

## Supplementary Information

for

### **Stimuli-responsive Prodrug of Non-Steroidal Anti-inflammatory Drug Diclofenac: Self-immolative Drug Release with Turn-On Near-Infrared Fluorescence**

**Abu Sufian,<sup>a</sup> Debojit Bhattacharjee,<sup>ab</sup> Pallavi Barman,<sup>a</sup> Abhay Srivastava,<sup>a</sup> Rajkumar P.  
Thummer,<sup>c</sup> and Krishna P. Bhabak<sup>\*abd</sup>**

<sup>a</sup>Department of Chemistry, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, India.

<sup>b</sup>Centre for the Environment, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, India.

<sup>c</sup>Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, India.

<sup>d</sup>Jyoti and Bhupat Mehta School of Health Sciences and Technology, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, India.

Email: kbhabak@iitg.ac.in

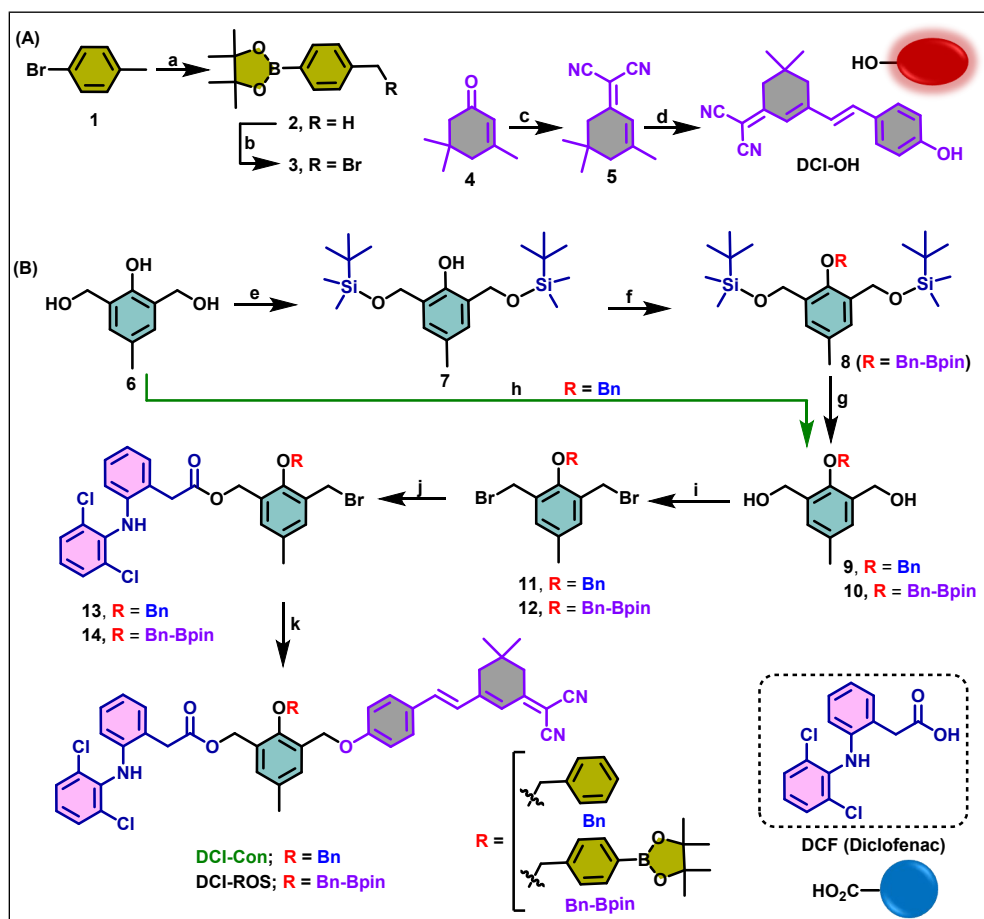
#### **Author Contributions**

K.P.B. conceptualized, designed and supervised the project. A.S. developed methodology and carried out the synthesis, purification, characterization of the intermediates and final prodrugs. A. S. carried out all the spectroscopic and HPLC studies in the aqueous medium. D.B. and P.B. carried out the cellular experiments. D.B. carried out western blot experiments fluorescent microscopy experiments and was supervised by K.P.B. and R.P.T. A. Srivastava helped in synthesizing some intermediates. Original draft of the manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

## Content

Sl No.	Contents	Page No.
1	Experimental section with synthetic details of the prodrugs	S3-S14
2	UV-Visible and fluorescence spectroscopic spectra of <b>DCI-Con</b>	S15
3	Determination of LOD of the prodrug <b>DCI-ROS</b>	S15-S16
4	Analysis of purity of prodrugs by HPLC	S16
5	ESI-MS spectrum of the intermediate <b>15</b> and <b>16</b>	S17
6	Reaction kinetics of prodrugs using HPLC method	S18-S19
7	Cellular viability of the <b>DCI-ROS</b> and <b>DCF</b>	S20
8	Fluorescence microscopic images of <b>DCI-ROS</b> and <b>DCI-Con</b>	S20-S21
9	Raw images of western blot analysis data	S21
10	NMR spectroscopic and ESI-MS spectrometric data	S22-S51
11	References	S52

## Experimental Section



**Scheme S1.** Synthetic schemes to (A) boronate ester precursor **3** and NIR fluorophore **DCI-OH** and (B) prodrugs **DCI-Con** and **DCI-ROS**. Reagents and conditions: (a) 2-Isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, n-BuLi, dry THF, -78 °C, 2 h; (b) NBS, AIBN, dry ACN, reflux, 3 h; (c) malononitrile, piperidine, glacial AcOH, EtOH, reflux, 12 h; (d) 4-hydroxy benzaldehyde, piperidine, dry ACN, reflux, 6 h; (e) TBDMSCl, imidazole, dry DMF, 0 °C – RT, 2 h; (f) **3**, K<sub>2</sub>CO<sub>3</sub>, dry DMF, 0 °C – RT, 12 h; (g) *p*-TsOH, MeOH, RT, 12 h; (h) Benzyl bromide, K<sub>2</sub>CO<sub>3</sub>, dry DMF, 0 °C – RT, 12 h; (i) PBr<sub>3</sub>, dry DCM, RT, 5 h; (j) **DCF**, DIPEA, dry ACN, 0 °C – RT, 12 h; (k) **DCI-OH**, K<sub>2</sub>CO<sub>3</sub>, acetone, 55 °C, 4 h.

**Materials and methods.** All the reagents were purchased either from Sigma Aldrich or from reputed local suppliers and used without further purification unless otherwise stated. Thin layer chromatographic (TLC) analyses were carried out on pre-coated silica gel on aluminum sheets

and the compounds were visualized by irradiation with UV or fluorescent light. Organic solvents used for chromatographic separations were distilled before use. Melting point of the synthesized compounds was recorded in a Büchi B540 melting point apparatus and the values are uncorrected. The NMR spectra ( $^1\text{H}$  and  $^{13}\text{C}$ ) were recorded on Bruker (400, 500 or 600 MHz) NMR spectrometers and the chemical shifts are cited with respect to TMS ( $\text{Me}_4\text{Si}$ ) as an internal standard. UV-Vis spectroscopic analyses were performed on a Lambda 45 UV-Vis spectrophotometer and fluorescence emission spectra were recorded on a Fluoromax-4 spectrophotometer (FluoroMax-4 - Horiba). Mass spectra were obtained using an Agilent 6520 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS spectrometer. The NMR spectral data of all the intermediates and products and ESI-MS spectra of the final prodrugs are included in the supplementary information section (Figures S13-S42, ESI). The final prodrugs (**DCI-ROS** and **DCI-Con**) were analyzed by reverse-phase HPLC method and they were found to have more than 95% purity (Figures S3-S4, ESI).

### Synthesis of compound **2**<sup>1</sup>

The compound **2** was synthesized following the reported procedure with a minor modification.<sup>1</sup> To a stirred solution of 4-bromotoluene (3.00 g, 17.54 mmol) in dry THF (80 mL) was added *n*-butyllithium solution (2.0 M in cyclohexane, 10.52 mL, 21.04 mmol) dropwise at  $-78\text{ }^\circ\text{C}$  under inert condition. After stirring for 30 min at the same condition, 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (4.23 g, 4.65 mL, 22.77 mmol) was added dropwise and the reaction was stirred at  $-78\text{ }^\circ\text{C}$  for 2 h. Progress of the reaction was monitored by TLC analysis. Upon completion, the reaction was quenched with saturated  $\text{NH}_4\text{Cl}$  solution (20 mL), water (10 mL) and the solution was allowed to attain room temperature. The biphasic mixture was extracted with petroleum ether ( $3\times 50\text{ mL}$ ). The combined organic layer was washed with brine solution ( $3\times 20\text{ mL}$ ), dried over anhydrous sodium sulfate and concentrated under reduced pressure to obtain the crude compound. The crude product was purified by flash chromatography using 100% petroleum ether as a mobile phase to afford the pure product **2** as white solid.  $R_f = 0.8$  (100% petroleum ether); Yield: 3.6 g (94%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 600 MHz):  $\delta$  (ppm) = 7.70 (d,  $J = 7.7\text{ Hz}$ , 2H), 7.18 (d,  $J = 7.6\text{ Hz}$ , 2H), 2.36 (s, 3H), 1.34 (s, 12H);  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 141.5, 134.9, 128.7, 83.8, 25.0, 21.9.

### Synthesis of compound **3**<sup>2</sup>

The compound **3** was synthesized following the reported procedure with a minor modification.<sup>2</sup> A mixture of compound **2** (2.20 g, 10.08 mmol), NBS (2.15 g, 12.10 mmol) and AIBN (0.16 g, 1.00 mmol) in dry acetonitrile (110 mL) was refluxed under inert condition for 3 h. The progress of the reaction was monitored by TLC analysis. Upon completion, the solvent was evaporated under reduced pressure. The reaction mixture was diluted with water (30 mL) and the crude product was extracted with ethyl acetate (3×30 mL). The combined organic layer was washed with brine solution (3×20 mL) and dried over anhydrous sodium sulfate. The organic layer was concentrated under reduced pressure to afford the crude product which was further purified by flash chromatography using ethyl acetate and petroleum ether as eluents to afford compound **3** as white solid.  $R_f$  = 0.6 (5% ethyl acetate in petroleum ether); Yield: 2.60 g (88%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) = 7.78 (d,  $J$  = 8.0 Hz, 2H), 7.39 (d,  $J$  = 8.0 Hz, 2H), 4.49 (s, 2H), 1.34 (s, 12H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) = 140.8, 135.4, 128.5, 84.1, 33.5, 25.0.

### Synthesis of compound **5**<sup>3</sup>

The compound **5** was synthesized following the reported procedure with a minor modification.<sup>3</sup> To a stirred solution of isophorone **4** (3.00 g, 21.69 mmol) and malononitrile (2.16 g, 32.55 mmol) in distilled ethanol (30 mL) was added piperidine (0.42 mL, 4.35 mmol) under inert atmosphere. Subsequently glacial acetic acid (75  $\mu$ L) was added into the reaction mixture and refluxed for 12 h. The progress of the reaction was monitored by TLC analysis. Upon completion of the reaction, the reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude product was purified by 60-120 mesh silica gel column chromatography using 30% DCM in petroleum ether as the eluent to afford the pure product **5** as white solid.  $R_f$  = 0.5 (50% DCM in petroleum ether), Yield: (2.30 g, 62%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) = 6.62 (s, 1H), 2.51 (s, 2H), 2.17 (s, 2H), 2.03 (s, 3H), 1.01 (s, 6H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) = 170.5, 159.9, 120.7, 113.3, 112.5, 78.3, 45.8, 42.7, 32.5, 27.9, 25.4.

### Synthesis of DCI-OH<sup>3</sup>

The fluorophore **DCI-OH** was synthesized following the reported procedure with a minor modification.<sup>3</sup> To a stirred solution of 4-hydroxybenzaldehyde (0.53g, 4.35 mmol) and

compound **5** (0.90 g, 4.83 mmol) in dry acetonitrile (20 mL) was added piperidine (100  $\mu$ L, 0.96 mmol) under inert atmosphere and the mixture was refluxed for 6 h. The progress of the reaction was monitored by TLC analysis. Upon completion, the solvent was evaporated under reduced pressure. The crude product was purified by neutral alumina column chromatography using 2% MeOH in DCM as eluent to afford the pure product **DCI-OH** as reddish solid.  $R_f$  = 0.2 (100% DCM), Yield: (0.90 g, 71%).  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ ):  $\delta$  (ppm) = 10.00 (s, 1H), 7.56 (d,  $J$  = 8.6 Hz, 2H), 7.22 (m, 2H), 6.80 (m, 3H), 2.60 (s, 2H), 2.53 (s, 2H), 1.01 (s, 6H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  (ppm) = 170.1, 159.3, 156.7, 138.3, 129.9, 127.1, 126.2, 121.4, 115.9, 114.1, 113.3, 74.8, 42.3, 38.2, 31.7, 27.4.

#### Synthesis of compound **7**<sup>4</sup>

To a mixture of compound **6** (4.00 g, 23.78 mmol), TBDMSCl (8.70 g, 57.72 mmol) and imidazole (4.00 g, 58.76 mmol) was added dry DMF (10 mL) at 0  $^\circ\text{C}$  under inert atmosphere. The reaction mixture was stirred at room temperature for 4 h. Progress of reaction was monitored by TLC analysis. After completion of the reaction, the mixture was diluted with 150 mL of petroleum ether and washed with water (5 $\times$ 20 mL) and saturated brine solution (2 $\times$ 20 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford the crude product. The crude compound was purified by flash chromatography using 100% petroleum ether to afford the pure product **7** as colorless oil.  $R_f$  = 0.5 (100% petroleum ether), Yield: (8.50 g, 90%).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 8.02 (s, 1H), 6.90 (s, 2H), 4.82 (s, 4H), 2.25 (s, 3H), 0.94 (s, 18H), 0.12 (s, 12H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 151.0, 128.4, 126.3, 125.9, 63.1, 26.0, 20.8, 18.5, -5.3.

#### Synthesis of compound **8**<sup>4</sup>

The solution of compound **7** (5.00 g, 12.60 mmol) and  $\text{K}_2\text{CO}_3$  (2.61 g, 18.90 mmol) in dry DMF (7 mL) was stirred at 0  $^\circ\text{C}$  under inert atmosphere. After 10 minutes, the solution of compound **3** (3.74 g, 12.60 mmol) in dry DMF (8 mL) was added to the solution in a dropwise manner. The reaction mixture was stirred at room temperature for 12 h. Progress of reaction was monitored by TLC study. Upon completion of the reaction, the reaction mixture was diluted with ethyl acetate (150 mL) and washed with water (3 $\times$ 10 mL) and brine (2 $\times$ 10 mL) successively. The organic phase was dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford the crude compound. The crude product was purified by flash chromatography using 100%

petroleum ether to afford the pure product as colorless liquid.  $R_f = 0.5$  (5% Ethyl acetate in petroleum ether), Yield: (6.5 g, 84%).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 7.83 (d,  $J = 8.5$  Hz, 2H), 7.42 (d,  $J = 7.7$  Hz, 2H), 7.17 (s, 2H), 4.88 (s, 2H), 4.70 (s, 4H), 2.34 (s, 3H), 1.35 (s, 12 H), 0.91 (s, 18H), 0.06 (s, 12 H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 151.3, 140.9, 135.2, 133.9, 133.8, 128.0, 127.1, 83.9, 76.3, 60.5, 26.1, 25.0, 21.3, 18.6, -5.1.

#### Synthesis of compound 9<sup>4</sup>

The solution of compound **6** (2.00 g, 11.89 mmol) and  $\text{K}_2\text{CO}_3$  (2.46 g, 17.84 mmol) in dry DMF (5 mL) was stirred at 0 °C under inert atmosphere. After 10 minutes, the benzyl bromide was added to the solution in a dropwise manner. The reaction mixture was stirred at room temperature for 12 h. Progress of reaction was monitored by TLC study. Upon completion of the reaction, the reaction mixture was diluted with ethyl acetate (100 mL) and washed with water (3×20 mL) and brine (2×20 mL) successively. The organic phase was dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford the crude compound. The crude product was purified by flash chromatography using 40% ethyl acetate in petroleum ether to afford the pure product as white solid.  $R_f = 0.5$  (40% Ethyl acetate in petroleum ether), Yield: (2.50 g, 81%).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) = 7.45 (d,  $J = 7.1$  Hz, 2H), 7.41 (t,  $J = 7.2$  Hz, 2H), 7.37 (d,  $J = 7.1$  Hz, 1H), 7.16 (s, 2H), 4.95 (s, 2H), 4.68 (d,  $J = 5.5$  Hz, 4H), 2.33 (s, 3H), 1.97 (m, 2H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 152.8, 137.0, 134.6, 134.0, 129.8, 128.9, 128.6, 128.3, 77.0, 61.3, 21.0.

#### Synthesis of compound 10<sup>4</sup>

To a stirred solution of compound **8** (2.00 g, 3.26 mmol) in 20 mL of distilled MeOH was added *p*-TsOH (0.15 g, 0.82 mmol) under inert atmosphere. The reaction mixture was stirred at room temperature for overnight. Upon completion of the reaction, the solvent was evaporated under reduced pressure and diluted with water. The crude compound was extracted with Ethyl acetate (3×30 mL). The combined organic phase was washed with brine (2×10 mL), and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to afford the crude compound which was further purified by flash chromatography using 50% Ethyl acetate in petroleum ether to afford the pure product **10** as white solid.  $R_f = 0.5$  (40% Ethyl acetate in petroleum ether), Yield: (1.10 g, 88%).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 7.83 (d,  $J = 7.8$  Hz, 2H), 7.41 (d,  $J = 7.7$  Hz, 2H), 7.14 (s, 2H), 4.92 (s, 2H), 4.64 (s, 4H), 2.31 (s, 3H), 1.35 (s,

12H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) = 152.6, 140.0, 135.3, 134.6, 133.9, 129.7, 127.3, 84.0, 76.9, 61.0, 25.0, 21.0.

### Synthesis of compound 11

To a stirred solution of compound **9** (2.00 g, 7.76 mmol) in 40 mL of dry DCM was added  $\text{PBr}_3$  (3.70 mL, 38.80 mmol) in a dropwise manner at 0 °C under inert condition and the mixture was stirred at room temperature for 12 h. Progress of the reaction was monitored by TLC analysis. Upon completion, reaction was quenched with water at 0 °C and the crude product was extracted with DCM (3×30 mL). The combined organic phase was washed with brine (3×30 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to obtain the crude compound which was further purified by flash chromatography using 5% Ethyl acetate in petroleum ether to afford the pure product **11** as white solid.  $R_f$  = 0.5 (5% Ethyl acetate in petroleum ether), Yield: (2.00 g, 70%).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 7.54 (d,  $J$  = 6.9 Hz, 2H), 7.43 (t,  $J$  = 7.3 Hz, 2H), 7.38 (t,  $J$  = 7.3 Hz, 1H), 7.20 (s, 2H), 5.16 (s, 2H), 4.52 (s, 4H), 2.32 (s, 3H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 153.2, 137.0, 135.0, 133.0, 132.0, 128.8, 128.5, 128.2, 77.4, 76.3, 28.1, 20.8. ESI-MS  $m/z$  calcd. for  $\text{C}_{16}\text{H}_{16}\text{Br}_2\text{O}$   $[\text{M}+\text{NH}_4]^+$  = 401.9891, obs. 401.9906.

### Synthesis of compound 12<sup>5</sup>

To a stirred solution of compound **10** (2.00 g, 5.20 mmol) in 40 mL of dry DCM (40 mL) was added  $\text{PBr}_3$  (2.50 mL, 26.00 mmol) in a dropwise manner at 0 °C under inert condition and the reaction mixture was stirred at room temperature for 5 h. Progress of the reaction was monitored by TLC analysis. Upon completion, the reaction was quenched with water at 0 °C and the crude product was extracted with DCM (3×30 mL). The combined organic phase was washed with brine (3×30 mL), and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to obtain the crude compound. The crude product was purified by flash chromatography using 5% Ethyl acetate in petroleum ether to afford the pure product **12** as white solid.  $R_f$  = 0.5 (5% Ethyl acetate in petroleum ether), Yield: (2.00 g, 75%).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) = 7.87 (d,  $J$  = 7.8 Hz, 2H), 7.53 (d,  $J$  = 7.8 Hz, 2H), 7.20 (s, 2H), 5.18 (s, 2H), 4.50 (s, 2H), 2.32 (s, 3H), 1.36 (s, 12H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) = 153.1, 140.0, 135.3, 135.0, 133.0, 131.9, 127.1, 84.0, 76.1, 28.1, 25.0, 20.8; ESI-MS  $m/z$  calcd. for  $\text{C}_{22}\text{H}_{27}\text{BBBr}_2\text{O}_3$   $[\text{M}+\text{NH}_4]^+$  = 528.0743, obs. 528.0745.



### Synthesis of compound 13

To a stirred solution of compound **11** (0.90 g, 2.45 mmol) in dry acetonitrile (40 mL) was added the solution of diclofenac (0.72 g, 2.45 mmol) and DIPEA (1.27 mL, 7.33 mmol) in dry acetonitrile (10 mL) at ice-cold condition under argon atmosphere. The reaction mixture was stirred at room temperature for overnight. Upon completion of the reaction, solvent was evaporated under reduced pressure and diluted with DCM (50 mL). The reaction mixture was washed with water (3×10 mL) and brine (2×20 mL) successively. The combined organic phase was dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure to afford the crude compound. The compound was purified by flash chromatography using 8% Ethyl acetate in petroleum ether as the eluent to afford the pure product **13** as white solid.  $R_f$  = 0.4 (5% Ethyl acetate in petroleum ether), Yield: (0.70 g, 47%).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 7.41 (t,  $J$  = 7.6 Hz, 2H), 7.35 (t,  $J$  = 7.3 Hz, 2H), 7.31 (d,  $J$  = 8.1 Hz, 2H), 7.21 (m, 2H), 7.12 -7.06 (m, 2H), 6.95 (t,  $J$  = 8.1 Hz, 3H), 6.91 (t,  $J$  = 7.4 Hz, 1H), 6.79 (s, 1H), 6.53 (d,  $J$  = 8.0 Hz, 1H), 5.21 (d,  $J$  = 3.2 Hz, 2H), 4.98 (s, 2H), 4.51 (s, 2H), 3.83 (s, 2H), 2.26 (s, 3H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 172.2, 153.5, 142.8, 137.9, 136.8, 134.7, 132.7, 132.0, 131.6, 131.0, 129.6, 129.4, 128.9, 128.7, 128.4, 128.2, 128.0, 124.3, 124.1, 122.2, 118.5, 76.8, 62.4, 38.7, 28.1, 20.8. ESI-MS  $m/z$  calcd. for  $\text{C}_{30}\text{H}_{26}\text{BrCl}_2\text{NO}_3$   $[\text{M}+\text{H}]^+ = 598.0551$ , obs. 598.0587.

### Synthesis of compound 14

To a stirred solution of compound **12** (0.65 g, 1.27 mmol) in dry acetonitrile (10 mL) was added the solution of diclofenac (0.38 g, 1.27 mmol) and DIPEA (0.6 mL, 3.82 mmol) in dry acetonitrile (5 mL) dropwise at 0 °C under argon atmosphere. The reaction mixture was stirred at room temperature for overnight. Upon completion of the reaction, the solvent was evaporated under reduced pressure and diluted with DCM (50 mL). The reaction mixture then washed with water (3×10 mL) and brine (2×10 mL). The combined organic phase was dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure to afford the crude compound. The compound was purified by silica gel column chromatography using 5% Ethyl acetate in petroleum ether as the eluent to afford the pure product **14** as white solid.  $R_f$  = 0.5 (10% Ethyl acetate in petroleum ether), Yield: (0.40 g, 43%).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) = 7.81 (d,  $J$  = 7.6 Hz, 2H), 7.41 (d,  $J$  = 7.7 Hz, 2H), 7.31 (d,  $J$  = 8.1 Hz, 2H), 7.19 (d,  $J$  = 9.8 Hz, 2H), 7.09 (t,  $J$  = 7.7 Hz, 1H), 7.07 (s, 1H), 6.96 (t,  $J$  = 8.1 Hz, 1H), 6.92 (t,  $J$  = 7.4 Hz,

1H), 6.78 (s, 1H), 6.53 (d,  $J$  = 8.0 Hz, 1H), 5.21 (s, 2H), 4.99 (s, 2H), 4.48 (s, 2H), 3.83 (s, 2H), 2.26 (s, 3H), 1.35 (s, 12H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) = 172.0, 153.6, 142.8, 139.9, 137.9, 135.2, 134.7, 132.7, 132.0, 131.6, 131.0, 129.6, 129.4, 128.9, 128.2, 127.0, 124.3, 124.1, 122.3, 118.5, 83.9, 76.7, 62.4, 38.8, 28.1, 25.0, 20.8; ESI-MS  $m/z$  calcd. for  $\text{C}_{36}\text{H}_{37}\text{BBBrCl}_2\text{NO}_5$   $[\text{M}+\text{Li}+\text{H}_2\text{O}]^+ = 748.1591$ , obs. 748.1608.

### Synthesis of DCI-Con

The mixture of compound **13** (0.50 g, 0.83 mmol), **DCI-OH** (0.29 g, 1.00 mmol) and  $\text{K}_2\text{CO}_3$  (0.17 g, 1.25 mmol) in acetone (2 mL) was stirred at 55 °C for 4 h. Progress of the reaction was monitored by TLC analysis. Upon completion of the reaction, the solvent was evaporated under reduced pressure, diluted with Ethyl acetate (30 mL) and washed with water ( $3 \times 10$  mL). The organic phase was washed with brine ( $2 \times 10$  mL) and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to obtain the crude compound which was further purified by flash chromatography with 100% DCM to obtain the pure product **DCI-Con** as red solid.  $R_f$  = 0.5 (15% Ethyl acetate in petroleum ether), Yield: (0.40 g, 59%). M.P. = 73-75 °C.  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 7.44 (d,  $J$  = 8.6 Hz, 2H), 7.32 (d,  $J$  = 8.1 Hz, 2H), 7.28 (d,  $J$  = 8.1 Hz, 6H), 7.21 (d,  $J$  = 7.2 Hz, 1H), 7.14 (s, 1H), 7.10 (t,  $J$  = 7.6 Hz, 1H), 7.02 (d,  $J$  = 16.0 Hz, 1H), 6.98 (t,  $J$  = 8.1 Hz, 1H), 6.95-6.91 (m, 3H), 6.88 (t,  $J$  = 14.5 Hz, 1H), 6.81 (s, 2H), 6.54 (d,  $J$  = 8.0 Hz, 2H), 5.25 (s, 2H), 5.01 (s, 2H), 4.88 (s, 2H), 3.85 (s, 2H), 2.59 (s, 2H), 2.46 (s, 2H), 2.29 (s, 3H), 1.08 (s, 6H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 172.2, 169.4, 160.1, 154.4, 153.7, 142.8, 137.9, 136.9, 136.8, 134.5, 131.6, 131.2, 131.0, 129.9, 129.6, 129.4, 129.1, 128.9, 128.7, 128.4, 128.2, 128.1, 127.3, 124.3, 124.1, 122.9, 122.2, 118.5, 115.4, 77.9, 77.8, 65.5, 62.5, 43.1, 39.4, 38.8, 32.2, 28.2, 20.9. ESI-MS  $m/z$  calcd. for  $\text{C}_{49}\text{H}_{43}\text{Cl}_2\text{N}_3\text{O}_4$   $[\text{M}+\text{NH}_4]^+ = 825.2974$ , obs. 825.2856.

### Synthesis of compound DCI-ROS

The mixture of compound **14** (0.28 g, 0.39 mmol), **DCI-OH** (0.09 g, 0.31 mmol) and  $\text{K}_2\text{CO}_3$  (0.05 g, 0.39 mmol) were dissolved in acetone (2 mL) under inert atmosphere and stirred at 55 °C for 4 h. Progress of the reaction was monitored by TLC analysis. Upon completion of the reaction, the solvent was evaporated under reduced pressure and diluted with Ethyl acetate (50 mL), washed with water ( $3 \times 10$  mL). The combined organic phase was washed with brine ( $2 \times 10$  mL) and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced

pressure to obtain the crude compound which was further purified by flash chromatography with 25% Ethyl acetate in petroleum ether to obtain the pure product **DCI-ROS** as red solid.  $R_f = 0.5$  (20% Ethyl acetate in petroleum ether), Yield: (0.14 g, 50%). M.P. = 92-94 °C.  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 7.74 (d,  $J = 7.8$  Hz, 2H), 7.43 (d,  $J = 8.6$  Hz, 2H), 7.32 (d,  $J = 8.1$  Hz, 2H), 7.28 (d,  $J = 7.7$  Hz, 2H), 7.20 (d,  $J = 7.5$  Hz, 1H), 7.13 (s, 1H), 7.10 (t,  $J = 7.6$  Hz, 1H), 7.01 (d,  $J = 16.0$  Hz, 1H), 6.97 (t,  $J = 8.1$  Hz, 1H), 6.95-6.85 (m, 4H), 6.80 (s, 2H), 6.54 (d,  $J = 8.0$  Hz, 1H), (5.26 (s, 2H), 4.97 (s, 2H), 4.88 (s, 2H), 3.85 (s, 2H), 2.60 (s, 2H), 2.46 (s, 2H), 2.28 (s, 3H), 1.34 (s, 12 H), 1.08 (s, 6H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 172.1, 169.4, 160.2, 154.5, 153.7, 142.8, 139.9, 137.9, 137.0, 135.2, 134.6, 131.6, 131.2, 131.0, 129.9, 129.6, 129.4, 129.1, 129.0, 128.8, 128.2, 127.3, 127.2, 124.4, 124.1, 122.9, 122.3, 118.5, 115.4, 113.9, 113.1, 84.0, 77.9, 77.6, 65.4, 62.5, 43.1, 39.4, 38.8, 32.2, 28.2, 25.0, 20.9. ESI-MS  $m/z$  calcd. for  $\text{C}_{55}\text{H}_{54}\text{BCl}_2\text{N}_3\text{O}_6$   $[\text{M}+\text{Li}]^+ = 940.3642$ , obs. 940.3642.

### UV-Vis and fluorescence spectroscopic studies

All the stock solutions of probes and the released fluorophore were prepared in spectroscopy grade DMSO and the stock solution of hydrogen peroxide was prepared in Milli-Q water. The pronounced absorption band at 550 nm and the emission in NIR range ( $\lambda_{\text{em}} = 670$  nm) of **DCI-OH** was observed in PBS buffer (20 mM, pH 7.5) in the presence of 50% DMSO (Figure S1, ESI).<sup>6</sup> Therefore, the absorption and emission spectra of the released fluorophore **DCI-OH** was collected in PBS with the added DMSO just prior to the measurements. Therefore, all the spectroscopic studies were performed in phosphate buffer (20 mM, pH = 7.5) only and DMSO (50% v/v) was added to the buffer solution just before the measurements at room temperature. DMSO was reported to enhance the dissociation of the phenolic hydroxyl group of **DCI-OH**.<sup>6</sup> Samples for absorption and emission spectroscopic measurements were carried out in quartz cuvettes (1.0 mL). Excitation wavelength ( $\lambda_{\text{ex}}$ ) = 550 nm and emission range ( $\lambda_{\text{em}}$ ) = 570-750 nm with slit width = 10/10 nm were used in the fluorescence emission spectroscopic studies.

### Analyte selectivity and pH variation studies

Analyte selectivity of the probe **DCI-ROS** (10  $\mu\text{M}$ ) was studied by measuring the emission intensity of the released fluorophore **DCI-OH** upon the incubation of the probe with different and bioanalytes (200  $\mu\text{M}$ ) in phosphate buffer (20 mM, pH 7.5) with 50% DMSO after incubating for 14 h. For the pH variation study, the emission intensity from **DCI-ROS** (10  $\mu\text{M}$ )

was measured in the presence/absence of H<sub>2</sub>O<sub>2</sub> (200 µM) with variable pH of the medium (pH: 5-11) in PBS buffer with 50% DMSO after an incubation time of 14 h. Control reactions with the probe in the absence of any added H<sub>2</sub>O<sub>2</sub> was studied under the identical reaction condition to understand the stability of the probes at different pH ranges.

#### **Determination of the limit of detection (LOD) of the prodrug**

The limit of detection (LOD) of the prodrug **DCI-ROS** was determined using the fluorescence titration.<sup>7</sup> The detection limit was calculated using the equation,  $LOD = 3\sigma/K$ , where  $\sigma$  is the standard deviation of blank measurements (without H<sub>2</sub>O<sub>2</sub>) and K is the slope of the plot of emission intensity versus H<sub>2</sub>O<sub>2</sub> concentration of the prodrug **DCI-ROS**. To get the standard deviation of the blank measurements, the emission intensity of pure **DCI-ROS** (10 µM) was measured 5 times in the absence of H<sub>2</sub>O<sub>2</sub>. For the determination of slope, the emission spectra from **DCI-ROS** were measured at different concentrations of H<sub>2</sub>O<sub>2</sub> (2.0 – 70.0 µM) after incubating the reaction mixture for 14 h.

#### **Reaction kinetics measurements by HPLC analysis**

Purity of the synthesized probes **DCI-ROS** and **DCI-Con** along with diclofenac (**DCF**) and the released fluorophore **DCI-OH** were analysed using analytical high-performance liquid chromatography (HPLC) Agilent 1220 infinity II LC system using reverse-phase C<sub>18</sub> column (Luna®, 150 × 4.6 mm, 5 µm). HPLC grade acetonitrile and water (Finar Ltd) were used as mobile phase and the absorbance profile of compounds were detected by PDA detector at wavelengths of 230, 254, 280 and 430 nm (as applicable). The stock solutions of the samples were prepared in acetonitrile and were injected into the HPLC system using the in-built autosampler at a flow rate of 1.0 mL/min using acetonitrile/water system as a mobile phase for 25 min (0-7 min: 75%-99% acetonitrile in water; 7-15 min: 99% acetonitrile in water; 15-25 min: 99%-75% acetonitrile in water). The reaction of **DCI-ROS** (100 µM) and **DCI-Con** (100 µM) with H<sub>2</sub>O<sub>2</sub> (2 mM) and sodium bicarbonate (500 µM) was prepared in acetonitrile/water system (60:40). The peaks due to the analytes, probes, reaction intermediates and the released drug and fluorophore were analyzed/identified at different wavelengths (230, 280 and 430 nm) at various time intervals.

#### **Cell Culture**

The triple-negative breast cancer (TNBC) cells (MDA-MB-231) and a macrophage-like, Abelson leukemia virus-transformed cell line (derived from BALB/c mice) (RAW 264.7) were obtained from the National Centre for Cell Science (NCCS), Pune, India. The cells were cultured in DMEM medium (Gibco) supplemented with 10% (v/v) FBS (Gibco) and 1% Pen-Strep (Gibco). Cells were cultured as a monolayer in a humidified incubator at 37 °C in the presence of 5% CO<sub>2</sub> level.

### Cell viability Assay

The synthesized probe **DCI-ROS** and the drug diclofenac (**DCF**) were screened for their anti-proliferative activities using the conventional MTT assay. MDA-MB- 231 cells and RAW 264.7 cells were seeded separately in 96-well culture plates at a density of  $2 \times 10^4$  cells/100  $\mu$ L/well and  $10^4$  cells/100  $\mu$ L/well, respectively. Cells were treated with the freshly prepared test compounds **DCI-ROS** and **DCF** (10.0, 25.0, 50.0 and 100.0  $\mu$ M) for 0 h (control) and 48 h (experimental), respectively. At the end of treatment period, 10.0  $\mu$ L of 5.0 mg/mL of MTT was added to the plate (control) and incubated for 4 h. Following the 4 h incubation, the reagent from the plate was removed and the purple formazan crystals were dissolved using 100  $\mu$ L of DMSO (Avra Synthesis Pvt Ltd) and the absorbance at 570 nm was measured using a microplate reader (MultiskanGo microplate reader, ThermoFisher Scientific). In the experimental set, similar MTT treatment protocol was followed only after 48 h. The mean  $\Delta$ OD values were calculated by the subtraction of mean OD values of 0 h plate (control) from the mean OD values of identical wells at 48 h plate (experimental) and the percentage proliferation was calculated keeping the mean  $\Delta$ OD of untreated control as 100%.

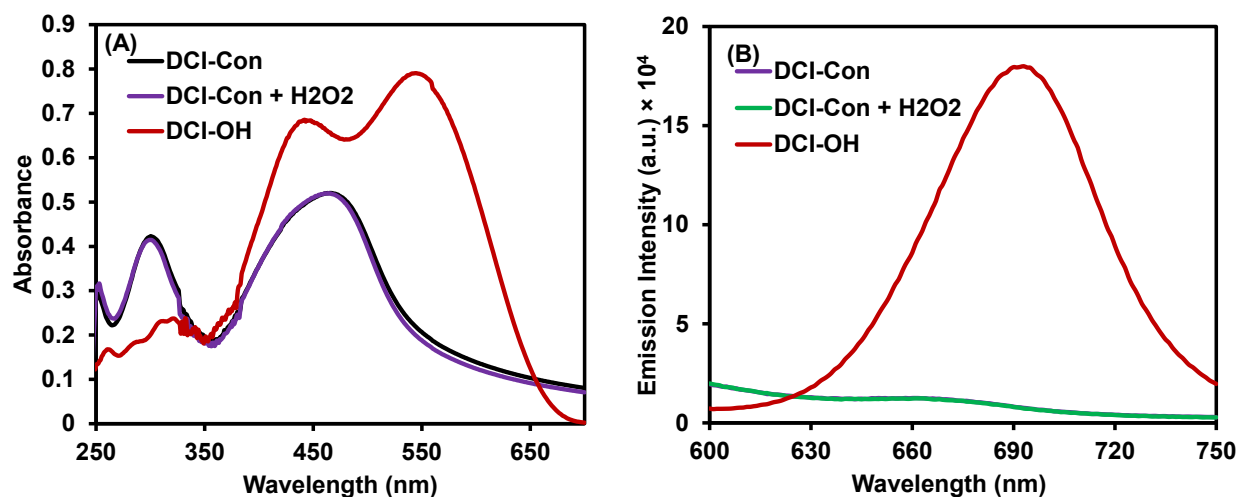
### Fluorescence Microscopic Studies

MDA-MB-231 cells and RAW 264.7 cells were separately cultured in high glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C in the incubator under 5% CO<sub>2</sub> atmosphere. MDA-MB-231 cells ( $3.0 \times 10^4$  cells/plate) and RAW 264.7 cells ( $2.0 \times 10^4$  cells/plate) were then plated in 35 mm cell culture petri dishes containing 2.0 mL of DMEM and incubated at 37 °C under 5% CO<sub>2</sub> for 24 h. The confluent cells (MDA-MB-231, RAW 264.7) were washed with DPBS and finally incubated with **DCI-ROS** (50.0  $\mu$ M) at 37 °C under 5% CO<sub>2</sub> for 5 h. After washing with DPBS (3 times), cellular morphology was carefully observed and imaged in a Bio-Rad ZOE<sup>TM</sup> fluorescent cell

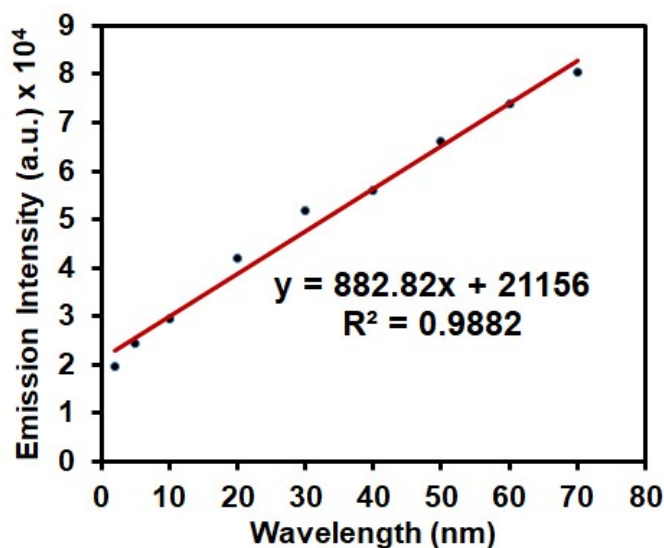
imager under bright field and red fluorescent emission filter. A negative control experiment was performed upon the pre-treatment of cells with *N*-acetyl cysteine (NAC, 2 mM) for 2 h to quench the endogenous ROS. After the pre-treatment, cells were washed with DPBS (3 times) and finally the cells were incubated with **DCI-ROS** (50.0  $\mu$ M) at 37 °C under 5% CO<sub>2</sub> for 5 h. The treated cells were finally washed with DPBS (3 times) and imaged in a Bio-Rad ZOE™ fluorescent cell imager under bright field and red fluorescent emission filter.

### Western Blot analysis

In order to estimate the COX-2 expression upon the release of the NSAID (**DCF**) from the probe **DCI-ROS**, immunoblotting experiment was performed. RAW 264.7 cells ( $5 \times 10^5$  cells per 10 mL) were plated in 60 mm cell culture dish and cultured in high glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C in the incubator under 5% CO<sub>2</sub> atmosphere. The overexpression of COX-2 level in RAW 264.7 cells was induced upon the pre-treatment of lipopolysaccharide (LPS, 50  $\mu$ g/mL, 14 h). After the treatment, cells were washed with DPBS (3 times) and further treated with compound **DCI-ROS** and **DCF** (50.0 and 100.0  $\mu$ M) and incubated for 24 h under 5% CO<sub>2</sub> atmosphere. After completion of the treatment, cells were washed with cold PBS (1X) and the protein extraction was performed. The whole cell protein was extracted using RIPA cell lysis buffer (150 mM of NaCl, 1% (v/v) NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 25 mM of Tris, 10 mg/mL of PMSF). Equal volume of protein was loaded and separated on 12% sodium dodecyl sulfate (SDS)–polyacrylamide gel by electrophoresis and transferred to a nitrocellulose membrane. Protein transfer was confirmed by Ponceau-S (HiMedia) staining. The blots were blocked with 5% non-fat dry milk in 1X TBST buffer for 1 h at room temperature. Finally, the primary antibody (1:5000) specific for COX-2 (Cell Signalling, D5H5) and GAPDH (Cell Signalling, 14C10) were incubated overnight at 4 °C. Following the primary antibody incubation, the blots were washed with TBST (1X) buffer and incubated with HRP-conjugated secondary antibodies (Abcam) for 1 h and developed with an ECL Detect Kit (Bio-Rad) and imaged using ChemiDoc™XRS System (Bio-Rad). The house-keeping gene GAPDH was used as a loading control.



**Figure S1.** (A) Absorption spectra of probe **DCI-Con** (50  $\mu\text{M}$ ) in the absence and presence of  $\text{H}_2\text{O}_2$  (1 mM) in phosphate buffer (20 mM, pH 7.5, with 50% DMSO v/v). The final UV-Vis spectra of the probes with  $\text{H}_2\text{O}_2$  were recorded after 14 h. (B) Emission spectra of probe **DCI-Con** (10  $\mu\text{M}$ ) in the absence and presence of  $\text{H}_2\text{O}_2$  (200  $\mu\text{M}$ ) after 14 h in phosphate buffer (20 mM, pH 7.5, with 50% DMSO v/v).  $\lambda_{\text{ex}}$  = 550 nm,  $\lambda_{\text{em}}$  = 670 nm, slit width = 10/10 nm.

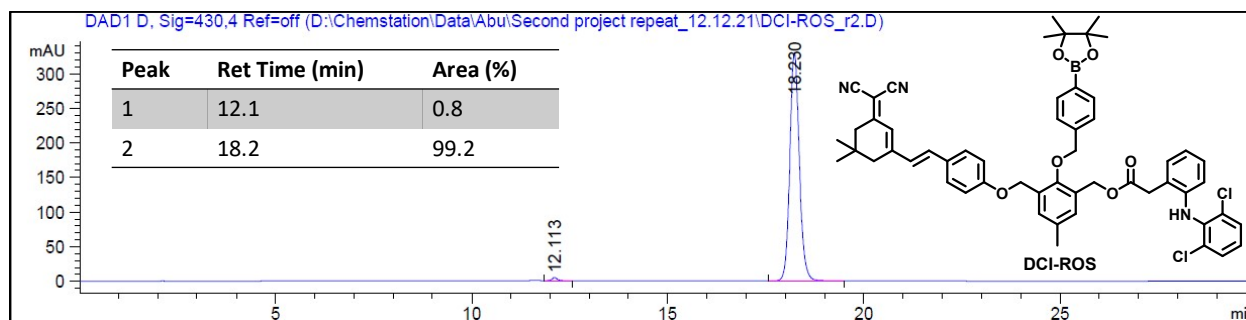


**Figure S2.** Emission spectra of prodrug **DCI-ROS** (10  $\mu\text{M}$ ) in the presence of  $\text{H}_2\text{O}_2$  (2.0 – 70.0  $\mu\text{M}$ ) after an incubation for 14 h in phosphate buffer saline (20 mM, pH 7.5, 50% DMSO v/v). From the expression of linear curve fitting, the value of k is 882.82.

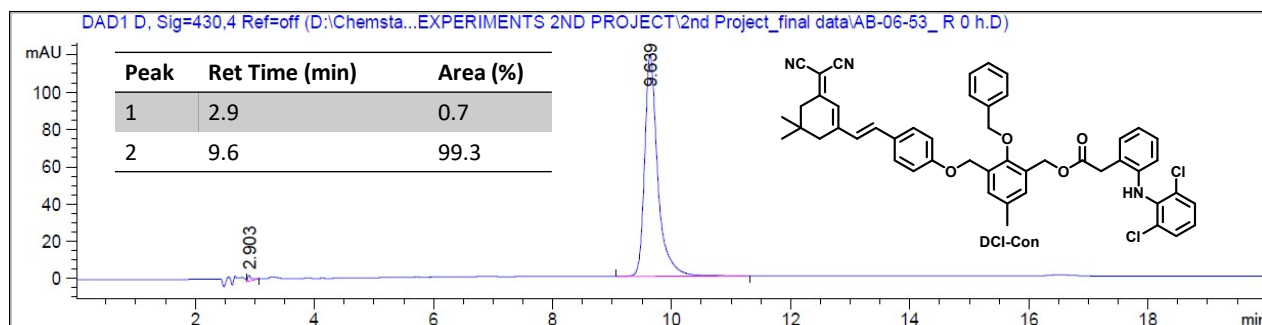
**Table S1. Determination of the standard deviation ( $\sigma$ ) from blank measurements**

Entry	Test 1	Test 2	Test 3	Test 4	Test 5	Mean	SD ( $\sigma$ )
<b>Emission Intensity (670 nm)</b>	13327.80	13172.33	13122.11	13063.63	13033.48	<b>13143.87</b>	<b>103.66</b>

$$\text{LOD} = 3\sigma / k = (3 \times 103.66) / 882.82 = \mathbf{352 \text{ nM}}$$

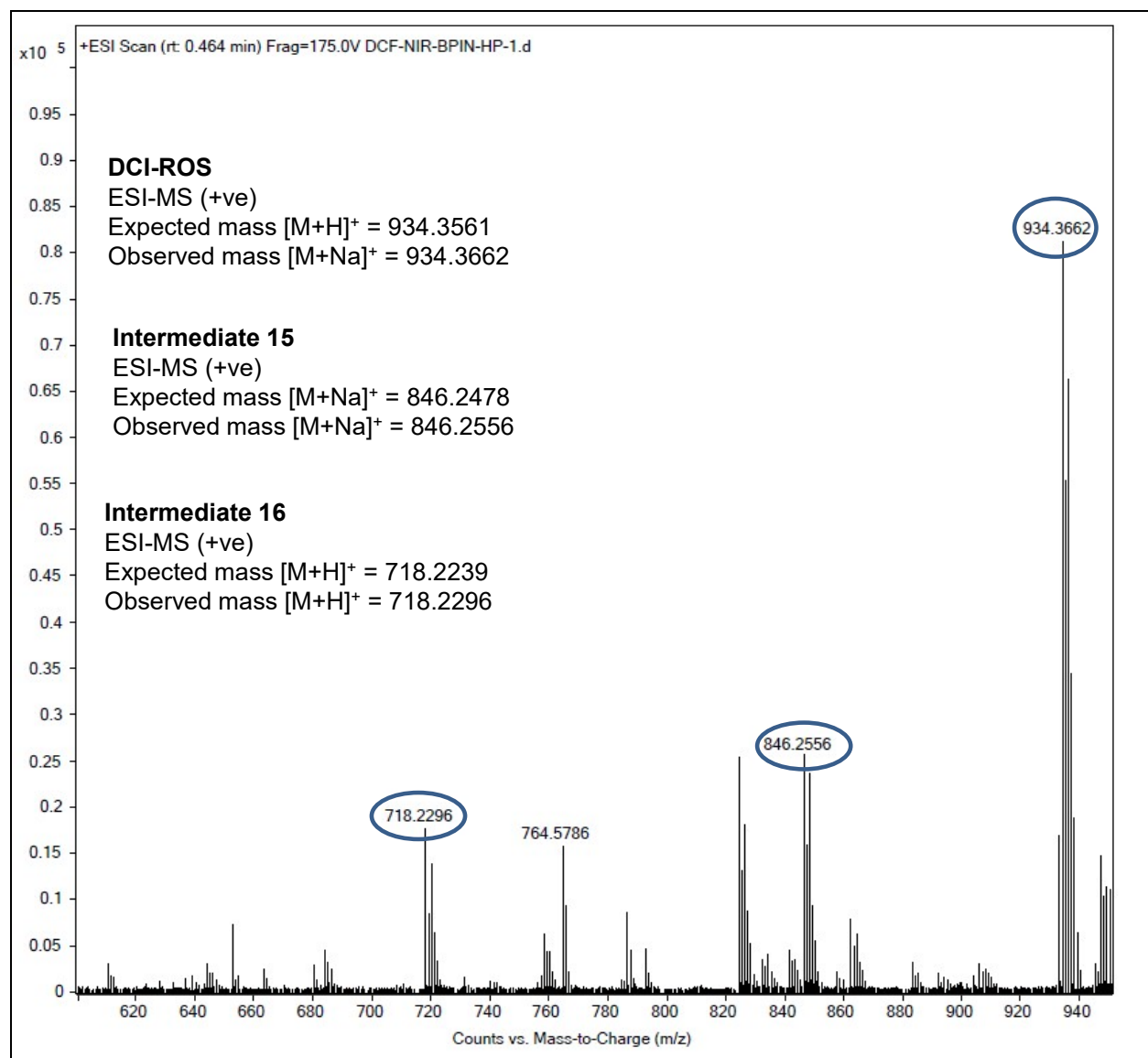


**Figure S3.** HPLC chromatogram of the prodrug **DCI-ROS** showing 99.2% purity.

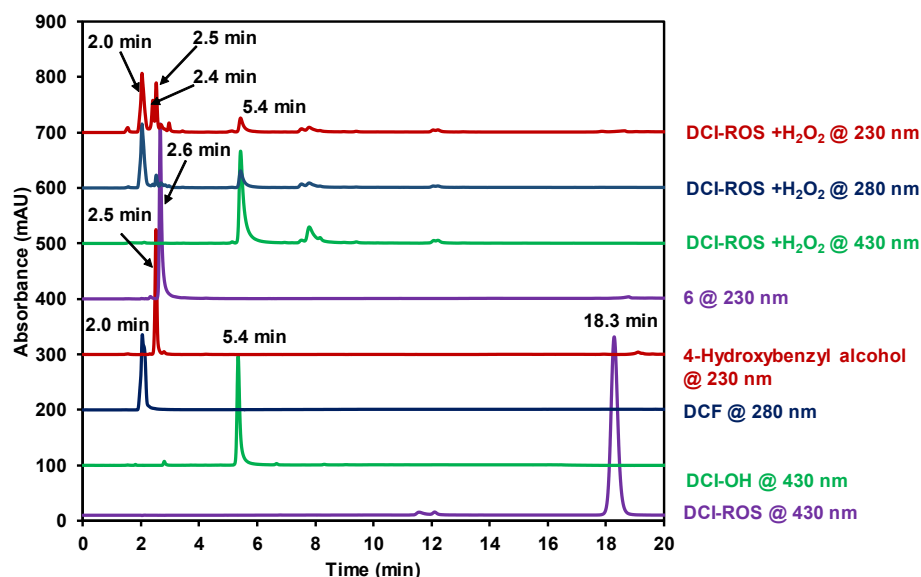


**Figure S4.** HPLC chromatogram of the prodrug **DCI-Con** showing 99.3% purity.

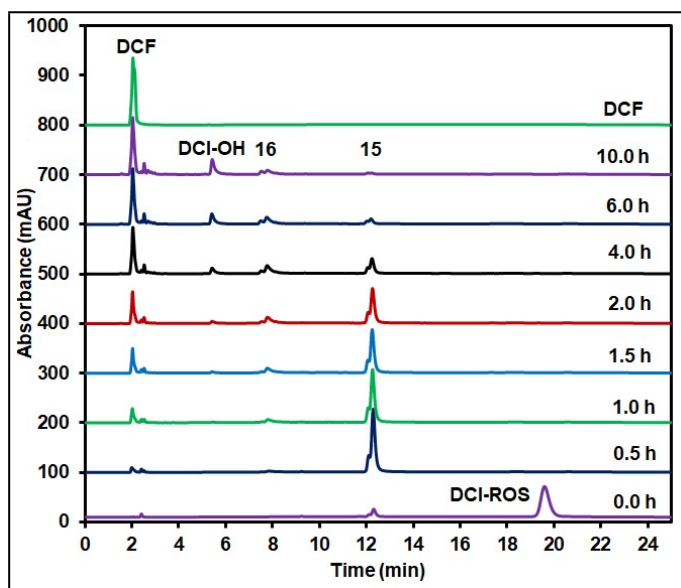




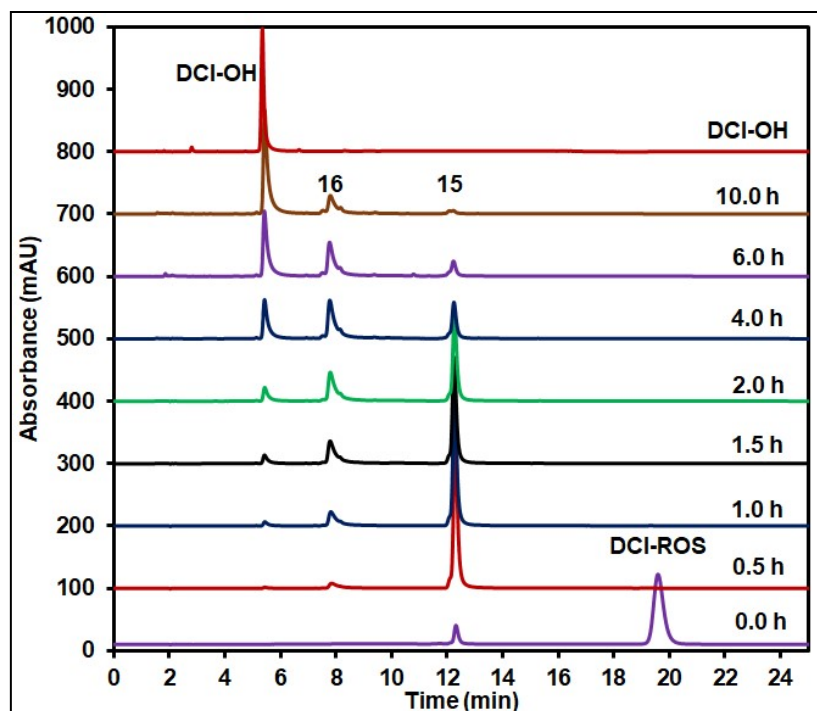
**Figure S5.** ESI-MS spectrum of the intermediate **15** and **16** in the reaction mixture of prodrug **DCI-ROS** with  $H_2O_2$  during HPLC analysis. ESI-MS (+ve)  $m/z$  calcd for  $C_{49}H_{43}Cl_2N_3O_5$   $[M + Na]^+$ : 846.2478; obs. 846.2556 (intermediate **15**), for  $C_{42}H_{37}Cl_2N_3O_4$   $[M + H]^+$ : 718.2239; obs. 718.2296 (intermediate **16**) and for  $C_{55}H_{54}BCl_2N_3O_6$   $[M + H]^+$ : 934.3561; Obs. 934.3662 (prodrug **DCI-ROS**).



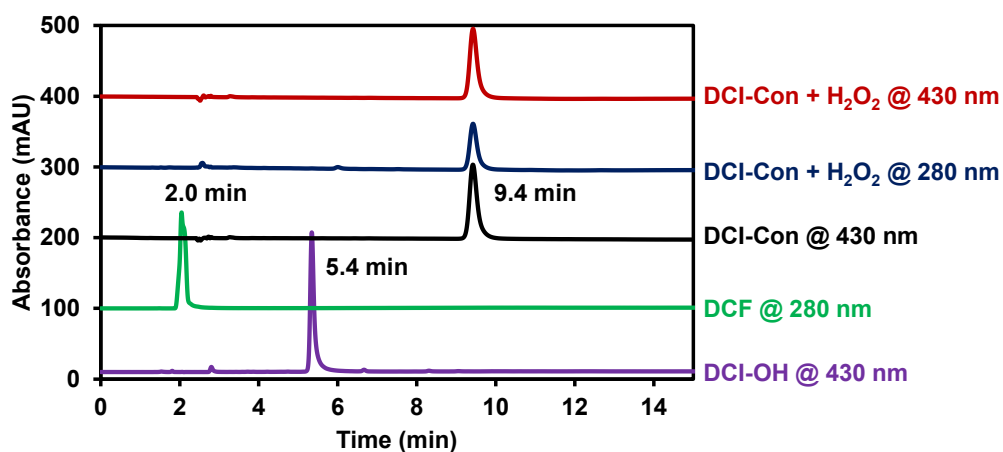
**Figure S6:** A complete HPLC overlay chromatogram for the reaction of **DCI-ROS** (100  $\mu$ M) with and without  $\text{H}_2\text{O}_2$  (2 mM), along with the released drug **DCF** (100  $\mu$ M, 280 nm), fluorophore **DCI-OH** (100  $\mu$ M, 430 nm), compound **6** (100  $\mu$ M, 230 nm), and 4-hydroxybenzyl alcohol (100  $\mu$ M, 230 nm). The chromatograms of the reaction mixture were extracted at three different wavelengths (230, 280 and 430 nm) after an incubation time of 12 h. The reaction profiles clearly indicate the release of the drug (**DCF**) as well as the fluorophore (**DCI-OH**) along with by-products. The peak at 2.4 min with almost fixed intensity appears from the stabilizers present in commercial  $\text{H}_2\text{O}_2$ .



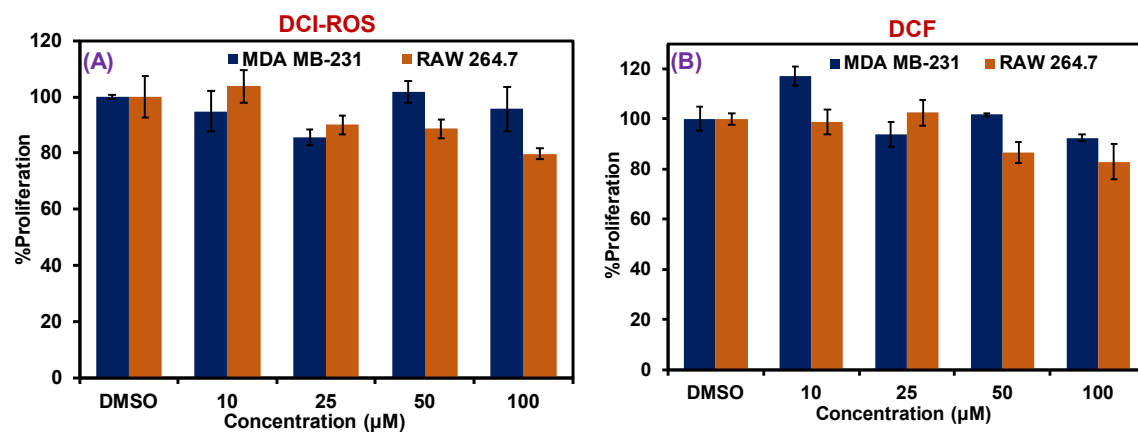
**Figure S7:** HPLC overlay chromatogram for the reaction of **DCI-ROS** (100  $\mu$ M) with  $\text{H}_2\text{O}_2$  (2 mM) over 12 h along with the chromatogram of released drug **DCF** (100  $\mu$ M). All the chromatograms were extracted at 280 nm. Increasing intensity of the released drug **DCF** (time = 2.0 min) was observed in the presence of  $\text{H}_2\text{O}_2$ .



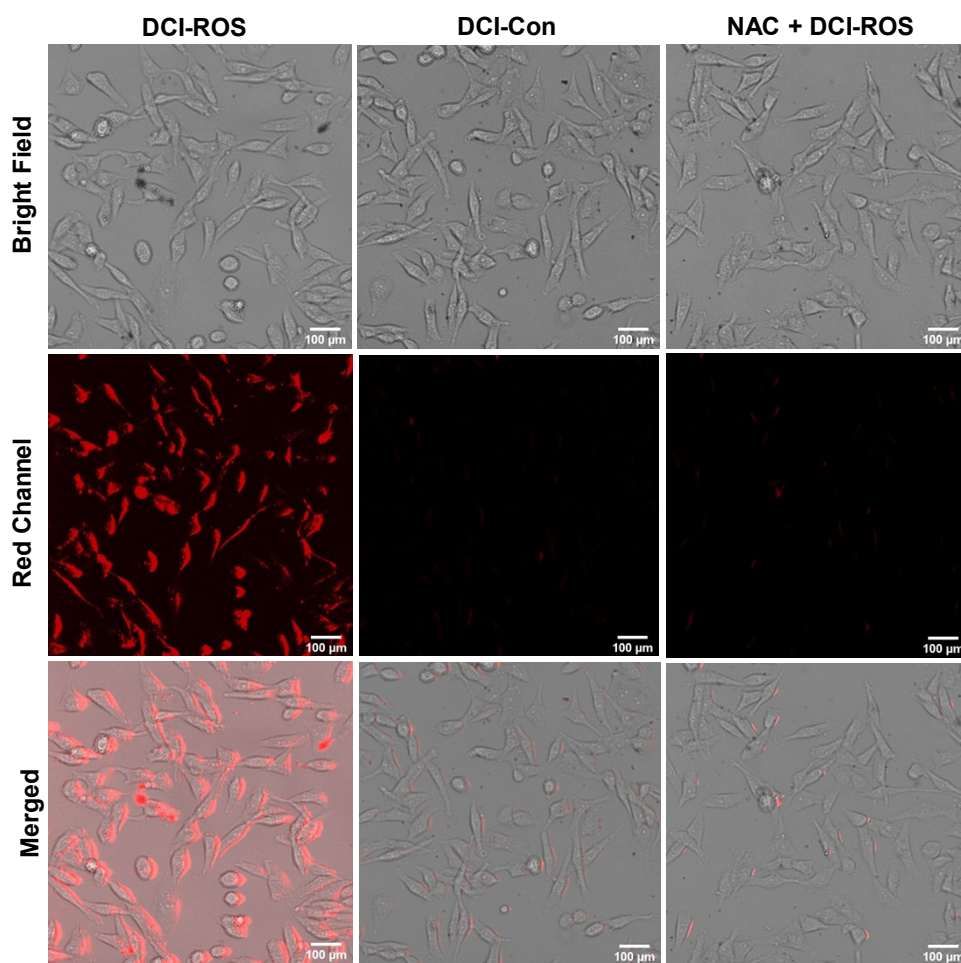
**Figure S8:** HPLC overlay chromatogram for the reaction of **DCI-ROS** with  $\text{H}_2\text{O}_2$  over 10 h along with the chromatogram of the released fluorophore **DCI-OH**. All the chromatograms were extracted at 430 nm. Increasing intensity of the released fluorophore **DCI-OH** (time = 5.4 min) was observed in the reaction over time.



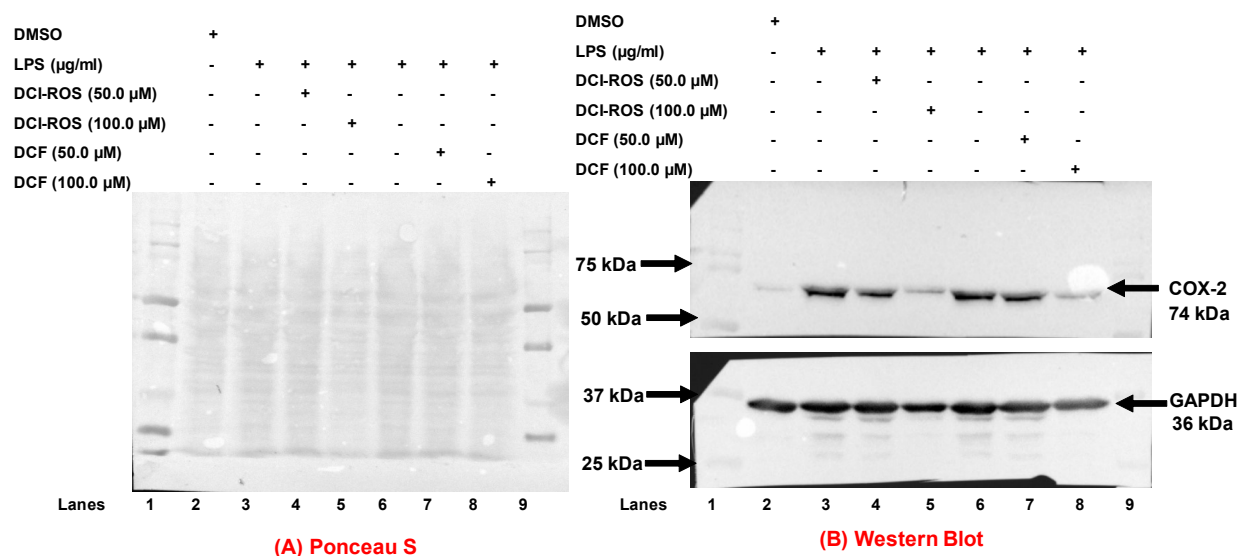
**Figure S9:** A complete HPLC overlay chromatogram for the reaction of **DCI-Con** (100  $\mu\text{M}$ ) with and without  $\text{H}_2\text{O}_2$  (2 mM), along with released drug **DCF** (100  $\mu\text{M}$ , 280 nm), and fluorophore **DCI-OH** (100  $\mu\text{M}$ , 430 nm). All the chromatograms were extracted at 280 and 430 nm wavelengths. Incubation time: 12 h. The chromatogram clearly indicates the non-reactive intact probe **DCI-Con** in the reaction mixture.



**Figure S10.** Dose-dependent cellular viability of MDA-MB-231 and RAW 264.7 cells in the presence of the prodrug **DCI-ROS** as well as the active drug **DCF** upto 100  $\mu\text{M}$  upon an incubation of 48 h.

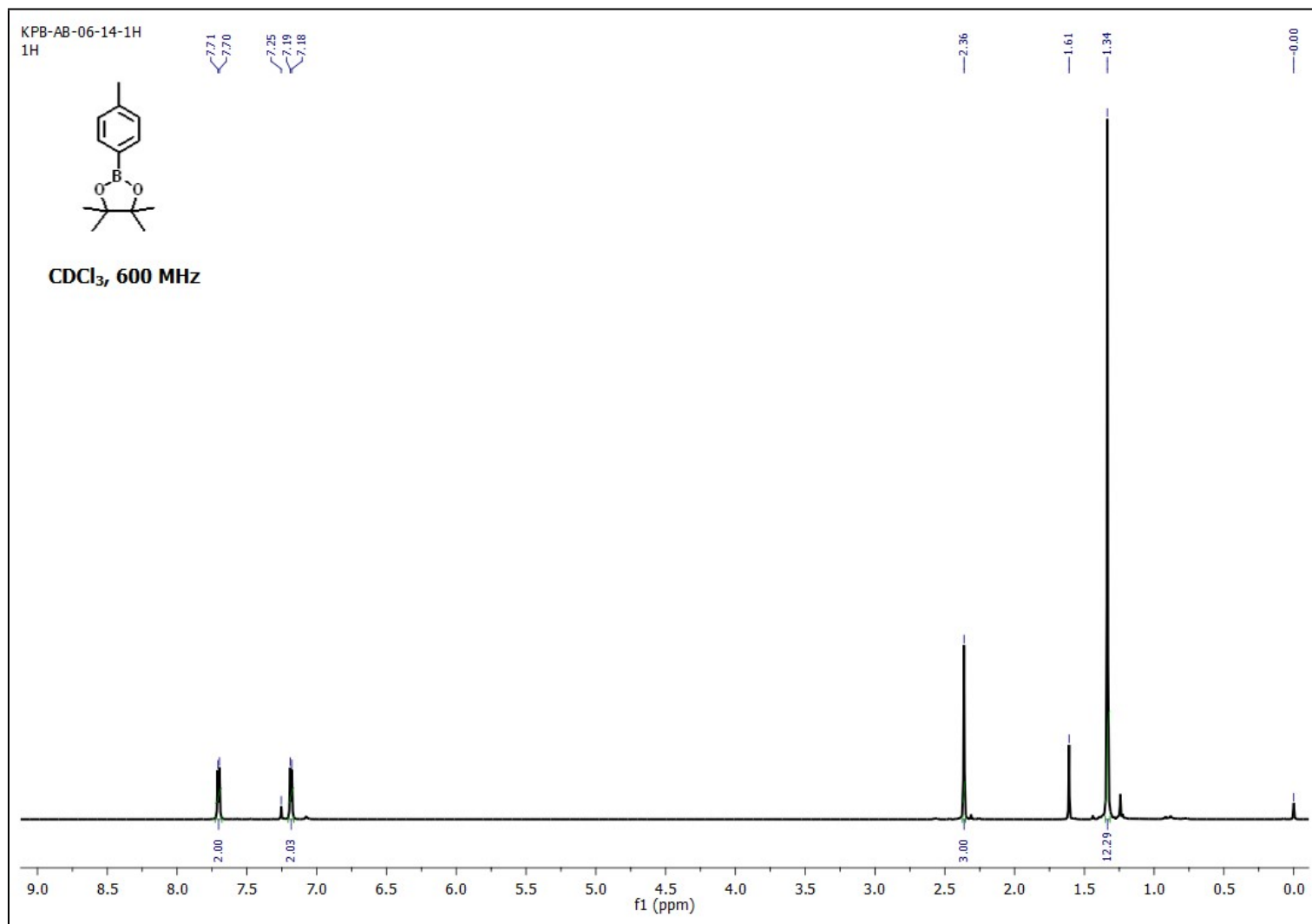


**Figure S11.** Fluorescence microscopy images (bright field, red channel and merged) of MDA-MB-231 cells in the presence of **DCI-ROS** (50  $\mu$ M) and **DCI-Con** (50  $\mu$ M) for the endogenous ROS-triggered fluorogenic drug release. The cells were pre-treated with NAC (2 mM) to quench the endogenous ROS. Scale bar: 100  $\mu$ m. Very negligible turn-on fluorescence emission from **DCI-Con** supports absence of ROS-triggering unit in the compound.

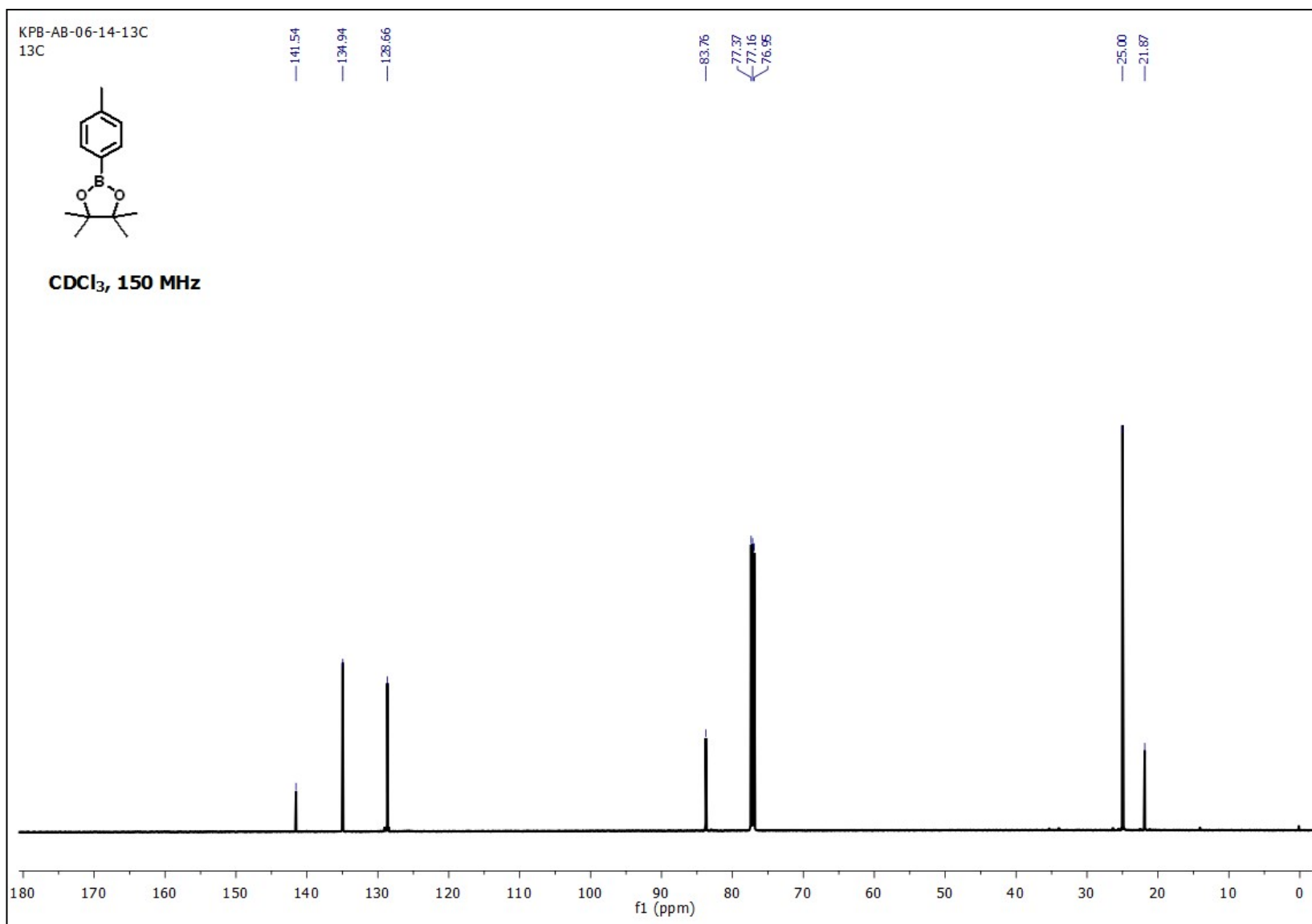


**Figure S12.** Raw images of the western blot for monitoring the inhibition of COX-2 level in the LPS-treated RAW 264.7 cells by **DCI-ROS** and **DCF**. The protein transfer on the membrane was confirmed using Ponceau S staining (A). The final western blot images (B) were taken after chemiluminescence using ECL kit. Lane details: (1) and (9) Molecular weight markers; (2) DMSO control; (3) and (6) LPS (50  $\mu$ g/ml) pre-treated cells; (4) LPS + **DCI-ROS** (50  $\mu$ M); (5) LPS + **DCI-ROS** (100  $\mu$ M); (7) LPS + **DCF** (50  $\mu$ M); (8) LPS + **DCF** (100  $\mu$ M).

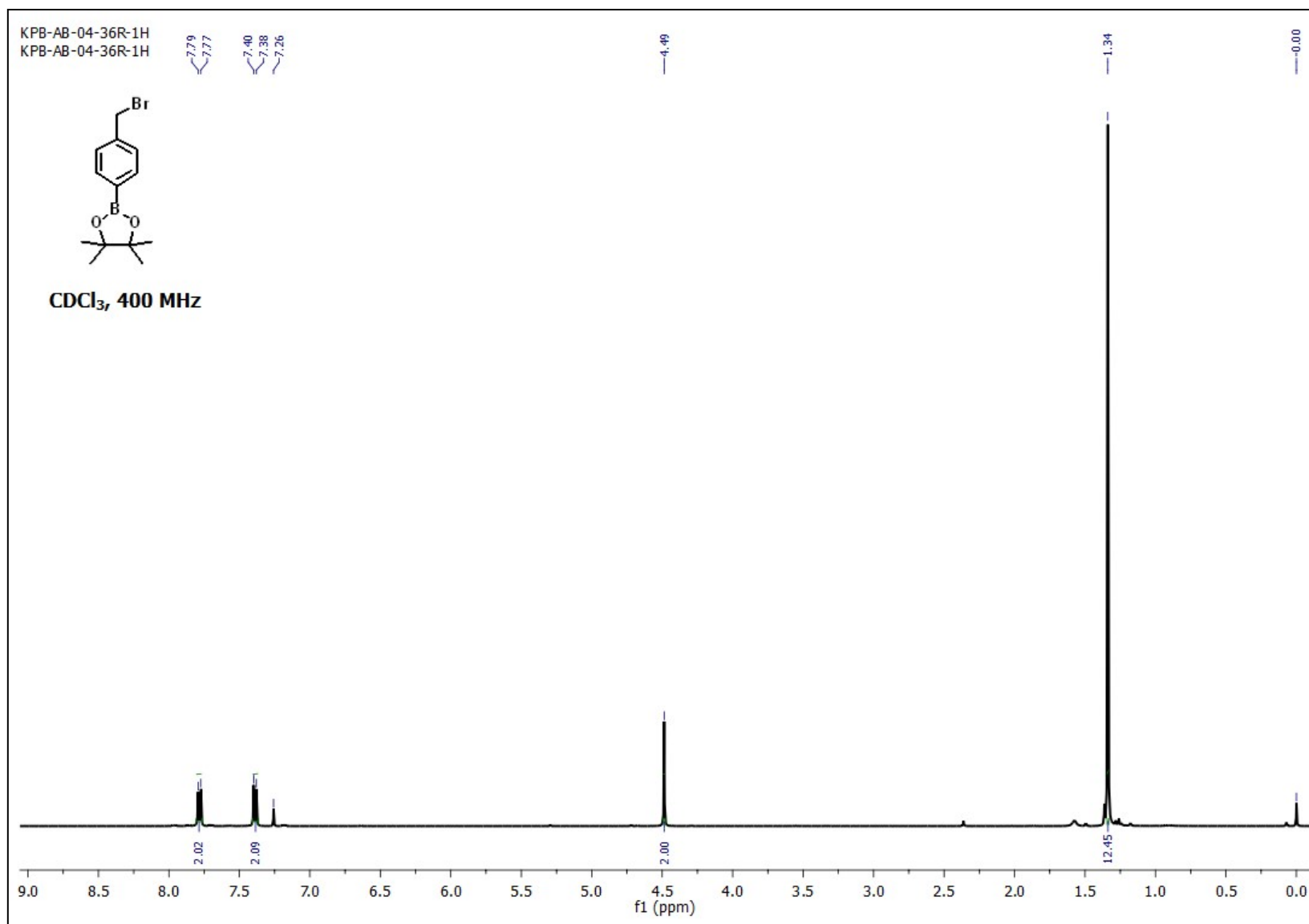
# NMR and ESI-MS spectra of the synthesized compounds



**Figure S13:** <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>, 600 MHz, ppm) of compound **2**.

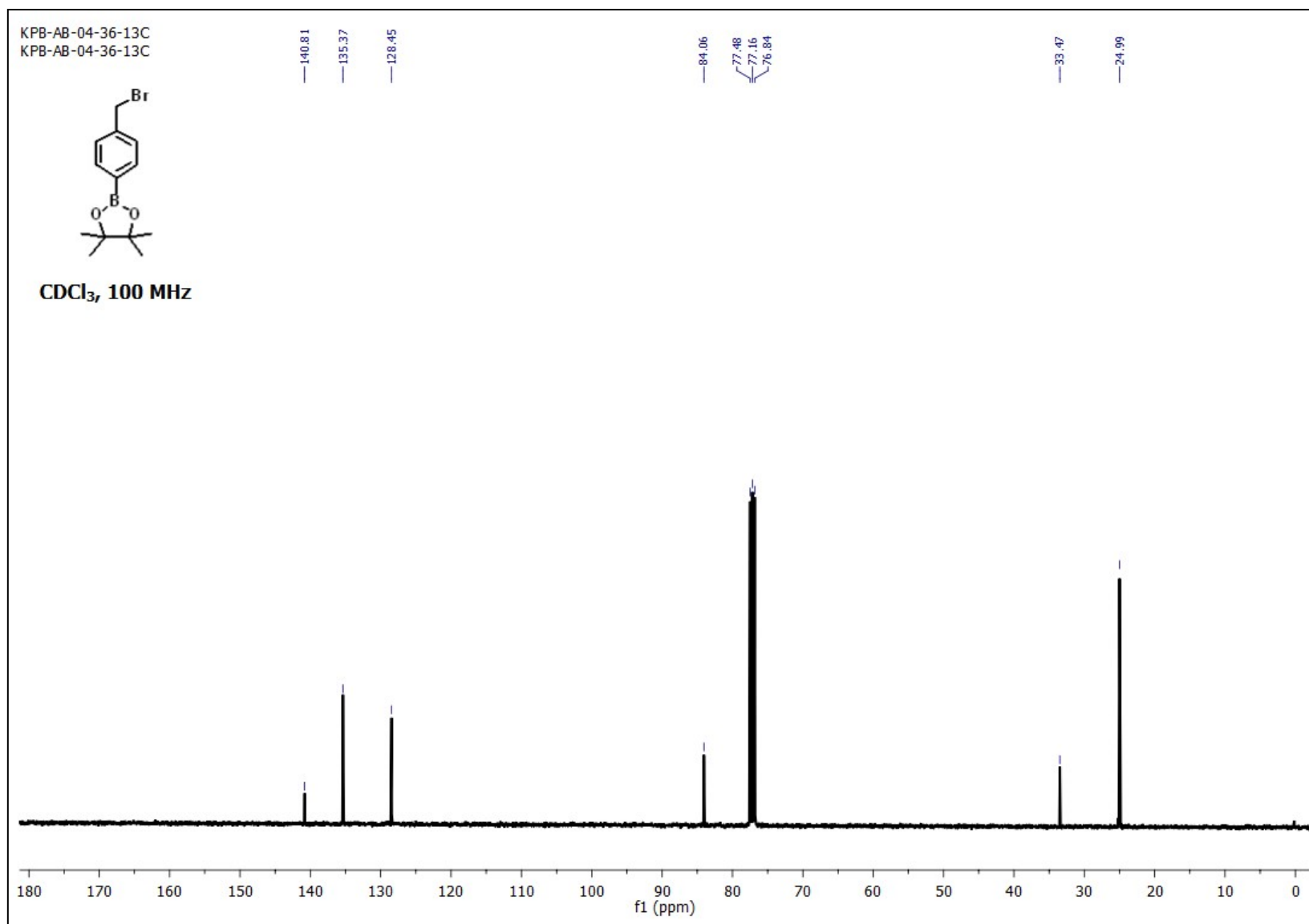


**Figure S14:** <sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>, 150 MHz, ppm) of compound **2**.

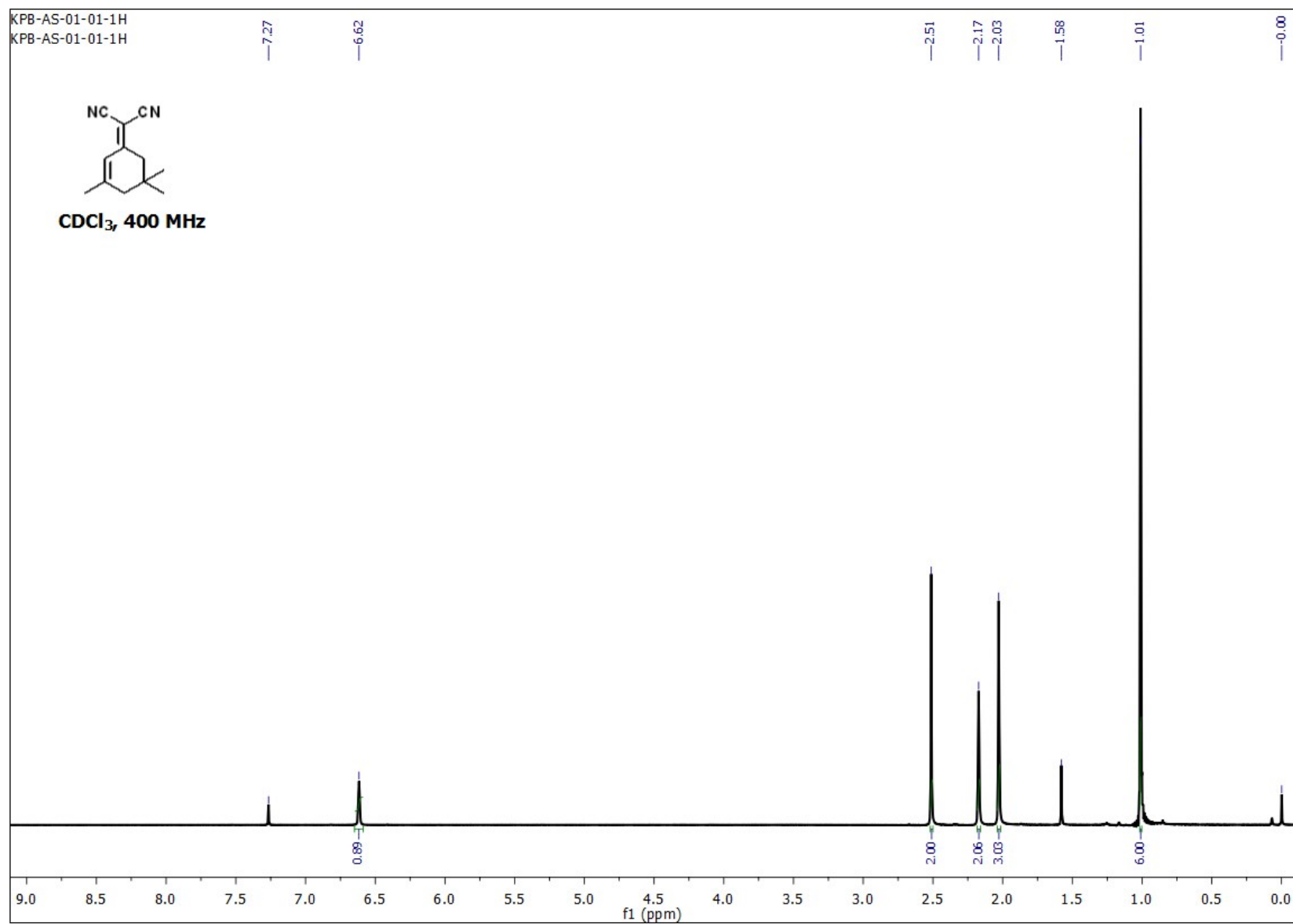


**Figure S15:**  $^1\text{H}$  NMR spectrum ( $\text{CDCl}_3$ , 400 MHz, ppm) of compound **3**.

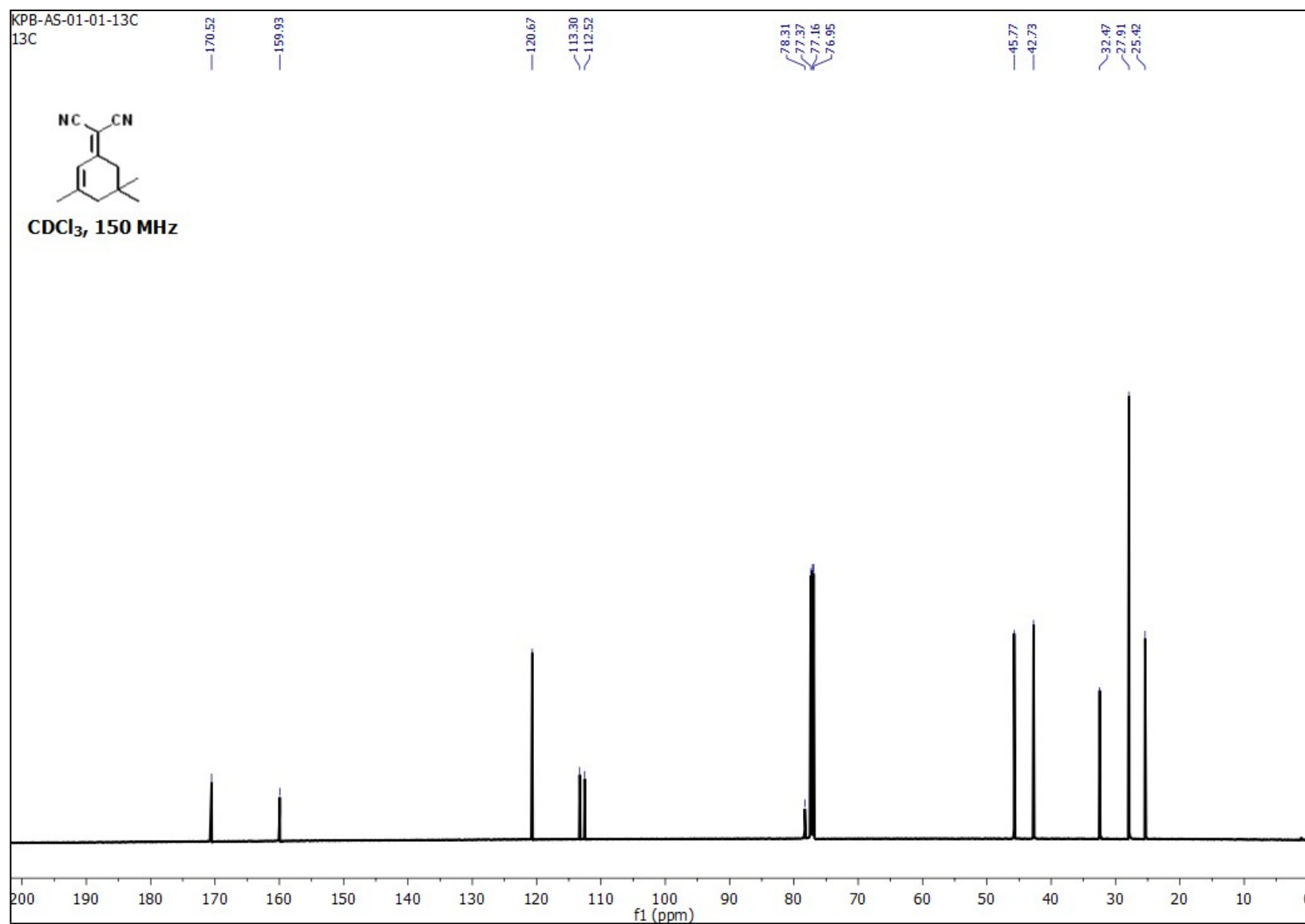




**Figure S16:** <sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>, 100 MHz, ppm) of compound **3**.



**Figure S17:** <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>, 400 MHz) of compound **5**.



**Figure S18:** <sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>, 150 MHz) of compound **5**.

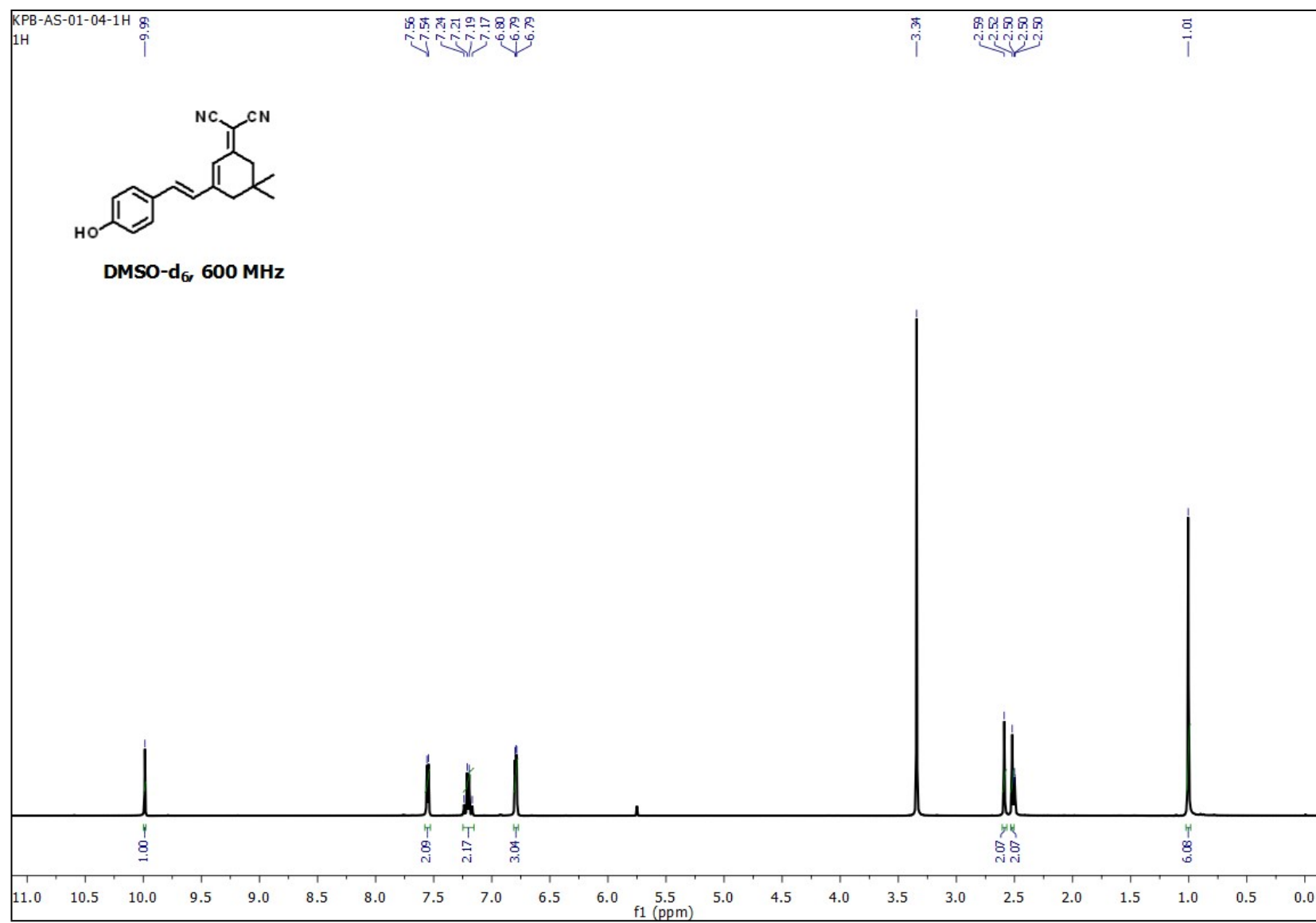


Figure S19:  $^1\text{H}$  NMR spectrum (DMSO- $d_6$ , 600 MHz) of DCI-OH.

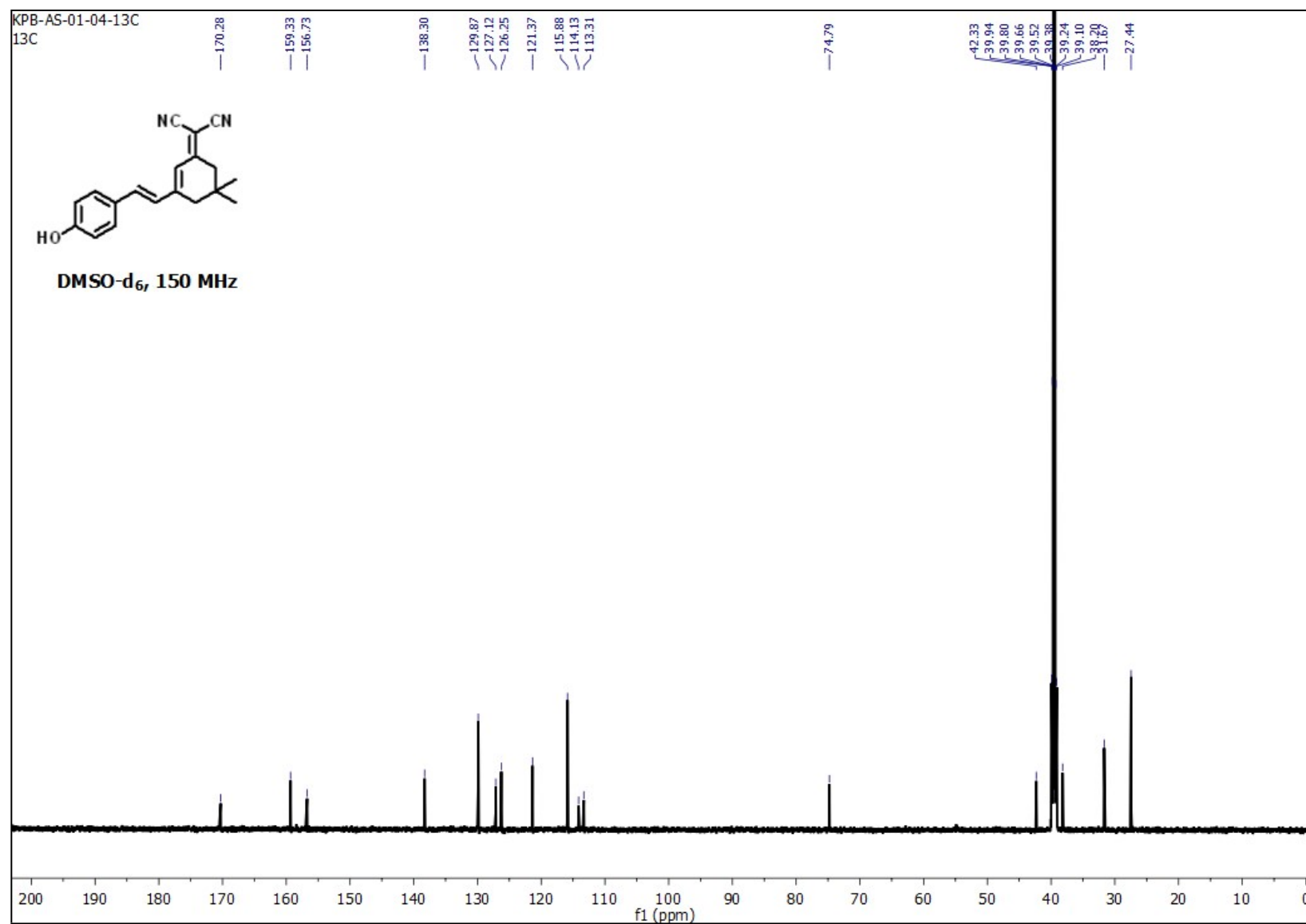
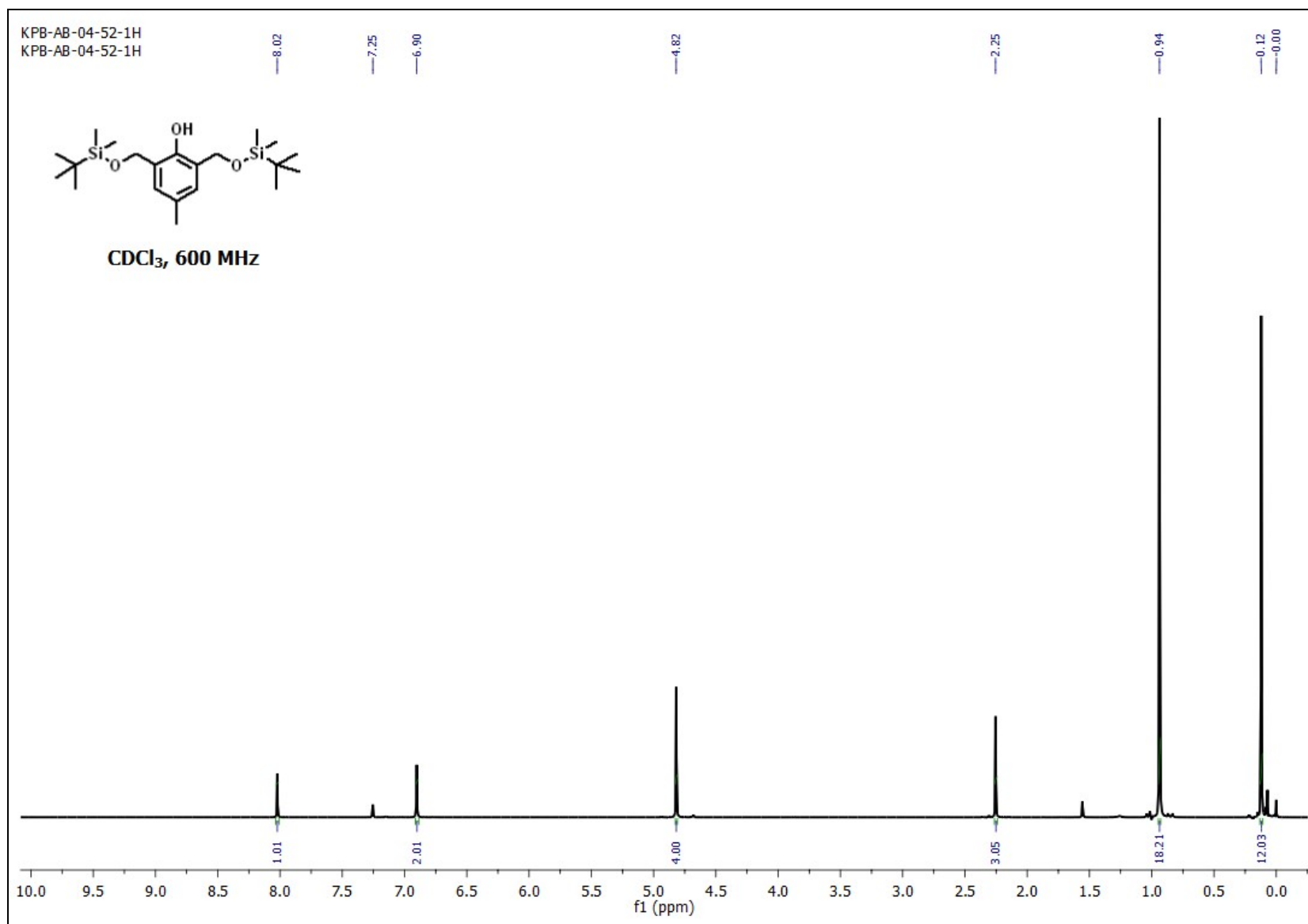
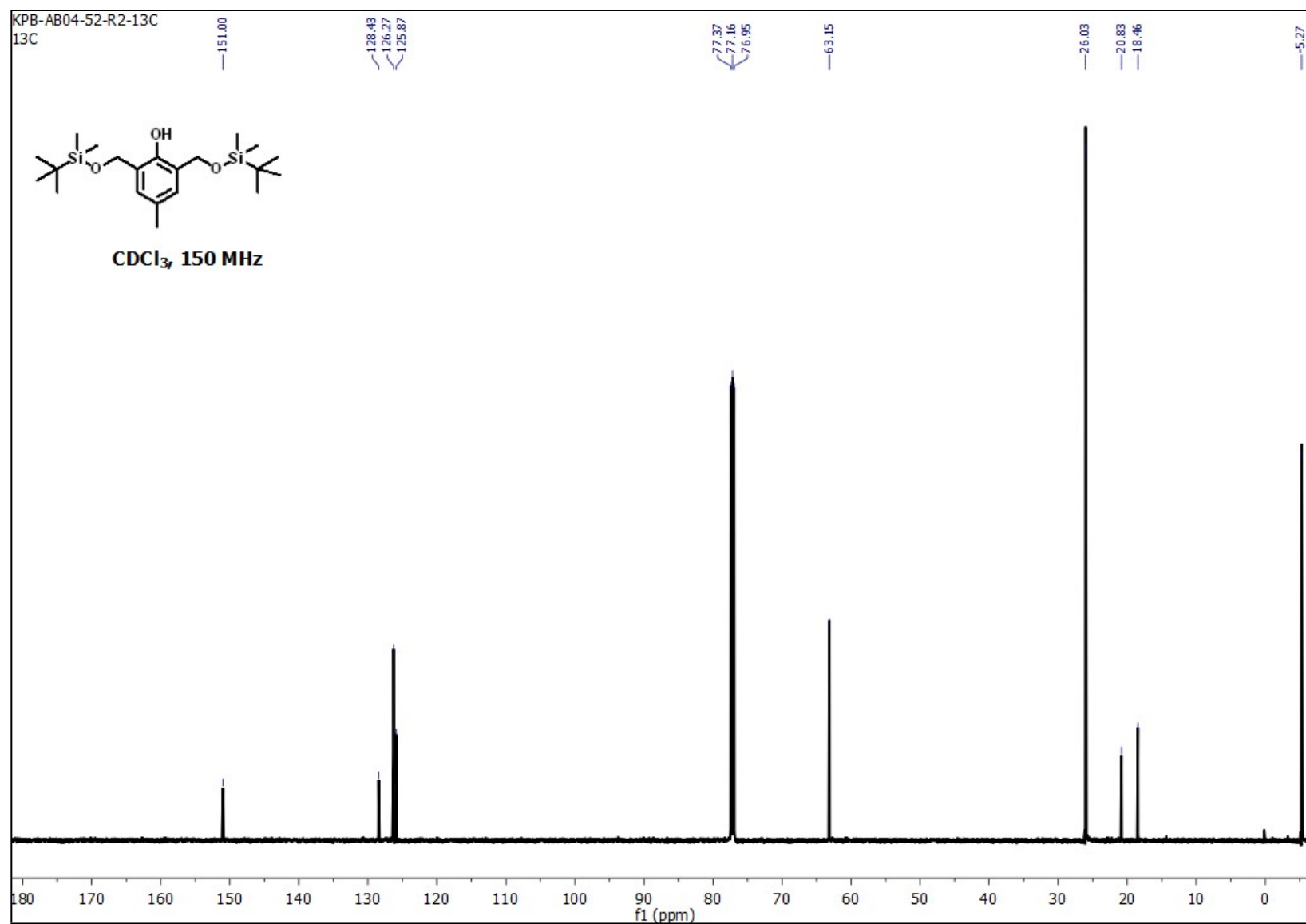


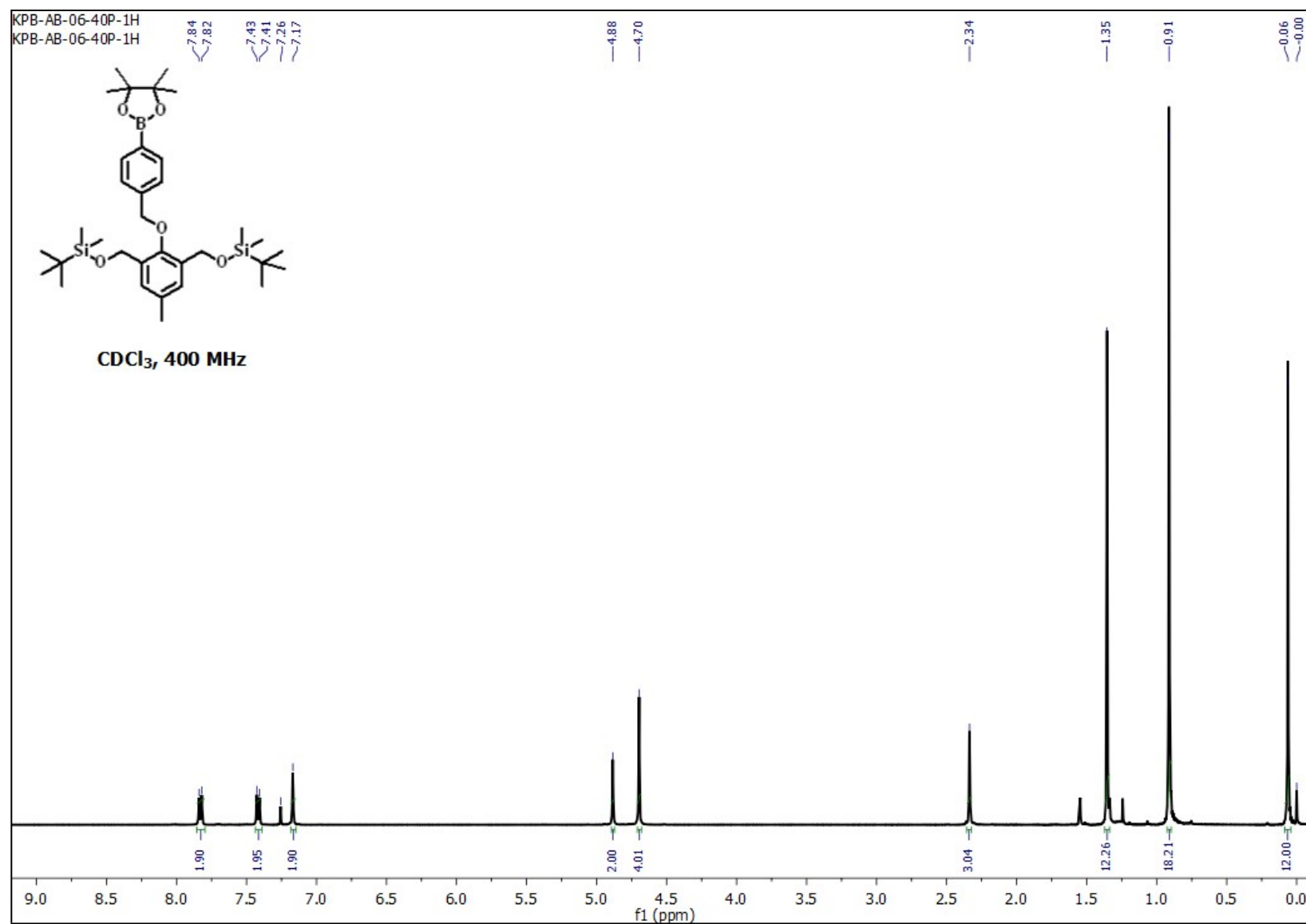
Figure S20: <sup>13</sup>C NMR spectrum (DMSO-*d*<sub>6</sub>, 150 MHz) of DCI-OH.



**Figure S21:** <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>, 600 MHz) of compound 7.

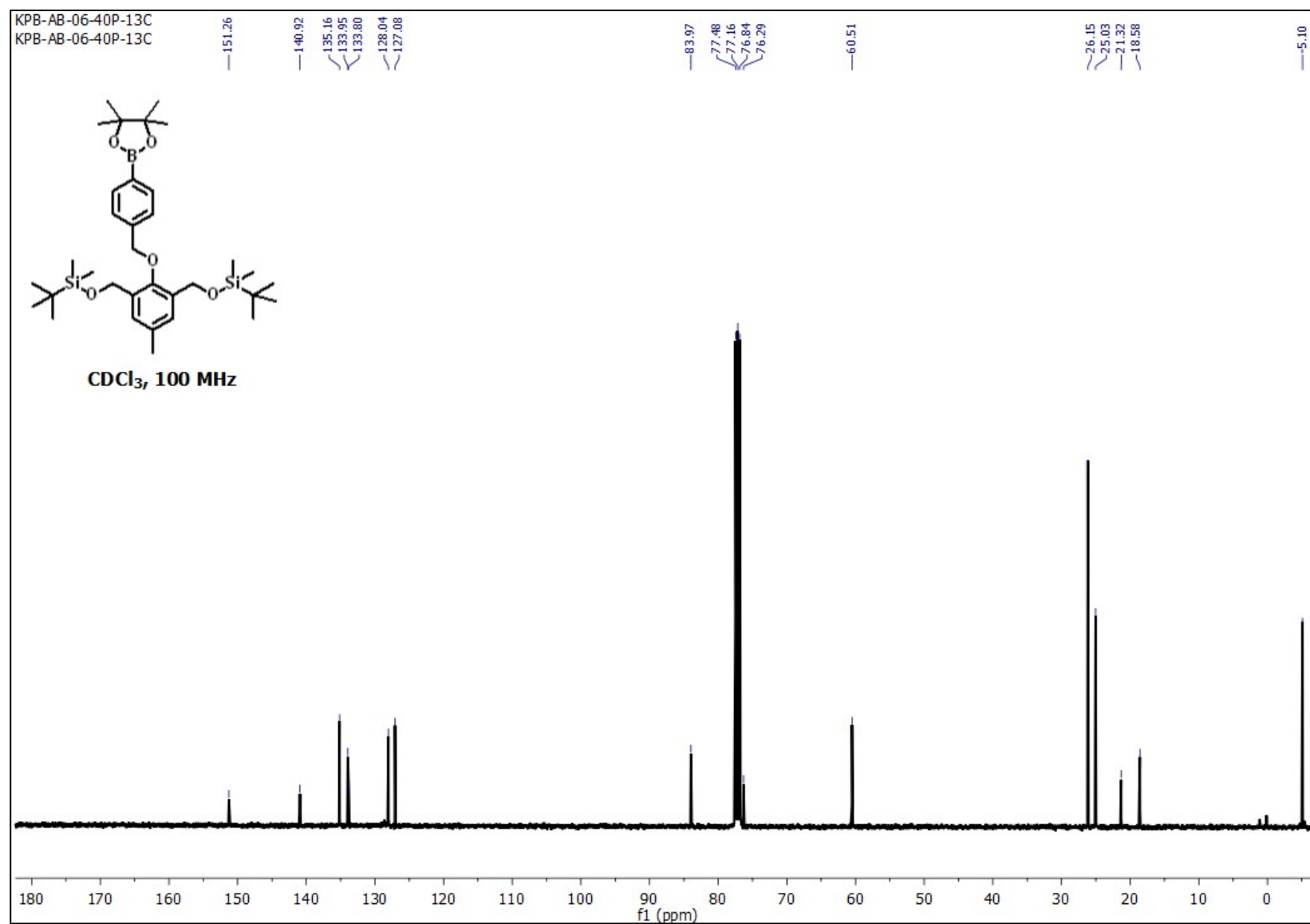


**Figure S22:** <sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>, 150 MHz) of compound 7.



**Figure S23:**  $^1\text{H}$  NMR spectrum ( $\text{CDCl}_3$ , 400 MHz) of compound **8**.





**Figure S24:** <sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>, 100 MHz) of compound **8**.

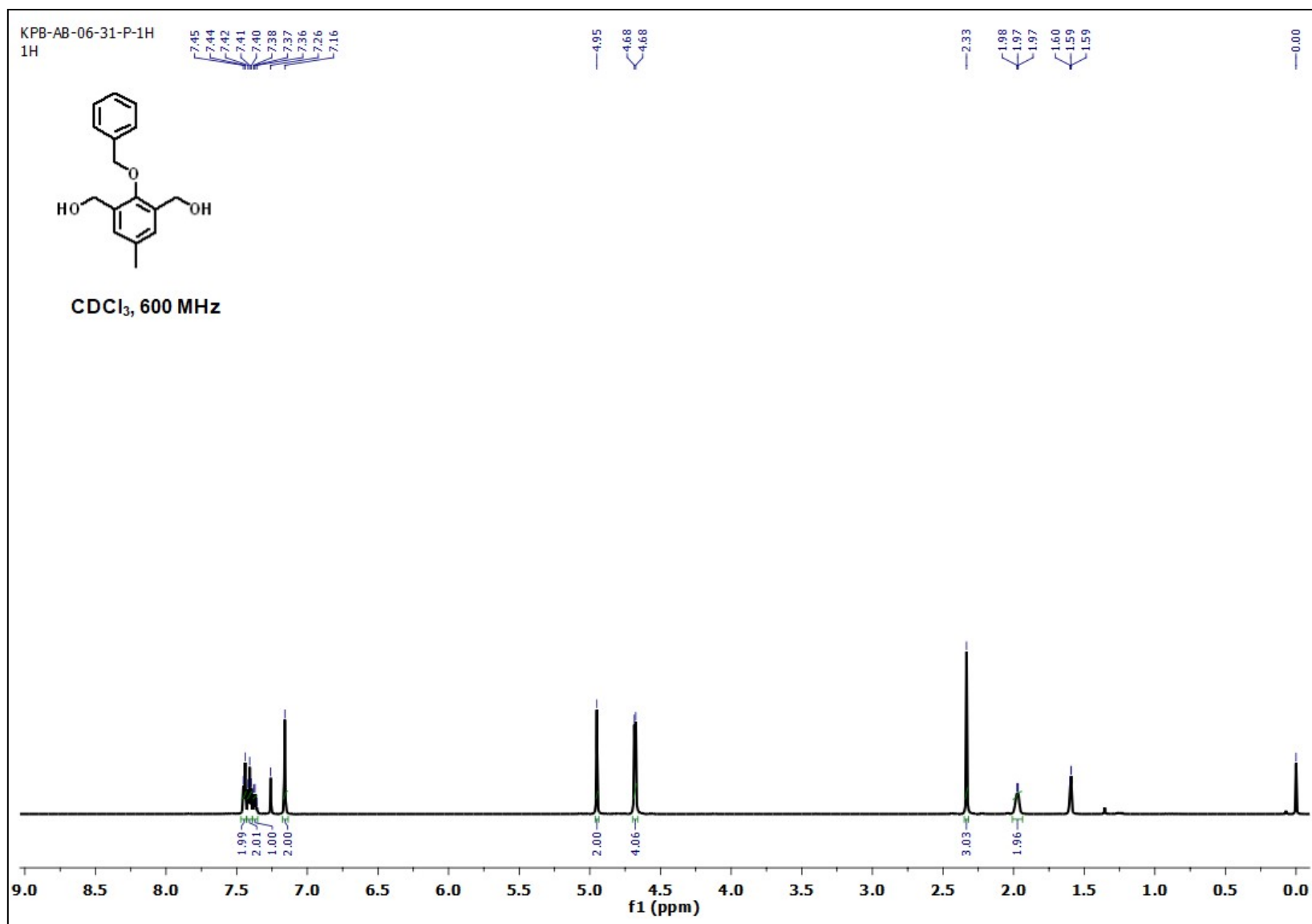
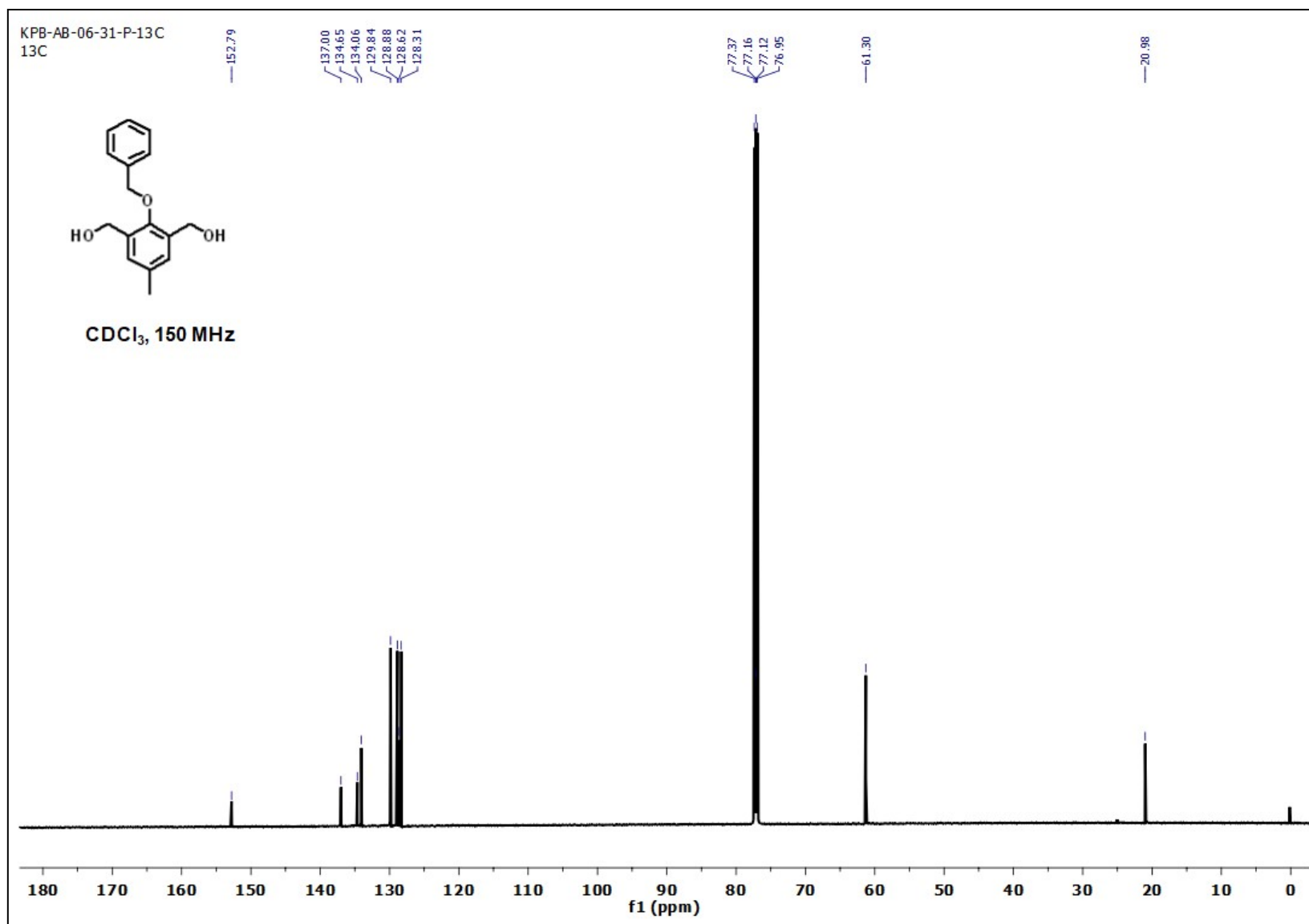


Figure S25: <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>, 600 MHz) of compound **9**.



**Figure S26:** <sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>, 150 MHz) of compound **9**.

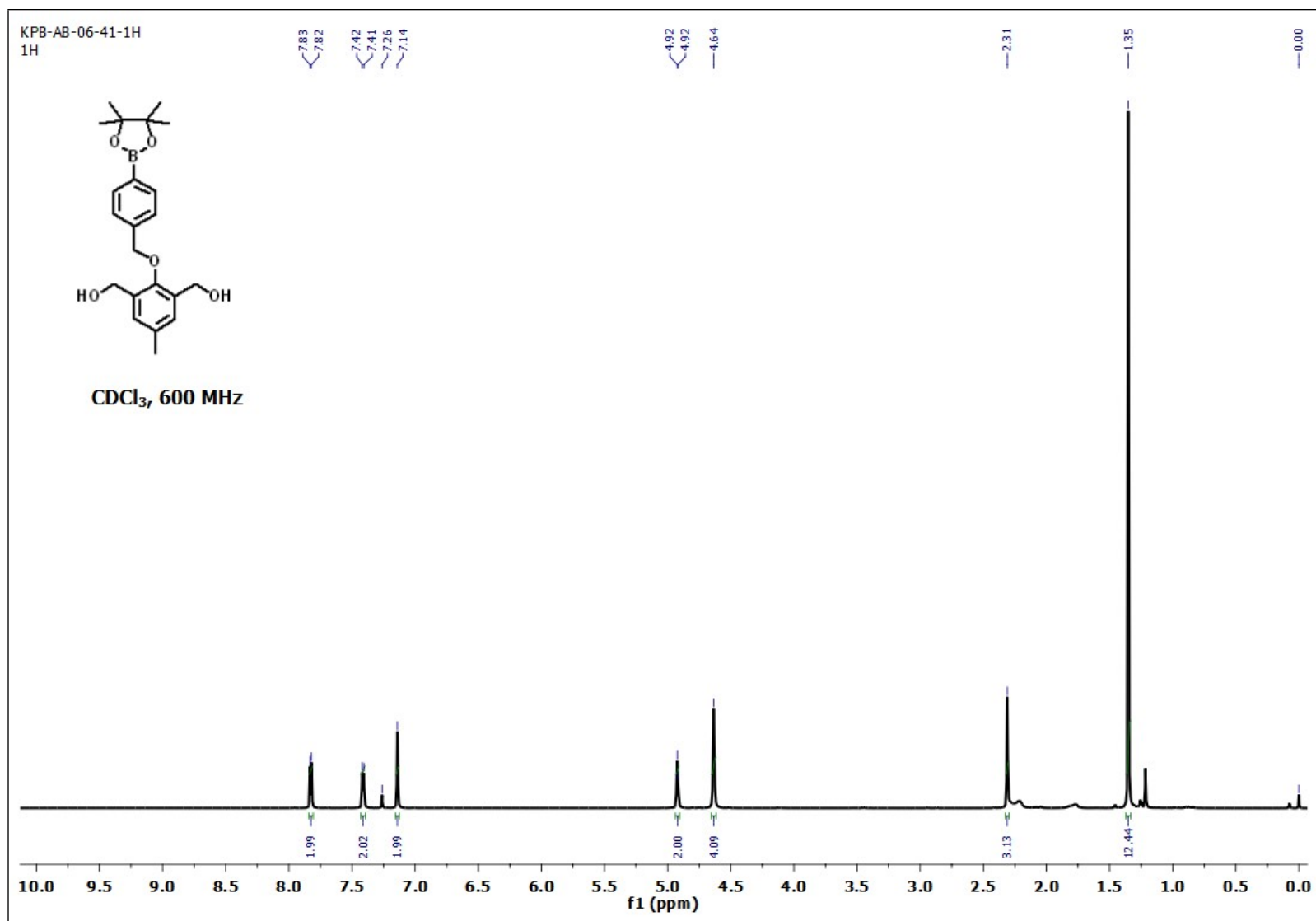
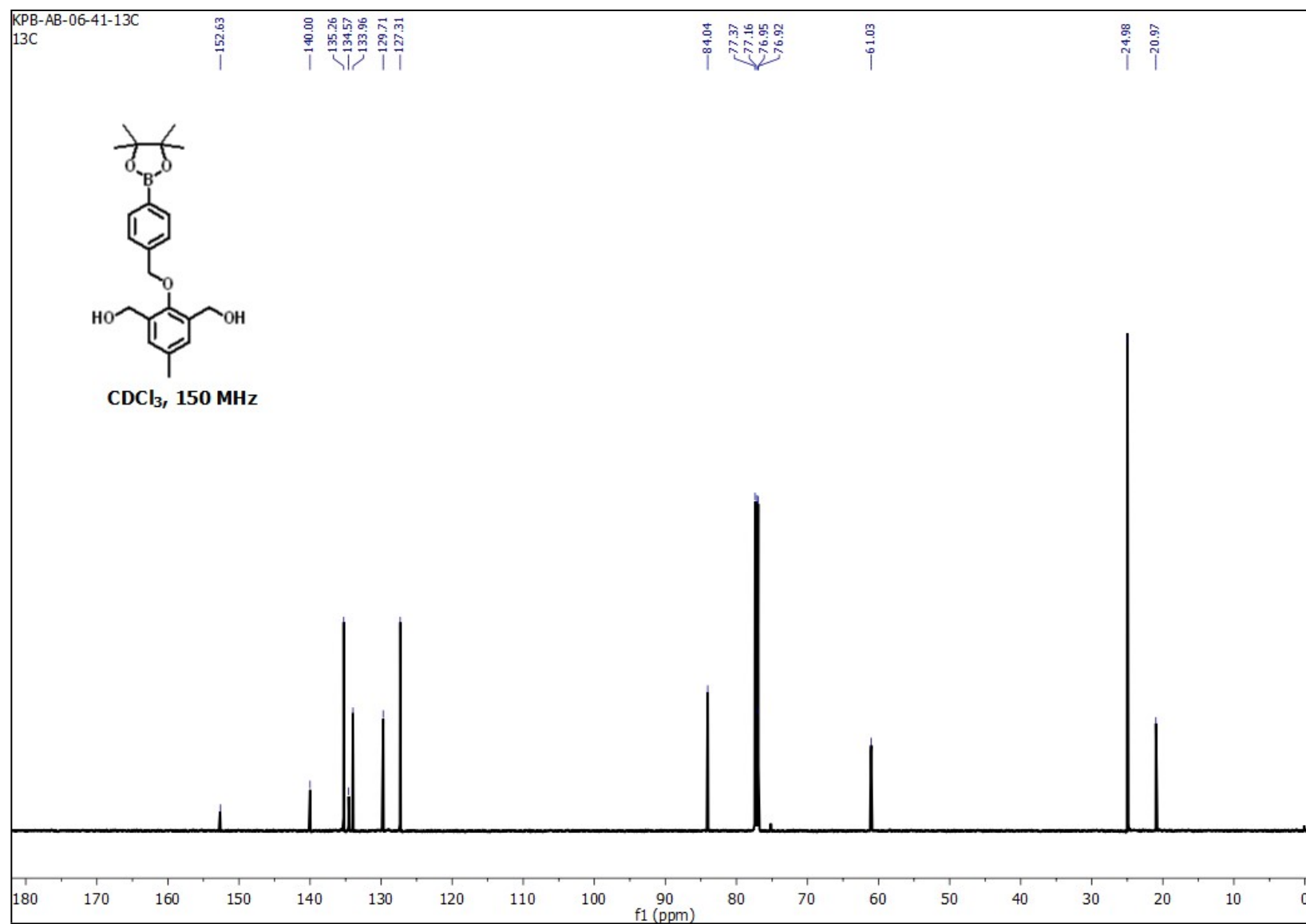


Figure S27: <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>, 600 MHz) of compound 10.



**Figure S28:** <sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>, 150 MHz) of compound **10**.

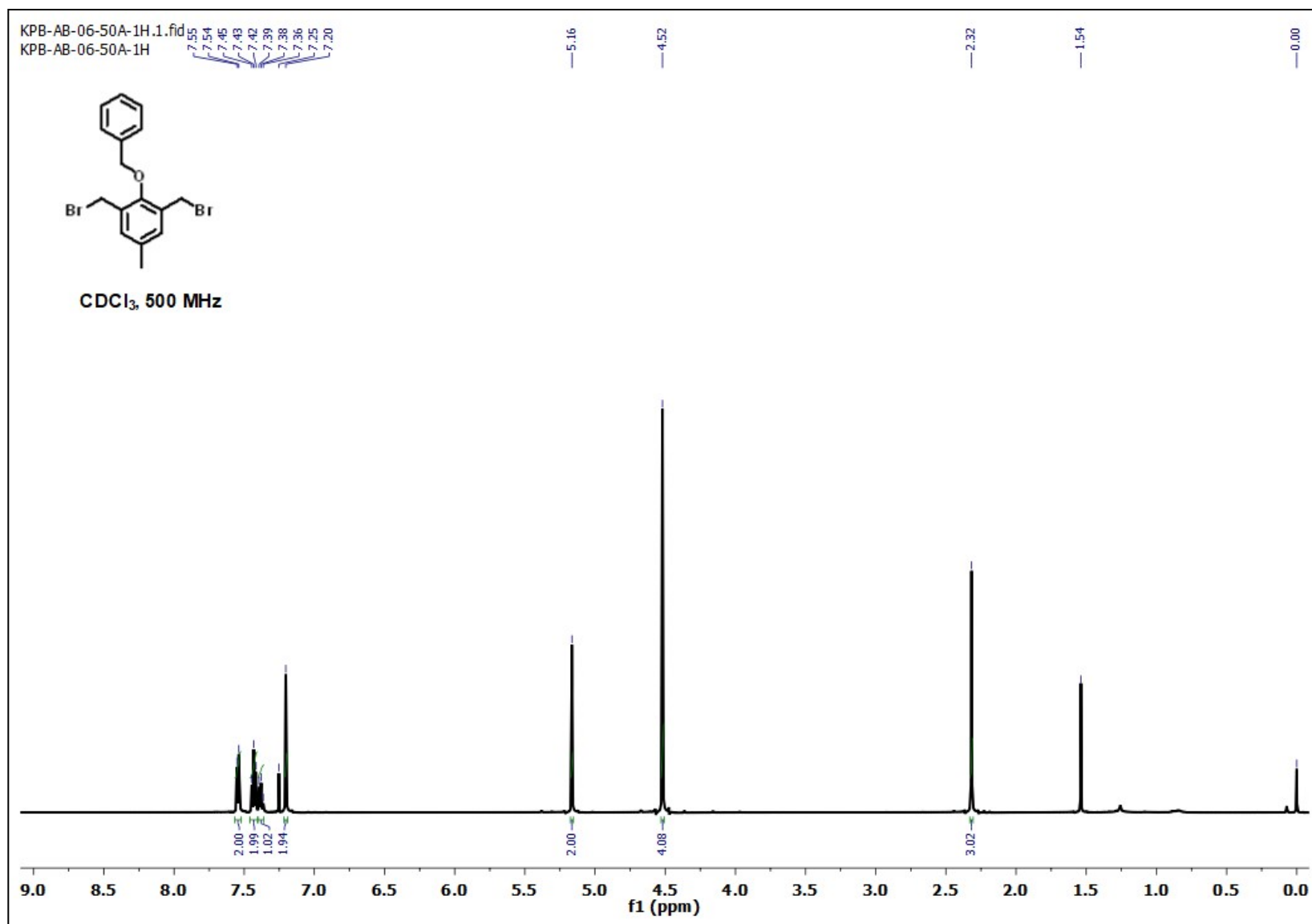


Figure S29: <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>, 500 MHz) of compound 11.

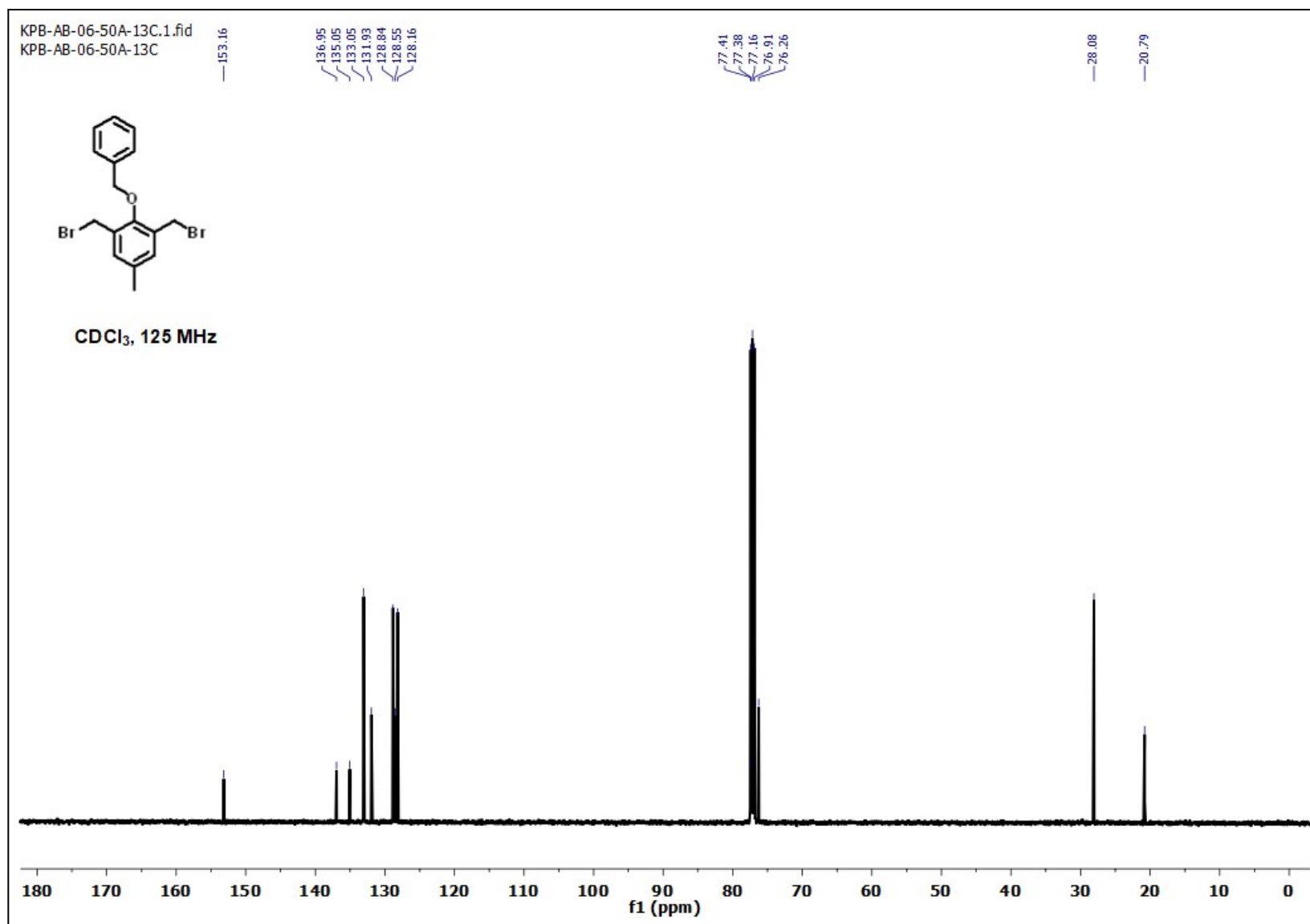
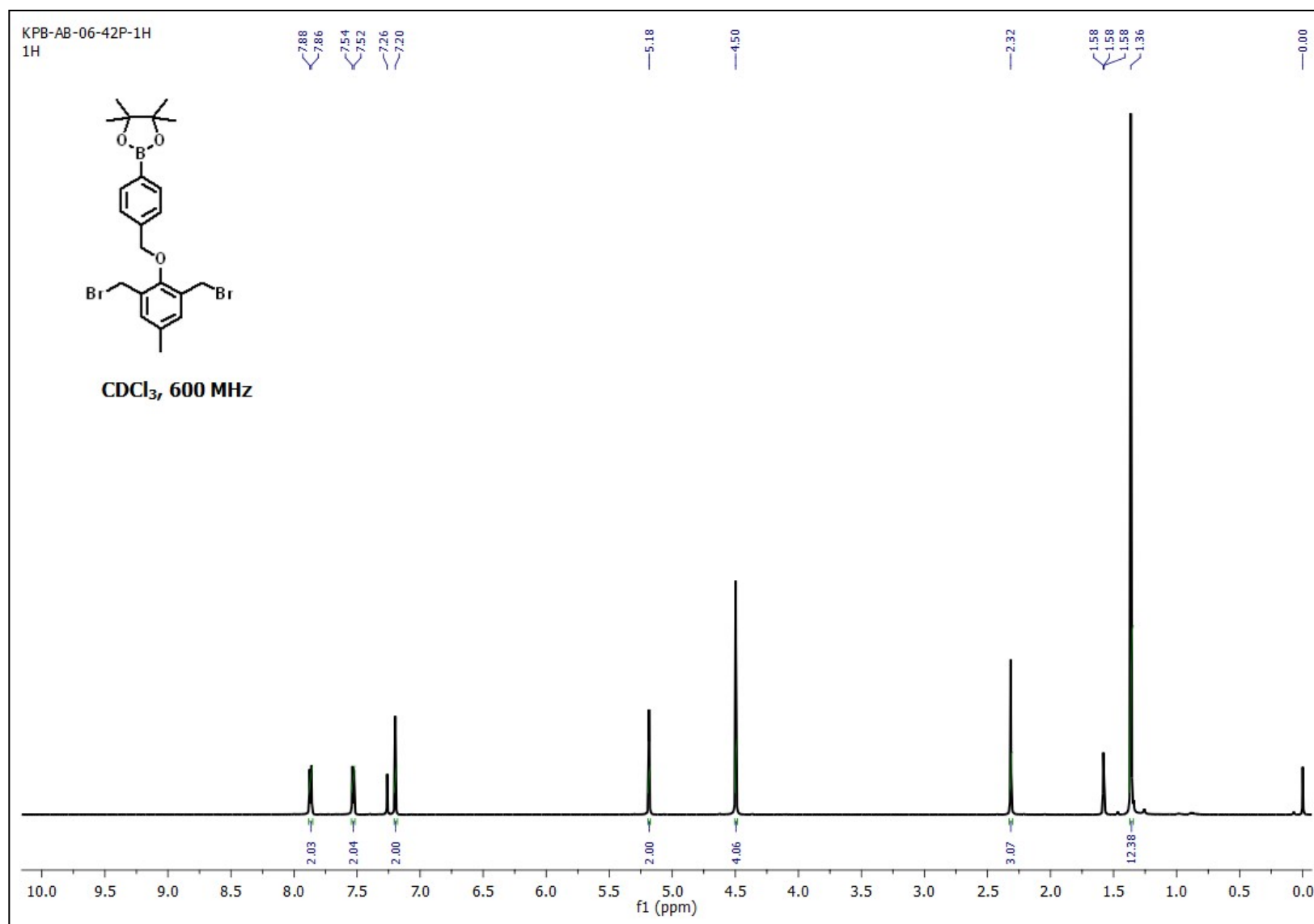
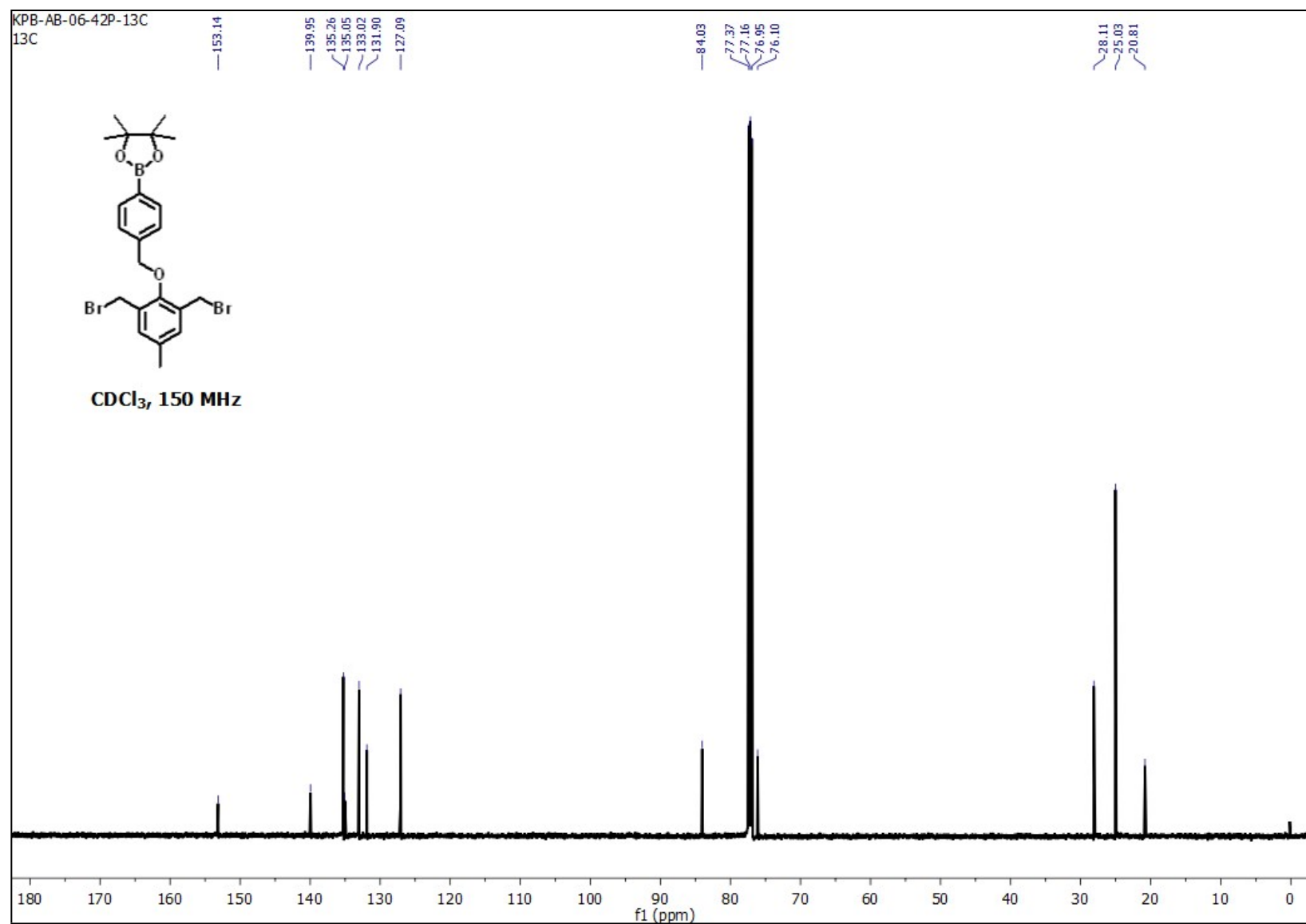


Figure S30: <sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>, 125 MHz) of compound **11**.



**Figure S31:** <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>, 600 MHz) of compound **12**.





**Figure S32:** <sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>, 150 MHz) of compound **12**.

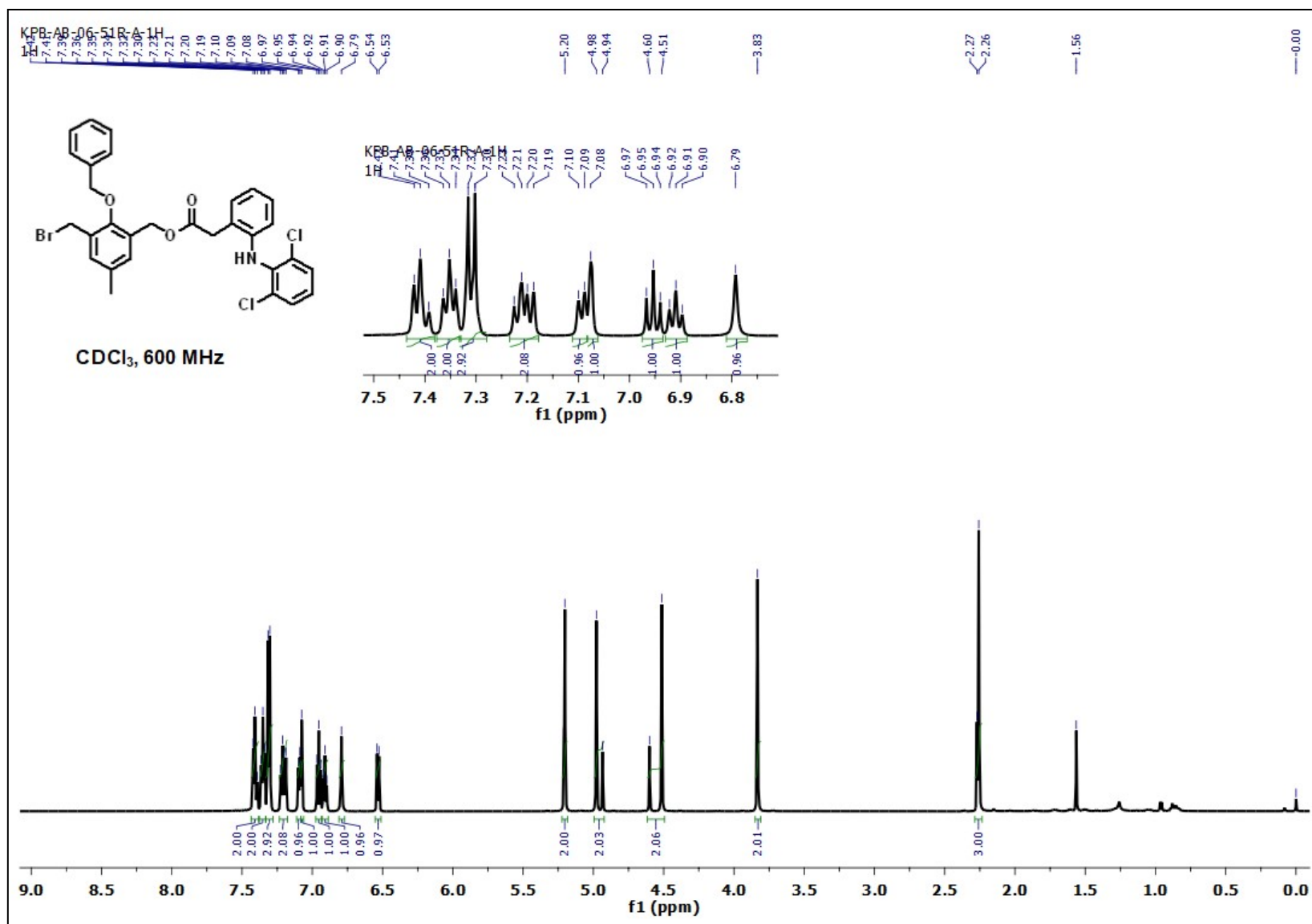


Figure S33:  $^1\text{H}$  NMR spectrum ( $\text{CDCl}_3$ , 600 MHz) of compound 13.

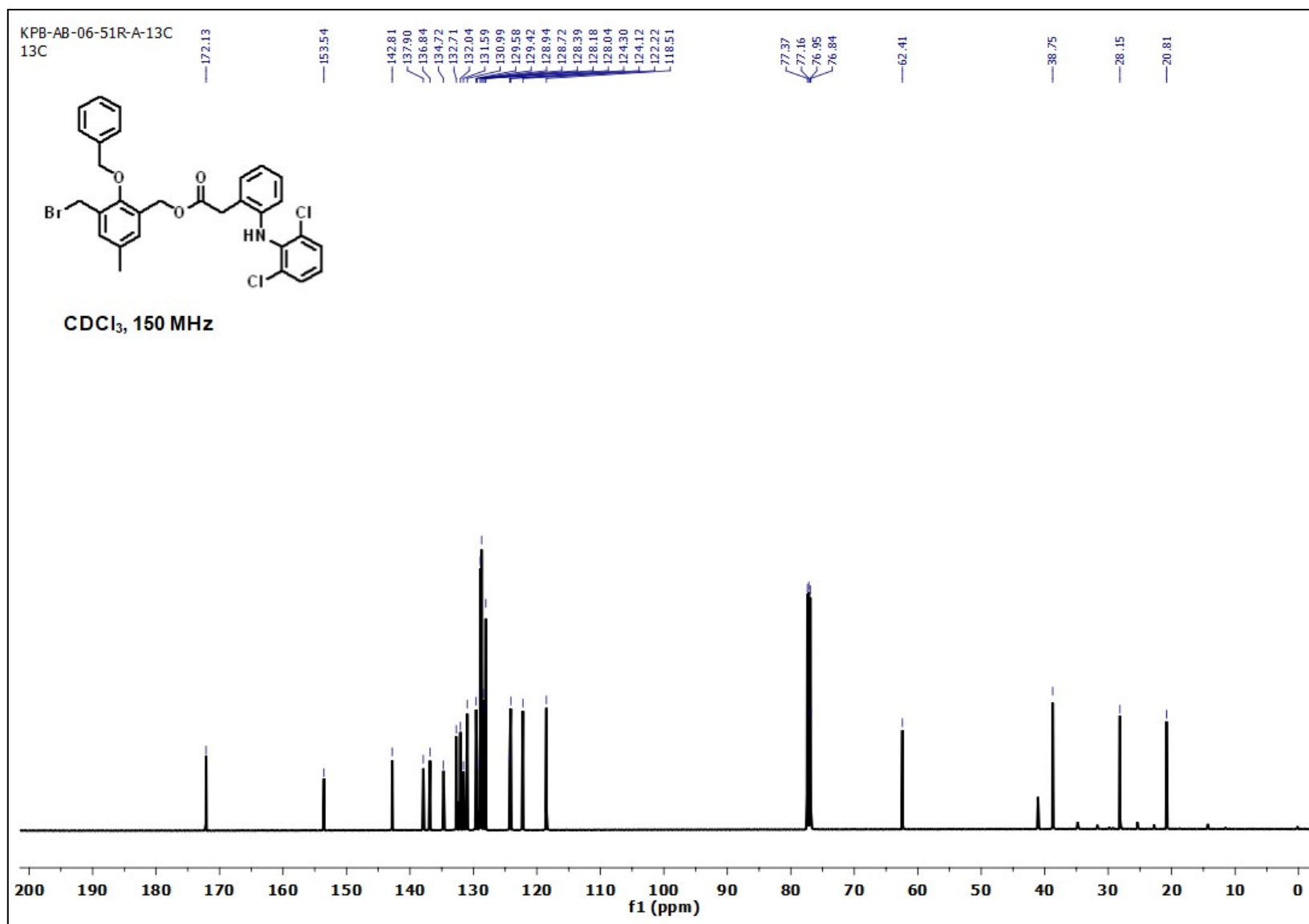


Figure S34: <sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>, 150 MHz) of compound 13.

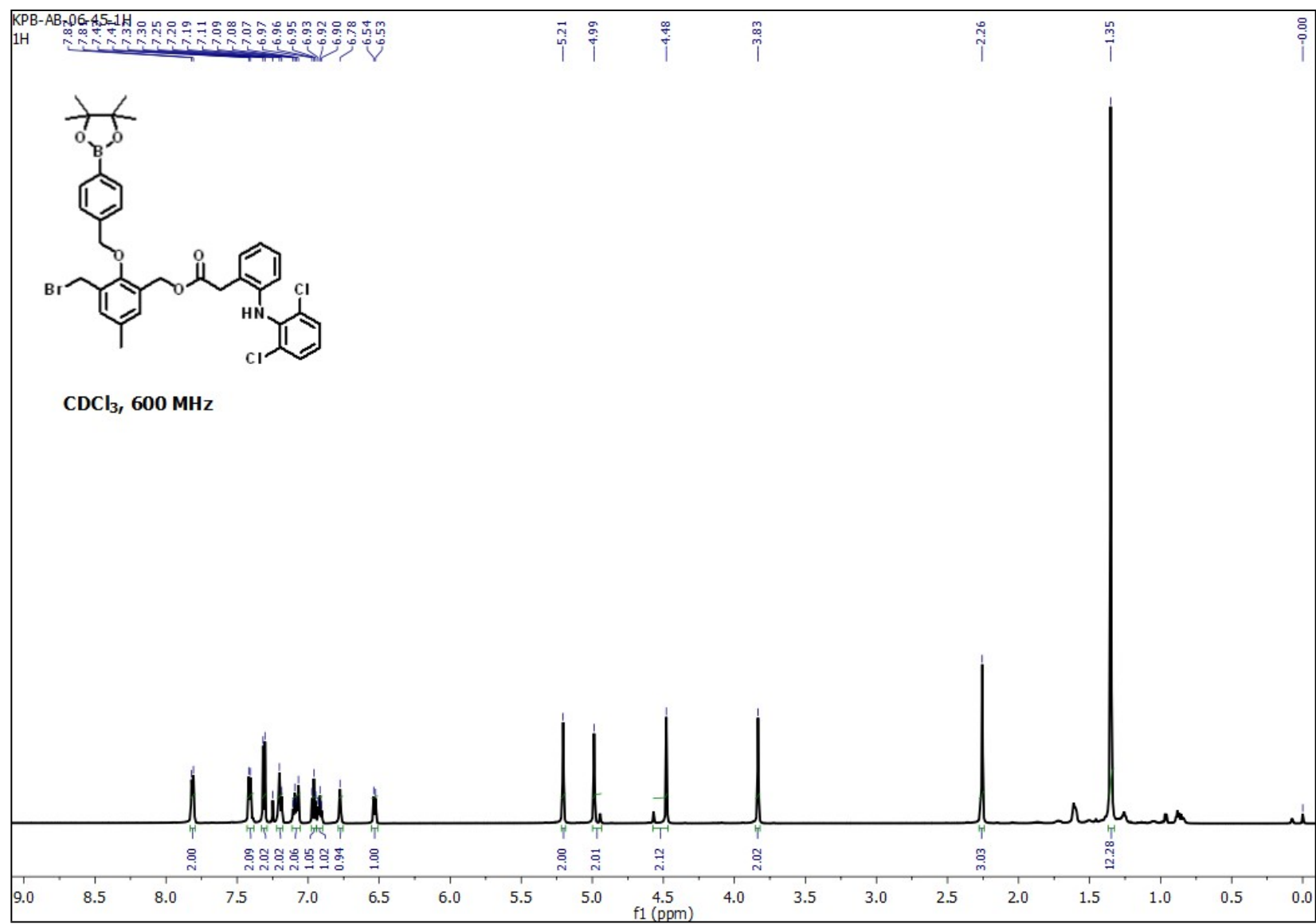
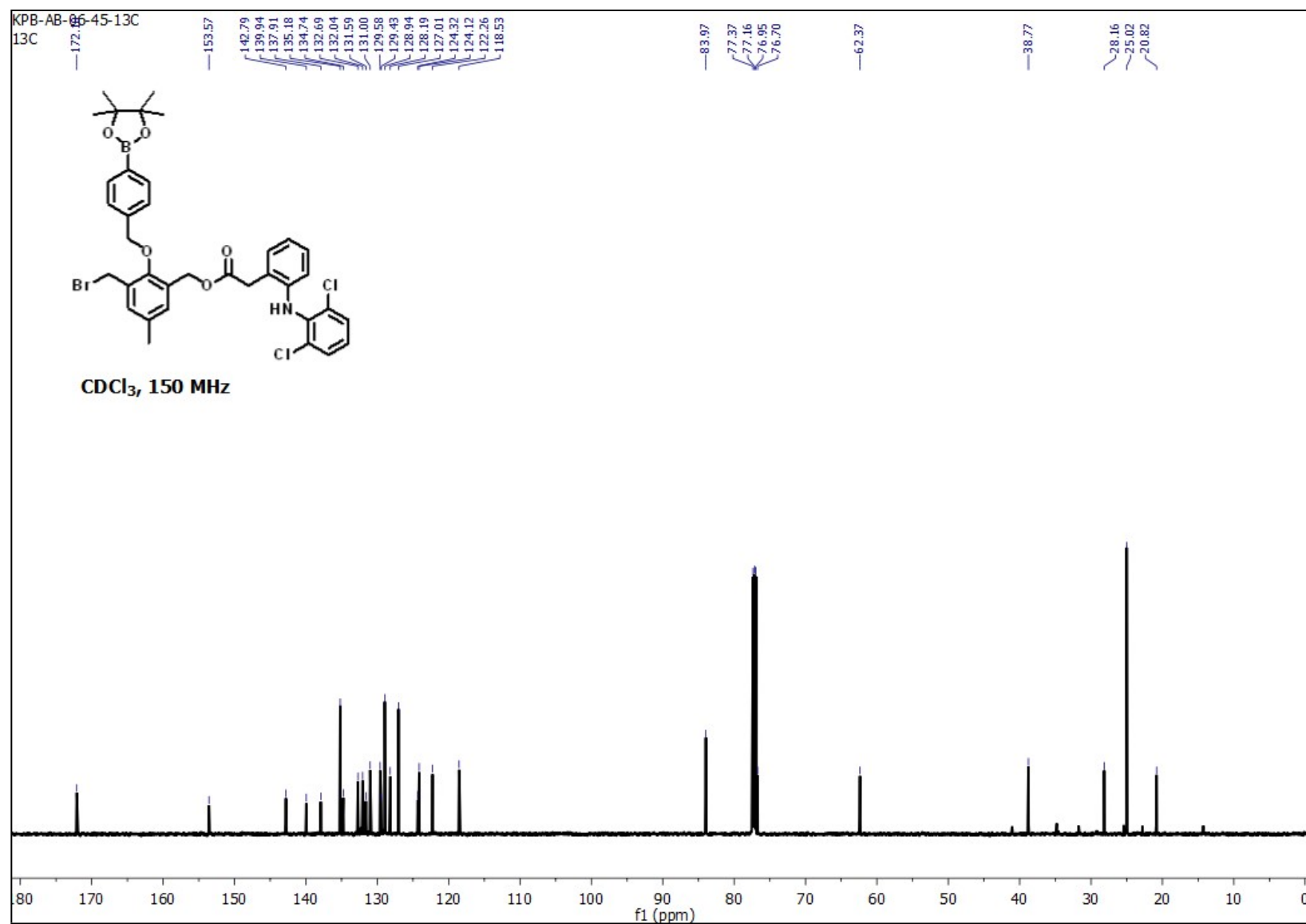


Figure S35: <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>, 600 MHz) of compound 14.



**Figure S36:** <sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>, 150 MHz) of compound **14**.

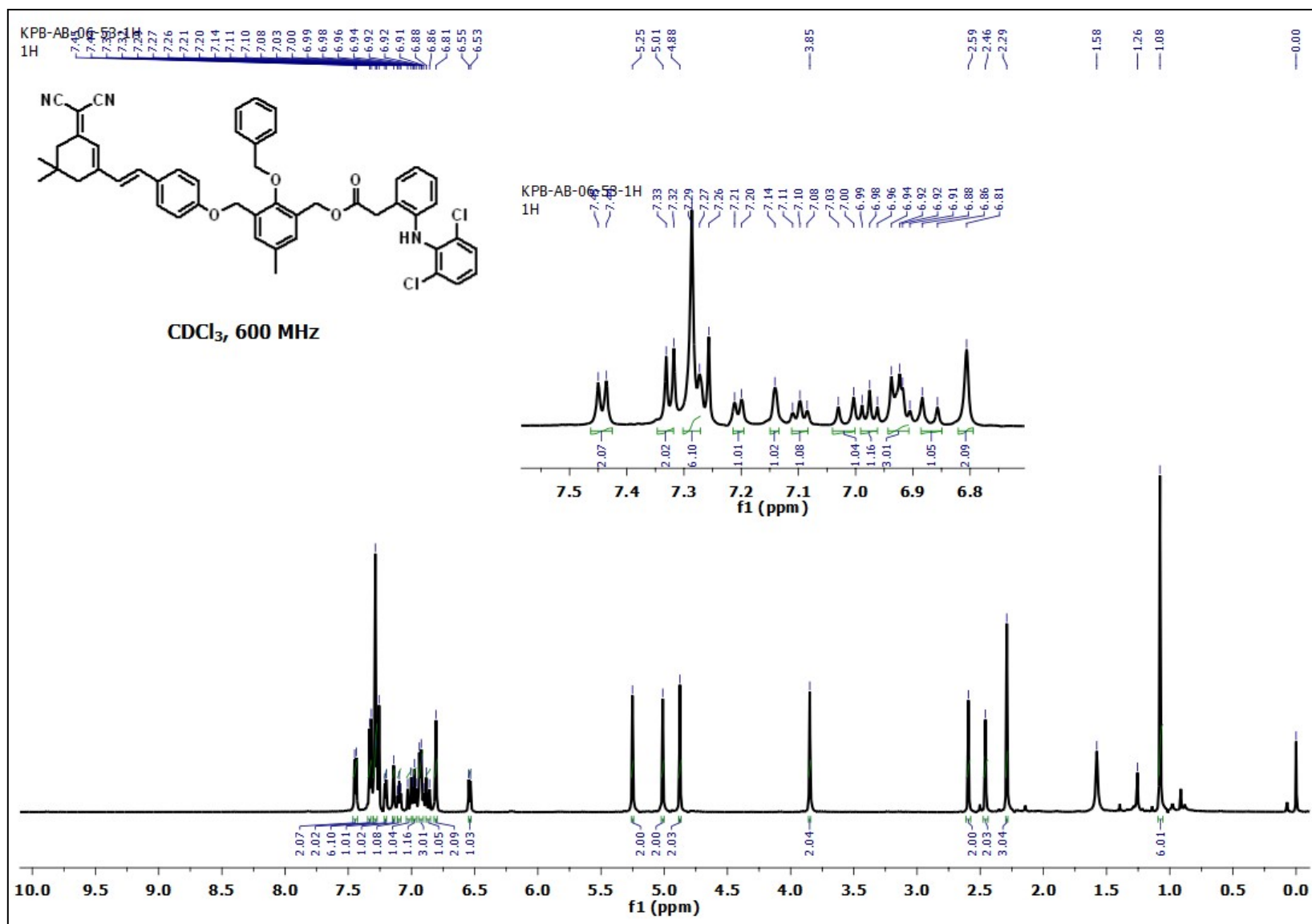


Figure S37: <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>, 600 MHz) of DCI-Con.

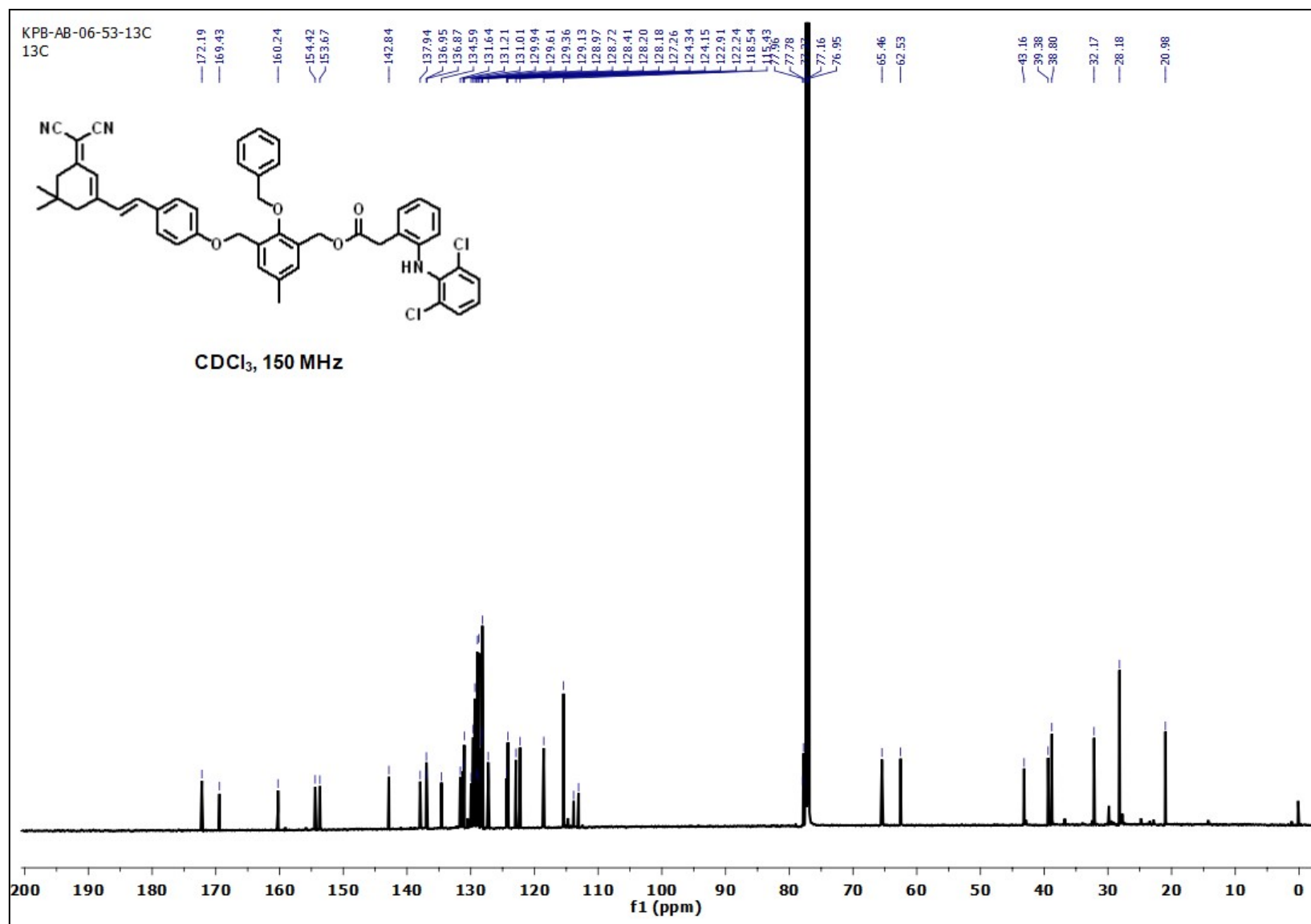
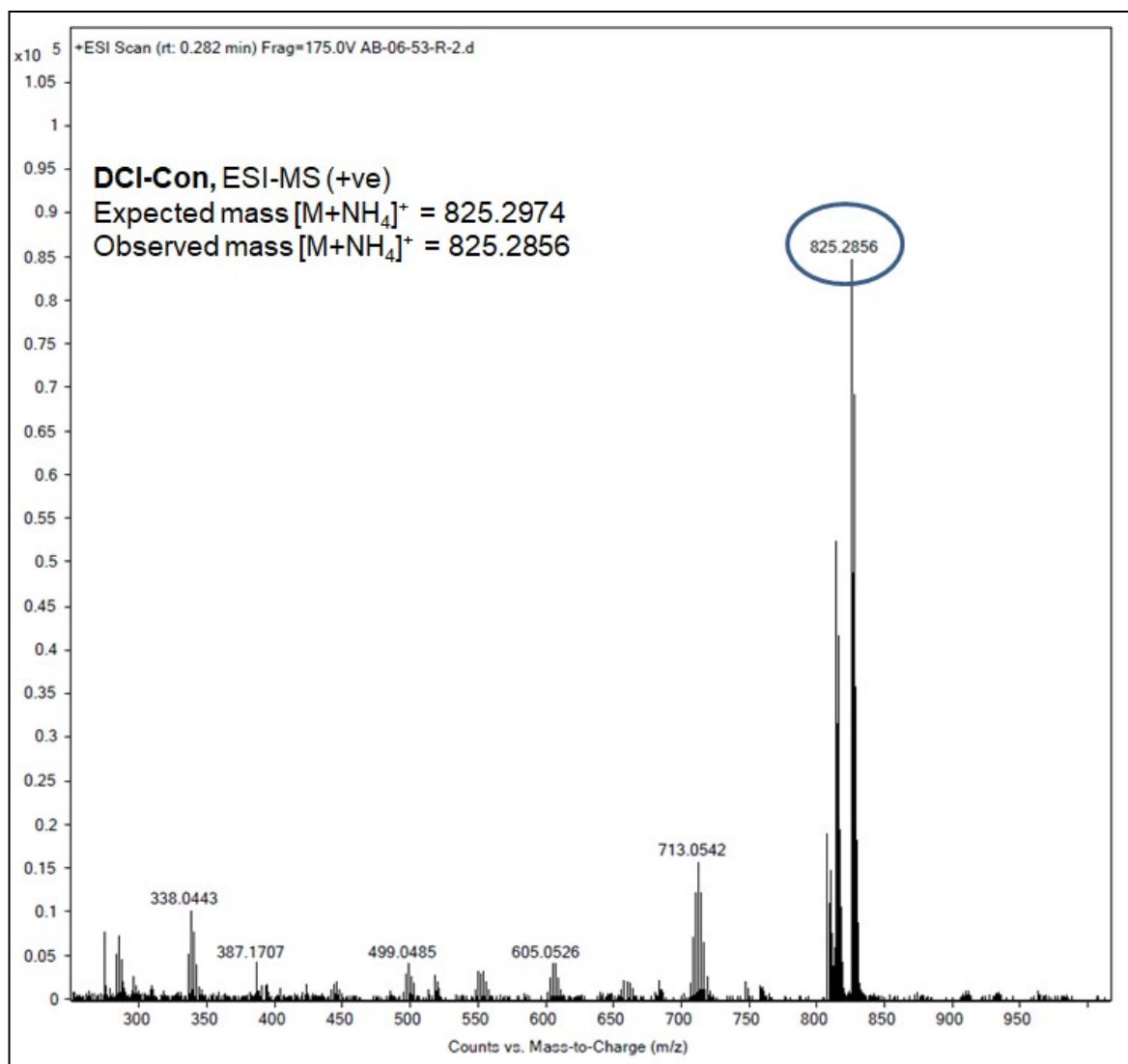


Figure S38: <sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>, 150 MHz) of DCI-Con.



**Figure S39:** ESI – MS spectrum of **DCI-Con**, ESI-MS (+ve)  $m/z$  calcd for  $C_{49}H_{43}Cl_2N_3O_4$   $[M + NH_4]^+$ : 825.2974; obs. 825.2856.



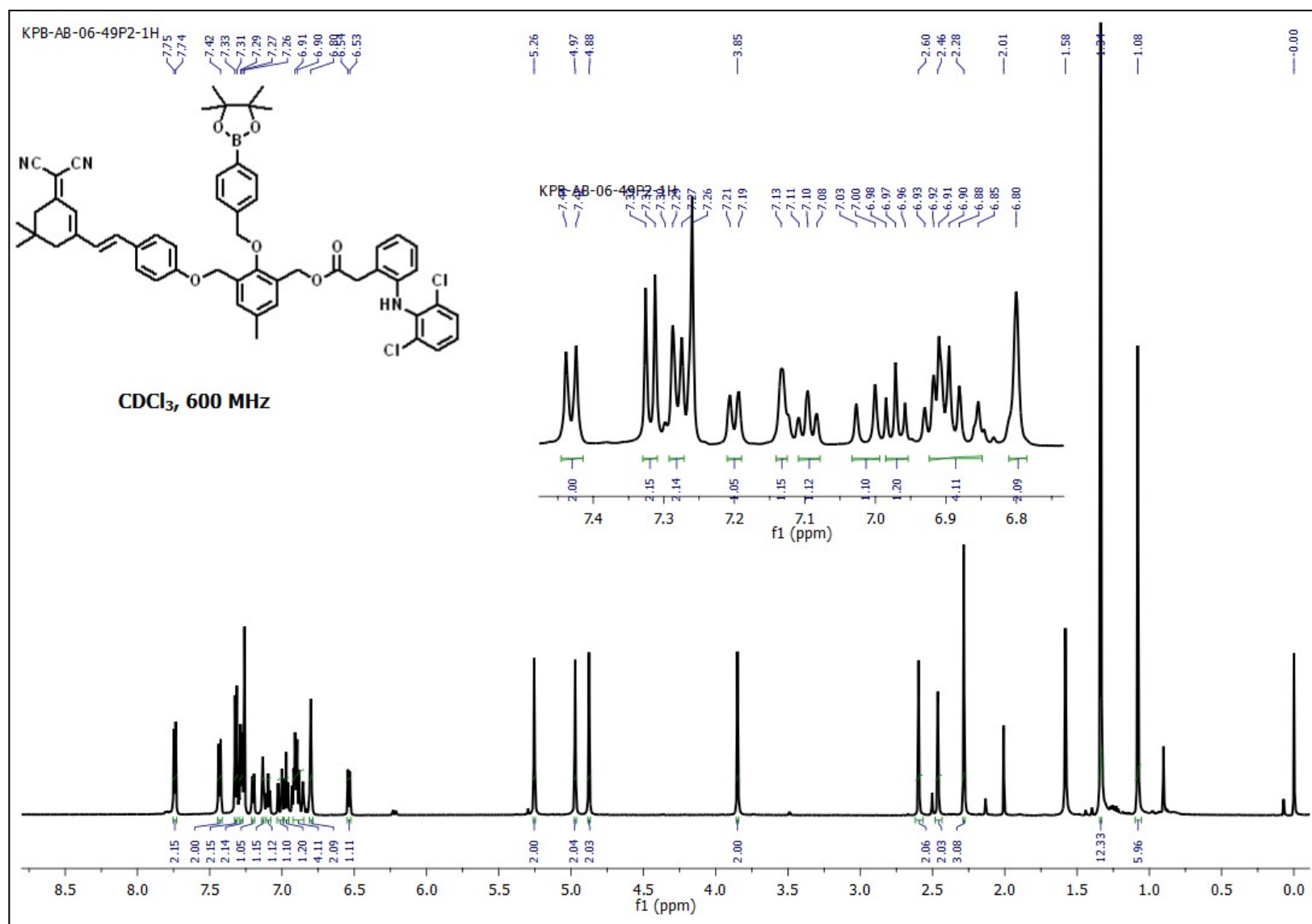


Figure S40: <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>, 600 MHz) of DCI-ROS.

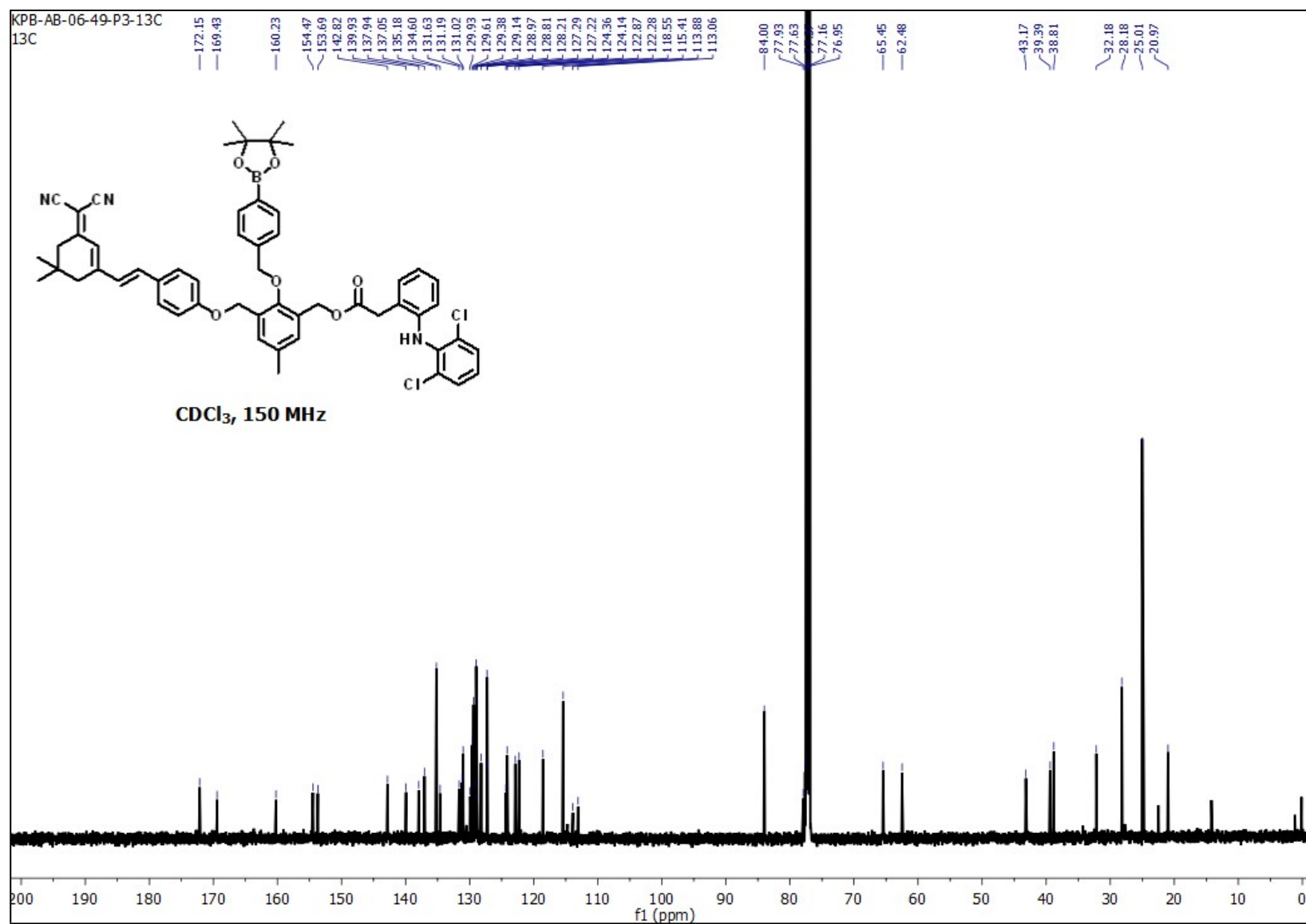
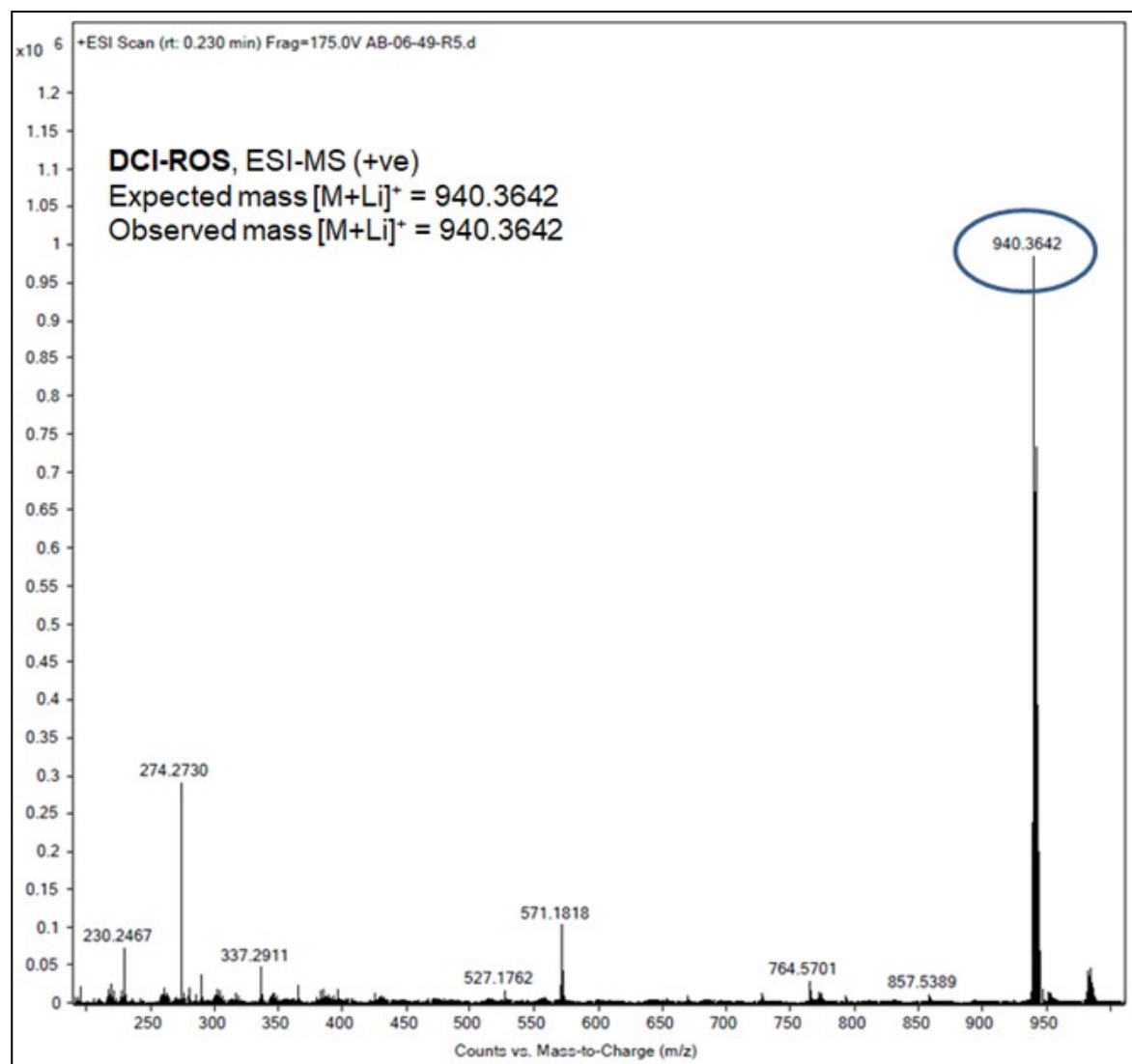


Figure S41: <sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>, 150 MHz) of DCI-ROS.



**Figure S42:** ESI – MS spectrum of **DCI-ROS**, ESI-MS (+ve)  $m/z$  calcd for  $C_{55}H_{54}BCl_2N_3O_6$   $[M + Li]^+$ : 940.3642; obs. 940.3642.

## References

1. Ebner, D. C.; Bagdanoff, J. T.; Ferreira, E. M.; McFadden, R. M.; Caspi, D. D.; Trend, R. M.; Stoltz, B. M., *Chem Eur J* **2009**, *15*, 12978-12992.
2. Zhai, W.; Chapin, B. M.; Yoshizawa, A.; Wang, H.-C.; Hodge, S. A.; James, T. D.; Anslyn, E. V.; Fossey, J. S., *Org Chem Front* **2016**, *3*, 918-928.
3. Yang, M.; Fan, J.; Sun, W.; Du, J.; Long, S.; Shao, K.; Peng, X., *Chem Commun* **2019**, *55*, 8583-8586.
4. Wu, S.; Tan, S. Y.; Ang, C. Y.; Luo, Z.; Zhao, Y., *Chem Commun* **2016**, *52*, 3508-3511.
5. Kastrati, A.; Bochet, C. G., *J Org Chem* **2019**, *84*, 7776-7785.
6. Wang, K.; Zhao, C.-X.; Leng, T.-H.; Wang, C.-Y.; Lu, Y.-X.; Shen, Y.-J.; Zhu, W.-H., *Dyes Pigments* **2018**, *151*, 194-201.
7. Xu, J.; Zhang, Y.; Yu, H.; Gao, X.; Shao, S., *Anal Chem* **2016**, *88*, 1455-1461.