SUPPORTING INFORMATION

Synthesis of Zephycandidine A from Haemanthamine

Patrick J. Murphy* and Jamie Tibble-Howlings, Radoslaw M. Kowalczyk, Kevin Stevens

General experimental procedures

Column chromatography was carried out on silica gel (60Å, 40-63 μm) and TLCs were conducted on pre-coated Kieselgel 60 F254 (Art. 5554; Merck) with the eluent specified in each case. All non-aqueous reactions were conducted in oven-dried apparatus under a static atmosphere of argon. Chemical shifts are reported in δ values relative to residual chloroform (7.26/77.16 ppm) and methanol (3.31/49.0 ppm) as internal standards. Proton and carbon NMR spectra were performed in the solvent stated on a Bruker 400 spectrometer unless indicated otherwise. High resolution NMR spectra for synthetic **1** were recorded on Bruker Avance III 700 MHz spectrometer operating at the Larmor frequency of 700.19 MHz for protons, 176.09 MHz for carbons and equipped with the four channel cryoprobe. The sample was prepared by adding 0.6 mL of CD_3OD to 3.01 mg of zephycandine **1** power. The sample solution was transferred to a standard 5mm NMR tube where the undissolved sample precipitated at the bottom. The tube was loaded to NMR magnet (16.44 T) and the constant temperature of 297 K was kept through all experiments. The standard 1D proton (^1H) and carbon (^{13}C) spectra were recorded with the spectral resolution of 0.038 Hz (0.00005 ppm) and 0.243 Hz (0.0014 ppm) and referenced to residue solvent signals at 3.31 ppm and 49.0 ppm, respectively. The 2D experiments: COSY, NOESY, HSQC and HMBC were also recorded using standard Bruker pulse sequences. Noticeably, to record full undistorted carbon spectra long relaxation delay (RD) of 10 s was required to avoid saturation of tertiary carbons even at 60^O tip angle. Infrared samples were obtained as thin films or solutions using sodium chloride plates on a Bruker Tensor 37 FT-IR. MS were determined on a Q Exactive Plus (Thermo Scientific) instrument run with positive electrospray ionization (ESI). Melting points were determined on a Stuart SMP10 apparatus and are uncorrected.

Trispheridine 2. *Typical procedure (Table 1, Entry 8)*: Haemanthamine **3**(i) (0.50 g, 1.66 mmol) was sealed in a Carius tube and heated at 190-195 °C for 24 h. Sequential trituration with acetone, ethyl acetate and chloroform followed by evaporation of the triturates gave the crude product. Purification by column chromatography (80% diethyl ether in hexane) gave **2** (74 mg, 0.33 mmol) as a white solid in 20% yield (99 % based on recovered **3**). Further elution (10 % methanol in chloroform) gave **3** (394 mg, 1.31 mmol, 79 % recovery).

Typical procedure with Zn Dust (Table 1, entry 1): Haemanthamine **3** (5.00 g, 16.6 mmol) was dissolved in methanol (50 ml) and added to finely ground commercial zinc powder (9.00 g, 598 mmol) and the mixture evaporated to dryness under vacuum. This mixture was heated at 190-195 °C for 24 h. Work up as above gave **2** (370 mg, 1.66 mmol) as a white solid in 10% yield (24% based on recovered **3**). Further elution (10 % methanol in chloroform) gave **3** (3.10 g, 10.6 mmol, 62 % recovery).

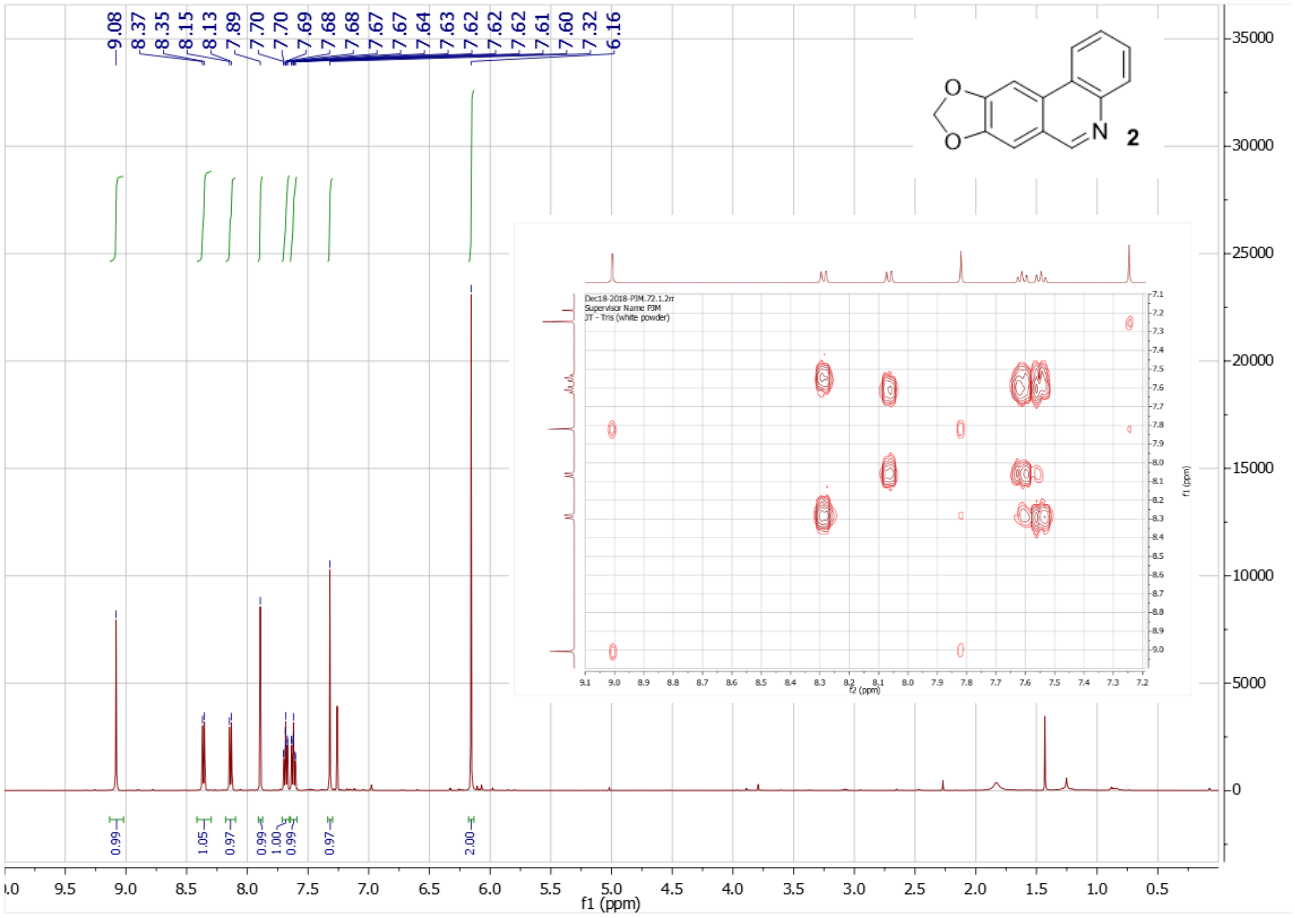
Typical procedure in decalin (Table 1, Entry 13): Haemanthamine **3** (2.00 g, 6.64 mmol) was added to cis/trans decalin (2 ml), sealed in a Carius tube and the mixture heated at 190-195 °C for 24 h. Work up as above gave **2** (191 mg, 0.857 mmol) as a white solid in 13% yield (39 % based on recovered **3**). Further elution (10 % methanol in chloroform) gave **3** (668 mg, 2.22 mmol, 33 % recovery). Data for **2** was in agreement with the literature. **Rf** 0.24 (40% EtOAc in PE); **Mp** 142-145 °C (lit.[5e] 142.5-144 °C); δ_H 9.08 (1H, s, CH), 8.36 (1H, br d, *J* 7.9 Hz, CH), 8.14 (1H, br d, *J* 8.1 Hz, CH), 7.89 (1H, s, CH), 7.68 (1H, ddd, *J* 1.2, 7.0, 8.1 Hz, CH), 7.62 (1H, ddd, *J* 1.2, 7.0, 7.9 Hz, CH), 7.32 (1H, s, CH), 6.16 (2H, s, CH_2); δ_C 151.9, 151.6, 148.3, 144.3, 130.4, 130.2, 128.1, 126.8, 124.4, 123.2, 122.1, 105.6, 102.0, 100.1; ν_{max}; 3031, 2960, 1620, 1581, 1528, 1497, 1486, 1462, 1394, 1382, 1293, 1254, 1227, 1198, 1111, 1094, 1034, 971, 938, 882, 847, 828, 785, 753, 718, 706, 673, 612, 544, 473, 432; **HRMS(ES)** found 224.0707, $C_{14}H_{10}NO_2$ ([M+H$^+$]) requires 224.0706.

5-(2-bromoethyl)-[1,3]dioxolo[4,5-j]phenanthridin-5-ium 4. Trispheridine **2** (1.00 g, 4.48 mmol) was suspended in freshly distilled 1,2-dibromoethane (30 mL) and heated at 70-80 °C for 72 hrs. After cooling the precipitate formed was removed by filtration and washed with 1,2-dibromoethane (5 ml) and ethyl acetate (2 x 5 mL). After drying under vacuum we obtained the salt **4** (1.56 g) as an off white solid which was contaminated with **2**.HBr (ca 20%). This mixture was used in the next reaction without further purification. An analytical sample was obtained by washing a small sample (ca 20 mg) in a pipette filter sequentially with small portions (5 x 0.6 ml) of CD_3OD to remove **2**.HBr. The 4th and 5th washings contained **4** (ca. 95% pure). **Mp** 263-266 °C (dec.); δ_H (CD_3OD) 9.79 (1H, s, CH), 8.99 (1H, dd, *J* 1.4, 8.3 Hz, CH), 8.48 (1H, s, CH) 8.47 (1H, br d, *J* 9.2 Hz, CH), 8.11 (1H, ddd, *J* 1.4, 7.2, 8.5 Hz, CH), 8.04 (1H, ddd, *J* 1.0, 7.2, 8.3 Hz, CH), 7.83 (1H, s, CH), 6.45 (2H, s, CH_2), 5.46 (2H, t, *J* 6.0 Hz, CH_2), 4.15 (2H, t, *J* 6.0 Hz, CH_2); δ_C (CD_3OD) 159.6, 154.8, 153.0, 152.5, 137.6, 133.3, 132.3, 131.1, 127.3, 126.5, 122.0, 119.8, 108.5, 106.0, 102.3, 59.2, 30.0; ν_{max}; 3476, 3401, 3071, 3021, 2944, 2879, 1653, 1612, 1565, 1538, 1503, 1475, 1427, 1408, 1392, 1353, 1329, 1284, 1257, 1211, 1180, 1154, 1131, 1114, 1036, 1020, 977, 943, 922, 892, 880, 859, 794, 780, 764, 729, 679, 610, 593, 559, 546, 503, 467, 454, 430; **HRMS(ES)** found 330.0124, $C_{16}H_{14}{}^{79}BrNO_2$ ([M+H$^+$]) requires 330.0124, found 332.0103, $C_{16}H_{14}{}^{81}BrNO_2$ ([M+H$^+$]) requires 330.0104.

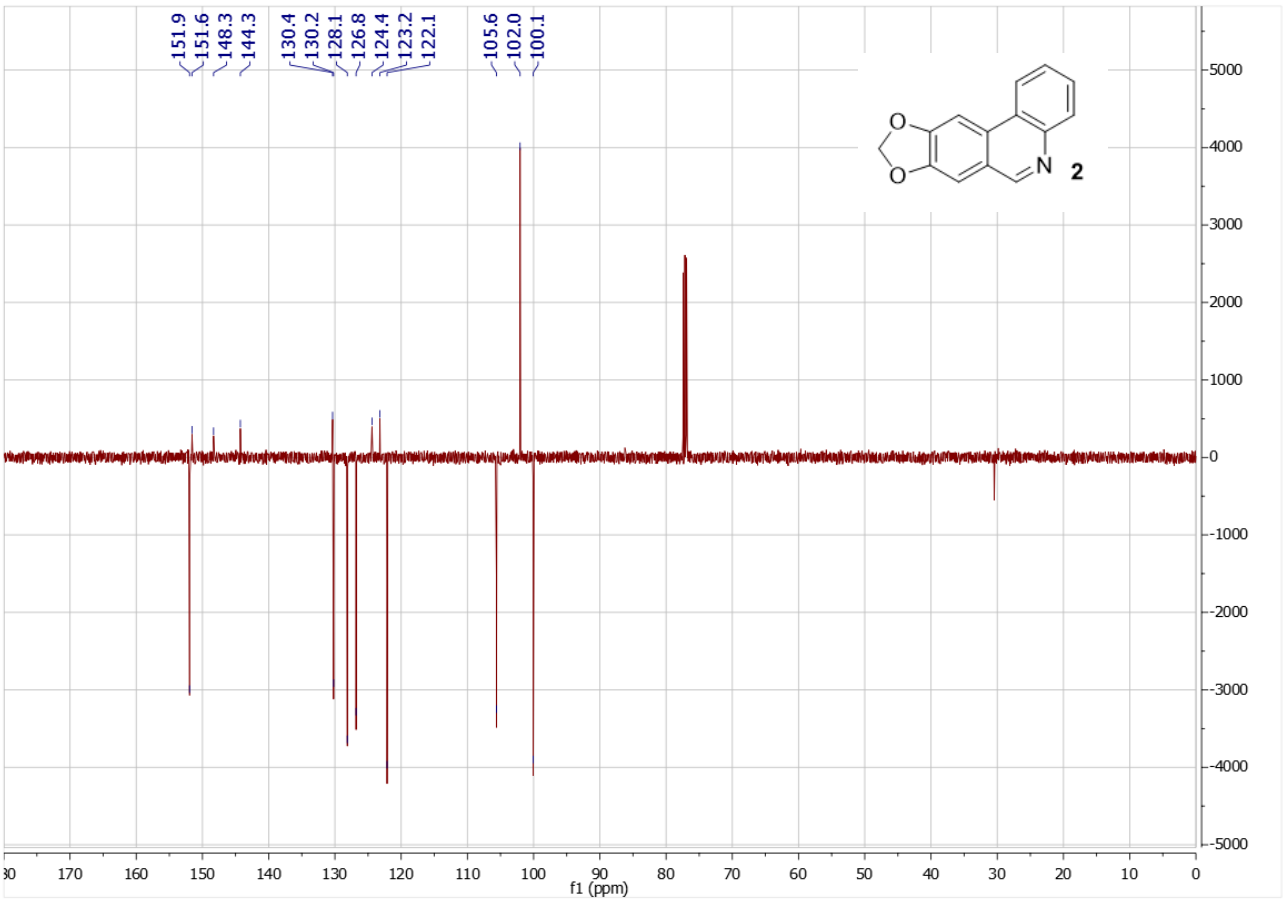
Zephycandidine A 1. Crude **4** (0.50 g, 1.05 mmol) was added to stirred liquid ammonia (ca 100 ml) at -70 °C and after removal of the cooling bath, the reaction mixture was warmed to -33 °C. The reaction was kept at this temperature for 1 h then cooled (-78°C) before adding Na_2CO_3 (603 mg; 5.69 mmol) and finely powdered MnO_2 (1.22 g; 14 mmol). After stirring for 1h, the cooling bath was removed to allow the ammonia to evaporate overnight. The residue was dried under vacuum for 10 minutes to remove any remaining ammonia, and then toluene (50 ml) was added. The reaction mixture was refluxed for 3 h, cooled and filtered with the solid inorganic residue being washed with acetone (3 x 10 ml). Concentration of the filtrates followed by column chromatography (30-50 % EtOAc in PE) gave **1** (0.15 g, 54 %) as an off white solid. Recovered trispheridine **2** (0.07 g) was also obtained. **Rf** 0.29 (50% EtOAc in PE); **Mp** 242-245 °C (dec.); δ_H (CD_3OD) 8.39 (1H, dd, *J* 1.2, 8.1 Hz, CH), 8.28 (1H, d, *J* 1.5 Hz, CH), 8.09 (1H, dd, *J* 1.2, 8.2 Hz, CH), 7.90 (1H, s, CH), 7.81 (1H, s, CH), 7.64 (1H, ddd, *J* 1.2, 7.2, 8.2 Hz, CH), 7.53 (1H, ddd, *J* 1.2, 7.2, 8.1 Hz, CH), 7.50 (1H, d, *J* 1.5 Hz, CH), 6.15 (2H, s, CH_2);δ_C (CD_3OD) 151.4, 150.5, 143.6, 132.1, 131.2, 129.6, 126.6, 125.23, 125.21, 122.9, 119.5, 117.3, 113.7, 102.9, 103.5, 102.8; δ_H ($CDCl_3$/CD_3OD, 10:1) 8.14 (1H, dd, *J* 0.8, 8.2 Hz, CH), 7.89 (1H, s, CH), 7.88 (1H, d, *J* 1.4 Hz, CH), 7.77 (1H, dd, *J* 0.8, 8.2 Hz, CH), 7.62 (1H, s, CH), 7.51 (1H, ddd, *J* 1.2, 7.2, 8.2 Hz, CH), 7.48 (1H, d, *J* 1.4 Hz, CH), 7.42 (1H, ddd, *J* 1.2, 7.2, 8.2 Hz, CH), 6.08 (2H, s, CH_2); δ_C ($CDCl_3$/CD_3OD, 10:1) 149.6, 148.9, 142.5, 130.9, 130.9, 128.1, 125.2, 123.9, 123.5, 121.7, 119.0, 115.9, 111.7, 102.8, 101.9, 101.4; ν_{max} 3117, 3090, 2917, 2851, 1618, 1536, 1403, 1390, 1313, 1260, 1206, 1174, 1144, 1122, 1034, 945, 929, 905, 848, 827, 768, 736, 692, 681, 620, 584, 472, 444, 423; λ_{max} (MeOH, log ε) 202 (4.62), 228 (4.63), 256 (4.86), 263 (4.89), 296 (4.37) nm; **HRMS(ES)** found 263.0818, $C_{16}H_{11}N_2O_2$ ([M+H$^+$]) requires 263.0815.

(i) Supplied by BioExtractions (Wales) Ltd., Unit 30, Tafarnaubach Industrial Estate, Tafarnaubach, Tredegar, Blaenau Gwent NP22 3AA, UK.

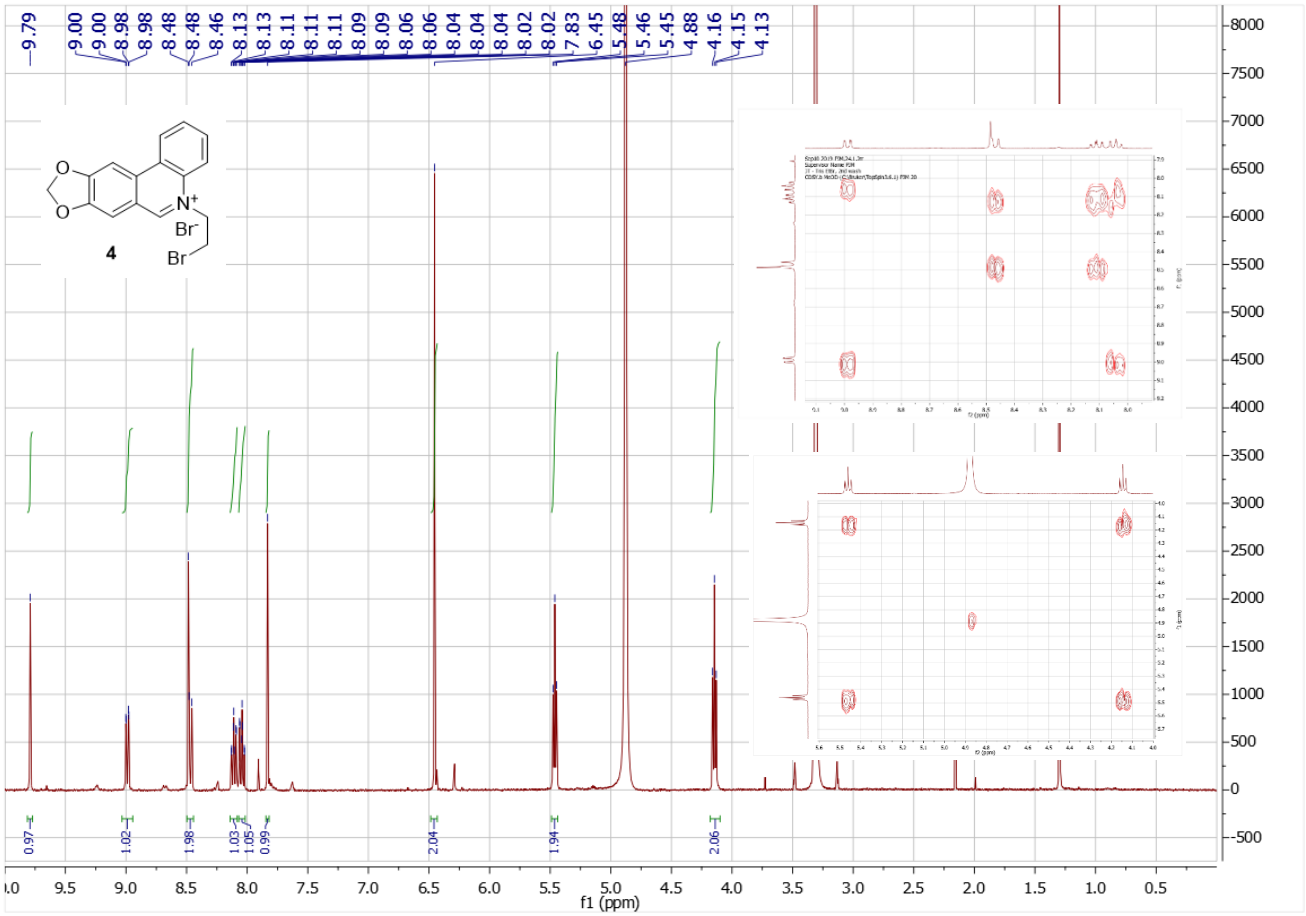
Trispheridine 2 ($CDCl_3$, 400 MHz, ^1H NMR, COSY (insert))



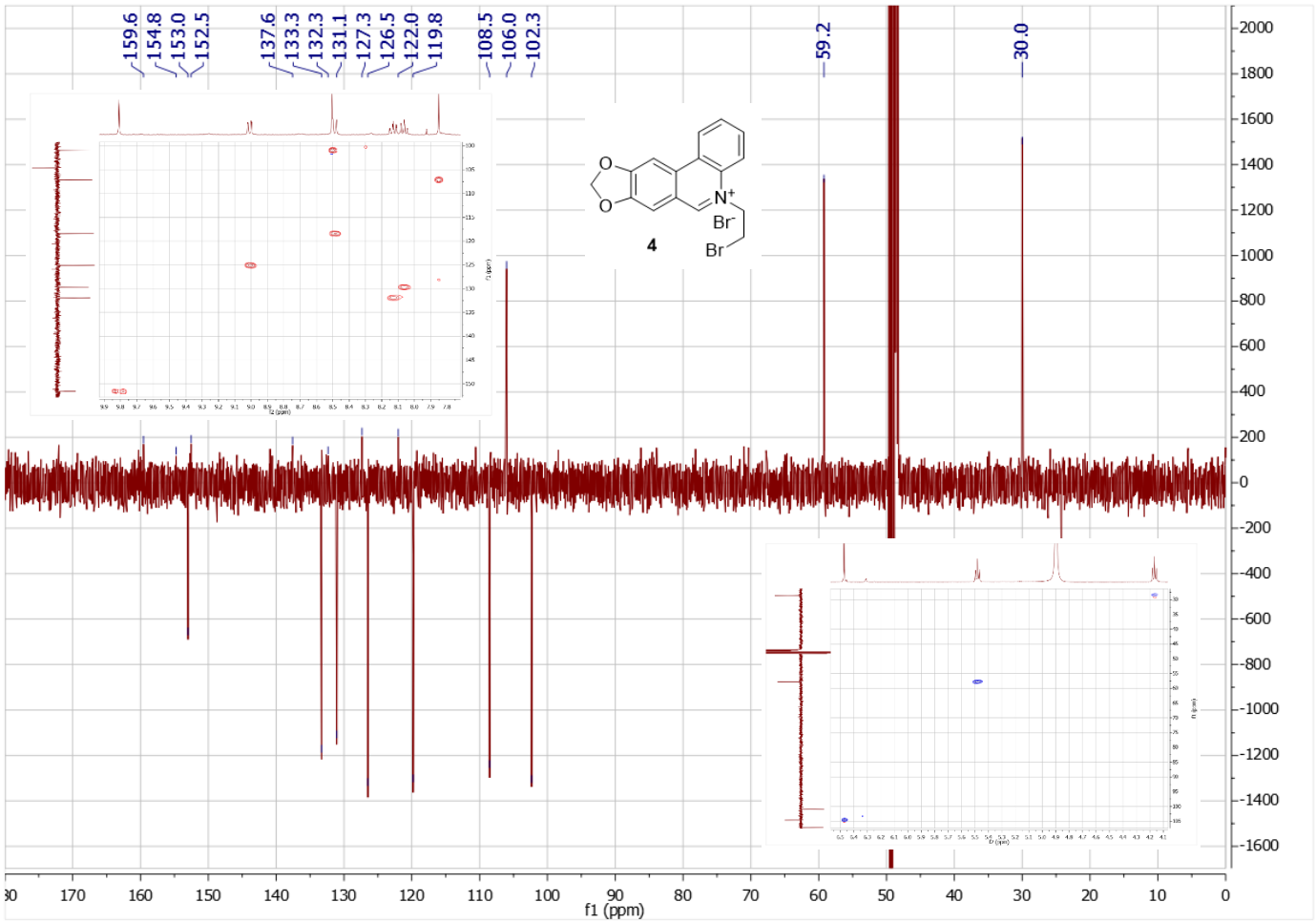
Trispheridine 2 ($CDCl_3$, 400 MHz, ^{13}C NMR)



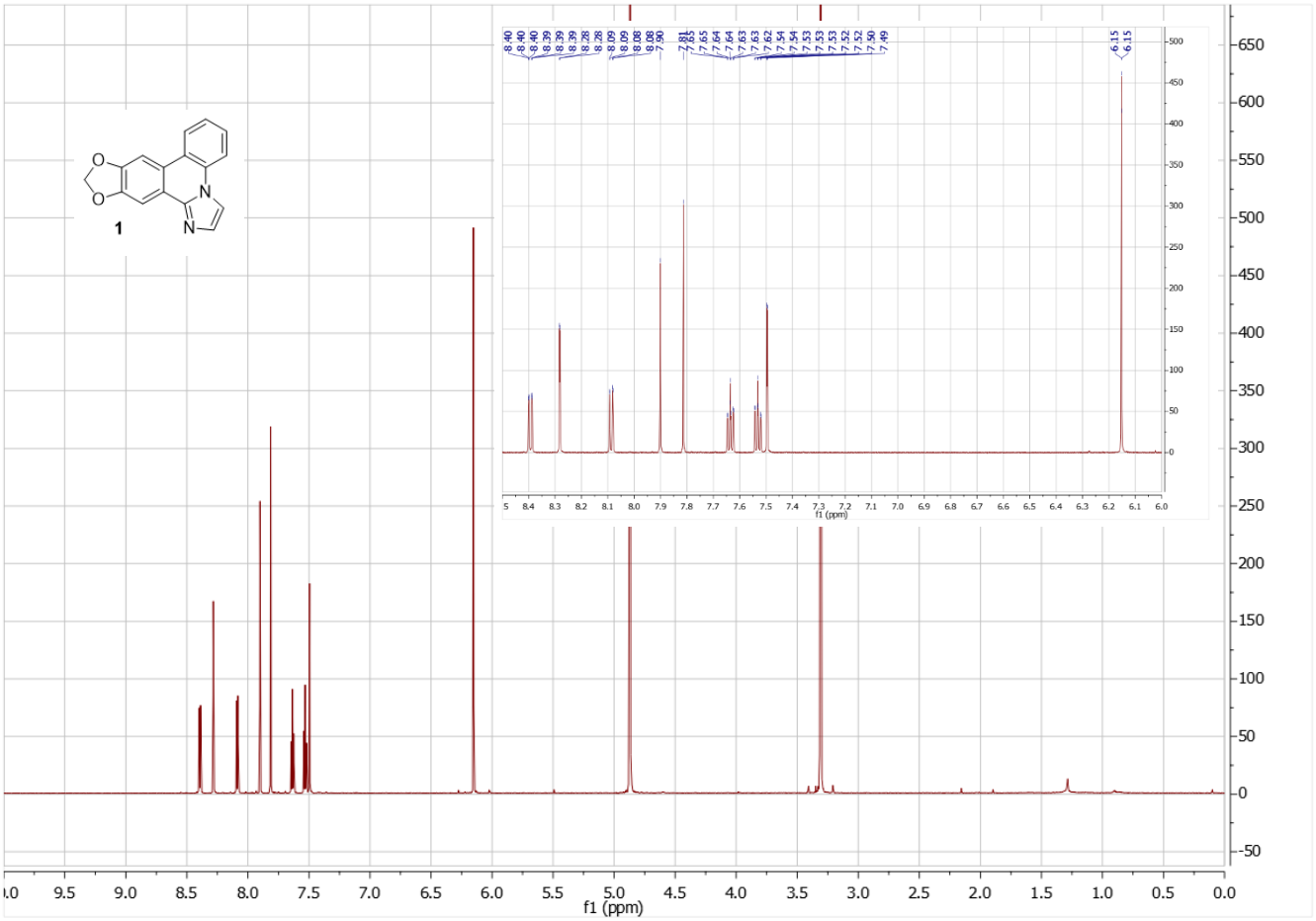
5-(2-bromoethyl)-[1,3]dioxolo[4,5-j]phenanthridin-5-ium 4 (CD_3OD, 400 MHz ^1H NMR, COSY (inserts))



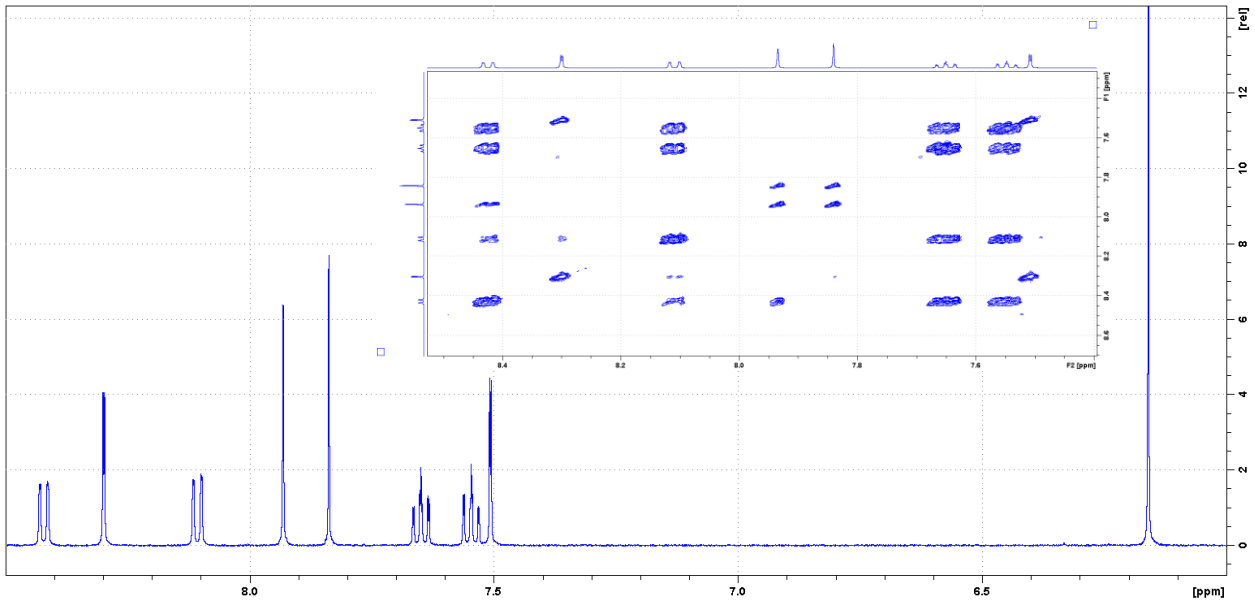
5-(2-bromoethyl)-[1,3]dioxolo[4,5-j]phenanthridin-5-ium 4 (CD_3OD, 400 MHz ^{13}C NMR, HSQC (inserts))



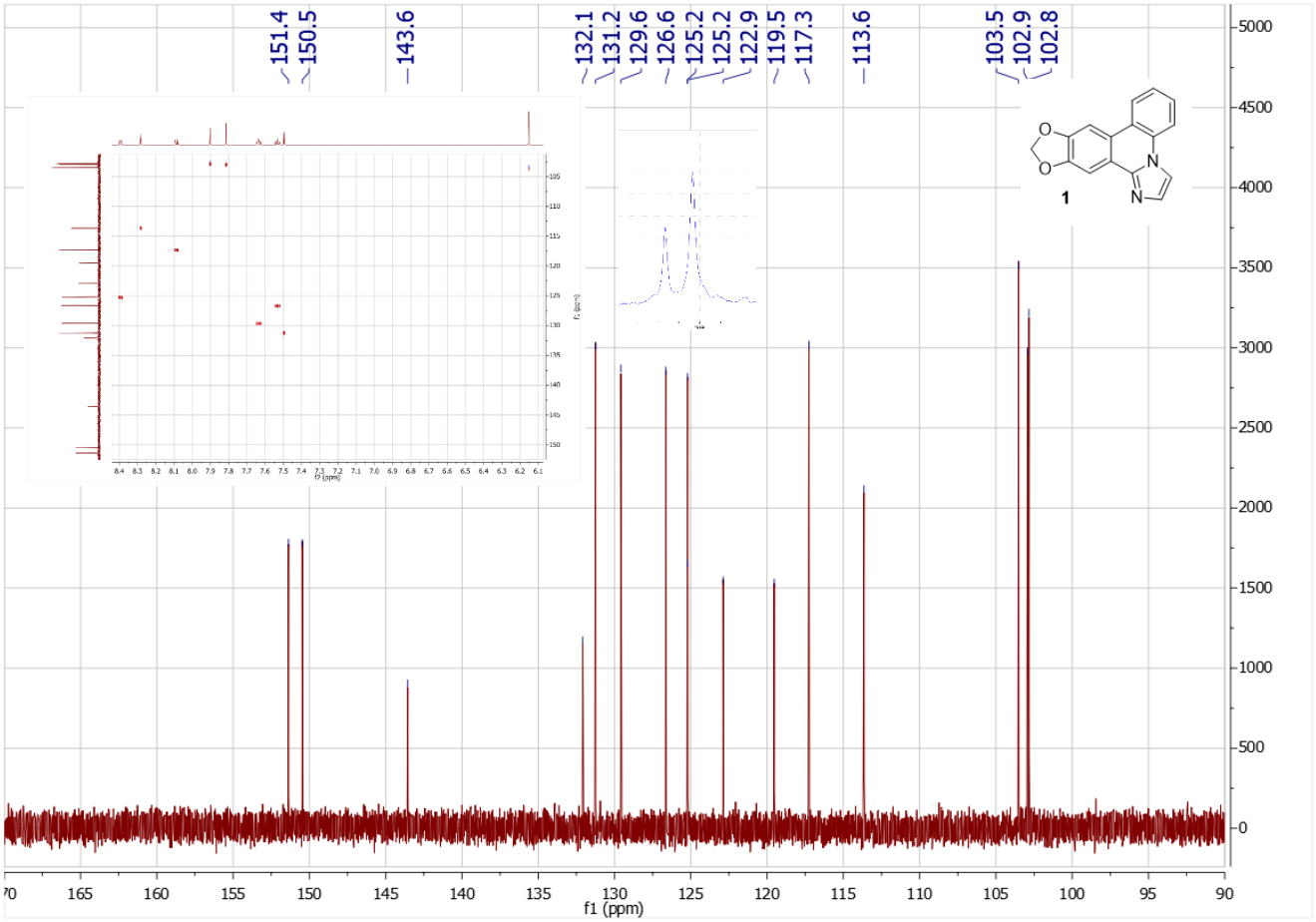
Zephycandidine A 1 (CD_3OD, 700 MHz, ^1H NMR) and expansion of 6.0-8.5 ppm (insert)



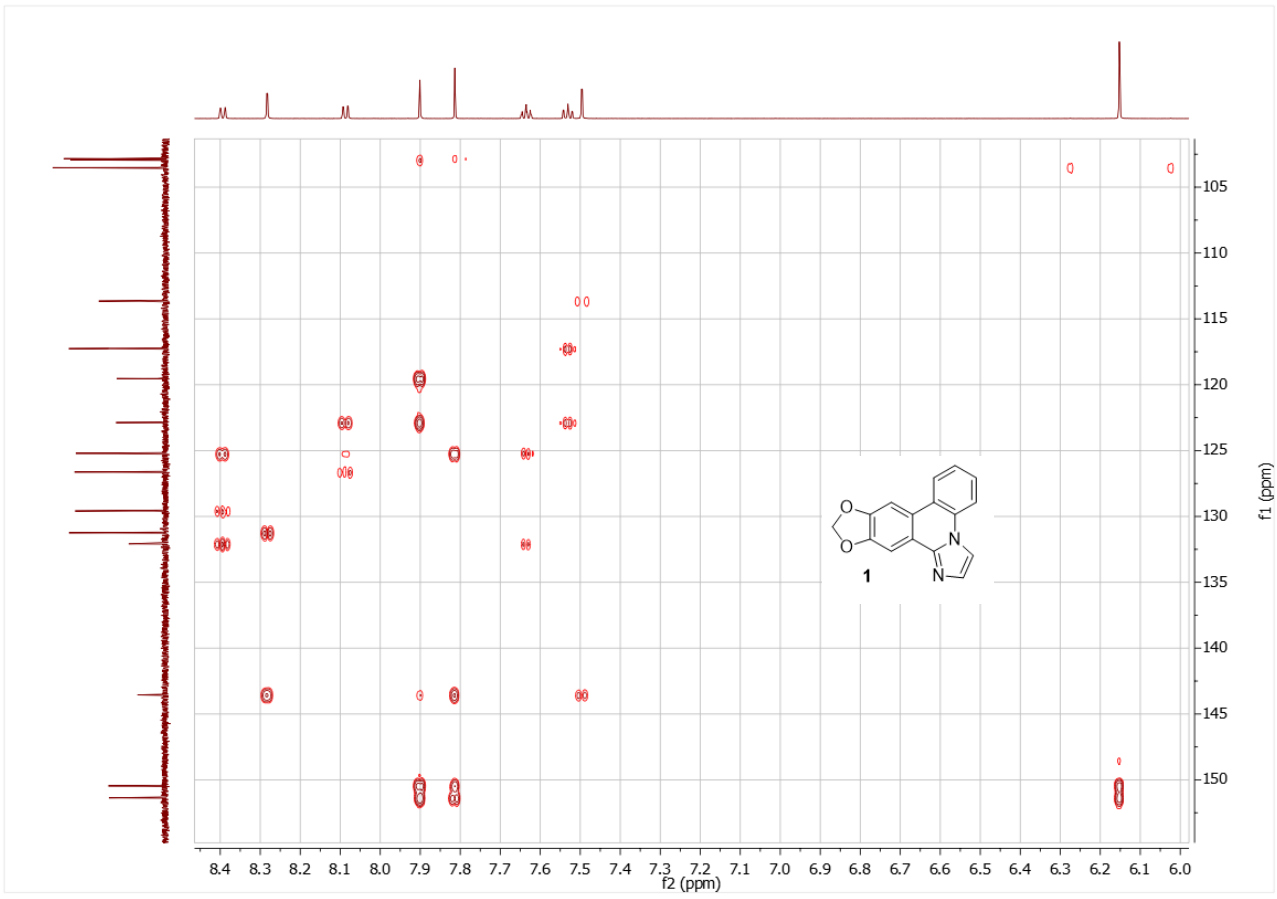
Zephycandidine A 1 (CD_3OD, 500 MHz, ^1H NMR, expansion of 6.0-8.5 ppm and COSY spectrum)



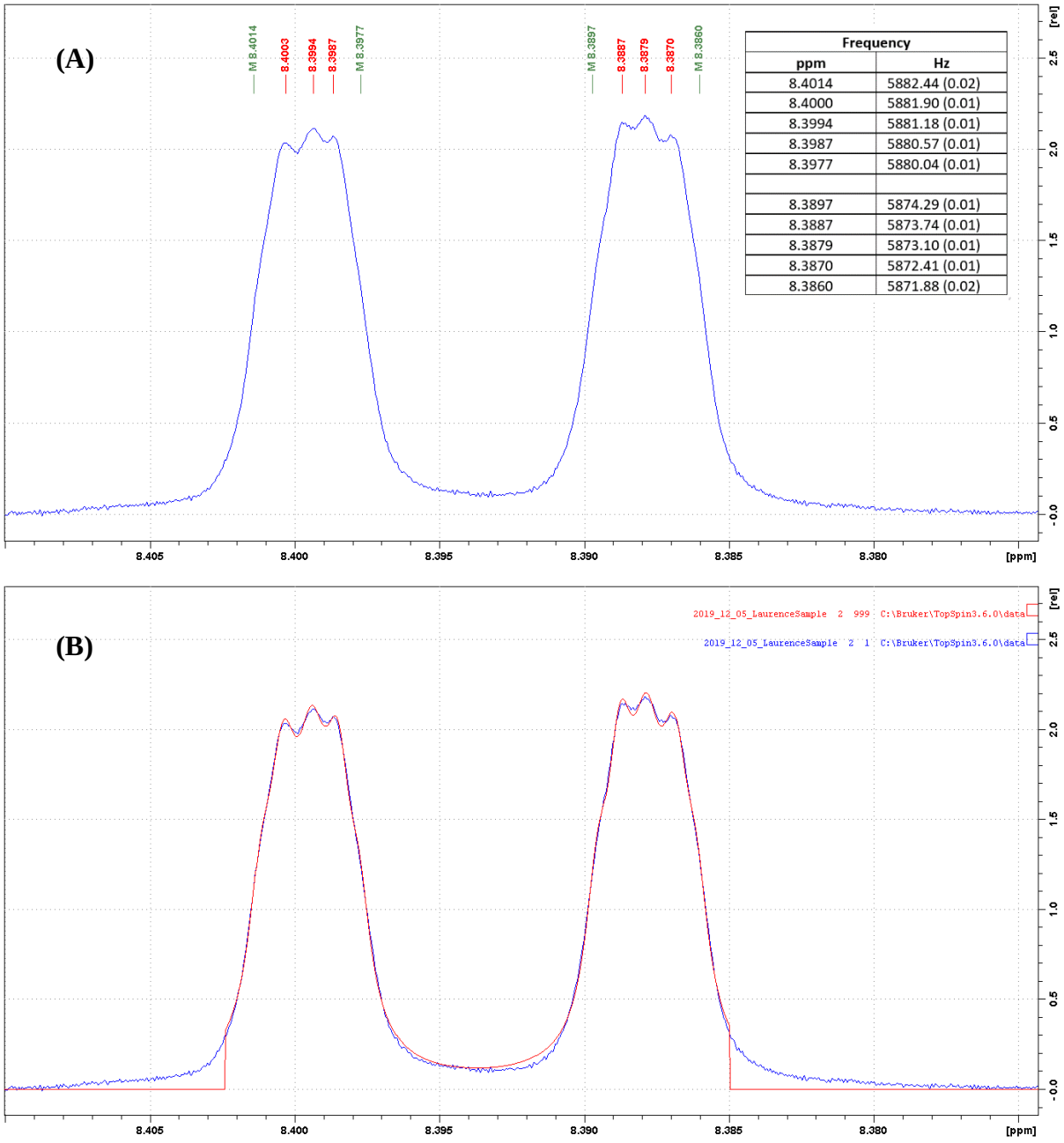
Zephycandidine A 1 (CD_3OD, 700 MHz, ^{13}C NMR, HSQC (insert))



Zephycandidine A 1. (CD_3OD, 700 MHz, HMBC)

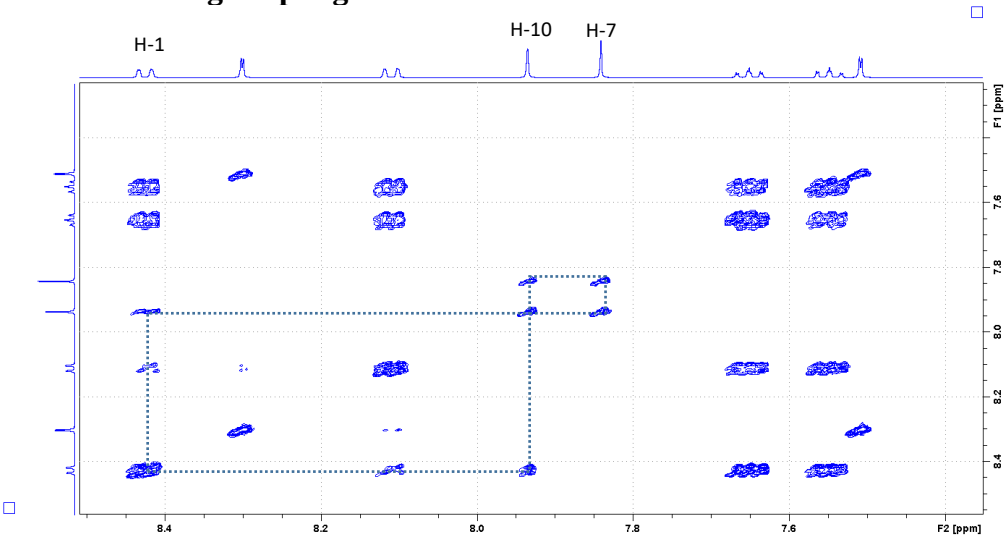


The elucidation of the couplings from the H-1 proton resonance (A) from the fit of five Lorenzian components (B).

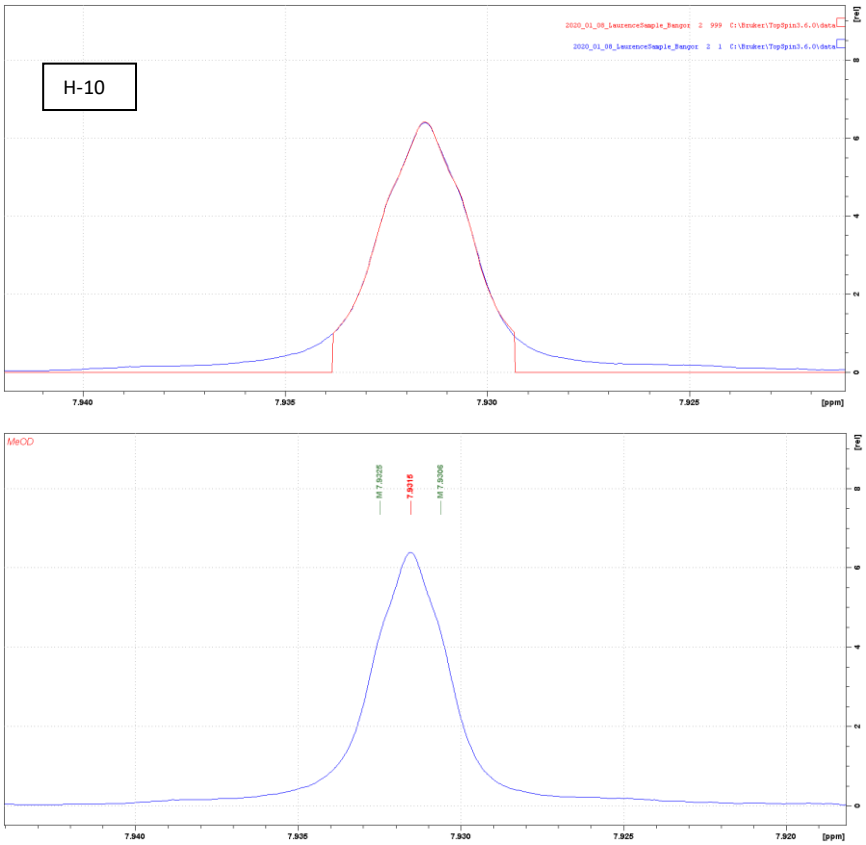


Frequency	
ppm	Hz
8.4014	5882.44 (0.02)
8.4000	5881.90 (0.01)
8.3994	5881.18 (0.01)
8.3987	5880.57 (0.01)
8.3977	5880.04 (0.01)
8.3897	5874.29 (0.01)
8.3887	5873.74 (0.01)
8.3879	5873.10 (0.01)
8.3870	5872.41 (0.01)
8.3860	5871.88 (0.02)

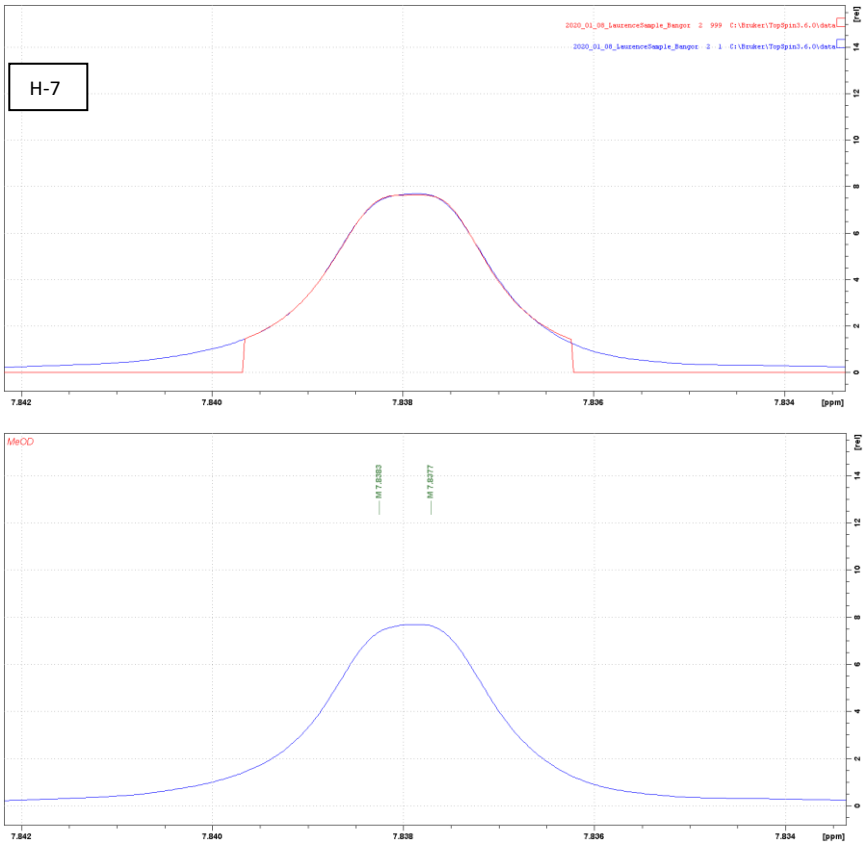
Correlation spectrum showing coupling between H-1/H-10 and H-10/H-7



The elucidation of the couplings from the H-10 proton resonance.

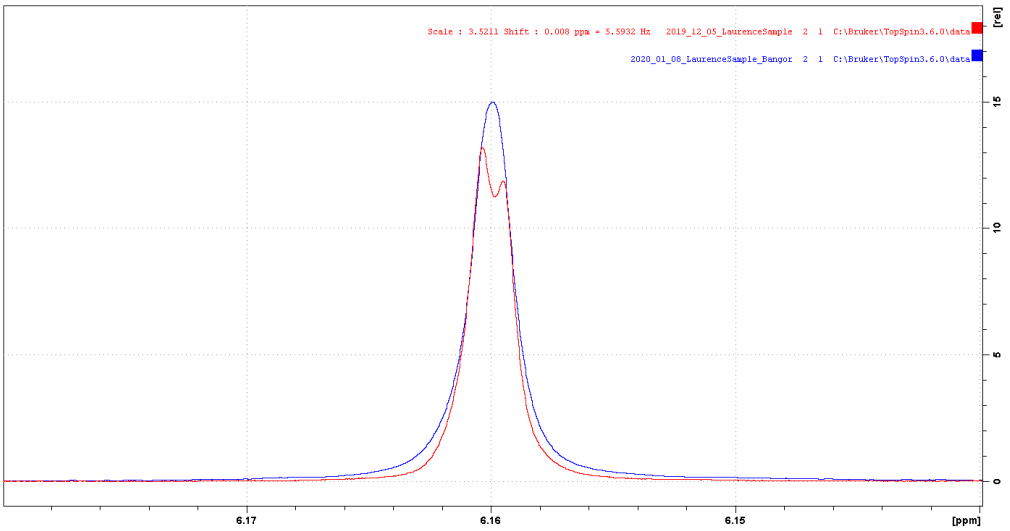


The elucidation of the couplings from the H-7 proton resonance.

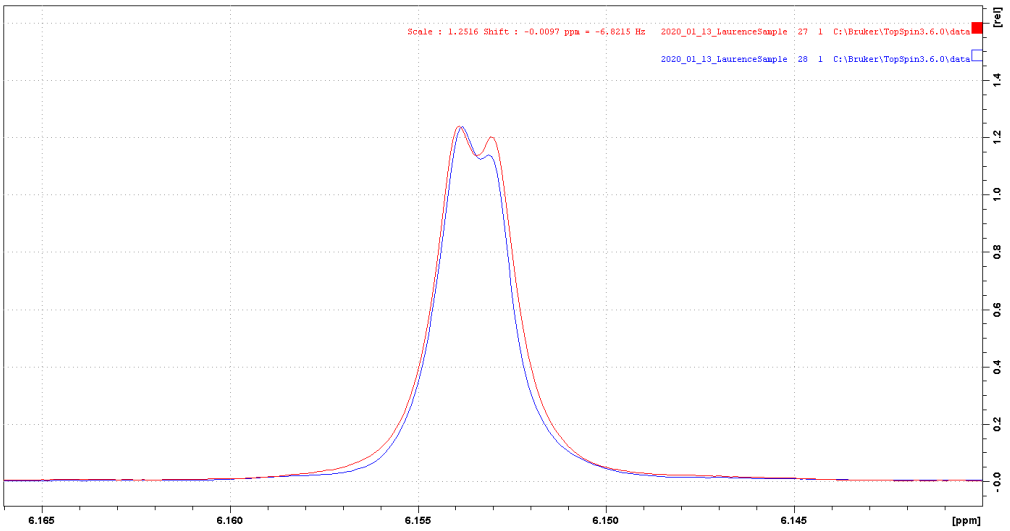


Residual dipolar coupling observed in 1.

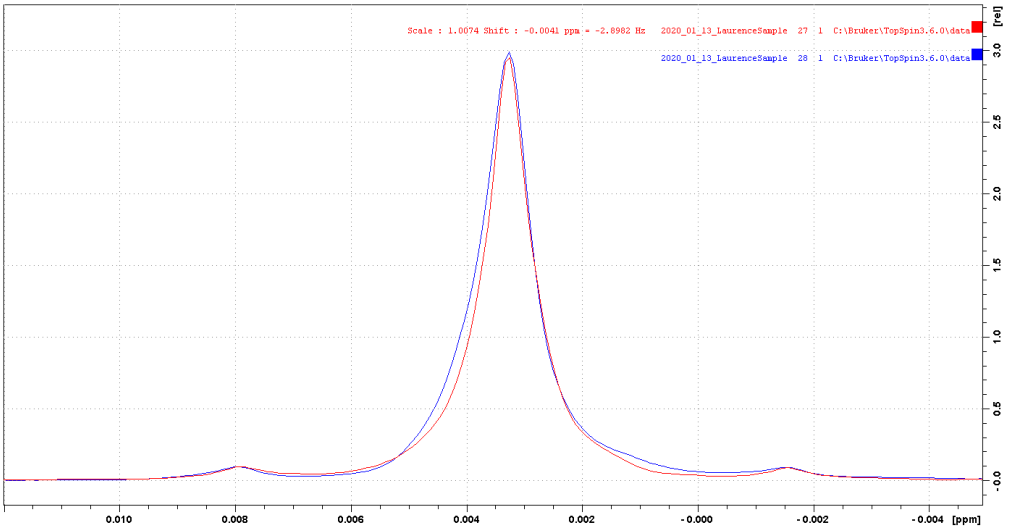
(A) The dipolar coupling splitting of the 6.15 ppm resonance is clearly visible in 700 MHz spectrum (red) but is not present in 500 MHz spectrum (blue). Data were recorded in CD_3OD solution.



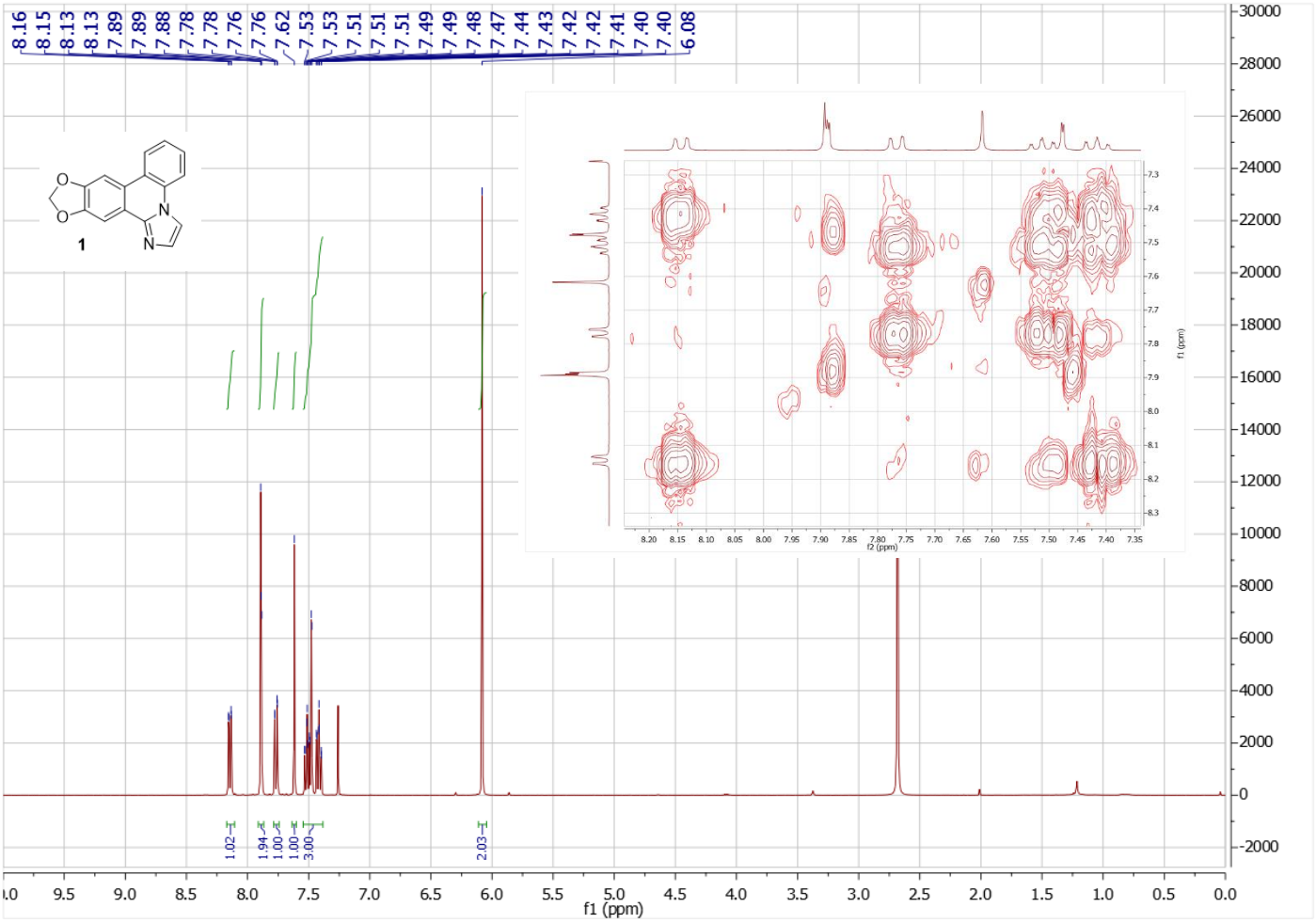
(B) The resonance at 6.15 ppm (700 MHz spectrometer) recorded at 297K (Blue line) and 277K (red line).



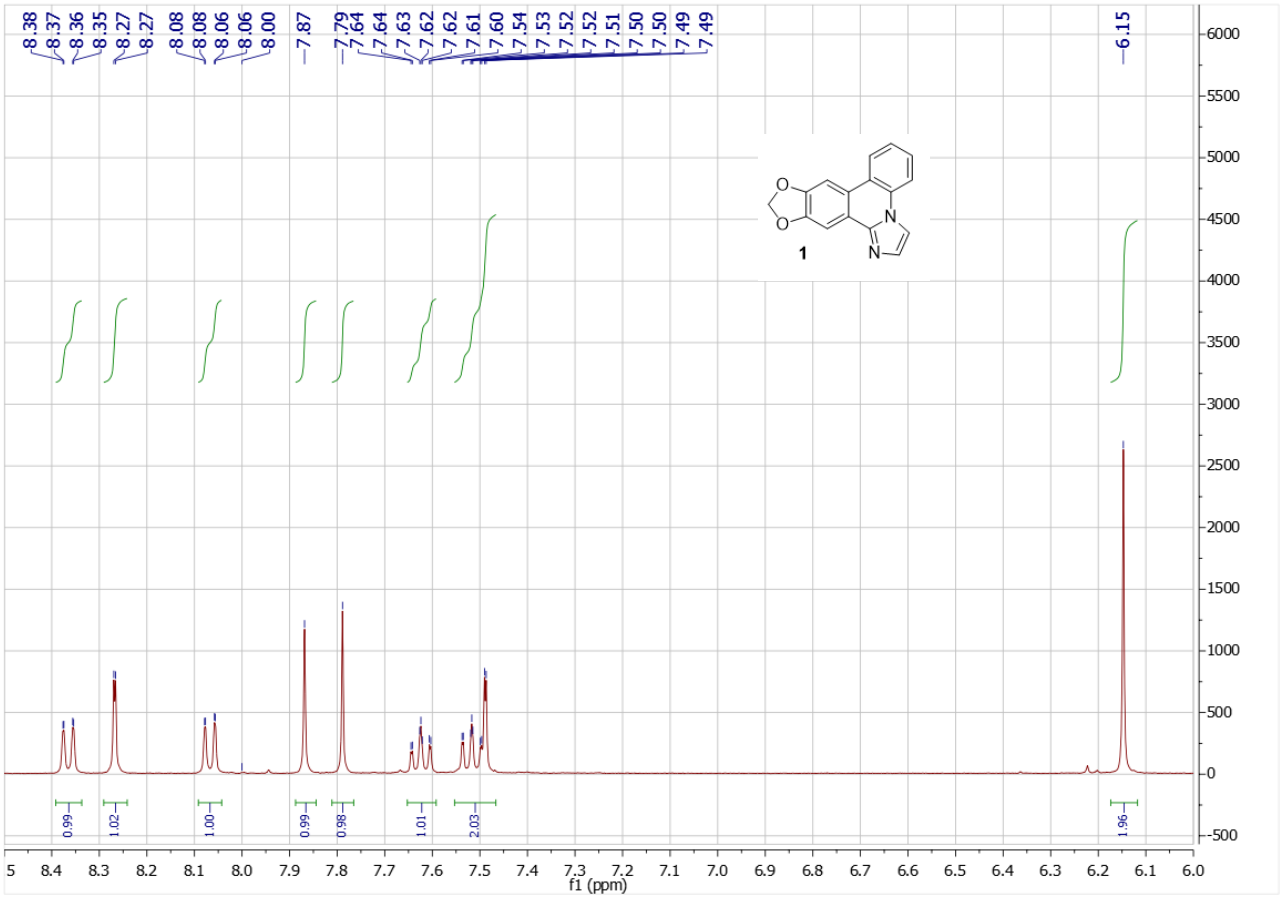
(C) The TMS reference resonance for the spectra in (B).



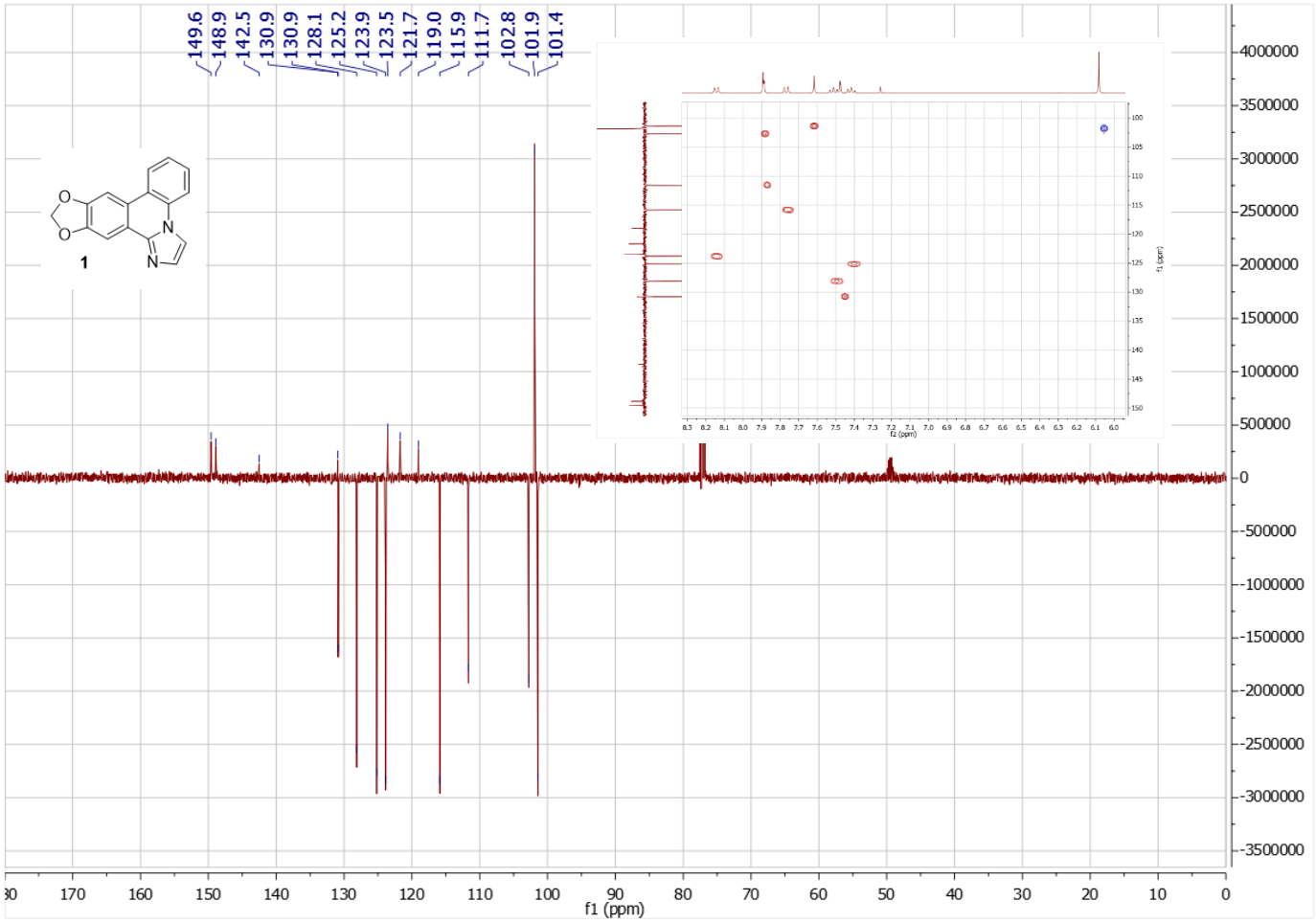
Zephycandidine A 1 ($CDCl_3/CD_3OD$ 10:1, 400 MHz, COSY (insert))



Zephycandidine A 1 ($CDCl_3/CD_3OD$ 10:1, 400 MHz, ^1H NMR, expansion of 6.0-8.5 ppm)

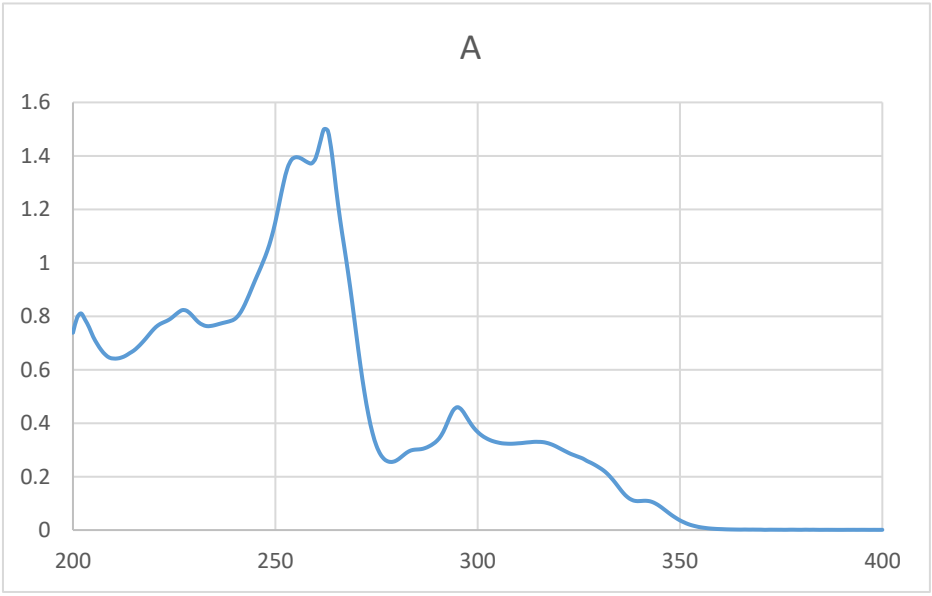


Zephycandidine A 1 ($CDCl_3/CD_3OD$ 10:1, 400 MHz, HSQC (insert))

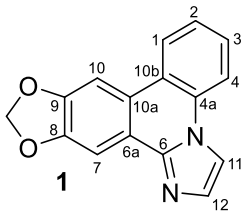


Zephycandidine A 1
UV (MeOH)/nm

Literature	Our data
λ_{max} (log ε)	λ_{max} (log ε)
207 (4.63)	202 (4.62),
220 (4.48)	228 (4.63)
256 (3.63)	256 (4.86)
	263 (4.89)
297 (4.18)	296 (4.37)



Data comparison with literature[1]



Proton and carbon spectra

	Reported data		Our Data			
	H	C	H	ΔH	C	ΔC
1	8.47 (dd, 8.2, 1.2)	125.4	**8.39** (dd, 8.2, 1.2, 0.6, 0.6)	-0.08	**125.2**	-0.2
2	7.58 (ddd, 8.2, 7.4, 1.0)	126.9	**7.53** (ddd, 8.1, 7.2, 1.2)	-0.05	**126.6**	-0.3
3	7.67 (ddd, 8.4, 7.4, 1.2)	129.8	**7.64** (ddd, 8.2, 7.2, 1.2)	-0.03	**129.6**	-0.2
4	8.14 (dd, 8.4, 1.0)	117.5	**8.09** (dd, 8.2, 1.2, 0.6)	-0.05	**117.2**	-0.2
4a		132.3			**132.1**	-0.2
6		143.8			**143.6**	-0.2
6a		119.8			**119.5**	-0.3
7	7.88 (s)	103.2	**7.81** (d, 0.6)	-0.07	**102.9**	-0.3
8		150.7			**150.5**	-0.2
9		151.6			**152.4**	-0.2
10	7.99 (s)	103.1	**7.90** (dd, 0.6, 0.6)	-0.09	**102.8**	-0.3
10a		125.5			**126.2**	-0.3
10b		123.1			**122.9**	-0.2
11	7.52 (d, 1.4)	113.8	**8.28** (d, 1.5)[i]	-0.04	**113.7**	-0.1
12	8.32 (d, 1.4)	131.5	**7.50** (d, 1.5)[i]	-0.02	**131.2**	-0.3
CH_2	6.16 (s)	103.7	**6.15** (d, 0.5 Hz)	-0.01	**103.5**	-0.2

i. Mis-assigned in original paper.[1]

1) Zhan, G.; Qu, X.; Liu, J.; Tong, Q.; Zhou, L.; Sun, B; Yao, G.; *Sci Rep*. **2016**; 6: 33990, doi: 10.1038/srep33990