Supporting Information

1,3-Oxazin-6-one Derivatives and Bohemamine-Type Pyrrolizidine Alkaloids from a Marine-Derived *Streptomyces spinoverrucosus*

Peng Fu, Scott La, and John B. MacMillan*

Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75390, United States

List of Supporting Information

Bioassay Protocols……..S3

Theory and Calculation Details……..S3

Figure S1. DFT optimized geometries of the four lowest-energy conformers of (4*S*, 5*S*)-**1**……S4

Table S1. The calculated ^{13}C NMR Data for **1** and **1a**……S5

Figure S2. ^{13}C and 2D NMR of **3a** (These data were from HMBC and HSQC spectra)……S5

Figure S3. HRESIMS spectrum of spinoxazine A (**1**)……S6

Figure S4. ^{1}H-NMR spectrum of spinoxazine A (**1**) in DMSO-d_6……S7

Figure S5. ^{13}C-NMR spectrum of spinoxazine A (**1**) in DMSO-d_6……S8

Figure S6. HSQC spectrum of spinoxazine A (**1**) in DMSO-d_6……S9

Figure S7. ^{1}H-^{1}H COSY spectrum of spinoxazine A (**1**) in DMSO-d_6……S10

Figure S8. HMBC spectrum of spinoxazine A (**1**) in DMSO-d_6……S11

Figure S9. NOESY spectrum of spinoxazine A (**1**) in DMSO-d_6……S12

Figure S10. HRESIMS spectrum of spinoxazine B (**2**)……S13

Figure S11. ^{1}H-NMR spectrum of spinoxazine B (**2**) in DMSO-d_6……S14

Figure S12. ^{13}C-NMR spectrum of spinoxazine B (**2**) in DMSO-d_6……S15

Figure S13. HSQC spectrum of spinoxazine B (**2**) in DMSO-d_6……S16

Figure S14. ^{1}H-^{1}H COSY spectrum of spinoxazine B (**2**) in DMSO-d_6……S17

Figure S15. HMBC spectrum of spinoxazine B (**2**) in DMSO-d_6……S18

Figure S16. HRESIMS spectrum of bohemamine D (**3**)……S19

Figure S17. ^{1}H-NMR spectrum of bohemamine D (**3**) in DMSO-d_6……S20

Figure S18. ^{13}C-NMR spectrum of bohemamine D (**3**) in DMSO-d_6……S21

Figure S19. HSQC spectrum of bohemamine D (**3**) in DMSO-d_6.....S22
Figure S20. ^{1}H-^{1}H COSY spectrum of bohemamine D (**3**) in DMSO-d_6.....S23
Figure S21. HMBC spectrum of bohemamine D (**3**) in DMSO-d_6.....S24
Figure S22. HRESIMS spectrum of compound **3a**.....S25
Figure S23. ^{1}H-NMR spectrum of compound **3a** in $CDCl_3$.....S26
Figure S24. HSQC spectrum of compound **3a** in $CDCl_3$.....S27
Figure S25. HMBC spectrum of compound **3a** in $CDCl_3$.....S28
Figure S26. NOESY spectrum of compound **3a** in $CDCl_3$.....S29
Figure S27. HRESIMS spectrum of bohemamine E (**4**) S30
Figure S28. ^{1}H-NMR spectrum of bohemamine E (**4**) in DMSO-d_6.....S31
Figure S29. ^{13}C-NMR spectrum of bohemamine E (**4**) in DMSO-d_6.....S32
Figure S30. ^{1}H-^{1}H COSY spectrum of bohemamine E (**4**) in DMSO-d_6.....S33
Figure S31. HSQC spectrum of bohemamine E (**4**) in DMSO-d_6.....S34
Figure S32. HMBC spectrum of bohemamine E (**4**) in DMSO-d_6.....S35
Figure S33. NOESY spectrum of bohemamine E (**4**) in DMSO-d_6.....S36
Figure S34. HRESIMS spectrum of bohemamine F (**5**) S37
Figure S35. ^{1}H-NMR spectrum of bohemamine F (**5**) in DMSO-d_6.....S38
Figure S36. ^{13}C-NMR spectrum of bohemamine F (**5**) in DMSO-d_6.....S39
Figure S37. ^{1}H-^{1}H COSY spectrum of bohemamine F (**5**) in DMSO-d_6.....S40
Figure S38. HSQC spectrum of bohemamine F (**5**) in DMSO-d_6.....S41
Figure S39. HMBC spectrum of bohemamine F (**5**) in DMSO-d_6.....S42
Figure S40. NOESY spectrum of bohemamine F (**5**) in DMSO-d_6.....S43
Figure S41. HRESIMS spectrum of bohemamine G (**6**) S44
Figure S42. ^{1}H-NMR spectrum of bohemamine G (**6**) in DMSO-d_6.....S45
Figure S43. ^{13}C-NMR spectrum of bohemamine G (**6**) in DMSO-d_6.....S46
Figure S44. ^{1}H-^{1}H COSY spectrum of bohemamine G (**6**) in DMSO-d_6.....S47
Figure S45. HSQC spectrum of bohemamine G (**6**) in DMSO-d_6.....S48
Figure S46. HMBC spectrum of bohemamine G (**6**) in DMSO-d_6.....S49
Figure S47. NOESY spectrum of bohemamine G (**6**) in DMSO-d_6.....S50
Figure S48. HRESIMS spectrum of bohemamine H (**7**) S51
Figure S49. ^{1}H-NMR spectrum of bohemamine H (**7**) in DMSO-d_6.....S52
Figure S50. ^{13}C-NMR spectrum of bohemamine H (**7**) in DMSO-d_6.....S53
Figure S51. ^{1}H-^{1}H COSY spectrum of bohemamine H (**7**) in DMSO-d_6.....S54
Figure S52. HSQC spectrum of bohemamine H (**7**) in DMSO-d_6.....S55
Figure S53. HMBC spectrum of bohemamine H (**7**) in DMSO-d_6.....S56
Figure S54. NOESY spectrum of bohemamine H (**7**) in DMSO-d_6.....S57

Figure S55. HRESIMS spectrum of bohemamine I (**8**) ……S58
Figure S56. ^{1}H-NMR spectrum of bohemamine I (**8**) in DMSO-d_6……S59
Figure S57. ^{13}C-NMR spectrum of bohemamine I (**8**) in DMSO-d_6……S60
Figure S58. ^{1}H-^{1}H COSY spectrum of bohemamine I (**8**) in DMSO-d_6……S61
Figure S59. HSQC spectrum of bohemamine I (**8**) in DMSO-d_6……S62
Figure S60. HMBC spectrum of bohemamine I (**8**) in DMSO-d_6……S63
Figure S61. NOESY spectrum of bohemamine I (**8**) in DMSO-d_6……S64

Bioassay Protocols

Antibiotic assays. The antibiotic activities against *Pseudomonas aeruginosa* and *Bacillus subtilis* were evaluated by an agar dilution method. The tested strains were cultivated in LB agar plates at 37 °C. Compounds **1**–**9**, and positive control (erythromycin) were dissolved in MeOH at different concentrations from 100 to 0.1 μg/mL by the continuous 10-fold dilution methods. A 10 μL quantity of test solution was absorbed by a paper disk (5 mm diameter) and placed on the assay plates. After 24 h incubation, zones of inhibition (mm in diameter) were recorded.

Cytotoxicity assays. Cell lines were cultivated in 10 cm dishes (Corning, Inc.) in NSCLC cell-culture medium: RPMI/L-glutamine medium (Invitrogen, Inc.), 1000 U/mL penicillin (Invitrogen, Inc.), 1 mg/mL streptomycin (Invitrogen, Inc.), and 5% fetal bovine serum (Atlanta Biologicals, Inc.). Cell lines were grown in a humidified environment in the presence of 5% CO_2 at 37 °C. For cell viability assays, HCC366, A549, HCC44 and HCC1171 cells (60 μL) were plated individually at a density of 1200, 750 and 500 cells/well, respectively, in 384-well microtiter assay plates (Bio-one; Greiner, Inc.). After incubating the assay plates overnight under the growth conditions described above, purified compounds were dissolved and diluted in DMSO and subsequently added to each plate with final compound concentrations ranging from 50 μM to 1 nM and a final DMSO concentration of 0.5%. After an incubation of 96 h under growth conditions, Cell Titer Glo reagent (Promega, Inc.) was added to each well (10 mL of a 1:2 dilution in NSCLC culture medium) and mixed. Plates were incubated for 10 min at room temperature, and luminescence was determined for each well using an Envision multimodal plate reader (Perkin-Elmer, Inc.). Relative luminescence units were normalized to the untreated control wells (cells plus DMSO only). Data were analyzed using the Assay Analyzer and Condoseo modules of the Screener Software Suite (GeneData, Inc.) as described previously.[S1]

Theory and Calculation Details. The calculations were performed by using the density functional theory (DFT) as carried out in the Gaussian 03.[S2] The preliminary conformational distributions search was performed by HyperChem 7.5 software. All ground-state geometries were optimized at the B3LYP/6-31G(d) level. Solvent effects of methanol solution were evaluated at the same DFT level by using the SCRF/PCM method.[S3] TDDFT[S4] at B3LYP/6-31G(d) was employed to calculate the electronic excitation energies and rotational strengths in methanol. The stable conformations obtained at the B3LYP/6-31G(d) level were further used in magnetic shielding constants at the B3LYP/6-311++G(2d,p) level.

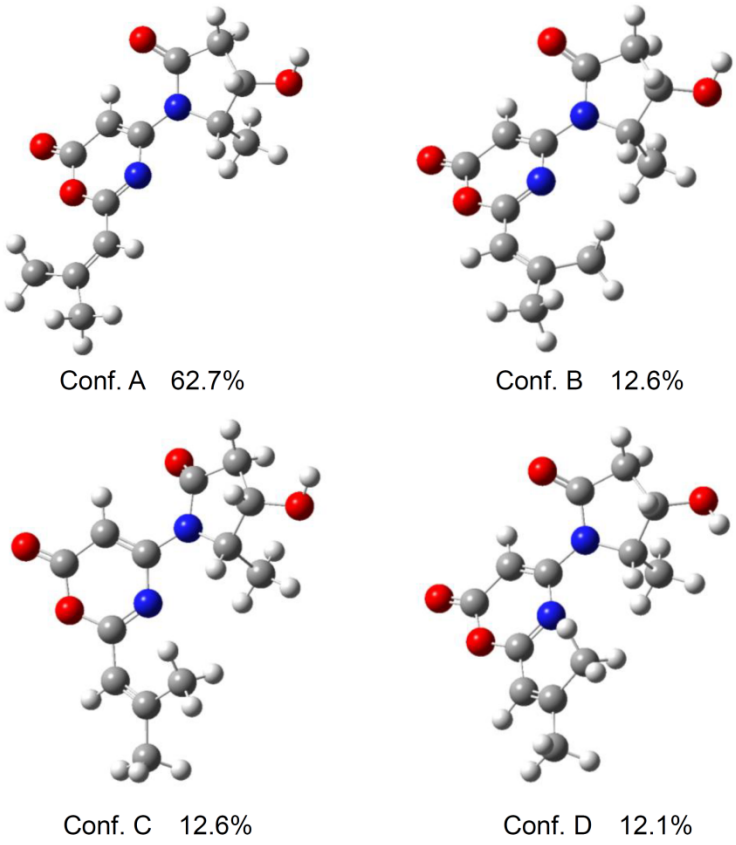

Figure S1. DFT optimized geometries of the four lowest-energy conformers of (4*S*, 5*S*)-**1**

(S1) Kim, H. S.; Mendiratta, S.; Kim, J.; Pecot, C. V.; Larsen, J. E.; Zubovych, I.; Seo, B. Y.; Kim, J.; Eskiocak, B.; Chung, H.; McMillan, E.; Wu, S.; De Brabander J; Komurov, K.; Toombs, J. E.; Wei, S.; Peyton, M.; Williams, N.; Gazdar, A. F.; Posner, B. A.; Brekken, R. A.; Sood, A. K.; Deberardinis, R. J.; Roth, M. G.; Minna, J. D.; White, M. A. *Cell* **2013**, *155*, 552–566.

(S2) Gaussian 03, Revision E.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, and J. A. Pople, Gaussian, Inc., Wallingford CT, 2004.

(S3) (a) Miertus, S.; Tomasi, J. *Chem. Phys.* **1982**, *65*, 239–245. (b) Tomasi, J.; Persico, M. *Chem. Rev.* **1994**, *94*, 2027–2094. (c) Cammi, R.; Tomasi, J. *J. Comp. Chem.* **1995**, *16*, 1449–1458.

(S4) (a) Casida, M. E. In Recent Advances in Density Functional Methods, part I; Chong, D. P., Eds.; World Scientific: Singapore, 1995; pp 155–192. (b) Gross, E. K. U.; Dobson, J. F.; Petersilka, M. Top. *Curr. Chem.* **1996**, *181*, 81–172. (c) Gross, E. K. U.; Kohn, W. Adv. Quantum Chem. **1990**, *21*, 255–291. (d) Runge, E.; Gross, E. K. U. *Phys. Rev. Lett.* **1984**, *52*, 997–1000.

Table S1. The calculated ^{13}C NMR Data for **1** and **1a**

position	measured **1**	Calcd. **1**	Corr. **1**	Error **1**	Calcd. **1a**	Corr. **1a**	Error **1a**
1	159.8	163.32	155.7235	4.076468	173.07	163.8759	-4.07586
2	89	95.78	90.54068	-1.54068	94.14	88.71082	0.289178
3	155.3	162.2	154.6426	0.65738	174.23	164.9805	-9.68053
4	57.7	62.76	58.67308	-0.97308	66.85	62.72256	-5.02256
5	64.1	72.98	68.5364	-4.4364	73.08	68.65538	-4.55538
6	39.3	45.32	41.84173	-2.54173	40.57	37.69611	1.603889
7	173.4	177.12	169.0419	4.358088	178.47	169.0183	4.381719
8	13.3	11.91	9.597741	3.702259	14.81	13.16486	0.135137
1'	163.7	173.55	165.5965	-1.89651	173.9	164.6663	-0.96627
2'	116.2	124.56	118.3163	-2.11626	122.83	116.0323	0.167691
3'	158.7	169	161.2053	-2.5053	170.71	161.6284	-2.92843
4'	28	30.57	27.60651	0.393493	30.02	27.64935	0.350654
5'	21	20.84	18.21608	2.783916	20.5	18.58345	2.41655

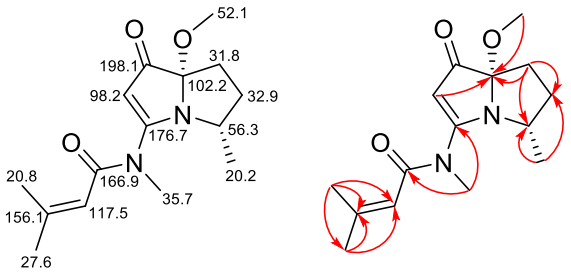

Figure S2. ^{13}C and 2D NMR of **3a** (These data were from HMBC and HSQC spectra).

Figure S3. HRESIMS spectrum of spinoxazine A (**1**)

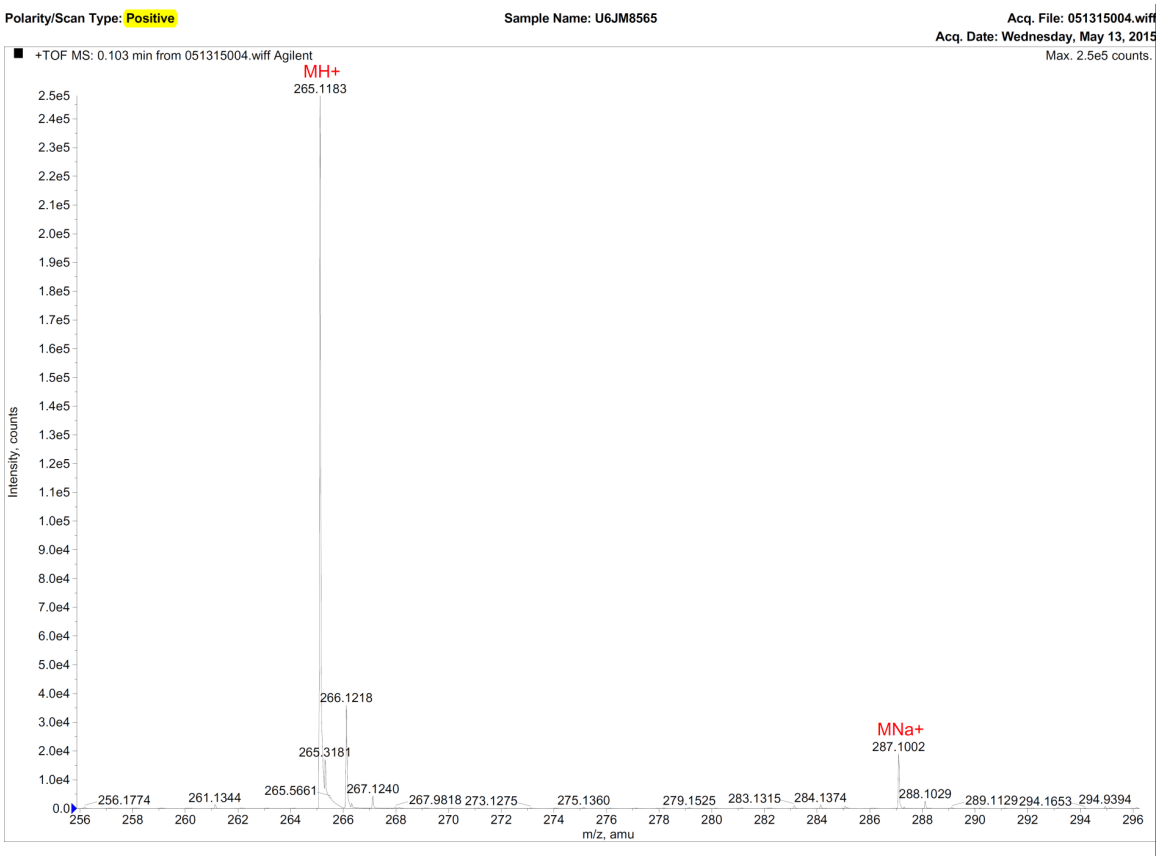

Figure S4. ^{1}H-NMR spectrum of spinoxazine A (**1**) in DMSO-d_6

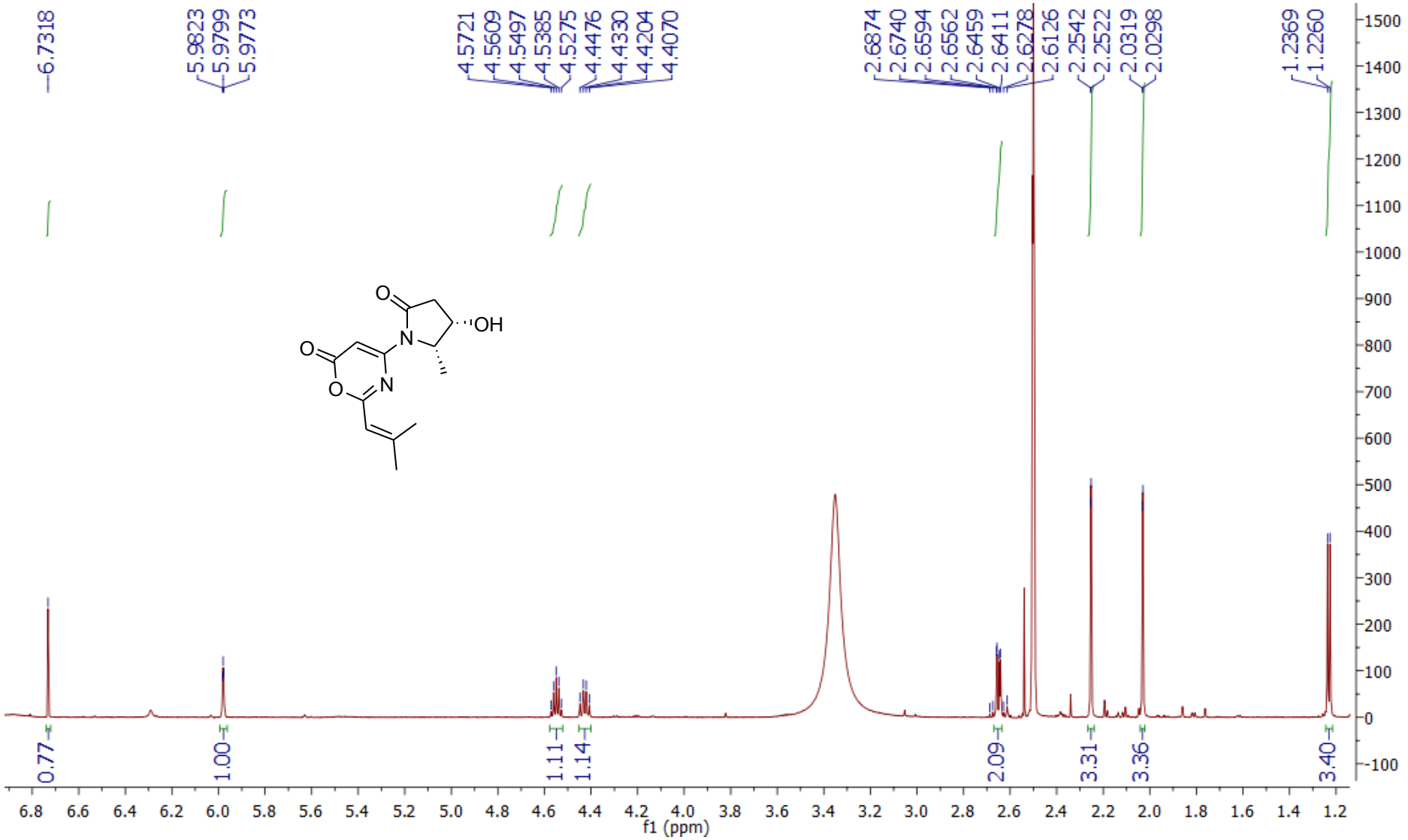

Figure S5. ^{13}C-NMR spectrum of spinoxazine A (**1**) in DMSO-d_6

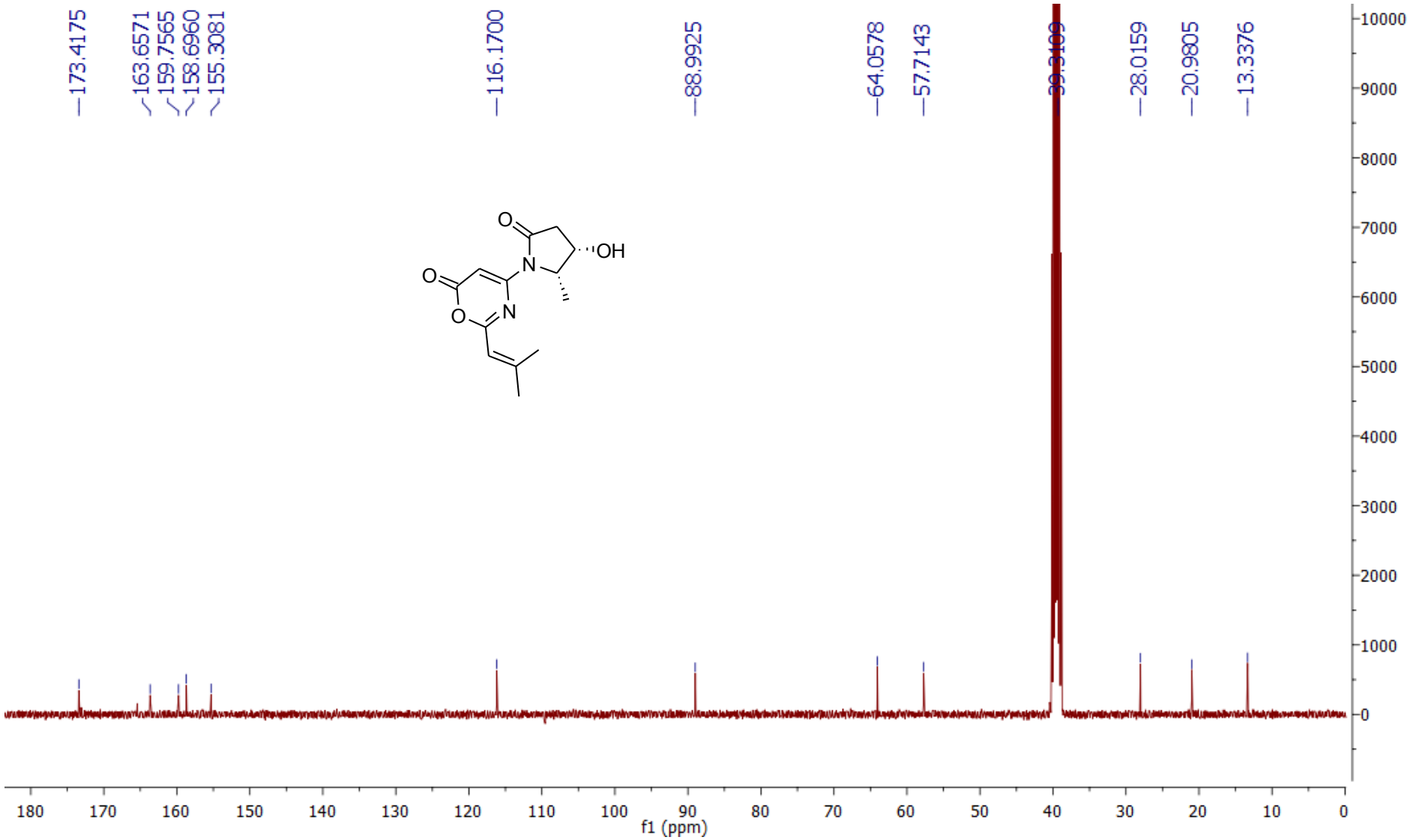

Figure S6. HSQC spectrum of spinoxazine A (**1**) in DMSO-d_6

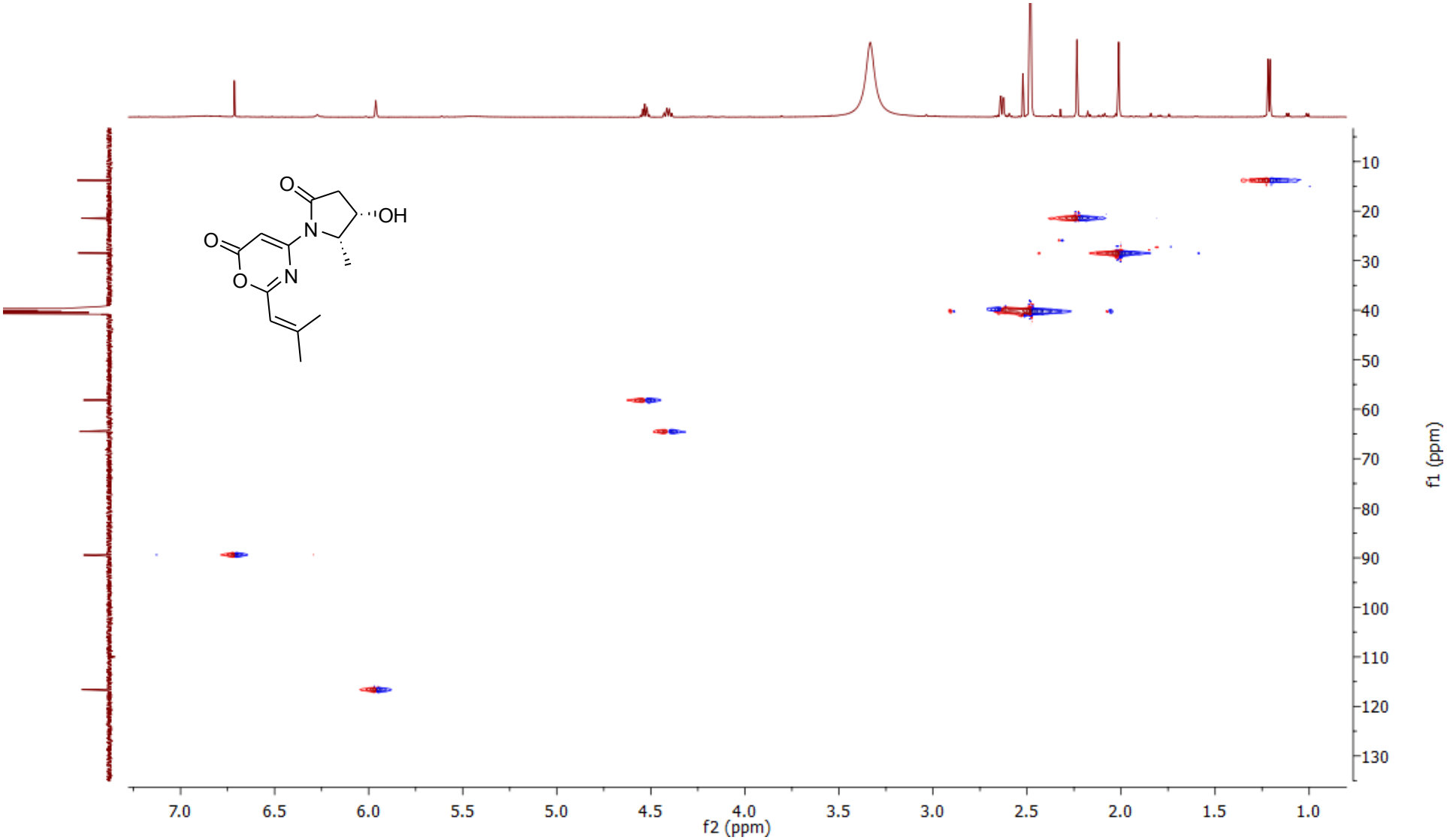

Figure S7. ^{1}H-^{1}H COSY spectrum of spinoxazine A (**1**) in DMSO-d_6

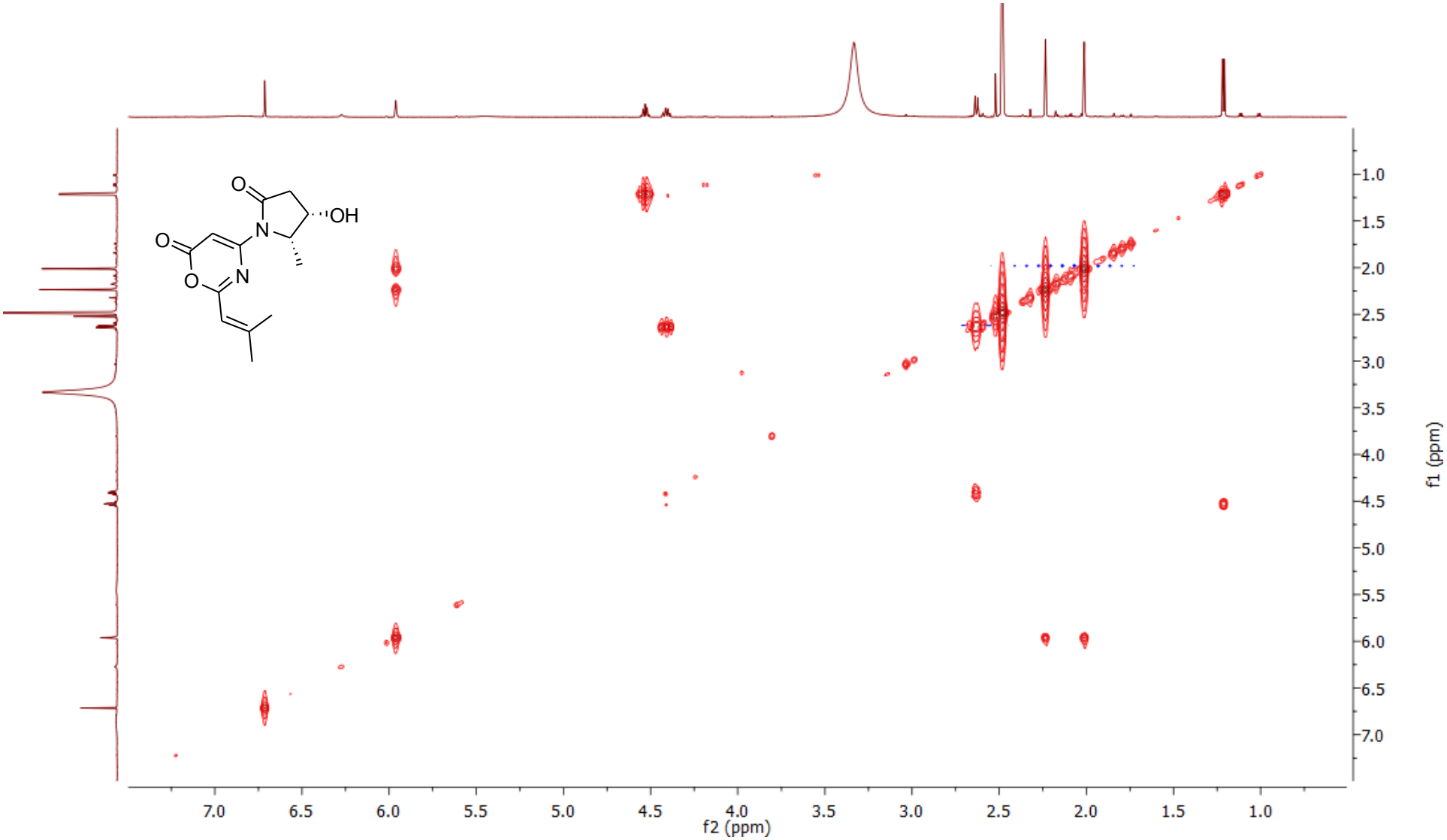

Figure S8. HMBC spectrum of spinoxazine A (**1**) in DMSO-d_6

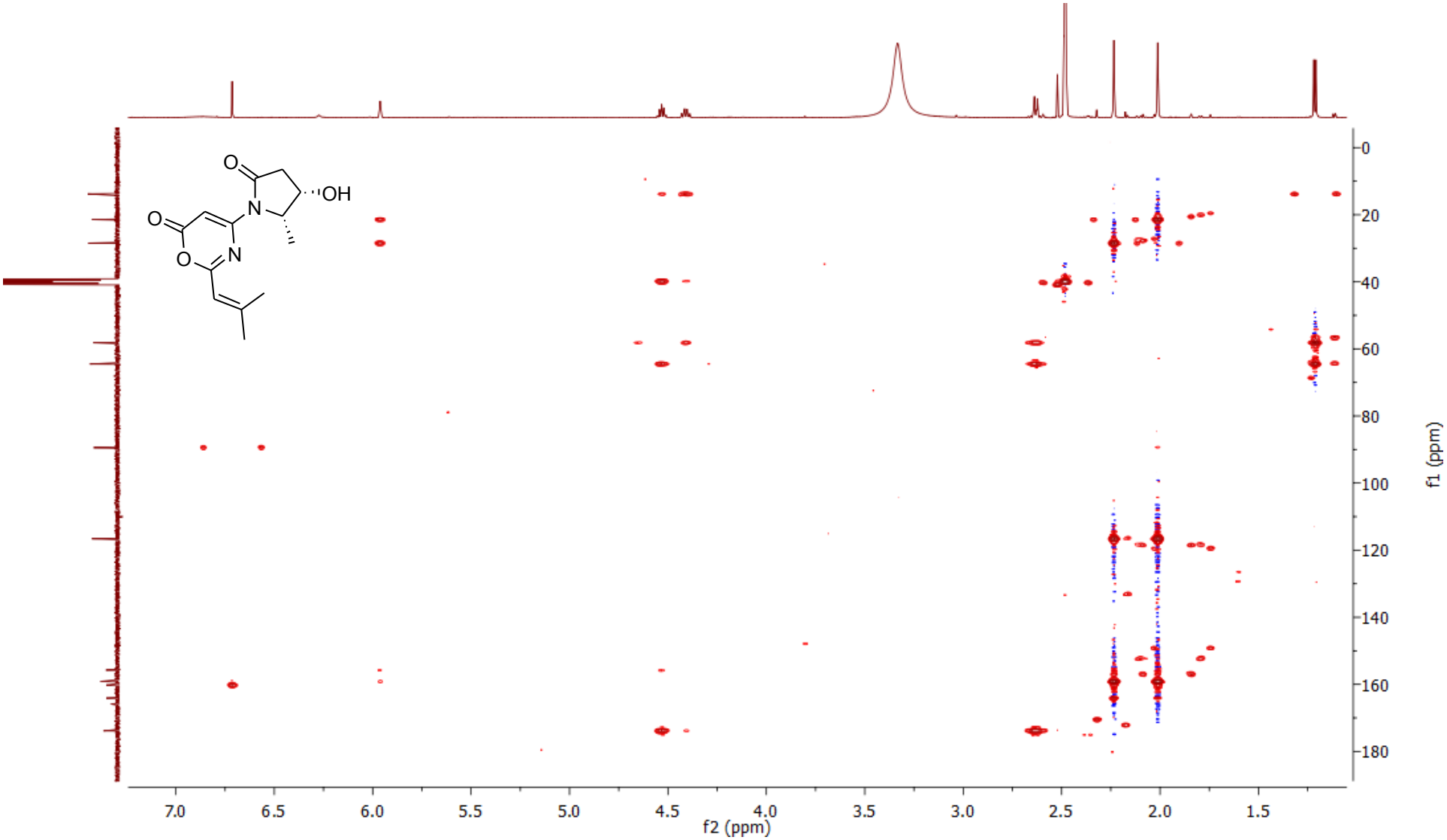

Figure S9. NOESY spectrum of spinoxazine A (**1**) in DMSO-d_6

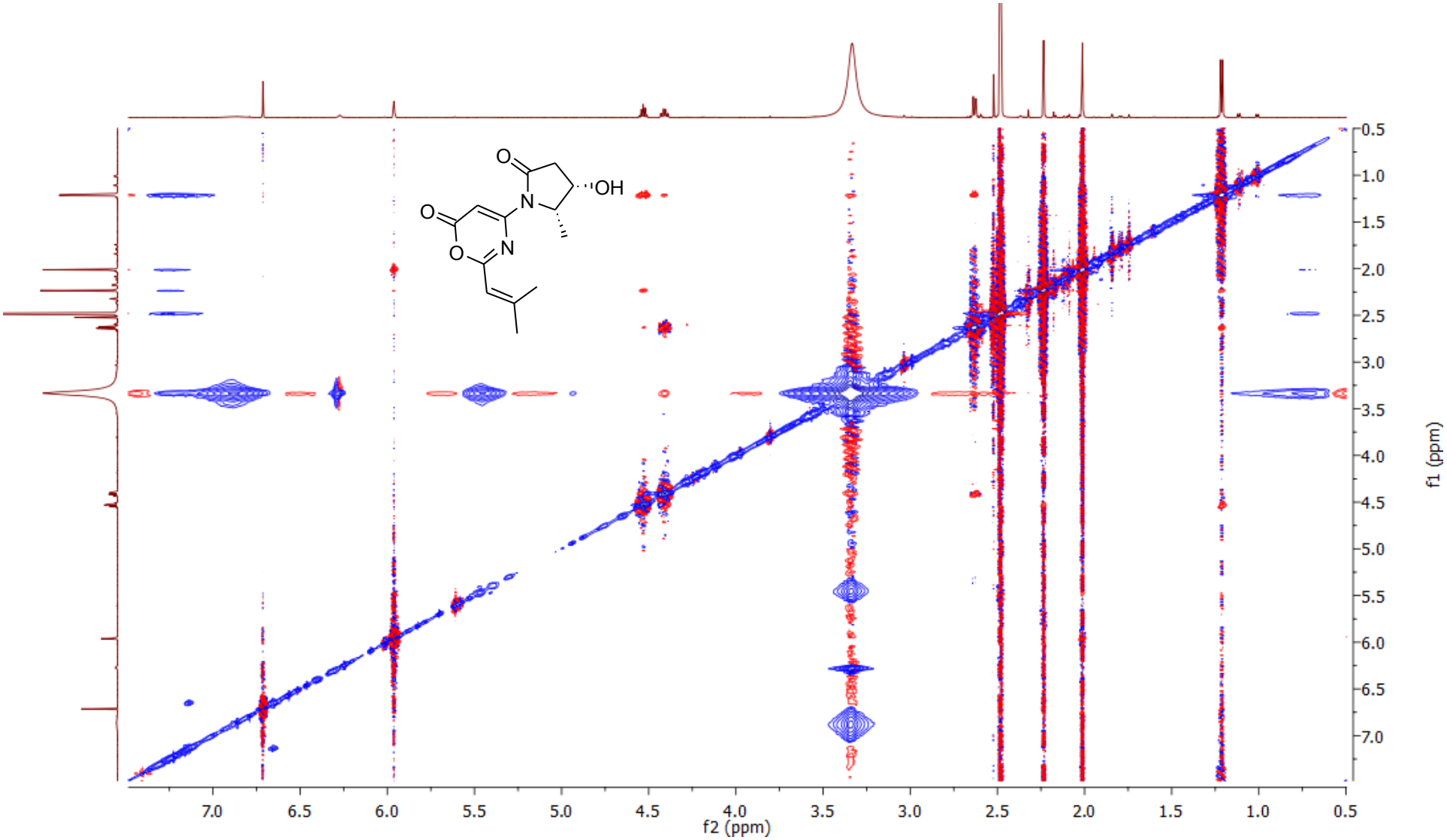

Figure S10. HRESIMS spectrum of spinoxazine B (**2**)

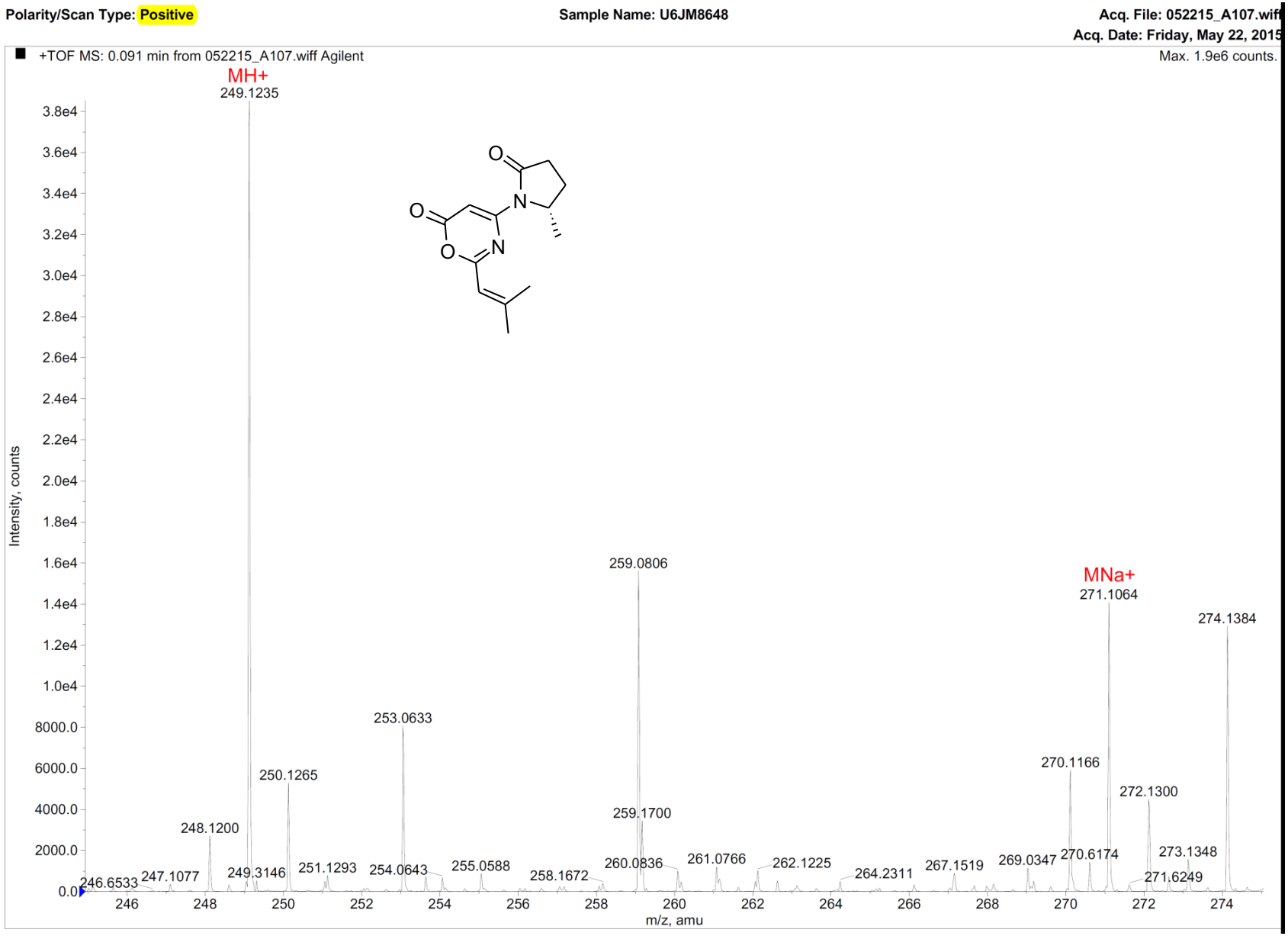

Figure S11. ^{1}H-NMR spectrum of spinoxazine B (**2**) in DMSO-d_6

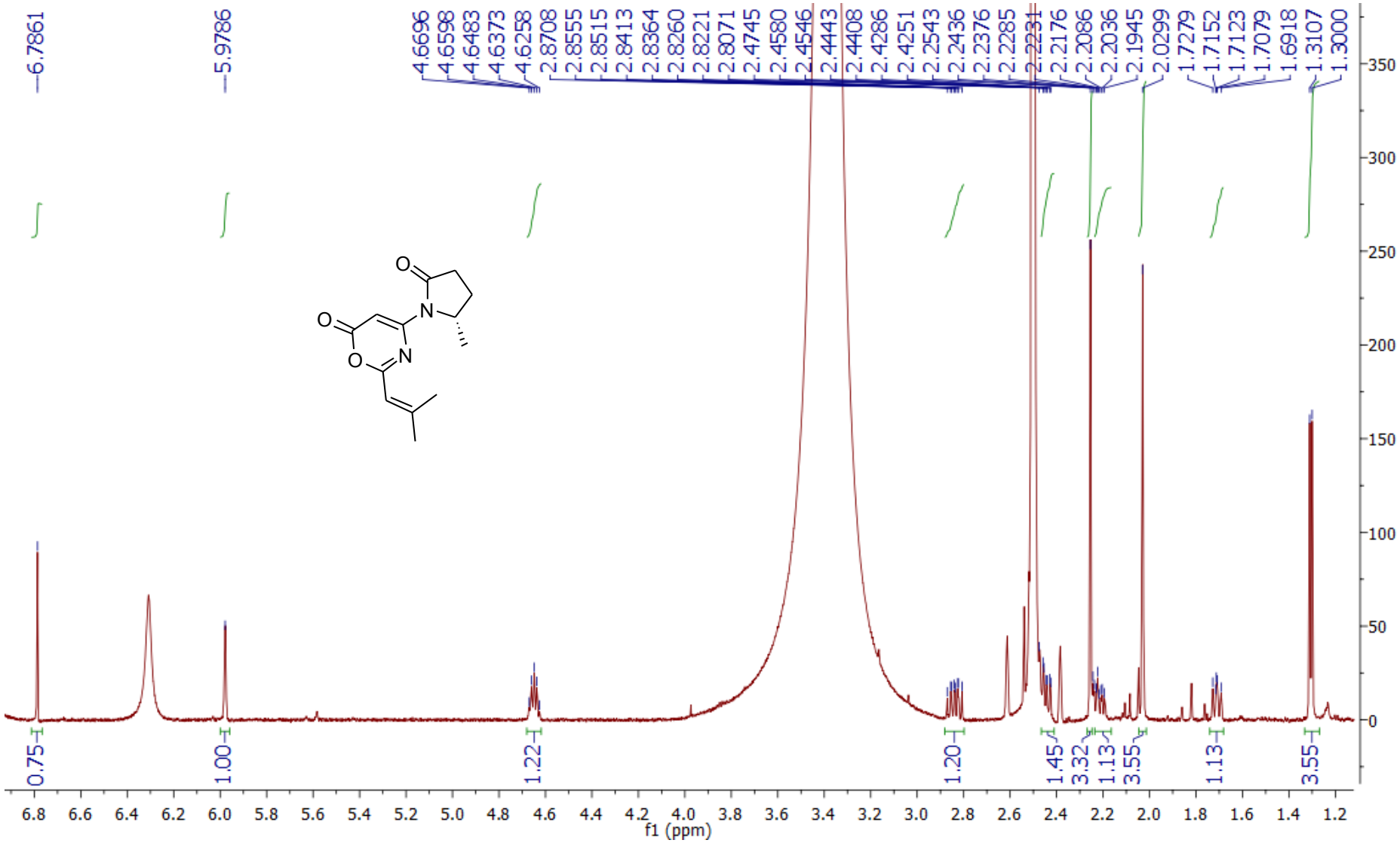

Figure S12. ^{13}C-NMR spectrum of spinoxazine B (**2**) in DMSO-d_6

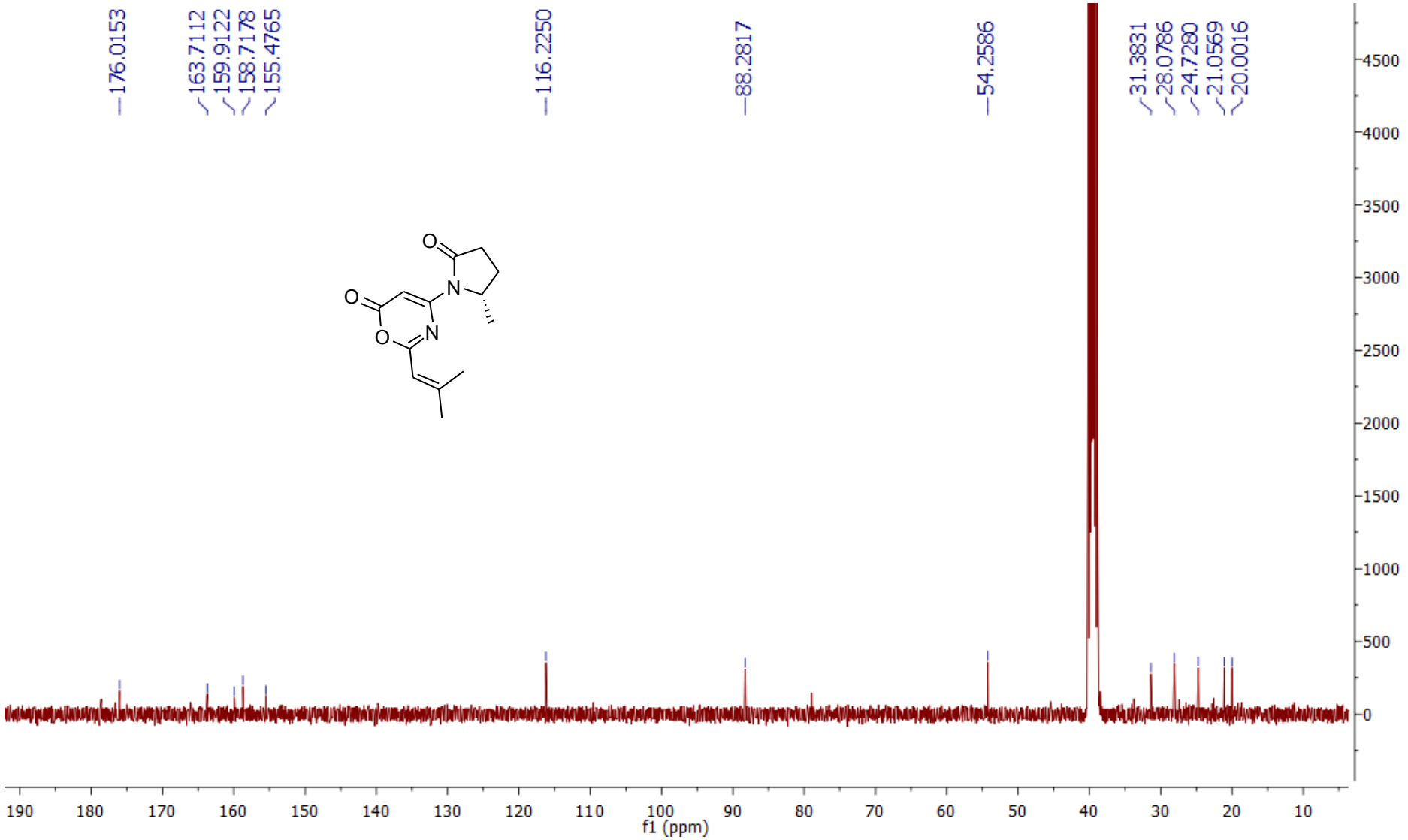

Figure S13. HSQC spectrum of spinoxazine B (**2**) in DMSO-d_6

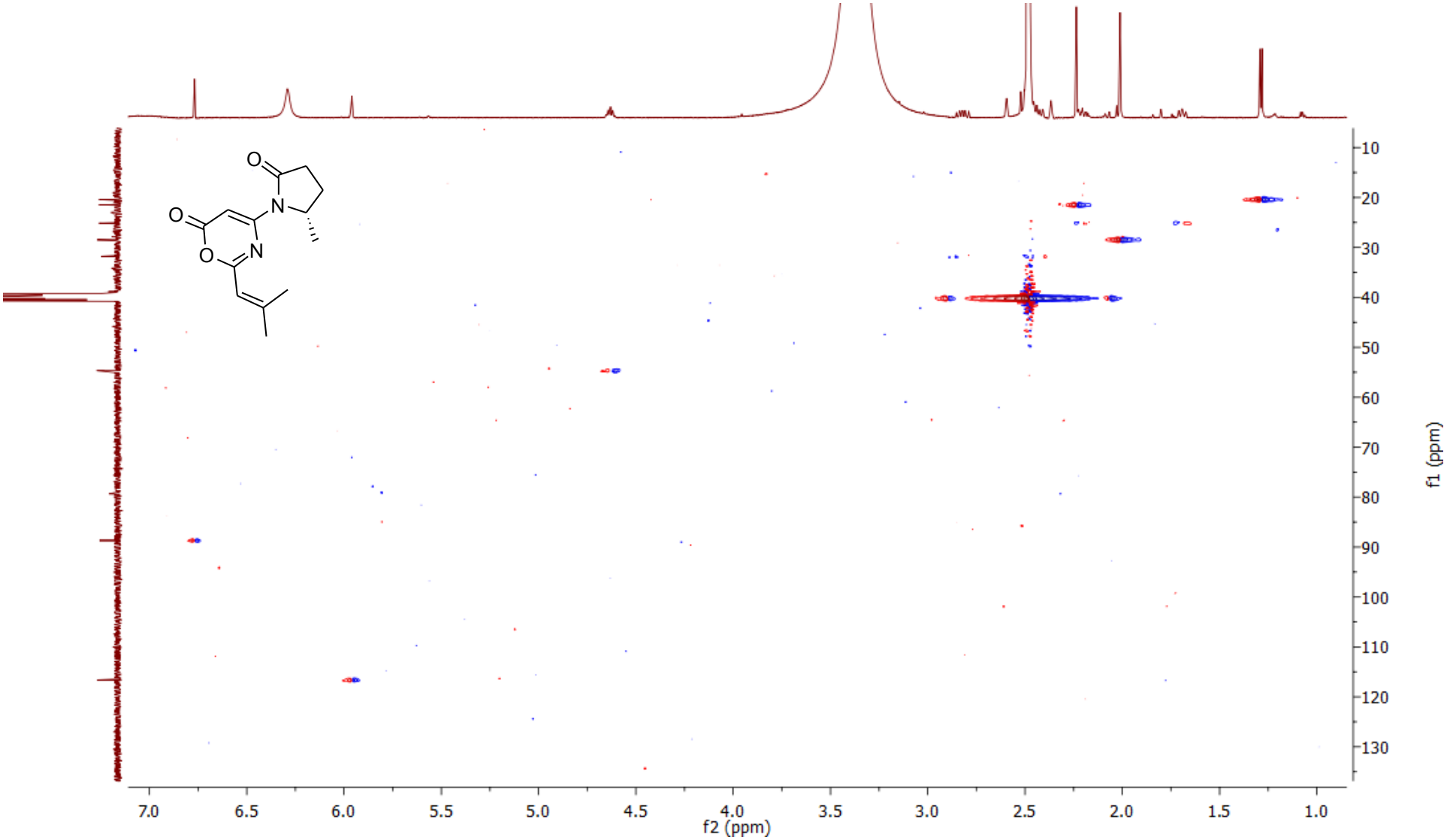

Figure S14. ^{1}H-^{1}H COSY spectrum of spinoxazine B (**2**) in DMSO-d_6

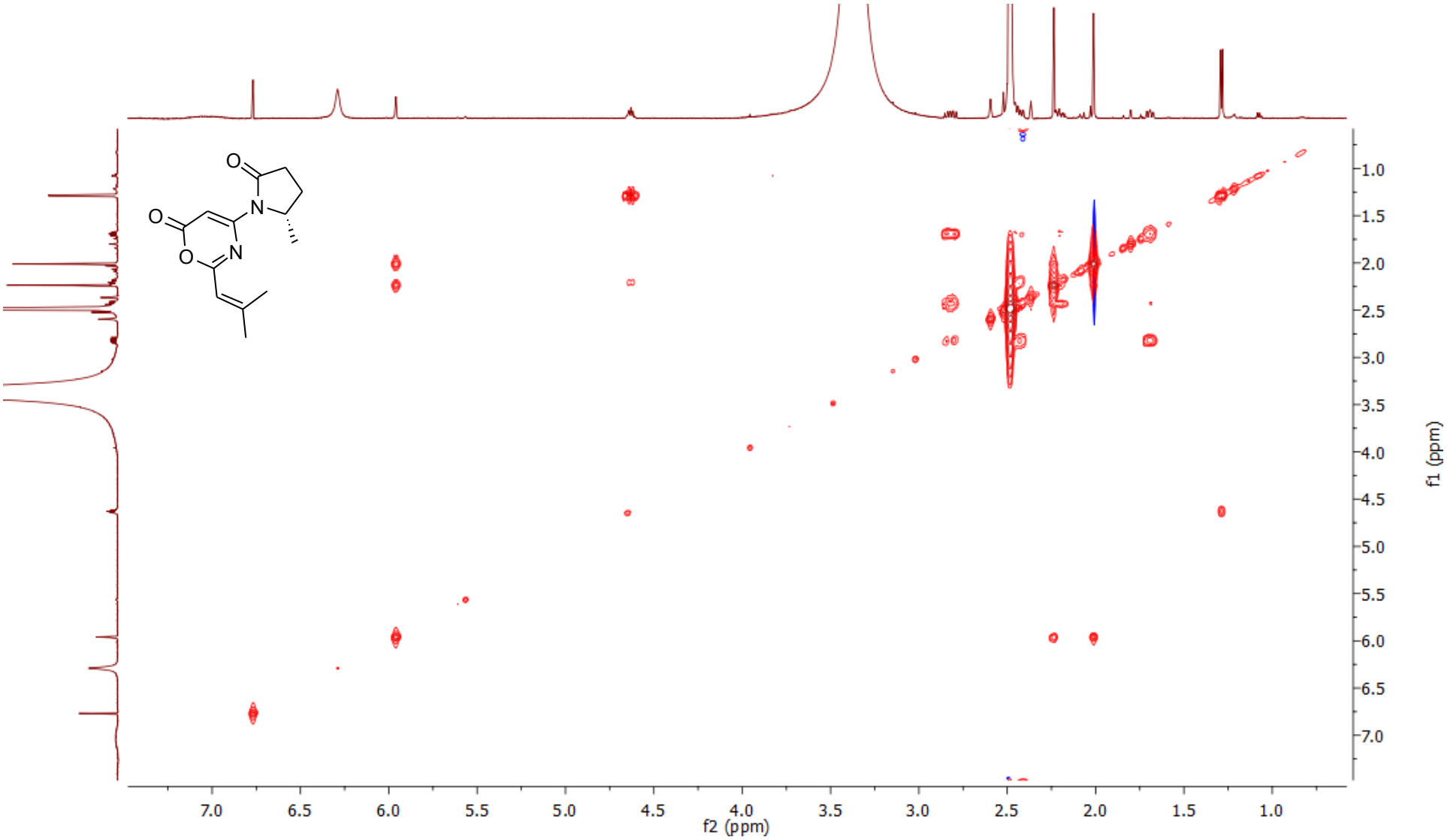

Figure S15. HMBC spectrum of spinoxazine B (**2**) in DMSO-d_6

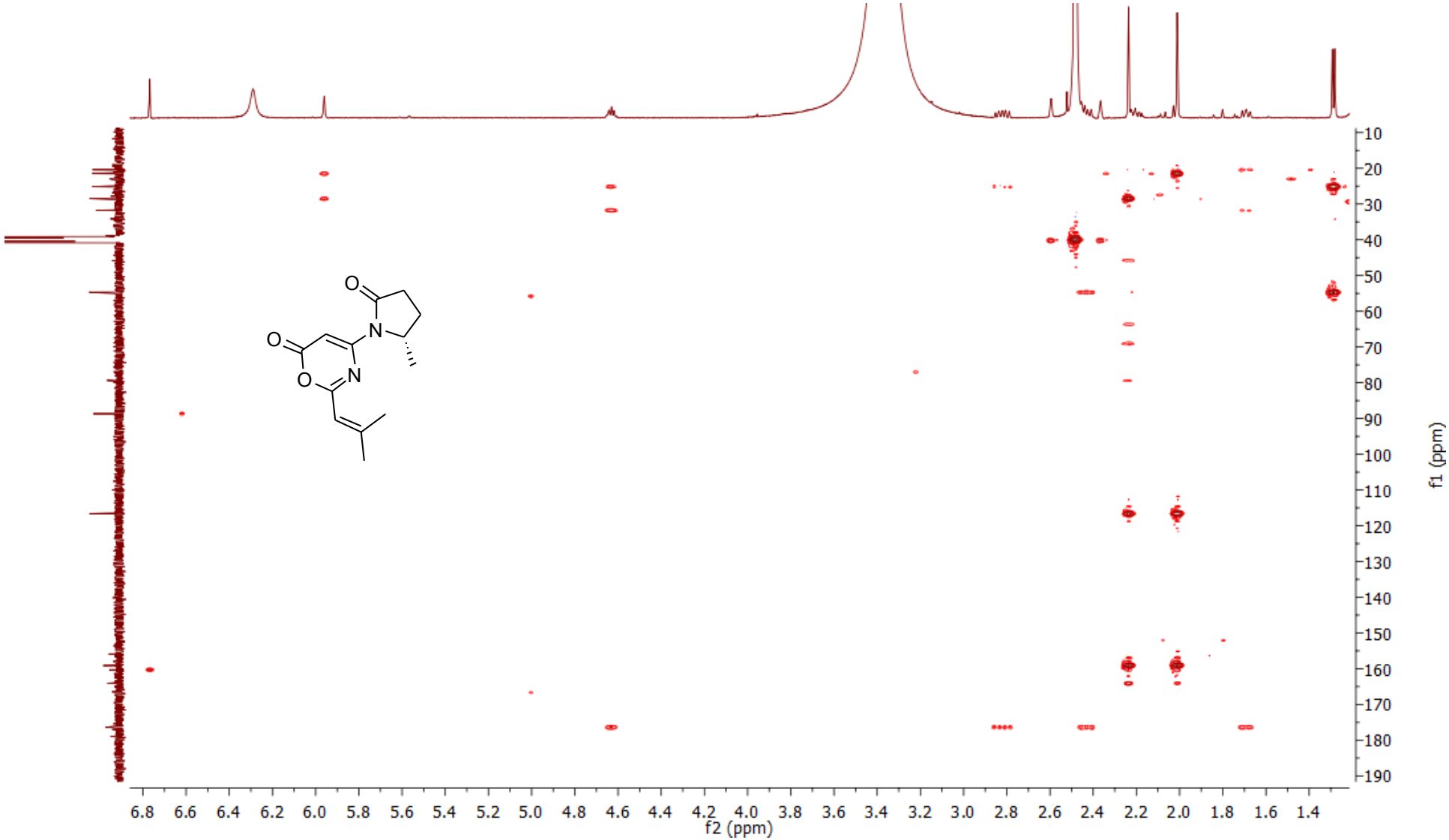

Figure S16. HRESIMS spectrum of bohemamine D (**3**)

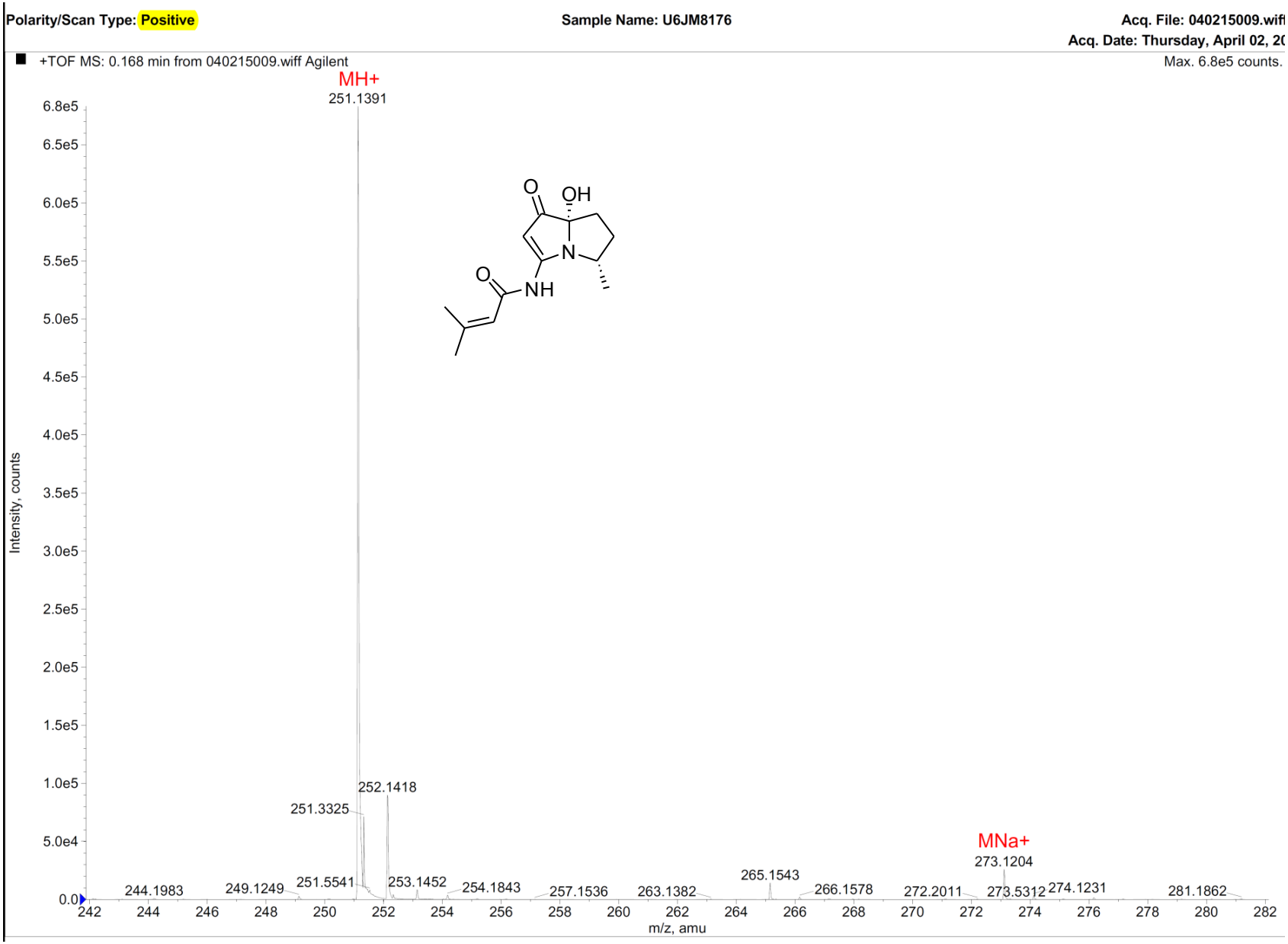

Figure S17. ^{1}H-NMR spectrum of bohemamine D (**3**) in DMSO-d_6

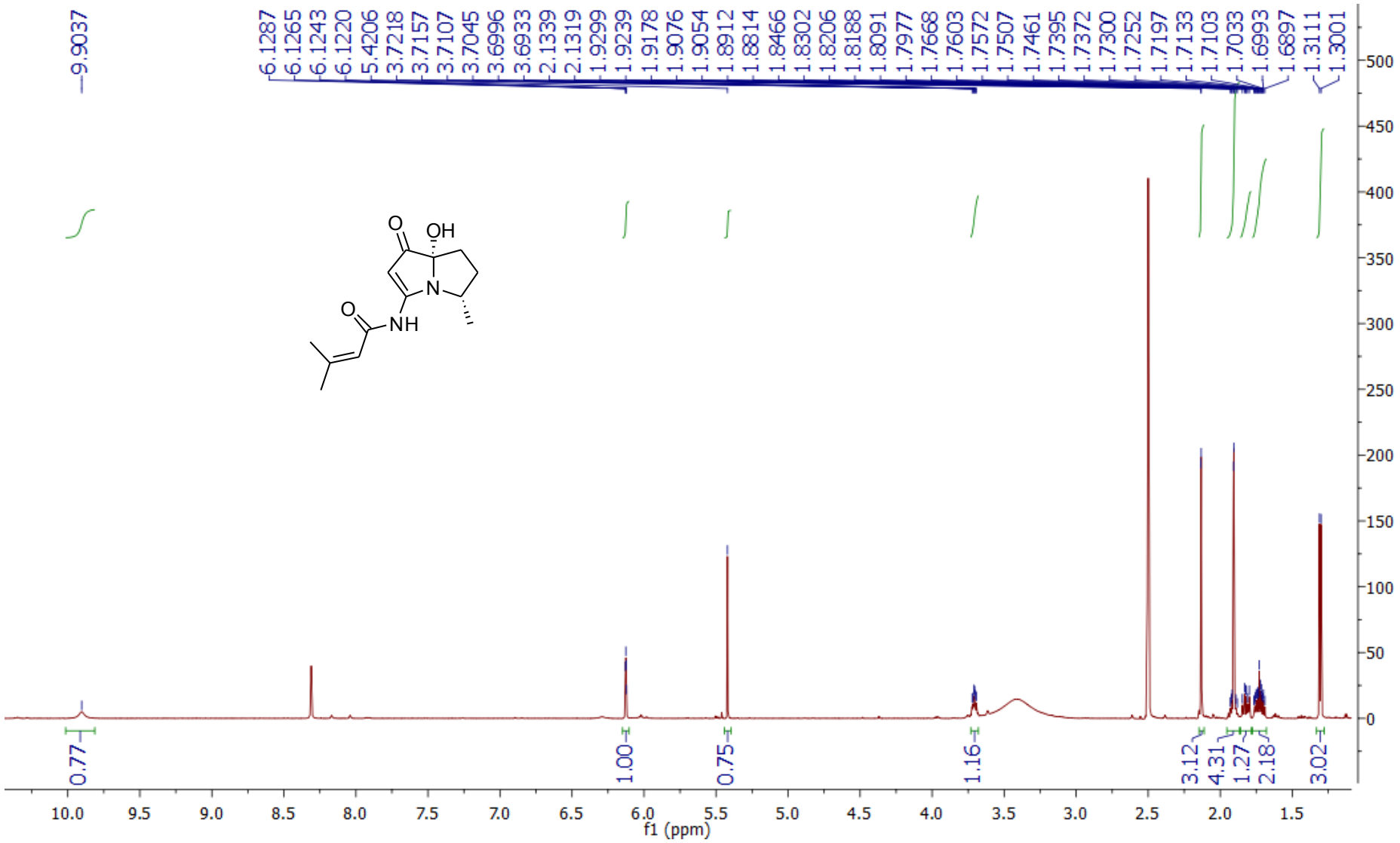

Figure S18. ^{13}C-NMR spectrum of bohemamine D (**3**) in DMSO-d_6

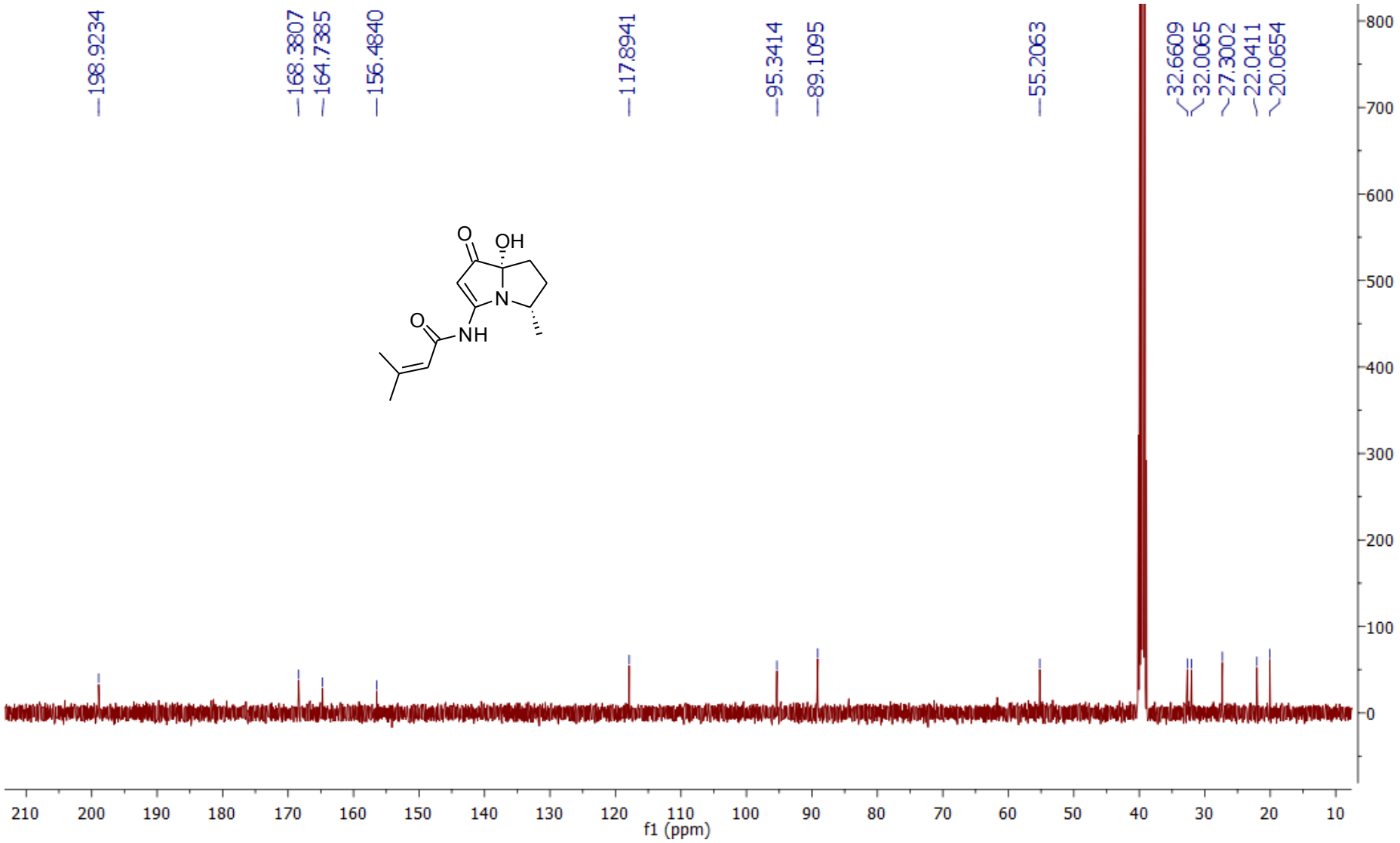

Figure S19. HSQC spectrum of bohemamine D (**3**) in DMSO-d_6

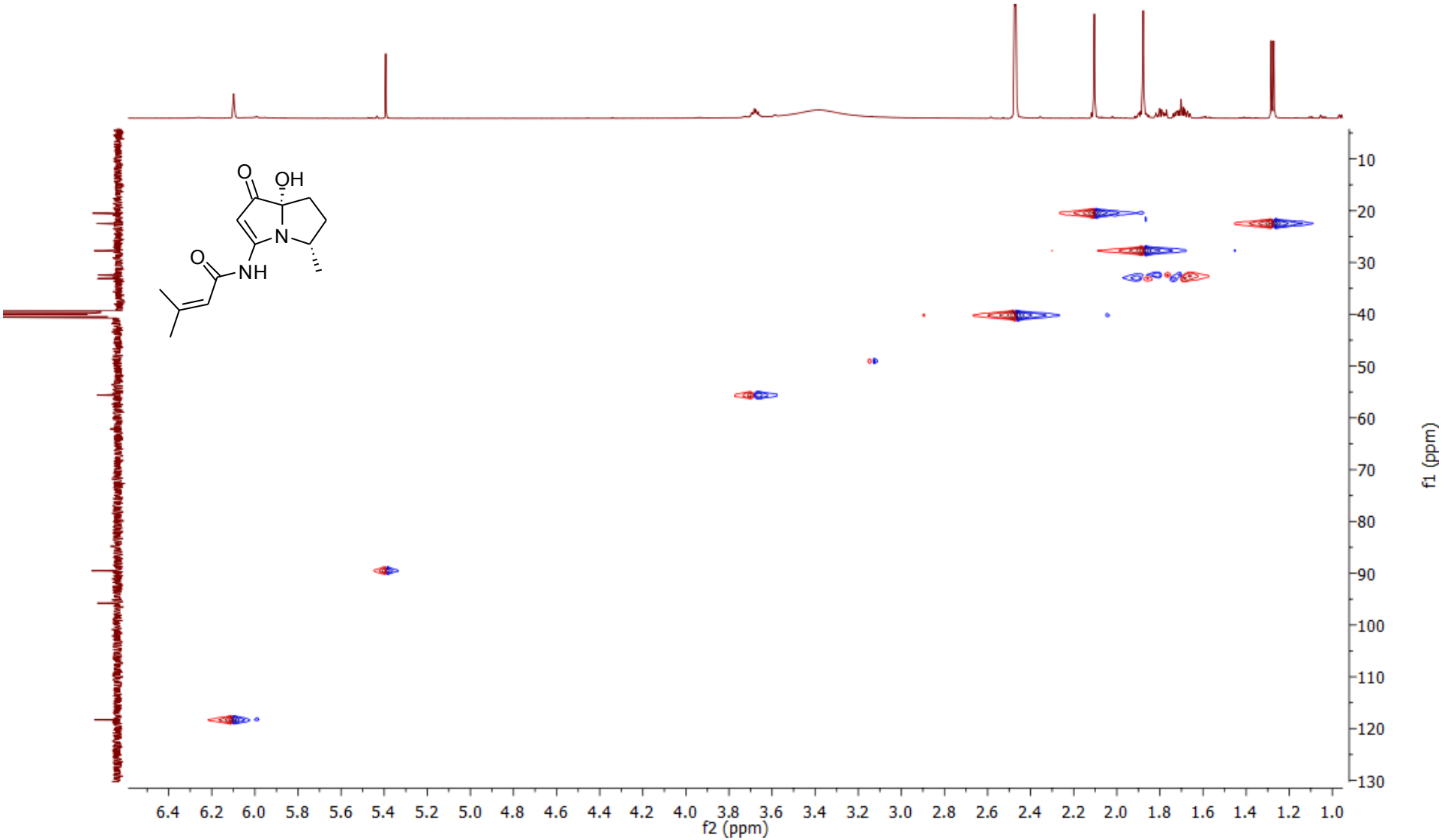

Figure S20. ^{1}H-^{1}H COSY spectrum of bohemamine D (**3**) in DMSO-d_6

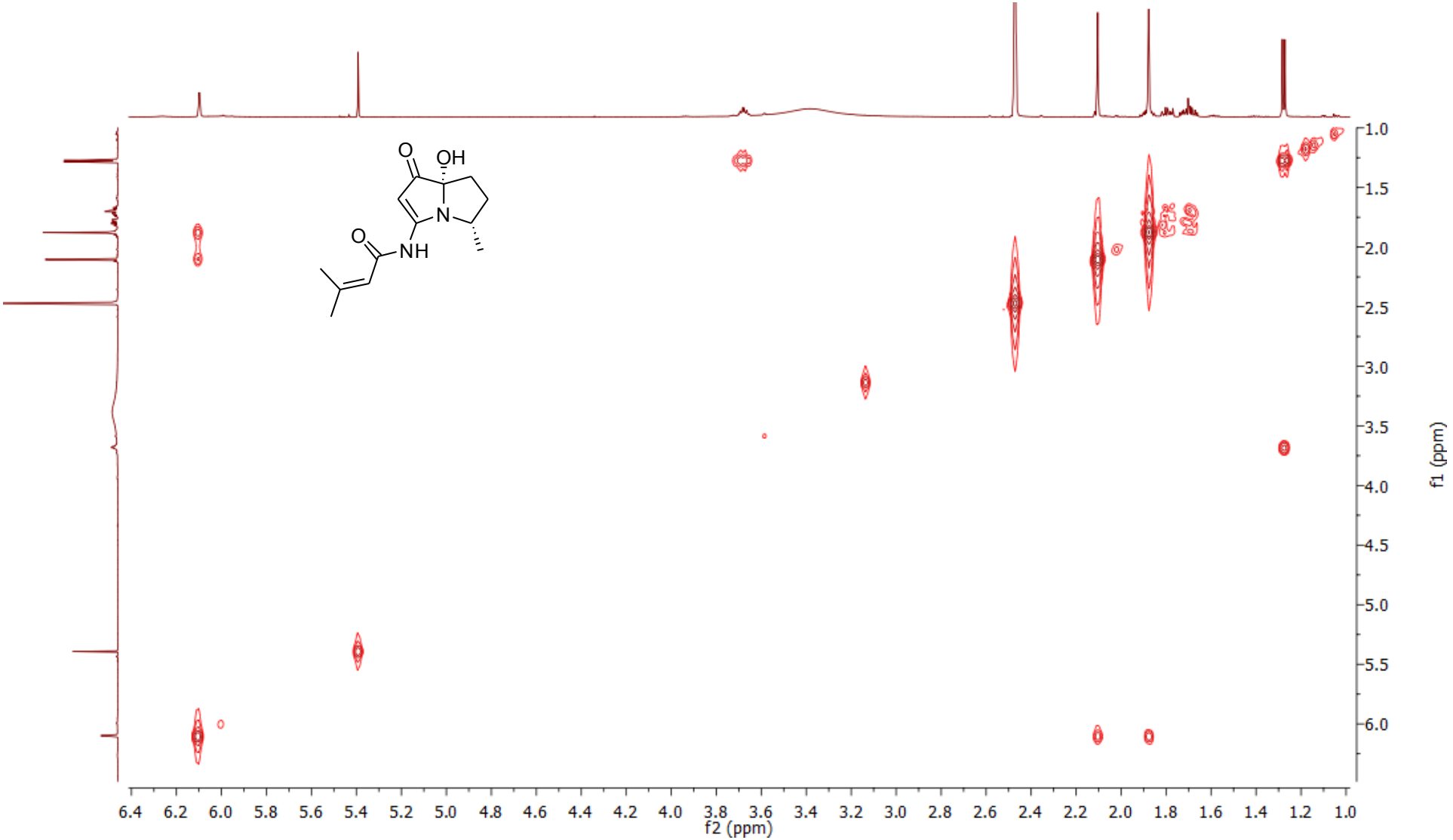

Figure S21. HMBC spectrum of bohemamine D (**3**) in DMSO-d_6

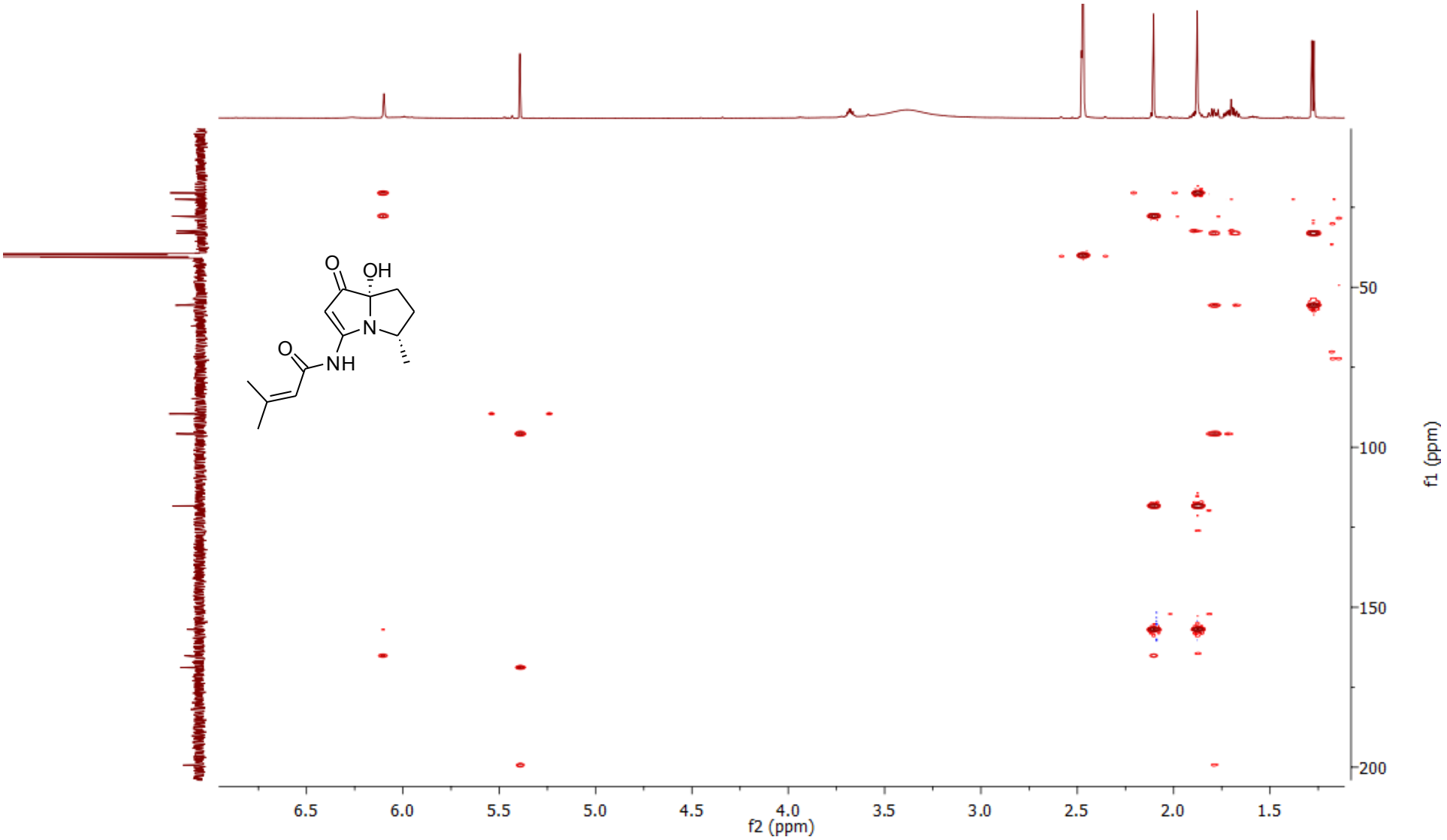

Figure S22. HRESIMS spectrum of compound **3a**

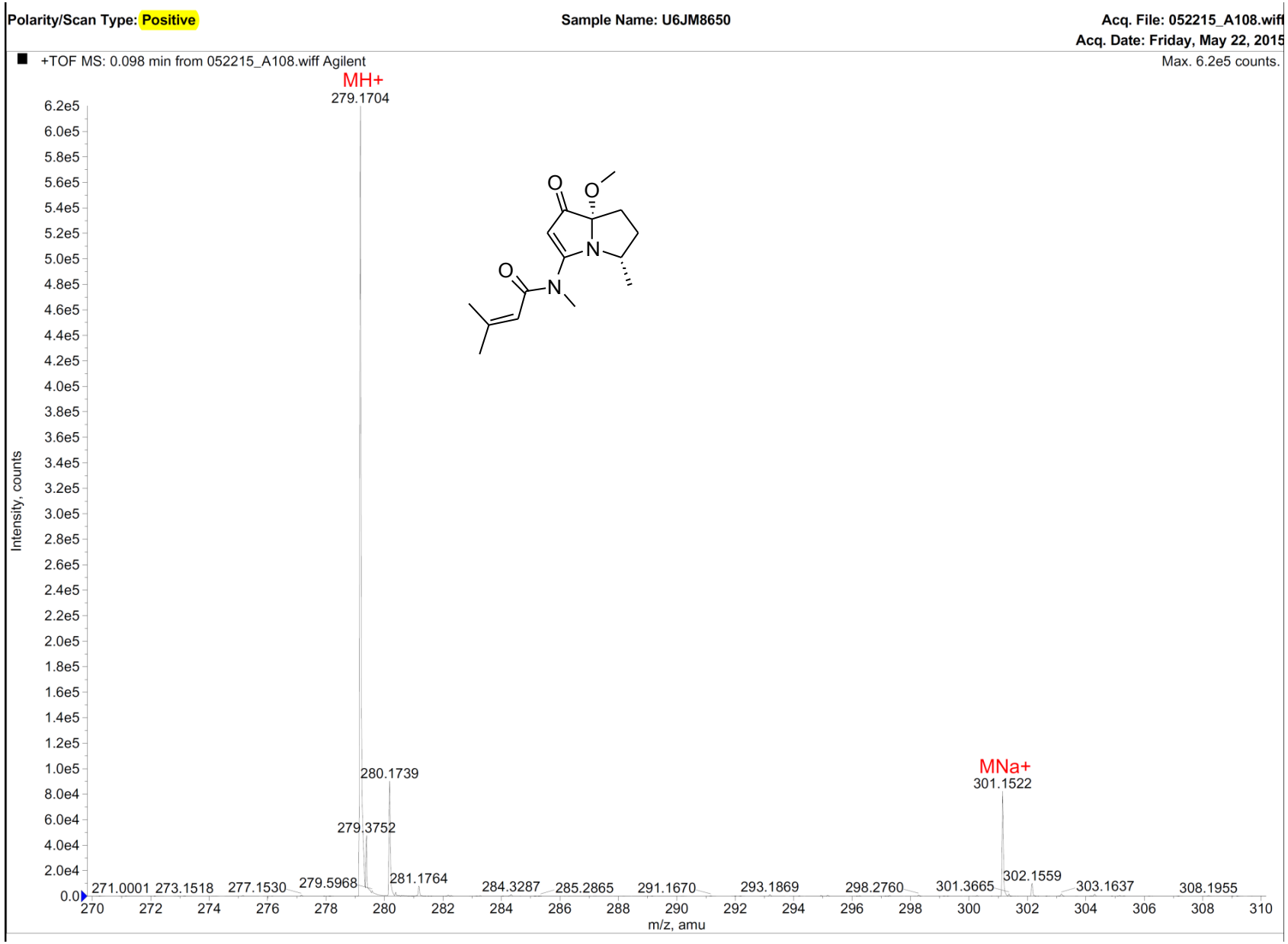

Figure S23. ^{1}H-NMR spectrum of compound **3a** in $CDCl_3$

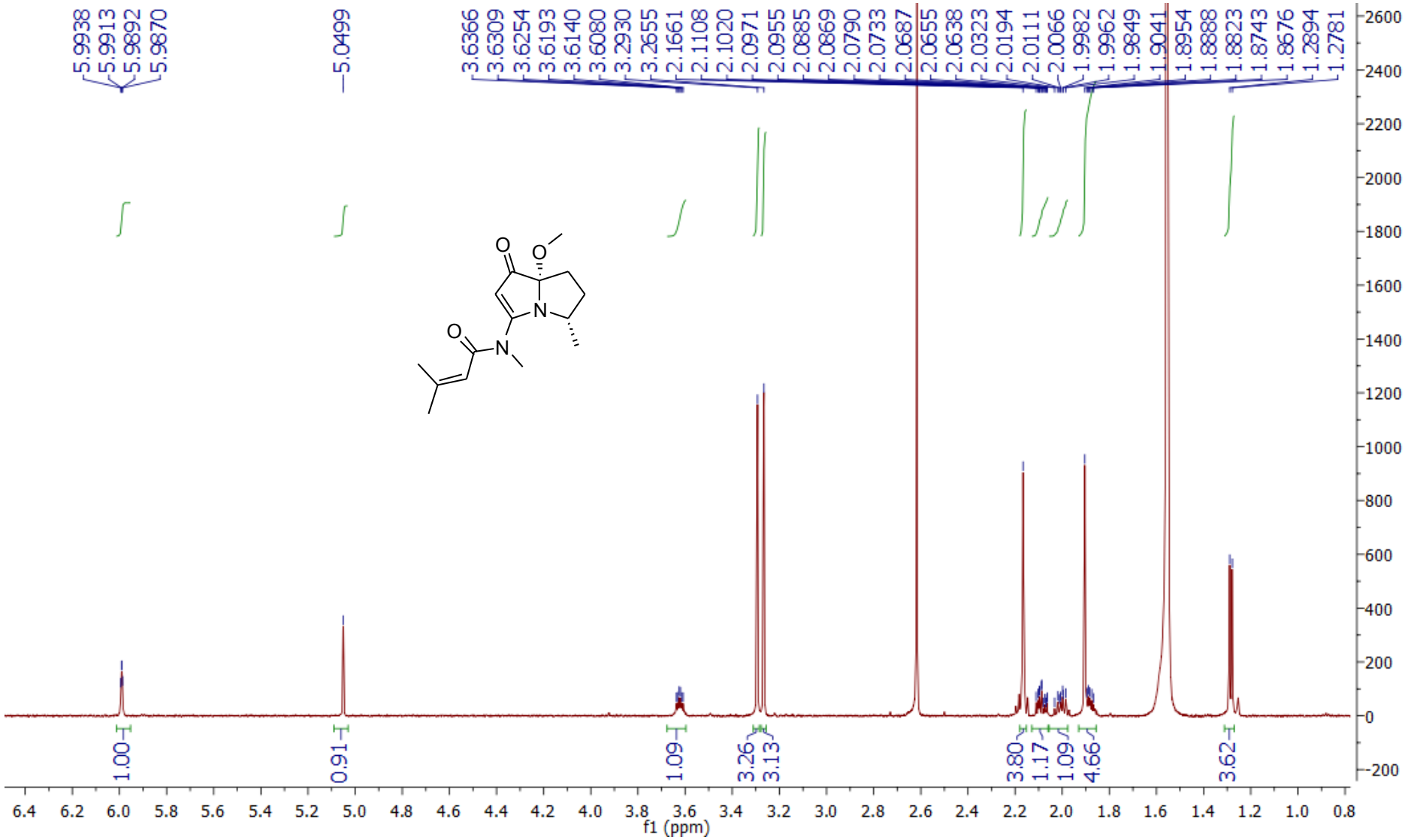

Figure S24. HSQC spectrum of compound **3a** in $CDCl_3$

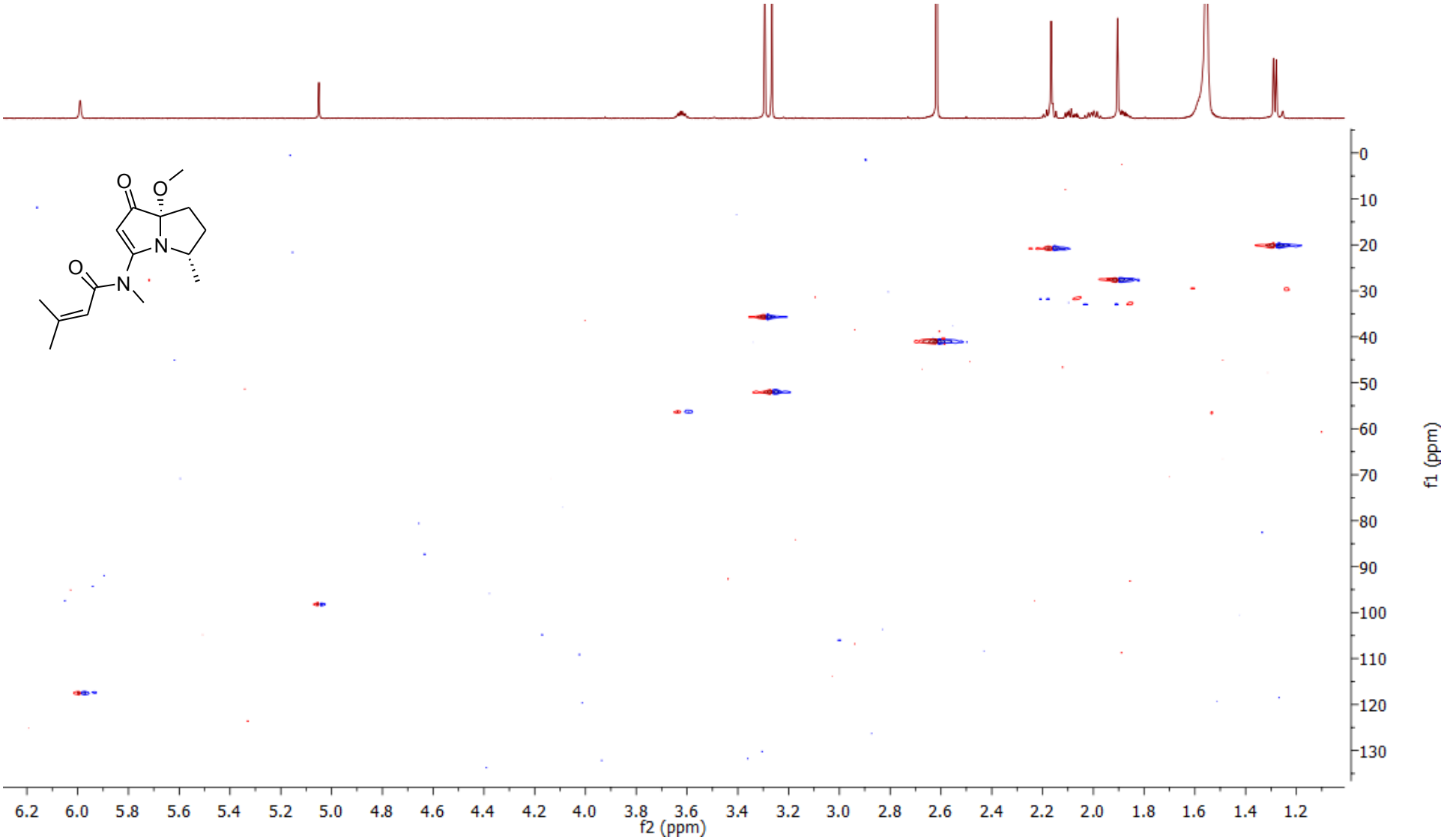

Figure S25. HMBC spectrum of compound **3a** in $CDCl_3$

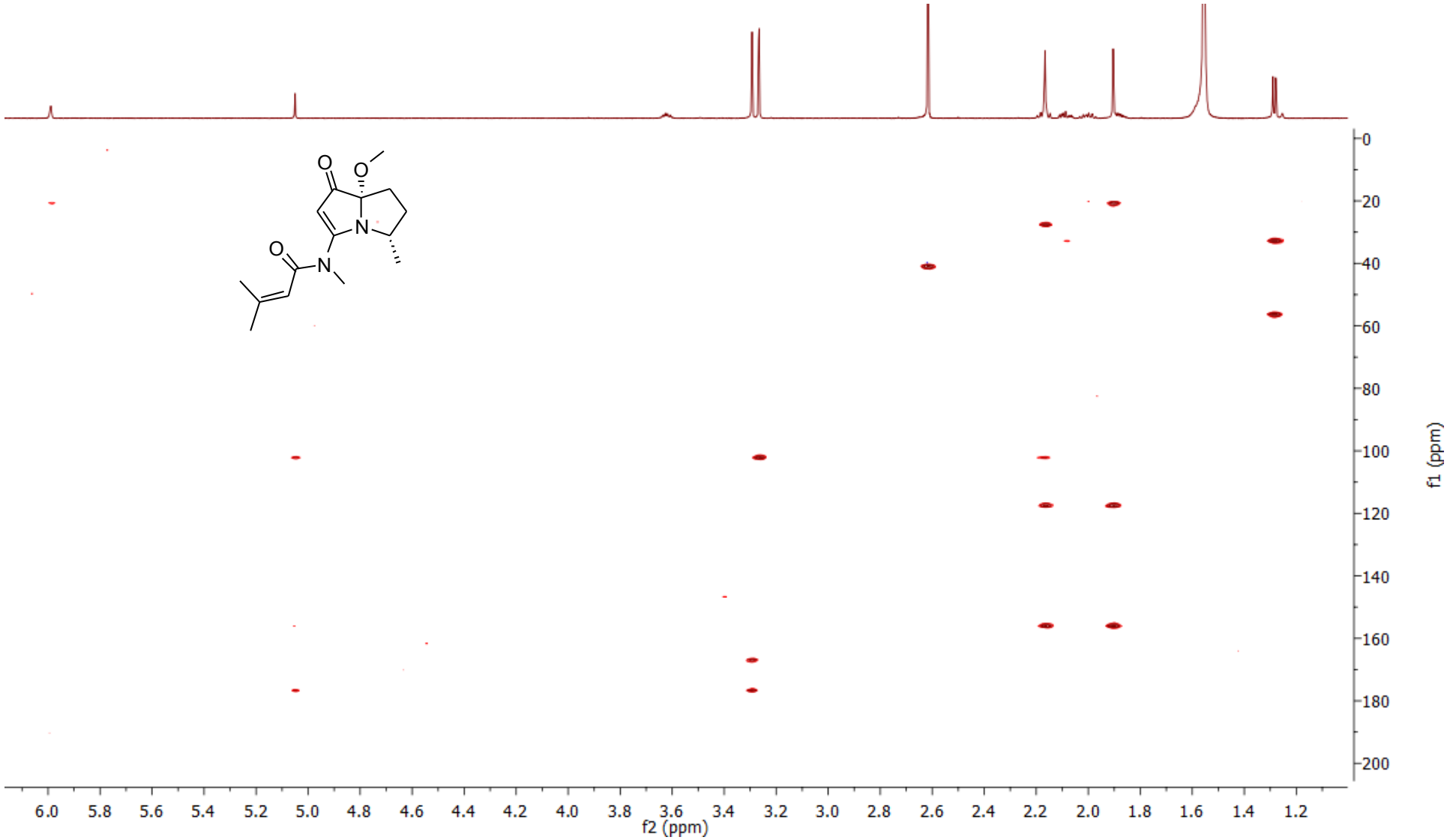

Figure S26. NOESY spectrum of compound **3a** in $CDCl_3$

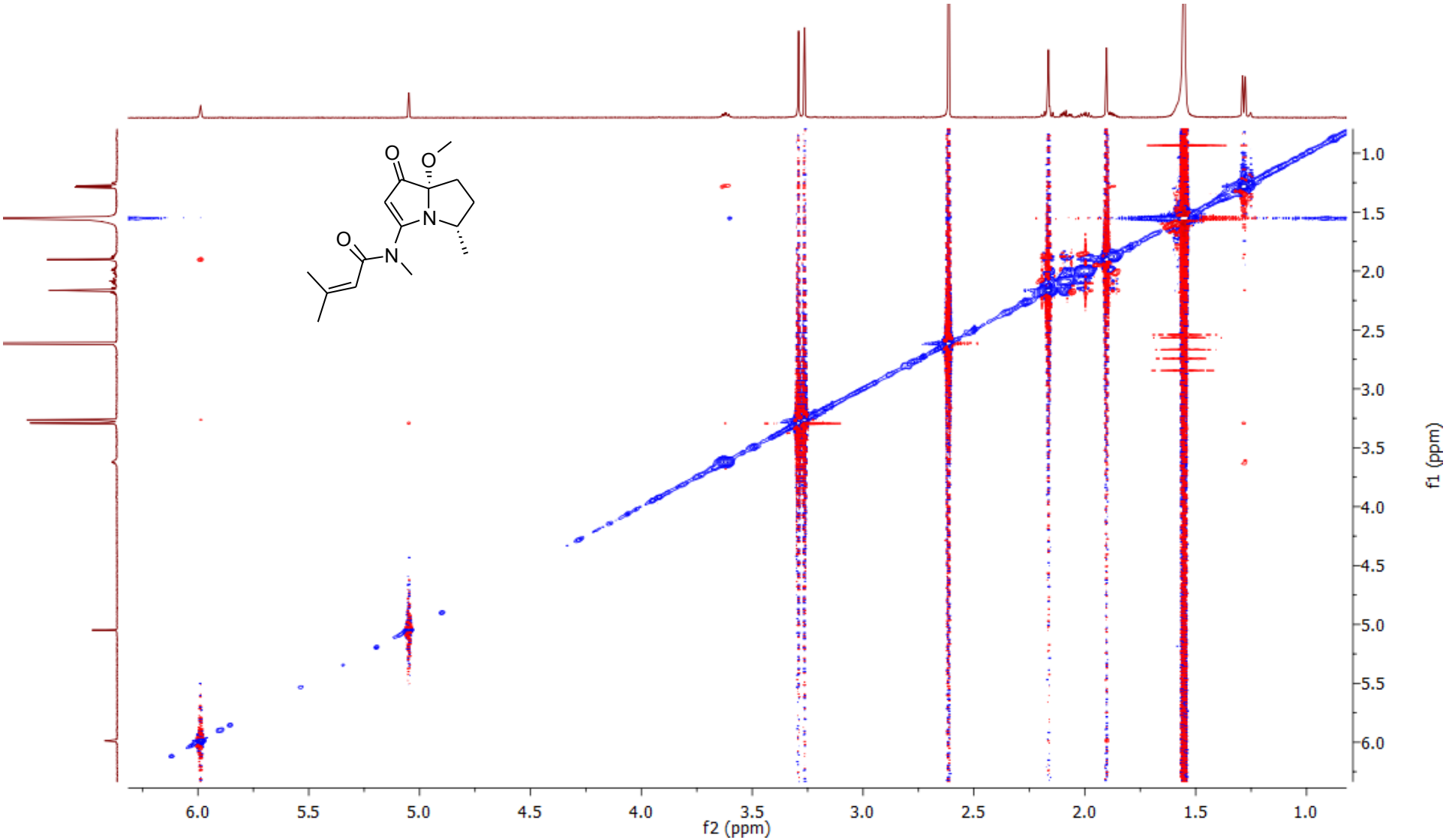

Figure S27. HRESIMS spectrum of bohemamine E (**4**)

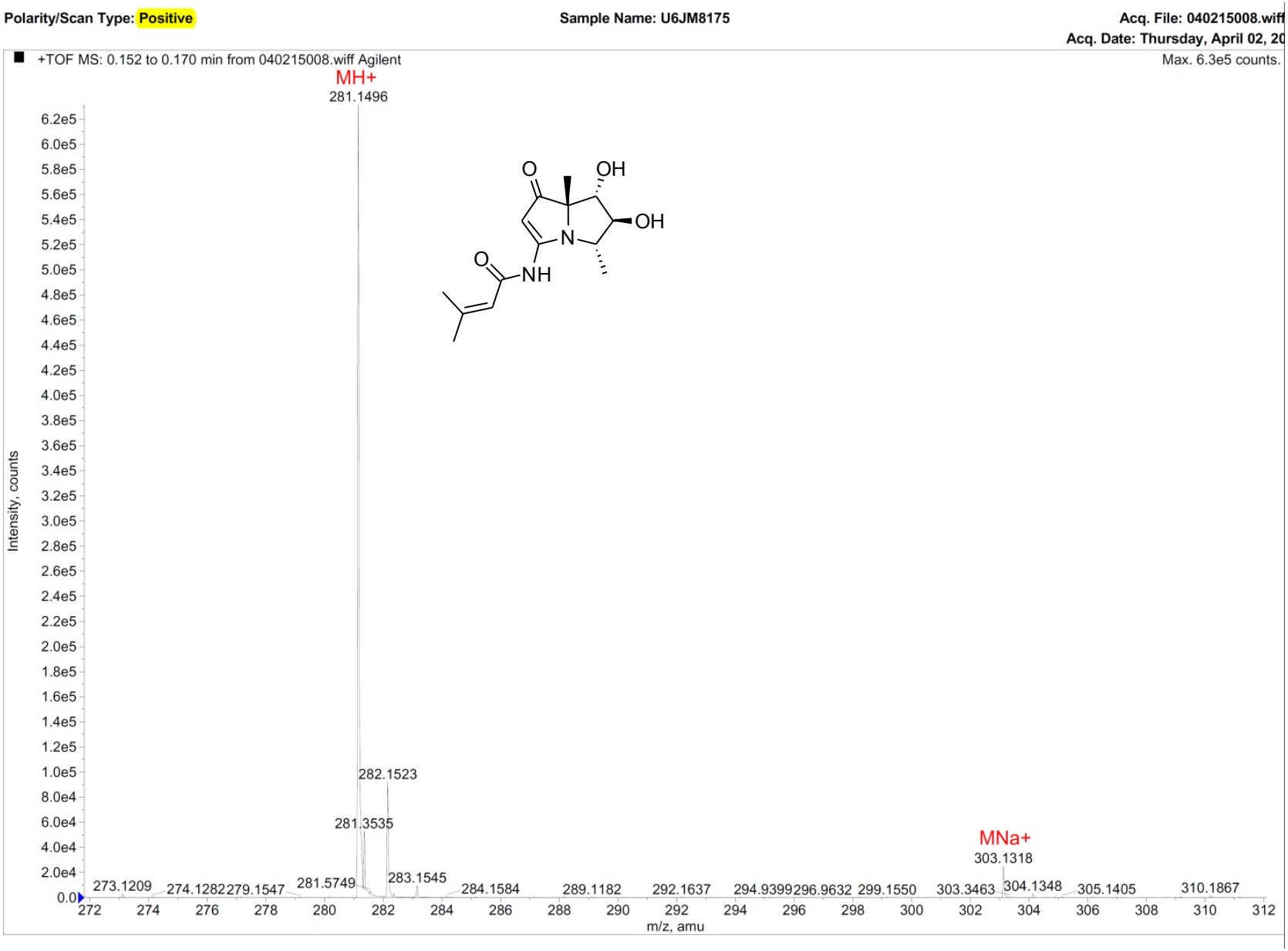

Figure S28. ^{1}H-NMR spectrum of bohemamine E (**4**) in DMSO-d_6

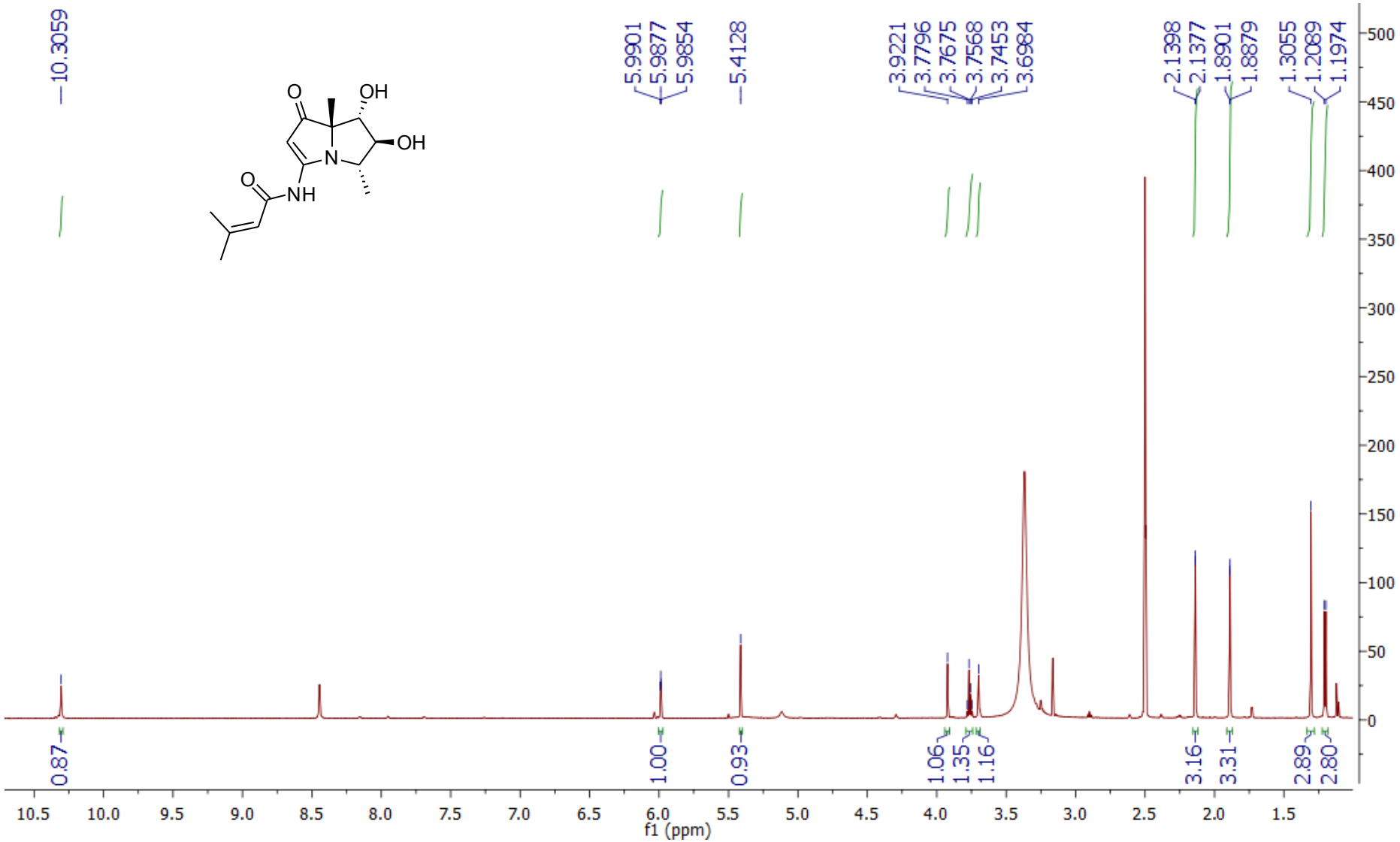

Figure S29. ^{13}C-NMR spectrum of bohemamine E (**4**) in DMSO-d_6

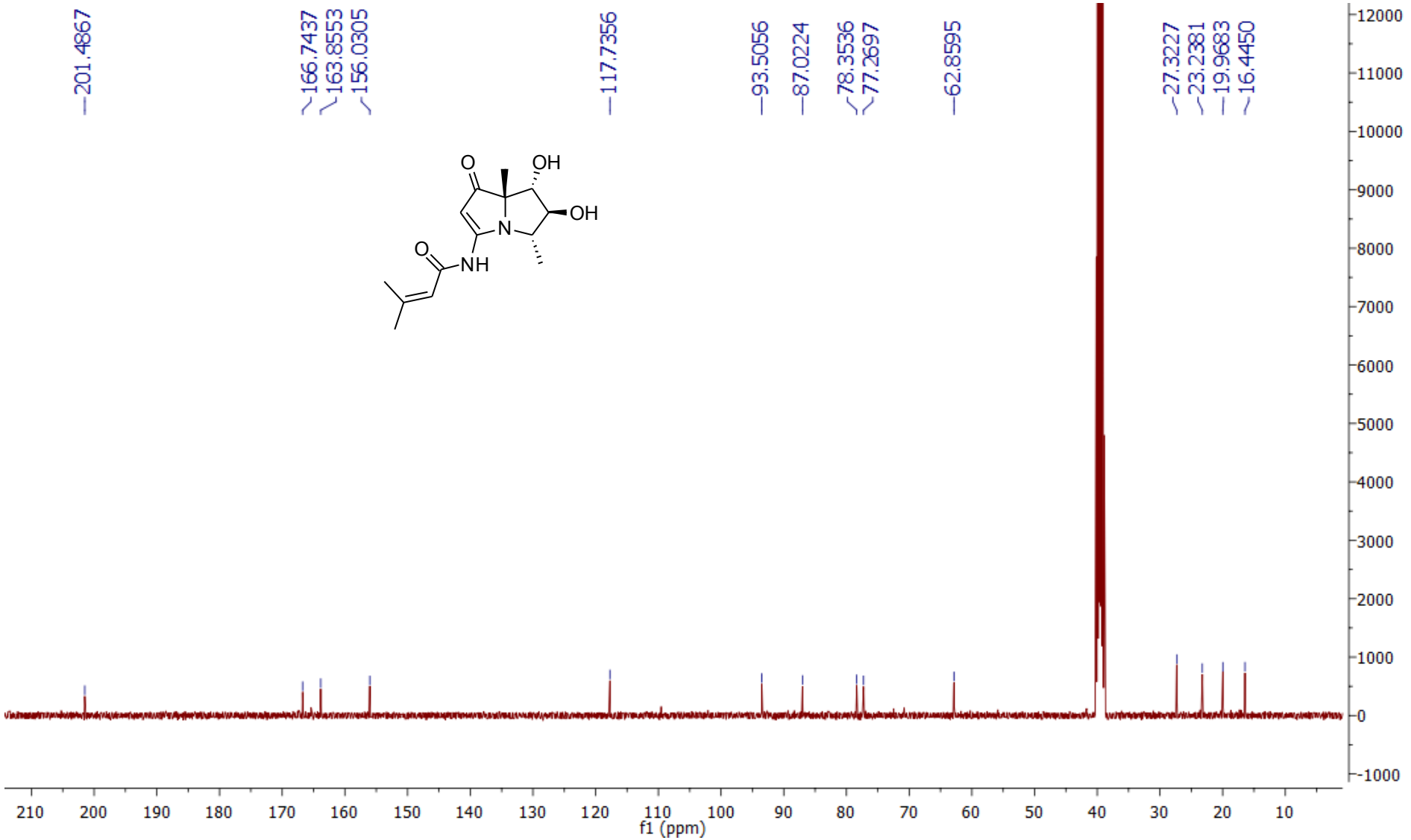

Figure S30. ^{1}H-^{1}H COSY spectrum of bohemamine E (**4**) in DMSO-d_6

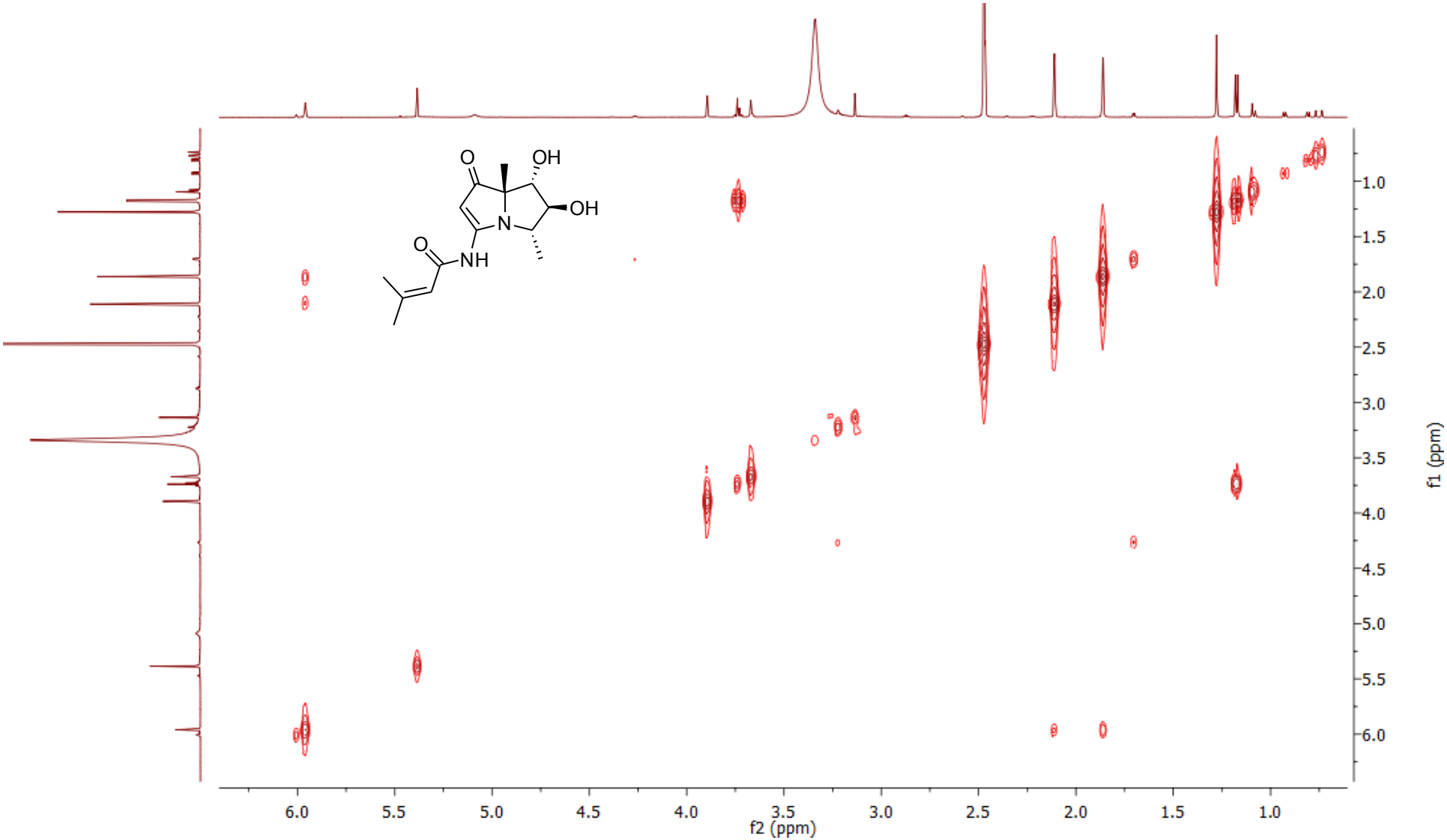

Figure S31. HSQC spectrum of bohemamine E (**4**) in DMSO-d_6

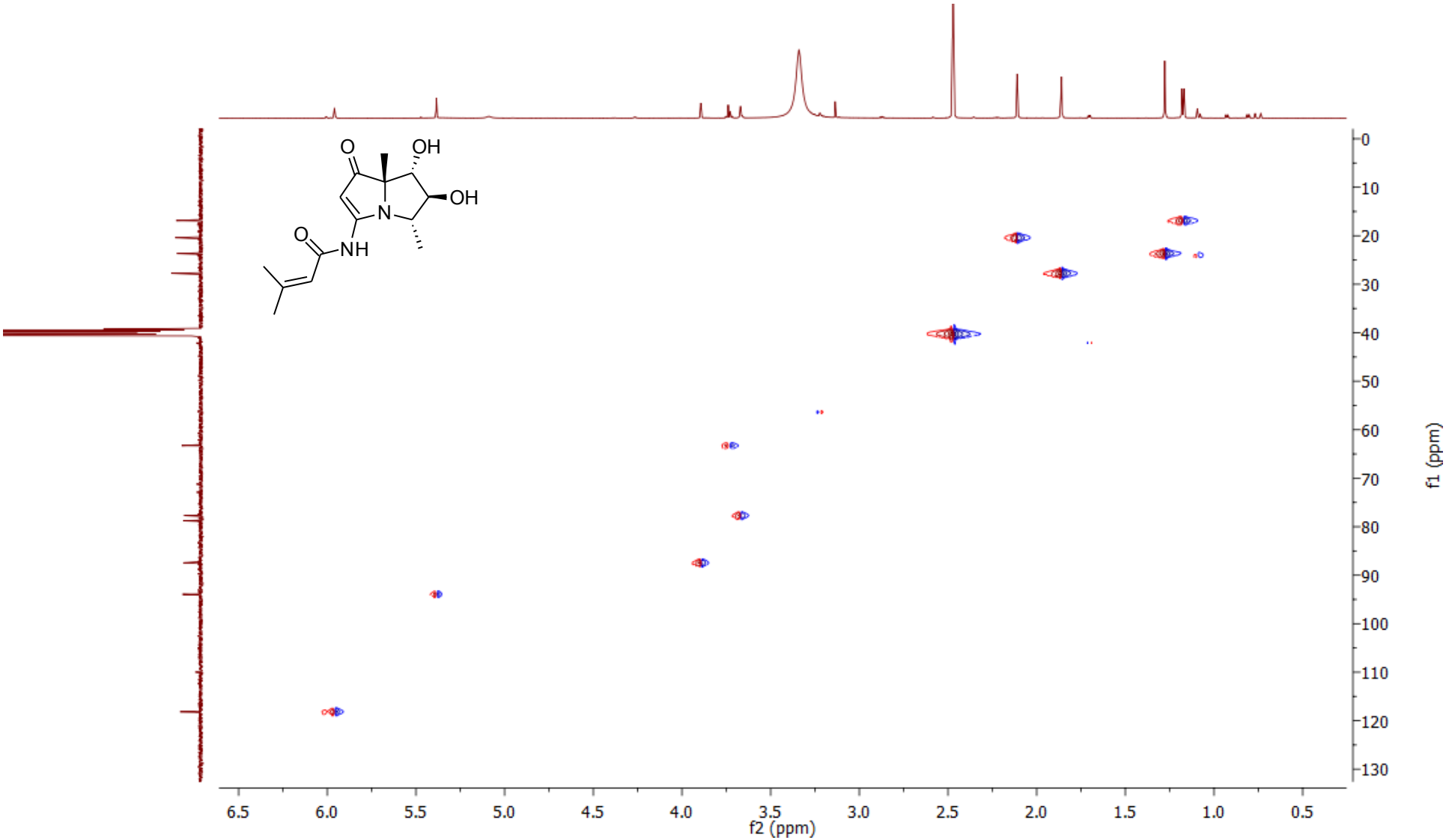

Figure S32. HMBC spectrum of bohemamine E (**4**) in DMSO-d_6

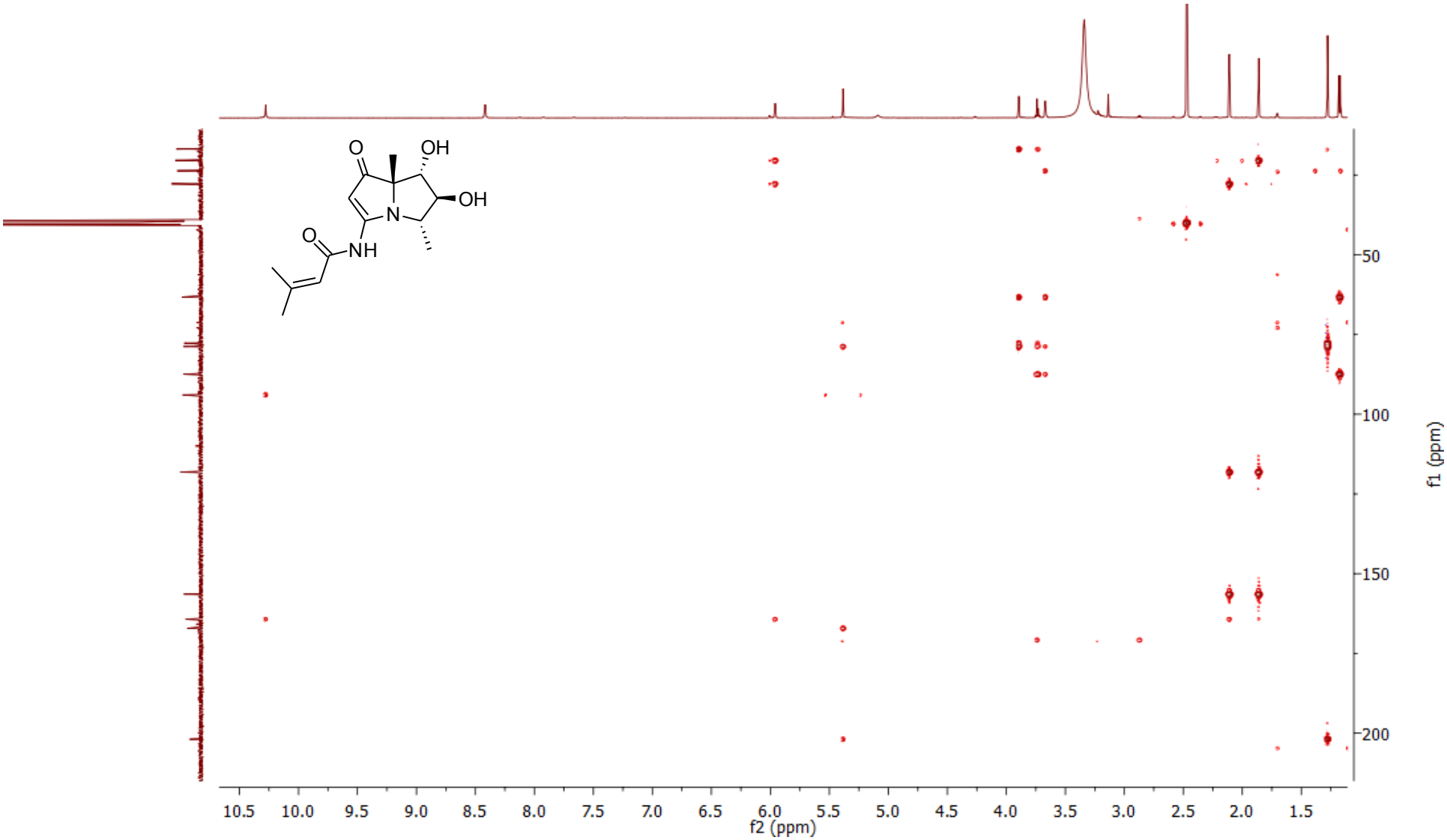

Figure S33. NOESY spectrum of bohemamine E (**4**) in DMSO-d_6

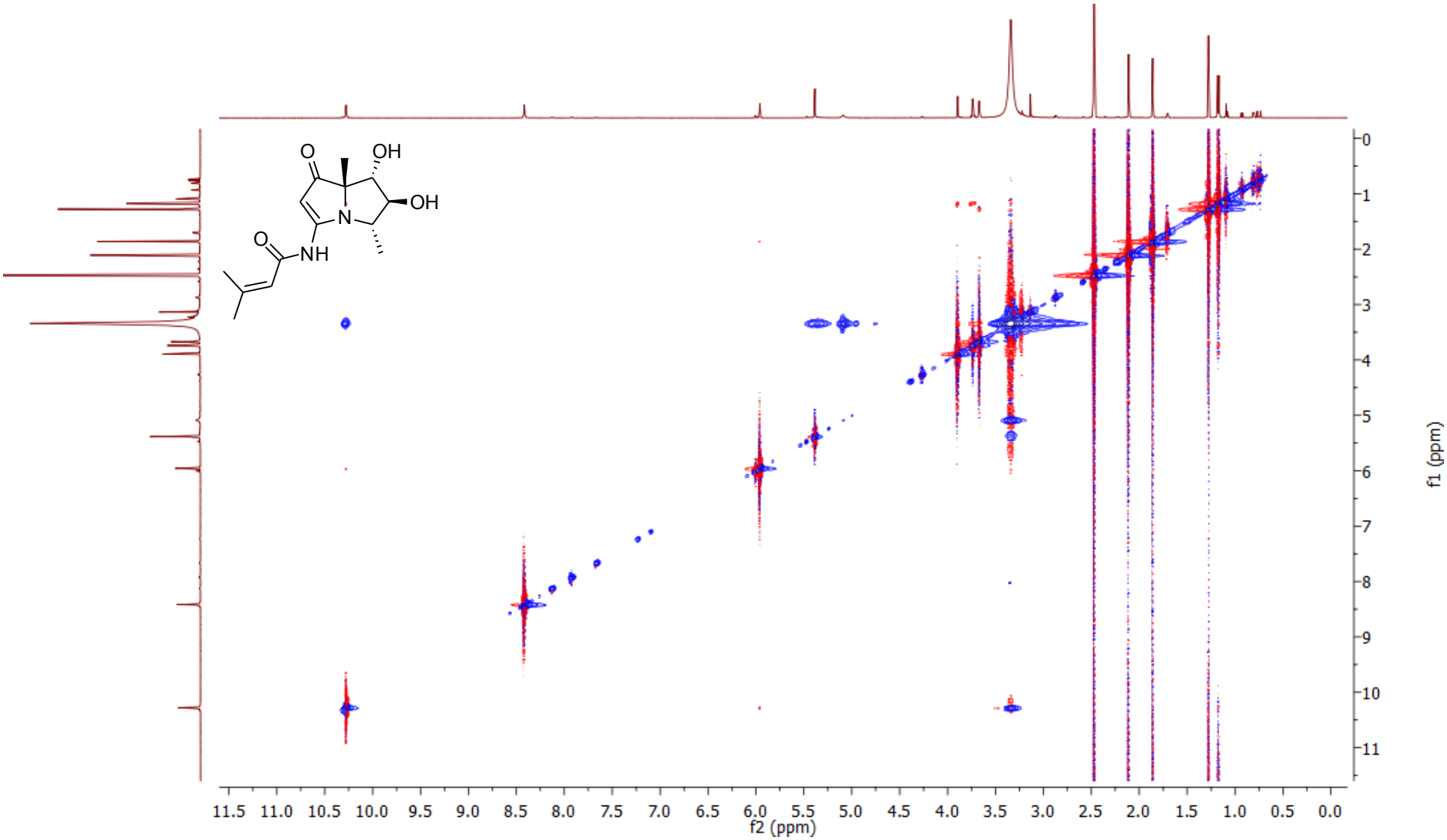

Figure S34. HRESIMS spectrum of bohemamine F (**5**)

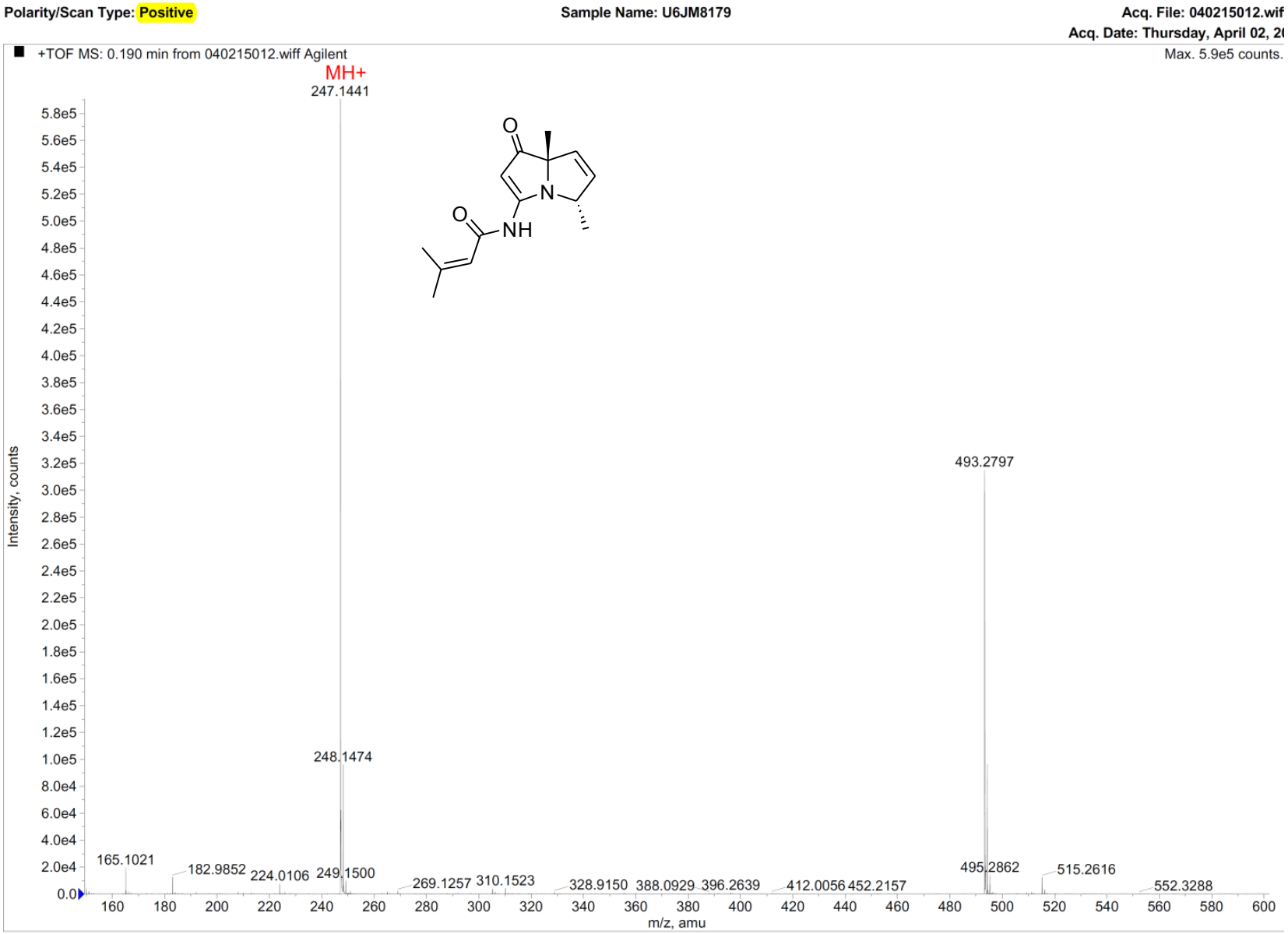

Figure S35. ^{1}H-NMR spectrum of bohemamine F (**5**) in DMSO-d_6

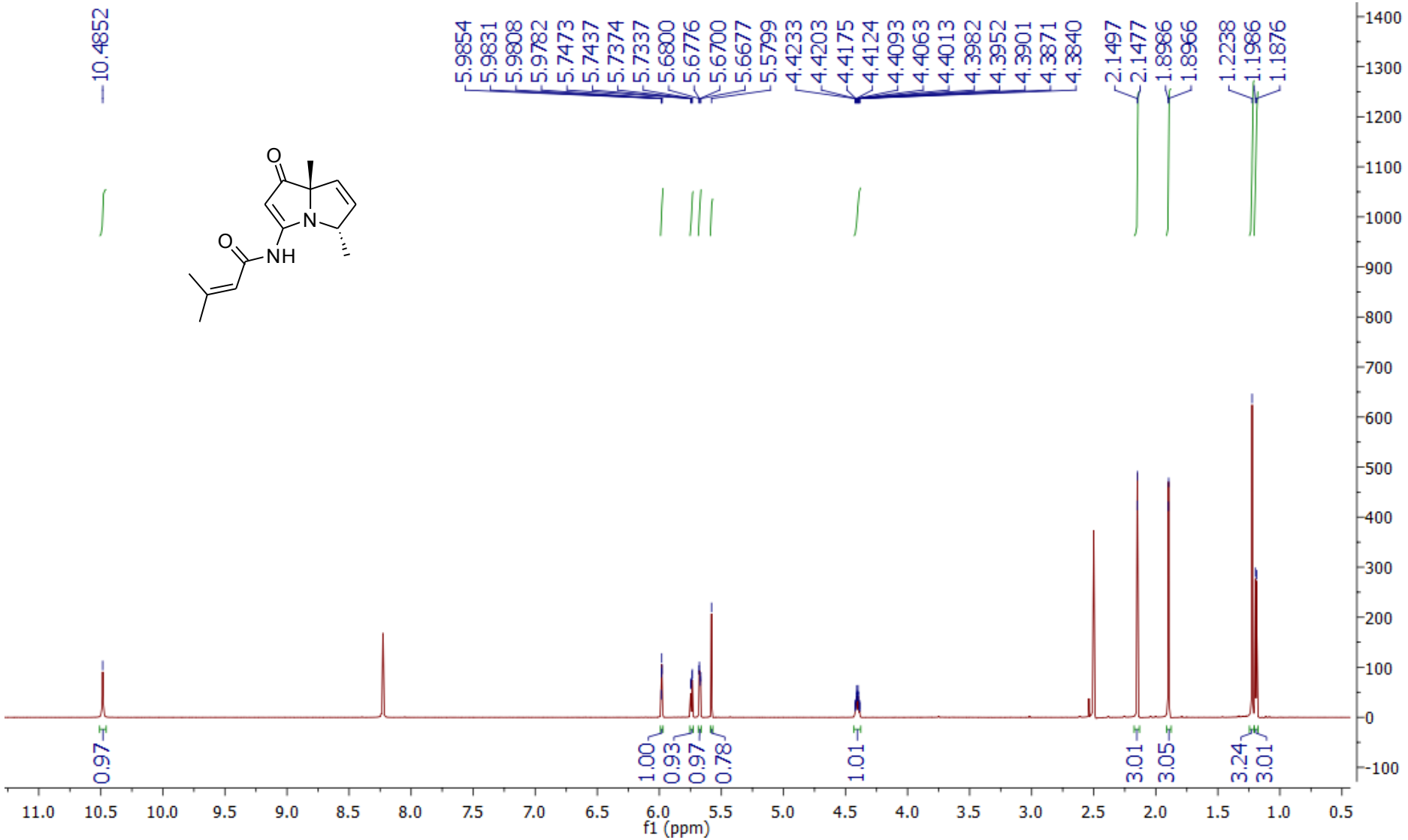

Figure S36. ^{13}C-NMR spectrum of bohemamine F (**5**) in DMSO-d_6

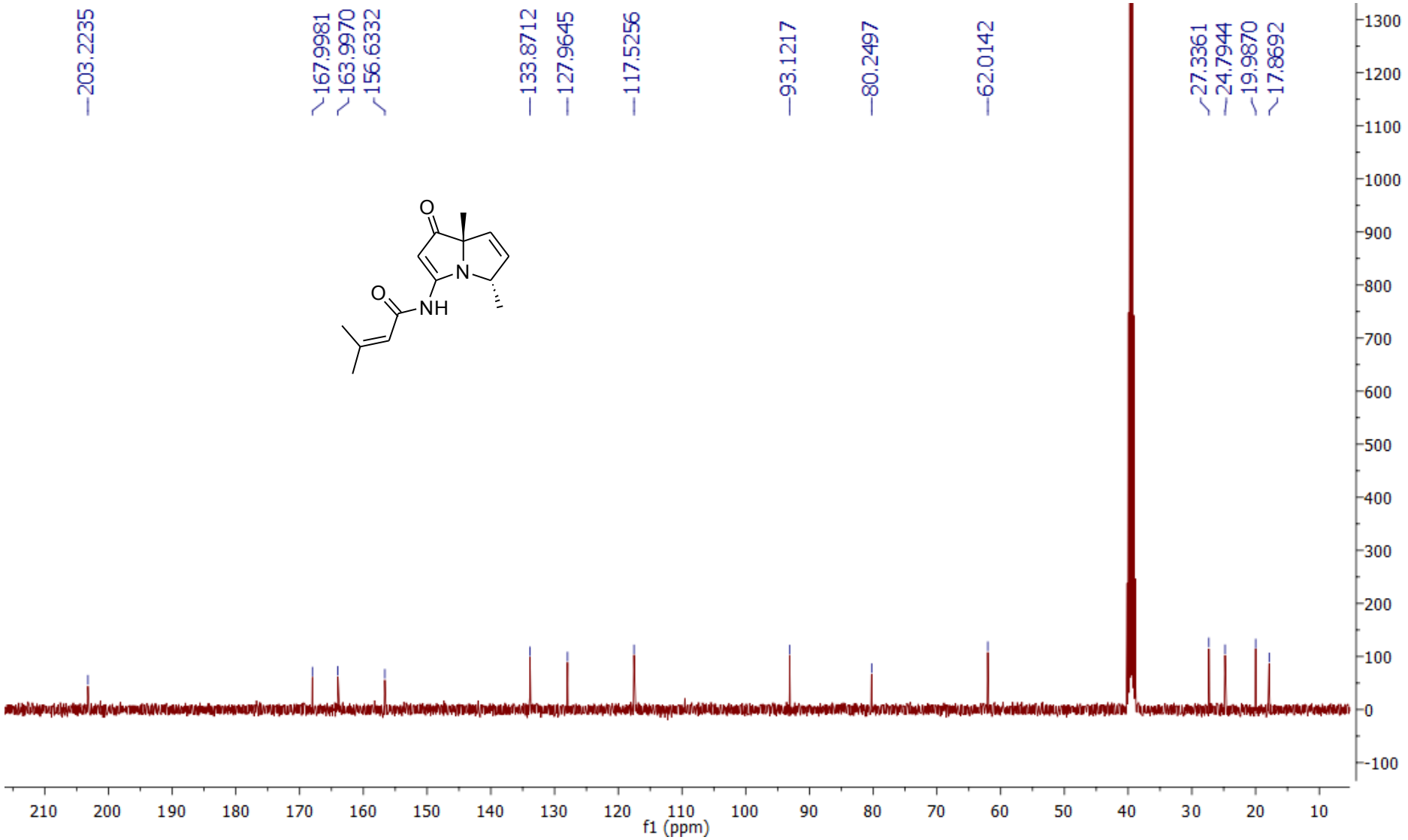

Figure S37. ^{1}H-^{1}H COSY spectrum of bohemamine F (**5**) in DMSO-d_6

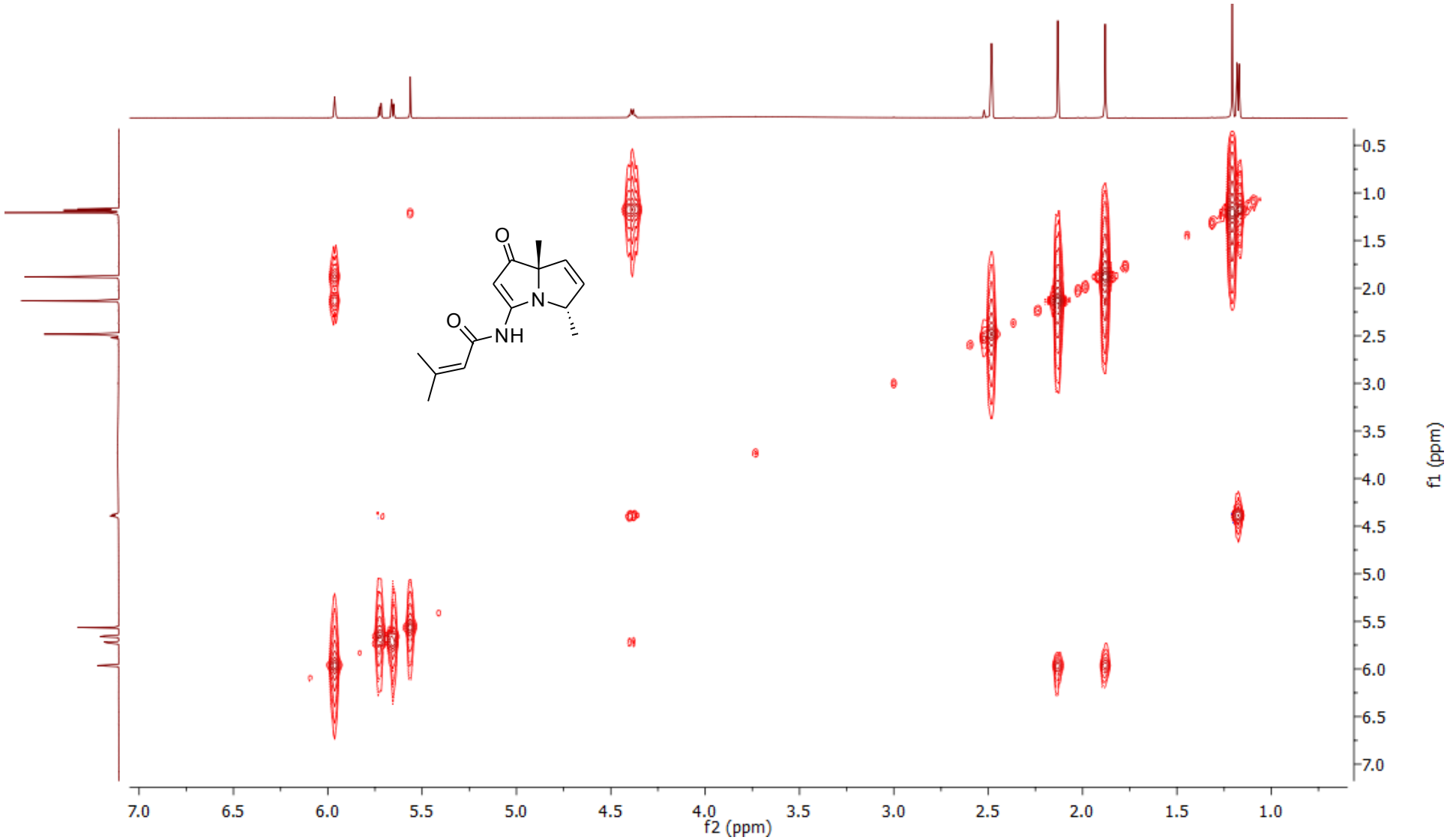

Figure S38. HSQC spectrum of bohemamine F (**5**) in DMSO-d_6

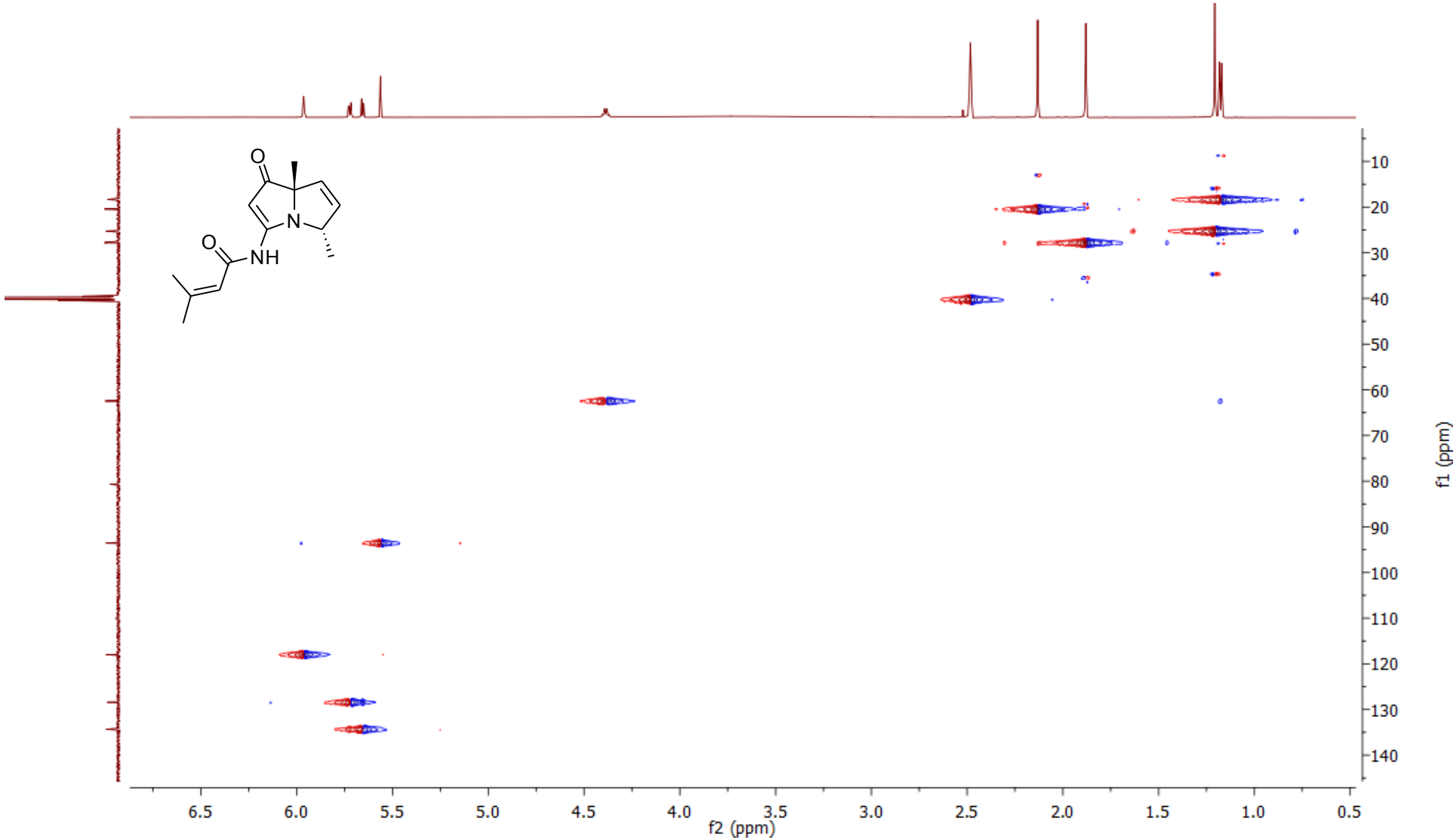

Figure S39. HMBC spectrum of bohemamine F (**5**) in DMSO-d_6

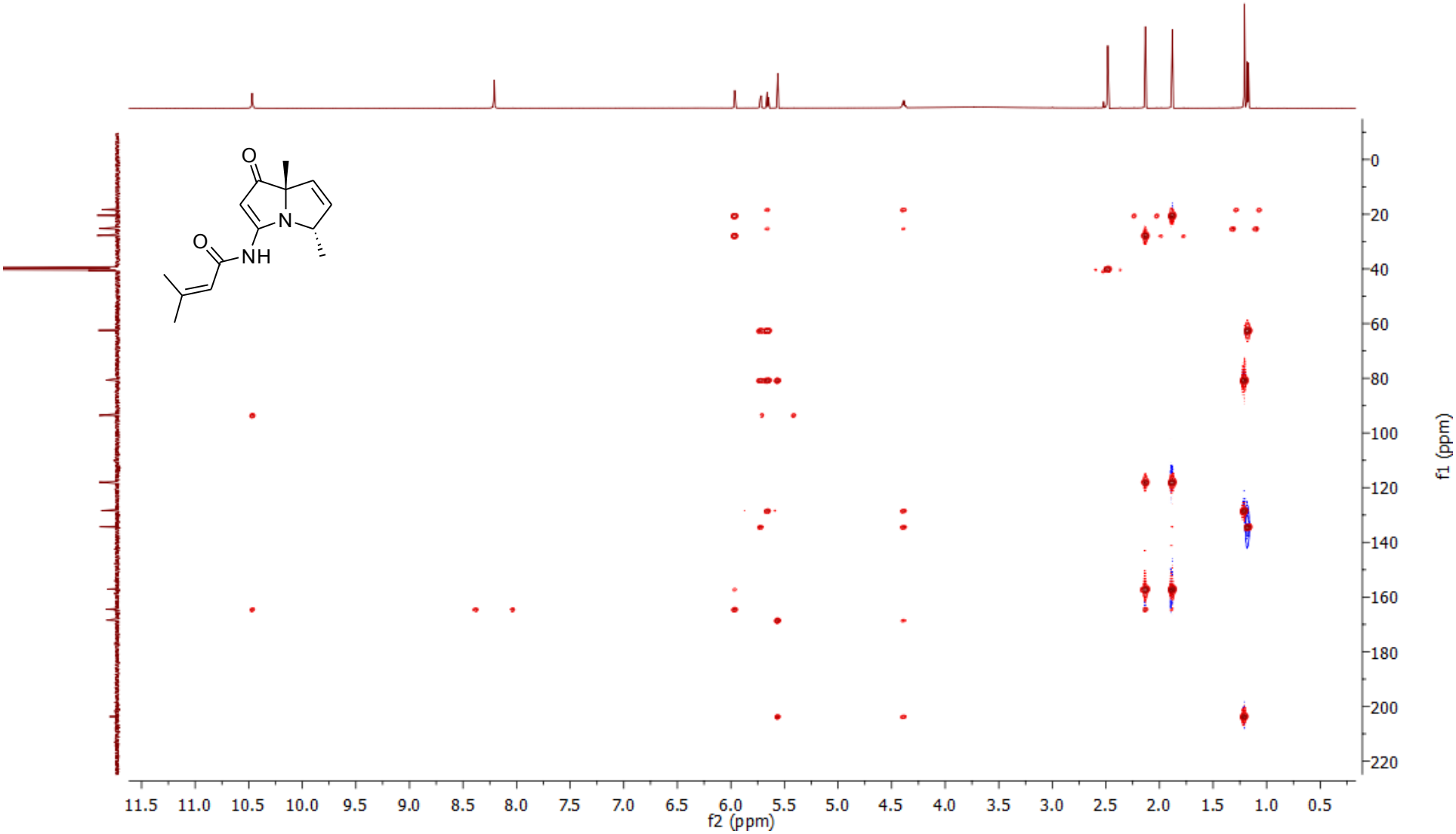

Figure S40. NOESY spectrum of bohemamine F (**5**) in DMSO-d_6

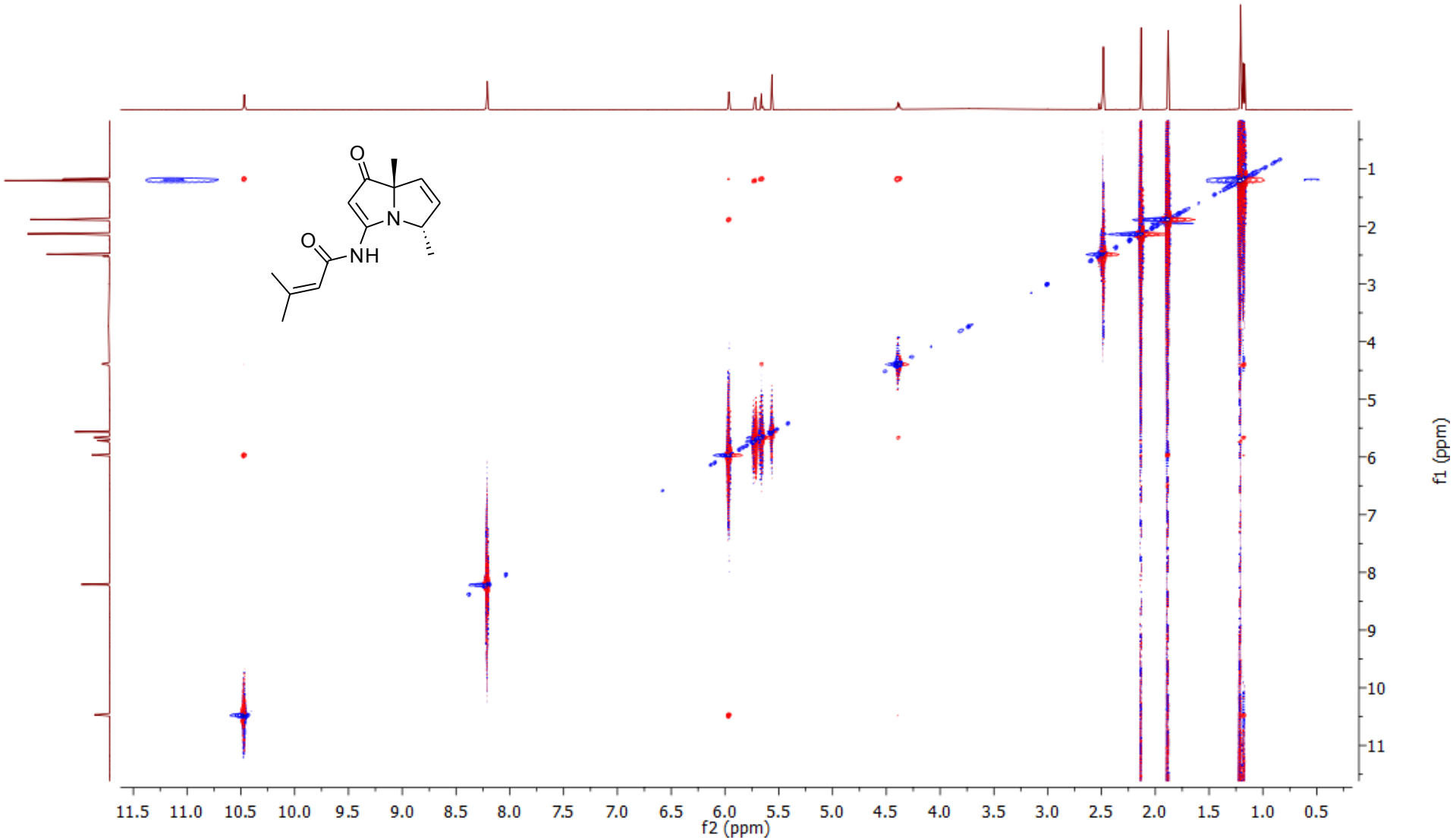

Figure S41. HRESIMS spectrum of bohemamine G (**6**)

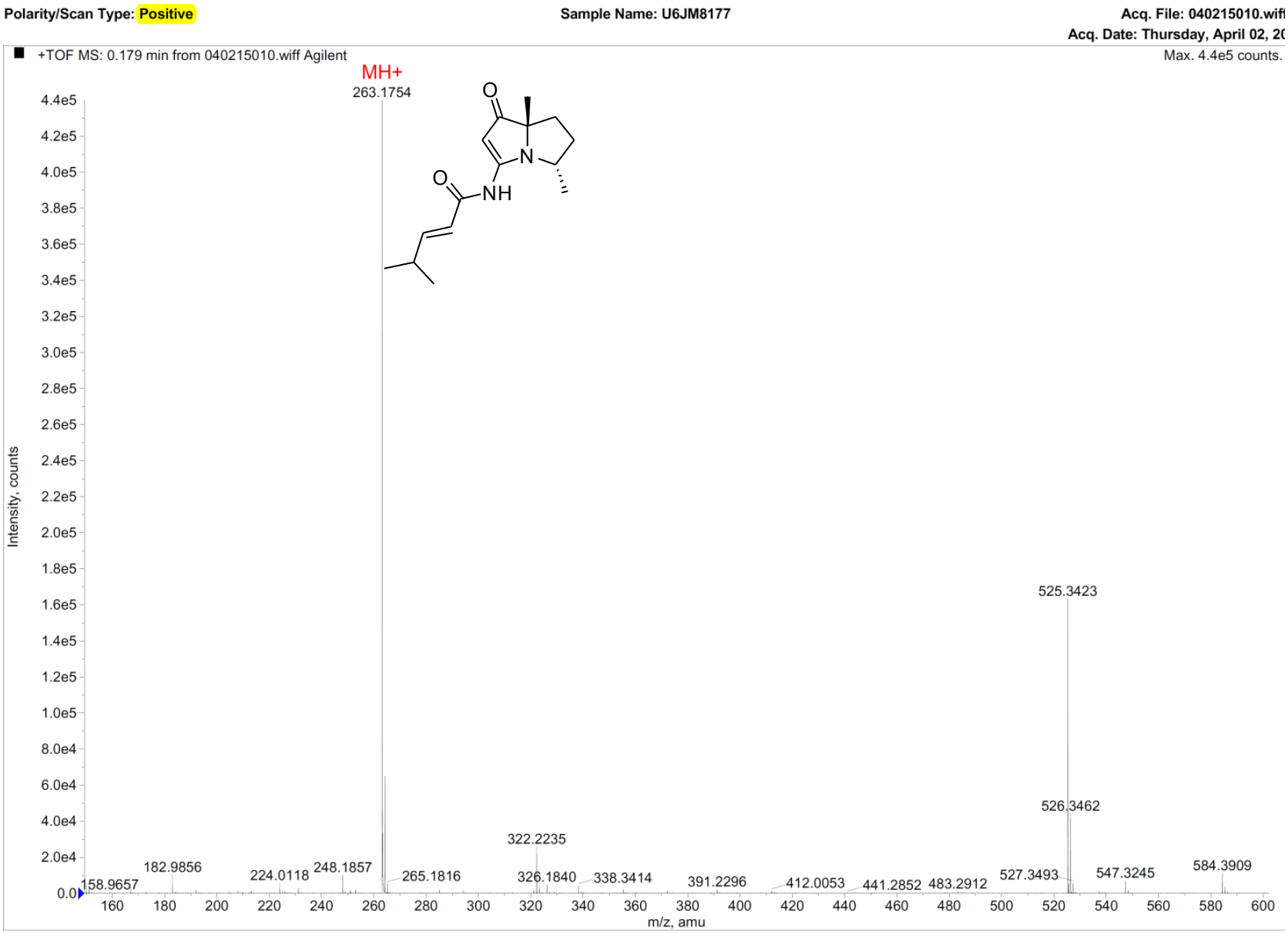

Figure S42. ^{1}H-NMR spectrum of bohemamine G (**6**) in DMSO-d_6

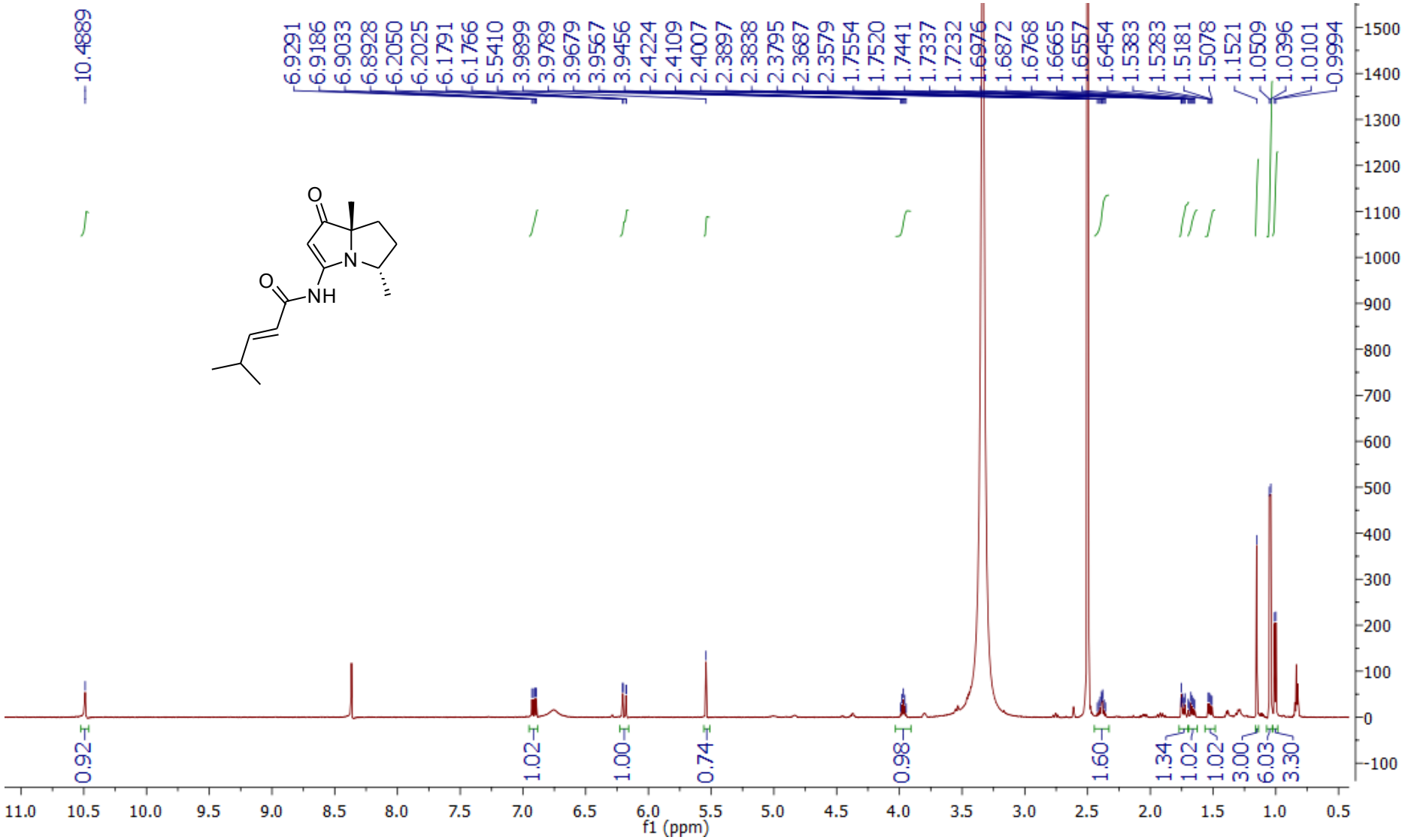

Figure S43. ^{13}C-NMR spectrum of bohemamine G (**6**) in DMSO-d_6

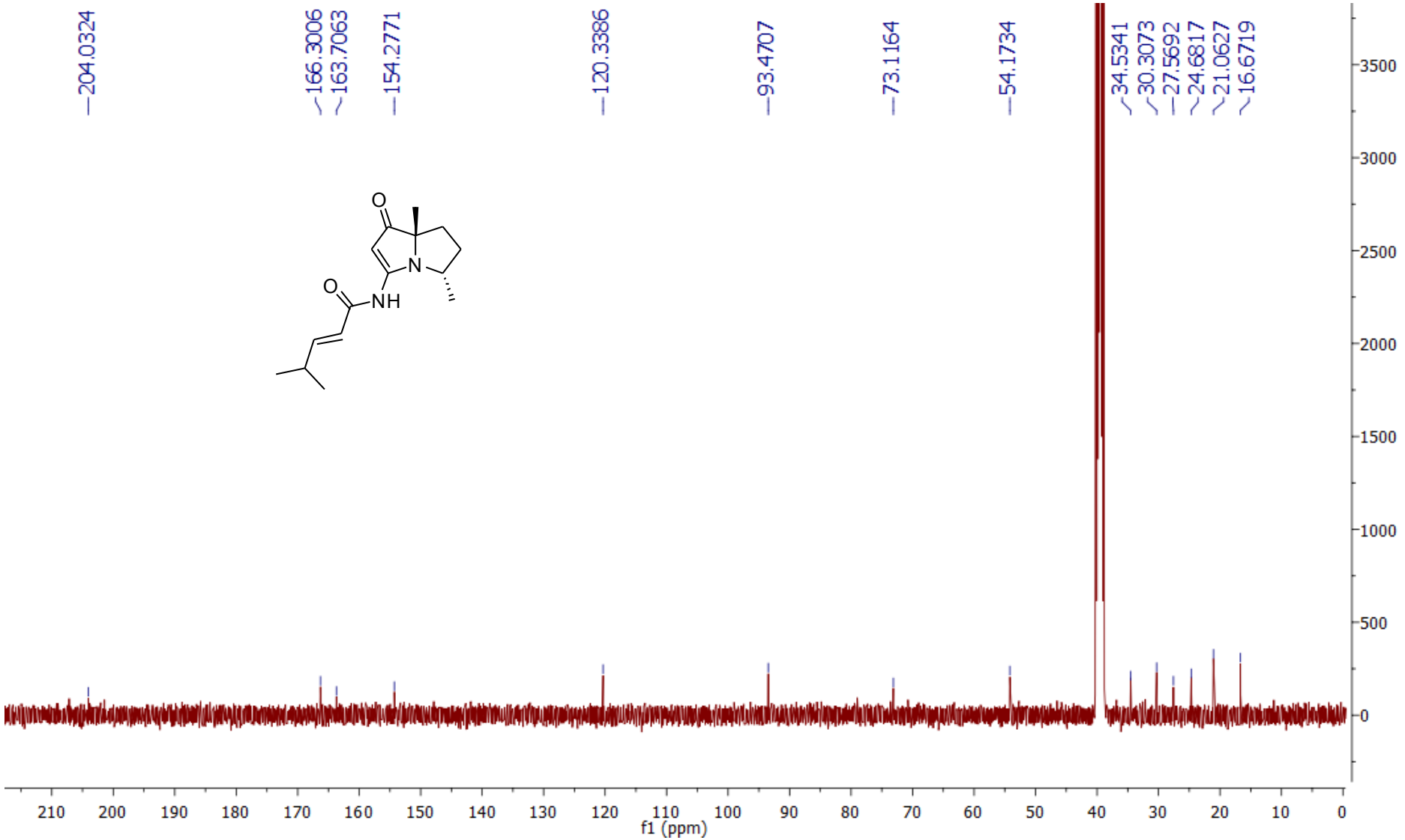

Figure S44. ^{1}H-^{1}H COSY spectrum of bohemamine G (**6**) in DMSO-d_6

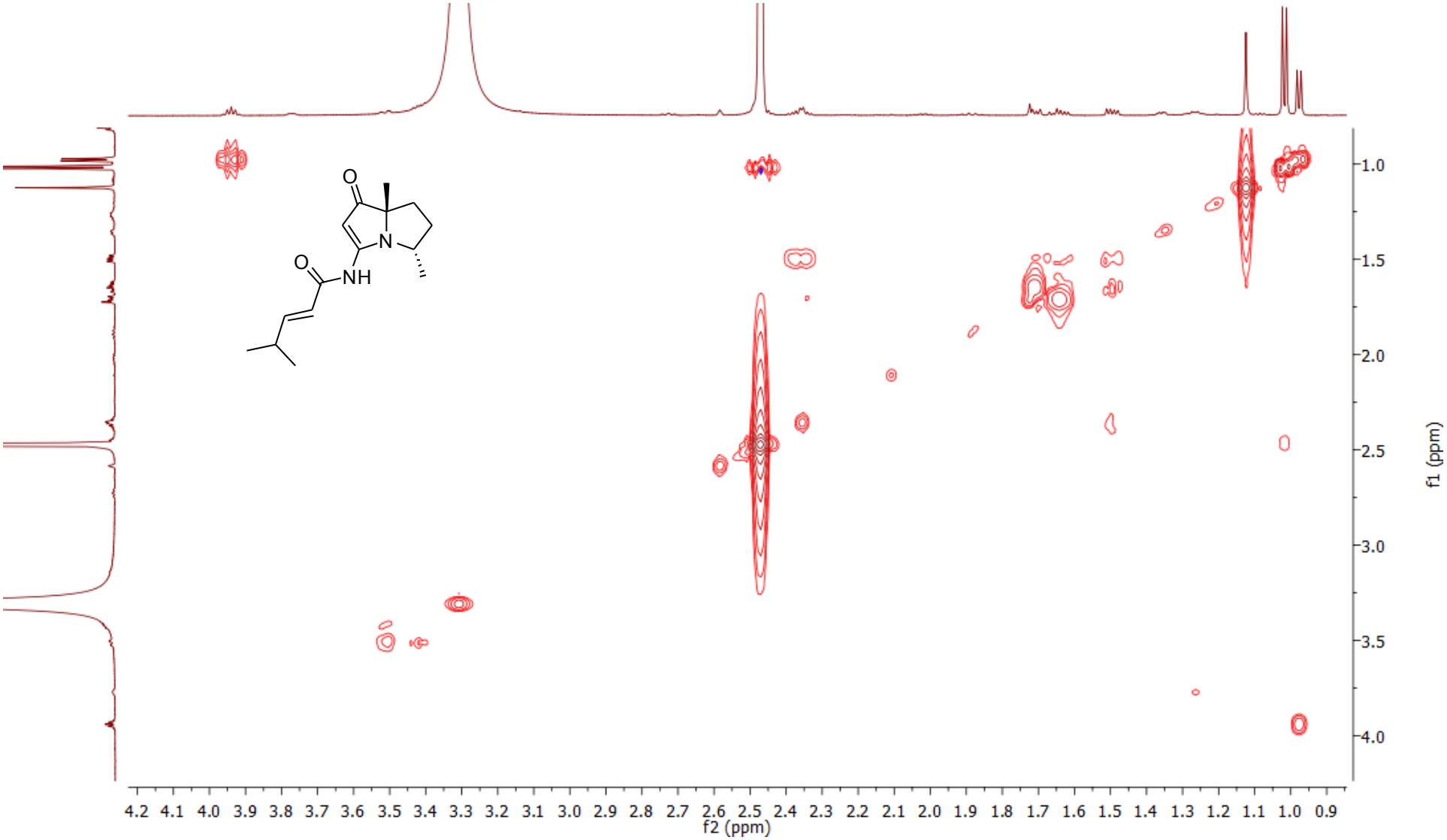

Figure S45. HSQC spectrum of bohemamine G (**6**) in DMSO-d_6

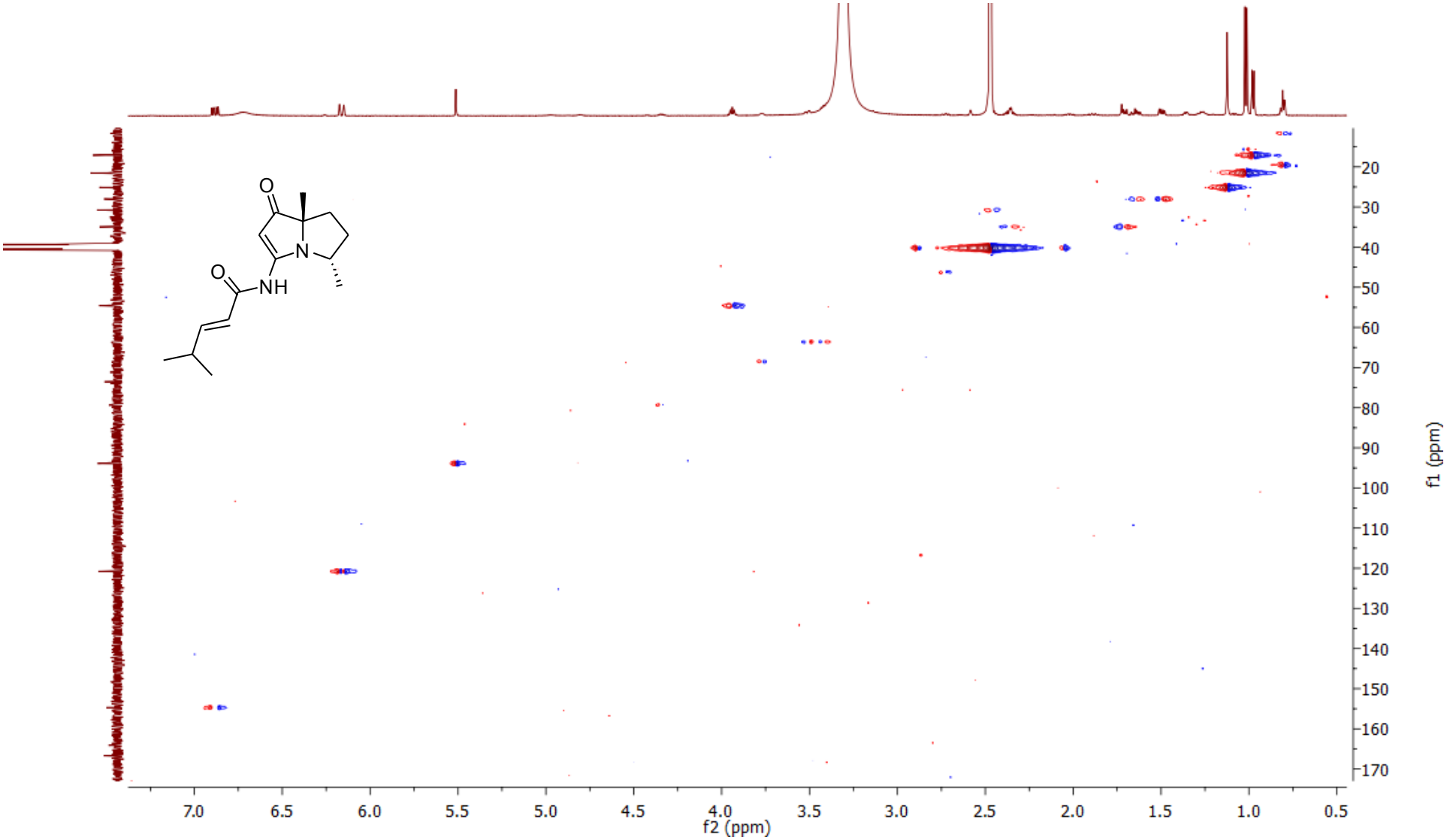

Figure S46. HMBC spectrum of bohemamine G (**6**) in DMSO-d_6

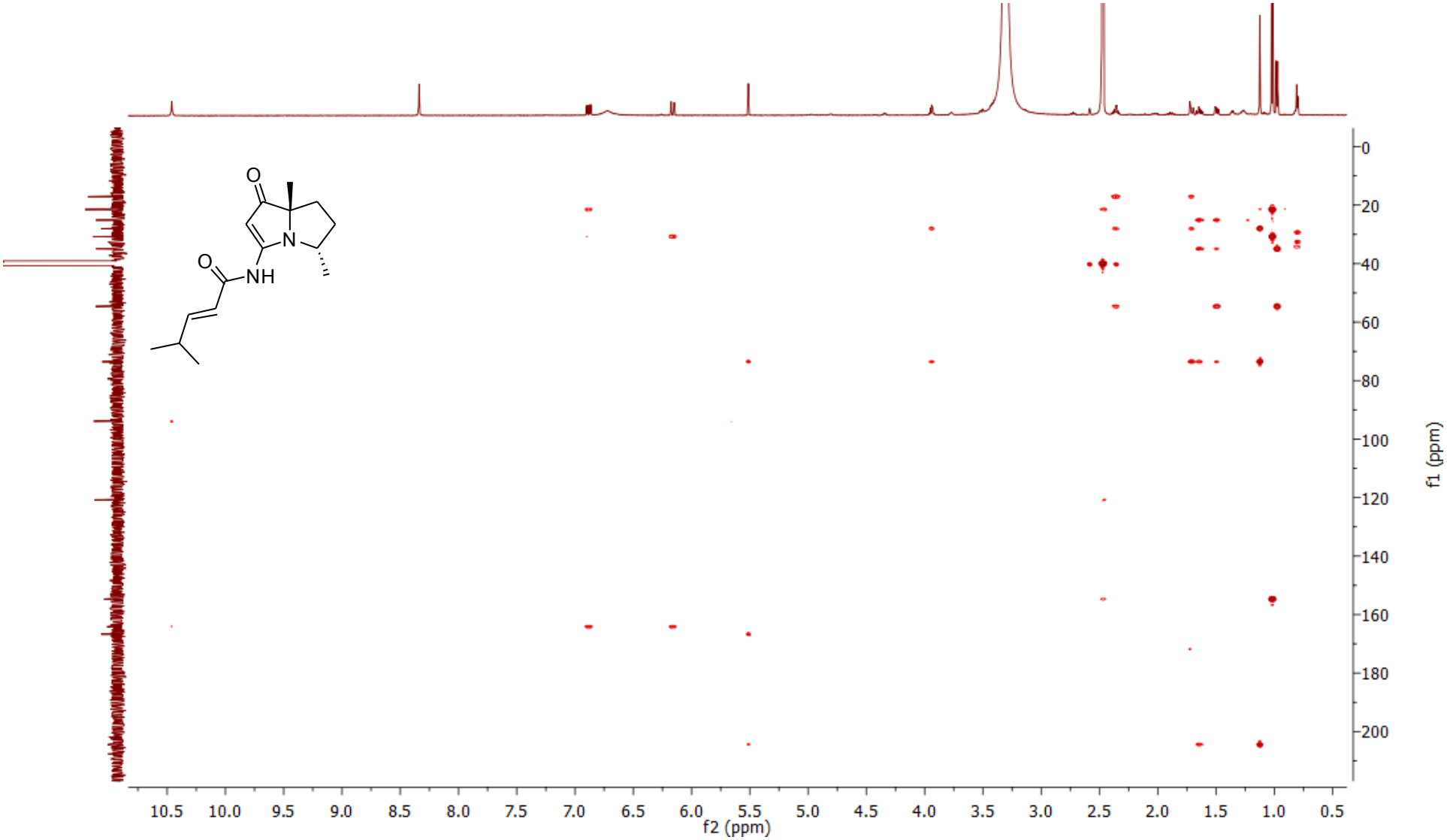

Figure S47. NOESY spectrum of bohemamine G (**6**) in DMSO-d_6

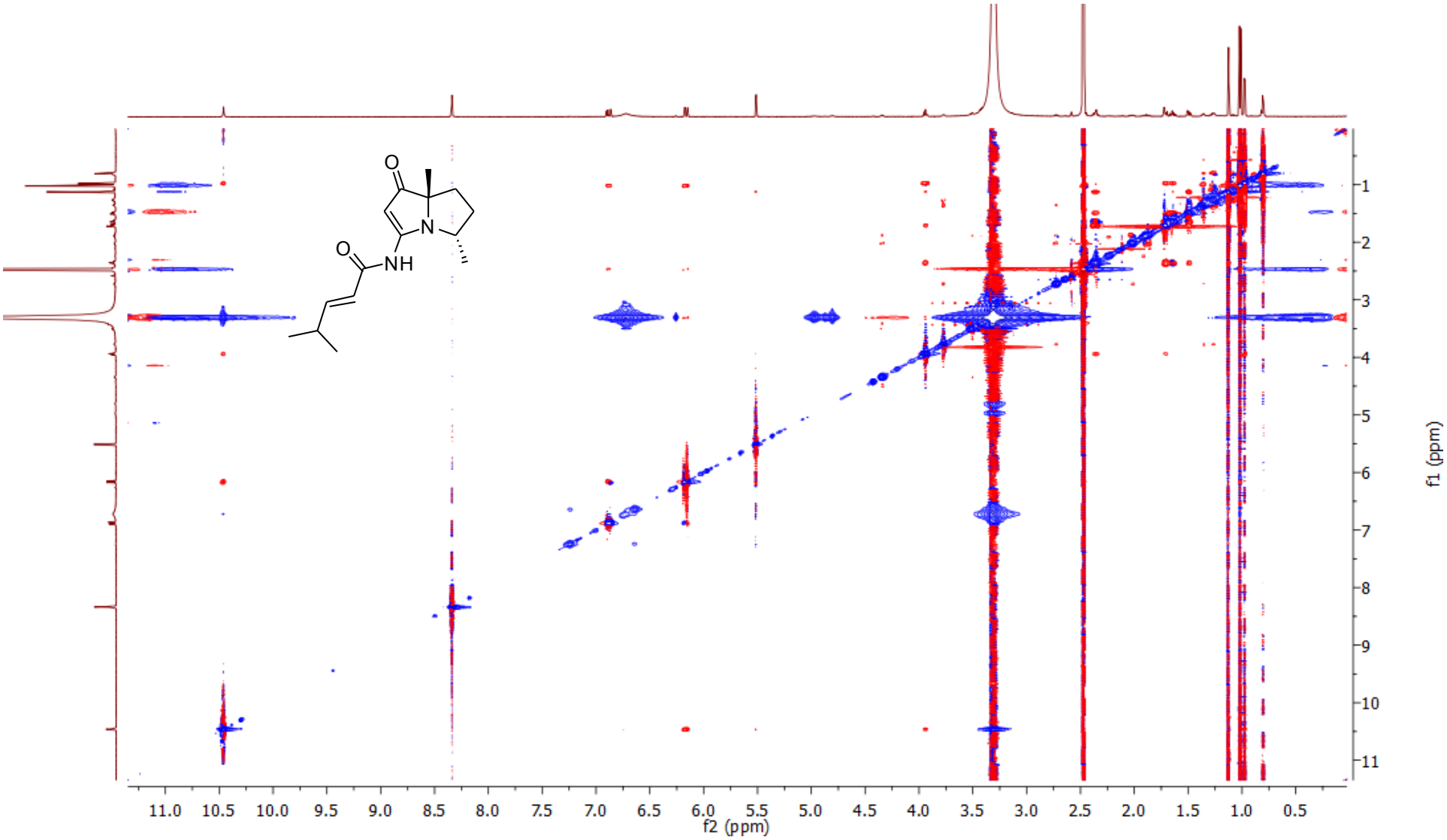

Figure S48. HRESIMS spectrum of bohemamine H (**7**)

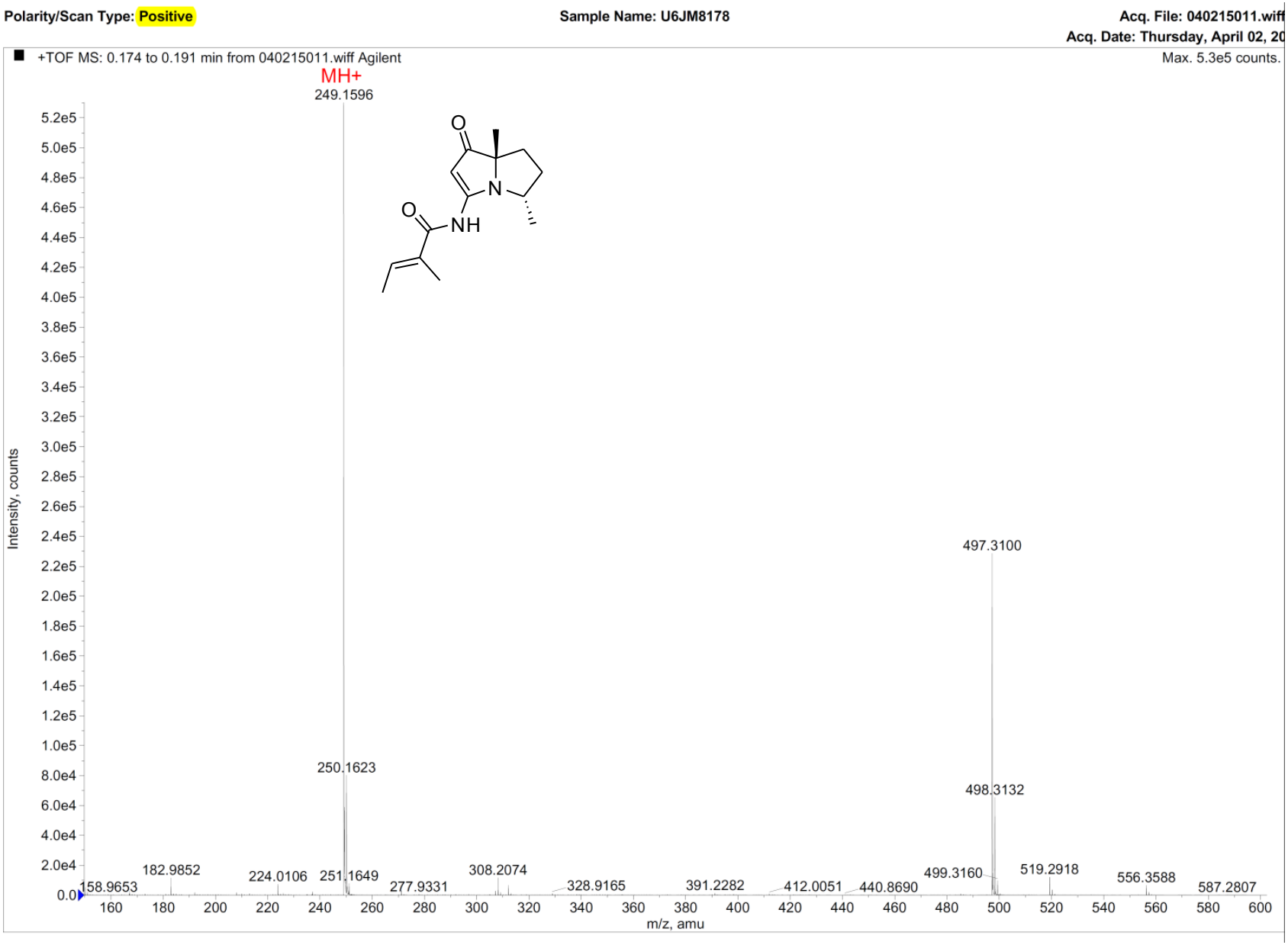

Figure S49. ^{1}H-NMR spectrum of bohemamine H (**7**) in DMSO-d_6

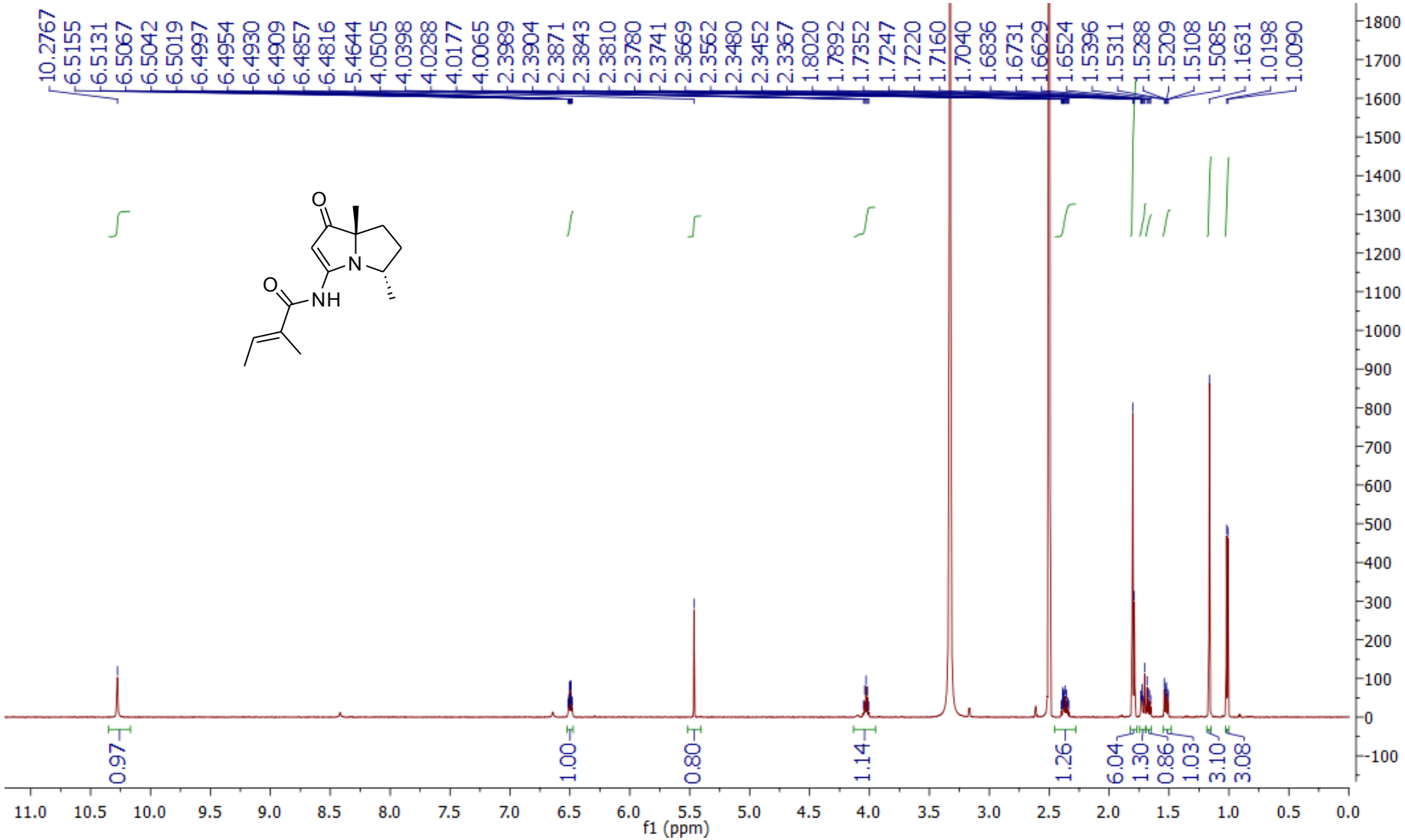

Figure S50. ^{13}C-NMR spectrum of bohemamine H (**7**) in DMSO-d_6

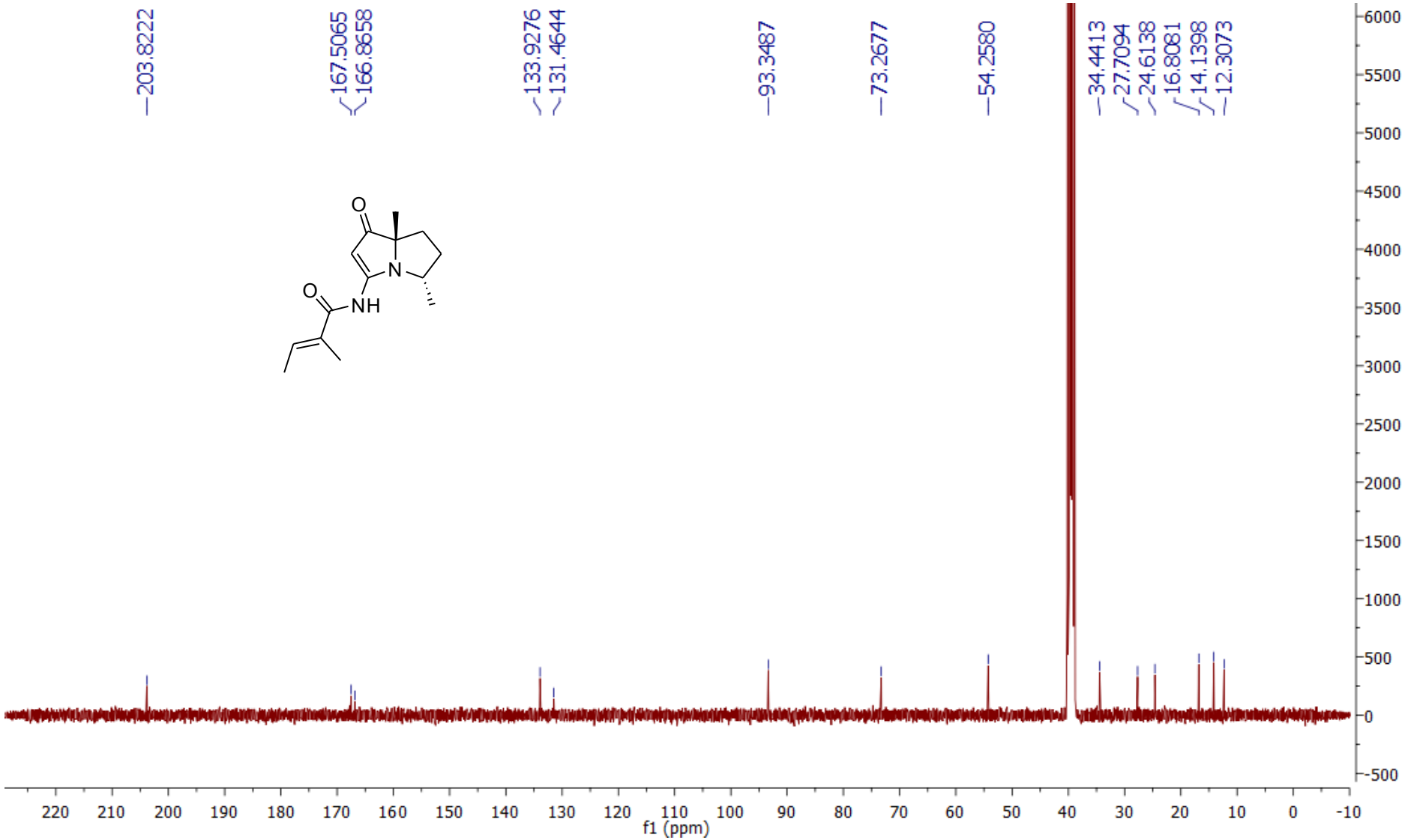

Figure S51. ^{1}H-^{1}H COSY spectrum of bohemamine H (**7**) in DMSO-d_6

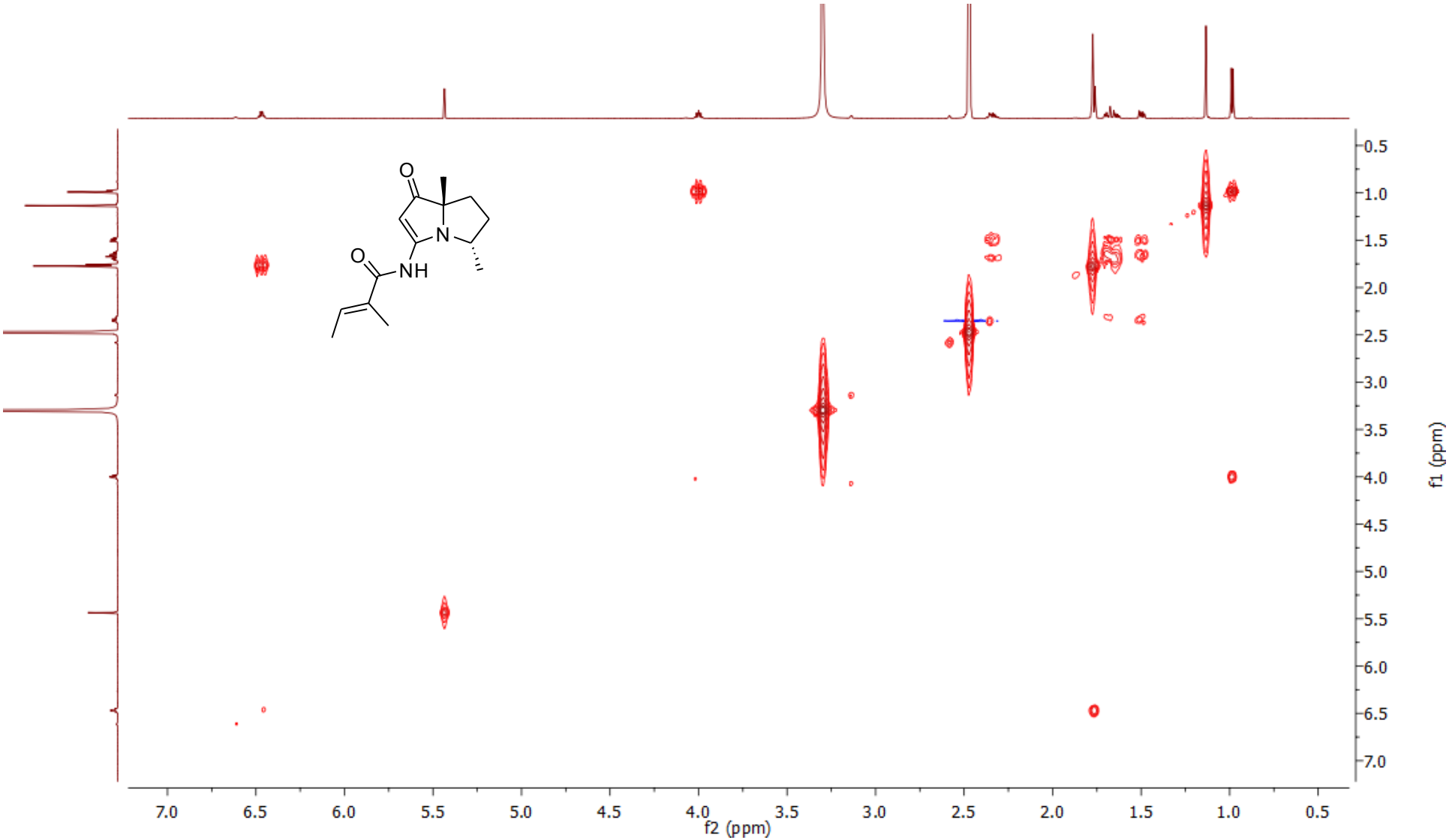

Figure S52. HSQC spectrum of bohemamine H (**7**) in DMSO-d_6

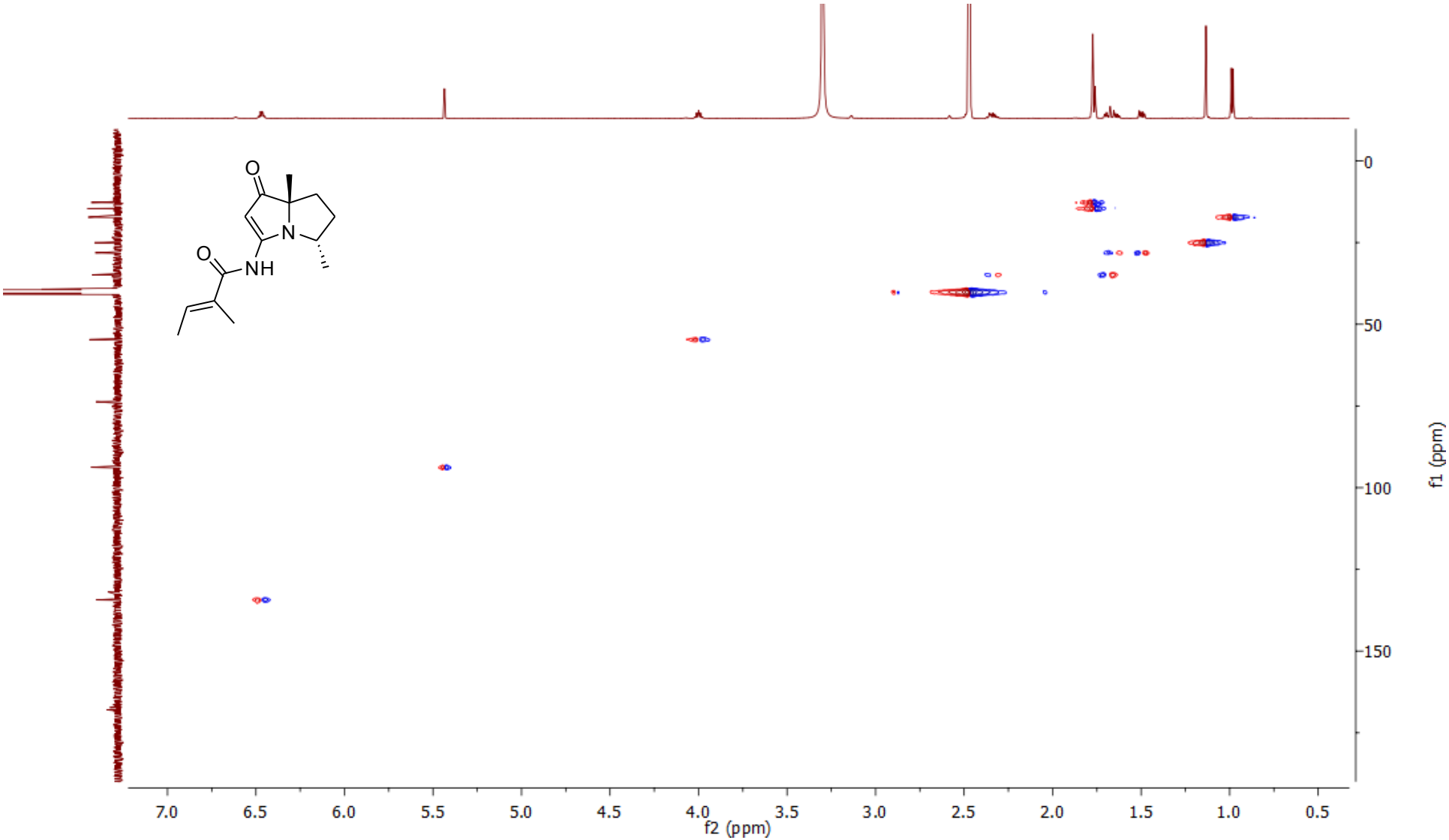

Figure S53. HMBC spectrum of bohemamine H (**7**) in DMSO-d_6

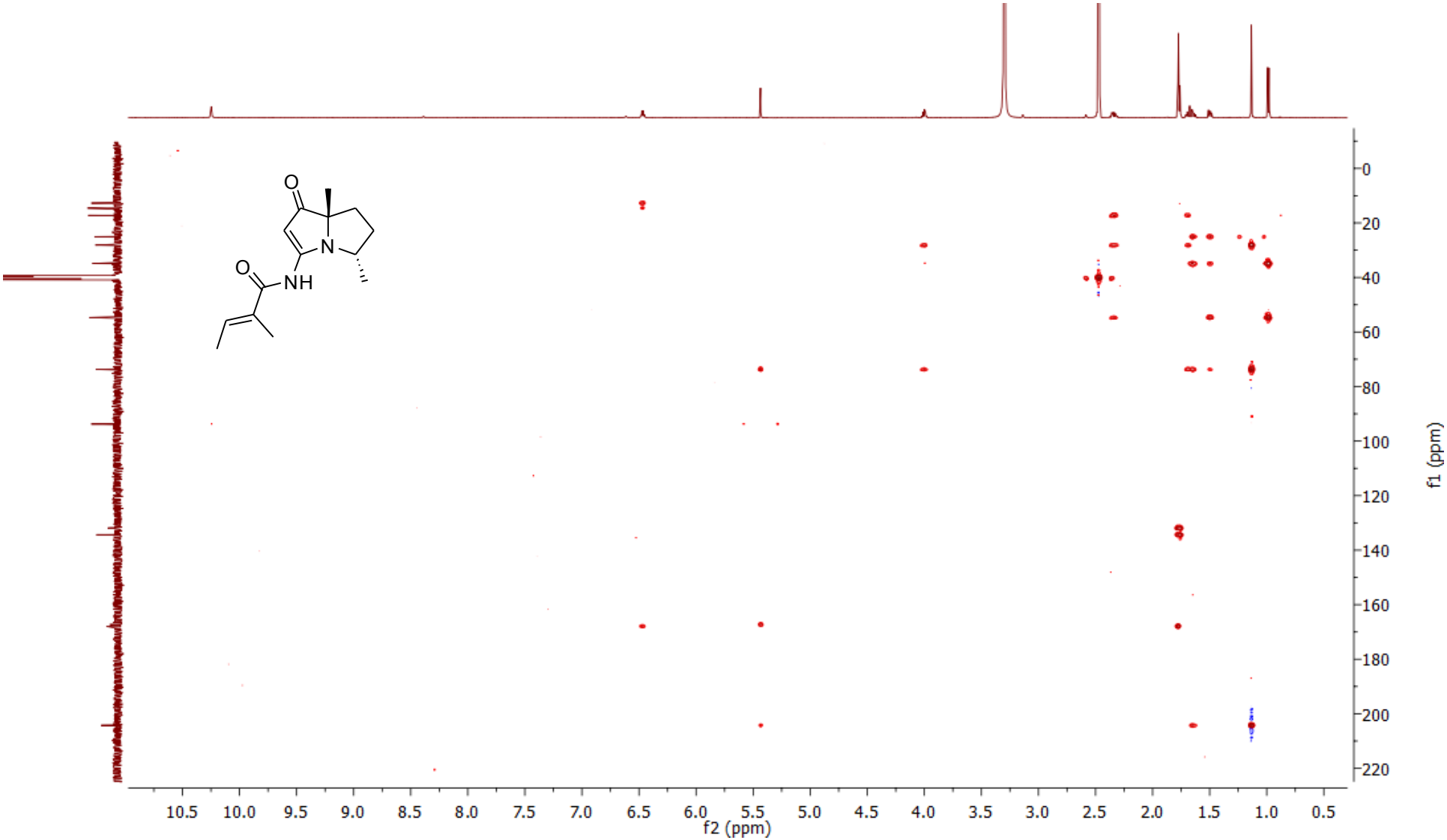

Figure S54. NOESY spectrum of bohemamine H (**7**) in DMSO-d_6

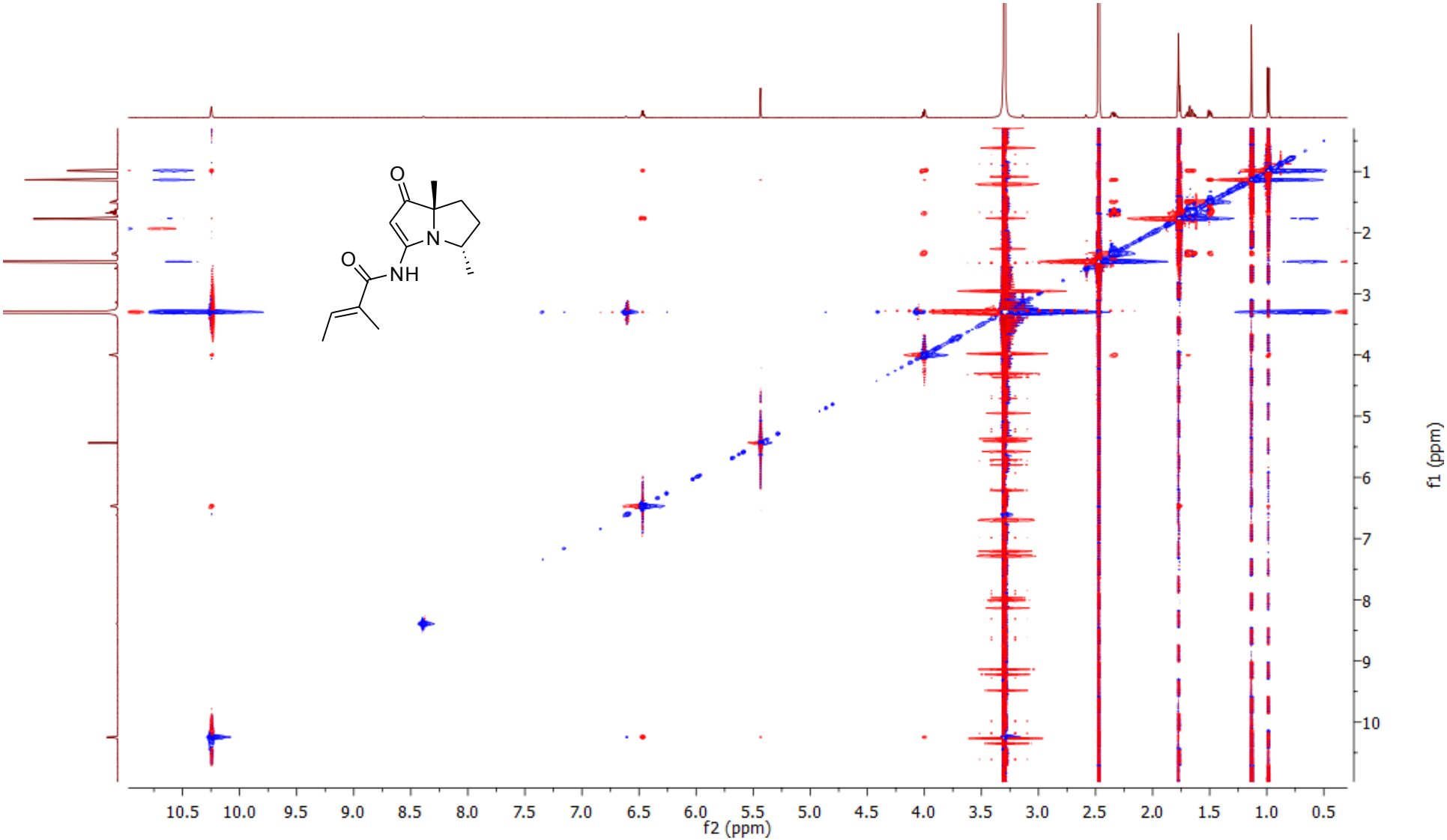

Figure S55. HRESIMS spectrum of bohemamine I (**8**)

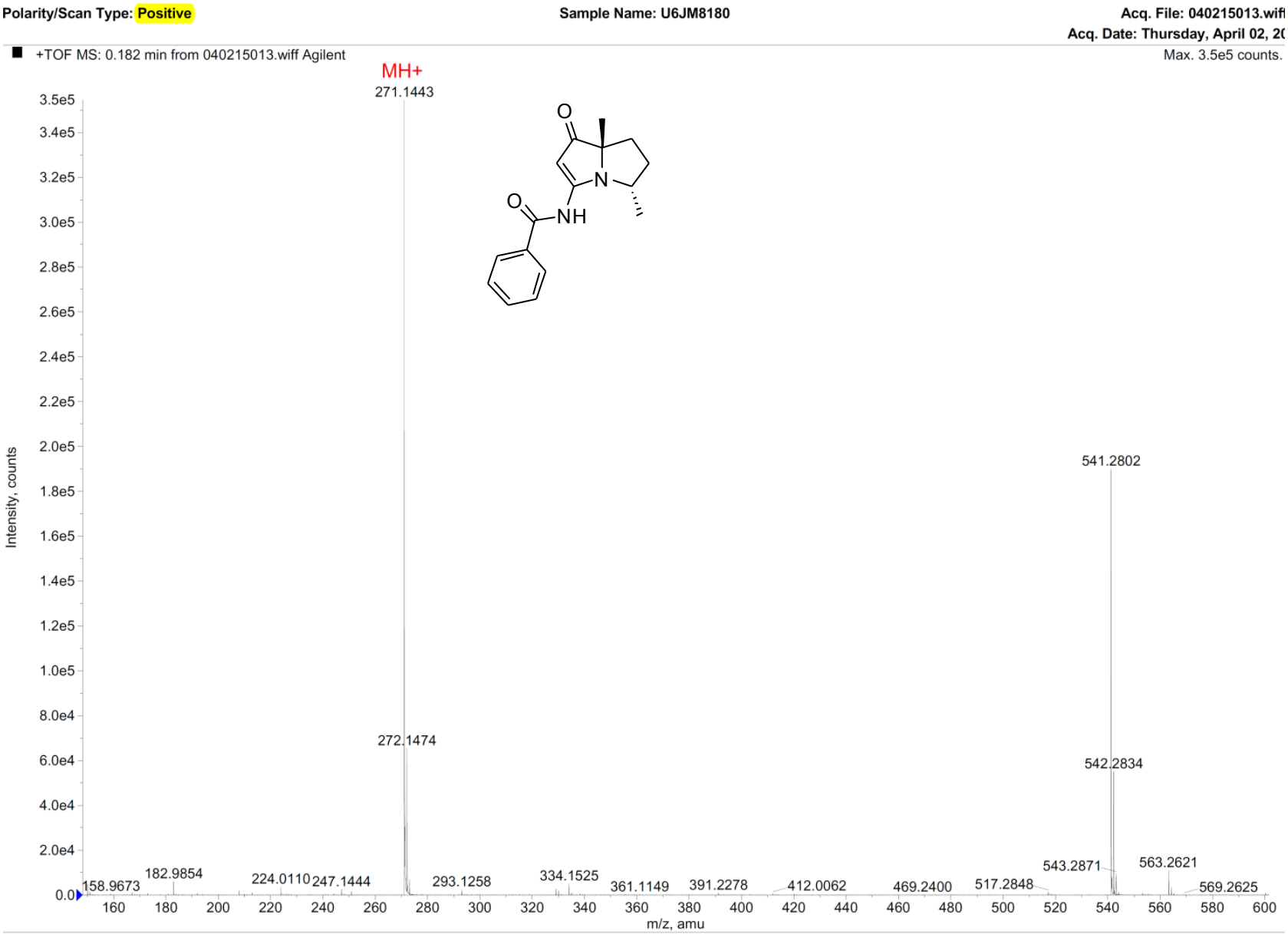

Figure S56. ^{1}H-NMR spectrum of bohemamine I (**8**) in DMSO-d_6

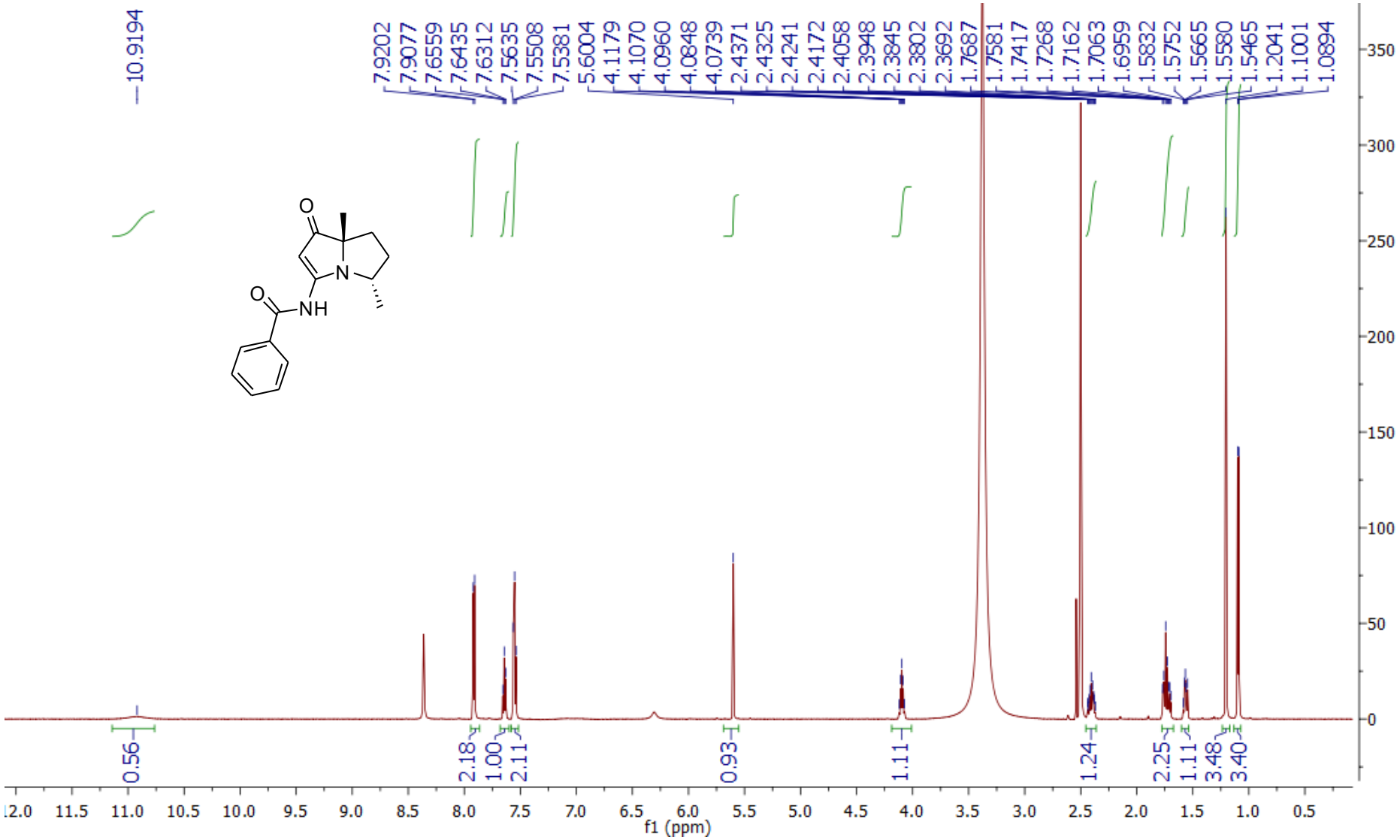

Figure S57. ^{13}C-NMR spectrum of bohemamine I (**8**) in DMSO-d_6

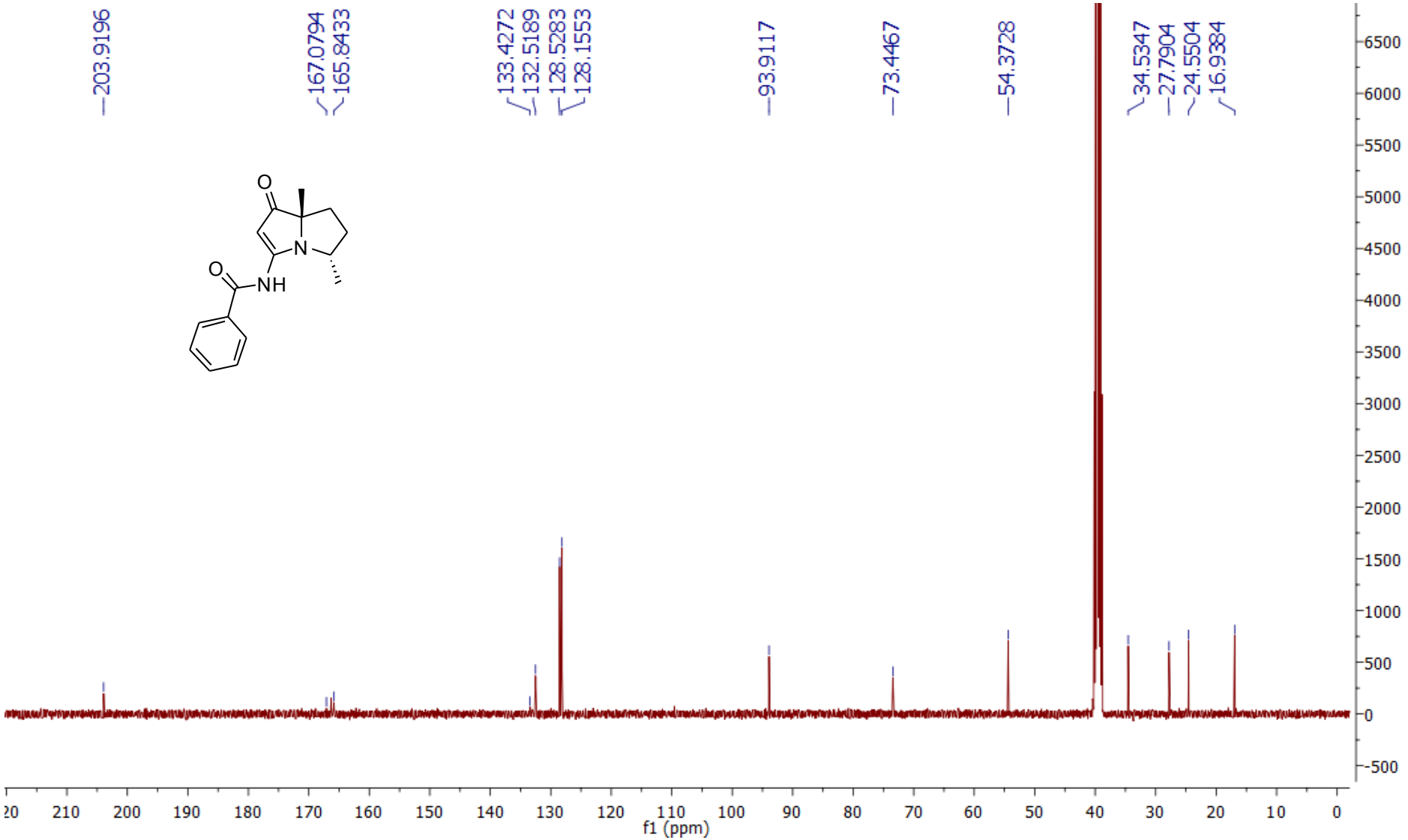

Figure S58. ^{1}H-^{1}H COSY spectrum of bohemamine I (**8**) in DMSO-d_6

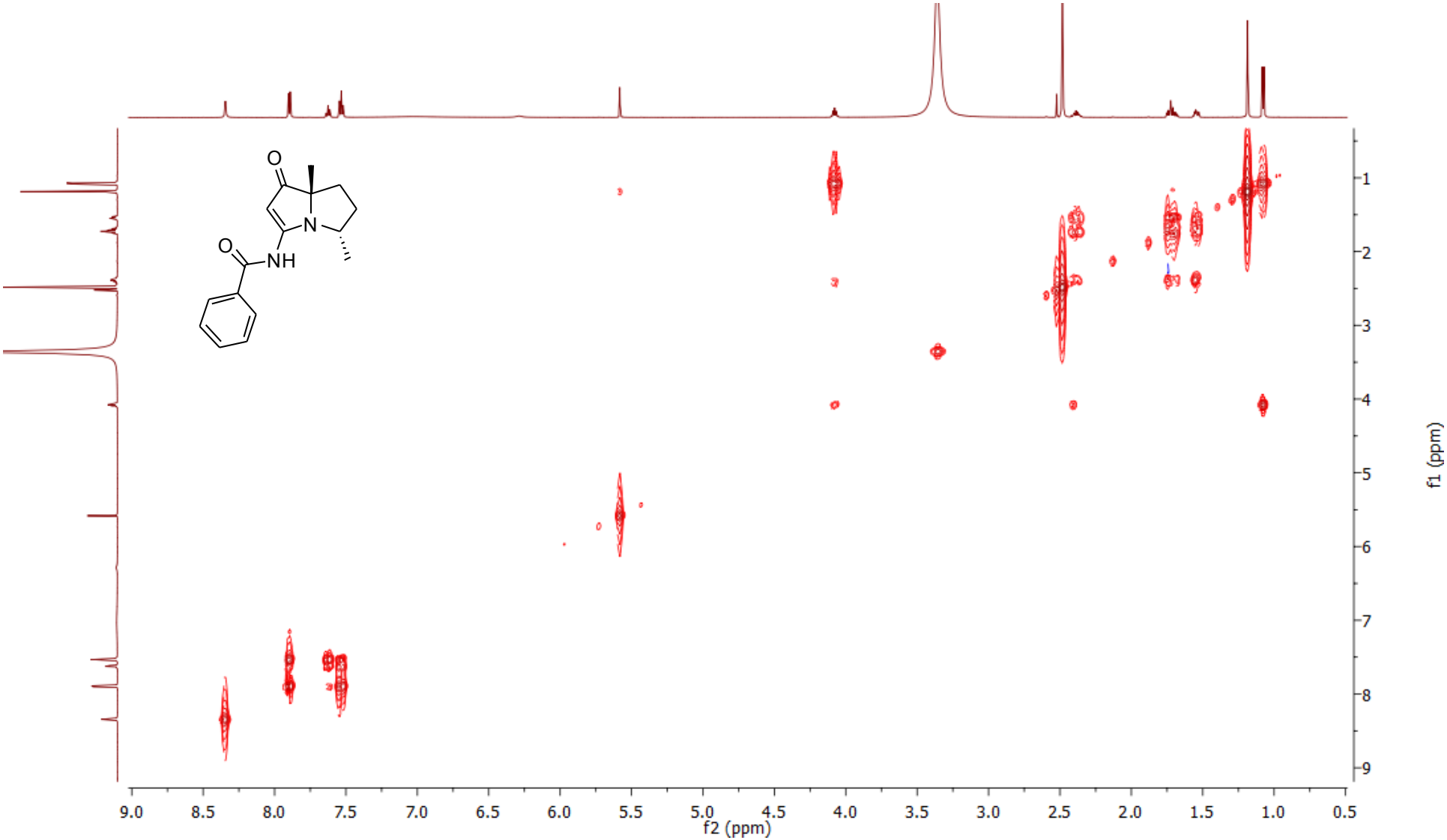

Figure S59. HSQC spectrum of bohemamine I (**8**) in DMSO-d_6

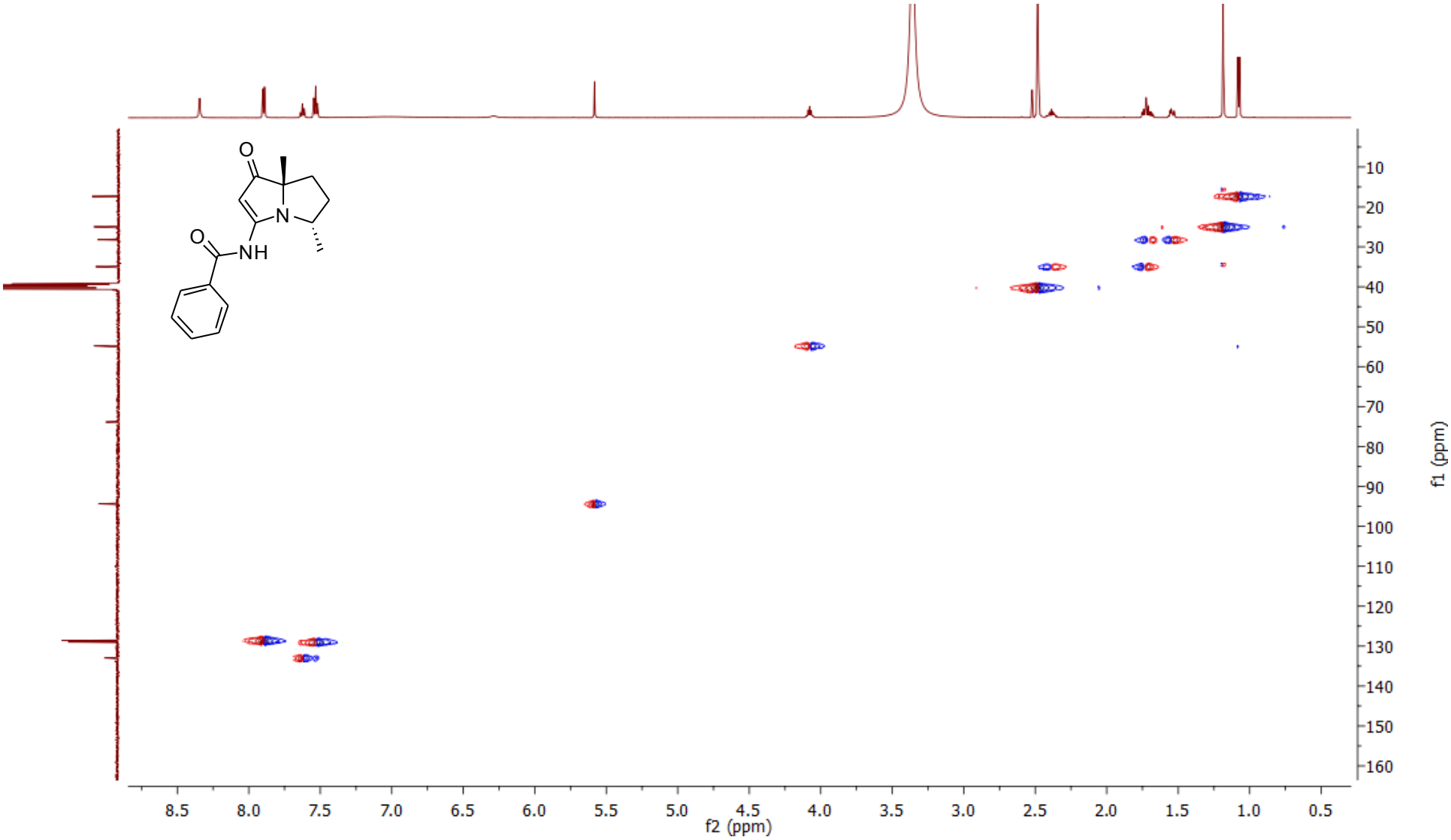

Figure S60. HMBC spectrum of bohemamine I (**8**) in DMSO-d_6

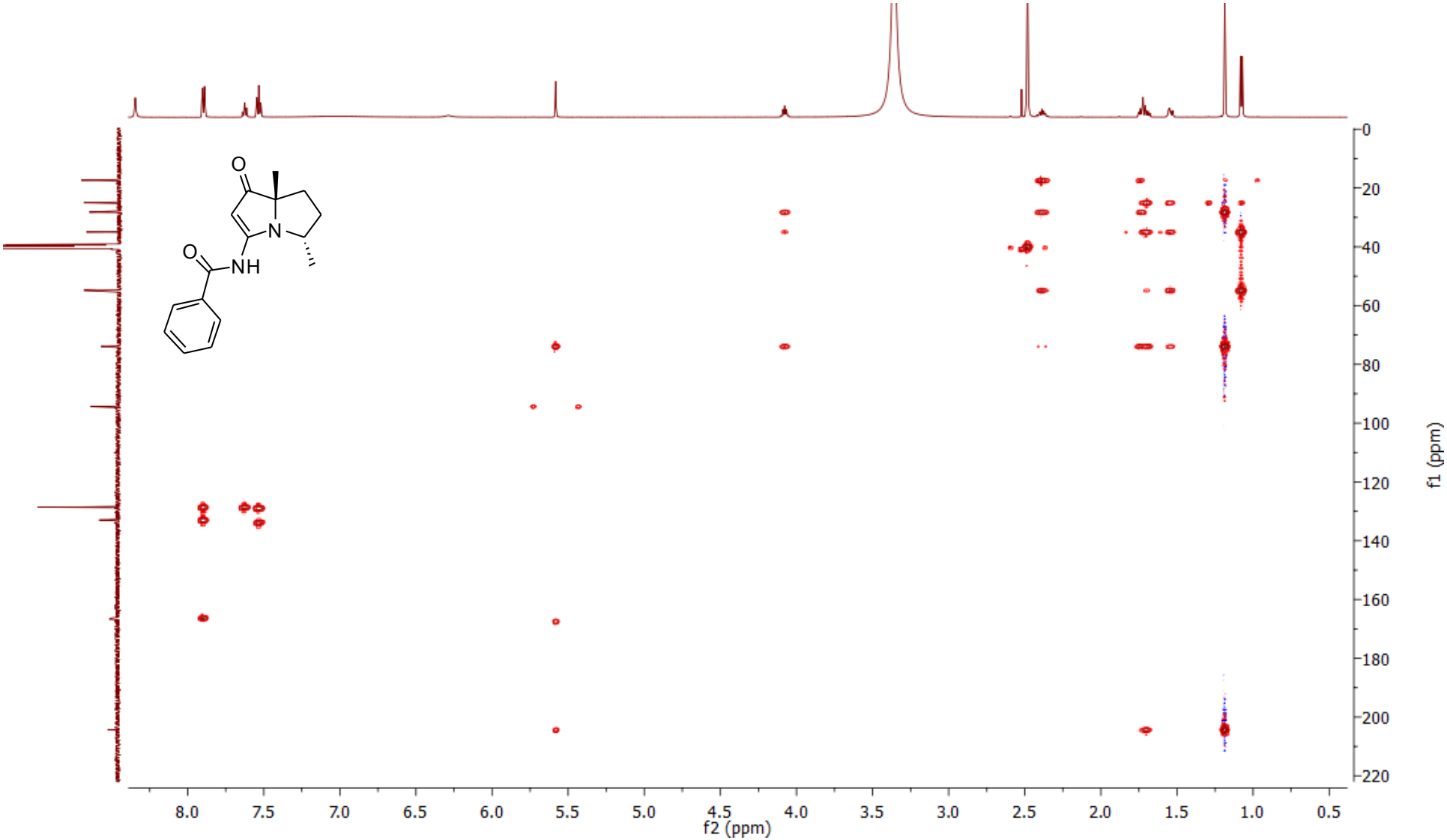

Figure S61. NOESY spectrum of bohemamine I (**8**) in DMSO-d_6