

## Supplementary Materials

# Benzofuran derivatives from Cortex Mori Radicis and their cho-linesterase inhibitory activity

Xiang Cui<sup>1,2</sup>, Ze-Hong Huang<sup>1</sup>, Shan-Shan Deng<sup>1</sup>, Yun-Xia Zhang<sup>1</sup>, Guo-Yin Li<sup>1</sup>, Li-Ning Wang<sup>2</sup>, Yan-Ru Deng<sup>2\*</sup> and Chang-Jing Wu<sup>1,3\*</sup>

<sup>1</sup> College of Life Sciences and Agronomy, Zhoukou Normal University, Zhoukou 466001, China

<sup>2</sup> College of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China

<sup>3</sup> Field Observation and Research Station of Green Agriculture in Dancheng County, Zhoukou 466001, China

\* Correspondence: dengyanru@tjutcm.edu.cn (Y.-R.D.); wucj2009@163.com (C.-J.W.)

## List of Supplementary Materials

Figure S1. HPLC detection of cis-trans-tautomerism of compounds 1 and 6 .....	1
Figure S2. Chiral HPLC analysis of compound 4.....	1
Figure S3. Secondary plots for the determination of the inhibitor constants.....	2
Figure S4. HRESIMS data of compound 1.....	3
Figure S5. <sup>1</sup> H-NMR spectrum of compound 1 in acetone- <i>d</i> <sub>6</sub> .....	3
Figure S6. <sup>13</sup> C-NMR and DEPT-135 spectra of compound 1 in acetone- <i>d</i> <sub>6</sub> .....	4
Figure S7. HSQC spectrum of compound 1 .....	4
Figure S8. <sup>1</sup> H- <sup>1</sup> H COSY spectrum of compound 1 .....	5
Figure S9. HMBC spectrum of compound 1 .....	5
Figure S10. ROESY spectrum of compound 1 .....	6
Figure S11. HRESIMS data of compound 2.....	6
Figure S12. <sup>1</sup> H-NMR spectrum of compound 2 in acetone- <i>d</i> <sub>6</sub> .....	7
Figure S13. <sup>13</sup> C-NMR and DEPT-135 spectra of compound 2 in acetone- <i>d</i> <sub>6</sub> .....	7
Figure S14. HSQC spectrum of compound 2 .....	8
Figure S15. <sup>1</sup> H- <sup>1</sup> H COSY spectrum of compound 2 .....	8
Figure S16. HMBC spectrum of compound 2 .....	9
Figure S17. ROESY spectrum of compound 2 .....	9
Figure S18. HRESIMS data of compound 3.....	10
Figure S19. <sup>1</sup> H-NMR spectrum of compound 3 in CD <sub>3</sub> OD .....	10
Figure S20. <sup>13</sup> C-NMR and DEPT-135 spectra of compound 3 in CD <sub>3</sub> OD .....	11
Figure S21. HSQC spectrum of compound 3 .....	11
Figure S22. <sup>1</sup> H- <sup>1</sup> H COSY spectrum of compound 3 .....	12
Figure S23. HMBC spectrum of compound 3 .....	12
Figure S24. CD spectrum of compound 3 .....	13
Figure S25. HRESIMS data of compound 4.....	13
Figure S26. <sup>1</sup> H-NMR spectrum of compound 4 in CD <sub>3</sub> OD .....	14
Figure S27. <sup>13</sup> C-NMR and DEPT-135 spectra of compound 4 in CD <sub>3</sub> OD .....	14
Figure S28. HSQC spectrum of compound 4 .....	15
Figure S29. <sup>1</sup> H- <sup>1</sup> H COSY spectrum of compound 4 .....	15
Figure S30. HMBC spectrum of compound 4 .....	16
Figure S31. CD spectrum of compound 4 .....	16
Figure S32. HRESIMS data of compound 5.....	17
Figure S33. <sup>1</sup> H-NMR spectrum of compound 5 in CD <sub>3</sub> OD .....	17
Figure S34. <sup>13</sup> C-NMR and DEPT-135 spectra of compound 5 in CD <sub>3</sub> OD .....	18
Figure S35. HSQC spectrum of compound 5 .....	18
Figure S36. <sup>1</sup> H- <sup>1</sup> H COSY spectrum of compound 5 .....	19
Figure S37. HMBC spectrum of compound 5 .....	19
Figure S38. <sup>1</sup> H-NMR spectrum of compound 6 in acetone- <i>d</i> <sub>6</sub> .....	20
Figure S39. <sup>13</sup> C-NMR and DEPT-135 spectra of compound 6 in acetone- <i>d</i> <sub>6</sub> .....	20
Figure S40. HSQC spectrum of compound 6 .....	21
Figure S41. <sup>1</sup> H- <sup>1</sup> H COSY spectrum of compound 6 .....	21
Figure S42. HMBC spectrum of compound 6 .....	22
Figure S43. <sup>1</sup> H-NMR spectrum of compound 14 in CD <sub>3</sub> OD .....	22
Figure S44. <sup>13</sup> C-NMR spectrum of compound 14 in CD <sub>3</sub> OD .....	23

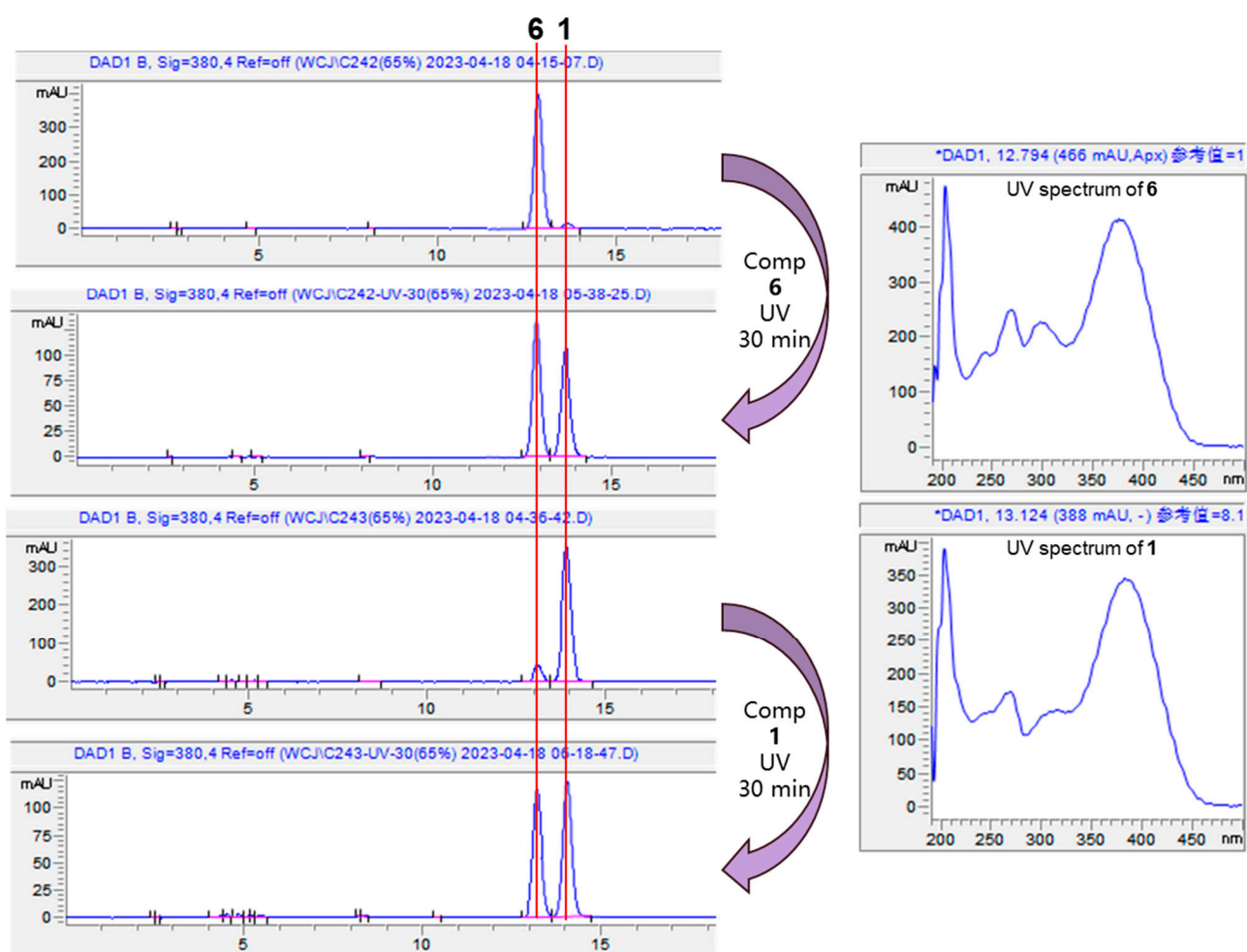


Figure S1. HPLC detection of cis-trans-tautomerism of compounds 1 and 6 promoted by UV exposure. Samples were analyzed on an Agilent 1100 HPLC system using an analytical Kromasil C-18 column (5  $\mu$ m, 100 Å, 4.6 mm  $\times$  250 mm; Akzo Nobel), with 65% aqueous methanol as mobile phase, detected at 380 nm.

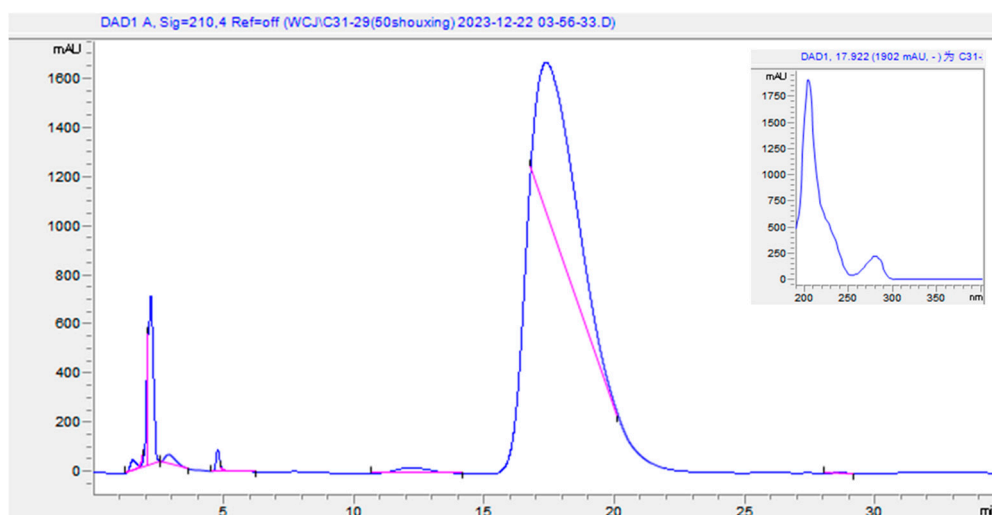


Figure S2. Chiral HPLC analysis of compound 4. The HPLC analysis was conducted with a CHIRALPAK® AD-RH column (5  $\mu$ m, 4.6 mm  $\times$  150 mm, Daicel Chiral Technologies (China) CO., LTD.) using 50% methanol as mobile phase with flow rate of 1 ml/min at 30°C.

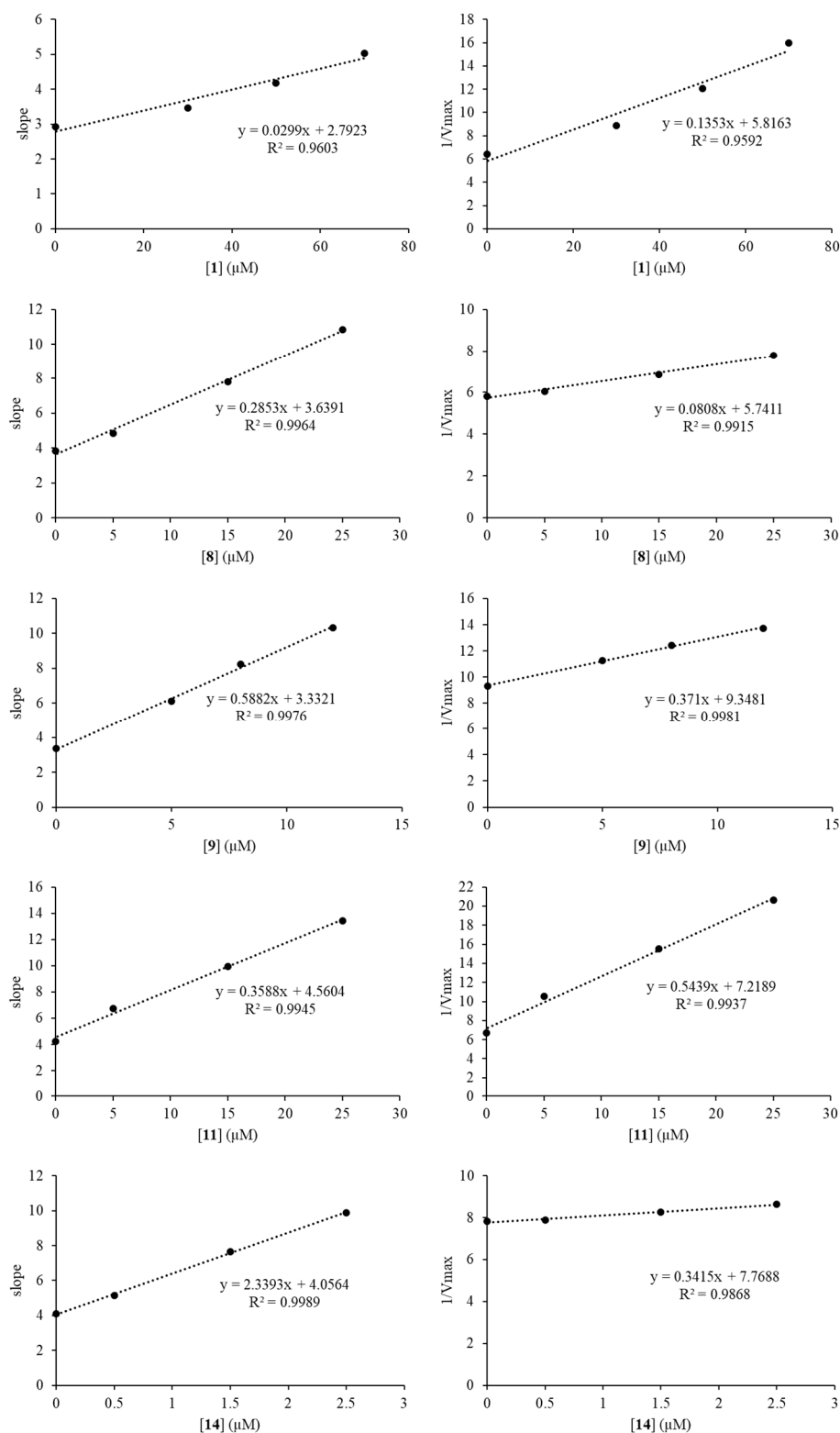


Figure S3. Secondary plots for the determination of the inhibitor constants for mixed-type inhibitors **1**, **8**, **9**, **11**, and **14**. 1/V is plotted as a function of [I], and the value of  $-\alpha K_i$  is determined from the x intercept of the line. The value of  $-K_i$  is determined from the x intercept of a plot of the slope of the lines from the double-reciprocal (Lineweaver — Burk) plot as a function of [I].

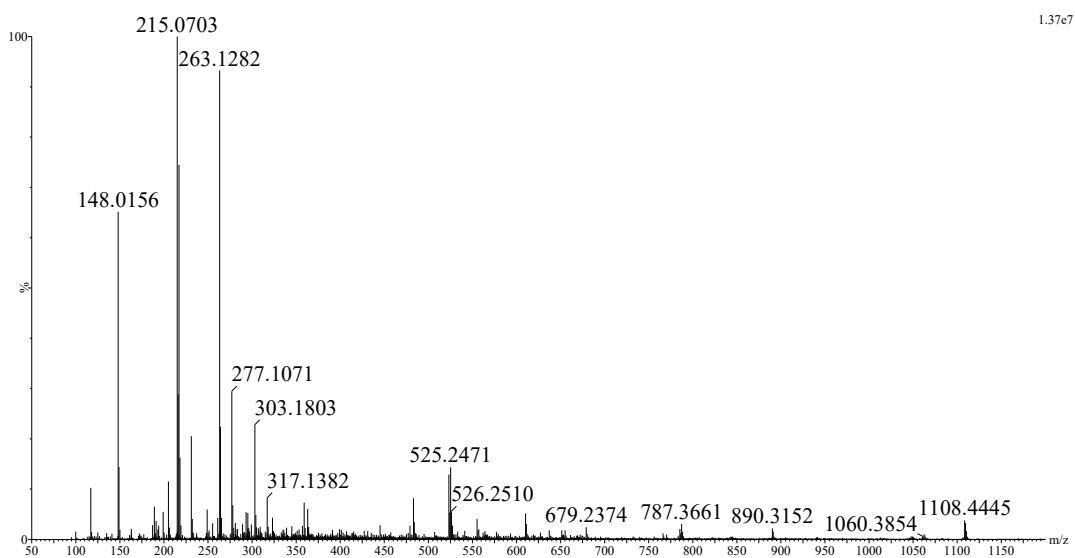


Figure S4. HRESIMS data of compound **1**

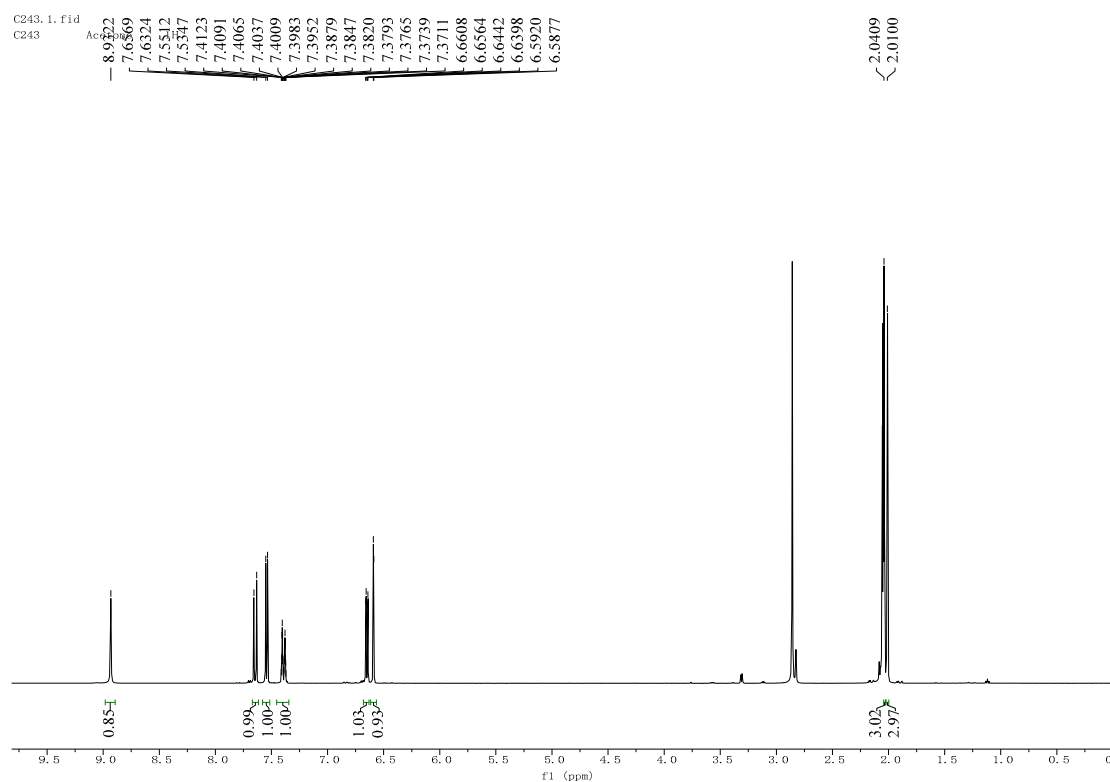


Figure S5. <sup>1</sup>H-NMR spectrum of compound **1** in acetone-*d*<sub>6</sub>

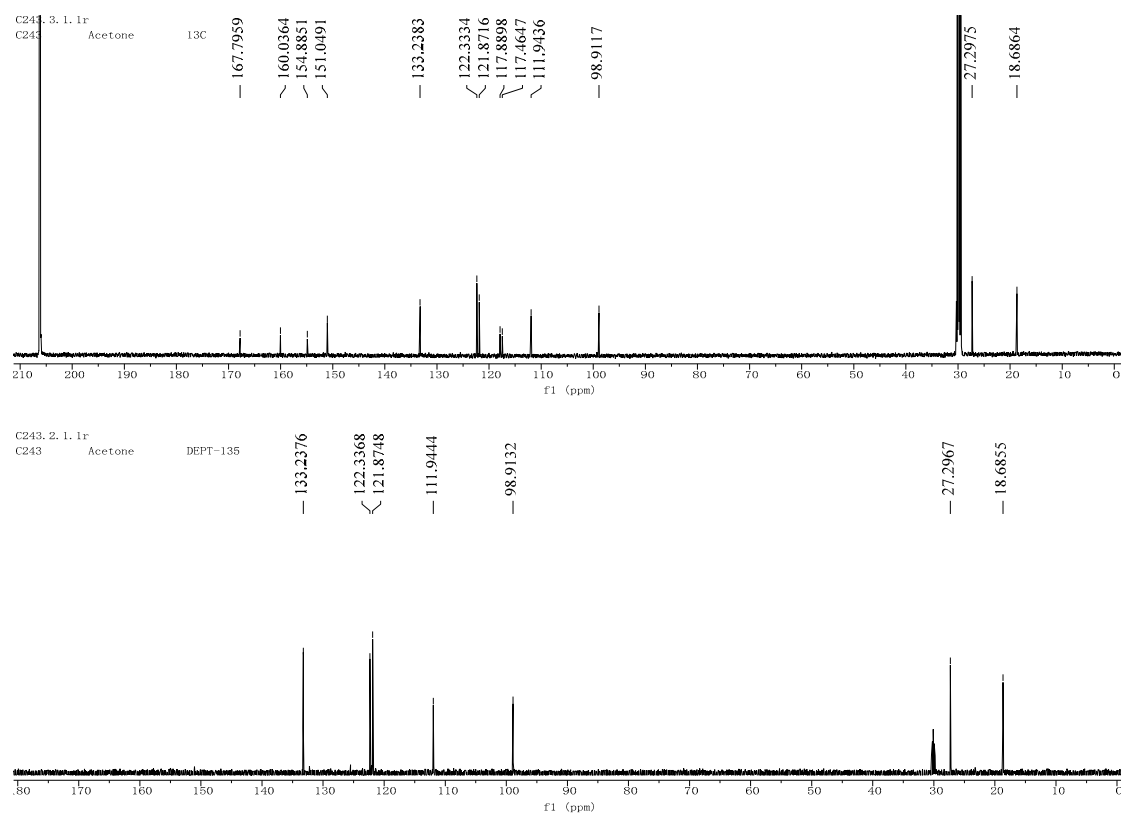


Figure S6. <sup>13</sup>C-NMR and DEPT-135 spectra of compound **1** in acetone-*d*<sub>6</sub>

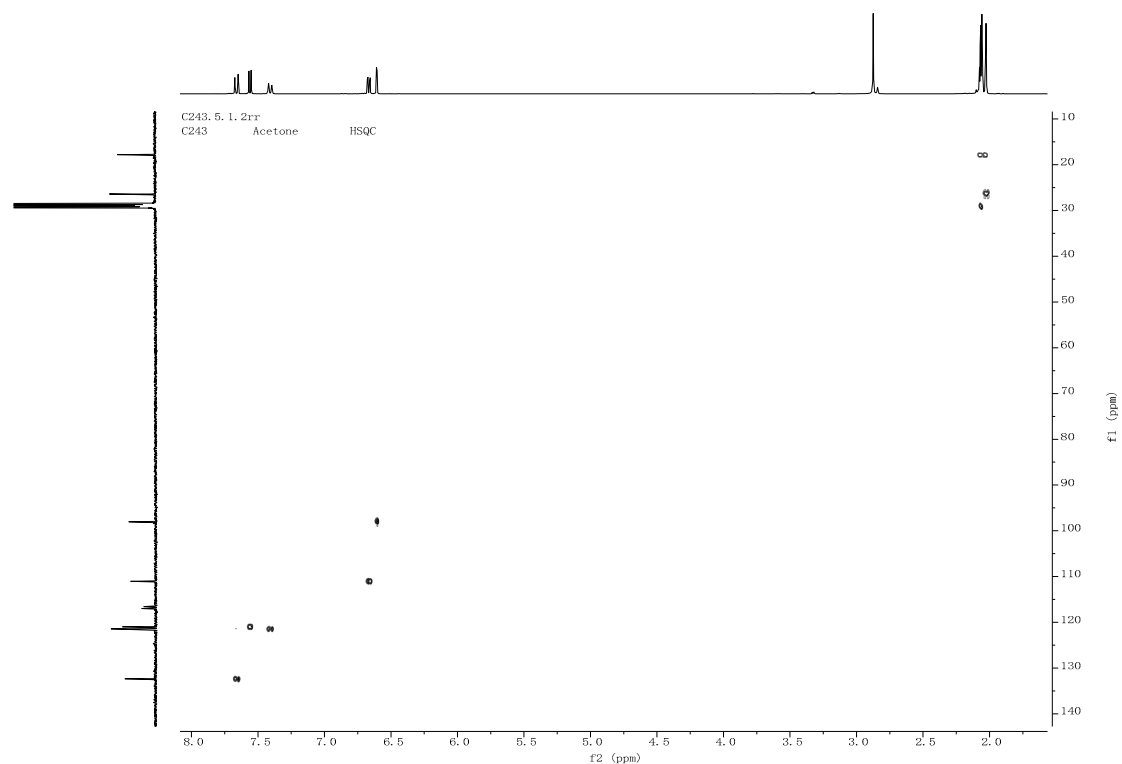


Figure S7. HSQC spectrum of compound **1**

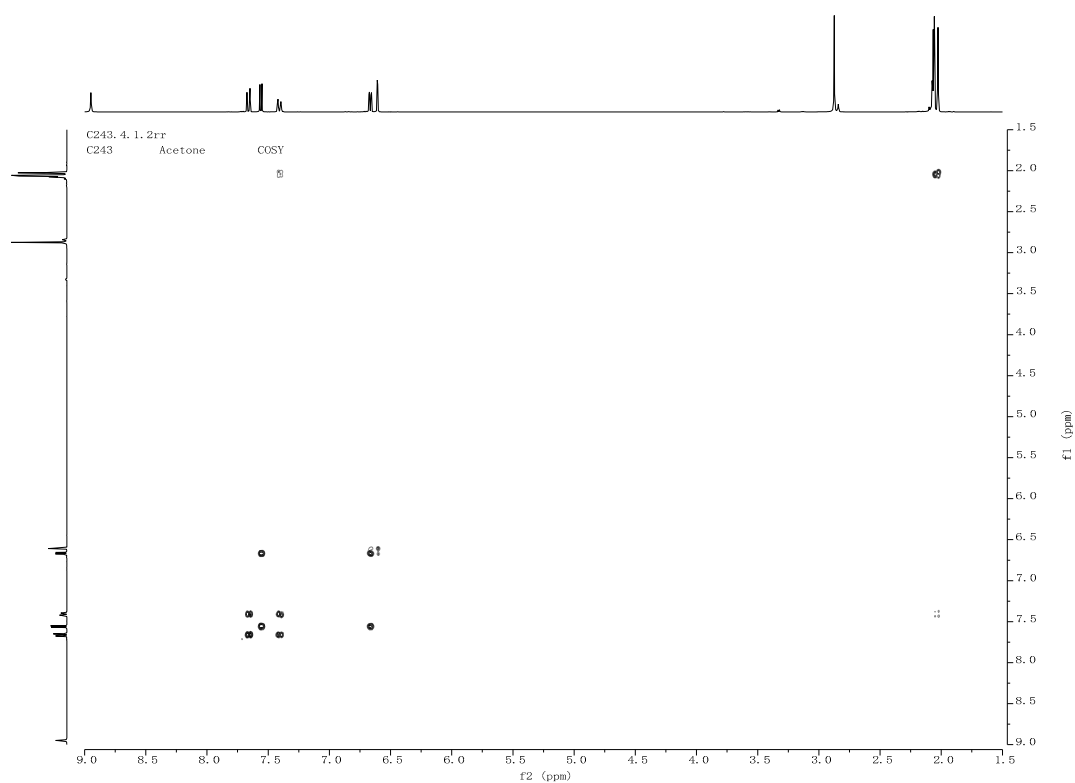


Figure S8.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound **1**

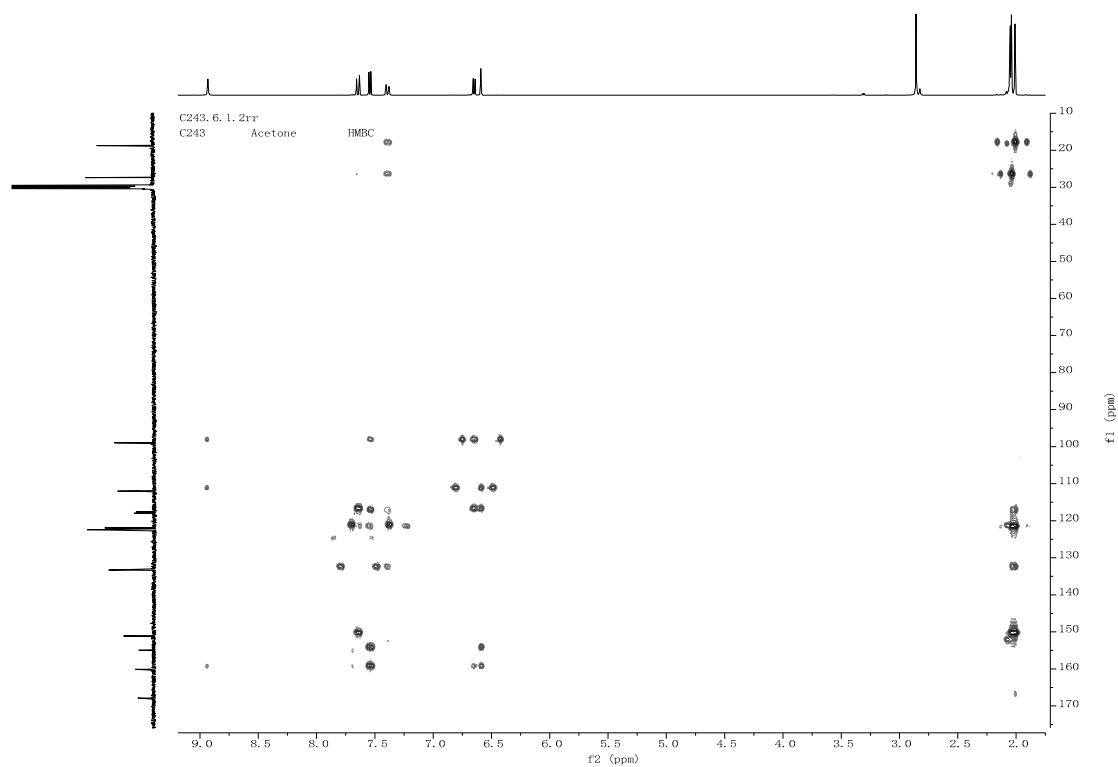


Figure S9. HMBC spectrum of compound **1**

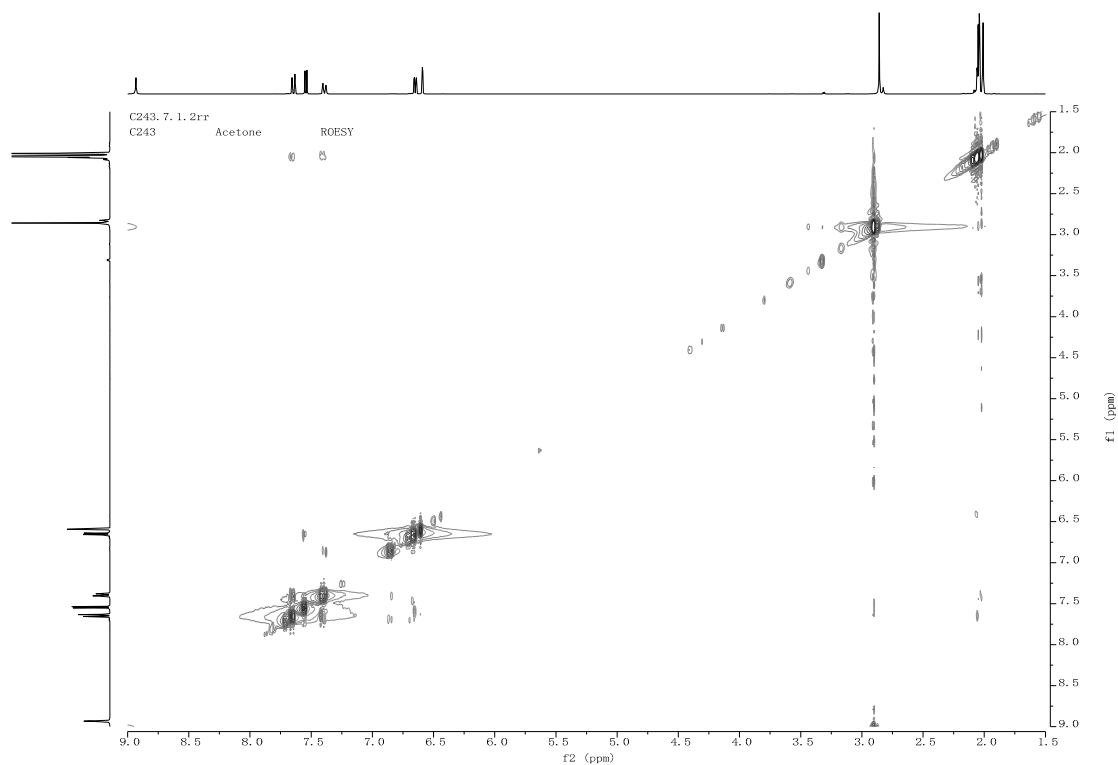


Figure S10. ROESY spectrum of compound **1**

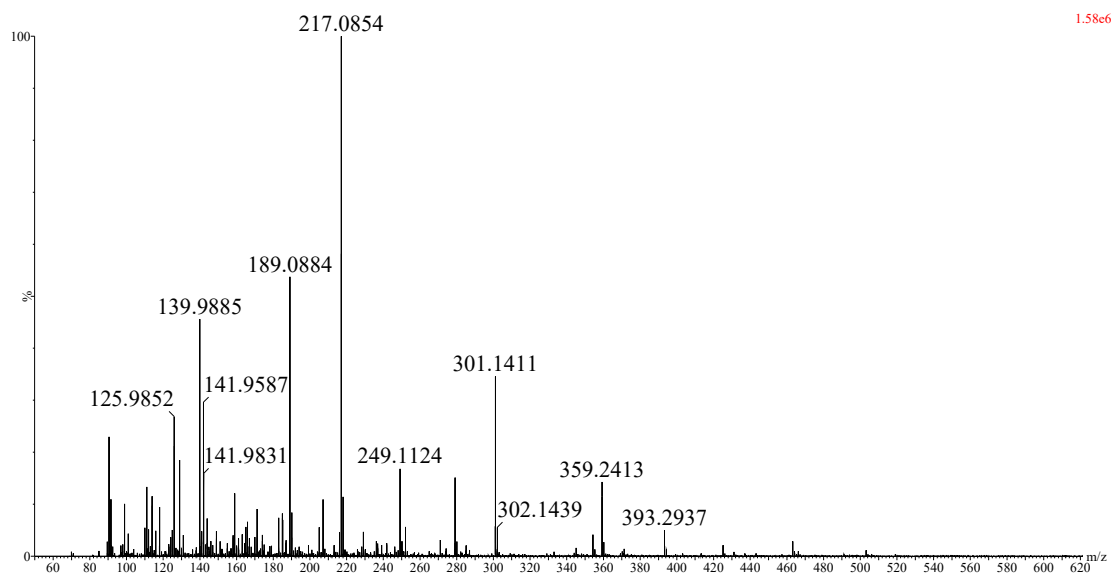


Figure S11. HRESIMS data of compound **2**



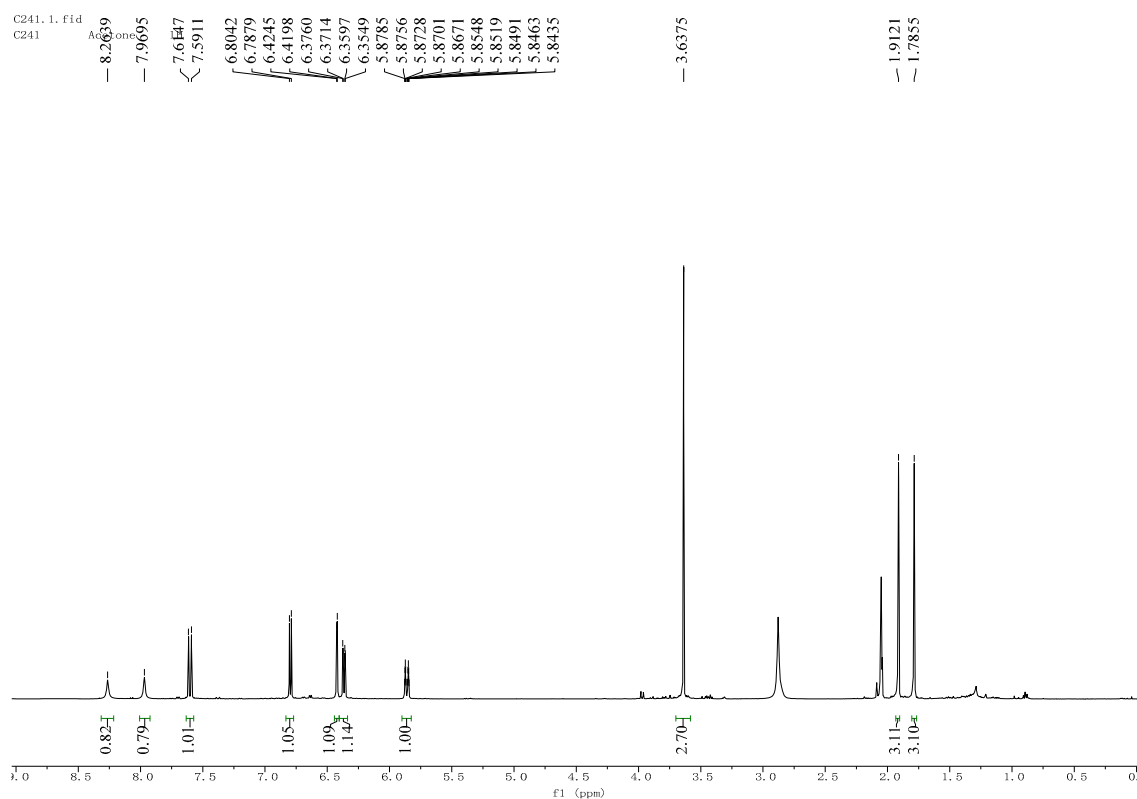


Figure S12.  $^1\text{H}$ -NMR spectrum of compound **2** in acetone- $d_6$

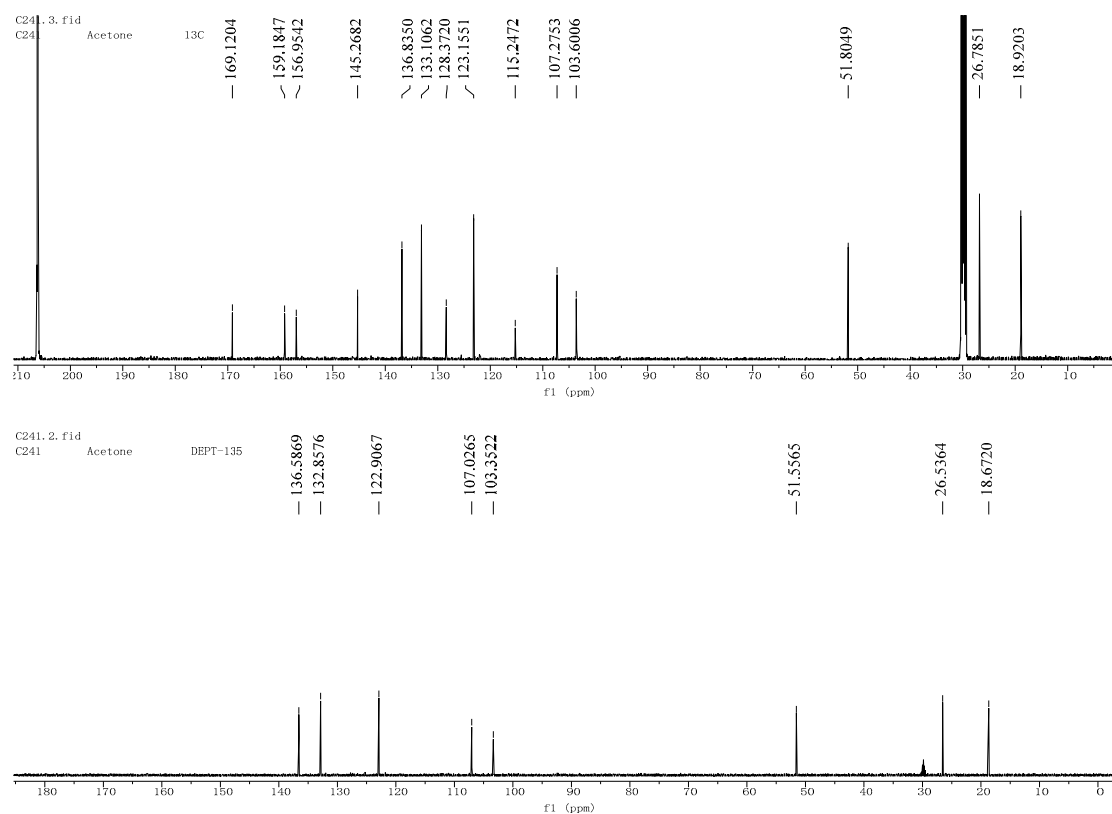


Figure S13.  $^{13}\text{C}$ -NMR and DEPT-135 spectra of compound **2** in acetone- $d_6$

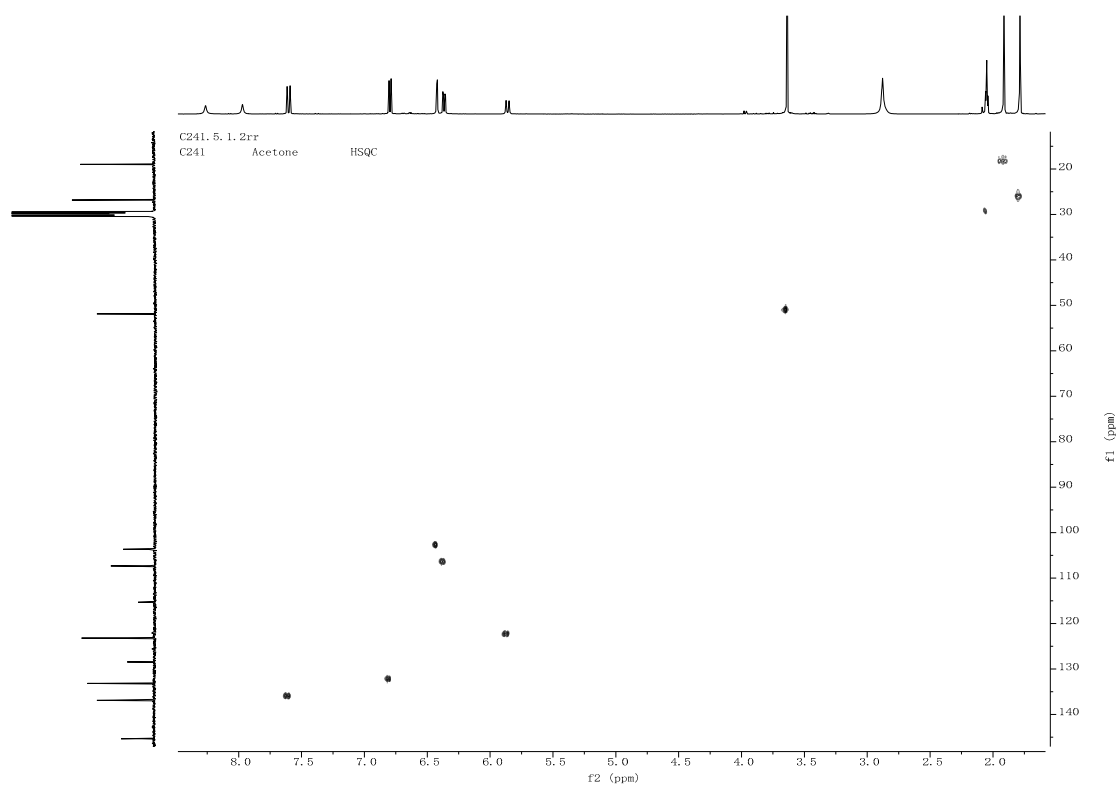


Figure S14. HSQC spectrum of compound 2

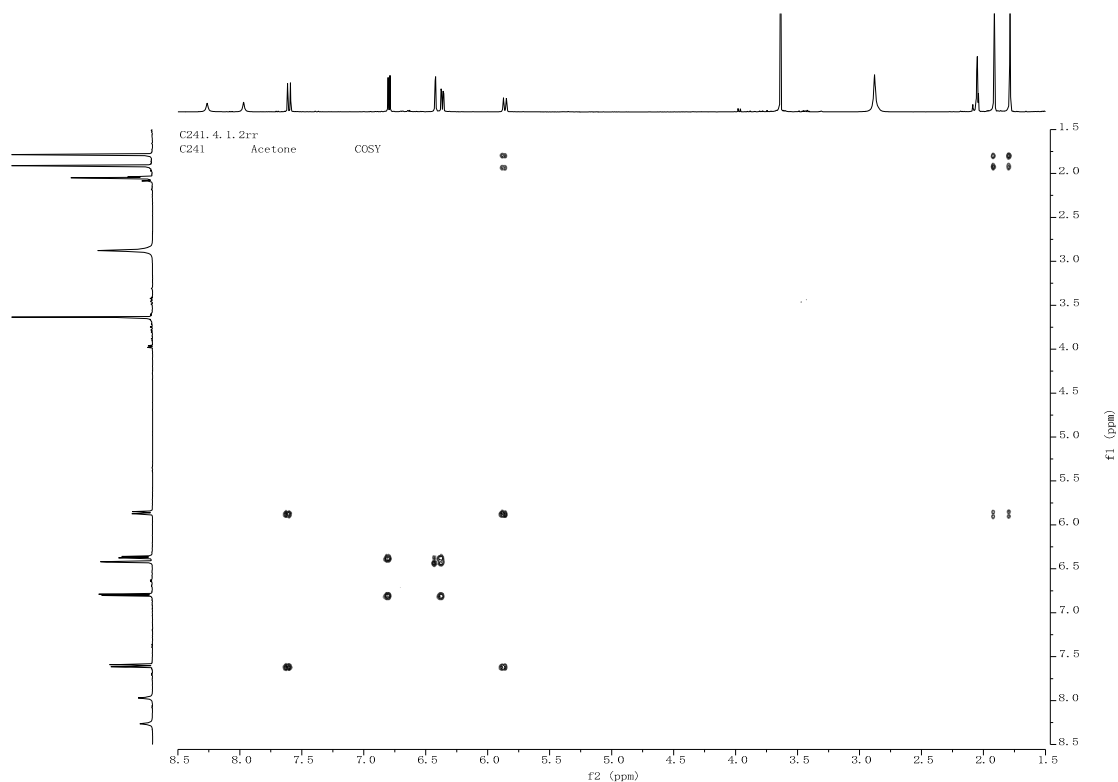


Figure S15.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound 2

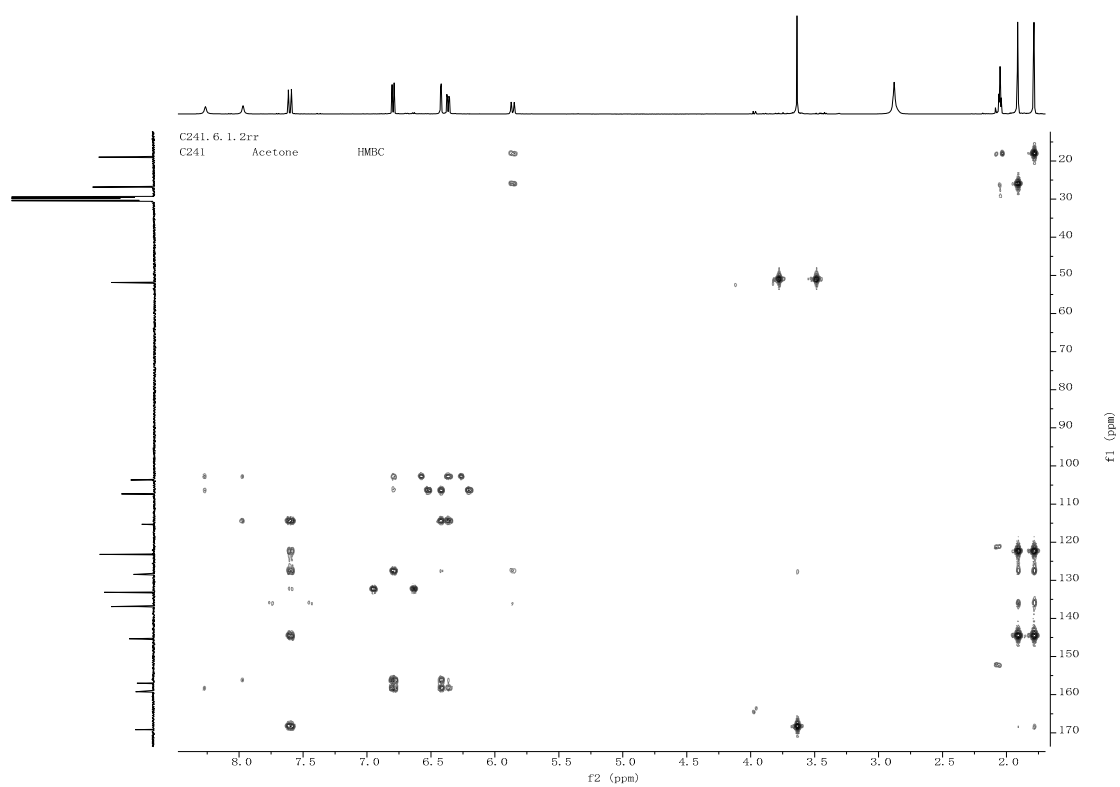


Figure S16. HMBC spectrum of compound 2

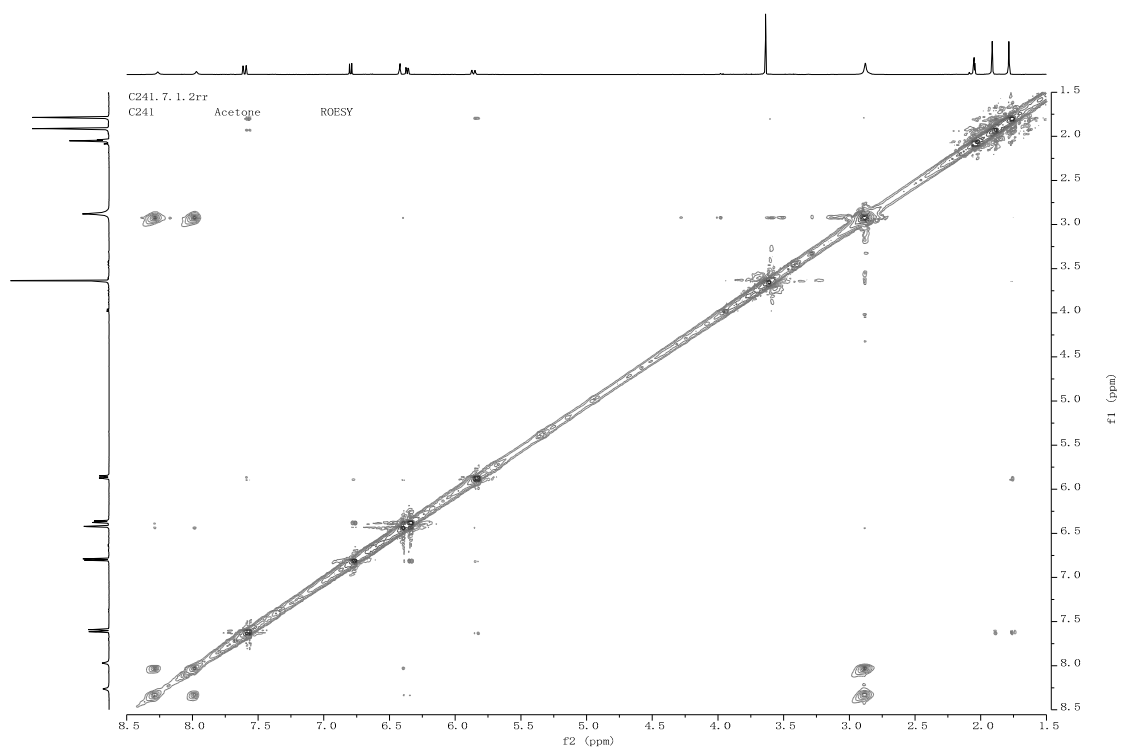


Figure S17. ROESY spectrum of compound 2

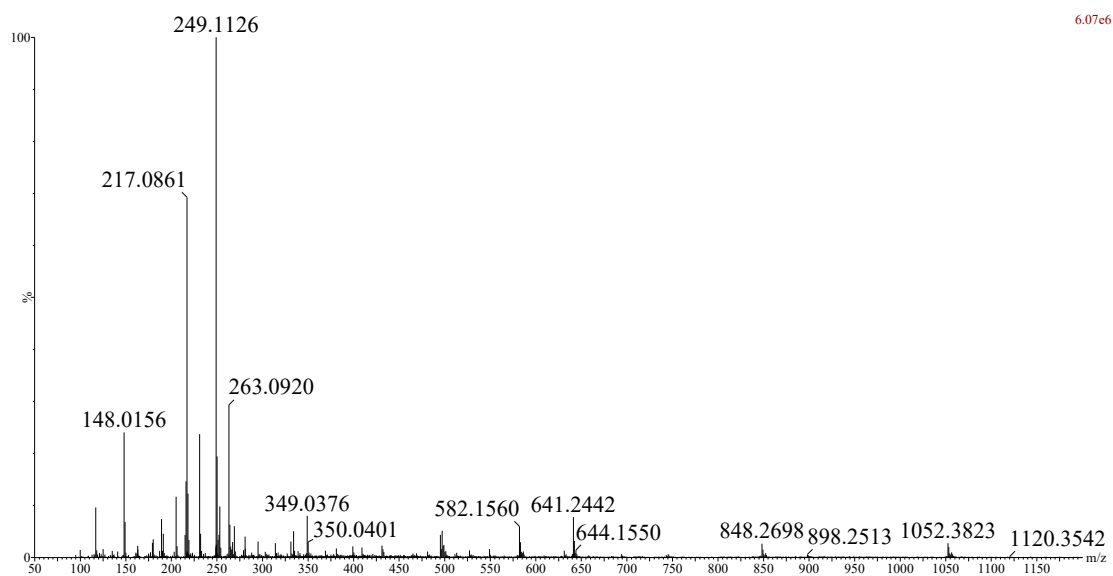


Figure S18. HRESIMS data of compound **3**

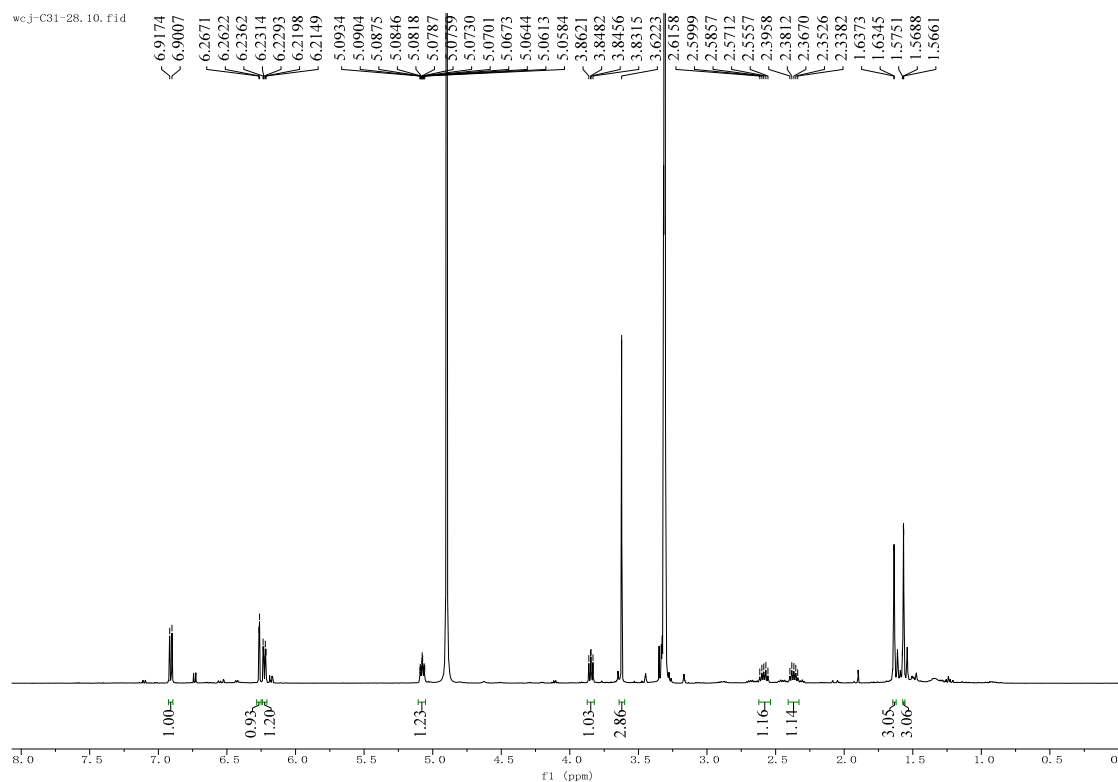


Figure S19. <sup>1</sup>H-NMR spectrum of compound **3** in CD<sub>3</sub>OD

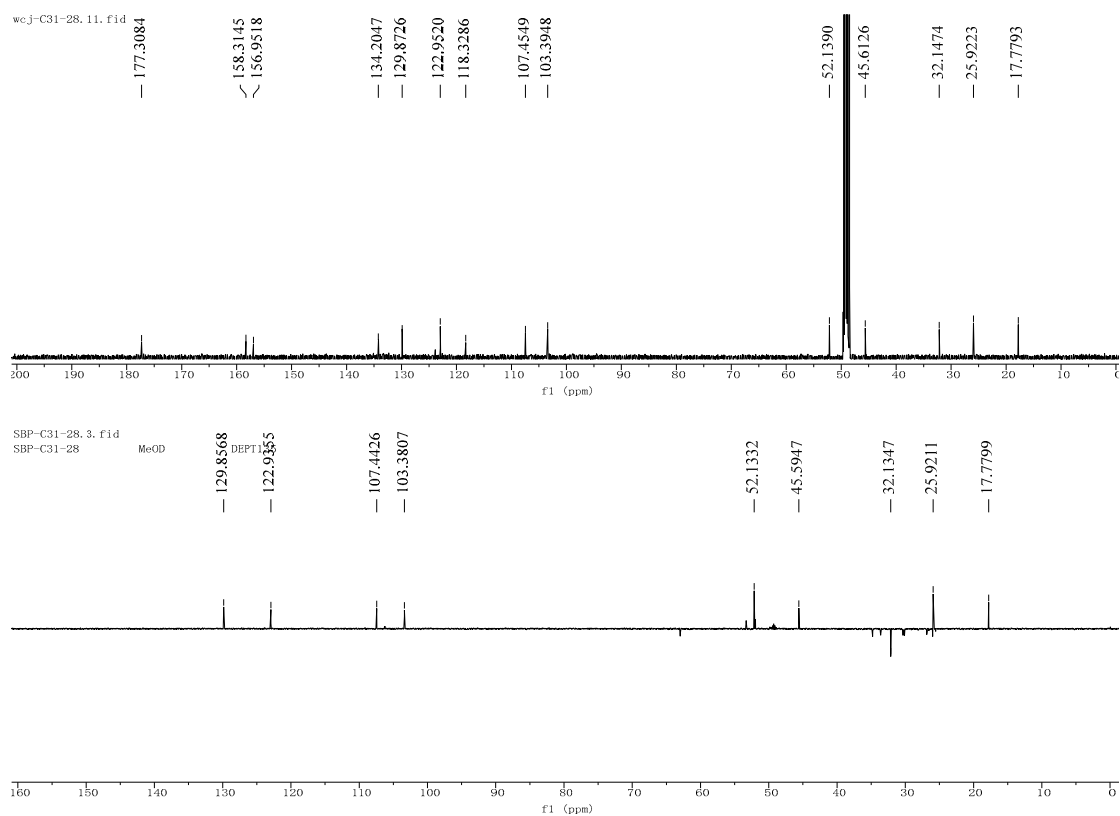


Figure S20.  $^{13}\text{C}$ -NMR and DEPT-135 spectra of compound 3 in  $\text{CD}_3\text{OD}$

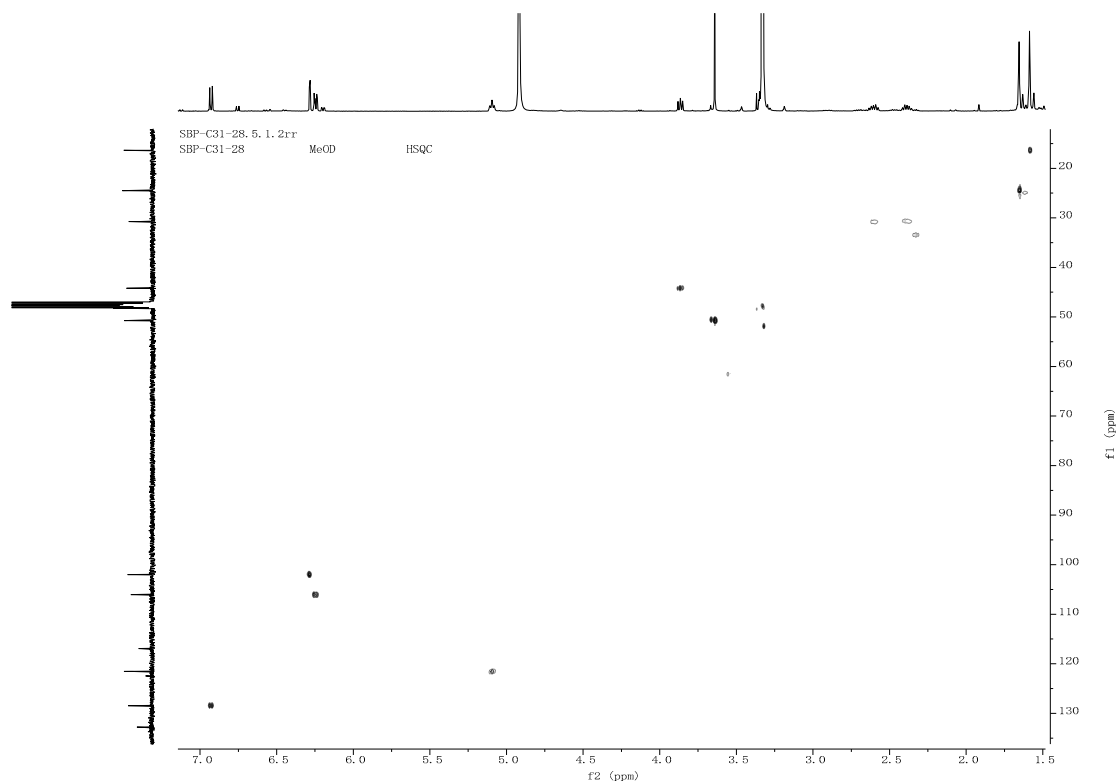


Figure S21. HSQC spectrum of compound 3

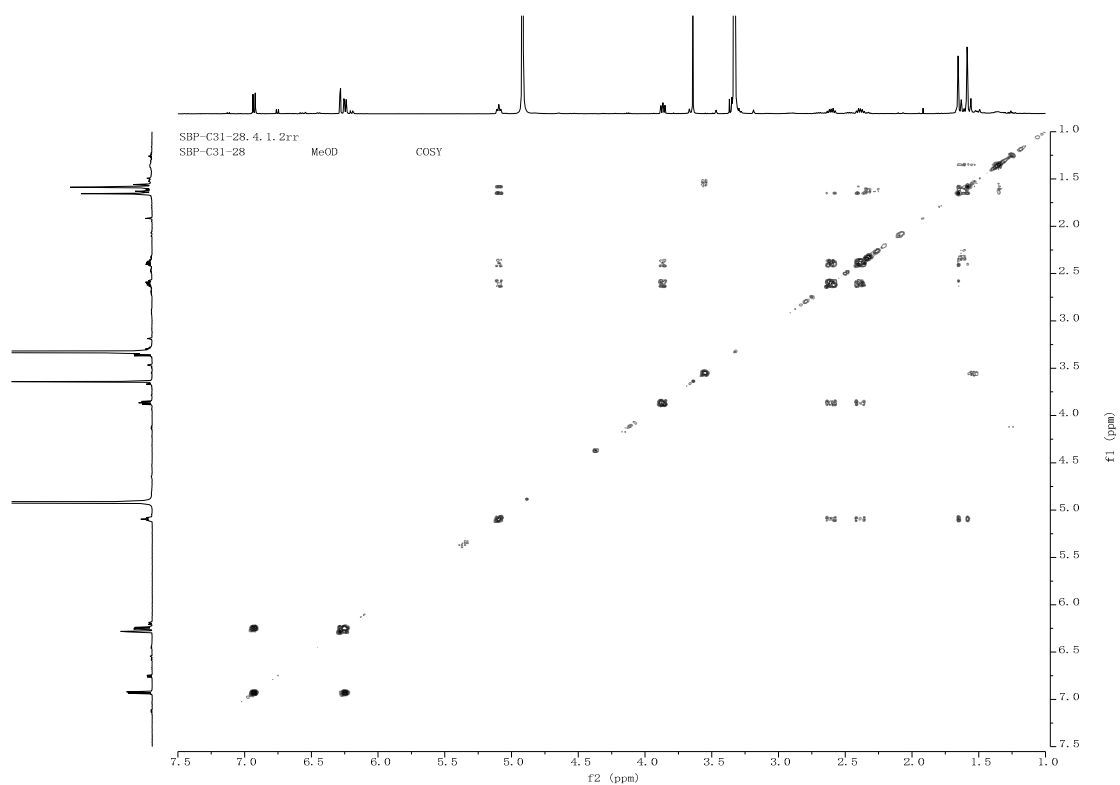


Figure S22.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound **3**

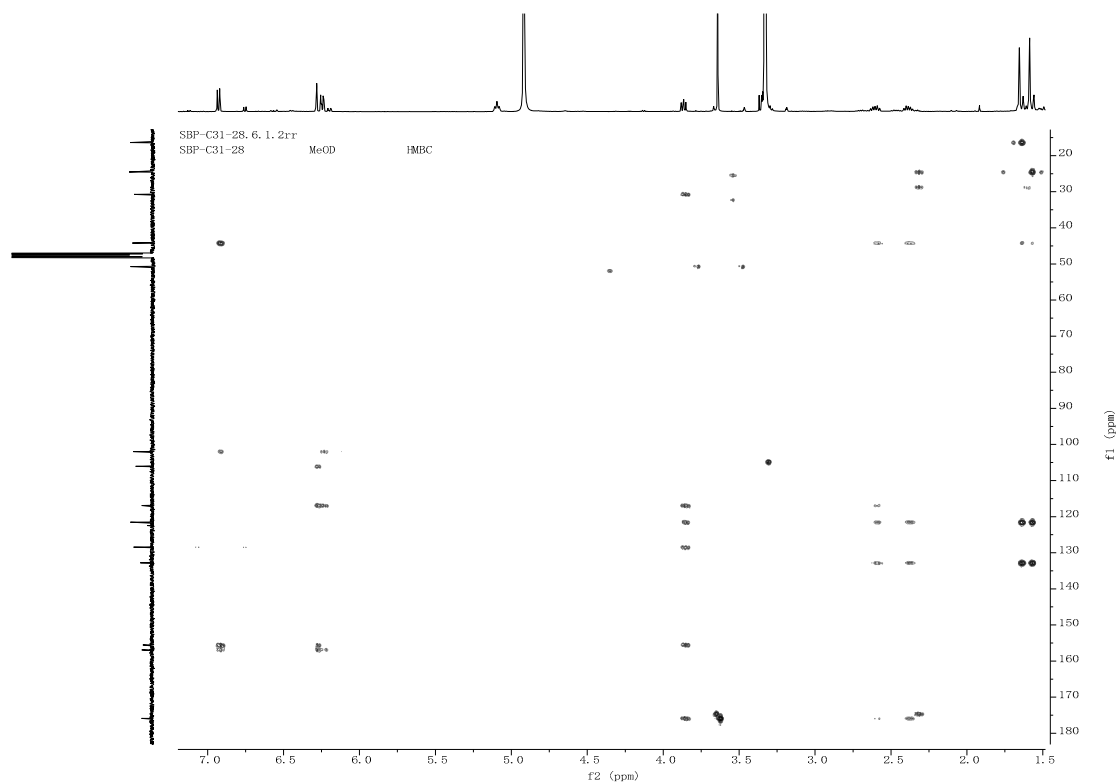


Figure S23. HMBC spectrum of compound **3**



Figure S24. CD spectrum of compound 3

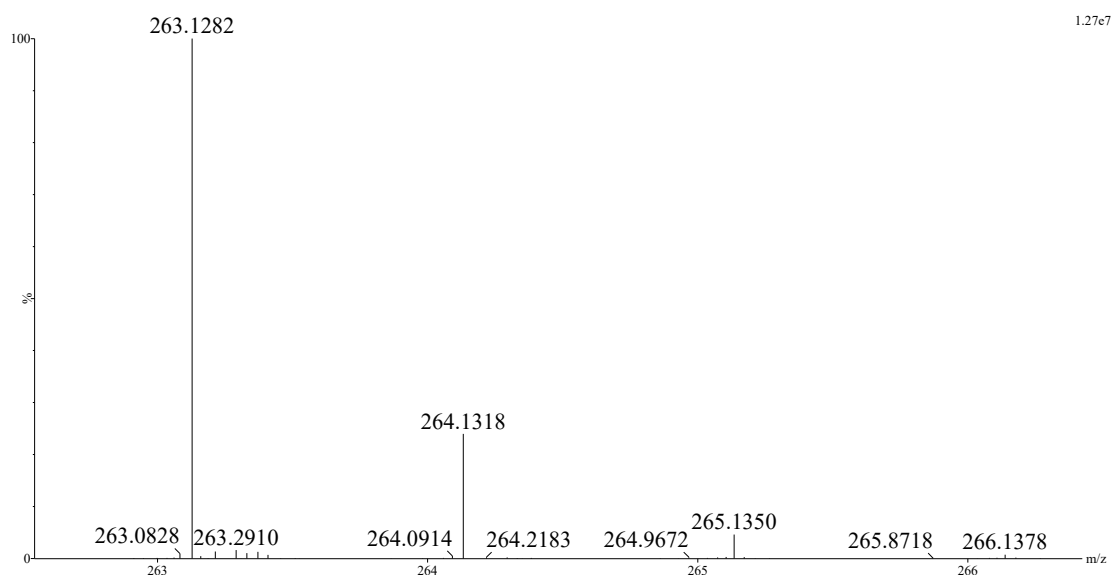


Figure S25. HRESIMS data of compound 4

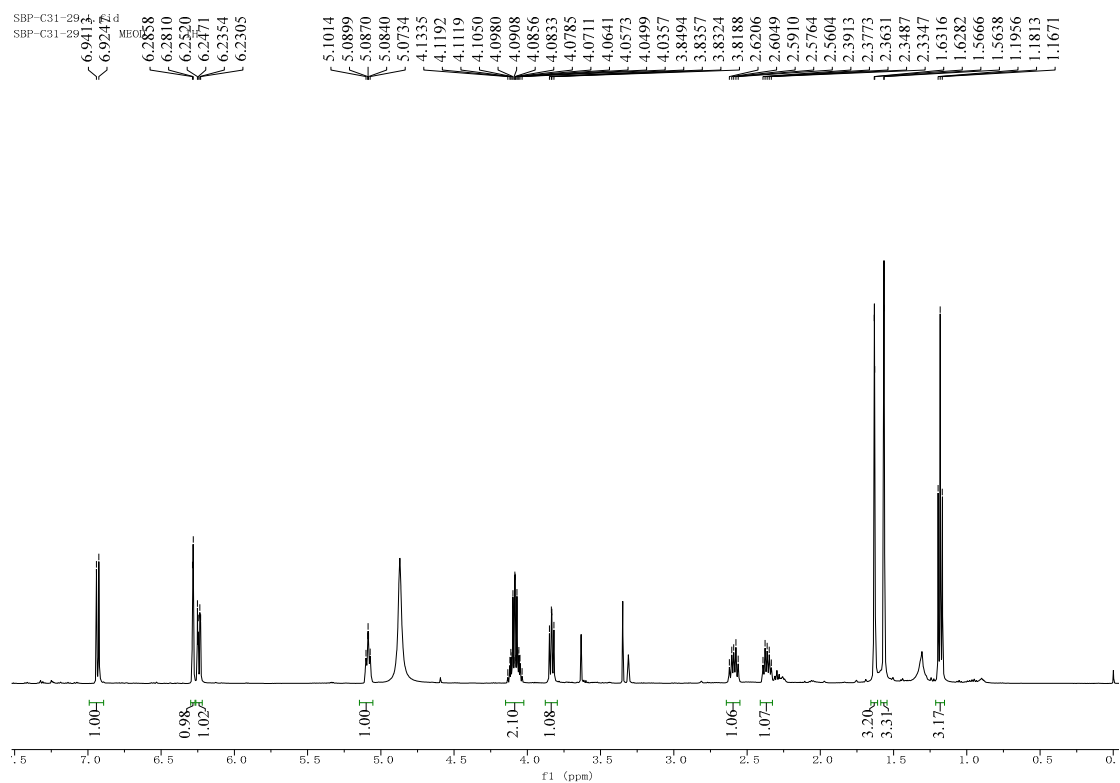


Figure S26.  $^1\text{H}$ -NMR spectrum of compound **4** in  $\text{CD}_3\text{OD}$

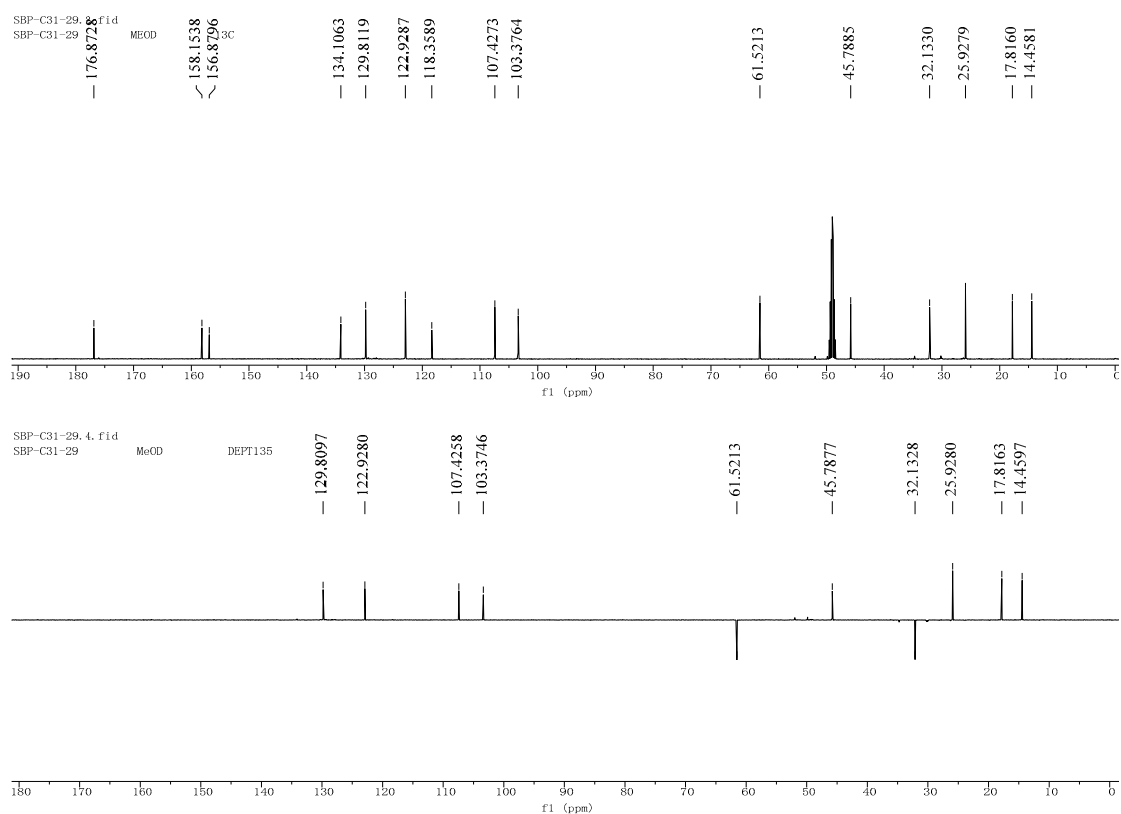


Figure S27.  $^{13}\text{C}$ -NMR and DEPT-135 spectra of compound **4** in  $\text{CD}_3\text{OD}$



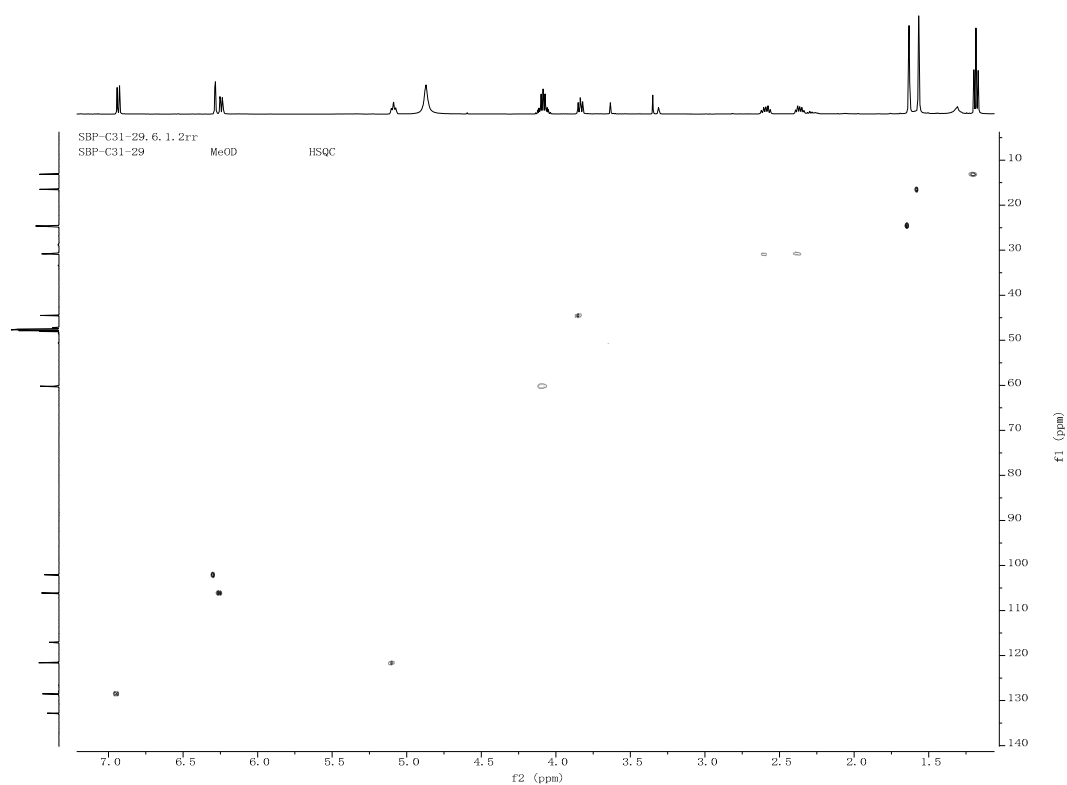


Figure S28. HSQC spectrum of compound 4

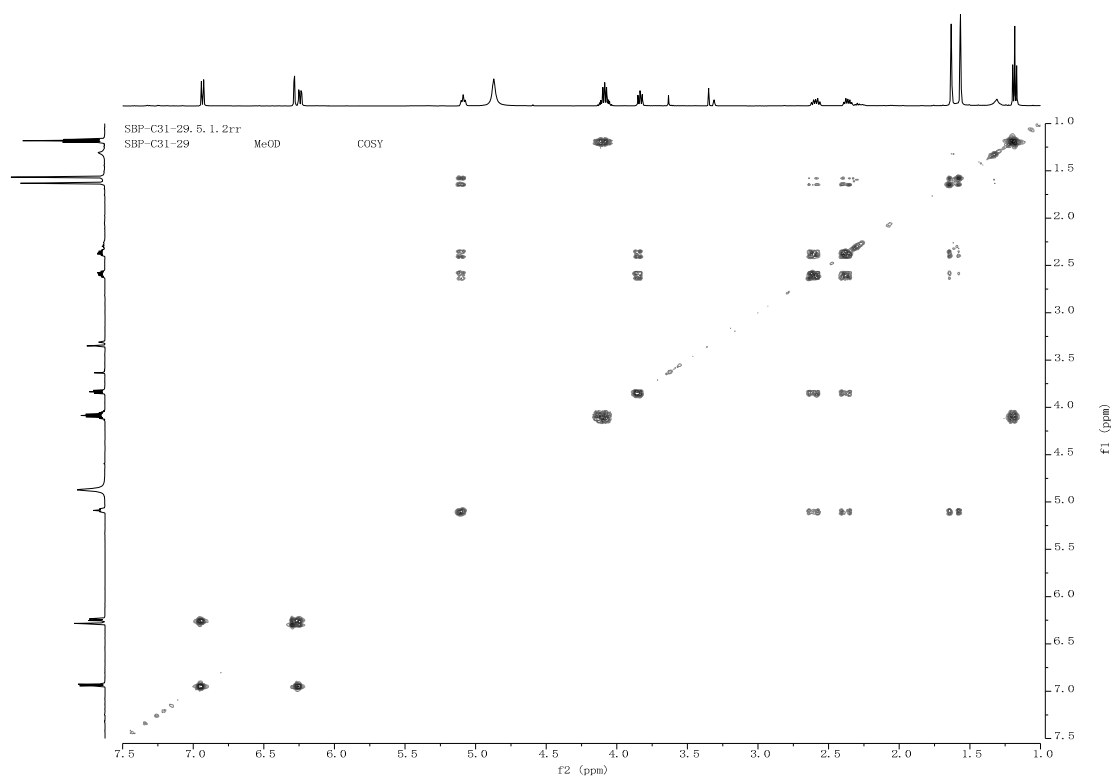


Figure S29.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound 4

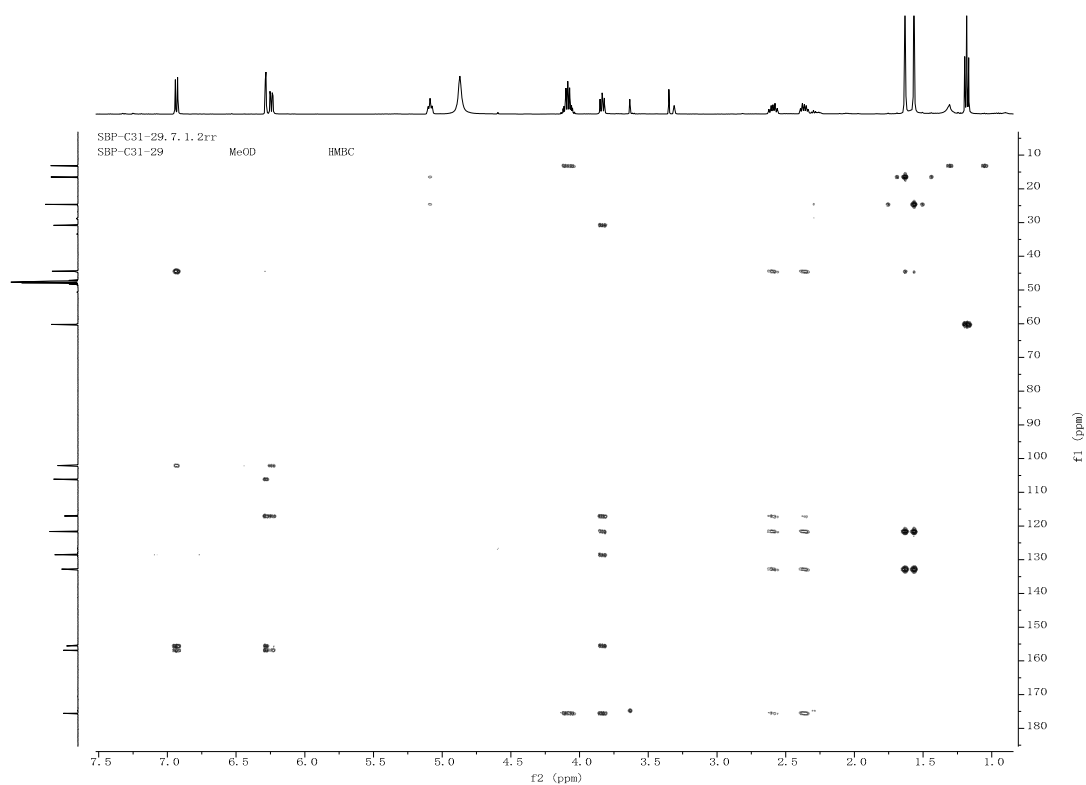


Figure S30. HMBC spectrum of compound **4**

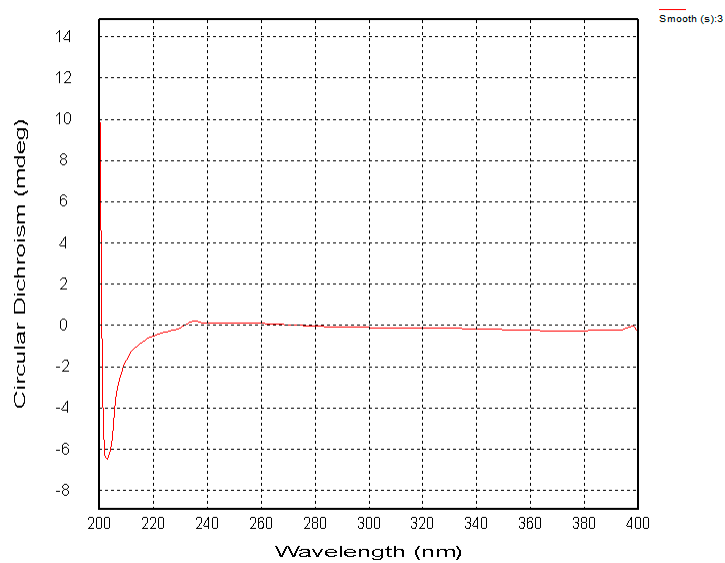


Figure S31. CD spectrum of compound **4**

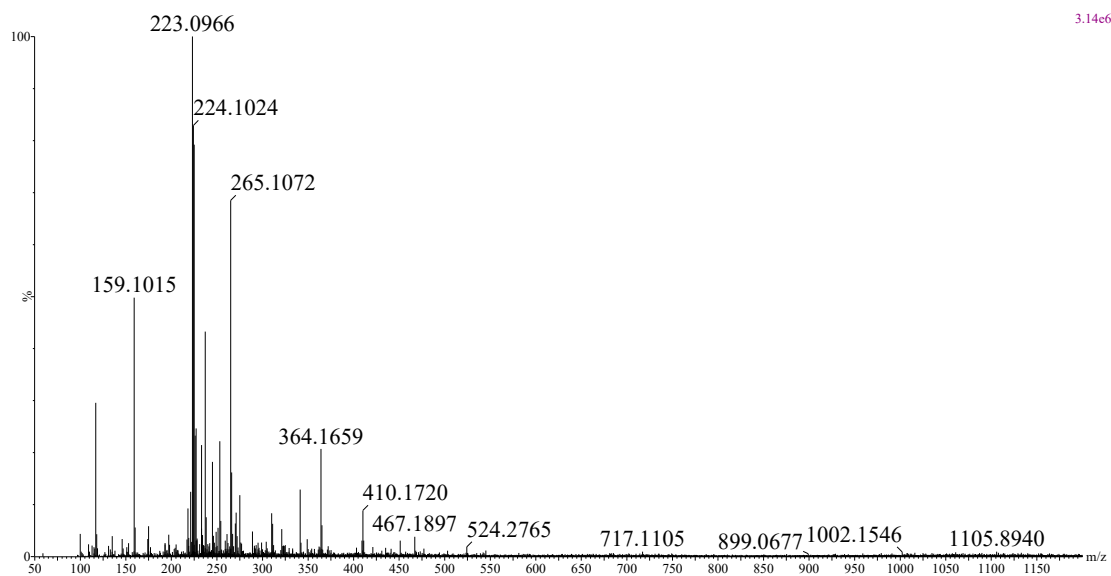


Figure S32. HRESIMS data of compound **5**

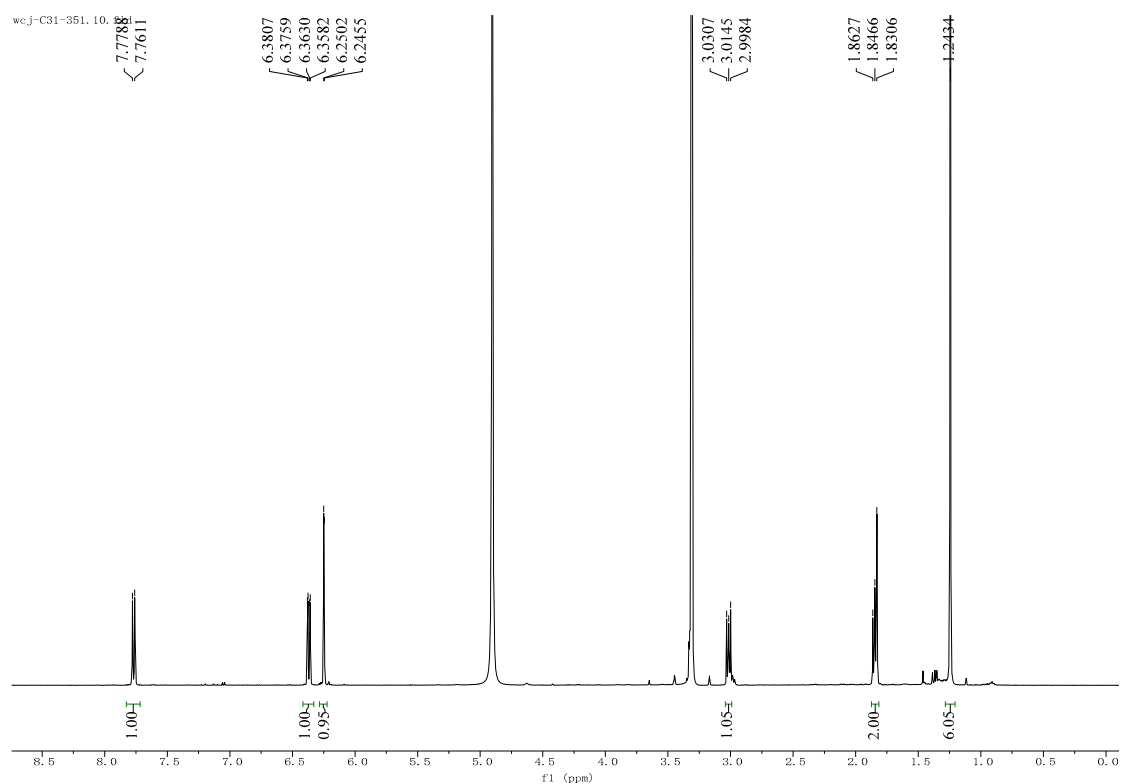


Figure S33. <sup>1</sup>H-NMR spectrum of compound **5** in CD<sub>3</sub>OD

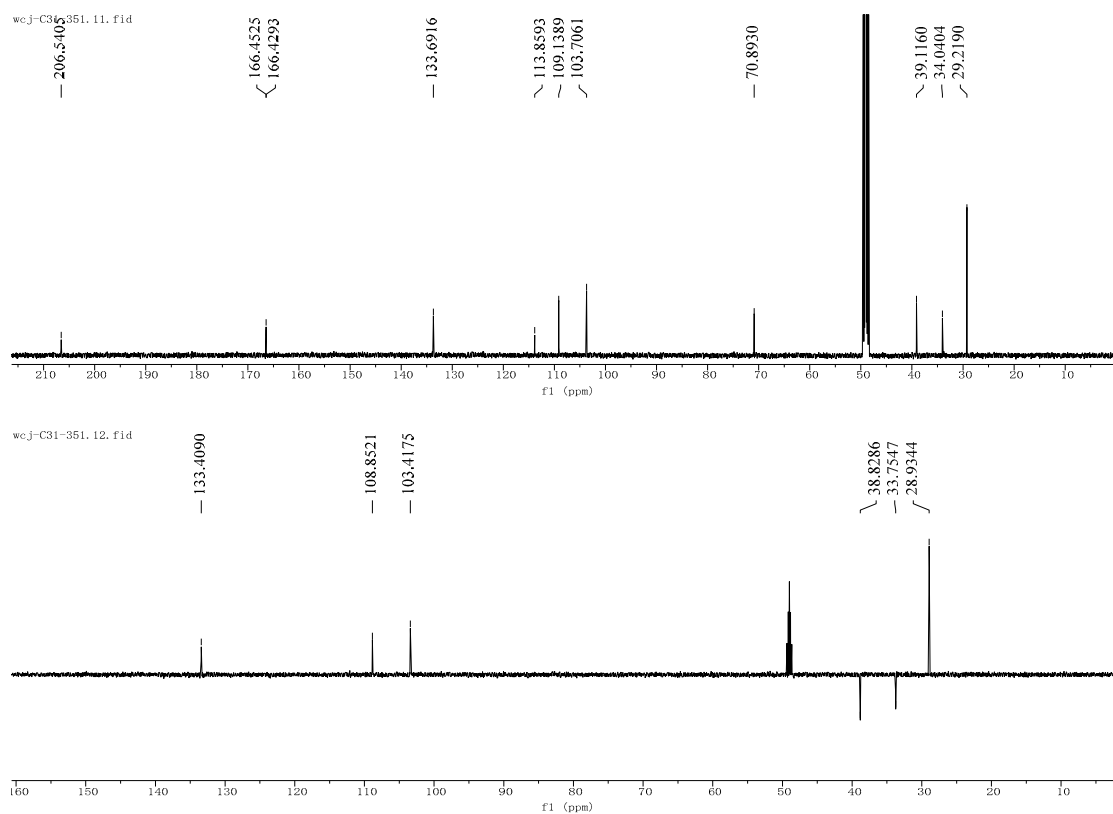


Figure S34. <sup>13</sup>C-NMR and DEPT-135 spectra of compound **5** in CD<sub>3</sub>OD

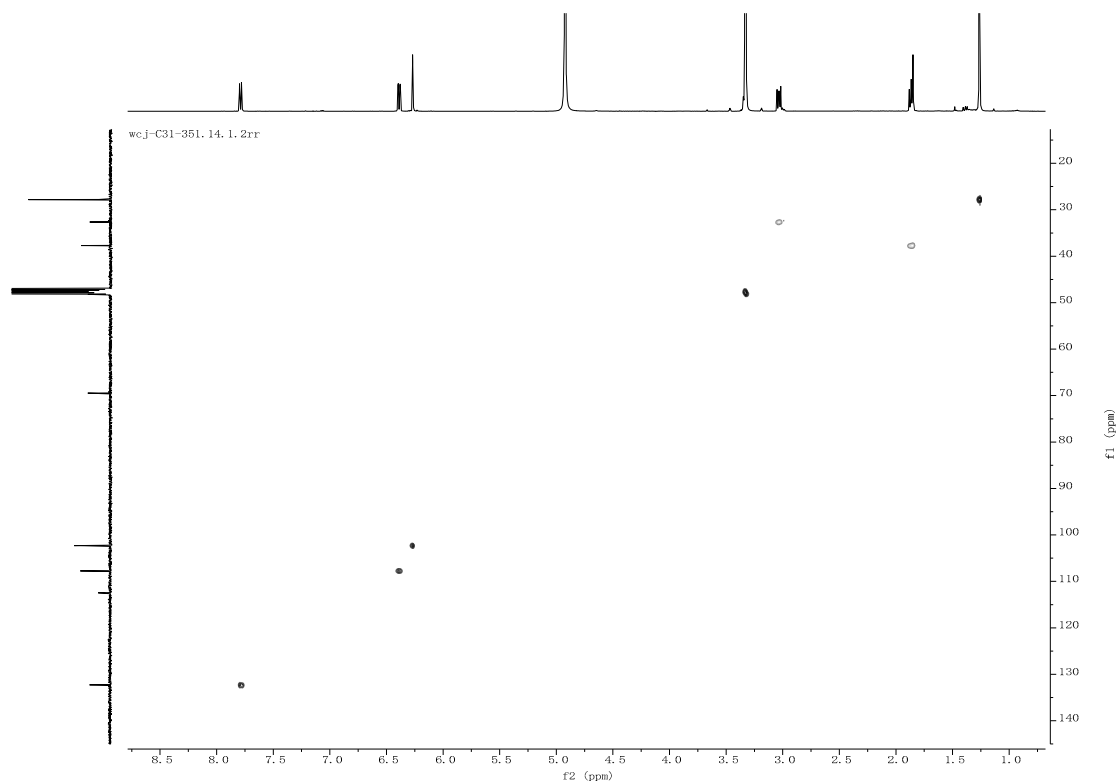


Figure S35. HSQC spectrum of compound **5**

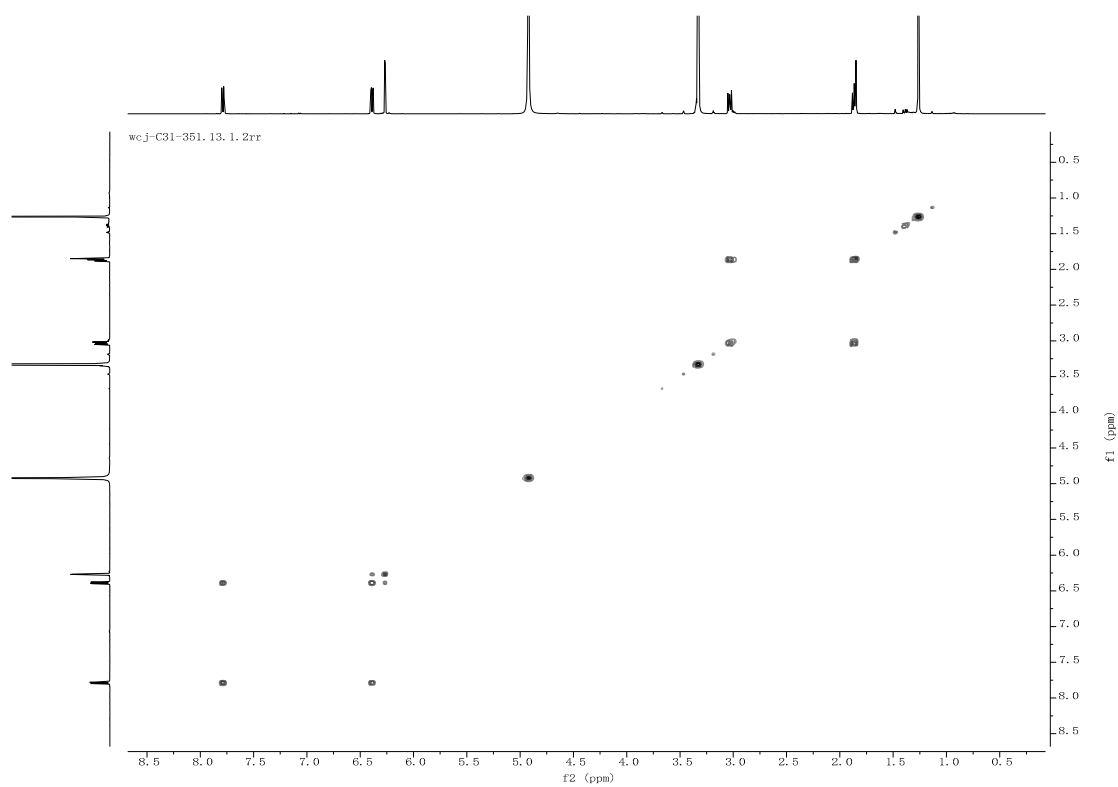


Figure S36.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound **5**

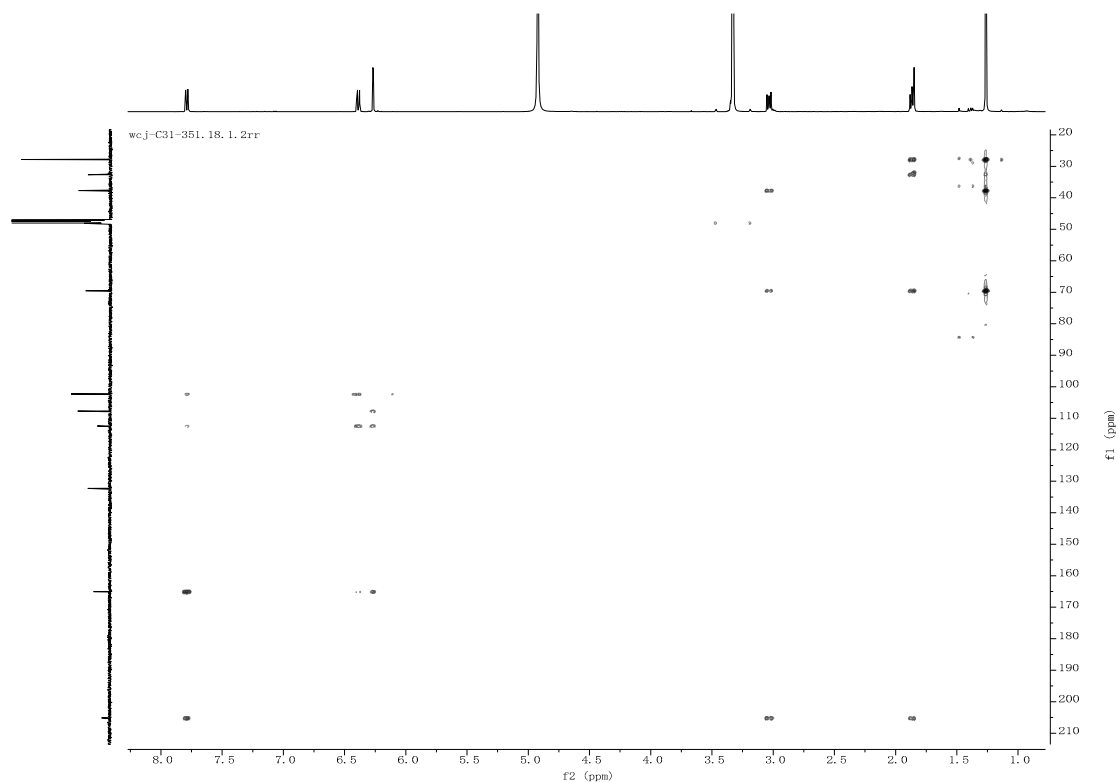


Figure S37. HMBC spectrum of compound **5**

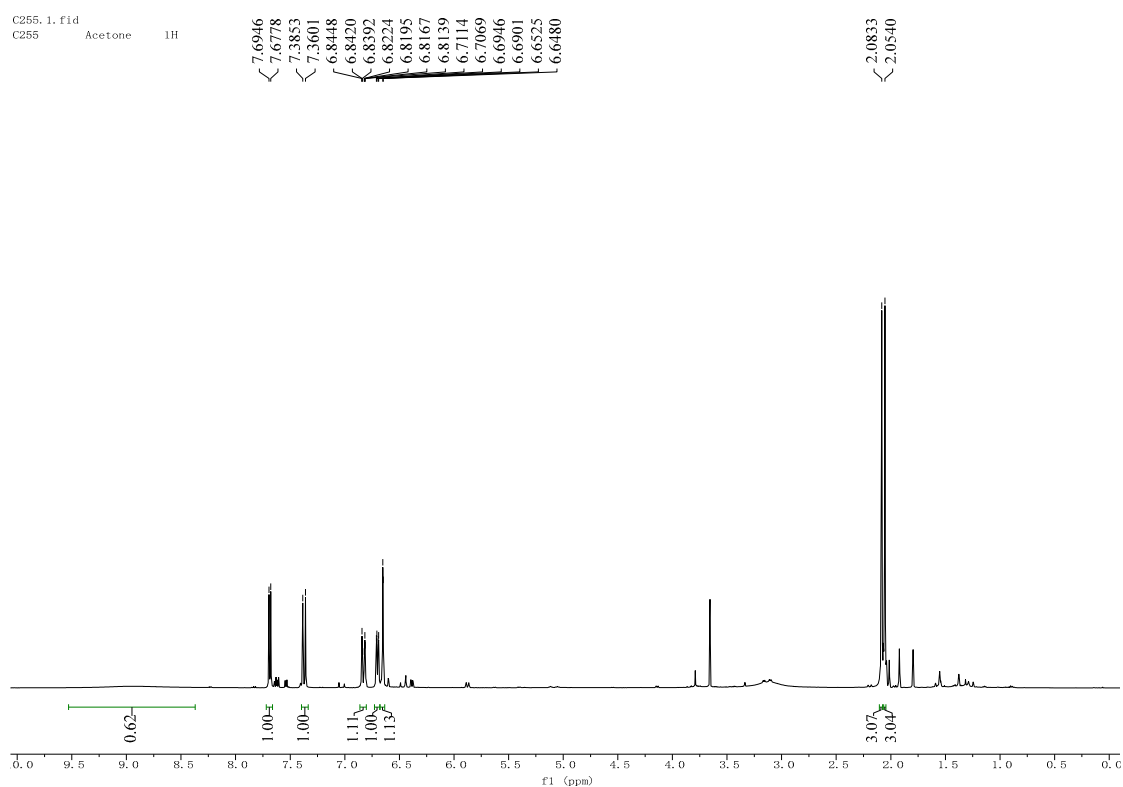


Figure S38.  $^1\text{H}$ -NMR spectrum of compound **6** in acetone- $d_6$

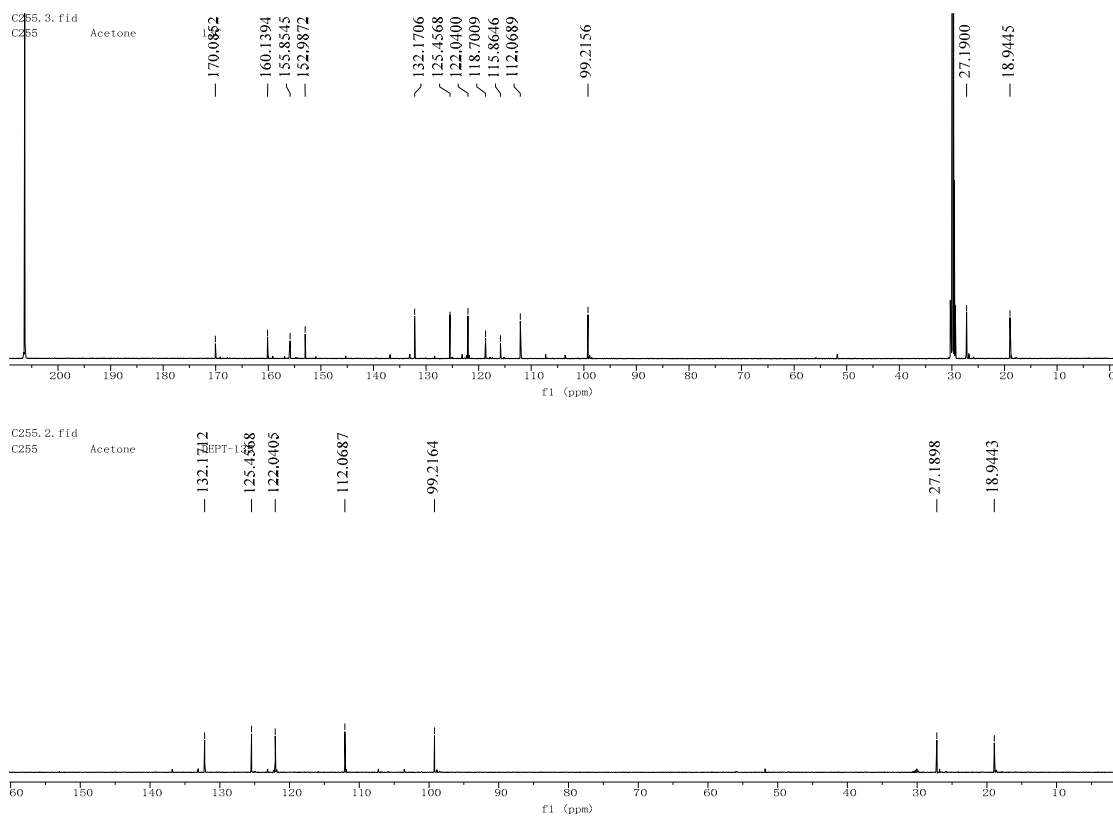


Figure S39.  $^{13}\text{C}$ -NMR and DEPT-135 spectra of compound **6** in acetone- $d_6$

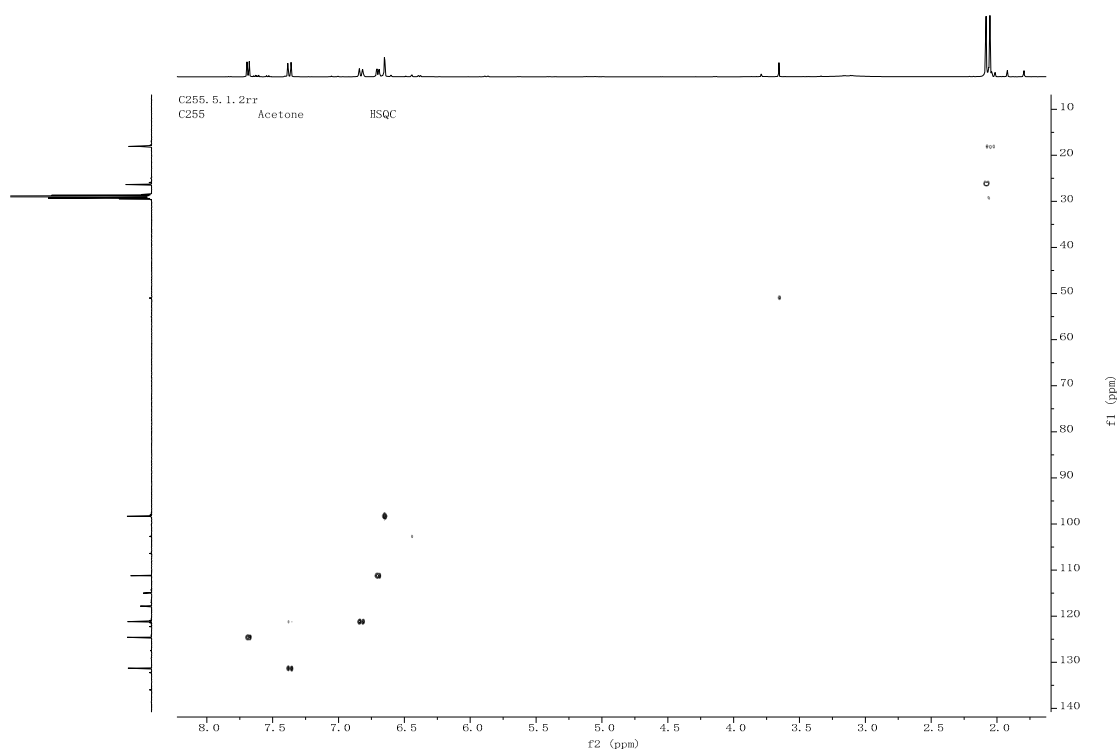


Figure S40. HSQC spectrum of compound 6

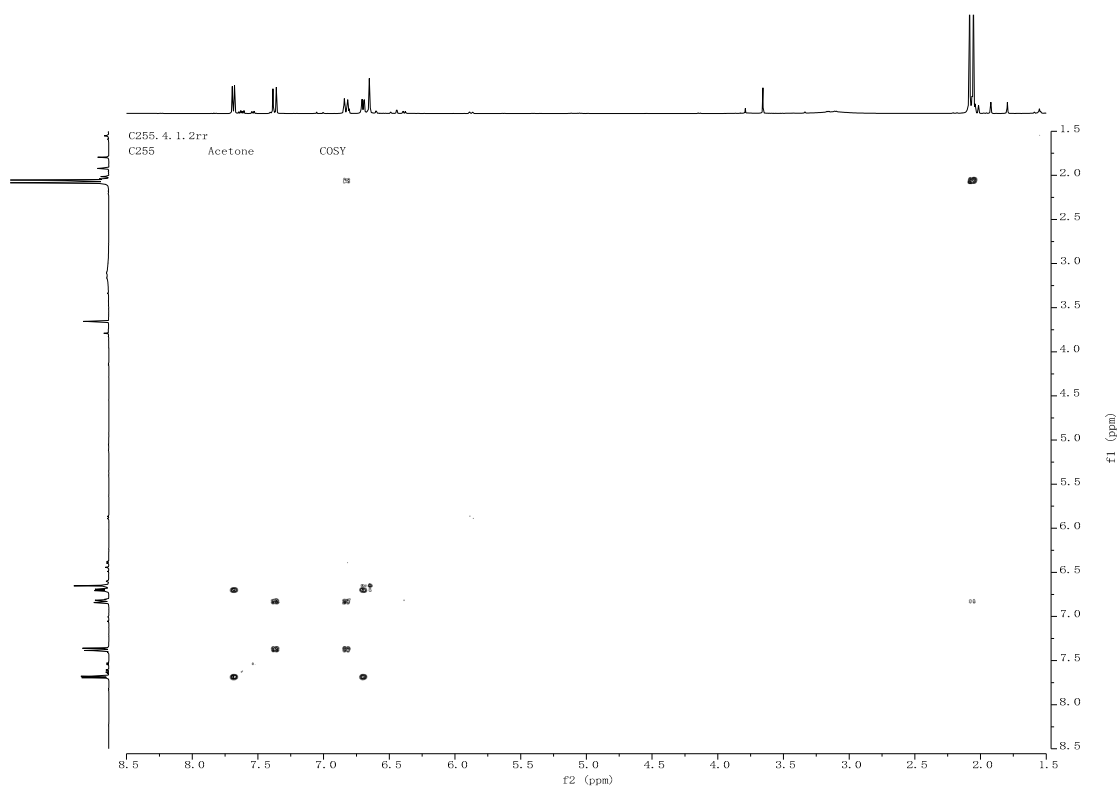


Figure S41.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound 6

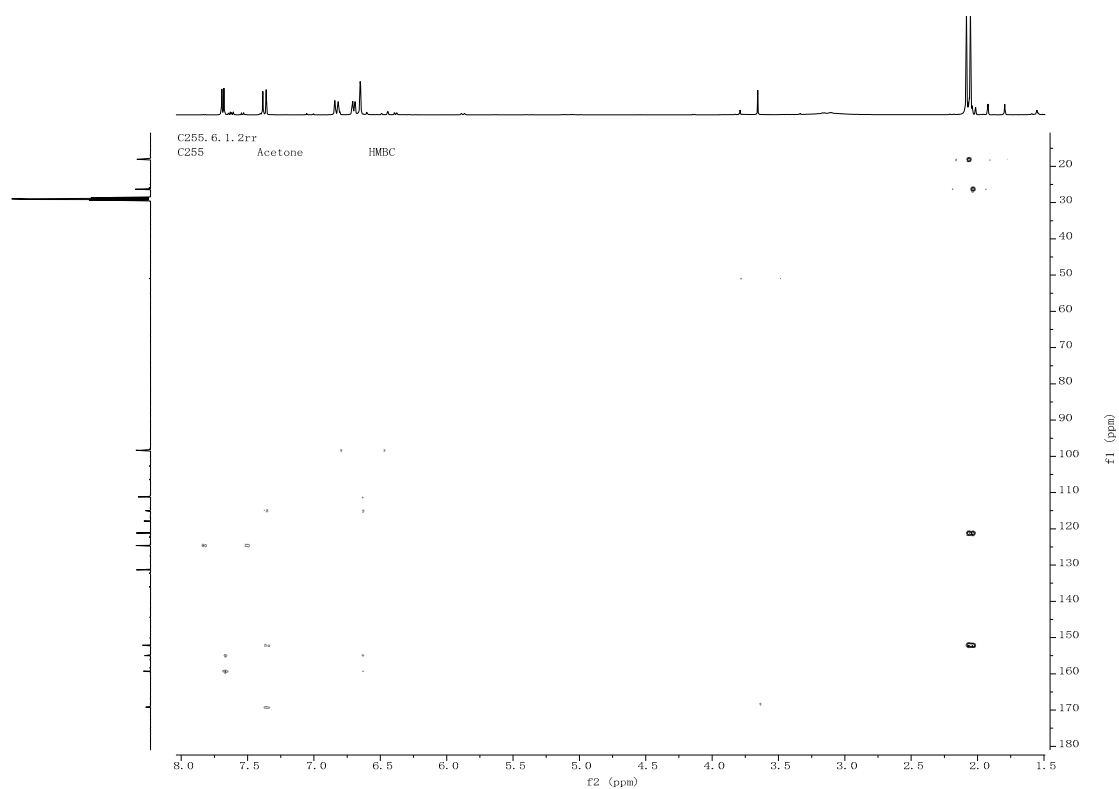


Figure S42. HMBC spectrum of compound **6**

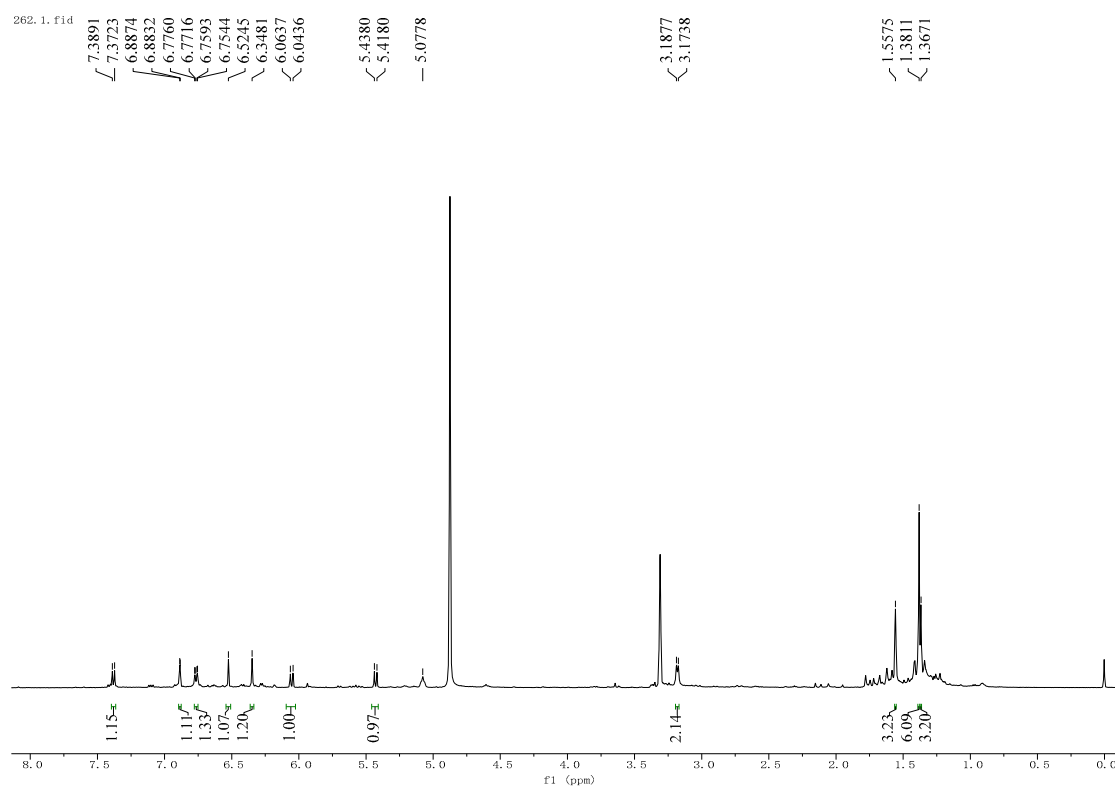


Figure S43.  $^1\text{H}$ -NMR spectrum of compound **14** in  $\text{CD}_3\text{OD}$



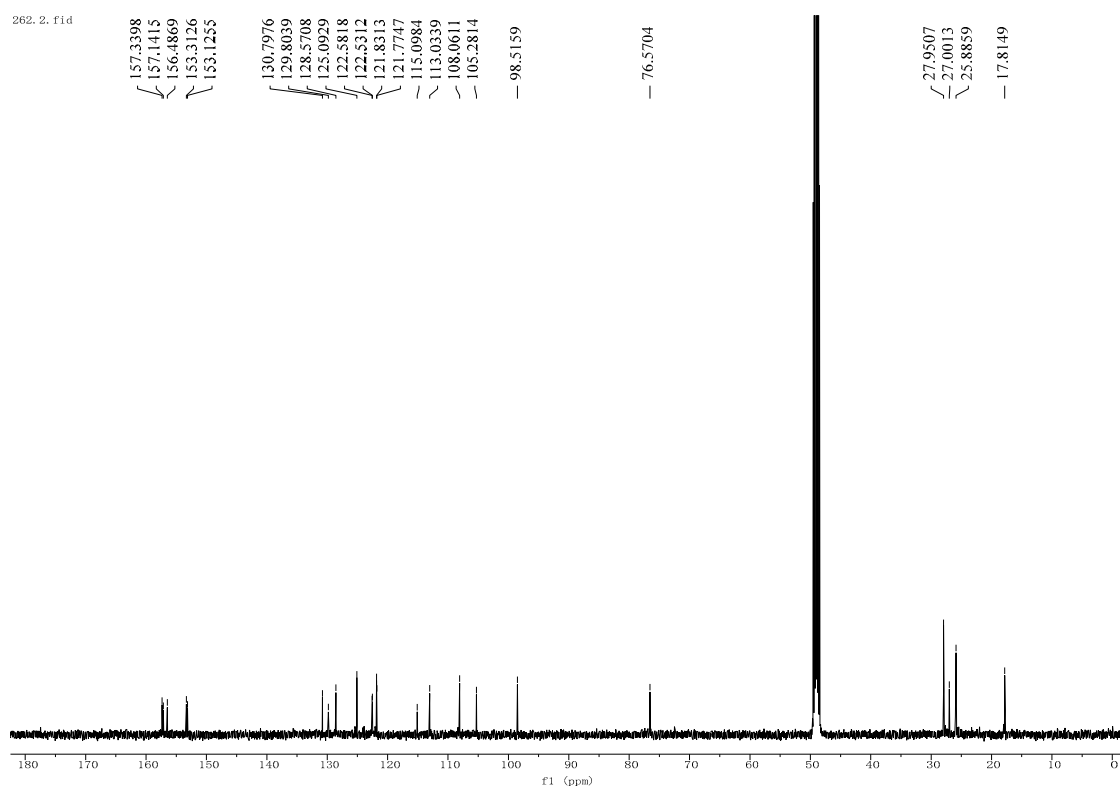


Figure S44.  $^{13}\text{C}$ -NMR spectrum of compound **14** in  $\text{CD}_3\text{OD}$