

Furostanol Saponins from *Trillium tschonoskii* Promote the Expansion of Human Cord Blood Hematopoietic Stem and Progenitor Cells

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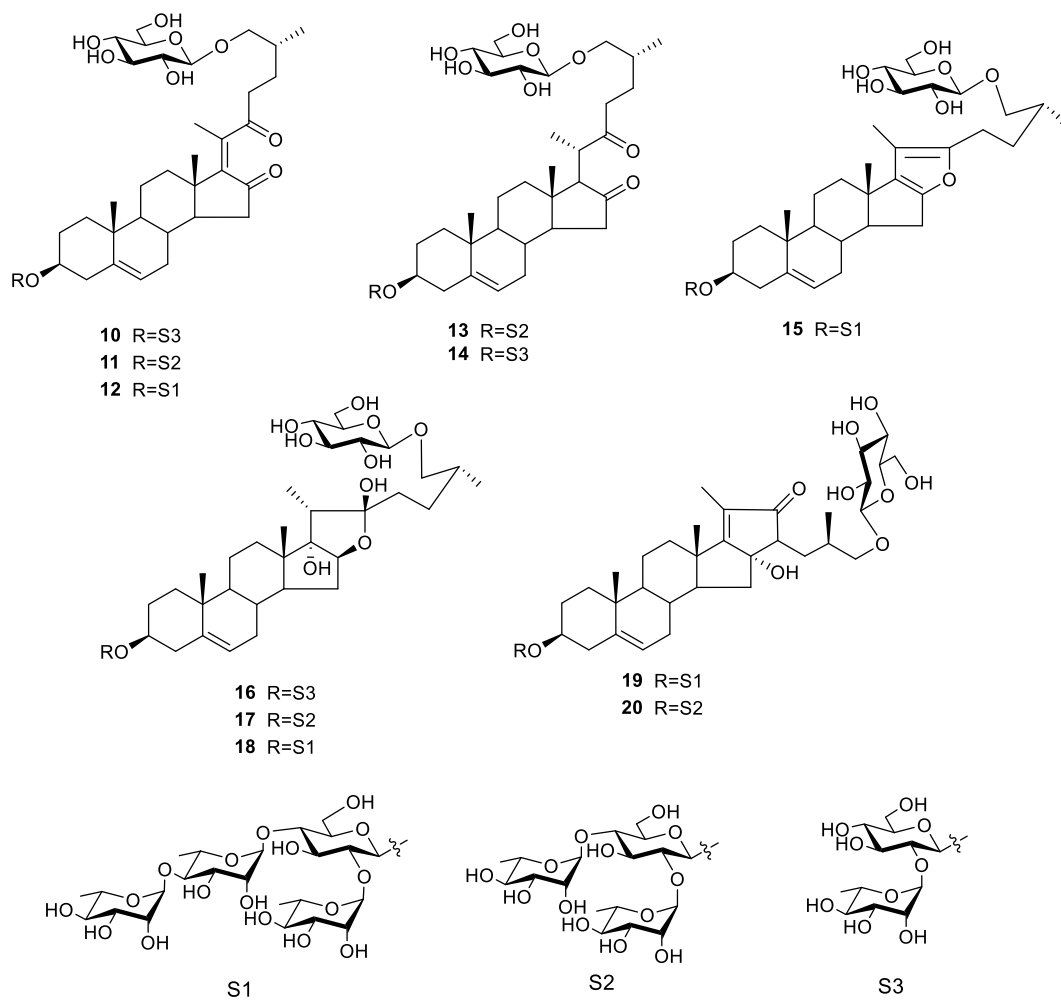
[#]These authors contributed equally to this work

The details of extraction and isolation.

5.0 kg of *T. tschonoskii* rhizomes were crushed and extracted with 50% aq. EtOH at reflux. (Three times, each for 2 hrs.). The filtered solution was concentrated and centrifuged to get the supernatants and sediments. The supernatants were subjected to a SP825 macroporous resin column, eluted with EtOH-H₂O (5:95→30:70→50:50→75:25→95:5) to yield five fractions (Fr.A~Fr.E). Fr.C (120.0 g) was subjected to silica-gel CC with CHCl₃-MeOH-H₂O (5:1:0.1→2:1:0.1) as the eluent, and five fractions (Fr.C1~Fr.C5) were obtained. Fr.C3 (35.0 g) was further subjected to MCI CC with (CH₃)₂CO-H₂O (10:90→50:50) as the eluent. As a result, a total of thirty fractions were collected (Fr.C3/1~Fr.C3/30). Fr.C3/6 was separated by preparative HPLC (pHPLC) with CH₃CN-H₂O (20:80) to give seven subfractions (Fr.C3/6-1~Fr.C3/6-7). Fr.C3/7~9 was separated by preparative HPLC (pHPLC) with CH₃CN-H₂O (20:80) to yield three fractions (Fr.C3/7~9/1~Fr.C3/7~9/3). Fr.C3/10~11 was separated by pHPLC with CH₃CN-H₂O (22:78) to yield five fractions (Fr.C3/10~11/1~Fr.C3/10~11/5). Fr.C3/12 was separated by pHPLC with CH₃CN-H₂O (22:78) to give five subfractions (Fr.C3/12/1~Fr.C3/12/5). Fr.C3/13~14 was separated by pHPLC with CH₃CN-H₂O (22:78) to yield five fractions (Fr.C3/13~14/1~Fr.C3/13~14/5). Fr.C3/6/3, Fr.C3/7~9/3, Fr.C3/10~11/3, Fr.C3/12/3 and Fr.C3/13~14/3 were separated by pHPLC with (CH₃)₂CO-H₂O (22:78) to give **11** (47.0 mg). Fr.C3/6~4/6, Fr.C3/10~11/4, Fr.C3/12/2, and Fr.C3/13~14/2 were separated by pHPLC with (CH₃)₂CO-H₂O (22:78) to give **16** (303.0 mg) and **17** (327.0 mg). Fr.C3/12/4 and Fr.C3/13~14/4 were separated by pHPLC with (CH₃)₂CO-H₂O (24:76) to give **12** (57.0 mg). Fr.C3/10~11/5, Fr.C3/12/5 and Fr.C3/13~14/5 were separated by pHPLC with (CH₃)₂CO-H₂O (24:76) to give **10** (42.0 mg). Fr.C4 (35.0 g) was subjected to a MCI gel column eluted with (CH₃)₂CO-H₂O (10:90→15:85→20:80→30:70→50:50) to afford nine fractions (Fr.C4/1~Fr.C4/9). Fr.C4/1 was separated by pHPLC with CH₃CN-H₂O (20:80) to give six fractions (Fr.C4/1/1~Fr.C4/1/6). Fr.C4/2 was separated by pHPLC with CH₃CN-H₂O (v/v, 20:80) to give six fractions (Fr.C4/2/1~Fr.C4/2/6). Fr.C4/3 was

separated by pHPLC with CH₃CN-H₂O (21:79) to give three fractions (Fr.C4/3/1~Fr.C4/3/1), Fr.C4/4 was separated by pHPLC with CH₃CN-H₂O (22:78) to give four fractions (Fr.C4/4/1~Fr.C4/4/4). Fr.C4/5 was separated by pHPLC with CH₃CN-H₂O (v/v, 22:78) to give five fractions (Fr.C4/5/1~Fr.C4/5/5). Fr.C4/6 was separated by pHPLC with CH₃CN-H₂O (23:77) to give five fractions (Fr.C4/6/1~Fr.C4/6/5). Fr.C4/1/6, Fr.C4/2/6 and Fr.C4/3/2 were separated by pHPLC with (CH₃)₂CO-H₂O (25:75) to give **2** (10.0 mg). Fr.C4/3/3, Fr.C4/4/4, Fr.C4/5/4 and Fr.C4/6/5 were separated by preparative HPLC with (CH₃)₂CO-H₂O (26:74) to give **18** (25.0 mg). Fr.C5 (35.0 g) was subjected to C₁₈ column with (CH₃)₂CO-H₂O (24:76) to afford six fractions (Fr.C5/1~Fr.C5/6). Fr.C5/1 was separated by pHPLC with CH₃CN-H₂O (16:84) to give five fractions (Fr.C5/1/1~Fr.C5/1/5). Fr.C5/2 was separated by pHPLC with (CH₃)₂CO-H₂O (22:78) to give four fractions (Fr.C5/2/1~Fr.C5/2/4). Fr.C5/3 was separated by pHPLC with (CH₃)₂CO-H₂O (22:78) to give five fractions (Fr.C5/3/1~Fr.C5/3/5). Fr.C5/4 was separated by pHPLC with (CH₃)₂CO-H₂O (22:78) to give five fractions (Fr.C5/4/1~Fr.C5/4/5). Fr.C5/5 was separated by pHPLC with CH₃CN-H₂O (20:80) to give six fractions (Fr.C5/5/1~Fr.C5/5/6). Fr.C5/6 was separated by pHPLC with CH₃CN-H₂O (22:78) to give seven fractions (Fr.C5/6/1~Fr.C5/6/7). Fr.C5/2/3, Fr.C5/3/3, Fr.C5/4/2 and Fr.C5/4/3 were separated by pHPLC with CH₃CN-H₂O (18:82) to give **1** (9.2 mg), **4** (14.6 mg) and **13** (141.0 mg). Fr.C5/2/4, Fr.C5/3/5 and Fr.C5/4/5 were separated by pHPLC with CH₃CN-H₂O (20:80) to give **7** (11.9 mg), **8** (4.8 mg), **9** (11.9 mg), **19** (12.0 mg). Fr.C5/5/2, Fr.C5/5/3 and Fr.C5/6/2 were separated by preparative HPLC with (CH₃)₂CO-H₂O (24:76) to give **3** (6.0 mg). The sediments (133.0 g) and Fr.D (95.0 g) were subjected to ODS column with CH₃CN-H₂O (45:55) to afford a mix fraction (Fr.F). Fr.F was subjected to ODS column with (CH₃)₂CO-H₂O (v/v, 40:60) to afford a five fractions (Fr.F/1~Fr.F/5). Fr.F/2 was separated by preparative HPLC with CH₃CN-H₂O (22:78) to give five fractions (Fr.F/2/1~Fr.F/2/5). Fr.F/3 was separated by pHPLC with CH₃CN-H₂O (24:76) to give six fractions (Fr.F/3/1~Fr.F/3/6). Fr.F/4 was separated by pHPLC with CH₃CN-H₂O (26:74) to give six fractions (Fr.F/4/1~Fr.F/4/6). Fr.F/2/2, Fr.F/2/3 and Fr.F/3/1 were separated by preparative

HPLC with (CH₃)₂CO-H₂O (25:75) to give **14** (40.0 mg) and **20** (34.0 mg). Fr.F/2/5, Fr.F/3/2, Fr.F/3/4 and Fr.F/4/1 were separated by pHPLC with (CH₃)₂CO-H₂O (29:71) to give **5** (122.0 mg), **6** (141.0 mg), **15** (14.5 mg).



Structures of the known compounds **10-20**.

¹³C NMR data of known compounds **10-20** (recorded in pyridine-d₅; δ in ppm).

Position	10	11	12	13	14	15	16	17	18	19	20
1	37.2	37.2	37.2	37.2	37.2	37.4	37.6	37.6	37.6	37.6	37.6
2	30.2	30.1	30.1	30.1	30.2	30.2	30.2	30.2	30.2	30.2	30.2
3	78.7	78.5	78.7	78.1	77.8	78.0	78.3	78.3	78.7	78.0	78.1
4	38.8	38.9	38.7	38.9	38.9	39.0	39.0	39.0	39.0	39.0	39.0
5	141.0	140.9	140.9	141.0	141.0	141.2	140.9	140.9	140.8	140.9	140.9
6	121.3	121.4	121.4	121.5	121.4	121.6	121.8	121.6	121.9	121.6	121.6
7	31.7	31.7	31.7	32.0	32.0	32.0	32.5	32.5	32.5	31.9	31.8
8	30.8	30.8	30.8	31.0	31.0	30.5	32.3	32.3	32.3	32.0	31.9
9	49.9	49.9	49.9	50.1	50.1	51.1	50.3	50.3	50.3	50.5	50.5
10	37.1	37.1	37.1	37.1	37.1	37.3	37.2	37.2	37.2	37.2	37.6
11	20.9	20.9	20.9	20.8	20.8	20.7	21.0	21.0	21.0	20.6	20.6
12	39.0	38.8	38.9	39.0	39.0	35.7	32.0	32.0	32.0	35.7	35.7
13	43.4	43.4	43.4	44.0	42.4	41.2	45.1	45.1	45.1	44.0	44.0
14	50.5	50.5	50.5	50.3	50.3	60.8	53.1	53.1	53.1	53.8	53.8
15	36.1	36.1	36.1	39.1	39.1	26.9	32.2	32.2	32.2	38.4	38.4
16	210.4	210.4	210.4	216.8	216.9	155.3	90.8	90.8	90.8	82.9	82.9
17	142.6	142.6	142.6	63.9	63.9	137.2	90.5	90.5	90.5	182.0	182.1
18	15.8	15.8	15.8	14.1	14.1	18.2	17.3	17.3	17.3	15.7	15.7
19	19.4	19.3	19.3	19.4	19.4	19.3	19.5	19.5	19.5	19.4	19.4
20	145.6	145.6	145.6	42.4	44.0	111.9	43.6	43.6	43.6	128.2	128.2
21	16.8	16.8	16.8	16.2	16.2	9.0	10.5	10.5	10.5	8.5	8.6
22	205.7	205.6	205.6	212.5	212.5	153.7	111.4	111.4	111.4	212.2	212.2
23	38.0	38.0	38.0	38.3	38.3	33.2	36.9	36.9	36.9	57.5	57.5
24	28.0	28.0	28.0	28.1	28.1	24.4	28.1	28.1	28.1	29.3	29.3
25	33.4	33.4	33.4	33.5	33.5	33.5	34.3	34.3	34.3	31.8	32.0
26	75.1	75.1	75.1	75.0	75.0	74.9	75.2	75.2	75.3	76.6	76.6
27	17.5	17.5	17.5	17.4	17.4	17.2	17.5	17.5	17.5	17.2	17.2
3'-O-Glc-1'	100.4	100.3	100.4	100.4	100.4	100.4	100.4	100.3	100.4	100.4	100.4
2'	79.7	78.0	80.4	78.0	79.7	77.8	79.7	78.6	80.4	77.8	78.0
3'	77.9	78.7	77.1	78.7	77.9	77.0	77.9	78.0	77.0	77.1	78.6
4'	71.9	77.8	78.0	77.8	71.9	78.1	71.7	78.1	78.1	77.8	77.8
5'	78.3	77.0	78.0	77.0	78.3	77.8	77.8	76.9	77.9	77.9	77.0
6'	62.7	61.4	61.3	61.3	62.7	61.3	62.9	61.3	61.2	61.3	61.3
2'-O-Rha-1''	102.1	102.0	102.2	102.0	102.1	102.3	102.1	102.0	102.2	102.2	102.1
2''	72.9	72.6	72.9	72.9	72.9	72.9	72.9	72.5	72.9	72.9	72.6
3''	72.6	72.8	72.7	72.8	72.6	72.7	72.6	72.8	72.7	72.7	72.8
4''	74.2	73.9	74.0	73.9	74.2	74.0	74.2	74.2	74.1	74.0	74.0
5''	69.5	70.4	70.4	70.4	69.5	70.4	69.5	69.5	70.4	70.4	69.6
6''	18.7	18.5	18.9	18.7	18.7	18.9	18.7	18.7	18.9	18.9	18.5
4'-O-Rha-1'''		102.9	103.3	102.9		103.3		102.9	103.3	103.3	103.0
2'''		72.5	73.3	72.5		73.3		72.6	73.3	73.3	72.6

3'''		72.9	72.9	72.6		72.9		72.8	72.9	72.9	72.9
4'''		74.2	77.8	74.2		80.4		73.9	77.8	80.4	74.2
5'''		69.5	68.4	69.5		68.4		70.4	68.4	68.4	70.5
6'''		18.7	18.7	18.5		18.7		18.5	18.7	18.7	18.7
4'''-O-Rha-1'''			102.3			102.3			102.3	102.3	
2'''			72.9			72.9			72.9	72.9	
3'''			72.5			72.5			72.5	72.5	
4'''			74.2			74.2			74.2	74.2	
5'''			69.5			69.6			69.6	69.6	
6'''			18.5			18.5			18.5	18.5	
26-O-Glc-1'''	104.9	104.9	104.9	104.9	104.9	104.9	105.0	105.1	105.0	105.3	105.3
2'''	75.3	75.2	75.2	75.2	75.2	75.2	75.2	75.2	75.2	75.3	75.3
3'''	78.5	78.7	78.5	78.6	78.6	78.7	78.5	78.7	78.6	78.5	78.5
4'''	71.8	71.7	71.7	71.8	71.8	71.8	71.8	71.7	71.8	71.7	71.7
5'''	78.5	78.0	78.7	78.7	78.7	78.5	78.6	78.5	78.5	78.6	78.6
6'''	62.9	62.9	62.9	62.9	62.9	62.9	62.7	62.9	62.9	62.8	62.8

NMR spectra of compounds 1-20

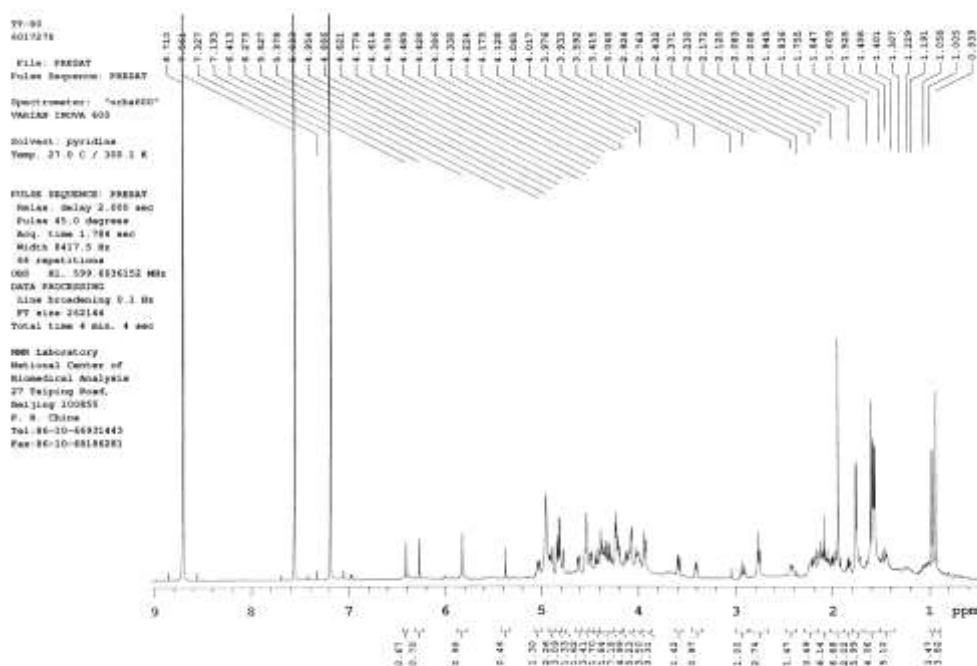


Figure S1. The ^1H NMR spectrum of compound **1**.

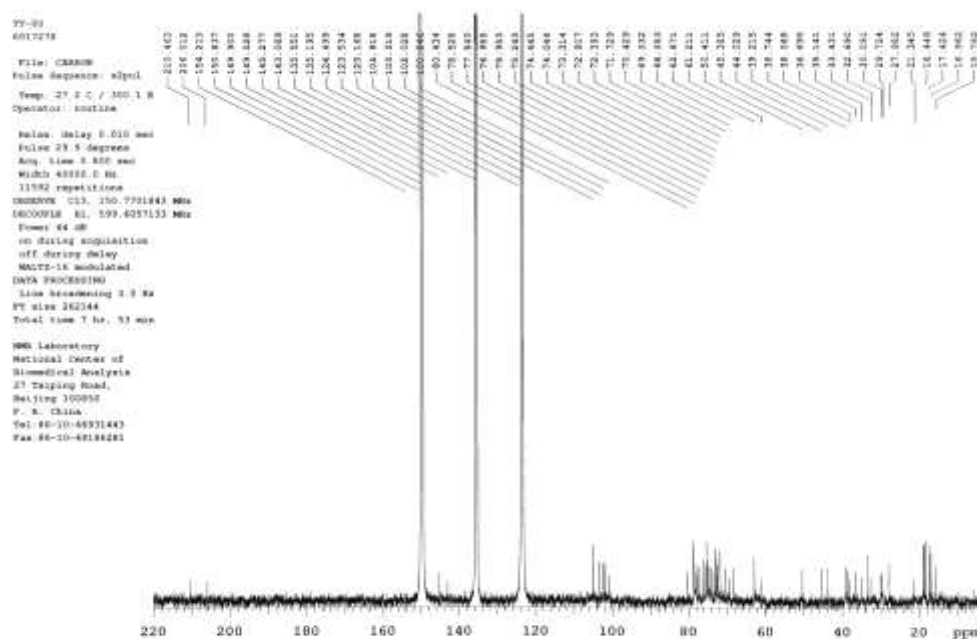


Figure S2. The ^{13}C NMR spectrum of compound **1**.

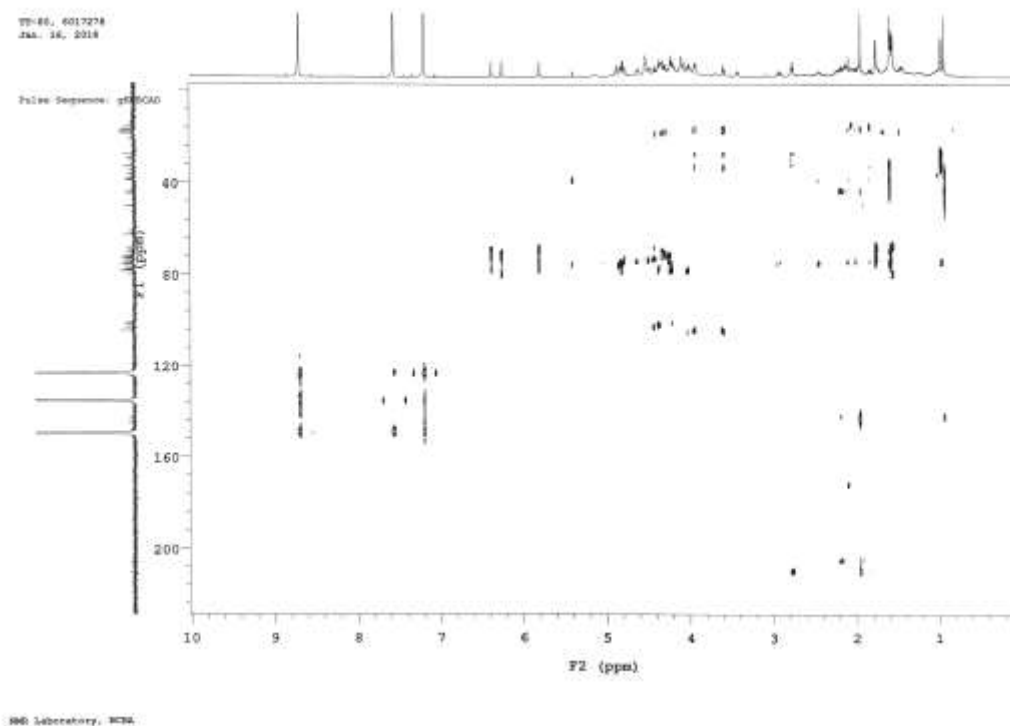


Figure S5. The HMBC spectrum of compound **1**.

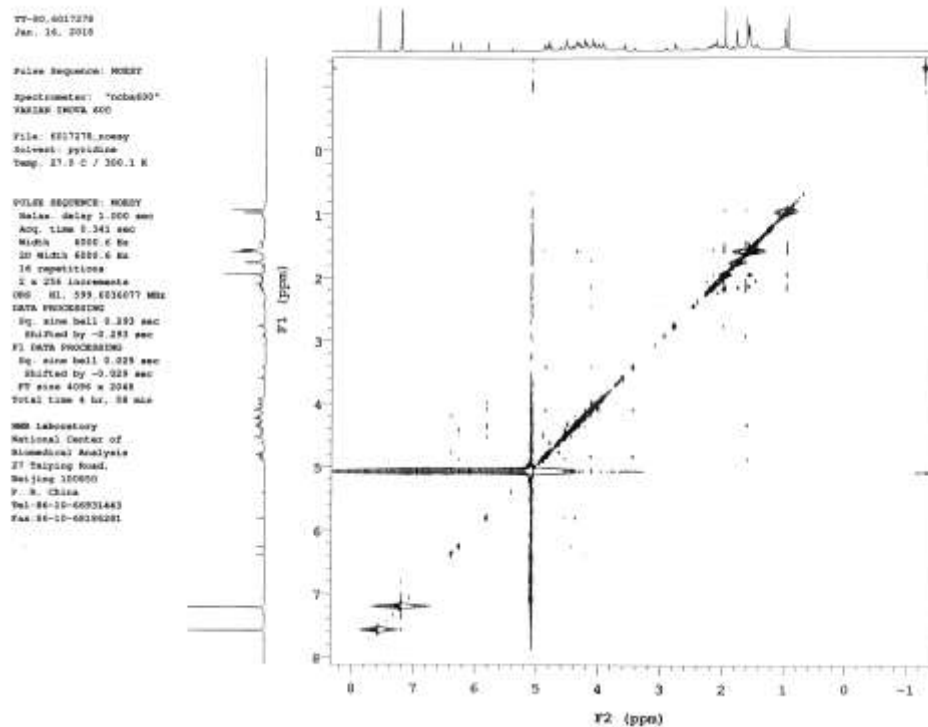


Figure S6. The NOESY of compound **1**.

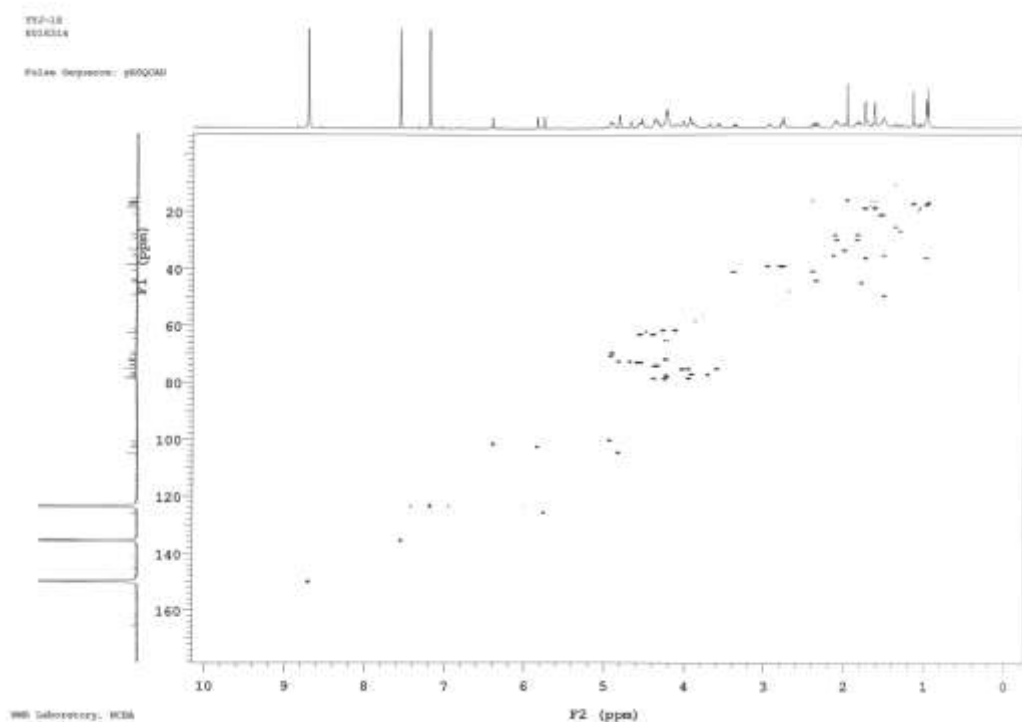


Figure S9. The HSQC spectrum of compound **2**.

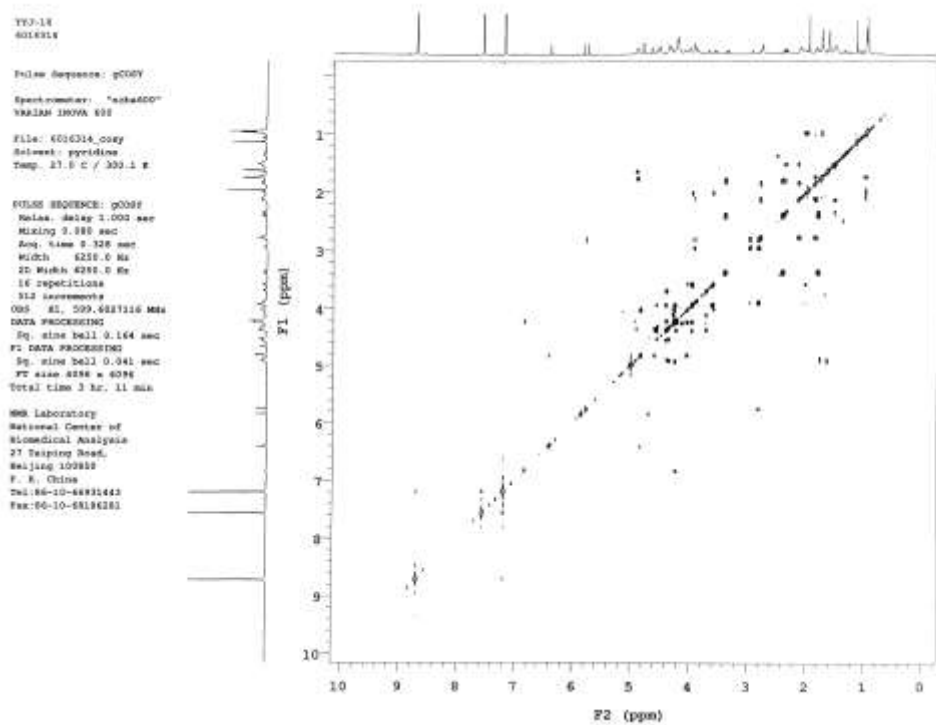


Figure S10. The ^1H - ^1H COSY of compound **2**.

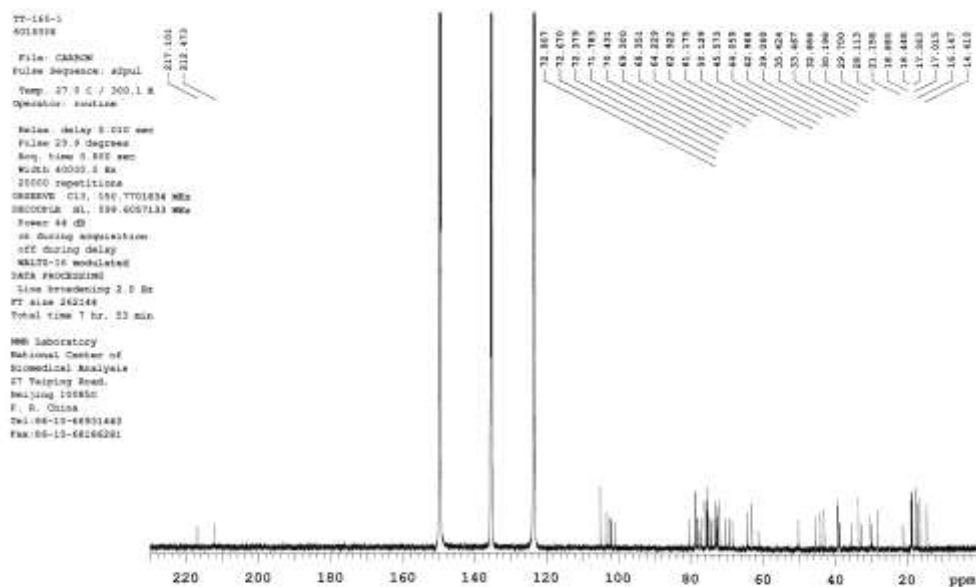


Figure S13. The ^{13}C NMR spectrum of compound **3**.

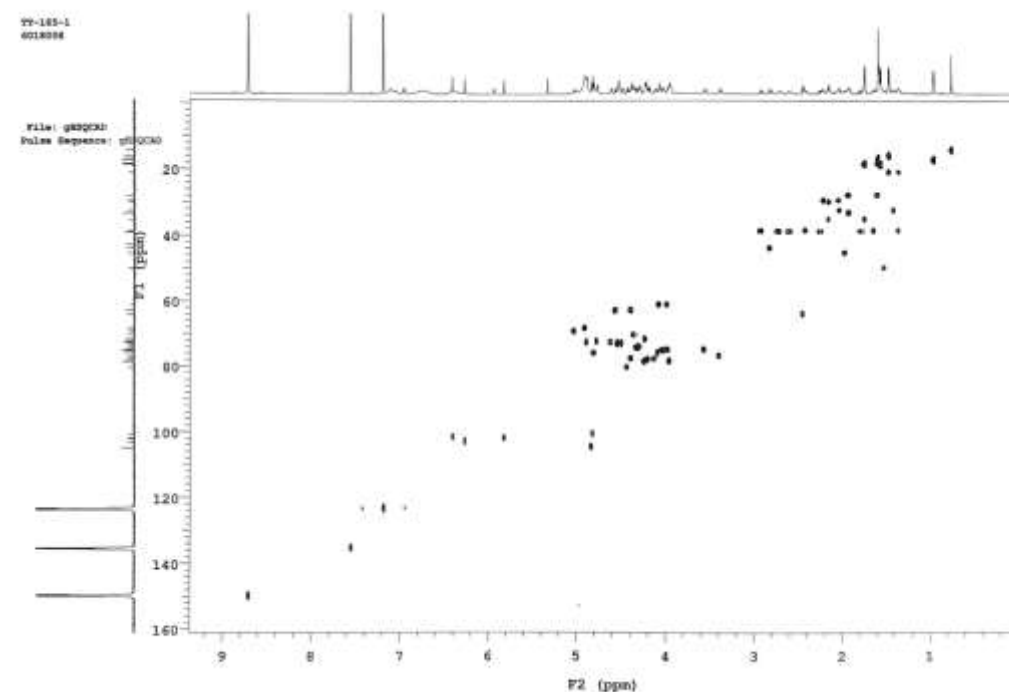


Figure S14. The HSQC spectrum of compound **3**.

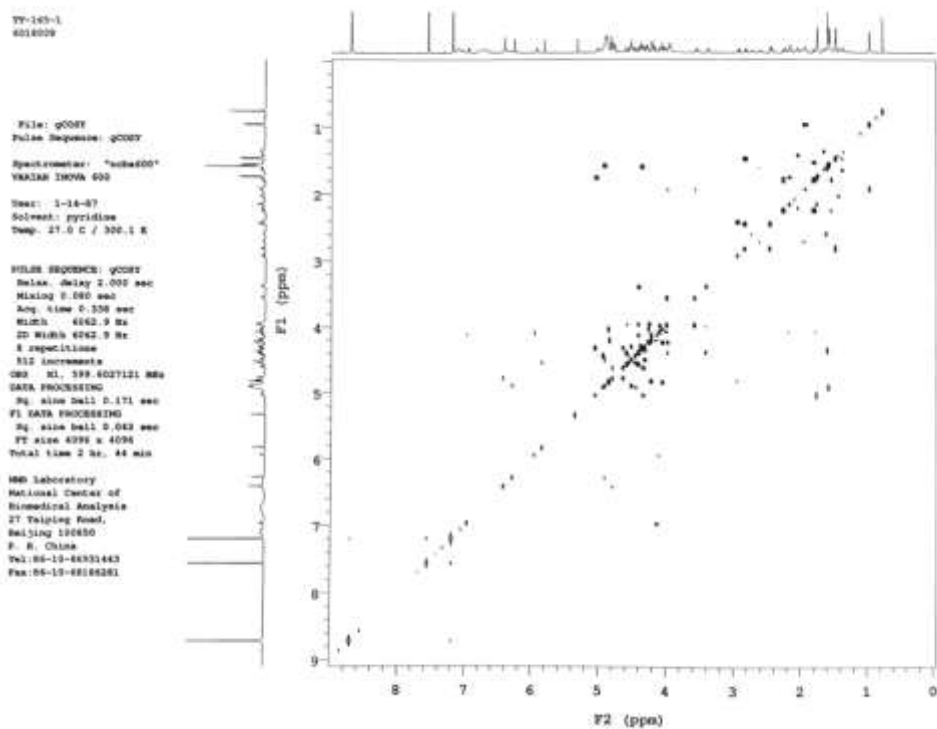


Figure S15. The ^1H - ^1H COSY of compound **3**.

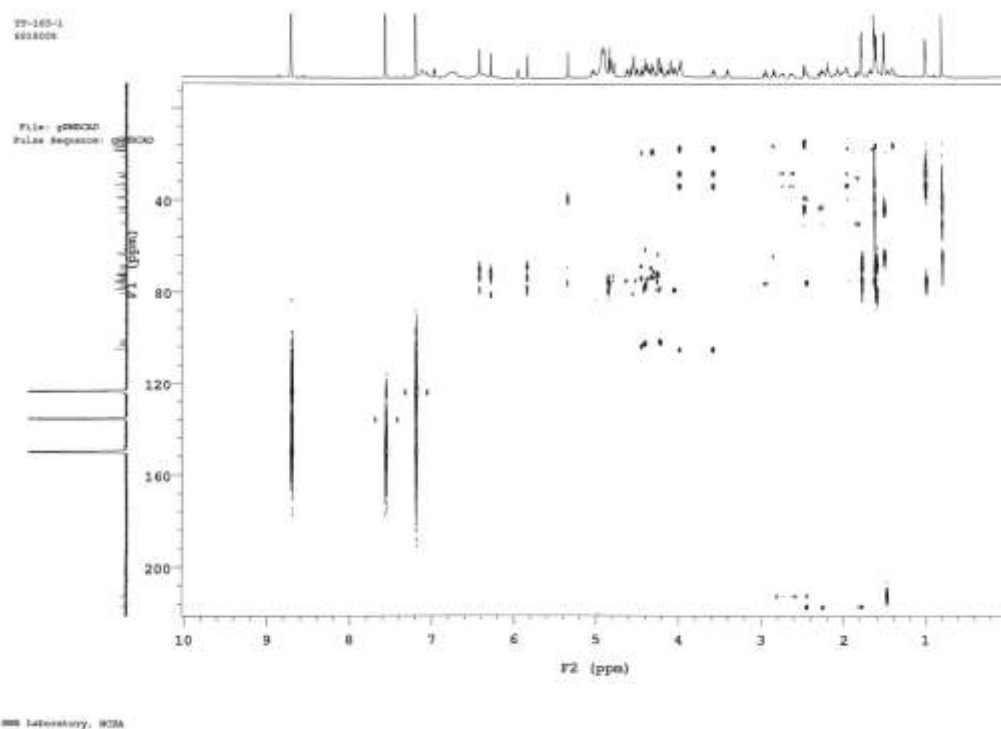


Figure S16. The HMBC spectrum of compound **3**.

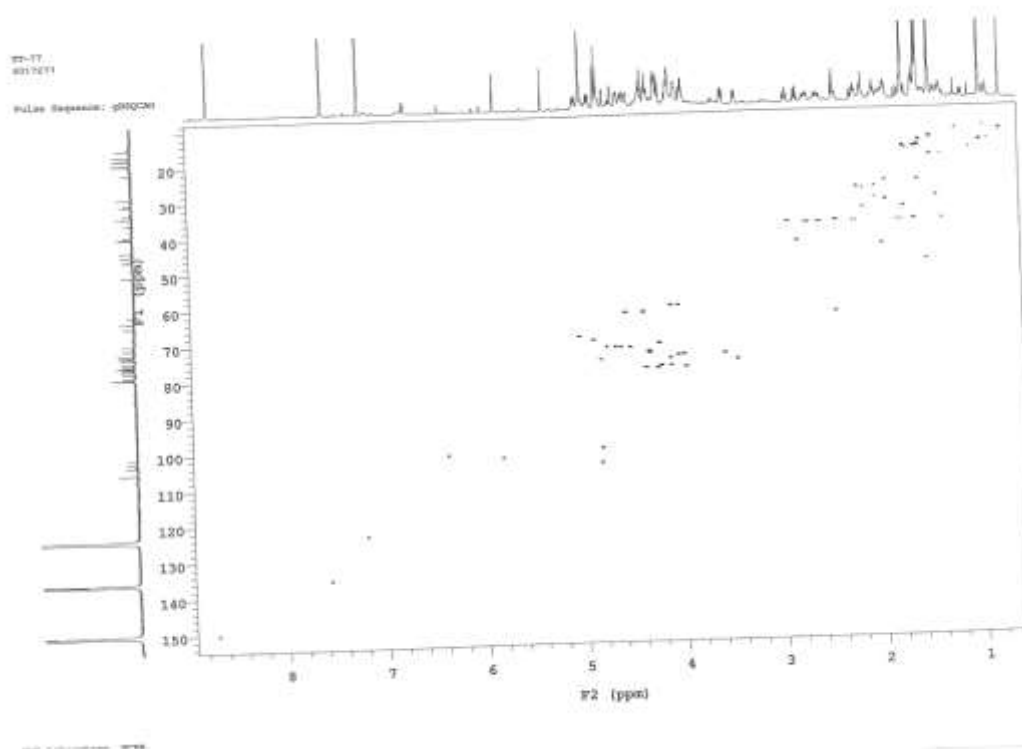


Figure S19. The HSQC spectrum of compound **4**.

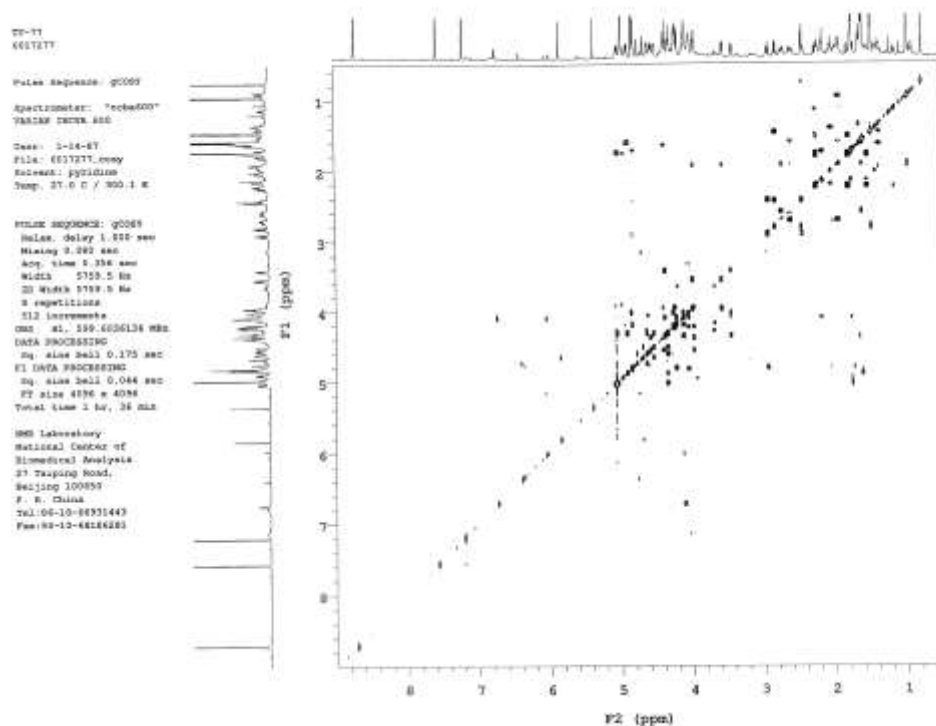


Figure S20. The ^1H - ^1H COSY of compound **4**.

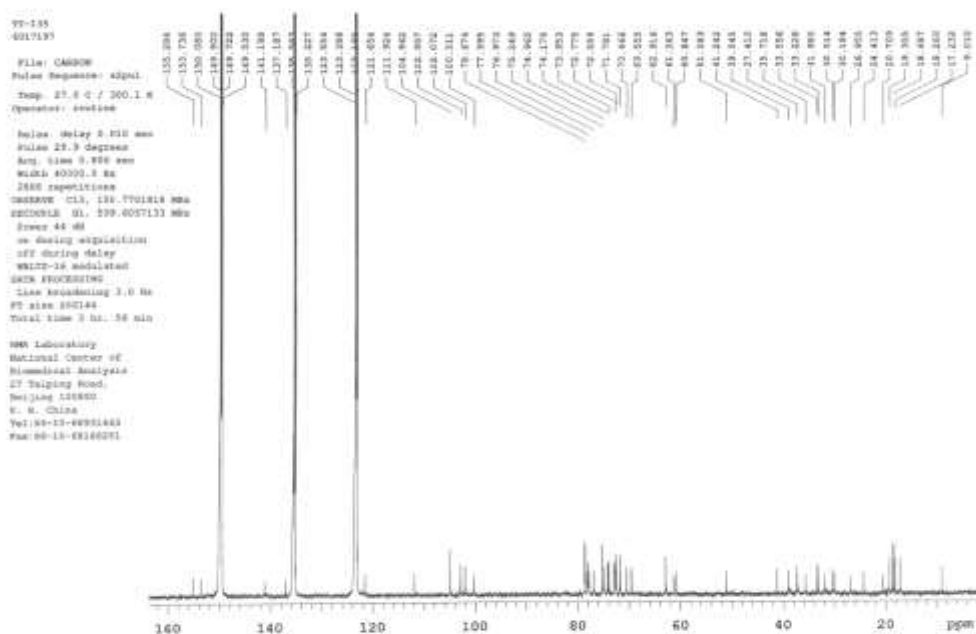


Figure S23. The ^{13}C NMR spectrum of compound **5**.

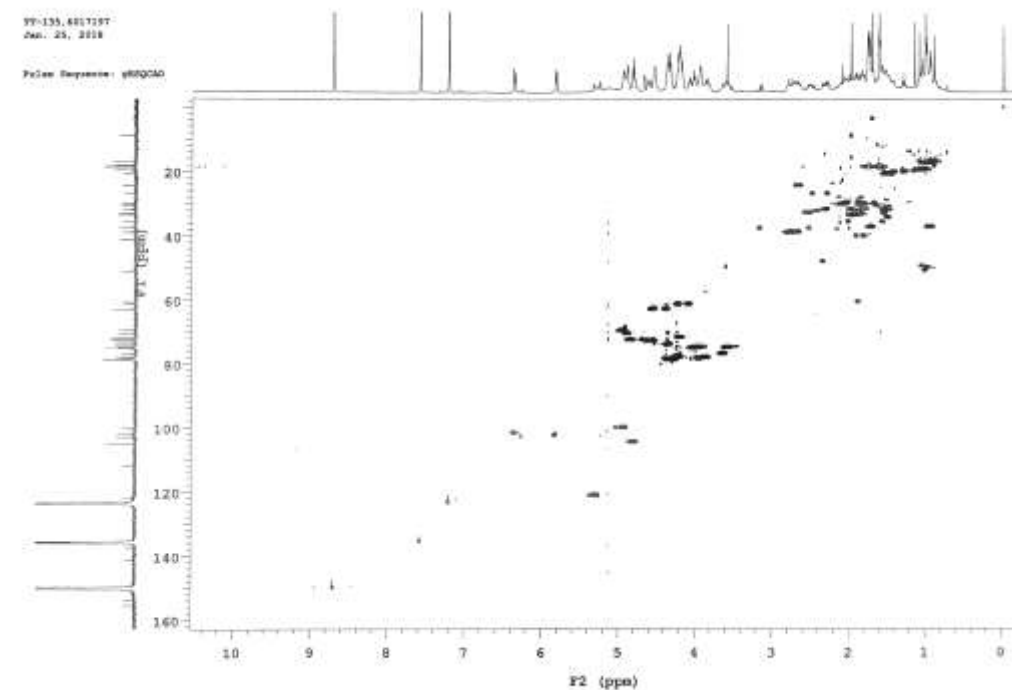


Figure S24. The HSQC spectrum of compound **5**.

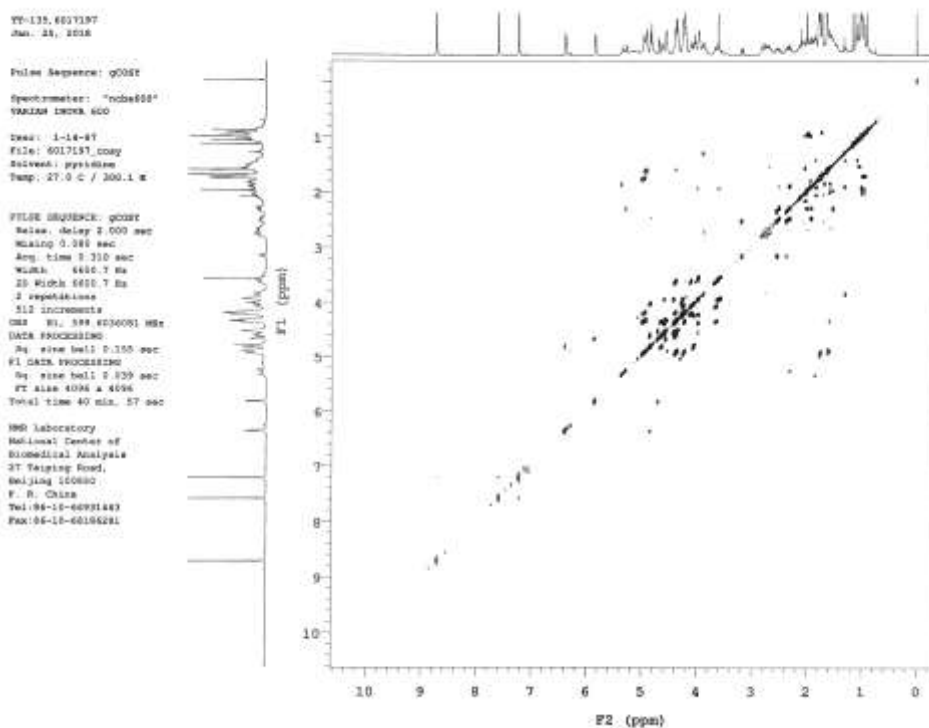


Figure S25. The ^1H - ^1H -COSY of compound **5**.

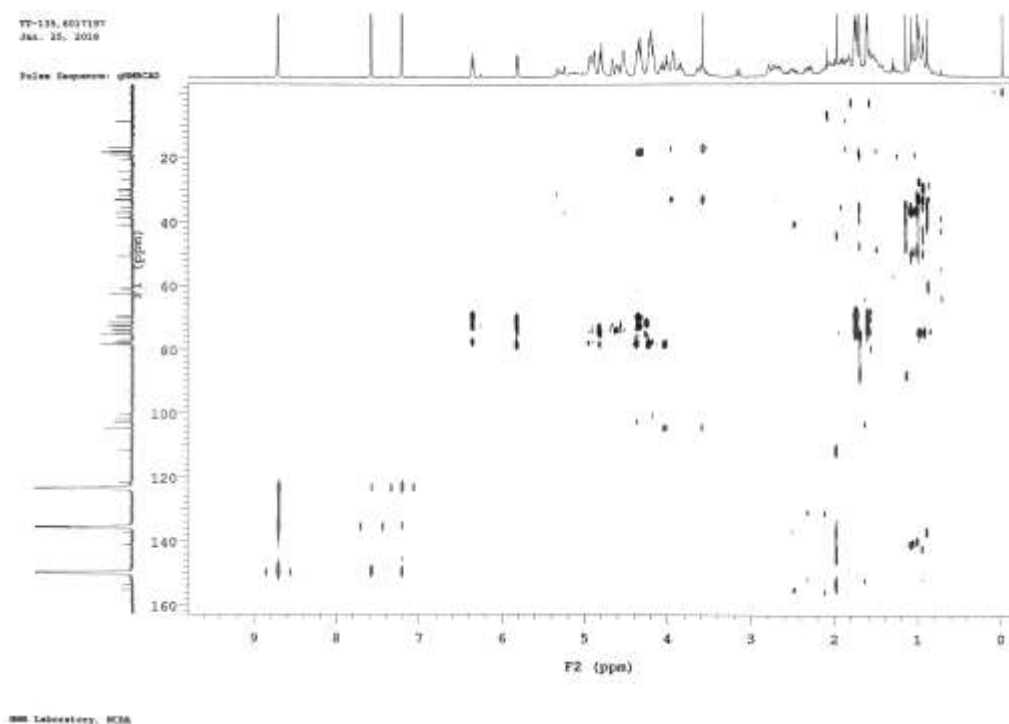


Figure S26. The HMBC spectrum of compound **5**.

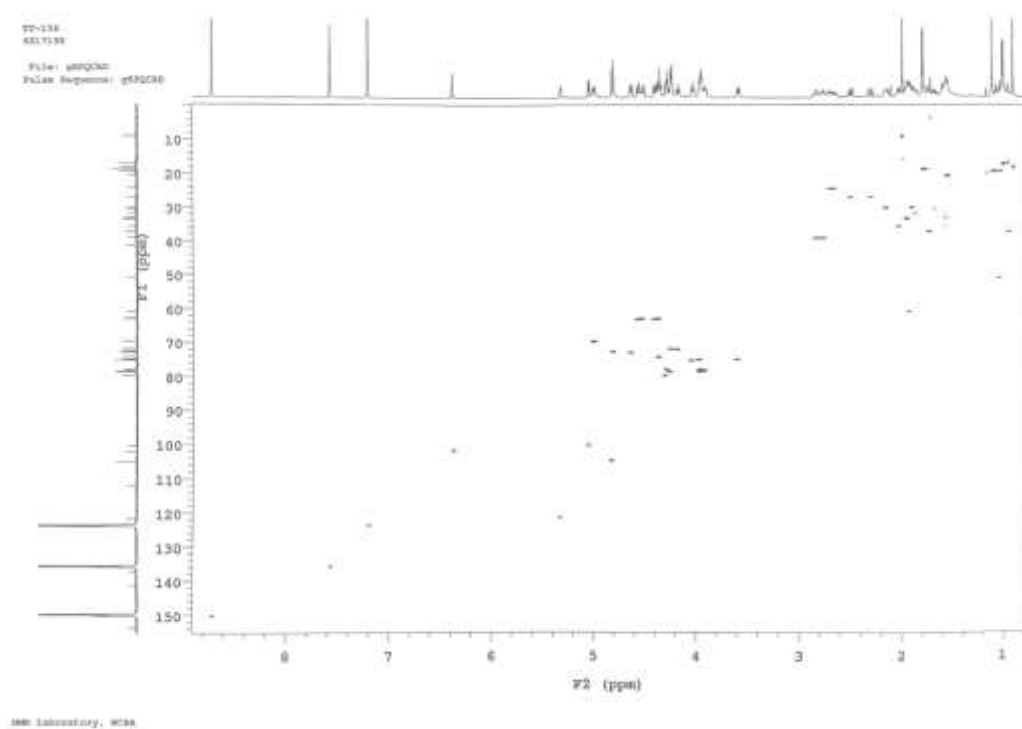


Figure S29. The HSQC spectrum of compound **6**.

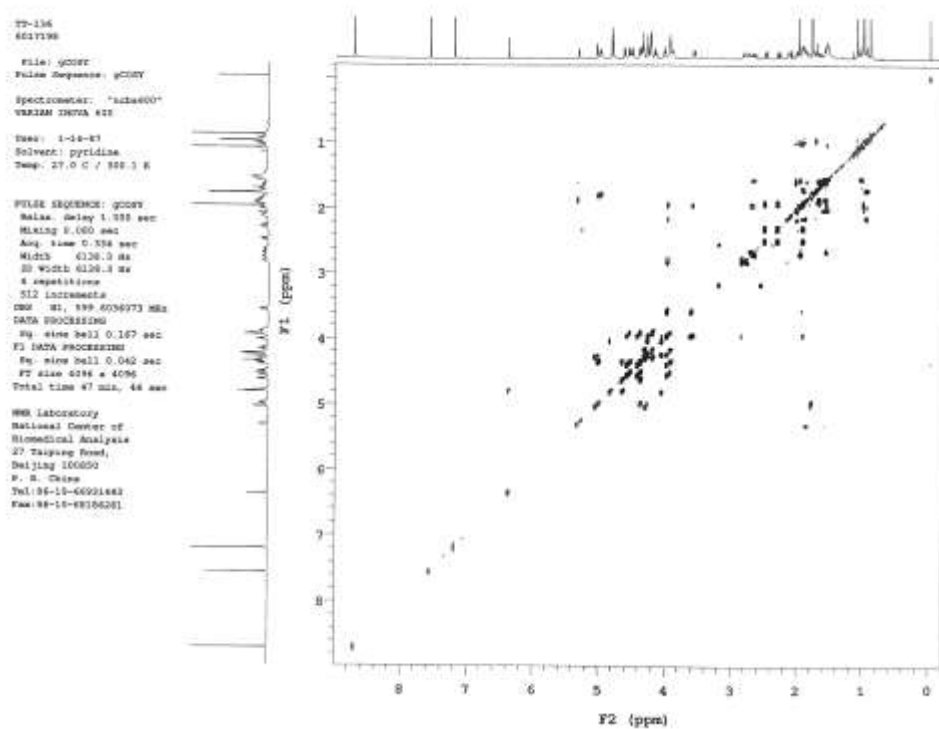


Figure S30. The ^1H - ^1H COSY spectrum of compound **6**.

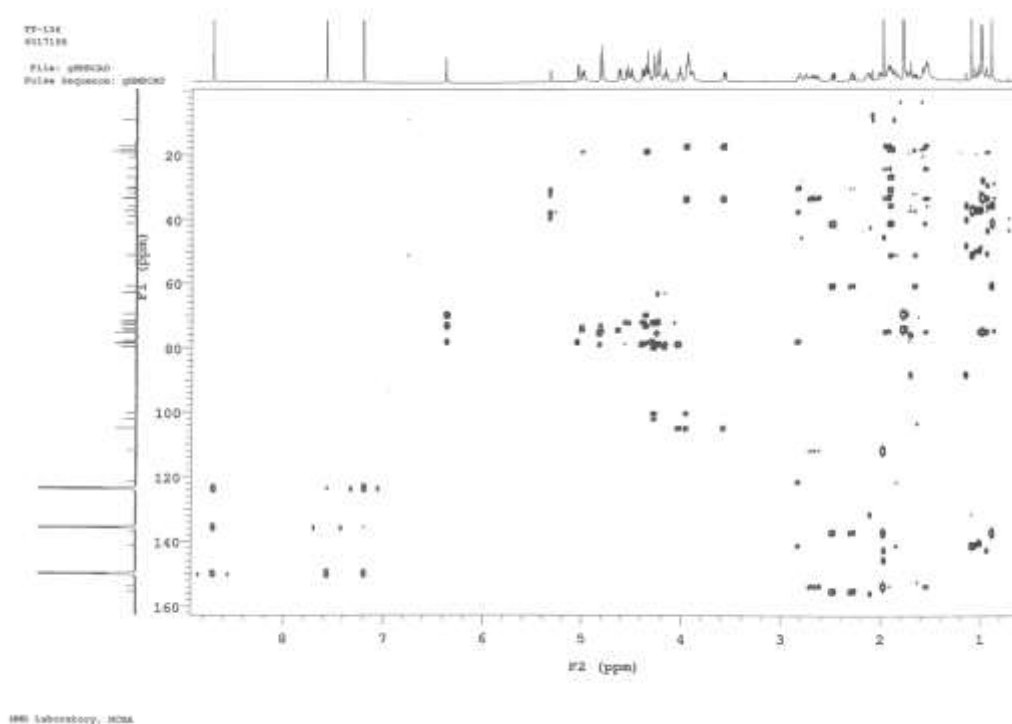


Figure S31. The HMBC spectrum of compound **6**.

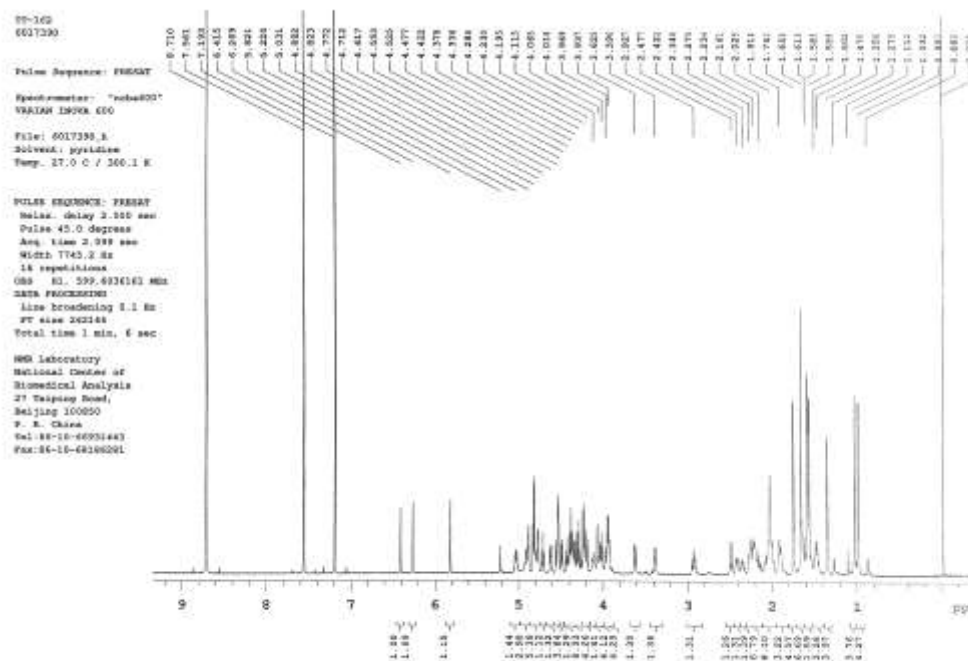


Figure S32. The ^1H NMR spectrum of compound **7**.

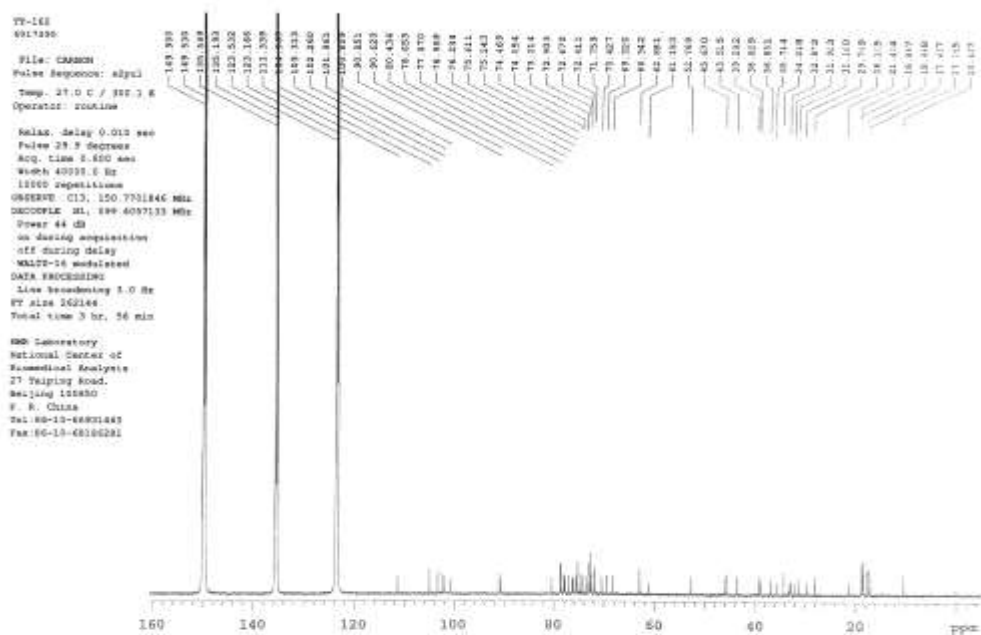


Figure S33. The ^{13}C NMR spectrum of compound **7**.

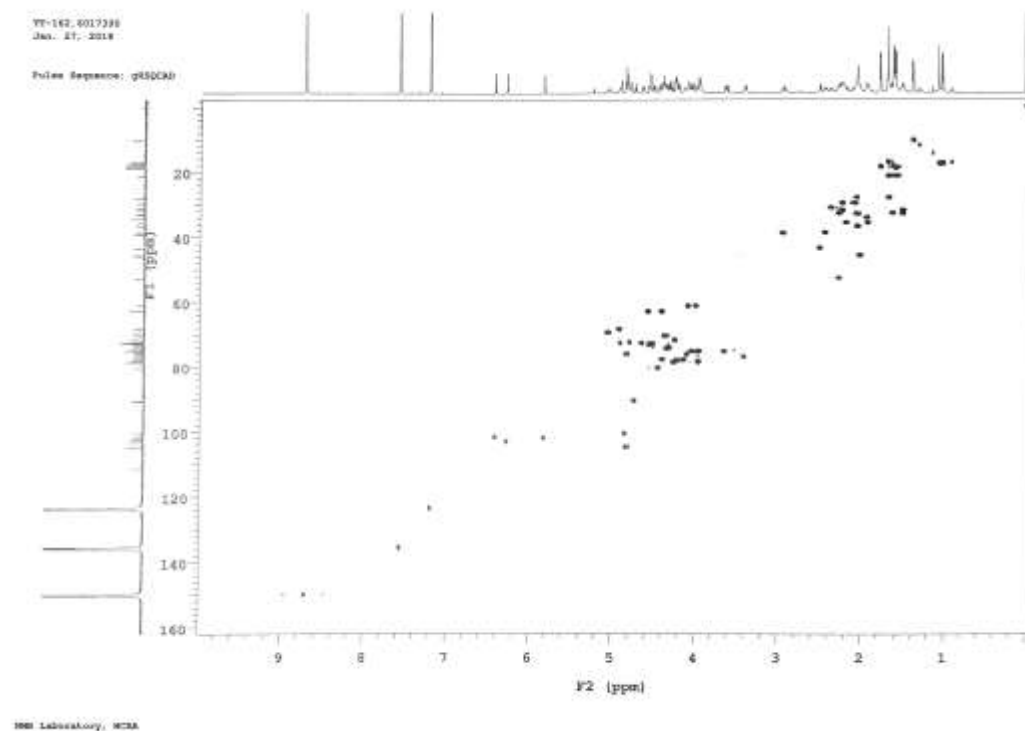


Figure S34. The HSQC spectrum of compound **7**.

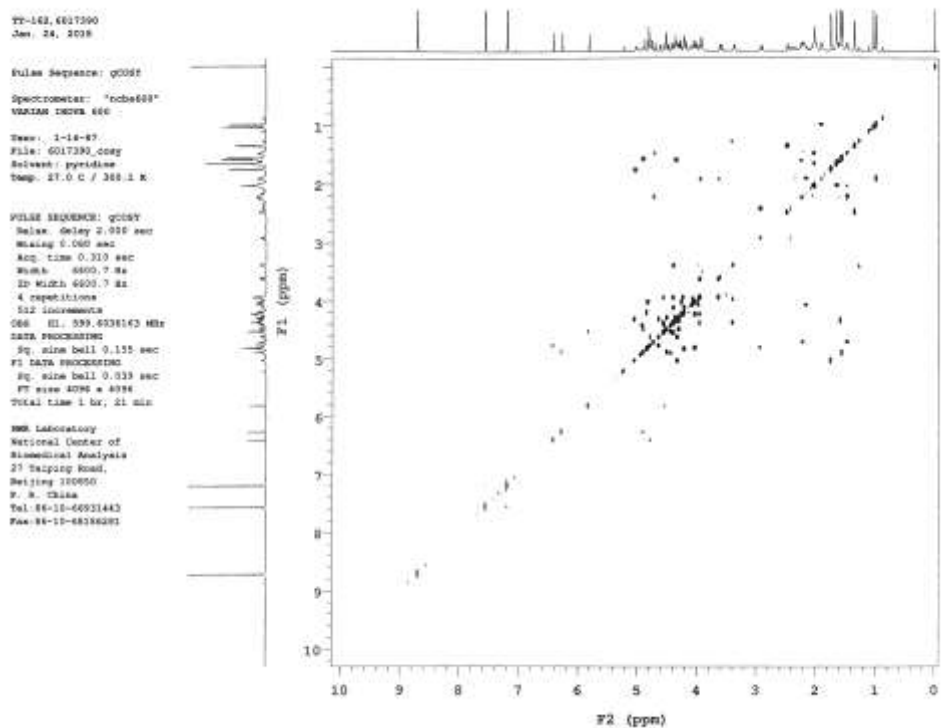


Figure S35. The ^1H - ^1H COSY spectrum of compound **7**.

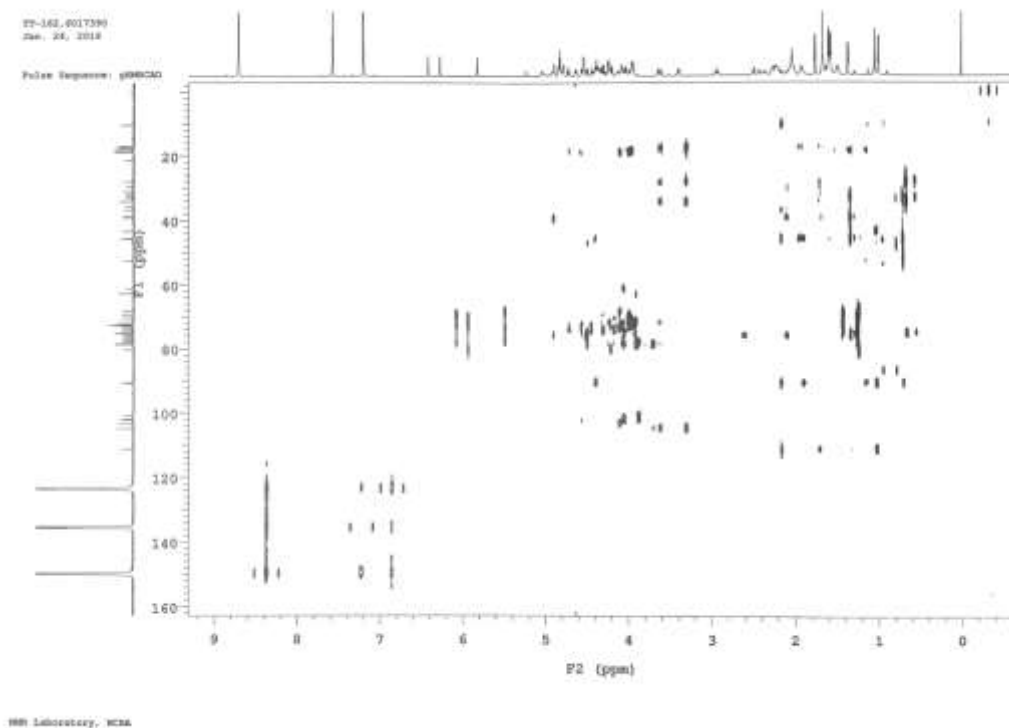


Figure S36. The HMBC spectrum of compound **7**.

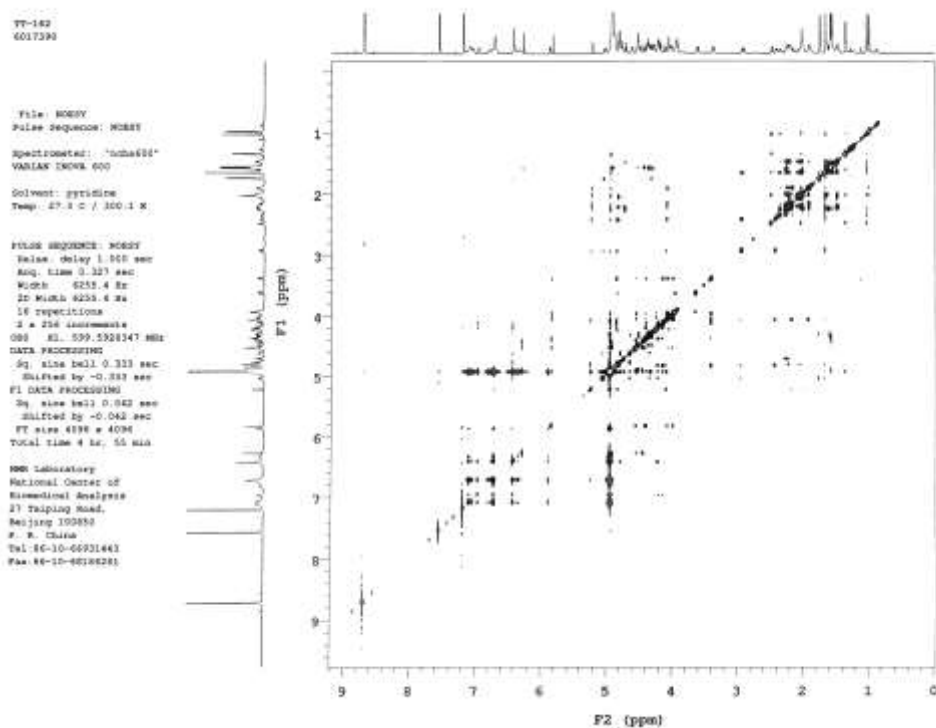


Figure S37. The NOESY of compound **7**.

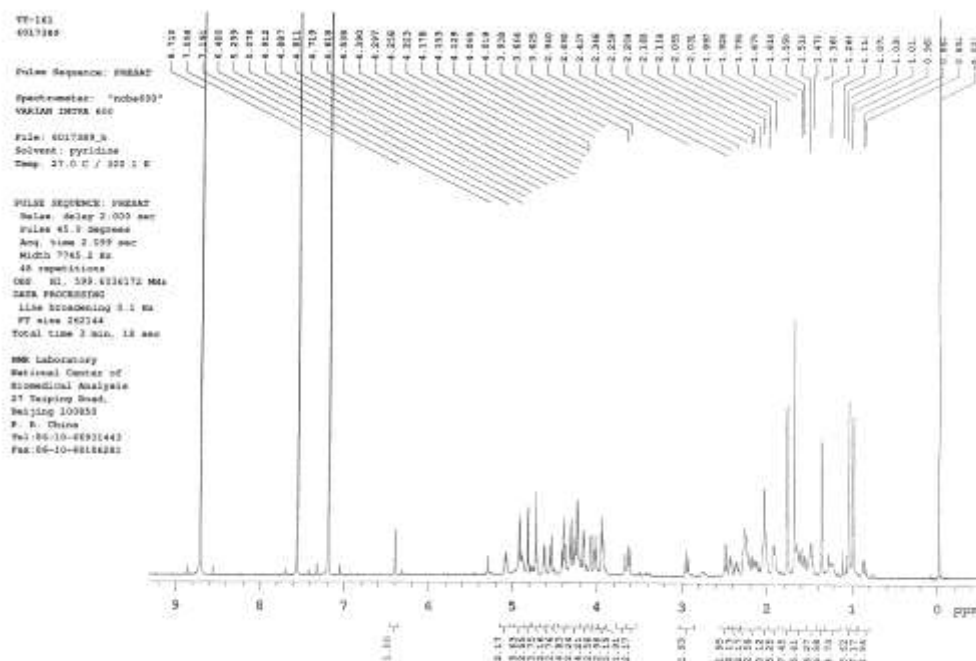


Figure S38. The ^1H NMR spectrum of compound **8**.

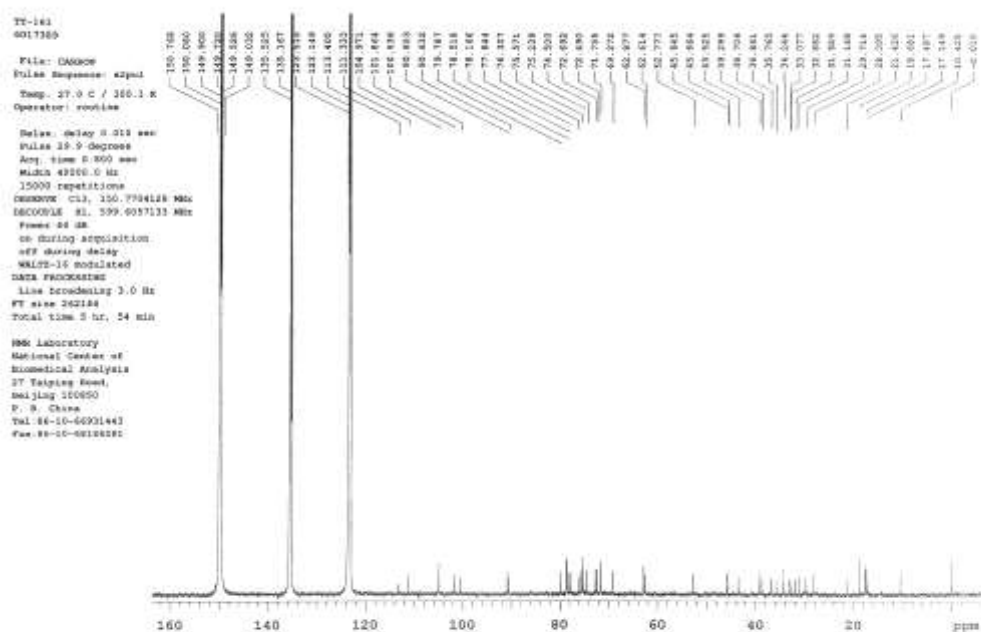


Figure S39. The ^{13}C NMR spectrum of compound **8**.

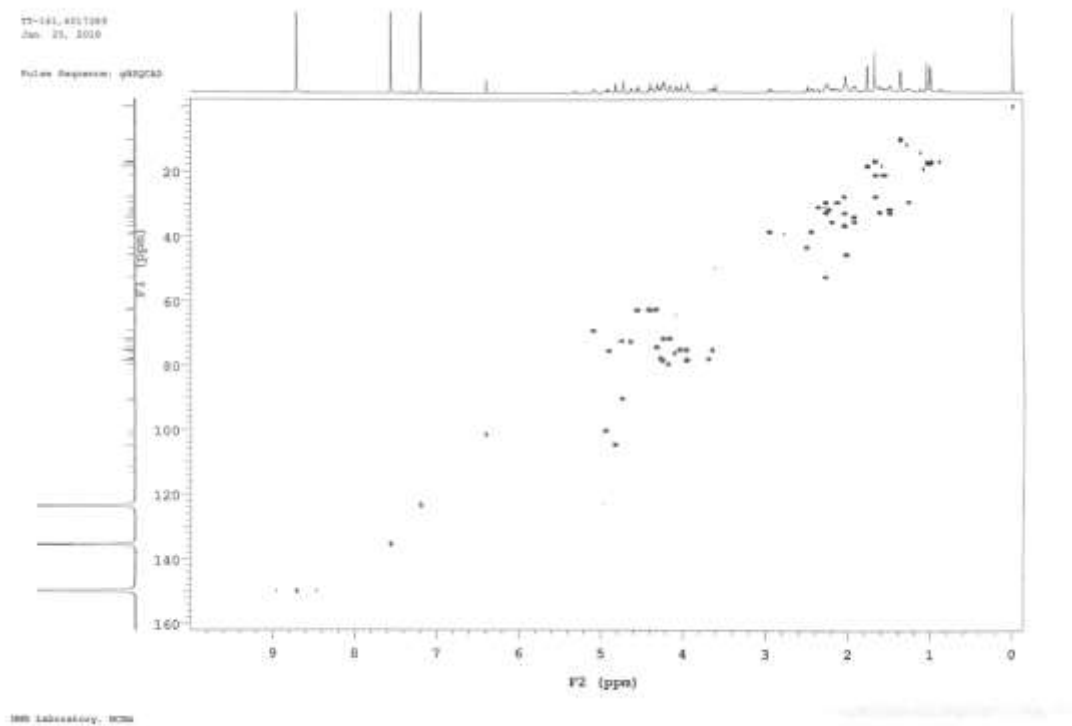


Figure S40. The HSQC spectrum of compound **8**.

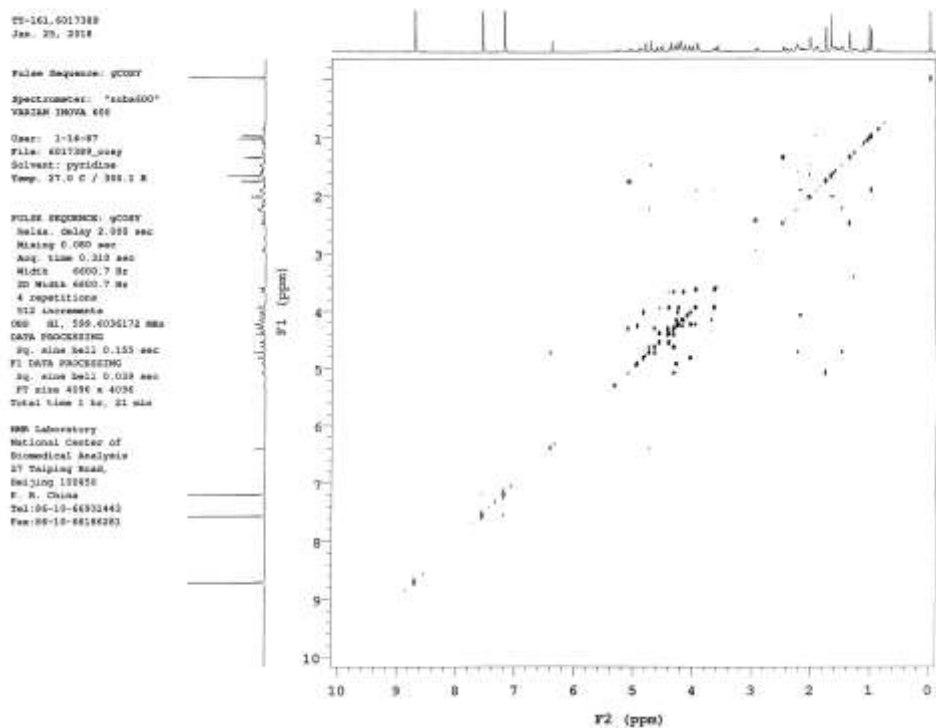


Figure S41. The ^1H - ^1H COSY spectrum of compound **8**.

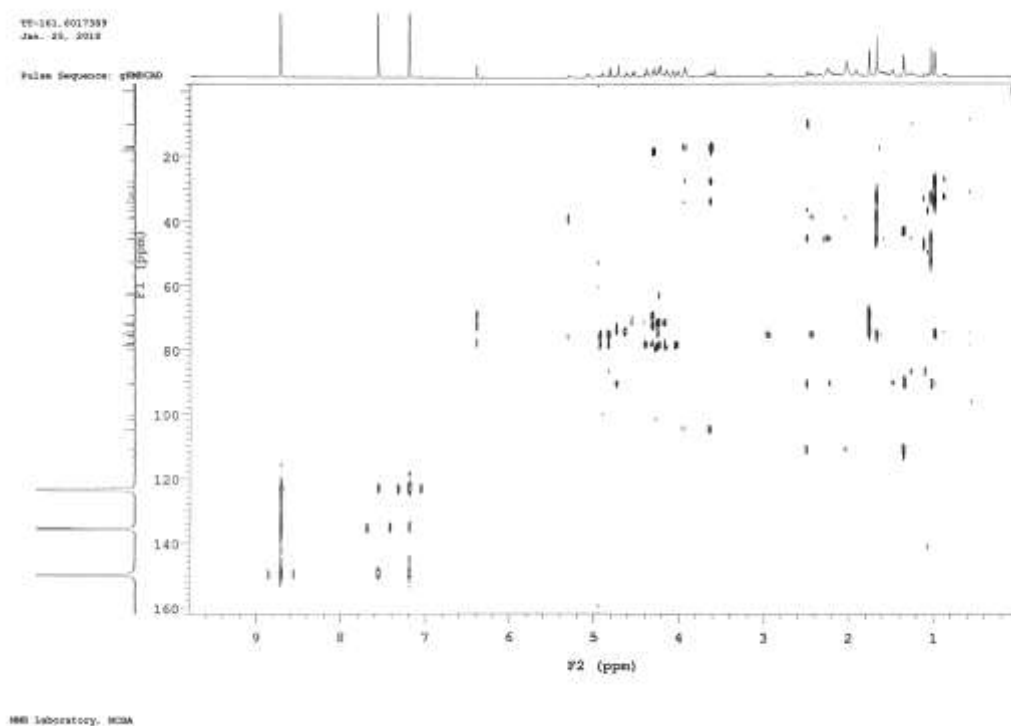


Figure S42. The HMBC spectrum of compound **8**.

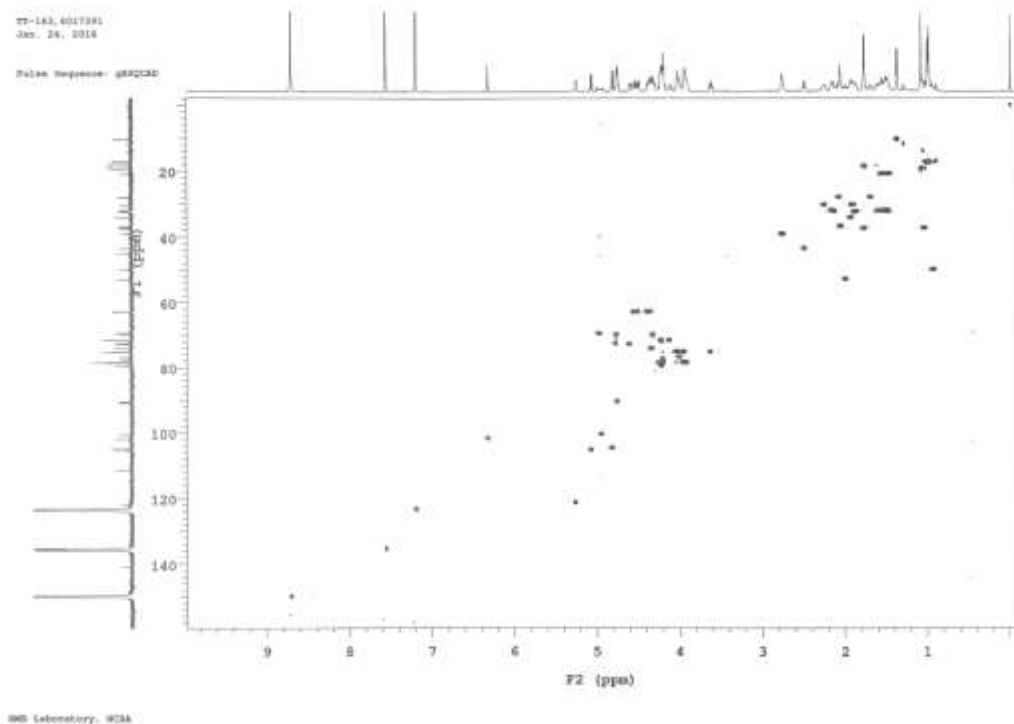


Figure S45. The HSQC spectrum of compound **9**.

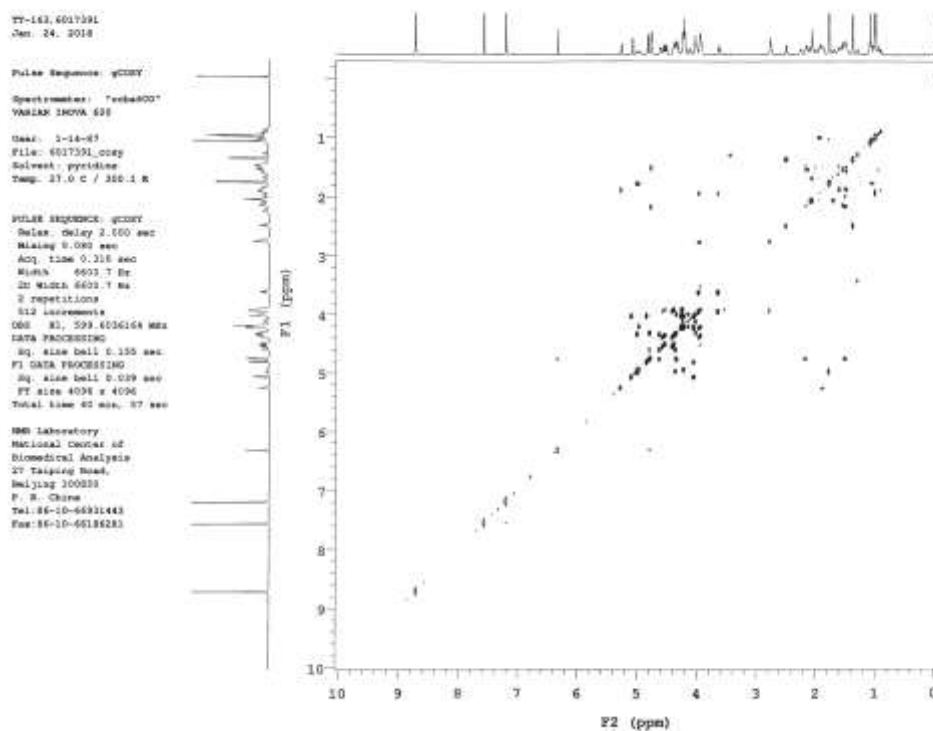


Figure S46. The ^1H - ^1H COSY spectrum of compound **9**.

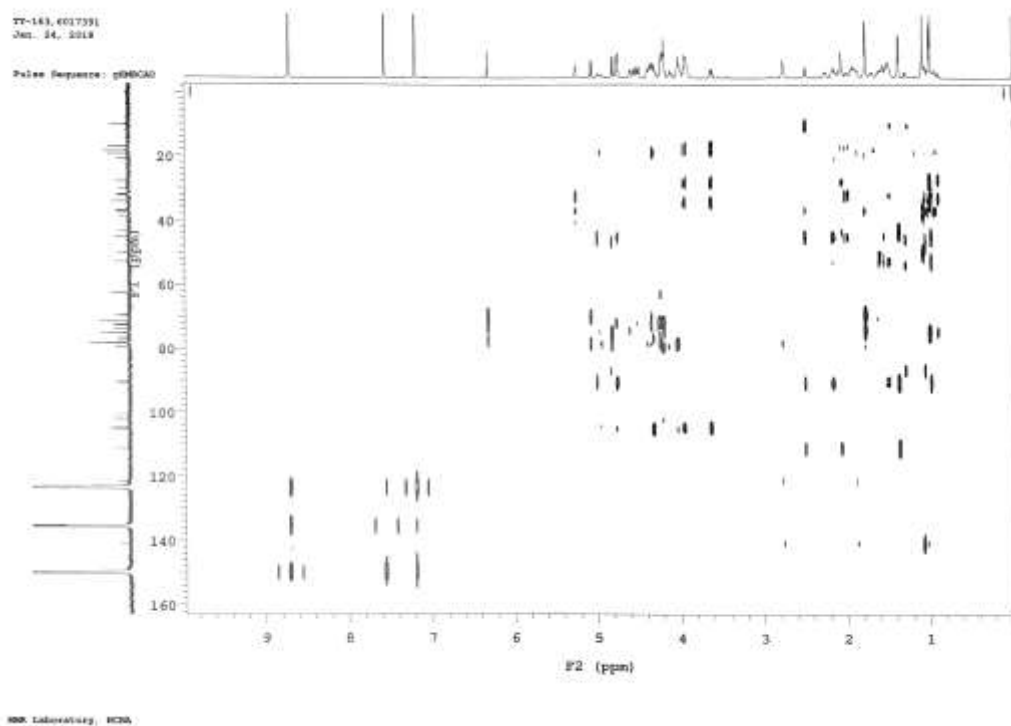


Figure S47. The HMBC spectrum of compound **9**.

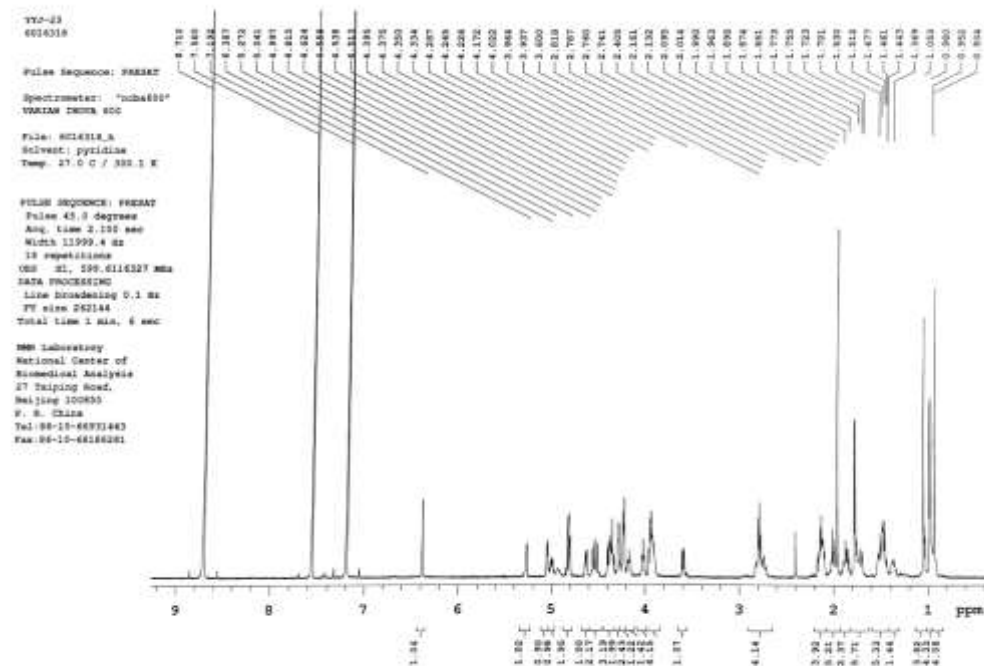


Figure S48. The ^1H NMR spectrum of compound **10**.

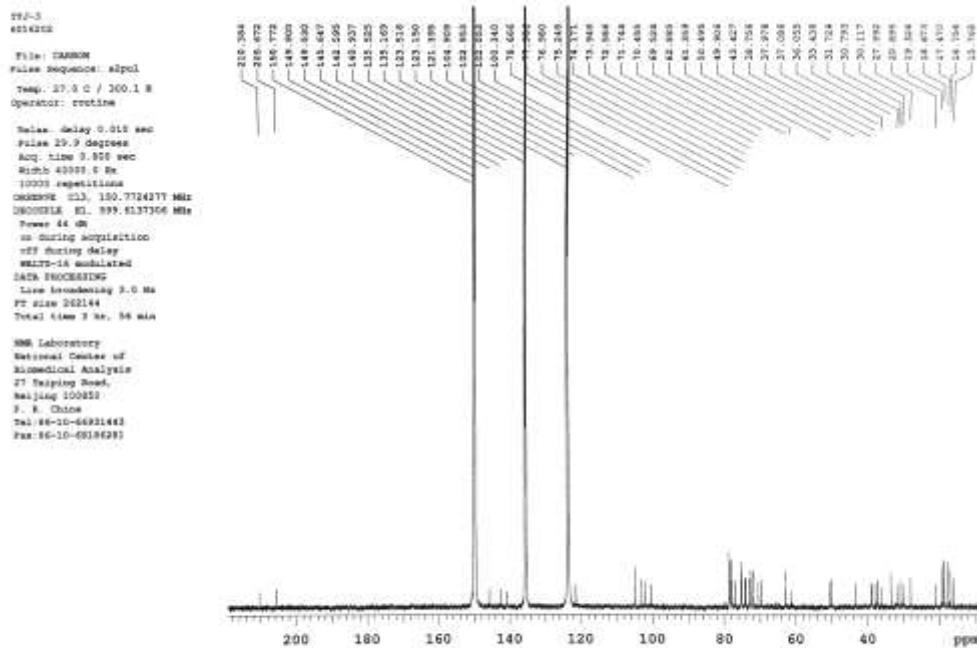


Figure S51. The ^{13}C NMR spectrum of compound **11**.

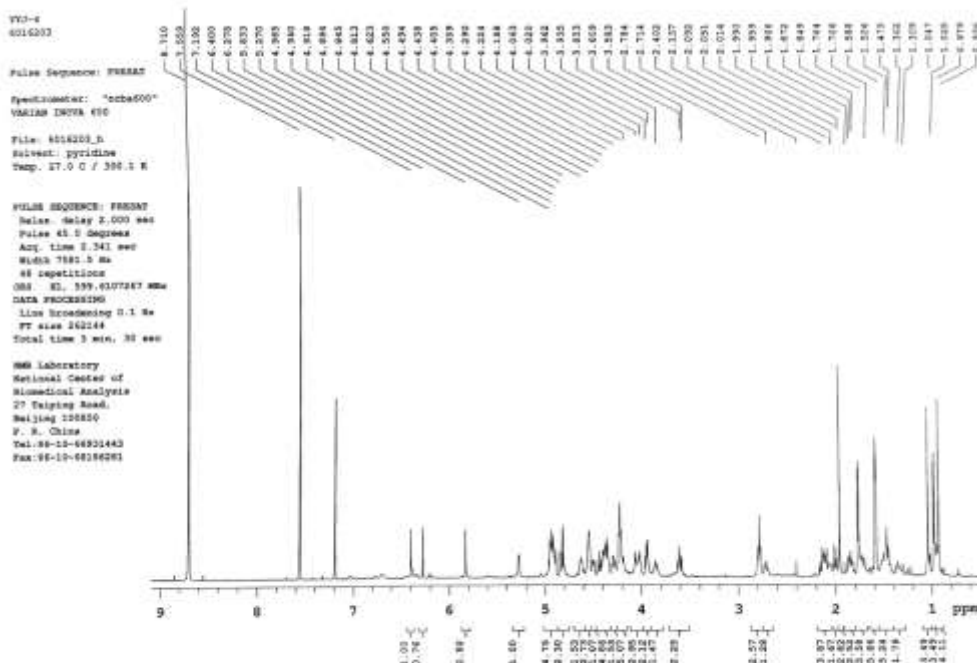


Figure S52. The ^1H NMR spectrum of compound **12**.

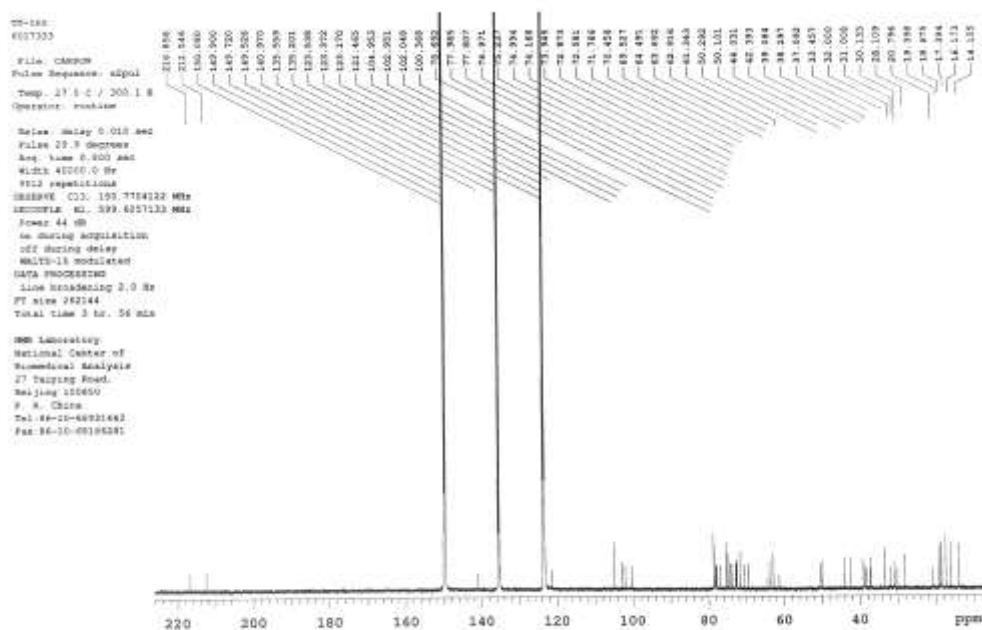


Figure S55. The ^{13}C NMR spectrum of compound **13**.

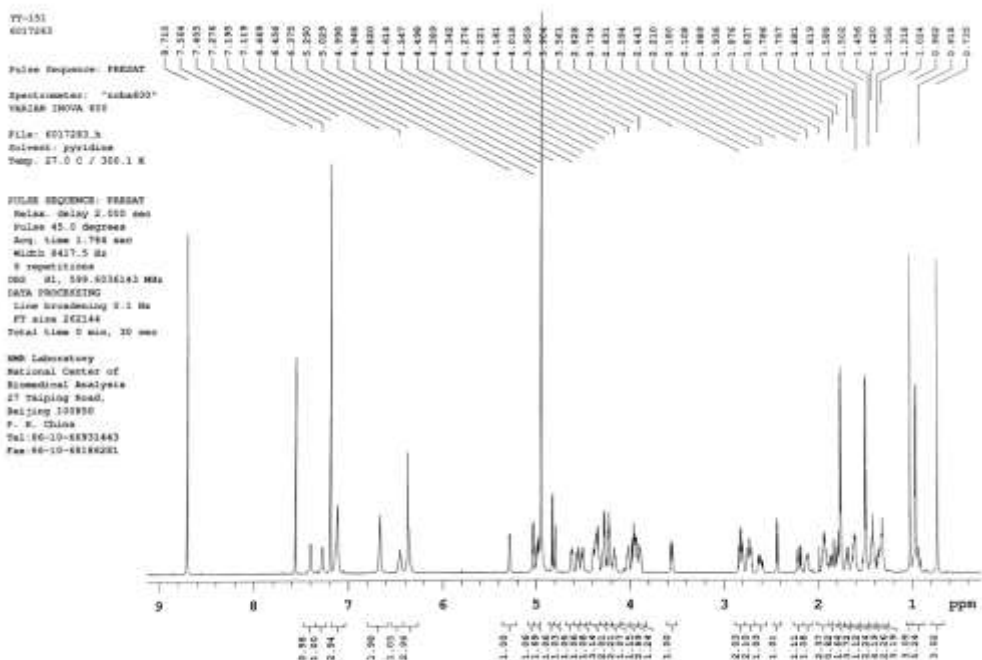


Figure S56. The ^1H NMR spectrum of compound **14**.

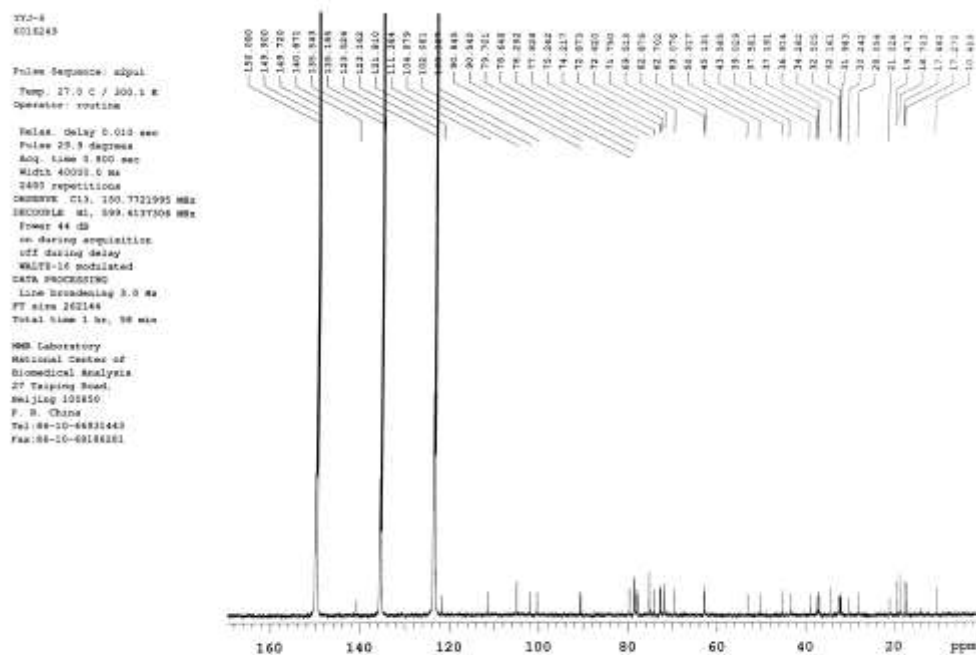


Figure S61. The ^{13}C NMR spectrum of compound **16**.

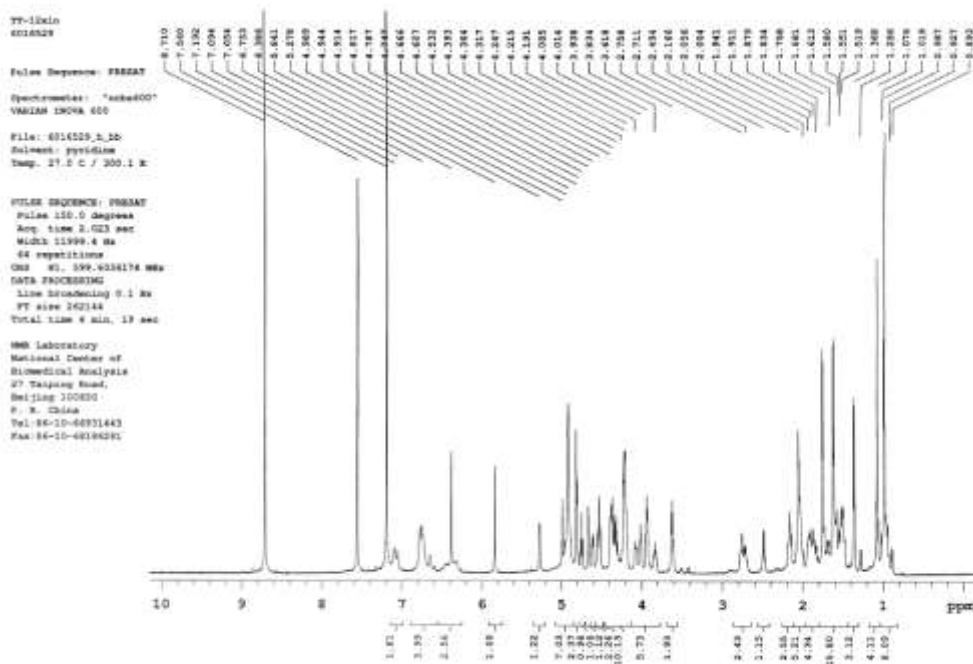


Figure S62. The ^1H NMR spectrum of compound **17**.

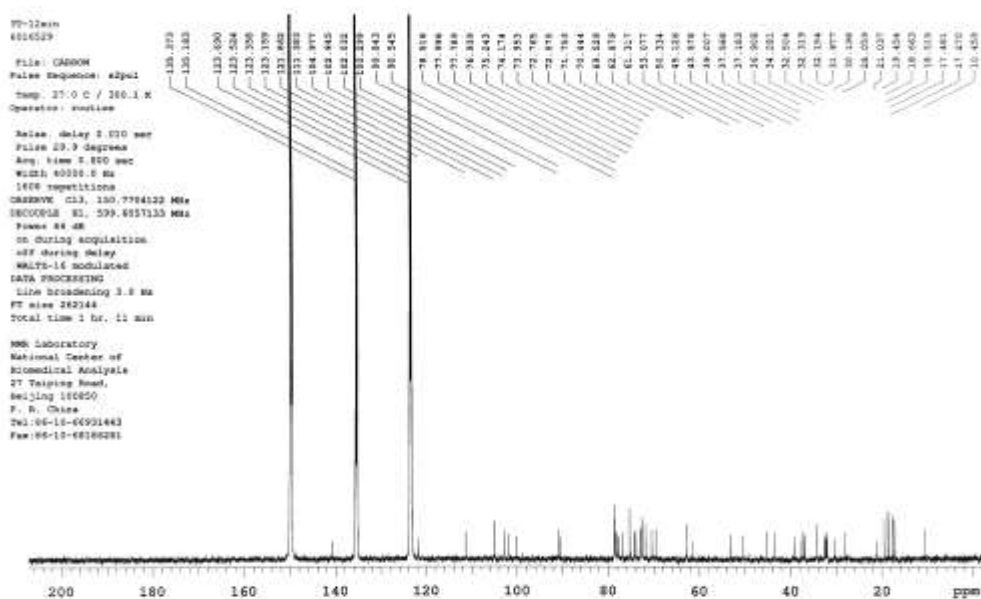


Figure S63. The ^{13}C NMR spectrum of compound 17.

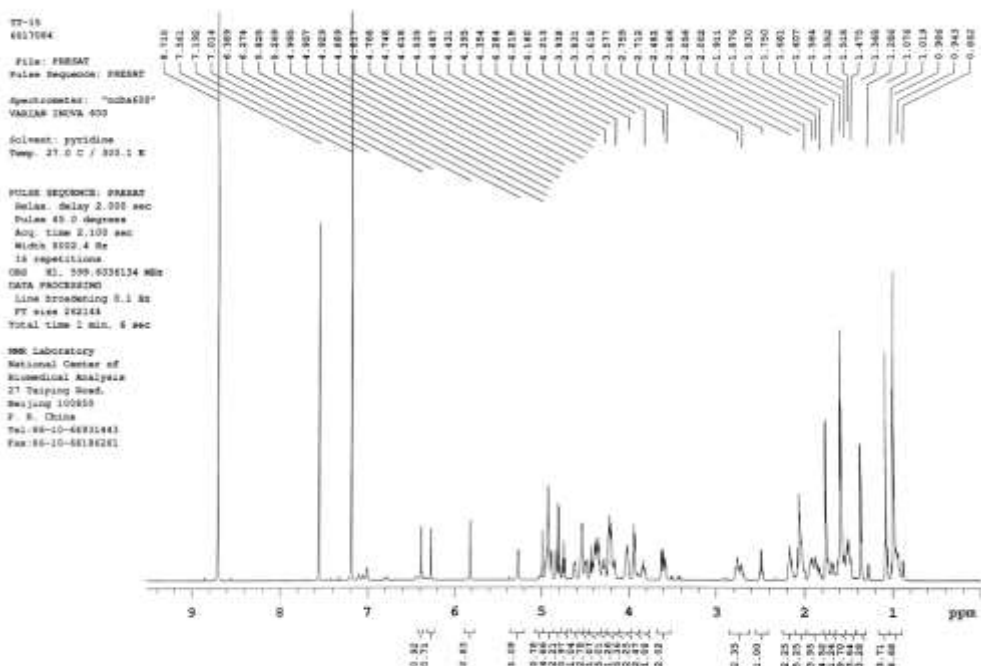


Figure S64. The ^1H NMR spectrum of compound 18.

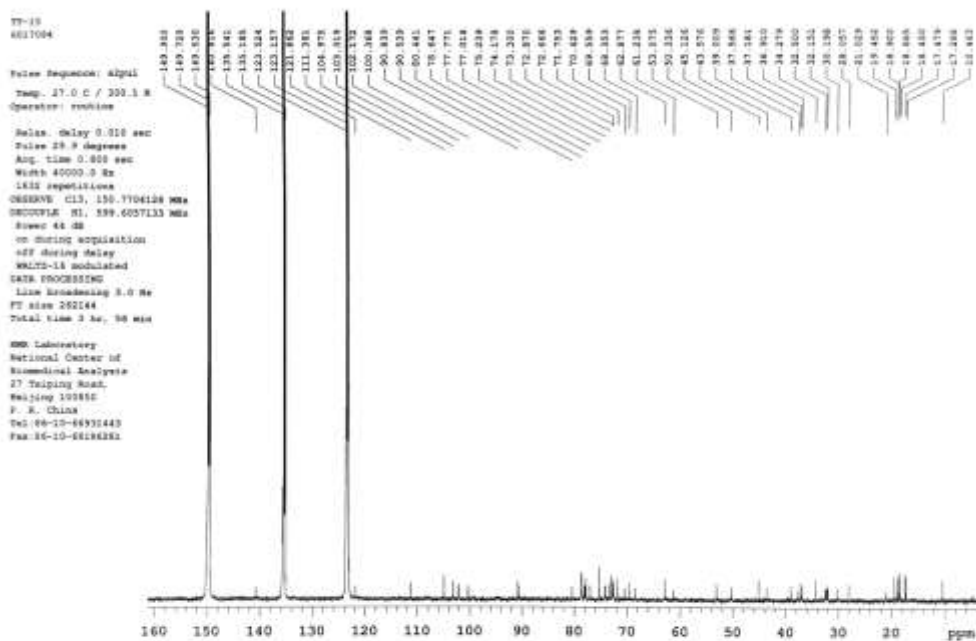


Figure S65. The ^{13}C NMR spectrum of compound **18**.

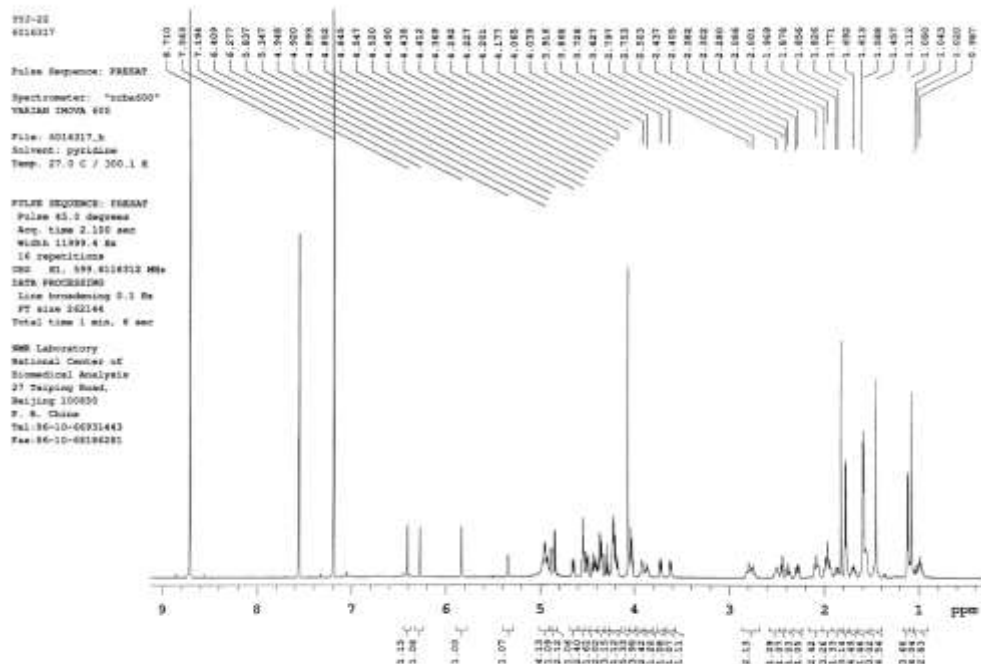


Figure S66. The ^1H NMR spectrum of compound **19**.

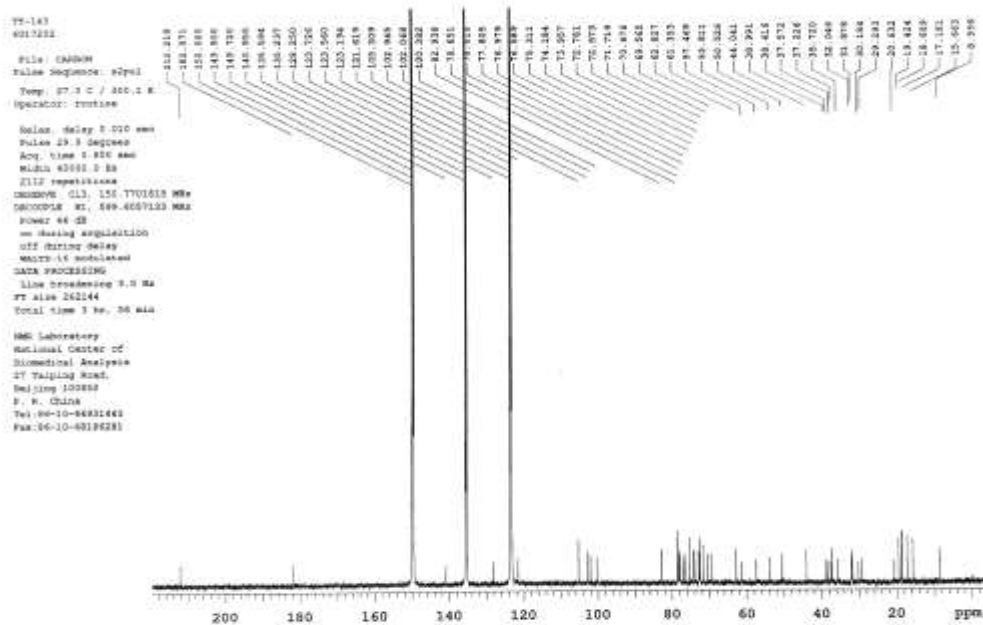


Figure S69. The ^{13}C NMR spectrum of compound **20**.