

Supporting Information

Alertoxins with quorum sensing inhibitory activities from the marine-derived fungus *Cladosporium* sp. KFD33

Fei Zhang^{1,2,†}, Li-Man Zhou^{1,†}, Fan-Dong Kong^{1,†}, Qing-Yun Ma¹, Qing-Yi Xie¹, Jiu-Hui Li¹, Hao-Fu Dai¹, Lei Guo^{2,*}, You-Xing Zhao^{1,*}

¹Hainan Key Laboratory of Research and Development of Natural Product from Li Folk Medicine, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical

Agricultural Sciences, Haikou 571101, China; MarchFay@163.com (F. Z);

zhouliman88@126.com (L.M. Z); kongfandong@itbb.org.cn (F.D. K); maqingyun@itbb.org.cn

(Q.Y. M); xieqingyi@itbb.org.cn (Q.Y. X); lijiahui@itbb.org.cn (J.H. L.); daihaofu@itbb.org.cn (H.F. D.);

²Jiangsu Key Laboratory of Marine Bioresources and Environment, Co-innovation Center of Jiangsu Marine Bio-industry Technology, Jiangsu Ocean University, Lianyungang 222005;

* Correspondence: zhaoyouxing@itbb.org.cn (Y.X. Z); guol@jou.edu.cn (L. G);

Tel.: +86-898-6698-9095 (Y.X. Z); +86-159-5072-6013 (L. G)

† These authors contributed equally to this paper.

List of Supporting Information

Figure S1. The ¹ H NMR Spectrum of Compound 1 in DMSO	3
Figure S2. The ¹³ C NMR Spectrum of Compound 1 in DMSO	4
Figure S3. The HMQC Spectrum of Compound 1 in DMSO	5
Figure S4. The HMBC Spectrum of Compound 1 in DMSO	6
Figure S5. The COSY Spectrum of Compound 1 in DMSO	7
Figure S6. The HRESIMS Spectroscopic Data of Compound 1	8
Figure S7. The IR Spectrum of Compound 1	9
Figure S8. The ¹ H NMR Spectrum of Compounds 2 and 3 in DMSO	10
Figure S9. The ¹³ C NMR Spectrum of Compounds 2 and 3 in DMSO	11
Figure S10. The DEPT Spectrum of Compounds 2 and 3 in DMSO	12
Figure S11. The HMQC Spectrum of Compounds 2 and 3 in DMSO	13
Figure S12. The HMBC Spectrum of Compounds 2 and 3 in DMSO	14
Figure S13. The COSY Spectrum of Compounds 2 and 3 in DMSO	15
Figure S14. The HRESIMS Spectroscopic Data of Compounds 2 and 3	16
Figure S15. The IR Spectrum of Compounds 2 and 3	17
Figure S16. The ¹ H NMR Spectrum of Compound 4 in DMSO	18

Figure S17. The ¹³ C NMR Spectrum of Compound 4 in DMSO	19
Figure S18. The DEPT Spectrum of Compound 4 in DMSO.....	20
Figure S19. The HMQC Spectrum of Compound 4 in DMSO.....	21
Figure S20. The HMBC Spectrum of Compound 4 in DMSO.....	22
Figure S21. The COSY Spectrum of Compound 4 in DMSO	23
Figure S22. The HRESIMS Spectroscopic Data of Compound 4	24
Figure S23. The IR Spectrum of Compound 4	25
Figure S24. The ¹ H NMR Spectrum of Compound 5 in DMSO.....	26
Figure S25. The ¹³ C NMR Spectrum of Compound 5 in DMSO	27
Figure S26. The DEPT Spectrum of Compound 5 in DMSO.....	28
Figure S27. The HMQC Spectrum of Compound 5 in DMSO.....	29
Figure S28. The HMBC Spectrum of Compound 5 in DMSO.....	30
Figure S29. The COSY Spectrum of Compound 5 in DMSO	31
Figure S30. The HRESIMS Spectroscopic Data of Compound 5	32
Figure S31. The IR Spectrum of Compound 5	33
Figure S32. A peak area ratio of compounds 2 and 3 over a chiral column	34
Figure S33. The strain of <i>Cladosporium</i> sp. KFD33.....	35
Figure S34. <i>C. violaceum</i> CV026 well diffusion assay.....	36
Figure S35. The energy minimized 3D chemical structures for 1-5	37
The 18S gene sequences of <i>Cladosporium</i> sp. KFD33.....	38
Theory and Calculation Details.	39

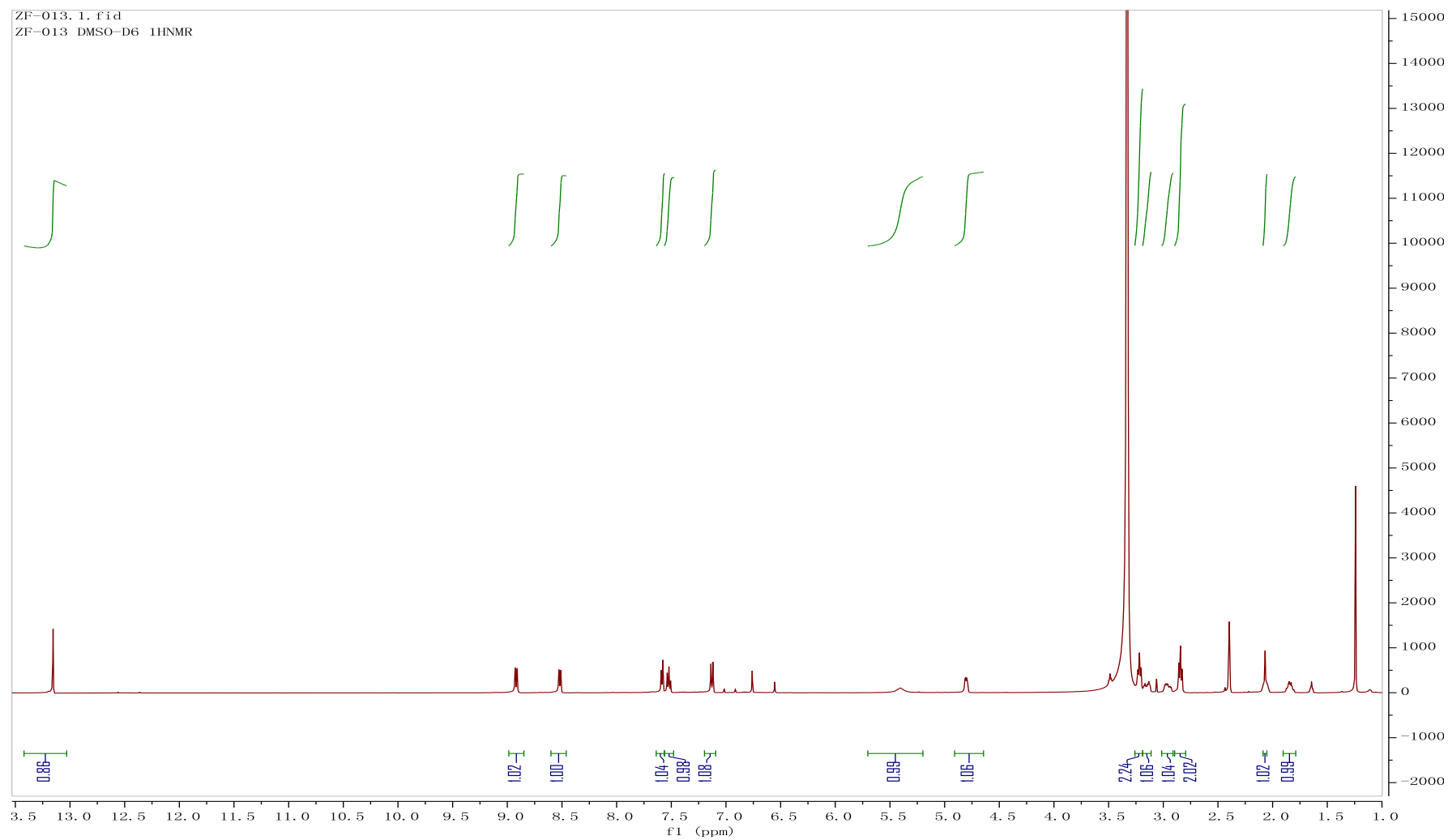


Figure S1. The ^1H NMR Spectrum of Compound **1** in DMSO

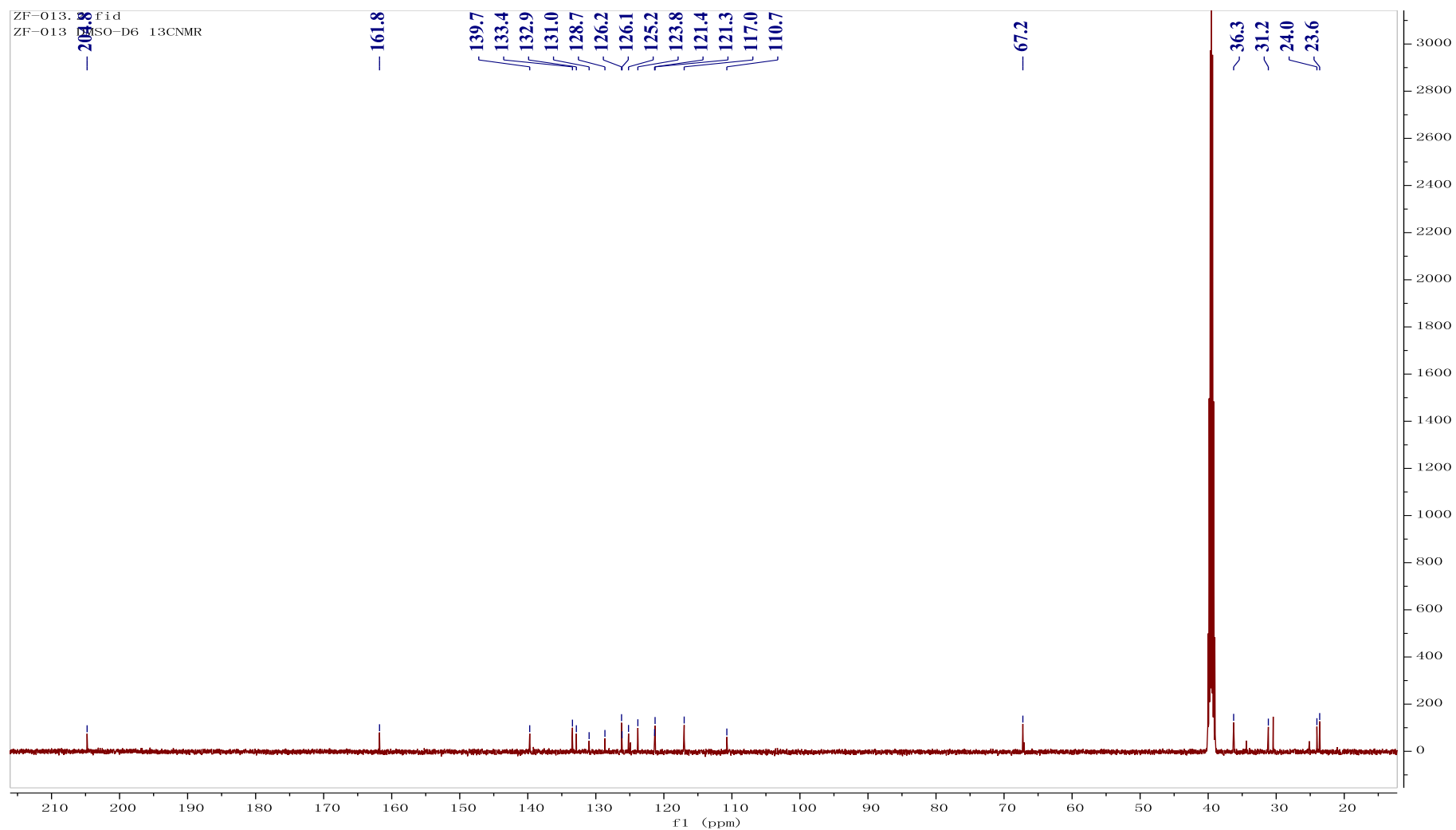


Figure S2. The ^{13}C NMR Spectrum of Compound **1** in DMSO

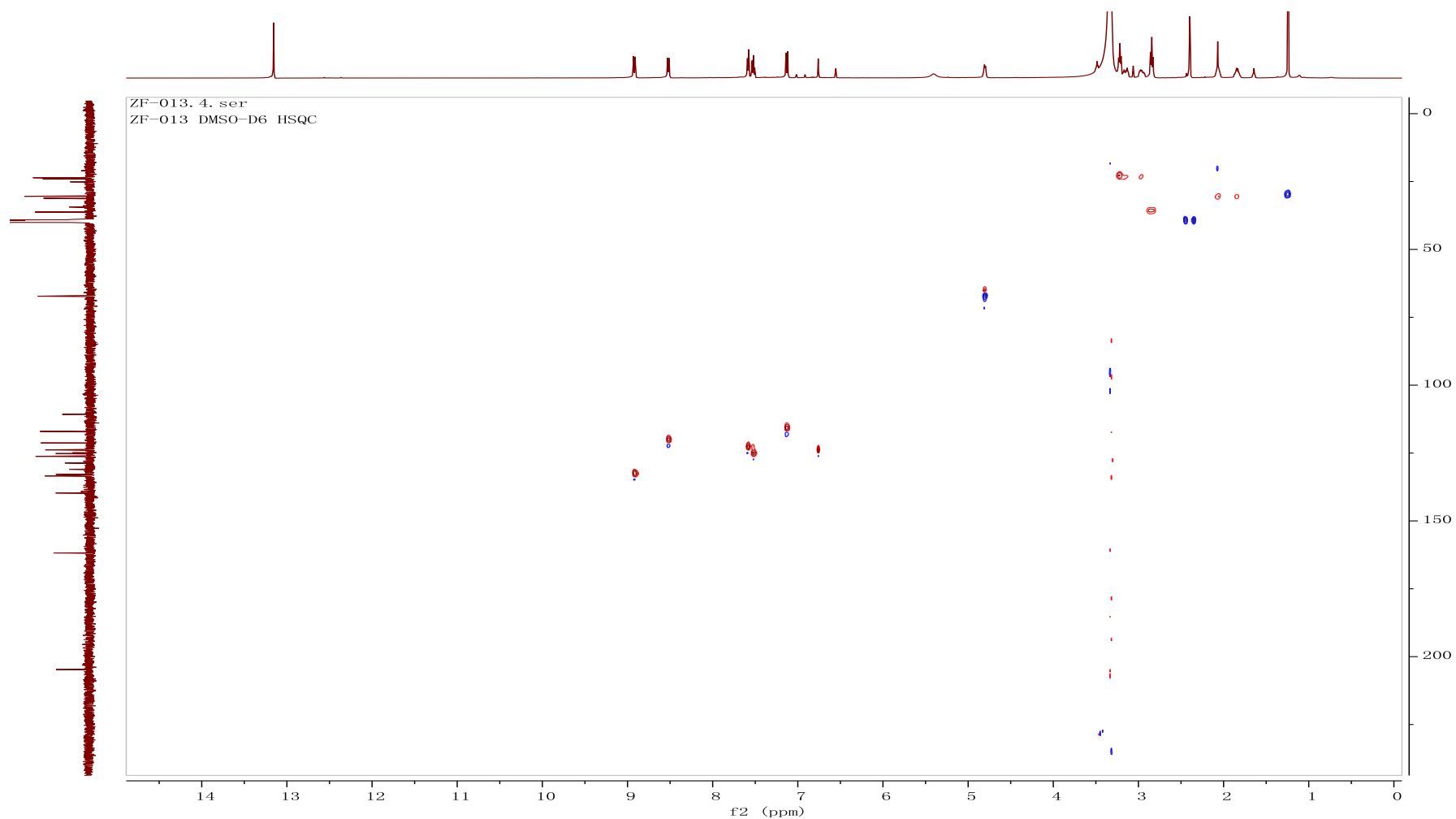


Figure S3. The HMQC Spectrum of Compound **1** in DMSO

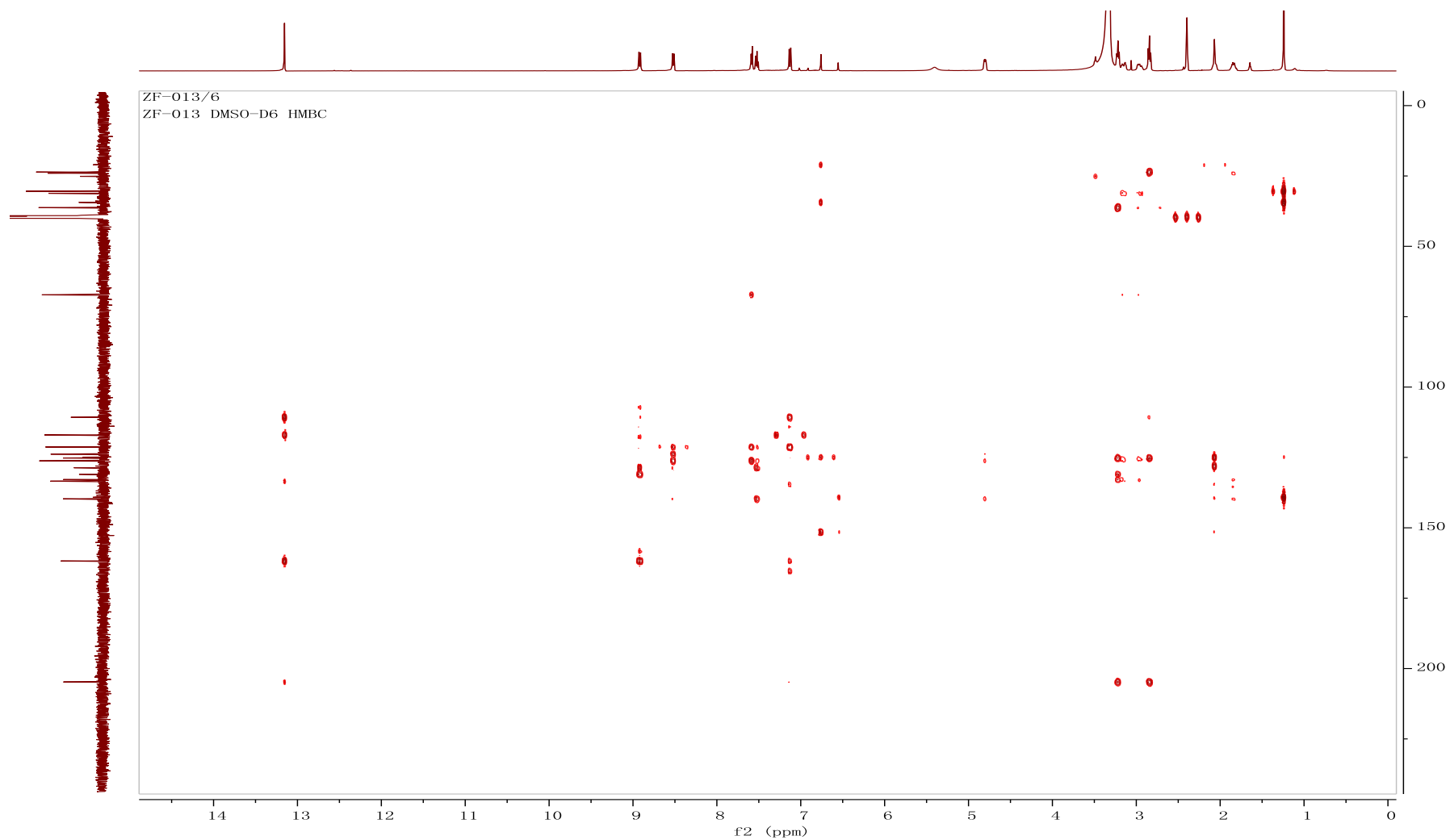


Figure S4. The HMBC Spectrum of Compound **1** in DMSO

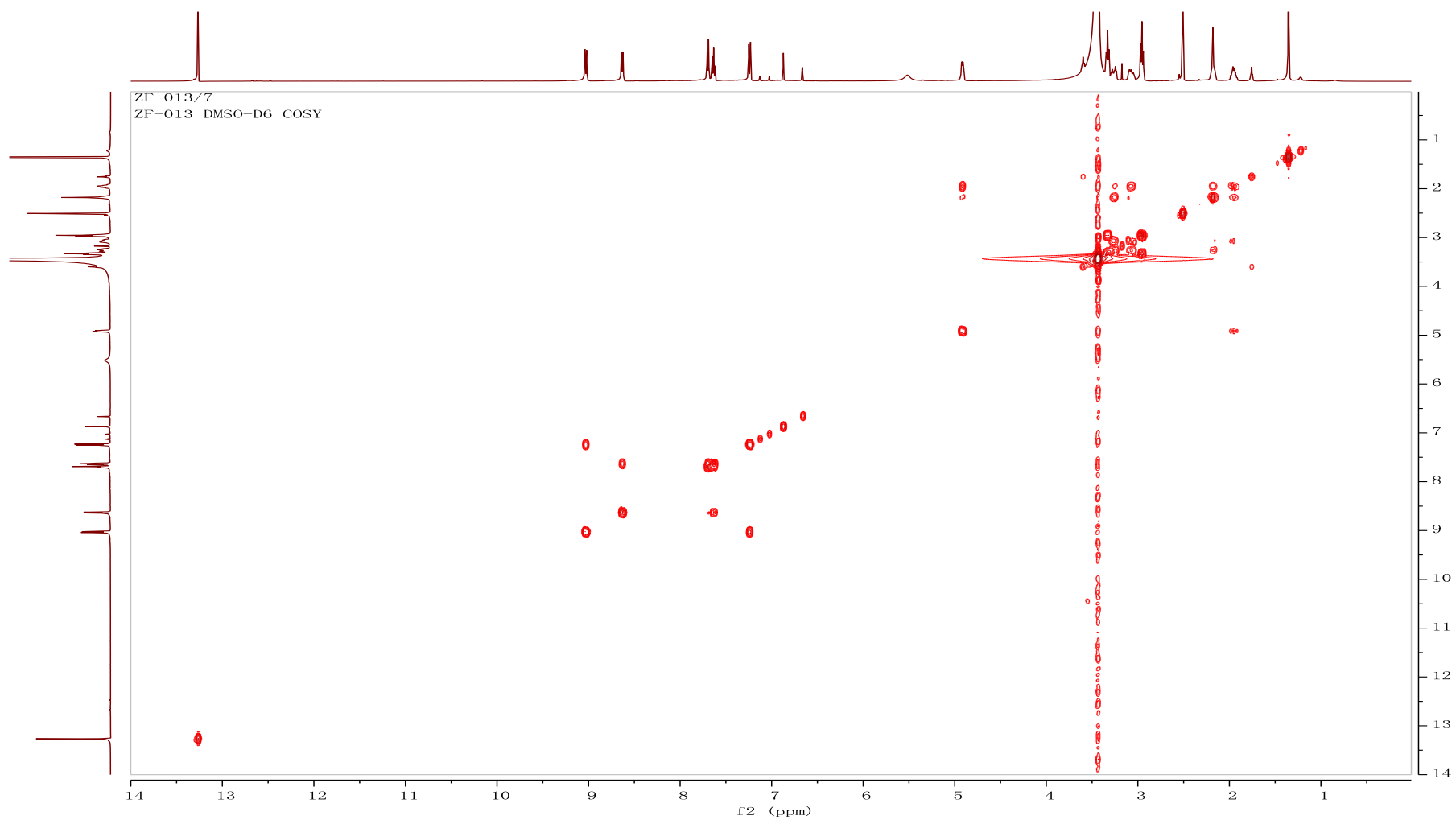


Figure S5. The COSY Spectrum of Compound **1** in DMSO

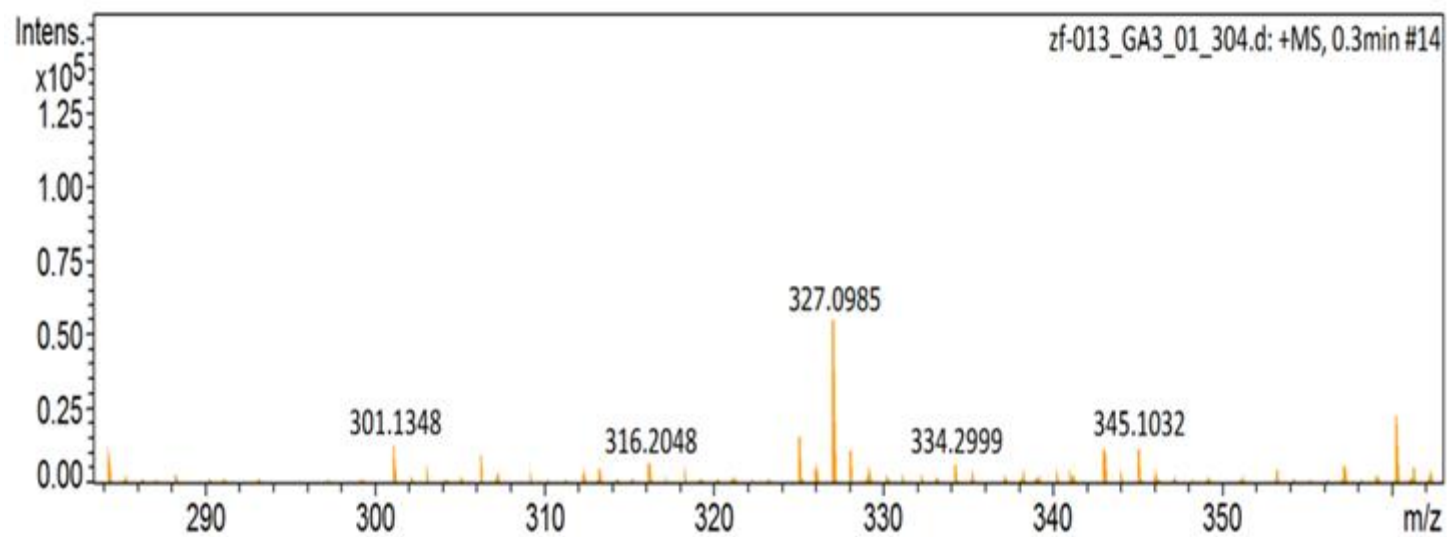


Figure S6. The HRESIMS Spectroscopic Data of Compound **1**

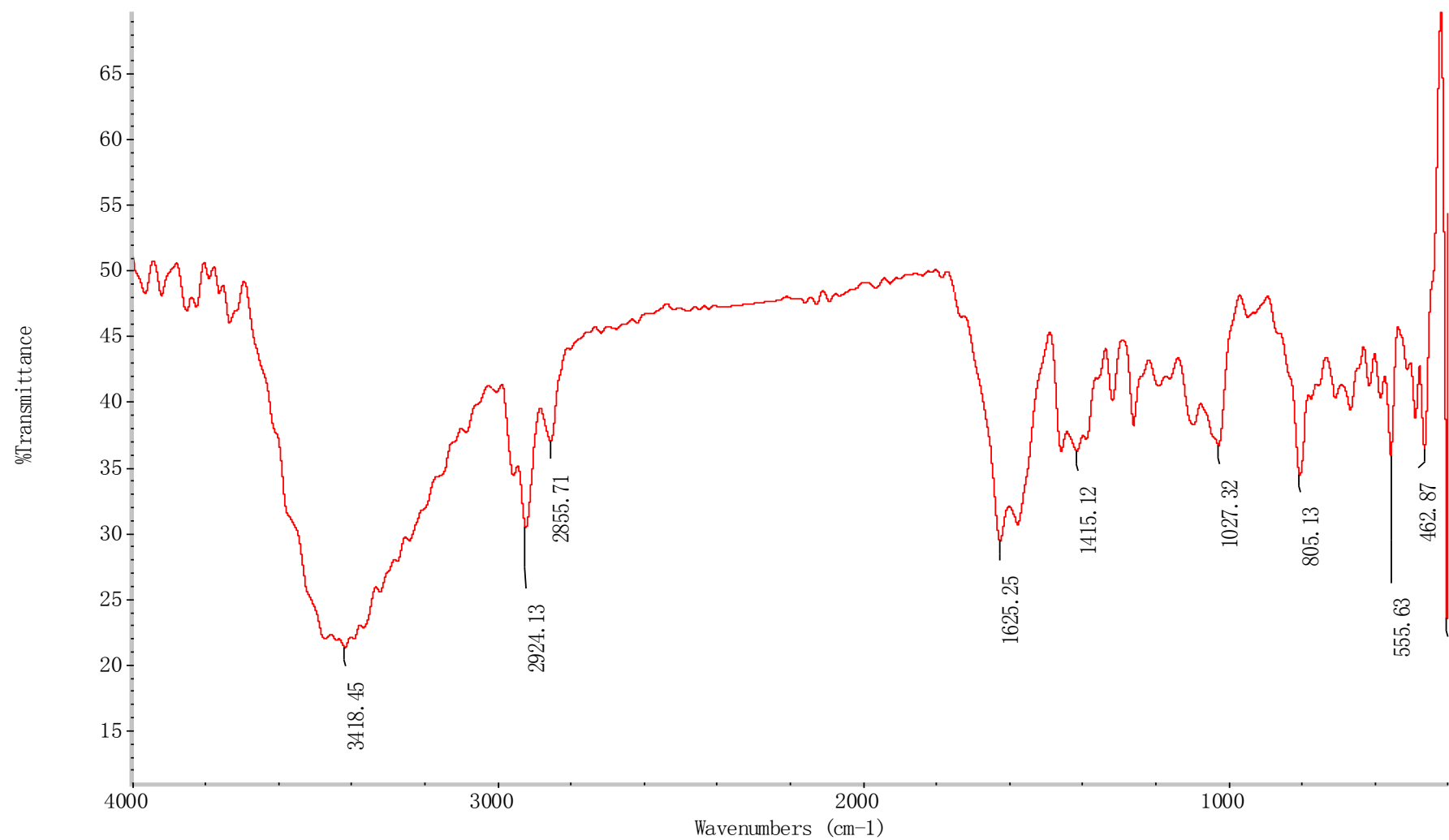


Figure S7. The IR Spectrum of Compound **1**

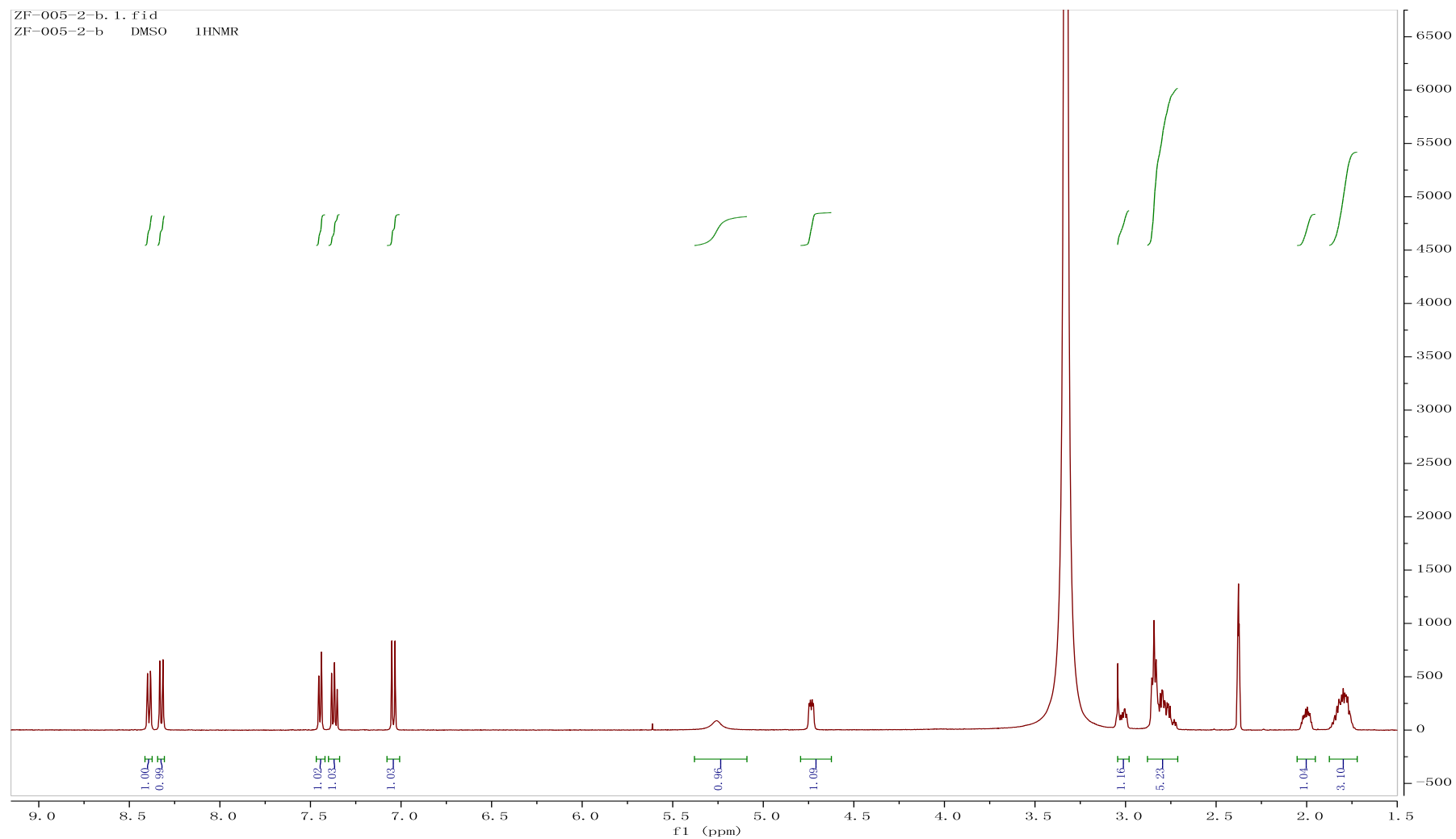


Figure S8. The ^1H NMR Spectrum of Compounds **2** and **3** in DMSO

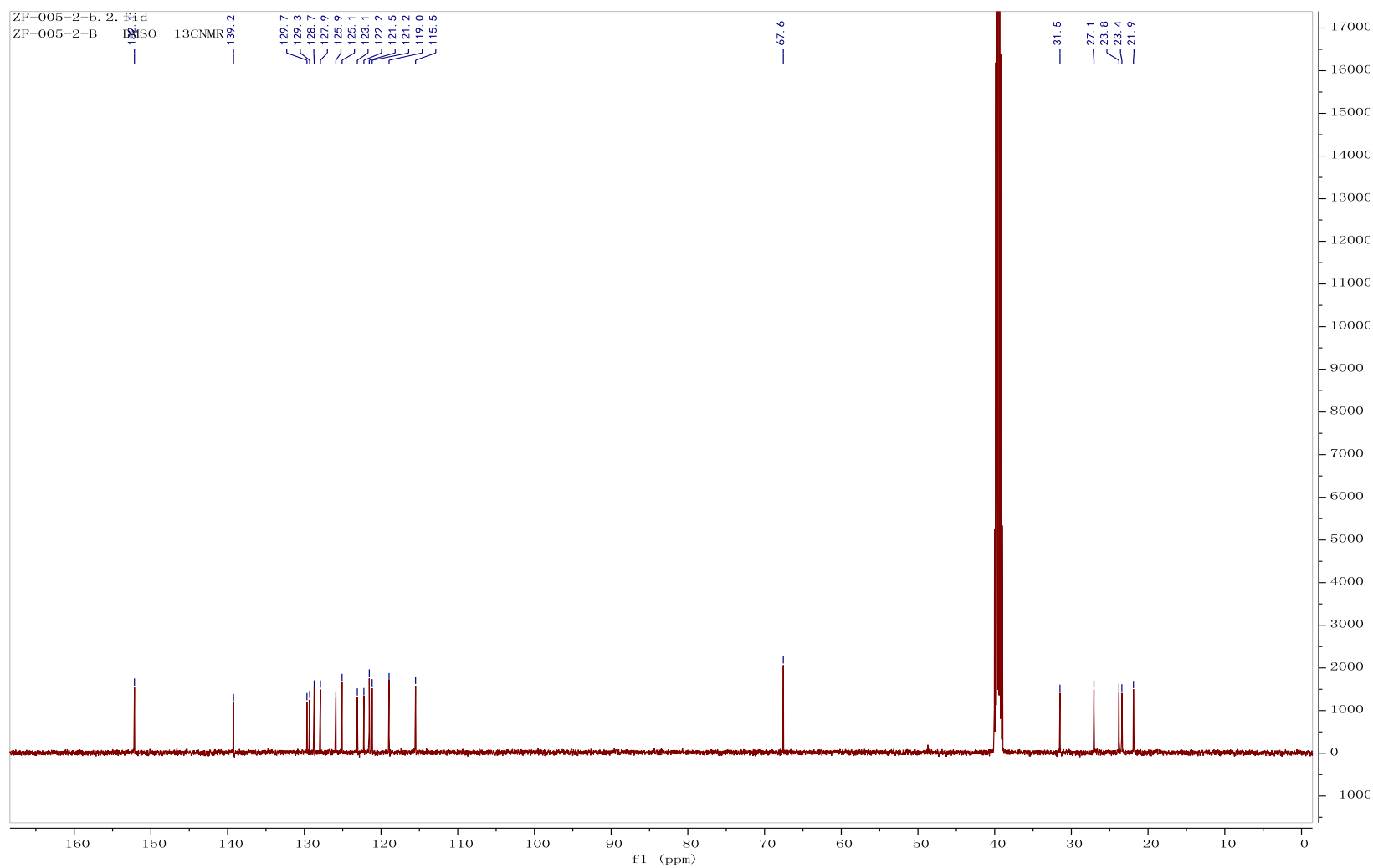


Figure S9. The ^{13}C NMR Spectrum of Compounds **2** and **3** in DMSO

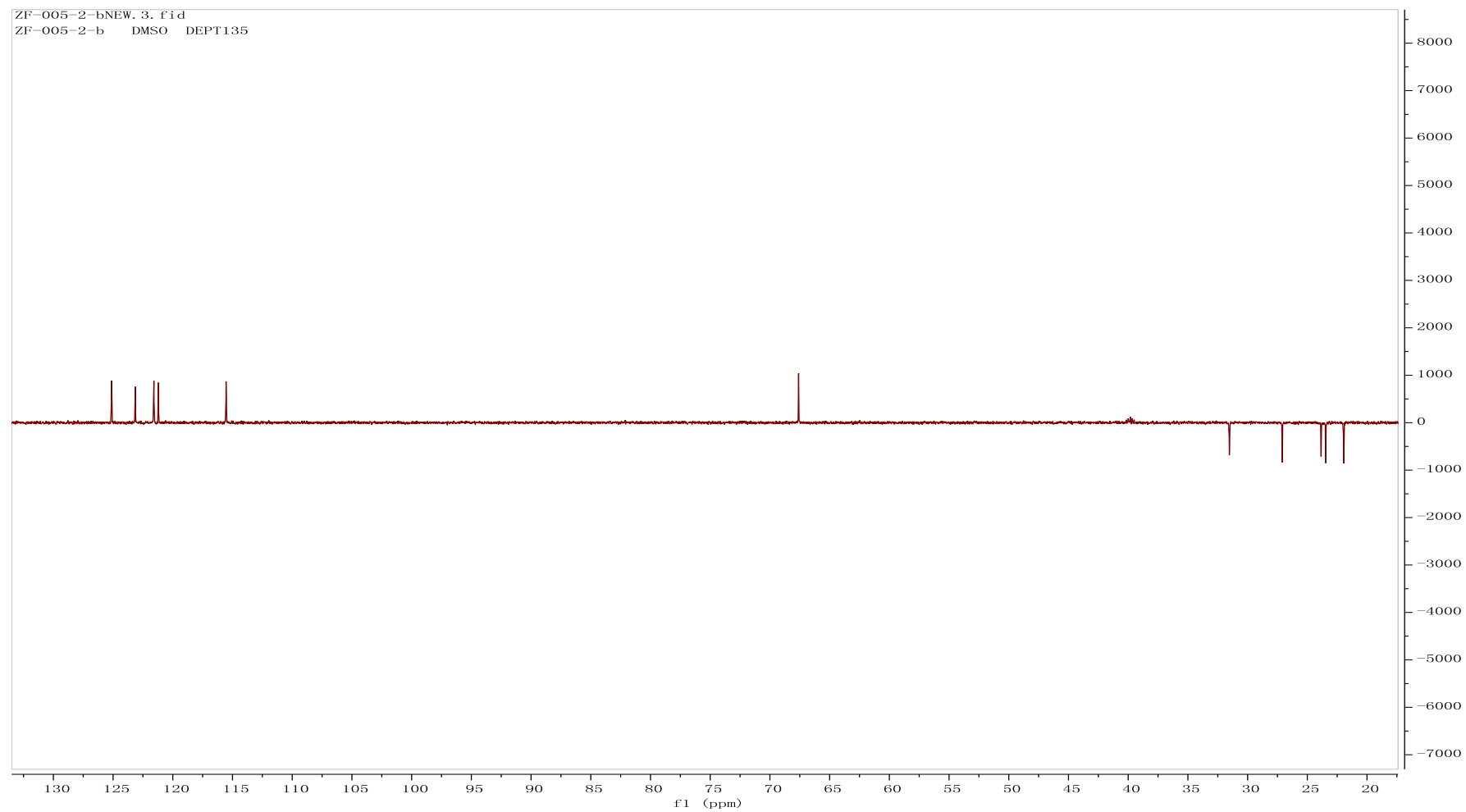


Figure S10. The DEPT Spectrum of Compounds **2** and **3** in DMSO

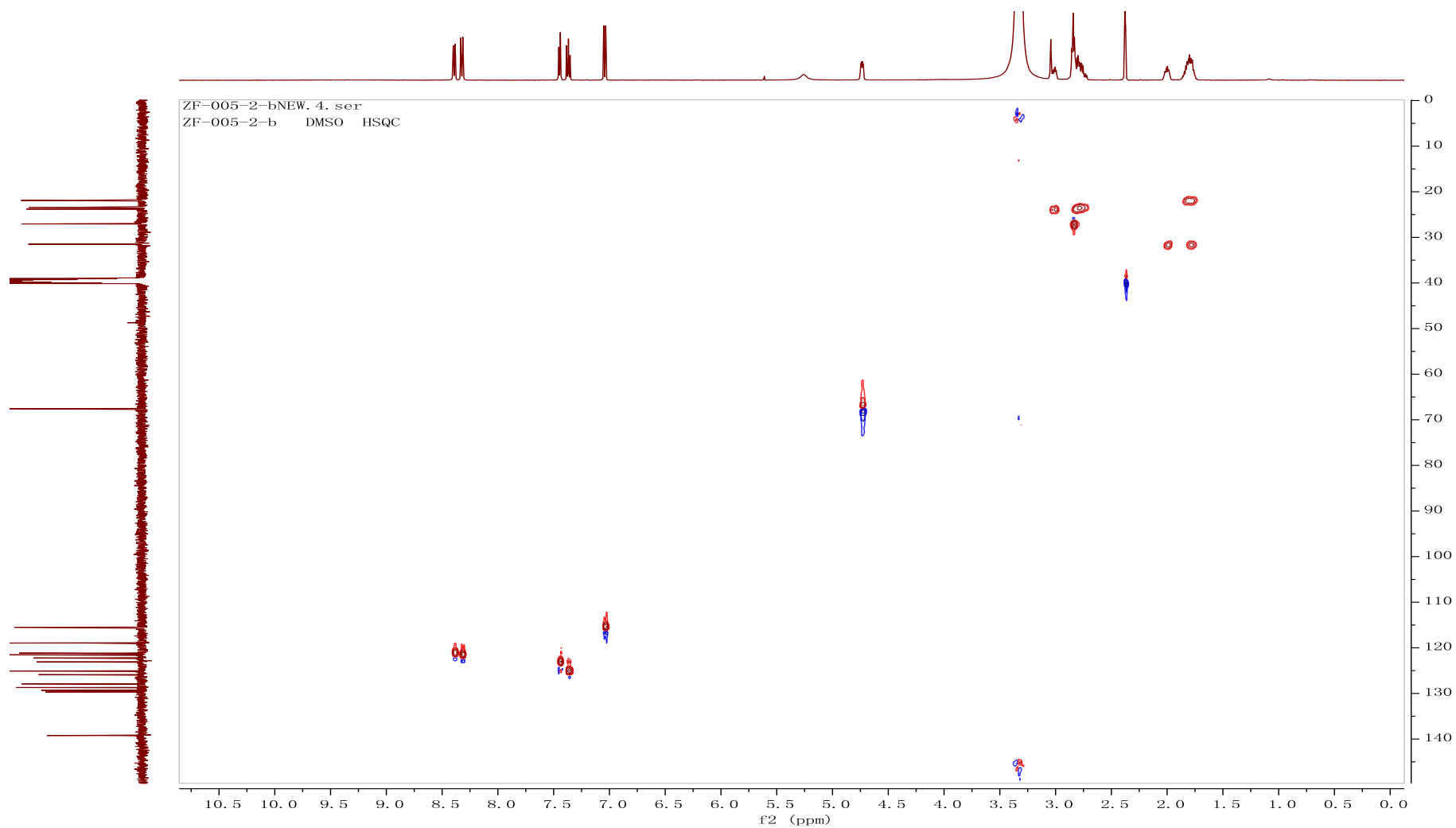


Figure S11. The HMQC Spectrum of Compounds **2** and **3** in DMSO

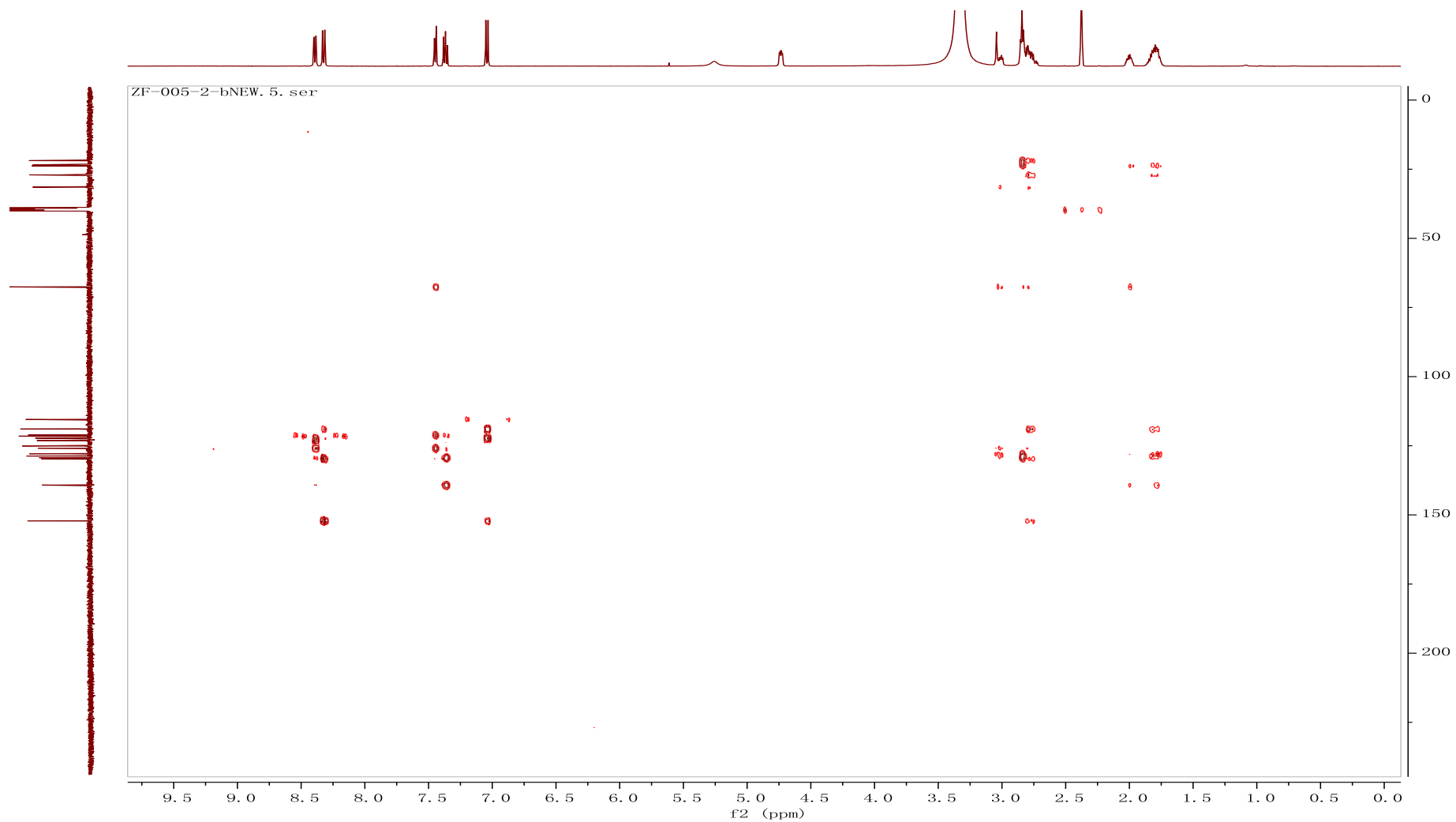


Figure S12. The HMBC Spectrum of Compounds **2** and **3** in DMSO

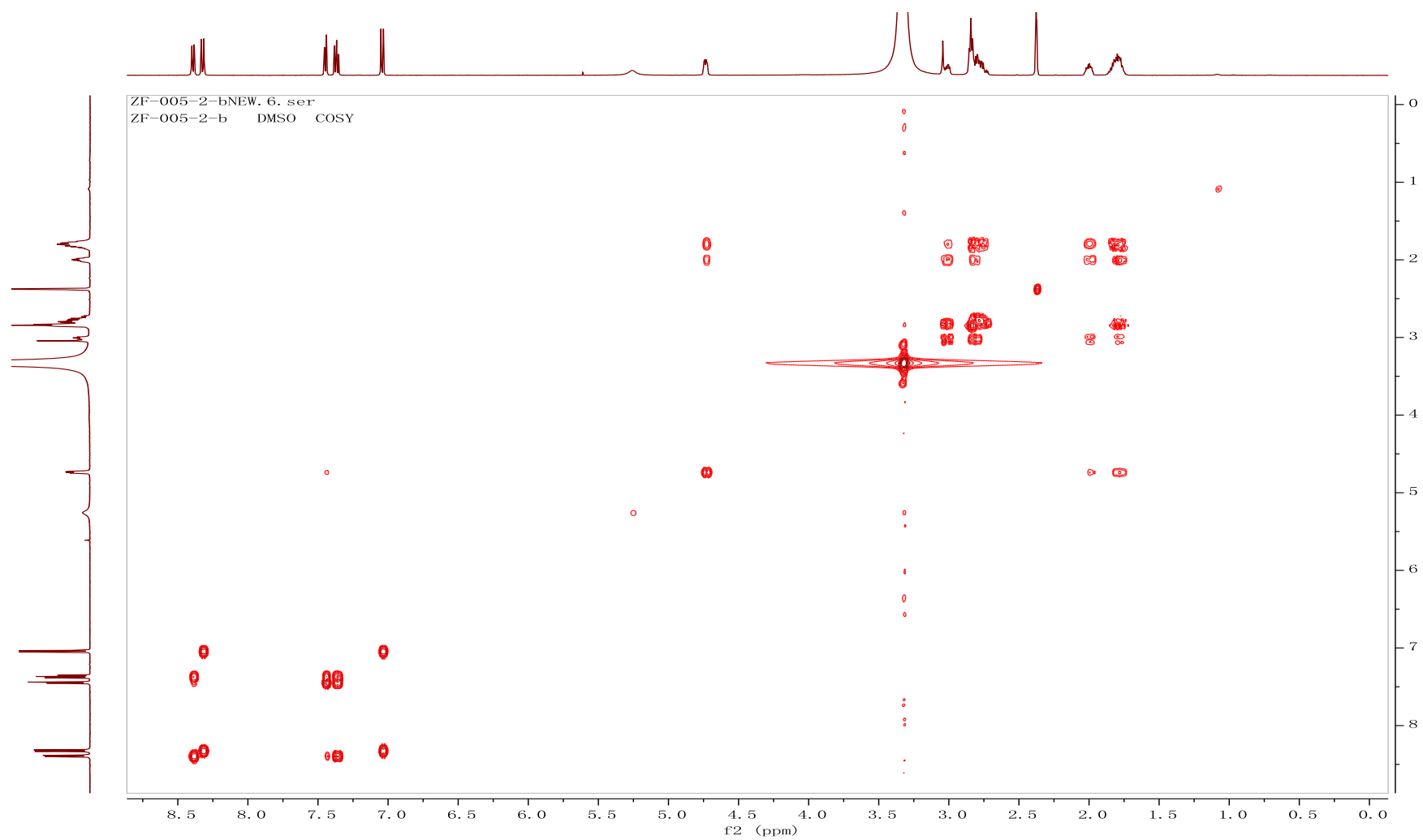


Figure S13. The COSY Spectrum of Compounds **2** and **3** in DMSO

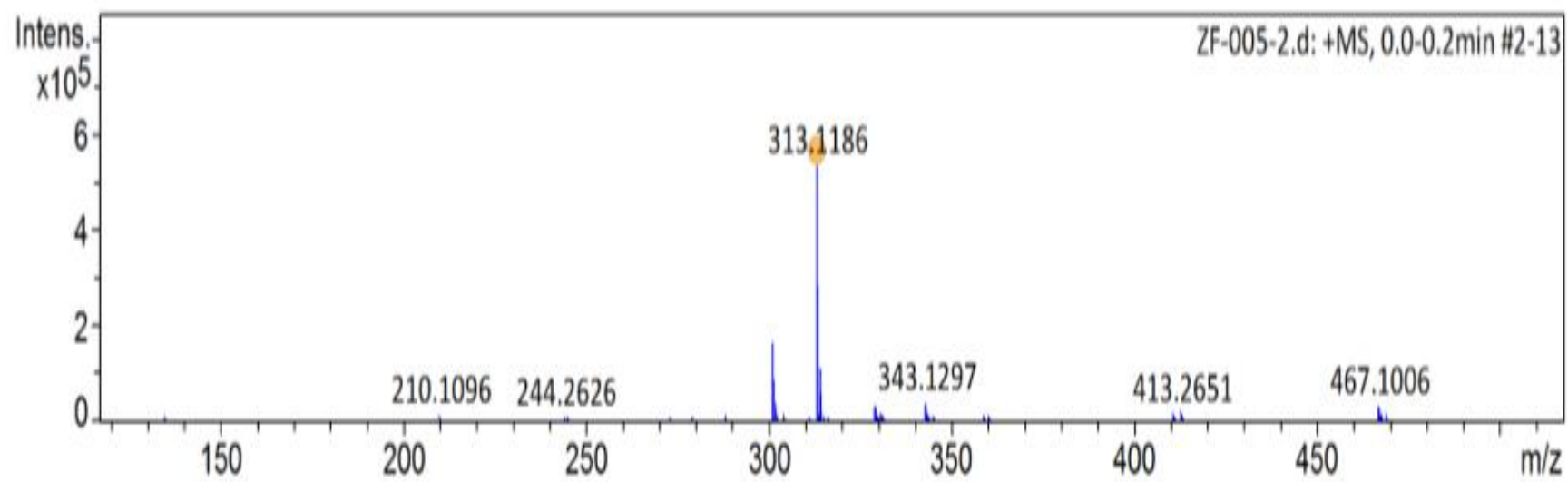


Figure S14. The HRESIMS Spectroscopic Data of Compounds **2** and **3**

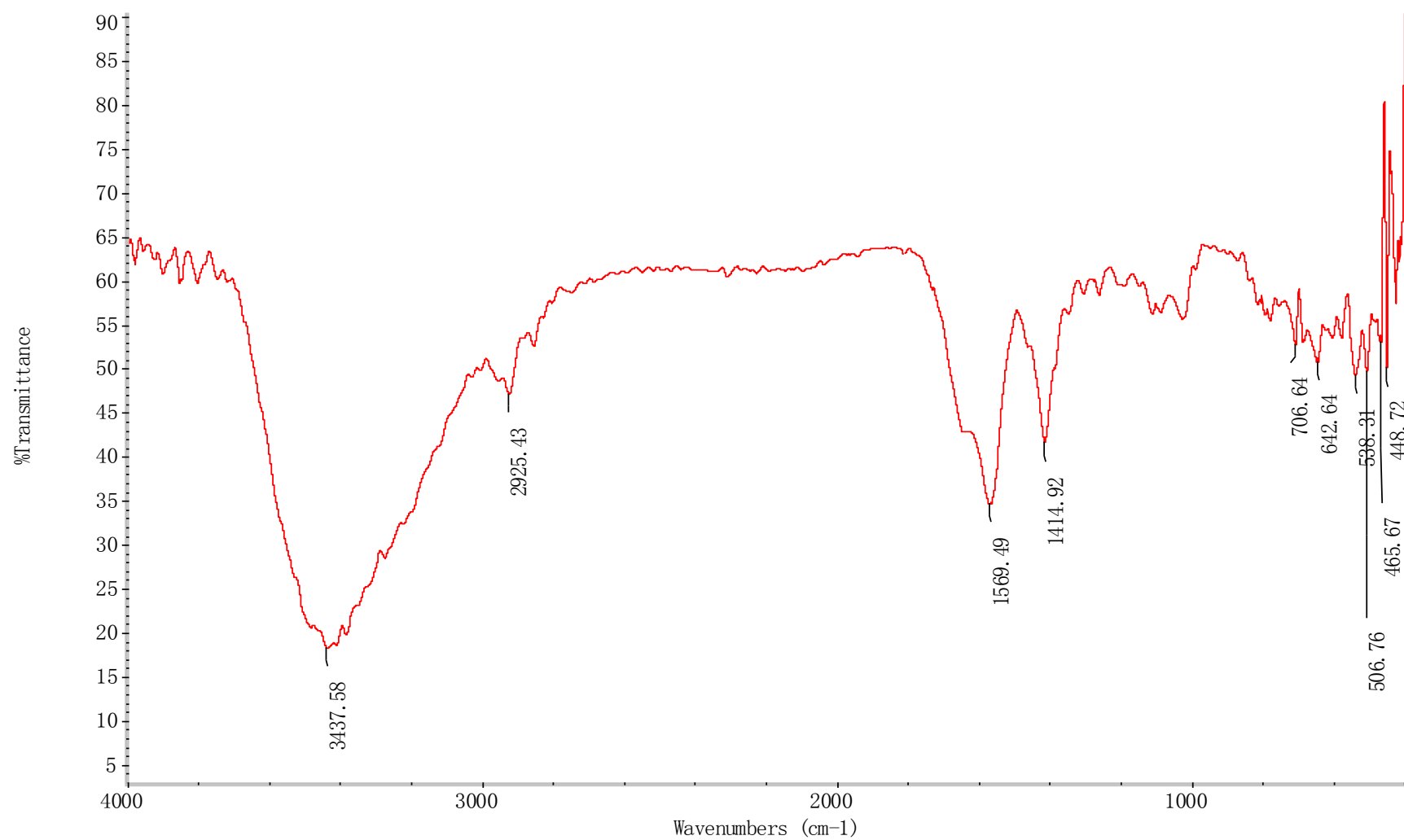


Figure S15. The IR Spectrum of Compounds **2** and **3**

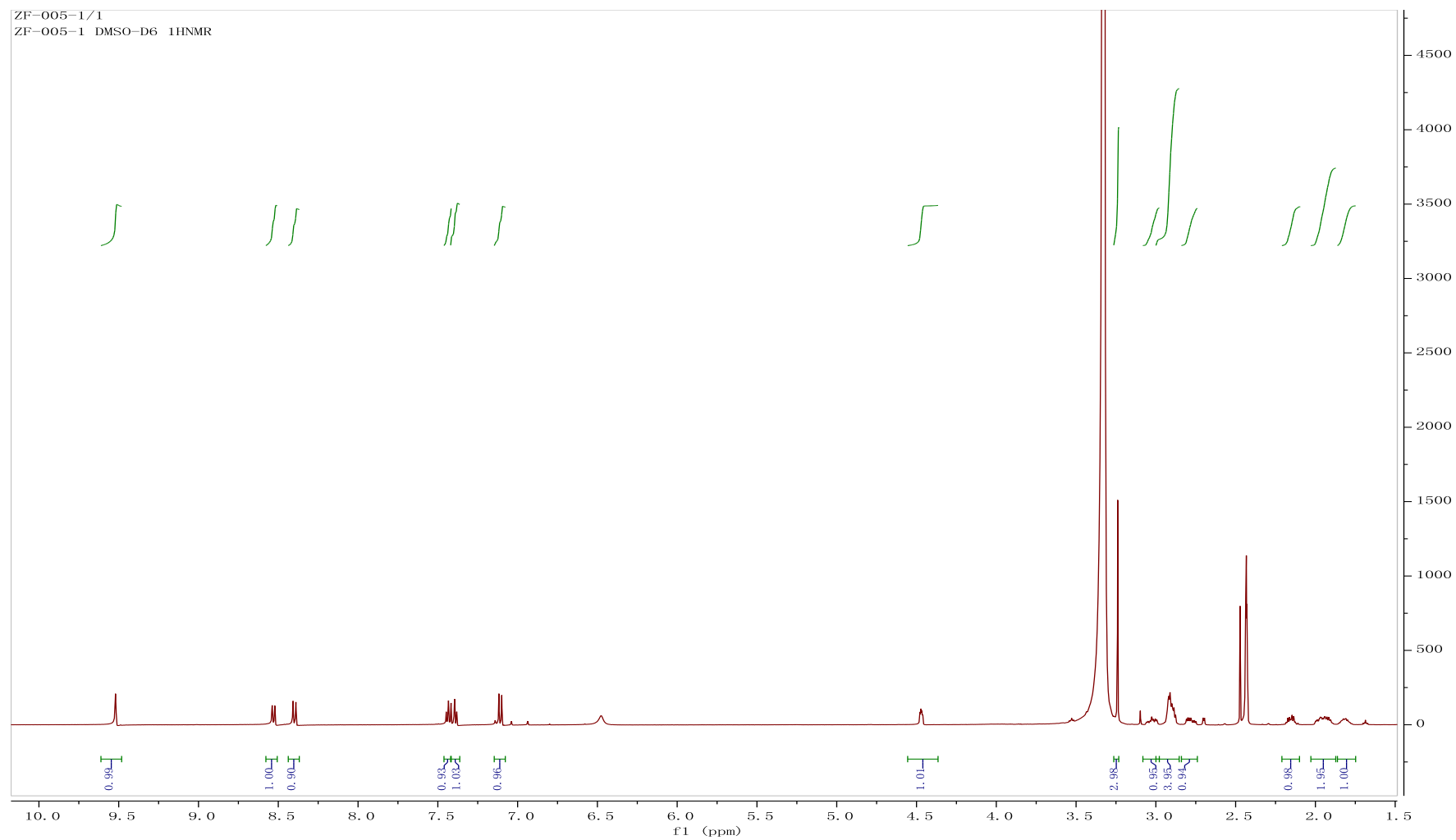


Figure S16. The ¹H NMR Spectrum of Compound **4** in DMSO

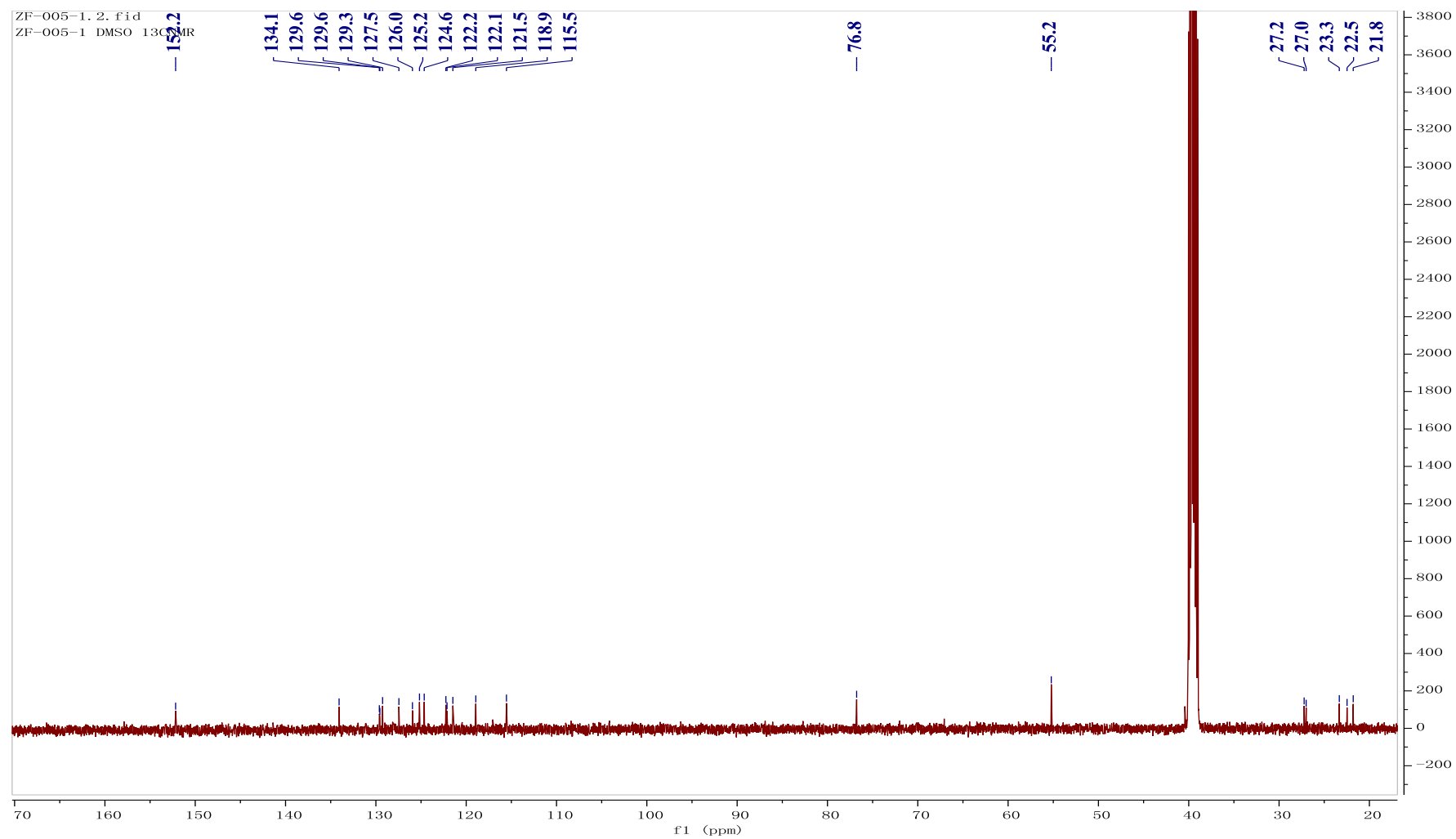


Figure S17. The ^{13}C NMR Spectrum of Compound 4 in DMSO

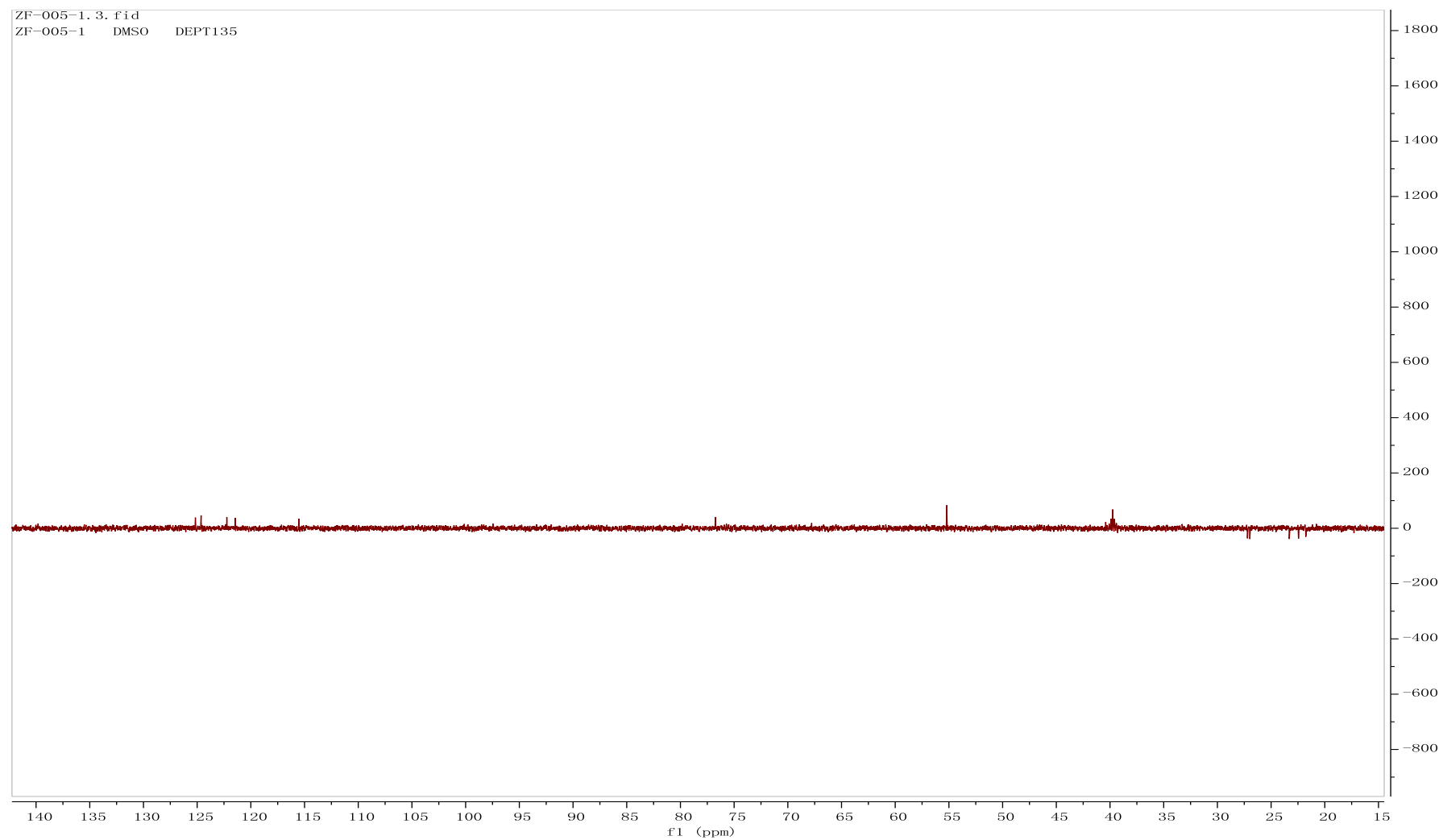


Figure S18. The DEPT Spectrum of Compound **4** in DMSO

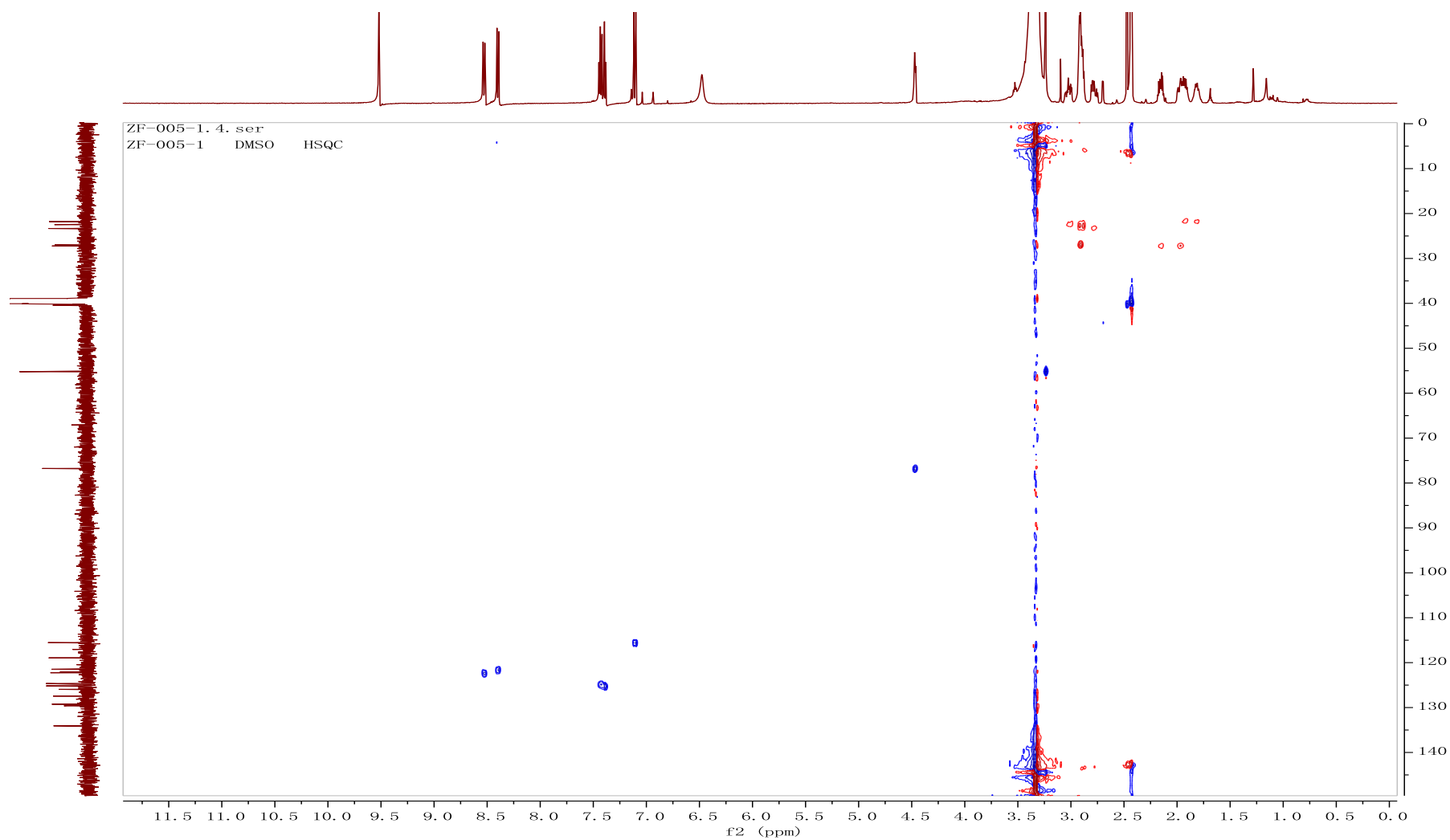


Figure S19. The HMQC Spectrum of Compound **4** in DMSO



Figure S20. The HMBC Spectrum of Compound **4** in DMSO

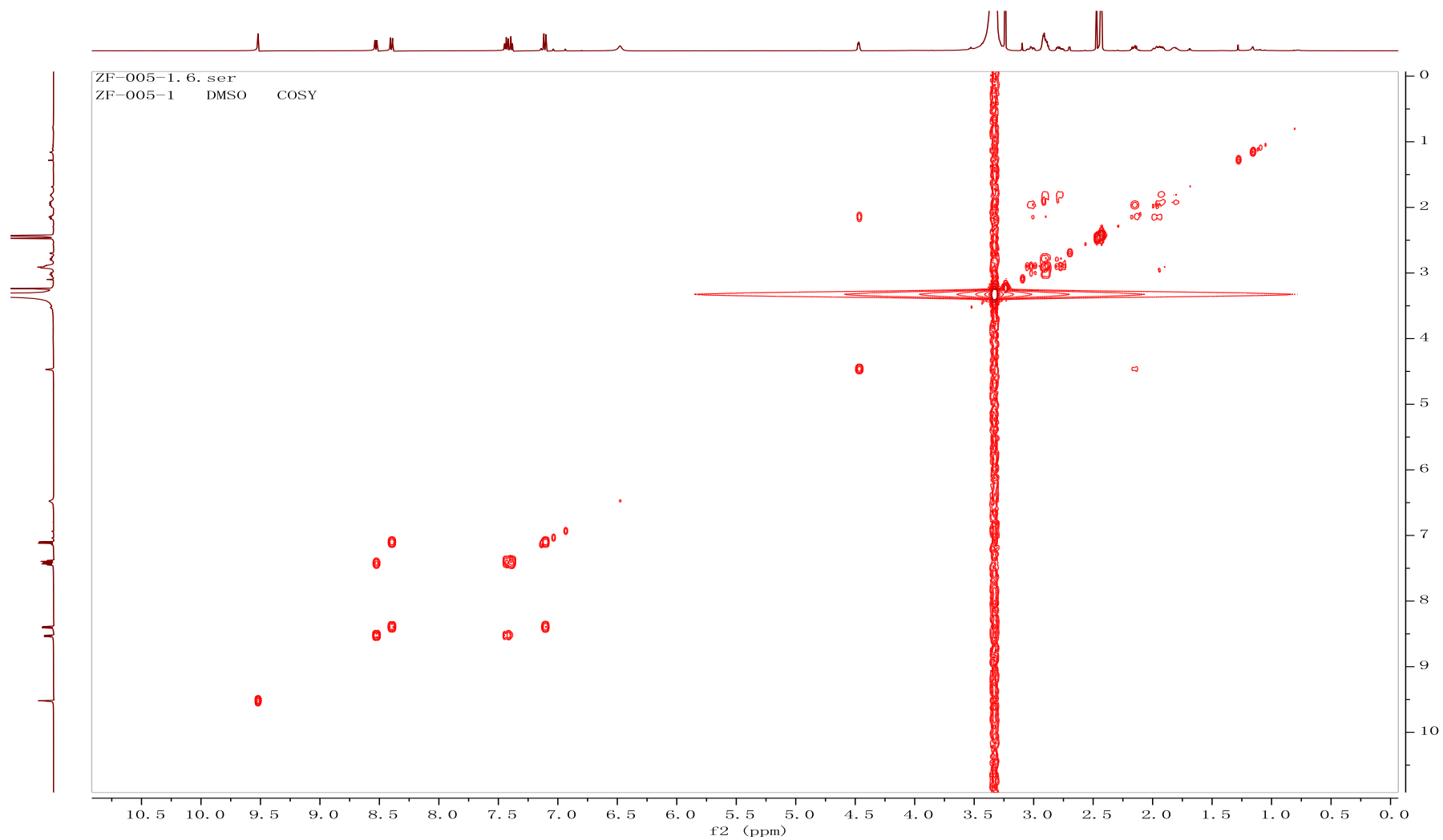


Figure S21. The COSY Spectrum of Compound **4** in DMSO

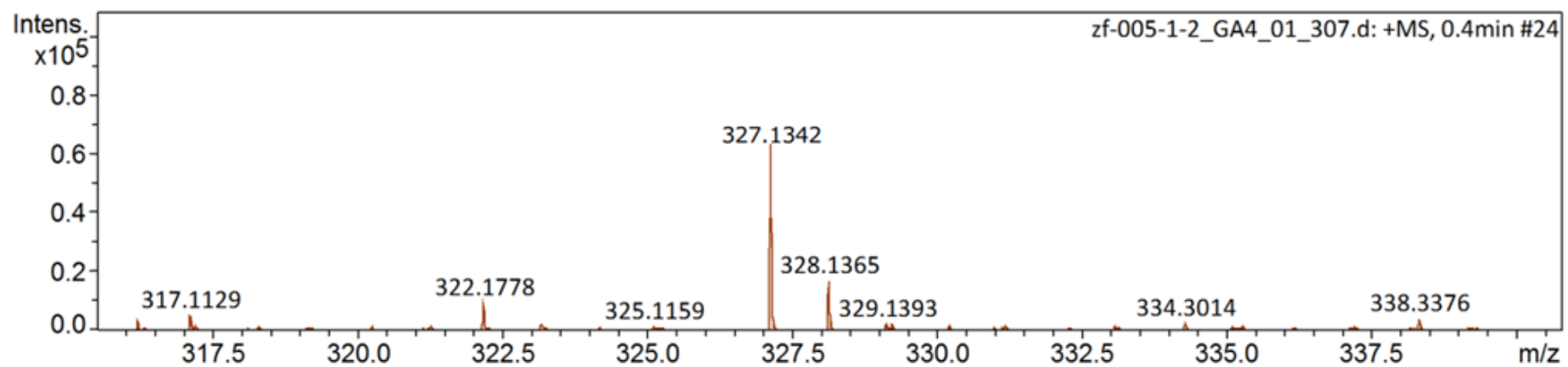


Figure S22. The HRESIMS Spectroscopic Data of Compound **4**

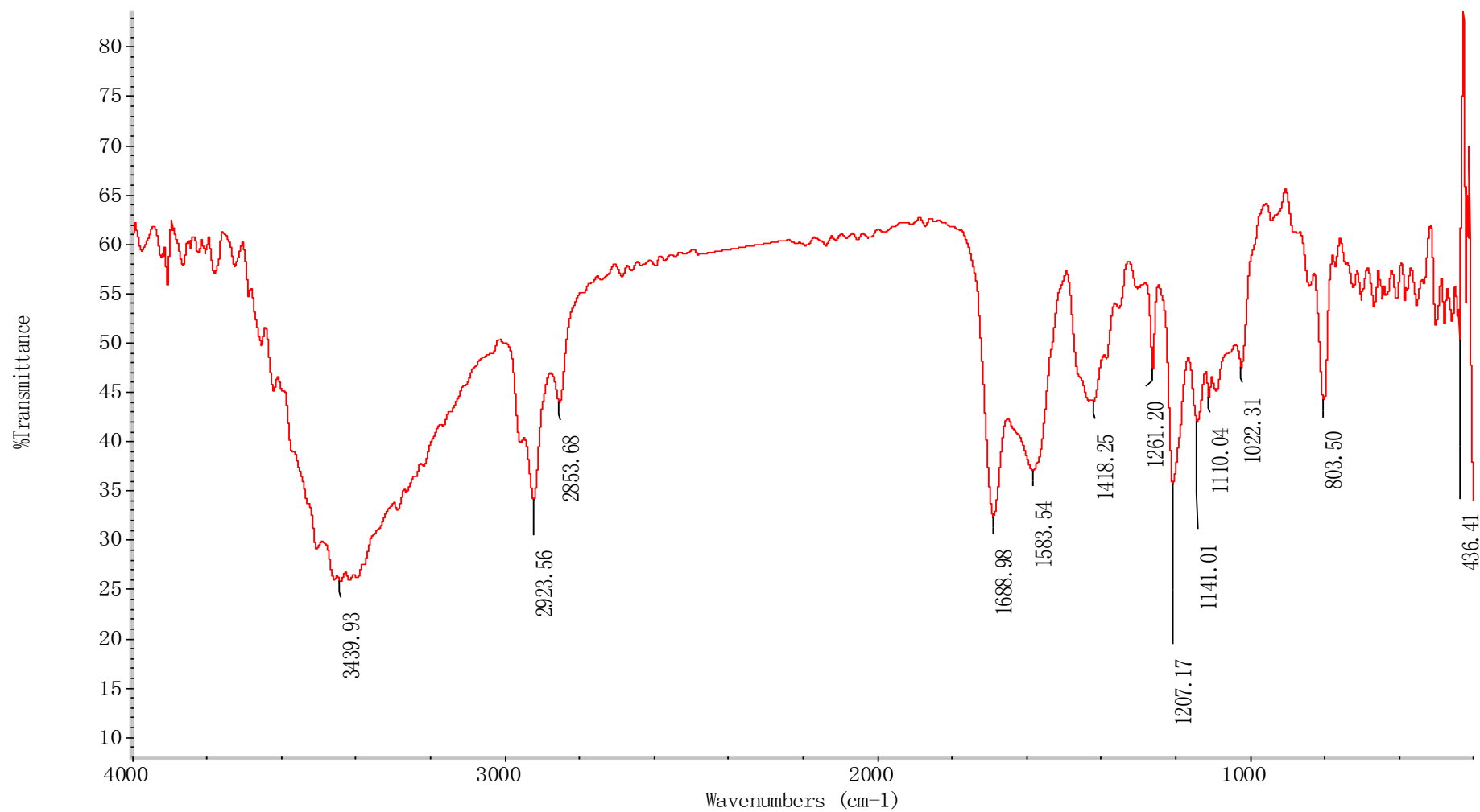


Figure S23. The IR Spectrum of Compound 4

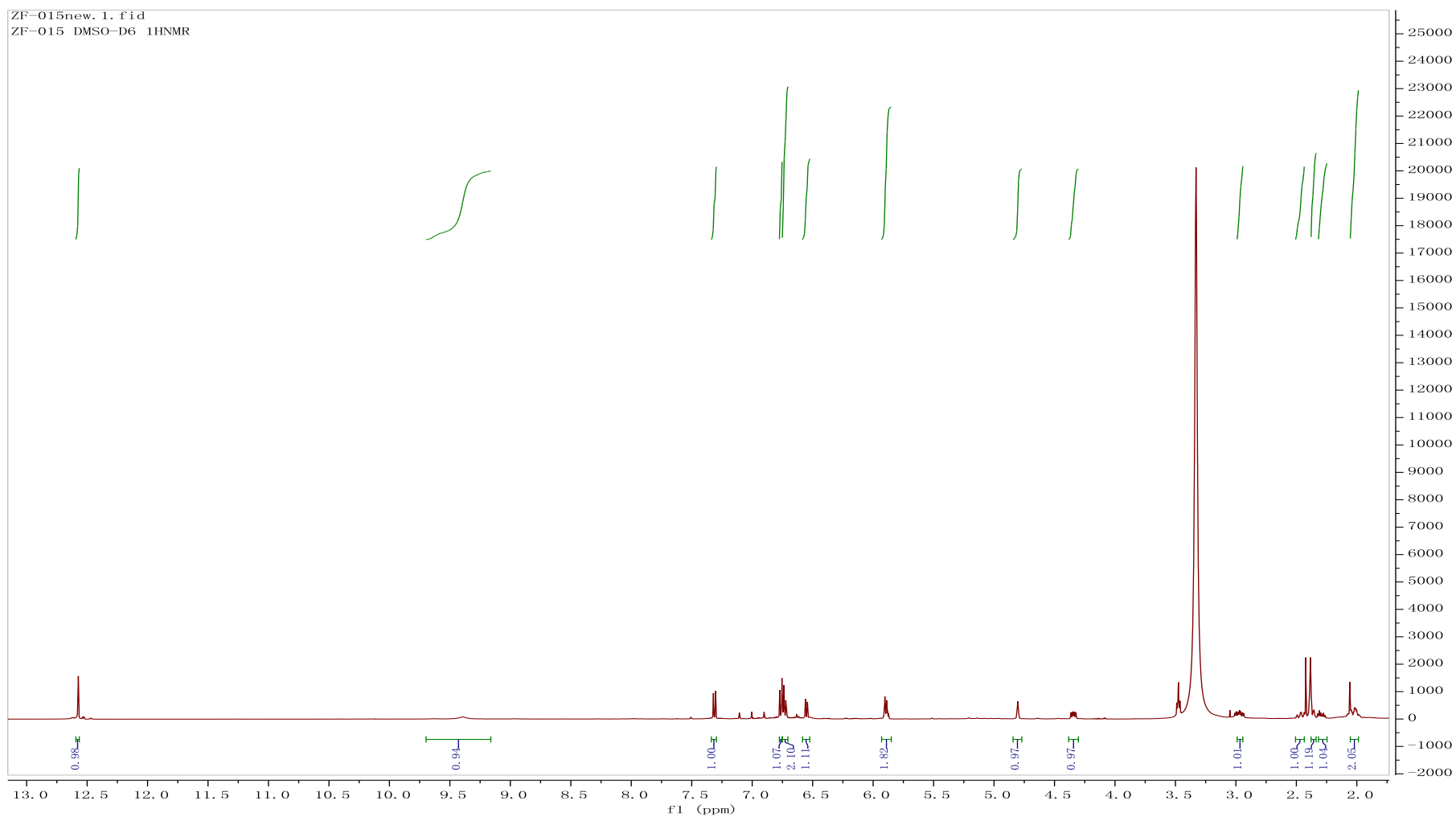


Figure S24. The ^1H NMR Spectrum of Compound **5** in DMSO

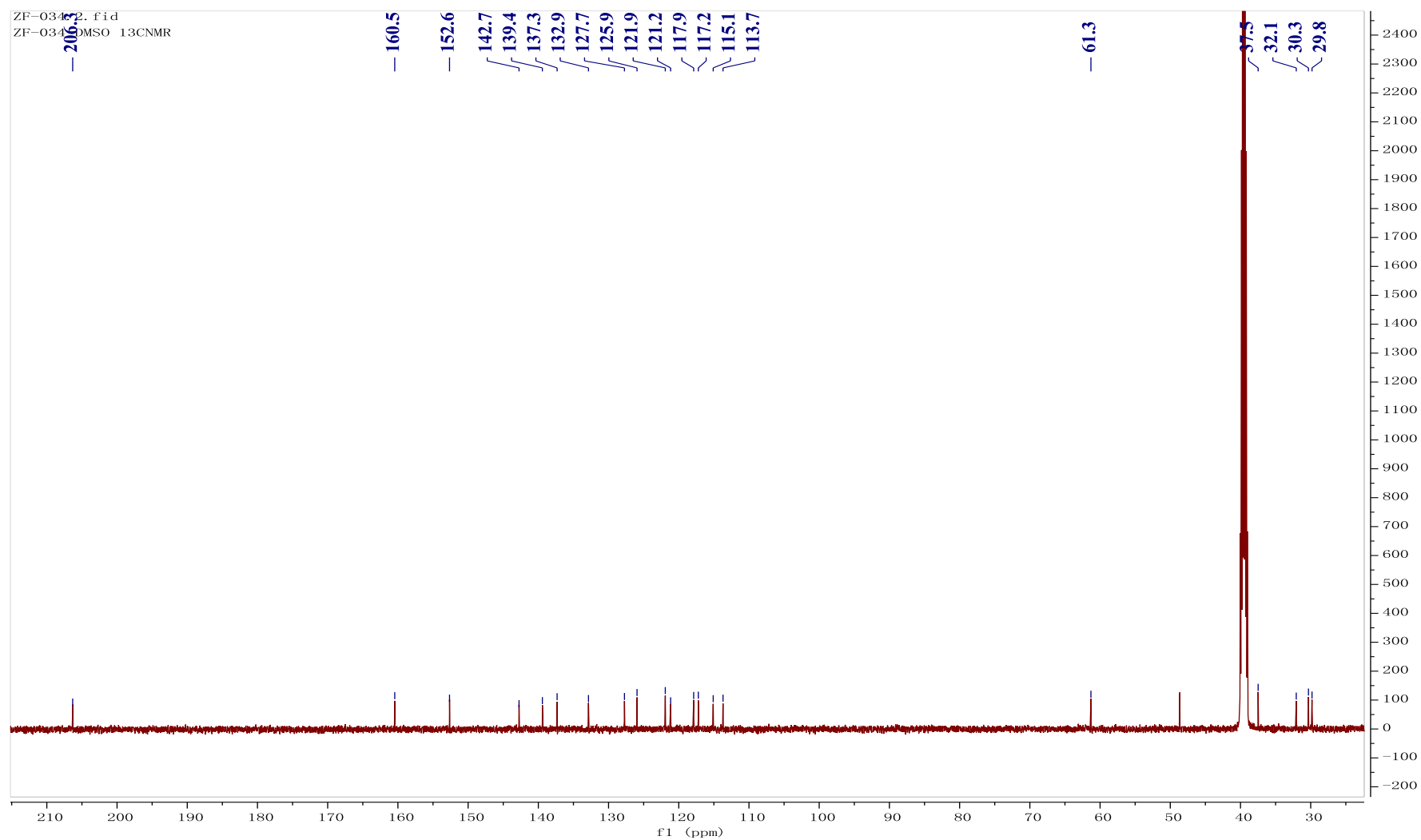


Figure S25. The ^{13}C NMR Spectrum of Compound **5** in DMSO

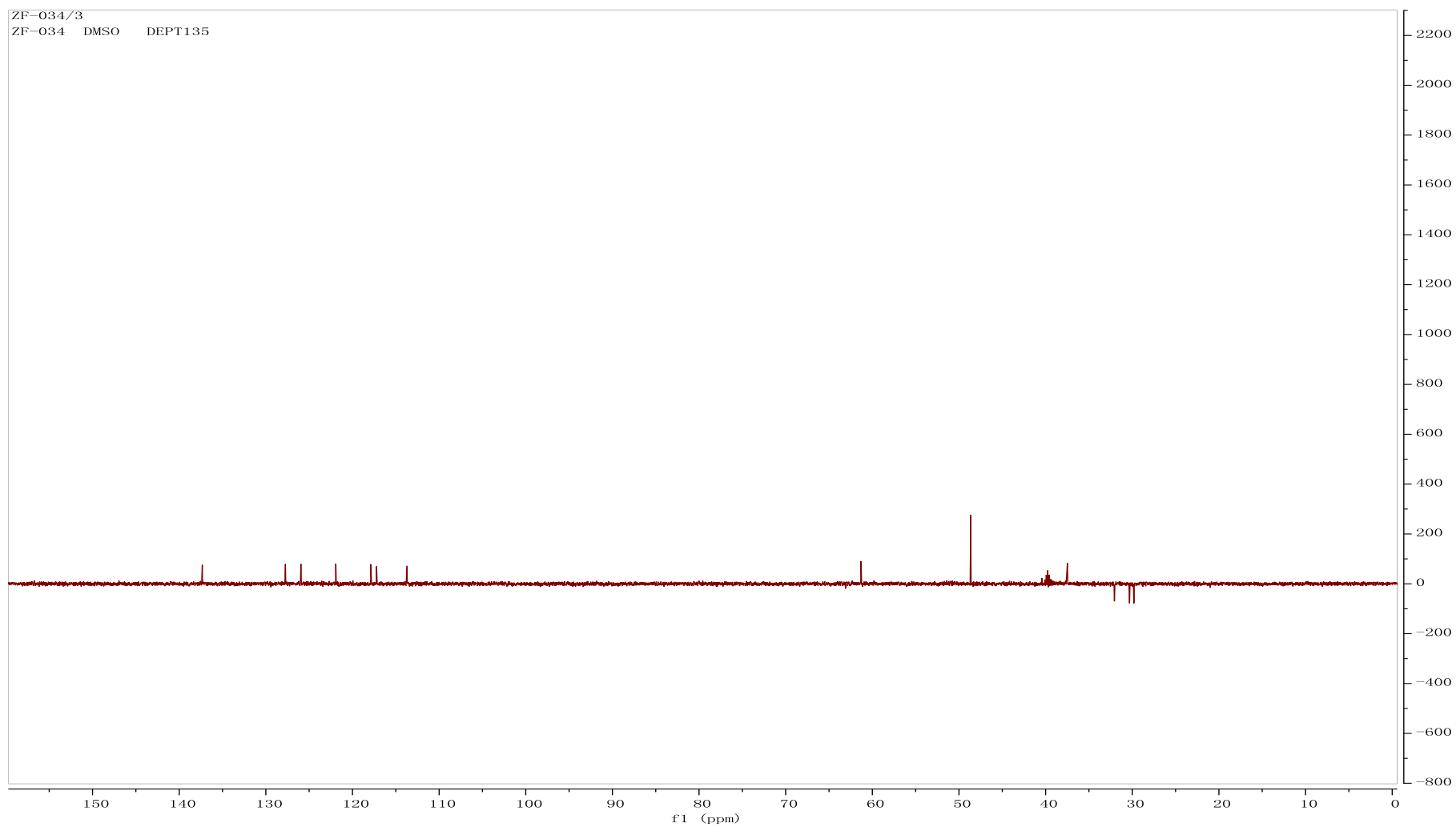


Figure S26. The DEPT Spectrum of Compound **5** in DMSO



29

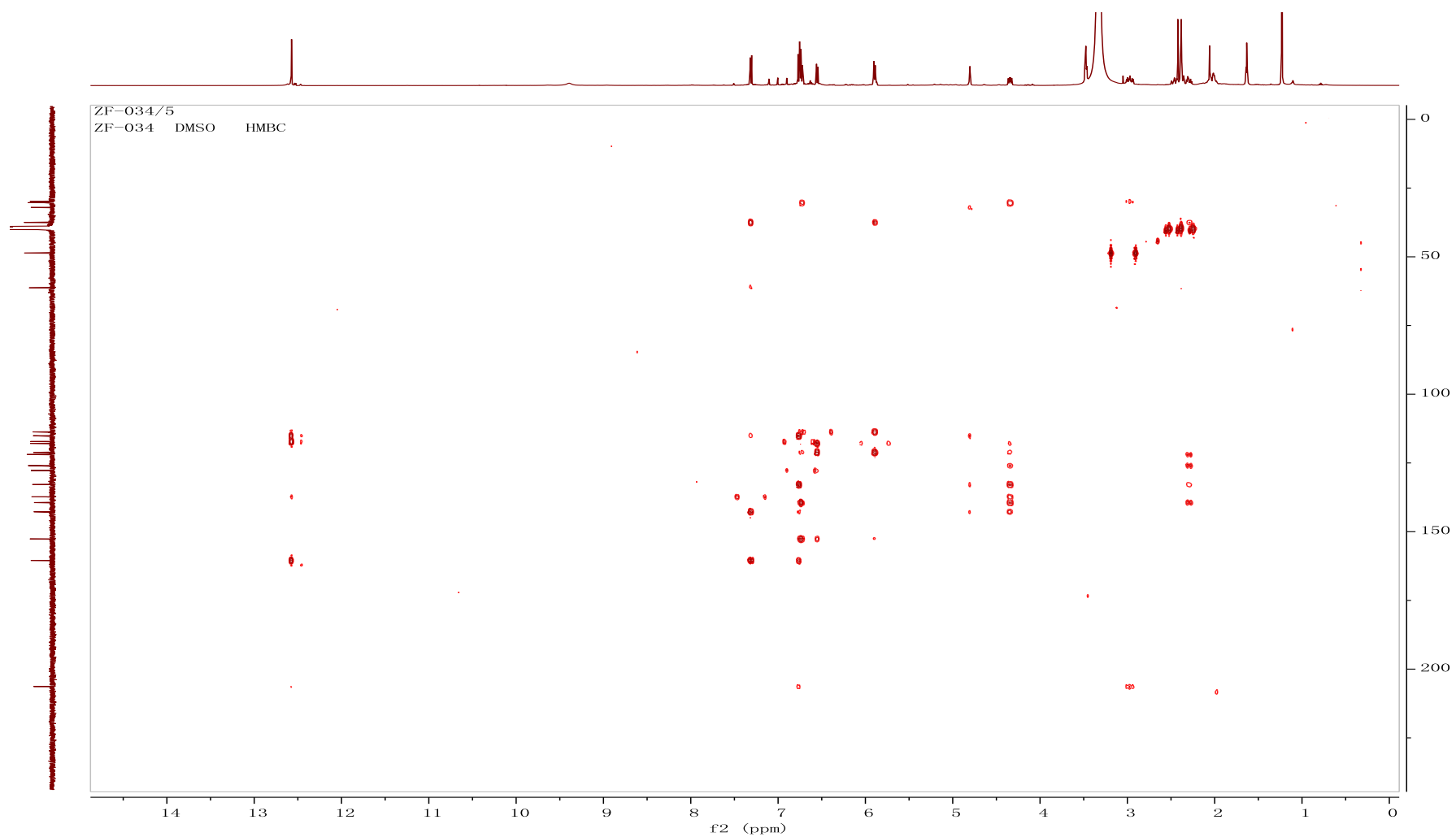


Figure S28. The HMBC Spectrum of Compound 5 in DMSO

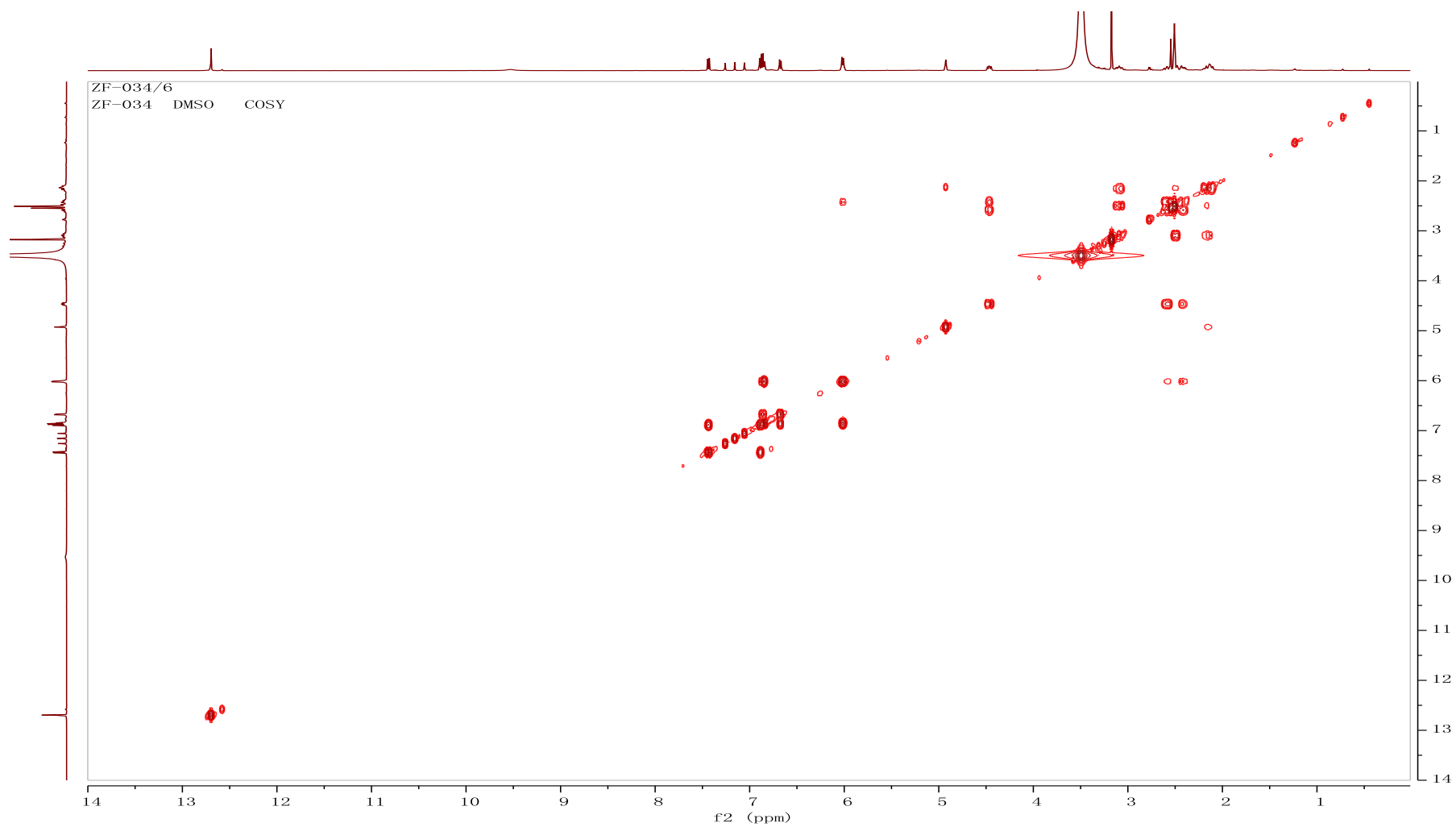


Figure S29. The COSY Spectrum of Compound **5** in DMSO

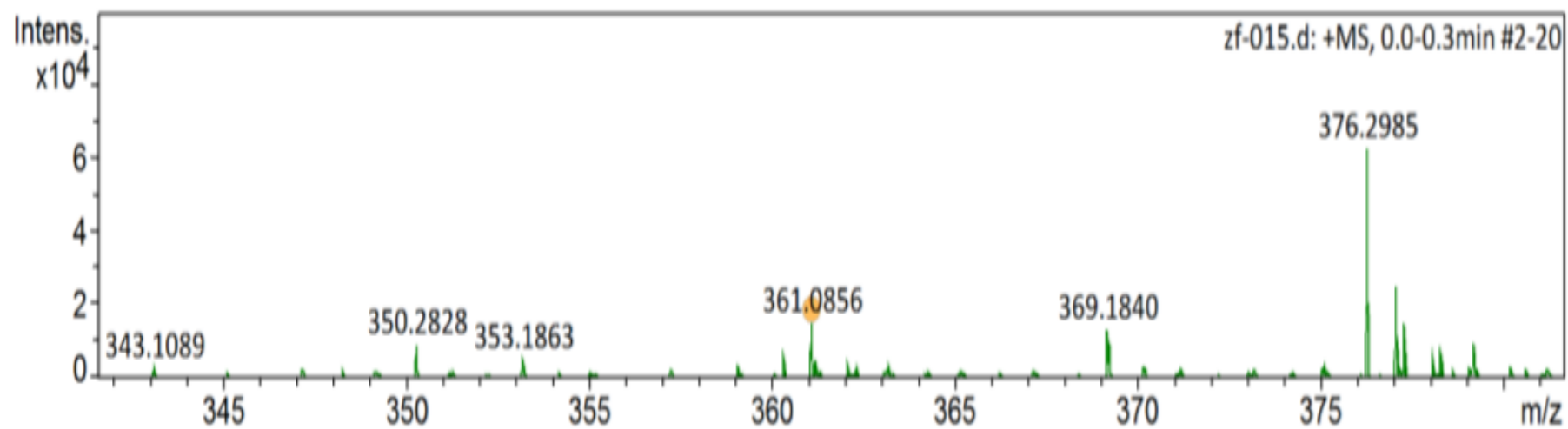


Figure S30. The HRESIMS Spectroscopic Data of Compound 5

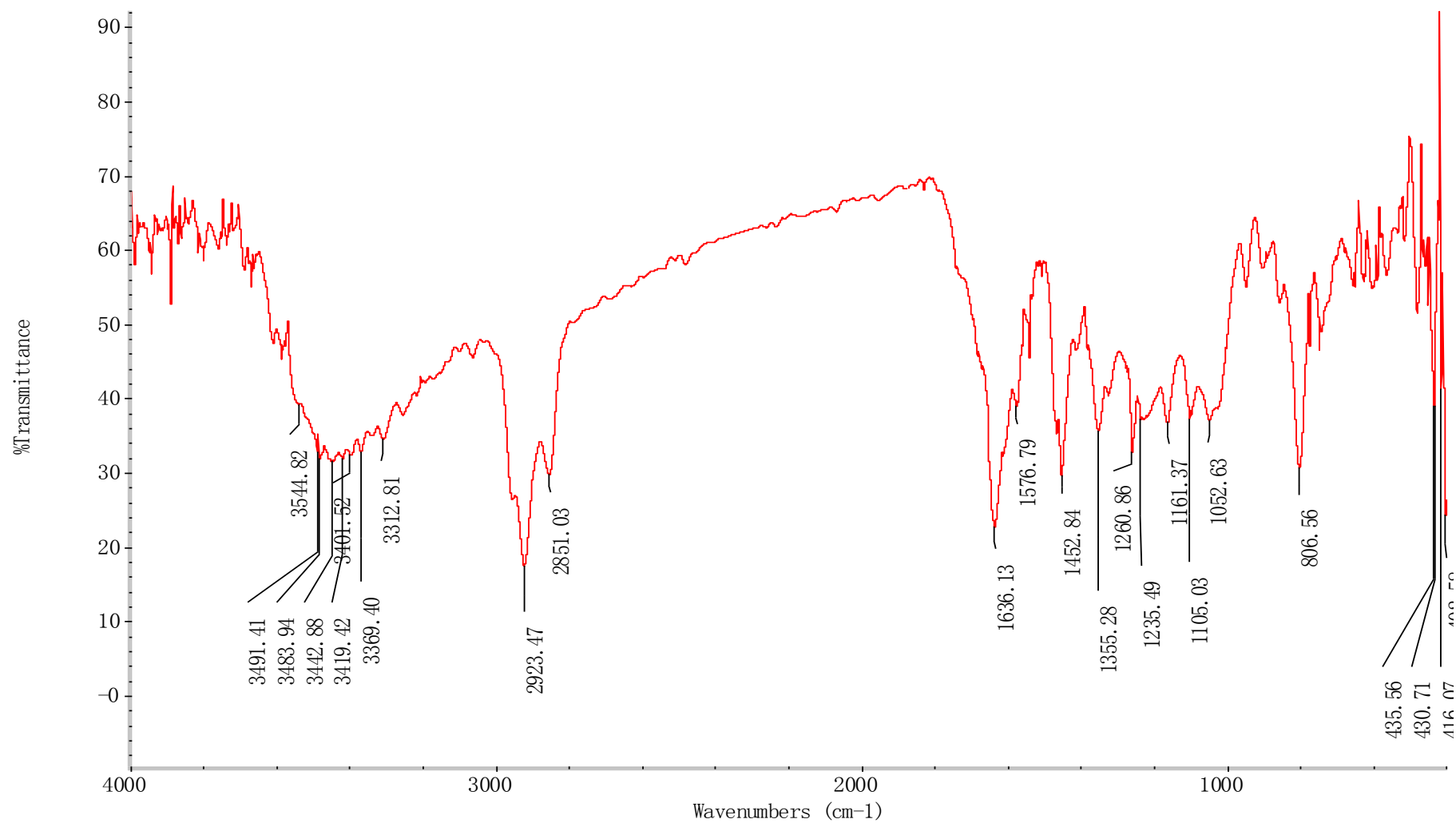


Figure S31. The IR Spectrum of Compound 5

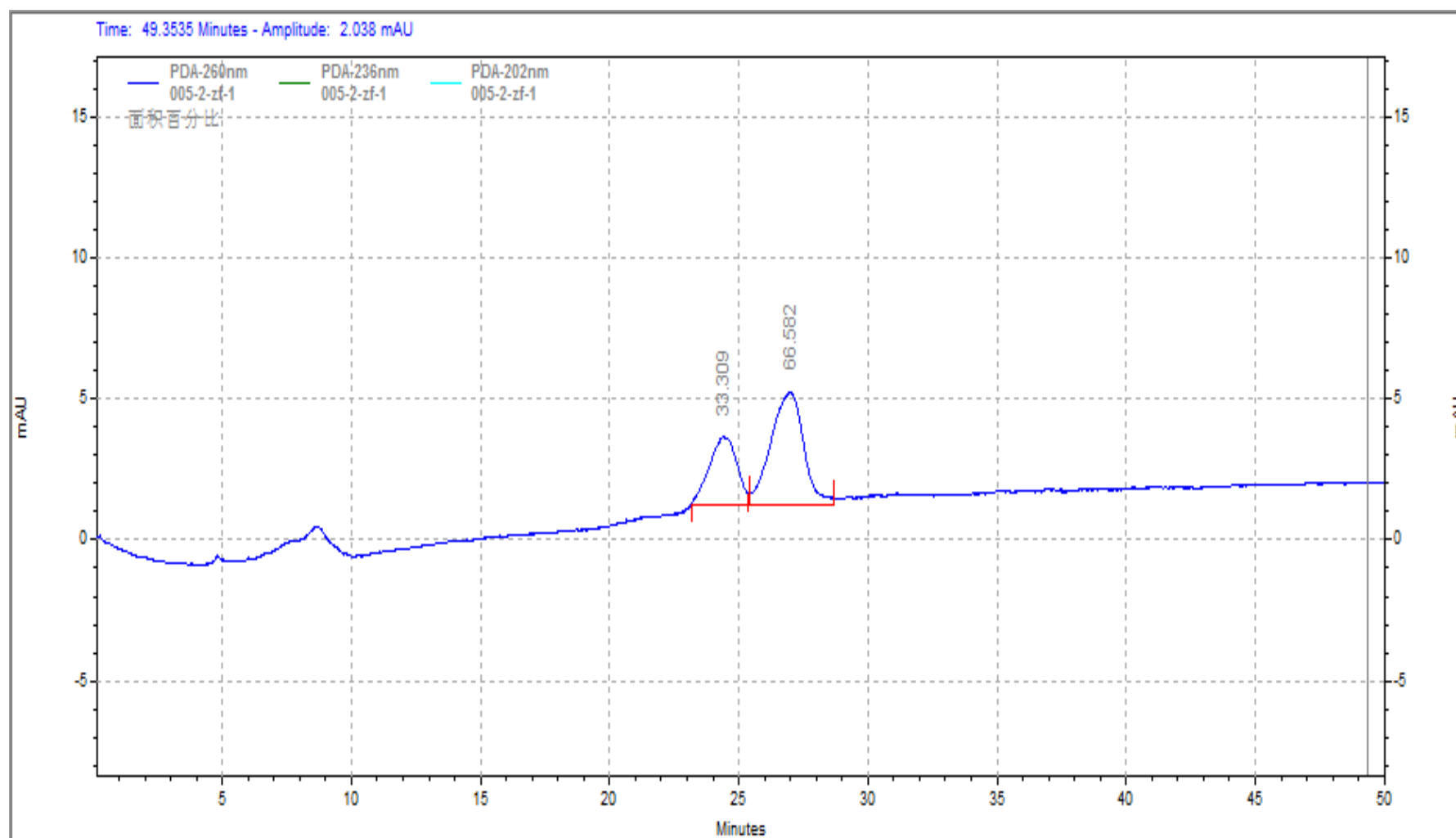


Figure S32. A peak area ratio of compounds **2** and **3** over a chiral column



Figure S33. The picture of strain *Cladosporium* sp. KFD33

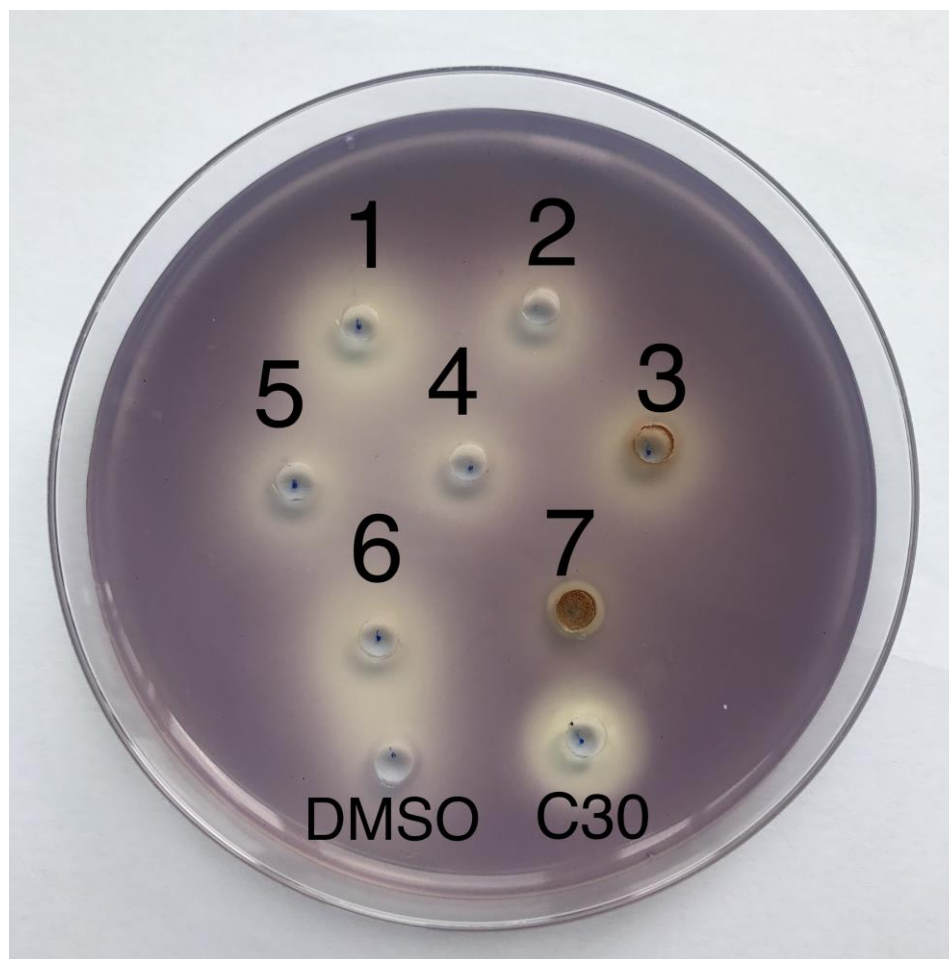


Figure S34. *C. violaceum* CV026 well diffusion assay
(Compounds 1-6 with 30, 30, 20, 30, 20 and 30 $\mu\text{g}/\text{well}$)

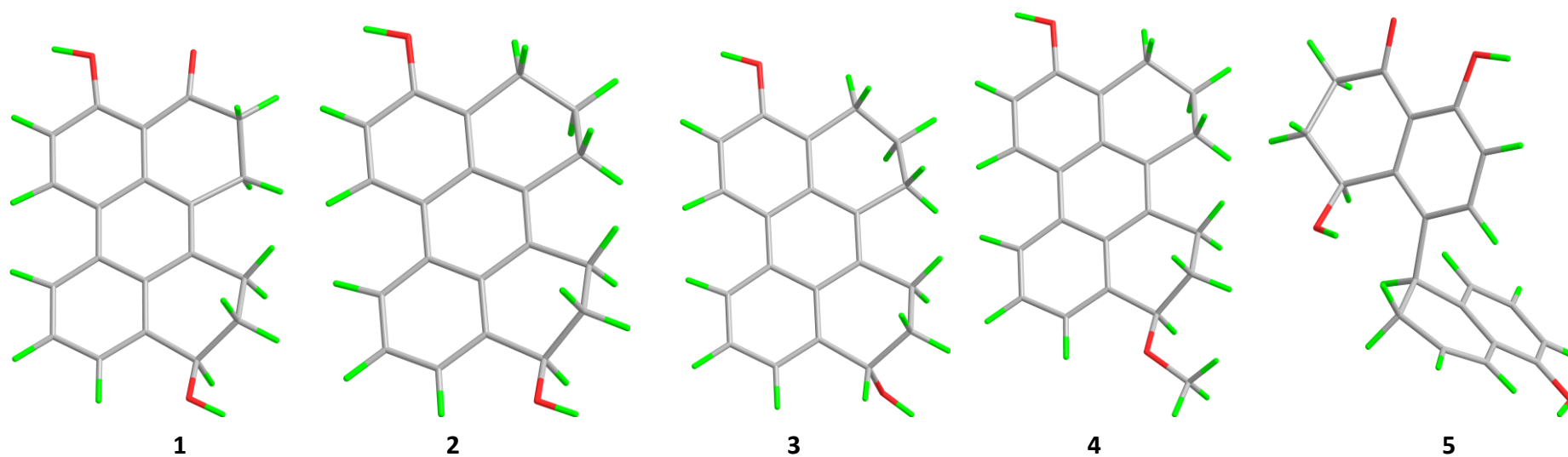


Figure S35. The energy minimized 3D chemical structures for 1-5.

18S rRNA gene sequences of *Cladosporium* sp. KFD33

GCACTATACGGTGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTTATTTGATAGTACCTTACTACATGGATAACCGTGGTAATTCTAGAG
CTAATACATGCTAAAAACCTCGACTTCGGAAGGGGTGATTTATTAGATAAAAAACCAATGCCCTTCGGGGCTCCTTGGTGAATCATAATAA
CTTAACGAATCGCATGGCCTTGCGCCGGCGATGGTTCATTCAAATTTCTGCCCTATCAACTTTTCGATGGTAGGATAGTGGCCTACCATGGTAT
CAACGGGTAACGGGGAATTAGGGTTCGACTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATT
ACCCAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATTGGAATGAGTACAATTTAAATCCCT
TAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAA
GCTCGTAGTTGAACCTTGGGCCTGGCTGGCCGGTCCGCCTCACCGCGTGTACTGGTCCGGCCGGGCCTTTCCTTCTGGGGAACCTCATGCCCTT
CACTGGGCGTGTTGGGGAACCAGGACTTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAAT
AGAATAGGACGTGTGGTTCTATTTTGTGGTTTCTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCATCAGTATTCAATCGTCAGAG
GTGAAATTCTTGGATTGATTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCAGTGAACGAAAGTTAGGGGATCGA
AGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGACGGTGTTAGTATTTGACCCGTTTCGGCACCTTACGA
GAAATCAAAGTTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGCGTGGAGCCT
GCGGCTTAATTTGACTCAACACGGGGAACTCACCAGGTCCAGACACAATAAGGATTGACAGATTGAGAGCTCTTTCTTGATTTTGTGGGTG
GTGGTGATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGATAACGAACGAGACCTTAACCTGCTAAATAGCCAGGCCCGCT
TTGGCGGGTTCGCCGGCTTCTTAGAGGGACTATC

The calculations were performed by using the density functional theory (DFT) as carried out in the Gaussian 03.^{S1} The preliminary conformational distributions search was performed using Frog2 online version^{S2}. Further geometrical optimization were performed 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.

(S1) 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.

(S2) Miteva, M. A.; Guyon, F.; and Tuffery, P.; *Nucleic Acids Res.*, **2010**, 38, W622–W627.

(S3) Sai, C.; Li, D.; Xue, C.; Wang, K.; Hu, P.; Pei, Y.; Bai, J.; Jing Y.; Li, Z.; Hua H. *Org. Lett.* **2015**, 17, 4102-5.

(S4) (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.