

Asperosin A, a [4+2] Diels–Alder Cycloaddition Polyketide Dimer from *Aspergillus rugulosa* with Immunosuppressive Activity

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Figure S1. HRESIMS spectrum of 1.

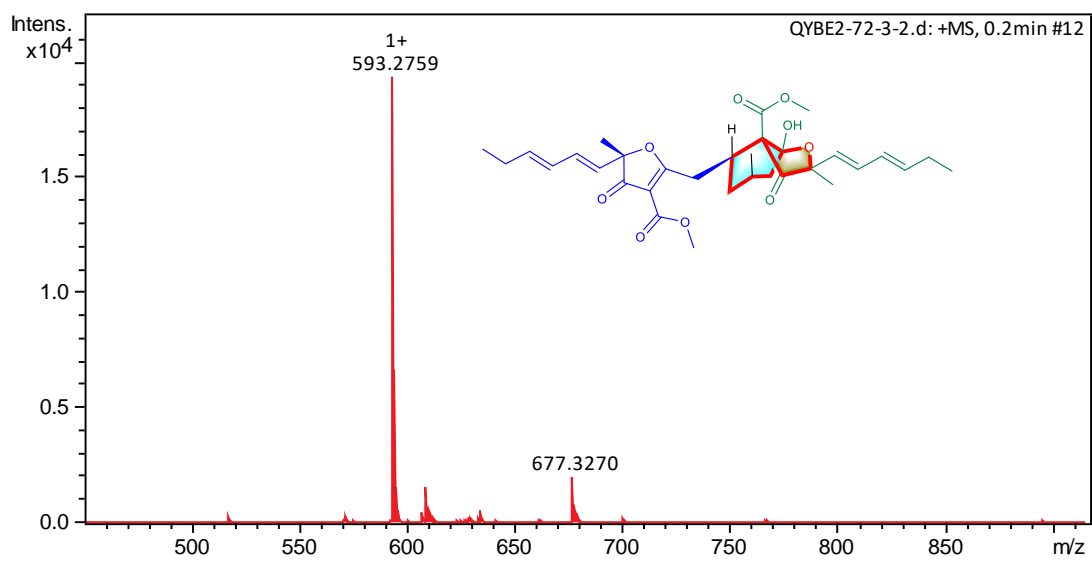


Figure S2. UV spectrum of 1.

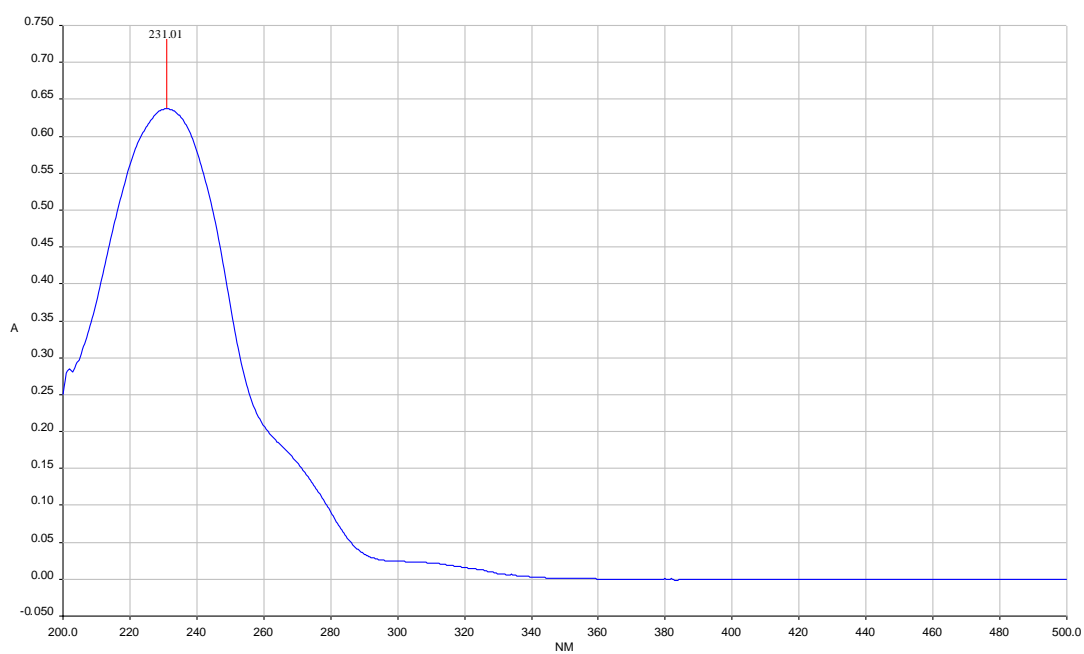


Figure S3. IR spectrum of 1.

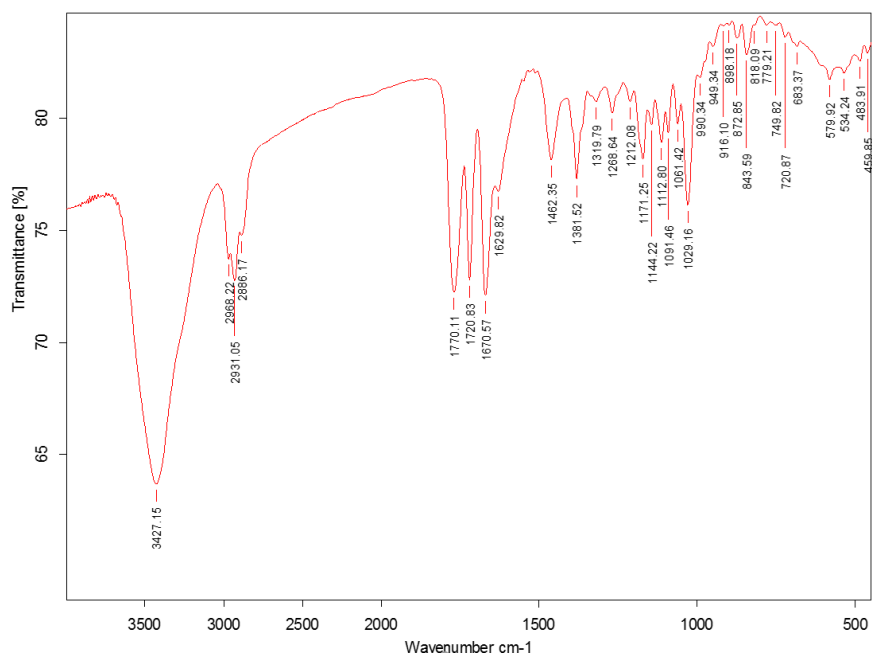


Figure S4. Experimental and calculated ECD spectra of 2 and *ent*-2.

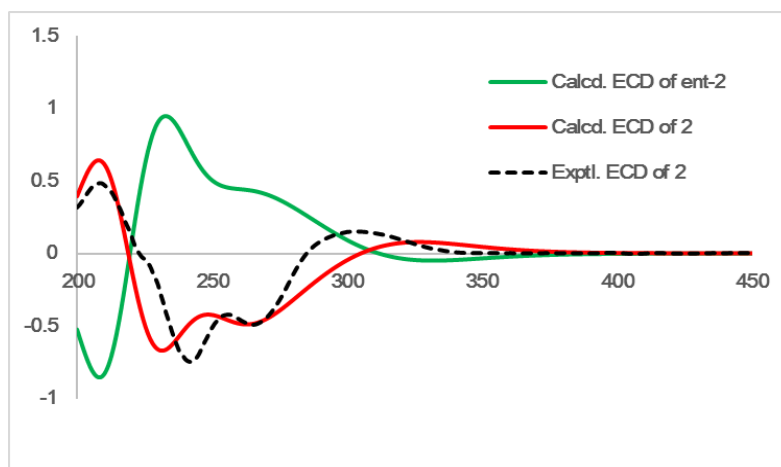
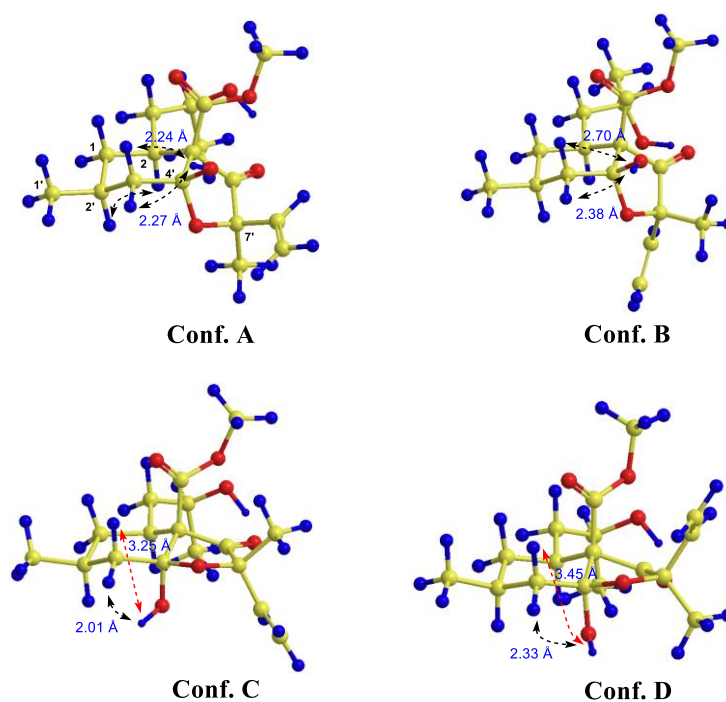


Figure S5. Simplified Chemdraw 3D structures and key NOEs of 1.



Figures S6–8. The bioactivities of 1.

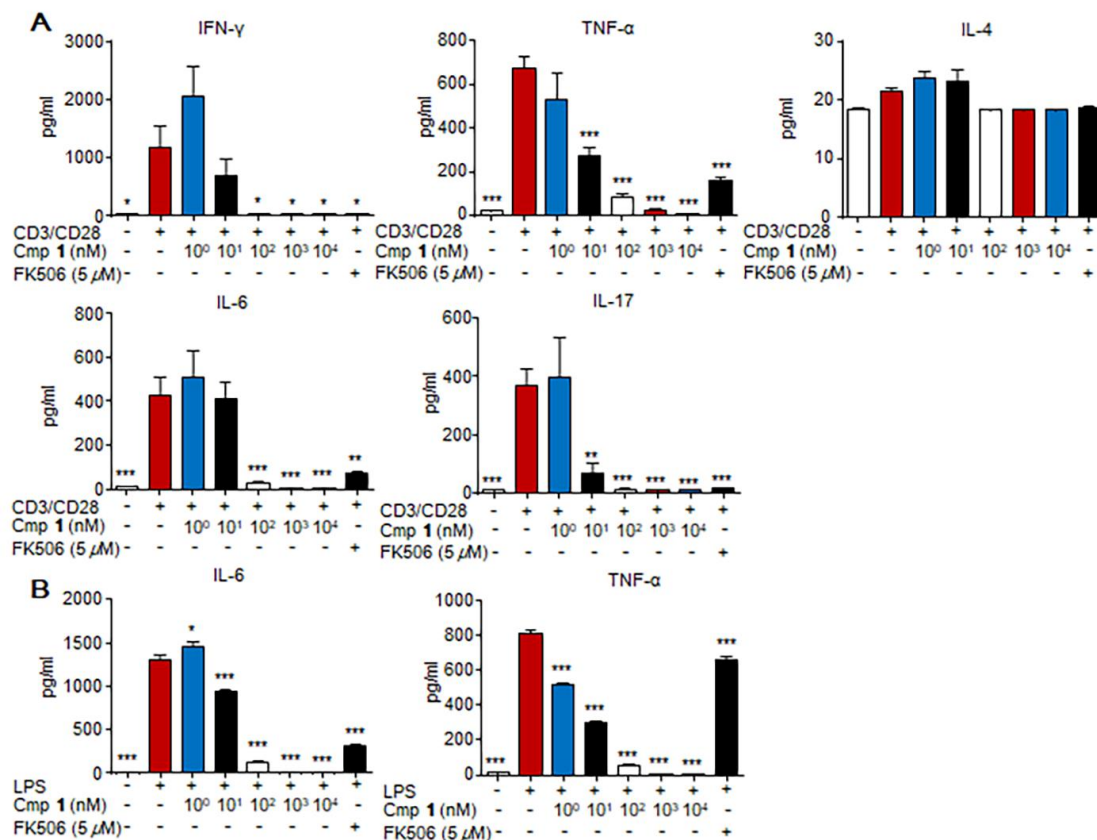


Figure S6. Compound 1 inhibited the level of inflammatory cytokines IFN- γ , TNF- α , IL-4, IL-6 and

IL-17 in cell cultures for murine splenocytes treated with anti-CD3/CD28 mAb or LPS. synthesis. Murine splenocytes were treated with **1** to 10000 nM of **1** for 1 h and subsequently stimulated with anti-CD3/CD28 mAb (A) or 10 $\mu\text{g}/\text{mL}$ LPS (B). 72 h after stimulation, supernatants were harvested and IFN- γ , TNF- α , IL-4, IL-6 and IL-17 protein level of were measured by cytometric bead array. Mean \pm SEM of three replicates are shown; * indicates $0.01 < p < 0.05$, ** indicates $0.001 \leq p \leq 0.01$, and *** indicates $p < 0.001$.

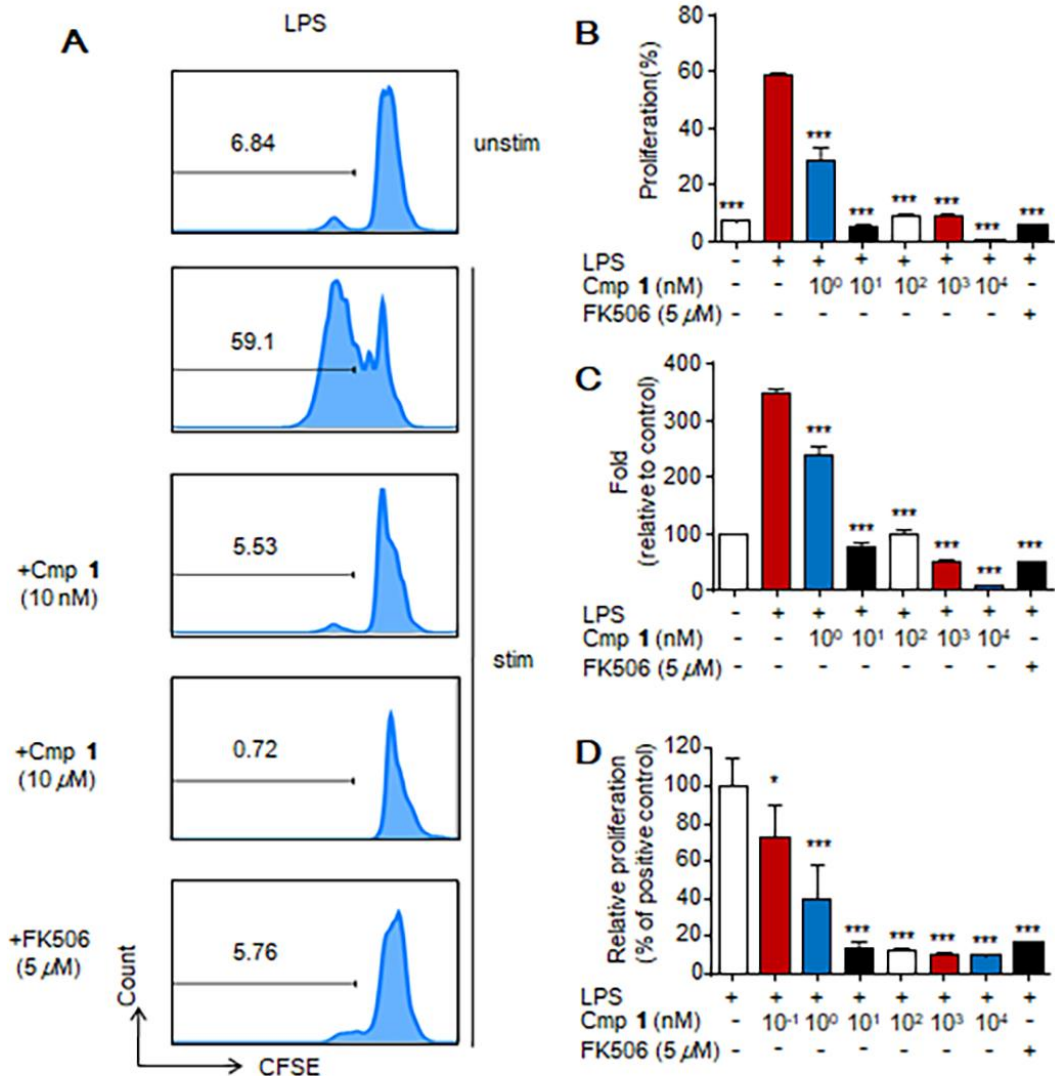


Figure S7. Impact of **1** on LPS stimulated murine splenocytes proliferation *in vitro*. (A) Murine splenocytes were labeled with CFSE and then cultured with stimuli, LPS with or without **1** as shown, for 72 h. CFSE dilutions indicate proliferation. (B) Proliferation of LPS stimulated murine splenocytes with different concentrates. (C) Viable cell counts of LPS stimulated murine splenocytes with different concentrates. (D) Relative proliferation of positive control measured by CCK method. Mean \pm SEM of three replicates are shown; * indicates $0.01 < p < 0.05$, and *** indicates $p < 0.001$.

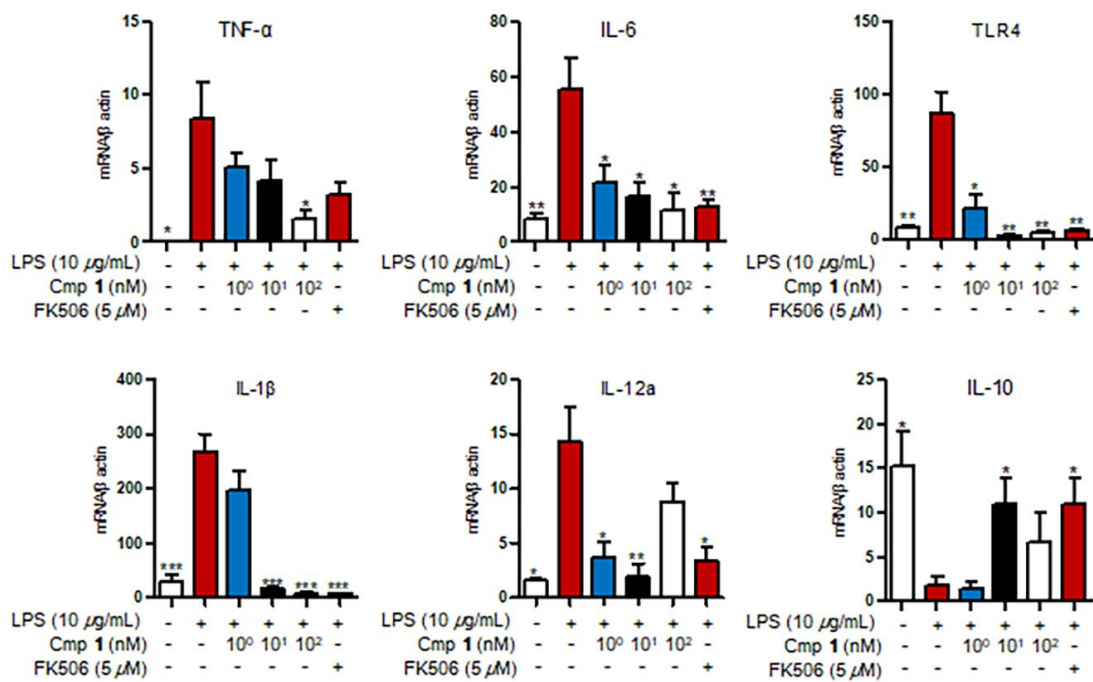


Figure S8. Impact of **1** on gene expression of LPS stimulated murine splenocytes. Murine splenocytes were treated with **1** to 100 nM of **1** for 1 h and subsequently stimulated with 10 μg/mL LPS. 72 h after stimulation, RNA were extracted and TNF-α, IL-6, TLR4, IL-1β, IL-12a and IL-10 transcription level of were measured by RT-PCR. Mean ± SEM of three replicates are shown; *indicates 0.01 < p < 0.05, ** indicates 0.001 ≤ p ≤ 0.01, and *** indicates p < 0.001

Figure S9. ¹H NMR spectrum of 1 record in 400 MHz (MeOH-d₄).

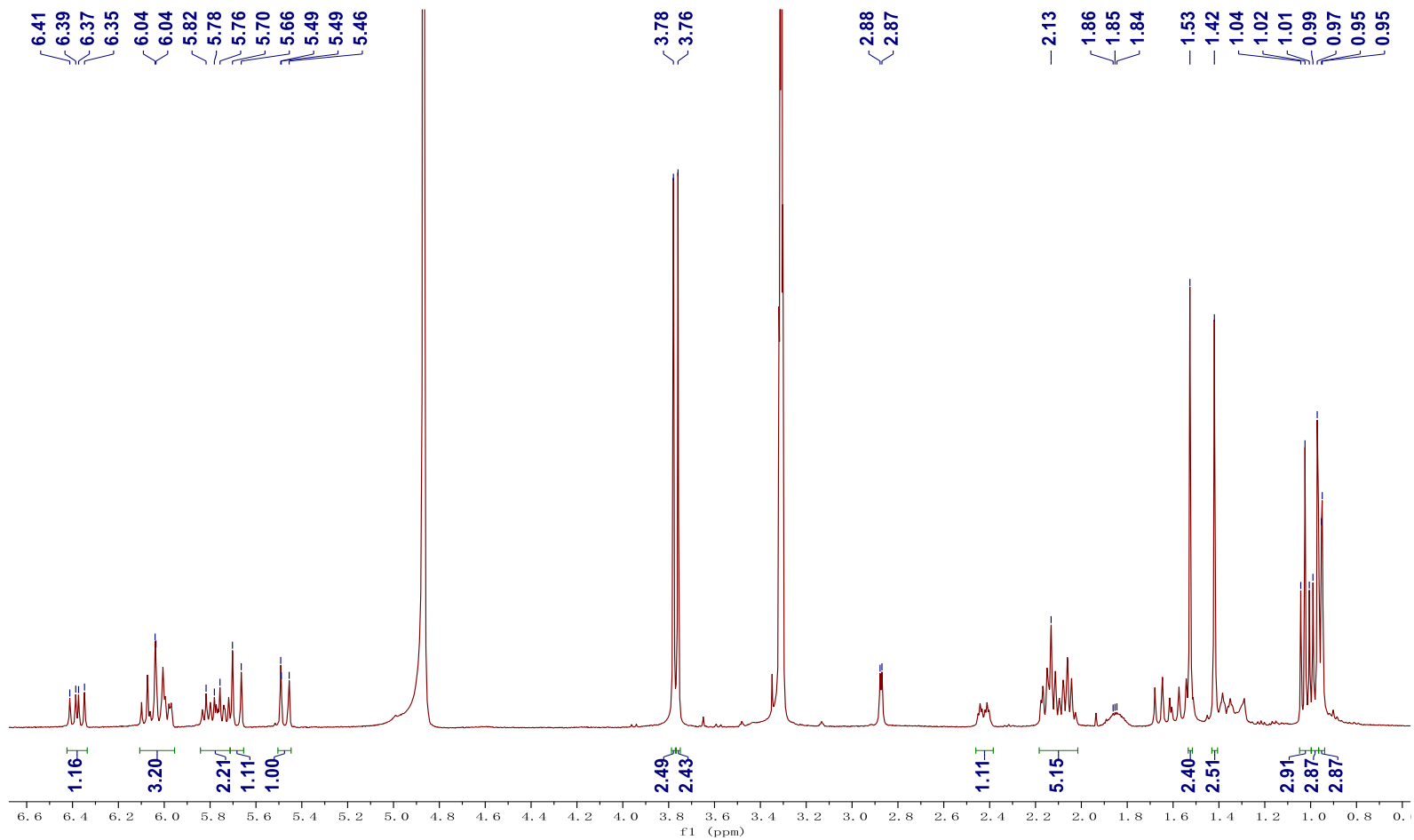


Figure S10. ^{13}C NMR and DEPT spectra of 1 record in 100 MHz ($\text{MeOH-}d_4$).

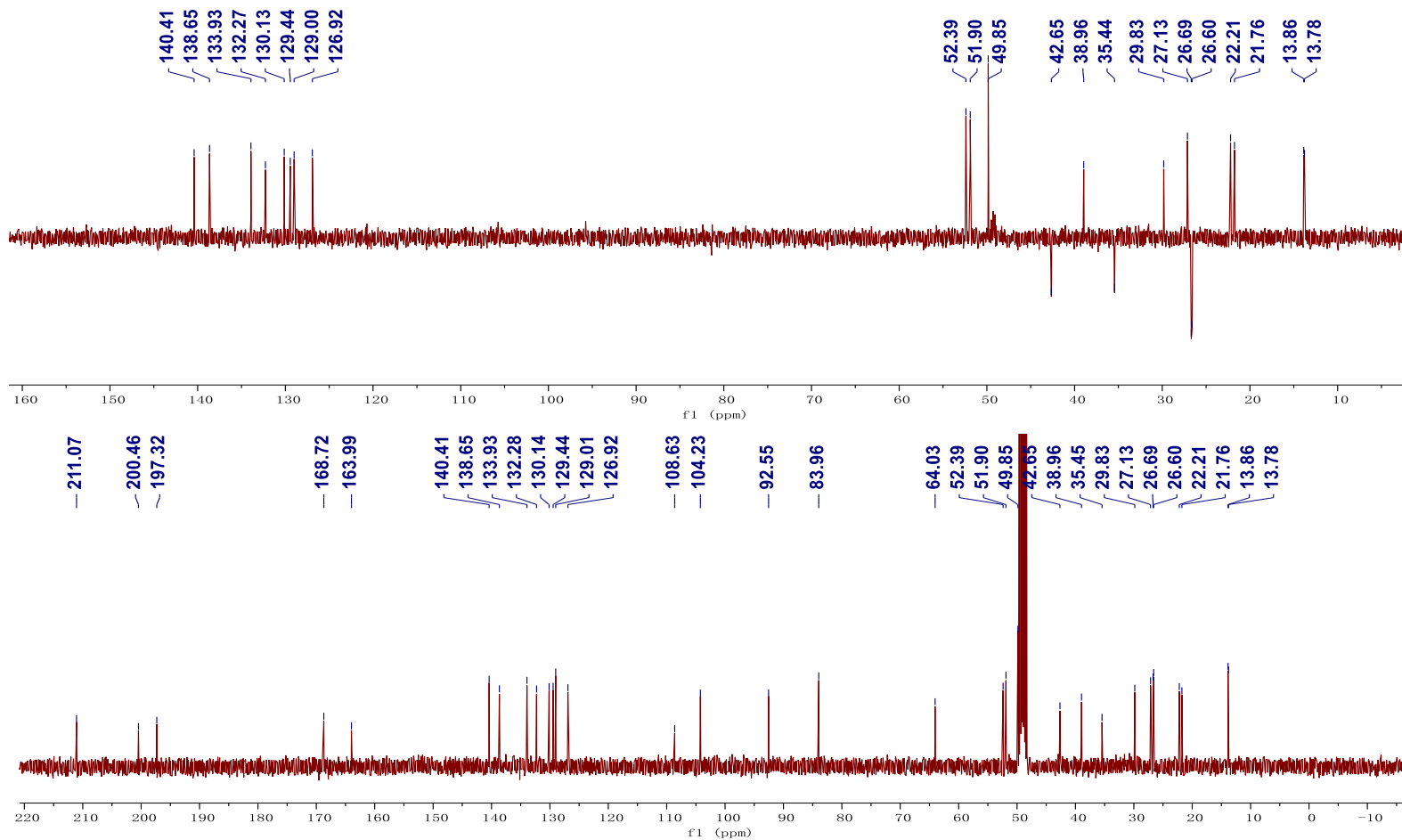


Figure S11. HSQC spectrum of 1 record in 400 MHz (MeOH-*d*4).

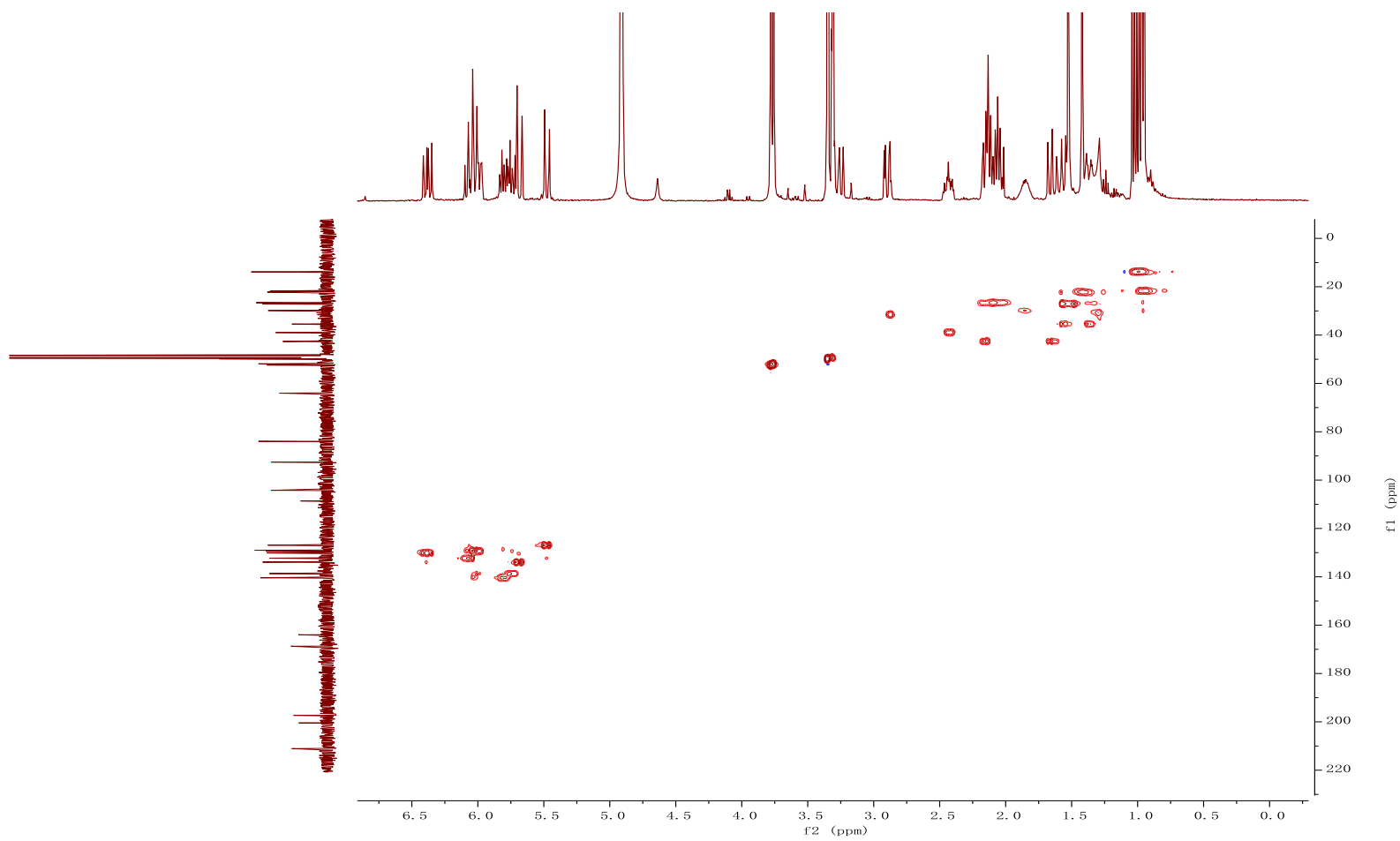


Figure S12. HMBC spectrum of 1 record in 400 MHz (MeOH- d_4).

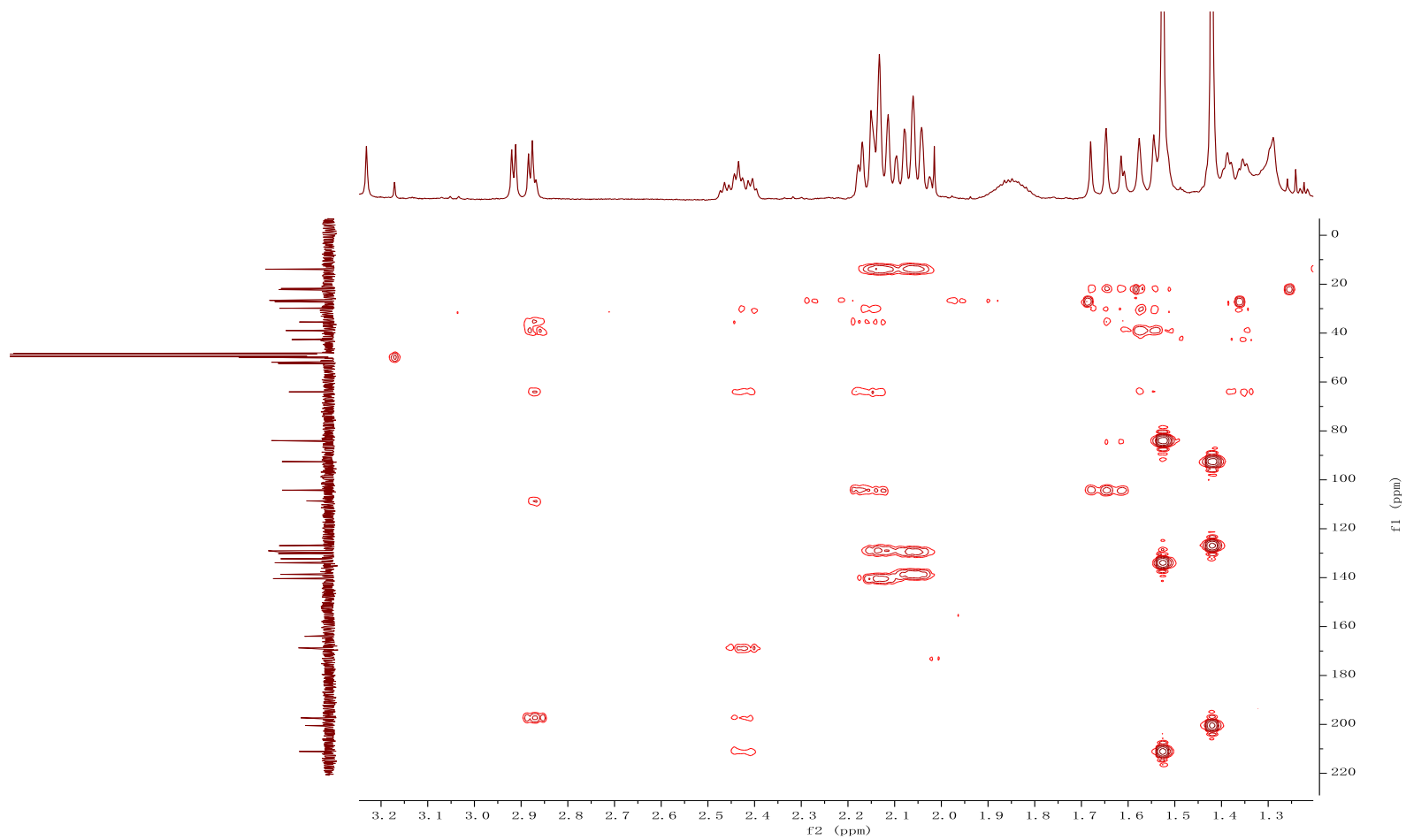


Figure S13. ^1H - ^1H COSY spectrum of 1 record in 400 MHz (MeOH- d_4).

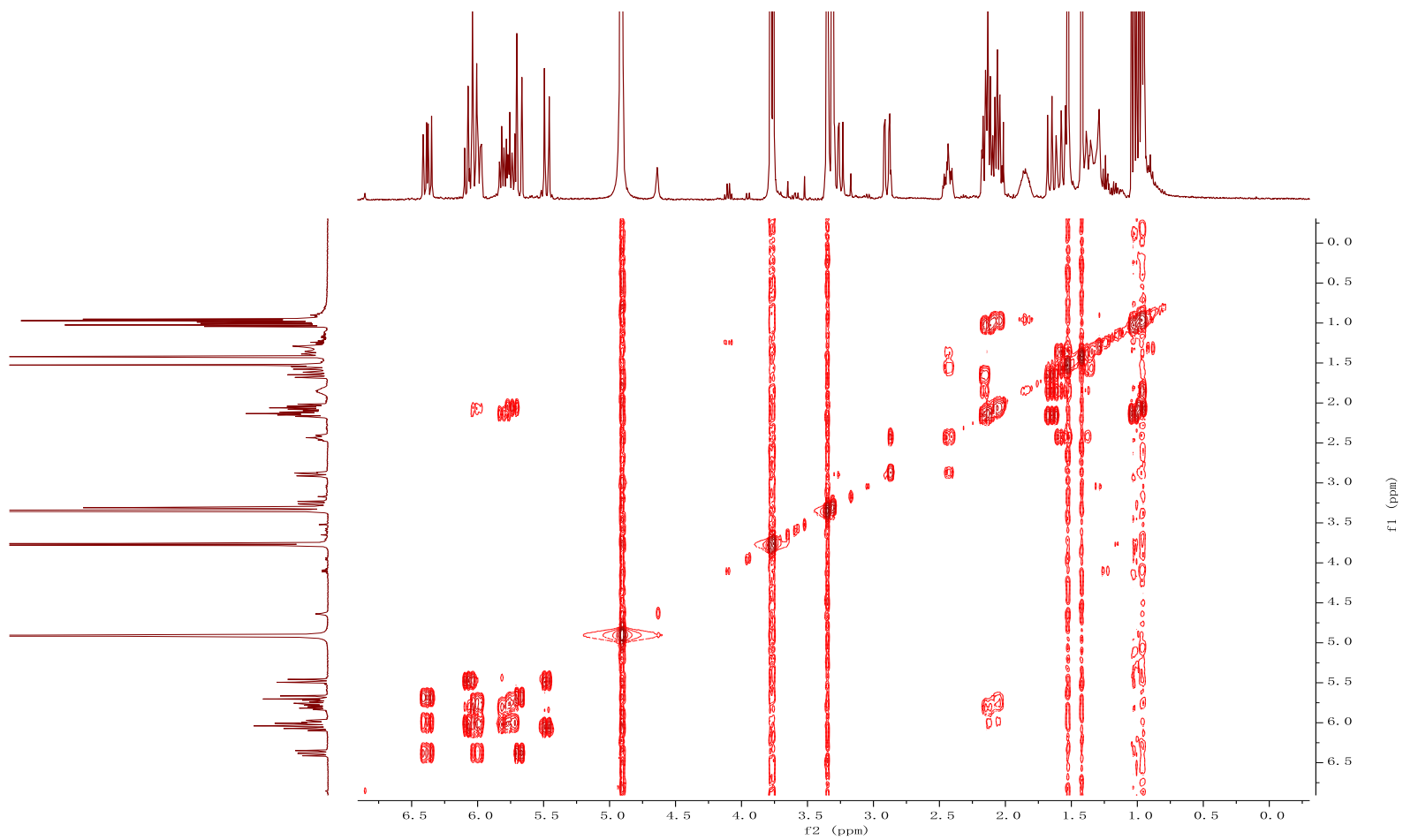


Figure S14. NOESY spectrum of 1 record in 400 MHz (MeOH- d_4).

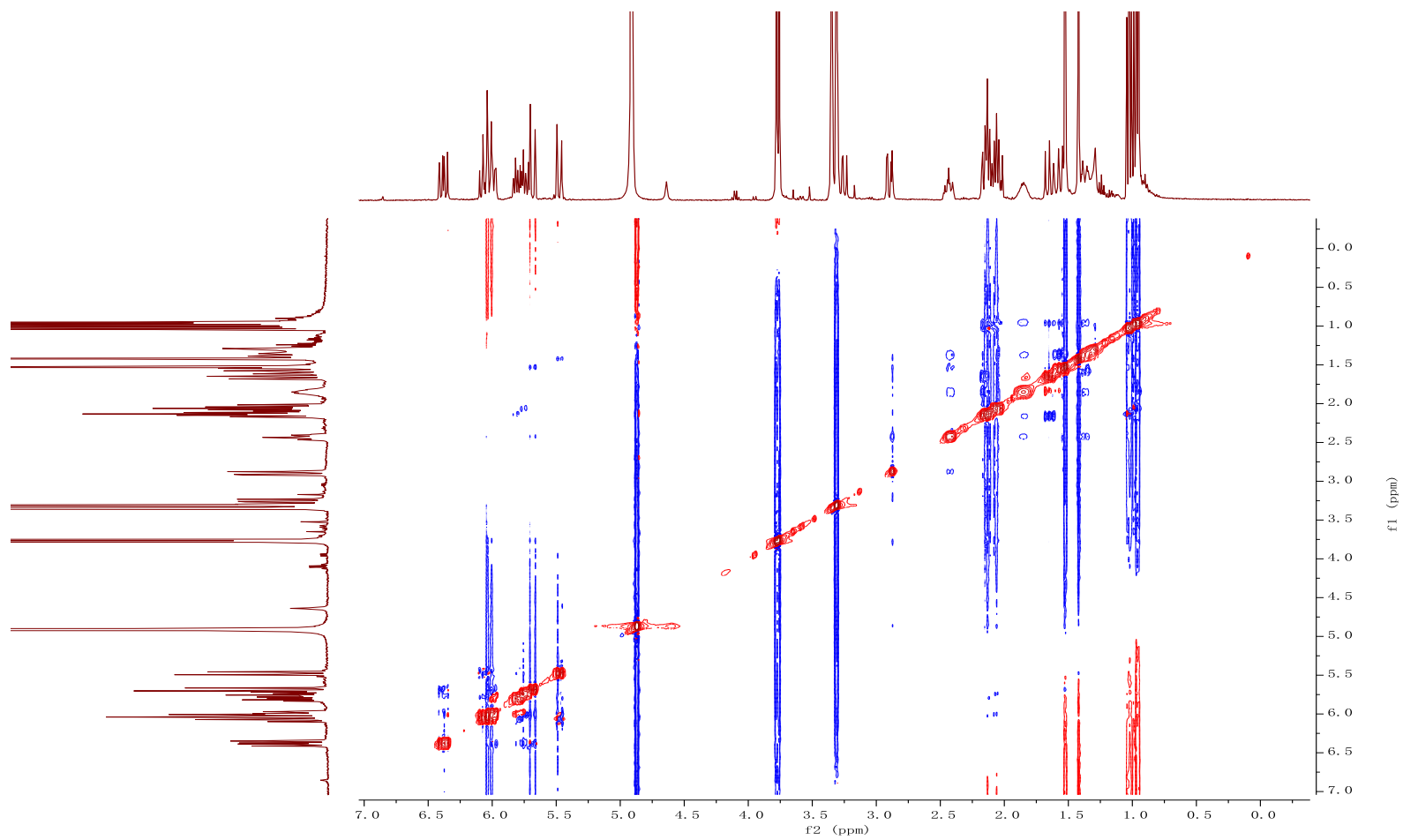


Figure S15. ¹H NMR spectrum of 1 record in 600 MHz (DMSO-d₆).

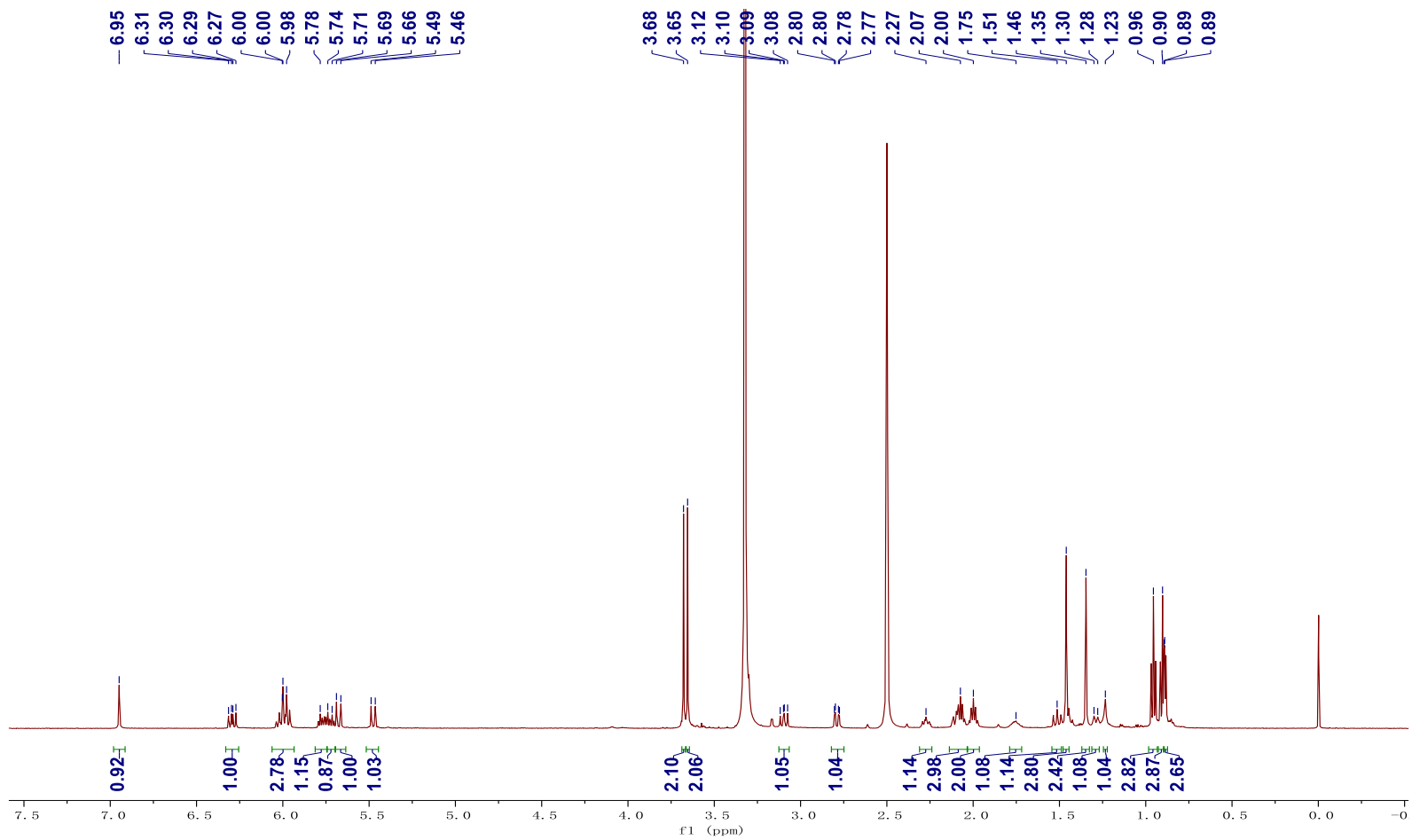


Figure S16. ^{13}C NMR and DEPT spectra of 1 record in 150 MHz ($\text{DMSO}-d_6$).

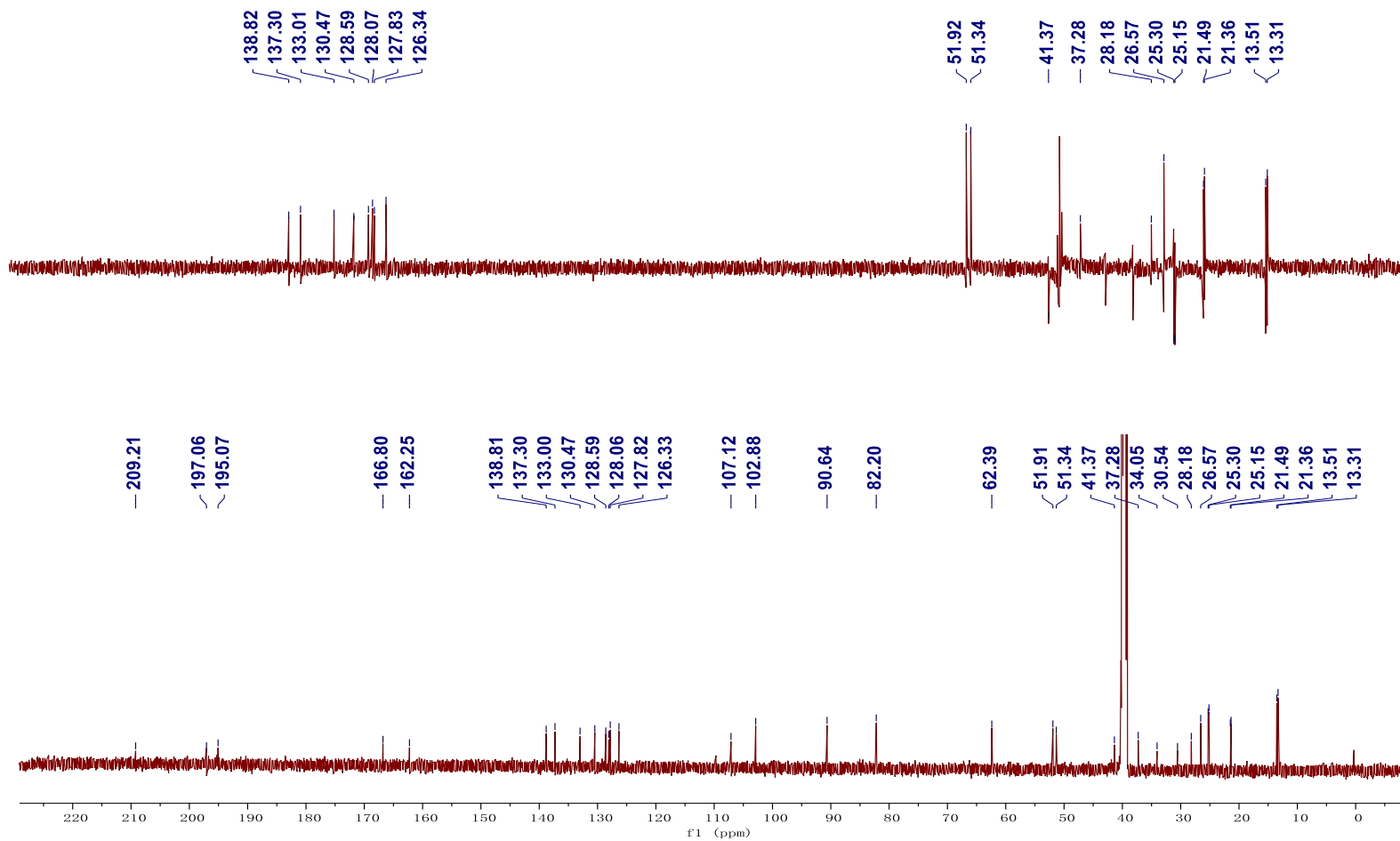


Figure S17. HSQC spectrum of 1 record in 600 MHz (DMSO- d_6).

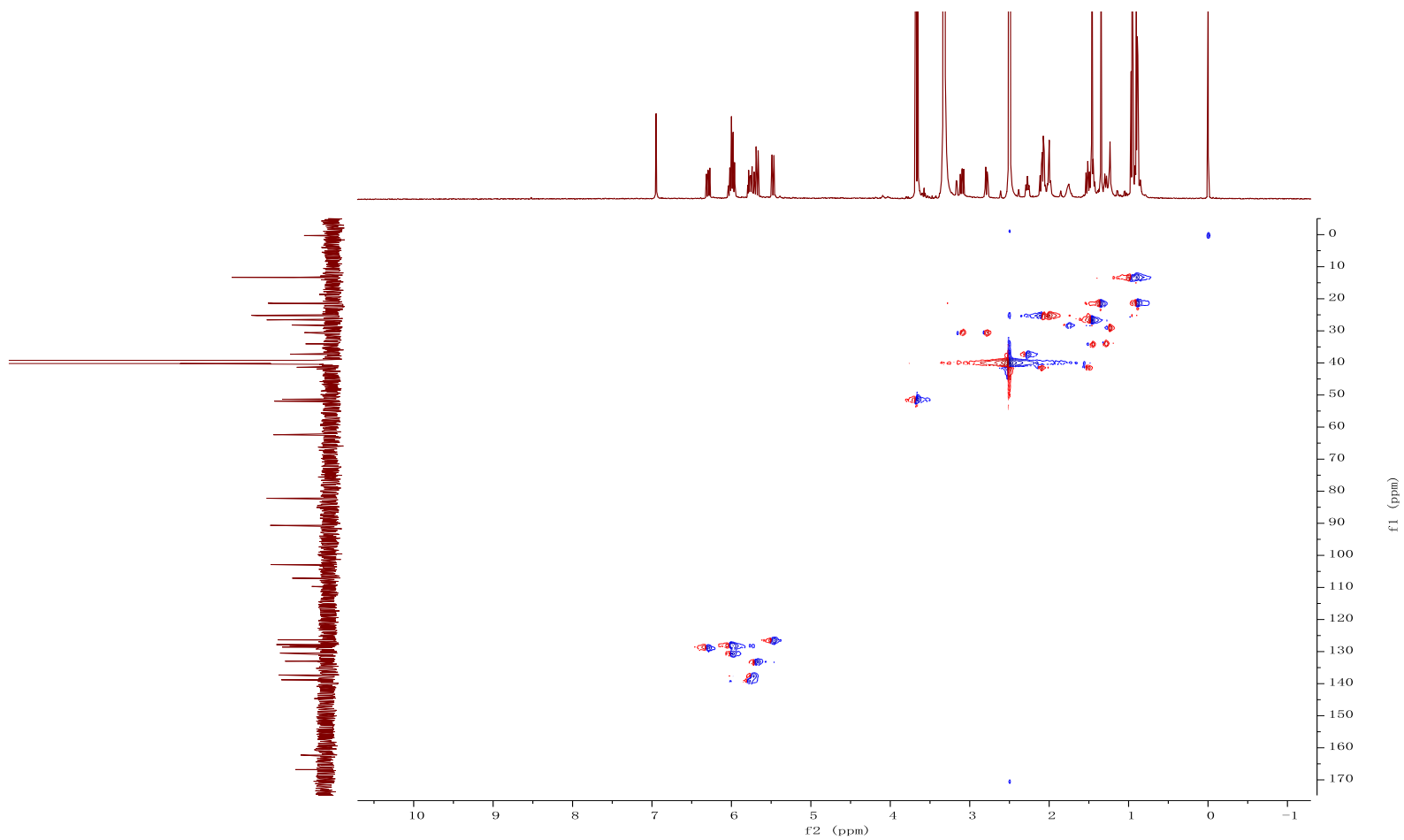


Figure S18. HMBC spectrum of 1 record in 600 MHz (DMSO- d_6).

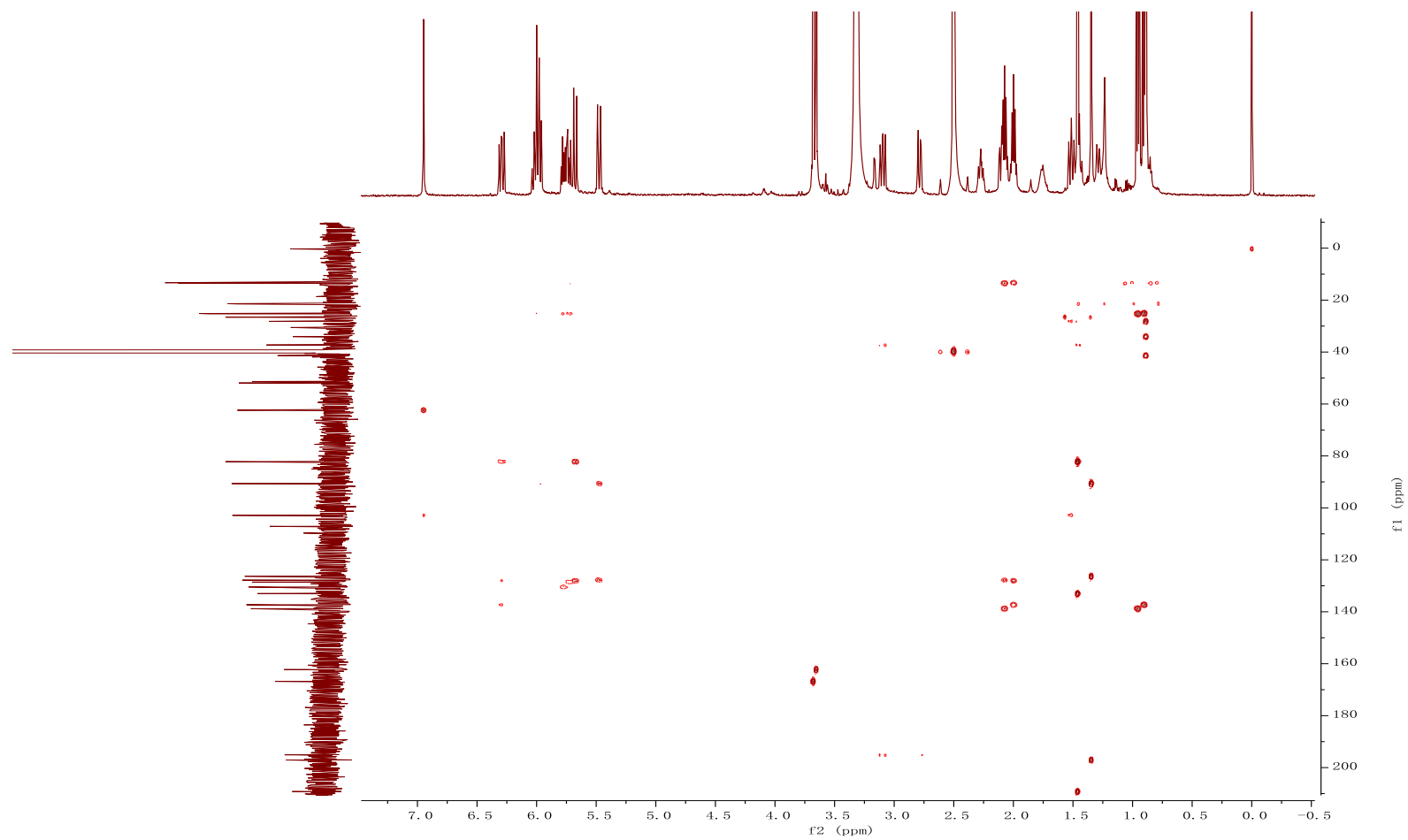


Figure S19. ^1H - ^1H COSY spectrum of 1 record in 600 MHz (DMSO- d_6).

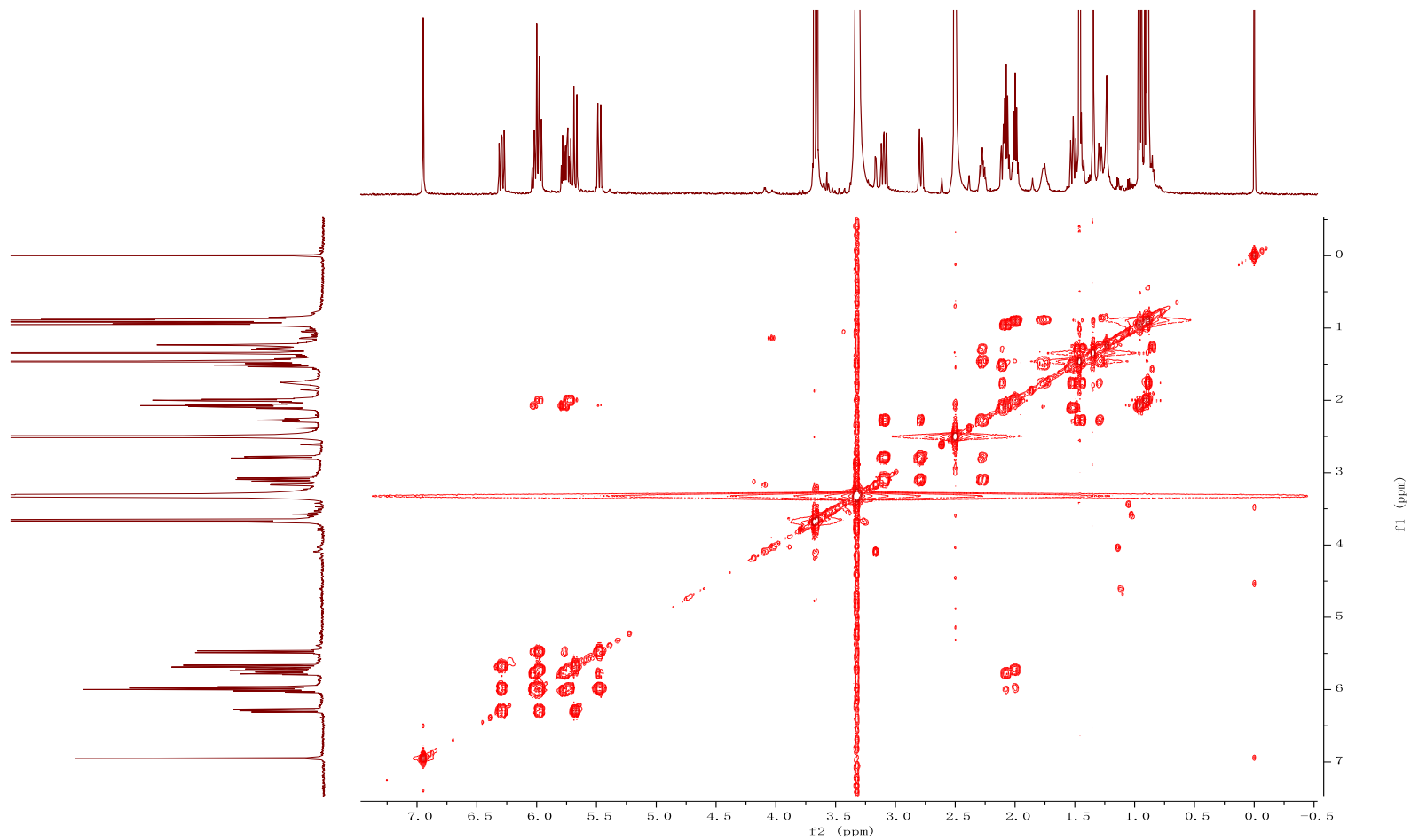


Figure S20. NOESY spectrum of 1 record in 600 MHz (DMSO- d_6).

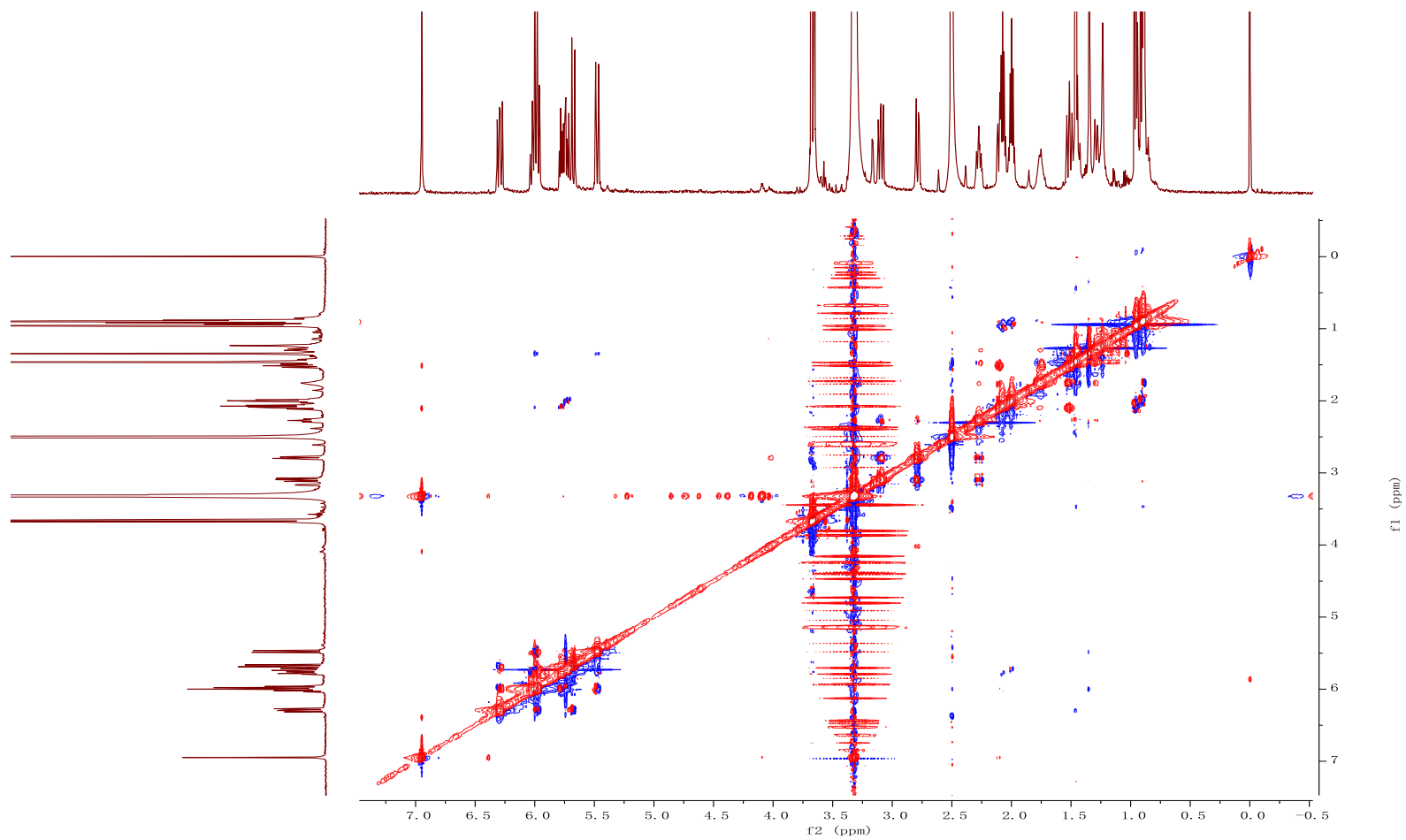


Figure S21. Partial enlarged drawing of NOESY spectrum of 1 record in 600 MHz (DMSO- d_6).

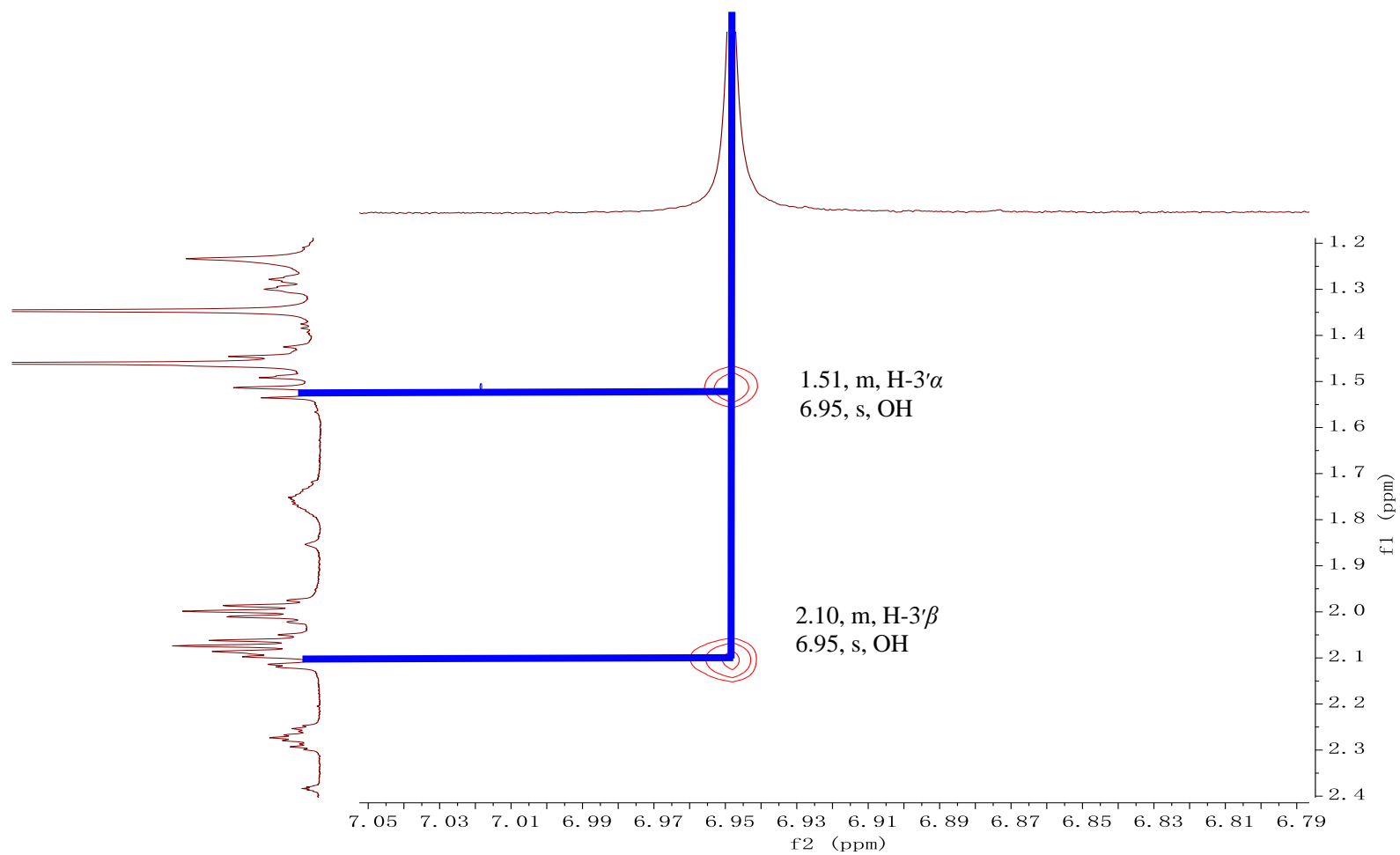


Figure S22. ¹H NMR spectrum of 2.

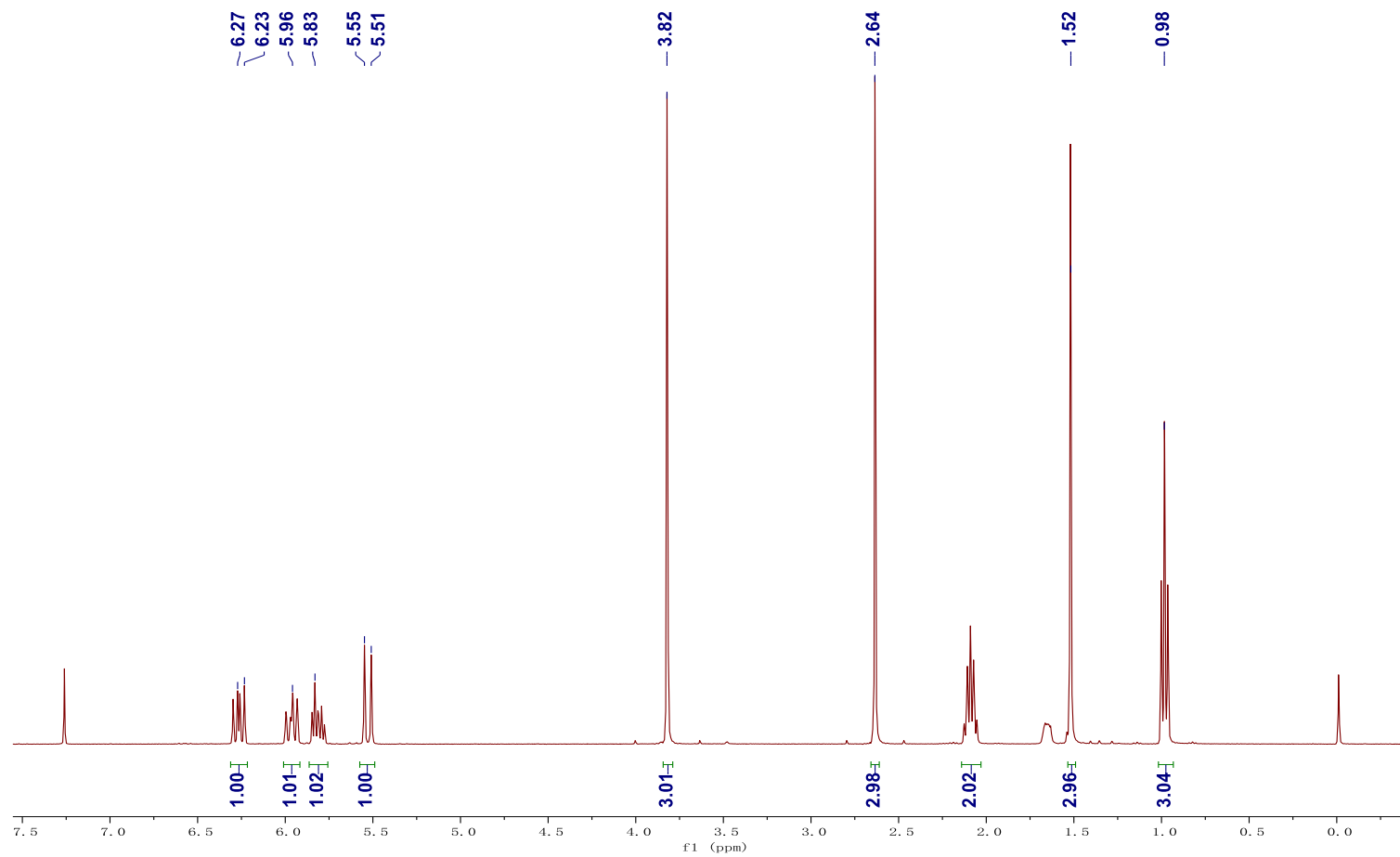


Figure S23. ^1H NMR spectrum of 3.

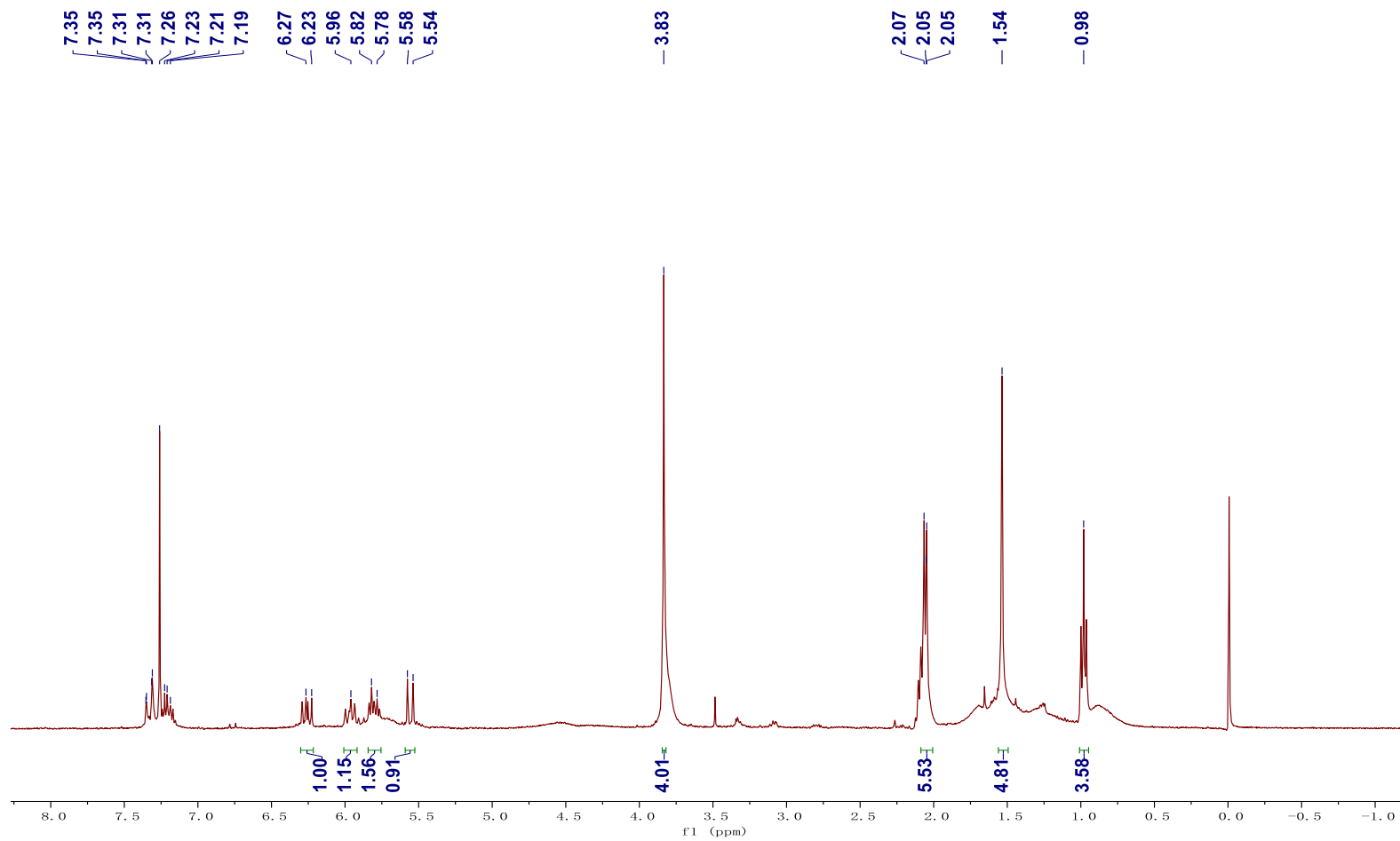


Figure S24. ^{13}C NMR spectrum of 3.

