Supporting Information

Germacrane Sesquiterpenoids as a New Type of Anticardiac Fibrosis Agent Targeting Transforming Growth Factor β Type I Receptor

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5. The purity analyses of **1**–**31** by HPLC ·····S134–S149

6. The analysis of **11** on pan assay interference compounds (PAINS) ····S150–S151

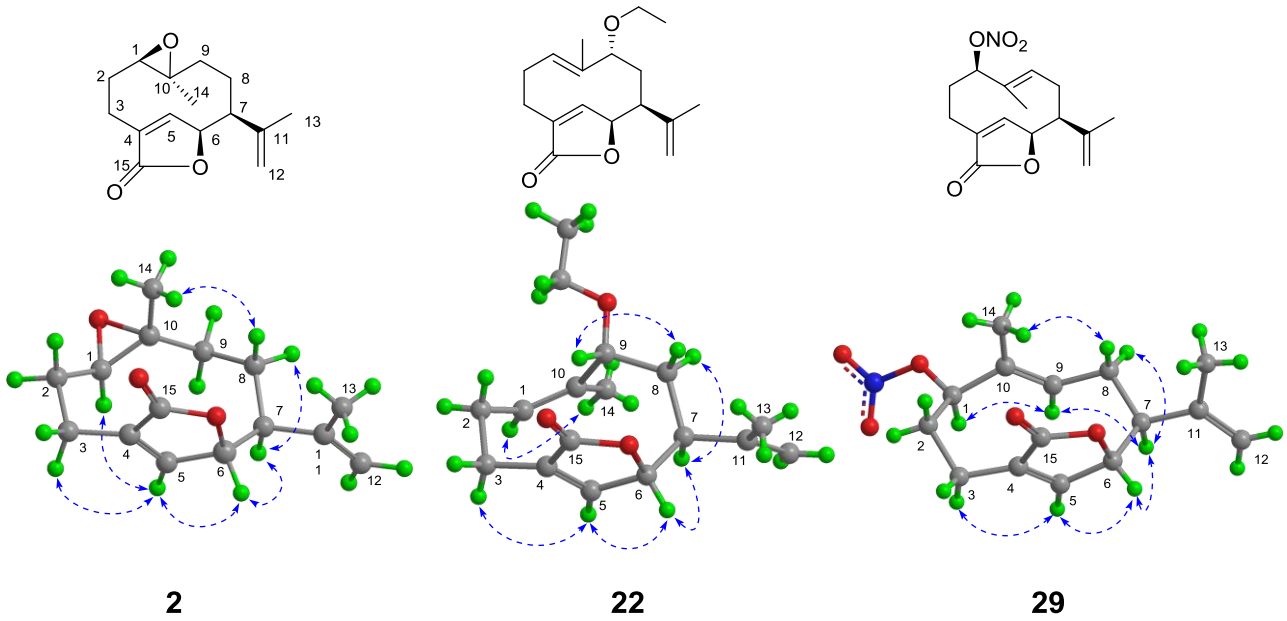


Figure S1. Key NOE correlations of the representative compounds **2**, **22**, and **29**.

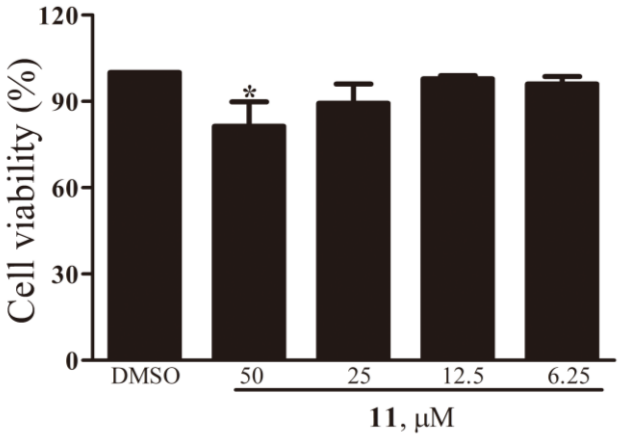


Figure S2. The cytotoxicity of **11** against cardiac fibroblasts. Cardiac fibroblasts were treated with the indicated concentrations of **11** for 48 h. Cell viability was detected by MTT assays. *$p < 0.05$ compared with DMSO group. $N = 3$ independent experiments.

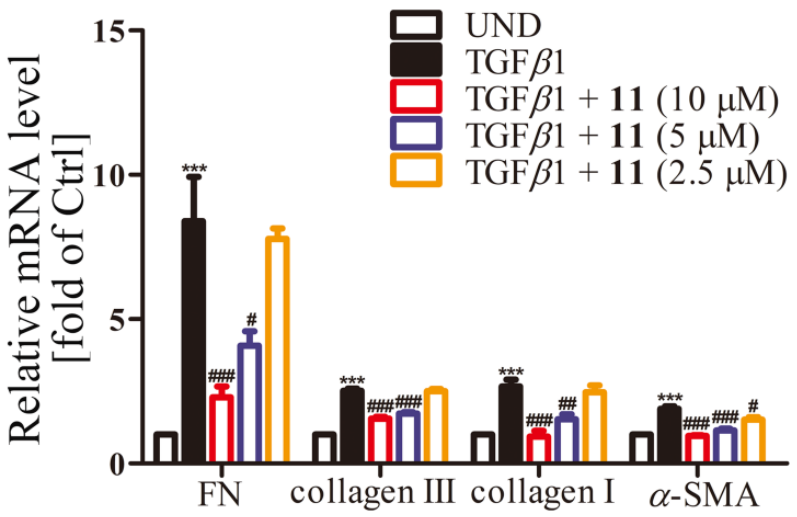


Figure S3. The inhibitory effects of **11** on the mRNA levels of FN, α-SMA, and collagens in TGFβ1-stimulated cardiac fibroblasts. Cardiac fibroblasts were treated with indicated concentrations of **11** and 10 ng/mL TGFβ1 for 12 h. mRNA levels were determined by quantitative real time reverse transcription polymerase chain reaction (qRT-PCR). The inhibitory effects of **11** on the mRNA levels of FN, α-SMA, and collagen I and III at the concentrations of 2.5, 5, and 10 μM. ***$P < 0.001$ *vs*. UND group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ *vs*. TGFβ1-treated group. N = 3 independent experiments.

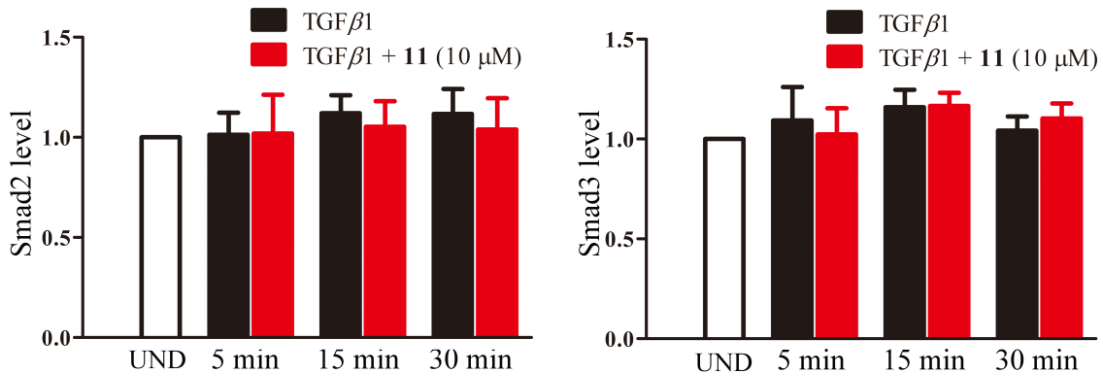


Figure S4. The effects of **11** on the total protein levels of Smad2/3 in TGFβ1-stimulated cardiac fibroblasts. Cardiac fibroblasts were treated with **11** (10 μM) and 10 ng/mL TGFβ1 for indicated time. The protein levels were determined by Western blot and then quantified by densitometry. $P > 0.05$ *vs*. UND group and TGFβ1-treated group. N = 3 independent experiments.

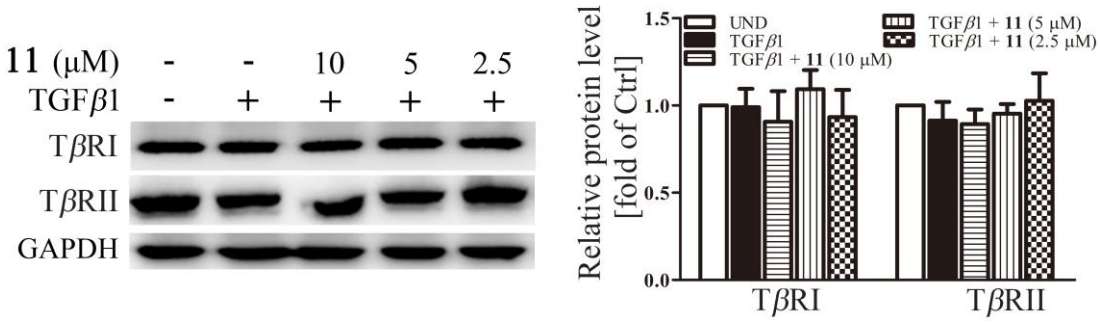


Figure S5. The influence of **11** on the expression of TβRI and TβRII in TGFβ1-stimulated cardiac fibroblasts. The cardiac fibroblasts were treated with indicated concentrations of **11** and 10 ng/mL TGFβ1 for 5 min. The protein levels of TβRI and TβRII were determined by Western blot with indicated antibodies. GAPDH was used as a loading control. $P > 0.05$ *vs.* UND group and TGFb1-treated group. $N = 3$ independent experiments.

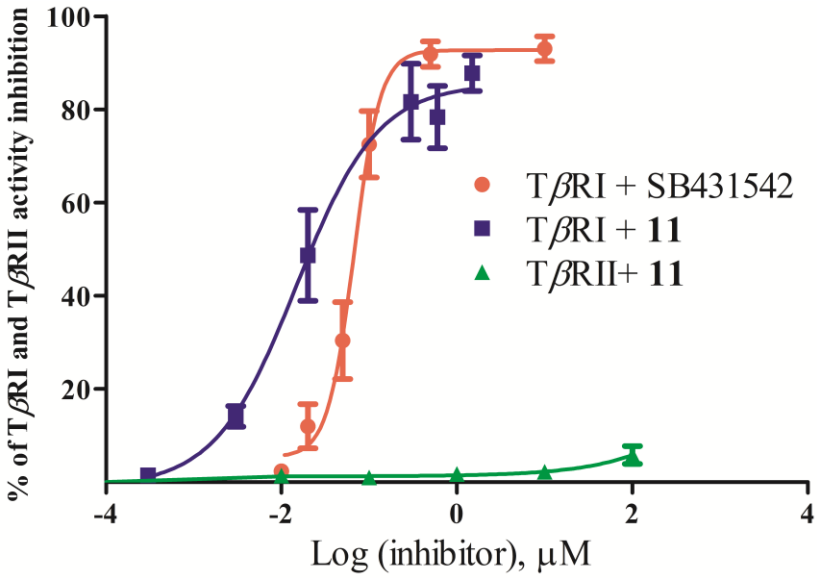


Figure S6. Inhibitory curves of **11** on human recombinant TβRI and TβRII. The solutions of **11** or SB431542 in different concentrations were mixed with reaction buffer including purified TβRI protein (4 ng/μL) or TβRII (10 ng/μL), 50 μM ATP, and 0.2 μg/μL casein (for TβRI) or 0.1 μg/μL MBP (for TβRII). The mixtures were incubated for 2 h at room temperature. After incubation, the ADP generated was measured, which is the indicative of kinase activity. The IC_{50} values of **11** and SB431542 were calculated from dose-response curves generated using Graphpad prism software. $N = 3$ independent experiments.

Table S1. The relative contents of FN under the treatment of **1–31** in TGFβ1-stimulated cardiac fibroblasts

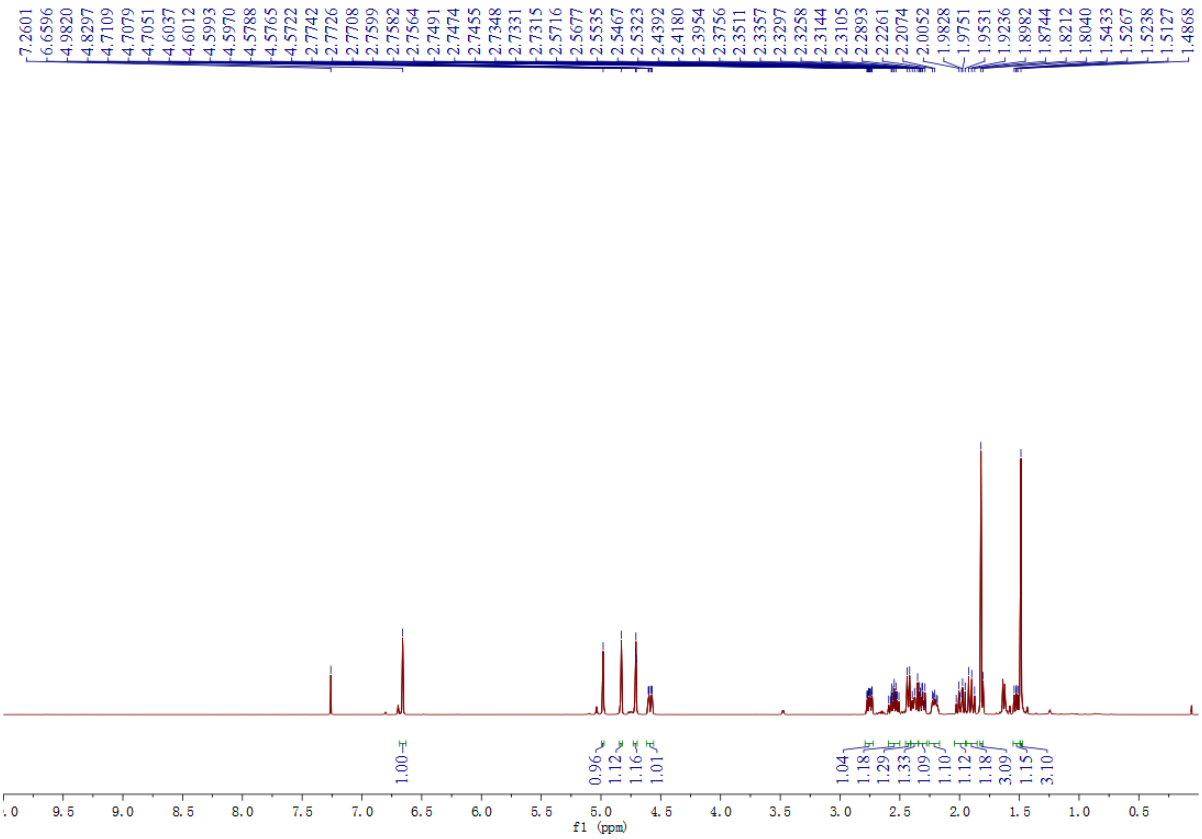
Compd.	FN level (%)		Compd.	FN level (%)	
	50 μM	10 μM		50 μM	10 μM
Nif	23.3 ± 2.5	46.3 ± 4.5	**15**	24.2 ± 2.7	43.5 ± 3.2
SB	12.0 ± 1.0	18.3 ± 2.5	**16**	34.1 ± 4.2	49.8 ± 7.3
1	66.0 ± 4.3	76.1 ± 6.0	**17**	39.1 ± 4.1	51.3 ± 2.1
2	44.7 ± 3.3	58.1 ± 2.1	**18**	76.6 ± 2.7	89.7 ± 3.9
3	27.3 ± 5.1	42.6 ± 7.6	**19**	78.8 ± 4.2	86.1 ± 4.1
4	32.2 ± 3.4	47.8 ± 2.6	**20**	27.2 ± 2.9	44.6 ± 4.1
5	46.6 ± 4.6	63.1 ± 5.4	**21**	62.6 ± 3.5	73.9 ± 4.3
6	43.1 ± 2.5	63.3 ± 3.3	**22**	62.1 ± 2.6	72.8 ± 3.9
7	78.3 ± 3.0	84.7 ± 4.1	**23**	62.5 ± 4.6	74.4 ± 1.3
8	76.2 ± 3.3	89.5 ± 3.4	**24**	68.3 ± 3.3	75.5 ± 3.9
9	50.1 ± 6.7	64.1 ± 5.9	**25**	62.2 ± 6.7	77.8 ± 2.1
10	55.0 ± 3.6	65.3 ± 2.1	**26**	61.1 ± 4.2	74.5 ± 5.6
11	13.3 ± 4.1	26.1 ± 4.1	**27**	57.6 ± 6.0	71.1 ± 9.7
12	25.0 ± 2.2	44.7 ± 5.4	**28**	26.9 ± 2.9	61.6 ± 5.8
13	43.3 ± 6.2	56.5 ± 3.1	**29**	56.6 ± 5.2	62.5 ± 8.0
14	34.1 ± 2.7	49.9 ± 2.8	**30**	59.7 ± 2.9	68.6 ± 4.6
Control[a]	100 %	100 %	**31**	59.1 ± 2.5	71.1 ± 6.7

[a] cells treated with 10 ng/mL TGFβ1

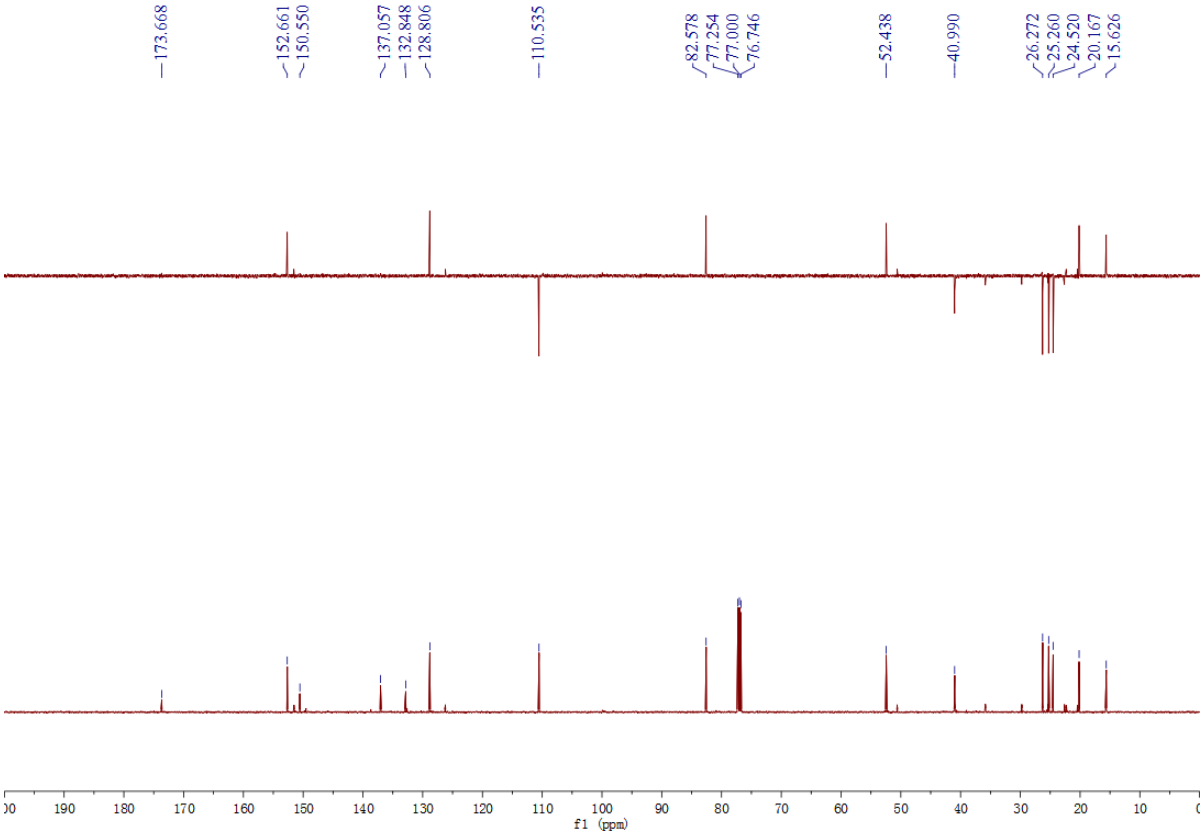
Table S2. Rat-specific primer sequences for qRT-PCR

Primers	Forward/Reverse	Sequences
GAPDH	Forward	5′-AGGTCGGTGTGAACGGATTTG-3′
	Reverse	5′-TGTAGACCATGTAGTTGAGGTCA-3′
FN	Forward	5′-GCTCAGCAAATCGTGCAGC-3′
	Reverse	5′-CTAGGTAGGTCCGTTCCCACT-3′
α-SMA	Forward	5′-GGCACCACTGAACCCTAAGG-3′
	Reverse	5′-ACAATACCAGTTGTACGTCCAGA-3′
Collagen Ⅰ	Forward	5′-GAGGGCCAAGACGAAGACATC-3′
	Reverse	5′-CAGATCACGTCATCGCACAAC-3′
Collagen Ⅲ	Forward	5′-TTGAAGGAGGATGTTCCCATCT-3′
	Reverse	5′-ACAGACACATATTTGGCATGGTT-3′

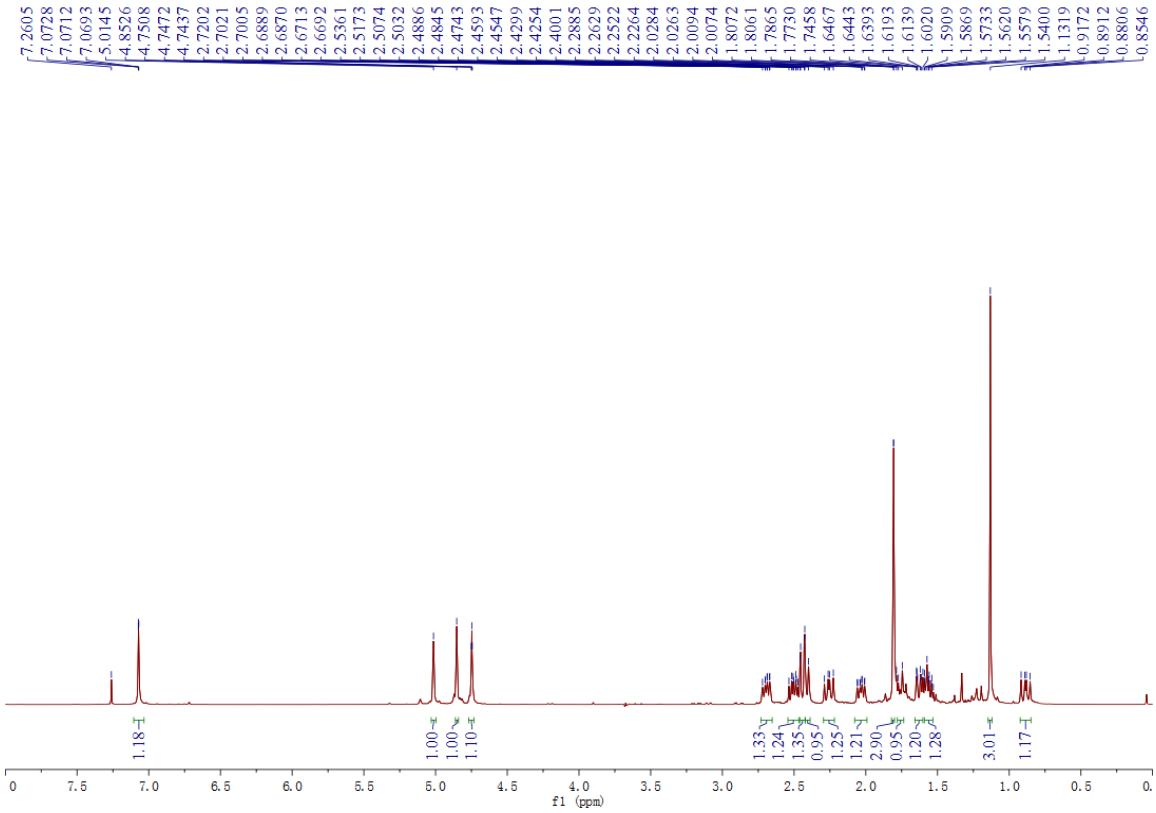
S2.1. ^{1}H NMR spectrum of **1**



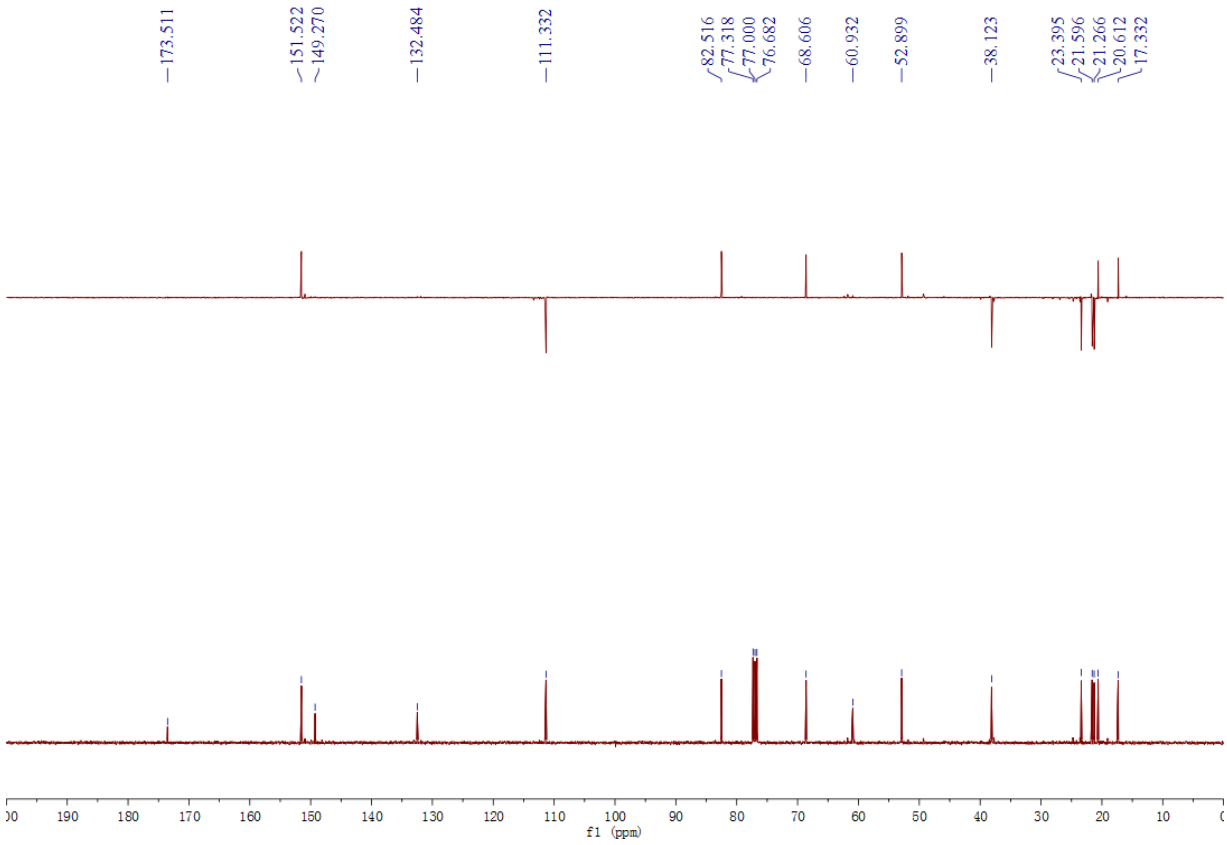
S2.2. ^{13}C NMR and DEPT spectra of **1**



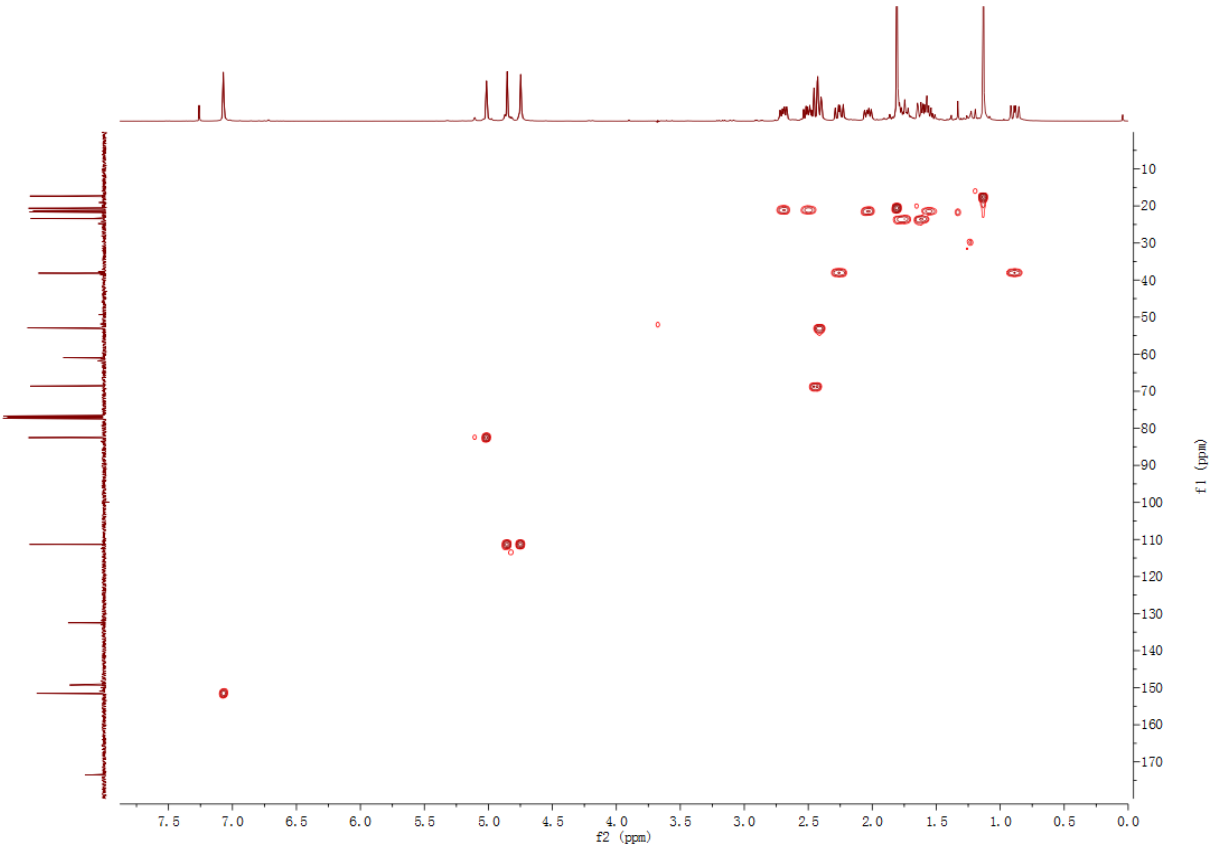
S2.3. ^{1}H NMR spectrum of **2**



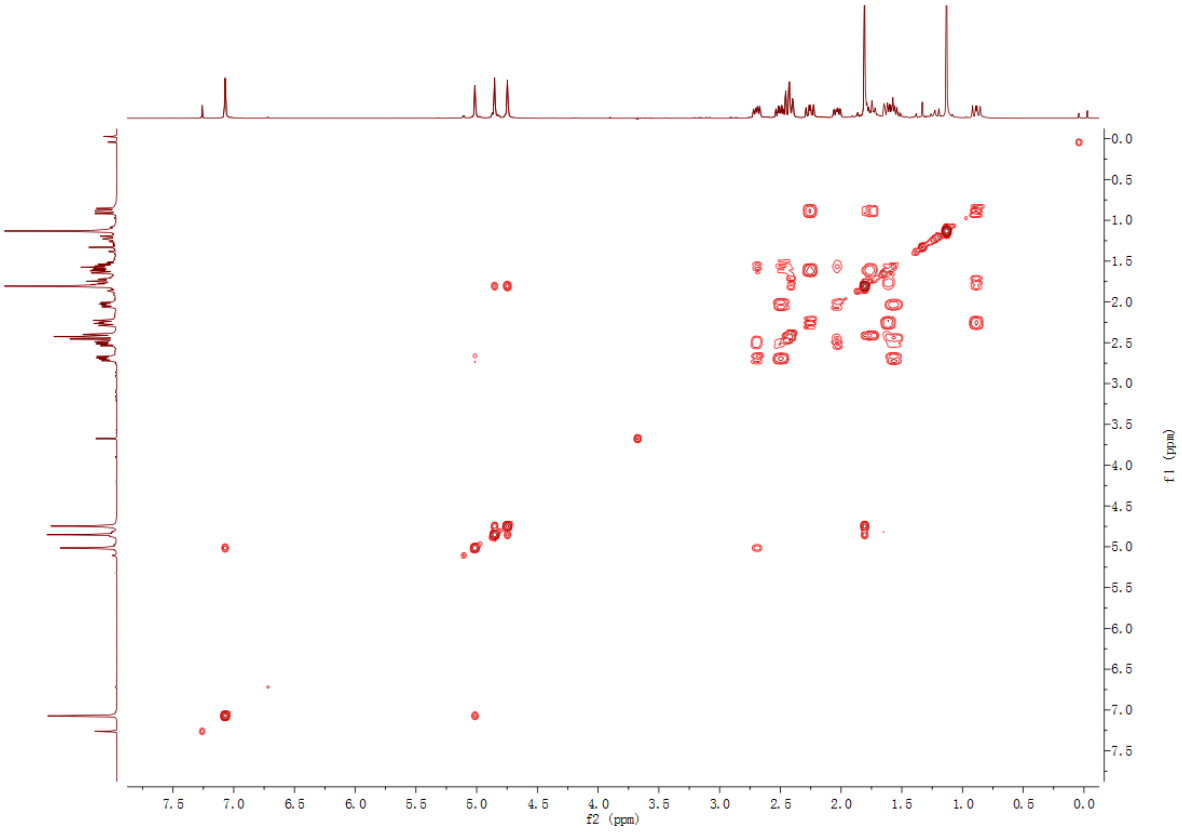
S2.4. ^{13}C NMR and DEPT spectra of **2**



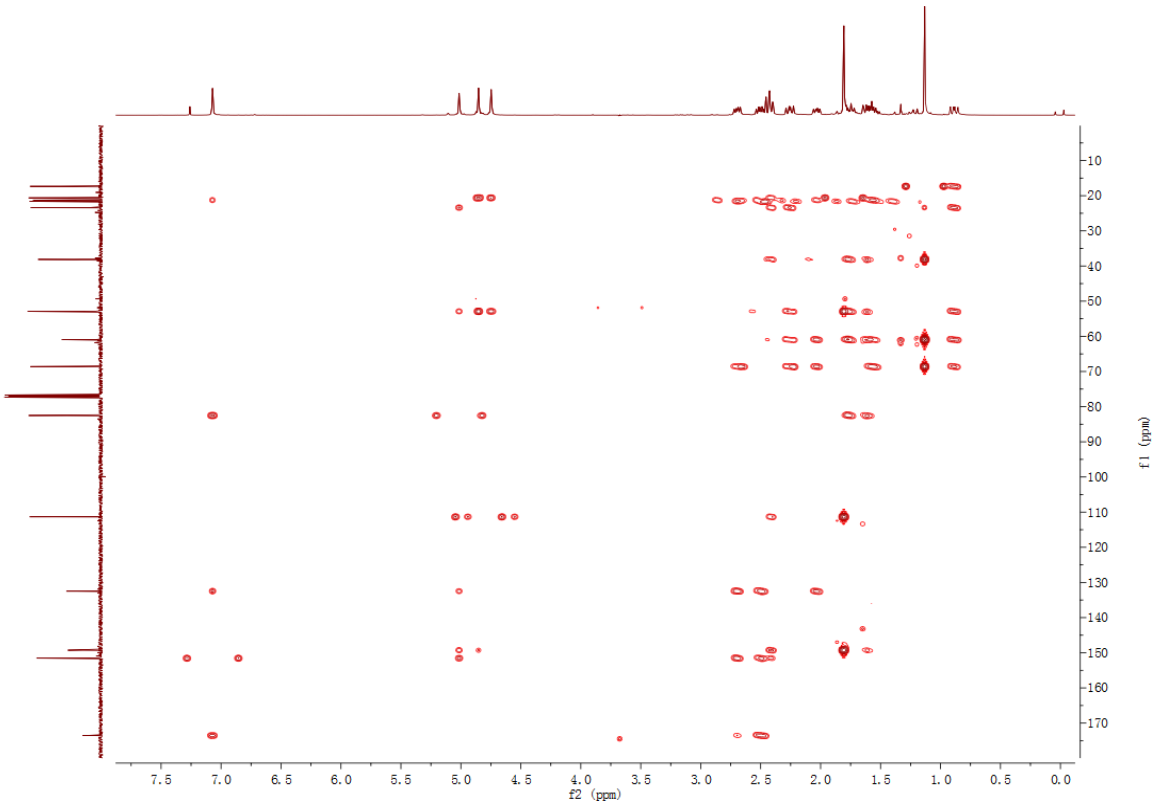
S2.5. HSQC spectrum of **2**



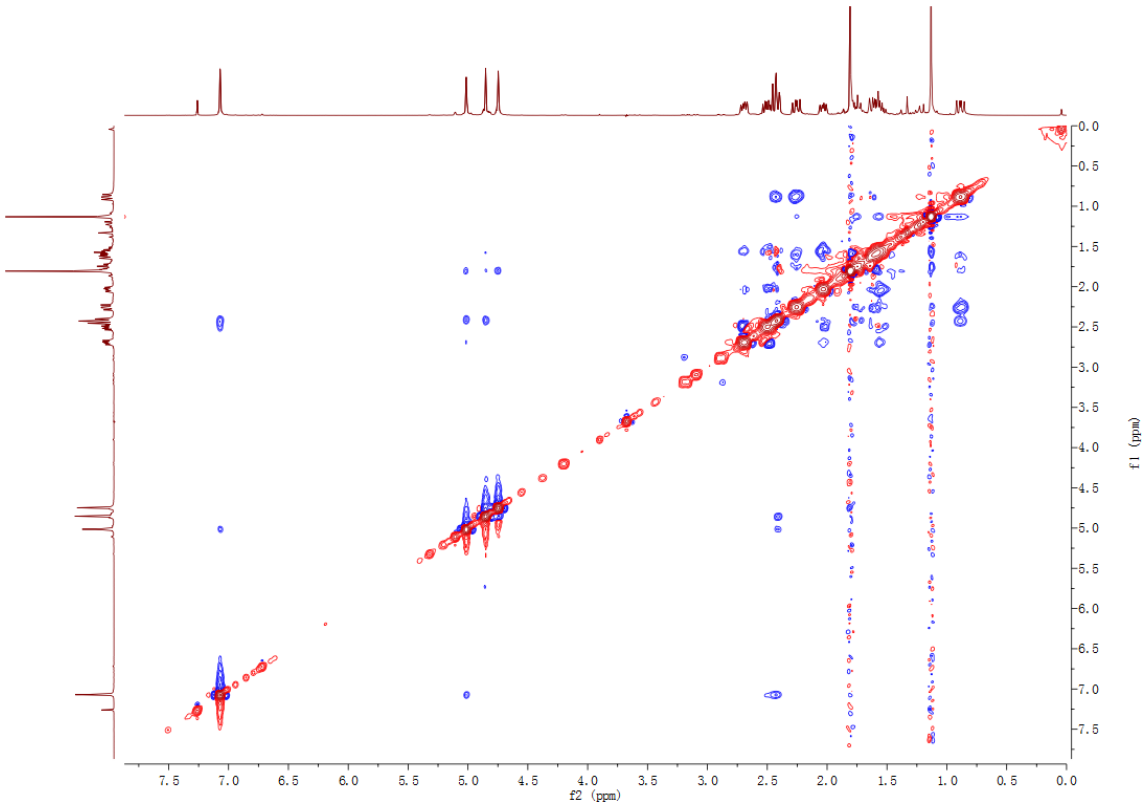
S2.6. ^{1}H–^{1}H COSY spectrum of **2**



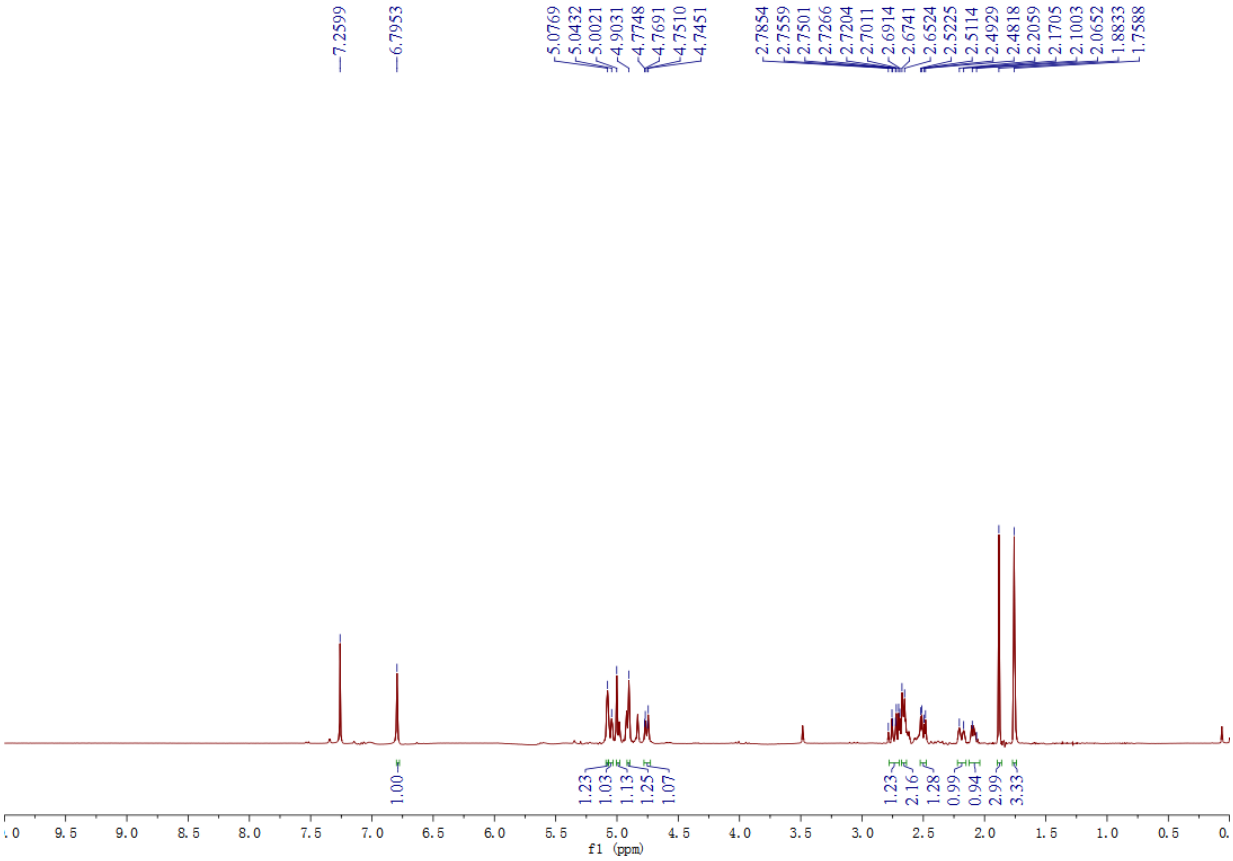
S2.7. HMBC spectrum of **2**



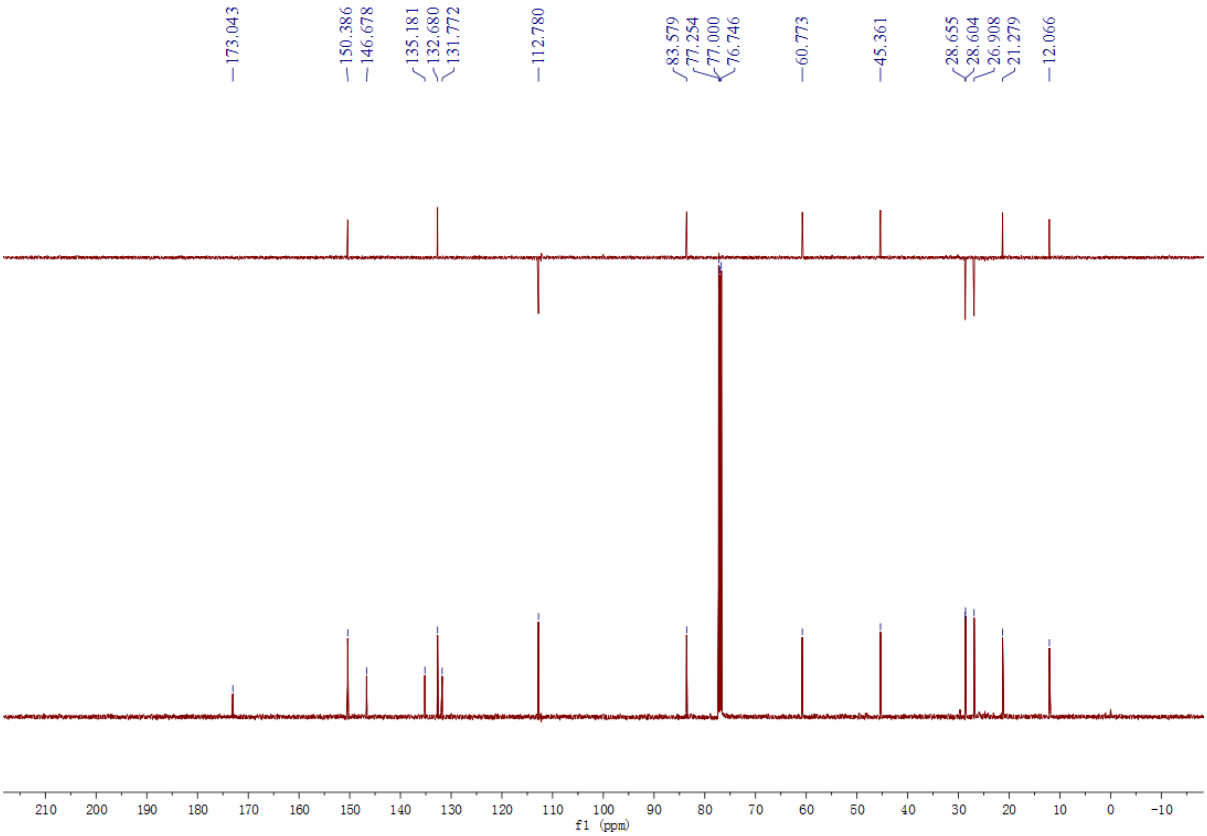
S2.8. NOESY spectrum of **2**



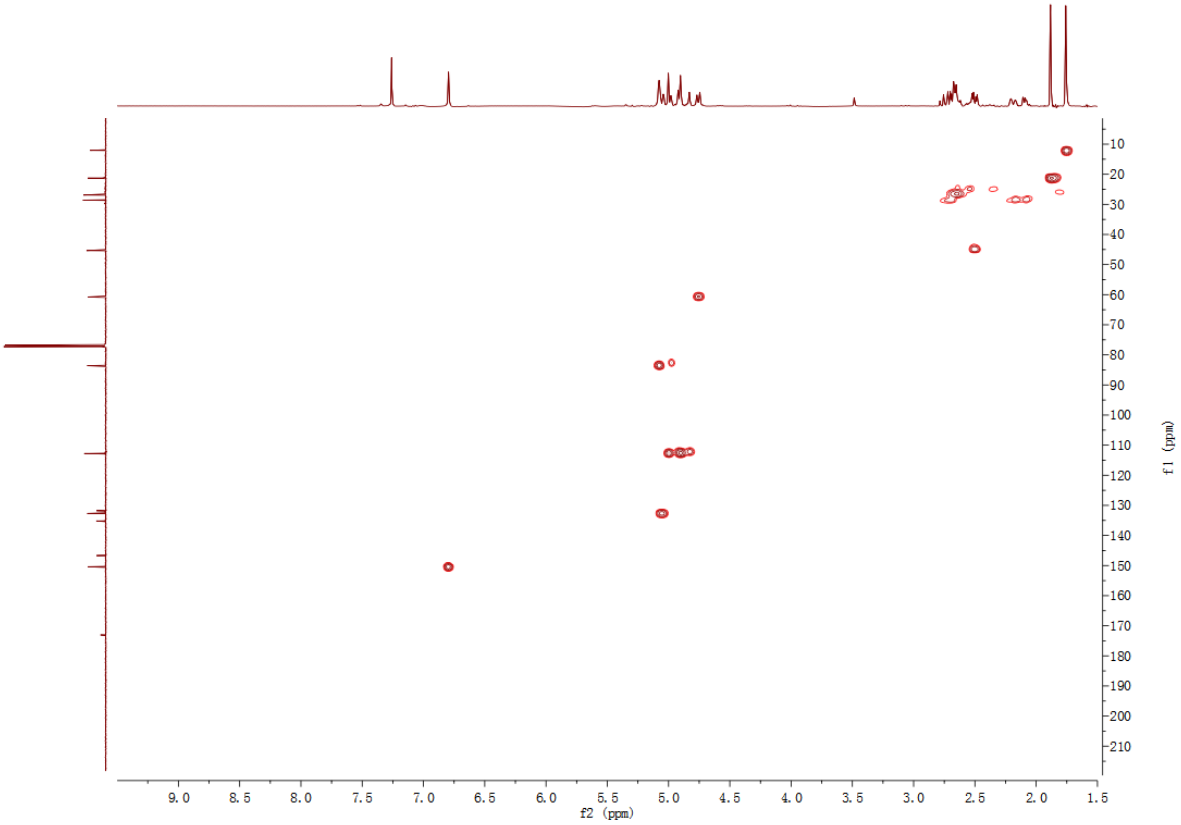
S2.9. ^{1}H NMR spectrum of **3**



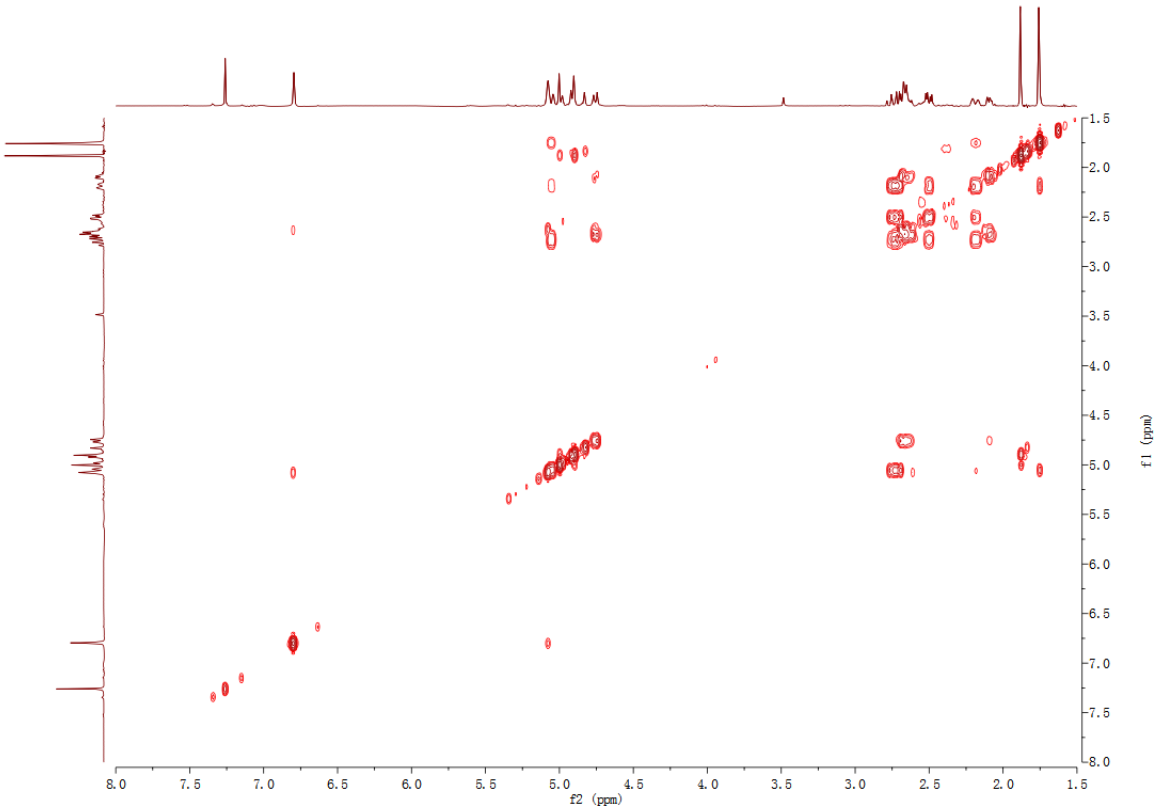
S2.10. ^{13}C NMR and DEPT spectra of **3**



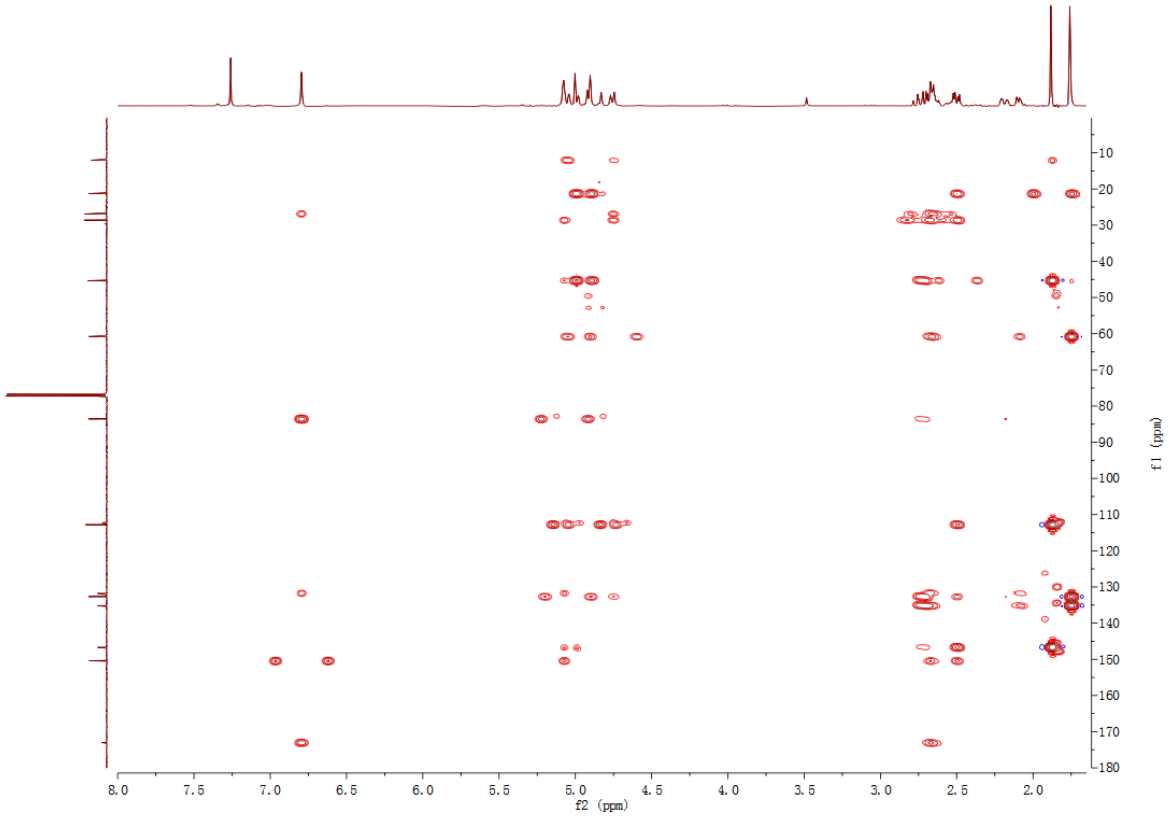
S2.11. HSQC spectrum of **3**



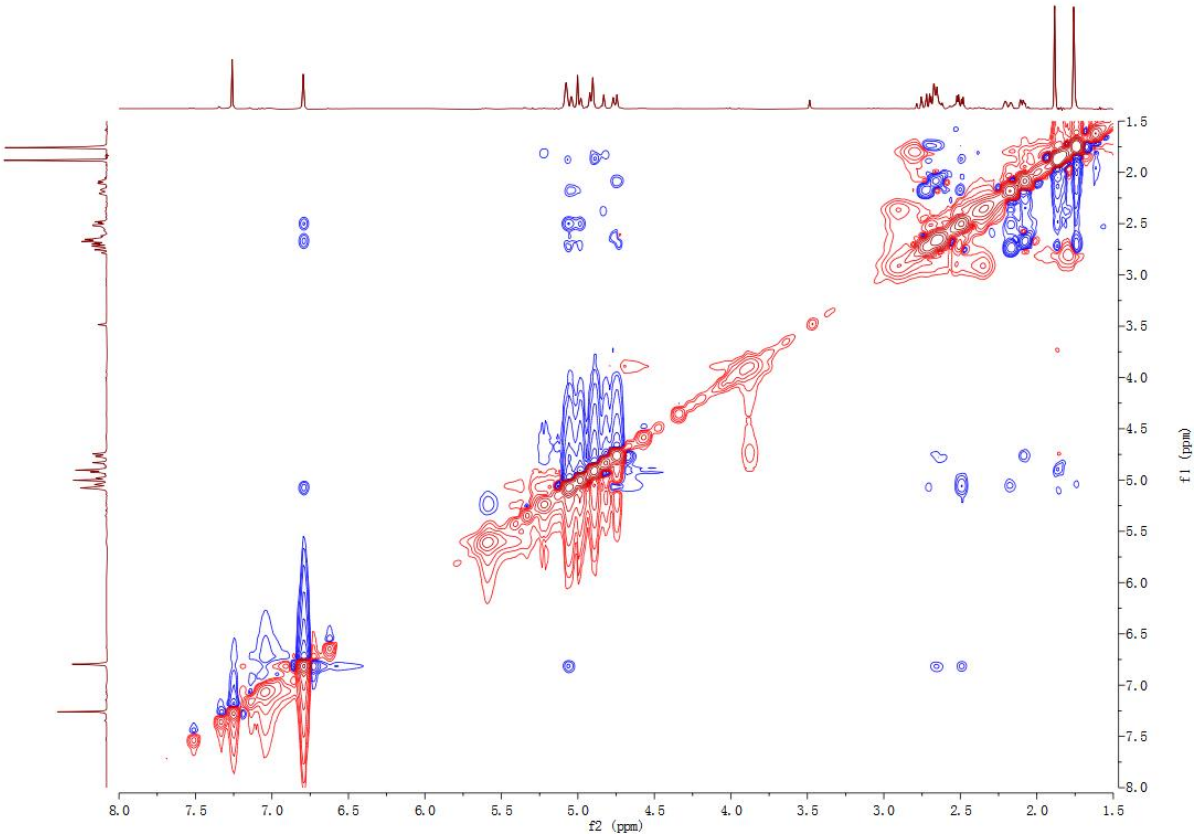
S2.12. ^{1}H–^{1}H COSY spectrum of **3**



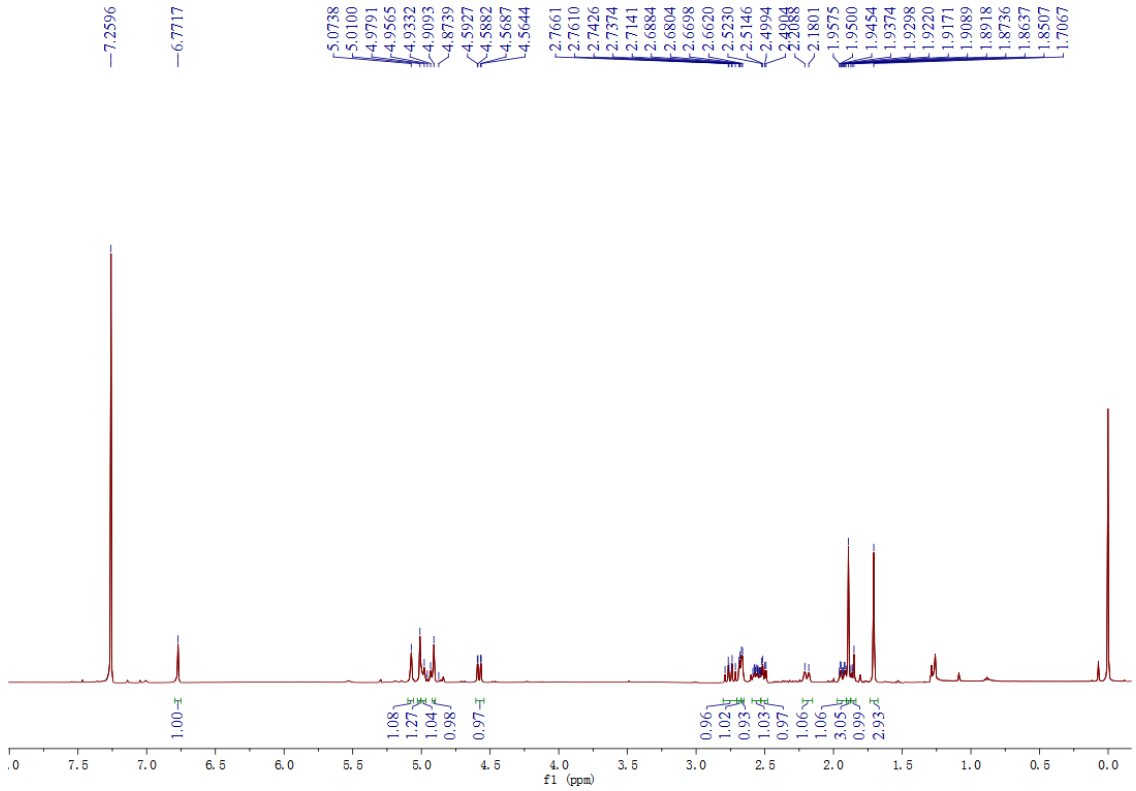
S2.13. HMBC spectrum of **3**



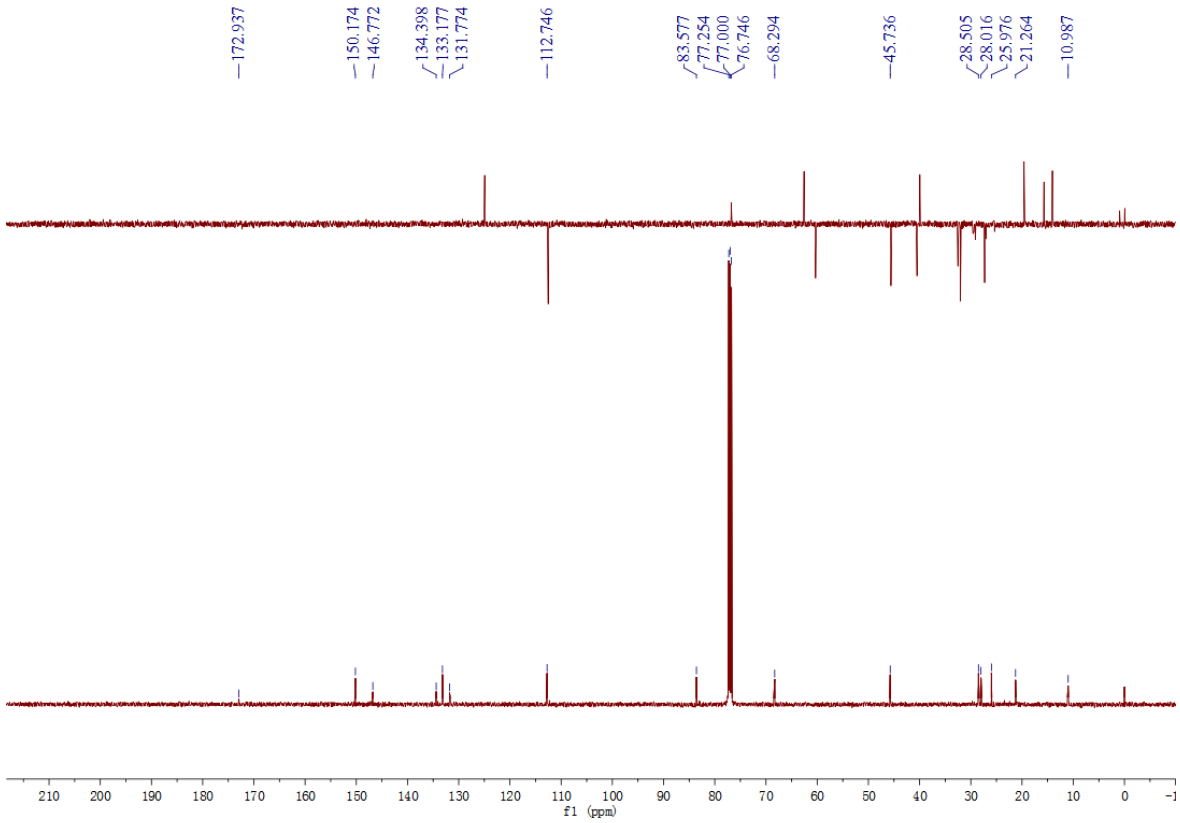
S2.14. NOESY spectrum of **3**



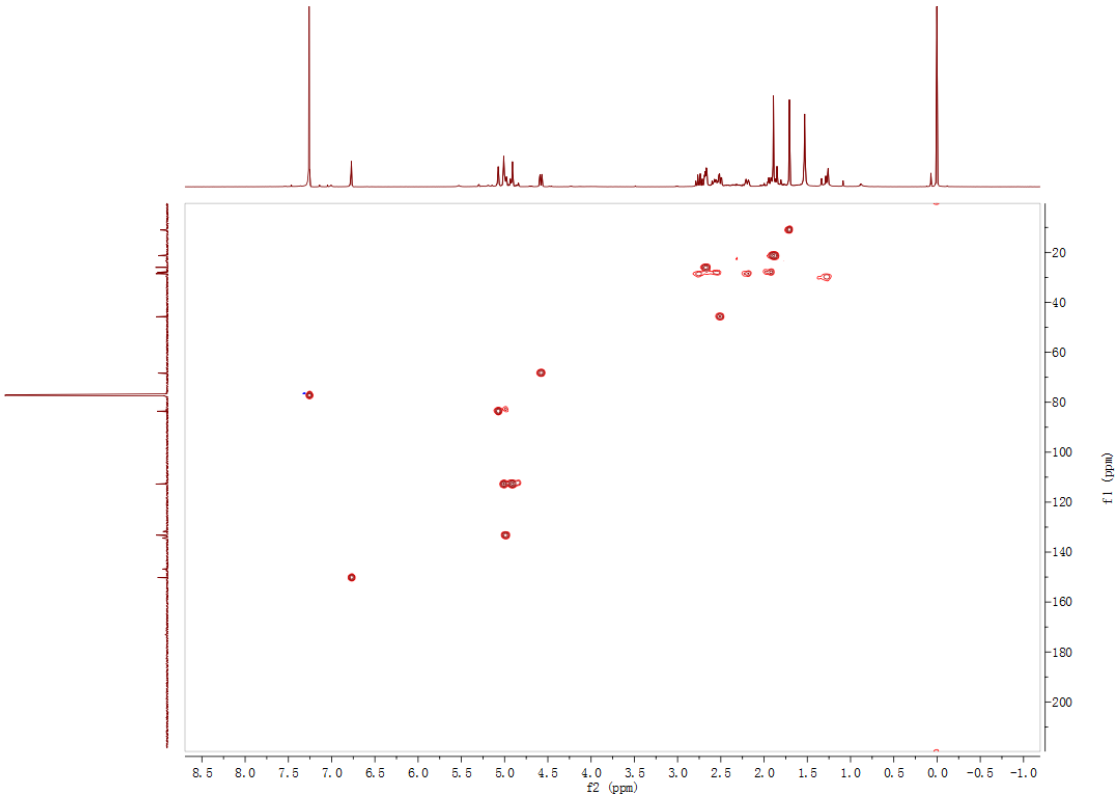
S2.15. ^{1}H NMR spectrum of **4**



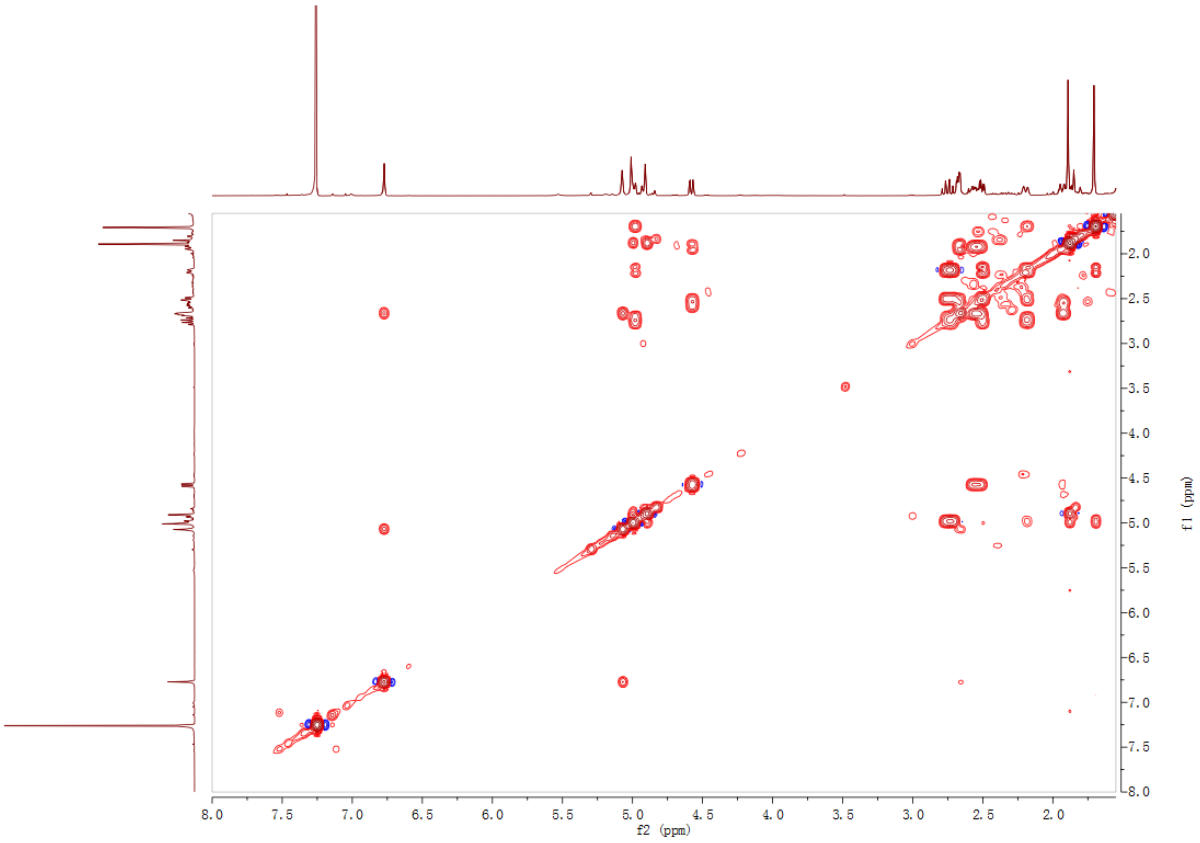
S2.16. ^{13}C NMR and DEPT spectra of **4**



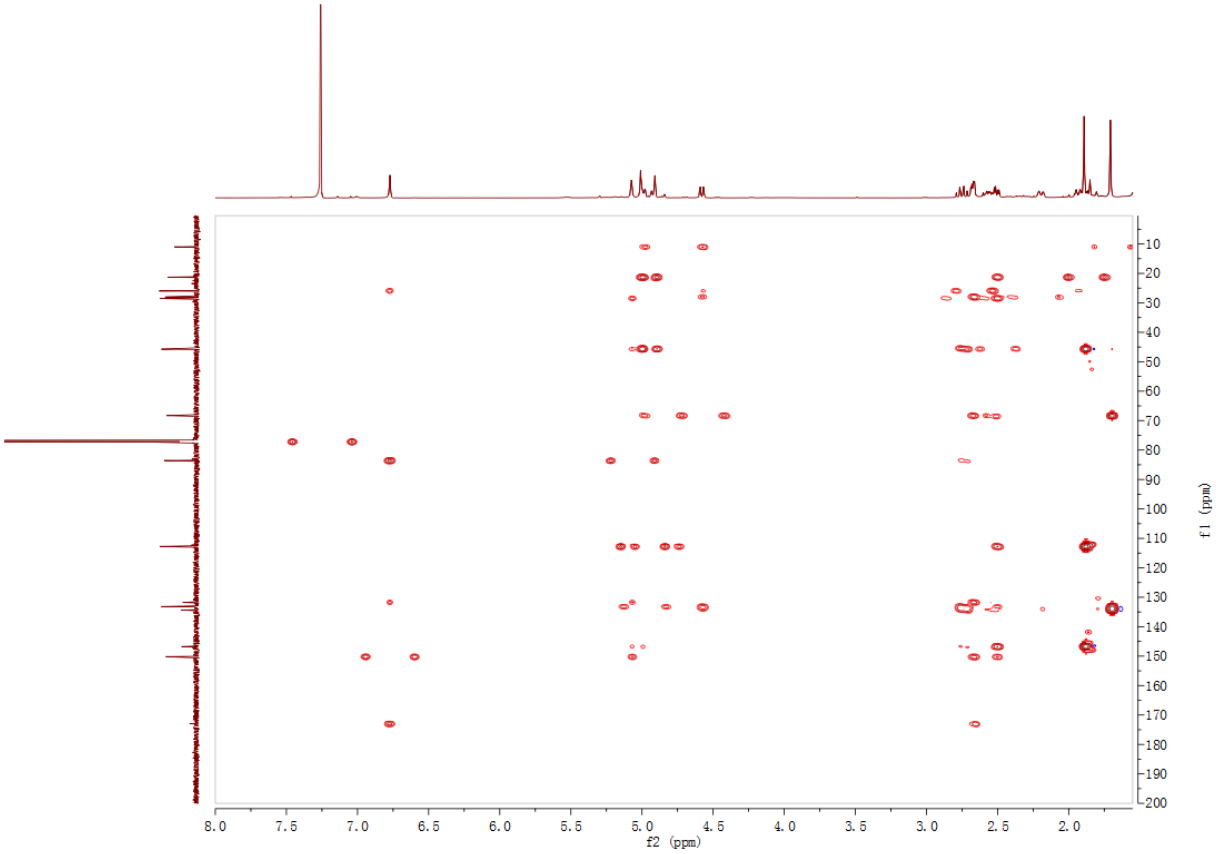
S2.17. HSQC spectrum of **4**



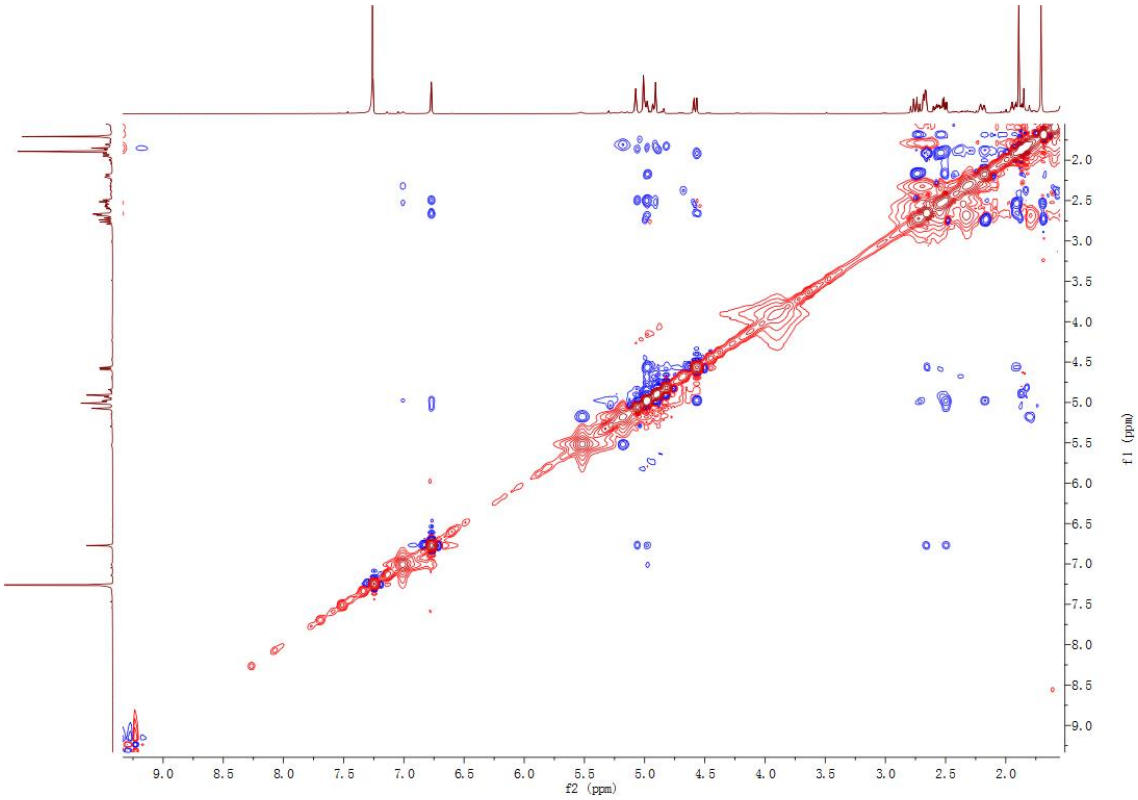
S2.18. ^{1}H–^{1}H COSY spectrum of **4**



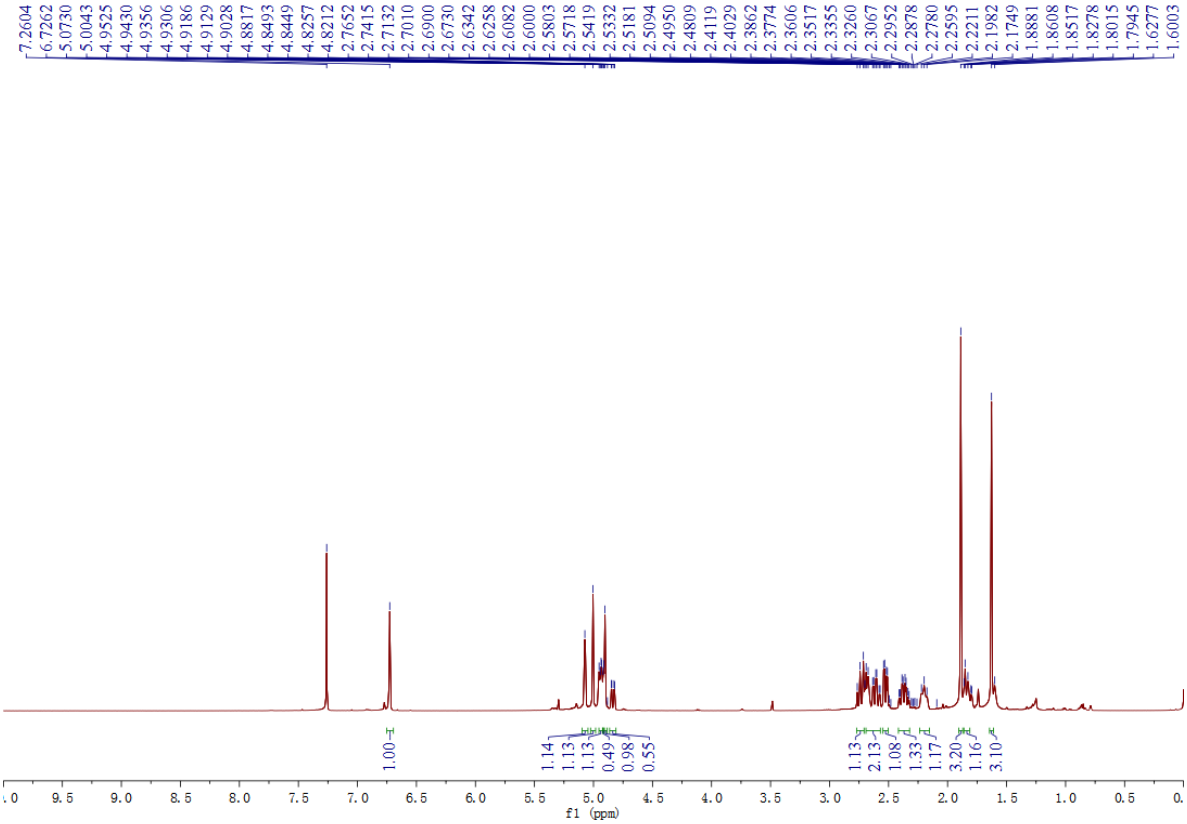
S2.19. HMBC spectrum of **4**



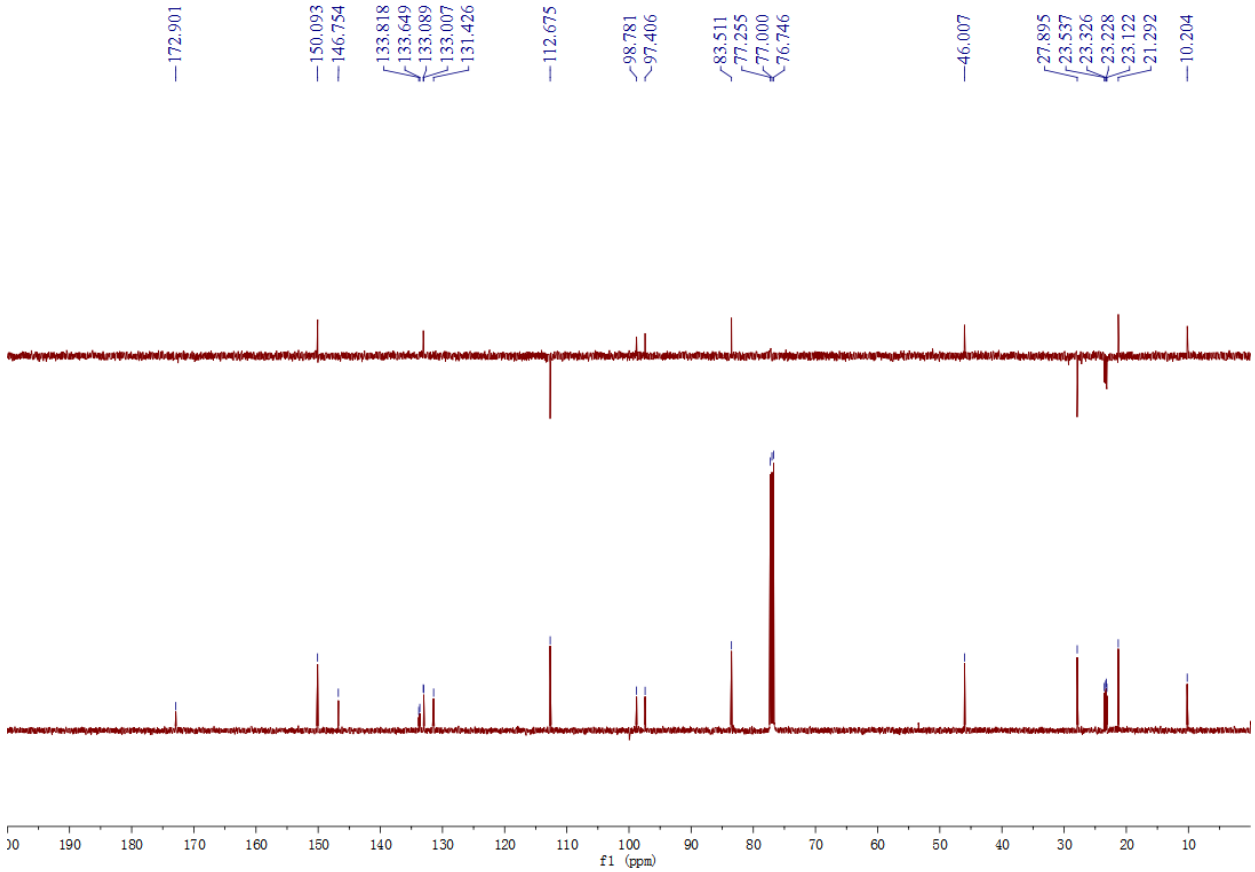
S2.20. NOESY spectrum of **4**



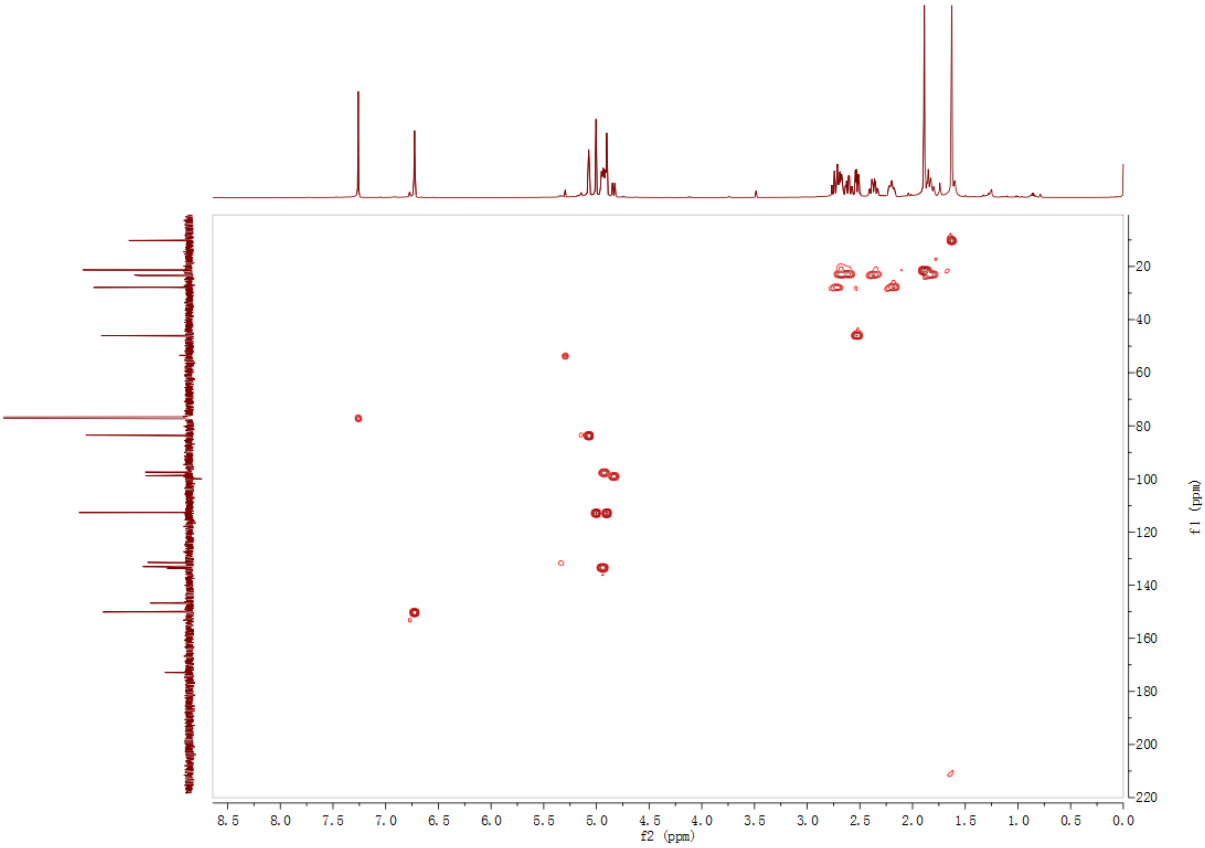
S2.21. ^{1}H NMR spectrum of **5**



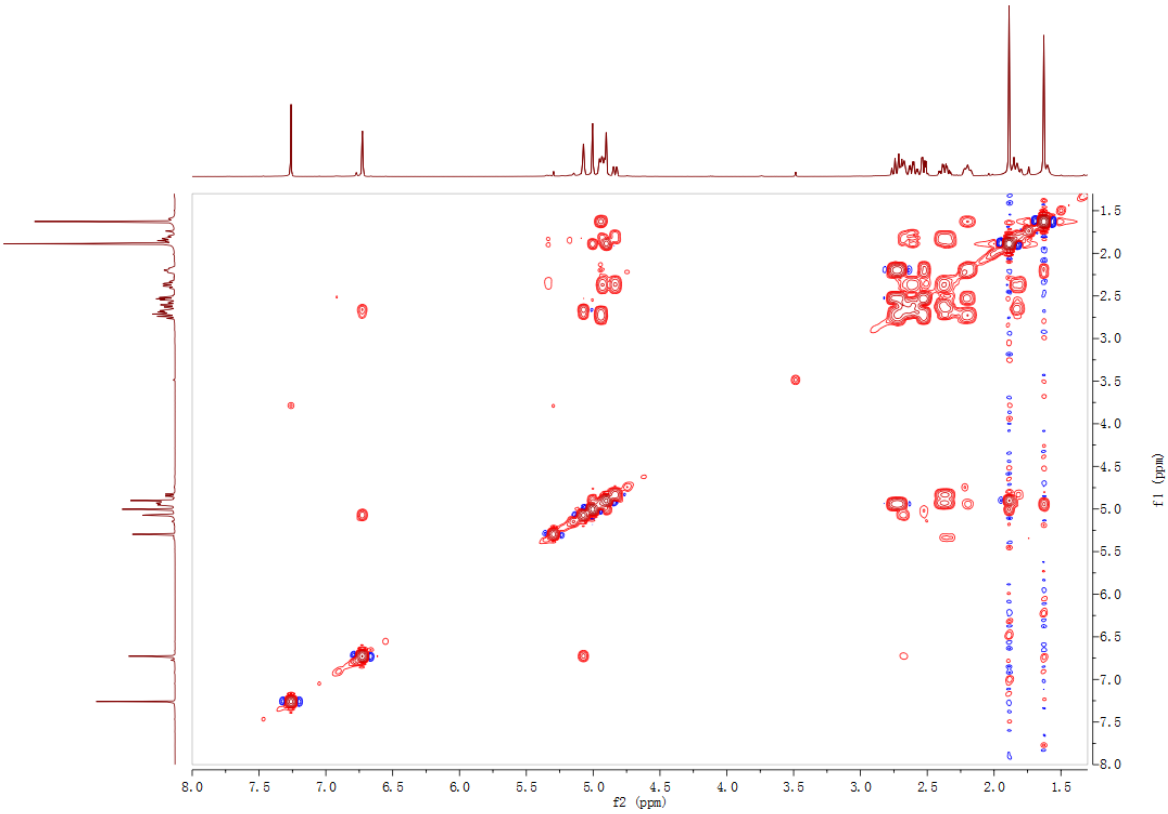
S2.22. ^{13}C NMR and DEPT spectra of **5**



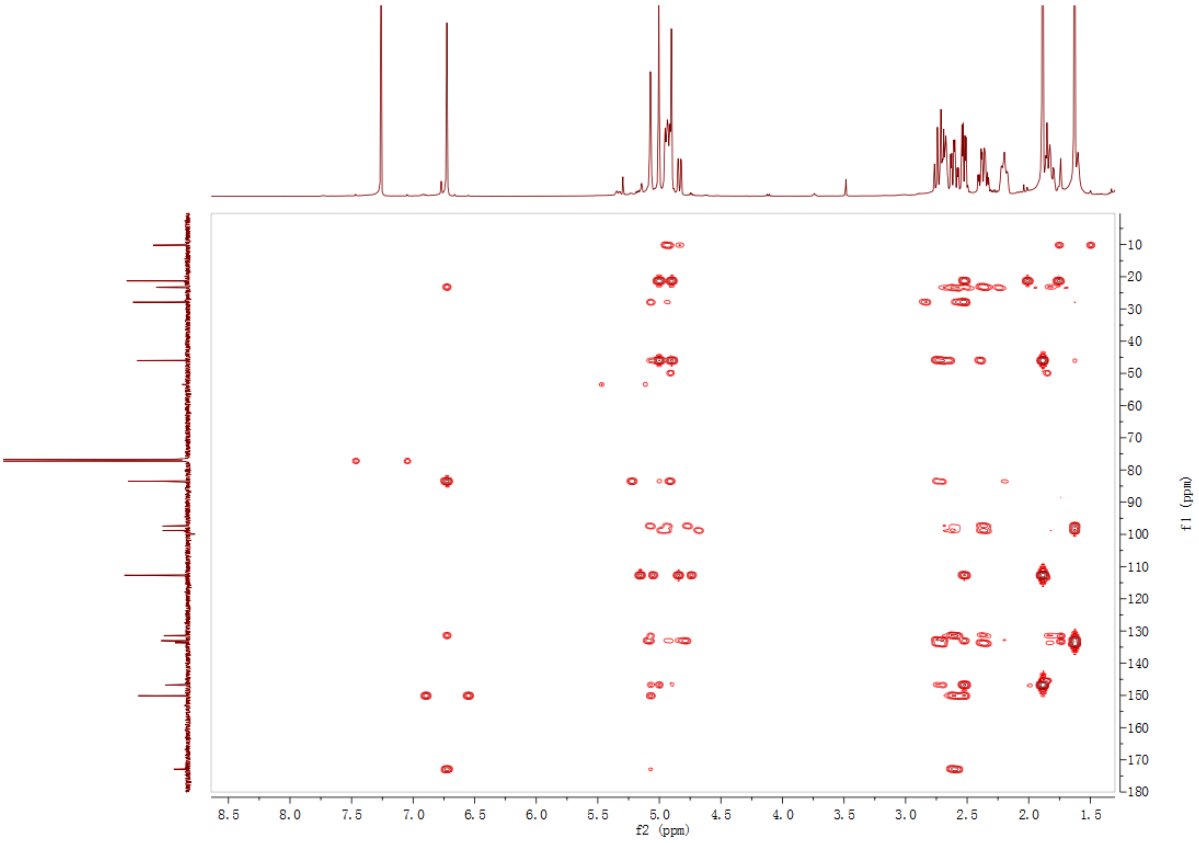
S2.23. HSQC spectrum of **5**



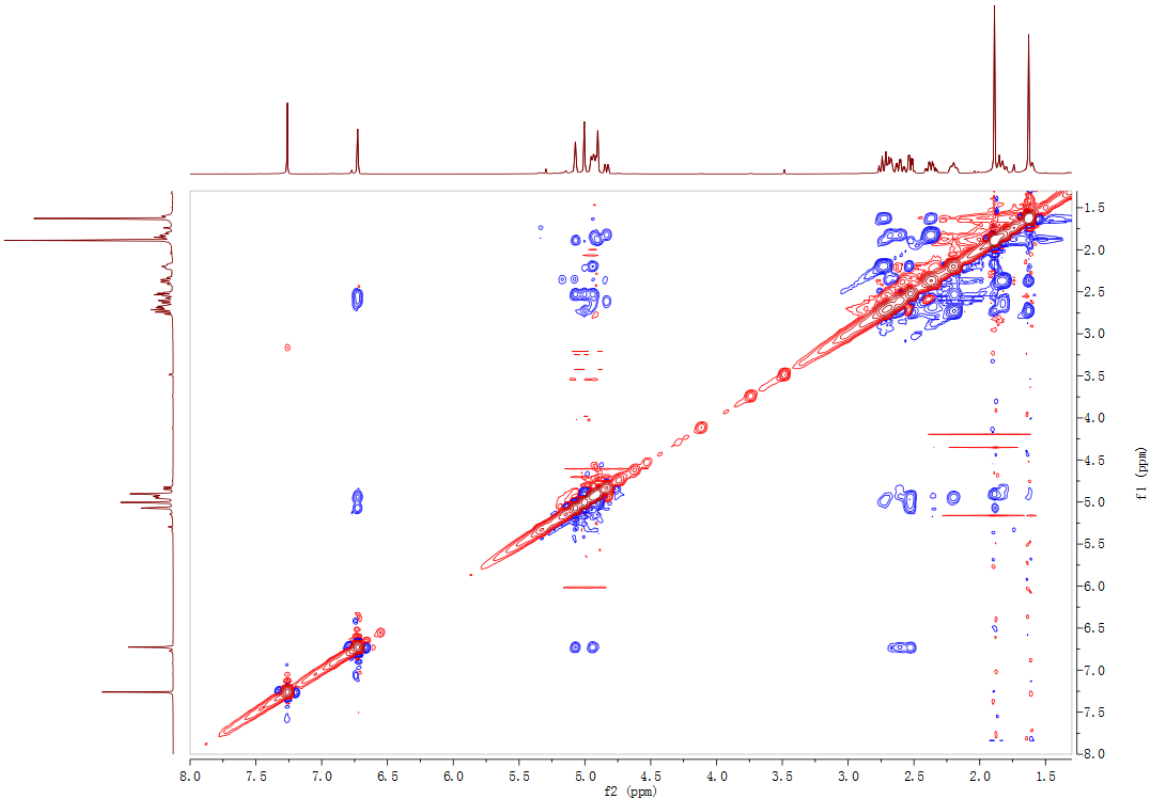
S2.24. ^{1}H–^{1}H COSY spectrum of **5**



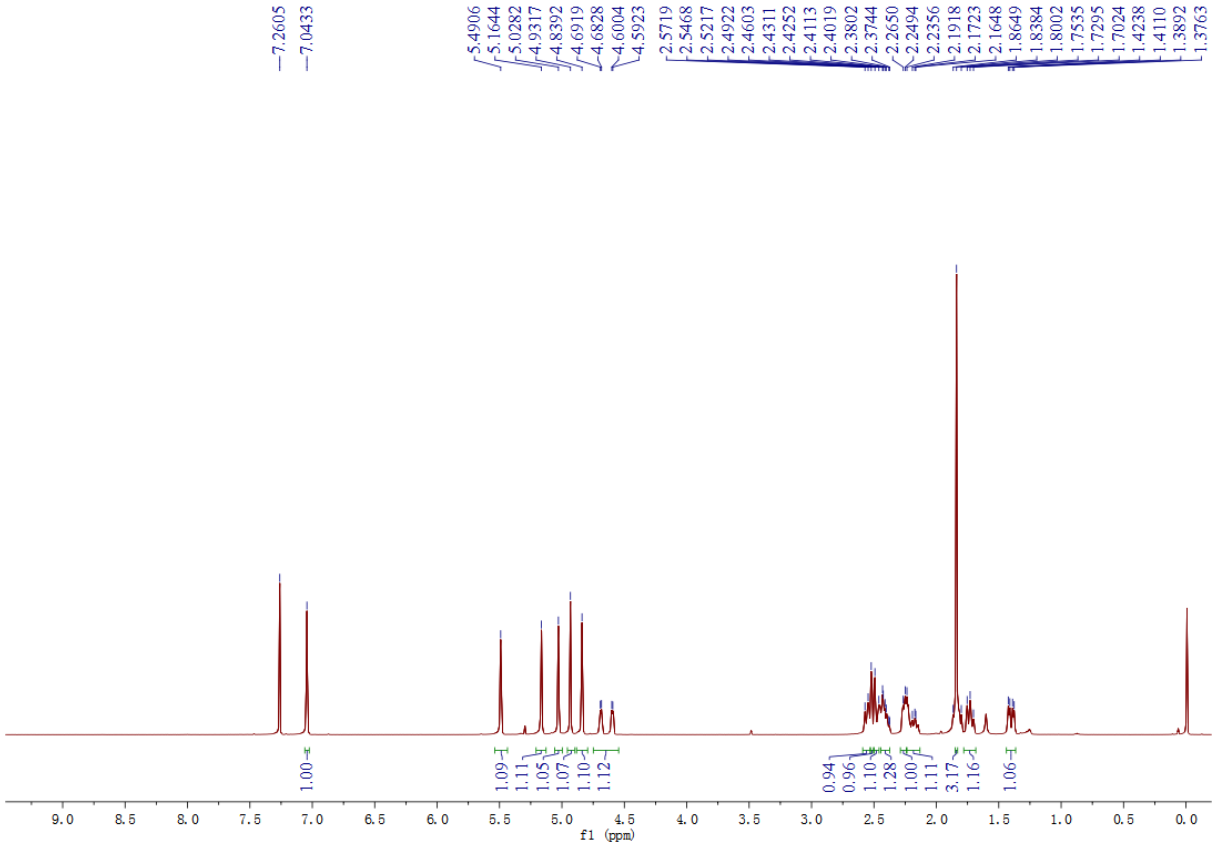
S2.25. HMBC spectrum of **5**



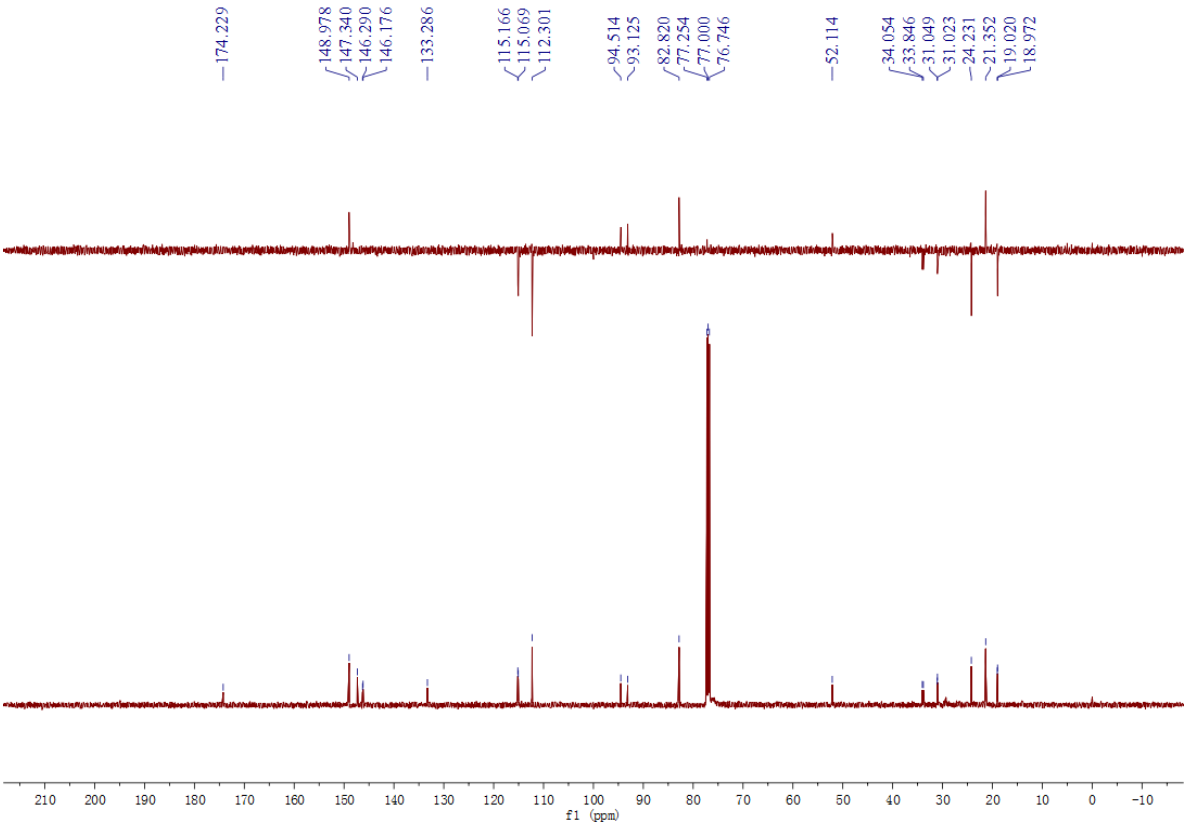
S2.26. NOESY spectrum of **5**



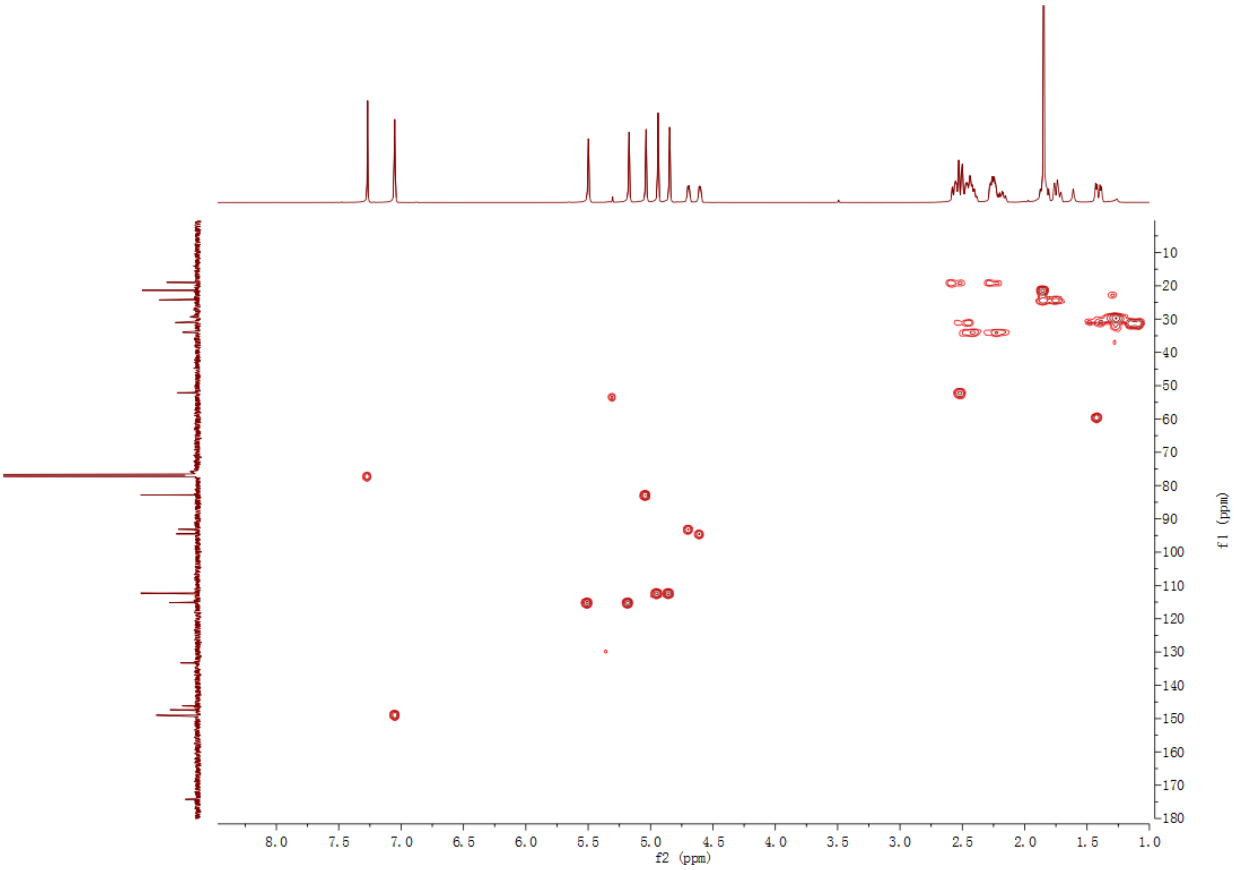
S2.27. ^{1}H NMR spectrum of **6**



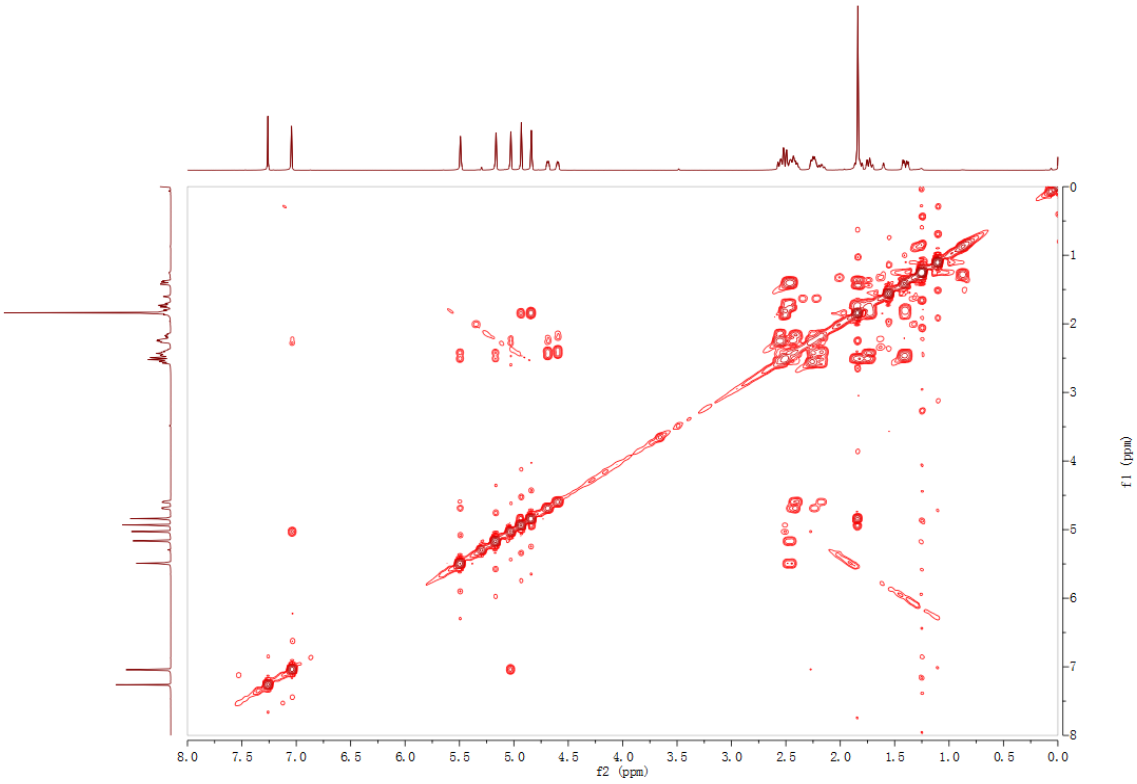
S2.28. ^{13}C NMR and DEPT spectra of **6**



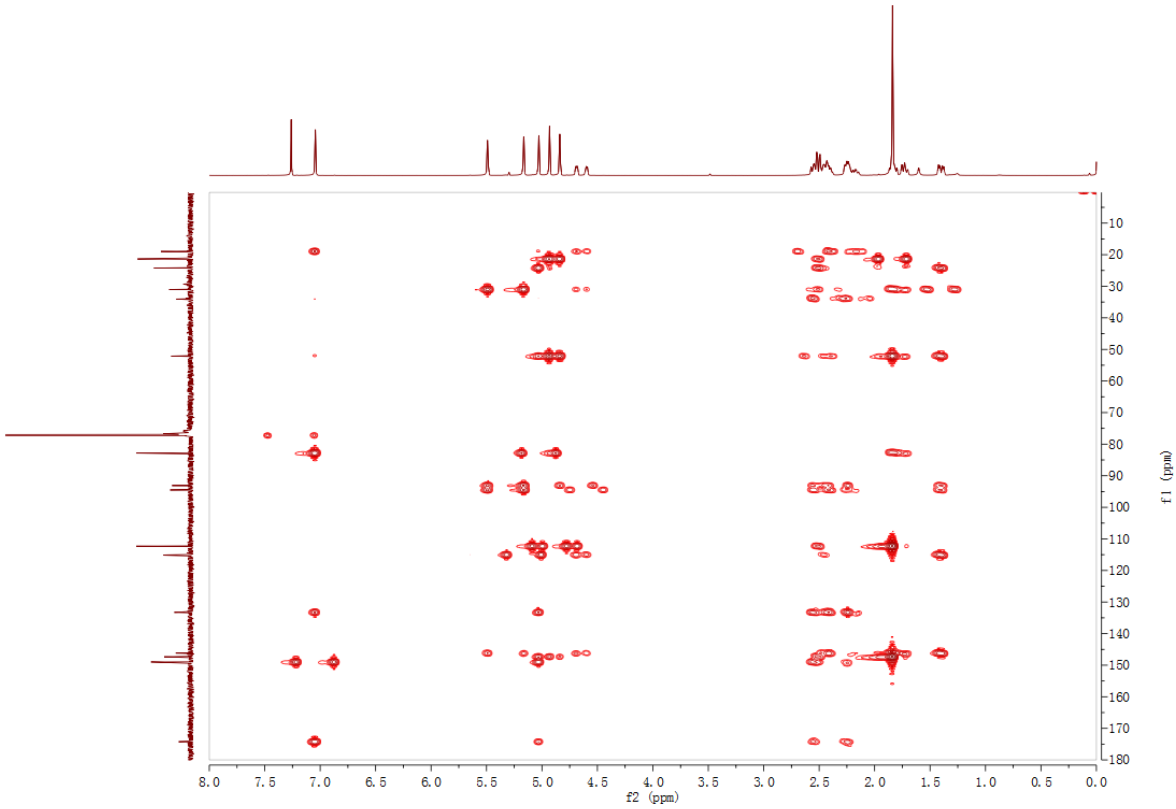
S2.29. HSQC spectrum of **6**



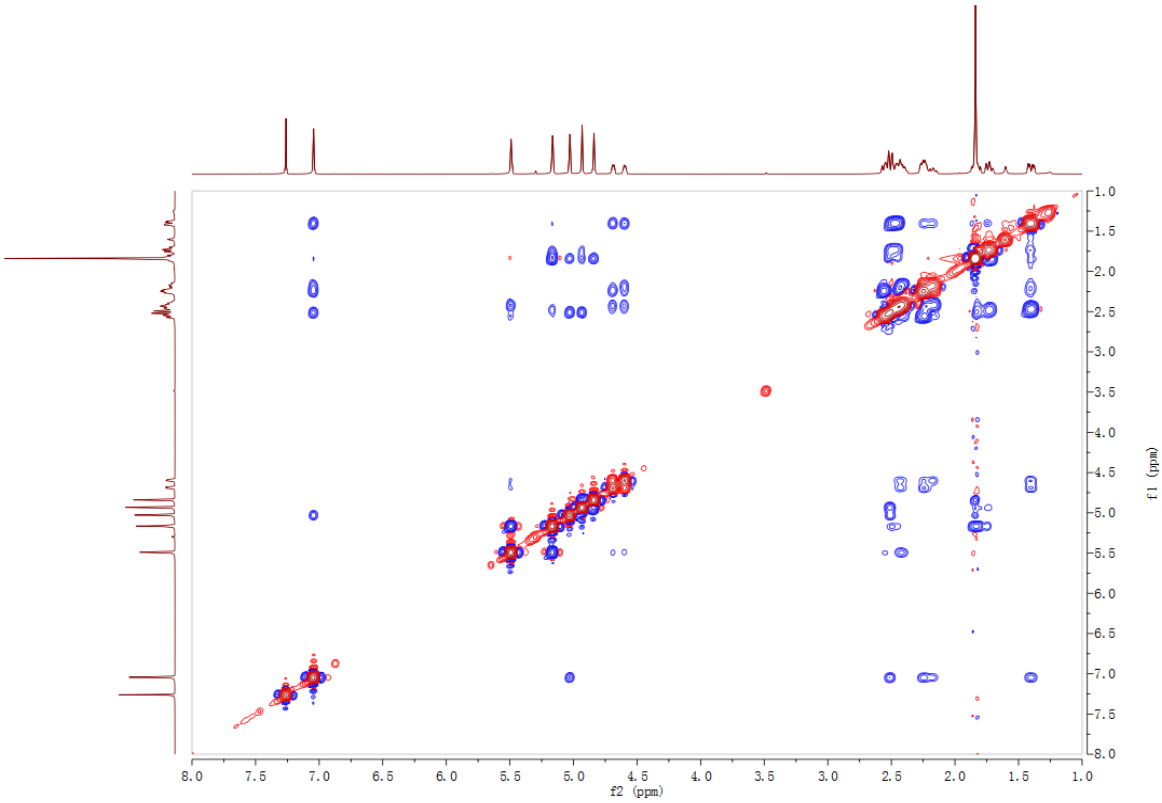
S2.30. ^{1}H–^{1}H COSY spectrum of **6**



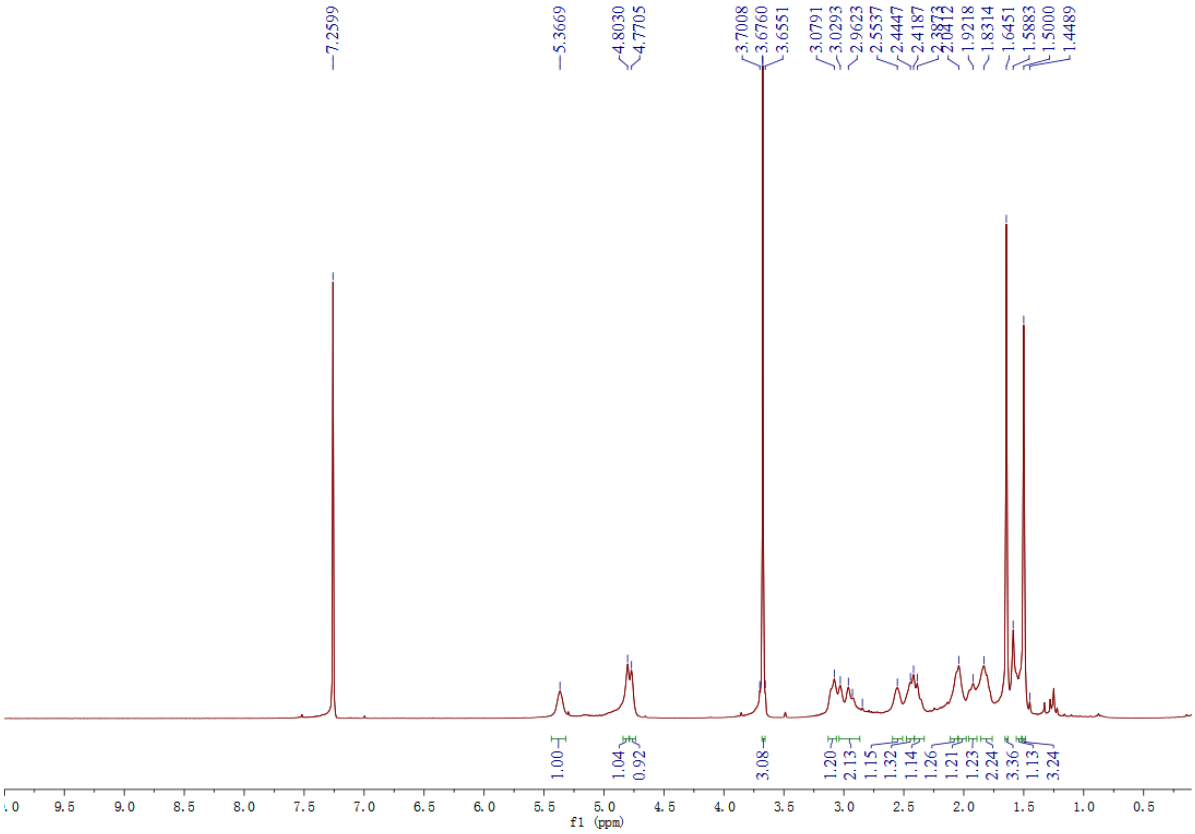
S2.31. HMBC spectrum of **6**



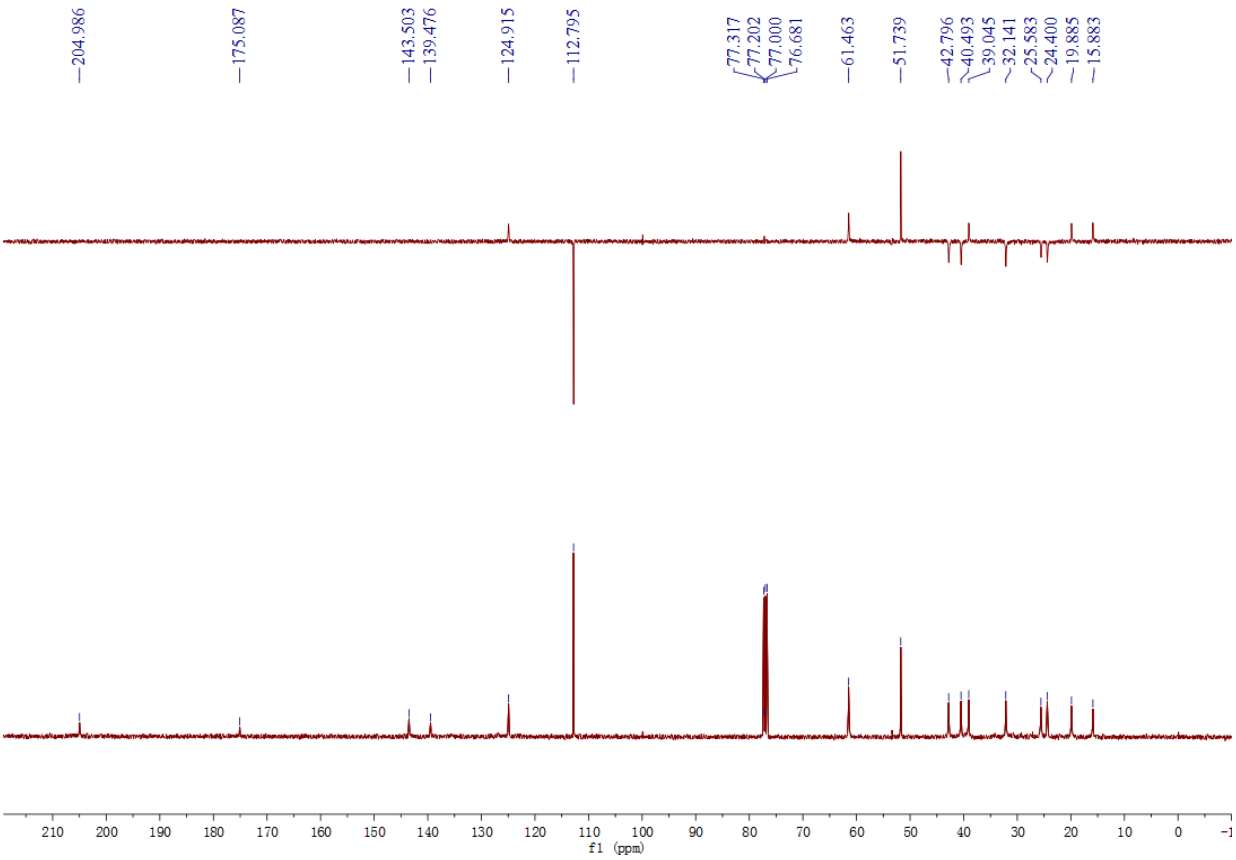
S2.32. NOESY spectrum of **6**



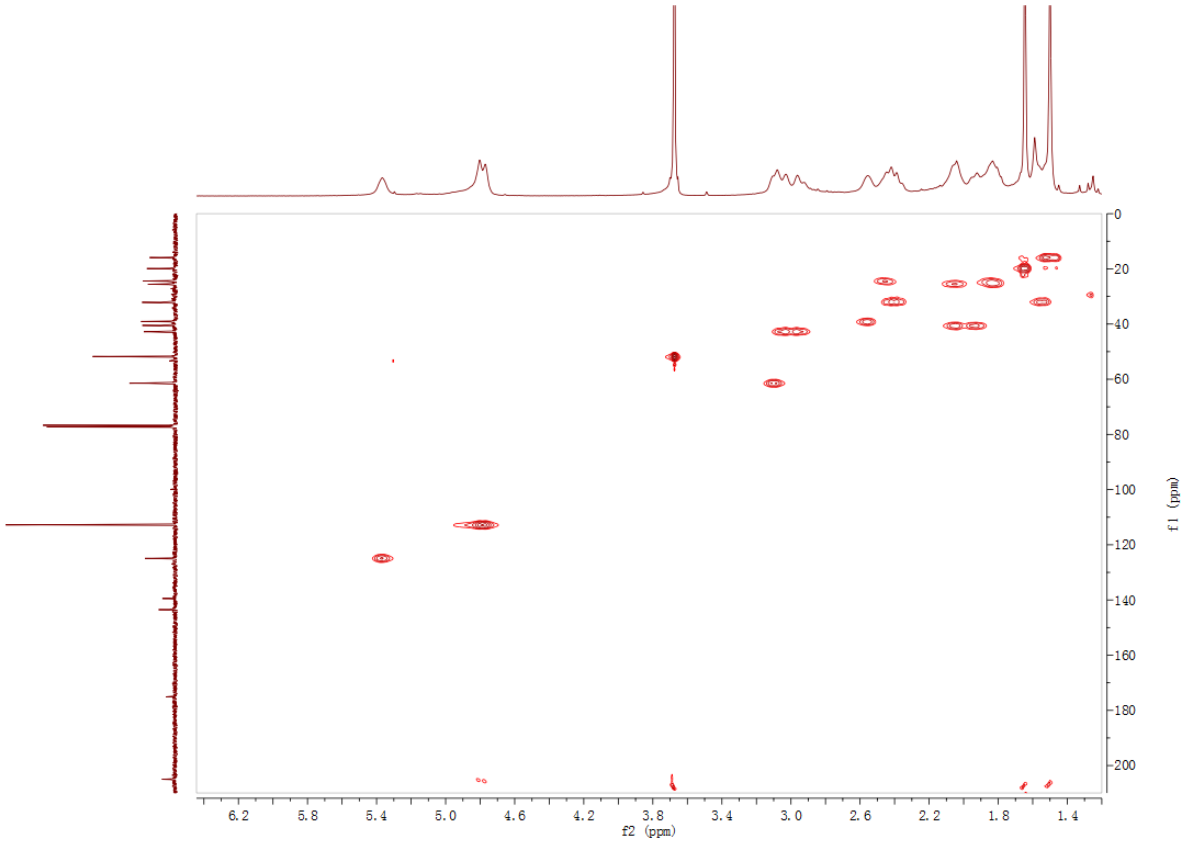
S2.33. ^{1}H NMR spectrum of **7**



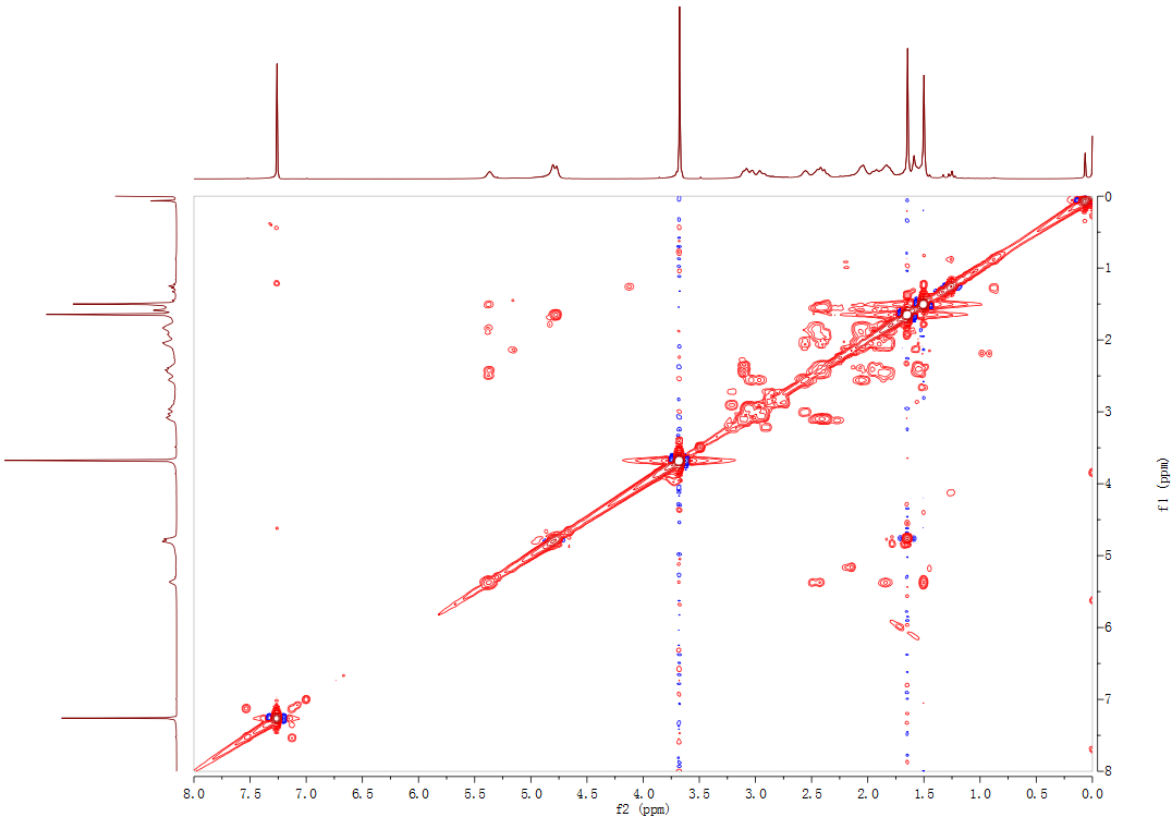
S2.34. ^{13}C NMR and DEPT spectra of **7**



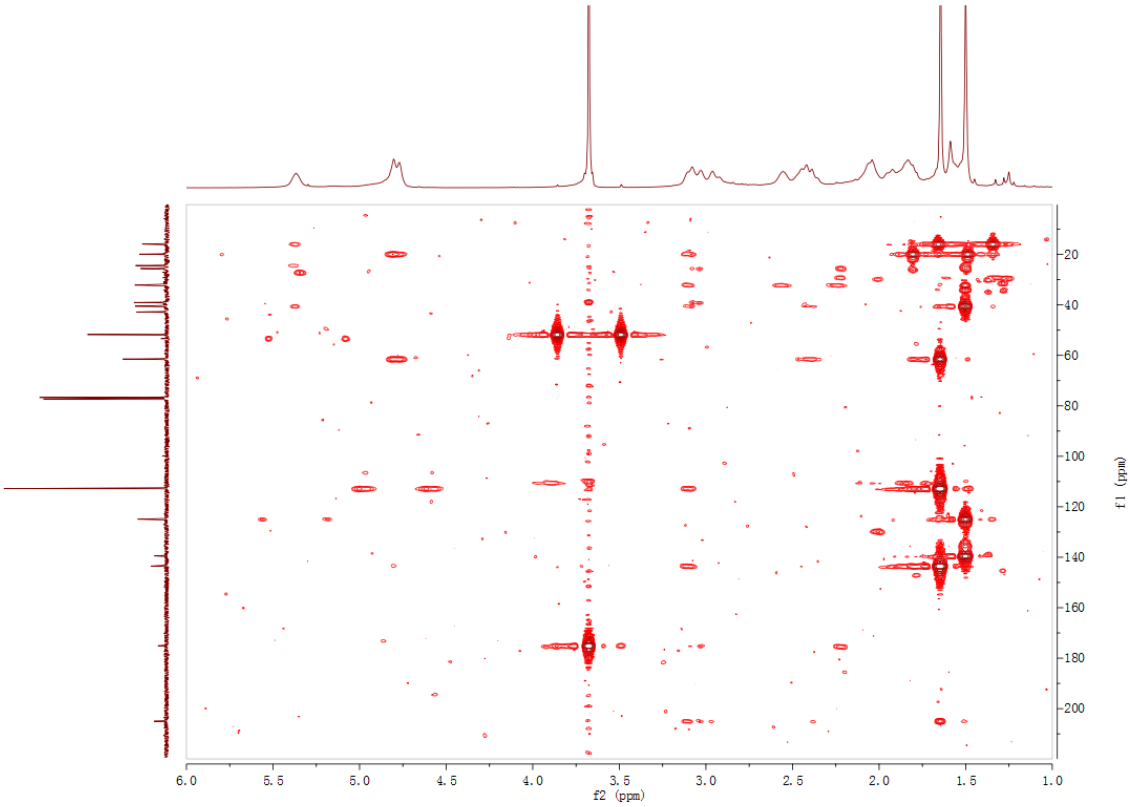
S2.35. HSQC spectrum of **7**



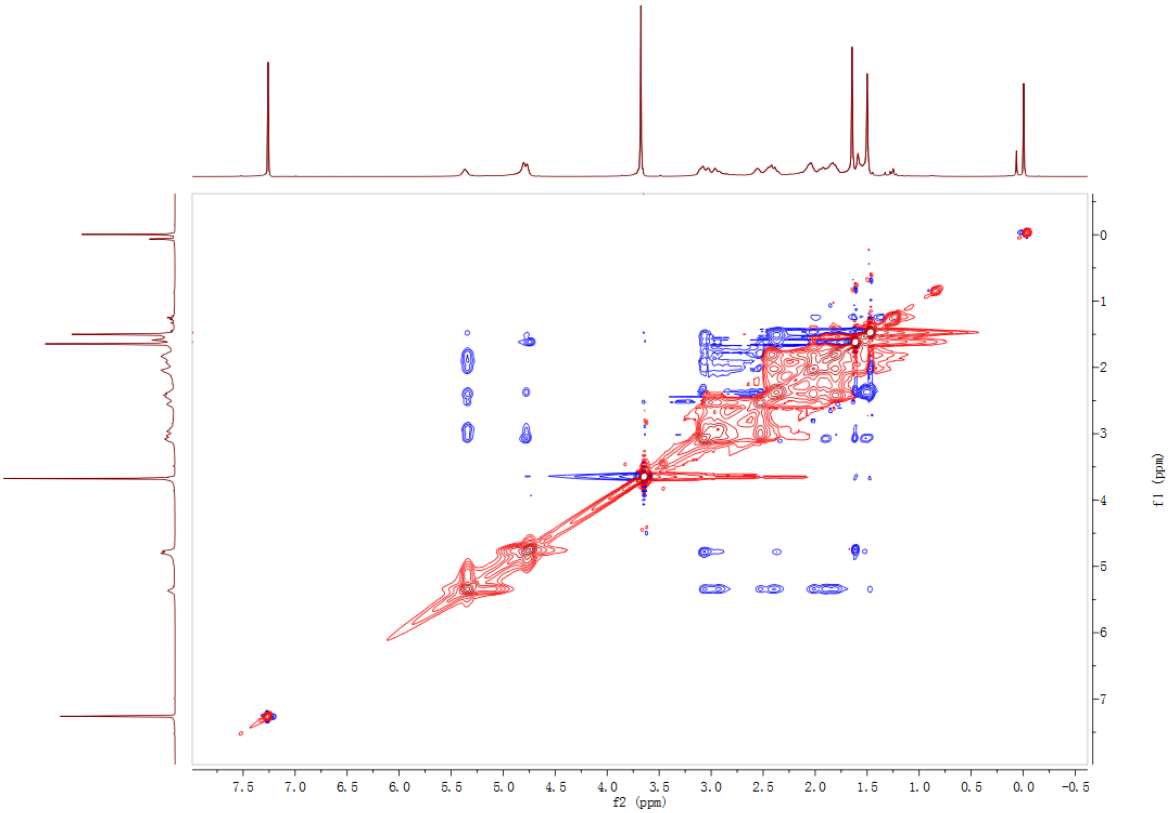
S2.36. ^{1}H–^{1}H COSY spectrum of **7**



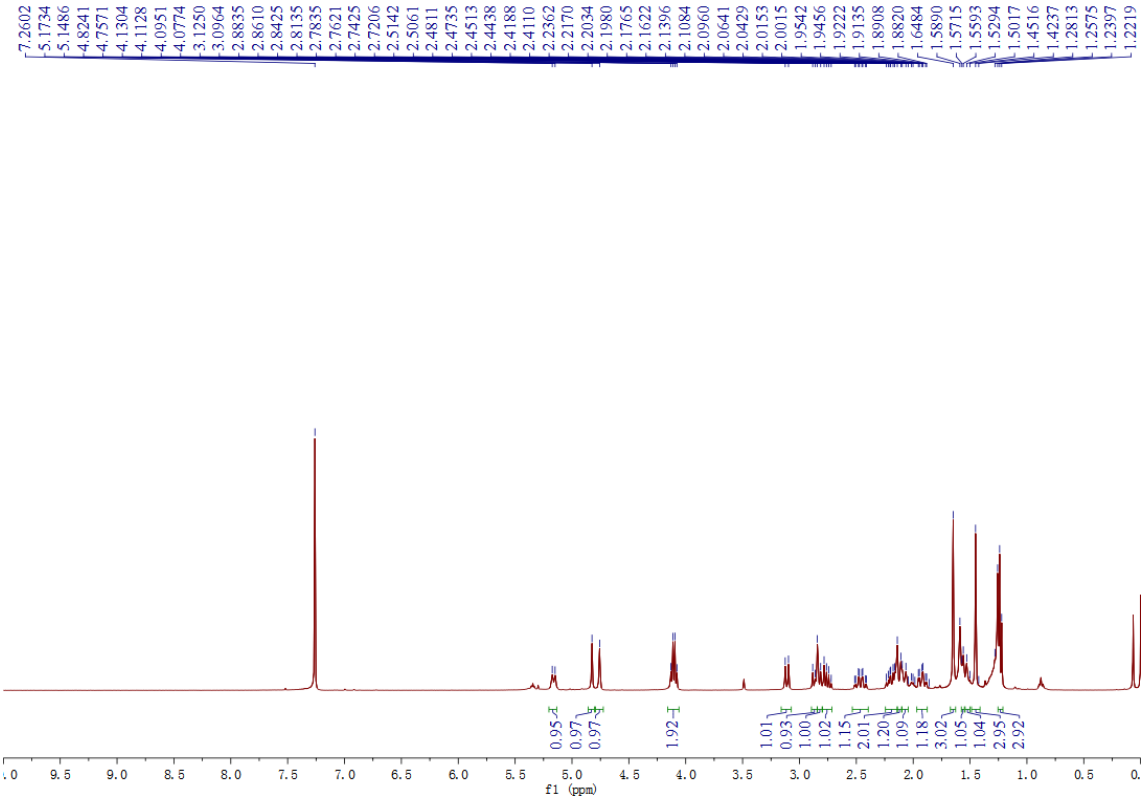
S2.37. HMBC spectrum of **7**



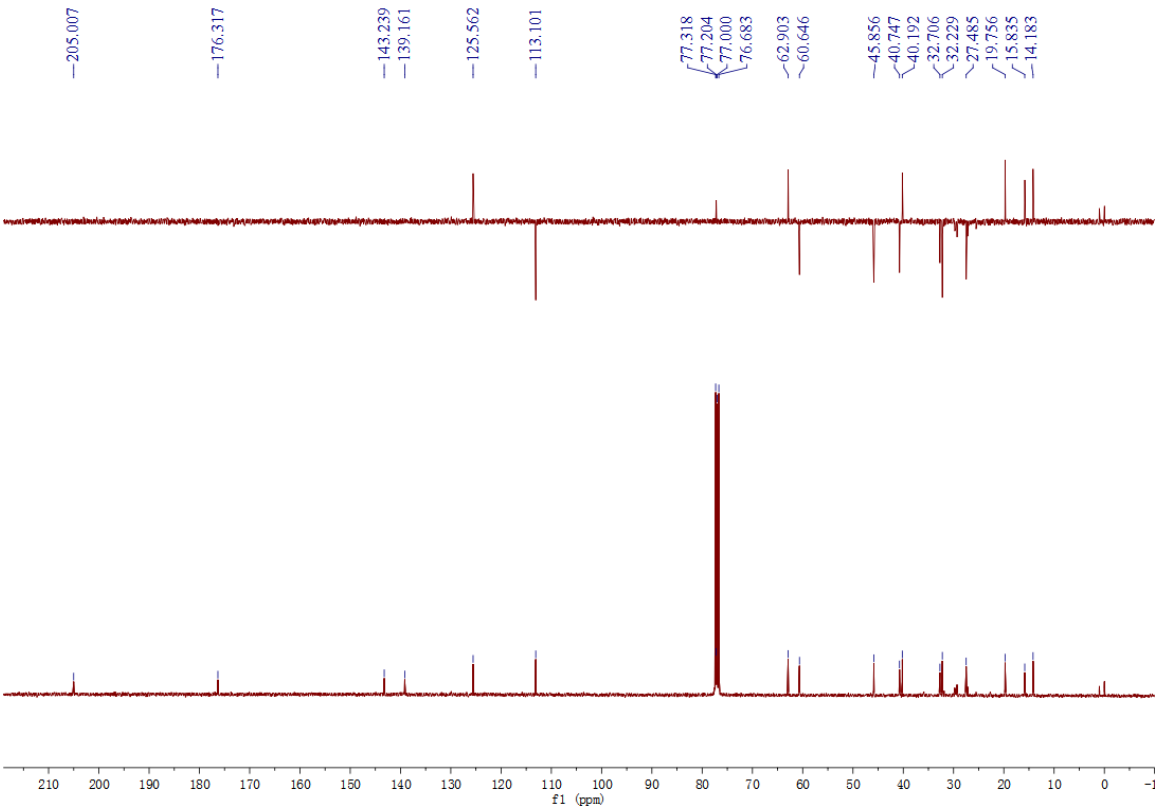
S2.38. NOESY spectrum of **7**



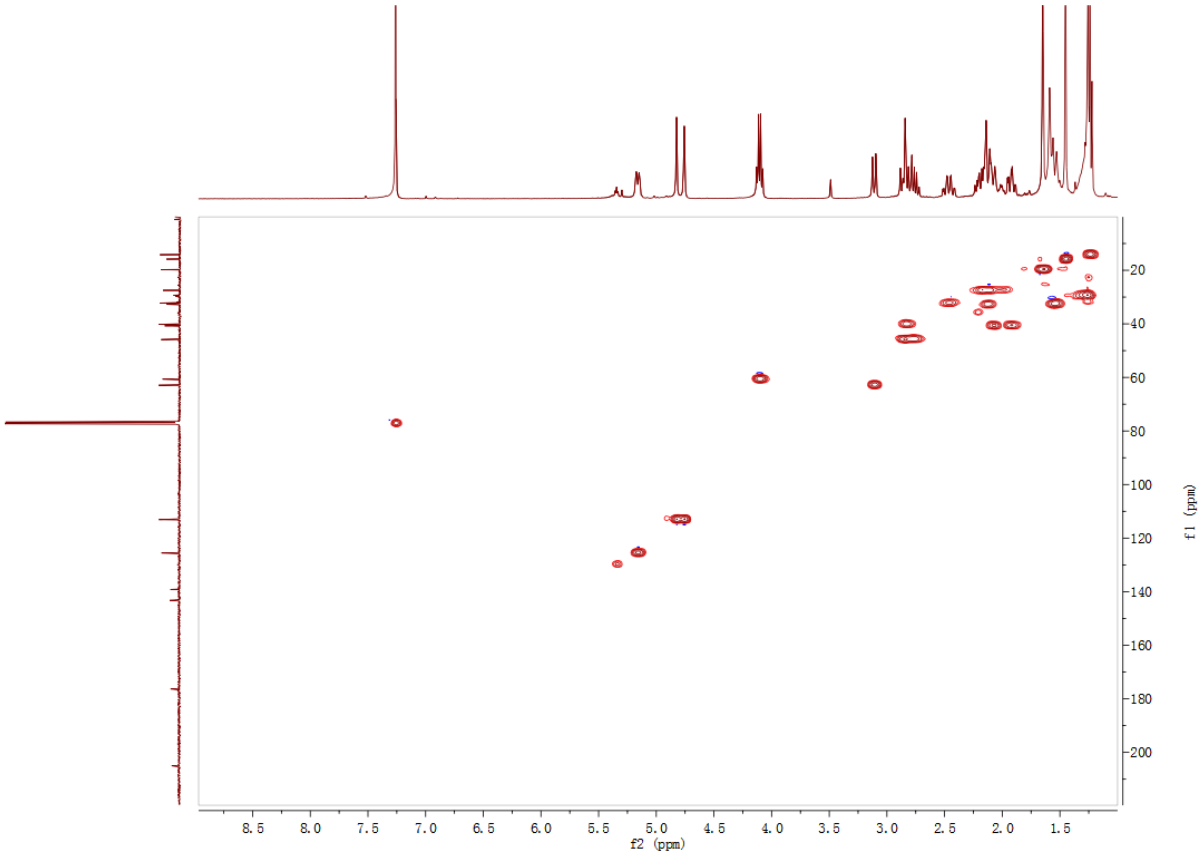
S2.39. ^{1}H NMR spectrum of **8**



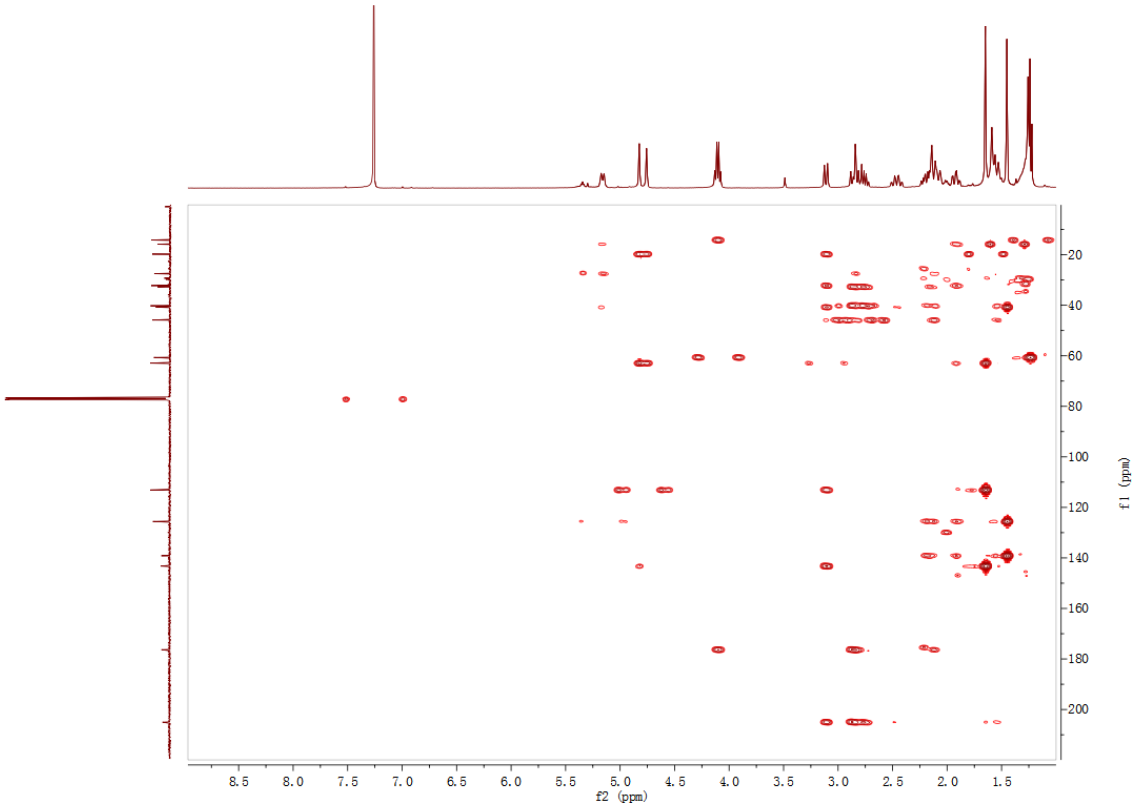
S2.40. ^{13}C NMR and DEPT spectra of **8**



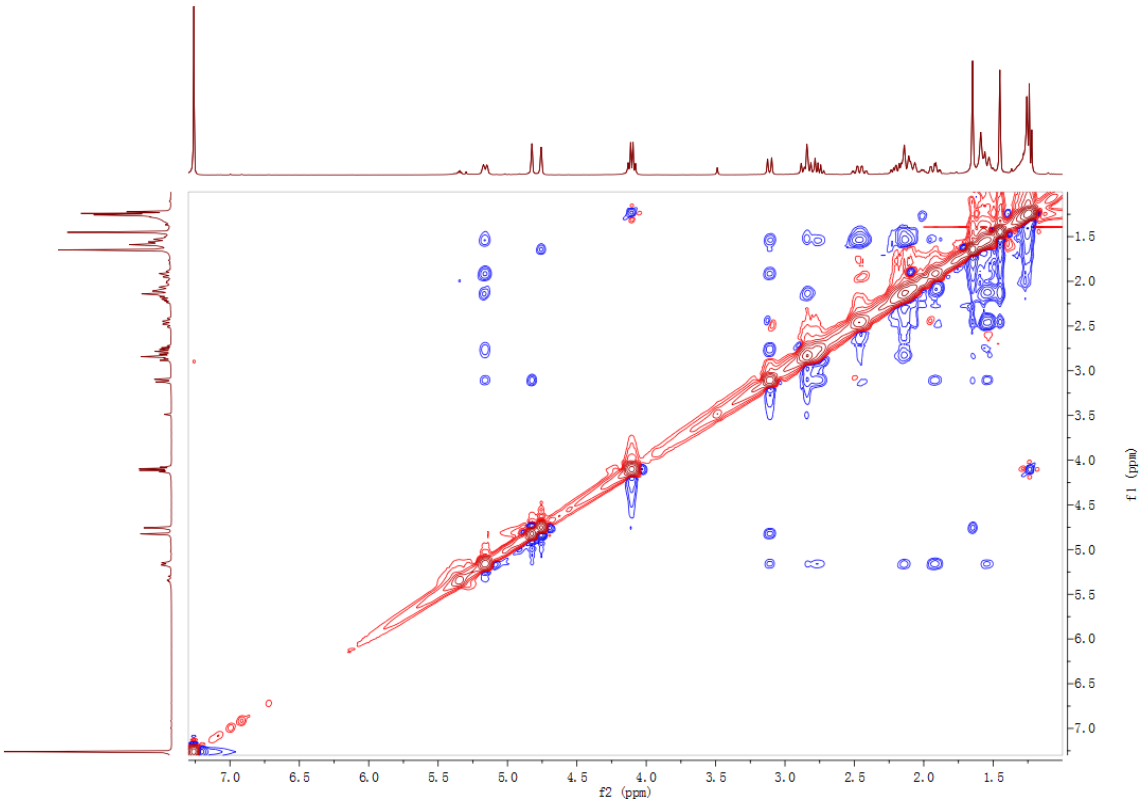
S2.41. HSQC spectrum of **8**



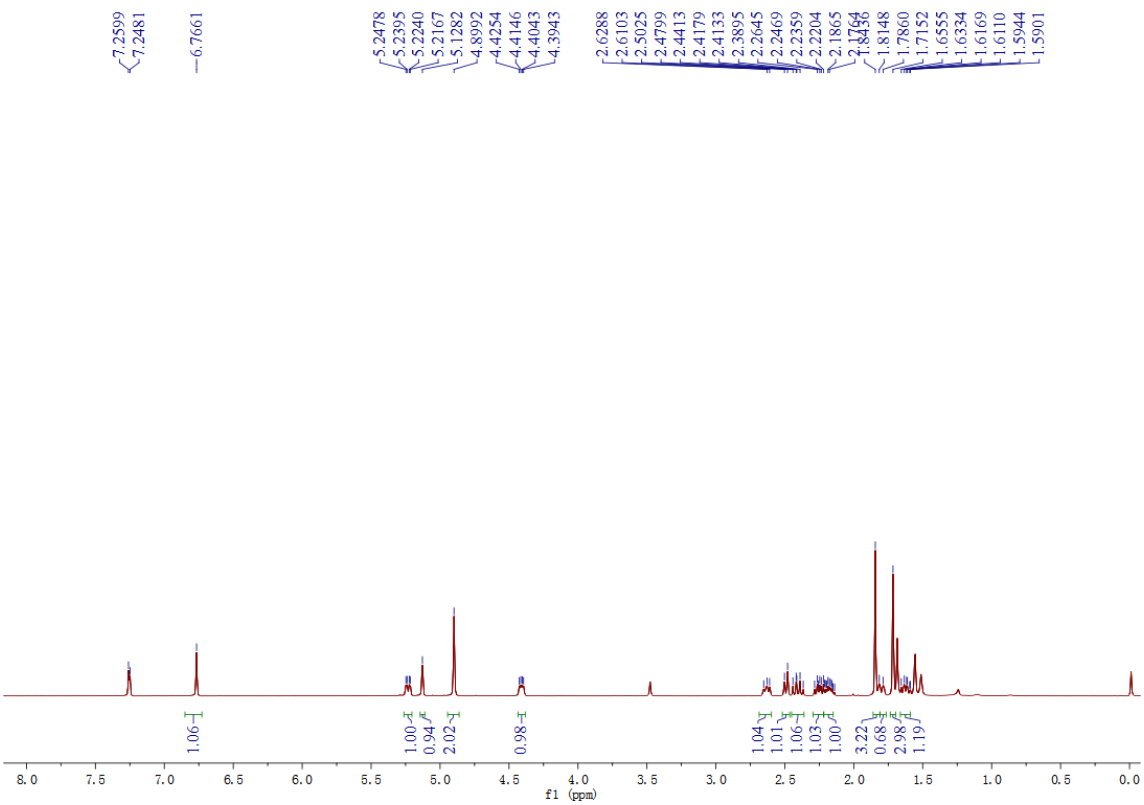
S2.42. HMBC spectrum of **8**



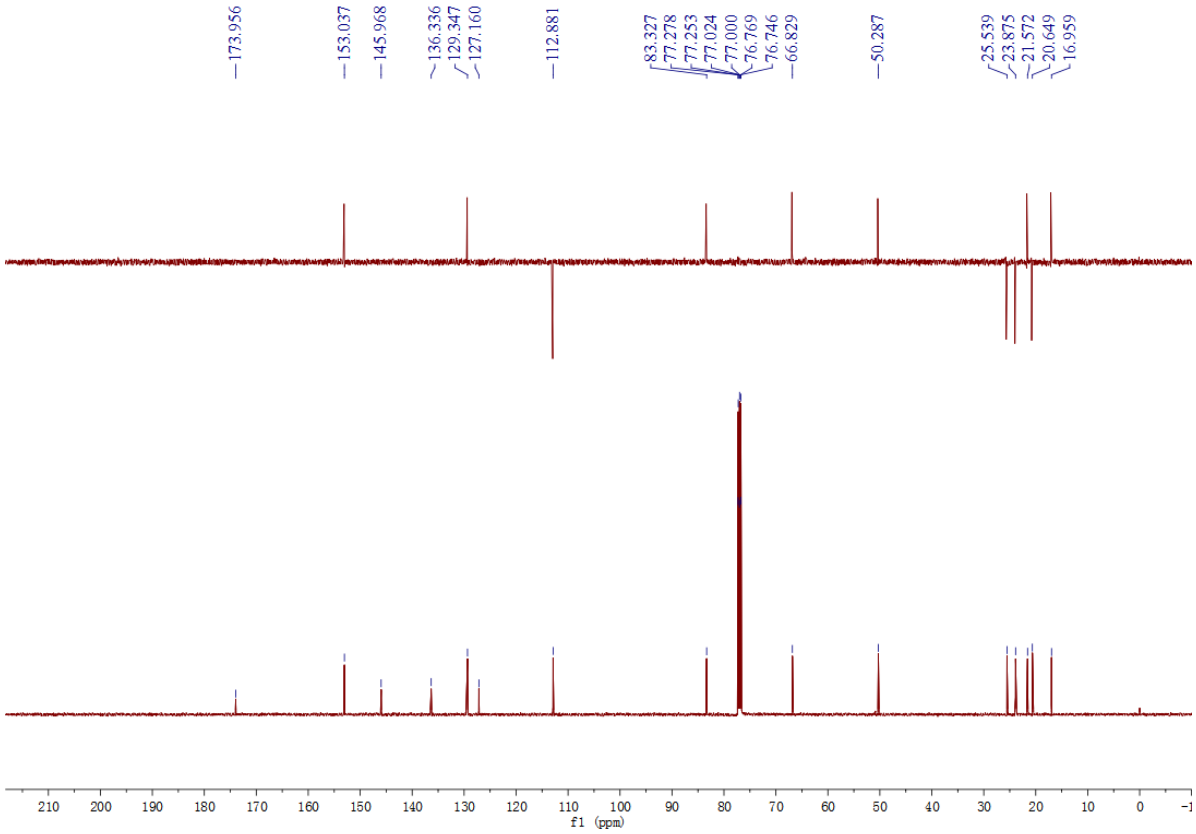
S2.43. NOESY spectrum of **8**



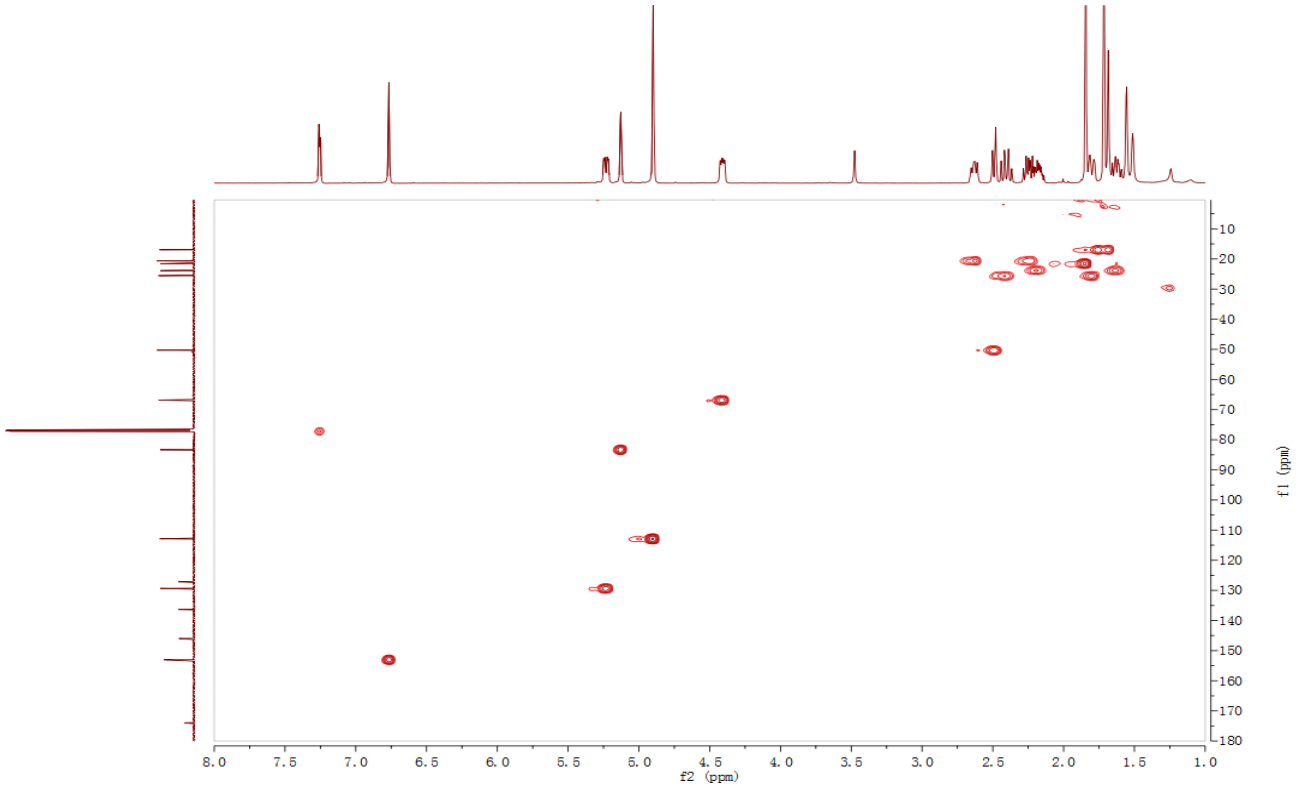
S2.44. ^{1}H NMR spectrum of **9**



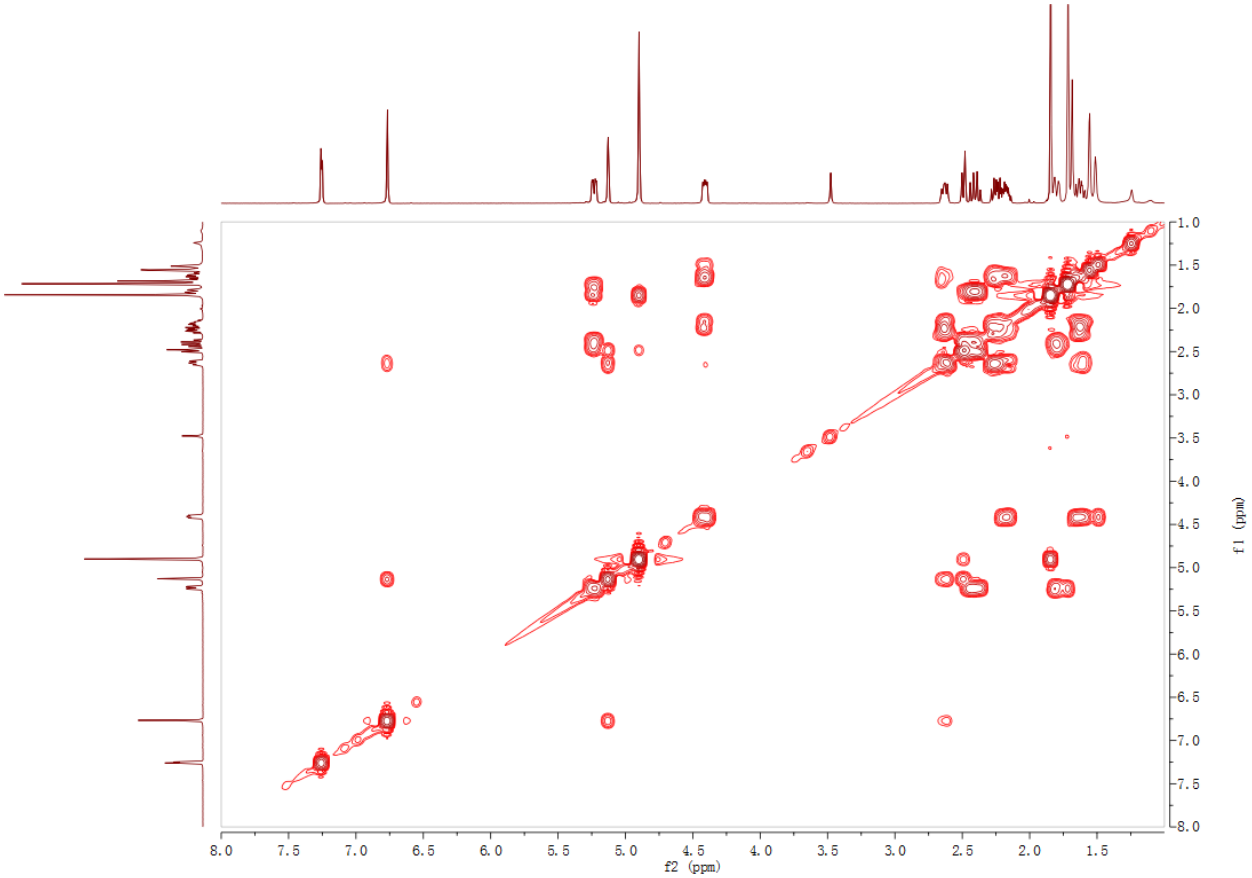
S2.45. ^{13}C NMR and DEPT spectra of **9**



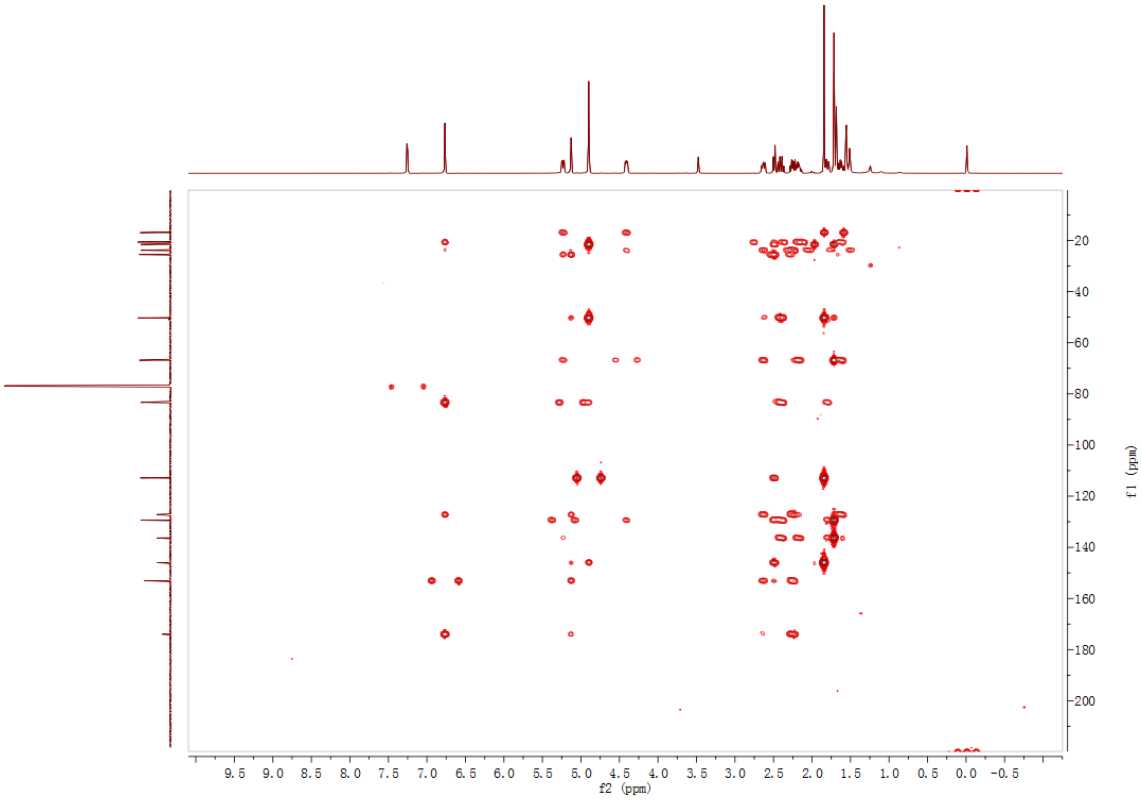
S2.46.HSQC spectrum of **9**



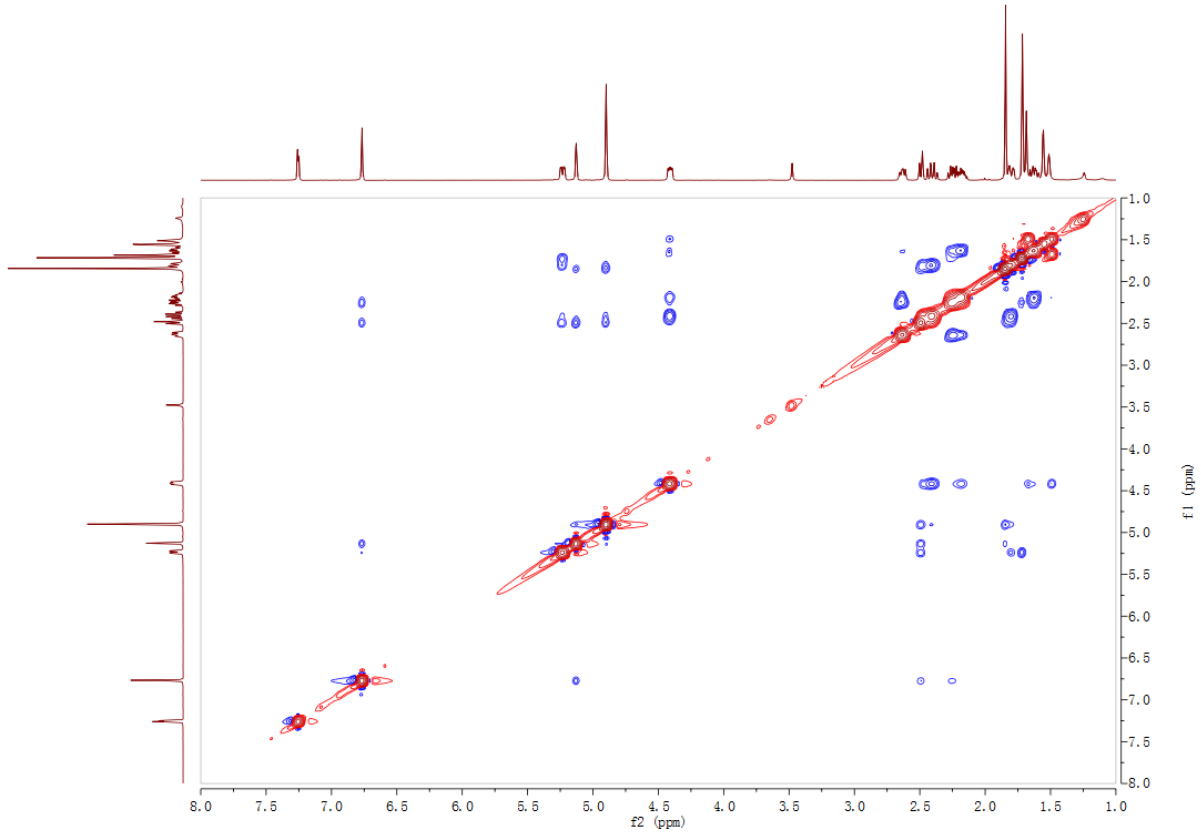
S2.47. $^{1}H–^{1}H$ COSY spectrum of **9**



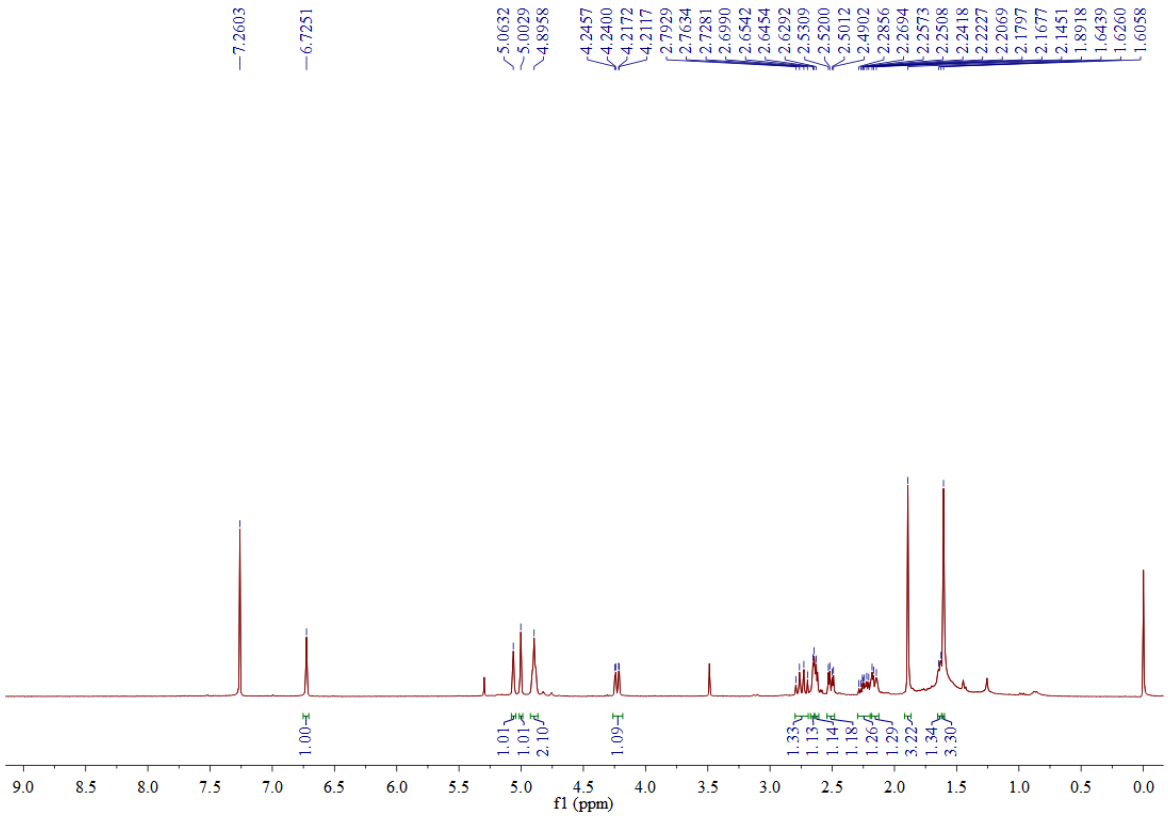
S2.48. HMBC spectrum of **9**



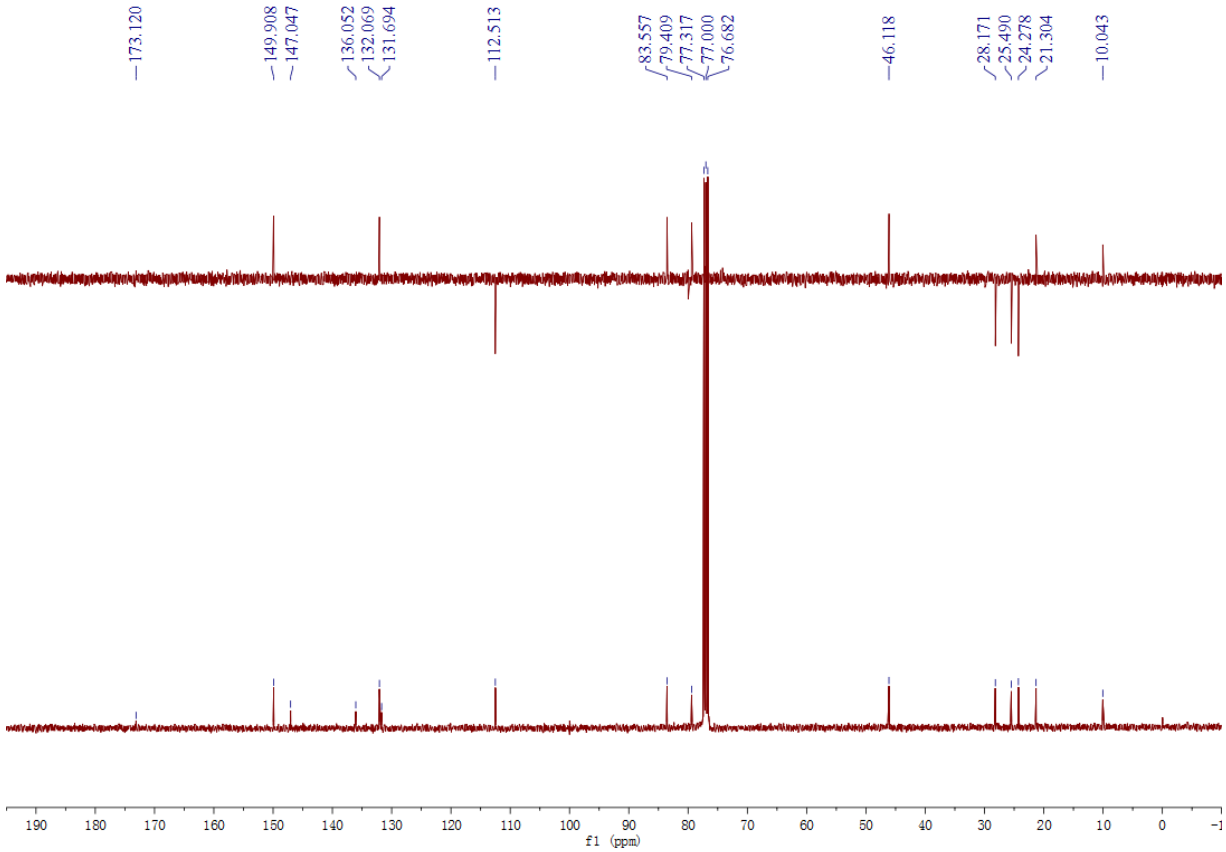
S2.49. NOESY spectrum of **9**



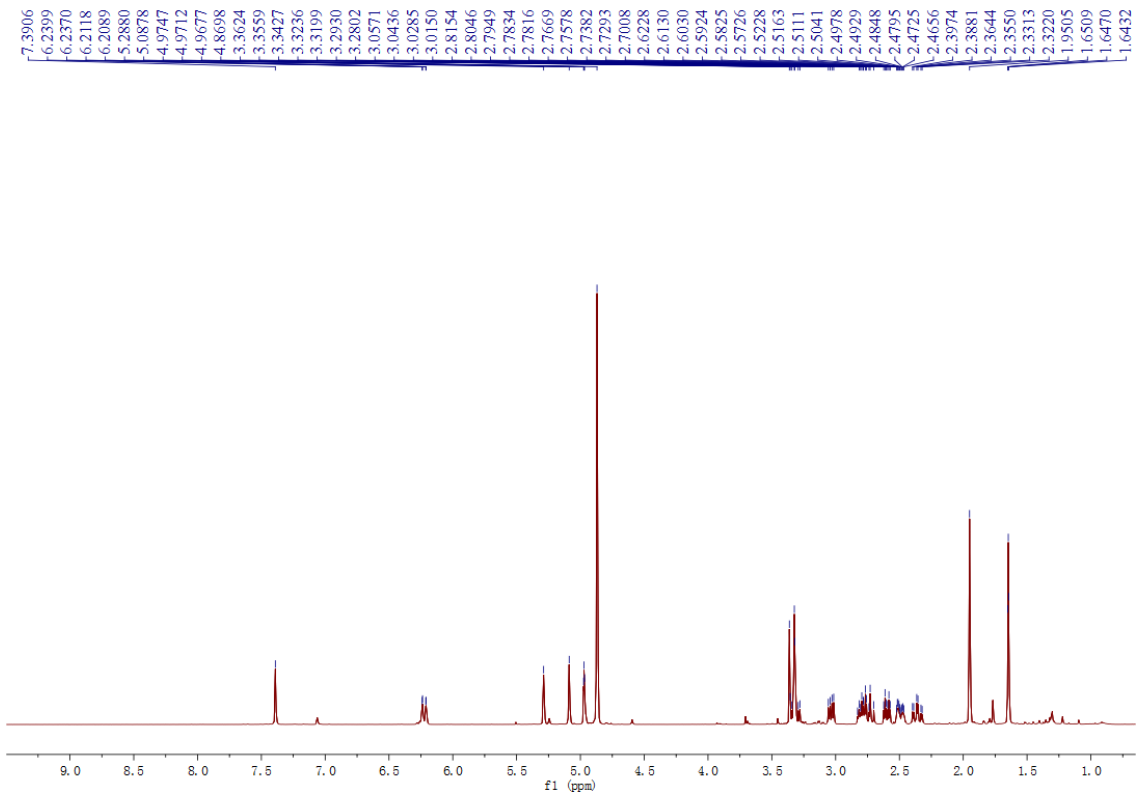
S2.50. ^{1}H NMR spectrum of **10**



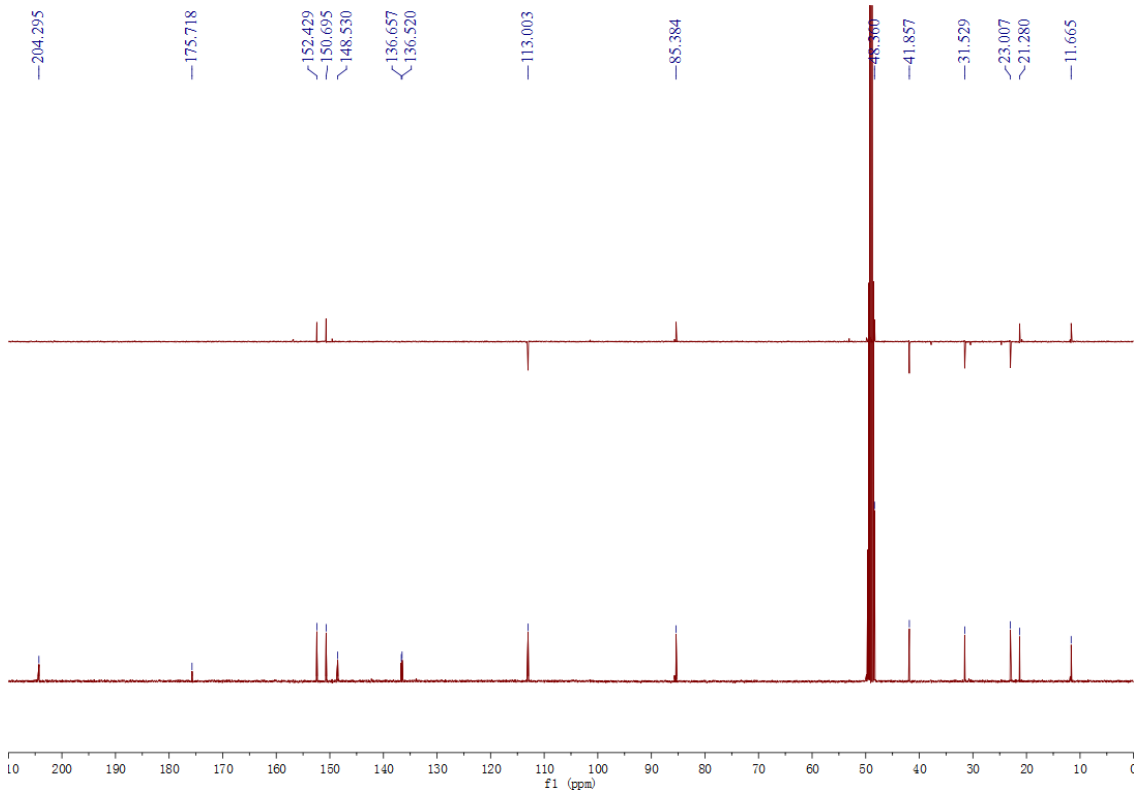
S2.51. ^{13}C NMR and DEPT spectra of **10**



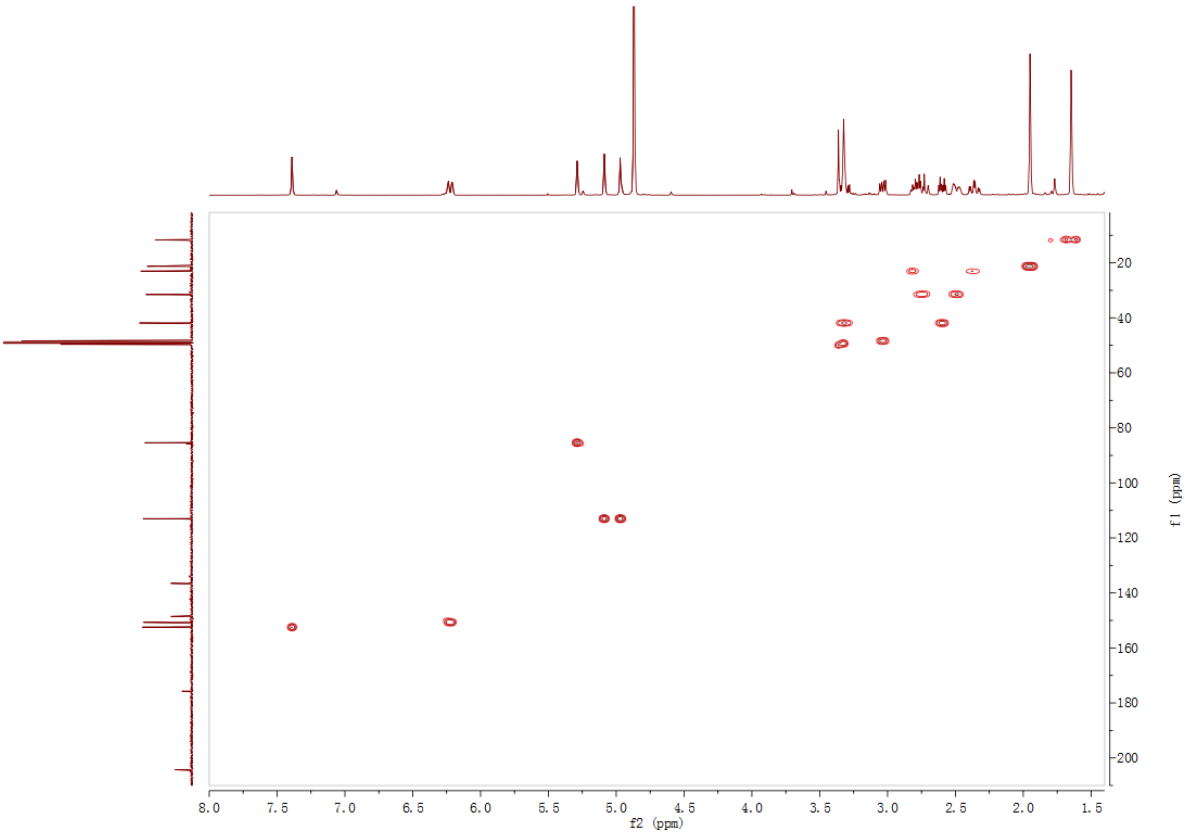
S2.52. ^{1}H NMR spectrum of **11**



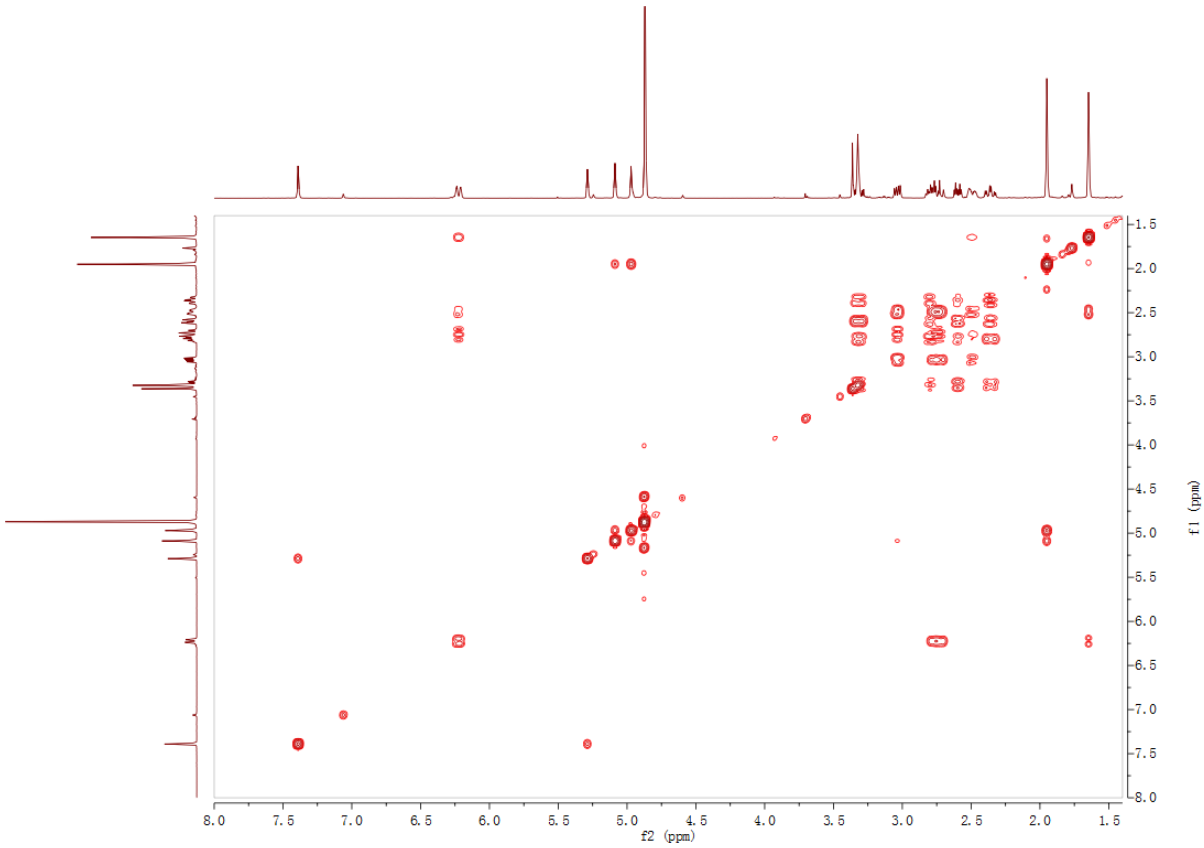
S2.53. ^{13}C NMR and DEPT spectra of **11**



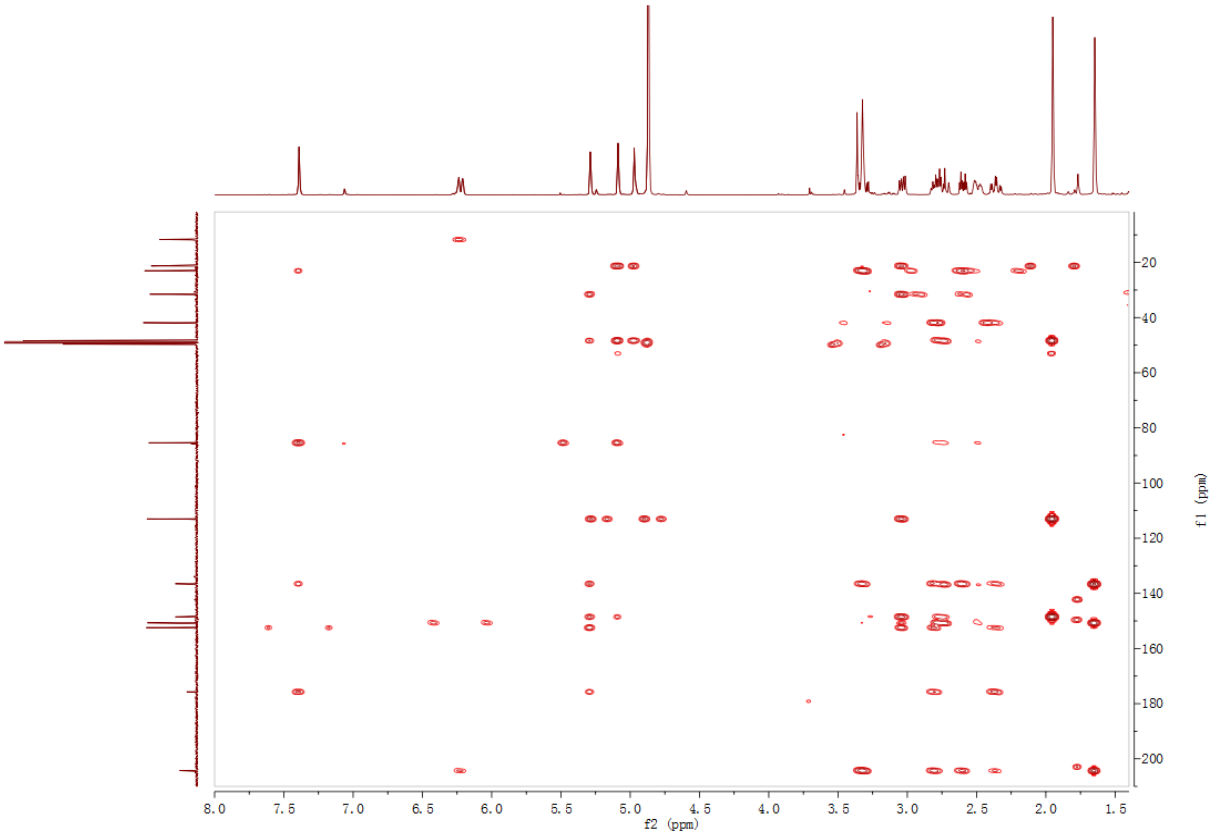
S2.54.HSQC spectrum of **11**



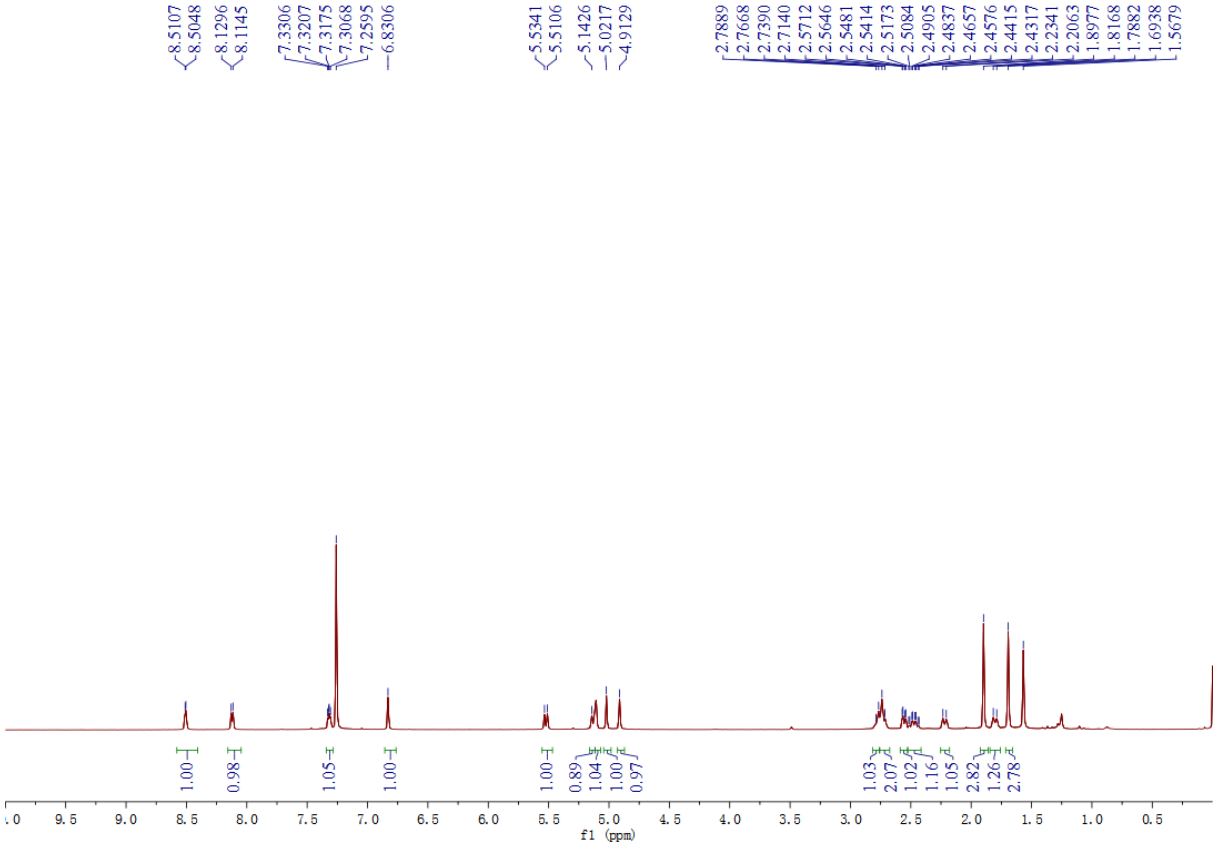
S2.55. $^{1}H–^{1}H$ COSY spectrum of **11**



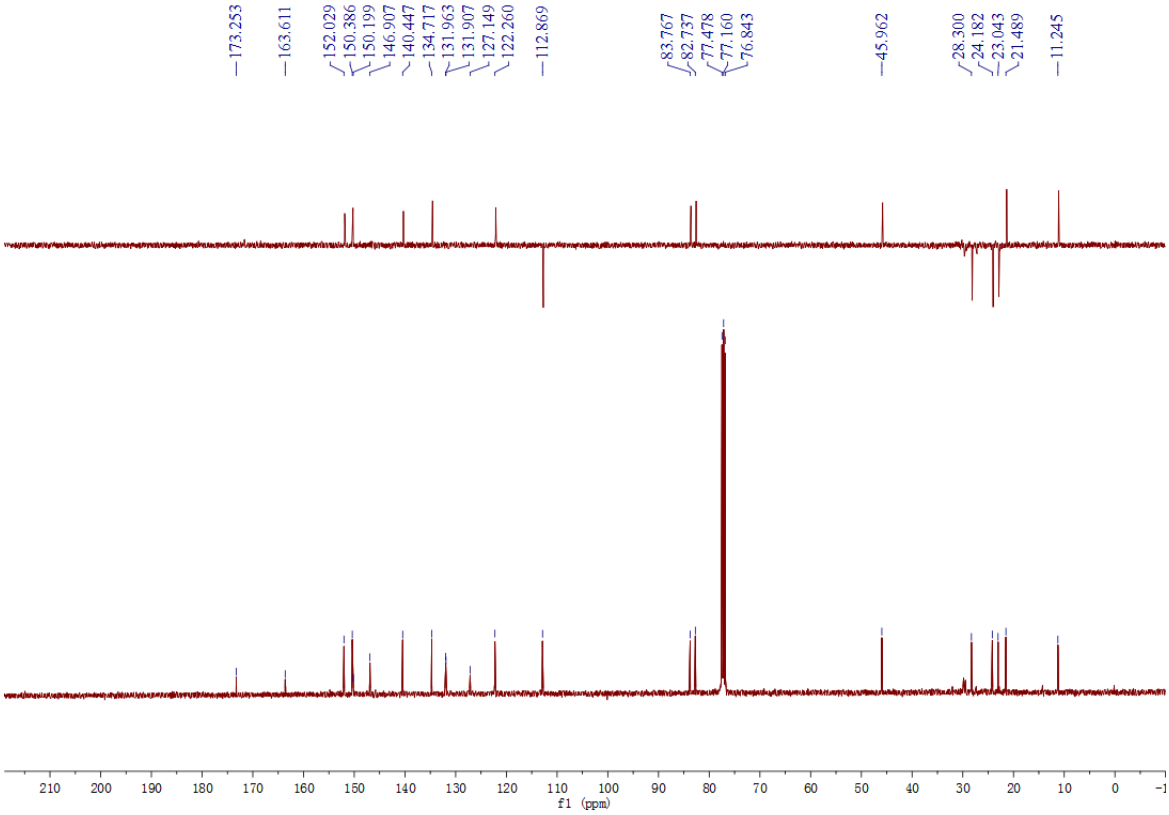
S2.56. HMBC spectrum of **11**



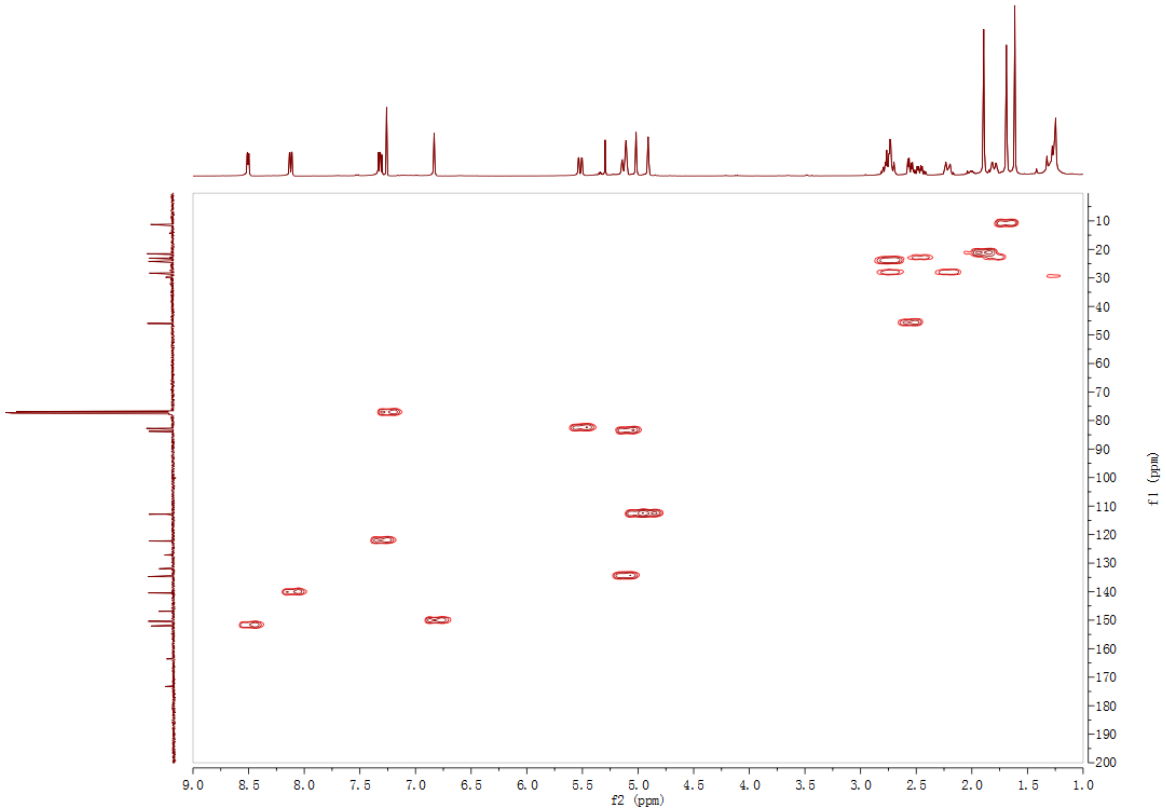
S2.57. ^{1}H NMR spectrum of **12**



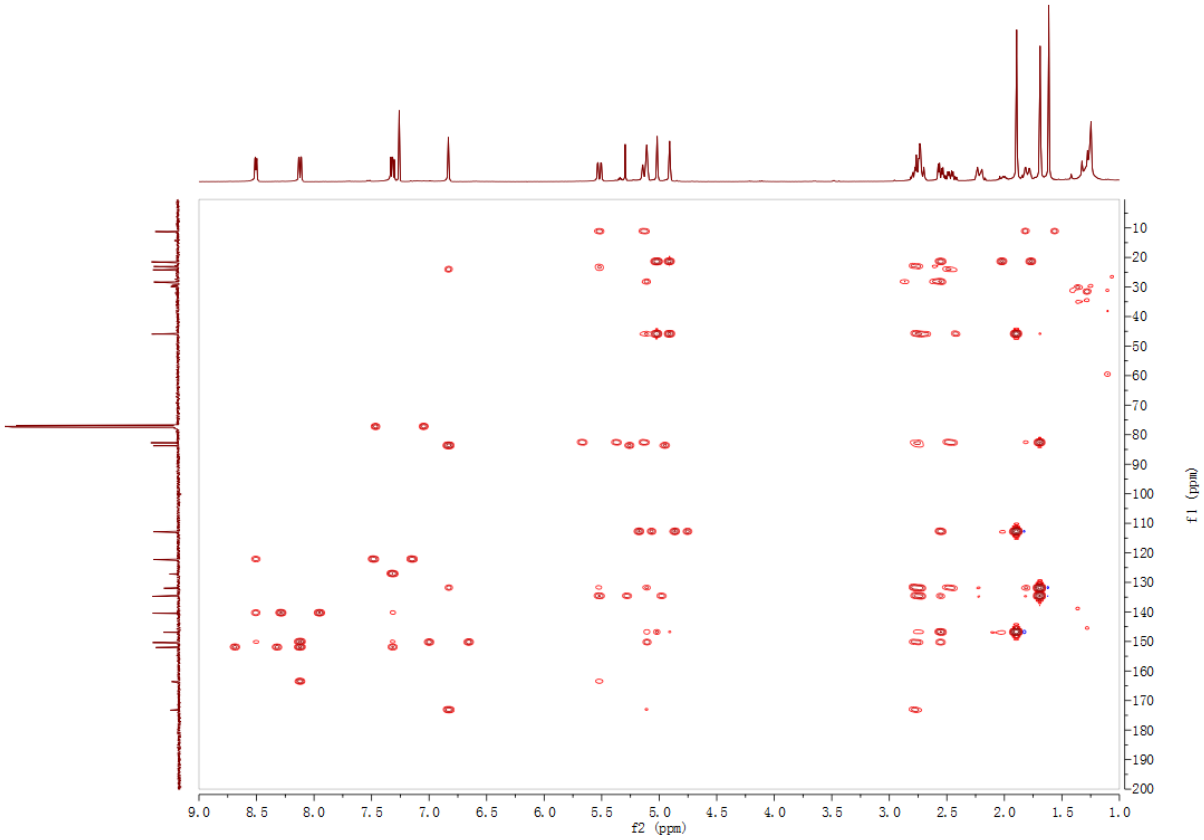
S2.58. ^{13}C NMR and DEPT spectra of **12**



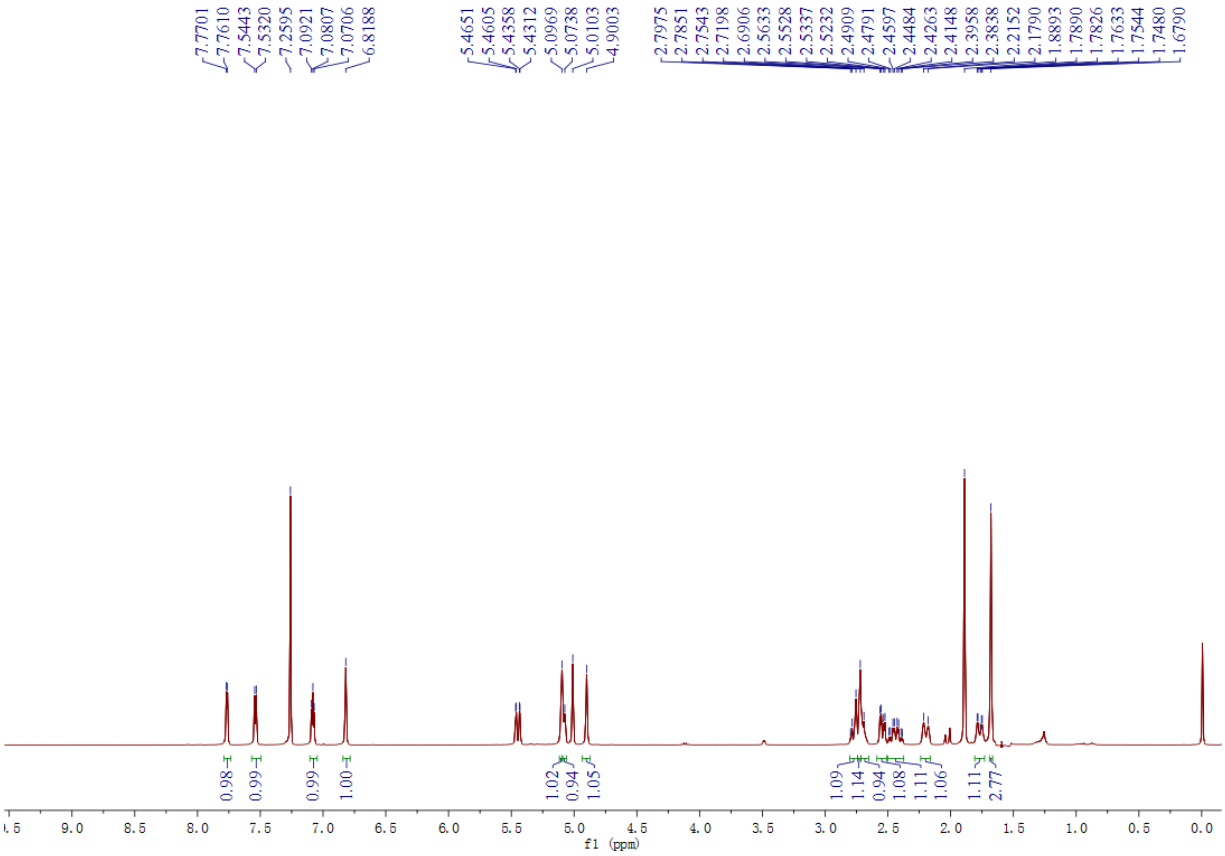
S2.59.HSQC spectrum of **12**



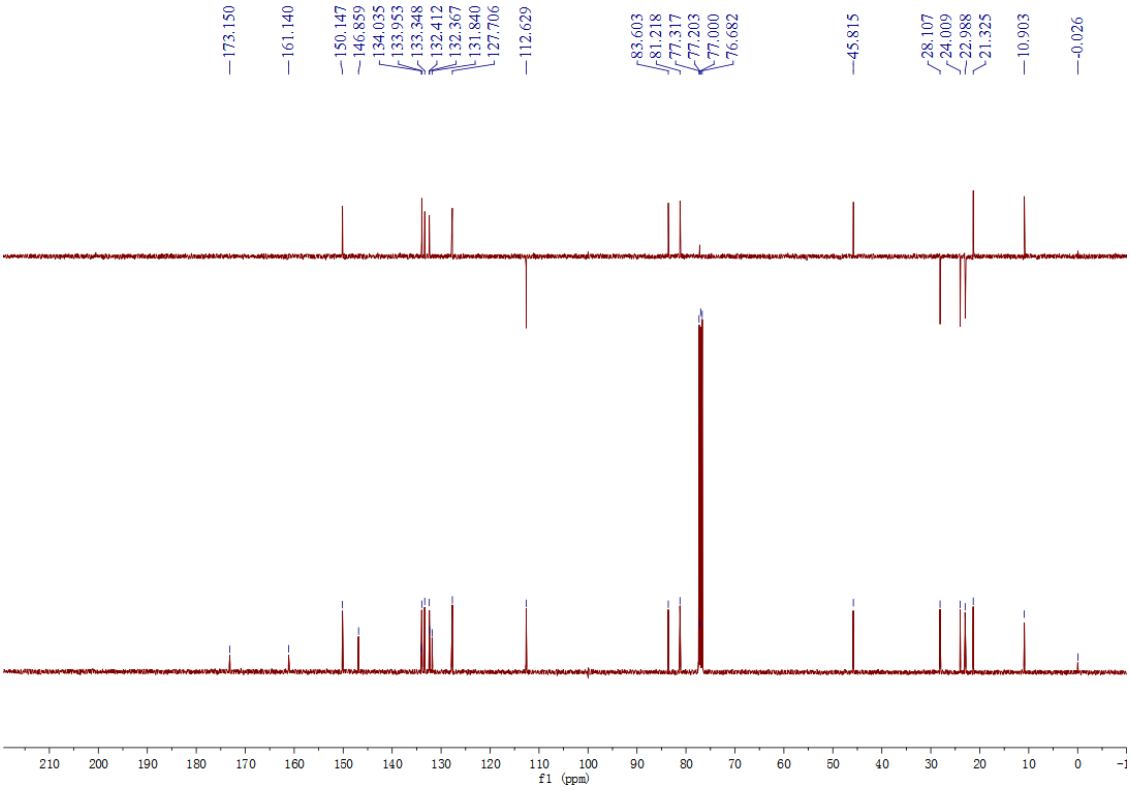
S2.60. HMBC spectrum of **12**



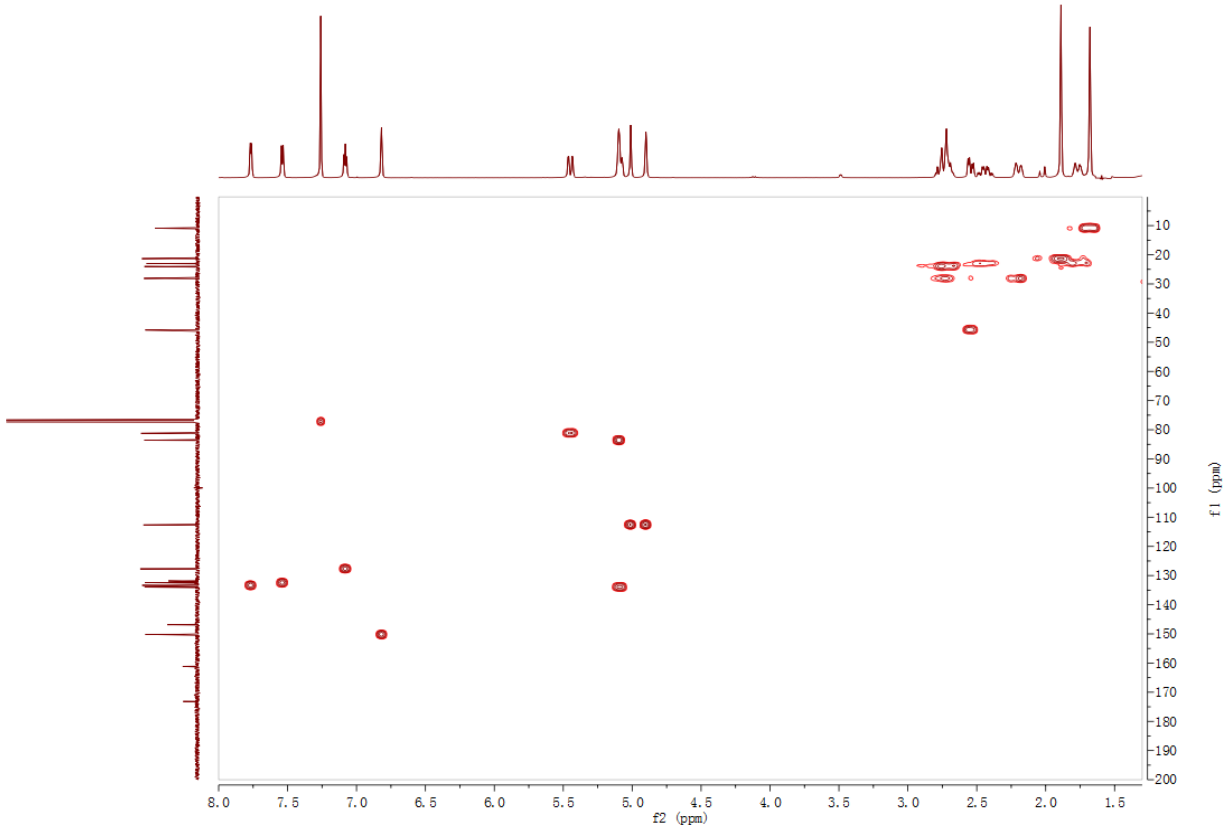
S2.61. ^{1}H NMR spectrum of **13**



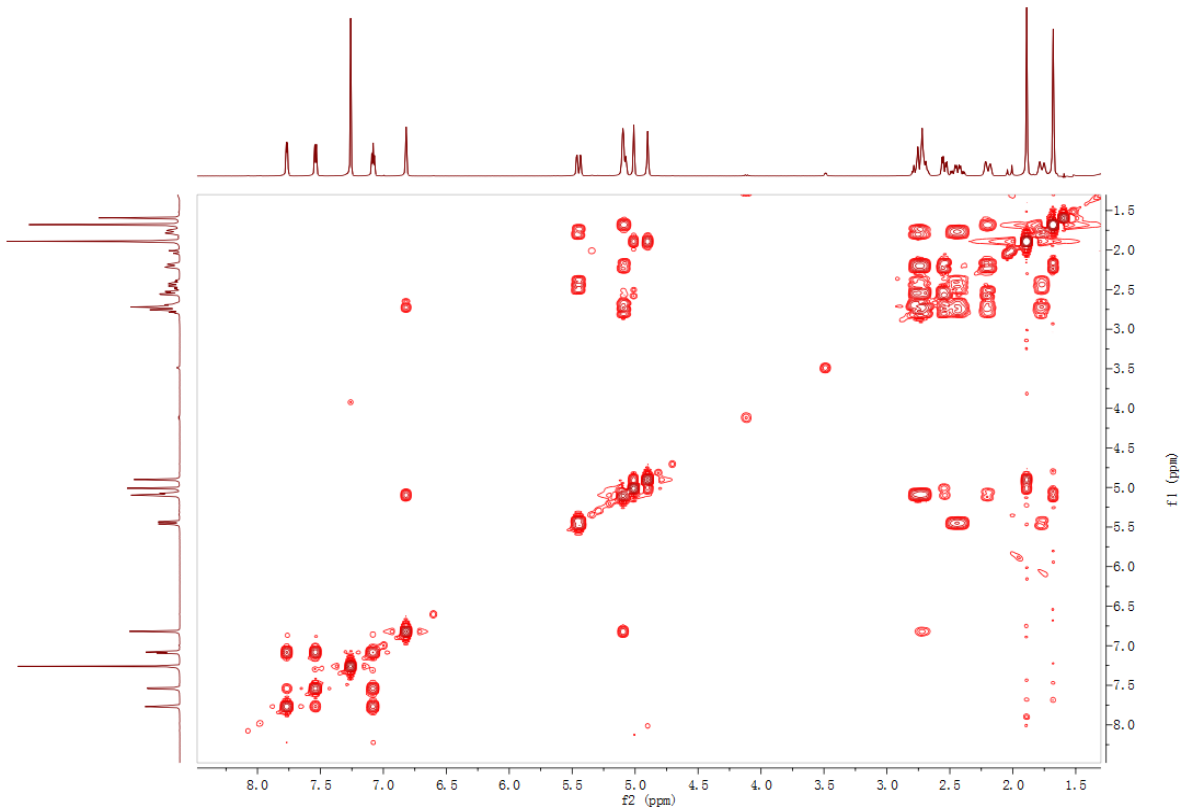
S2.62. ^{13}C NMR and DEPT spectra of **13**



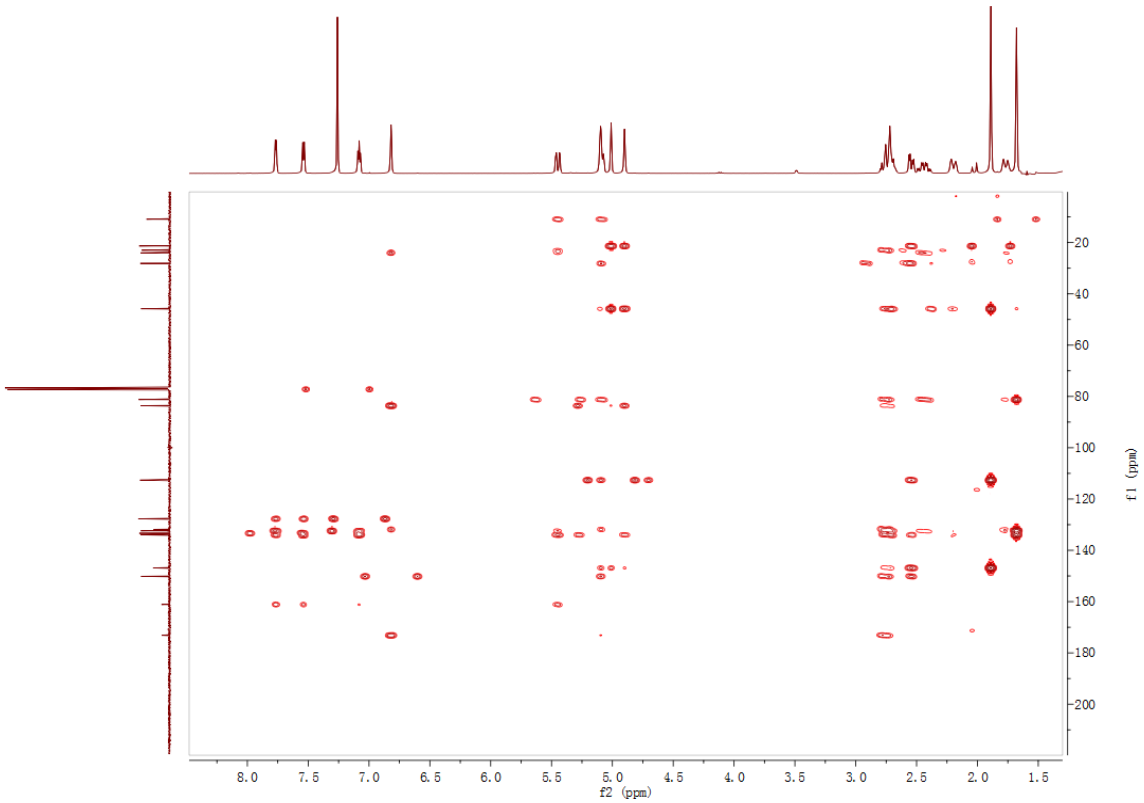
S2.63.HSQC spectrum of **13**



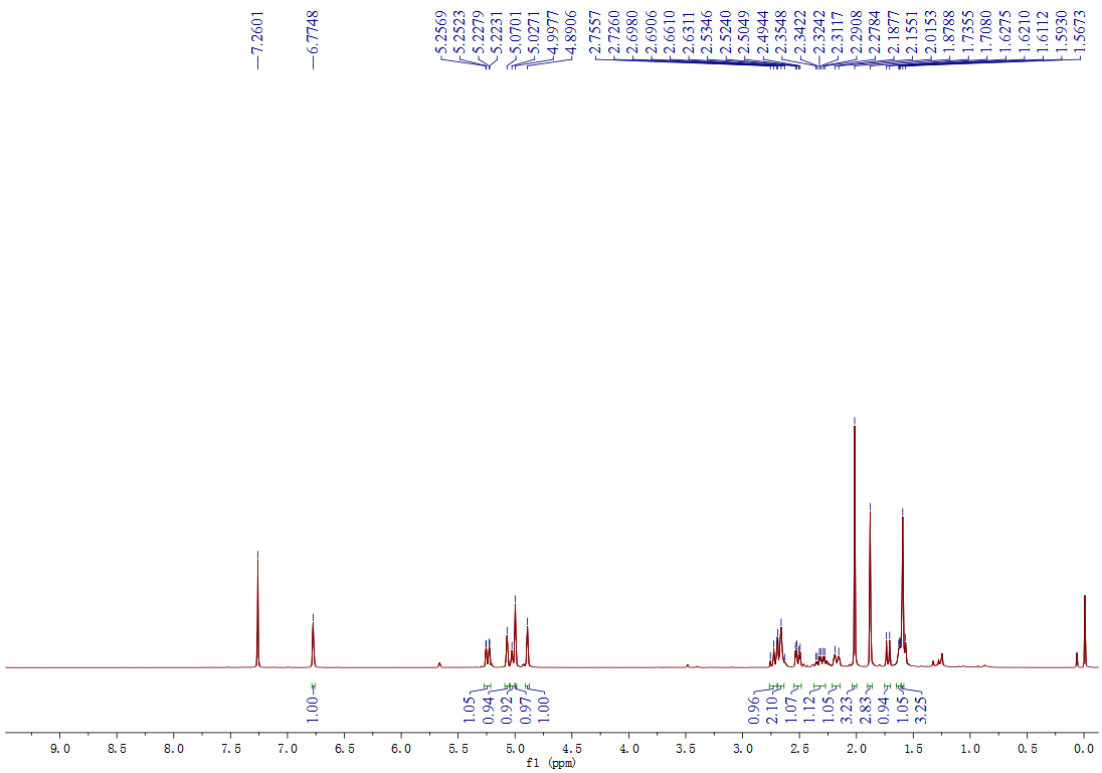
S2.64. ^{1}H–^{1}H COSY spectrum of **13**



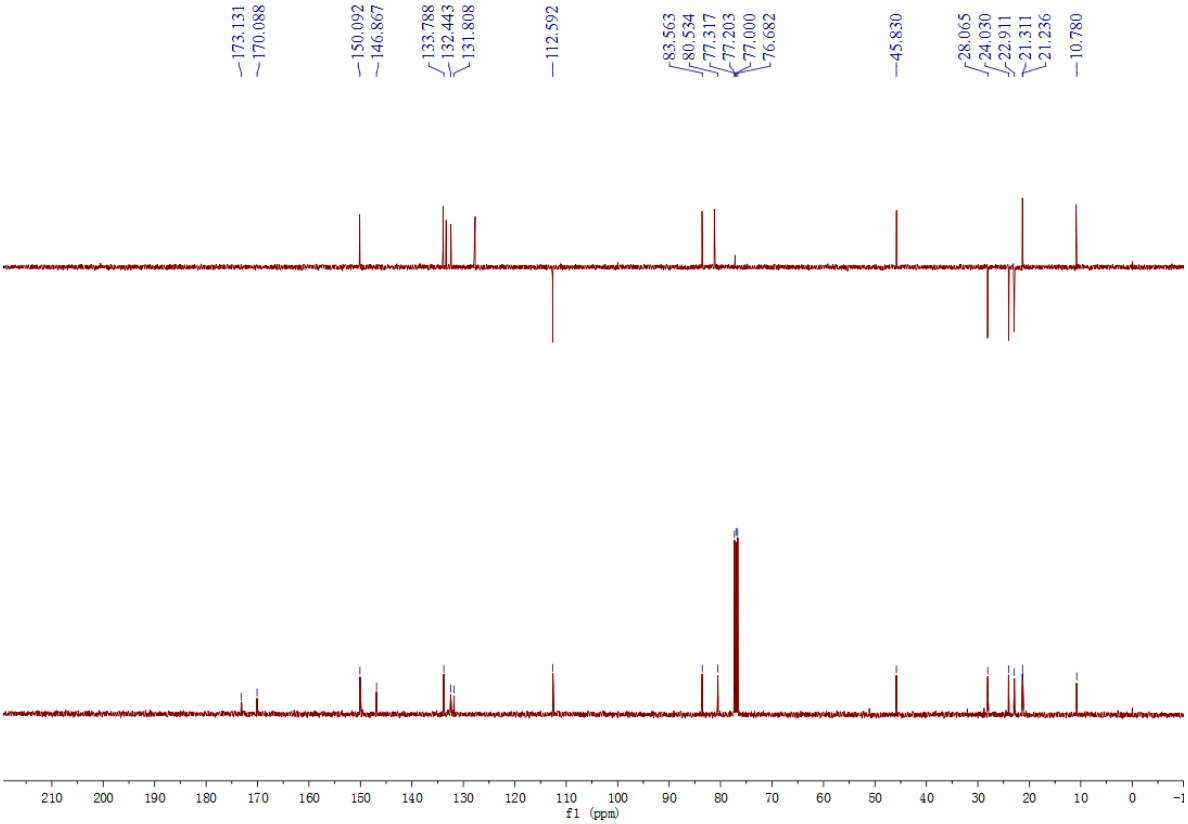
S2.65. HMBC spectrum of **13**



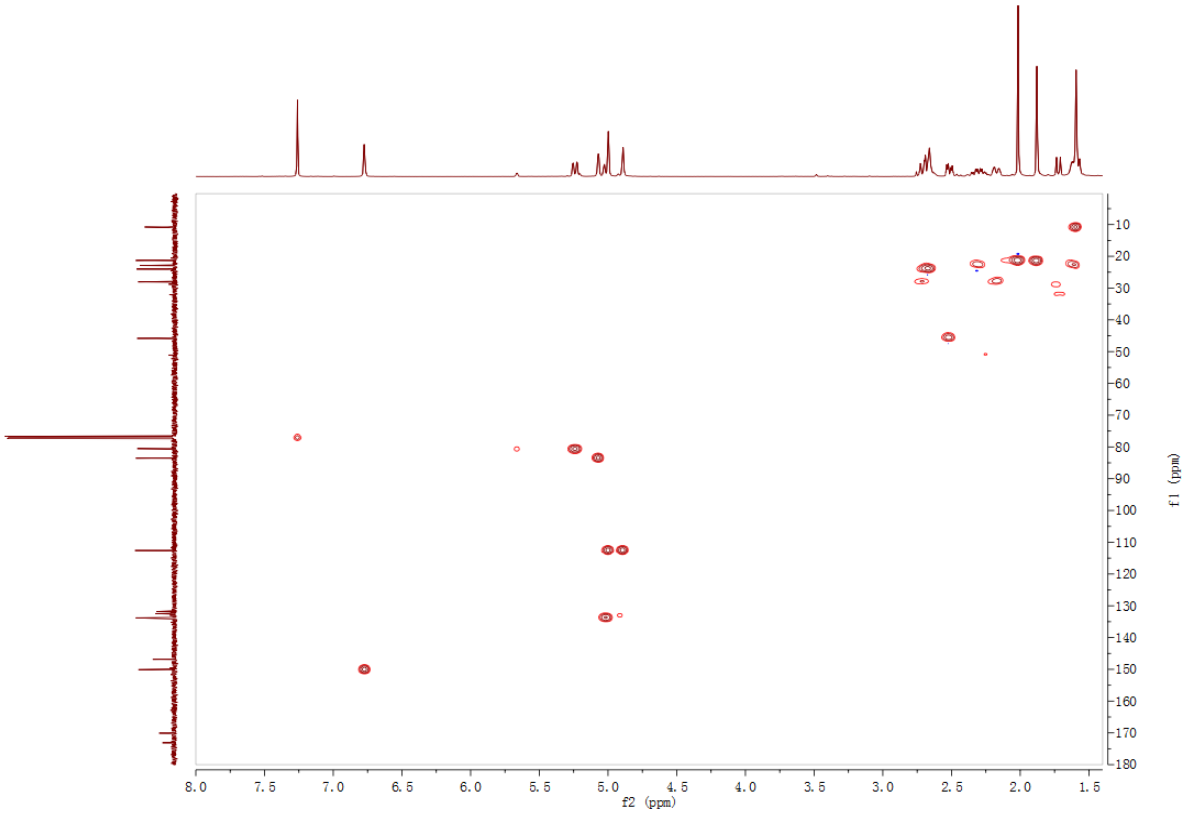
S2.66. ^{1}H NMR spectrum of **14**



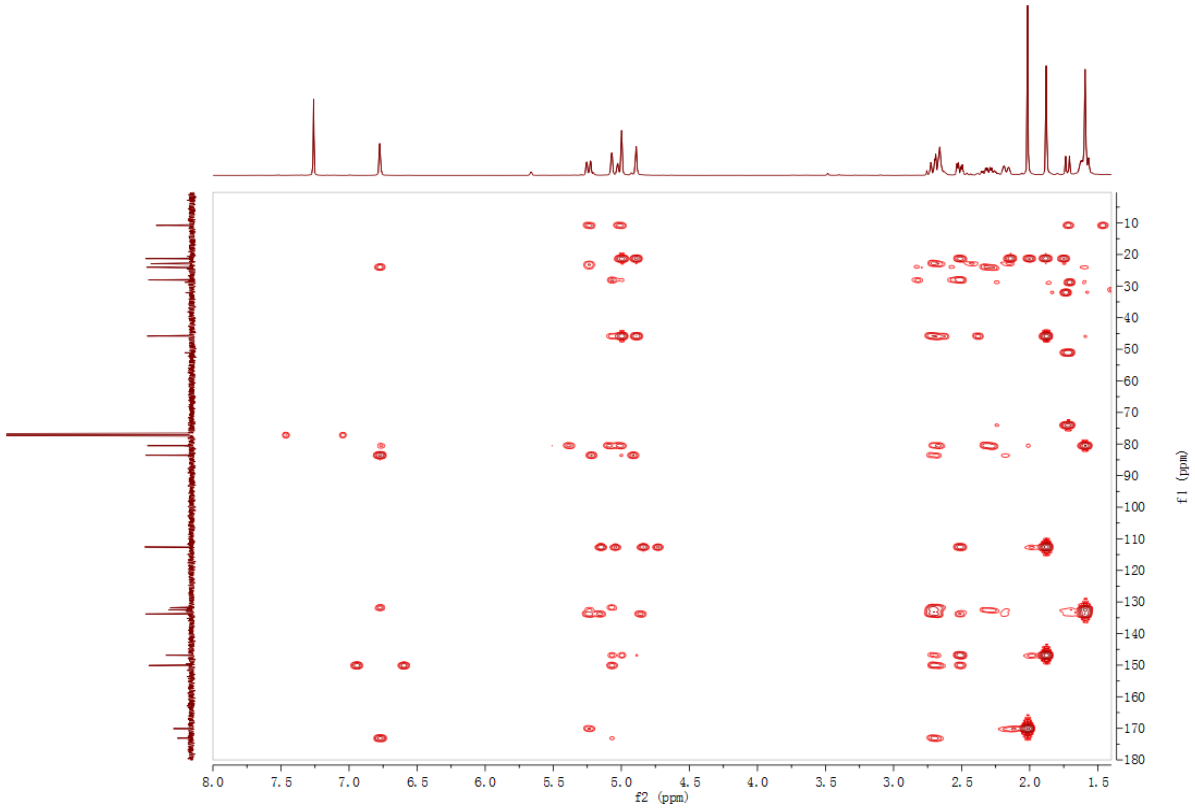
S2.67. ^{13}C NMR and DEPT spectra of **14**



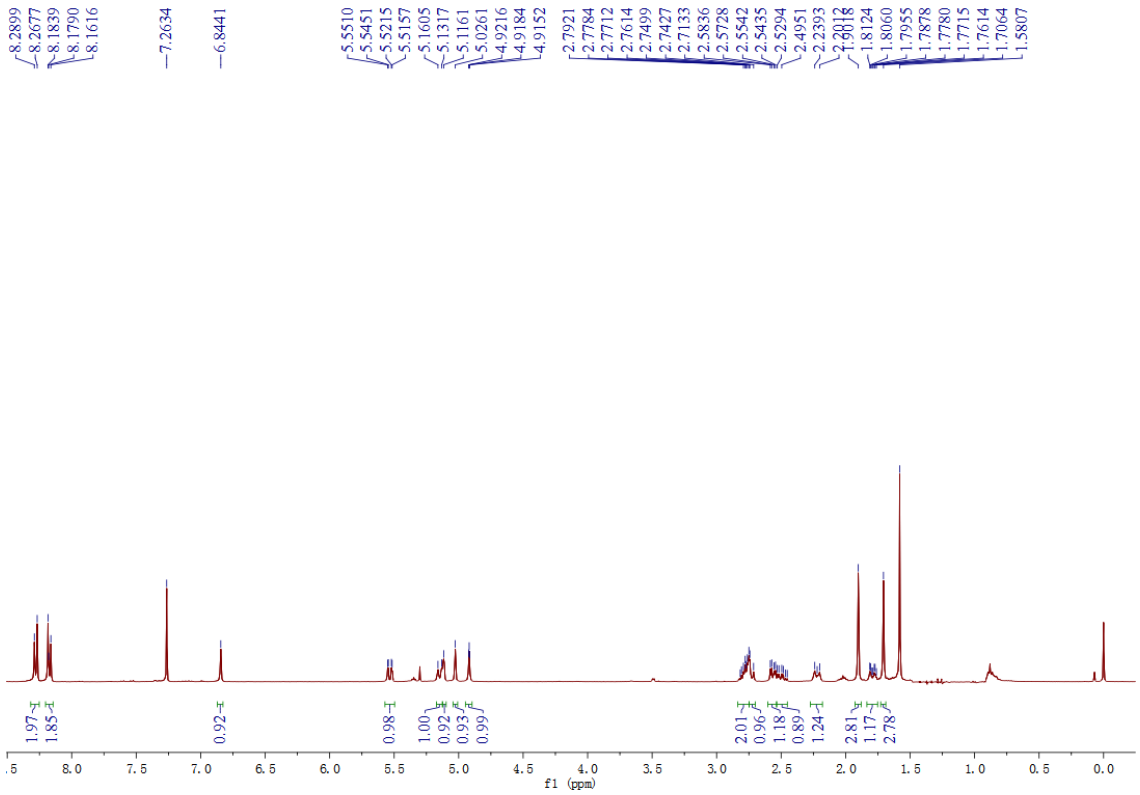
S2.68.HSQC spectrum of **14**



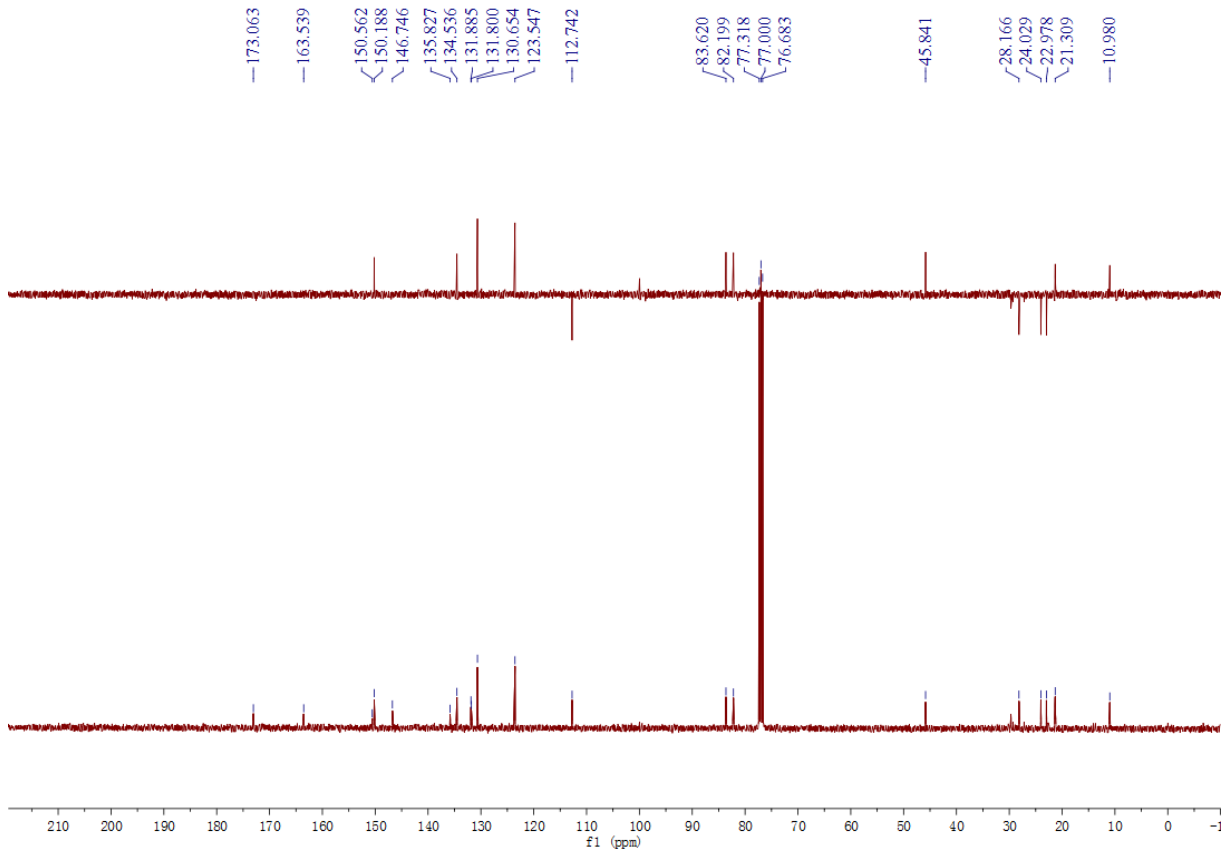
S2.69. HMBC spectrum of **14**



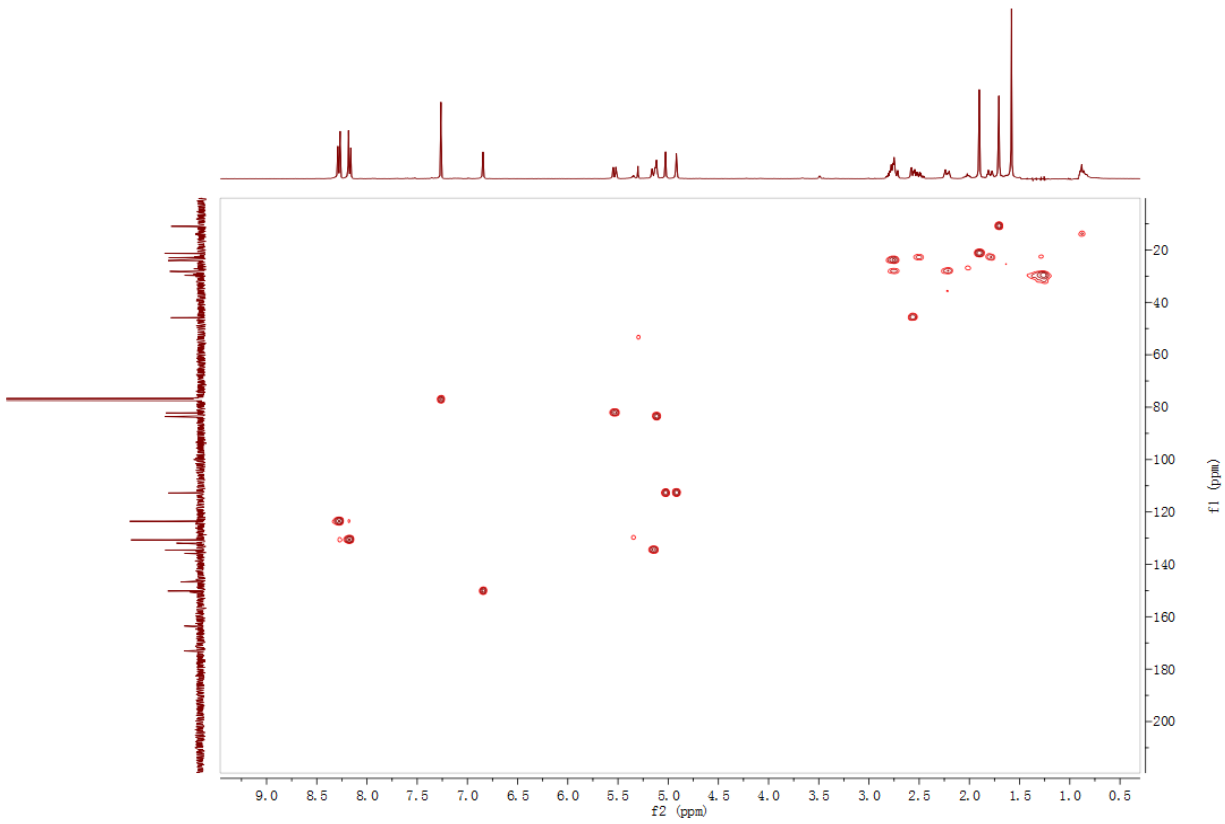
S2.70. ^{1}H NMR spectrum of **15**



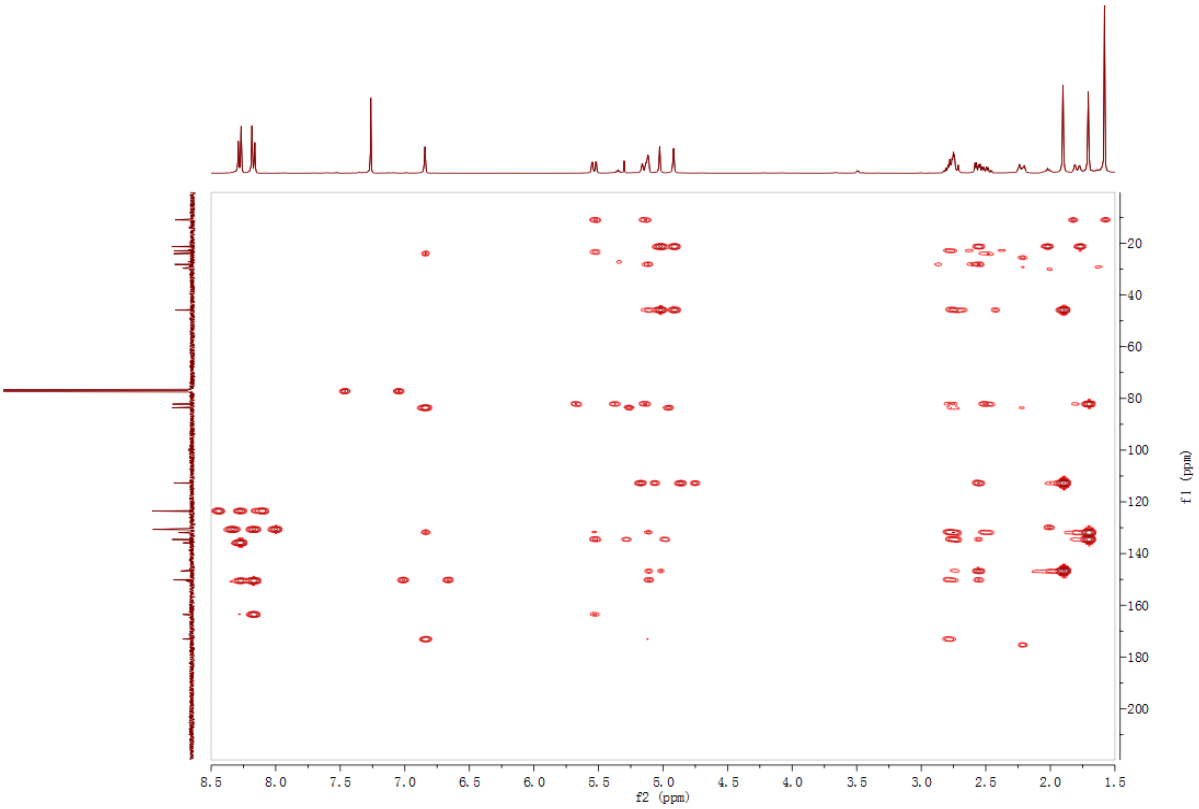
S2.71. ^{13}C NMR and DEPT spectra of **15**



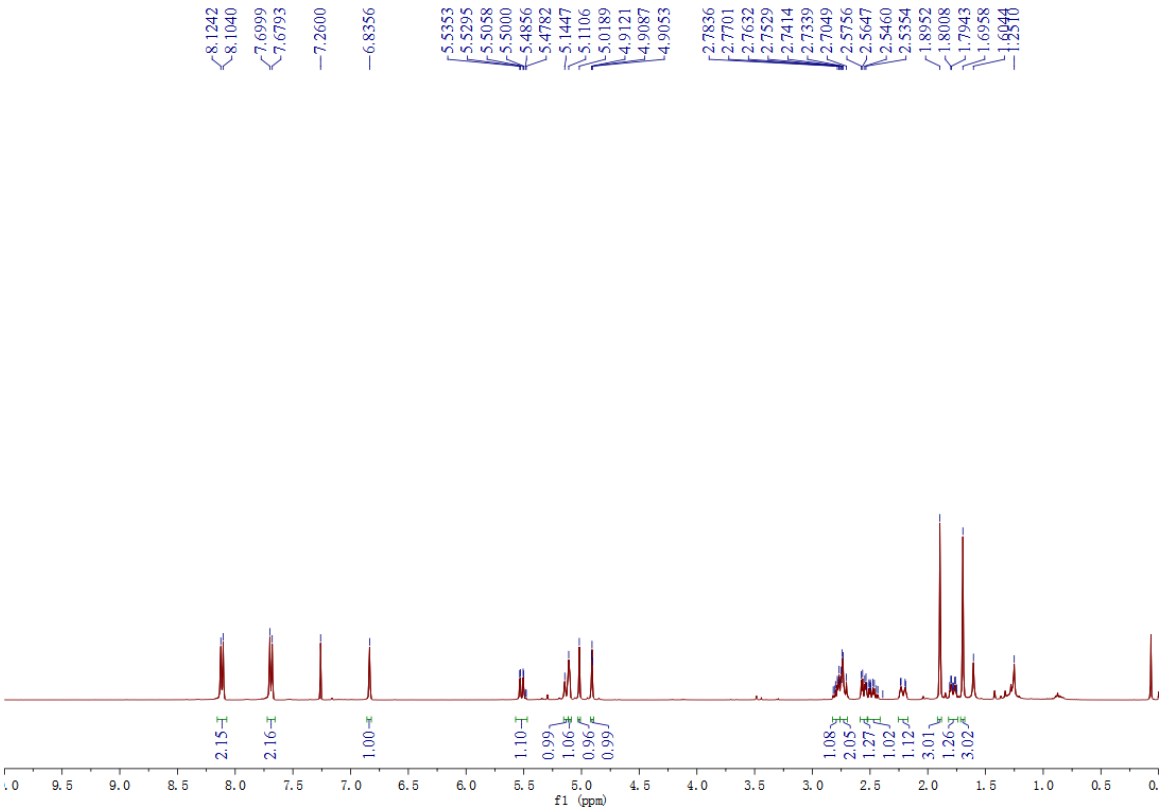
S2.72. HSQC spectrum of **15**



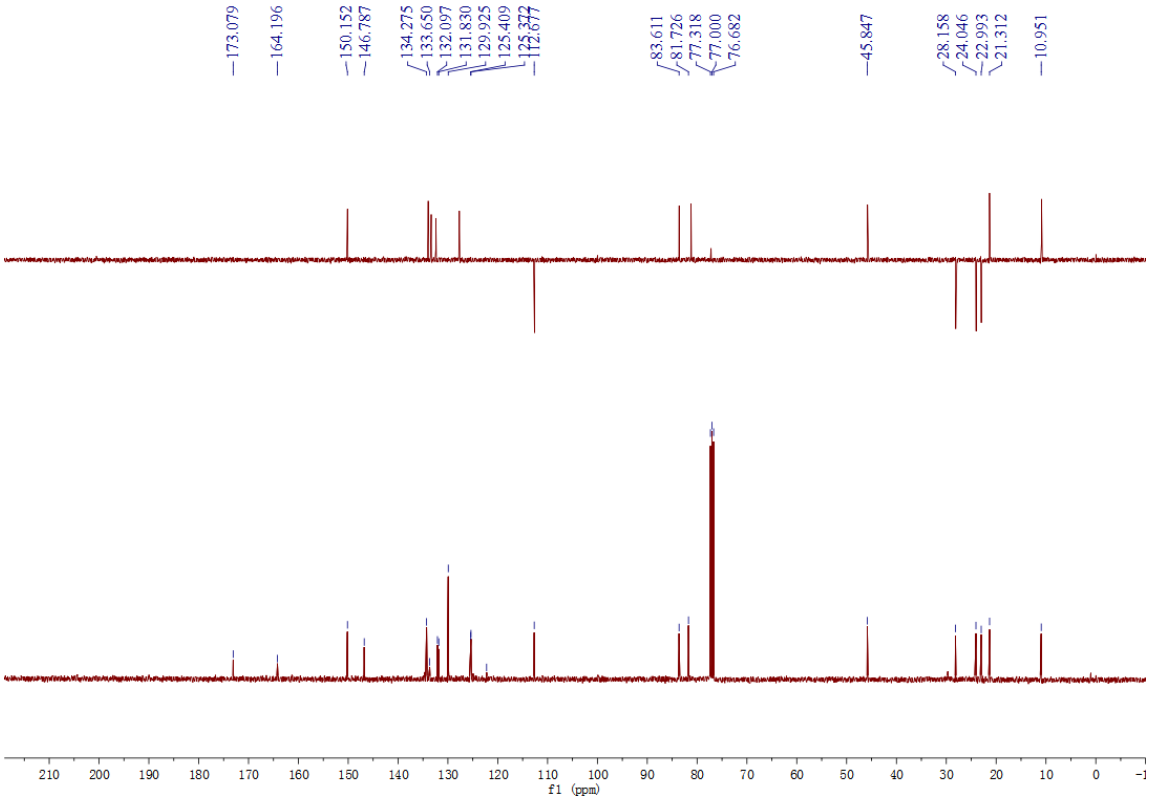
S2.73. HMBC spectrum of **15**



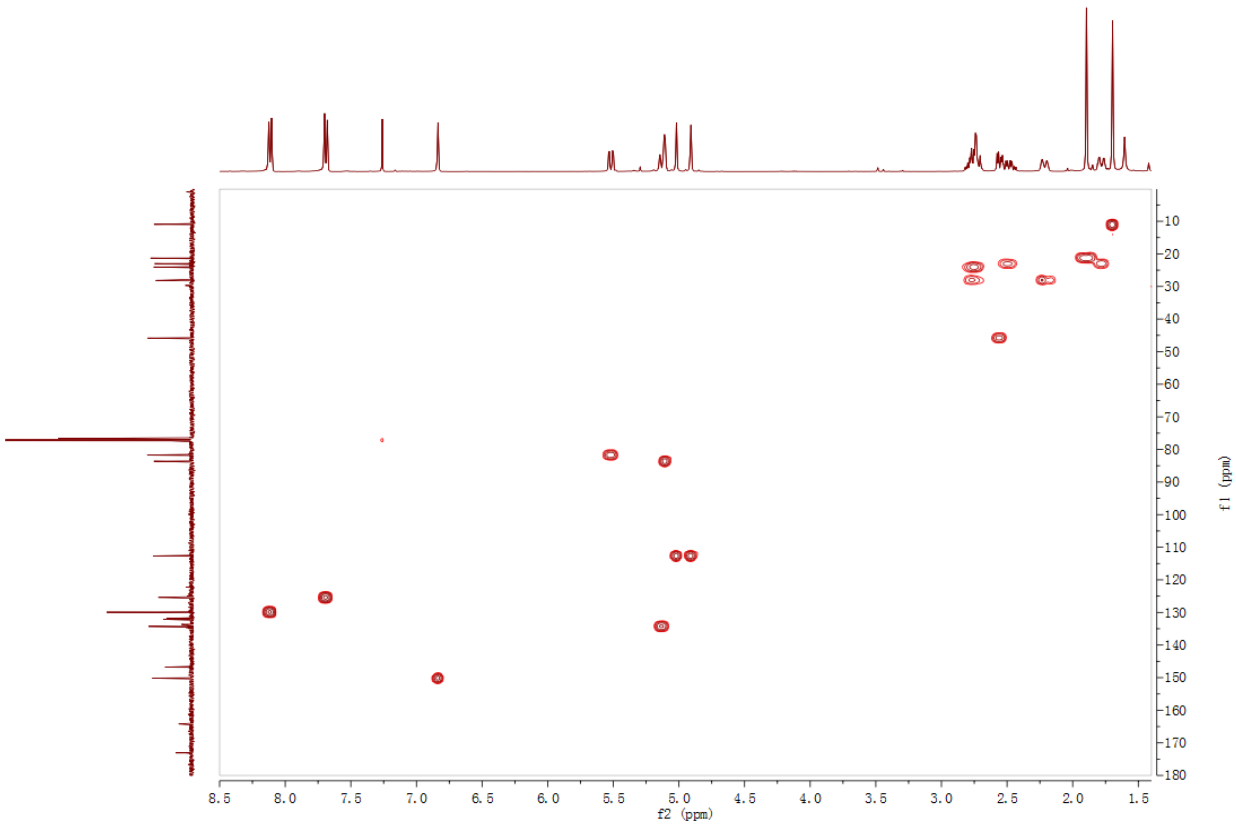
S2.74. ^{1}H NMR spectrum of **16**



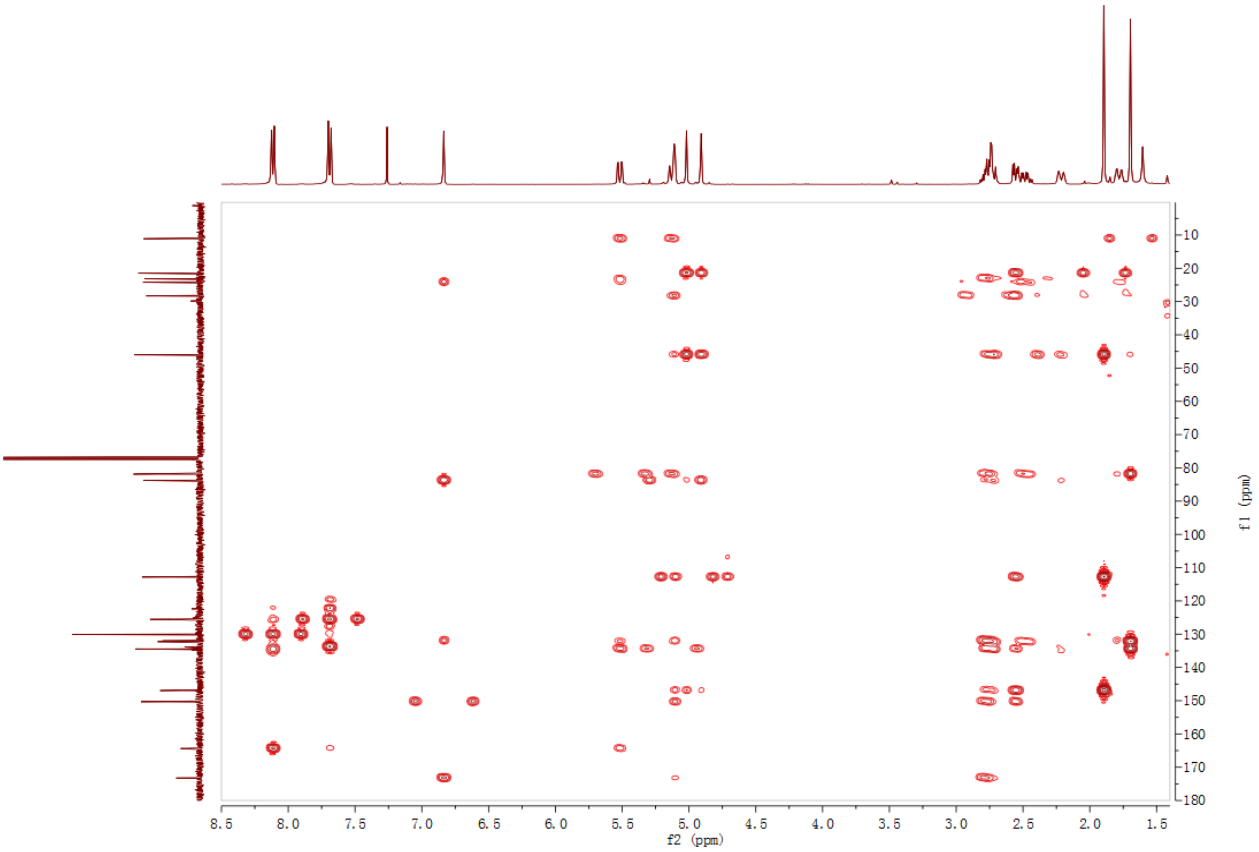
S2.75. ^{13}C NMR and DEPT spectra of **16**



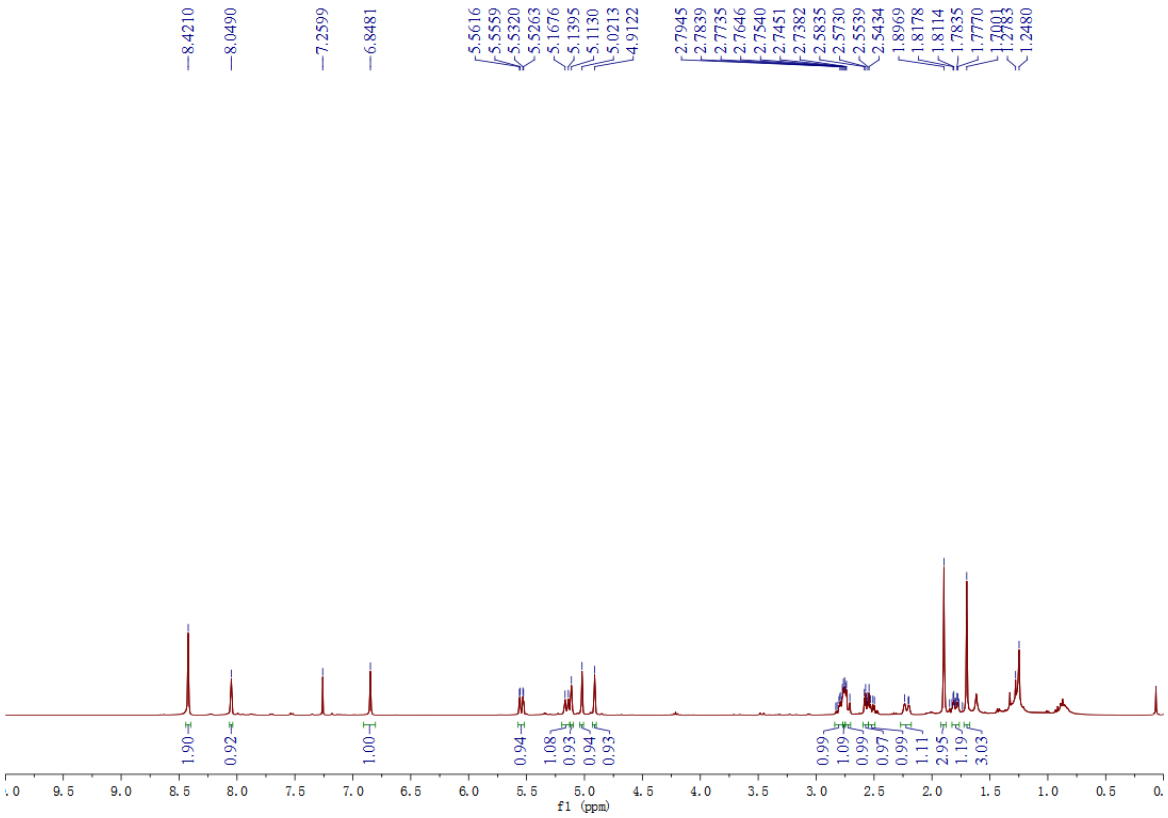
S2.76. HSQC spectrum of **16**



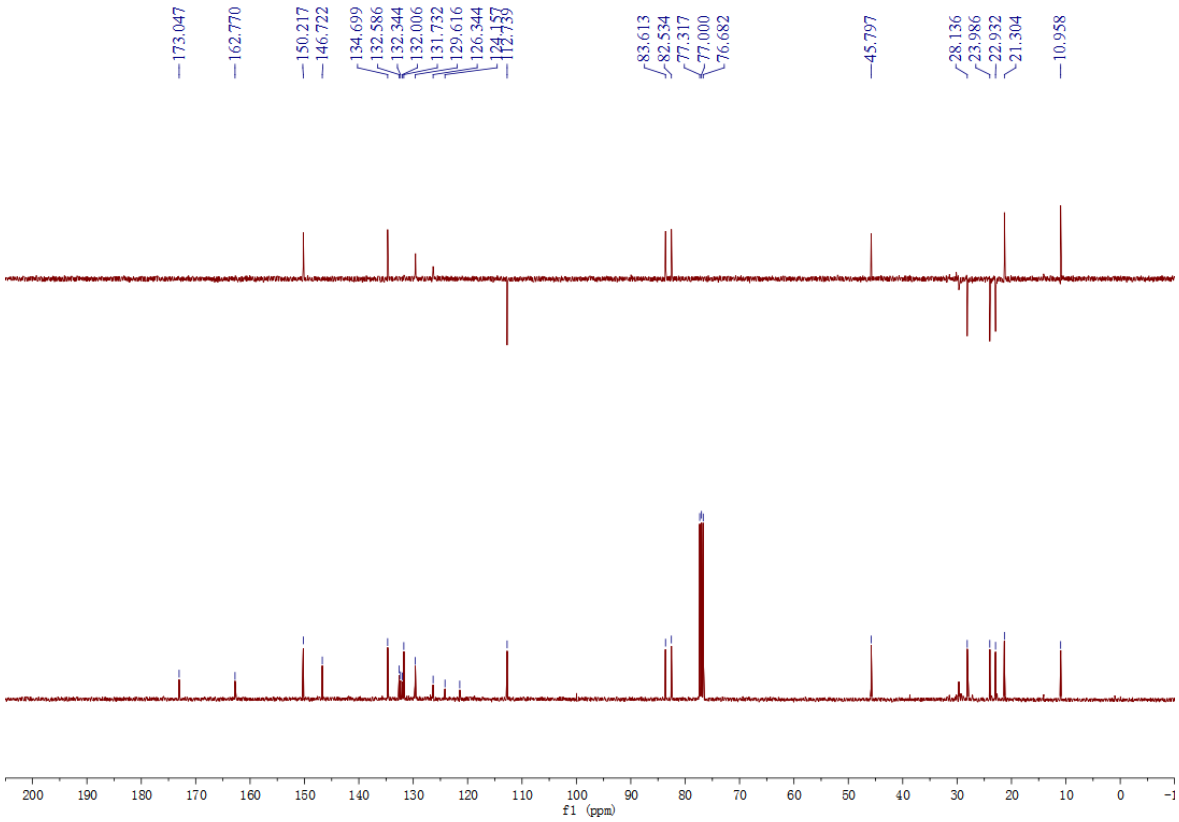
S2.77. HMBC spectrum of **16**



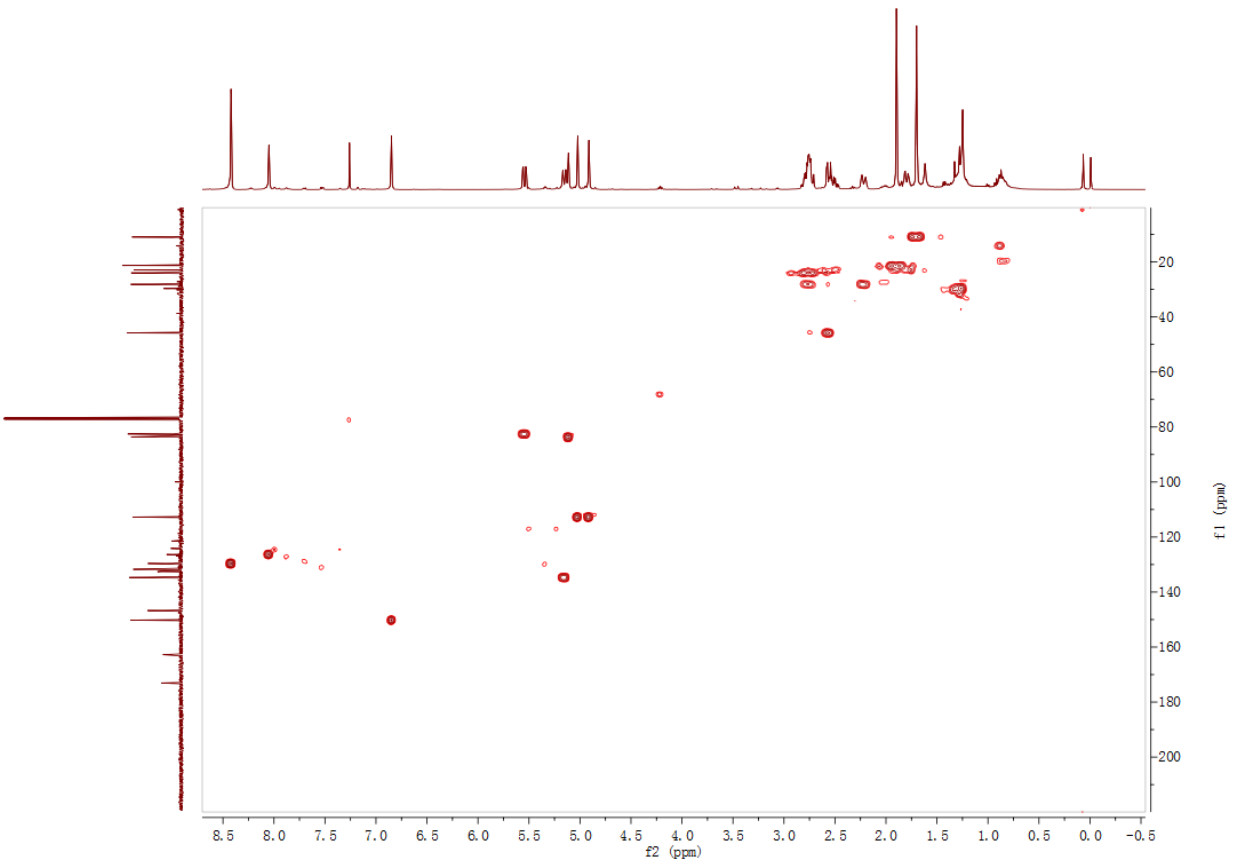
S2.78. ^{1}H NMR spectrum of **17**



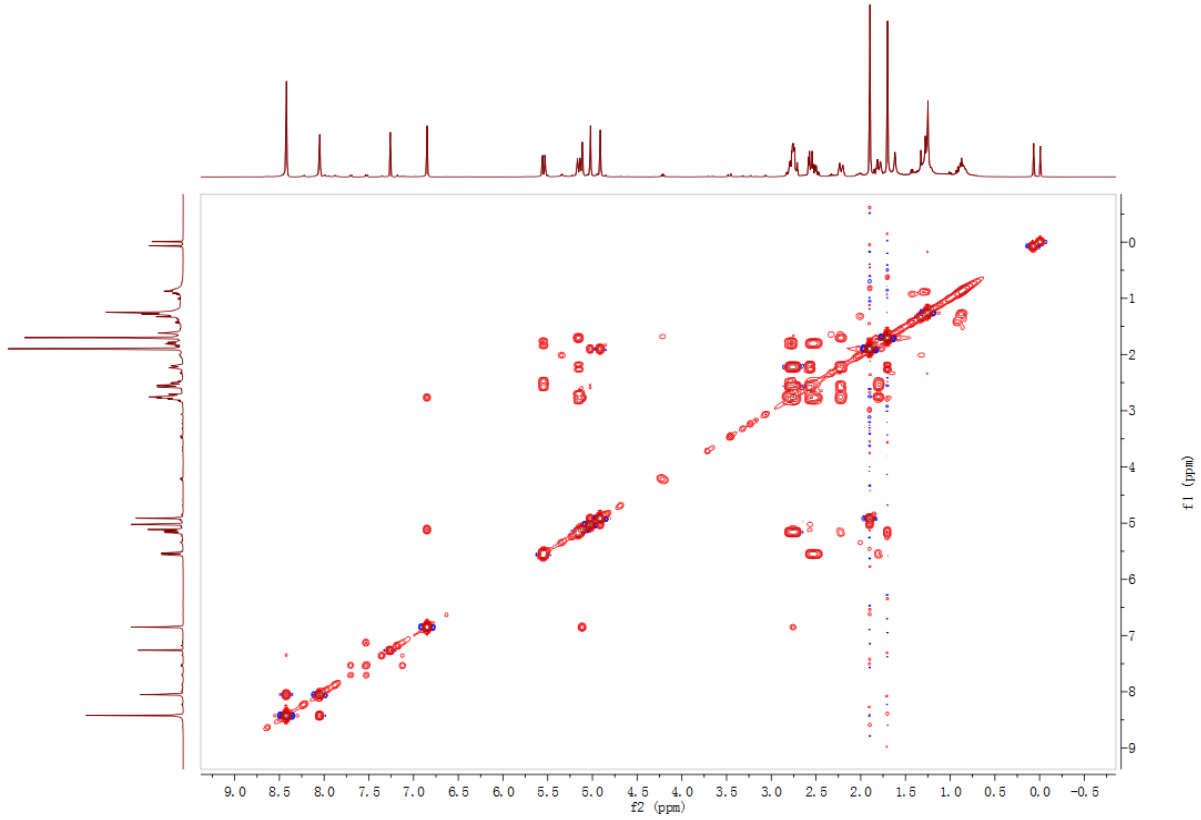
S2.79. ^{13}C NMR and DEPT spectra of **17**



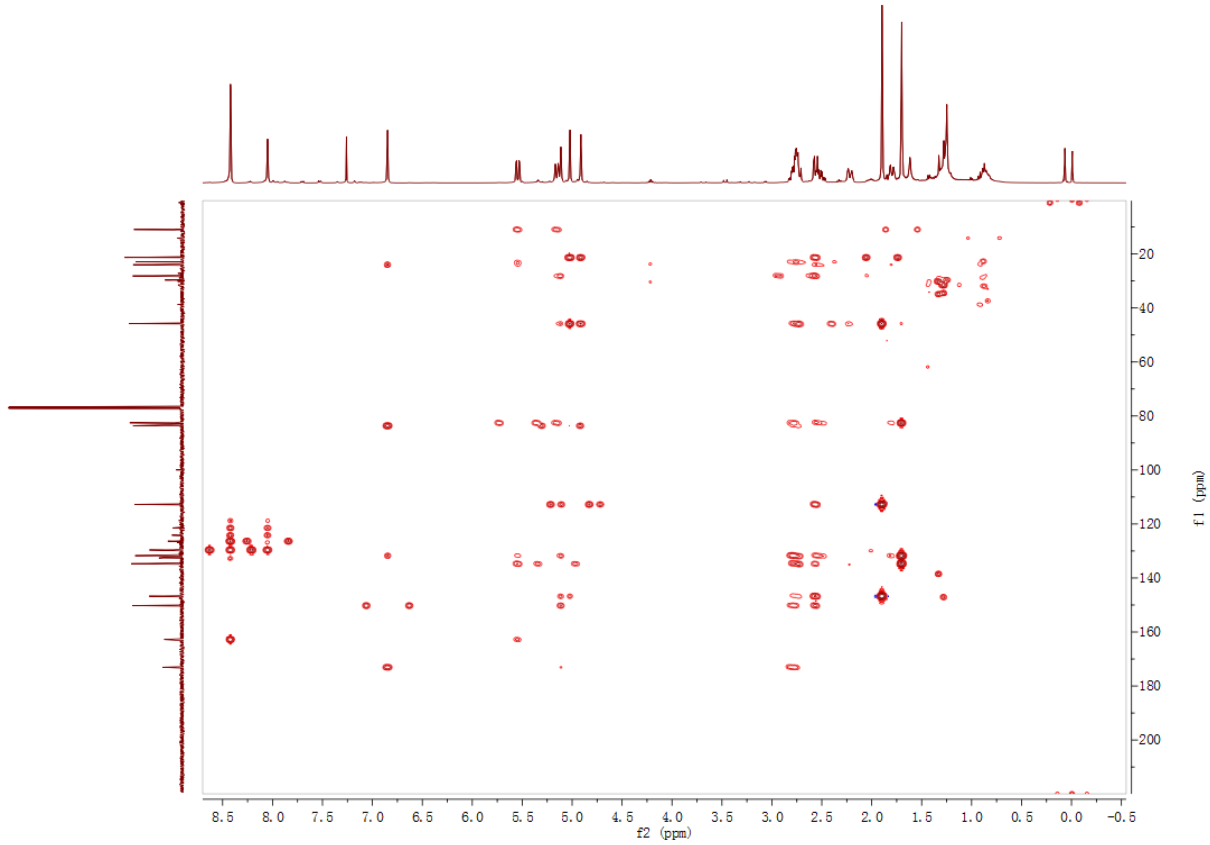
S2.80. HSQC spectrum of **17**



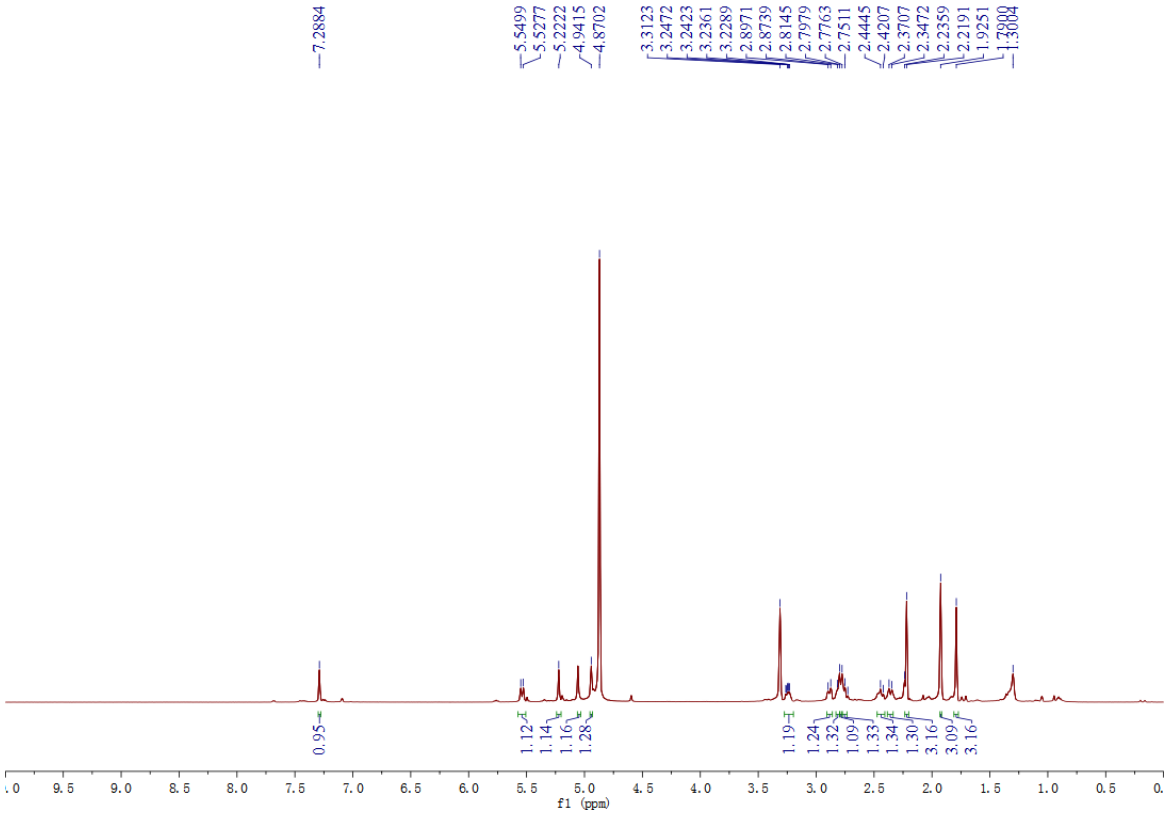
S2.81. $^{1}H–^{1}H$ COSY spectrum of **17**



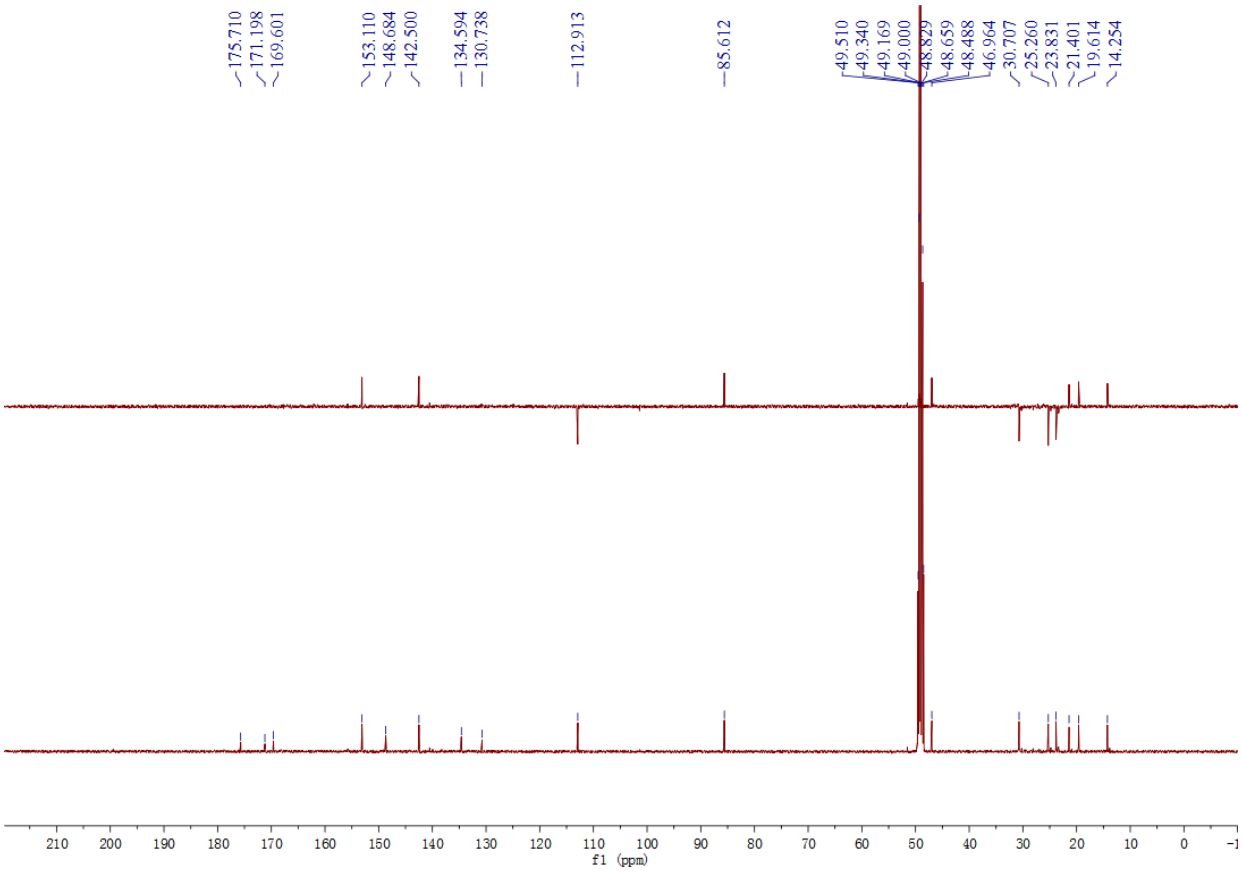
S2.82. HMBC spectrum of **17**



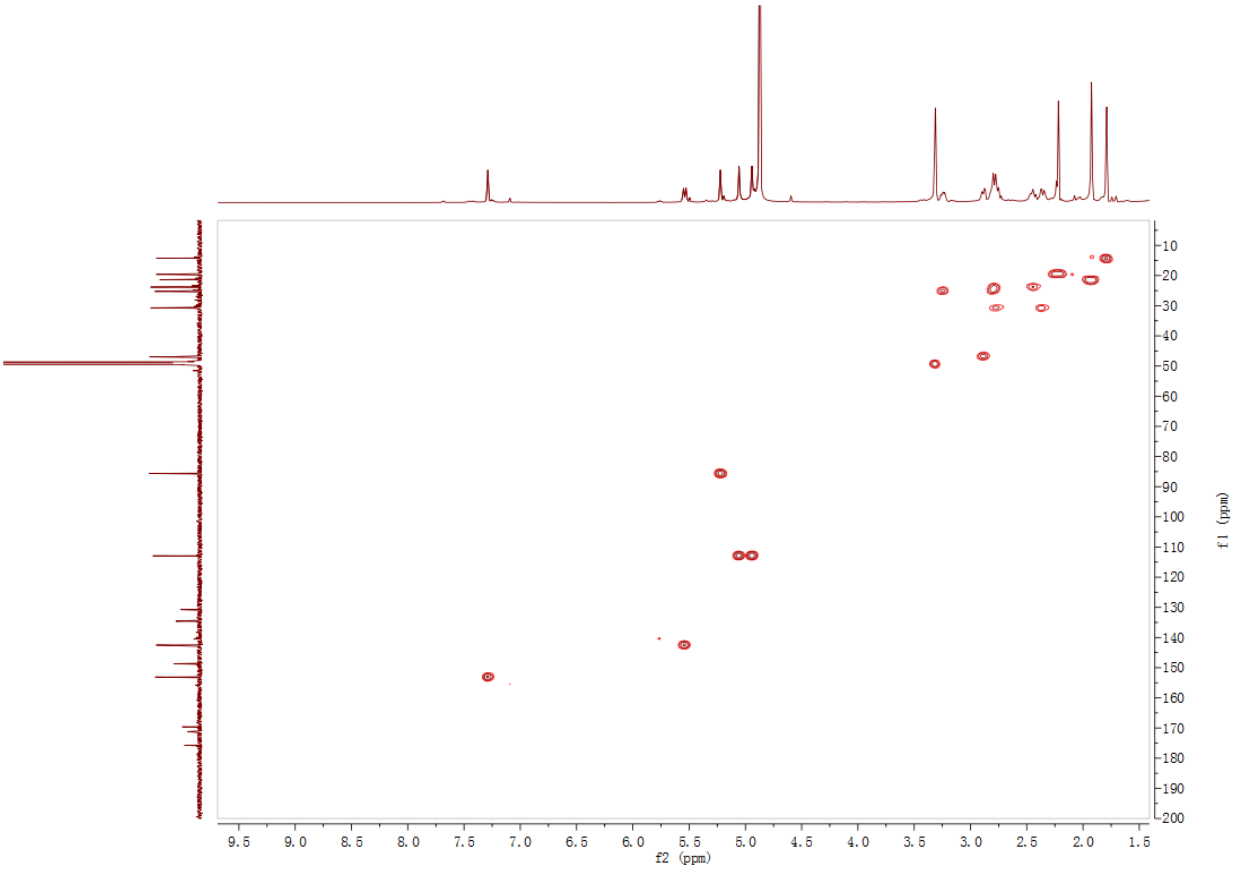
S2.83. ^{1}H NMR spectrum of **18**



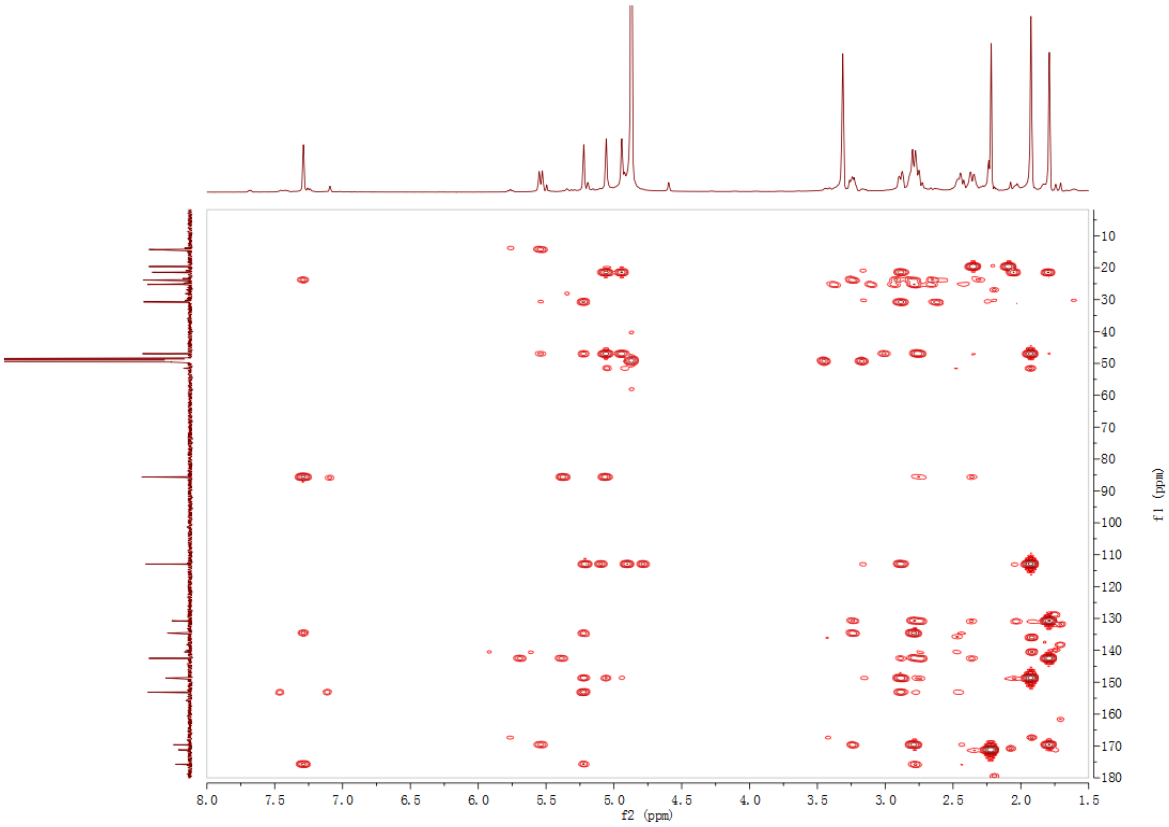
S2.84. ^{13}C NMR and DEPT spectra of **18**



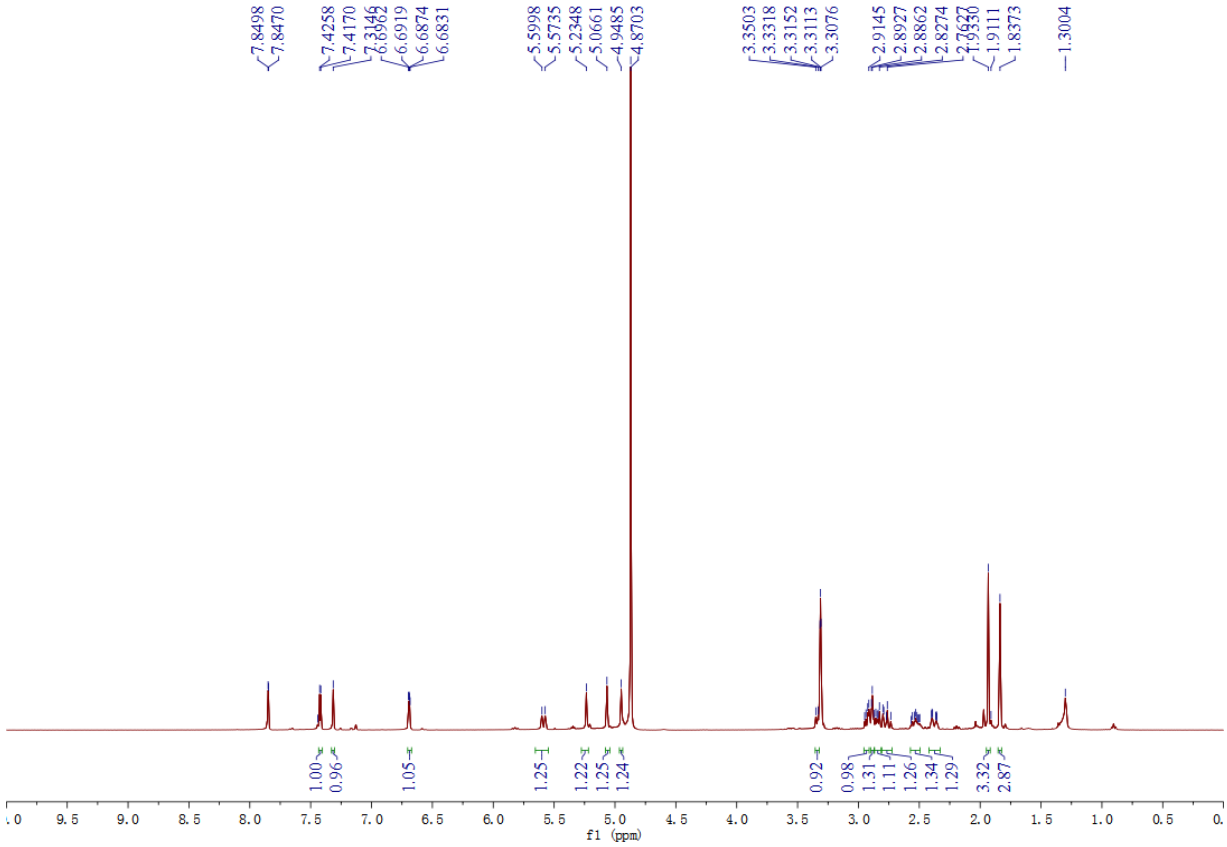
S2.85. HSQC spectrum of **18**



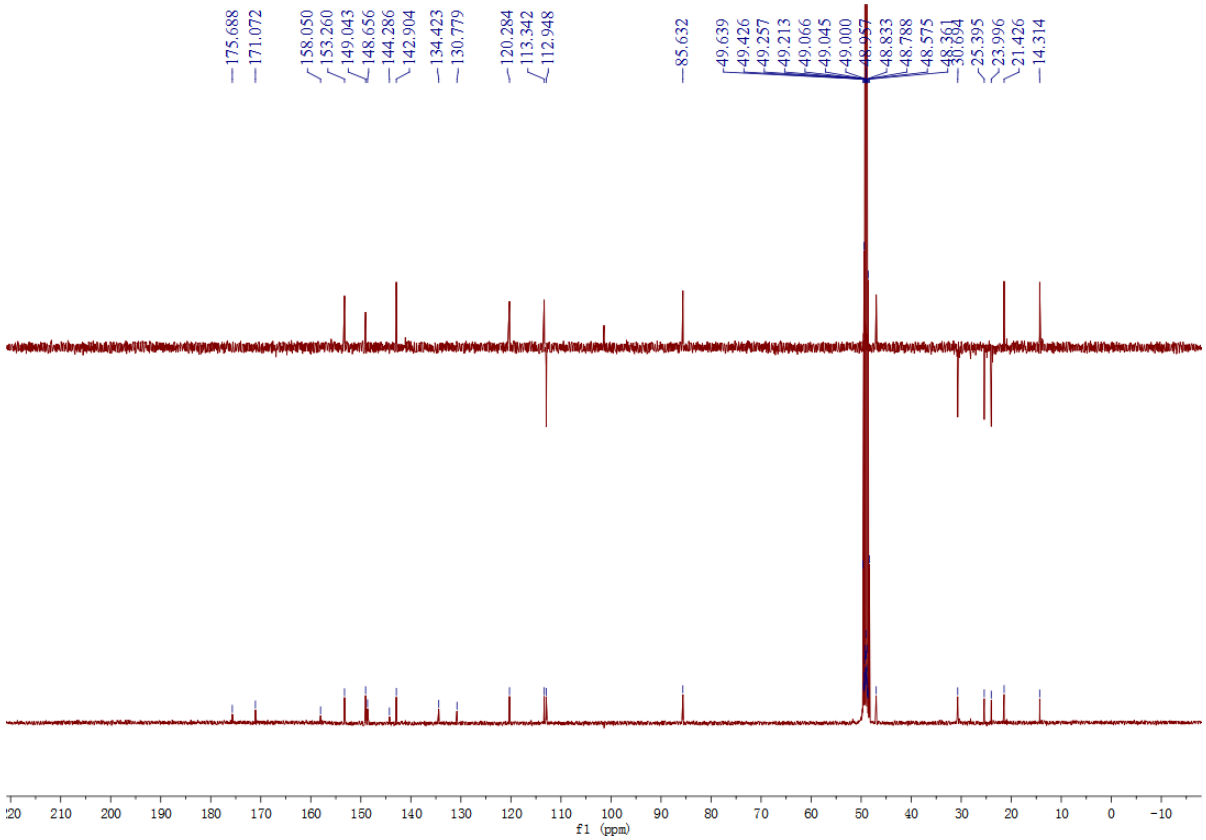
S2.86. HMBC spectrum of **18**



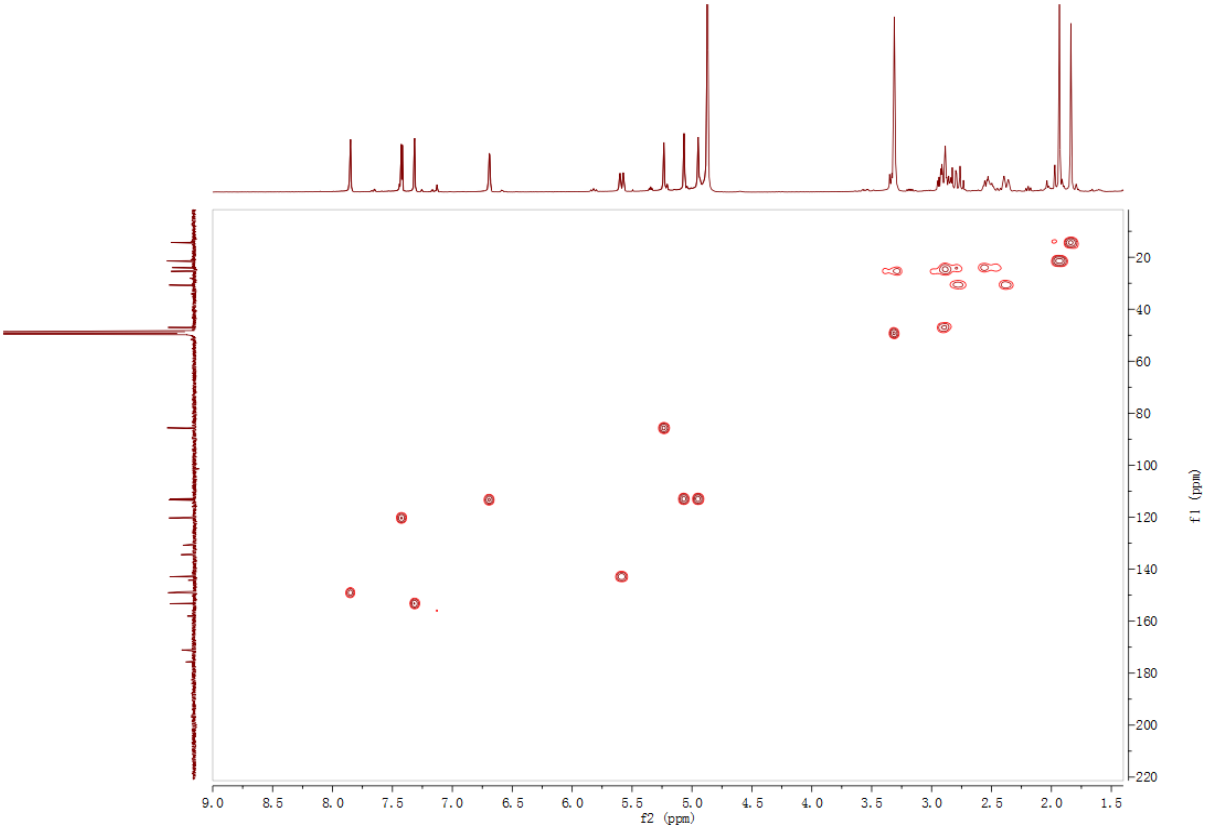
S2.87. ^{1}H NMR spectrum of **19**



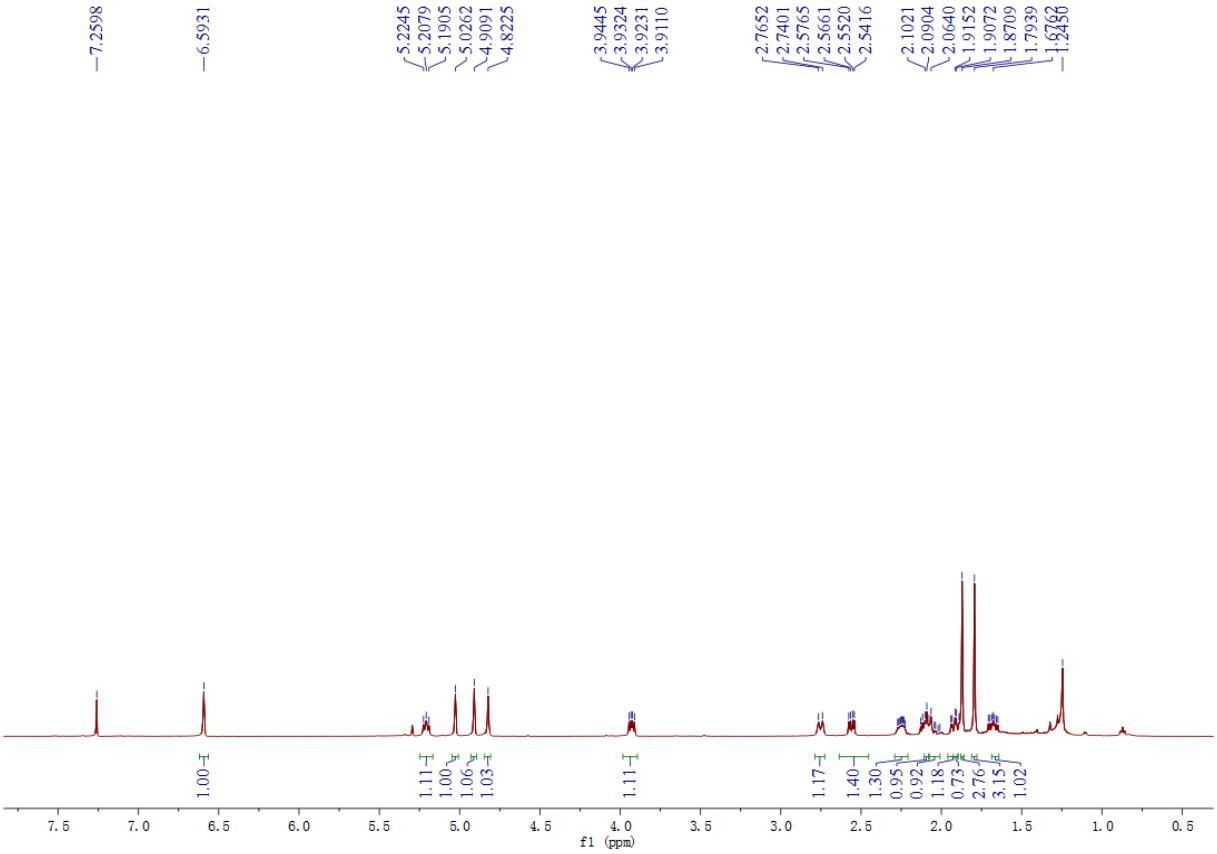
S2.88. ^{13}C NMR and DEPT spectra of **19**



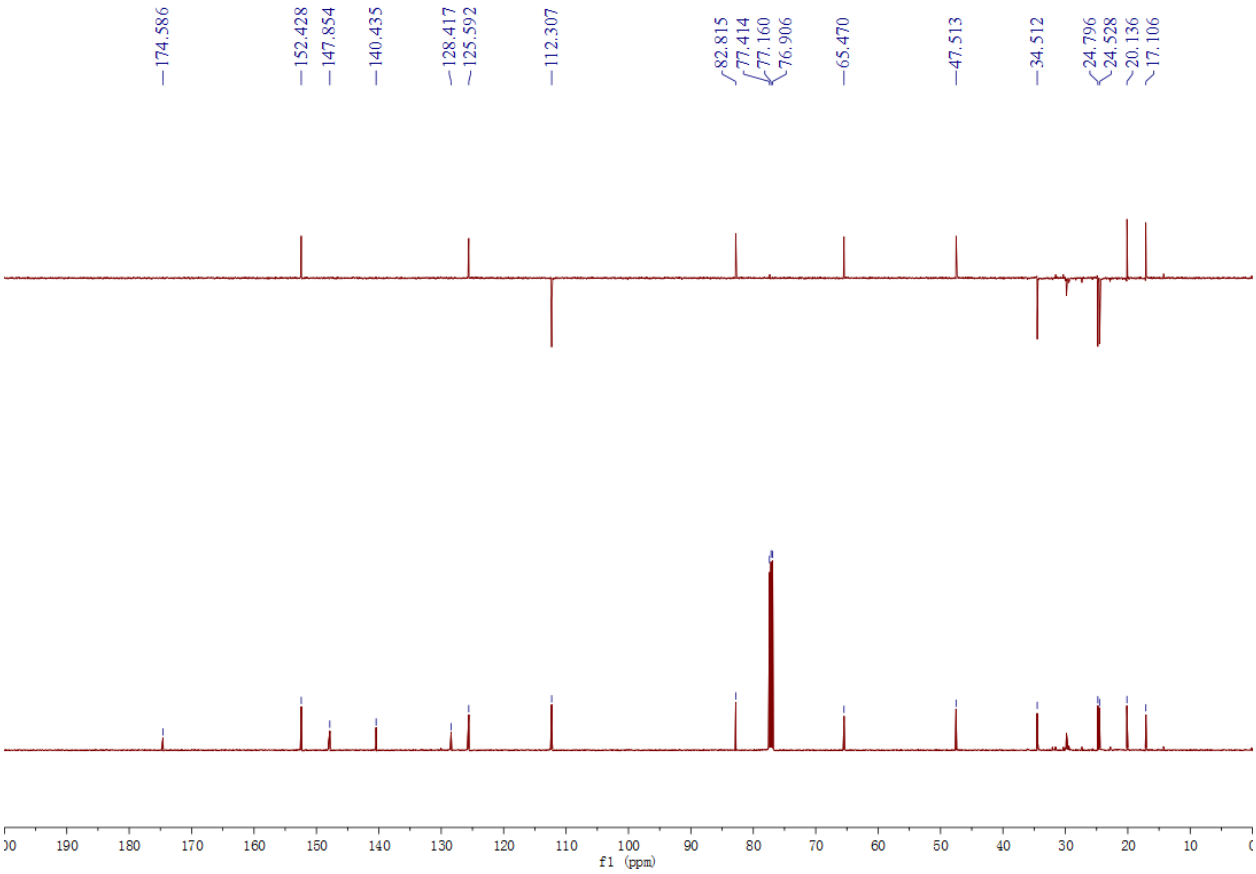
S2.89. HSQC spectrum of **19**



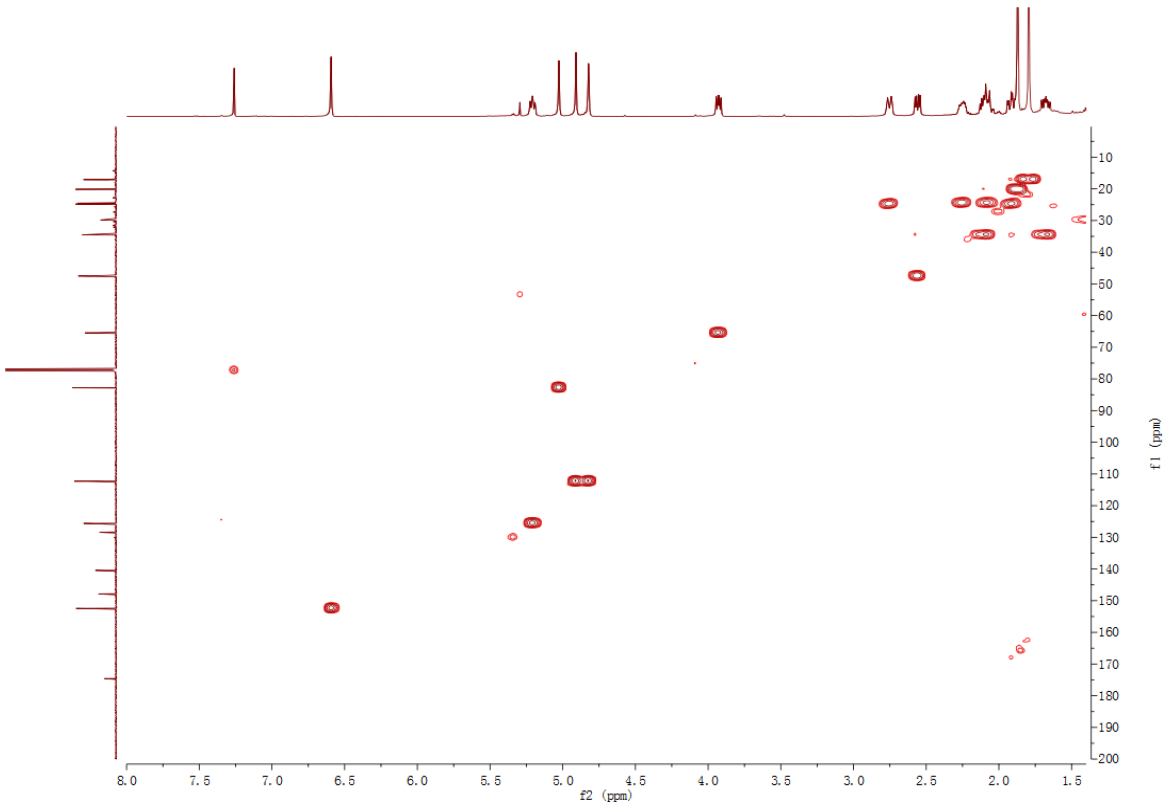
S2.90. ^{1}H NMR spectrum of **20**



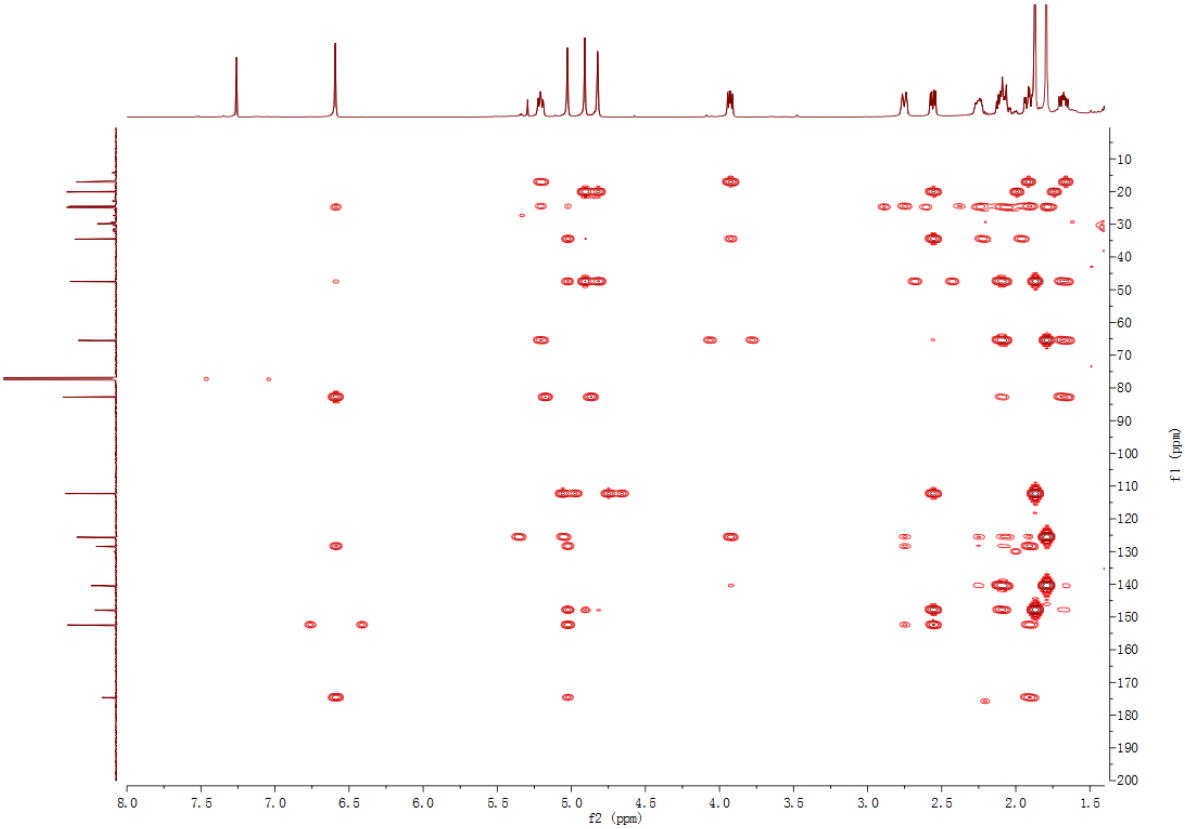
S2.91. ^{13}C NMR and DEPT spectra of **20**



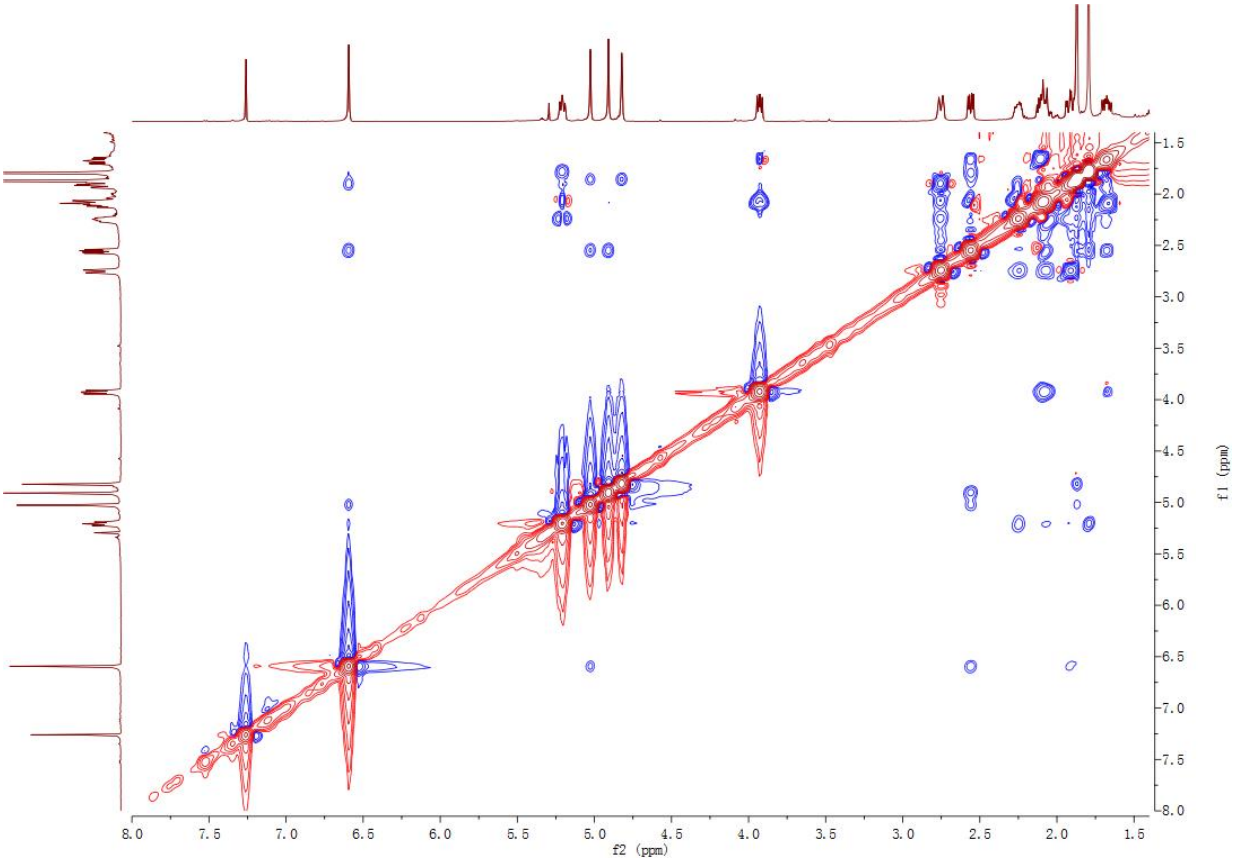
S2.92. HSQC spectrum of **20**



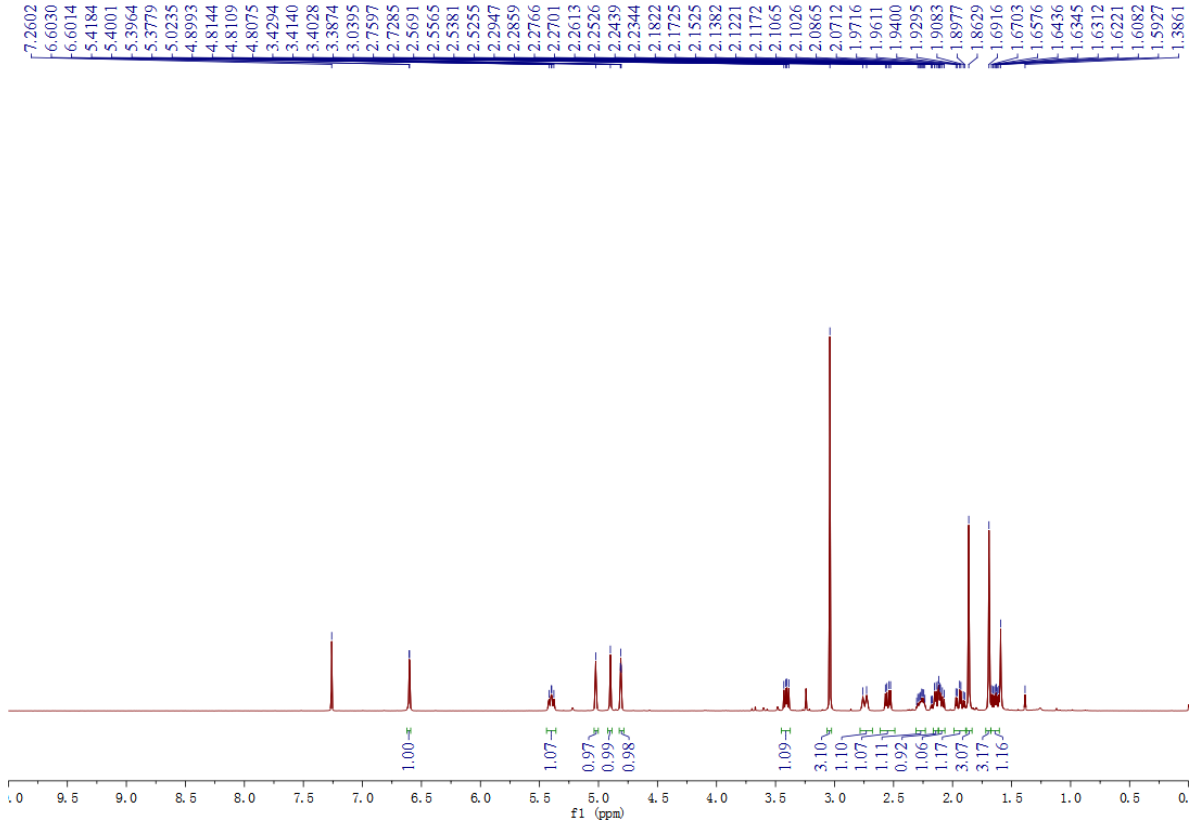
S2.93. HMBC spectrum of **20**



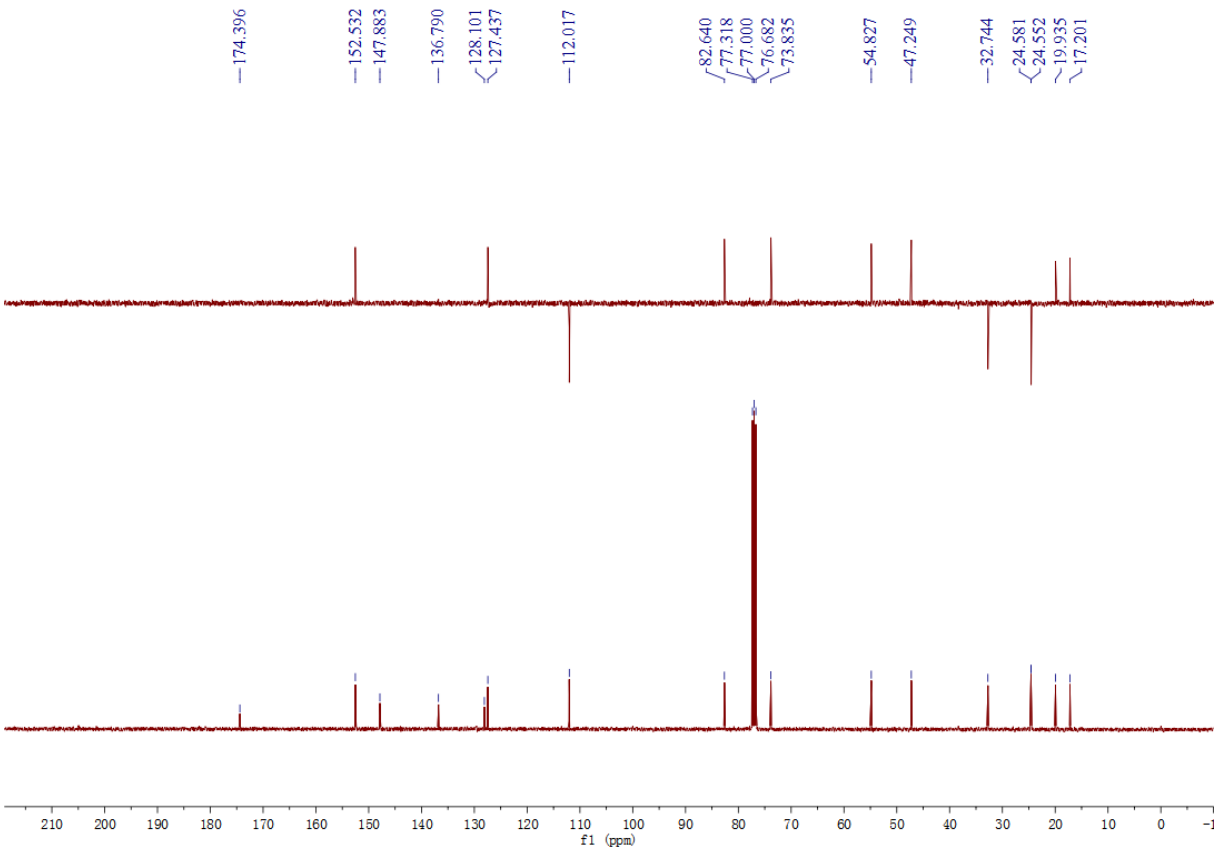
S2.94. NOESY spectrum of **20**



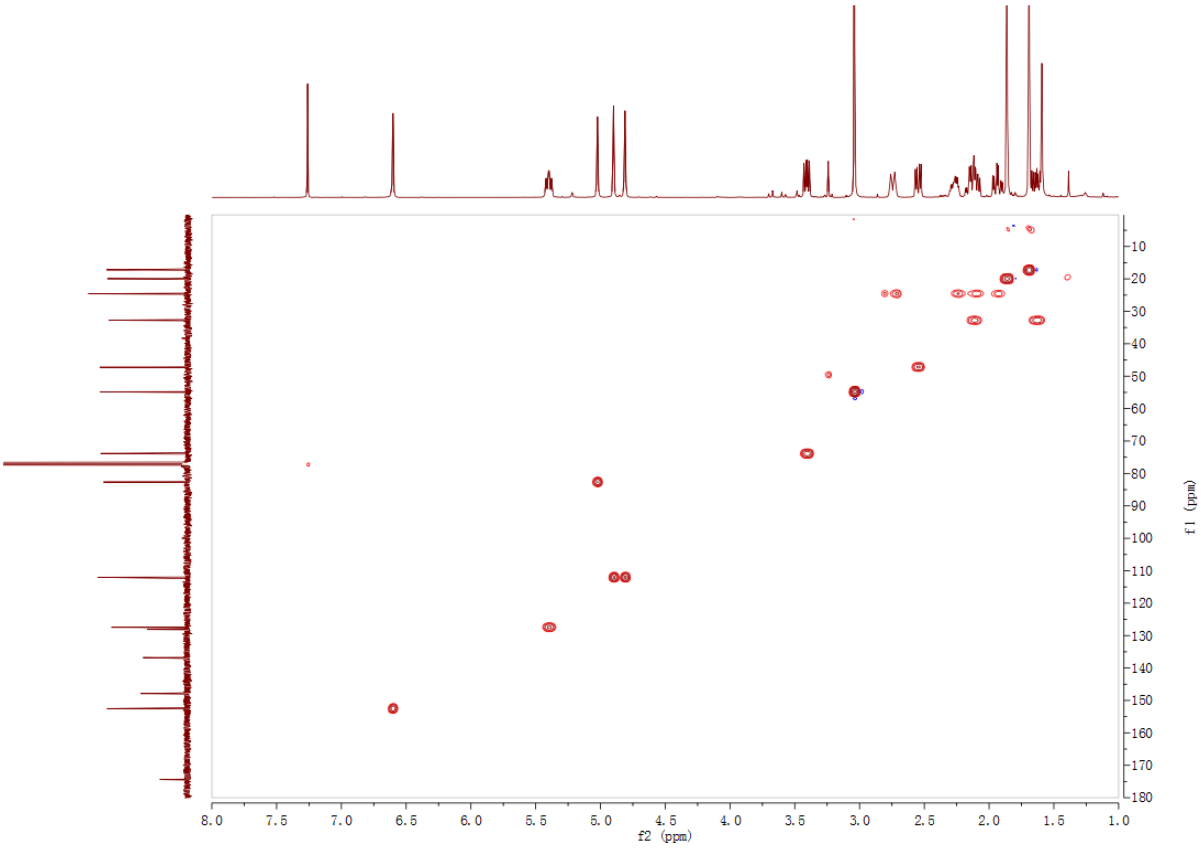
S2.95. ^{1}H NMR spectrum of **21**



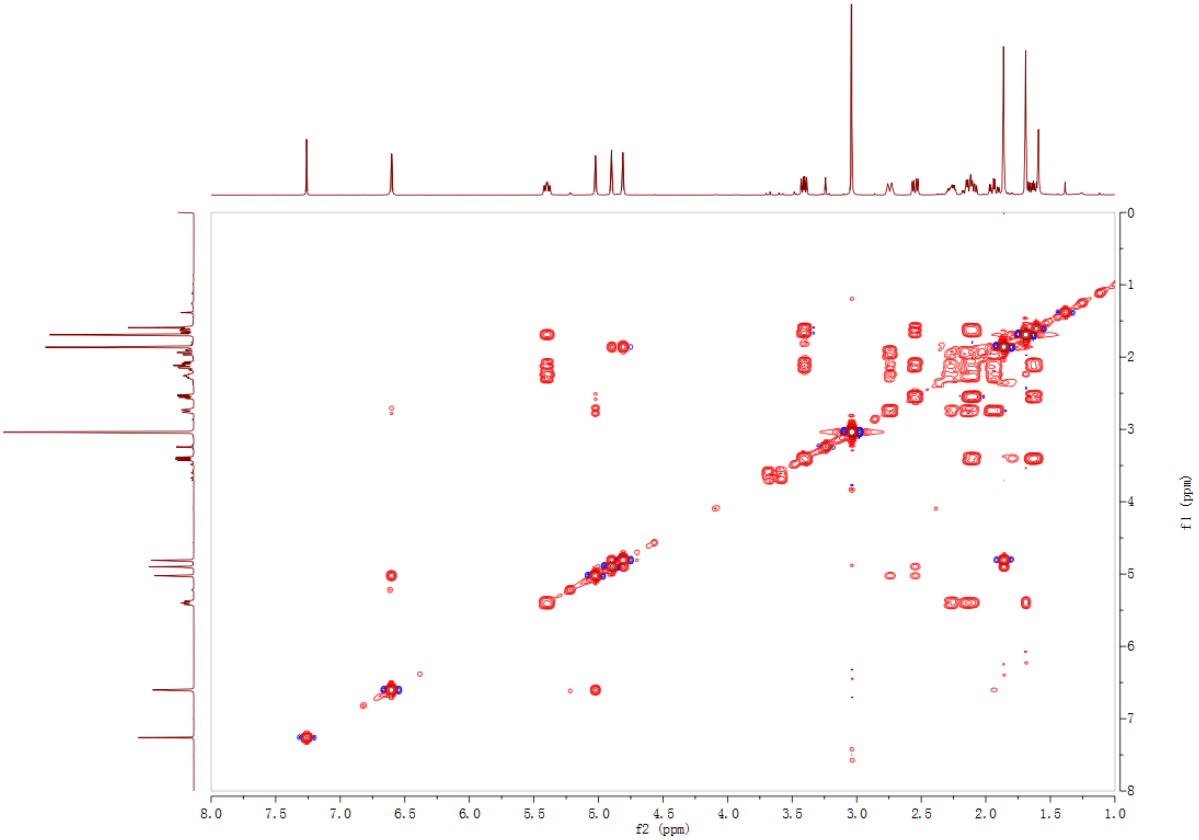
S2.96. ^{13}C NMR and DEPT spectra of **21**



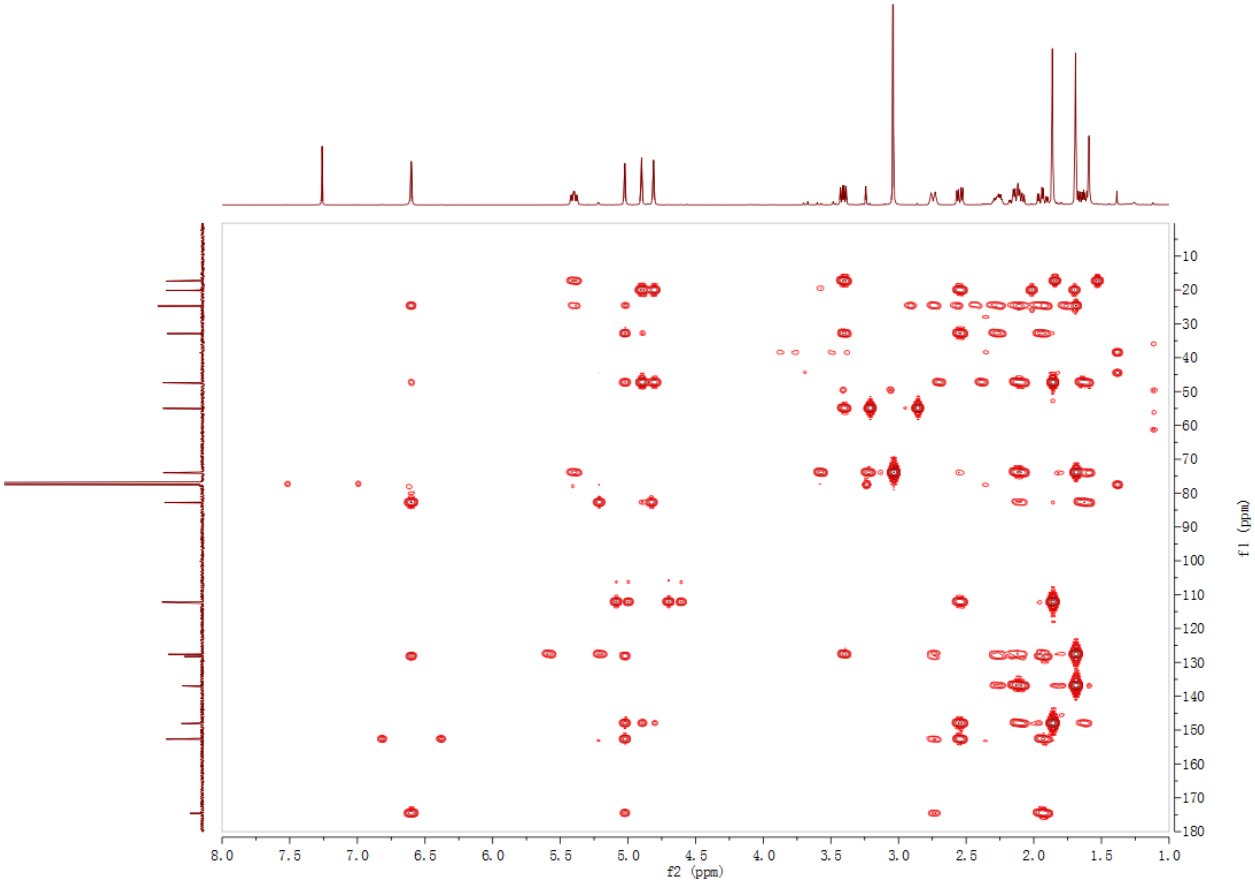
S2.97. HSQC spectrum of **21**



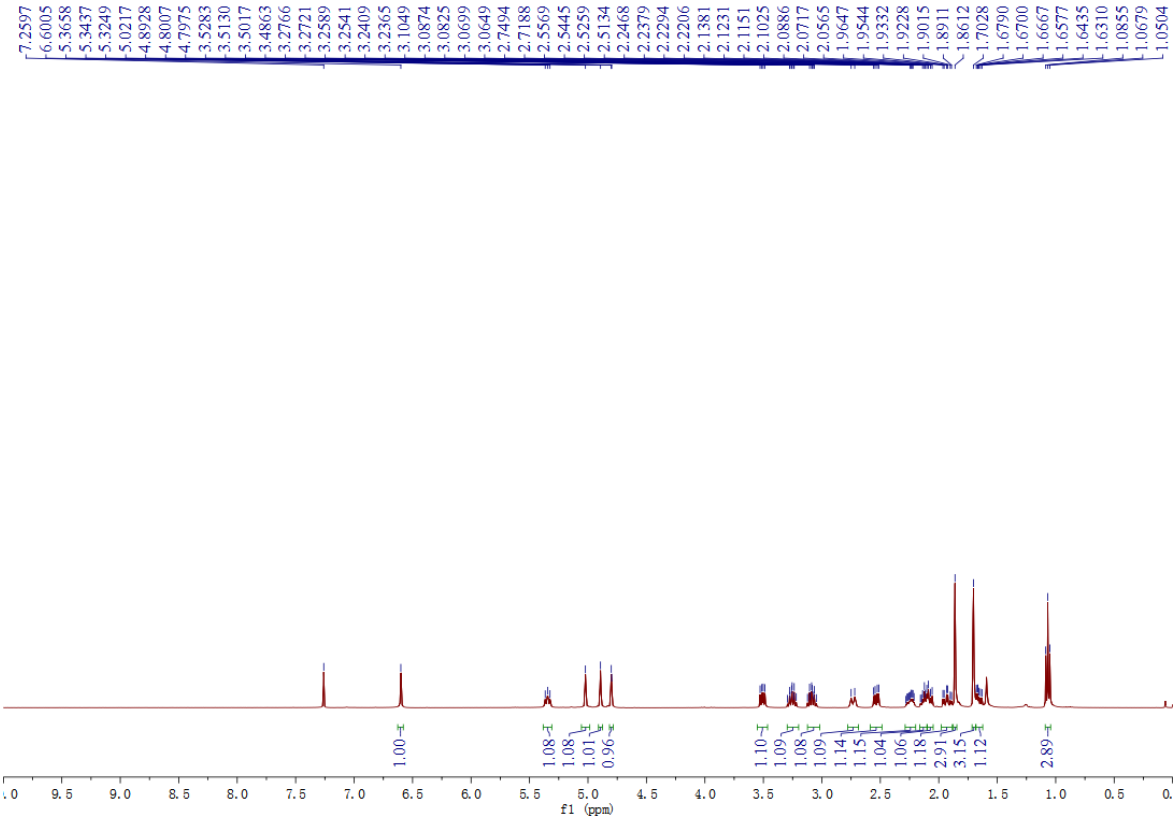
S2.98. ^{1}H–^{1}H COSY spectrum of **21**



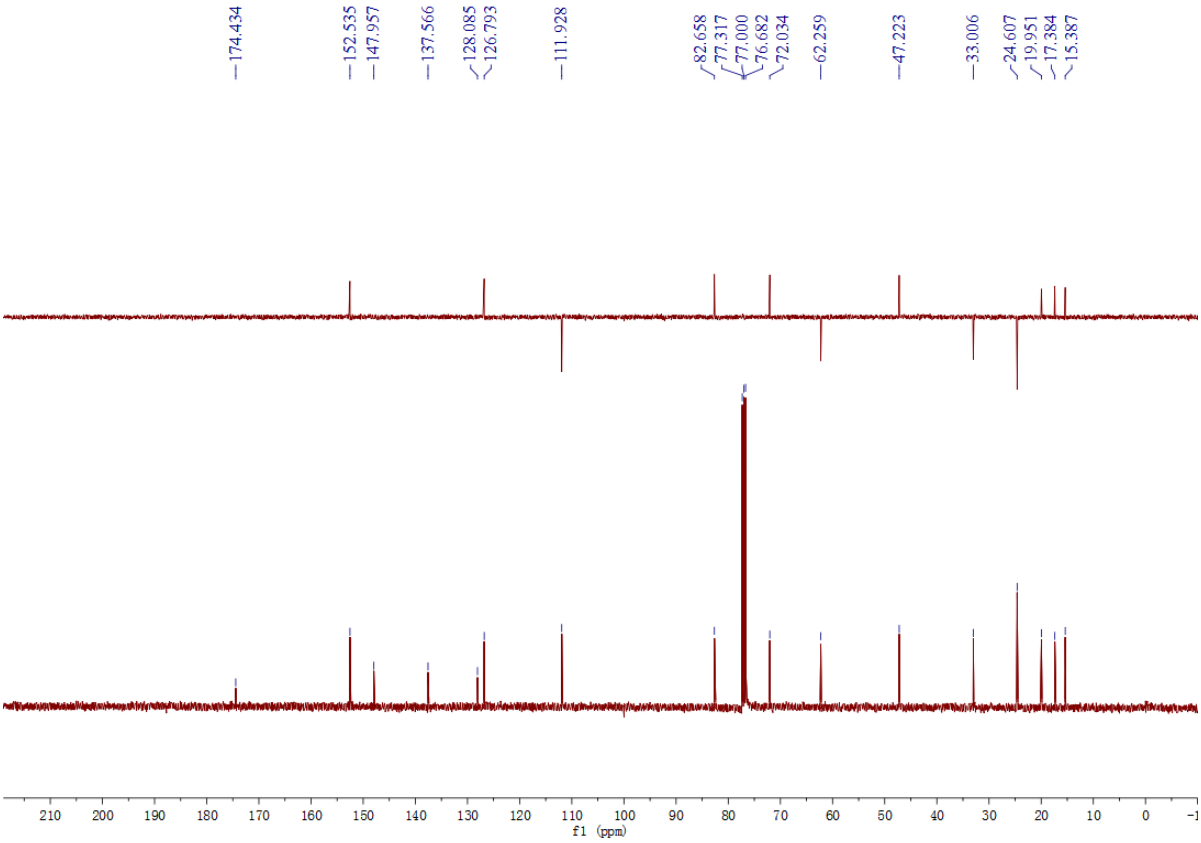
S2.99. HMBC spectrum of **21**



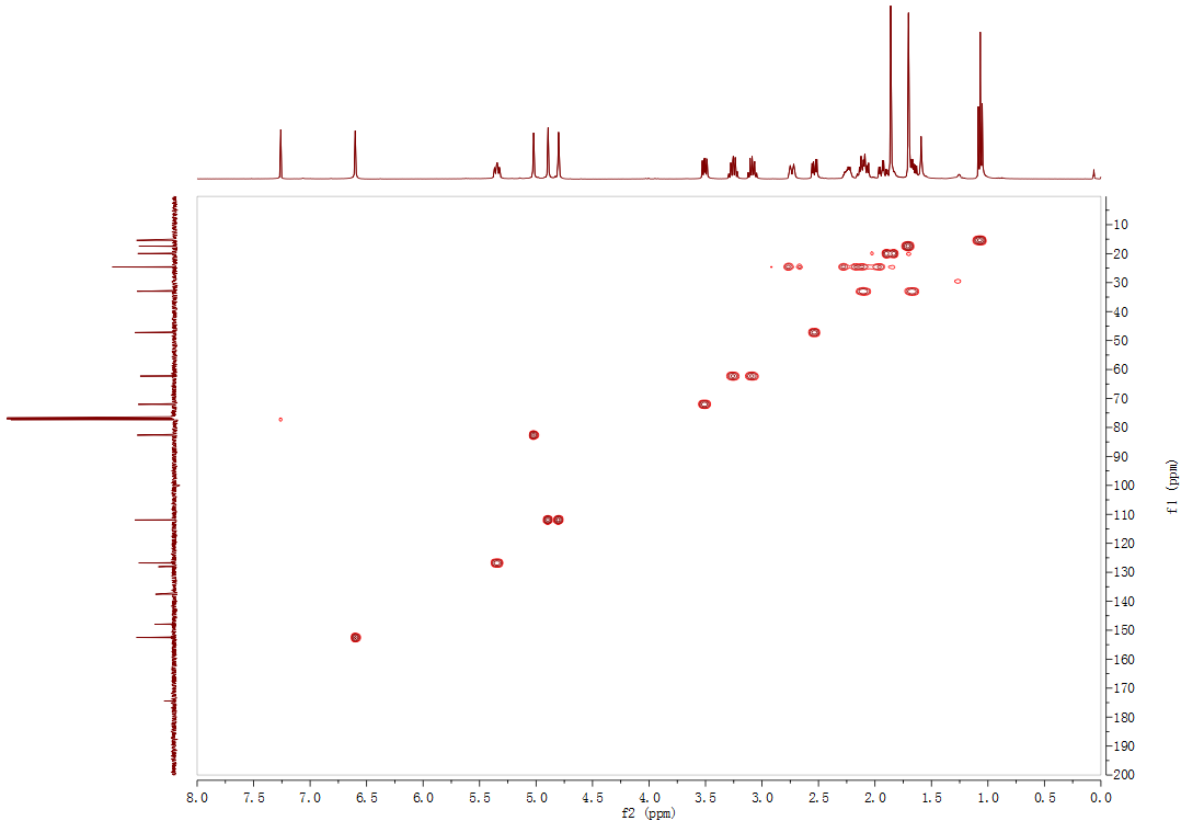
S2.100. ^{1}H NMR spectrum of **22**



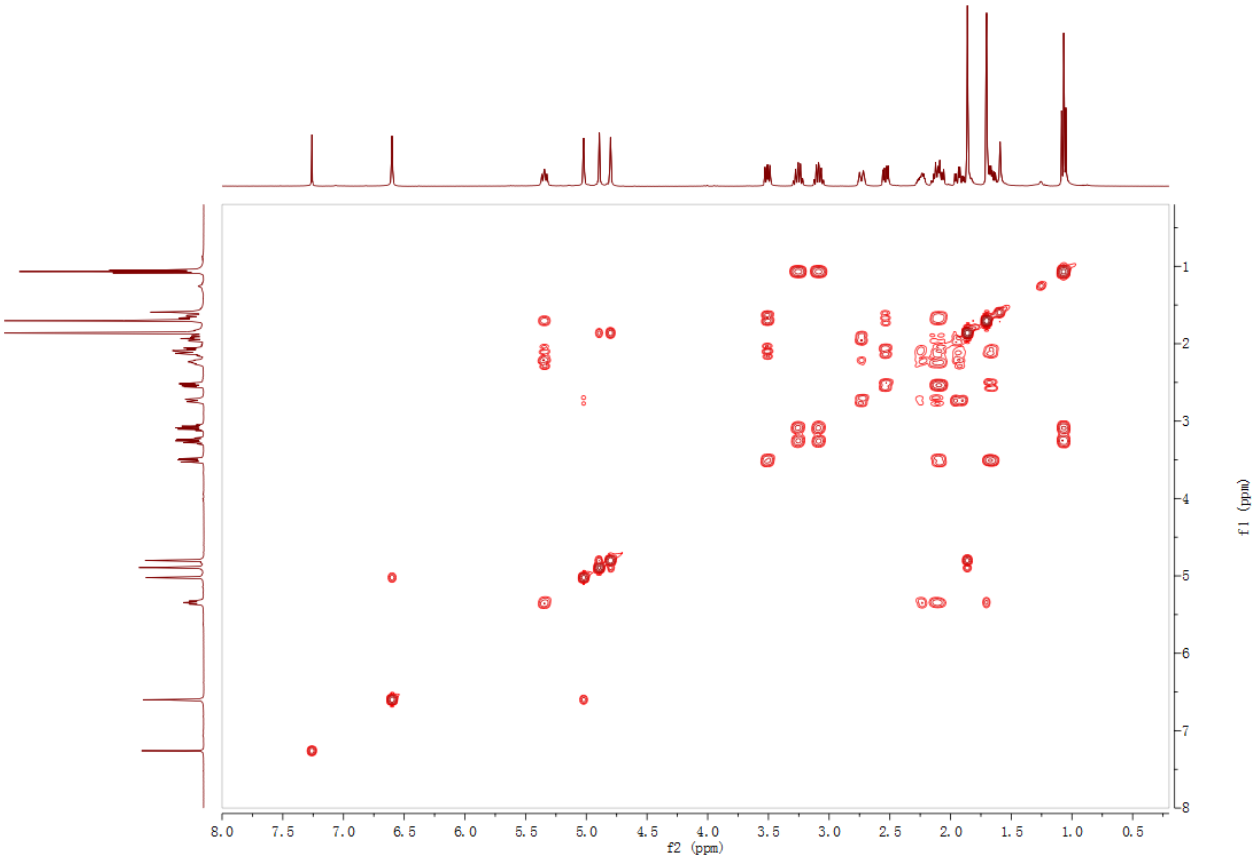
S2.101. ^{13}C NMR and DEPT spectra of **22**



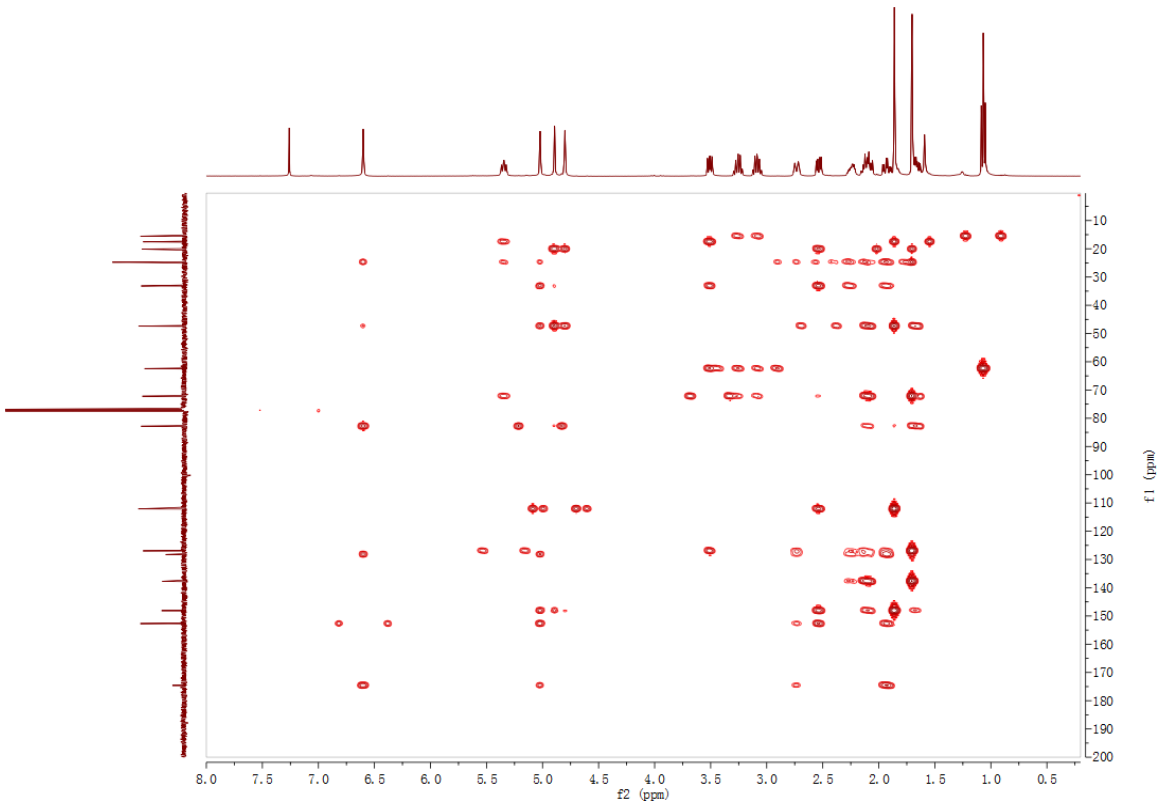
S2.102. HSQC spectrum of **22**



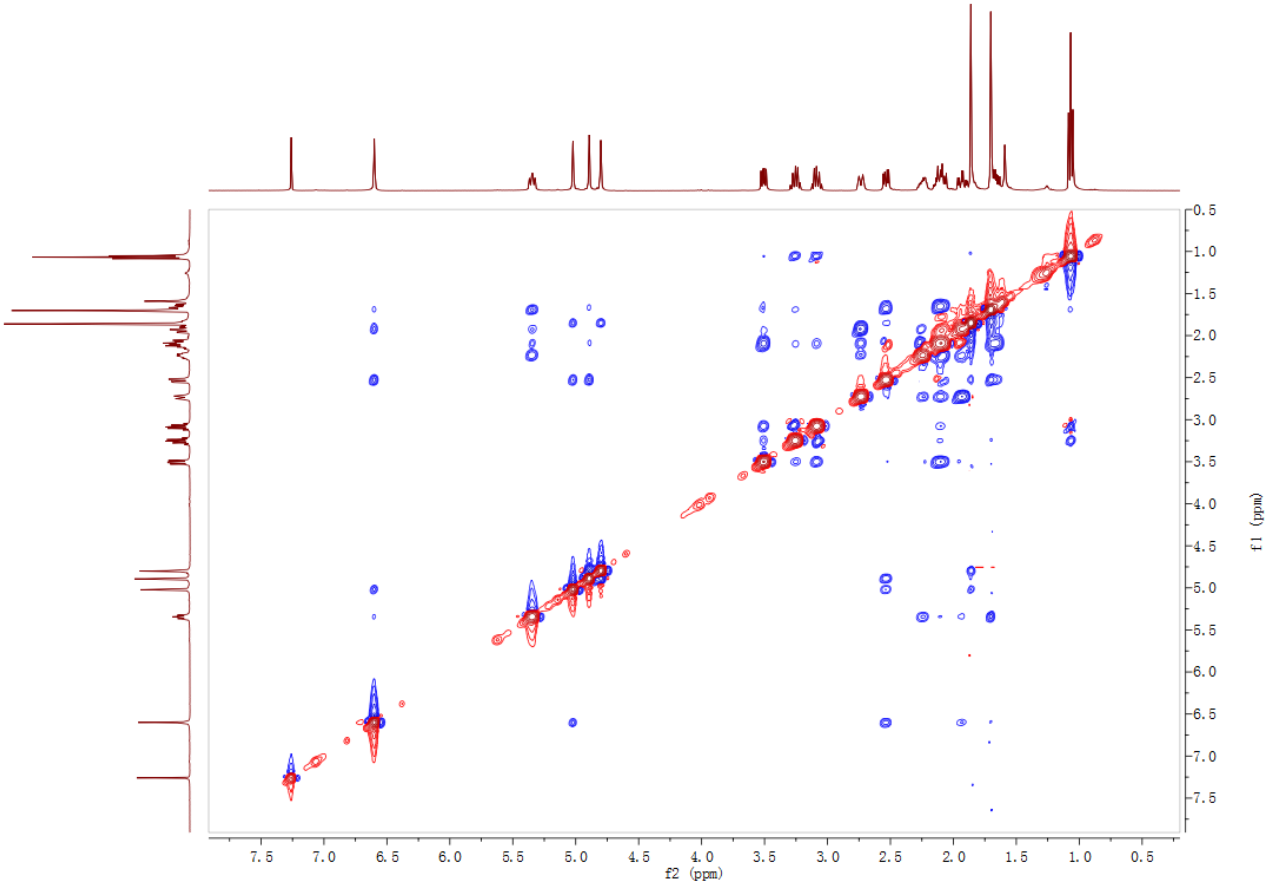
S2.103. $^{1}H–^{1}H$ COSY spectrum of **22**



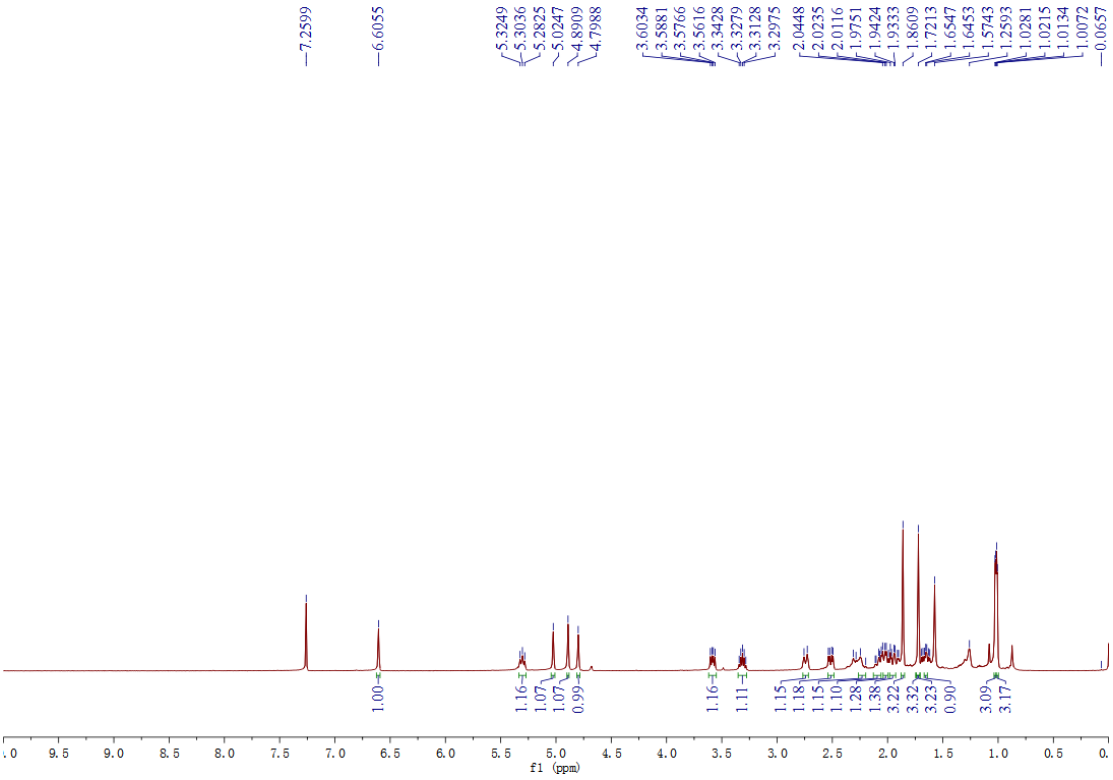
S2.104. HMBC spectrum of **22**



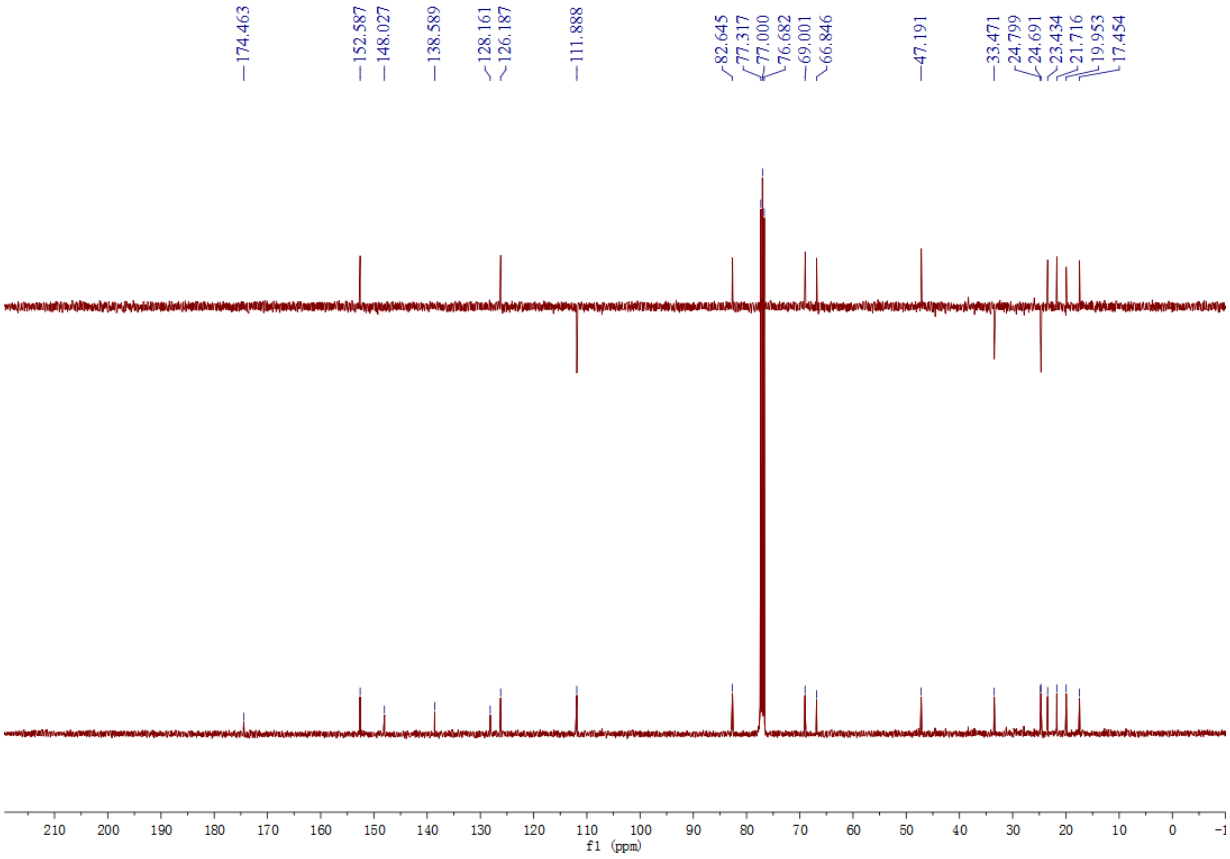
S2.105. NOESY spectrum of **22**



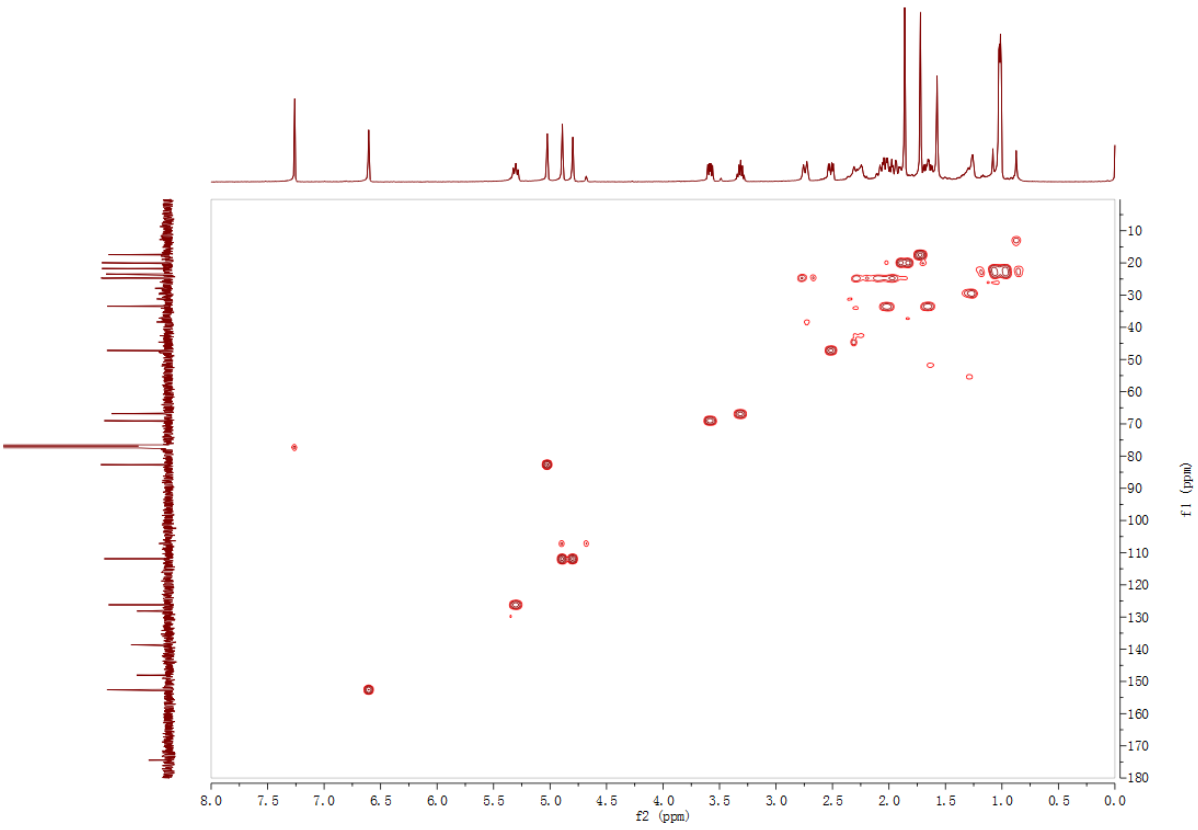
S2.106. ^{1}H NMR spectrum of **23**



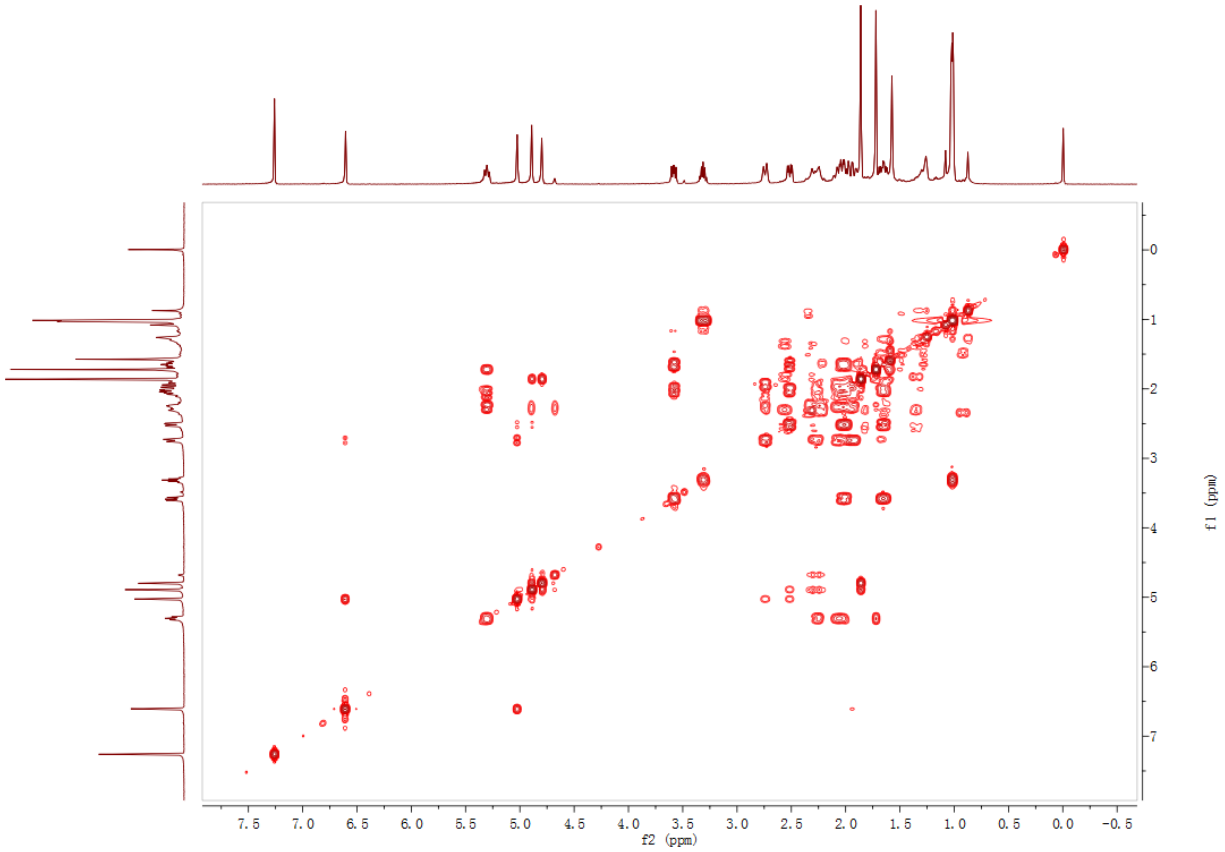
S2.107. ^{13}C NMR and DEPT spectra of **23**



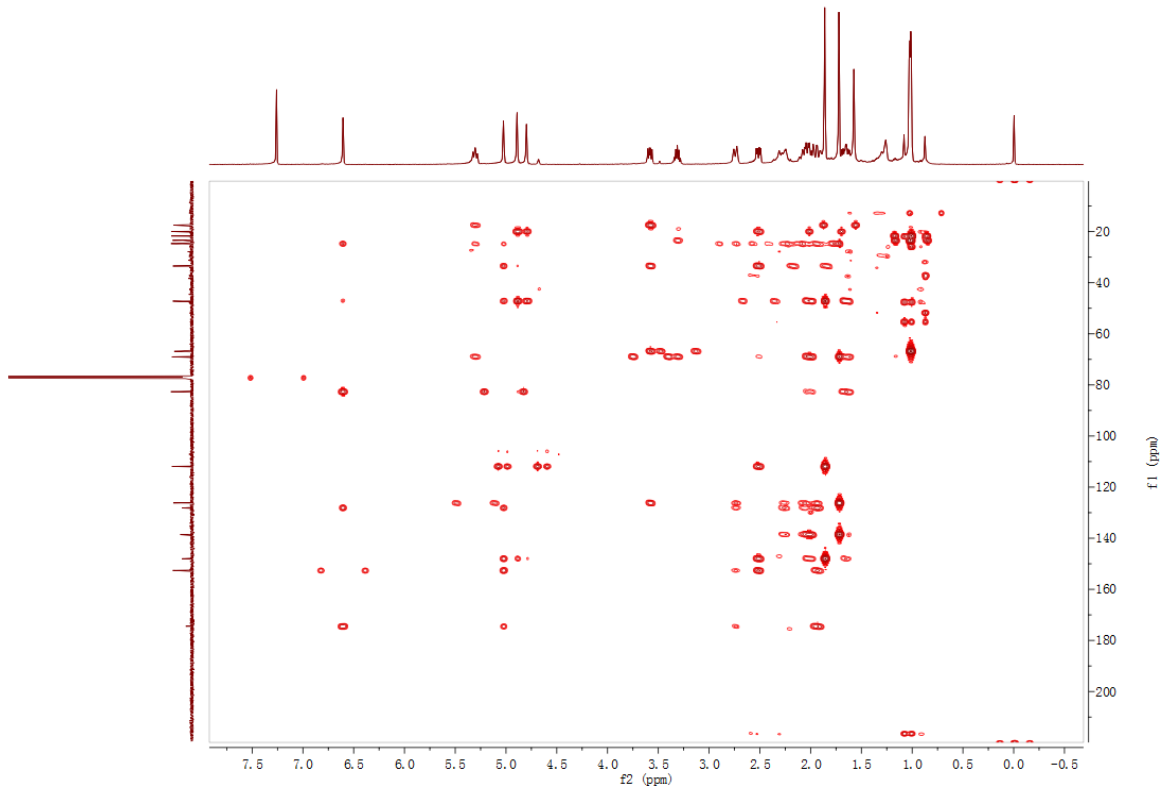
S2.108. HSQC spectrum of **23**



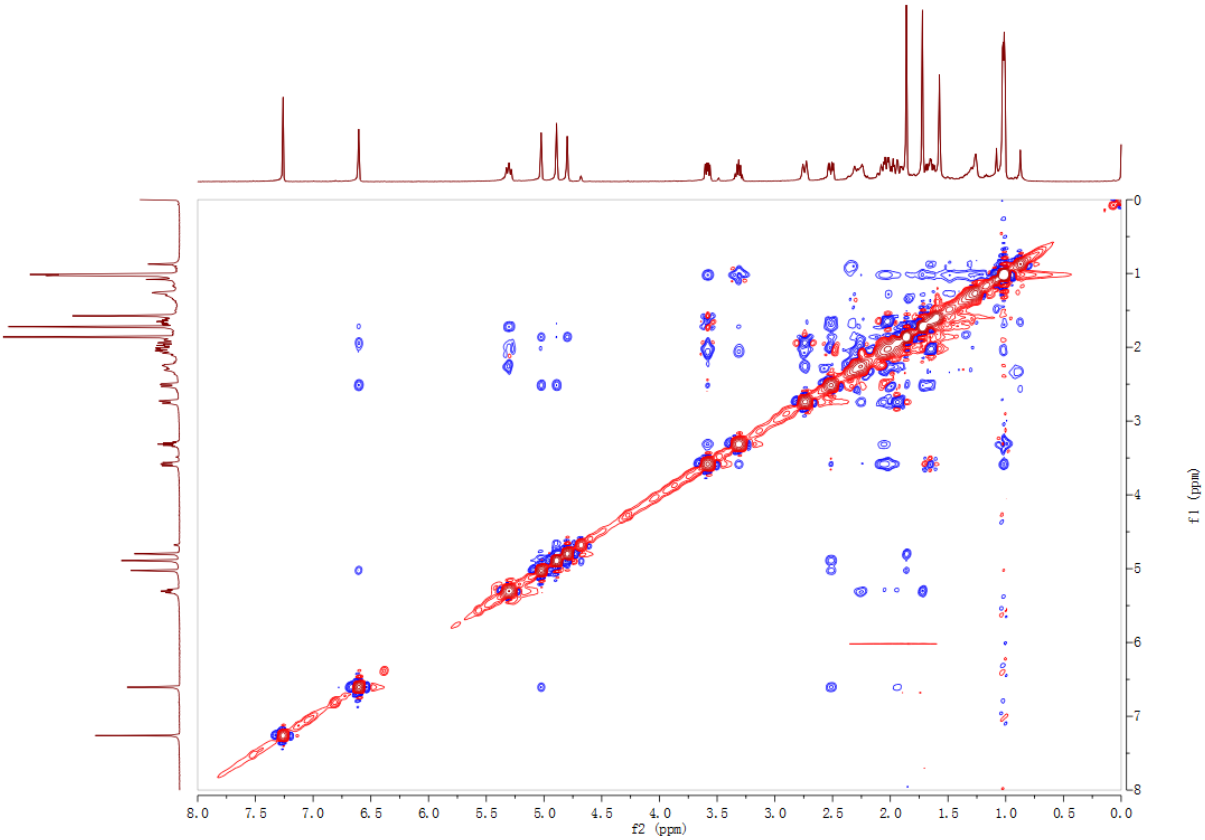
S2.109. $^{1}H–^{1}H$ COSY spectrum of **23**



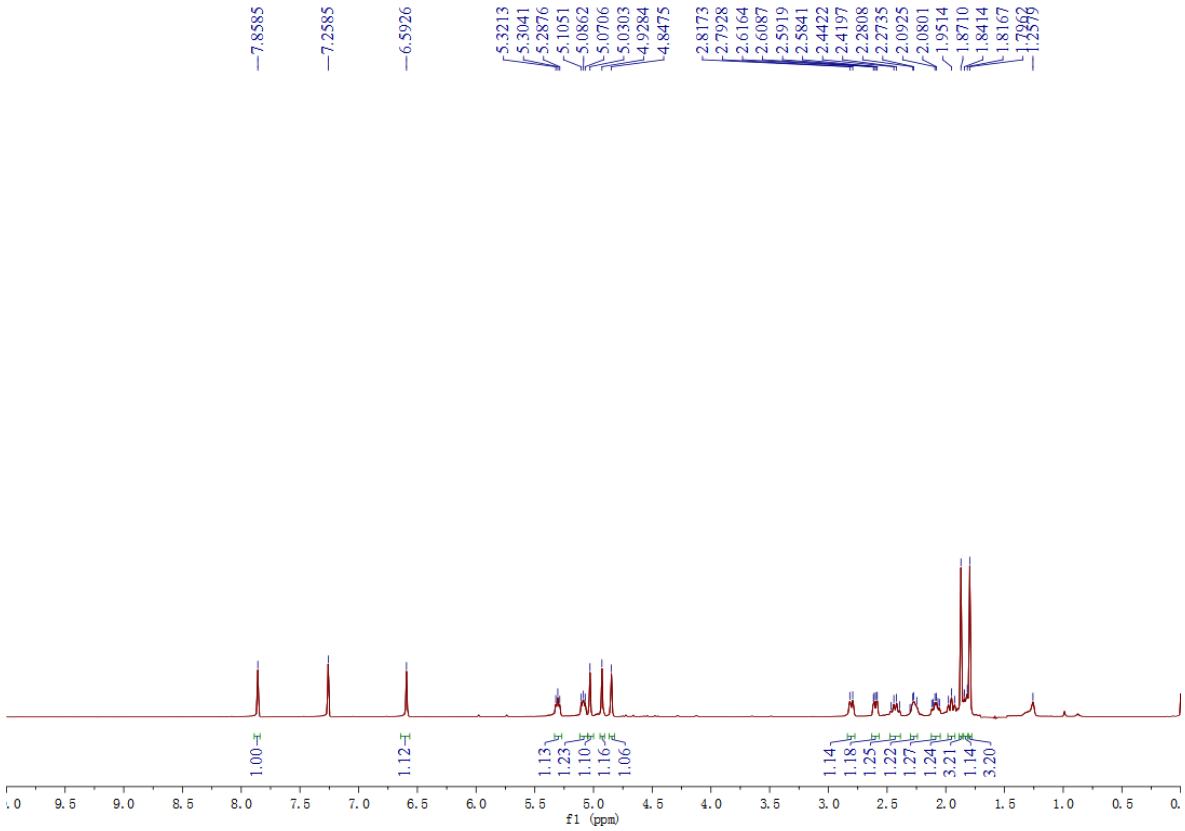
S2.110. HMBC spectrum of **23**



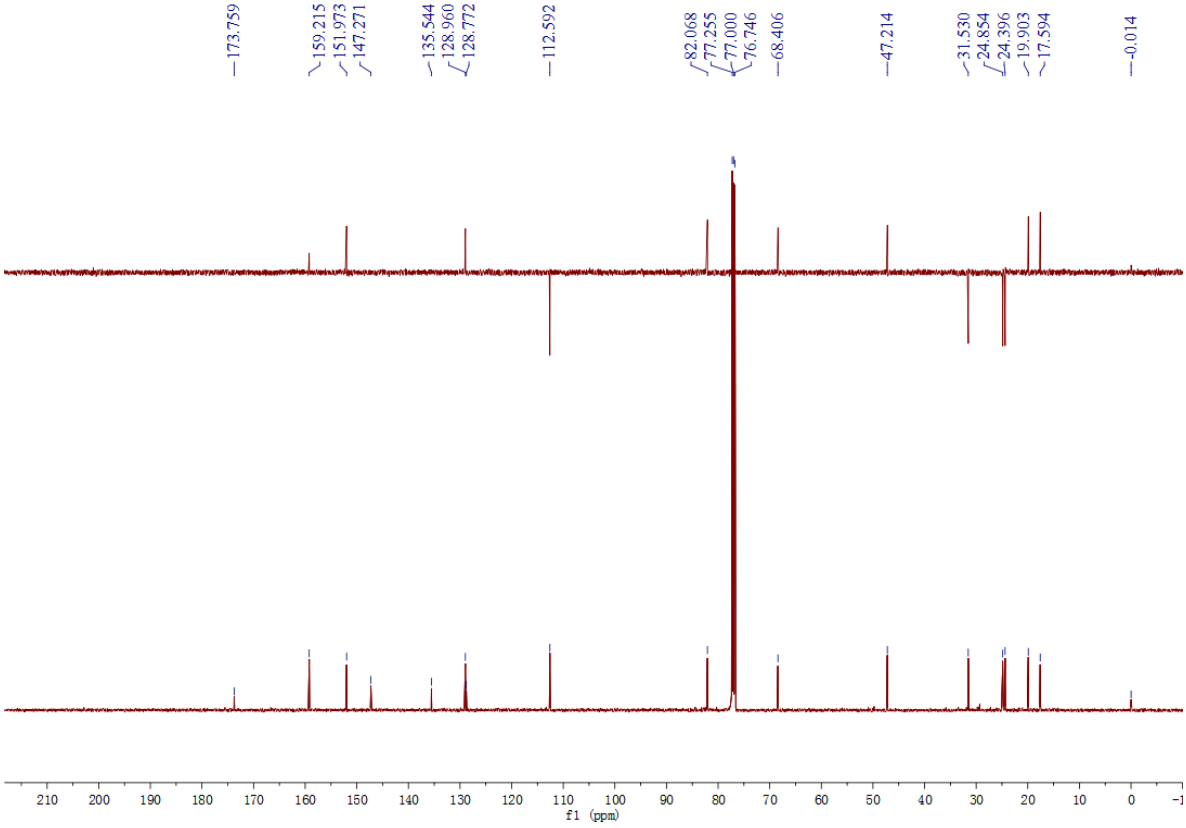
S2.111. NOESY spectrum of **23**



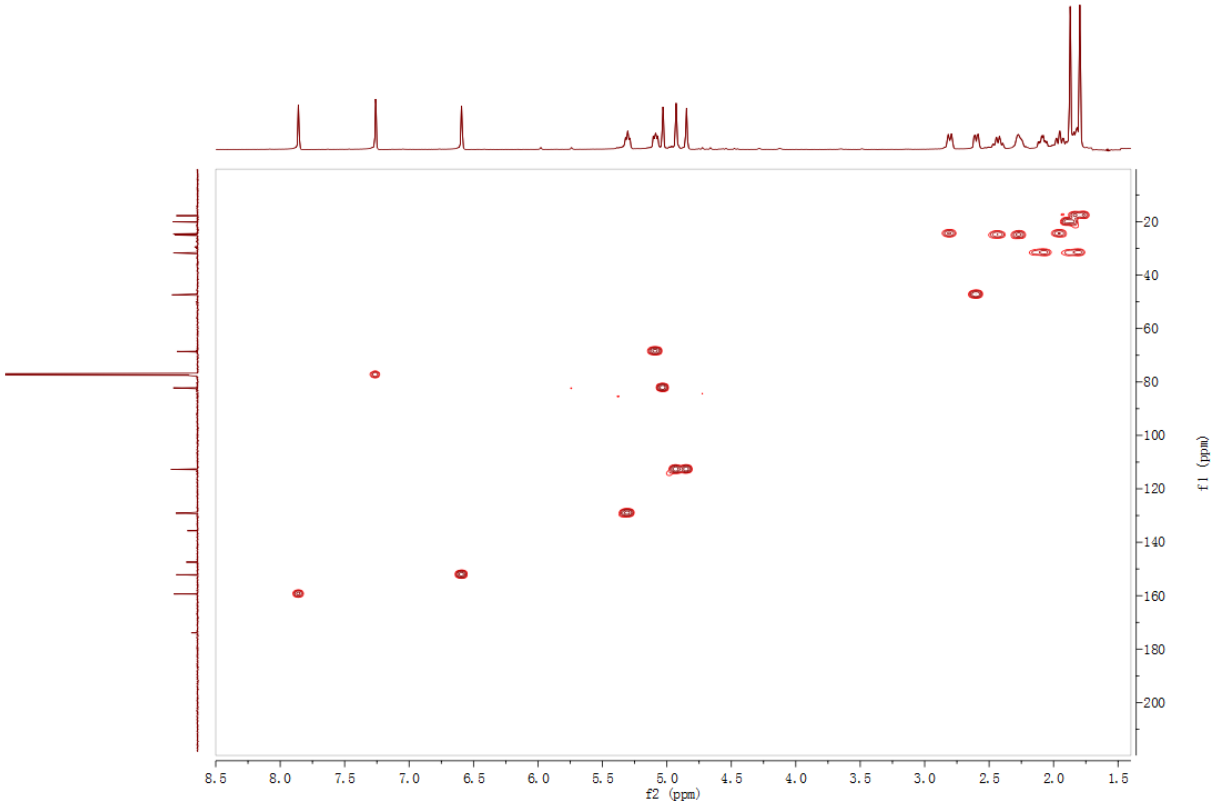
S2.112. ^{1}H NMR spectrum of **24**



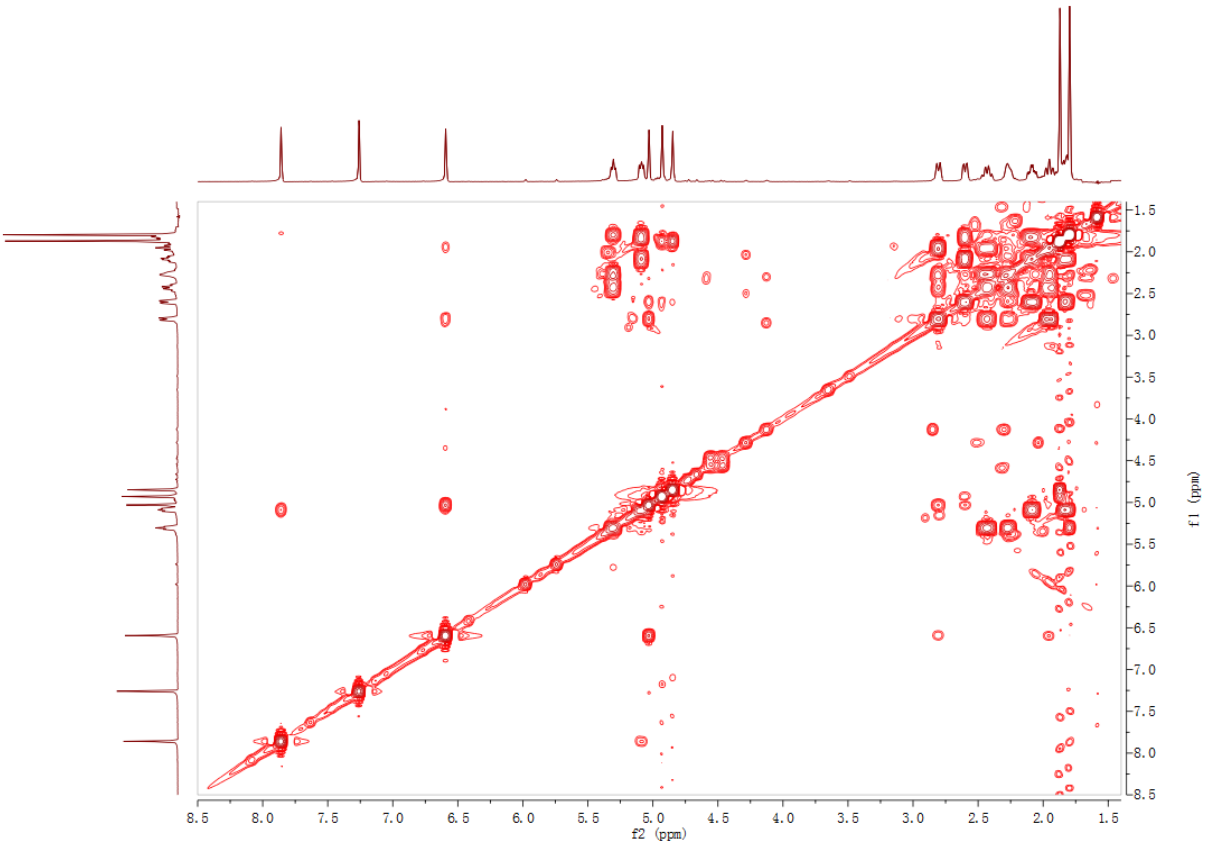
S2.113. ^{13}C NMR and DEPT spectra of **24**



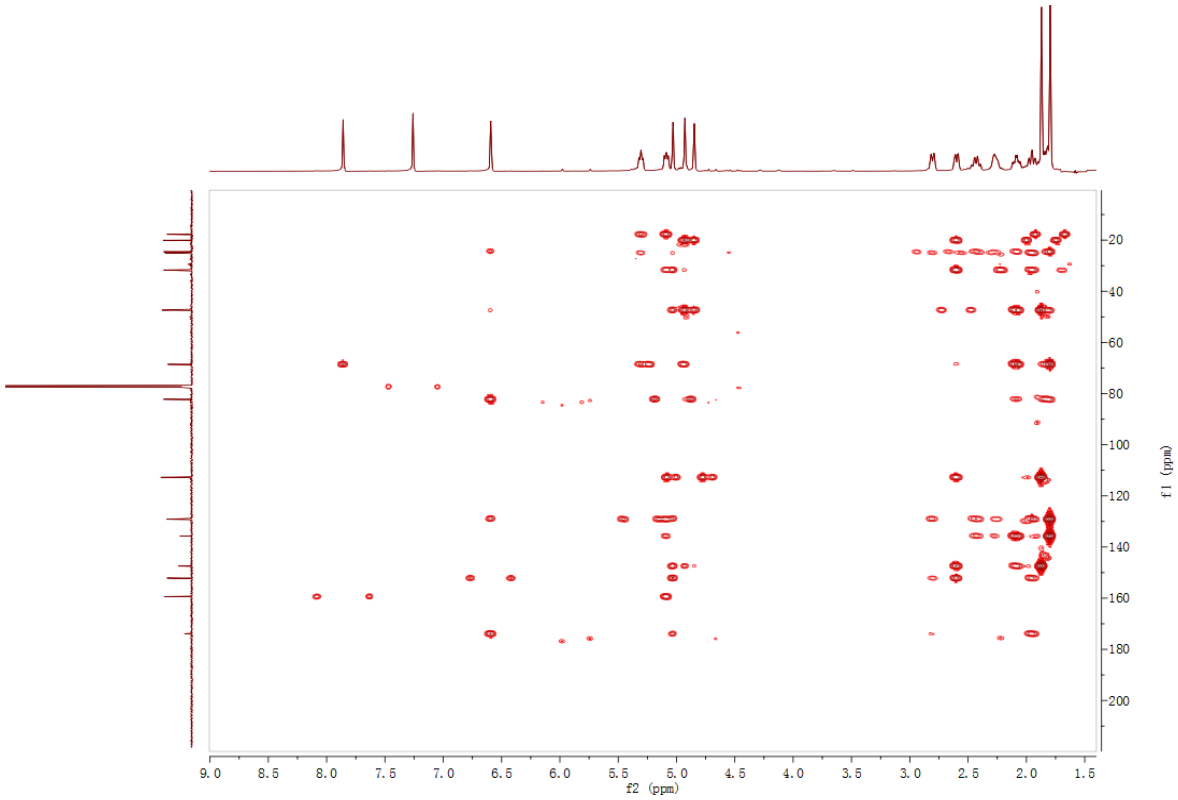
S2.114. HSQC spectrum of **24**



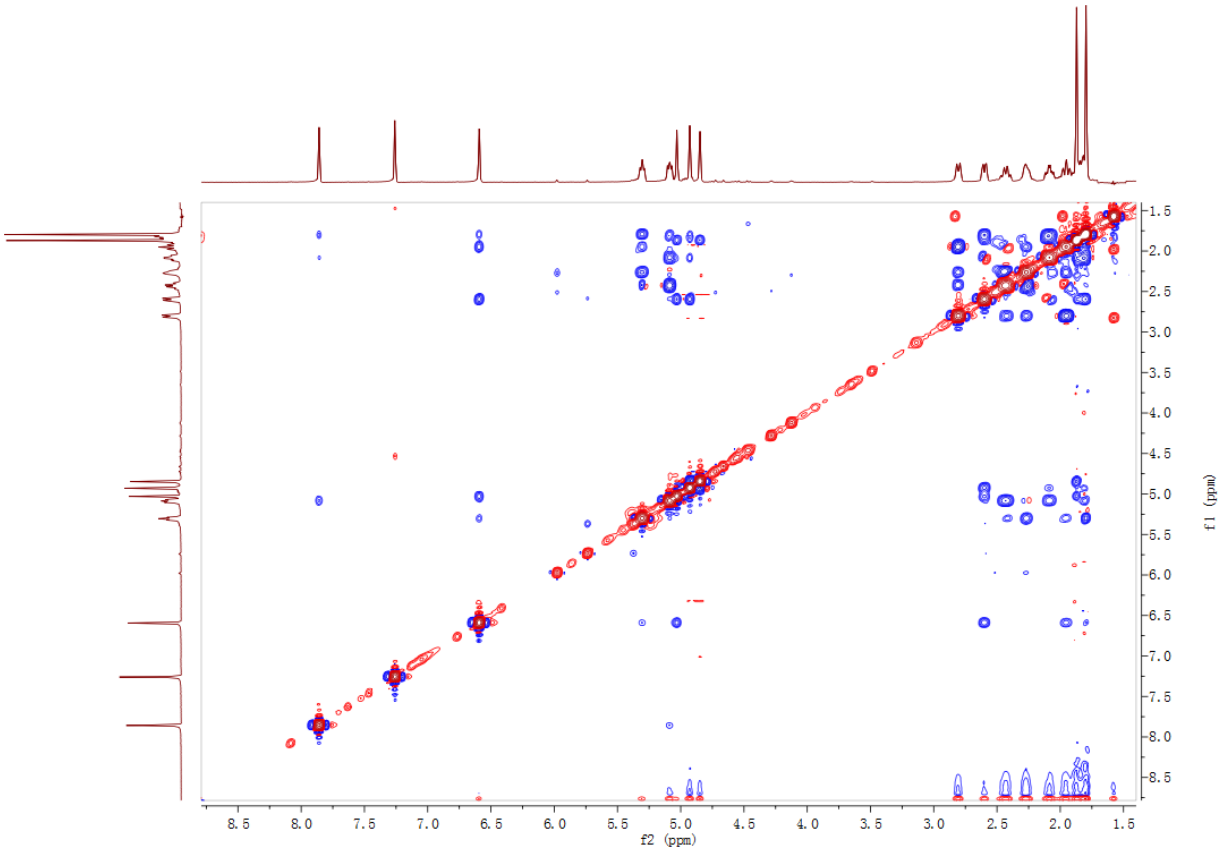
S2.115. $^{1}H–^{1}H$ COSY spectrum of **24**



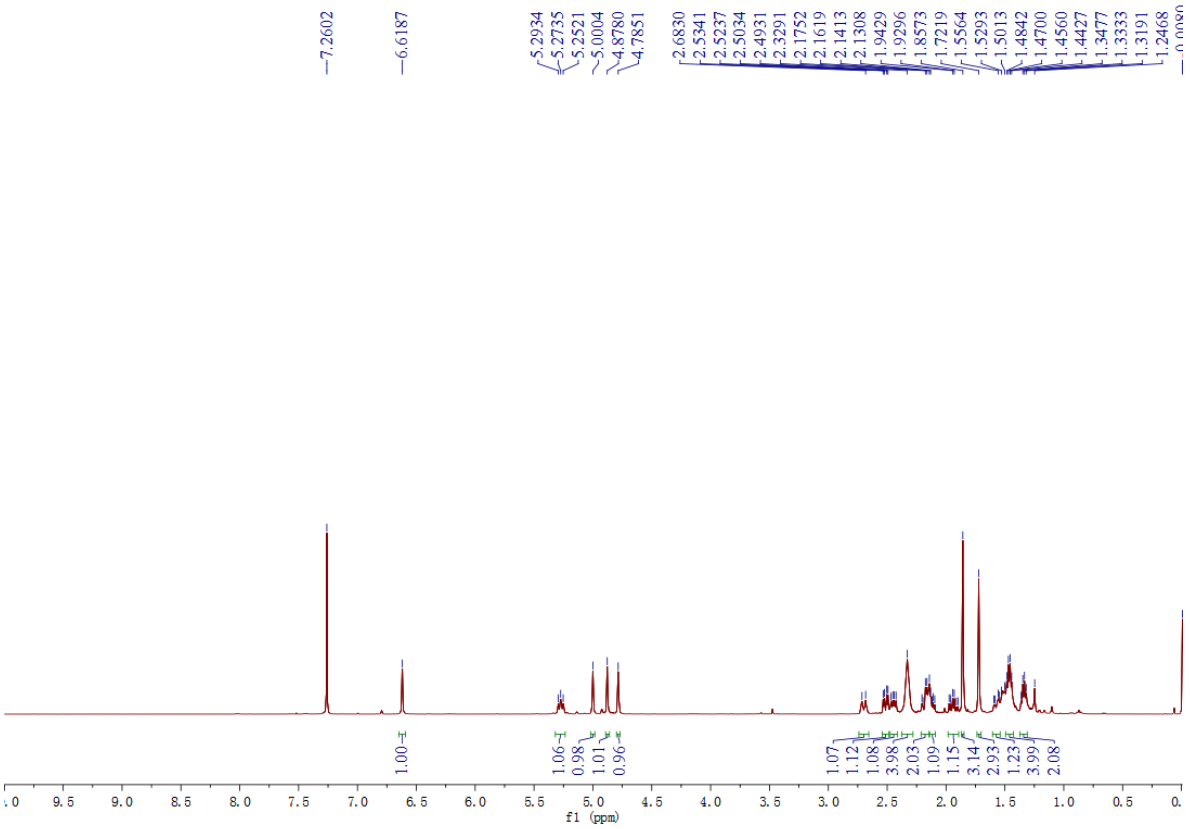
S2.116. HMBC spectrum of **24**



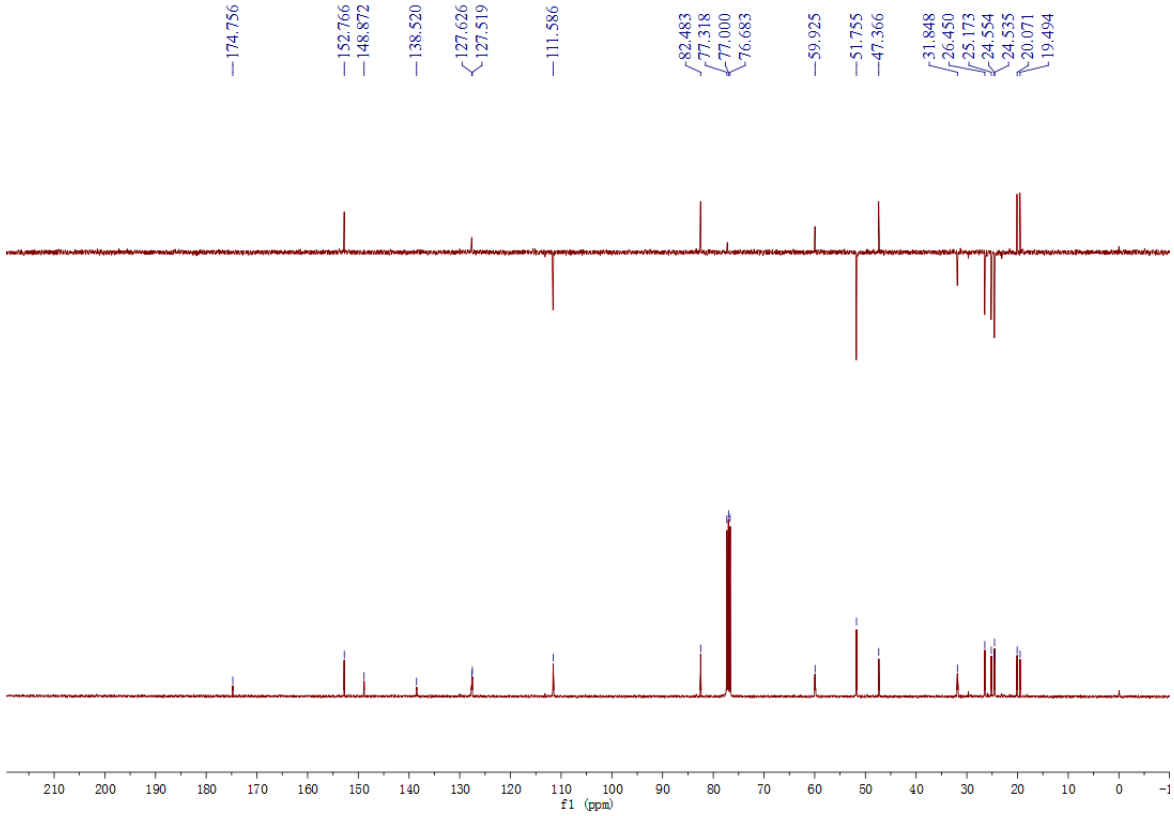
S2.117. NOESY spectrum of **24**



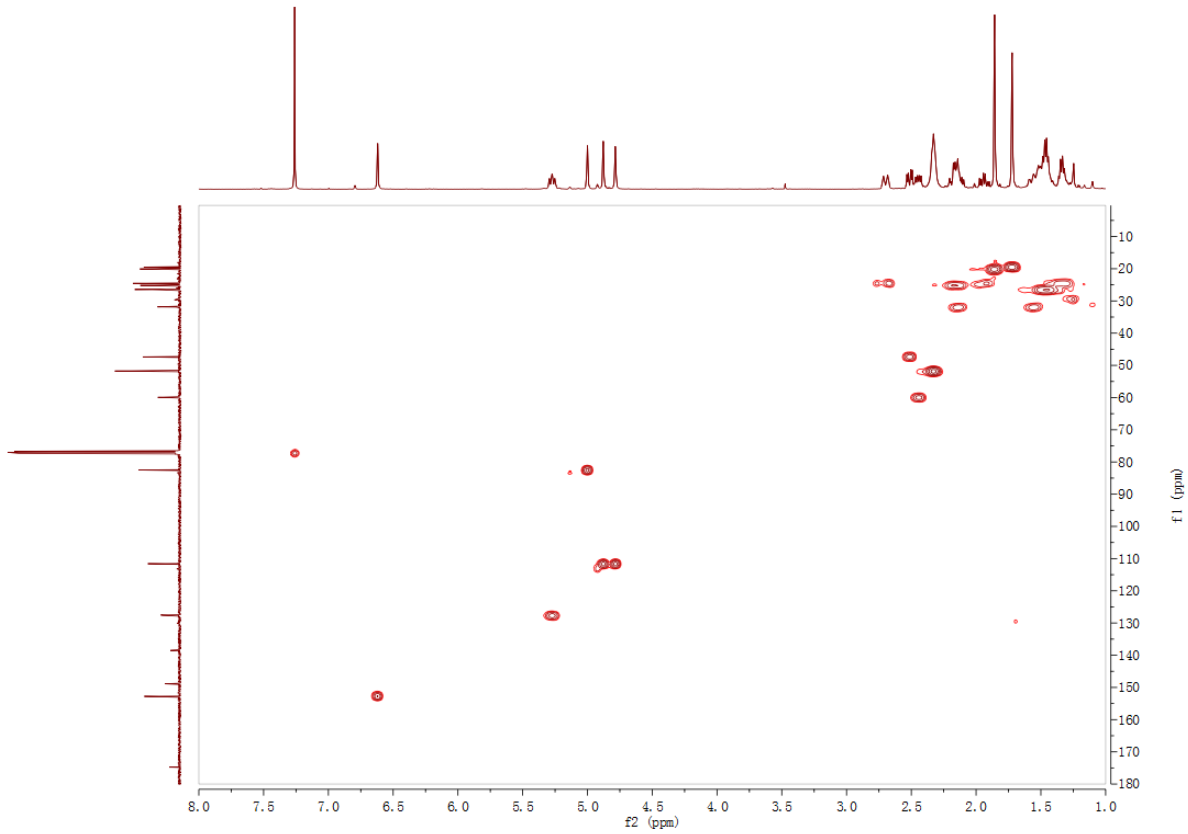
S2.118. ^{1}H NMR spectrum of **25**



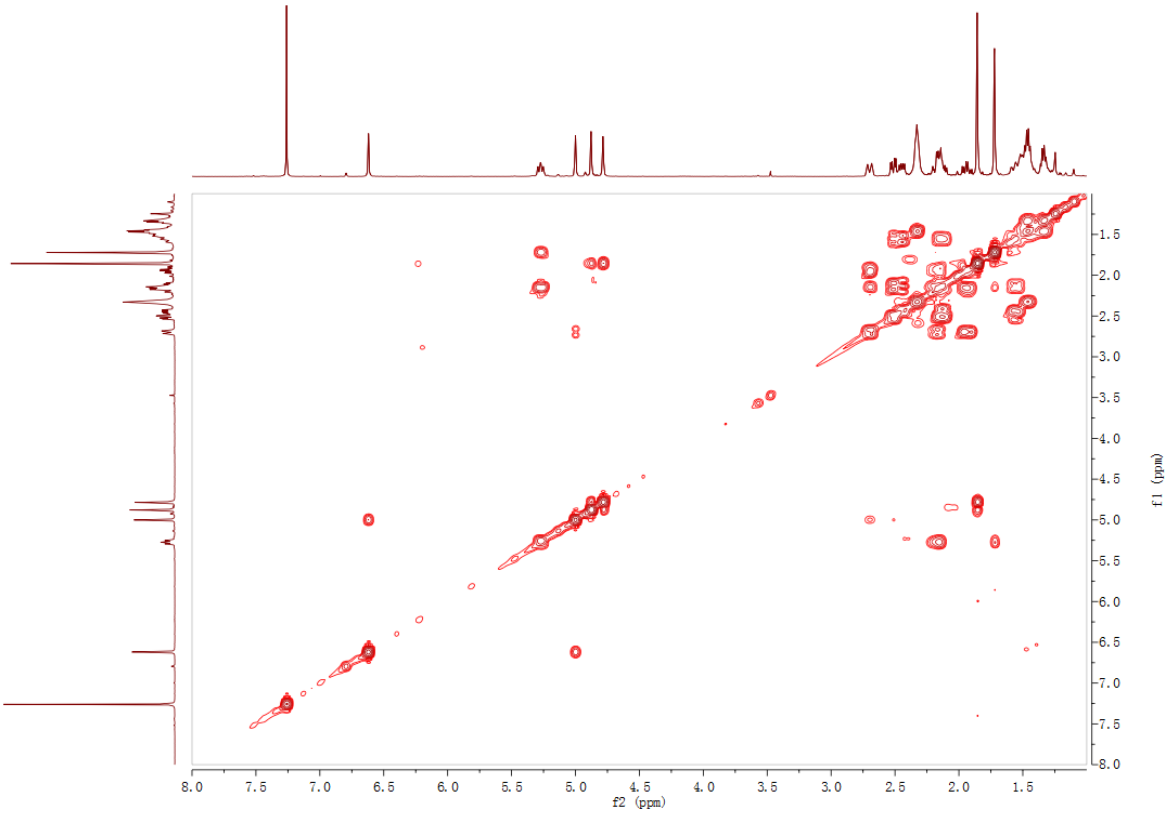
S2.119. ^{13}C NMR and DEPT spectra of **25**



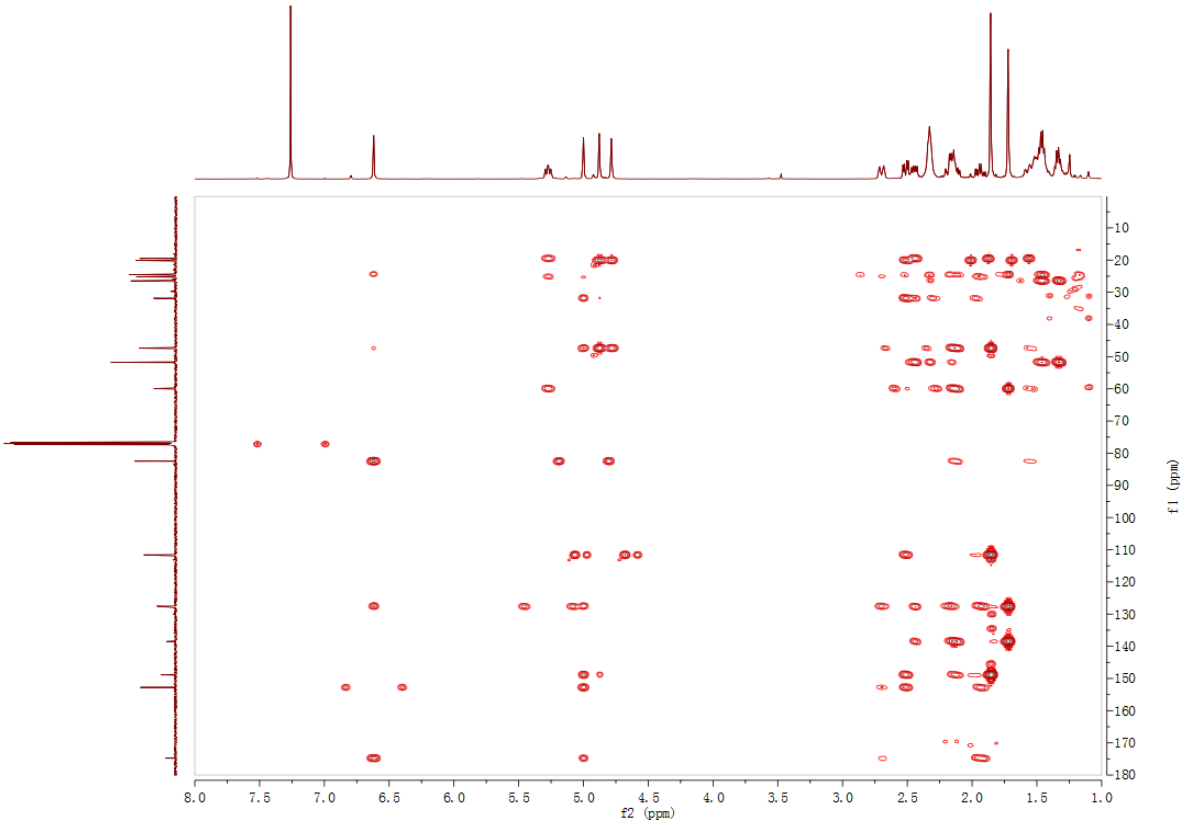
S2.120. HSQC spectrum of **25**



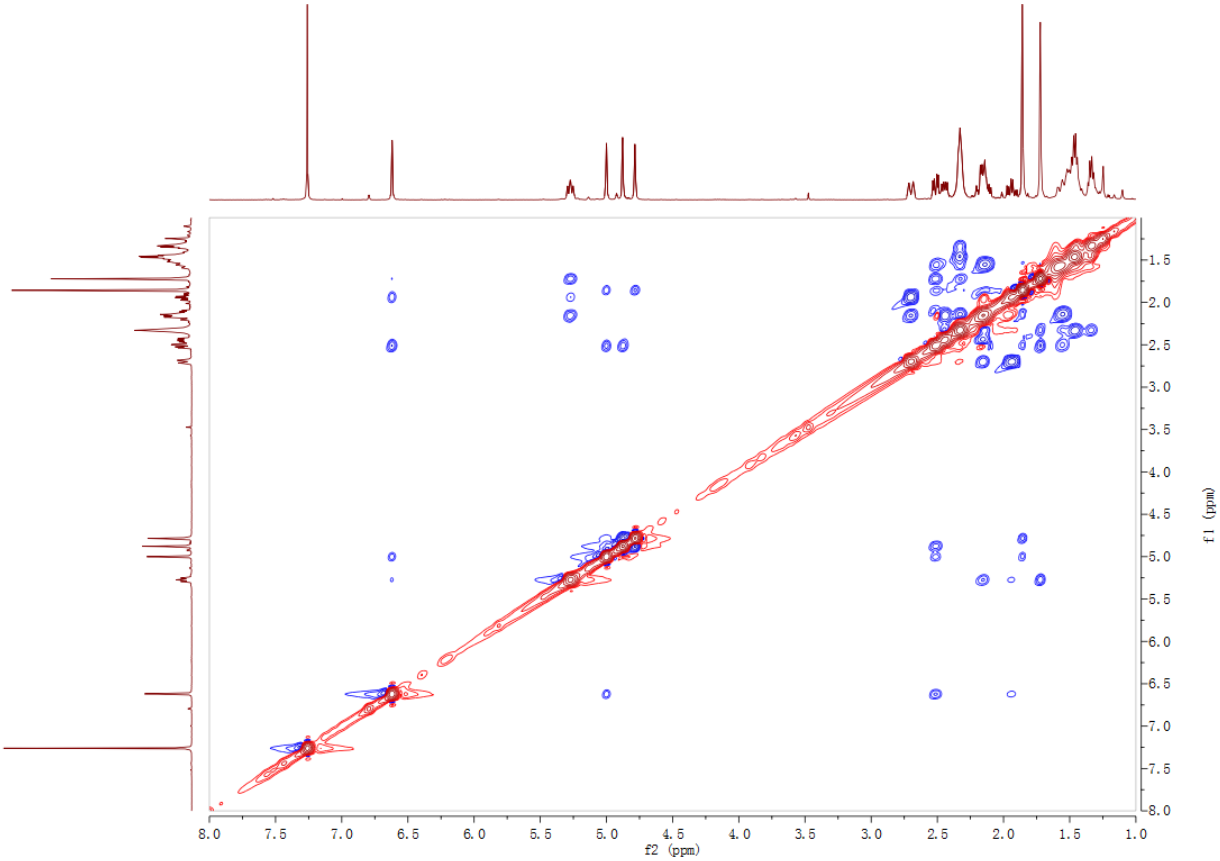
S2.121. $^{1}H–^{1}H$ COSY spectrum of **25**



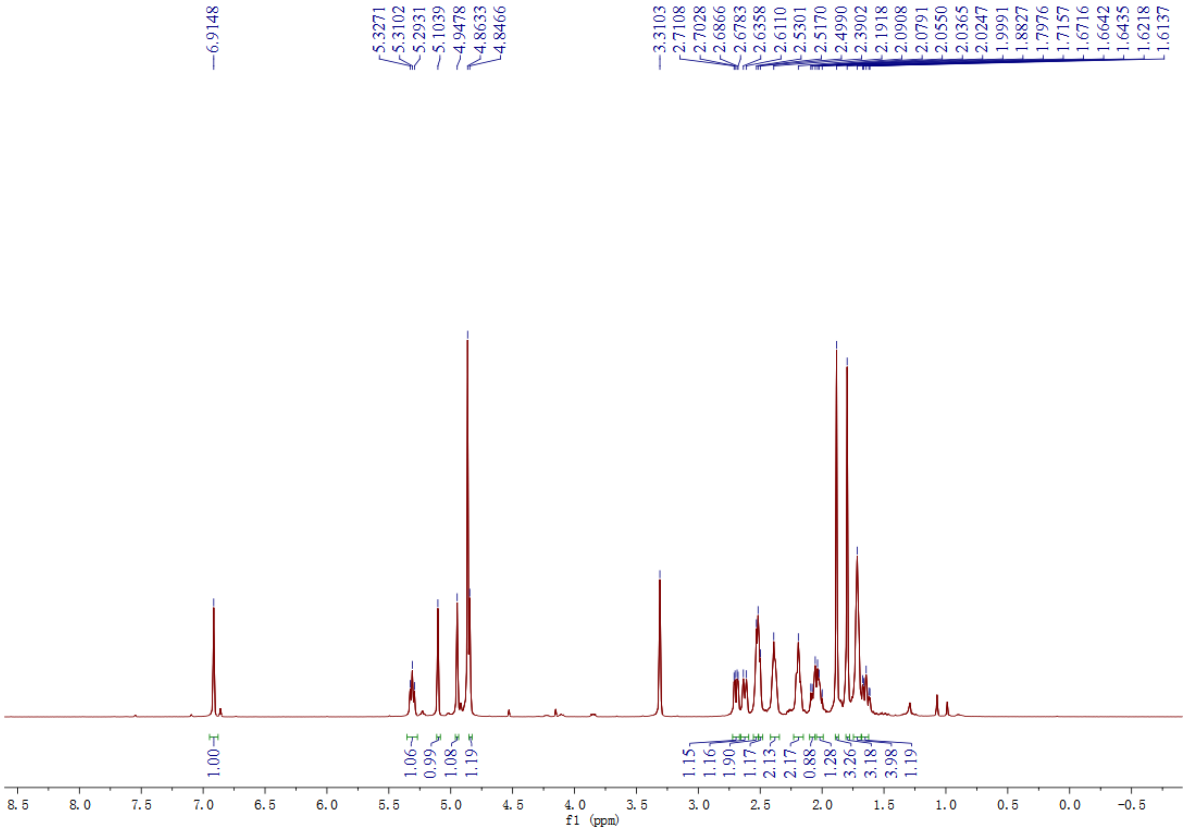
S2.122. HMBC spectrum of **25**



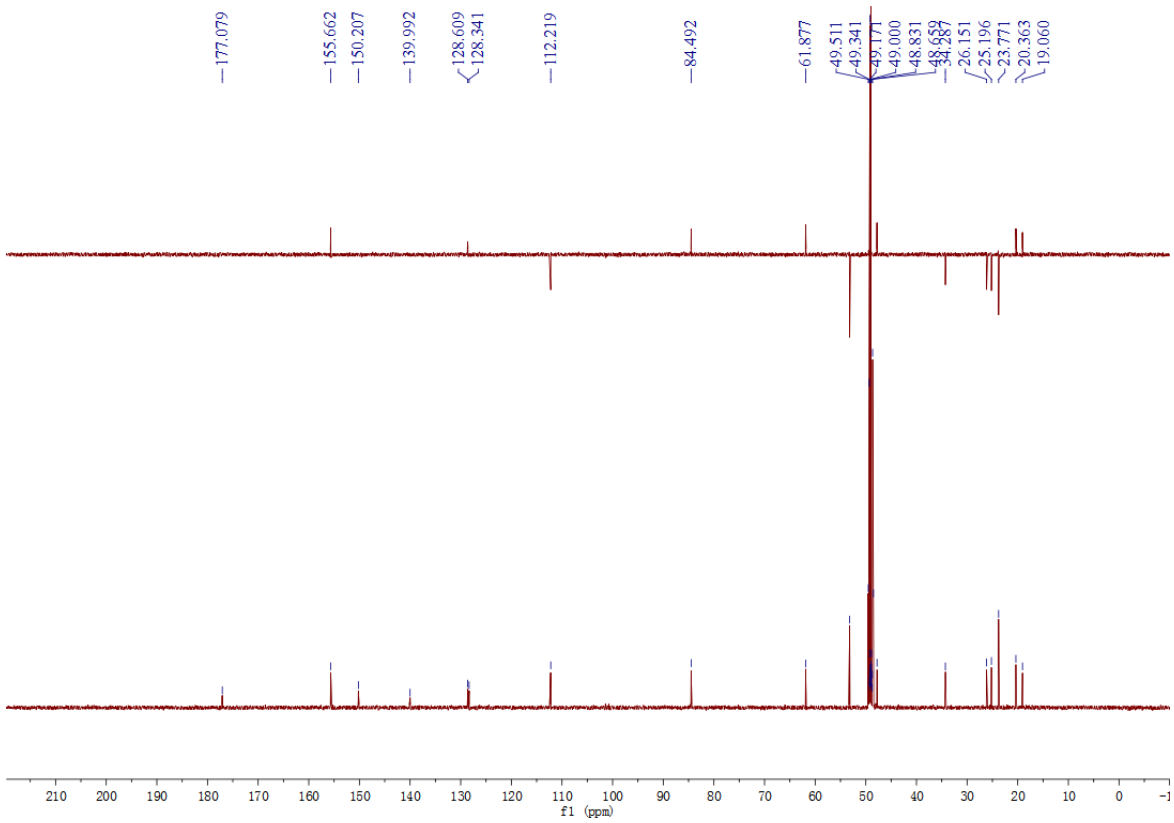
S2.123. NOESY spectrum of **25**



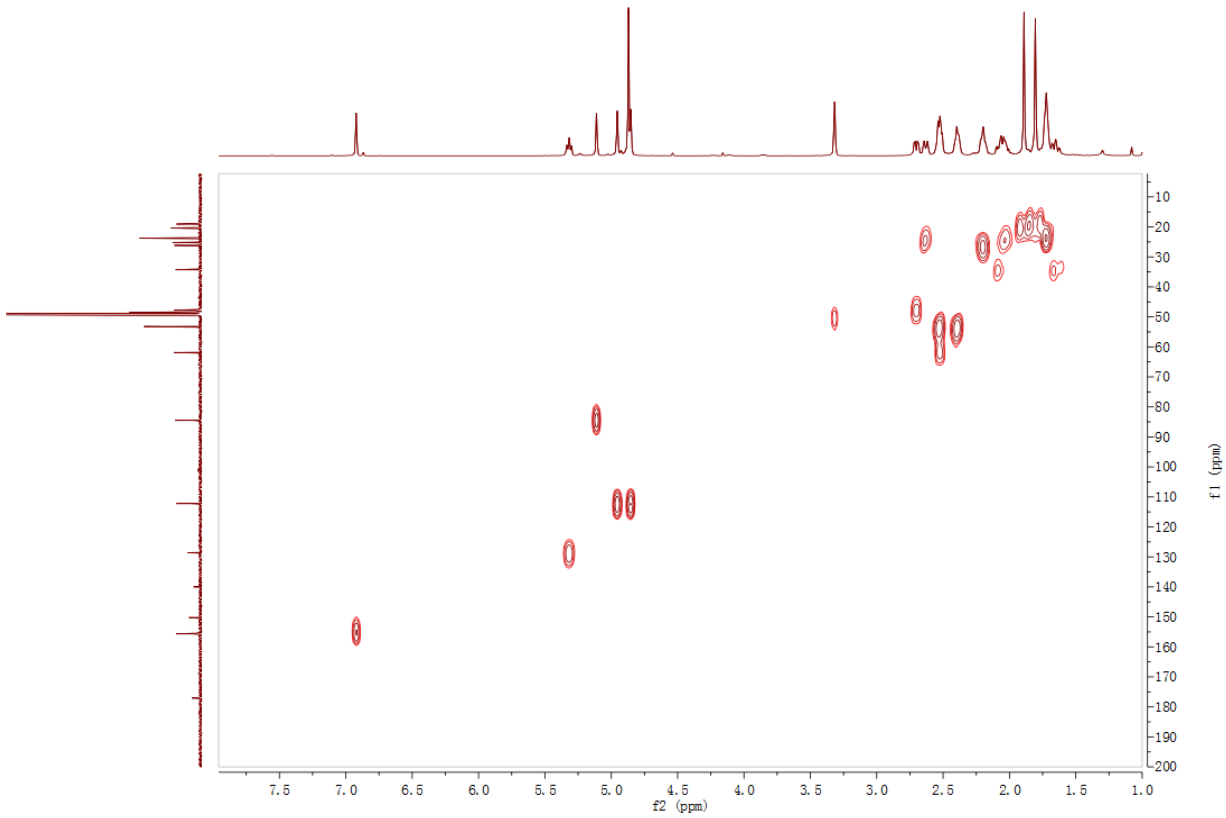
S2.124. ^{1}H NMR spectrum of **26**



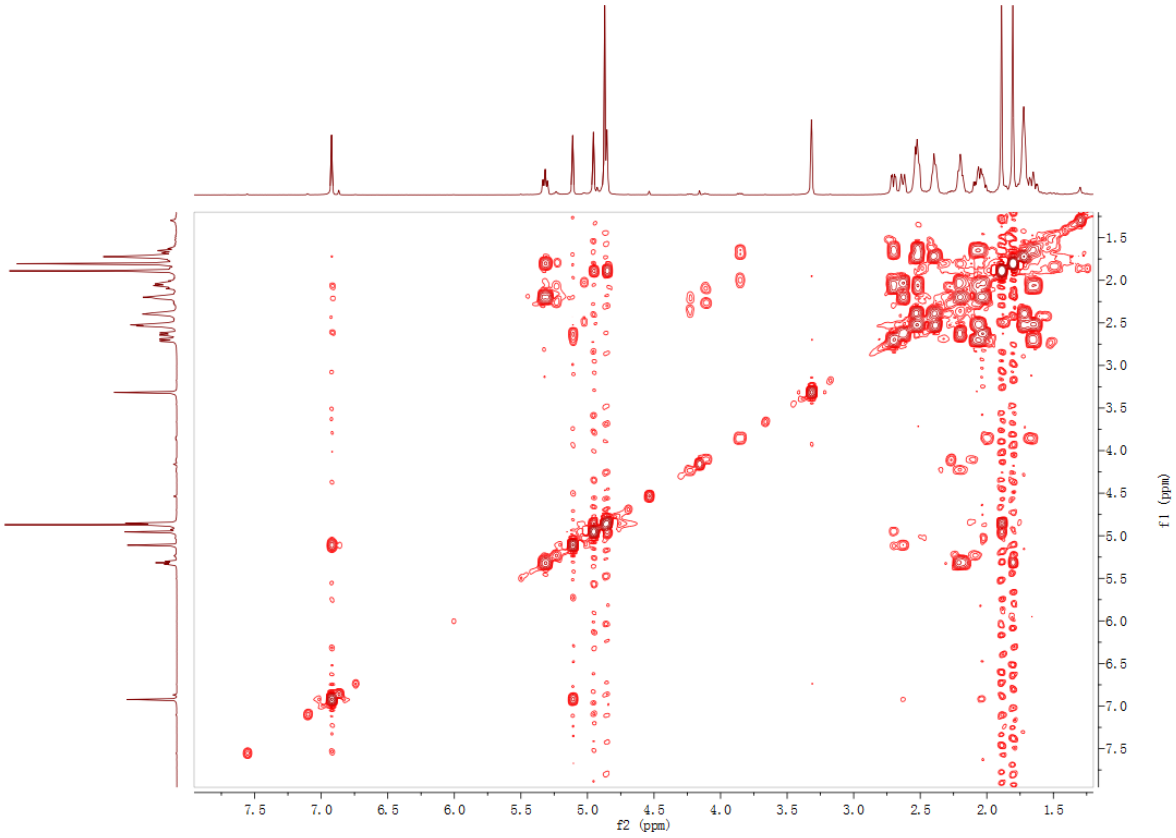
S2.125. ^{13}C NMR and DEPT spectra of **26**



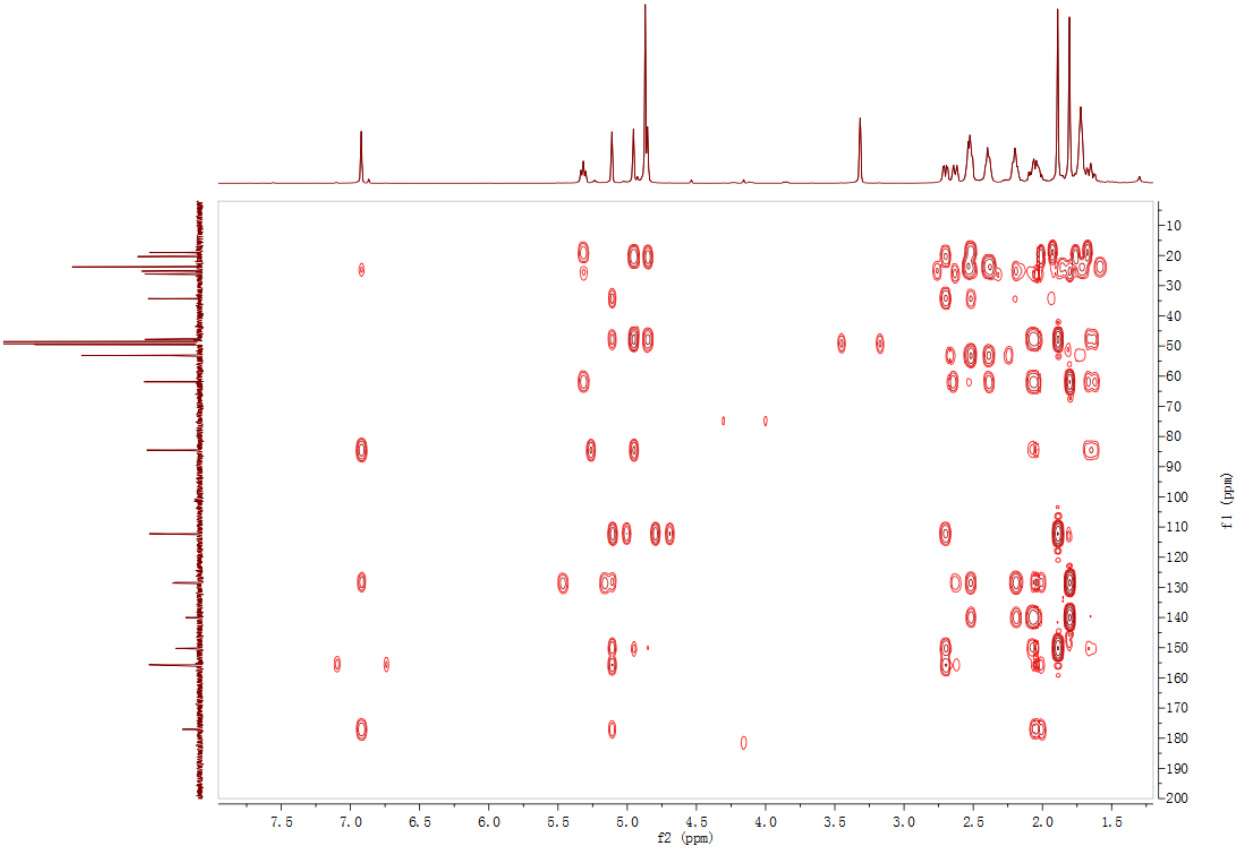
S2.126. HSQC spectrum of **26**



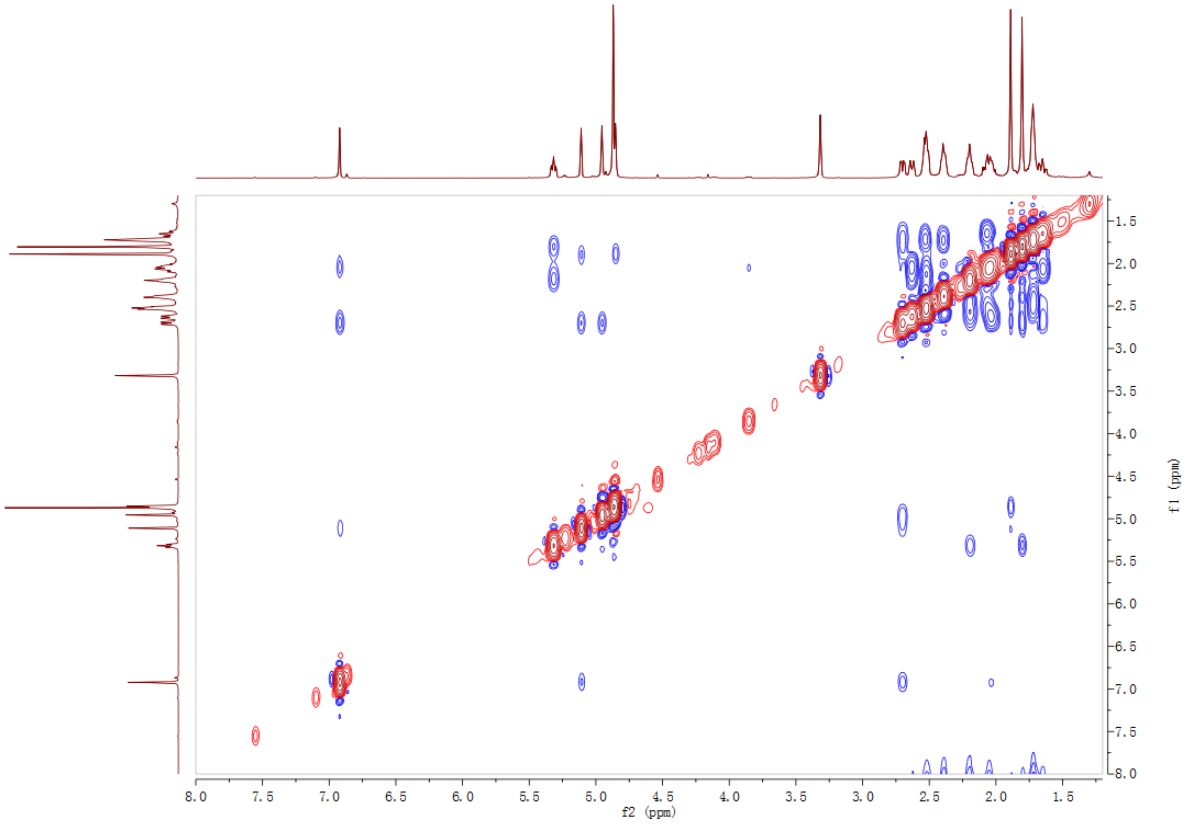
S2.127. $^{1}H–^{1}H$ COSY spectrum of **26**



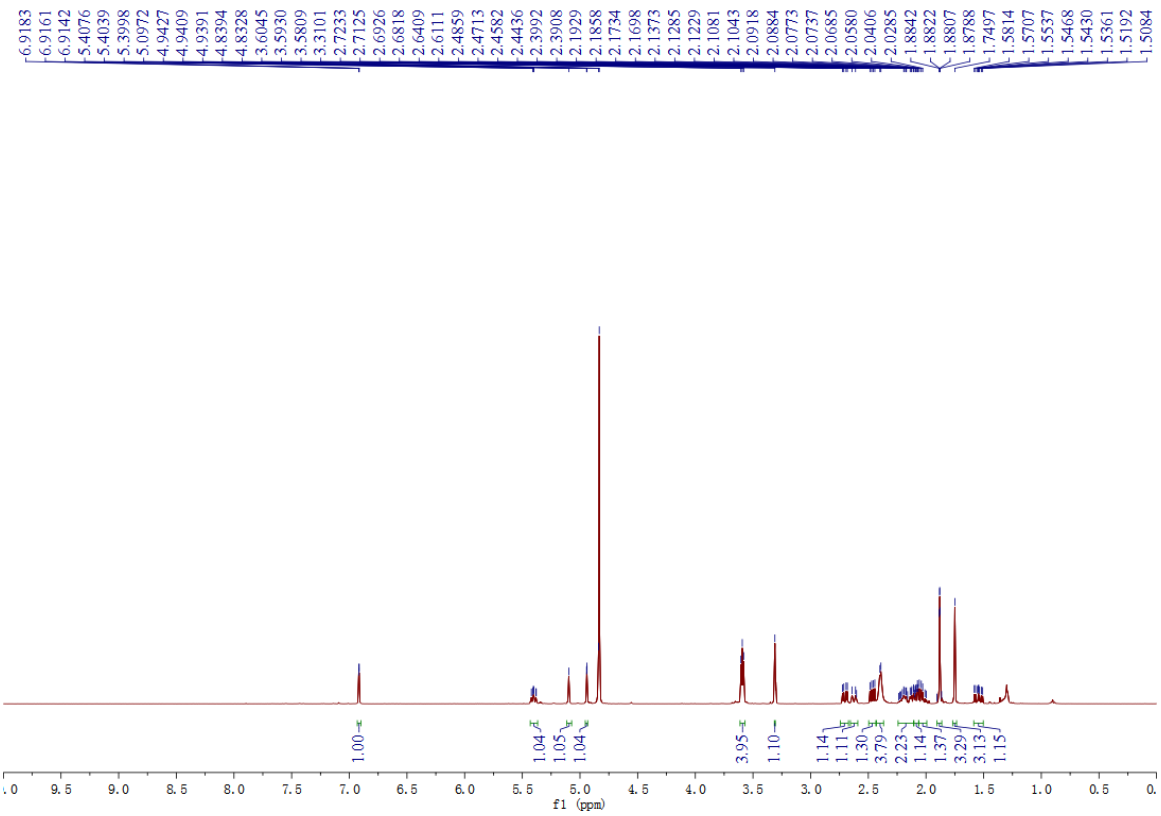
S2.128. HMBC spectrum of **26**



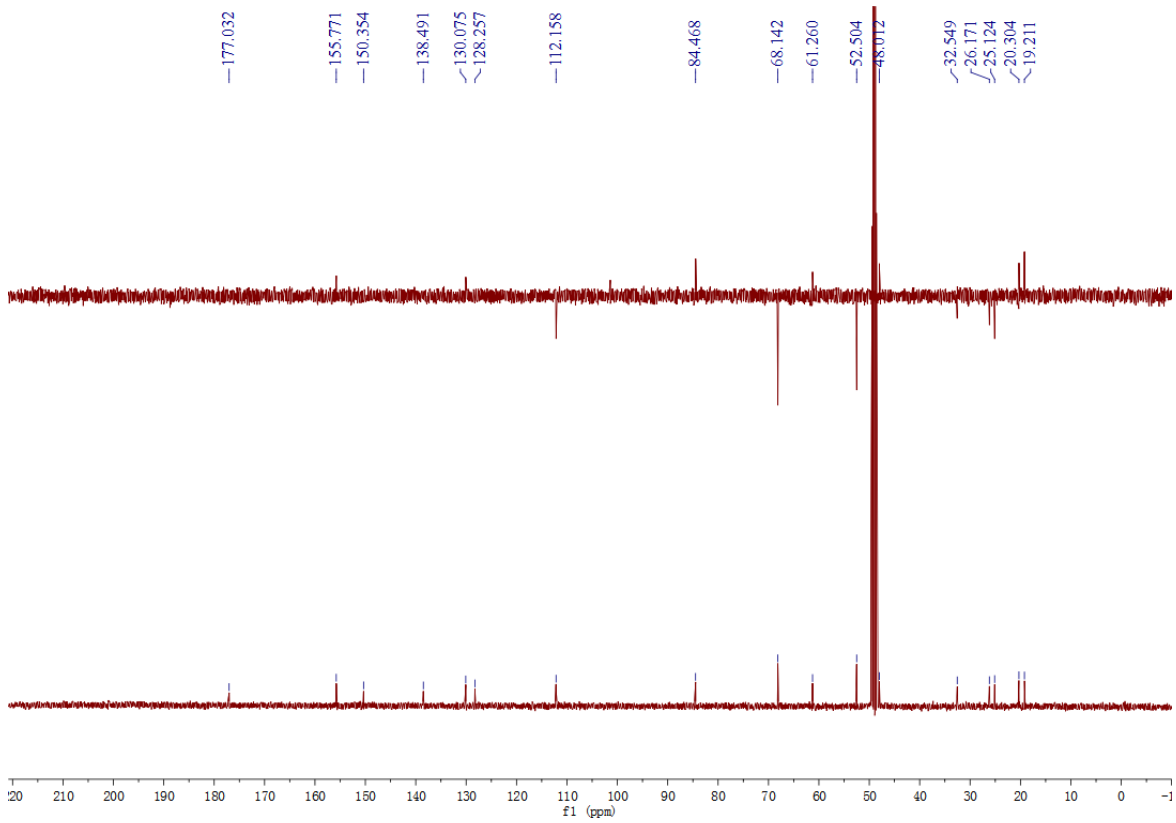
S2.129. NOESY spectrum of **26**



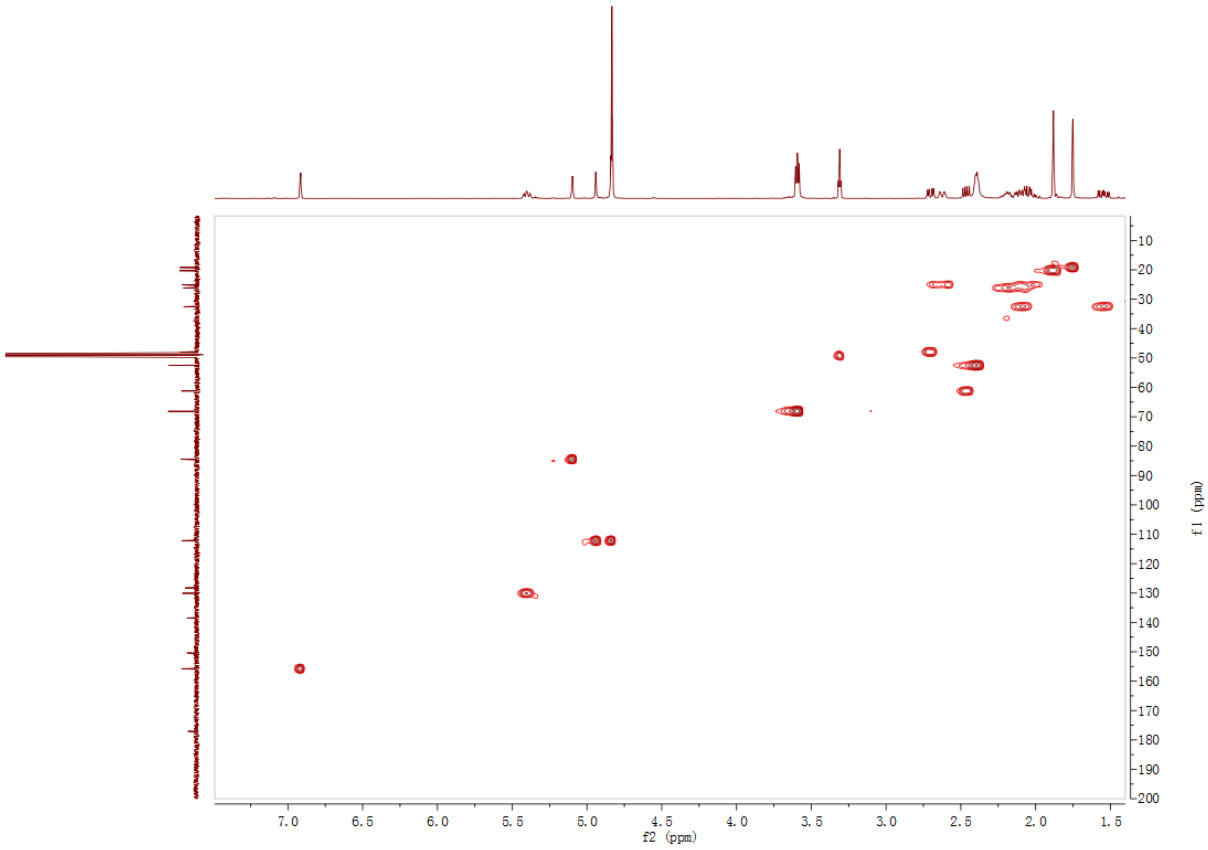
S2.130. ^{1}H NMR spectrum of **27**



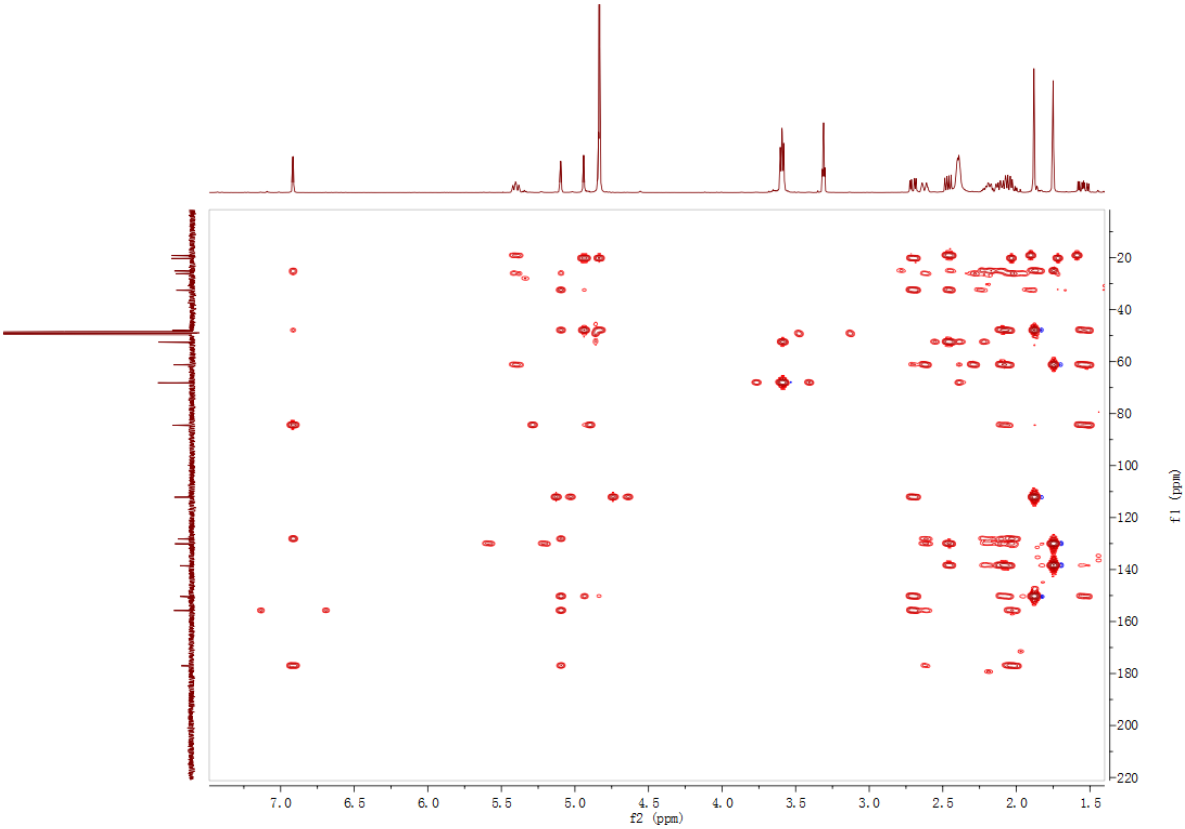
S2.131. ^{13}C NMR and DEPT spectra of **27**



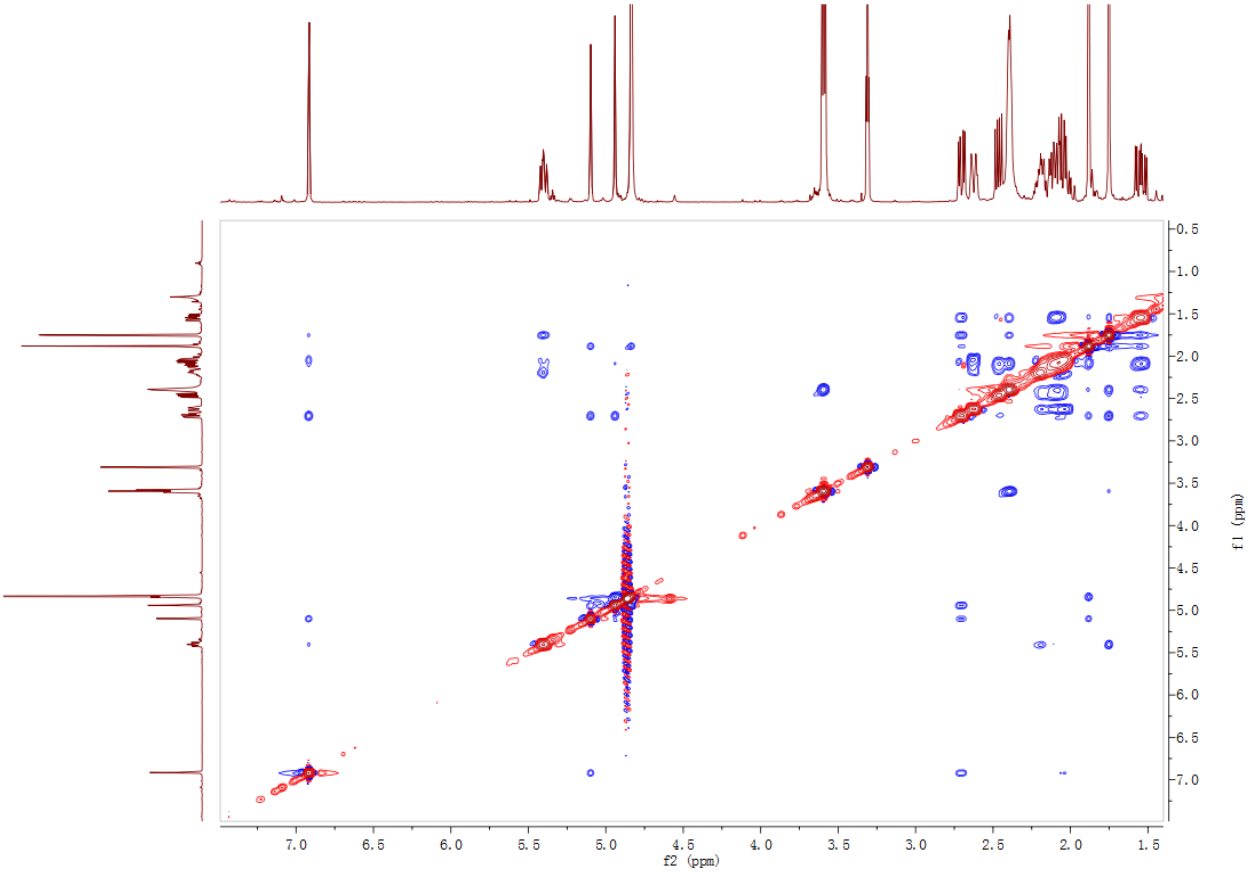
S2.132. HSQC spectrum of **27**



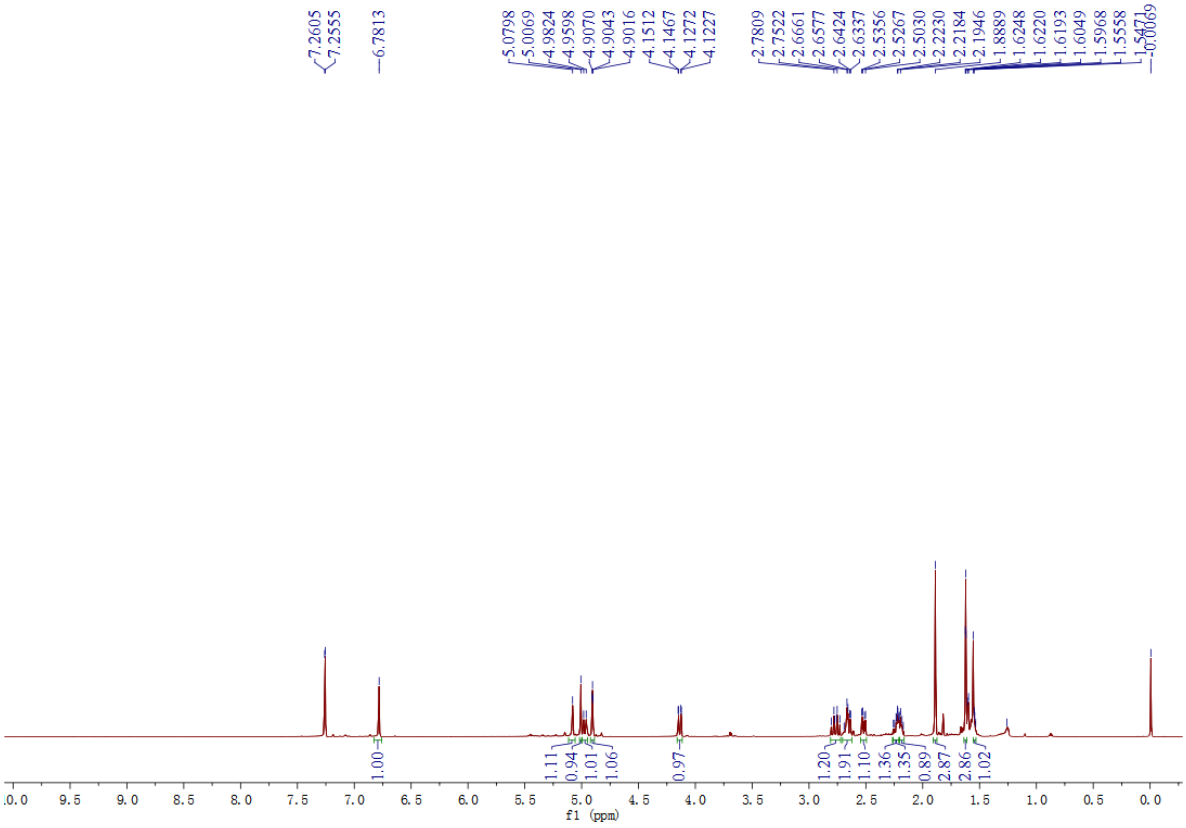
S2.133. HMBC spectrum of **27**



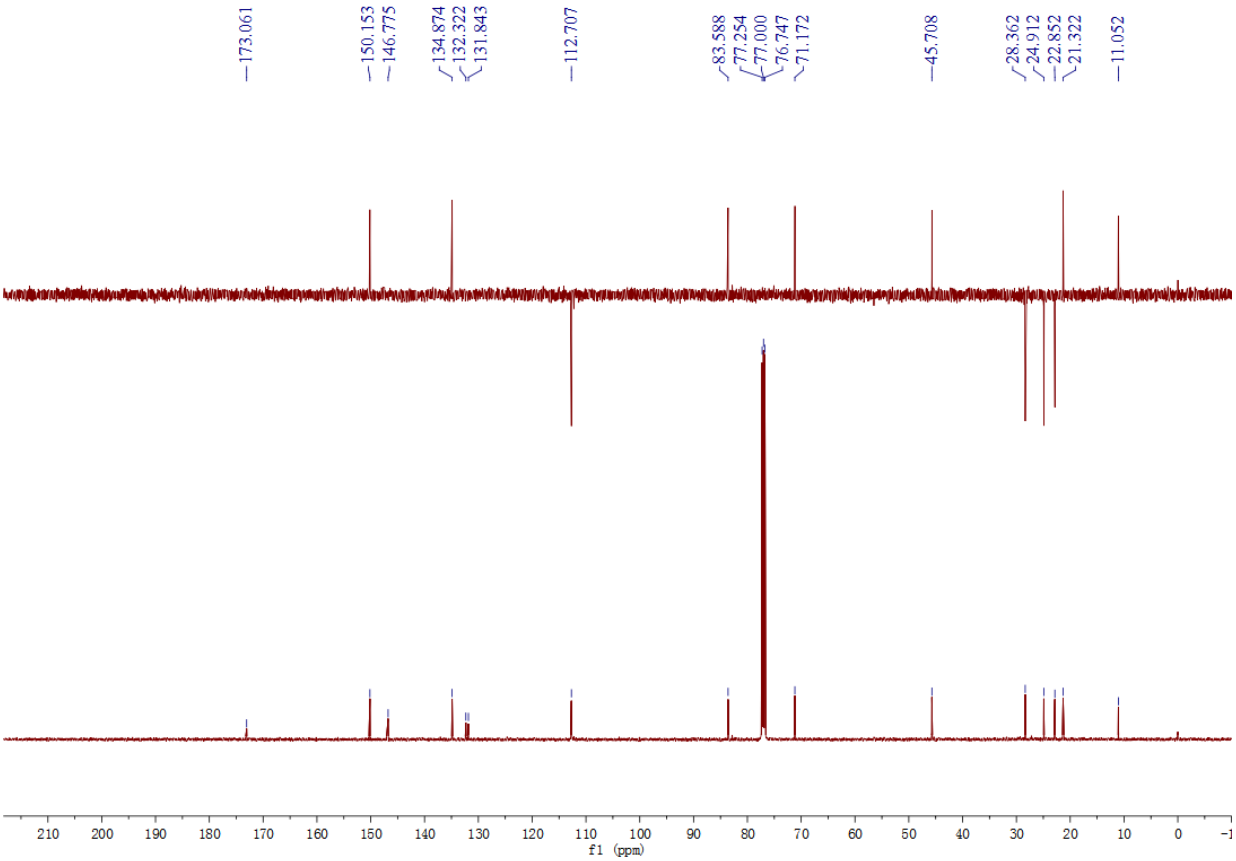
S2.134. NOESY spectrum of **27**



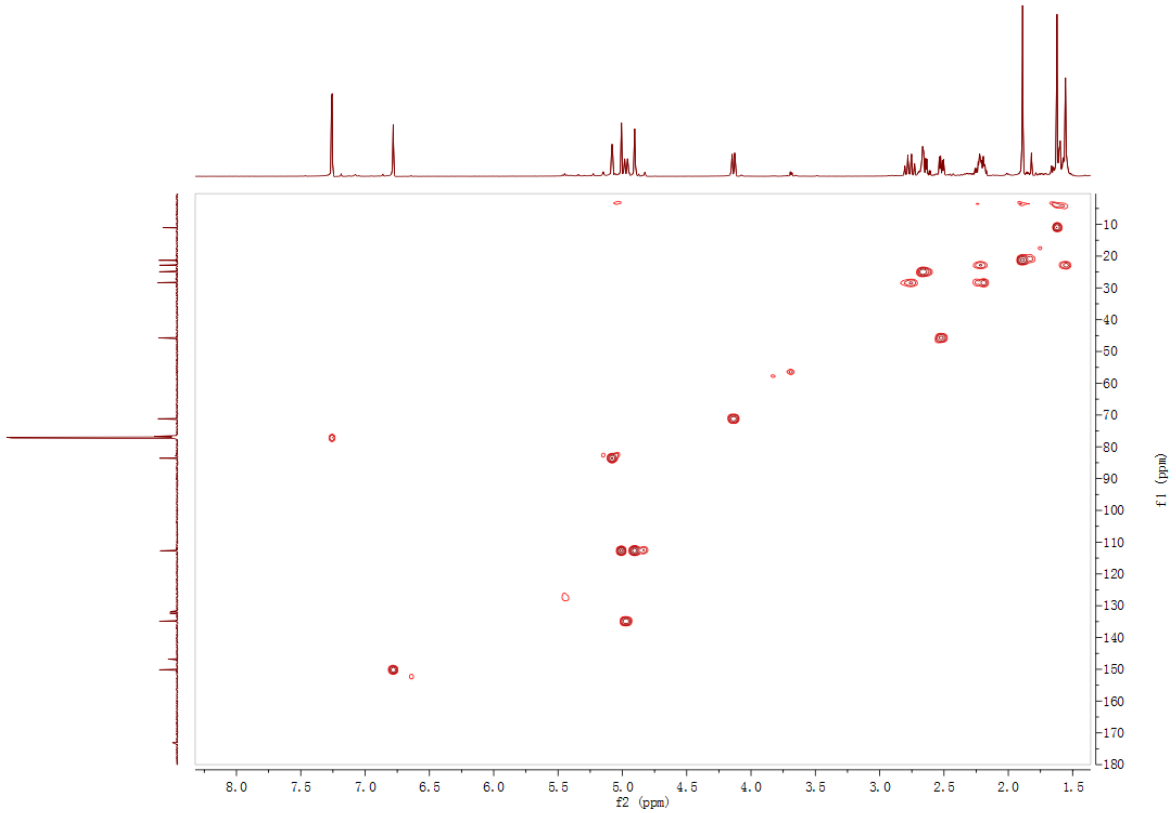
S2.135. ^{1}H NMR spectrum of **28**



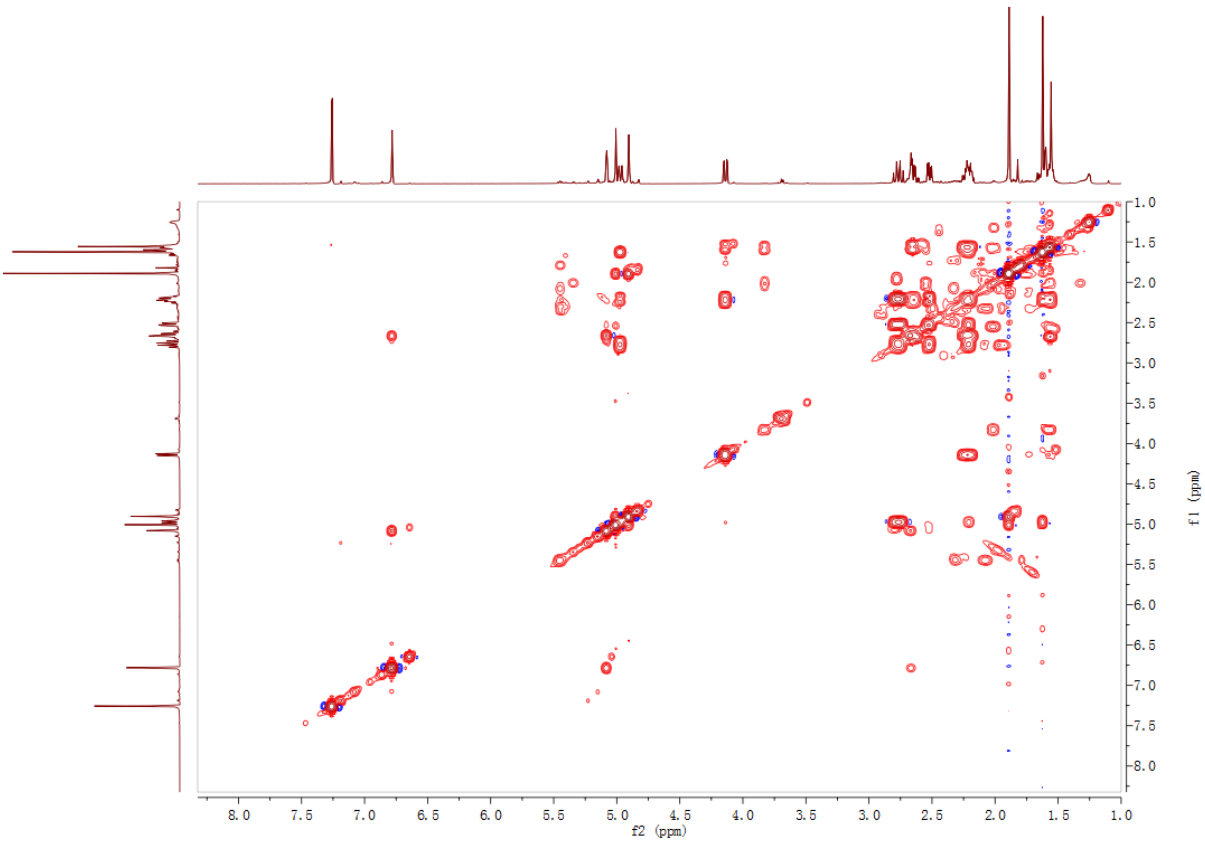
S2.136. ^{13}C NMR and DEPT spectra of **28**



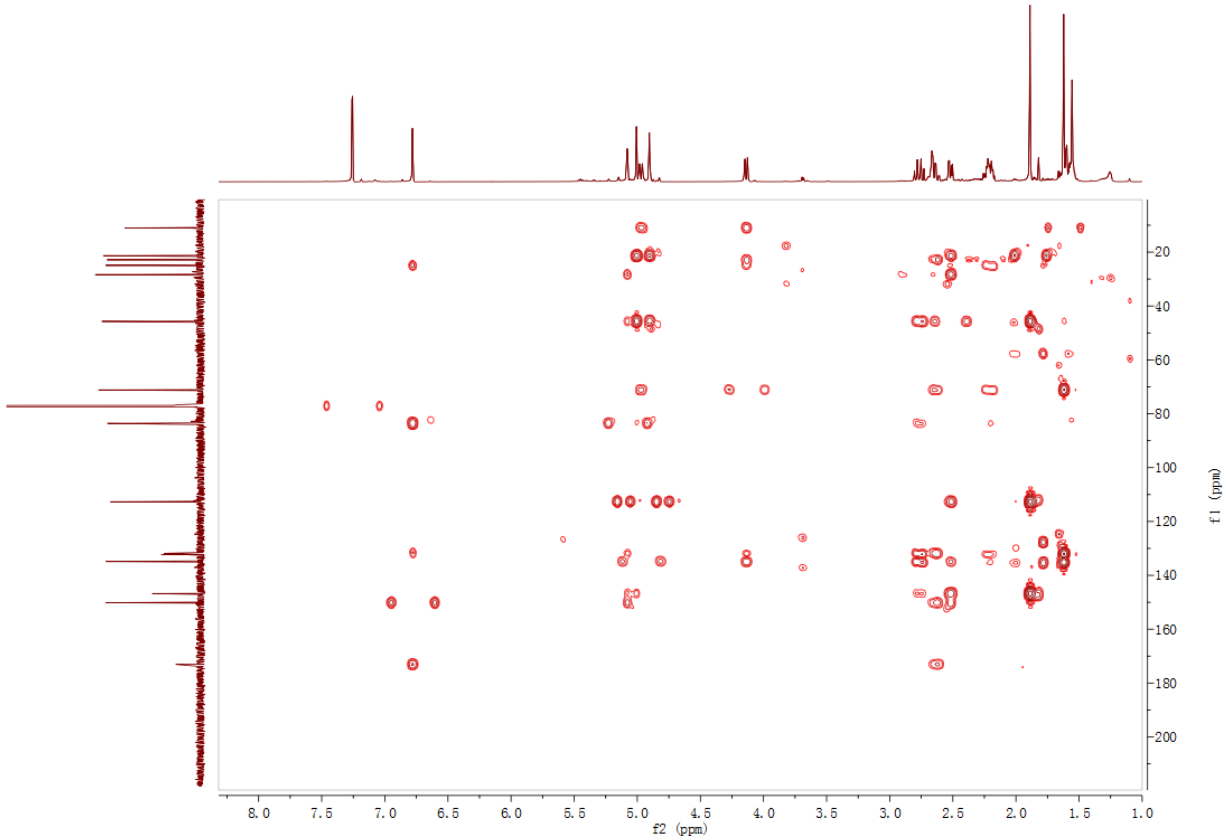
S2.137. HSQC spectrum of **28**



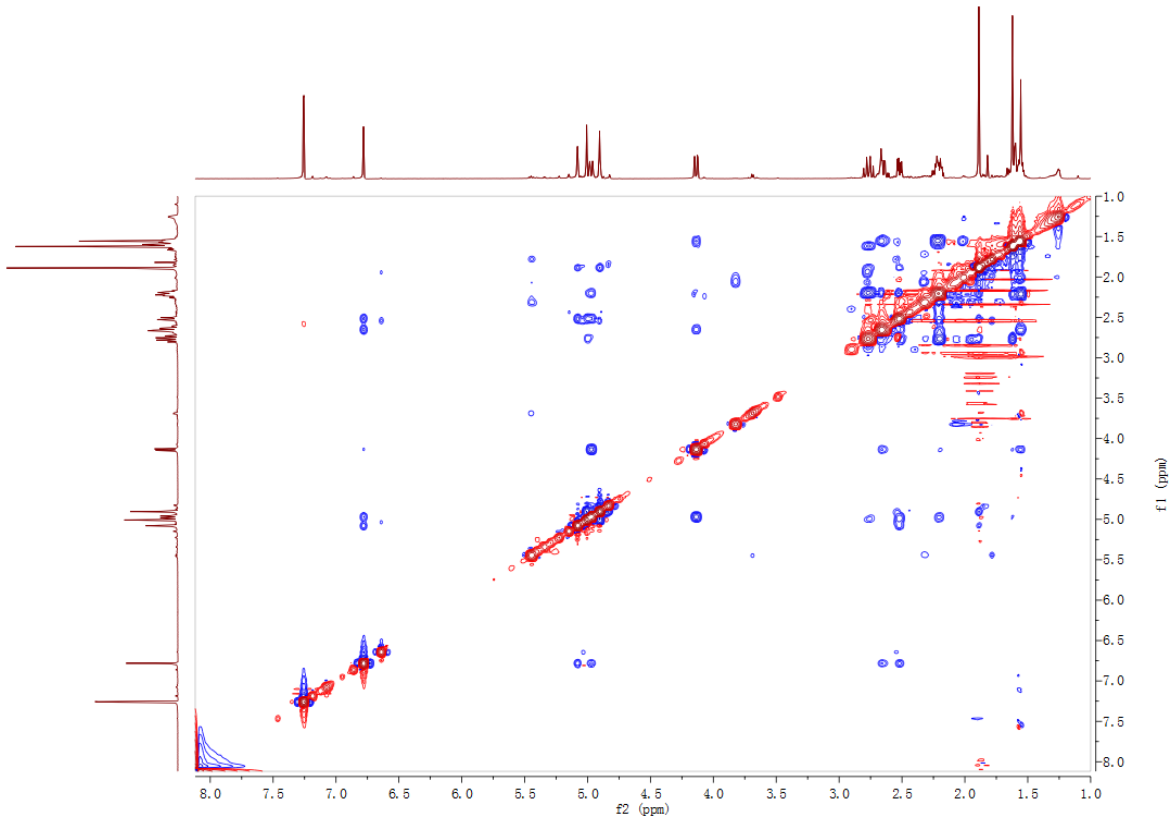
S2.138. ^{1}H–^{1}H COSY spectrum of **28**



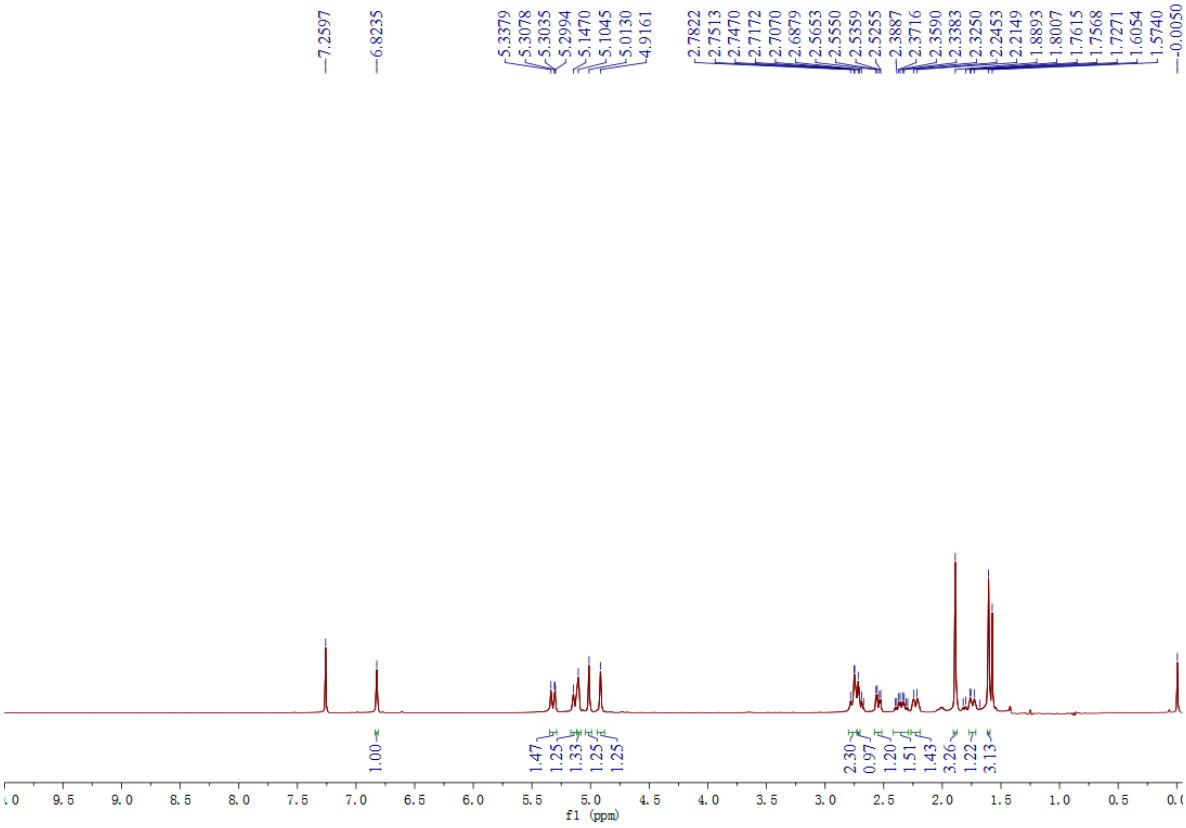
S2.139. HMBC spectrum of **28**



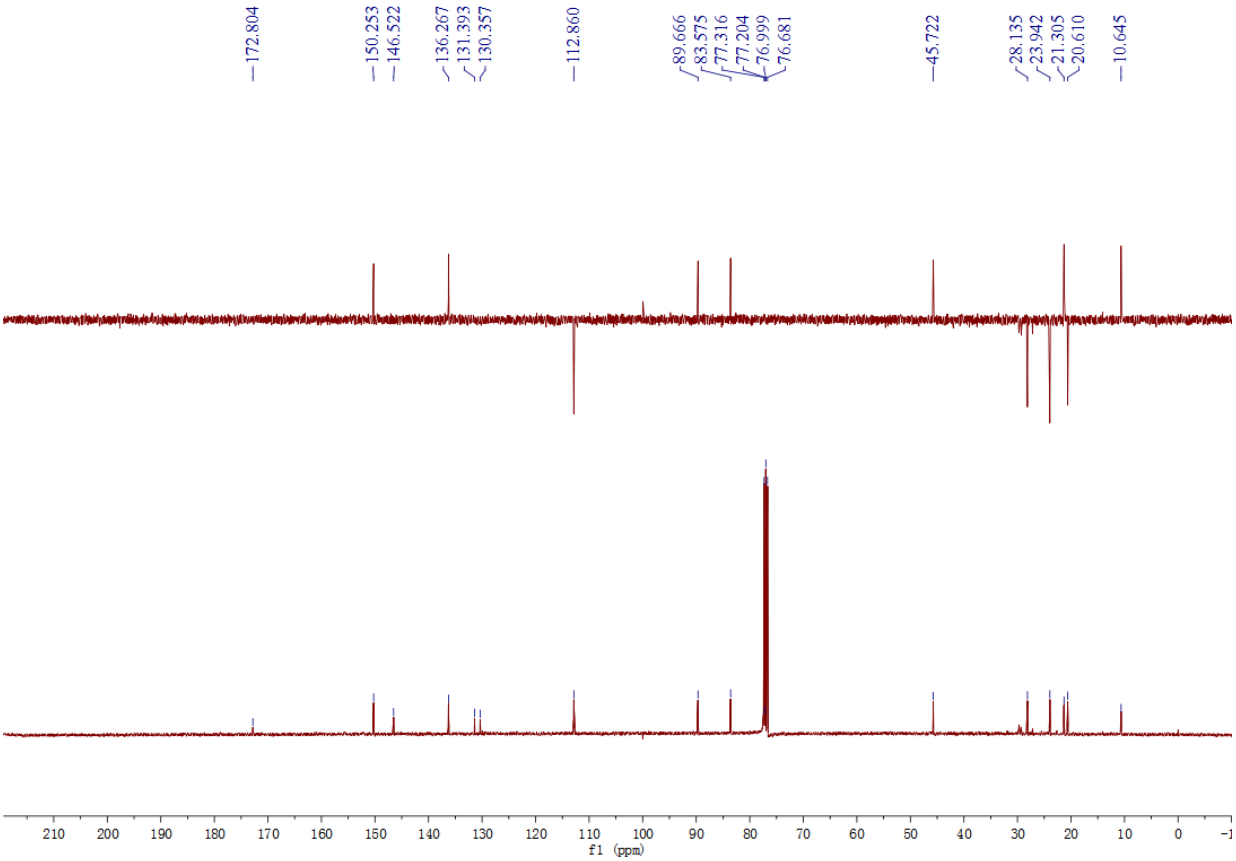
S2.140. NOESY spectrum of **28**



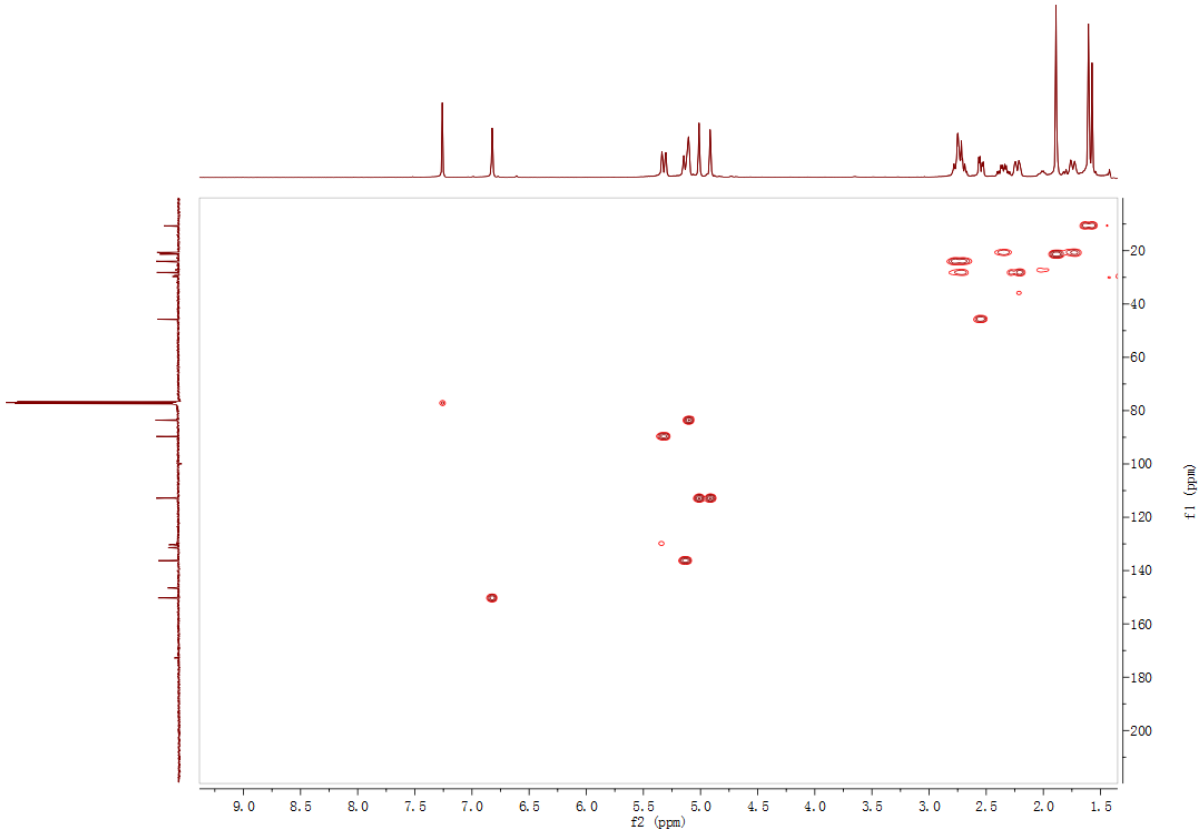
S2.141. ^{1}H NMR spectrum of **29**



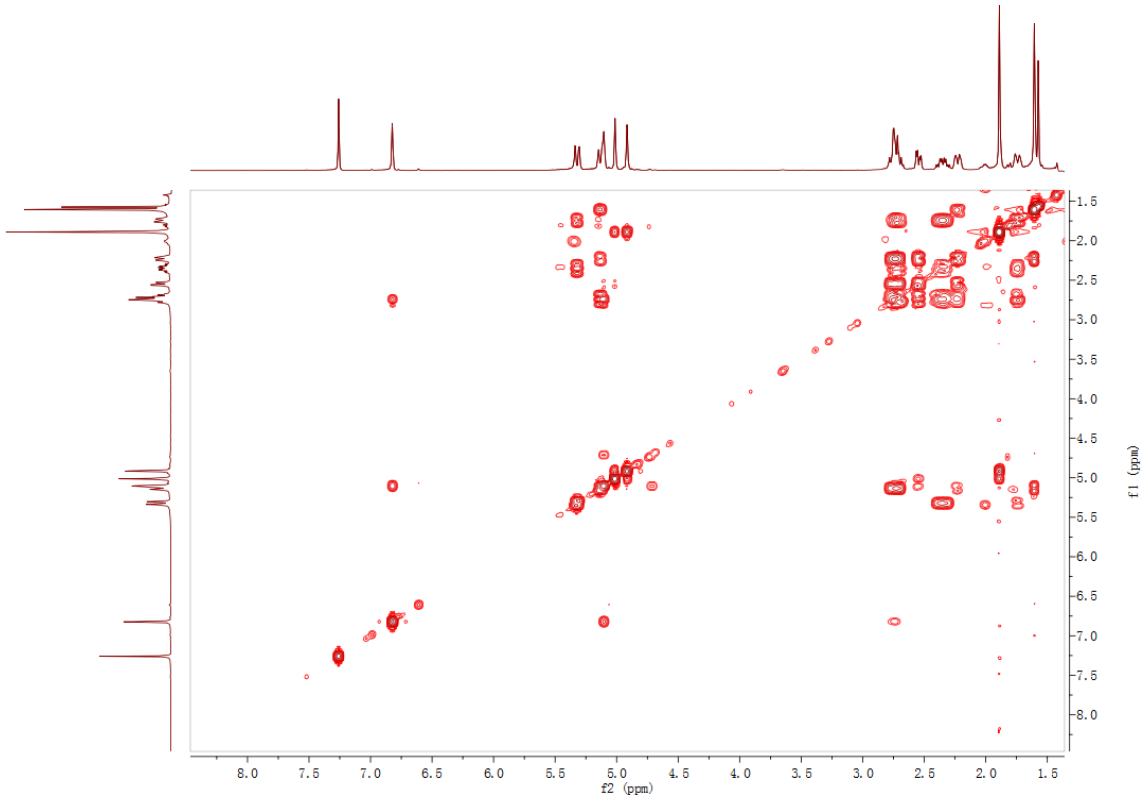
S2.142. ^{13}C NMR and DEPT spectra of **29**



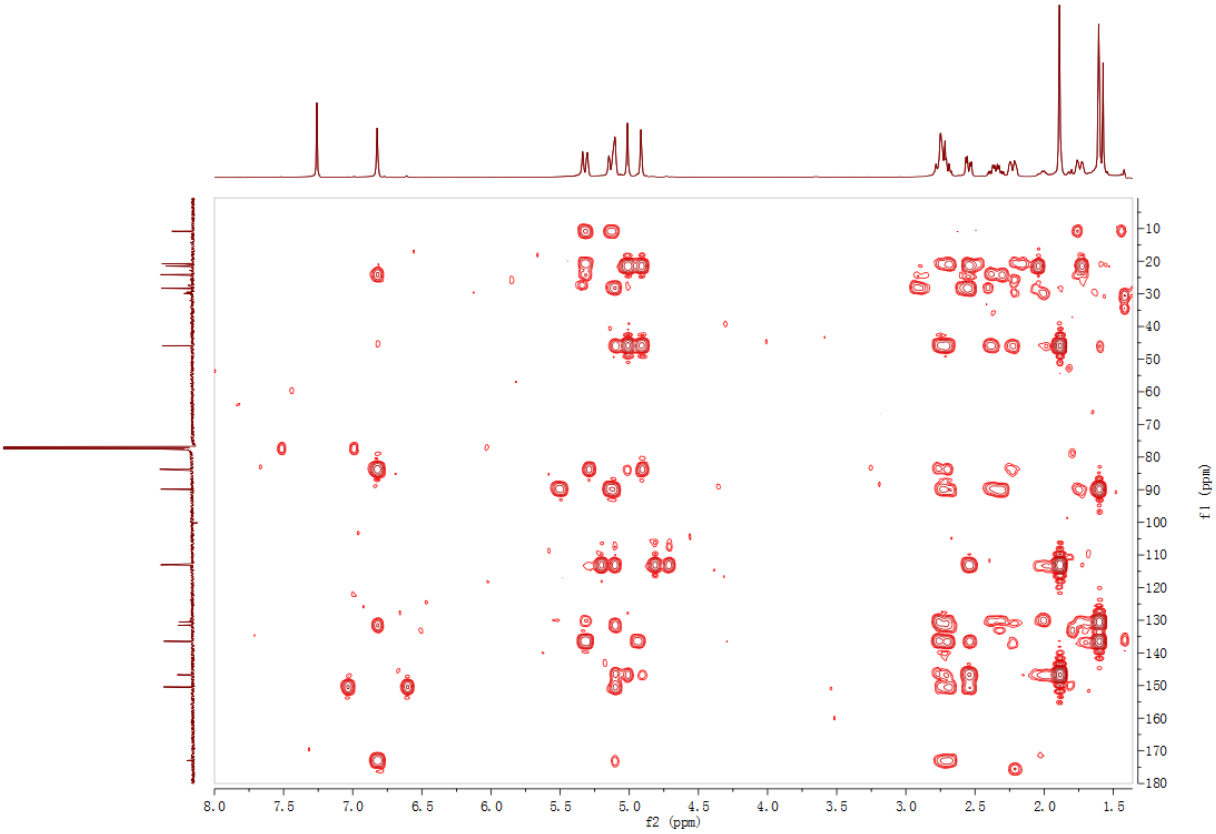
S2.143. HSQC spectrum of **29**



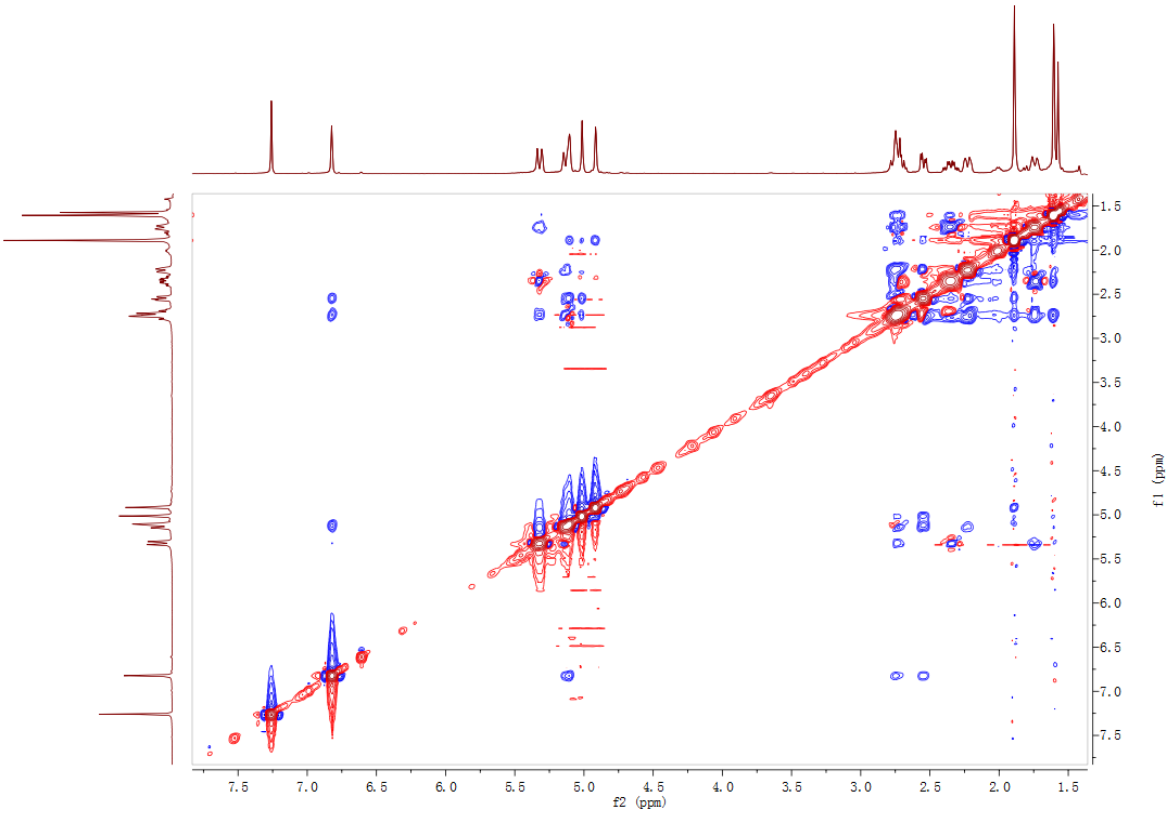
S2.144. ^{1}H–^{1}H COSY spectrum of **29**



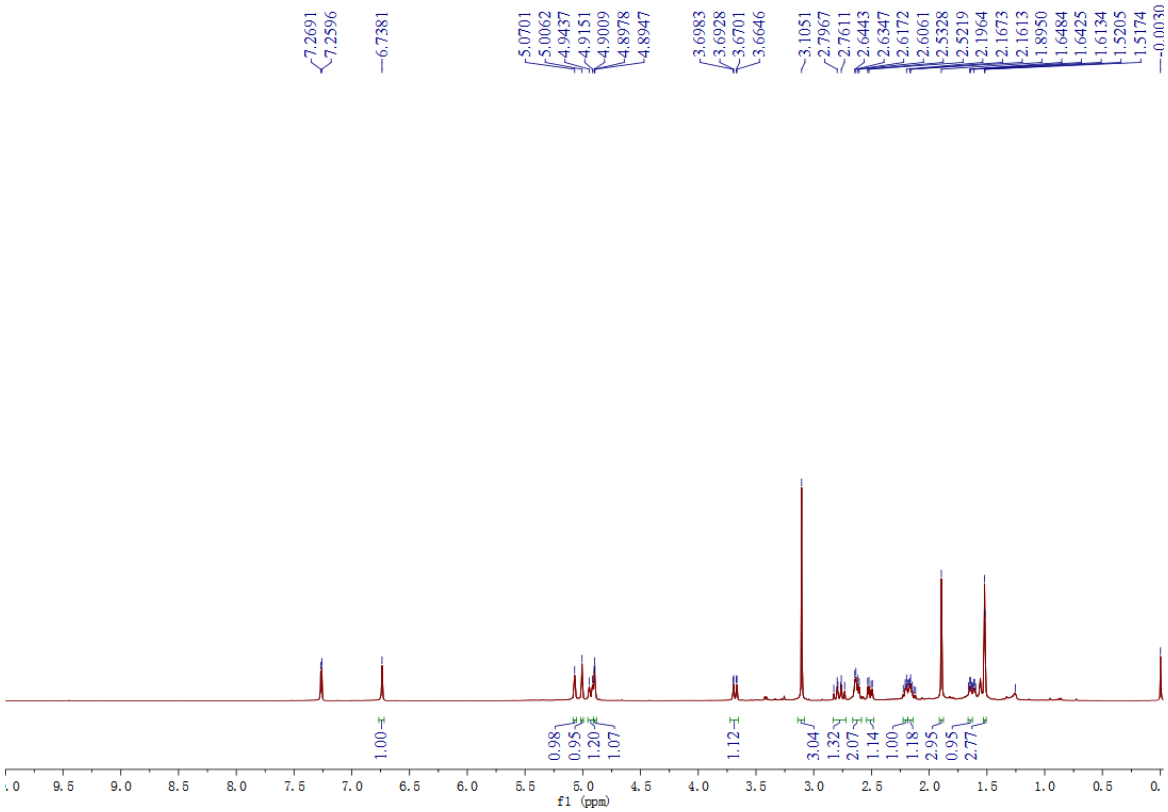
S2.145. HMBC spectrum of **29**



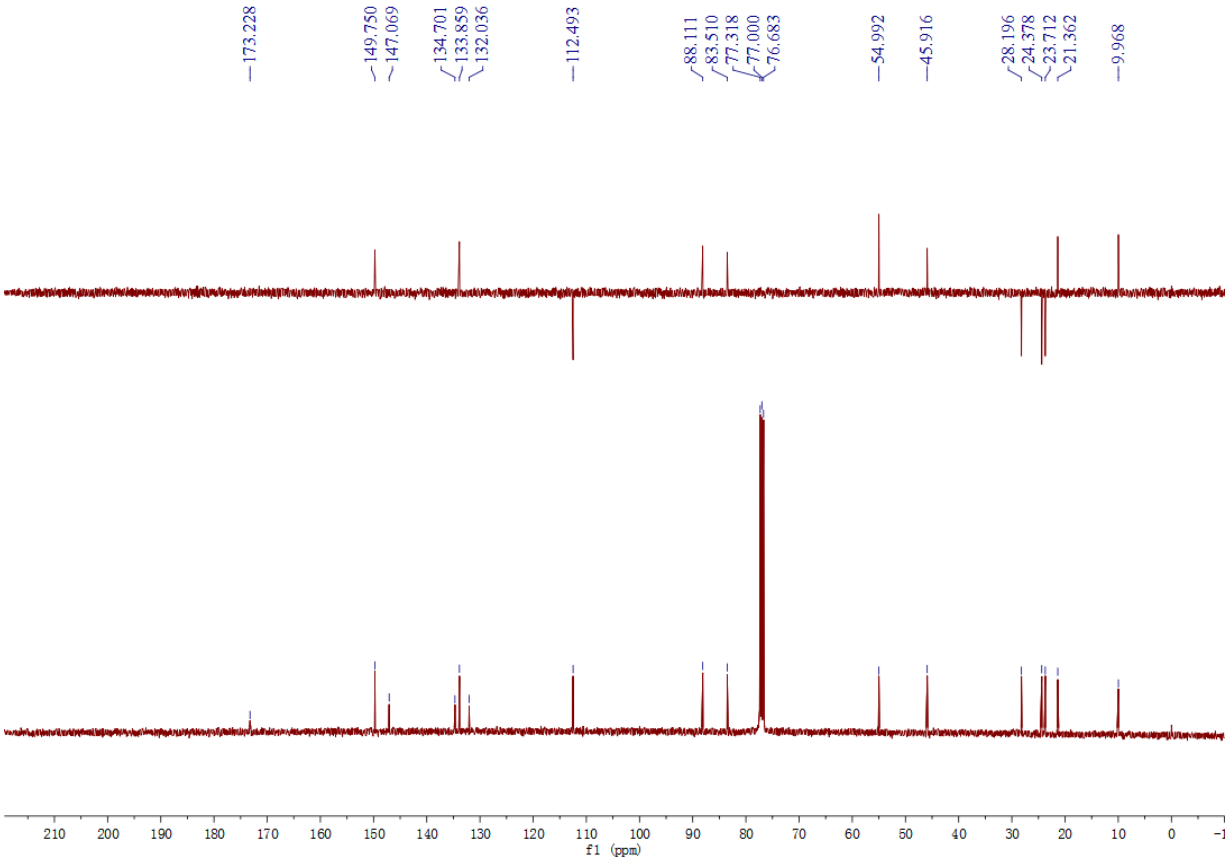
S2.146. NOESY spectrum of **29**



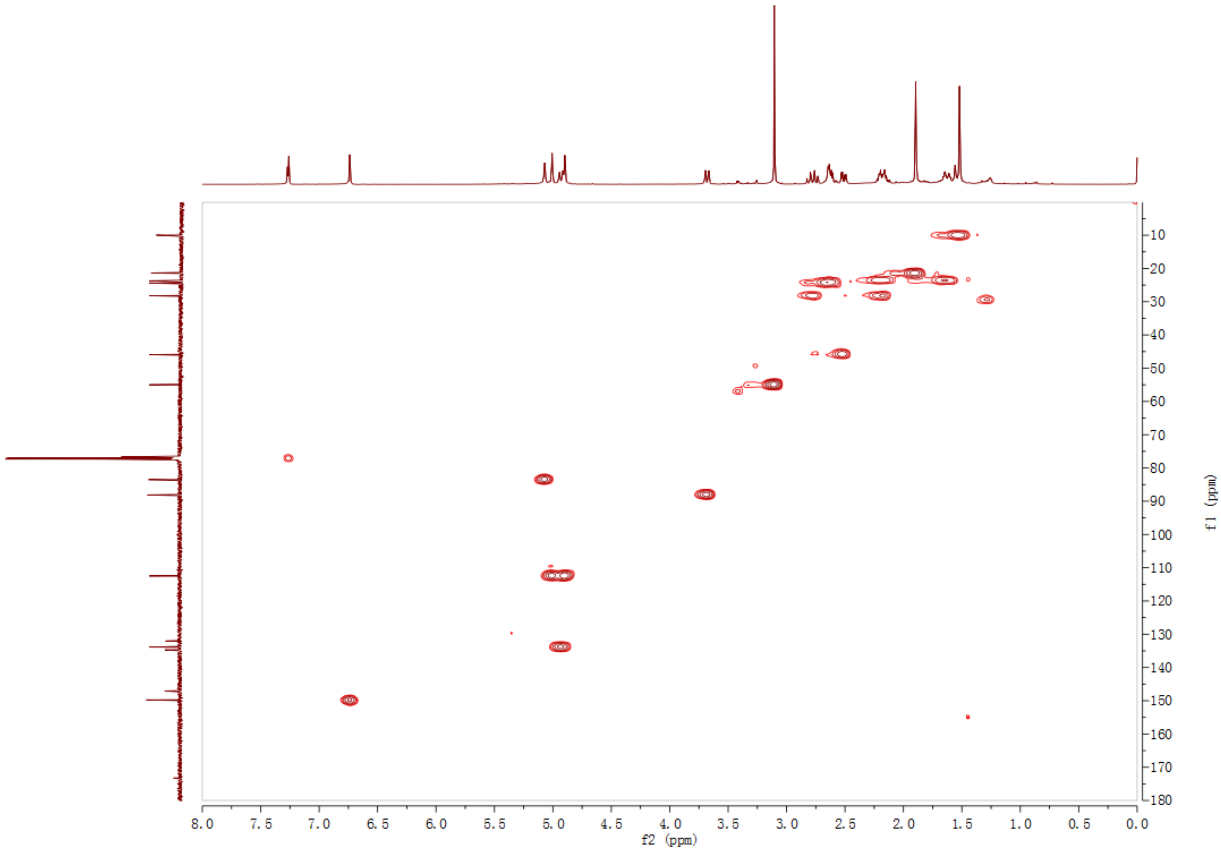
S2.147. ^{1}H NMR spectrum of **30**



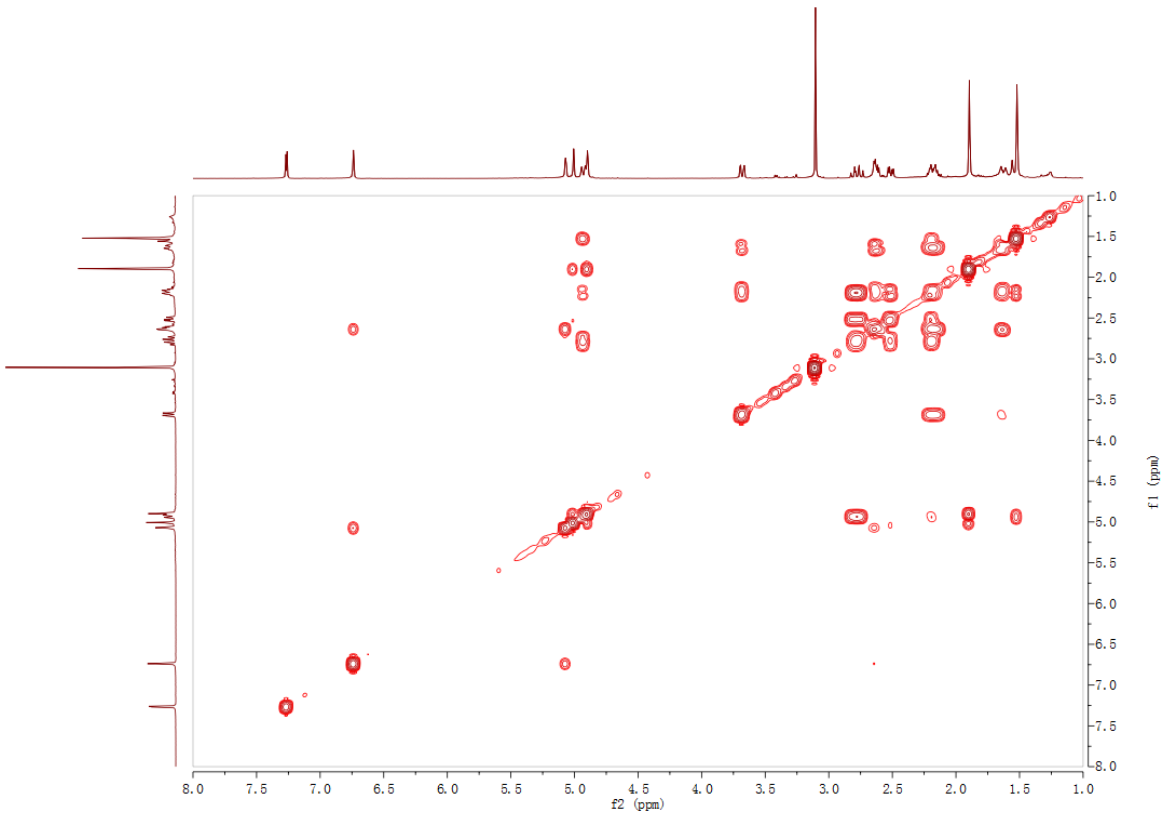
S2.148. ^{13}C NMR and DEPT spectra of **30**



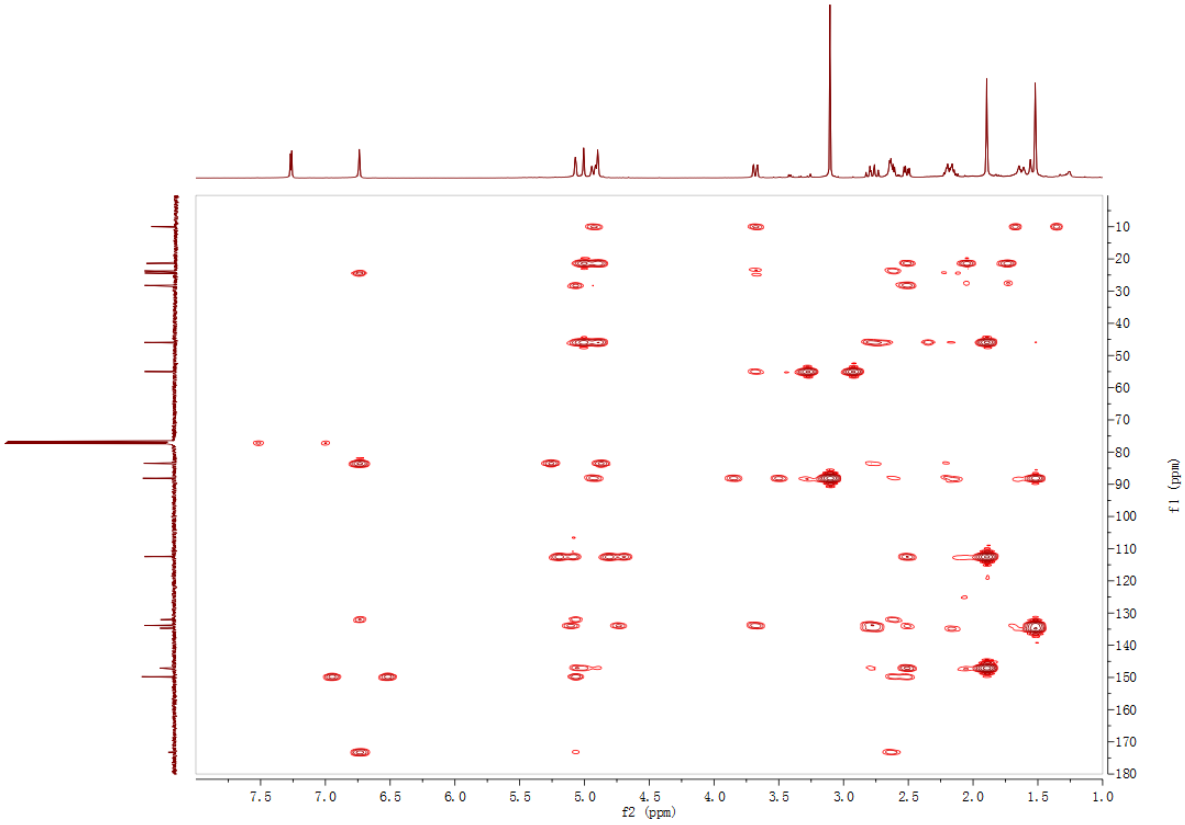
S2.149. HSQC spectrum of **30**



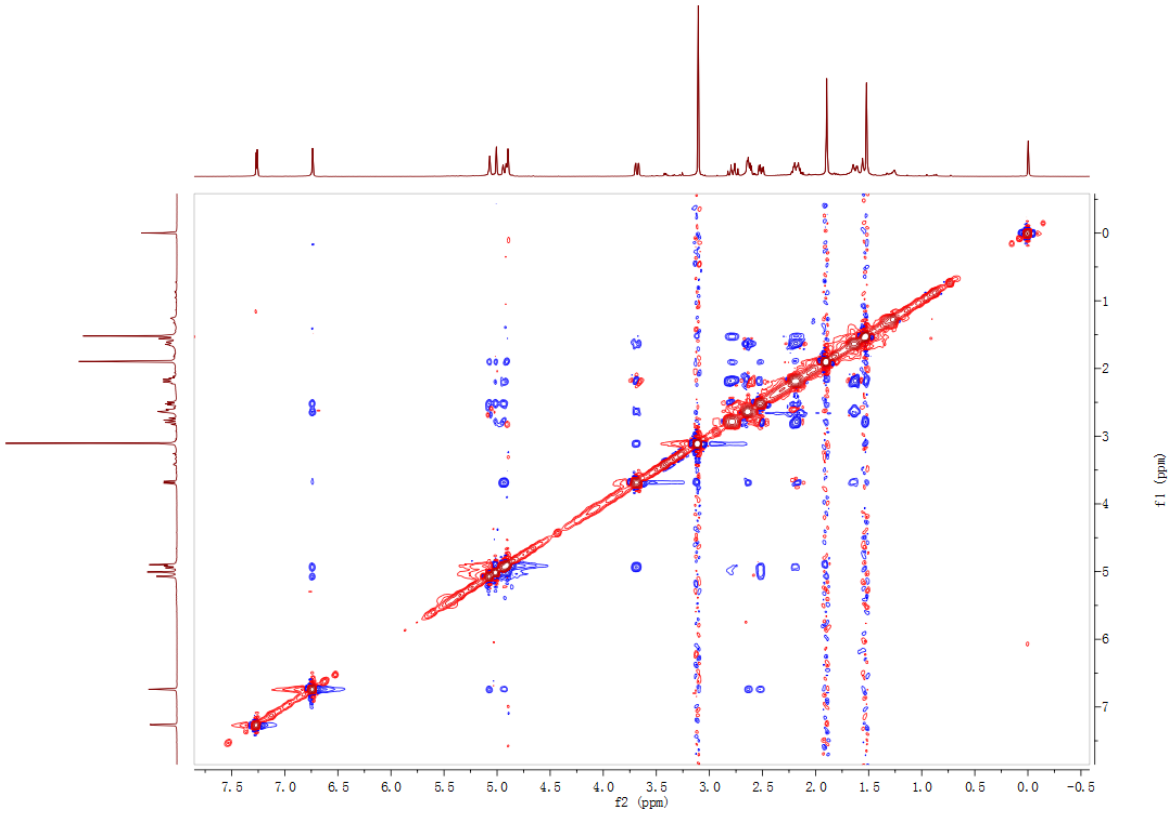
S2.150. ^{1}H–^{1}H COSY spectrum of **30**



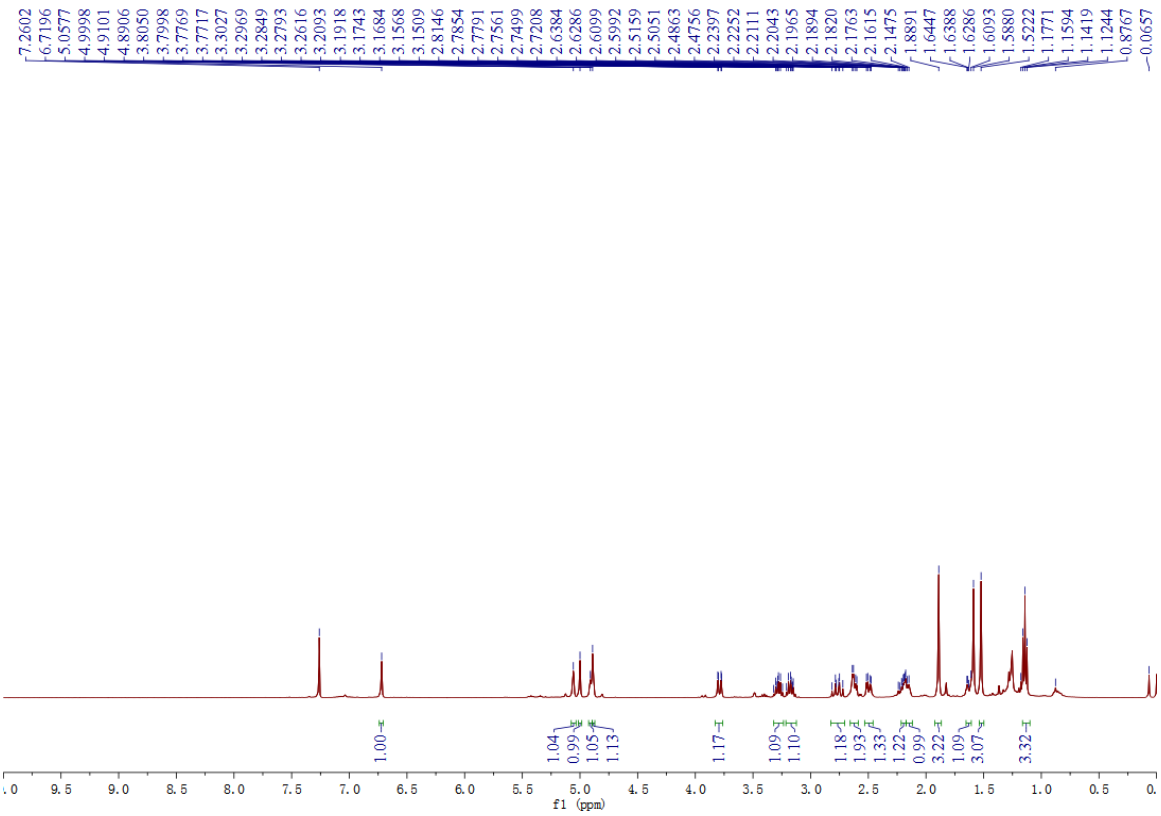
S2.151. HMBC spectrum of **30**



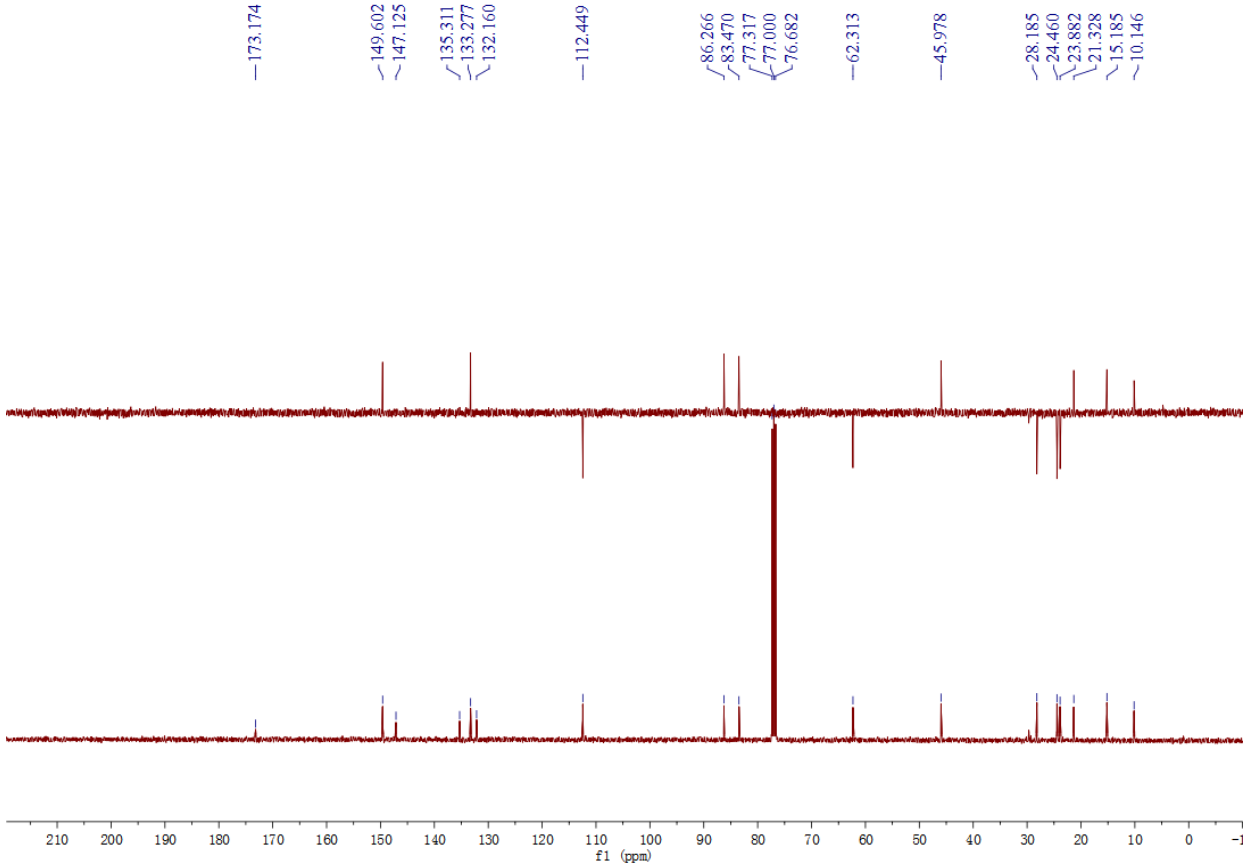
S2.152. NOESY spectrum of **30**



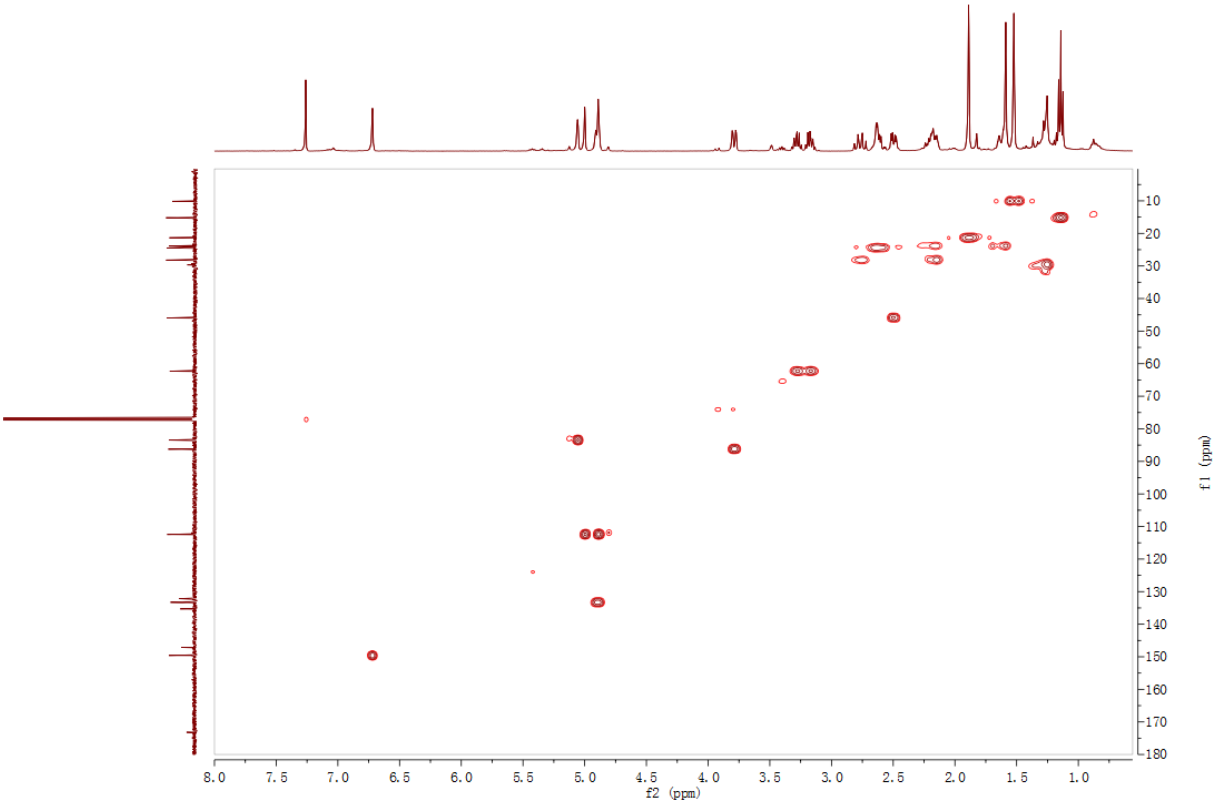
S2.153. ^{1}H NMR spectrum of **31**



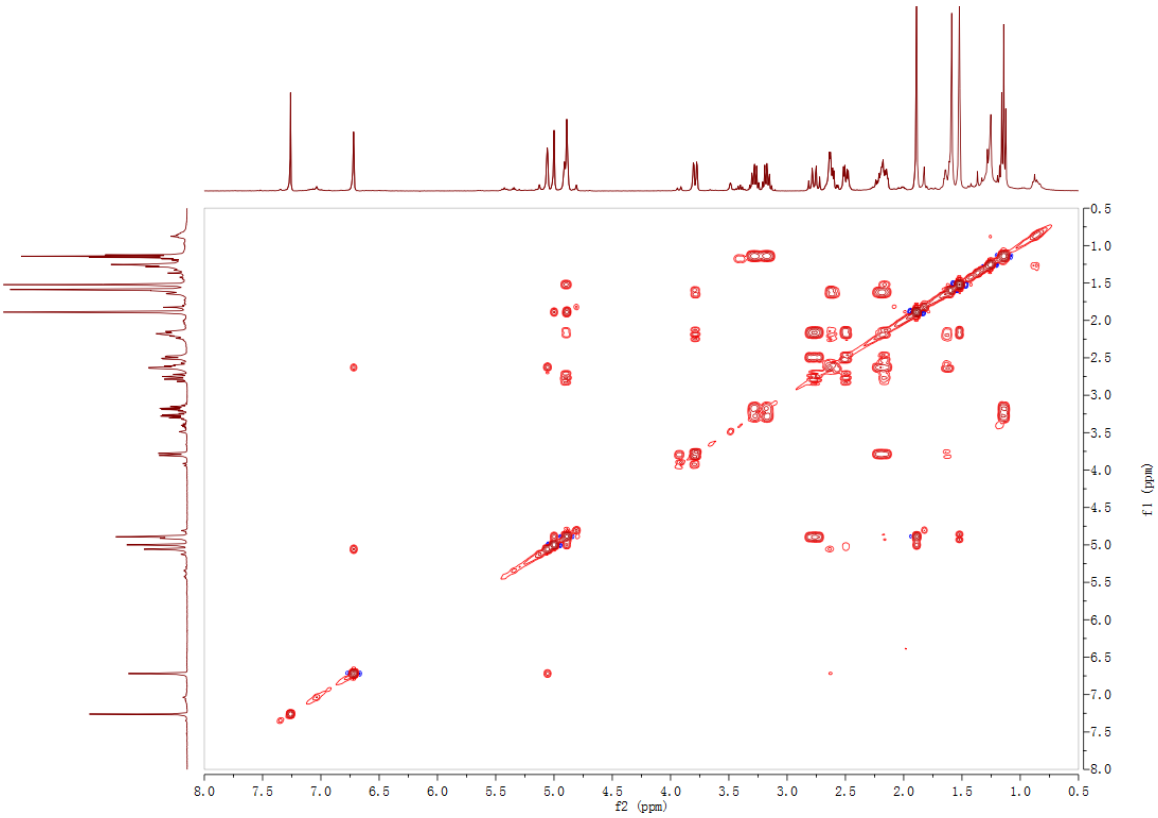
S2.154. ^{13}C NMR and DEPT spectra of **31**



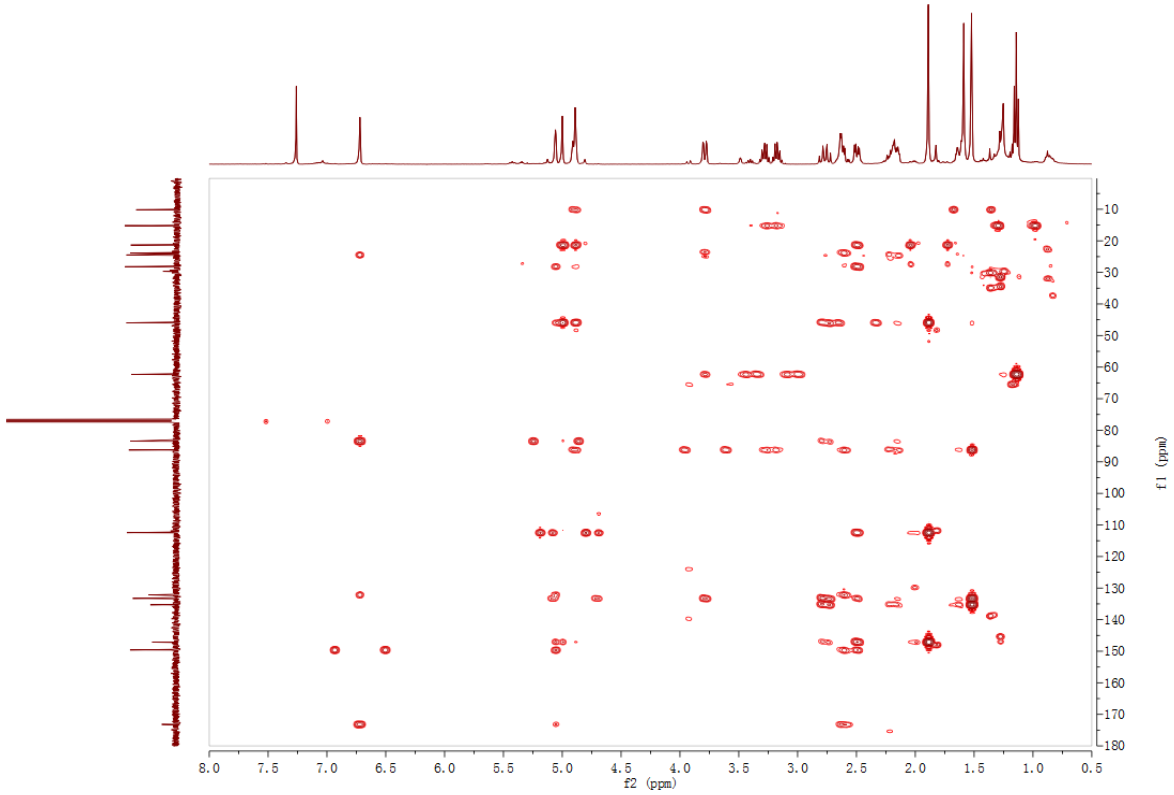
S2.155. HSQC spectrum of **31**



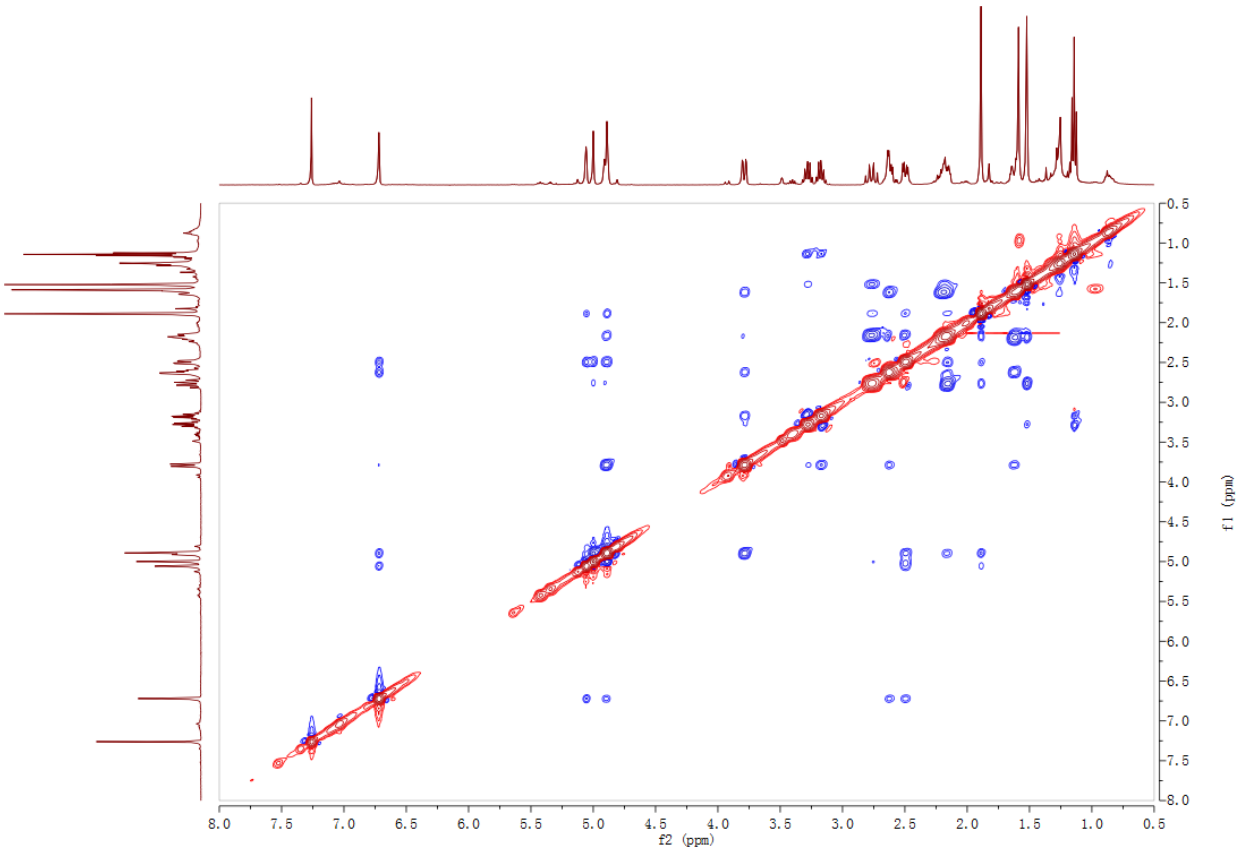
S2.156. ^{1}H–^{1}H COSY spectrum of **31**



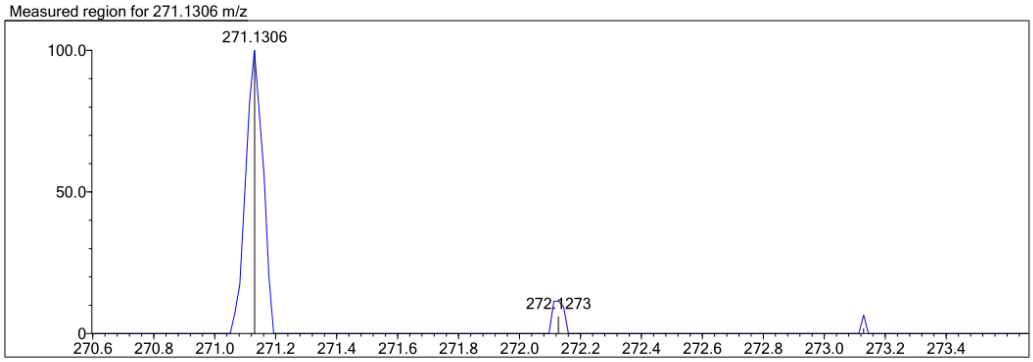
S2.157. HMBC spectrum of **31**

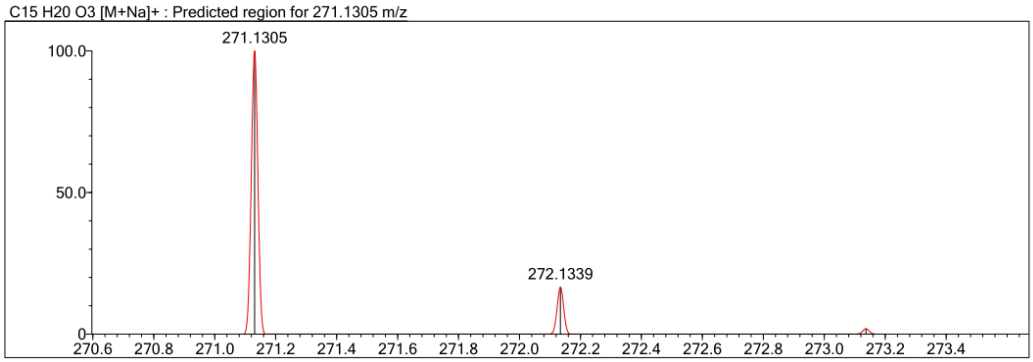


S2.158. NOESY spectrum of **31**



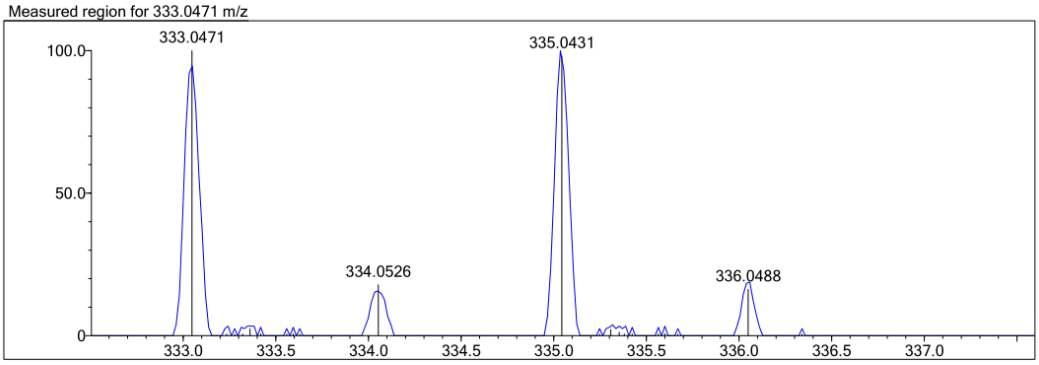
S3.1. HRESIMS data of **2**

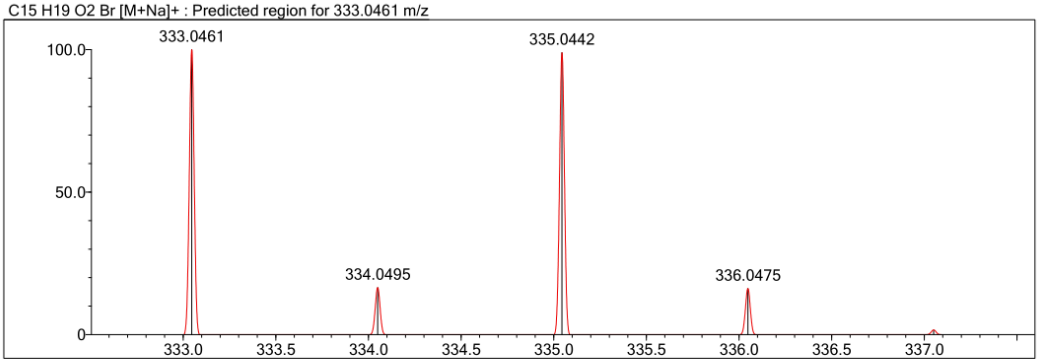




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	54.94	C15 H20 O3	[M+Na]+	271.1306	271.1305	0.1	0.37	54.94	6.0

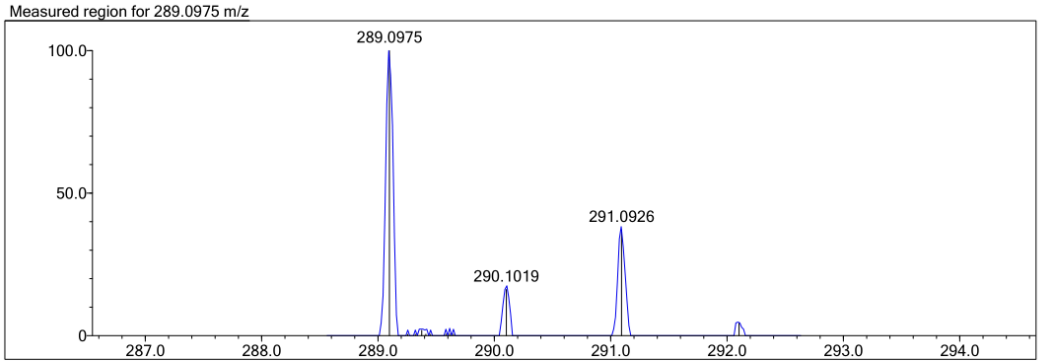
S3.2. HRESIMS data of **3**

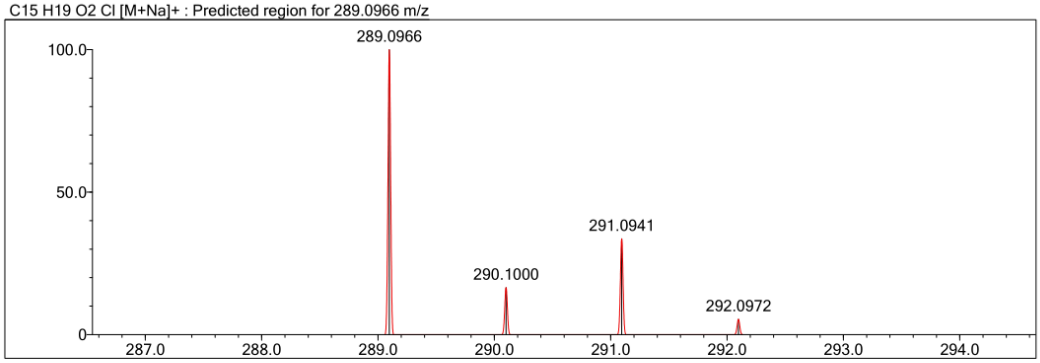




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	67.54	C15 H19 O2 Br	[M+Na]+	333.0471	333.0461	1.0	3.00	71.10	6.0

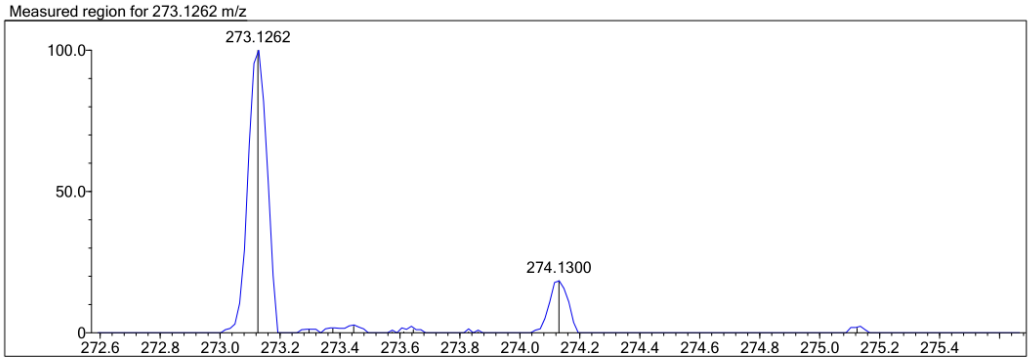
S3.3. HRESIMS data of **4**

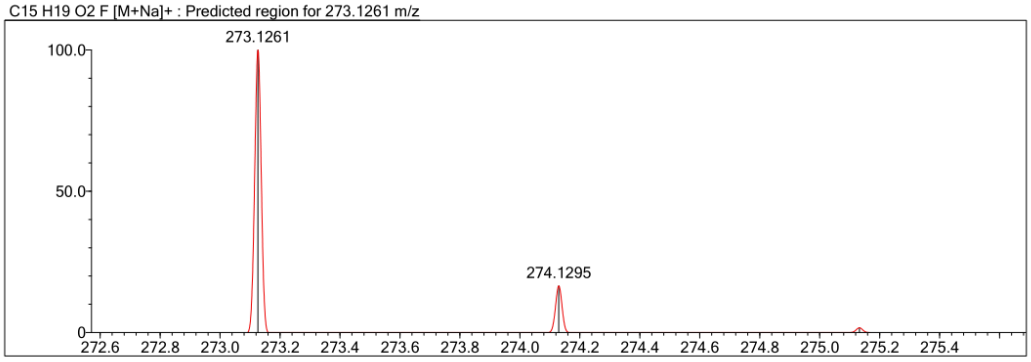




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	83.35	C15 H19 O2 Cl	[M+Na]+	289.0975	289.0966	0.9	3.11	87.99	6.0

S3.4. HRESIMS data of **5**

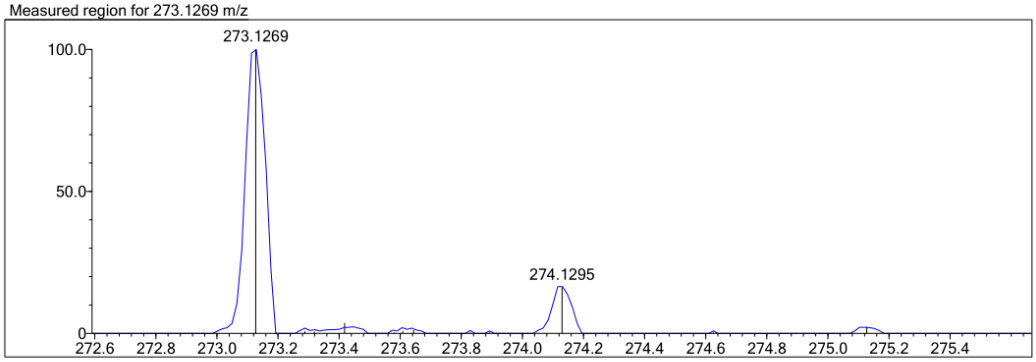




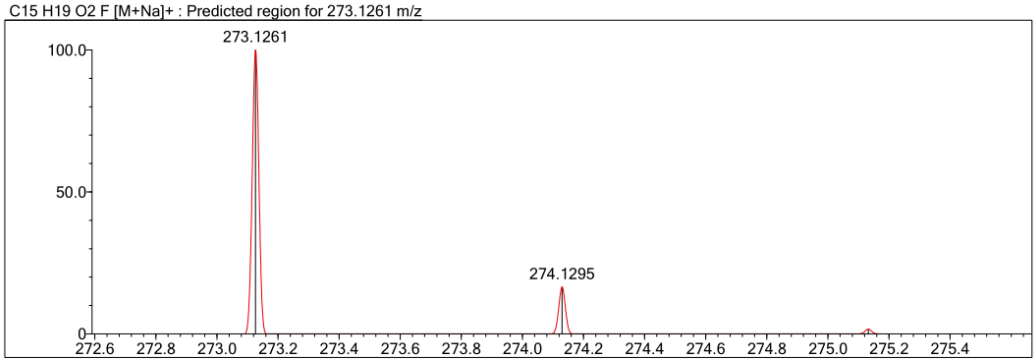
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	84.50	C15 H19 O2 F	[M+Na]+	273.1262	273.1261	0.1	0.37	84.50	6.0

S3.5. HRESIMS data of **6**

Measured region for 273.1269 m/z



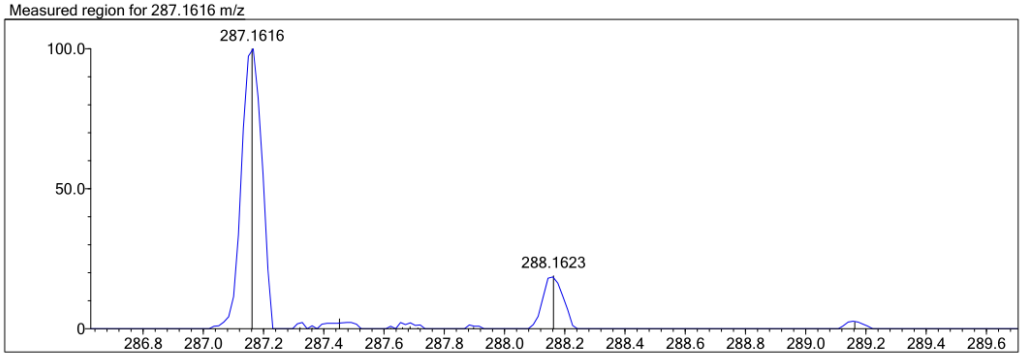
C15 H19 O2 F [M+Na]+ : Predicted region for 273.1261 m/z



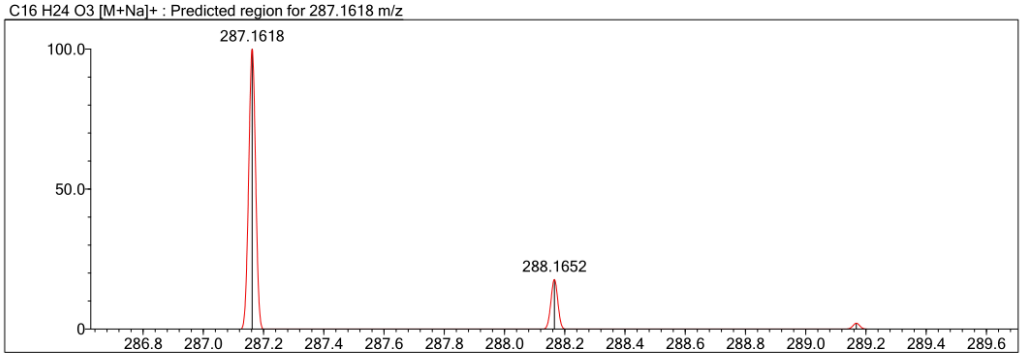
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	63.46	C15 H19 O2 F	[M+Na]+	273.1269	273.1261	0.8	2.93	66.67	6.0

S3.6. HRESIMS data of **7**

Measured region for 287.1616 m/z

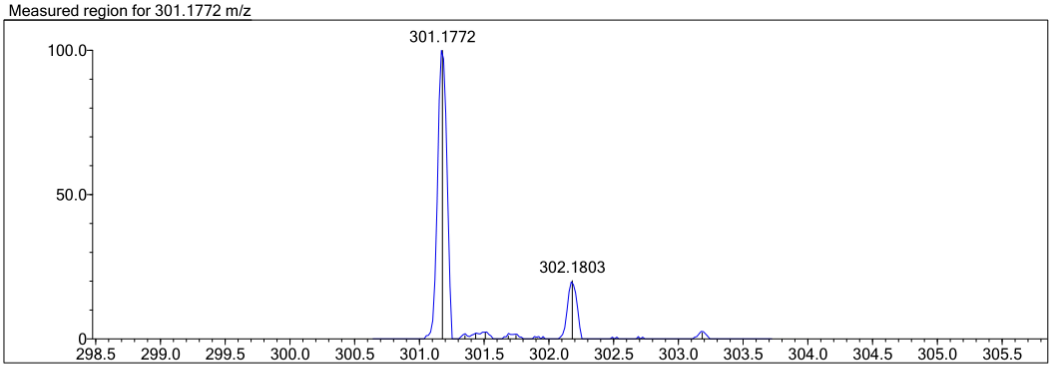


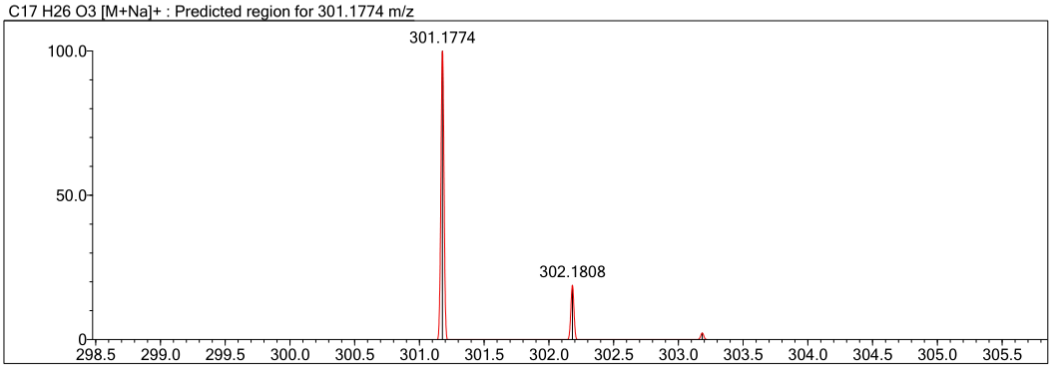
C16 H24 O3 [M+Na]+ : Predicted region for 287.1618 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	69.78	C16 H24 O3	[M+Na]+	287.1616	287.1618	-0.2	-0.70	69.78	5.0

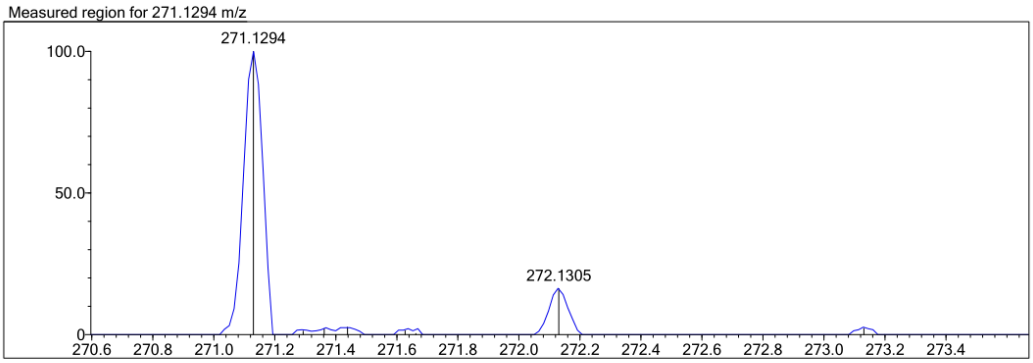
S3.7. HRESIMS data of **8**

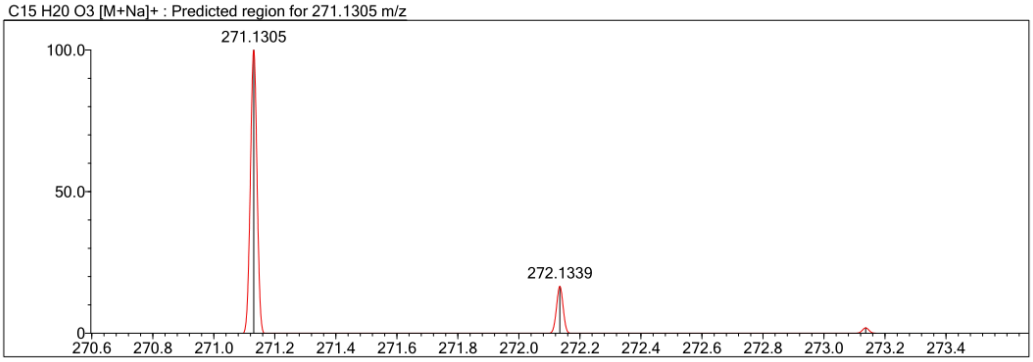




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	73.70	C17 H26 O3	[M+Na]+	301.1772	301.1774	-0.2	-0.66	73.70	5.0

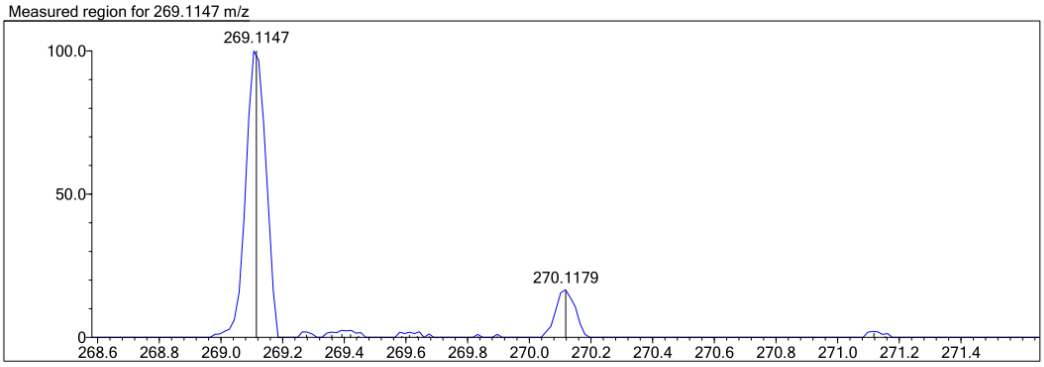
S3.8. HRESIMS data of **9**

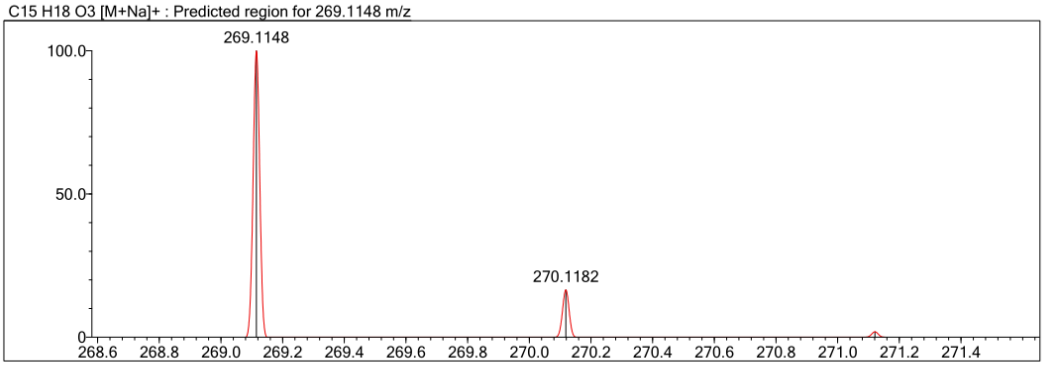




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	68.16	C15 H20 O3	[M+Na]+	271.1294	271.1305	-1.1	-4.06	73.81	6.0

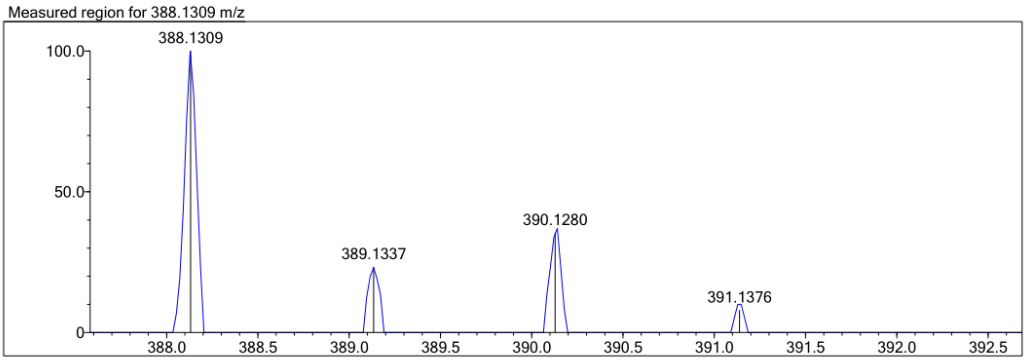
S3.9. HRESIMS data of **11**

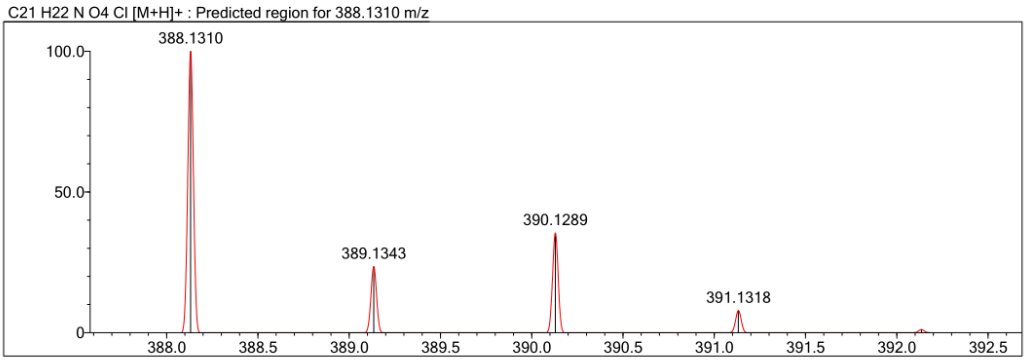




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	80.72	C15 H18 O3	[M+Na]+	269.1147	269.1148	-0.1	-0.37	80.72	7.0

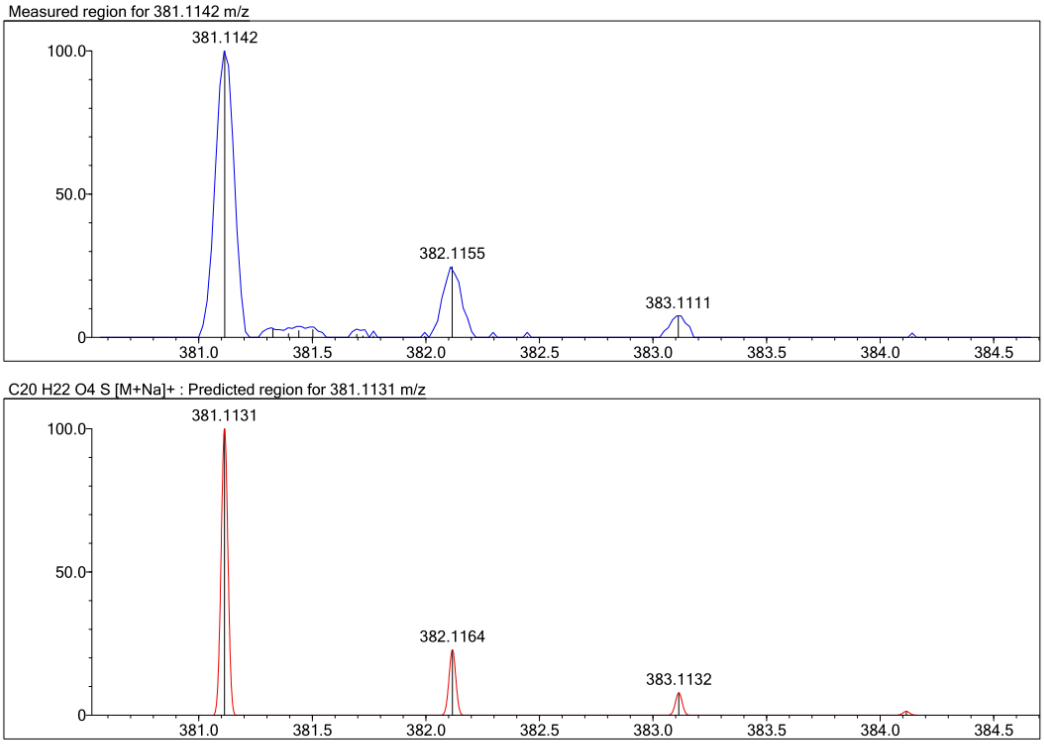
S3.10. HRESIMS data of **12**





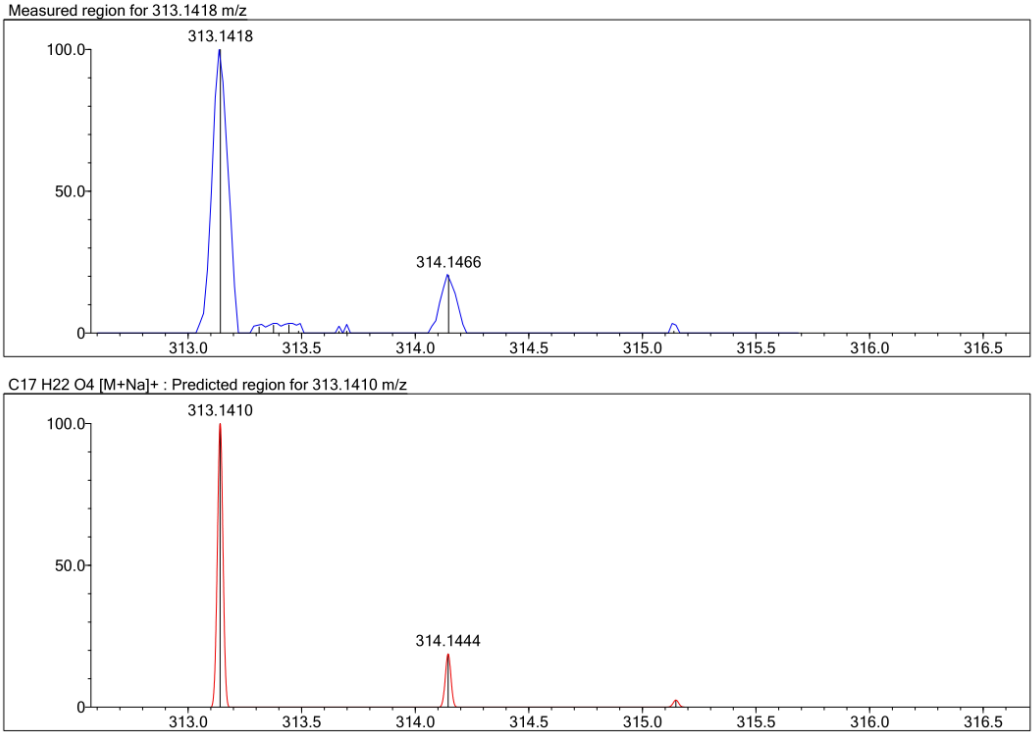
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	91.10	C21 H22 N O4 Cl	[M+H]+	388.1309	388.1310	-0.1	-0.26	91.10	11.0

S3.11. HRESIMS data of **13**



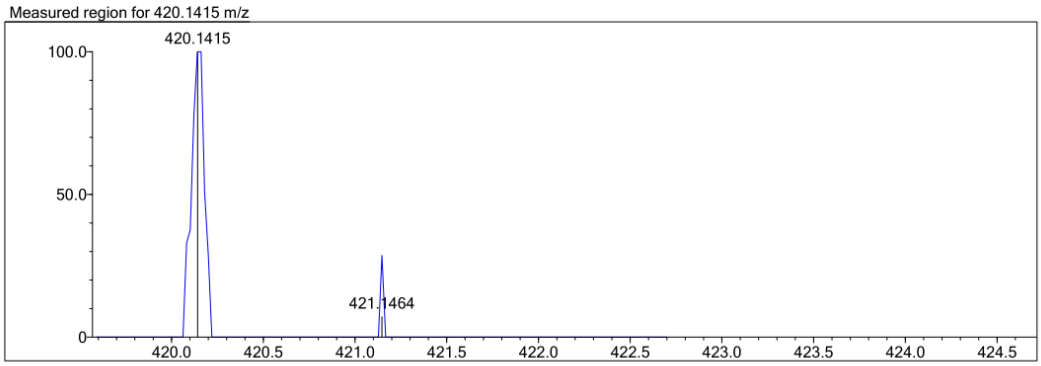
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	83.42	C20 H22 O4 S	[M+Na]+	381.1142	381.1131	1.1	2.89	87.56	10.0

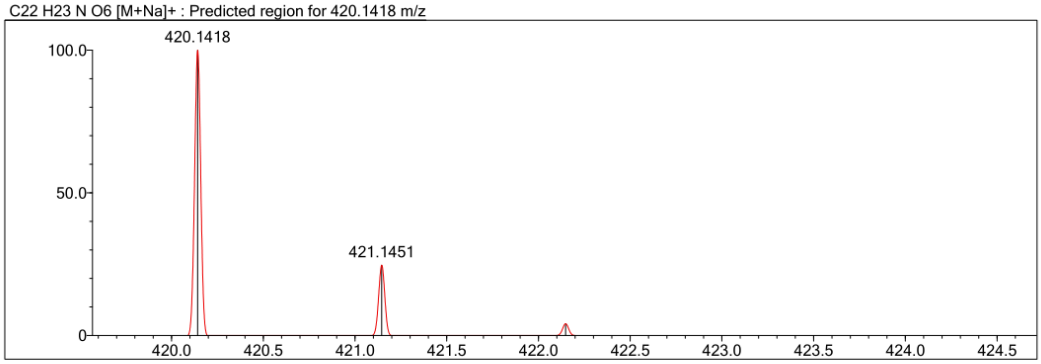
S3.12. HRESIMS data of **14**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	65.29	C17 H22 O4	[M+Na]+	313.1418	313.1410	0.8	2.55	67.92	7.0

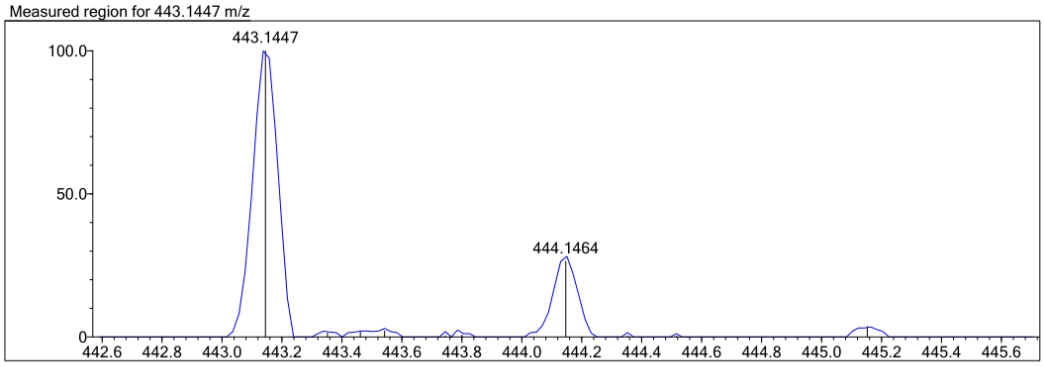
S3.13. HRESIMS data of **15**

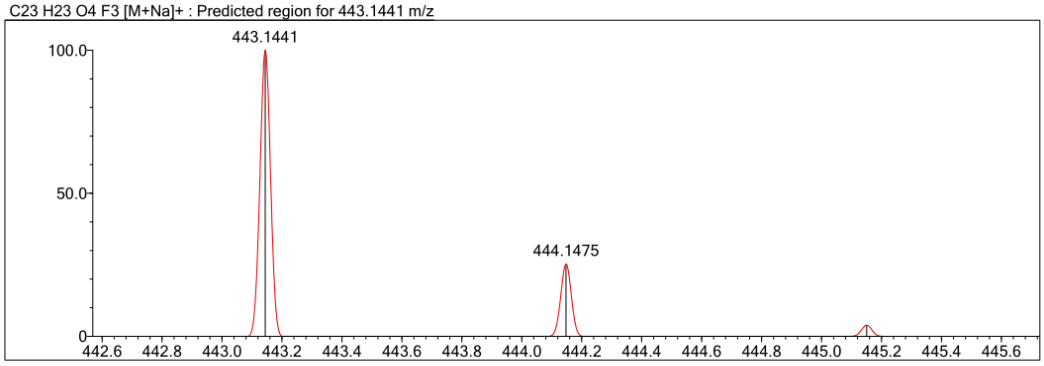




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	91.43	C22 H23 N O6	[M+Na]+	420.1415	420.1418	-0.3	-0.71	91.43	12.0

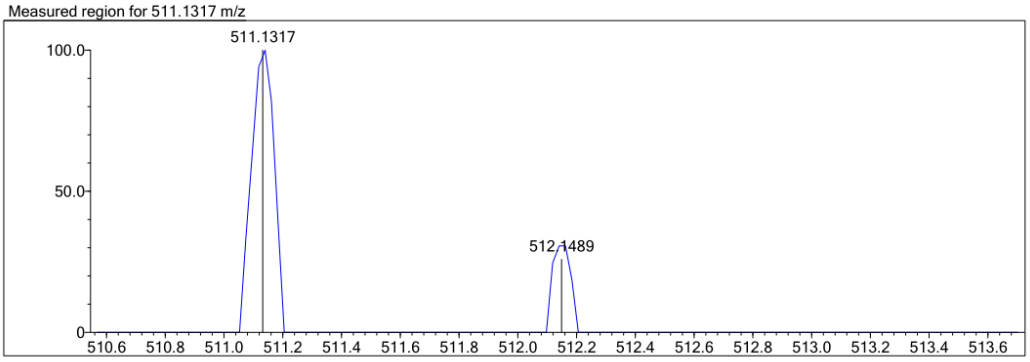
S3.14. HRESIMS data of **16**

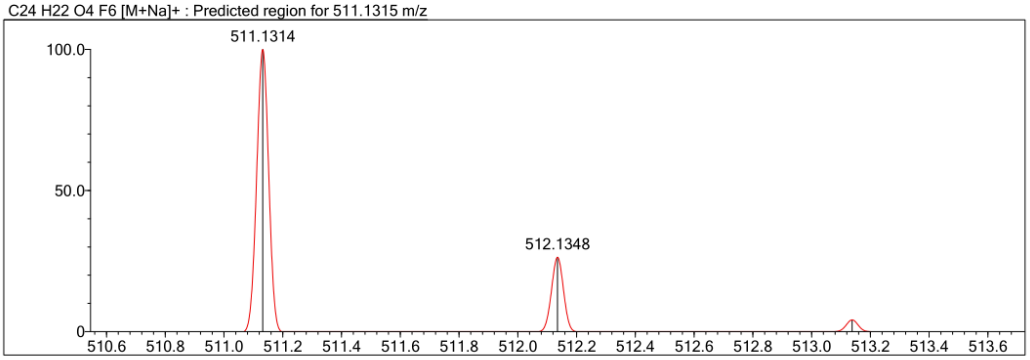




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	91.61	C23 H23 O4 F3	[M+Na]+	443.1447	443.1441	0.6	1.35	92.42	11.0

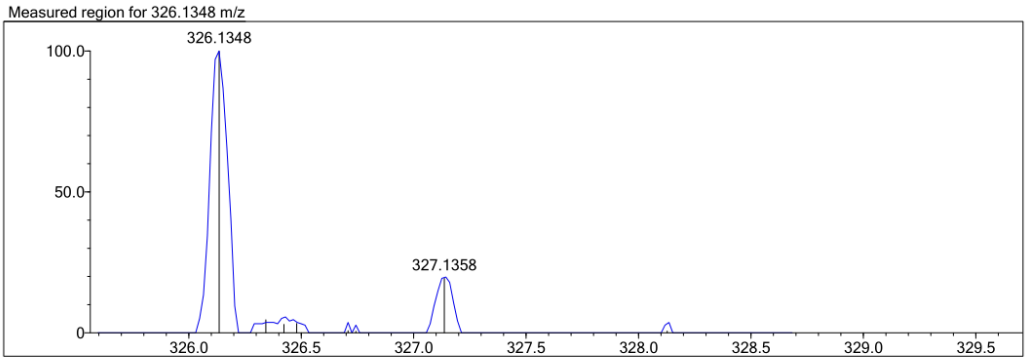
S3.15. HRESIMS data of **17**

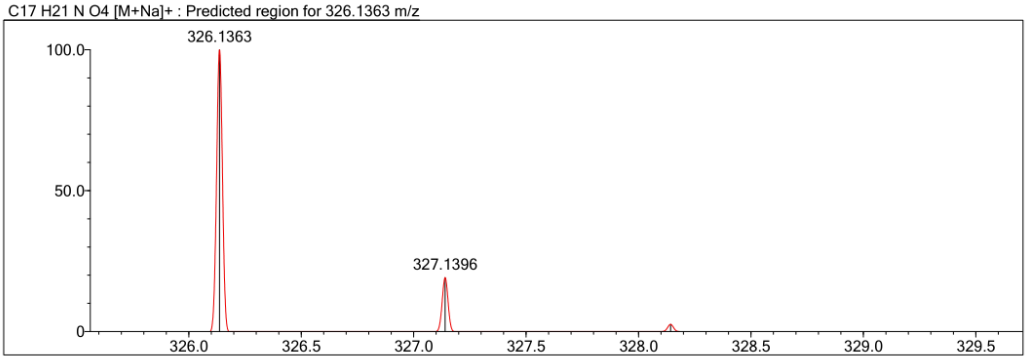




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
4	60.93	C24 H22 O4 F6	[M+Na]+	511.1317	511.1315	0.2	0.39	60.93	11.0

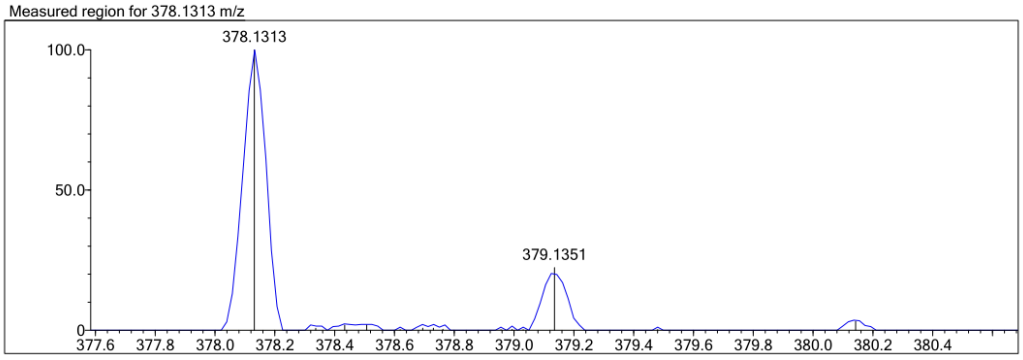
S3.16. HRESIMS data of **18**

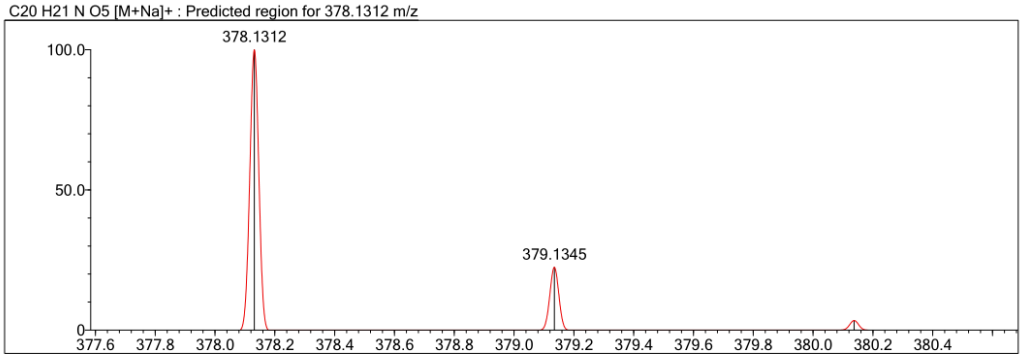




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	57.82	C17 H21 N O4	[M+Na]+	326.1348	326.1363	-1.5	-4.60	63.53	8.0

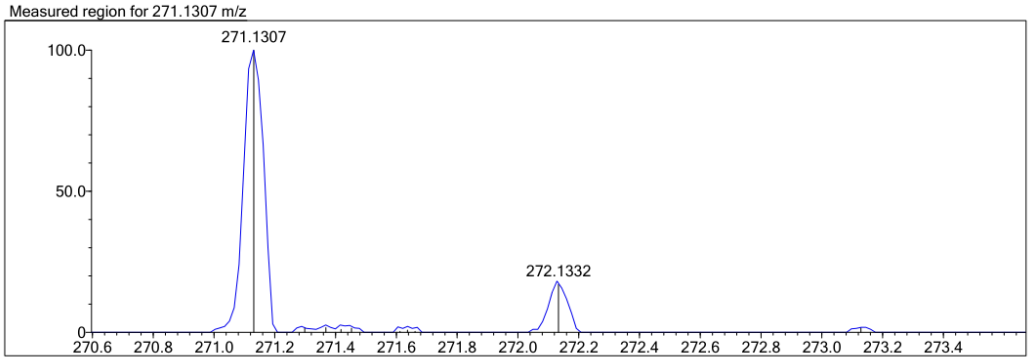
S3.17. HRESIMS data of **19**

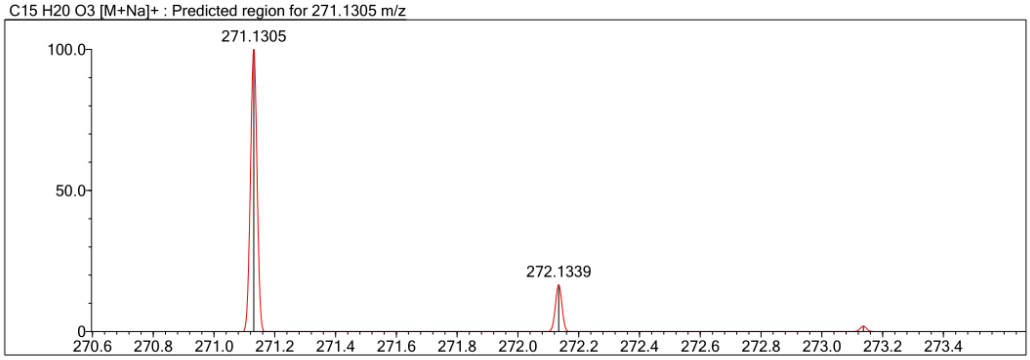




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	87.07	C20 H21 N O5	[M+Na]+	378.1313	378.1312	0.1	0.26	87.07	11.0

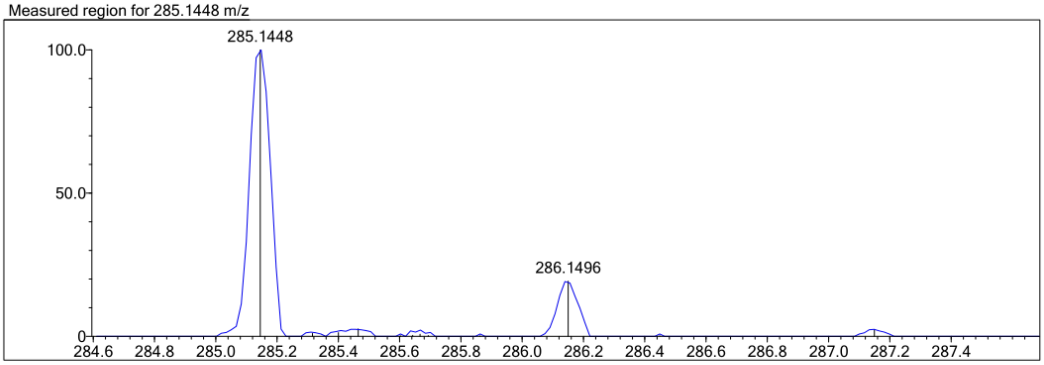
S3.18. HRESIMS data of **20**

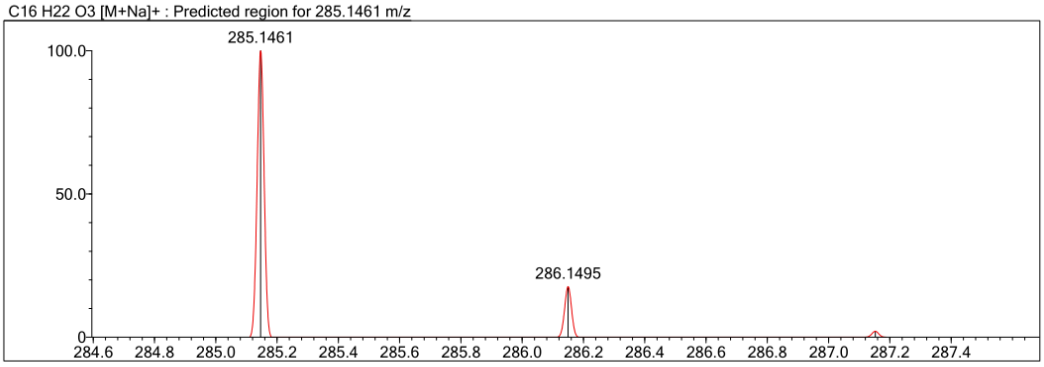




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	72.95	C15 H20 O3	[M+Na]+	271.1307	271.1305	0.2	0.74	72.95	6.0

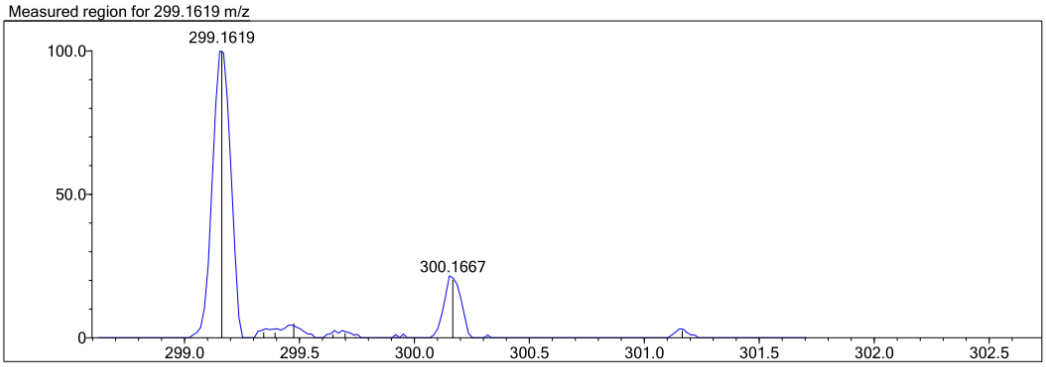
S3.19. HRESIMS data of **21**

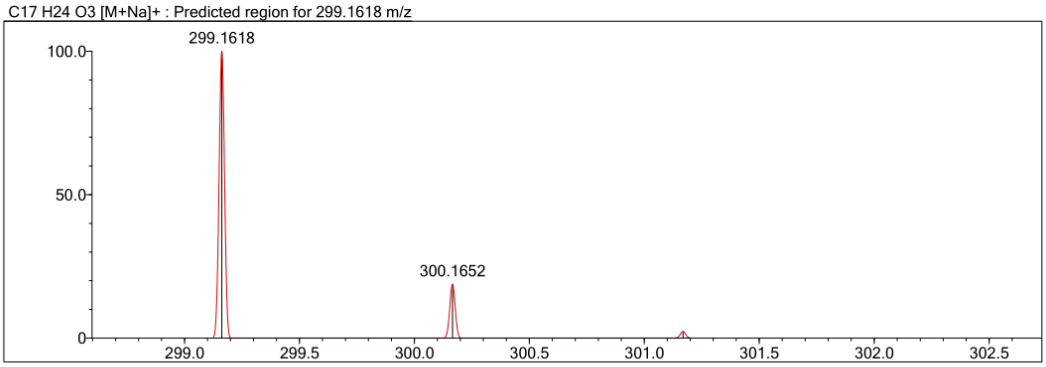




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	68.26	C16 H22 O3	[M+Na]+	285.1448	285.1461	-1.3	-4.56	74.93	6.0

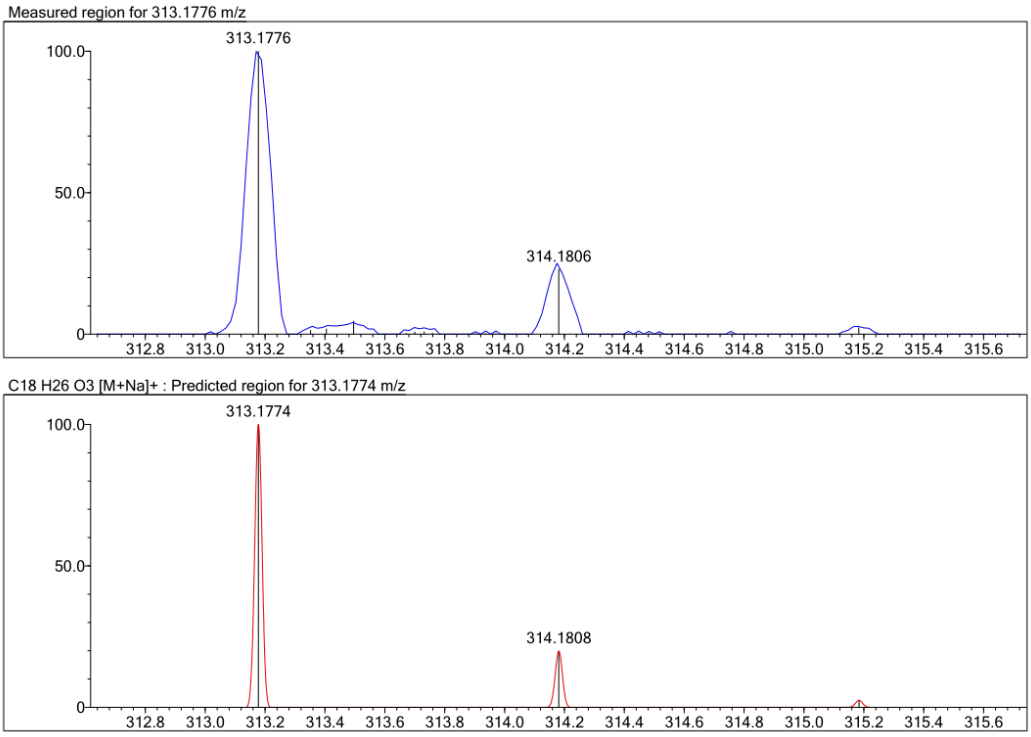
S3.20. HRESIMS data of **22**





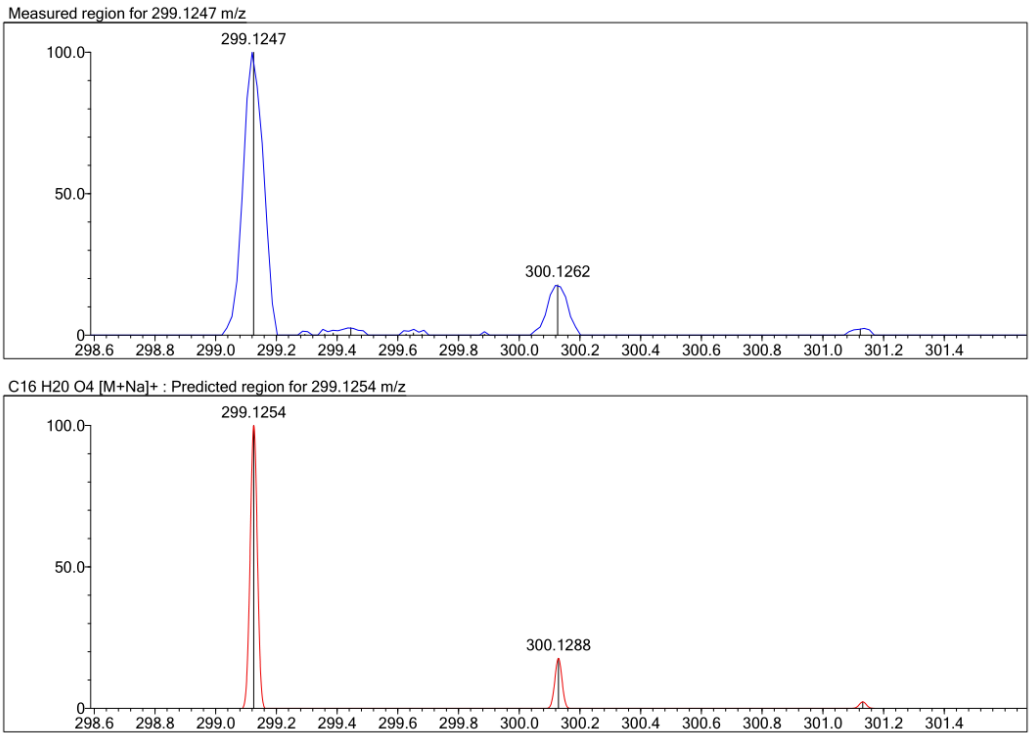
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	76.05	C17 H24 O3	[M+Na]+	299.1619	299.1618	0.1	0.33	76.05	6.0

S3.21. HRESIMS data of **23**



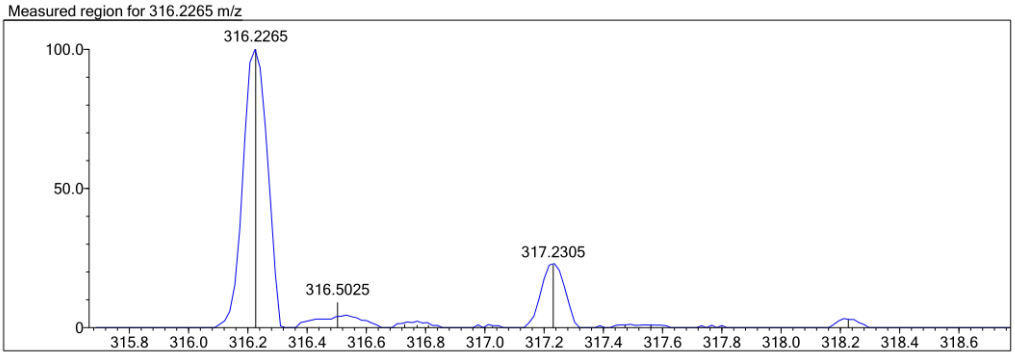
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	74.46	C18 H26 O3	[M+Na]+	313.1776	313.1774	0.2	0.64	74.46	6.0

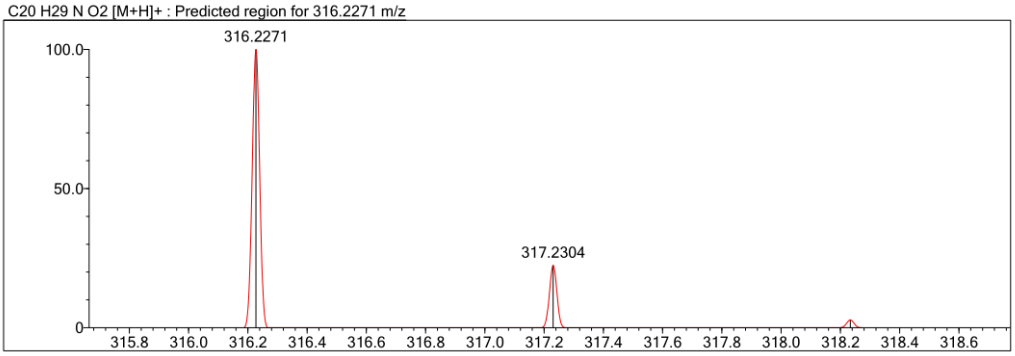
S3.22. HRESIMS data of **24**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	66.75	C16 H20 O4	[M+Na]+	299.1247	299.1254	-0.7	-2.34	69.07	7.0

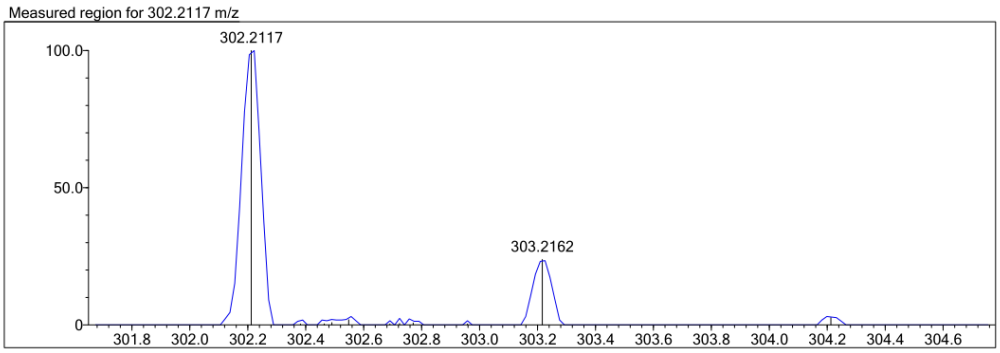
S3.23. HRESIMS data of **25**

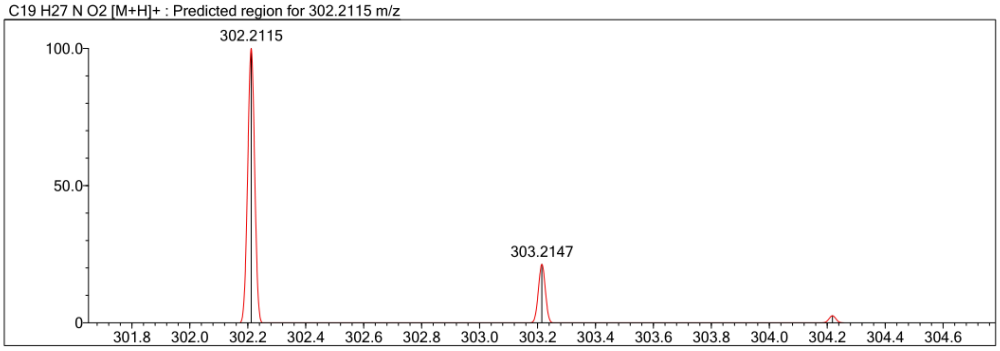




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	70.43	C20 H29 N O2	[M+H]+	316.2265	316.2271	-0.6	-1.90	72.05	7.0

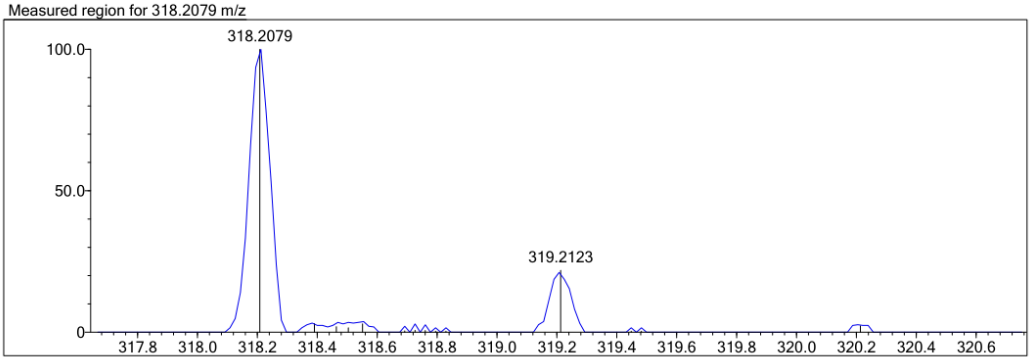
S3.24. HRESIMS data of **26**

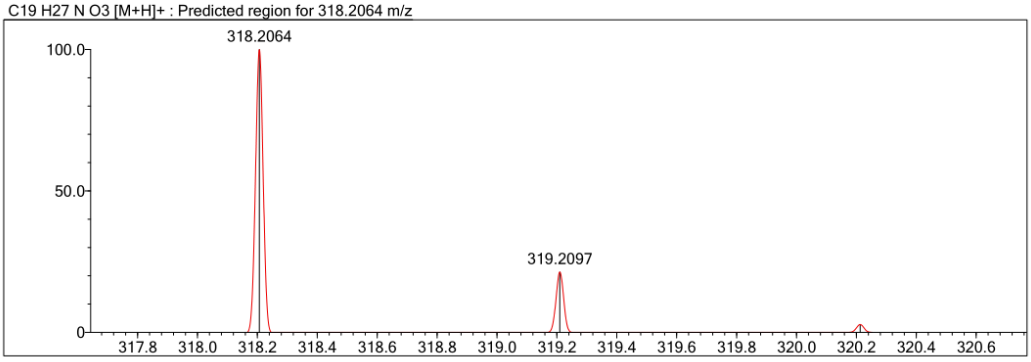




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	78.44	C19 H27 N O2	[M+H]+	302.2117	302.2115	0.2	0.66	78.44	7.0

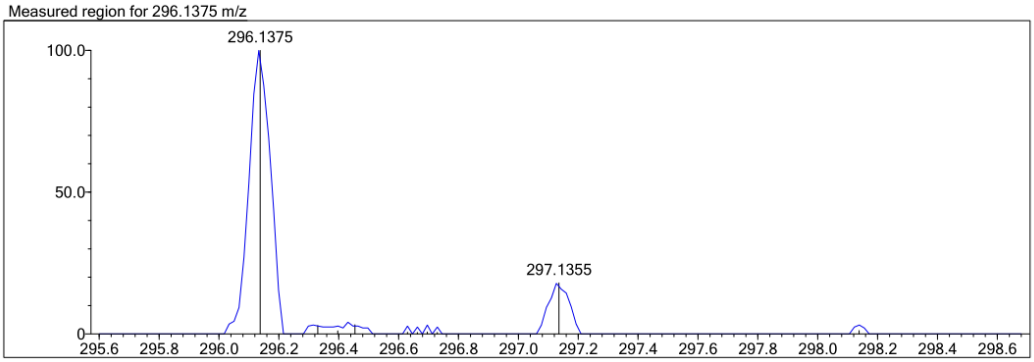
S3.25. HRESIMS data of **27**

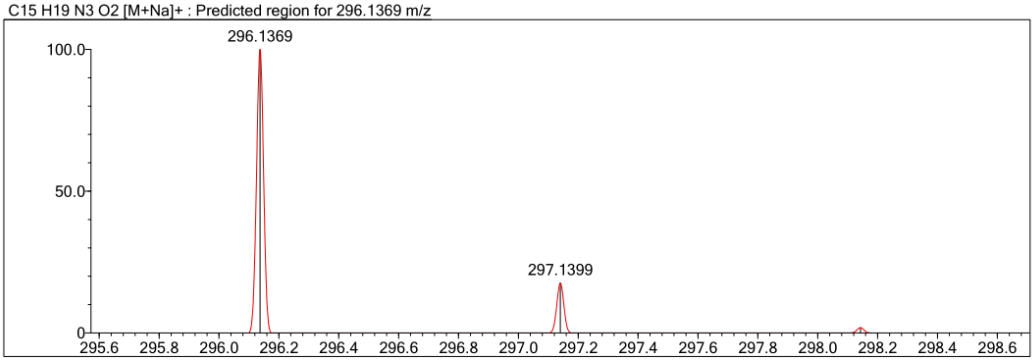




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	70.97	C19 H27 N O3	[M+H]+	318.2079	318.2064	1.5	4.71	78.23	7.0

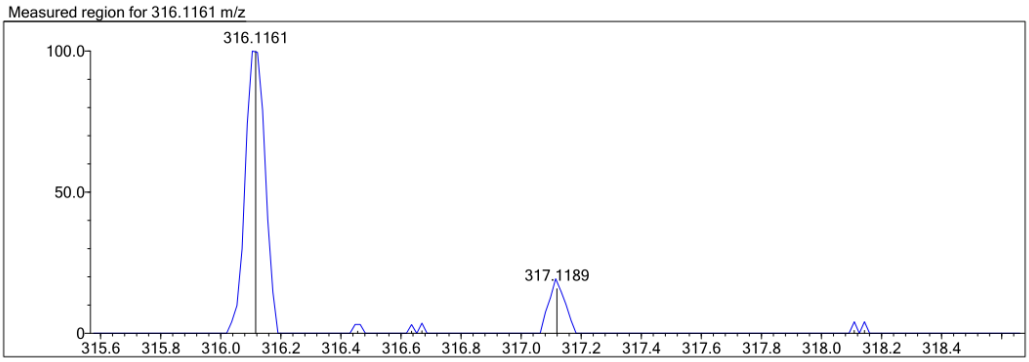
S3.26. HRESIMS data of **28**

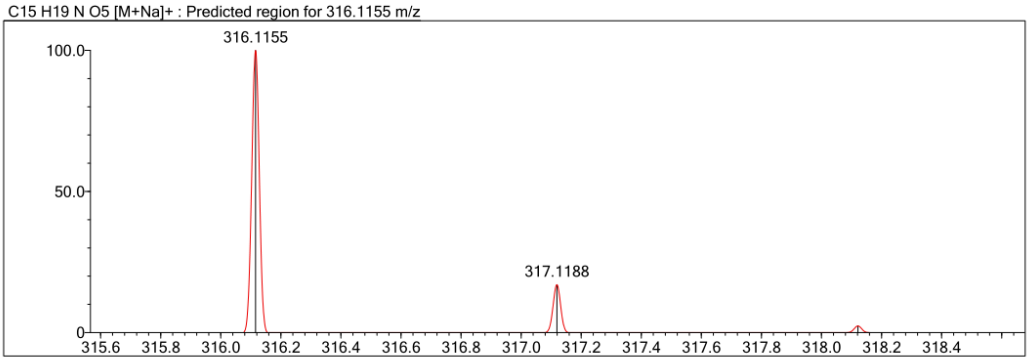




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	75.96	C15 H19 N3 O2	[M+Na]+	296.1375	296.1369	0.6	2.03	77.97	8.0

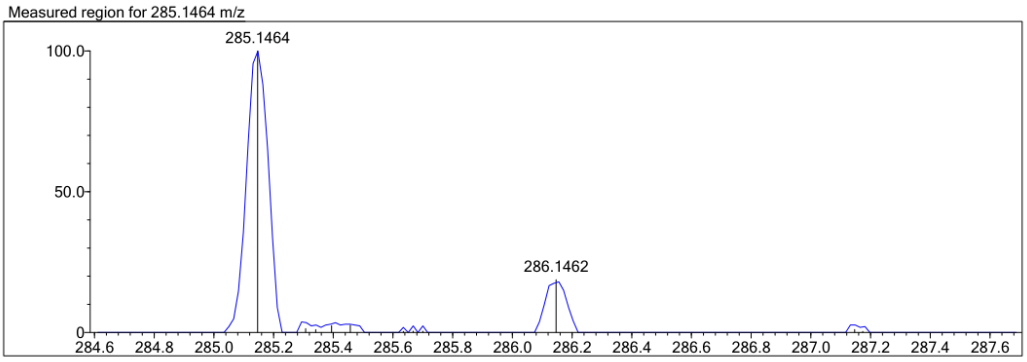
S3.27. HRESIMS data of **29**

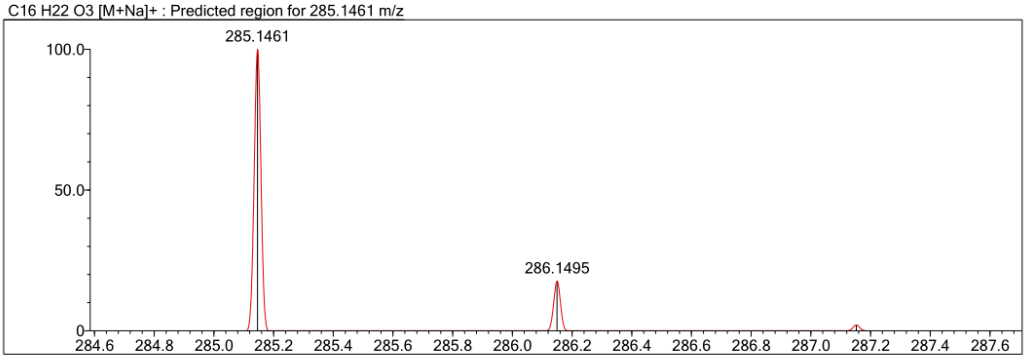




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	63.39	C15 H19 N O5	[M+Na]+	316.1161	316.1155	0.6	1.90	64.85	7.0

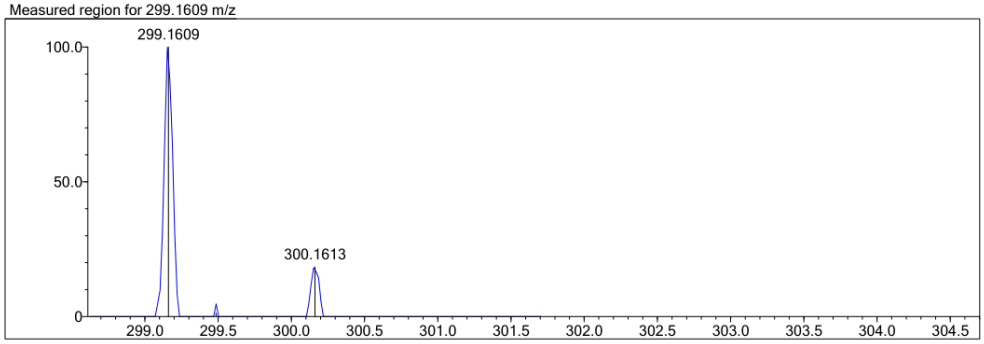
S3.28. HRESIMS data of **30**

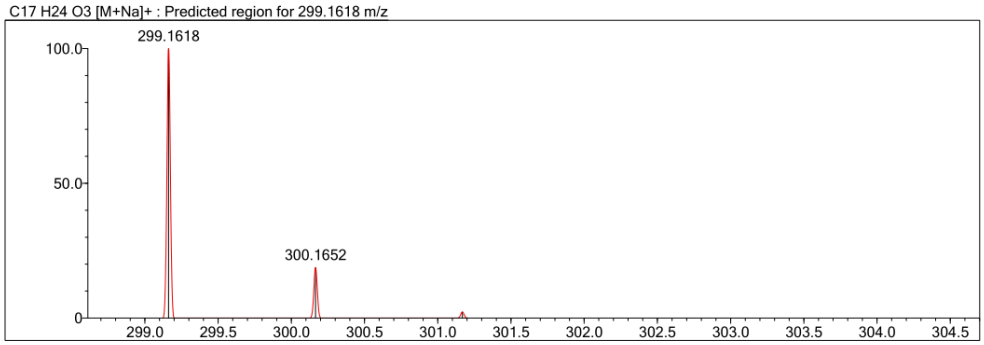




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	59.50	C16 H22 O3	[M+Na]+	285.1464	285.1461	0.3	1.05	59.58	6.0

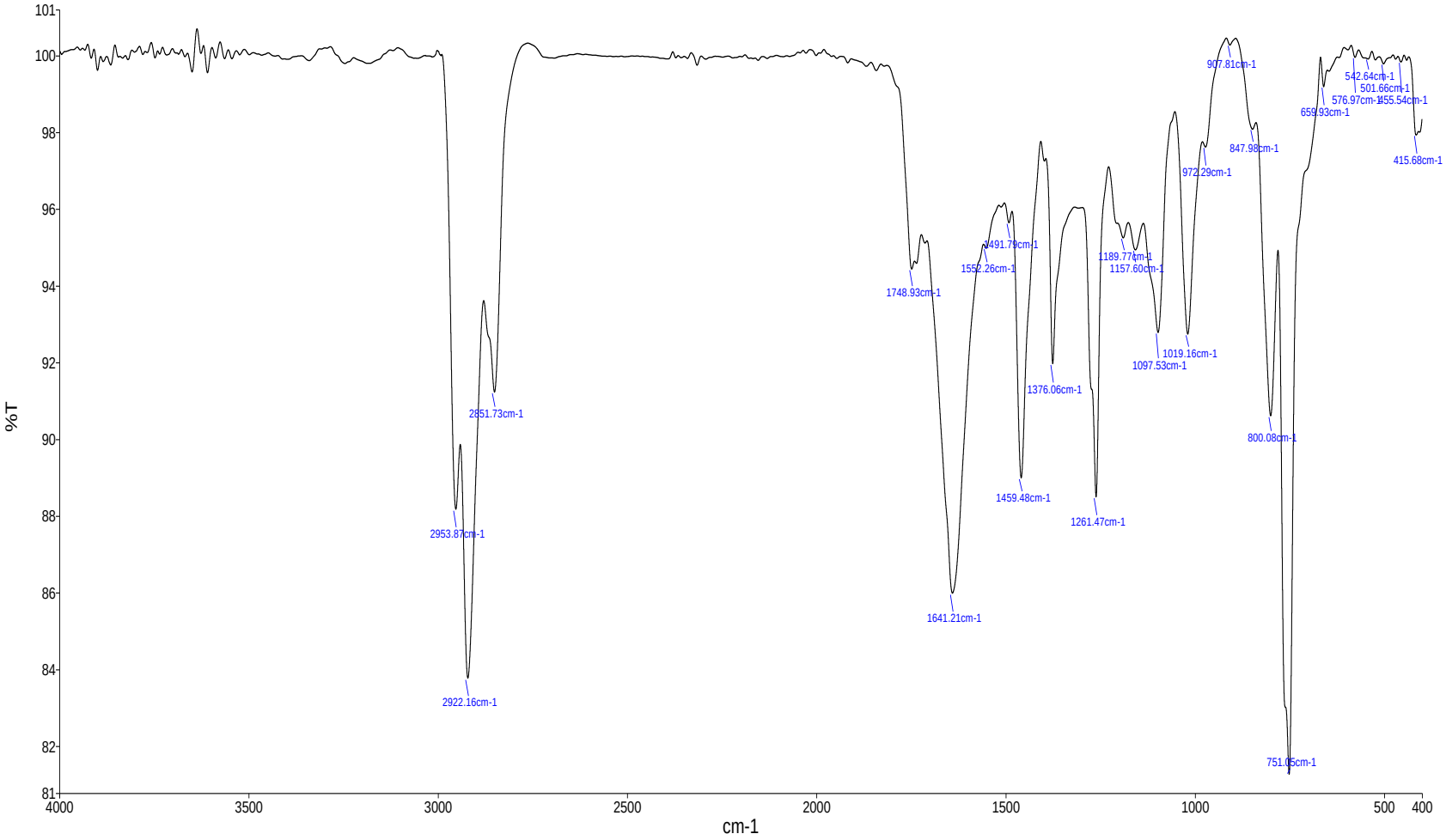
S3.29. HRESIMS data of **31**



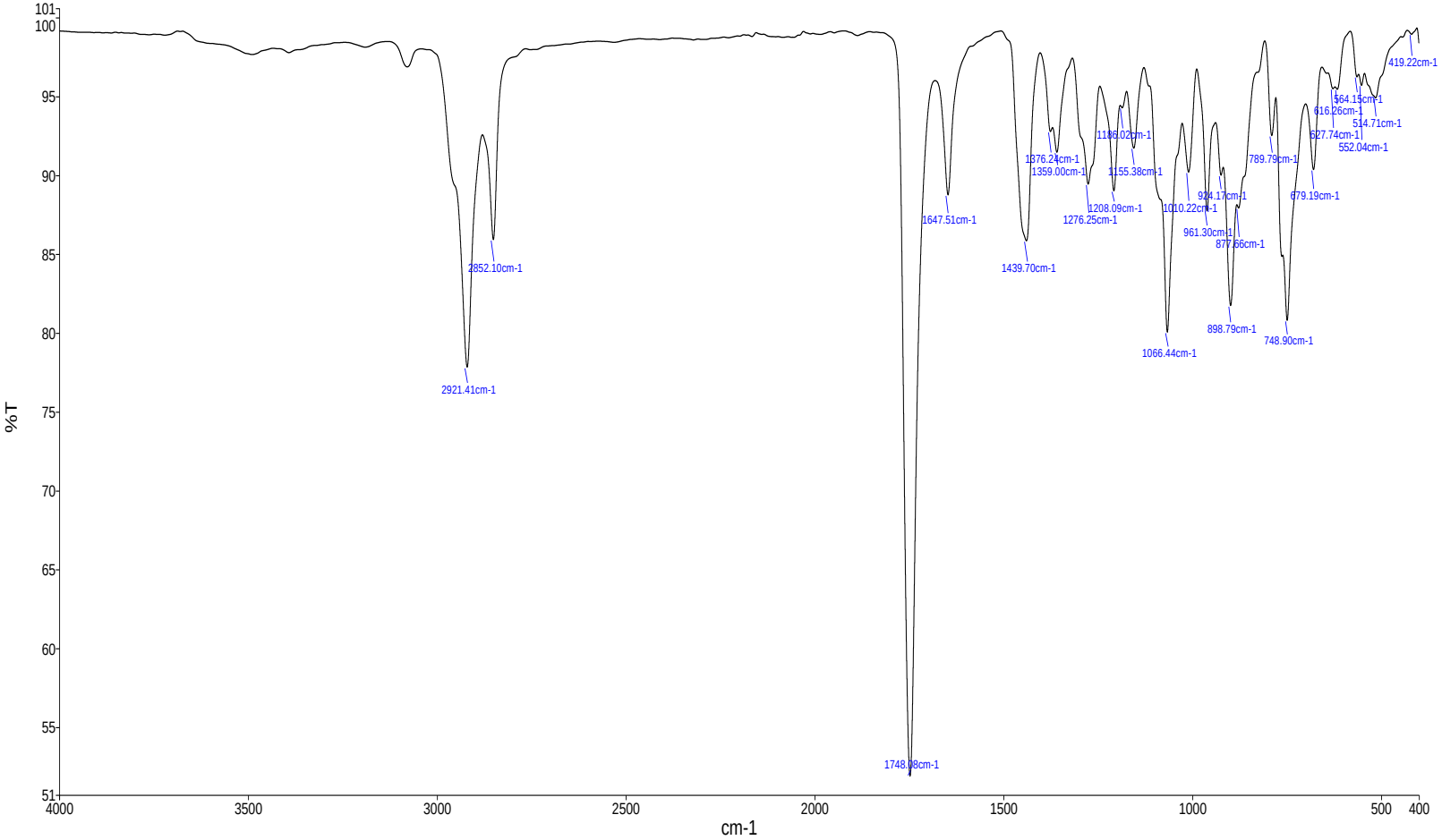


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	59.29	C17 H24 O3	[M+Na]+	299.1609	299.1618	-0.9	-3.01	62.43	6.0

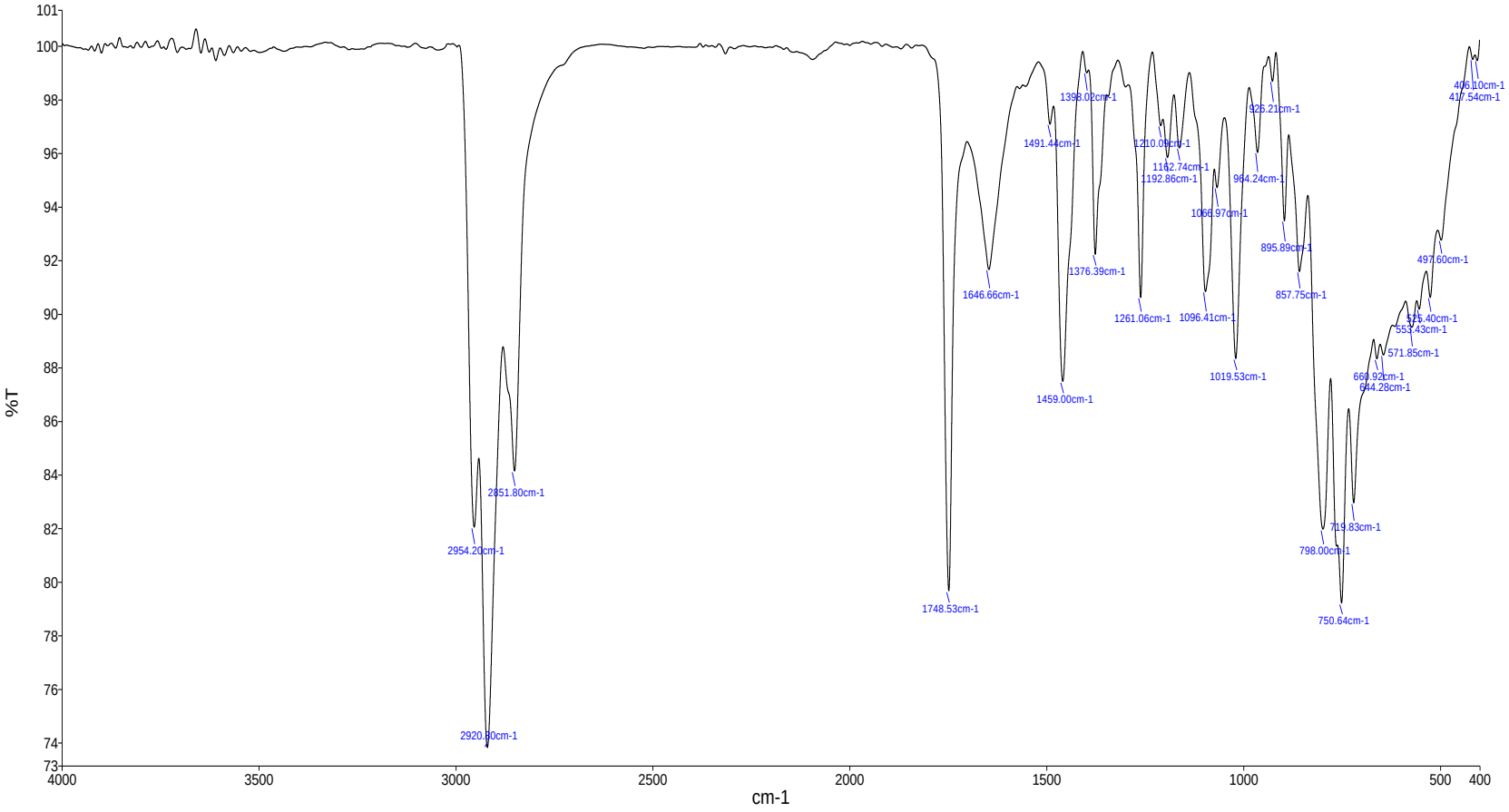
S4.1. IR (KBr disc) spectrum of **2**



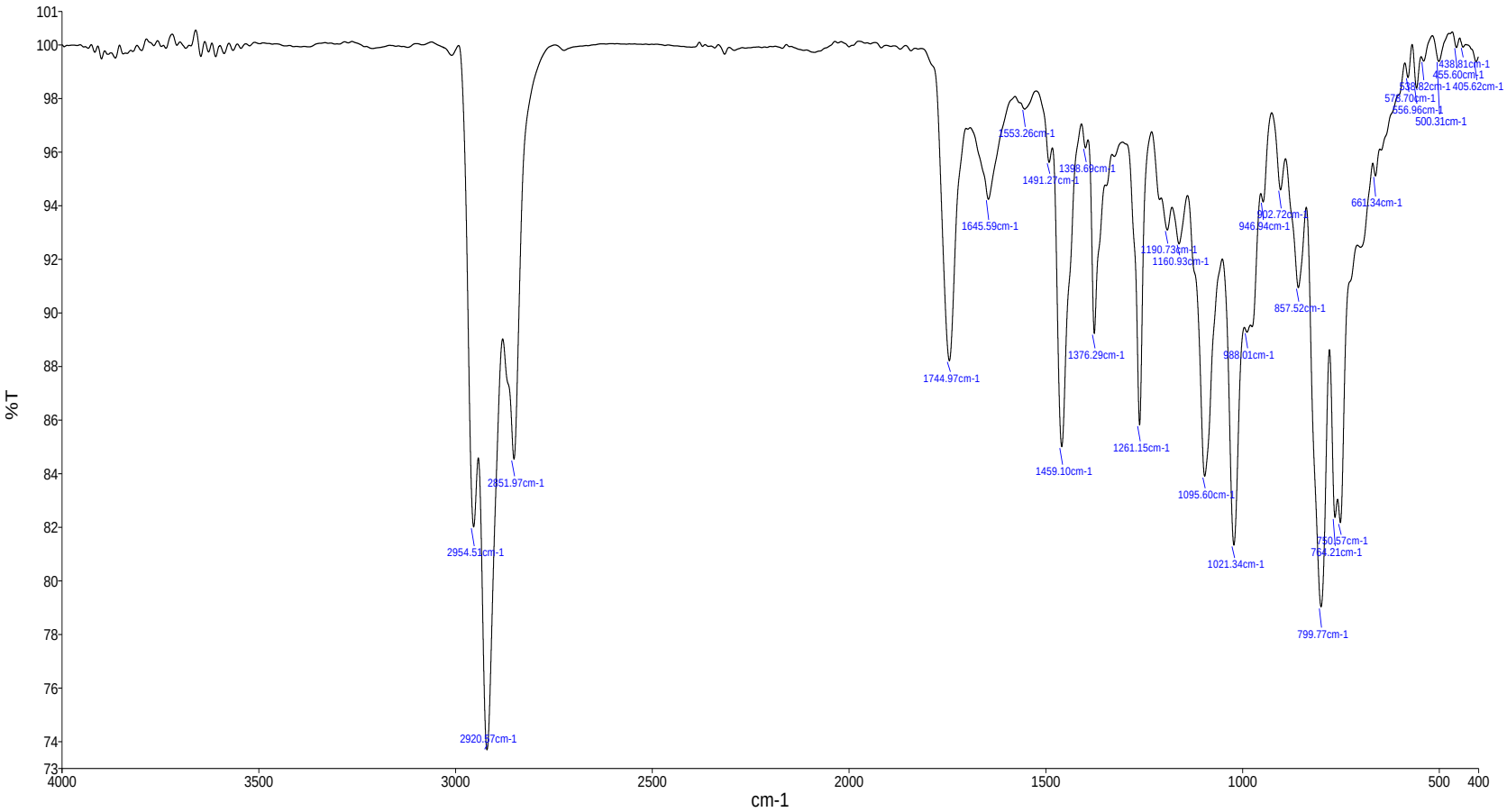
S4.2. IR (KBr disc) spectrum of **3**



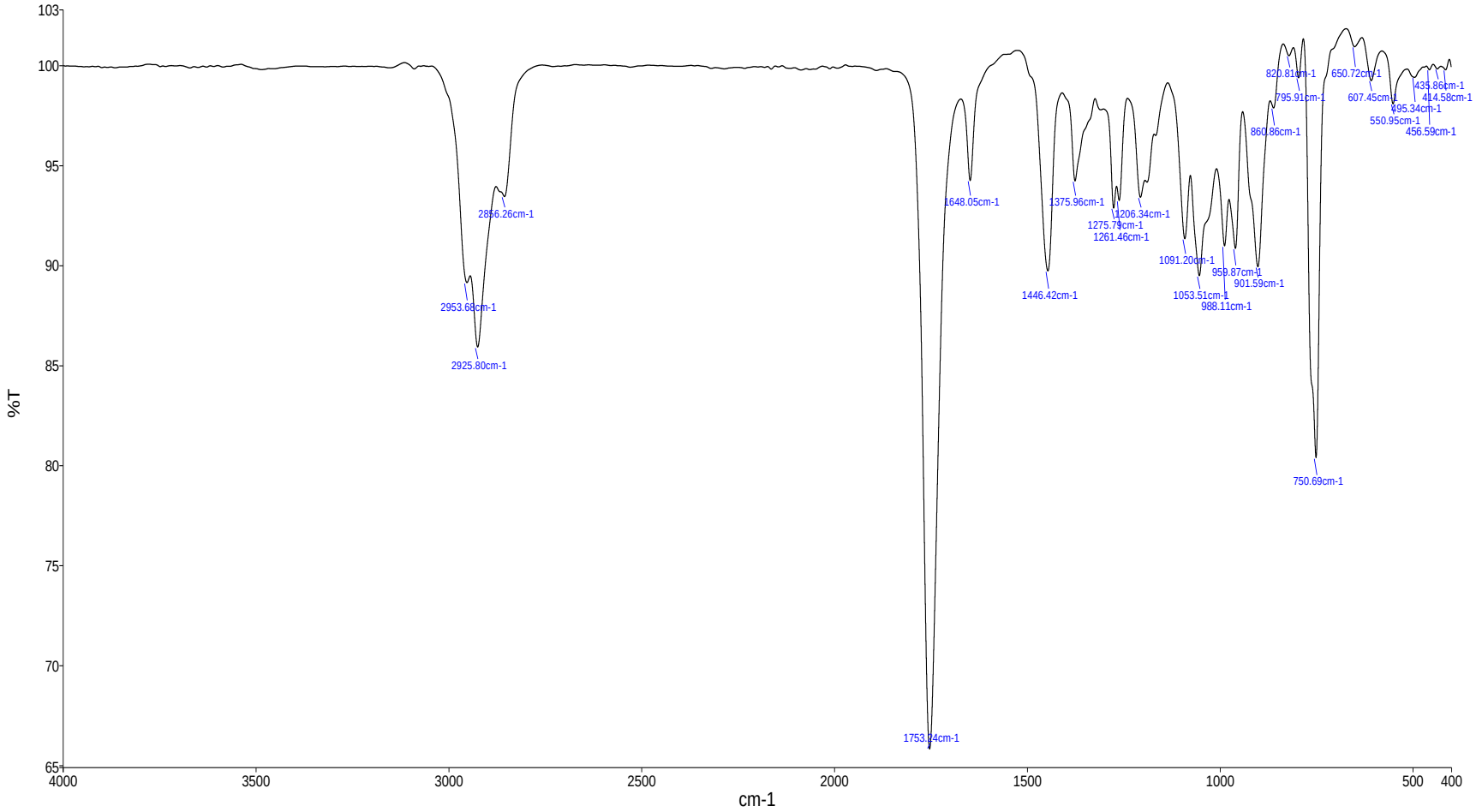
S4.3. IR (KBr disc) spectrum of **4**



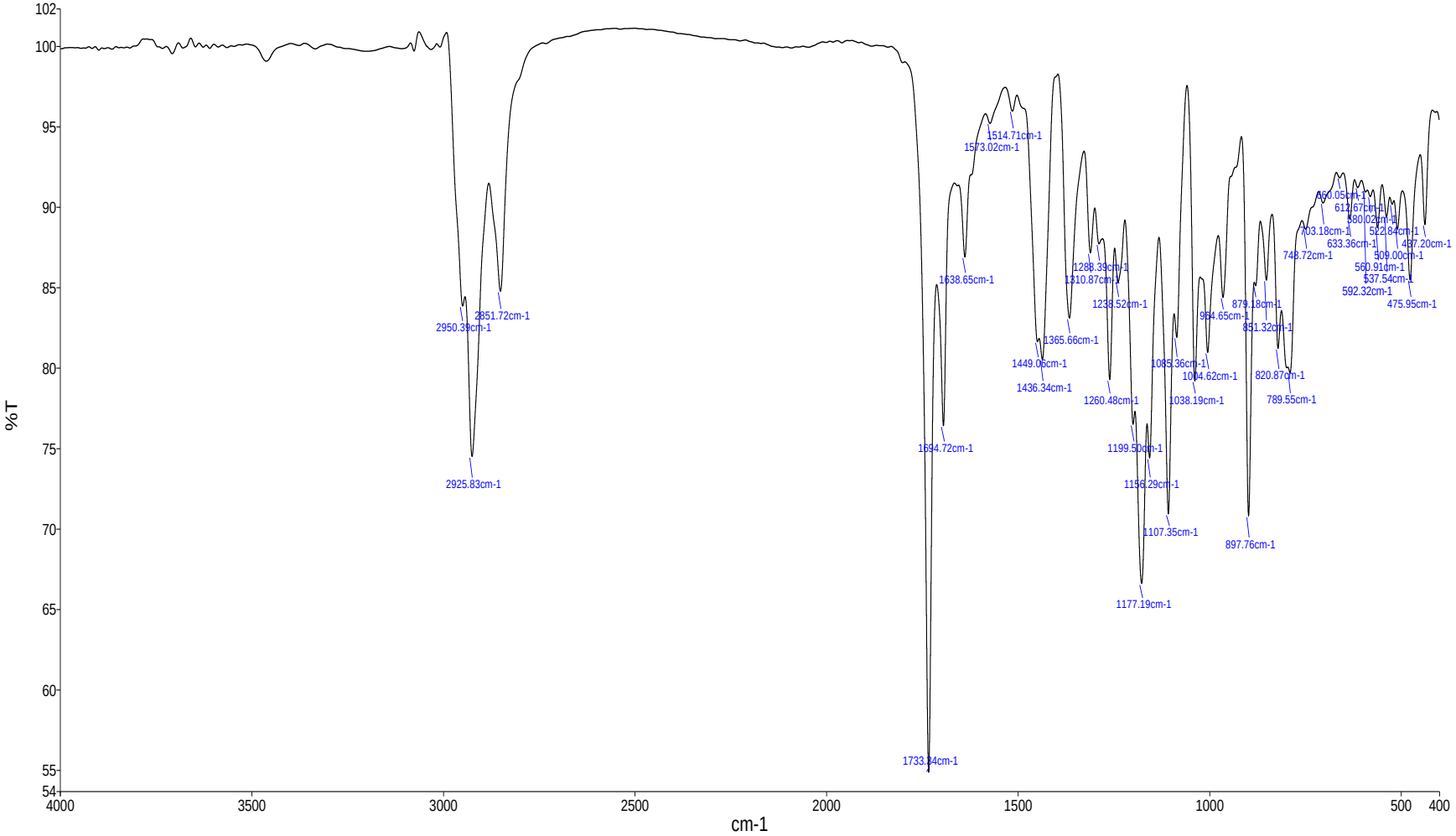
S4.4. IR (KBr disc) spectrum of **5**



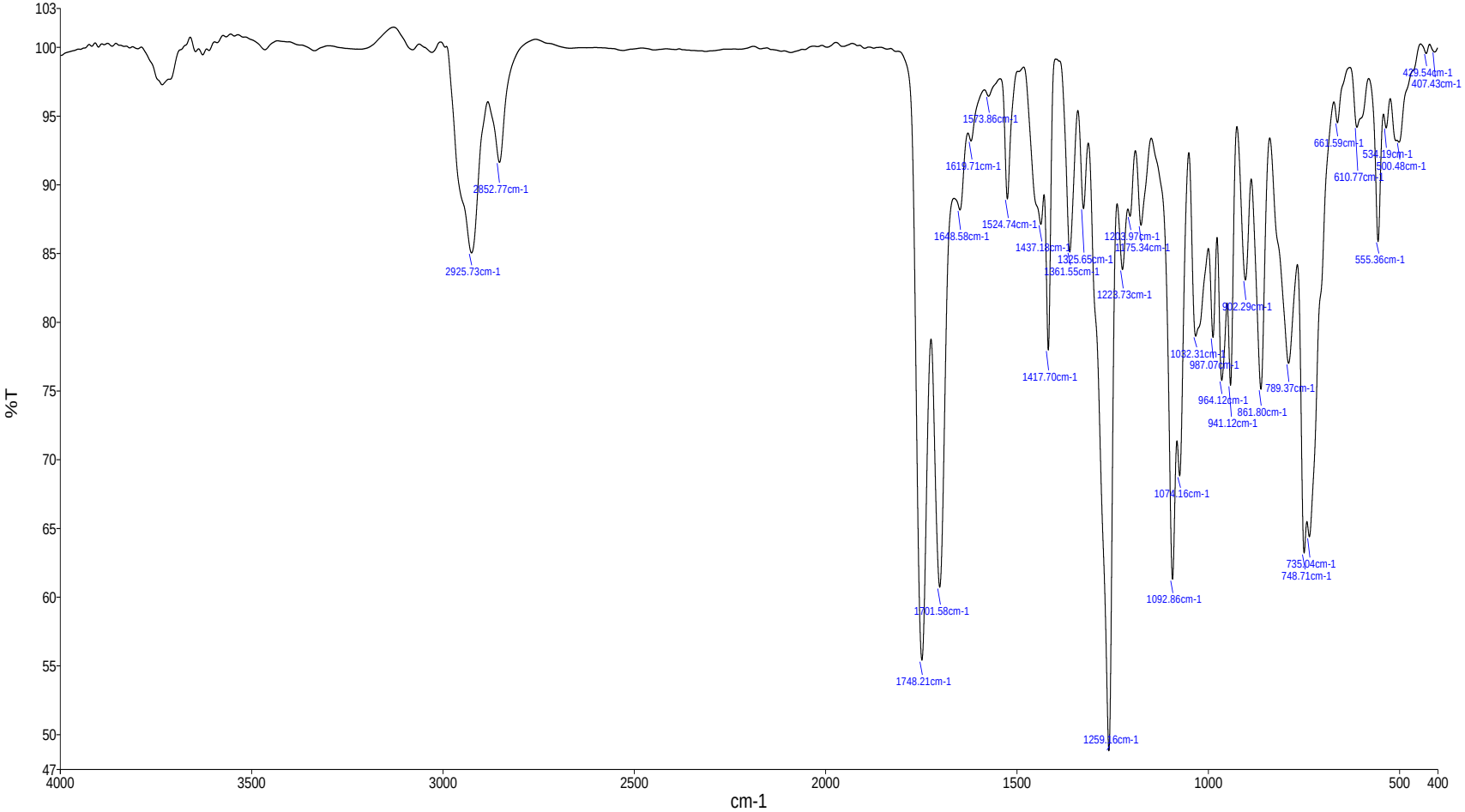
S4.5. IR (KBr disc) spectrum of **6**



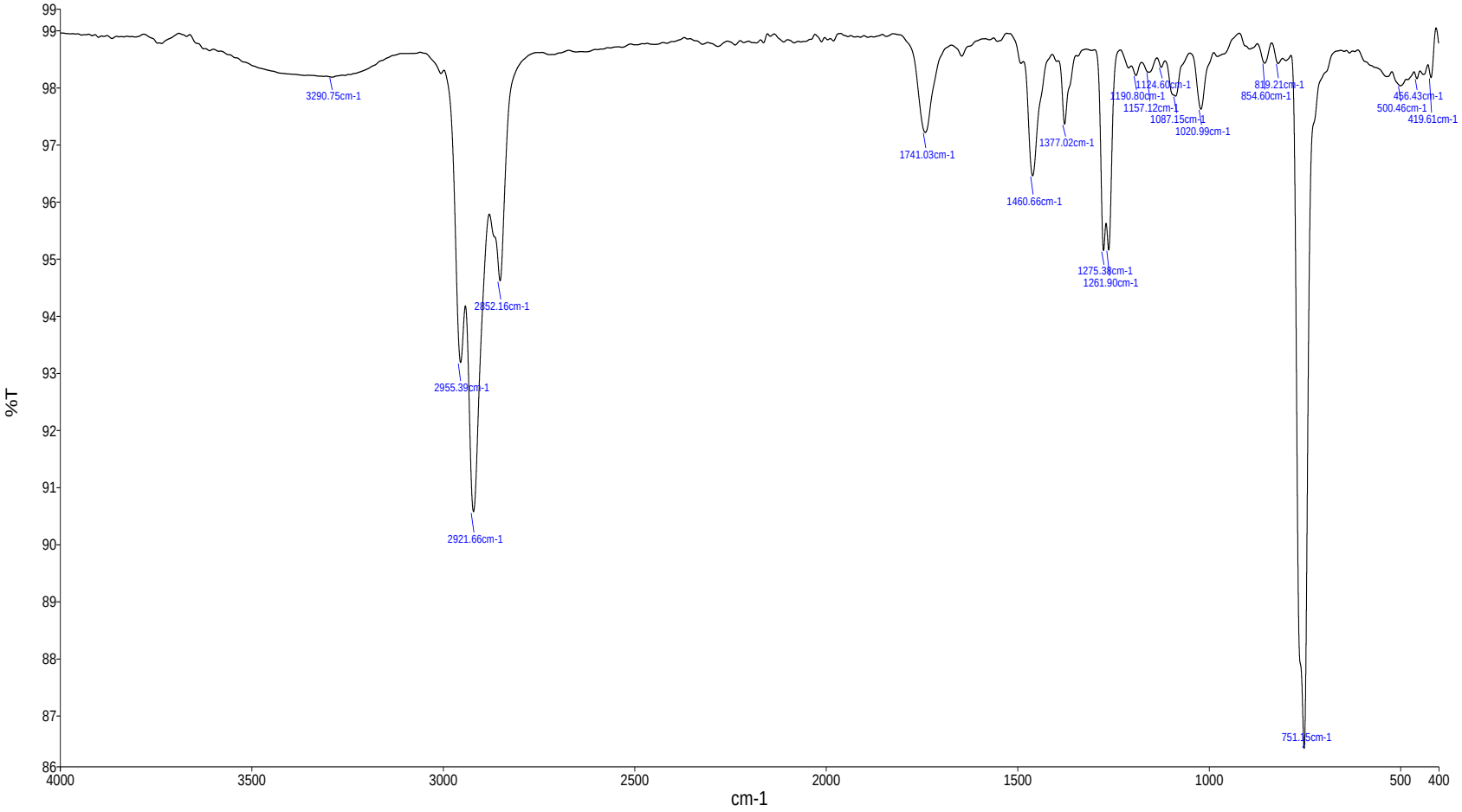
S4.6. IR (KBr disc) spectrum of **7**



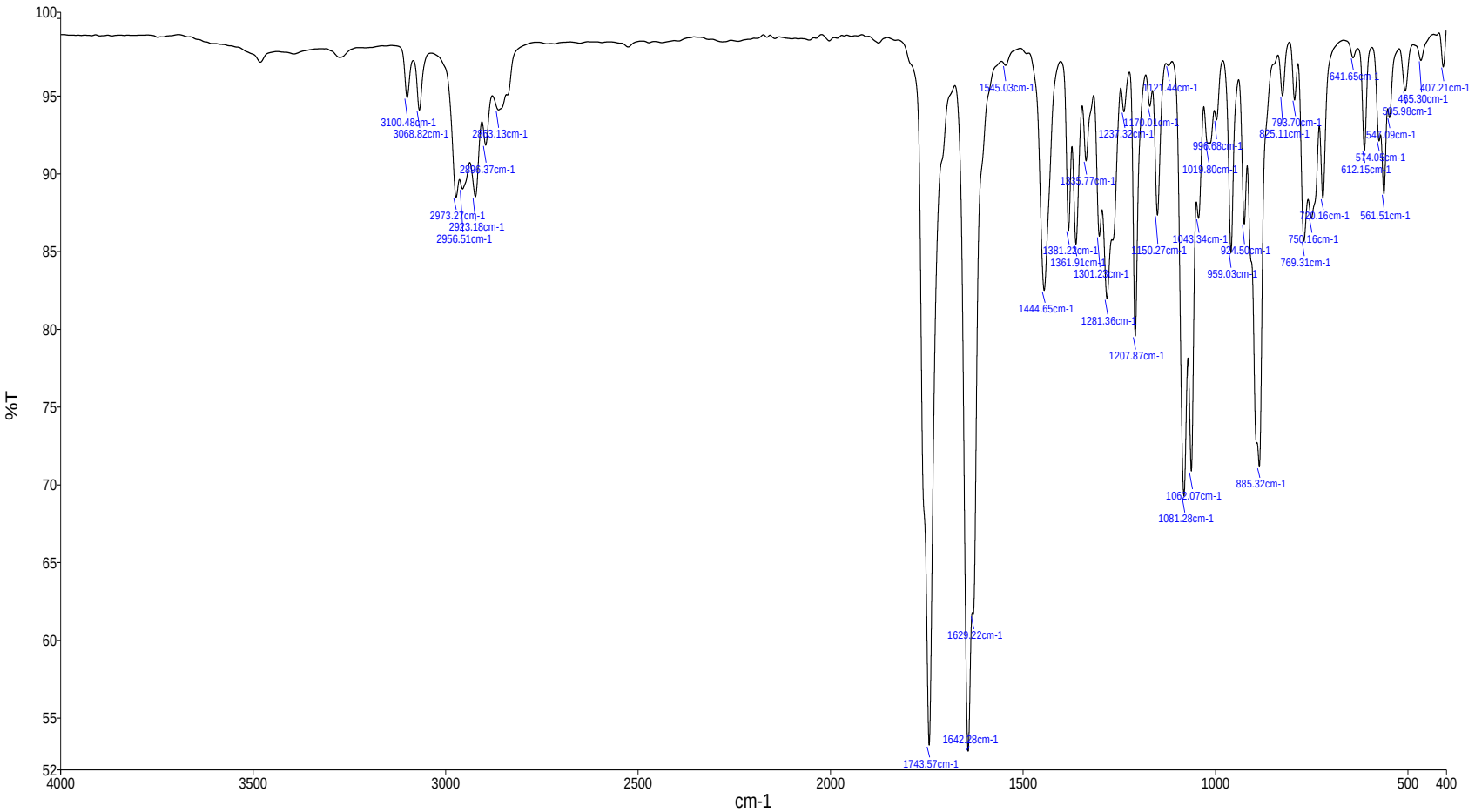
S4.7. IR (KBr disc) spectrum of **8**



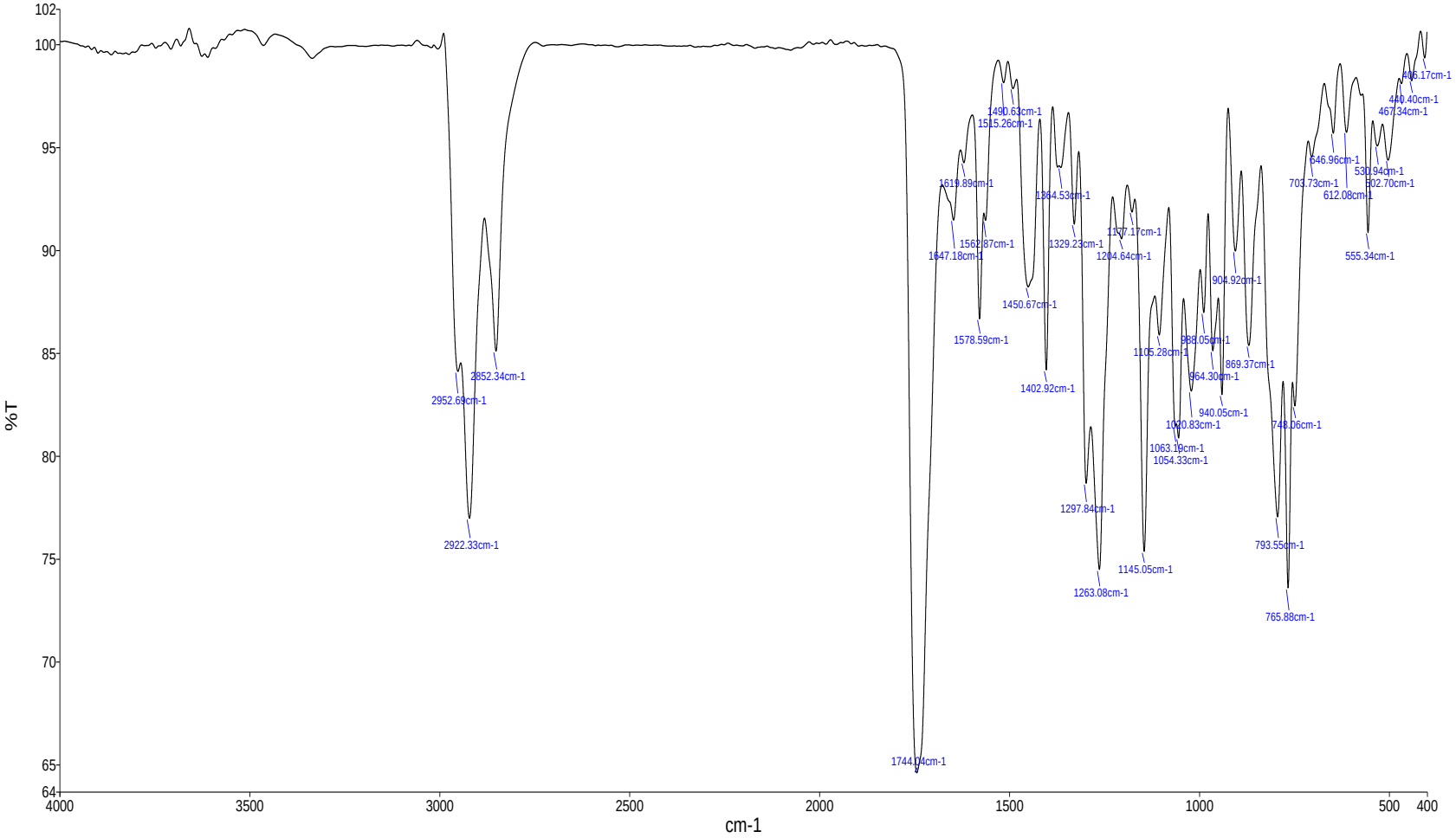
S4.8. IR (KBr disc) spectrum of **9**



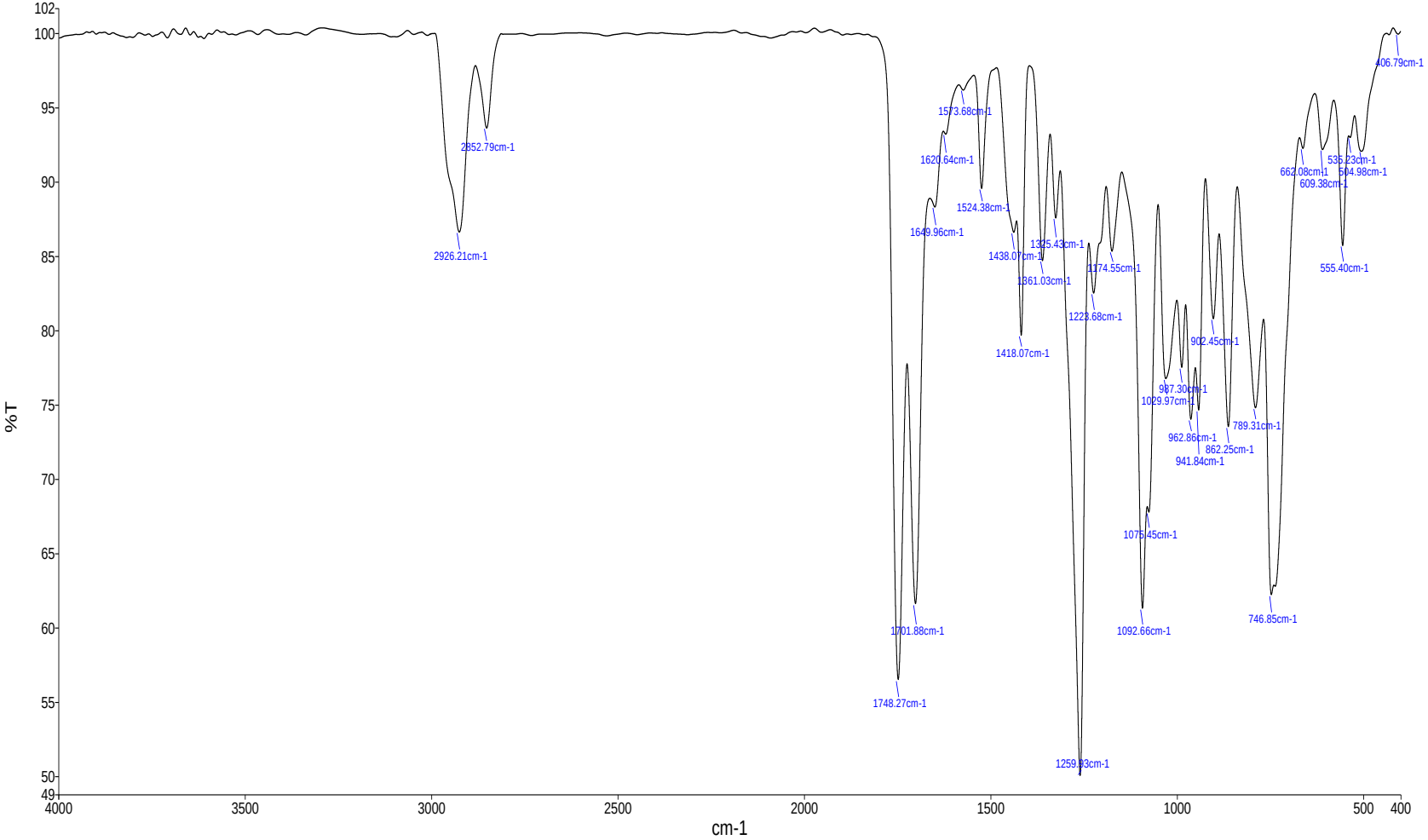
S4.9. IR (KBr disc) spectrum of **11**



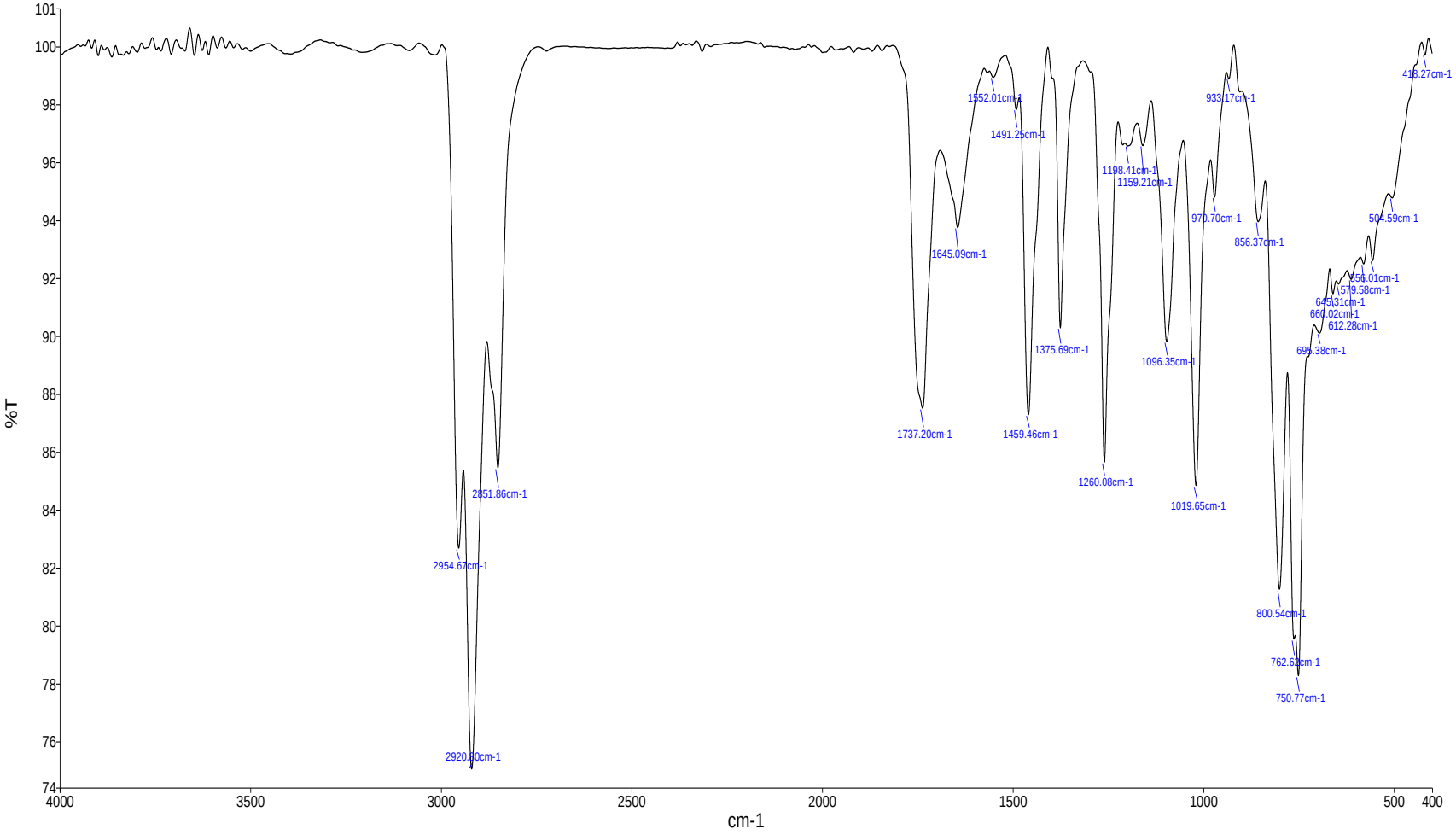
S4.10. IR (KBr disc) spectrum of **12**



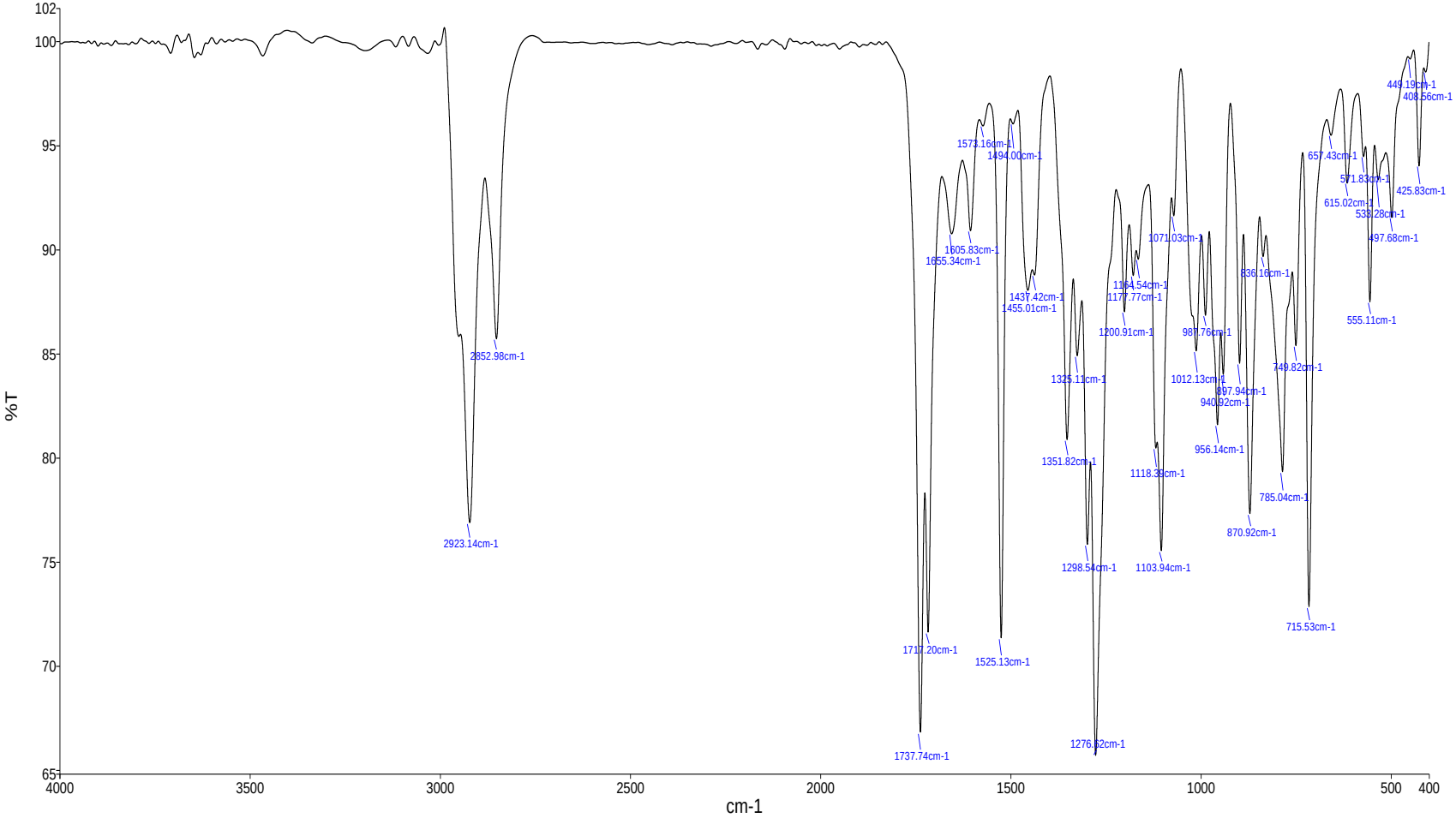
S4.11. IR (KBr disc) spectrum of **13**



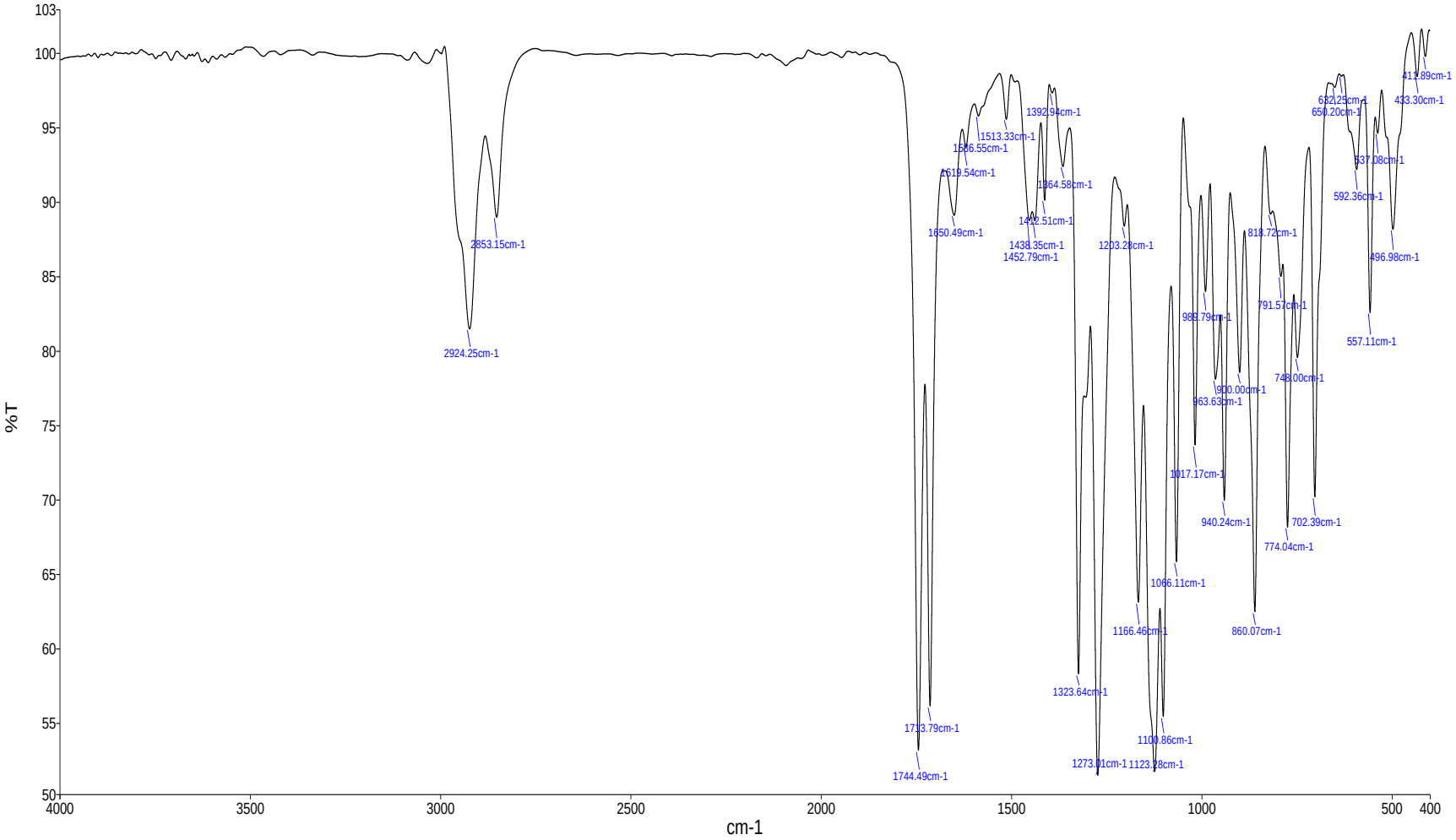
S4.12. IR (KBr disc) spectrum of **14**



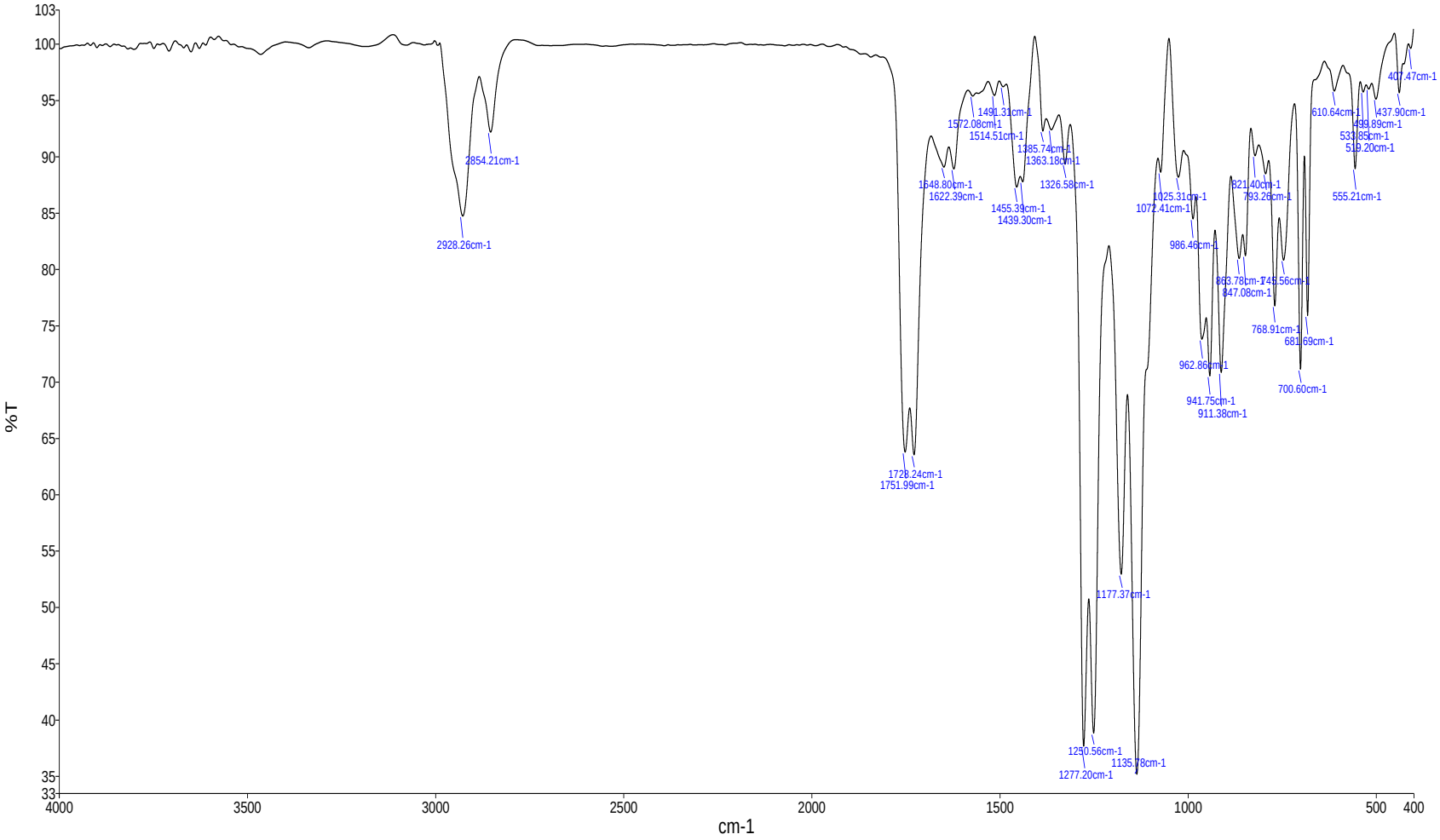
S4.13. IR (KBr disc) spectrum of **15**



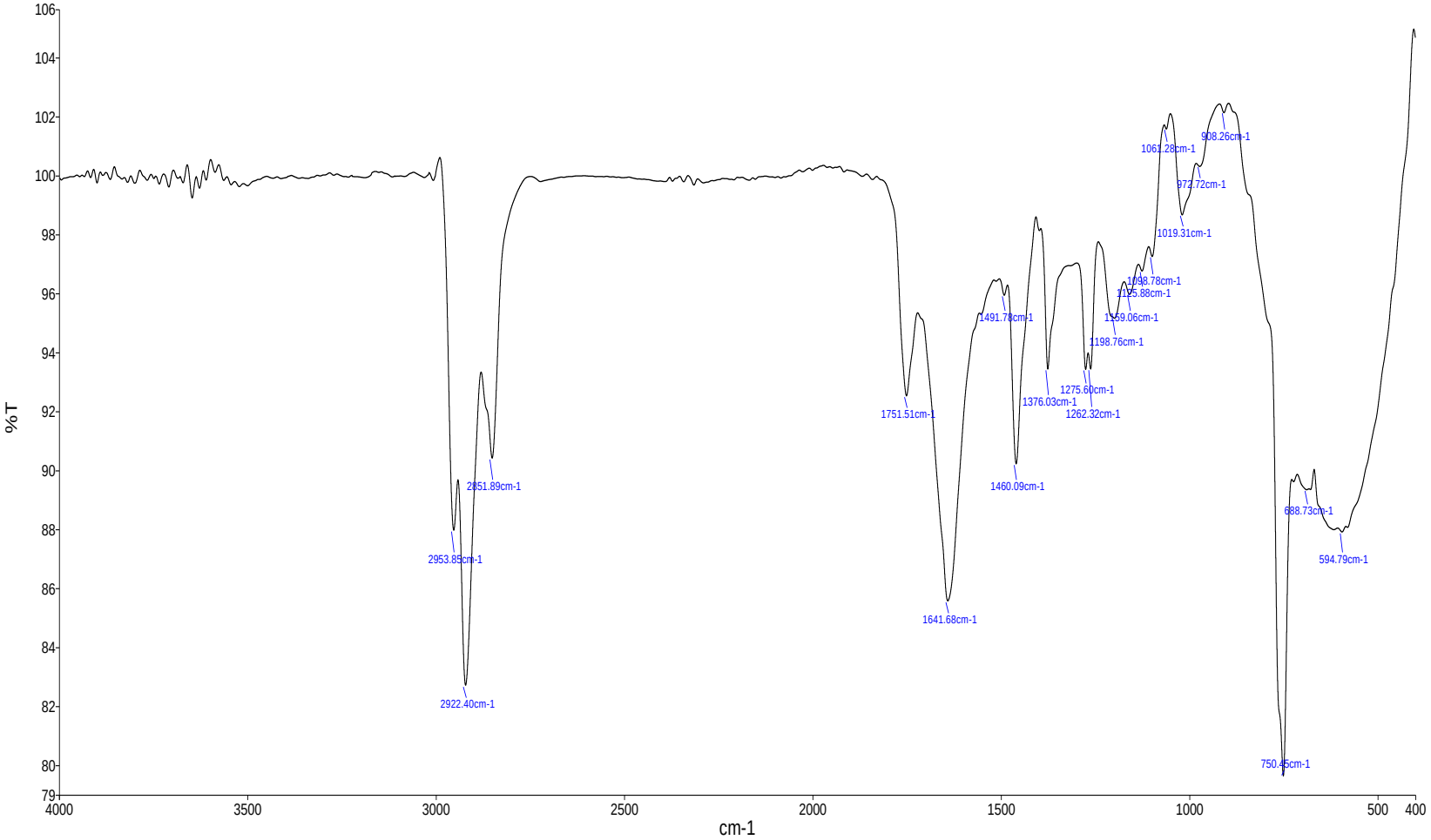
S4.14. IR (KBr disc) spectrum of **16**



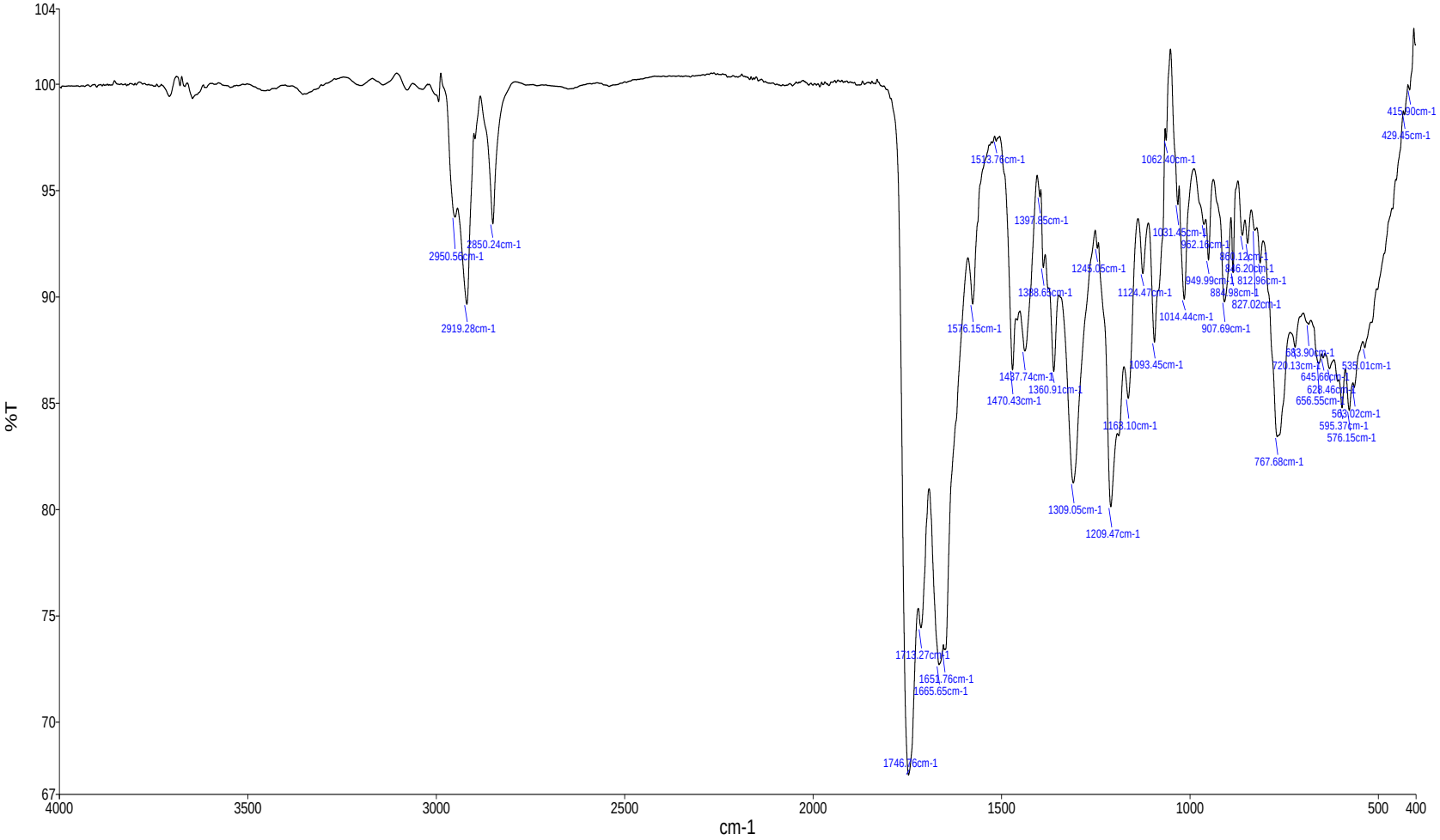
S4.15. IR (KBr disc) spectrum of **17**



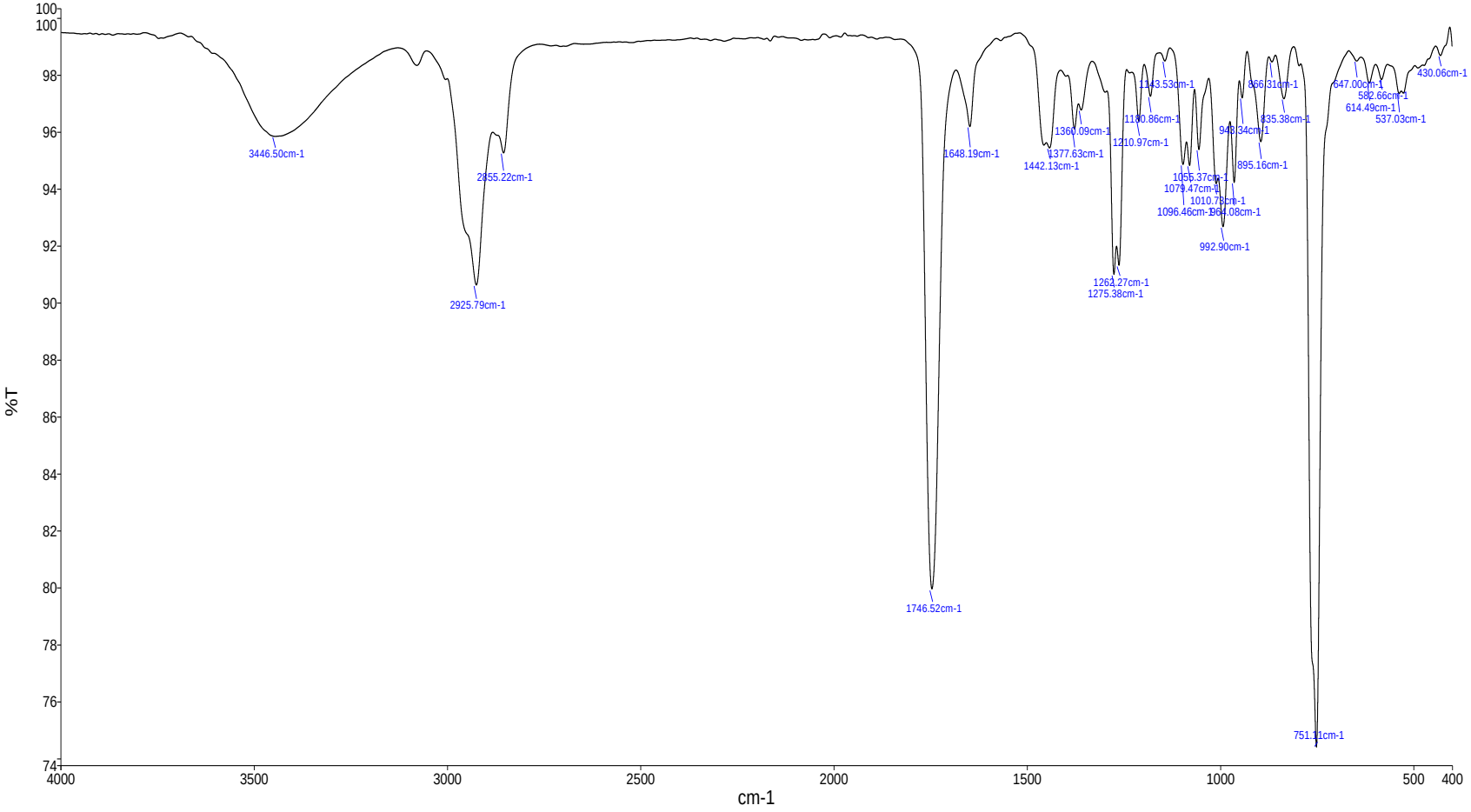
S4.16. IR (KBr disc) spectrum of **18**



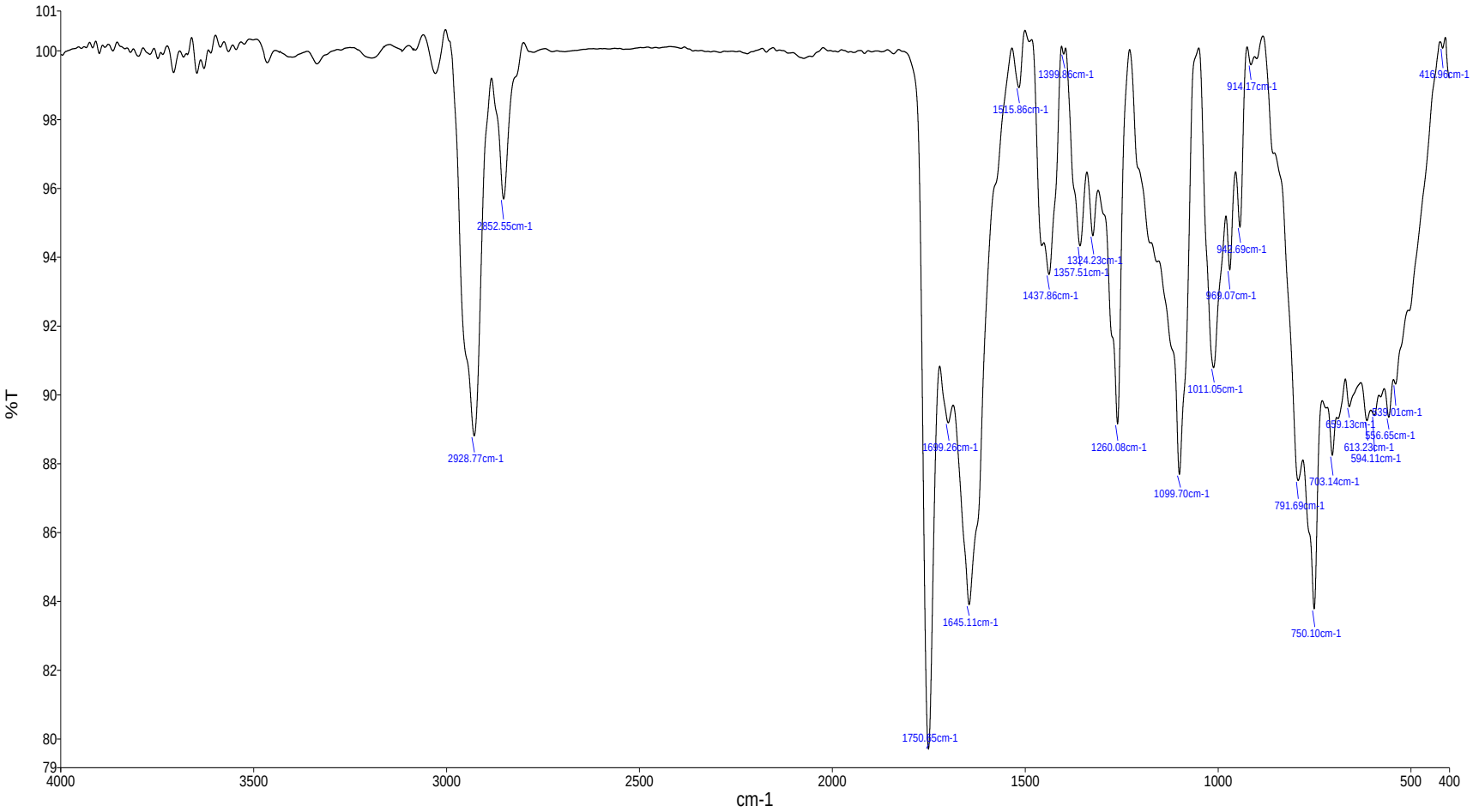
S4.17. IR (KBr disc) spectrum of **19**



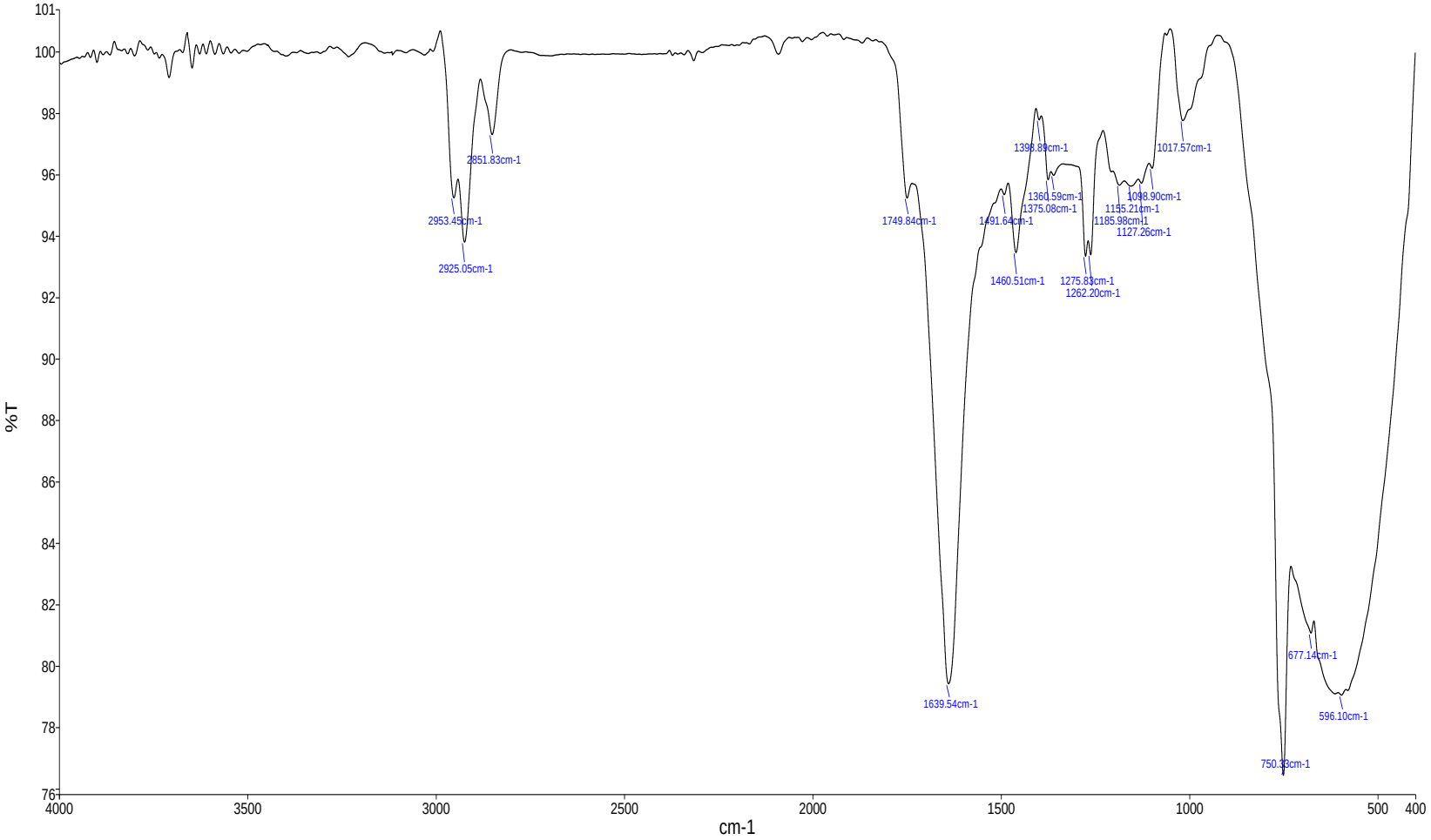
S4.18. IR (KBr disc) spectrum of **20**



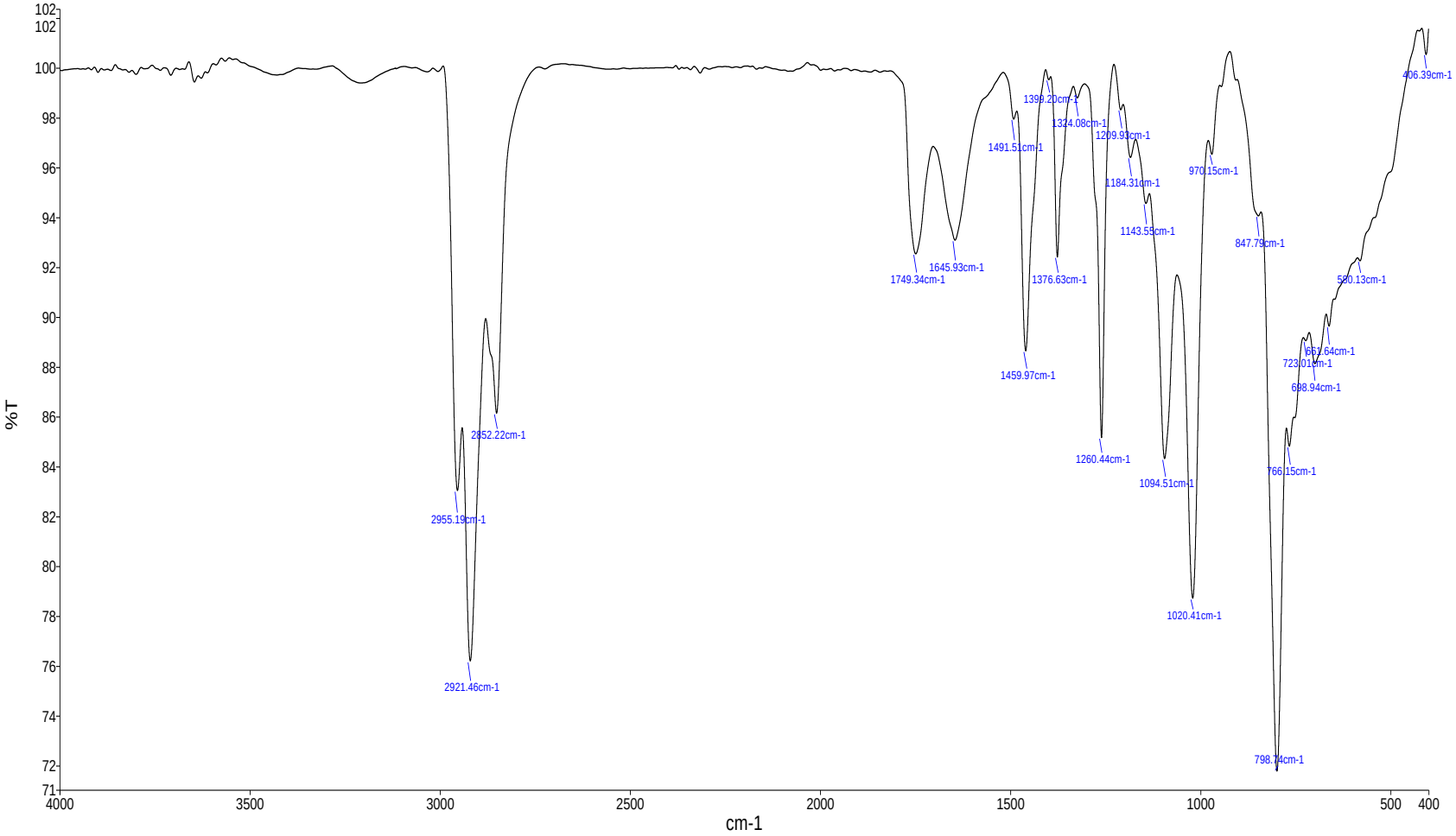
S4.19. IR (KBr disc) spectrum of **21**



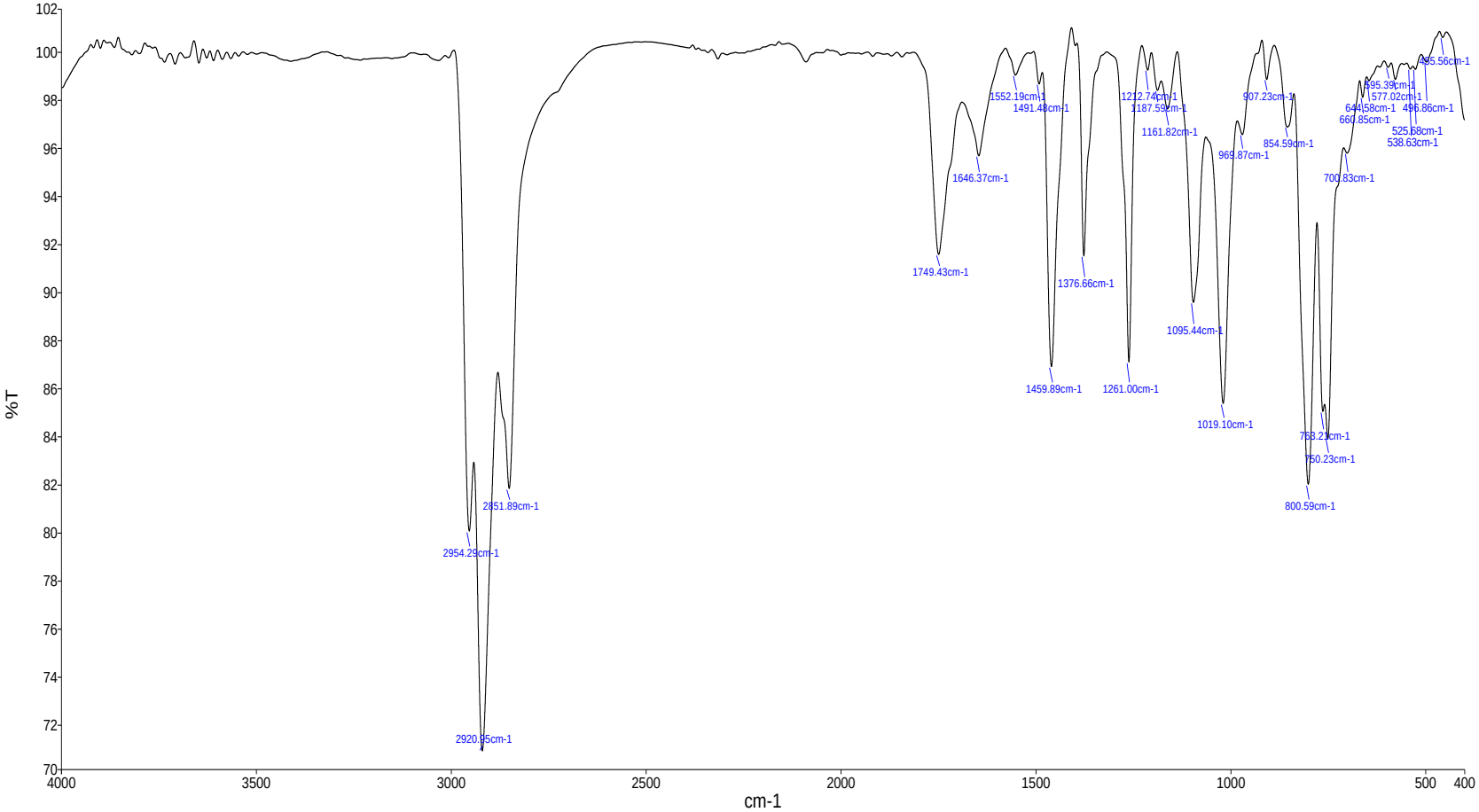
S4.20. IR (KBr disc) spectrum of **22**



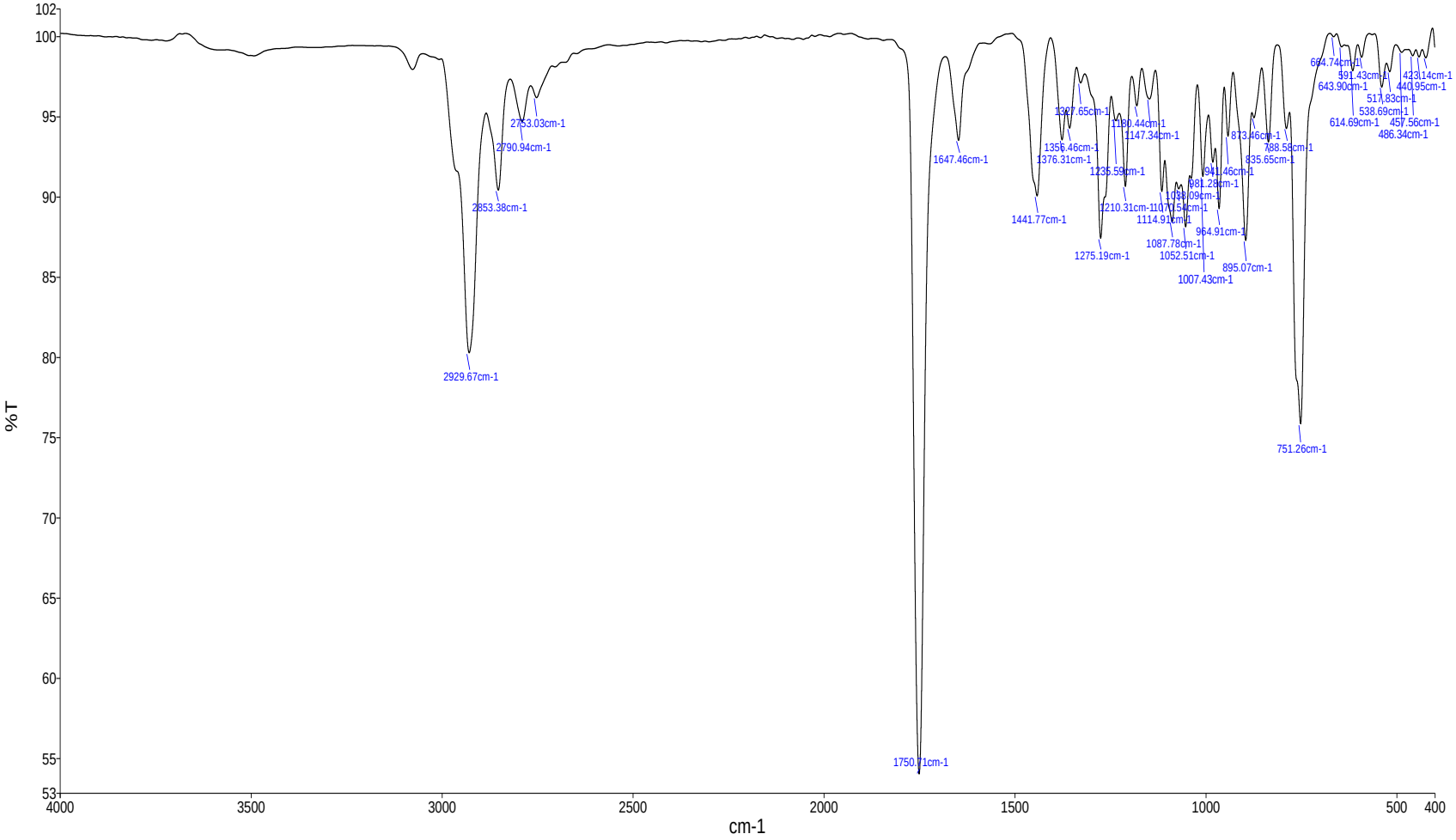
S4.21. IR (KBr disc) spectrum of **23**



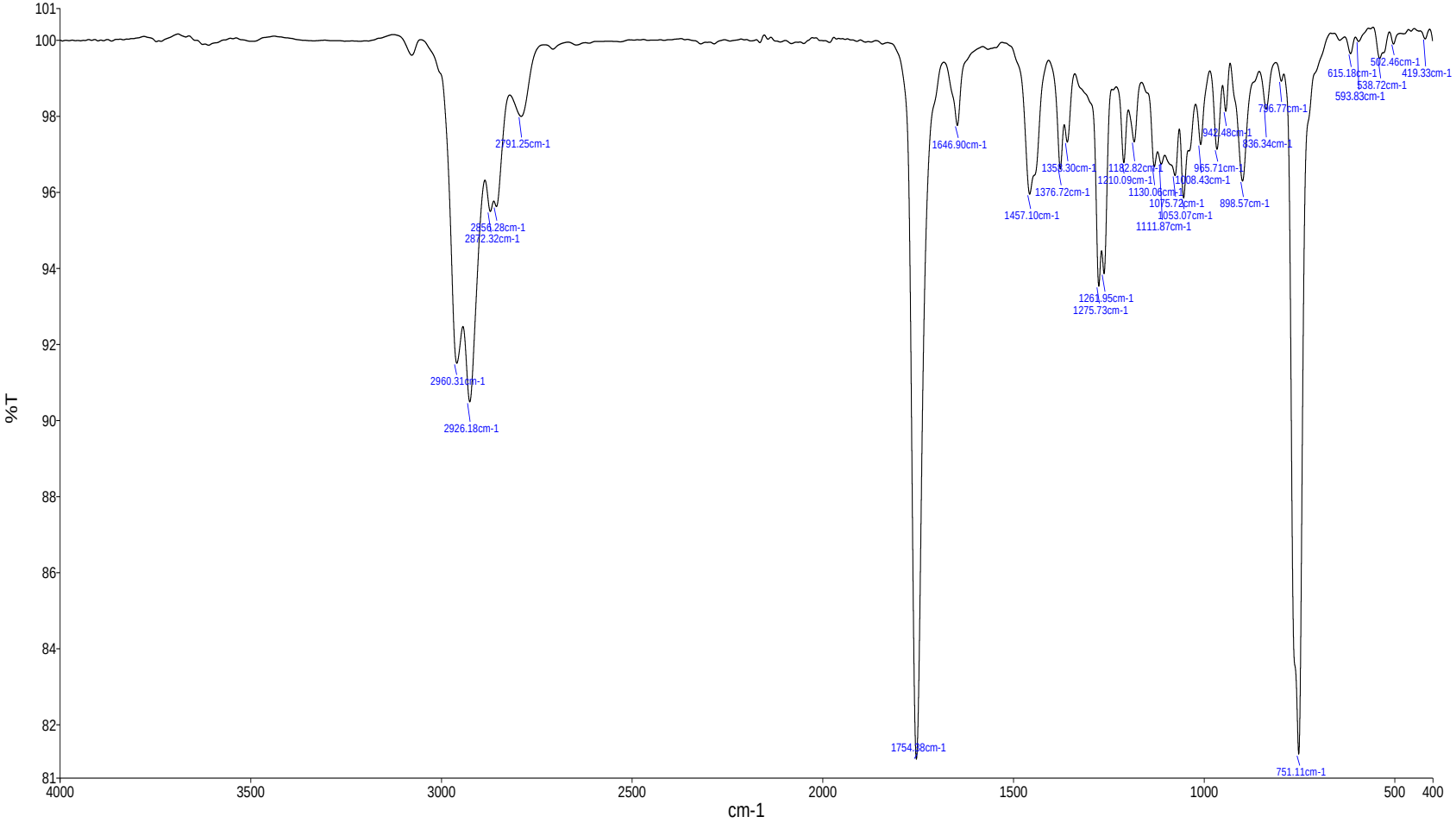
S4.22. IR (KBr disc) spectrum of **24**



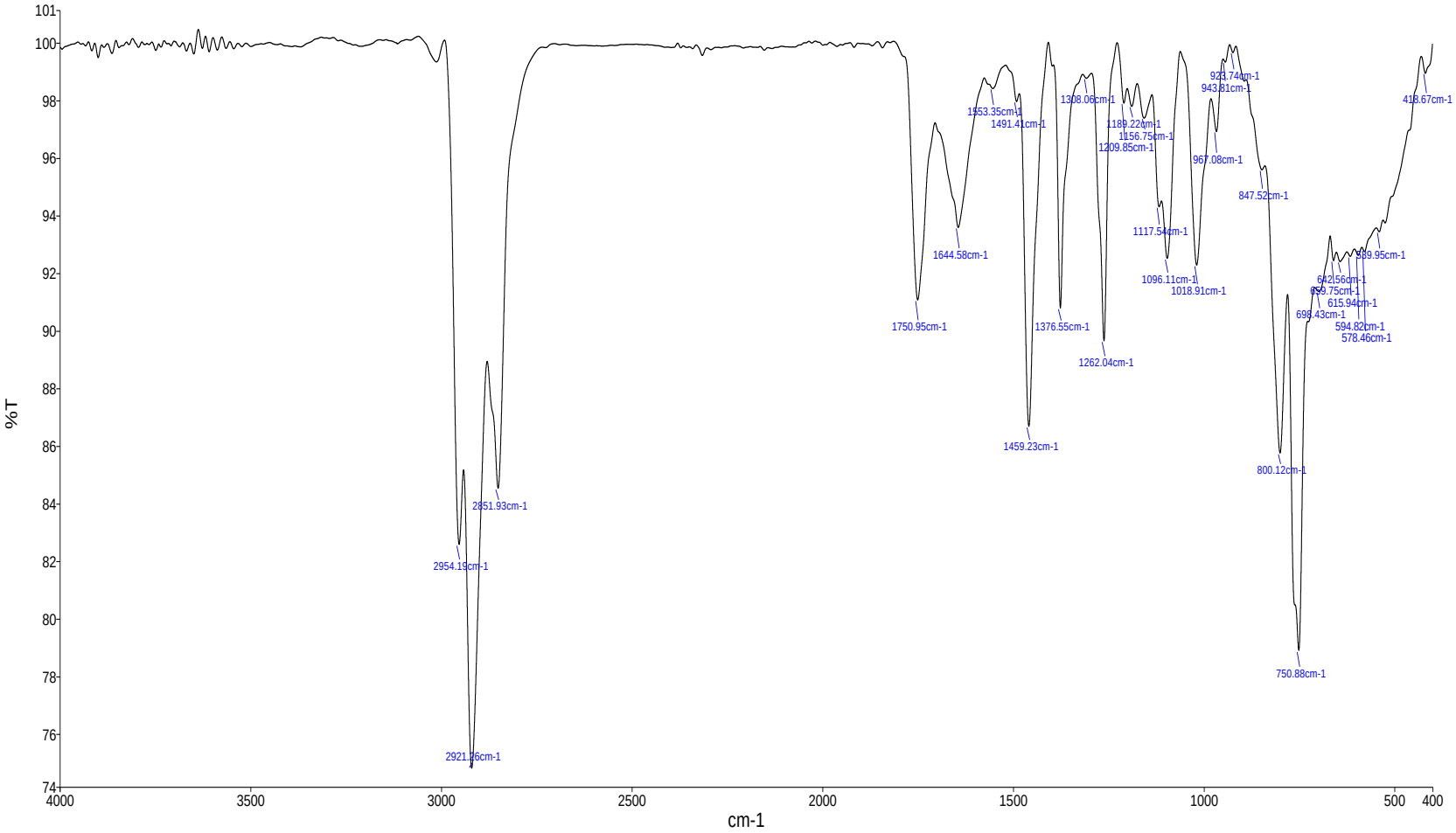
S4.23. IR (KBr disc) spectrum of **25**



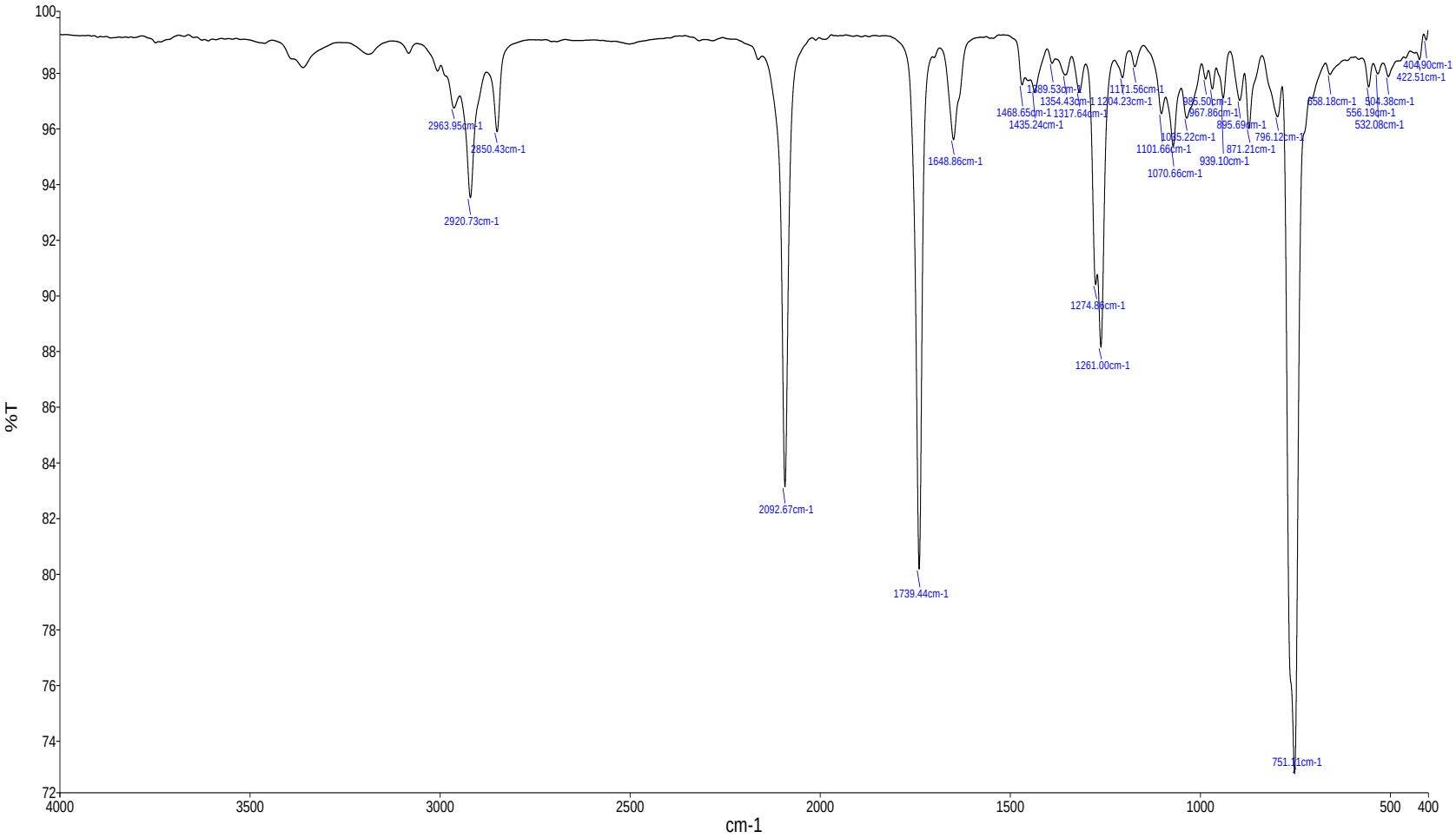
S4.24. IR (KBr disc) spectrum of **26**



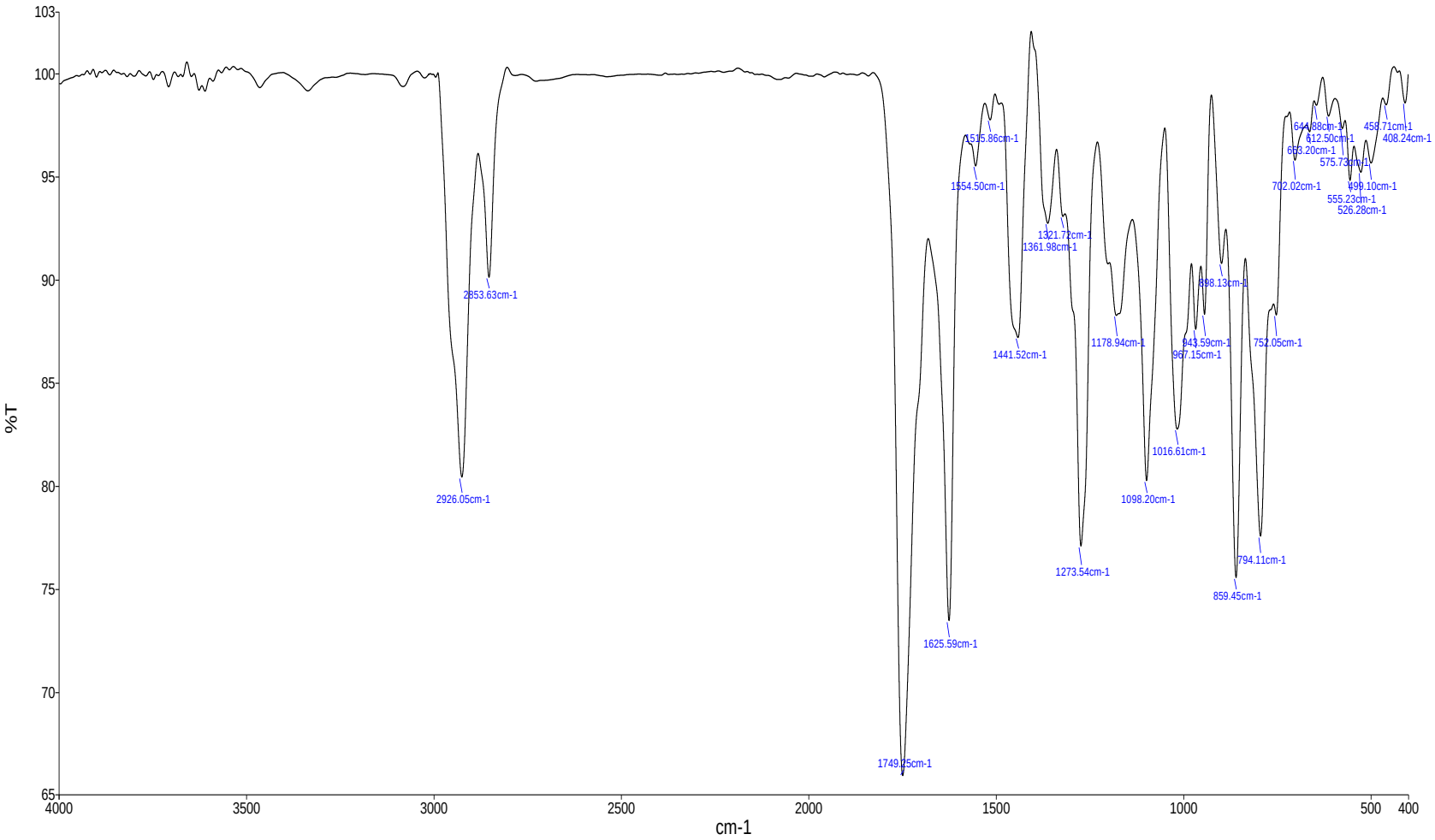
S4.25. IR (KBr disc) spectrum of **27**



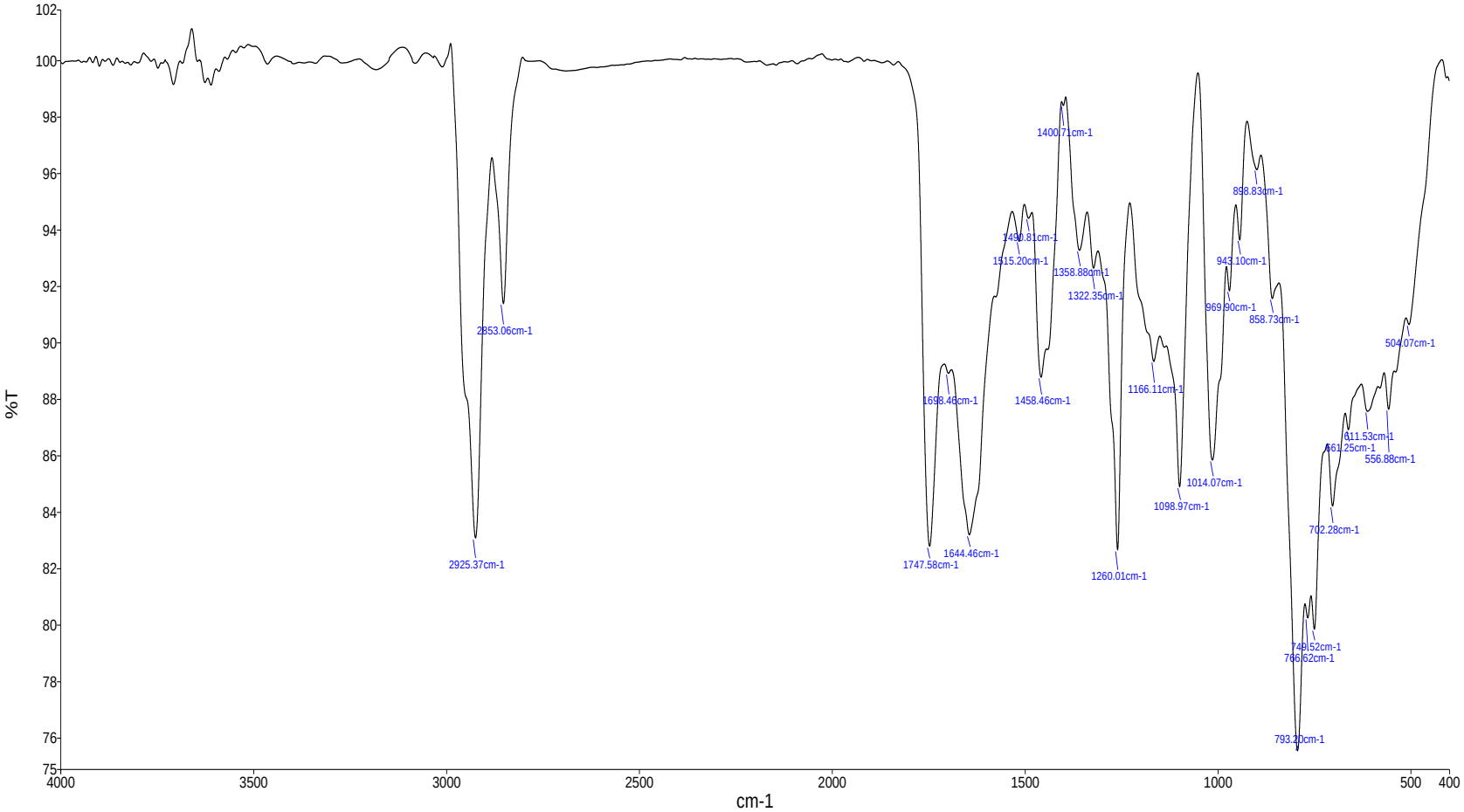
S4.26. IR (KBr disc) spectrum of **28**



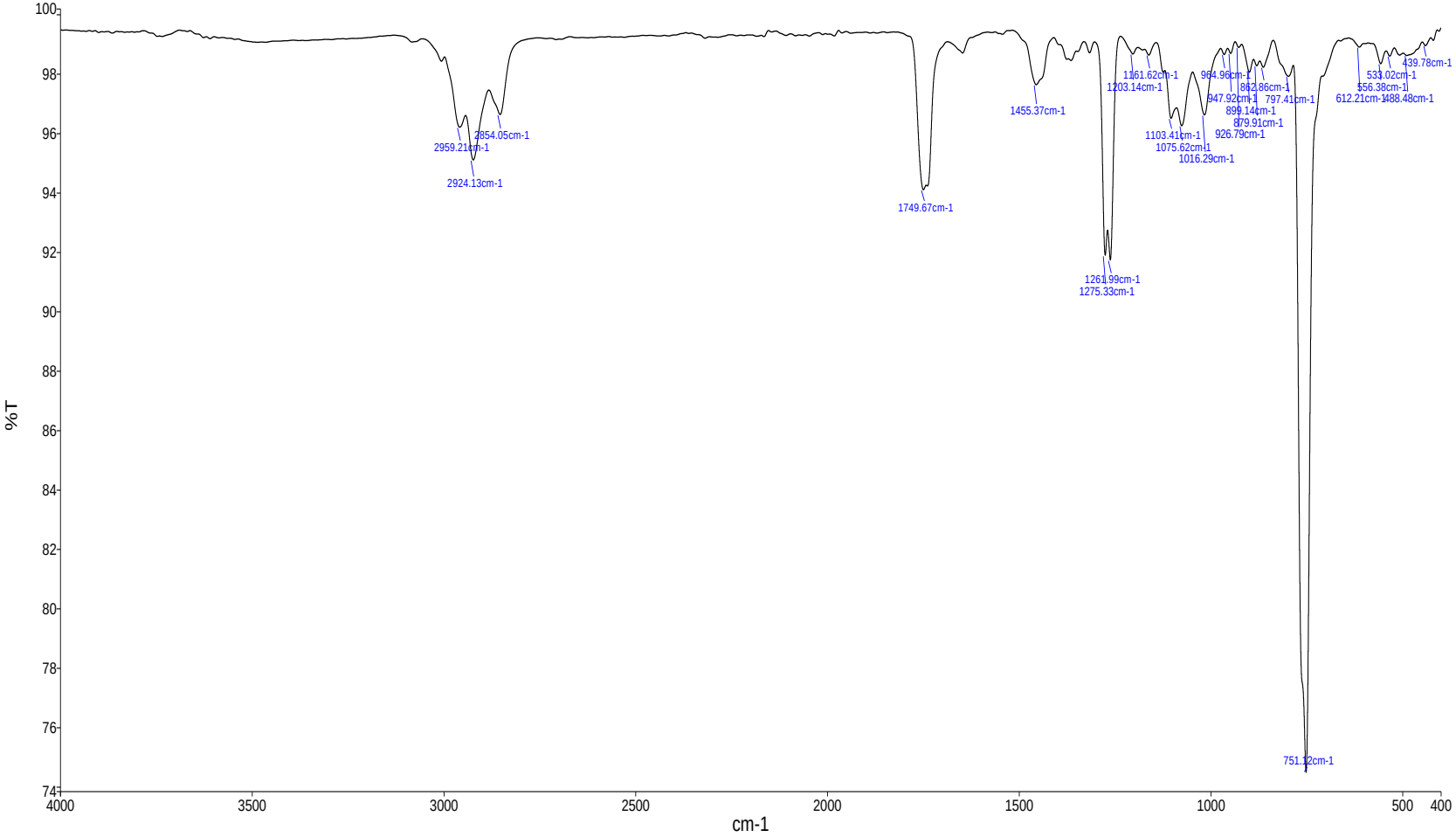
S4.27. IR (KBr disc) spectrum of **29**



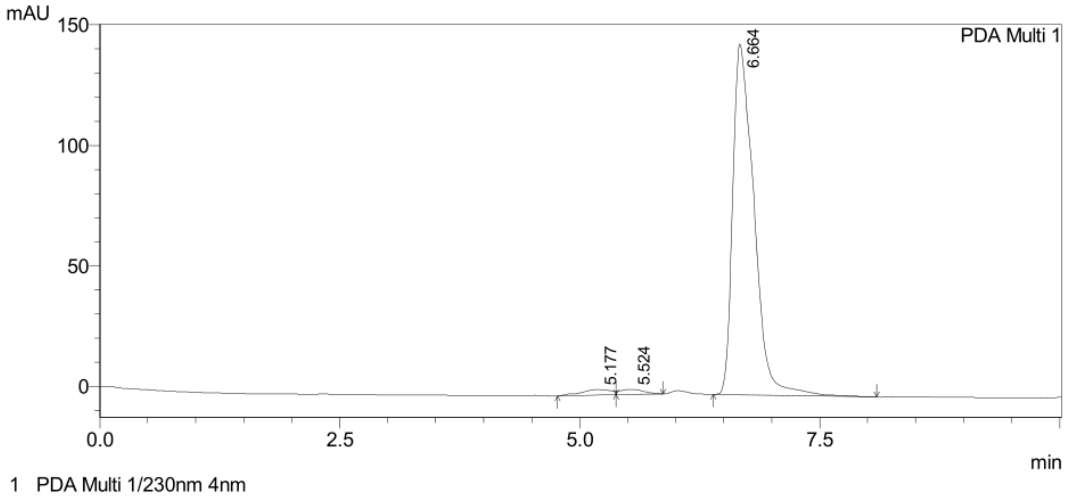
S4.28. IR (KBr disc) spectrum of **30**



S4.29. IR (KBr disc) spectrum of **31**



S5.1**.** HPLC chromatogram of **1**

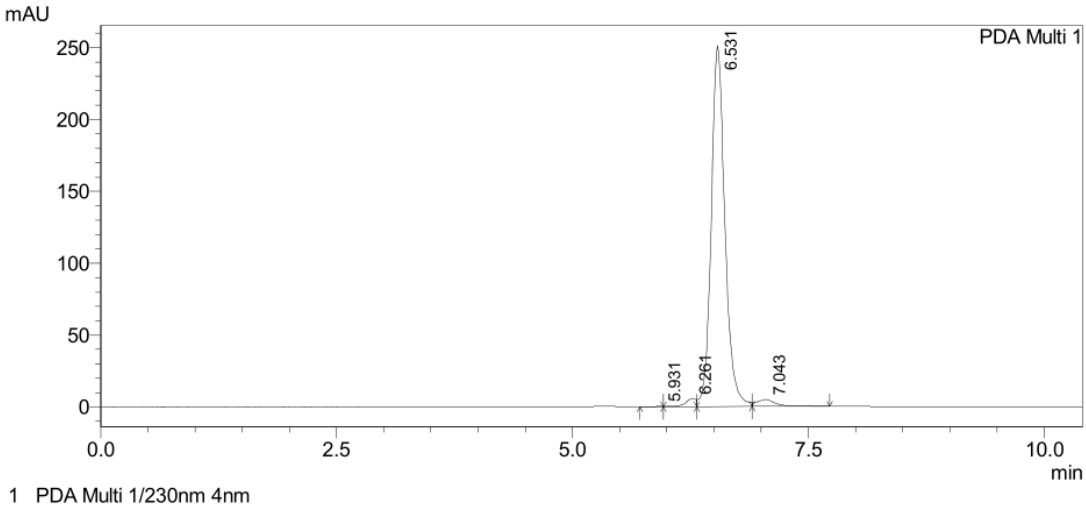


PeakTable

Ch1 230nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.177	49305	2223	2.121	1.482
2	5.524	35998	2128	1.548	1.418
3	6.664	2239796	145660	96.331	97.099
Total		2325099	150012	100.000	100.000

S5.2. HPLC chromatogram of **2**

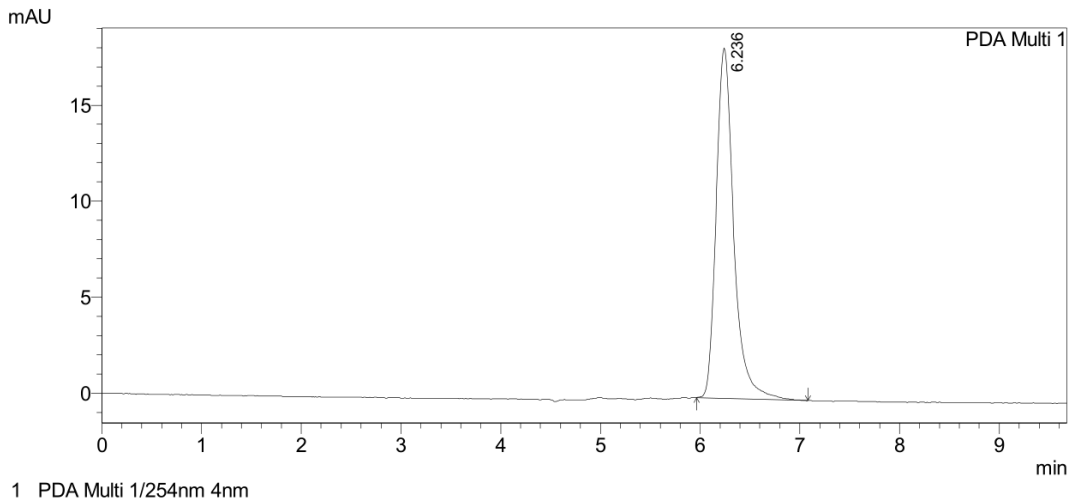


PeakTable

Ch1 230nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.931	1438	204	0.054	0.078
2	6.261	44495	5495	1.679	2.102
3	6.531	2535451	251000	95.659	96.029
4	7.043	69130	4681	2.608	1.791
Total		2650514	261380	100.000	100.000

S5.3. HPLC chromatogram of **3**

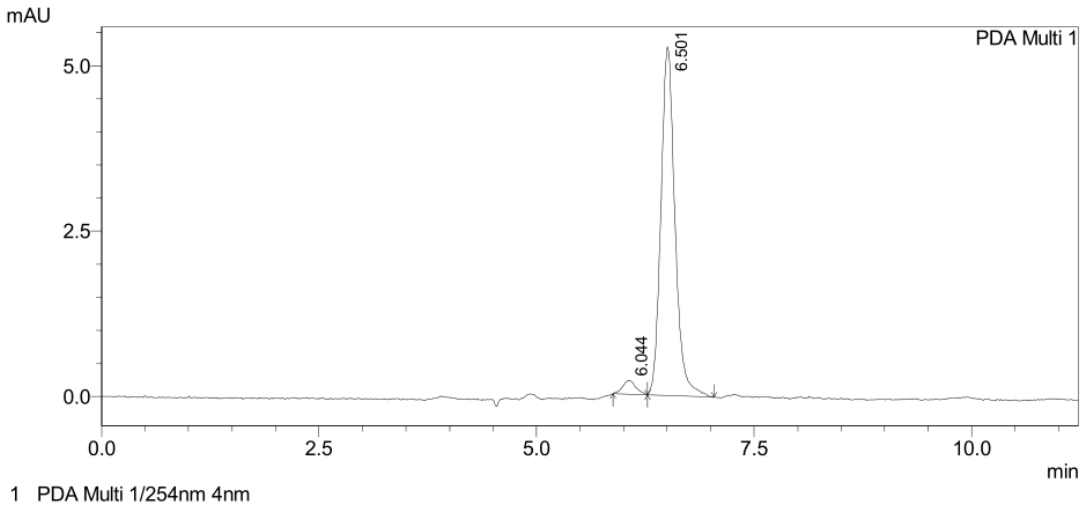


PeakTable

Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.236	223861	18256	100.000	100.000
Total		223861	18256	100.000	100.000

S5.4. HPLC chromatogram of **4**

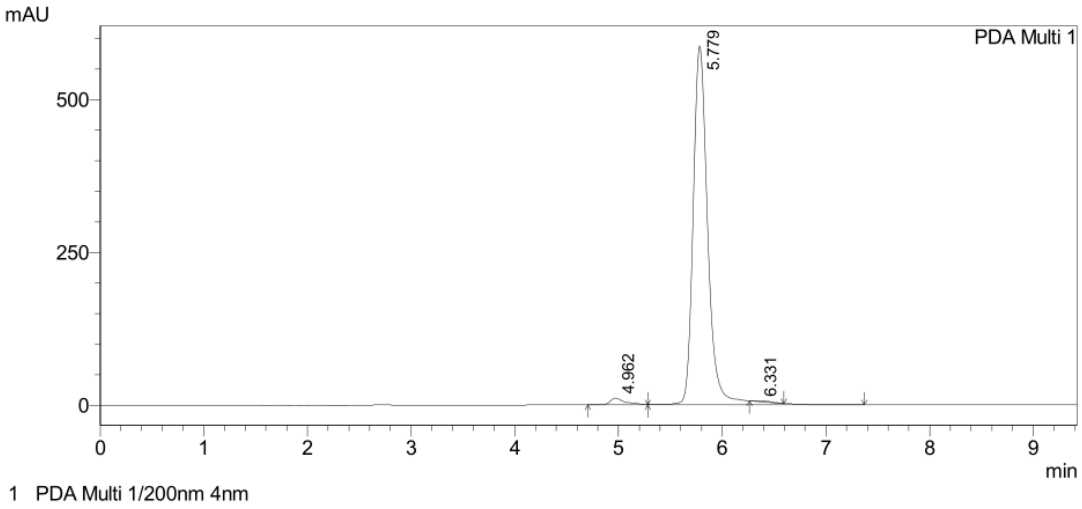


PeakTable

Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.044	2193	206	3.577	3.763
2	6.501	59121	5274	96.423	96.237
Total		61315	5480	100.000	100.000

S5.5. HPLC chromatogram of **5**

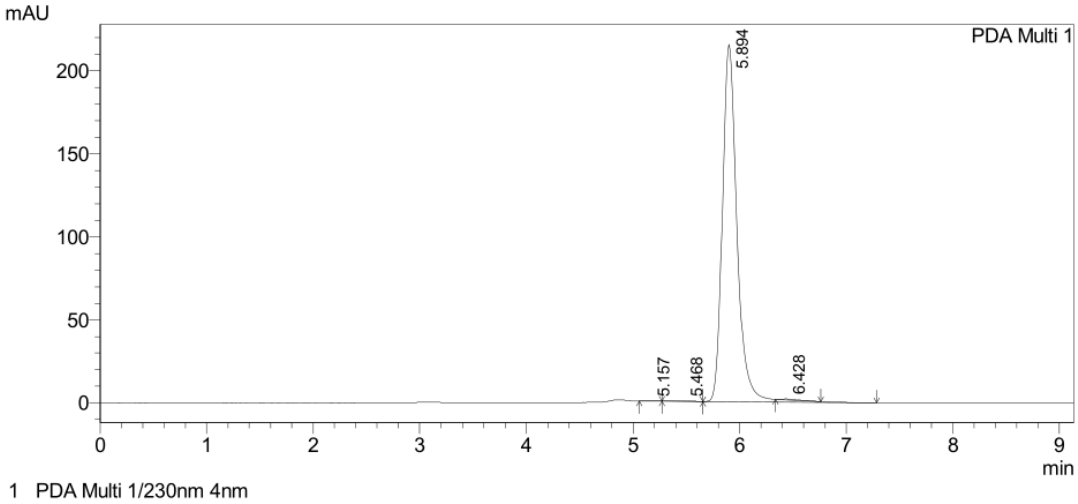


PeakTable

Ch1 200nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.962	113318	10792	1.917	1.801
2	5.779	5783236	587203	97.814	98.008
3	6.331	15905	1140	0.269	0.190
Total		5912459	599135	100.000	100.000

S5.6. HPLC chromatogram of **6**

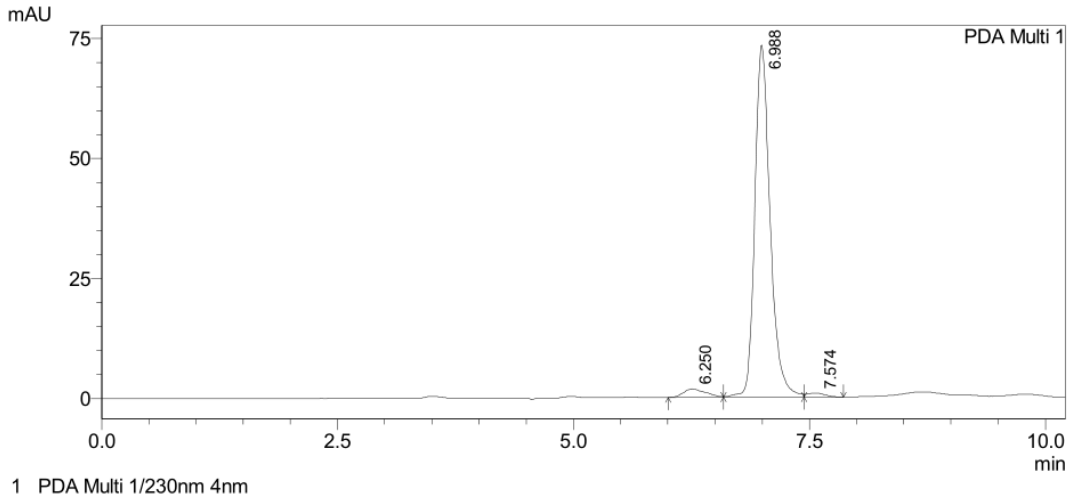


PeakTable

Ch1 230nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.157	1670	222	0.080	0.103
2	5.468	5940	402	0.286	0.186
3	5.894	2062941	215060	99.333	99.485
4	6.428	6251	489	0.301	0.226
Total		2076802	216174	100.000	100.000

S5.7. HPLC chromatogram of **7**

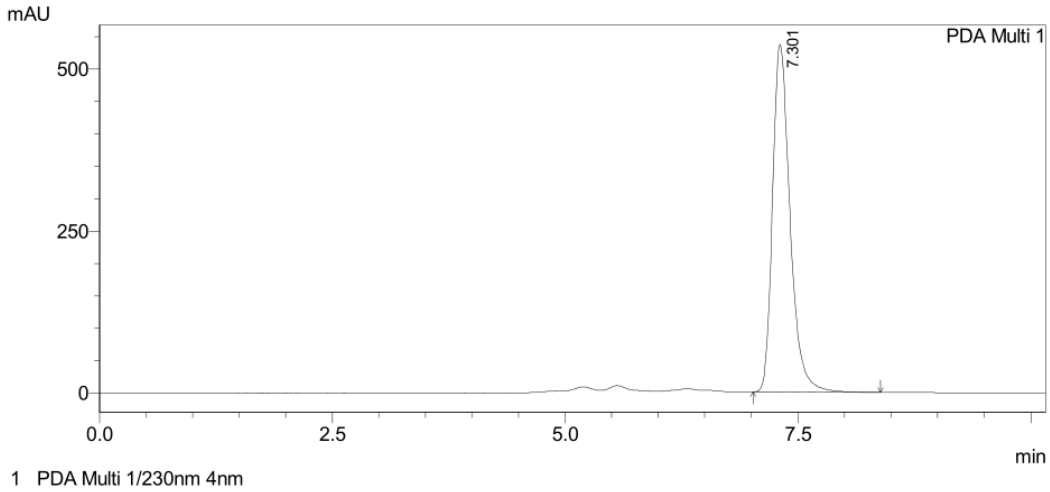


PeakTable

Ch1 230nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.250	28290	1722	3.247	2.270
2	6.988	832119	73328	95.516	96.647
3	7.574	10775	822	1.237	1.083
Total		871184	75873	100.000	100.000

S5.8. HPLC chromatogram of **8**

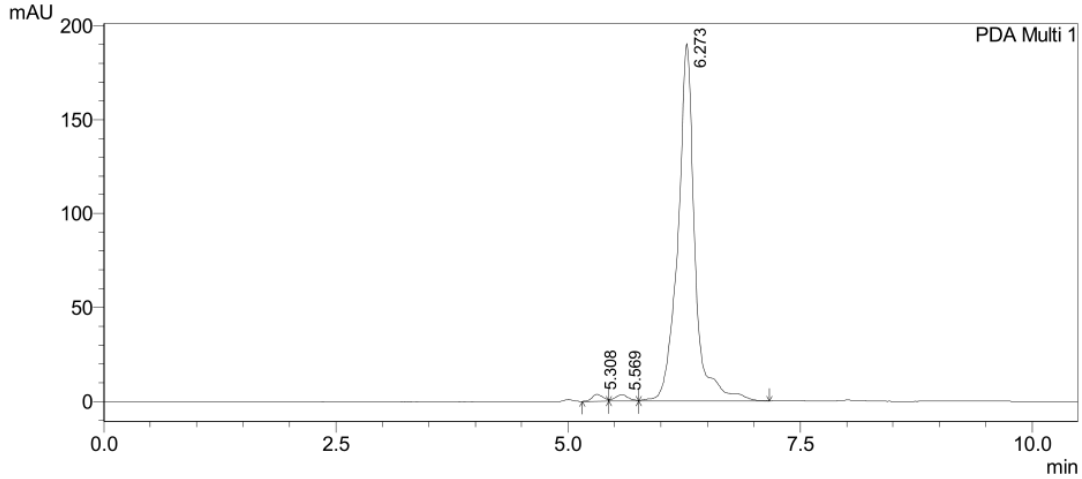


PeakTable

Ch1 230nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	7.301	6994123	535810	100.000	100.000
Total		6994123	535810	100.000	100.000

S5.9. HPLC chromatogram of **9**



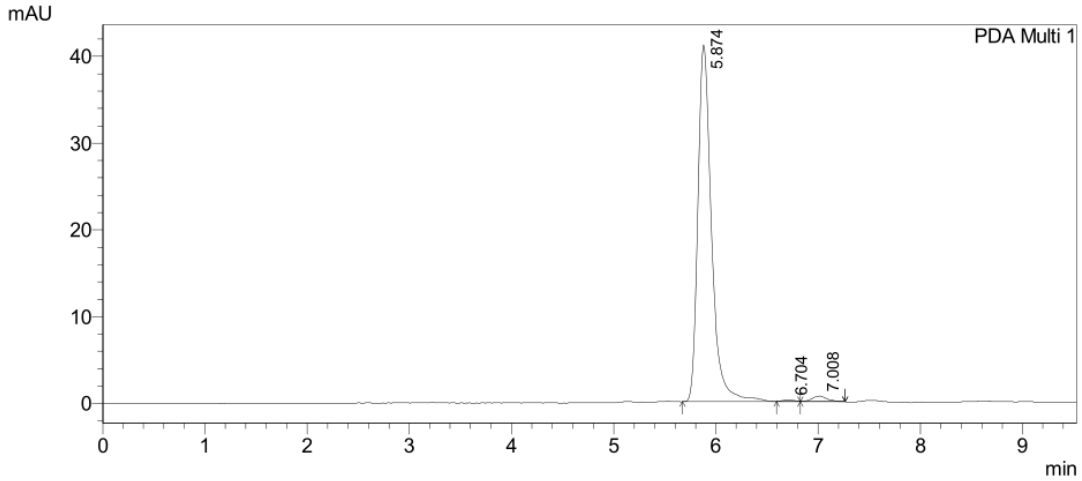
1 PDA Multi 1/200nm 4nm

PeakTable

Ch1 200nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.308	30888	3698	1.242	1.873
2	5.569	35182	3497	1.415	1.771
3	6.273	2420480	190238	97.343	96.356
Total		2486551	197433	100.000	100.000

S5.10. HPLC chromatogram of **10**



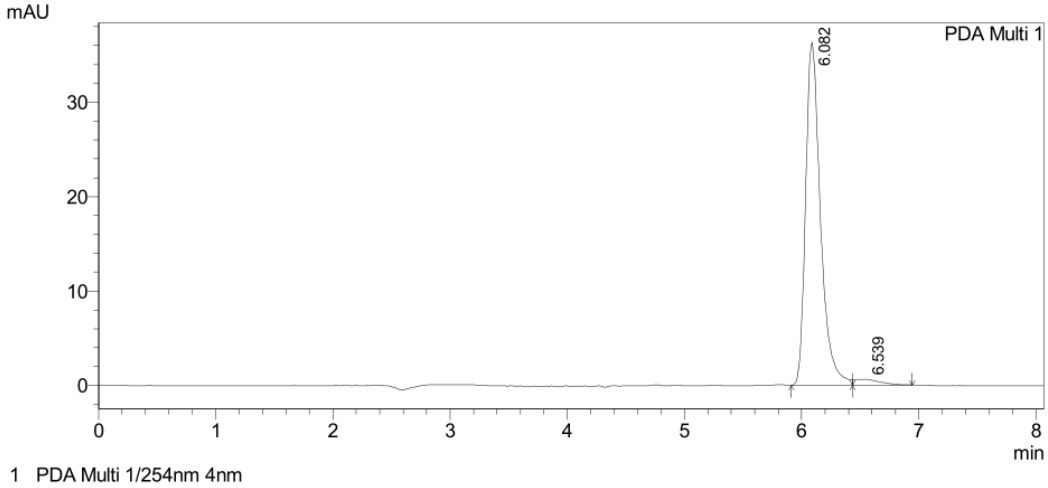
1 PDA Multi 1/230nm 4nm

PeakTable

Ch1 230nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.874	381196	41161	97.879	98.092
2	6.704	1448	182	0.372	0.433
3	7.008	6812	619	1.749	1.475
Total		389456	41962	100.000	100.000

S5.11. HPLC chromatogram of **11**

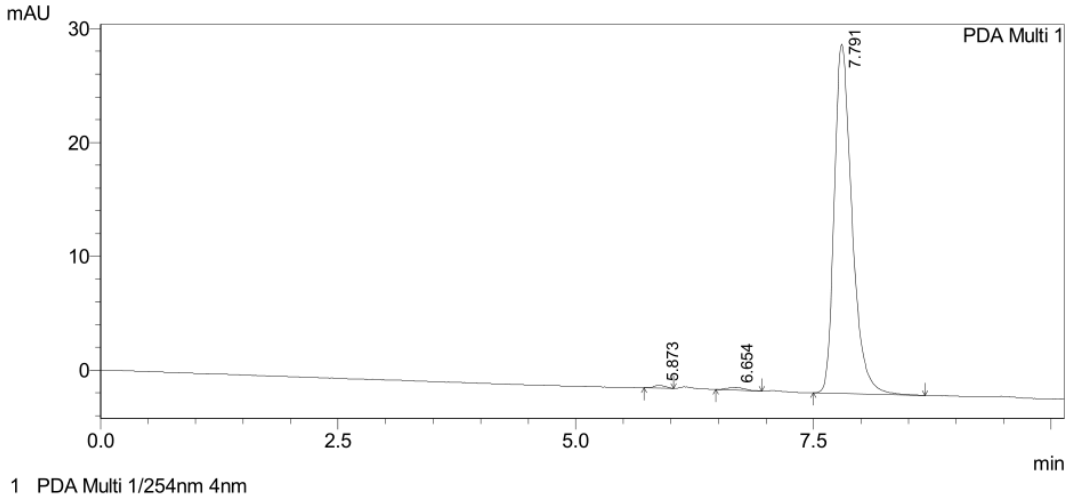


PeakTable

Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.082	319429	36323	96.800	98.237
2	6.539	10559	652	3.200	1.763
Total		329988	36975	100.000	100.000

S5.12. HPLC chromatogram of **12**

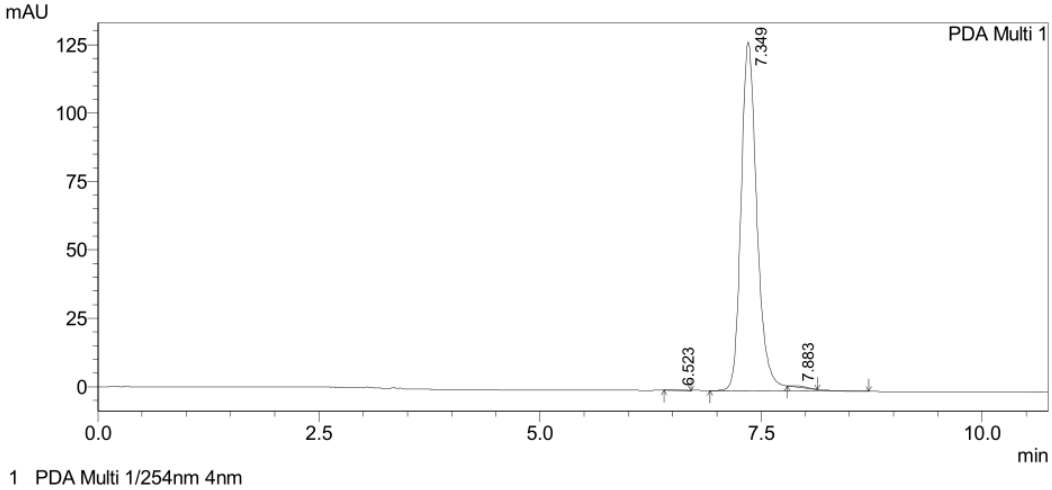


PeakTable

Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.873	1939	239	0.482	0.768
2	6.654	3103	234	0.770	0.752
3	7.791	397730	30676	98.748	98.480
Total		402772	31149	100.000	100.000

S5.13. HPLC chromatogram of **13**

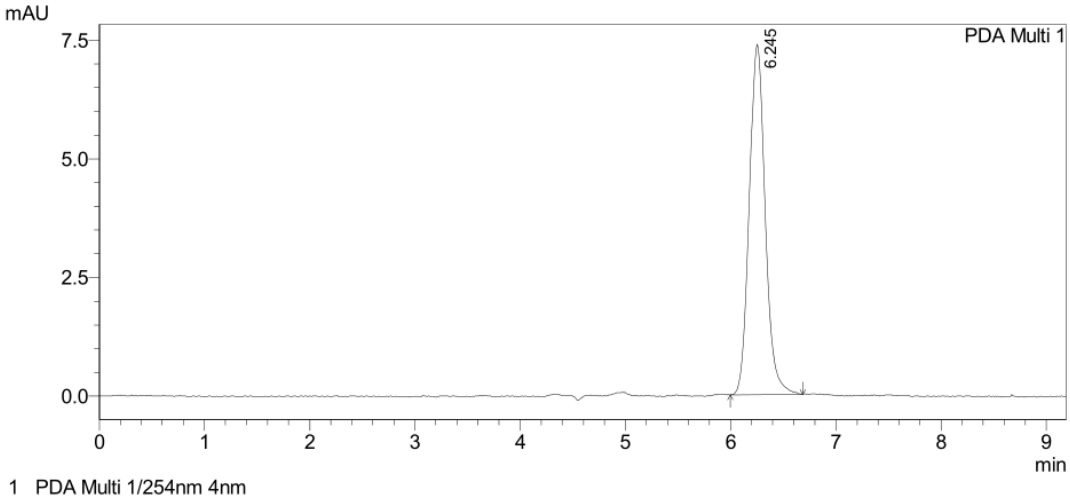


PeakTable

Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.523	1130	141	0.069	0.110
2	7.349	1627630	127370	99.663	99.586
3	7.883	4366	388	0.267	0.304
Total		1633126	127899	100.000	100.000

S5.14. HPLC chromatogram of **14**

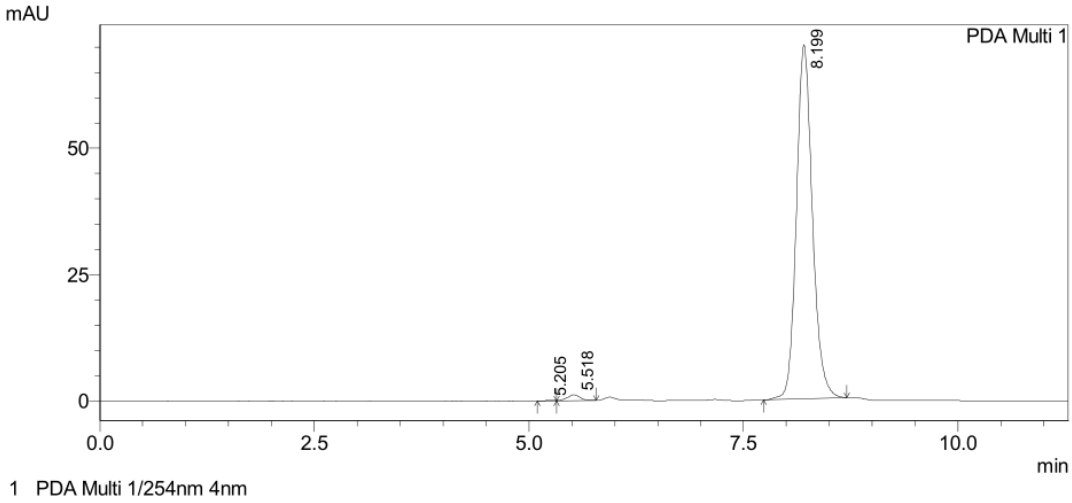


PeakTable

Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.245	77573	7378	100.000	100.000
Total		77573	7378	100.000	100.000

S5.15. HPLC chromatogram of **15**

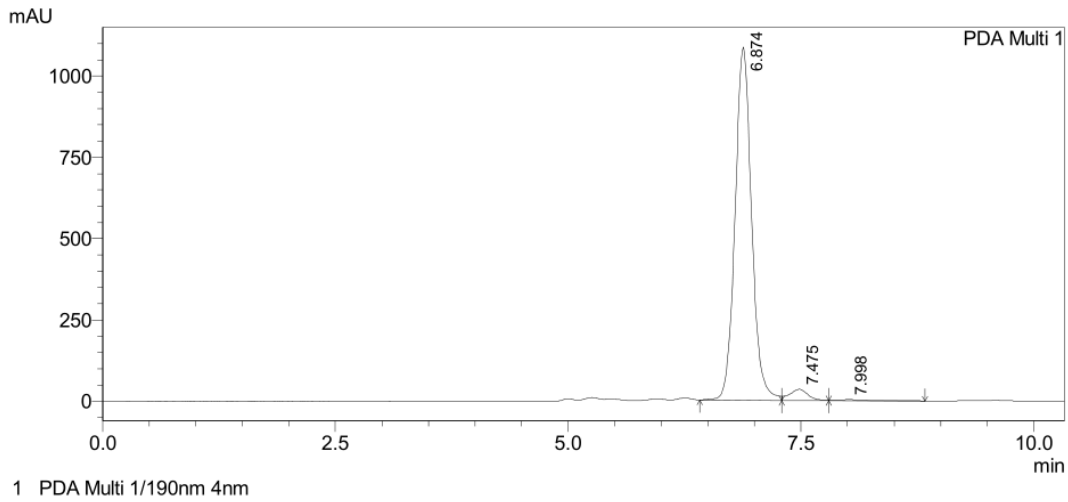


PeakTable

Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.205	809	91	0.088	0.128
2	5.518	12126	1183	1.315	1.659
3	8.199	909071	70061	98.597	98.213
Total		922006	71336	100.000	100.000

S5.16. HPLC chromatogram of **16**

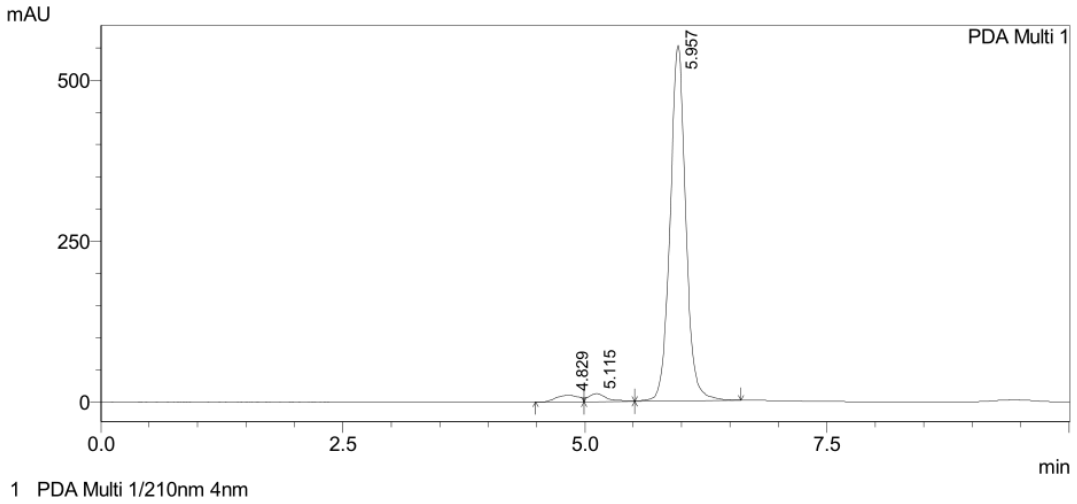


PeakTable

Ch1 190nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.874	13394627	1085114	96.249	96.719
2	7.475	460501	34039	3.309	3.034
3	7.998	61513	2774	0.442	0.247
Total		13916642	1121927	100.000	100.000

S5.17. HPLC chromatogram of **17**

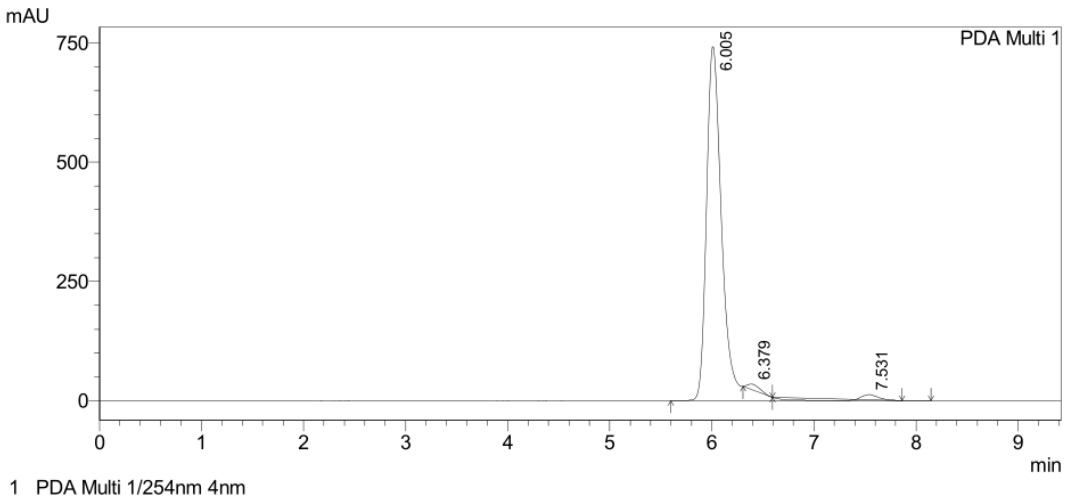


PeakTable

Ch1 210nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.829	167596	10320	2.563	1.795
2	5.115	157650	12358	2.410	2.150
3	5.957	6214901	552183	95.027	96.055
Total		6540147	574861	100.000	100.000

S5.18. HPLC chromatogram of **18**

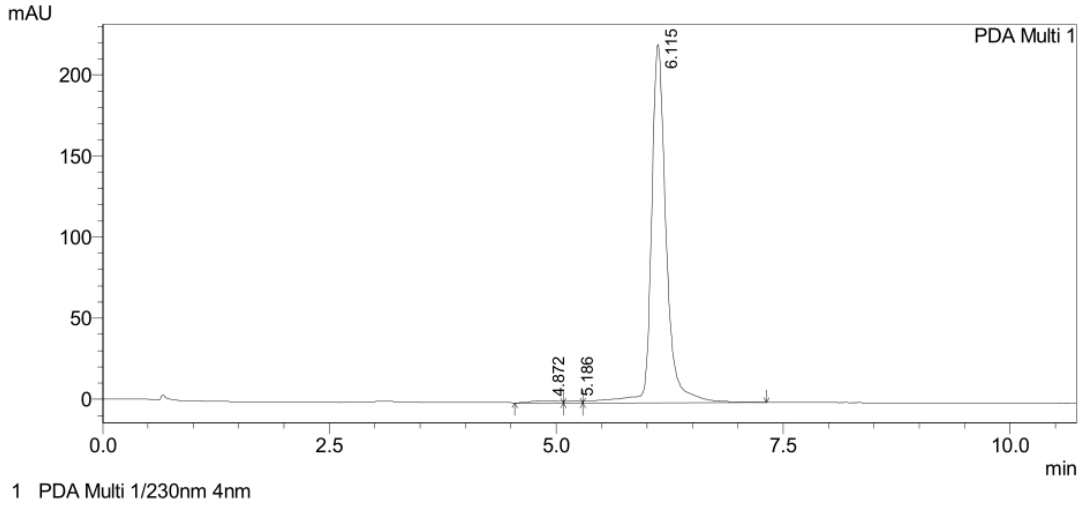


PeakTable

Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.005	8121922	741852	99.508	97.197
2	6.379	97366	10818	1.193	1.417
3	7.531	-57208	10578	-0.701	1.386
Total		8162079	763248	100.000	100.000

S5.19. HPLC chromatogram of **19**

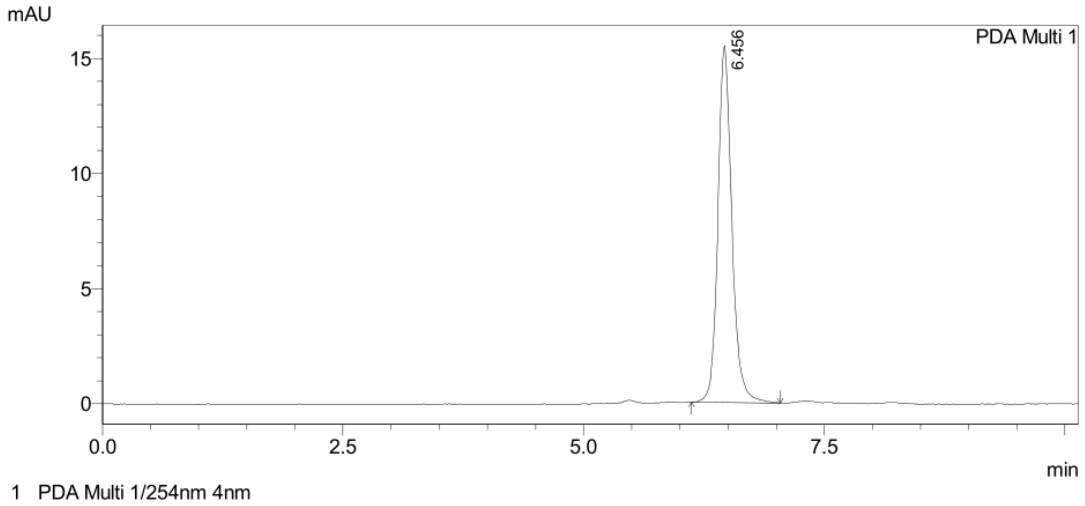


PeakTable

Ch1 230nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.872	27288	1353	1.076	0.606
2	5.186	13230	1110	0.522	0.497
3	6.115	2496045	220817	98.403	98.897
Total		2536563	223280	100.000	100.000

S5.20. HPLC chromatogram of **20**

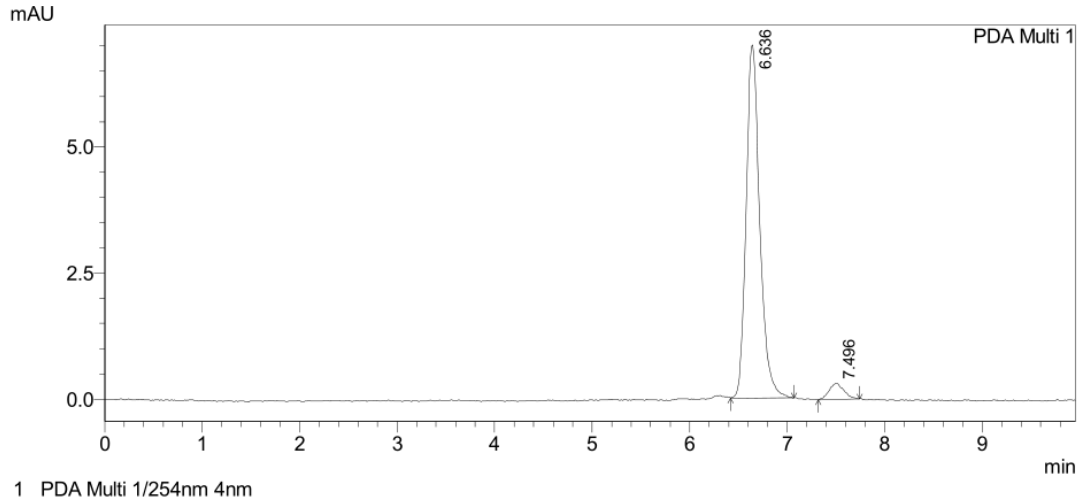


PeakTable

Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.456	156440	15501	100.000	100.000
Total		156440	15501	100.000	100.000

S5.21. HPLC chromatogram of **21**

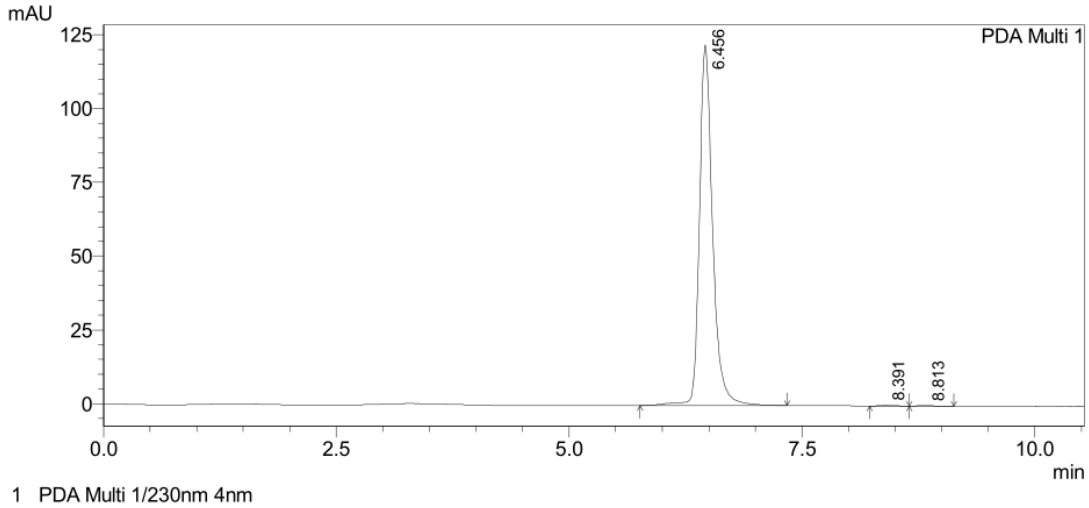


PeakTable

Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.636	68171	6999	95.296	95.673
2	7.496	3365	317	4.704	4.327
Total		71535	7315	100.000	100.000

S5.22. HPLC chromatogram of **22**

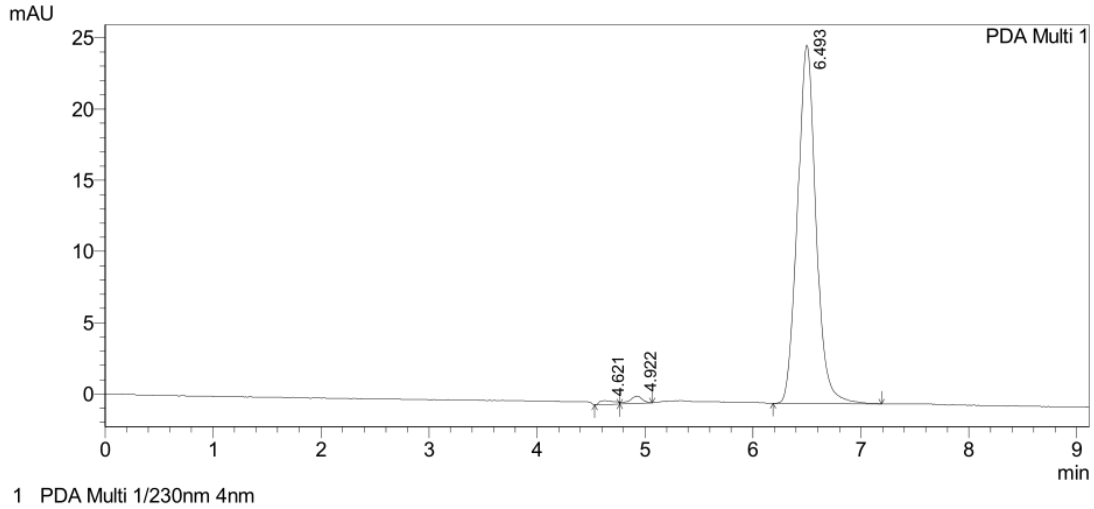


PeakTable

Ch1 230nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.456	1208097	122209	99.581	99.626
2	8.391	2922	275	0.241	0.224
3	8.813	2162	183	0.178	0.149
Total		1213181	122667	100.000	100.000

S5.23. HPLC chromatogram of **23**

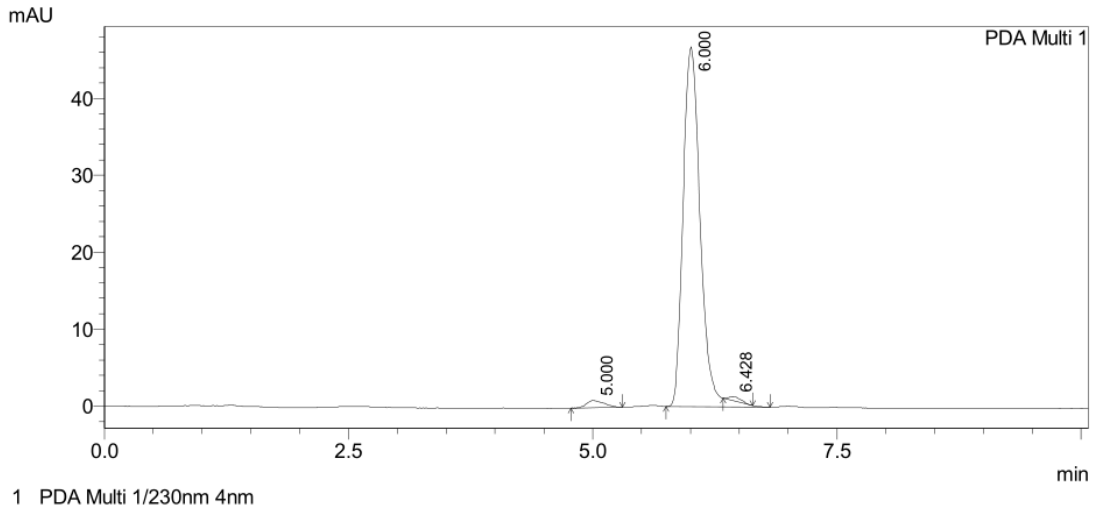


PeakTable

Ch1 230nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.621	2860	295	0.924	1.137
2	4.922	4183	515	1.352	1.984
3	6.493	302457	25162	97.724	96.879
Total		309500	25973	100.000	100.000

S5.24. HPLC chromatogram of **24**

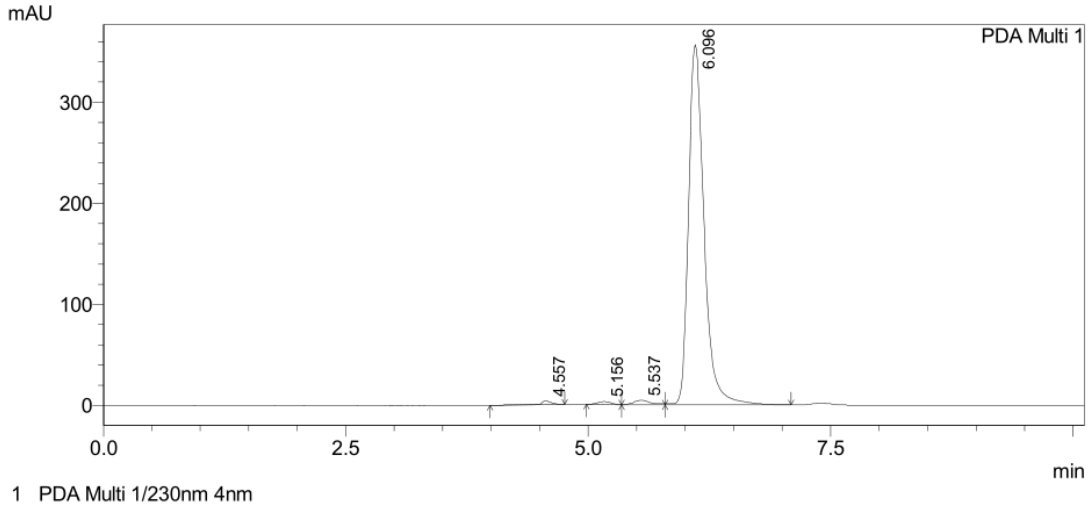


PeakTable

Ch1 230nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.000	11325	917	1.909	1.903
2	6.000	577321	46796	97.297	97.068
3	6.428	4713	496	0.794	1.030
Total		593359	48210	100.000	100.000

S5.25. HPLC chromatogram of **25**

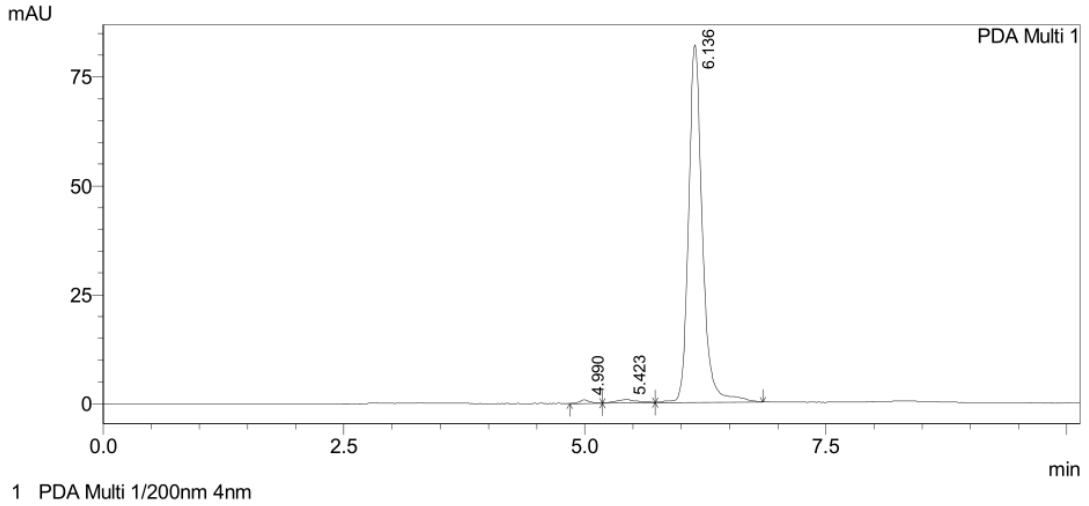


PeakTable

Ch1 230nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.557	30229	3889	0.742	1.059
2	5.156	26459	2833	0.650	0.771
3	5.537	53624	4300	1.317	1.171
4	6.096	3962494	356172	97.292	96.998
Total		4072806	367194	100.000	100.000

S5.26. HPLC chromatogram of **26**

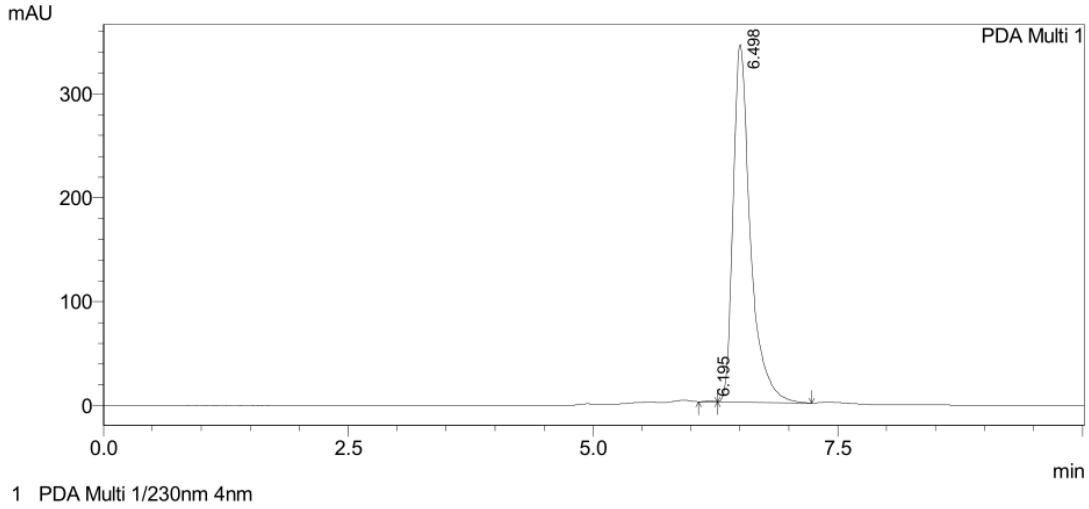


PeakTable

Ch1 200nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.990	6140	812	0.735	0.970
2	5.423	12907	831	1.544	0.992
3	6.136	816780	82116	97.721	98.038
Total		835827	83759	100.000	100.000

S5.27. HPLC chromatogram of **27**

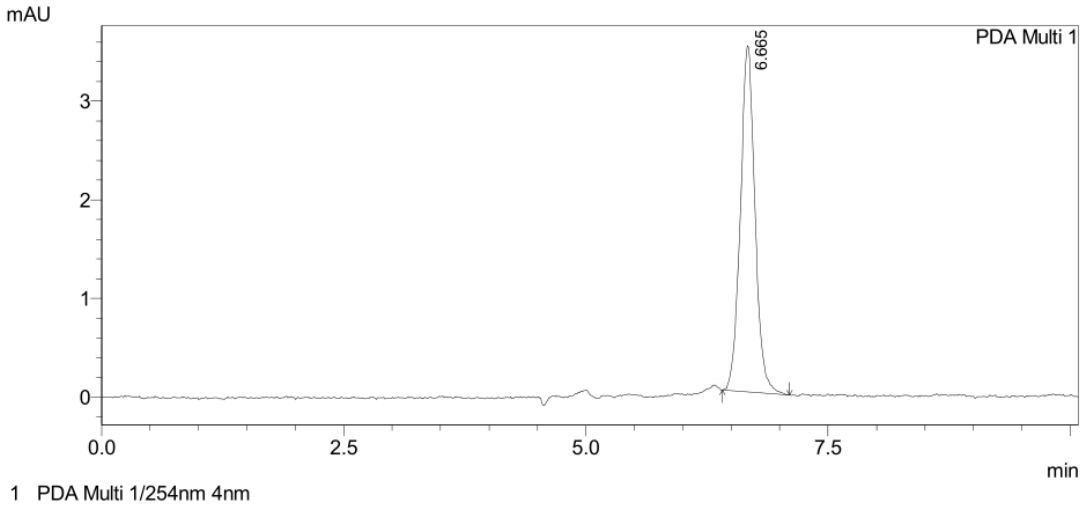


PeakTable

Ch1 230nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.195	6196	927	0.146	0.269
2	6.498	4228068	344236	99.854	99.731
Total		4234264	345164	100.000	100.000

S5.28. HPLC chromatogram of **28**

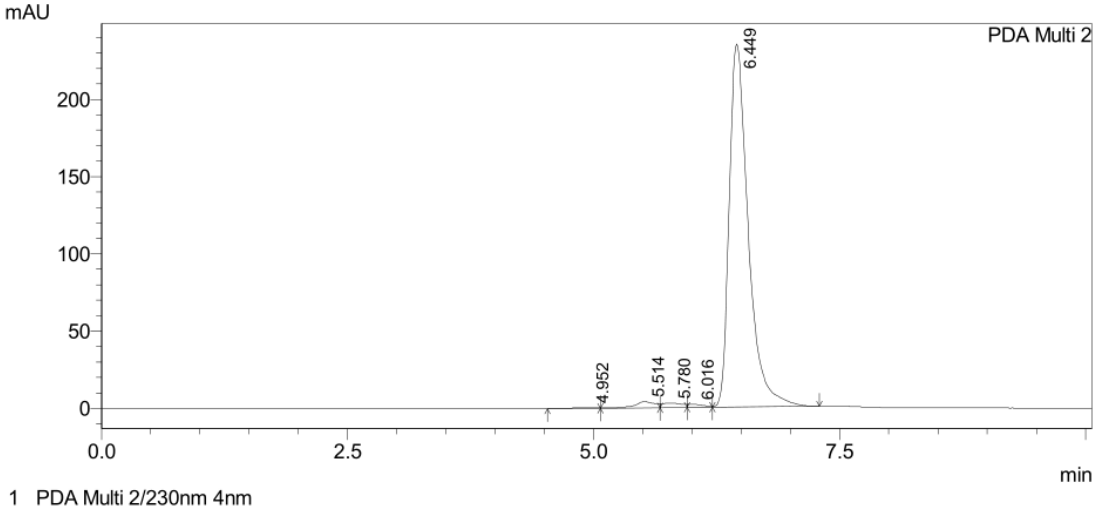


PeakTable

Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.665	36884	3510	100.000	100.000
Total		36884	3510	100.000	100.000

S5.29. HPLC chromatogram of **29**

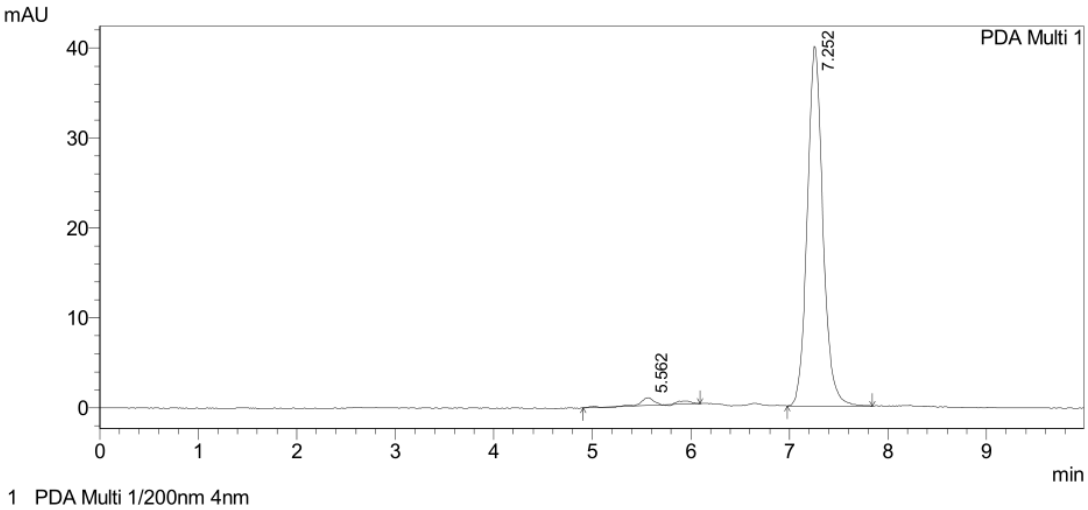


PeakTable

Ch2 230nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.952	7853	476	0.238	0.195
2	5.514	59691	4039	1.809	1.654
3	5.780	41394	2925	1.255	1.198
4	6.016	20937	1843	0.635	0.755
5	6.449	3169035	234912	96.063	96.199
Total		3298910	244195	100.000	100.000

S5.30. HPLC chromatogram of **30**

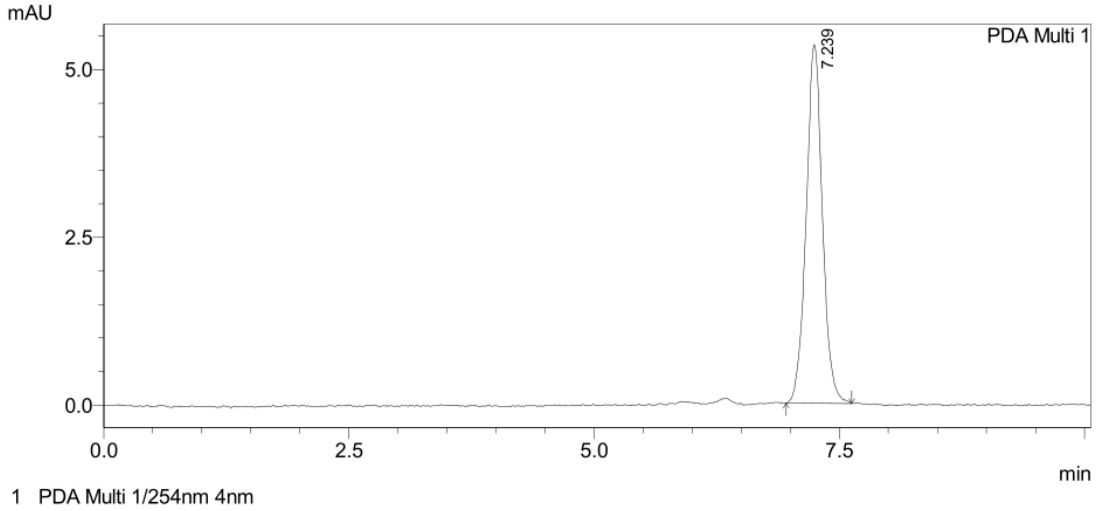


PeakTable

Ch1 200nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.562	11883	869	2.624	2.127
2	7.252	440917	39991	97.376	97.873
Total		452801	40860	100.000	100.000

S5.31. HPLC chromatogram of **31**



PeakTable

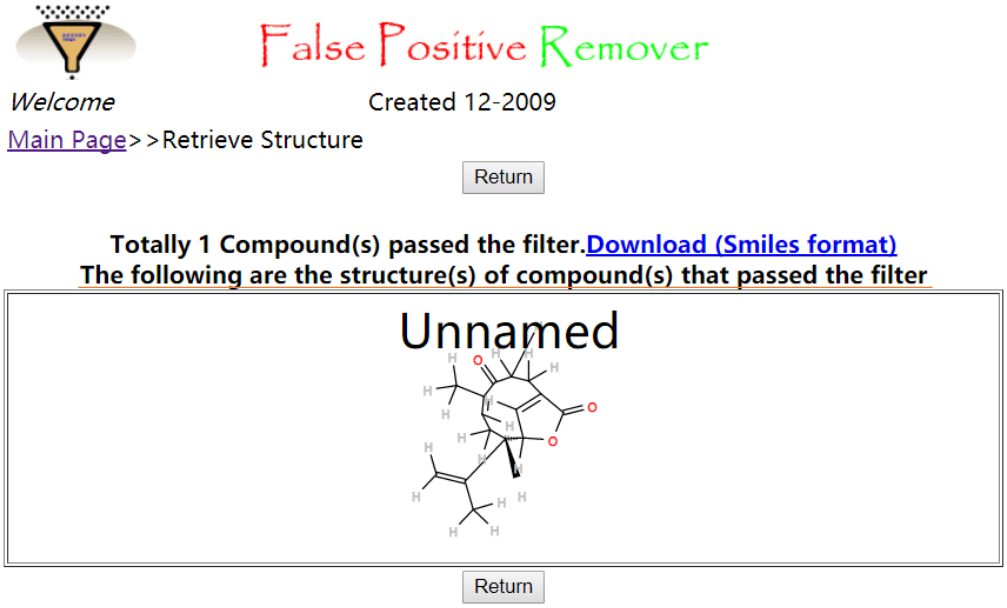
Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	7.239	61646	5343	100.000	100.000
Total		61646	5343	100.000	100.000

6. The analysis of **11** on pan assay interference compounds (PAINS)

Under the guidance of the article "The Ecstasy and Agony of Assay Interference Compounds *J. Med. Chem.* 2017, *12*, 575–578", the potential risk of **11** as PAINS were analyzed:

1) The preliminary screening of active compounds was phenotypic-based (in cardiac fibroblasts) rather than the target-based, and only compound **11** was selected as the promising candidate for further study.
2) Compound **11** was nontoxic to cardiac fibroblasts under all testing concentrations, and the inhibitory effects to fibronectin (FN), α-smooth muscle actin (α-SMA), and collagens in TGFβ1-stimulated cardiac fibroblasts were dose-dependent.
3) The structure of **11** dose not contain any fluorophores and the inhibition of FN has a clear structure–activity relationships.
4) Virtual screening by PAINS-Remover (https://www.cbligand.org/PAINS) showed that **11** dose not contain the PAINS features.



5) The colloidal aggregation potentials of **11** were virtually and experimentally assessed, and no colloidal aggregation risk were observed.

① Virtual screening by Aggregator Advisor: http://advisor.docking.org

② Dynamic light scattering (DLS) of **11** in potassium phosphate (KPi) buffer.[a]

Concentration of **11** (μM)	DLS scanning diameter ranges (2–2000 nm)
10	no particles
100	no particles

[a] Measurements were conducted by using a DynaPro Plate Reader II with a 50 mW laser at 830 nm, using a detector angle of 150°. The solution of 10 mM of **11** in DMSO was diluted to the denoted concentrations by 50 mM KPi, pH 7.0, at room temperature. Data were filtered using a baseline limit of 1 ± 0.01. Independent experiments involved triplicate analyses for each sample conditions. The detailed procedures were according to *J. Med. Chem*, **2015**, *58*, 7076–7087.

These evidences combined the efficacious animal experiments suggested that **11** is a genuine active compound rather than a PAINS.