# Supplementary Materials

# Bioactivity-Guided Isolation of Antistroke Compounds from Gymnadenia conopsea (L.) R. Br.

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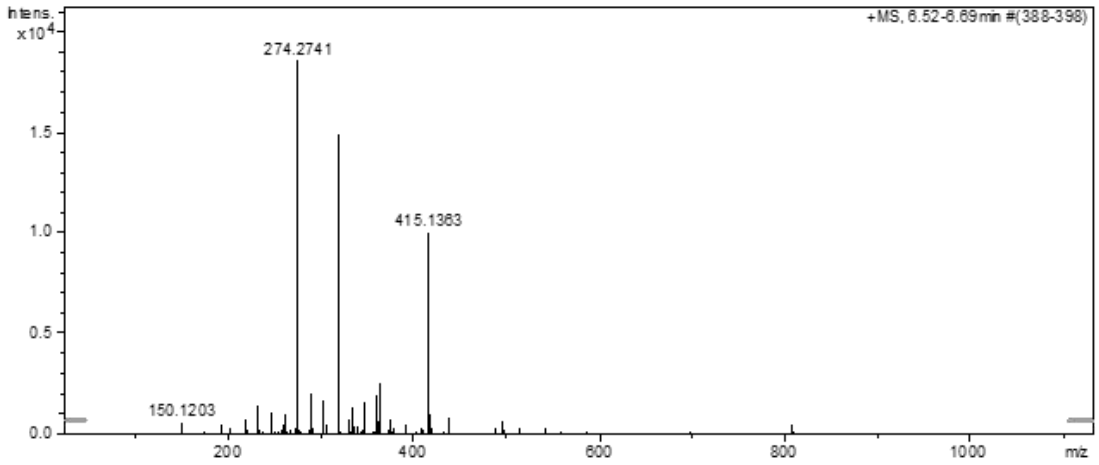
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| Meas. m/z# | | Formula | Score | m/z | err [mDa] | err [ppm] | mSigma | rdb | e⁻ Conf | N-Rule |
|---|---|---|---|---|---|---|---|---|---|---|
| 415.1363 | 1 | C 13 H 28 Na 0 13 | 0.98 | 415.1422 | 5.9 | 14.2 | 38.9 | -0.5 | even | ok |
| | 2 | C 20 H 24 Na 0 8 | 100 | 415.1363 | 0 | 0.1 | 73 | 8.5 | even | ok |
| | 3 | C 22 H 23 0 8 | 14.57 | 415.1387 | 2.4 | 5.8 | 84.1 | 11.5 | even | ok |
| | 4 | C 27 H 20 Na 0 3 | 0.06 | 415.1305 | -5.9 | -14.1 | 111 | 17.5 | even | ok |
| | 5 | C 29 H 19 0 3 | 0.86 | 415.1329 | -3.4 | -8.3 | 122.2 | 20.5 | even | ok |

**Figure S1.** HRESIMS spectrum of **1**.

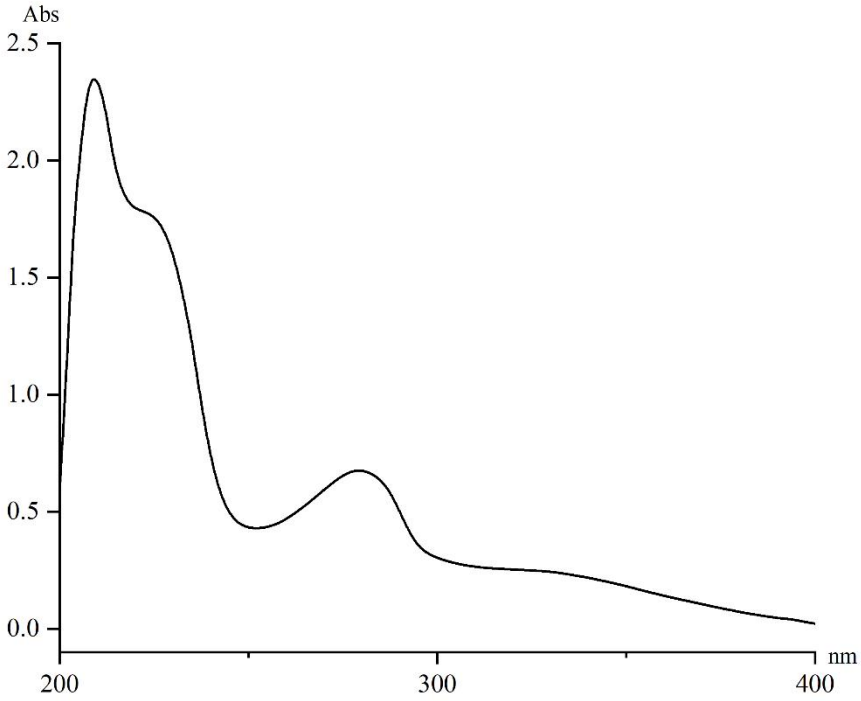



**Figure S2.** UV spectrum of **1**.

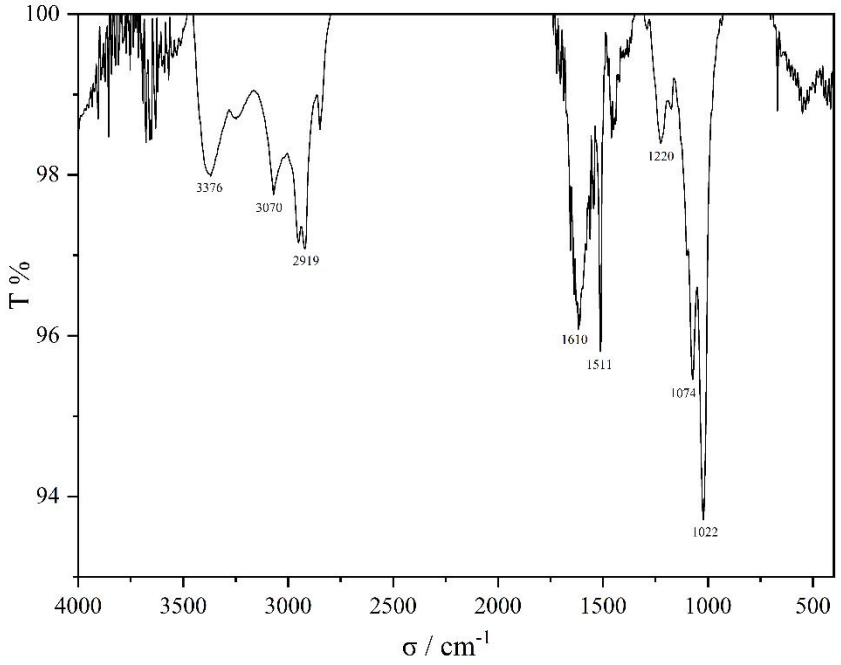



**Figure S3.** IR spectrum of **1**.

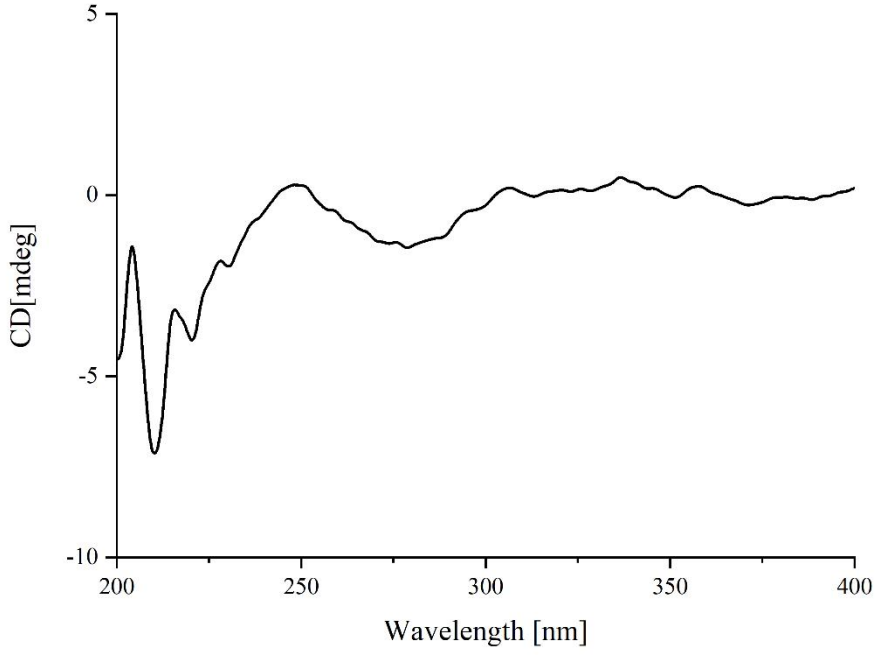



**Figure S4.** CD spectrum of **1**.

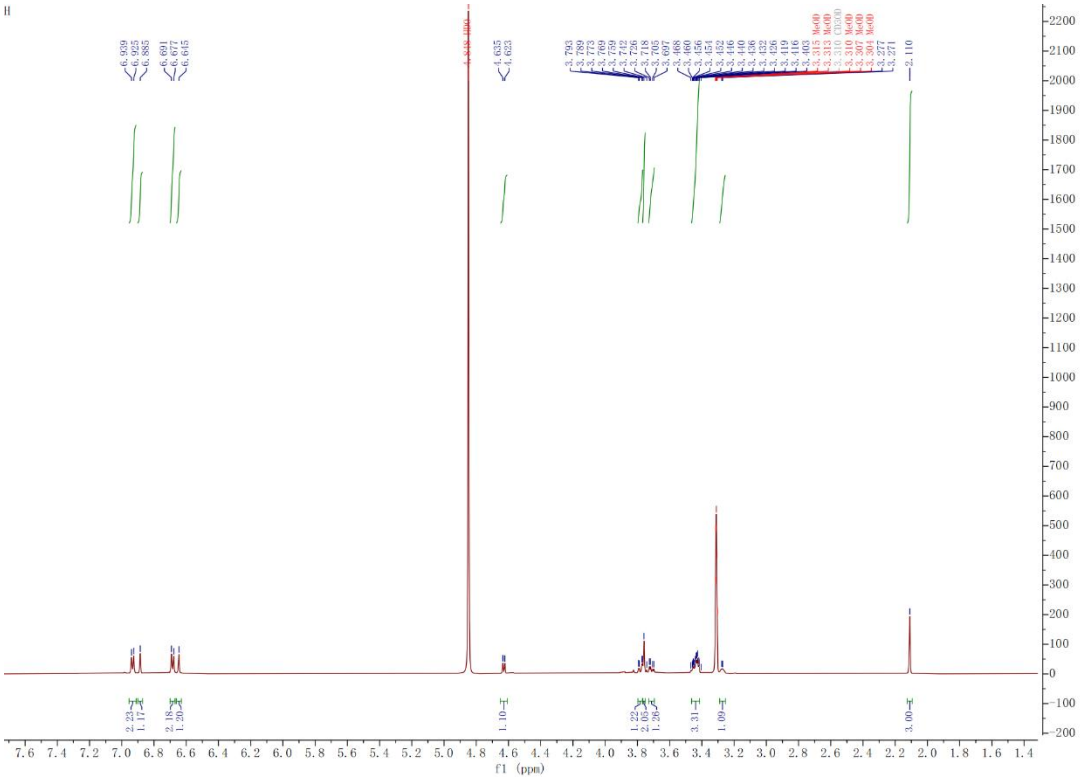


**Figure S5.** $^{1}$H NMR spectrum of **1**.

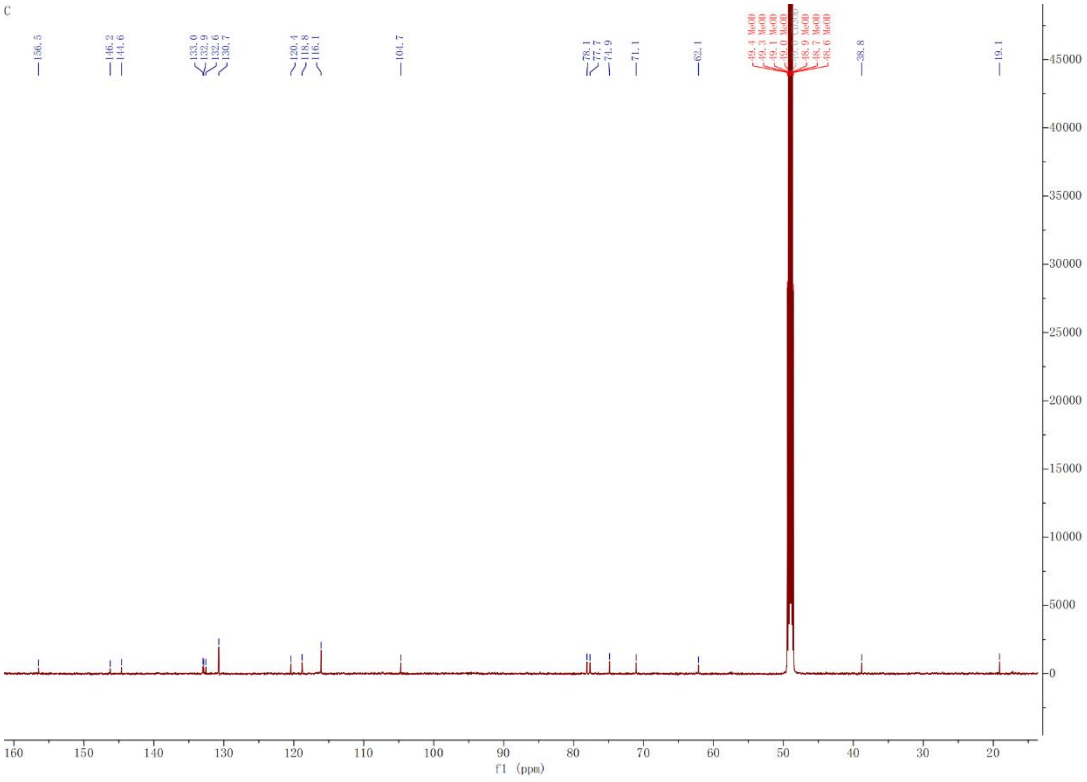


**Figure S6.** $^{13}$C NMR spectrum of **1**.

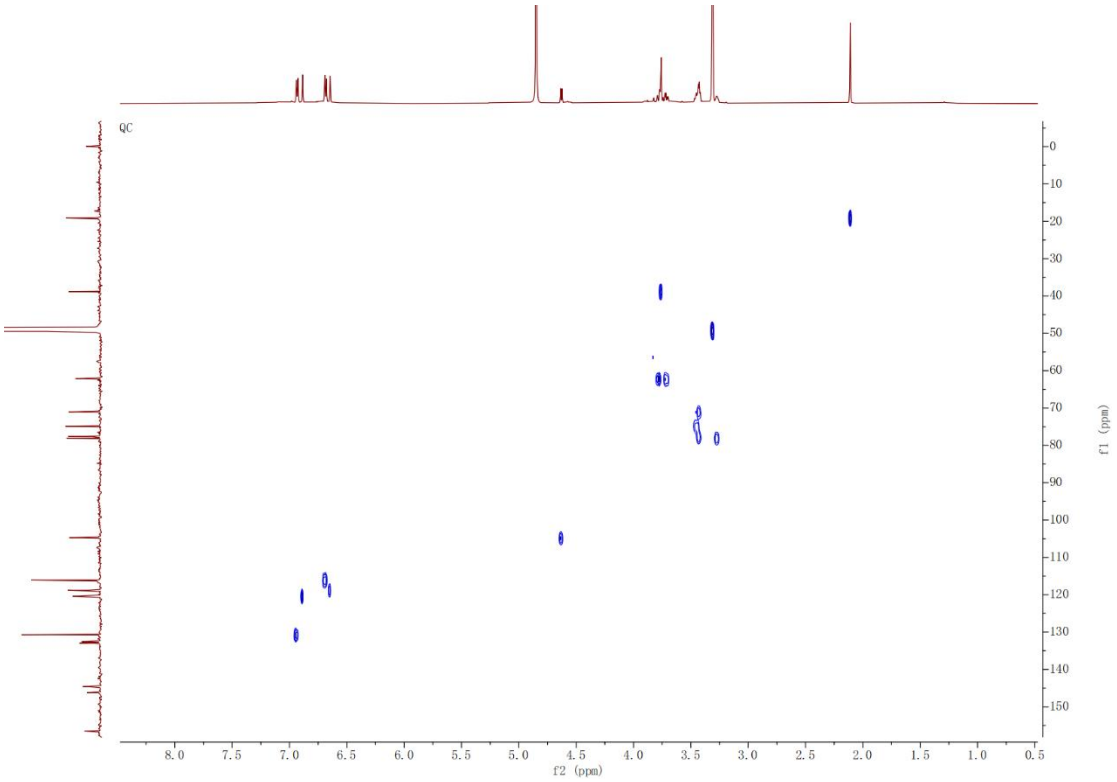



**Figure S7.** HSQC spectrum of **1**.

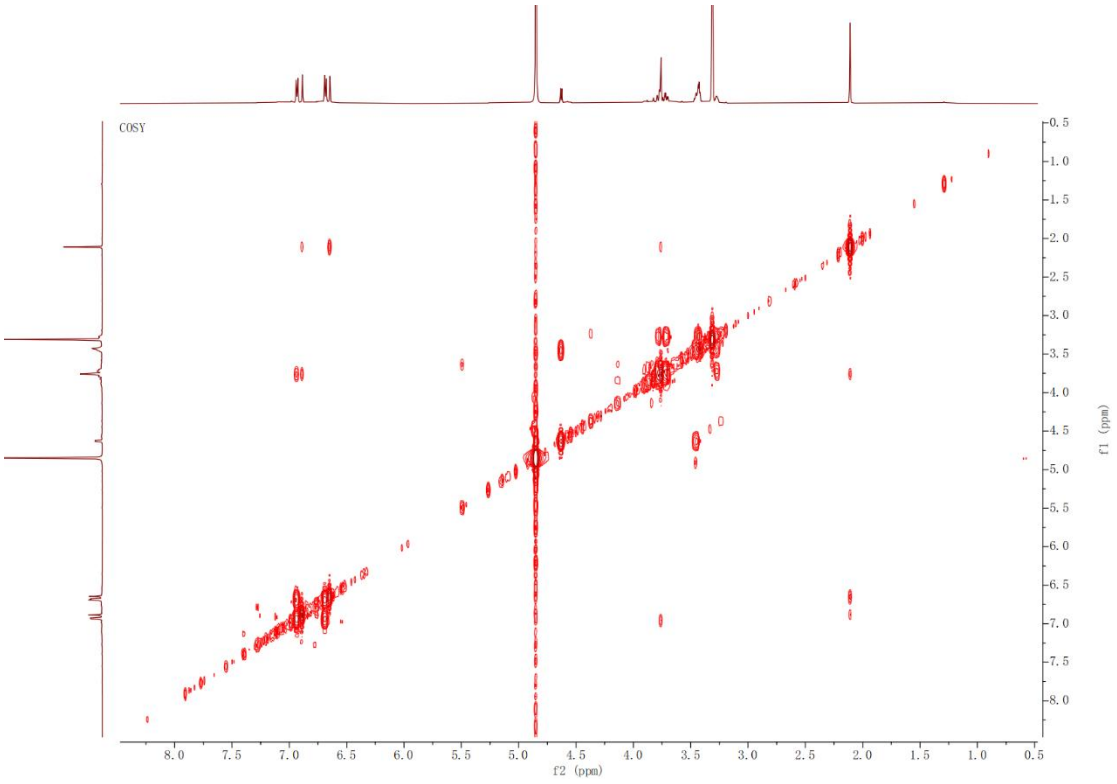



**Figure S8.** $^{1}$H-$^{1}$H COSY spectrum of **1**.

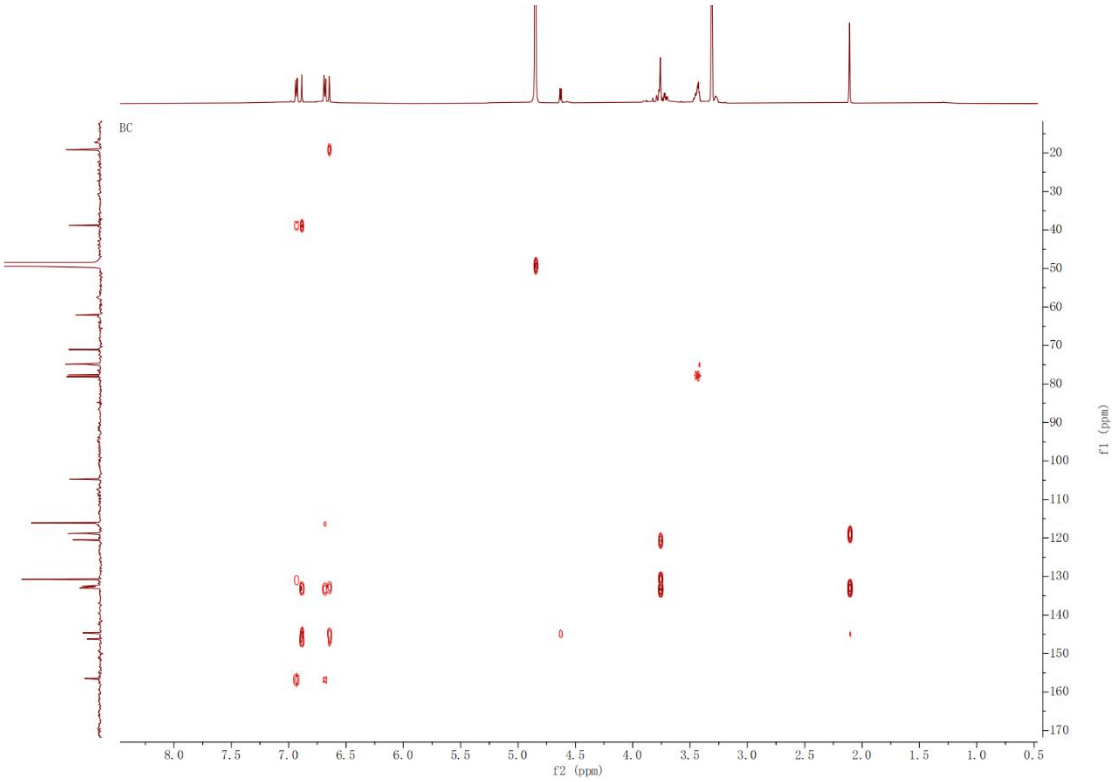



**Figure S9.** HMBC spectrum of **1**.

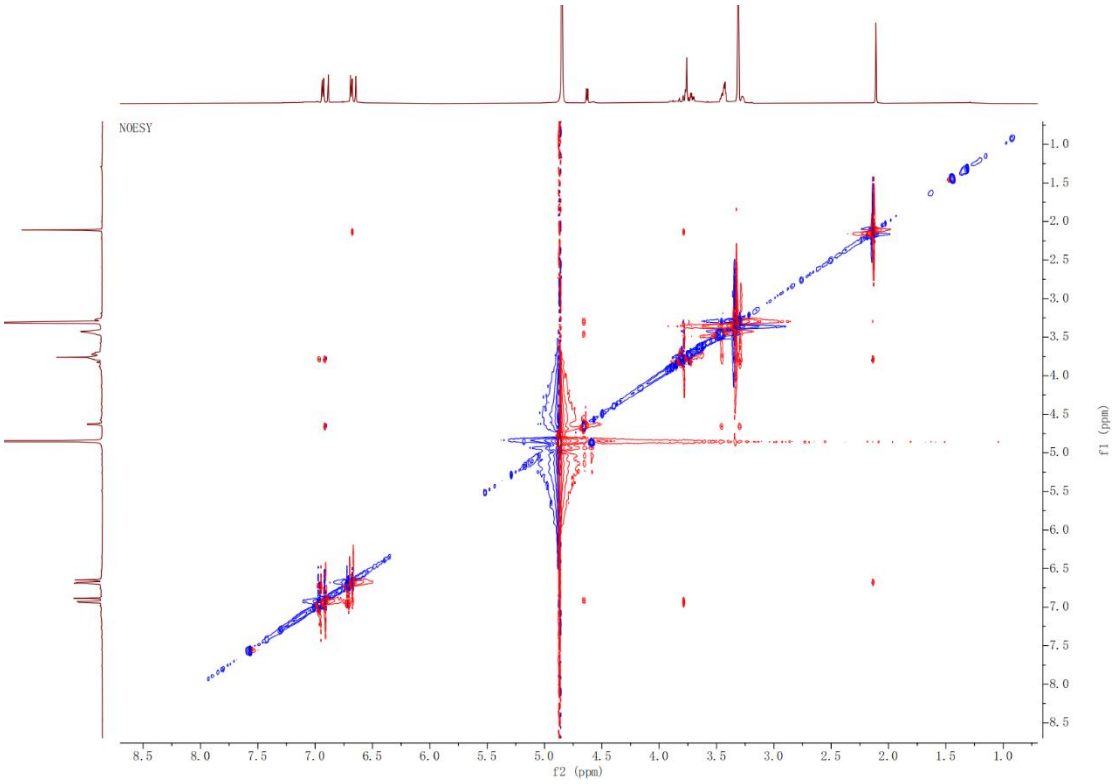
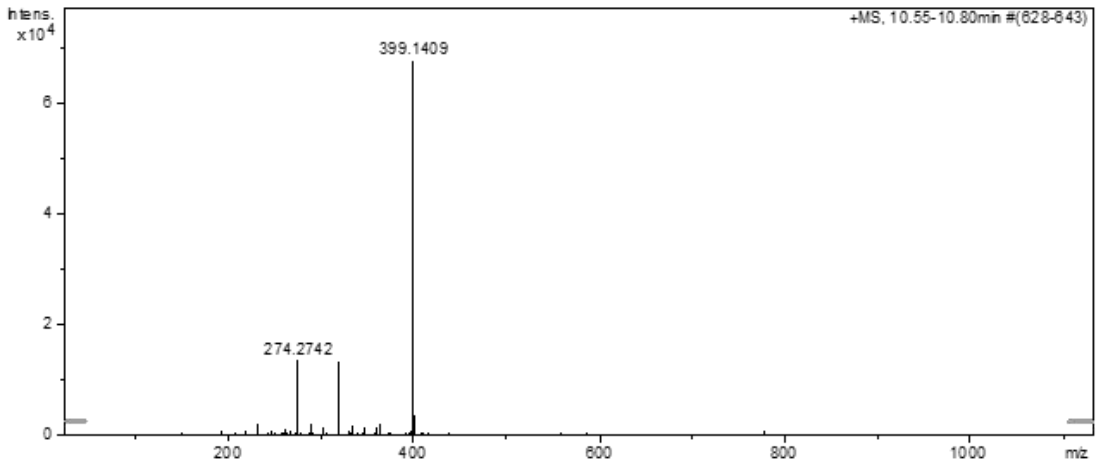



**Figure S10.** NOESY spectrum of **1**.




| Meas. m/z | # | Formula | Score | m/z | err [mDa] | err [ppm] | mSigma | rdb | e⁻ Conf | N-Rule |
|---|---|---|---|---|---|---|---|---|---|---|
| 399.1409 | 1 | C 13 H 28 Na 0 12 | 0.84 | 399.1473 | 6.4 | 16 | 57.6 | -0.5 | even | ok |
| | 2 | C 20 H 24 Na 0 7 | 100 | 399.1414 | 0.5 | 1.3 | 97.5 | 8.5 | even | ok |
| | 3 | C 22 H 23 O 7 | 9.64 | 399.1438 | 2.9 | 7.3 | 110.3 | 11.5 | even | ok |
| | 4 | C 27 H 20 Na 0 2 | 0.27 | 399.1356 | -5.3 | -13.4 | 122.4 | 17.5 | even | ok |
| | 5 | C 29 H 19 O 2 | 2.62 | 399.138 | -2.9 | -7.4 | 133.6 | 20.5 | even | ok |

**Figure S11.** HRESIMS spectrum of **2**.

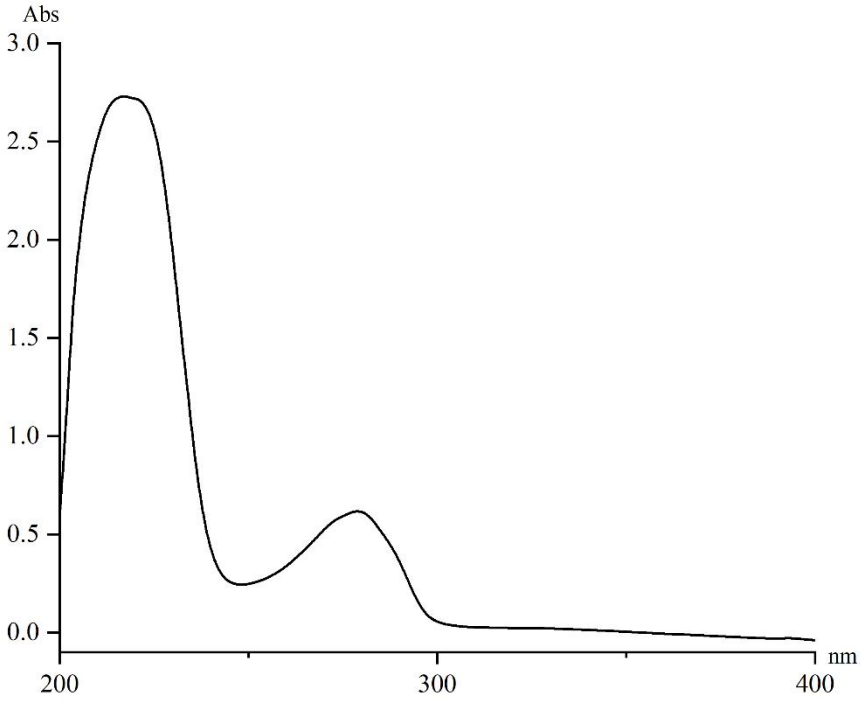



**Figure S12.** UV spectrum of **2**.

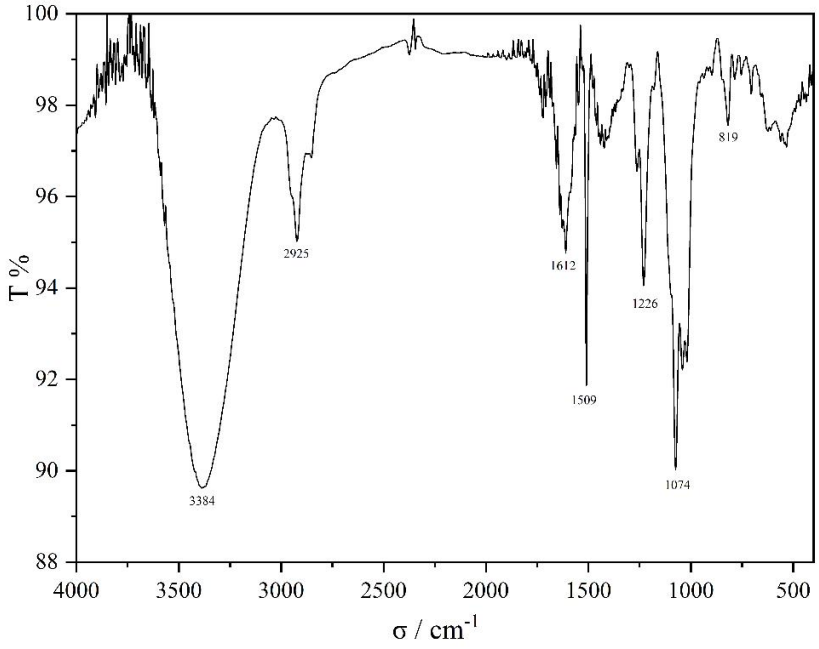



**Figure S13.** IR spectrum of **2**.

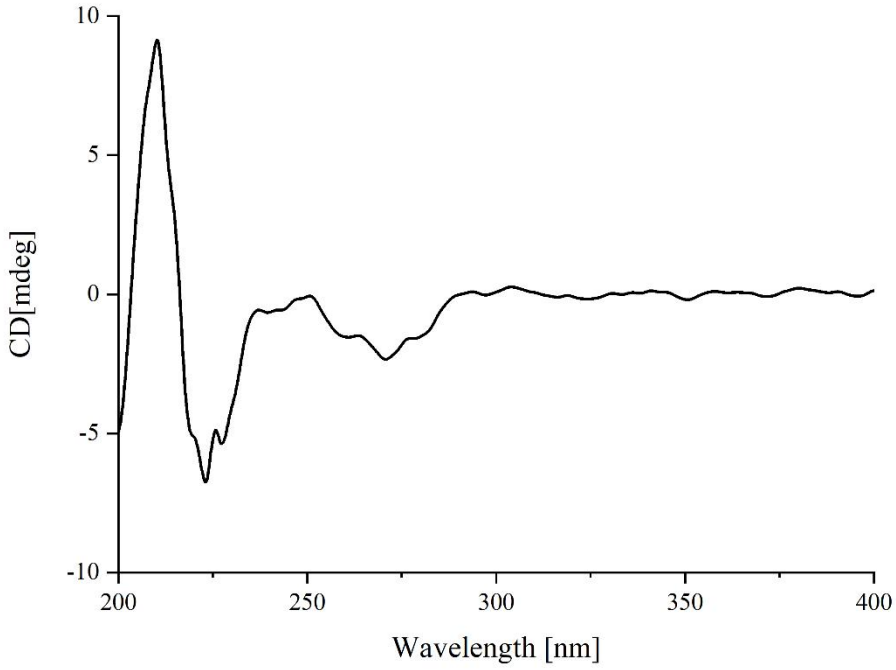



**Figure S14.** CD spectrum of **2**.

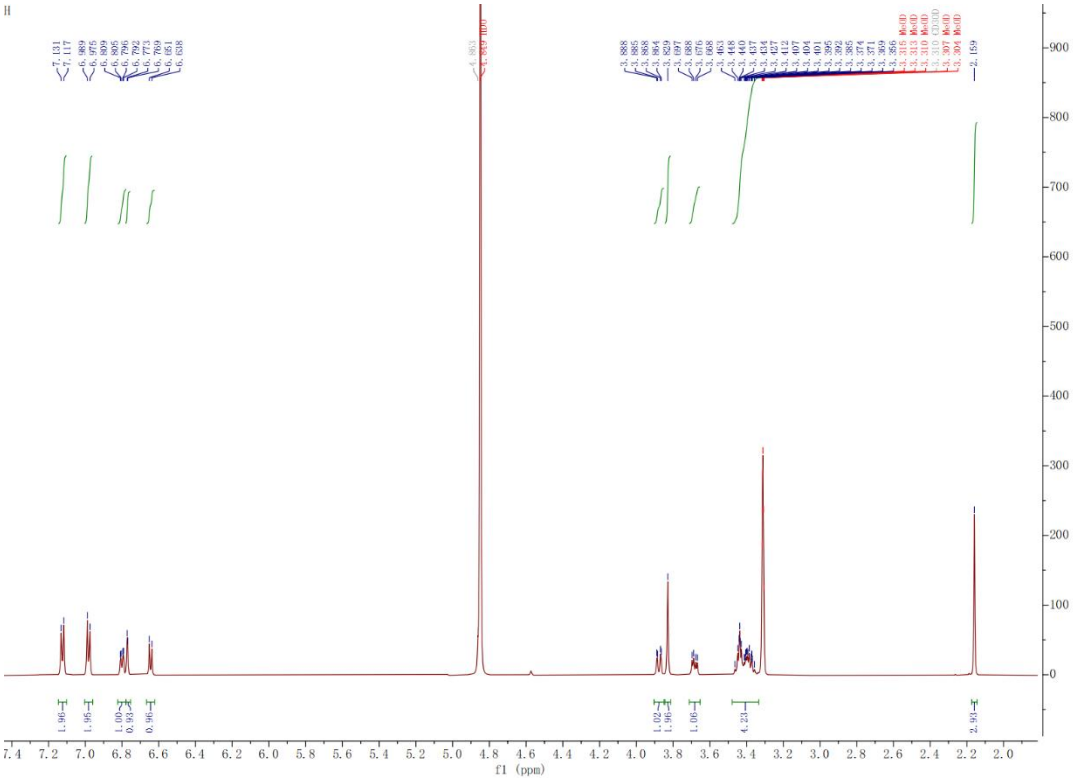


**Figure S15.** $^{1}$H NMR spectrum of **2**.

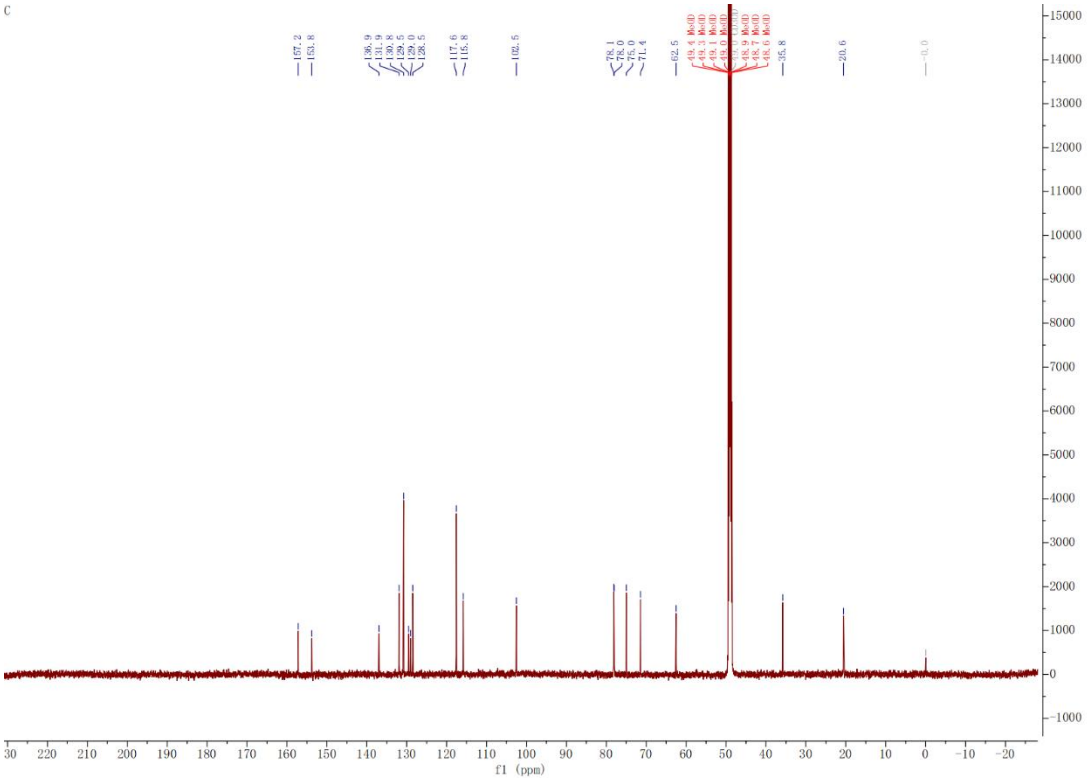


**Figure S16.** $^{13}$C NMR spectrum of **2**.

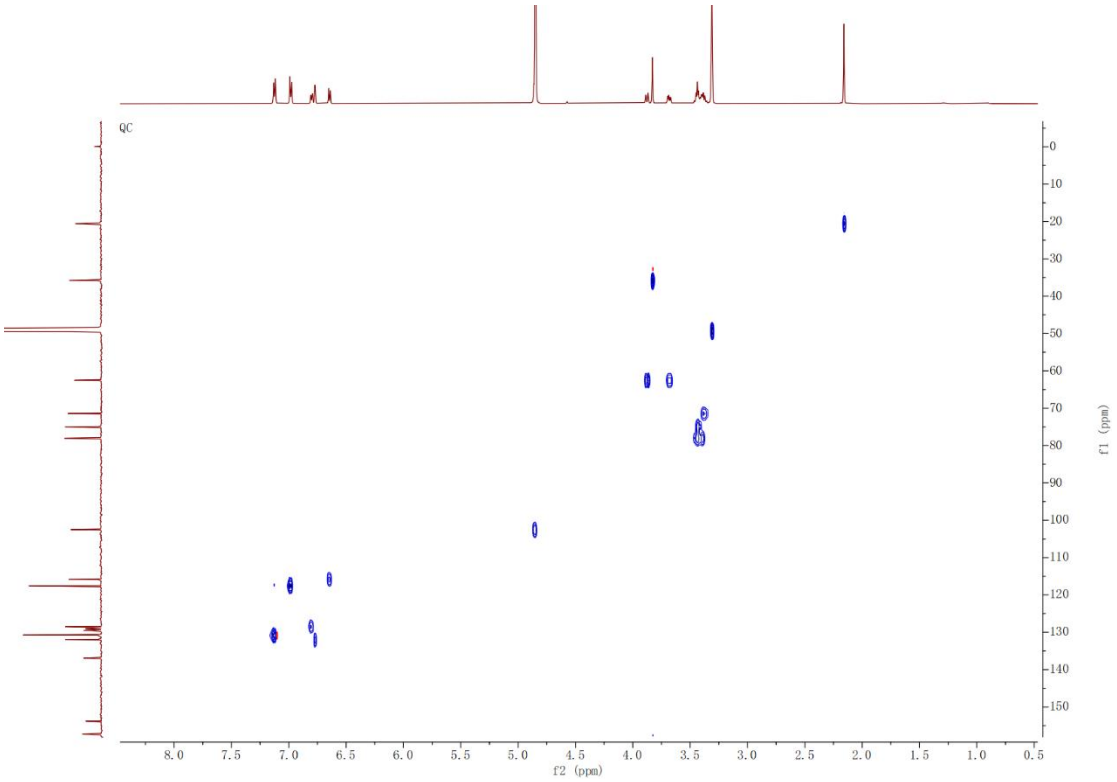



**Figure S17.** HSQC spectrum of **2**.

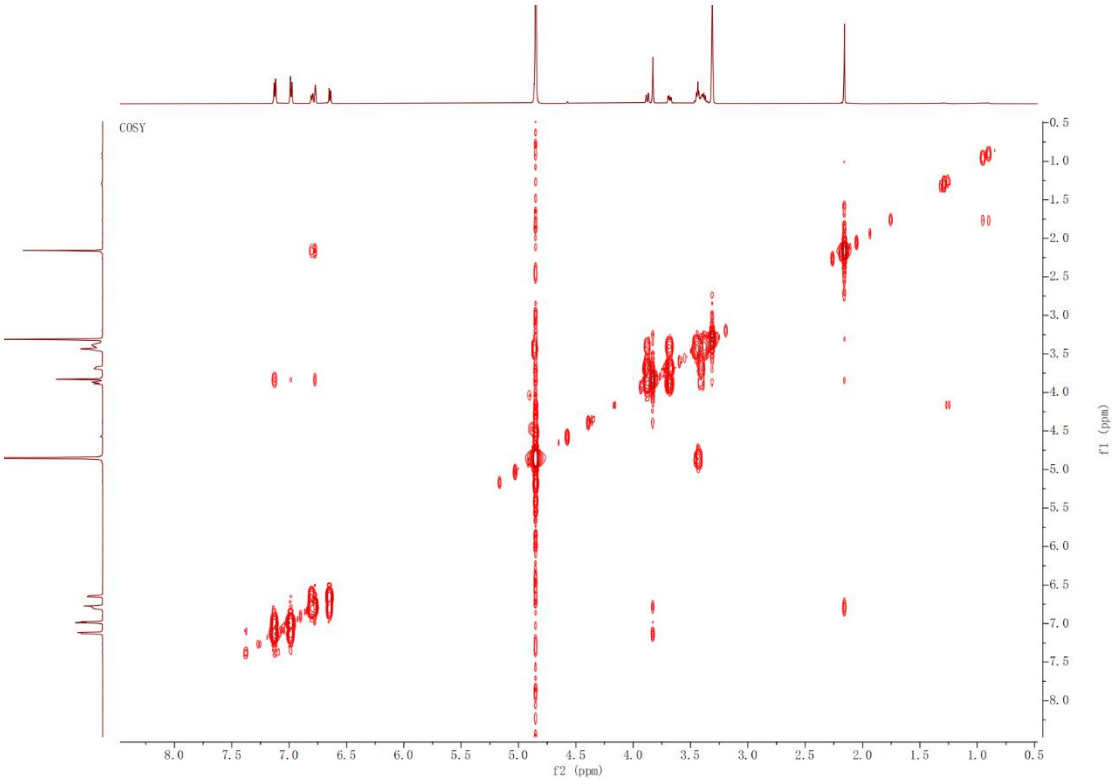



**Figure S18.** $^{1}$H-$^{1}$H COSY spectrum of **2**.

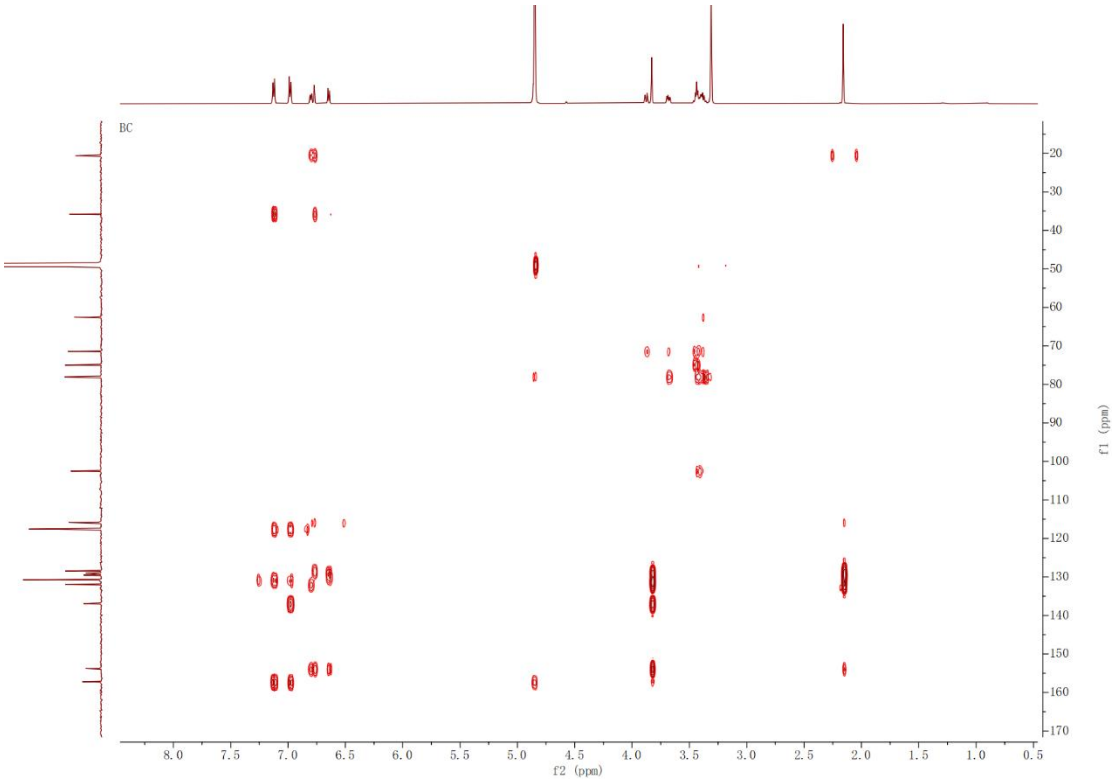



**Figure S19.** HMBC spectrum of **2**.

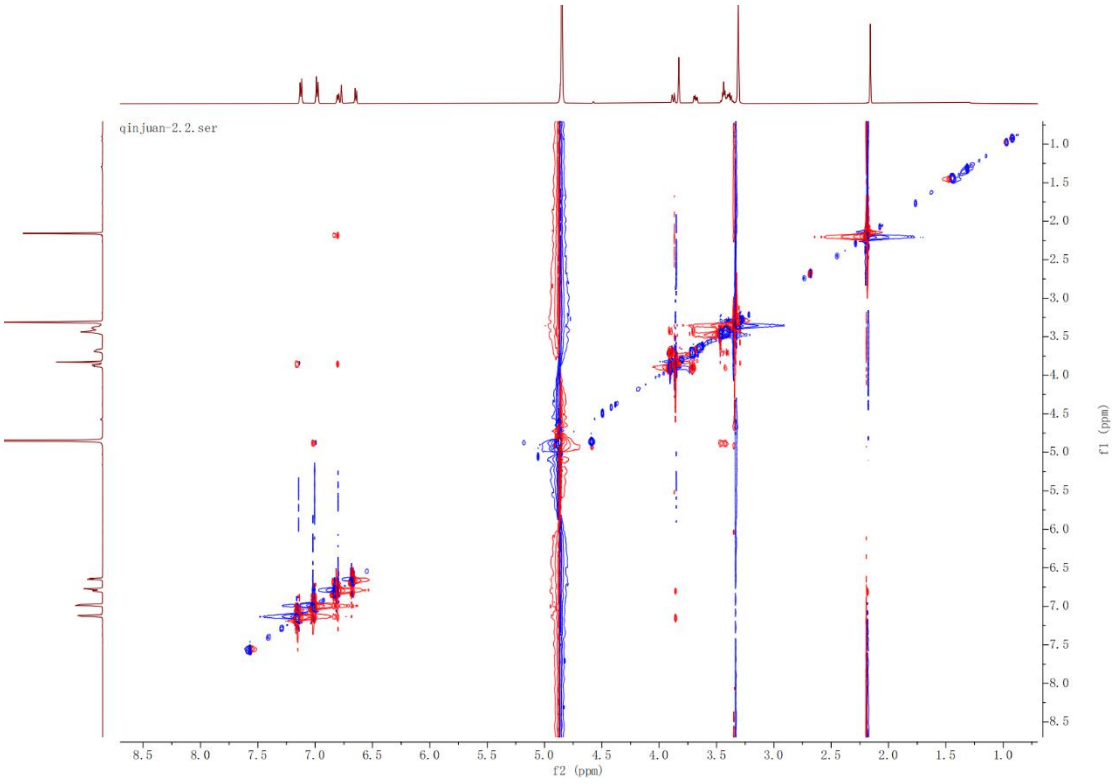
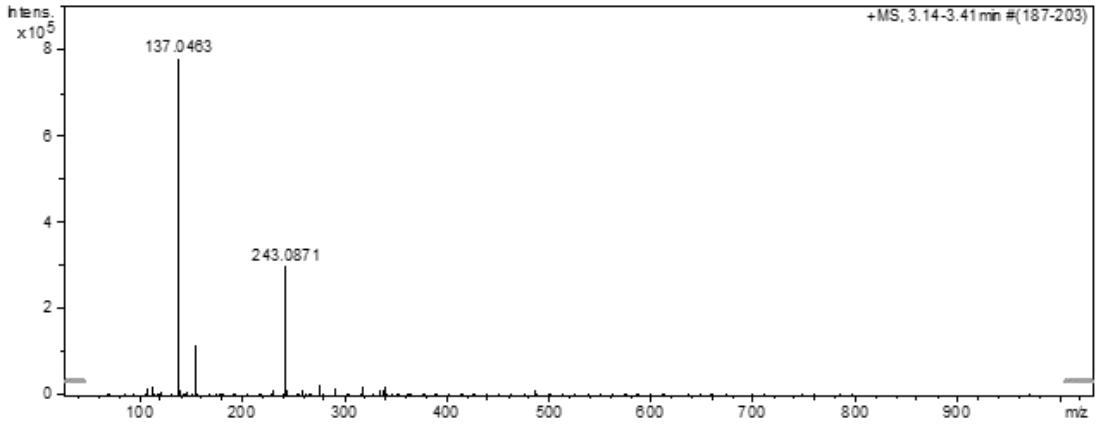



**Figure S20.** NOESY spectrum of **2**.




| Meas. m/z# | | Formula | Score | m/z | err [mDa] | err [ppm] | mSigma | rdb | e⁻ Conf | N-Rule |
|---|---|---|---|---|---|---|---|---|---|---|
| 243.0871 | 1 | C 6 H 15 N 2 O 8 | 4.67 | 243.0823 | -4.8 | -19.8 | 23.6 | 0.5 | even | ok |
| | 2 | C 7 H 11 N 6 O 4 | 17.17 | 243.0836 | -3.5 | -14.3 | 37 | 5.5 | even | ok |
| | 3 | C 9 H 16 Na O 6 | 22.75 | 243.0839 | -3.2 | -13.1 | 37.3 | 1.5 | even | ok |
| | 4 | C 6 H 8 N 10 Na | 4.58 | 243.0826 | -4.5 | -18.7 | 39 | 7.5 | even | ok |
| | 5 | C 11 H 15 O 6 | 100 | 243.0863 | -0.8 | -3.2 | 49.8 | 4.5 | even | ok |
| | 6 | C 10 H 12 N 4 Na O 2 | 48.56 | 243.0852 | -1.9 | -7.6 | 51.4 | 6.5 | even | ok |
| | 7 | C 8 H 7 N 10 | 39.17 | 243.085 | -2.1 | -8.8 | 51.5 | 10.5 | even | ok |
| | 8 | C 12 H 11 N 4 O 2 | 70.67 | 243.0877 | 0.6 | 2.3 | 64 | 9.5 | even | ok |
| | 9 | C 15 H 12 N 2 Na | 14.63 | 243.0893 | 2.2 | 8.9 | 78.4 | 10.5 | even | ok |
| | 10 | C 17 H 11 N 2 | 0.68 | 243.0917 | 4.6 | 18.8 | 91 | 13.5 | even | ok |

**Figure S21.** HRESIMS spectrum of **13**.

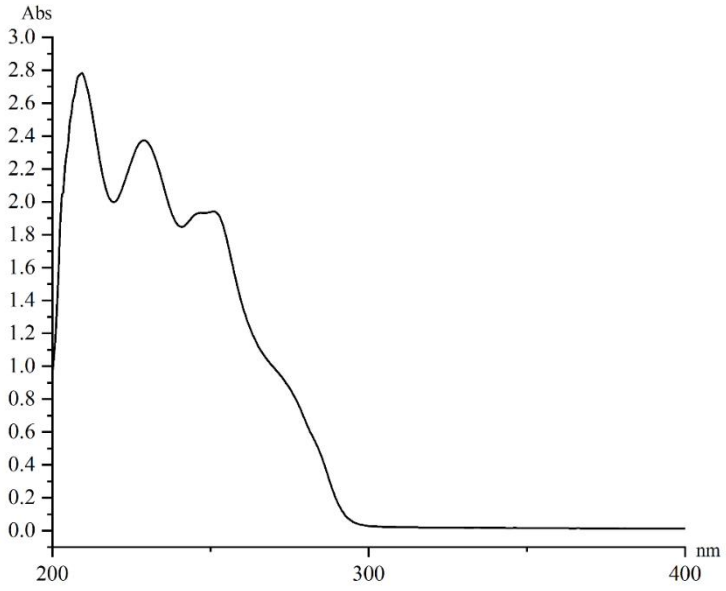



**Figure S22.** UV spectrum of **13**.

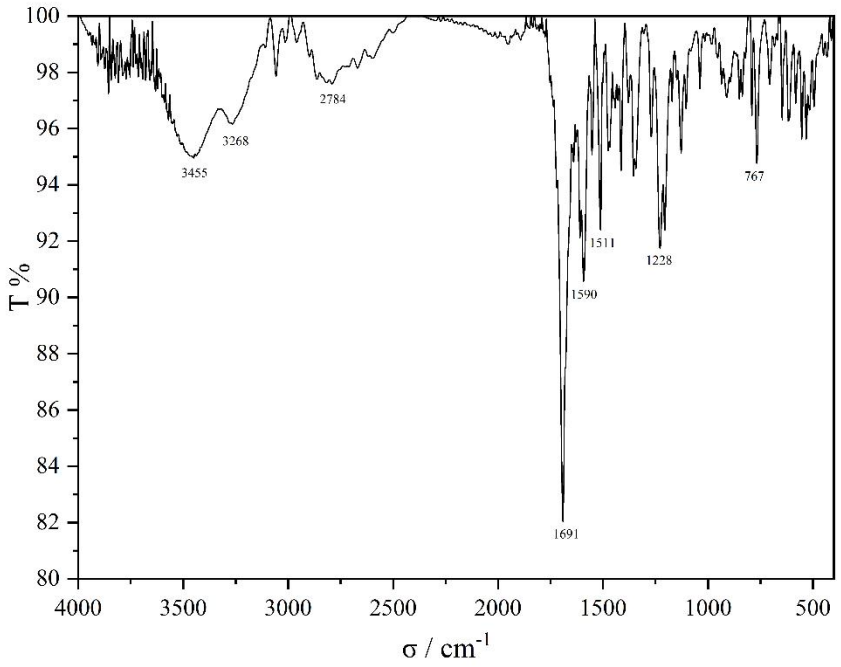



**Figure S23.** IR spectrum of **13**.

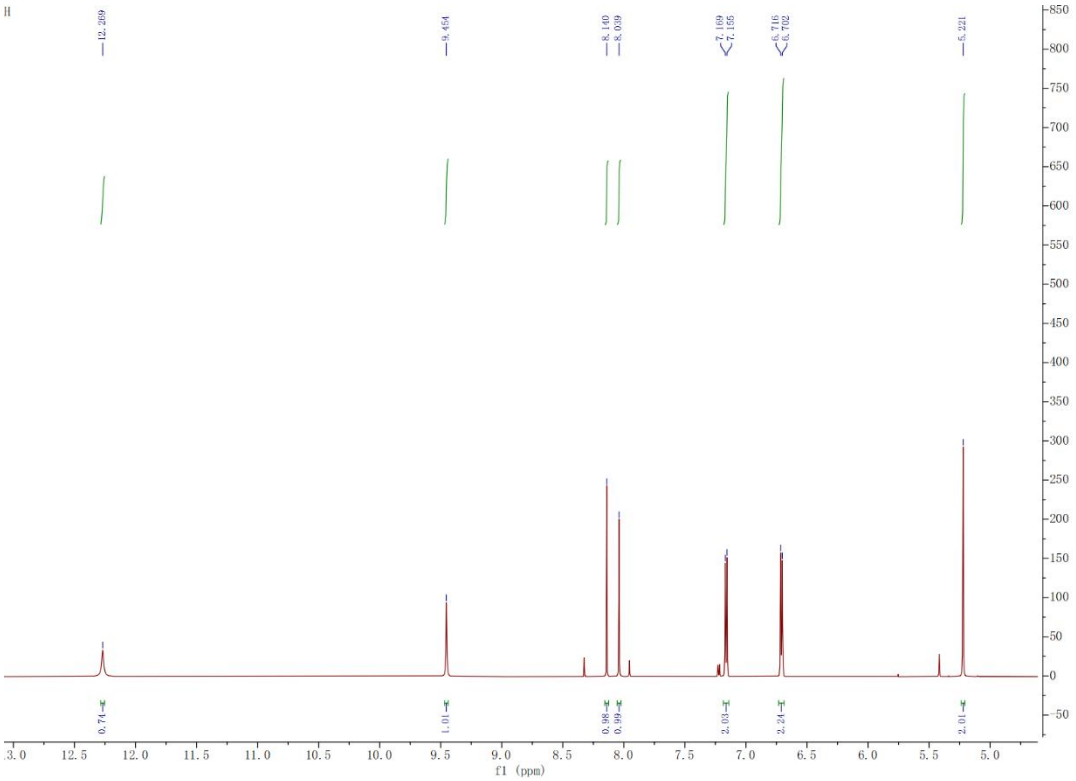



**Figure S24.** $^{1}$H NMR spectrum of **13**.

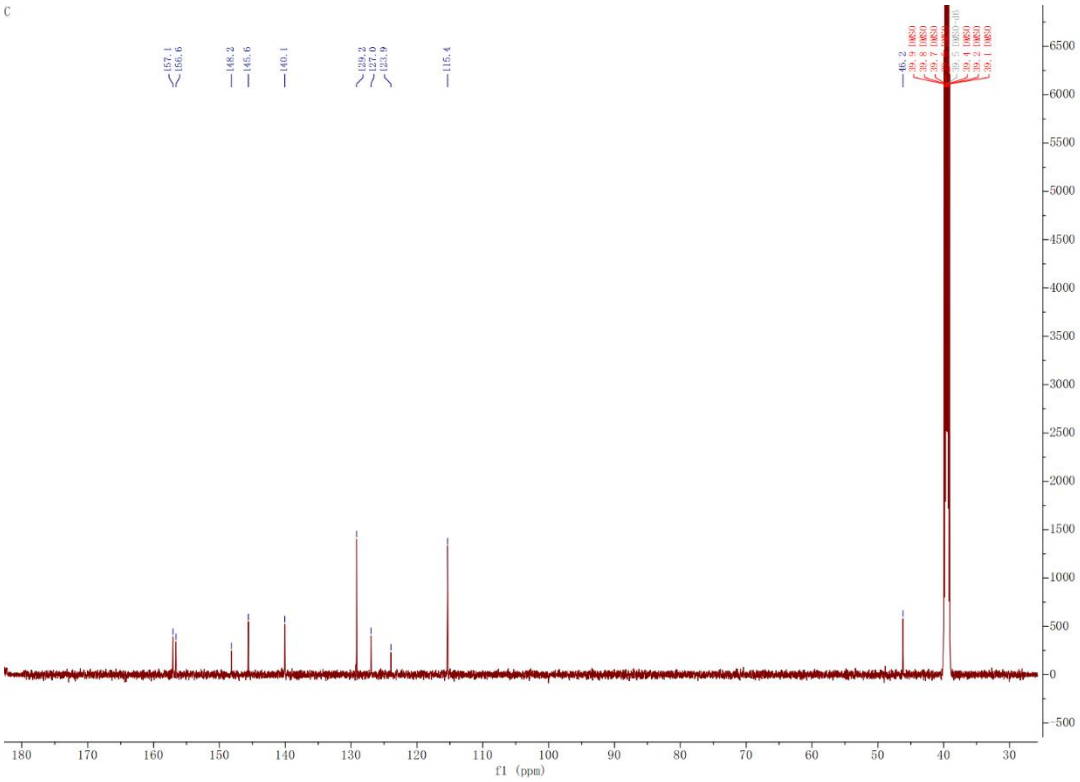



**Figure S25.** $^{13}$C NMR spectrum of **13**.

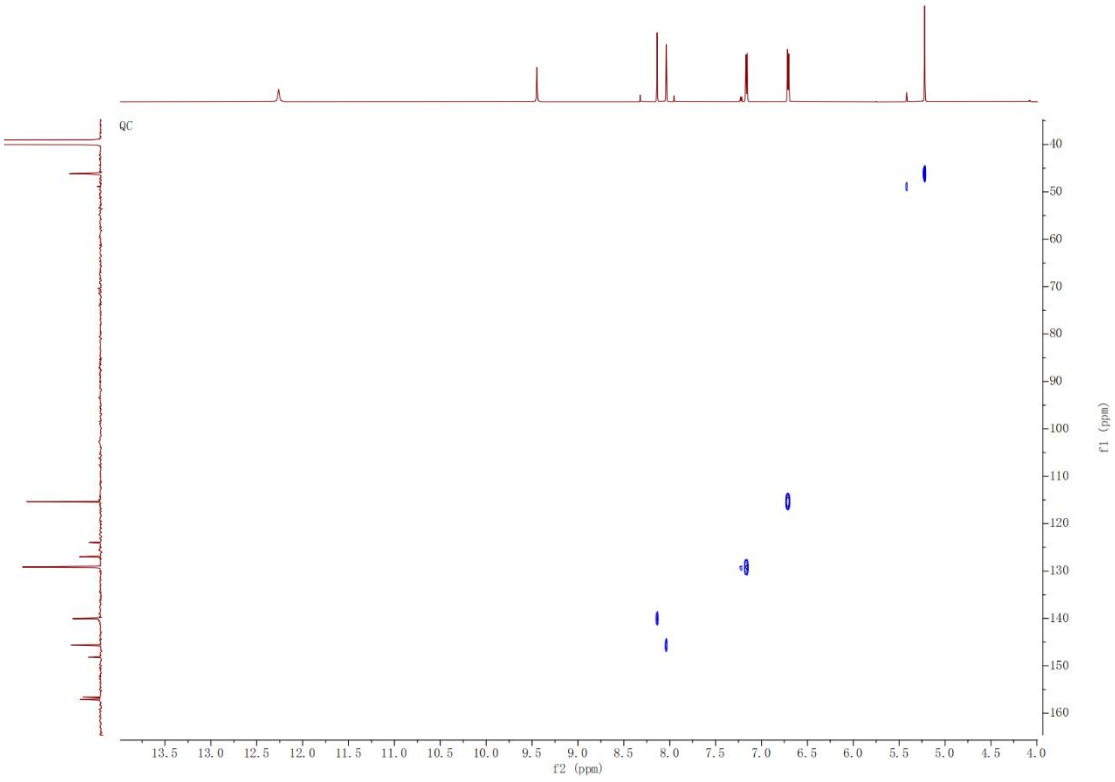



**Figure S26.** HSQC spectrum of **13**.

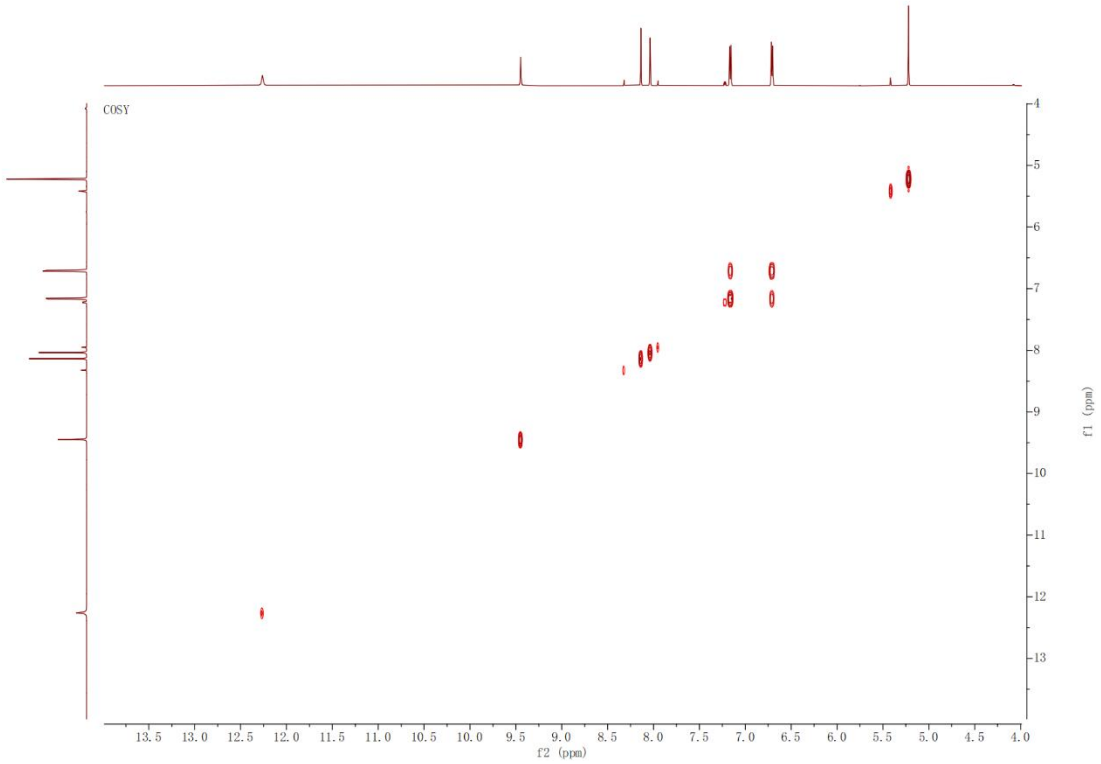



**Figure S27.** $^{1}$H-$^{1}$H COSY spectrum of **13**.

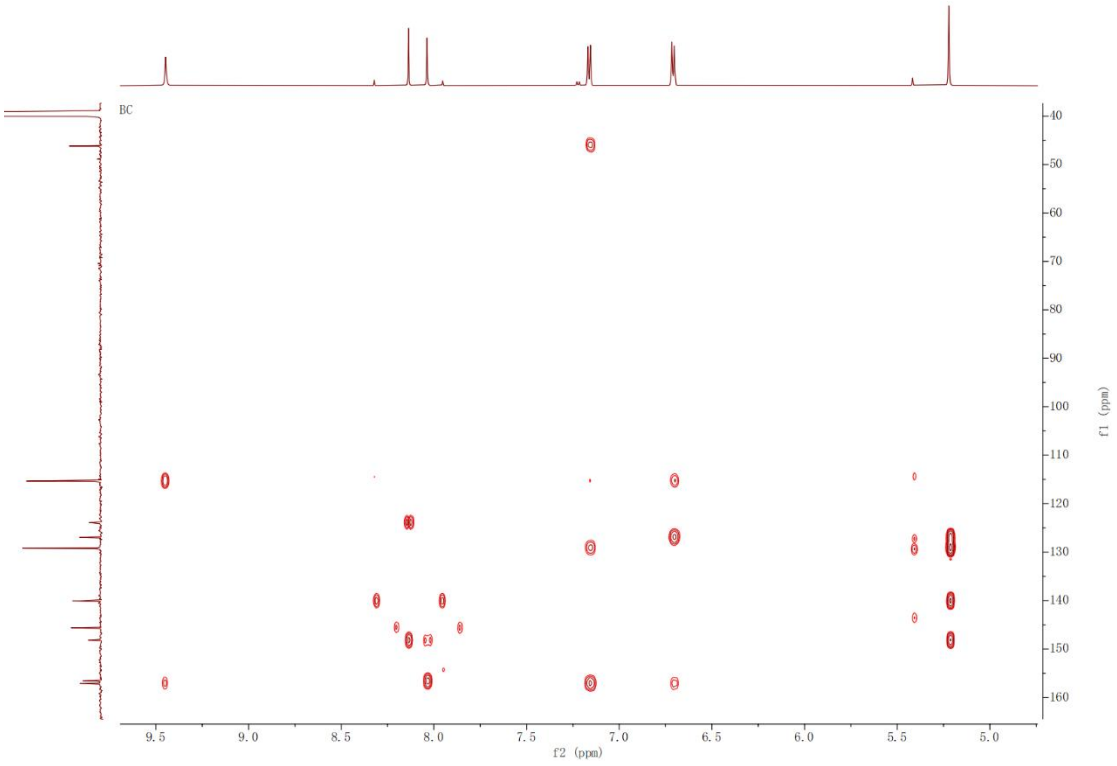



**Figure S28.** HMBC spectrum of **13**.

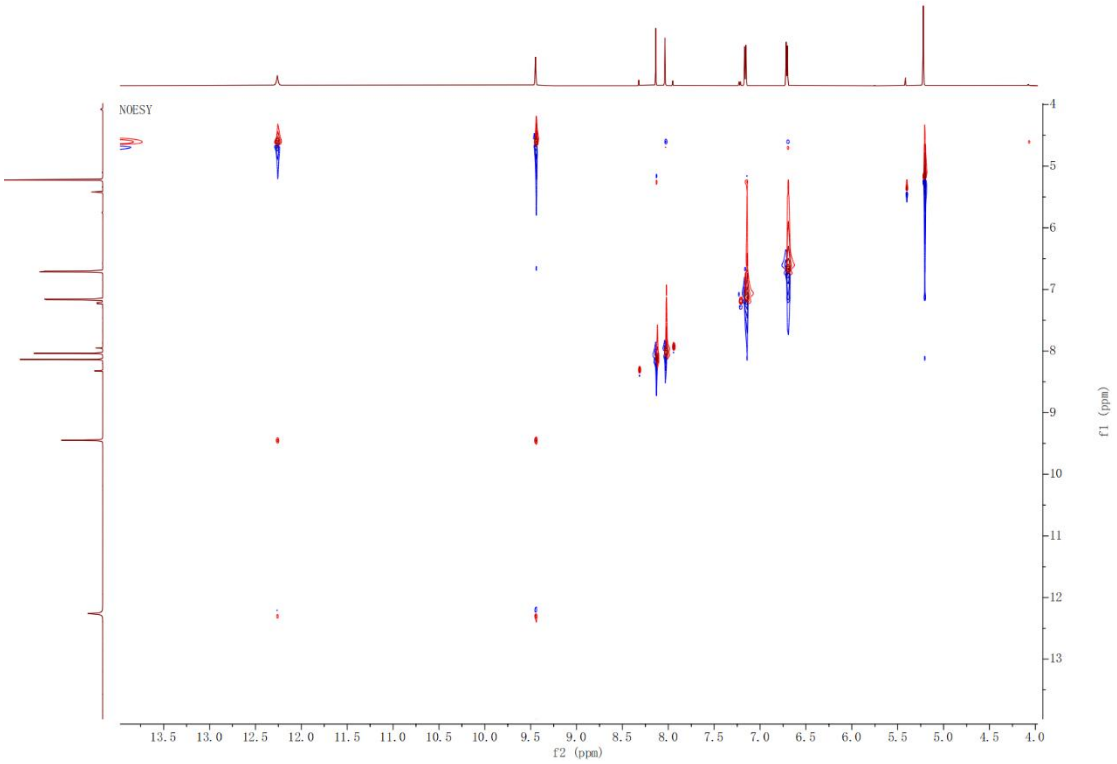
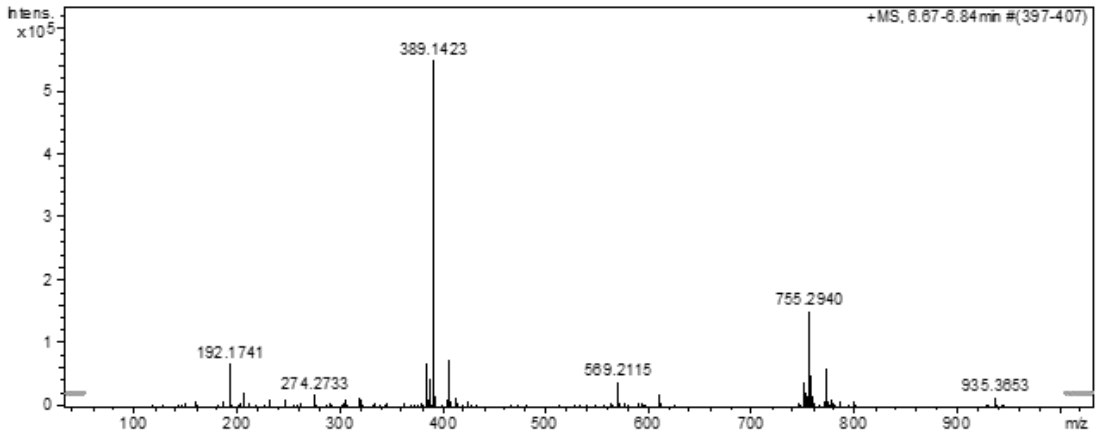



**Figure S29.** NOESY spectrum of **13**.




| Meas. m/z# | | Formula | Score | m/z | err [mDa] | err [ppm] | mSigma | rdb | e⁻ Conf | N-Rule |
|---|---|---|---|---|---|---|---|---|---|---|
| 389.1423 | 1 | C 11 H 22 N 6 Na 0 8 | 18.07 | 389.1391 | -3.2 | -8.2 | 1.5 | 3.5 | even | ok |
| | 2 | C 8 H 14 N 16 Na 0 2 | 3.22 | 389.1378 | -4.5 | -11.6 | 11 | 9.5 | even | ok |
| | 3 | C 15 H 26 Na 0 10 | 100 | 389.1418 | -0.5 | -1.3 | 16 | 2.5 | even | ok |
| | 4 | C 12 H 18 N 10 Na 0 4 | 43.97 | 389.1405 | -1.8 | -4.7 | 16.3 | 8.5 | even | ok |
| | 5 | C 16 H 22 N 4 Na 0 6 | 64.35 | 389.1432 | 0.8 | 2.2 | 28.1 | 7.5 | even | ok |
| | 6 | C 13 H 14 N 14 Na | 70.51 | 389.1418 | -0.5 | -1.3 | 31.3 | 13.5 | even | ok |
| | 7 | C 17 H 18 N 8 Na 0 2 | 17.97 | 389.1445 | 2.2 | 5.6 | 42.9 | 12.5 | even | ok |
| | 8 | C 21 H 22 N 2 Na 0 4 | 0.67 | 389.1472 | 4.9 | 12.5 | 55.2 | 11.5 | even | ok |

**Figure S30** HRESIMS spectrum of **17**.

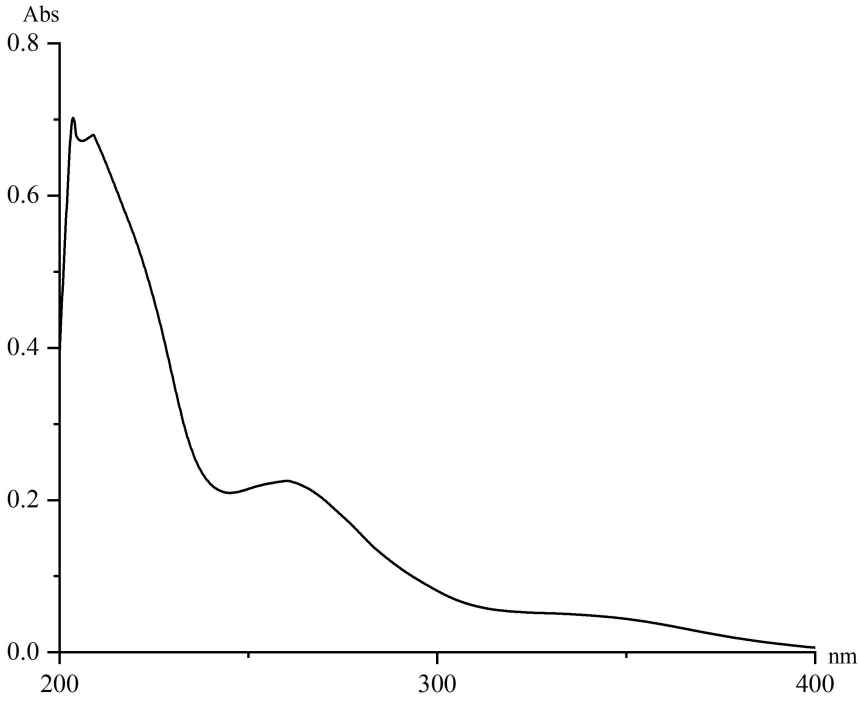



**Figure S31** UV spectrum of **17**.

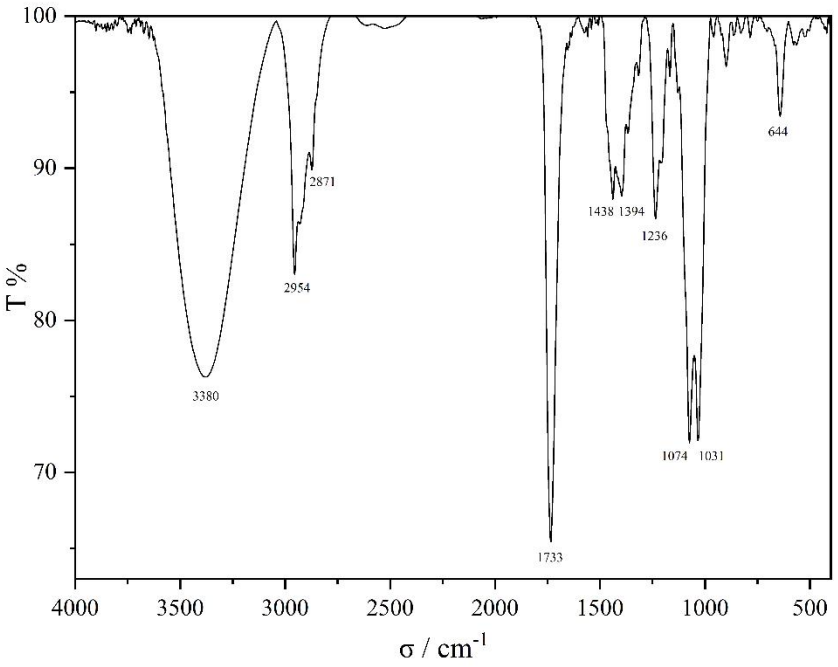



**Figure S32** IR spectrum of **17**.

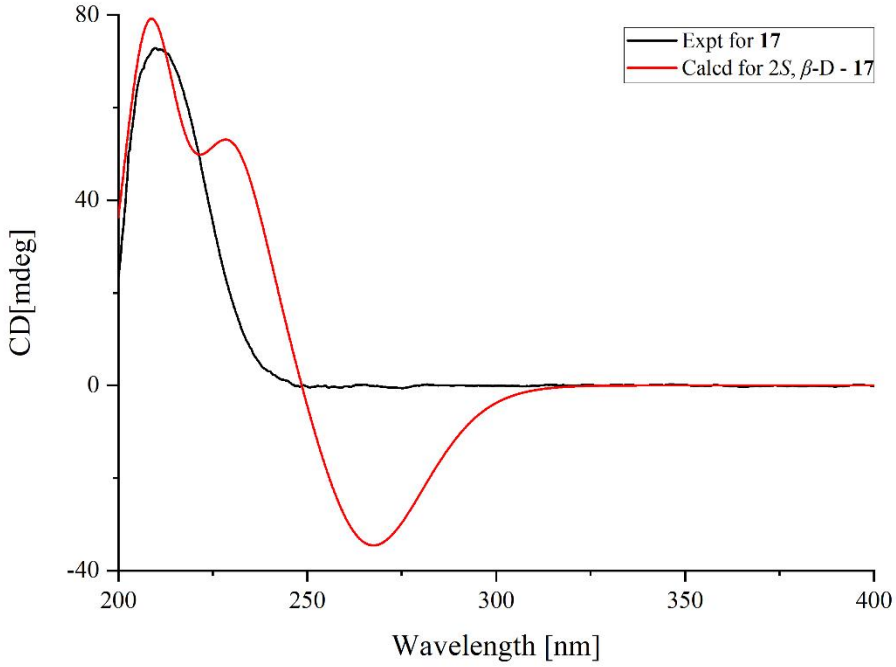



**Figure S33** CD spectrum of **17**.

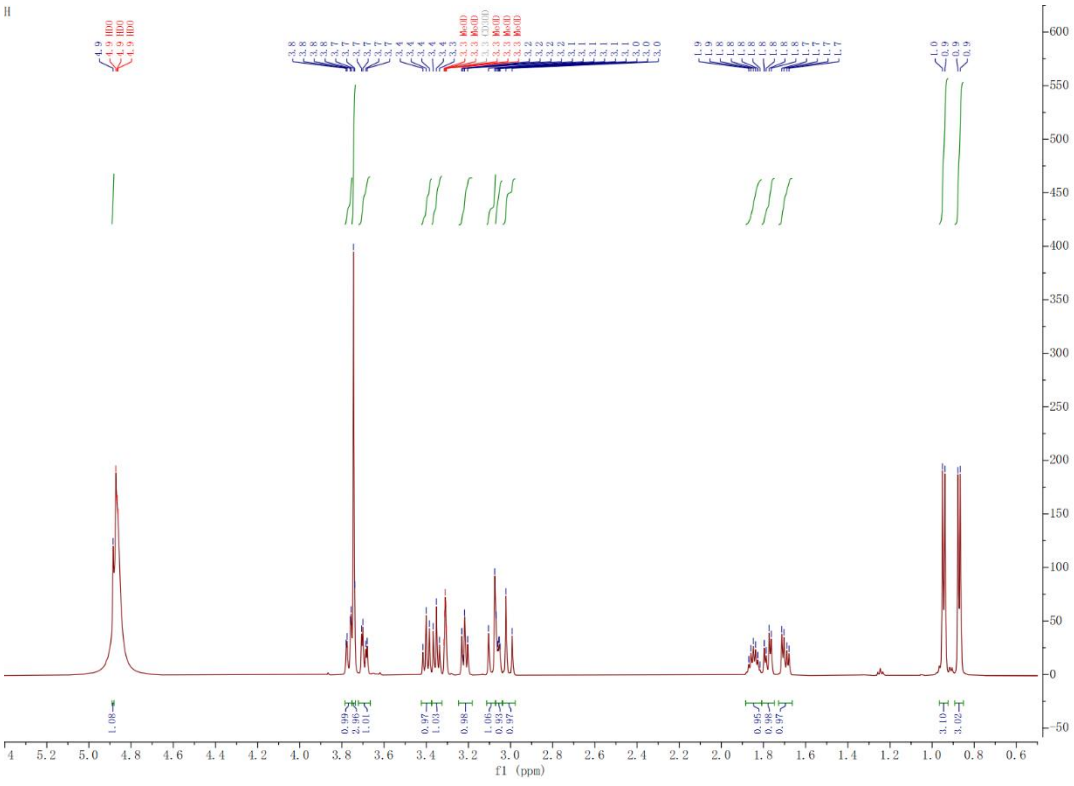



**Figure S34** $^{1}$H NMR spectrum of **17**.

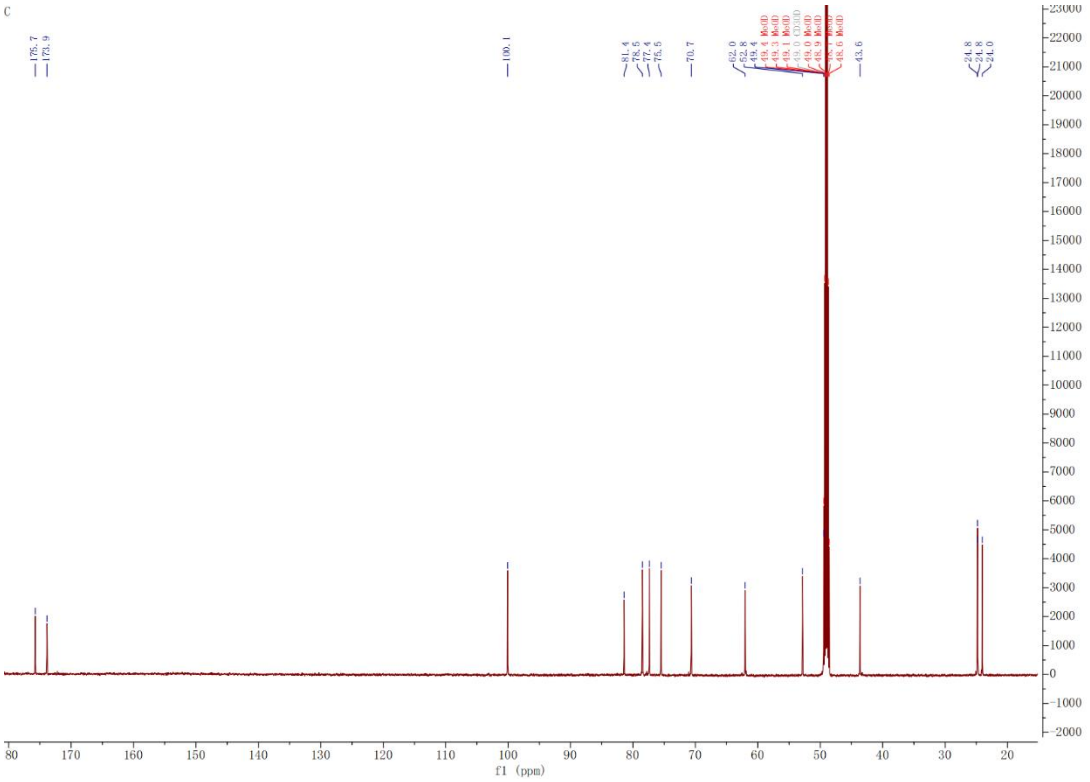



**Figure S35** $^{13}$C NMR spectrum of **17**.

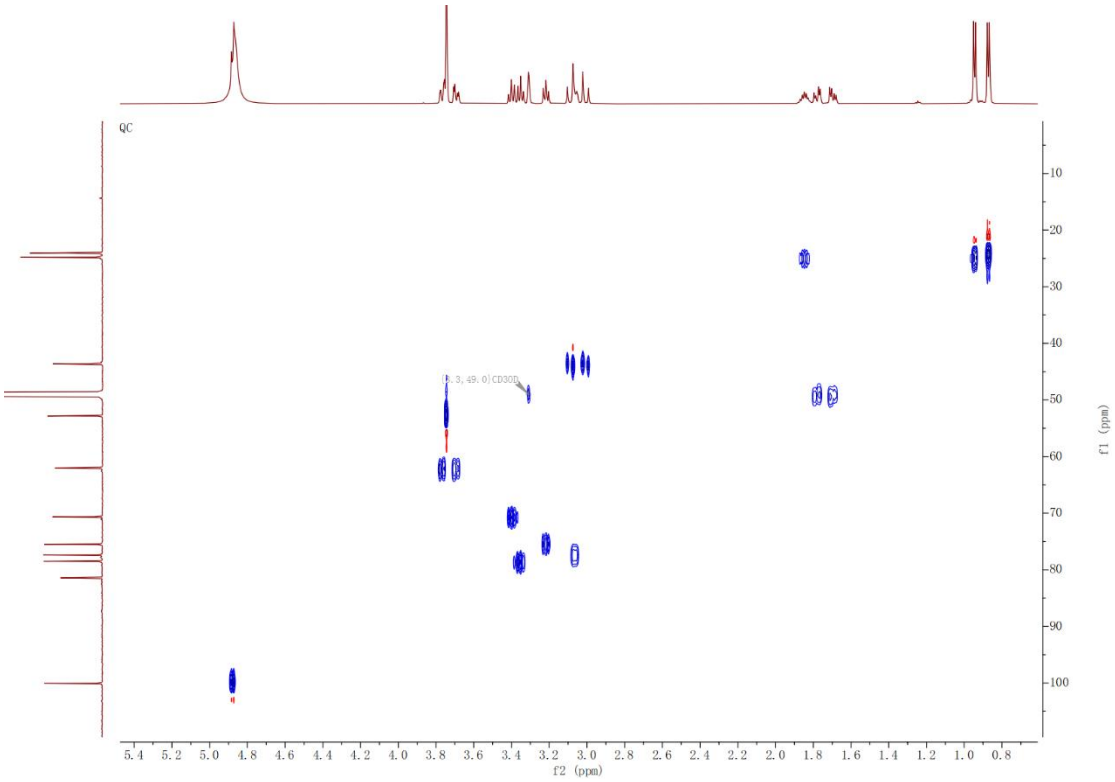



**Figure S36** HSQC spectrum of **17**.

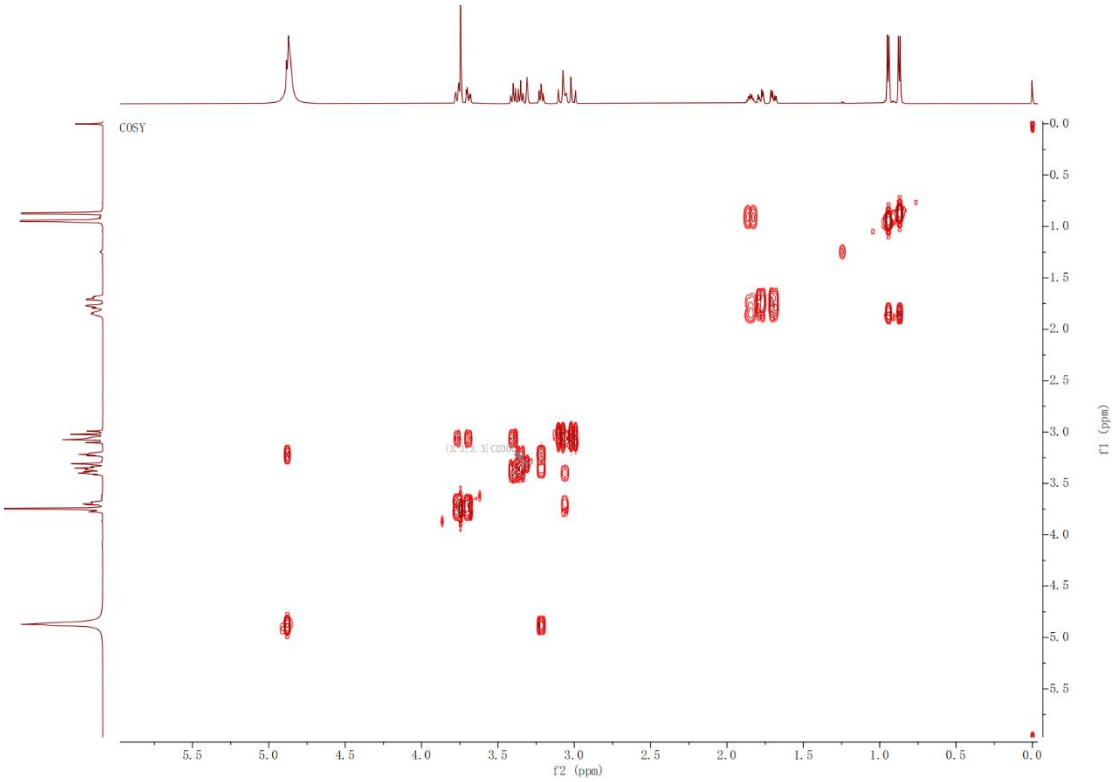



**Figure S37** $^{1}$H-$^{1}$H COSY spectrum of **17**.

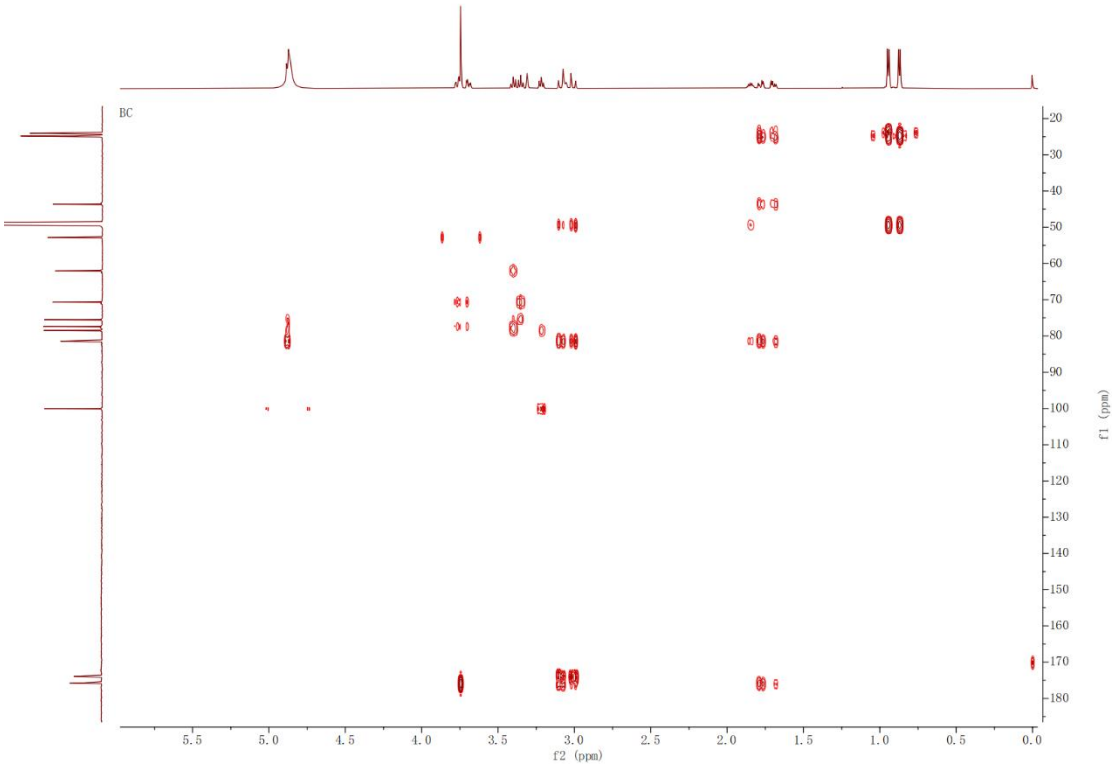



**Figure S38** HMBC spectrum of **17**.

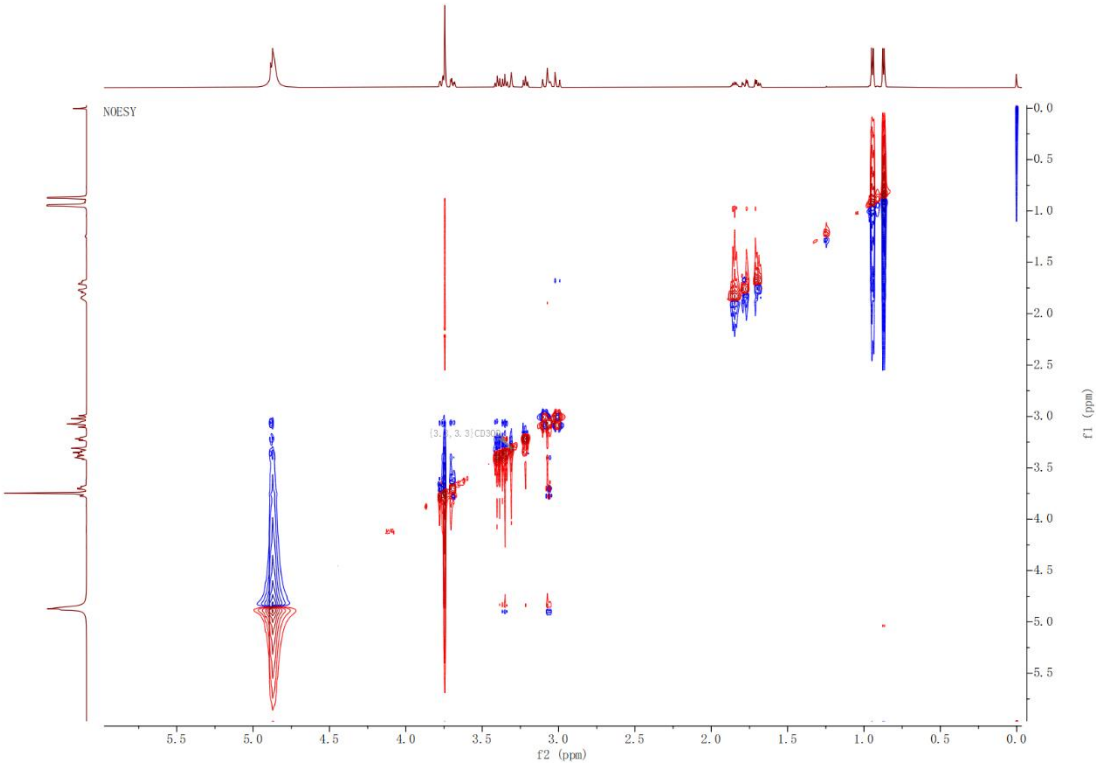



**Figure S39** NOESY spectrum of **17**.

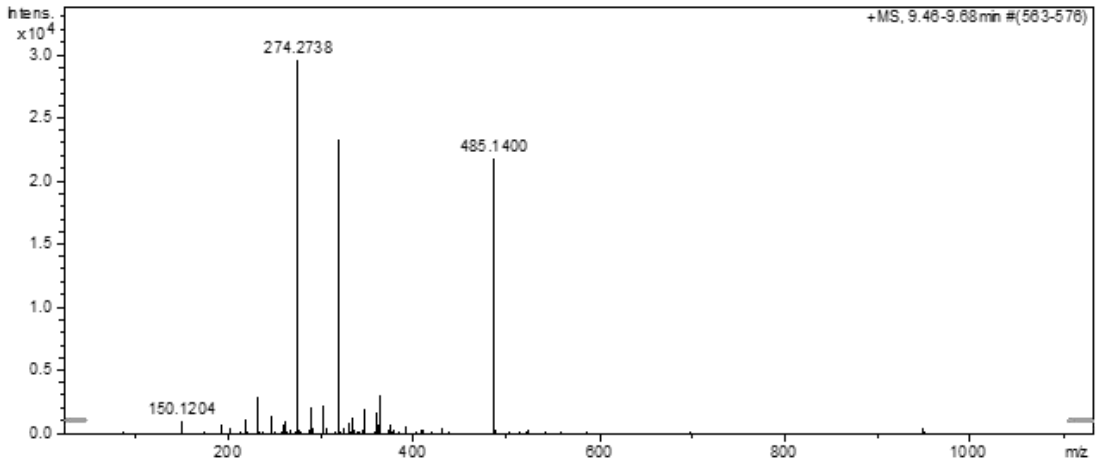



**Figure S40.** HRESIMS spectrum of **3**.

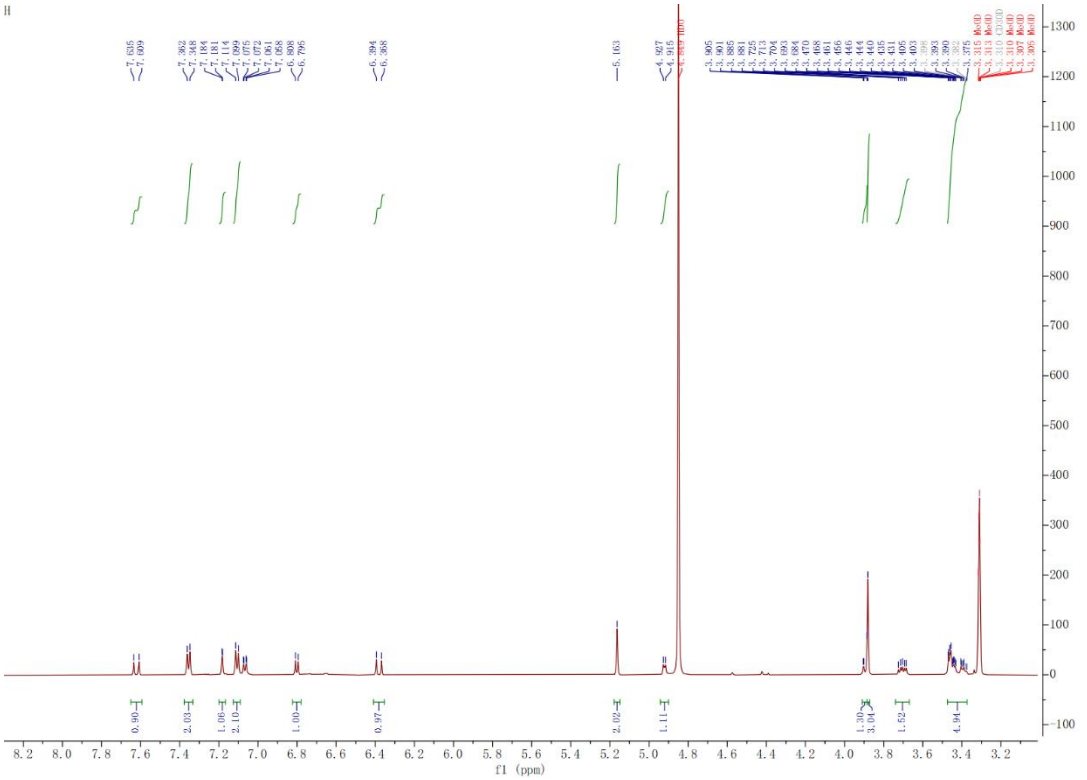


**Figure S41.** $^{1}$H NMR spectrum of **3**.

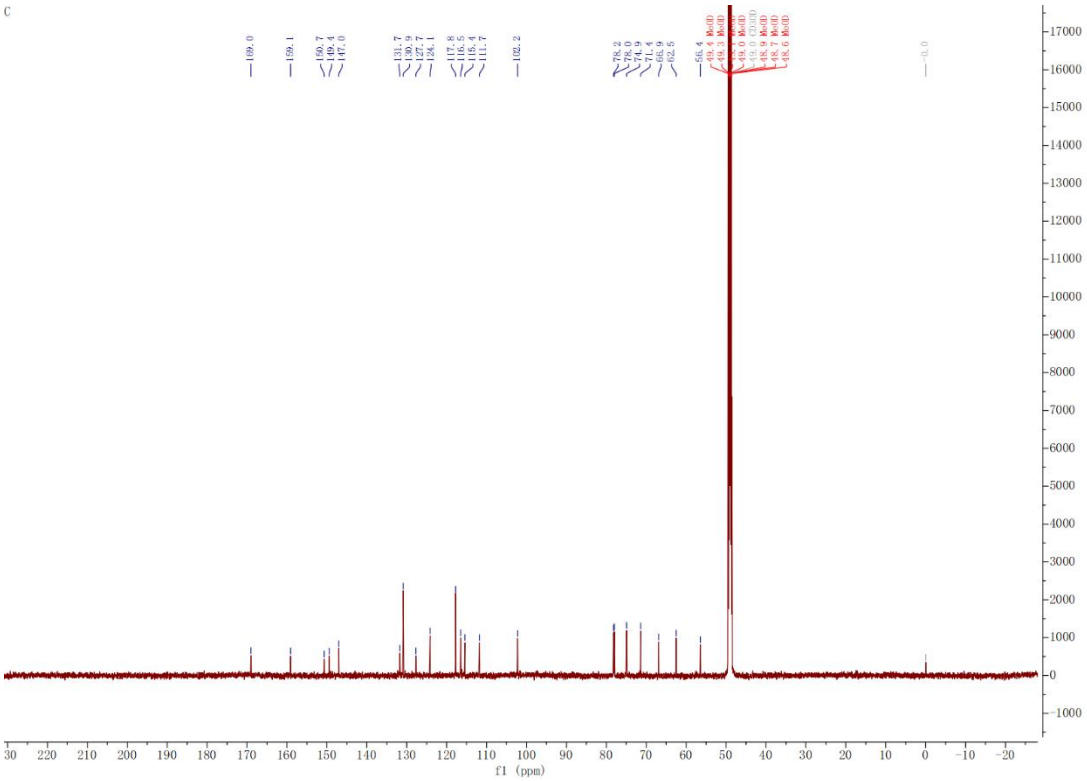


**Figure S42.** $^{13}$C NMR spectrum of **3**.

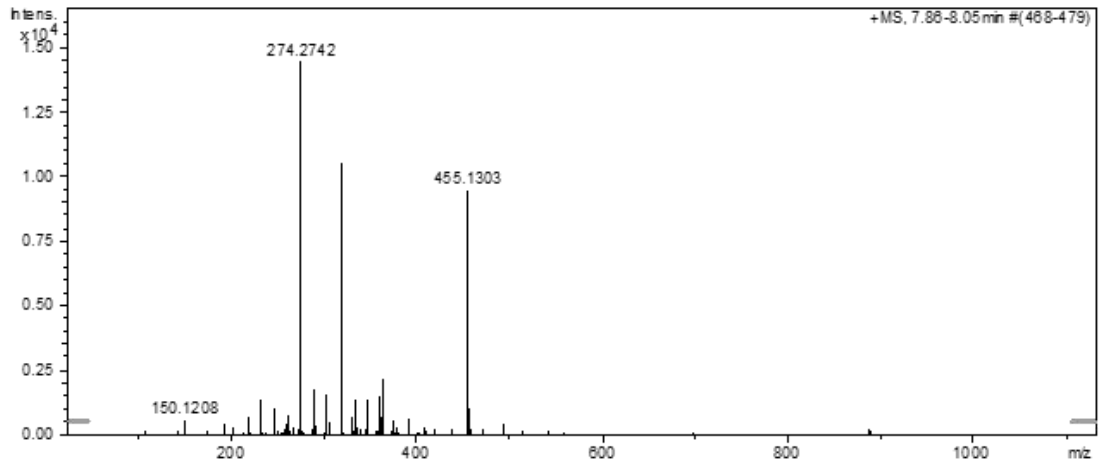



**Figure S43.** HRESIMS spectrum of **4**.

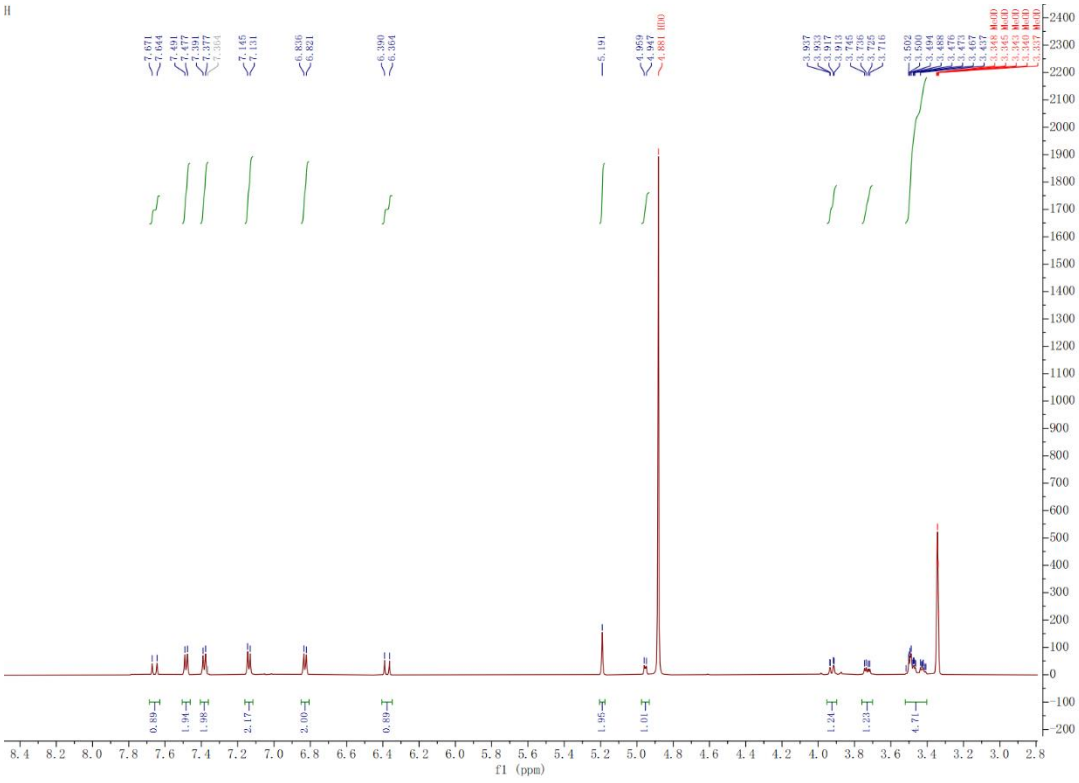



**Figure S44.** $^{1}$H NMR spectrum of **4**.

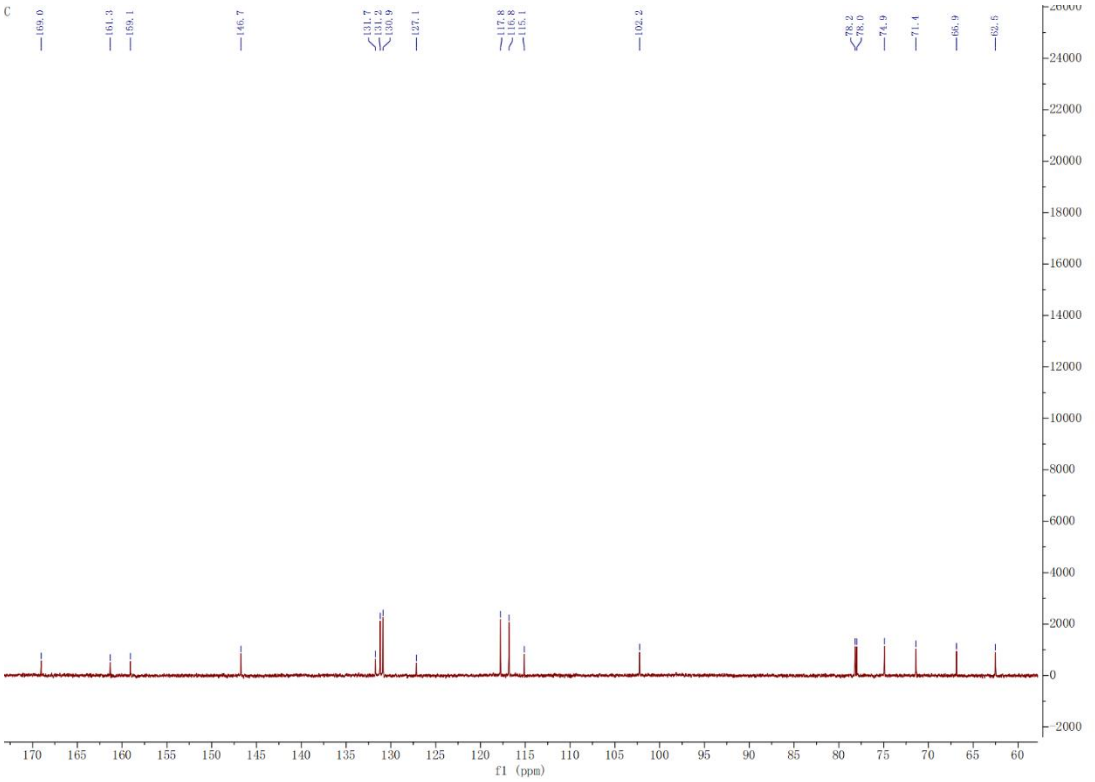



**Figure S45.** $^{13}$C NMR spectrum of **4**.

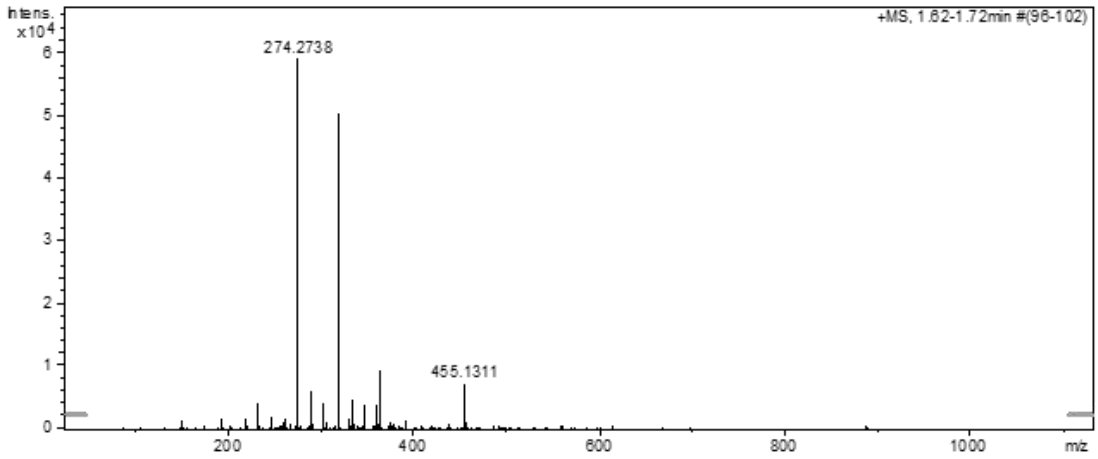



**Figure S46.** HRESIMS spectrum of **5**.

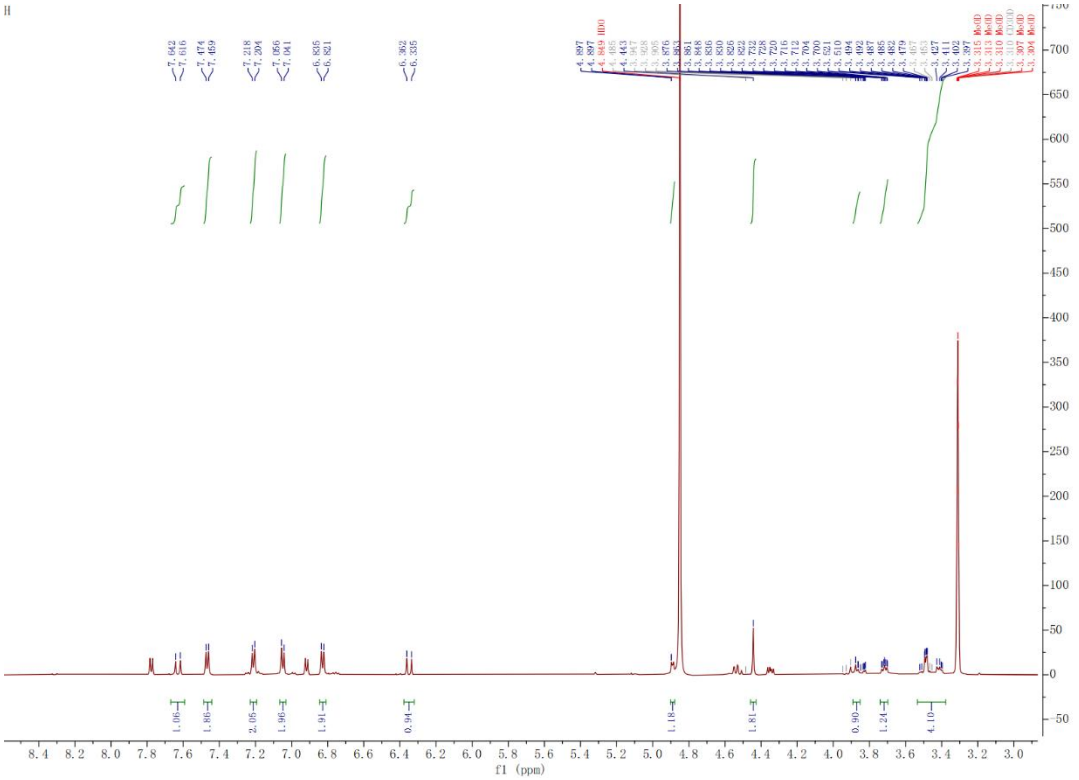


**Figure S47.** $^{1}$H NMR spectrum of **5**.

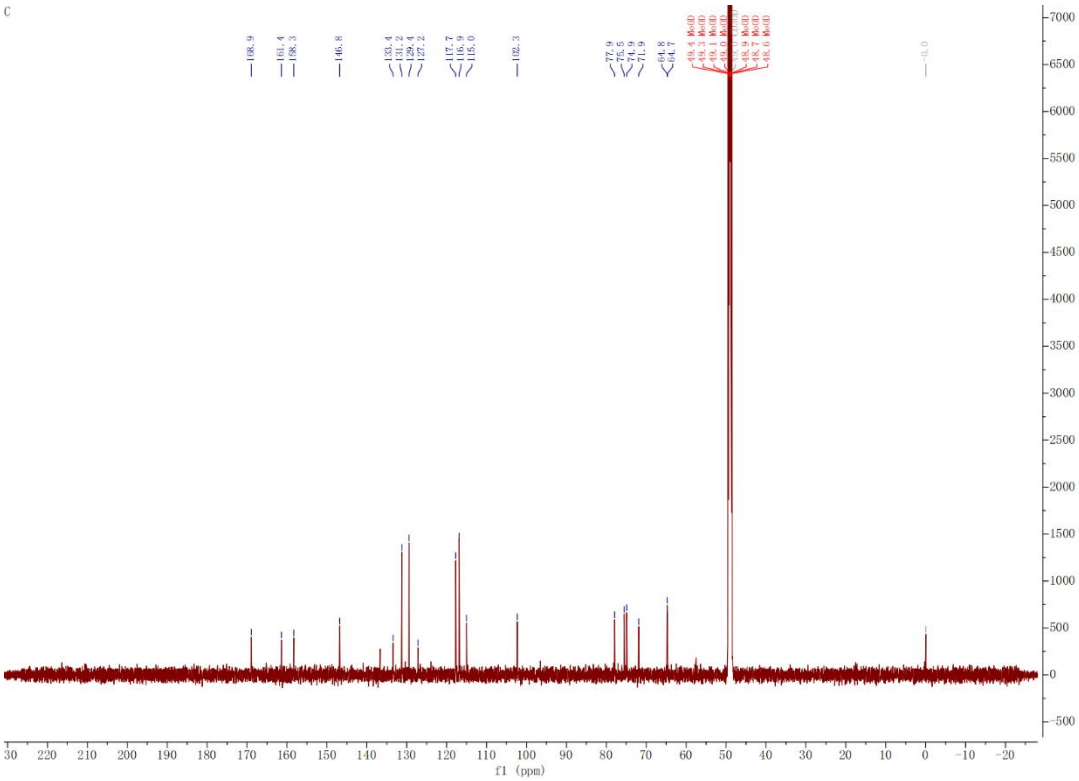


**Figure S48.** $^{13}$C NMR spectrum of **5**.

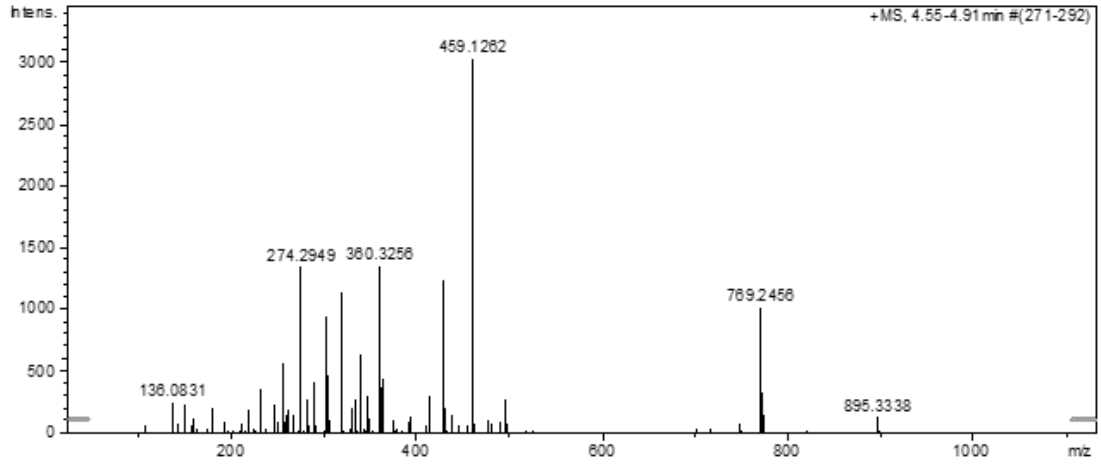



**Figure S49.** HRESIMS spectrum of **6**.

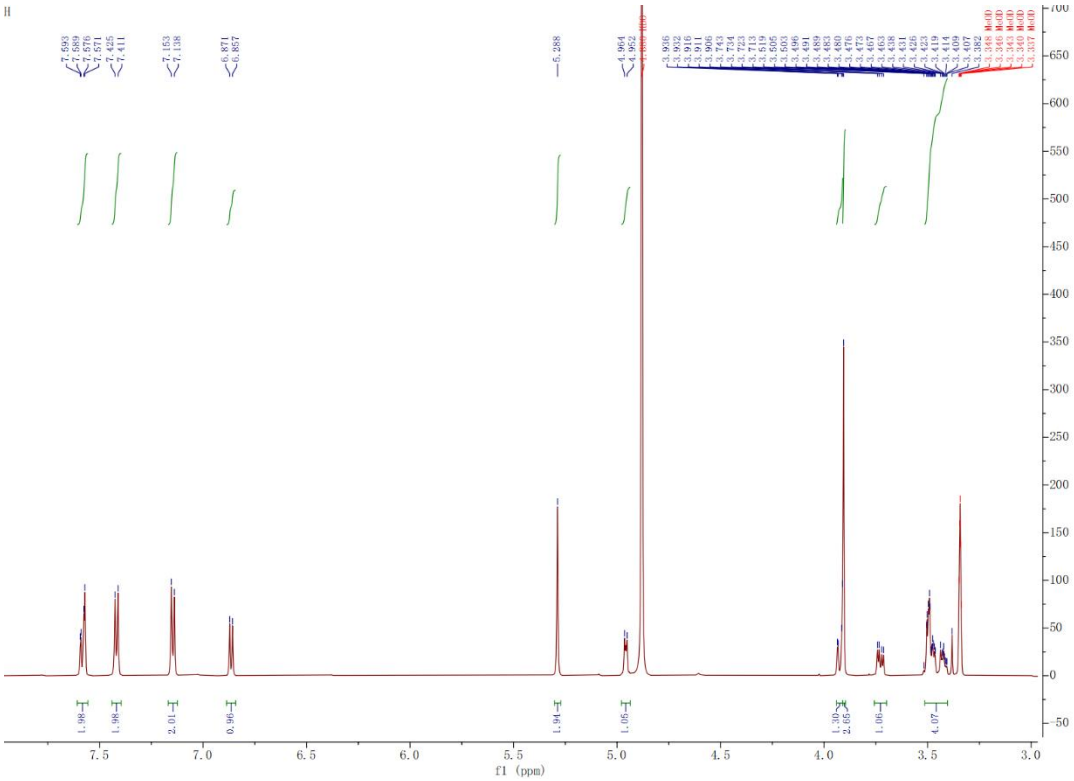



**Figure S50.** $^{1}$H NMR spectrum of **6**.

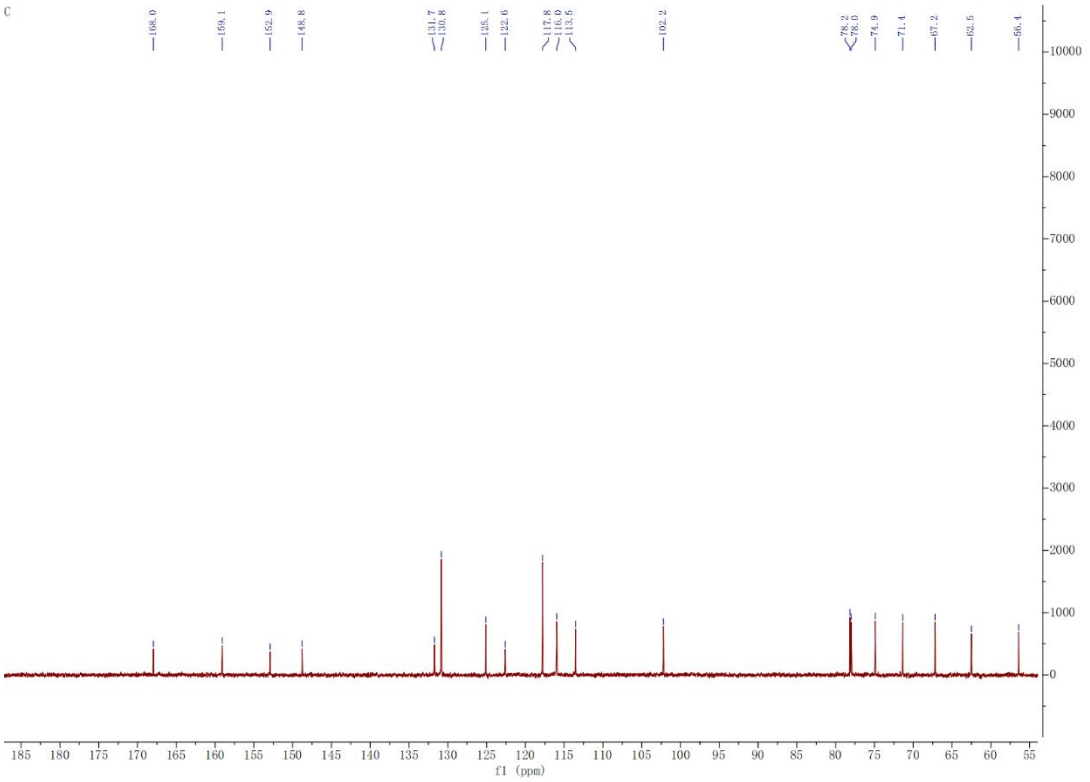



**Figure S51.** $^{13}$C NMR spectrum of **6**.

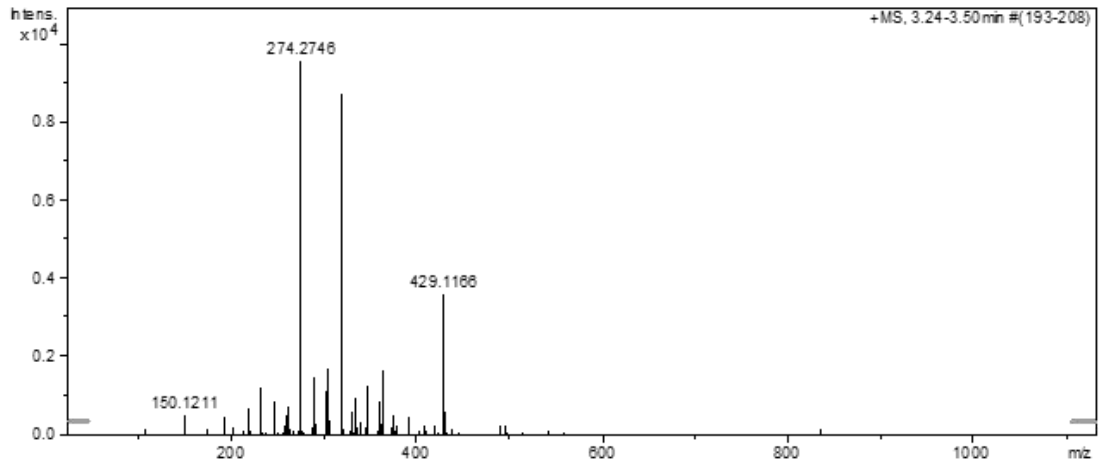



**Figure S52.** HRESIMS spectrum of **7**.

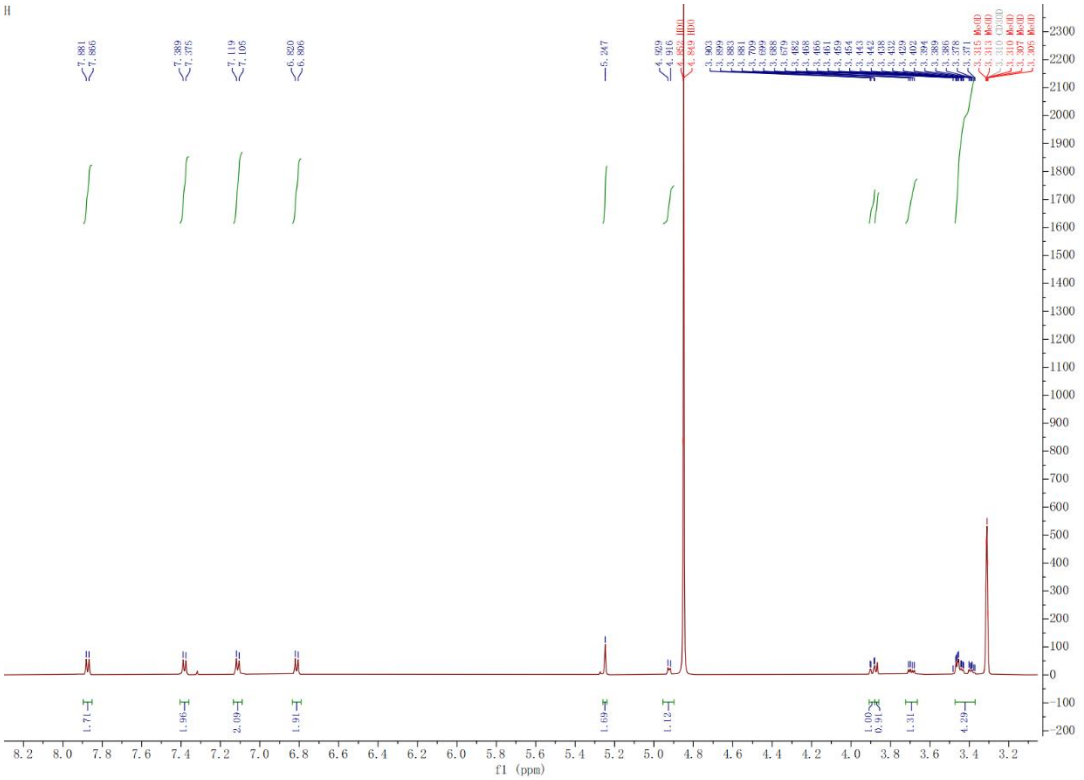


**Figure S53.** $^{1}$H NMR spectrum of **7**.

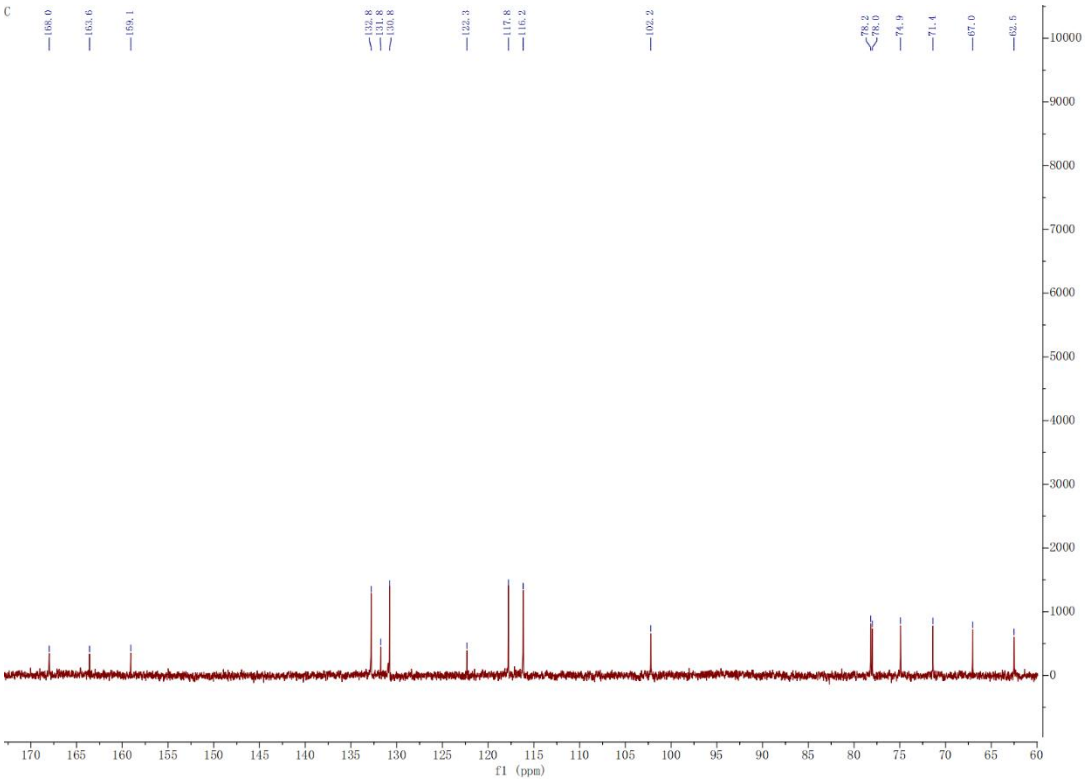


**Figure S54.** $^{13}$C NMR spectrum of **7**.

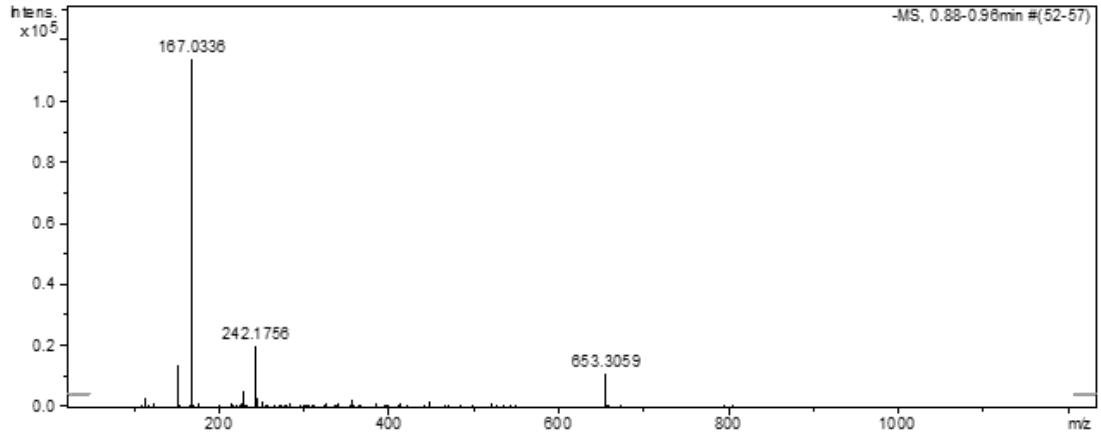



**Figure S55.** HRESIMS spectrum of **8**.

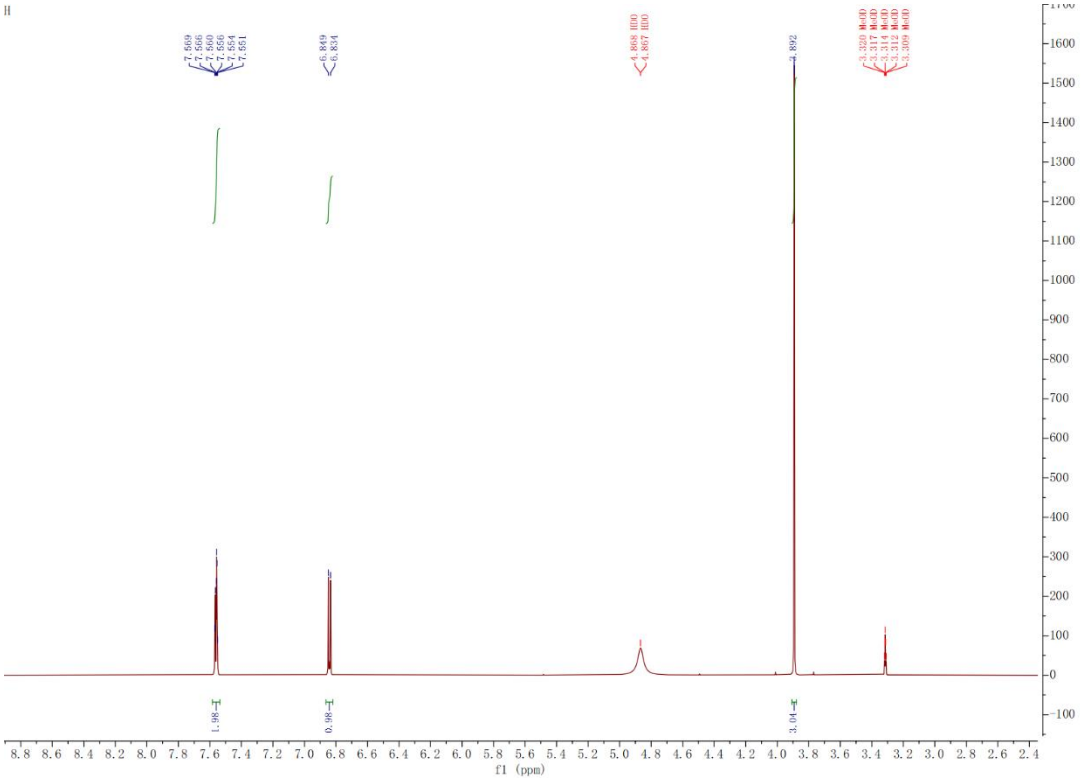



**Figure S56.** $^{1}$H NMR spectrum of **8**.

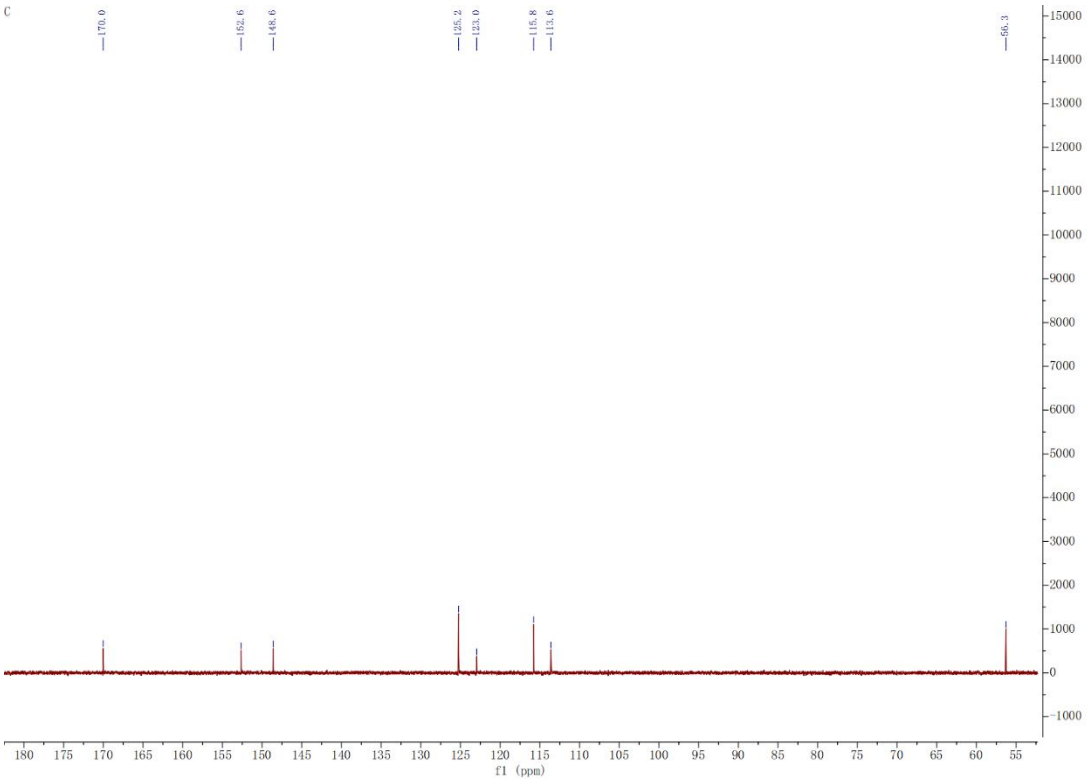



**Figure S57.** $^{13}$C NMR spectrum of **8**.

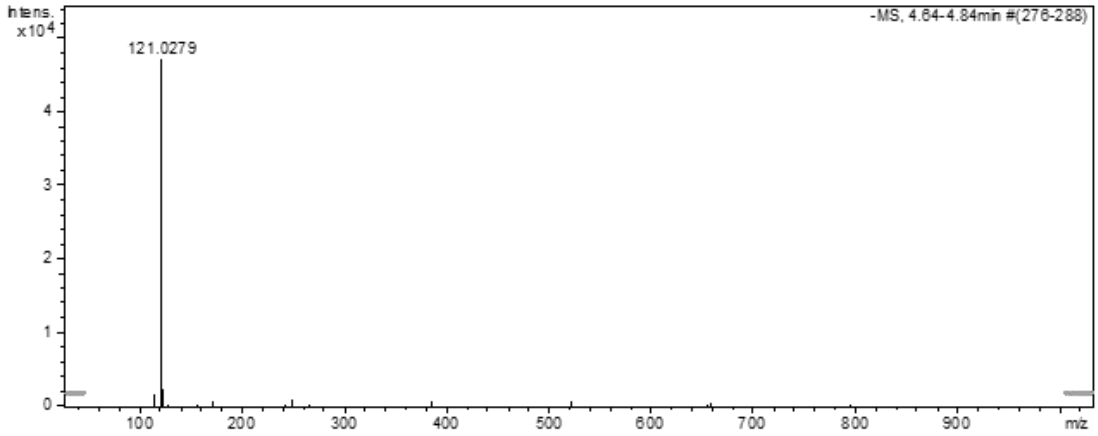



**Figure S58.** HRESIMS spectrum of **9**.

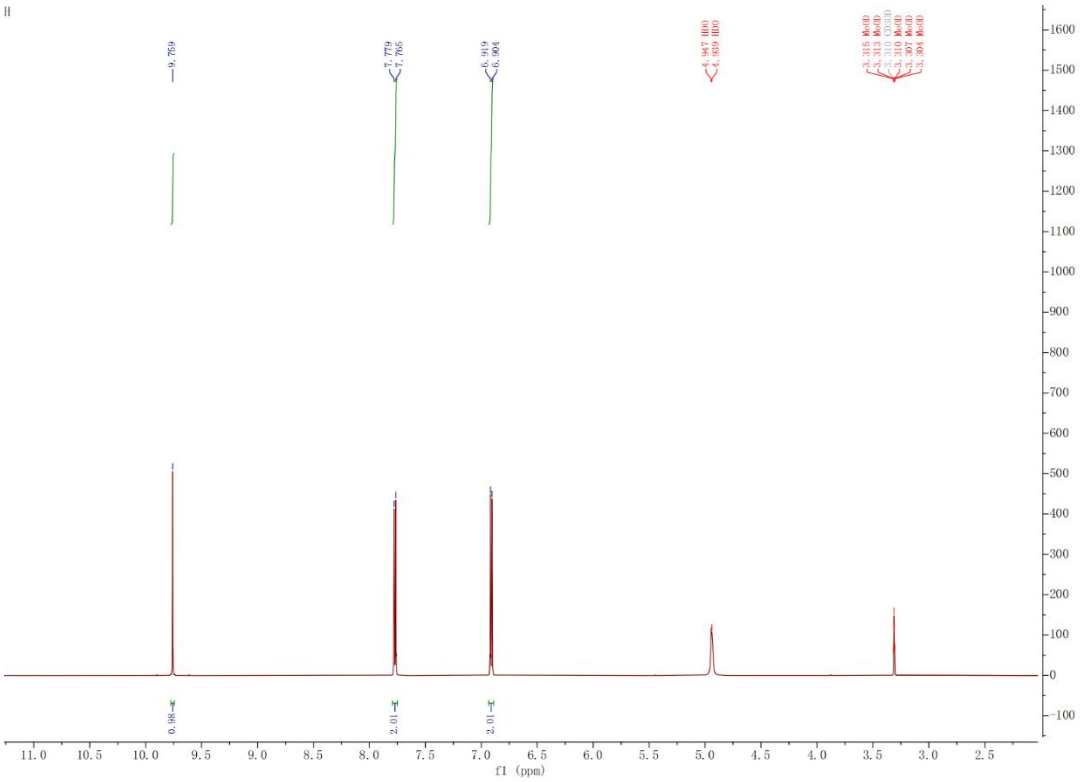



**Figure S59.** $^{1}$H NMR spectrum of **9**.

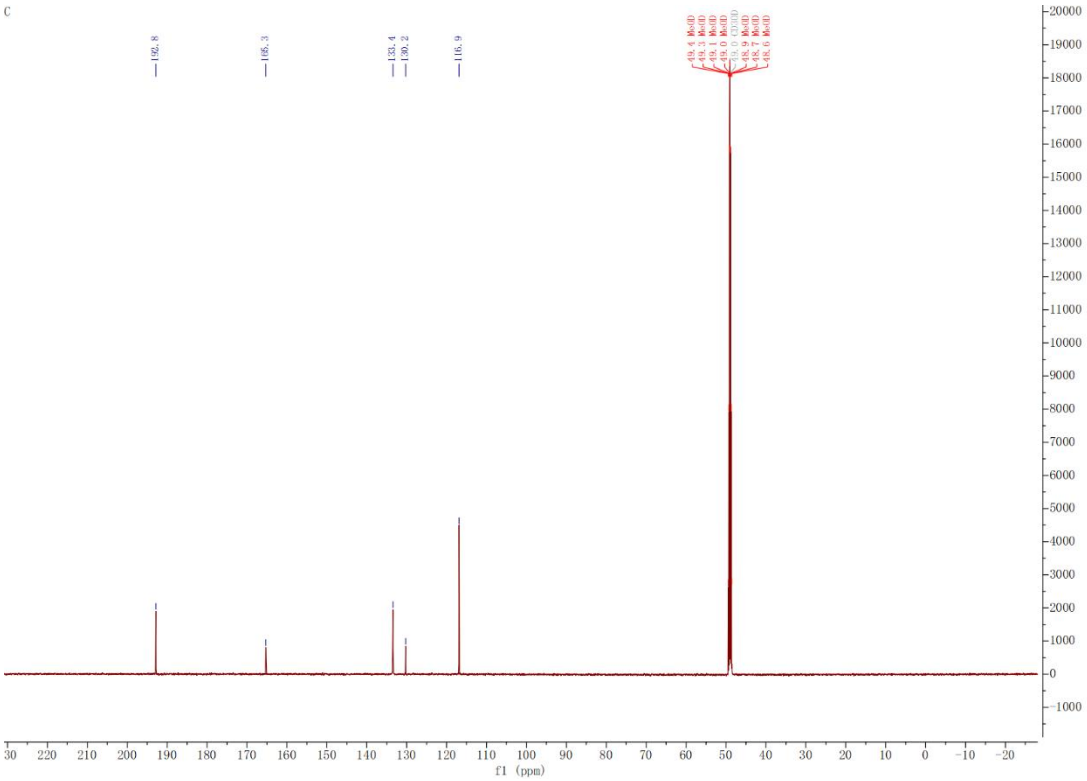



**Figure S60.** $^{13}$C NMR spectrum of **9**.

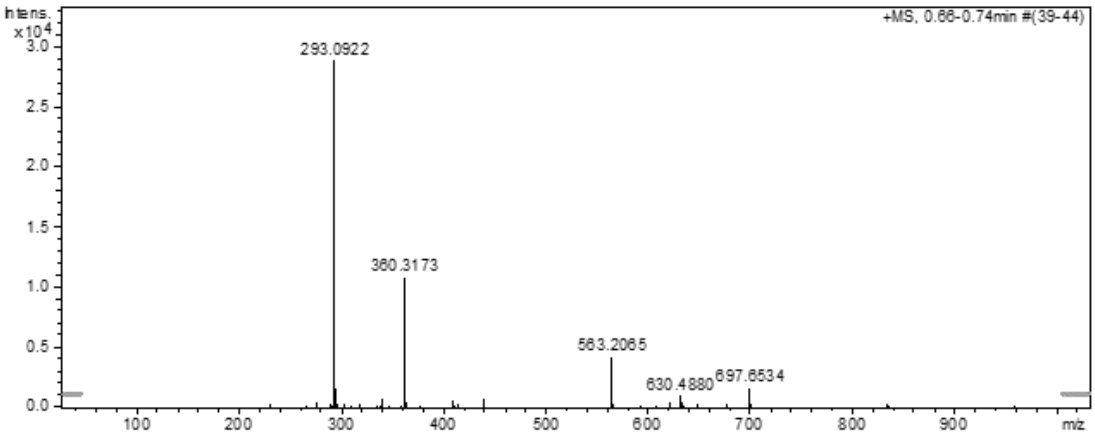



**Figure S61.** HRESIMS spectrum of **10**.

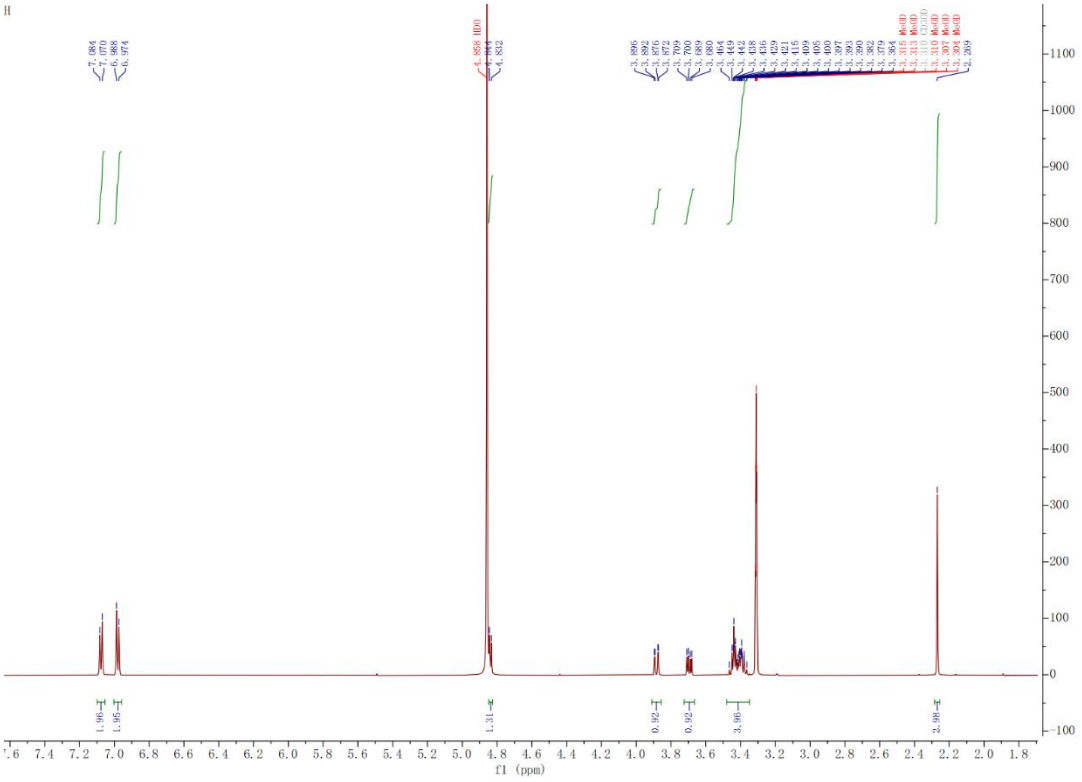



**Figure S62.** $^{1}$H NMR spectrum of **10**.

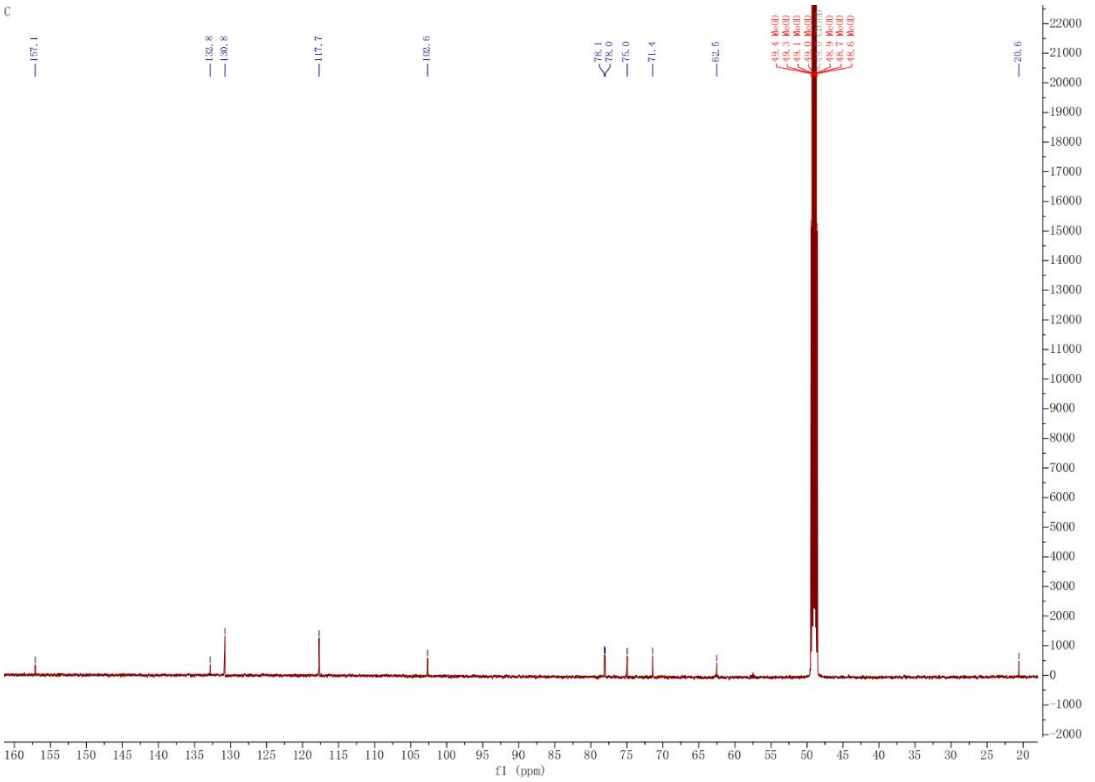



**Figure S63.** $^{13}$C NMR spectrum of **10**.

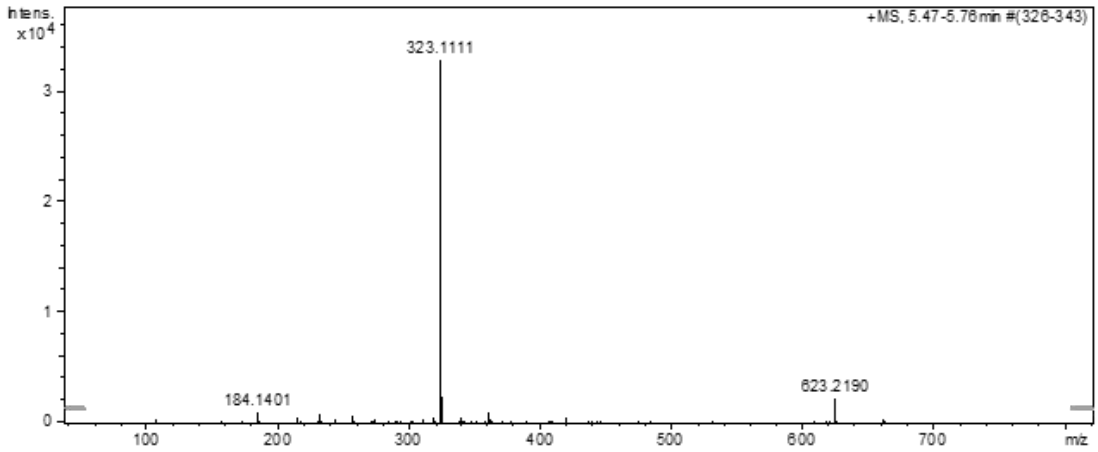



**Figure S64.** HRESIMS spectrum of **11**.

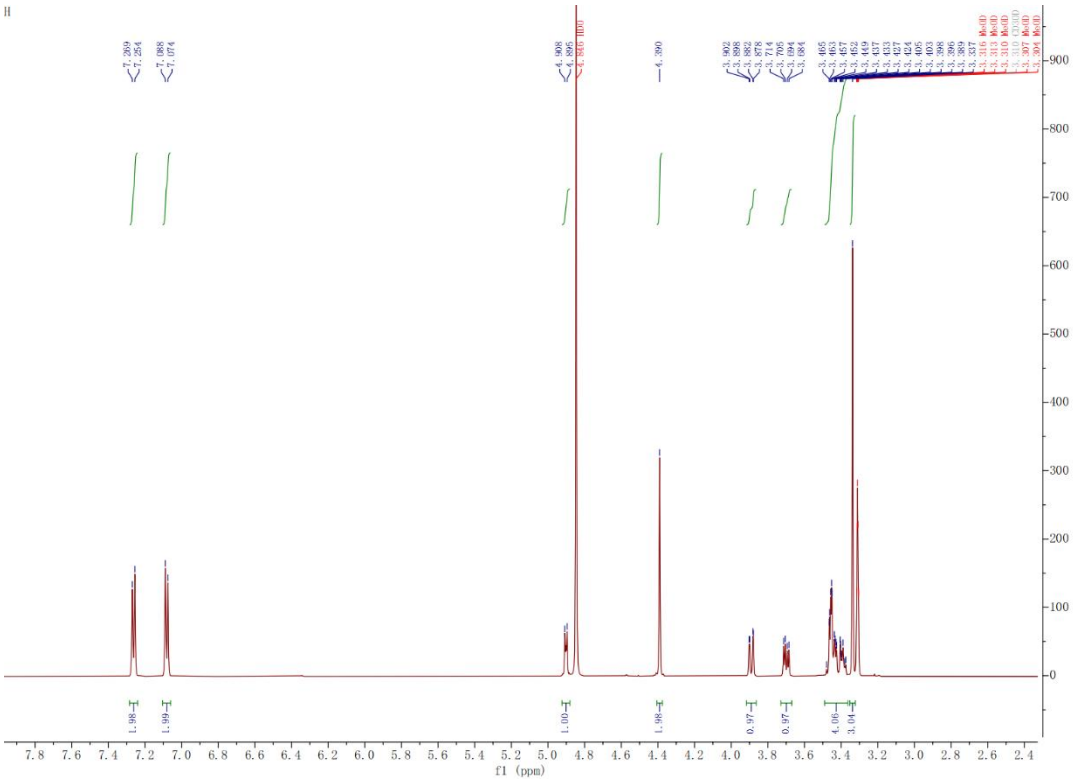


**Figure S65.** $^{1}$H NMR spectrum of **11**.

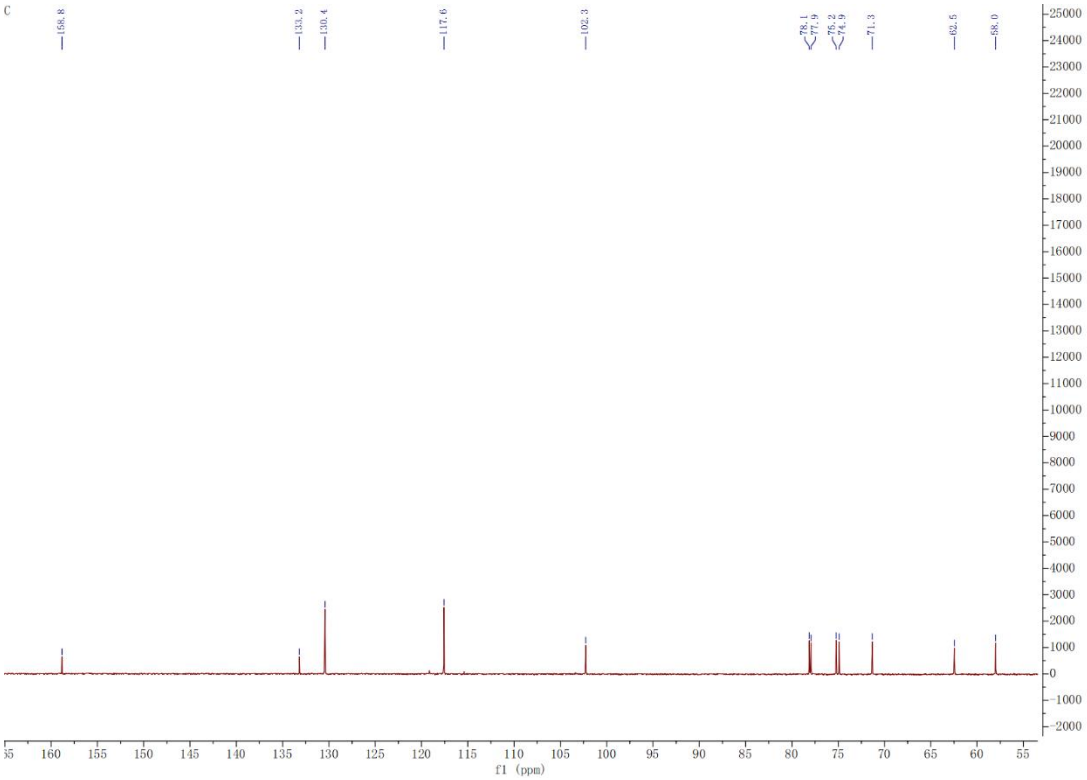


**Figure S66.** $^{13}$C NMR spectrum of **11**.

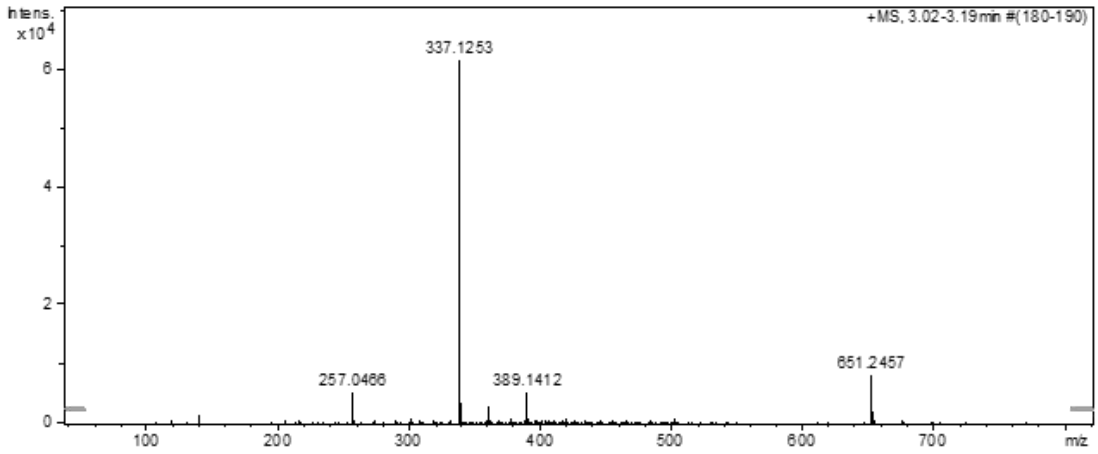



**Figure S67.** HRESIMS spectrum of **12**.

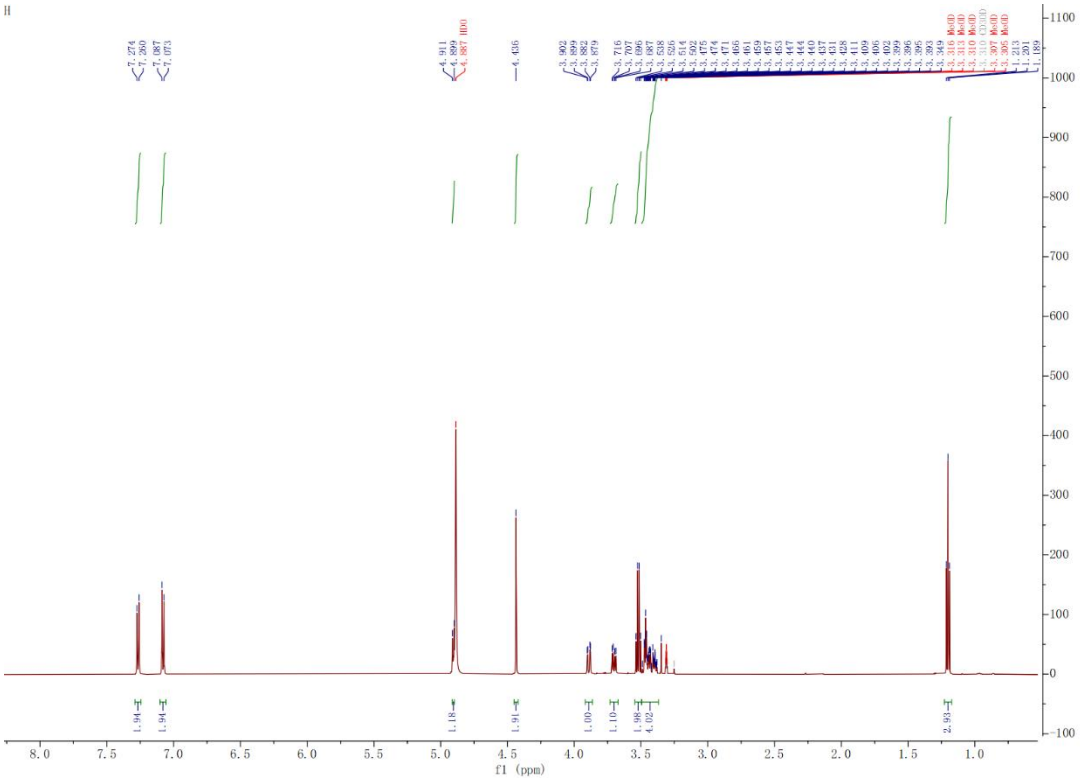



**Figure S68.** $^{1}$H NMR spectrum of **12**.

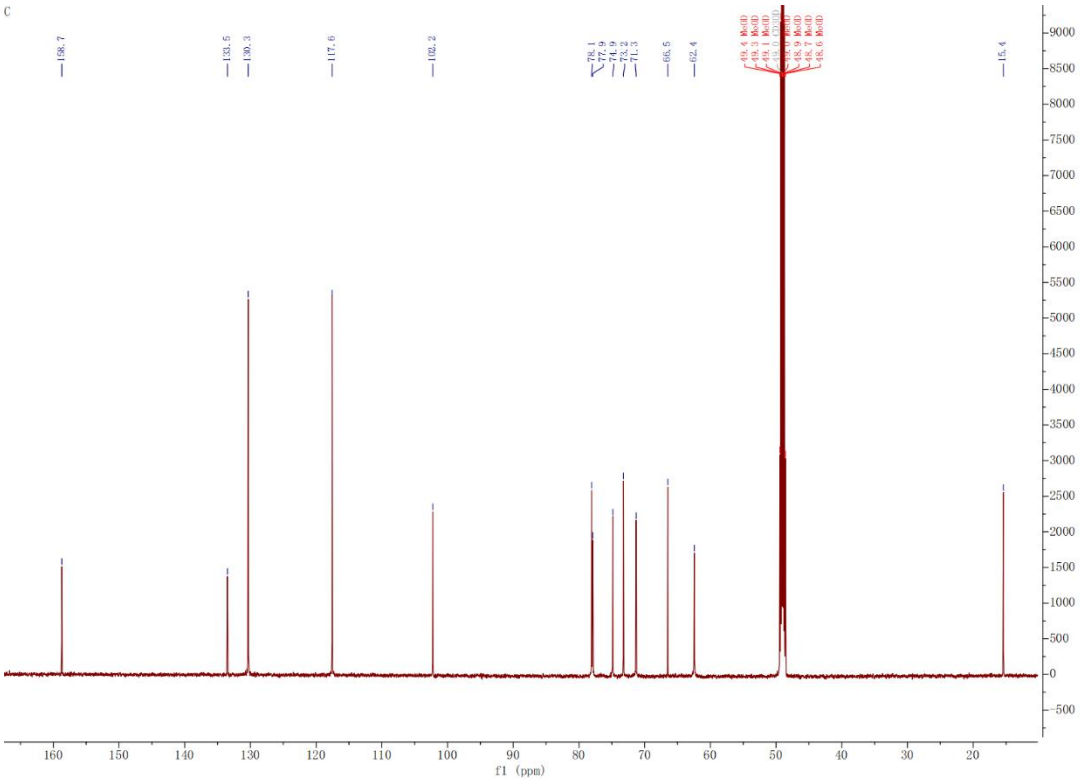



**Figure S69.** $^{13}$C NMR spectrum of **12**.

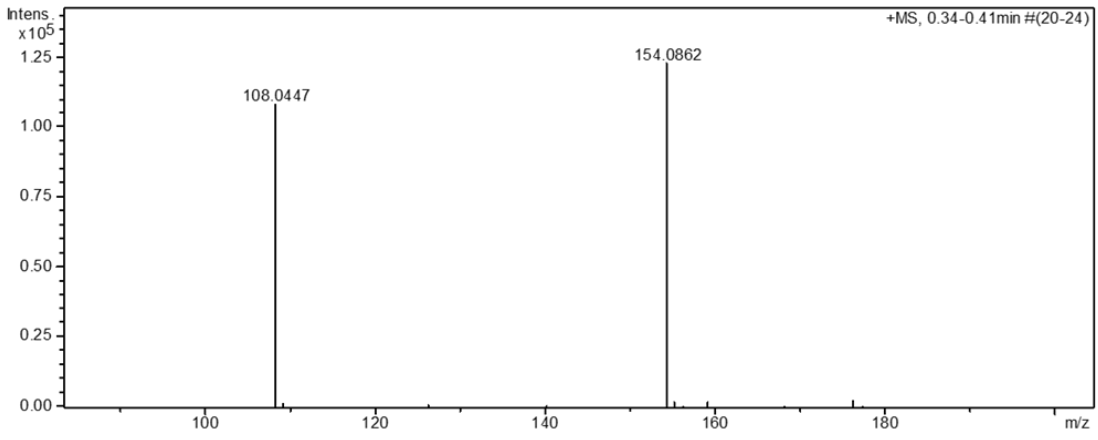



**Figure S70.** HRESIMS spectrum of **14**.

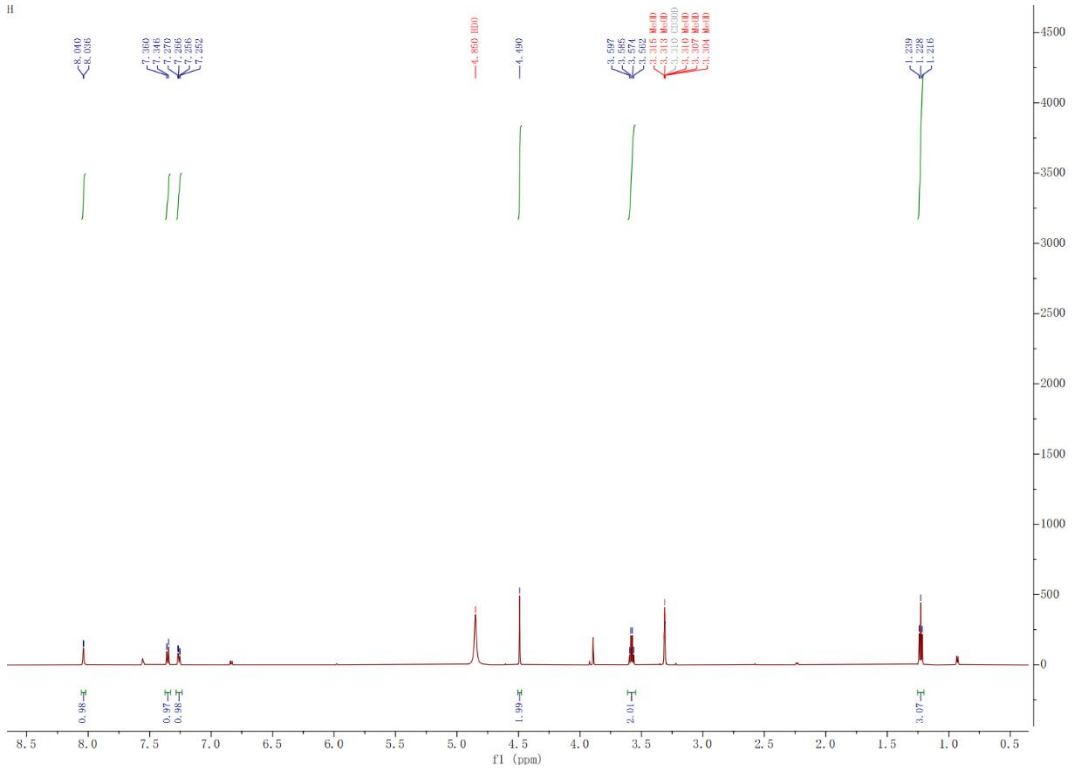


**Figure S71.** $^{1}$H NMR spectrum of **14**.

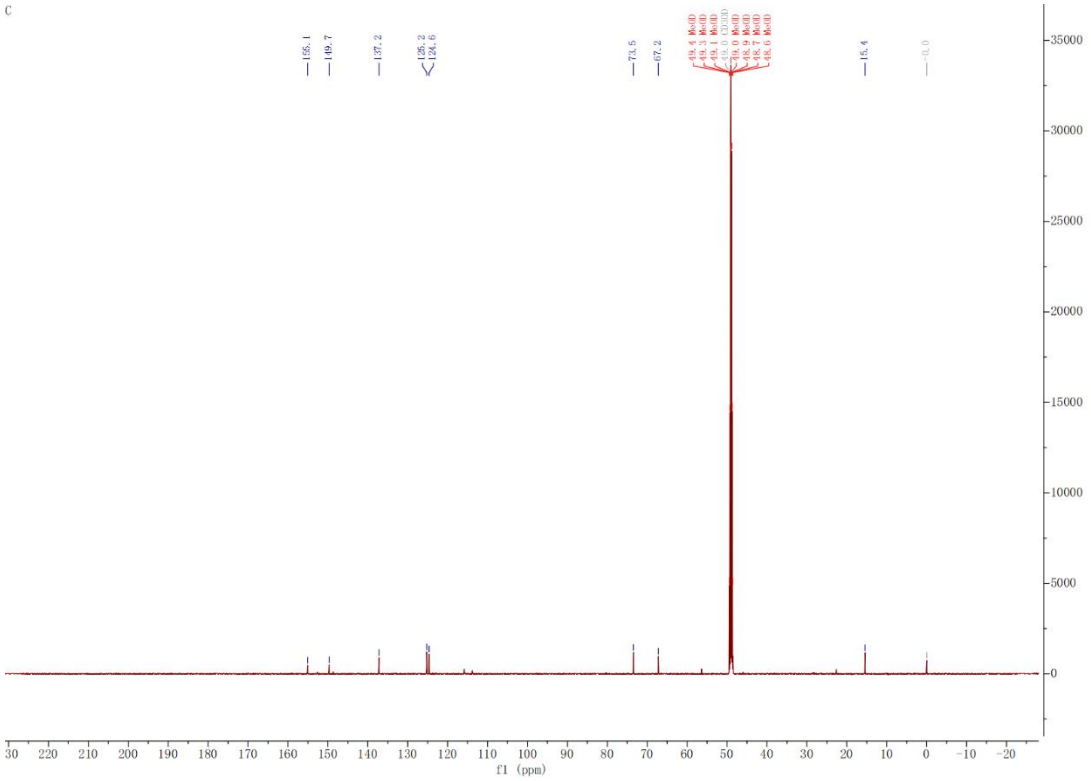


**Figure S72.** $^{13}$C NMR spectrum of **14**.

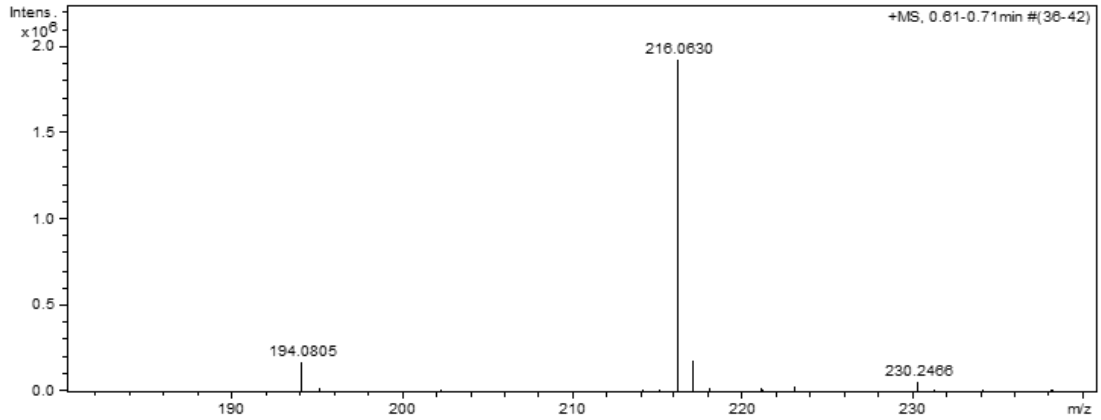



**Figure S73.** HRESIMS spectrum of **15**.

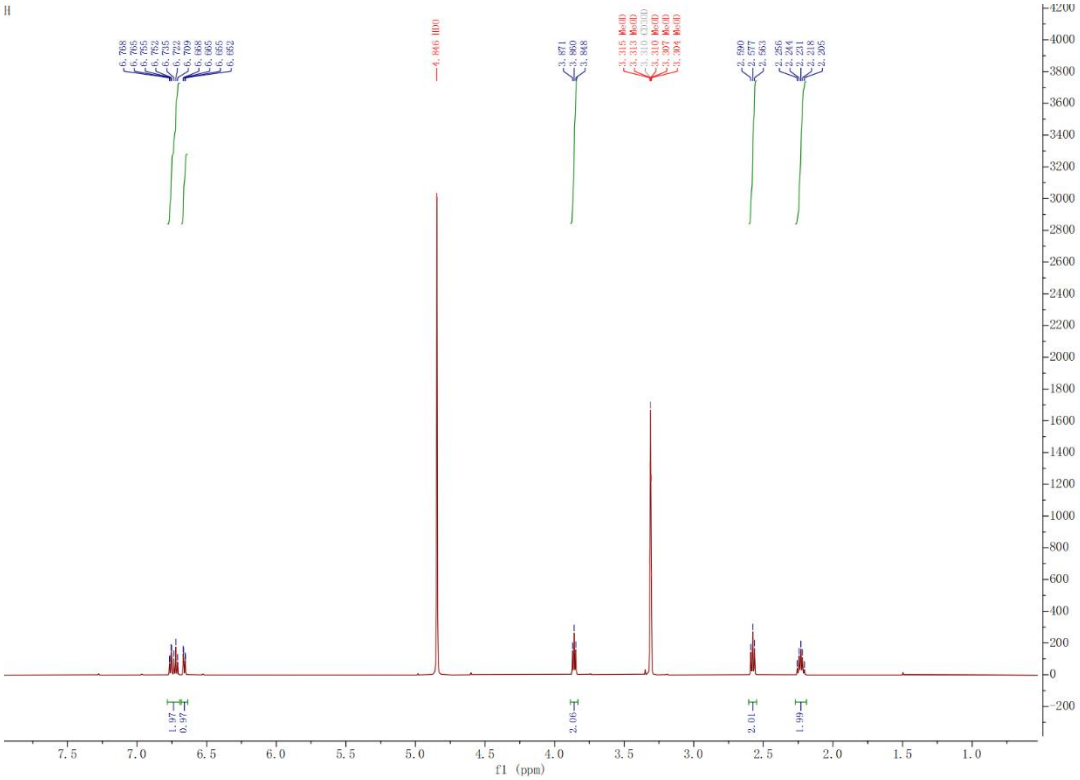



**Figure S74.** $^{1}$H NMR spectrum of **15**.

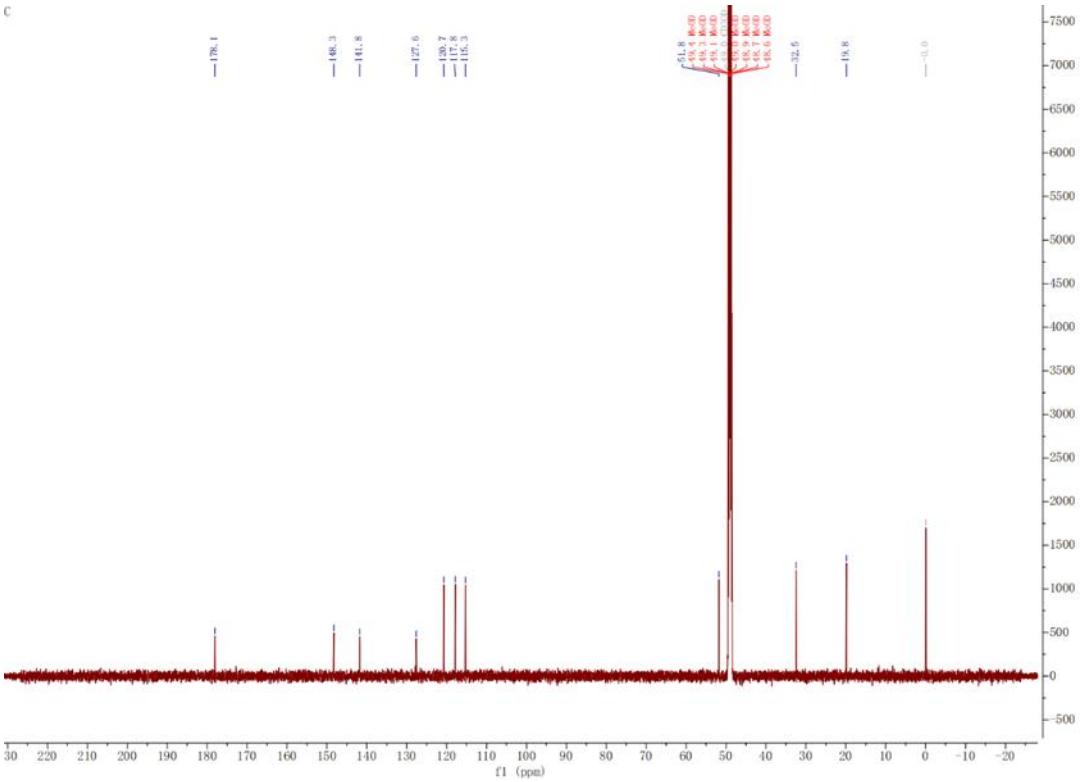


**Figure S75.** $^{13}$C NMR spectrum of **15**.

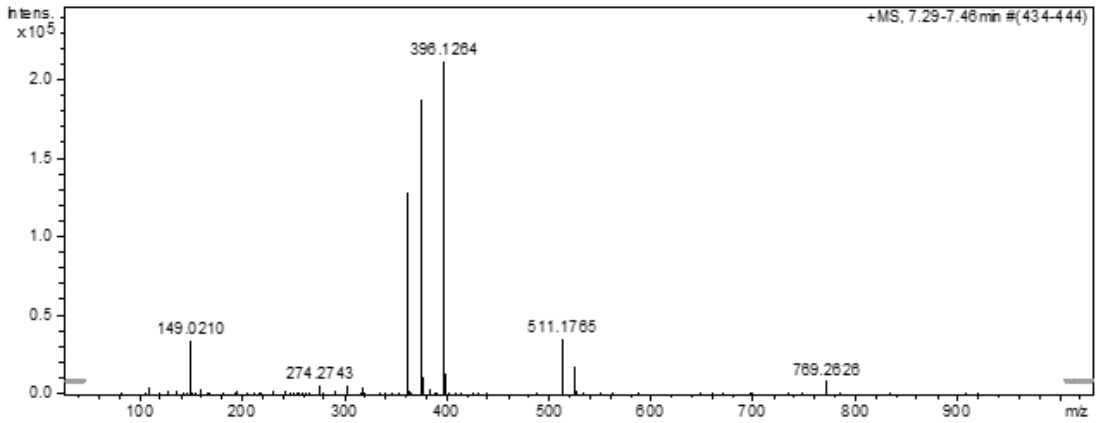



**Figure S76.** HRESIMS spectrum of **16**.

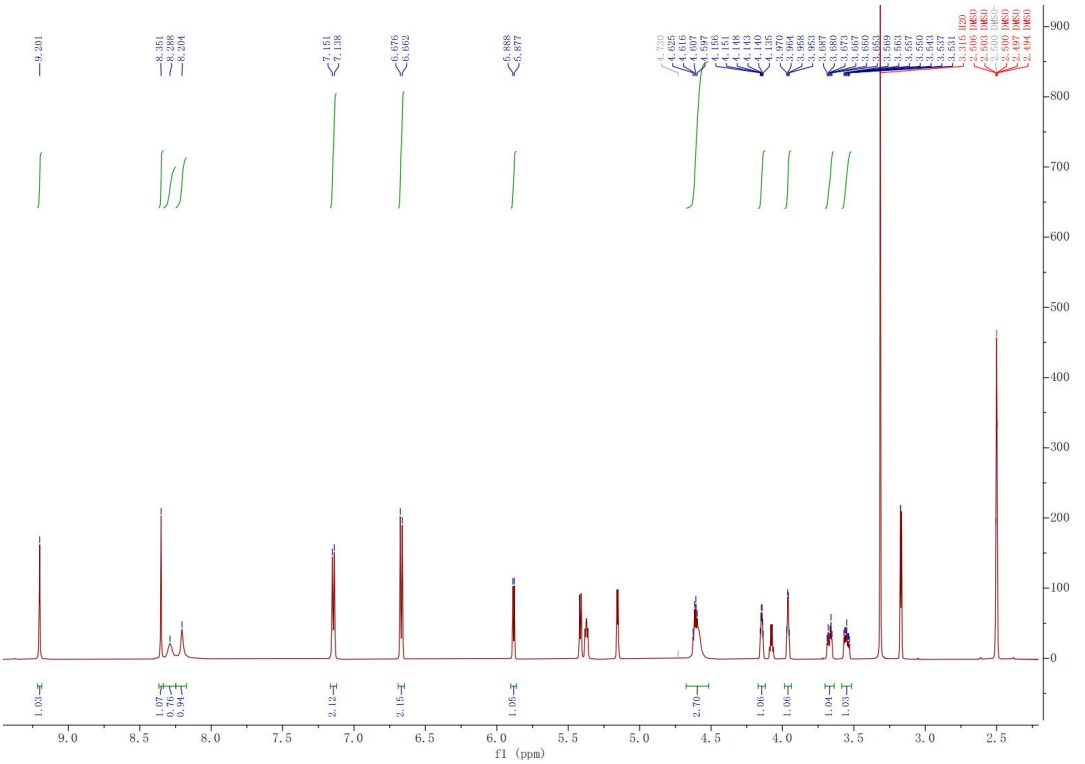


**Figure S77.** $^{1}$H NMR spectrum of **16**.

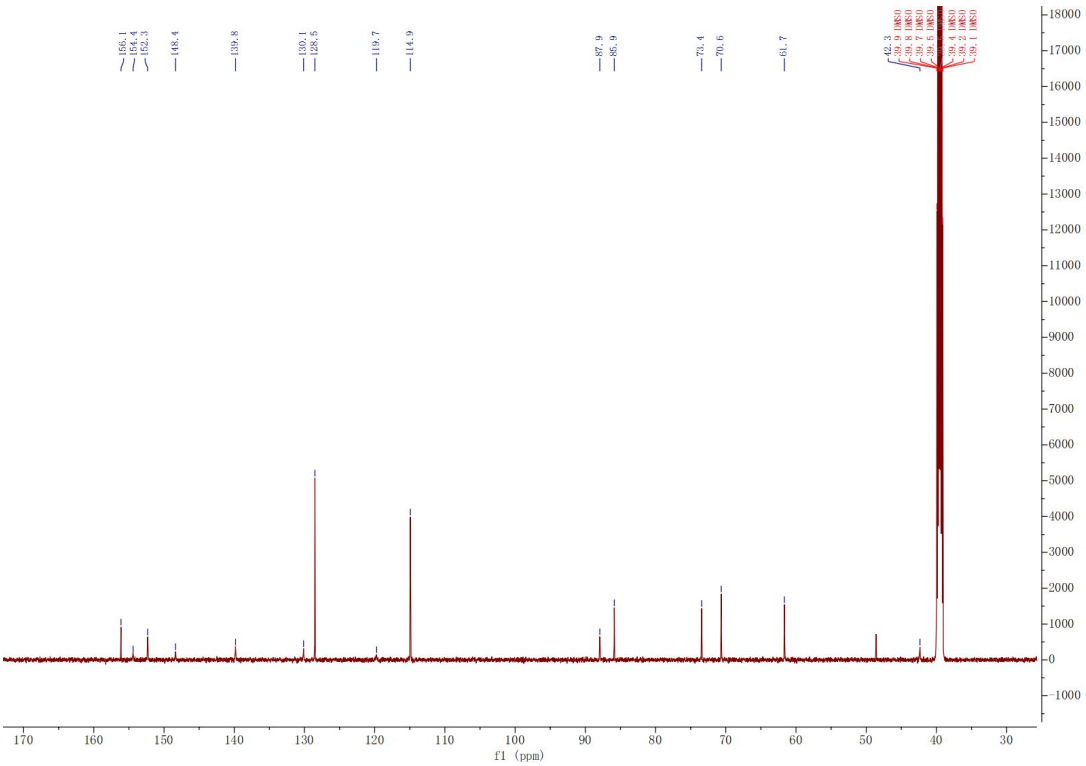


**Figure S78.** $^{13}$C NMR spectrum of **16**.

**Table S1** $^{1}$H and $^{13}$C-NMR date of compounds **1** and **2** recorded in $CD_3OD$ ($\delta$ in ppm, $J$ in Hz)

| NO. | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta_H$ (nH, m, $J$, Hz) | $\delta_C$ (ppm) | $\delta_H$ (nH, m, $J$, Hz) | $\delta_C$ (ppm) |
| 1' | | 133.0 | | 136.9 |
| 2', 6' | 6.93, d, $J$ = 8.4 Hz | 130.7 | 7.12, d, $J$ = 8.4 Hz | 130.8 |
| 3', 5' | 6.68, d, $J$ = 8.4 Hz | 116.1 | 6.98, d, $J$ = 8.4 Hz | 117.6 |
| 4' | | 156.5 | | 157.2 |
| 7' | 3.76, s | 38.8 | 3.83, s | 35.8 |
| 1 | | 146.2 | | 129.0 |
| 2 | | 144.6 | | 153.8 |
| 3 | 6.89, s | 118.8 | 6.64, d, $J$ = 7.8 Hz | 115.8 |
| 4 | | 132.6 | 6.80, dd, $J$ = 7.8, 2.4Hz | 128.5 |
| 5 | | 132.9 | | 129.5 |
| 6 | 6.65, s | 120.4 | 6.77, d, $J$ = 2.4 Hz | 131.9 |
| CH3 | 2.11, s | 19.1 | 2.16, s | 20.6 |
| 1'' | 4.63, d, $J$ = 7.2 Hz | 104.7 | 4.86, s | 102.5 |
| 2'' | 3.44, m | 74.9 | 3.42, m | 75.0 |
| 3'' | 3.44, m | 77.7 | 3.42, m | 78.0 |
| 4'' | 3.44, m | 71.1 | 3.42, m | 71.4 |
| 5'' | 3.27, m | 78.1 | 3.37, m | 78.1 |
| 6'' | 3.89, dd, $J$ = 12.0, 2.4Hz, H-6''a; 3.70, dd, J=12.6, 4.8 Hz, H-6'''b | 62.1 | 3.88, dd, $J$ = 12.0, 2.4Hz, H-6''a; 3.68, dd, J=12.6, 5.4 Hz, H-6'''b | 62.5 |

**Table S2** $^{1}$H and $^{13}$C-NMR date of compound **13** recorded in $CD_3OD$ ($\delta$ in ppm, $J$ in Hz)

| NO. | 13 | |
|---|---|---|
| | $\delta_H$ (nH, m, $J$, Hz) | $\delta_C$ (ppm) |
| 2 | 8.04, s | 145.6 |
| 4 | | 148.2 |
| 5 | | 123.9 |
| 6 | | 156.6 |
| 8 | 8.14, s | 140.1 |
| 1' | | 127.0 |
| 2', 6' | 7.16, d, $J$ = 8.4 Hz | 129.2 |
| 3', 5' | 6.71, d, $J$ = 8.4 Hz | 115.4 |
| 4' | | 157.1 |
| 7' | 5.22, s | 46.2 |

**Table S3** $^{1}H$ and $^{13}C$-NMR date of compounds **17** recorded in $CD_3OD$ ($\delta$ in ppm, $J$ in Hz)

| NO. | **17** | |
|---|---|---|
| | $\delta_H$ (nH, m, $J$, Hz) | $\delta_C$ (ppm) |
| 1 | - | 175.7 |
| 2 | - | 81.4 |
| 3 | 3.09, d, J=17.4 Hz; 3.01, d, $J$ = 17.4 Hz | 43.6 |
| 4 | - | 173.9 |
| 5 | 1.78, m; 1.70, m | 49.4 |
| 6 | 1.85, m | 24.8 |
| 7 | 0.87, d, $J$ = 6.6 Hz | 24 |
| 8 | 0.95, d, $J$ = 6.6 Hz | 24.8 |
| 9 | 3.75, s | 52.8 |
| 1' | 4.89, s | 100.1 |
| 2' | 3.21, t, $J$ = 7.8 Hz | 75.5 |
| 3' | 3.06, m | 77.4 |
| 4' | 3.40, t, $J$ = 9.0 Hz | 70.7 |
| 5' | 3.35, t, $J$ = 8.4 Hz | 78.5 |
| 6' | 3.77, dd, $J$ = 12.0, 2.4 Hz, H-6'a; 3.69, dd, $J$ = 12.0, 4.2 Hz, H-6'b | 62 |

**Table S4** The primer sequence of genes for qPCR

| Gene | Sequence of the primer | Length of products (bp) |
|---|---|---|
| *Col27a1* | F: 5'-CGAACAGAAGATGGCGGATGGT-3'<br>R: 5'-CCGTGATAGGCTTCAGGCAAGT-3' | 122 |
| *Banp* | F: 5'-GCCAGCCAGGAAGTCAGAACA-3'<br>R: 5'-CCTCACGGTGGAACAGGTAGTC-3' | 219 |
| *Hmgcs1* | F: 5'-ACGGTTCCCTTGCTTCTGTTCT-3'<br>R: 5'-ATCCTGGTGTGGCATCTTGTGT-3' | 138 |
| *Insig1* | F: 5'-ATCACCATCGCCTTCCTAGCC-3'<br>R: 5'-CCACTGTGACACCTCCTGAGAA-3' | 136 |
| *Sytl3* | F: 5'-CTCCTCCATCCAGTCCAACACA-3'<br>R: 5'-GGCAGCAGGTAGGTCTTGACAT-3' | 256 |
| *Gsta5* | F: 5'-CCTTGTAGGCAACAGGCTGAC-3'<br>R: 5'-GGCTGCTGATTCTGCTCTTGA-3' | 131 |
| *Egr1* | F: 5'-CTGGAGGAGATGATGCTGCTGAG-3'<br>R: 5'-TGCTGCTGCTGCTGCTGTTAT-3' | 100 |
| *Armcx5* | F: 5'-ACAGAACTCCTGGCAGAACCT-3'<br>R: 5'-CCTTGACAGATGGCTCACCTC-3' | 127 |
| *GAPDH* | F: 5'-AAGTTCAACGGCACAGTCAAG-3'<br>R: 5'-ACATACTCAGCACCAGCATCA-3' | 122 |