: 7.26 ppm [^{1}H], 77.16 ppm [^{13}C] and DMSO-d6: 2.5 ppm [^{1}H], 39.52 ppm [^{13}C]) were used as an internal chemical shift reference. All chemical shifts are given in ppm (δ) and coupling constants (J) in Hz as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), or broad (b). High-resolution mass spectrometry was performed on an Agilent LCMS-TOF-DART at Duke University's Mass Spectrometry Facility.

Details of SMFS Measurements.

The AFM pulling experiments were conducted in toluene at an ambient temperature (~23˚C) in the same manner as described previously[1-5] using a homemade AFM, which was constructed using a Bruker (previously Digital Instruments) Multimode AFM head mounted on top of a piezoelectric positioner (Physik Instrumente, GmbH), similar to the one described in detail previously.[6] Sharp Microlever silicon probes (MSNL) were purchased from Bruker (Camarillo, CA) and the force curves used for analysis were obtained with rectangular-shaped cantilevers (205 μm x 15 μm, nominal tip radius ~2 nm, nominal spring constant k ~ 0.02 N/m, frequency ~ 15 kHz). Multiple probes of the same type were used throughout the course of the experiments. The spring constant of each cantilever was calibrated in air, using the thermal noise method, based on the energy equipartition theorem as described previously.[7] Cantilever tips were prepared by soaking in piranha solution for ~15 min at room temperature. Silicon surfaces were prepared by soaking ~30 min in hot piranha solution, followed by washing with DI-water and drying under a stream of nitrogen. The surface and cantilever were then placed in a UVO cleaner (ozone produced through UV light) for 15 min. After ozonolysis, the cantilever was mounted, and ~20 μL of a ~0.1-0.05

mg mL^{-1} polymer solution was added to the silicon surface and allowed to dry. Measurements were carried out in a fluid cell with scanning set for a series of constant velocity approaching/retracting cycles. To collect 'Force clamp' data cantilever deflection was monitored during each retraction cycle. If it reached threshold value corresponding to 200pN the system was switched to the force-control mode with the selected set point force value, which it attempted to keep for a set period of time (10-20sec), after which force-control mode was switched off and constant velocity retraction resumed to finish the 'pull'. During acquisition data were filtered at 500Hz. Force curves were collected in dSPACE (dSPACE Inc., Wixom, MI) and Matlab (The MathWorks, Inc., Natick, MA) and analyzed later using Matlab. .

Synthetic Procedures for P1-P3



Synthesis of bisalkenes 2a-c: Synthesis of bisalkene **2a** was performed according to prior literature.[8] The same procedure was adapted to synthesize bisalkenes **2b** and **2c**. Generally, compound **1** (2.56 g, 20 mmol, 1 eq.), prepared according to literature precedent,[9] was charged to a 250 mL quartz round bottom flask equipped with a magnetic stir bar along with maleic anhydride (4.31g, 20 mmol, 2.2 eq.) and benzophenone (1 g). Then, 100 mL of acetonitrile was added to dissolve all components and the colorless solution was sparged with N_2(g) for 30 minutes. Then, the N_2(g) inlet was removed and the sealed reaction flask was placed in a Rayonet photoreaction chamber and irradiated with UV light (254nm) for 50 hours with stirring. Then, the reaction was removed from the chamber and DMAP (0.54 g, 4.4 mmol, 0.2 eq.), EDC (16.8 g, 88 mmol, 4.4 eq.), and the corresponding alcohol (132 mmol, 6.6 eq.) were added to the reaction flask. The reaction was allowed to stir at room temperature overnight under ambient conditions. The solvent was then evaporated under reduced pressure and the remaining brown sludge was dissolved in 200 mL of ethyl acetate. The solution was washed with water (200 mL x 3) and brine (200 mL). The organic solution was then dried over Na_2SO_4. Solvent was removed under reduced pressure and purification by flash chromatography (SiO_2, 9:1 hexanes:EtOAc) furnished the desired product as a colorless oil with an average isolated yield of 10%.

(2a):

Physical State: colorless oil

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.77 (ddt, J = 17.0, 10.3, 6.7 Hz, 2H), 5.15 – 5.03 (m, 4H), 4.19 – 4.06 (m, 4H), 3.86 (dt, J = 13.1, 2.4 Hz, 2H), 3.75 – 3.67 (m, 2H), 3.24 (s, 2H), 2.85 (s, 2H), 2.37 (qt, J = 6.8, 1.4 Hz, 4H), 1.38 – 1.37 (m, 6H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 172.65, 133.84, 117.27, 117.22, 63.86, 61.41, 40.04, 39.42, 32.95, 30.91.

HRMS-ESI (m/z): [M + H]+ calcd for $C_{19}H_{28}O_6$, 353.1959; found, 353.1952.

TLC: R_f = 0.33 (4:1 hexanes:EtOAc), visualized with $KMnO_4$ stain.

(2b):

Physical State: colorless oil

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.82 – 5.74 (m, 2H), 5.05 – 4.93 (m, 4H), 4.13 – 4.01 (m, 4H), 3.91 – 3.83 (m, 2H), 3.76 – 3.67 (m, 2H), 3.24 (b, 2H), 2.86 (b, 2H), 2.09 – 2.04 (m, 4H), 1.64 – 1.58 (m, 4H), 1.48 – 1.35 (m, 10H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 172.74, 138.25, 114.89, 114.86, 64.78, 61.49, 40.12, 39.38, 33.24, 30.91, 27.98, 25.13.

HRMS-ESI (m/z): [M + H]+ calcd for $C_{23}H_{36}O_6$, 409.2585; found, 409.2587.

TLC: R_f = 0.4 (4:1 hexanes:EtOAc), visualized with $KMnO_4$ stain.

(2c):

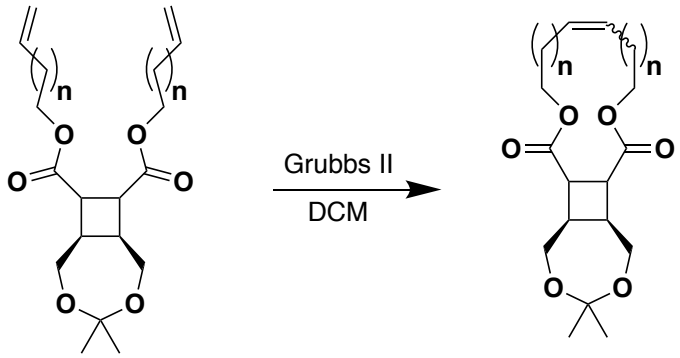
Physical State: colorless oil

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.85 – 5.77 (m, 2H), 5.03 – 4.89 (m, 4H), 4.11 – 3.99 (m, 4H), 3.91 – 3.82 (m, 2H), 3.75 – 3.68 (m, 2H), 3.26 – 3.21 (m, 1H), 2.89 – 2.84 (m, 1H), 2.04 (q, J = 7.1 Hz, 4H), 1.59 (m, 4H), 1.42 – 1.24 (m, 34H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 172.69, 139.17, 114.13, 102.46, 64.87, 61.85, 39.12, 33.78, 29.44, 29.40, 29.23, 29.09, 28.91, 28.57, 25.88, 25.41, 23.72.

HRMS-ESI (m/z): [M + H]+ calcd for $C_{33}H_{56}O_6$, 549.415; found, 549.4156.

TLC: R_f = 0.45 (4:1 hexanes:EtOAc), visualized with $KMnO_4$ stain.



2a: n = 1
2b: n = 3
2c: n = 8

3a: n = 1
3b: n = 3
3c: n = 8

Synthesis of macrocycles 3a-c: A solution of Grubbs catalyst 2nd generation (0.2 mmol, 0.1 eq.) in 1000 mL dichloromethane was sparged with N_2(g) for 30 minutes while stirring. To this solution was added dropwise a solution of bisalkene **2** (2 mmol, 1 eq.) in 5 mL of dichloromethane and the reaction was heated to 40 °C until disappearance of the starting material was observed by TLC. The reaction was cooled to room temperature, then opened to atmosphere and quenched with 3 mL of ethyl vinyl ether. Purification by flash chromatography (SiO_2, 7:3 hexanes:EtOAC) furnished macrocycles **3a-c** as white solids with an average isolated yield of 70%.

(3a, mixture of isomers):

Physical State: white solid

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.54 – 5.34 (m, 2H), 4.46 – 4.41 (m, 2H), 4.10 – 3.96 (m, 2H), 3.87 – 3.84 (m, 2H), 3.72 – 3.67 (m, 2H), 3.24 (s, 2H), 2.90 (d, J = 19.0 Hz, 2H), 2.57 – 2.25 (m, 4H), 1.39 – 1.32 (m, 6H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 172.38, 172.25, 128.95, 128.57, 102.42, 63.52, 62.64, 61.84, 61.80, 40.68, 40.34, 39.04, 38.70, 31.98, 27.25, 25.40, 23.69.

HRMS-ESI (m/z): [M + Na]+ calcd for $C_{17}H_{24}O_6$, 347.1465; found, 347.1467.

TLC: R_f = 0.2 (4:1 hexanes:EtOAc), visualized with $KMnO_4$ stain.

(3b, mixture of isomers):

Physical State: white solid

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.36 – 5.29 (m, 2H), 4.21 – 4.18 (m, 2H), 3.96 – 3.84 (m, 4H), 3.72 – 3.67 (m, 2H), 3.24 (s, 2H), 2.88 (s, 2H), 2.17 – 1.98 (m, 4H), 1.63 – 1.57 (m, 4H), 1.42 – 1.32 (m, 10H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 172.50, 131.14, 102.43, 64.69, 64.08, 61.88, 40.35, 40.33, 40.30, 40.27, 38.99, 38.91, 31.48, 30.55, 27.50, 27.21, 26.15, 25.42, 24.76, 23.87, 23.69.

HRMS-ESI (m/z): [M + Na]+ calcd for $C_{21}H_{32}O_6$, 403.2091; found, 403.2098.

TLC: R_f = 0.25 (4:1 hexanes:EtOAc), visualized with $KMnO_4$ stain.

(3c, mixture of isomers):

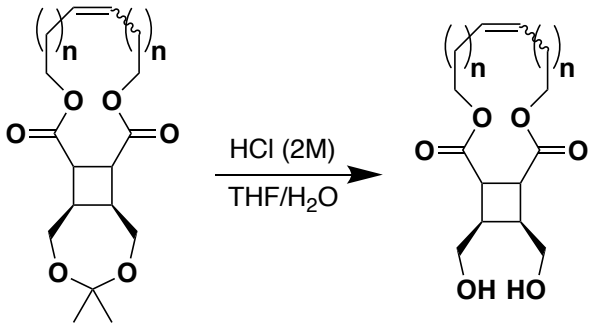
Physical State: white solid

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.37 – 5.30 (m, 2H), 4.11 – 3.95 (m, 4H), 3.88 – 3.85 (m, 2H), 3.75 – 3.67 (m, 2H), 3.25 (b, 2H), 2.88 (b, 2H), 2.07 – 1.97 (m, 4H), 1.60 – 1.55 (m, 4H), 1.40 – 1.22 (m, 30H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 172.64, 172.61, 130.81, 130.07, 102.46, 65.02, 64.89, 61.90, 40.35, 40.32, 39.02, 32.06, 29.60, 29.55, 29.38, 29.30, 29.27, 29.17, 28.87, 28.70, 28.65, 28.63, 28.06, 26.70, 26.23, 25.91, 25.46, 23.72.

HRMS-ESI (m/z): [M + H]+ calcd for $C_{31}H_{52}O_6$, 521.3837; found, 521.3842.

TLC: R_f = 0.38 (3:1 hexanes:EtOAc), visualized with $KMnO_4$ stain.



3a: n = 1
3b: n = 3
3c: n = 8

4a: n = 1
4b: n = 3
4c: n = 8

Synthesis of diols 4a-c: Compound **3** (0.85 mmol) was dissolved in 25 mL of THF and cooled to 0 °C in an ice-water bath. Then, 12.5 mL of a 2M HCl solution was added dropwise. The reaction was stirred for 1 hour at 0 °C and then quenched by the dropwise addition of saturated $NaHCO_3$ (aq) until a pH of 7 was achieved. The solution was then allowed to warm to room temperature and was extracted with ethyl acetate (100 mL x 2). The combined organic layers were washed with brine and subsequently dried over Na_2SO_4. Purification by flash chromatography (SiO_2, 3:7 hexanes:EtOAC) furnished compounds **4a-c** as white solids with an average isolated yield of 60%.

(4a, mixture of isomers):

Physical State: white solid

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.57 – 5.34 (m, 2H), 4.47 – 4.41 (m, 2H), 4.10 – 3.96 (m, 2H), 3.84 – 3.77 (m, 4H), 3.24 – 3.16 (m, 2H), 3.11 – 2.92 (m, 4H), 2.60 – 2.24 (m, 4H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 172.66, 172.47, 128.92, 128.55, 63.73, 62.91, 61.43, 61.40, 40.62, 40.42, 39.42, 39.07, 31.74, 27.23.

HRMS-ESI (m/z): [M + H]+ calcd for $C_{14}H_{20}O_6$, 285.1333; found, 285.1337.

TLC: R_f = 0.15 (4:1 hexanes:EtOAc), visualized with $KMnO_4$ stain.

(4b, mixture of isomers):

Physical State: white solid

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.37 – 5.28 (m, 2H), 4.25 – 4.18 (m, 2H), 3.98 – 3.77 (m, 6H), 3.25 – 3.14 (m, 2H), 3.06 – 3.01 (m, 2H), 2.79 (b, 2H), 2.21 – 2.06 (m, 3H), 2.01 – 1.85 (m, 1H), 1.62 – 1.57 (m, 4H), 1.49 – 1.35 (m, 4H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 172.77, 172.72, 131.10, 129.91, 64.82, 64.23, 61.27, 61.25, 40.19, 40.17, 39.24, 39.19, 31.40, 27.38, 27.10, 26.10, 24.65, 23.80.

HRMS-ESI (m/z): [M + H]+ calcd for $C_{18}H_{28}O_6$, 341.1959; found, 341.1962.

TLC: R_f = 0.2 (1:1 hexanes:EtOAc), visualized with $KMnO_4$ stain.

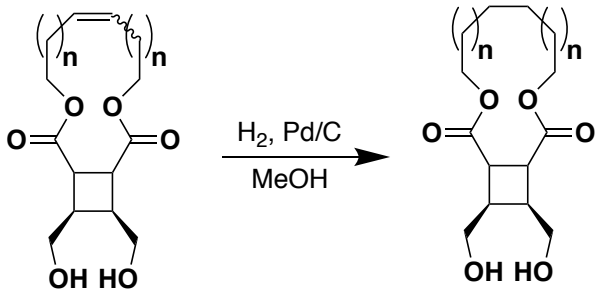
(4c, mixture of isomers):

Physical State: white solid

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.37 – 5.29 (m, 2H), 4.08 – 4.02 (m, 4H), 3.88 – 3.78 (m, 4H), 3.24 – 3.17 (m, 2H), 3.04 – 3.02 (m, 2H), 2.72 – 2.69 (m, 2H), 2.05 – 2.00 (m, 4H), 1.62 – 1.57 (m, 4H), 1.37 – 1.24 (m, 24H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 172.81, 130.79, 65.17, 61.53, 40.22, 39.30, 32.04, 29.59, 29.37, 29.27, 28.84, 28.60, 28.03, 26.21.

TLC: R_f = 0.25 (7:3 hexanes:EtOAc), visualized with $KMnO_4$ stain.



4a: n = 1
4b: n = 3
4c: n = 8

5a: n = 1
5b: n = 3
5c: n = 8

Hydrogenation of diols 4a-c: Diol **4** (1 mmol) was dissolved in 10 mL of dry methanol in a 25 mL flame-dried, 2-neck round bottom flask and sparged with Ar(g) for 15 minutes. Then, 2 mg of palladium on carbon (10 wt. % loading) was added and the suspension was further sparged with Ar(g) for 15 minutes. The suspension was then placed under vacuum for 10 seconds and backfilled with H_2(g) via balloon. The suspension was then sparged with H_2(g) for 6 hours. Then, the outlet needle was removed and the suspension was allowed to stir overnight under an atmosphere of H_2(g). The hydrogen balloon was then removed and the suspension was sparged with Ar(g) for 10 minutes and then poured over Celite® and washed with MeOH (100 mL). Purification by flash chromatography (SiO_2, 3:7 hexanes:EtOAC) furnished compounds **5a-c** as white solids with an average isolated yield of 65%.

(5a):

Physical State: white solid

^{1}H NMR (500 MHz, $CDCl_3$): δ 4.45 – 4.41 (m, 2H), 4.03 – 3.94 (m, 2H), 3.85 – 3.79 (m, 4H), 3.29 – 3.21 (m, 2H), 3.13 – 2.92 (m, 4H), 1.73 – 1.50 (m, 8H), 1.48 – 1.36 (m, 2H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 172.87, 65.04, 61.35, 40.61, 38.96, 26.31, 24.32.

TLC: R_f = 0.24 (4:1 hexanes:EtOAc), visualized with $KMnO_4$ stain.

(5b):

Physical State: white solid

^{1}H NMR (500 MHz, $CDCl_3$): δ 4.21 – 4.19 (m, 2H), 4.02 – 3.98 (m, 2H), 3.80 – 3.74 (m, 4H), 3.43 (s, 2H), 3.18 (d, J = 5.5 Hz, 2H), 3.00 – 2.90 (m, 2H), 1.71 – 1.51 (m, 4H), 1.42 – 1.18 (m, 12H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 172.82, 63.58, 61.47, 39.96, 39.57, 27.93, 26.44, 25.48, 24.11.

HRMS-ESI (m/z): [M + H]+ calcd for $C_{18}H_{30}O_6$, 343.2115; found, 343.2112.

TLC: R_f = 0.25 (1:1 hexanes:EtOAc), visualized with $KMnO_4$ stain.

(5c):

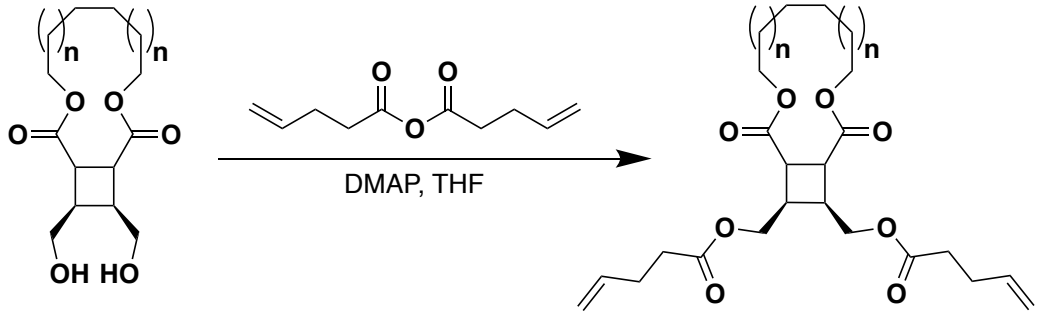
Physical State: white solid

^{1}H NMR (500 MHz, $CDCl_3$): δ 4.11 – 3.99 (m, 4H), 3.84 – 3.83 (m, 4H), 3.49 (s, 1H), 3.20 (d, J = 5.7 Hz, 2H), 3.04 – 3.02 (m, 2H), 2.72 (m, 1H), 1.62 – 1.58 (m, 4H), 1.34 – 1.29 (m, 32H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 172.77, 65.06, 61.53, 40.23, 39.32, 29.04, 29.00, 28.69, 28.54, 28.36, 27.98, 27.50, 27.47, 25.72.

HRMS-ESI (m/z): [M + H]+ calcd for $C_{28}H_{50}O_6$, 483.3680; found, 483.3691.

TLC: R_f = 0.27 (7:3 hexanes:EtOAc), visualized with $KMnO_4$ stain.



5a: n = 1
5b: n = 3
5c: n = 8

6a: n = 1
6b: n = 3
6c: n = 8

Synthesis of bisalkenes 6a-c: Compound **5** (1 mmol, 1 eq.) and 4-dimethylaminopyridine (1.8 mmol, 1.8 equivalent) were dissolved in anhydrous THF (10 mL) in a 20 mL scintillation vial.

Under an atmosphere of N_2(g), 4-pentenoic anhydride (4.4 mmol, 4.4 eq.) was added dropwise via syringe and the reaction was stirred at room temperature overnight. Excess anhydride was quenched with 1 mL MeOH. Purification by flash chromatography (SiO_2, 4:1 hexanes:EtOAC) furnished compounds **6a-c** as colorless oils with an average yield of 60%.

(6a):

Physical State: colorless oil

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.85 – 5.78 (m, 2H), 5.10 – 4.97 (m, 4H), 4.50 – 4.39 (m, 2H), 4.27 – 4.16 (m, 4H), 3.99 – 3.95 (m, 2H), 3.30 – 3.15 (m, 4H), 2.43 – 2.33 (m, 8H), 1.69 – 1.65 (m, 4H), 1.61 – 1.53 (m, 4H), 1.45 – 1.37 (m, 2H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 172.76, 171.81, 136.47, 115.65, 65.14, 63.08, 41.63, 35.59, 33.42, 28.72, 26.37, 24.30.

HRMS-ESI (m/z): [M + H]+ calcd for $C_{24}H_{34}O_8$, 451.2326; found, 451.2335.

TLC: R_f = 0.56 (1:1 hexanes:EtOAc), visualized with $KMnO_4$ stain.

(6b):

Physical State: colorless oil

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.85 – 5.77 (m, 2H), 5.10 – 4.98 (m, 4H), 4.27 – 4.20 (m, 6H), 4.07 – 4.02 (m, 2H), 3.25 – 3.18 (m, 2H), 3.14 – 3.11 (m, 2H), 2.51 – 2.33 (m, 8H), 1.71 – 1.56 (m, 4H), 1.42 – 1.24 (m, 12H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 172.73, 171.89, 136.47, 115.66, 63.77, 63.11, 40.89, 36.33, 33.44, 28.73, 27.88, 26.36, 25.47, 24.05.

HRMS-ESI (m/z): [M + H]+ calcd for $C_{28}H_{42}O_8$, 507.2952; found, 502.2965.

TLC: R_f = 0.4 (3:1 hexanes:EtOAc), visualized with $KMnO_4$ stain.

(6c):

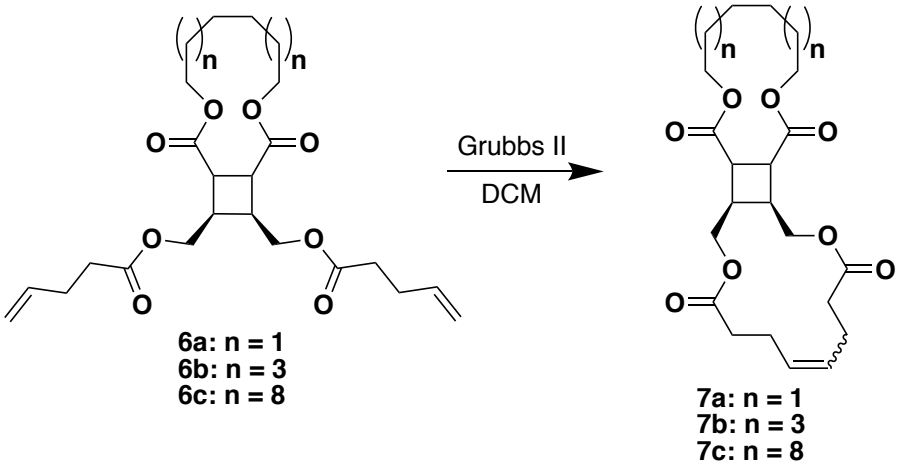
Physical State: colorless oil

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.85 – 5.77 (m, 2H), 5.09 – 4.98 (m, 4H), 4.27 – 4.18 (m, 4H), 4.16 – 3.99 (m, 5H), 3.25 – 3.19 (m, 2H), 3.15 – 3.13 (m, 2H), 2.45 – 2.32 (m, 8H), 1.63 – 1.58 (m, 5H), 1.34 – 1.24 (m, 34H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 172.72, 171.85, 136.45, 115.65, 65.19, 63.11, 41.17, 35.98, 33.42, 29.02, 28.99, 28.72, 28.67, 28.51, 28.35, 27.97, 27.48, 27.47, 25.69.

HRMS-ESI (m/z): [M + H]+ calcd for $C_{38}H_{62}O_8$, 647.4518; found, 647.4527.

TLC: R_f = 0.5 (3:1 hexanes:EtOAc), visualized with $KMnO_4$ stain.



Synthesis of ROMP monomers 7a-c: An identical procedure to that of compounds **3a-c** was followed, yielding compounds **7a-c** as white solids with an average yield of 70%.

(7a):

Physical State: white solid

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.47 – 5.54 (m, 2H), 4.48 – 4.43 (m, 2H), 4.27 – 4.16 (m, 4H), 4.03 – 3.94 (m, 2H), 3.30 – 3.16 (m, 4H), 2.37 – 2.23 (m, 8H), 1.70 – 1.66 (m, 4H), 1.61 – 1.53 (m, 4H), 1.45 – 1.39 (m, 2H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 173.24, 171.89, 130.36, 65.22, 63.57, 40.02, 35.94, 34.56, 27.70, 26.29, 24.31.

HRMS-ESI (m/z): [M + H]+ calcd for $C_{22}H_{30}O_8$, 432.2013; found, 432.2015.

TLC: R_f = 0.44 (1:1 hexanes:EtOAc), visualized with $KMnO_4$ stain.

(7b):

Physical State: white solid

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.47 – 5.45 (m, 2H), 4.30 – 4.17 (m, 6H), 4.08 – 4.03 (m, 2H), 3.26 – 3.19 (m, 2H), 3.13 – 3.10 (m, 2H), 2.34 – 2.24 (m, 8H), 1.72 – 1.58 (m, 4H), 1.40 – 1.26 (m, 12H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 173.22, 171.97, 130.34, 63.79, 63.60, 39.39, 36.66, 34.57, 27.89, 27.70, 26.40, 25.48, 24.10.

HRMS-ESI (m/z): [M + H]+ calcd for $C_{26}H_{38}O_8$, 479.2639; found, 479.2654.

TLC: R_f = 0.3 (3:1 hexanes:EtOAc), visualized with $KMnO_4$ stain.

(7c):

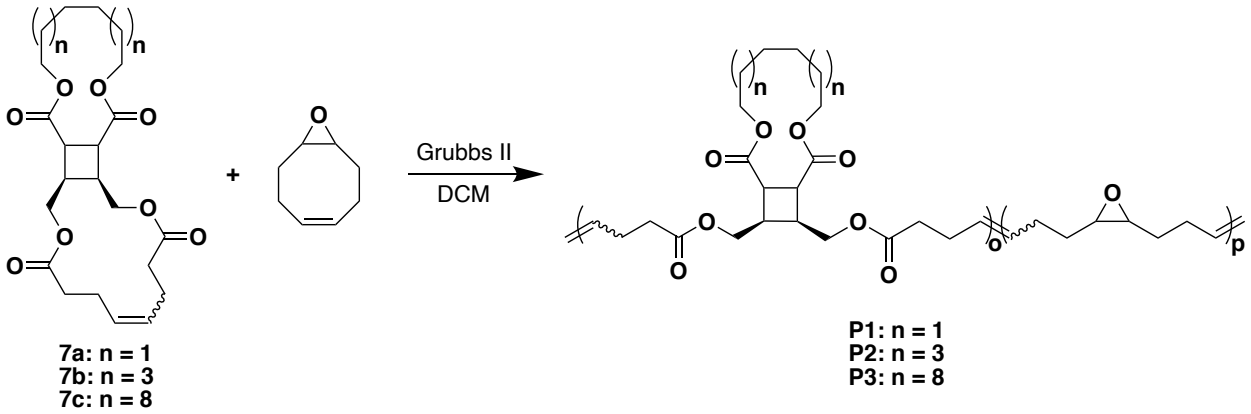
Physical State: white solid

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.47 – 5.45 (m, 2H), 4.26 – 4.19 (m, 4H), 4.11 – 4.01 (m, 4H), 3.23 – 3.22 (m, 2H), 3.16 – 3.10 (m, 2H), 2.35 – 2.24 (m, 8H), 1.63 – 1.58 (m, 4H), 1.34 – 1.29 (m, 34H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 173.23, 171.98, 130.36, 65.22, 63.62, 39.63, 36.39, 34.57, 28.99, 28.99, 28.68, 28.51, 28.37, 28.00, 27.70, 27.53, 27.51, 25.69.

HRMS-ESI (m/z): [M + H]+ calcd for $C_{36}H_{58}O_8$, 619.4205; found, 619.4215.

TLC: R_f = 0.35 (3:1 hexanes:EtOAc), visualized with $KMnO_4$ stain.



Synthesis of ROMP polymers P1-P3: Cyclobutane macrocycles were co-polymerized with freshly distilled 9-oxabicyclo[6.1.0]non-4-ene using Grubbs Catalyst 2nd Generation at a total monomer concentration of 1 M in dry DCM under N_2(g). A 2 mL crimp top vial was charged with macrocycle **7** and freshly distilled 9-oxabicyclo[6.1.0]non-4-ene under N_2(g). A stock solution of Grubbs Catalyst 2nd Generation in dry DCM was prepared and sparged with N_2(g) for 15 min. Then, the Grubbs Catalyst solution (0.00067 equiv.) was added via air-tight syringe to dissolve the monomers and initiate the polymerization. After 16 hours, the polymerization was quenched with 10 drops of ethyl vinyl ether and then precipitated into methanol to give the crude polymer. Polymers were purified via one additional precipitation into MeOH and one reverse precipitation from DCM according to literature precedent. The polymer was dried on the high vac for at least 1 hour prior to use.

(P1):

Physical State: white, gummy polymer

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.54 – 5.35 (m, 2H), 4.45 – 4.41 (m, 0.54H), 4.24 – 4.15 (m, 1.07H), 3.97 – 3.93 (m, 0.54H), 3.28 – 3.25 (m, 0.55H), 3.19 – 3.16 (m, 0.54H), 2.92 – 2.89 (m 1.41H), 2.39 – 2.06 (m, 5.15H), 1.69 – 1.47 (m, 4.87H), 1.46 – 1.18 (m, 4.75H).

(P2):

Physical State: white, gummy polymer

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.56 – 5.34 (m, 2H), 4.29 – 4.17 (m, 1.11H), 4.05 – 4.01 (m, 0.37H), 3.22 – 3.21 (m, 0.37H), 3.12 – 3.10 (m, 0.37H), 2.93 – 2.89 (m, 1.57H), 2.40 – 2.07 (m, 4.75H), 1.71 – 1.49 (m, 4.11H), 1.42 – 1.21 (m, 2.28H).

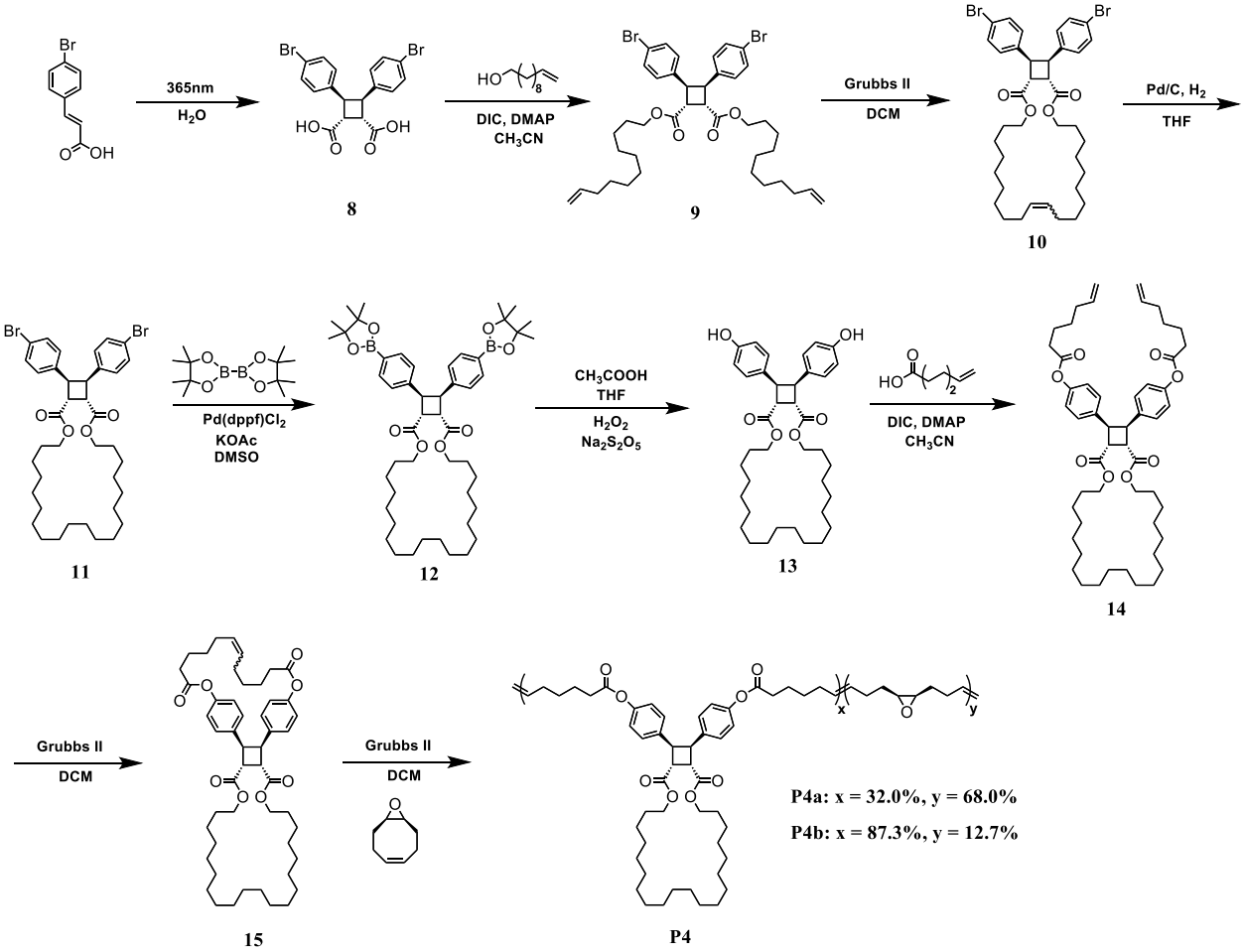
(P3):

Physical State: white, gummy polymer

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.51 – 5.42 (m, 2H), 4.25 – 4.20 (m, 0.4H), 4.10 – 3.99 (m, 0.41H), 3.22 – 3.21 (m, 0.2H), 3.15 – 3.09 (m, 0.2H), 2.93 – 2.90 (m, 1.78H), 2.38 – 2.07 (m, 4.54H), 1.65 – 1.53 (m, 4.1H), 1.33 – 1.25 (m, 3.27H).

Further polymer characterization for P1-P3 are listed in Table 1 of the manuscript.

Synthetic Procedures for P4



Scheme S1. Synthetic route of **P4**.

Synthesis of 8. To a 2 L conical flask, around 1 L acetone was added and heated to boiling point. About 15 g of trans-4-bromo-cinnamic acid was added portion-wise to the boiling acetone. The mixture was stirred and heated until the solid was completely dissolved (more acetone was needed if the solid did not completely dissolve). The solution was cooled down at room temperature overnight to yield needle like β-trans-4-bromo-cinnamic acid crystal (metastable). The crystal was left at the bottom of the flask, while the acetone was poured into another conical flask for the future preparation of β-trans-4-bromo-cinnamic acid crystal. To a 500 mL pyrex conical flask, 400 mL DI water was added and stirred vigorously with a stir bar. The collected crystal was suspended in the DI water. The suspension was irradiated with 365nm UV light for overnight to obtain a cloudy suspension, which was filtrated afterwards to obtained 8 g of white powder. ^{1}H NMR confirmed the white powder was β-truxinic acid (**8**). ^{1}H NMR (500 MHz, DMSO-d6): δ 12.48 (s, 2H), 7.13-

7.29 (d, J = 8.5 Hz, 4H), 7.03-7.01 (d, J = 8.5 Hz, 4H), 4.20-4.19 (m, 2H), 3.79-3.78 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d6): δ 173.75, 138.63, 130.68, 130.16, 119.23, 43.73, 42.36. HRMS-ESI (m/z): $[M + H]^+$ calculated for $C_{18}H_{14}Br_2O_4$, 452.9932; observed 452.9319.

Synthesis of 9. To a 250 mL round bottom flask (RBF), compound **8** (5 g, 11 mmol) was mixed with 100 mL acetonitrile. DIC (7 mL, 44 mmol) was added dropwise. The solid first dissolved, and then the solution became cloudy again. DMAP (380 mg, 17 mmol) and 10-Undecen-1-ol (8.8 mL, 44 mmol) were then added to the solution. The reaction was stirred at r.t. for overnight. After the reaction completed, the solution was filtrated to obtain a yellow solution. The yellow solution was concentrated using rotary evaporator and diluted with 200 mL DCM. The solution was washed with DI water (100 mL×2) and brine (100mL×1). DCM phase was collected and dried with Na_2SO_4. After filtration, the solution was concentrated onto silica. Column chromatography (SiO_2, 0 ~ 10% EtOAC / hexane gradient eluent) gave compound **9** as a colorless oil (7.28 g). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.26 - 7.25 (d, J = 8.5 Hz, 4H), 6.80 - 6.78 (d, J = 8.5 Hz, 4H), 5.85 - 5.76 (ddt, J = 16.9, 10.1, 6.6 Hz, 2H), 5.01 - 4.97 (dd, J = 17.1, 1.9 Hz, 2H), 4.94 - 4.91 (dd, J = 10.2, 2.1 Hz, 2H), 4.32 - 4.31 (m, 2H), 4.16-4.07 (m, 4H), 3.73 - 3.72 (m, 2H), 2.05 - 2.01 (m, 4H), 1.65 - 1.59 (m, 4H), 1.38 - 1.27 (m, 24H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.20, 139.28, 137.61, 131.44, 129.56, 120.66, 114.29, 65.51, 44.44, 43.57, 33.91, 29.58, 29.52, 29.35, 29.22, 29.03, 28.67, 25.99. HRMS-ESI (m/z): $[M + H]^+$ calculated for $C_{40}H_{54}Br[81Br]O_4$, 757.2462; observed 759.2427.

Synthesis of 10. To a 2 L RBF, compound **9** (2 g, 2.6 mmol) was dissolved in 1.3 L dichloromethane. The solution was heated to reflux, and Grubbs II (112 mg, 0.13 mmol) was added portion-wise. The reaction was left to react for overnight. After the reaction completed, the solution was cooled to room temperature, then opened to atmosphere and quenched with 10 mL of ethyl vinyl ether. Purification by flash chromatography (SiO_2, 0 ~ 10% EtOAC in hexane, gradient eluent) furnished **10** (1.7 g) as a white solid with an isolated yield of 88%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.27 - 7.25 (d, J = 8.4 Hz, 4H), 6.80 - 6.78 (d, J = 8.2 Hz, 4H), 5.35 - 5.31 (m, 2H), 4.34 - 4.33 (m, 2H), 4.16 - 4.03 (m, 4H), 3.74 - 3.73 (m, 2H), 2.06 – 2.00 (m, 4H), 1.66 – 1.60 (m, 4H), 1.36 – 1.27 (m, 20H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.02, 137.52, 131.33, 130.83, 129.47,

120.54, 65.57, 44.13, 43.48, 32.05, 29.63, 29.40, 29.30, 28.86, 28.63, 28.06, 26.27. HRMS-ESI (m/z): $[M + H]^+$ calculated for $C_{38}H_{50}Br_2O_4$, 729.2149; found, 729.2141.

Synthesis of 11. Compound **10** (1.7 g, 2.3 mmol) was dissolved in 20 mL of dry THF in a 50 mL flame-dried, 3-neck round bottom flask and sparged with Ar (g) for 15 minutes. Then, 40 mg of palladium on carbon (10 wt. % loading) was added and the suspension was further sparged with Ar (g) for 15 minutes. The suspension was then placed under vacuum for 10 seconds and backfilled with H_2 (g) via balloon. The suspension was then sparged with H_2(g) for 6 hours. Then, the outlet needle was removed, and the suspension was allowed to stir overnight under an atmosphere of H_2(g). This procedure was repeated for one more day to assure complete reduction. The hydrogen balloon was then removed, and the suspension was sparged with Ar(g) for 10 minutes and then poured over Celite® and washed with DCM (100 mL). Purification by flash chromatography (SiO_2, 0 ~ 10% EtOAC in hexane, gradient eluent) furnished compounds **11** (1 g) as white solid with an isolated yield of 60%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.27 - 7.25 (d, J = 8.5 Hz, 4H), 6.80 - 6.78 (d, J = 8.5 Hz, 4H), 4.34 – 4.32 (m, 2H), 4.16 - 4.07 (m, 4H), 3.74 - 3.72 (m, 2H), 1.67 – 1.61 (m, 4H), 1.35 – 1.29 (m, 32H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.15, 137.66, 131.47, 129.59, 120.67, 65.58, 44.32, 43.65, 29.20, 29.17, 28.84, 28.70, 28.52, 28.14, 27.66, 27.63, 25.89. HRMS-ESI (m/z): $[M + H]^+$ calculated for $C_{38}H_{52}Br_2O_4$, 731.2305; found, 731.2285.

Synthesis of 12. To a 50 mL RBF, compound 1b (1 g, 1.3 mmol), $(Bpin)_2$ (770 mg, 3 mmol), Pd(dppf)Cl_2·DCM (107 mg, 0.13 mmol), KOAc (853 mg, 7.8 mmol) were mixed with 30 mL anhydrous DMSO. The mixture was sparged with N_2 for 10 minutes and heated up to 110 °C. The solids completely dissolved when the temperature reached ~70°C, and the solution became dark purple. The reaction was stirred at 110 °C for overnight. After the reaction was completed, the solution was cooled down to r.t. and diluted with DCM (200 mL). The dilute solution was filtered with Celite®. The filtrate was washed with DI water (200 mL×3) and brine (200 mL×1). DCM phase was collected and dried with Na_2SO_4. After filtration, the solution was concentrated onto silica. Column chromatography (SiO_2, 0 ~ 25% EtOAC / hexane gradient eluent) gave compound **12** (0.7 g) as bluish white solid. ^{1}H NMR suggests there were about 15% monofunctional byproduct, but the crude product of 12 was used in next step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.54 - 7.52 (d, J = 7.8 Hz, 4H), 6.95 - 6.93 (d, J = 7.8 Hz, 4H), 4.42 - 4.41

(m, 2H), 4.17 - 4.07 (m, 4H), 3.82 - 3.81 (m, 2H), 1.67 - 1.61 (m, 4H), 1.30 - 1.29 (m, 56H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.20, 141.93, 134.46, 127.06, 83.54, 65.12, 44.82, 43.57, 28.92, 28.89, 28.57, 28.44, 28.26, 27.89, 27.42, 27.39, 25.63, 24.73. HRMS-ESI (m/z): $[M + H]^+$ calculated for $C_{50}H_{76}[11B]_2O_8$, 825.5872; found, 827.5793.

Synthesis of 13. To a 50 mL RBF, compound **12** (0.7 g, 0.85 mmol) and acetic acid (0.8 mL) were dissolved in 20 mL THF. The solution was cooled down in ice water bath. 30% H_2O_2 (2 mL) was added dropwise. The reaction was stirred at r.t for overnight. After the reaction was completed, sodium metabisulfite (1 g) was added. The solution was concentrated with rotary evaporator and diluted with EtOAc (200 mL). The solution was washed with DI water (100 mL×2) and brine (100mL×1). EtOAc phase was collected and dried with Na_2SO_4. After filtration, the solution was concentrated onto silica. Column chromatography (SiO_2, 0 ~ 40% EtOAC in hexane, gradient eluent, RediSep Rf Gold Silica Flash Chromatography Column, Teledyne ISCO) gave compound **13** as a colorless sticky oil (460 mg). The product turned solid under vacuum. Note that compound **13** should be stored in the freezer (side products were found by NMR after stored at r.t. for a week). ^1H NMR (500 MHz, DMSO-d6): δ 9.06 (s, 2H), 6.80 – 6.78 (d, J = 8.6 Hz, 4H), 6.49 – 6.47 (d, J = 8.6 Hz, 4H), 4.06 – 3.96 (m, 6H), 3.79 – 3.76 (m, 2H), 1.57 – 1.52 (m, 4H), 1.27 (m, 32H). ^{13}C NMR (126 MHz, DMSO-d6) δ 172.47, 155.39, 129.19, 128.90, 114.56, 64.23, 44.01, 42.64, 28.53, 28.48, 28.09, 28.09, 27.71, 27.22, 26.69, 26.63, 25.27. HRMS-ESI (m/z): $[M + H]^+$ calculated for $C_{38}H_{54}O_6$, 607.3993; observed 607.3999.

Synthesis of 14. To a 50 mL round bottom flask (RBF), compound **13** (460 mg, 0.76 mmol), 6-heptenoic acid (388 mg, 3 mmol) and DMAP (129 mg, 1 mmol) were dissolved with 20 mL DCM. DIC (0.48 mL, 3 mmol) was added dropwise. The reaction was stirred at r.t. for overnight. After the reaction was completed, the solution was filtrated and diluted with 20 mL DCM. The solution was washed with DI water (50 mL×2) and brine (50mL×1). DCM phase was collected and dried with Na_2SO_4. After filtration, the solution was concentrated onto silica. Column chromatography (SiO_2, 0 ~ 5% EtOAC in DCM, gradient eluent) gave compound **14** (377 mg) as a white solid. ^1H NMR (500 MHz, $CDCl_3$): δ 6.93 - 6.92 (d, J = 8.5 Hz, 4H), 6.86 – 6.84 (d, J = 8.5 Hz, 4H), 5.84 – 5.76 (ddt, J = 16.9, 10.2, 6.6 Hz, 2H), 5.04 – 5.00 (dd, J = 17.1, 1.9 Hz, 2H), 4.98 – 4.95 (dd, J = 10.2, 1.9 Hz, 2H), 4.39 – 4.37 (m, 2H), 4.16 – 4.07 (m, 4H), 3.77 – 3.76 (m, 2H), 2.51 – 2.48 (t,

J = 7.5 Hz, 4H), 2.12 – 2.07 (m, 4H), 1.76 – 1.69 (m, 4H), 1.66 – 1.63 (m, 4H), 1.51 – 1.45 (m, 4H), 1.37 – 1.30 (m, 32H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.09, 171.86, 149.08, 138.19, 136.01, 128.67, 121.08, 114.73, 65.23, 44.12, 43.62, 34.07, 33.20, 28.95, 28.92, 28.60, 28.46, 28.27, 28.16, 27.89, 27.41, 27.39, 25.64, 24.20. HRMS-ESI (m/z): $[M + H]^+$ calculated for $C_{52}H_{74}O_8$, 827.5457; observed 827.5456.

Synthesis of 15. An identical procedure to that of compounds **10** was followed, yielding compounds **15** (255 mg) as white solids with a yield of 70%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.84 – 6.77 (m, 8H), 5.39 (m, 2H), 4.36 – 4.34 (m, 2H), 4.17 – 4.07 (m, 4H), 3.82 – 3.81 (m, 2H), 2.48 – 2.44 (m, 4H), 2.04 – 2.03 (m, 4H), 1.66 – 1.65 (m, 8H), 1.45 – 1.30 (m, 36H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.44, 171.93, 149.51, 135.64, 130.43, 128.72, 121.20, 65.47, 44.91, 42.67, 34.42, 32.22, 29.21, 29.18, 28.86, 28.73, 28.68, 28.53, 28.16, 27.68, 27.65, 25.91, 24.39. HRMS-ESI (m/z): $[M + H]^+$ calculated for $C_{50}H_{70}O_8$, 799.5144; found, 799.5141.

Synthesis of P4a and P4b. A 2 mL crimp top vial was charged with **15** (0.33 equiv. for P4a, 0.8 equiv. for P4b) and freshly distilled 9-oxabicyclo[6.1.0]non-4-ene (0.67 equiv. for P4a, 0.2 equiv. for P4b) under N_2 (g). A stock solution of Grubbs Catalyst 2nd Generation in dry DCM was prepared and sparged with N_2 (g) for 10 min. Then, the stock solution that contains Grubbs Catalyst (1/1500 equiv.) was added via air-tight syringe to dissolve the monomers to the concentration of 1 M and initiate the polymerization. After 16 hours, the polymerization was quenched with 5 drops of ethyl vinyl ether and then precipitated into methanol to give the crude polymer. Polymers were purified via two additional precipitation into MeOH and one reverse precipitation from DCM according to literature precedent. The polymer was dried on the high vac for at least 1 hour prior to use. ^{1}H NMR (500 MHz, $CDCl_3$): P4a: δ 6.93 – 6.84 (m, 8H), 5.51 – 5.44 (m, 6.25H), 4.38 – 4.37 (m, 1.9H), 4.14 – 4.08 (m, 3.89H), 3.77 – 3.75 (m, 1.9H), 2.92 (m, 4.33H), 2.49 – 2.46 (m, 3.85H), 2.18 – 2.01 (m, 12.75H), 1.71 – 1.63 (m, 24.39H), 1.45 – 1.30 (m, 37.32H). P4b: P4a: δ 6.93 – 6.84 (m, 8H), 5.51 – 5.44 (m, 2.29H), 4.38 – 4.37 (m, 2.01H), 4.14 – 4.08 (m, 4.15H), 3.77 – 3.75 (m, 2.10H), 2.92 (m, 0.66H), 2.49 – 2.46 (m, 4.04H), 2.18 – 2.01 (m, 5.23H), 1.71 – 1.30 (m, 52.44H).

Further polymer characterization for P4 is listed in Table 1 of the manuscript.

Details of Constant-Velocity SMFS Analysis

Determination of Polymer Extension by Fitting to a FJC Model

The contour lengths of the polymers before and after transition were determined by fitting the pre- and post- transition force curves to an extended freely jointed chain (FJC) model as described previously.[1-2] Such a fit allows the determination of polymer chain lengths corresponding to the initial state, when active mechanophores are intact (*L1*), and the final state, when all mechanophores have undergone an irreversible ring-opening reaction (*L2*).

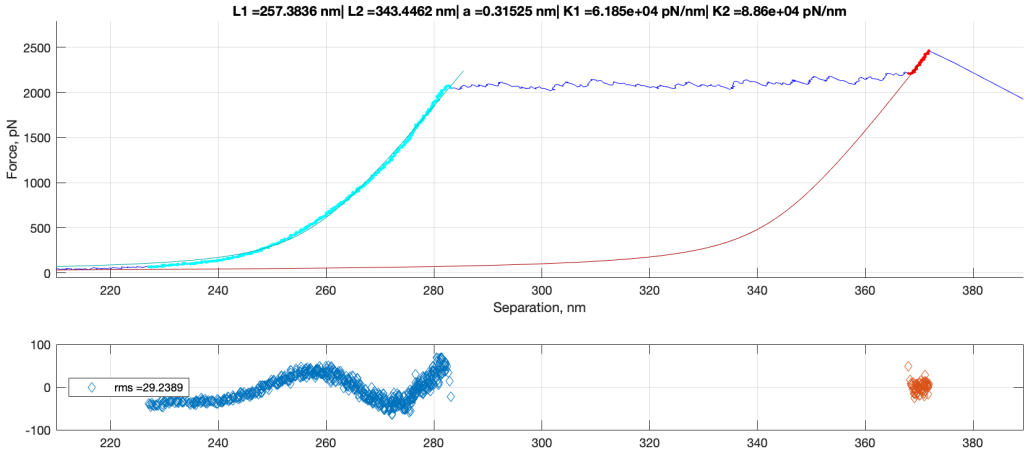


Figure S1: Representative FJC fitting of polymer P1.

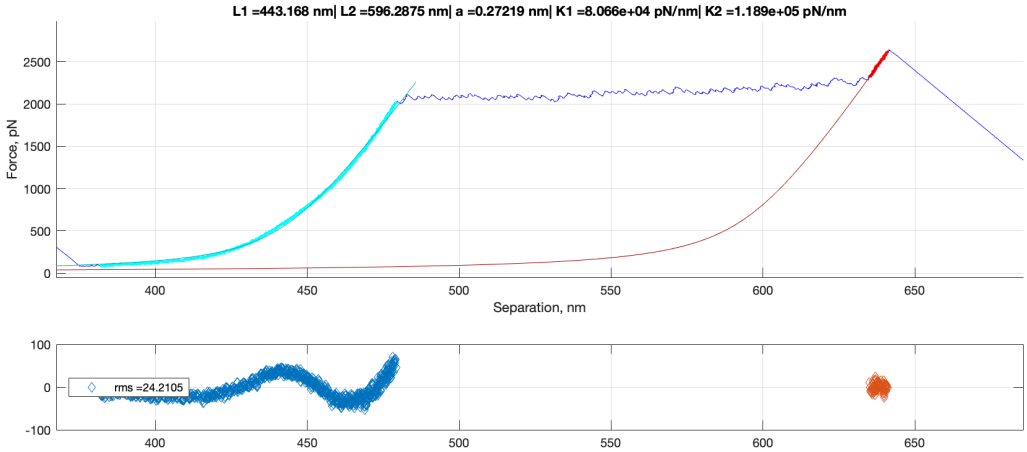


Figure S2: Representative FJC fitting of polymer P2.

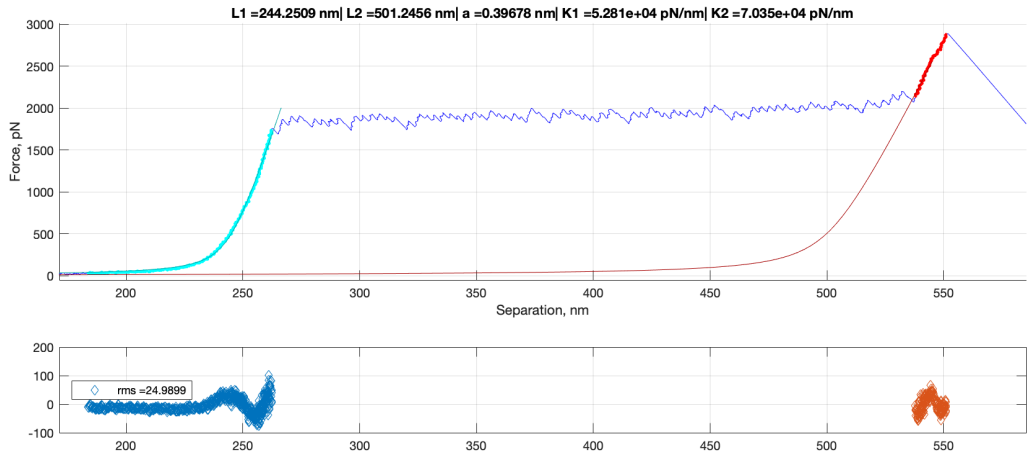


Figure S3: Representative FJC fitting of polymer P3.

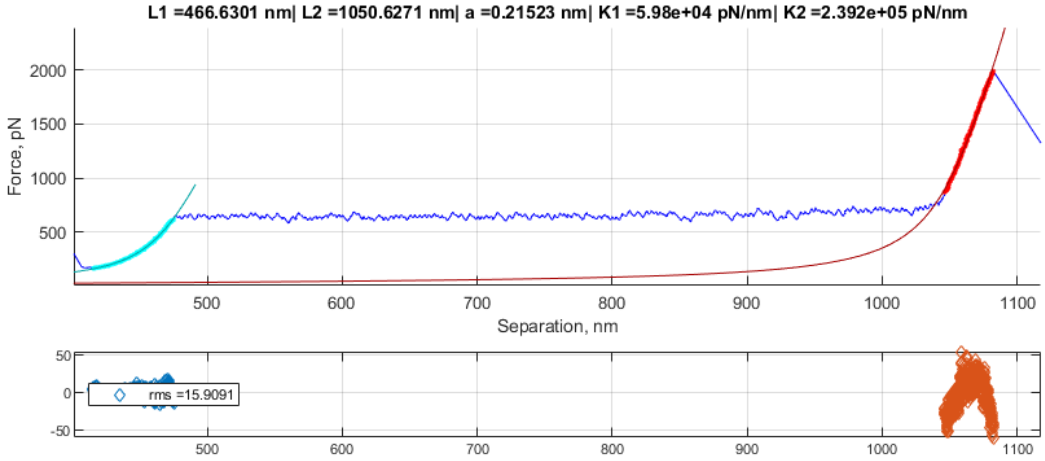


Figure S4: Representative FJC fitting of polymer P4.

A similar FJC fitting was performed to determine the change in polymer contour length after individual rupture events, as shown in **Figures S5-S6** for polymers **P3** and **P4**, respectively. For this analysis, the segment elasticity of the unreacted chain (K1) was determined from the FJC fitting, and this value was used for K2 (as has been done previously[10]). In the figures below, L2-L1 is taken to be the change in contour length for an individual event. L2-L1 was calculated for all rupture events that could successfully be fit within a given plateau and across multiple pulls. Histograms of the aggregate data can be seen in Figure 4 of the manuscript.

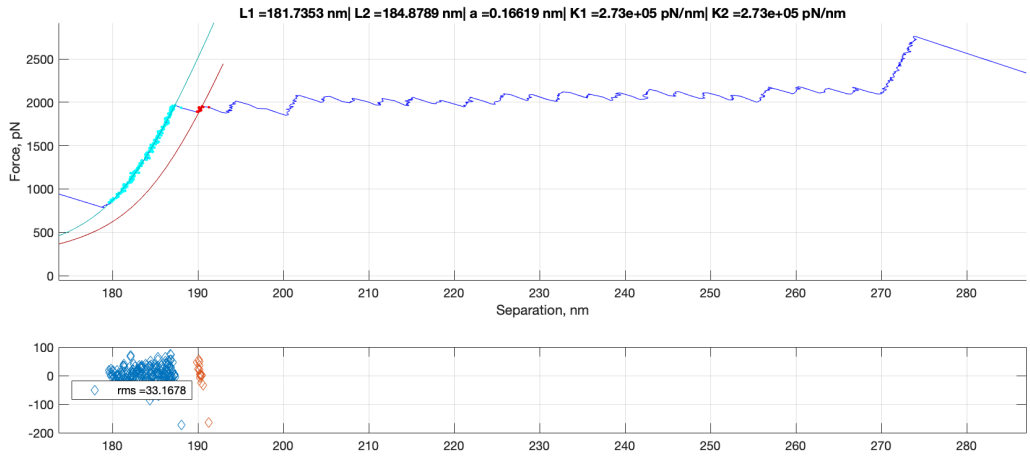


Figure S5: Representative FJC fitting of a single rupture event for polymer P3.

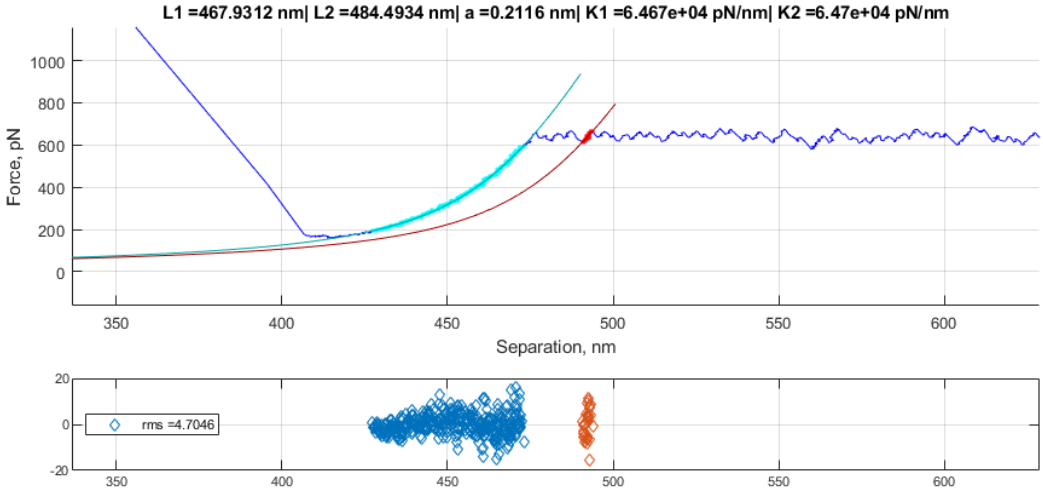


Figure S6: Representative FJC fitting of a single rupture event for polymer P4.

Spreadsheet Analysis - Counting Bonds/Ruptures Within a Strand

For the spreadsheet analysis of force dependent kinetics for polymers **P3** and **P4**, we need to count the number of rupture events that occurs within a given force range, $N(f)$, and the total time spent by cyclobutane mechanophores within a given force range, $t(f)$. To do this, we use Matlab's *Findpeaks* function to first select all of the ruptures that can be resolved within a given plateau region of the force vs. separation curve (**Figure S7**). Matlab then tabulates the separation value for all rupture events (all red triangles). Slightly different analyses were performed for counting the ruptures in **P3** vs **P4**, but the concept is the same.

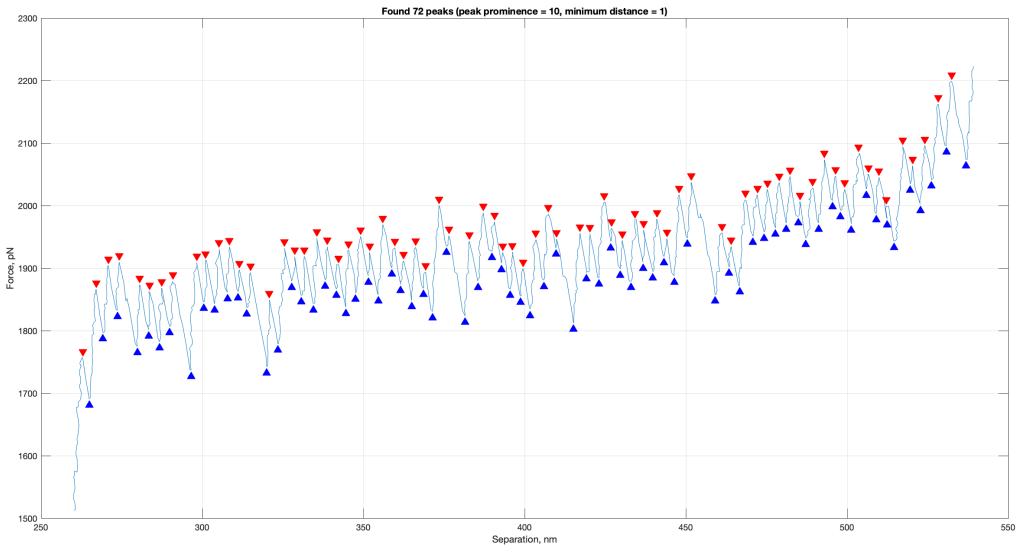


Figure S7. Representative result for using Matlab to select peaks in the plateau region for polymer **P3**, using the same curve as shown in **Figure S3**.

For polymer **P3**, we first calculate the distance between detected peaks, $\Delta d = d_n - d_{n-1}$ (red triangle to red triangle). We then sort Δd into 0.4 nm bin sizes and generate frequency histograms (**Figure S8**). Fitting the histograms with a Gaussian distribution provides average Δd values, Δd_{avg}, of 3.46 ± 0.66 nm for **P3**.

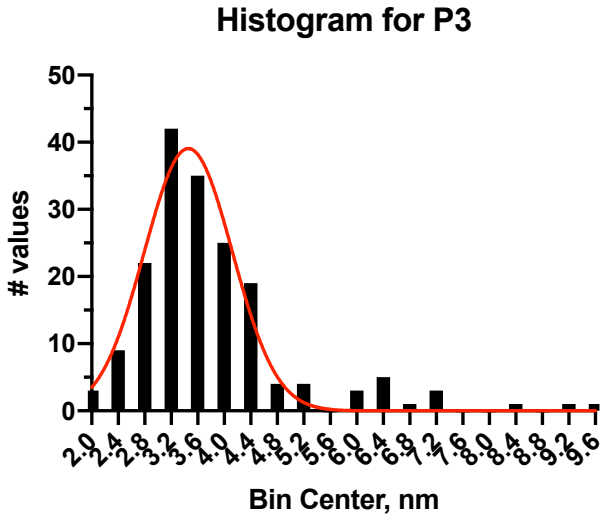


Figure S8. Histogram of Δd across all pulls for polymer **P3**, which was fit with a Gaussian distribution (red line).

So that we can more accurately determine the total number of cyclobutane bonds/ruptures within a given strand, we compare Δd_{avg} to all individual Δd values to estimate if the Δd value corresponds to a single, double, or triple activation event. More specifically, if Δd falls within two standard deviations of Δd_{avg}, then we record that event as a single activation event. If Δd is larger than Δd_{avg} by two standard deviations, but within two standard deviations of $2*\Delta d_{avg}$, then we record that

event as a double activation event. Finally, if Δd is larger than $2*\Delta d_{avg}$ by two standard deviations, then we record that event as a triple activation event.

For polymer **P4**, since there are more peaks that cannot be resolved by the *Findpeaks* function (as compared to **P3**), the grid of the Matlab plot was used to assist in the determination of whether a peak contains a single, double or triple rupture event. An illustration is shown in **Figure S9**. Peaks **c** and **e** with 2 rupture events can be identified by looking at the intercepts between their loading curves and a chosen horizontal line (e.g., 630 pN in **Figure S9**). Based on peaks **a** and **c** and their intercept with 630 pN (horizontal grid), we can determine the intercept separation (by vertical grids) of a double event. We then compare it with the intercept separation between peak **c** and **d** to discern that there are 2 rupture events between peak **c** and **d**. Using this method peak by peak, we are able to determine the number of rupture events for each peak. Note that **P3** has also been analyzed with this same method (not shown here), and the final results of the rate-force dependence from this method are within error of the results obtained using the aforementioned method.

For each CV curve of **P3** and **P4**, the total number of rupture events of that curve N can be obtained.

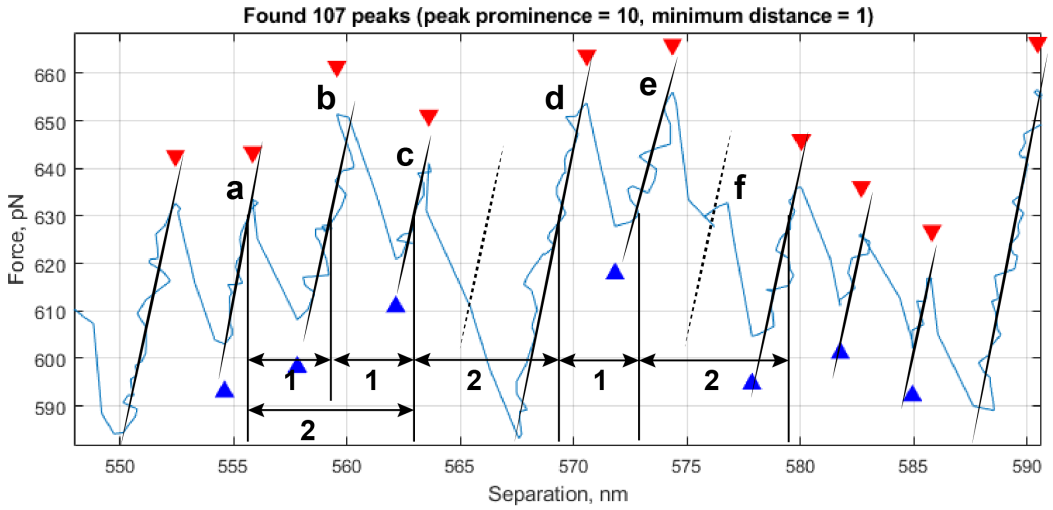


Figure S9. Representative illustration of counting rupture events for each peak for P4. Solid lines are added as a guide for the loading curve for each peak, dashed lines are added as a guide to show the loading curve of unresolved rupture events.

Spreadsheet Analysis - Calculating Time a Mechanophore Spends Within a Force Range

In order to determine the total amount of time a cyclobutane bond spends within a given force range, $t(f)$, we first code Matlab to count the total number of digital data points, ddp, that are collected within a given force range between two rupture events (from red triangle to red triangle). We chose a force range of 20 pN for **P3** and 50 pN for **P4**. We then multiply the ddp between rupture events by the number of cyclobutane bonds that are still intact at that time to obtain an effective number of ddp. We know how many bonds are still intact because we counted the total number of bonds, N (see above), and we determined how many bonds were broken for each detected rupture, so we can keep track of how many bonds remain as we count from rupture to

rupture. For a given force bin, *t(f)* can be calculated by dividing the total effective number of ddp in that force bin with the sampling rate (5000 ddp / s).

Spreadsheet Analysis - Counting Ruptures Within a Force Bin

To determine which force bin a rupture should be counted in for any particular pulling experiment, we simply look at the ddps that were collected between ruptures (red triangle to red triangle) and see which force bin the last ddp was recorded in for a given rupture. For **P3**, since multi-rupture events (see above) are very rare (<15%), and the force bin size is large, then all multiple rupture events (2 or 3) are assumed to have occurred within the same force bin as the identified peak. For **P4**, multi-rupture events (see above) are relatively frequent (25 ~ 28%), and the force bin size is relatively small (20 pN). Therefore, peaks that can be identified by looking at the curves but cannot be captured by the *Findpeaks* function (e.g. peak **f** in **Figure S9**) are counted in the corresponding force bin. Peaks that cannot be identified by looking at the curves (e.g. the peak that is supposed to be between peak **c** and **d** in **Figure S9**) are counted to be in the same bin as the previously identified peak (peak **c**) as if these two rupture events happened at the same time. For a given force bin, *N(f)* can be calculated by summing the number of all rupture events in the corresponding force bin.

Spreadsheet Analysis - Summary Tables

The operations described above have been used for 8 CV curves of **P3** and 6 CV curves of **P4**. Since some curves do not have enough number of rupture events for analysis, we then combine the number of rupture events of several curves to obtain a group of *N(f)* data with a total number of rupture events across all bins more than 100. The corresponding *t(f)* were also totaled together. 8 curves of **P3** were combined to obtain three groups of *N(f)* and *t(f)* data, while 6 curves of **P4** were combined to obtained 4 groups of *N(f)* and *t(f)* data. Next, the rate constant *k(f)* was calculated for each group, and the average and the standard error of *k(f)* can be obtained. Tables S1 and S2 below summarize all constant velocity data that was collected and analyzed for polymers **P3** and **P4**, respectively.

We note again that for both **P3** and **P4**, SMFS was performed on multiple polymer formulations (varying amounts of mechanophore content) and across multiple days and AFM cantilevers. Overlays of all pulls used in the aforementioned analyses are shown in **Figure S10**.

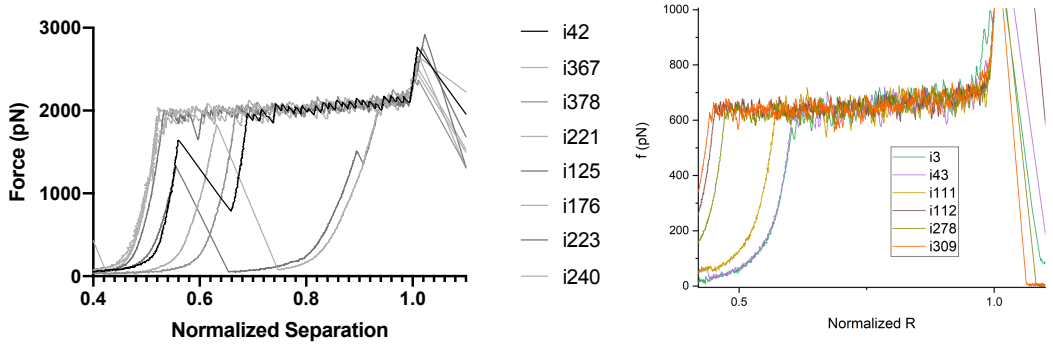


Figure S10. Overlay of normalized force vs separation curves for polymers P3 (left) and P4 (right).

Table S1. Constant velocity data for **P3**. Shaded columns were used for rate-force dependence plot.

Group	Bin center (pN)	1925	1975	2025	2075	2125	2175	2225	2275	2325	2375
	N(f)	3	18	30	25	19	7	1	0	0	0
1	t(f) (s)	6.6414	14.8056	11.6046	3.2592	0.8316	0.099	0.0018	0	0	0
	k(f) (s^{-1})	0.45171	1.21576	2.58518	7.67059	22.8475	70.7071	555.556	0	0	0
	N(f)	0	2	29	27	30	9	3	2	0	1
2	t(f) (s)	1.074	8.2836	11.9718	7.0728	2.526	0.3714	0.0756	0.0108	0	0.0042
	k(f) (s^{-1})	0	0.24144	2.42236	3.81744	11.8765	24.2326	39.6825	185.185	0	238.095
	N(f)	7	18	22	29	13	6	2	0	0	0
3	t(f) (s)	5.2818	4.8312	3.8568	1.8324	0.3348	0.0714	0.0084	0	0	0
	k(f) (s^{-1})	1.32531	3.72578	5.70421	15.8262	38.8292	84.0336	238.095	0	0	0
k(f)_AVG				3.57058	9.10476	24.5177	59.6578				
k(f)_SE				1.06784	3.54002	7.82525	18.1255				

Table S2. Constant velocity data for **P4**. Shaded columns were used for rate-force dependence plot.

Group	Bin center (pN)	590	610	630	650	670	690	710	730	750	770	790	810	830
	N(f)		1	18	52	45	25	15	9	1	2			
1	t(f) (s)		15.186	41.328	52.591	22.902	5.601	1.5864	0.3462	0.024	0.0096			
	k(f) (s^{-1})		0.0658	0.4355	0.9887	1.9648	4.4634	9.4553	25.996	41.666	208.33			
	N(f)		5	24	36	34	19	14	8	0	0	1		
2	t(f) (s)		21.521	31.333	23.464	10.161	4.3938	1.314	0.225	0.0066	0.006	0.0006		
	k(f) (s^{-1})		0.2323	0.7659	1.5342	3.3459	4.3242	10.654	35.555	0	0	1666.6		
	N(f)	2	5	22	59	47	22	17	4	2	3	1	1	1
3	t(f) (s)	7.756	22.495	52.818	53.956	19.525	5.3352	2.0598	0.3048	0.1164	0.0936	0.0234	0.012	0.0036
	k(f) (s^{-1})	0.257	0.2222	0.4165	1.0934	2.4071	4.1235	8.2532	13.123	17.182	32.051	42.735	83.333	277.77
	N(f)		3	10	22	28	42	38	10	7	1	1	1	
4	t(f) (s)		6.174	10.114	10.917	8.4	4.653	1.8492	0.2364	0.0564	0.0078	0.0078	0.003	
	k(f) (s^{-1})		0.4859	0.9887	2.0152	3.3333	9.0264	20.549	42.301	124.11	128.20	128.20	333.33	
k(f)_AVG (s^{-1})				0.6516	1.4079	2.7628	5.4844	12.228	29.244					
k(f)_SE (s^{-1})				0.2760	0.4688	0.6901	2.3654	5.6334	12.658					

Details of Constant-Force SMFS Analysis

Representative Curves

Extension vs. time curves and force vs. time curves are obtained according to the procedure described above. Representative curves are shown in **Figure S11** and **Figure S12** for polymers **P3** and **P4**, respectively. The extension vs. time curves are used to obtain both the change in polymer contour length per activation event (by fitting each "step" with Matlab, see Figure 3 of manuscript) and to obtain the rate of unfolding at a given force (by fitting the entire curve with an exponential decay function, see **Figures S13-14**). The value "p2" in the force vs. time curves was used as the force at which the polymer was clamped, obtained by fitting the curve using Matlab. The "spikes" of decreasing force in the force vs. time curves correspond to individual rupture events and line up with the "steps" that can be seen in the distance vs. time curves.

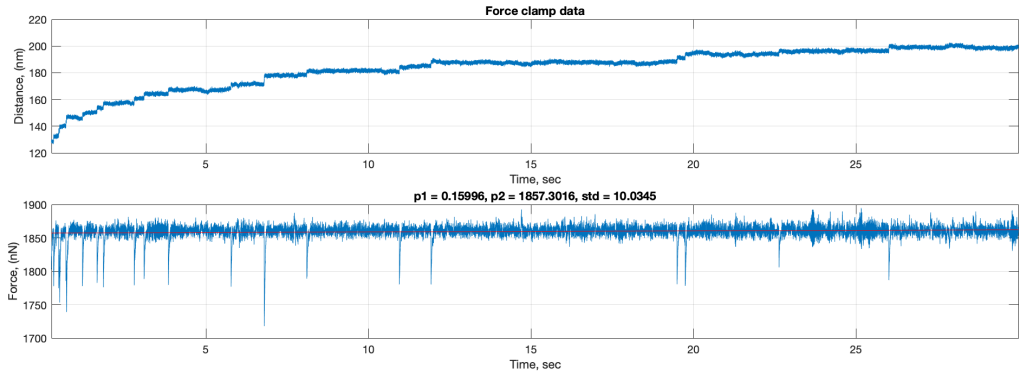


Figure S11. Representative extension vs. time (top) and force vs. time (bottom) curves for polymer P3.

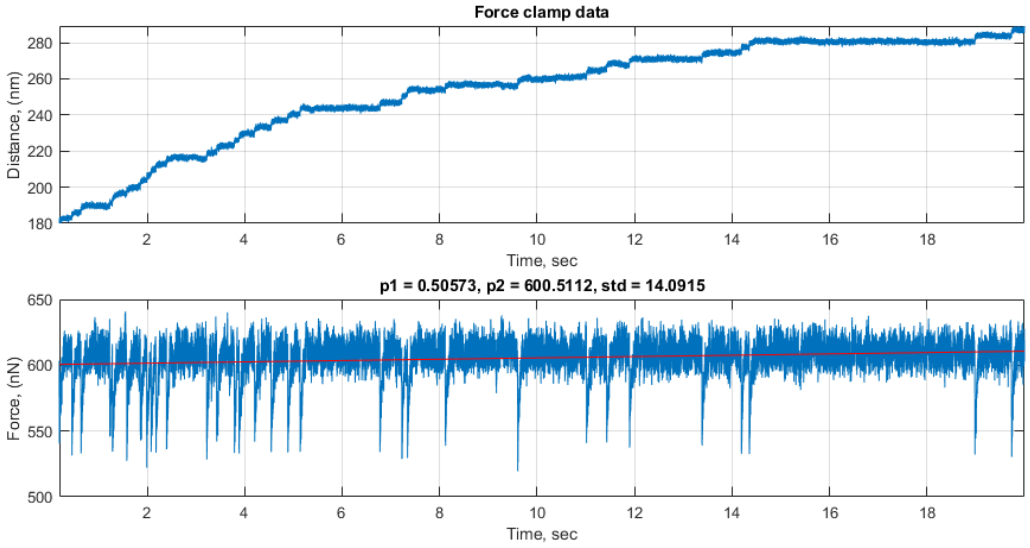


Figure S12. Representative extension vs. time (top) and force vs. time (bottom) curves for polymer P4.

Curve Fitting

The extension vs. time curves are fit with an exponential decay ($y = a + b * e^{-c*x}$) curve using Matlab (**Figures S13-S14**). The "c" value, obtained from the fit, corresponds to the rate ($k(f)$) that is used in constructing the rate vs. force plots shown in Figure 5 of the manuscript.

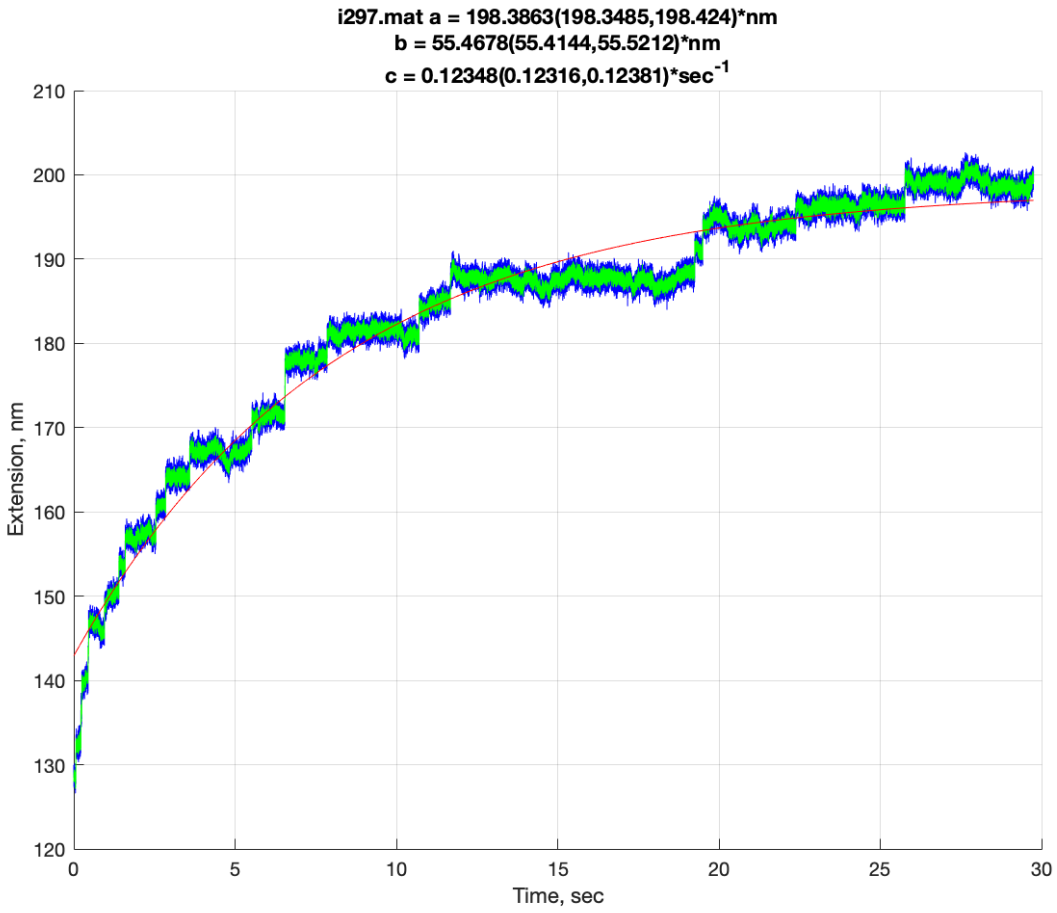


Figure S13. Representative fit of the extension vs. time curve with an exponential decay function for polymer P3.

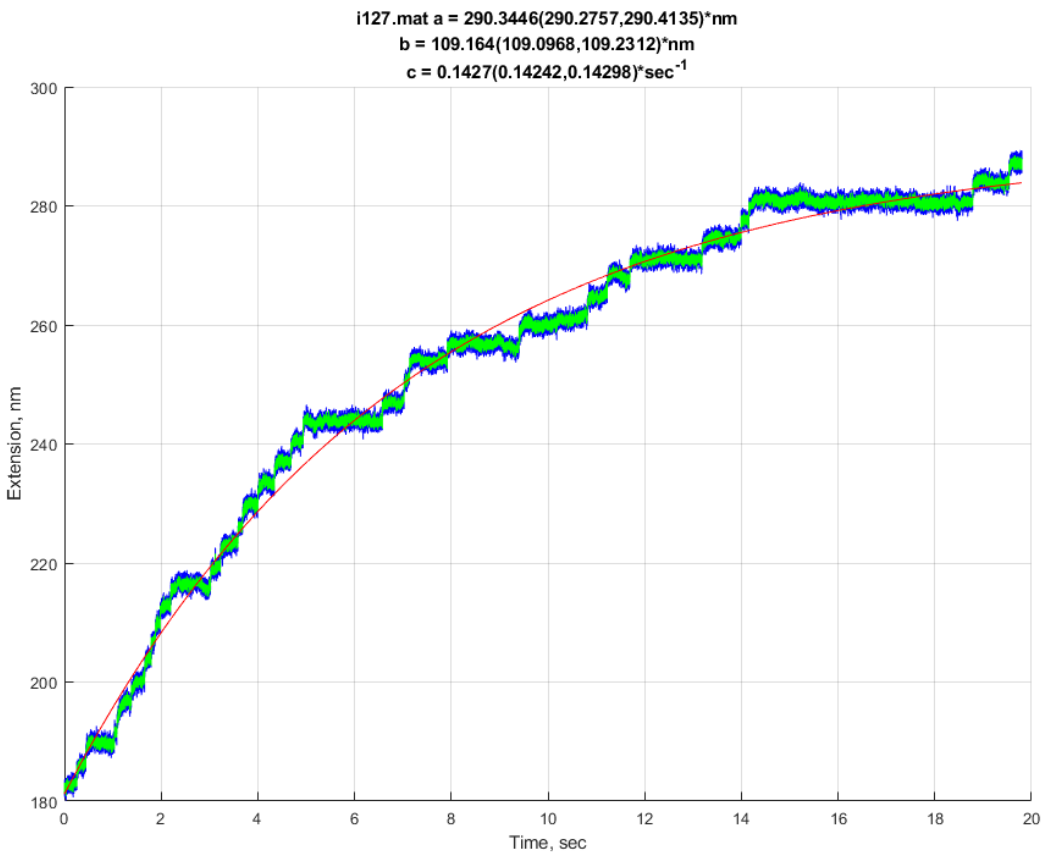


Figure S14. Representative fit of the extension vs. time curve with an exponential decay function for polymer P4.

Modeling of Mechanophore-Embedded Polymer Extension

Modeling of Monomer Contour Lengths

The detailed procedure of modeling of the contour lengths of the repeating units has been described previously.[2] The modeling was performed using Spartan® software. The equilibrium conformers of the molecules were minimized at the molecular mechanics level of theory for **CB3** and the semi-empirical (PM3) level of theory for **CB4**. The end-to-end distance of the molecule was constrained until the bonding geometries were noticeably distorted. CoGEF (constrained geometry simulates external force)[11] plots of energy as a function of displacement (blue-dot to blue-dot, below) was then obtained by shortening the constraint in 0.1 Å increments. The incremental change in energy (E_n - E_{n-1}) vs. change in distance (d_n - d_{n-1}) was taken as the force at the midpoint of the increment, and the resulting force vs. displacement curve was extrapolated to zero force to give a force-free contour length, (l_1) or (l_2).

Summary of Monomer Extension Data.

Mechanophore	Reactant (Å)	*E,E* Ring-Opened Product (Å)
CB3	14.1	45.4
CB4	19.1	53.2

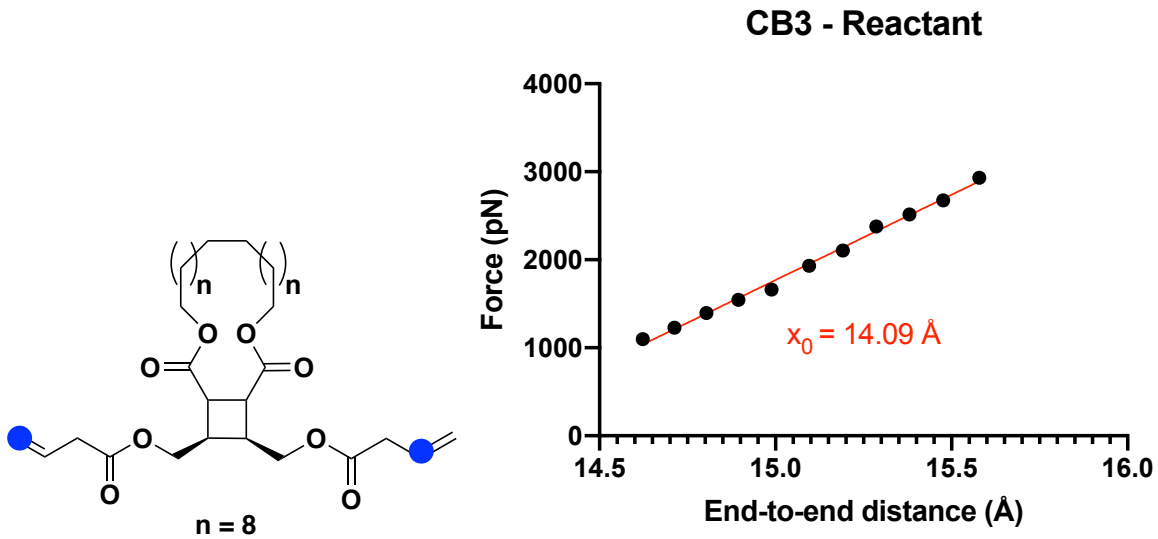


Figure S15. Monomer CB3 in its unreacted form (left) and the corresponding force vs. displacement curve (right) obtained by CoGEF. Fitting the curve (red line) provides l_1 as the x-intercept: l_1 = 14.1 Å.

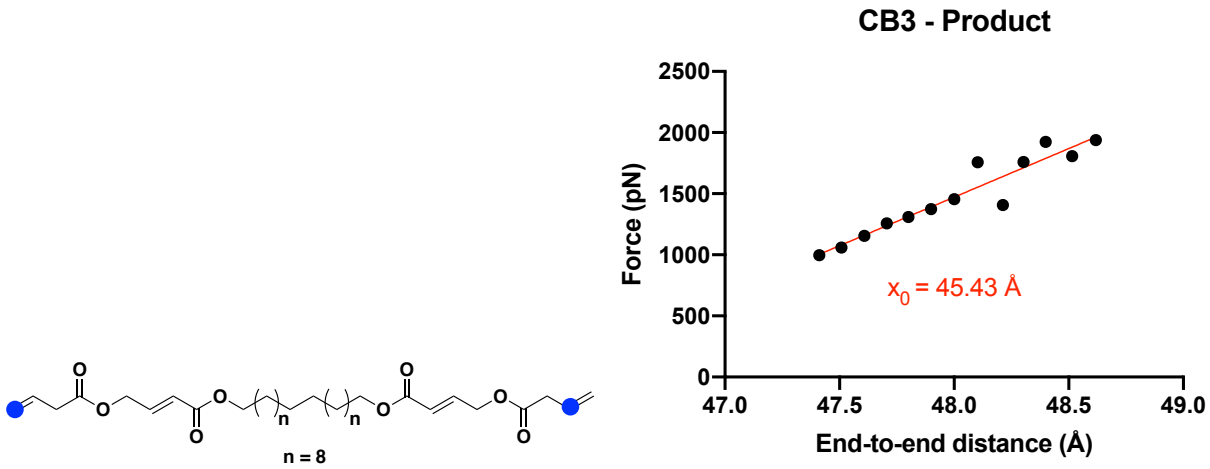


Figure S16. Monomer CB3 in its fully unravelled form (left) and the corresponding force vs. displacement curve (right) obtained by CoGEF. Fitting the curve (red line) provides l_2 as the x-intercept: l_2 = 45.4 Å.

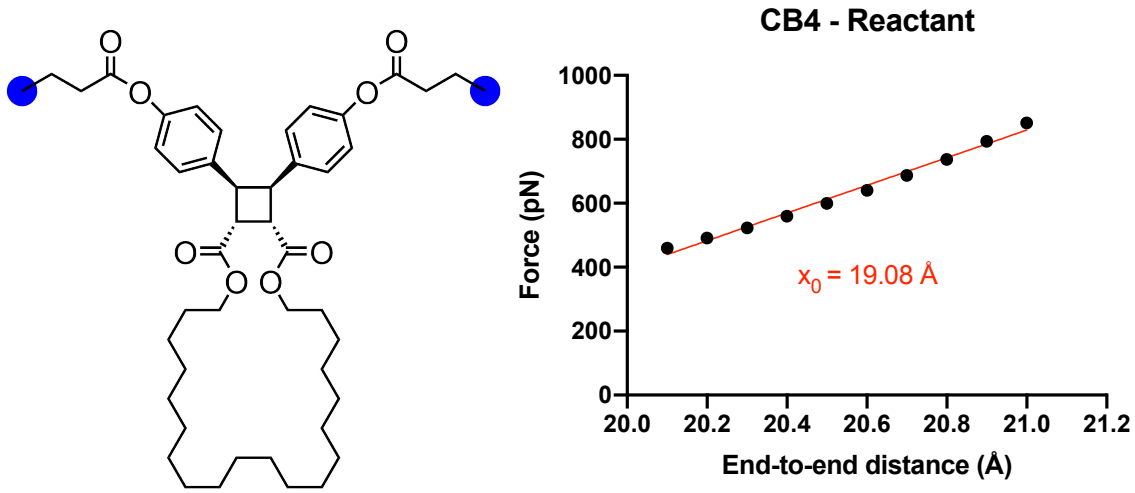


Figure S17. Monomer CB4 in its unreacted form (left) and the corresponding force vs. displacement curve (right) obtained by CoGEF. Fitting the curve (red line) provides l_1 as the x-intercept: l_1 = 19.1 Å.

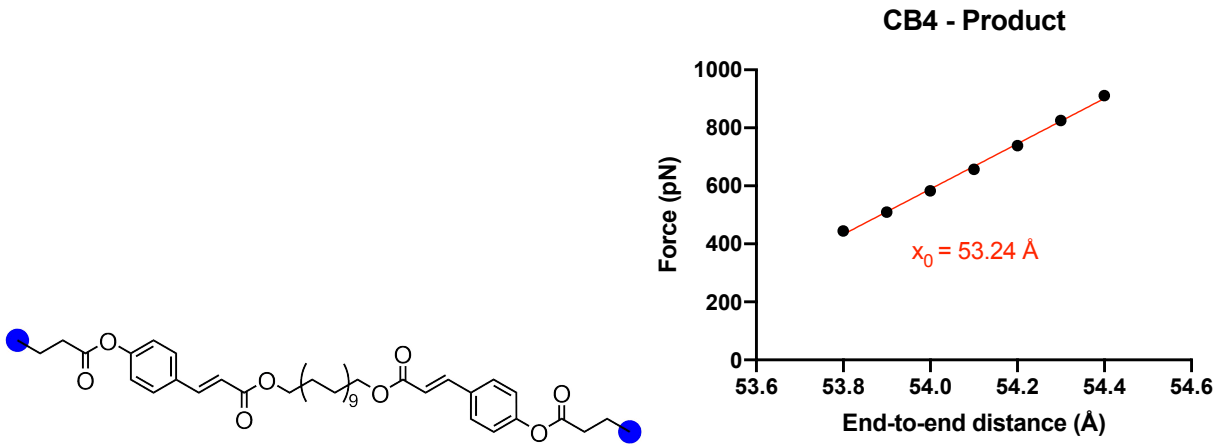


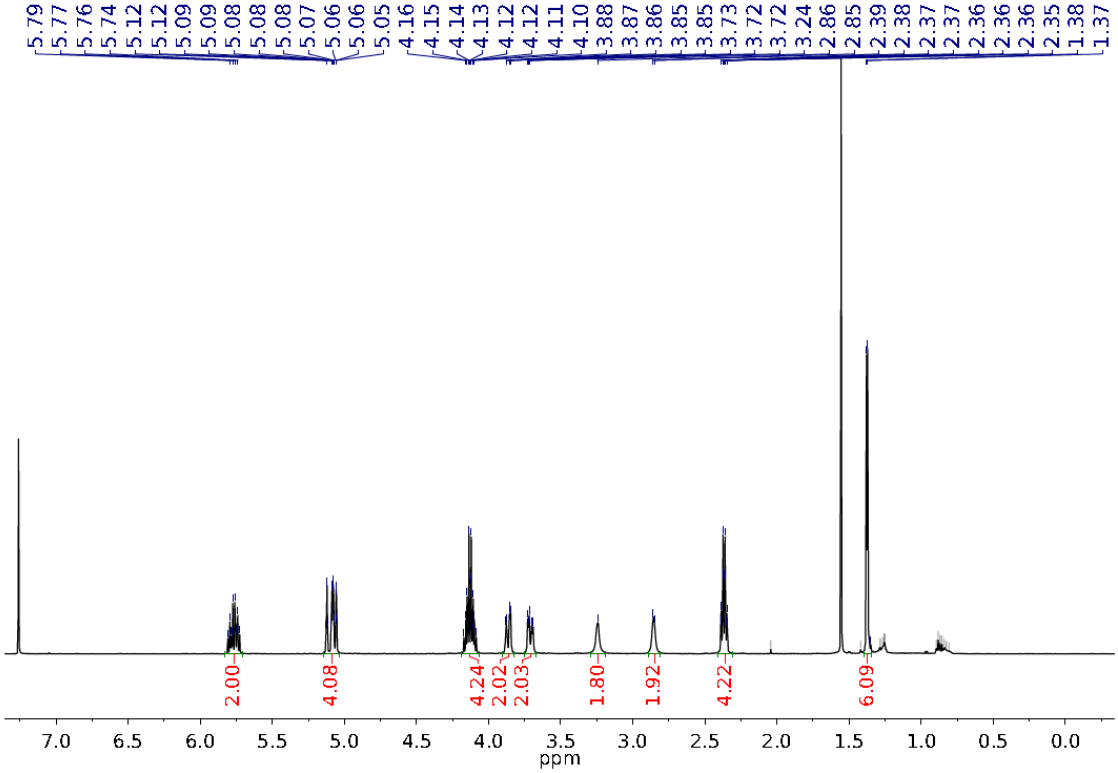
Figure S18. Monomer CB4 in its fully unravelled form (left) and the corresponding force vs. displacement curve (right) obtained by CoGEF. Fitting the curve (red line) provides l_2 as the x-intercept: l_2 = 53.24 Å.

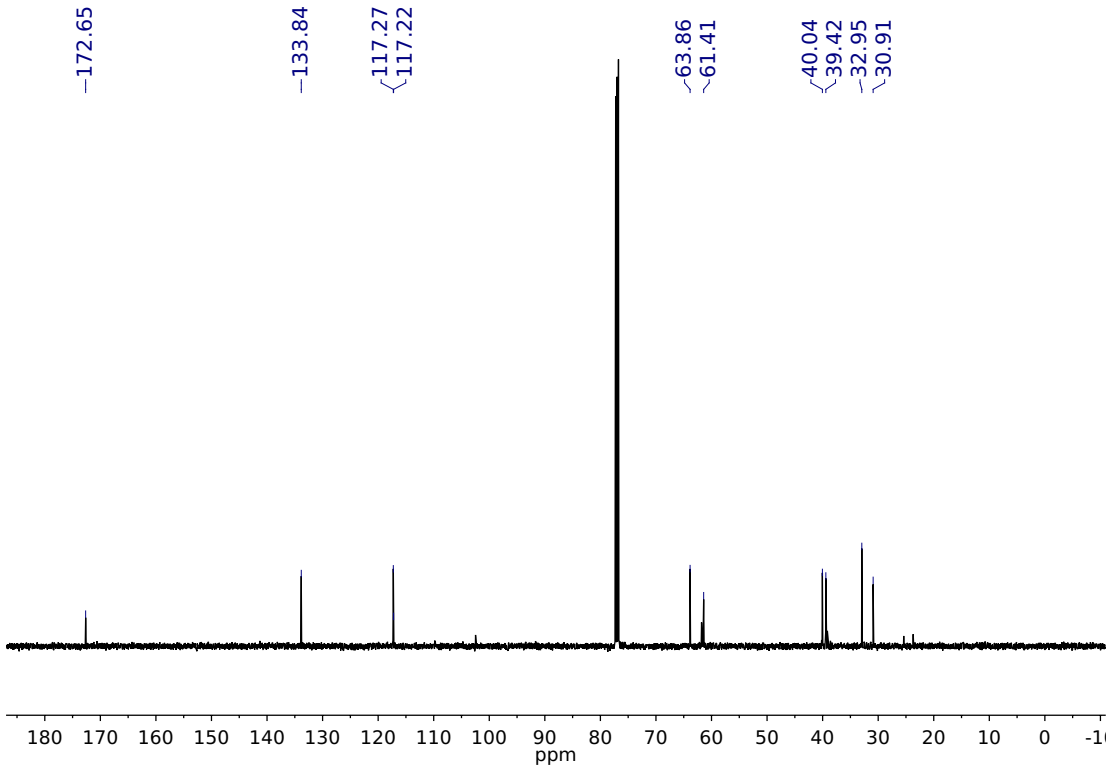
References

1. Wu, D.; Lenhardt, J. M.; Black, A. L.; Akhremitchev, B. B.; Craig, S. L., Molecular Stress Relief through a Force-Induced Irreversible Extension in Polymer Contour Length. *J. Am. Chem. Soc.* **2010,** *132* (45), 15936-15938.
2. Klukovich, H. M.; Kouznetsova, T. B.; Kean, Z. S.; Lenhardt, J. M.; Craig, S. L., A backbone lever-arm effect enhances polymer mechanochemistry. *Nat. Chem.* **2013,** *5* (2), 110-114.
3. Wang, J.; Kouznetsova, T. B.; Niu, Z.; Ong, M. T.; Klukovich, H. M.; Rheingold, A. L.; Martinez, T. J.; Craig, S. L., Inducing and quantifying forbidden reactivity with single-molecule polymer mechanochemistry. *Nature Chem.* **2015,** *7* (4), 323-327.
4. Wang, J.; Kouznetsova, T. B.; Niu, Z.; Rheingold, A. L.; Craig, S. L., Accelerating a Mechanically Driven anti-Woodward–Hoffmann Ring Opening with a Polymer Lever Arm Effect. *J. Org. Chem.* **2015,** *80* (23), 11895-11898.
5. Kouznetsova, T. B.; Wang, J.; Craig, S. L., Combined Constant-Force and Constant-Velocity Single-Molecule Force Spectroscopy of the Conrotatory Ring Opening Reaction of Benzocyclobutene. *ChemPhysChem* **2016**, n/a-n/a.
6. Oberhauser, A. F.; Marszalek, P. E.; Erickson, H. P.; Fernandez, J. M., The molecular elasticity of the extracellular matrix protein tenascin. *Nature* **1998,** *393* (6681), 181-185.
7. Florin, E. L.; Rief, M.; Lehmann, H.; Ludwig, M.; Dornmair, C.; Moy, V. T.; Gaub, H. E., Sensing specific molecular interactions with the atomic force microscope. *Biosensors and Bioelectronics* **1995,** *10* (9), 895-901.
8. Lin, Y.; Kouznetsova, T. B.; Craig, S. L., Mechanically Gated Degradable Polymers. *J. Am. Chem. Soc.* **2020,** *142* (5), 2105-2109.
9. Szpera, R. K., N.; Natarajan, K.; Paillard, N.; Linclau, B., The synthesis of the 2,3-difluorobutan-1,4-diol diastereomers. *Beilstein J. Org. Chem.* **2017,** *13*, 2883–2887.
10. Chung, J.; Kushner, A. M.; Weisman, A. C.; Guan, Z., Direct correlation of single-molecule properties with bulk mechanical performance for the biomimetic design of polymers. *Nature Materials* **2014,** *13* (11), 1055-1062.
11. Beyer, M. K., The mechanical strength of a covalent bond calculated by density functional theory. *J. Chem. Phys.* **2000,** *112* (17), 7307-7312.

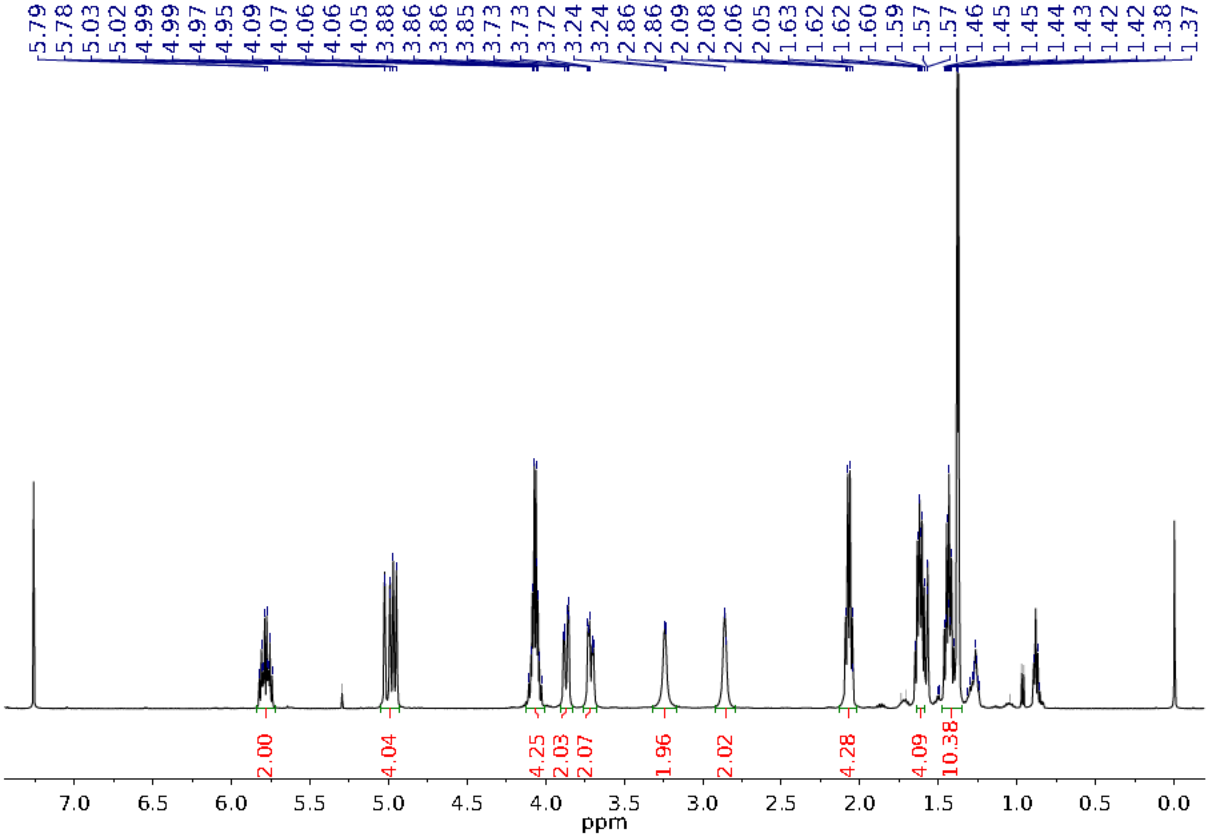
NMR Spectra

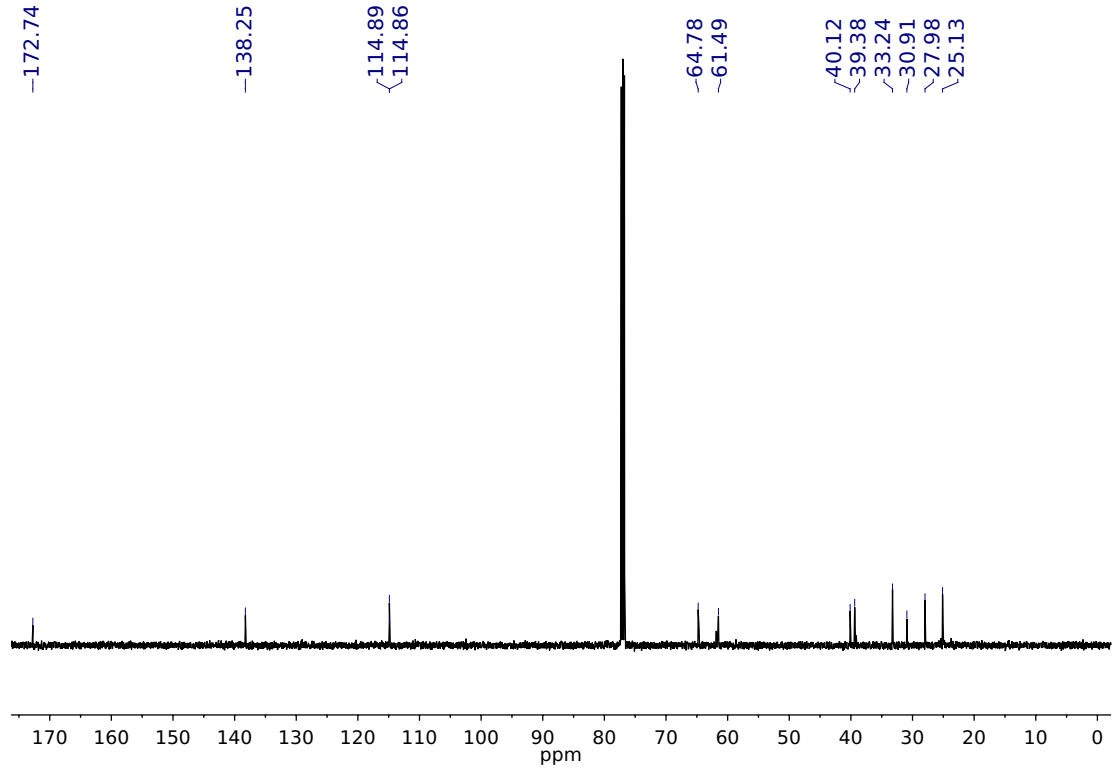
Synthesis of 2a



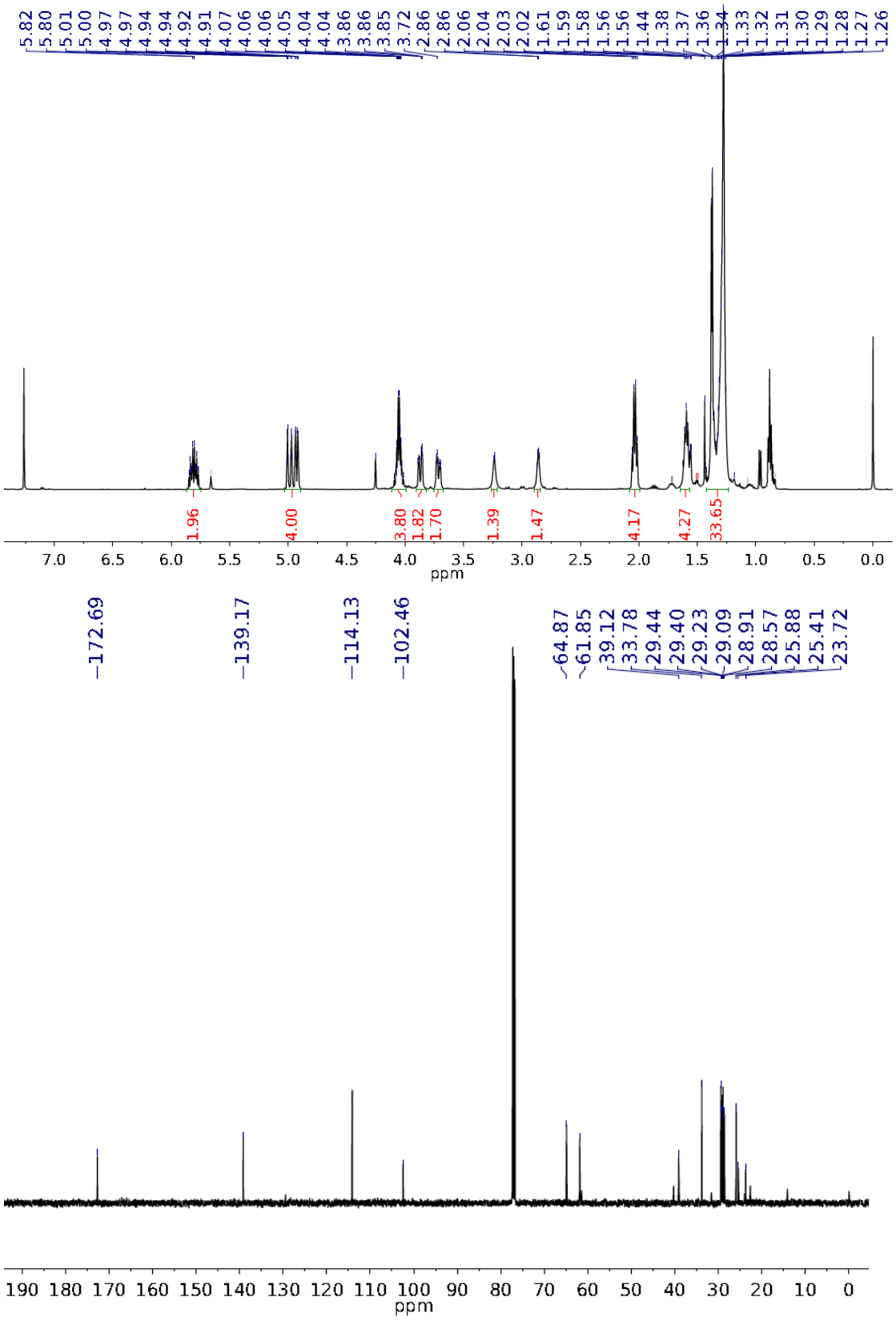


Synthesis of 2b

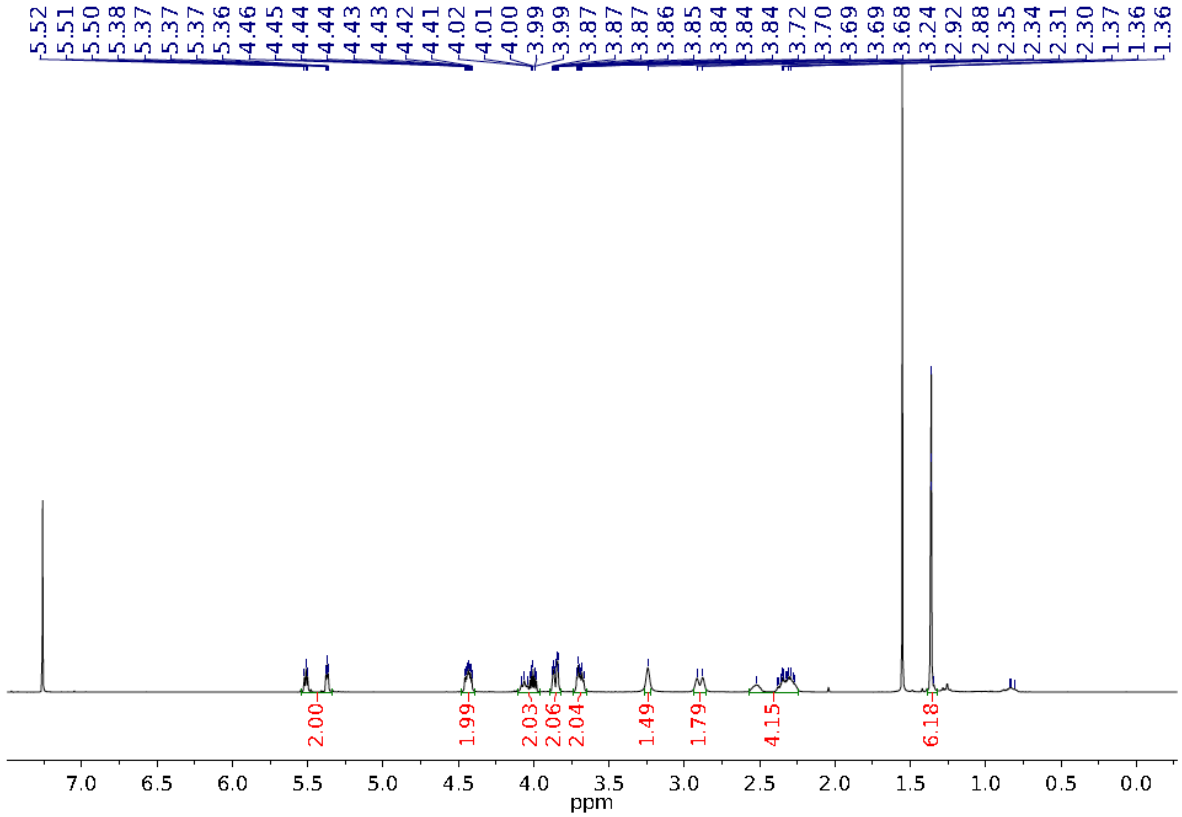


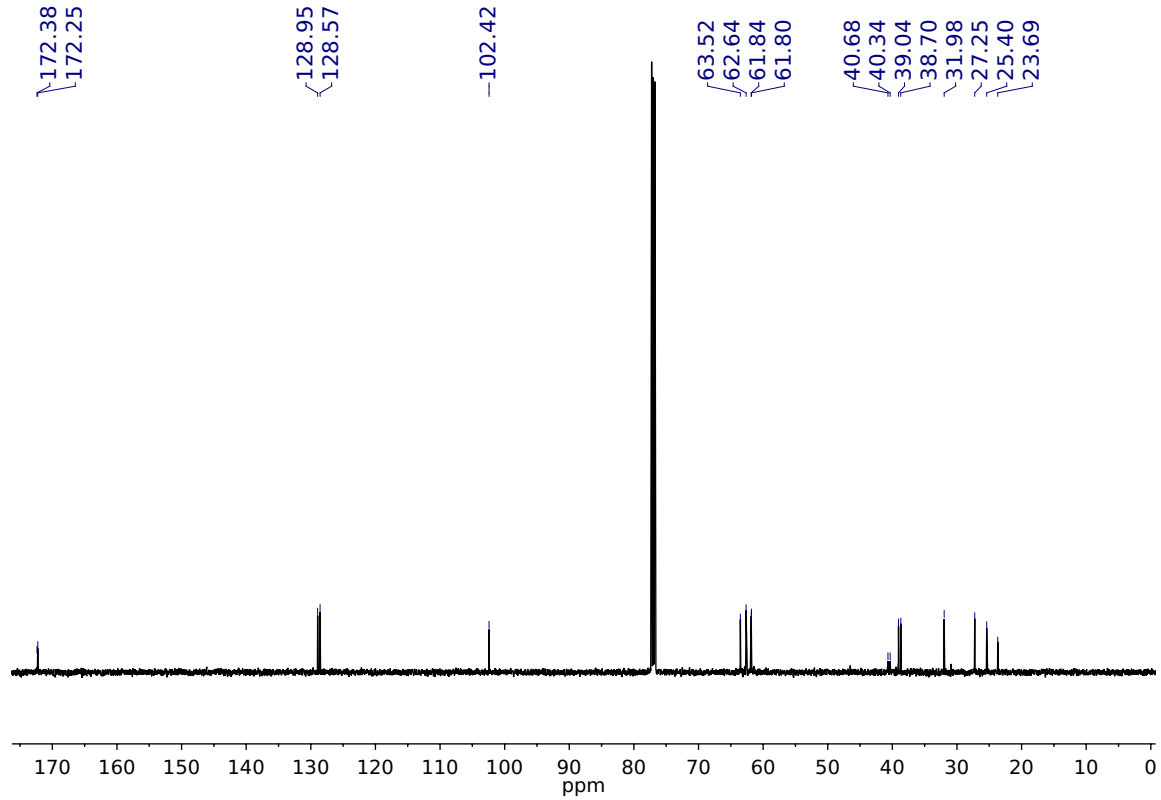


Synthesis of 2c

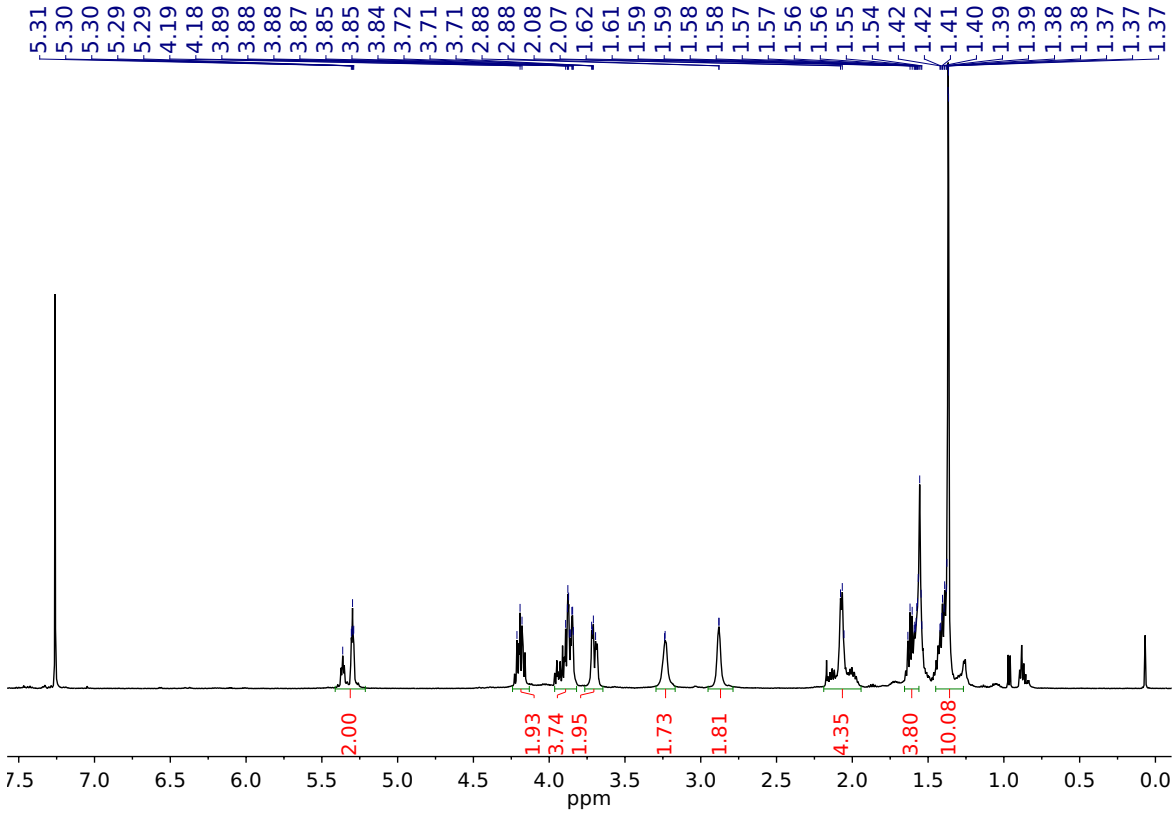


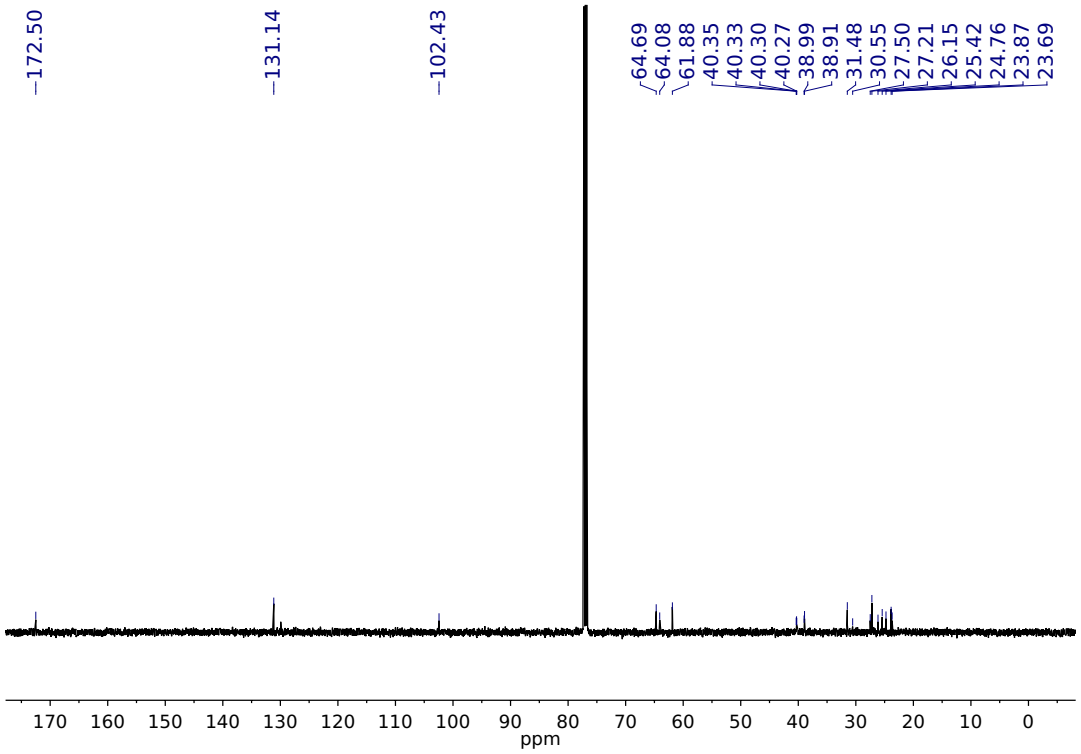
Synthesis of 3a



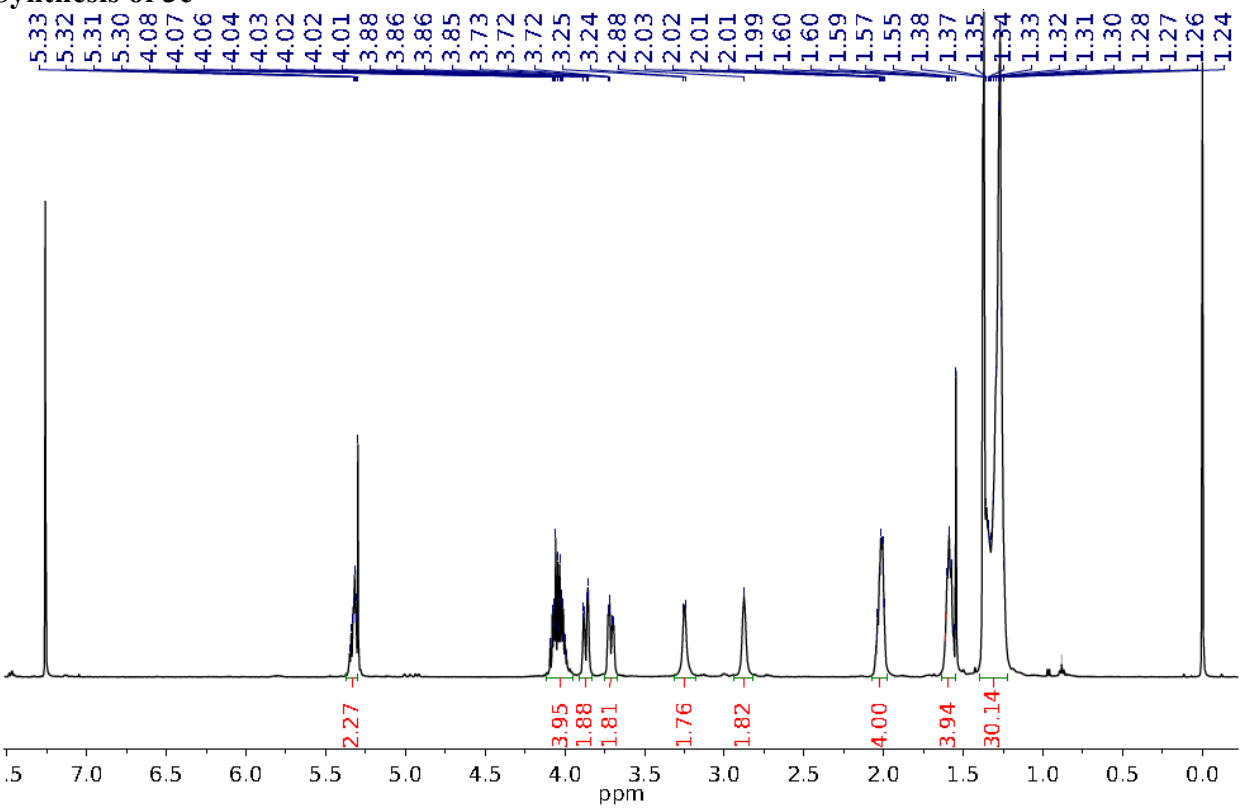


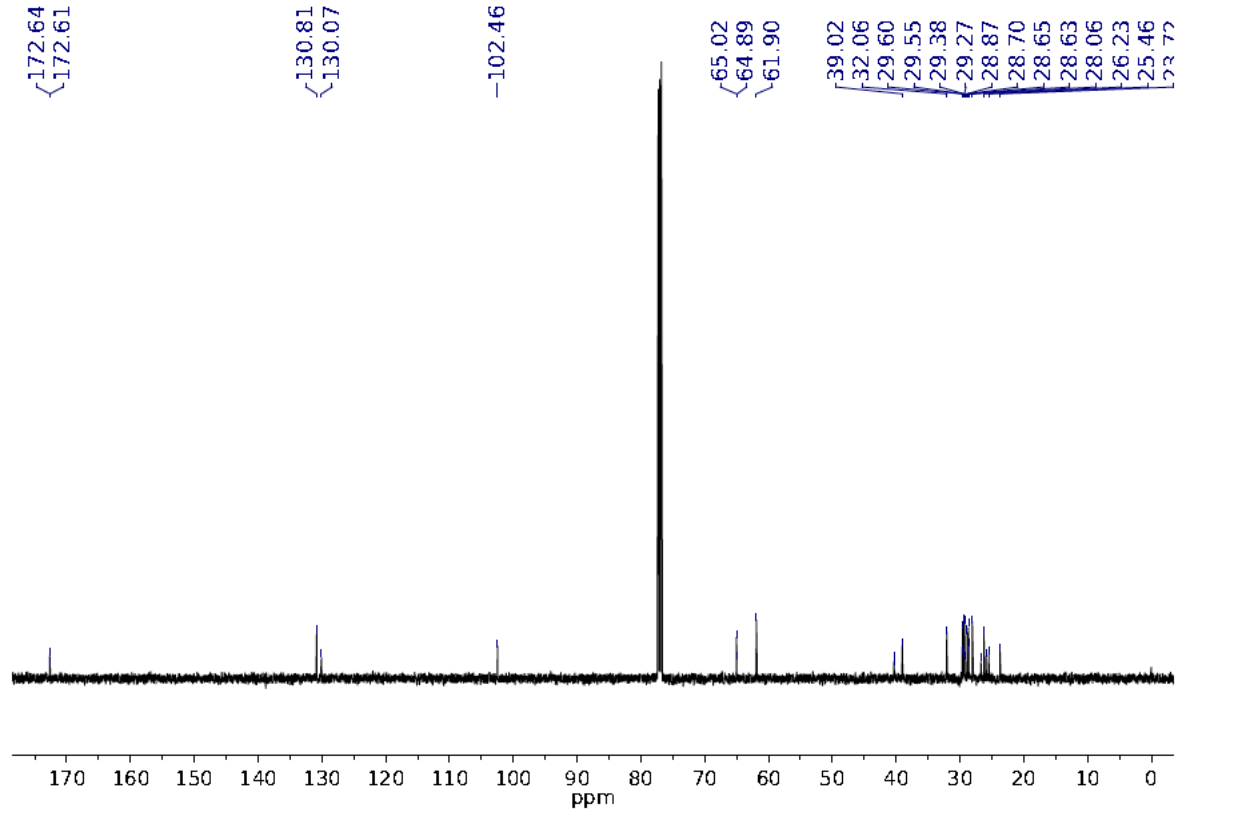
Synthesis of 3b



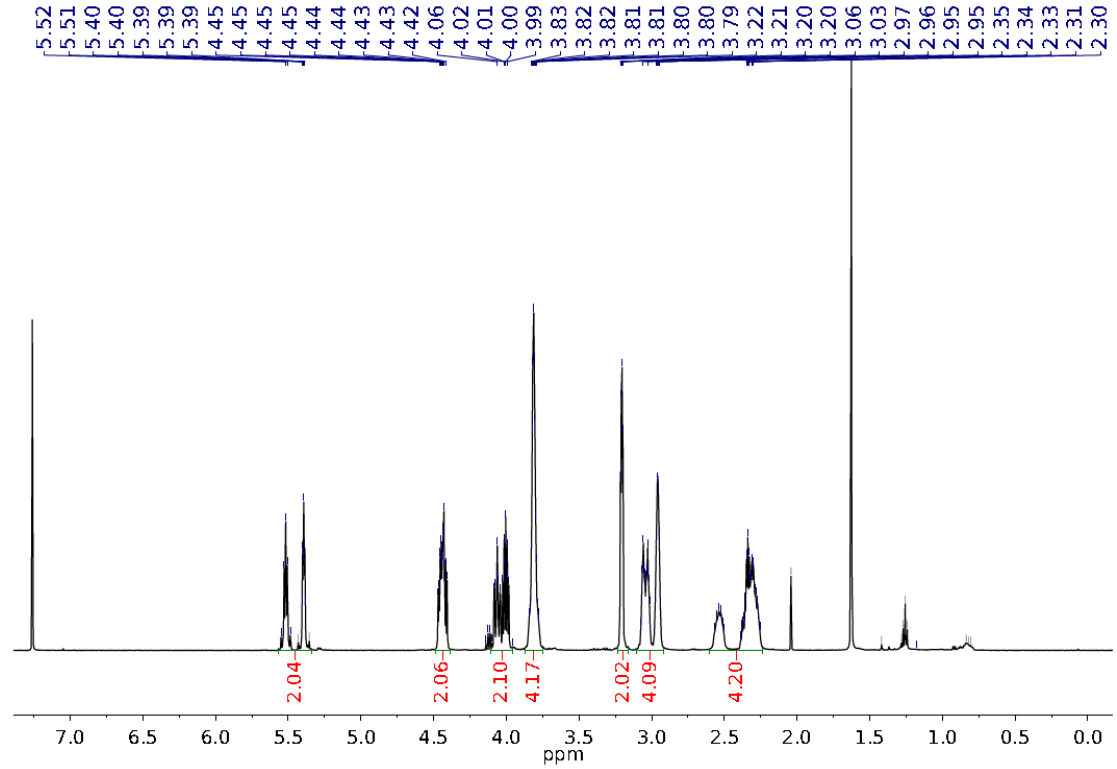


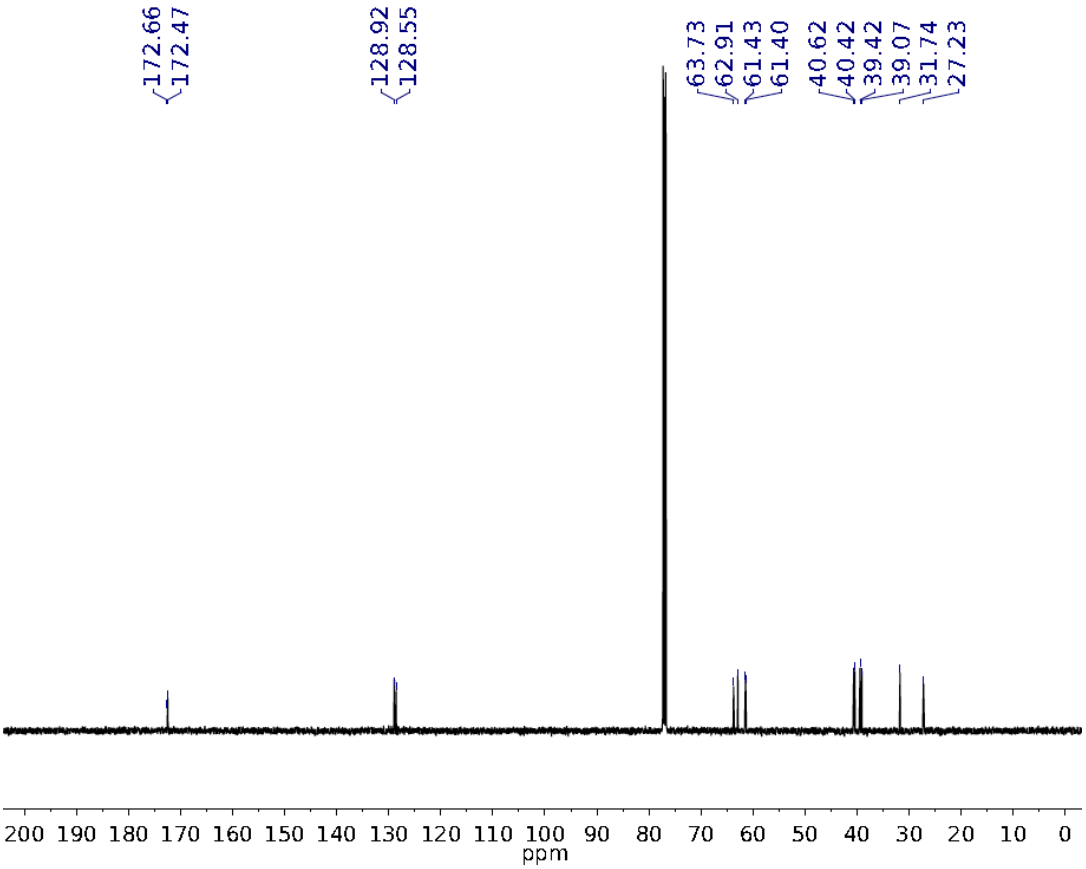
Synthesis of 3c



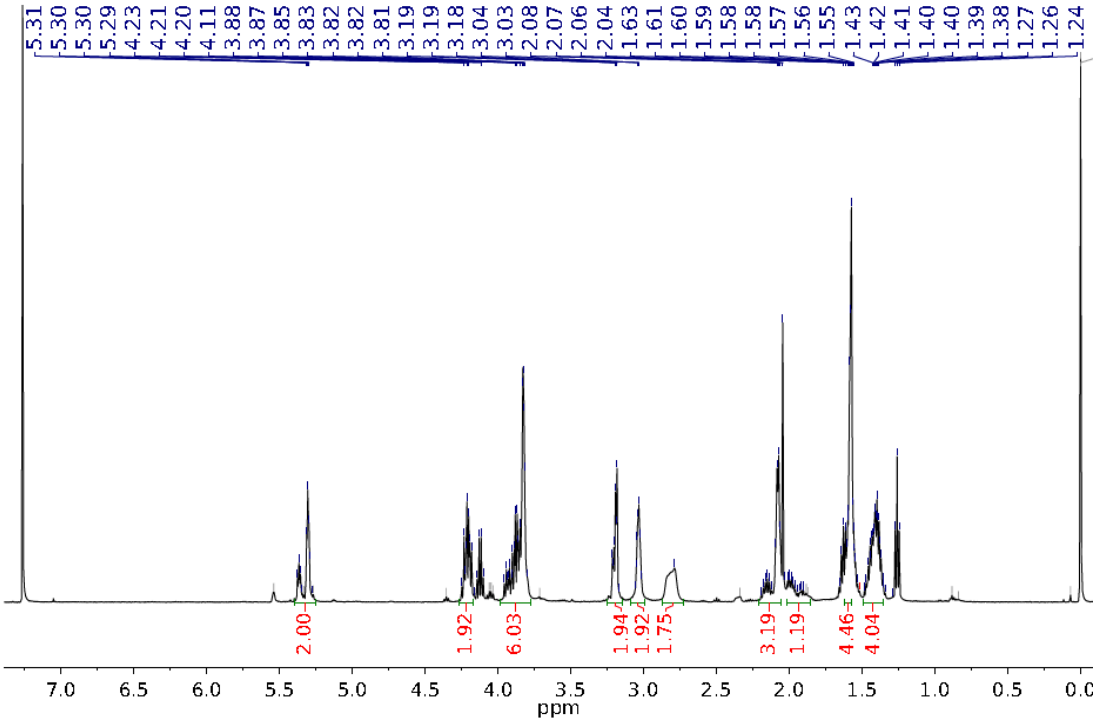


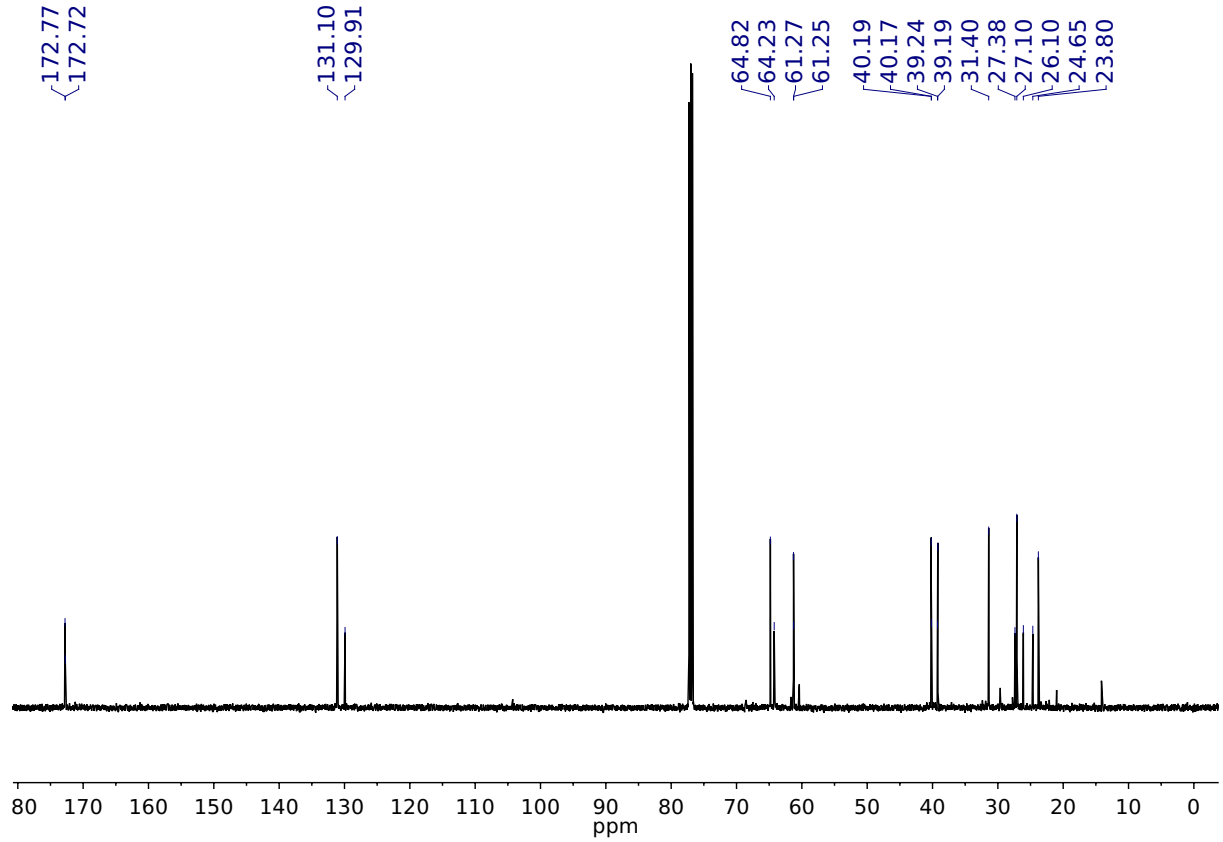
Synthesis of 4a



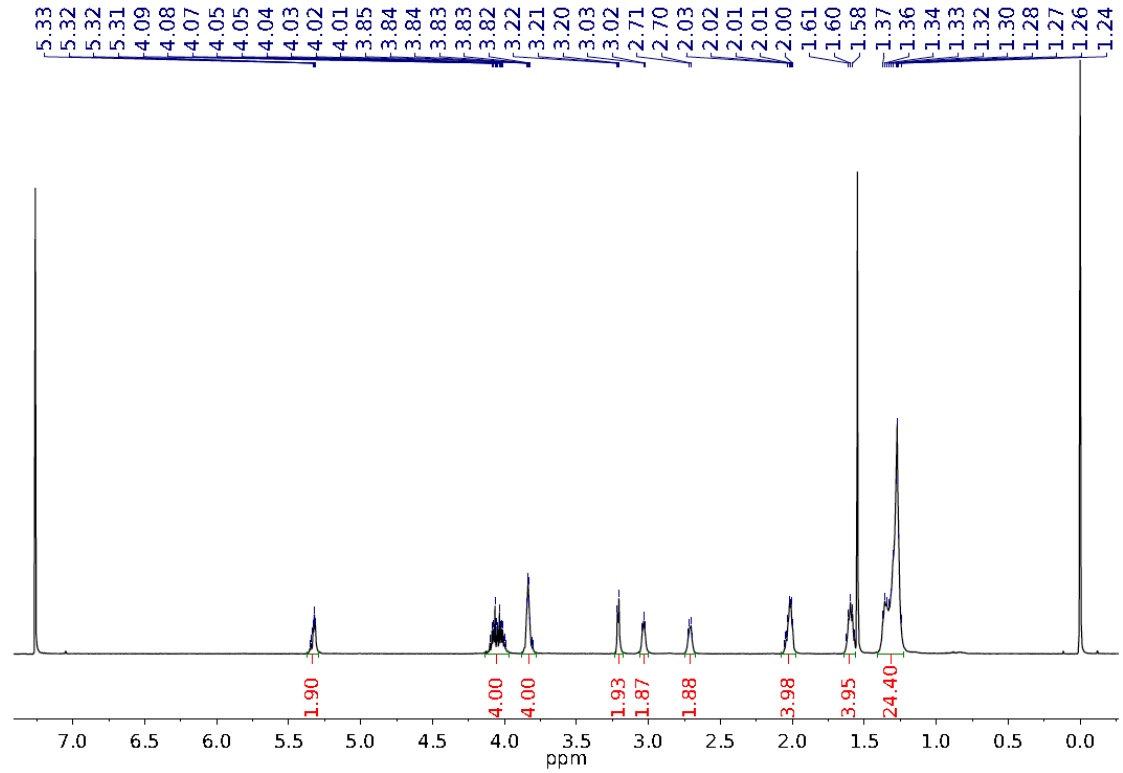


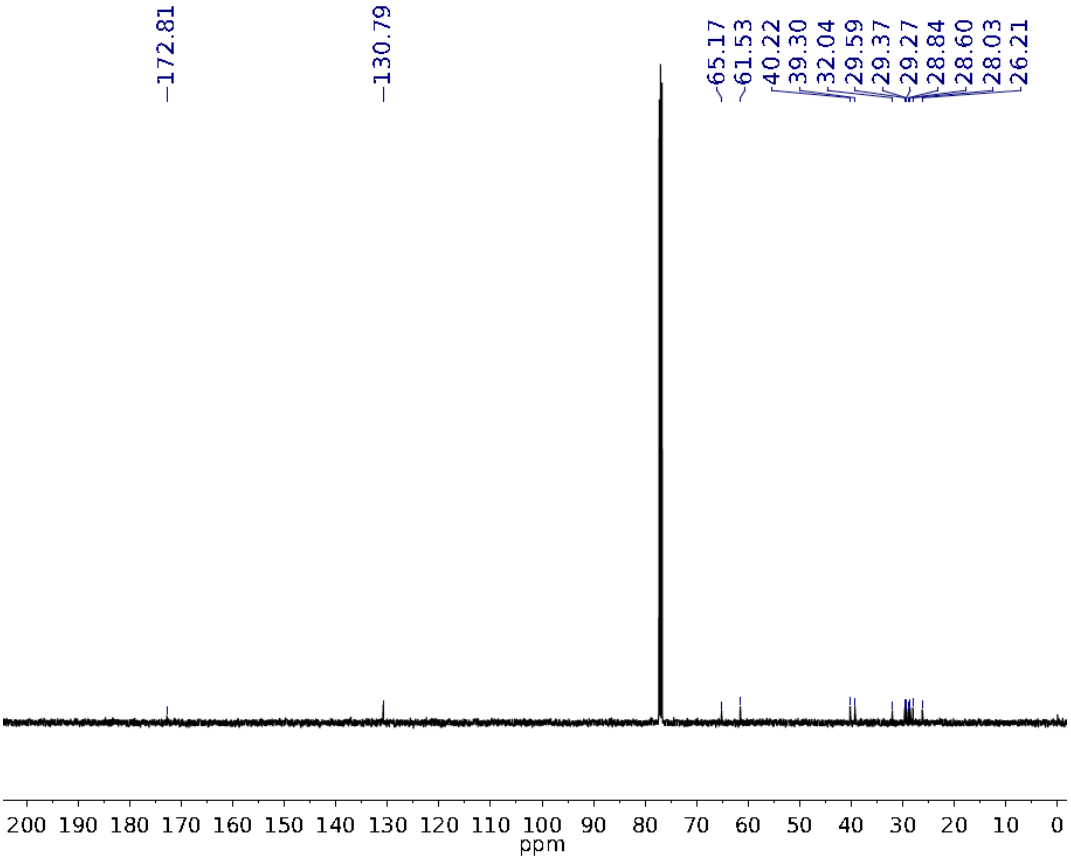
Synthesis of 4b



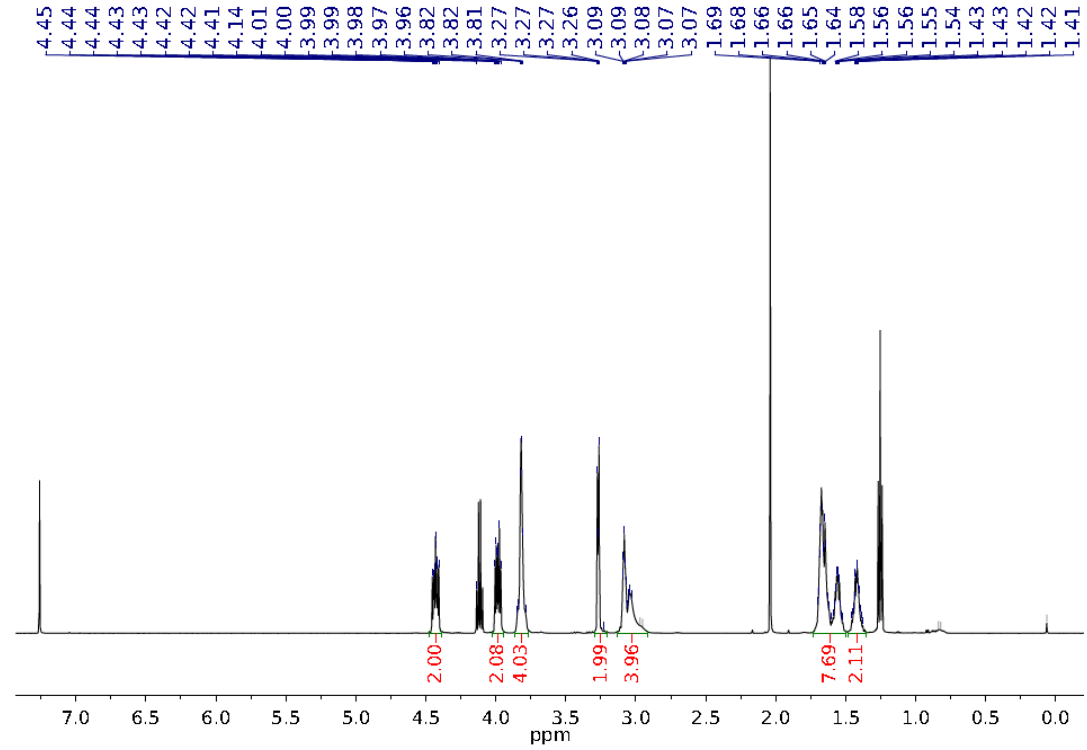


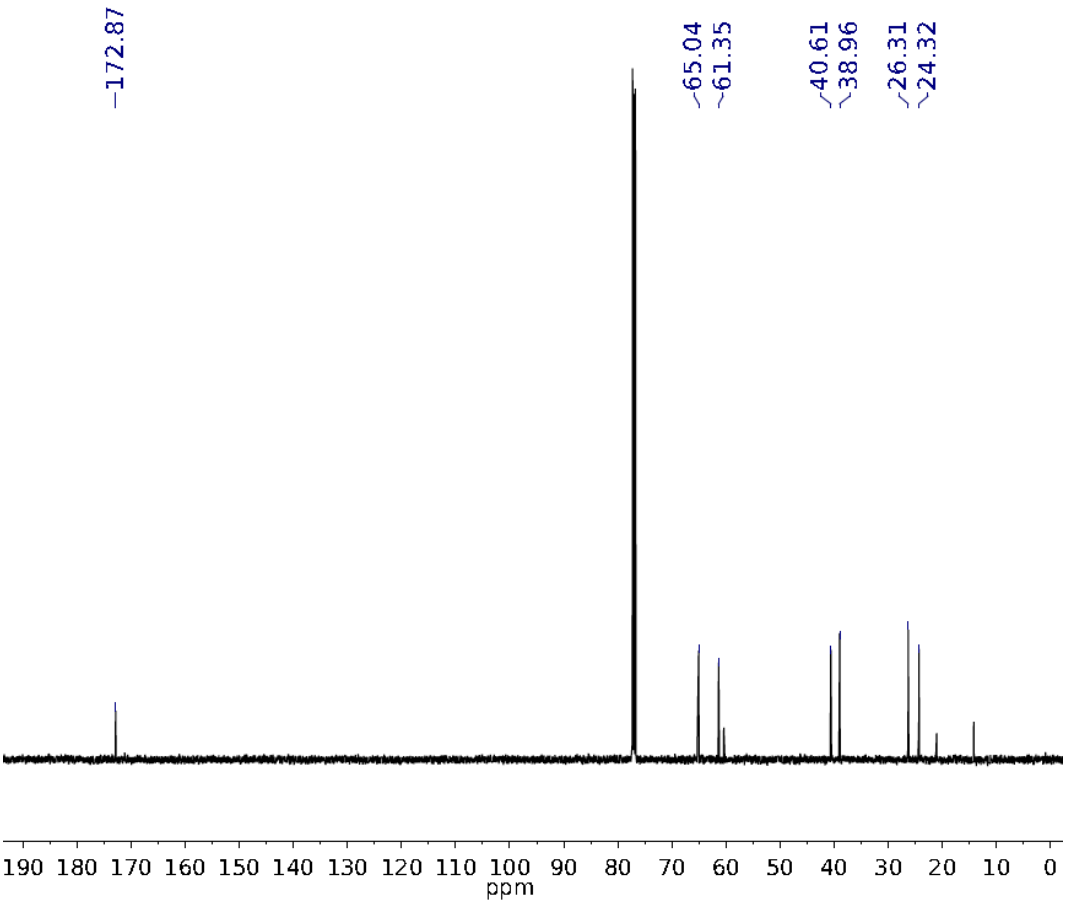
Synthesis of 4c



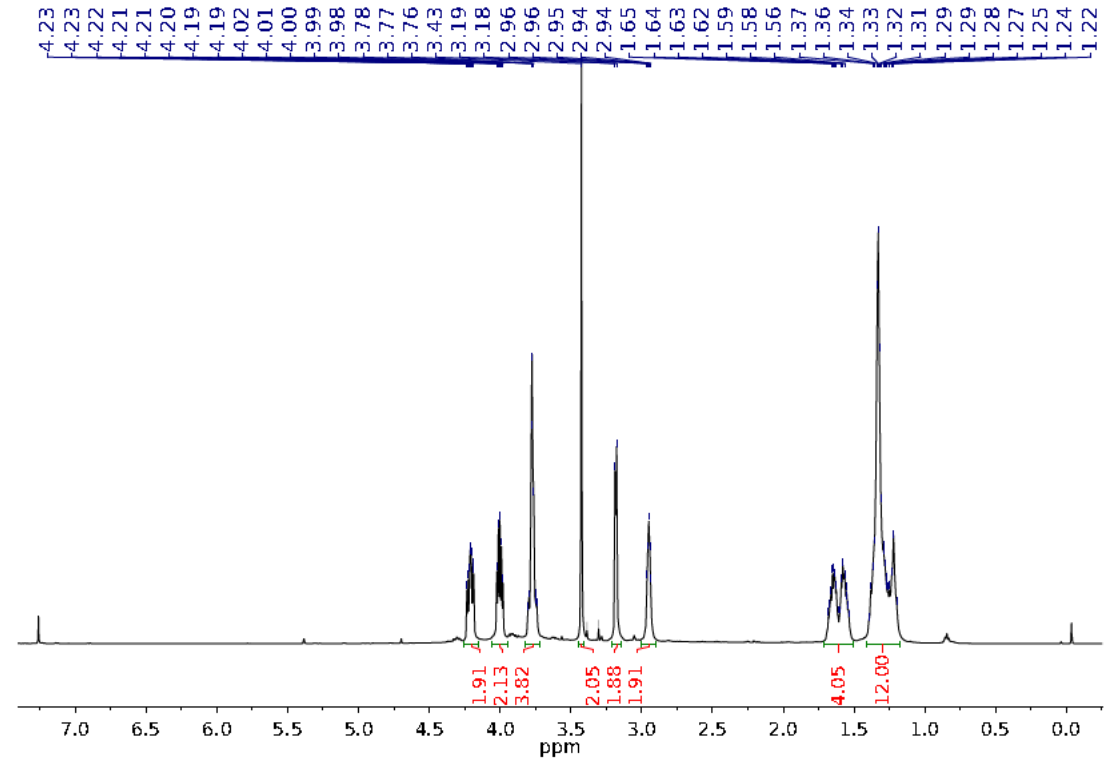


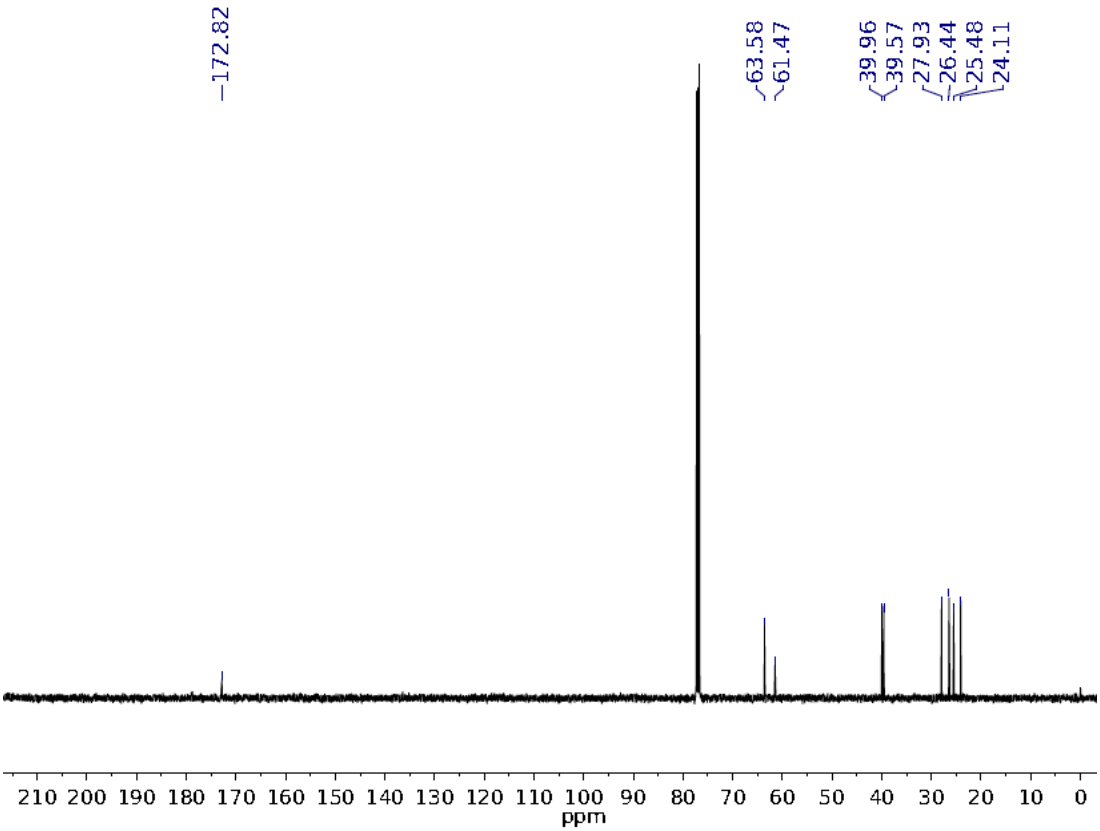
Synthesis of 5a



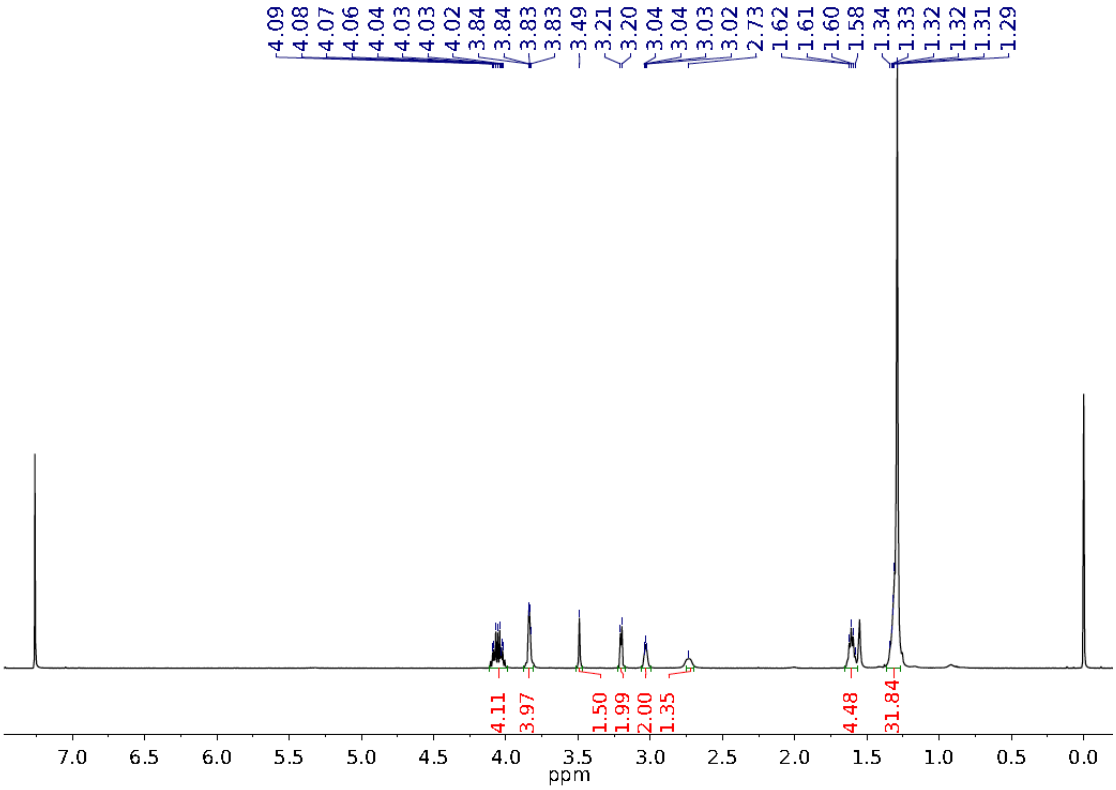


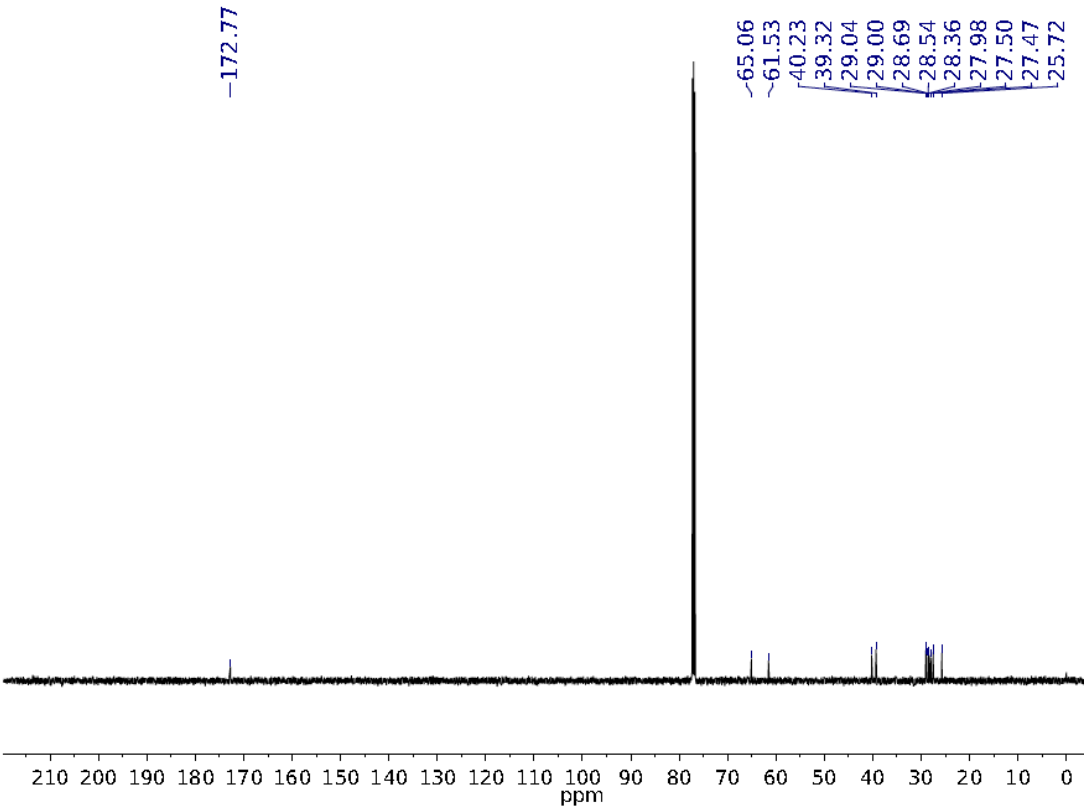
Synthesis of 5b



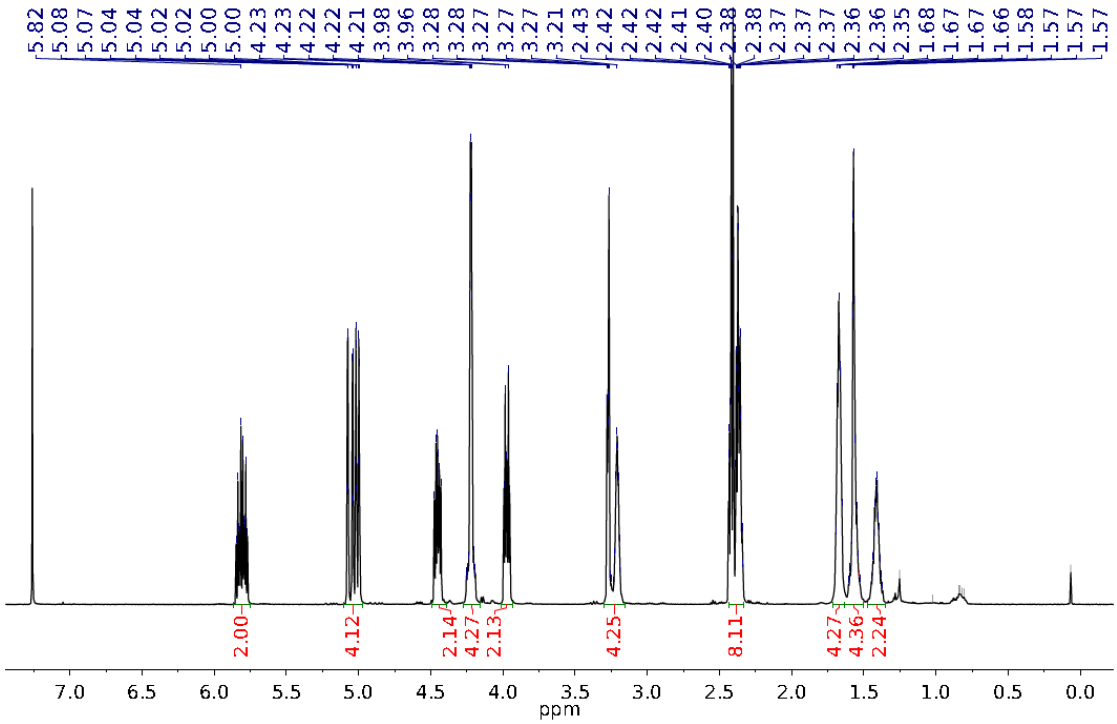


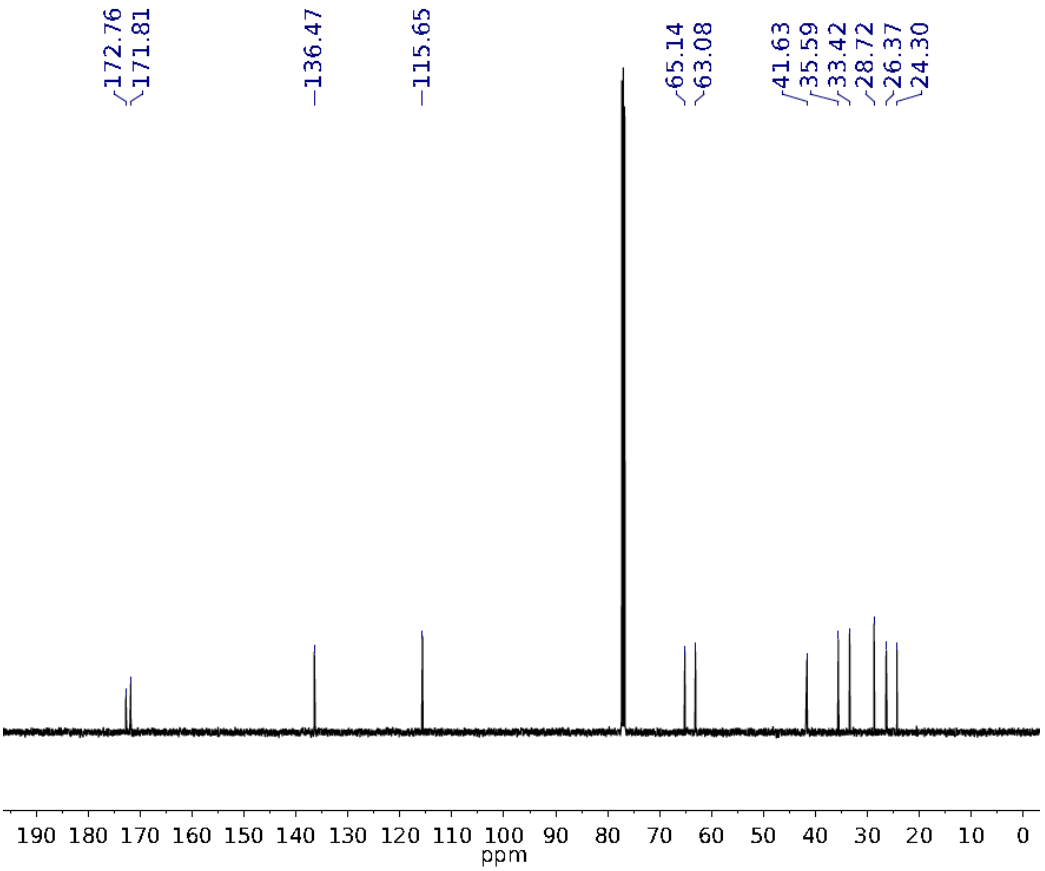
Synthesis of 5c



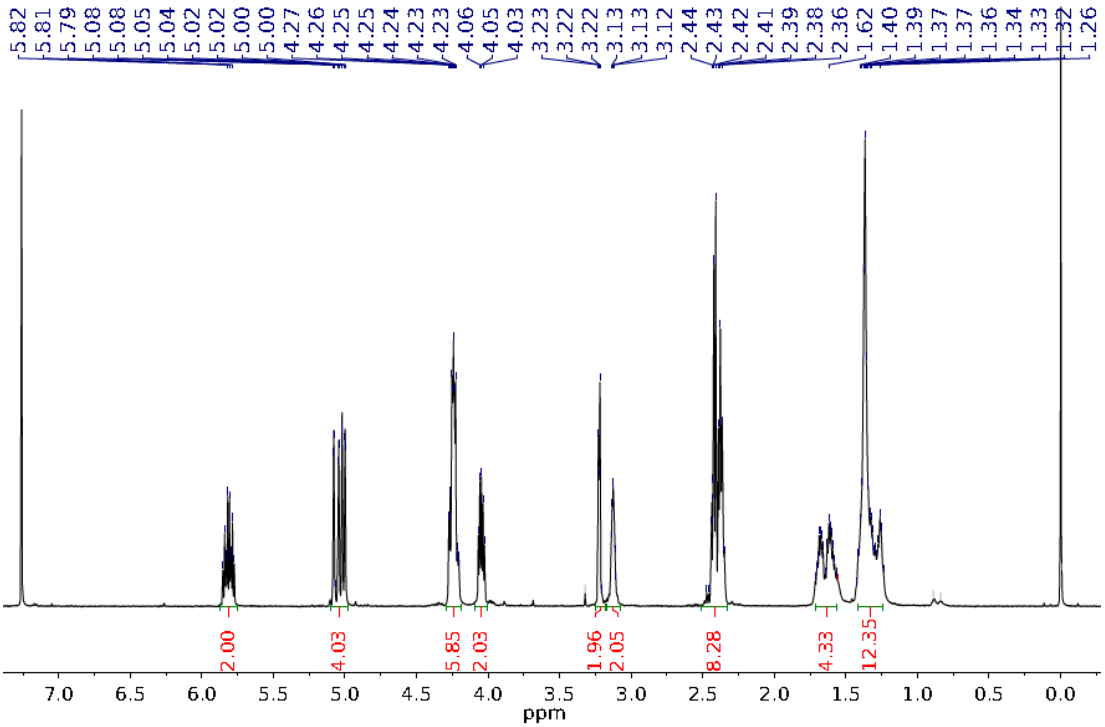


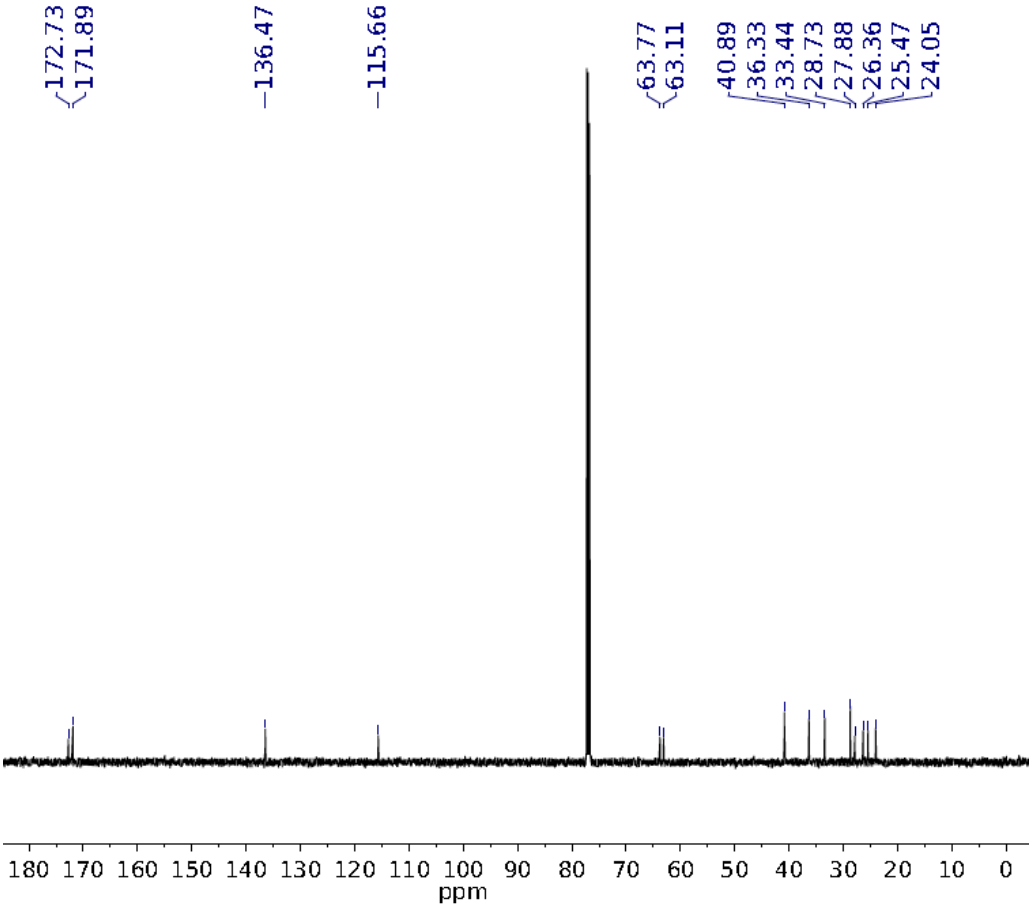
Synthesis of 6a



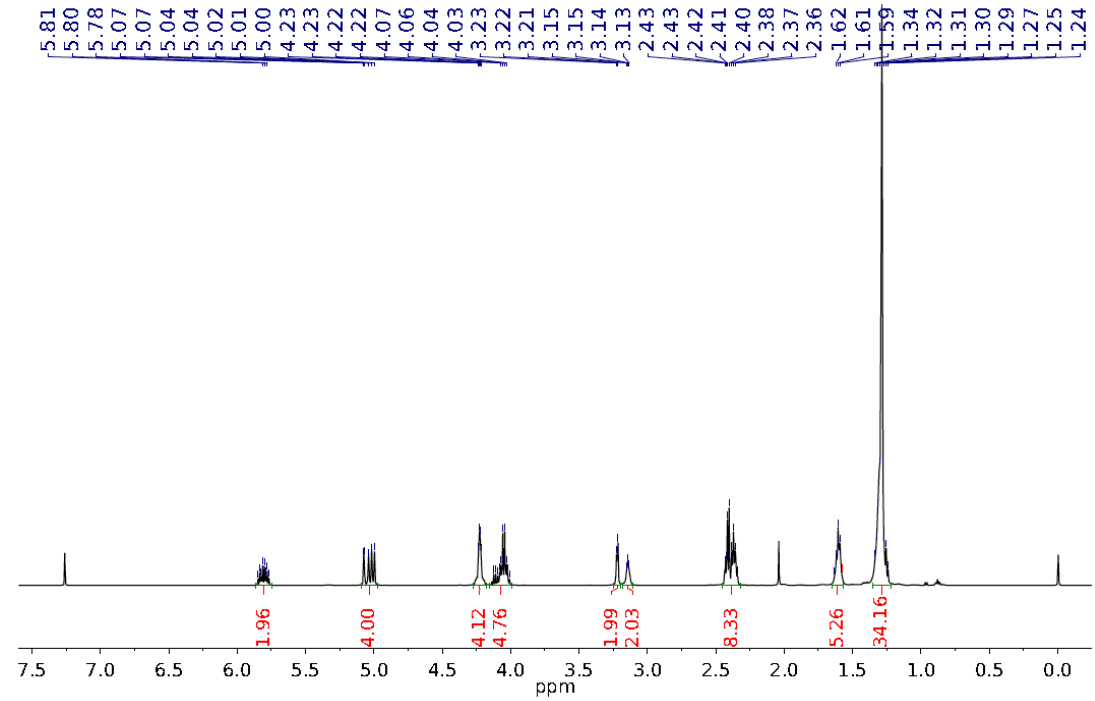


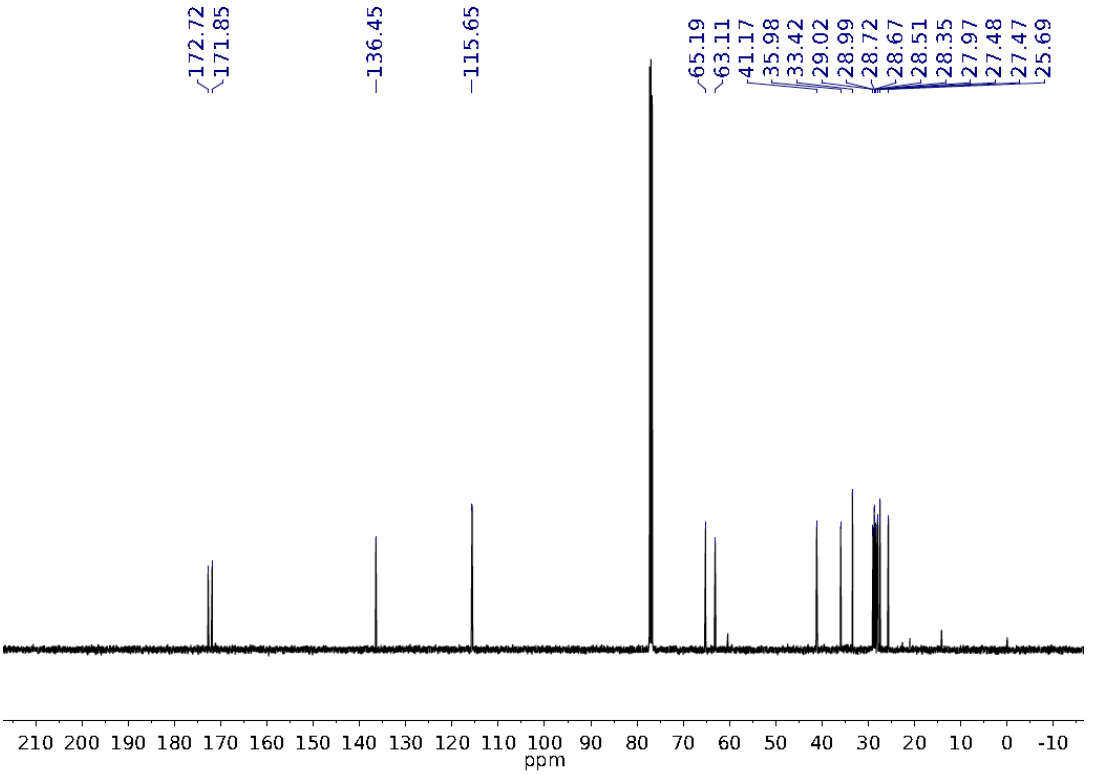
Synthesis of 6b



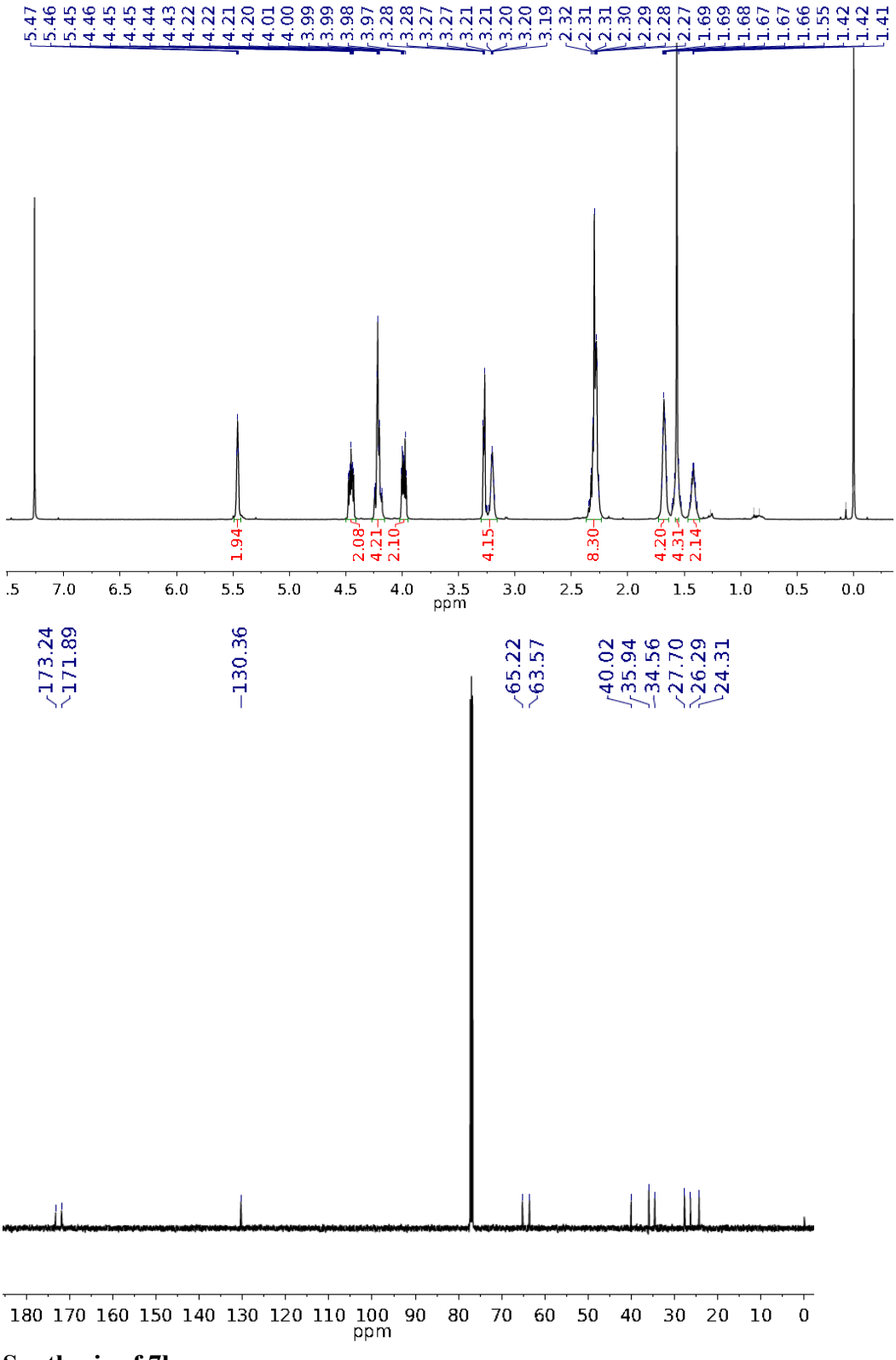


Synthesis of 6c

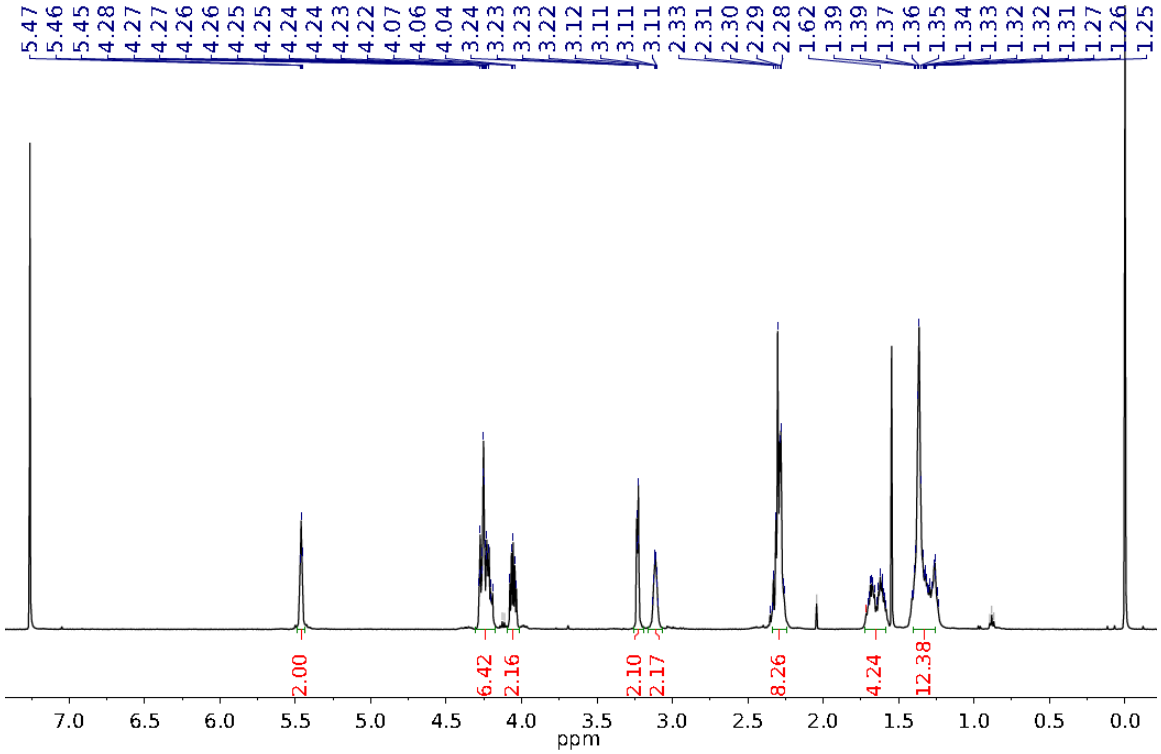


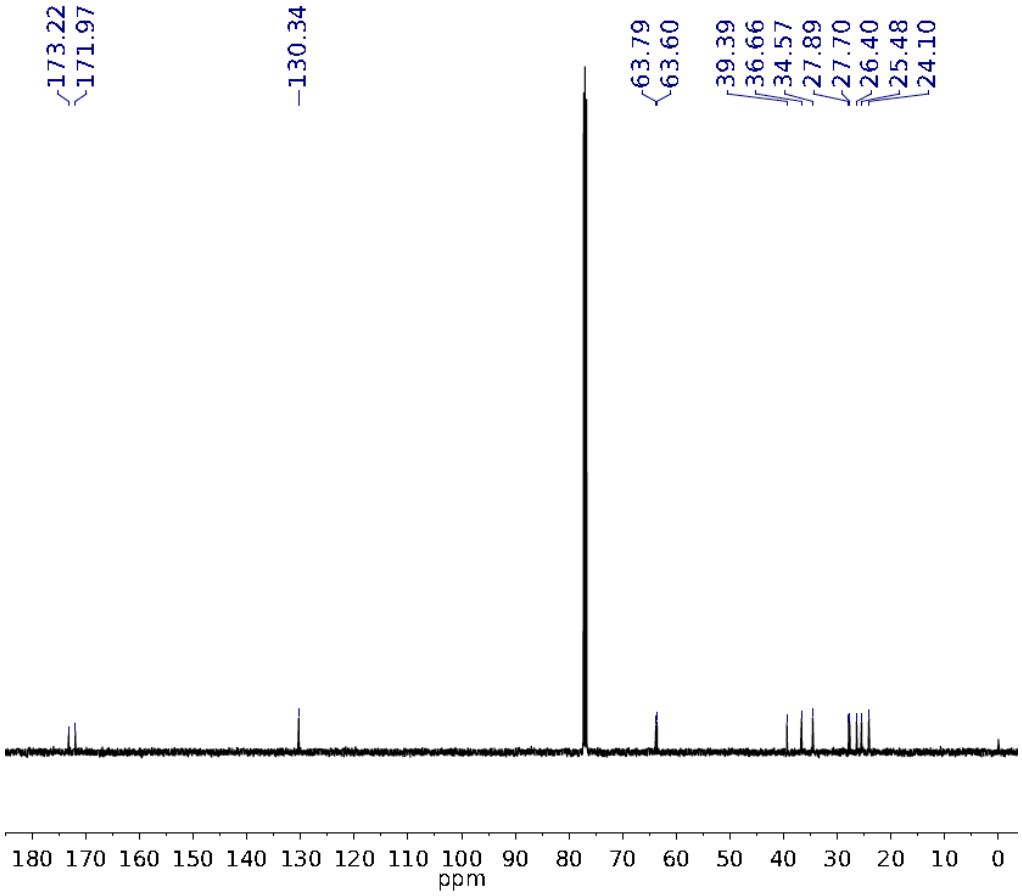


Synthesis of 7a

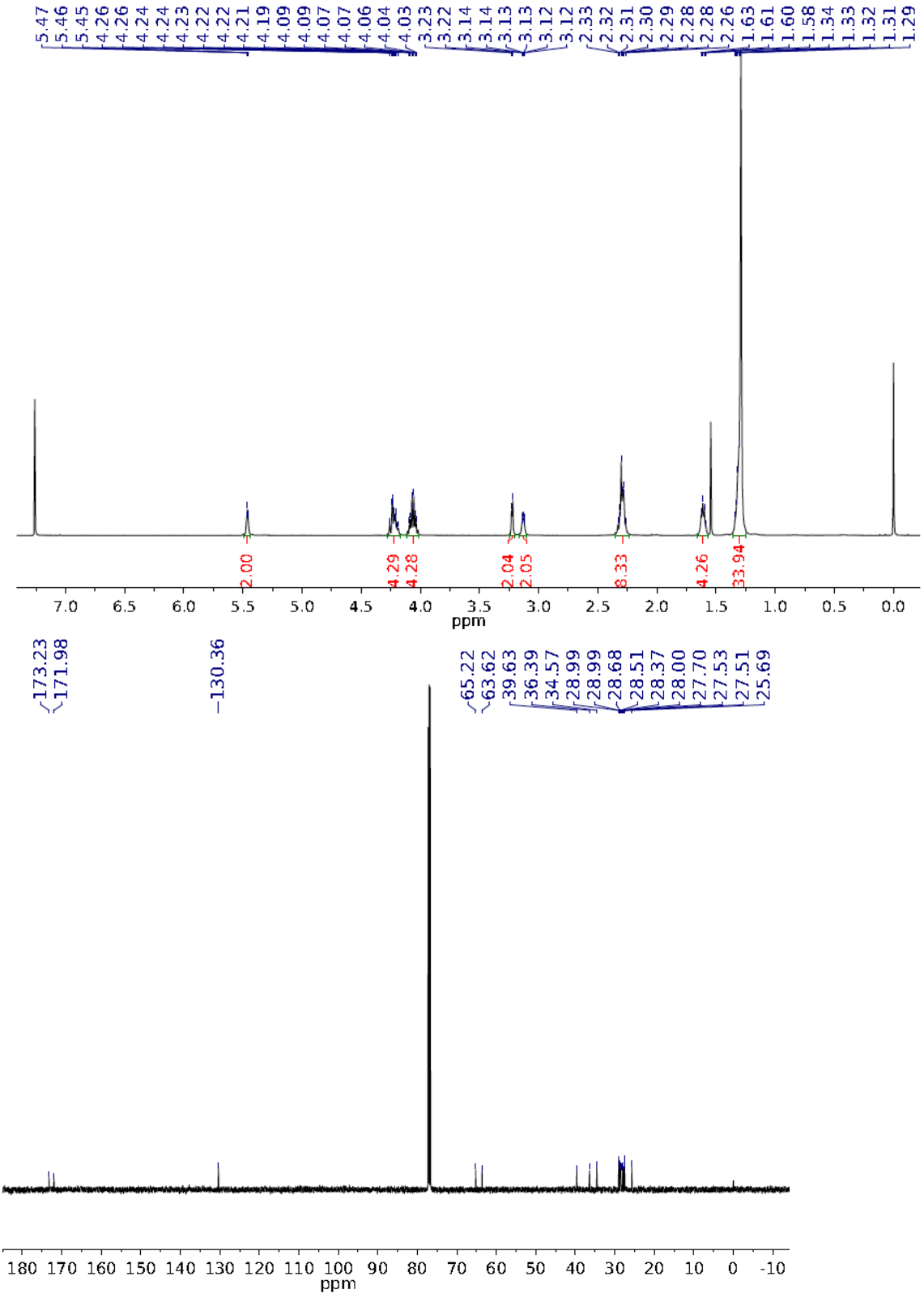


Synthesis of 7b

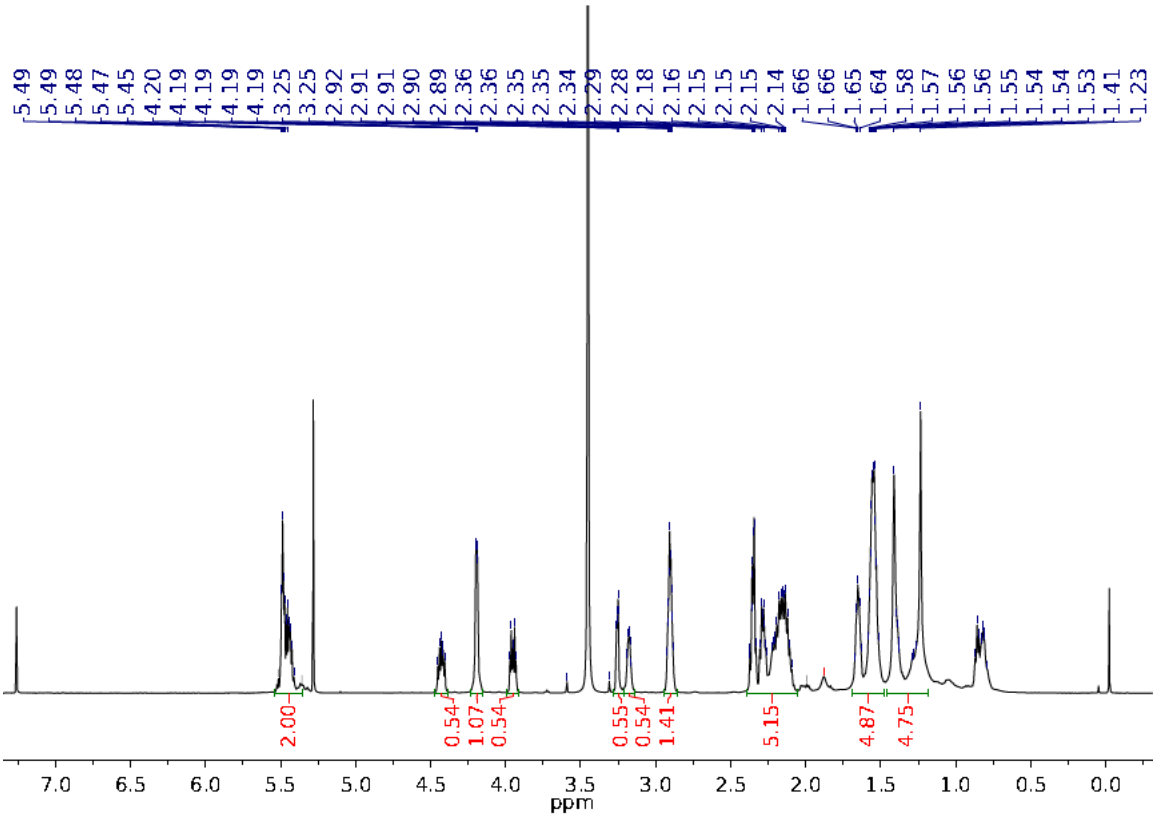

5.47
5.46
5.45
4.28
4.27
4.27
4.26
4.26
4.25
4.25
4.24
4.24
4.23
4.22
4.07
4.06
4.04
3.24
3.23
3.23
3.22
3.12
3.11
3.11
3.11
2.33
2.31
2.30
2.29
2.28
1.62
1.39
1.39
1.37
1.36
1.35
1.34
1.33
1.32
1.32
1.31
1.27
1.26
1.25
2.00
6.42
2.16
2.10
2.17
8.26
4.24
12.38
7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
ppm


173.22
171.97
130.34
63.79
63.60
39.39
36.66
34.57
27.89
27.70
26.40
25.48
24.10
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

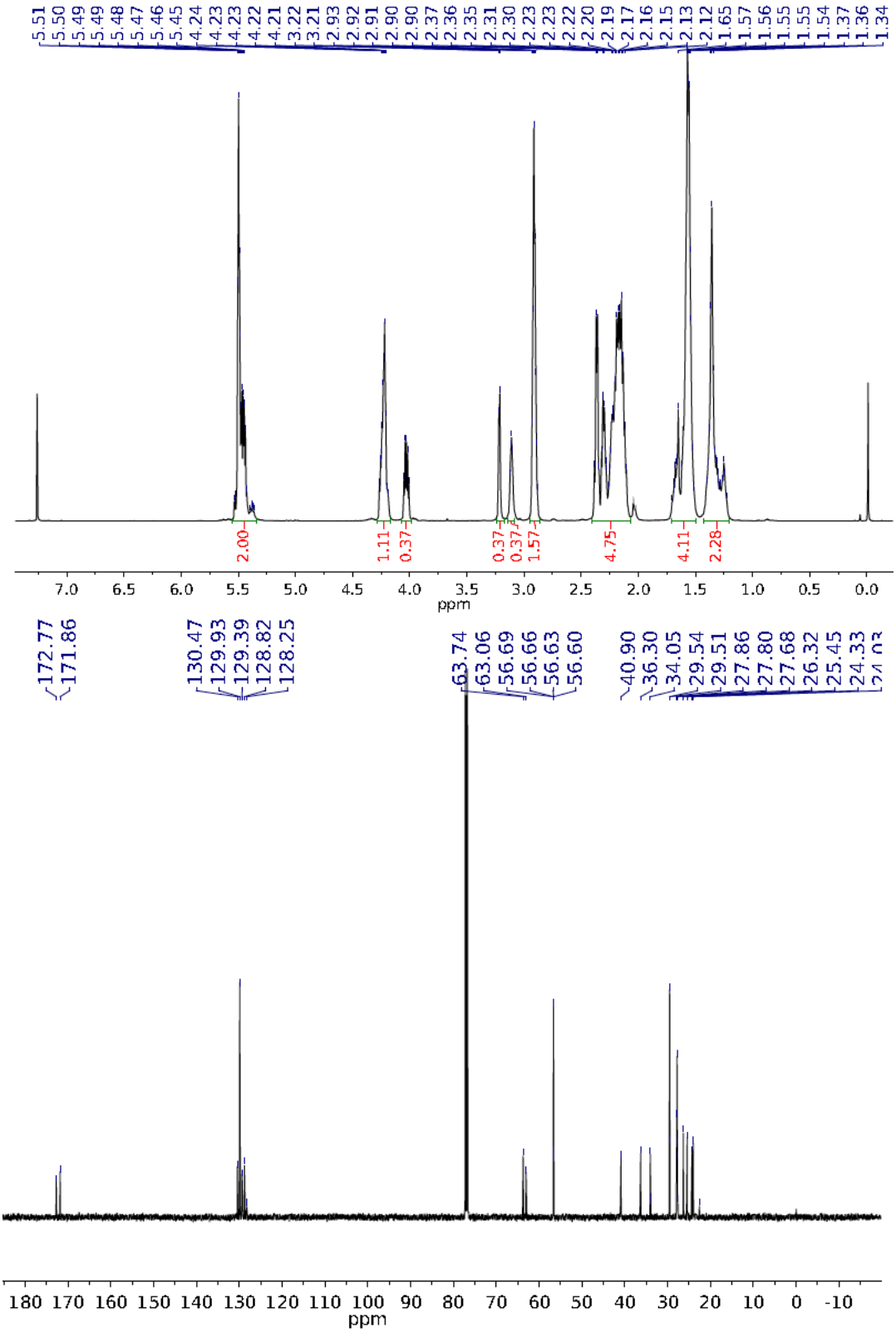
Synthesis of 7c



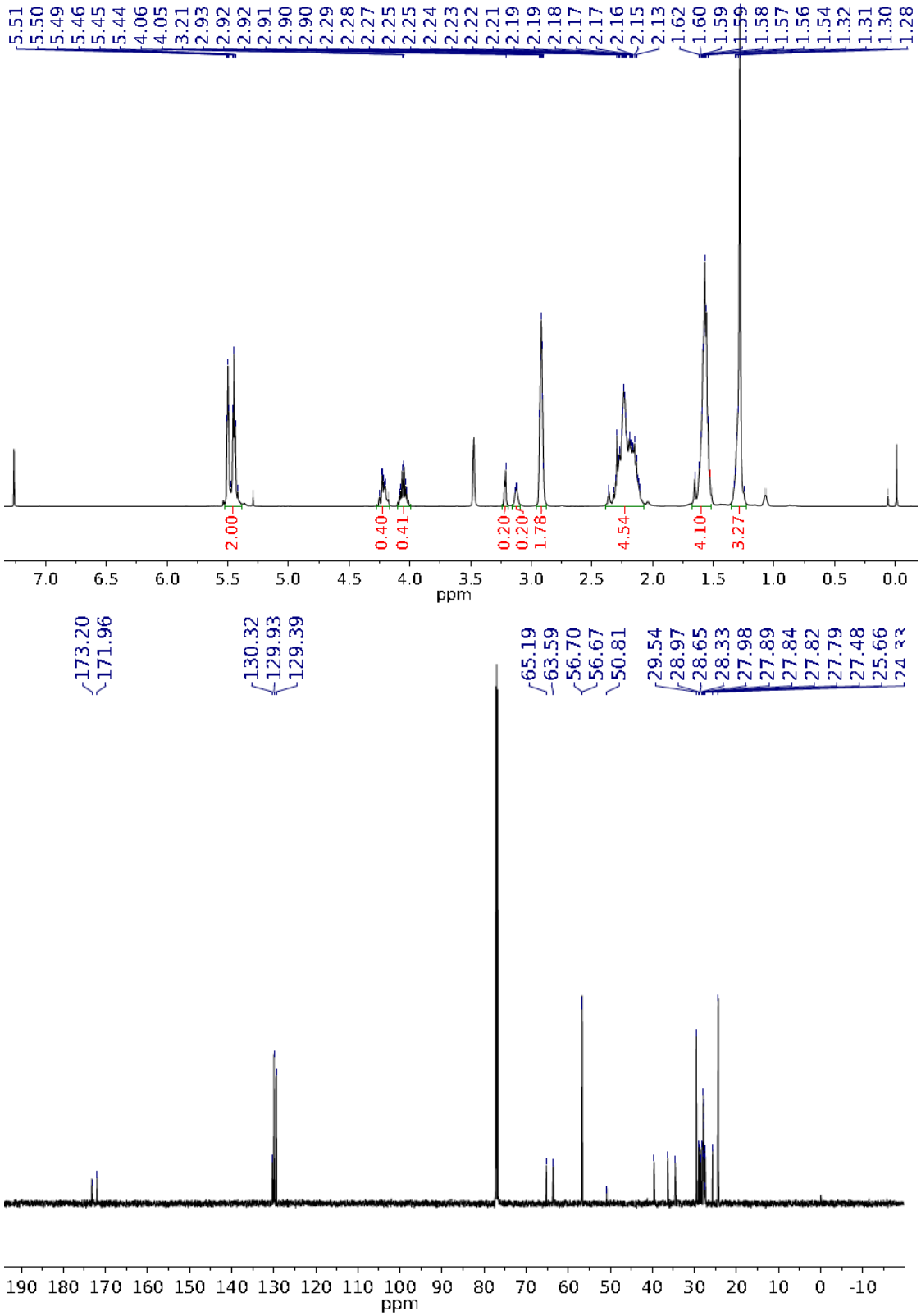
Synthesis of P1a



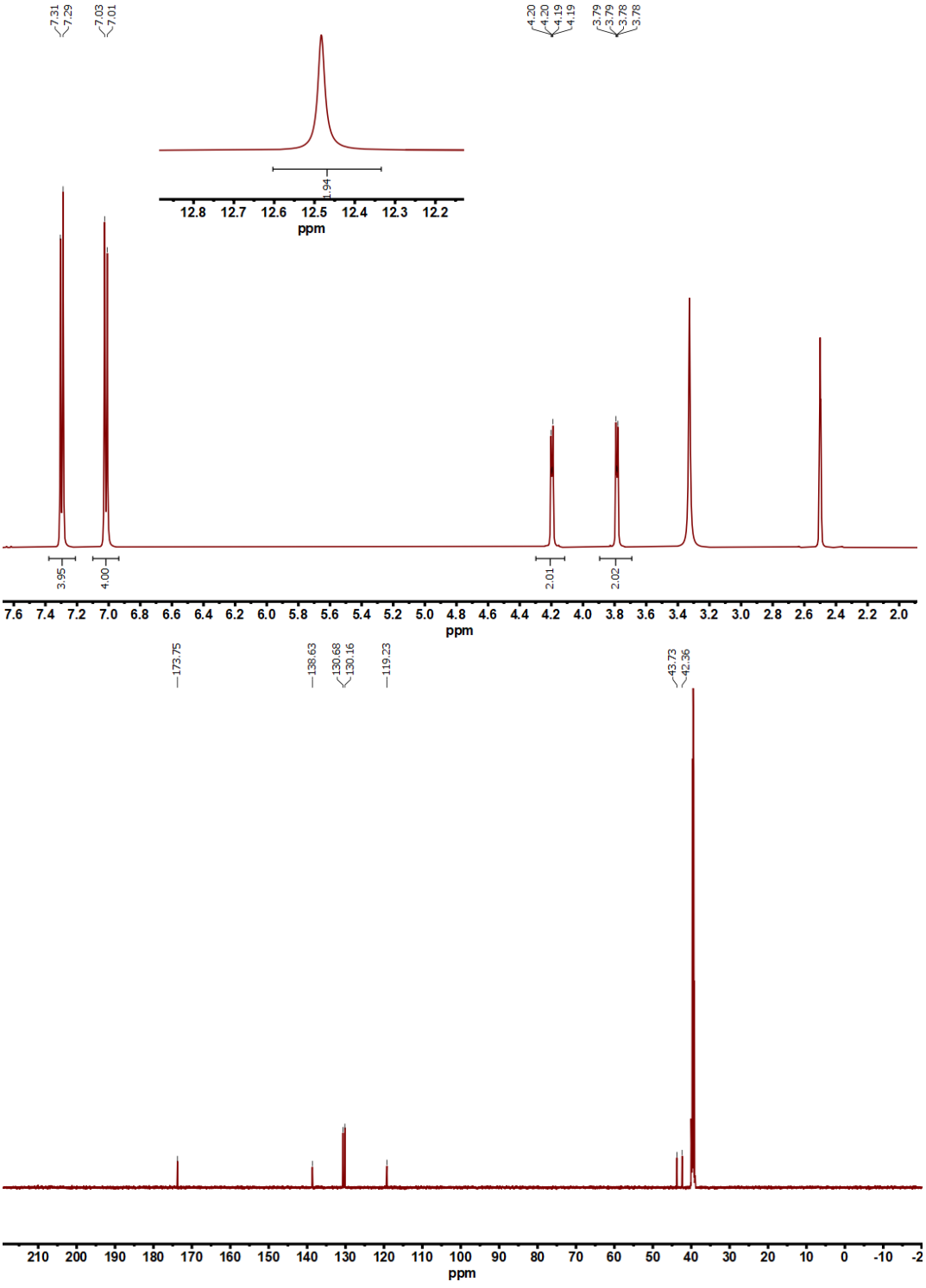
Synthesis of P1b



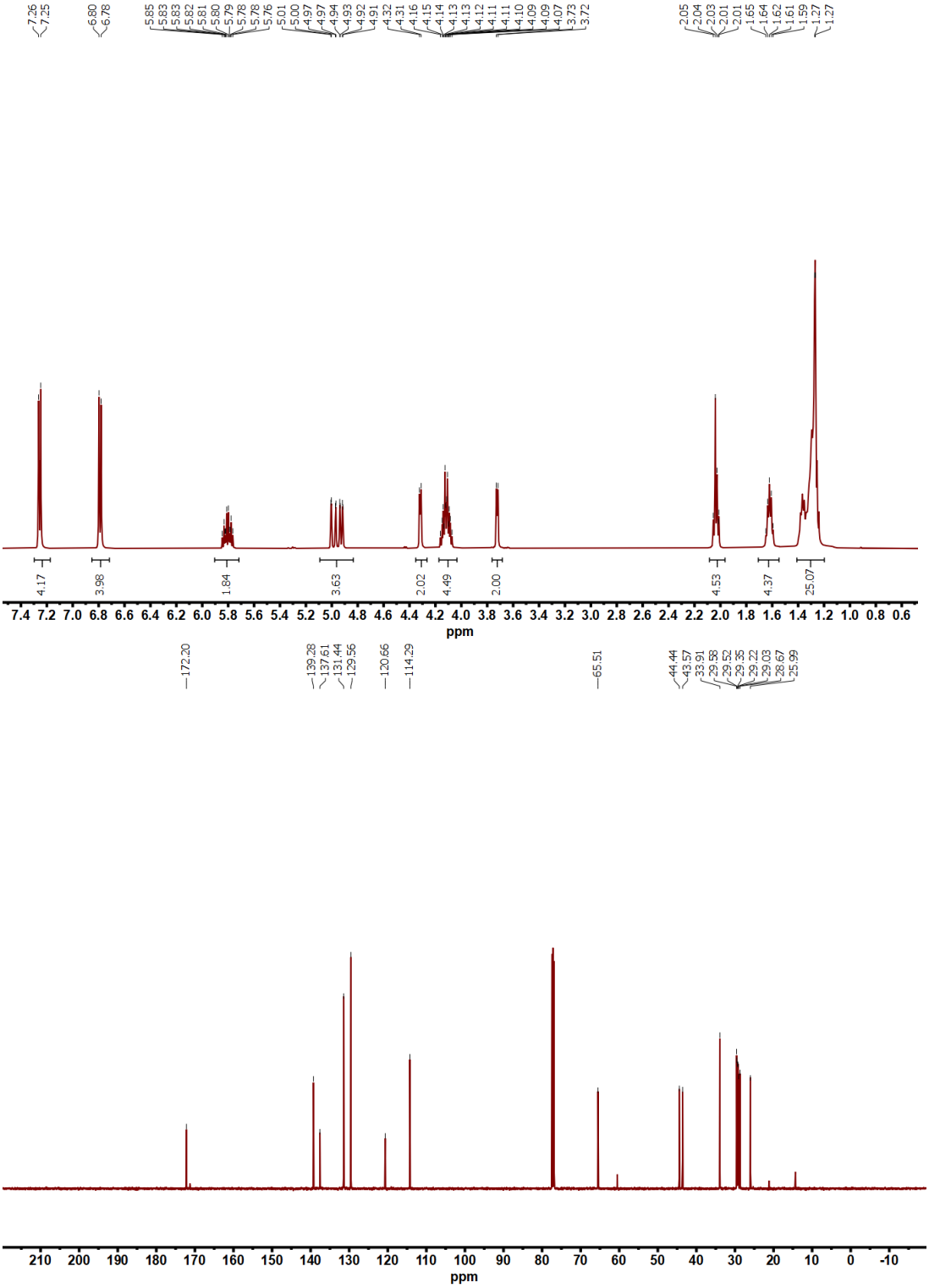
Synthesis of P1c



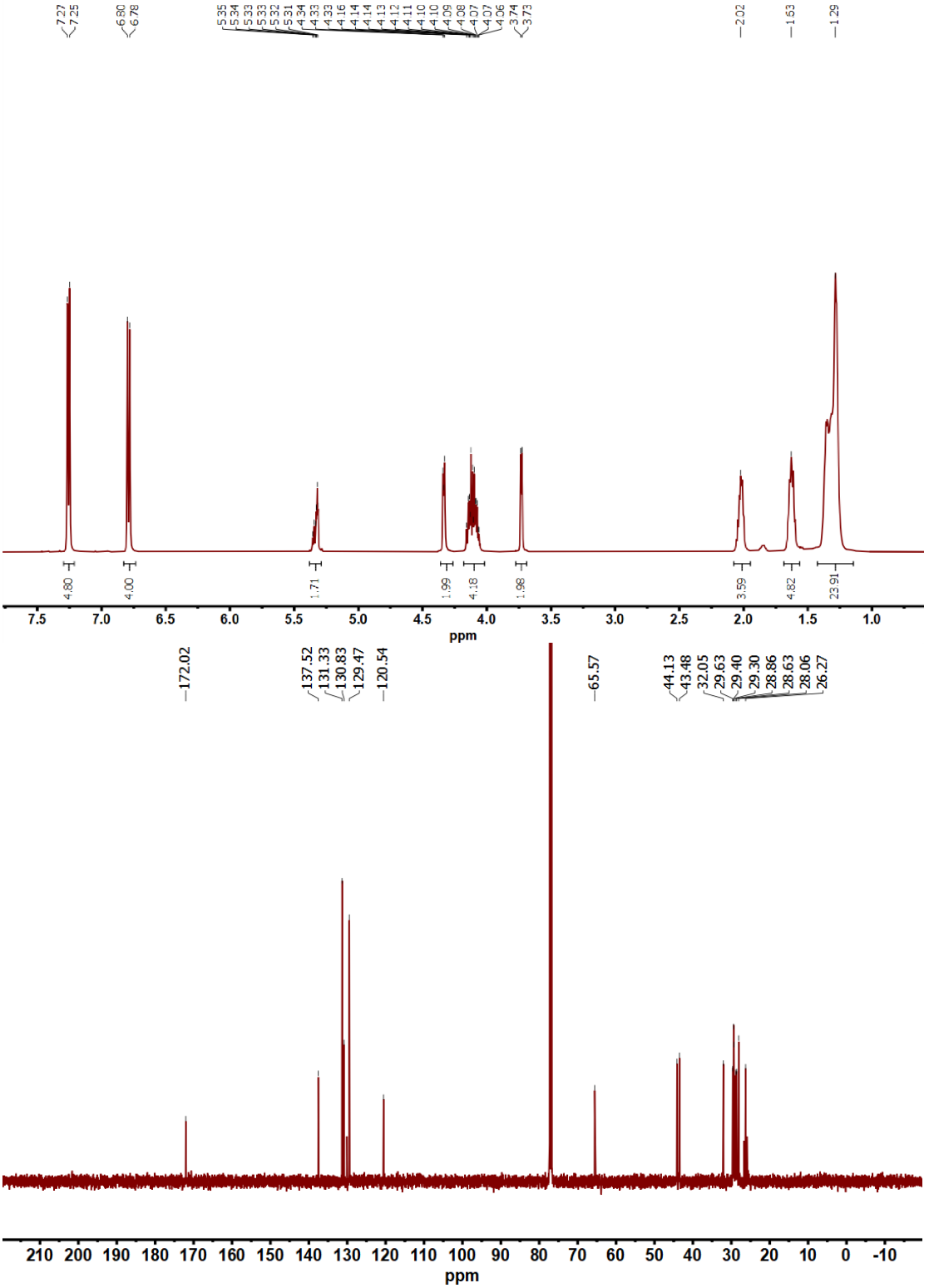
Synthesis of 8



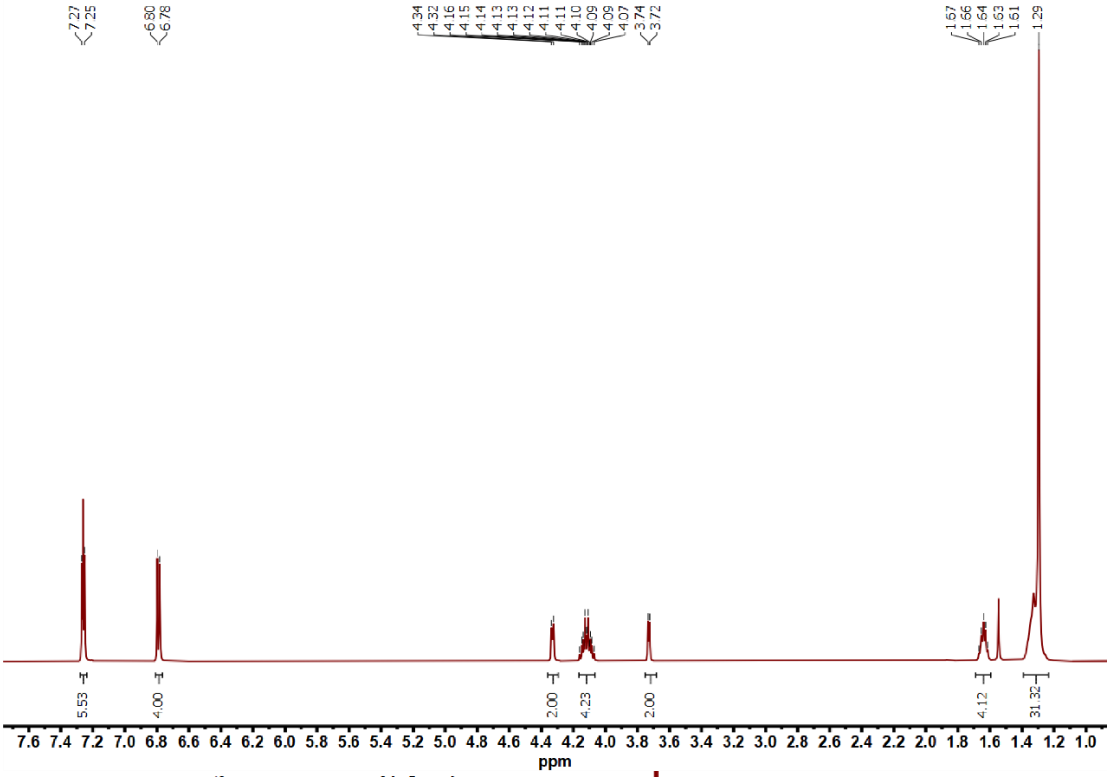
Synthesis of 9

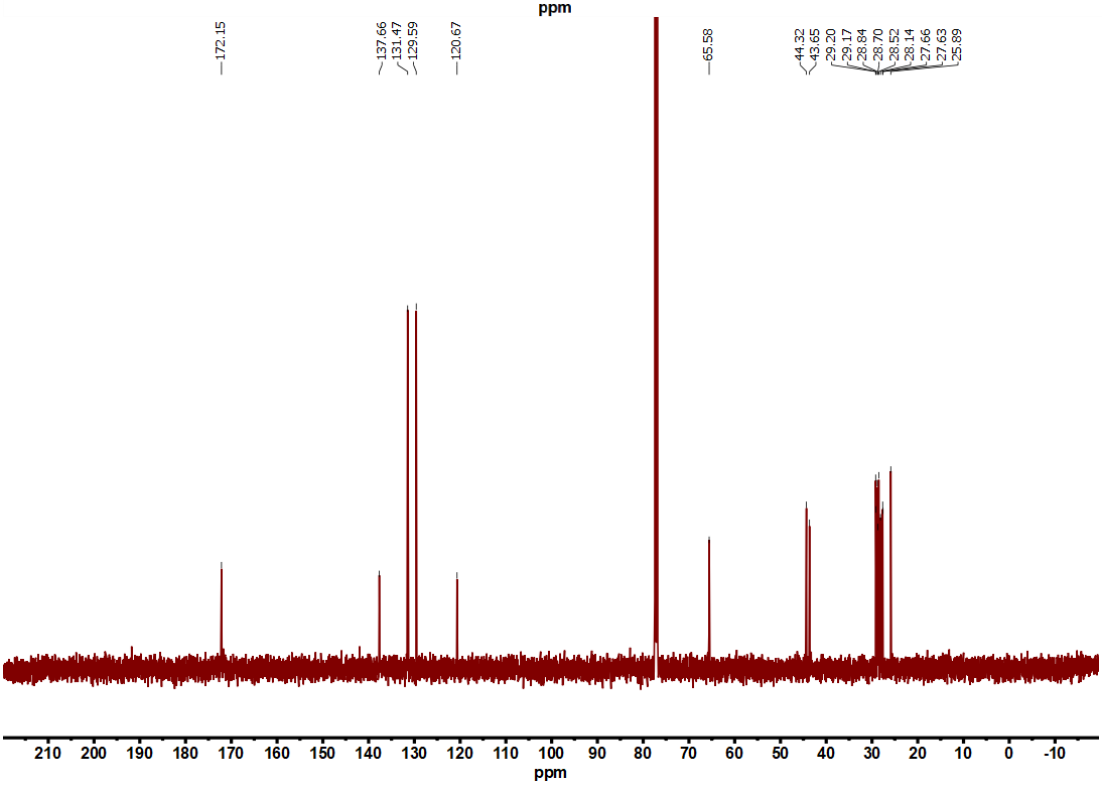


Synthesis of 10

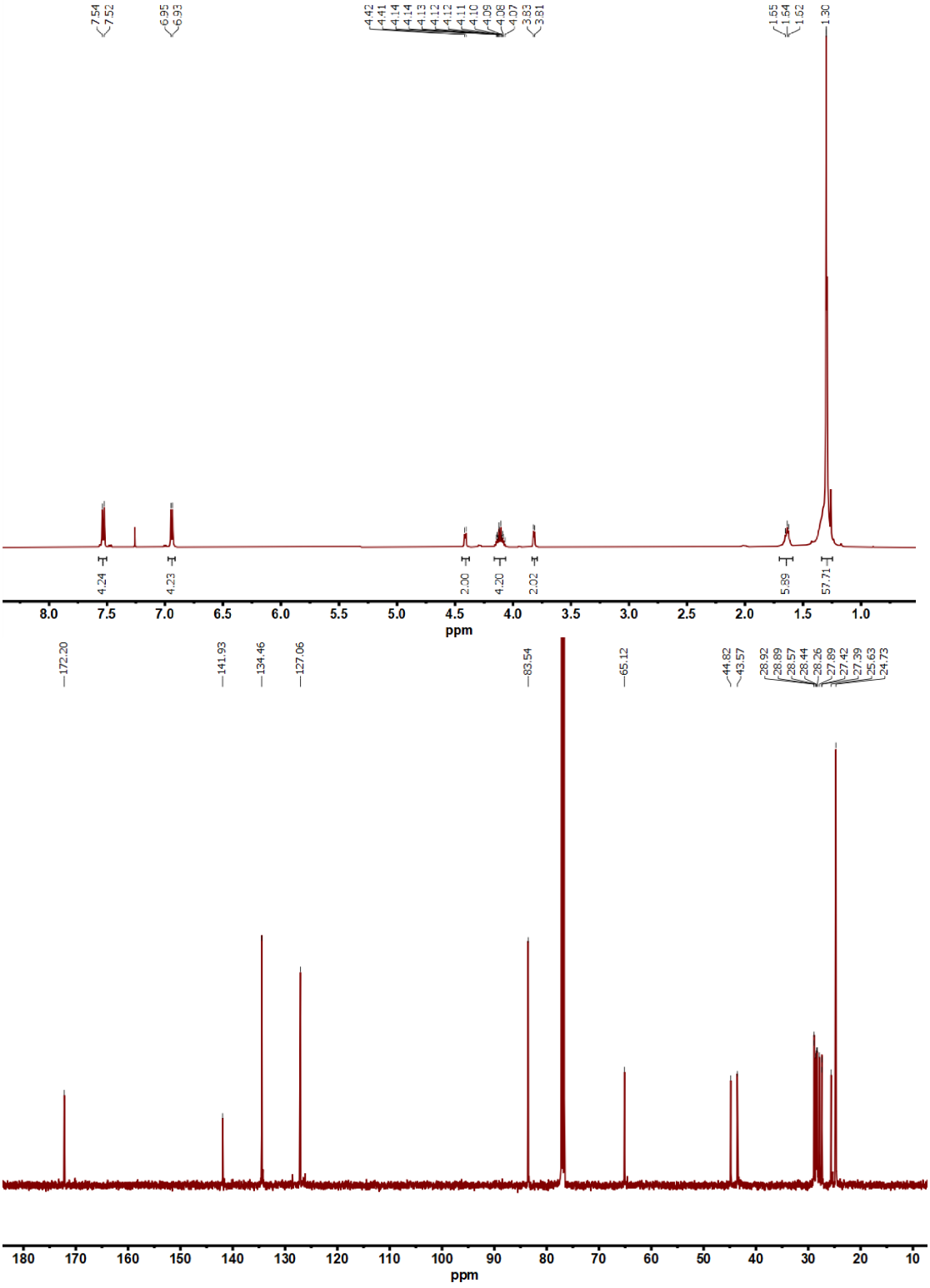


Synthesis of 11

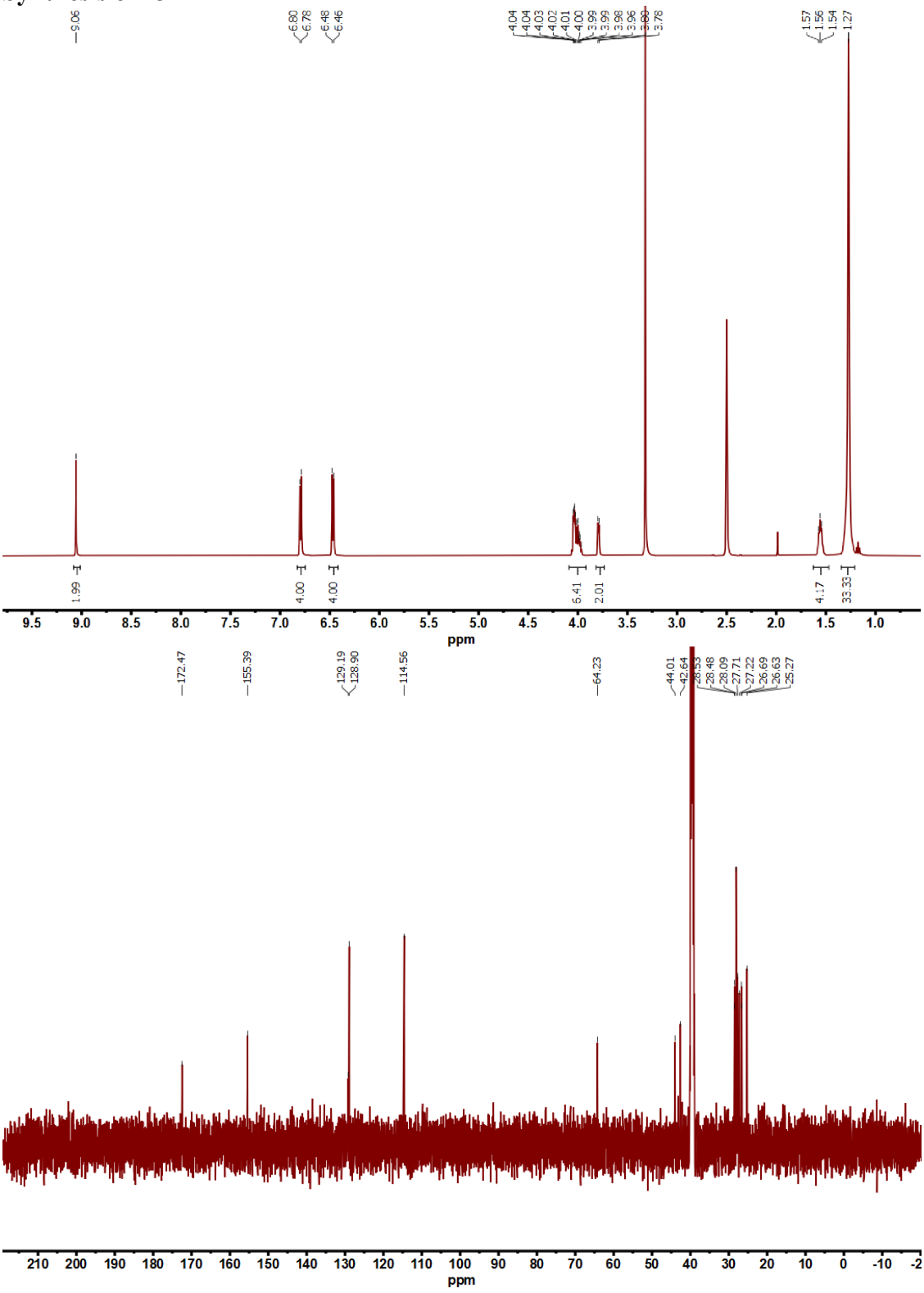




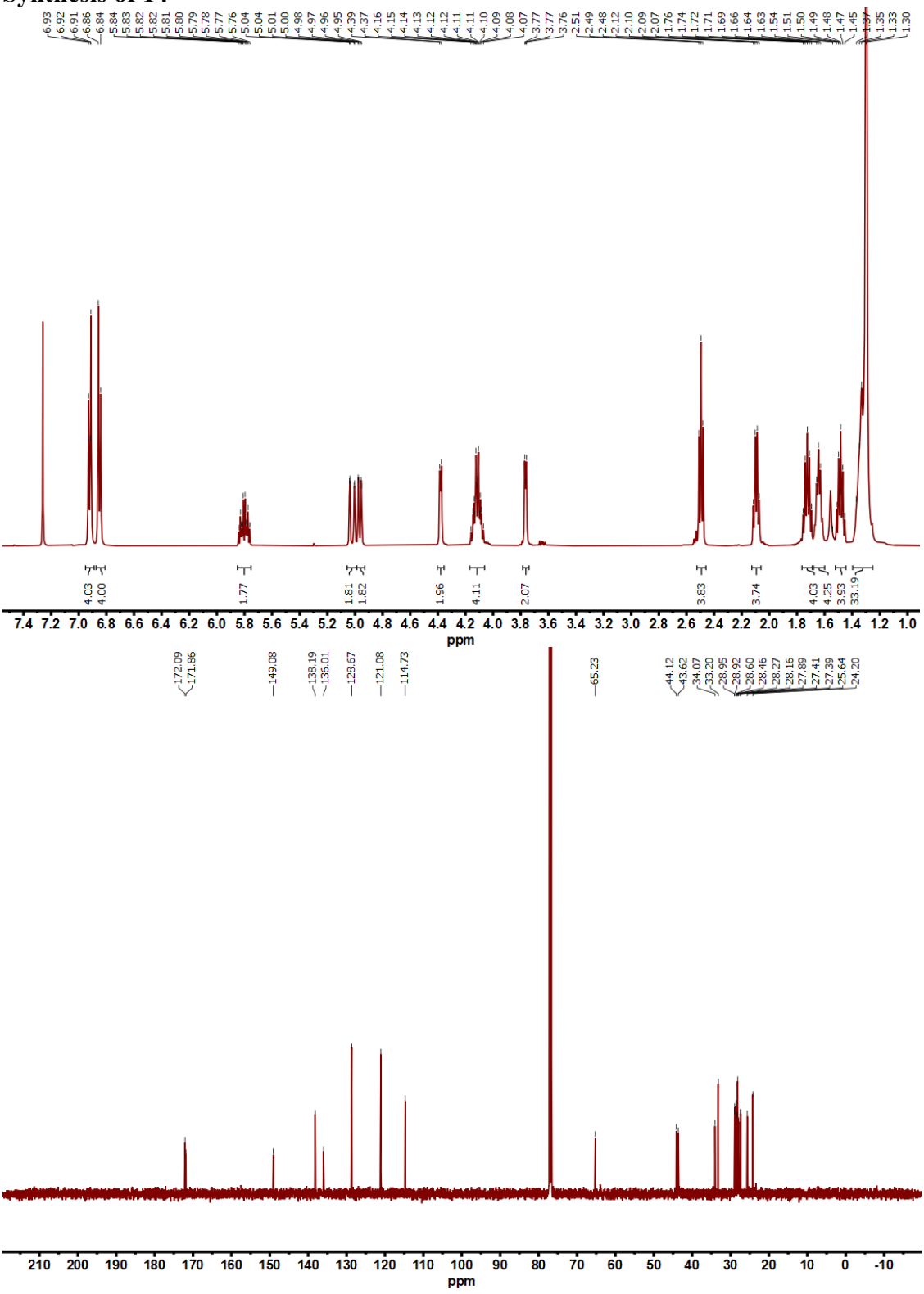
Synthesis of 12



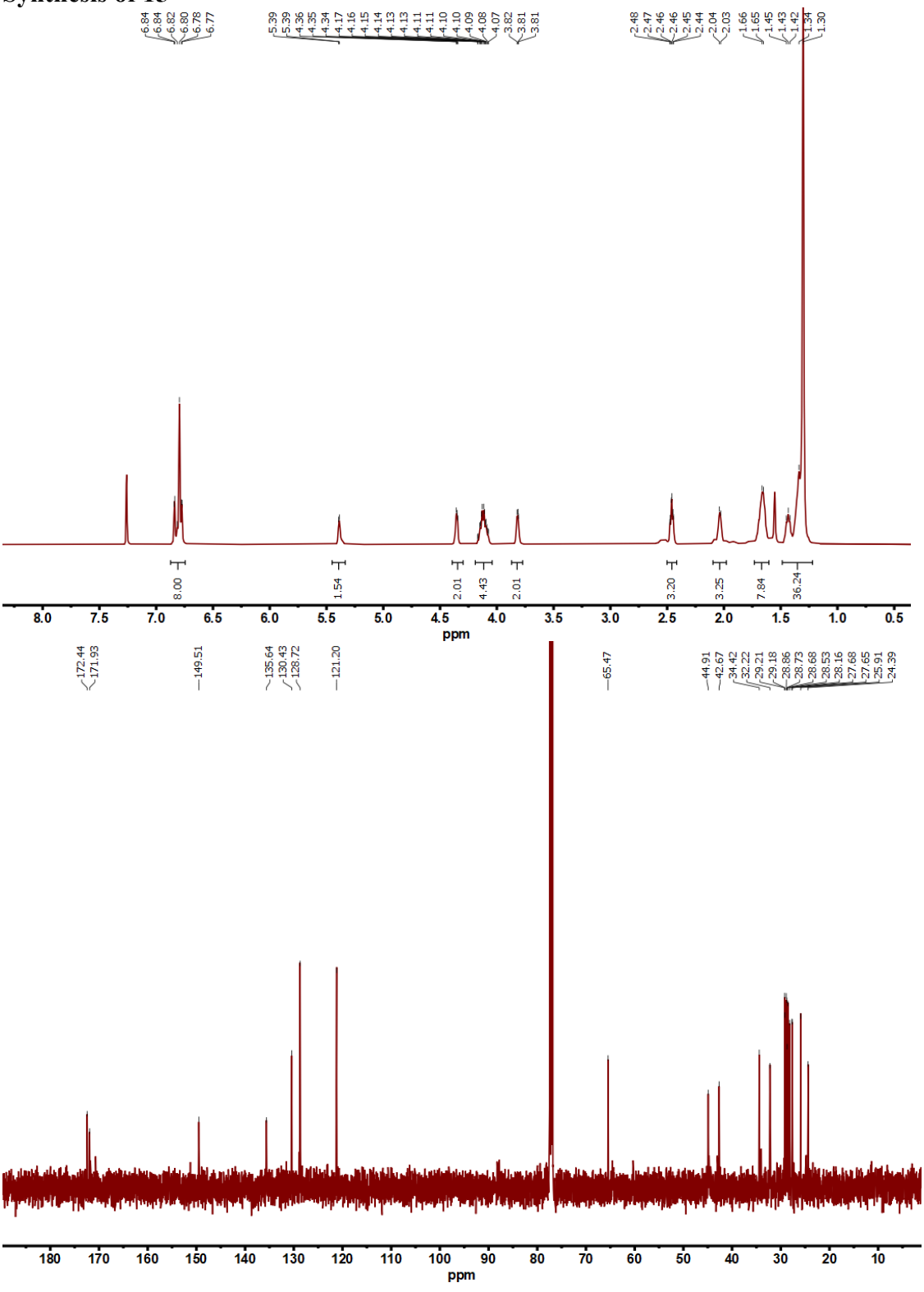
Synthesis of 13



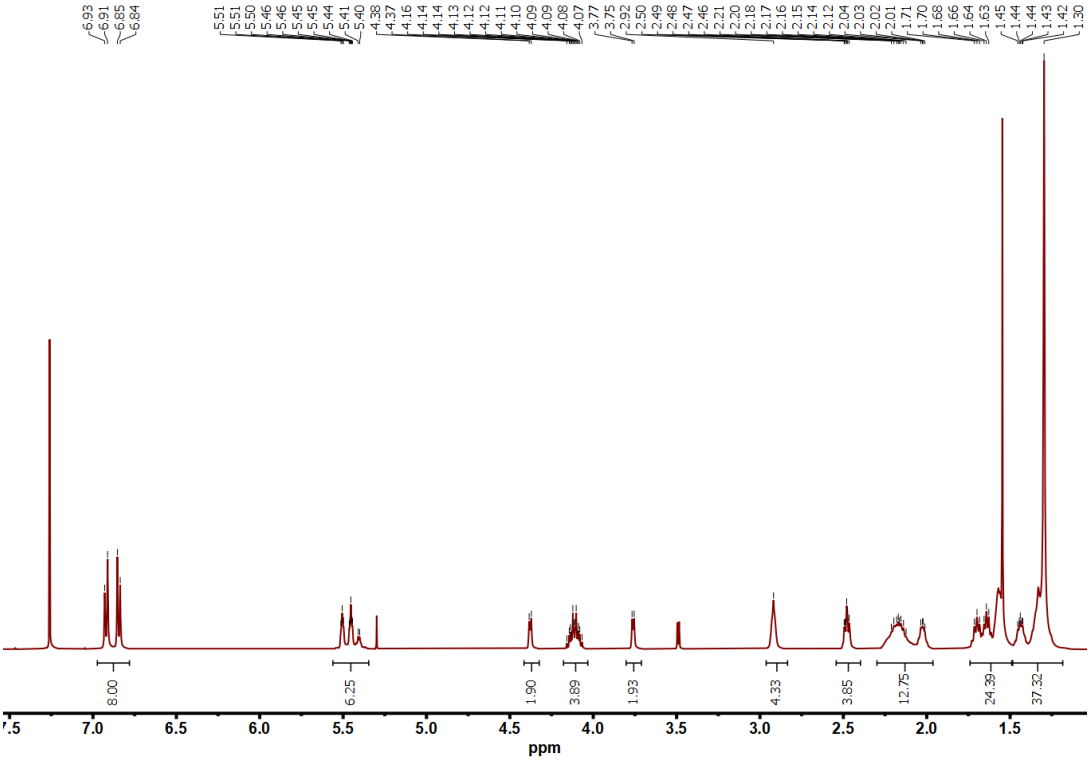
Synthesis of 14



Synthesis of 15



Synthesis of P4a



Synthesis of P4b