

Supporting Information

Daryamide Analogues from a Marine-Derived *Streptomyces* species

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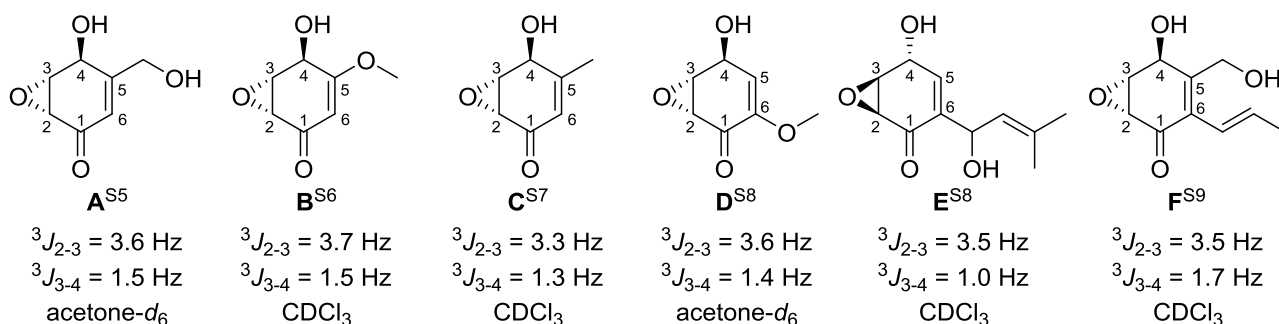
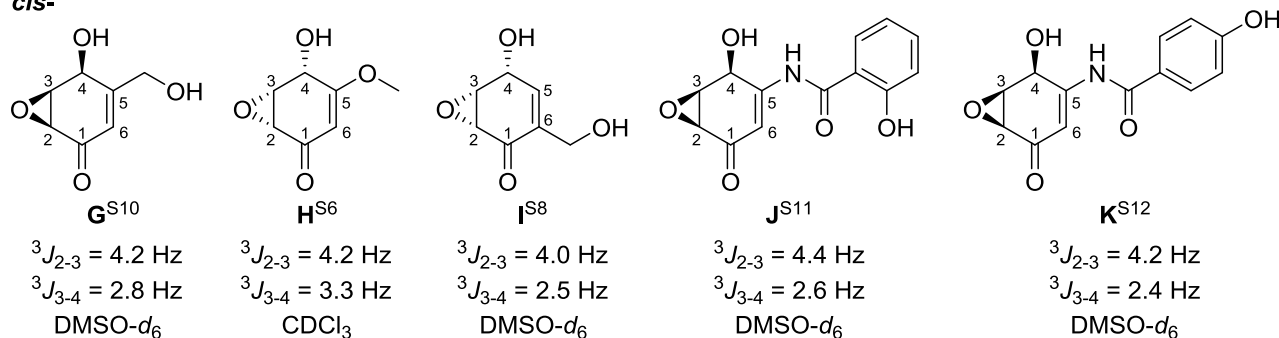
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Bioassay Protocols

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 1–5, 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% CO₂ at 37 °C. For cell viability assays, HCC366, A549, HCC44 and H2122 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.

trans-**cis-****Figure S1.** Analysis of coupling constants for some example of cyclohexenes with an epoxide moiety.**References:**

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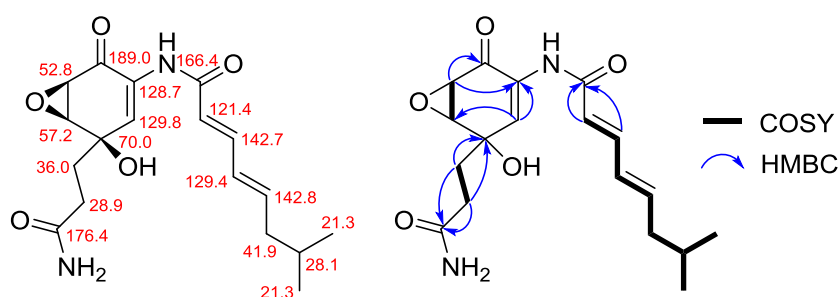


Figure S2. ¹³C chemical shifts and key 2D NMR correlations of compound **1d**.

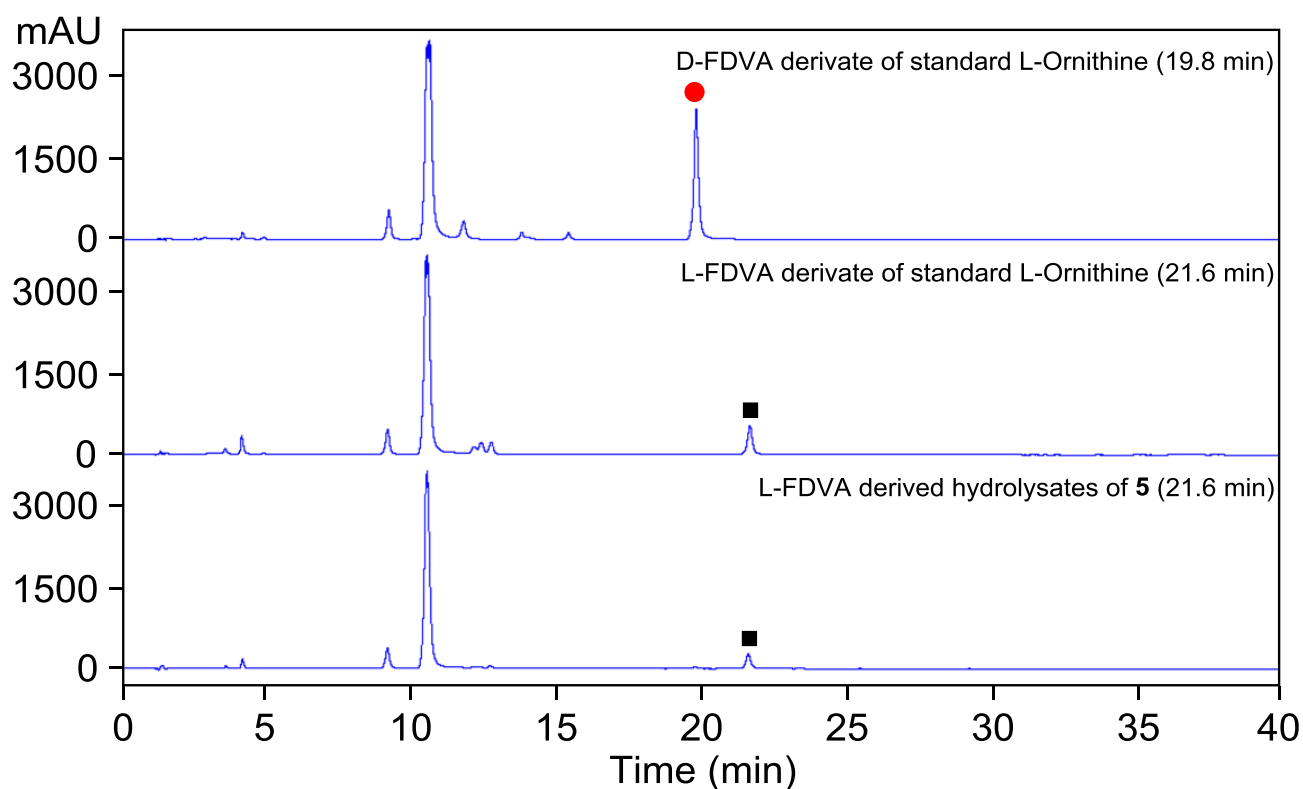


Figure S3. Marfey's method to determine the absolute configuration of **5**.

Figure S4. ^1H -NMR spectrum of daryamide D (**1**) in CD_3OD

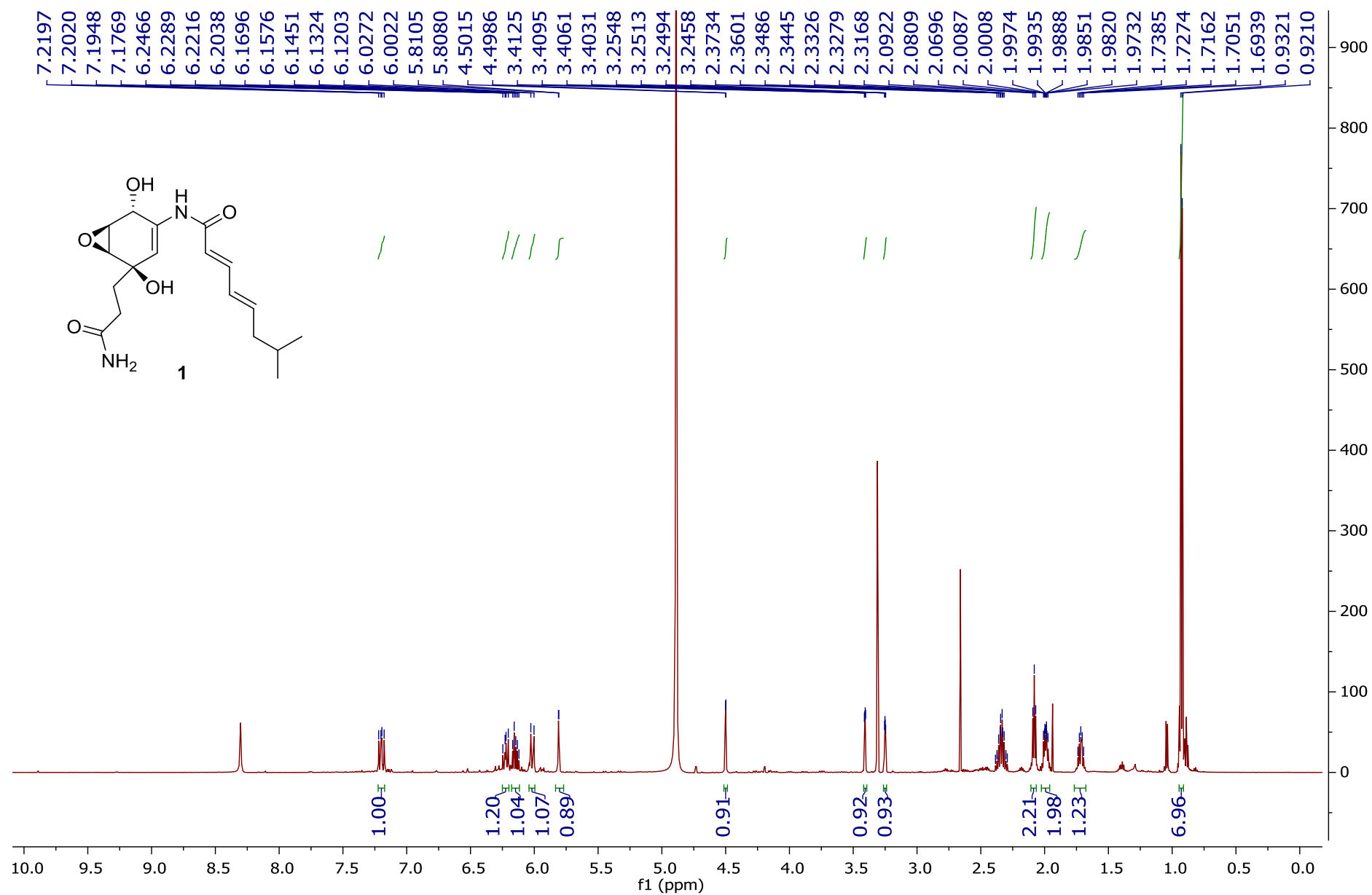


Figure S5. ^{13}C -NMR spectrum of daryamide D (**1**) in CD_3OD

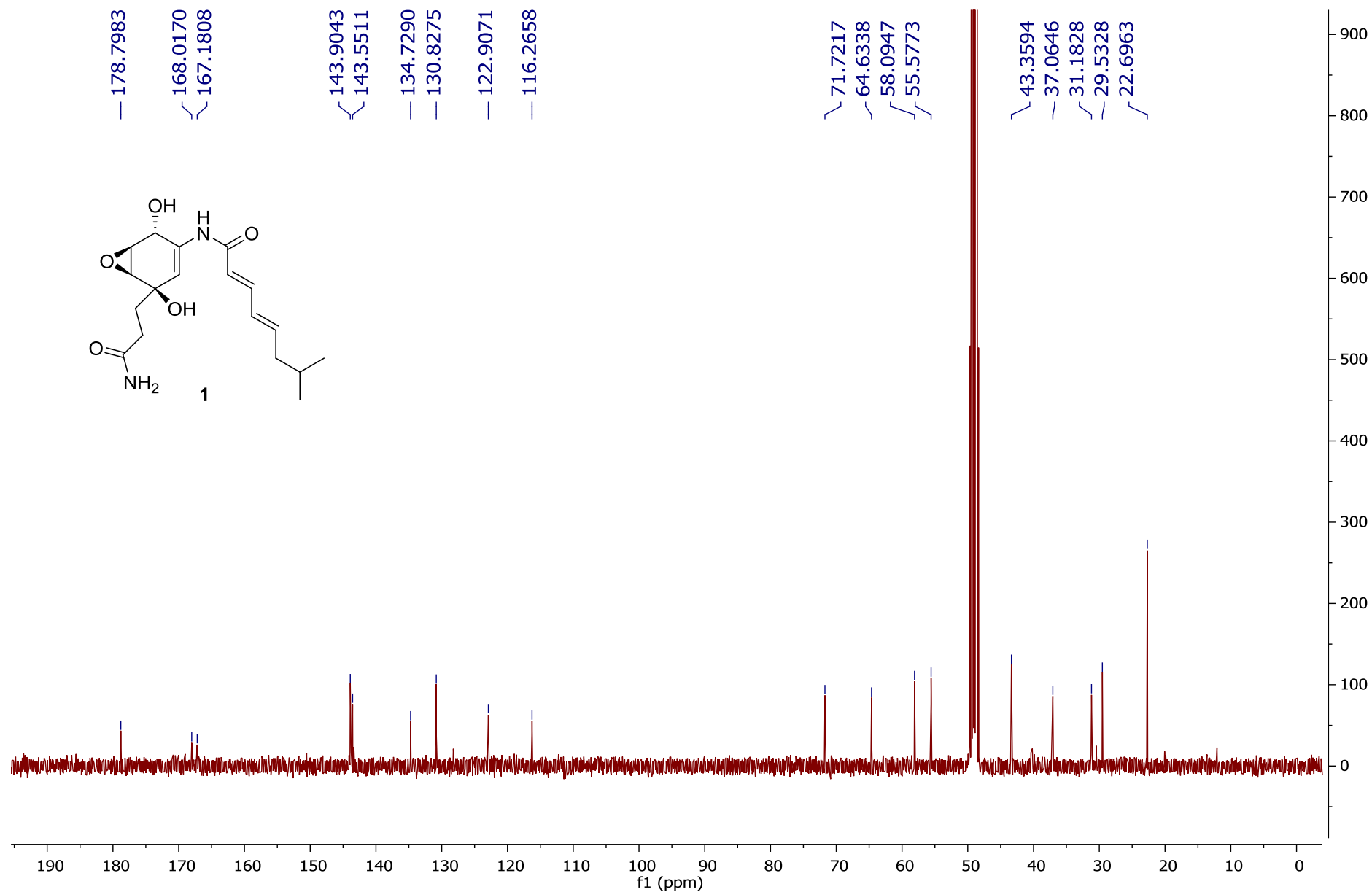


Figure S6. HSQC spectrum of daryamide D (**1**) in CD₃OD

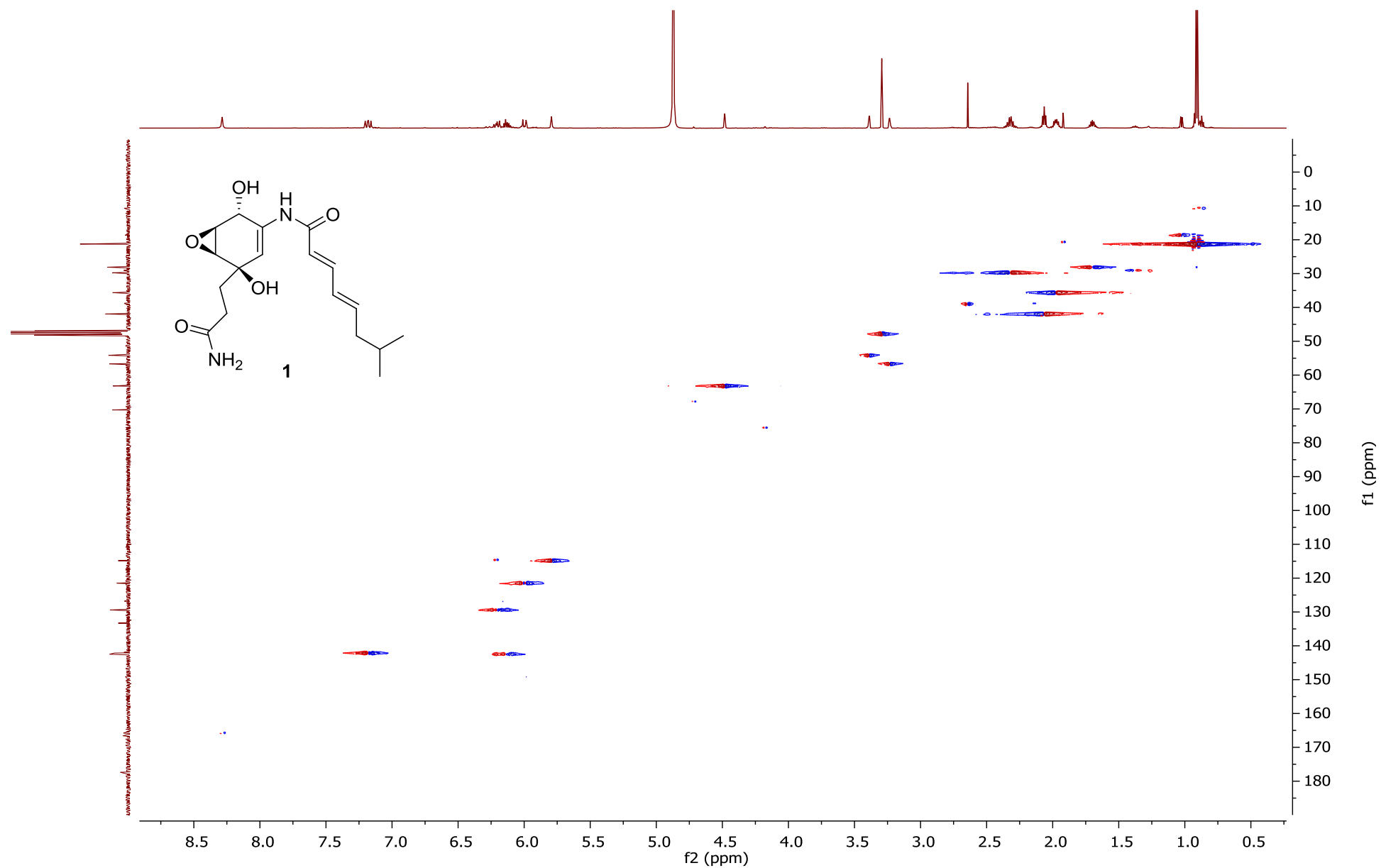


Figure S7. ^1H - ^1H COSY spectrum of daryamide D (**1**) in CD_3OD

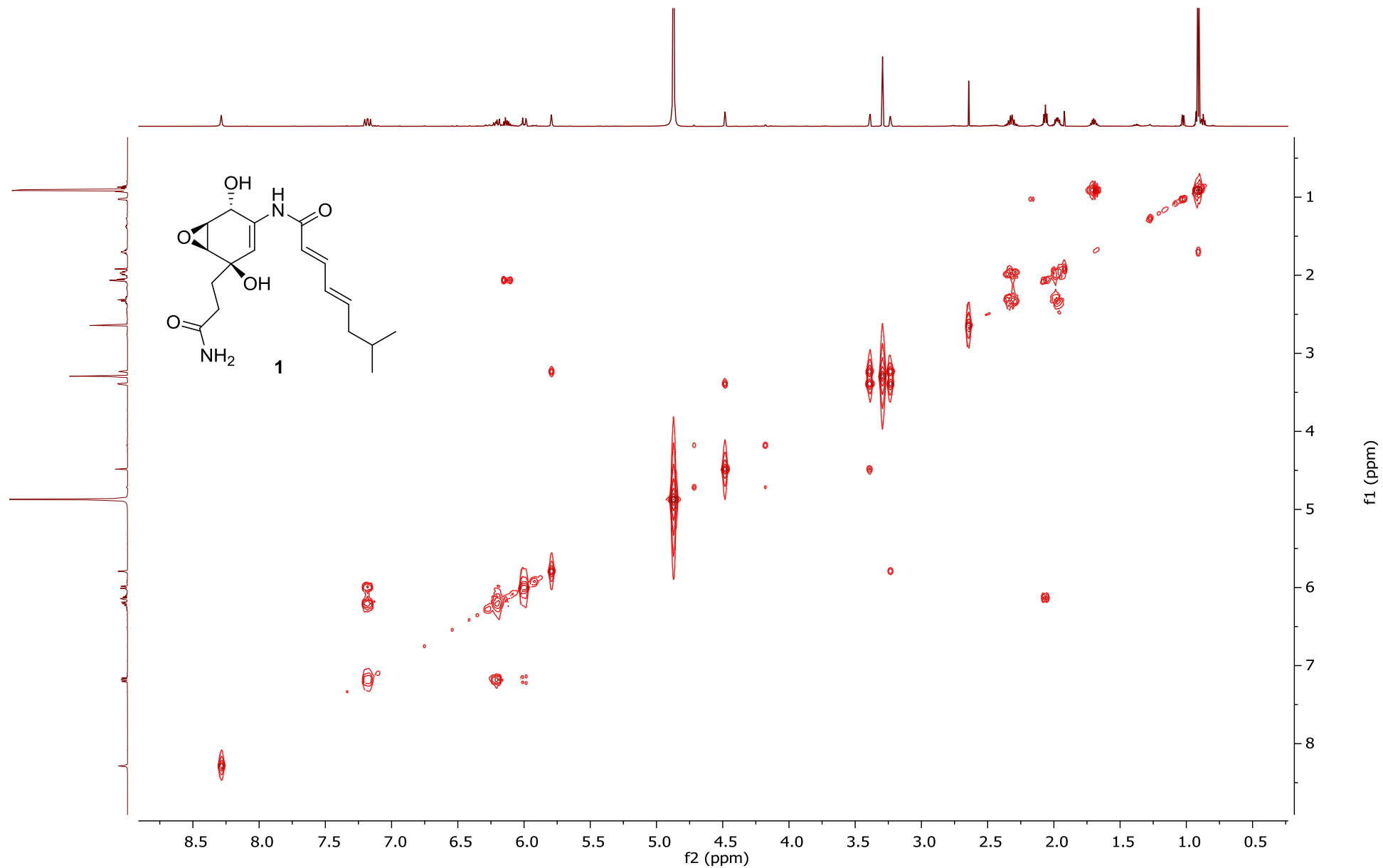


Figure S8. HMBC spectrum of daryamide D (**1**) in CD₃OD

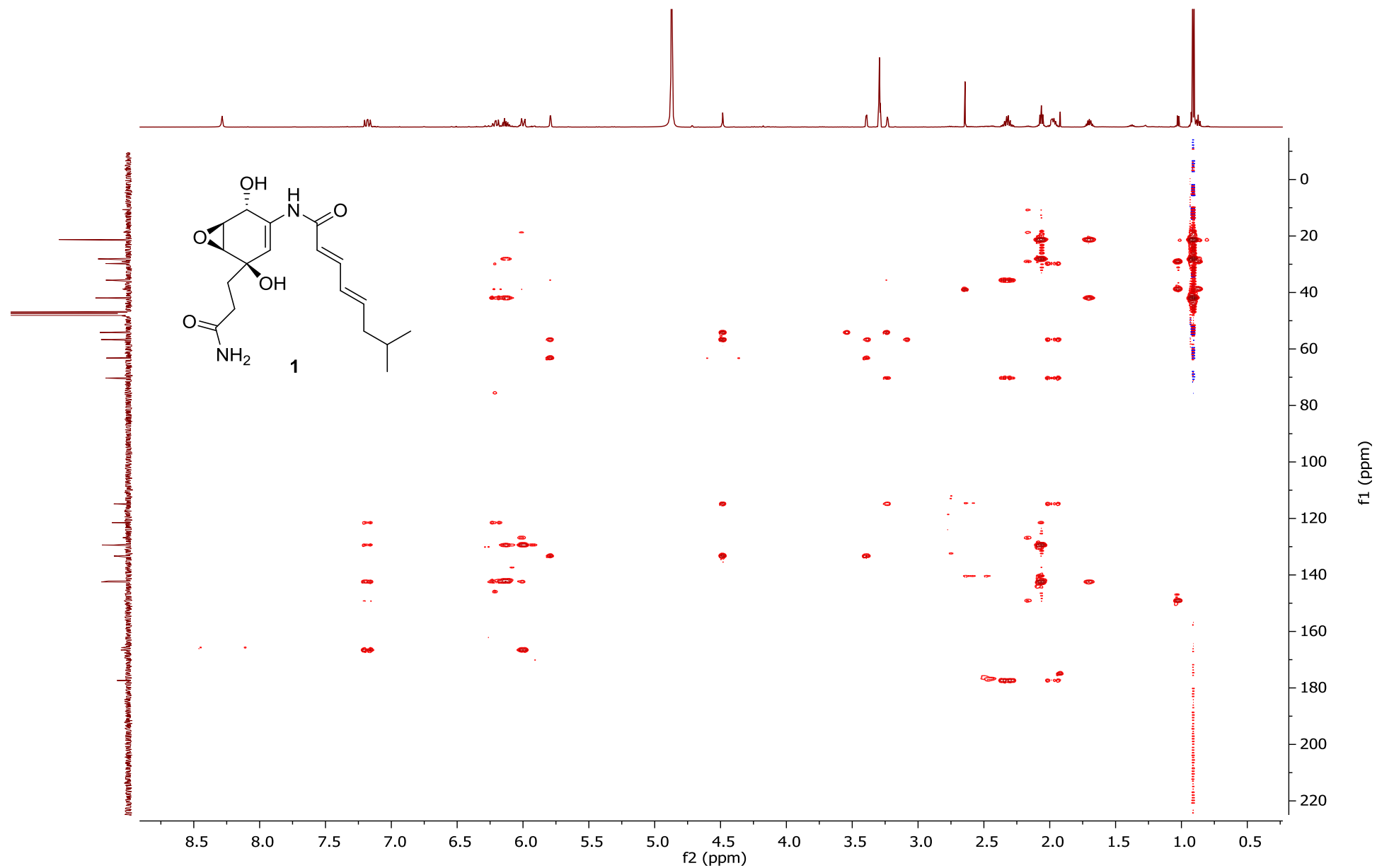


Figure S9. NOESY spectrum of daryamide D (1) in CD₃OD

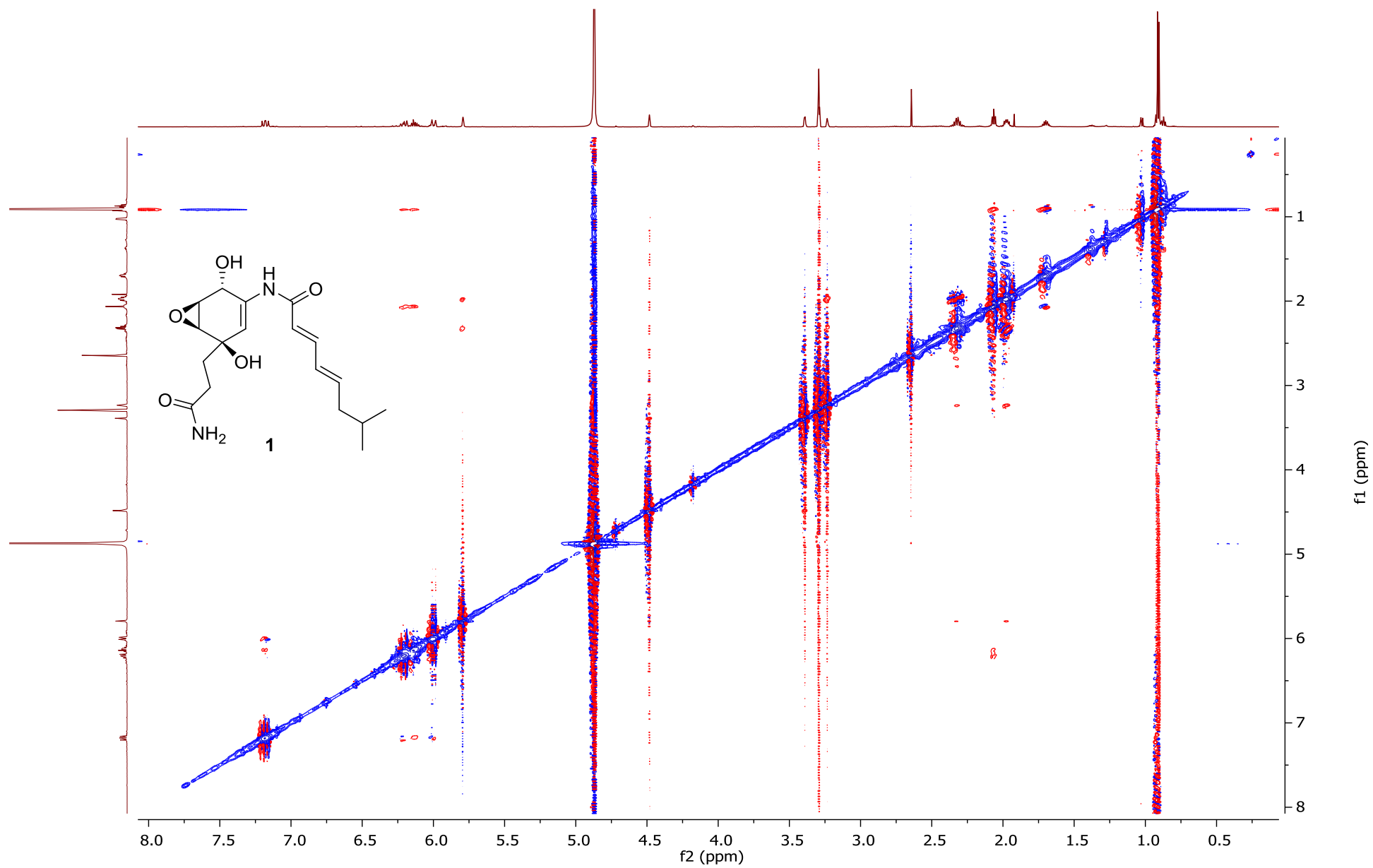


Figure S10. ^1H -NMR spectrum of daryamide D (1) in $\text{DMSO}-d_6$

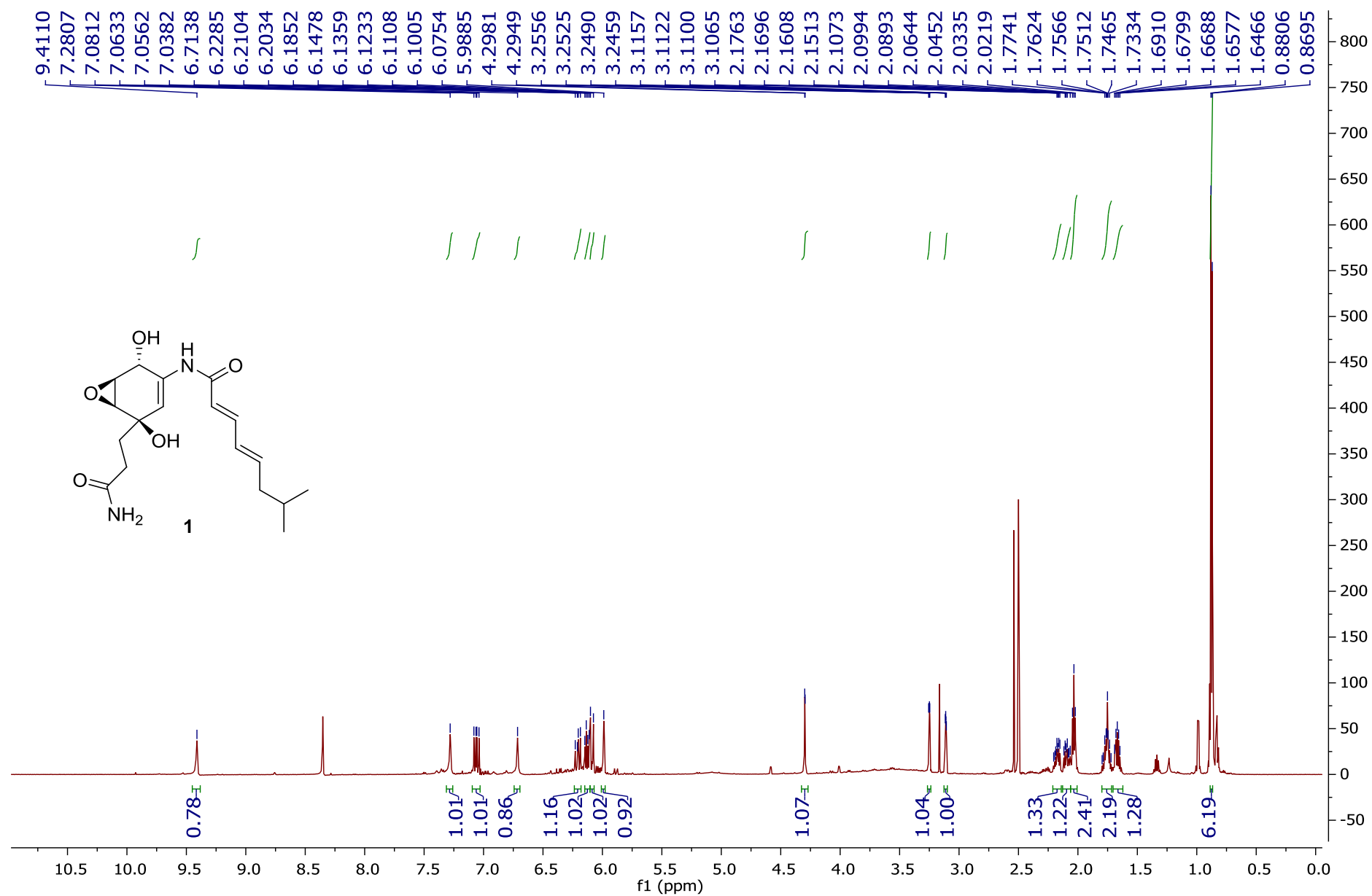


Figure S11. ^{13}C -NMR spectrum of daryamide D (**1**) in $\text{DMSO}-d_6$

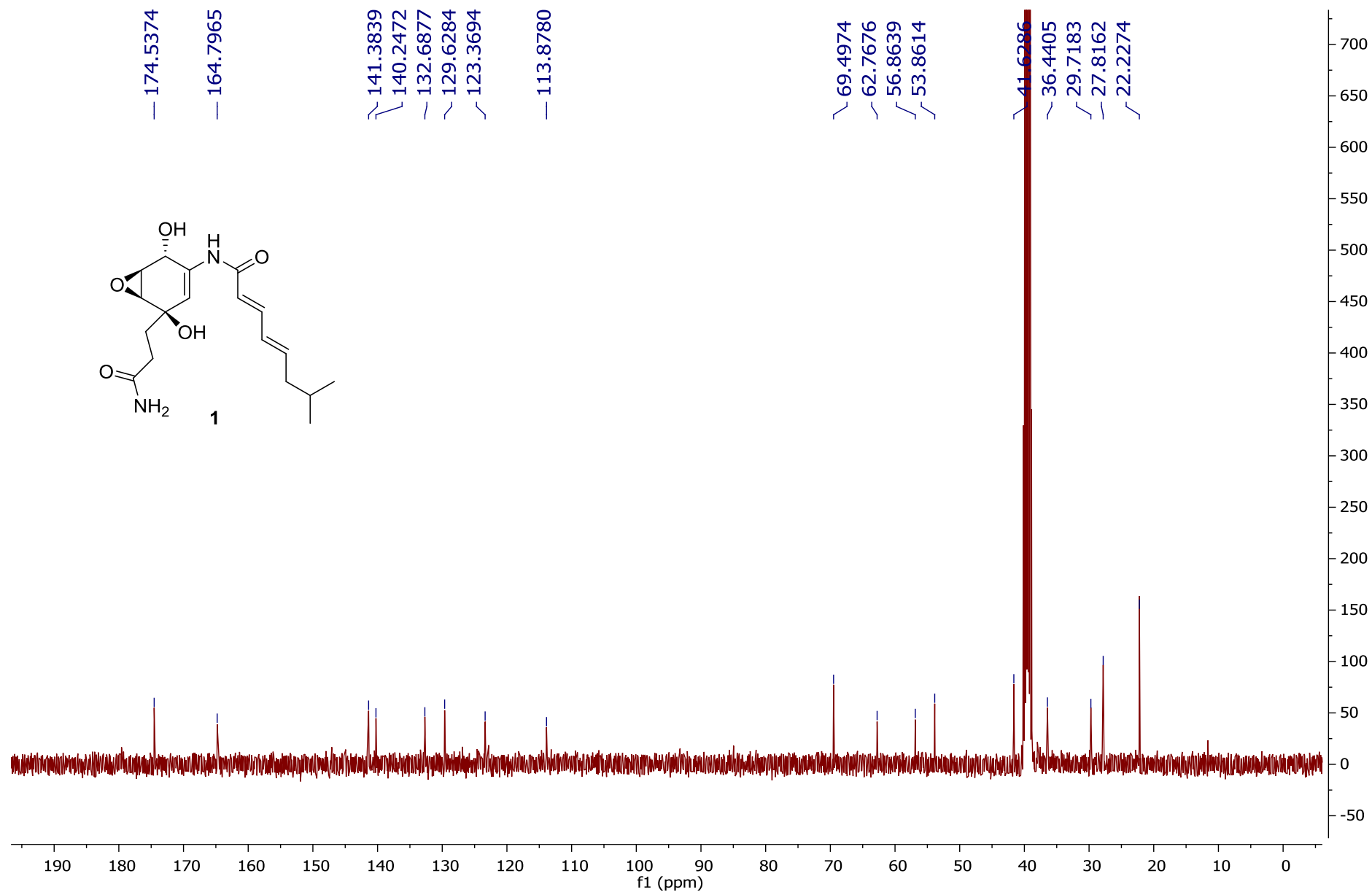


Figure S12. HSQC spectrum of daryamide D (**1**) in DMSO- d_6

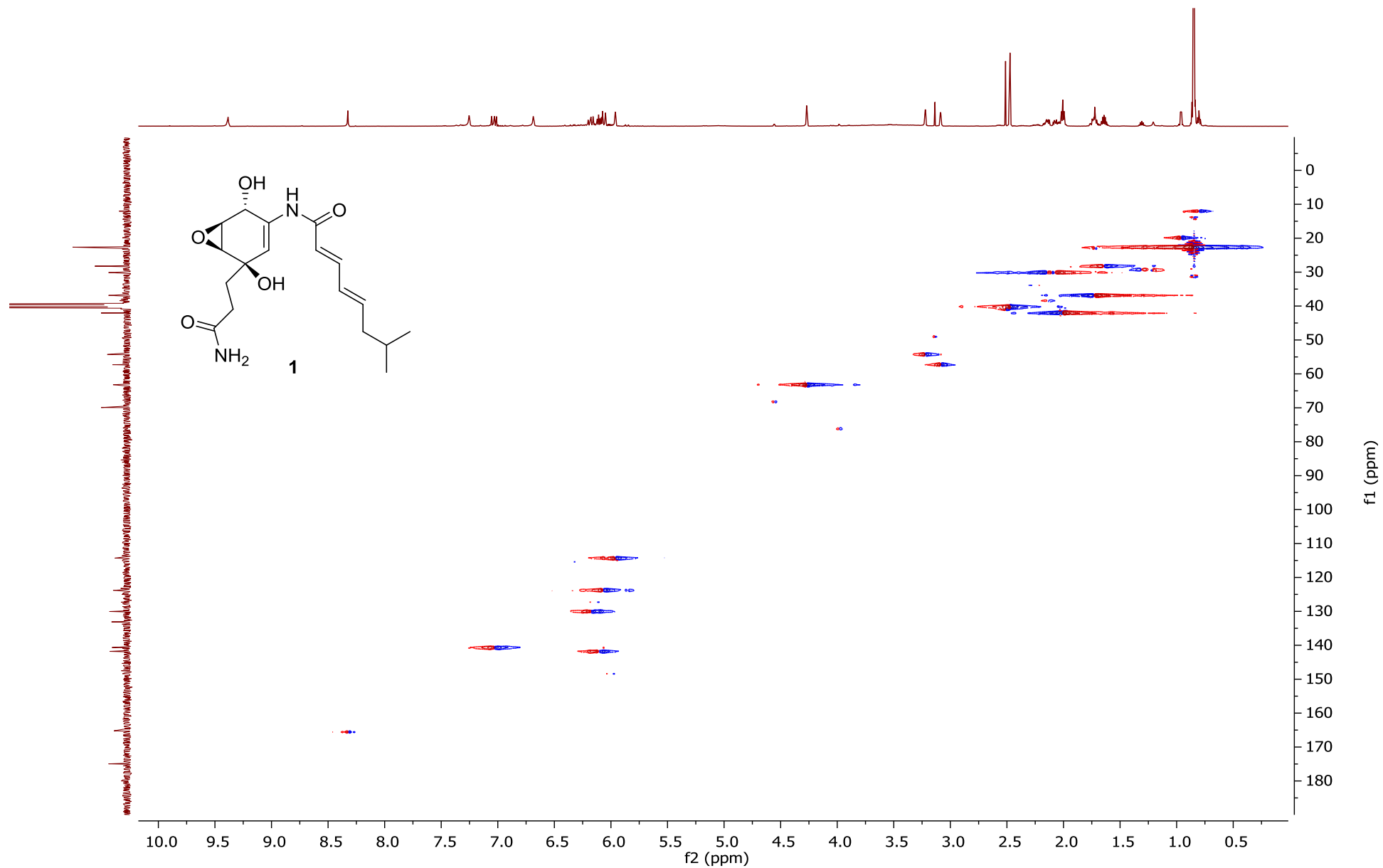


Figure S13. ^1H - ^1H COSY spectrum of daryamide D (1) in $\text{DMSO-}d_6$

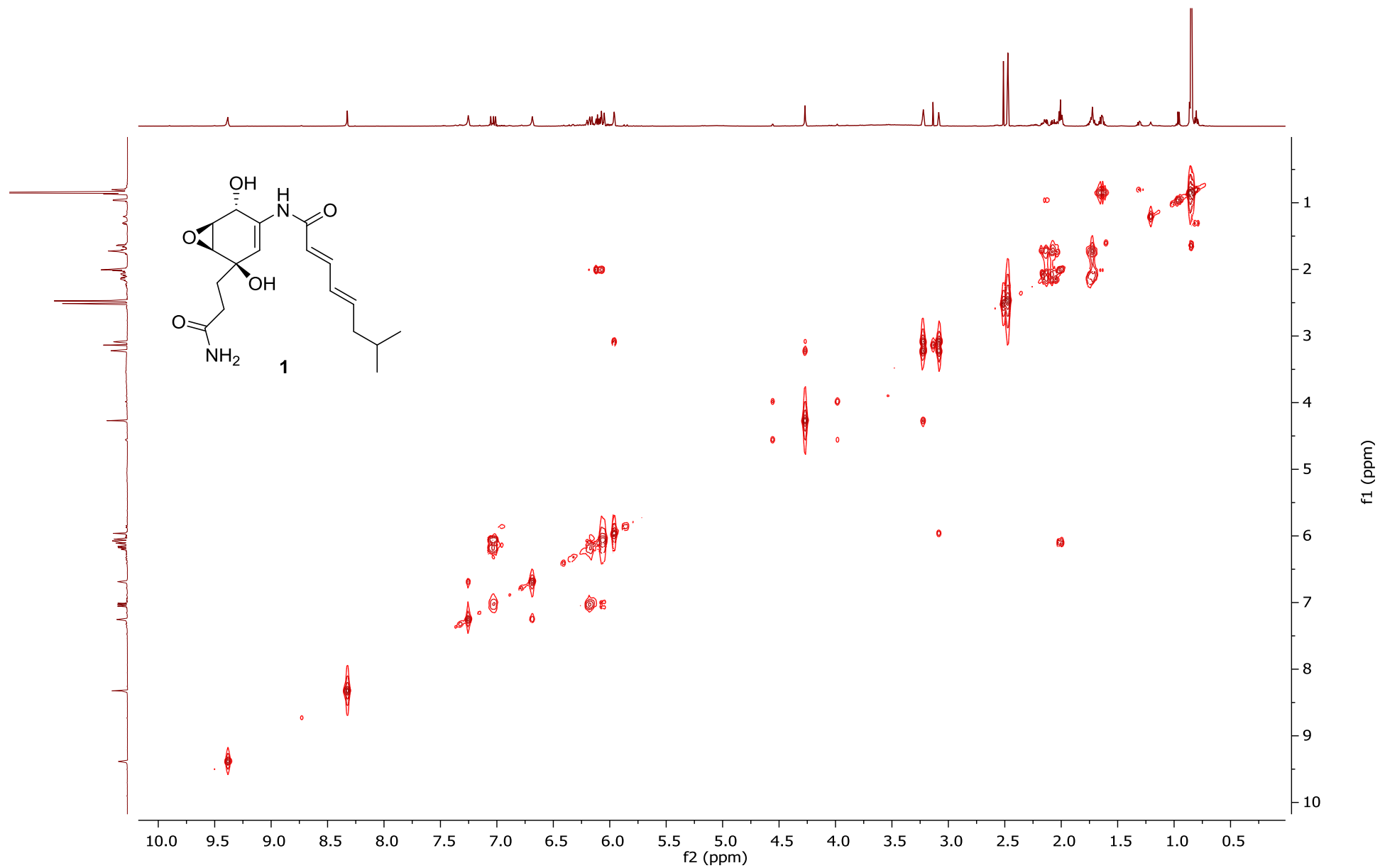


Figure S14. HMBC spectrum of daryamide D (**1**) in DMSO- d_6

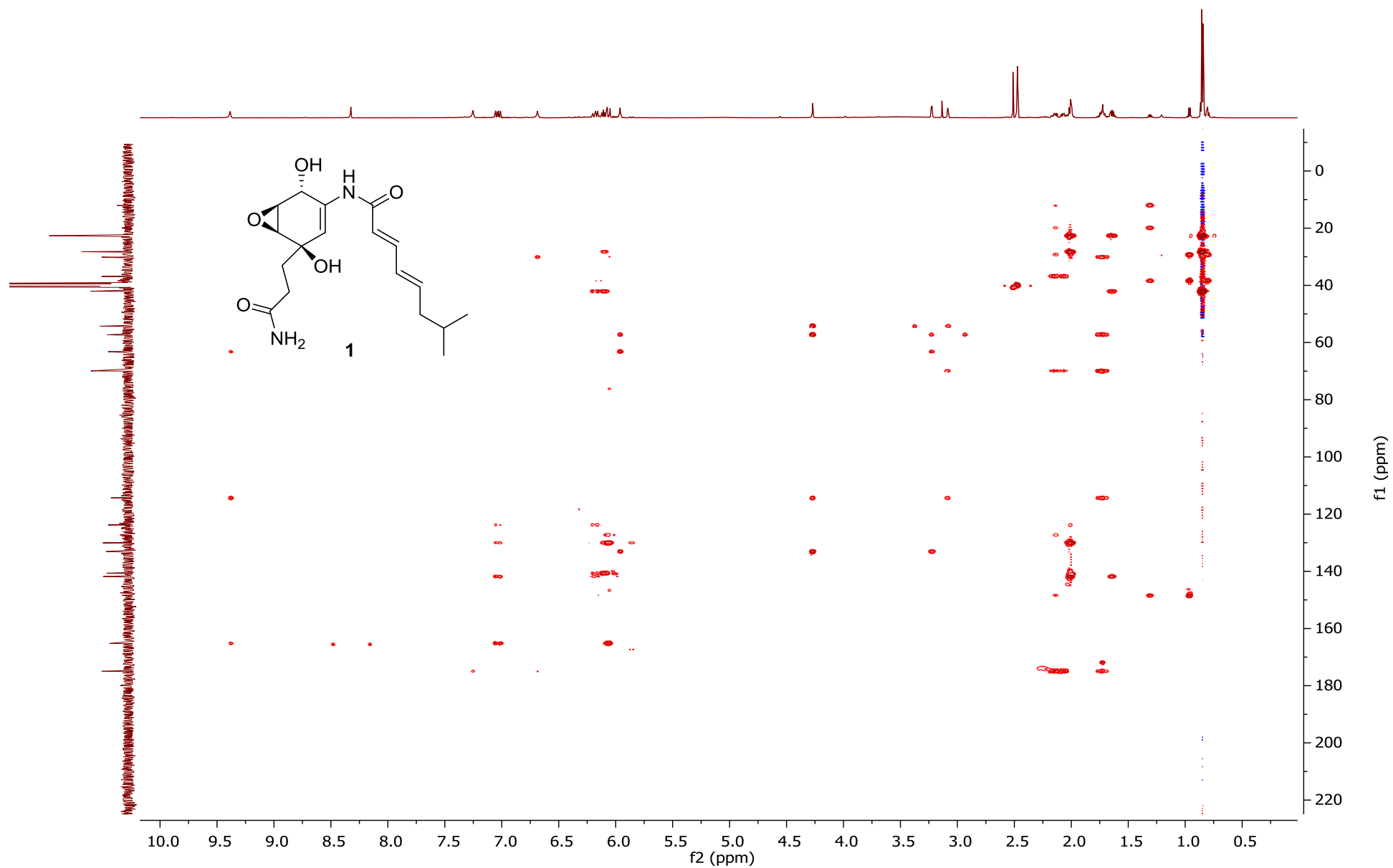


Figure S15. NOESY spectrum of daryamide D (**1**) in DMSO- d_6

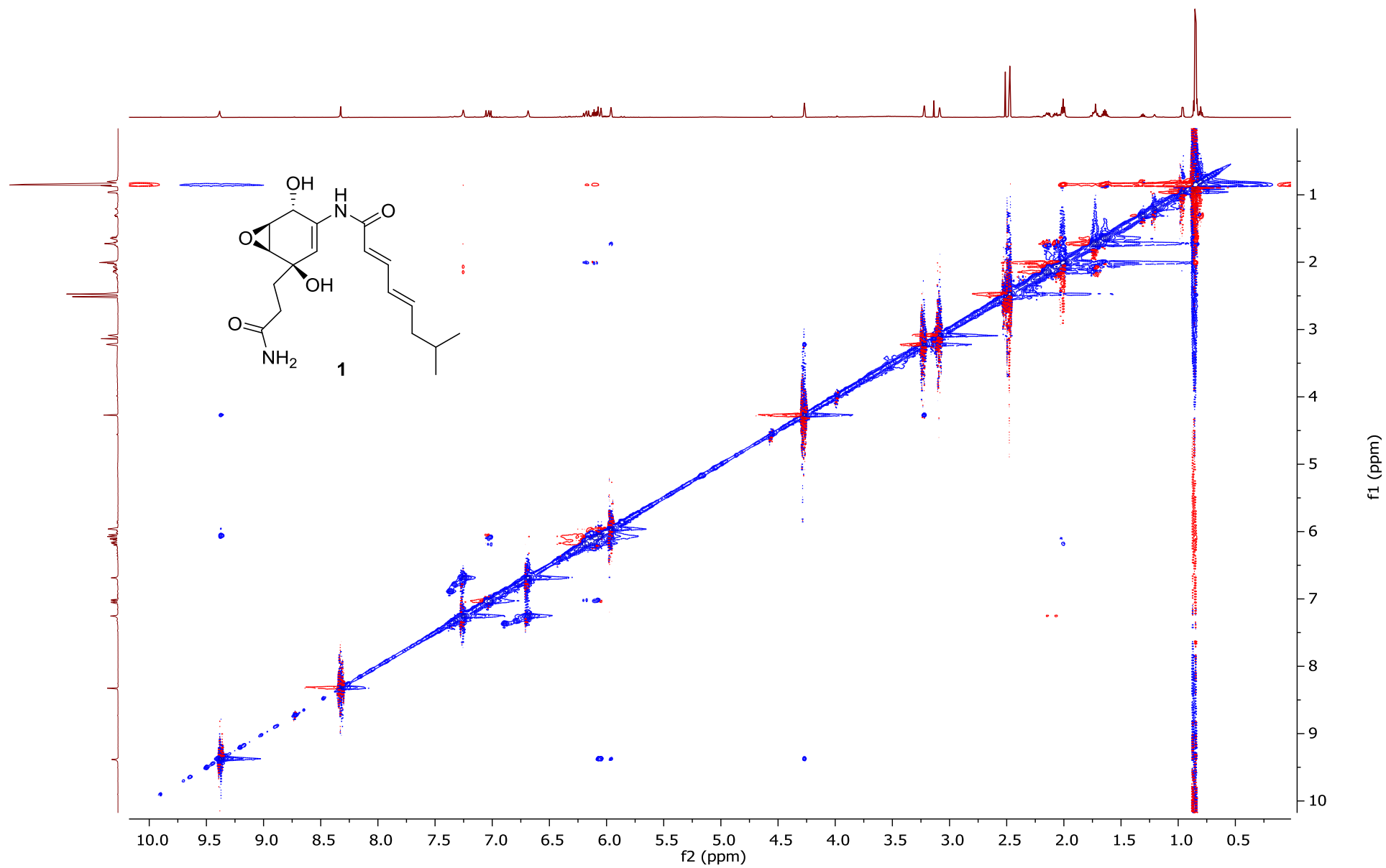


Figure S16. ^1H -NMR spectrum of daryamide D (**1**) in CDCl_3

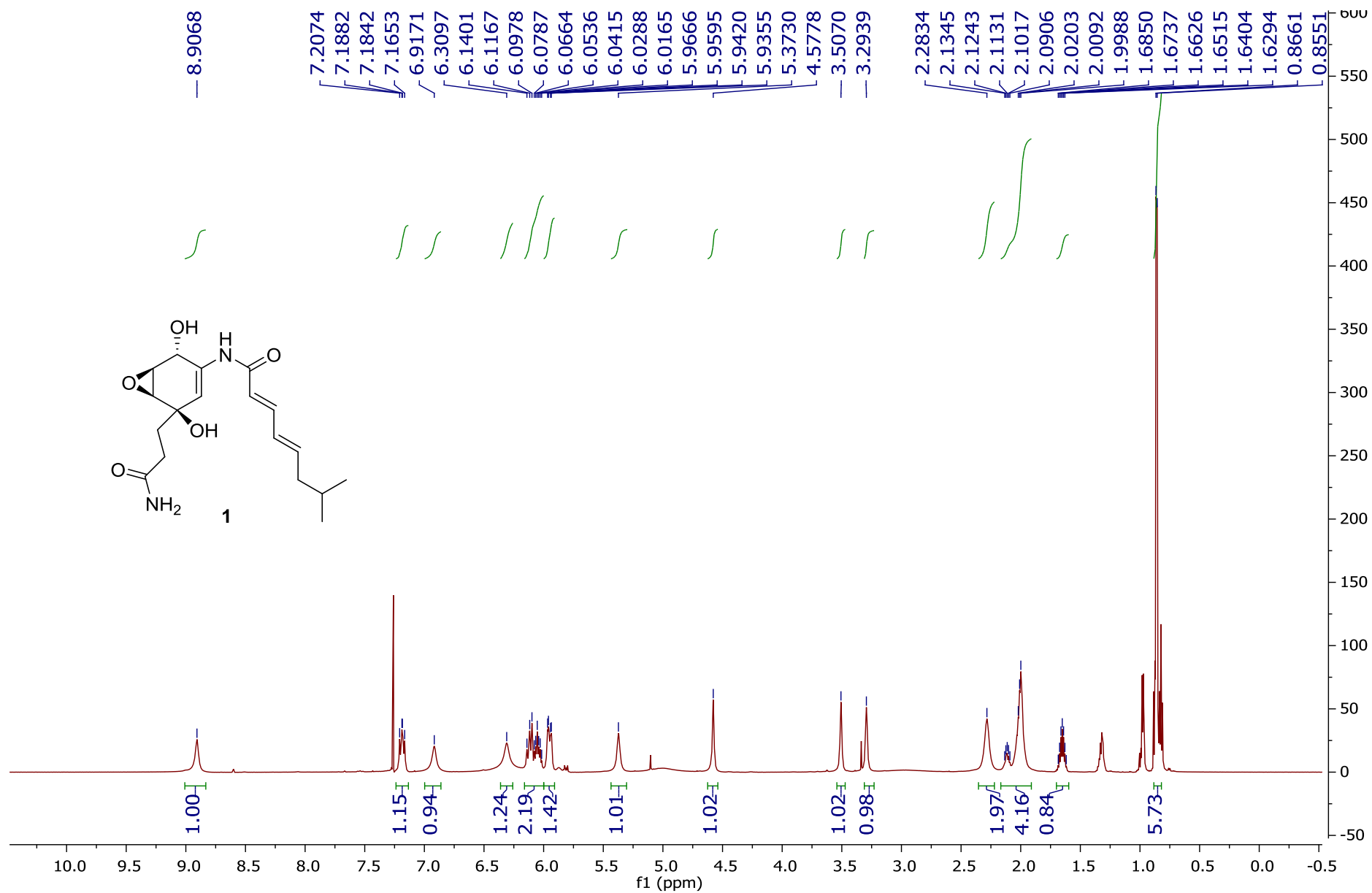


Figure S17. ^1H -NMR spectrum of daryamide D (**1**) in pyridine- d_5

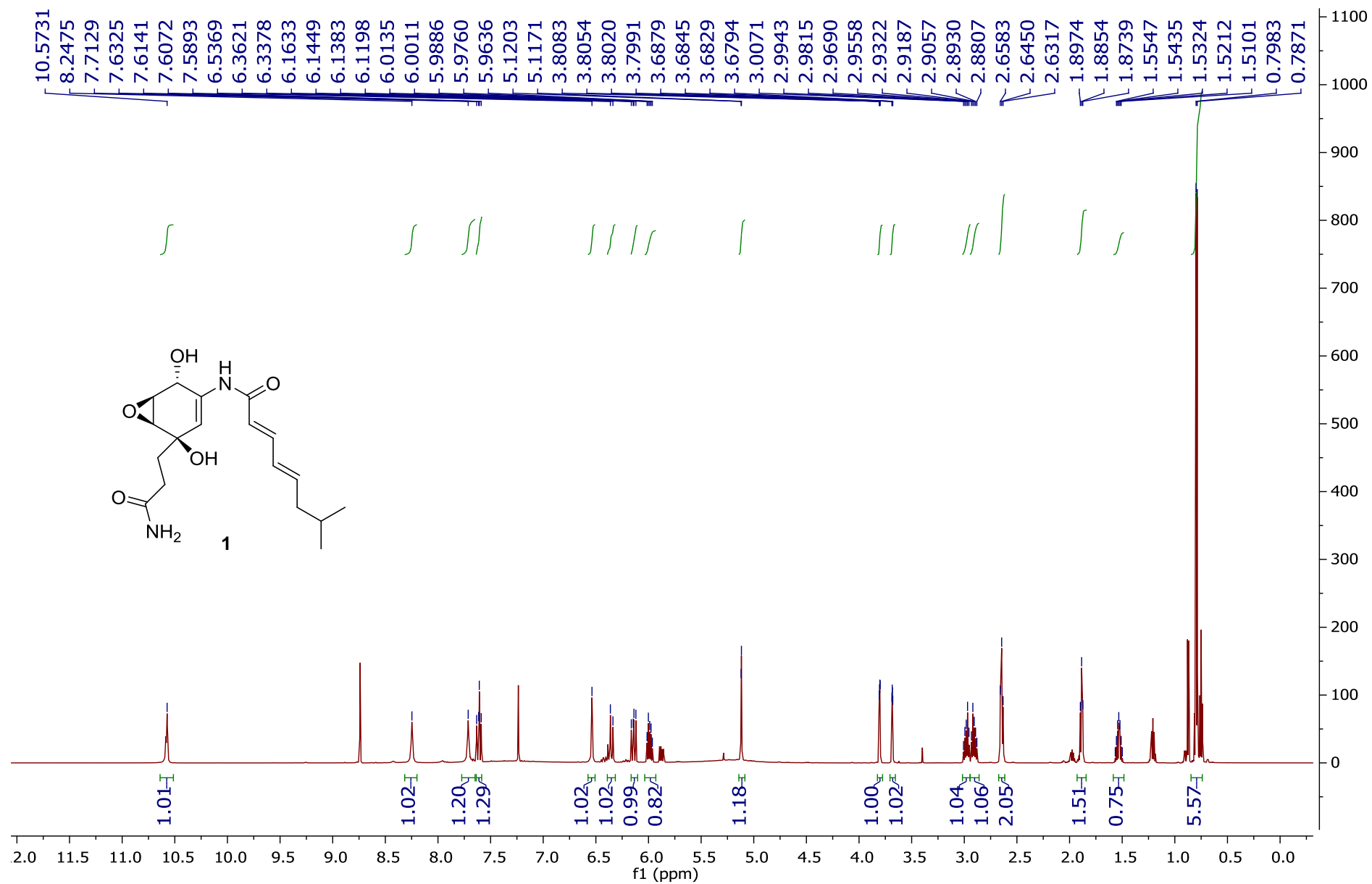


Figure S18. ^1H -NMR spectrum of compound **1a** in CDCl_3

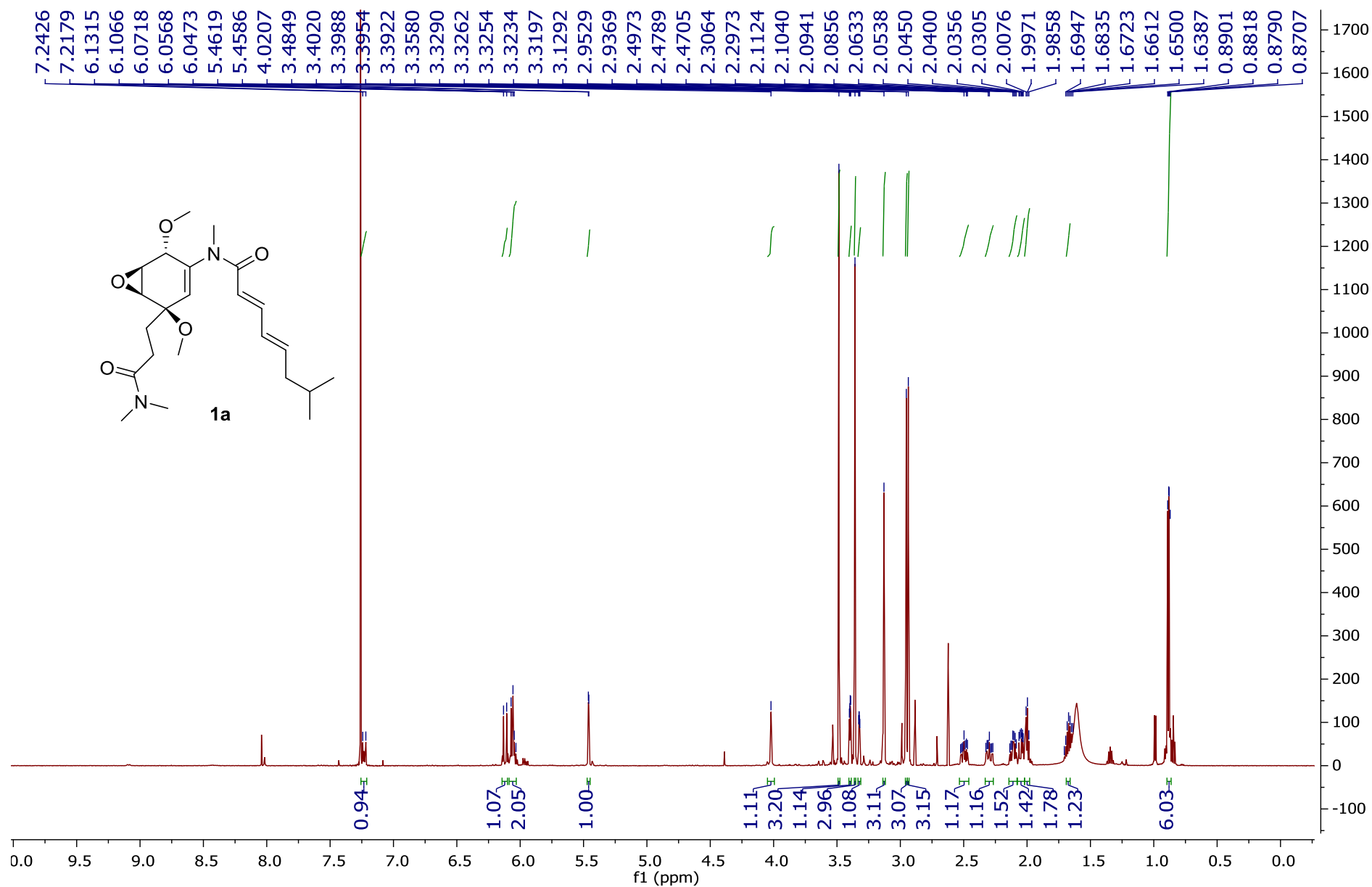


Figure S19. ^{13}C -NMR spectrum of compound **1a** in CDCl_3

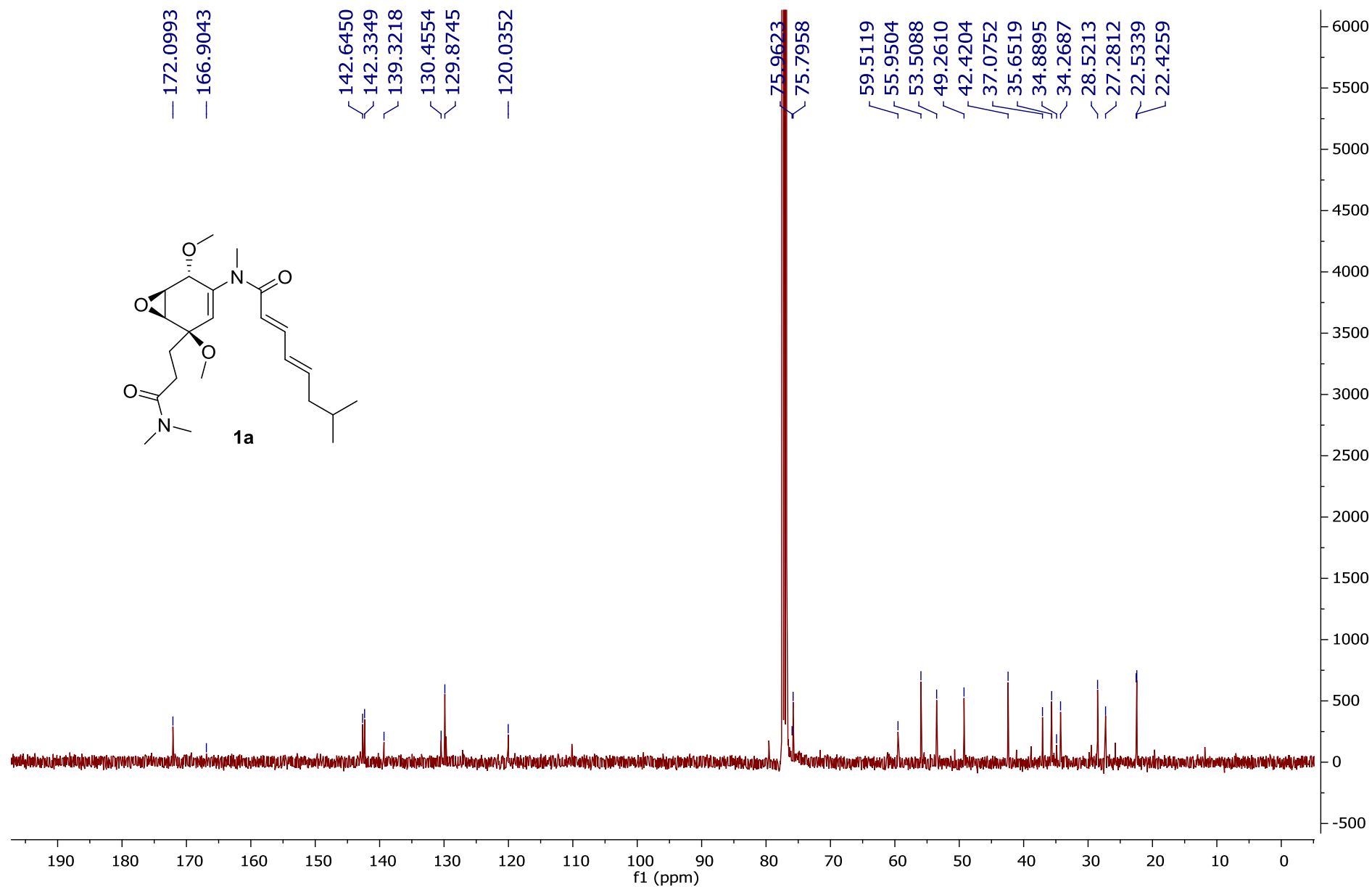


Figure S20. HSQC spectrum of compound **1a** in CDCl₃

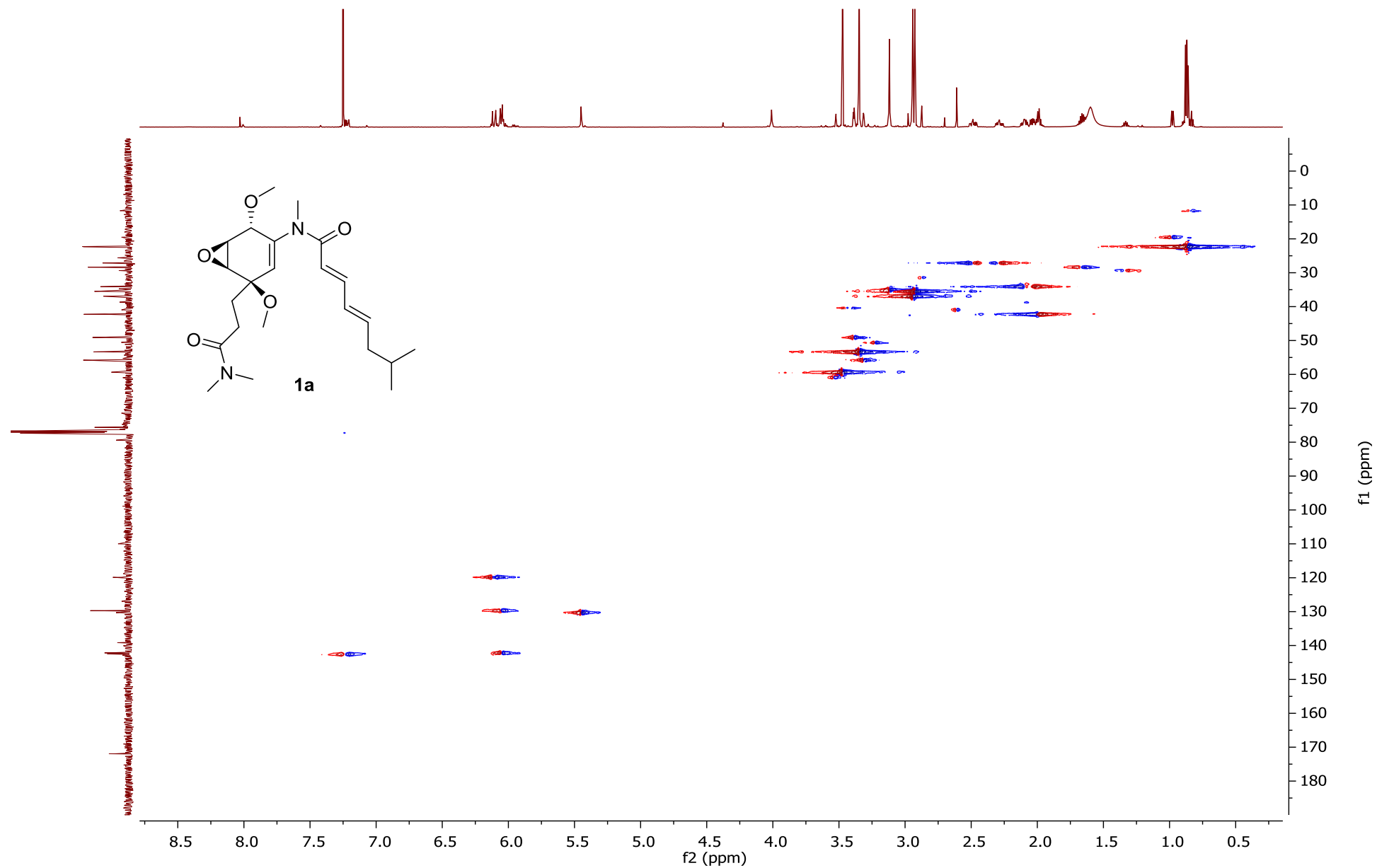


Figure S21. ^1H - ^1H COSY spectrum of compound **1a** in CDCl_3

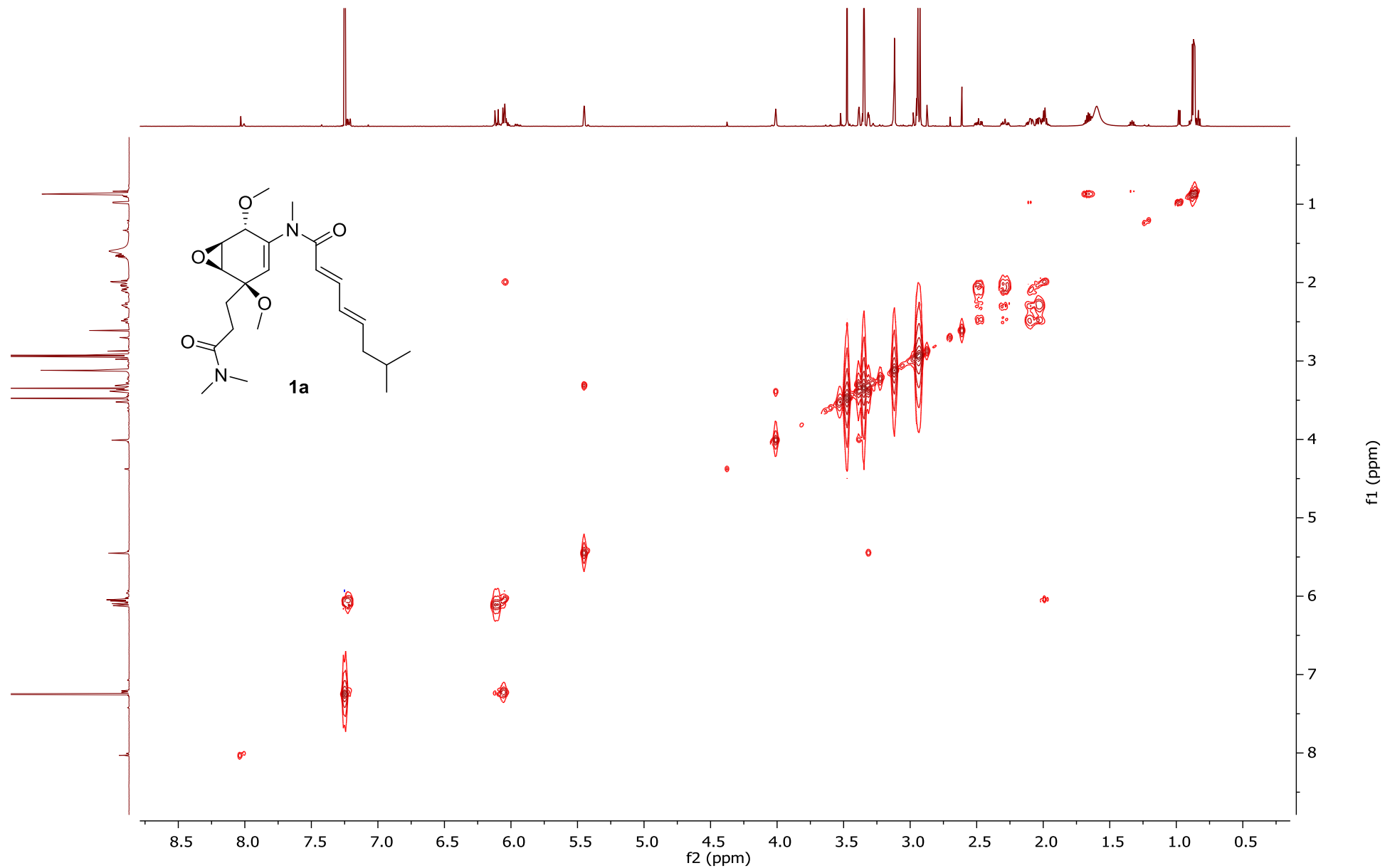


Figure S22. HMBC spectrum of compound **1a** in CDCl₃

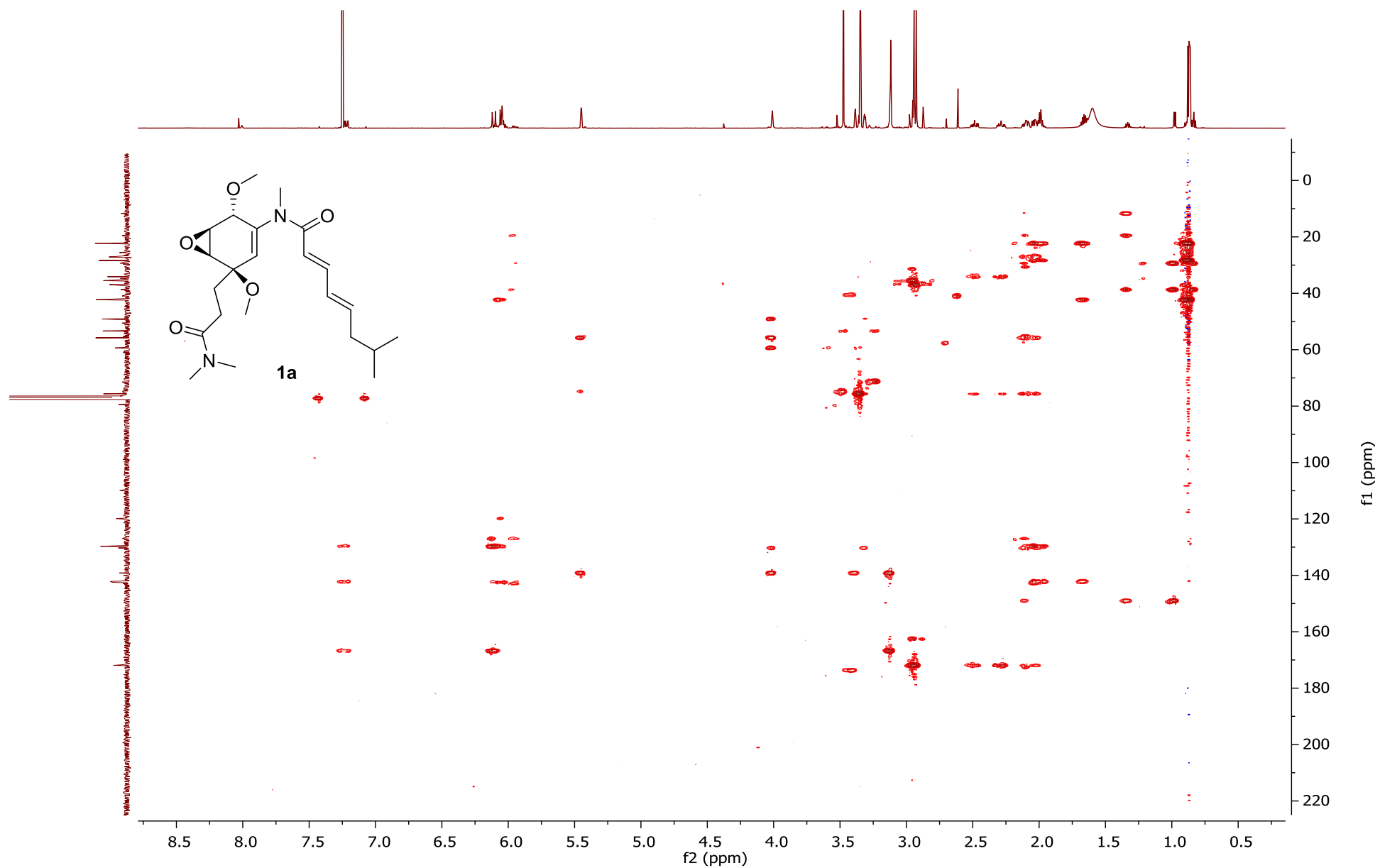


Figure S23. NOESY spectrum of compound **1a** in CDCl₃

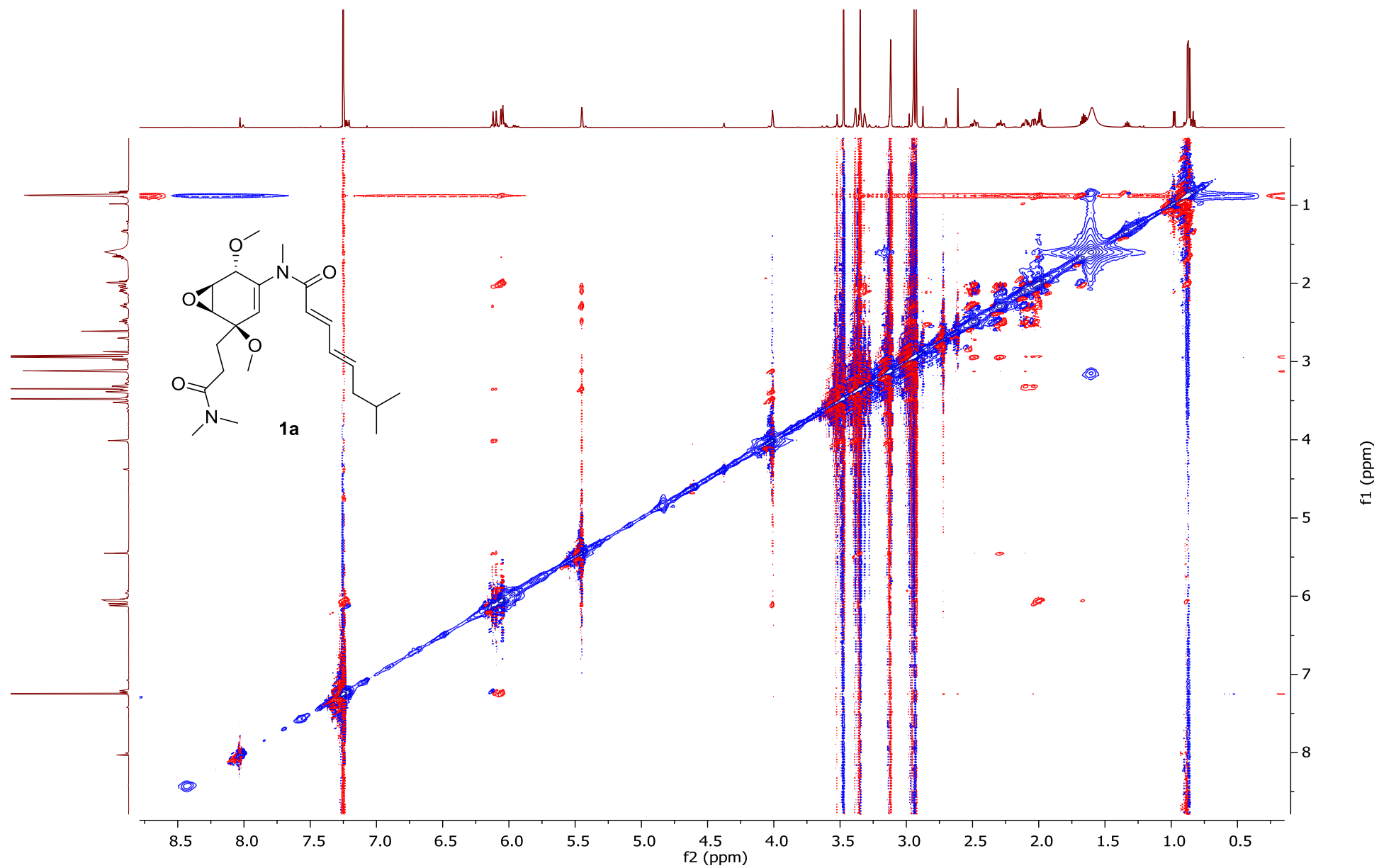


Figure S24. 1D NOE spectrum of compound **1a** in CDCl₃

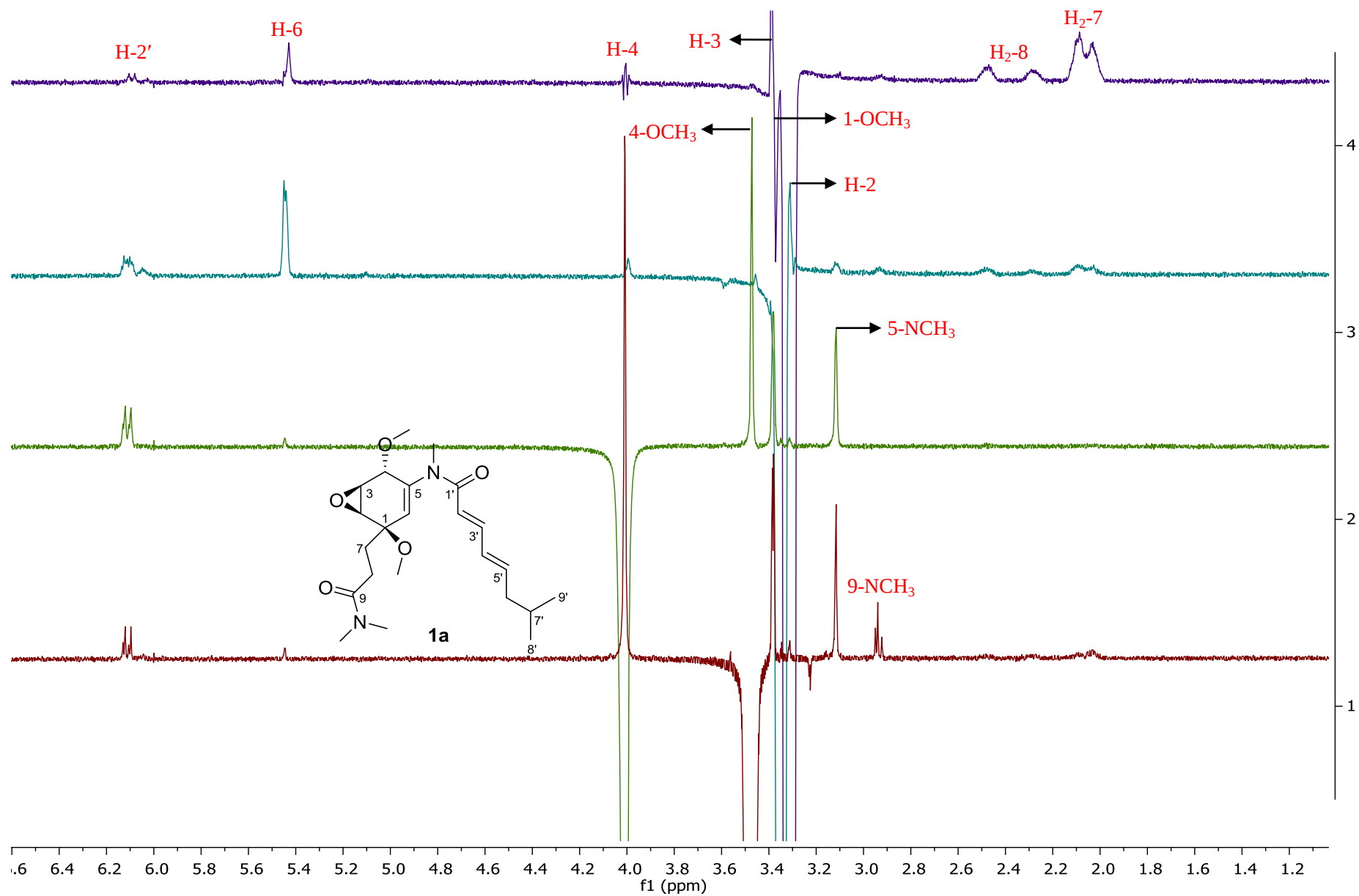


Figure S25. ^1H -NMR spectrum of compound **1b** in CDCl_3

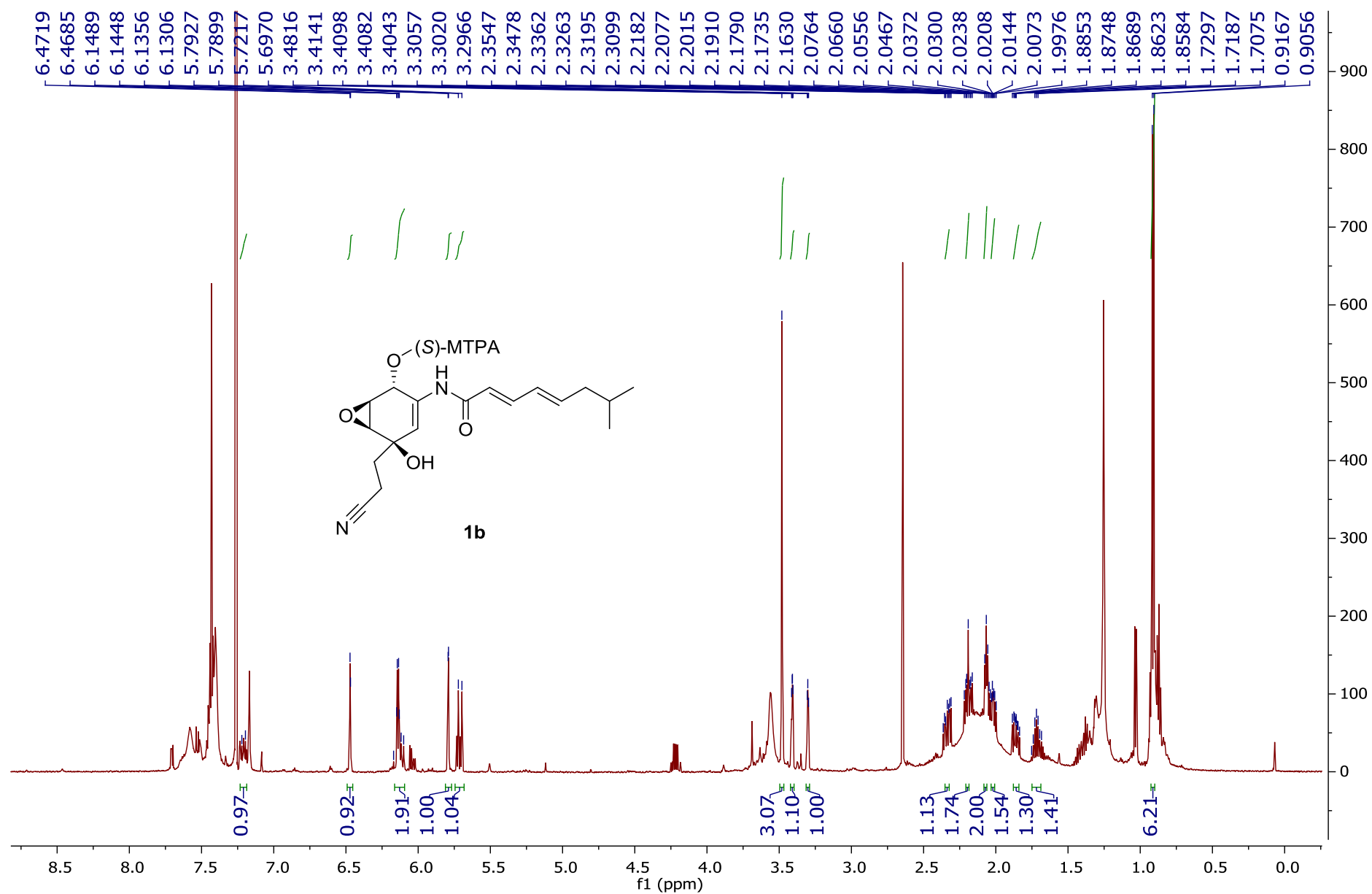


Figure S26. ^1H - ^1H COSY spectrum of compound **1b** in CDCl_3

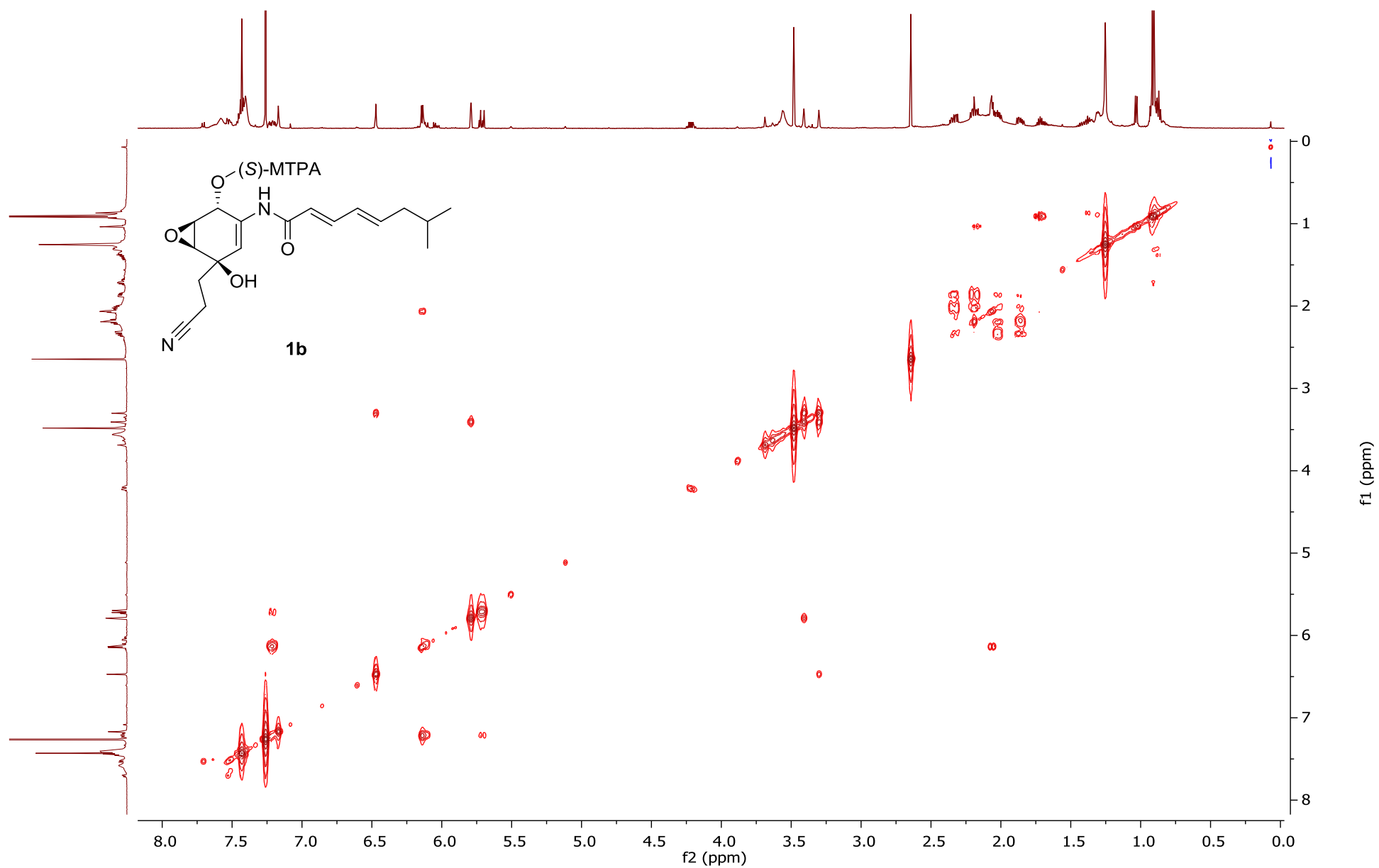


Figure S27. HSQC spectrum of compound **1b** in CDCl₃

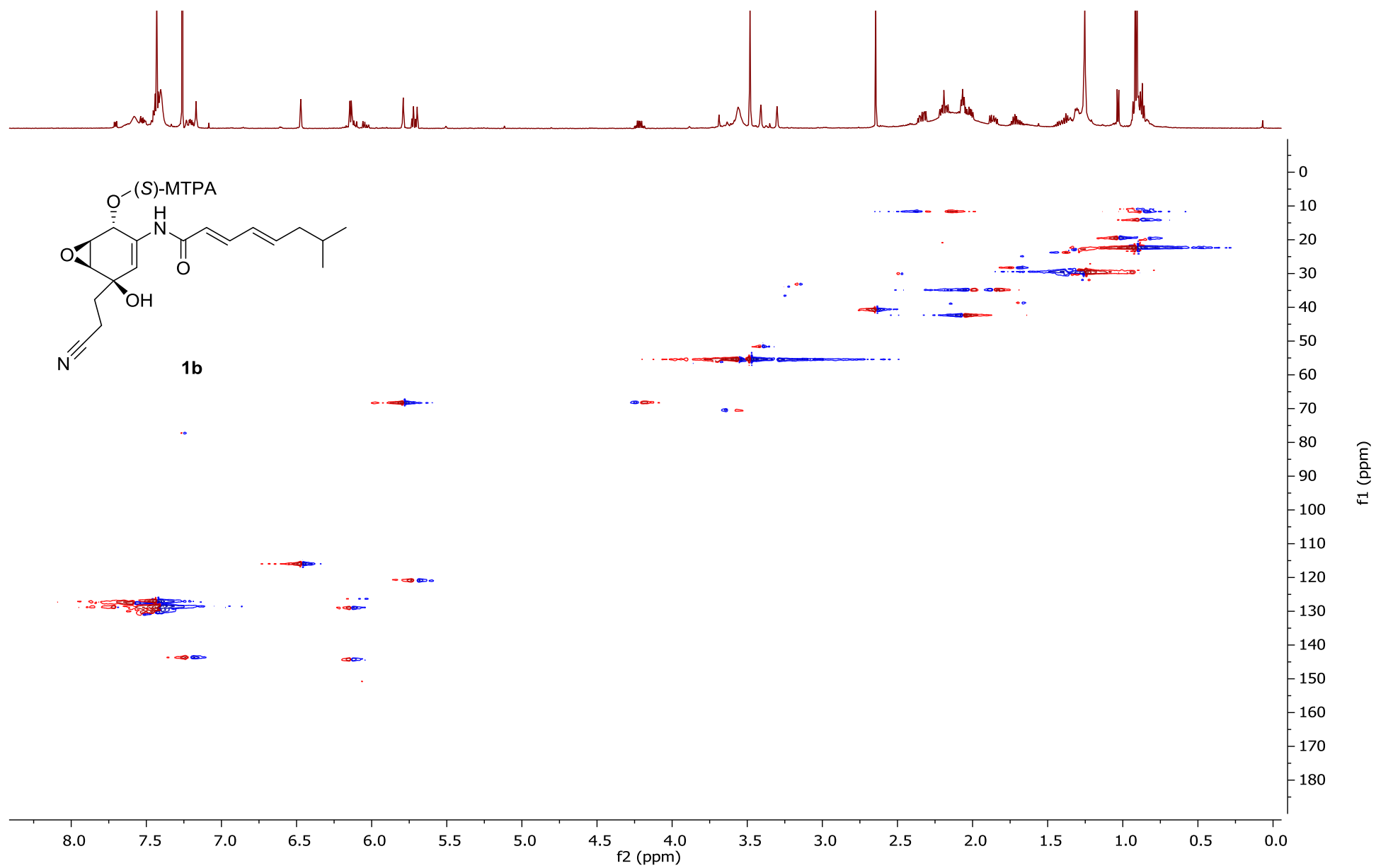


Figure S28. HMBC spectrum of compound **1b** in CDCl₃

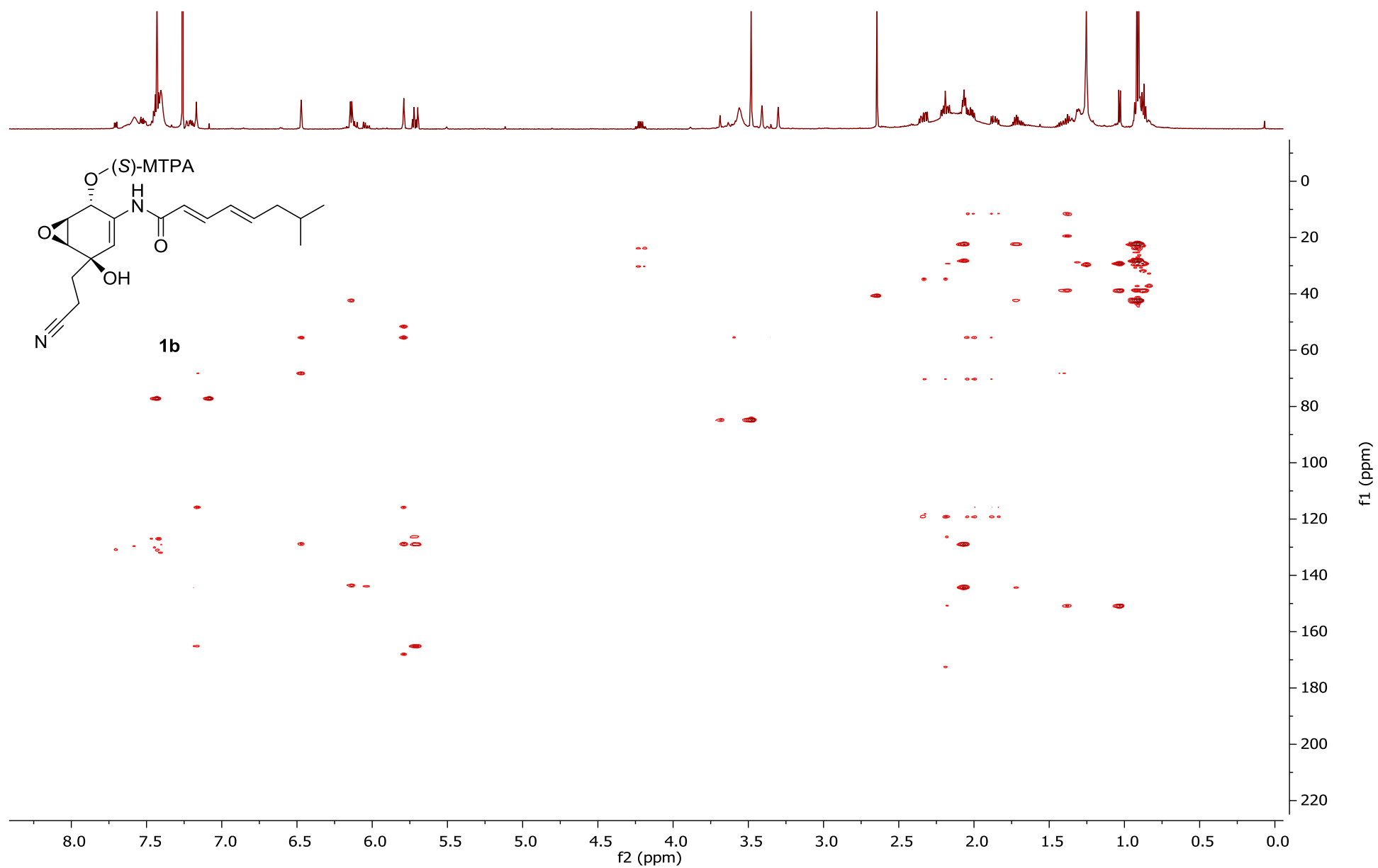


Figure S29. ^1H -NMR spectrum of compound **1c** in CDCl_3

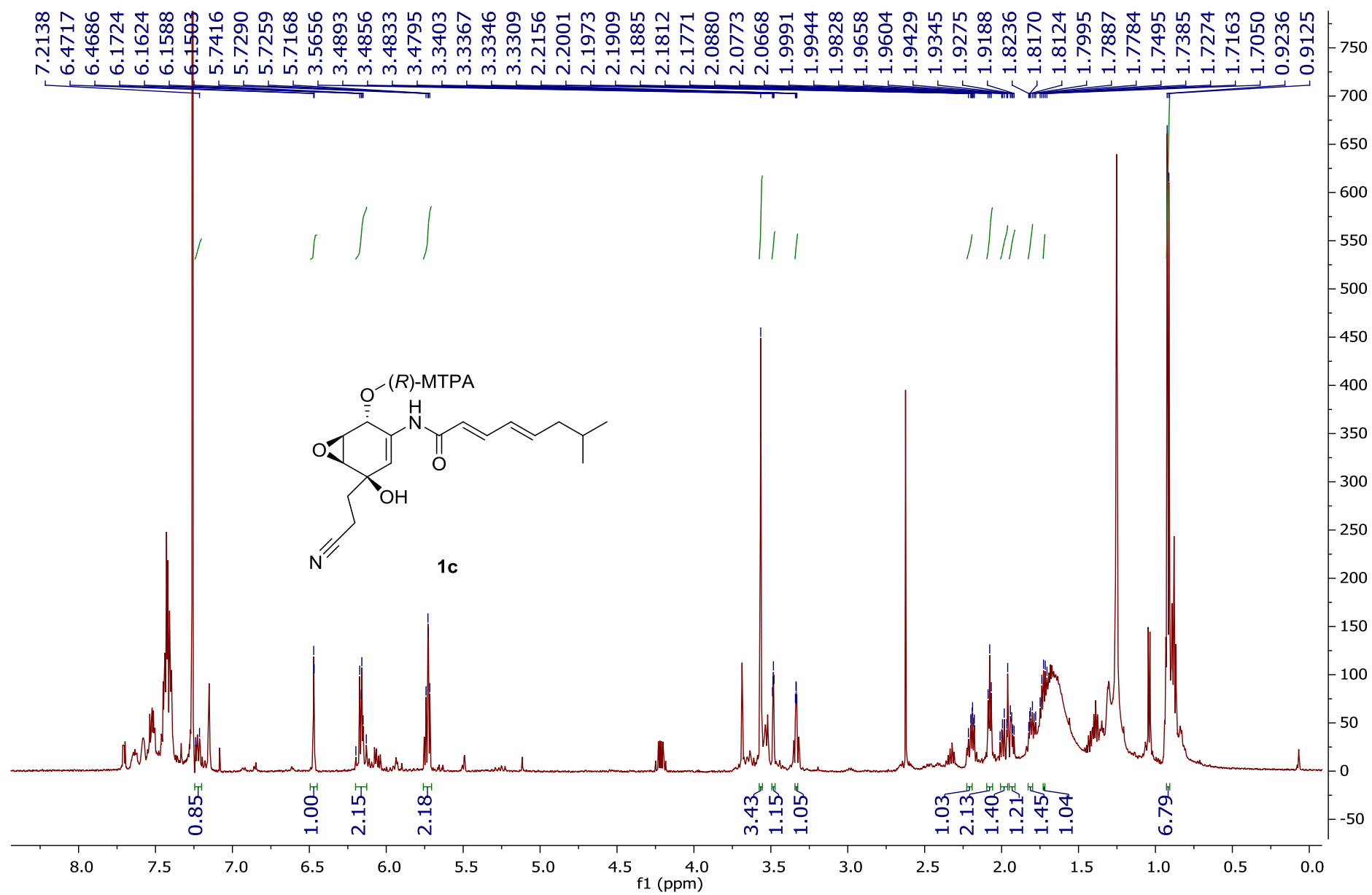


Figure S30. ^1H - ^1H COSY spectrum of compound **1c** in CDCl_3

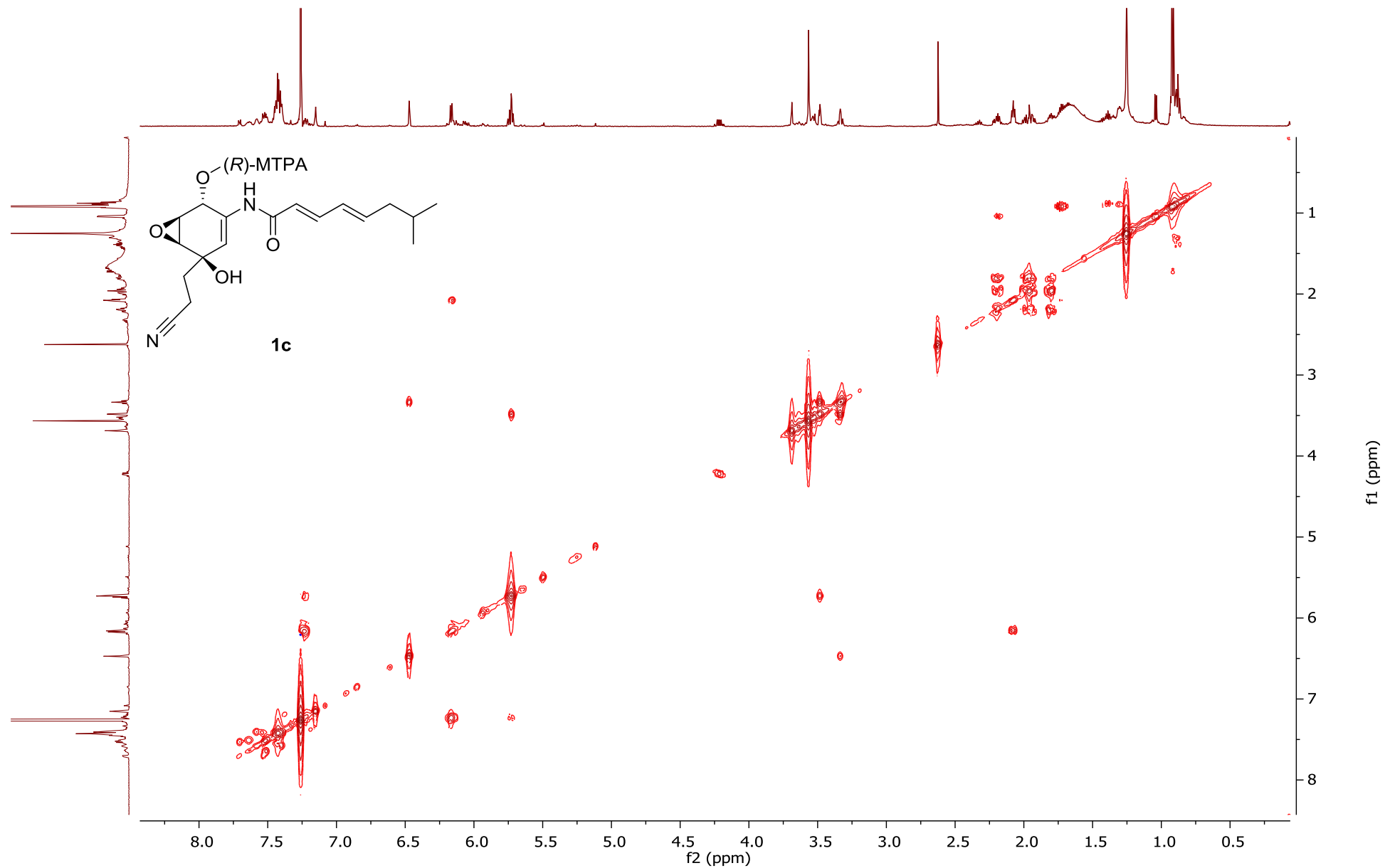


Figure S31. ^1H -NMR spectrum of compound **1d** in CD_3OD

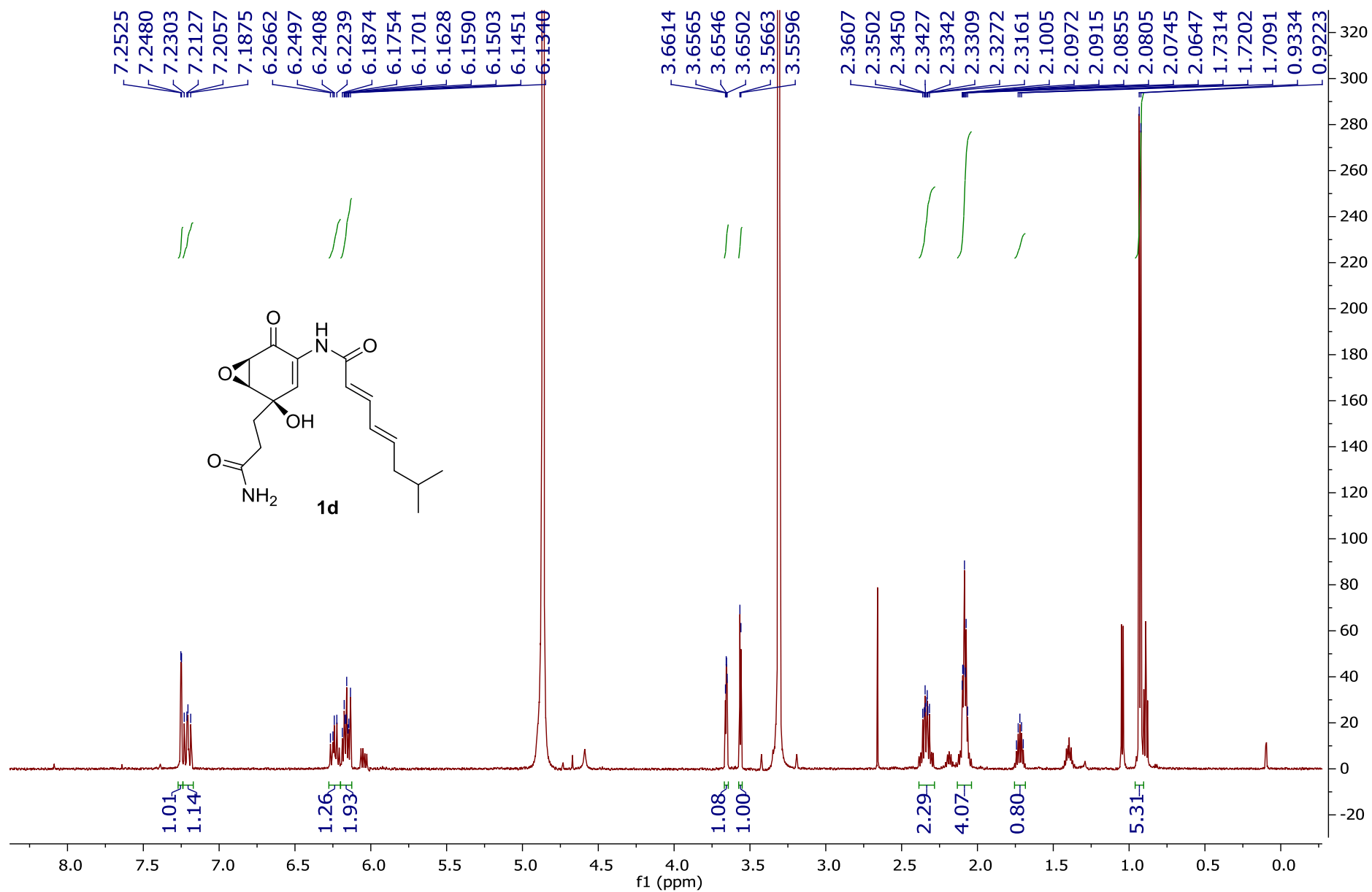


Figure S32. HSQC spectrum of compound **1d** in CD₃OD

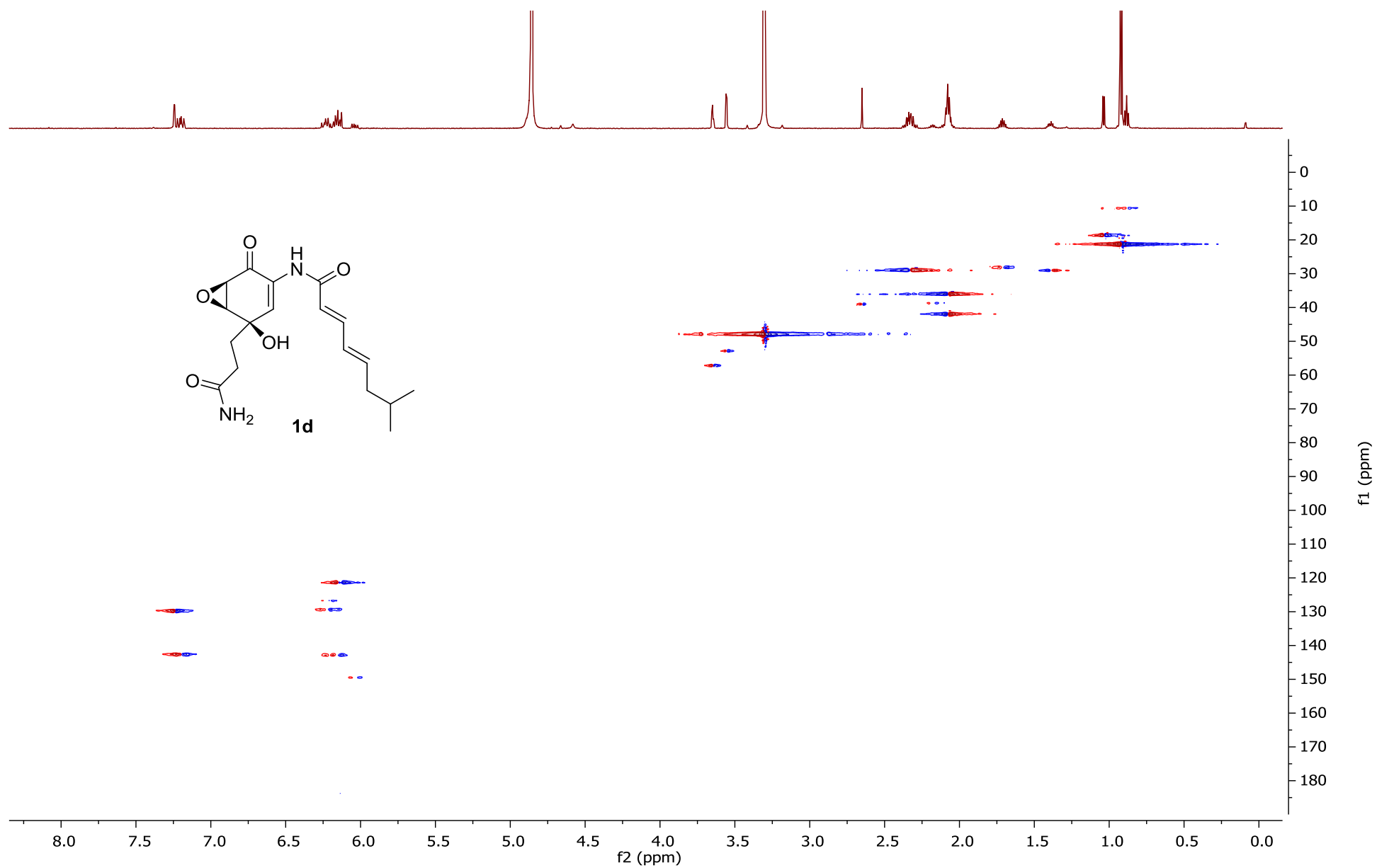


Figure S33. ^1H - ^1H COSY spectrum of compound **1d** in CD_3OD

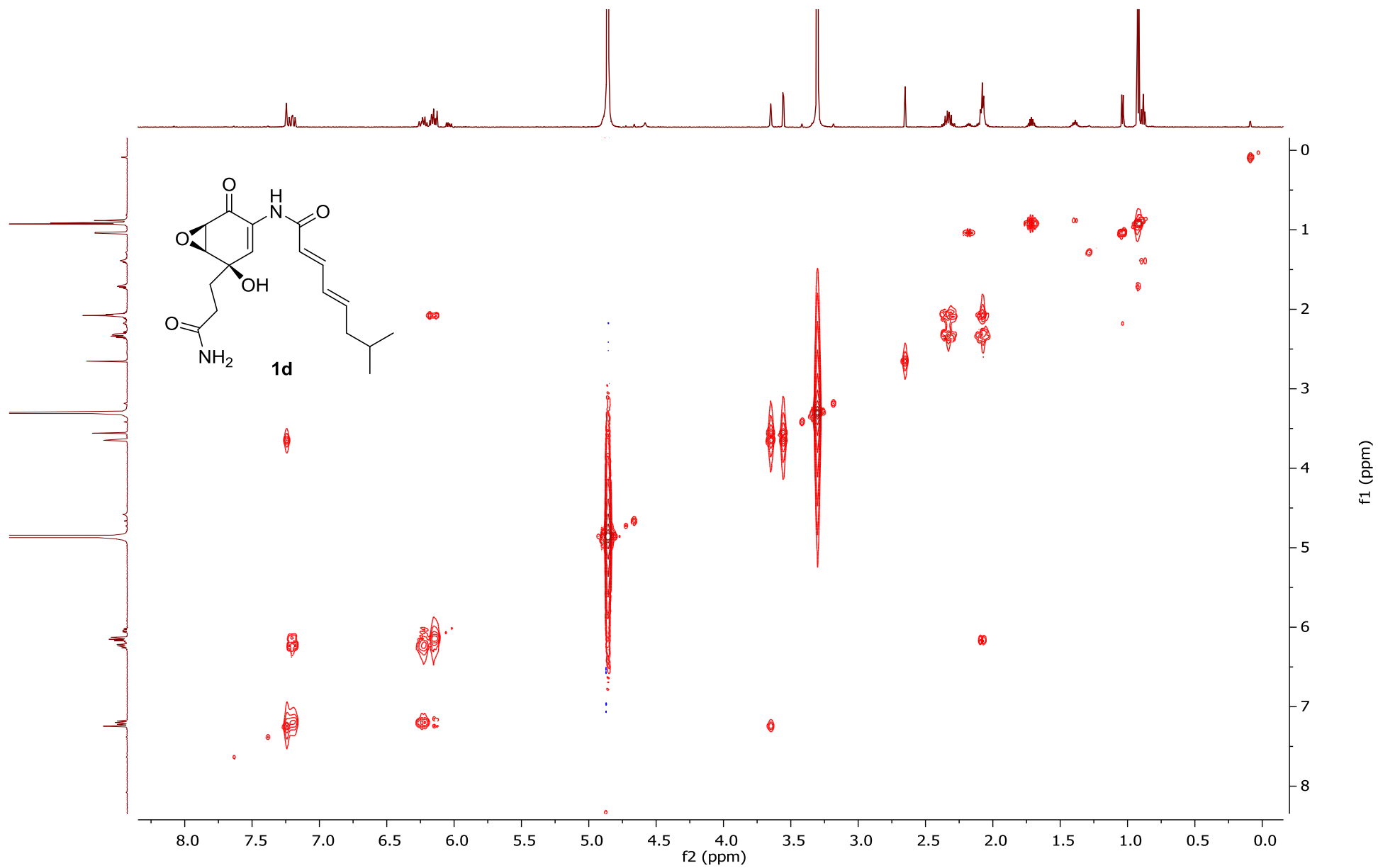


Figure S34. HMBC spectrum of compound **1d** in CD₃OD

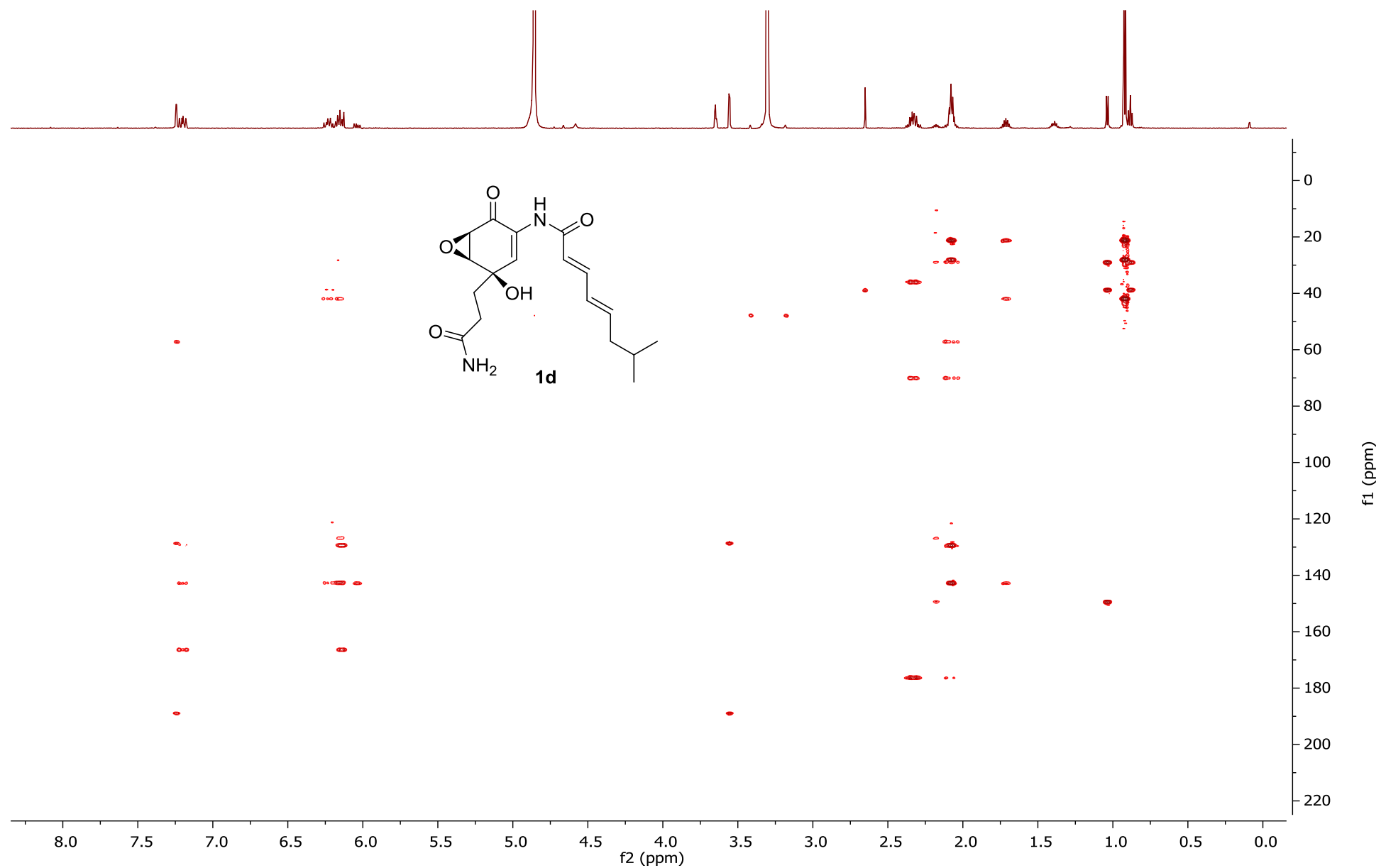


Figure S35. ^1H -NMR spectrum of daryamide E (2) in CD_3OD

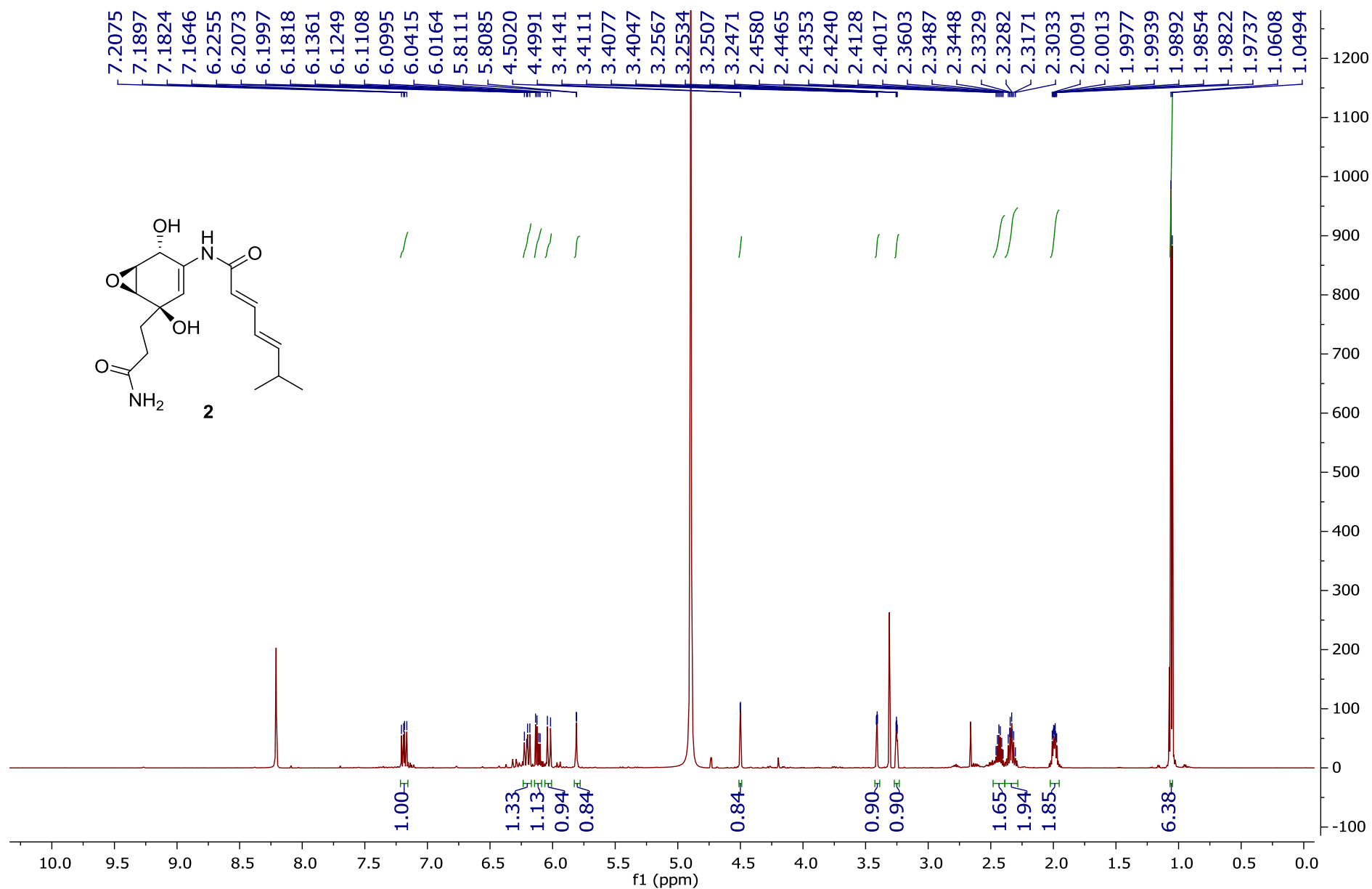


Figure S36. ^{13}C -NMR spectrum of daryamide E (**2**) in CD_3OD

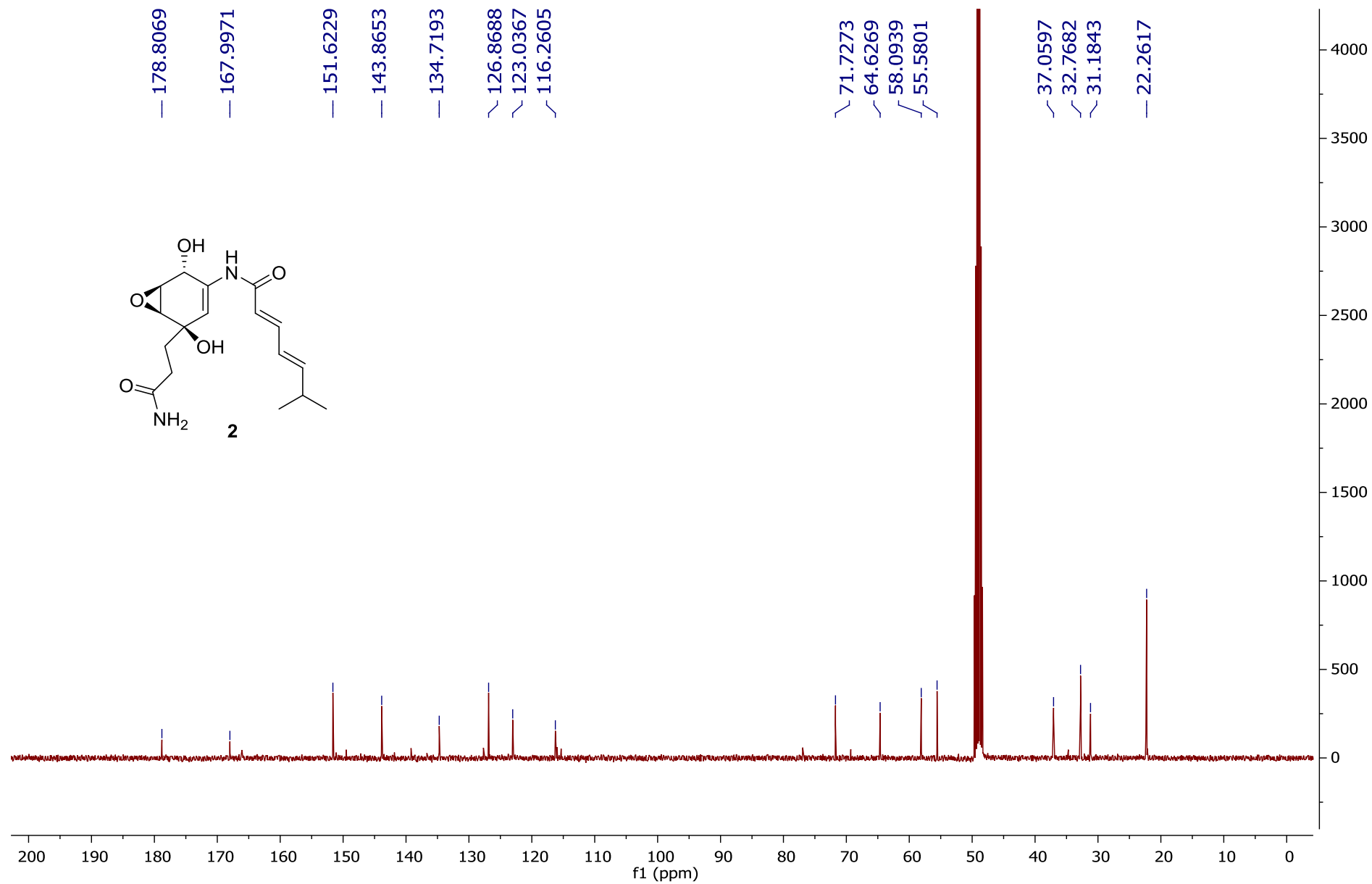


Figure S37. HSQC spectrum of daryamide E (2) in CD₃OD

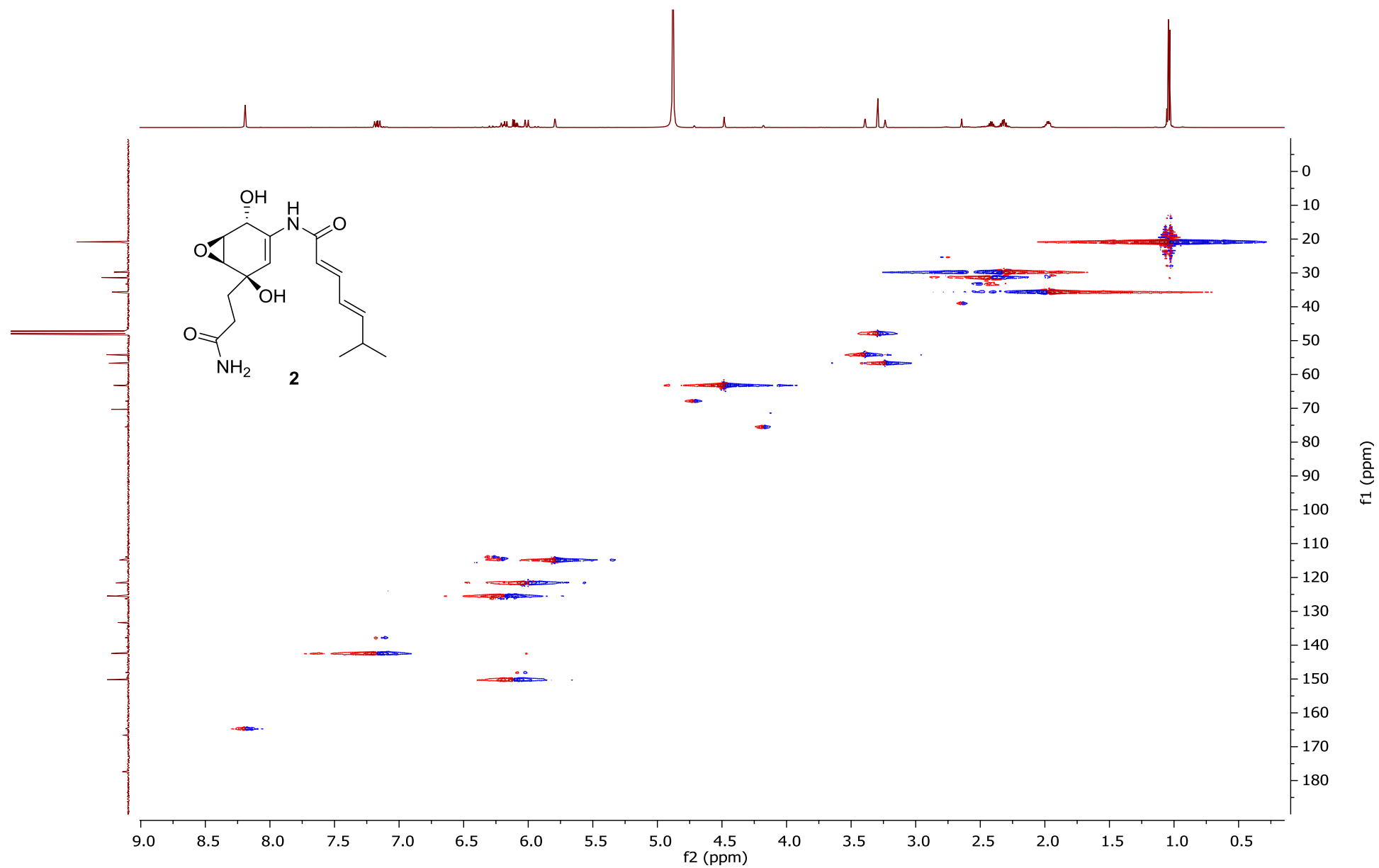


Figure S38. ^1H - ^1H COSY spectrum of daryamide E (2) in CD_3OD

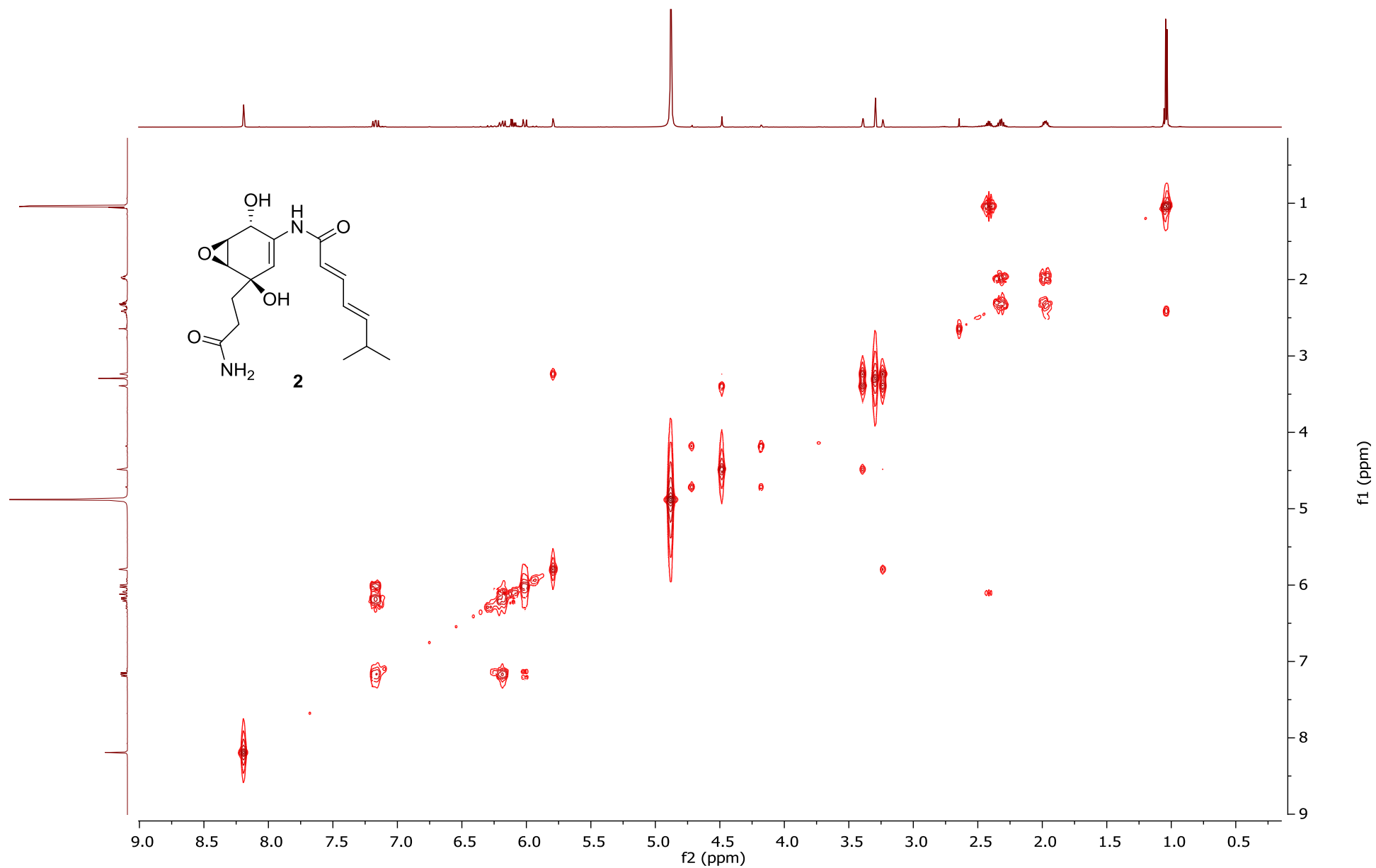


Figure S39. HMBC spectrum of daryamide E (2) in CD₃OD

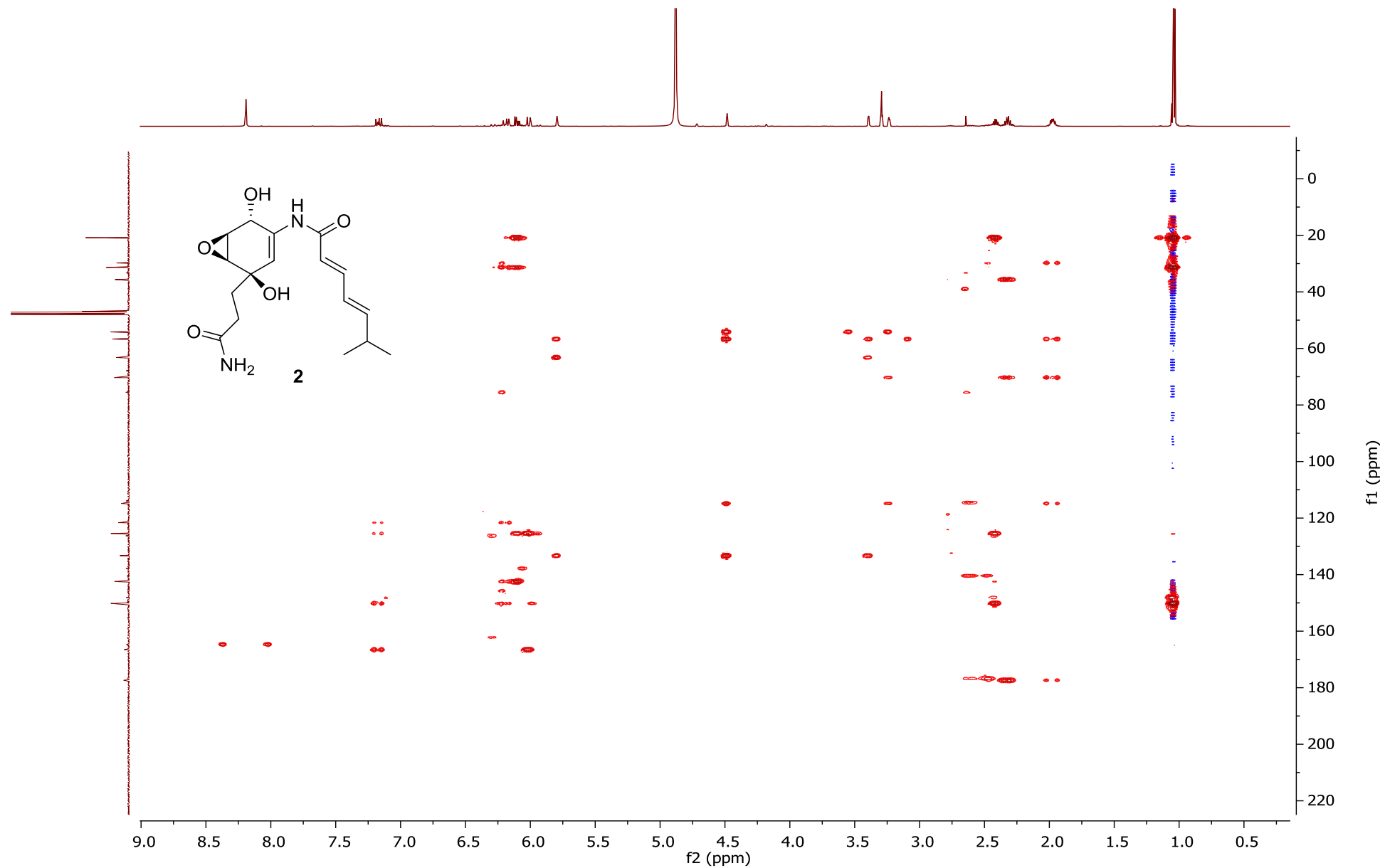


Figure S40. ^1H -NMR spectrum of daryamide F (**3**) in CD_3OD

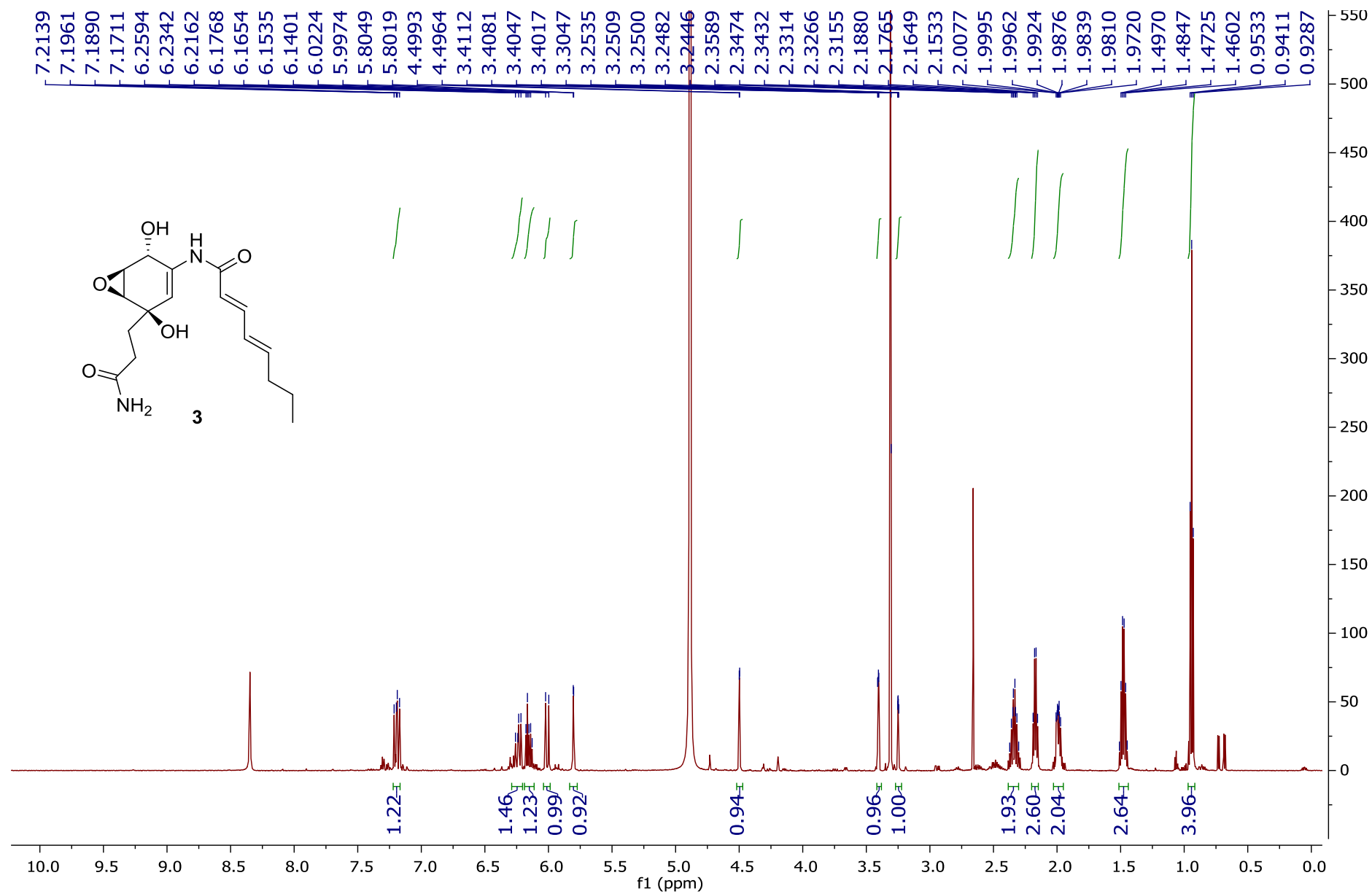


Figure S41. ^{13}C -NMR spectrum of daryamide F (**3**) in CD_3OD

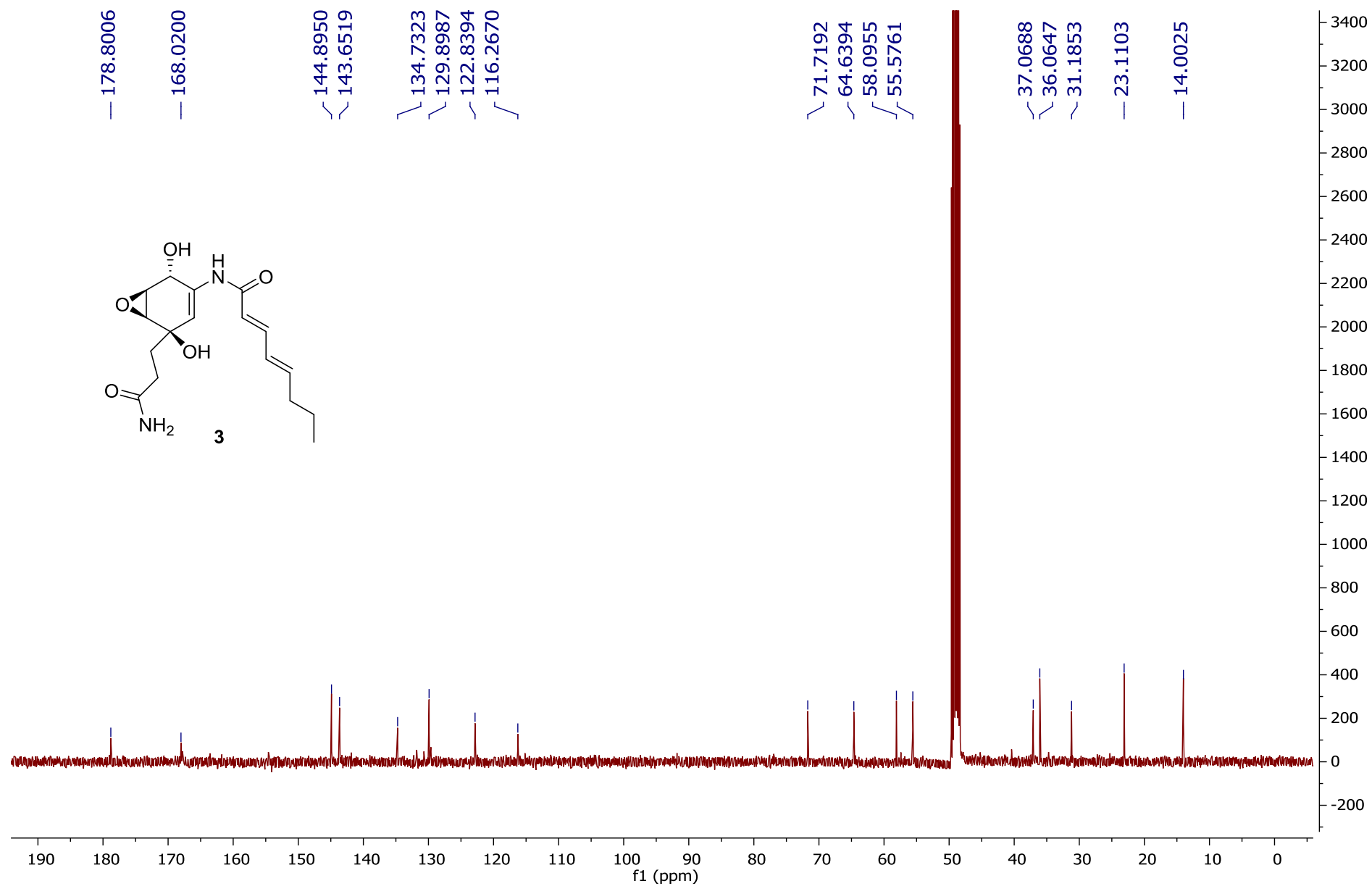


Figure S42. HSQC spectrum of daryamide F (3) in CD₃OD

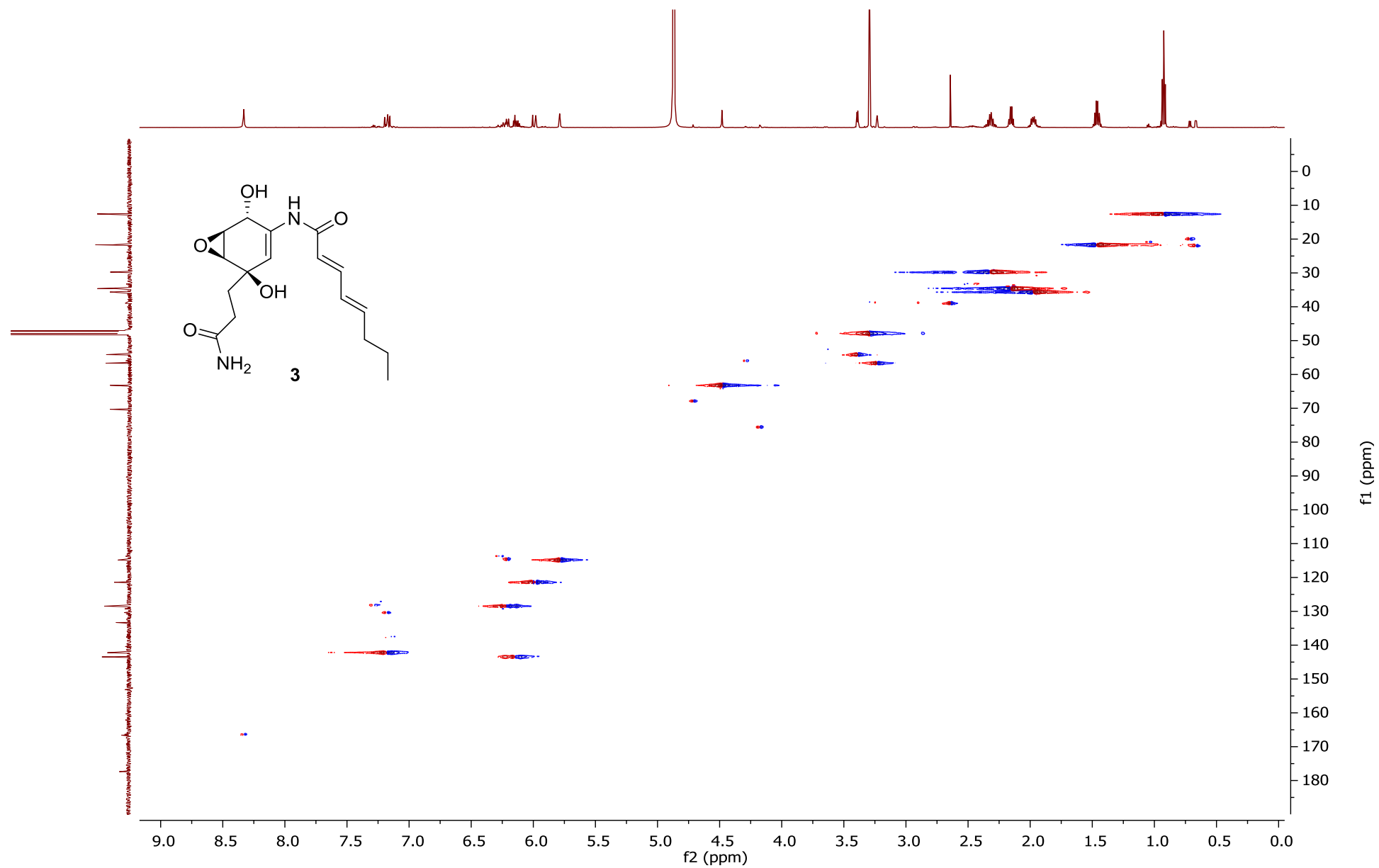


Figure S43. ^1H - ^1H COSY spectrum of daryamide F (**3**) in CD_3OD

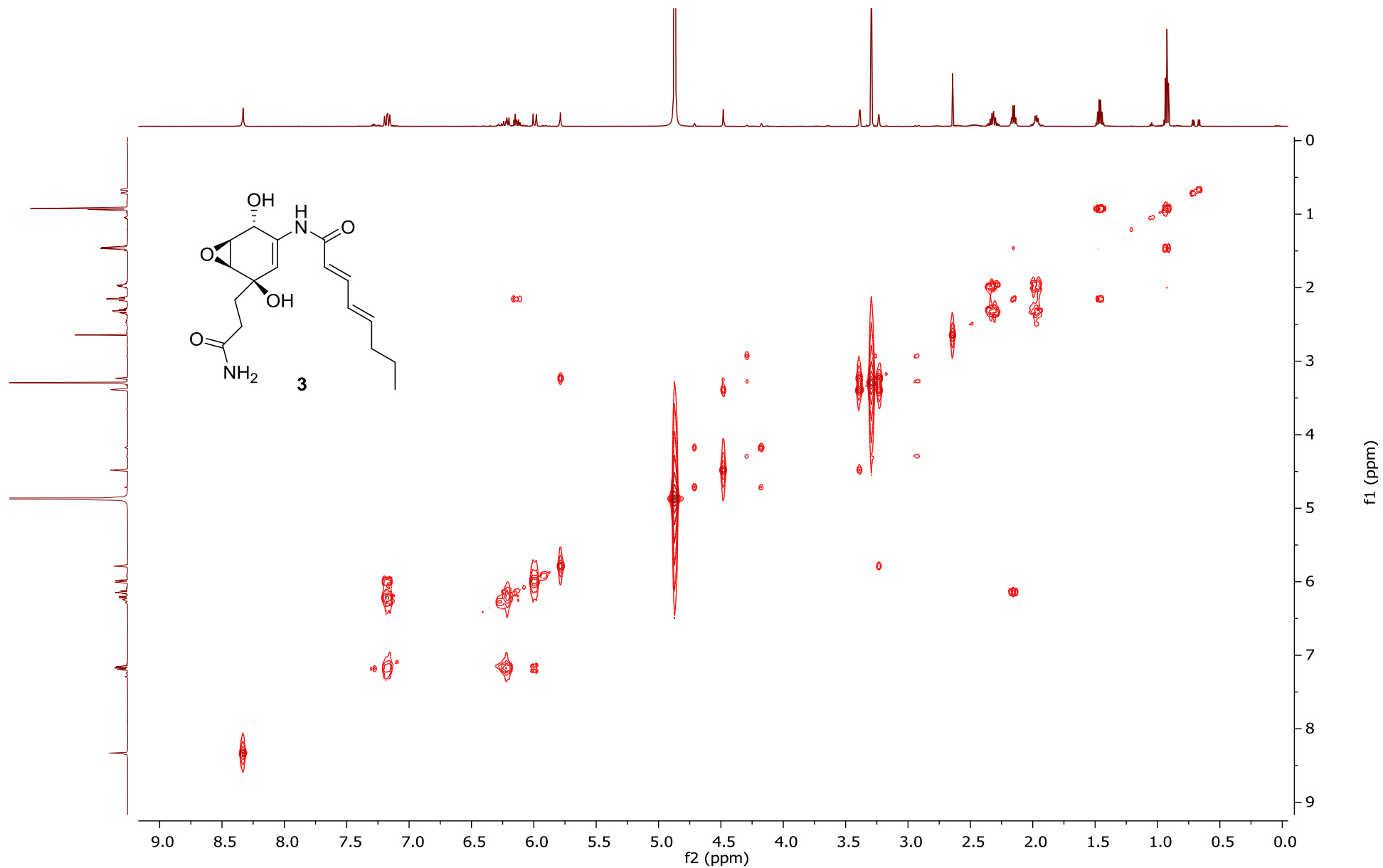


Figure S44. HMBC spectrum of daryamide F (3) in CD₃OD

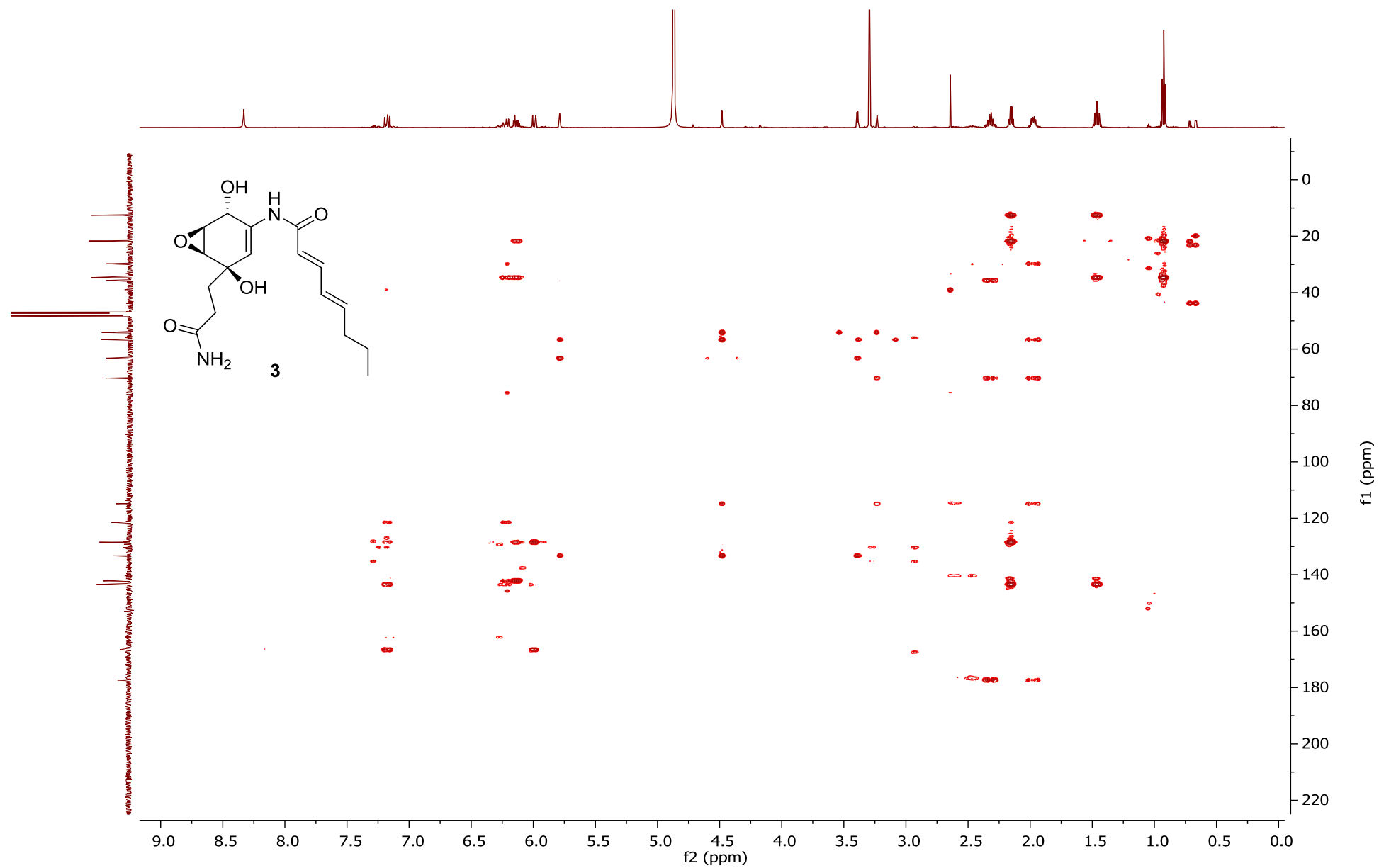


Figure S45. ^1H -NMR spectrum of carpatamide D (**4**) in CD_3OD

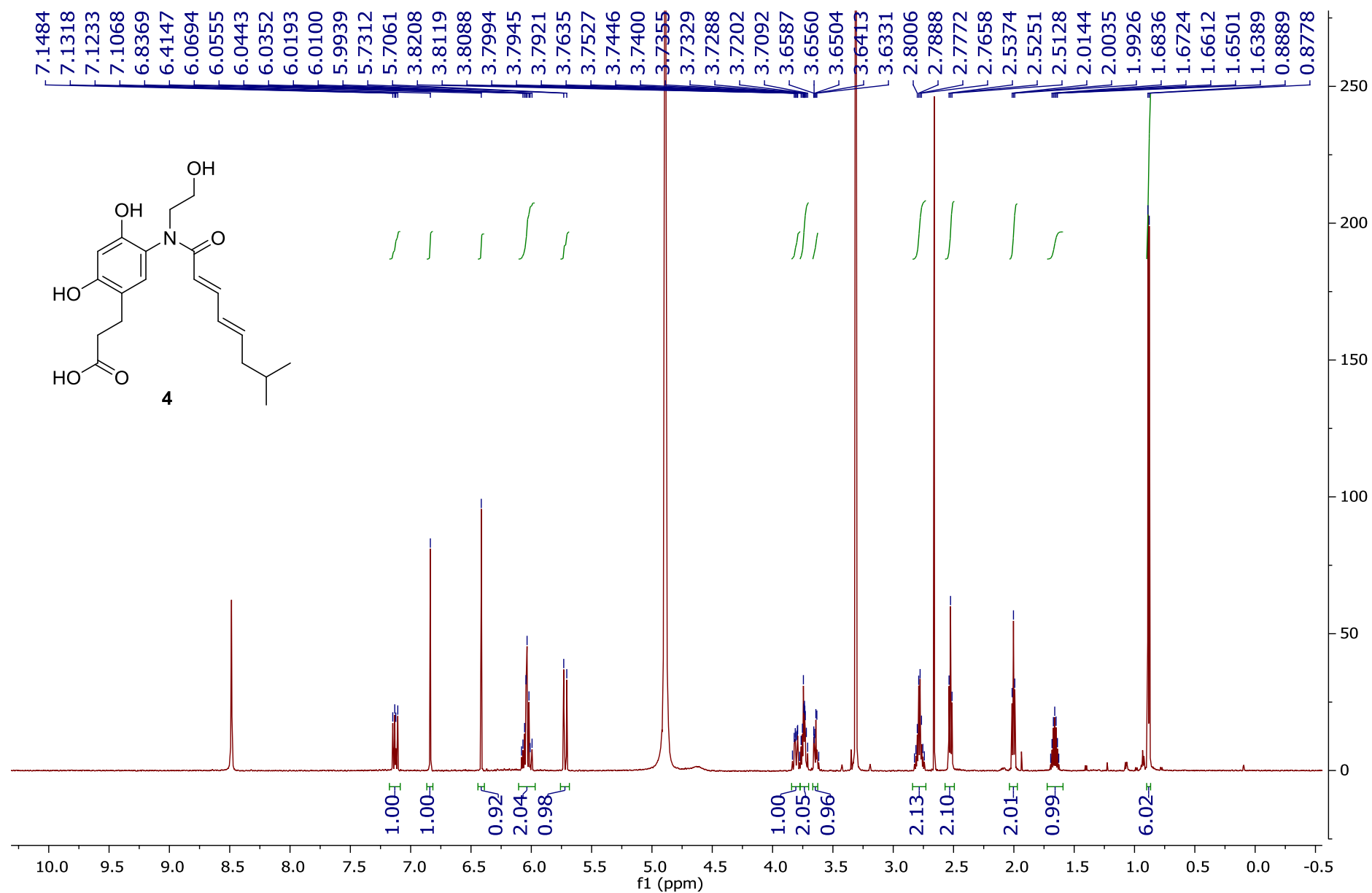


Figure S46. ^{13}C -NMR spectrum of carpatamide D (**4**) in CD_3OD

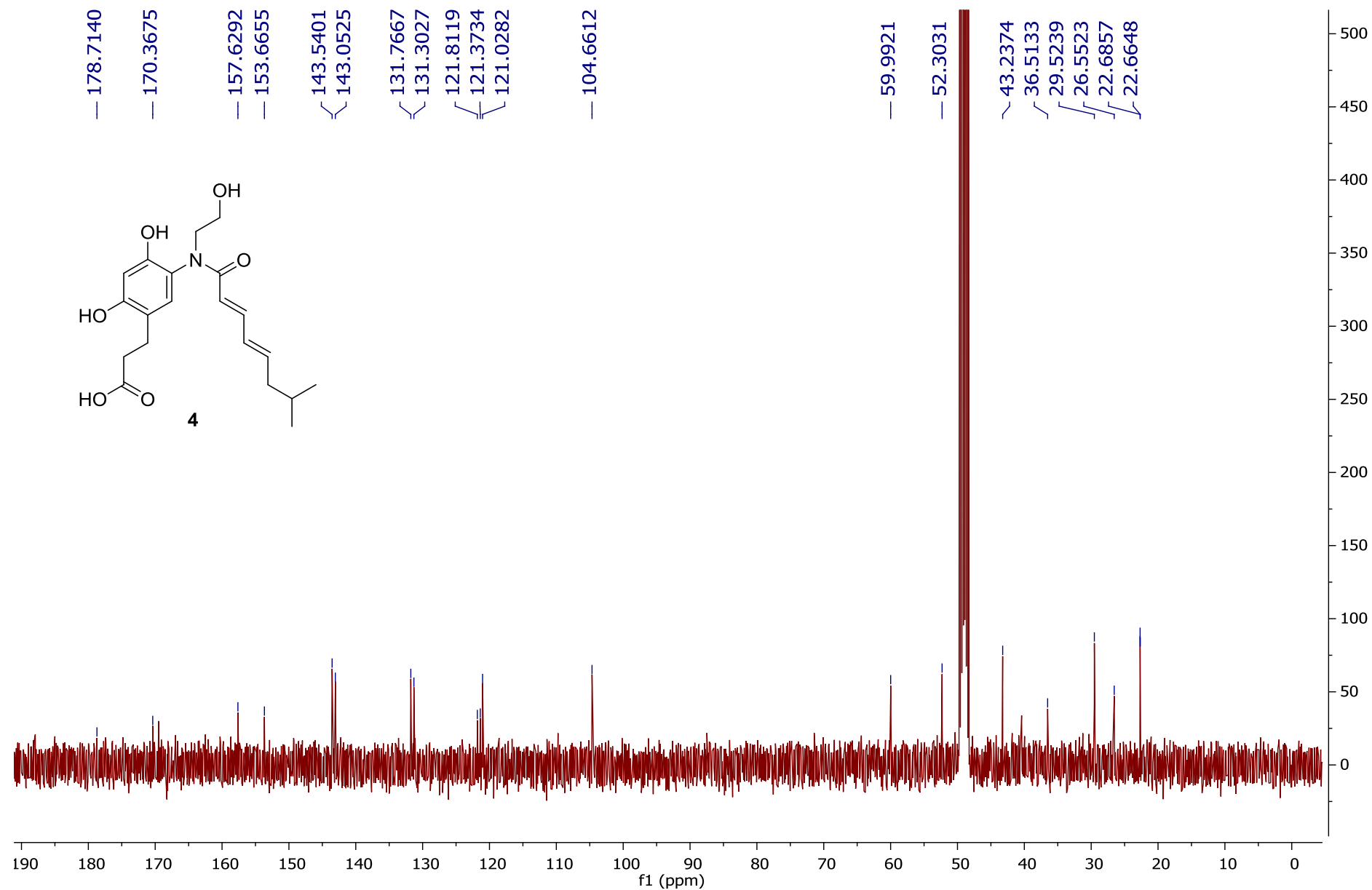


Figure S47. HSQC spectrum of carpatamide D (4) in CD₃OD

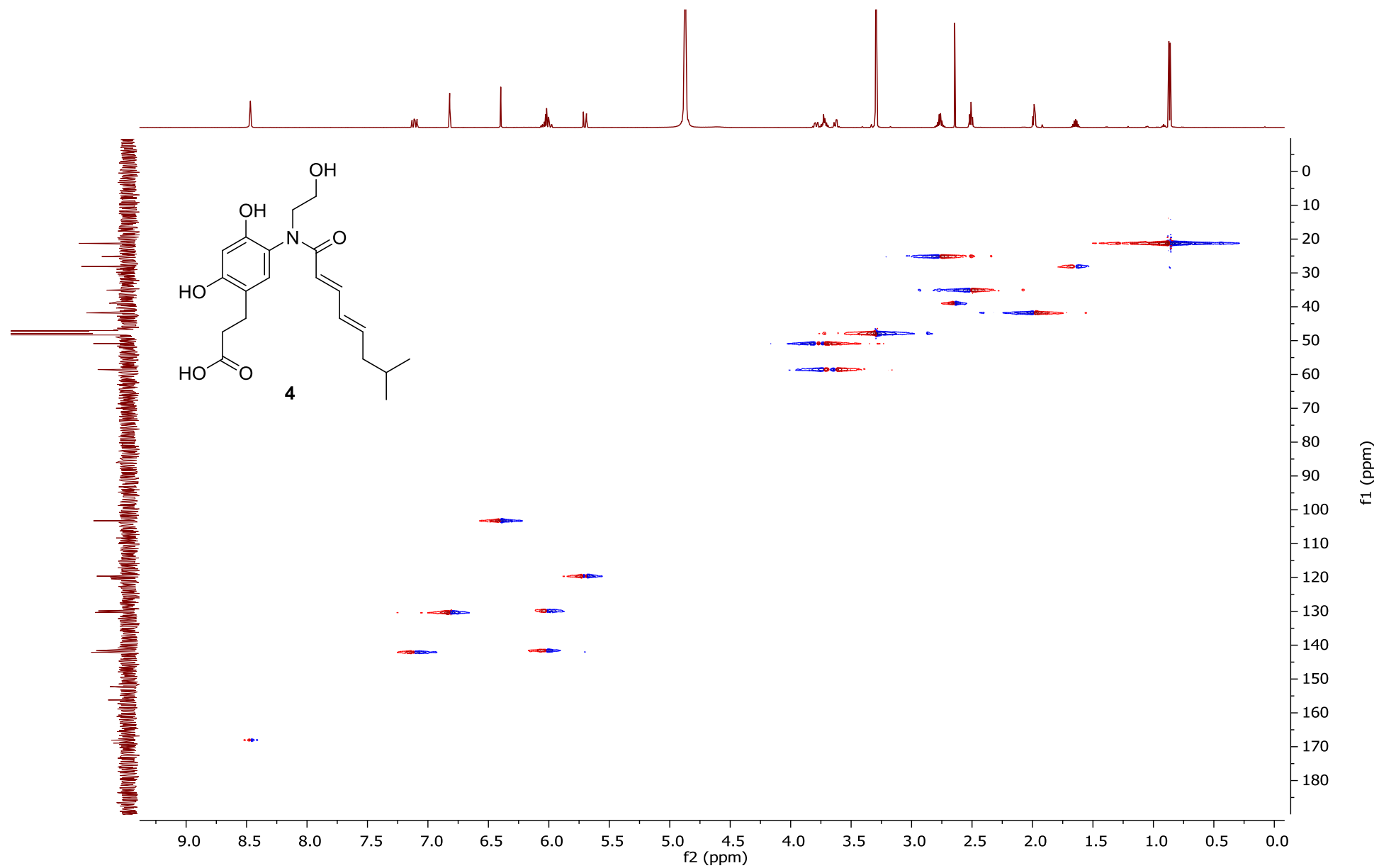


Figure S48. ^1H - ^1H COSY spectrum of carpatamide D (**4**) in CD_3OD

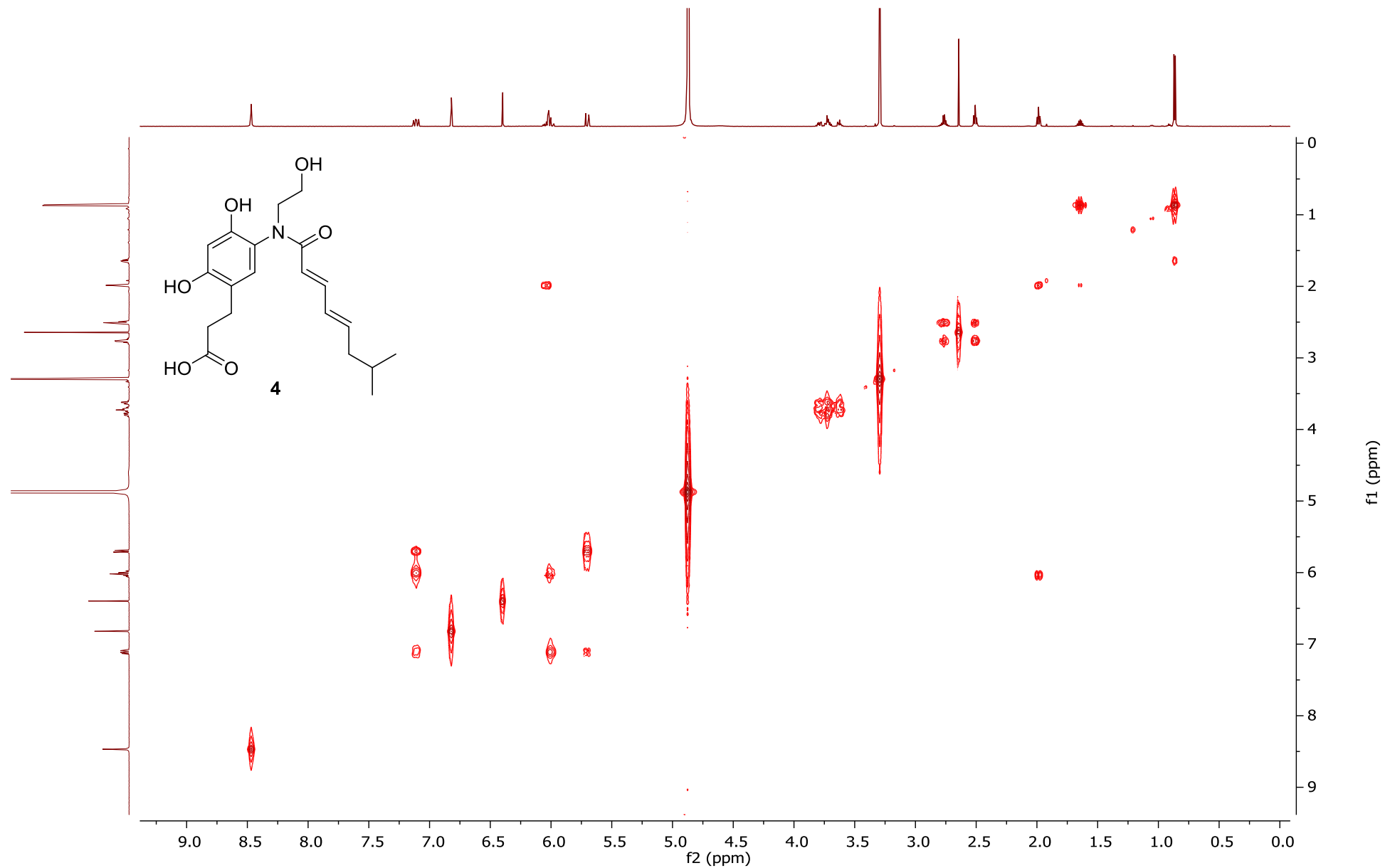


Figure S49. HMBC spectrum of carpatamide D (**4**) in CD₃OD

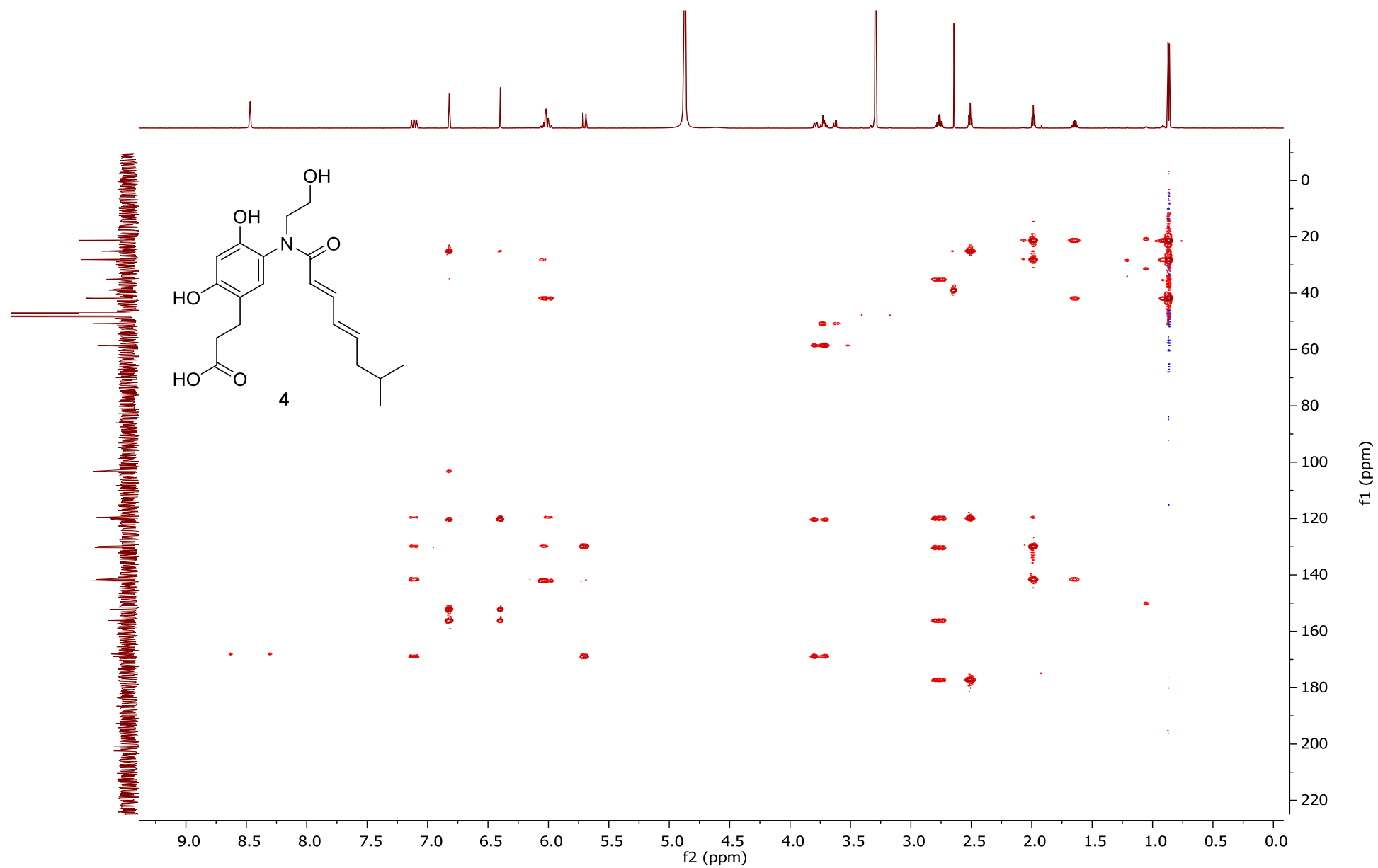


Figure S50. NOESY spectrum of carpatamide D (**4**) in CD₃OD

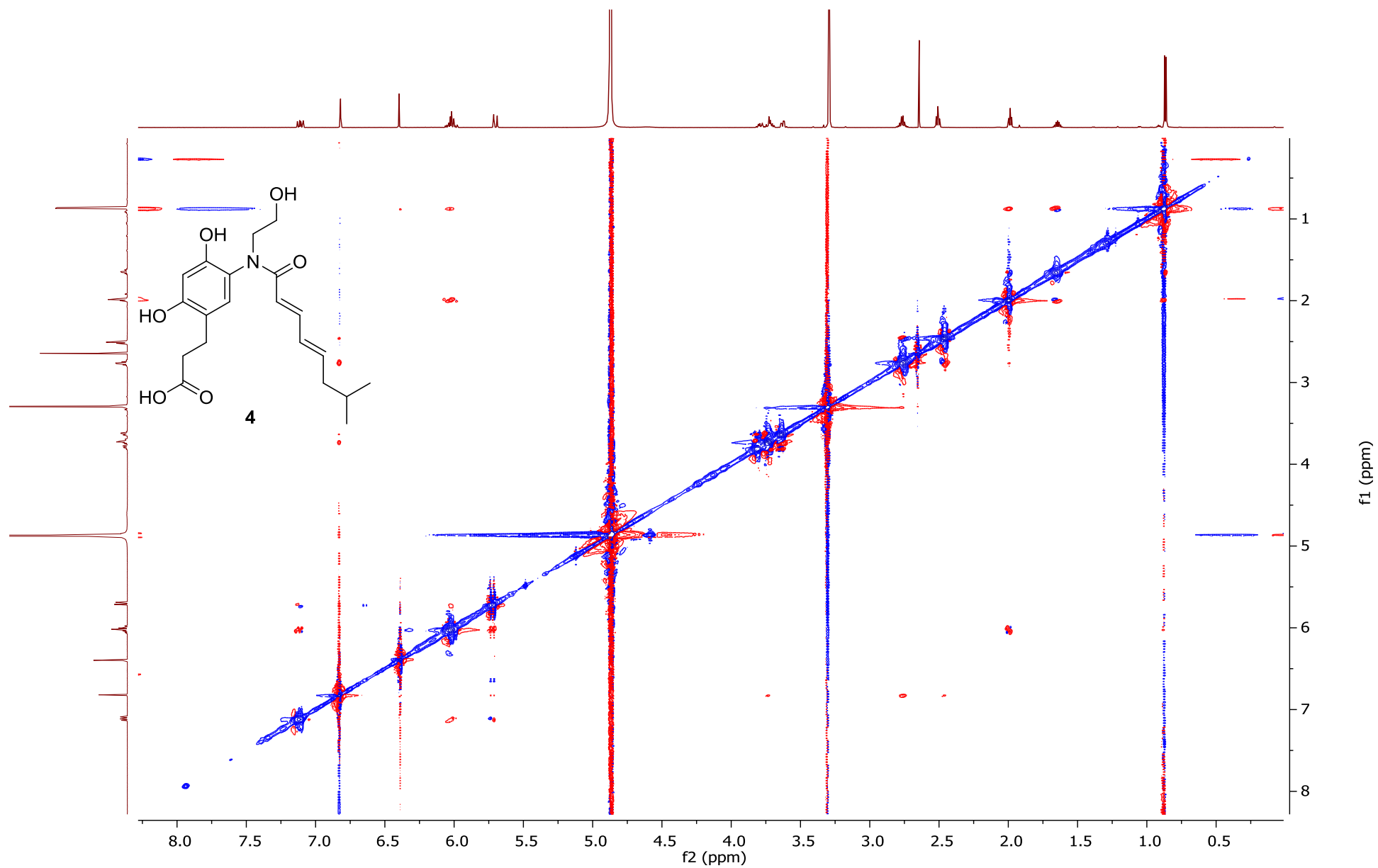


Figure S51. ^1H -NMR spectrum of ornilactam A (**5**) in CD_3OD

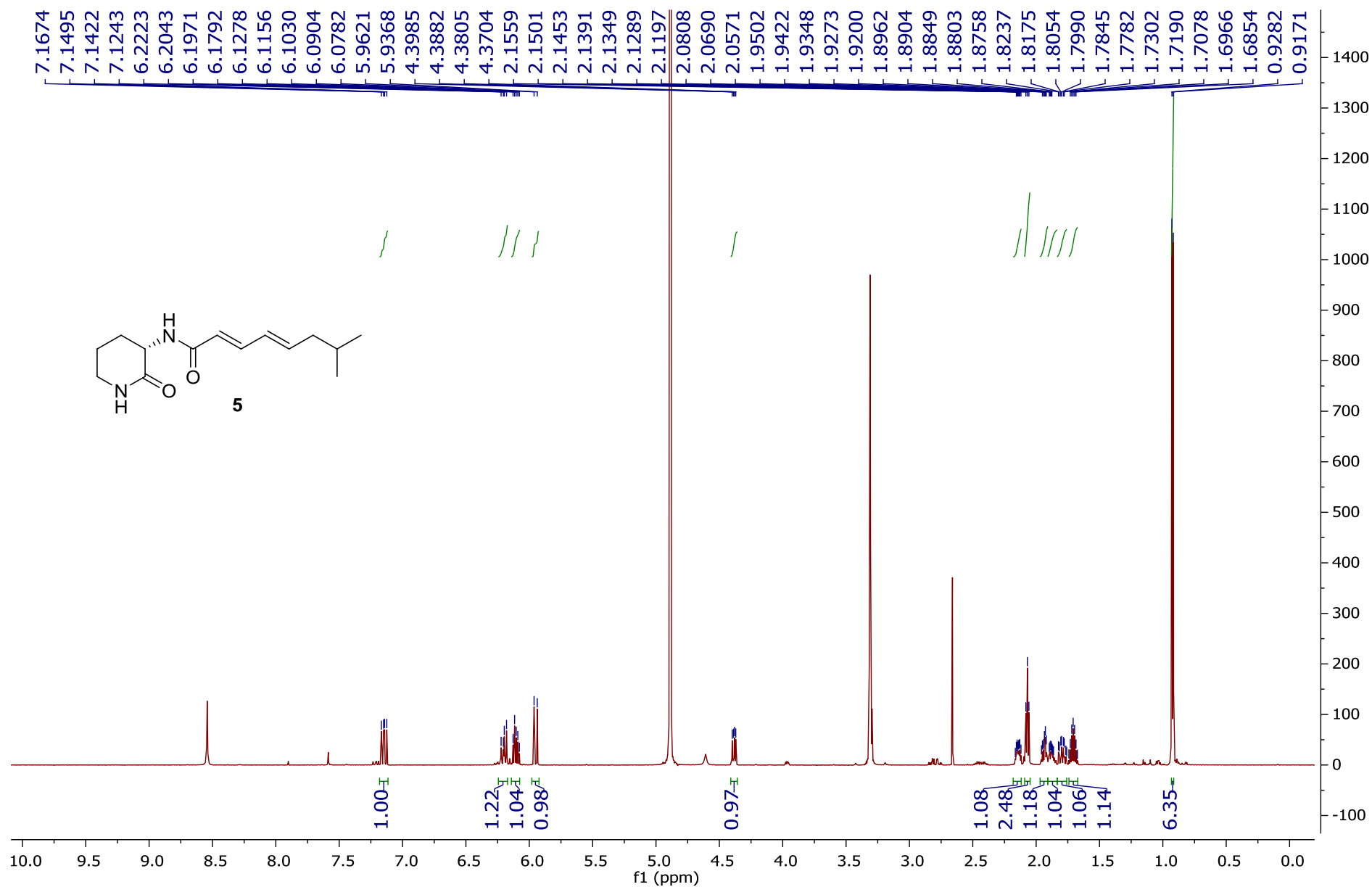


Figure S52. ^{13}C -NMR spectrum of ornilactam A (**5**) in CD_3OD

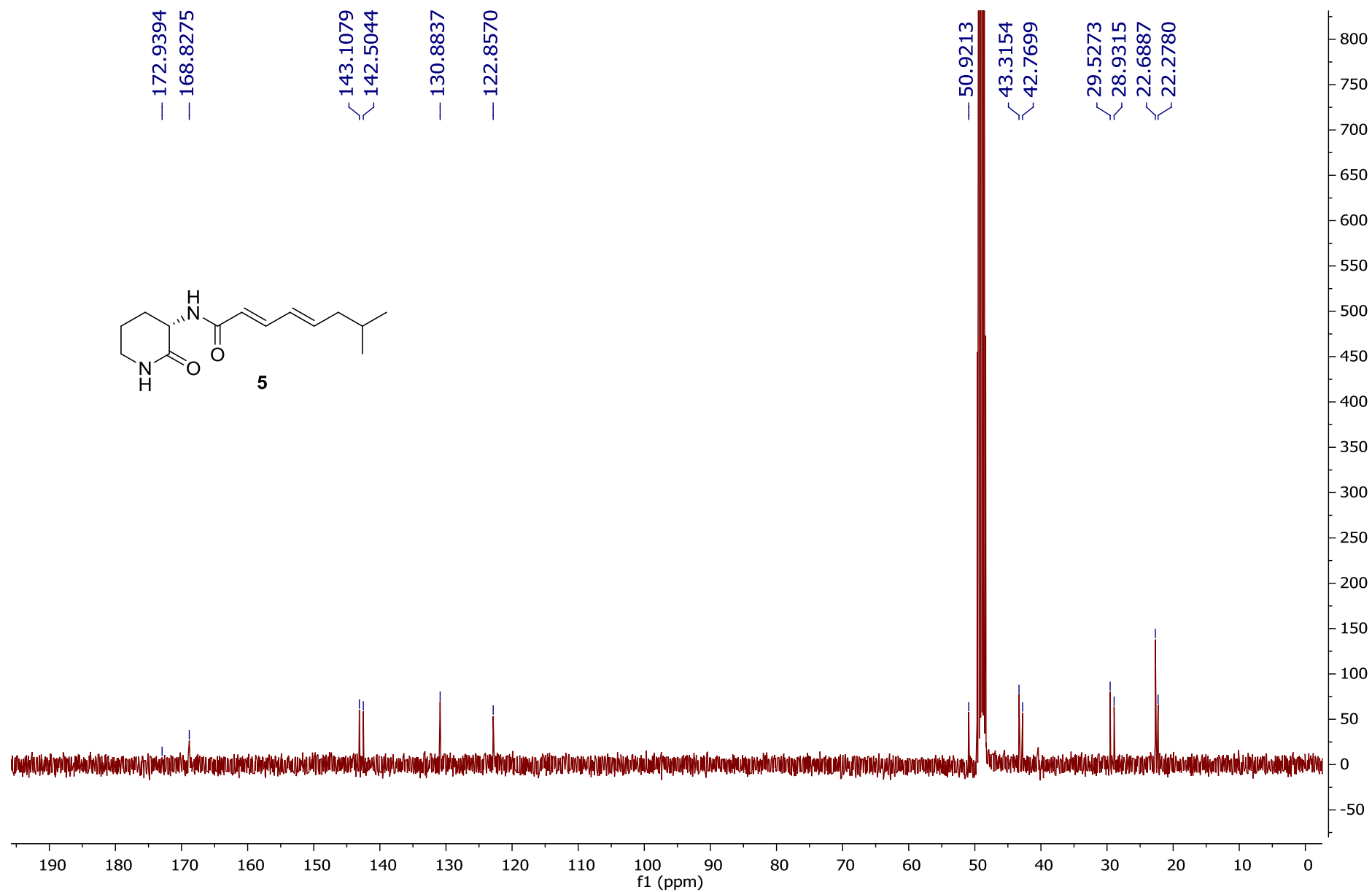


Figure S53. HSQC spectrum of ornilactam A (5) in CD₃OD

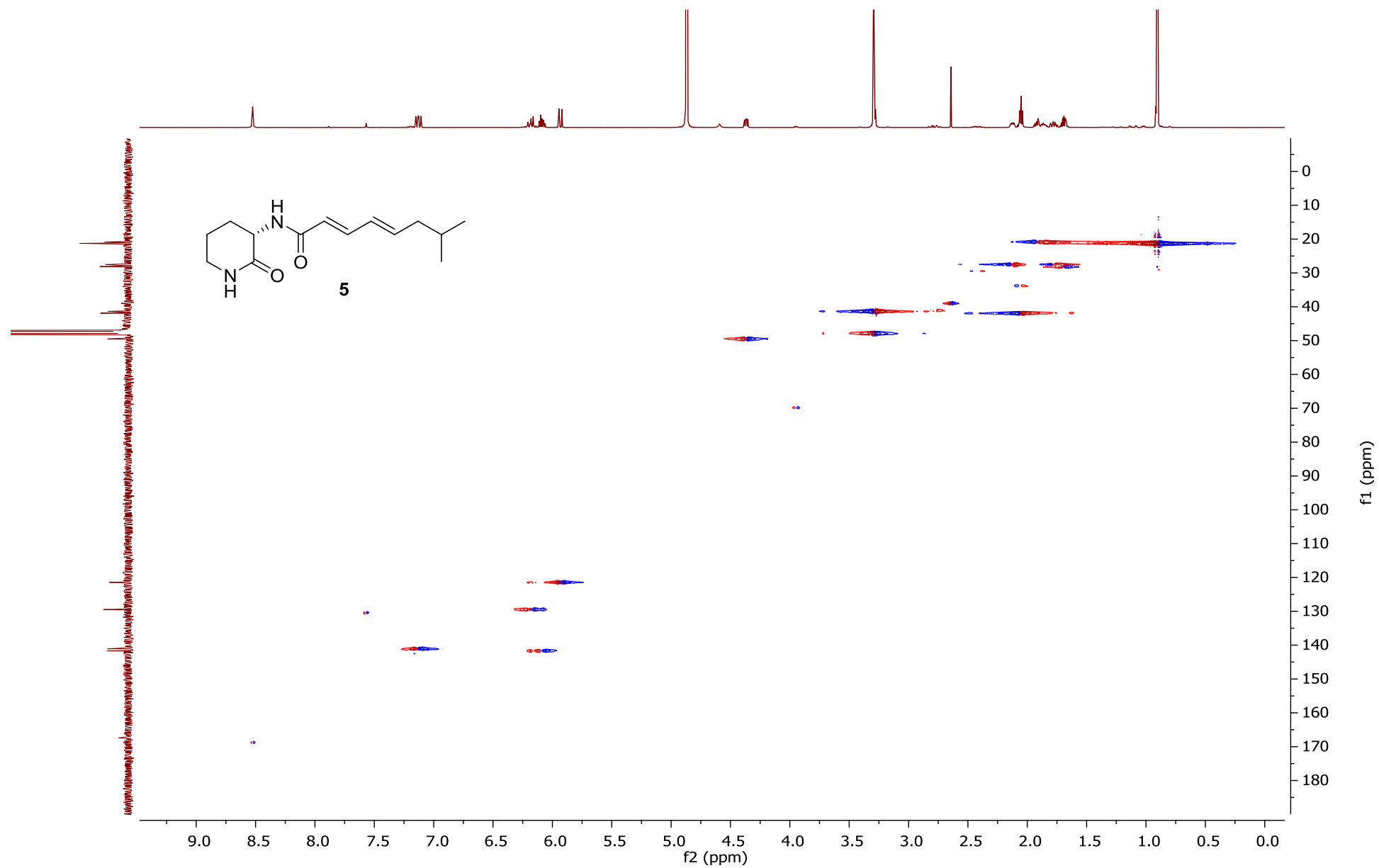


Figure S54. ^1H - ^1H COSY spectrum of ornilactam A (**5**) in CD_3OD

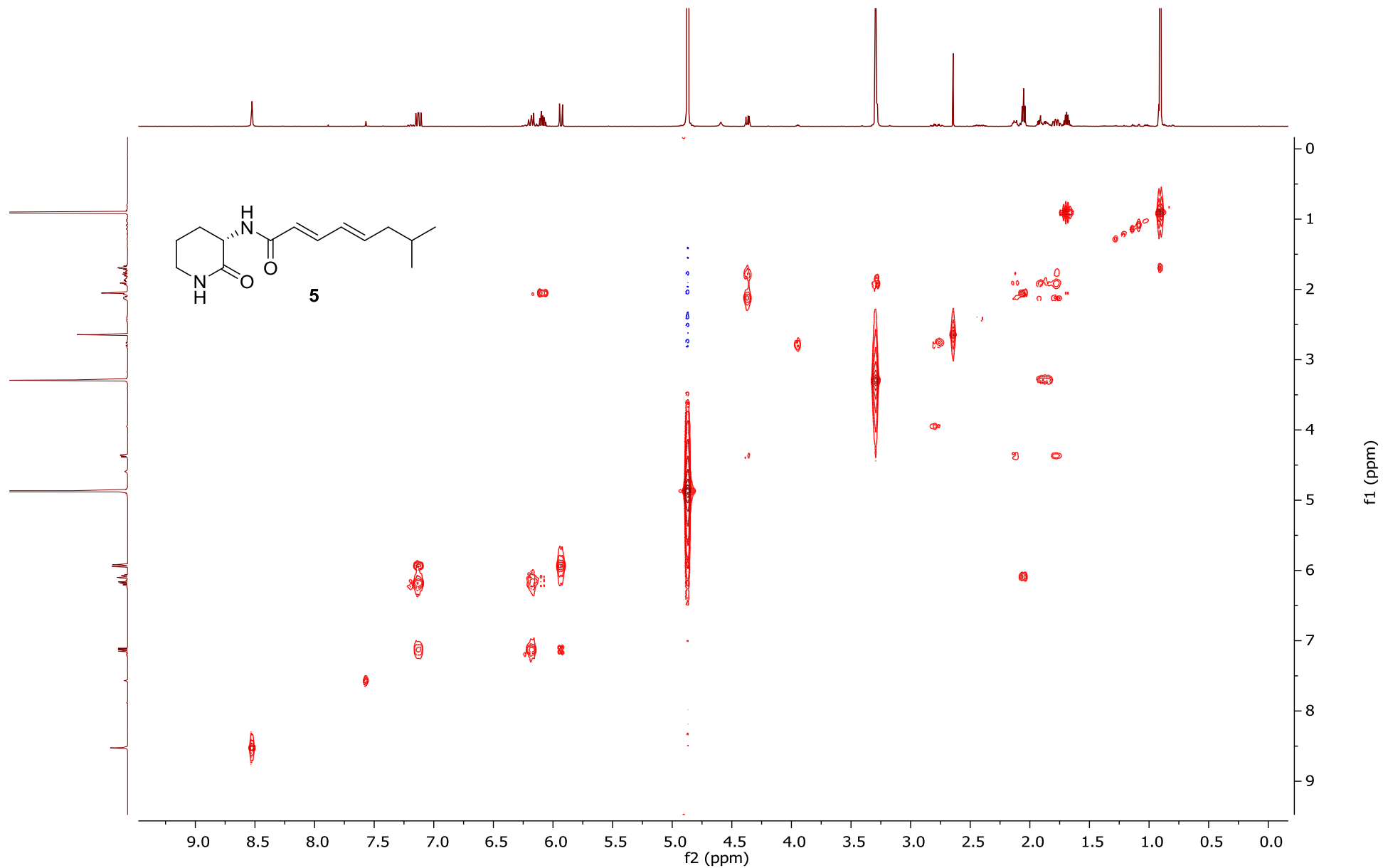


Figure S55. HMBC spectrum of ornilactam A (5) in CD₃OD

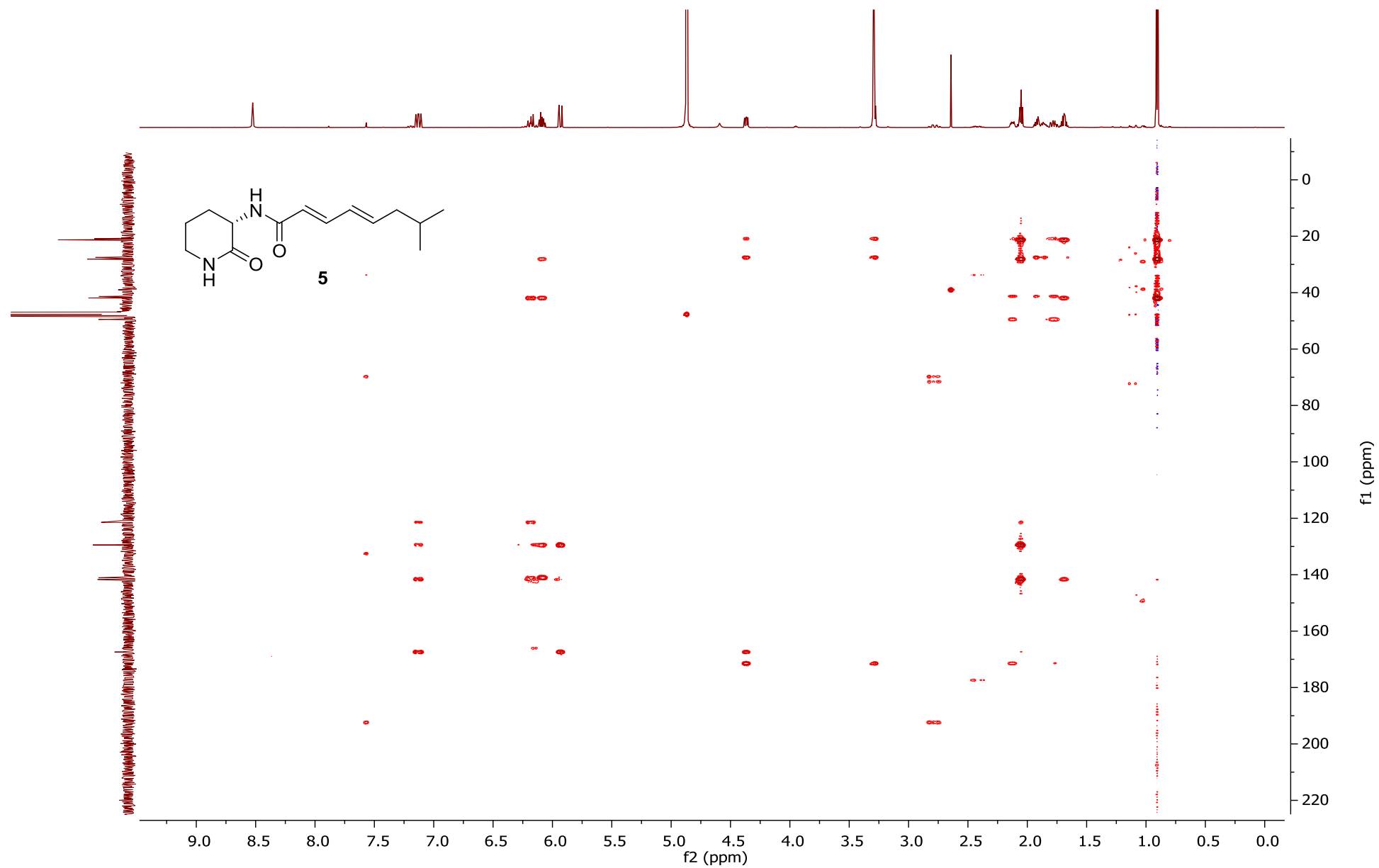


Figure S56. ^1H -NMR spectrum of compound **1b** in $\text{DMSO}-d_6$

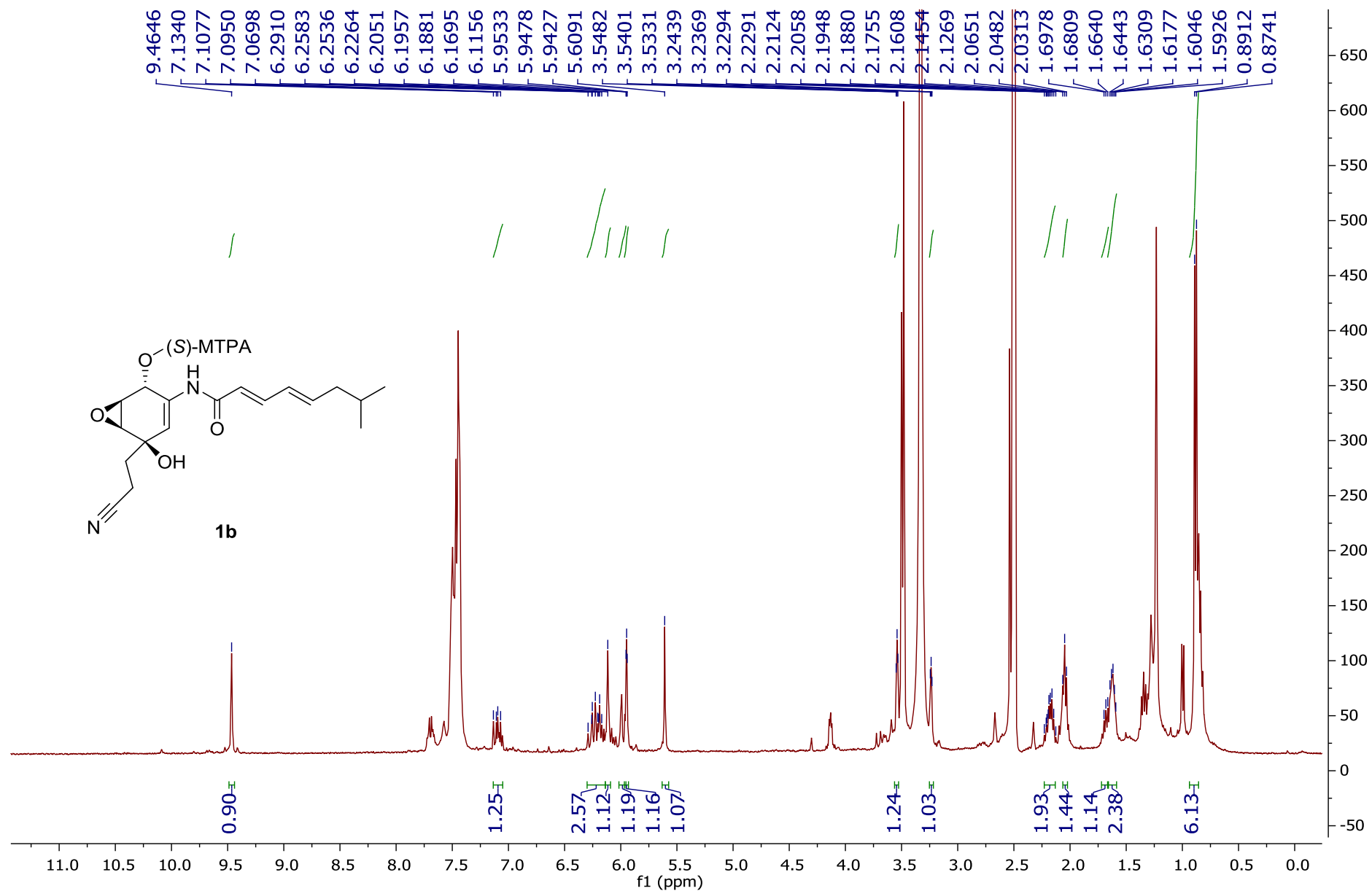


Figure S57. ^1H -NMR spectrum of compound **1c** in $\text{DMSO}-d_6$

