

**Sclerotides C-E and Scopularide I, New Cyclic Peptides from the Soft-Coral
Derived Fungus *Aspergillus sclerotiorum***

Jieyi Long^{1,2}, Yaqi Chen³, Weihao Chen^{1,2}, Junfeng Wang¹, Xuefeng Zhou¹, Bing
Yang^{1*}, and Yonghong Liu^{1,2*}

¹ CAS Key Laboratory of Tropical Marine Bio-resources and Ecology/Guangdong
Key Laboratory of Marine Materia Medica/Innovation Academy of South China Sea
Ecology and Environmental Engineering, South China Sea Institute of Oceanology,
Chinese Academy of Sciences, Guangzhou 510301, P. R. China;

² University of Chinese Academy of Sciences, 19 Yuquan Road, Beijing 100049, P. R.
China;

³ State Key Laboratory of Chemical Oncogenomics, Key Laboratory of Chemical
Genomics, Peking University Shenzhen Graduate School, Shenzhen, 518055 China;

Contents

| | |
|--|----|
| ITS sequence of the strain <i>Aspergillus sclerotiorum</i> SCSIO 41031 | 4 |
| Figure S1. ^1H NMR spectrum of 1 (DMSO- d_6 , 700 MHz) | 5 |
| Figure S2. ^{13}C NMR spectrum of 1 (DMSO- d_6 , 175 MHz) | 5 |
| Figure S3. DEPT spectrum of 1 (DMSO- d_6)..... | 6 |
| Figure S4. HSQC spectrum of 1 (DMSO- d_6) | 6 |
| Figure S5. ^1H - ^1H COSY spectrum of 1 (DMSO- d_6)..... | 7 |
| Figure S6. HMBC spectrum of 1 (DMSO- d_6) | 7 |
| Figure S7. HRESIMS spectrum of 1..... | 8 |
| Figure S8. IR spectrum of 1 | 8 |
| Figure S9. UV spectrum of 1 | 9 |
| Figure S10. CD spectrum of 1 | 9 |
| Figure S11. ^1H NMR spectrum of 2 (DMSO- d_6 , 700 MHz)..... | 10 |
| Figure S12. ^{13}C NMR spectrum of 2 (DMSO- d_6 , 175 MHz) | 10 |
| Figure S13. DEPT spectrum of 2 (DMSO- d_6)..... | 11 |
| Figure S14. HSQC spectrum of 2 (DMSO- d_6) | 11 |
| Figure S15. ^1H - ^1H COSY spectrum of 2 (DMSO- d_6)..... | 12 |
| Figure S16. HMBC spectrum of 2 (DMSO- d_6) | 12 |
| Figure S17. HRESIMS spectrum of 2..... | 13 |
| Figure S18. IR spectrum of 2..... | 13 |
| Figure S19. UV spectrum of 2 | 14 |
| Figure S20. CD spectrum of 2 | 14 |
| Figure S21. ^1H NMR spectrum of 3 (DMSO- d_6 , 500 MHz) | 15 |
| Figure S22. ^{13}C NMR spectrum of 3 (DMSO- d_6 , 125 MHz) | 15 |
| Figure S23. DEPT spectrum of 3 (DMSO- d_6)..... | 16 |
| Figure S24. HSQC spectrum of 3 (DMSO- d_6) | 16 |
| Figure S25. ^1H - ^1H COSY spectrum of 3 (DMSO- d_6)..... | 17 |
| Figure S26. HMBC spectrum of 3 (DMSO- d_6) | 17 |
| Figure S27. TOCSY spectrum of 3..... | 18 |
| Figure S28. HRESIMS spectrum of 3..... | 18 |
| Figure S29. HRESIMS/MS fragmentation of 3..... | 19 |
| Figure S30. IR spectrum of 3 | 20 |

| | |
|---|----|
| Figure S31. UV spectrum of 3 | 20 |
| Figure S32. CD spectrum of 3 | 21 |
| Figure S33. ¹ H NMR spectrum of 4 (DMSO- <i>d</i> ₆ , 500 MHz) | 21 |
| Figure S34. ¹³ C NMR spectrum of 4 (DMSO- <i>d</i> ₆ , 125 MHz) | 22 |
| Figure S35. DEPT spectrum of 4 (DMSO- <i>d</i> ₆) | 22 |
| Figure S36. HSQC spectrum of 4 (DMSO- <i>d</i> ₆) | 23 |
| Figure S37. ¹ H- ¹ H COSY spectrum of 4 (DMSO- <i>d</i> ₆) | 23 |
| Figure S38. HMBC spectrum of 4 (DMSO- <i>d</i> ₆) | 24 |
| Figure S39. NOESY spectrum of 4 (DMSO- <i>d</i> ₆) | 25 |
| Figure S40. HRESIMS spectrum of 4 | 25 |
| Figure S41. IR spectrum of 4 | 26 |
| Figure S42. UV spectrum of 4 | 26 |
| Figure S43. CD spectrum of 4 | 27 |
| Figure S44. HPLC analysis of FDAA derivatives of standard amino acids (YMC-Pack ODS-A column, 250*4.6 mmI.D., S-5 μm, 12 nm). | 28 |
| Figure S45. HPLC analysis of FDAA derivatives of compound 2 and 3 (YMC-Pack ODS-A column, 250*4.6 mmI.D., S-5 μm, 12 nm) | 29 |
| Table S1. Test concentration and OD value of compound 4 against AChE | 29 |
| Figure S46. IC ₅₀ curve of compound 4 against AChE | 30 |
| Table S2. Test concentration and OD value of compound 4 against HONE1-EBV and HONE1. | 30 |
| Figure S47. IC ₅₀ curve of compound 4 against HONE1-EBV and HONE1. | 30 |

ITS sequence of the strain *Aspergillus sclerotiorum* SCSIO 41031

TGCGGAAGGATCATTACTGAGTGAGGGTCCCTCGGGGCCCAACCTCCCACCCGTGTAT
ACCGTACCTTGTTGCTTCGGCGGGGCCCGCCGCGCAAGCGGCCGCCGGGGGGGGGCGT
CAAACCCCCCTCCCTAGGCGAGCGCCCGCCGGAGACACCAACGTGAACACTGTCTGA
AGTTTTGTTGTCTGAGTTCGATTGTATCGCAATCAGTTAAACTTTCAACAATGGATCTC
TTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAATTAATGTGAATTGCAGAA
TTCAGTGAATCATCGAGTCTTTGAACGCACATTGCACCCCCTGGTATTCCGGGGGGGTAT
GCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGTCGTCGTCCC
CCCGGGGACGGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGG
GGCTTTGTCACCCGCTCTTGTAAGGCCCGGCCGGCGCTGGCCGACGCTGAAAAGCAACC
AACTATTTCTCCAGGTTGACCTCGGATCAGGTAGGGATAACCGCTGAACTTAAGCATAT
C

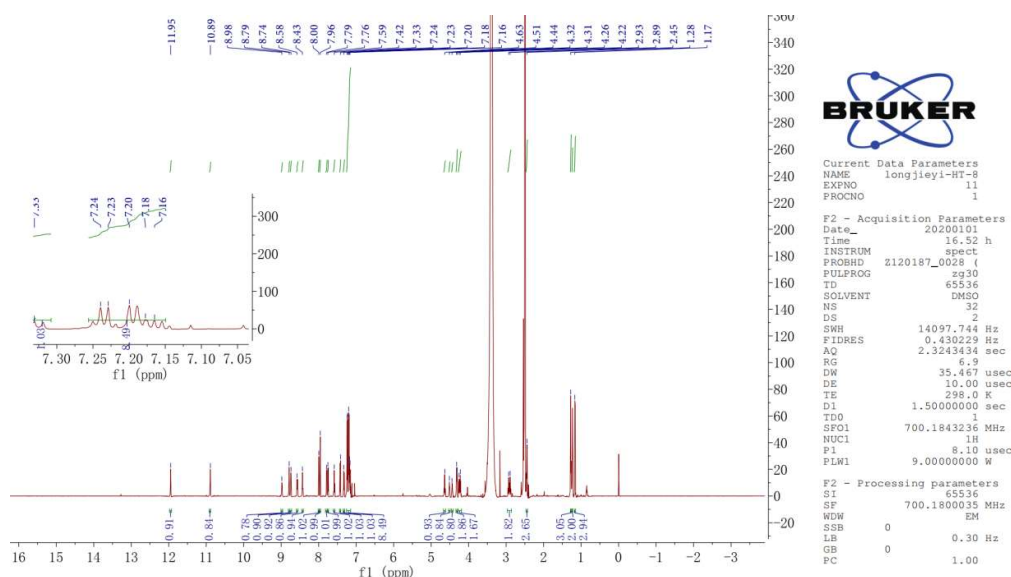


Figure S1. ^1H NMR spectrum of **1** (DMSO- d_6 , 700 MHz)

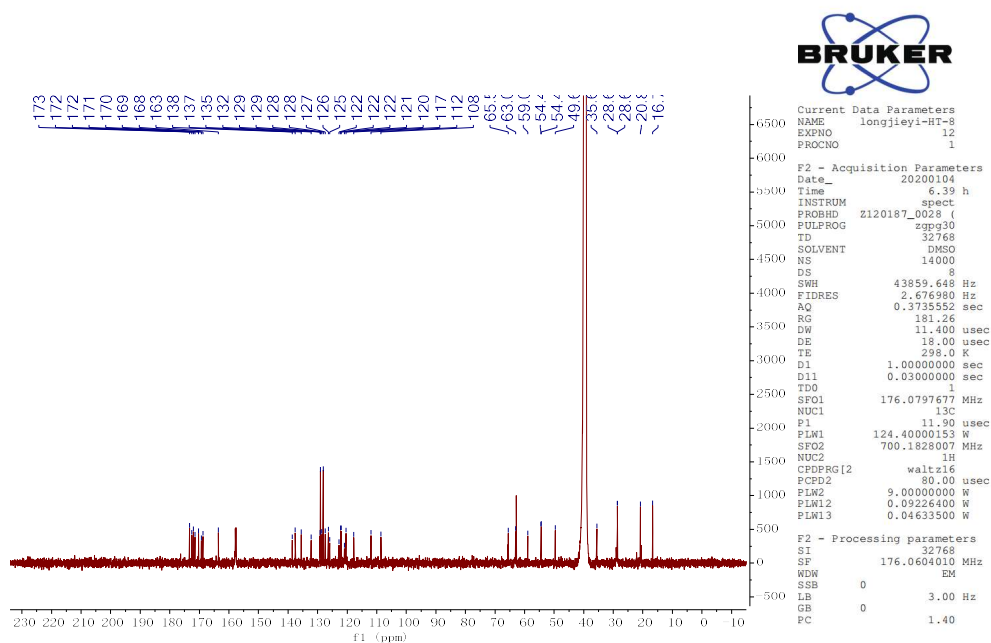


Figure S2. ^{13}C NMR spectrum of **1** (DMSO- d_6 , 175 MHz)

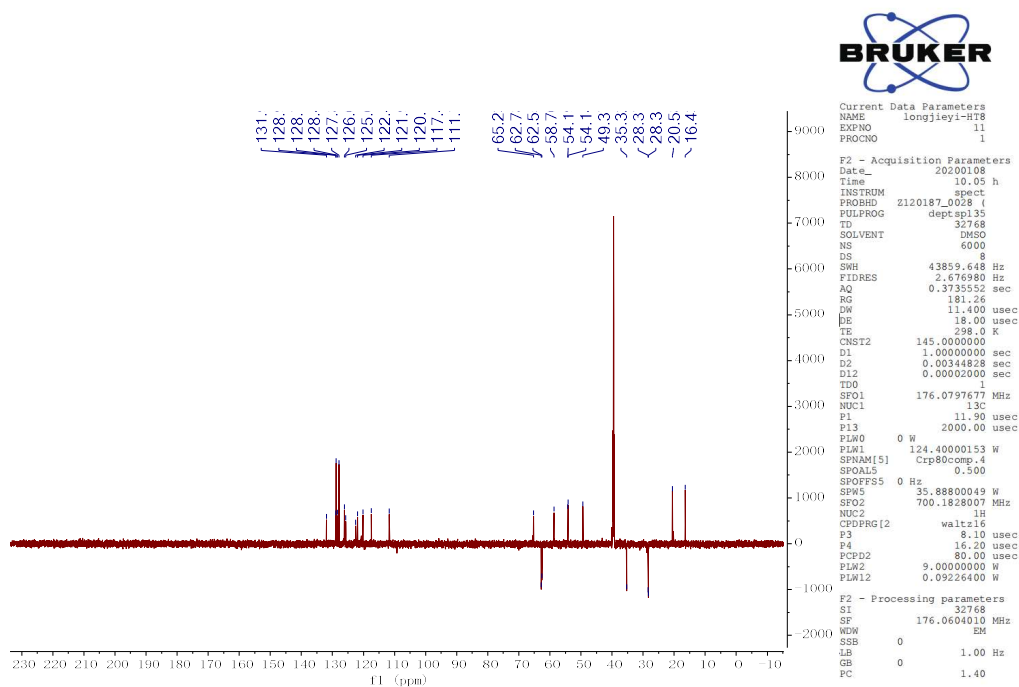


Figure S3. DEPT spectrum of **1** (DMSO- d_6)

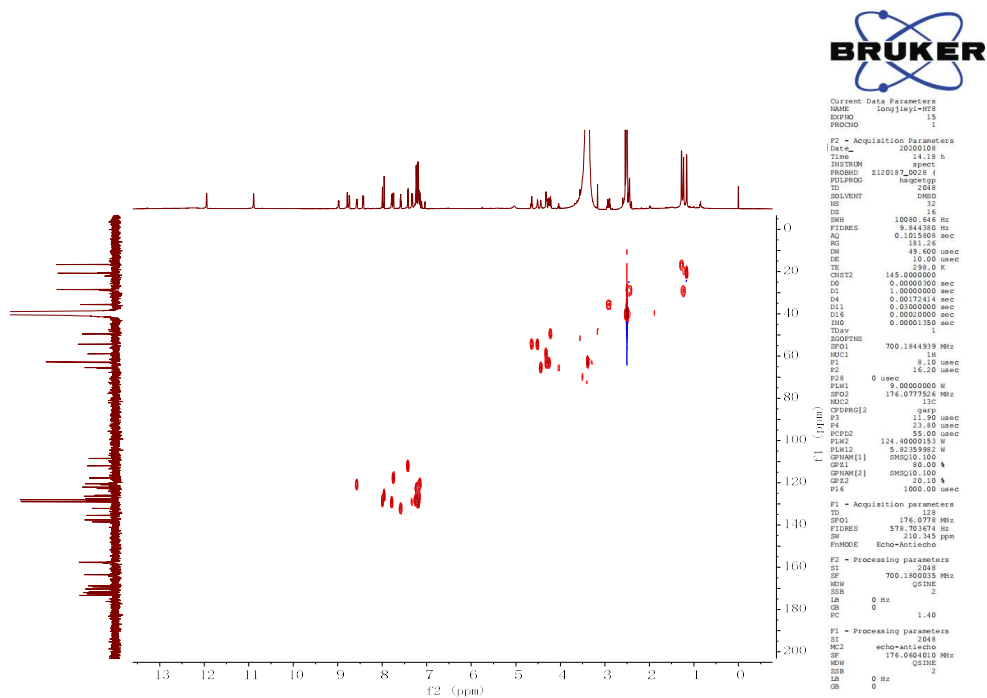


Figure S4. HSQC spectrum of **1** (DMSO- d_6)

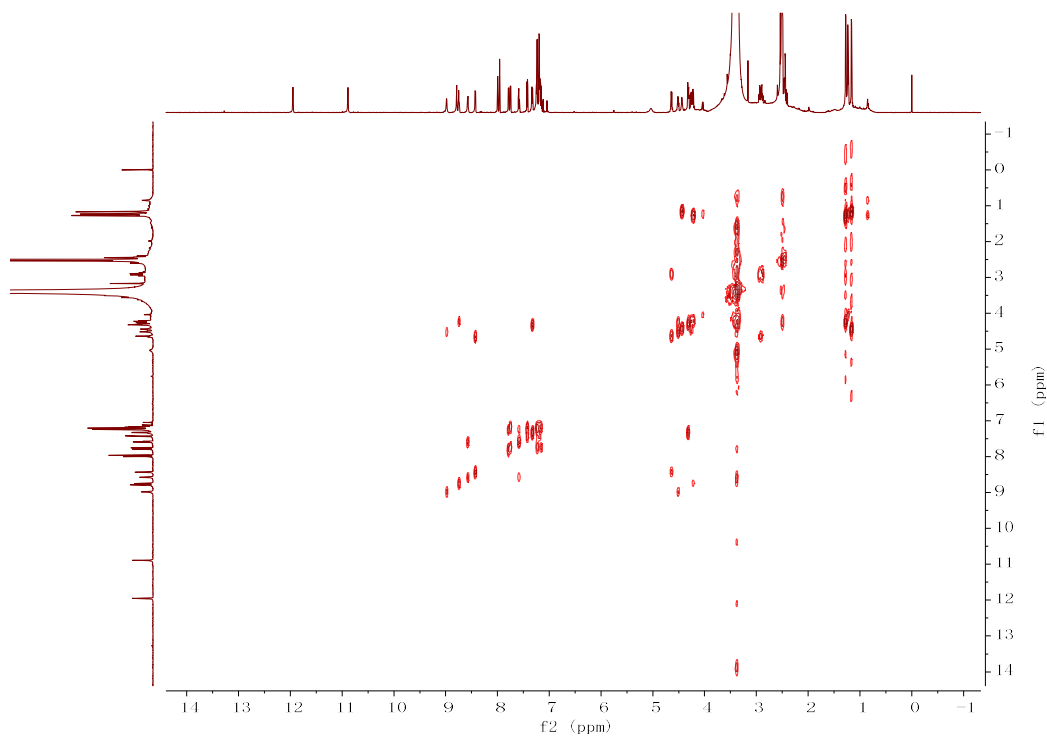


Figure S5. ^1H - ^1H COSY spectrum of **1** ($\text{DMSO}-d_6$)

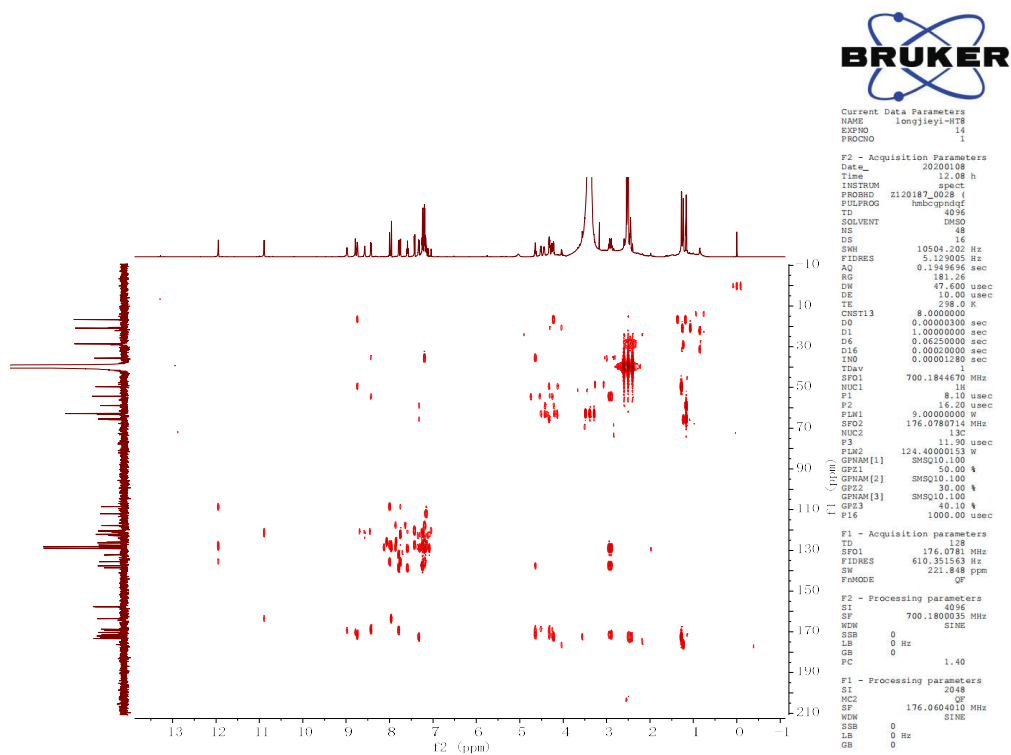


Figure S6. HMBC spectrum of **1** ($\text{DMSO}-d_6$)

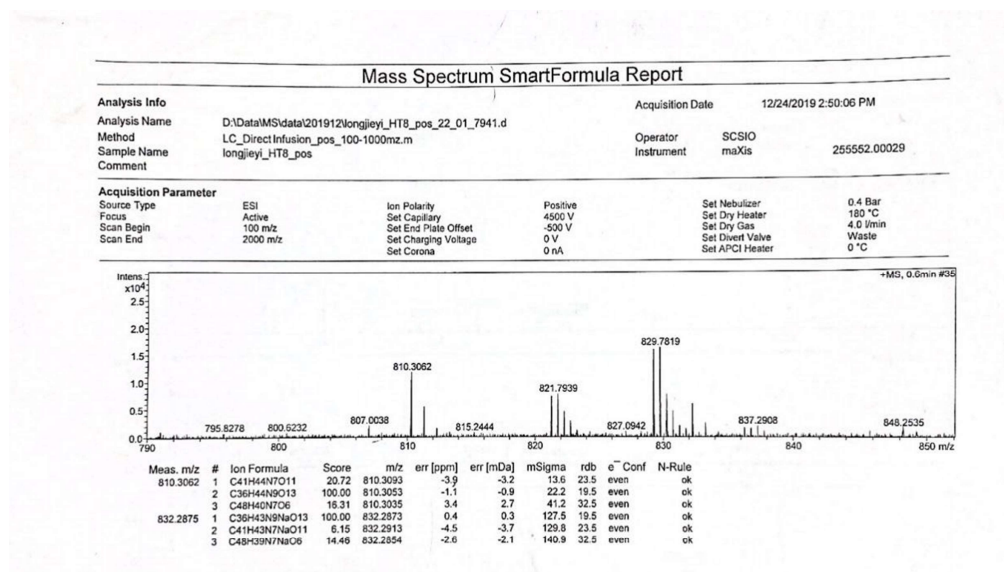


Figure S7. HRESIMS spectrum of **1**

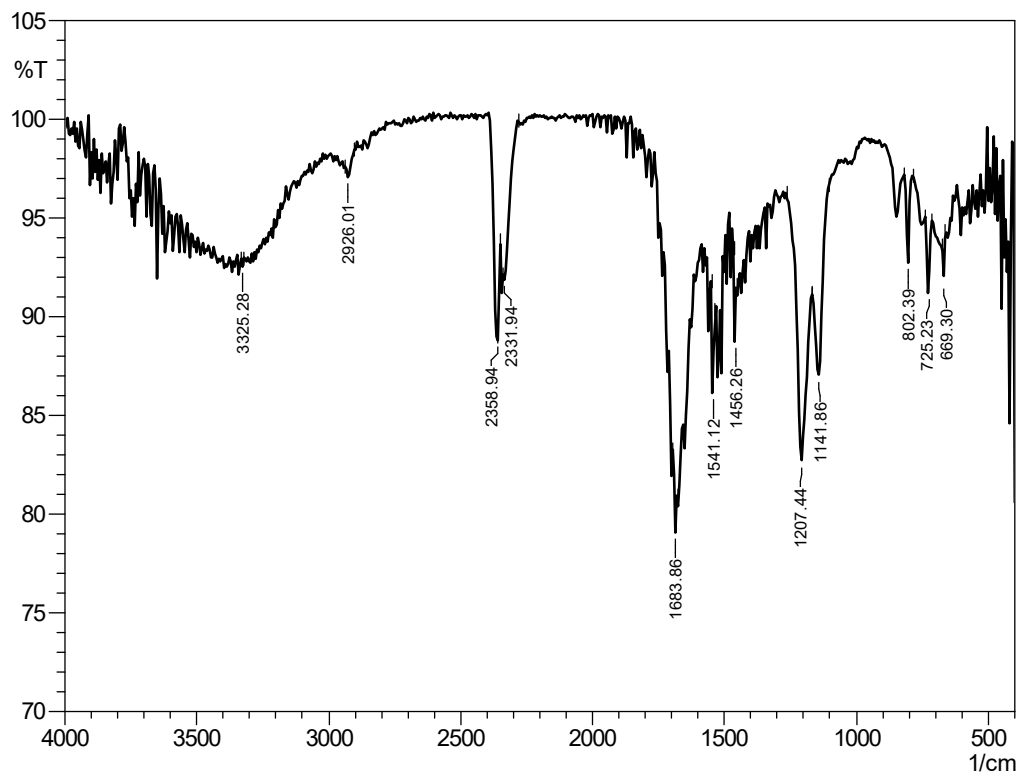


Figure S8. IR spectrum of **1**

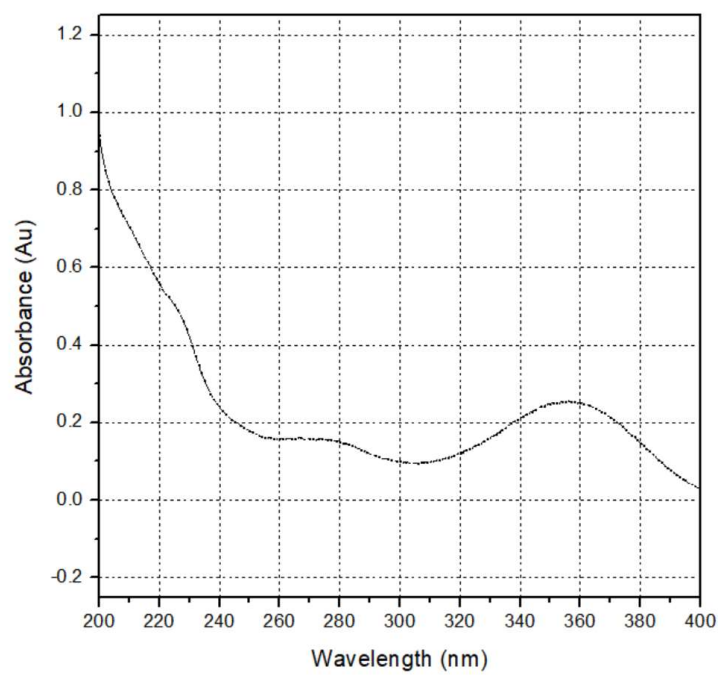


Figure S9. UV spectrum of **1**

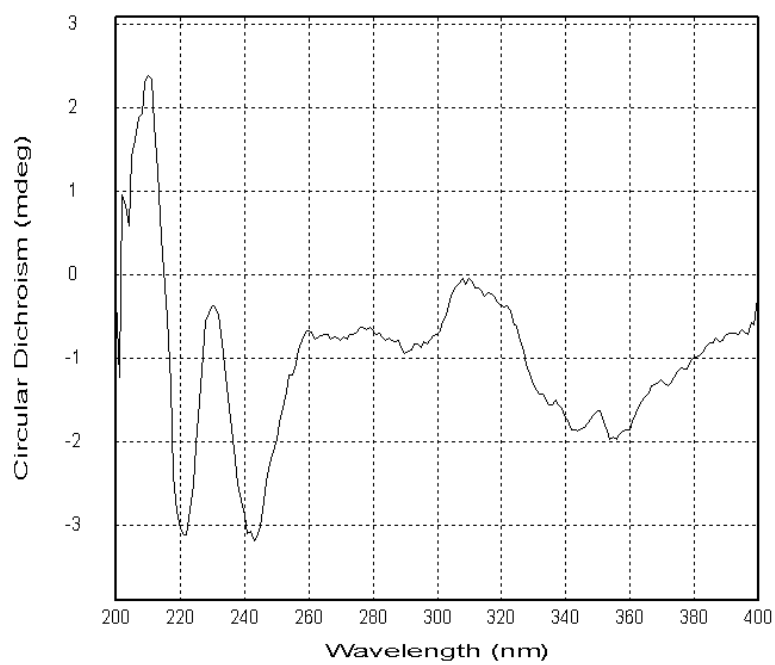


Figure S10. CD spectrum of **1**

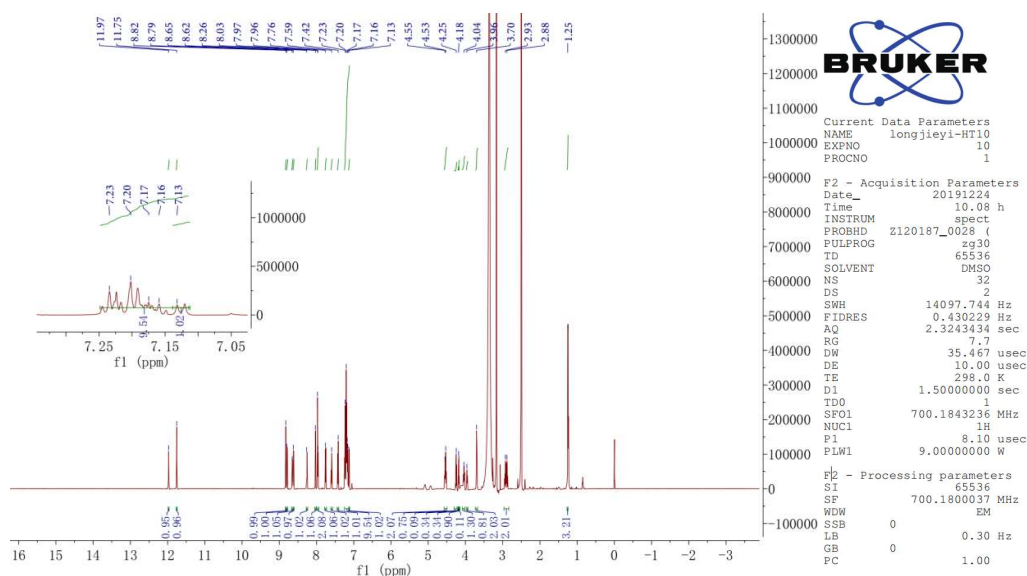


Figure S11. ^1H NMR spectrum of **2** (DMSO- d_6 , 700 MHz)

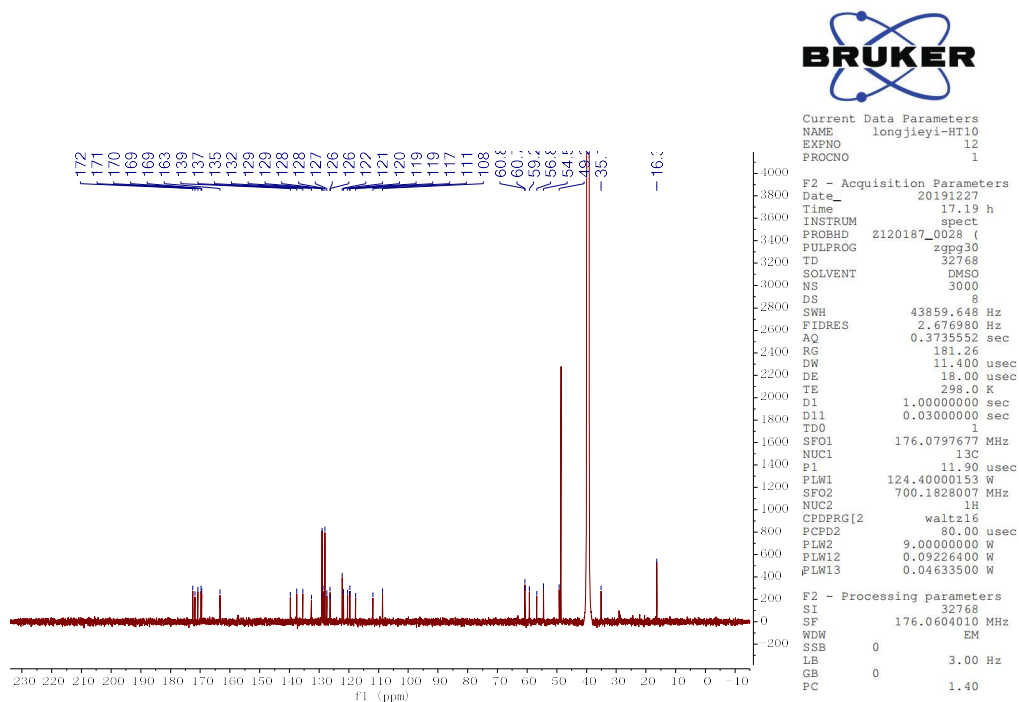


Figure S12. ^{13}C NMR spectrum of **2** (DMSO- d_6 , 175 MHz)

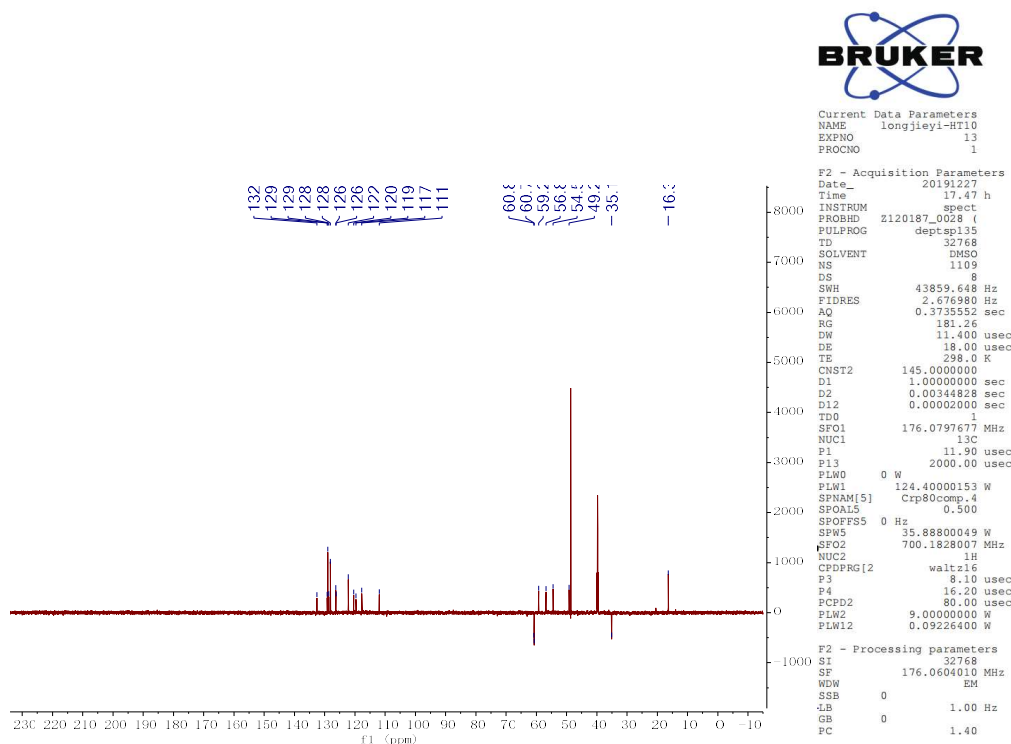


Figure S13. DEPT spectrum of **2** (DMSO-*d*₆)

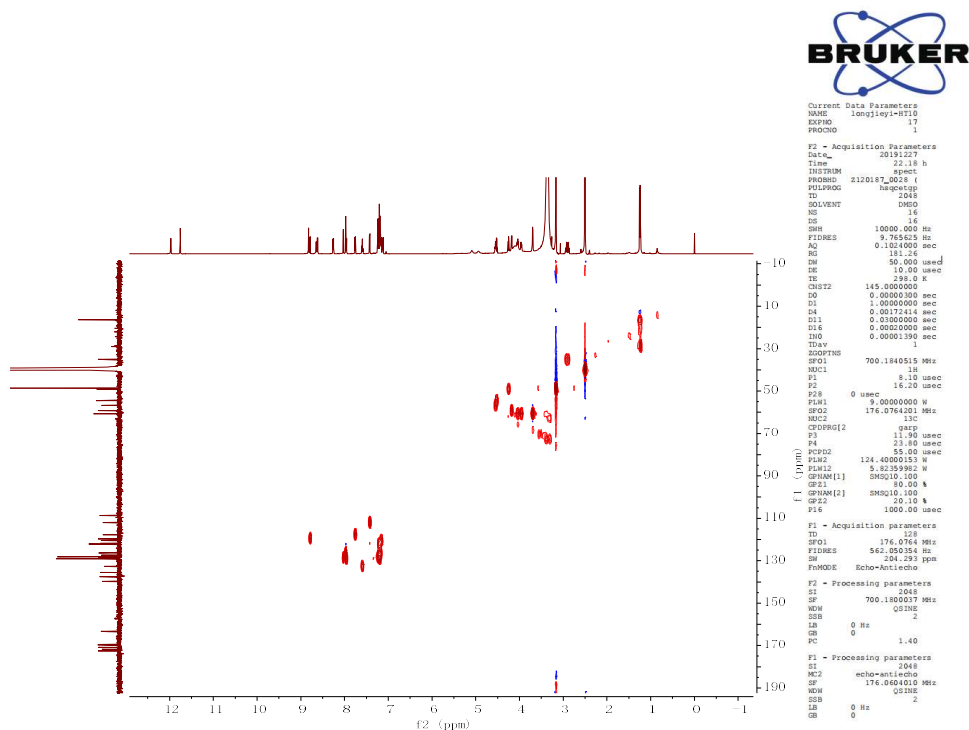


Figure S14. HSQC spectrum of **2** (DMSO-*d*₆)

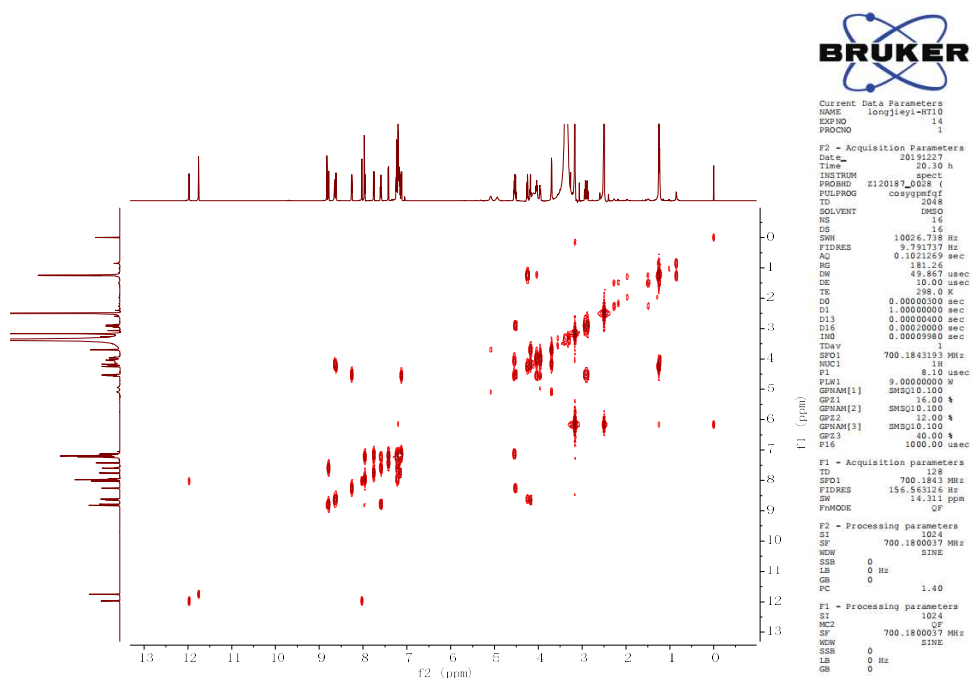


Figure S15. ^1H - ^1H COSY spectrum of **2** (DMSO- d_6)

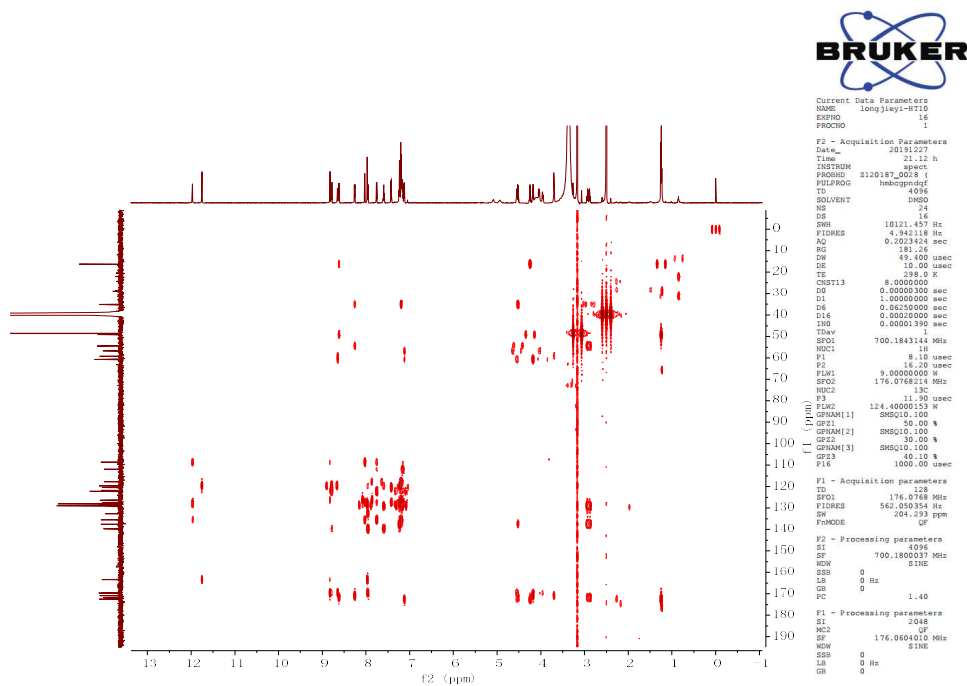


Figure S16. HMBC spectrum of **2** (DMSO- d_6)

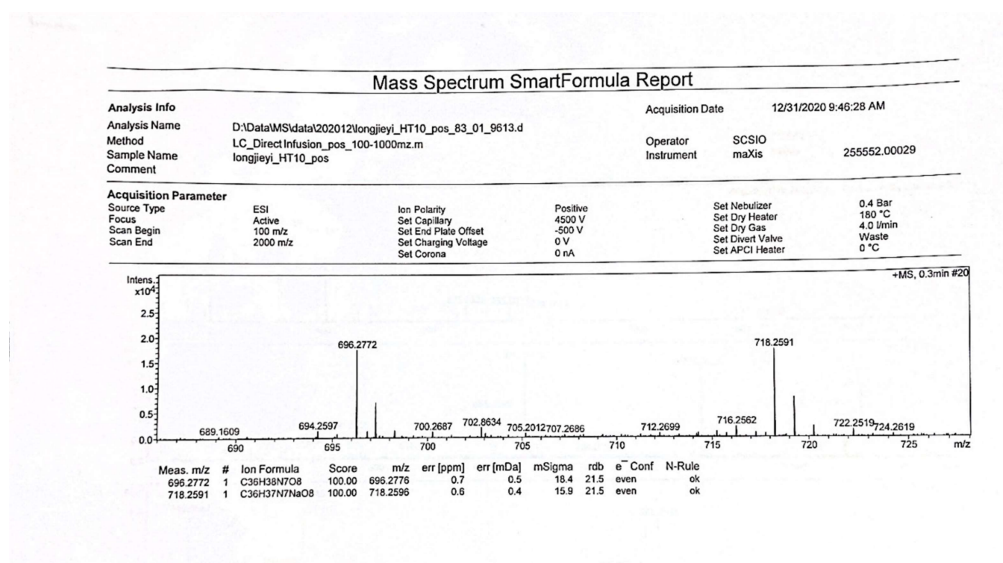


Figure S17. HRESIMS spectrum of **2**

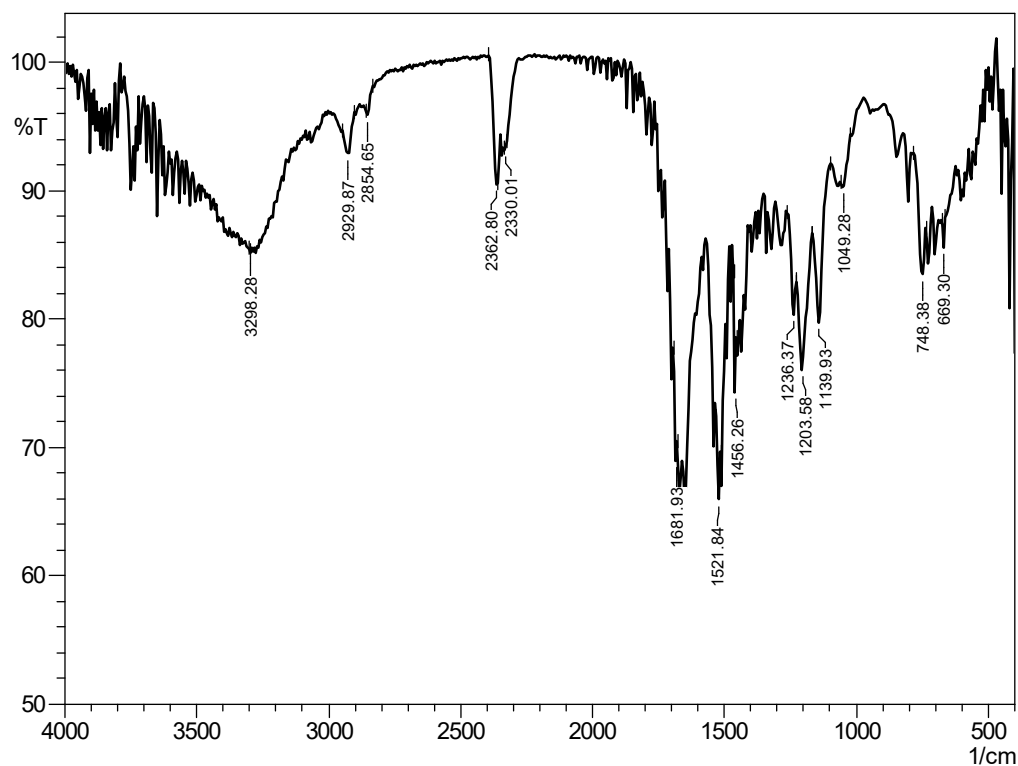


Figure S18. IR spectrum of **2**

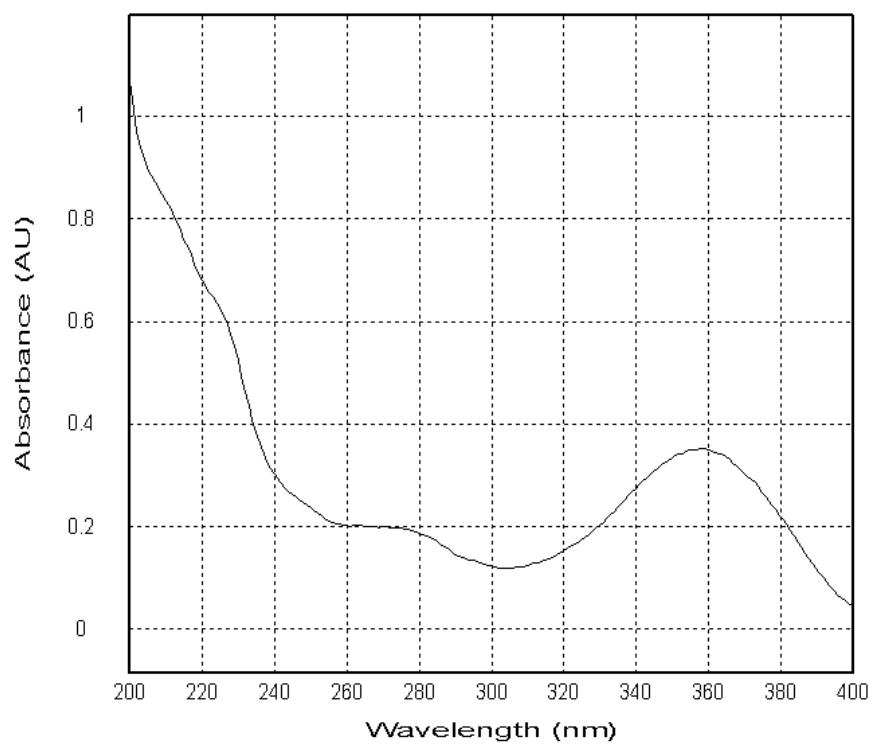


Figure S19. UV spectrum of **2**

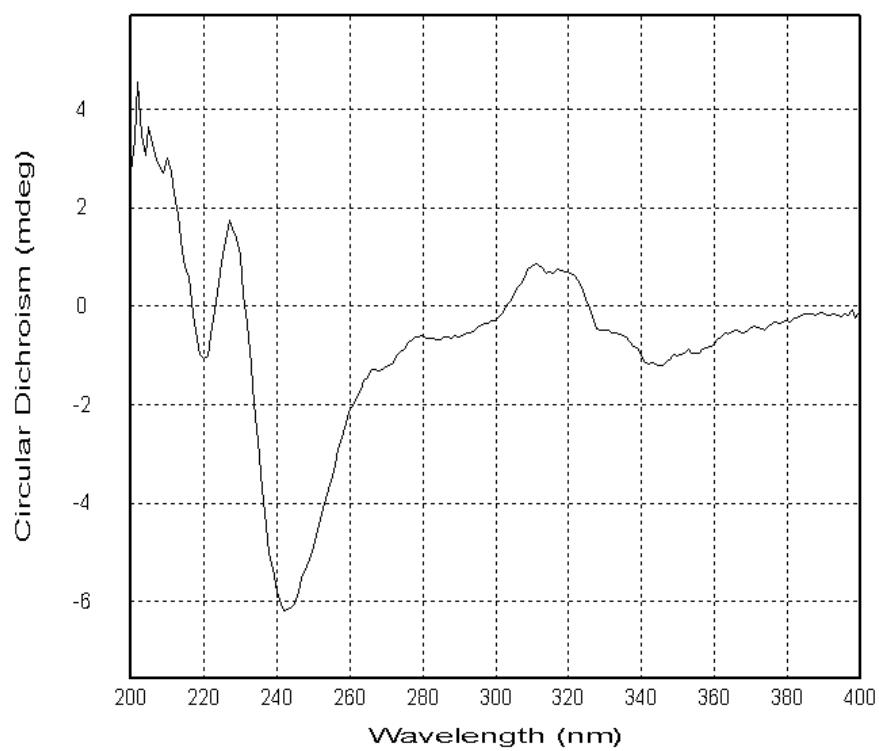


Figure S20. CD spectrum of **2**

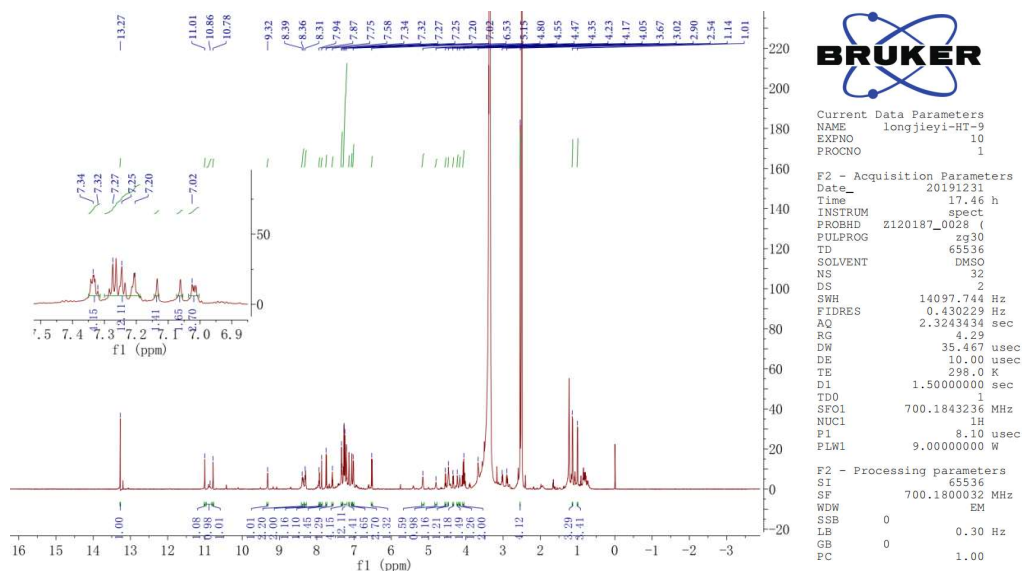


Figure S21. ^1H NMR spectrum of **3** (DMSO- d_6 , 500 MHz)

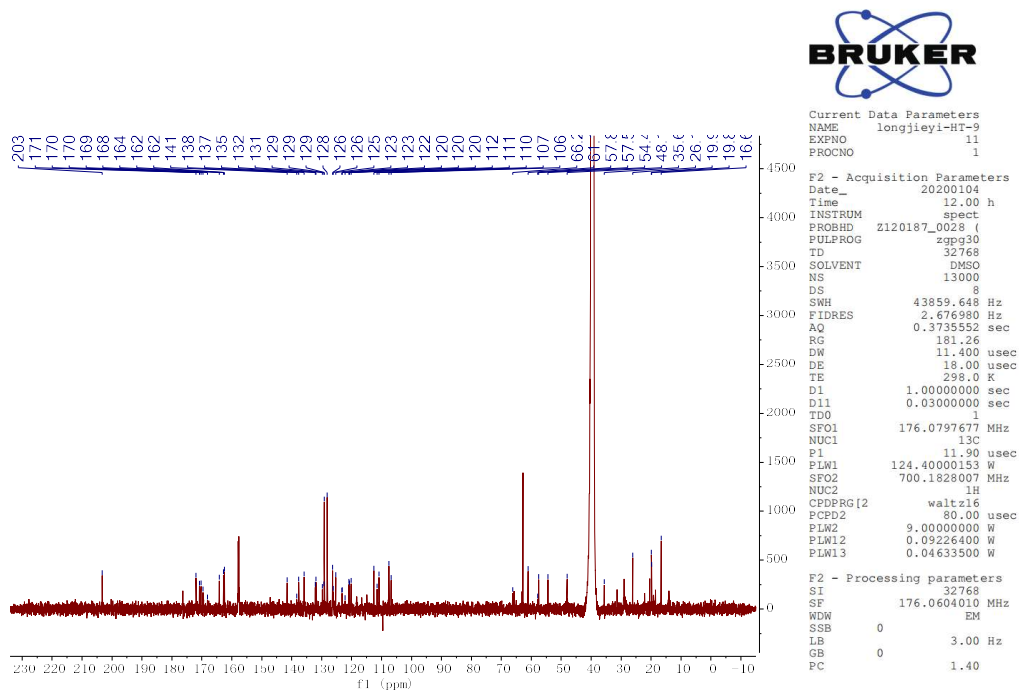


Figure S22. ^{13}C NMR spectrum of **3** (DMSO- d_6 , 125 MHz)

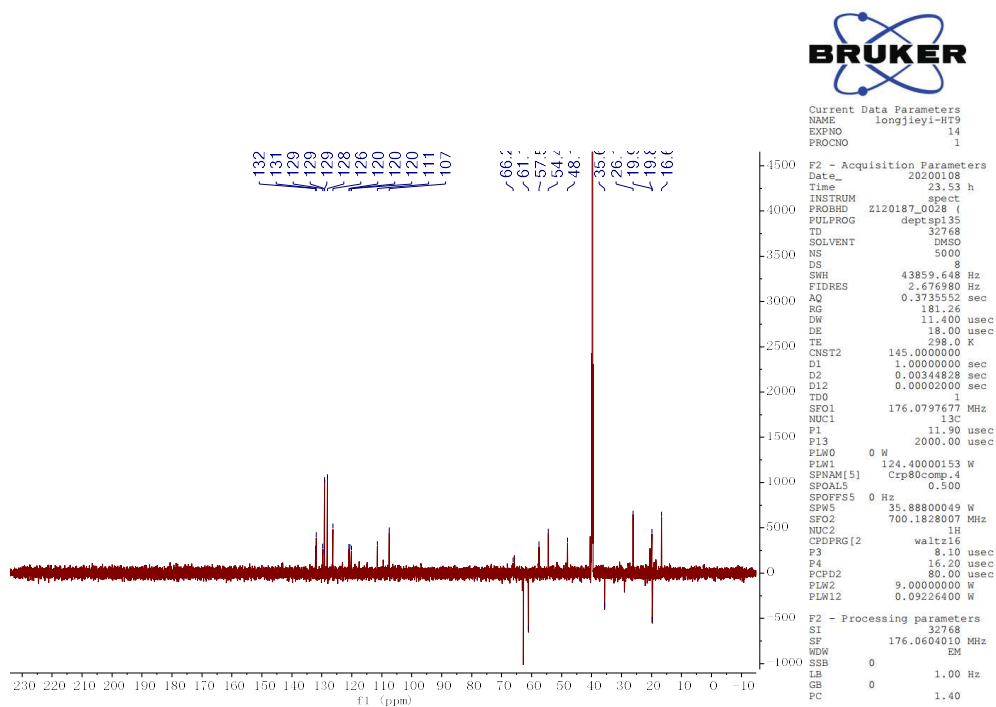


Figure S23. DEPT spectrum of **3** (DMSO- d_6)

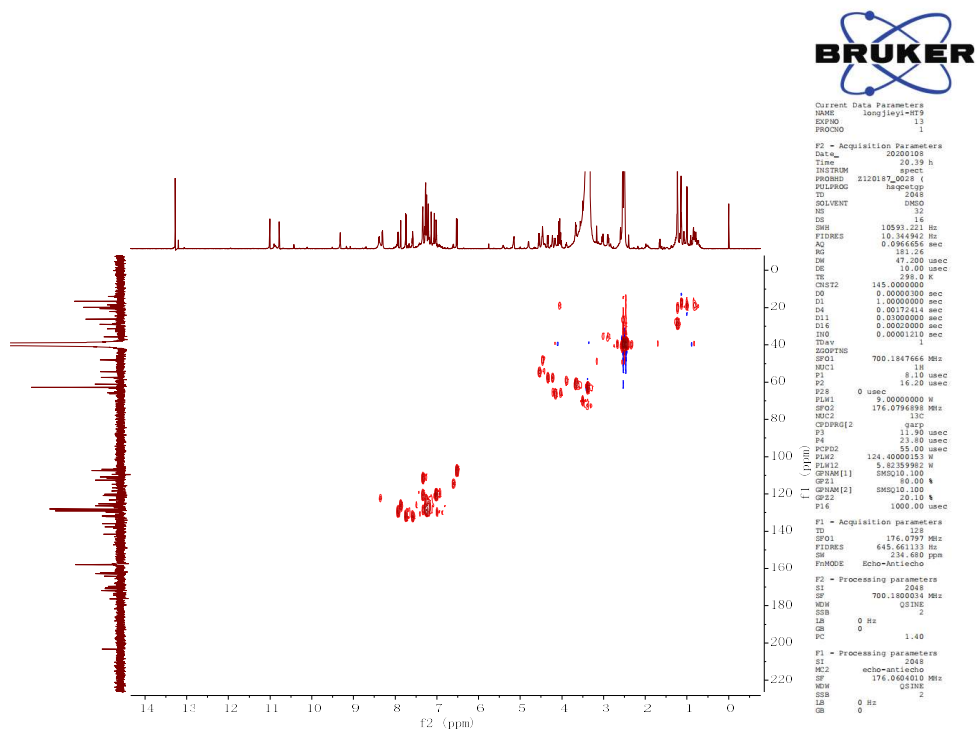


Figure S24. HSQC spectrum of **3** (DMSO- d_6)

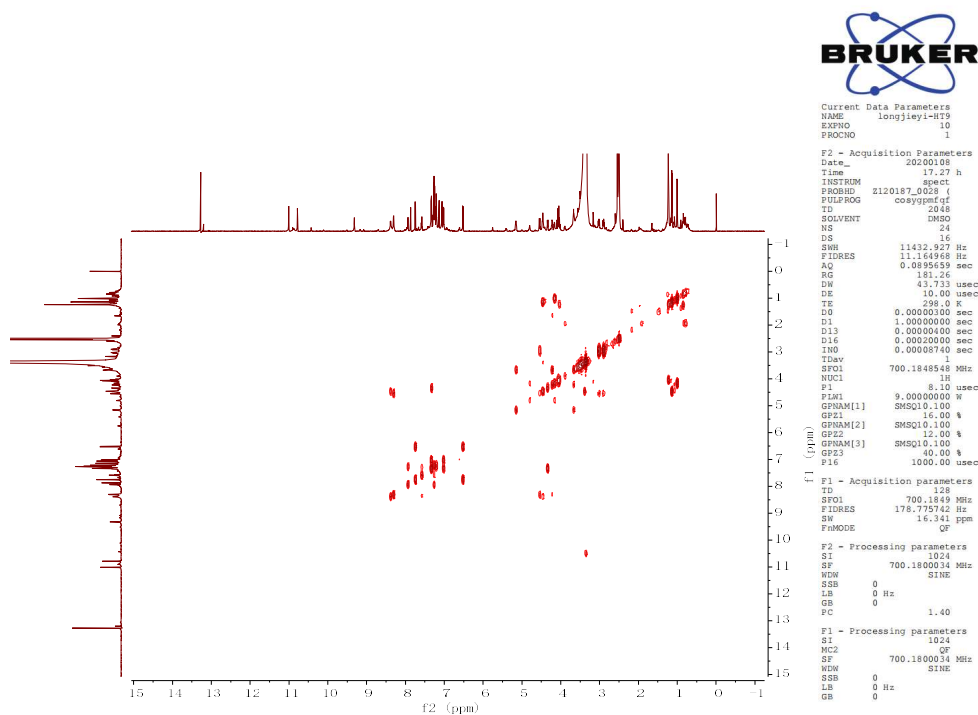


Figure S25. ^1H - ^1H COSY spectrum of **3** (DMSO- d_6)

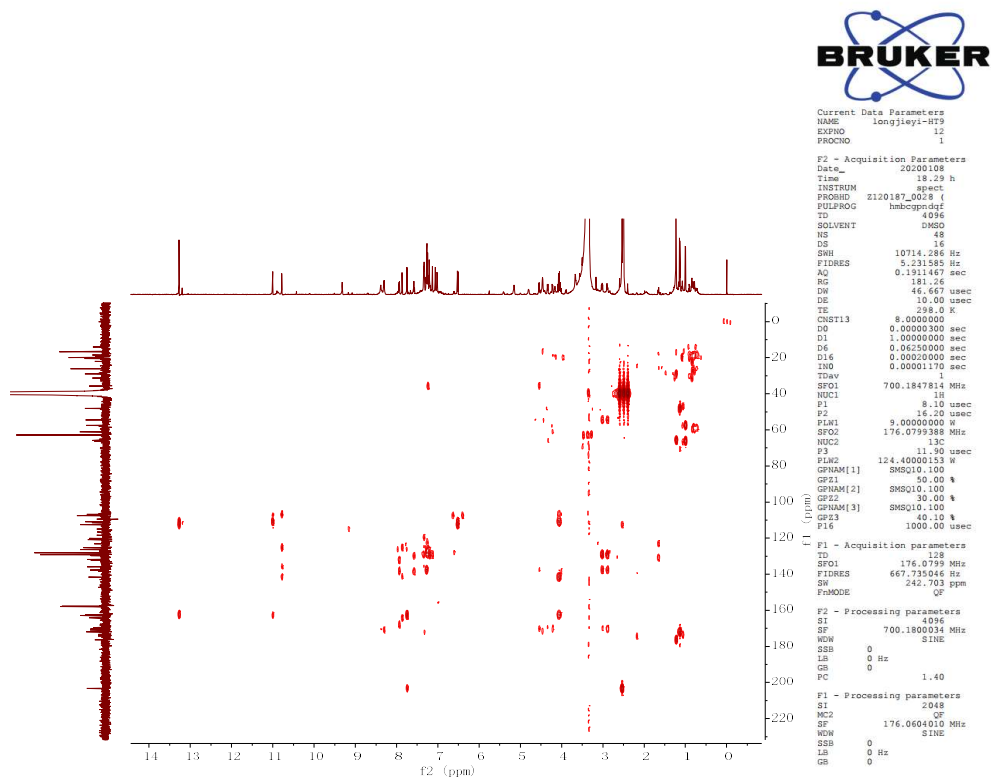


Figure S26. HMBC spectrum of **3** (DMSO- d_6)

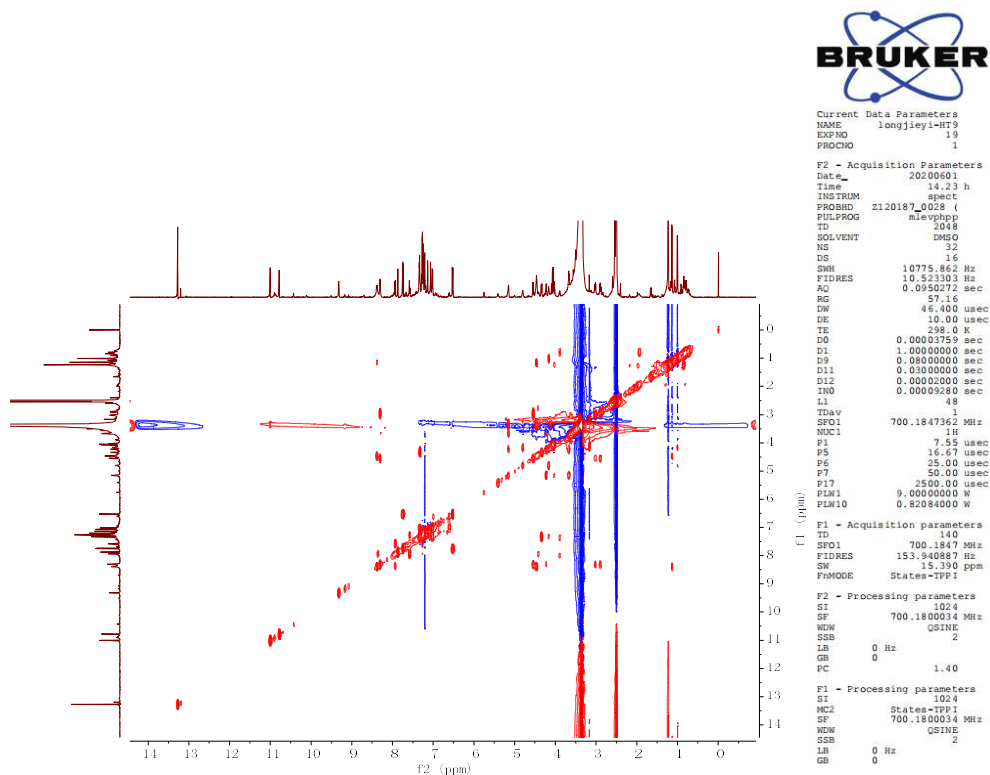


Figure S27. TOCSY spectrum of 3

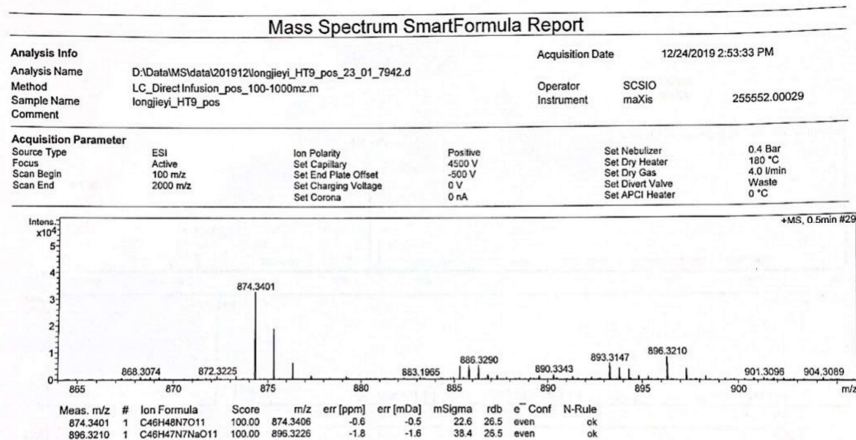


Figure S28. HRESIMS spectrum of 3

Window Display Report

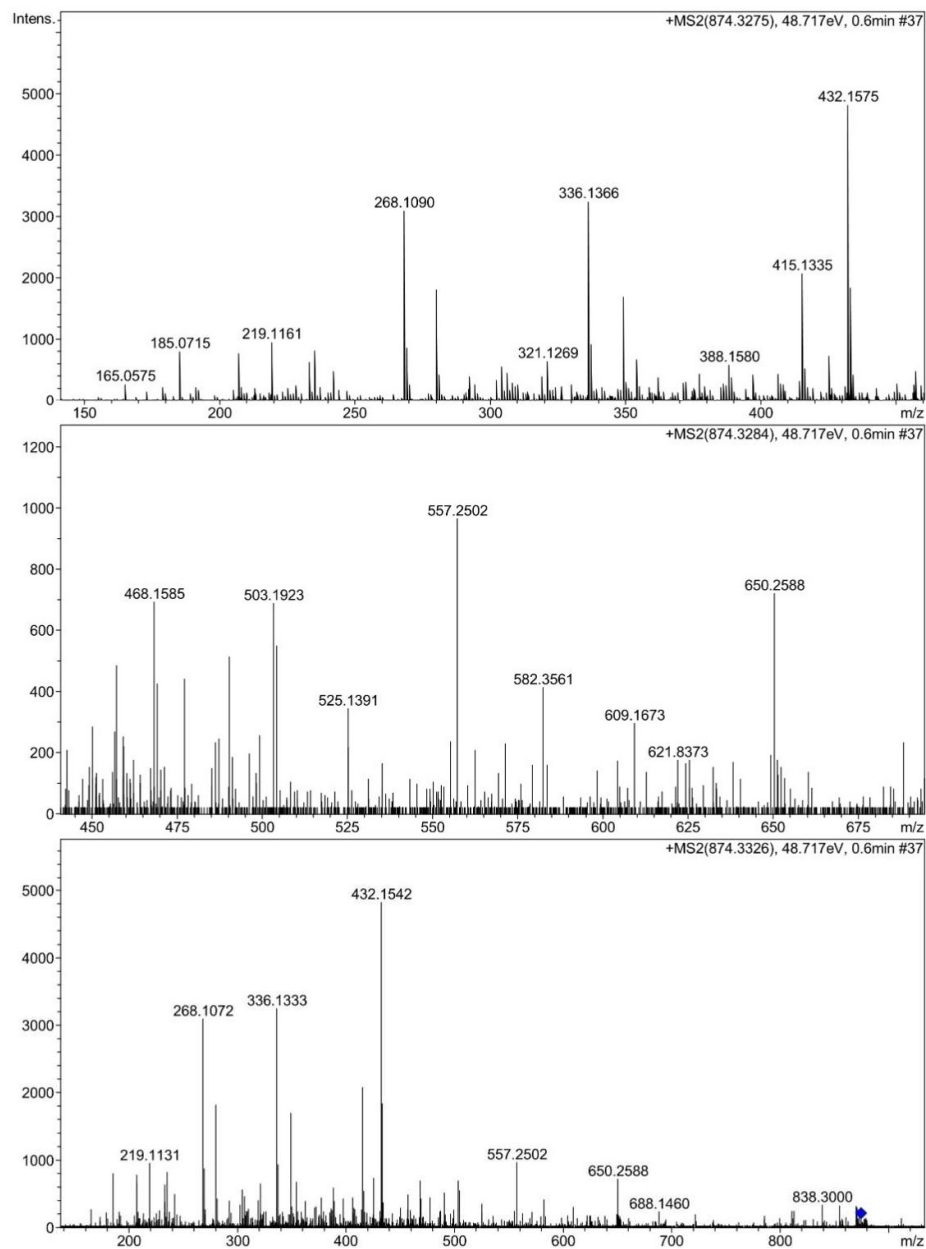


Figure S29. HRESIMS/MS fragmentation of **3**

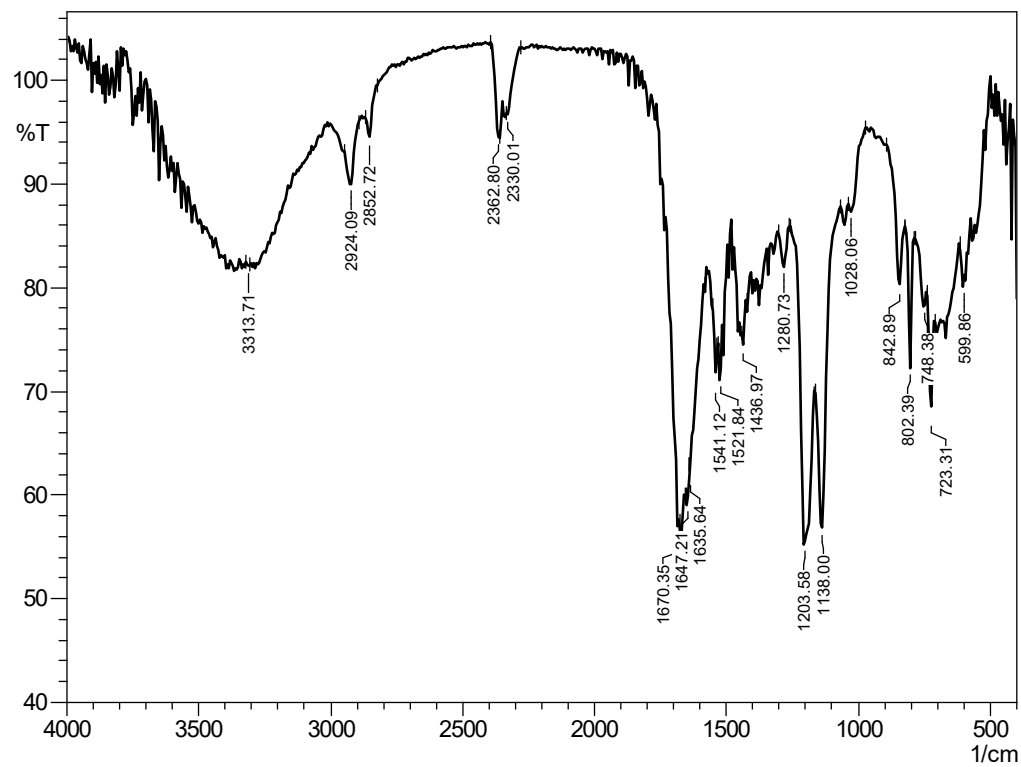


Figure S30. IR spectrum of **3**

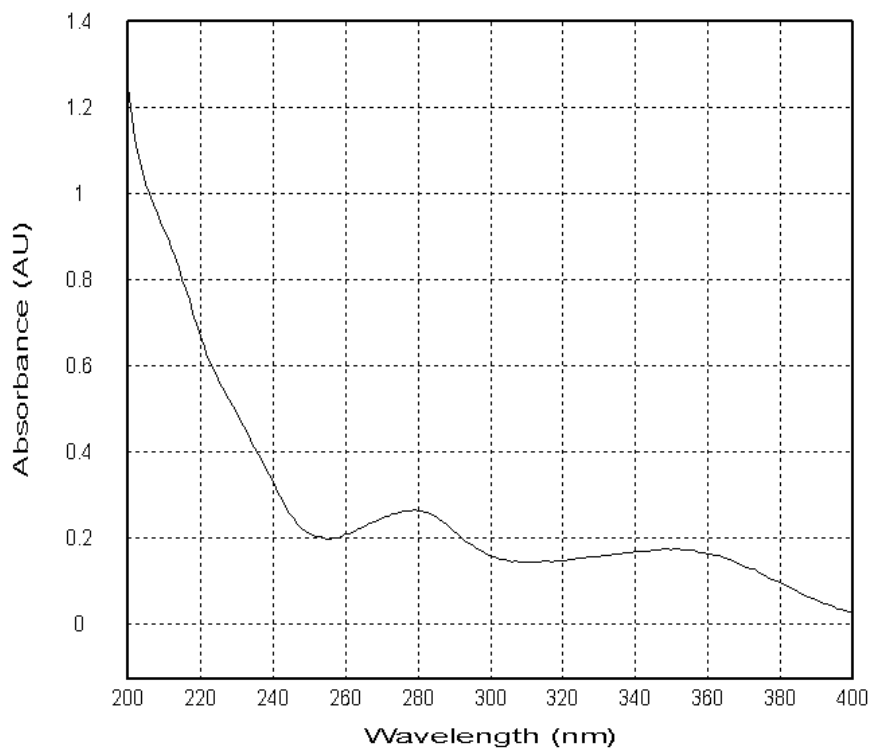


Figure S31. UV spectrum of **3**

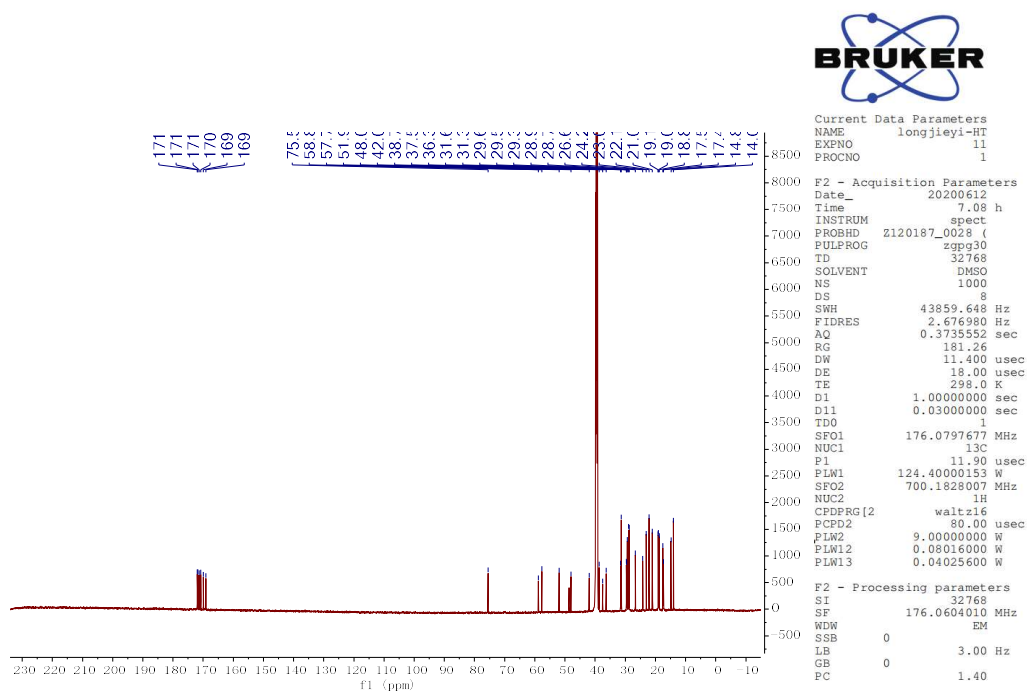


Figure S34. ^{13}C NMR spectrum of **4** (DMSO- d_6 , 125 MHz)

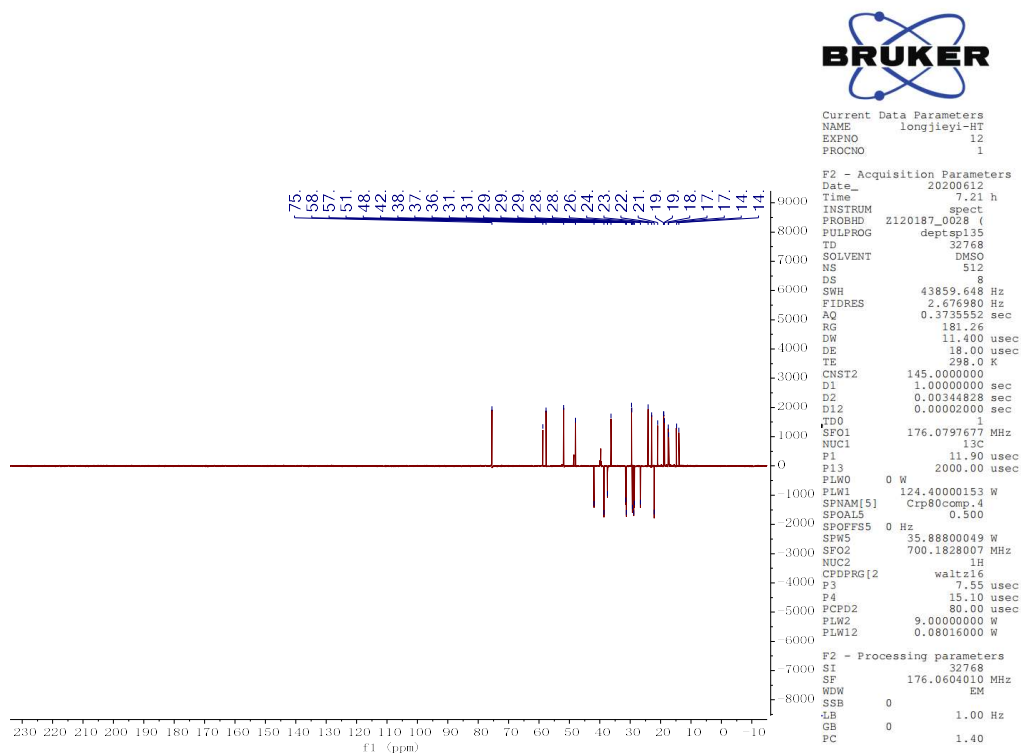


Figure S35. DEPT spectrum of **4** (DMSO- d_6)

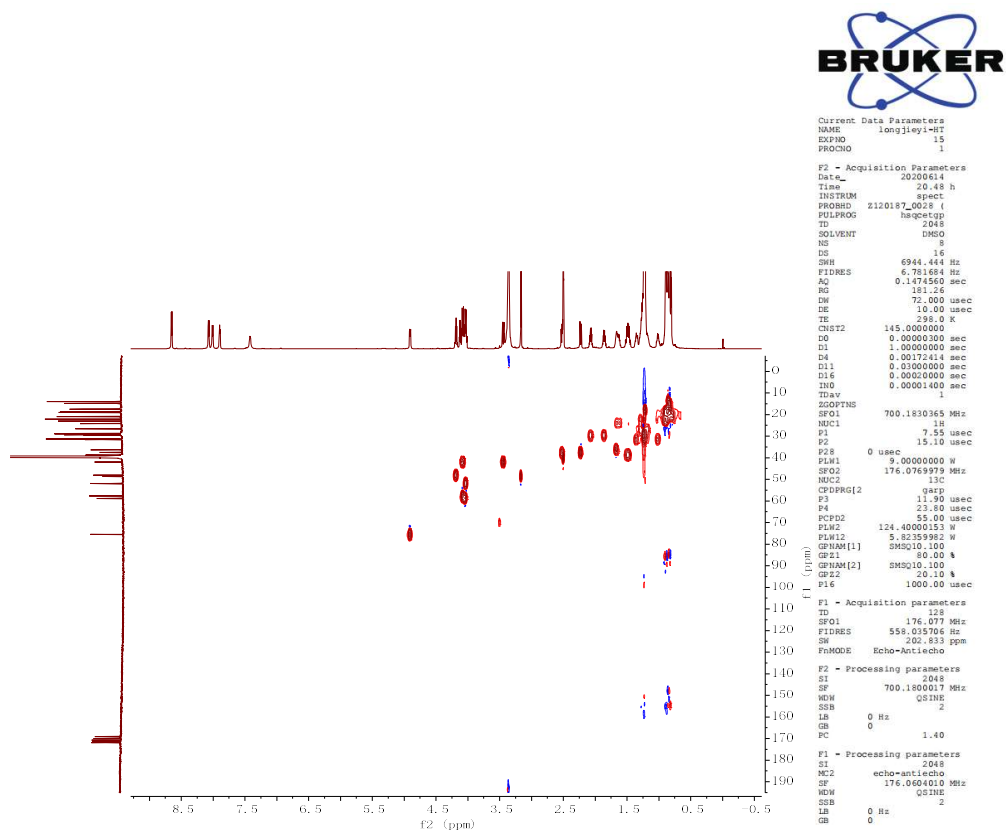


Figure S36. HSQC spectrum of **4** (DMSO- d_6)

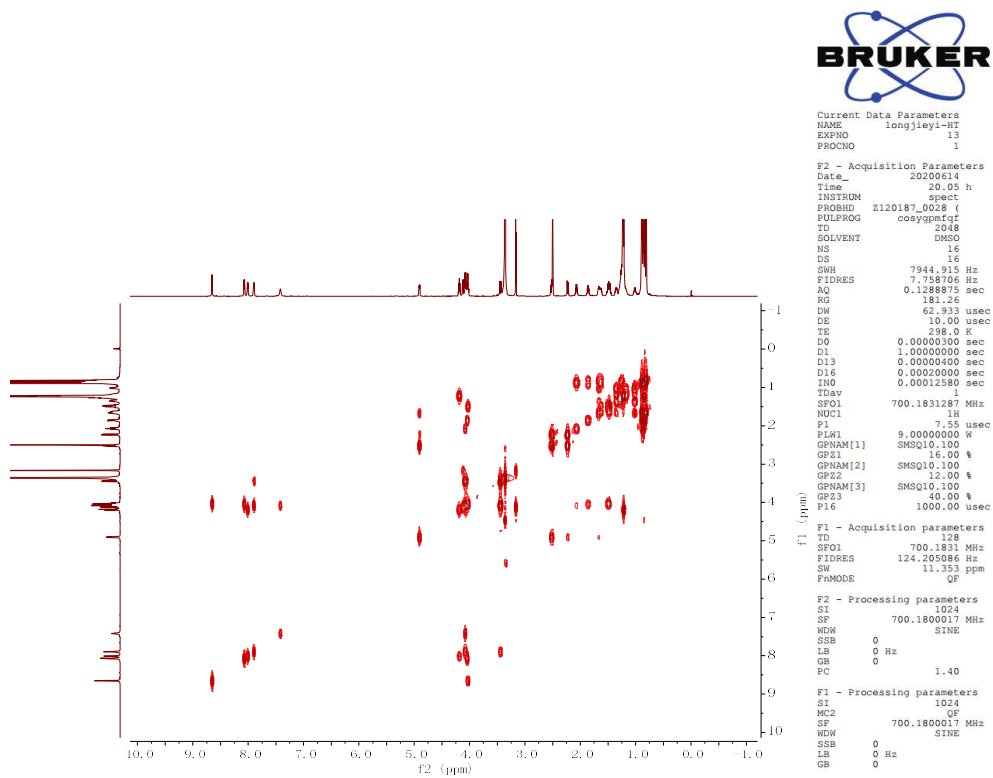


Figure S37. ^1H - ^1H COSY spectrum of **4** (DMSO- d_6)



| P2 - Acquisition Parameters | |
|-----------------------------|-----------------|
| DATE_ | 20020614 |
| TIME | 21.11 h |
| INSTRUM | HRMS |
| PROBHD | Z120187_0028 |
| NUCLEO1 | hmbicqcd300 |
| PULPROG | zgpg30 |
| TD | 4096 |
| SOLVENT | DMSO |
| DS | 16 |
| SWH | 7211.513 Hz |
| FIDRES | 3.512529 Hz |
| AQ | 0.2839893 sec |
| RG | 181.26 |
| DE | 69.93313 usec |
| TE | 300.2 usec |
| TD0 | 728 |
| TD1 | 298.0 K |
| CN1S74 | 120.000000 sec |
| CN1S77 | 165.000000 sec |
| CN1S73 | 8.000000 sec |
| C16 | 0.00000000 sec |
| T1 | 1.00000000 sec |
| T2 | 0.00250000 sec |
| T3 | 0.00000000 sec |
| T4 | 0.00001460 sec |
| FO1 | 700.1835050 sec |
| NUC1 | 13C |
| NUC2 | 1H |
| P1 | 7.55 usec |
| P2 | 15.50 usec |
| P3 | 97.00000000 sec |
| FO2 | 176.076868 sec |
| US2 | 1.00000000 sec |
| P4 | 31.90 usec |
| P5 | 2000.00 usec |
| P6 | 124.000000 sec |
| SPF07 | Cp30comp.4 |
| SPF07 | 0.500 |
| SPF07 | 35.8880004 usec |
| SPF07 | 1.00000000 sec |
| CP1 | 80.00 usec |
| CP2 | SM10.00 usec |
| CP3 | 140.00 usec |
| CP4 | 100.00 usec |
| CP5 | SM10.00 usec |
| CP6 | SM10.00 usec |
| CP7 | SM10.00 usec |
| CP8 | 100.00 usec |
| CP9 | 100.00 usec |
| F1 - Acquisition parameters | |
| TD | 128 |
| FSRES | 726.676 Hz |
| TDRES | 535.12529 Hz |
| SW | 1494.99 ppm |
| NAME | Ecto-Anticido |
| P2 - Processing parameters | |
| SI | 32768 |
| PC | 700.1800017 MHz |
| NUC1 | 13C |
| NUC2 | 1H |
| LB | 4 |
| SB | 0 Hz |
| GB | 0 |
| PC | 1.40 |
| F1 - Processing parameters | |
| SI | 1024 |
| NUC1 | ecto-anticido |
| NUC2 | Q27H |
| GB | 2 |
| LB | 0 Hz |
| SB | 0 |

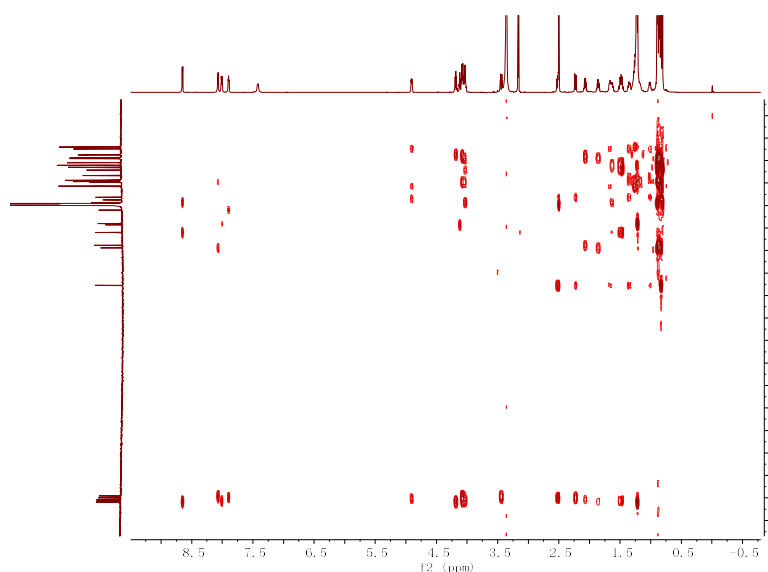


Figure S38. HMBC spectrum of **4** (DMSO-*d*₆)

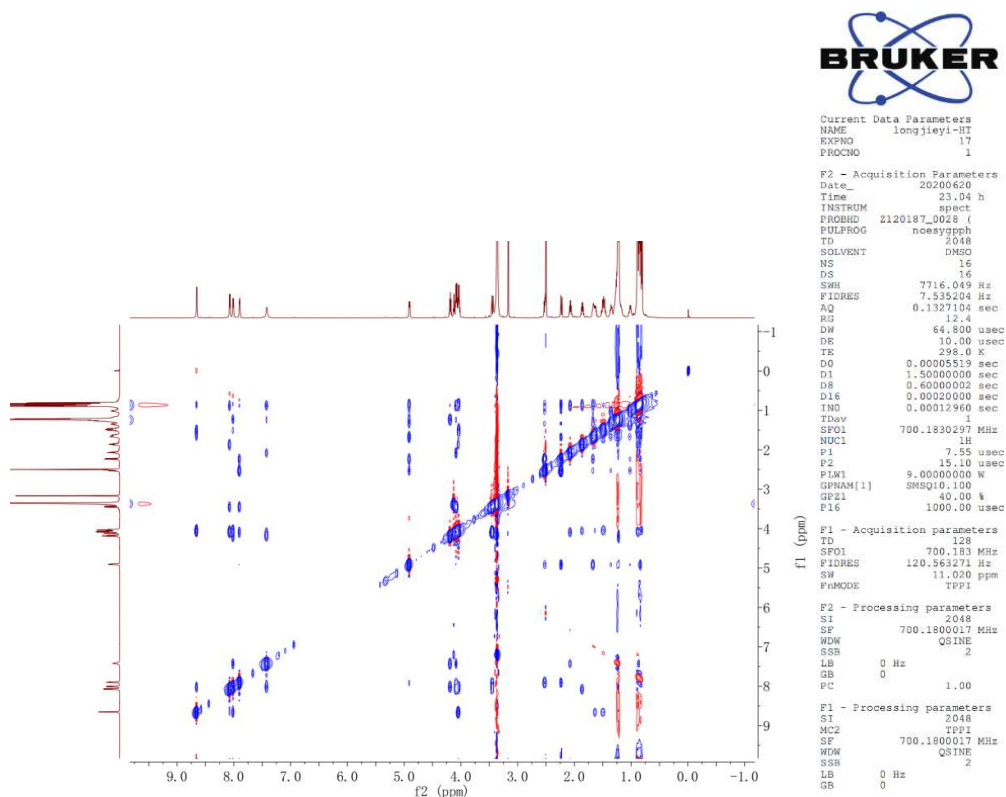


Figure S39. NOESY spectrum of **4** (DMSO- d_6)

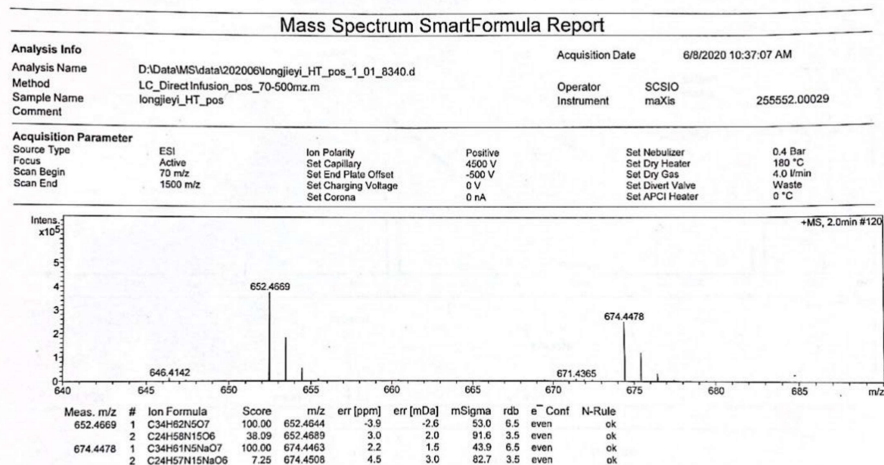


Figure S40. HRESIMS spectrum of **4**

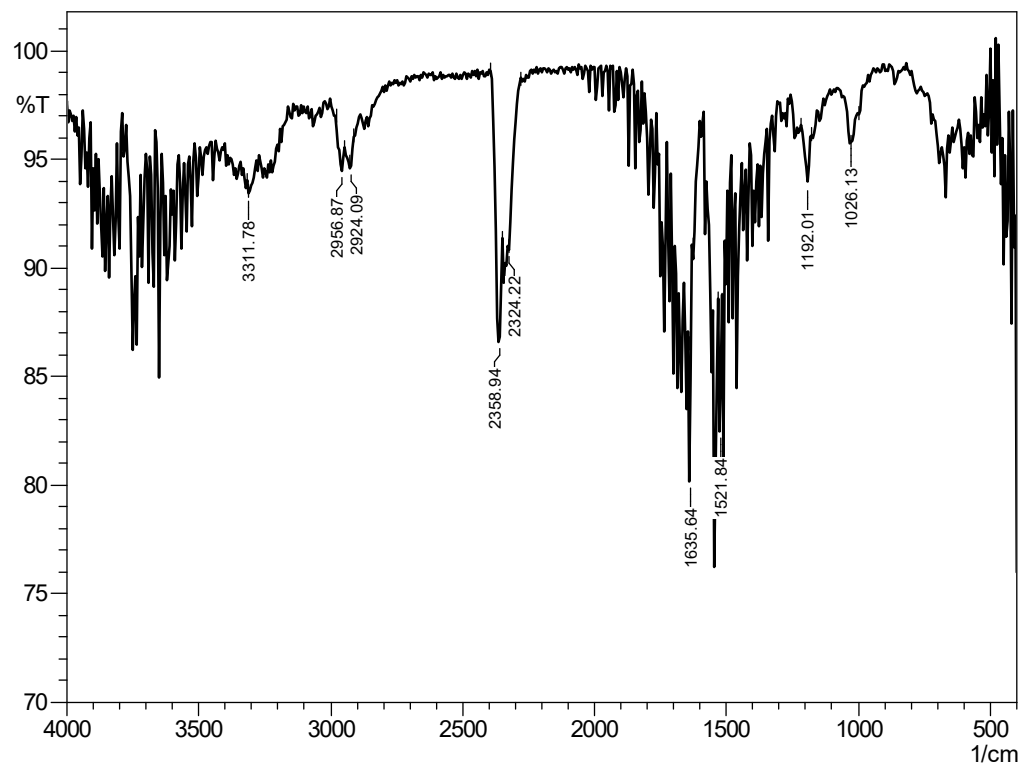


Figure S41. IR spectrum of 4

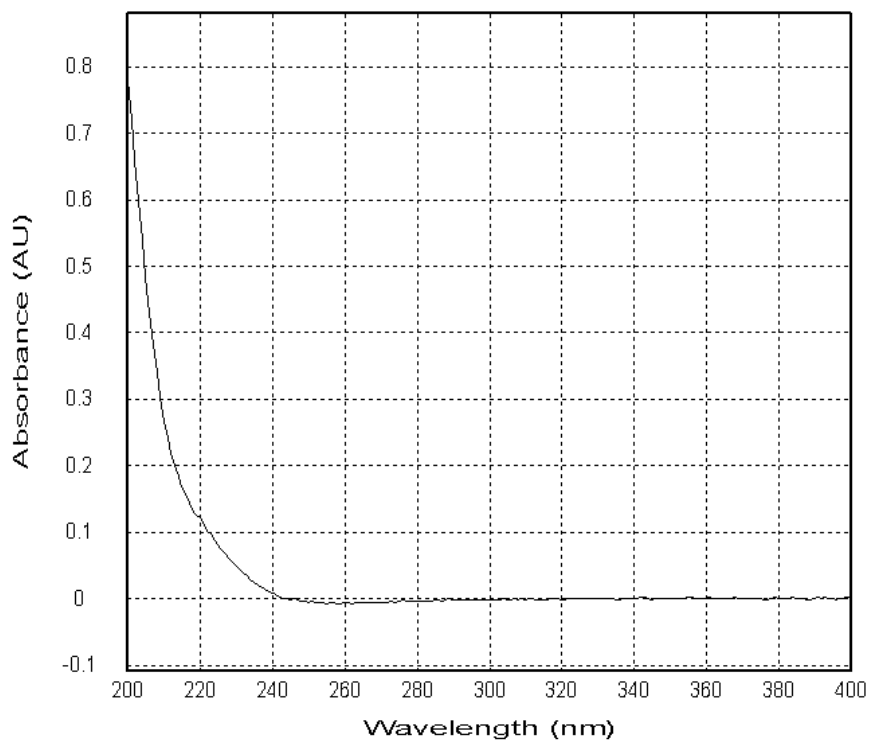


Figure S42. UV spectrum of 4

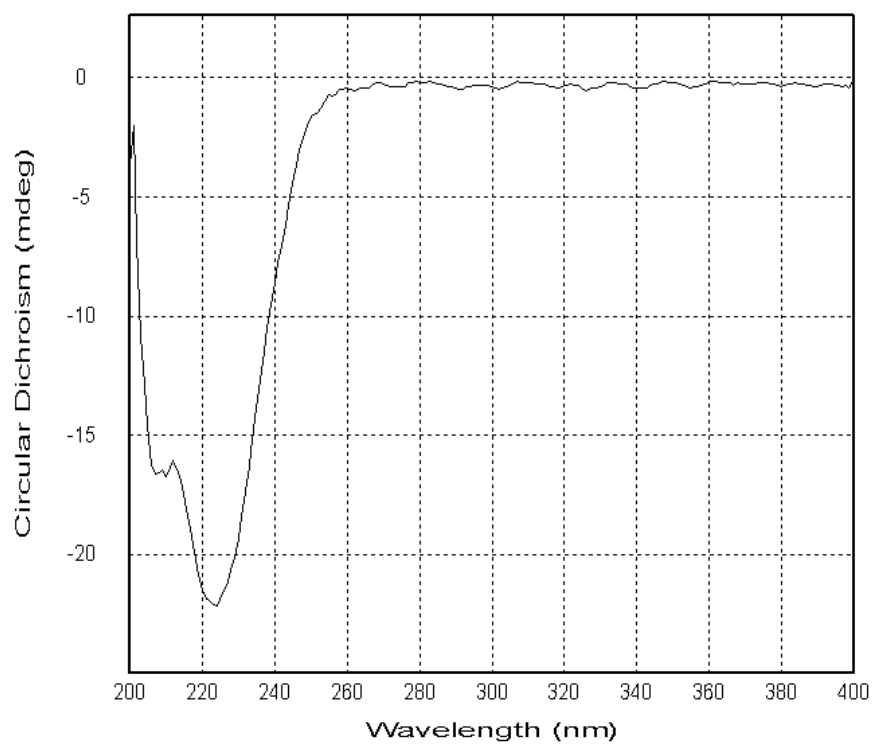
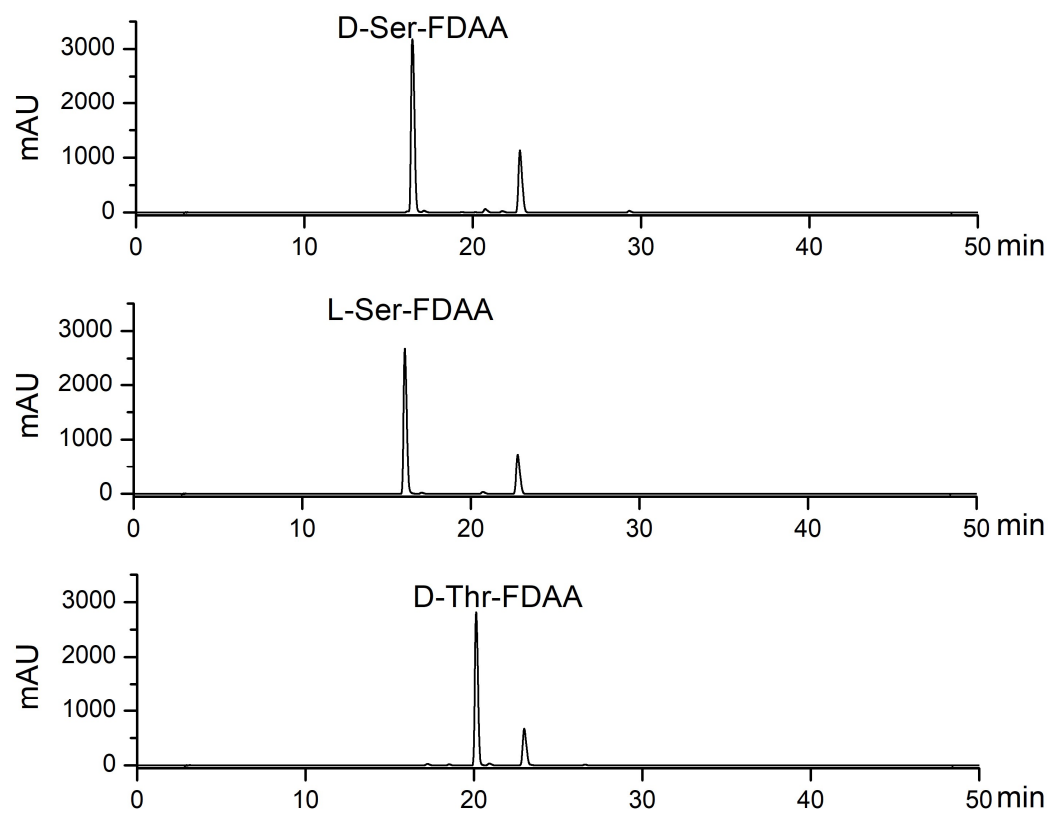


Figure S43. CD spectrum of **4**



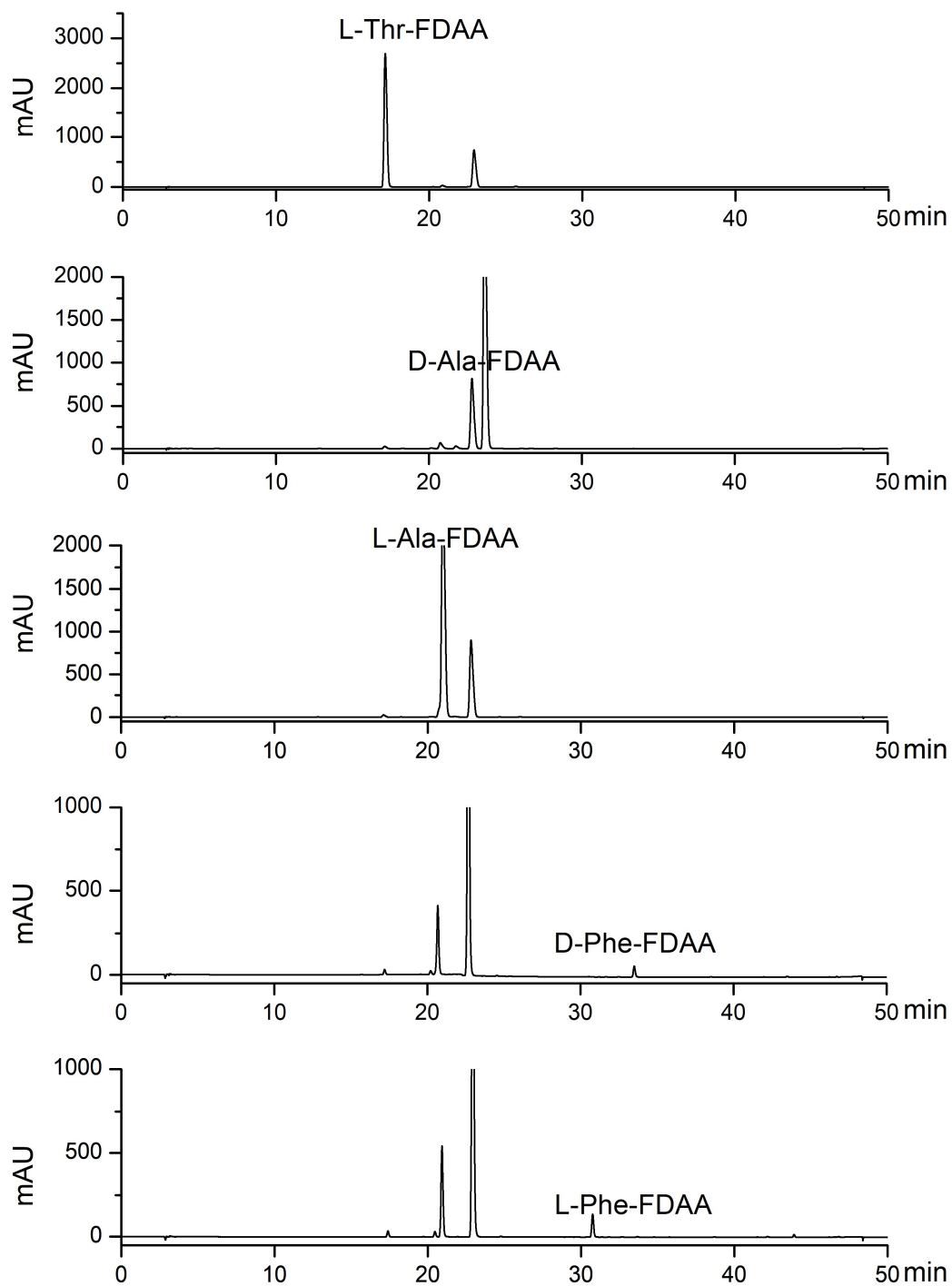


Figure S44. HPLC analysis of FDAA derivatives of standard amino acids (YMC-Pack ODS-A column, 250*4.6 mm I.D., S-5 μ m, 12 nm).

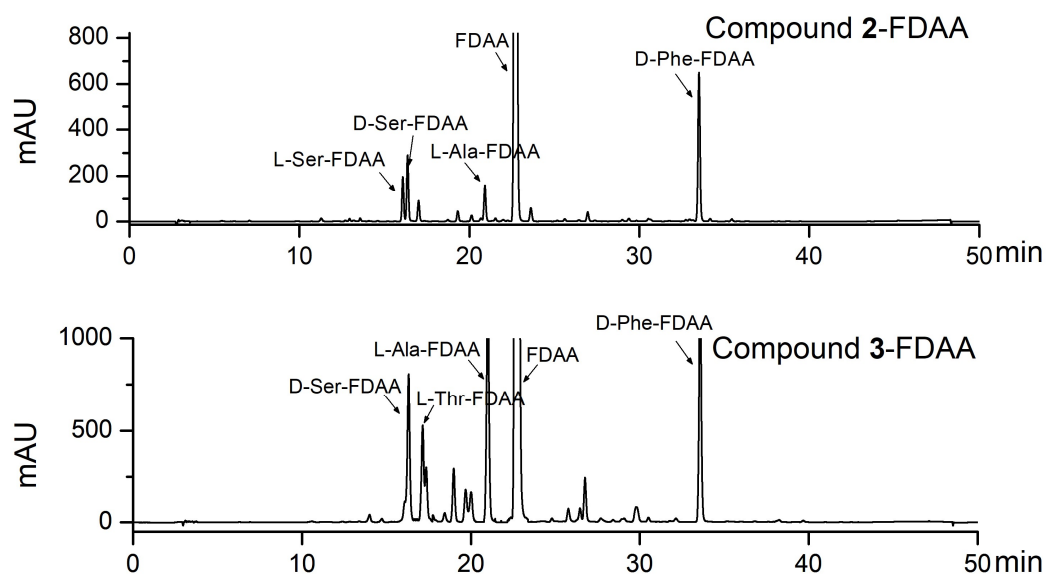


Figure S45. HPLC analysis of FDAA derivatives of compound **2** and **3** (YMC-Pack ODS-A column, 250*4.6 mmI.D., S-5 μ m, 12 nm)

| Test concentration of 4 μ g/mL | Average OD |
|------------------------------------|------------|
| 100 | 0.54 |
| 75 | 0.56 |
| 50 | 0.61 |
| 25 | 0.71 |
| 13 | 1.62 |

Table S1. Test concentration and OD value of compound **4** against AChE.

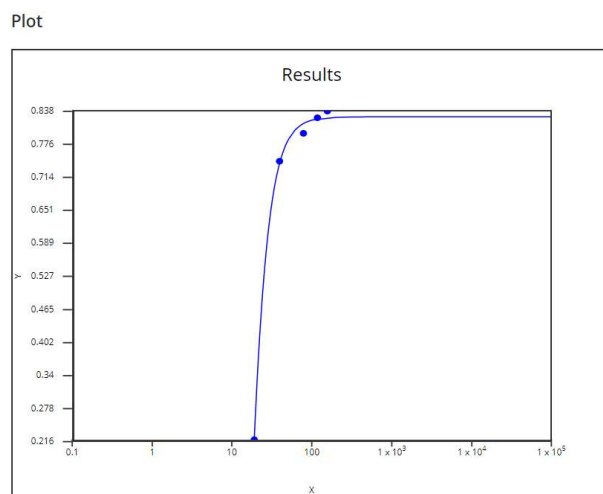


Figure S46. IC₅₀ curve of compound **4** against AChE.

| Test concentration | OD value of HONE1-EBV | Test concentration | OD value of HONE1 |
|--------------------|-----------------------|--------------------|-------------------|
| 50.000 | 0.400 | 100.000 | 0.328 |
| 16.667 | 0.329 | 33.333 | 0.305 |
| 5.556 | 0.528 | 11.111 | 0.729 |
| 1.852 | 0.968 | 3.704 | 0.880 |
| 0.617 | 1.074 | 1.235 | 0.922 |
| 0.206 | 1.111 | 0.412 | 1.066 |
| 0.068 | 1.092 | 0.137 | 1.153 |
| 0.023 | 1.047 | 0.046 | 1.020 |
| 0.008 | 0.997 | 0.015 | 1.084 |
| 0.003 | 0.976 | 0.005 | 1.021 |

Table S2. Test concentration and OD value of compound **4** against HONE1-EBV and HONE1.

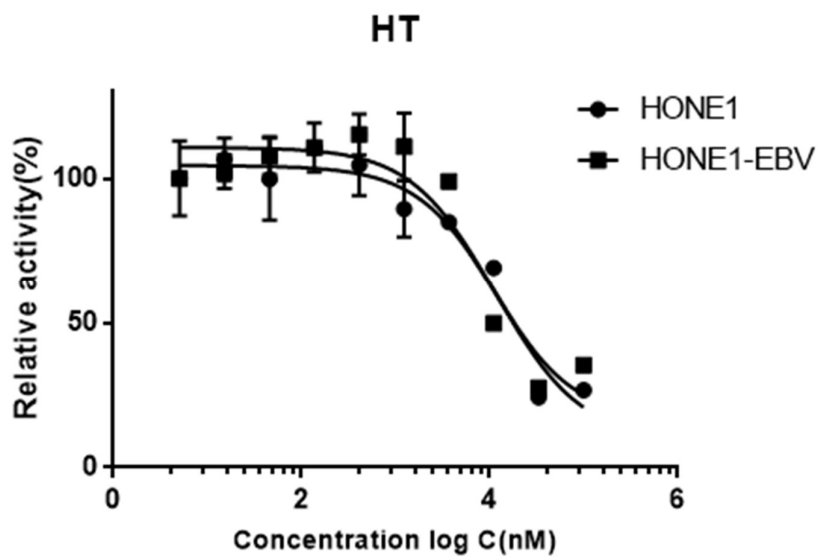


Figure S47. IC₅₀ curve of compound **4** against HONE1-EBV and HONE1.