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Supplementary Information

Antimalarial Activity of Piperidine Alkaloids from *Senna spectabilis* and Semisynthetic Derivatives

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[d]*Departamento de Química, Instituto de Ciências Exatas, Naturais e Educação, Universidade Federal do Triângulo Mineiro, 38064-200 Uberaba-MG, Brazil*

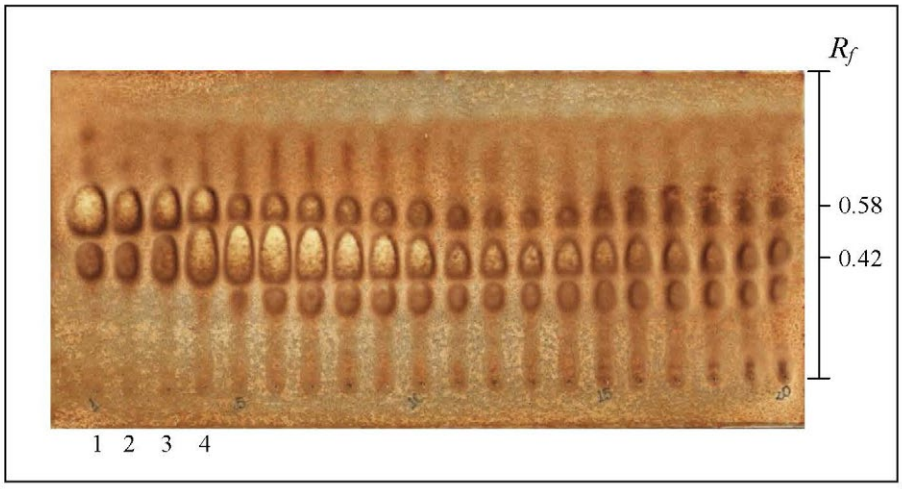

Figure S1. Thin-layer chromatography (TLC, SiO_2) of fractions from column chromatography (CC) of alkaloidal CH_2Cl_2 extract developed with $CHCl_3$-MeOH-NH_4OH (9:1:0.25) and revealed with iodochloroplatinate reagent.

*e-mail: pivatto@iqufu.ufu.br, cgarcia@usp.br, bolzaniv@iq.unesp.br

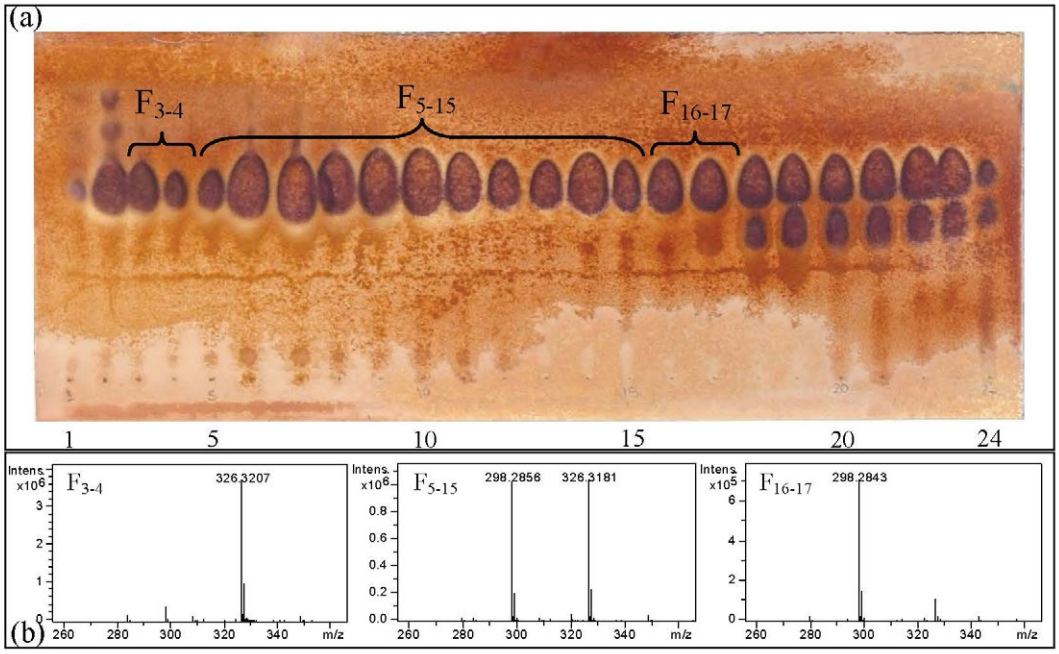

Figure S2. (a) TLC (SiO_2) of fractions from CC of F_{1-4} developed with $CHCl_3$-MeOH-NH_4OH (9:1:0.25) and revealed with iodochloroplatinate reagent. (b) ESI-MS of F_{3-4}, F_{5-15} and F_{16-17}.

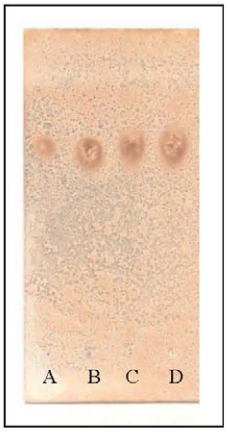

Figure S3. TLC (SiO_2) of **1** (A: free base, B: hydrochloride) and **2** (C: free base, D: hydrochloride) developed with $CHCl_3$-MeOH-NH_4OH (9:1:0.25) and revealed with iodochloroplatinate reagent.

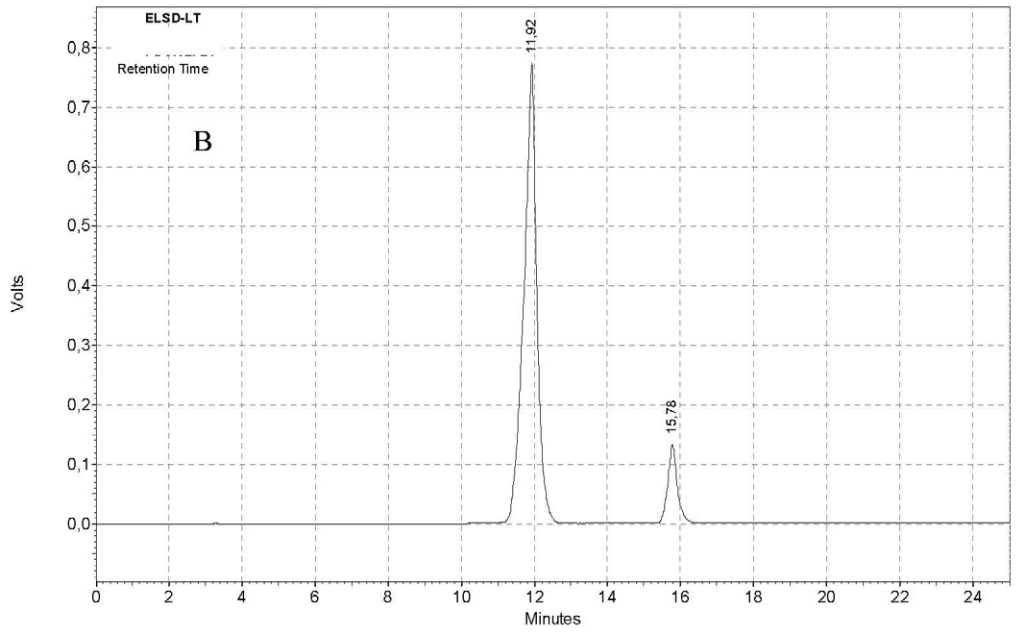

Figure S4. Analytical HPLC chromatogram on RP-C_{18} of a mixture of **1** and **2** ($F_{5\text{-}15}$) eluted with a gradient of increasing MeOH in 0.1% HOAc aqueous solution (35-100, flow rate 8.0 mL min^{-1}, 25 min) and light scattering detector.

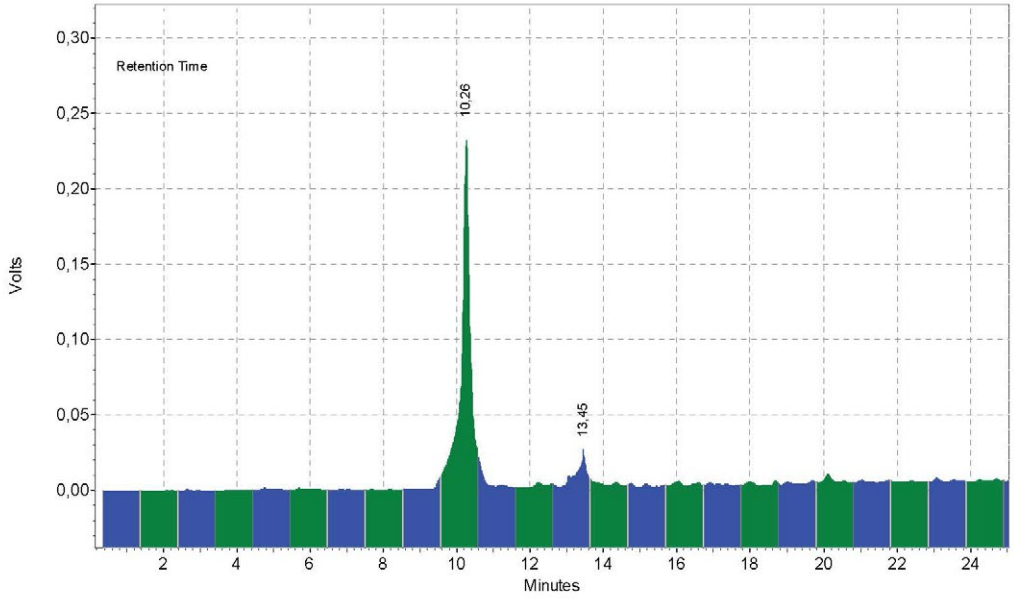

Figure S5. Preparative HPLC chromatogram on RP-C_{18} of a mixture of **1** and **2** ($F_{5\text{-}15}$) eluted with a gradient of increasing MeOH in 0.1% HOAc aqueous solution (35-100, flow rate 8.0 mL min^{-1}, 25 min) and 284 nm UV detector.

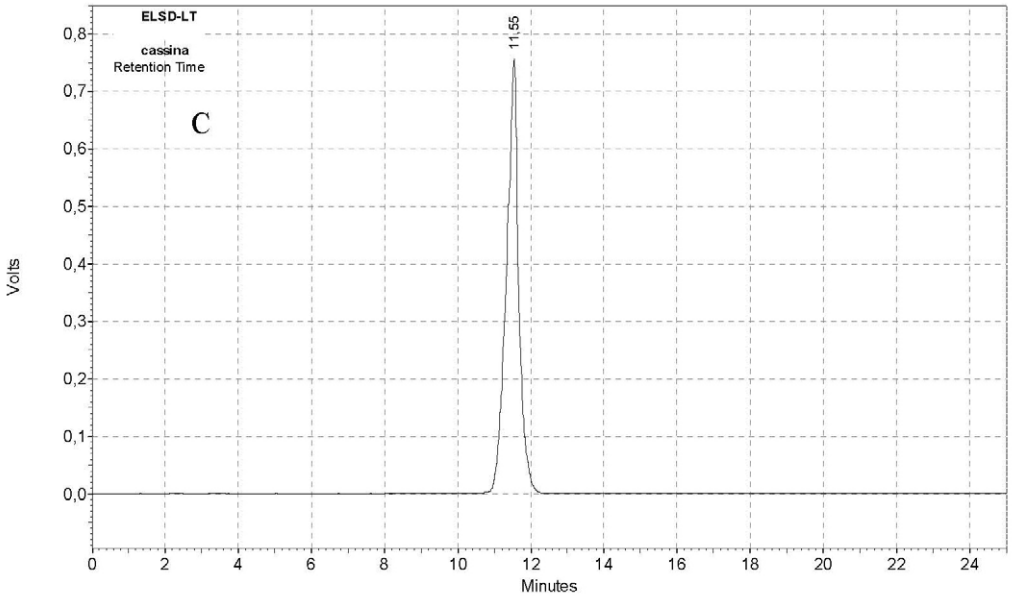

Figure S6. Analytical HPLC chromatogram on RP-C_{18} of **1** (F_{16-17}) eluted with a gradient of increasing MeOH in 0.1% HOAc aqueous solution (35-100, flow rate 8.0 mL min^{-1}, 25 min) and light scattering detector.

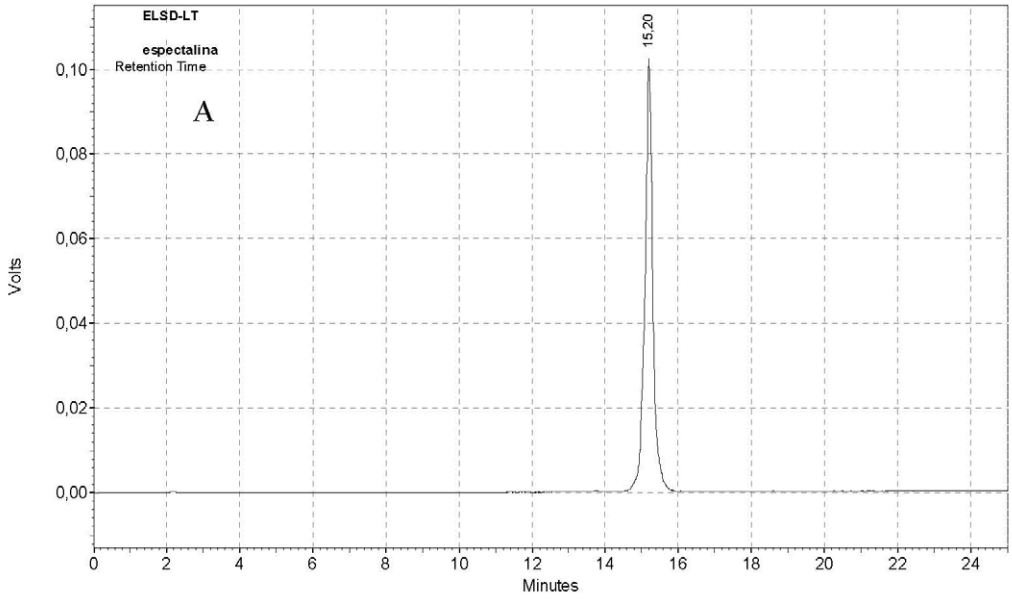

Figure S7. Analytical HPLC chromatogram on RP-C_{18} of **2** (F_{3-4}) eluted with a gradient of increasing MeOH in 0.1% HOAc aqueous solution (35-100, flow rate 8.0 mL min^{-1}, 25 min) and light scattering detector.

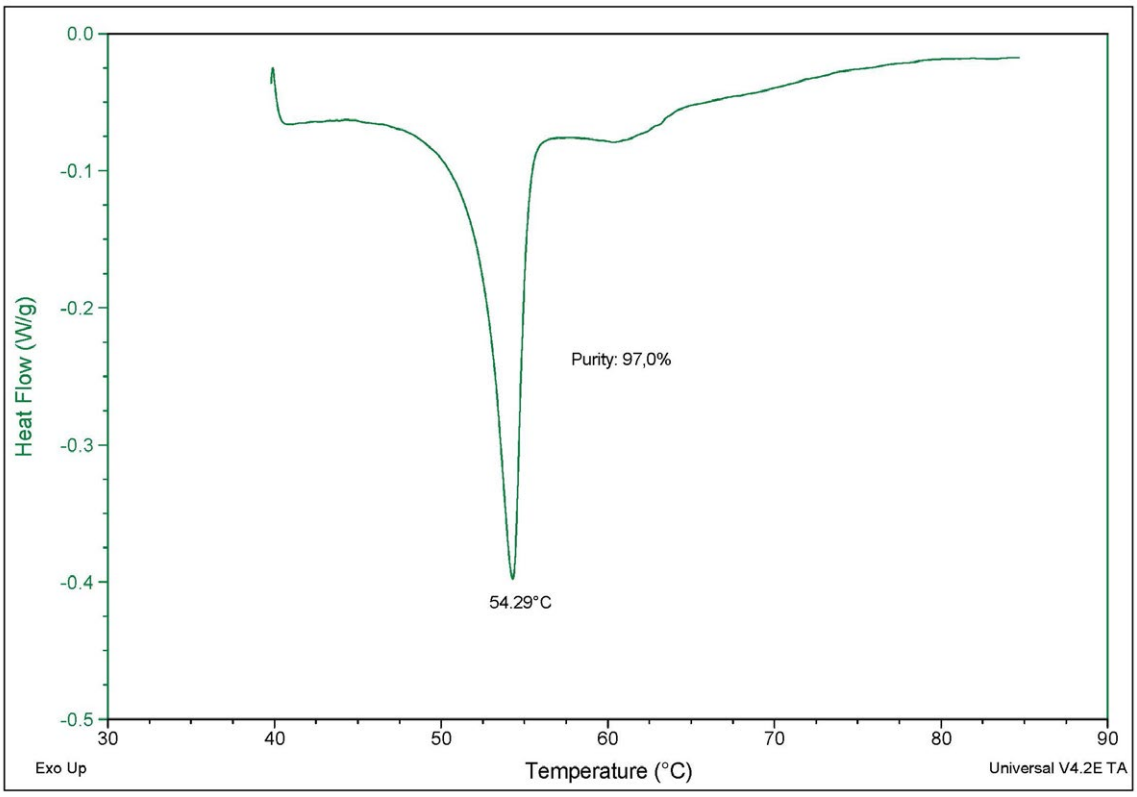

Figure S8. DSC analysis of **1**.

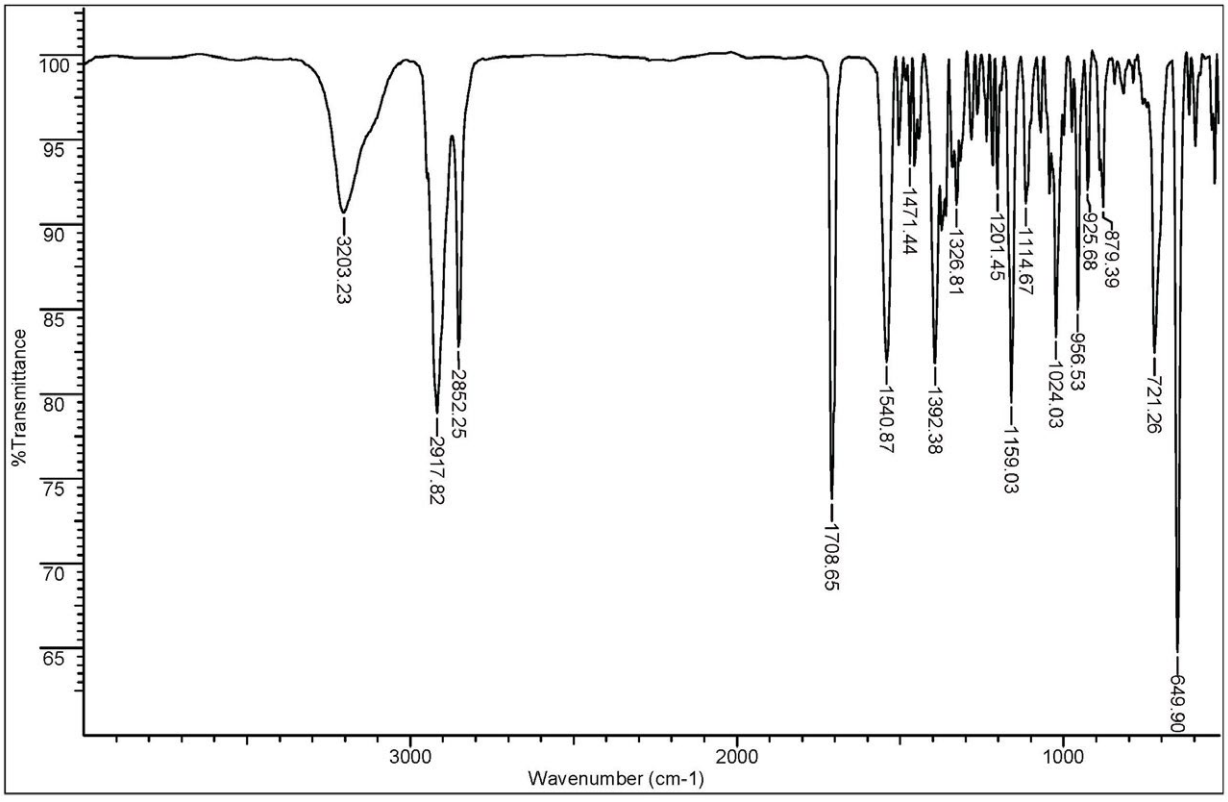

Figure S9. IR spectrum (film) of **1**.

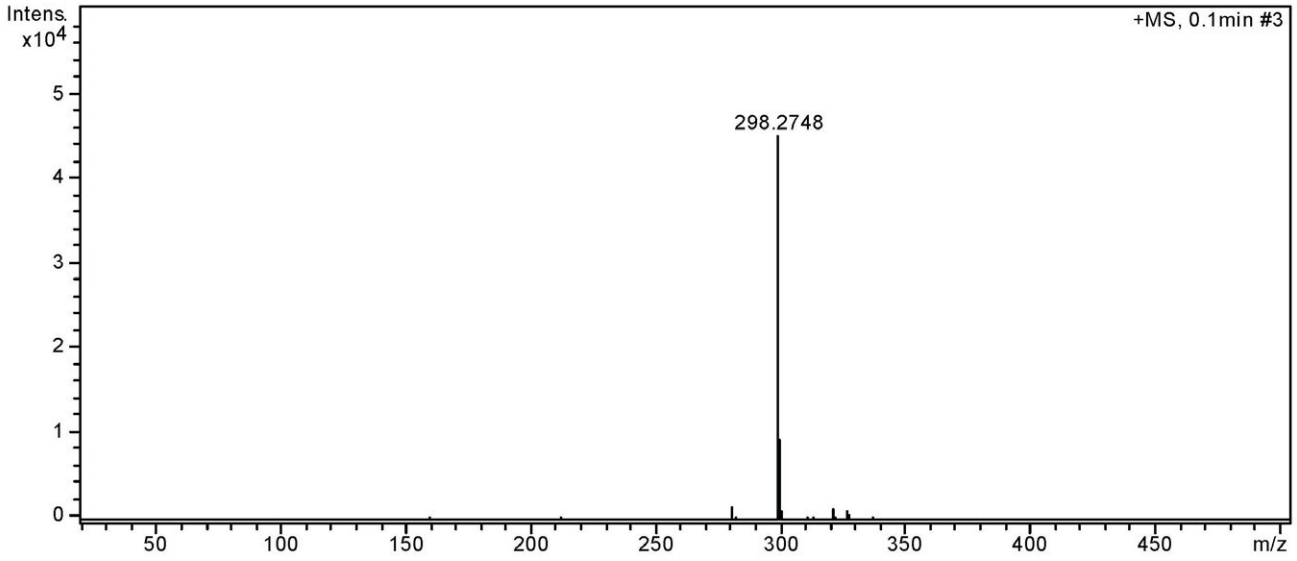

Figure S10. ESI-MS of **1** (HRMS *m/z* 298.2748 $[M + H]^+$ (calcd for $C_{18}H_{36}NO_2$: 298.2746)).

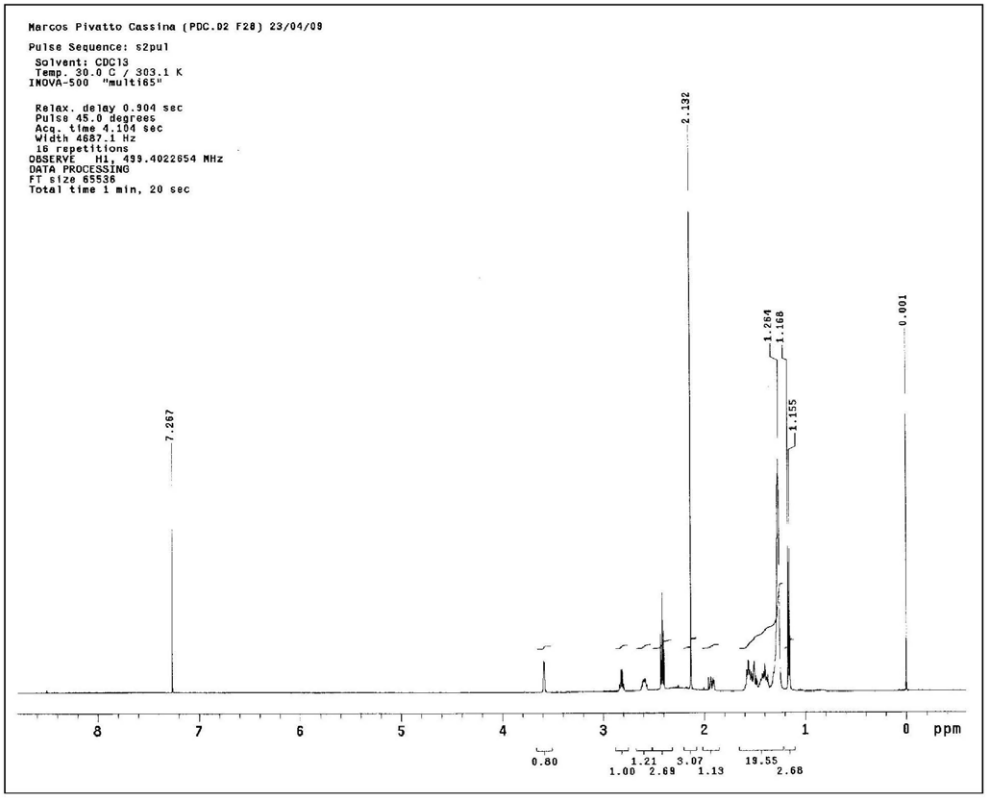

Figure S11. 500 MHz ^{1}H NMR spectrum of **1** in chloroform-*d*.

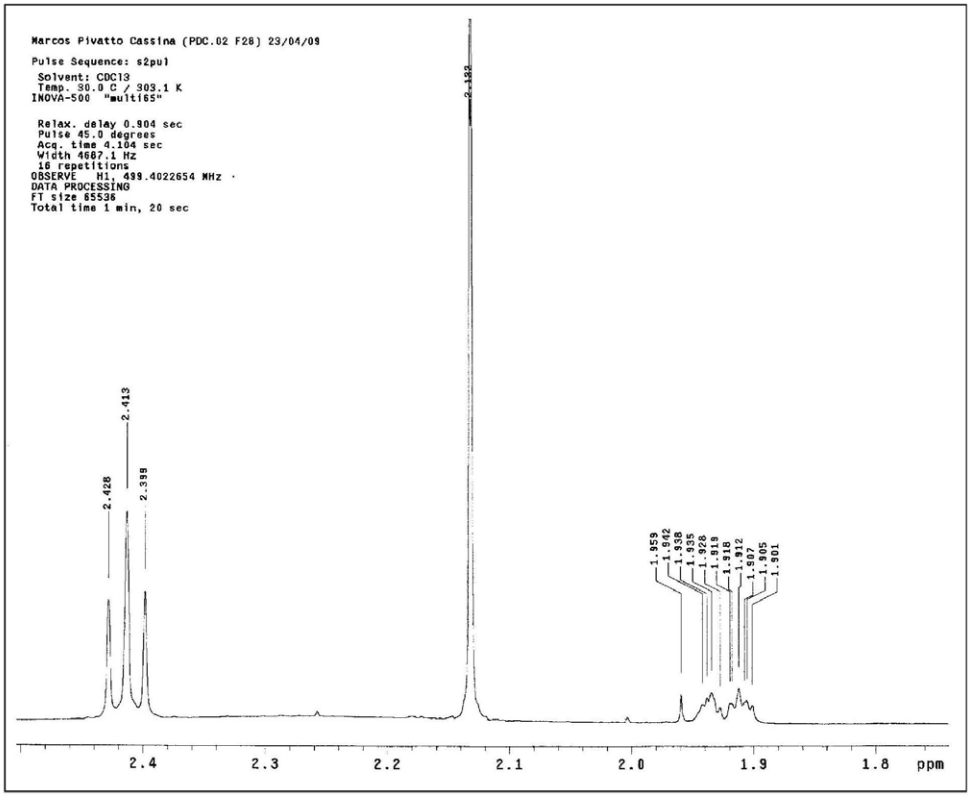

Figure S12. 500 MHz ^{1}H NMR spectrum (expansion δ 1.8-2.4) of **1** in chloroform-*d*.

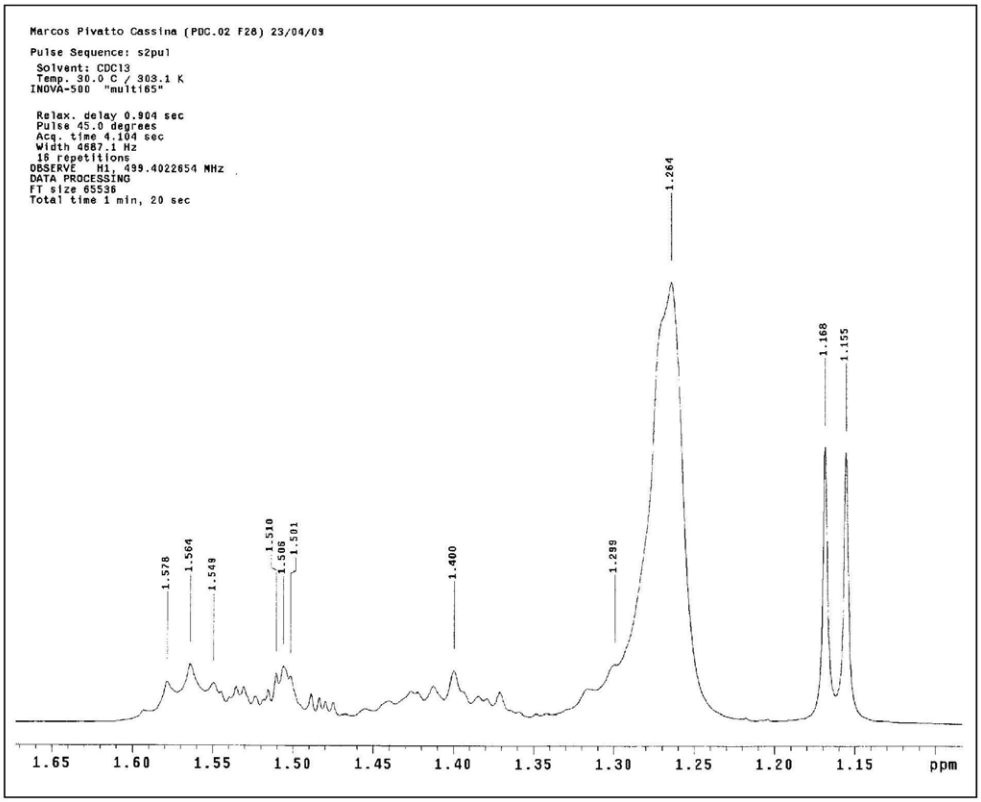

Figure S13. 500 MHz ^{1}H NMR spectrum (expansion δ 1.15-1.65) of **1** in chloroform-*d*.

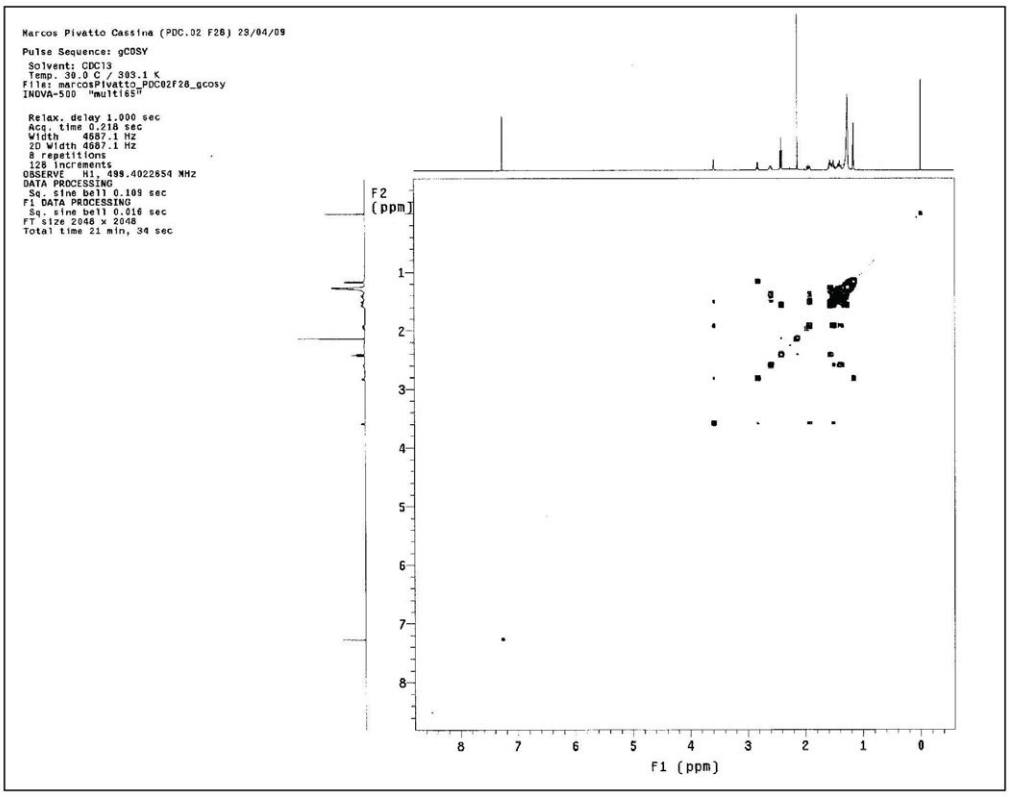

Figure S14. COSY spectrum of **1** in chloroform-*d*.

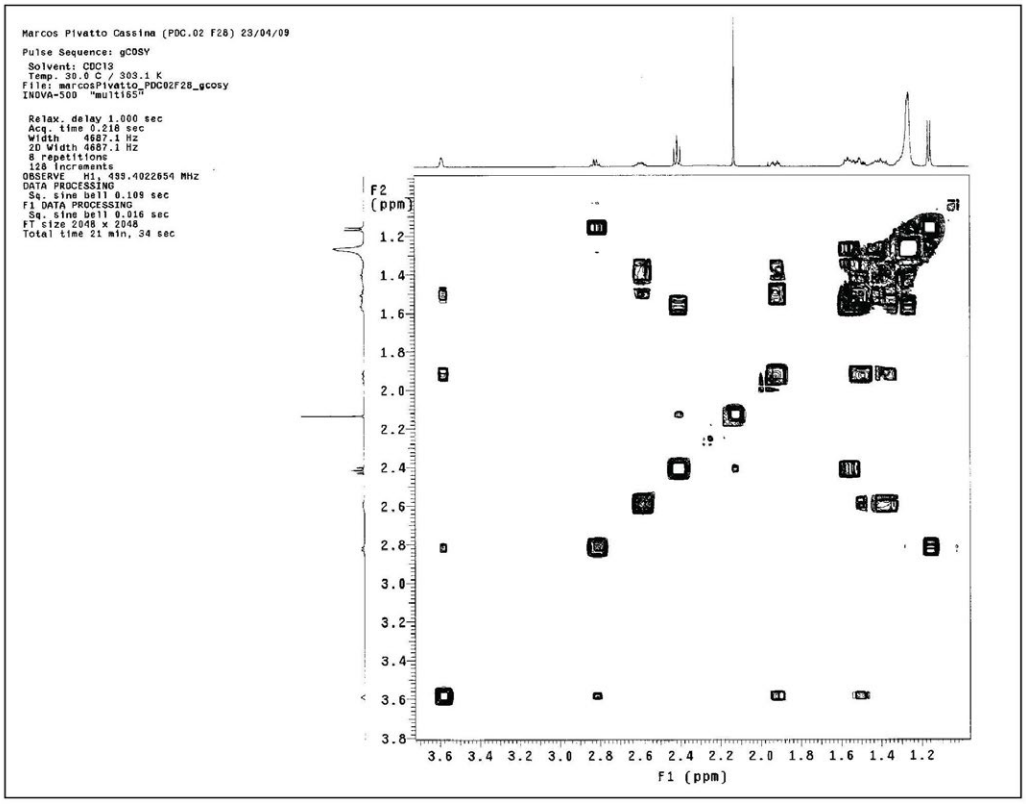

Figure S15. COSY spectrum (expansion δ 1.2-3.6) of **1** in chloroform-*d*.

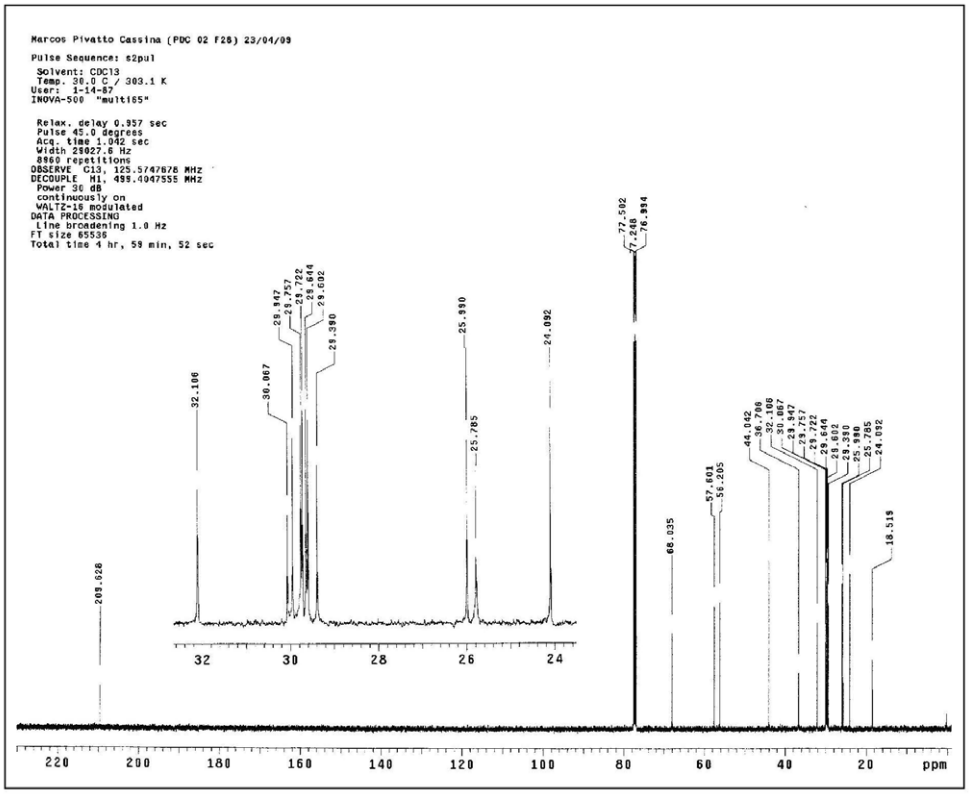

Figure S16. 125 MHz ^{13}C NMR spectrum of **1** in chloroform-*d*.

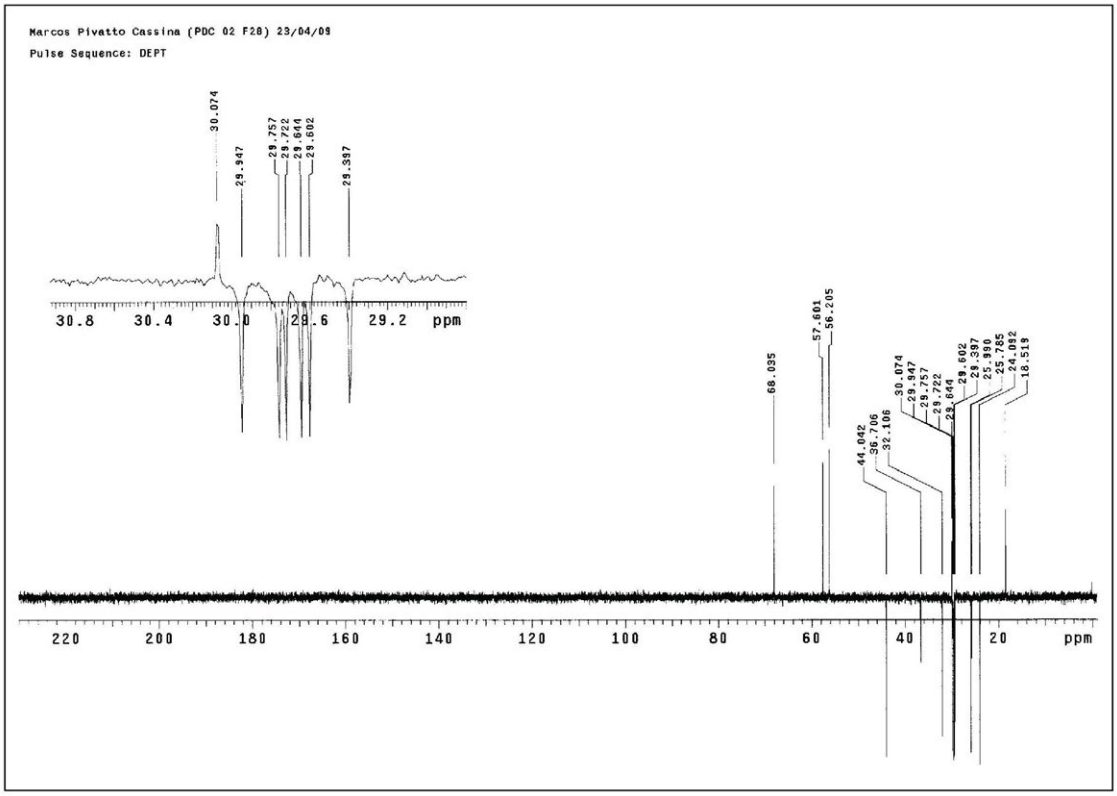

Figure S17. 125 MHz DEPT 135 NMR spectrum of **1** in chloroform-*d*.

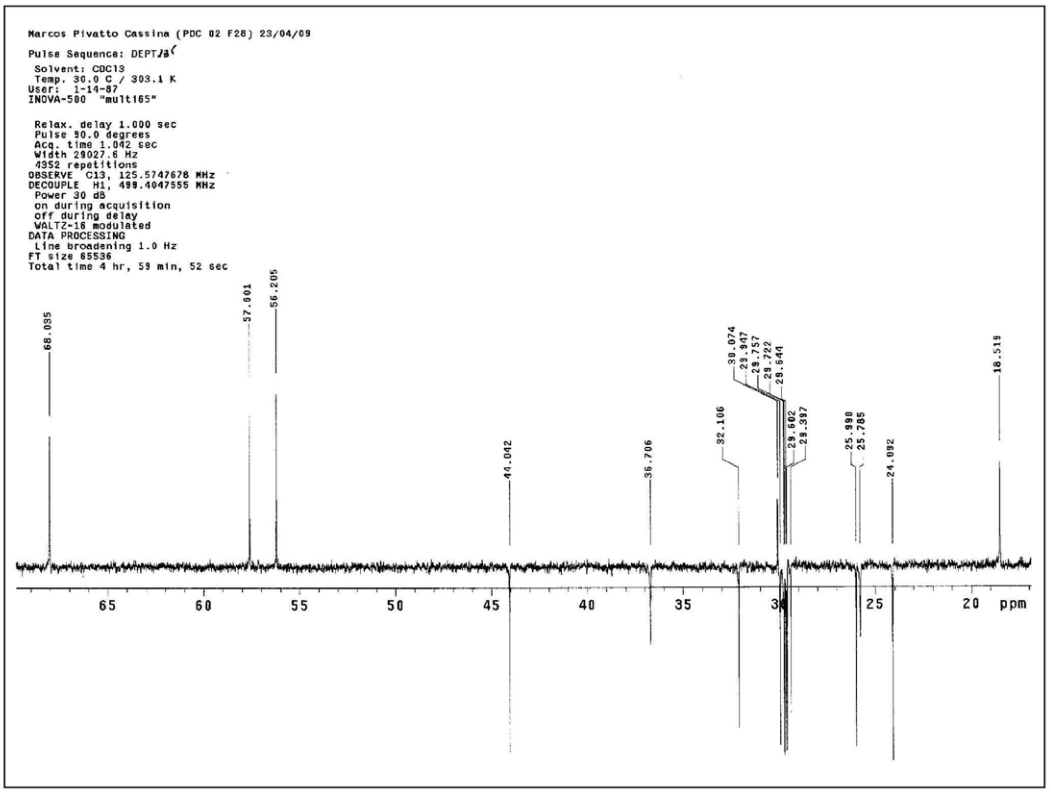

Figure S18. 125 MHz DEPT 135 NMR spectrum (expansion δ 20-65) of **1** in chloroform-*d*.

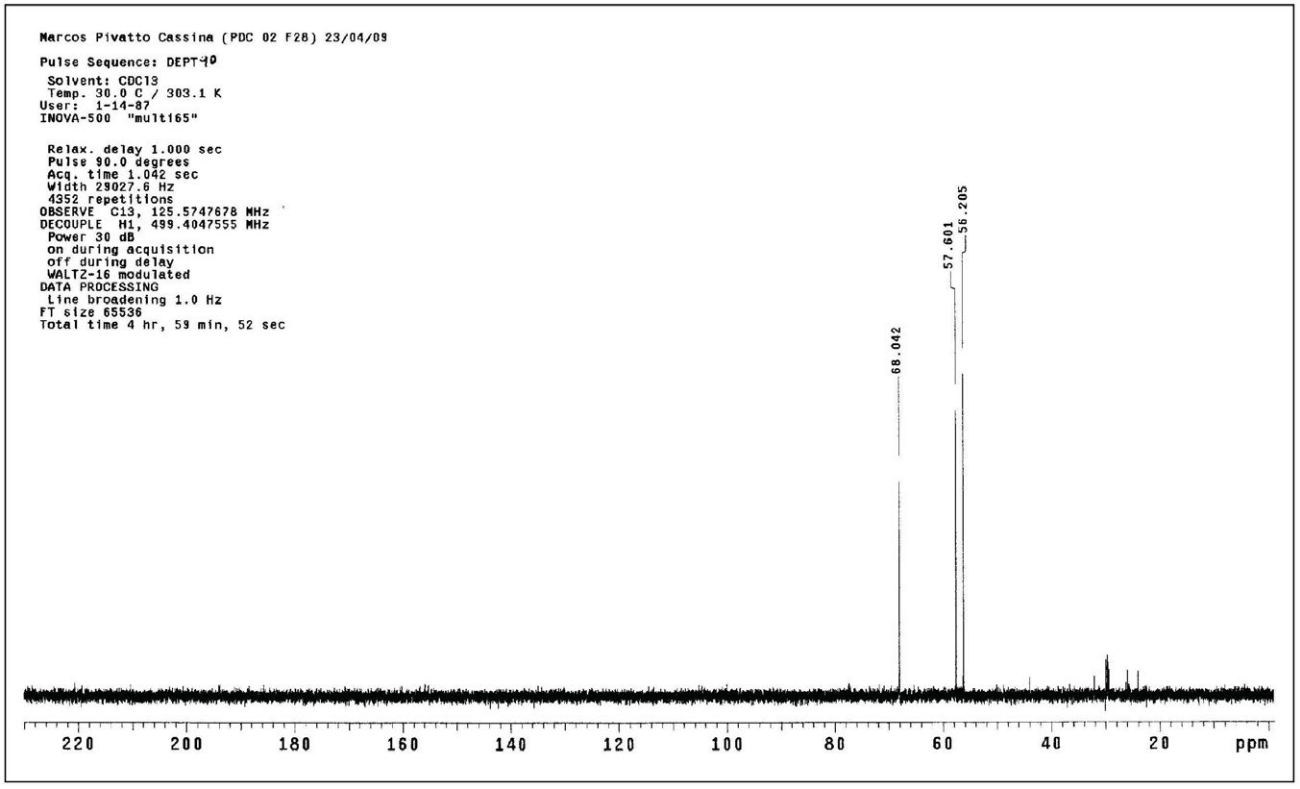

Figure S19. 125 MHz DEPT 90 NMR spectrum of **1** in chloroform-*d*.

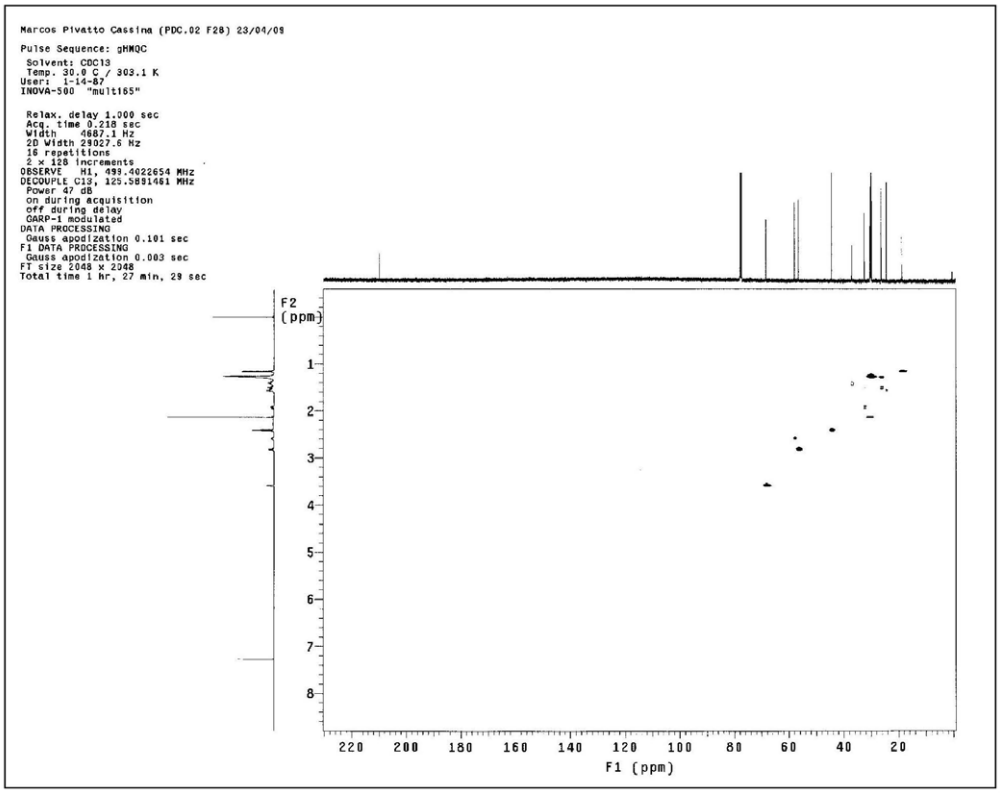

Figure S20. HMQC spectrum of **1** in chloroform-*d*.

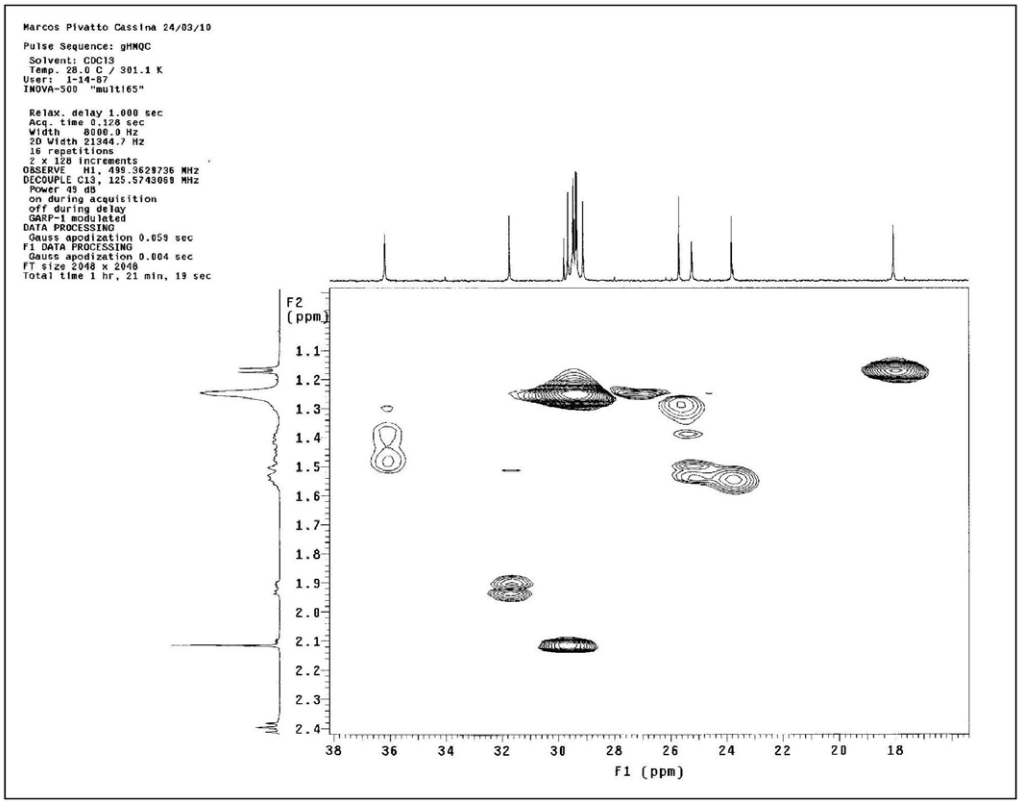

Figure S21. HMQC spectrum (expansion δ 18-38) of **1** in chloroform-*d*.

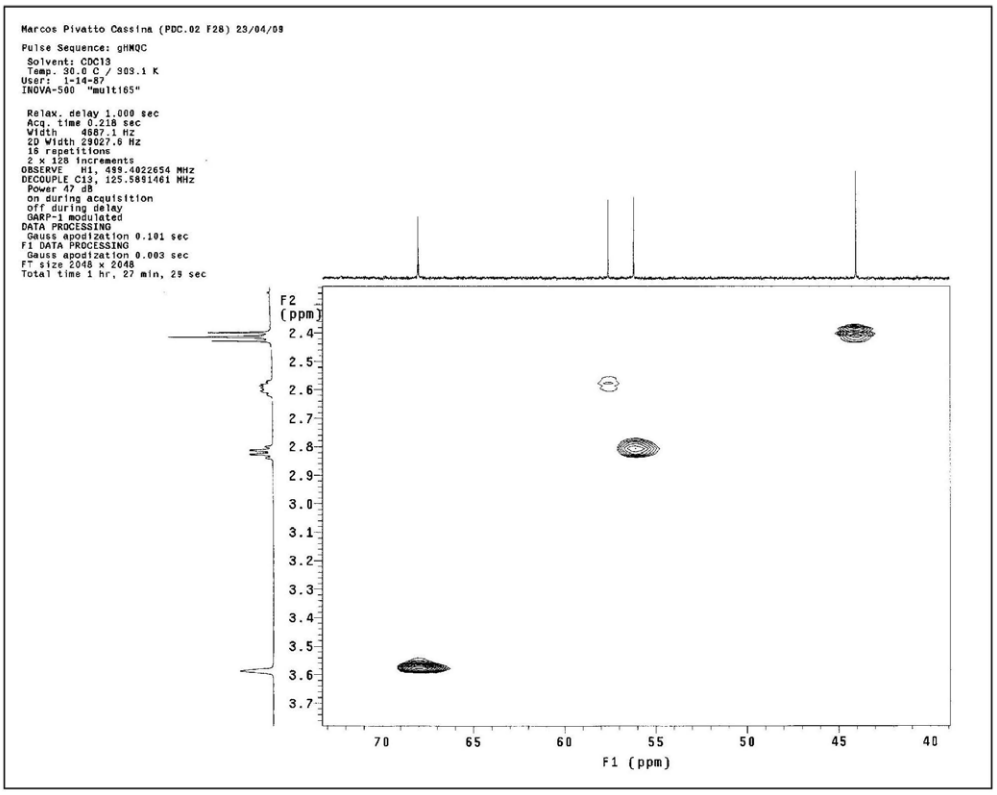

Figure S22. HMQC spectrum (expansion δ 40-70) of **1** in chloroform-*d*.

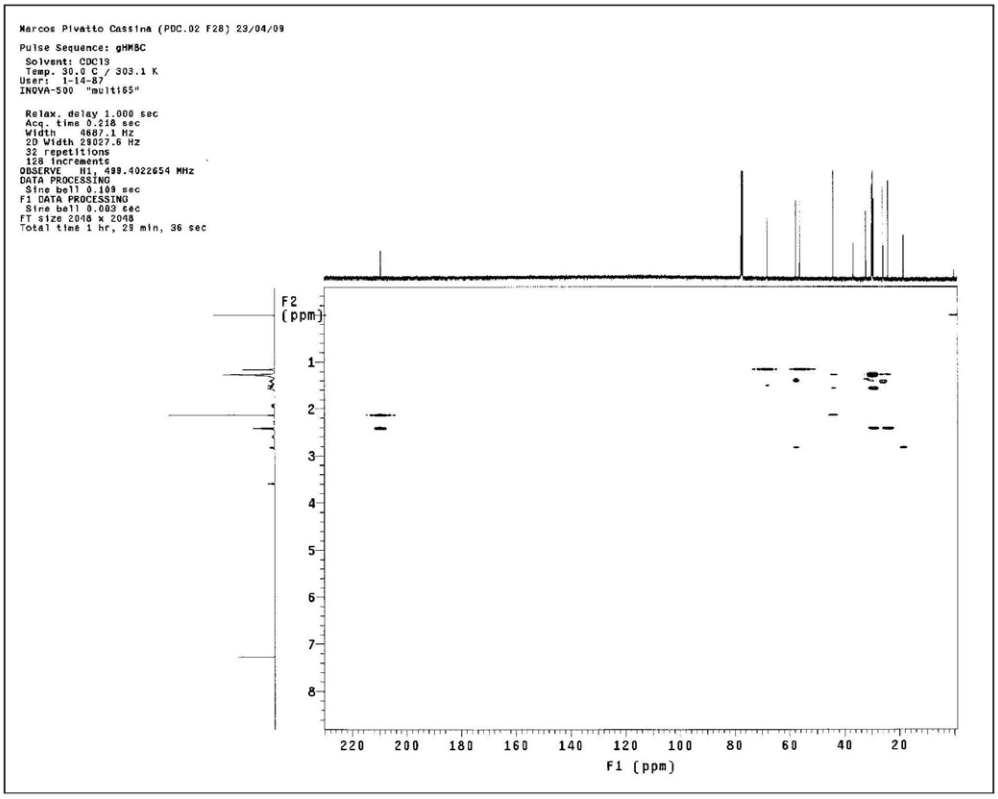

Figure S23. HMBC spectrum of **1** in chloroform-*d*.

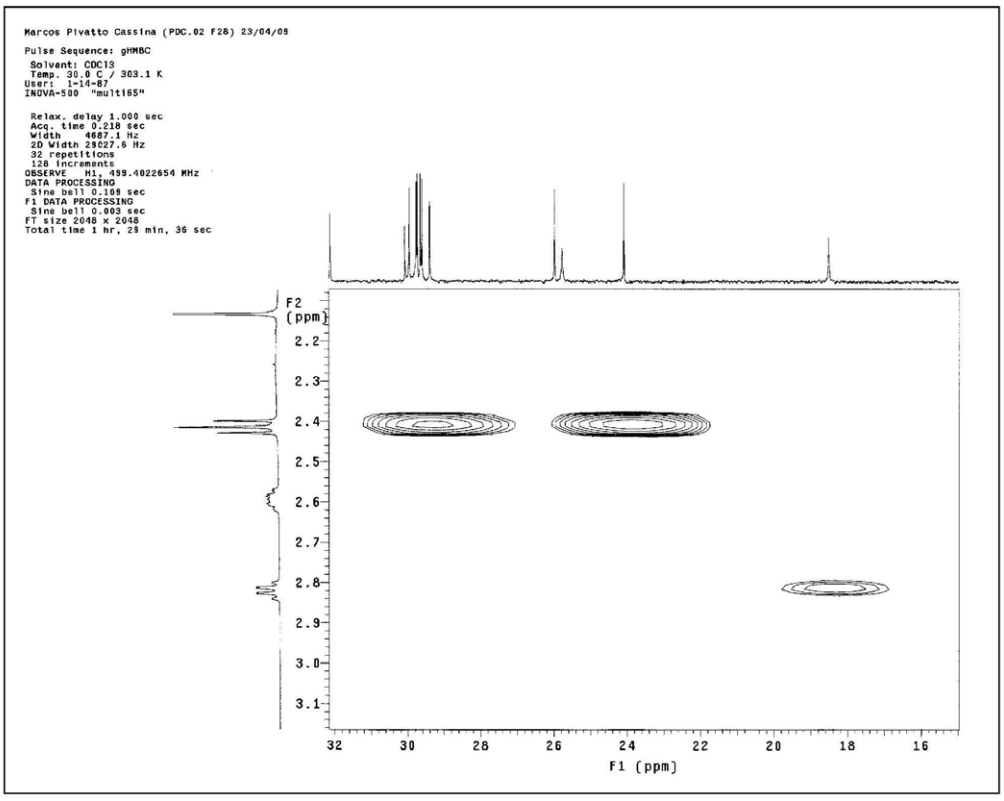

Figure S24. HMBC spectrum (expansion δ 16-32) of **1** in chloroform-*d*.

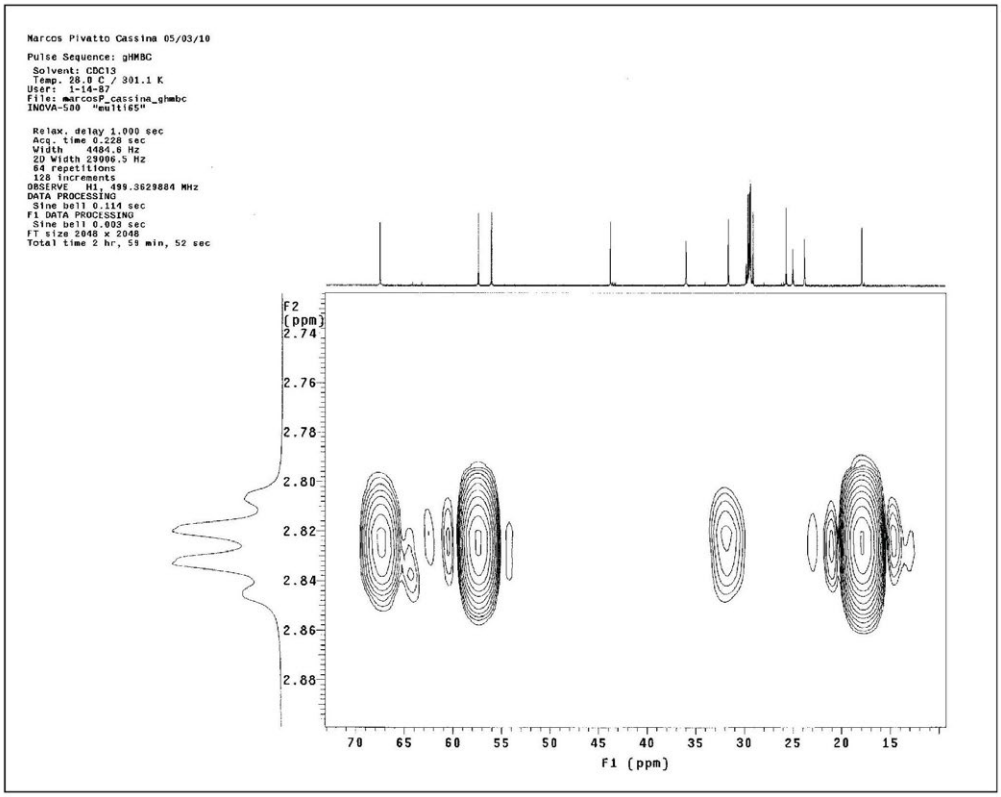

Figure S25. HMBC spectrum (expansion δ 15-70) of **1** in chloroform-*d*.

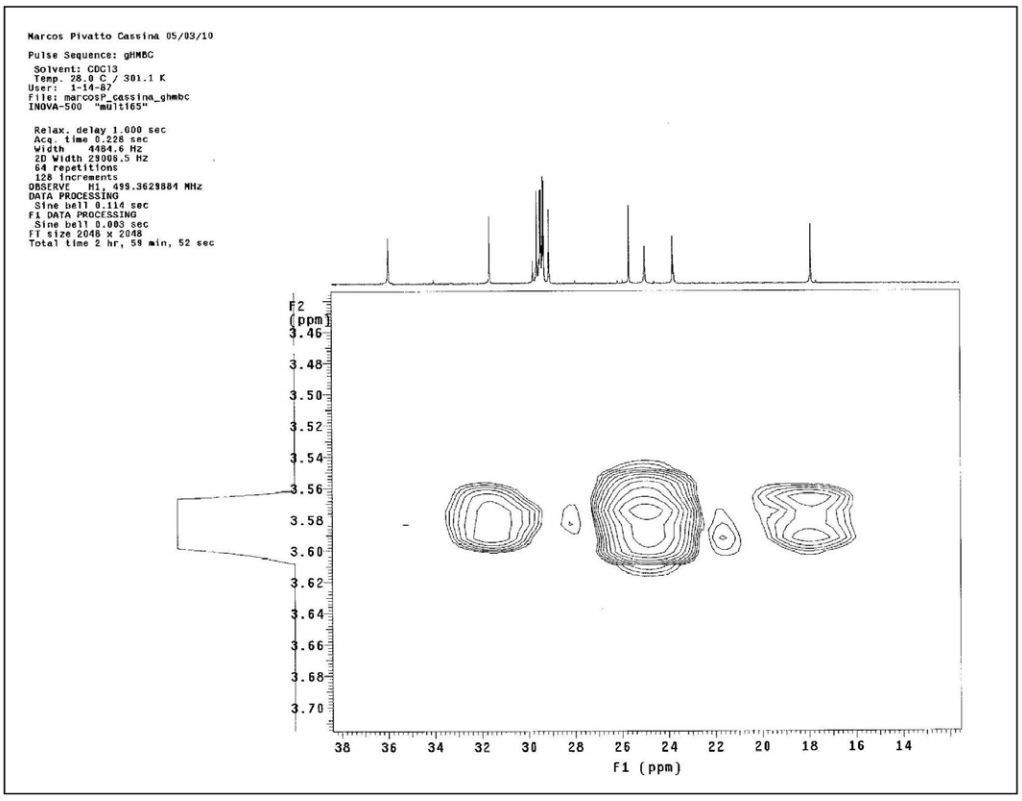

Figure S26. HMBC spectrum (expansion δ 14-38) of **1** in chloroform-*d*.

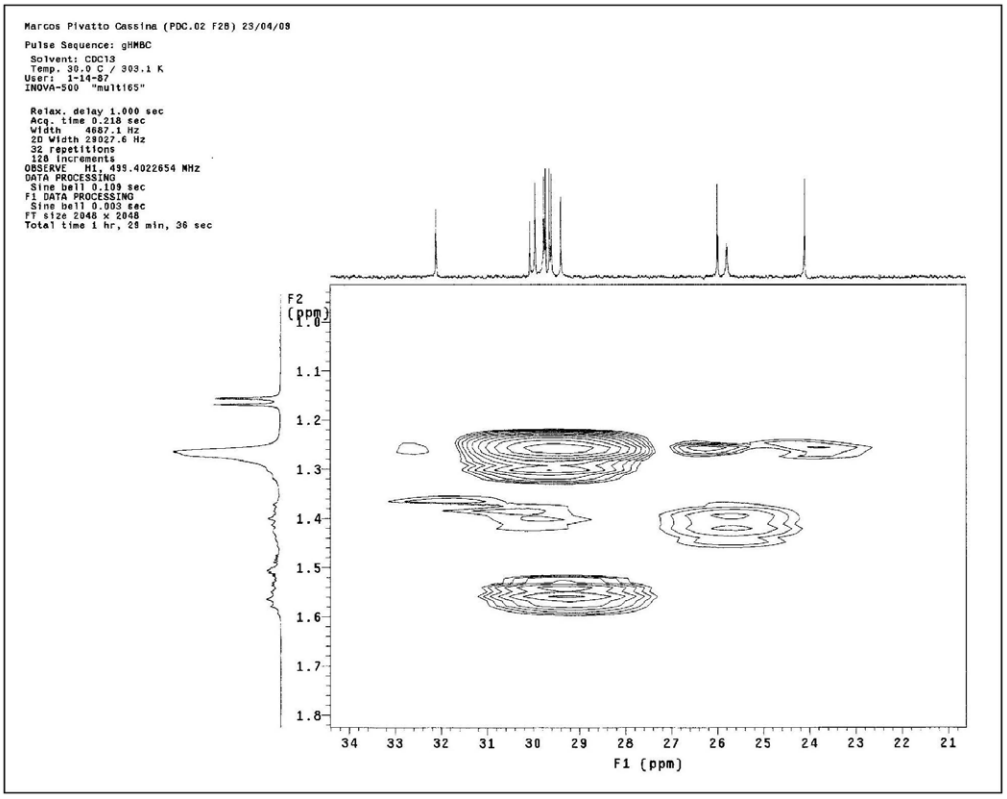

Figure S27. HMBC spectrum (expansion δ 21-34) of **1** in chloroform-*d*.

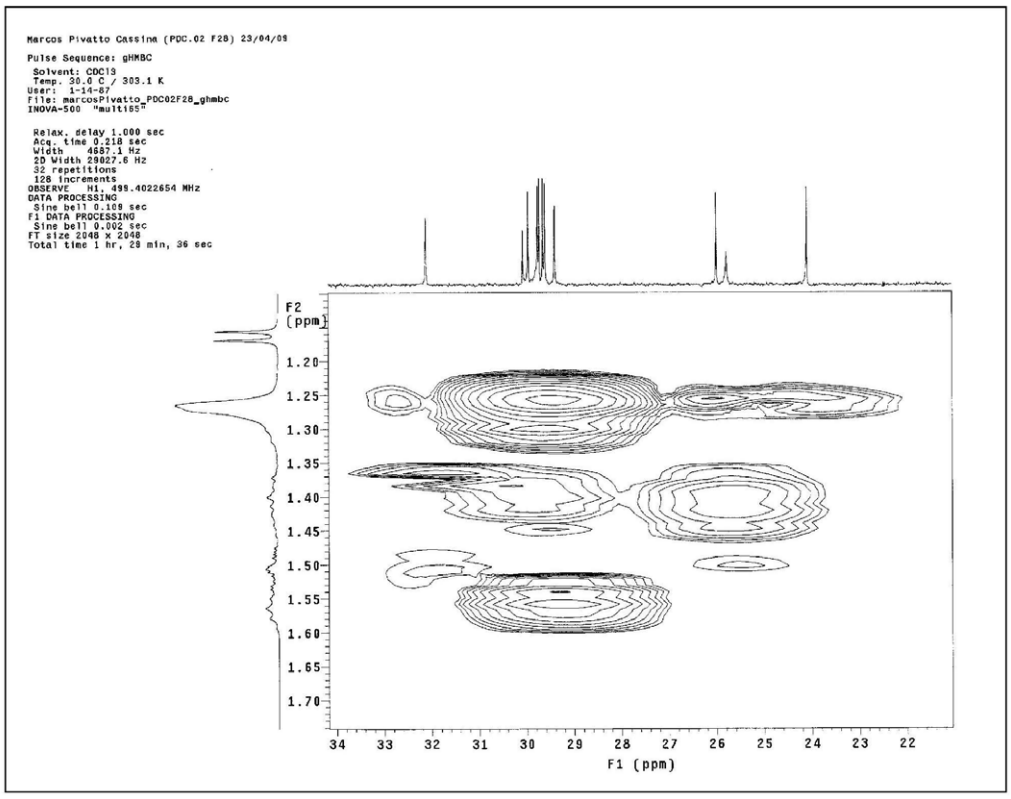

Figure S28. HMBC spectrum (expansion δ 22-34) of **1** in chloroform-*d*.

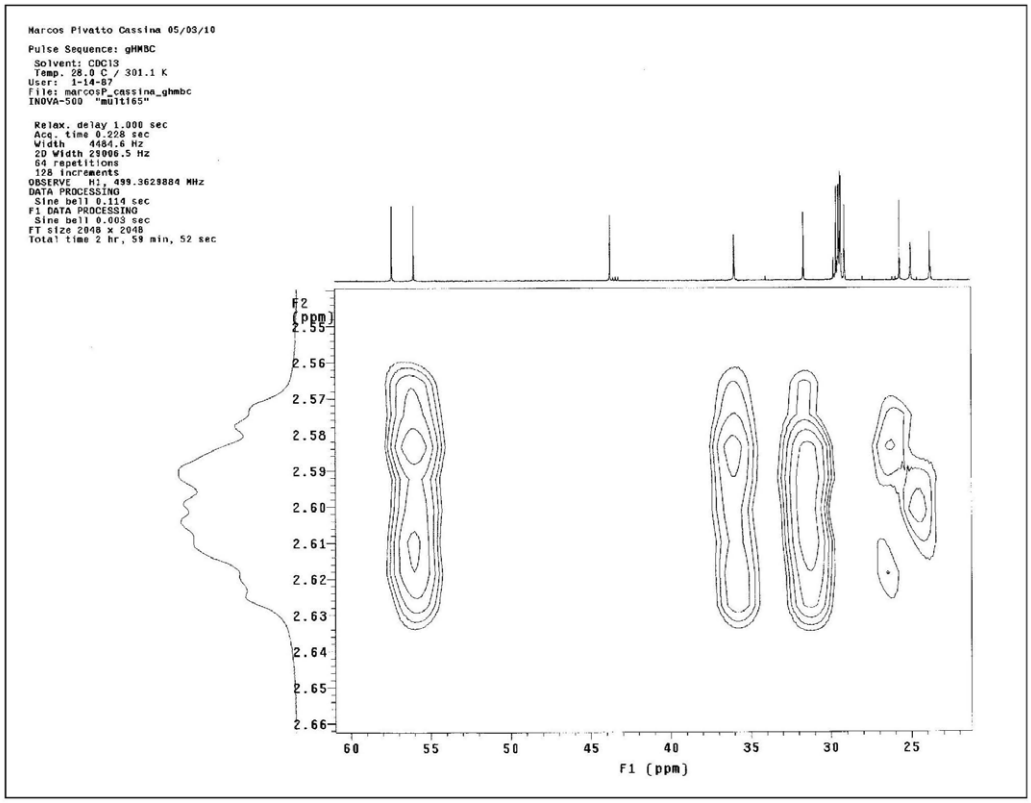

Figure S29. HMBC spectrum (expansion δ 25-60) of **1** in chloroform-*d*.

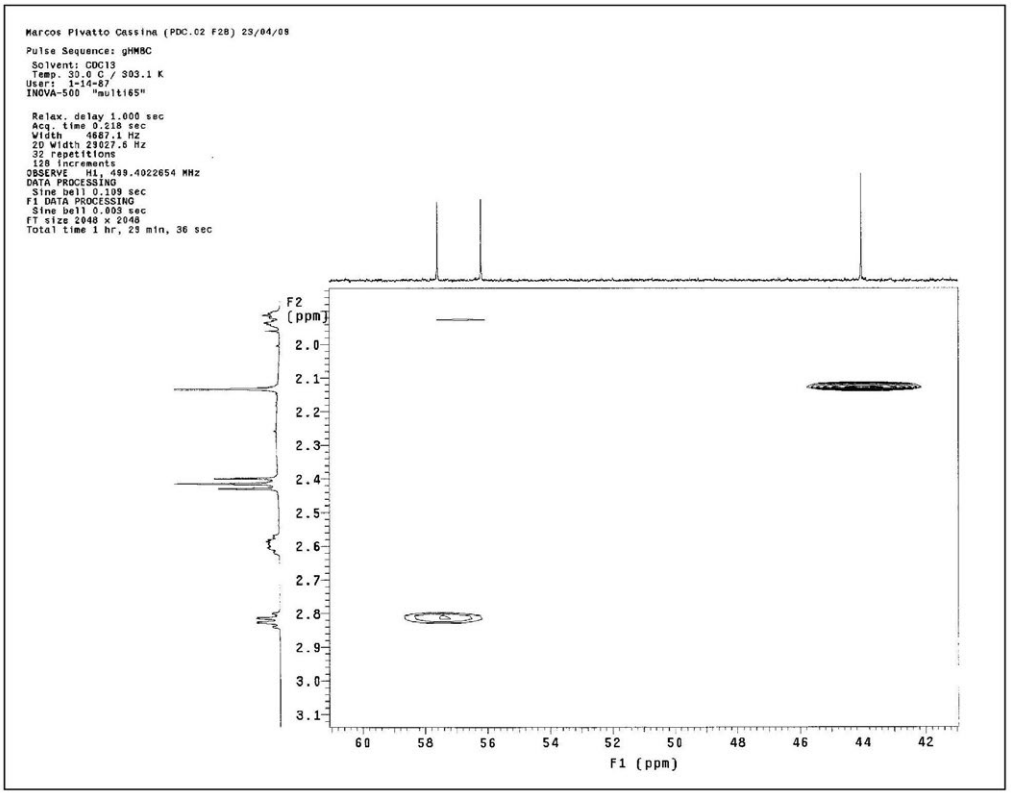

Figure S30. HMBC spectrum (expansion δ 42-60) of **1** in chloroform-*d*.

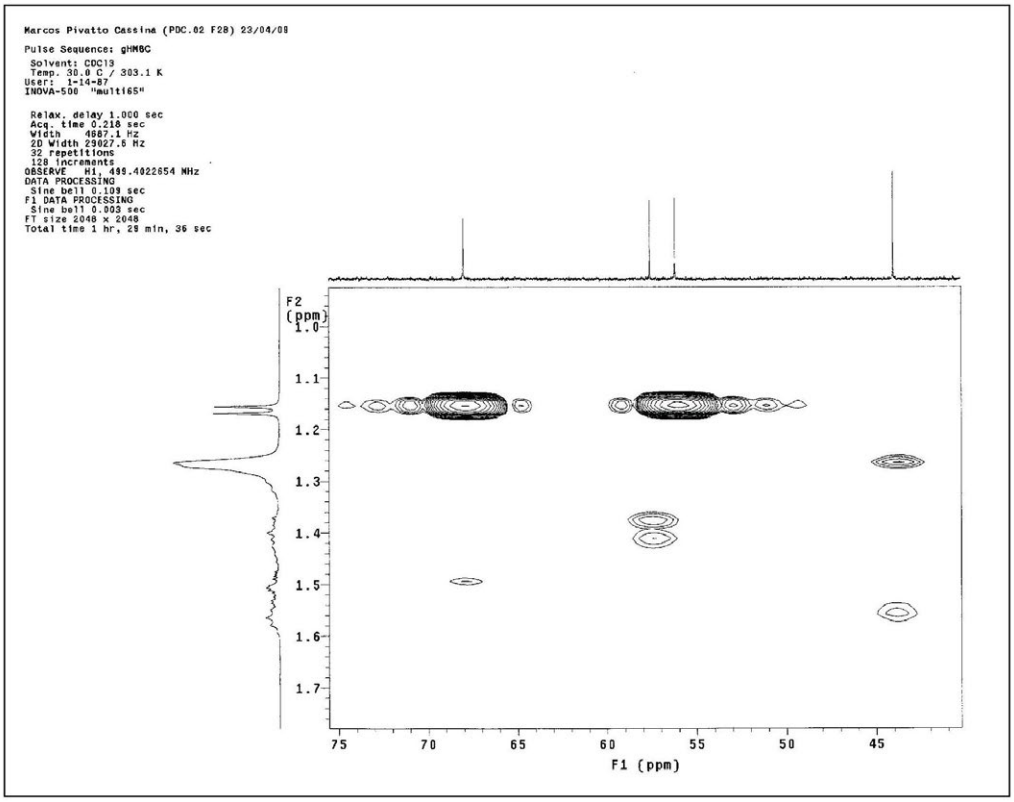

Figure S31. HMBC spectrum (expansion δ 45-75) of **1** in chloroform-*d*.

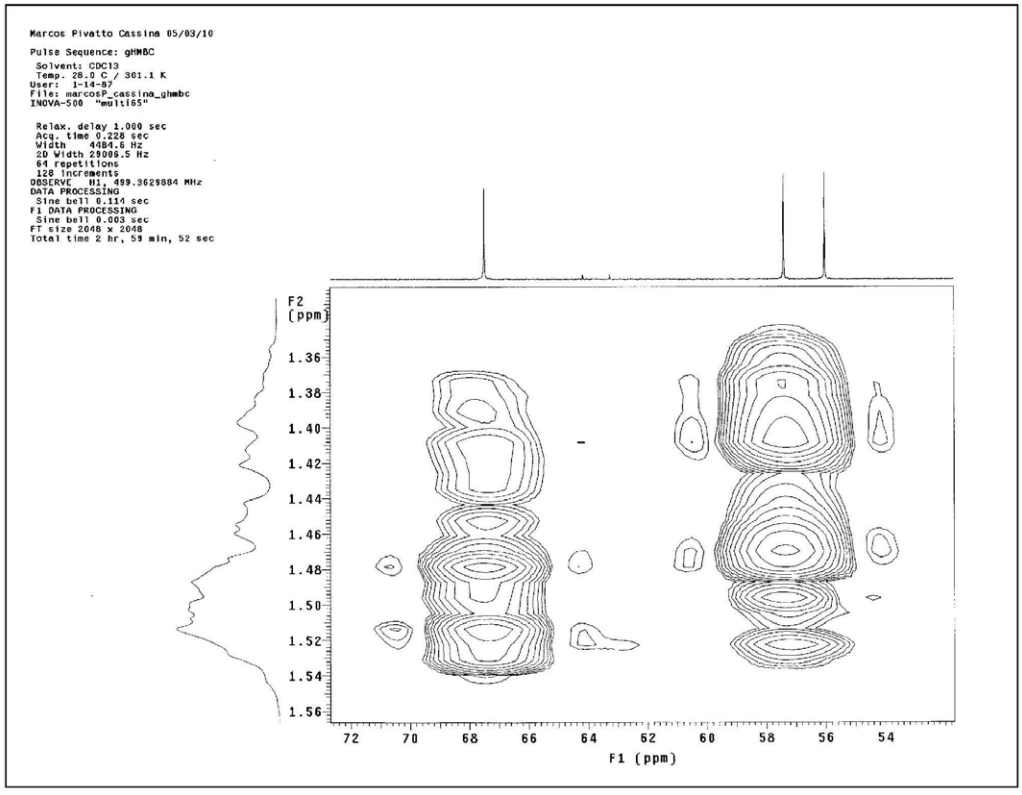

Figure S32. HMBC spectrum (expansion δ 54-72) of **1** in chloroform-*d*.

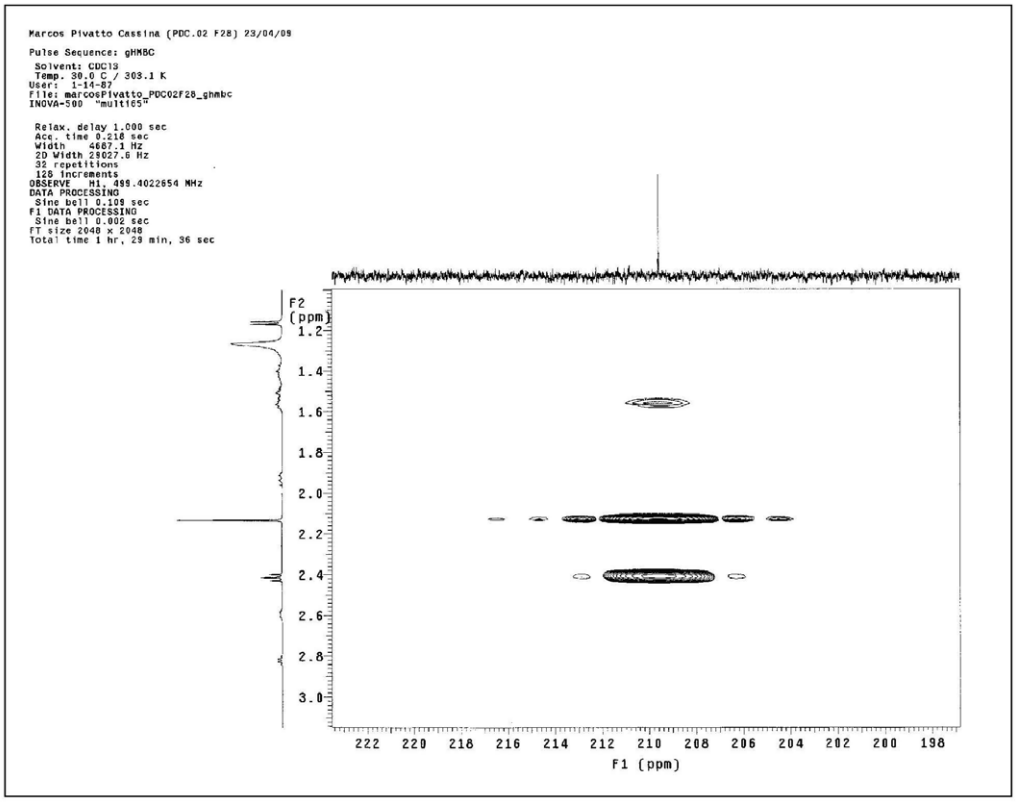

Figure S33. HMBC spectrum (expansion δ 198-222) of **1** in chloroform-*d*.

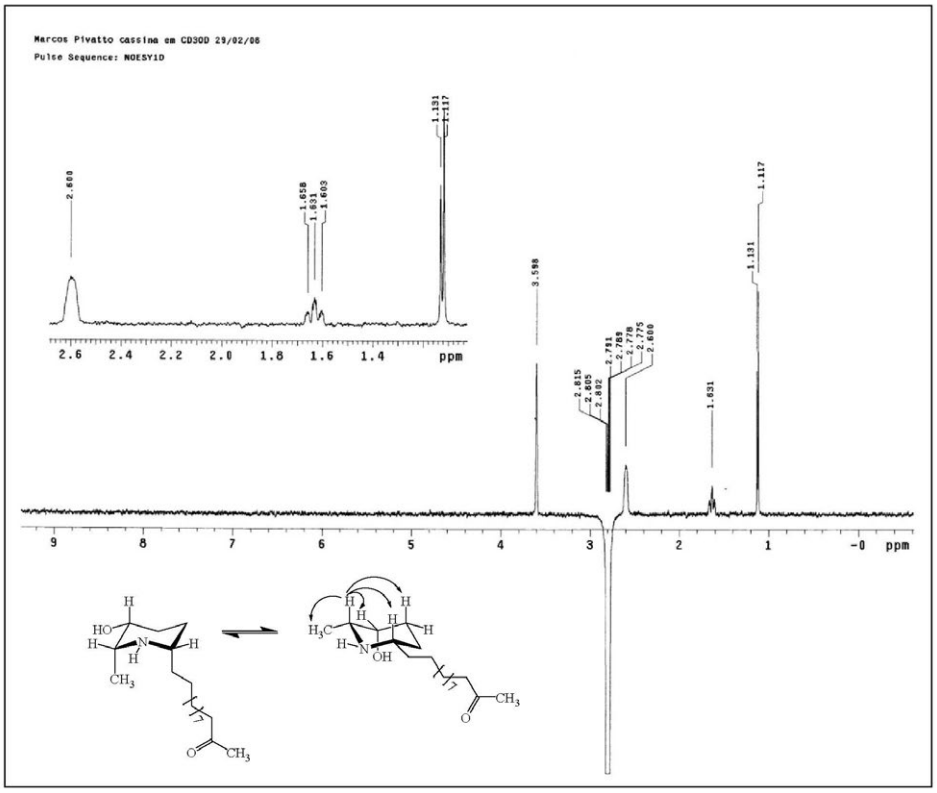

Figure S34. 1D NOESY spectrum of **1** in methanol-d_4 irradiating δ 2.82.

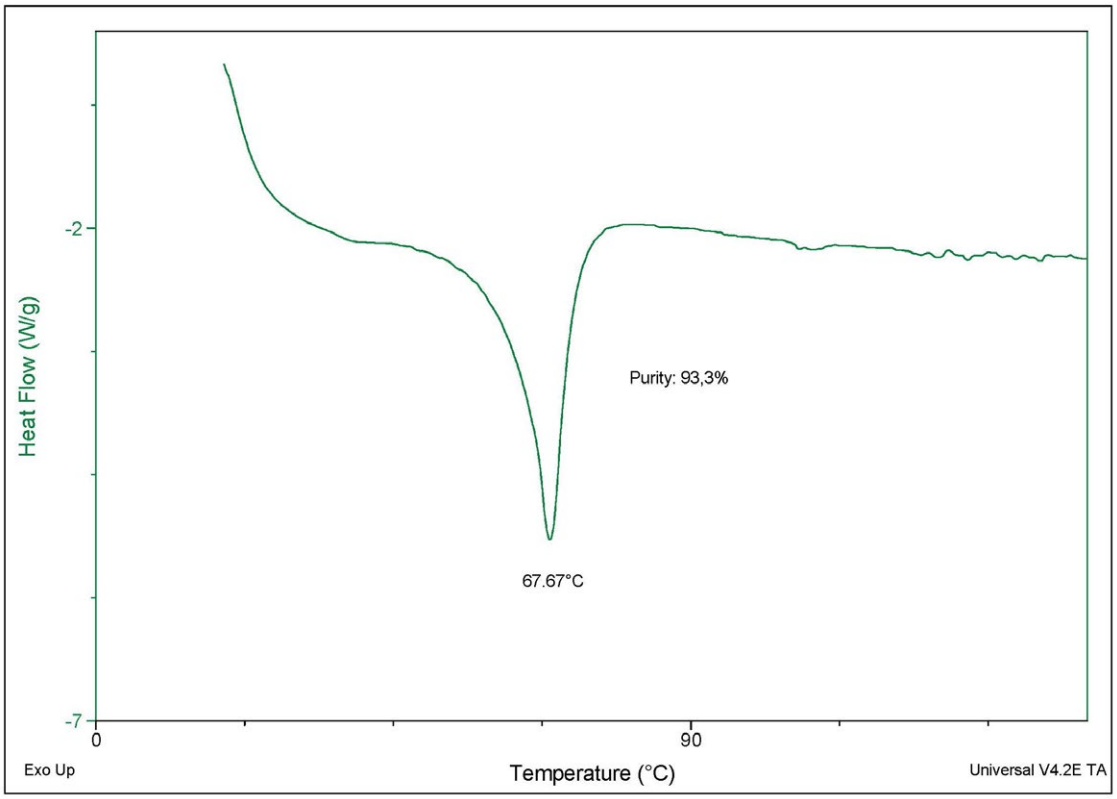

Figure S35. DSC analysis of **2**.

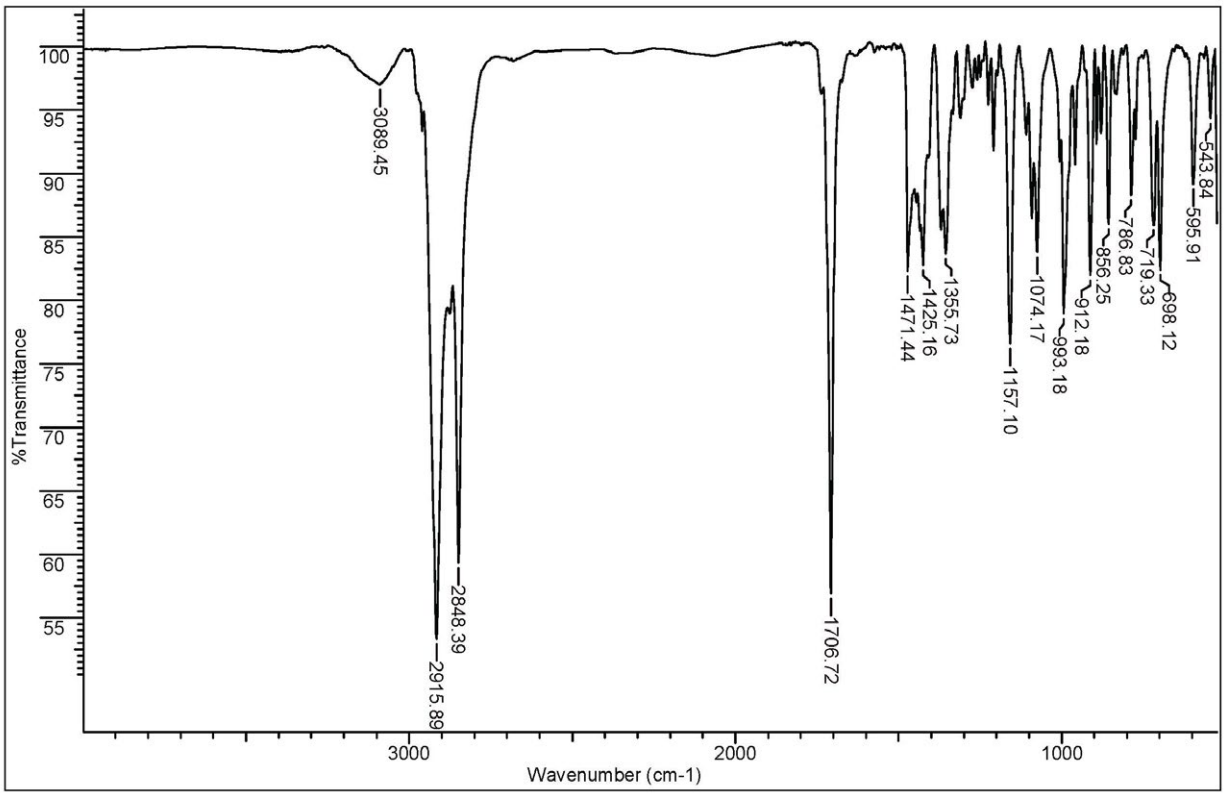

Figure S36. IR spectrum (film) of **2**.

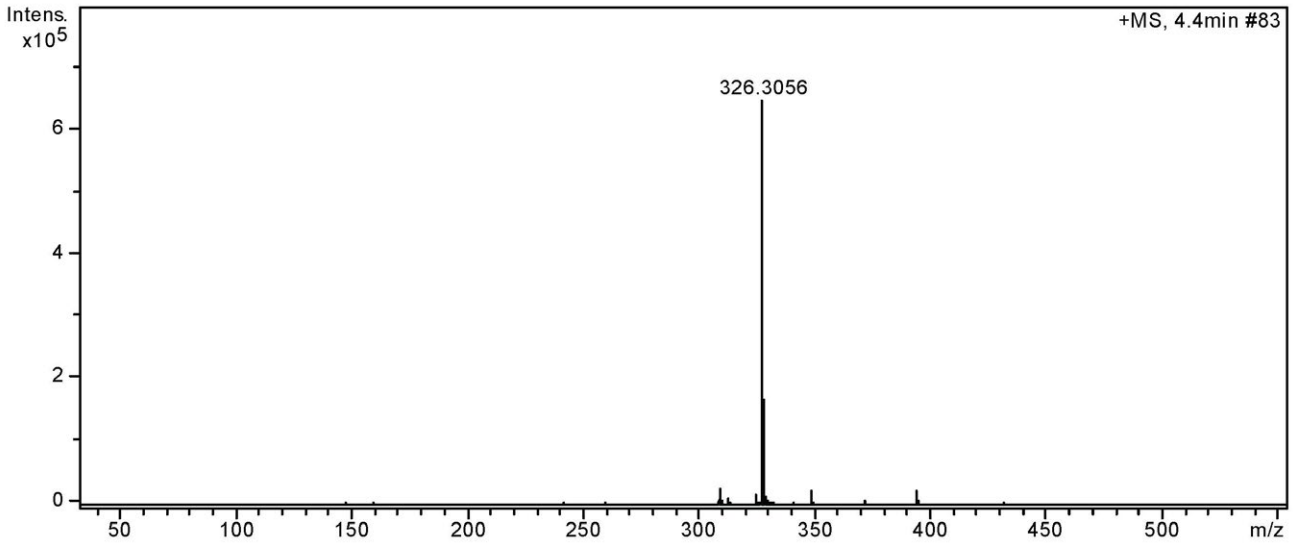

Figure S37. ESI-MS of **2** (HRMS *m/z* 326.3056 $[M + H]^+$ (calcd for $C_{20}H_{40}NO_2$: 326.3054)).

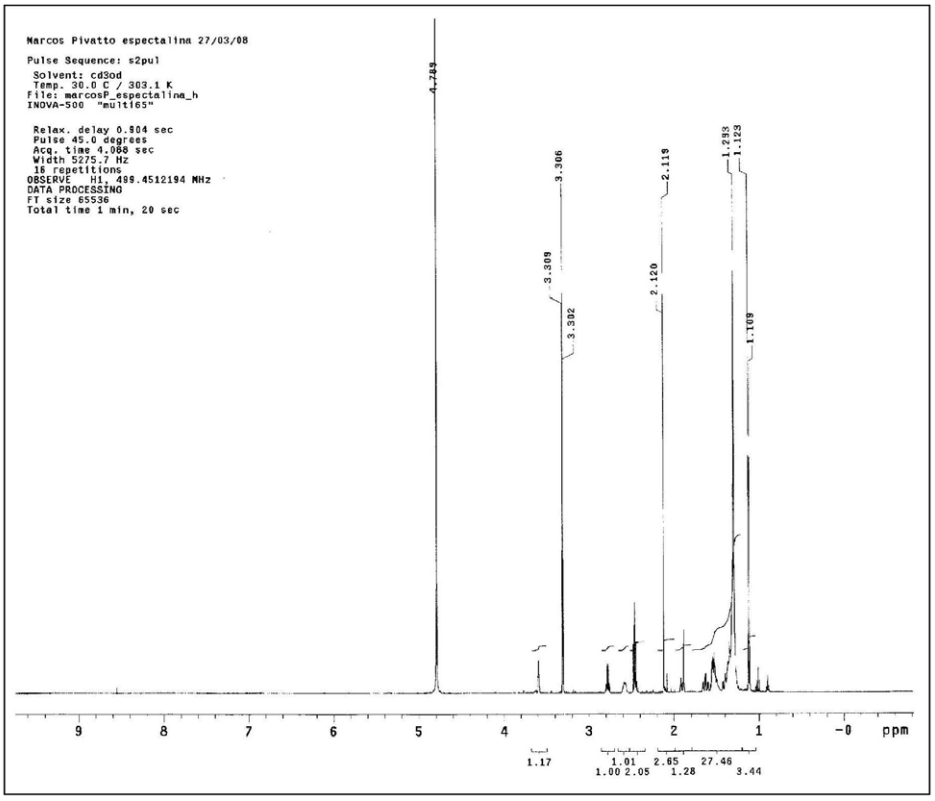

Figure S38. 500 MHz ^{1}H NMR spectrum of **2** in methanol-d_4.

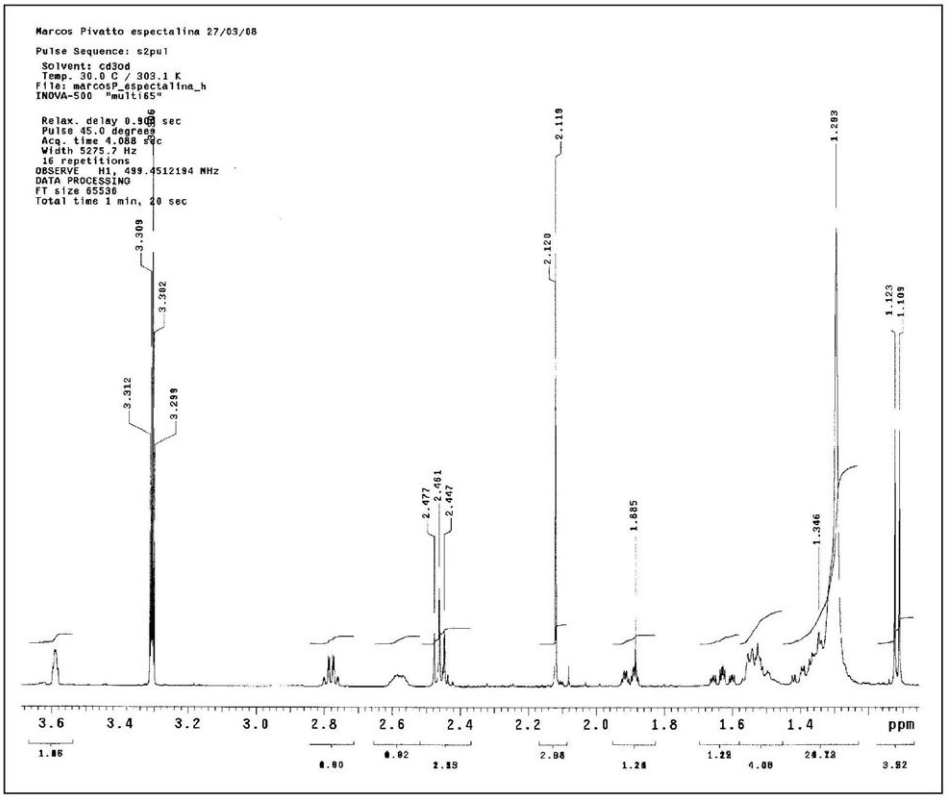

Figure S39. 500 MHz ^{1}H NMR spectrum (expansion δ 1.2-3.6) of **2** in methanol-d_4.

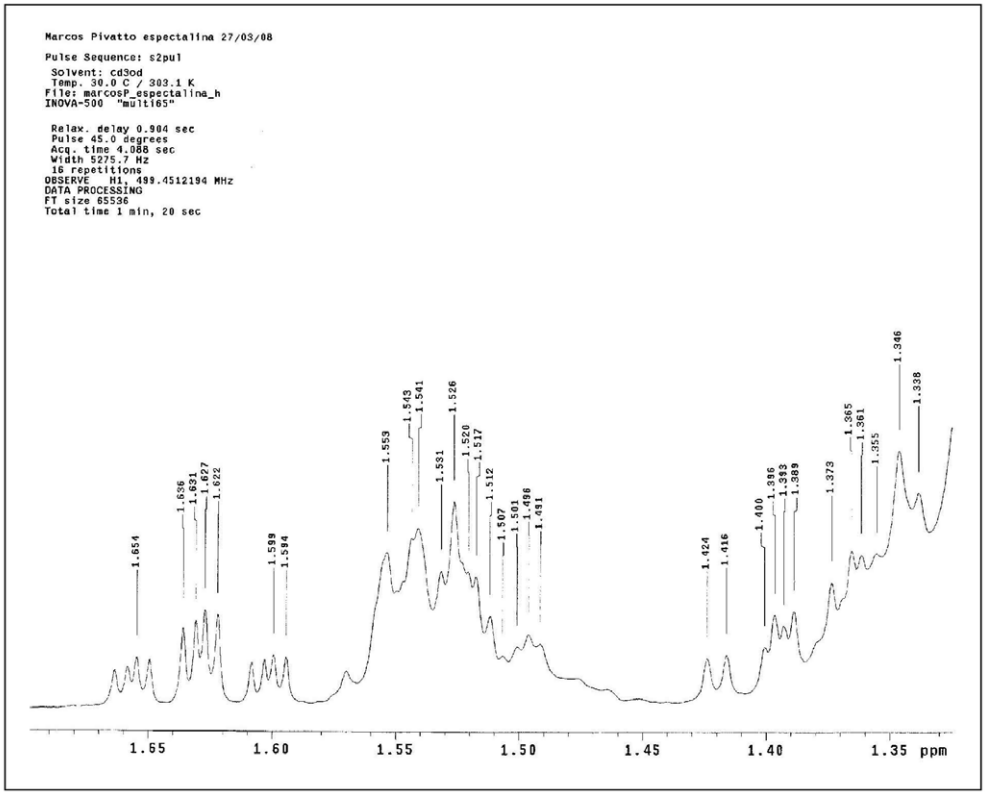

Figure S40. 500 MHz ^{1}H NMR spectrum (expansion δ 1.35-1.65) of **2** in methanol-d_4.

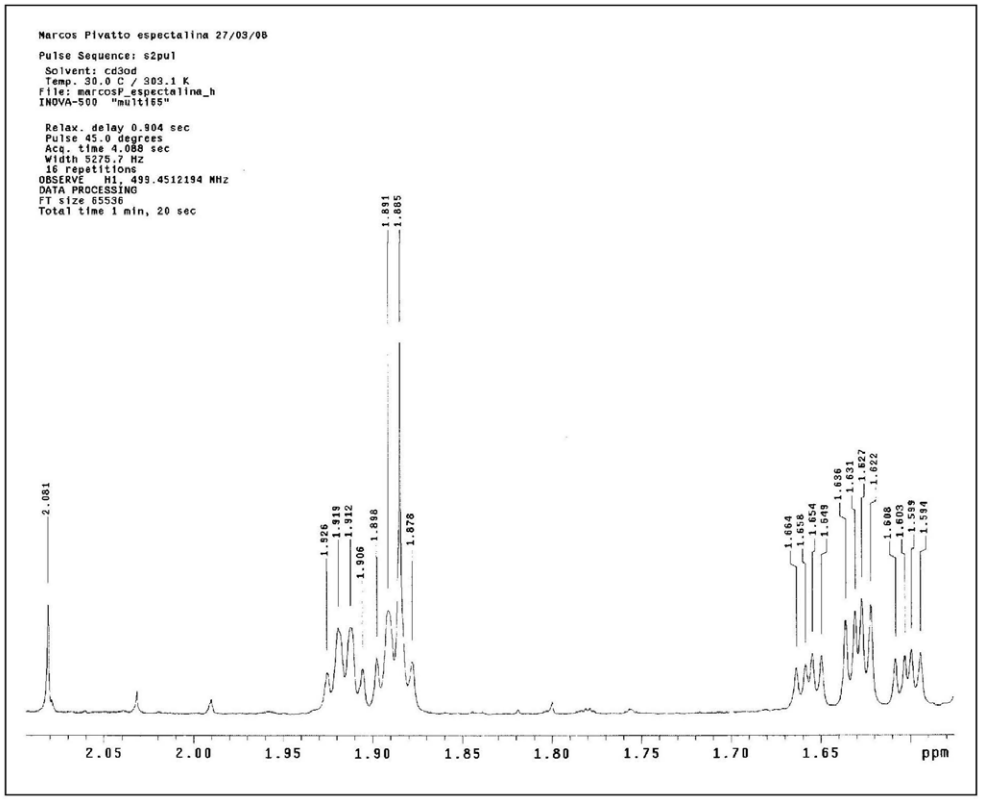

Figure S41. 500 MHz ^{1}H NMR spectrum (expansion δ 1.60-2.05) of **2** in methanol-d_4.

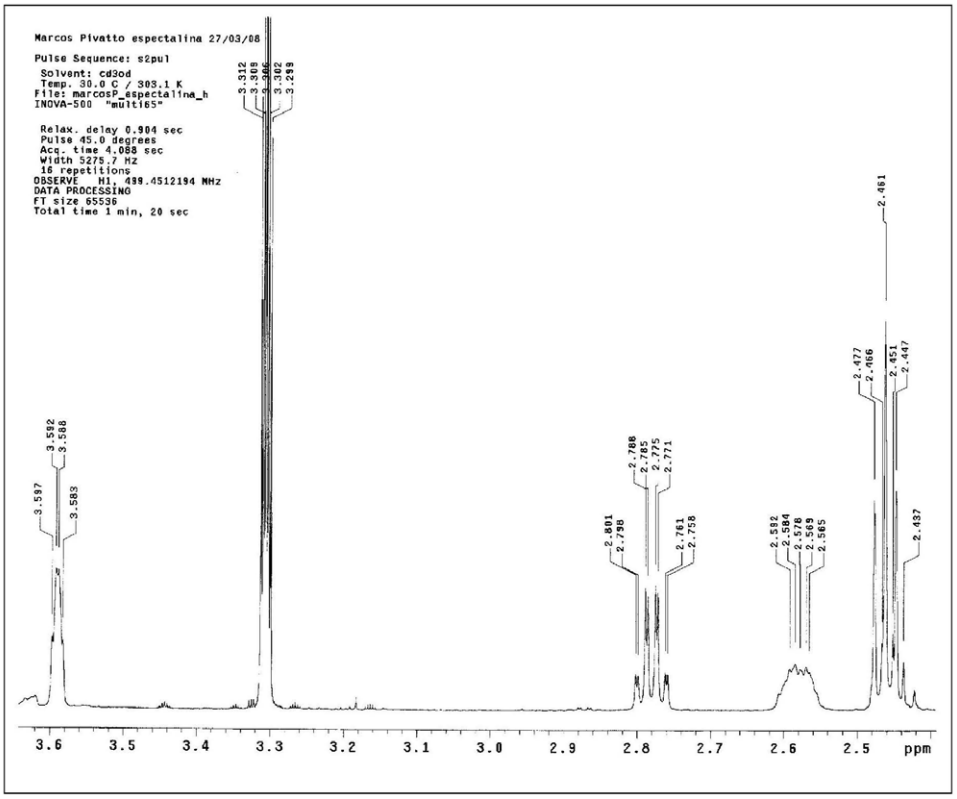

Figure S42. 500 MHz ^{1}H NMR spectrum (expansion δ 2.4-3.6) of **2** in methanol-d_4.

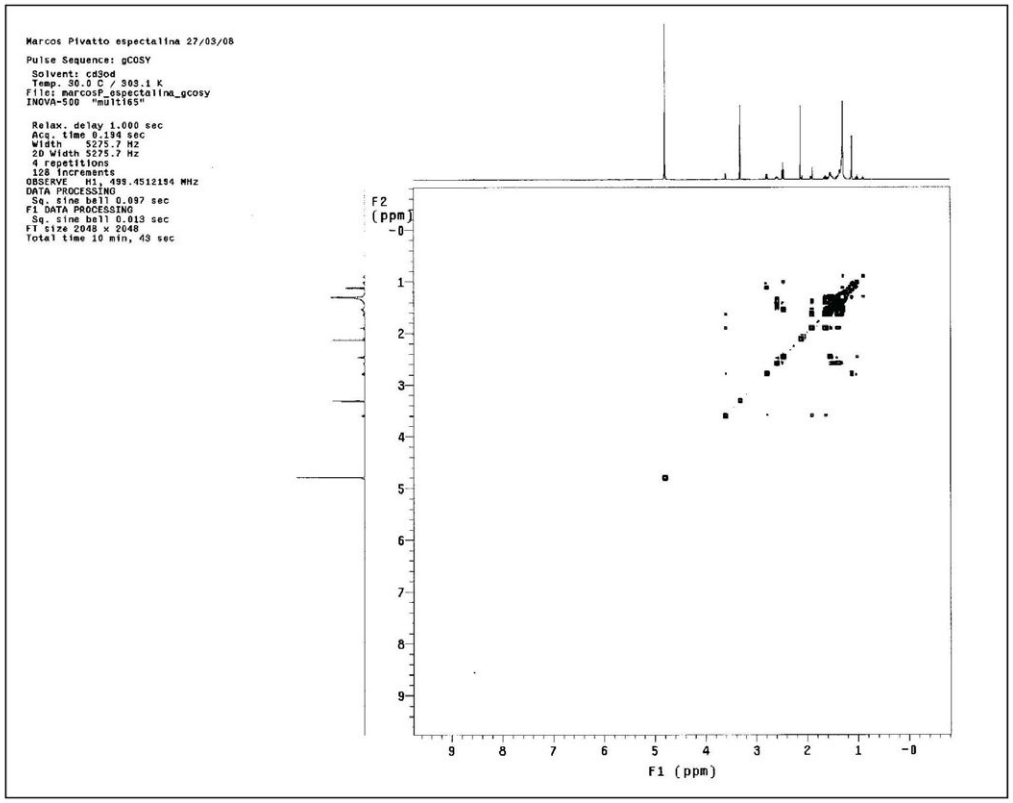

Figure S43. COSY spectrum of **2** in methanol-d_4.

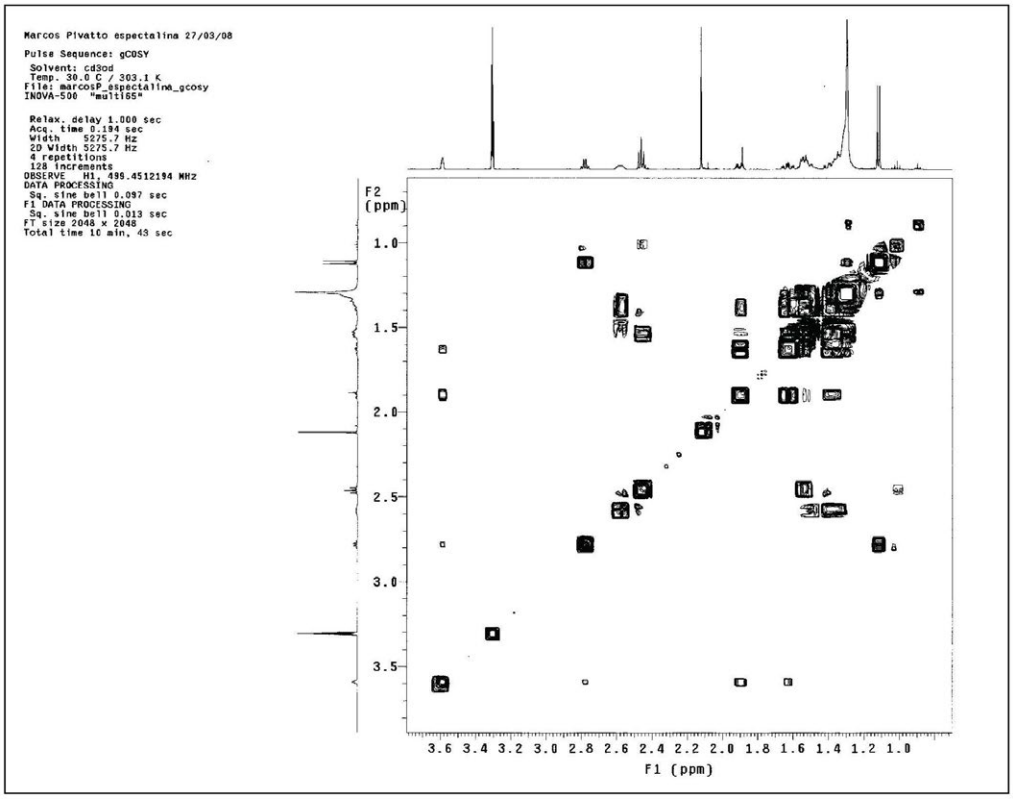

Figure S44. COSY spectrum (expansion δ 0.0-3.6) of **2** in methanol-d_4.

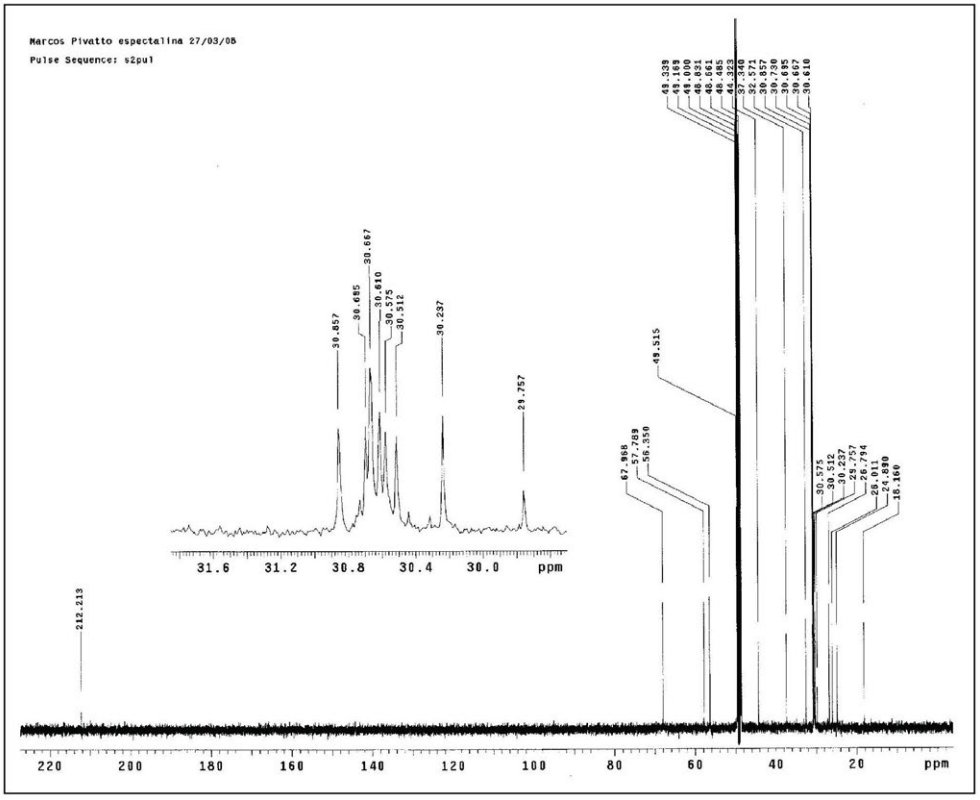

Figure S45. 125 MHz ^{13}C NMR spectrum of **2** in methanol-d_4.

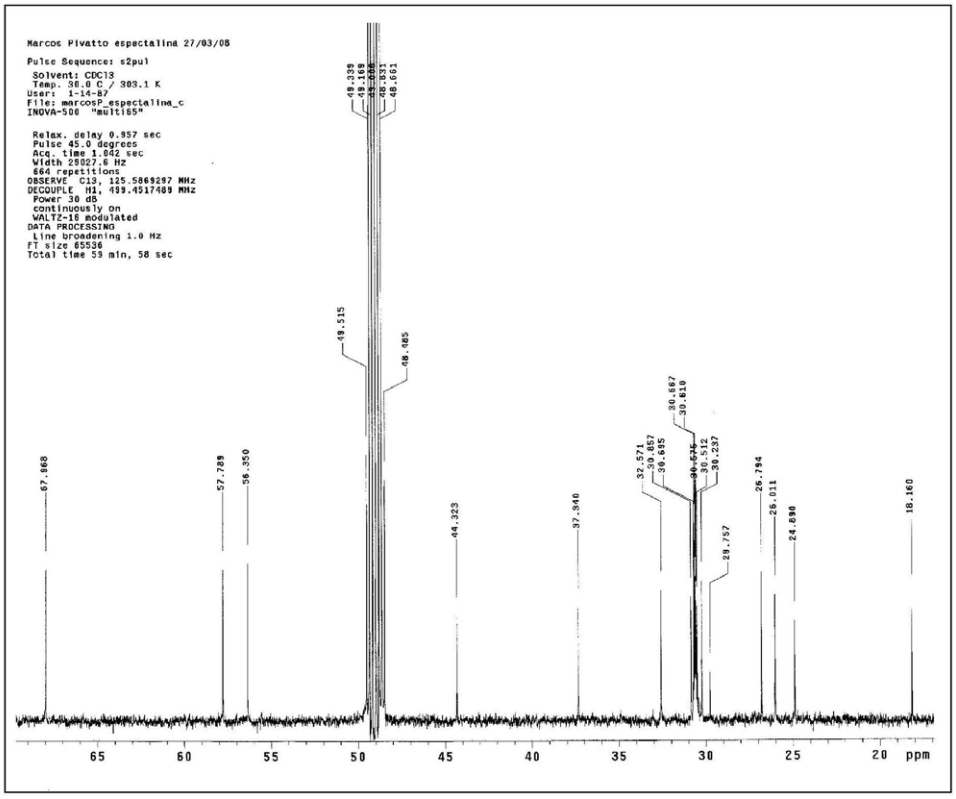

Figure S46. 125 MHz ^{13}C NMR spectrum (expansion δ 20-65) of **2** in methanol-d_4.

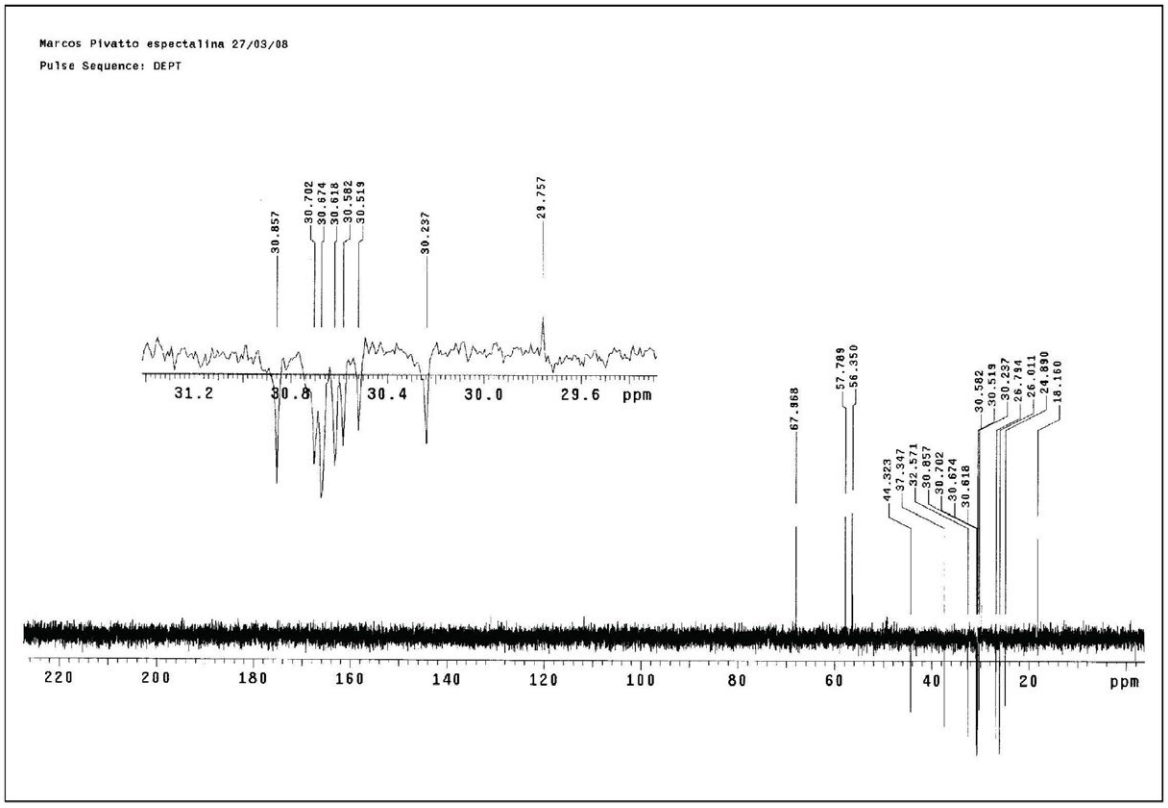

Figure S47. 125 MHz DEPT 135 NMR spectrum of **2** in methanol-d_4.

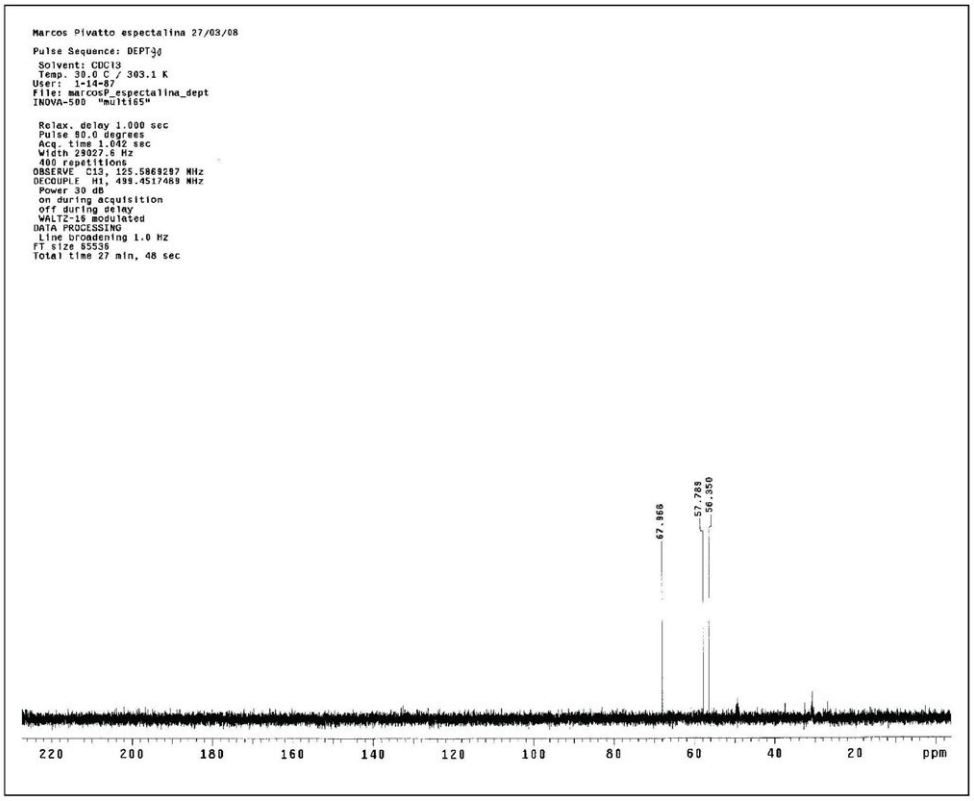

Figure S48. 125 MHz DEPT 90 NMR spectrum of **2** in methanol-d_4.

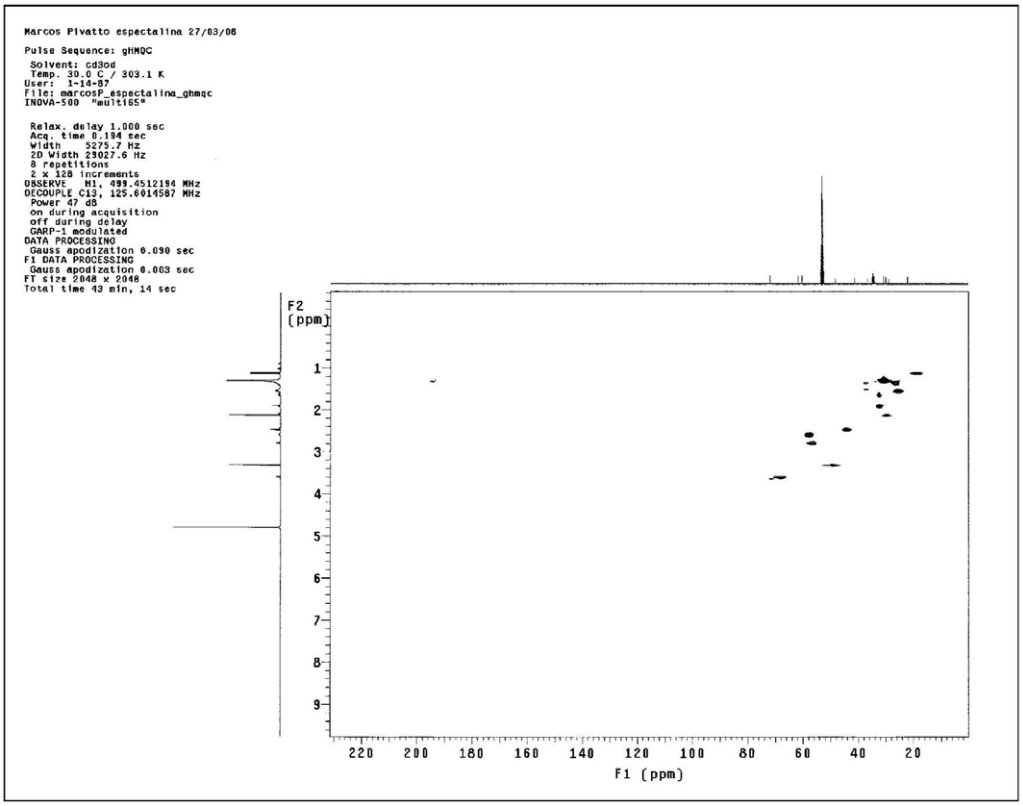

Figure S49. HMQC spectrum of **2** in methanol-d_4.

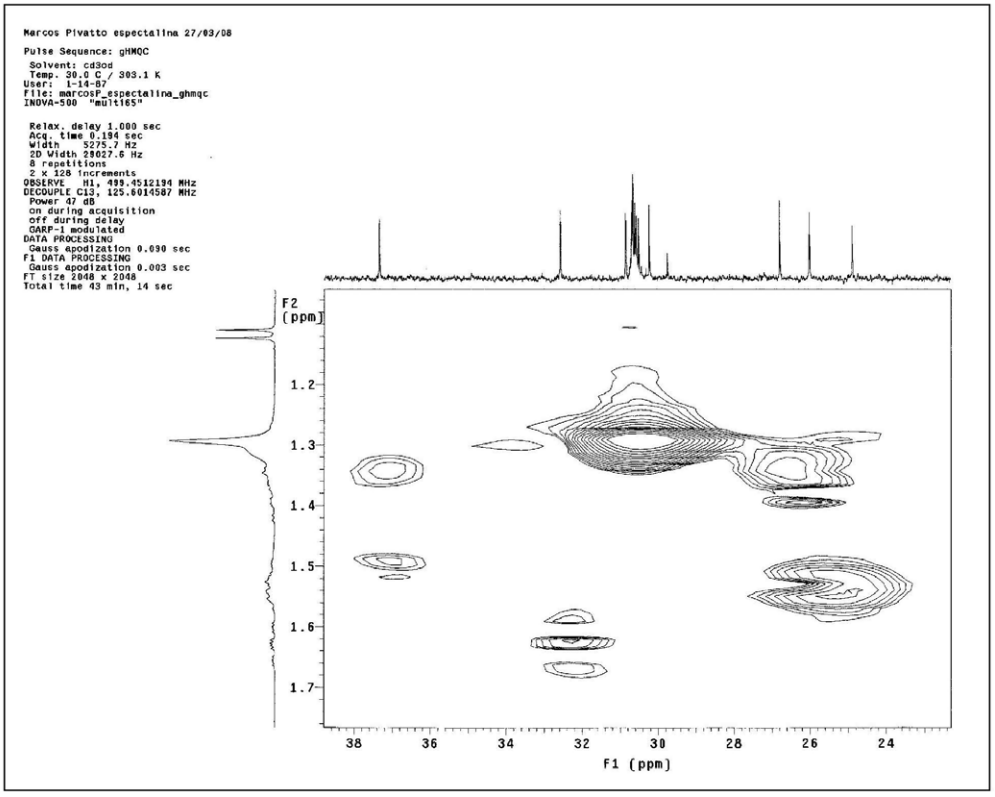

Figure S50. HMQC spectrum (expansion δ 24-38) of **2** in methanol-d_4.

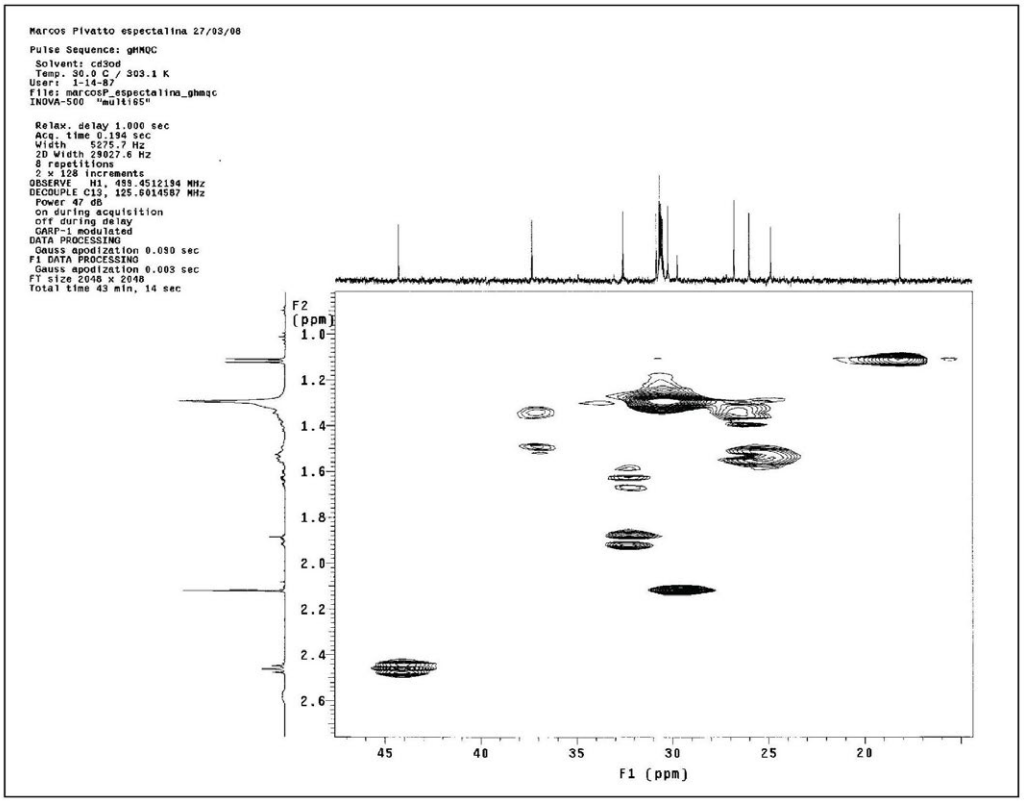

Figure S51. HMQC spectrum (expansion δ 20-45) of **2** in methanol-d_4.

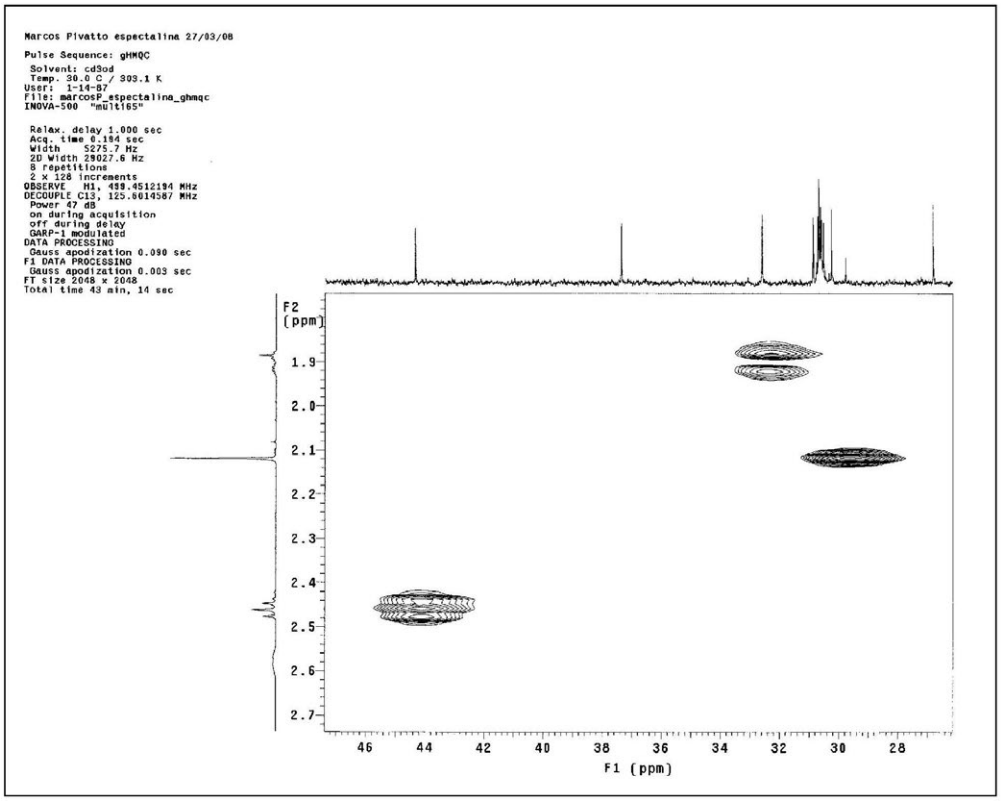

Figure S52. HMQC spectrum (expansion δ 28-46) of **2** in methanol-d_4.

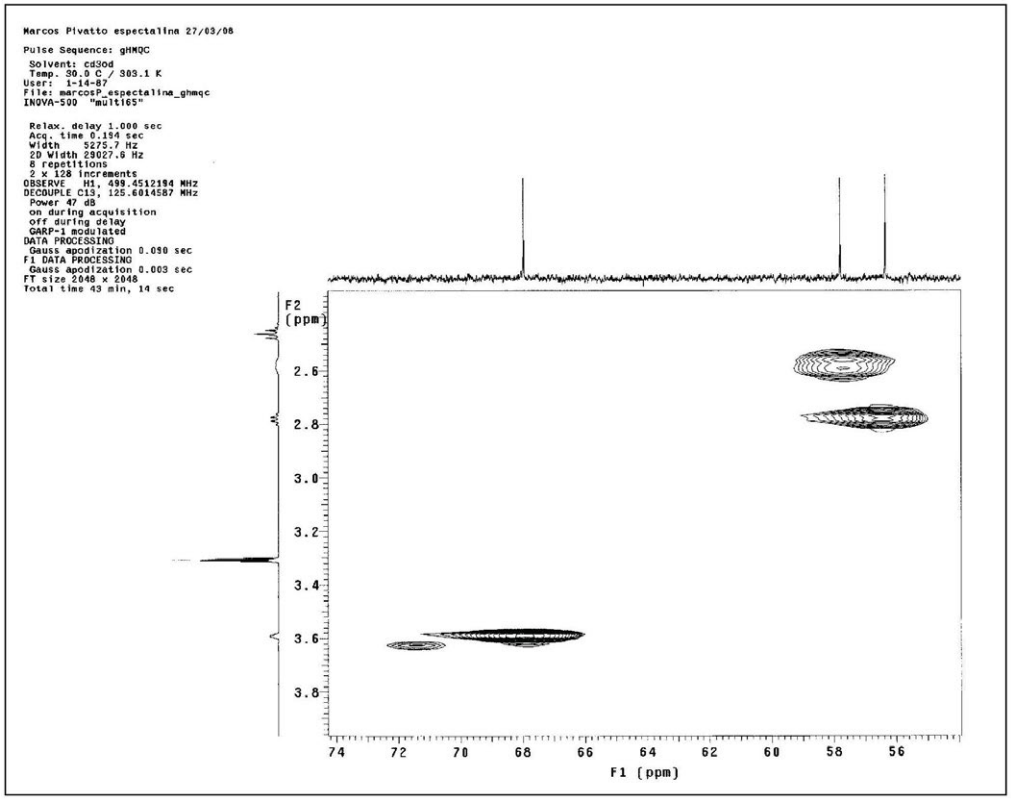

Figure S53. HMQC spectrum (expansion δ 54-74) of **2** in methanol-d_4.

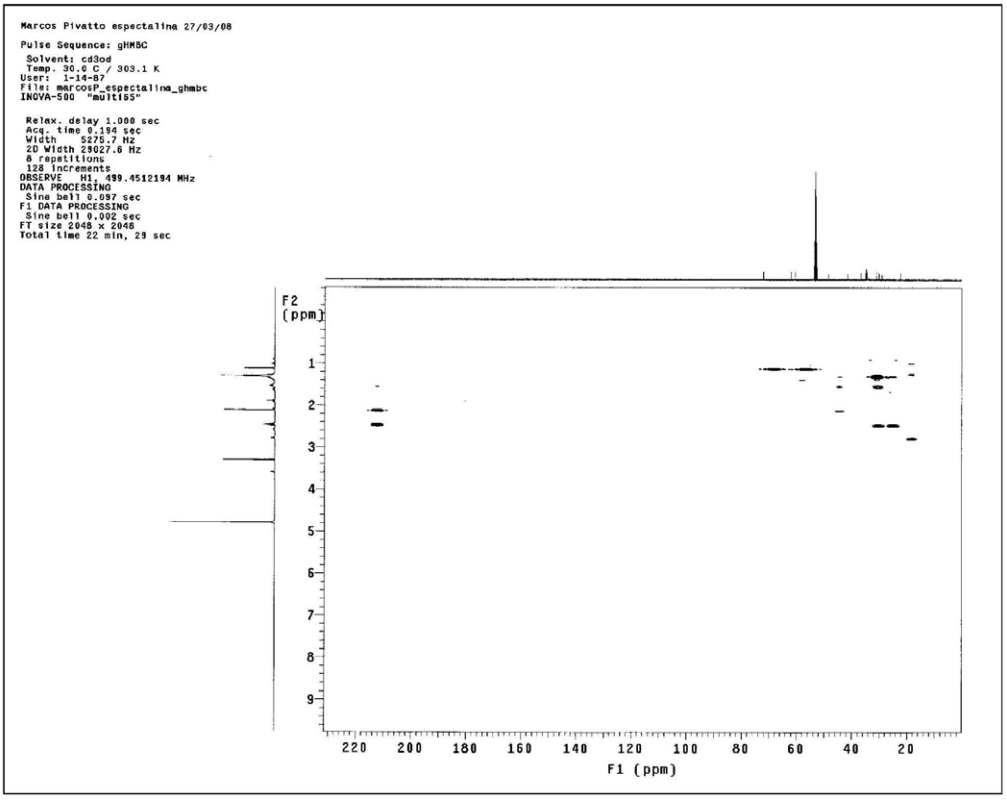

Figure S54. HMBC spectrum of **2** in methanol-d_4.

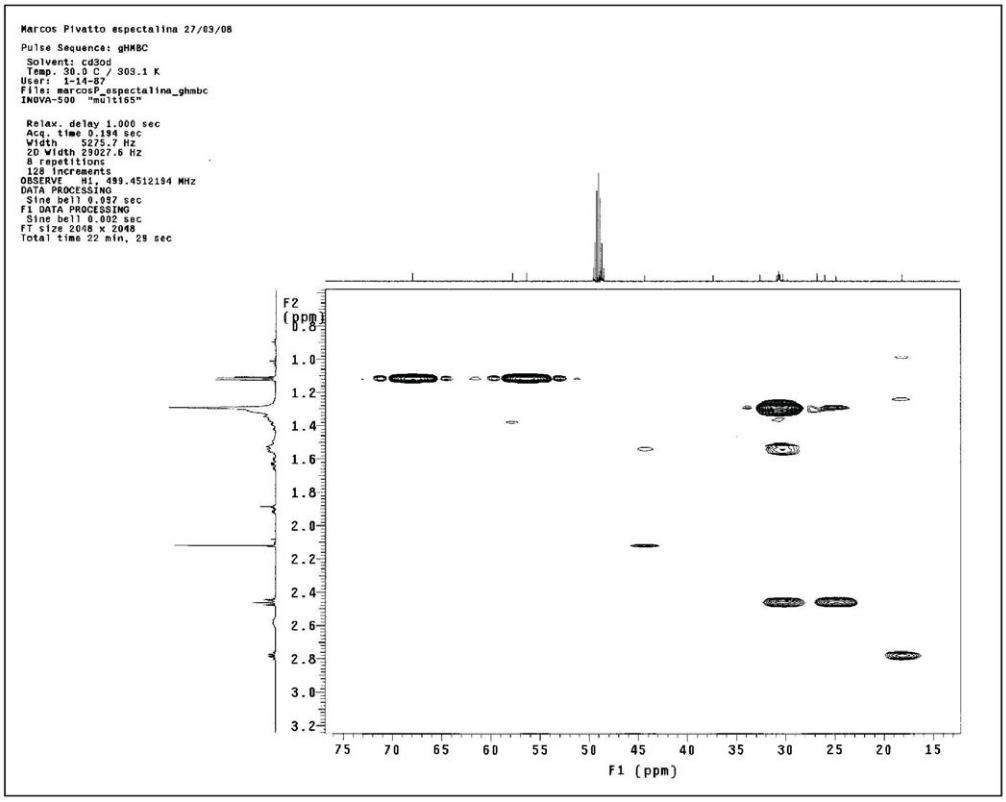

Figure S55. HMBC spectrum (expansion δ 15-75) of **2** in methanol-d_4.

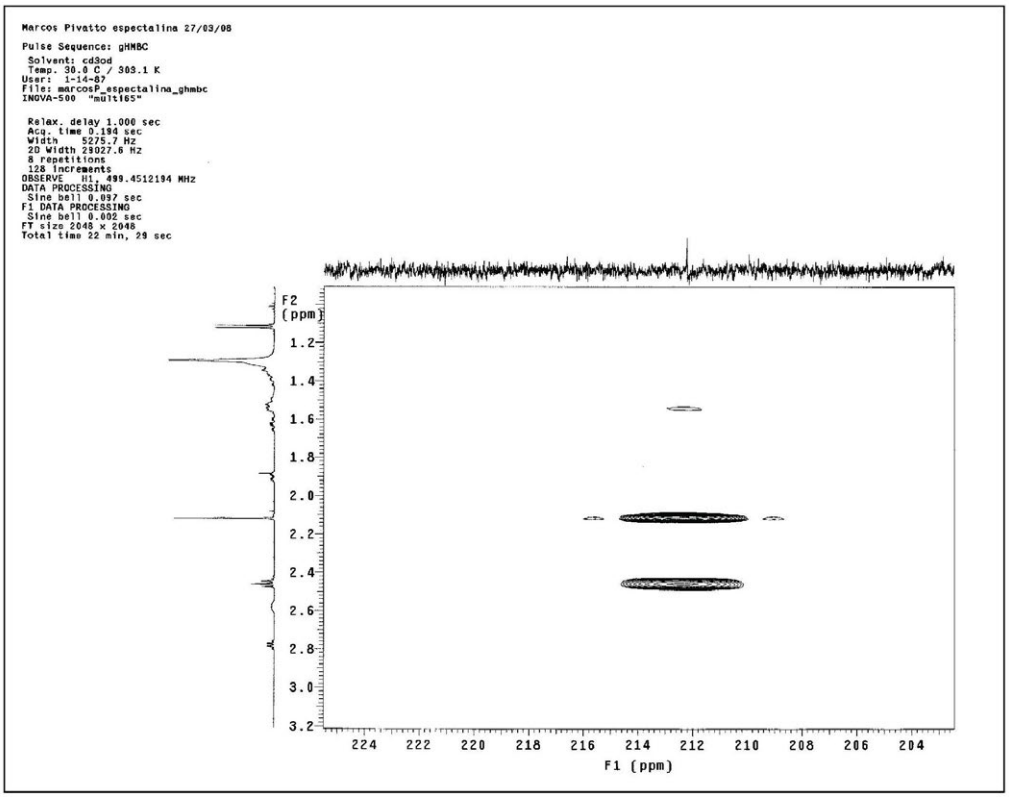

Figure S56. HMBC spectrum (expansion δ 204-224) of **2** in methanol-d_4.

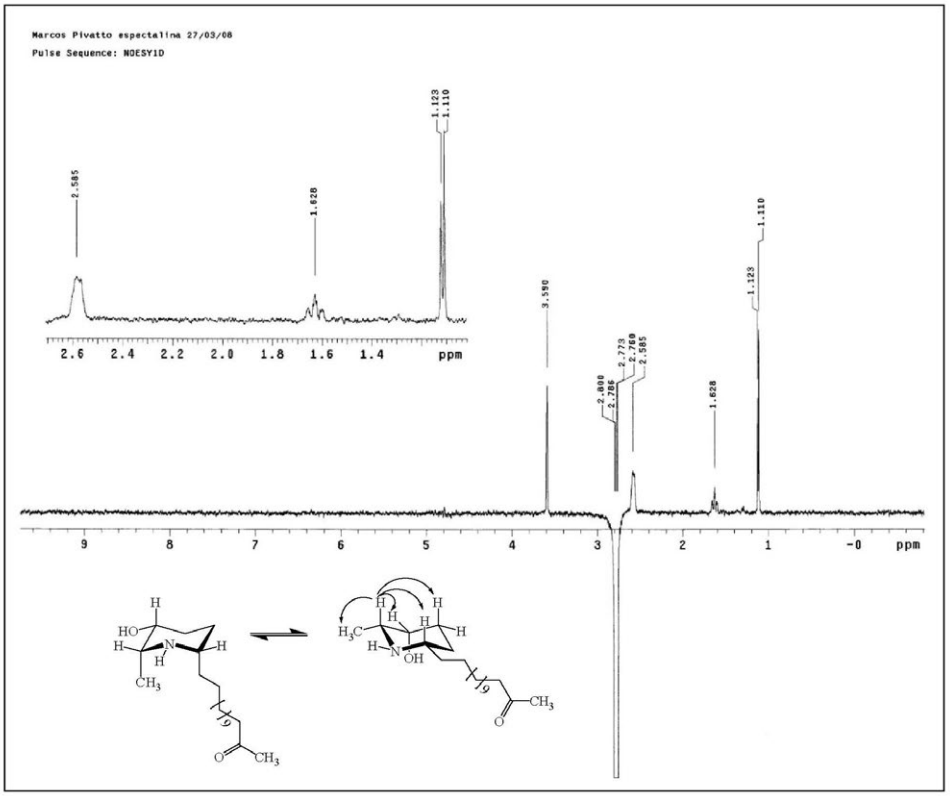

Figure S57. 1D NOESY spectrum of **2** in methanol-d_4 irradiating δ 2.78.

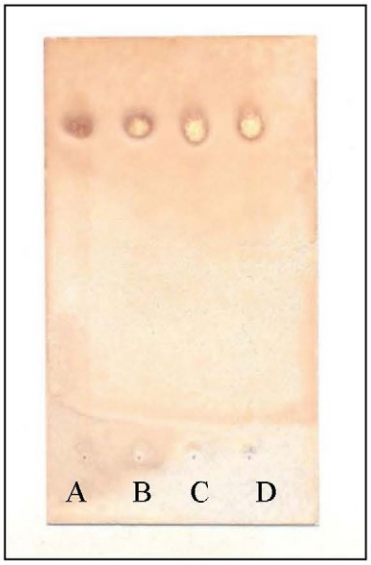

Figure S58. TLC (SiO_2) of **3** (A: free base, B: hydrochloride) and **4** (C: free base, D: hydrochloride) developed with $CHCl_3$-MeOH-NH_4OH (9:1:0.25) and revealed with iodochloroplatinate reagent.

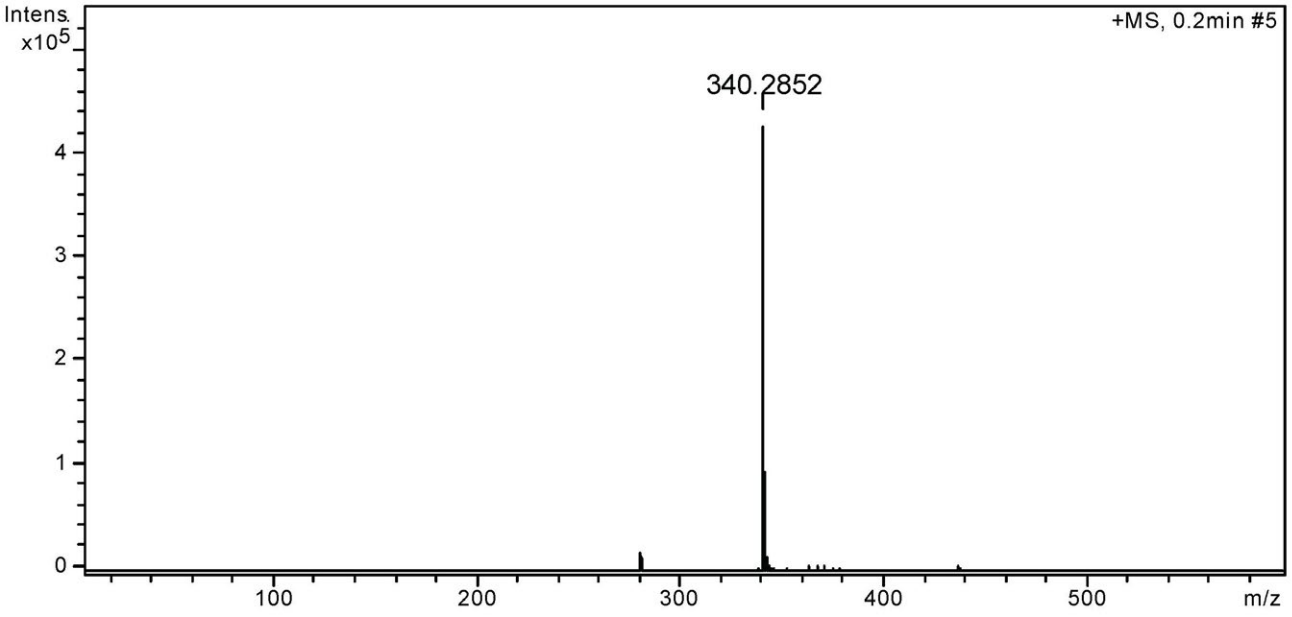

Figure S59. ESI-MS of **2** (HRMS *m/z* 340.2852 $[M + H]^+$ (calcd for $C_{20}H_{40}NO_2$: 340.2852)).

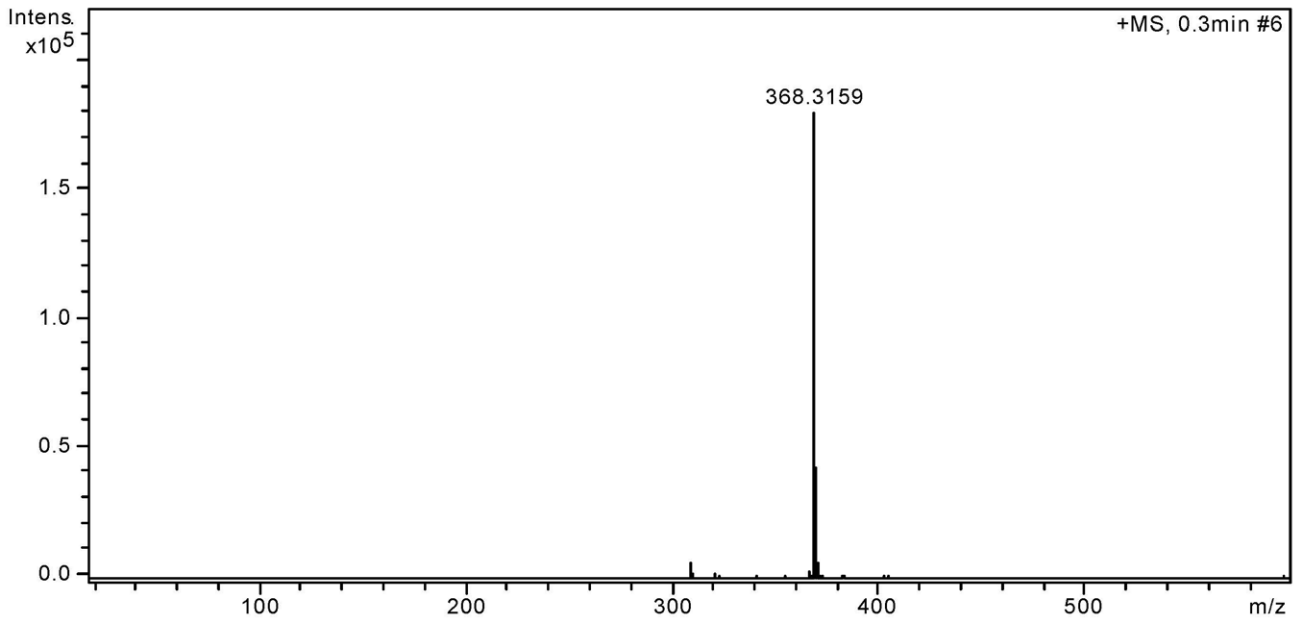

Figure S60. ESI-MS of **2** (HRMS *m/z* 368.3159 $[M + H]^+$ (calcd for $C_{20}H_{40}NO_2$: 368.3165)).

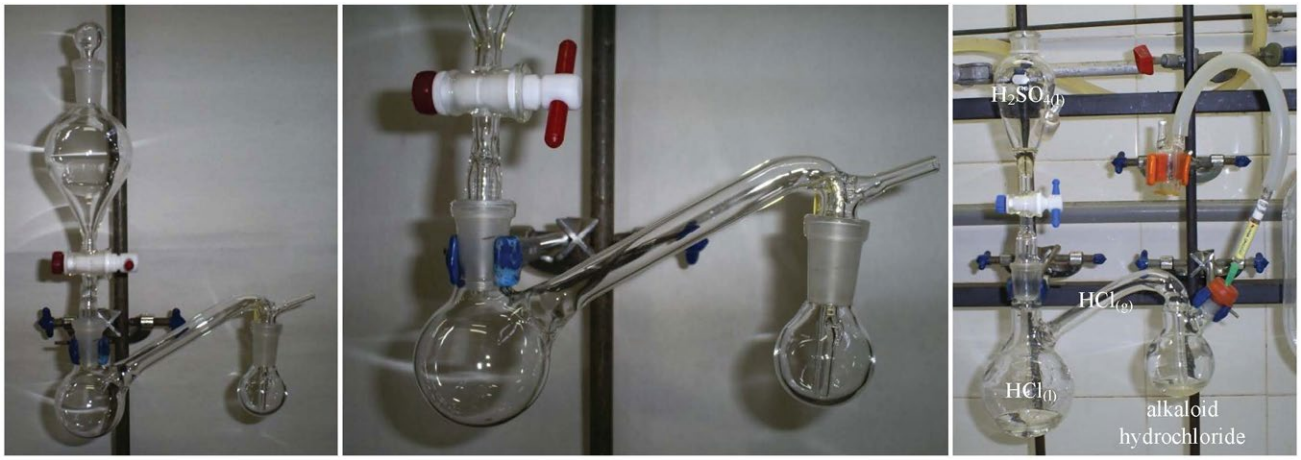

Figure S61. Modified Kipp's apparatus.

Table S1. Data to plot graph of the antimalarial activity of (–)-cassine (**1**) (IC_{50} 1.82 μM)

Concentration / M	Mean survival / %			Standard deviation
	Experiment 1	Experiment 2	Experiment 3	
0.000000001	107.2505	100.8985	105.1969	3.241435369
0.00000001	104.8964	98.56245	104.252	3.485808208
0.0000001	100.9416	94.51932	100.0787	3.485612878
0.000001	84.27496	78.34681	84.80315	3.584835498
0.00001	39.17138	39.1734	48.97638	5.660336356
0.0001	28.15442	39.3531	54.48819	13.21582905

Table S2. Data to plot graph of the antimalarial activity of (–)-spectaline (**2**) (IC_{50} 2.76 μM)

Concentration / M	Mean survival / %			Standard deviation
	Experiment 1	Experiment 2	Experiment 3	
0.000000001	99.81413	107.9892	95.7431	6.236613
0.00000001	102.881	97.12747	99.25317	2.909263
0.0000001	114.4052	95.51167	111.6505	10.20634
0.000001	89.68401	90.0359	86.78118	1.786218
0.00001	44.42379	43.62657	47.94623	2.298645
0.0001	40.61338	32.58528	48.17028	7.793687

Table S3. Data to plot graph of the antimalarial activity of (–)-3-*O*-acetylcassine (**3**) (IC_{50} 24.47 μM)

Concentration / M	Mean survival / %			Standard deviation
	Experiment 1	Experiment 2	Experiment 3	
1.28×10^{-09}	90.54	85.65	73.32	8.873832
6.40×10^{-09}	92.63	87.76	69.74	12.05812
3.20×10^{-08}	80.18	79.23	67.709	6.942163
1.60×10^{-07}	84	78.43	68.72	7.73291
0.0000008	84.81	78.43	66.92	9.066758
0.000004	82.63	72.11	57.623	12.55583
0.00002	73.54	58.17	38.85	17.38244
0.0001	45.818	38.31	21.18	12.62826

Table S4. Data to plot graph of the antimalarial activity of (–)-3-*O*-acetylspectaline (**4**) (IC_{50} 25.14 μM)

Concentration / M	Mean survival / %			Standard deviation
	Experiment 1	Experiment 2	Experiment 3	
1.28×10^{-09}	89.09	88.86	73.96	8.669677
6.40×10^{-09}	91.63	88.46	68.96	12.27618
3.20×10^{-08}	90.63	92.27	68.1	13.50604
1.60×10^{-07}	85.9	90.07	67.005	12.29095
0.0000008	80.18	81.54	62.392	10.68417
0.000004	88.36	74.52	58.092	15.15243
0.00002	69.45	54.56	38.15	15.65615
0.0001	40.27	38.61	12.9	15.34534

Table S5. Data to plot graph of the antimalarial activity of chloroquine (IC_{50} 0.30 μM)

Concentration / M	Mean survival / %			Standard deviation
	Experiment 1	Experiment 2	Experiment 3	
1.28×10^{-09}	94	84.18	95.75	6.23644931
6.40×10^{-09}	84.22274	84.27	94.96	6.1855623
3.20×10^{-08}	84.57076	80.45	88.67	4.110004695
1.60×10^{-07}	71.34571	67.63	87.88	10.78001041
0.0000008	48.14385	36.9	45.08	5.81268354
0.000004	46.98376	41.9	44.21	2.545403055
0.00002	29.00232	32.81	43.27	7.387790824
0.0001	17.74942	38.36	40.51	12.56624122