10.1071/CH15434_AC
©CSIRO 2015
Australian Journal of Chemistry 2015, 68 (12), 1880-1884

Supplementary Material

Synthesis and Properties of Photoswitchable Carbohydrate Fluorosurfactants

Yingxue Hu,[A,D] Joshua B. Marlow,[A,D] Rajesh Ramanathan,[B]
Wenyue Zou,[B] Hui Geok Tiew,[C] Matthew J. Pottage,[A] Vipul Bansal,[B]
Rico F. Tabor,[A] and Brendan L. Wilkinson[A,E]

[A]School of Chemistry, Monash University, PO Box 23, Clayton, Vic. 3800, Australia.
[B]Ian Potter NanoBioSensing Facility, NanoBiotechnology Research Laboratory, School of Applied Sciences, RMIT University, PO Box 2476, Melbourne, Vic. 3001, Australia.
[C]School of Chemical and Life Sciences, Nanyang Polytechnic, 180 Ang Mo Kio Avenue 8, Singapore 569830.
[D]These authors contributed equally to this work.
[E]Corresponding author. Email: brendan.wilkinson@monash.edu

Contents

1.0 Materials and Methods

General

Analytical thin layer chromatography (TLC) was performed on commercially prepared silica plates (Merck Kieselgel 60 0.25 mm F254). Flash column chromatography was performed using 230-400 mesh Kieselgel 60 silica eluting with distilled solvents as described. Solvents and reagents were purchased from Sigma-Aldrich and Merck and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer at frequencies of 400 MHz and 100 MHz respectively. Chemical shift is reported as parts per million (ppm) downfield shift. The data are reported as chemical shift (δ), multiplicity, relative integral, coupling constant (*J* Hz) and assignment where possible. IR spectra were recorded on a Bruker ATR spectrometer. Optical rotation was measured on an Optical Activity Polaer 2001 (546 nm) polarimeter using a 1 mL cell.

Nutrient broth (NB) and Brain Heart Infusion (BHI) bacterial growth media were purchased from Amyl Media PTY LTD and Becton, Dickinson and Company, respectively. All media components were used as received. Gram negative *Escherichia coli* (*E. coli* DH5α) and Gram positive *Staphylococcus aureus* (*S. aureus* ATCC 1698) were procured from Southern Biologicals and maintained on Nutrient Agar plates, as per standard microbiological protocol.

LC-MS

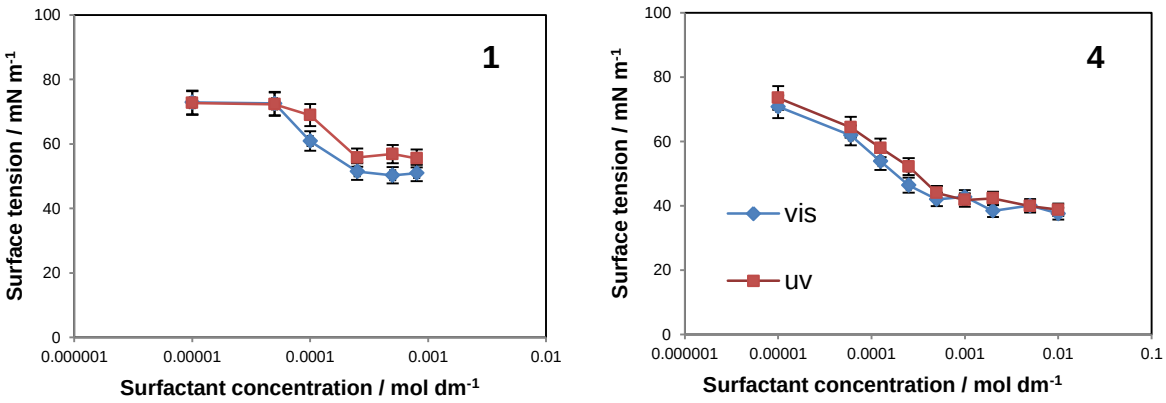
LC-MS was recorded on an Agilent 6120 LC-MS system operating in positive ion mode. Separations were performed on an Agilent Poroshell-120 2.7 µm (3.0 mm x 50 mm) C18 column using a linear gradient of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B) as the mobile phase. Separations were performed using a linear gradient of 20% solvent B to 100% solvent B over 15 minutes, operating at a flow rate of 0.3 mL/min.

Preparative HPLC

Deprotected carbohydrate-based fluorosurfactants **1-6**, were purified by reversed-phase (C18) preparative HPLC using an Agilent 1260 preparative HPLC system equipped with an automated fraction collector. Separations were performed on an Agilent Zorbax SB300 5 µm (20 mm x 150 mm) C18 column using a linear gradient of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B) as mobile phase, operating at a flow rate of 10 mL/min. Fluorosurfactants **1-6** were purified using a linear gradient of 20% solvent B to 100% solvent B over 40 minutes (monitoring at 280 nm). Purified fractions were subsequently combined and lyophilized.

Surface tension measurements

Surface tension measurements were made on a custom-designed pendant drop instrument.[1] A time series was taken (that is, surface tension as a function of time) and values were noted for 100 s to ensure full equilibration of interfacial adsorption. Once a stable surface tension had been attained, this was recorded. Drop volumes were measured throughout and changes of <5% throughout the course of a measurement were a requirement for the data shown. Critical micelle concentration (CMC) values were obtained from the intersection of lines extrapolated from surface tension values in the near pre- and post-CMC regions. Surface tension data for surfactants not shown in the main paper is provided in Figure S0.



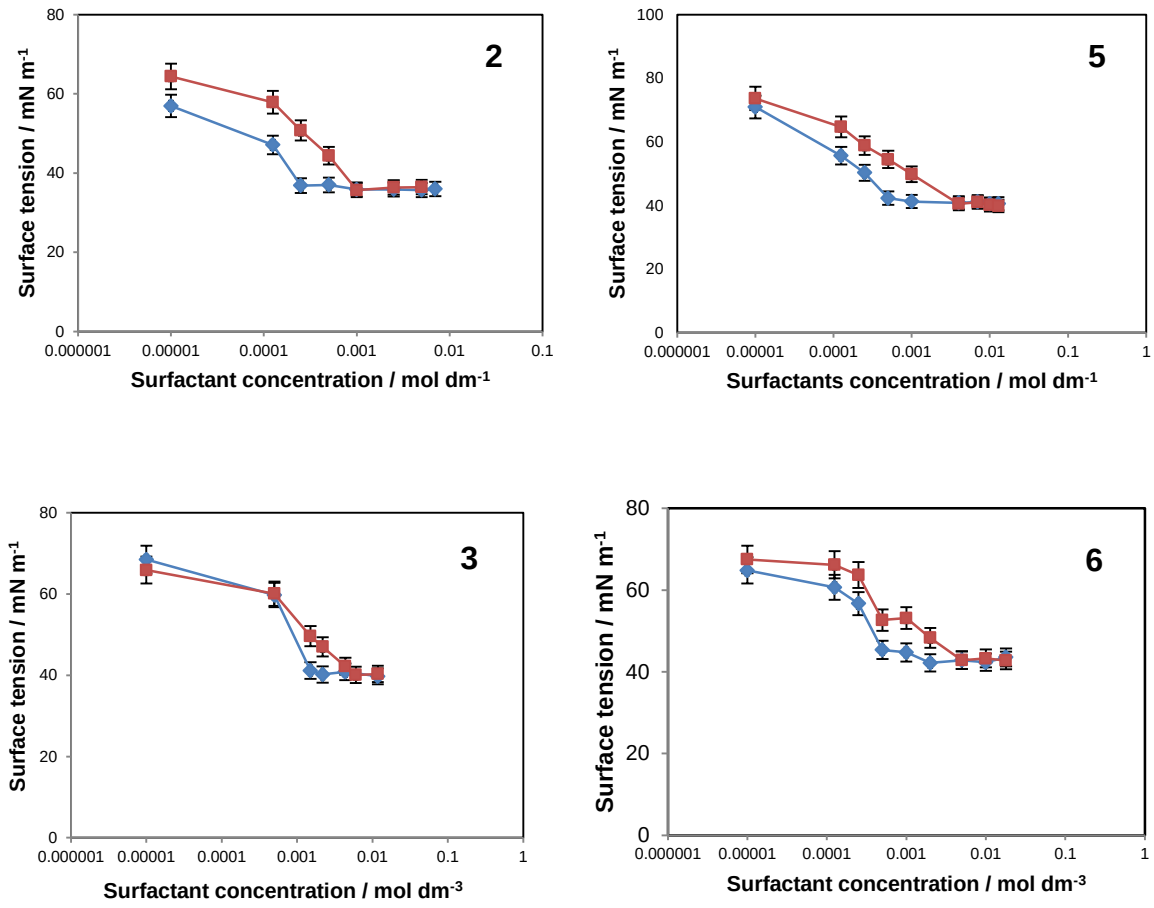


Figure S0: Surface tension data (air-water) for fluorosurfactants **1-6** in both pre- and post-UV irradiation conditions.

UV illumination/photoswitching

The UV-vis measurements were performed using a Cary 60 spectrophotometer in dual beam mode. The *trans*-dominated species were samples analysed immediately after dilution; this *trans* dominance was further confirmed by blue-adapting the samples *via* irradiation for 20 minutes under a 440 nm LED (3 W radiant power) and observing a negligible change in the spectra. The *cis*-dominated species were illuminated under a fluorescent tube lamp (peak wavelength 365 nm, 6.5 W radiant power) for 15 minutes before analysis.

The *trans* fraction f_t is defined by

(1) $f_t = \frac{R_s - R_c}{R_t - R_c}$

where R_s, R_t and R_c are the values of the ratio of the absorbance at the higher wavelength peak in the spectrum (at 435 nm in all samples) to the absorbance at the lower wavelength peak (at 325 nm in samples 1 and 2, at 320 nm in sample 3), in the *trans*-dominated state and in the *cis*-dominated state respectively. This equation assumes a linear relationship between peak intensity ratio and *trans/cis* concentration. It was derived by assigning the *cis*-dominated state a *trans*-fraction of 0, and the *trans*-dominated state a *trans*-fraction of **1**; although this is technically inaccurate (as *per* literature reports these values will be closer to 0.1 and 0.9)[2] it allows for facile calculation of the rate of change from the *cis*-dominated (and experimentally achievable) photostationary state.

Assuming a first order process, the kinetics for the thermal reversion from the *cis* photostationary state is expressed by:

(2) $\ln \frac{f_t(t=0) - f_t(t=\infty)}{f_t(t) - f_t(t=\infty)} = k_{c-t} t$

where f_t, $f_t(t=0)$ and $f_t(t=\infty)$ are the fractions of the *trans*-isomer at time *t*, in the *cis*-dominated photo-stationary state and in the trans dominated state respectively.[UEDA,] The half-life, $t_{1/2}$, of the reversion from the photo-stationary state is then found by:

(3) $t_{1/2} = \frac{\ln(2)}{k_{c-t}}$.

The resultant value was confirmed by comparison to a graph of f_t versus *t*.

UV-Vis spectroscopy

UV-visible spectroscopy was performed using a Cary 60 spectrophotometer. The molar extinction coefficients of the two main peaks, at roughly 450 nm and 350 nm in the samples in each photo-stationary state, were calculated for

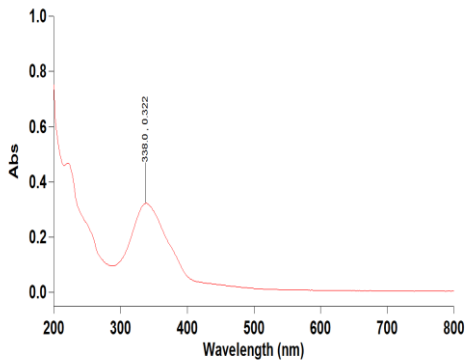
samples 1, 2 and 3 *via* analysis of five samples with a concentration ratio of 1:2:3:4:5, where the absorbance of the most concentrated sample was just below 1.0 to ensure the applicability of the Beer-Lambert law. In the photoillumination experiments, the *trans*-dominated and the *cis*-dominated spectra were taken from samples immediately after dilution and after 15 minutes of irradiation under a fluorescent tube lamp (peak wavelength 365 nm, 6.5 W radiant power).

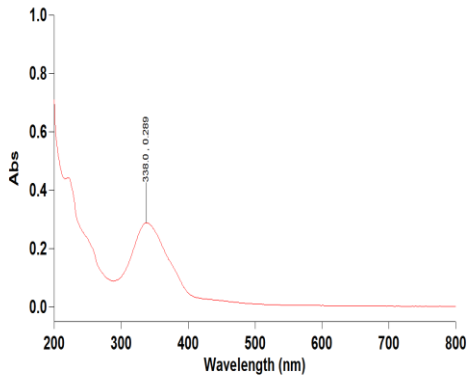
Octanol/water partition coefficient (Log P)

The log *P* of fluorosurfactants was determined by preparing a 50 μM solution of **1-3** in MilliQ water and adding an equal volume of 1-octanol. The UV-vis spectra (350 nm) was taken of the water layer before (t = 0) and after diffusion equilibrium (t = 15 h). The larger the log *P* value, the higher the solubility in the hydrophobic solvent. The log *P* was calculated by the following:

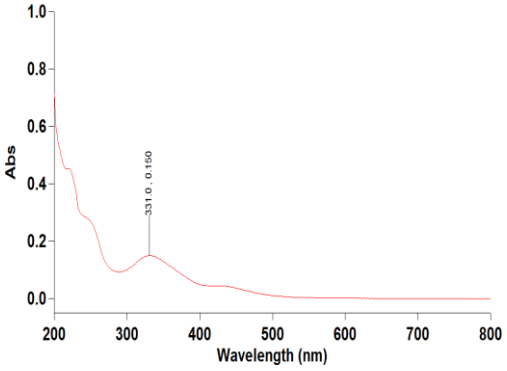
$$\text{Log } P = [(\text{Abs before} - \text{Abs after})/\text{Abs after})]$$

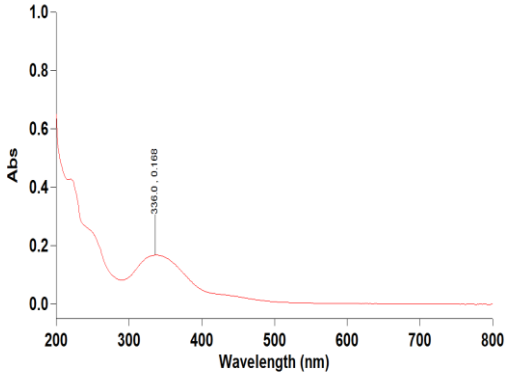
Fluorosurfactant **1** (*trans*)



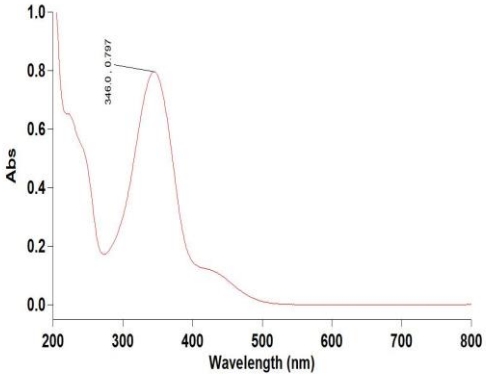


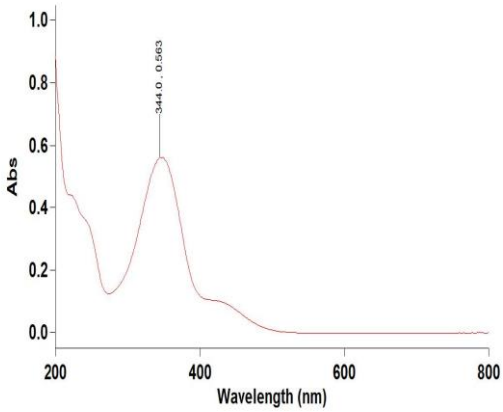
Fluorosurfatant **1** (*cis*)



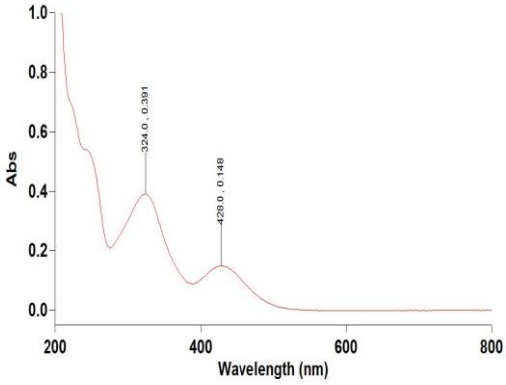


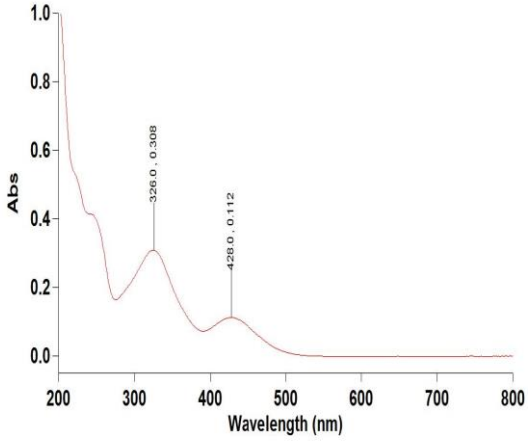
Fluorosurfactant **2** (*trans*)



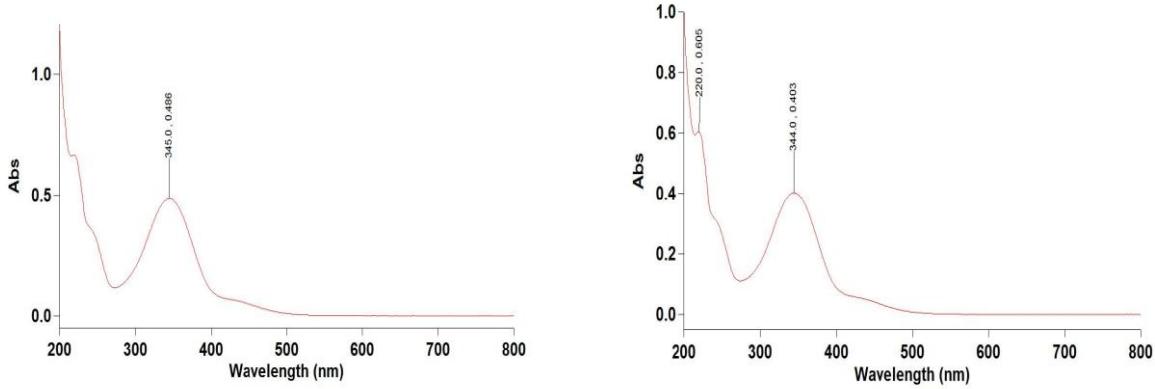


Fluorosurfactant **2** (*cis*)





Fluorosurfactant **3** (*trans*)



Flurosurfactant **3** (*cis*)

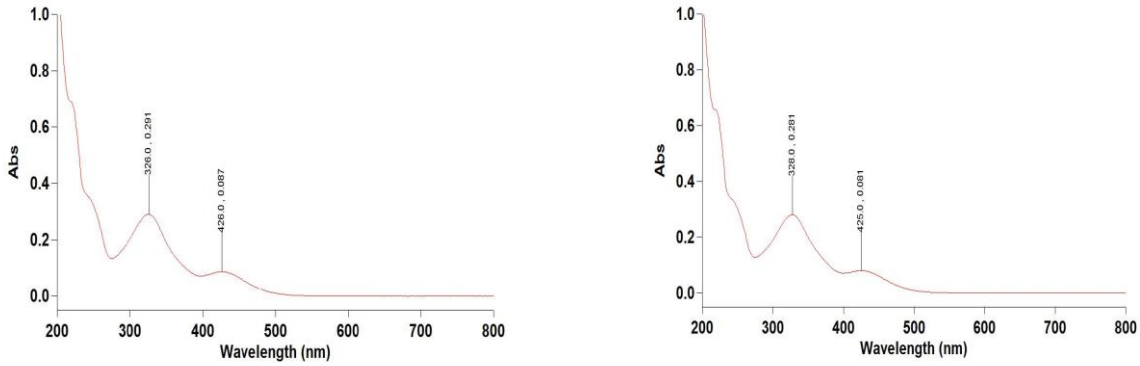


Figure S1: UV-Vis spectra of fluorosurfactants **1-3** (bottom) before (left) and after (right) diffusion equilibrium.

Antibacterial assays

The antibacterial activity of the fluorosurfactants **1-6** was tested against Gram-negative *E. coli* and Gram positive *S. aureus*. A fixed concentration of (OD_{600} – 0.1: log phase 10^8 CFU/mL) of freshly grown cultures were used for antibacterial assay, after determining the bacterial concentration using a GENESYS 10S UV-Vis Spectrophotometer. 100 µL of the bacterial suspension was incubated with 100 µL of the fluorosurfactants **1-6** at 37 °C. Different concentrations of the **1-6** (0, 1, 10, 25, 50, 100, 250 µg/mL) were used to determine the concentration-dependent influence on bacterial growth and/or inhibition. Appropriate controls were chosen wherein one of the controls contained no fluorosurfactants while the other contained a mixture of

different concentrations of **1-6** with the bacteria-free growth media. The bacterial growth curves were monitored at OD_{600} over a period of 24 hours (0 h, 1.5 h, 3 h, 5 h, 21 h and 24 h) in 96-well format using a Perkin Elmer EnVision^{TM} 2104 Multilabel Plate Reader. All antibacterial assays performed in triplicates; tests were repeated independently three times; each well was read five times. The average of 45 readings (3 x 3 x 5) in each case was calculated and plotted along with standard deviation. The data was background corrected by subtracting the OD_{600} value obtained from the control containing a mixture of **1-6** and the bacteria-free media growth.

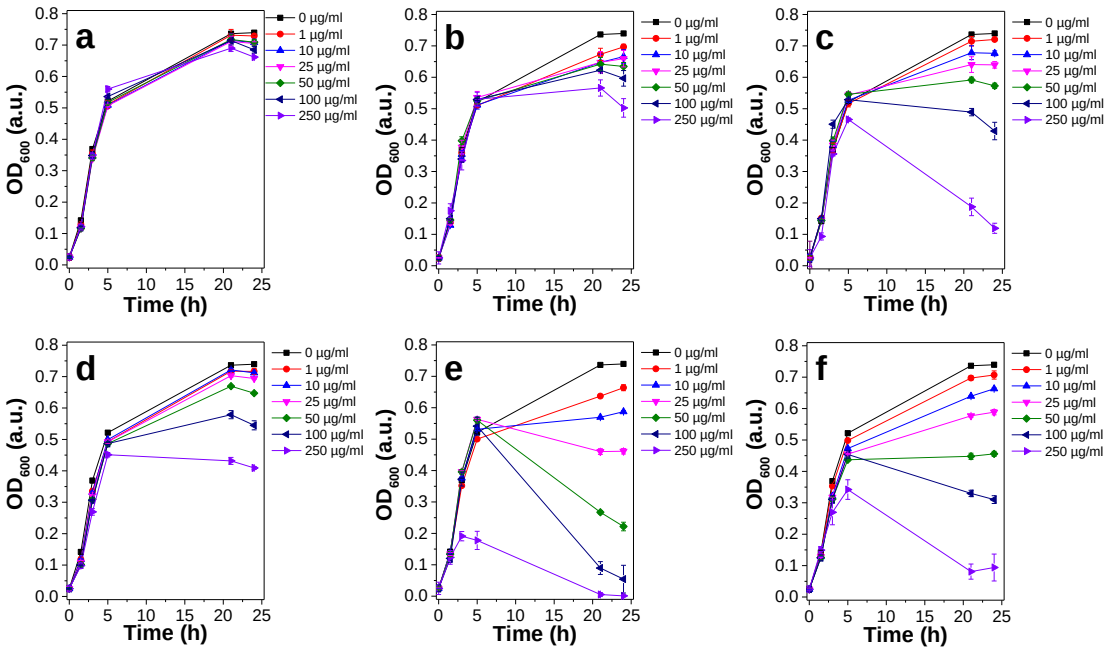


Figure S1: Dose dependent antibacterial activity of (a) **6** (b) **5** (c) **4** (d) **3** (e) **2** and (f) **1** against Gram positive bacteria *S. aureus* over a period of 24 hours.

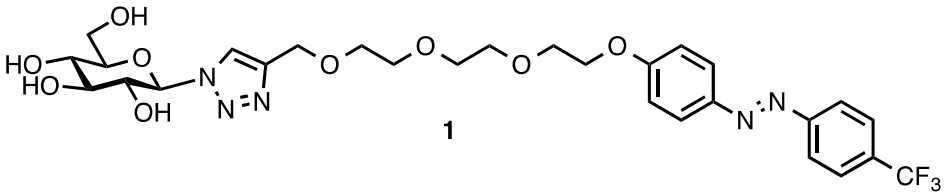
General procedure for the synthesis of carbohydrate fluorosurfactants 1-6

A mixture of the corresponding β-D-glycopyranosyl azide (1.0 equiv) and propargyl ether **9-11** (1.0 equiv.) was suspended in *tert*-BuOH/H_2O (2:1 v/v, 0.5 mM). Sodium ascorbate (0.4 equiv.) and $CuSO_4.5H_2O$ (0.2 equiv.) was added and the deep yellow suspension was stirred vigorously for 2h at 40 ^oC.

TLC analysis (ethyl acetate/petroleum ether 40-60, 4:1 v/v) showed complete consumption of the azide and alkyne, with formation of a polar product (R_f ca. 0.3). The reaction mixture was extracted into ethyl acetate (10 mL), and washed with water (10 mL) and brine (10 mL). The organic layer was dried (Na_2SO_4), filtered and evaporated to dryness. The resulting yellow oil was re-dissolved in dry methanol. A 0.5M solution of sodium methoxide in methanol (0.1 equiv.) was then added and the reaction stirred at room temperature for one hour, at which time LC-MS analysis (20 to 100%B over 15 min) showed reaction completion. The reaction was neutralized with Amberlite IR-120 acidic ion exchange resin, filtered and concentrated under reduced pressure. Surfactants **1-6** were subsequently purified by reversed-phase preparative HPLC (See materials and methods) and isolated as deep yellow solids following lyophilization (21-67% yields).

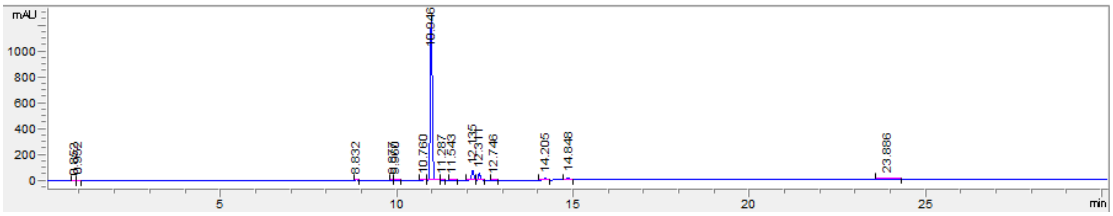
2.0 Analytical data for compounds 1-6, 9-11.

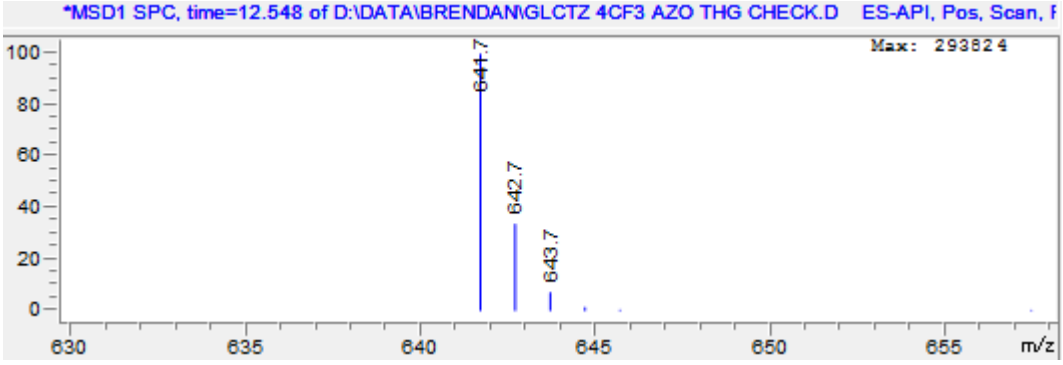
2-[2-[2-(4-trifluoromethylazophenyl phenoxy)ethoxy)ethoxy]ethyl β-D-glucopyranosyl-1,2,3-triazole **1**



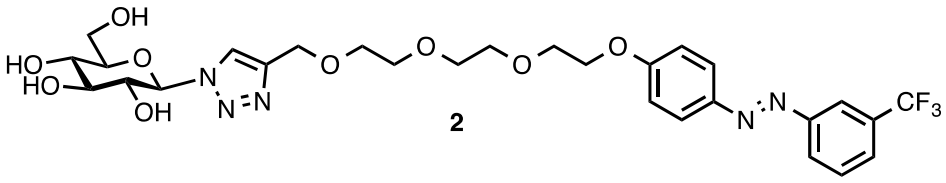
1

Yield 42%; Mp = 95.7°C; $[\alpha]_D^{20}$ -20.6 (c, 0.15 in CH_3OH); FT-IR: v_{max}/cm^{-1} 2859, 1596, 1421, 1037, 762; ^1H NMR (600MHz, CD_3OD) δ 7.95 (2H, d, J=8.93Hz), 7.86 (1H, d, J=8.93Hz), 7.82 (1H, d, J=8.93Hz), 7.75-7.71 (2H, m), 7.62 (1H, t, J=7.88Hz), 7.24 (2H, d, J=8.93Hz), 6.97-6.95 (1H, m), 4.70-4.61 (4H, m), 4.68-4.63 (4H, m), 4.29-4.21 (3H, m), 3.92-3.87 (5H, m), 3.32-3.30 (12H, m); ^{13}C NMR (600MHz, CD_3OD) δ 179.0, 169.1, 162.1, 159.9, 149.5, 147.6, 142.0, 140.4, 132.6, 129.9, 128.5, 126.1, 125.8, 125.0, 124.0, 116.1, 114.8, 114.3, 88.6, 79.8, 77.7, 73.1, 69.8, 69.2, 67.7, 64.1, 63.5, 61.1; LC-MS: m/z 641.7

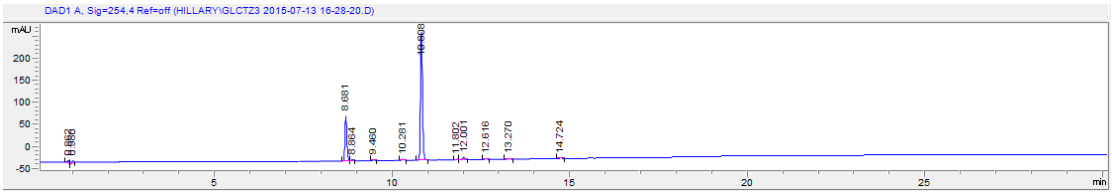


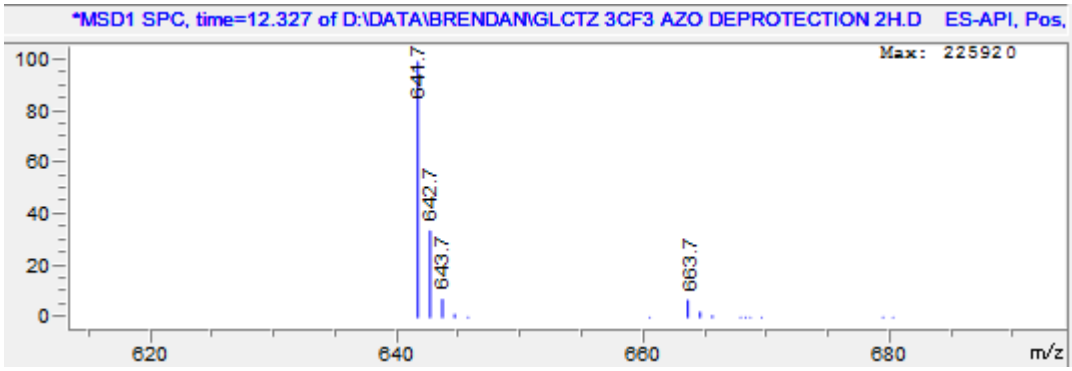


2-[2-[2-(3-trifluoromethylazophenyl phenoxy)ethoxy)ethoxy]ethyl β-D-glucopyranosyl-1,2,3-triazole **2**

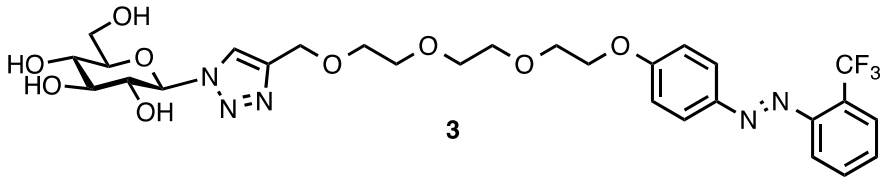


Yield 67%; Mp=97.7°C; $[\alpha]_D^{20}$ -14.1 (c, 0.17 in CH_3OH); FT-IR: v_{max}/cm^{-1} 3351, 1599, 1498, 1325, 1247, 1055, 691; ^1H NMR (600MHz, CD_3OD) δ 8.04 (2H, d, J=8.74Hz), 7.98 (2H, d, J=9.41Hz), 7.86 (2H, d, J=8.07Hz), 7.51 (1H, s), 7.18 (1H, d, J=9.08Hz), 7.15 (1H, d, J=8.98Hz), 5.58 (1H, d, J=9.15Hz), 4.72-4.67 (3H, m), 4.31-4.28 (4H, m), 3.94-3.91 (4H, m), 3.78-3.66 (13H, m); ^{13}C NMR (600MHz, CD_3OD) δ 190.7, 179.1, 169.1, 162.2, 154.7, 146.9, 140.4, 129.9, 126.2, 125.1, 122.4, 115.1, 89.0, 79.9, 78.6, 73.8, 70.2, 69.9, 69.8, 69.2, 68.2, 67.6, 64.2, 63.5, 61.4; LC-MS: m/z 641.7

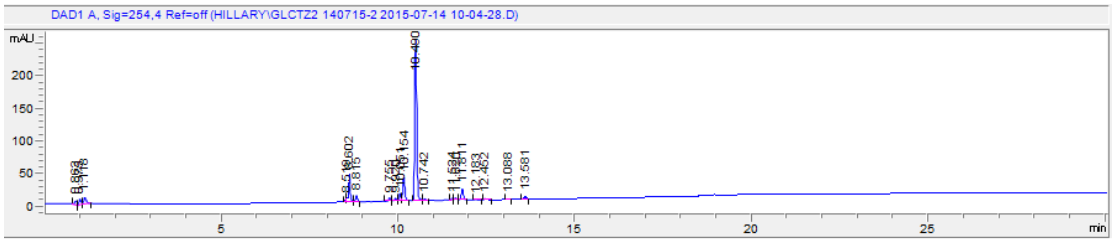


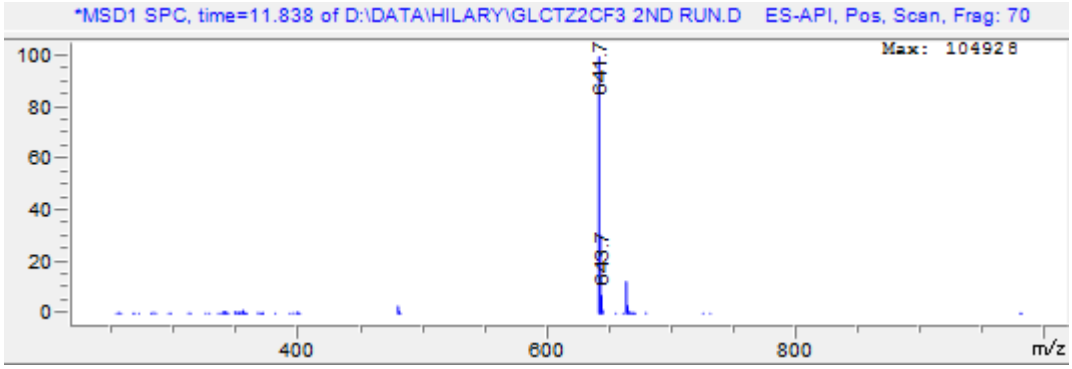


2-[2-[2-(2-trifluoromethylazophenyl phenoxy)ethoxy)ethoxy]ethyl β-D-glucopyranosyl-1,2,3-triazole **3**

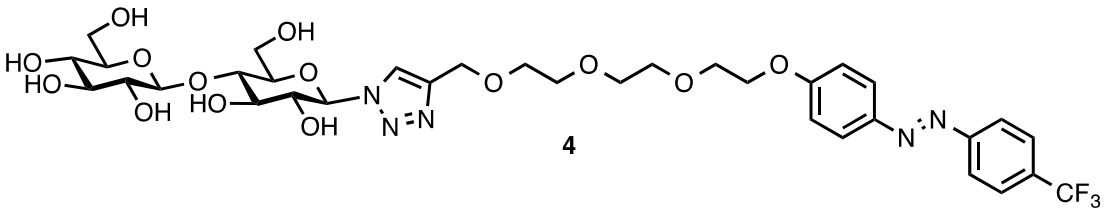


Yield 52%; Mp=189.9°C; $[\alpha]_D^{20}$ -26.4 (c, 0.13 in CH_3OH); FT-IR: v_{max}/cm^{-1} 2859, 1593, 1421, 1322, 1063, 854; ^1H NMR (600MHz, CD_3OD) δ 8.09 (1H, s), 8.01-8.00 (2H, m), 7.85 (2H, d, J=9.06Hz), 7.67-7.61 (2H, m), 7.01 (2H, d, J=9.06Hz), 5.50 (1H, d, J=9.07Hz), 4.56 (2H, s), 4.13 (2H, t, J=4.65Hz), 3.83-3.74 (5H, m), 3.62-3.59 (4H, m), 3.58-3.55 (6H, m), 3.49-3.39 (4H, m); ^{13}C NMR (600MHz, CD_3OD) δ 162.1, 152.7, 146.6, 144.6, 124.9, 124.8, 123.0, 118.3, 114.8, 88.2, 79.7, 77.1, 72.6, 70.4, 70.2, 69.5, 69.4, 69.3, 67.7, 63.5, 60.9; LC-MS: m/z 641.7.

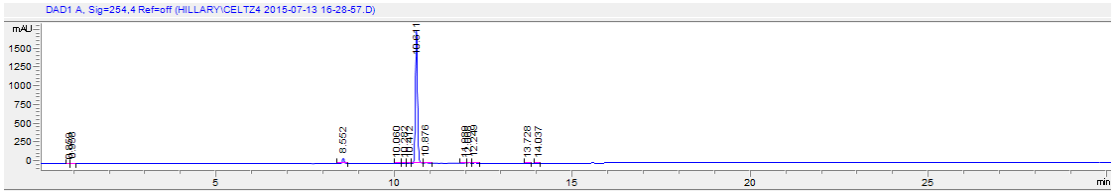


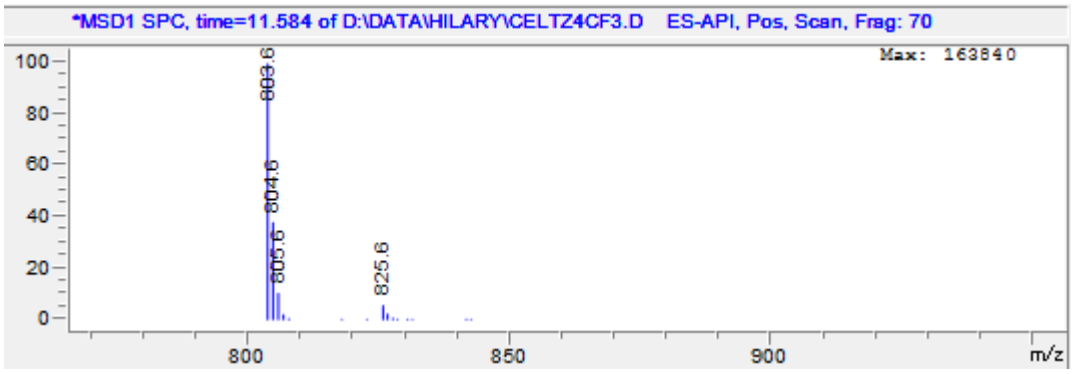


2-[2-[2-(4-trifluoromethylazophenyl phenoxy)ethoxy)ethoxy]ethyl β-D-cellobiosyl-1,2,3-triazole **4**

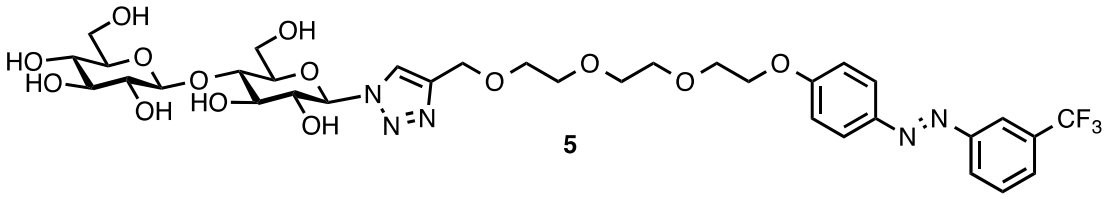


Yield 42%; Mp=154.6°C (Decomp); $[\alpha]_D^{20}$ -10.0 (c, 0.13 in CH_3OH); FT-IR: v_{max}/cm^{-1} 2858, 1629, 1424, 1061, 824; ^1H NMR (400MHz, CD_3OD) δ 8.14 (1H, s), 7.97 (2H, d, J=8.27Hz), 7.91 (2H, d, J=8.97Hz), 7.79 (2H, d, J=8.27Hz), 7.09 (2H, d, J=8.97Hz), 5.57 (1H, d, J=9.24Hz), 4.63-4.61 (2H, m), 4.44 (1H, d, J=7.99Hz), 4.23-4.20 (2H, m), 3.93-3.59 (21H, m), 3.28-3.26 (5H, m); ^{13}C NMR (600MHz, CD_3OD) δ169.1, 162.3, 160.3, 154.8, 146.8, 126.1, 124.9, 122.5, 114.7, 103.1, 87.9, 78.2, 76.8, 76.5, 73.5, 72.5, 70.3, 70.1, 69.9, 69.4, 69.3, 67.7, 63.5, 61.0, 60.0; LC-MS: m/z 803.6.



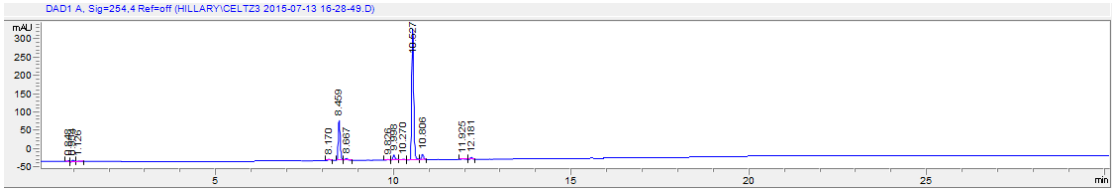


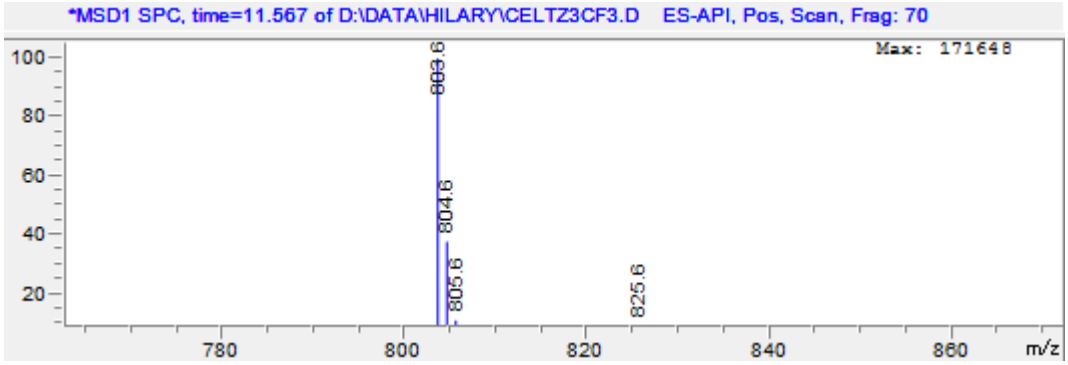
2-[2-[2-(3-trifluoromethylazophenyl phenoxy)ethoxy)ethoxy]ethyl β-D-cellobiosyl-1,2,3-triazole **5**



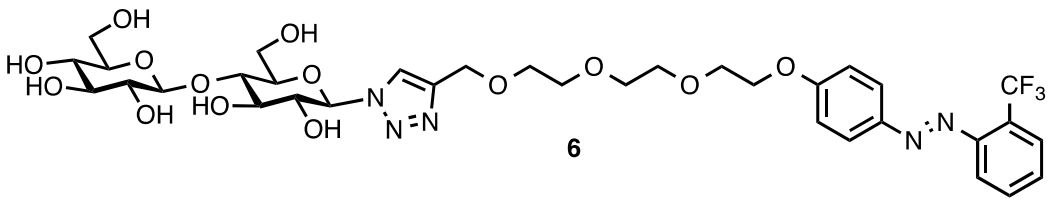
5

Yield 41%; Mp=150.6°C (Decomp); $[\alpha]_D^{20}$ -8.7 (c, 0.16 in CH_3OH); FT-IR: ν_{max}/cm^{-1} 3574, 2845, 1627, 1426, 1022, 779; ^1H NMR (400MHz, CD_3OD) δ 8.13-8.07 (3H, m), 7.93 (2H, d, J=9.04Hz), 7.74-7.67 (2H, m), 7.09 (2H, d, J=9.04Hz), 5.55 (1H, d, J=9.38Hz), 4.63-4.61 (2H, m), 4.44 (1H, d, J=7.92Hz), 4.23-4.21 (2H, m), 3.92-3.83 (7H, m), 3.67-3.61 (10H, m), 3.28-3.26 (12H, m); ^{13}C NMR (600MHz, CD_3OD) δ 170.6, 163.7, 161.7, 154.4, 148.3, 145.8, 131.4, 127.7, 127.4, 126.2, 124.8, 119.6, 116.2, 115.7, 104.5, 89.9, 79.7, 79.1, 78.2, 78.1, 77.5, 74.8, 74.6, 71.7, 71.5, 71.4, 70.7, 69.1, 64.9, 62.3, 61.5, 58.3; LC-MS: m/z 803.6.

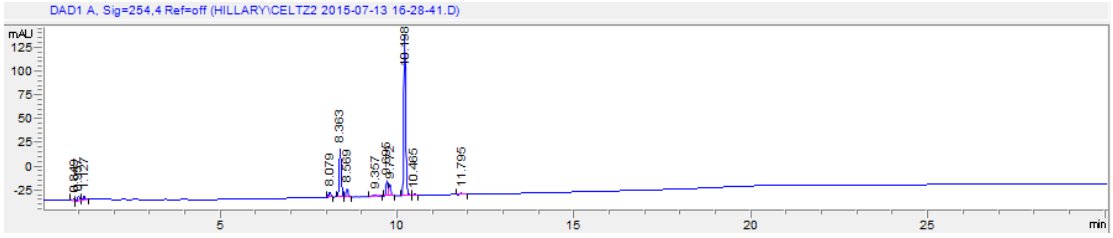


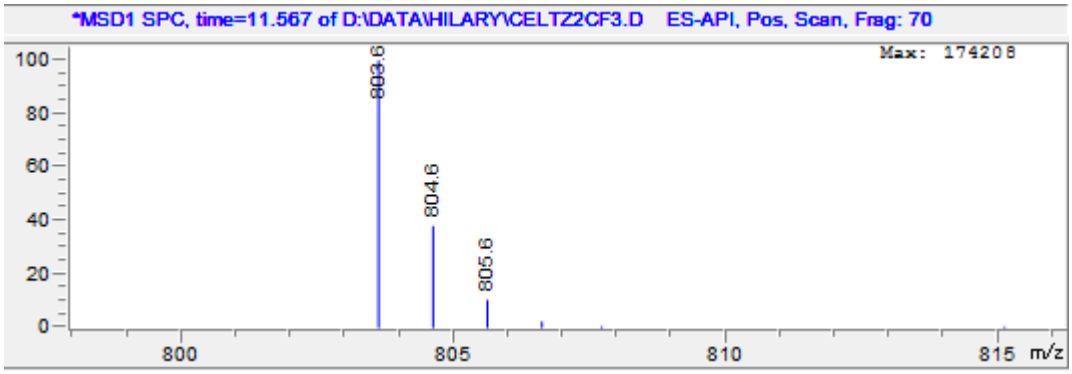


2-[2-[2-(2-trifluoromethylazophenyl phenoxy)ethoxy)ethoxy]ethyl β-D-cellobiosyl-1,2,3-triazole **6**



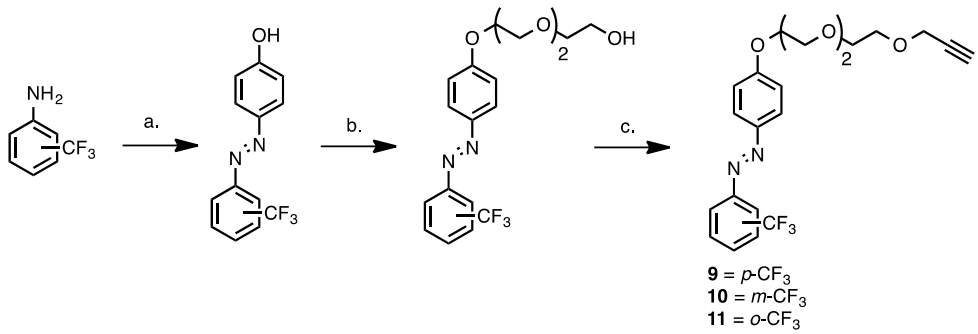
Yield 40%; Mp=206.8°C (Decomp); $[\alpha]_D^{20}$ -10.7 (c, 0.14 in CH_3OH); FT-IR: v_{max}/cm^{-1} 2829, 1596, 1428, 1038, 878; ^1H NMR (400MHz, CD_3OD) δ 7.91 (2H, d, J=9.50Hz), 7.83-7.77(2H, m), 7.71-7.66(2H, m), 7.58 (1H, t, J=7.60Hz), 7.11(2H, d, J=9.50Hz), 5.54 (1H, d, J=9.08Hz), 4.64-4.62 (2H, m), 4.47-4.44 (2H, m), 4.24 (2H, m), 3.89-3.84 (7H, m), 3.67-3.62 (13H, m), 3.28-3.26 (8H, m); ^{13}C NMR (600MHz, CD_3OD) δ 179.0, 169.0, 162.2, 160.1, 149.6, 147.3, 141.9, 140.3, 132.6, 129.9, 128.6, 125.6, 125.1, 115.9, 114.8, 103.2, 88.3, 78.3, 77.9, 76.8, 76.6, 73.5, 70.2, 70.0, 69.6, 69.2, 67.7, 63.5, 60.9, 60.1; LC-MS: m/z 803.6.





General procedure for the synthesis of azobenzene propargyl ethers 9-11 (Scheme S1).

Azobezene-functionalized propargyl ethers **9-11** were synthesized over four steps using known literature procedures.[1,3,4]

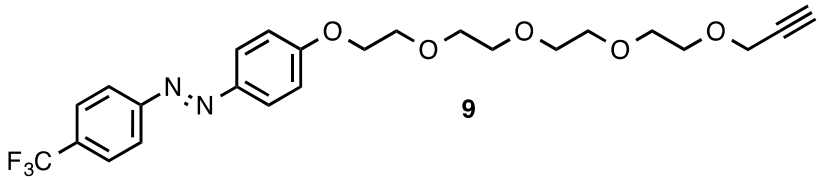


Conditions

a) i) $NaNO_2$, HCl, ii) phenol, NaOH, 0oC to rt.. b) triethyleneglycol monotosylate, K_2CO_3, LiCl, EtOH, Δ . c) NaH, propargyl bromide DMF, r.t.

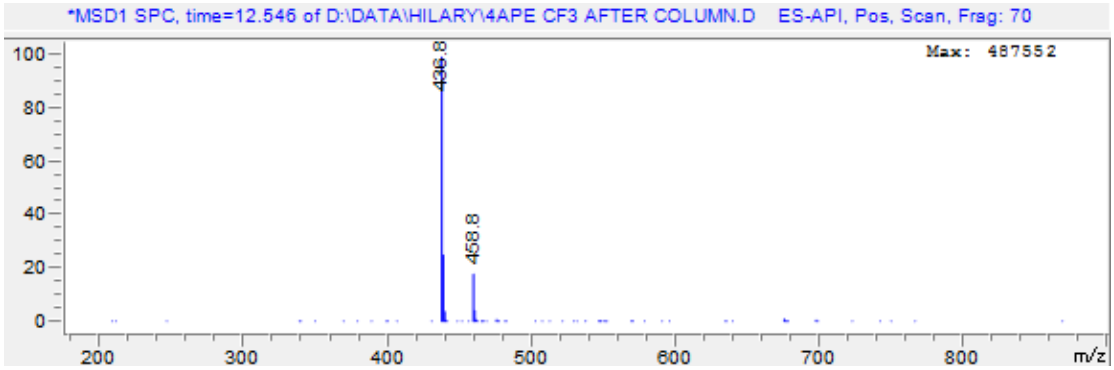
Scheme S1. Synthesis of azobenzene alkynes **9-11**.[1,3,4]

(E)-1-(4-trifluoromethylphenyl)-2-(4-(2-(2-(2-prop-2-yn-1-yloxy)ethoxy)ethoxy)ethoxy)phenyl diazene **9**

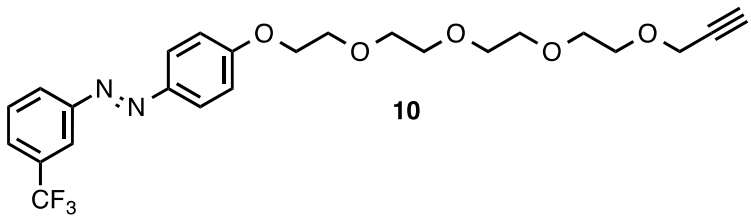


9

v_{max}/cm^{-1} 2931, 2114, 1741, 1227, 1033, 852; ^{1}H NMR (400MHz, $CDCl_3$) δ 7.96-7.93 (4H, m), 7.75 (2H, d, J=8.49Hz), 7.05 (2H, d, J=9.03Hz), 4.24 (2H, t, J=4.72Hz), 4.21 (2H, d, J=2.45Hz), 3.91 (2H, t, J=5.50Hz), 3.77-3.75 (2H, m), 3.72-3.69 (6H, m), 2.42 (1H, t, J=2.46Hz); 13C NMR (400Hz, CDCl3) δ 161.9, 154.6, 146.9, 126.3, 125.2, 122.7, 114.9, 79.7, 74.5, 70.9, 70.7, 70.5, 69.6, 69.1, 67.8, 58.4; LC-MS: m/z 436.8; Yield 76%.

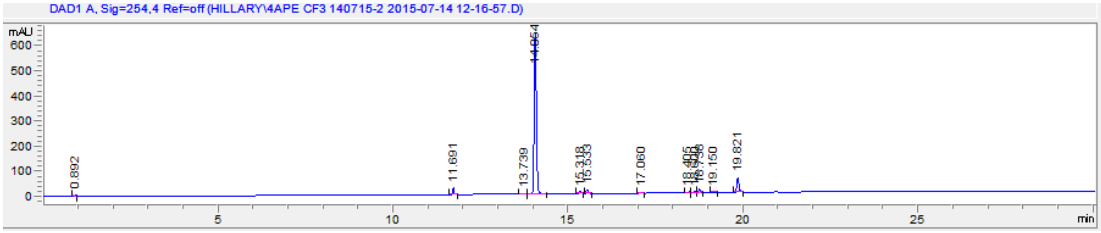


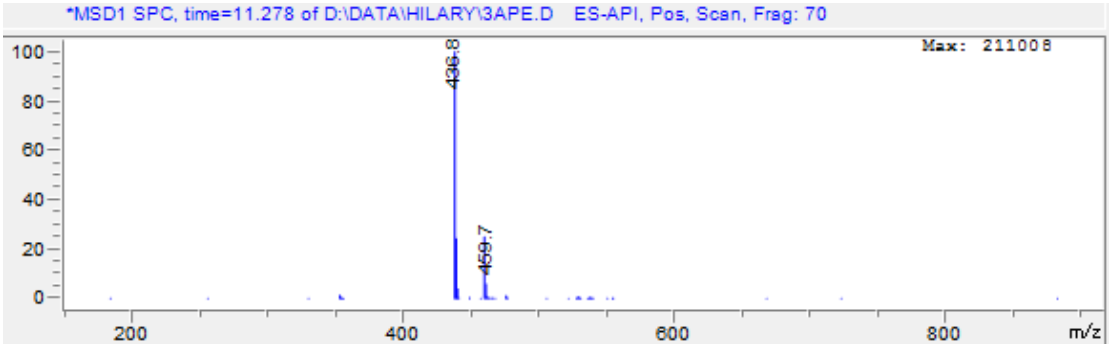
(E)-1-(3-trifluoromethylphenyl)-2-(4-(2-(2-(2-prop-2-yn-1-yloxy)ethoxy)ethoxy)ethoxy)phenyl diazene **10**



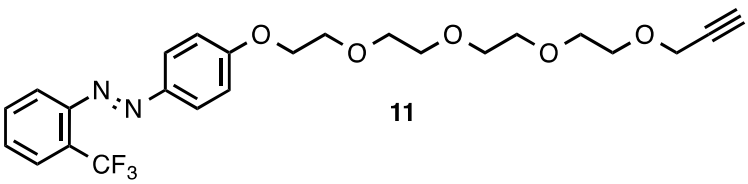
10

v_{max}/cm^{-1} 2862, 1503, 1329, 1092, 841; ^{1}H NMR (400MHz, $CDCl_3$) δ 8.14 (1H, s), 8.05 (1H, d, J=8.01Hz), 7.93 (2H, d, J=9.04Hz), 7.69-7.59 (2H, m), 7.04 (2H, d, J=9.04), 4.25-4.20 (4H, m), 3.90 (2H, t, J=4.90Hz), 3.77-3.66 (8H, m), 2.42 (1H, t, J=2.44Hz); 13C NMR (400Hz, CDCl3) δ 161.9, 152.7, 146.8, 129.6, 126.1, 125.2, 119.4, 115.1, 79.8, 74.6, 70.9, 70.7, 70.5, 69.6, 69.1, 67.9, 58.4; LC-MS: m/z 436.8; Yield 46%.



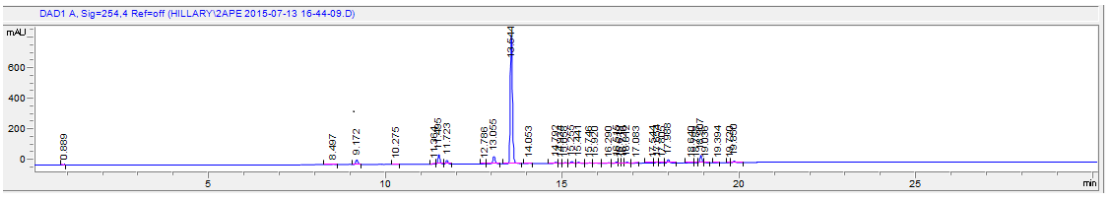


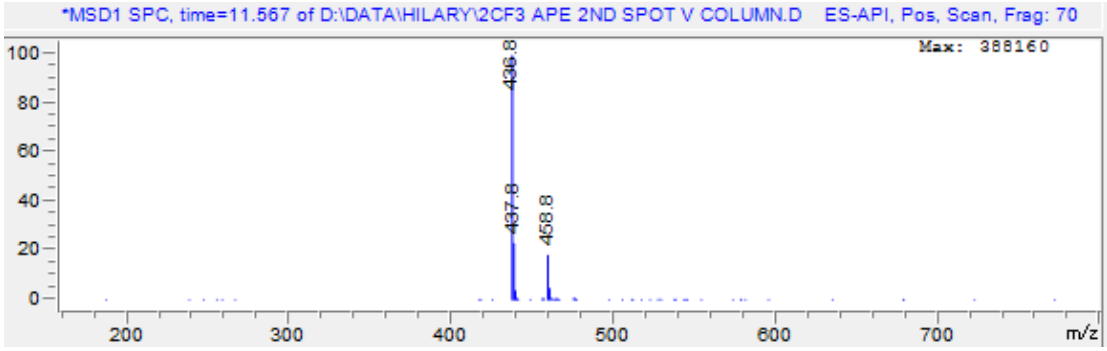
(E)-1-(2-trifluoromethylphenyl)-2-(4-(2-(2-(2-prop-2-yn-1-yloxy)ethoxy)ethoxy)ethoxy)phenyl diazene **11**



11

v_{max}/cm^{-1} 2876, 1598, 1314, 1096, 764; ^{1}H NMR (400MHz, CD_3OD) δ 7.58 (2H, d, J=9.02Hz), 7.50 (1H, t, J=7.96Hz), 7.36 (1H, t, J=7.51Hz), 7.25 (1H, t, J=7.51Hz), 7.08 (1H, d, J=7.96Hz), 6.76 (2H, d, J=9.02Hz), 3.91-3.87 (2H, m), 3.54-3.52 (2H, m), 3.38-3.35 (2H, m), 3.30-3.23 (8H, m), 2.97-2.95 (1H, m); 13C NMR (400 MHz, CD_3OD) δ 162.3, 149.6, 147.1, 145.1, 132.7, 129.8, 129.7, 127.7, 126.1, 125.1, 115.9, 114.8, 79.2, 74.5, 70.2, 69.5, 69.3, 68.7, 68.3, 67.7, 57.6; LC-MS: m/z 436.8; Yield 33%.





3.0 References

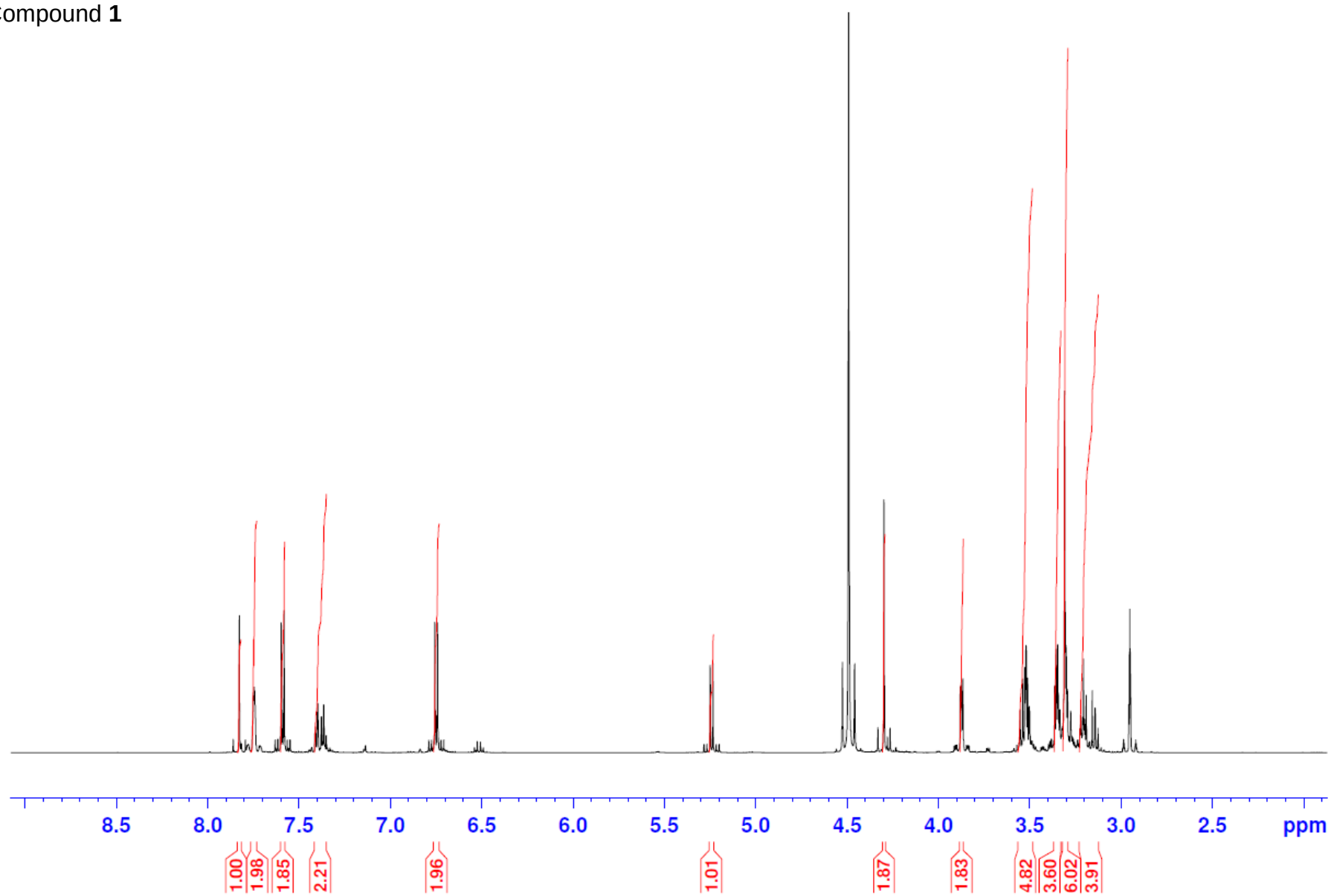
1. Tabor, R. F. T., D. D.; Han, S. S.; Young, S. A.; Seeger, Z. L. E.; Pottage, M. J.; Garvey, C. J.; Wilkinson, B. L. , Reversible pH- and Photocontrollable Carbohydrate-Based Surfactants. *Chem. Eur. J.* **2014,** *20* (43), 13881-13884

2. a) Mita, I.; Horie, K.; Hirao, K. *Macromolecules*, **1989**, *22*, 558-563; b) Ueda, M. *J. Non-cryst. Solids*, **1993**, *163*, 125-132; c) Gille, K.; Knoll, H.; Quitzsch, K. Int. J. Chem. Kinetics, 1999, *31* (5), 337-350.

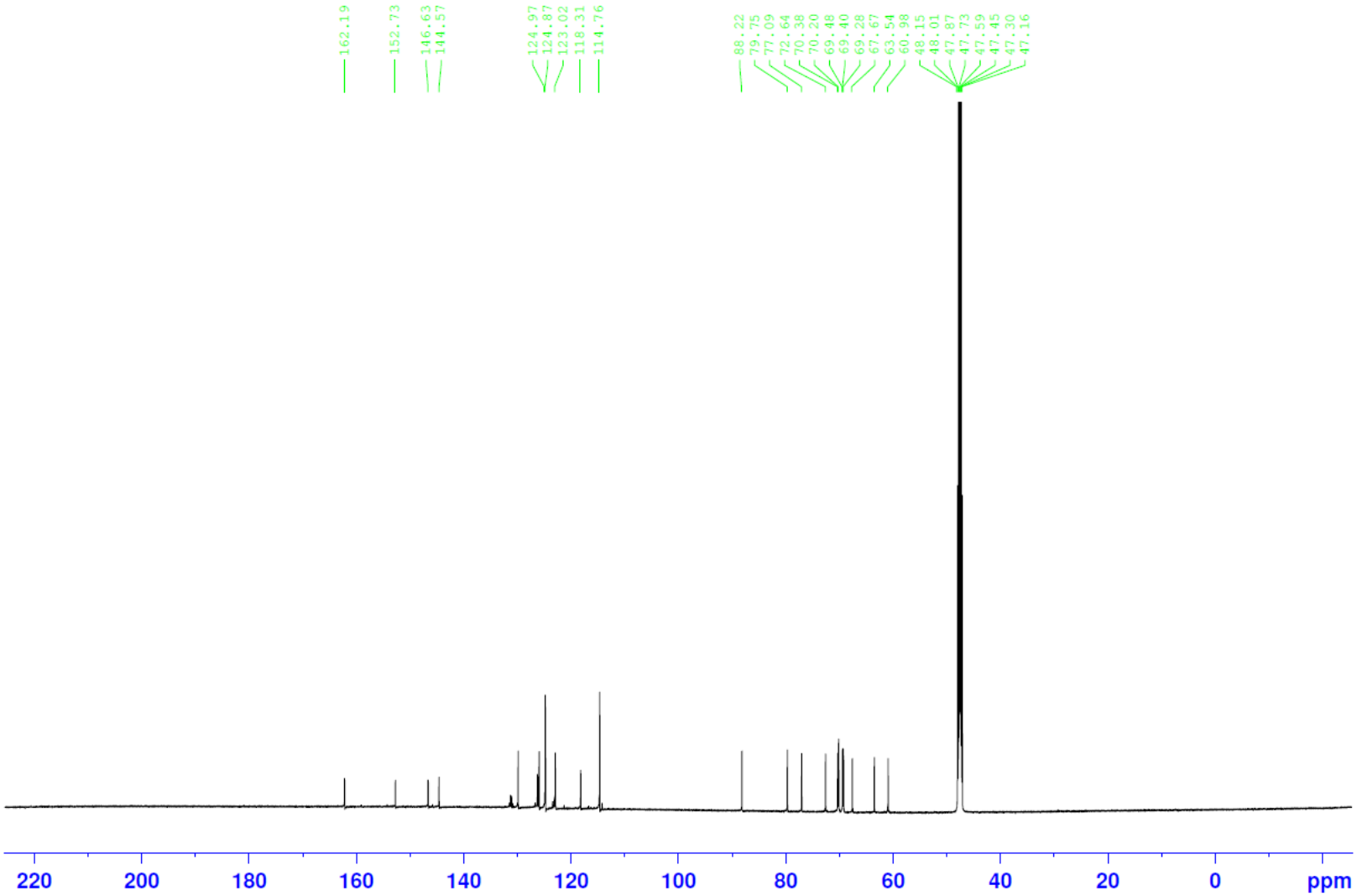
3. Billamboz, M.; Mangin, F.; Drillaud, N.; Chevrin-Villette, C.; Banaszak-Leónard, E.; Len, C. *J. Org. Chem.* **2014**, *79*, 493–500.

4. Drillaud, N.; Banaszak-Leónard, E.; Pezron, I.; Len, C. *J. Org. Chem.* **2012**, *77* (*21*), 9553-9561

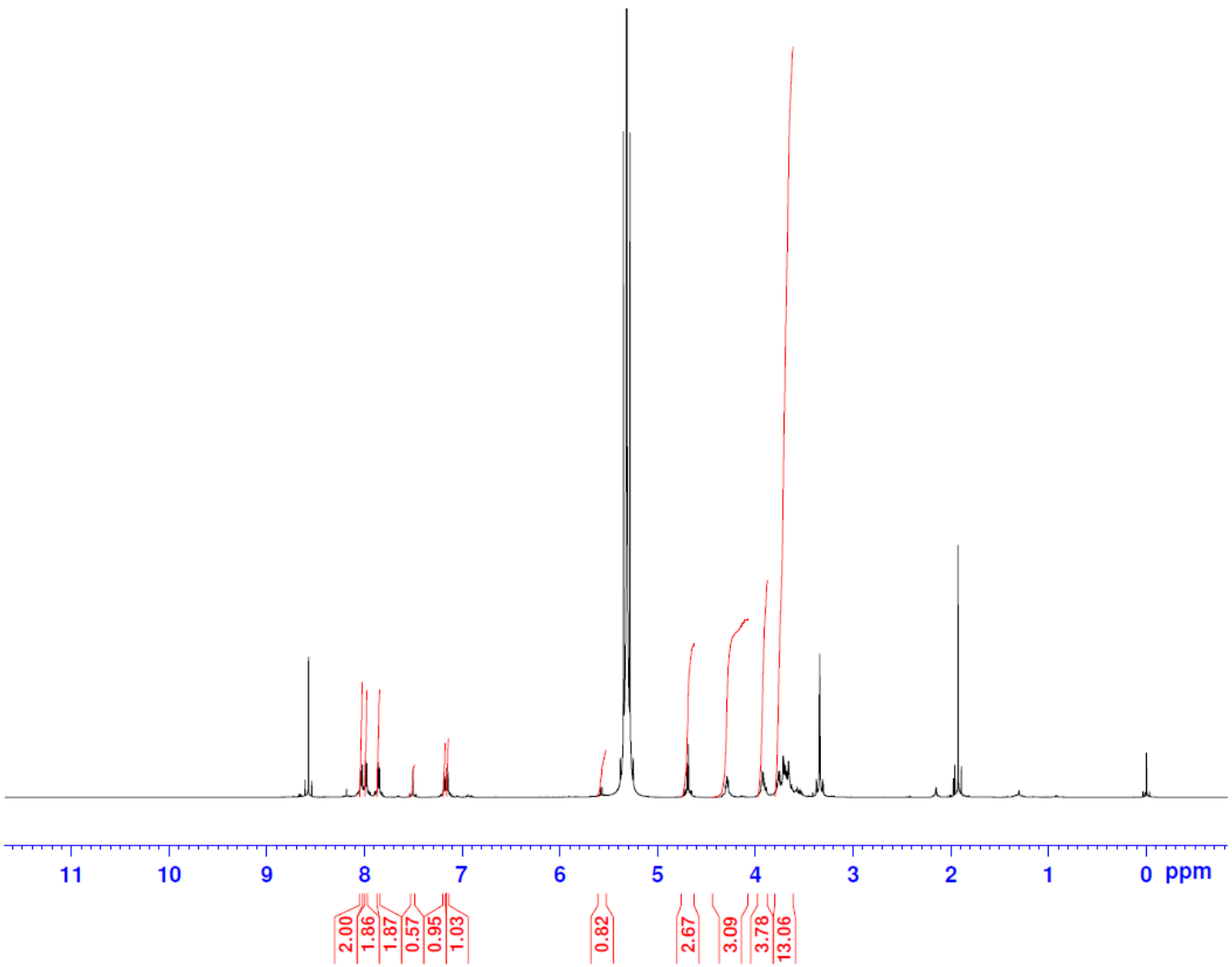
Compound **1**


8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm
1.00
1.98
1.85
2.21
1.96
1.01
1.87
1.83
4.82
3.60
6.02
3.91

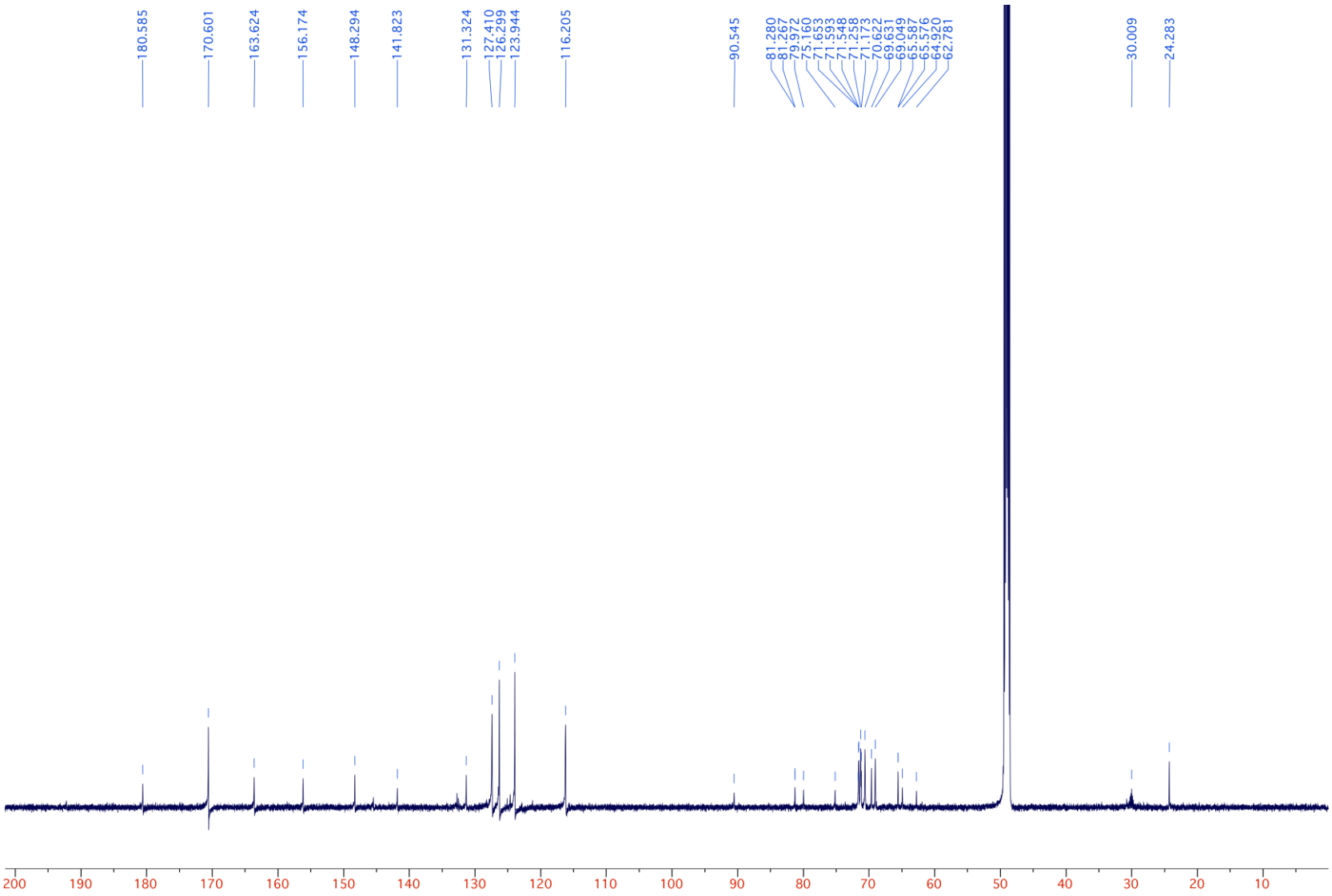
Compound **1**


162.19
152.73
146.63
144.57
124.97
124.87
123.02
118.31
114.76
88.22
79.75
77.09
72.64
70.38
70.20
69.48
69.40
69.28
67.67
63.54
60.98
48.15
48.01
47.87
47.73
47.59
47.45
47.30
47.16
220
200
180
160
140
120
100
80
60
40
20
0
ppm

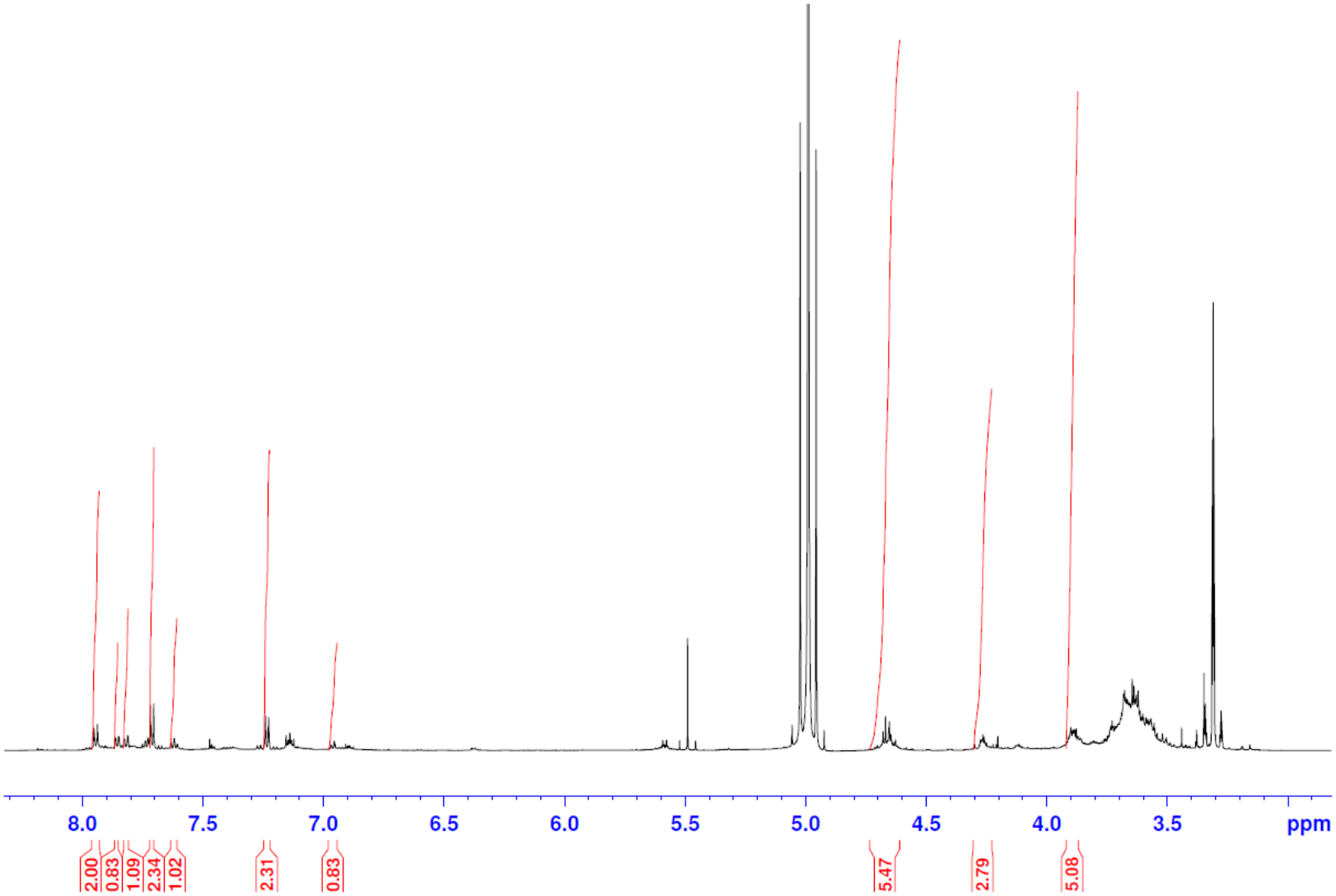
Compound **2**


11
10
9
8
7
6
5
4
3
2
1
0 ppm
2.00
1.86
1.87
0.57
0.95
1.03
0.82
2.67
3.09
3.78
13.06

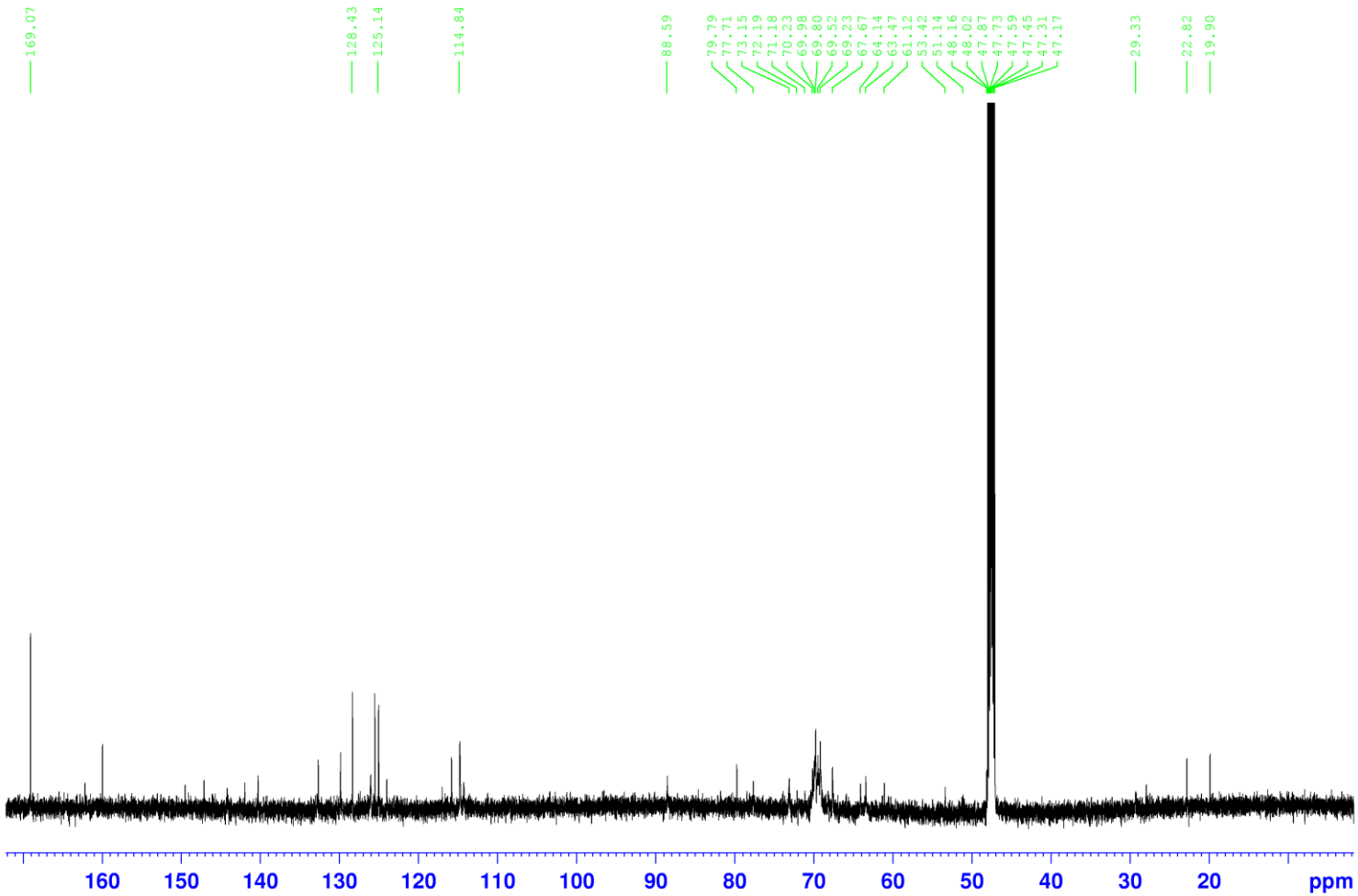
Compound **2**


180.585
170.601
163.624
156.174
148.294
141.823
131.324
127.410
126.299
123.944
116.205
90.545
81.280
81.267
79.972
75.160
71.653
71.593
71.548
71.258
71.173
70.622
69.631
69.049
65.587
65.576
64.920
62.781
30.009
24.283
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

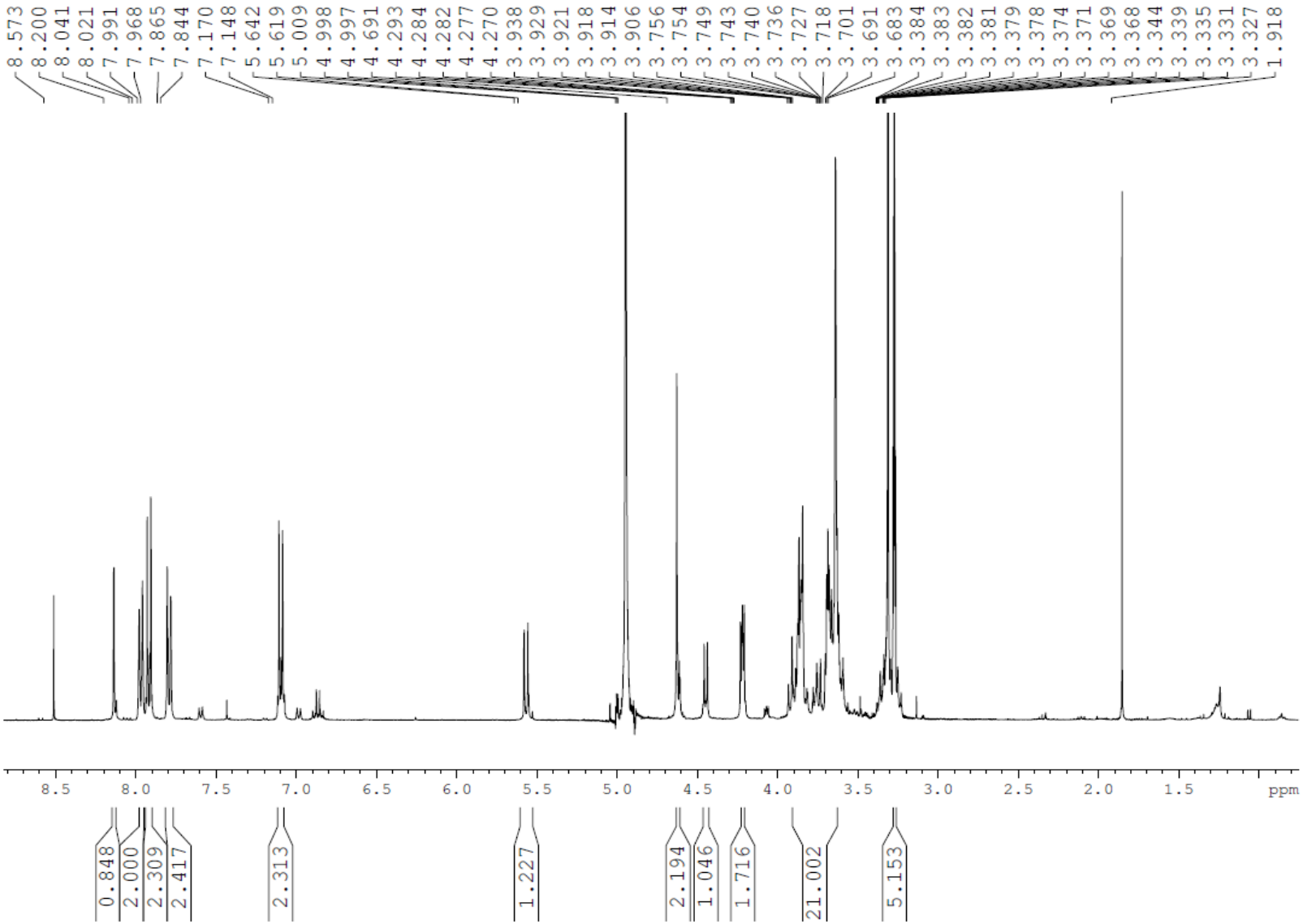
Compound **3**


8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
ppm
2.00
0.83
1.09
2.34
1.02
2.31
0.83
5.47
2.79
5.08

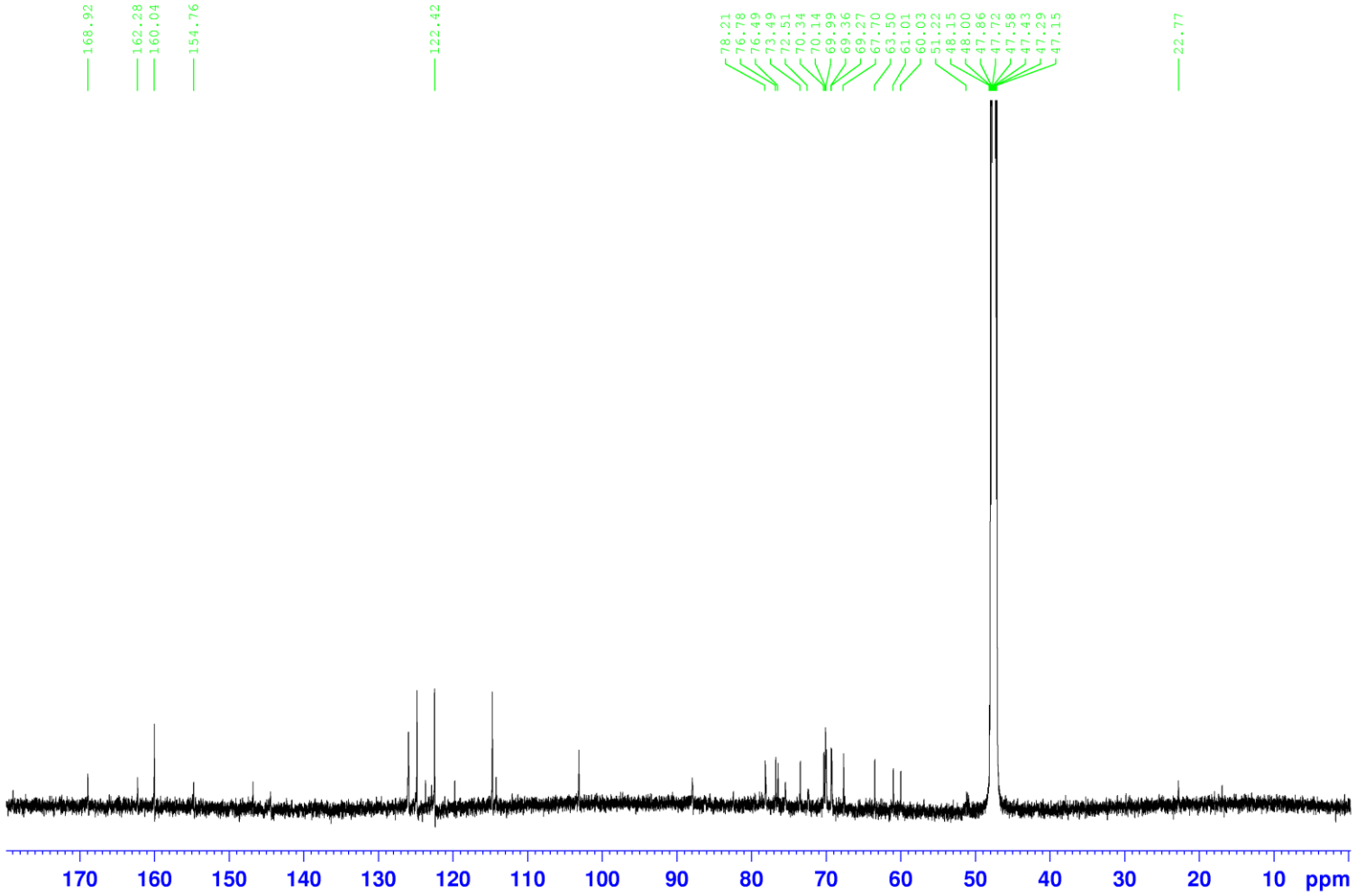
Compound **3**


169.07
128.43
125.14
114.84
88.59
79.79
77.71
73.15
72.19
71.18
70.23
69.98
69.80
69.52
69.23
67.67
64.14
63.47
61.12
53.42
51.14
48.16
48.02
47.87
47.73
47.59
47.45
47.31
47.17
29.33
22.82
19.90
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

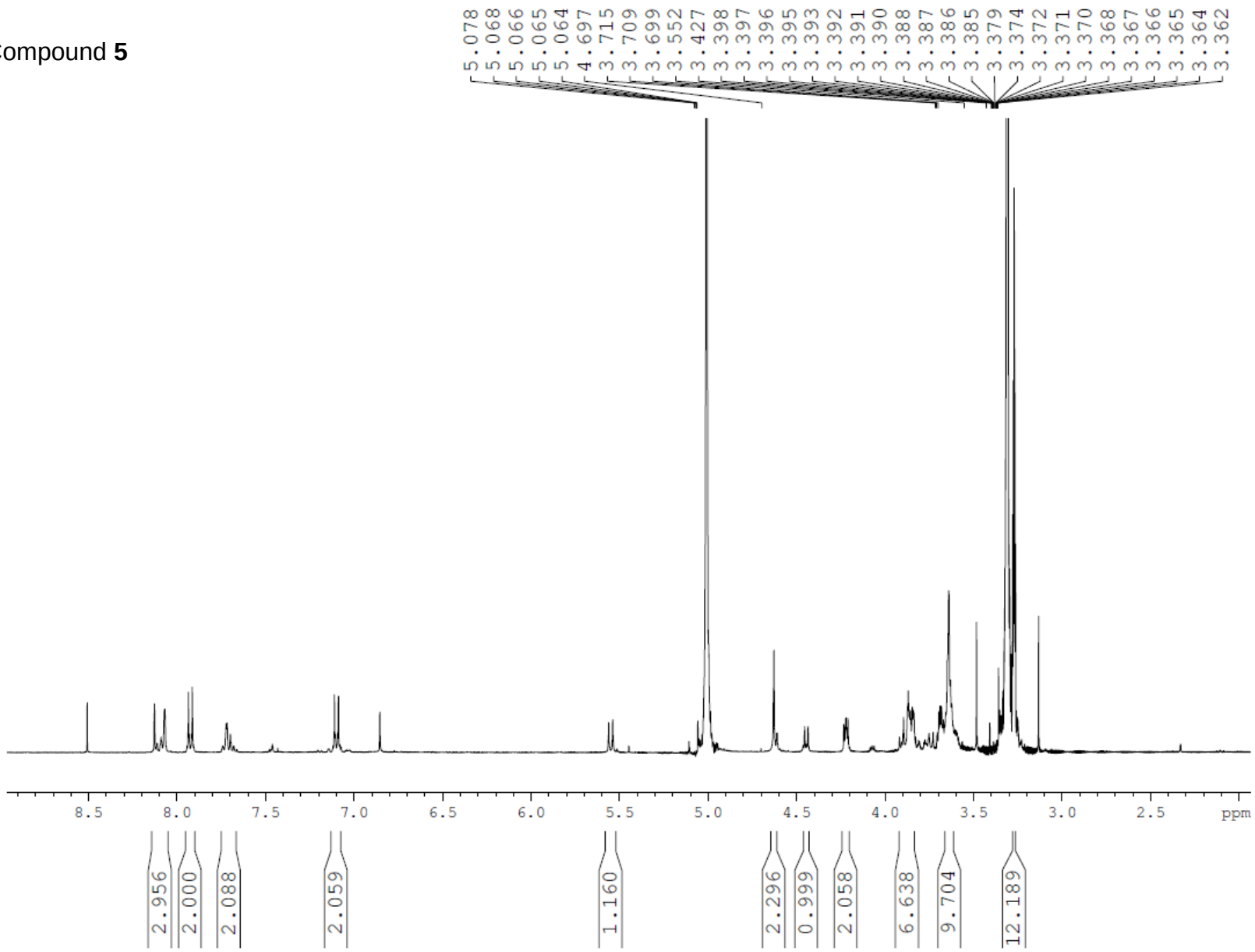
Compound **4**


8.573
8.200
8.041
8.021
7.991
7.968
7.865
7.844
7.170
7.148
5.642
5.619
5.009
4.998
4.997
4.691
4.293
4.284
4.282
4.277
4.270
3.938
3.929
3.921
3.918
3.914
3.906
3.756
3.754
3.749
3.743
3.740
3.736
3.727
3.718
3.701
3.691
3.683
3.384
3.383
3.382
3.381
3.379
3.378
3.374
3.371
3.369
3.368
3.344
3.339
3.335
3.331
3.327
1.918
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
0.848
2.000
2.309
2.417
2.313
1.227
2.194
1.046
1.716
21.002
5.153

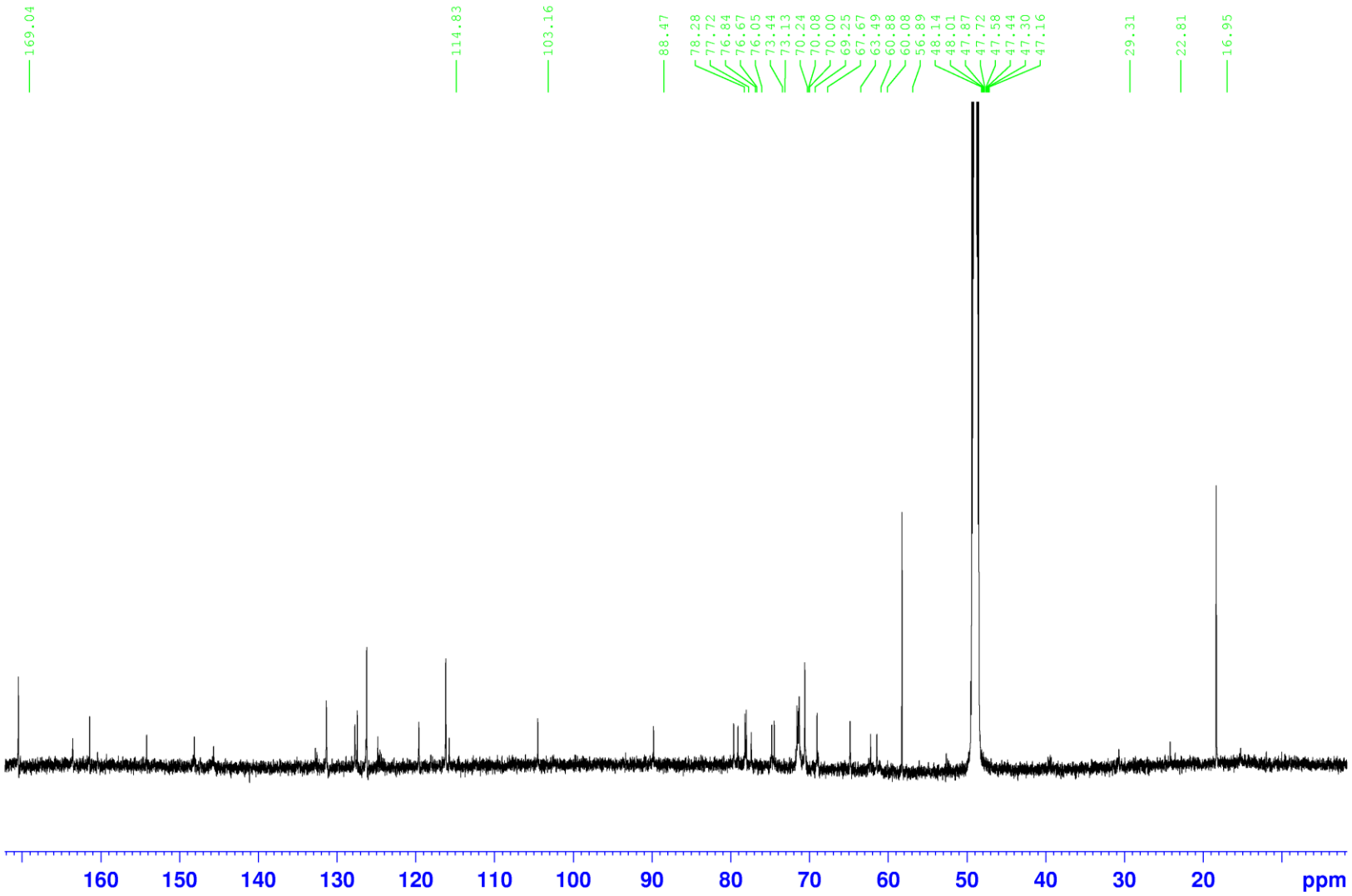
Compound **4**


168.92
162.28
160.04
154.76
122.42
78.21
76.78
76.49
73.49
72.51
70.34
70.14
69.99
69.36
69.27
67.70
63.50
61.01
60.03
51.22
48.15
48.00
47.86
47.72
47.58
47.43
47.29
47.15
22.77
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

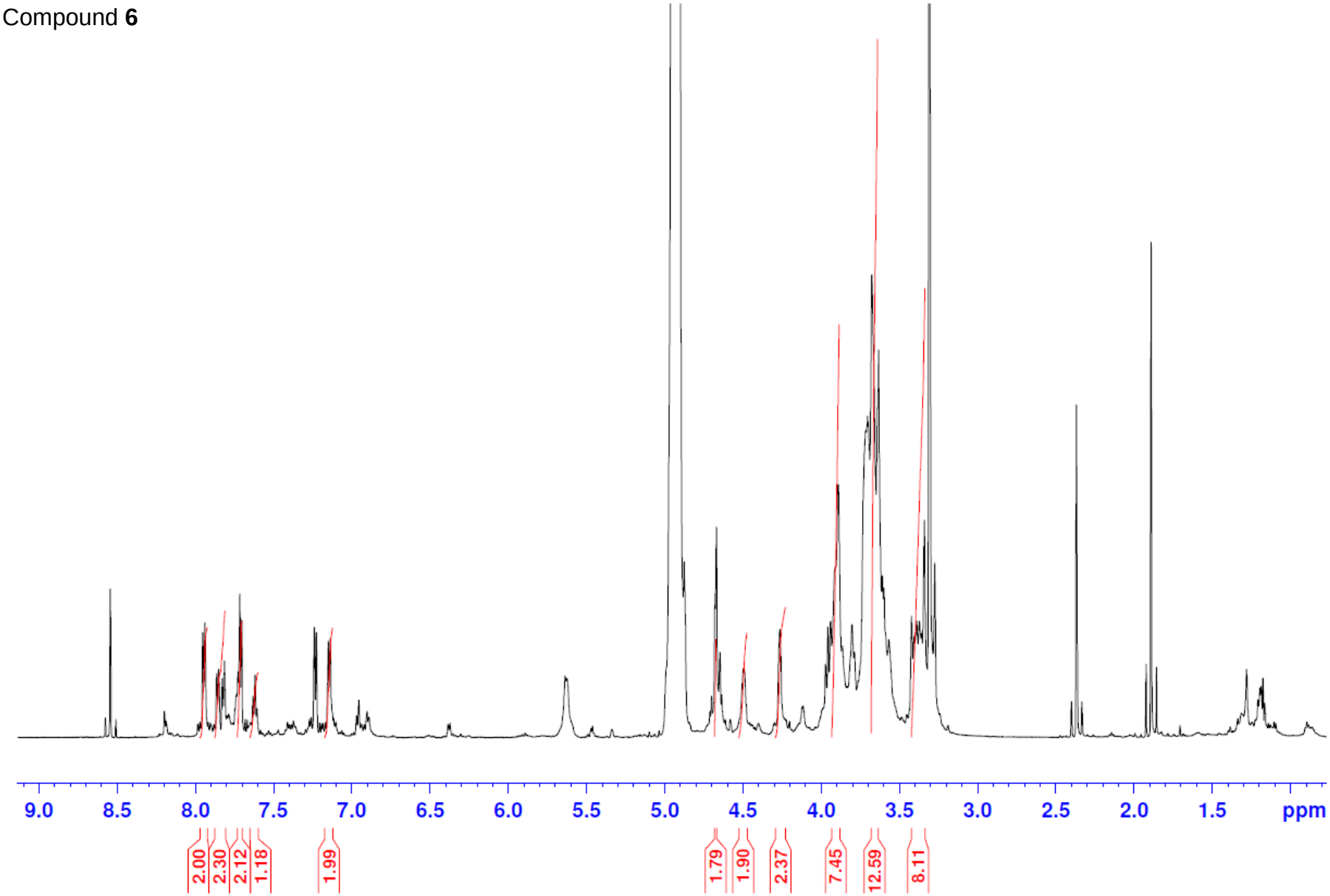
Compound **5**


5.078
5.068
5.066
5.065
5.064
4.697
3.715
3.709
3.699
3.552
3.427
3.398
3.397
3.396
3.395
3.393
3.392
3.391
3.390
3.388
3.387
3.386
3.385
3.379
3.374
3.372
3.371
3.370
3.368
3.367
3.366
3.365
3.364
3.362
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm
2.956
2.000
2.088
2.059
1.160
2.296
0.999
2.058
6.638
9.704
12.189

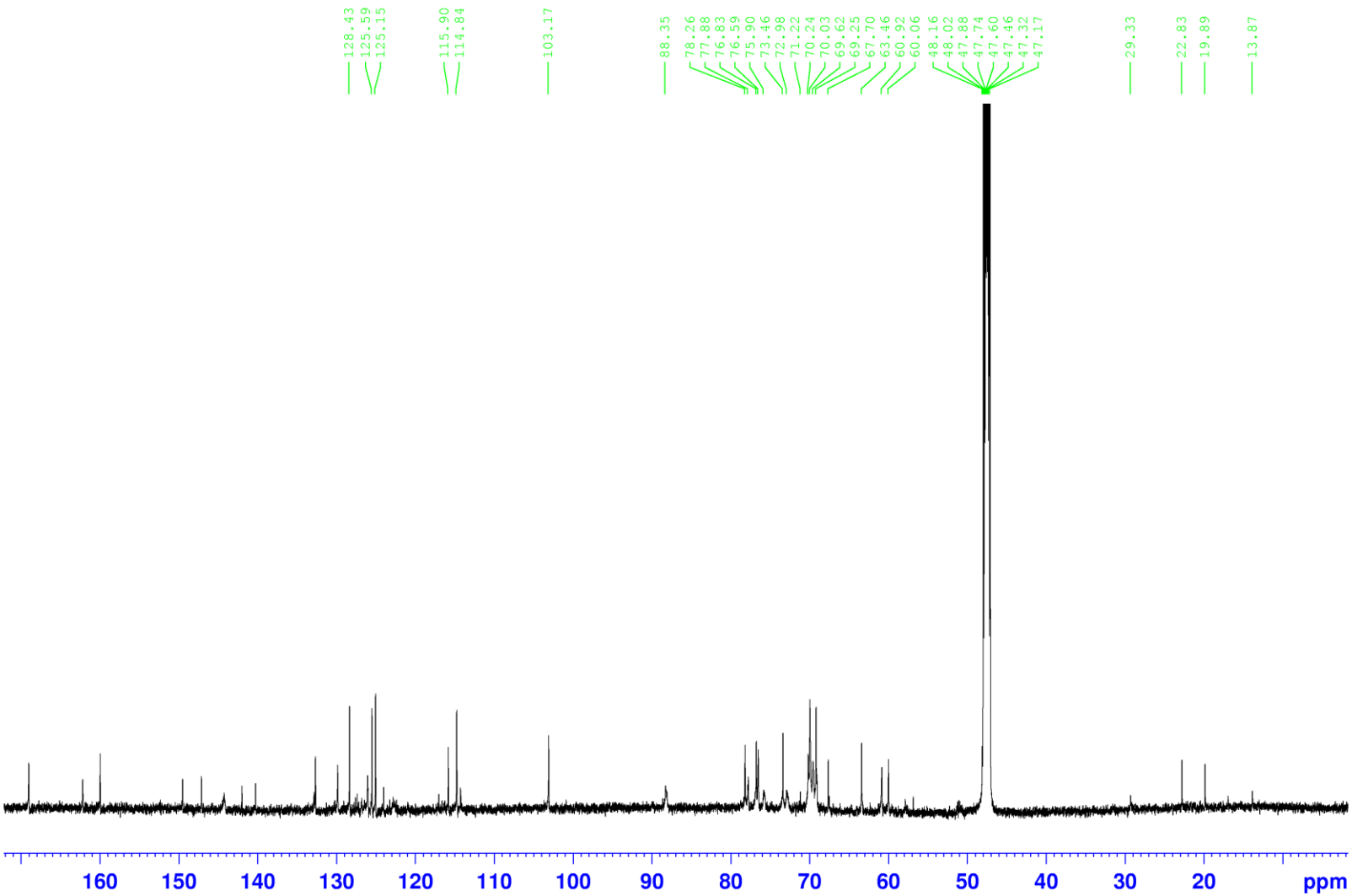
Compound **5**


169.04
114.83
103.16
88.47
78.28
77.72
76.84
76.67
76.05
73.44
73.13
70.24
70.08
70.00
69.25
67.67
63.49
60.88
60.08
56.89
48.14
48.01
47.87
47.72
47.58
47.44
47.30
47.16
29.31
22.81
16.95
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

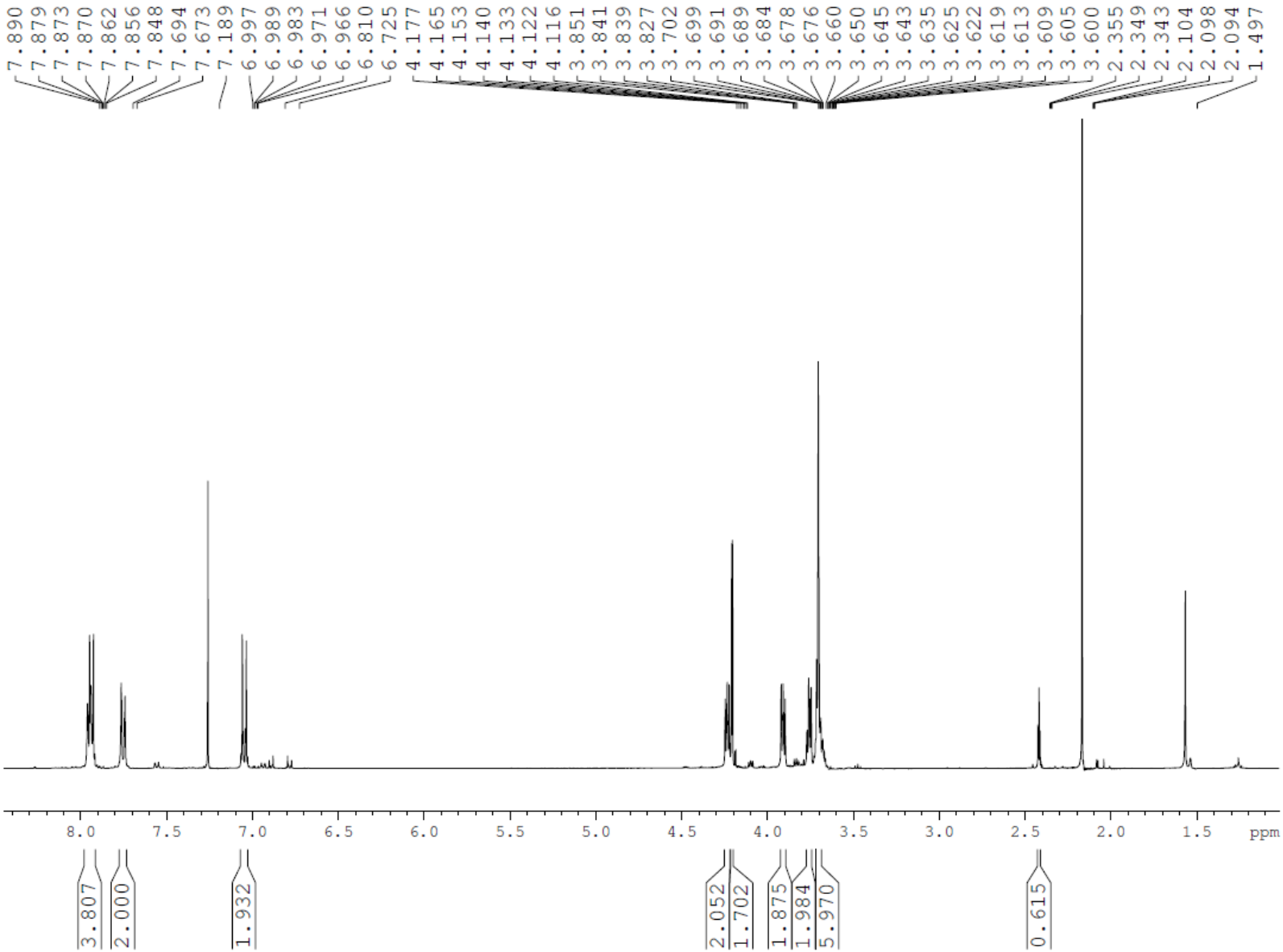
Compound **6**


9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
2.00
2.30
2.12
1.18
1.99
1.79
1.90
2.37
7.45
12.59
8.11

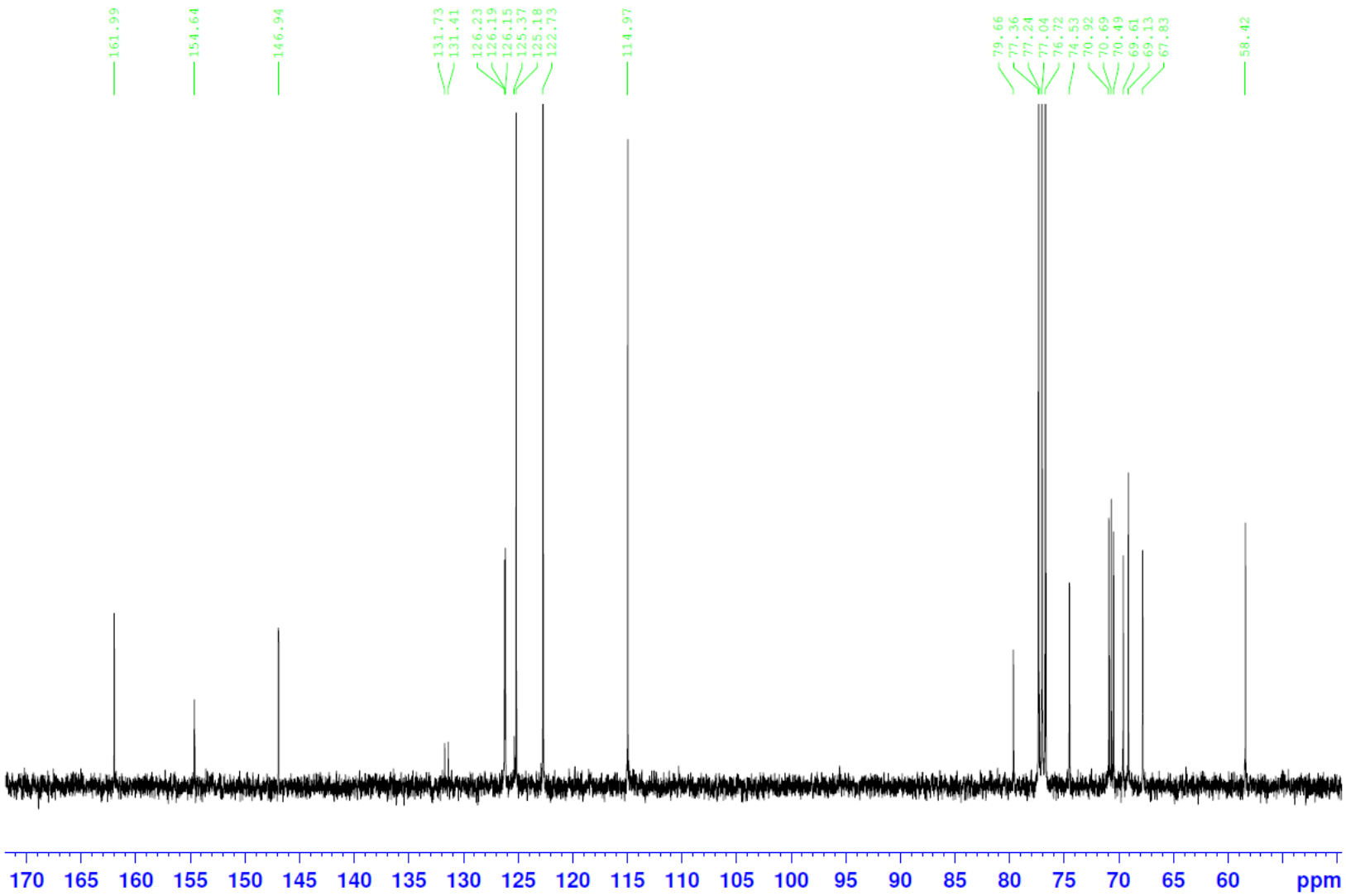
Compound **6**


128.43
125.59
125.15
115.90
114.84
103.17
88.35
78.26
77.88
76.83
76.59
75.90
73.46
72.98
71.22
70.24
70.03
69.62
69.25
67.70
63.46
60.92
60.06
48.16
48.02
47.88
47.74
47.60
47.46
47.32
47.17
29.33
22.83
19.89
13.87
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

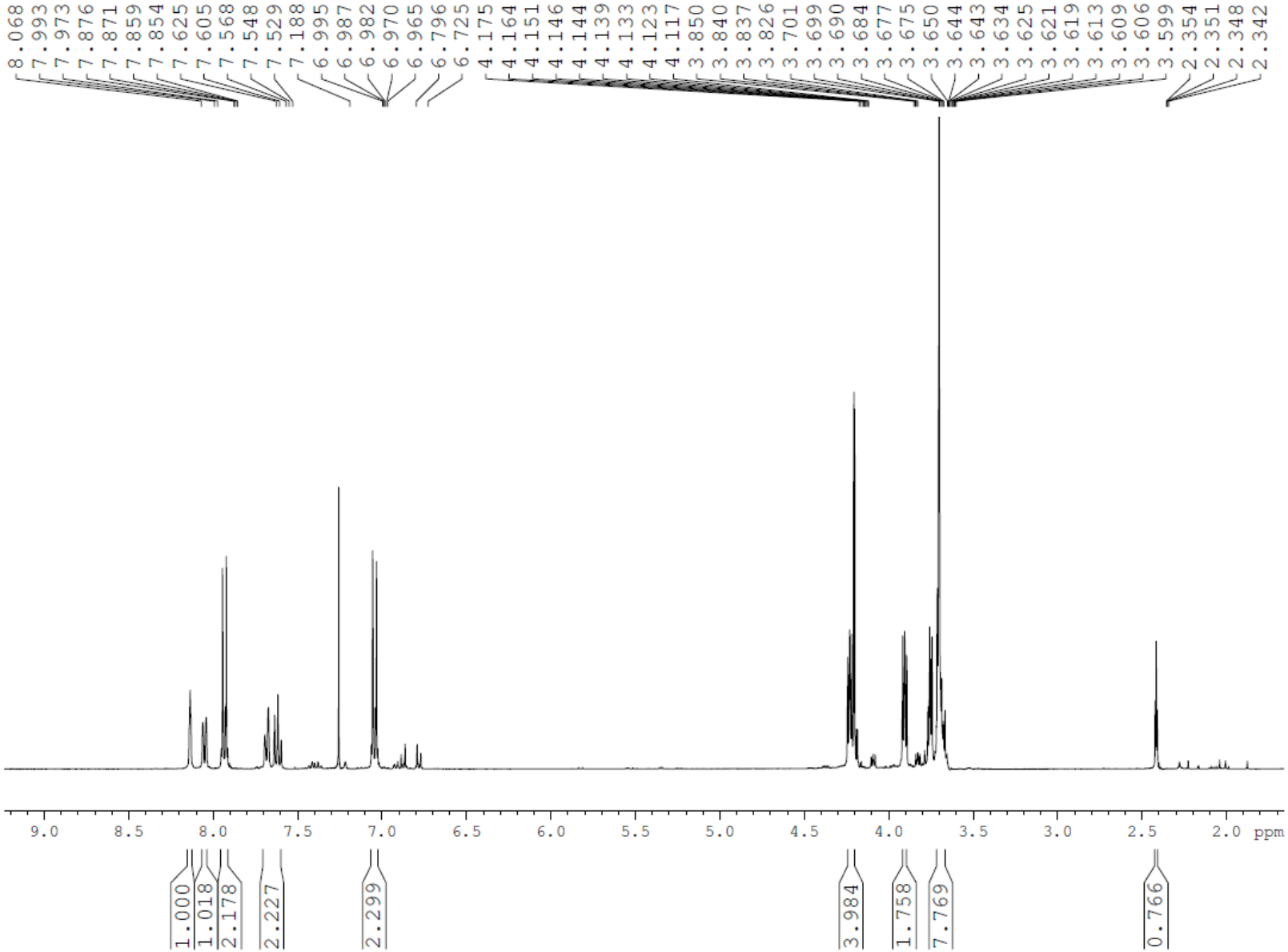
Compound **9**


7.890
7.879
7.873
7.870
7.862
7.856
7.848
7.694
7.673
7.189
6.997
6.989
6.983
6.971
6.966
6.810
6.725
4.177
4.165
4.153
4.140
4.133
4.122
4.116
3.851
3.841
3.839
3.827
3.702
3.699
3.691
3.689
3.684
3.678
3.676
3.660
3.650
3.645
3.643
3.635
3.625
3.622
3.619
3.613
3.609
3.605
3.600
2.355
2.349
2.343
2.104
2.098
2.094
1.497
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
3.807
2.000
1.932
2.052
1.702
1.875
1.984
5.970
0.615

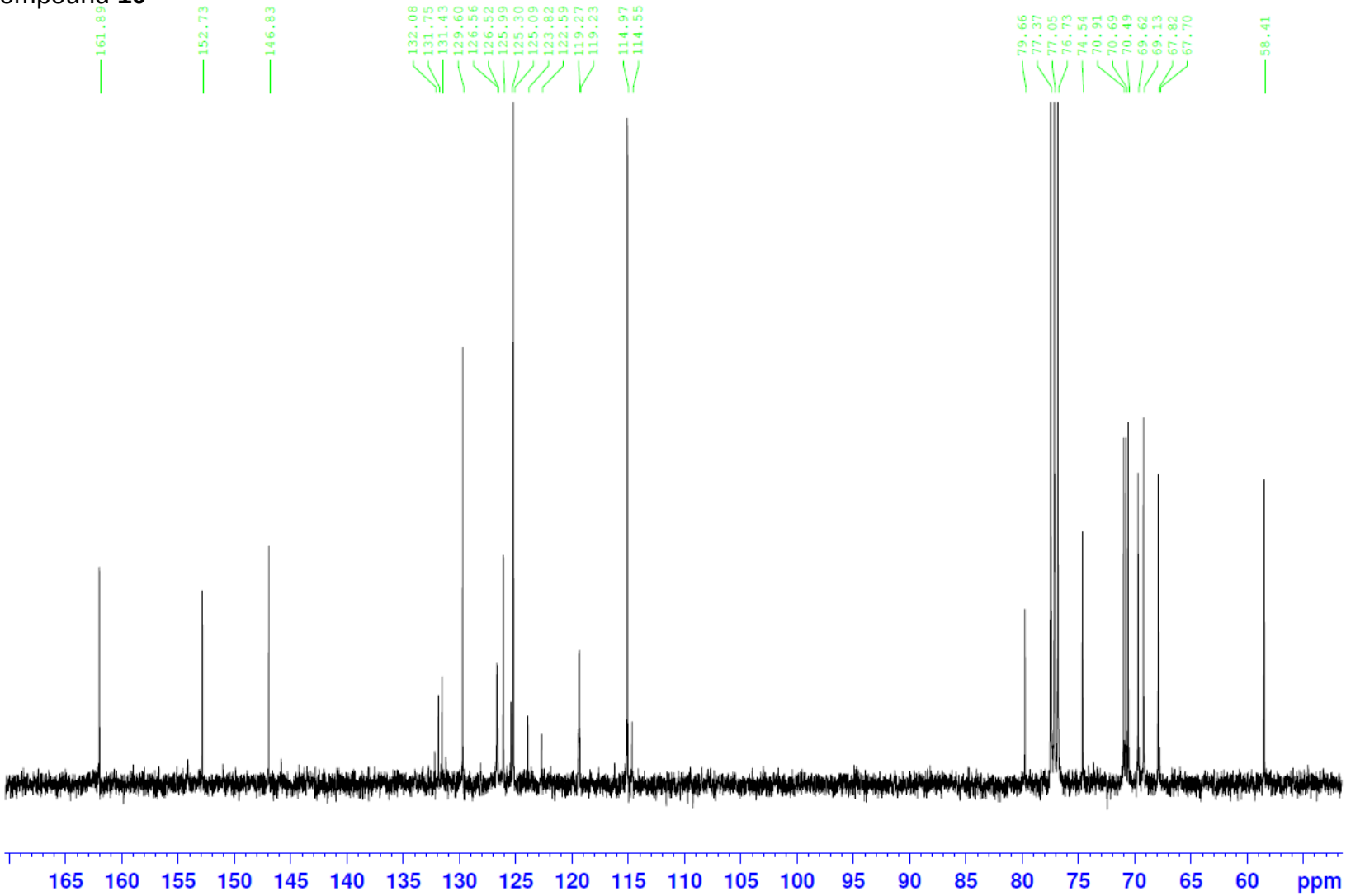
Compound **9**


161.99
154.64
146.94
131.73
131.41
126.23
126.19
126.15
125.37
125.18
122.73
114.97
79.66
77.36
77.24
77.04
76.72
74.53
70.92
70.69
70.49
69.61
69.13
67.83
58.42
170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 ppm

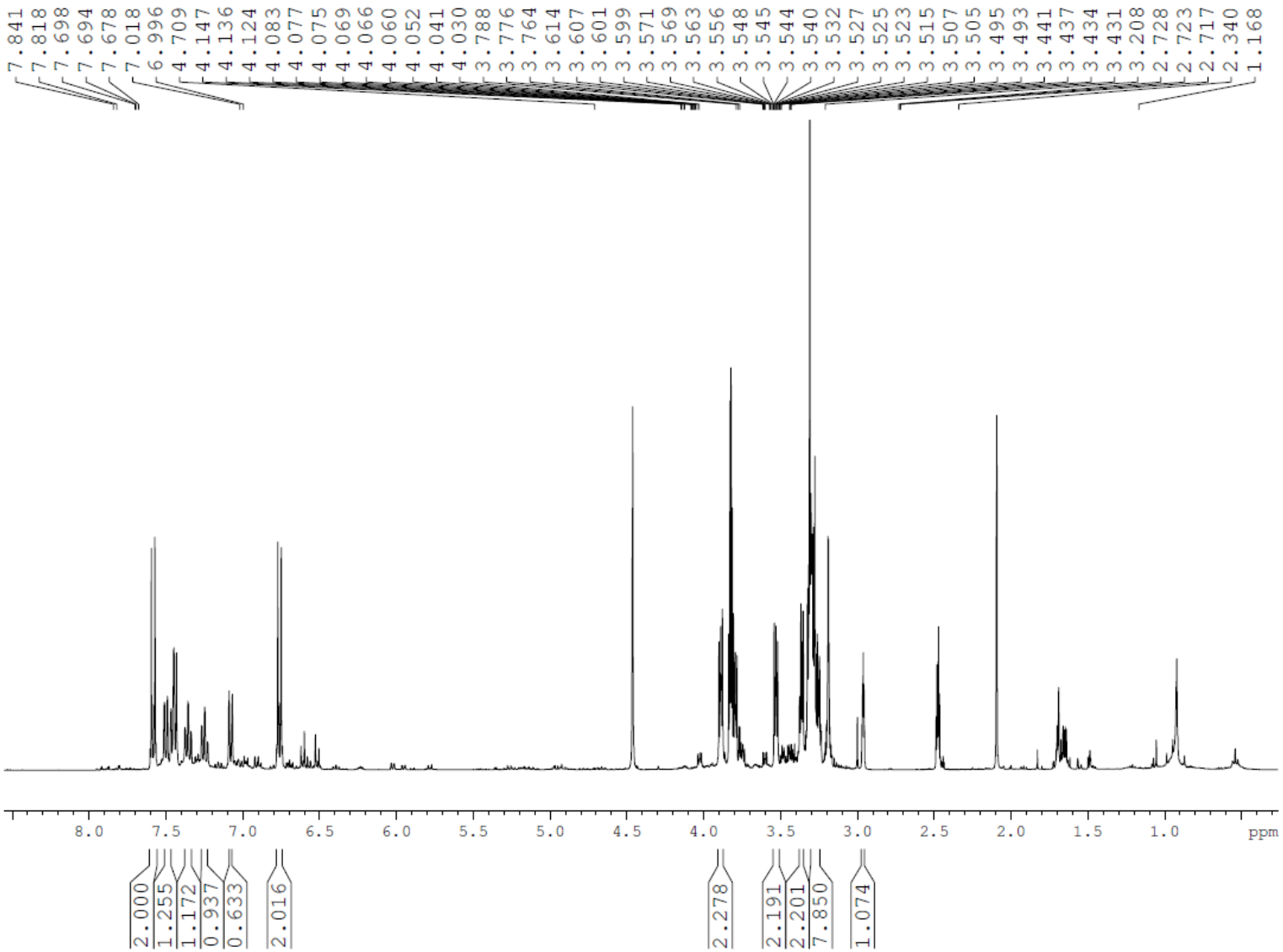
Compound **10**


8.068
7.993
7.973
7.876
7.871
7.859
7.854
7.625
7.605
7.568
7.548
7.529
7.188
6.995
6.987
6.982
6.970
6.965
6.796
6.725
4.175
4.164
4.151
4.146
4.144
4.139
4.133
4.123
4.117
3.850
3.840
3.837
3.826
3.701
3.699
3.690
3.684
3.677
3.675
3.650
3.644
3.643
3.634
3.625
3.621
3.619
3.613
3.609
3.606
3.599
2.354
2.351
2.348
2.342
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
1.000
1.018
2.178
2.227
2.299
3.984
1.758
7.769
0.766

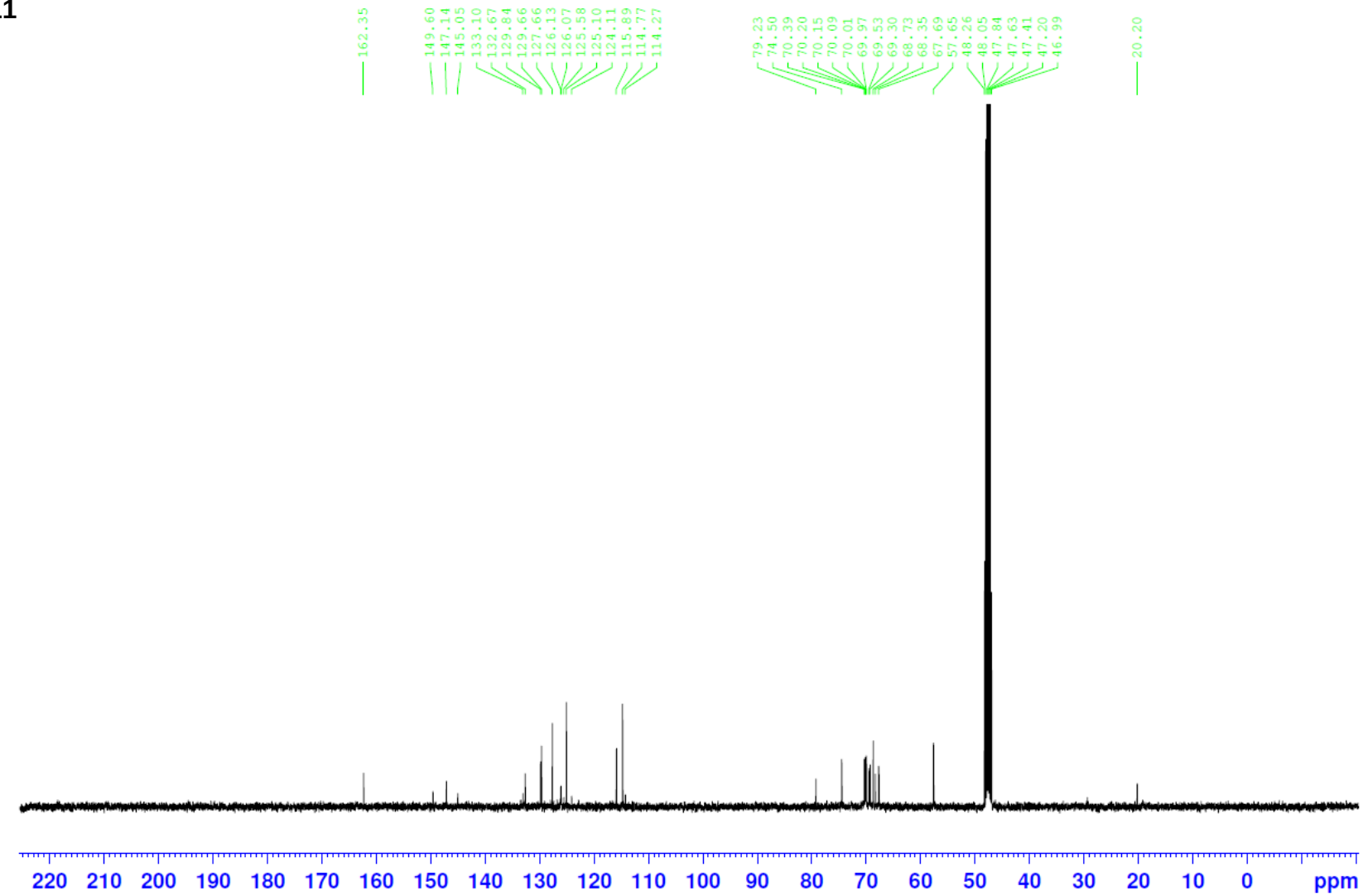
Compound **10**


161.89
152.73
146.83
132.08
131.75
131.43
129.60
126.56
126.52
125.99
125.30
125.09
123.82
122.59
119.27
119.23
114.97
114.55
79.66
77.37
77.05
76.73
74.54
70.91
70.69
70.49
69.62
69.13
67.82
67.70
58.41
165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 ppm

Compound **11**


7.841
7.818
7.698
7.694
7.678
7.018
6.996
4.709
4.147
4.136
4.124
4.083
4.077
4.075
4.069
4.066
4.060
4.052
4.041
4.030
3.788
3.776
3.764
3.614
3.607
3.601
3.599
3.571
3.569
3.563
3.556
3.548
3.545
3.544
3.540
3.532
3.527
3.525
3.523
3.515
3.507
3.505
3.495
3.493
3.441
3.437
3.434
3.431
3.208
2.728
2.723
2.717
2.340
1.168
8.0
7.5
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
ppm
2.000
1.255
1.172
0.937
0.633
2.016
2.278
2.191
2.201
7.850
1.074

Compound **11**


162.35
149.60
147.14
145.05
133.10
132.67
129.84
129.66
127.66
126.13
126.07
125.58
125.10
124.11
115.89
114.77
114.27
79.23
74.50
70.39
70.20
70.15
70.09
70.01
69.97
69.53
69.30
68.73
68.35
67.69
57.65
48.26
48.05
47.84
47.63
47.41
47.20
46.99
20.20
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm