Supporting Information

Antiplasmodial alkaloids from bulbs of *Amaryllis belladonna*

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Experimental Section

General experimental procedures

UV spectra were measured on a Shimadzu UV-1201 spectrophotometer and specific rotations were recorded on a JASCO P-2000 polarimeter. NMR spectra were obtained on a Bruker Avance 400 spectrometer; chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. Mass spectra were obtained on an Agilent 6220 LC-TOF-MS in the positive ion mode. ECD spectra were obtained on a JASCO J-810 spectropolarimeter with a 1 cm cell in MeOH at room temperature with constant nitrogen flushing under the following conditions: speed 100 nm/min, time constant 1 s, bandwidth 1.0 nm. Semipreparative HPLC was performed using Shimadzu LC-10AT instrument with a semipreparative C_{18} Phenomenex Luna column (5 μm, 250 × 10 mm). Solid phase extractions were performed with Thermo Scientific HyperSep C_{18} 500 mg, 3 mL SPE cartridges, and all RP TLC was conducted with Sorbtech C_{18}-W silica TLC plates. All isolated compounds were purified to 95% or better, as judged by HPLC (UV and ELSD detection) and NMR before antiplasmodial assay.

Plant material

Specimens of bulbs of *Amaryllis belladonna* Steud. (C. Morenburg 128) were collected and identified by Cori Morenberg of the New York Botanical Garden in the succulent garden of the Quail Botanical Gardens, Encinitas, CA on September 6, 1995. http://sweetgum.nybg.org/science/vh/specimen_details.php?irn=269183

Extraction and isolation

Dried, powdered plant material (1.17 Kg) was exhaustively extracted with MeOH at room temperature to give 39 g of an extract which was partitioned into aqueous MeOH and dichloromethane soluble portions. The aqueous MeOH-soluble portion of the crude extract was passed through a polyvinyl pyrrolidone column to give a detanninized extract designated CM00128a (X-4283), and 2.0 g of this extract was made available to Virginia Tech. A 1.5 g portion of this extract, which had an IC_{50} against *P. falciparum* Dd2 of 1.25 μg/mL, was suspended in aqueous MeOH (MeOH–H_2O, 9:1, 100 mL) and extracted with hexane (4 × 100 mL portions) to give an inactive hexane-soluble fraction (165.0 mg, IC_{50} >10 μg/mL). The aqueous fraction was then diluted to MeOH–H_2O, 6:4 (150 mL) and further extracted with CH_2Cl_2 (4 × 100 mL portions) and EtOAc (4 × 100 mL portions) to yield the CH_2Cl_2-soluble (310.0 mg, IC_{50} approximately 2.5 μg/mL) and EtOAc-soluble fractions (210.0 mg, IC_{50} approximately 2.5 μg/mL). The active CH_2Cl_2 fraction was chromatographed by open column on a normal silica gel with the solvent system from 30:1 to 1:1 CH_2Cl_2:MeOH. Five fractions (AC1-AC5) were collected based on TLC analyses. The active fraction AC2 (IC_{50} < 1.25 μg/mL) was further separated by HPLC on a semi-preparative C_{18}

column (Phenomenex Luna column, 5 μm, 25 × 10 mm) eluted with a solvent gradient from 60:40 to 80:20 $CH_3CN:H_2O$ over 25 min at a flow rate of 2.5 mL/min. This process gave compounds 5 (10.4 mg) and 6 (0.4 mg), and semi-pure compound 2 (5.1 mg). The collected semi-pure compound **2** was then further purified by HPLC on the same column eluted with an isocratic $CH_3OH:H_2O$ flow of 75:25 for 15 min, which yielded compound **2** (1.0 mg) and compound **3** (0.8 mg). The active EtOAc fraction was chromatographed by open column on a normal Silica gel with the solvent system from 20:1 to 3:1 CH_2Cl_2:MeOH. Four fractions (AE1-AE4) were collected according to TLC analyses and the fraction AE2 ($2.5 < IC50 < 5.0$ μg/mL) was further separated by HPLC on a semi-preparative C_{18} column eluted with isocratic 50:50 $CH_3CN:H_2O$ for 25 min, which yielded compound **1** (6.4 mg). The active fraction AE3 ($2.5 < IC_{50} < 5.0$ μg/mL) was chromatographed by open column on a RP C_{18} Silica gel with the solvent system from 20:80 to 60:40 $MeOH:H_2O$, which yielded compound **4** (7.0 mg).

Antimalarial and antiproliferative bioassays

Dose-dependent growth inhibition of each fraction and pure compounds was assessed against *P. falciparum* strain Dd2 (chloroquine-resistant) in a 72h growth assay in the presence of inhibitor as described previously with minor modifications. Ring stage parasite cultures (100 μL/well, 1% hematocrit and 1% parasitaemia) were grown for 72 h in the presence of increasing concentrations of the inhibitor in a humidified chamber at 37 °C and low oxygen conditions. After 72 h in culture, parasite viability was determined by DNA quantitation using SYBR Green I. Artemisinin was used as a positive control with an IC_{50} value of 12.1 ± 0.1 nM. Parasite growth was normalized to untreated controls. The IC_{50} calculations were performed with GraphPad Prism software using a nonlinear regression curve fitting, and the reported values represent averages of three independent determinations with each determination in duplicate, and are expressed ± SEM. The A2780 ovarian cancer cell line assay was performed by the protocol of Virginia Polytechnic Institute and State University, as previously reported.

3.5. Compound information

1,4-Dihydroxy-3-methoxypowellan (**1**): white amorphous powder; $[\alpha]^{25}_D$ -37 (c 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 254 nm; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 1 and Figures S1 – S6; HRESIMS found *m/z* 349.1492 $[M]^+$ (calcd for $C_{18}H_{23}NO_6$ 349.1520).

Distichamine (**2**): yellow amorphous powder; $[\alpha]^{25}_D$ -49 (c 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 254 nm; ^{1}H NMR (400 MHz, DMSO) and ^{13}C NMR (100 MHz, DMSO) data, see Figures S7 – S8; HRESIMS found *m/z* 329.1269 $[M]^+$ (calcd for $C_{18}H_{19}NO_5$ 329.1263).

11-O-Acetylambelline (**3**): white amorphous powder; $[\alpha]^{25}_D$ +25 (c 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 254 nm; ^{1}H NMR (400 MHz, DMSO) and ^{13}C NMR (100 MHz, DMSO) data, see Table 1 and Figures S9 – S10. HRESIMS found *m/z* 374.1621 $[M+H]^+$ (calcd for $C_{20}H_{24}NO_6$ 374.1604).

Ambelline (**4**): white amorphous powder; $[\alpha]^{25}_D$ +37 (c 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 254 nm; ^{1}H NMR (400 MHz, DMSO) and ^{13}C NMR (100 MHz, DMSO) data, see Figures S11 – S12. HRESIMS found *m/z* 332.1497 $[M+H]^+$ (calcd for $C_{18}H_{22}NO_5$ 332.1492).

Acetylcaranine (**5**): yellow amorphous powder; $[\alpha]^{25}_D$ -30 (c 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 254 nm; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 1 and Figures S13 – S15; HRESIMS found *m/z* 313.1413 $[M+H]^+$ (calcd for $C_{18}H_{20}NO_4$ 313.1387).

Hippadine (**6**): yellow amorphous powder; $[\alpha]^{25}_D$ +17 (c 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 254 nm; ^{1}H NMR (400 MHz, DMSO) data, see Figure S16; HRESIMS found *m/z* 264.0633 $[M+H]^+$ (calcd for $C_{16}H_{10}NO_3$ 264.0661).

List of Supporting Information

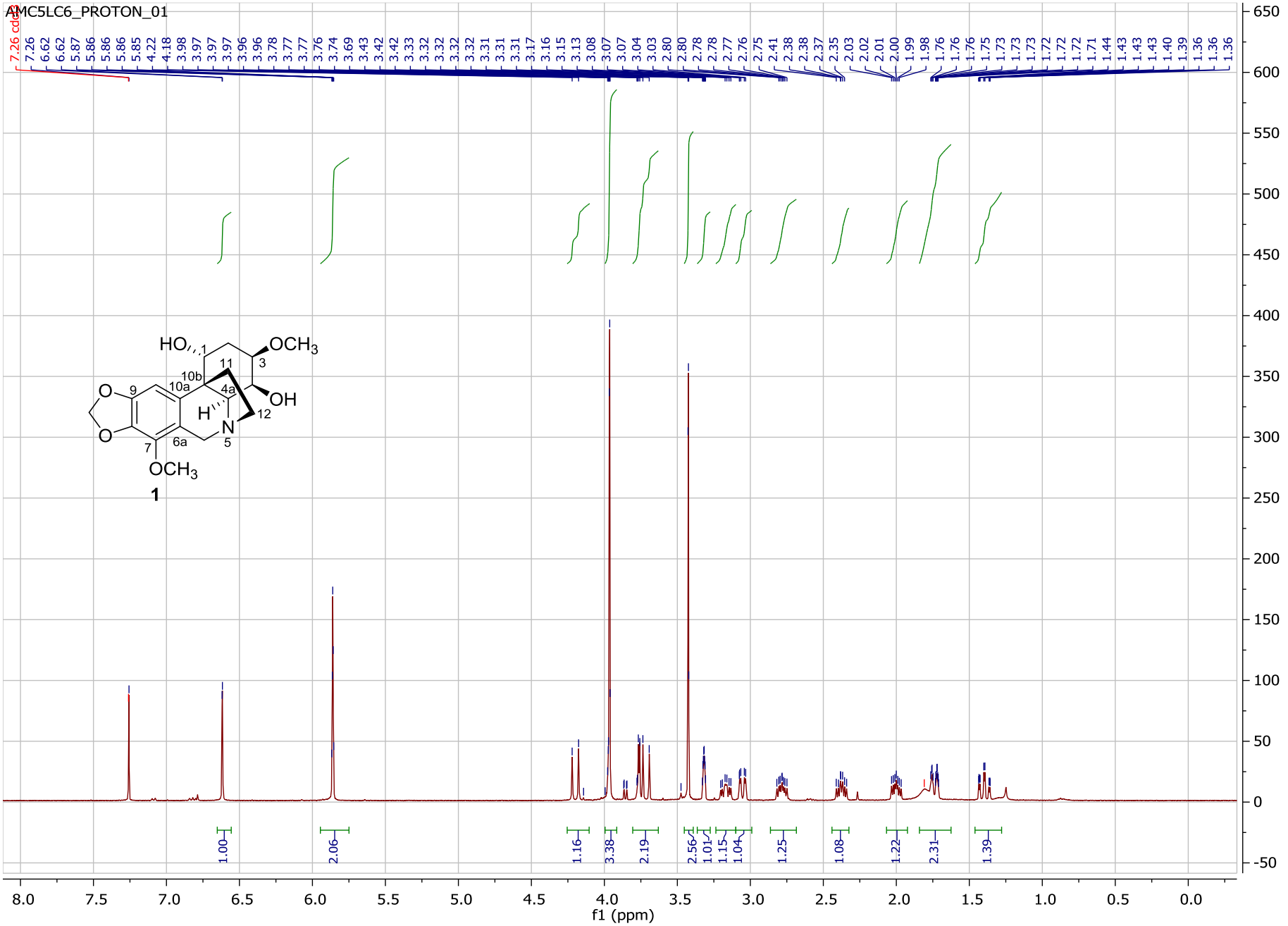


Figure S1. ^{1}H NMR spectrum (400 MHz) of compound **1** in $CDCl_3$

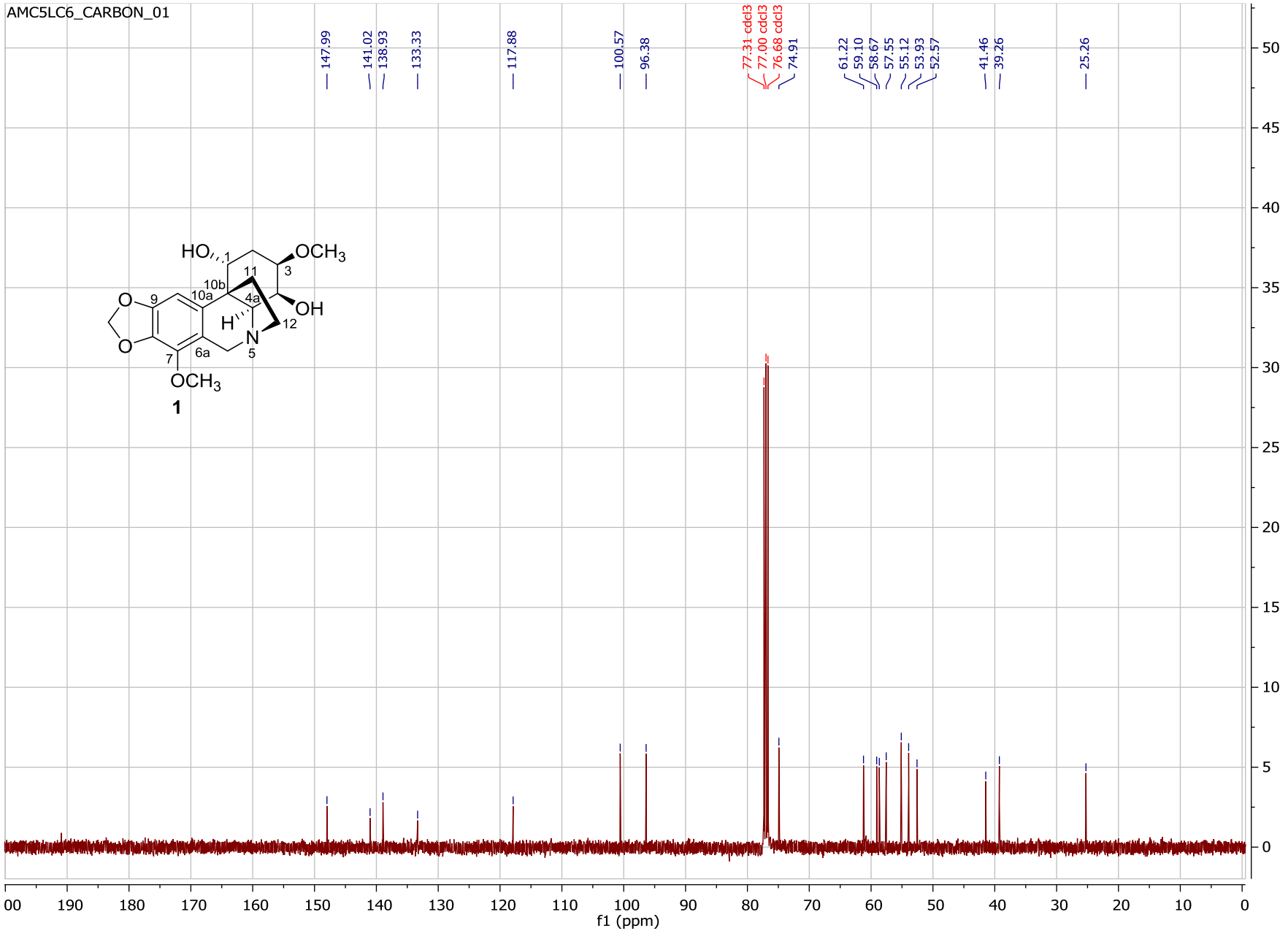


Figure S2. ^{13}C NMR spectrum (400 MHz) of compound **1** in $CDCl_3$

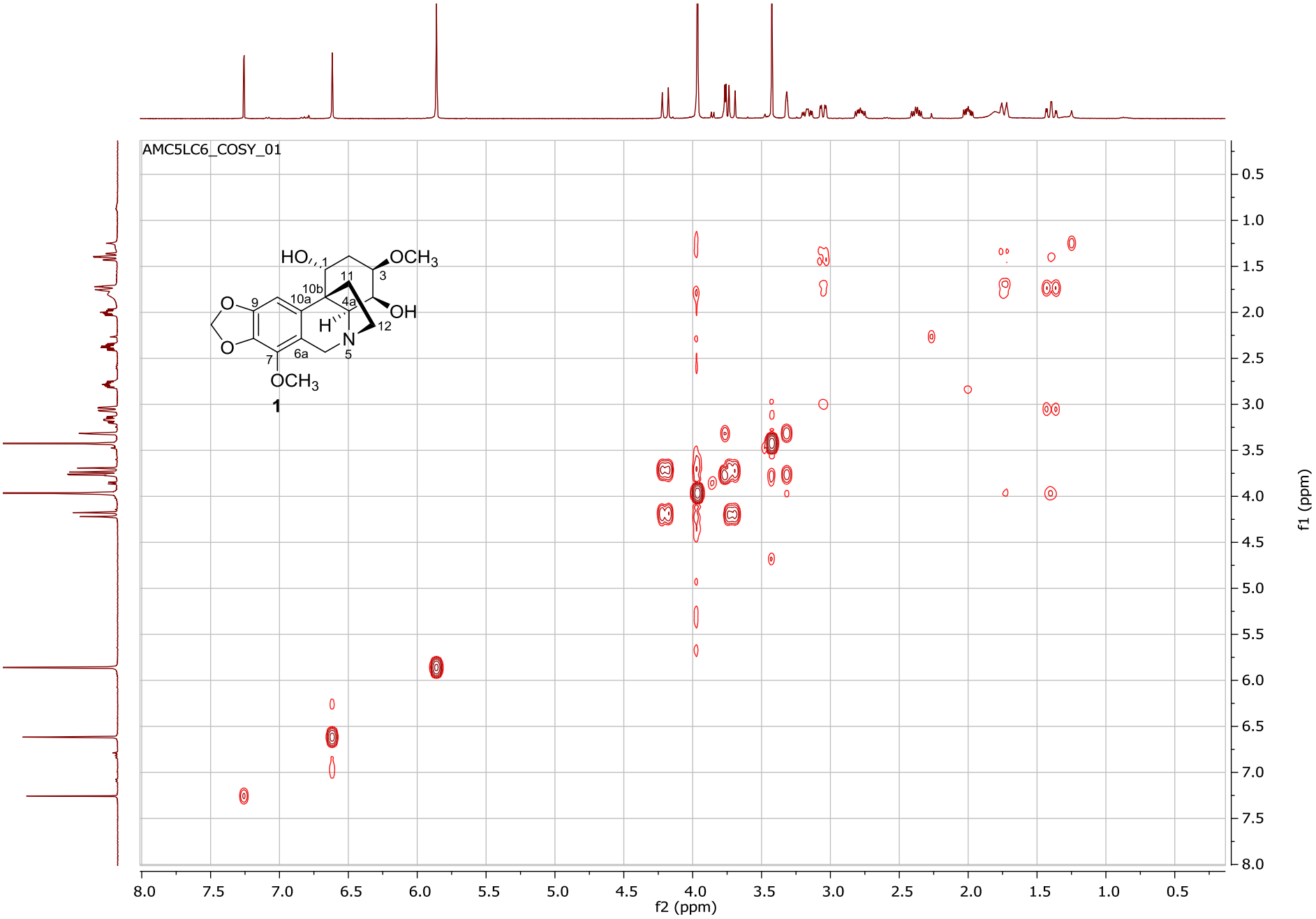


Figure S3. COSY spectrum of compound **1** in $CDCl_3$

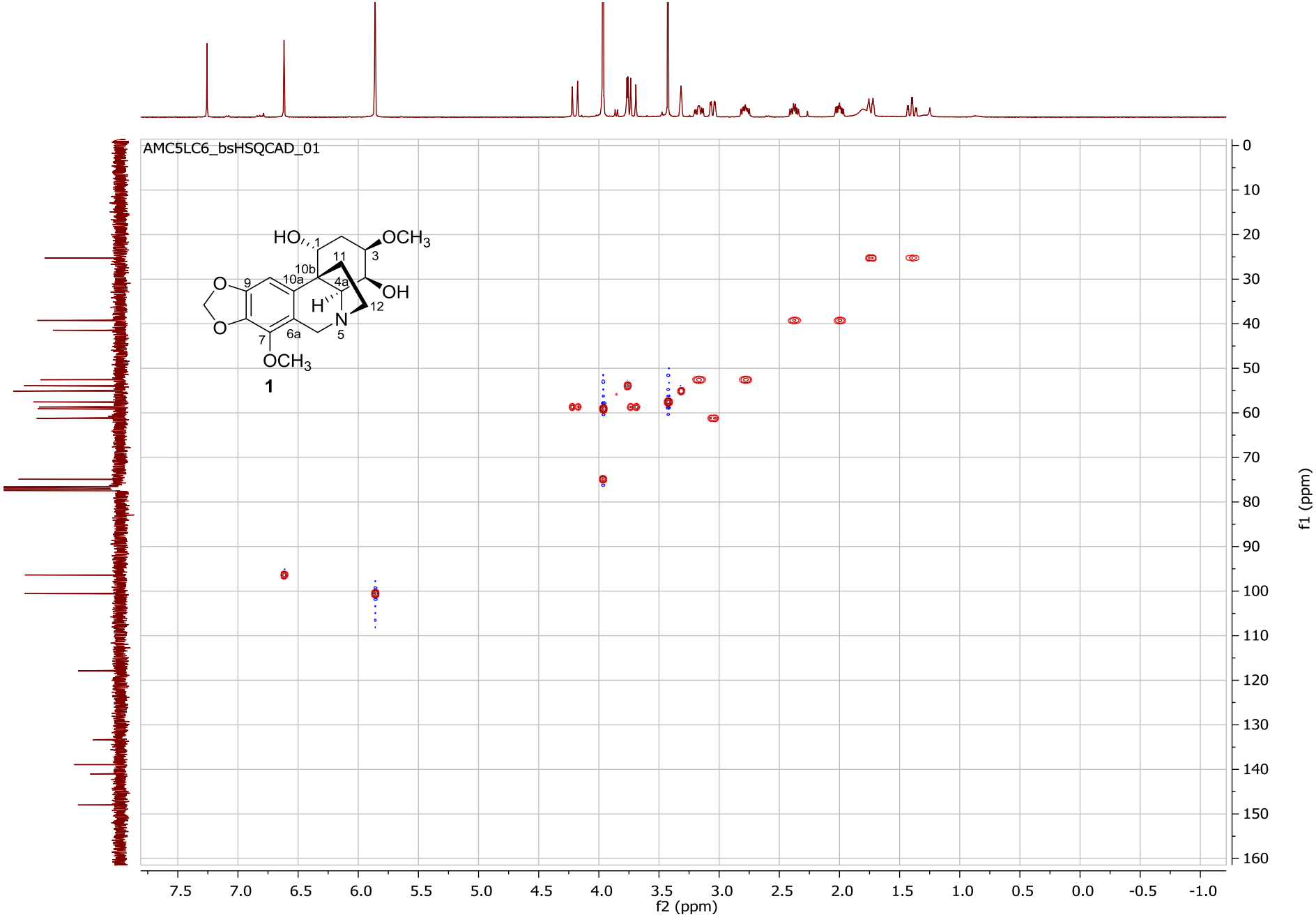


Figure S4. HSQC spectrum of compound **1** in $CDCl_3$

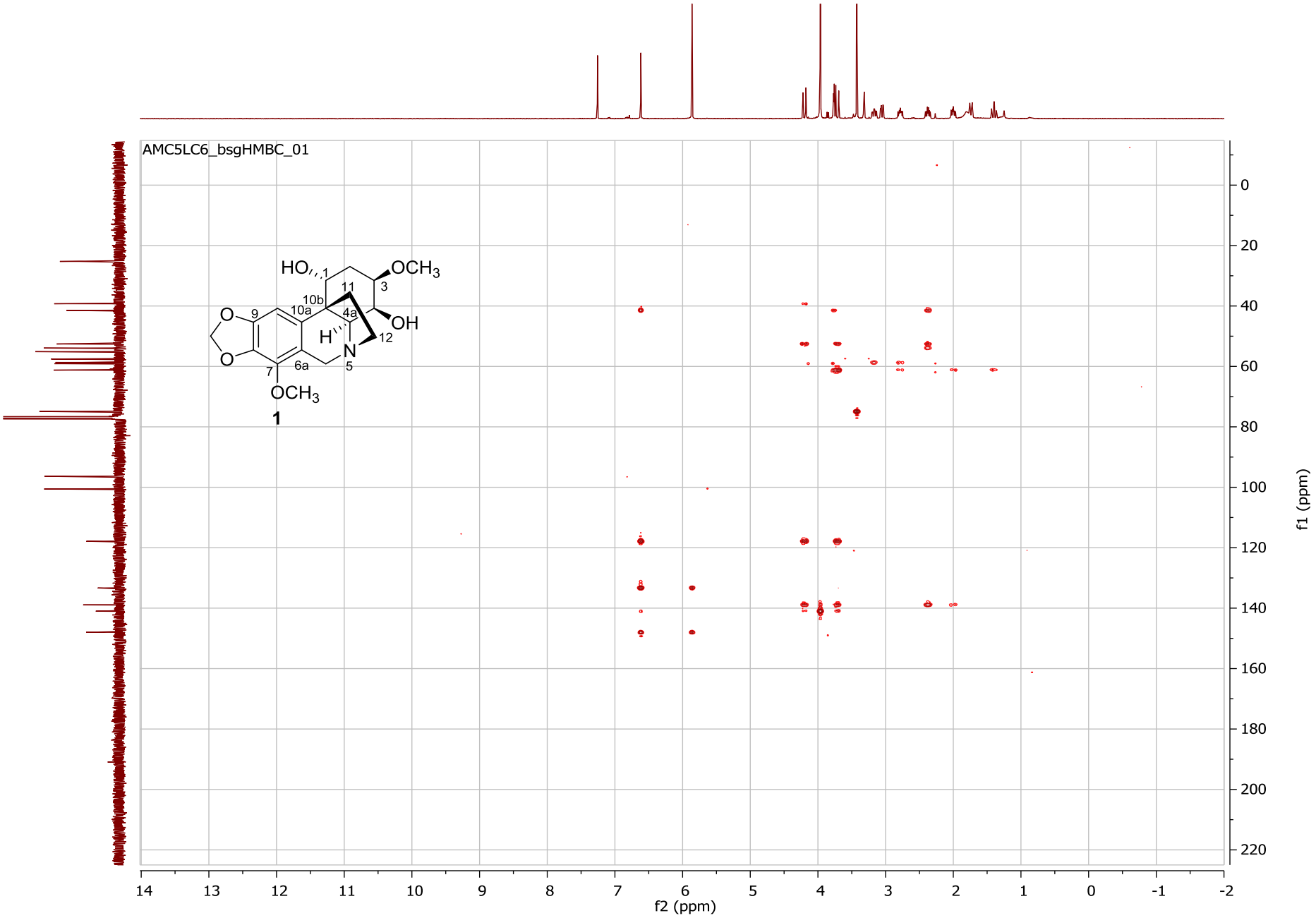


Figure S5. HMBC spectrum of compound **1** in $CDCl_3$

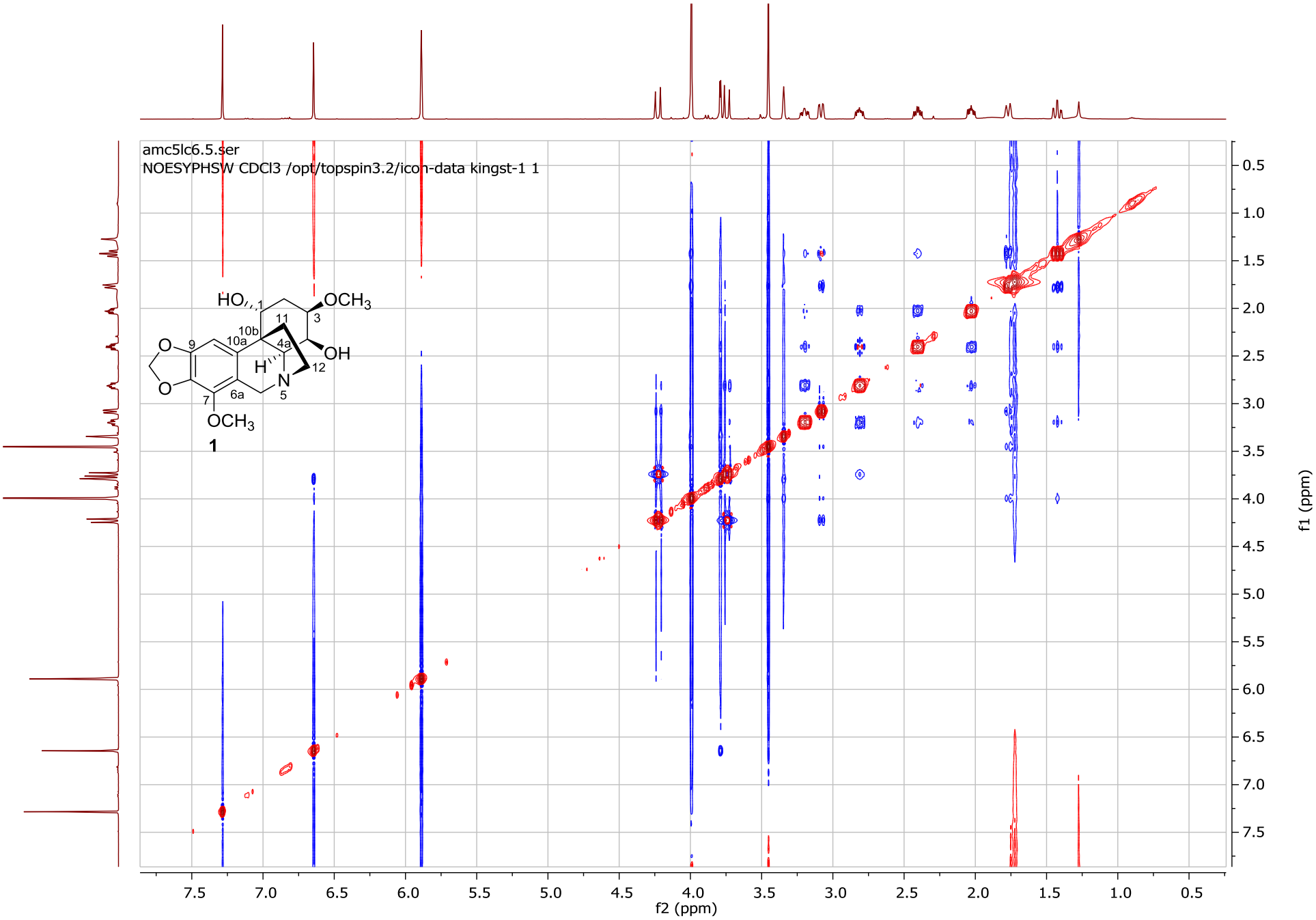


Figure S6. NOESEY spectrum of compound **1** in $CDCl_3$

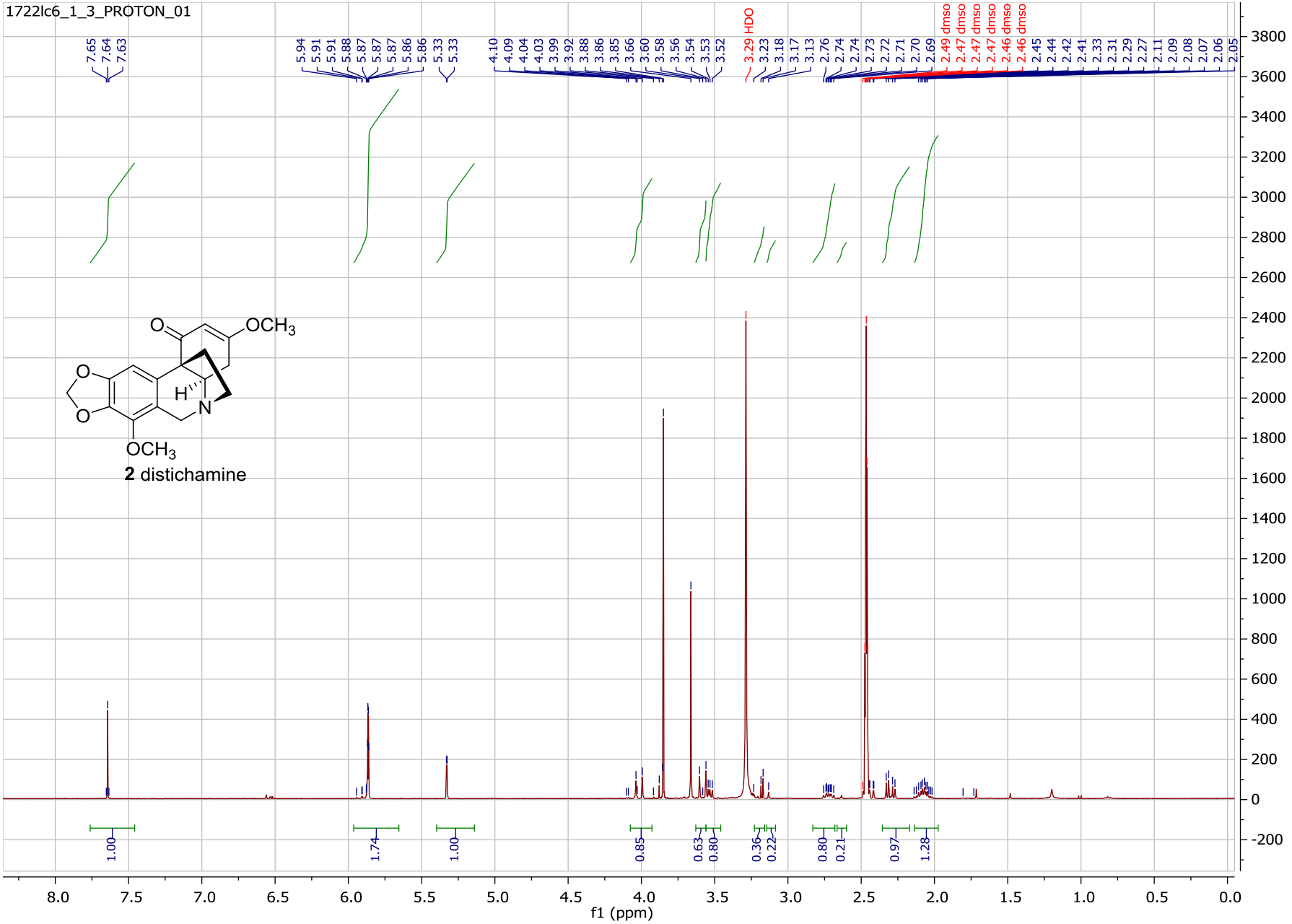


Figure S7. ^{1}H NMR spectrum (400 MHz) of compound **2** in DMSO

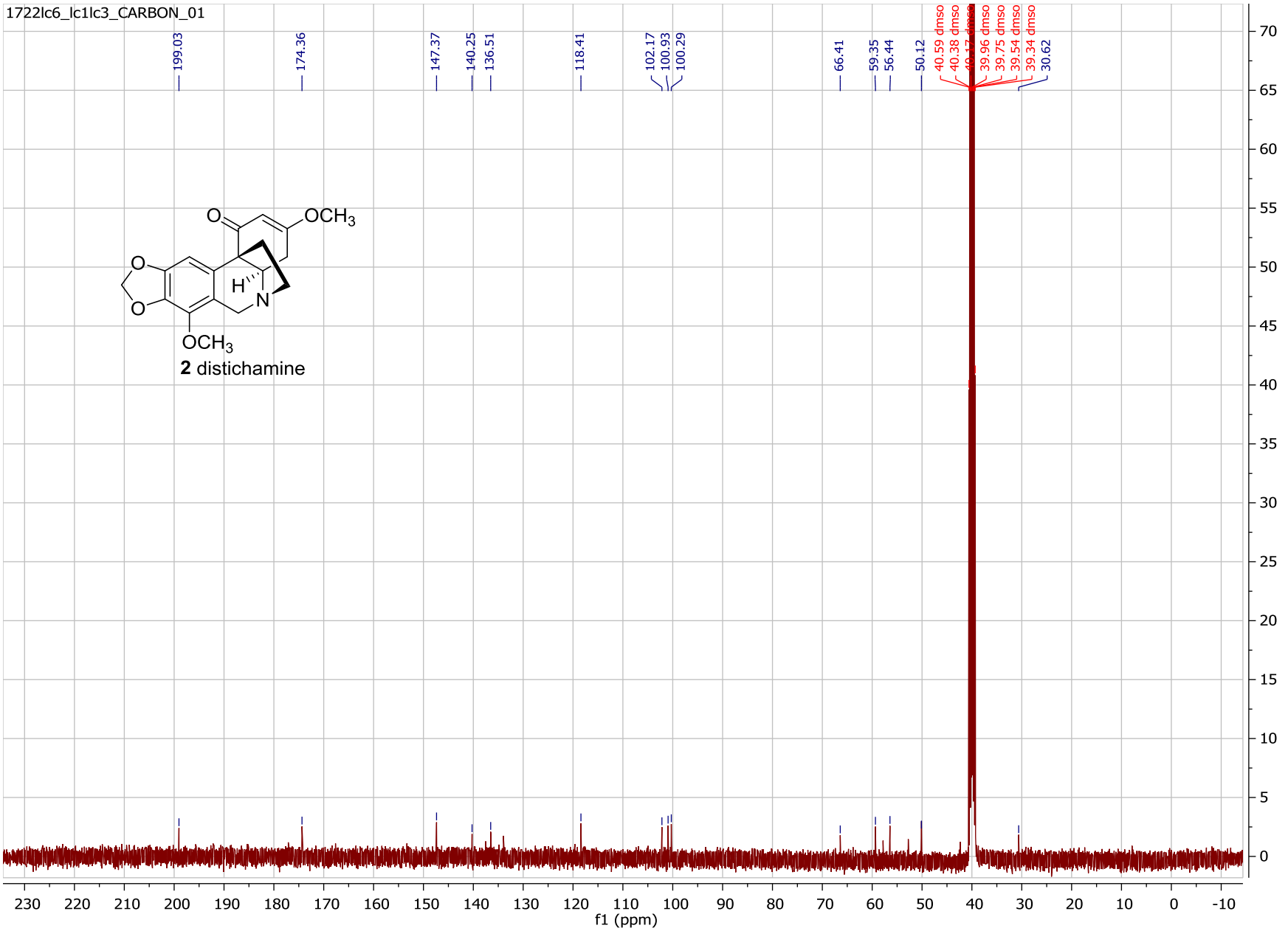


Figure S8. ^{13}C NMR spectrum (400 MHz) of compound **2** in DMSO

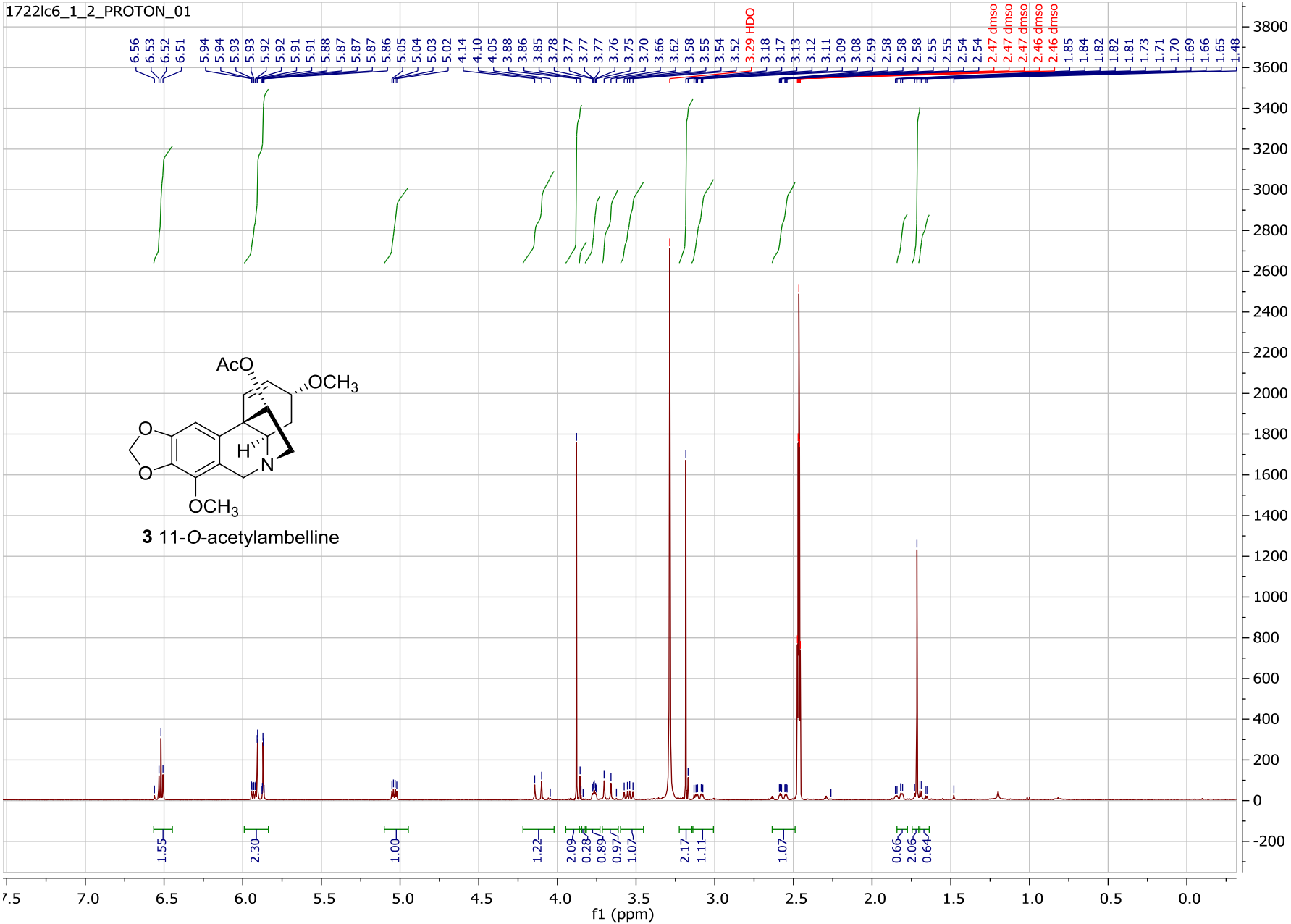


Figure S9. ^{1}H NMR spectrum (400 MHz) of compound **3** in DMSO

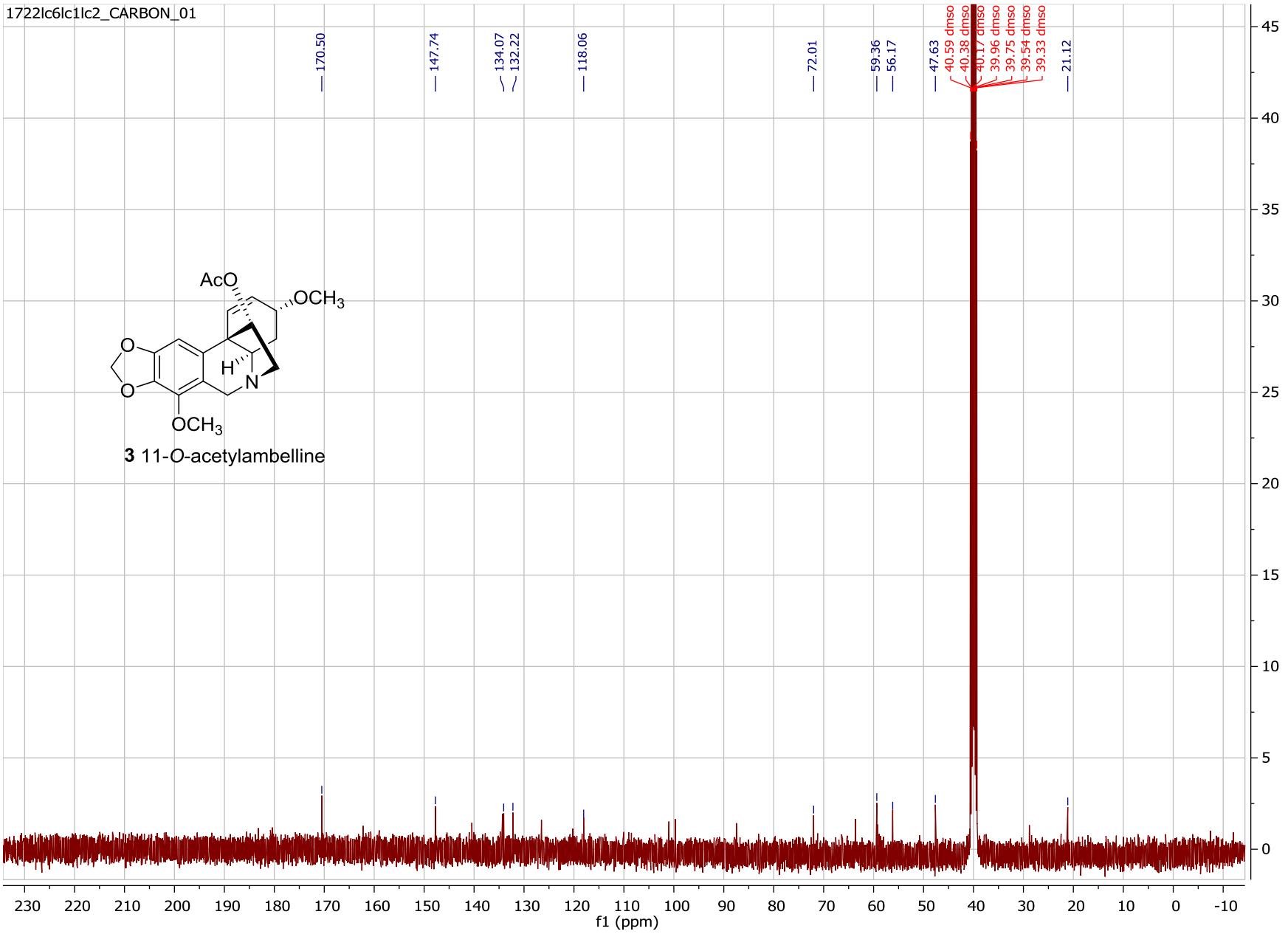


Figure S10. ^{13}C NMR spectrum (400 MHz) of compound **3** in DMSO

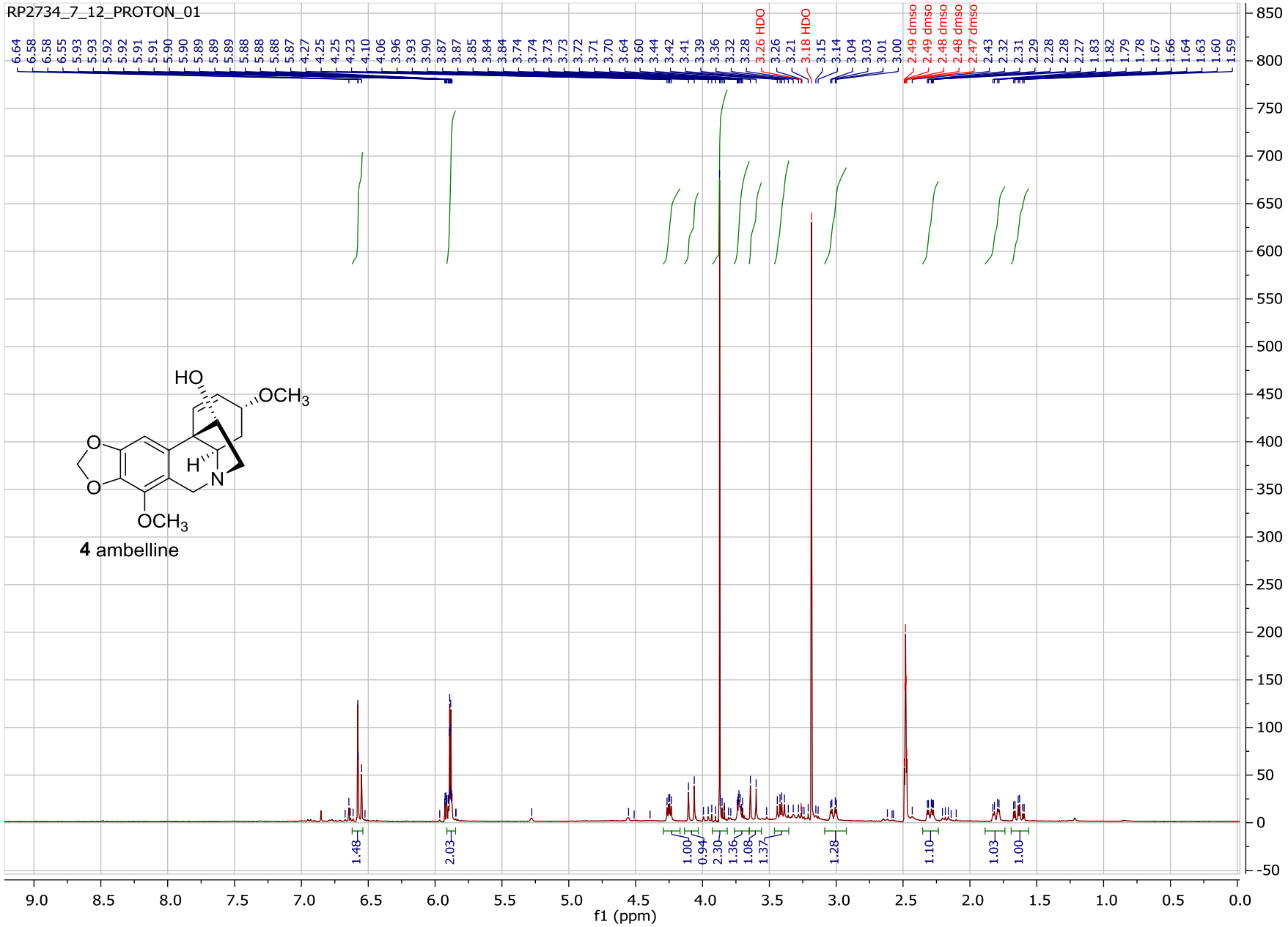


Figure S11. [1]H NMR spectrum (400 MHz) of compound **4** in DMSO

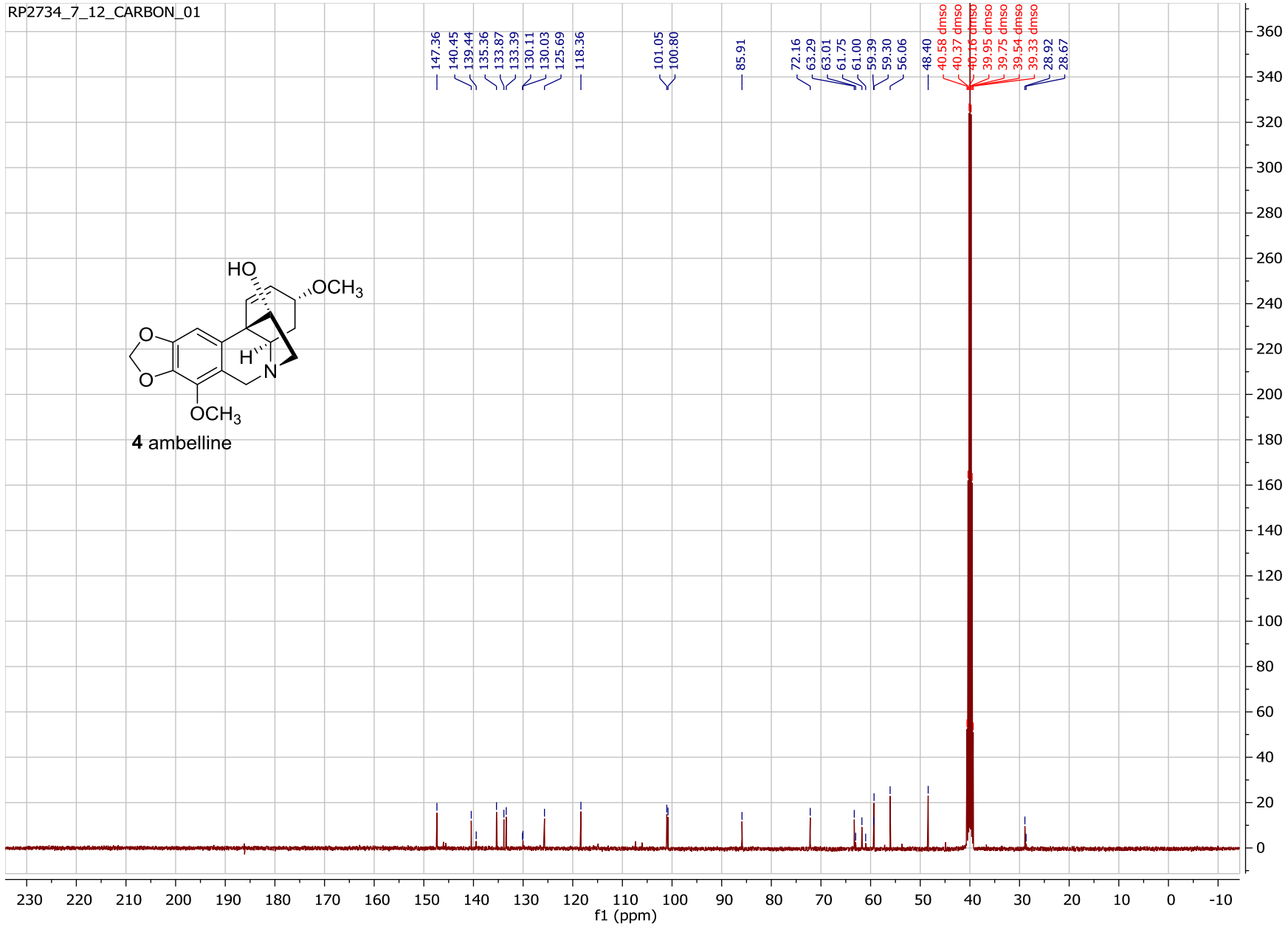


Figure S12. ^{13}C NMR spectrum (400 MHz) of compound **4** in DMSO

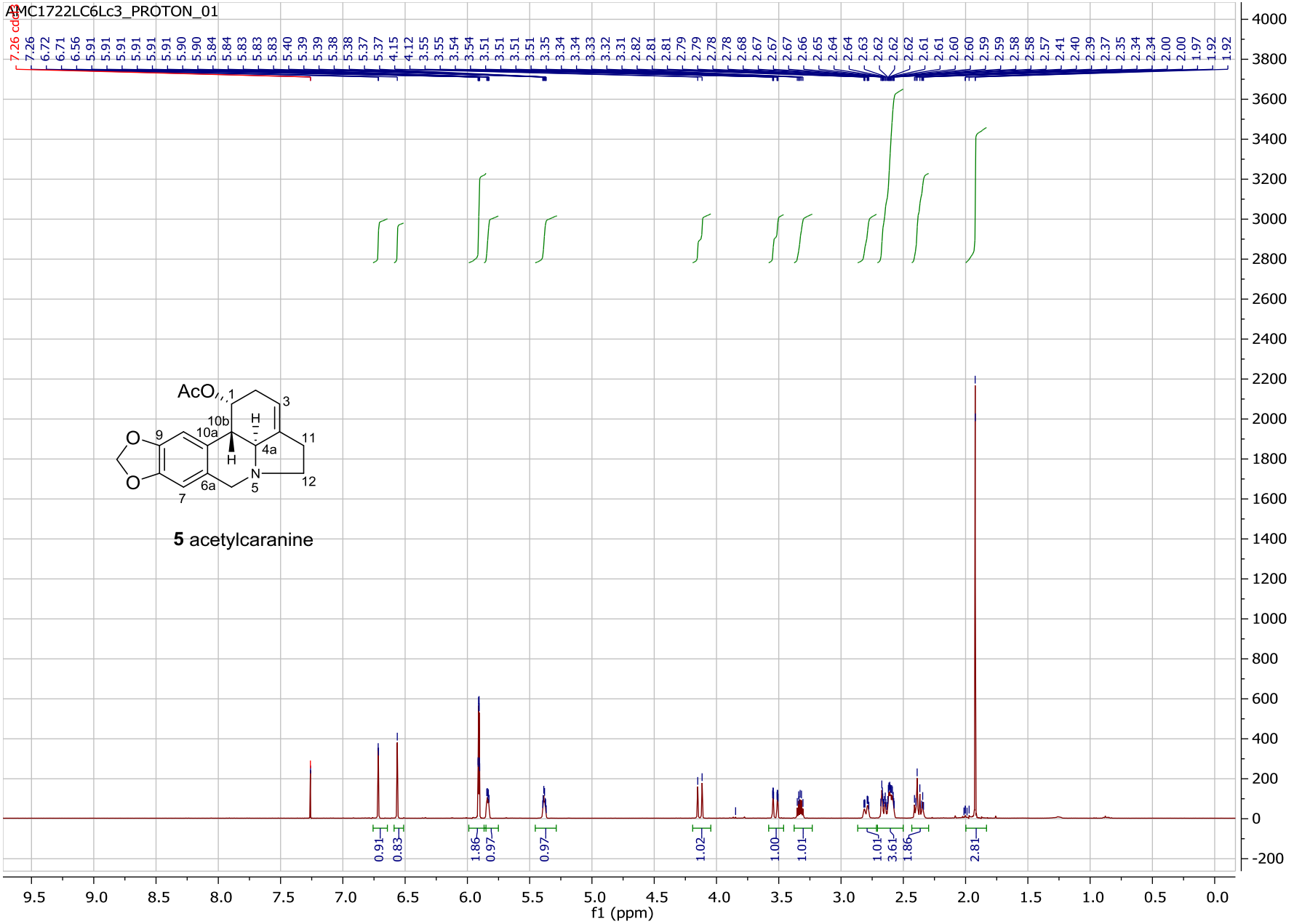


Figure S13. ^{1}H NMR spectrum (400 MHz) of compound **5** in $CDCl_3$

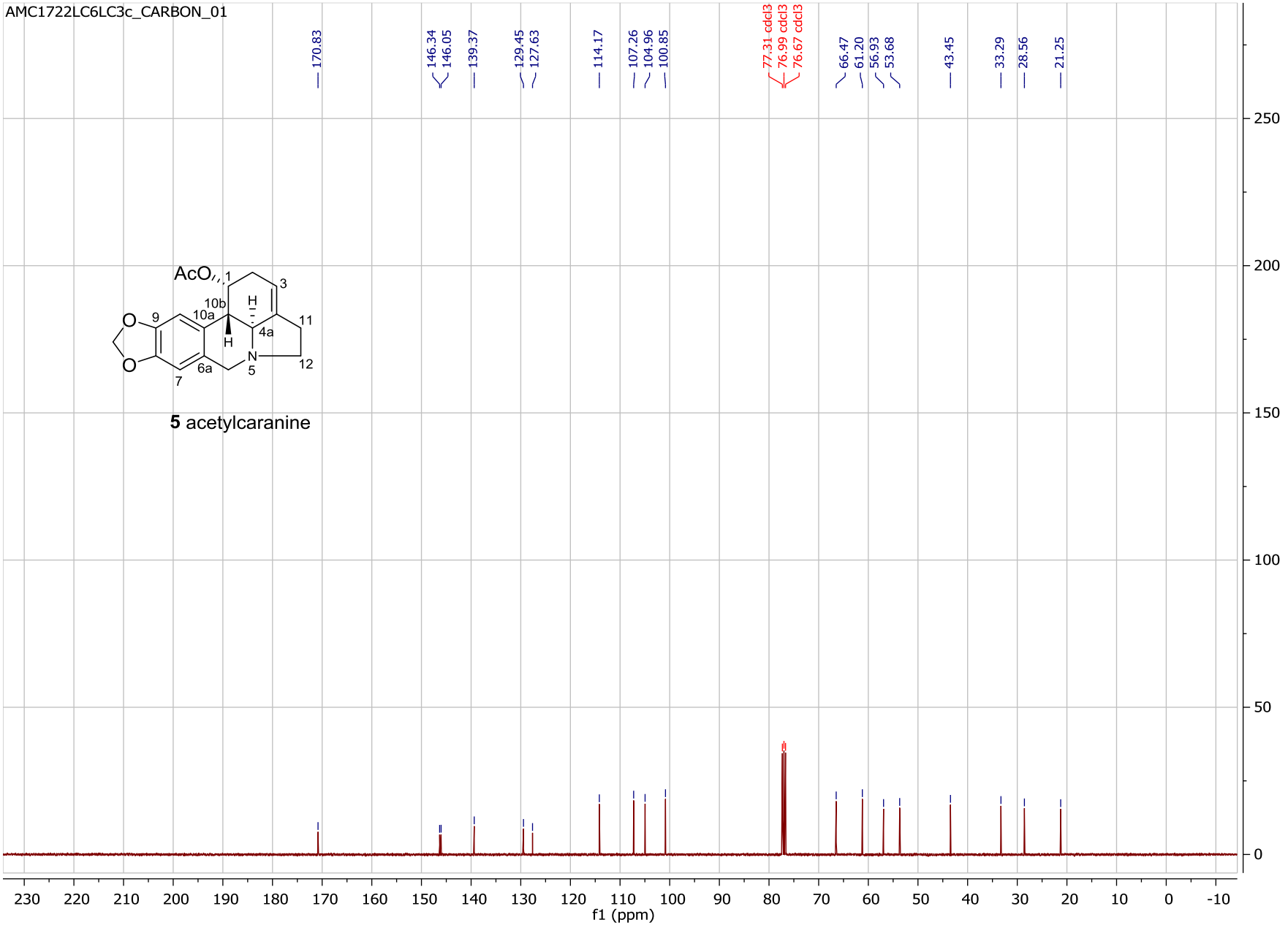


Figure S14. ^{13}C NMR spectrum (400 MHz) of compound **5** in $CDCl_3$

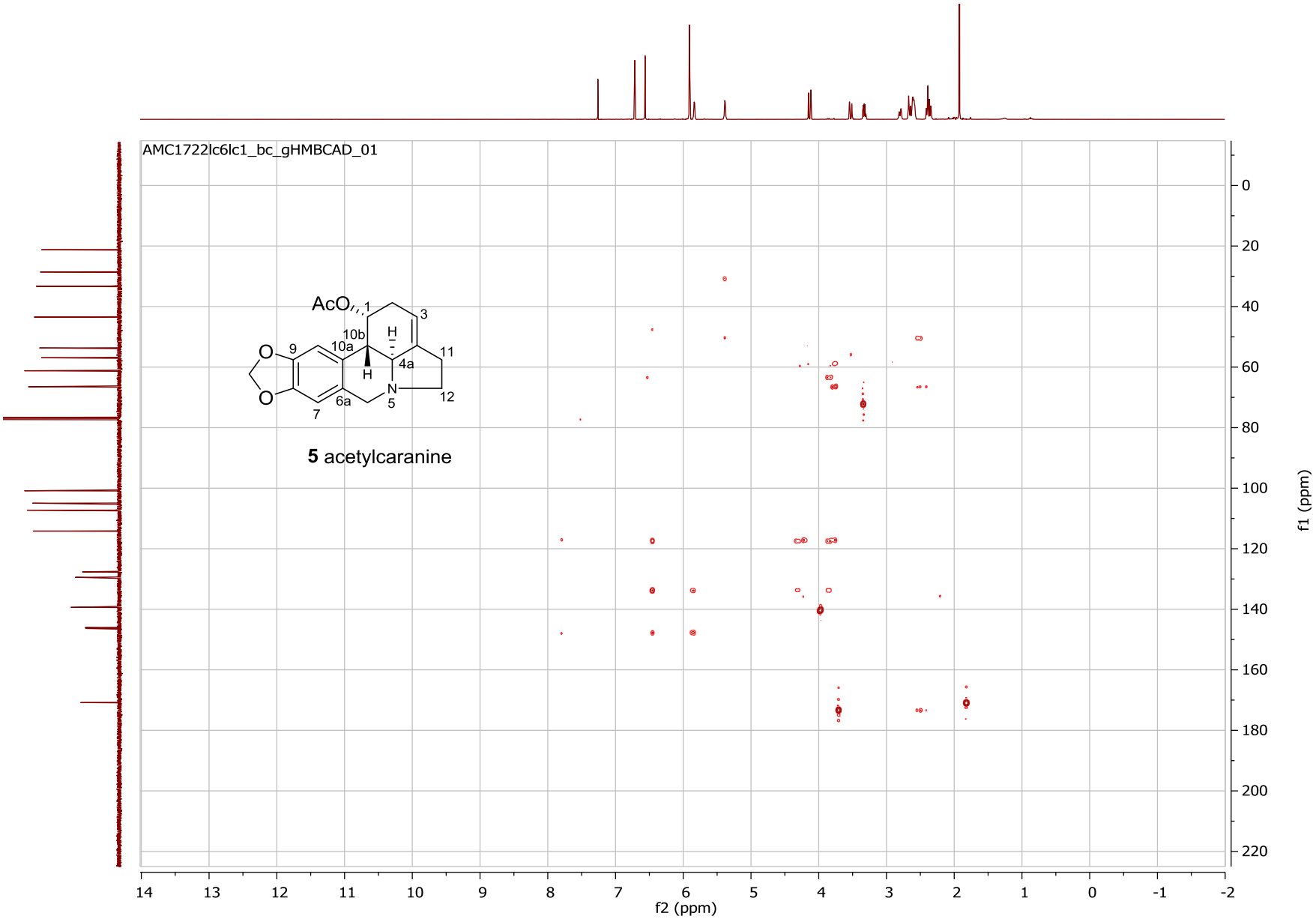


Figure S15. HMBC spectrum of compound **5** in $CDCl_3$

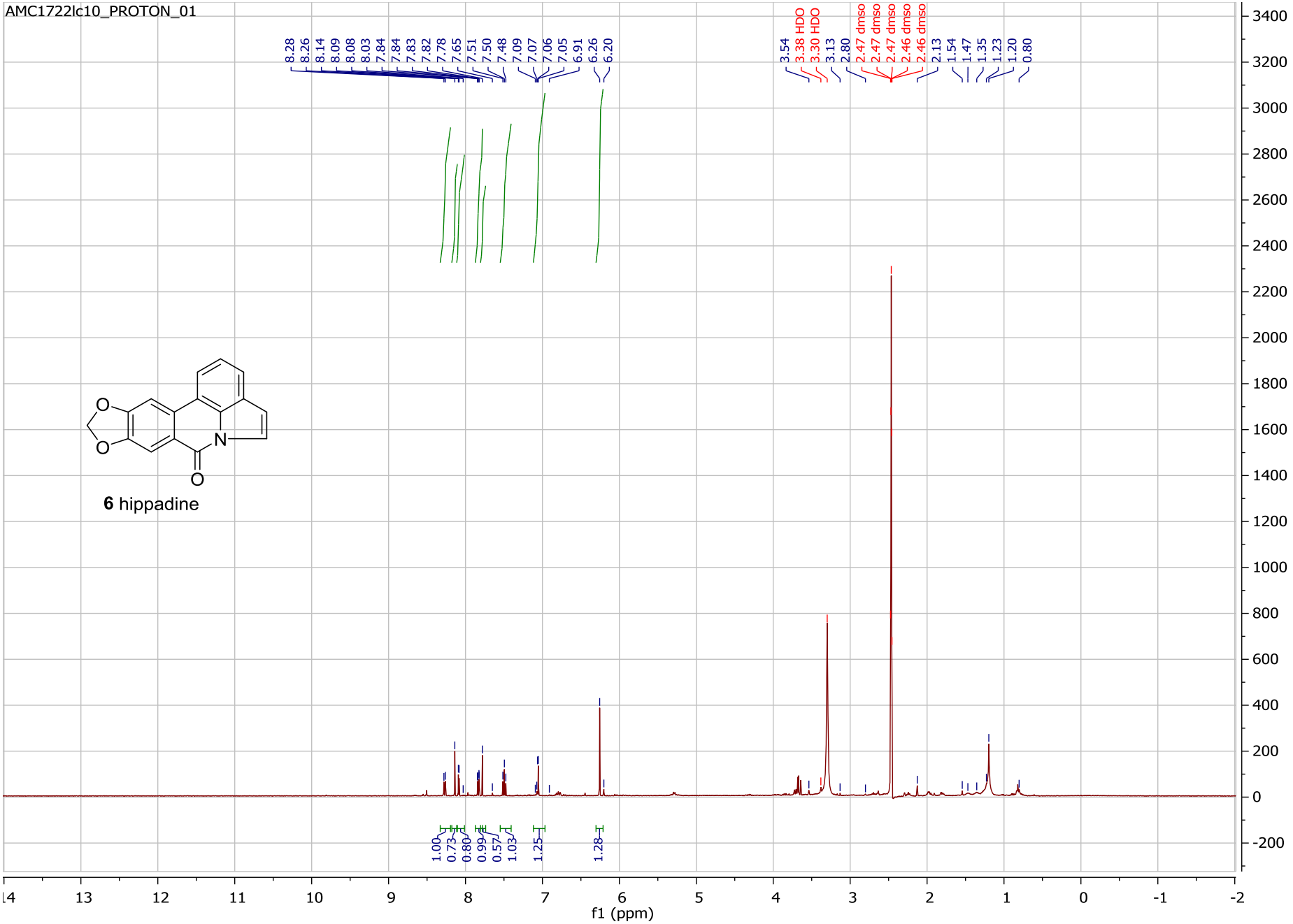
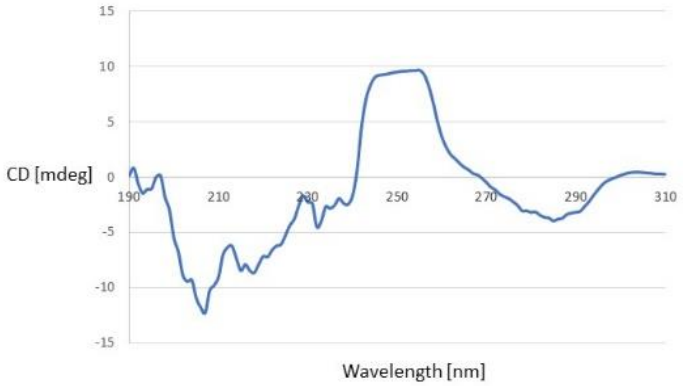


Figure S16. [1]H NMR spectrum (400 MHz) of compound **6** in DMSO



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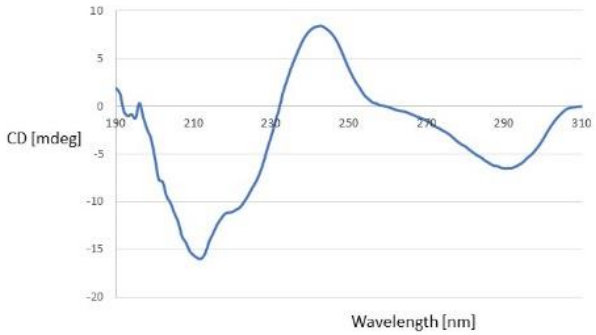

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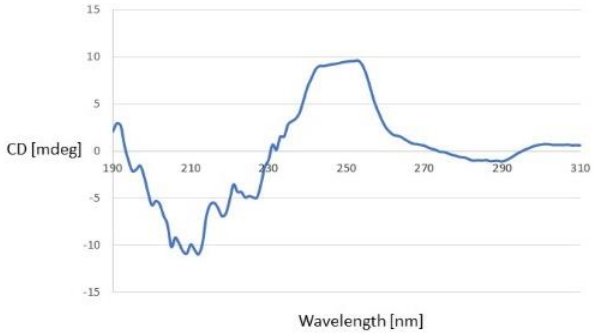

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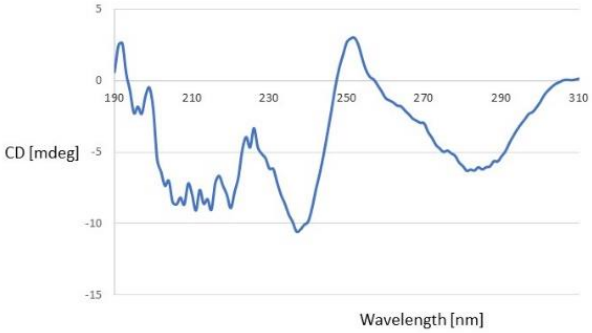

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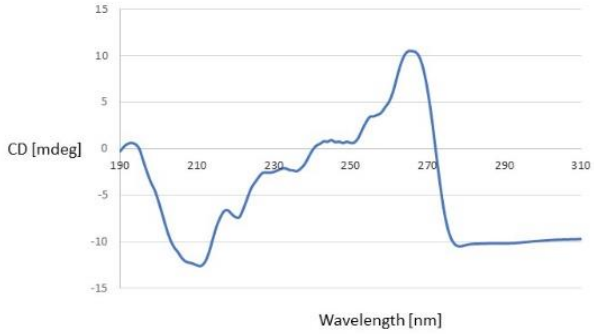

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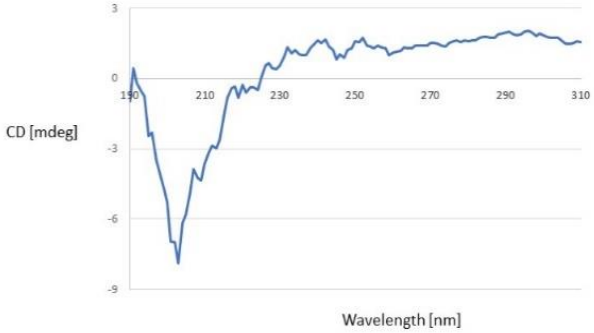

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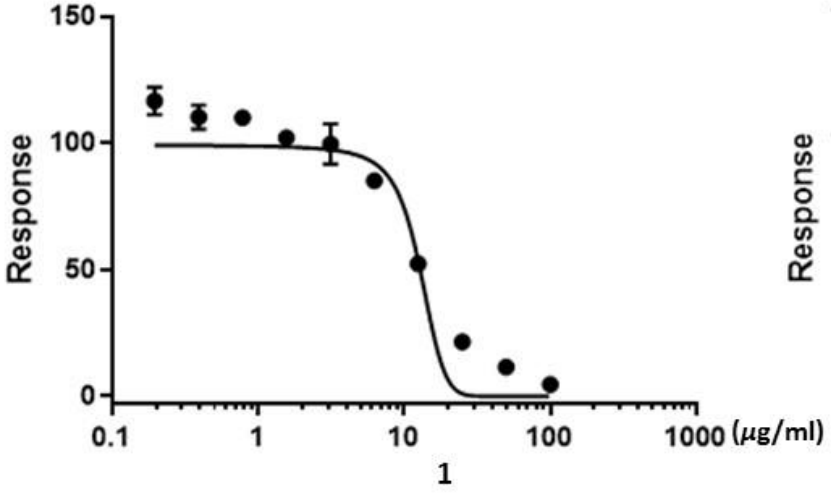
Figure S17. CD spectra of compounds **1-6**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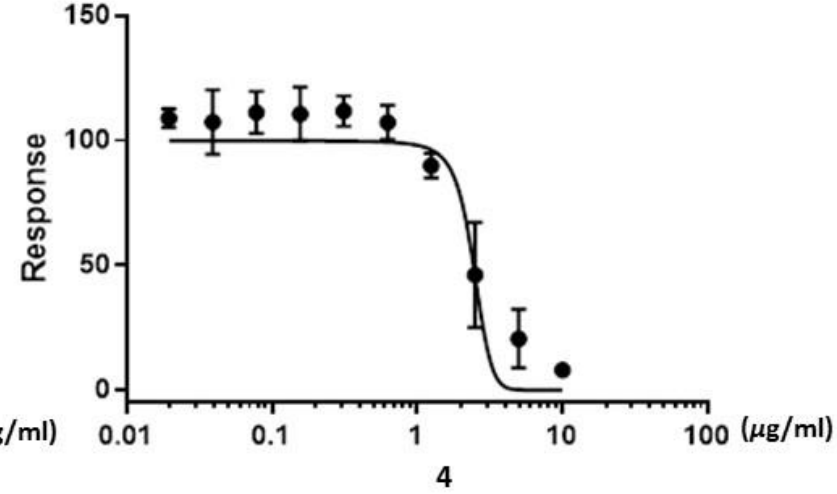
100
50
0
Response
0.1
1
10
100
1000 (μg/ml)

1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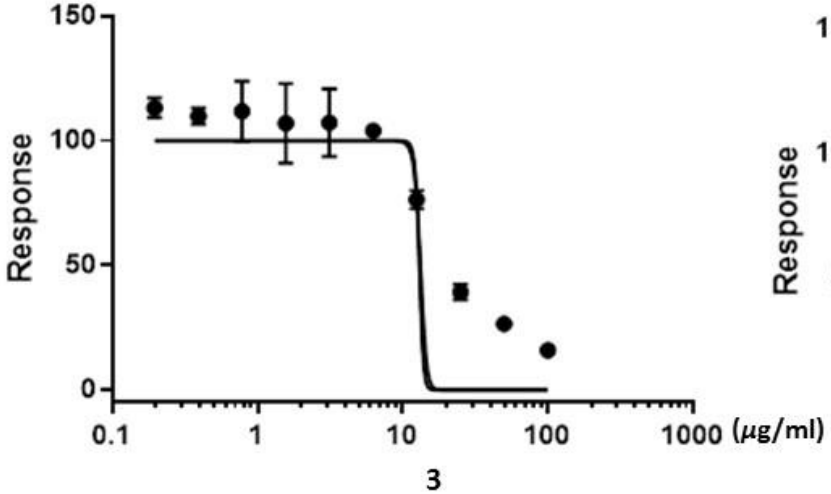
100
50
0
Response
0.01
0.1
1
10
100 (μg/ml)

4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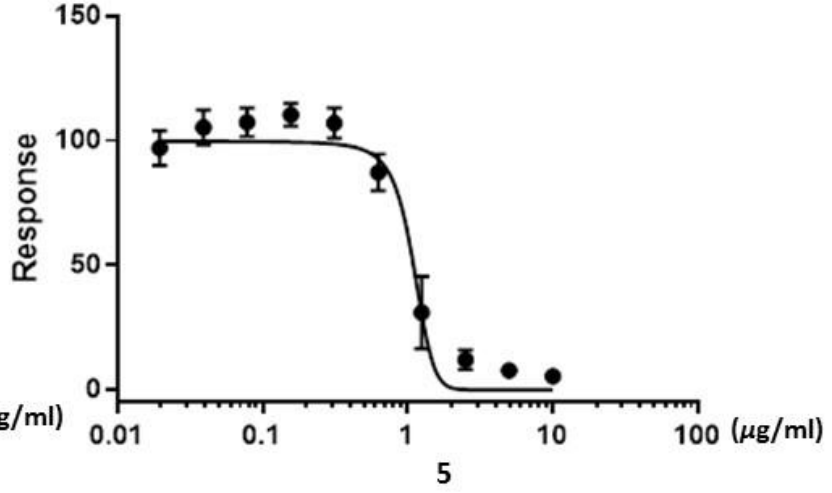
100
50
0
Response
0.1
1
10
100
1000 (μg/ml)

3


150
100
50
0
Response
0.01
0.1
1
10
100 (μg/ml)

5

Figure S18. Dose-response curves for antiplasmodial assays of compounds **1**, **3**, **4**, and **5**.