

Cytotoxic polyhydroxylated pregnane glycosides from *Cissampelos pareira* var. *hirsuta*

Yan-Jun Sun,^{*abc} Hao-Jie Chen,^{ab} Rui-Jie Han,^{ab} Chen Zhao,^{ab} Ying-Ying Si,^{ab} Meng Li,^{ab} Kun Du,^{ab} Hui Chen,^{ab} Wei-Sheng Feng^{*ab}

Fourteen new polyhydroxylated pregnane glycosides, cissasteroid A–N (**1–14**), and five known analogues (**15–19**), were isolated from the dried whole plant of *Cissampelos pareira* var. *hirsuta*. Their structures and stereochemistry were elucidated by extensive spectroscopic data, chemical hydrolysis, and ECD measurements. All the compounds were tested for their cytotoxicity against five human cancer cell lines, and inhibitory activity against NO release in LPS-induced RAW 264.7 cells. Compared with cisplatin, compound **7** showed more potent cytotoxicities against the HL-60, A549, SMMC-7721, MCF-7, and SW480 cell lines, with IC₅₀ values of 2.19, 14.38, 2.00, 7.58, and 7.44 μM, respectively. The preliminary study of structure-activity relationship indicated that benzoic acid esterification at C-20 may have a negative effect on the cytotoxic activity of polyhydroxylated pregnane derivatives in these five human cancer cell lines. These results revealed the potential of compound **7** as an ideal antitumor lead compound.

^aCo-construction Collaborative Innovation Center for Chinese Medicine and Respiratory Diseases by Henan & Education Ministry of P. R. China, Henan University of Traditional Chinese Medicine, Zhengzhou 450046, P. R. China. E-mails: sunyanjunily@126.com; fwsh@hactcm.edu.cn

^bSchool of Pharmacy, Henan University of Traditional Chinese Medicine, Zhengzhou 450046, P. R. China

^cHenan Research Center for Special Processing Technology of Chinese Medicine, Zhengzhou, 450046, P. R. China

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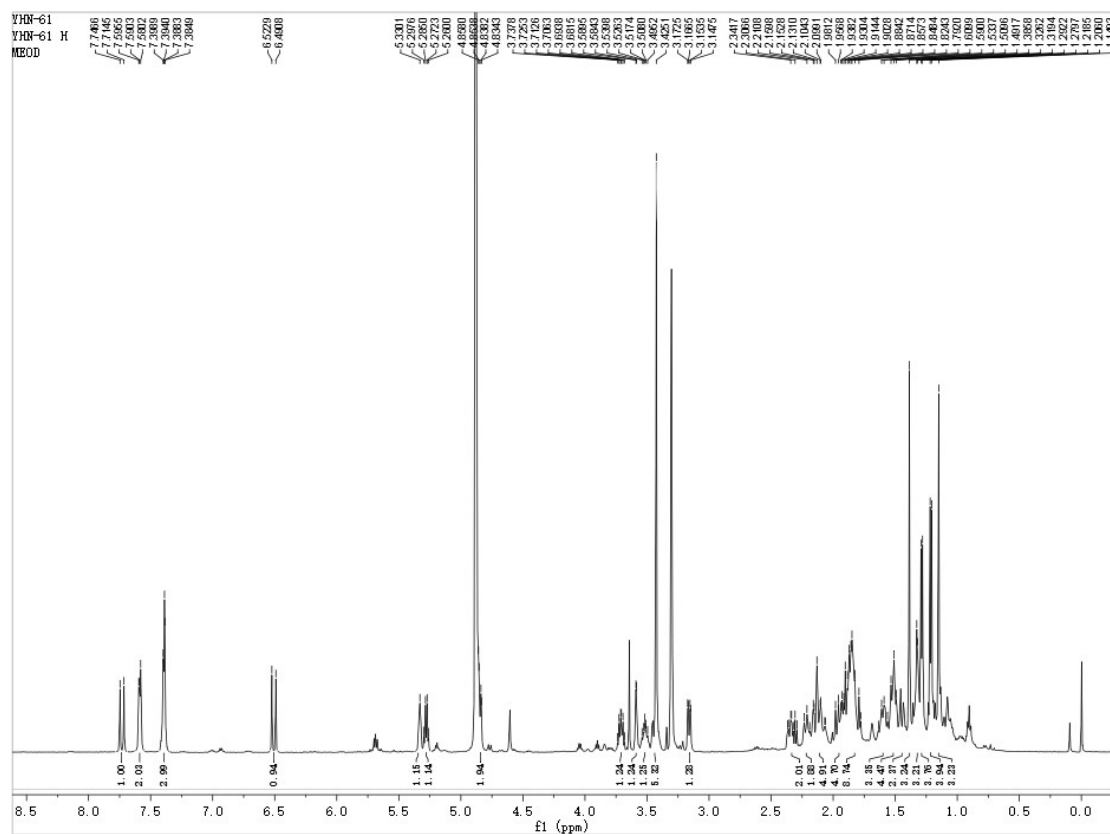


Figure S1. ^1H NMR spectrum (500 MHz) of **1** in CD_3OD

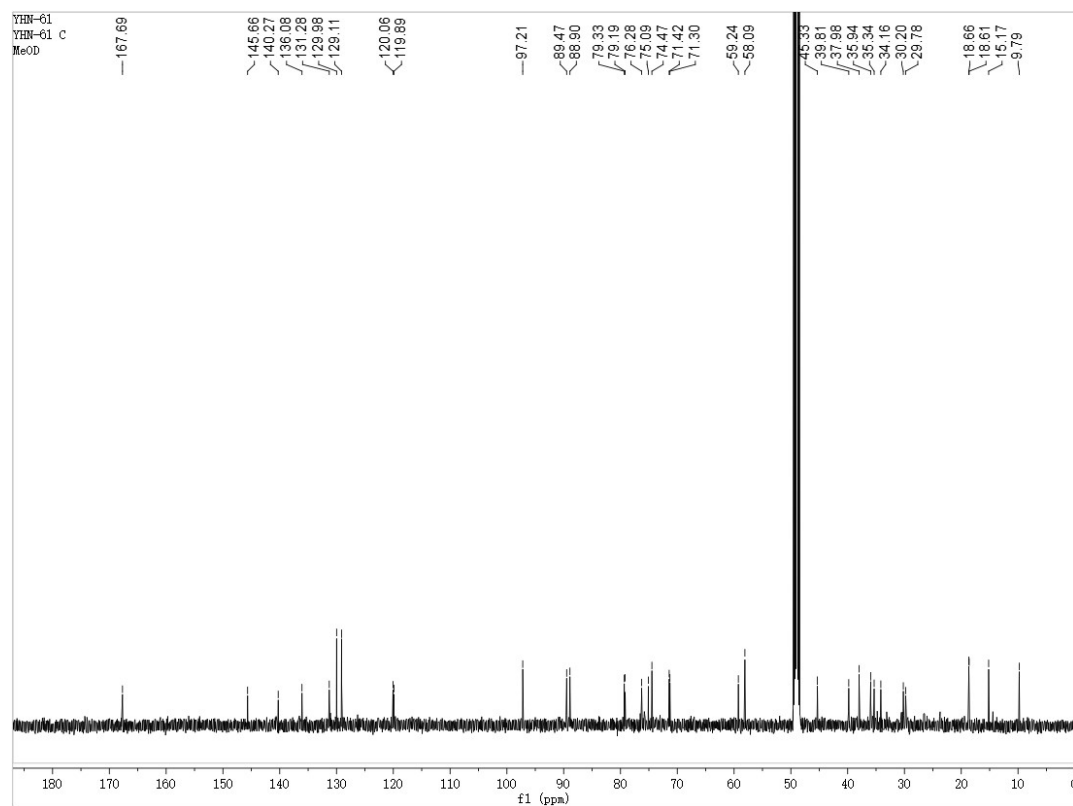


Figure S2. ^{13}C NMR spectrum (125 MHz) of **1** in CD_3OD

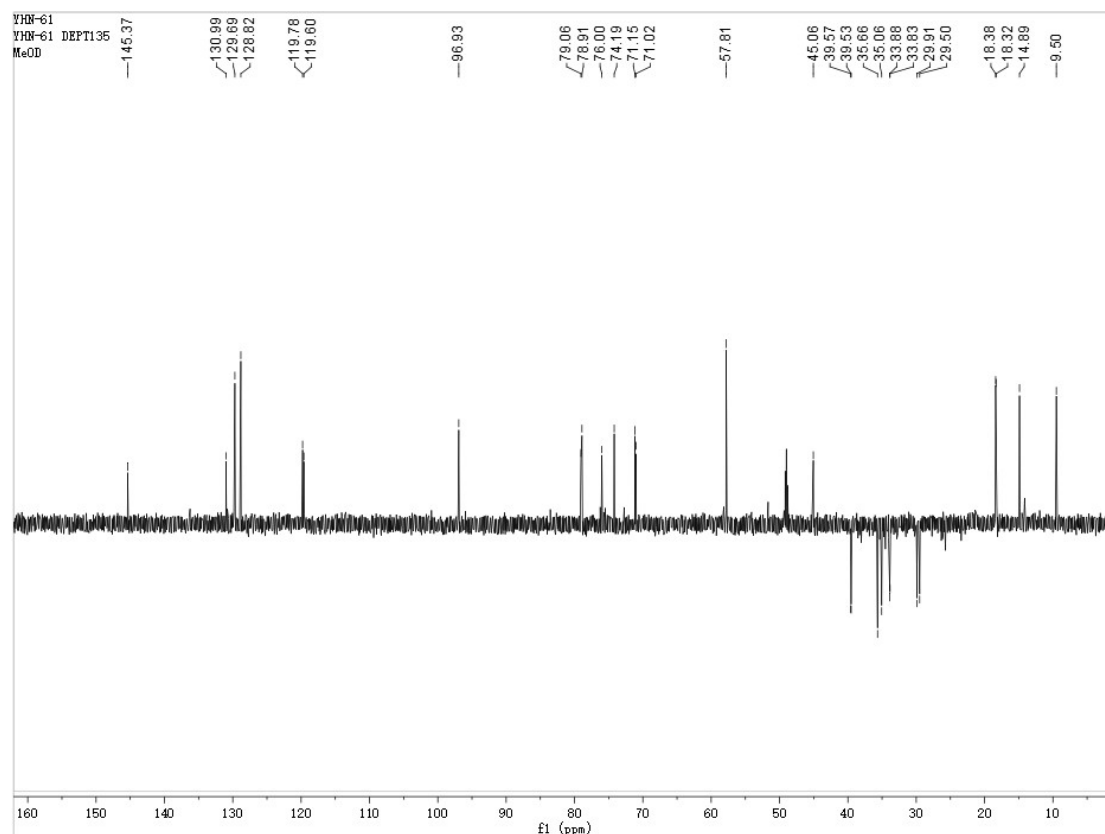


Figure S3. DEPT spectrum of **1** in CD₃OD

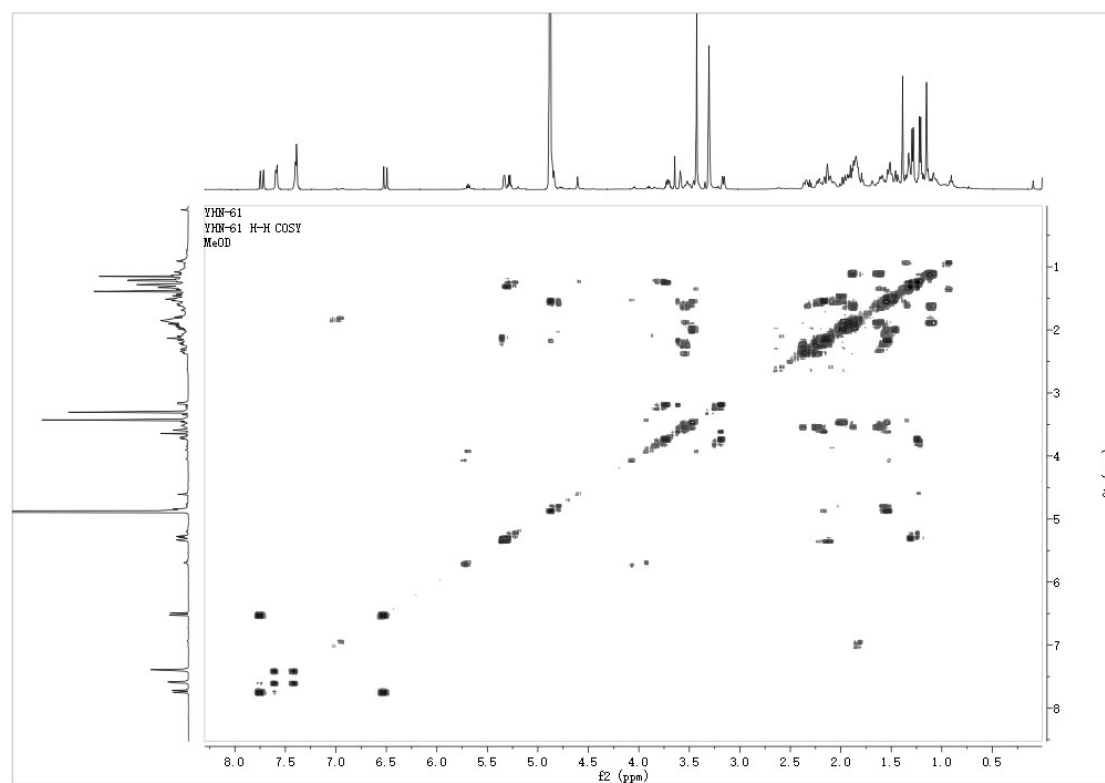


Figure S4. ¹H-¹H COSY of **1** in CD₃OD

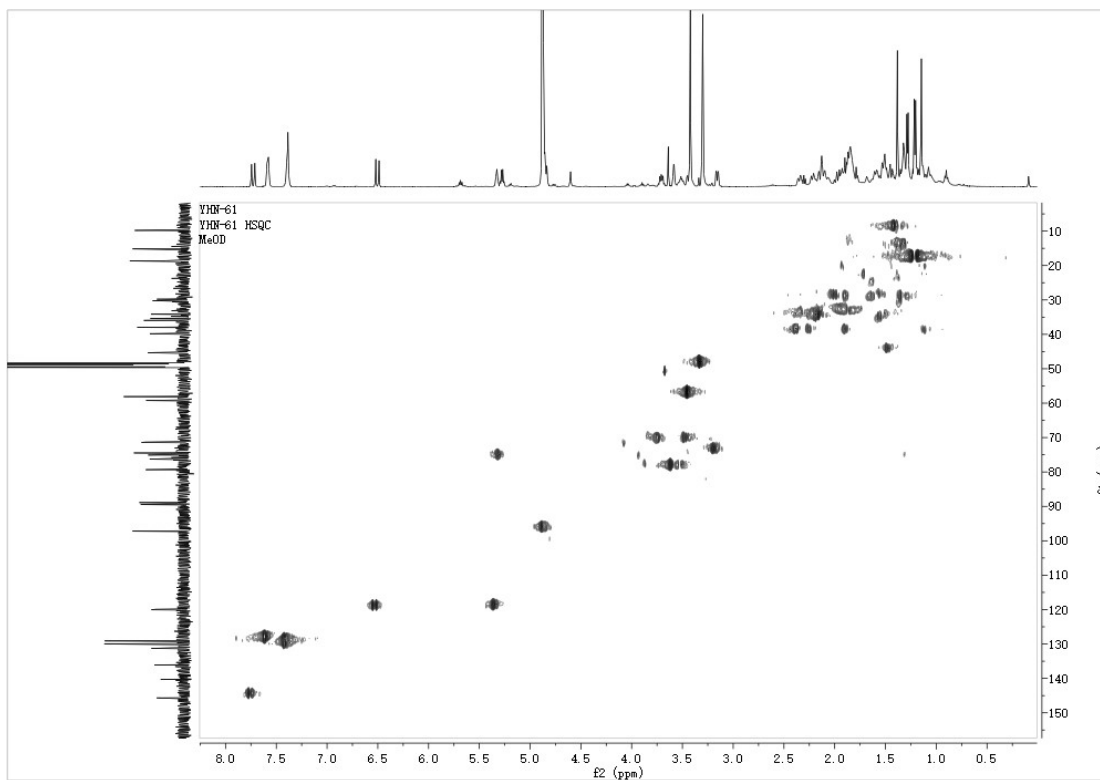


Figure S5. HSQC of **1** in CD₃OD

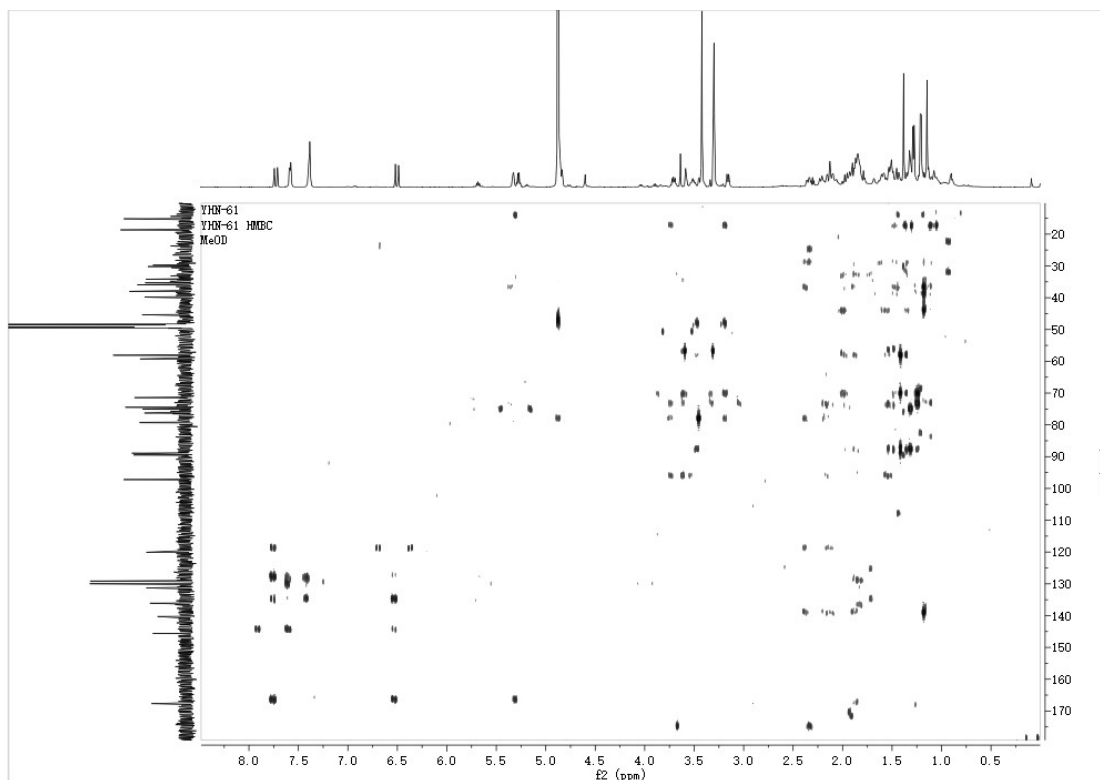


Figure S6. HMBC of **1** in CD₃OD

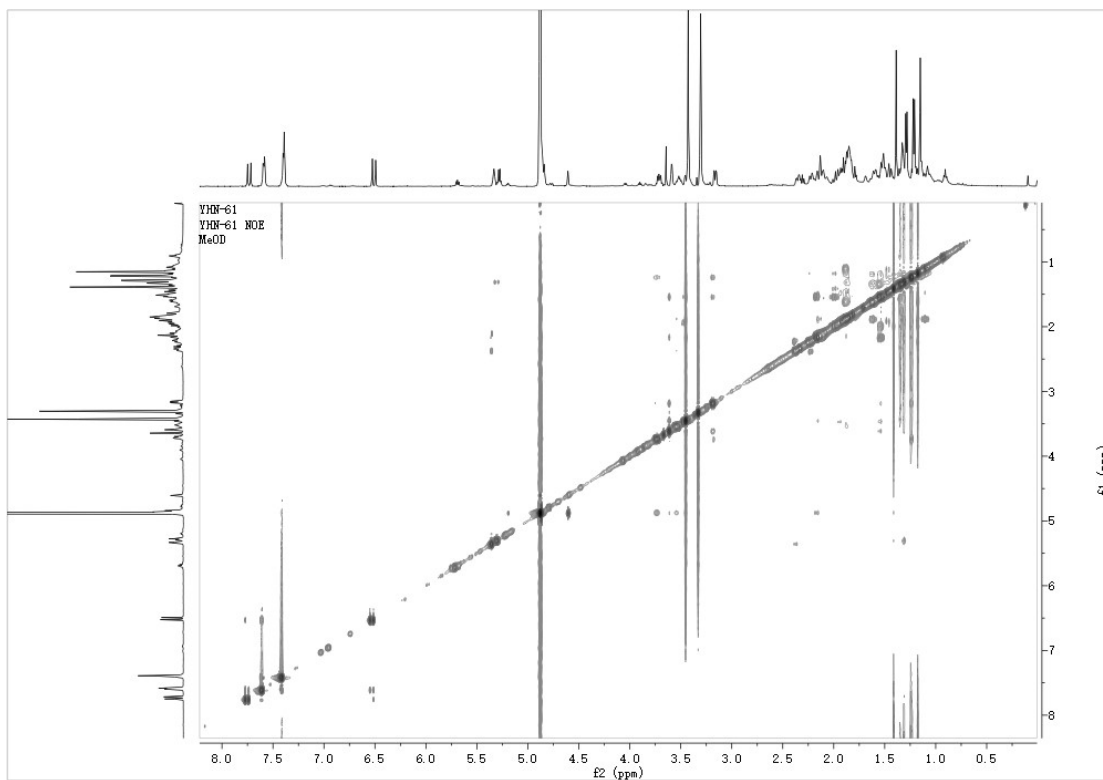


Figure S7. NOESY of 1 in CD₃OD

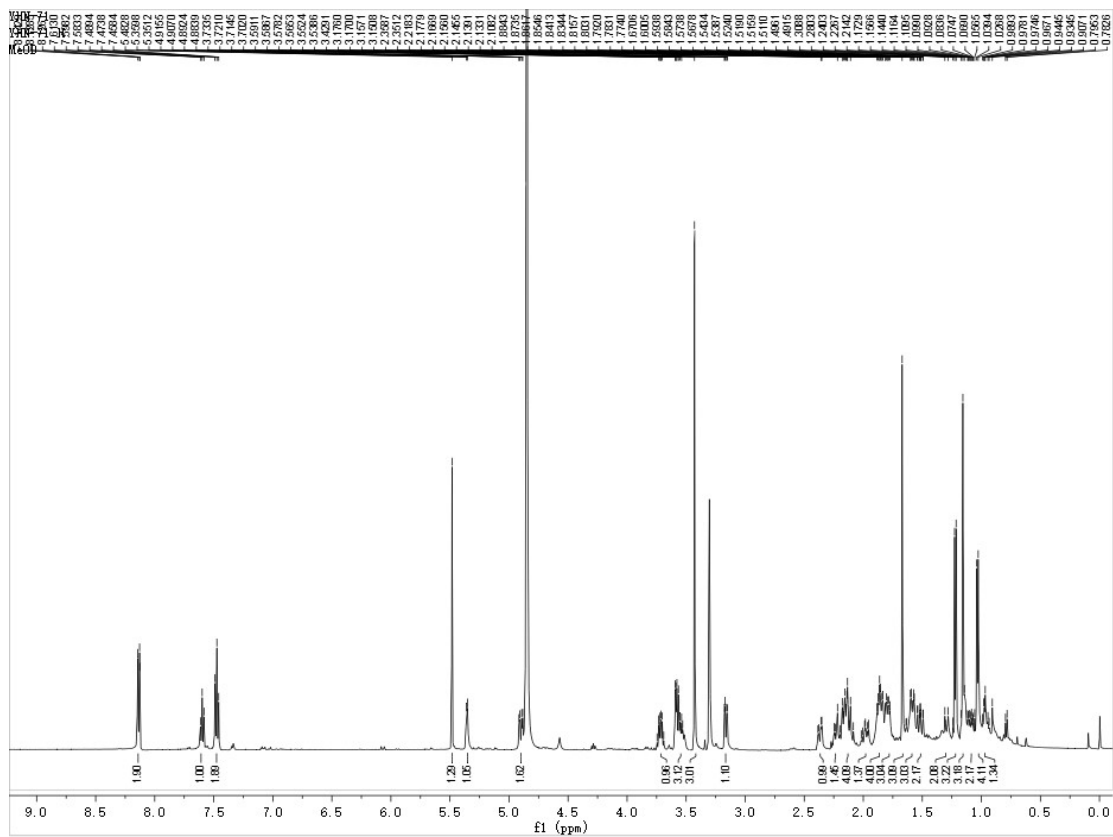


Figure S8. ¹H NMR spectrum (500 MHz) of 2 in CD₃OD

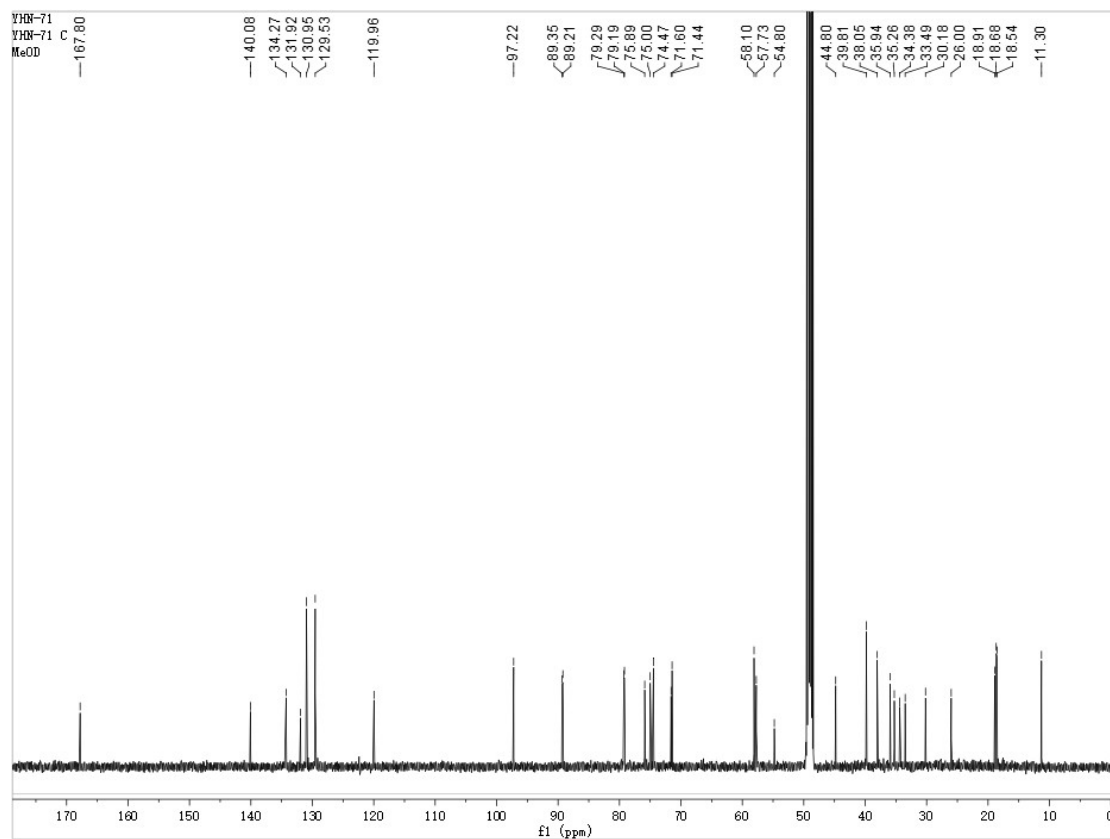


Figure S9. ^{13}C NMR spectrum (125 MHz) of **2** in CD_3OD

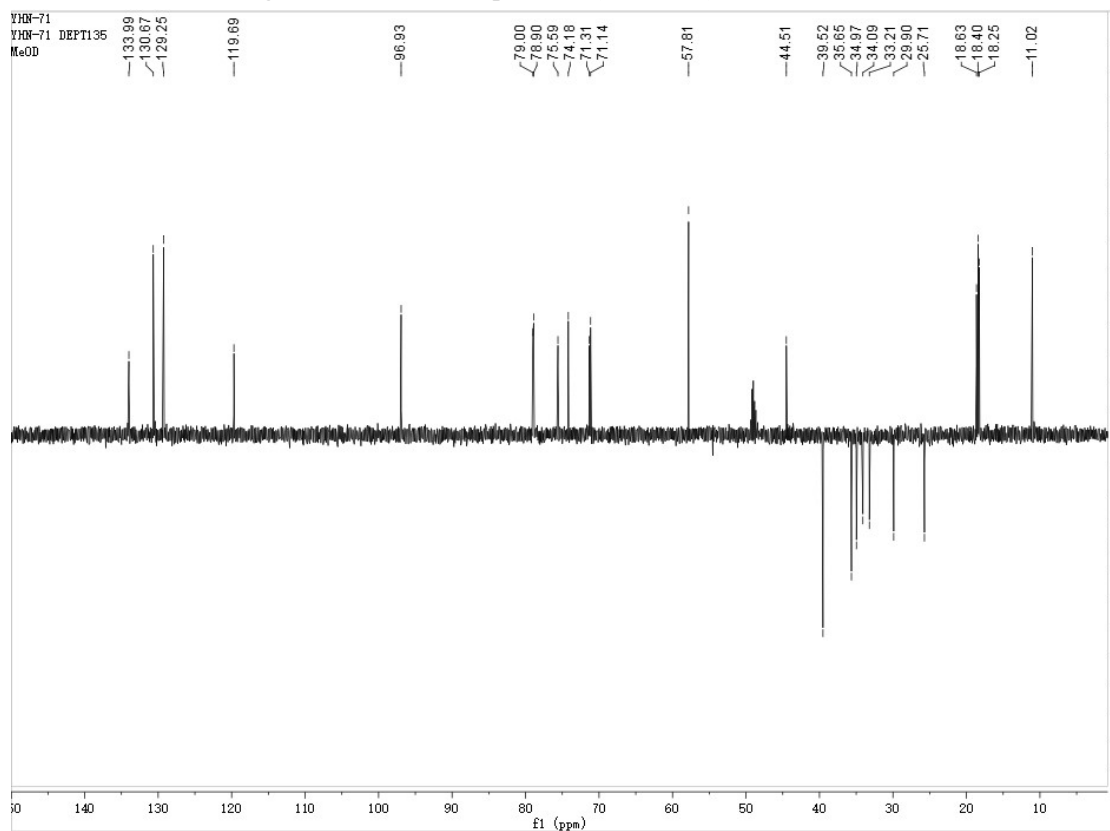


Figure S10. DEPT spectrum of **2** in CD_3OD

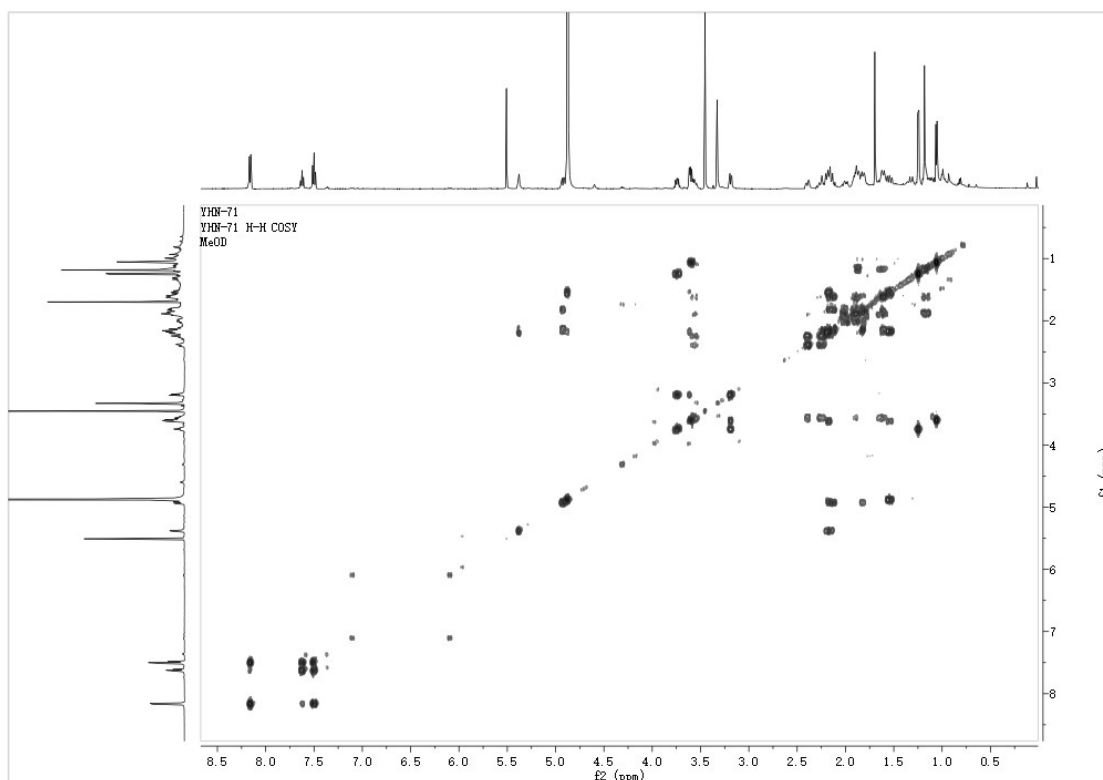


Figure S11. ^1H - ^1H COSY of **2** in CD_3OD

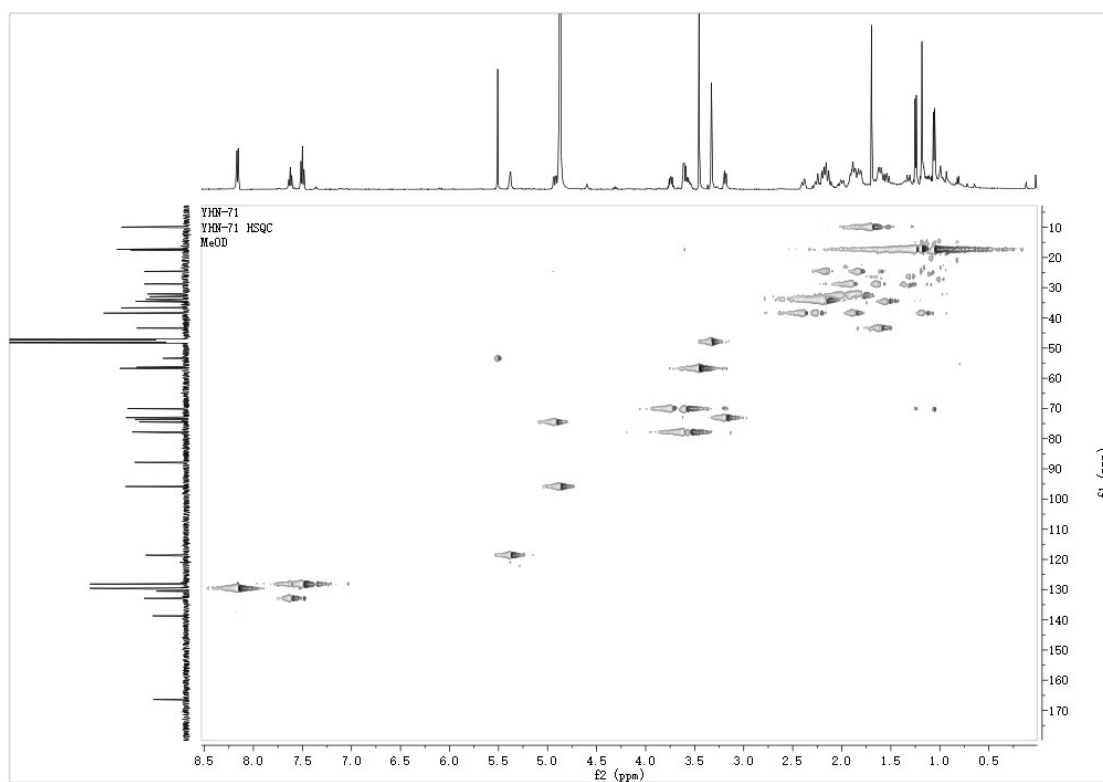


Figure S12. HSQC of **2** in CD_3OD

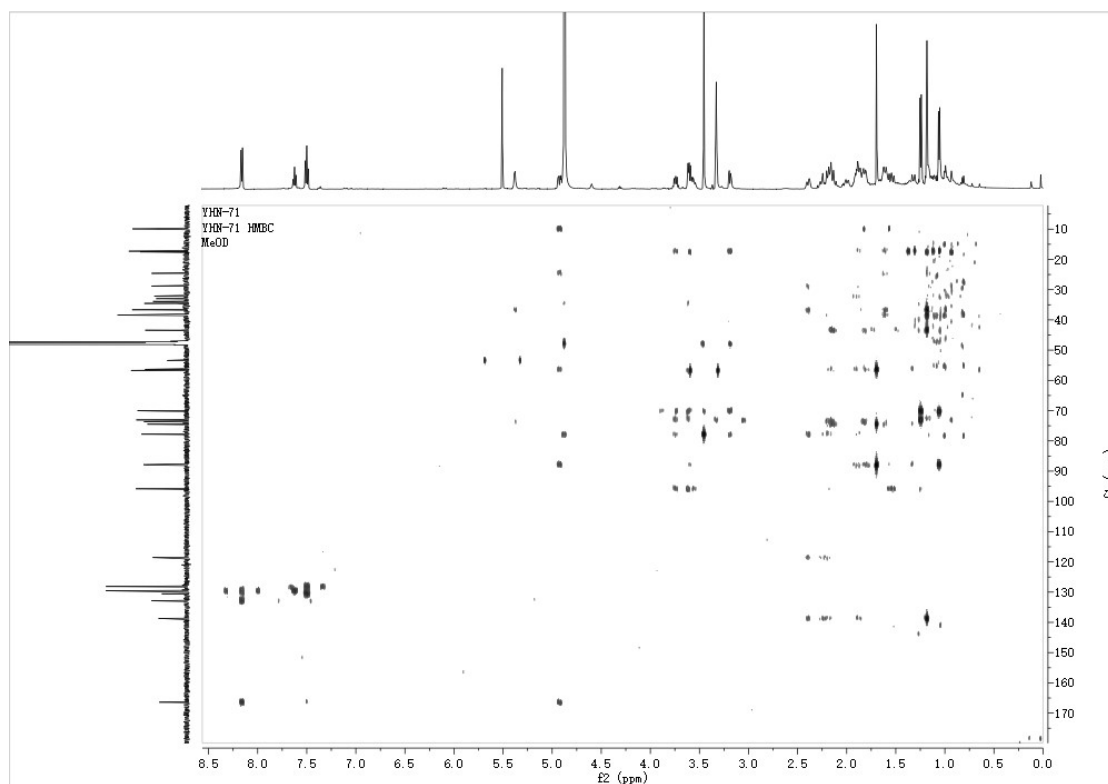


Figure S13. HMBC of **2** in CD₃OD

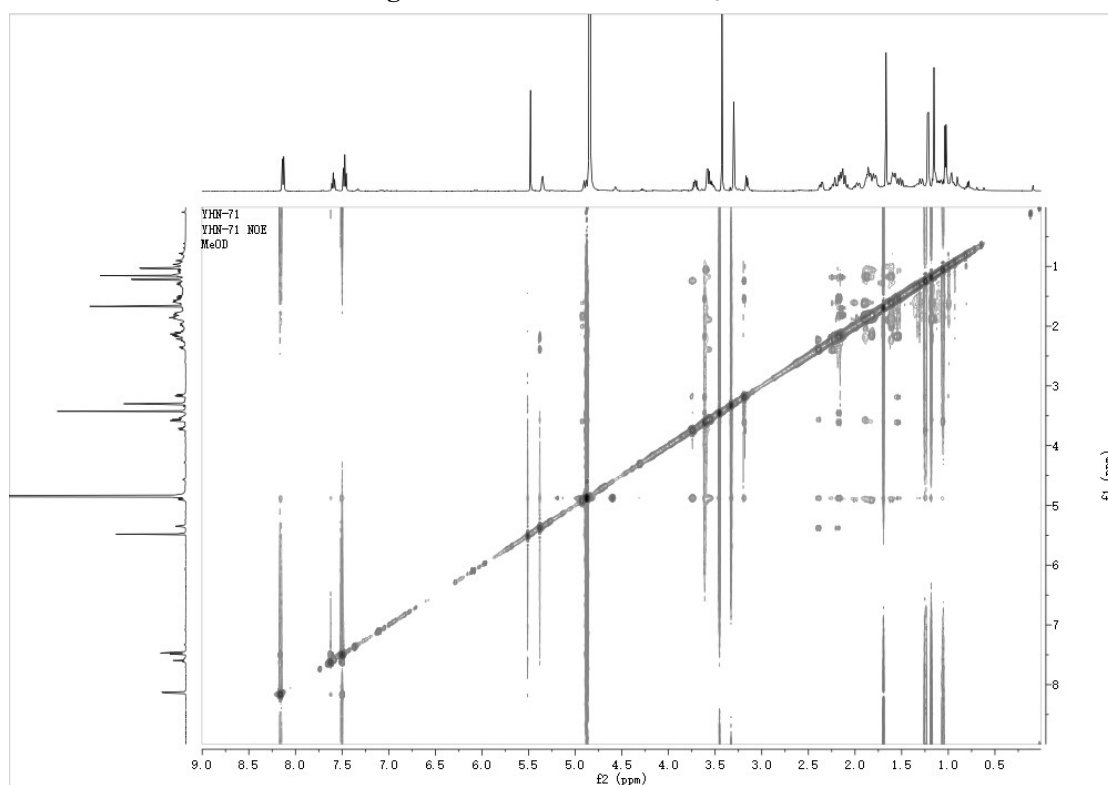
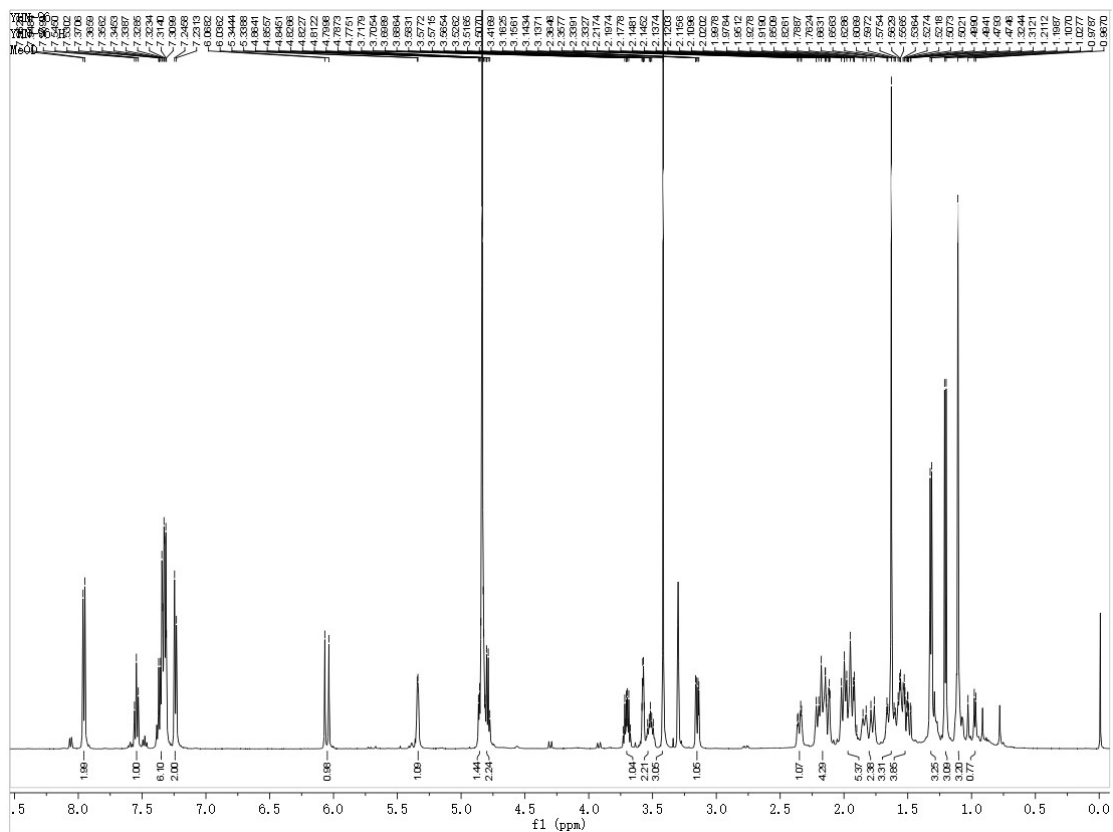


Figure S14. NOESY of **2** in CD₃OD



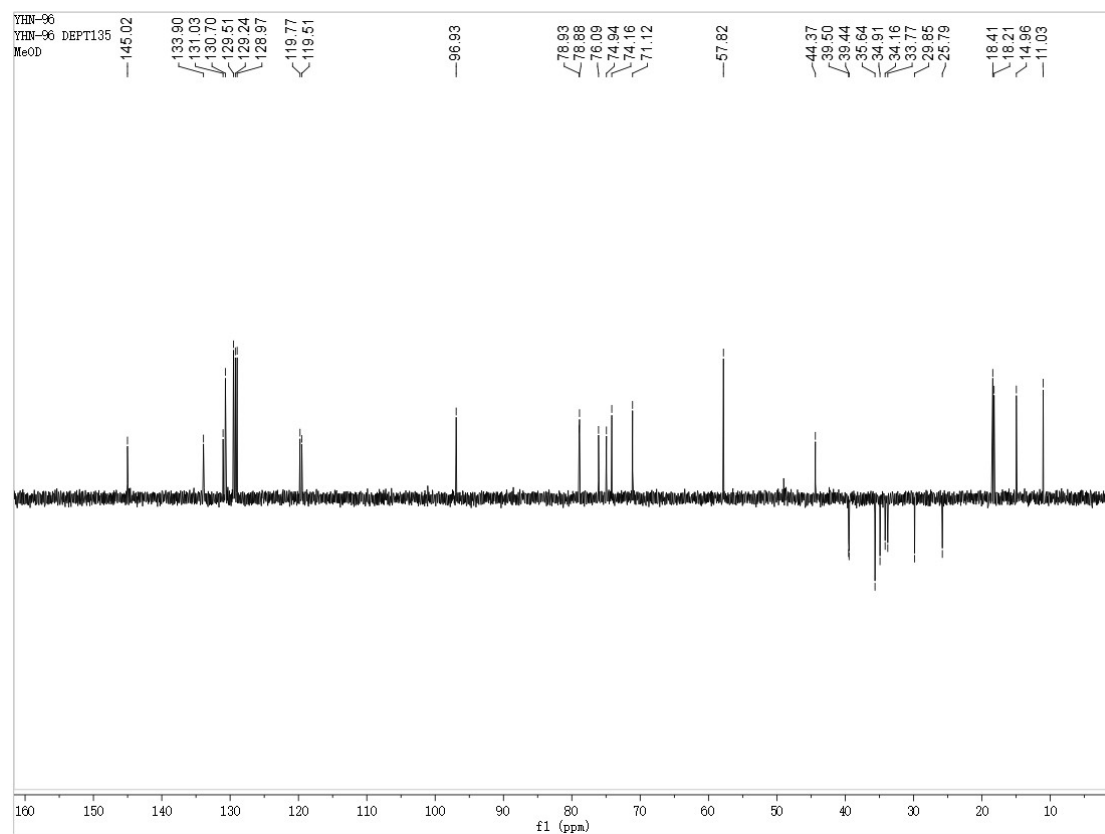


Figure S17. DEPT spectrum of **3** in CD₃OD

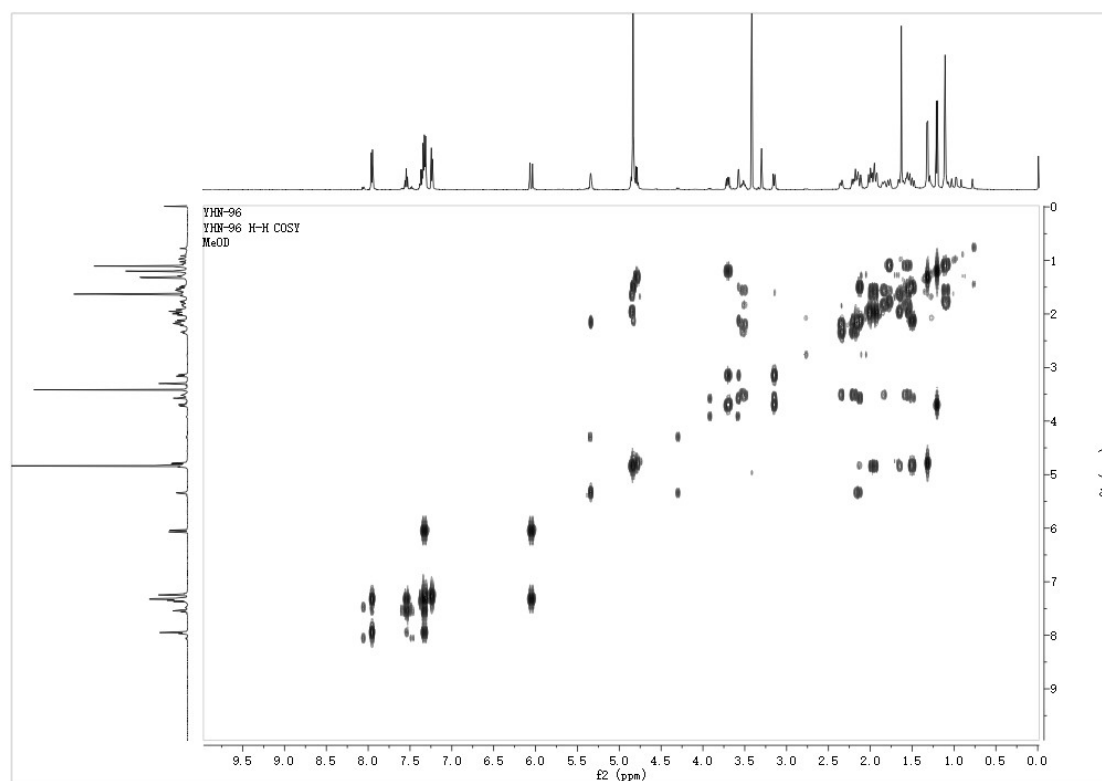


Figure S18. ¹H-¹H COSY of **3** in CD₃OD

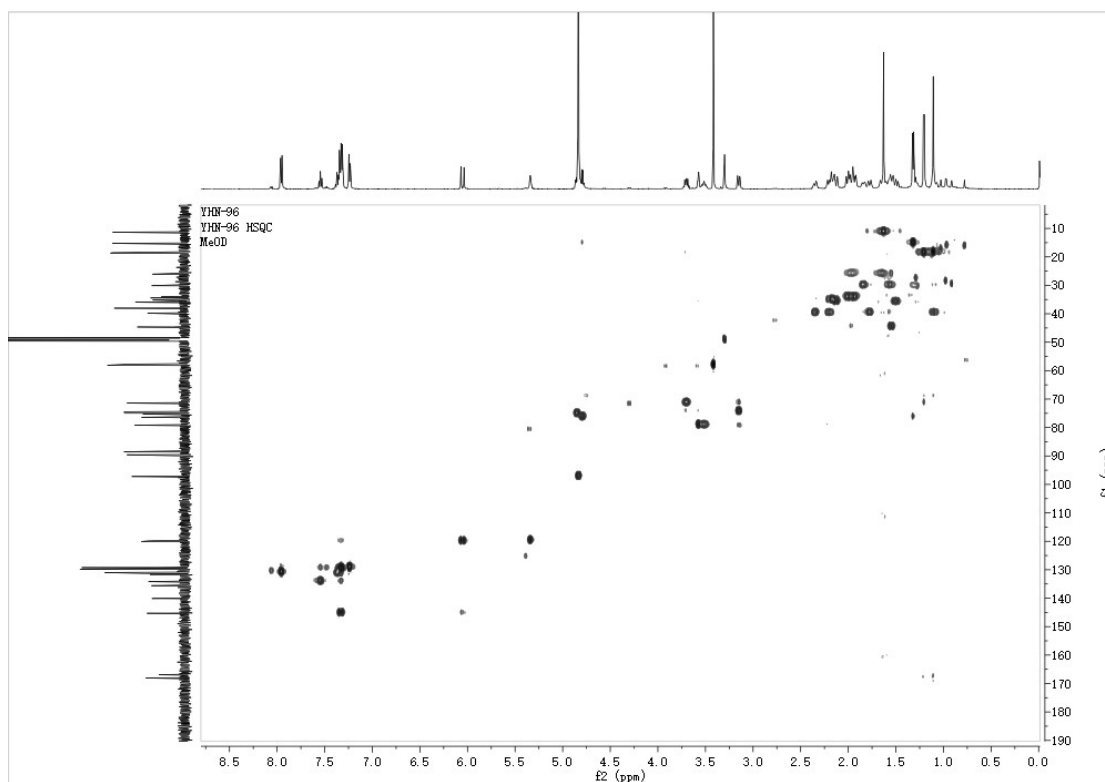


Figure S19. HSQC of 3 in CD₃OD

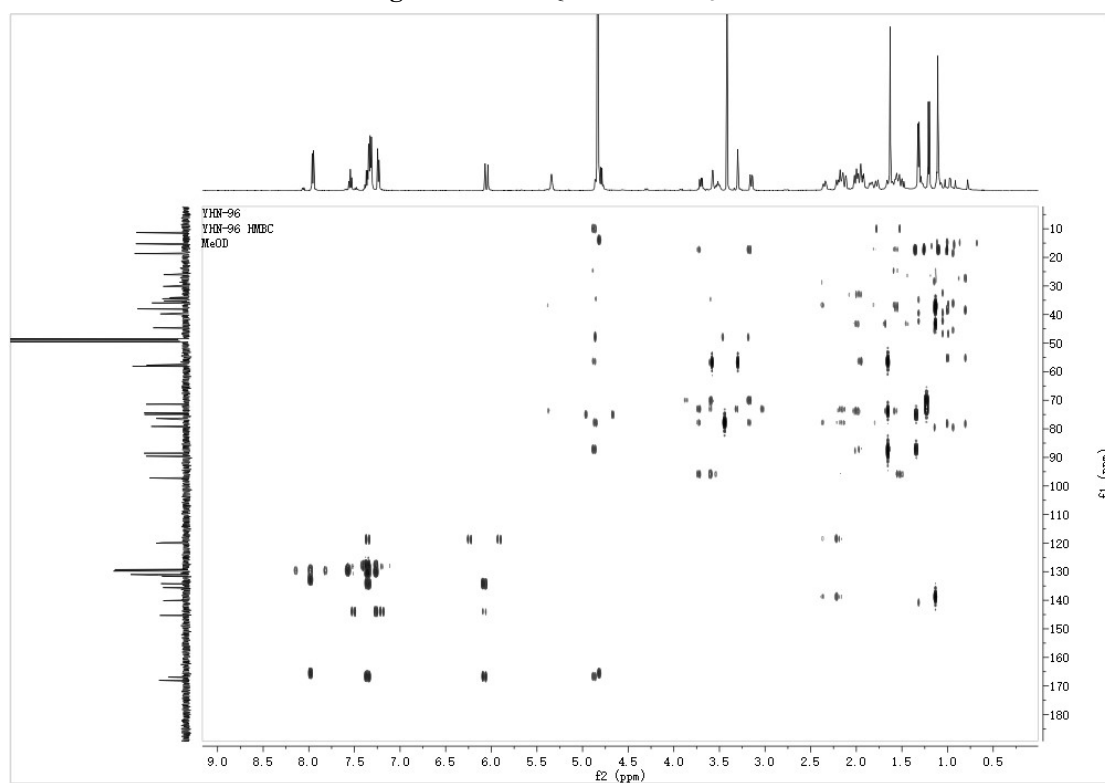


Figure S20. HMBC of 3 in CD₃OD

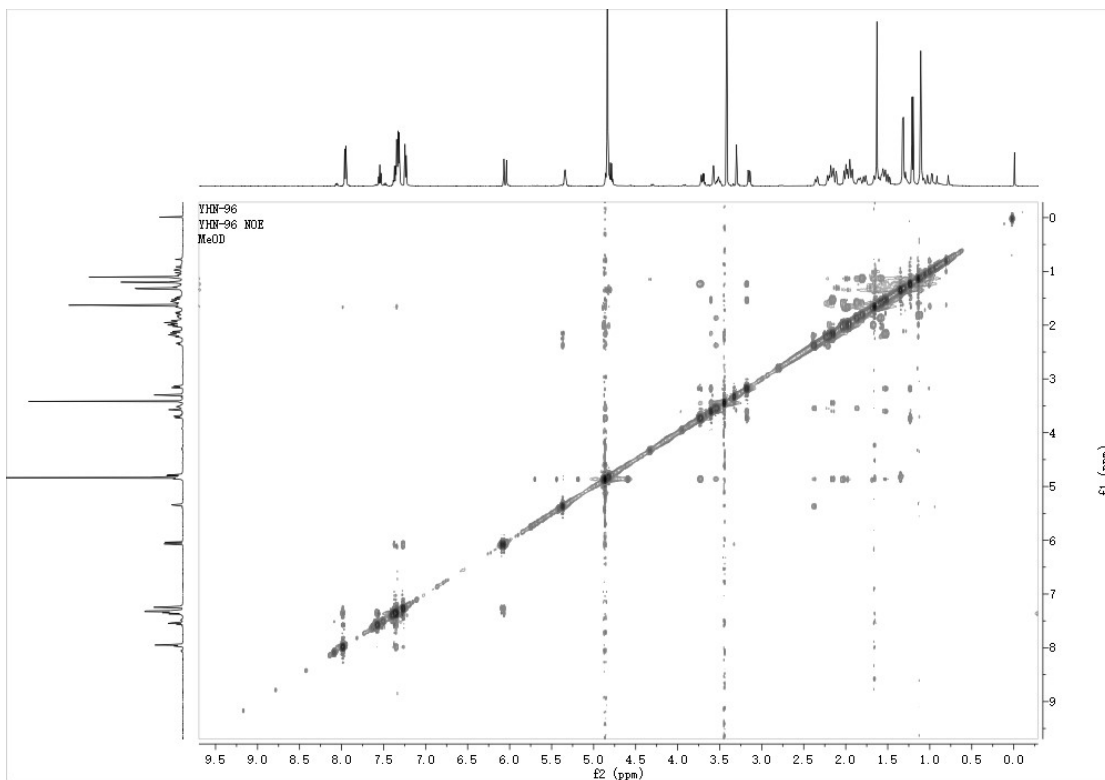


Figure S21. NOESY of 3 in CD₃OD

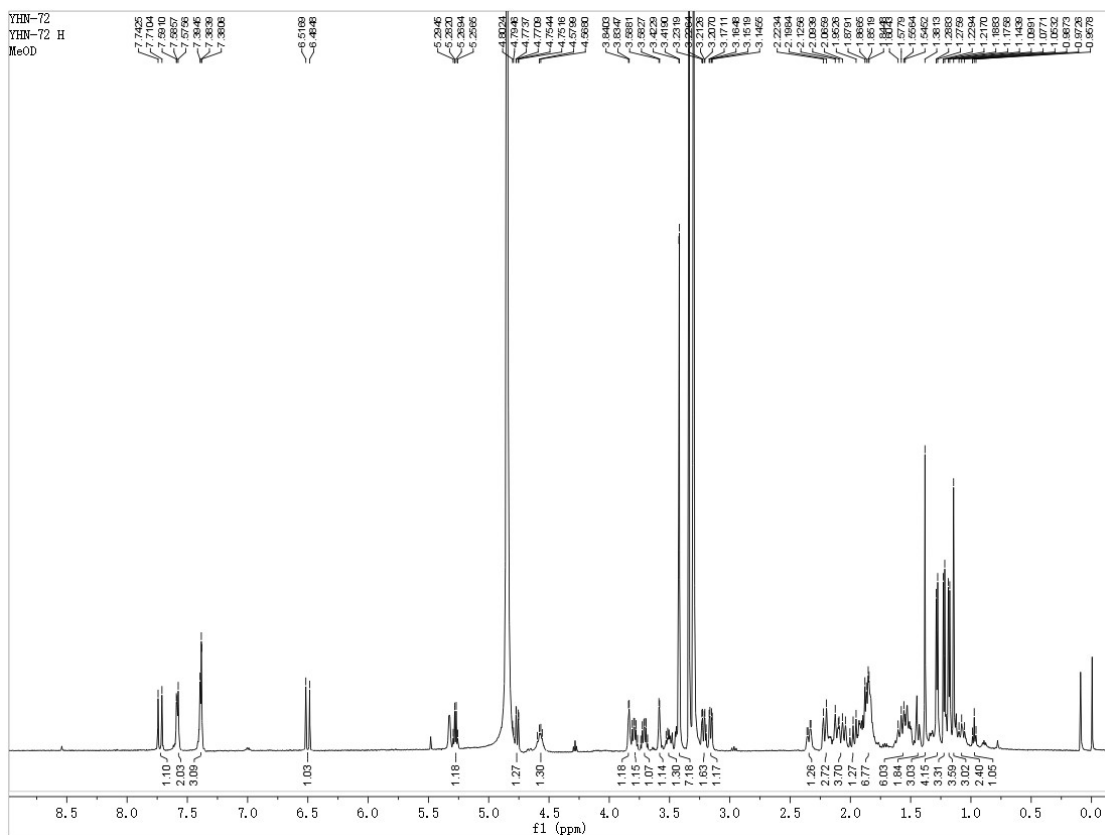


Figure S22. ¹H NMR spectrum (500 MHz) of 4 in CD₃OD

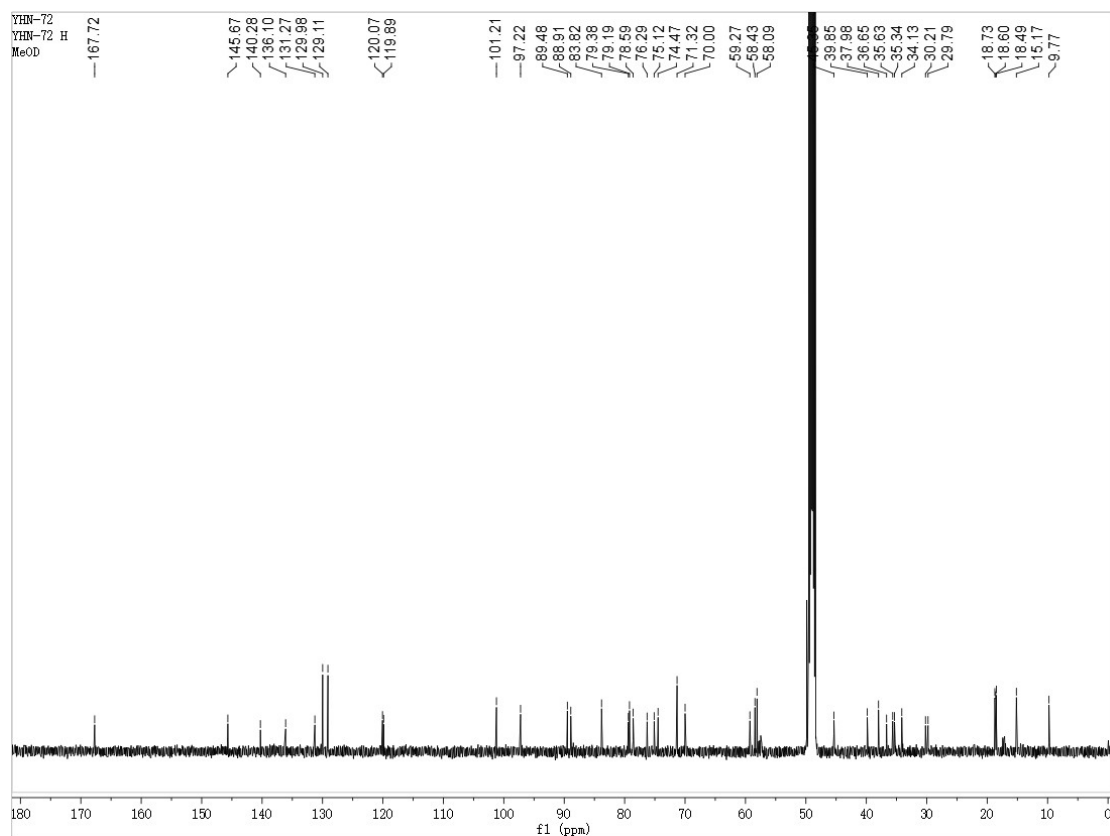


Figure S23. ^{13}C NMR spectrum (125 MHz) of **4** in CD_3OD

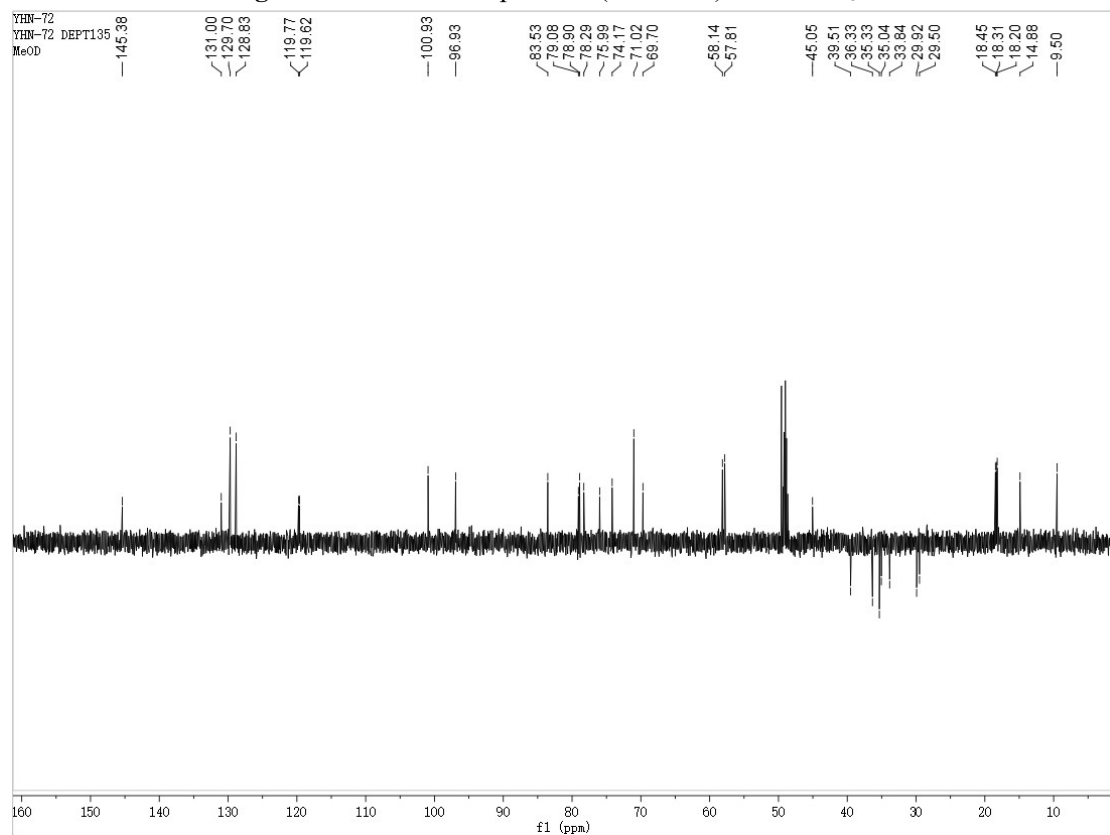


Figure S24. DEPT spectrum of **4** in CD_3OD

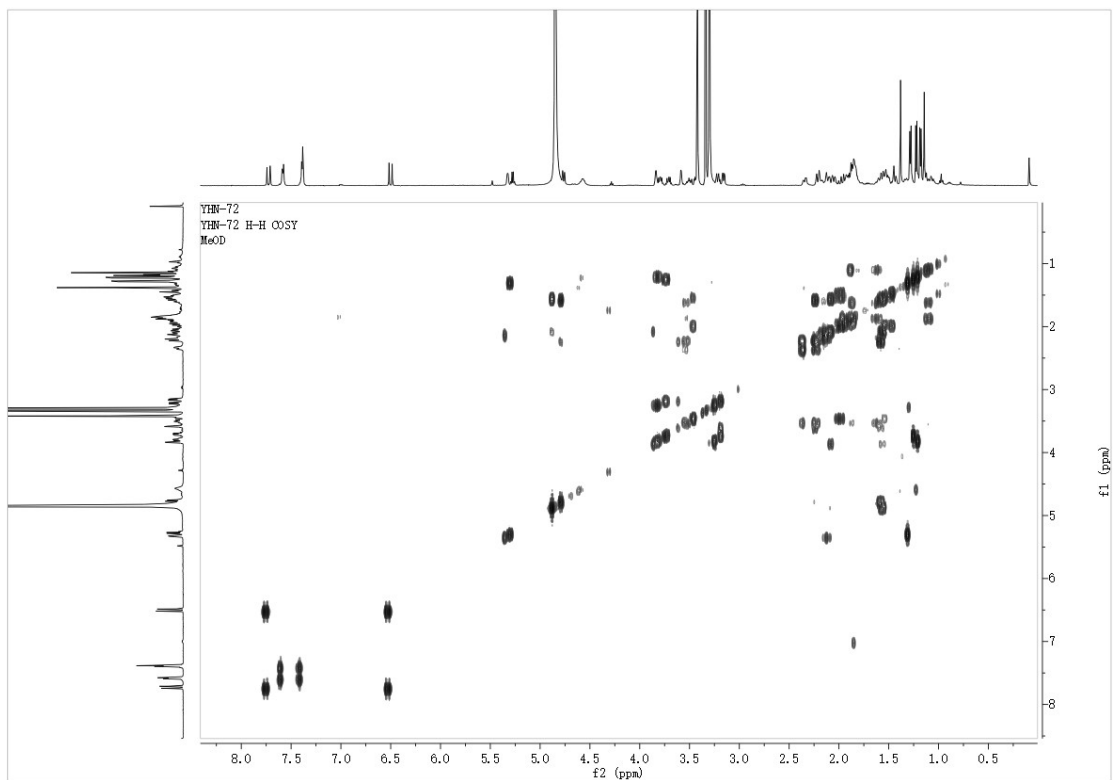


Figure S25. ^1H - ^1H COSY of **4** in CD_3OD

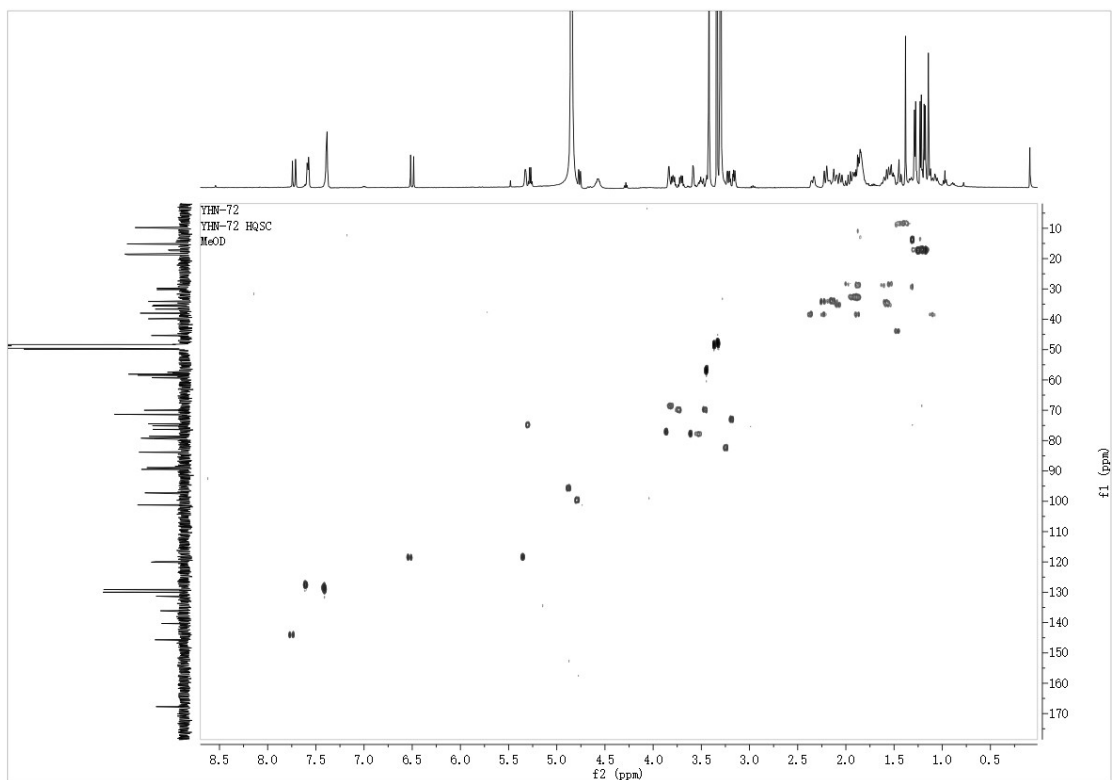


Figure S26. HSQC of **4** in CD_3OD

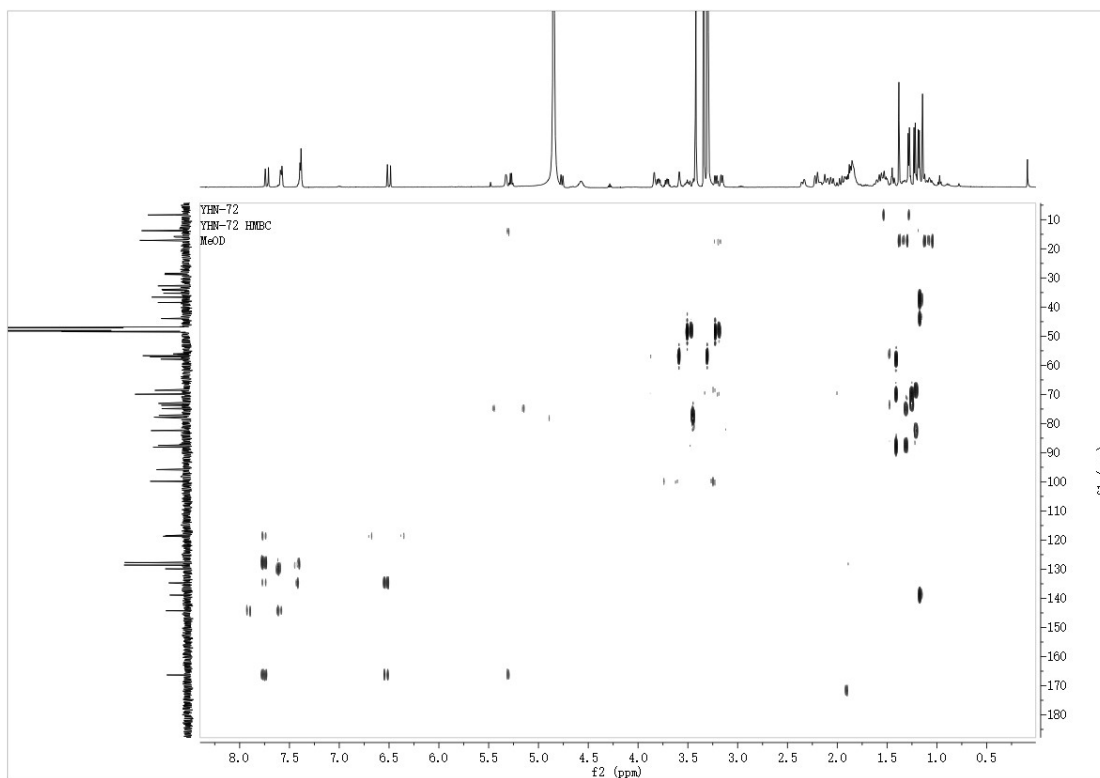


Figure S27. HMBC of 4 in CD₃OD

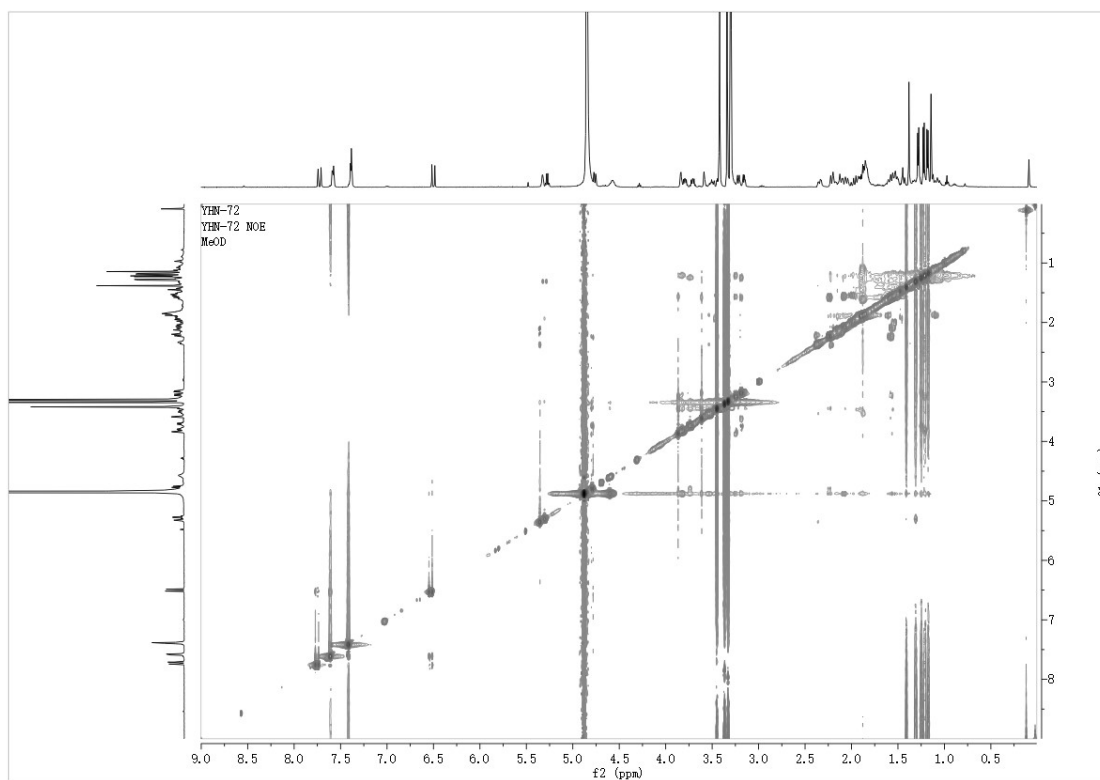


Figure S28. NOESY of 4 in CD₃OD

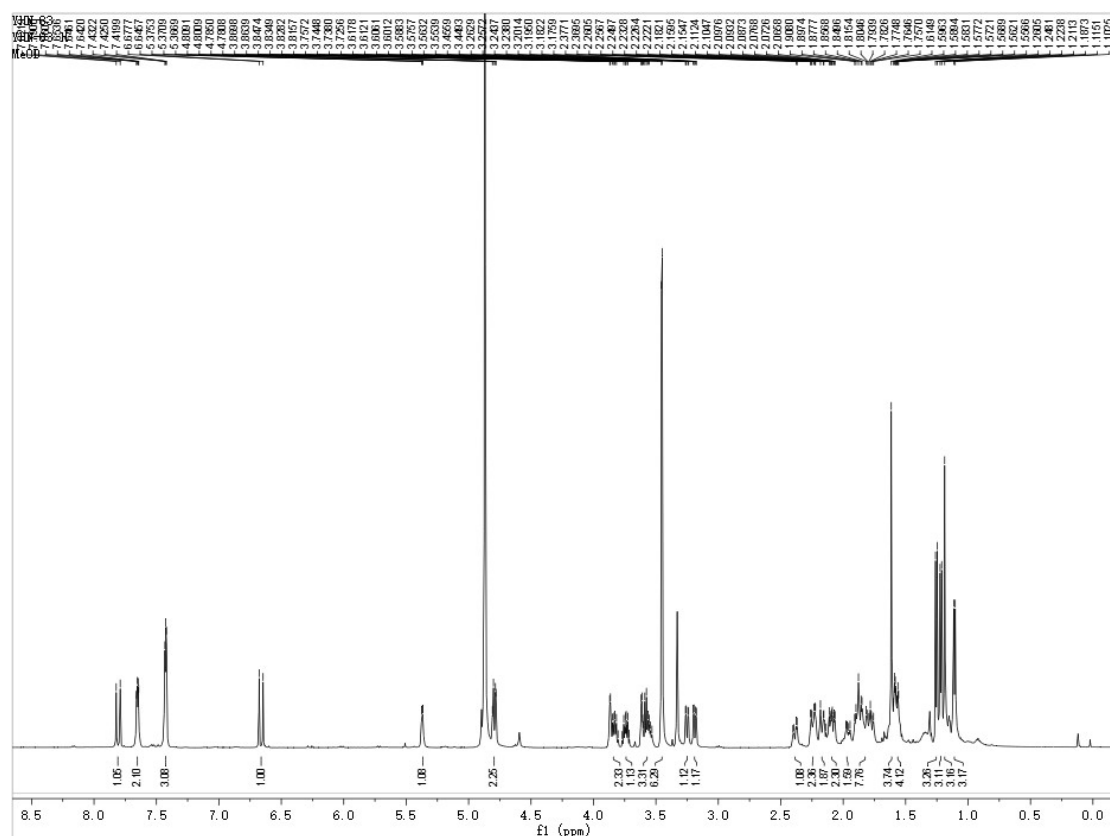


Figure S29. ^1H NMR spectrum (500 MHz) of **5** in CD_3OD

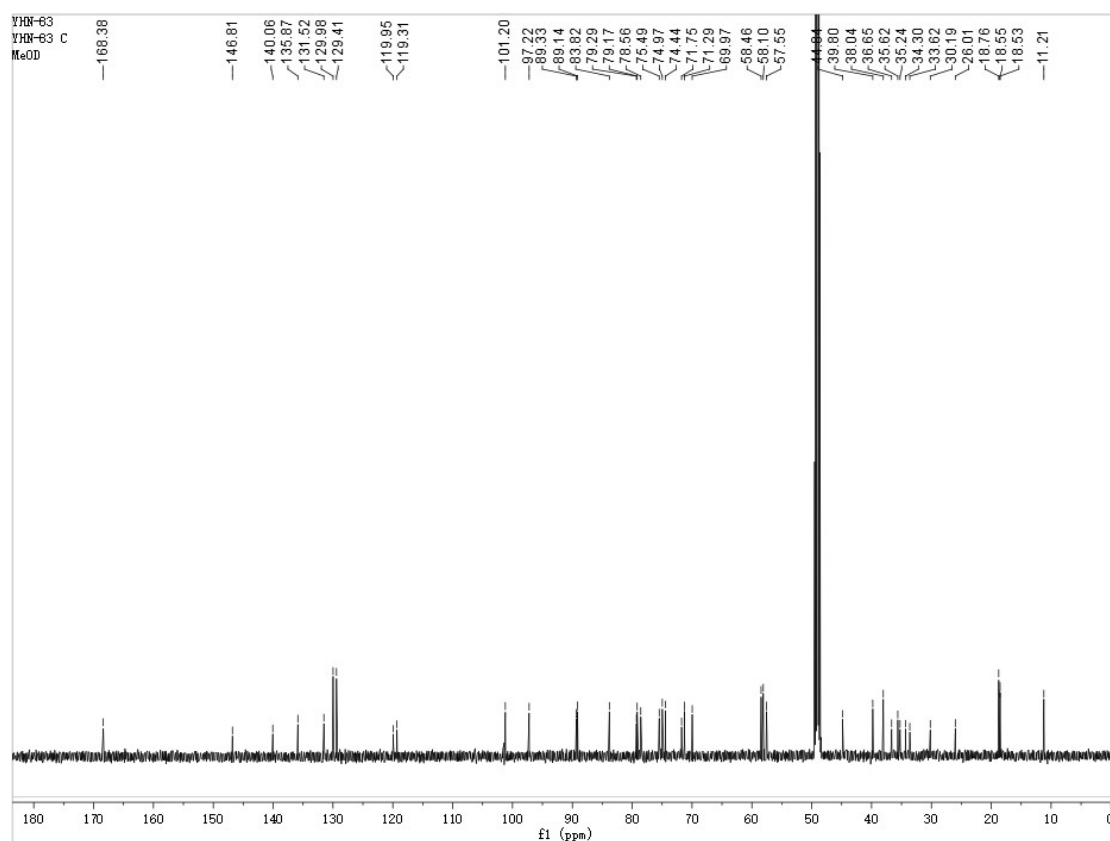


Figure S30. ^{13}C NMR spectrum (125 MHz) of **5** in CD_3OD

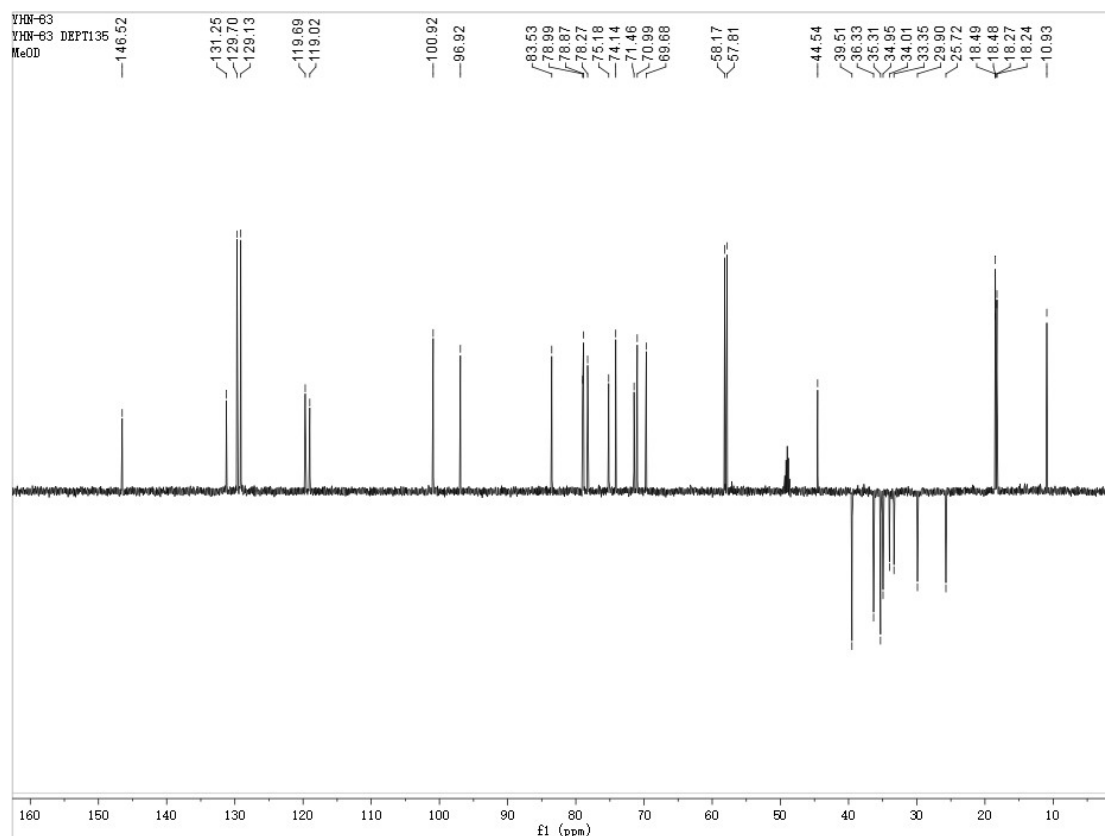


Figure S31. DEPT spectrum of **5** in CD₃OD

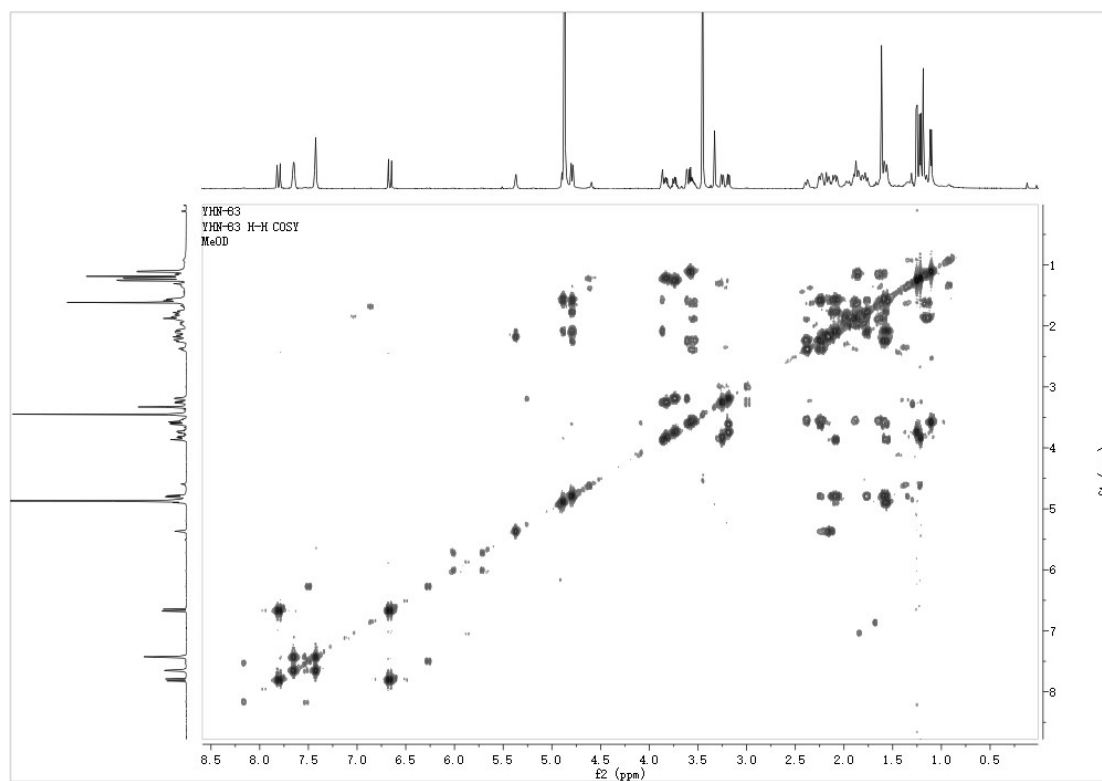


Figure S32. ¹H-¹H COSY of **5** in CD₃OD

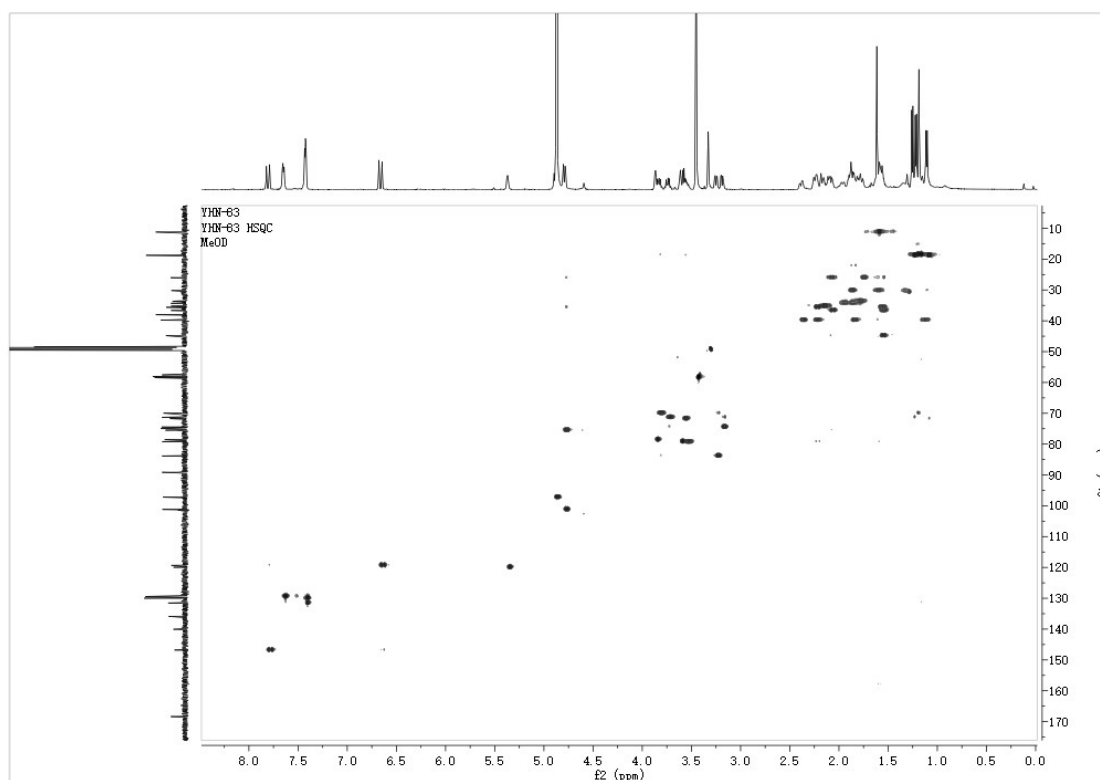


Figure S33. HSQC of 5 in CD₃OD

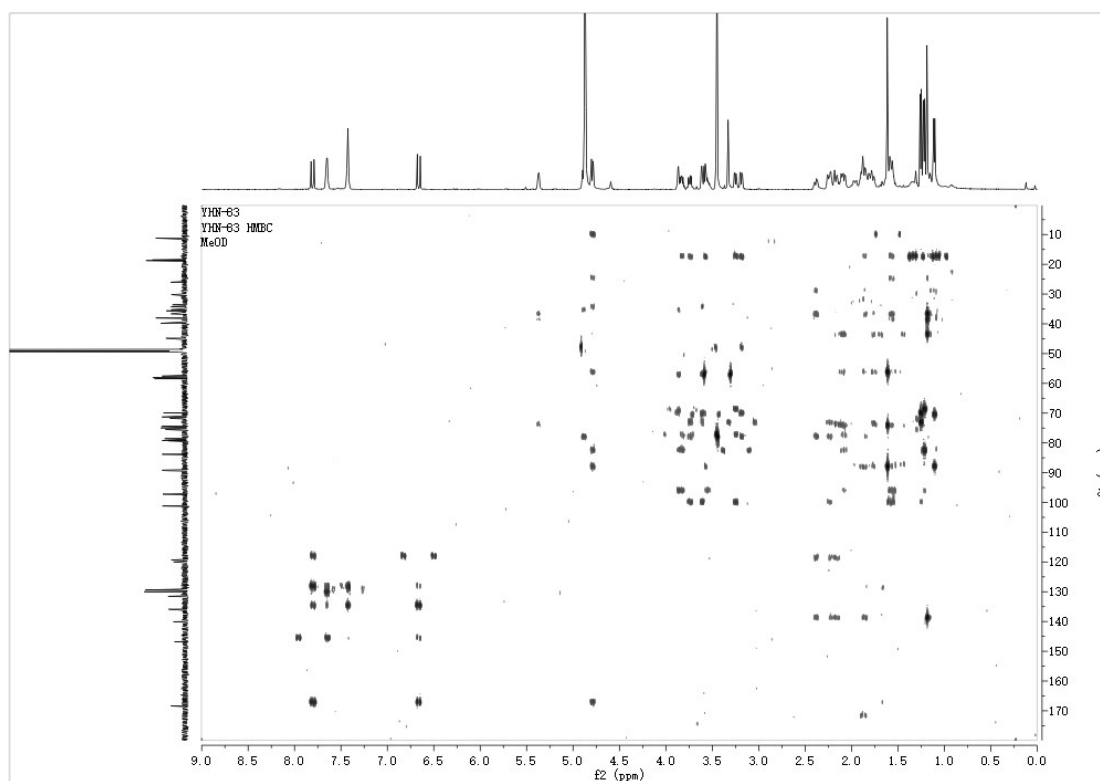


Figure S34. HMBC of 5 in CD₃OD

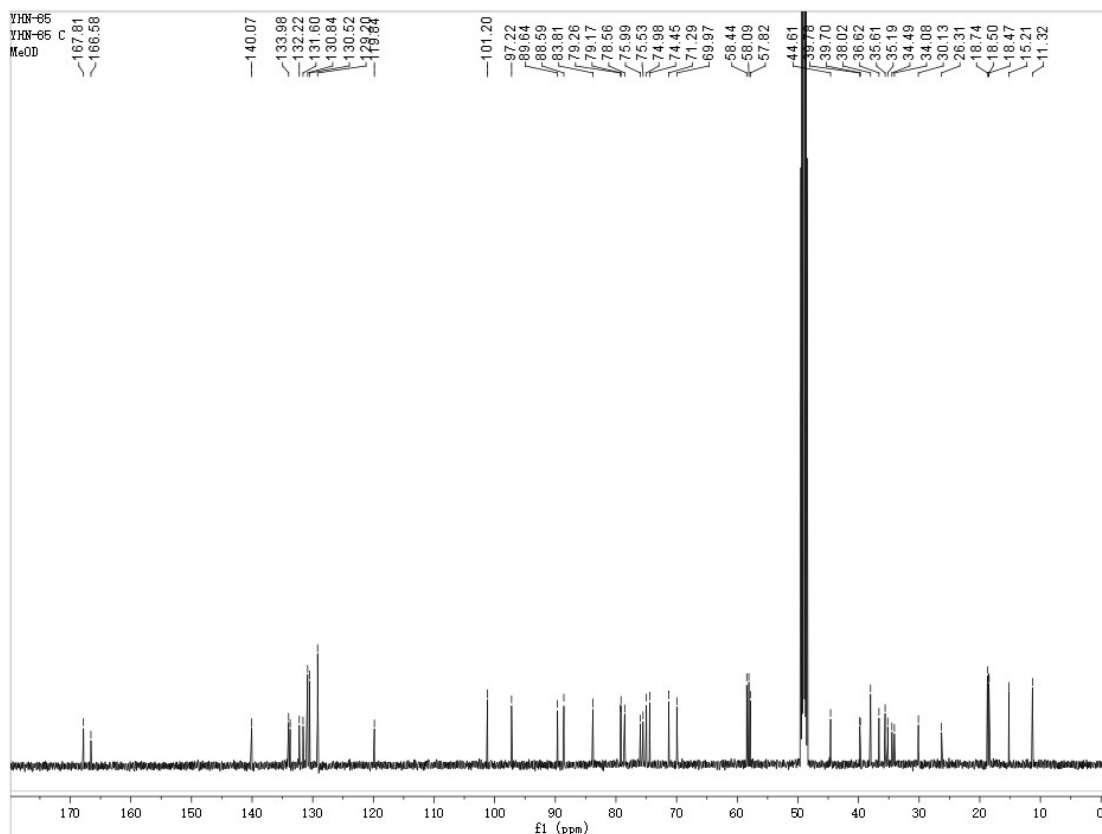


Figure S37. ^{13}C NMR spectrum (125 MHz) of **6** in CD_3OD

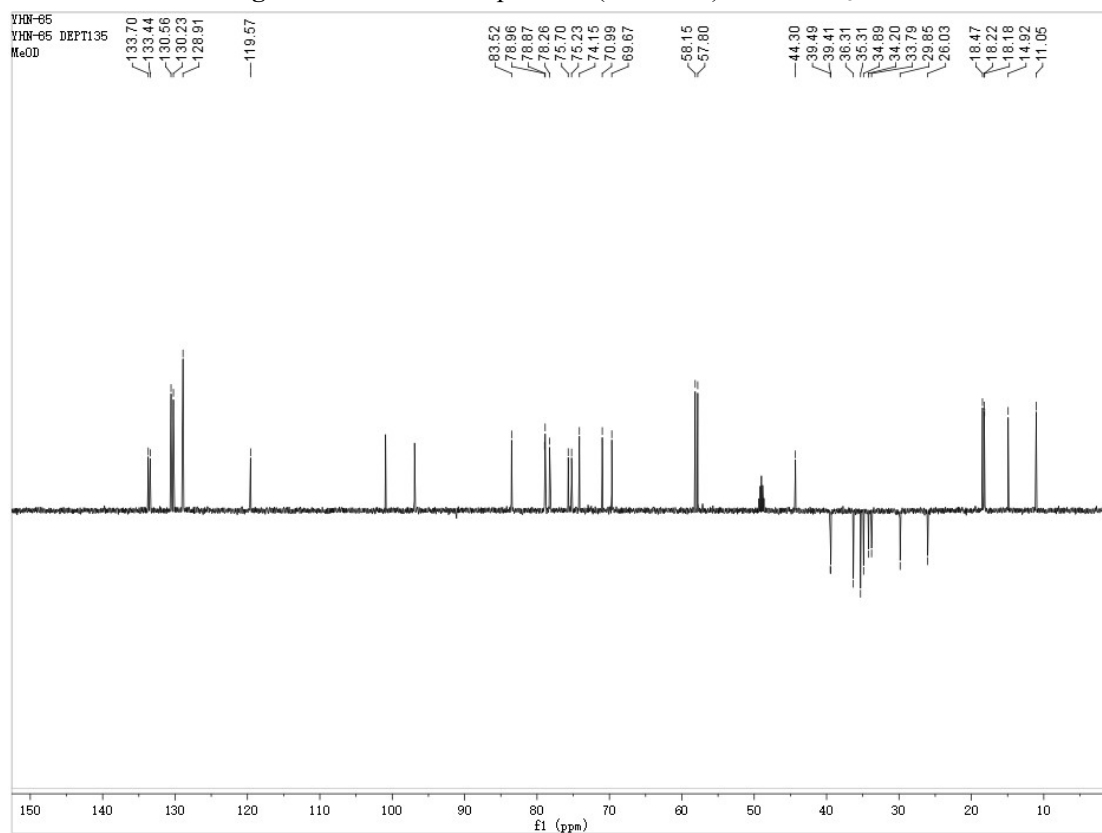


Figure S38. DEPT spectrum of **6** in CD_3OD

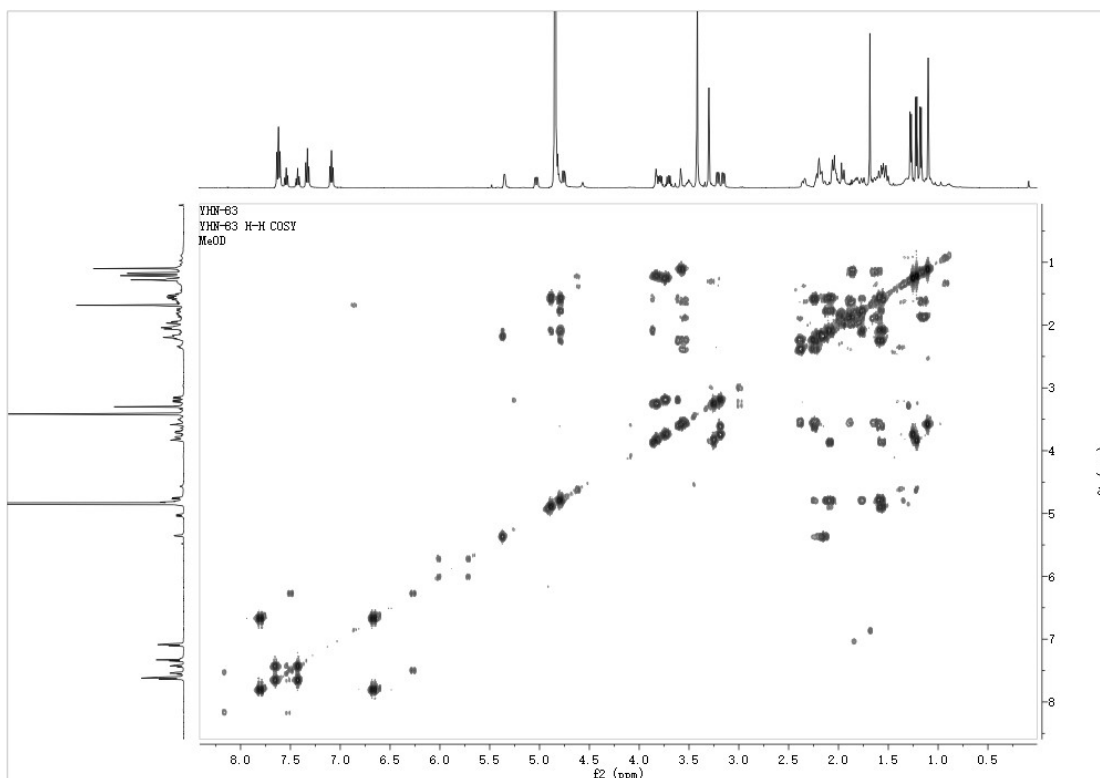


Figure S39. ^1H - ^1H COSY of **6** in CD_3OD

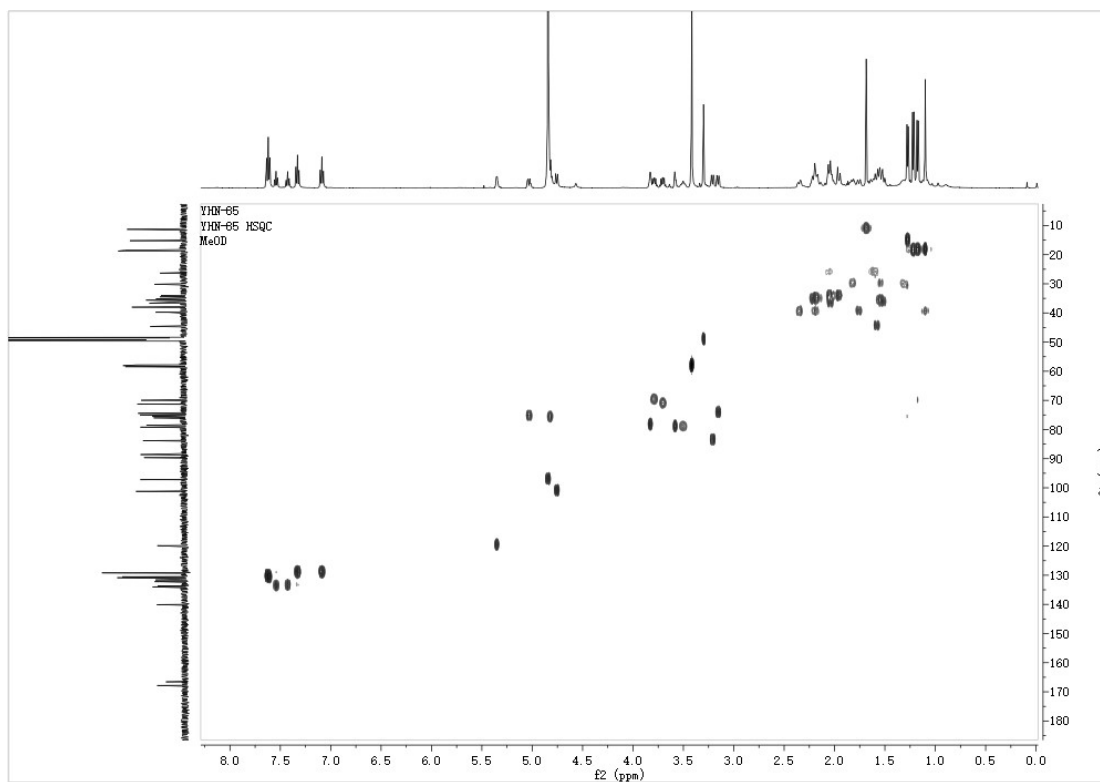


Figure S40. HSQC of **6** in CD_3OD

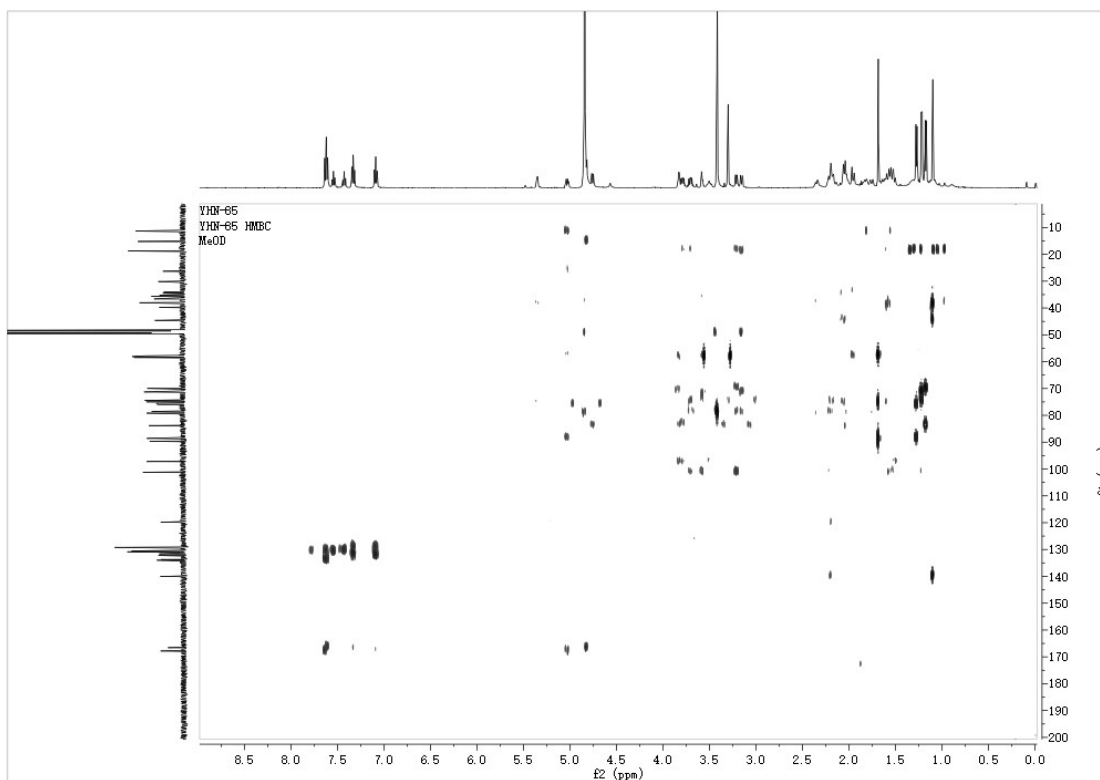


Figure S41. HMBC of 6 in CD₃OD

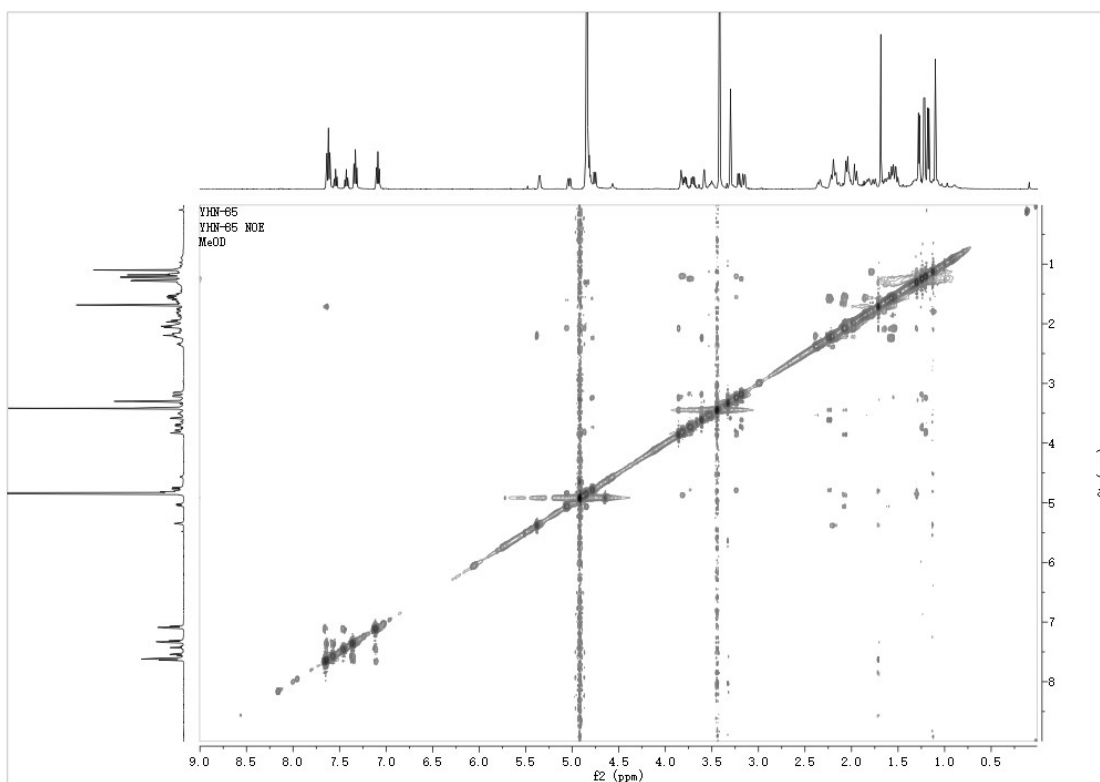


Figure S42. NOESY of 6 in CD₃OD

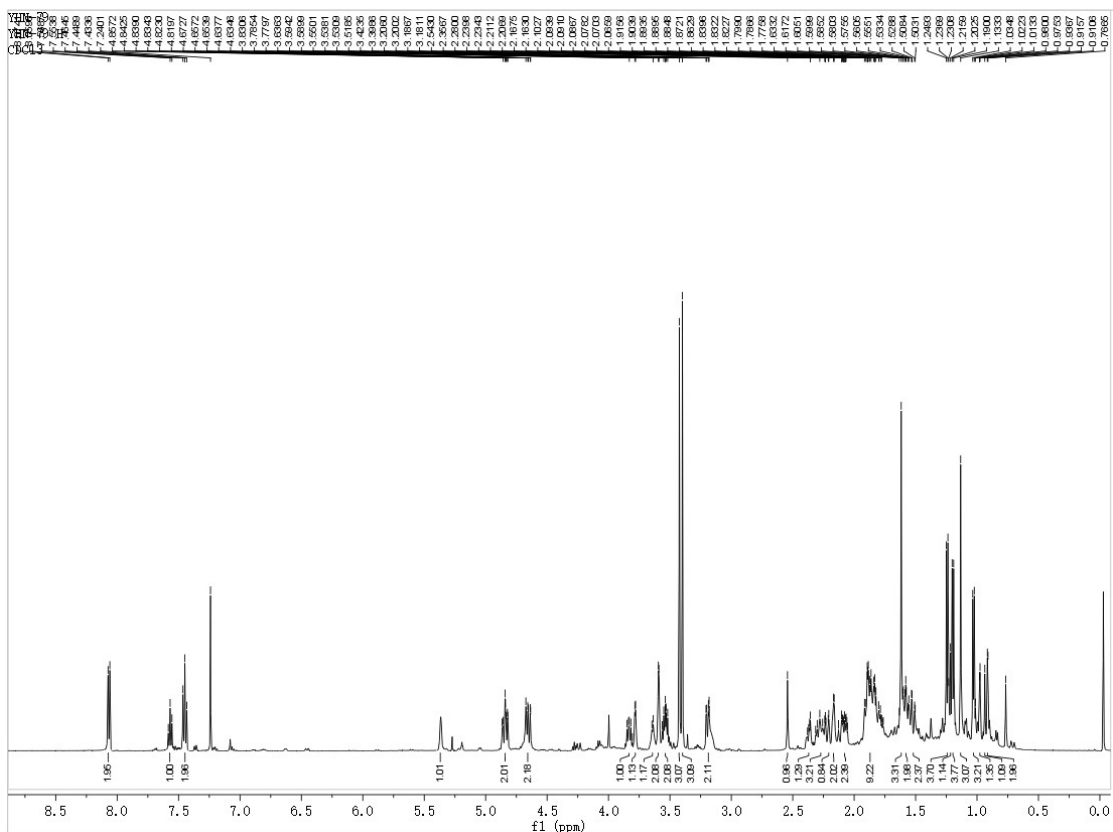


Figure S43. ^1H NMR spectrum (500 MHz) of **7** in CDCl_3

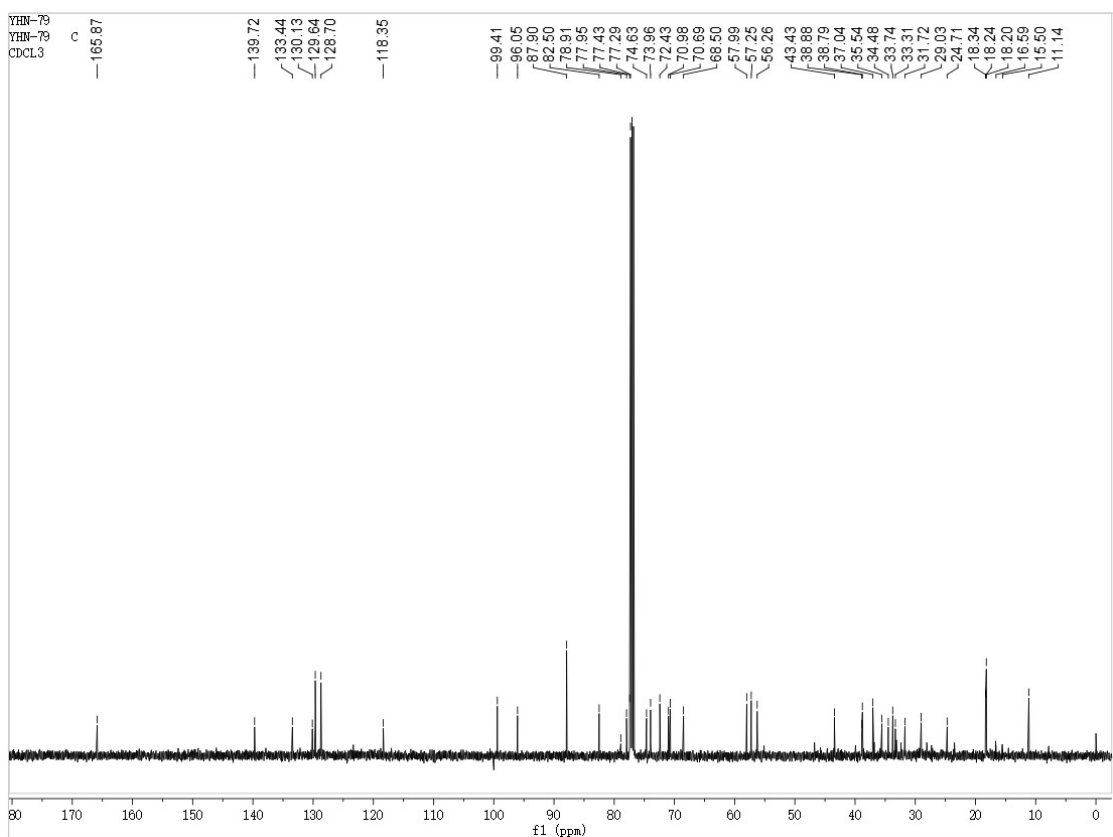


Figure S44. ^{13}C NMR spectrum (125 MHz) of **7** in CDCl_3

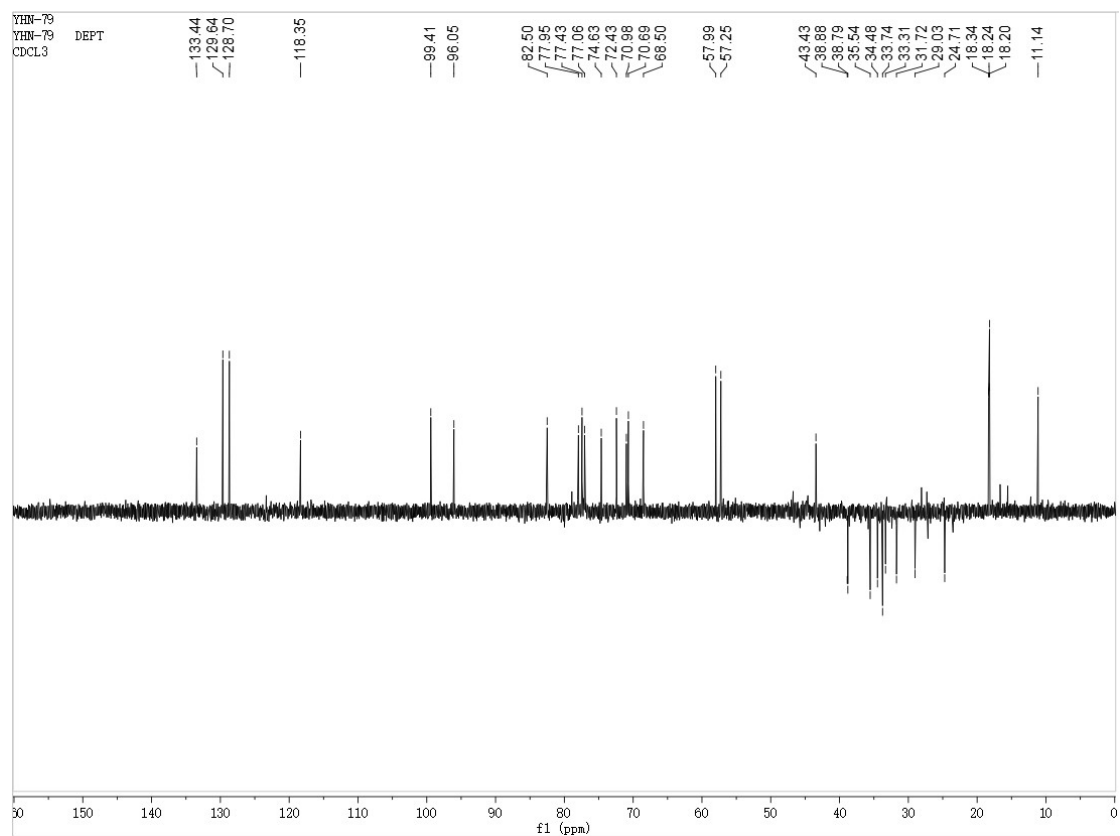


Figure S45. DEPT spectrum of **7** in CDCl₃

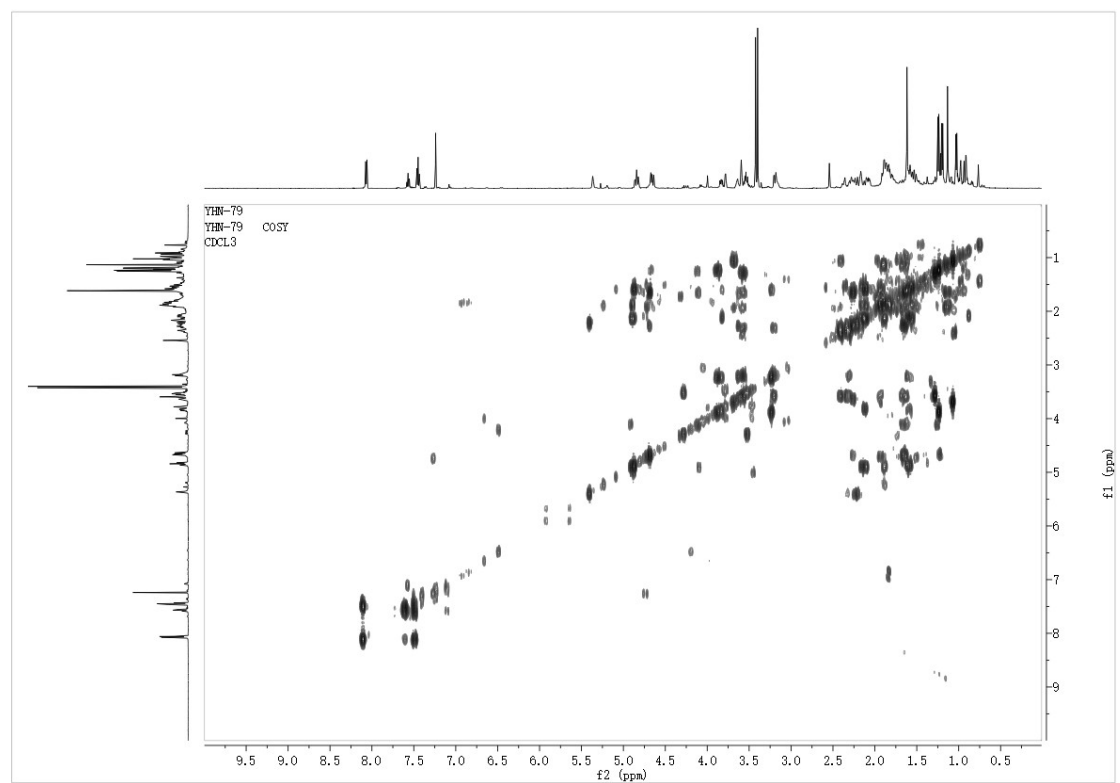


Figure S46. ¹H-¹H COSY of **7** in CDCl₃

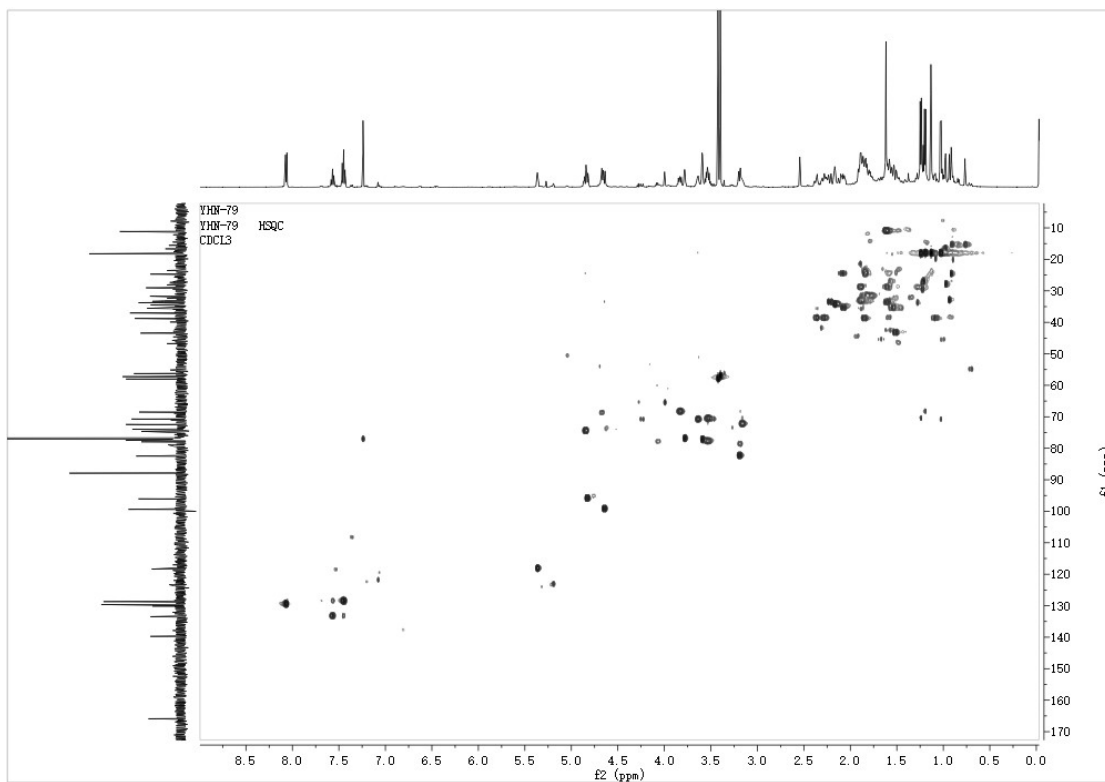


Figure S47. HSQC of 7 in CDCl₃

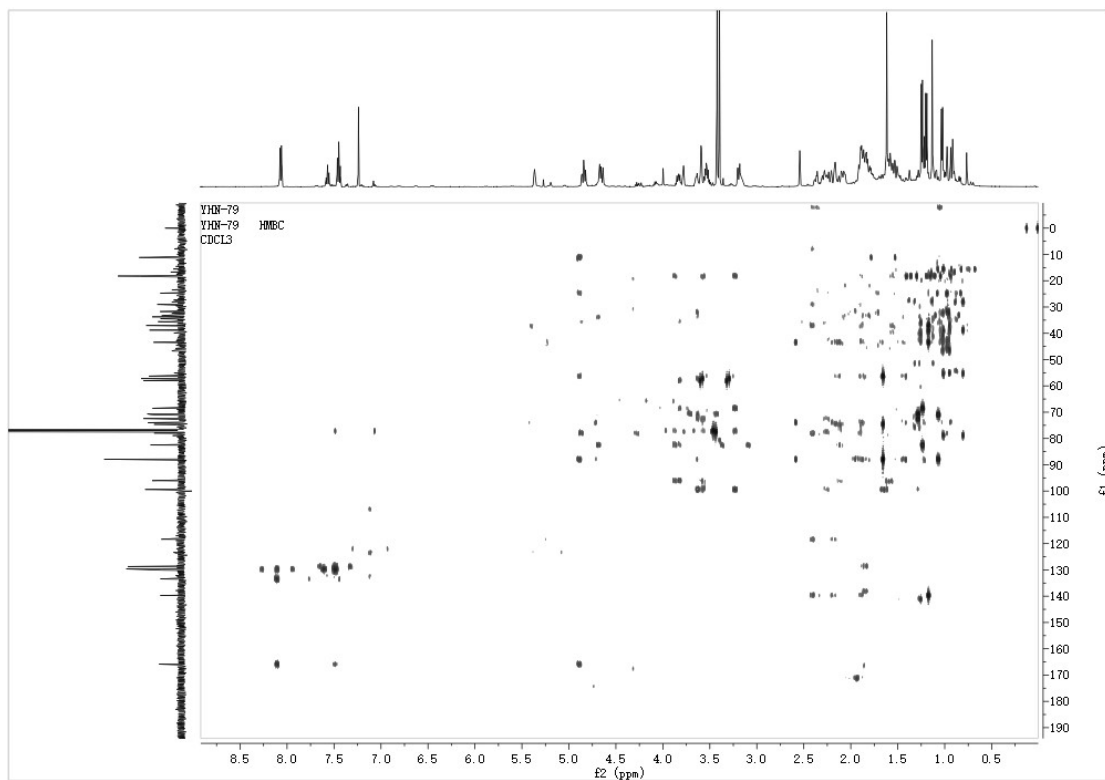


Figure S48. HMBC of 7 in CDCl₃

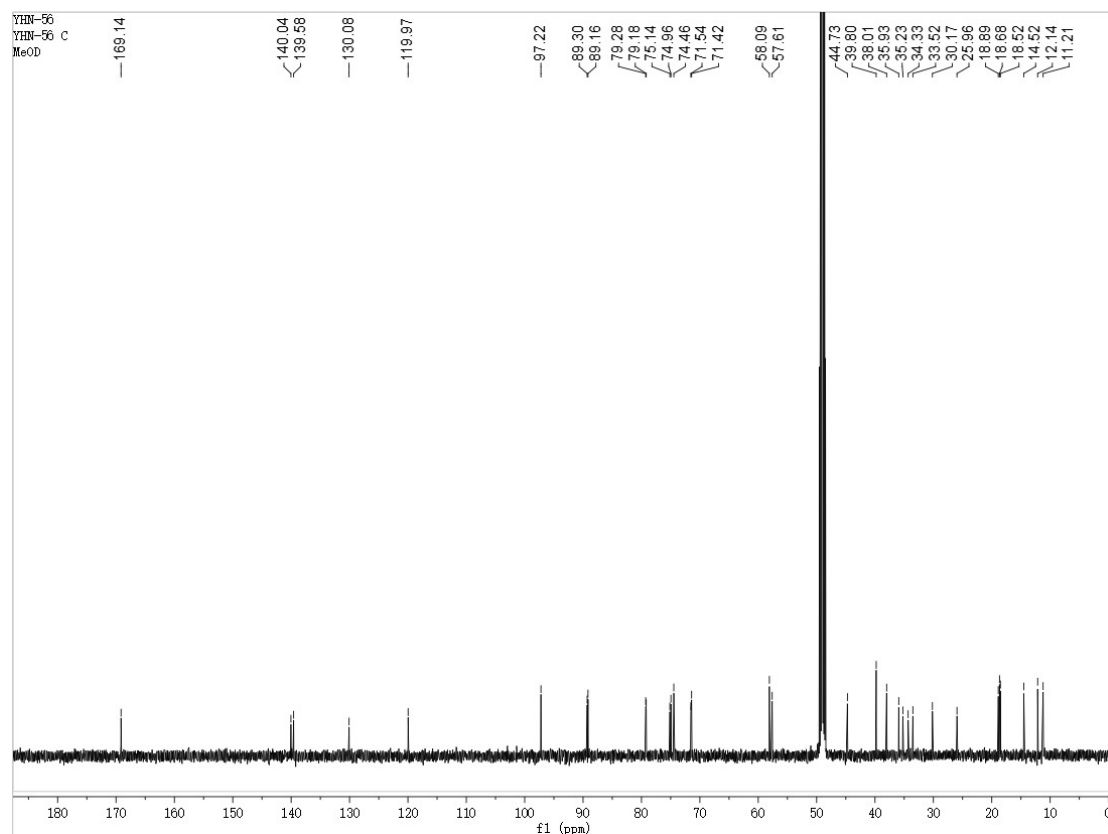


Figure S51. ^{13}C NMR spectrum (125 MHz) of **8** in CD_3OD

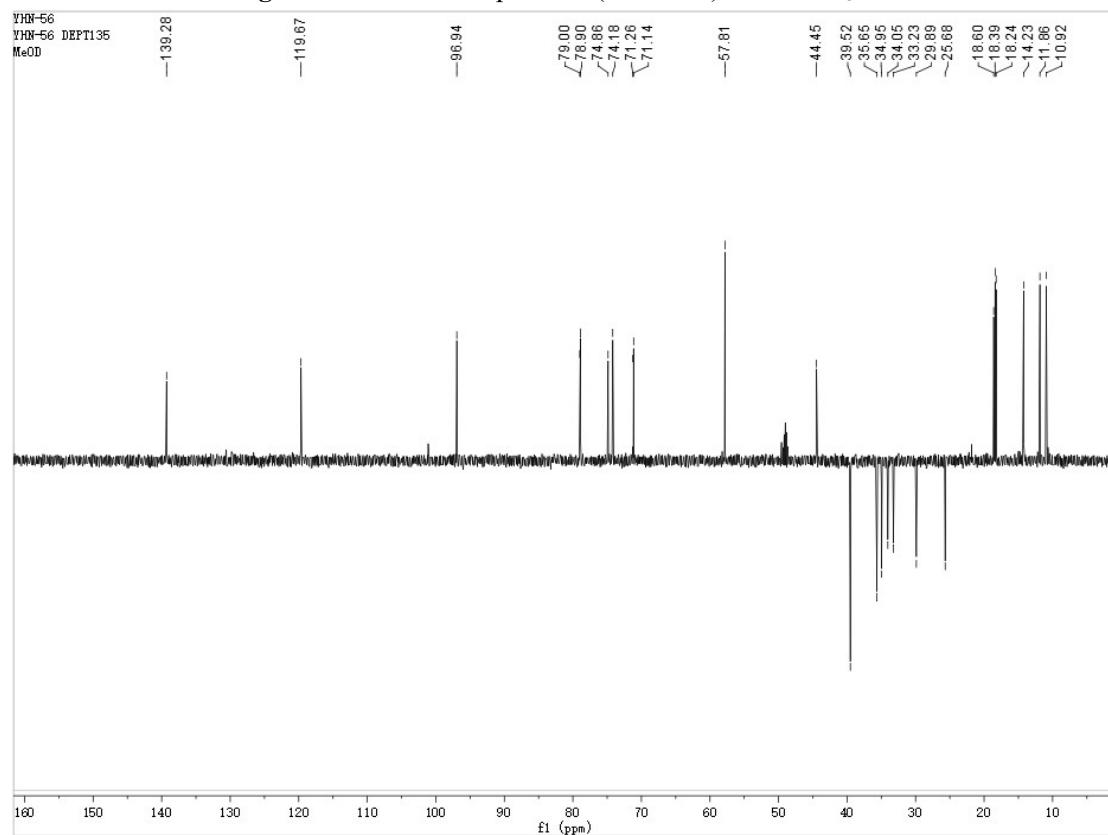


Figure S52. DEPT spectrum (125 MHz) of **8** in CD_3OD

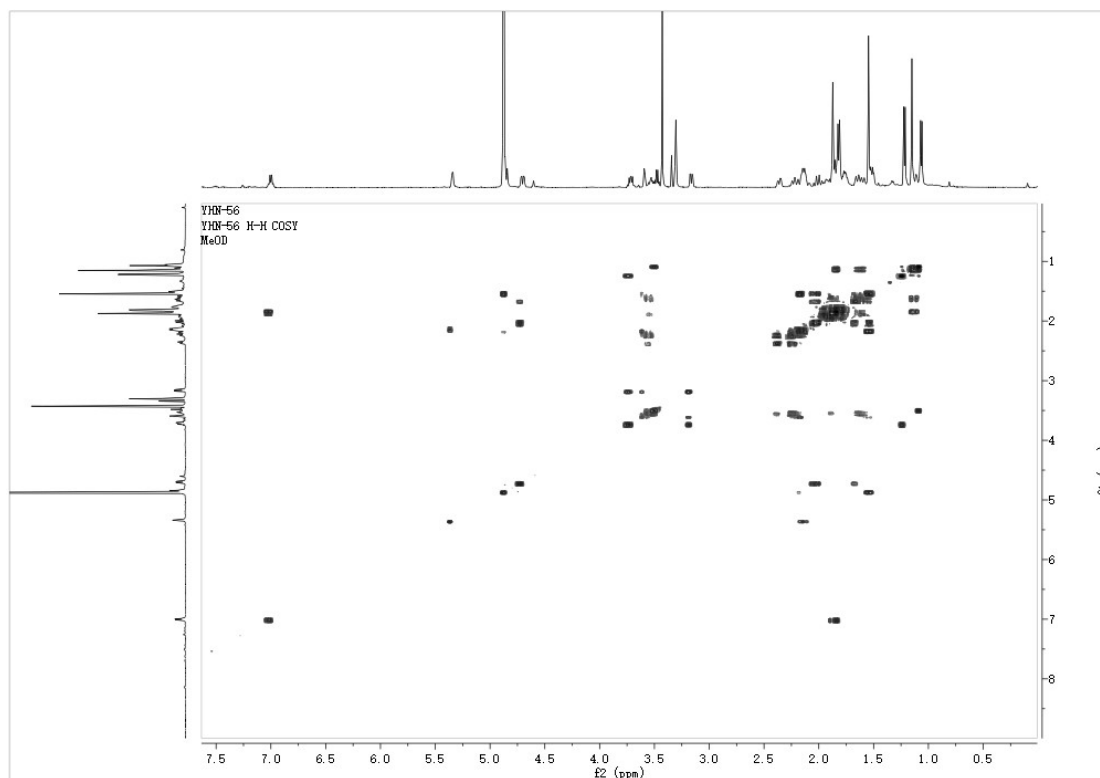


Figure S53. ^1H - ^1H COSY of **8** in CD_3OD

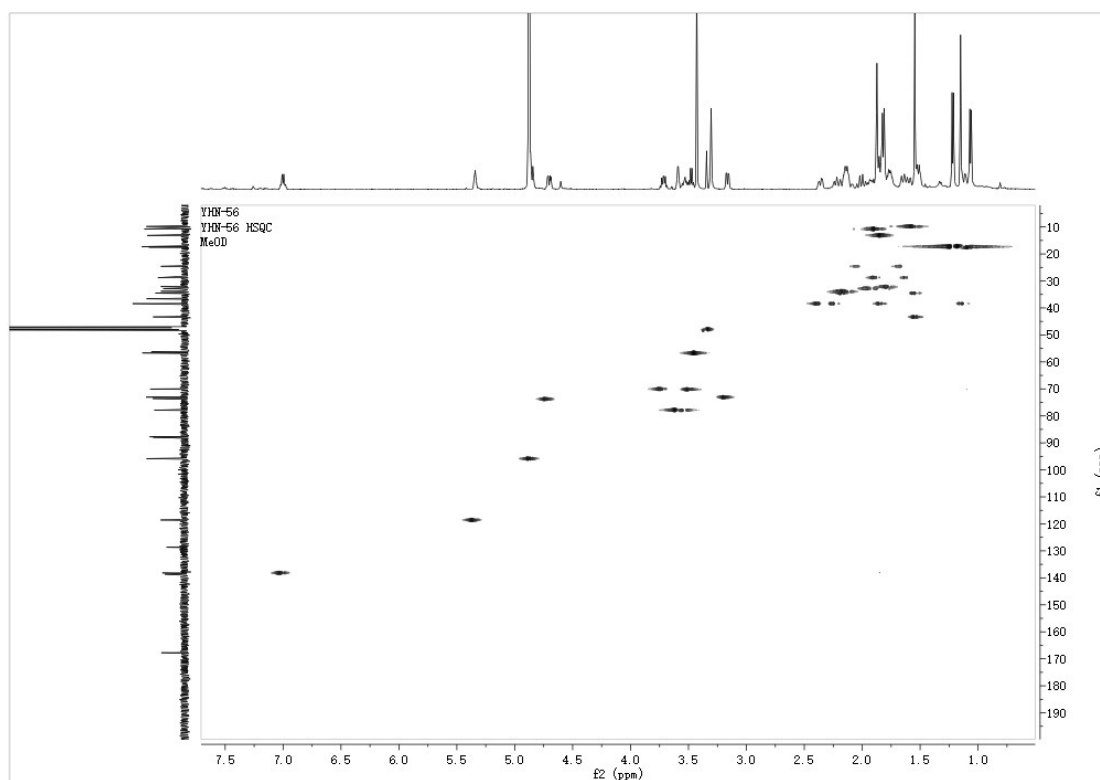


Figure S54. HSQC of **8** in CD_3OD

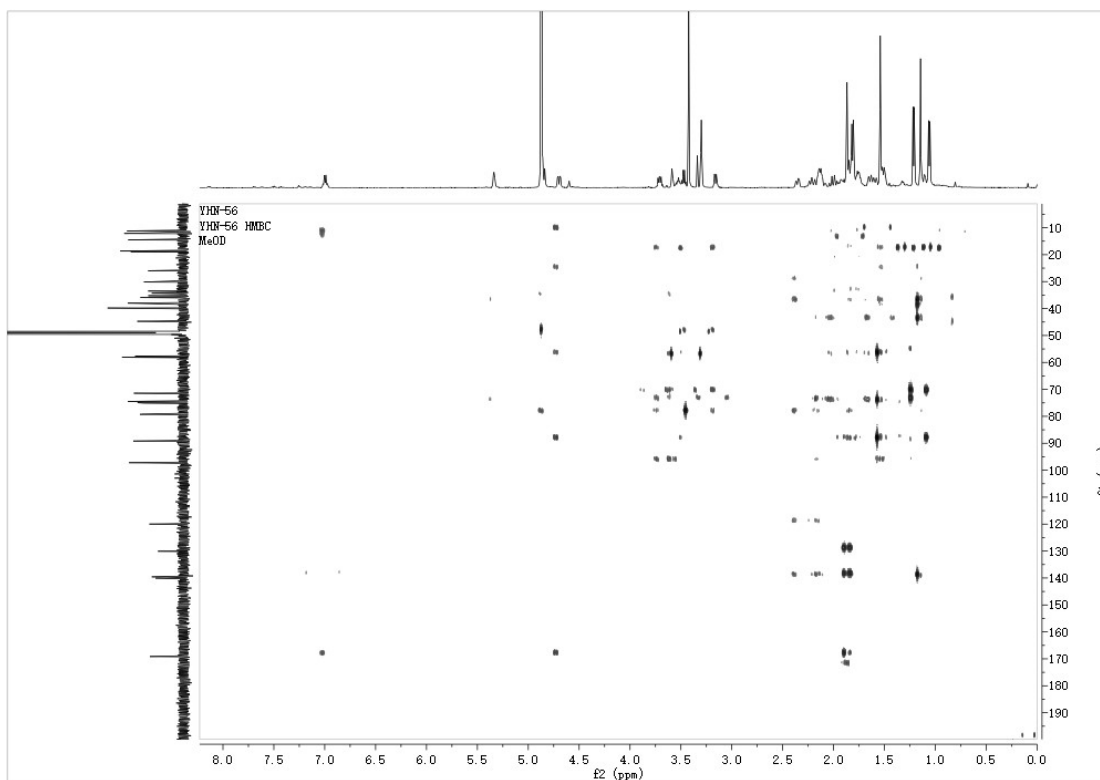


Figure S55. HMBC of **8** in CD₃OD

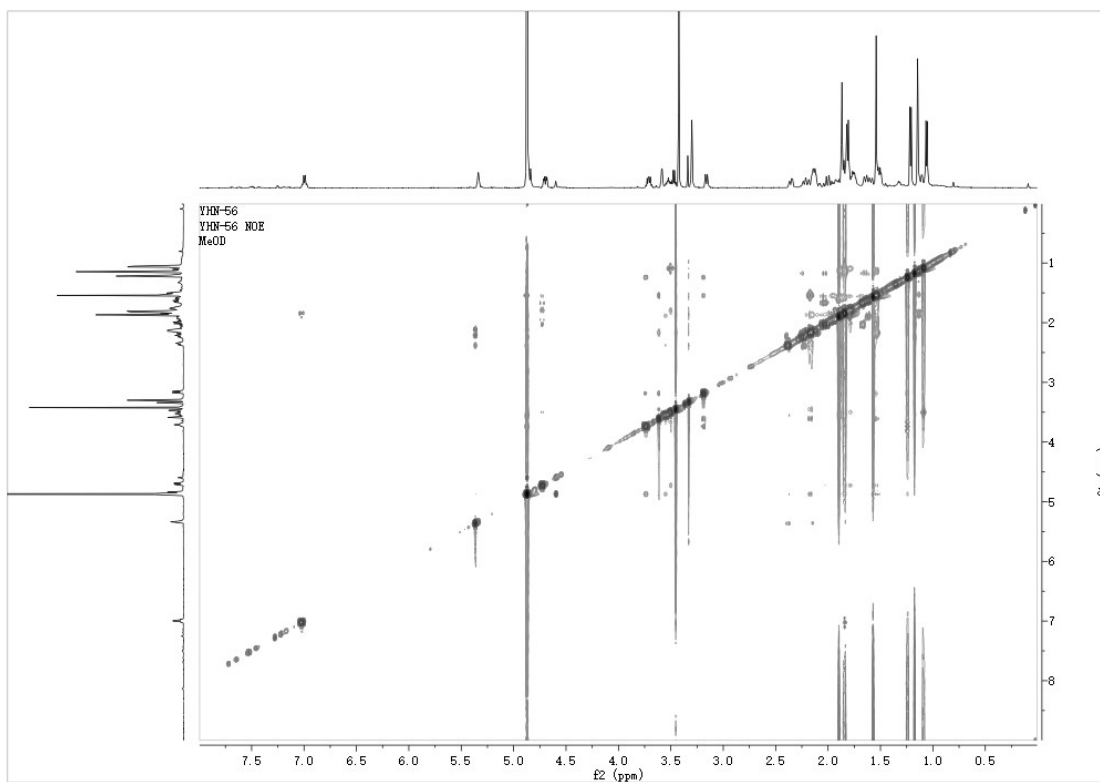


Figure S56. NOESY of **8** in CD₃OD

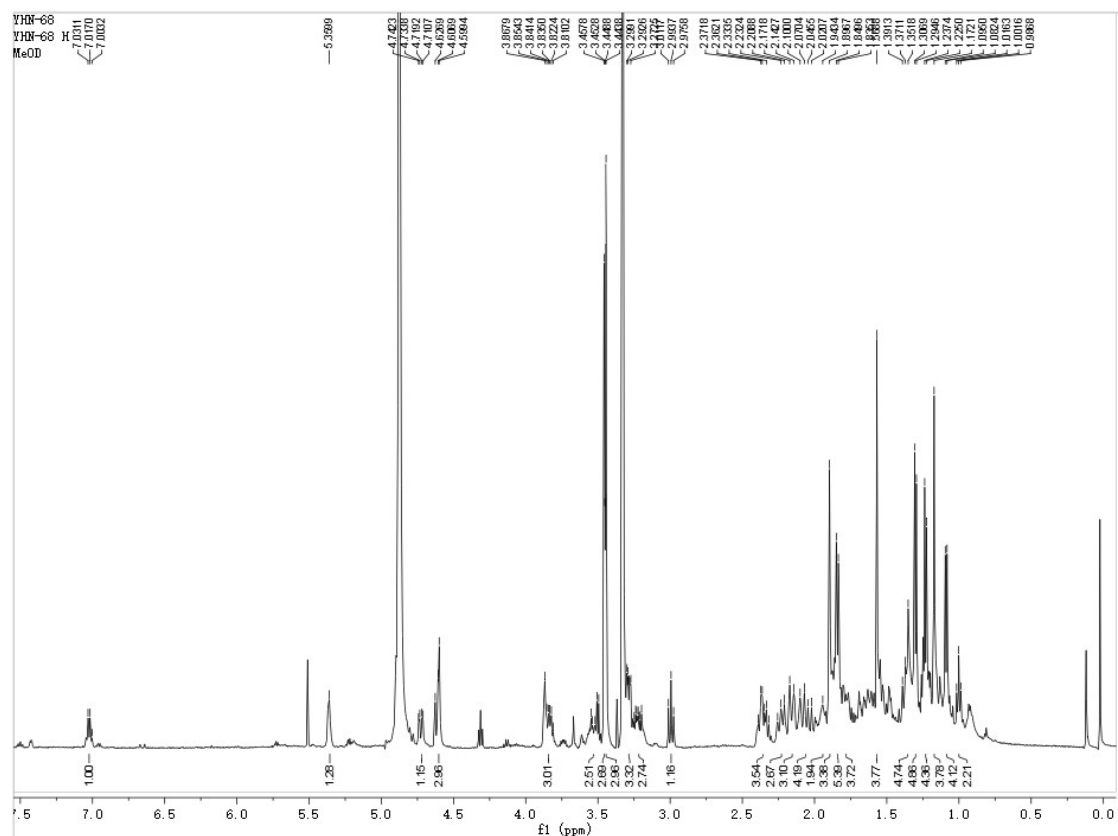


Figure S57. ^1H NMR spectrum (500 MHz) of **9** in CD_3OD

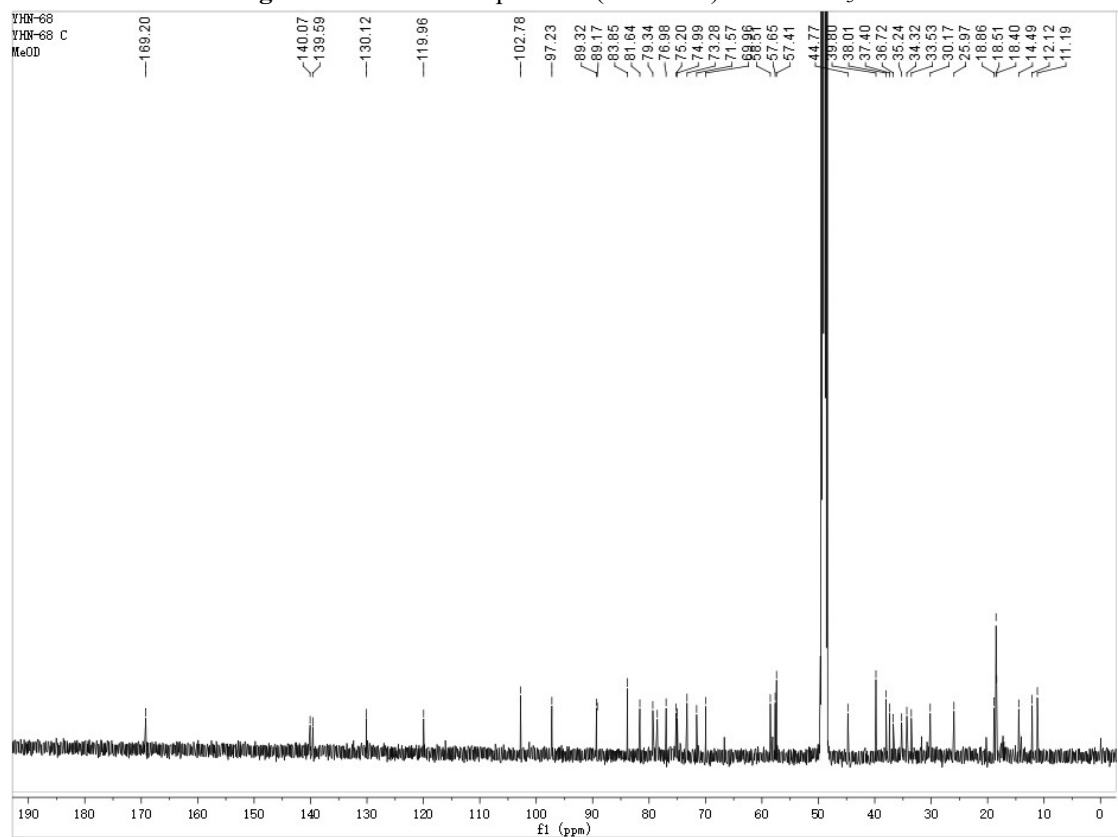


Figure S58. ^{13}C NMR spectrum (125 MHz) of **9** in CD_3OD

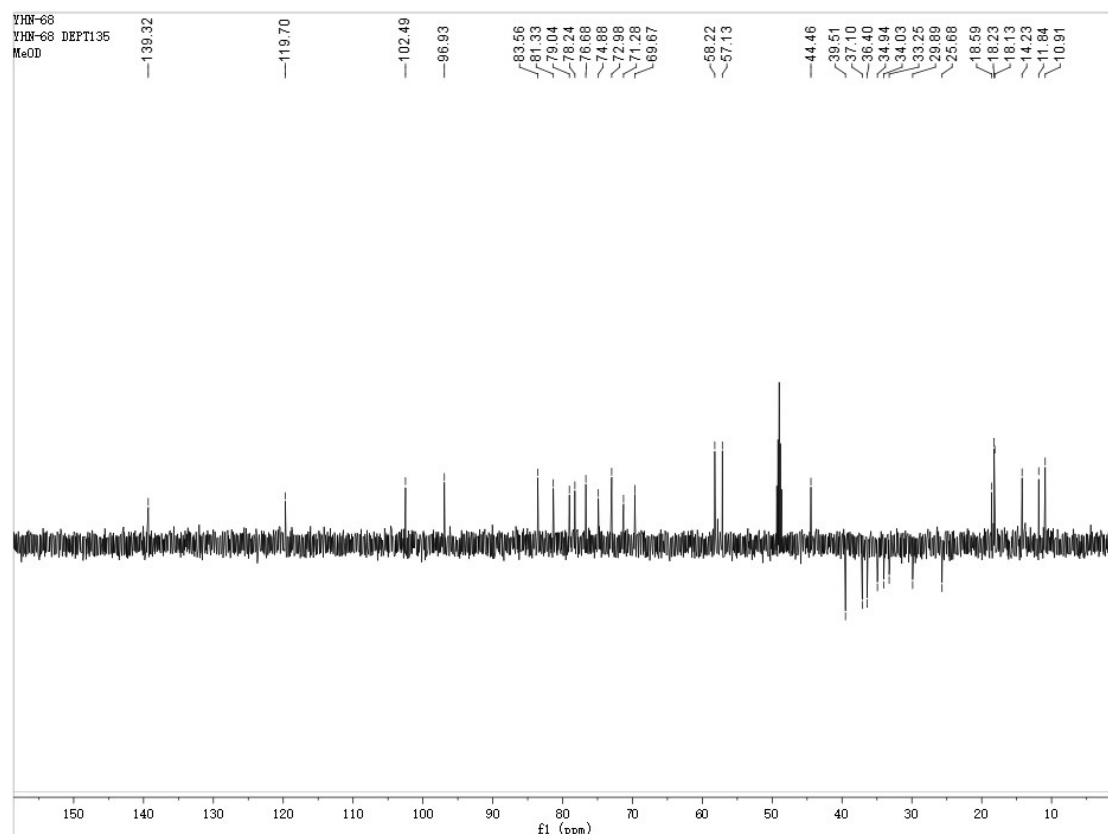


Figure S59. DEPT spectrum of **9** in CD₃OD

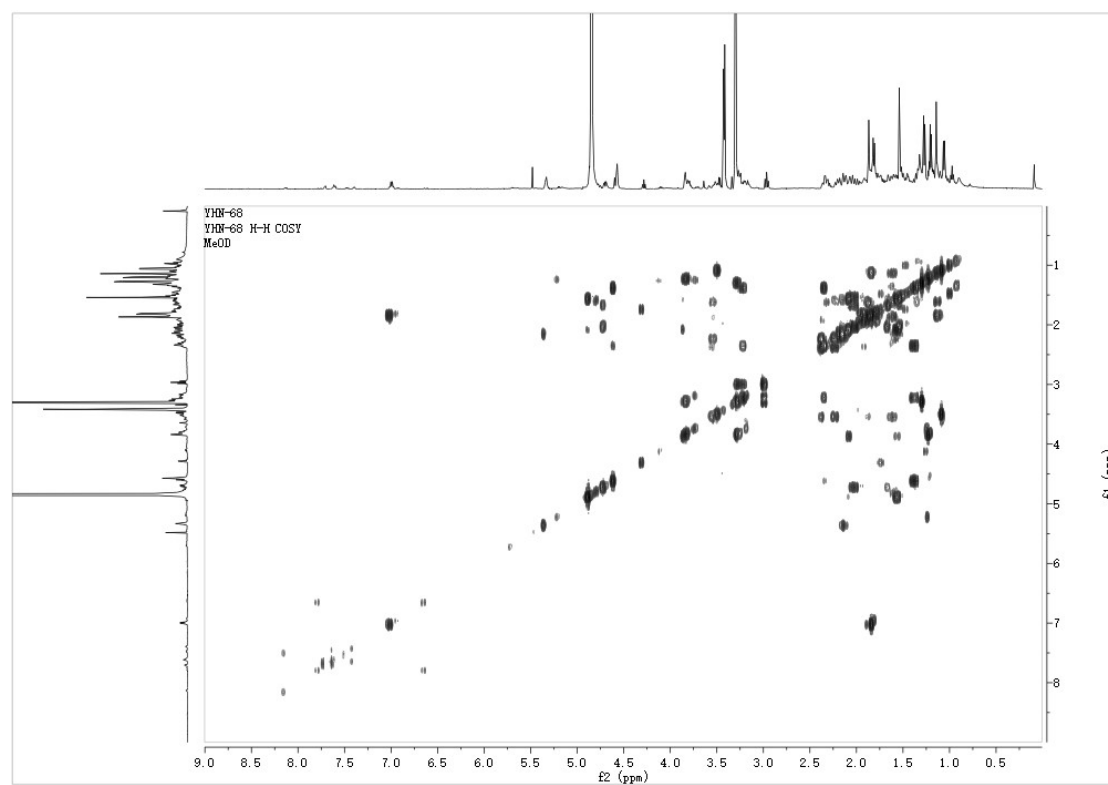


Figure S60. ¹H-¹H COSY of **9** in CD₃OD

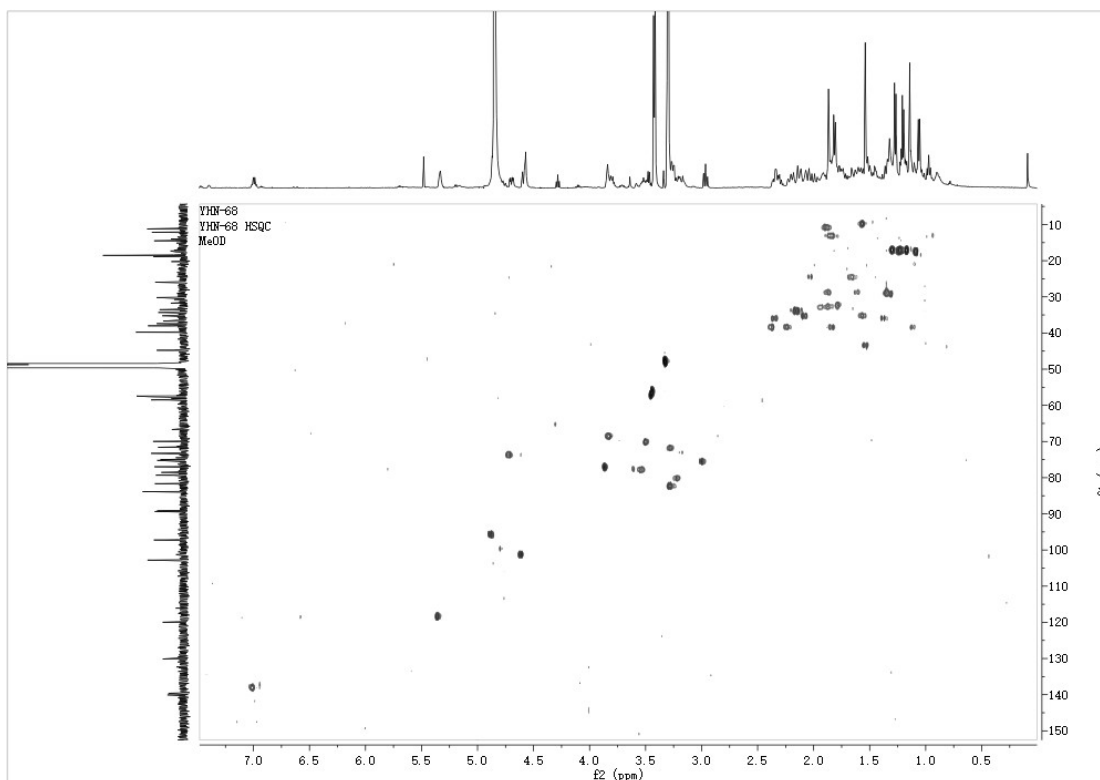


Figure S61. HSQC of **9** in CD₃OD

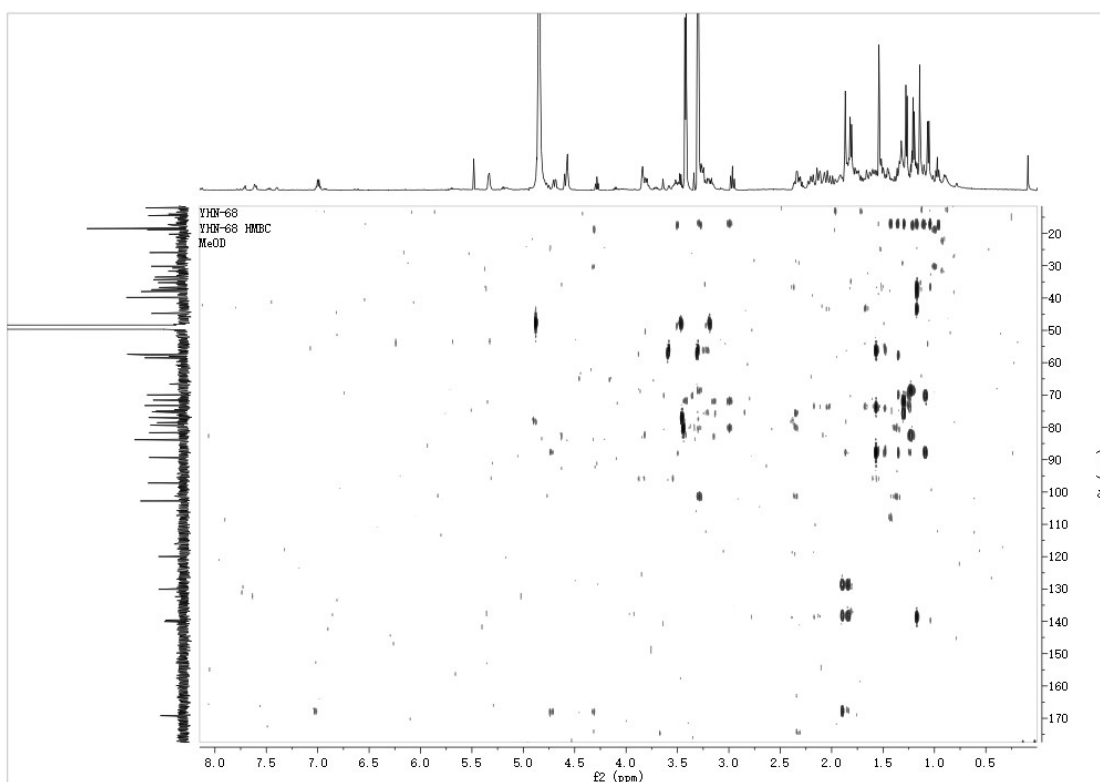


Figure S62. HMBC of **9** in CD₃OD

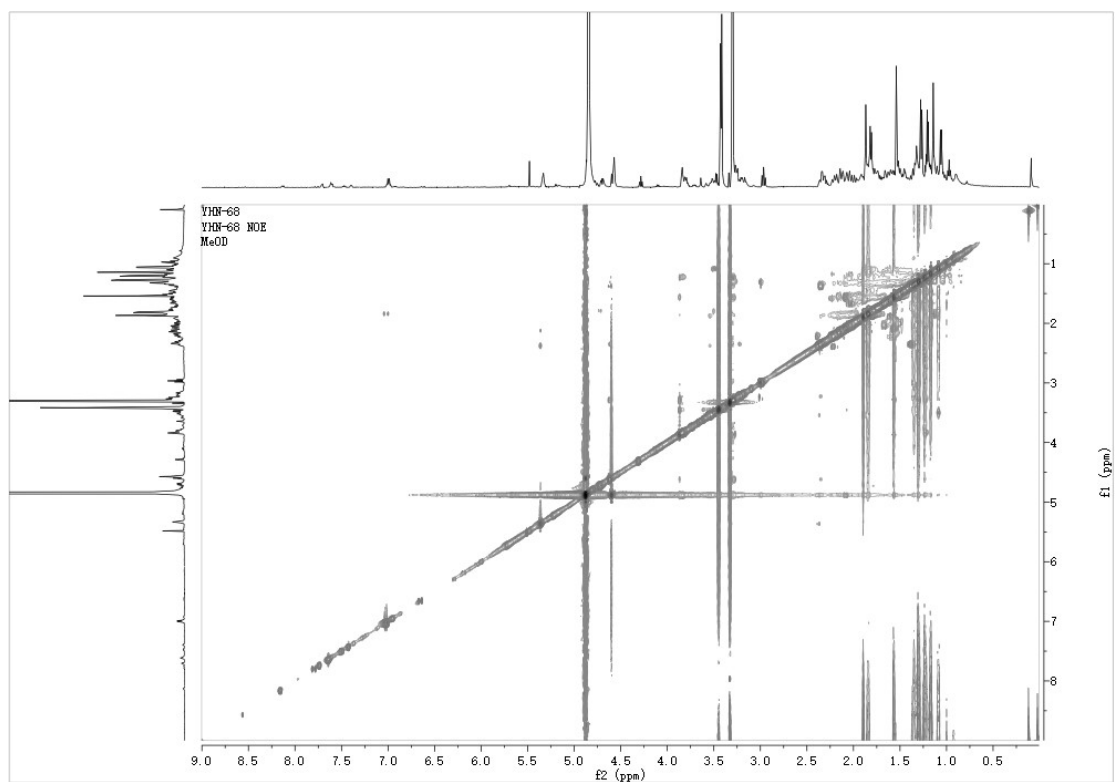


Figure S63. NOESY of **9** in CD₃OD

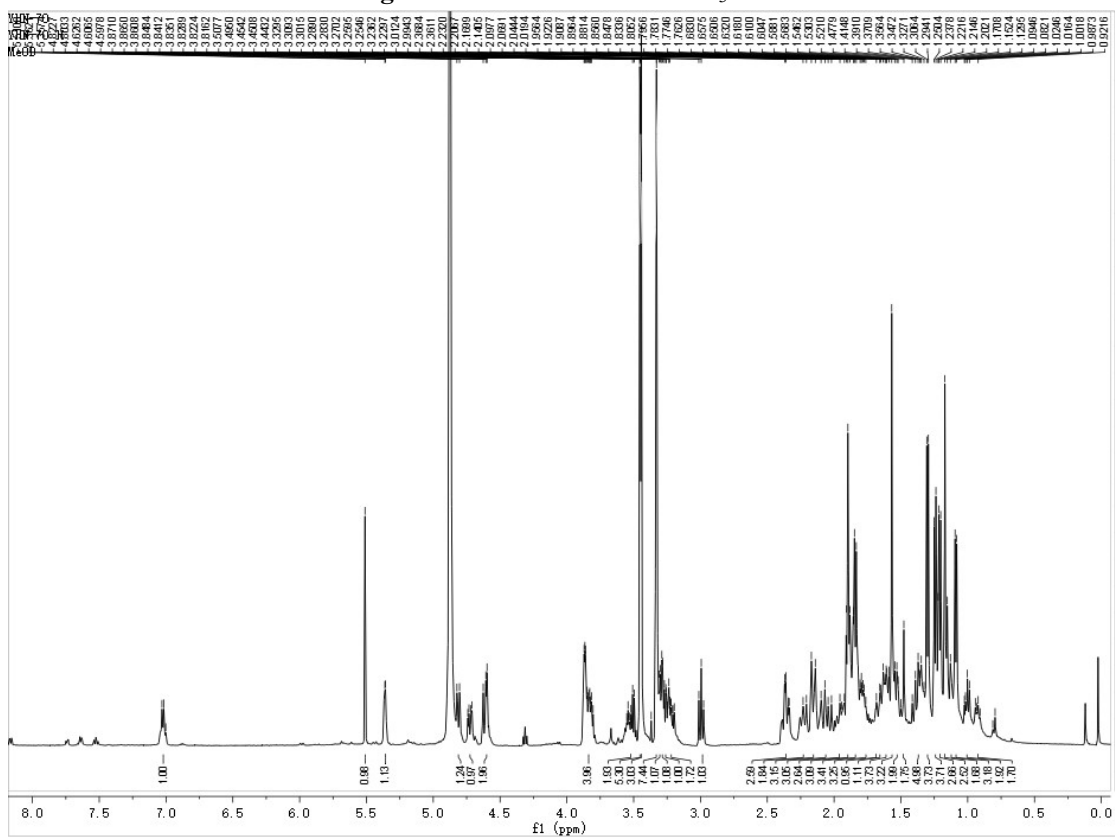


Figure S64. ¹H NMR spectrum (500 MHz) of **10** in CD₃OD

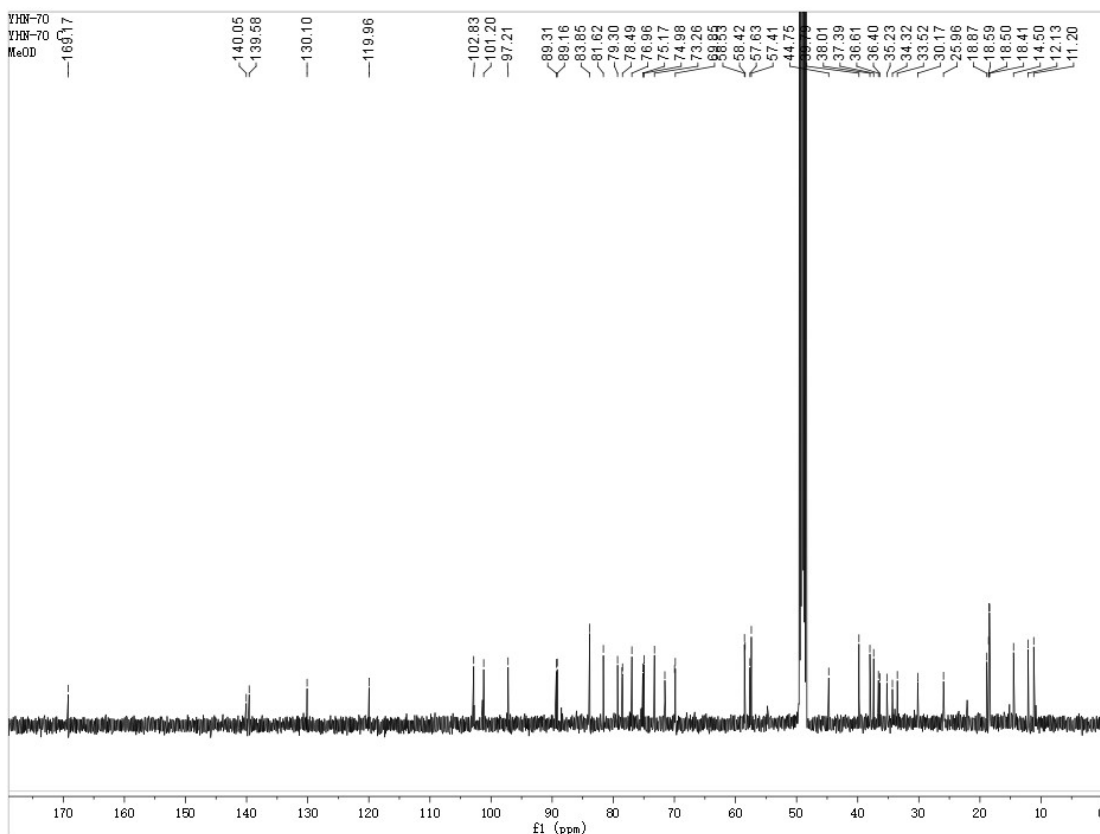


Figure S65. ^{13}C NMR spectrum (125 MHz) of **10** in CD_3OD

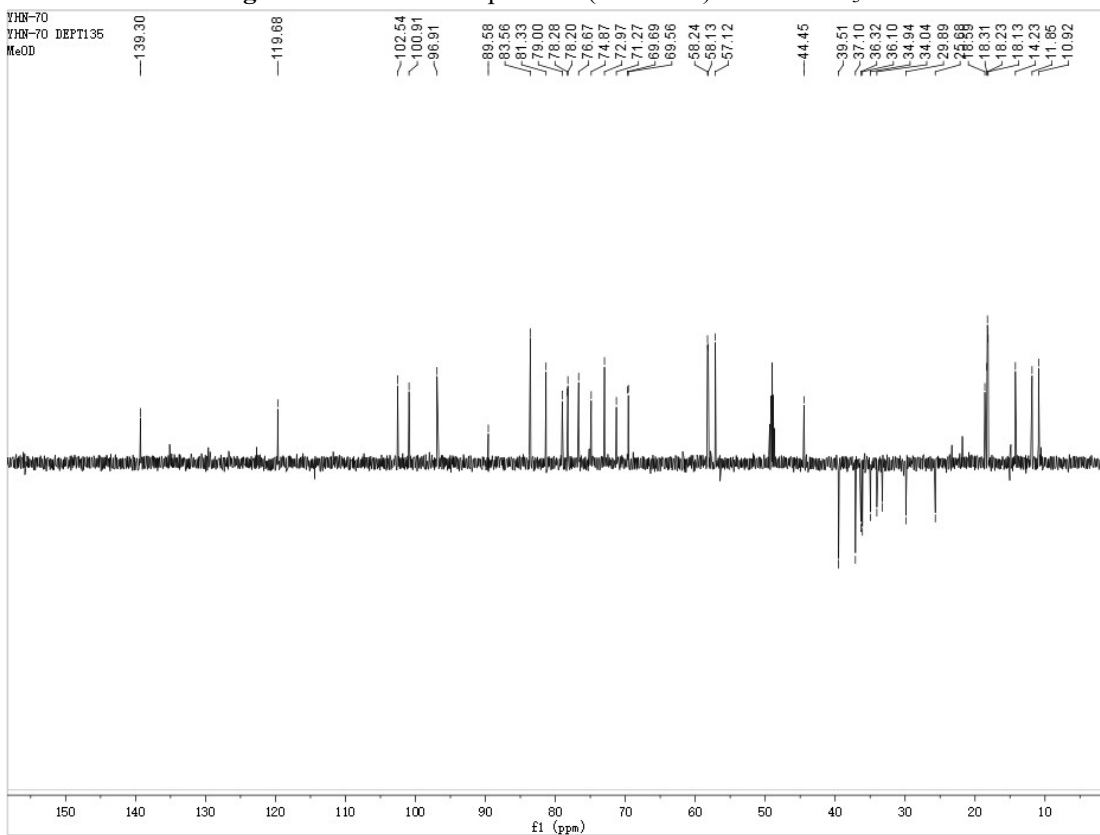


Figure S66. DEPT spectrum of **10** in CD_3OD

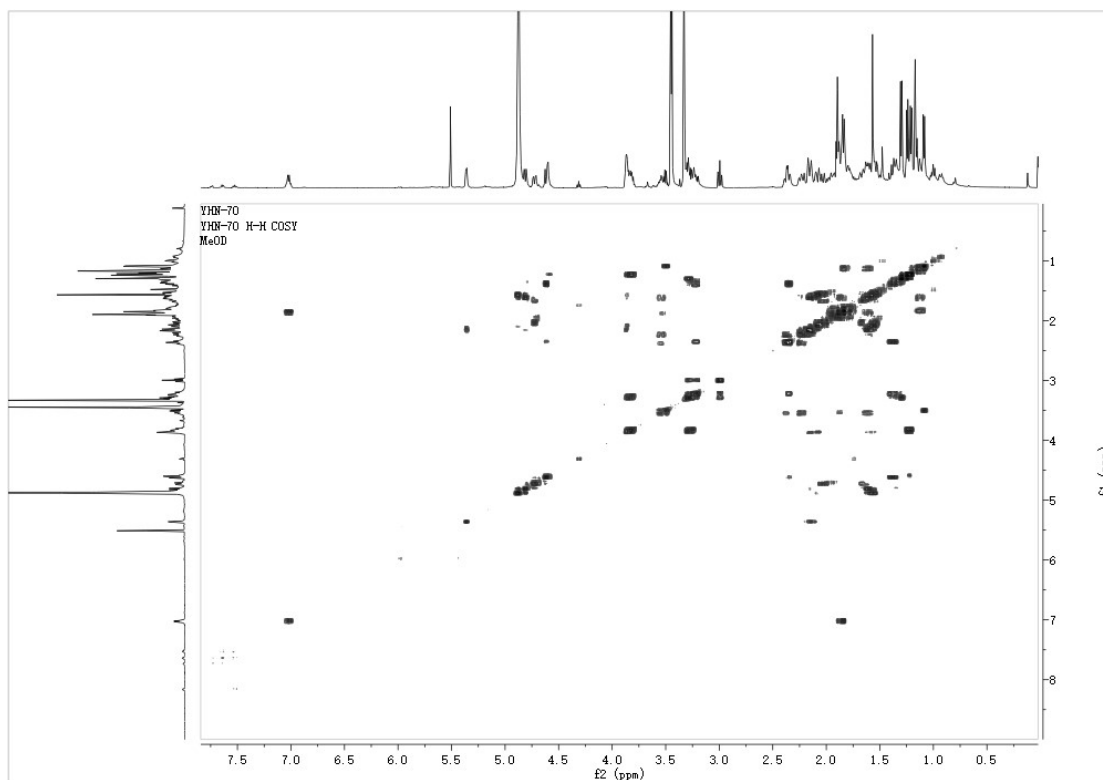


Figure S67. ^1H - ^1H COSY of **10** in CD_3OD

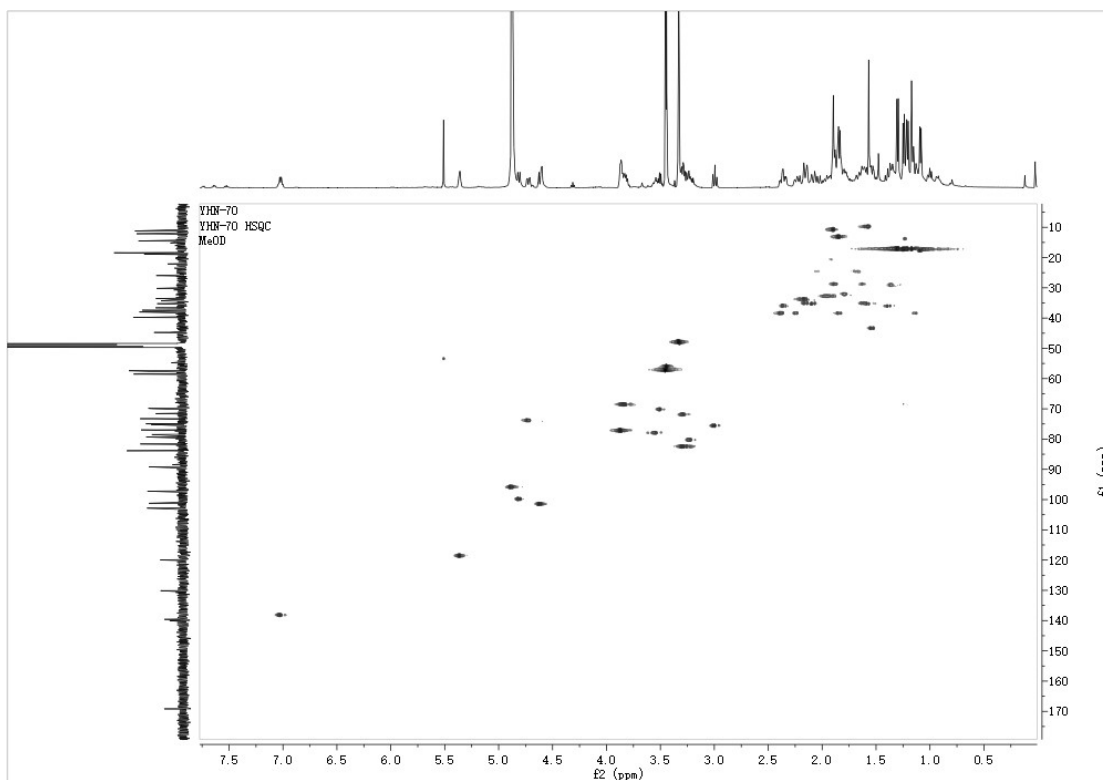


Figure S68. HSQC of **10** in CD_3OD

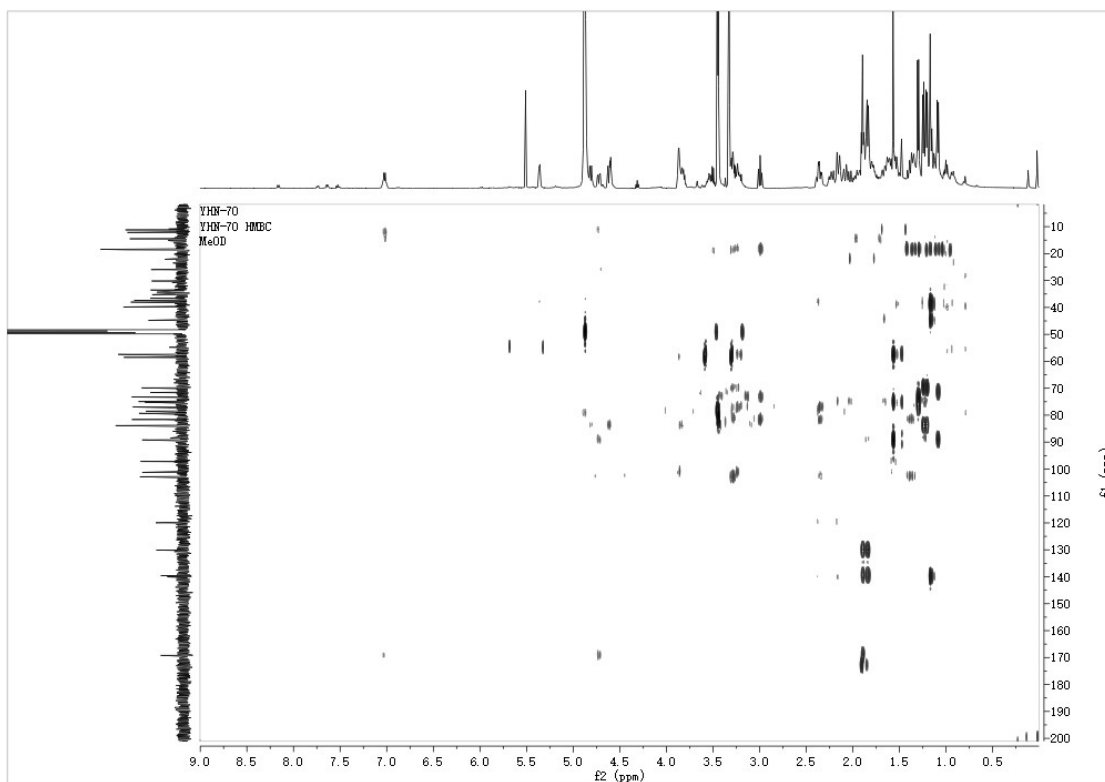


Figure S69. HMBC of 10 in CD₃OD

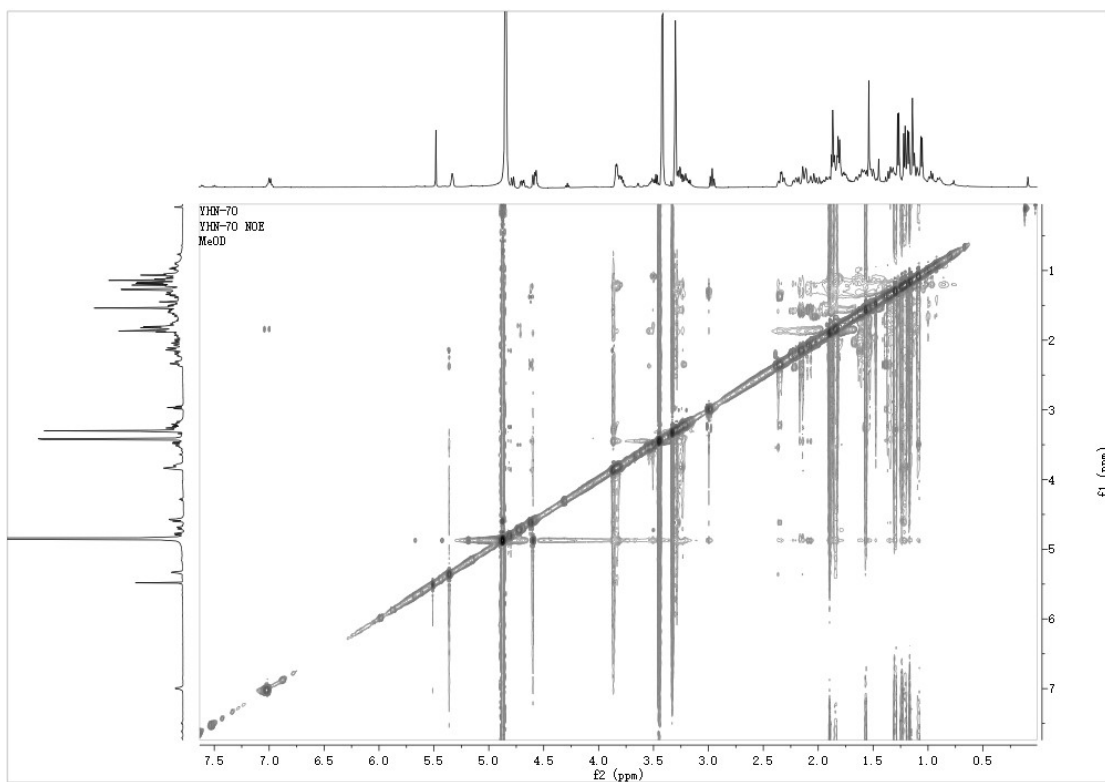


Figure S70. NOESY of 10 in CD₃OD

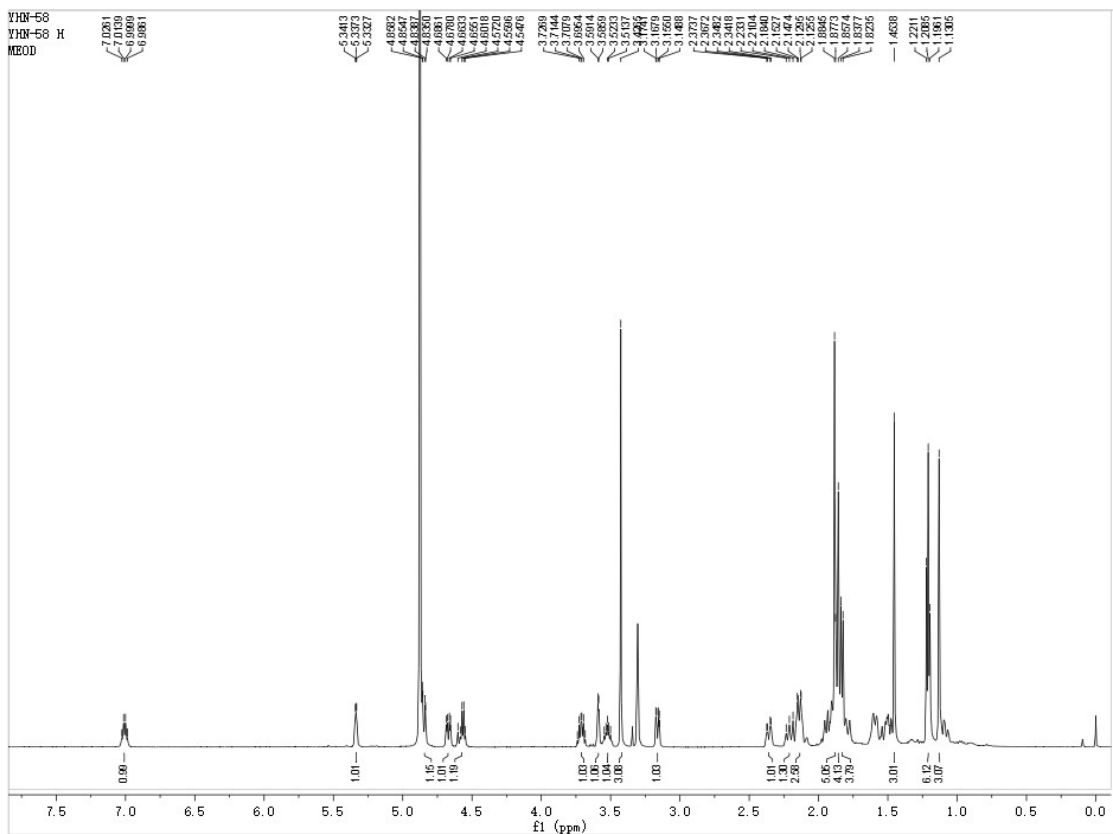


Figure S71. ^1H NMR spectrum (500 MHz) of **11** in CD_3OD

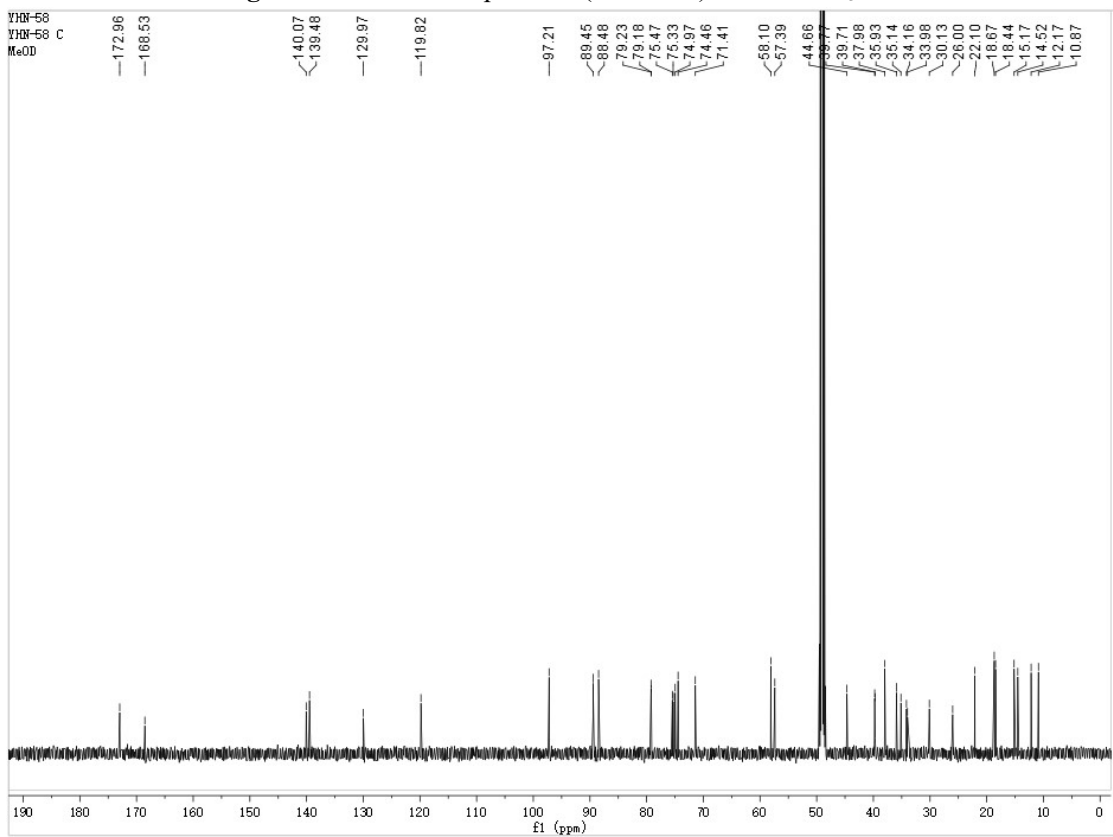


Figure S72. ^{13}C NMR spectrum (125 MHz) of **11** in CD_3OD

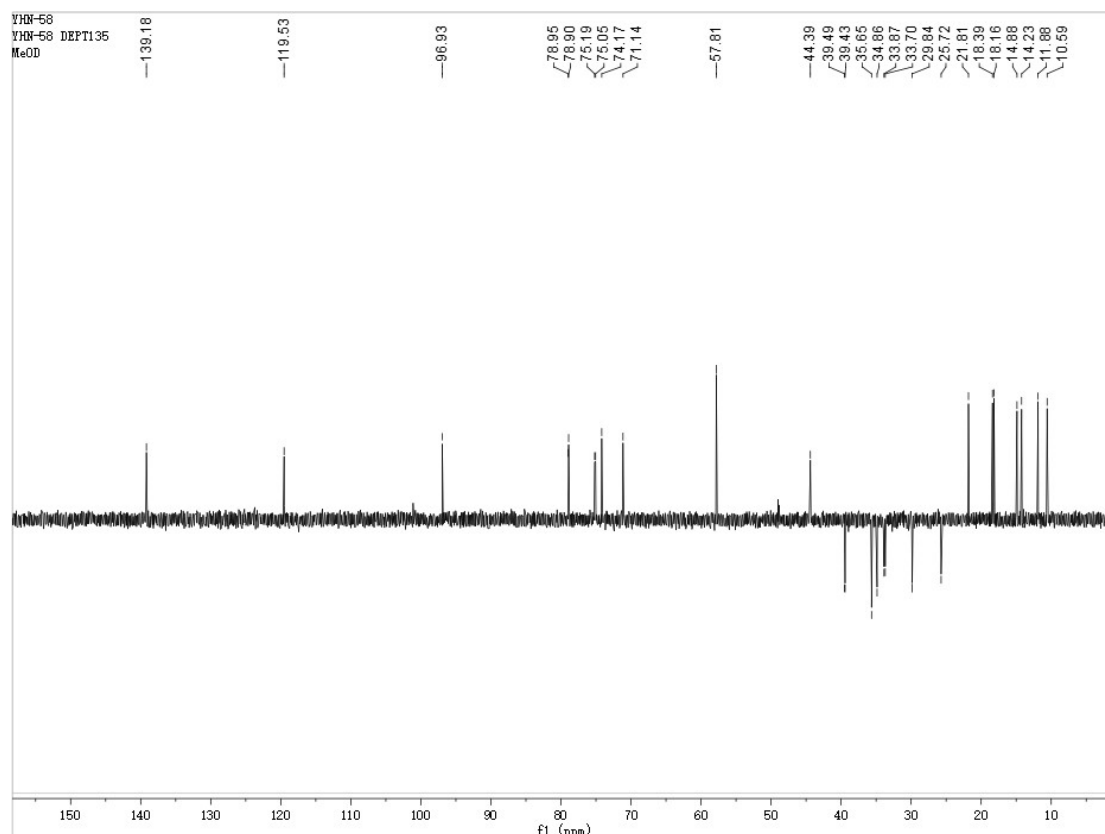


Figure S73. DEPT spectrum (125 MHz) of **11** in CD₃OD

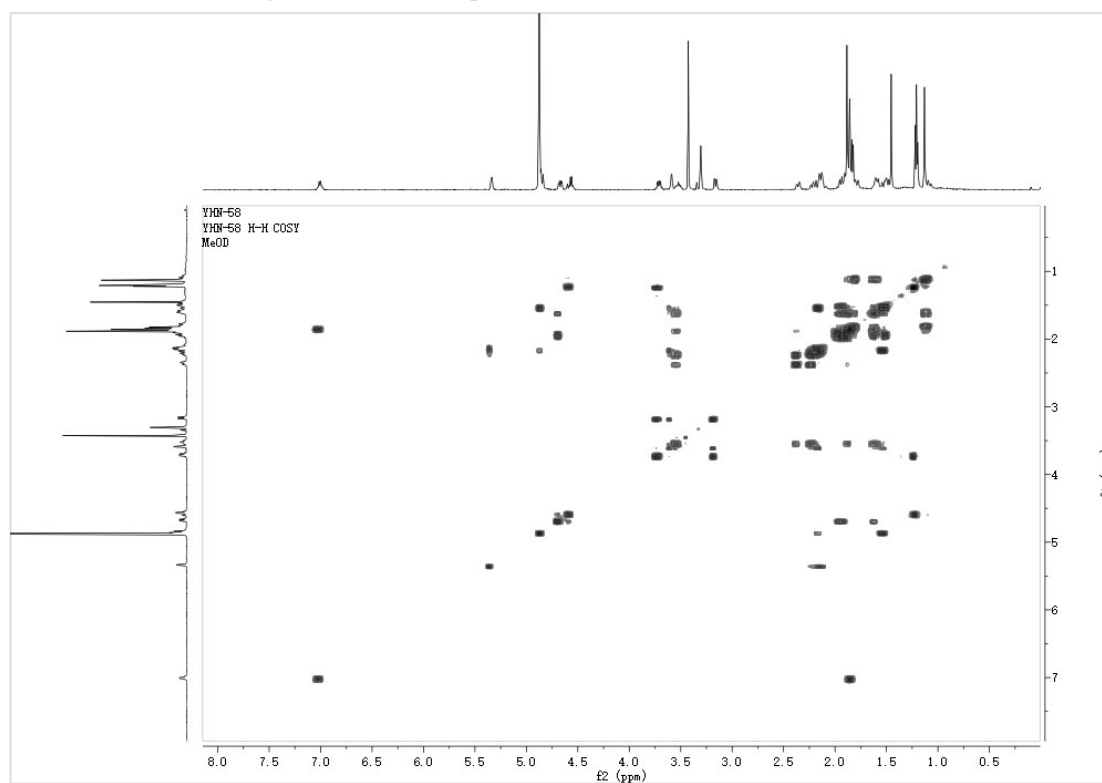


Figure S74. ¹H-¹H COSY (500 MHz) of **11** in CD₃OD

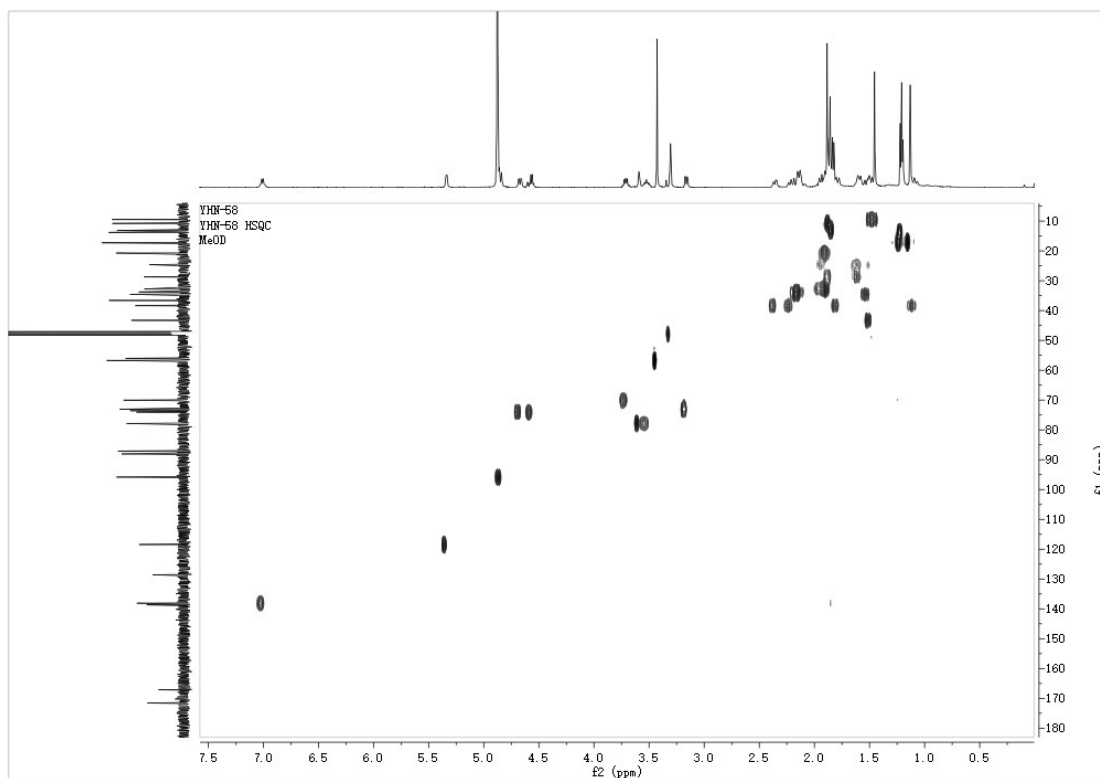


Figure S75. HSQC of 11 in CD₃OD

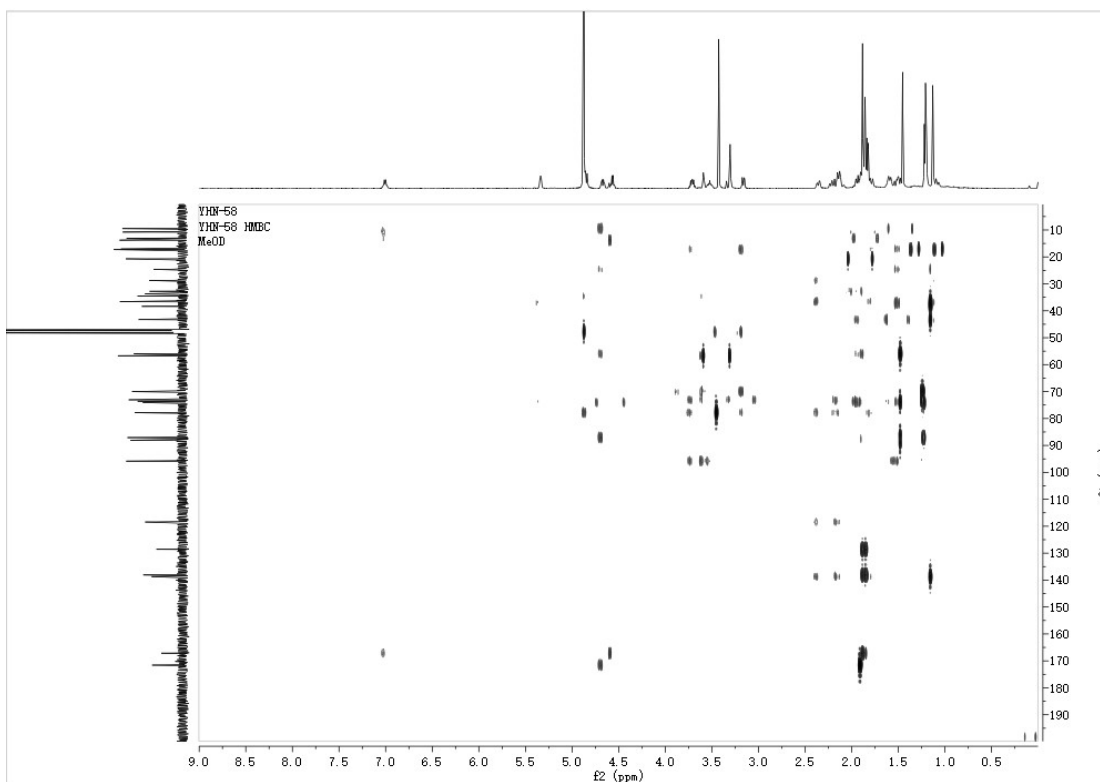


Figure S76. HMBC of 11 in CD₃OD

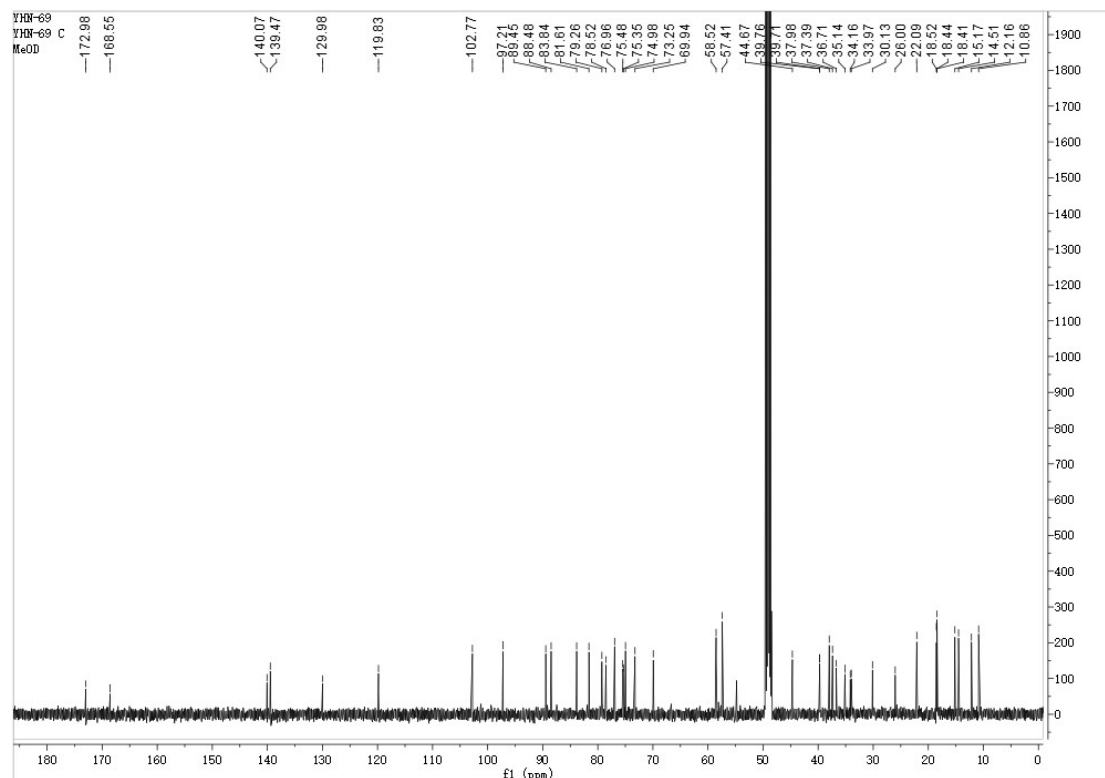


Figure S79. ^{13}C NMR spectrum (125 MHz) of **12** in CD_3OD

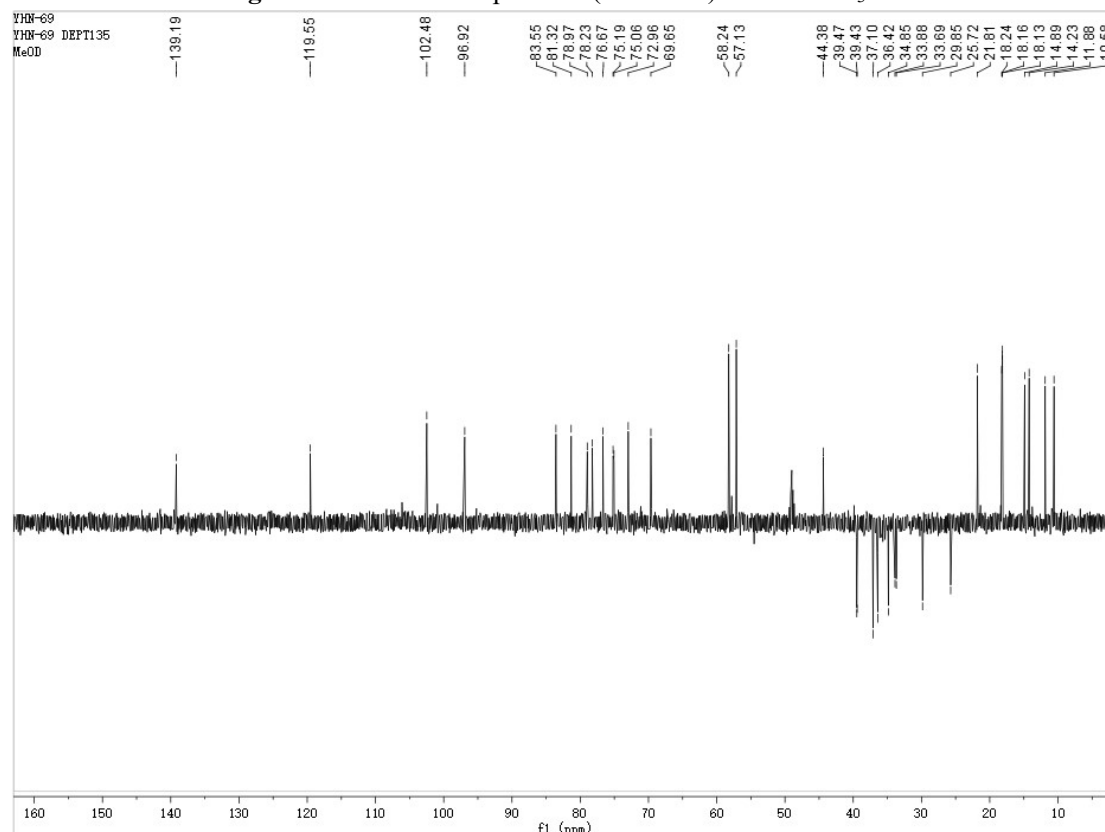


Figure S80. DEPT spectrum of **12** in CD_3OD

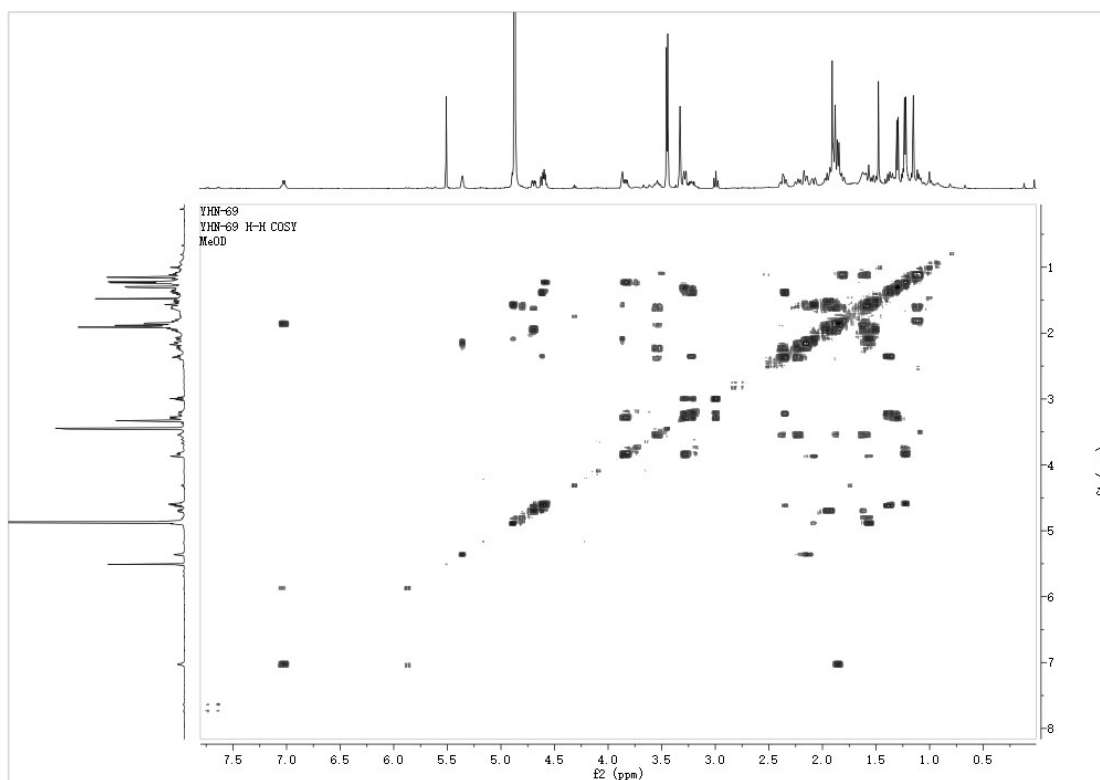


Figure S81. ^1H - ^1H COSY of **12** in CD_3OD

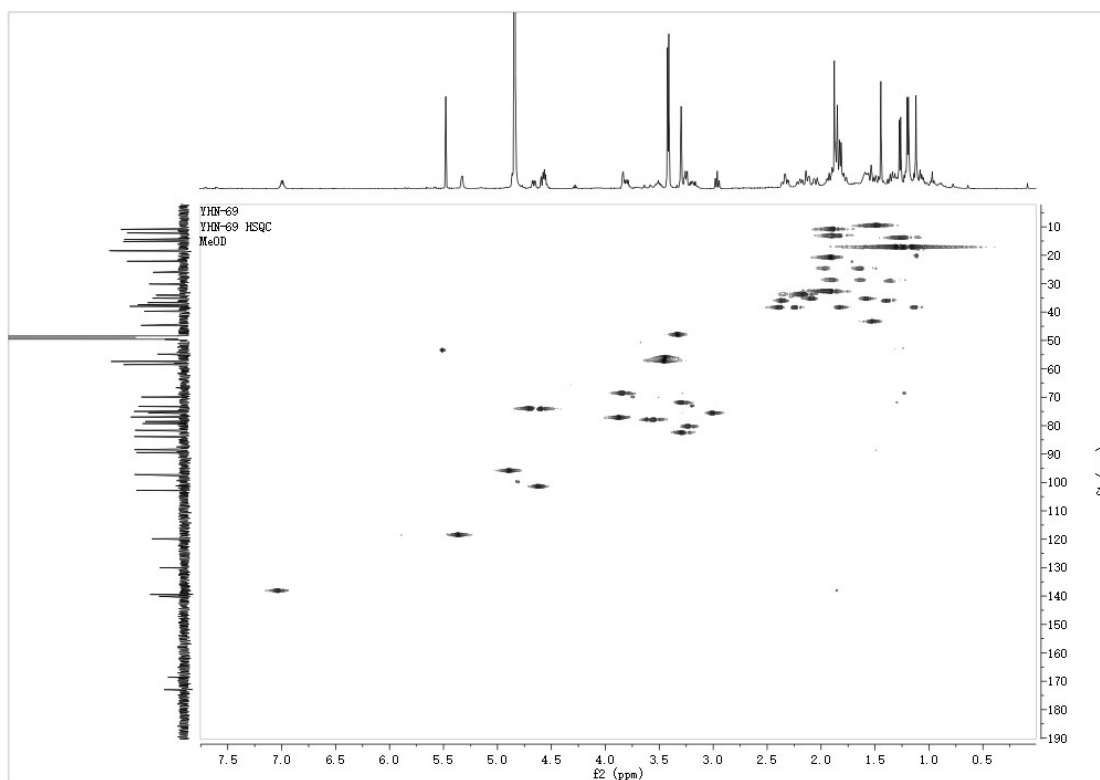


Figure S82. HSQC of **12** in CD_3OD

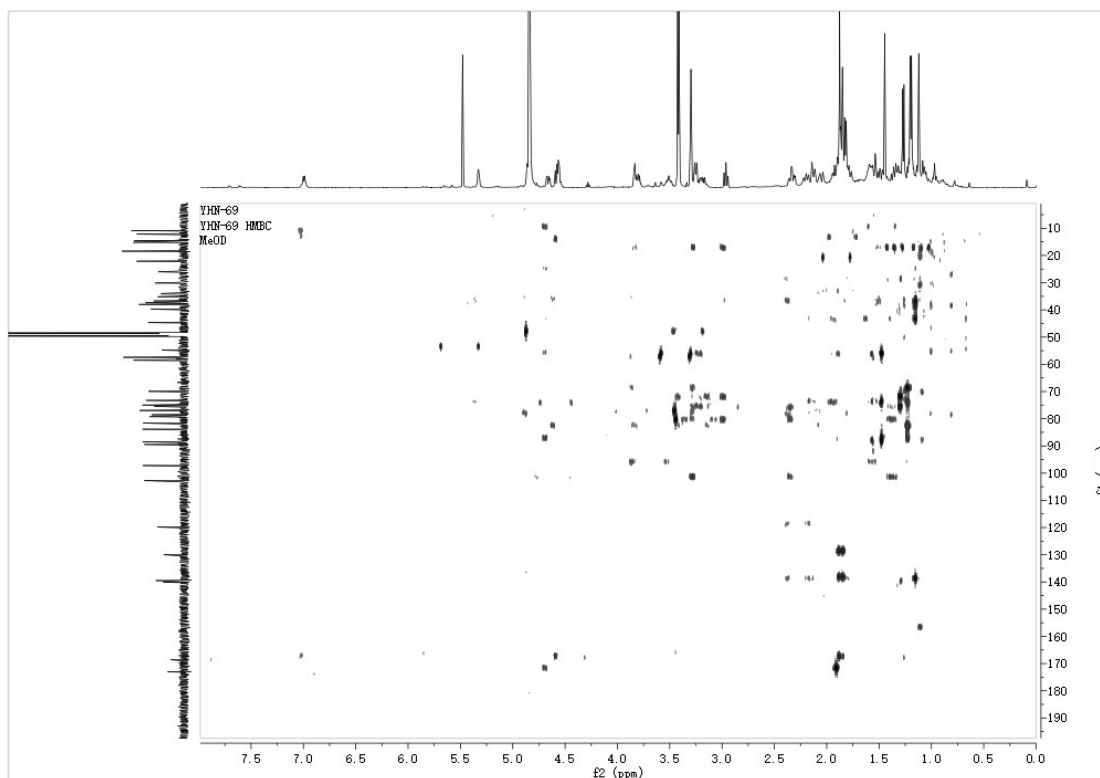


Figure S83. HMBC of 12 in CD₃OD

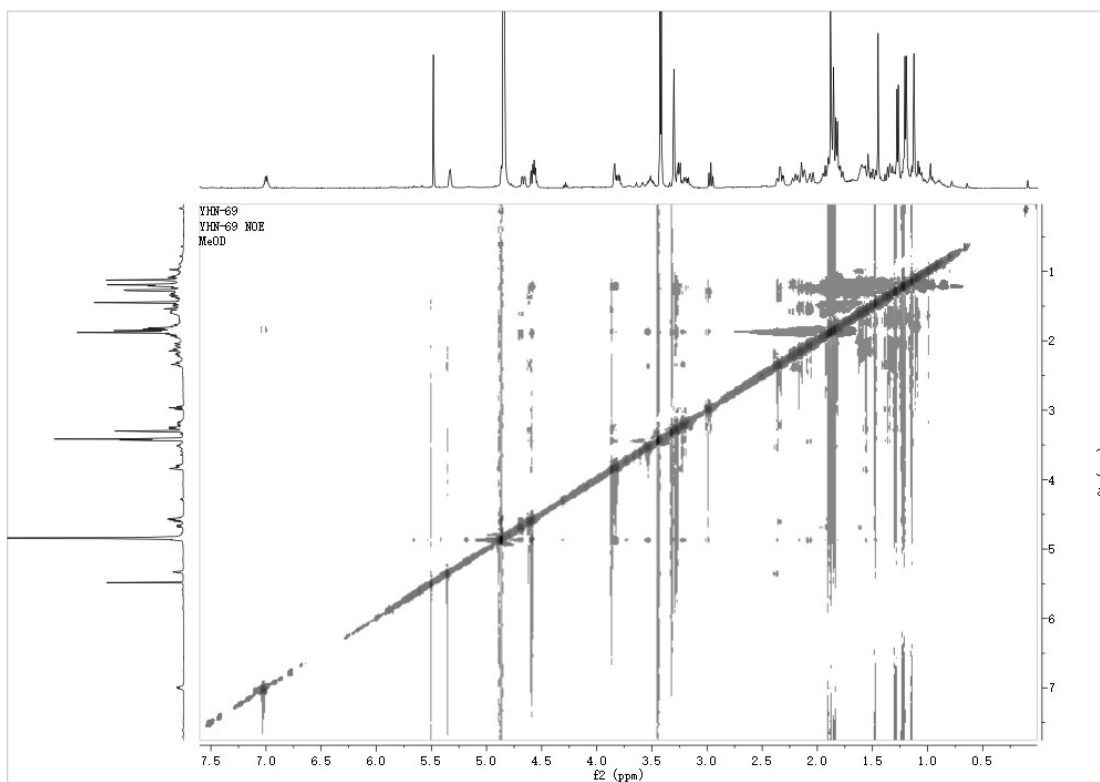


Figure S84. NOESY of 12 in CD₃OD

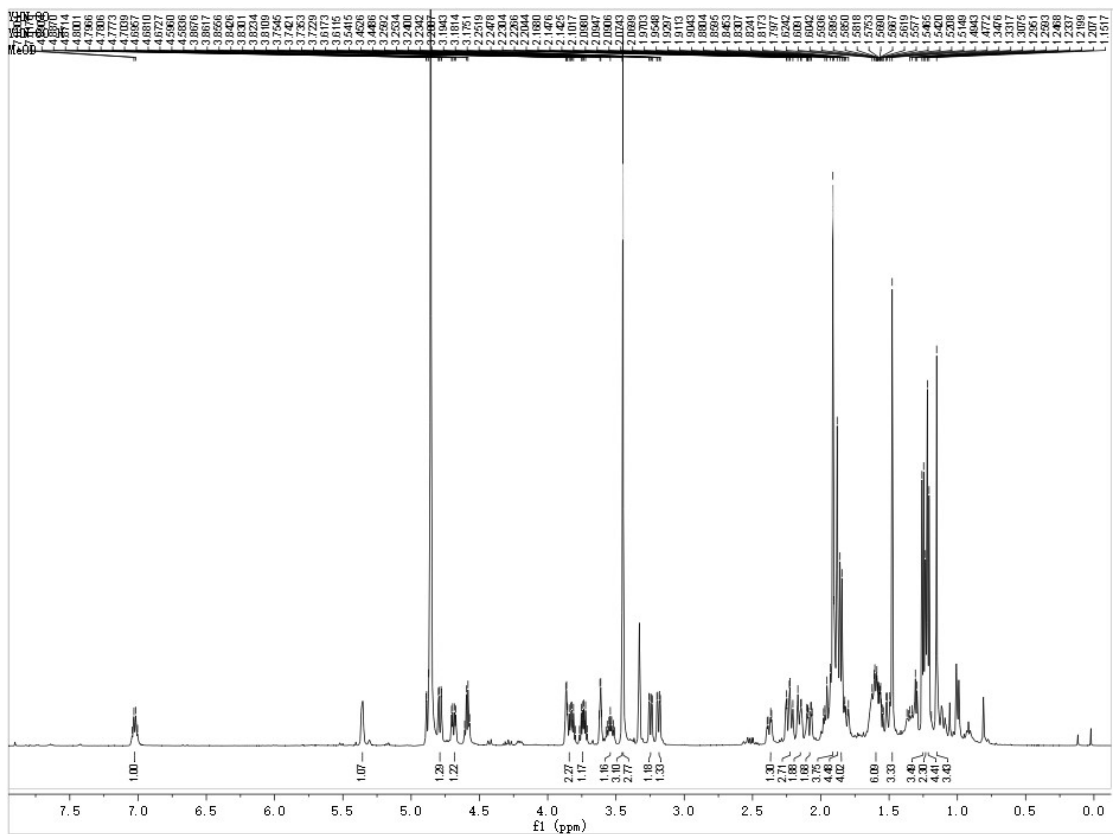


Figure S85. ¹H NMR spectrum (500 MHz) of **13** in CD₃OD

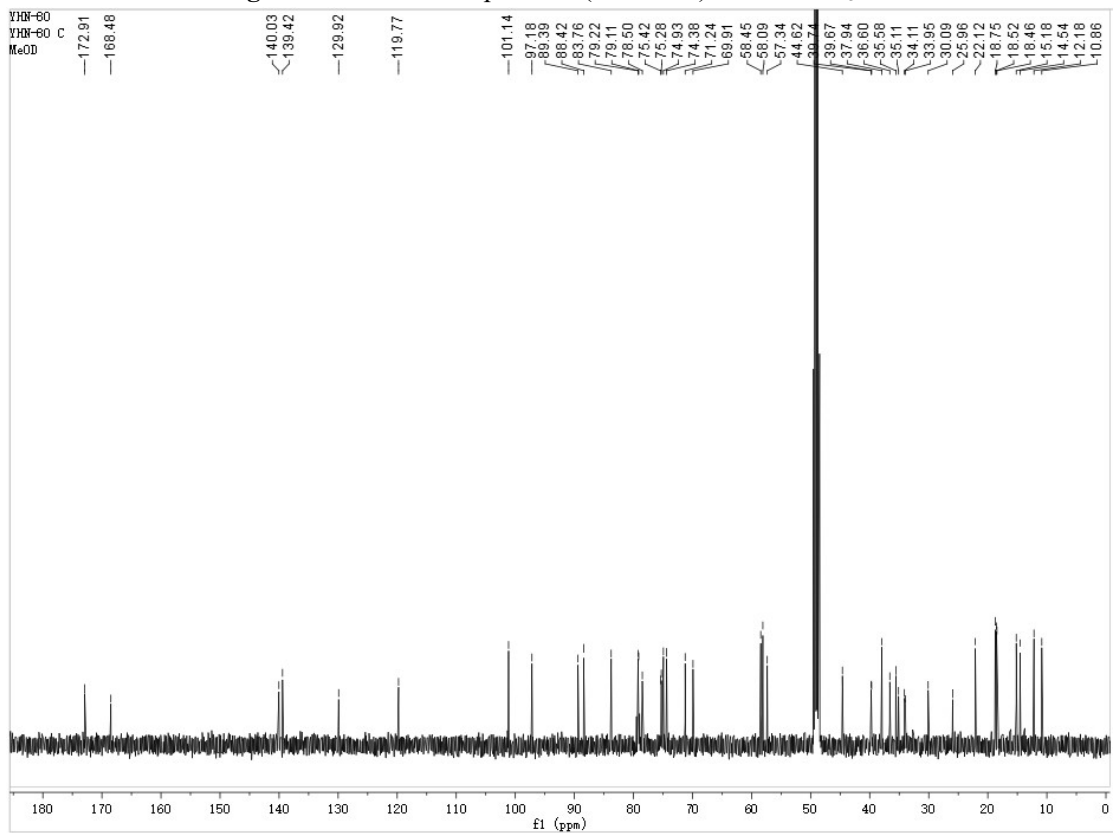


Figure S86. ¹³C NMR spectrum (125 MHz) of **13** in CD₃OD

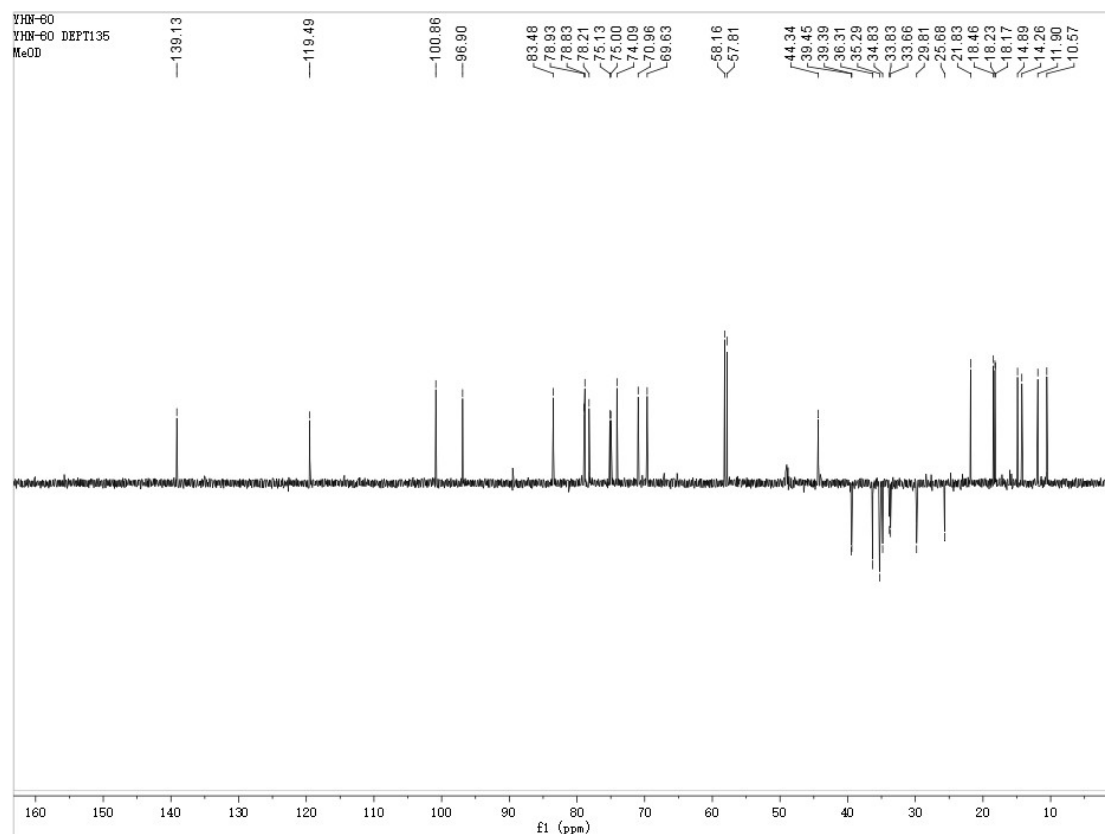


Figure S87. DEPT spectrum of 13 in CD₃OD

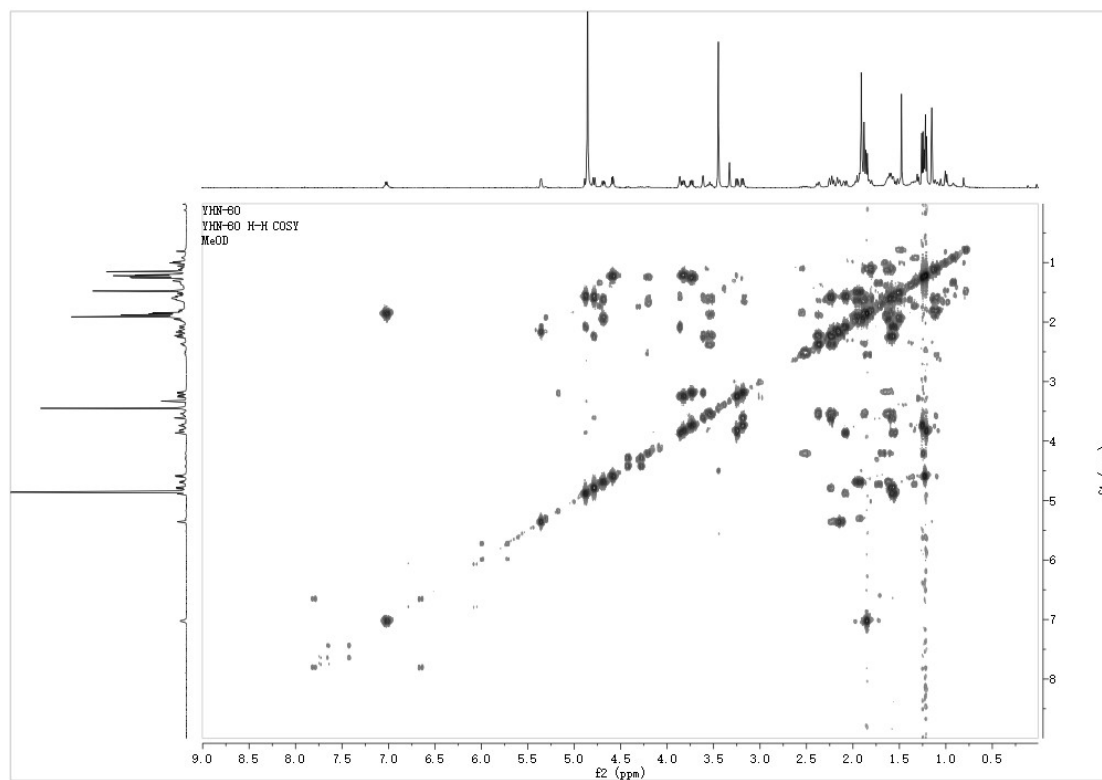


Figure S88. ¹H-¹H COSY of 13 in CD₃OD

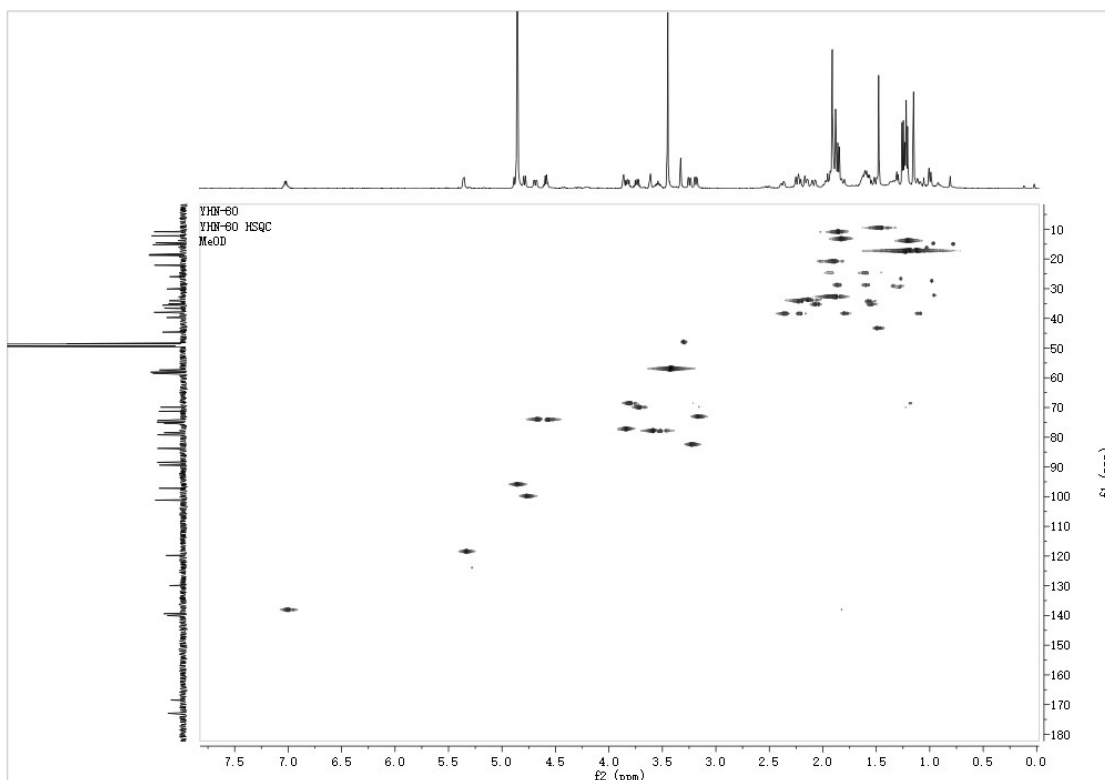


Figure S89. HSQC of 13 in CD₃OD

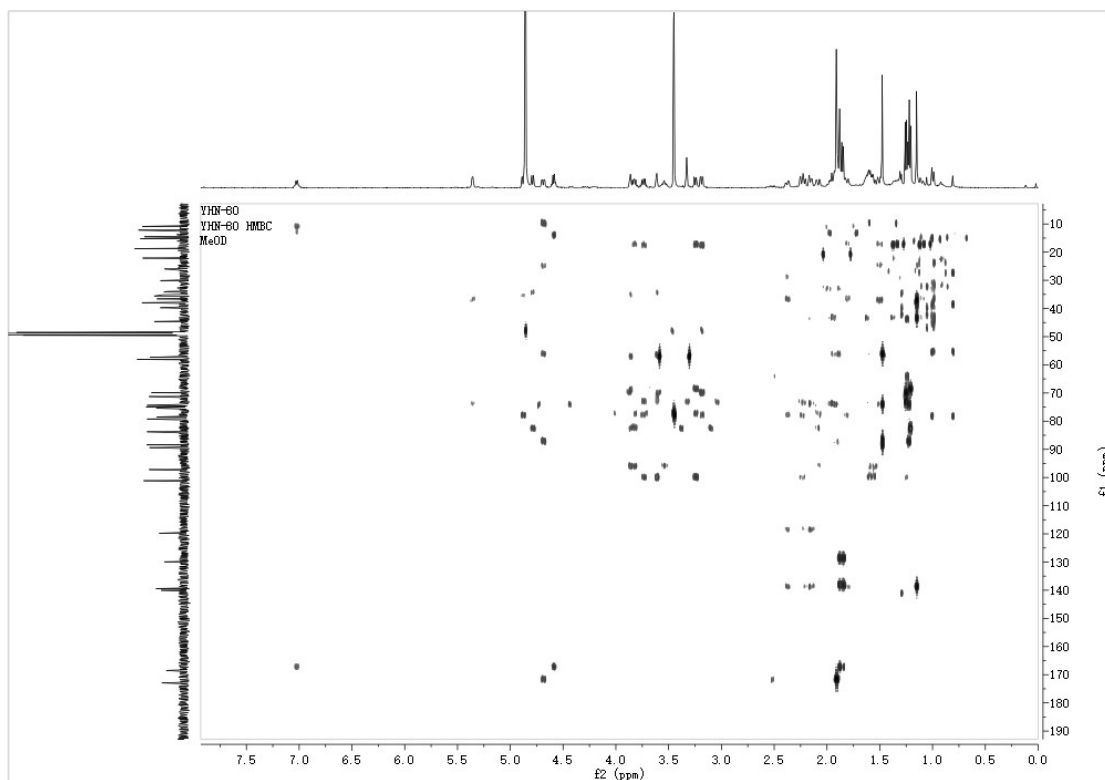


Figure S90. HMBC of 13 in CD₃OD

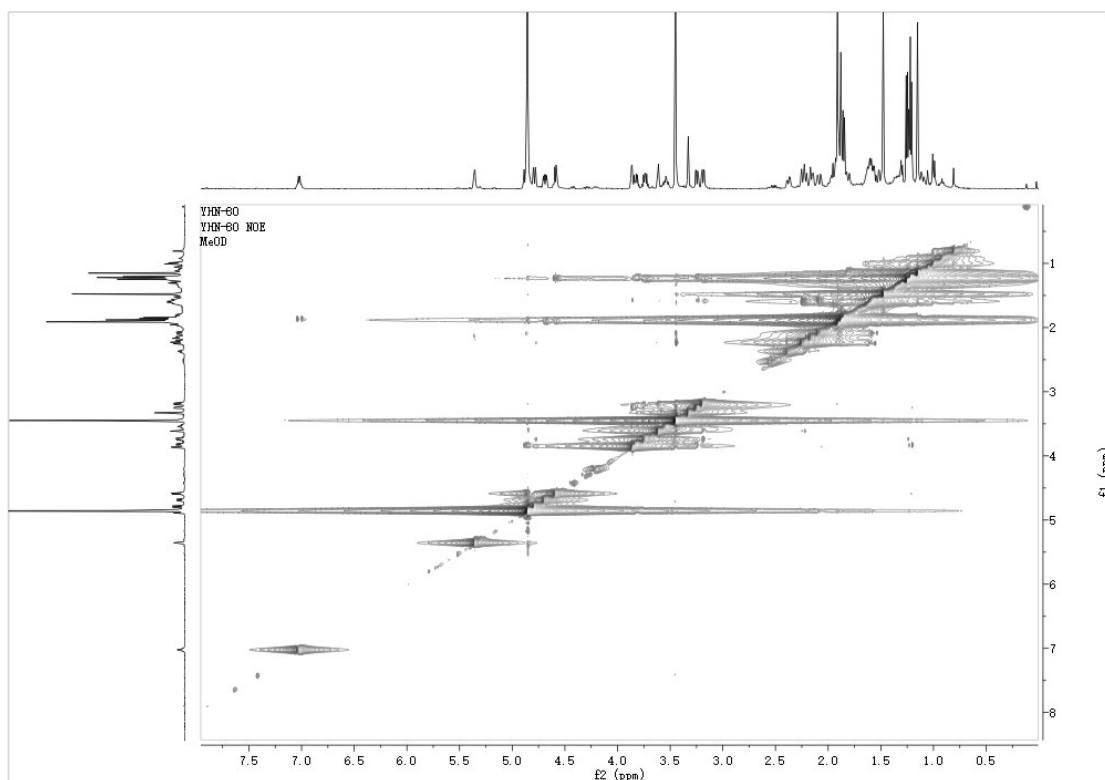


Figure S91. NOESY of 13 in CD₃OD

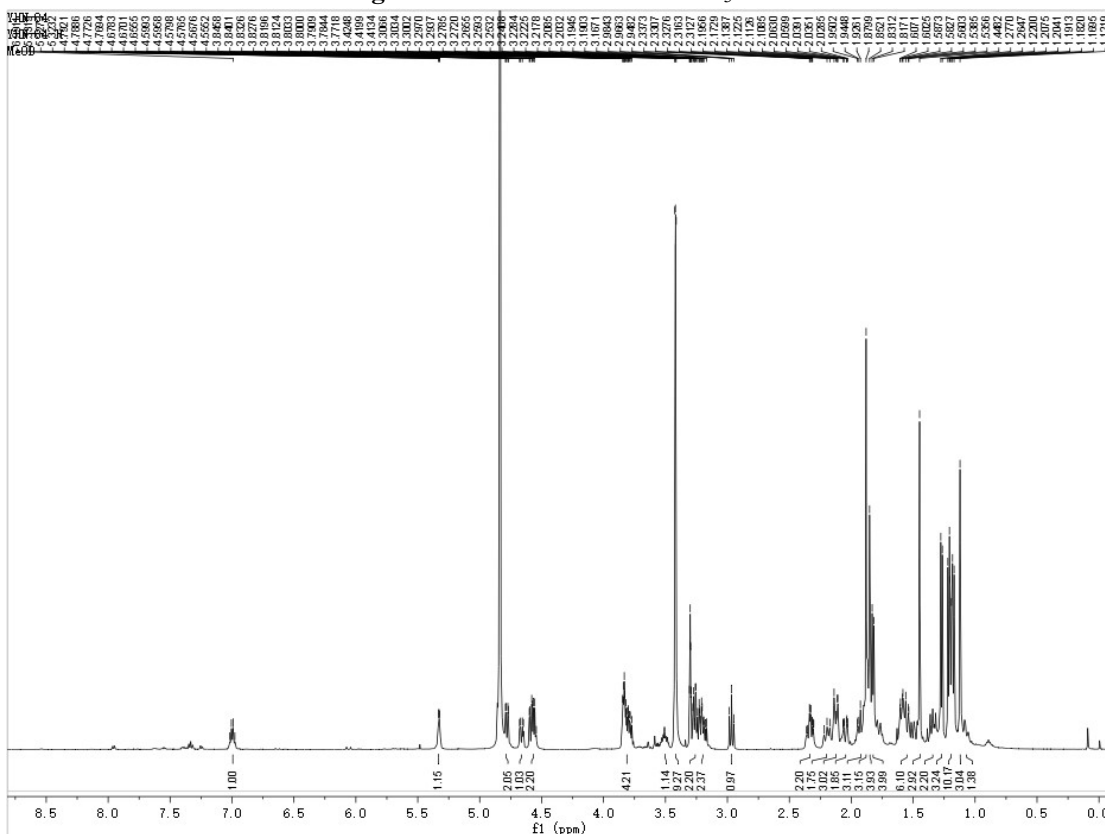


Figure S92. ¹H NMR spectrum (500 MHz) of 14 in CD₃OD

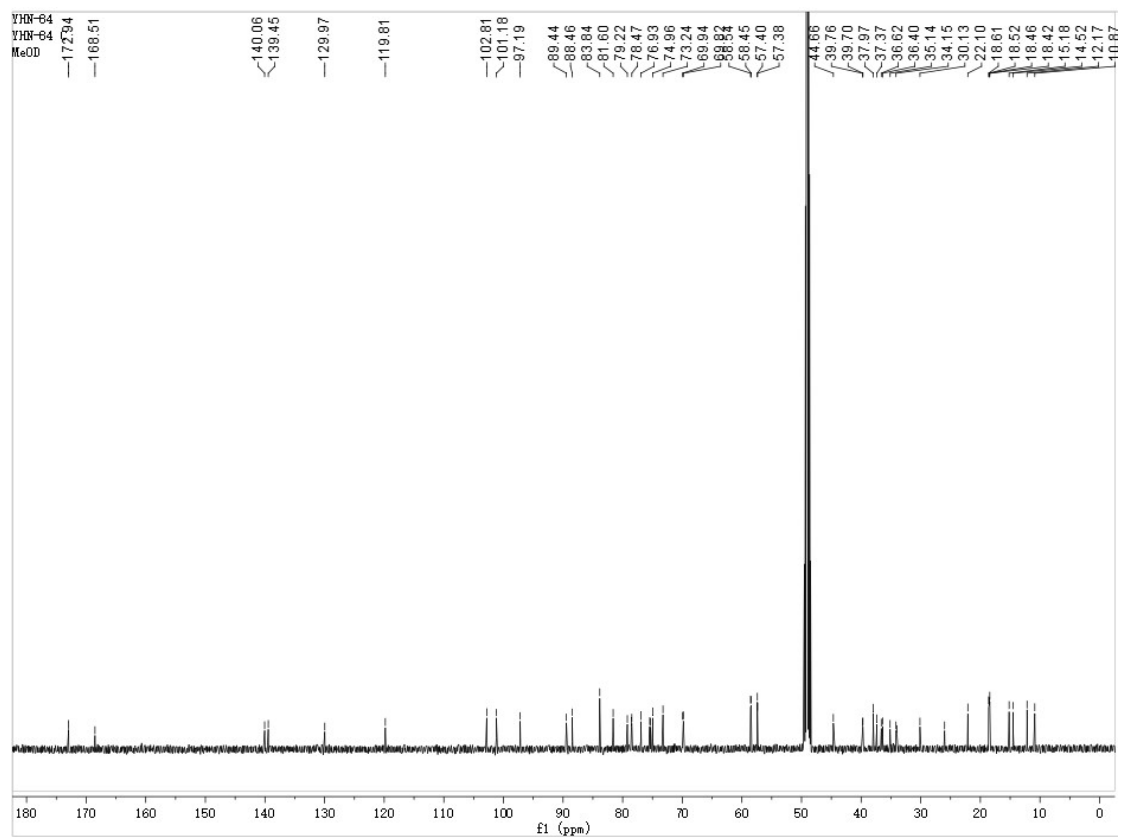


Figure S93. ^{13}C NMR spectrum (125 MHz) of **14** in CD_3OD

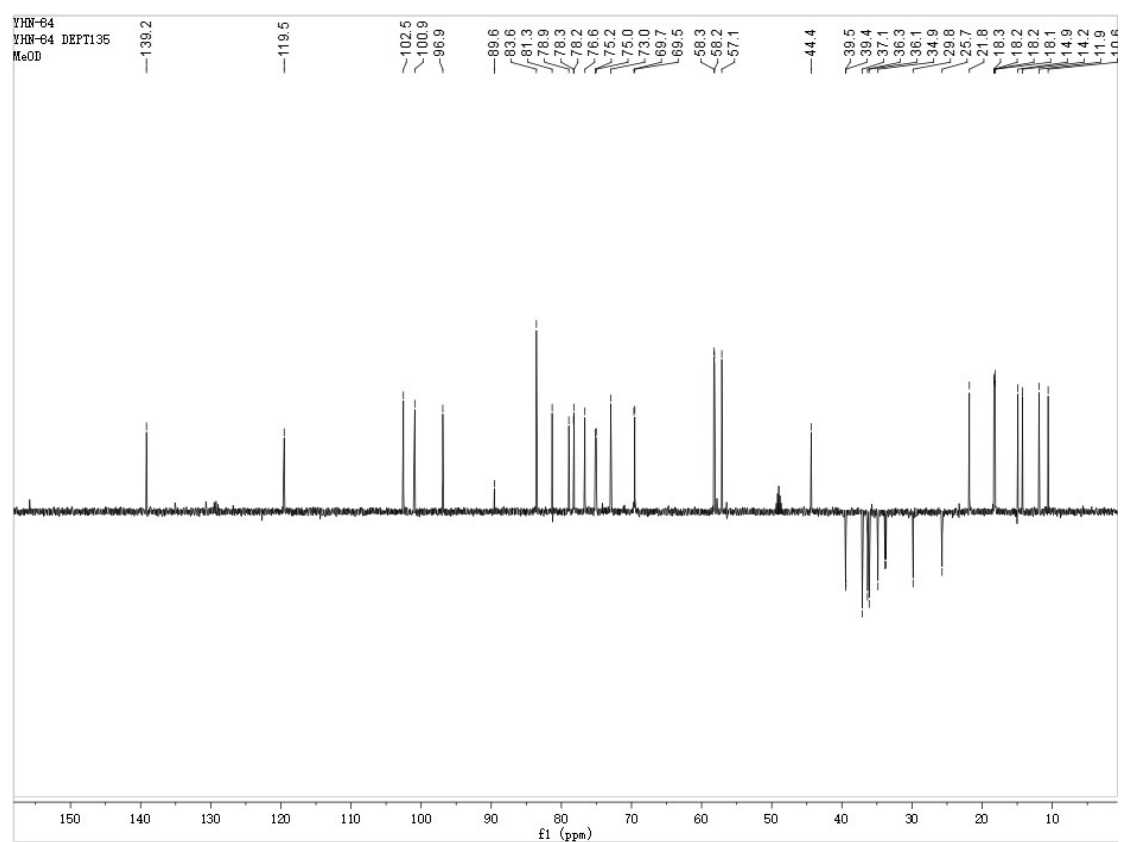


Figure S94. DEPT spectrum of **14** in CD_3OD

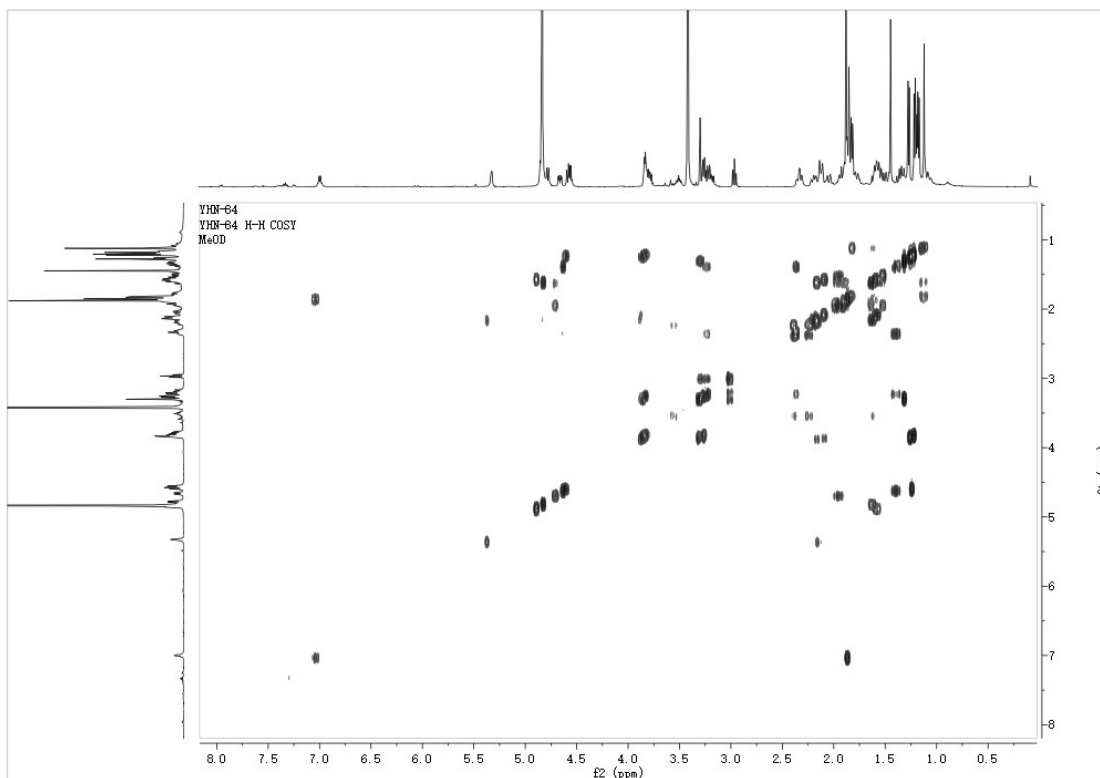


Figure S95. ^1H - ^1H COSY of **14** in CD_3OD

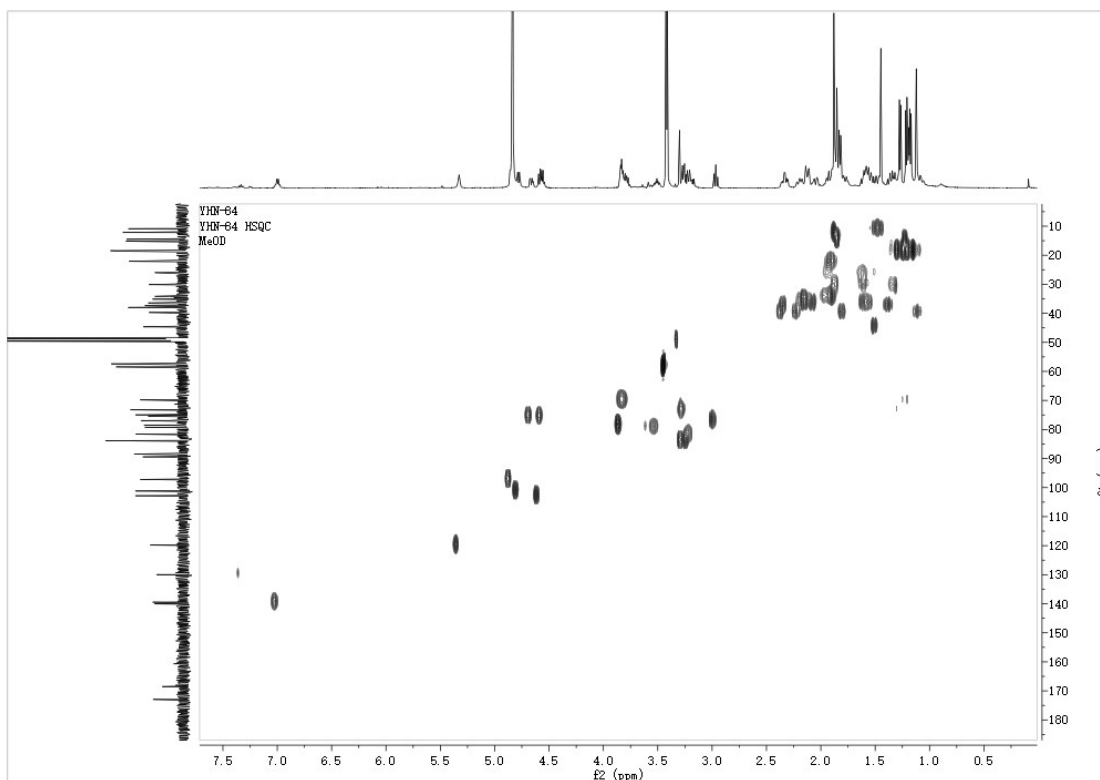


Figure S96. HSQC of **14** in CD_3OD

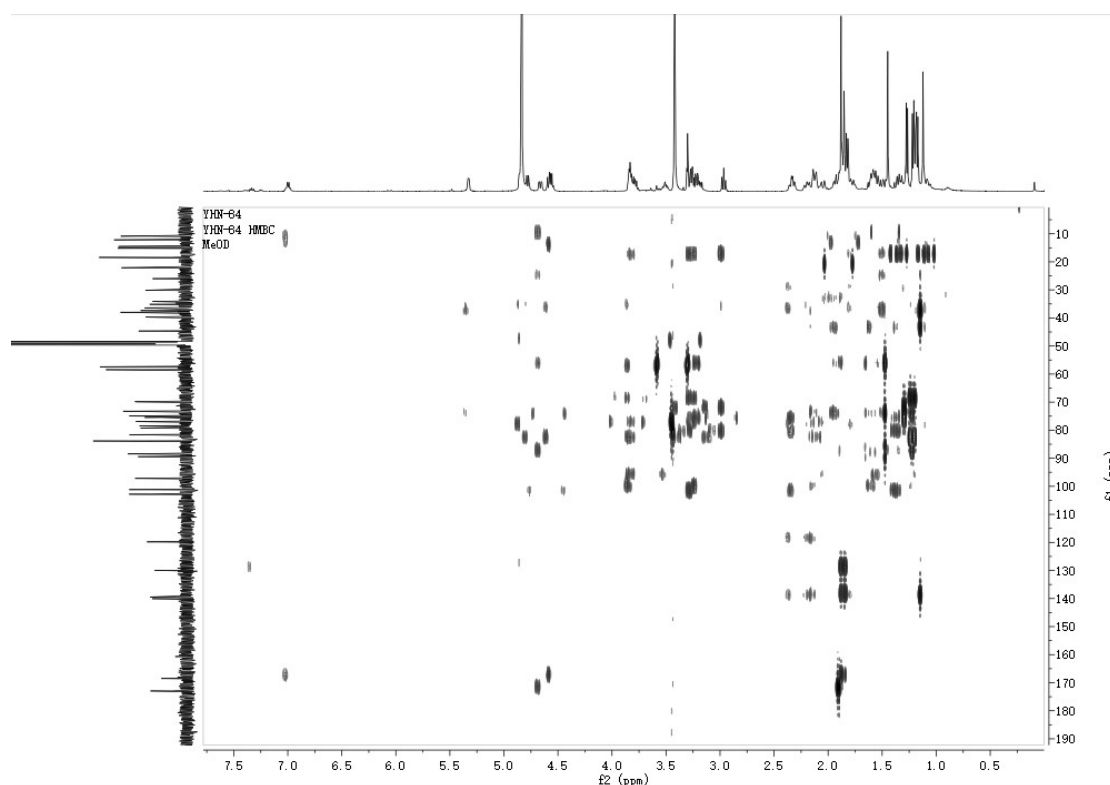


Figure S97. HMBC of 14 in CD₃OD

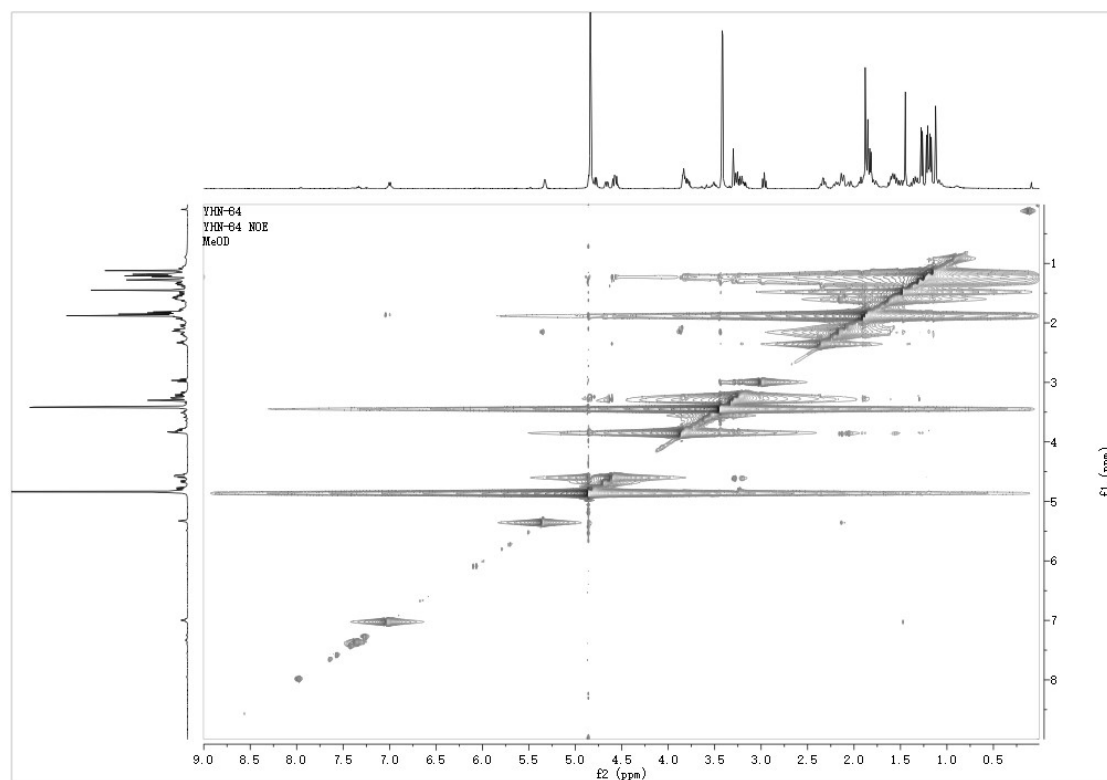


Figure S98. NOESY of 14 in CD₃OD