

Four new phenylethanoid and flavonoid glycoside dimers from the fruits of *Forsythia suspensa* and their neuroprotective activities

Fan Zhang,^{a,b,†} Ya-Nan Yang,^{a,‡} Zi-Ming Feng,^a Jian-Shuang Jiang,^a and Pei-Cheng
Zhang^{a*}

^a*State Key Laboratory of Bioactive Substance and Function of Natural Medicines,
Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union
Medical College, Beijing 100050, People's Republic of China*

^b*Beijing Changping Hospital of Integrated Chinese and Western Medicine, Beijing
102208, People's Republic of China*

Supporting information

The List of Contents

No	Content	Page
1	Figure S1. Two optimized conformations of 1 with the population (%)	S3
2	Figure S2. The IR spectrum of compound 1*	S4
3	Figure S3. The UV spectrum of compound 1*	S4
4	Figure S4. The ¹ H NMR spectrum of compound 1* in acetic acid- <i>d</i> ₄	S5
5	Figure S5. The ¹³ C NMR spectrum of compound 1* in acetic acid- <i>d</i> ₄	S5
6	Figure S6. The HSQC spectrum of compound 1* in acetic acid- <i>d</i> ₄	S6
7	Figure S7. The HMBC spectrum of compound 1* in acetic acid- <i>d</i> ₄	S6
8	Figure S8. The ¹ H- ¹ H COSY spectrum of compound 1* in acetic acid- <i>d</i> ₄	S7
9	Figure S9. The ROESY spectrum of compound 1* in acetic acid- <i>d</i> ₄	S7
10	Figure S10. The TOCSY spectrum of compound 1* in acetic acid- <i>d</i> ₄	S8
11	Figure S11. The CD spectrum of 1* in MeOH	S8
12	Figure S12. The HRESIMS of compound 1*	S9
13	Figure S13. The IR spectrum of compound 2*	S9
14	Figure S14. The UV spectrum of compound 2*	S10
15	Figure S15. The ¹ H NMR spectrum of compound 2* in methanol- <i>d</i> ₄	S10
16	Figure S16. The ¹³ C NMR spectrum of compound 2* in methanol- <i>d</i> ₄	S11
17	Figure S17. The HSQC spectrum of compound 2* in methanol- <i>d</i> ₄	S11
18	Figure S18. The HMBC spectrum of compound 2* in methanol- <i>d</i> ₄	S12
19	Figure S19. The ¹ H- ¹ H COSY spectrum of compound 2* in methanol- <i>d</i> ₄	S12
20	Figure S20. The ROESY spectrum of compound 2* in methanol- <i>d</i> ₄	S13
21	Figure S21. The CD spectrum of 2* in MeOH : H ₂ O 1:1	S13
22	Figure S22. The HRESIMS of compound 2*	S14
23	Figure S23. The IR spectrum of compound 3*	S14
24	Figure S24. The UV spectrum of compound 3*	S15
25	Figure S25. The ¹ H NMR spectrum of compound 3* in acetic acid- <i>d</i> ₄	S15

26	Figure S26. The ^{13}C NMR spectrum of compound 3* in acetic acid- d_4	S16
27	Figure S27. The HSQC spectrum of compound 3* in acetic acid- d_4	S16
28	Figure S28. The HMBC spectrum of compound 3* in acetic acid- d_4	S17
29	Figure S29. The ^1H - ^1H COSY spectrum of compound 3* in acetic acid- d_4	S17
30	Figure S30. The ROESY spectrum of compound 3* in acetic acid- d_4	S18
31	Figure S31. The CD spectrum of 3* in MeOH : H ₂ O 1:1	S18
32	Figure S32. The overlay CD spectrum of 2* and 3*	S19
33	Figure S33. The HRESIMS of compound 3*	S19
34	Figure S34. The IR spectrum of compound 4*	S20
35	Figure S35. The UV spectrum of compound 4*	S20
36	Figure S36. The ^1H NMR spectrum of compound 4* in acetic acid- d_4	S21
37	Figure S37. The ^{13}C NMR spectrum of compound 4* in acetic acid- d_4	S21
38	Figure S38. The HSQC spectrum of compound 4* in acetic acid- d_4	S22
39	Figure S39. The HMBC spectrum of compound 4* in acetic acid- d_4	S22
40	Figure S40. The ^1H - ^1H COSY spectrum of compound 4* in acetic acid- d_4	S23
41	Figure S41. The ROESY spectrum of compound 4* in acetic acid- d_4	S23
42	Figure S42. The CD spectrum of 4* in MeOH : H ₂ O 1:1	S24
43	Figure S43. The HRESIMS of compound 4*	S24

Experimental section

Neuroprotective effects of compounds 1-4. Compounds 1-4 were tested for neuroprotective effects against rotenone-induced injury in PC12 cells using an MTT assay. The PC12 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 5% horse serum, 5% fetal bovine serum. Then, 100 μ l cells with an initial density of 5×10^4 cells/ml were seeded in each well of a poly-L-lysine coated 96-well culture plates and precultured for 24h at 37 °C under a 5% CO₂ atmosphere. Afterwards, the medium were placed by different fresh medium including the control (complete medium), the model (complete medium with 4 μ M rotenone) and the sample (complete medium with 4 μ M rotenone and 1 μ M test samples), and the cells were cultured for 48 h. Then, 10 μ l MTT (0.5 mg/ml) was added to each well. After incubation for 4 h, the medium was removed and 100 μ l DMSO was added to dissolve formazan crystals. The optical density (OD) of the PC12 cells was measured on a microplate reader at 550 nm. The cell viability (%) of each sample was calculated by the following formula: Cell viability (%) = $OD_{(\text{model or sample})} / OD_{\text{control}} \times 100$

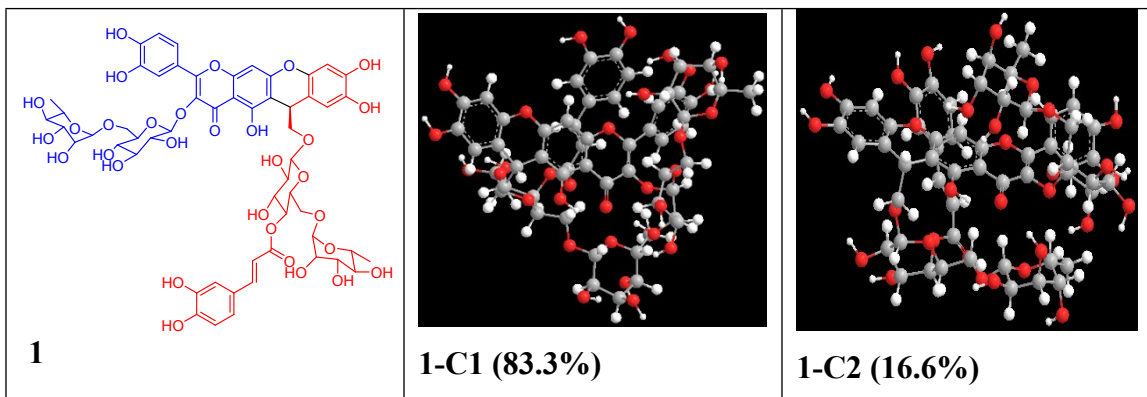


Figure S1 Two Optimized Conformations of **1** with the Population (%)

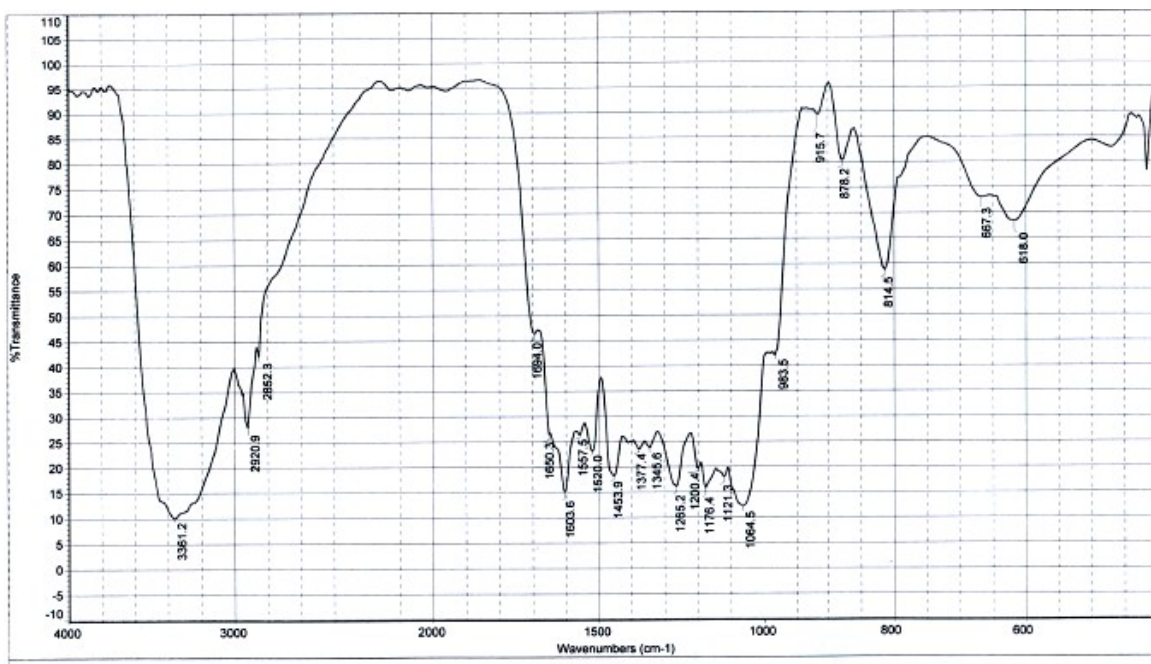


Figure S2. The IR spectrum of compound **1***

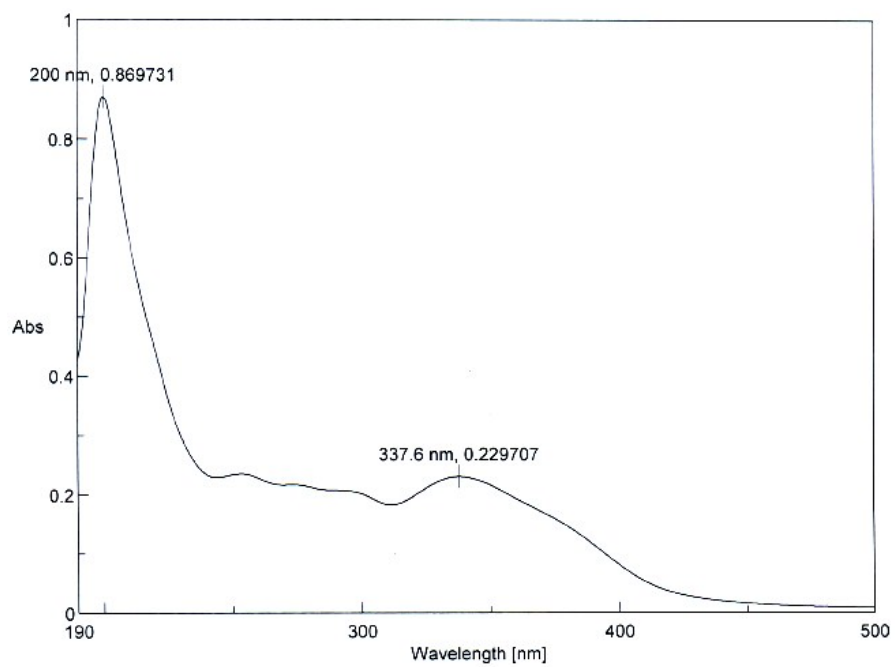


Figure S3. The UV spectrum of compound **1***

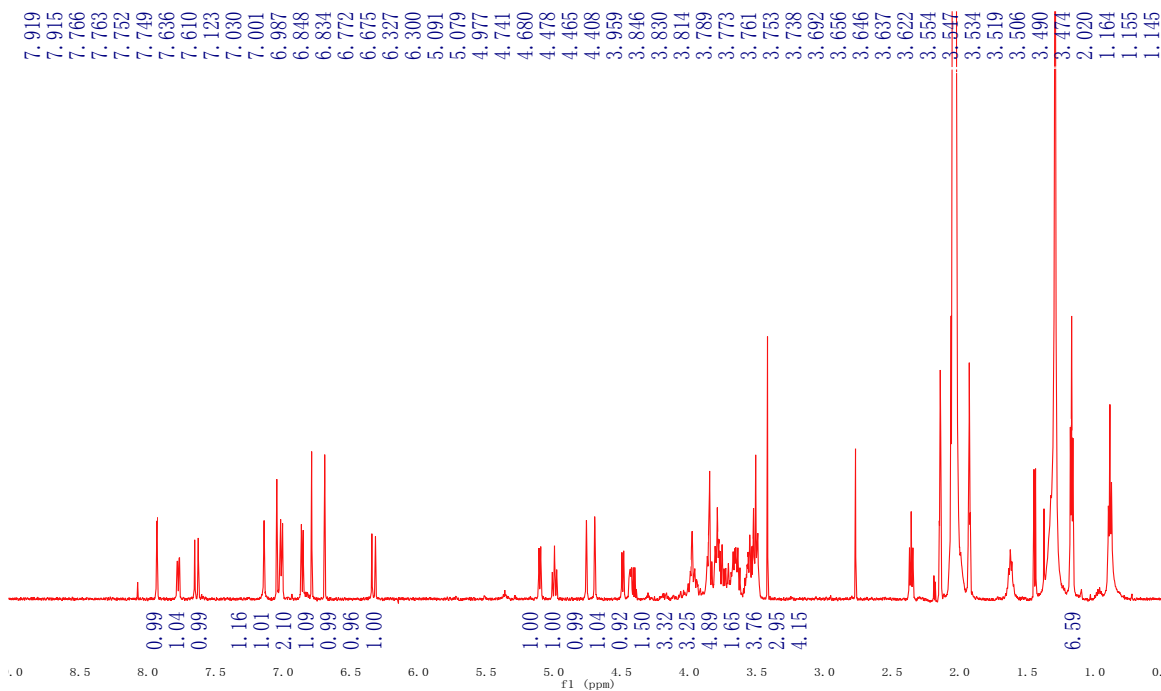


Figure S4. The ^1H NMR spectrum of compound **1*** in acetic acid- d_4

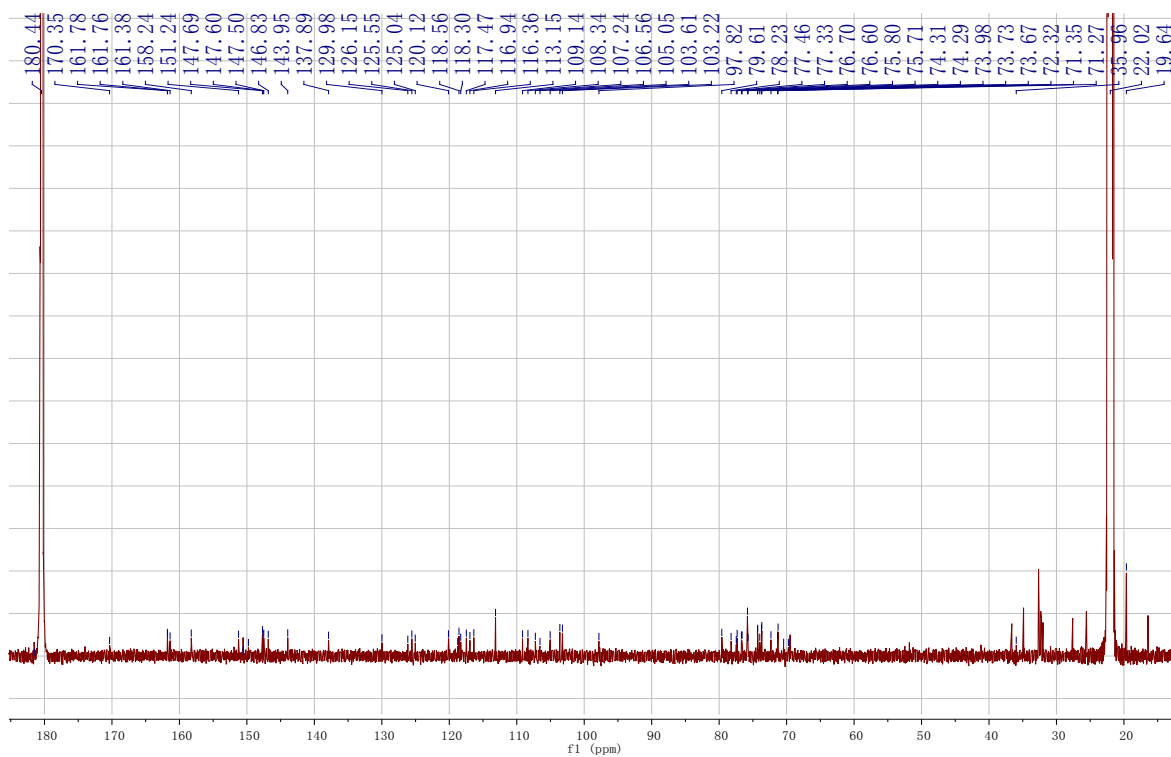


Figure S5. The ^{13}C NMR spectrum of compound **1*** in acetic acid- d_4

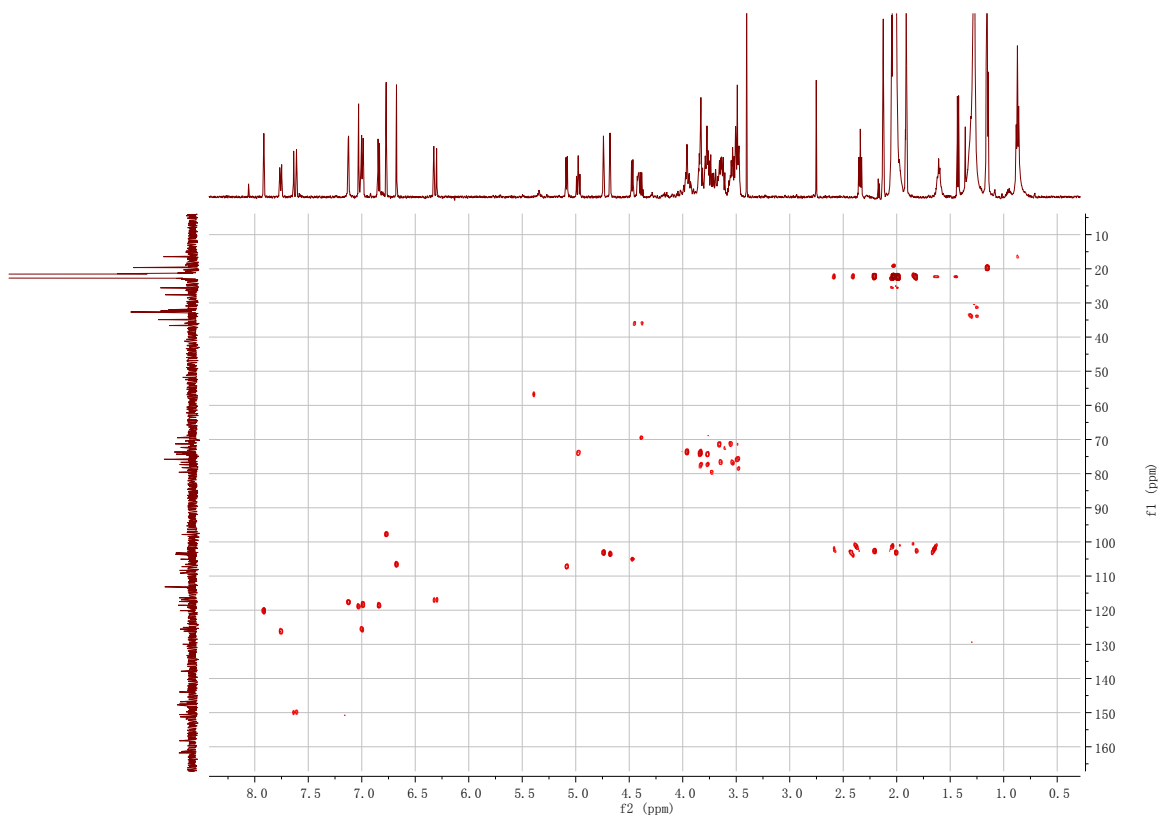


Figure S6. The HSQC spectrum of compound **1*** in acetic acid- d_4

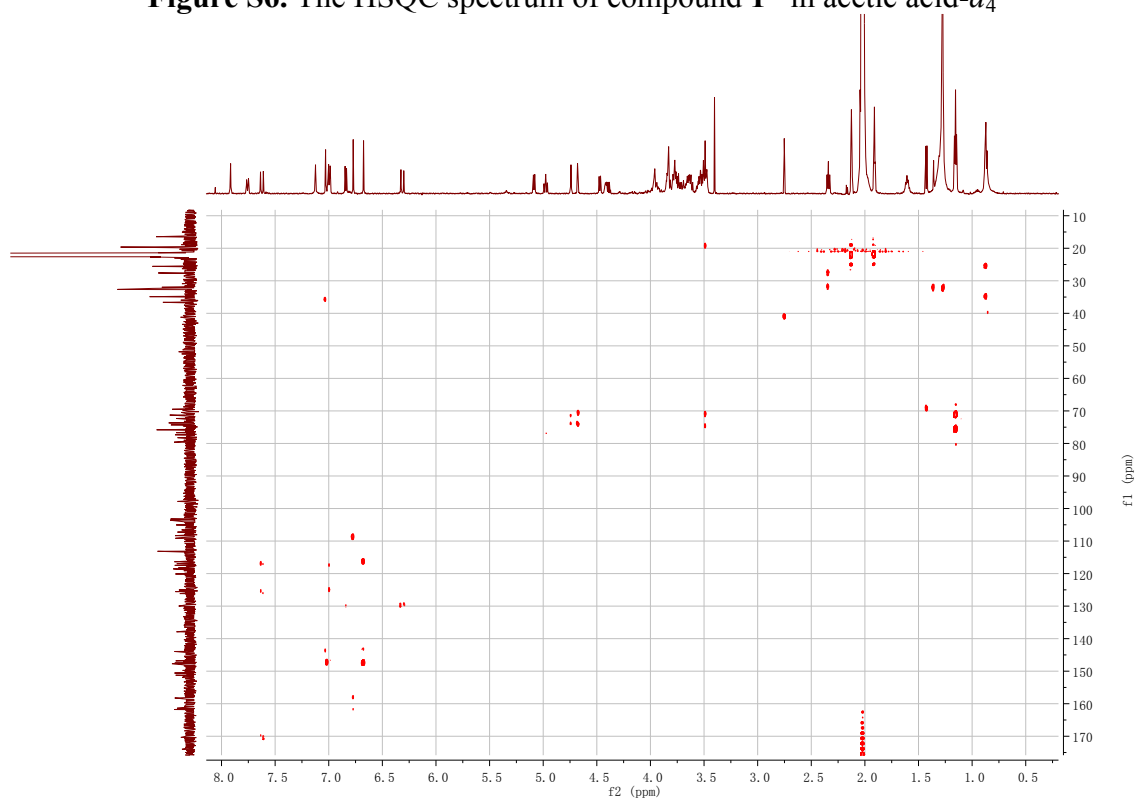


Figure S7. The HMBC spectrum of compound **1*** in acetic acid- d_4

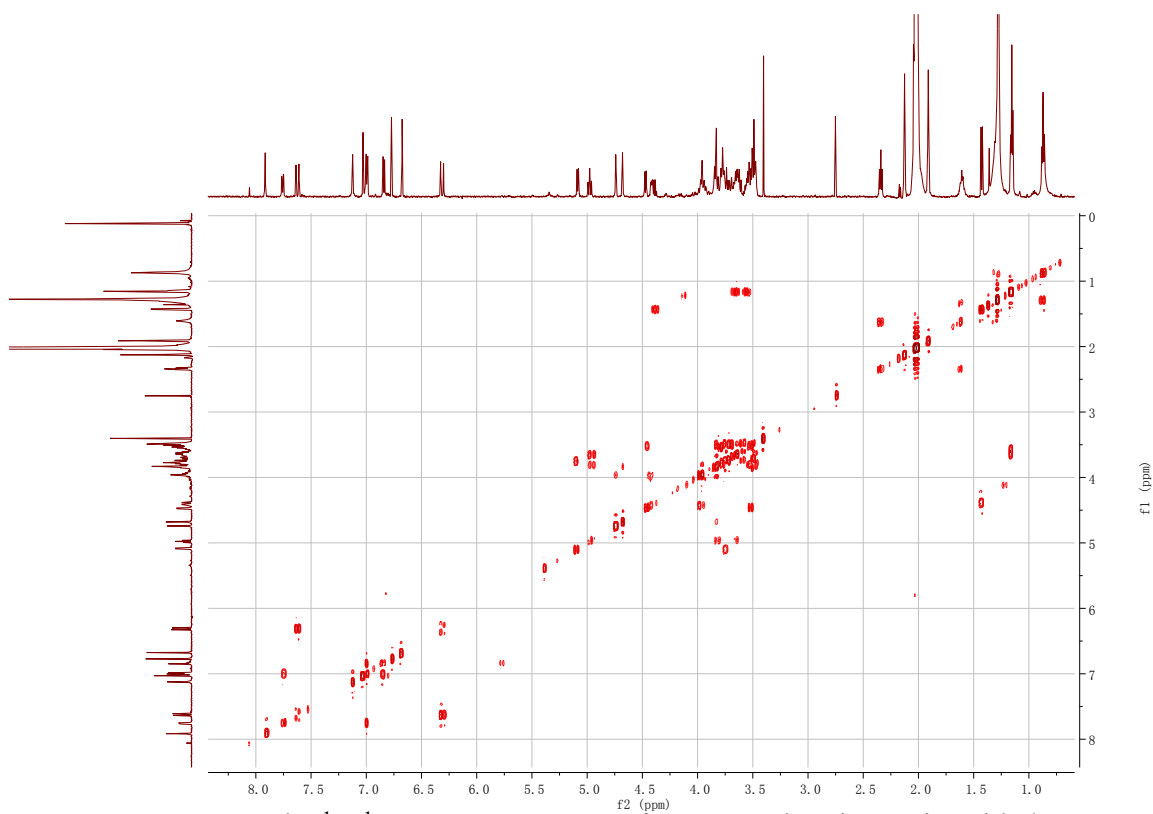


Figure S8. The ^1H - ^1H COSY spectrum of compound **1*** in acetic acid- d_4

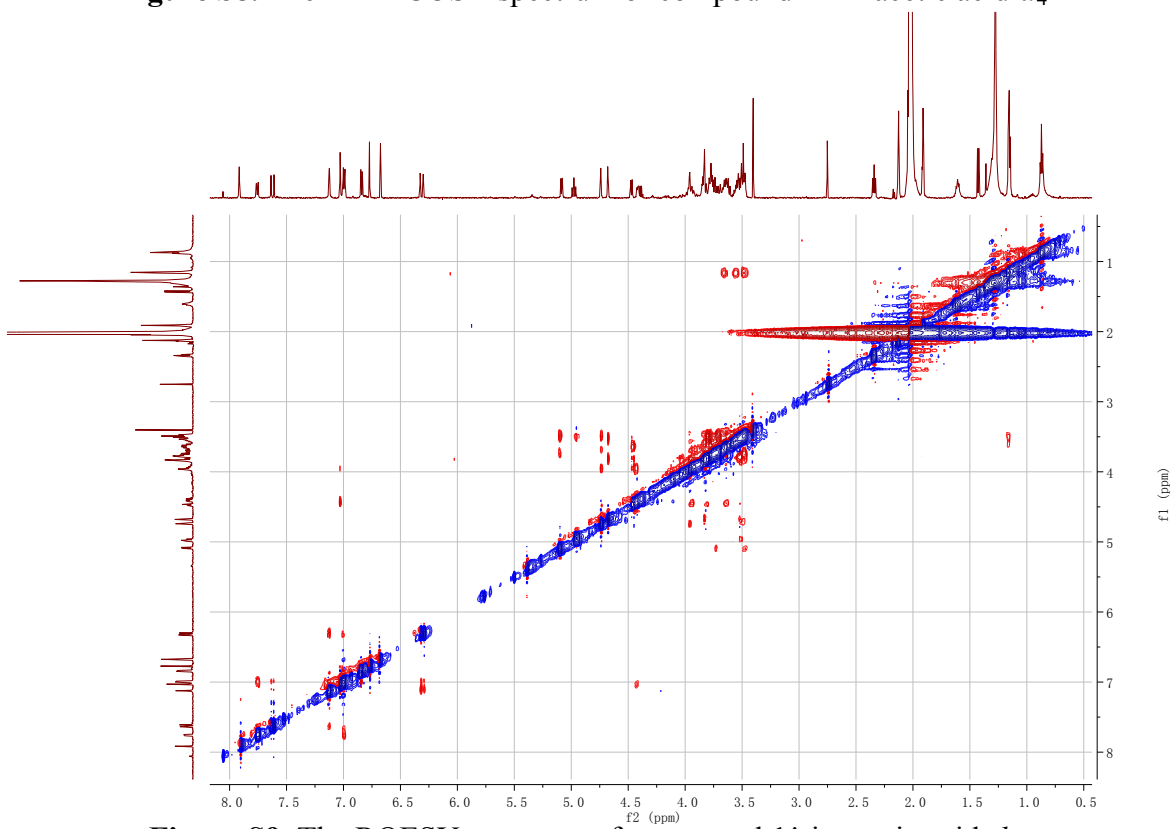


Figure S9. The ROESY spectrum of compound **1*** in acetic acid- d_4

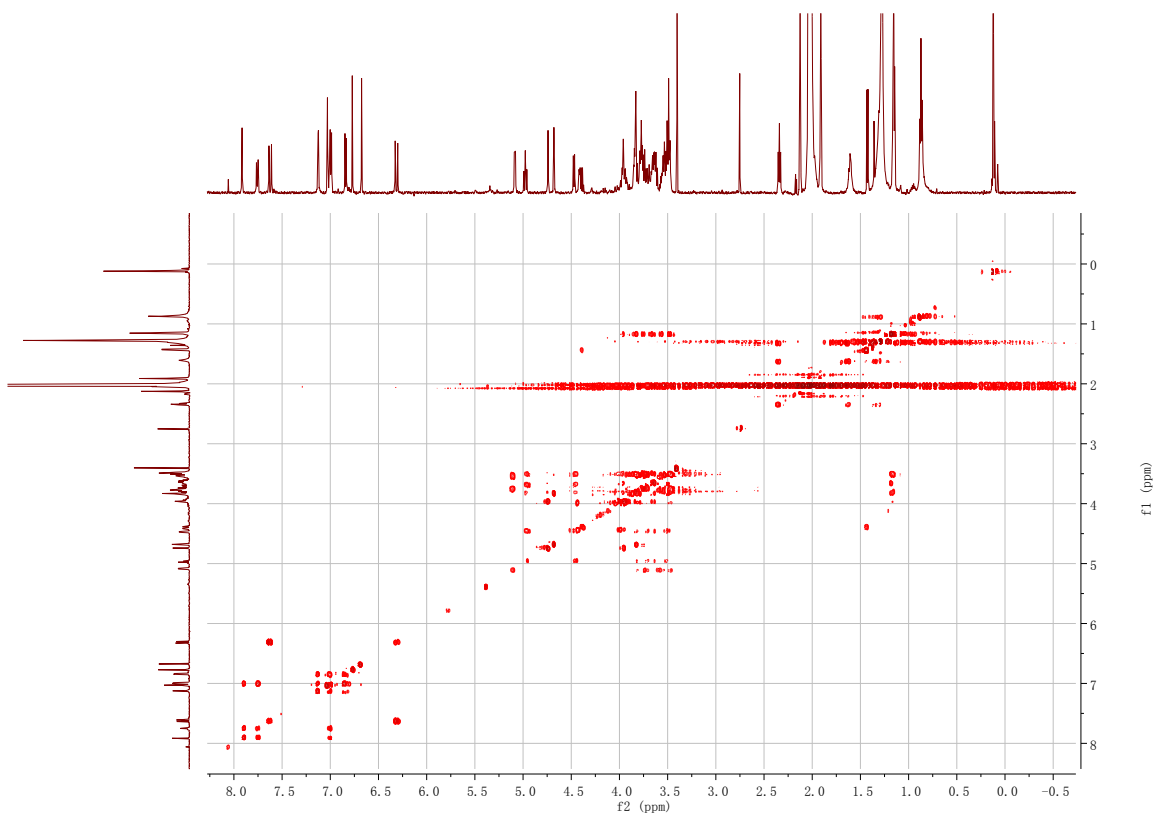


Figure S10. The TOCSY spectrum of compound **1*** in acetic acid- d_4

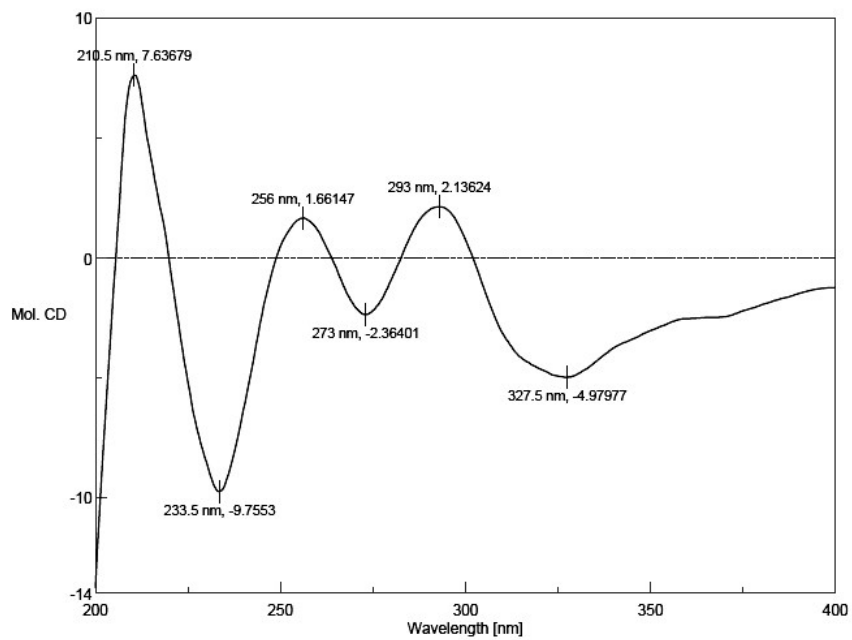


Figure S11. The CD spectrum of **1*** in MeOH

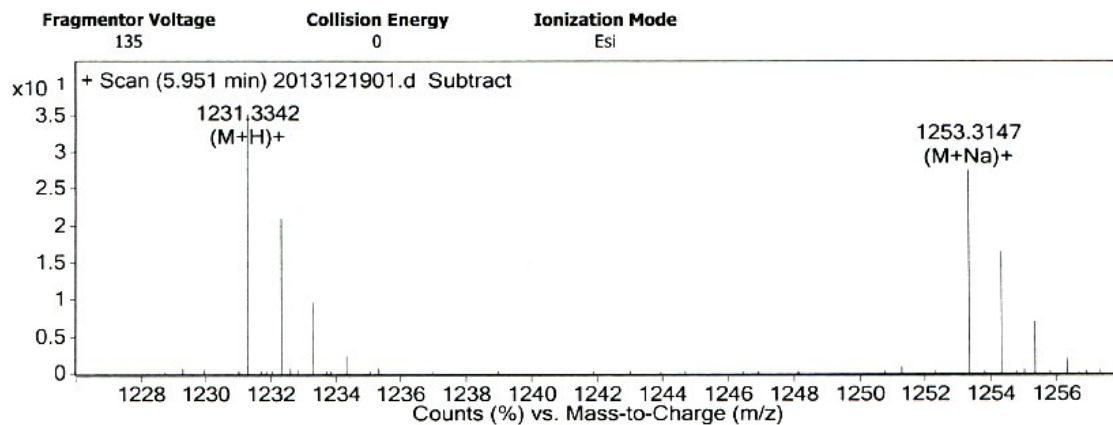


Figure S12. The HRESIMS of compound 1*

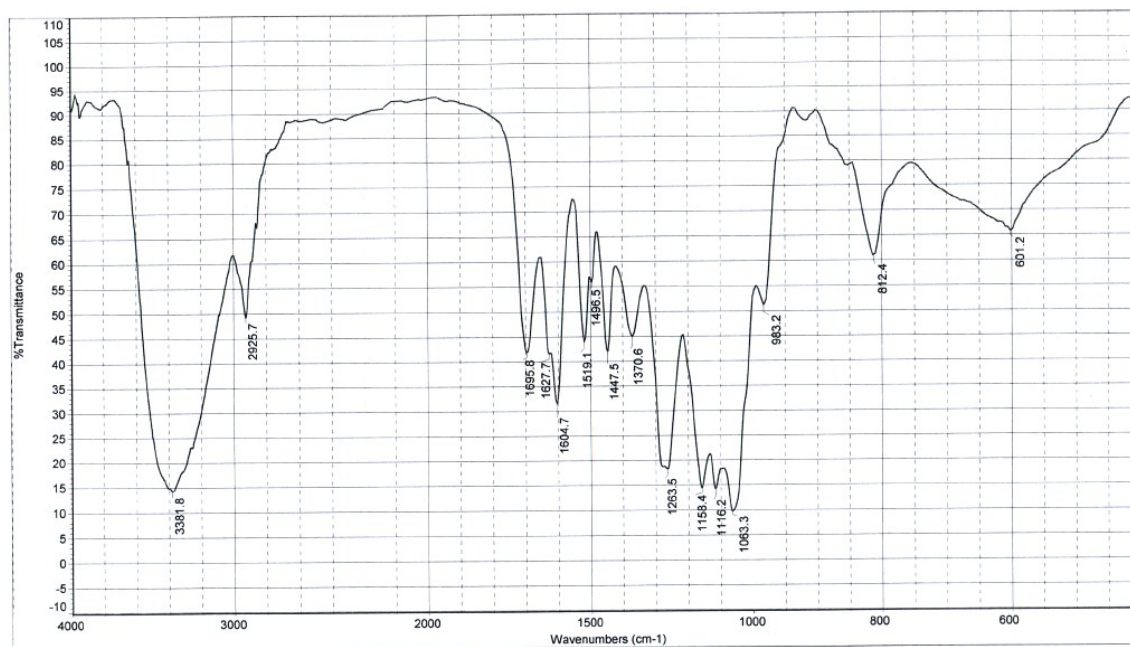


Figure S13. The IR spectrum of compound 2*

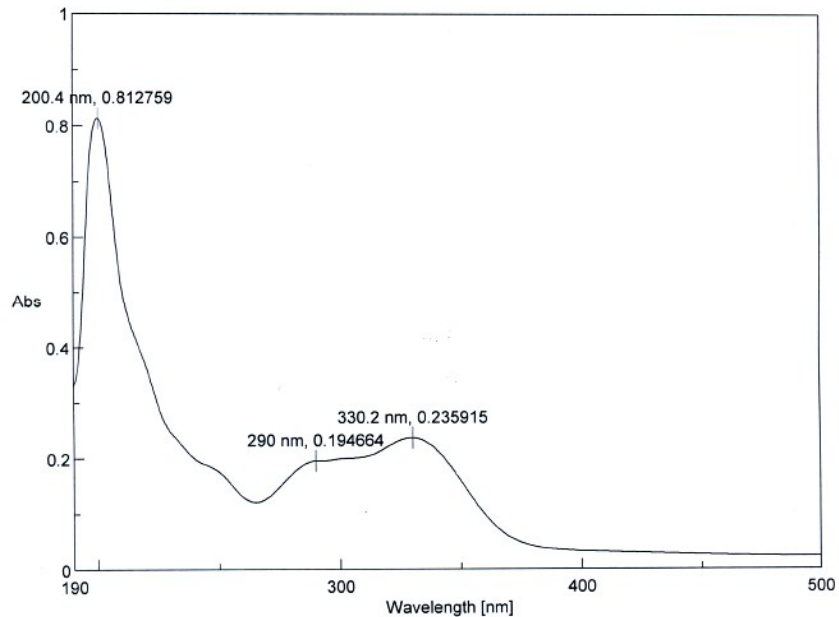


Figure S14. The UV spectrum of compound 2*

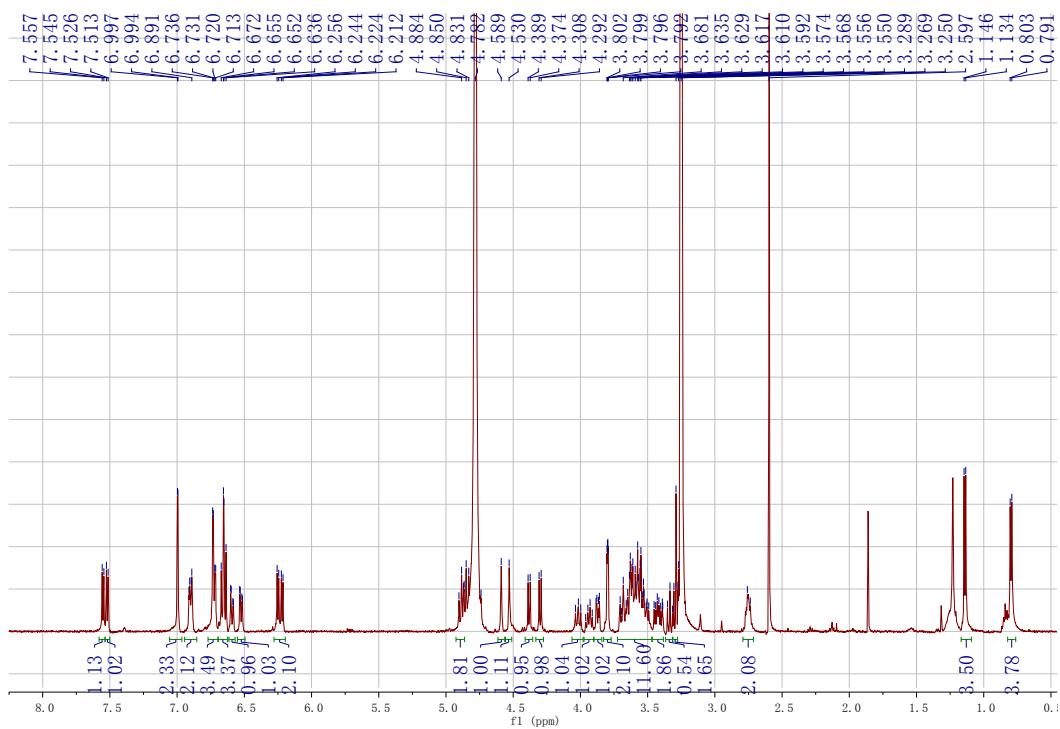


Figure S15. The ¹H NMR spectrum of compound 2* in methanol-*d*₄

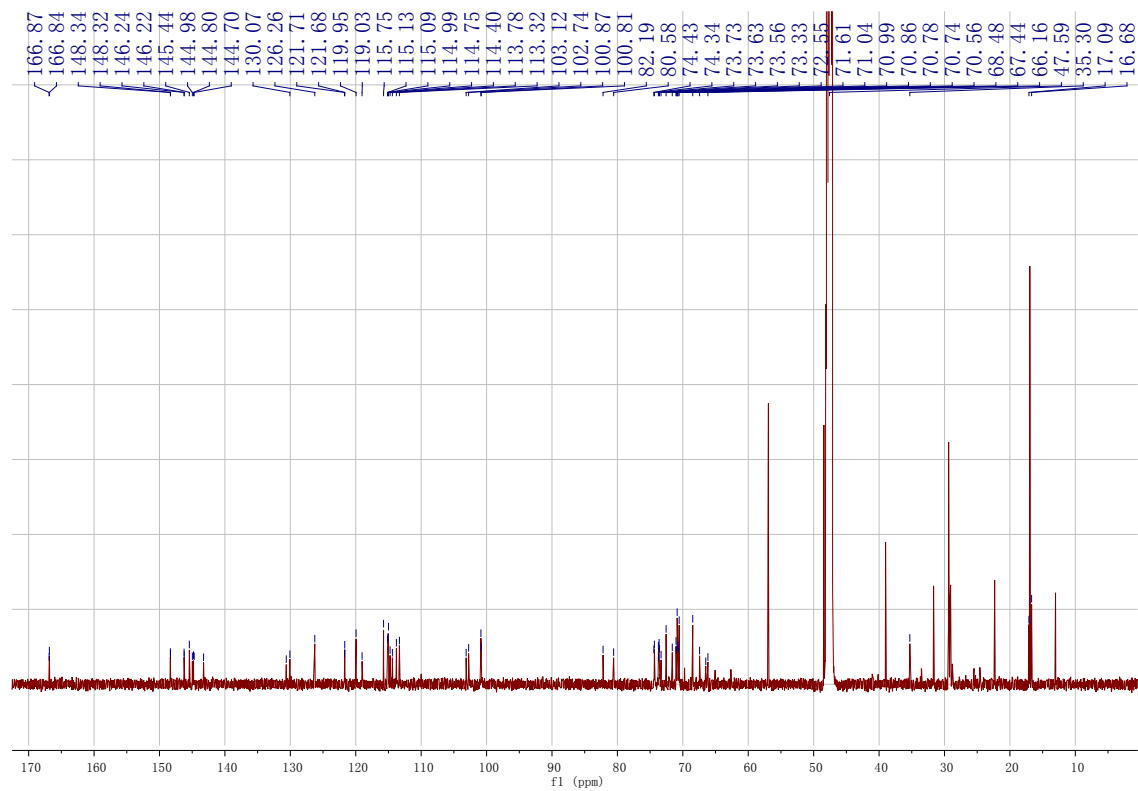


Figure S16. The ^{13}C NMR spectrum of compound **2*** in methanol- d_4

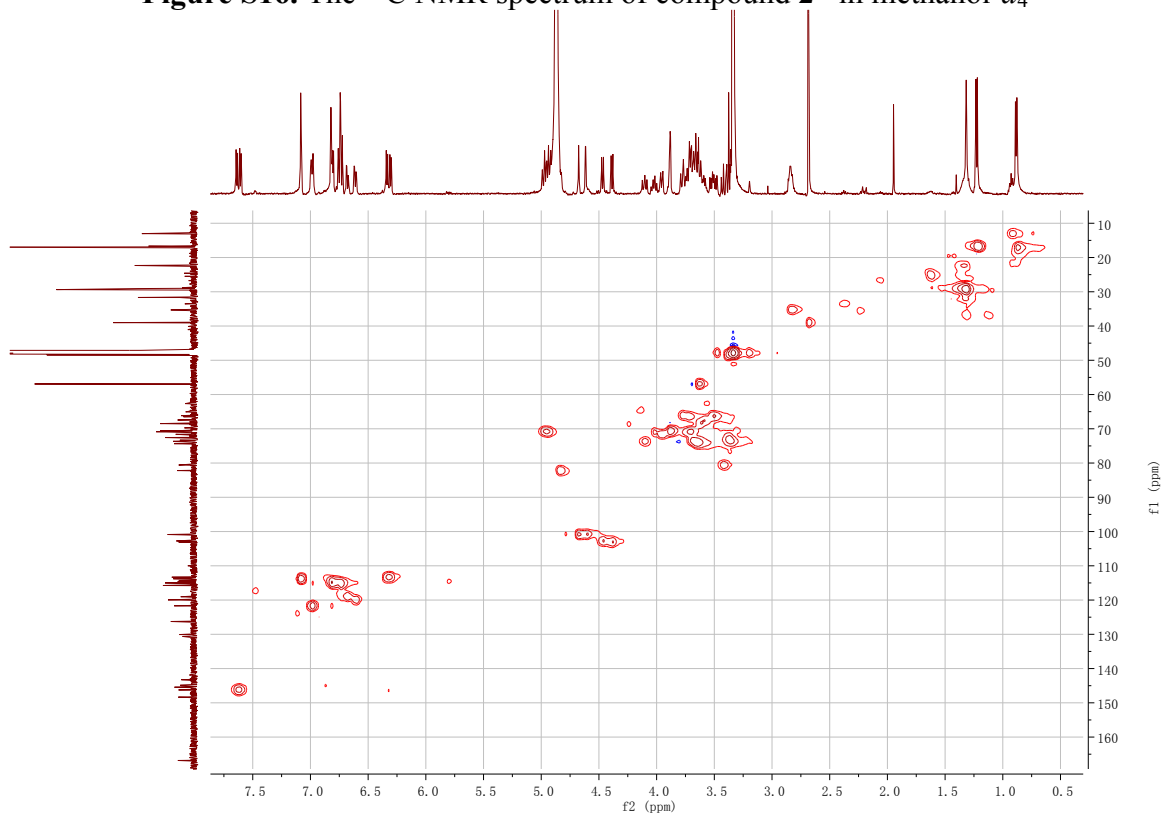


Figure S17. The HSQC spectrum of compound **2*** in methanol- d_4

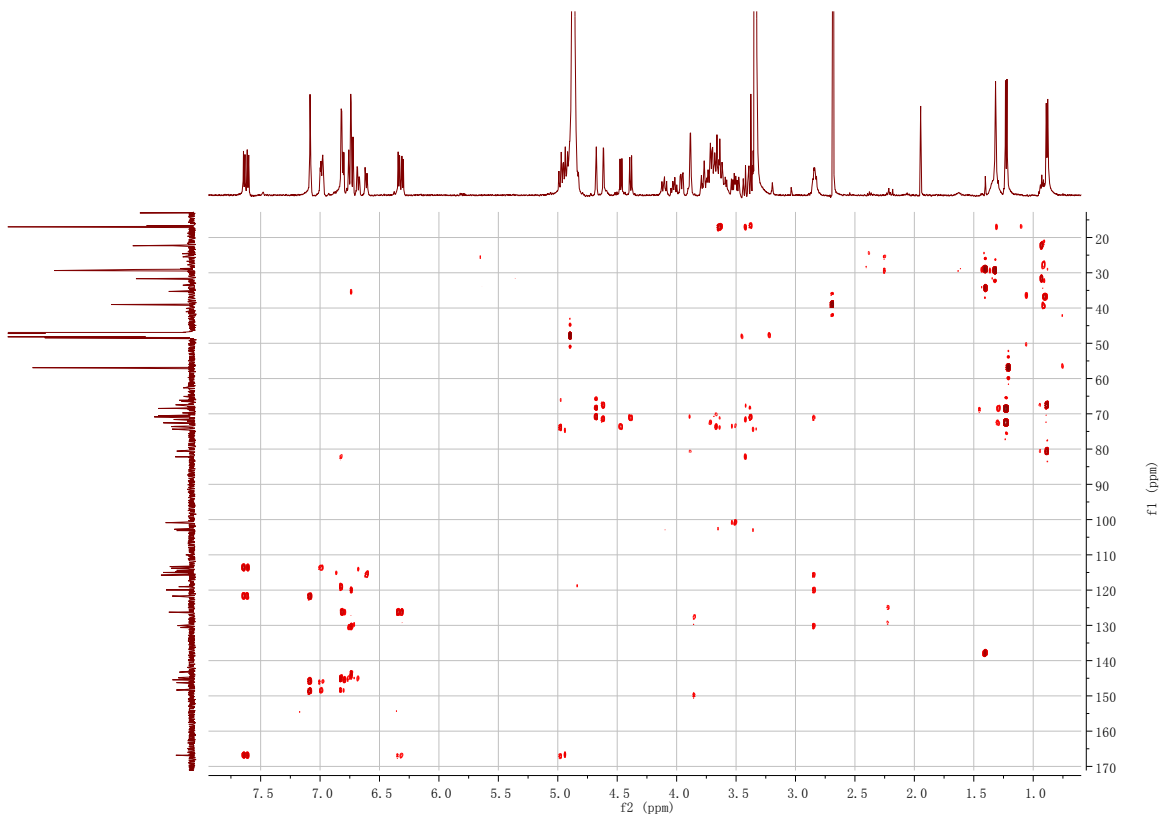


Figure S18. The HMBC spectrum of compound **2*** in methanol- d_4

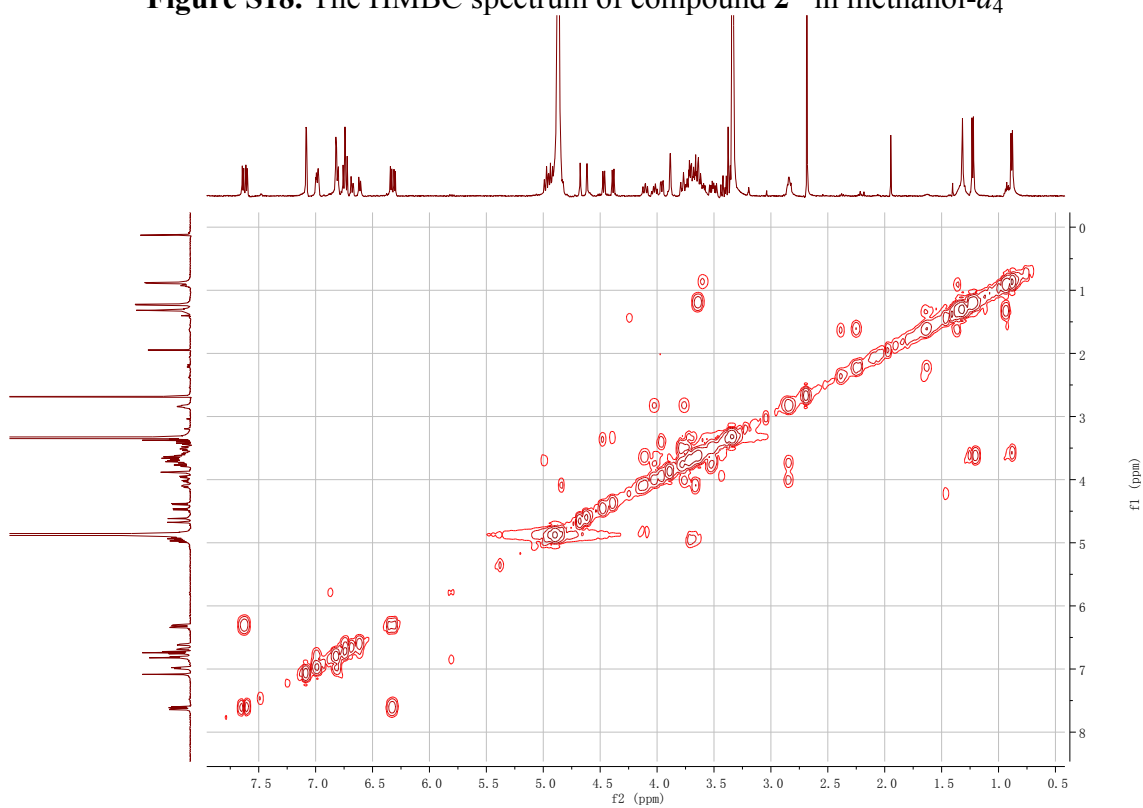


Figure S19. The ^1H - ^1H COSY spectrum of compound **2*** in methanol- d_4

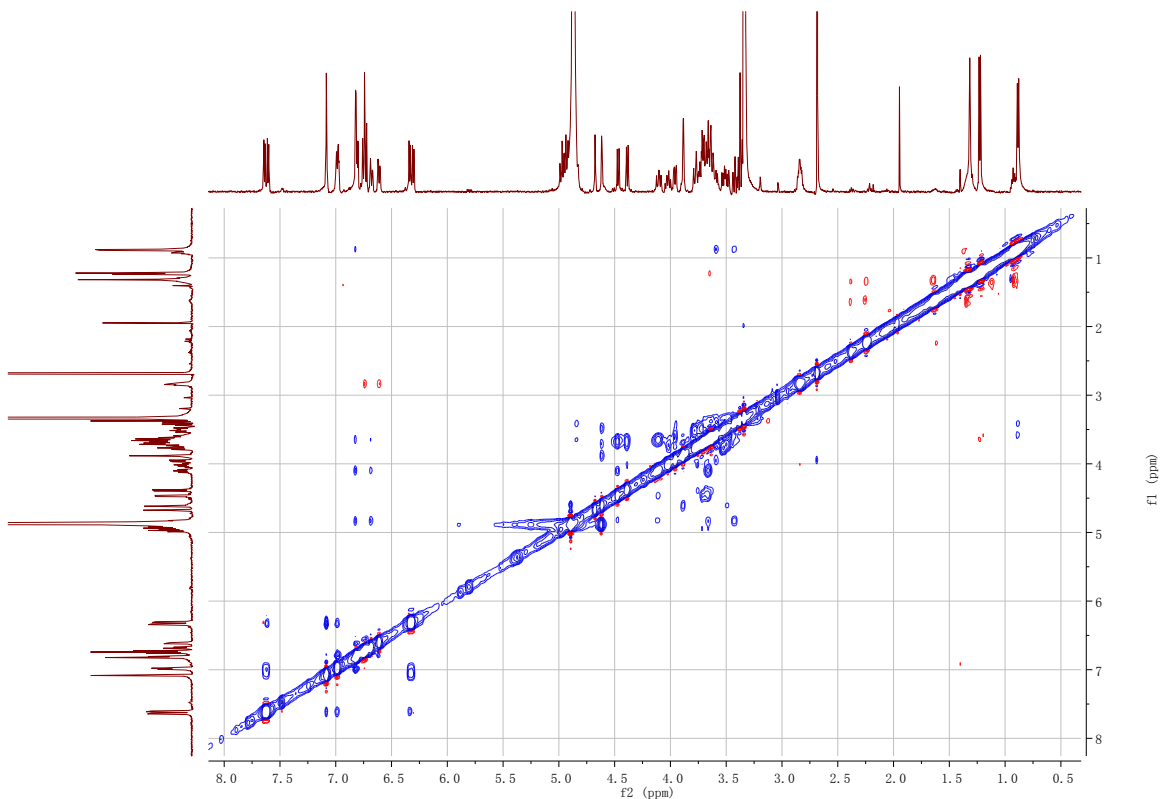


Figure S20. The ROESY spectrum of compound **2*** in methanol- d_4

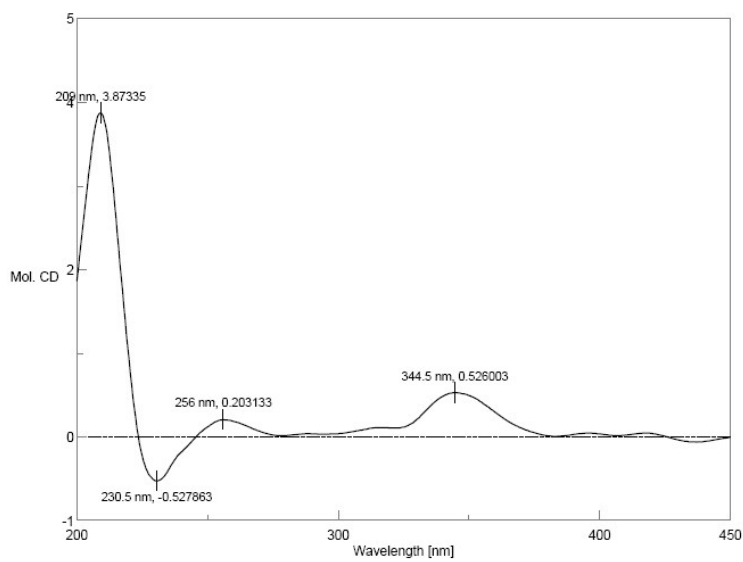


Figure S21. The CD spectrum of **2*** in MeOH : H₂O 1:1

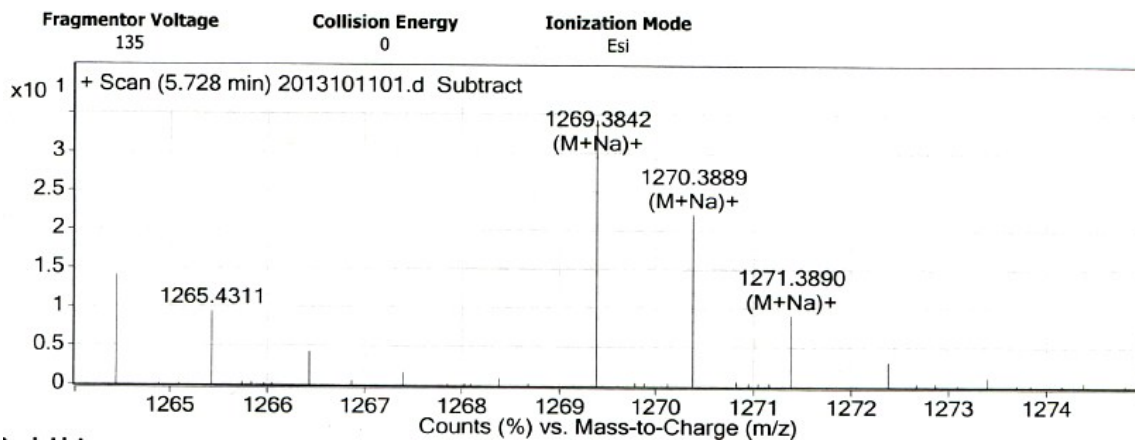


Figure S22. The HRESIMS of compound 2*

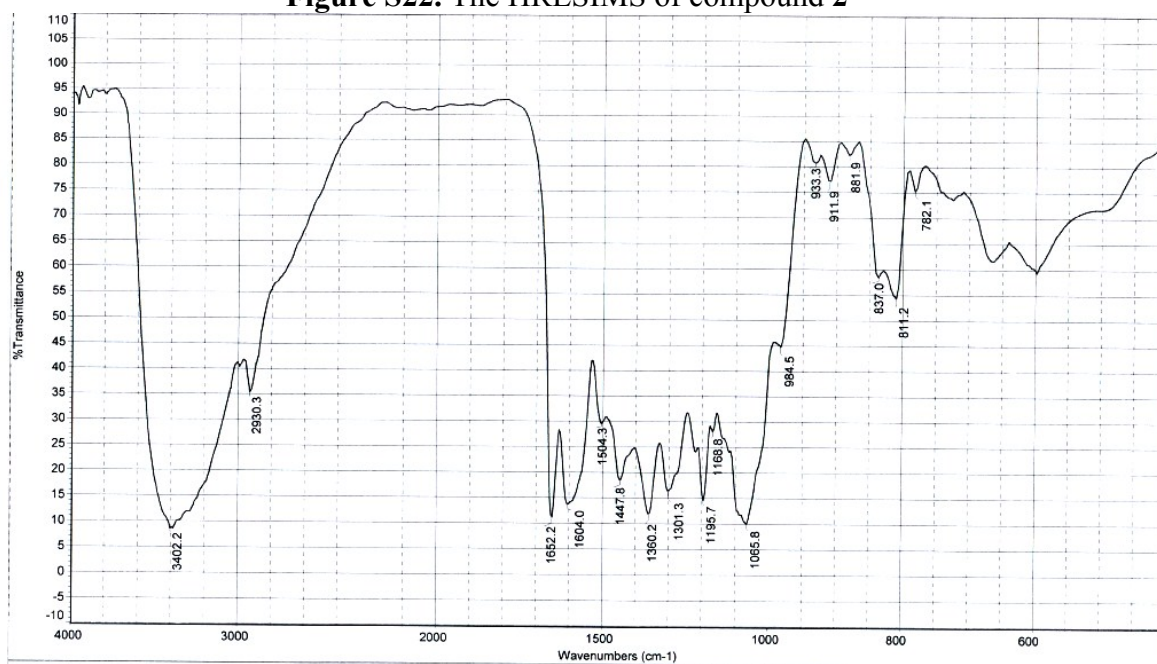


Figure S23. The IR spectrum of compound 3*

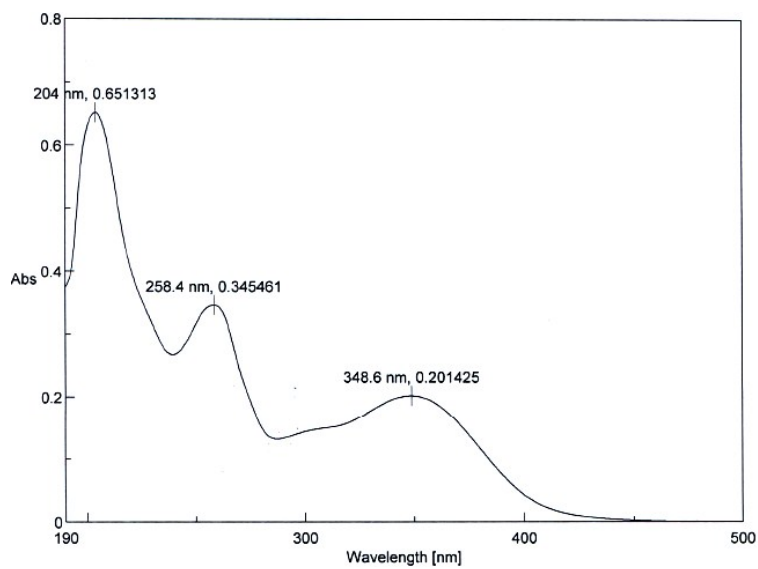


Figure S24. The UV spectrum of compound 3*

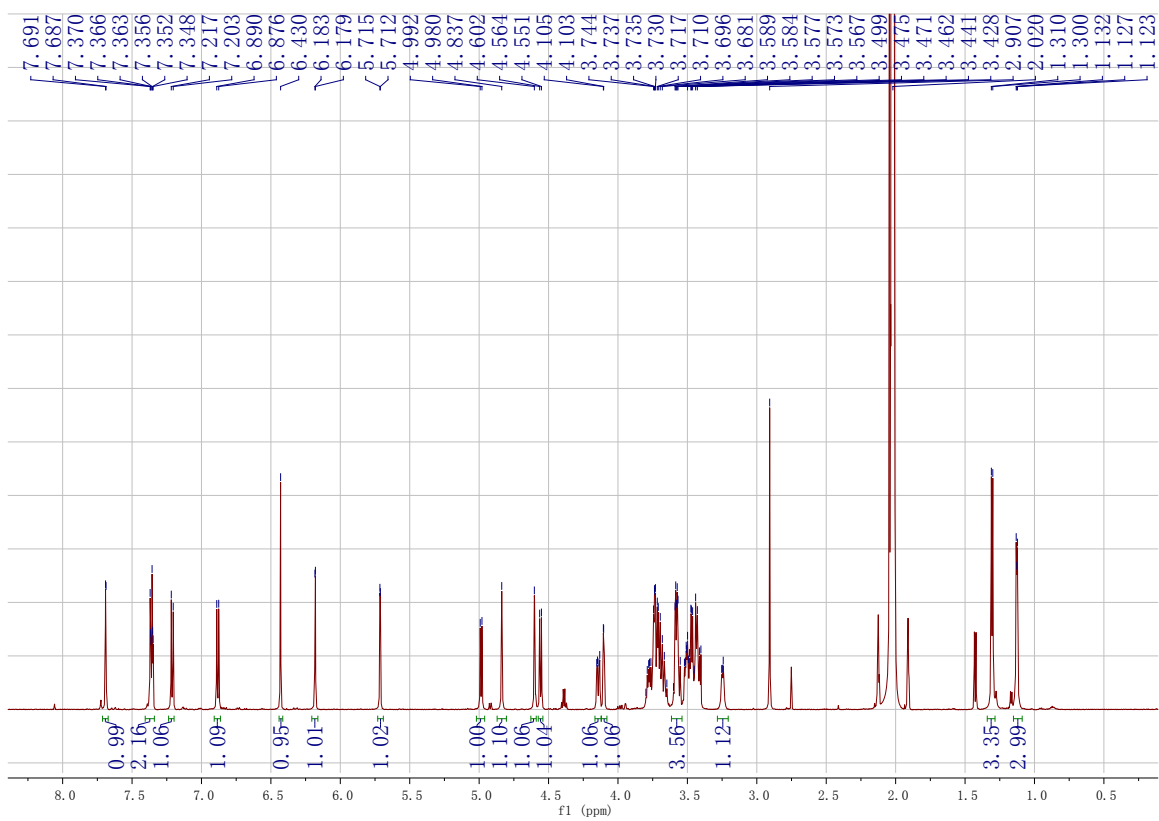


Figure S25. The ¹H NMR spectrum of compound 3* in acetic acid-d₄

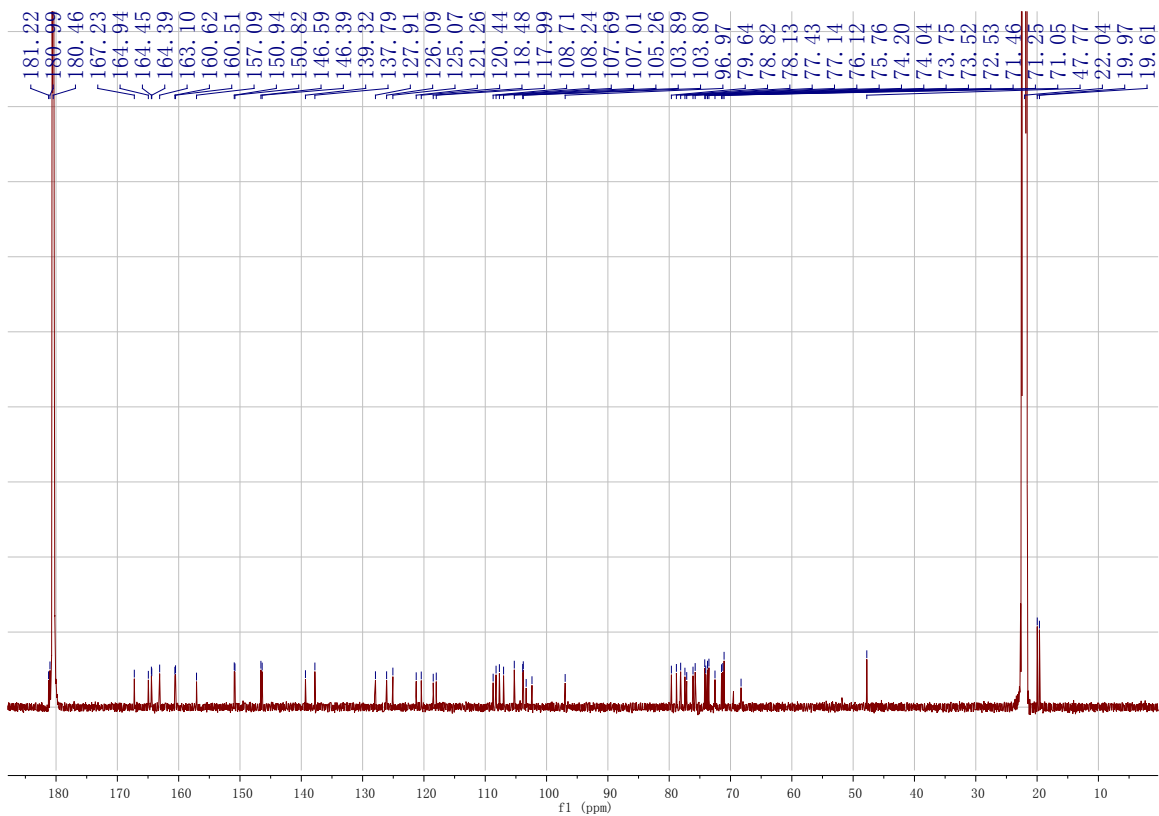


Figure S26. The ^{13}C NMR spectrum of compound 3* in acetic acid- d_4

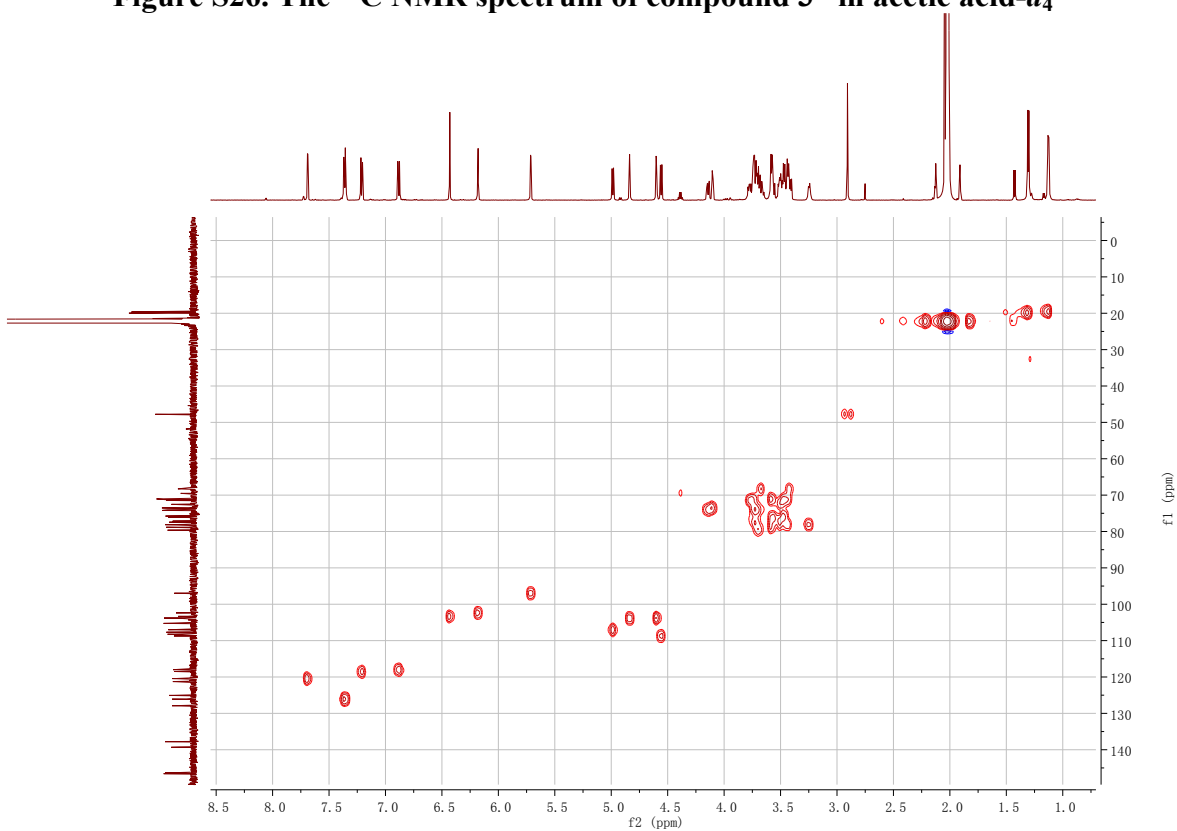


Figure S27. The HSQC spectrum of compound 3* in acetic acid- d_4

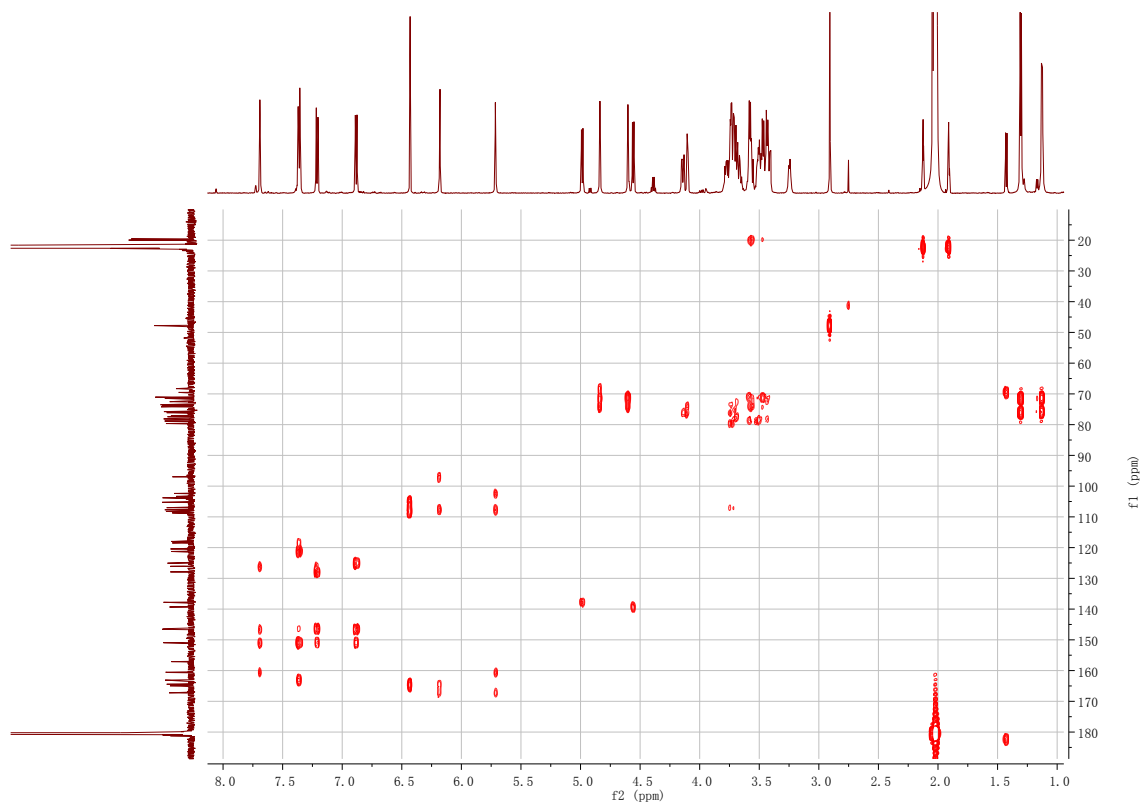


Figure S28. The HMBC spectrum of compound 3* in acetic acid- d_4

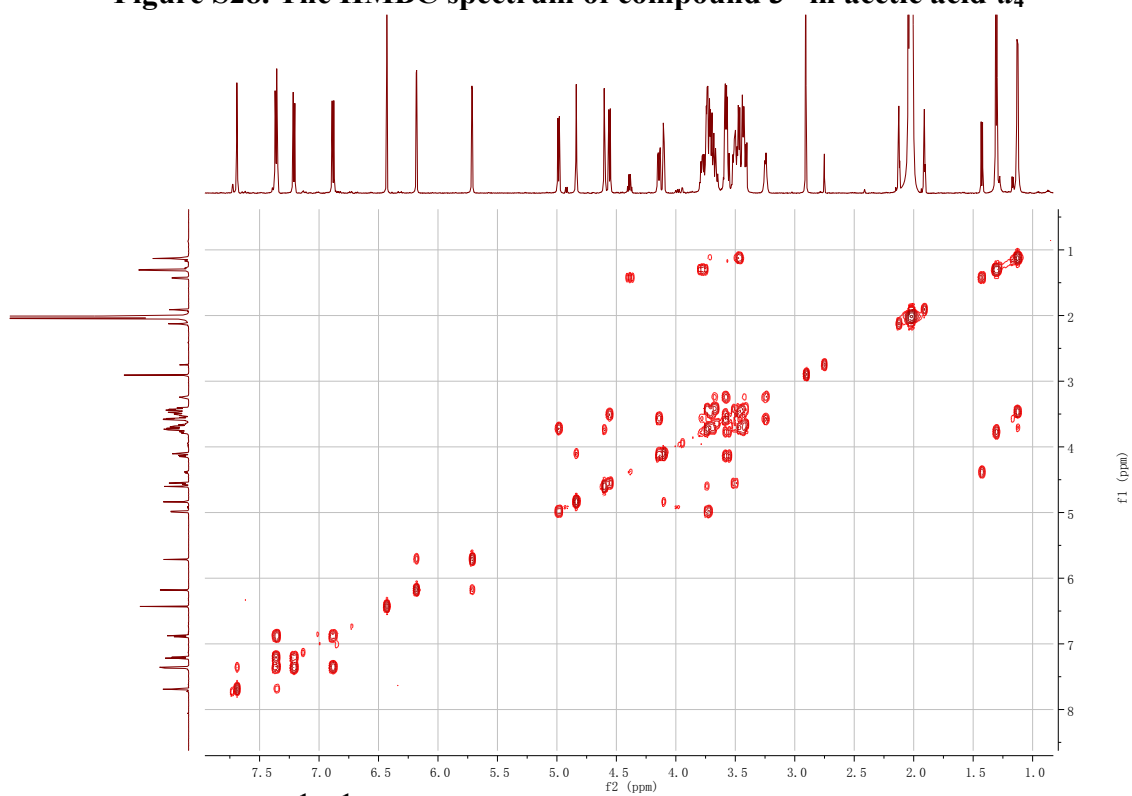


Figure S29. The ^1H - ^1H COSY spectrum of compound 3* in acetic acid- d_4

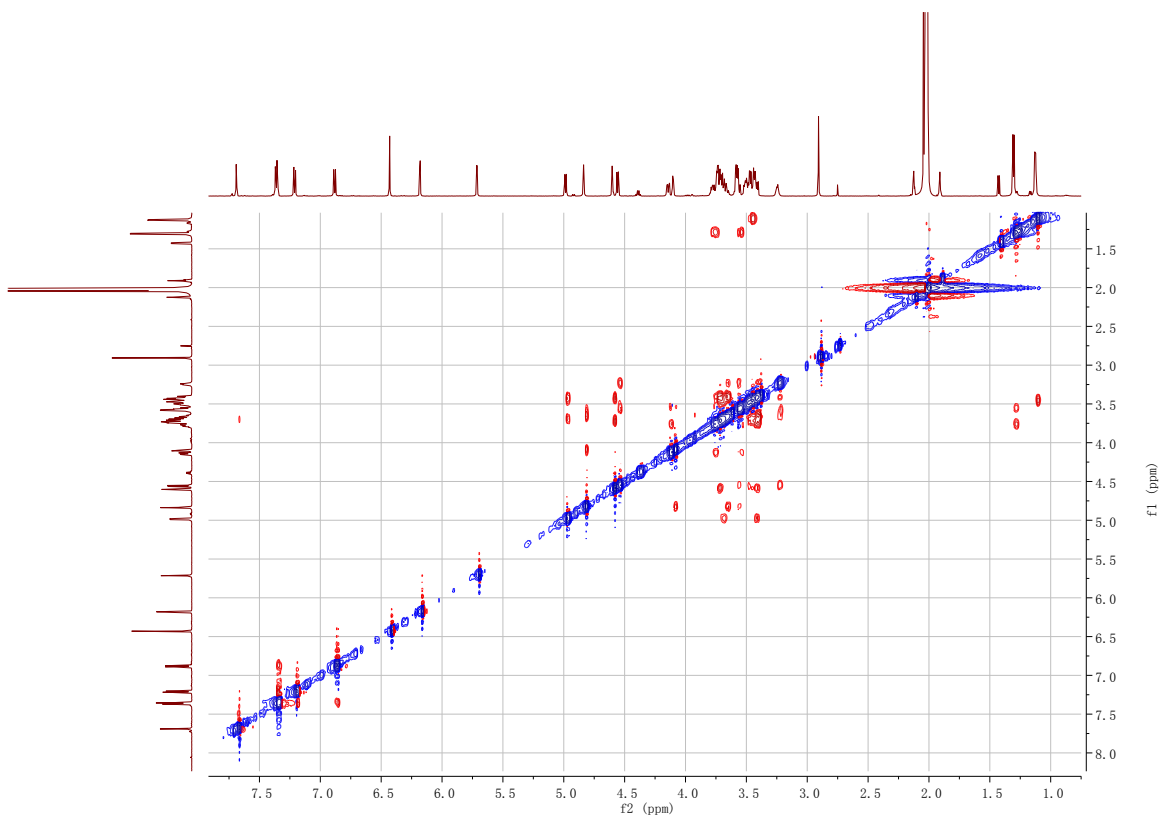


Figure S30. The ROESY spectrum of compound **3*** in acetic acid-*d*₄

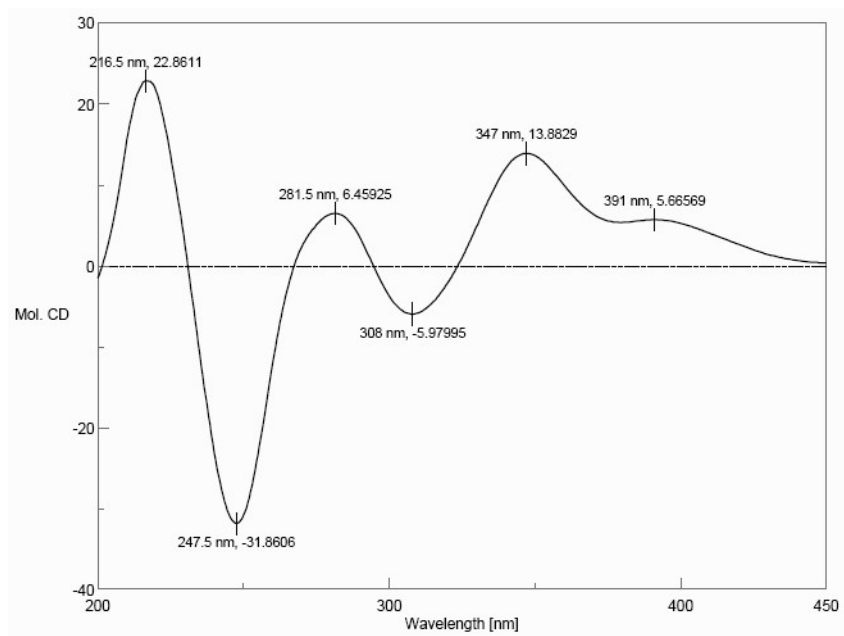


Figure S31. The CD spectrum of **3*** in MeOH : H₂O 1:1

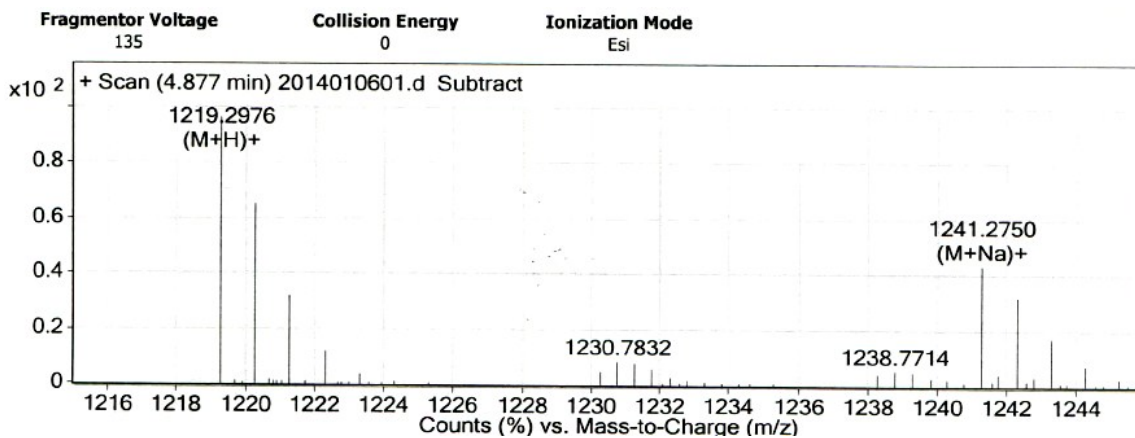


Figure S32. The HRESIMS of compound 3*

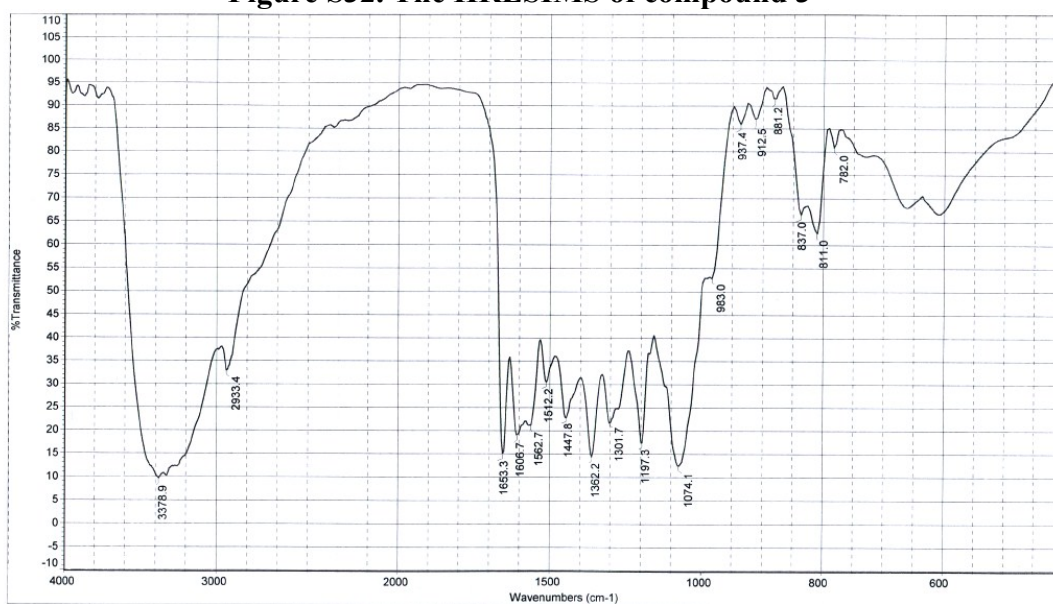


Figure S33. The IR spectrum of compound 4*

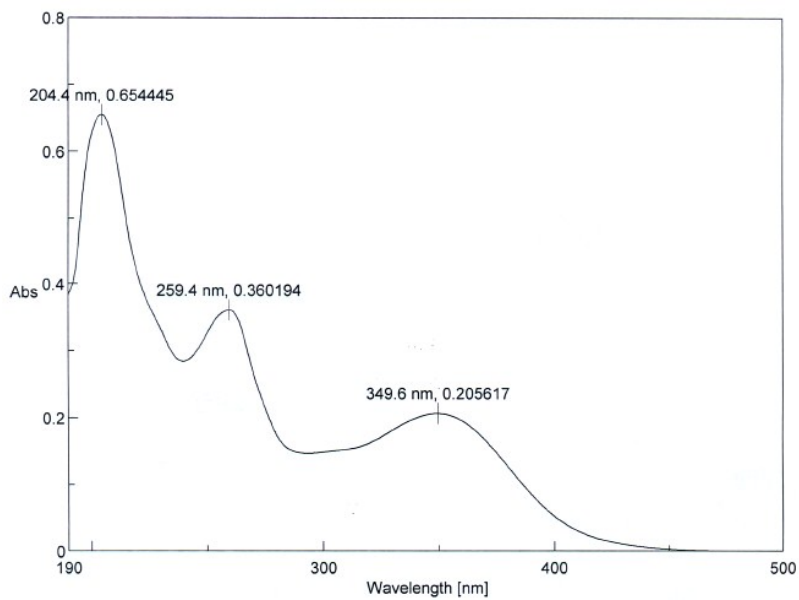


Figure S34. The UV spectrum of compound 4*

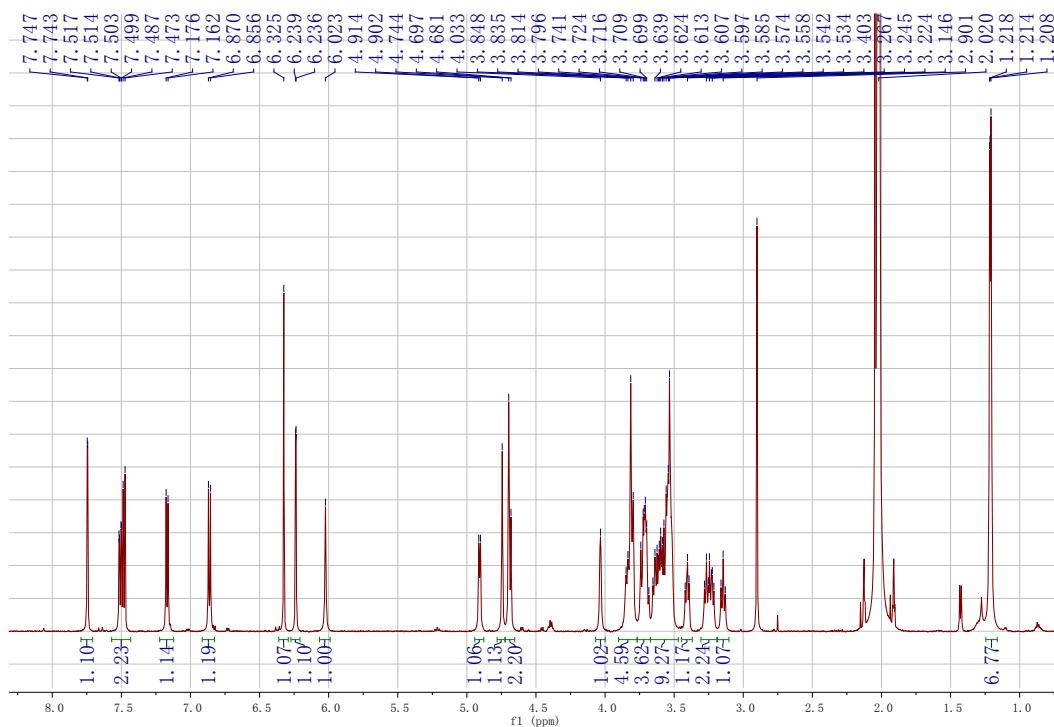


Figure S35. The ¹H NMR spectrum of compound 4* in acetic acid-*d*₄

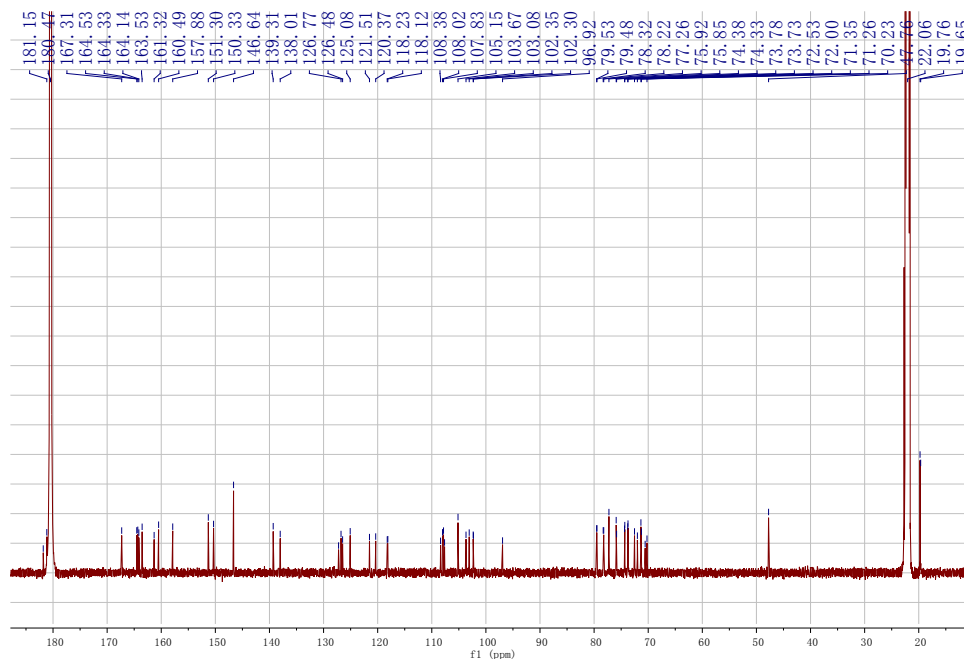


Figure S36. The ^{13}C NMR spectrum of compound 4^* in acetic acid- d_4

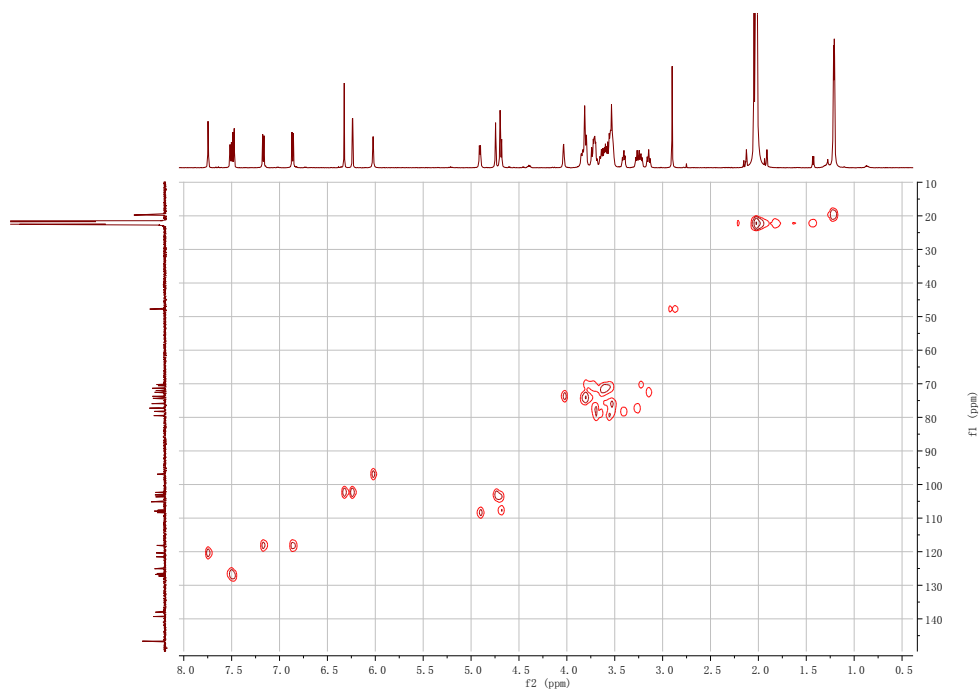


Figure S37. The HSQC spectrum of compound 4^* in acetic acid- d_4

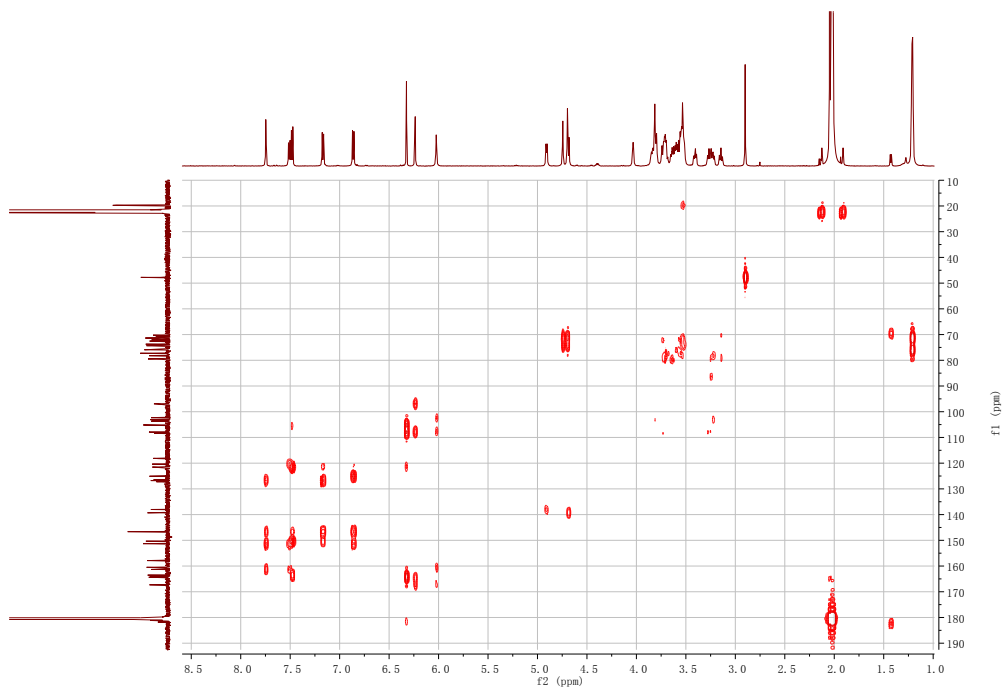


Figure S38. The HMBC spectrum of compound 4* in acetic acid- d_4

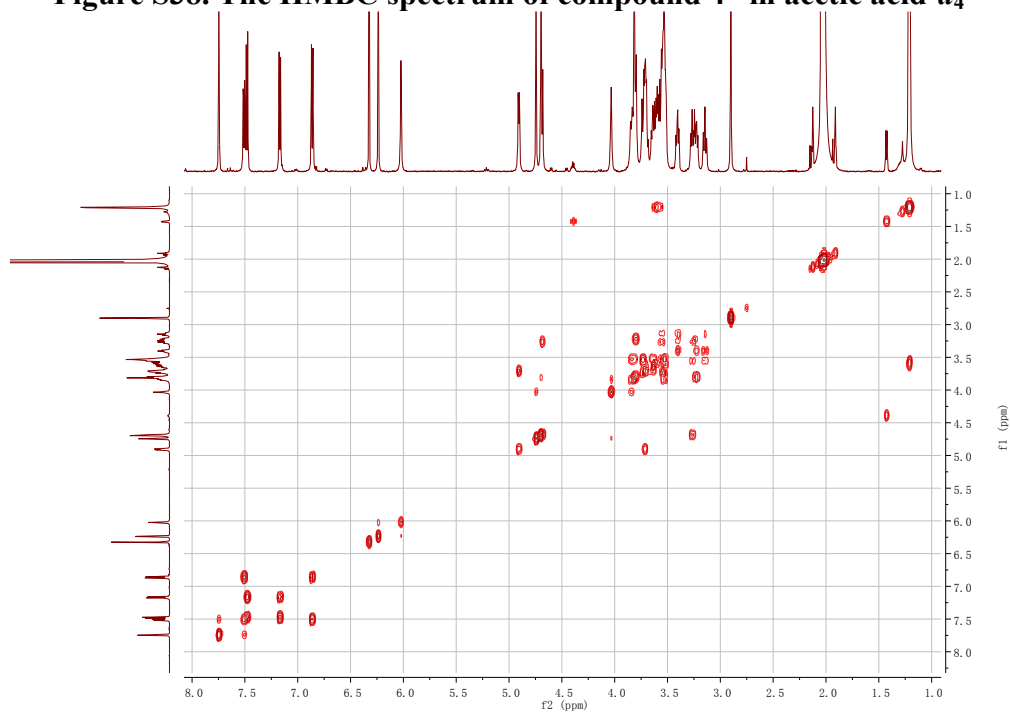


Figure S39. The ^1H - ^1H COSY spectrum of compound 4* in acetic acid- d_4

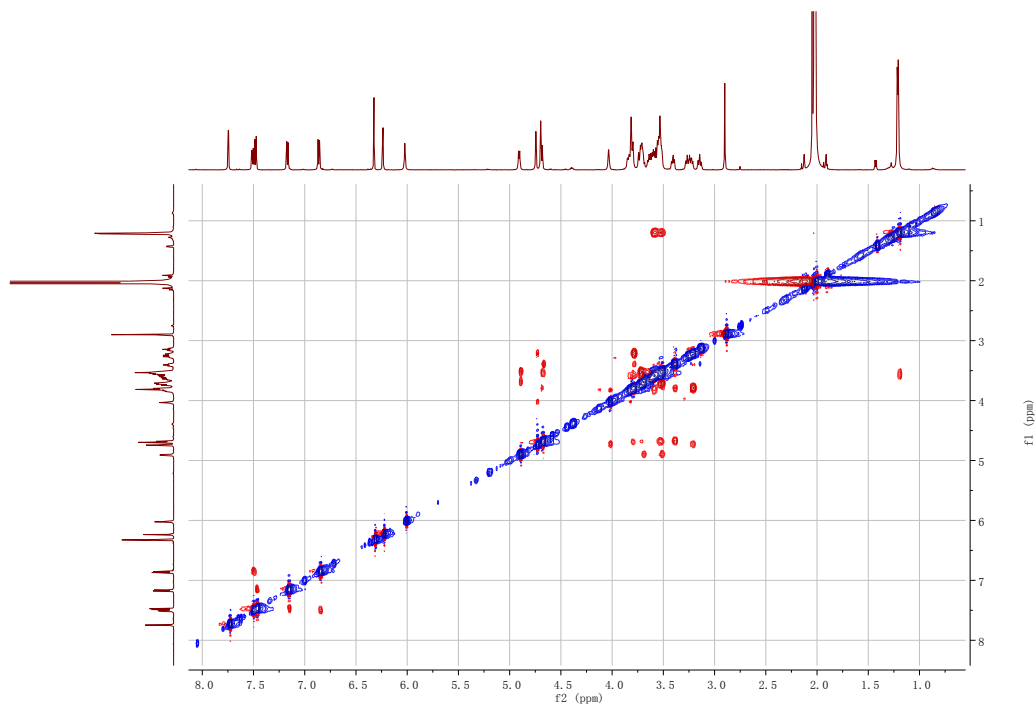


Figure S40. The ROESY spectrum of compound 4* in acetic acid- d_4

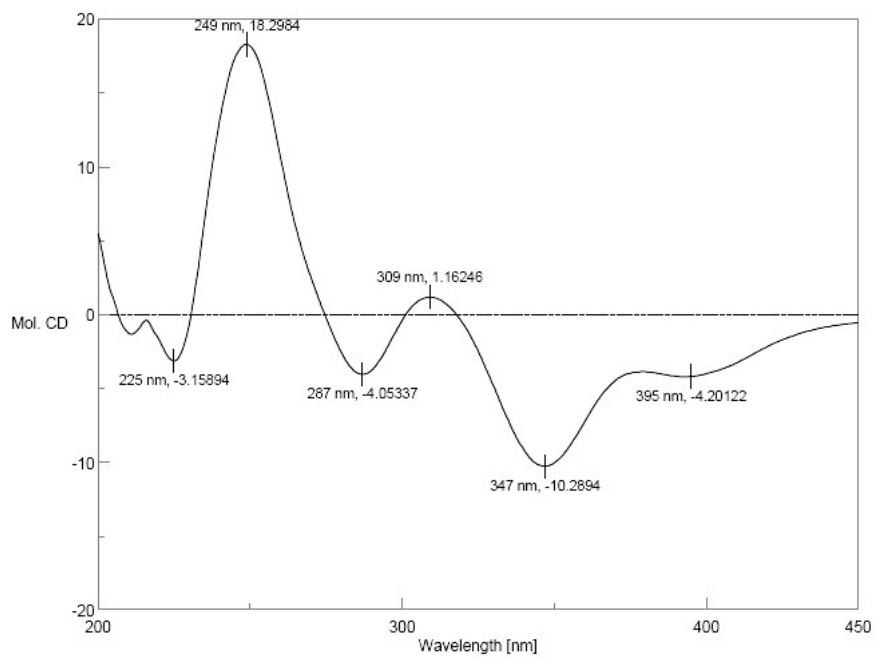


Figure S41. The CD spectrum of 4* in MeOH : H₂O 1:1

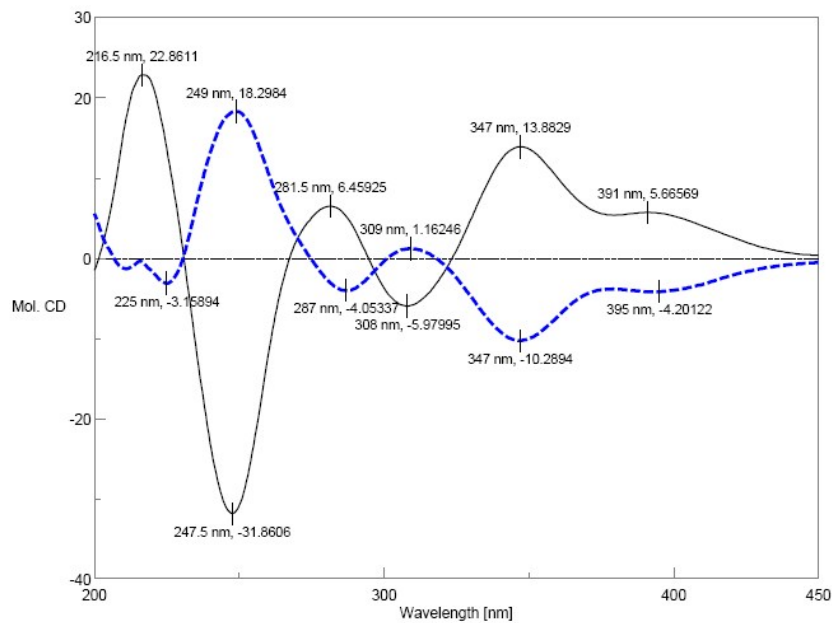


Figure S42. The overlay CD spectrum of 3* and 4*

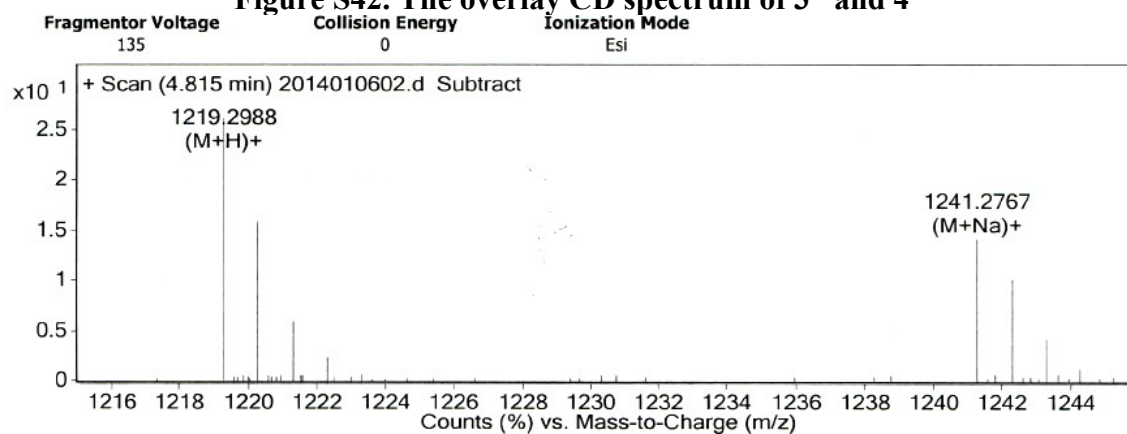


Figure S43. The HRESIMS of compound 4*