

Supporting Information

Total Synthesis and Biological Profiling of Putative (±)-Marinoaziridine B and (±)-N-Methyl Marinoaziridine A

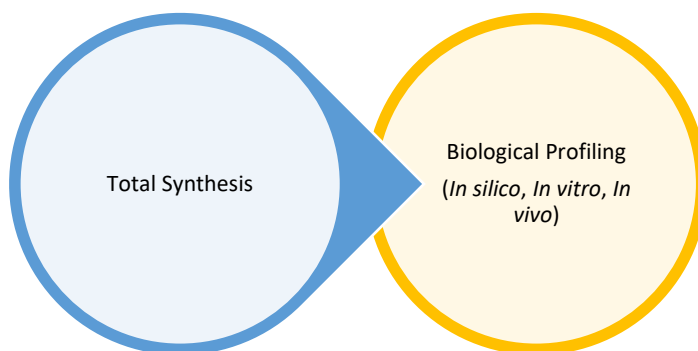
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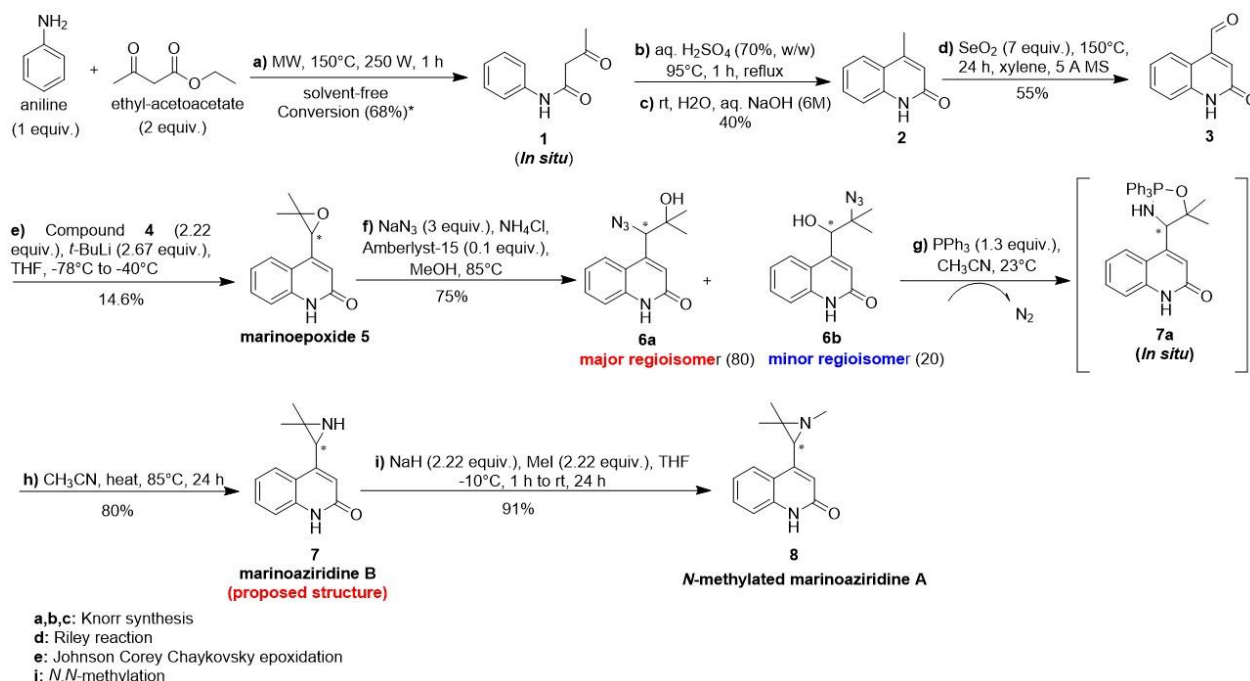
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1. Research Methodology



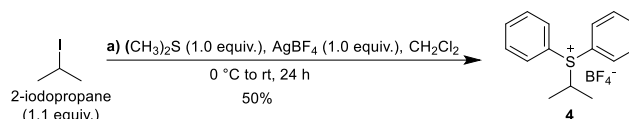
2. Summary of the total synthesis

The compounds **1-5** were synthesized to following our reported procedure [1].



Scheme S1. Total synthesis of the proposed structure of marinoaziridine **B** (**7**) and *N*-methyl marinoaziridine **A** (**8**). Reagents and conditions: **a**) microwave-assisted synthesis, 150 °C, 1 h, keeping the power constant at 250 W, solvent-free, conversion of the starting material determined by HPLC analysis of the crude reaction mixture (68%), compound **1** was used in the next step without purification; **b**) aq. H₂SO₄ (70%, w/w, 2 equiv.), 95 °C, 1 h, **c**) then H₂O, aq. NaOH (6M), 23 °C, pH = 7, 40% (2 steps); **d**) SeO₂ (7 equiv.), xylene, 5 Å, powder, activated, 150 °C, 24 h, 55%; **e**) compound **4** (2.22 equiv.), *t*-BuLi (2.67 equiv.), THF, -78 °C to -50 °C, then H₂O, aq. NH₄Cl, 14.6%; **f**) NaN₃ (3 equiv.), NH₄Cl (3 equiv.), Amberlyst®-15 (0.1 equiv.), MeOH, 85 °C, 24 h, 75% (**6a**:**6b** = 80:20, rr); **g**) PPh₃ (1.3 equiv.), CH₃CN, 23 °C; **h**) CH₃CN, heat, 85 °C, 24 h, 80%; **i**) i) NaH (2.22 equiv.), MeI (2.22 equiv.), THF, -10 °C, 1 h to rt, 24 h, 91%.

a. Synthesis of compound 4



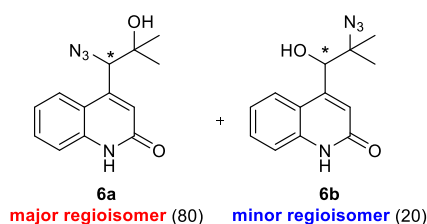
Scheme S2. Synthesis of compound **4**. Reagents and conditions: a) 2-iodopropane (1.1 equiv.), (CH₃)₂S (1.0 equiv.), AgBF₄ (1.0 equiv.), CH₂Cl₂, 0 °C to rt, 24 h, 50% [1].

3. Experimental Section

3.1. Experimental procedures

The compounds **1-5** were synthesized to following our reported procedure [1].

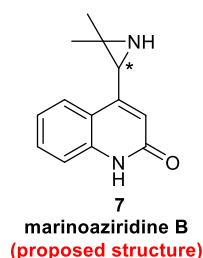
❖ Preparation of racemic quinolin-2(1*H*)-one azido alcohol **6a-b**:



A closed tube was charged with compound **5** (0.160 g, 0.7 mmol), sodium azide (0.136 g, 2.1 mmol), ammonium chloride (0.112 g, 2.1 mmol) and Amberlyst®-15 (0.031 g, 0.1 equiv.) in methanol (3 mL) and was stirred at 85 °C until HPLC indicated complete consumption of starting materials. The mixture was then diluted with water. The aqueous layer was extracted with ethyl acetate (3 x 15 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The regioisomeric side product was observed on the crude NMR (**6a:6b** = 80:20, *rr*). The crude product was purified *via* column chromatography (*flash* silica gel, dichloromethane/methanol = 25/1). Pure products **6a/6b** (0.144 g, 75%, 80:20, *rr*) were isolated as a light-yellow oils.

- **Data for 4-(1-azido-2-hydroxy-2-methylpropyl)quinolin-2(1*H*)-one **6a** (major regioisomer):** light yellow oil; ¹H NMR (CDCl₃, 600 MHz) 11.23 – 11.10 (1H, m, H-10), 7.87 (1H, d, *J*₁ = 8.4 Hz, H-5), 7.55 (1H, ddd, *J*₁ = 8.8 Hz, *J*₂ = 7.0 Hz, *J*₃ = 1.2 Hz, H-7), 7.38 (1H, dd, *J*₁ = 7.7 Hz, *J*₂ = 1.1 Hz, H-8), 7.28 (1H, ddd, *J*₁ = 8.8 Hz, *J*₂ = 7.3 Hz, *J*₃ = 1.5 Hz, H-6), 6.94 (1H, s, H-2), 5.10 (1H, s, H-12), 1.93 (1H, s, H-14), 1.31 (3H, s, H-15), 1.30 (3H, s, H-16); ¹³C (CDCl₃, 151 MHz) 138.08 (C, C-9), 130.99 (CH, C-7), 124.84 (CH, C-5), 122.77 (CH, C-6), 121.98 (CH, C-2), 119.25 (C, C-4), 116.71 (CH, C-8), 73.62 (C, C-13), 68.62 (CH, C-12), 27.03 (CH₃, C-15), 26.17 (CH₃, C-16); HRMS *m/z* 259.11968 (calculated for C₁₃H₁₄N₄O₂ [M+H]⁺ 259.11950).
- **Data for 4-(2-azido-1-hydroxy-2-methylpropyl)quinolin-2(1*H*)-one **6b** (minor regioisomer):** light yellow oil; ¹H NMR (CDCl₃, 600 MHz) 10.98 – 11.08 (1H, m, H-10), 7.83 (1H, d, *J*₁ = 8.1 Hz, H-4), 7.52 (1H, br t, *J*₁ = 8.1 Hz, H-7), 7.34 (1H, d, *J*₁ = 8.1 Hz, H-8), 7.25 (1H, t, *J*₁ = 7.3 Hz, H-6), 6.99 (1H, s, H-2), 5.19 (1H, d, *J*₁ = 2.9 Hz, H-12), 2.57 (1H, br d, *J*₁ = 3.7 Hz, H-14), 1.35 – 1.40 (6H, m, H-16, H-15); ¹³C (CDCl₃, 75 MHz) 131.25 (CH, C-7), 125.07 (CH, C-5), 122.54 (CH, C-6), 121.04 (CH, C-2), 120.60 (C, C-4), 116.56 (CH, C-8), 74.64 (CH, C-12), 65.01 (C, C-13), 22.49 (CH₃, C-16), 22.33 (CH₃, C-15); HRMS *m/z* 259,11968 (calculated for C₁₃H₁₄N₄O₂ [M+H]⁺ 259,11950).

❖ Preparation of racemic 4-(3,3-dimethylaziridin-2-yl)quinolin-2(1*H*)-one, compound **7**:

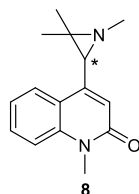


A mixture of azido alcohols **6a/6b** (0.091 g, 0.33 mmol) and triphenylphosphine (0.102 g, 0.40 mmol) in dry acetonitrile (5 mL) was stirred at room temperature for 1h, and then the resulting mixture was stirred 24 h at 85°C, until TLC indicated complete consumption of starting materials. The mixture was then diluted with water. The aqueous layer was extracted with dichloromethane (3 x 15 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified *via* column chromatography (*flash* silica gel, dichloromethane/methanol = 25/1). Pure product **7** (0.051 g, 80%) was isolated as yellow oil.

- **Data for racemic 4-(3,3-dimethylaziridin-2-yl)quinolin-2(1*H*)-one, compound **7**:** yellow oil; ¹H NMR (CDCl₃, 600 MHz) 12.14 (NH, br s, H-10), 7.71 (1H, br d, *J*₁ = 7.7 Hz, H-5), 7.52 (1H, m, H-7), 7.50 (1H, m, H-8), 7.25 (1H, t, *J*₁ = 7.0 Hz, H-6),

6.81 (1H, s, H-2), 3.25 (1H, br s, H-12), 1.64 (3H, s, H-16), 0.98 (3H, s, H-15); ^{13}C (CDCl_3 , 151 MHz) 164.16 (C, C-1), 149.03 (C, C-3), 138.32 (C, C-9), 130.63 (CH, C-7), 123.83 (CH, C-5), 122.70 (CH, C-6), 119.79 (C, C-4), 119.58 (CH, C-2), 116.74 (CH, C-8), 43.03 (CH, C-12), 39.67 (C, C-14), 26.75 (CH_3 , C-16), 19.16 (CH_3 , C-15); HRMS m/z 215.11857 (calculated for $\text{C}_{13}\text{H}_{14}\text{N}_2\text{O}$ $[\text{M}+\text{H}]^+$ 215.11844).

❖ Preparation of racemic 1-methyl-4-(1,3,3-trimethylaziridin-2-yl)quinolin-2(1H)-one, compound 8:



N-methylated marinoaziridine A

To a flame dried Schlenk flask was added compound **7** (0.021 g, 0.09 mmol) which was stirred under vacuum for 0.5 h, and then suspended in dry THF (5 mL) under an atmosphere of argon. The mixture was cooled to -10 and then was added NaH (0.003 g, 0.20 mmol). The reaction mixture was stirred for 0.5 h before the addition of the methyl iodide (6 μL , 0.20 mmol). After the addition was complete, the solution was heated to room temperature until TLC indicated complete consumption of starting materials. The reaction was quenched by the addition of water (10 mL). The aqueous layer was extracted with dichloromethane (3 x 30 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous Na_2SO_4 and filtered. The solvent was removed in *vacuo* to give a yellow oil which was purified *via* column chromatography (*flash* silica gel, dichloromethane/methanol = 25/1). Pure product **8** (0.021, 91%) was isolated as a white solid.

- **Data for racemic racemic 1-methyl-4-(1,3,3-trimethylaziridin-2-yl)quinolin-2(1H)-one, compound 8:** white solid; mp 135–136 °C; ^1H NMR (CDCl_3 , 600 MHz) 7.70 (1H, dd, $J_1 = 7.9$ Hz, $J_2 = 1.3$ Hz, H-5), 7.58 (1H, ddd, $J_1 = 8.5$ Hz, $J_2 = 7.2$ Hz, $J_3 = 1.5$ Hz, H-7), 7.39 (1H, d, $J_1 = 8.4$ Hz, H-8), 7.25 (1H, ddd, $J_1 = 8.1$ Hz, $J_2 = 7.0$ Hz, $J_3 = 1.2$ Hz, H-6), 6.85 (1H, d, $J_1 = 1.1$ Hz, H-2), 3.72 (3H, s, H-11), 2.61 (3H, s, -13H), 2.44 (1H, s, H-12), 1.51 (3H, s, H-15), 0.90 (3H, s, H-16); ^{13}C (CDCl_3 , 151 MHz) 162.34 (C, C-1), 146.93 (C, C-3), 139.88 (C, C-9), 130.34 (CH, C-7), 124.56 (CH, C-5), 121.86 (CH, C-6), 120.76 (C, C-4), 119.91 (C, C-2), 114.53 (CH, C-8), 51.27 (CH, C-12), 43.09 (C, C-14), 39.47 (NCH_3 , C-13), 29.25 (NCH_3 , C11), 21.35 (CH_3 , C-16), 17.34 (CH_3 , C-15); HRMS m/z 243.14974 (calculated for $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}$ $[\text{M}+\text{H}]^+$ 243,14974).

3.2. NMR analysis of synthetic intermediates and final products

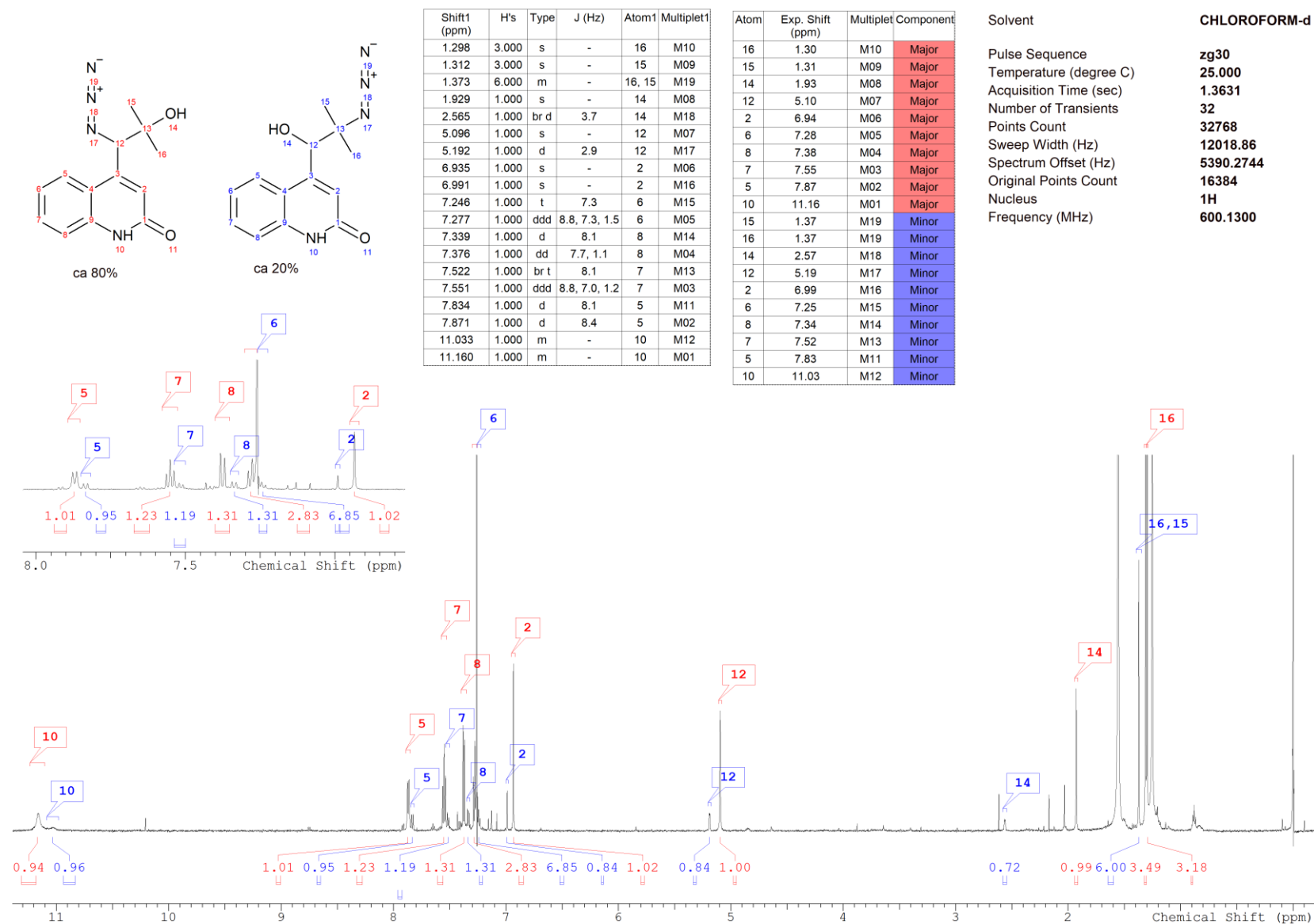
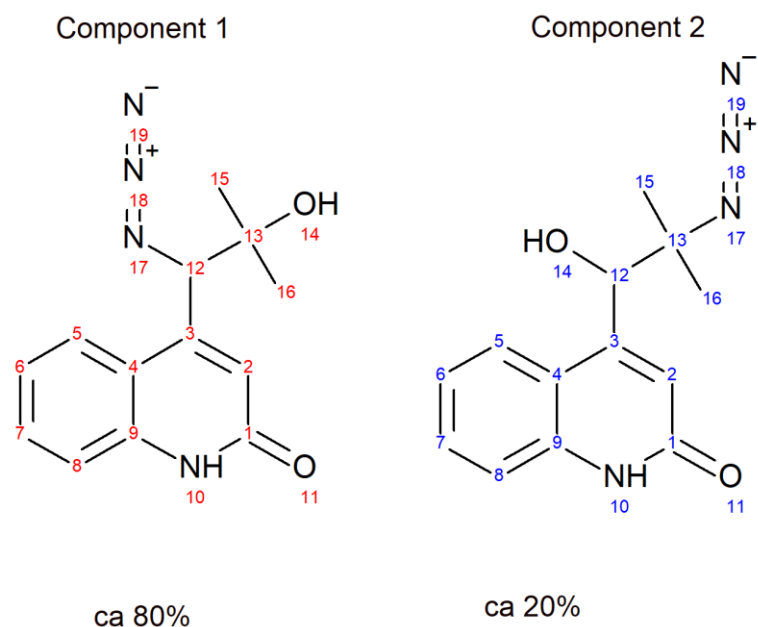


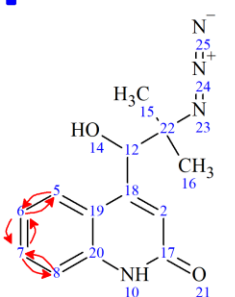
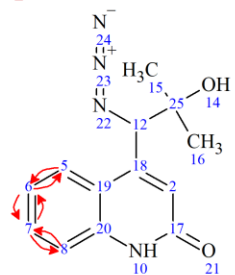
Figure S1. Structure, numbering and full assignment of ¹H NMR spectrum for **6a,b** in CDCl₃ at 25 °C.



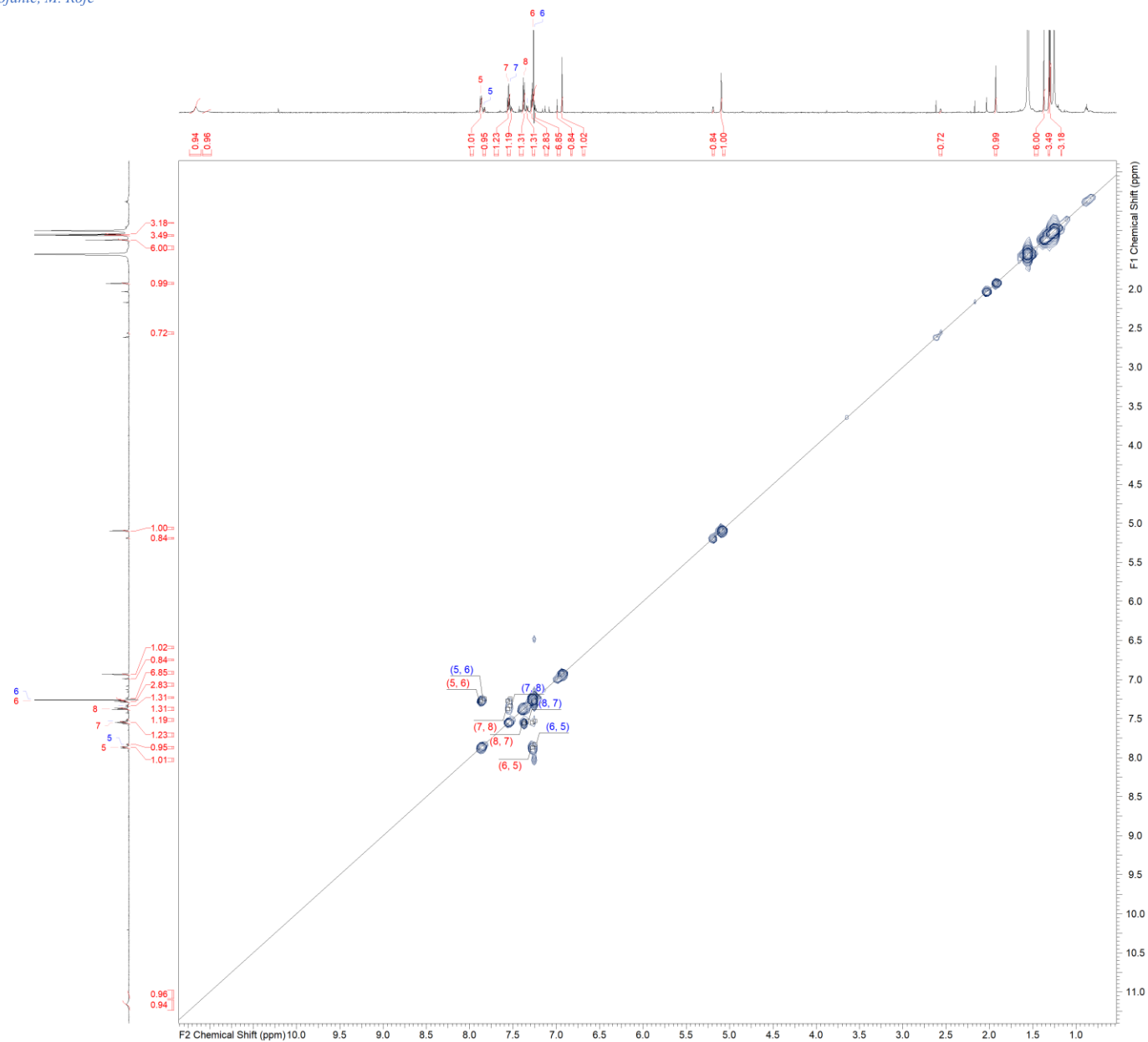
^{13}C NMR (151 MHz, CHLOROFORM-d): δ = 22.3 (15), 23.4 (16), 26.2 (16), 27.0 (15), 65.0 (13), 68.6 (12), 73.6 (13), 74.1 (12), 116.6 (8), 116.7 (8), 119.2 (4), 120.6 (4), 121.0 (2), 122.0 (2), 122.5 (6), 122.8 (6), 124.8 (5), 125.1 (5), 130.6 (7), 131.3 (7), 138.1 (9), 147.6 (3) ppm

Atom	Exp. Shift (ppm)	Component
16	26.2	Component 1
15	27.0	Component 1
12	68.6	Component 1
13	73.6	Component 1
8	116.7	Component 1
4	119.2	Component 1
2	122.0	Component 1
6	122.8	Component 1
5	124.8	Component 1
7	130.6	Component 1
9	138.1	Component 1
1	-	Component 2
9	-	Component 2
3	-	Component 2
15	22.3	Component 2
16	23.4	Component 2
13	65.0	Component 2
12	74.1	Component 2
8	116.6	Component 2
4	120.6	Component 2
2	121.0	Component 2
6	122.5	Component 2
5	125.1	Component 2
7	131.3	Component 2

Figure S2. Structure, numbering and full assignment of ^{13}C chemical shifts extracted from ^1H - ^{13}C HSQCe and ^1H - ^{13}C HMBC NMR spectra for **6a,b** in CDCl_3 at 25 °C.

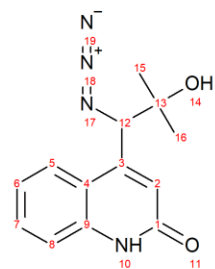


Acquisition Time (sec)	(0.1065, 0.0267)
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Frequency (MHz)	(600.1300, 600.1300)
Nucleus	(1H, 1H)
Points Count	(2048, 2048)
Pulse Sequence	cosygppg
Solvent	CHLOROFORM-d
Sweep Width (Hz)	(9610.69, 9597.45)
Number of Transients	4
Spectrum Type	COSY
Temperature (degree C)	25.000

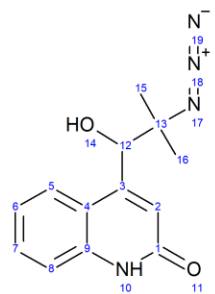


S7

Component 1 ca 80%



Component 2 ca 20%



Acquisition Time (sec)	(0.1139, 0.0094)
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Nucleus	(1H, 13C)
Origin	spect
Original Points Count	(1024, 256)
Points Count	(1024, 1024)
Pulse Sequence	hsqcetgpgpsisp.2
Solvent	CHLOROFORM-d
Sweep Width (Hz)	(8984.02, 27138.29)

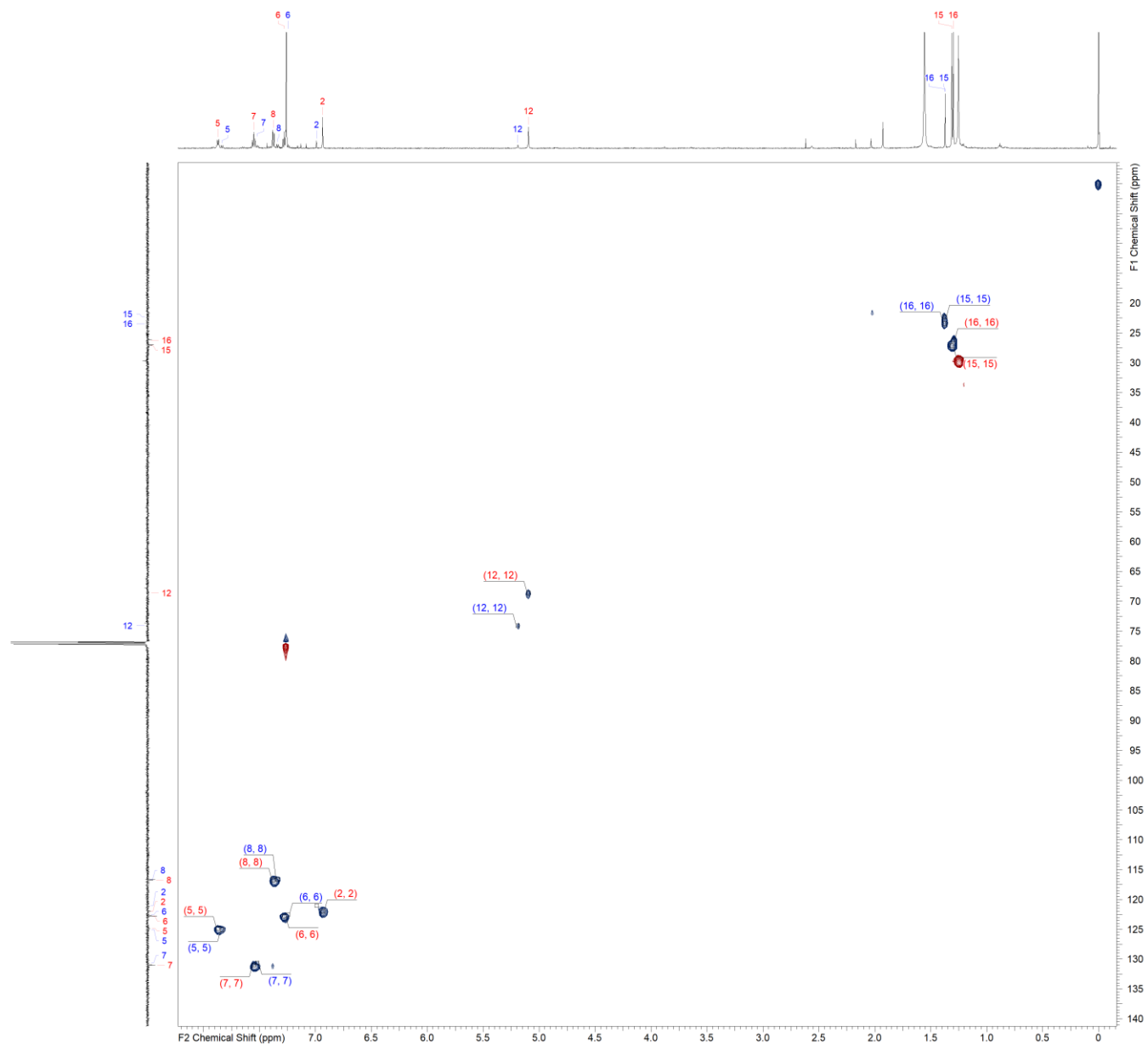


Figure S4. Fully assigned ^1H - ^{13}C HSQC NMR spectrum of **6a,b** in CDCl_3 at 25°C .

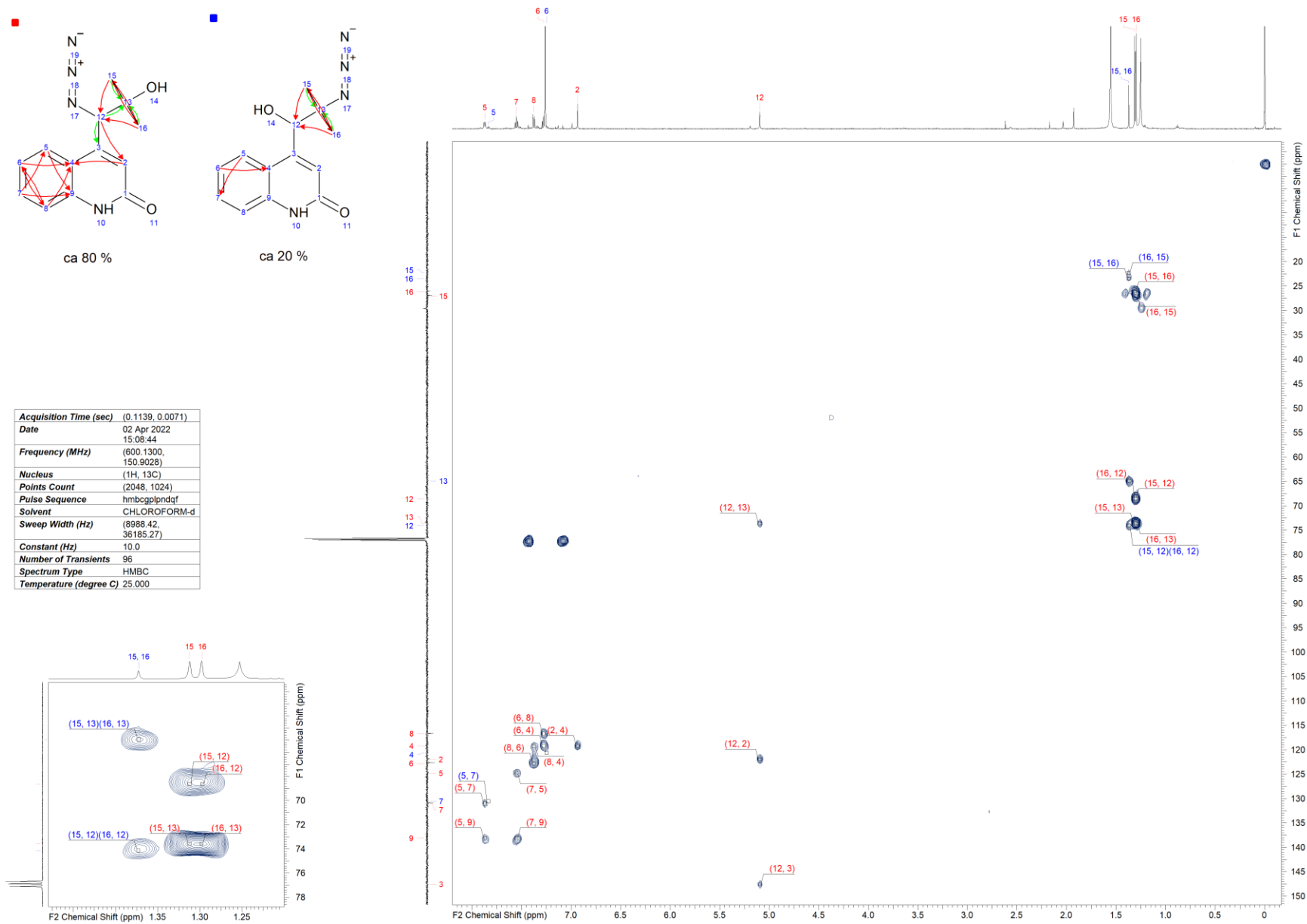


Figure S5. Fully assigned ^1H - ^{13}C HMBC NMR spectrum of **6a,b** in CDCl_3 at 25 $^\circ\text{C}$.

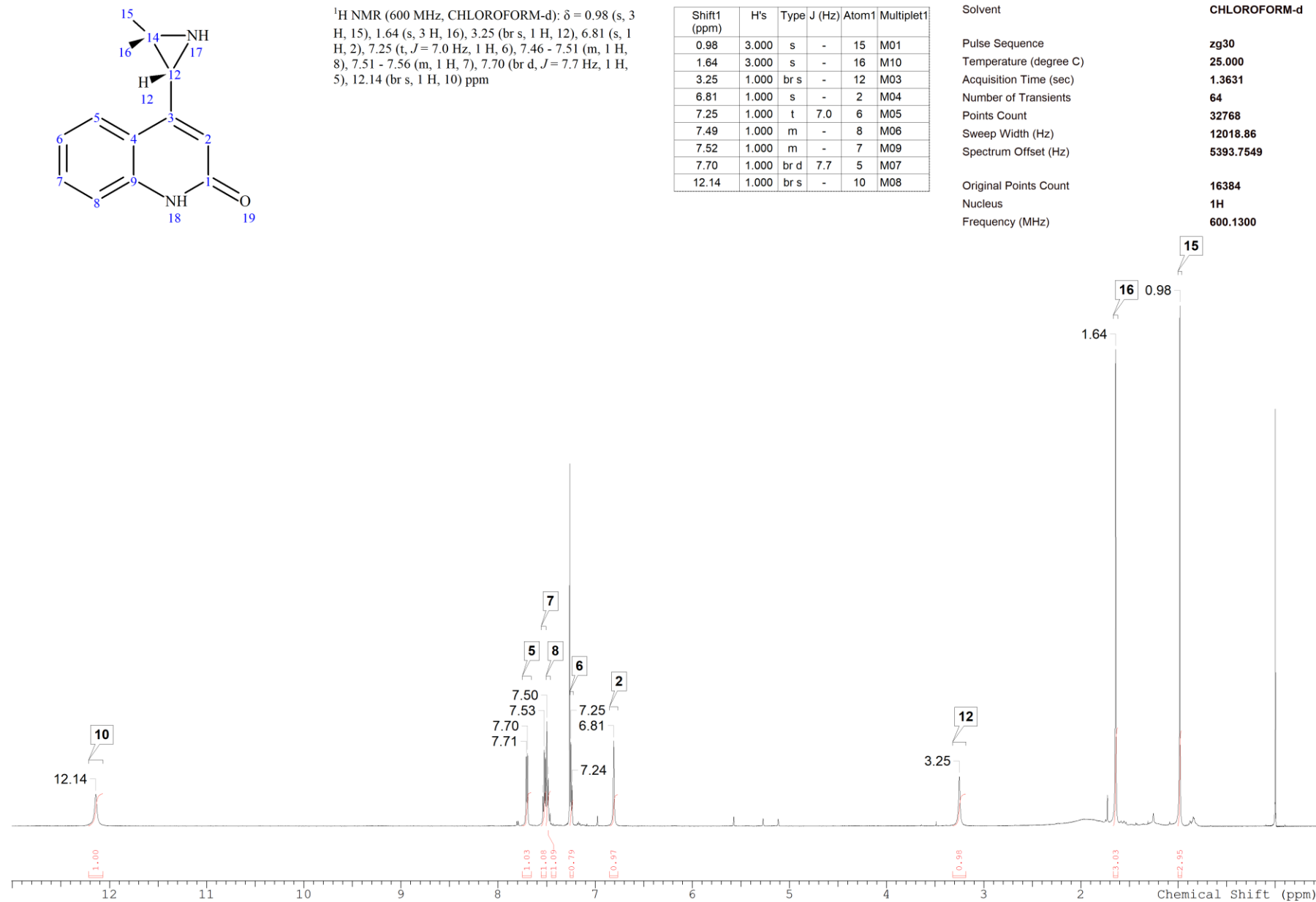


Figure S6. Structure, numbering and full assignment of ¹H NMR spectrum for compound **7** in CDCl₃ at 25 °C.

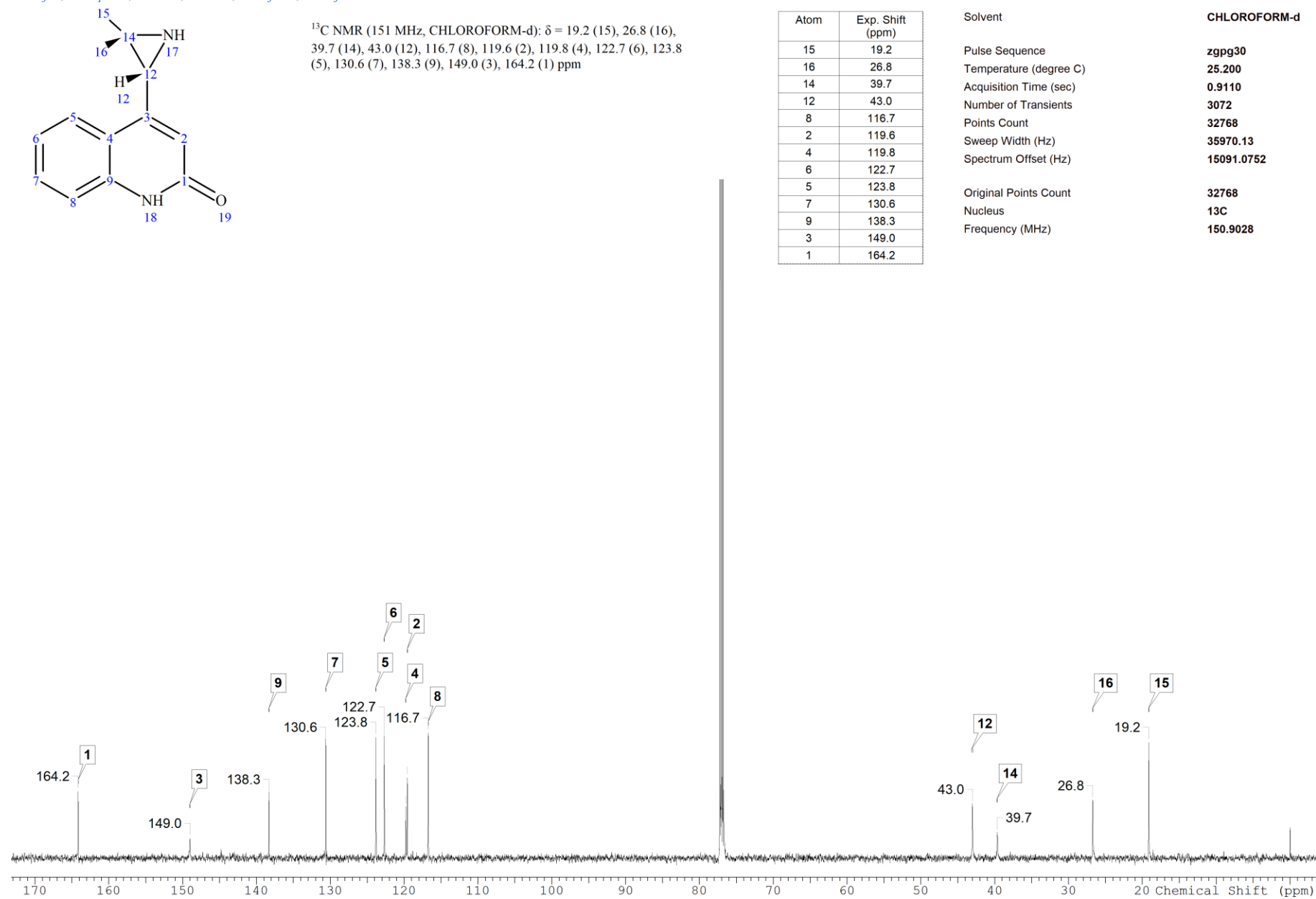


Figure S7. Structure, numbering and full assignment of ¹³C NMR spectrum for compound **7** in CDCl₃ at 25 °C.

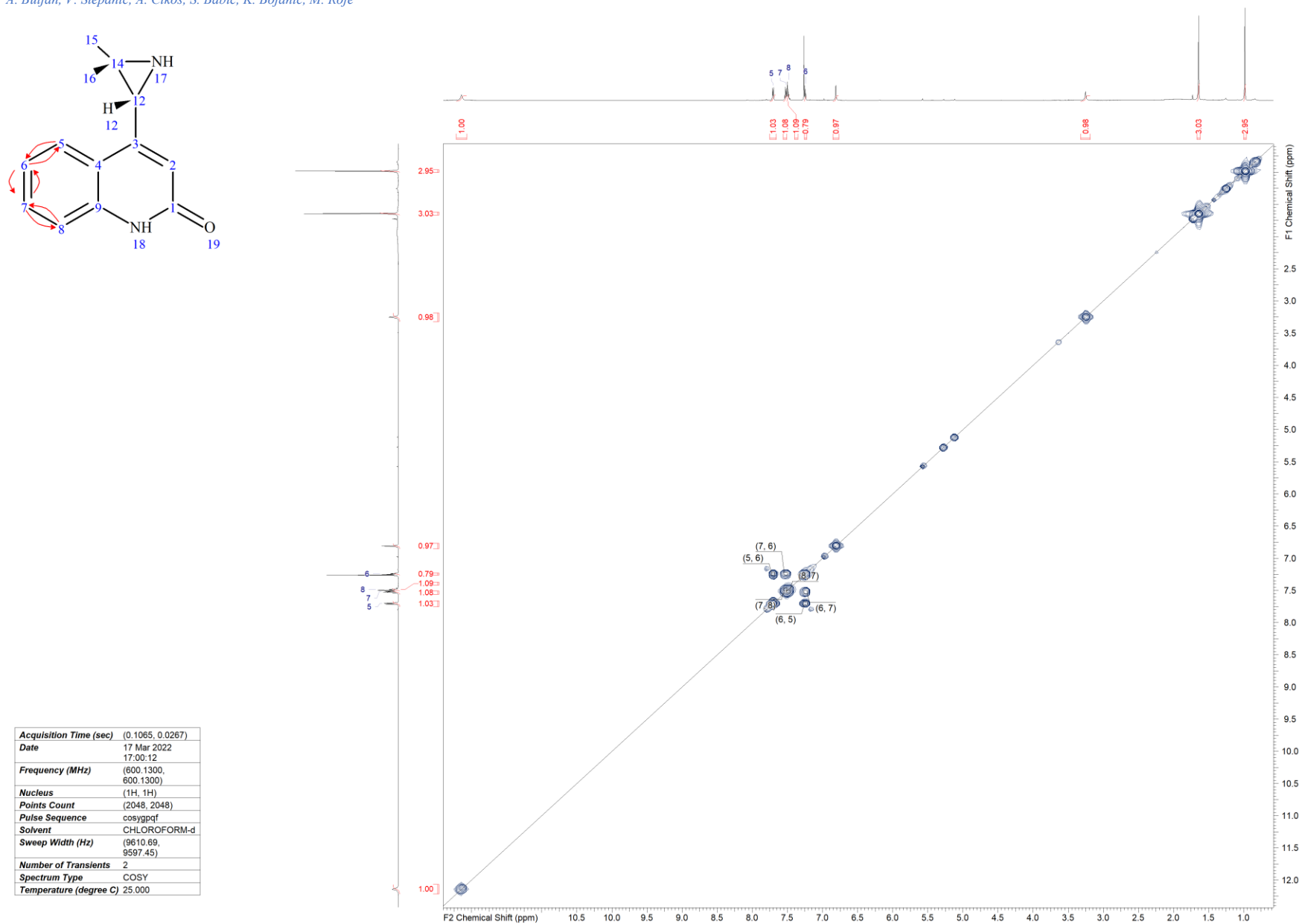


Figure S8. Fully assigned ¹H-¹H COSY NMR spectrum of compound **7** in CDCl₃ at 25 °C.

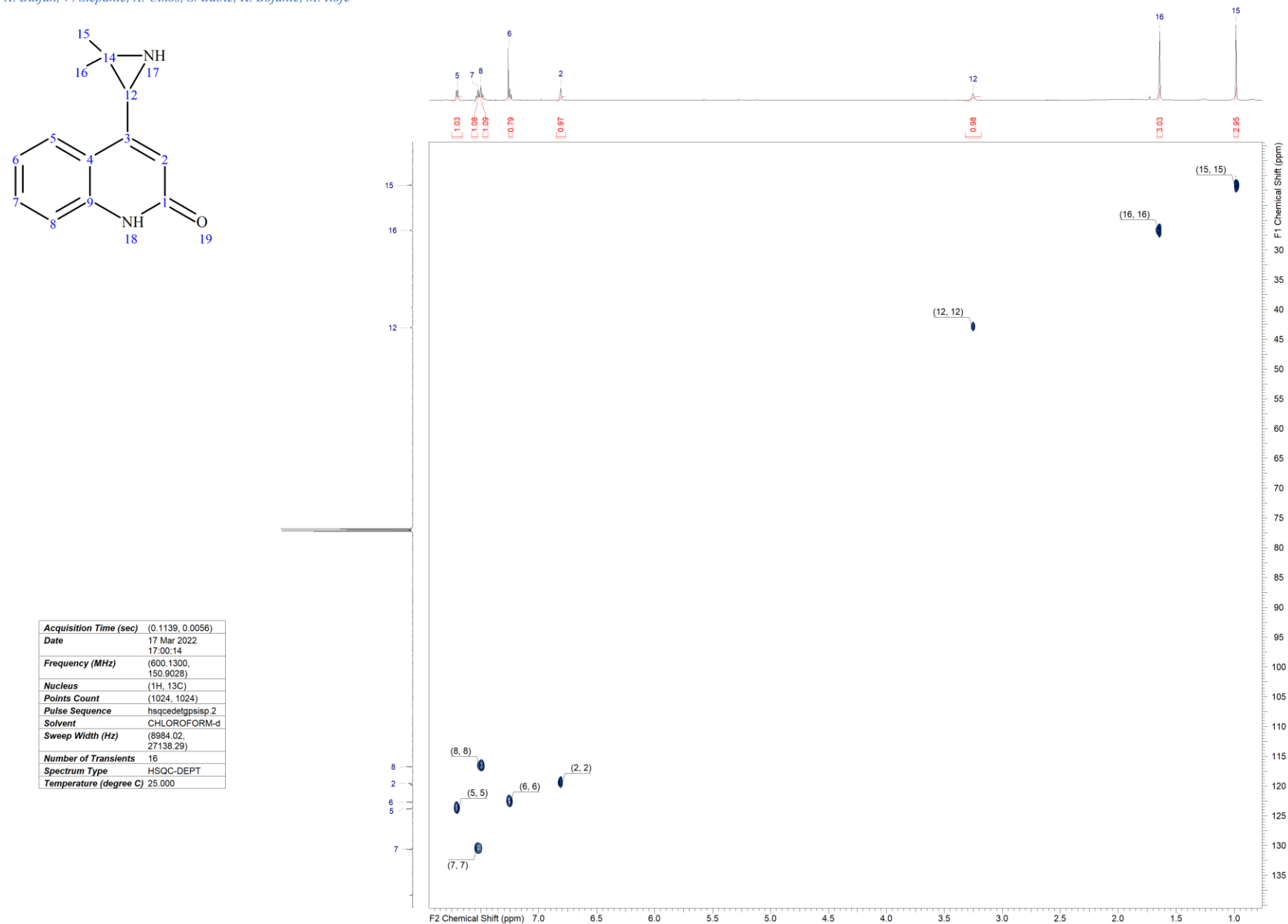


Figure S9. Fully assigned ¹H-¹³C HSQC NMR spectrum of compound **7** in CDCl₃ at 25 °C.

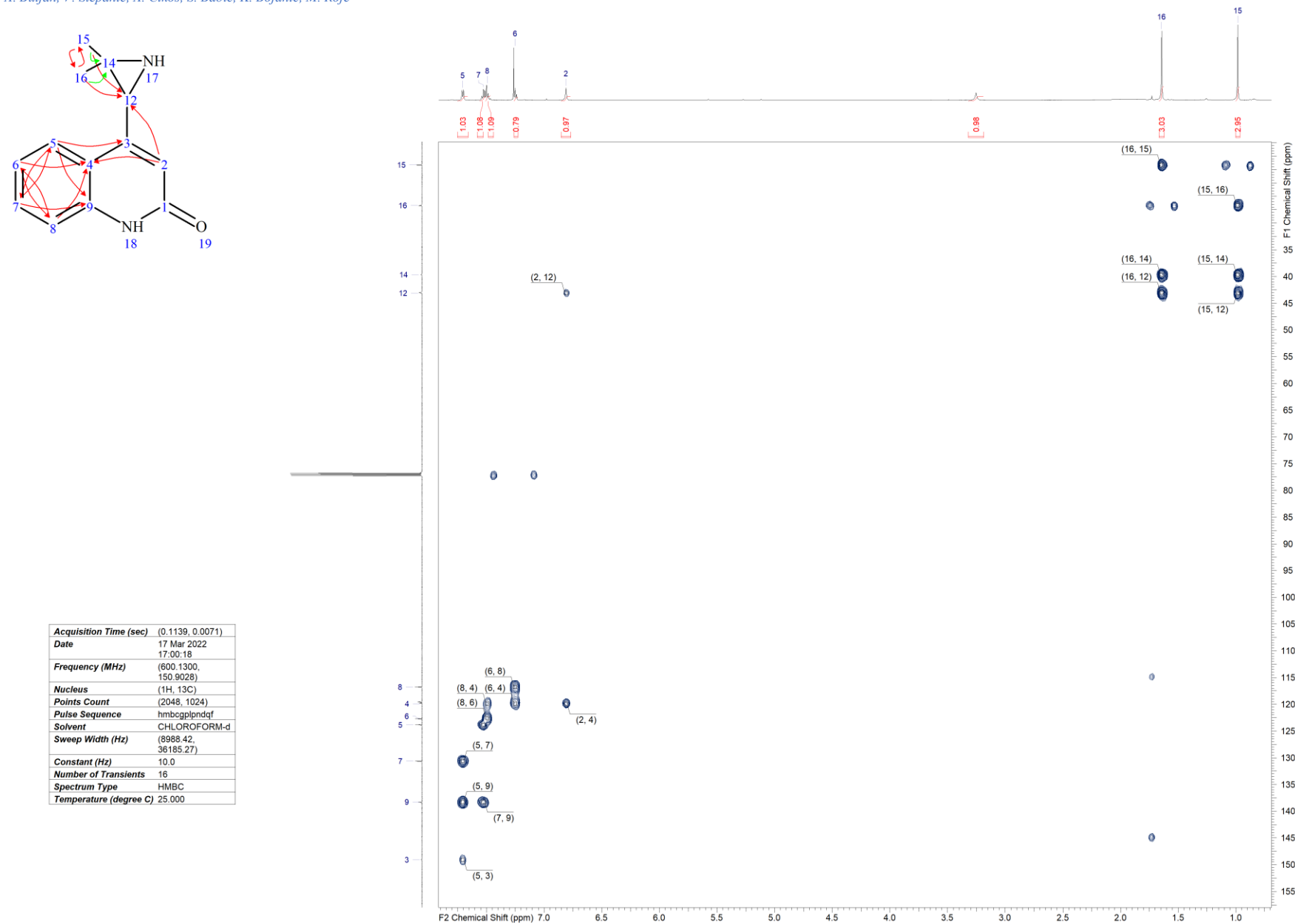


Figure S10. Fully assigned ¹H-¹³C HMBC NMR spectrum of compound **7** in CDCl₃ at 25 °C.

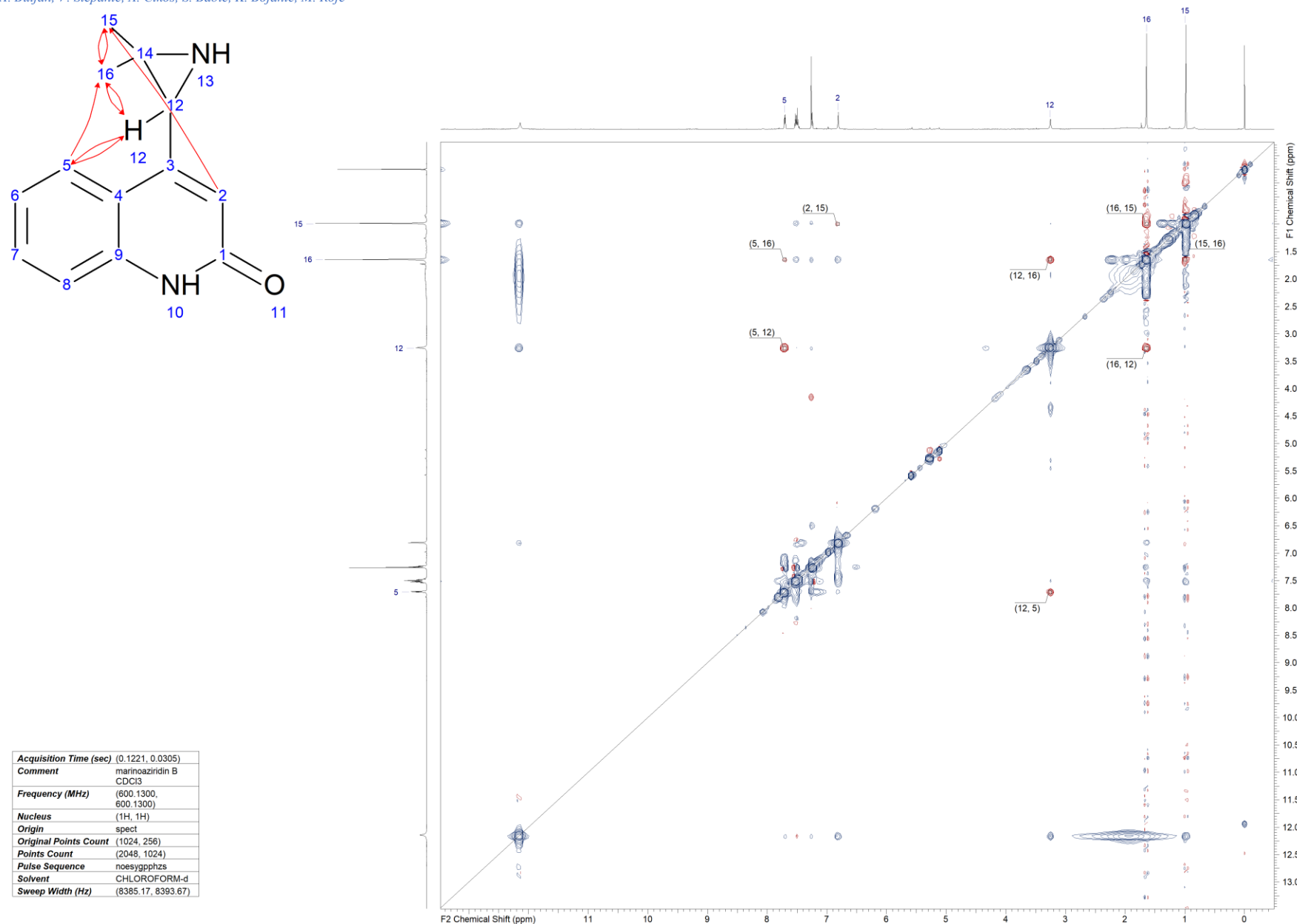


Figure S11. Fully assigned ¹H-¹H NOESY NMR spectrum of compound 7 in CDCl₃ at 25 °C.

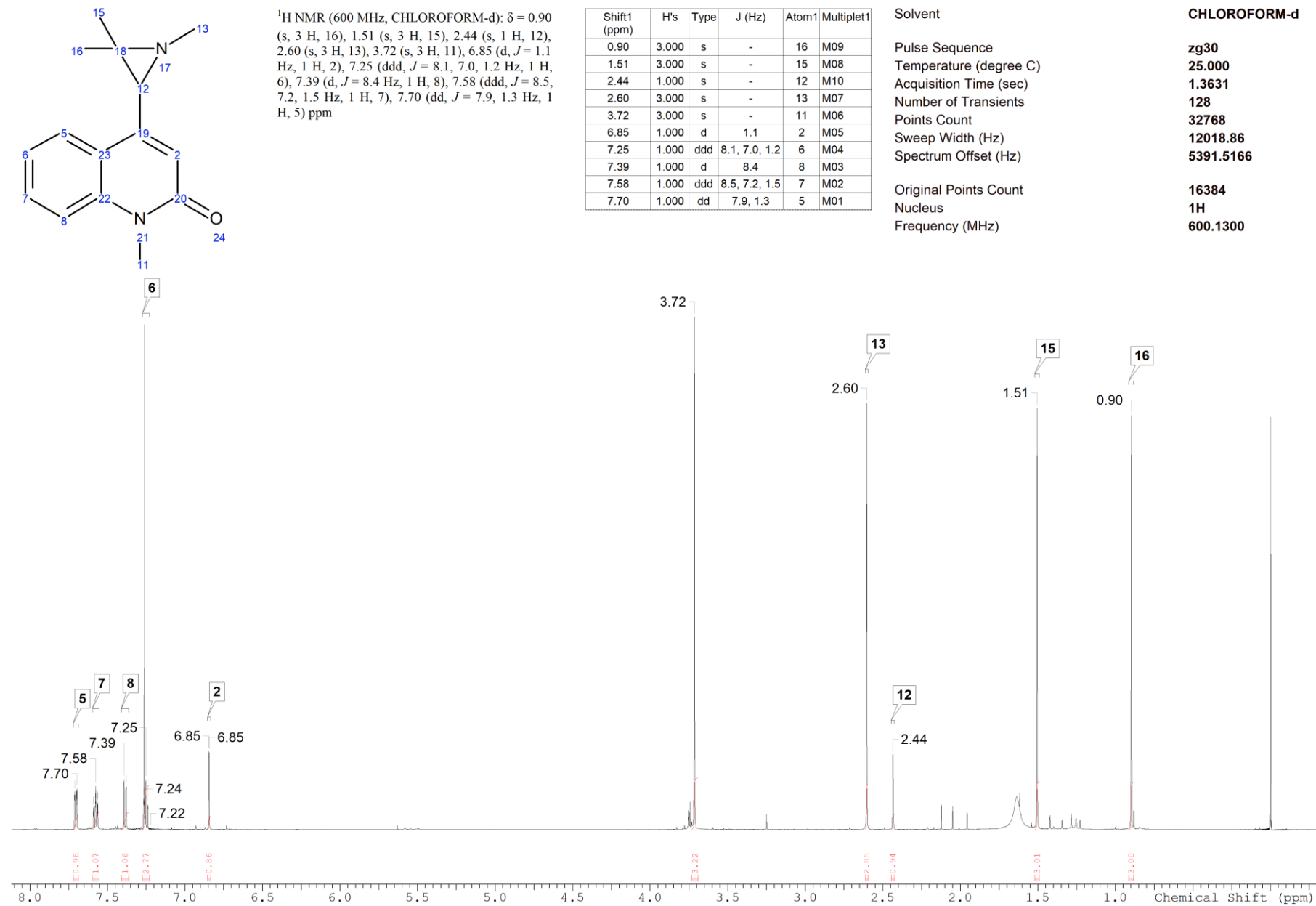


Figure S12. Structure, numbering and full assignment of ¹H NMR spectrum for compound **8** in CDCl₃ at 25 °C.

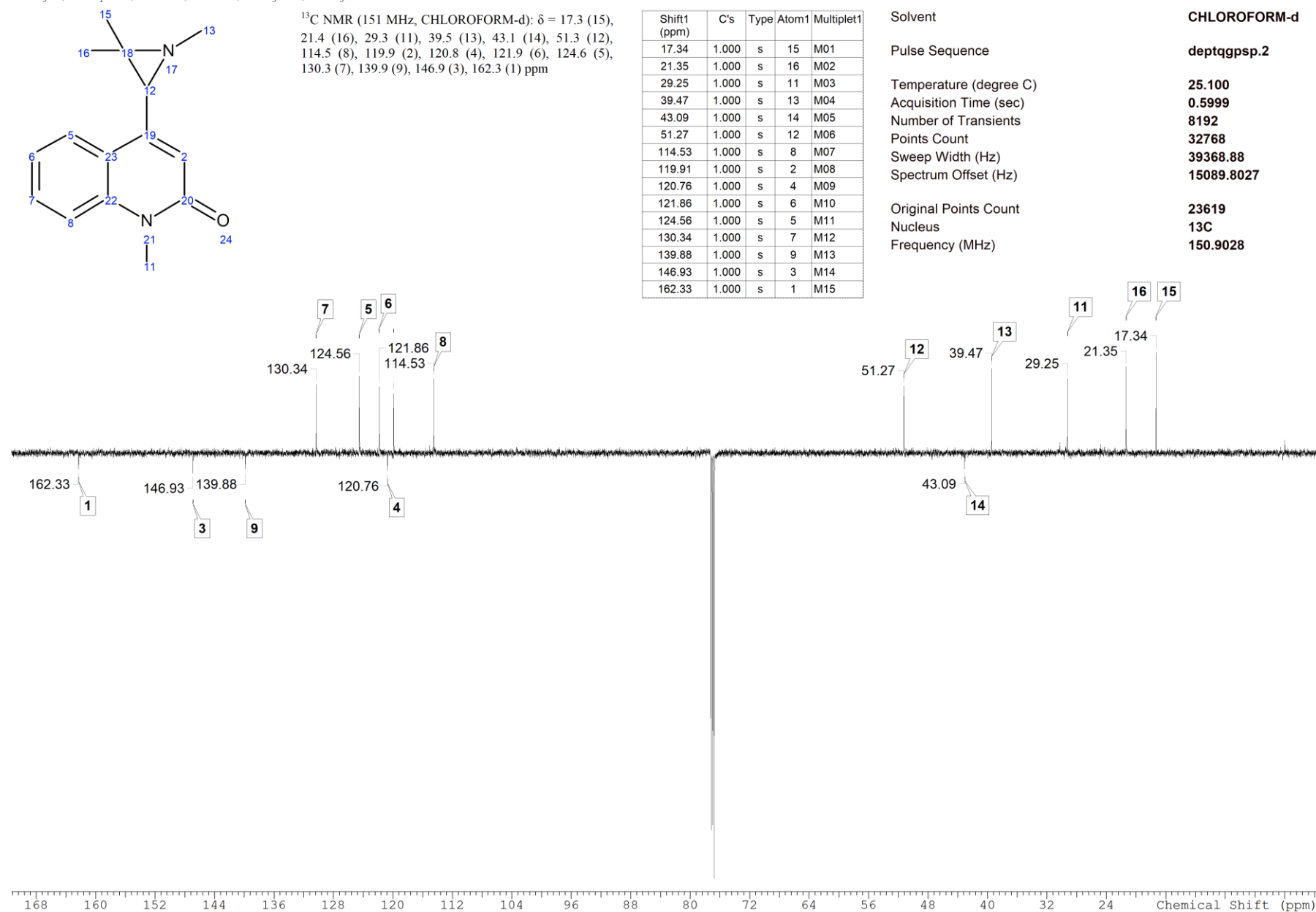


Figure S13. Structure, numbering and full assignment of ¹³C NMR spectrum for compound **8** in CDCl₃ at 25 °C.

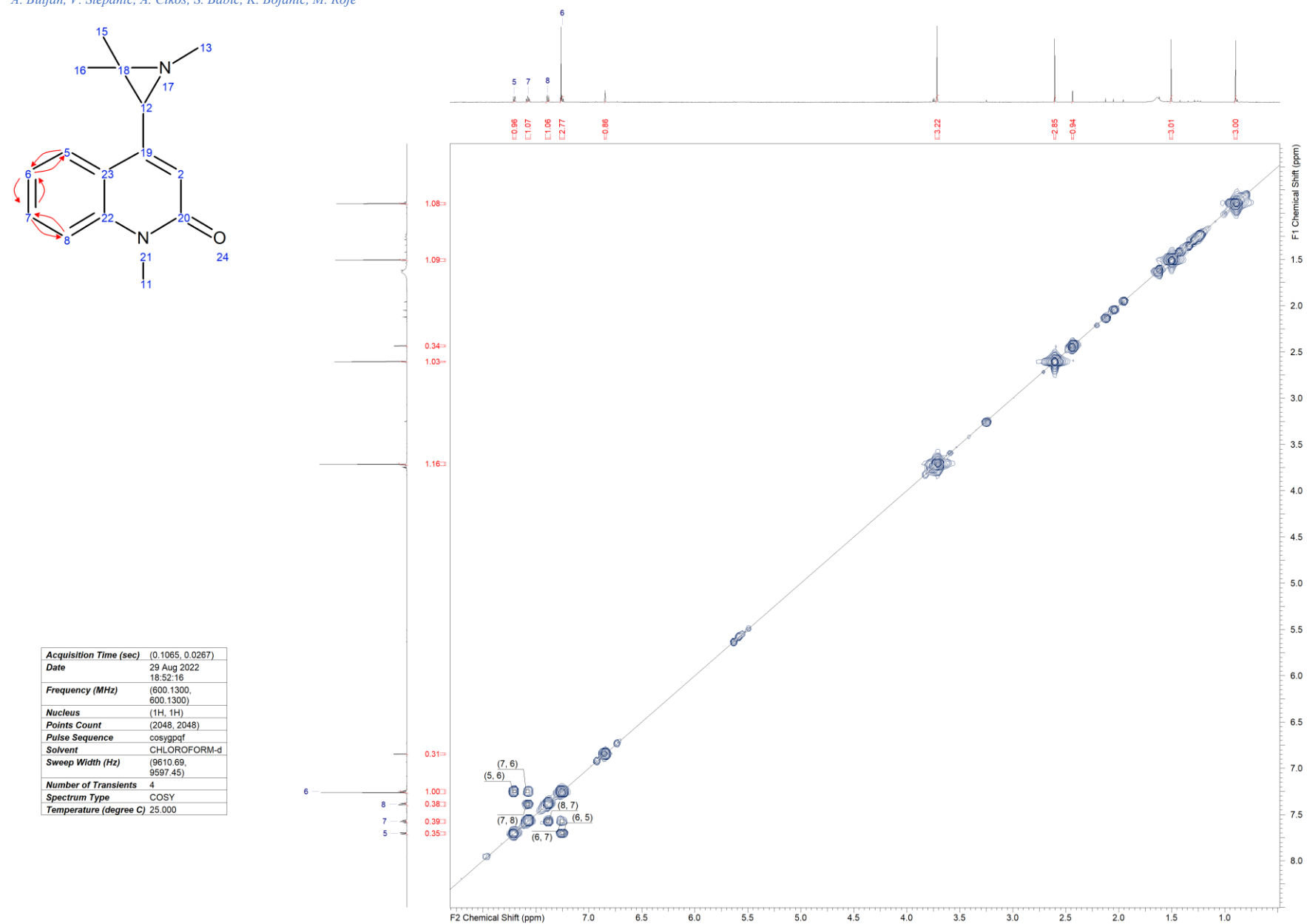


Figure S14. Fully assigned ^1H - ^1H COSY NMR spectrum of compound **8** in CDCl_3 at 25 °C.

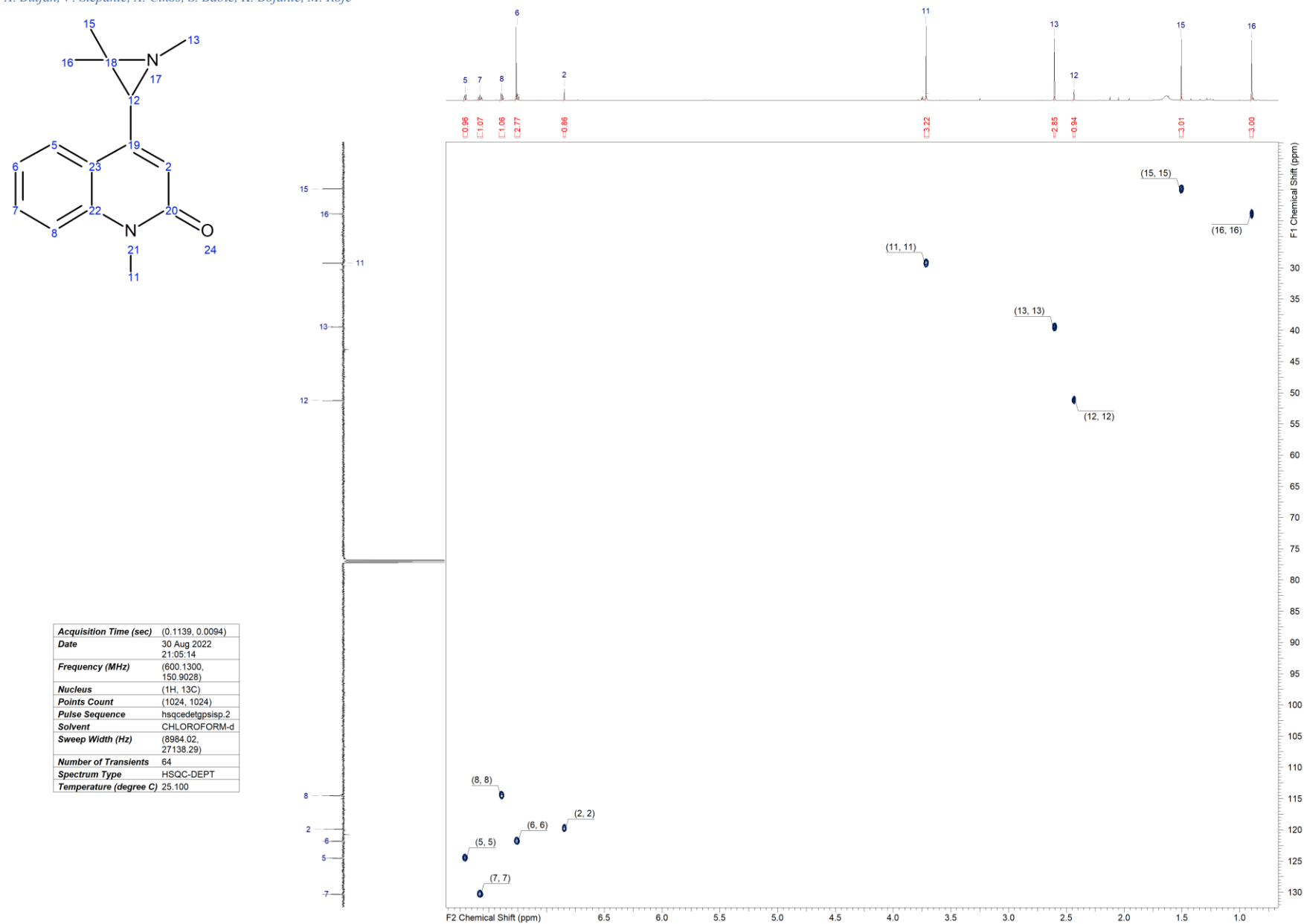


Figure S15. Fully assigned ¹H-¹³C HSQC NMR spectrum of compound **8** in CDCl₃ at 25 °C.

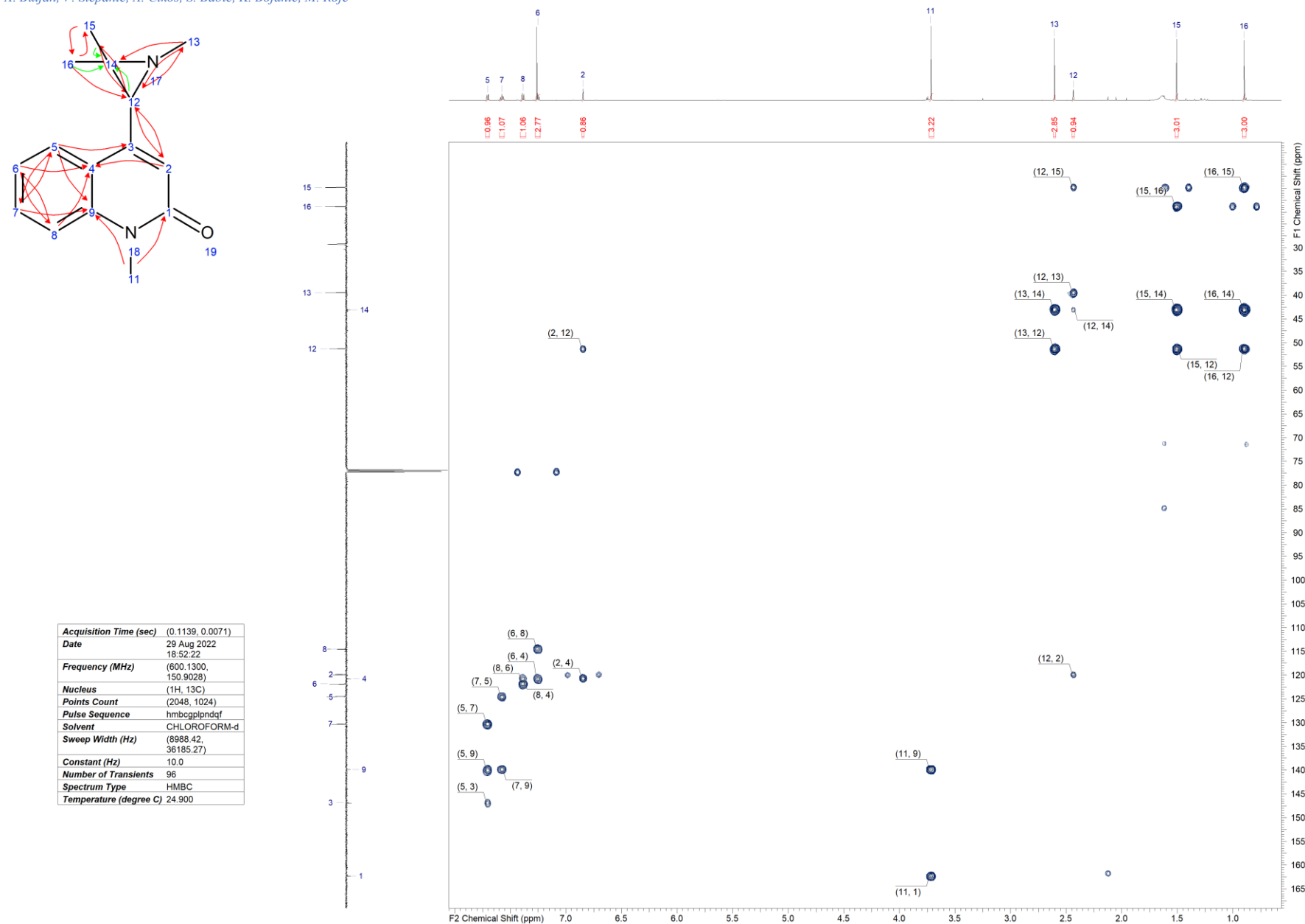


Figure S16. Fully assigned ^1H - ^{13}C HMBC NMR spectrum of compound **8** in CDCl_3 at 25 °C.

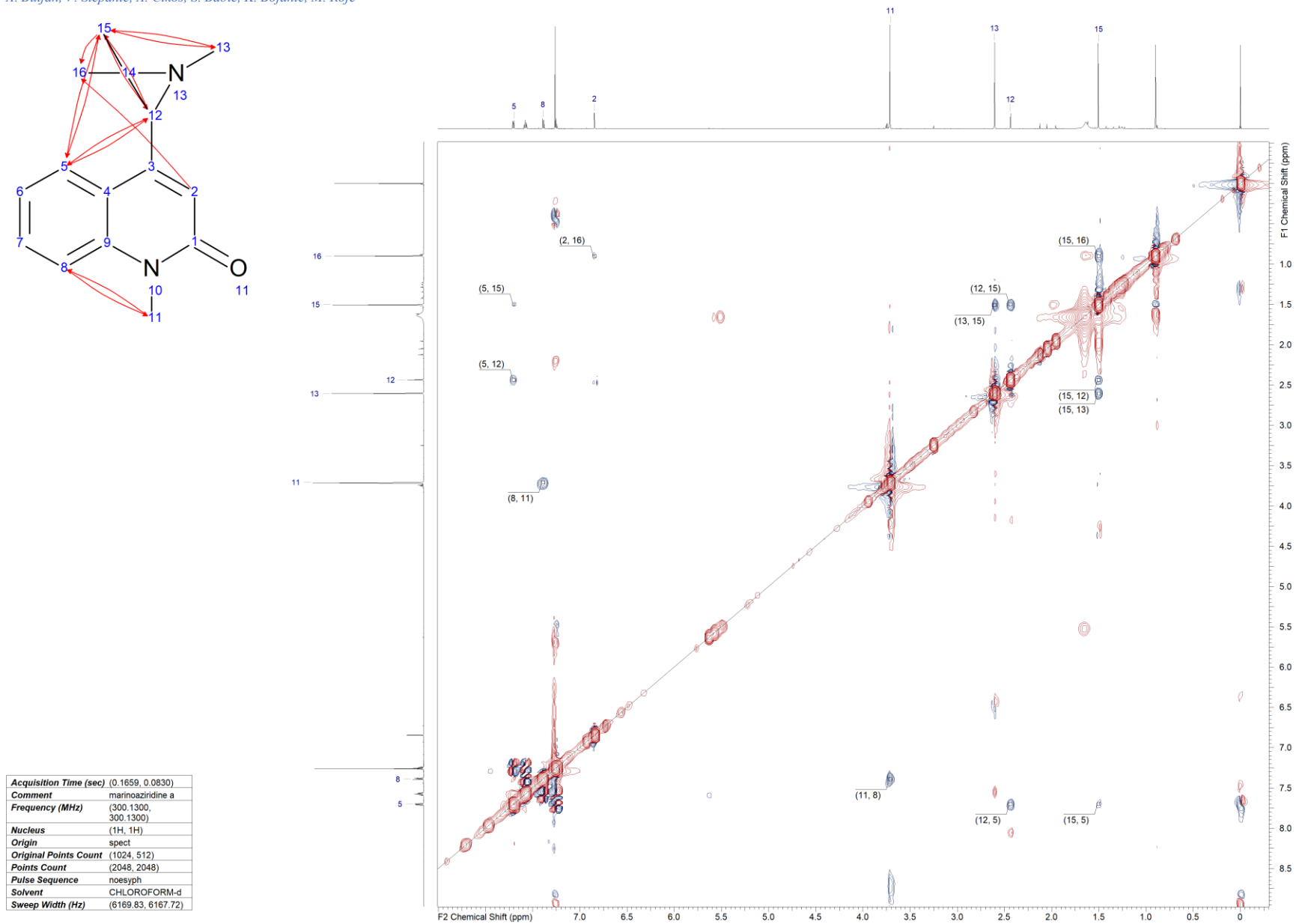


Figure S17. Fully assigned ¹H-¹H NOESY NMR spectrum of compound 8 in CDCl₃ at 25 °C.

3.3. Comparison of synthetic and isolated marinoaziridine B with literature data

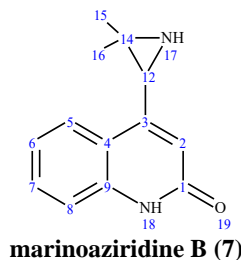
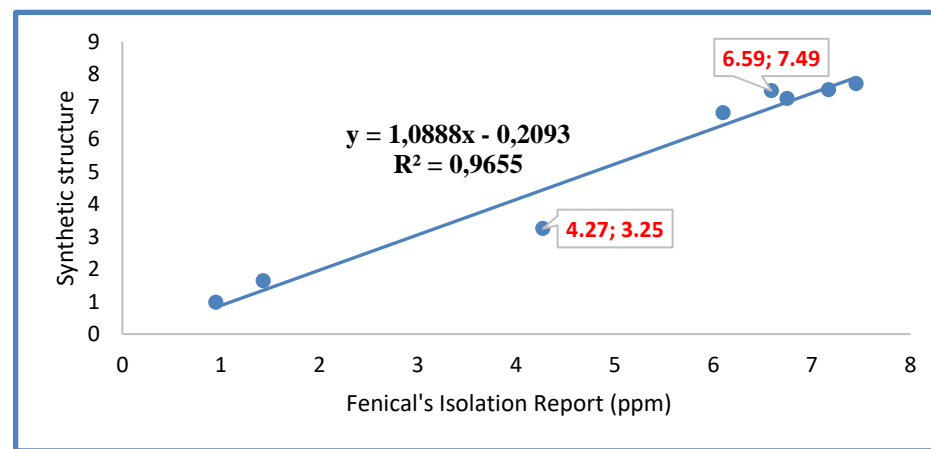


Table S1. Comparison of ^1H NMR chemical shifts^a of synthetic structure and literature data (Fenical's isolation report) [2]

position	Fenical's isolation report (ppm)[2]	Synthetic structure (ppm)	$\Delta\delta^b$
15	0.95	0.98	0.03
16	1.43	1.64	0.21
12	4.27	3.25	-1.02
2	6.10	6.81	0.71
6	6.75	7.25	0.50
8	6.59	7.49	0.90
7	7.17	7.52	0.35
5	7.45	7.71	0.26



^aNMR spectra were recorded in CDCl_3 at 25°C , ^b $\Delta\delta_1 = \delta_{(\text{synthetic structure})} - \delta_{(\text{Fenical's Isolation Report})}$

Figure S18. Linear correlations of ^1H NMR chemical shifts (CDCl_3) for Fenical's isolation report and synthetic structure.

Table S2. Comparison of ^{13}C NMR data^a of synthetic structure and literature data (Fenical's Isolation Report) [2].

position	Fenical's isolation report (ppm) [2] ^{Error!} Reference source not found.	Proposed structure (synthetic) (ppm)	$\Delta\delta^b$
15	19.2	19.16	-0.04
16	29.7	26.75	-2.95
14	56.2	39.67	-16.53
12	63.5	43.03	-20.47
8	115.5	116.74	1.24
2	113.7	119.58	5.88
4	114.1	119.79	5.69
6	119.3	122.70	3.40
5	126.8	123.83	-2.97
7	132.0	130.63	-1.37
9	144.1	138.32	-5.78
3	156.3	149.03	-7.27
1	177.0	164.16	-12.84

^aNMR spectra were recorded and calculated in CDCl_3 at

25°C; ^b $\Delta\delta_1 = \delta_{(\text{proposed structure})} - \delta_{(\text{Fenical's Isolation Report})}$.

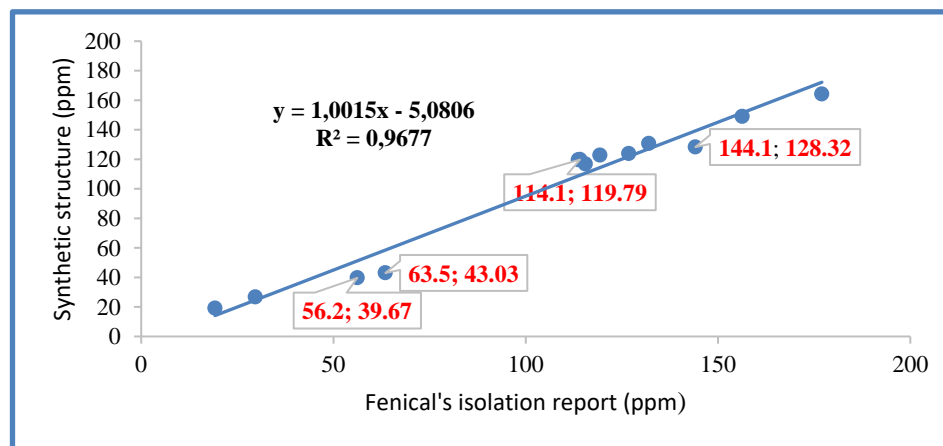


Figure S19. Linear correlations of ^{13}C NMR chemical shifts (CDCl_3) for: a) Fenical's isolation report and synthetic structure.

4. Biological Activity

a. *In silico* ADMET and bioactivity profiling

Table S3. Physicochemical parameters all predicted by ADMET Predictor ver. 10.0.0.11.

Identifier ¹	Basic_pKa ²	FCation ³	logP ⁴	logD ⁵	Peff ⁶	MDCK ⁷	Sw ⁸	BBB_Filter ⁹	LogBB ¹⁰	hum_fup% ¹¹	rat_fup% ¹²	Vd ¹³	RBP ¹⁴	RBP_rat ¹⁵	Fumic ¹⁶
rac-7	8.240	0.873	1.625	0.736	2.009	372.070	2.192	High (99%)	0.078	67.635	40.166	2.720	1.173	1.427	0.777
rac-8	8.430	0.916	1.855	0.785	3.662	933.479	4.071	High (99%)	0.453	68.371	44.718	2.449	1.092	1.473	0.749

¹Compound identifier. ²Predicted macroscopic pKa that appear to be dominated by basic functional groups. ³Cumulative contribution of purely cationic species to fraction ionized at pH defined on input (pH = 7.4). ⁴Admet predictor model for log P with RMSE/MAE = 0.30/0.23. ⁵log D at 7.4 RMSE/MAE = 0.56/0.41. ⁶Effective human jejunal permeability (cm/s x 10⁴). RMSE/MAE = 0.31/0.25 log units. ⁷Apparent MDCK Transwell permeability (cm/s x 10⁷). RMSE/MAE = 0.47/0.48 (2D and 3D) log units. ⁸Water solubility (mg/mL). RMSE/MAE = 0.59/0.45 (2D) and 0.57/0.43 (3D) in log units. ⁹Predicts whether or not a compound can penetrate the Blood Brain Barrier. Overall accuracy = 92%. ¹⁰Logarithm of the Brain/Blood partition coefficient. RMSE/MAE = 0.37/0.28 (2D and 3D). ¹¹Percent UNBOUND to blood plasma proteins in human. RMSE/MAE = 0.43/0.32 (2D) log units. ¹²Percent UNBOUND to blood plasma proteins in Rat. RMSE/MAE = 0.30/0.24 log units. ¹³Volume of distribution (L/kg) in human at steady state. RMSE/MAE = 0.40/0.30 log units (2D and 3D). ¹⁴Blood to plasma concentration ratio in human. RMSE/MAE = 0.09/0.07 (2D) log units. ¹⁵Blood to plasma concentration ratio in rat. RMSE/MAE = 0.10/0.08 (2D) log units. ¹⁶Fraction unbound in human liver microsomes at 1 mg/mL microsomal protein concentration. RMSE/MAE = 0.18/0.13.

Table S4. Toxicity parameters predicted by ADMET Predictor ver. 10.0.0.11.

Identifier ¹	Estro_Filter ²	Andro_Filter ³	Minnow_LC50 ⁴	Th_pyr_pIGC50 ⁵	Daphnia_LC50 ⁶	Bioconcn ⁷	Biodegradn ⁸	hERG_Filter ⁹	Rat_Acute ¹⁰	Rat_TD50 ¹¹	Mouse_TD50 ¹²	Chrom_Aberr ¹³	PLipidosis ¹⁴	Repro_Tox ¹⁵	MUT_NIHS ¹⁶
rac-7	Nontoxic (80%)	Nontoxic (57%)	38.965	0.209	1.026	2.634	No (95%)	No (64%)	332.625	18.919	205.33	Nontoxic (54%)	Nontoxic (69%)	Toxic (57%)	Positive (24%)
rac-8	Nontoxic (51%)	Toxic (53%)	13.609	0.653	4.899	6.351	No (95%)	Yes (99%)	361.796	29.323	99.433	Nontoxic (46%)	Toxic (69%)	Nontoxic (79%)	Positive (26%)

¹Compound identifier. ²Predicts whether or not the compound possesses estrogen receptor toxicity in rat. Overall accuracy = 90% (2D) and 95% (3D). ³Predicts whether or not the compound possesses androgen receptor toxicity in rat. Overall accuracy = 82%. ⁴LC₅₀ for fathead minnow lethal toxicity (mg/L) after 96 hours of exposure. RMSE/MAE = 0.50/0.38 (2D and 3D) in molar log units. ⁵Acute toxicity in Tetrahymena pyriformis protozoa expressed as -log of the 50% growth inhibitory concentration, (pIGC50) in mmol/L. RMSE/MAE = 0.32/0.25 (2D) and 0.32/0.24 (3D) in log units. ⁶Acute toxicity in Daphnia magna (water fleas) expressed as the 50% lethality after 48 hours, (LC50) in mg/L. Overall RMSE/MAE = 0.79/0.62 (2D and 3D) in molar log units. ⁷Bioconcentration factor - a partition coefficient between fish tissues and environmental water at steady state as concentration ratio (C_{fish}/C_{water}). RMSE/MAE = 0.47/0.36 (2D and 3D) in log units. ⁸Predicts whether or not the compound undergoes biodegradation readily in terms of relative biological oxygen demand (BOD>60%). Overall accuracy = 84%. ⁹Predicts whether or not the compound blocks the hERG potassium channel. Overall accuracy = 88%. ¹⁰LD₅₀ for rat acute toxicity (mg/kg in oral dose that would be lethal to 50% of the rats). RMSE/MAE = 0.60/0.45 (2D) and 0.58/0.44 (3D) in log units. ¹¹TD₅₀ for rat carcinogenicity (mg/kg/day in oral dose) over a standard lifetime. RMSE/MAE = 0.54/0.42 (2D) and 0.52/0.43 (3D) in log units. ¹²TD₅₀ for mouse carcinogenicity (mg/kg/day in oral dose) over a standard lifetime. RMSE/MAE = 0.47/0.38 (2D) and 0.46/0.38 (3D) in log units. ¹³Predicts whether or not the compound will trigger the mutagenic chromosomal aberrations. Overall accuracy = 80%. ¹⁴Qualitative estimation of causing phospholipidosis. Overall accuracy = 96%. ¹⁵Qualitative estimation of reproductive / developmental toxicity. Overall accuracy = 90%. ¹⁶Predicts the mutagenicity of the compound based on data compiled in 2017 by the National Institute of Health Sciences (NIHS) of Japan. Overall accuracy = 76%. Prediction confidence estimates are listed in parentheses.

b. Antiproliferative effect

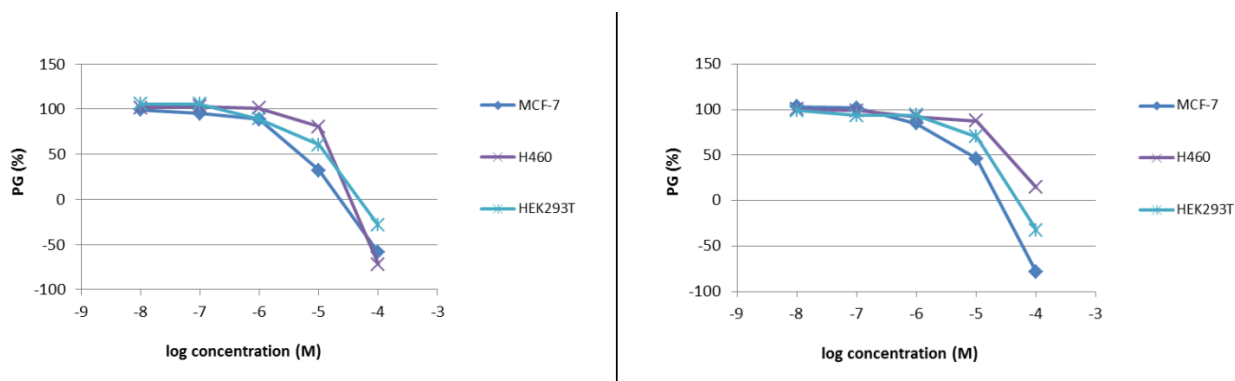


Figure S20. Dose-response profiles for compound *rac-7* and compound *rac-8* tested *in vitro* on H460, HEK293T and MCF-7.

Table S5. GI₅₀ values (in μ M).

Compound ID	GI ₅₀ ^a (μ M)		
	Cell lines		
	H460	HEK293T	MCF7
compound 7	16 \pm 0.2	13 \pm 0.1	5 \pm 1.0
compound 8	33 \pm 1.0	15 \pm 4.0	7 \pm 5.0

^aGI₅₀; the concentration that causes 50% growth inhibition.

c. Testing of embryotoxicity using the zebrafish model organism (*Danio rerio*)

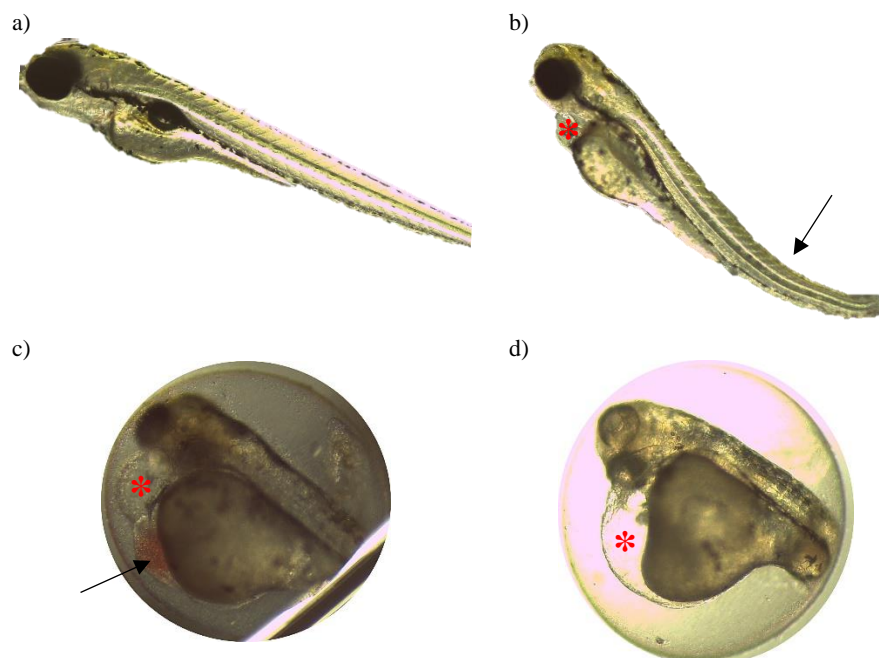


Figure S21. Developmental abnormalities in *Danio rerio* zebrafish exposed to the tested samples of compound *rac-7* and compound *rac-8* for 96 hours: a) normally developed larvae in the control groups, b) scoliosis (arrow), pericardial edema (star), c) bleeding in the yolk sac area (arrow), pericardial edema (star) d) edema in the area of the yolk sac (asterisk).

Table S6. Rate (%) of mortality, developmental abnormalities and hatching success of zebrafish *D. rerio* ($N = 30$) after 96 h of exposure to different concentrations of the tested compounds. Results are presented as mean value \pm standard deviation (SD).

Compound		Concentration		
		0.1mM	0.2 mM	0.4 mM
<i>rac-7</i>	mortality rate (%)	100.00 \pm 0.00	100.00 \pm 0.00	100.00 \pm 0.00
	abnormality rate (%)	-	-	-
	hatching rate (%)	-	-	-
<i>rac-8</i>	mortality rate (%)	0.00 \pm 0.00	0.00 \pm 0.00	76.67 \pm 5.77
	abnormality rate (%)	0.00 \pm 0.00	6.67 \pm 11.55	100.00 \pm 0.00
	hatching rate (%)	100.00 \pm 0.00	23.33 \pm 5.77	0.00 \pm 0.00

5. References

1. Buljan A.; Roje M. Application of green chiral chromatography in enantioseparation of newly synthesized racemic marinoepoxides. *Mar. Drugs* **2022**, *20*, 530.
2. Choi E. J.; Nam S.-J.; Paul L.; Beatty D.; Kauffman C. A.; Jensen P. R.; Fenical W. Previously uncultured marine bacteria linked to novel alkaloid production. *Chem. and Biol.* **2015**, *22*, 1270–1279.