SUPPORTING INFORMATION

Evaluating Nitrogen Containing Biosynthetic Products Produced by Saltwater Culturing of Several California Littoral Zone Gram-Negative Bacteria

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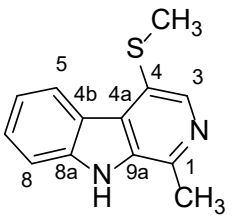
Table S1: Media and artificial seawater recipes

Seawater Salts (SWS)	Per liter: 8.0g $MgSO_4 \cdot 7H_2O$; 1.0g $CaCl_2 \cdot 2H_2O$; 0.5g KCl; 0.16 g $NaHCO_3$; 0.02 g H_3BO_3; 0.08 g KBr; 0.03g $SrCl_2 \cdot 6H_2O$; 0.01g β-glycerophosphate-2Na; 0.1 g $FeC_6H_5O_7$; 1.0 mL trace element solution; 20.0g NaCl; pH 8.0.
Trace Elements Solution	Per liter: 100 mg $MnCl_2 \cdot 4H_2O$; 20 mg $CoCl_2$; 10 mg $CuSO_4$; 10mg $Na_2MoO_4 \cdot 2H_2O$; 20 mg $ZnCl_2$; 5mg LiCl; 5mg $SnCl_2 \cdot 2H_2O$; 10mg H_3BO_3; 20 mg KBr; 20 mg KI; 8g EDTA·Na-Fe3+ salt.
Medium 1 (M1)	Yeast extract 2.0g/L; Mannitol 4.0g/L; Peptone 2.0g/L; 75% SWS to volume; pH6.8.
Medium 2 (M2)	Glycerol 15 ml/L; L-glutamine 5.0g/L; K_2HPO_4 1.5g/L; $MgSO_4$ 0.2g/L; Di-H_2O to volume; pH7.0.
Medium 3 (M3)	Galactose 1.0g/L; Peptone 6.0g/L; Glycerol 0.8ml/L; Yeast Extract 2.5g/L; 75% SWS to volume; pH6.8.
Medium 4 (M4)	Casitone 3.0g/L; $MgSO_4 \cdot 7H_2O$ 2.0g/L; $CaCl_2$ 0.5g/L; Trace Elements Solution 1.0 ml/L; Vitamin B12 Solution; 75% SWS to volume; pH6.8.
Medium 5 (M5)	K_2HPO_4 2.0g/L; NH_4Cl 1.5g/L; $MgSO_4 \cdot 7H_2O$ 0.5g/L; Glycerol 8.0 ml/L; Myo-inositol 0.4g/L; Monosodium L-glutamate 5.0g/L; NaF 0.084g/L; $FeSO_4 \cdot 7H_2O$ 0.025g/L; $ZnSO_4 \cdot 7H_2O$ 0.01g/L; $CoCl_2.6H_2O$ 0.01g/L; $CaCO_3$ 0.25g/L; p-aminobenzoate 0.001g/L; DiH_2O to volume; pH 7.0.

Table S2. Experimental ^{13}C NMR data (125MHz, DMSO-d_6, 25°C) for 1-methyl-4-methylthio-β-carboline (**1**) and literature data for related compounds: 1-ethyl-4-methylsulfone-β-carboline (**3**)[2], 1-methyl-β-carboline (aka harman) (**4**)[1], and 1-ethyl-4-methoxy-β-carboline (aka crenatine) (**5**)[1]

Carbon	1	3	4	5
1	139.6 (C)	152.7	142.2	140.6
3	134.8 (CH)	139.9	137.6	120.0
4	125.2 (C)	119.2	112.6	151.0
4a	124.3 (C)	no data	121.2	118.3
4b	120.9 (C)	126.7	127.0	119.6
5	123.4 (CH)	125.9	121.7	124.2
6	119.5 (CH)	121.7	119.2*	127.2
7	127.5 (CH)	129.8	127.8*	121.4
8	111.9 (CH)	111.9	112.0	111.3
8a	140.2 (C)	140.6	140.5	140.1
9a	133.8 (C)	133.7	134.6	135.3
1'	20.1 (CH_3)	27.5	no data	26.8
2'		12.2		13.3
4'	15.2 (CH_3)	43.2		56.1

*Literature value errors corrected.



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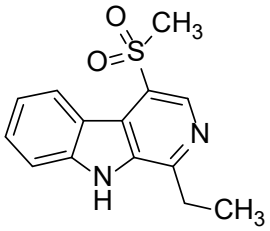

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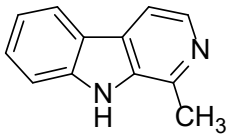

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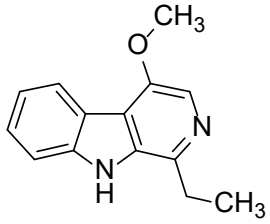

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(1) Koike, K.; Sakamoto, Y.; Ohmoto, T. A 13C NMR Study of β-Carboline Alkaloids. Org. Magn. Reson. 1984, 22 (7), 471–473.

(2) Prinsep, M. R.; Blunt, J. W.; Munro, M. H. G. New Cytotoxic β-Carboline Alkaloids from the Marine Bryozoan, Cribricellina Cribraria. J. Nat. Prod. 1991, 54 (4), 1068–1076.

Figure S1. ESI-FTMS accurate mass spectra. Left panel: molecular ion cluster of (**1**) (top) compared to simulated spectra with and without sulfur (middle and bottom). Right panel: accurate MS^2 spectrum (top) showing loss of -SCH_3 and comparison of measured vs predicted ion cluster for m/z 182.0838(bottom). In the MS^2 experiment, a capture width of +/- 6 Da centered on 229.08 was used so that all isotopic variants of (**1**) were captured for fragmentation.

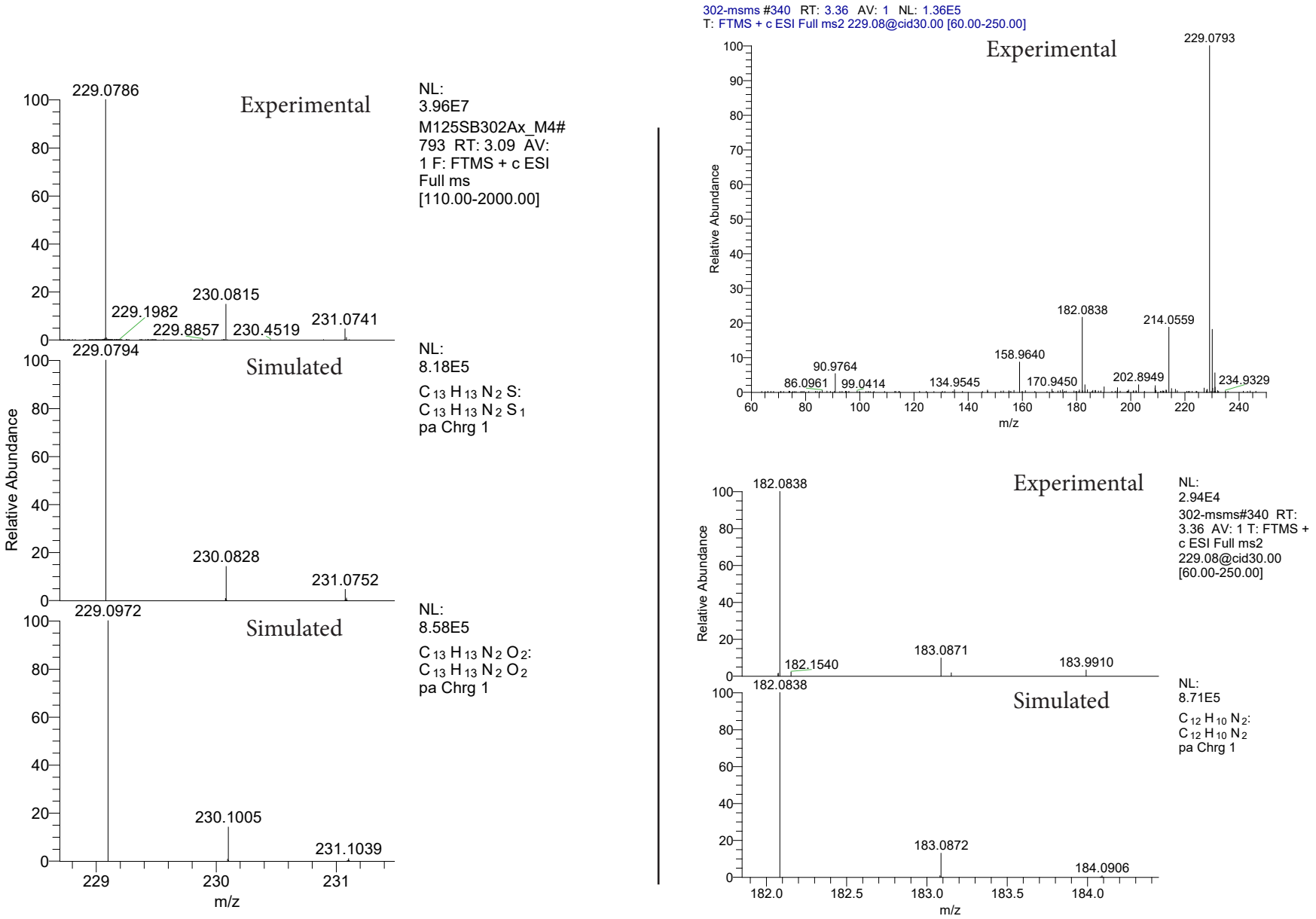


Figure S2. [1]H NMR spectrum of 1-methyl-4methylthio-β-carboline (1), (600 MHz, DMSO-d6).

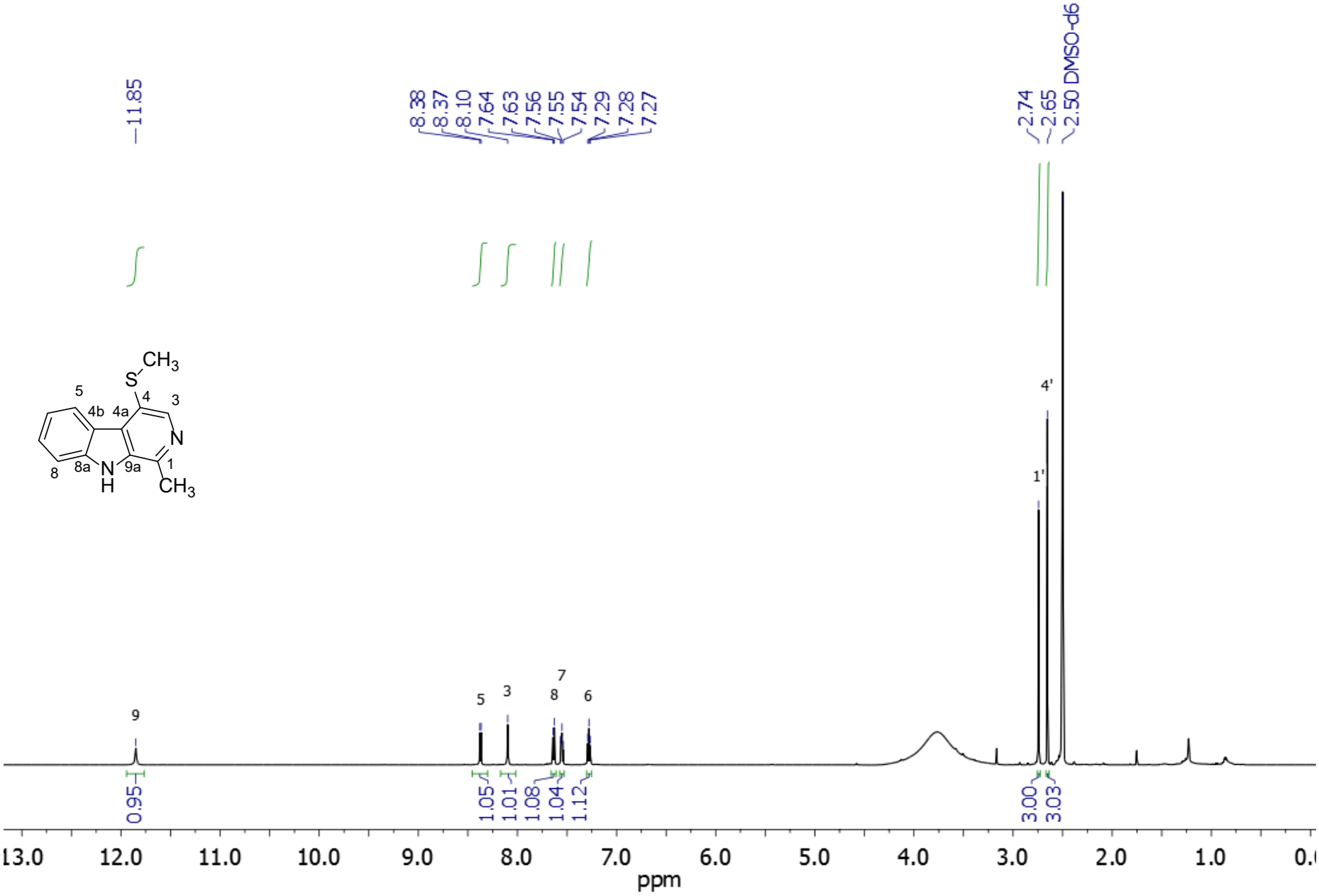


Figure S3. ^{13}C NMR spectrum of 1-methyl-4methylthio-β-carboline (1), (150 MHz, DMSO-d6).

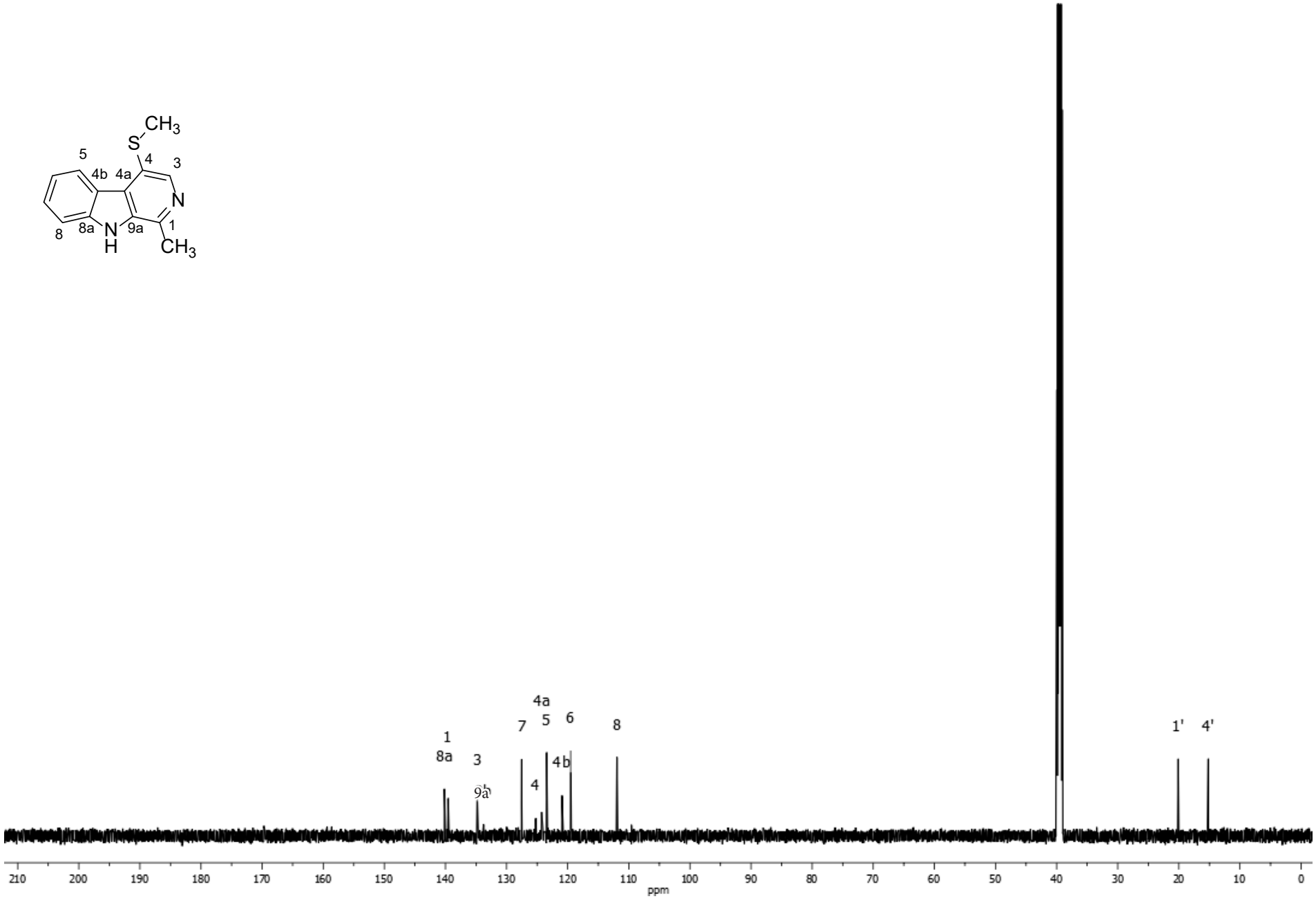


Figure S4. gHSQC NMR spectrum of 1-methyl-4methylthio-β-carboline (1), (600 MHz, DMSO-d6)

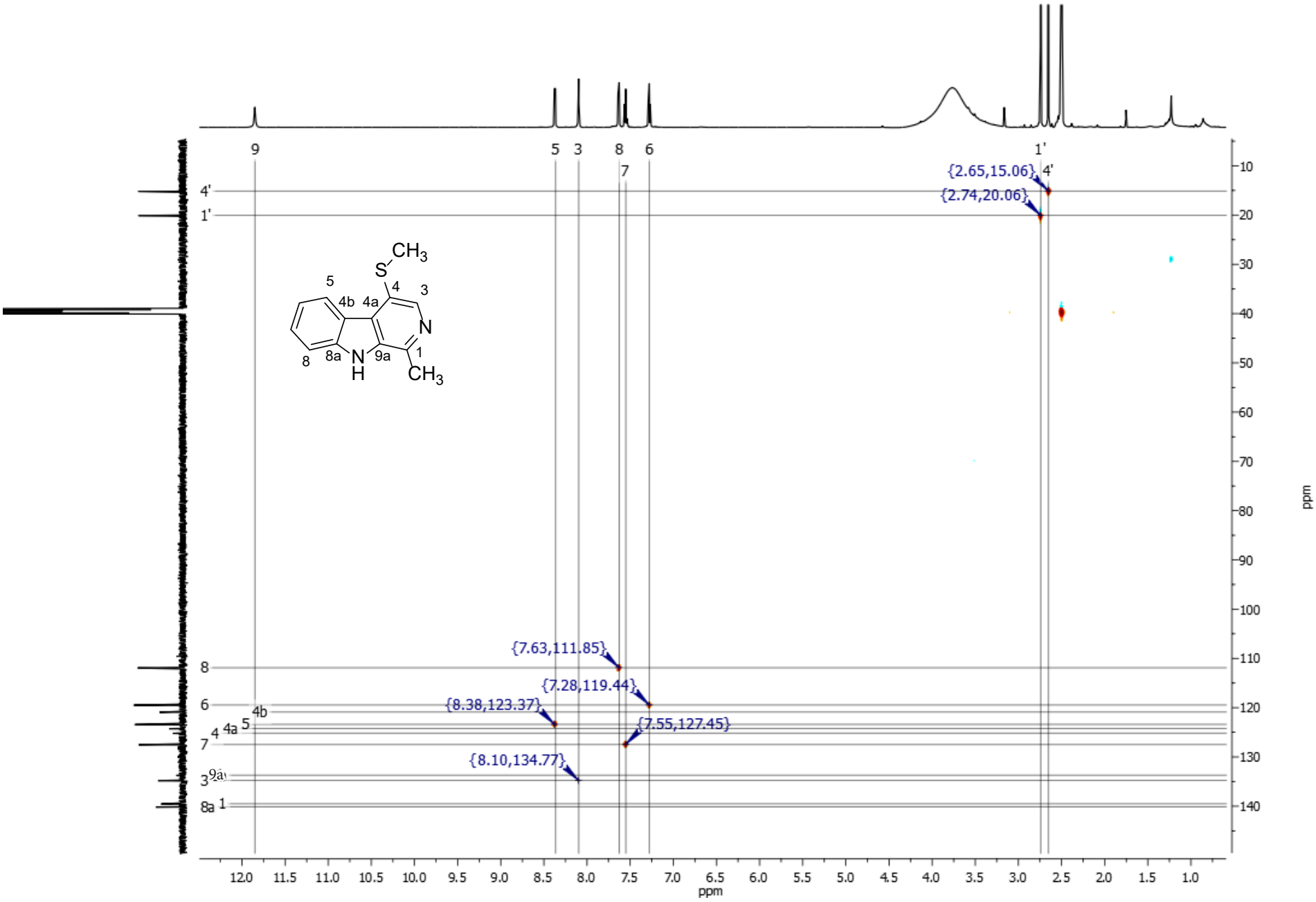


Figure S5. gHMBCAD NMR spectrum of 1-methyl-4methylthio-β-carboline (1), (600 MHz, DMSO-d6)

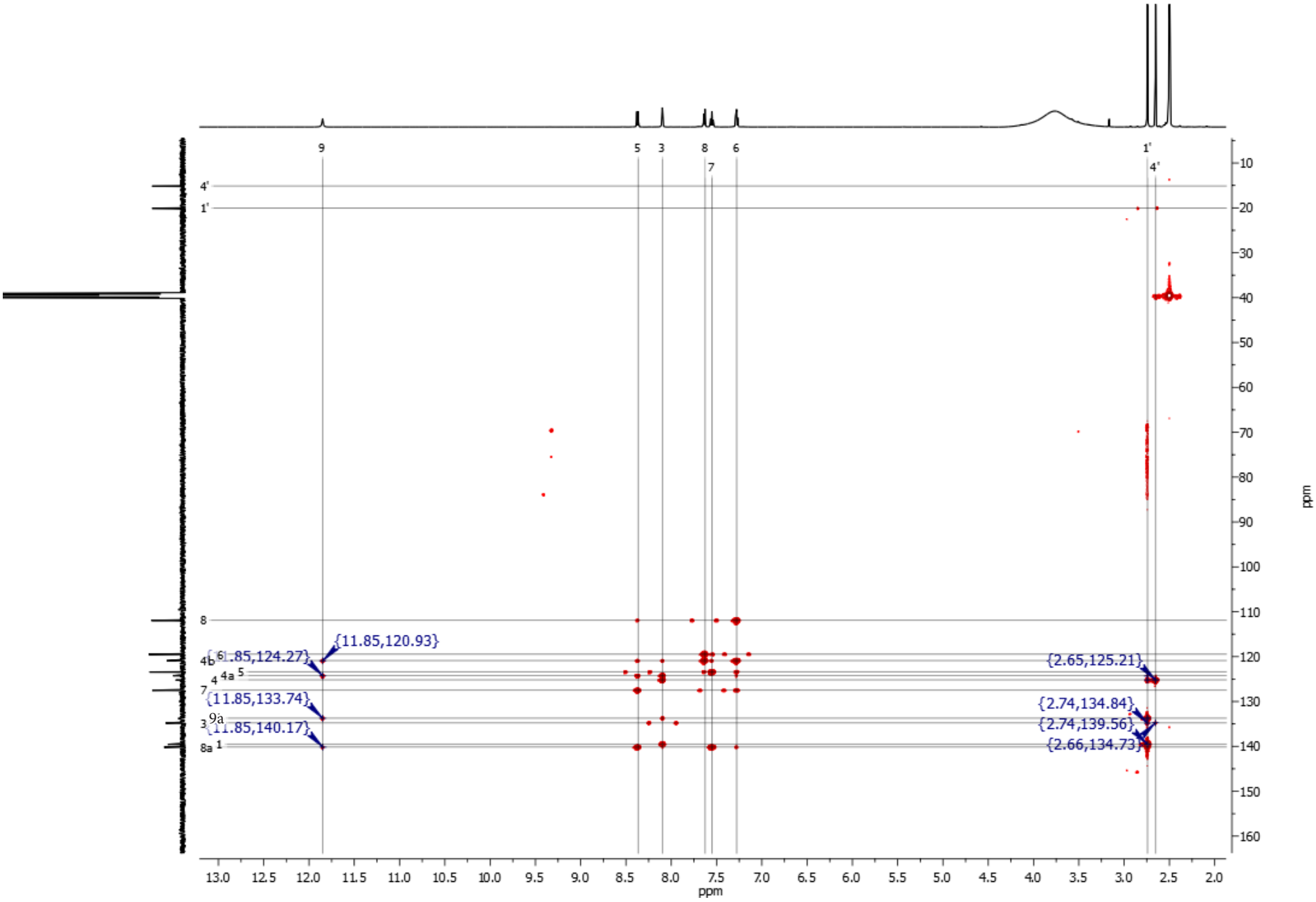


Figure S6. gHMBCAD NMR spectrum (downfield expansion) of 1-methyl-4methylthio-β-carboline (1), (600 MHz, DMSO-d6)

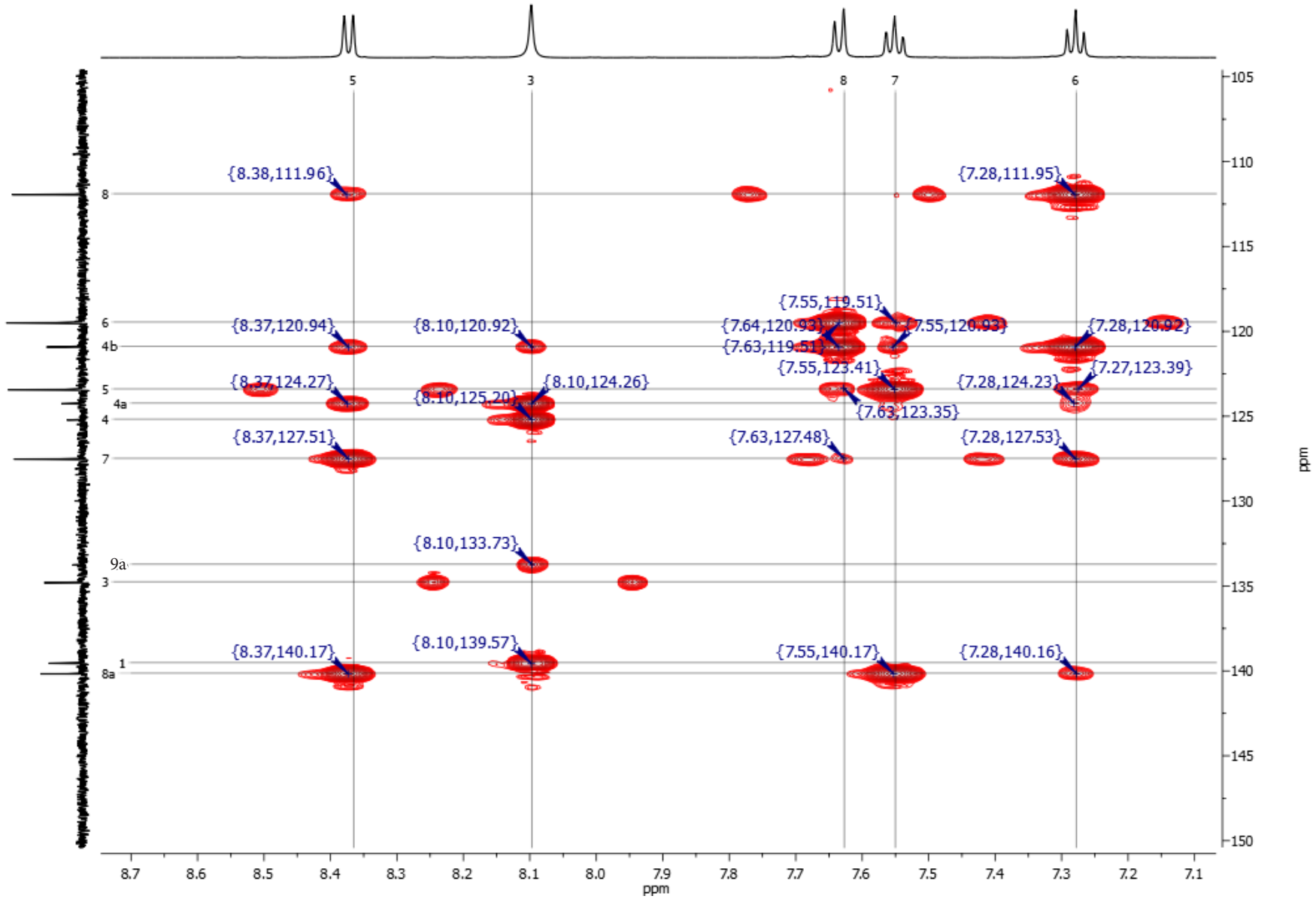


Figure S7. gHMBCAD NMR spectrum ($^{1}J_{CH}$ measurements) of 1-methyl-4methylthio-β-carboline (1), (600 MHz, DMSO-d6)

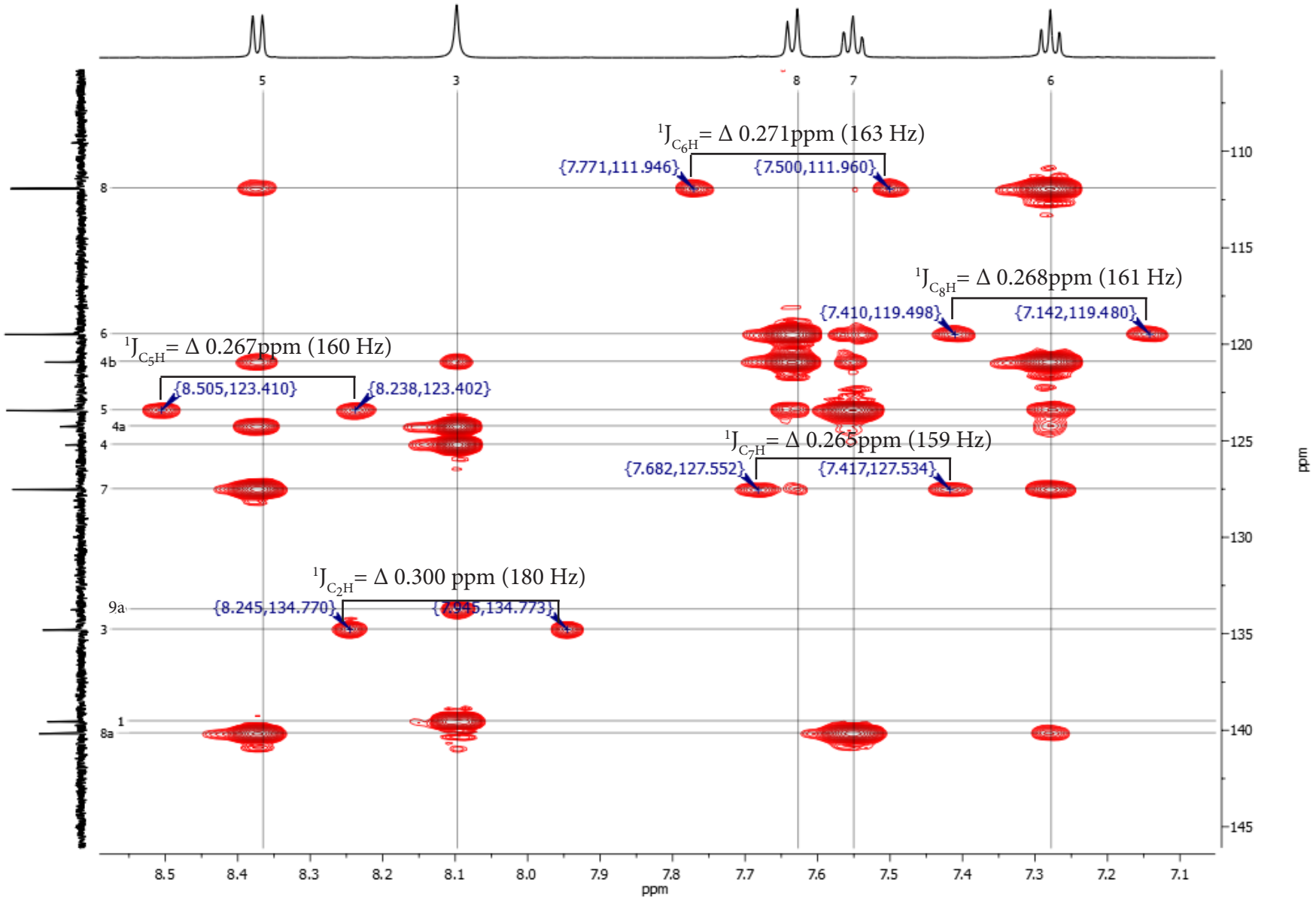


Figure S8. gHMBCAD NMR spectrum ($^{1}J_{CH}$ measurements) of 1-methyl-4methylthio-β-carboline (1), (600 MHz, DMSO-d6)

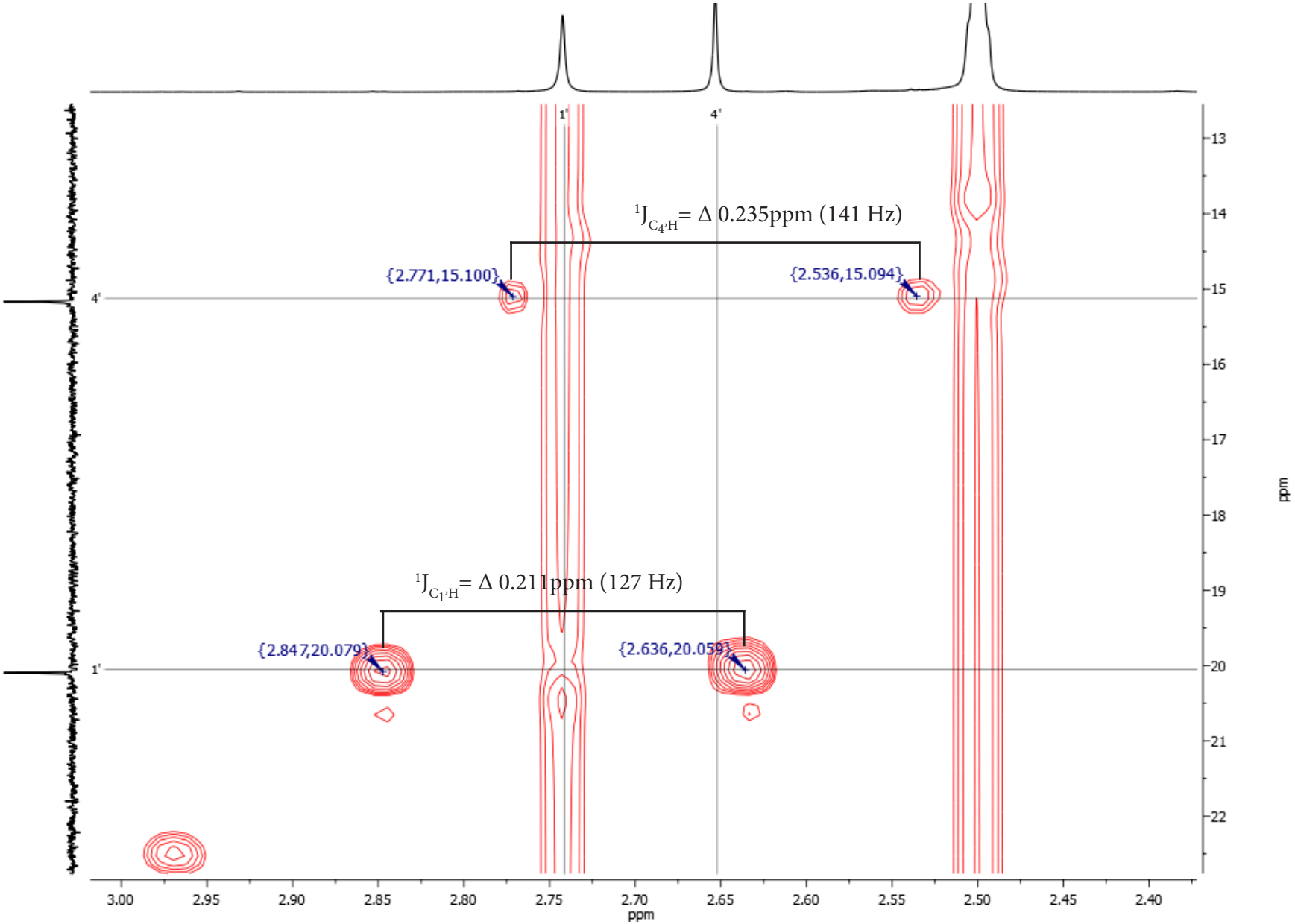


Figure S9. ^{1}H-^{1}H gCOSY of 1-methyl-4methylthio-β-carboline (1), (600 MHz, DMSO-d6)

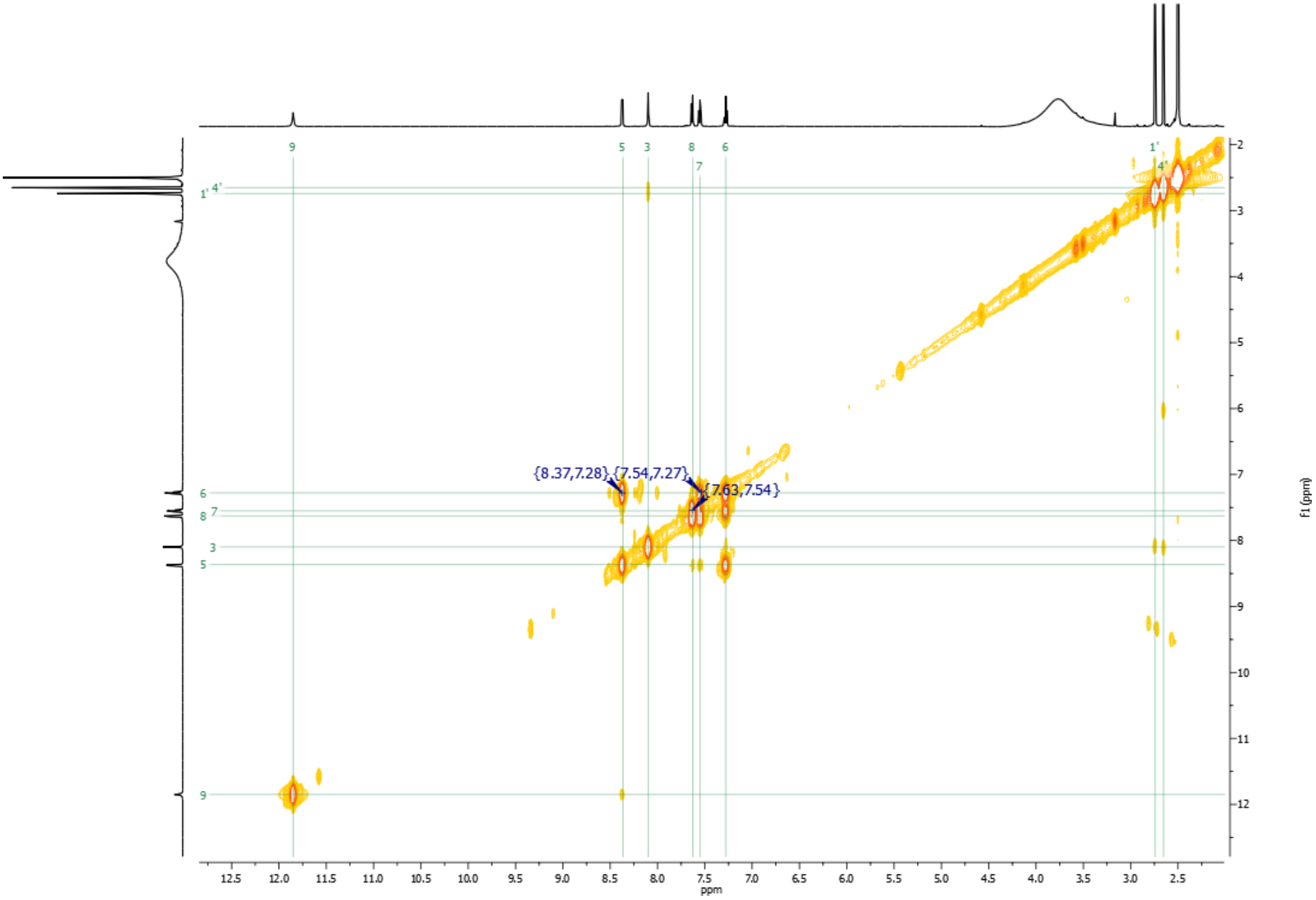


Figure S10. NOESY NMR spectrum of 1-methyl-4methylthio-β-carboline (1), (600 MHz, DMSO-d6)

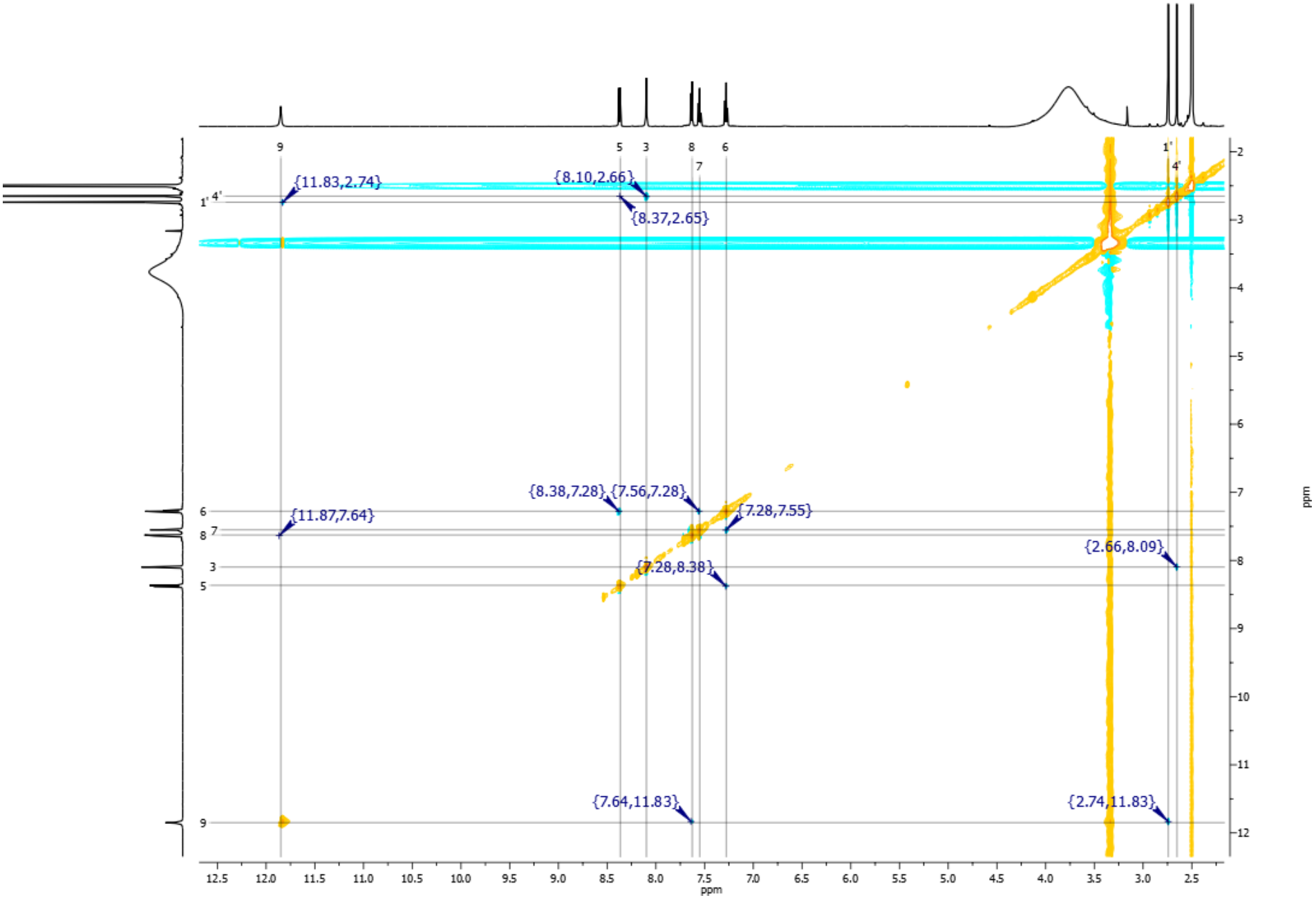


Figure S11. 1D nOe NMR spectra of 1-methyl-4methylthio-β-carboline (1) irradiated at 2.65 ppm (50Hz width), (600 MHz, DMSO-d6)
Key: 1 - full proton; 2 - 500 ms mixing time; 3 - 600ms; 4 - 700 ms; 5 - 800 ms

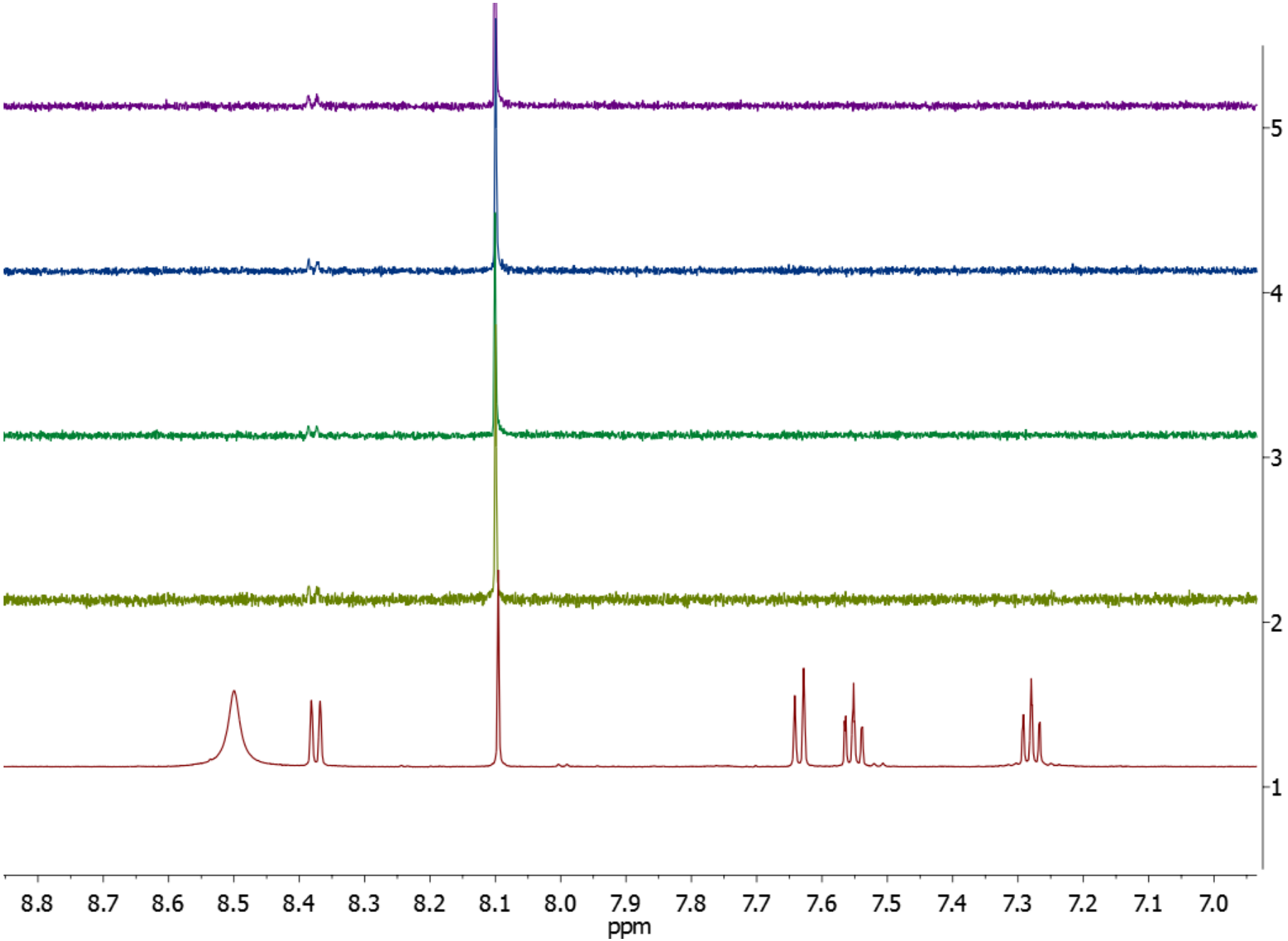


Figure S12. UV spectrum of 1-methyl-4-methylthio-β-carboline (1)

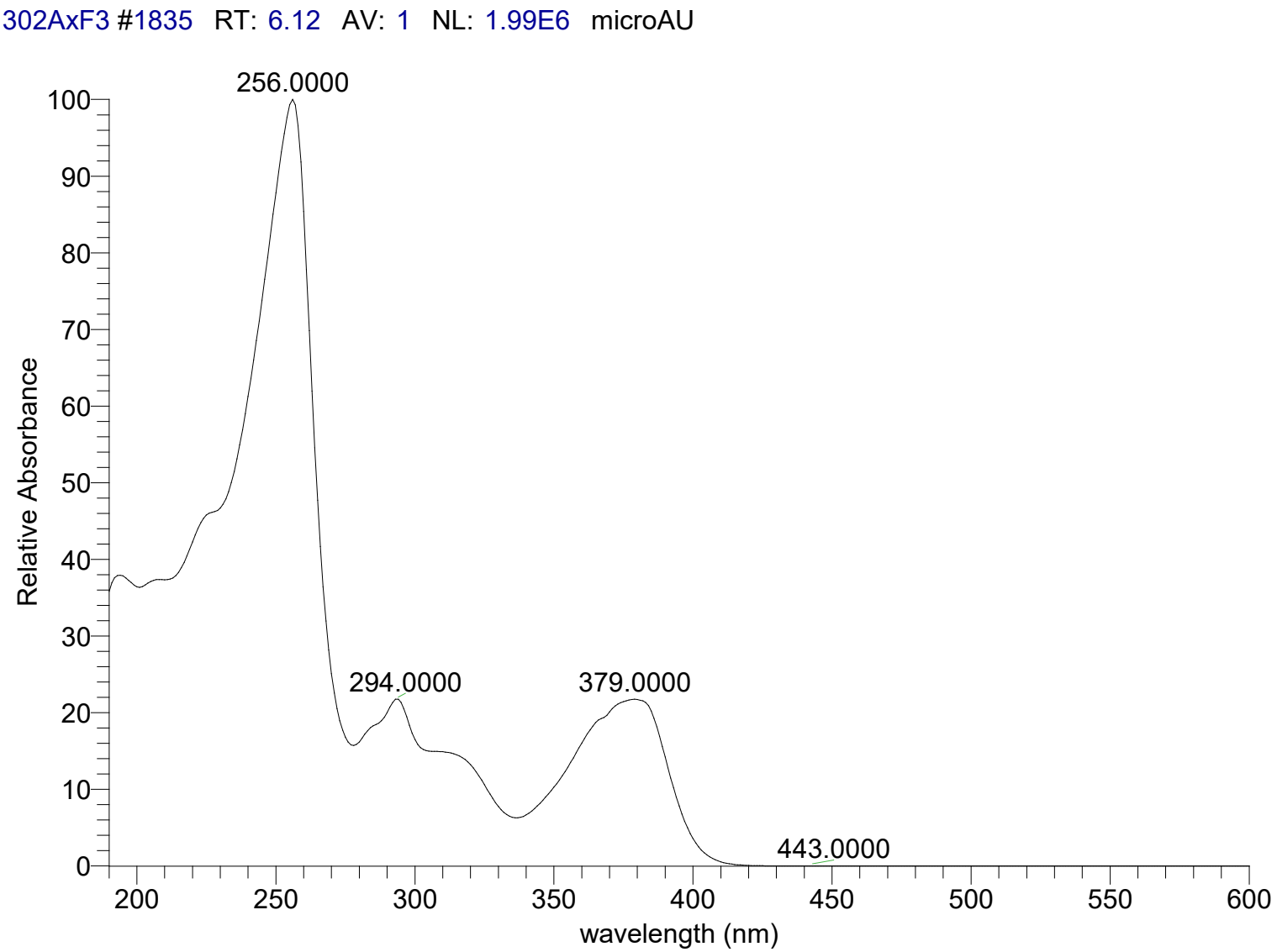
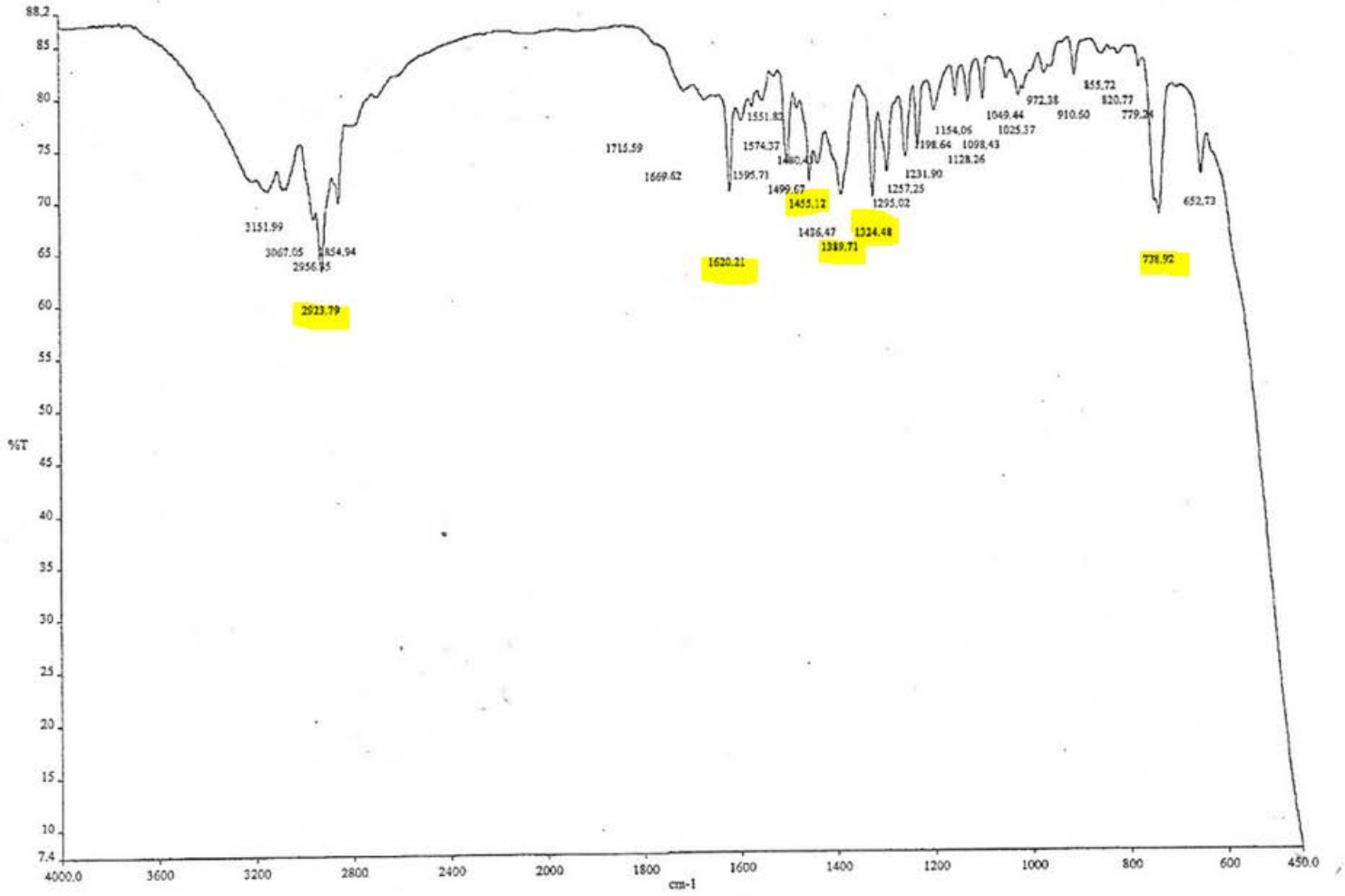


Figure S13. IR spectrum of 1-methyl-4-methylthio-β-carboline (1)



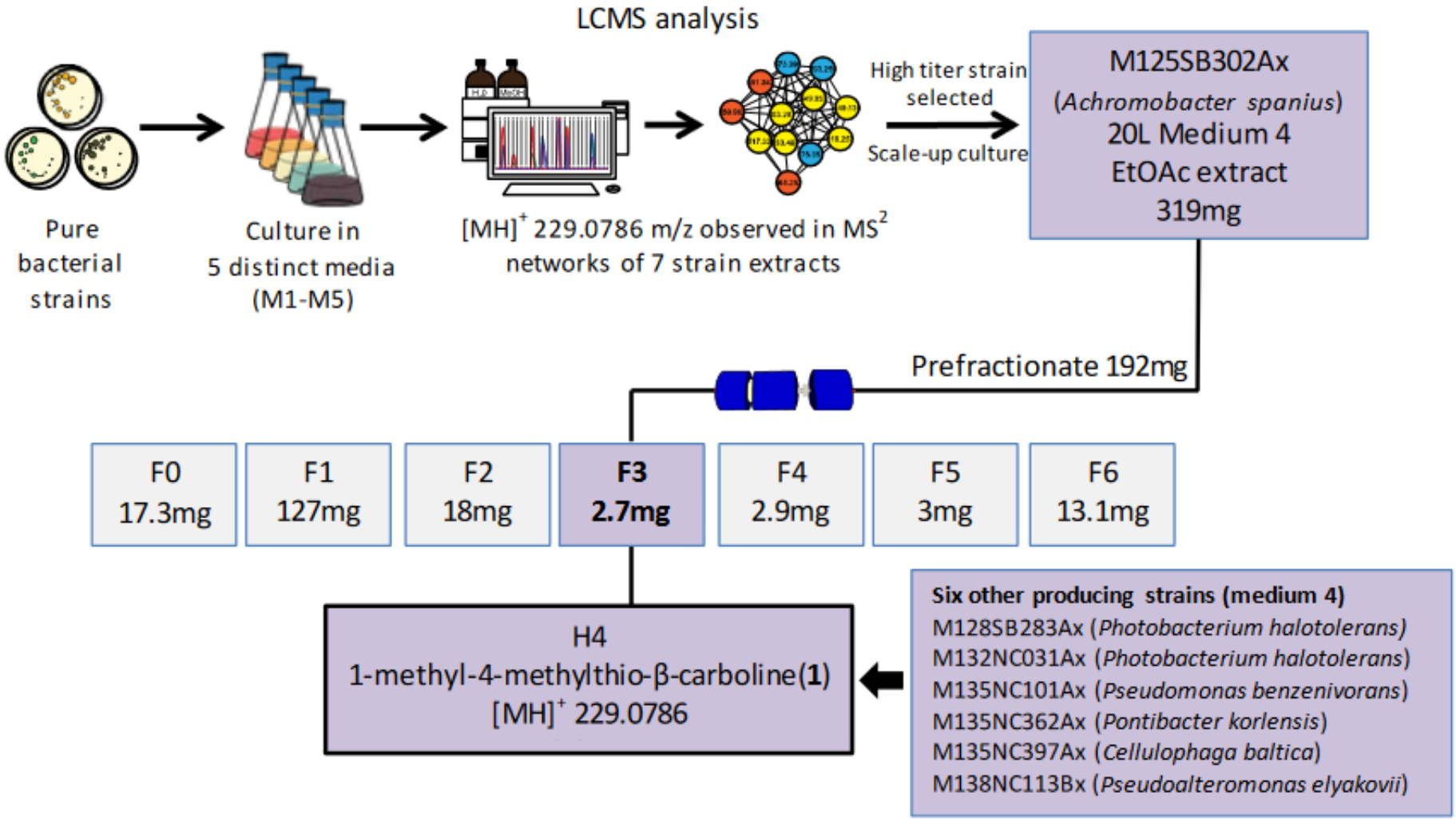


Figure S14. Survey of seven CA littoral zone Gram-negative bacteria strains assessed by LCMS for their capacity to produce 1-methyl-4-methylthio-β-carboline (**1**) and subsequent steps for its purification from strain M125SB302Ax. See Table S1 for media composition.

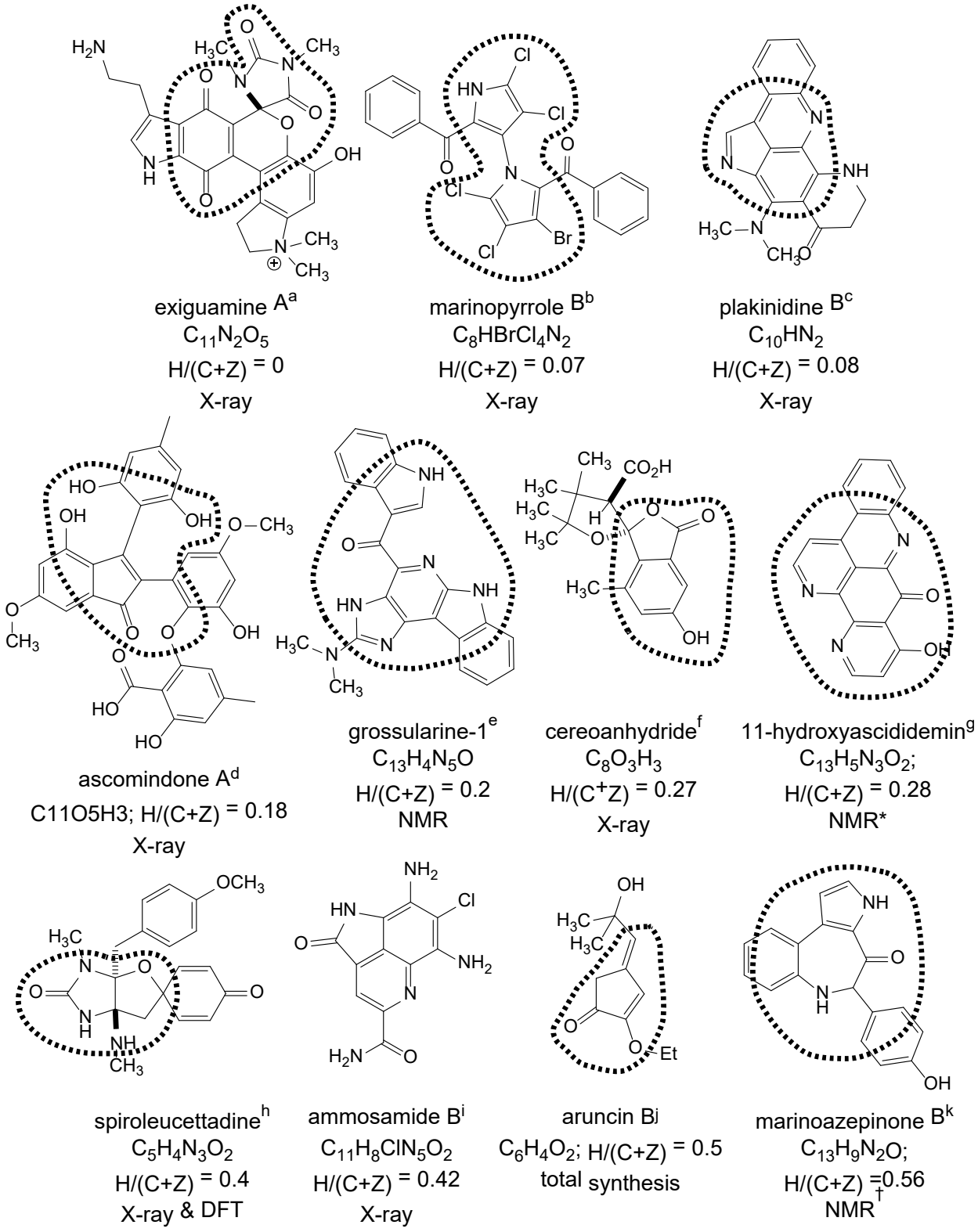


Figure S15. Select examples of structure assignment outcomes of natural products that possess a core H/(C+Z) < 0.6 (C+Z = Σ #C + # heteroatoms). These molecules are particularly challenging to elucidate using 2D NMR data as denoted by "Crews Rule." Molecular formulas represent highlighted region of molecules only. *data does not rule out other structures. [†]based on comparison to marinoazepinone A, which has an additional N-CH_3 and H/(C+Z)= 0.65. Reference letters correspond to reference 26 in the main text.

Figure S16. Querying medium 4 control for m/z 229.0794 (i.e. **1**) via extracted ion chromatography (XIC) searches. The normalization level (NL) shown at the right of each panel provides an ion current value for a peak with 100% relative abundance. The top trace [A] shows total ion chromatogram (TIC) from the media 4 control; a culture blank not inoculated with any Gram-negative bacterium and not expected to contain secondary metabolites. The trace [B] shows and XIC for the sample shown in [A] and confirms that the peaks at 3.00-3.07 minutes are devoid of the m/z = 229.07 compound. The trace [C] documents, by XIC, that retention time of 1 (m/z = 229.07), produced by the indicated strain, is 3.07 minutes.

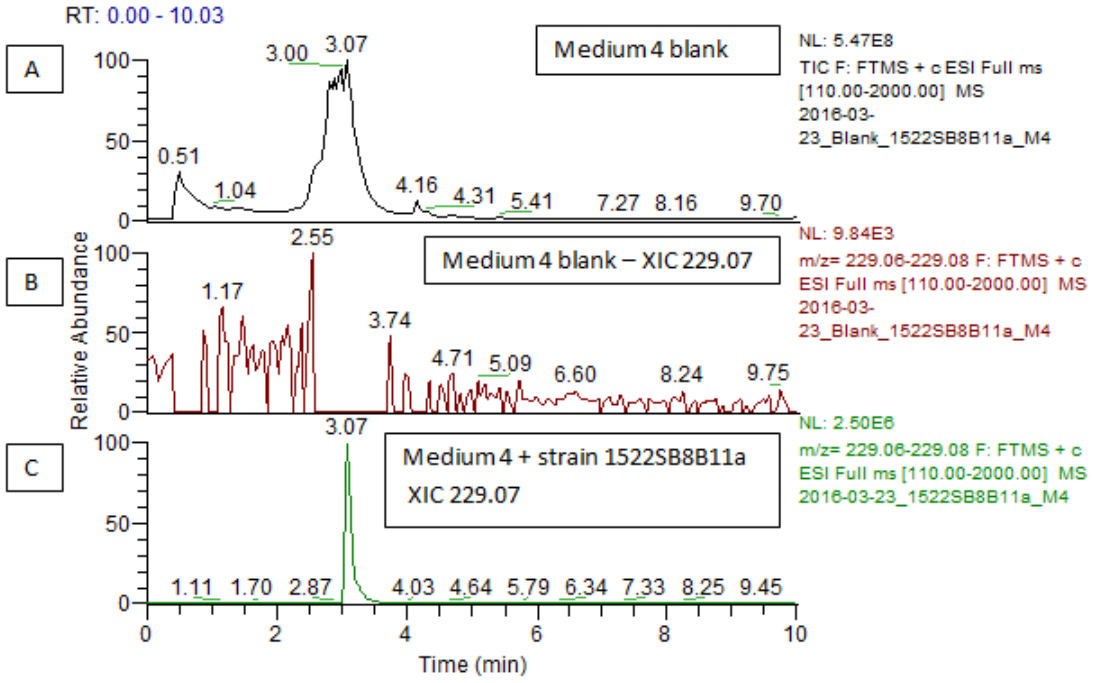
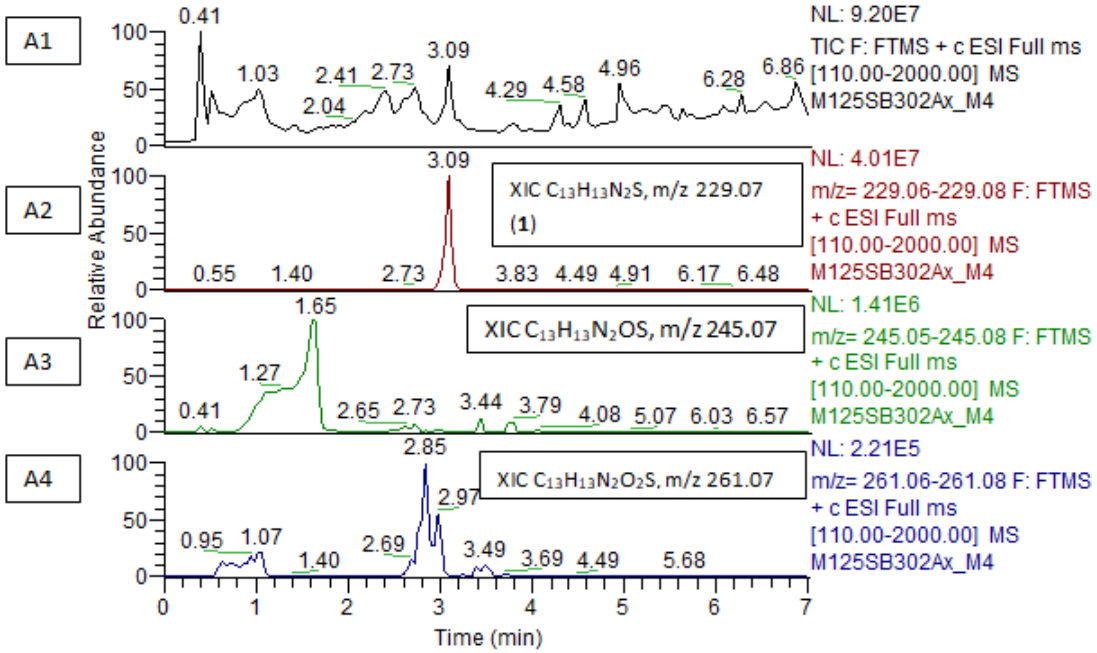


Figure S17. Querying for oxidized analogues of 1 via extracted ion chromatography (XIC) searches. The normalization level (NL) shown at the right of each panel provides an ion current value for a peak with 100% relative abundance. The top trace [A1] shows total ion chromatogram (TIC) peaks from the media 4 plus Gram-negative strain M125SB302Ax of the parent crude extract containing **1**. The traces [A2-A4] show three different XICs of the same parent crude extract. The trace [A2] documents that the retention time of **1** is 3.09 minutes (also observed in trace [A1]). Though panel [A3] shows XIC peaks (rt = 1.2-1.65) their mass spectra (not shown here) are not consistent with compounds of formula $C_{13}H_{13}N_2OS$. Trace [A4] also shows possible evidence for the presence of a doubly oxidized **1** ($C_{13}H_{13}N_2O_2S_2^+$) in the XIC peaks at rt = 2.85 min. and 2. 97 min and is supported by the experimental vs. predicted MS shown in [B]. However the lower ion count indicates a compound of this formula is possibly present but in a minute amount: approximately 200 fold less abundant than **1** based on their relative ion counts (NL) between trace [A2] and [A4].



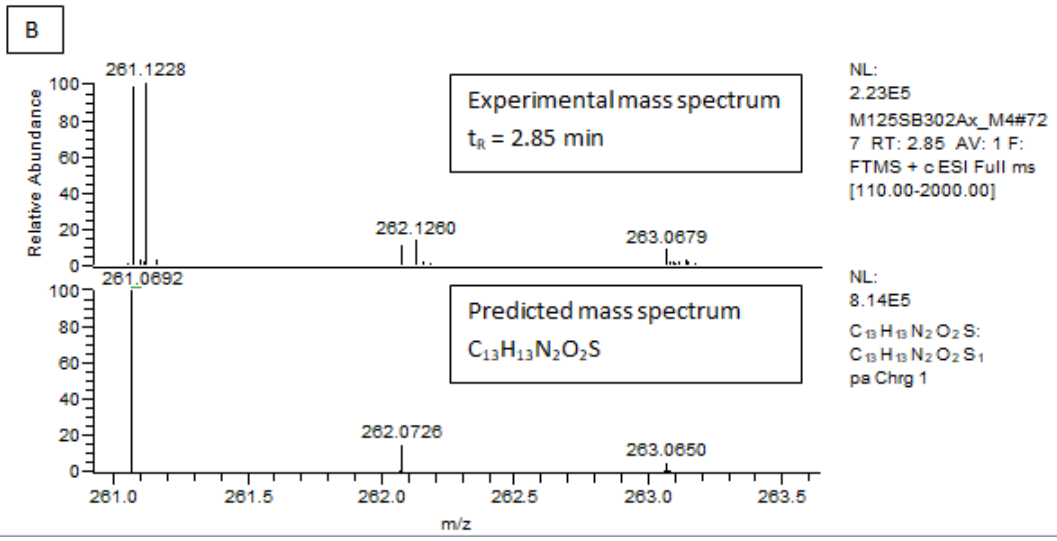
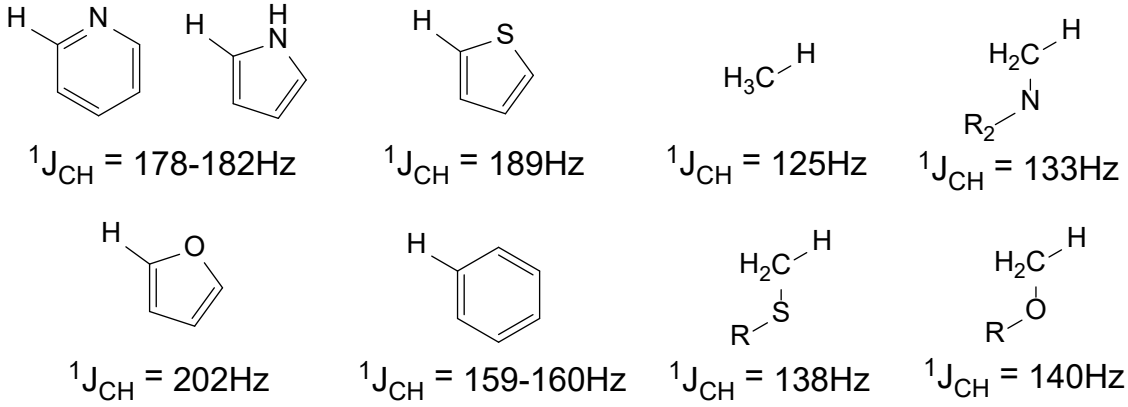


Chart S1. Reference $^{1}J_{CH}$ values for relevant sp^2 and sp^3 carbons



References:
(1) Crews, P.; Rodríguez, J.; Jaspars, M. Organic Structure Analysis, 2nd ed.; p. 150; Oxford University Press: New York, 2010.
(2) Reich, H. C-H Coupling Constants. Structure Determination Using NMR. http://www.chem.wisc.edu/areas/reich/nmr/10-cdata-05-jch.htm (accessed Mar 28, 2017).