Caged *Garcinia* xanthones: a novel chemical scaffold with potent antimalarial activity

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Running Head: Caged *Garcinia* Xanthones as novel antimalarial agents

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SUPPLEMETARY MATERIAL

1. General procedures

Unless indicated, all commercially available reagents were purchased at the highest commercial quality and were used as received without further purification. All nonaqueous reactions were carried out under argon atmosphere using dry glassware that had been flame-dried under a stream of argon unless otherwise noted. Anhydrous tetrahydrofuran (THF) and dimethylformamide (DMF) were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. Flash column chromatography was performed on silica gel (Merck Kieselgel 60, 230-400 mesh). The progress of all the reactions was monitored by thin-layer chromatography (TLC) using glass plates precoated with silica gel-60 F254 to a thickness of 0.5 mm (Merck), and compounds were visualized by irradiation with UV light and/or by treatment with a solution of CAM stain followed by heating. ^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 or 500 MHz Varian or 500 MHz JEOL instrument. $CDCl_3$ was treated with anhydrous K_2CO_3, chemical shifts (δ) are quoted in parts per million (ppm) referenced to the appropriate residual solvent peak reference ($CDCl_3$), with the abbreviations s, d, t, dd, m, denoting singlet, doublet, triplet, doublet of doublets, multiplet, respectively. J = coupling constants given in Hertz (Hz). High-resolution Mass spectra (HRMS) were recorded on a trisector WG AutoSpecQ spectrometer.

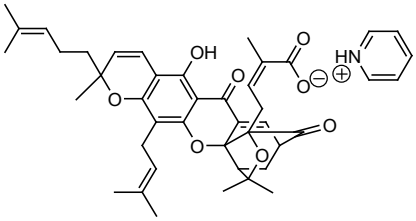
- Gambogic acid (**GBA**) was prepared as reported in: Guizzunti, G.; Batova, A.;

Chantarasriwong, O.; Dakanali, M.; Theodorakis, E. A. “Subcellular localization and activity of gambogic acid” *ChemBioChem* **2012**, *13*, 1191-1198.

- Cluvenone (**CLV**) was prepared as reported in: Chantarasriwong, O.; Cho, W. C.; Batova, A.; Chavasiri, W.; Moore, C.; Rheingold, A. L.; Theodorakis, E. A. “Evaluation of the pharmacophoric motif of the caged Garcinia xanthones” *Org. Biomol. Chem*. **2009**, *7*, 4886-4894.
- **MAD28** was prepared as reported in: Elbel, K. M.; Guizzunti, G.; Theodoraki, M. A.; Xu, J.; Batova, A.; Dakanali, M.; Theodorakis, E. A. “A-ring oxygenation modulates the chemistry and bioactivity of caged Garcinia xanthones” *Org. Biomol. Chem.* **2013**, *11*, 3341–3348.
- **CR135** and **CR142** were prepared by scaling-up the procedure reported in: Theodoraki, M. A.; Rezende Jr., C. O.; Chantarasriwong, O.; Corben, A. D.; Theodorakis, E. A.; Alpaugh, M. L. “Spontaneously-forming spheroids as an in vitro cancer cell model for anticancer drug screening” *Oncotarget* **2015**, *6*, 21255–21267.

2. Isolation of GBA (gambogic acid) from gamboge

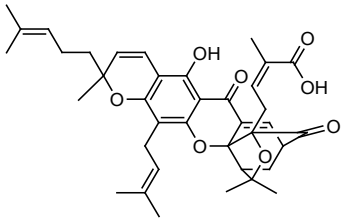
Pyridine salt of GBA



Dried powder of gamboge (20.0 g) from *G. hanburyi* trees was extracted with MeOH (80 mL) at room temperature for a day. The mixture was filtered and the residue was re-extracted two more times with MeOH (80 mL). The combined filtrate was concentrated under reduced pressure to give crude extract as a yellow powder (13.0 g, 75%). The crude extract (13.0 g) was dissolved in pyridine (13 mL), and then warm water (5 mL) was added to the stirred solution. The reaction mixture was cooled to room temperature and some precipitate was observed. Hexane (10 mL) was added to the mixture and the mixture was filtered. The solid was collected and washed with hexane and dried to yield pyridine salt of GBA as a yellow solid (2.2 g, 17%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.78 (s, 1H), 8.63-8.62 (m, 2H), 7.76 (t, J = 7.6 Hz, 1H), 7.54 (d, J = 6.9 Hz, 1H), 7.37-7.34 (m, 2H), 6.59 (d, J = 10.1 Hz, 1H), 6.08 (t, J = 7.4 Hz, 1H), 5.37 (d, J = 10.1 Hz, 1H), 5.04 (br s, 2H), 3.49-3.46 (m, 1H), 3.31-3.27 (m, 1H), 3.17-3.14 (m, 1H), 2.98-2.93 (m, 2H), 2.52 (d, J = 9.3 Hz, 1H), 2.31 (dd, J = 13.4,

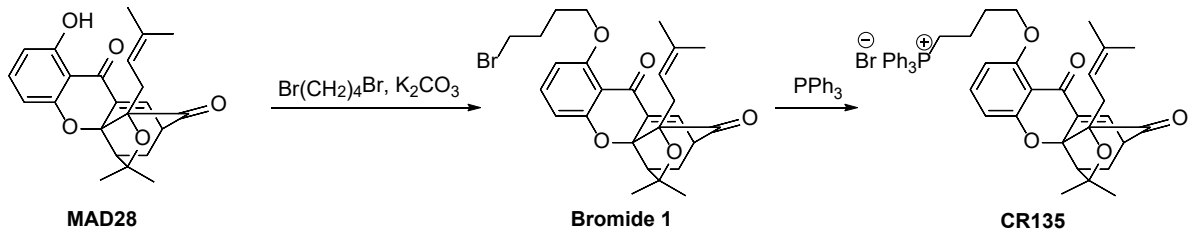
4.6 Hz, 1H), 2.00-1.98 (m, 2H), 1.75-1.54 (m, 20H), 1.41-1.39 (m, 1H), 1.37 (s, 3H), 1.29 (s, 3H).

GBA (Gambogic acid)



To a solution of the pyridine salt of GBA (1.26 g, 1.77 mmol) in ether (20 mL) was added aqueous HCl (1N, 12.6 mL). After 1 h of continued stirring at room temperature, the ether solution was washed with water (3 x 3 mL), dried over $MgSO_4$, and concentrated by rotary evaporation to give **GBA** as a yellow solid (1.10 mg, 99%). R_f = 0.38 (silica gel, 25% EtOAc-hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.77 (s, 1H), 7.55 (d, J = 6.9 Hz, 1H), 6.60 (d, J = 10.1 Hz, 1H), 6.09 (t, J = 7.3 Hz, 1H), 5.38 (d, J = 10.2 Hz, 1H), 5.04-5.02 (m, 2H), 3.50-3.47 (m, 1H), 3.33-3.27 (m, 1H), 3.16-3.13 (m, 1H), 2.96-2.94 (m, 2H), 2.52 (d, J = 9.3 Hz, 1H), 2.31 (dd, J = 13.7, 4.7 Hz, 1H), 2.02-2.00 (m, 2H), 1.75-1.62 (m, 17H), 1.54 (s, 3H), 1.41-1.38 (m, 1H), 1.38 (s, 3H), 1.29 (s, 3H).

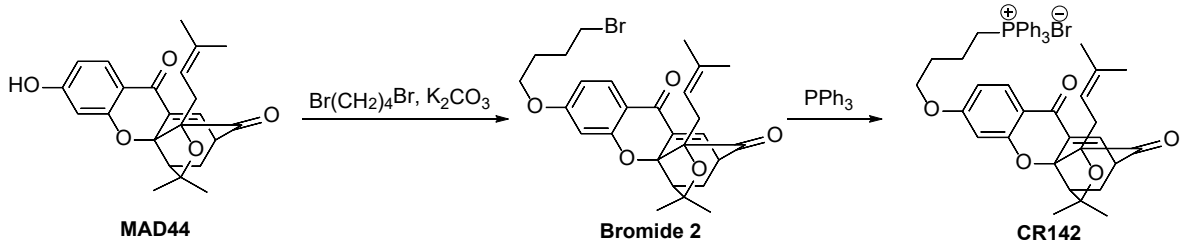
3. Synthesis of CR135



To a solution of **MAD28** (22 mg, 57.8 μmol) in DMF (1 mL), potassium carbonate (16 mg, 115.6 μmol) and 1, 4-dibromobutane (35μL, 289 μmol) were added. The mixture was left stirring at room temperature overnight. Upon completion, the reaction mixture was quenched with water (3 mL) and extracted with diethyl ether (2 × 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated in *vacuo*. The residue was purified by column chromatography (silica gel, 25 % EtOAc-hexane) to afford **Bromide 1** (26.7 mg, 90%) as a colorless liquid.

To a solution of **Bromide 1** (26.7 mg, 51.8 μmol) in acetonitrile (1 mL), triphenylphosphine (68 mg, 0.259 mmol) was added. The mixture was stirred at 150 °C overnight. Upon completion, the reaction mixture was cooled to room temperature and the excess acetonitrile was removed by rotary evaporation. The crude was dissolved in DCM (1 mL) and hexane (10 mL) was added. The solid was filtered and washed with hexane to yield **CR135** (34.2 mg, 85%) as a white solid. TLC: R_f = 0.1 (silica gel, 20% MeOH-DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93-7.86 (m, 6H), 7.80-7.75 (m, 3H), 7.70-7.65 (m, 6H), 7.42 (t, *J* = 8.3 Hz, 1H), 7.04 (d, *J* = 6.9 Hz, 1H), 6.65 (dd, *J* = 8.4, 2.2 Hz, 2H), 4.42 (dd, *J* = 8.4, 7.0 Hz, 1H), 4.27-4.02 (m, 4H), 3.43 (dd, *J* = 6.8, 4.2 Hz, 1H), 2.60 (d, *J* = 7.8 Hz, 2H), 2.40 (d, *J* = 9.6 Hz, 1H), 2.36-2.21 (m, 3H), 2.20-2.06 (m, 2H), 1.70 (s, 3H), 1.32-1.28 (m, 4H), 1.15 (s, 3H), 1.07 (s, 3H).

4. Synthesis of CR142



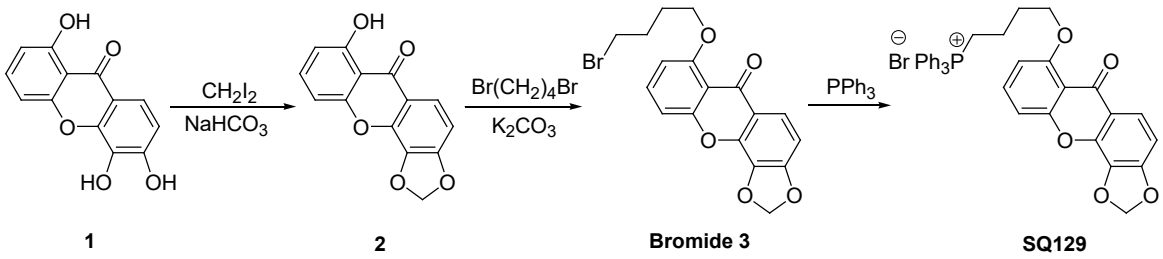
MAD44 **Bromide 2** **CR142**

To a solution of **MAD44** (0.10 g, 0.26 mmol) in DMF (2 mL), potassium carbonate (72 mg, 0.52 mmol) and 1, 4-dibromobutane (0.28 g, 1.31 mmol) were added. The mixture was left stirring at room temperature during 8 h. Upon completion, the reaction mixture was quenched with water (10 mL) and extracted with diethyl ether (2 × 20 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated in *vacuo*. The residue was purified by flash column chromatography (silica gel, 25% EtOAc-hexane) to afford **Bromide 2** (0.11 g, 85%) as a colorless liquid.

To a solution of **Bromide 2** (0.10 g, 0.19 mmol) in acetonitrile (5 mL), triphenylphosphine (0.25 g, 0.97 mmol) was added. The mixture was stirred under at 150 °C overnight.Upon completion, the reaction mixture was cooled to room temperature and the excess acetonitrile was removed by rotary evaporation. The crude was dissolved in DCM (3 mL) and hexane (30 mL) was added. The

solid was filtered and washed with hexane to yield **CR142** (0.15 g, 98%) as a white solid. R_f = 0.10 (silica gel, 20% MeOH-DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.91-7.85 (m, 7H), 7.82-7.78 (m, 3H), 7.72-7.67 (m, 6H), 7.38 (d, *J* = 6.9 Hz, 1H), 6.52 (dd, *J* = 8.8, 2.2 Hz, 1H), 6.41 (d, *J* = 1.9 Hz, 1H), 4.44 (t, *J* = 7.1 Hz, 1H), 4.16 (ddd, *J* = 20.6, 10.3, 5.4 Hz, 2H), 4.10-4.00 (m, 2H), 3.49-3.44 (m, 1H), 2.59 (d, *J* = 8.0 Hz, 2H), 2.43 (d, *J* = 9.5 Hz, 1H), 2.32 (dd, *J* = 12.9, 4.2 Hz, 3H), 1.91-1.84 (d, *J* = 5.8 Hz, 2H), 1.72 (s, 3H), 1.34-1.28 (m, 7H), 0.97 (s, 3H).

5. Synthesis of SQ129



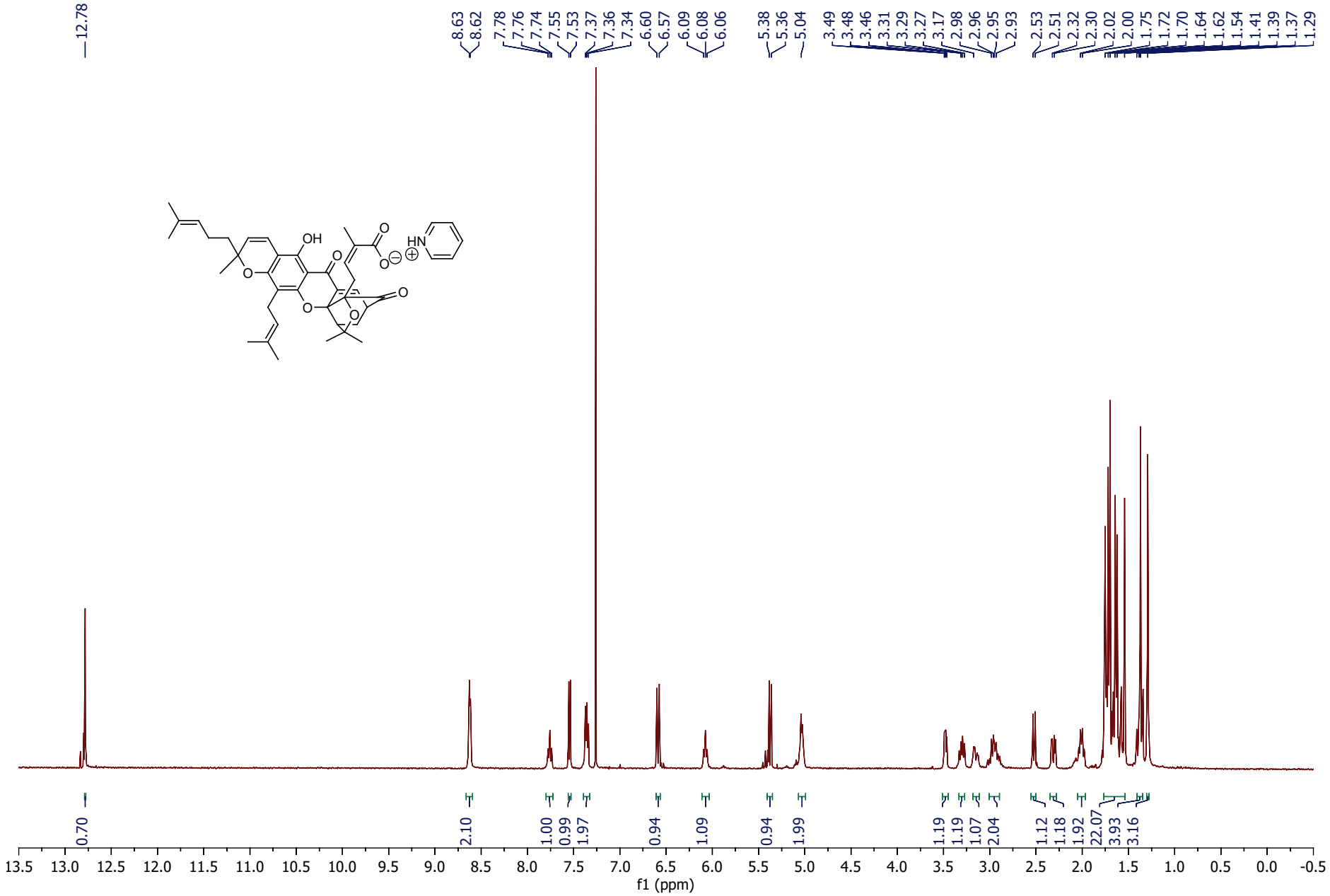
1 **2** **Bromide 3** **SQ129**

To a solution of **1** (244.2 mg, 1 mmol) in DMF (10 mL), CH_2I_2 (88.6 µL, 1.1 mmol) and $NaHCO_3$ (210 mg, 2.5 mmol) were added. The mixture was left stirring at 80 ^{o}C overnight. Upon completion, the reaction mixture was quenched with water (10 mL) and extracted with ethyl acetate (2 × 30 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated in *vacuo*. The residue was purified by flash column chromatography (silica gel, 17% EtOAc-hexane) to afford **2** (166.5 mg, 65%) as a white solid. R_f = 0.30 (silica gel, 17% EtOAc-hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.73 (s, 1H), 7.88 (d, *J* = 8.5 Hz, 1H), 7.56 (t, *J* = 8.3 Hz, 1H), 6.95-6.90 (m, 2H), 6.79 (dd, *J* = 8.3, 0.9 Hz, 1H), 6.23 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 181.34, 162.25, 155.94, 154.04, 141.05, 136.81, 134.26, 121.43, 116.80, 111.14, 108.47, 106.95, 106.38, 103.51 ppm; HRMS (*m/z*): calculated for $C_{14}H_9O_5^+$ $[M + H]^+$: 257.0444, found: 257.0447.

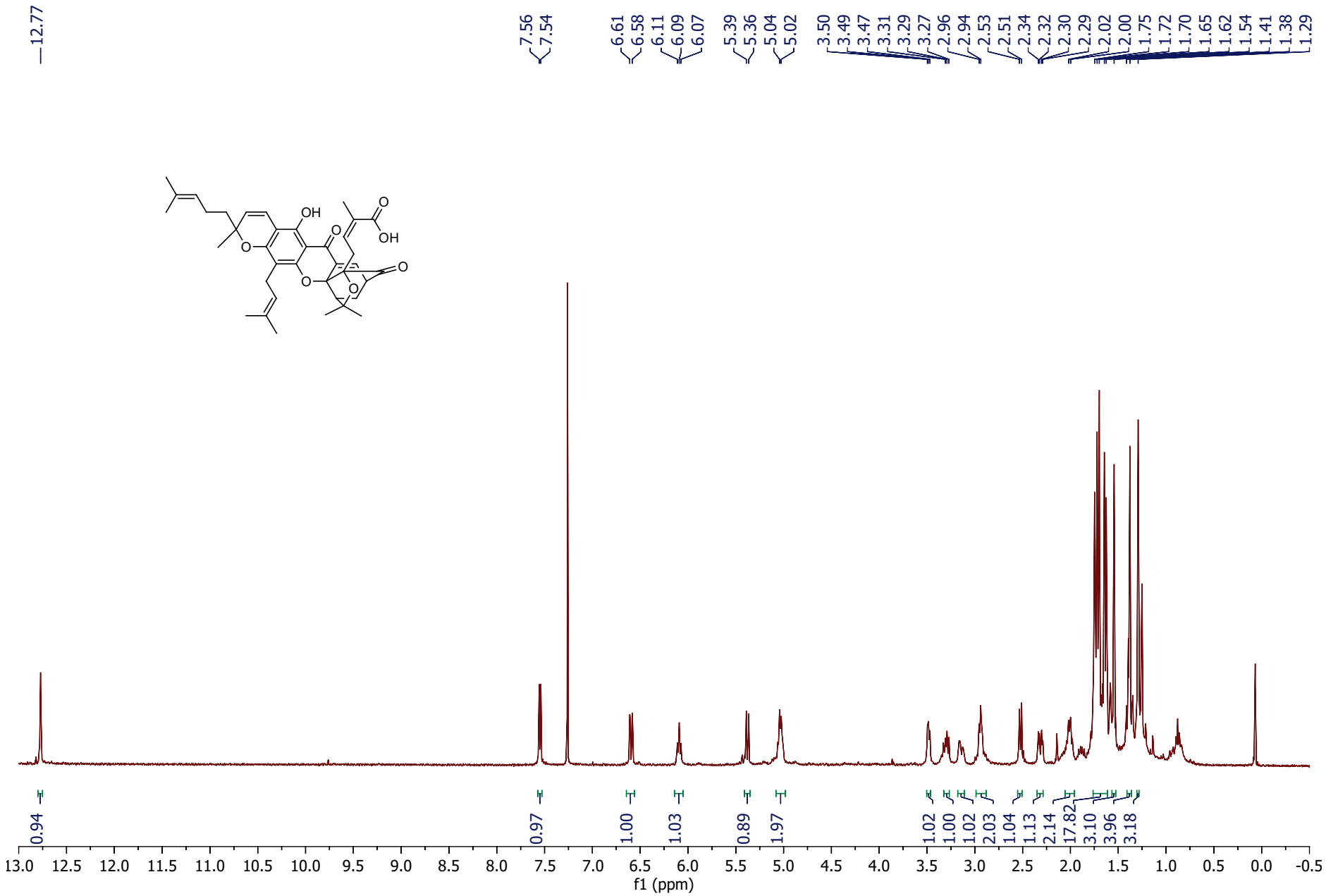
To a solution of **2** (49.1 mg, 0.19 mmol) in DMF (2 mL), potassium carbonate (52.9 mg, 0.38 mmol) and 1, 4-dibromobutane (114 µL, 0.96 mmol) were added. The mixture was left stirring at room

temperature during 8 h. Upon completion, the reaction mixture was quenched with water (10 mL) and extracted with diethyl ether (2 × 20 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated in *vacuo*. The residue was purified by flash column chromatography (silica gel, EtOAc/hexane = 1:3) to afford **Bromide 3** (67.3 mg, 90%) as a white solid. R_f = 0.30 (silica gel, 20% EtOAc-hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (d, J = 8.5 Hz, 1H), 7.53 (t, J = 8.4 Hz, 1H), 7.02 (dd, J = 8.4, 0.8 Hz, 1H), 6.85 (d, J = 8.5 Hz, 1H), 6.76 (d, J = 8.3 Hz, 1H), 6.17 (s, 2H), 4.14 (t, J = 6.0 Hz, 2H), 3.59 (t, J = 6.4 Hz, 2H), 2.29-2.21 (m, 2H), 2.15-2.06 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.22, 160.24, 157.79, 152.76, 140.10, 134.81, 133.82, 121.66, 119.37, 112.33, 109.90, 106.91, 105.89, 103.23, 68.29, 34.14, 29.41, 27.72; HRMS (*m/z*): calculated for $C_{18}H_{16}BrO_5^+$ $[M + H]^+$: 391.0176, found: 391.0177.

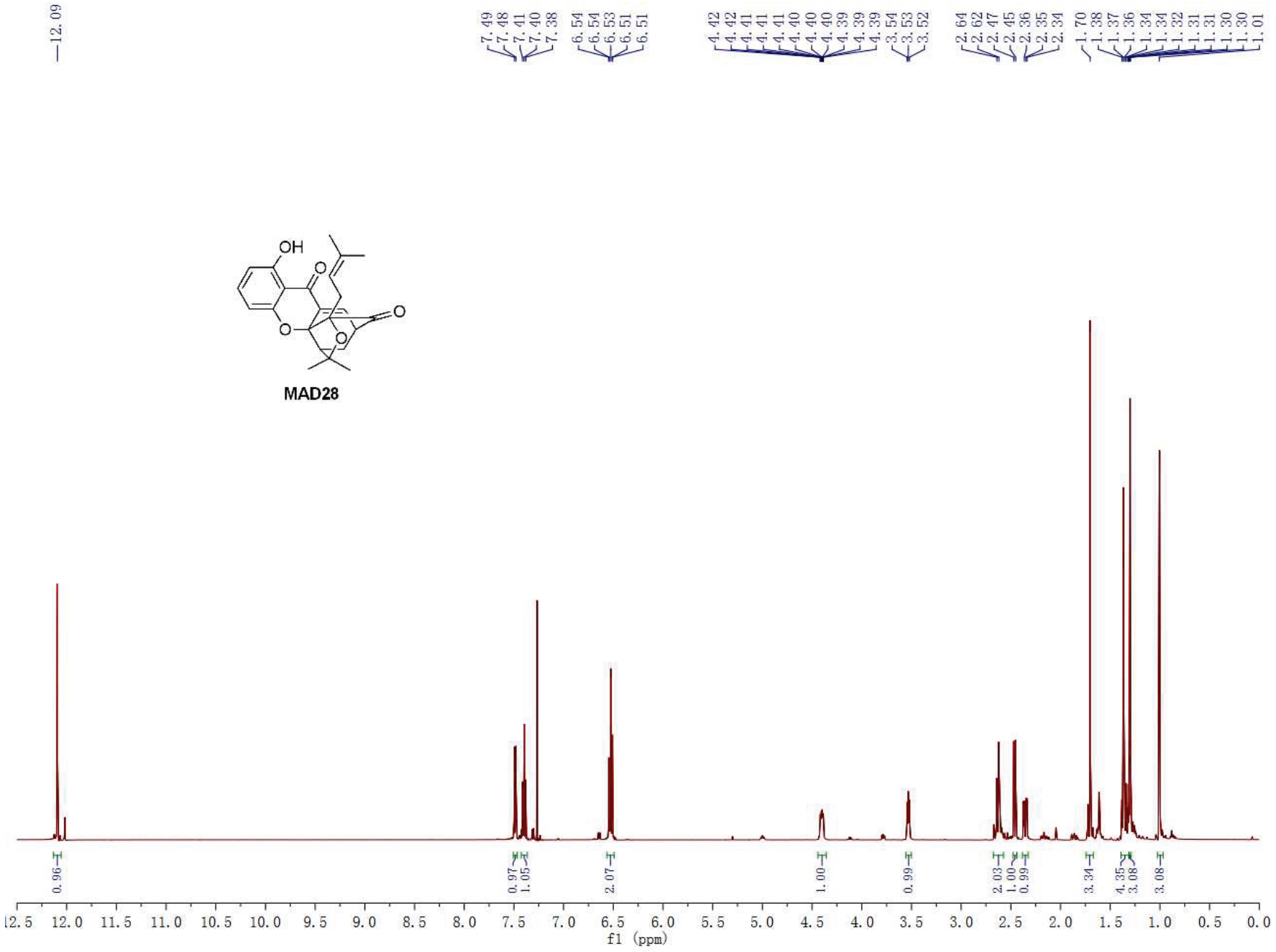
To a solution of **Bromide 3** (20.0 mg, 51 μmol) in acetonitrile (1 mL), triphenylphosphine (66.9 g, 255 μmol) was added. The mixture was stirred under at 150 °C overnight.Upon completion, the reaction mixture was cooled to room temperature and the excess acetonitrile was removed by rotary evaporation. The crude was dissolved in DCM (3 mL) and hexane (30 mL) was added. The solid was filtered and washed with hexane to yield **SQ129** (25.0 mg, 75%) as a white solid. R_f = 0.7 (silica gel, 20% MeOH-DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (dd, J = 12.7, 7.5 Hz, 6H), 7.75-7.70 (m, 3H), 7.66-7.57 (m, 8H), 7.05 (d, J = 8.3 Hz, 2H), 6.88 (dd, J = 14.1, 8.4 Hz, 2H), 6.22 (s, 2H), 4.34 (t, J = 5.3 Hz, 2H), 4.16-4.07 (m, 2H), 2.39-2.29 (m, 2H), 2.23-2.10 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.06, 159.98, 157.68, 152.87, 140.22, 135.45, 134.83, 134.80, 134.05, 133.95, 133.87, 130.46, 130.36, 121.09, 119.33, 119.07, 118.39, 111.95, 109.83, 107.42, 105.80, 103.30, 68.73, 28.90, 28.76, 22.29, 21.89, 20.07, 20.04; HRMS (*m/z*): calculated for $C_{36}H_{30}PO_5^+$ $[M – Br]^+$: 573.1825, found: 573.1823.



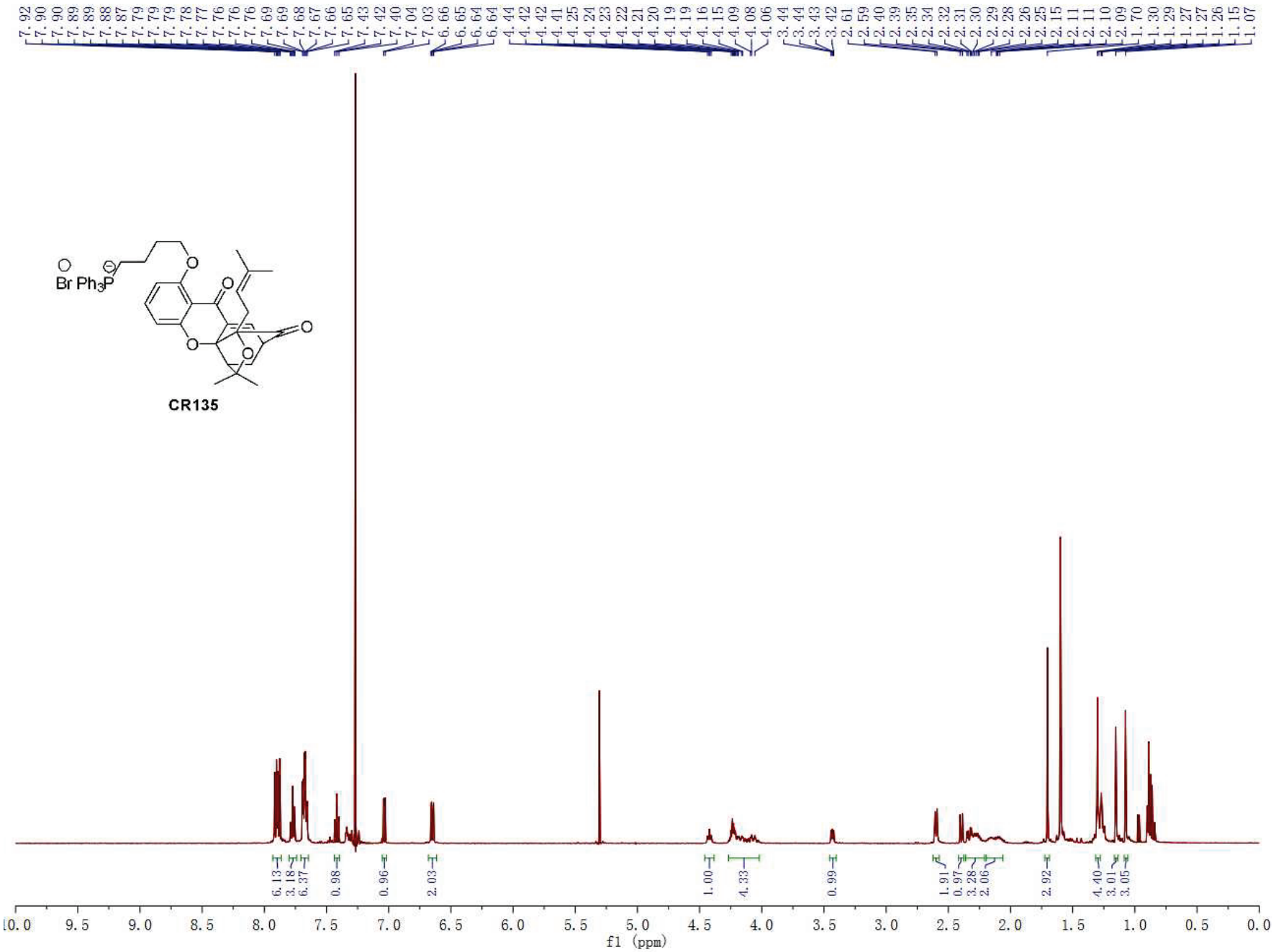
Spectrum 1: ^{1}H NMR ($CDCl_3$, 400 MHz) of pyridine salt of GBA



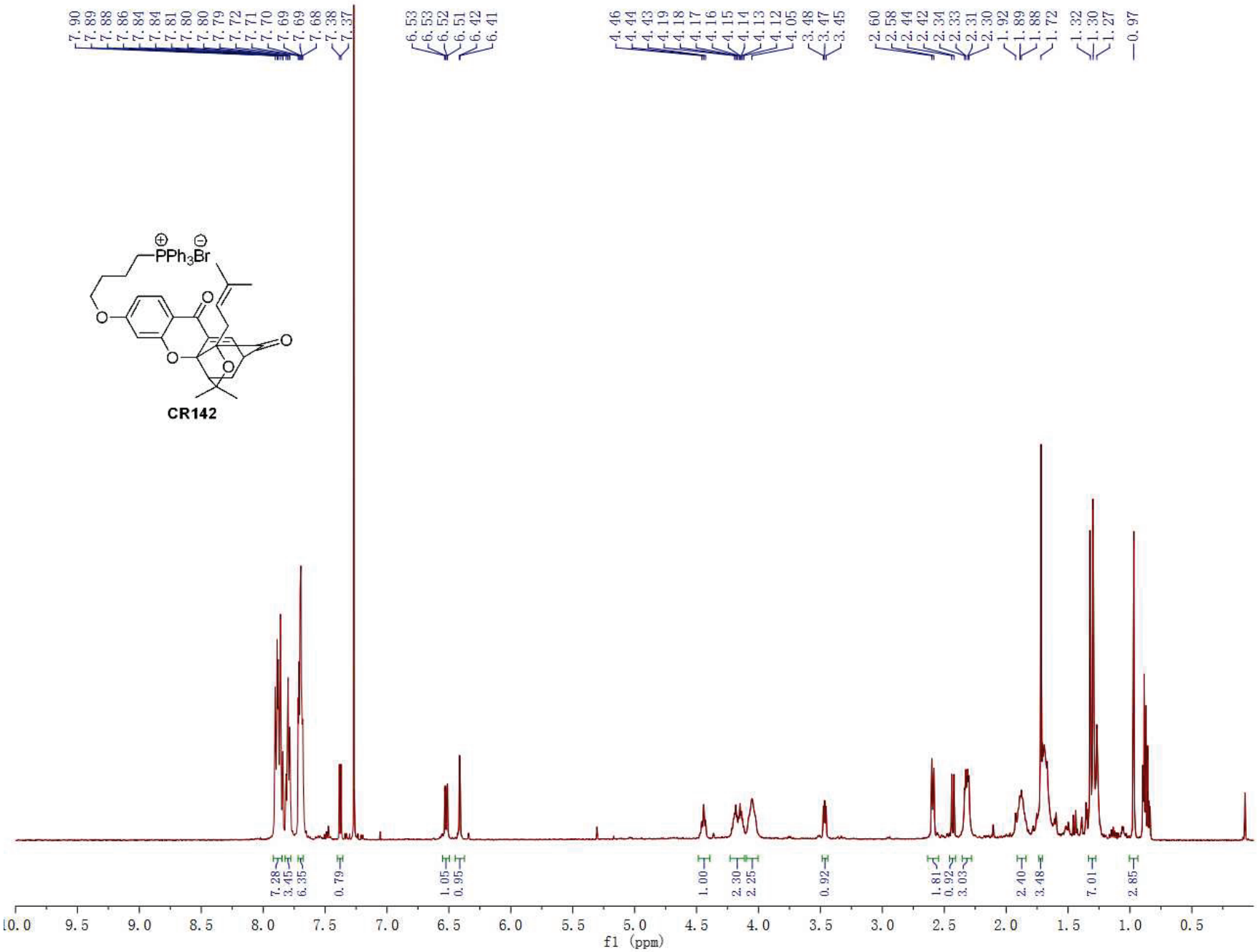
Spectrum 2: ^{1}H NMR ($CDCl_3$, 400 MHz) of GBA



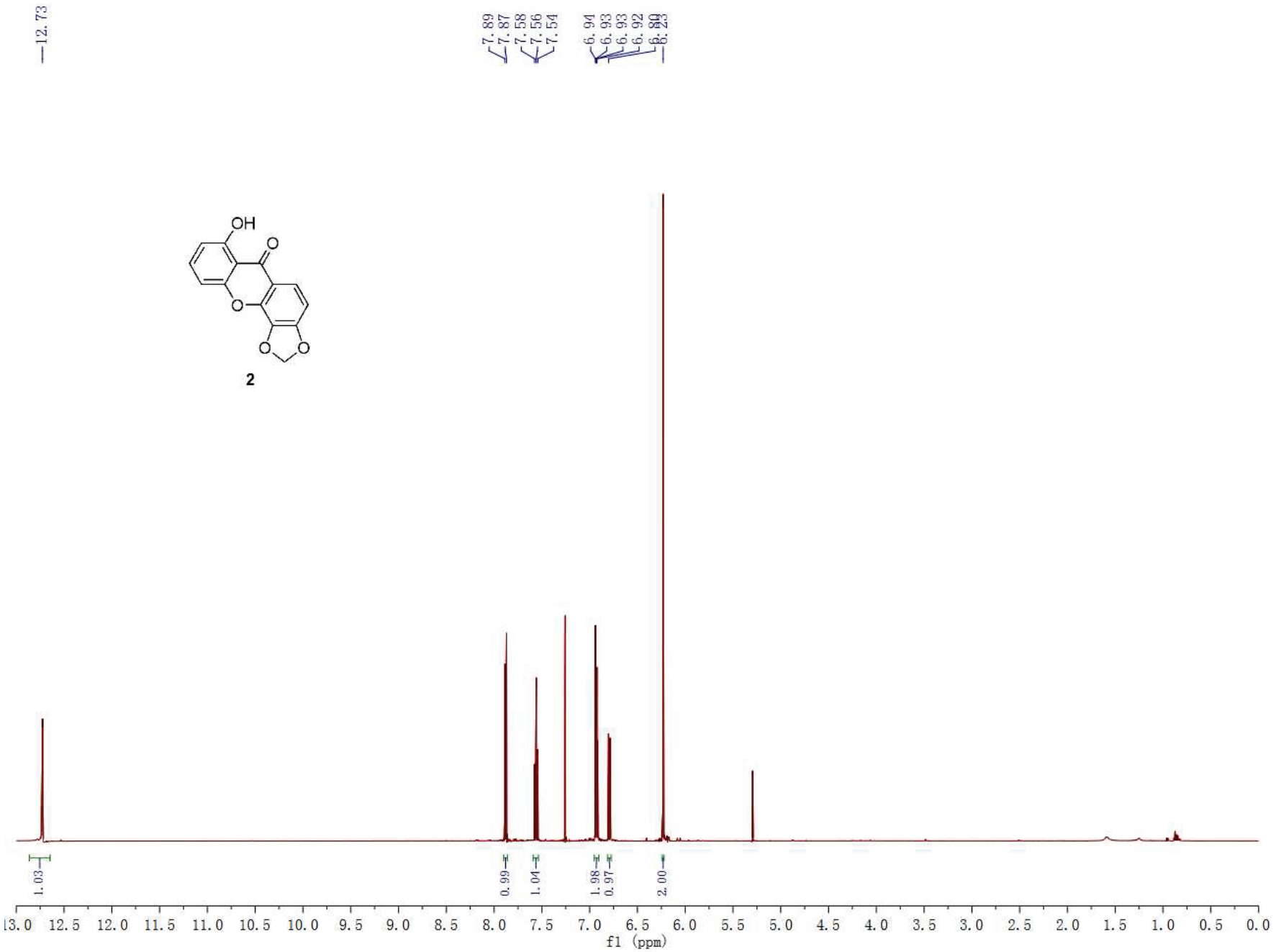
Spectrum 3: ^{1}H NMR ($CDCl_3$, 500 MHz) of MAD28



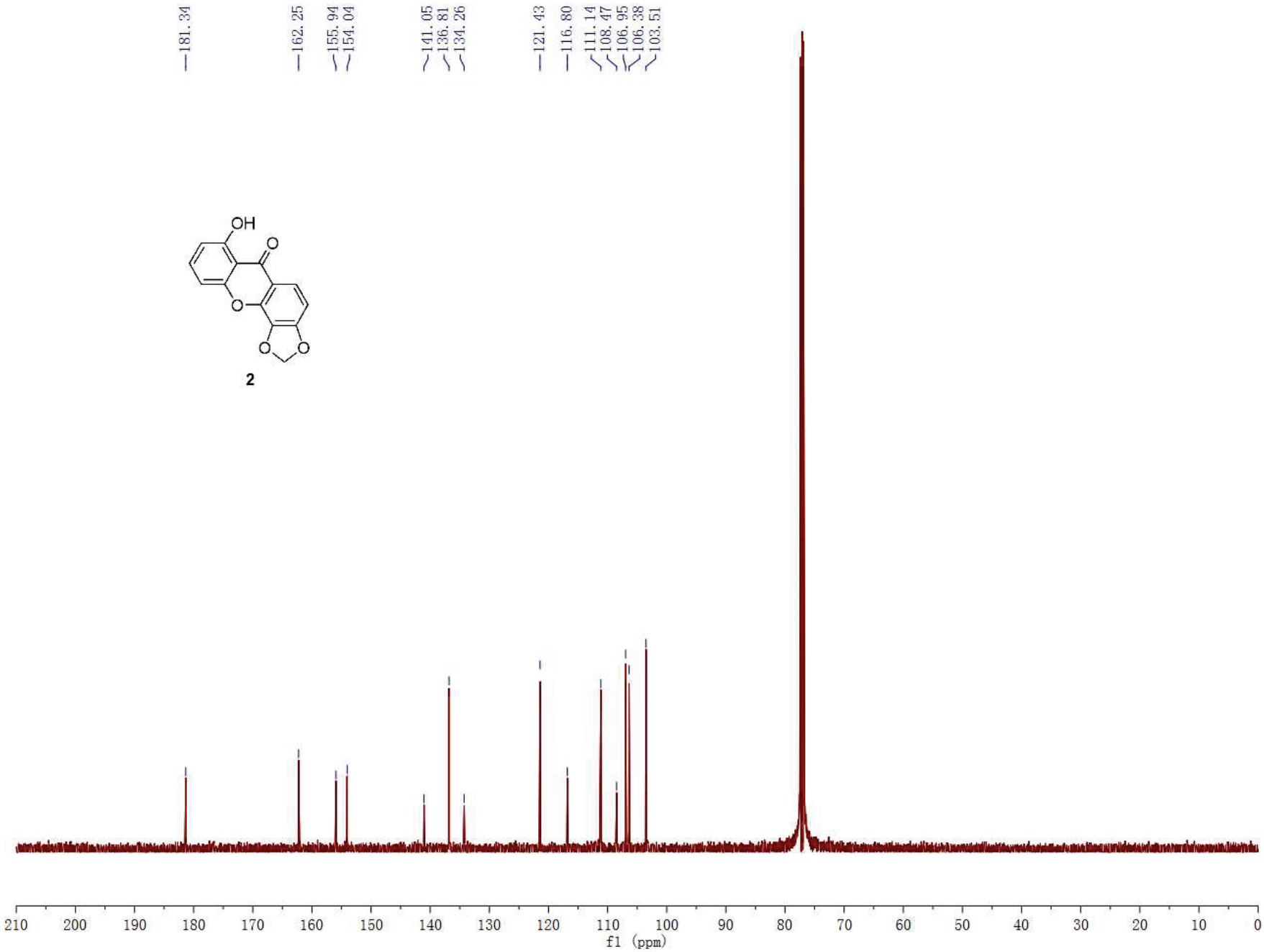
Spectrum 4: ^{1}H NMR ($CDCl_3$, 500 MHz) of CR135



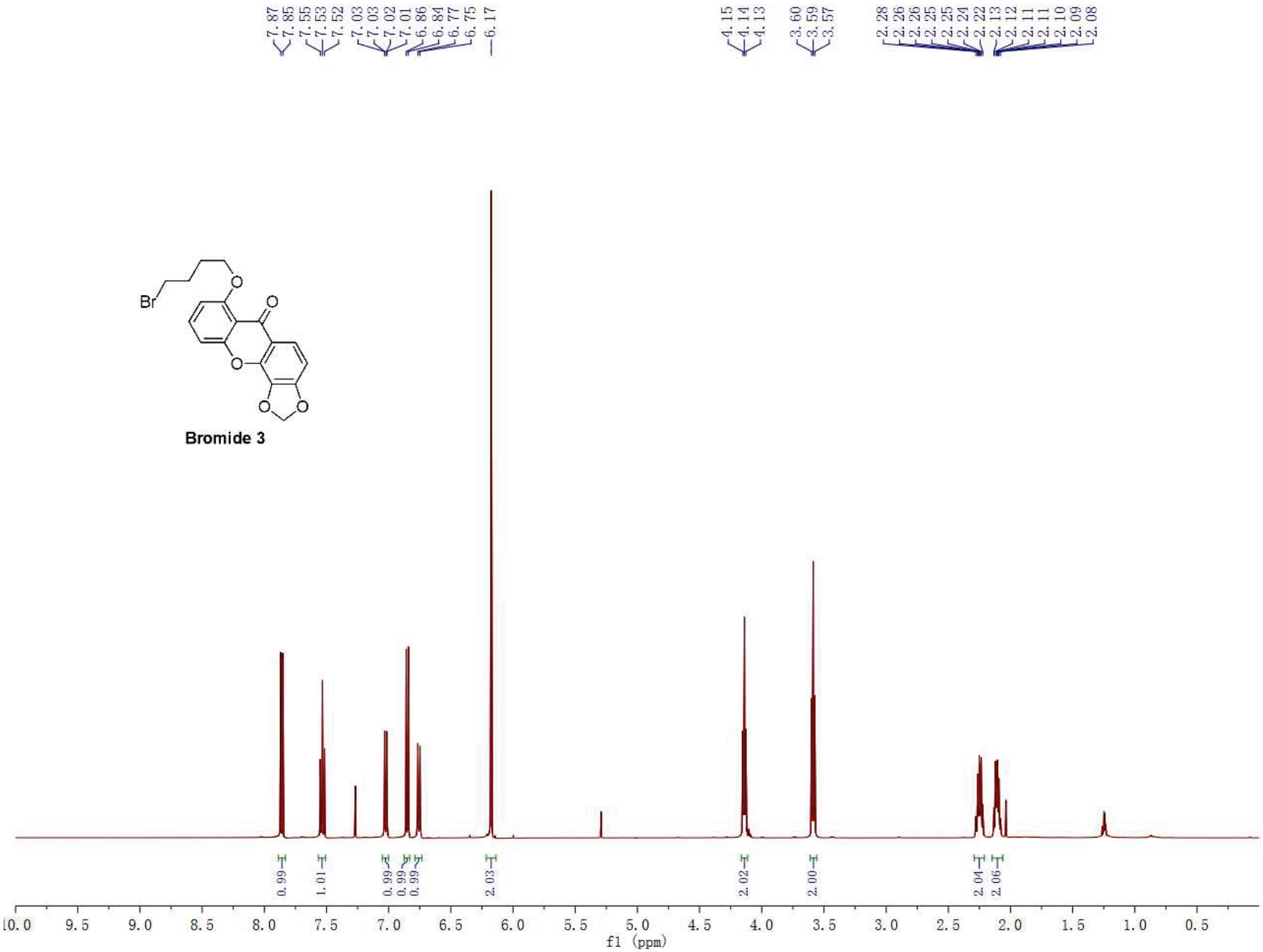
Spectrum 5: ^{1}H NMR ($CDCl_3$, 500 MHz) of CR142



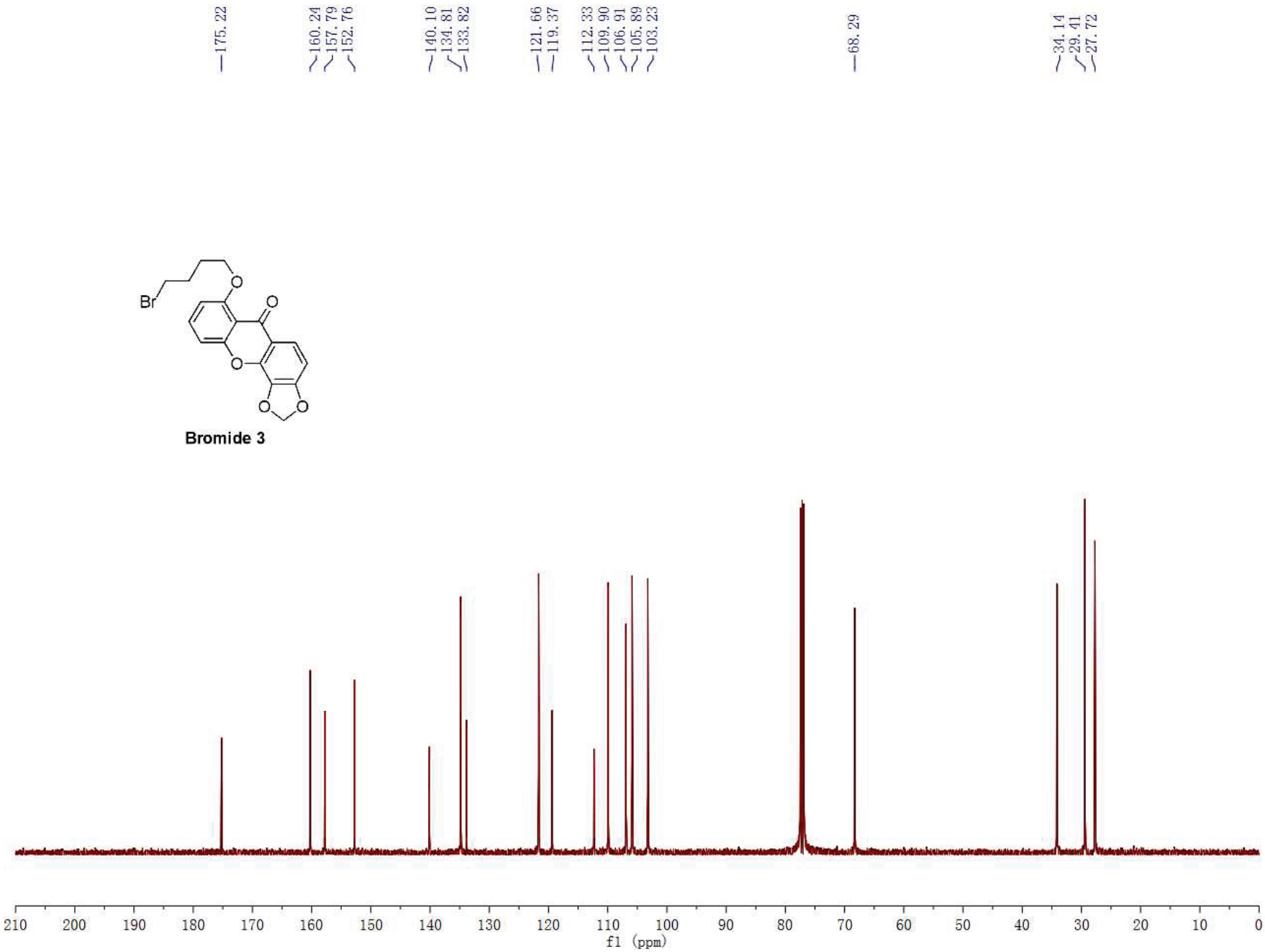
Spectrum 6: ^{1}H NMR ($CDCl_3$, 500 MHz) of 2



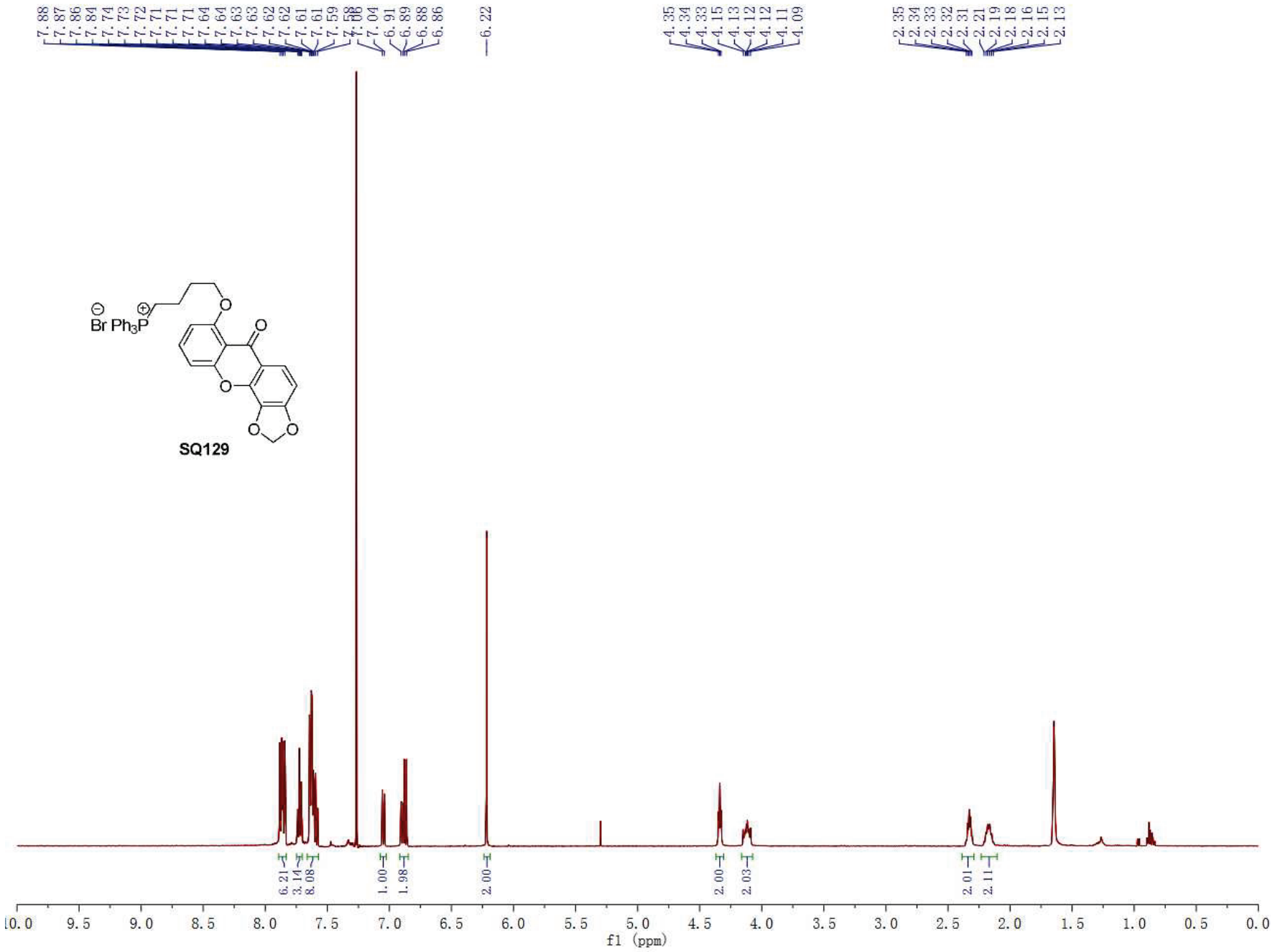
Spectrum 7: ^{13}C NMR ($CDCl_3$, 500 MHz) of 2



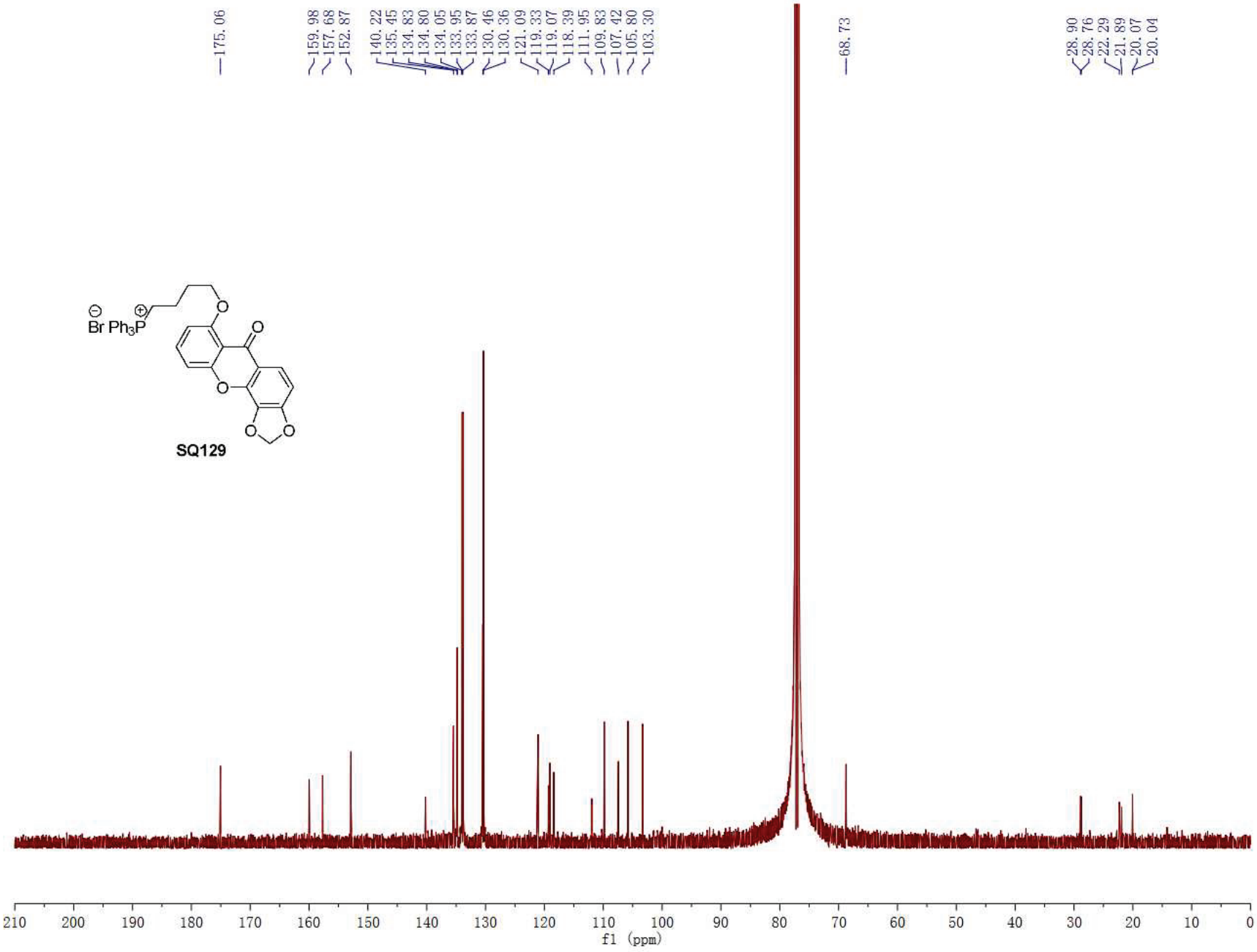
Spectrum 8: ^{1}H NMR ($CDCl_3$, 500 MHz) of Bromide 3



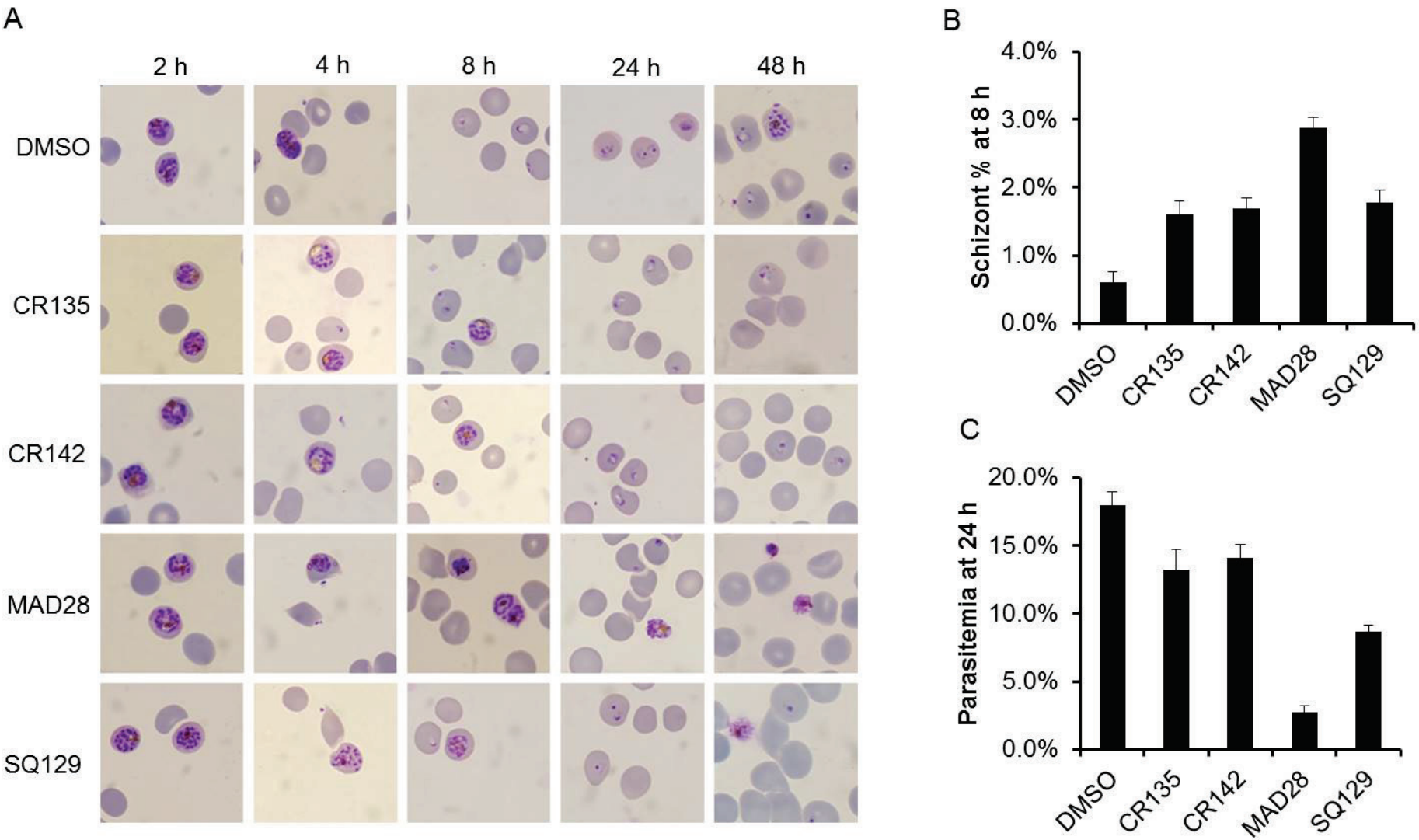
Spectrum 9: ^{13}C NMR ($CDCl_3$, 500 MHz) of Bromide 3



Spectrum 10: ^{1}H NMR ($CDCl_3$, 500 MHz) of SQ129

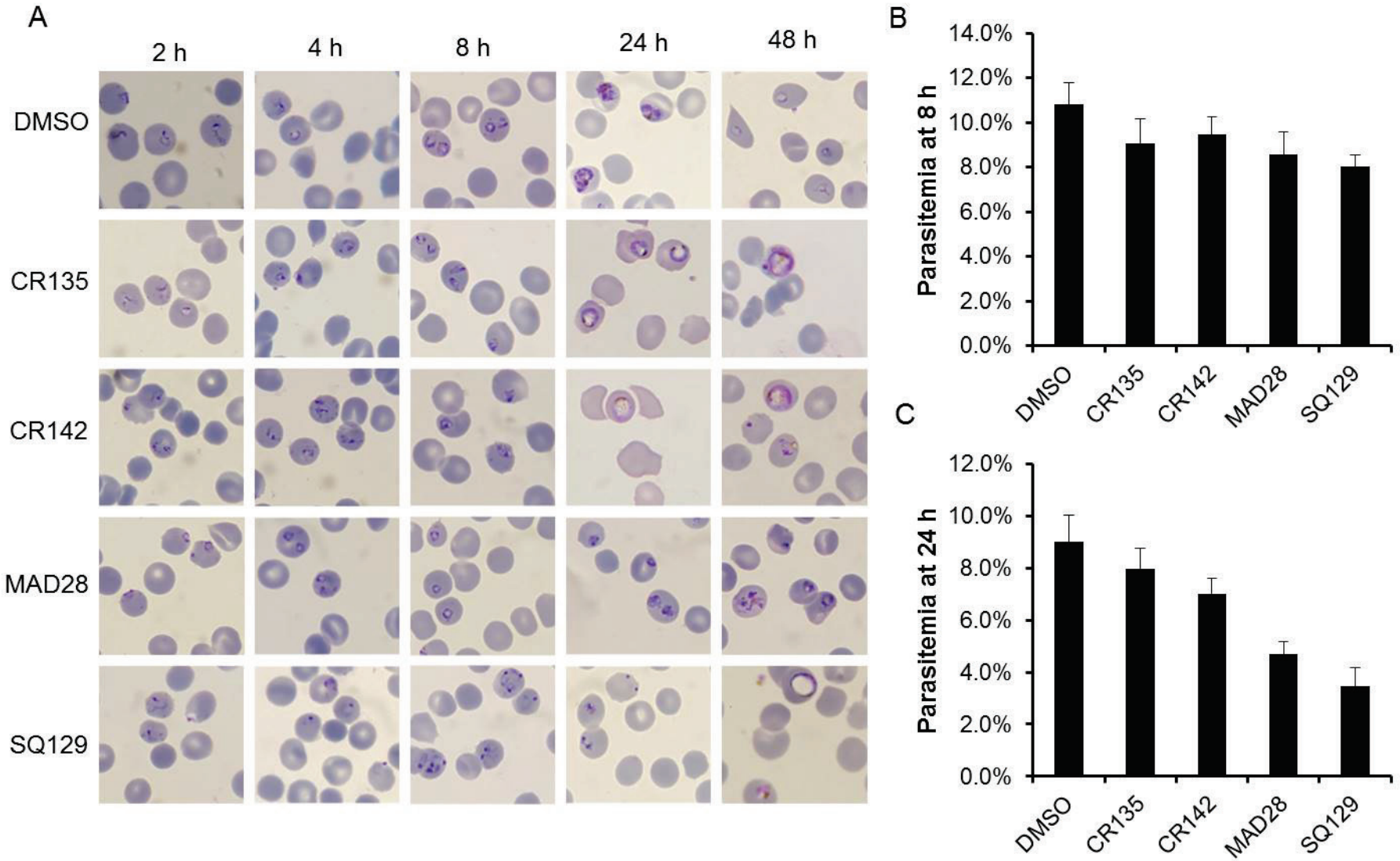


Spectrum 11: ^{13}C NMR ($CDCl_3$, 500 MHz) of SQ129



Supplementary Figure 1. Viability of schizont stage *P. falciparum* parasites treated with CGX derivatives.

Supplementary Figure 1. Viability of schizont stage *P. falciparum* parasites treated with CGX derivatives. Dd2 schizonts were treated with vehicle or compounds and sampled periodically over 48 h in culture. (A) Representative Giemsa stained slides are presented, showing morphological changes over time. (B) The percentage of schizonts in each treated parasite culture was determined by microscopic counting of 1000 red blood cells. (C) The parasitemia of each treatment after a 24 h exposure was determined by microscopic counting of 1000 red blood cells. This experiment was repeated three times, and error bars in (B) and (C) indicate the standard error of three biological replicates.



Supplementary Figure 2. Viability of ring stage *P. falciparum* parasites treated with CGX derivatives.

Supplementary Figure 2. Viability of ring stage *P. falciparum* parasites treated with CGX derivatives. Dd2 ring stage parasites were treated with vehicle or compounds and sampled periodically over 48 h in culture. (A) Representative Giemsa stained slides showing morphological changes over time. The parasitemia of each sample determined by microscopic counting at 8 h post treatment (B) or 24 h post treatment (C) is presented. Error bars in (B) and (C) indicate the standard error of three biological replicates.