

## Supporting Information

### Using UPLC-LTQ-Orbitrap-MS and HPLC-CAD to Identify Impurities in Cycloastragenol, which is a Pre-Clinical Candidate for COPD

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|               |                |             |                      |                 |              |                        |                     |
|---------------|----------------|-------------|----------------------|-----------------|--------------|------------------------|---------------------|
| Sample Name   | W200994-0003   | Position    | P1-B5                | Instrument Name | Instrument 1 | User Name              |                     |
| Inj Vol       | 1              | InjPosition |                      | SampleType      | Sample       | IRM Calibration Status | Success             |
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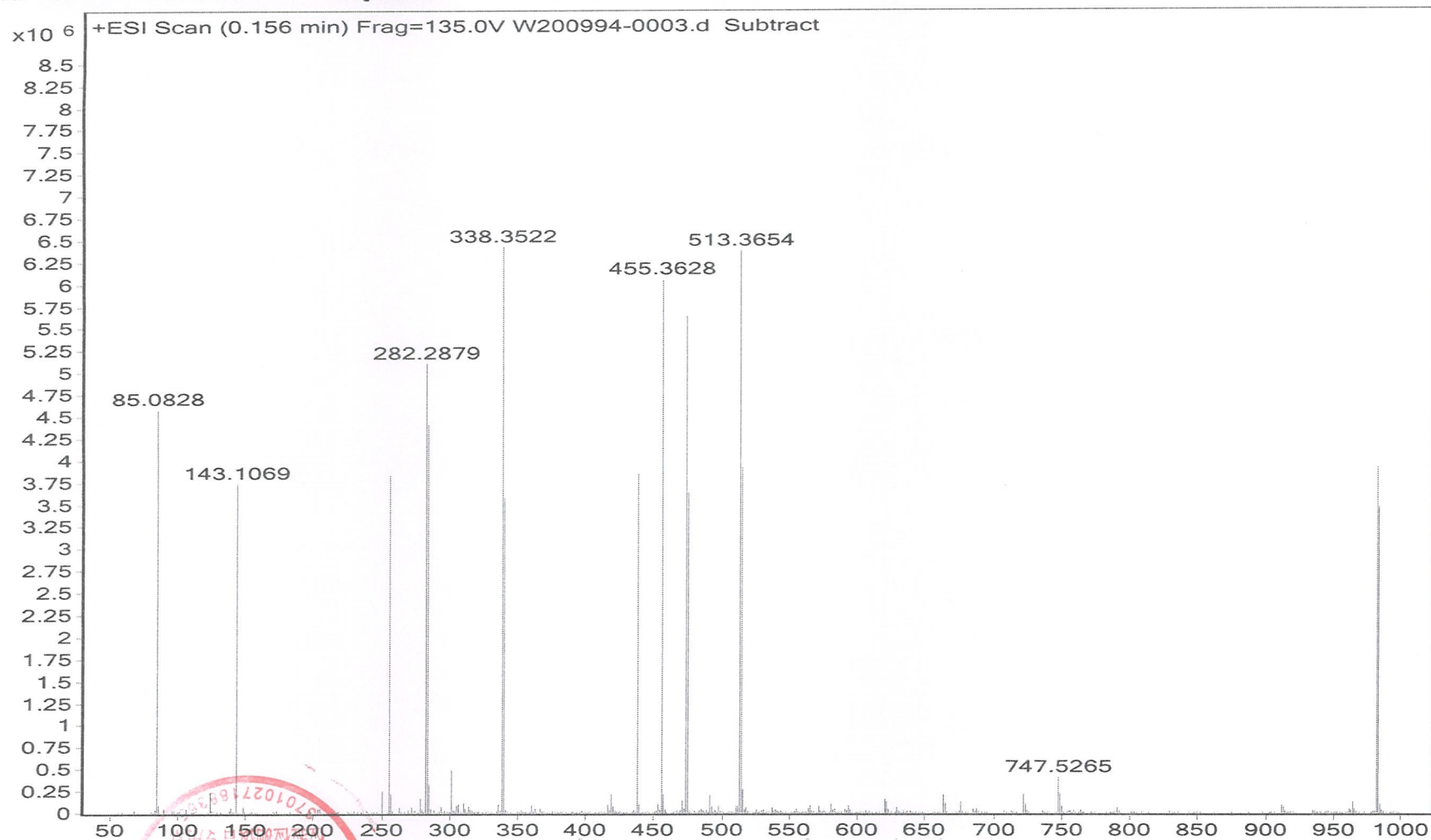


Figure S1 The positive HRESIMS spectrum of compound 9

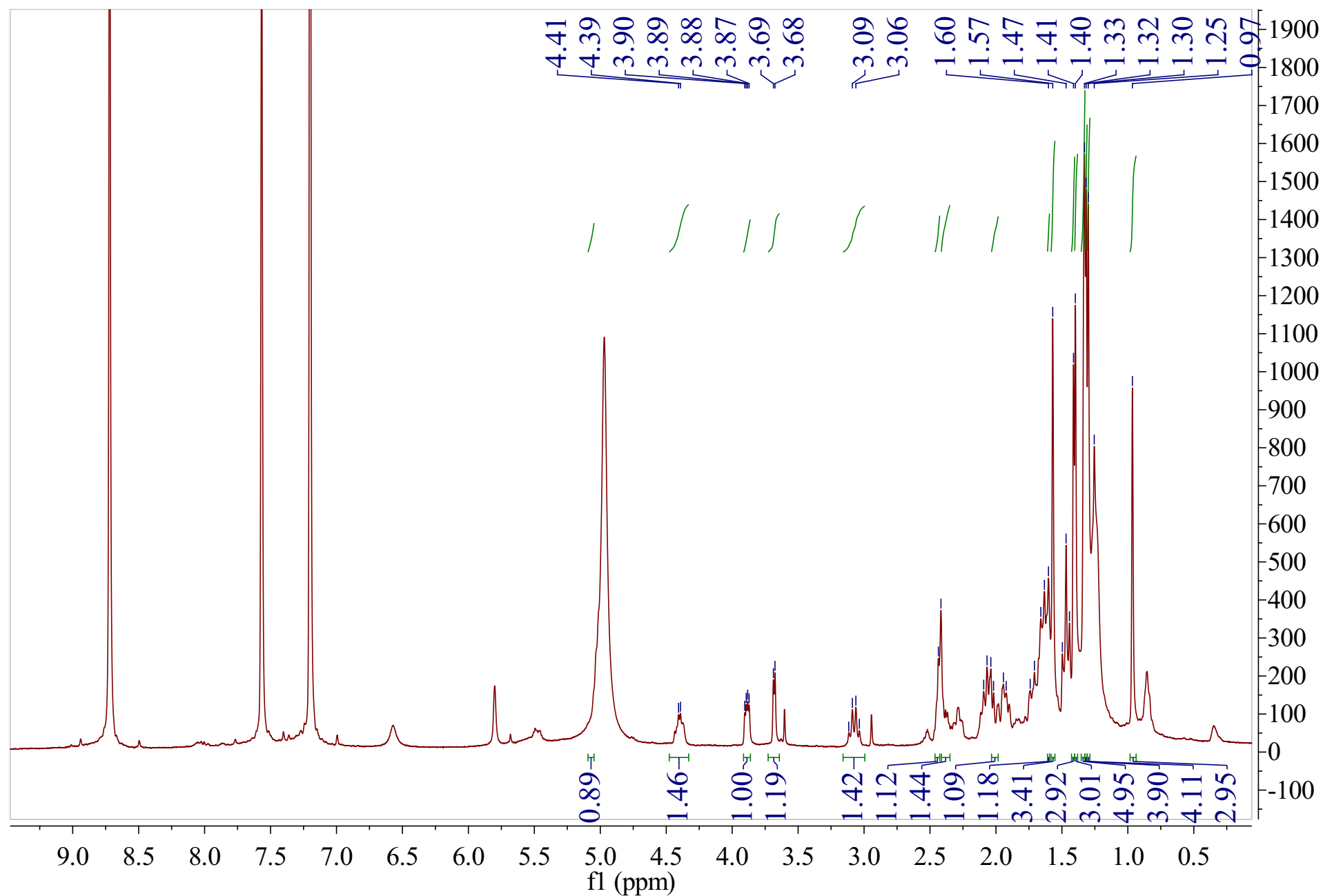


Figure S2 <sup>1</sup>H NMR (400 MHz, C<sub>5</sub>D<sub>5</sub>N) spectrum of compound 9

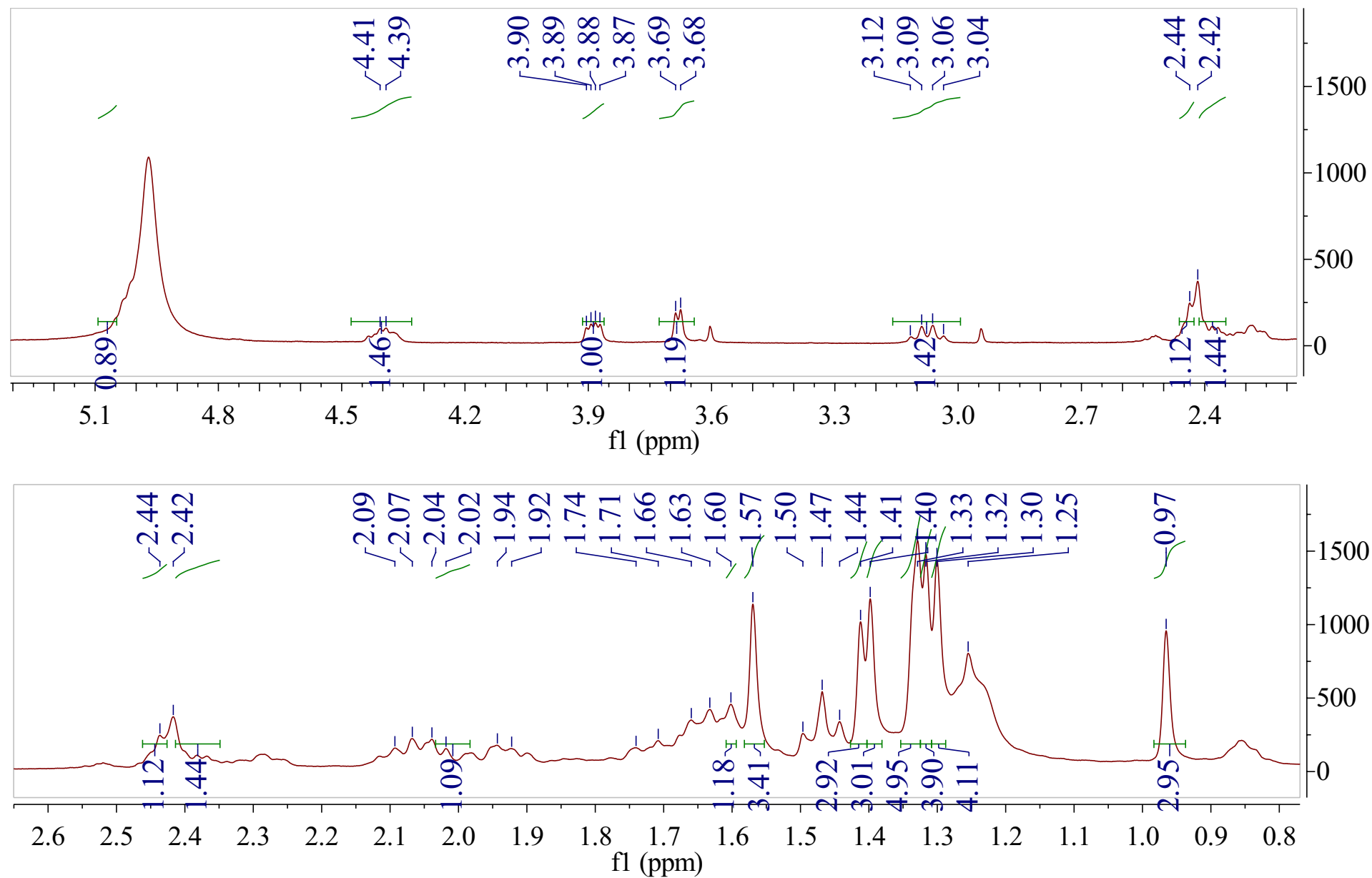
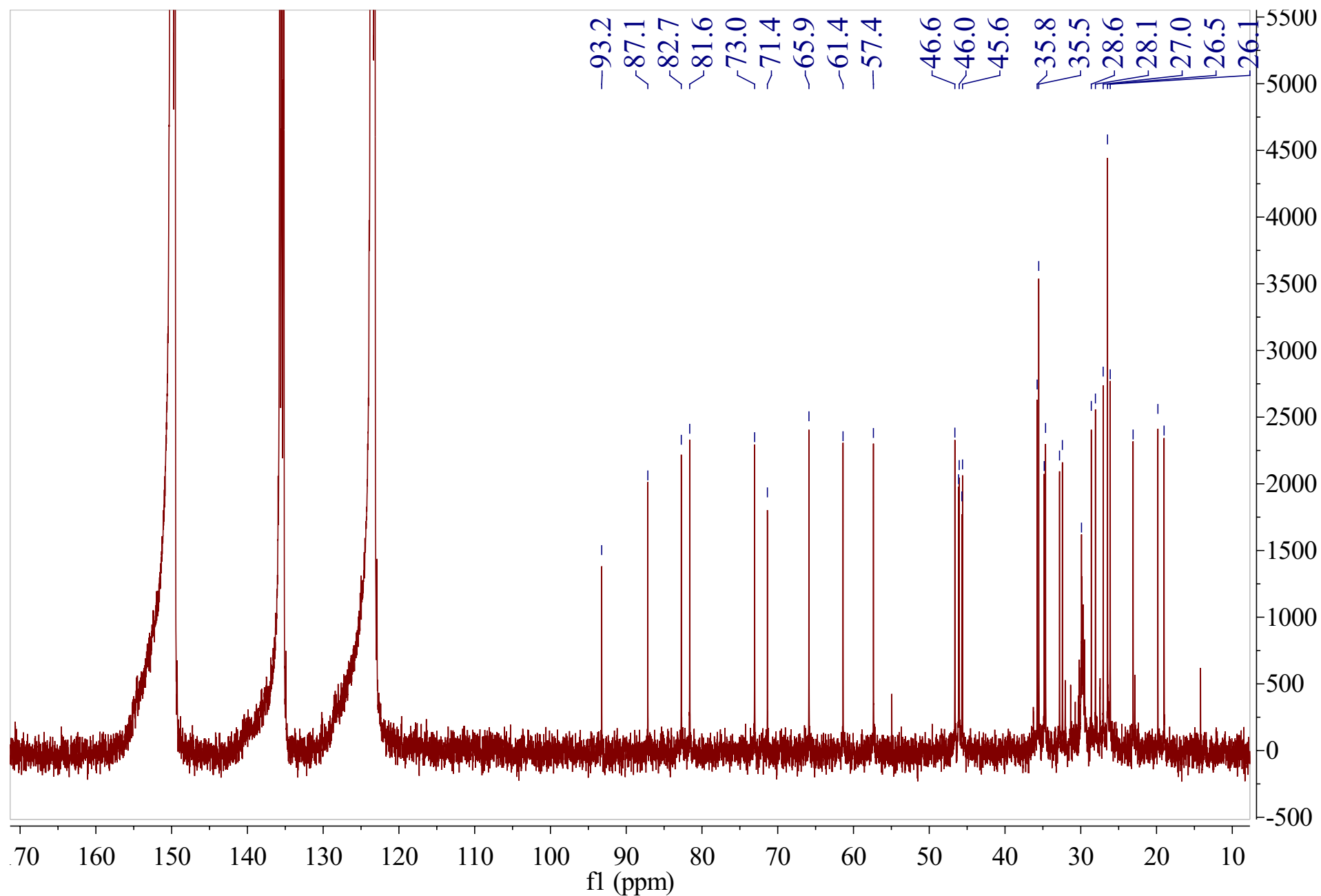
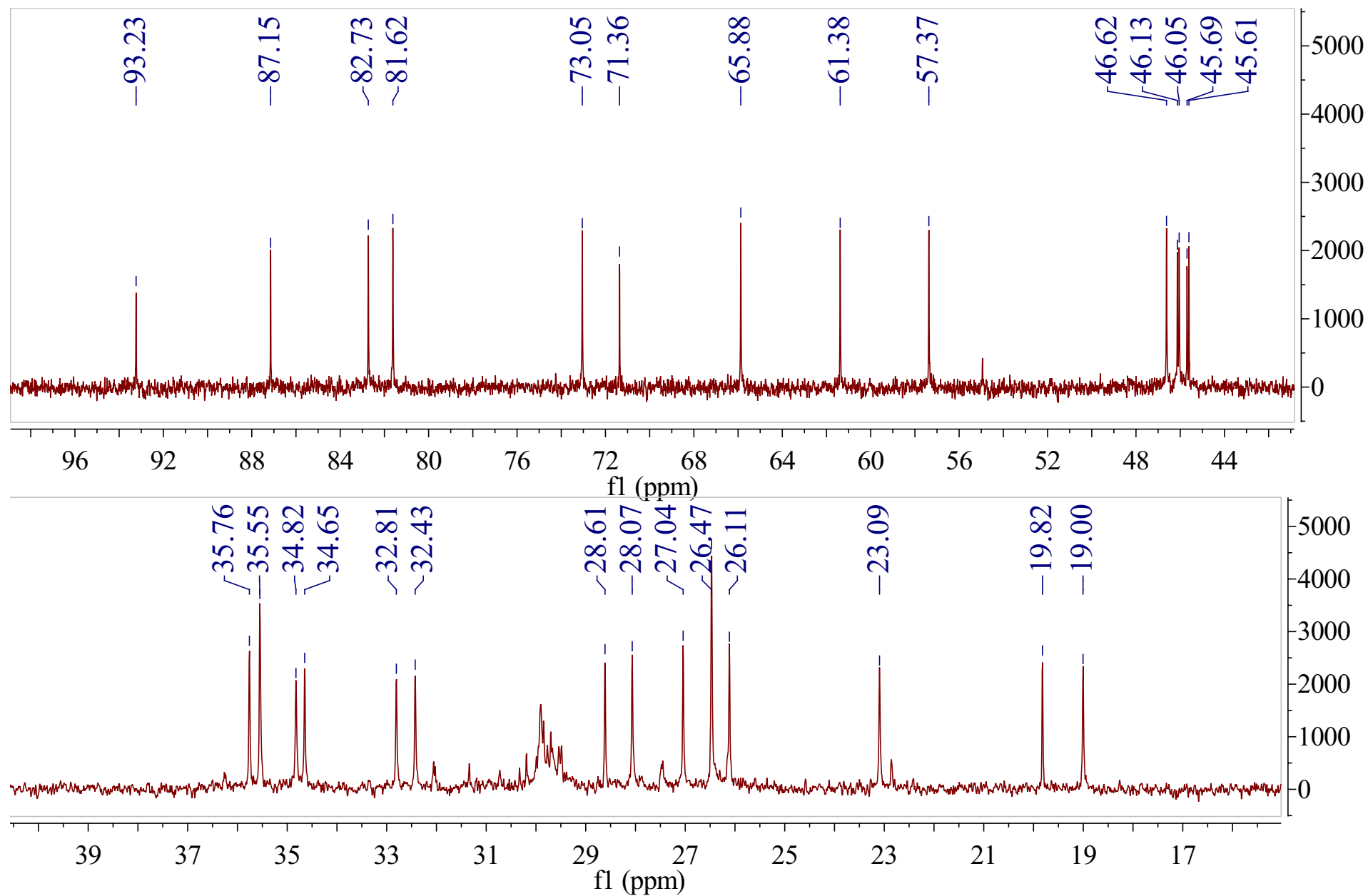


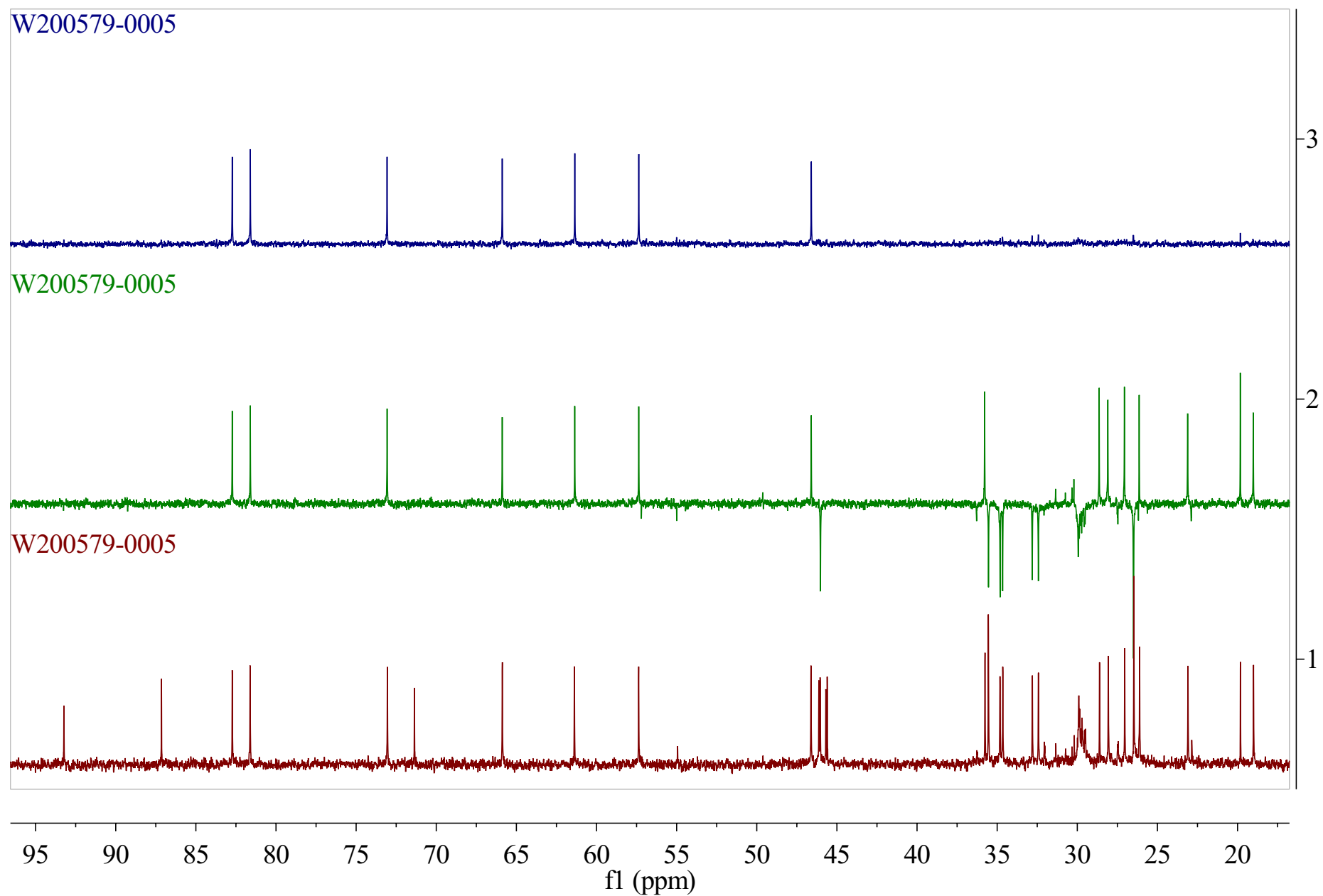
Figure S3 Amplificatory  $^1\text{H}$  NMR spectrum of compound 9



**Figure S4**  $^{13}\text{C}$  NMR (100 MHz,  $\text{C}_5\text{D}_5\text{N}$ ) spectrum of compound 9

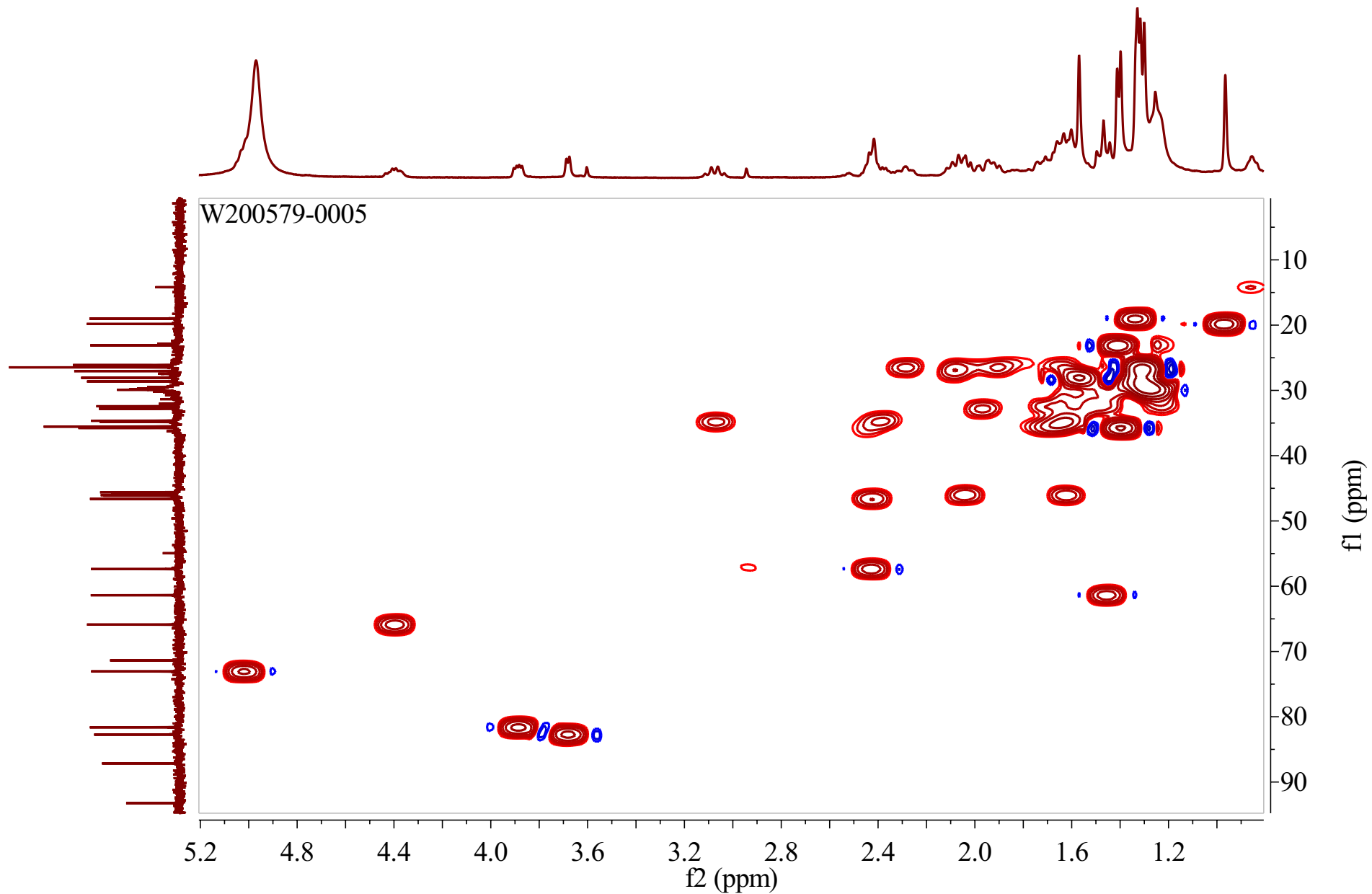


**Figure S5 Amplificatory  $^{13}\text{C}$  NMR spectrum of compound 9**

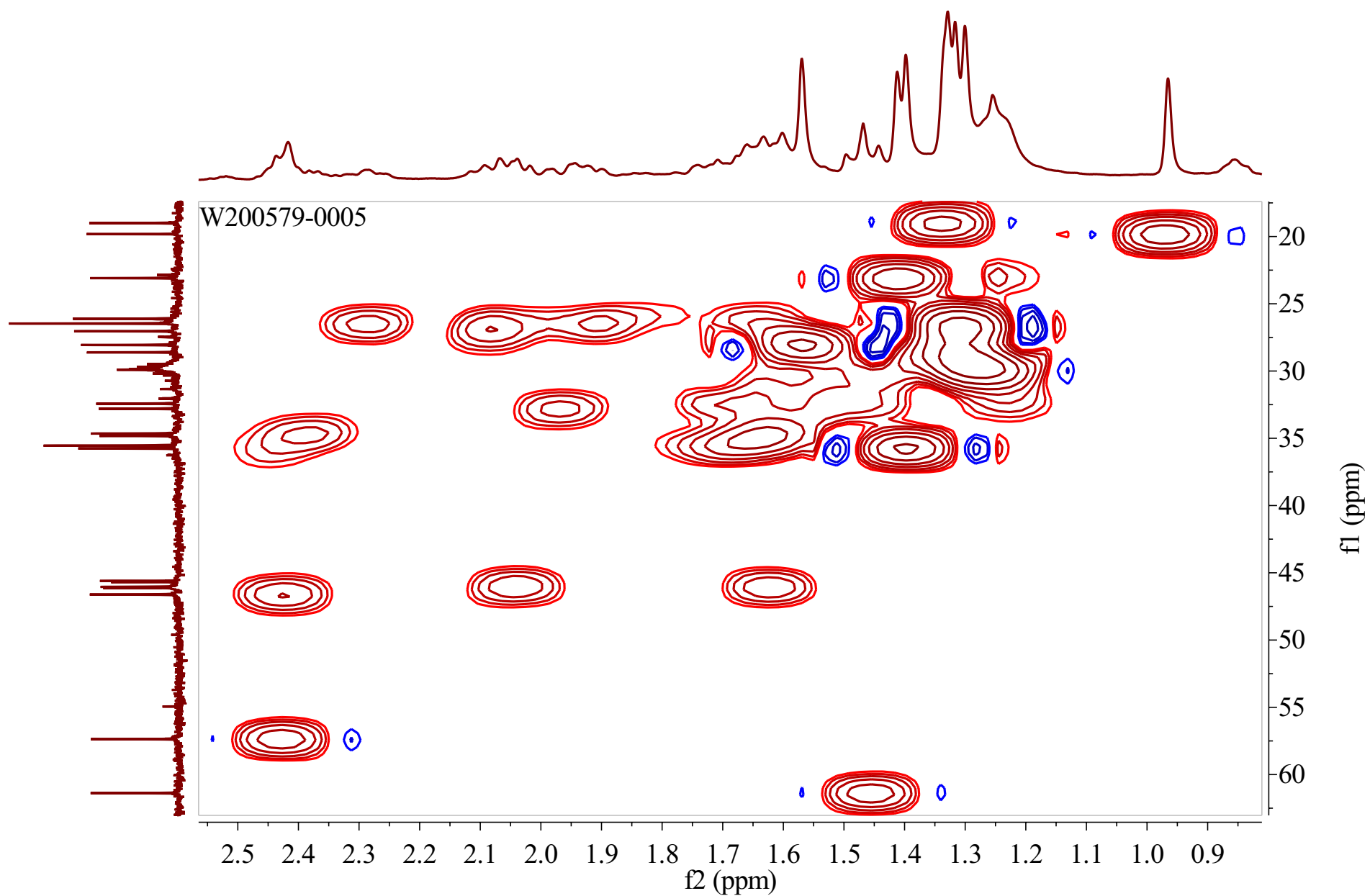


**Figure S6 DEPT spectrum of compound 9**

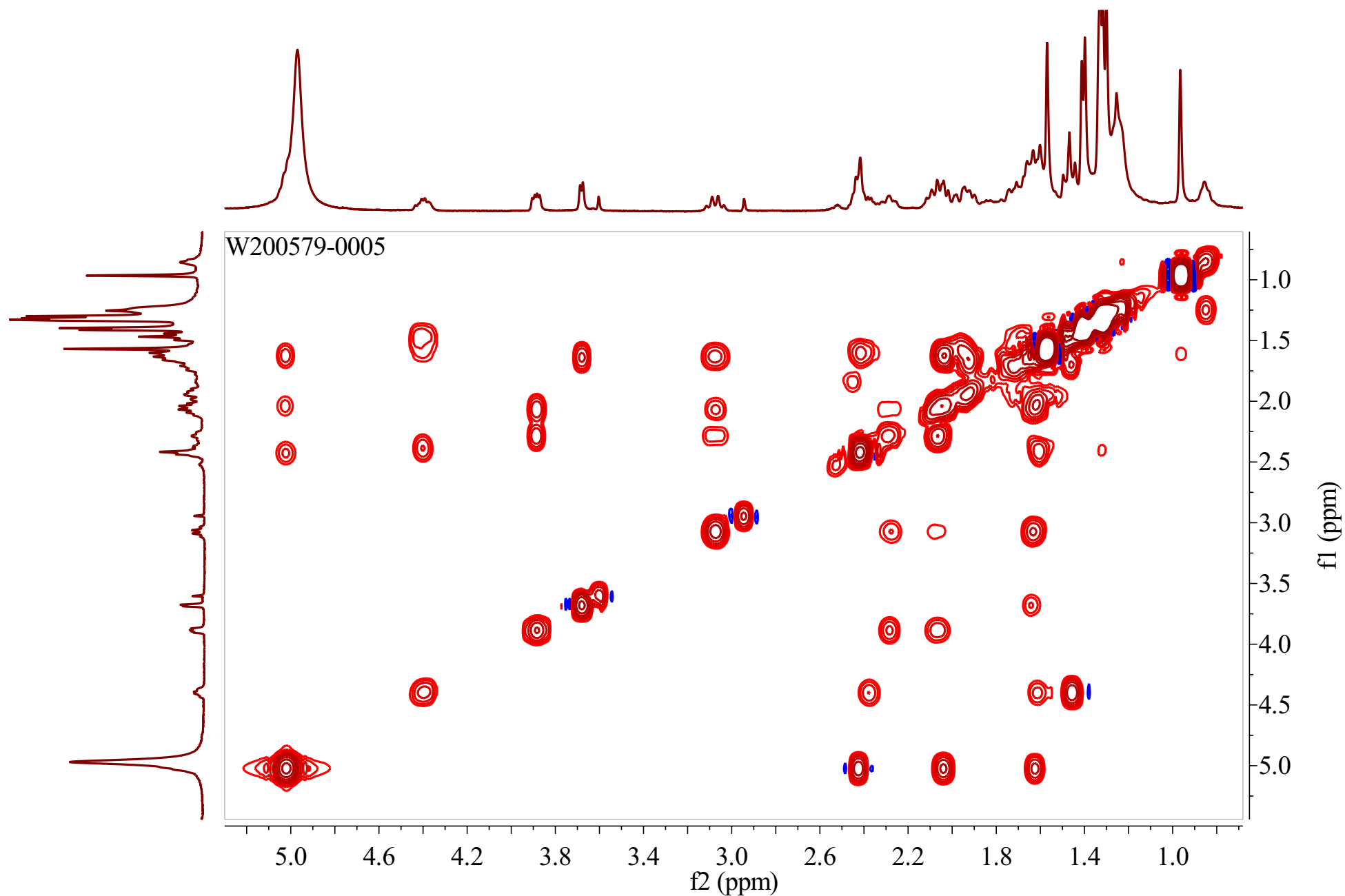




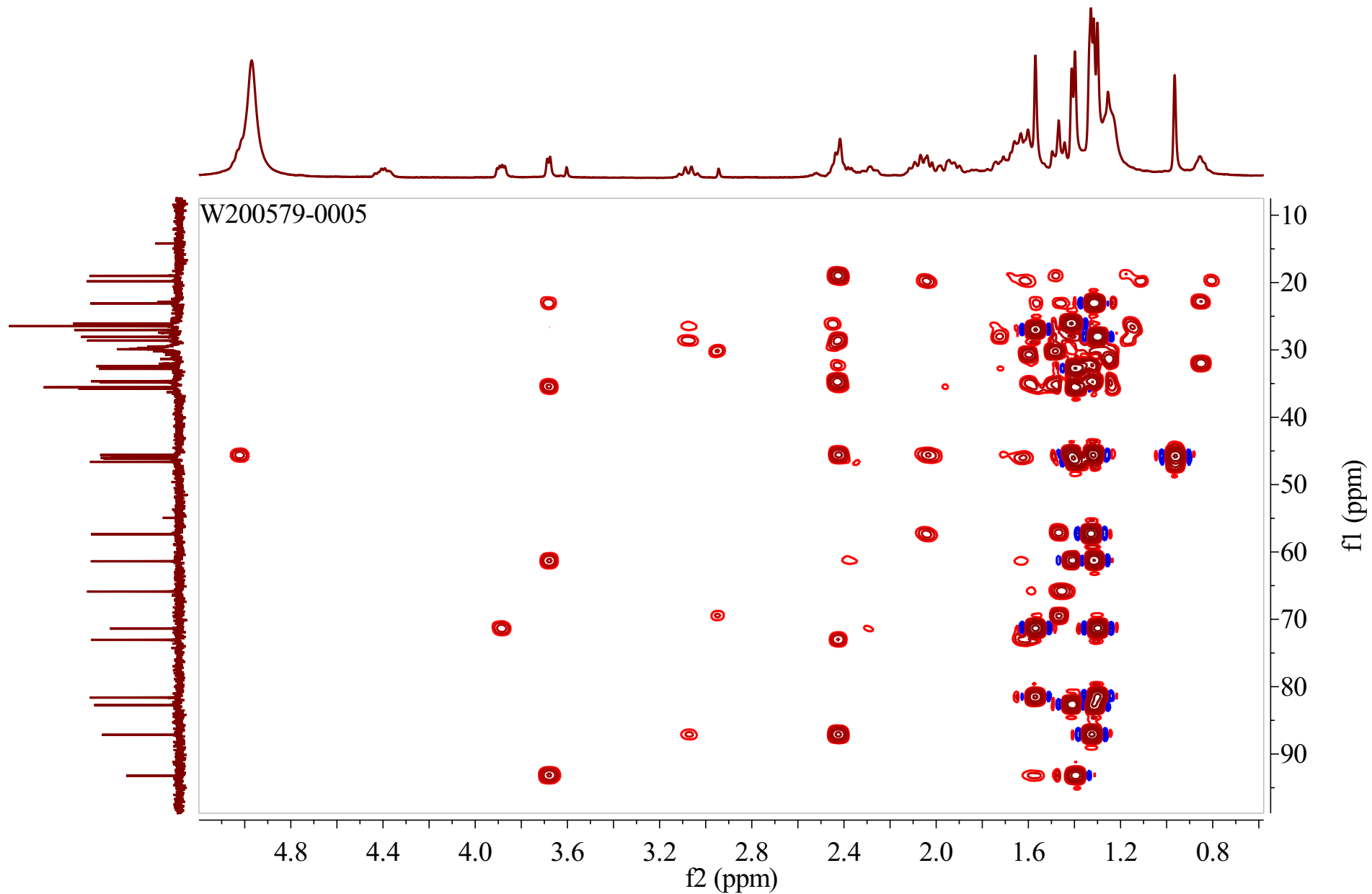
**Figure S7 HMQC spectrum of compound 9**



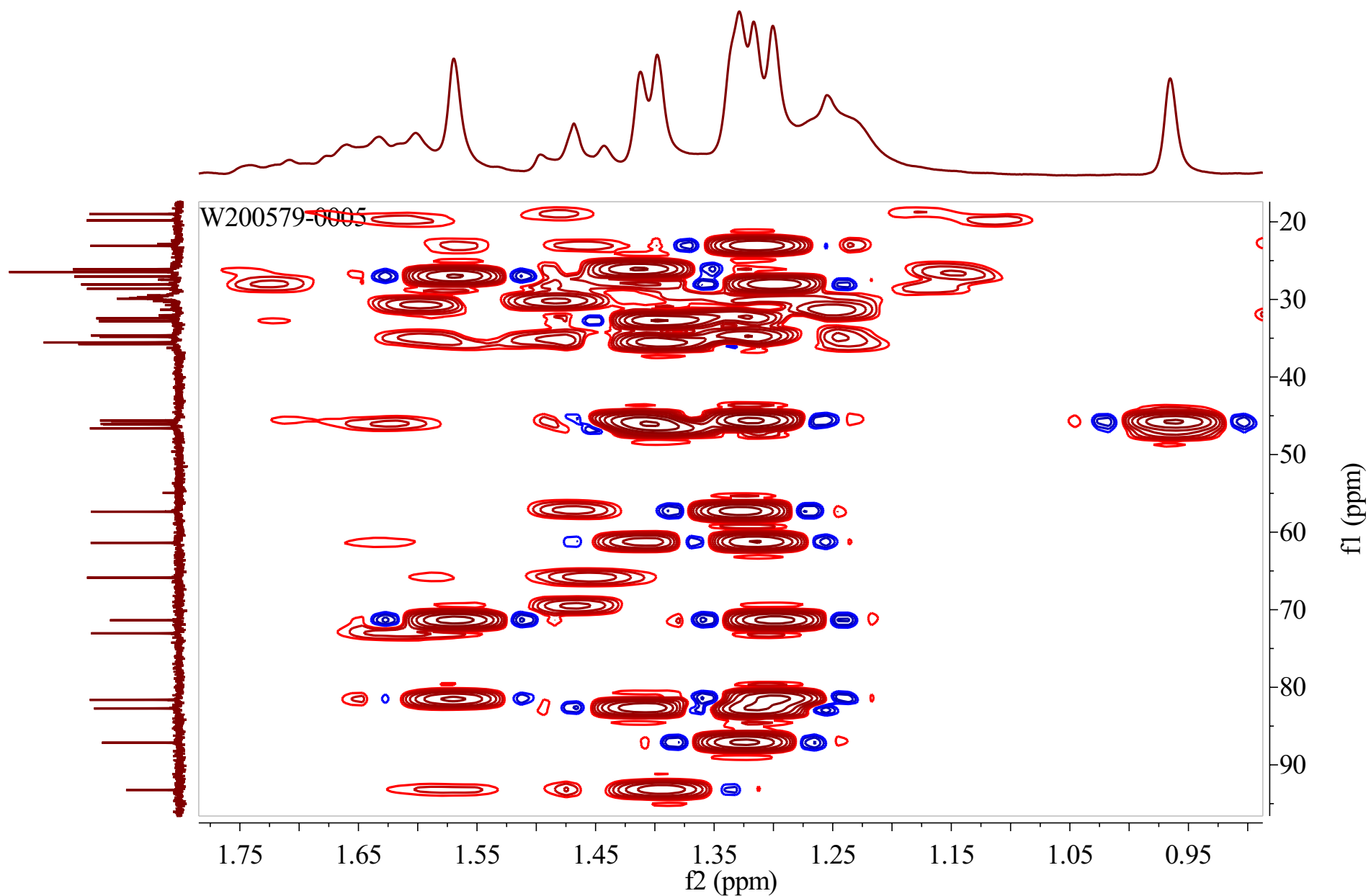
**Figure S8 Amplificatory HMQC spectrum of compound 9**



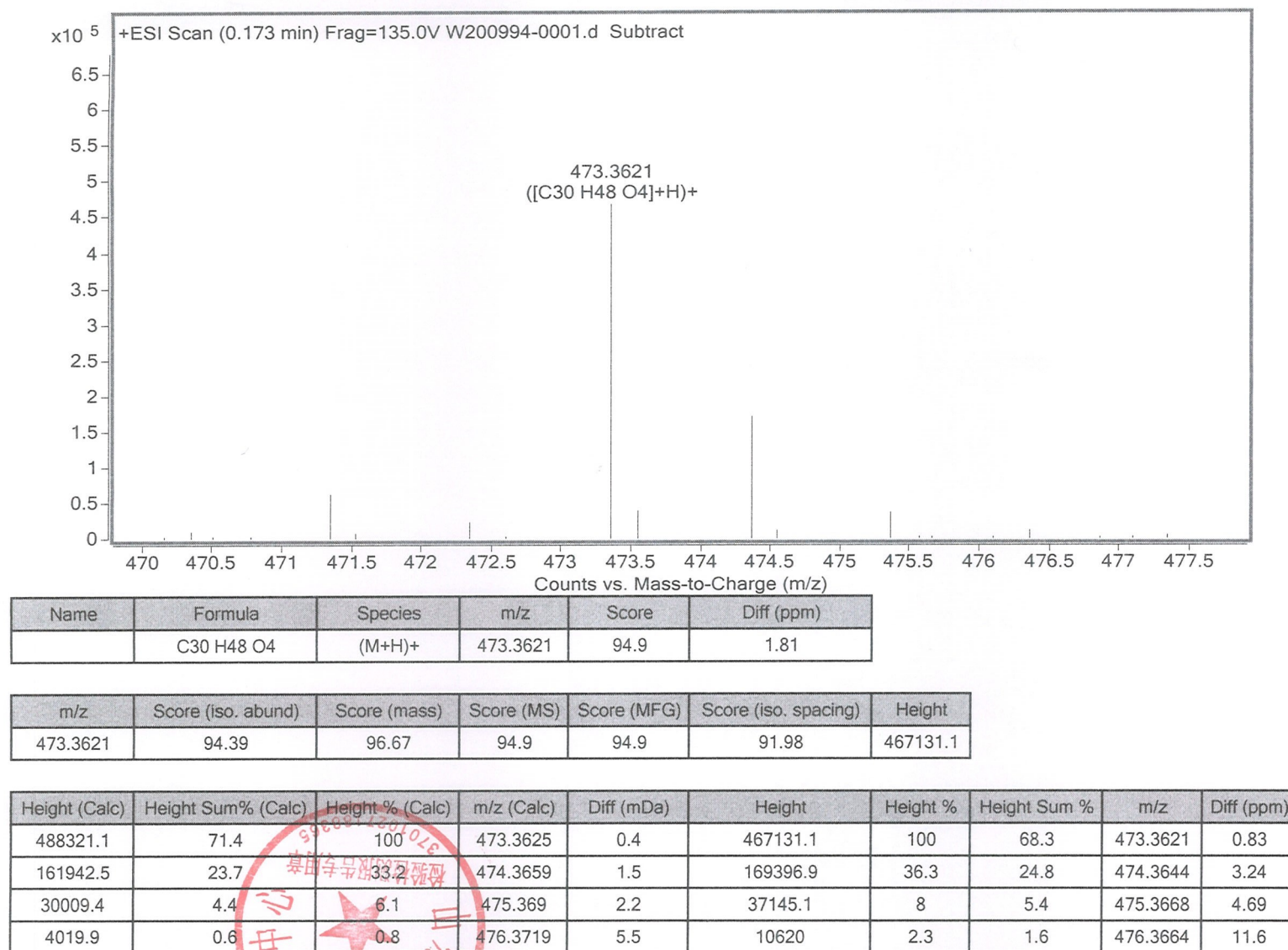
**Figure S9  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound 9**



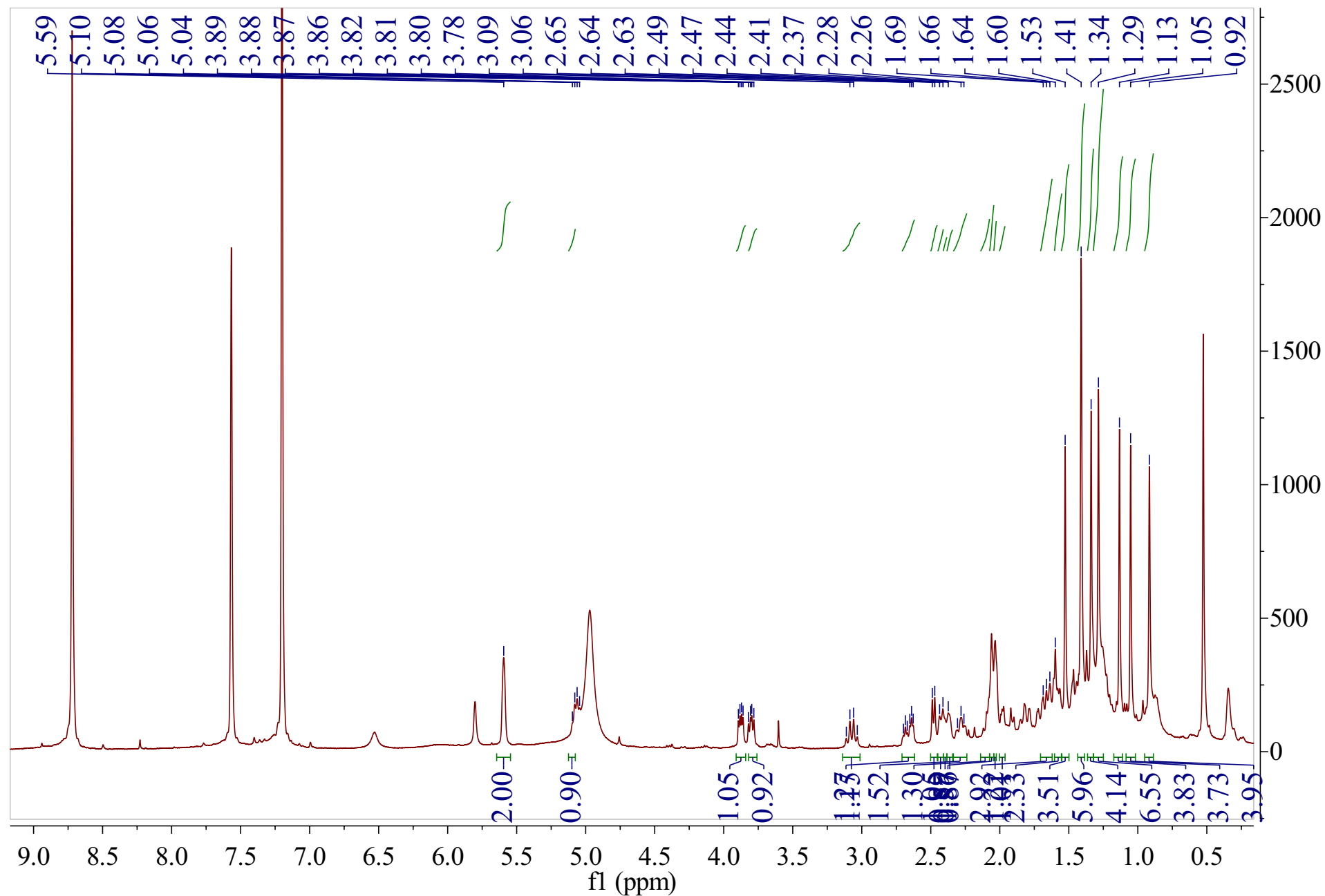
**Figure S10 Key HMBC spectrum of compound 9**



**Figure S11 Amplificatory HMBC spectrum of compound 9**



**Figure S12 The positive HRESIMS spectrum of compound 14**



**Figure S13  $^1\text{H}$  NMR (400 MHz,  $\text{C}_5\text{D}_5\text{N}$ ) spectrum of compound 14**

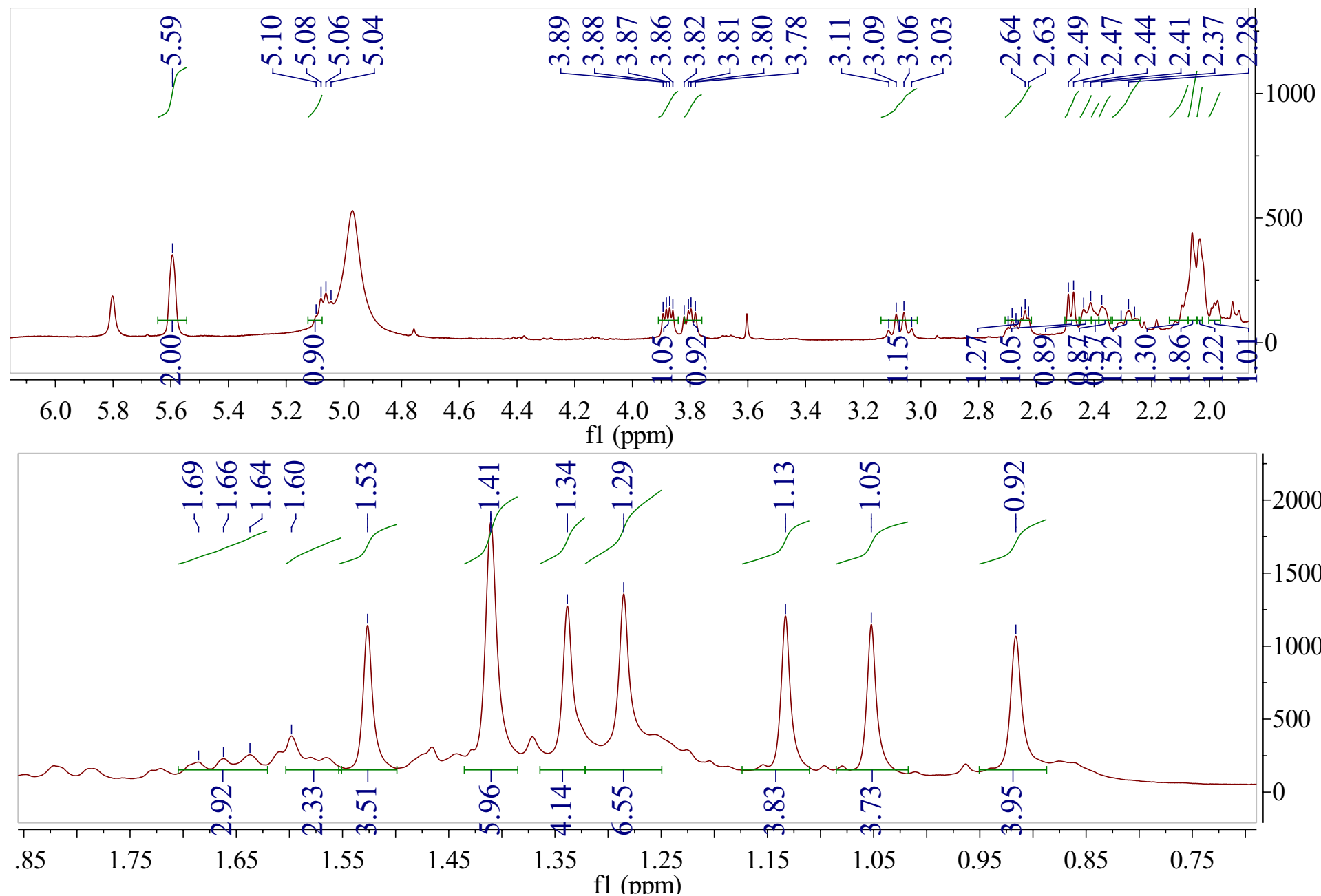


Figure S14 Amplificatory  $^1\text{H}$  NMR spectrum of compound 14



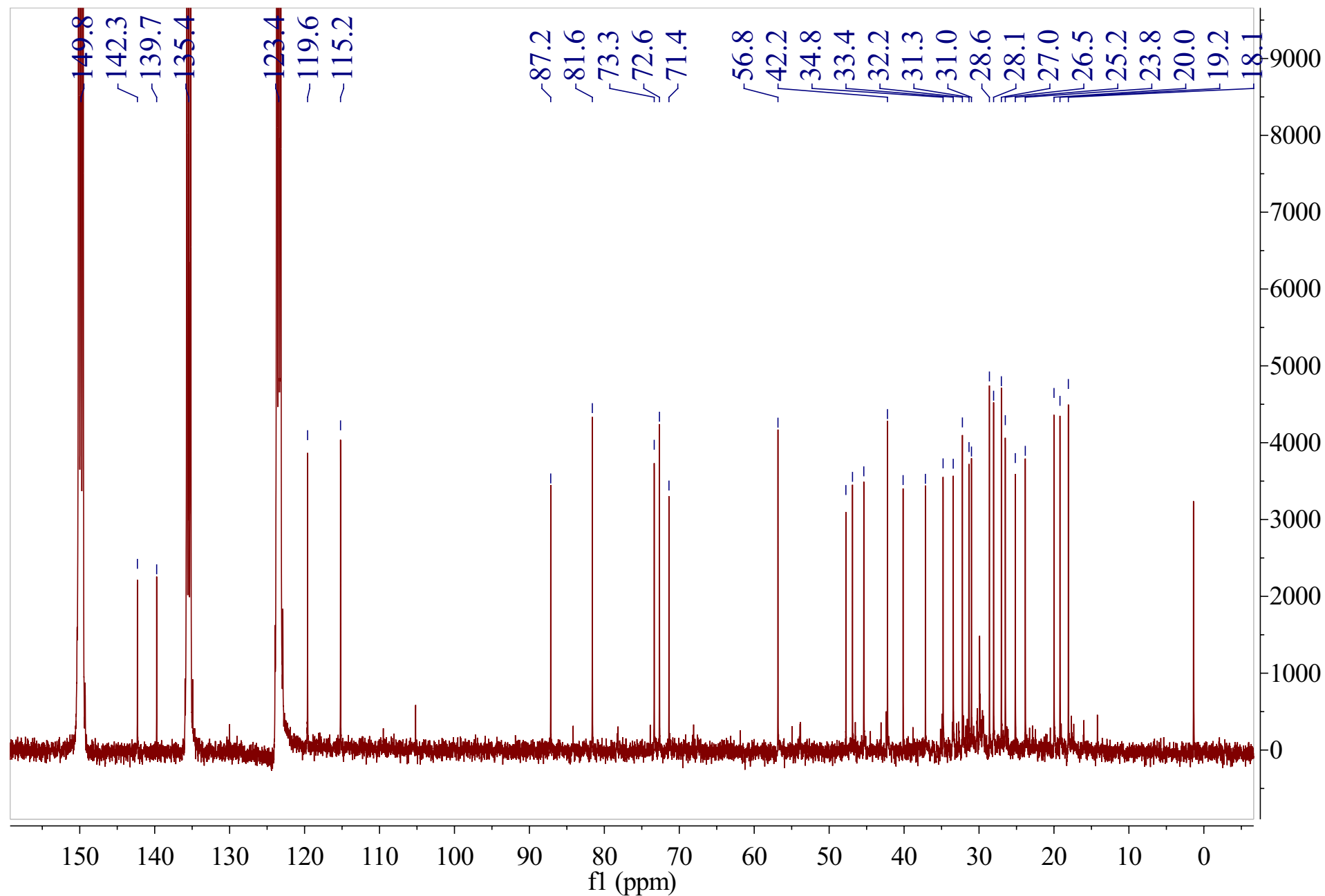


Figure S15  $^{13}\text{C}$  NMR (100 MHz,  $\text{C}_5\text{D}_5\text{N}$ ) spectrum of compound 14

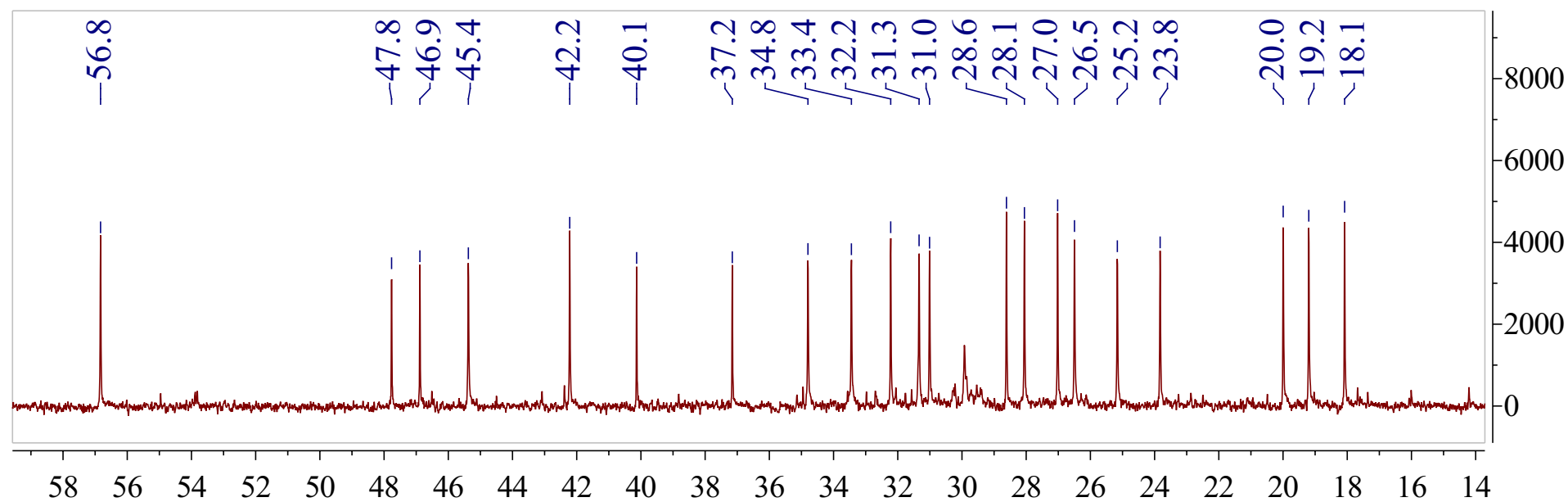
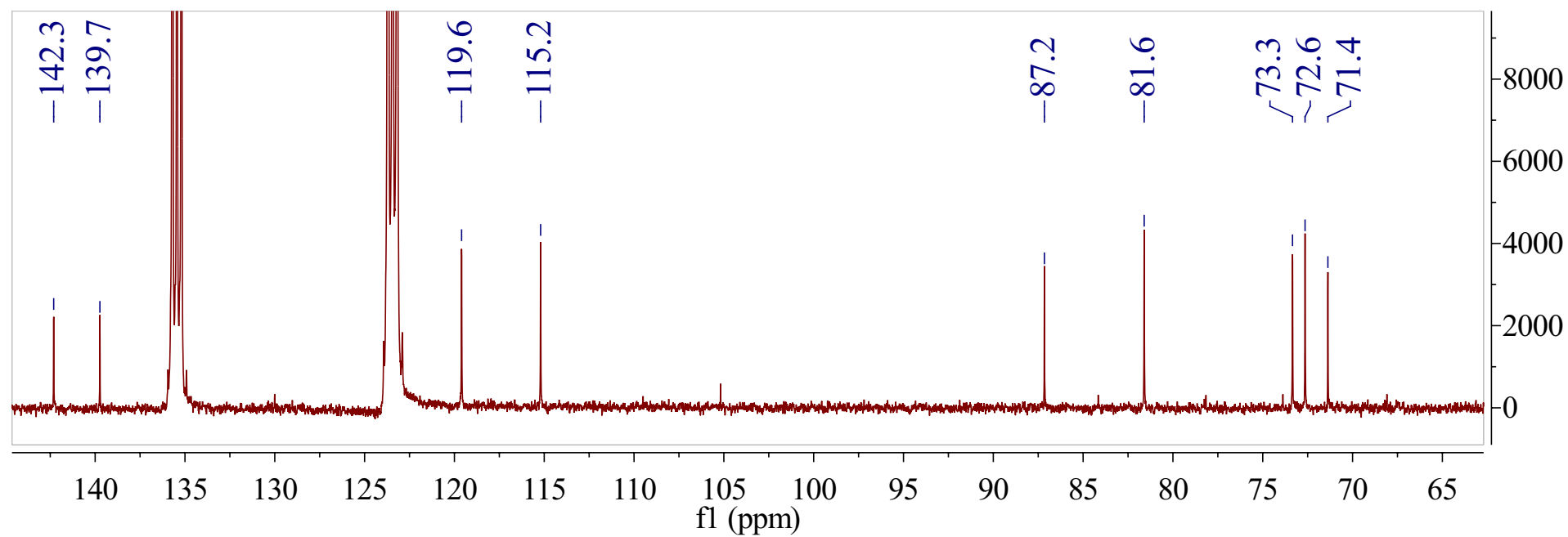
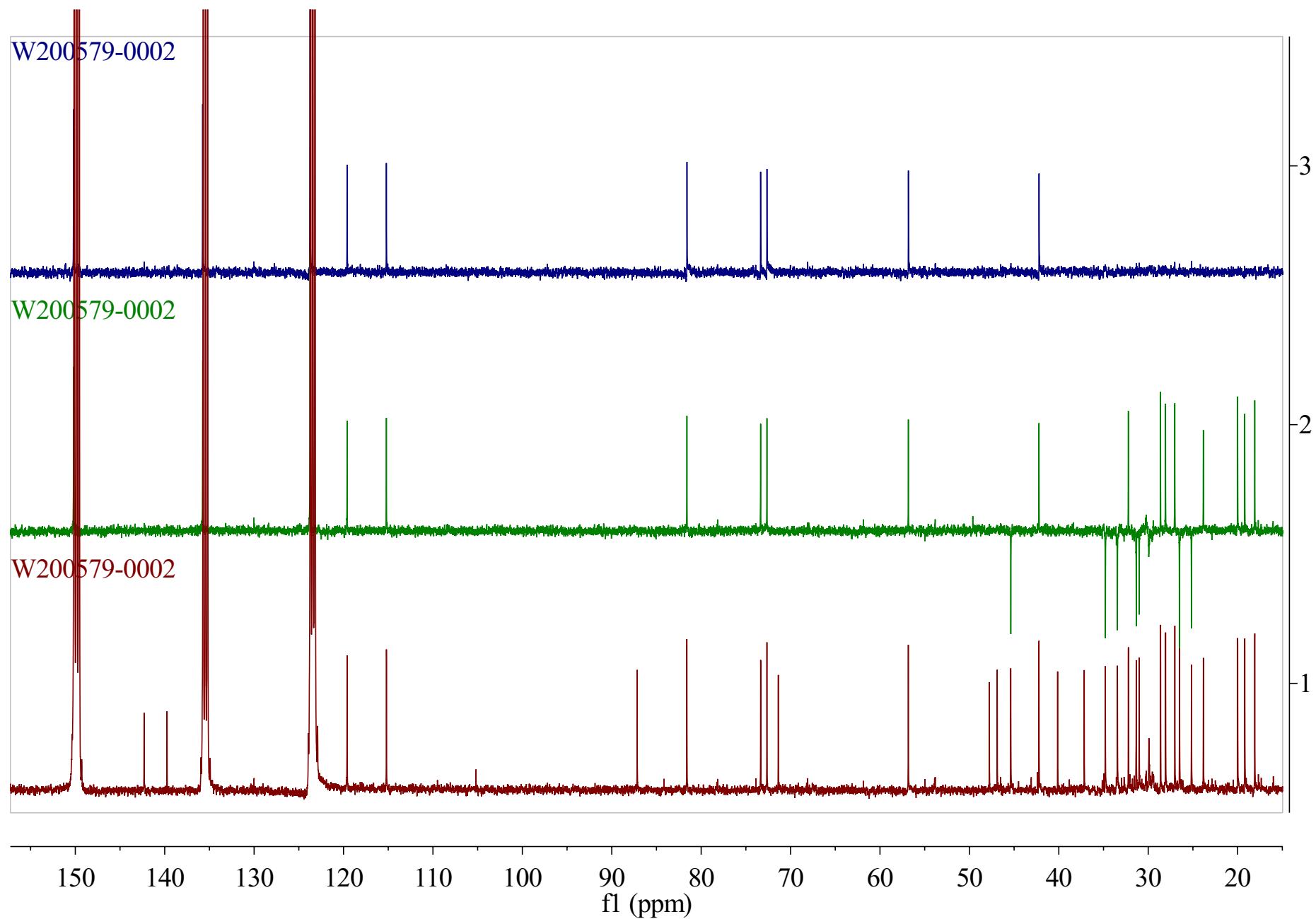
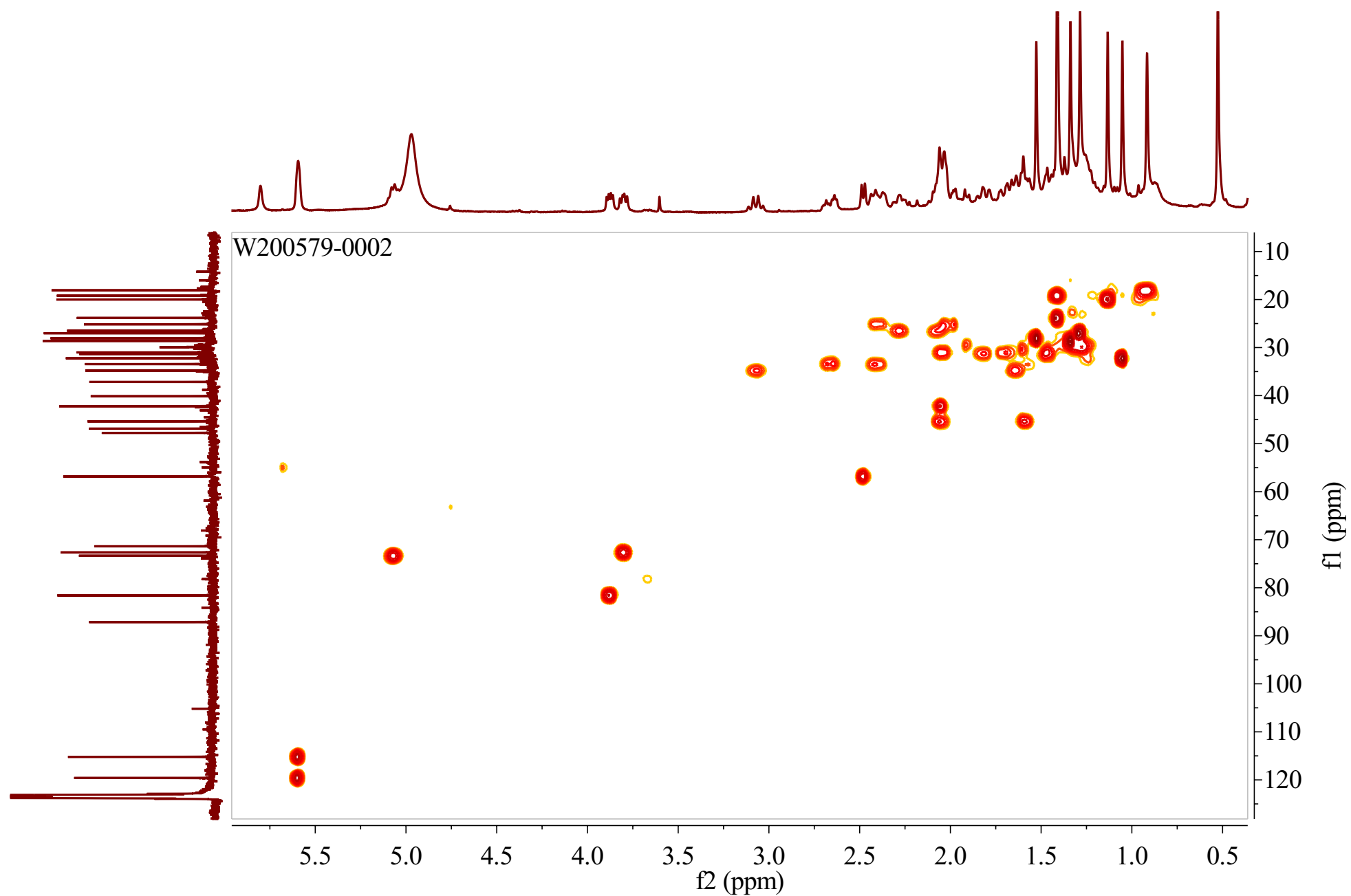


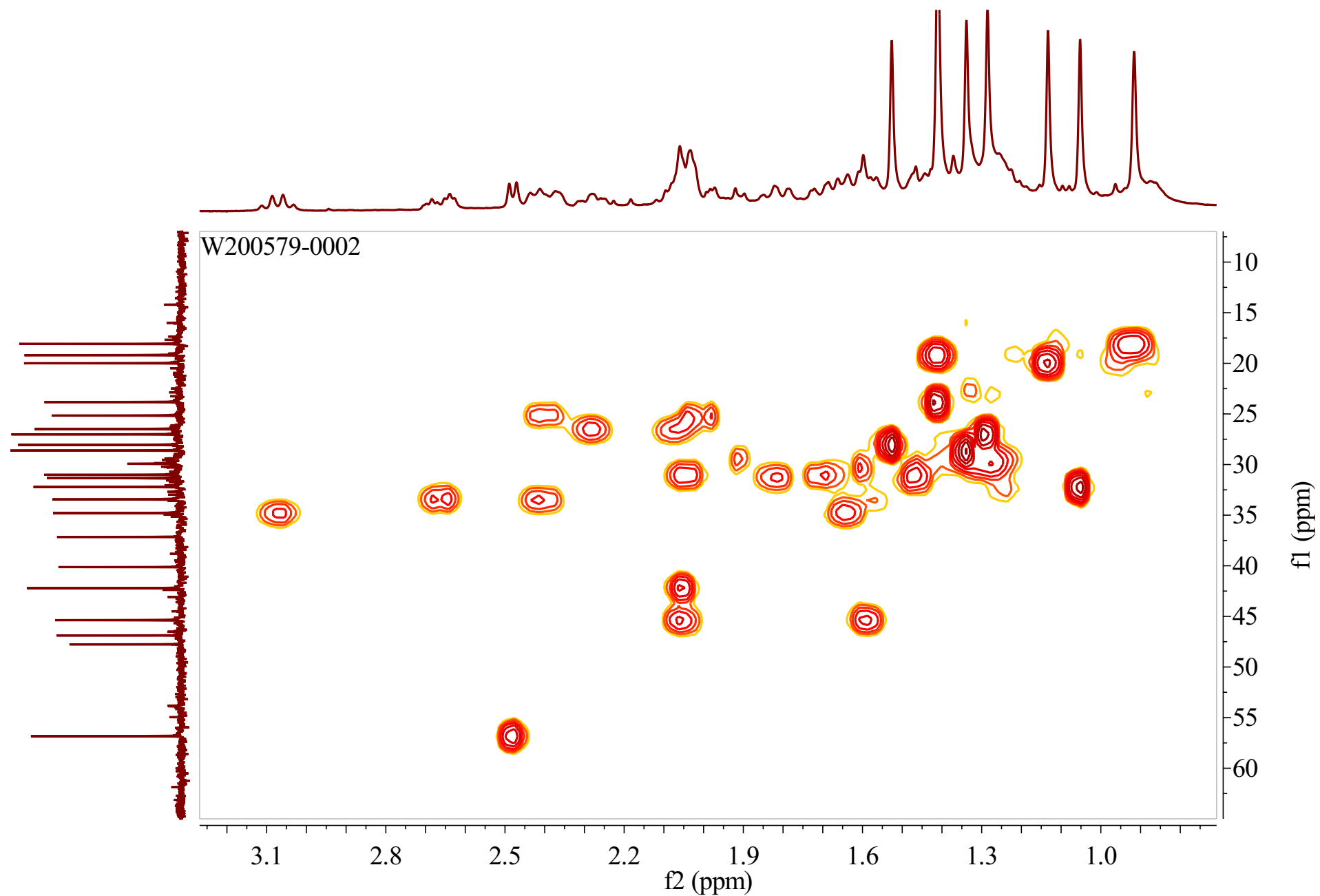
Figure S16 Amplificatory  $^{13}\text{C}$  NMR spectrum of compound 14



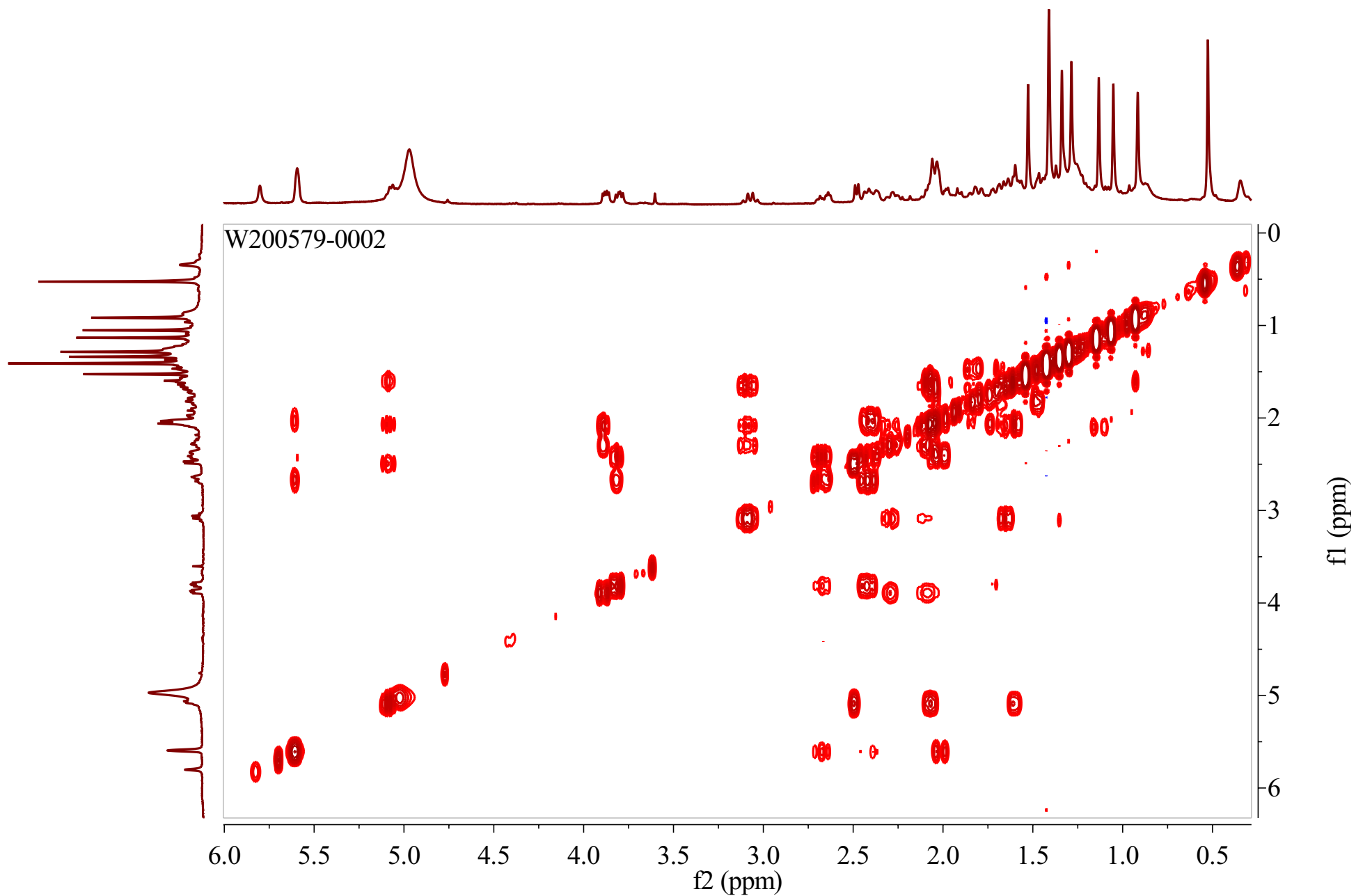
**Figure S17 DEPT spectrum of compound 14**



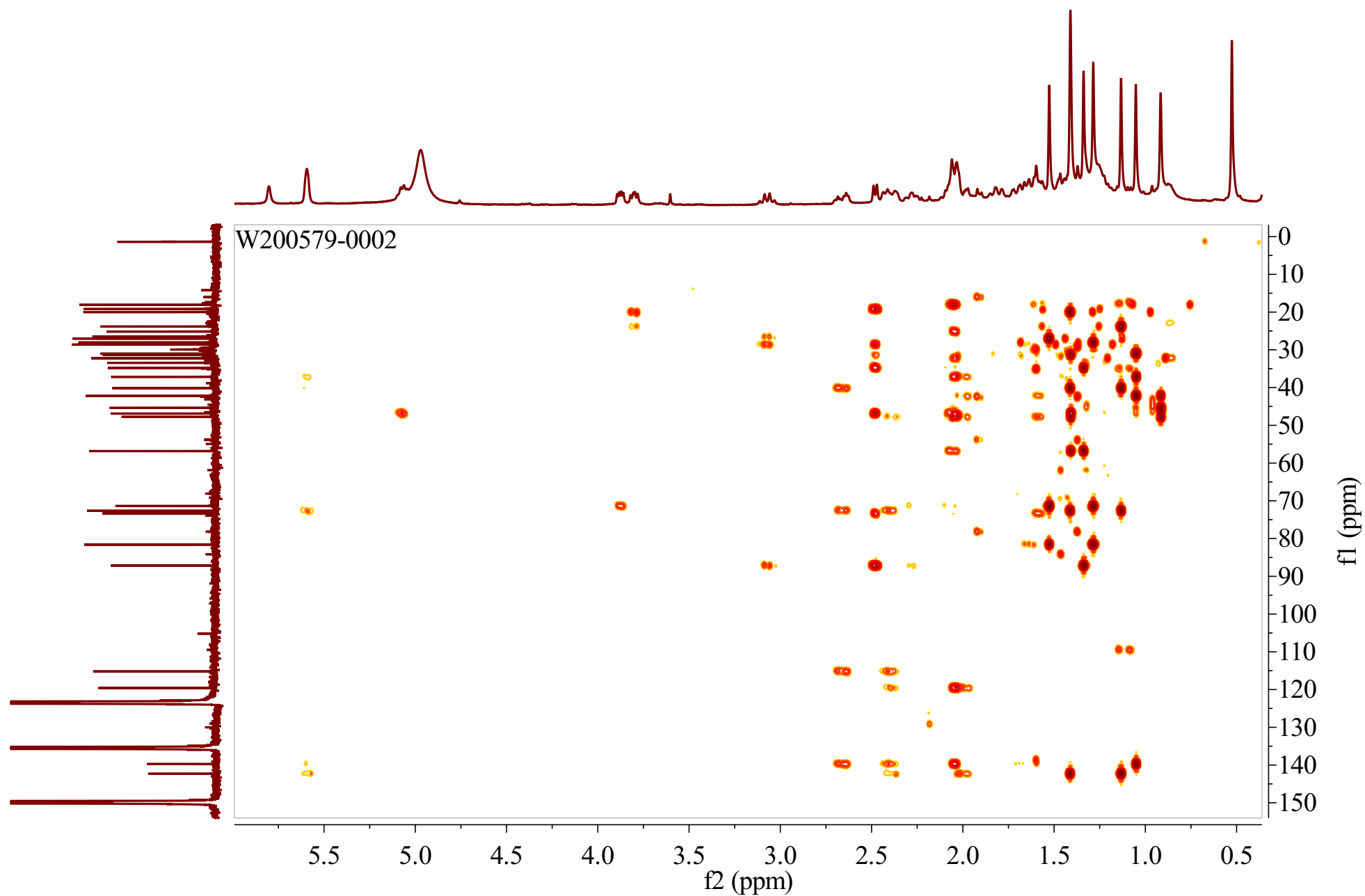
**Figure S18 HMQC spectrum of compound 14**



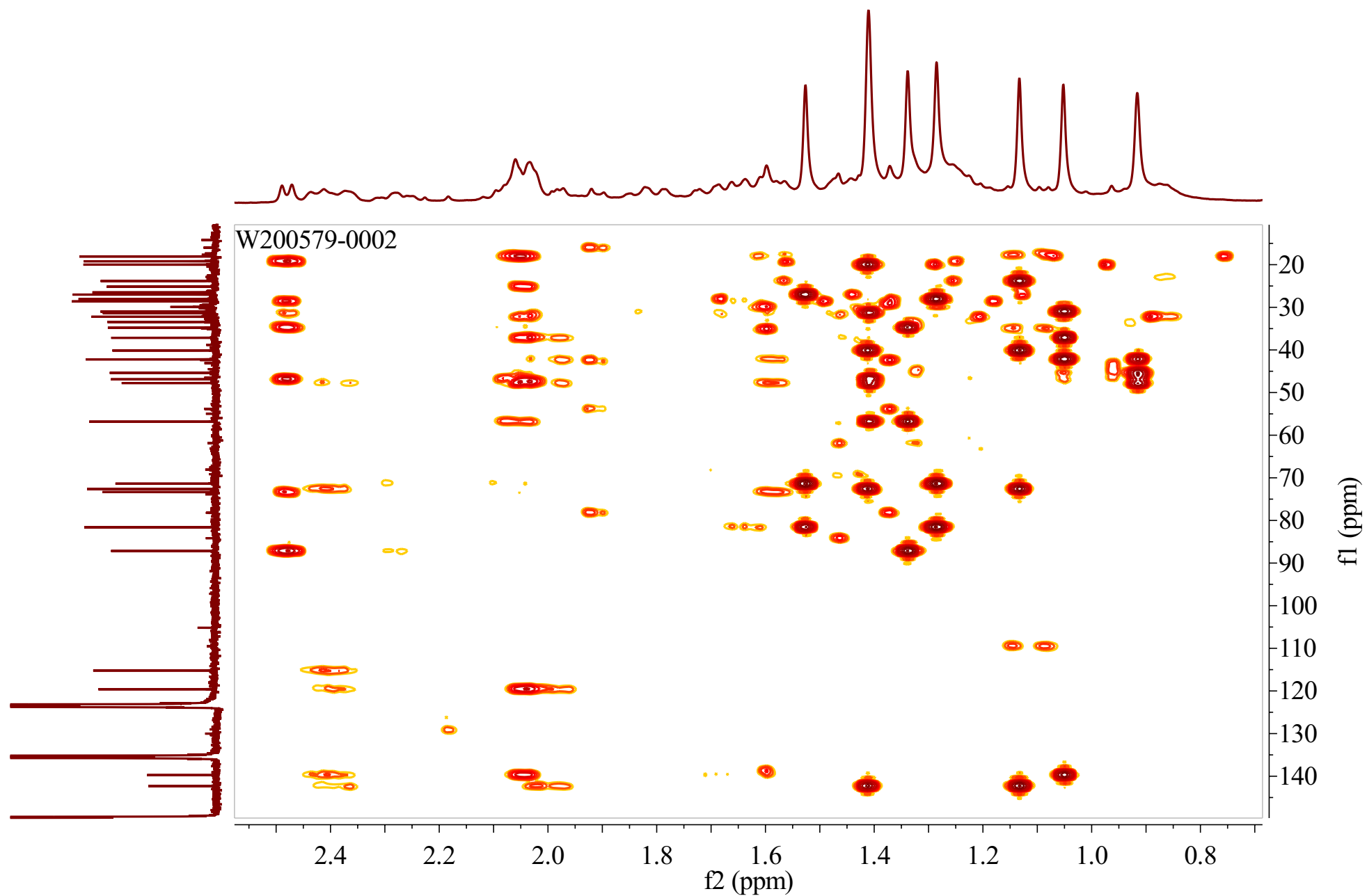
**Figure S19** Amplificatory HMQC spectrum of compound 14



**Figure S20  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound 14**

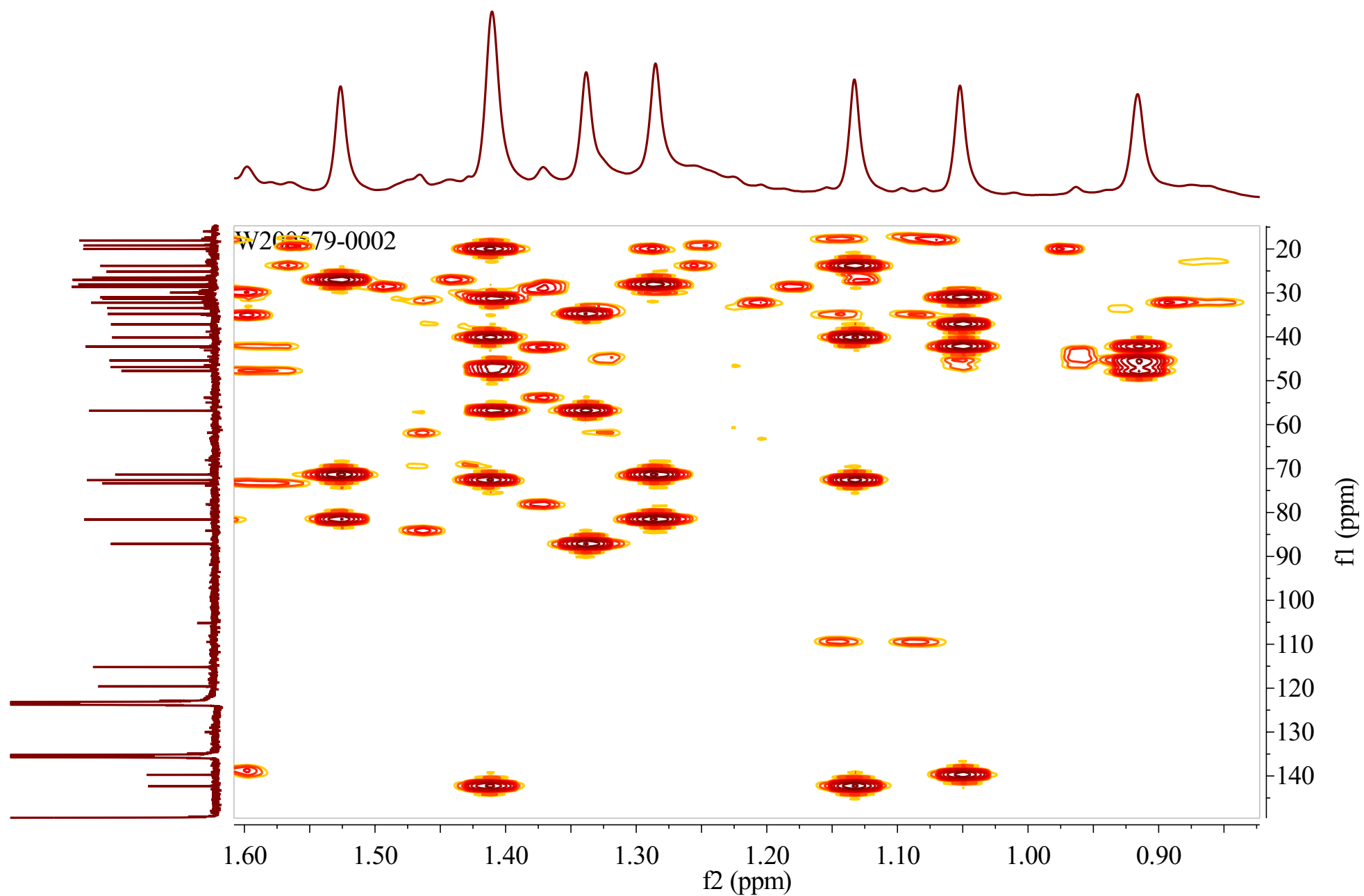


**Figure S21 Key HMBC spectrum of compound 14**



**Figure S22** Amplificatory HMBC spectrum of compound 14 (A)





**Figure S23 Amplificatory HMBC spectrum of compound 14 (B)**

**Table S1.**  $^{13}\text{C}$  NMR spectral data of compounds **9**, **14** and CAG.

| C  | 9                       | 14                      | CAG                     | C  | 9                       | 14                      | CAG                     |
|----|-------------------------|-------------------------|-------------------------|----|-------------------------|-------------------------|-------------------------|
| 1  | 35.5 (CH <sub>2</sub> ) | 115.2 (CH)              | 31.5 (CH <sub>2</sub> ) | 16 | 73.0 (CH)               | 73.3 (CH)               | 73.4 (CH)               |
| 2  | 26.5 (CH <sub>2</sub> ) | 33.4 (CH <sub>2</sub> ) | 32.8 (CH <sub>2</sub> ) | 17 | 57.4 (CH)               | 56.8 (CH)               | 58.4 (CH)               |
| 3  | 82.7 (CH)               | 72.6 (CH)               | 78.3 (CH)               | 18 | 19.0 (CH <sub>3</sub> ) | 19.2 (CH <sub>3</sub> ) | 21.6 (CH <sub>3</sub> ) |
| 4  | 46.1 (C)                | 40.1 (C)                | 42.4 (C)                | 19 | 35.8 (CH <sub>3</sub> ) | 32.2 (CH <sub>3</sub> ) | 31.0 (CH <sub>2</sub> ) |
| 5  | 61.4 (CH)               | 142.3 (C)               | 54.0 (CH)               | 20 | 87.1 (C)                | 87.2 (C)                | 87.2 (C)                |
| 6  | 65.9 (CH)               | 119.6 (CH)              | 68.3 (CH)               | 21 | 28.6 (CH <sub>3</sub> ) | 28.6 (CH <sub>3</sub> ) | 28.2 (CH <sub>3</sub> ) |
| 7  | 34.6 (CH <sub>2</sub> ) | 25.2 (CH <sub>2</sub> ) | 38.8 (CH <sub>2</sub> ) | 22 | 34.8 (CH <sub>2</sub> ) | 34.8 (CH <sub>2</sub> ) | 34.9 (CH <sub>2</sub> ) |
| 8  | 46.6 (CH)               | 42.2 (CH)               | 47.3 (CH)               | 23 | 26.5 (CH <sub>2</sub> ) | 26.5 (CH <sub>2</sub> ) | 26.4 (CH <sub>2</sub> ) |
| 9  | 35.5 (C)                | 37.2 (C)                | 21.0 (C)                | 24 | 81.6 (CH)               | 81.6 (CH)               | 81.7 (CH)               |
| 10 | 93.2 (C)                | 139.7 (C)               | 29.9 (C)                | 25 | 71.4 (C)                | 71.4 (C)                | 71.2 (C)                |
| 11 | 32.8 (CH <sub>2</sub> ) | 31.0 (CH <sub>2</sub> ) | 26.3 (CH <sub>2</sub> ) | 26 | 27.0 (CH <sub>3</sub> ) | 27.0 (CH <sub>3</sub> ) | 27.1 (CH <sub>3</sub> ) |
| 12 | 32.4 (CH <sub>2</sub> ) | 31.3 (CH <sub>2</sub> ) | 33.4 (CH <sub>2</sub> ) | 27 | 28.1 (CH <sub>3</sub> ) | 28.1 (CH <sub>3</sub> ) | 28.6 (CH <sub>3</sub> ) |
| 13 | 45.6 (C)                | 46.9 (C)                | 45.0 (C)                | 28 | 23.1 (CH <sub>3</sub> ) | 23.8 (CH <sub>3</sub> ) | 29.4 (CH <sub>3</sub> ) |
| 14 | 45.7 (C)                | 47.8 (C)                | 46.2 (C)                | 29 | 26.1 (CH <sub>3</sub> ) | 20.0 (CH <sub>3</sub> ) | 16.1 (CH <sub>3</sub> ) |
| 15 | 46.0 (CH <sub>2</sub> ) | 45.4 (CH <sub>2</sub> ) | 46.8 (CH <sub>2</sub> ) | 30 | 19.8 (CH <sub>3</sub> ) | 18.1 (CH <sub>3</sub> ) | 20.2 (CH <sub>3</sub> ) |

**Table S2** Anti-inflammatory of compound **9** activities against LPS-induced lymphocyte cells

| NO.               | Cell survival (%) |
|-------------------|-------------------|
| DMSO Control      | 100.00            |
| LPS Control       | 77.11             |
| Compound <b>9</b> |                   |
| 10 nM             | 75.18             |
| 100 nM            | 85.03             |
| 1 $\mu$ M         | 89.57             |

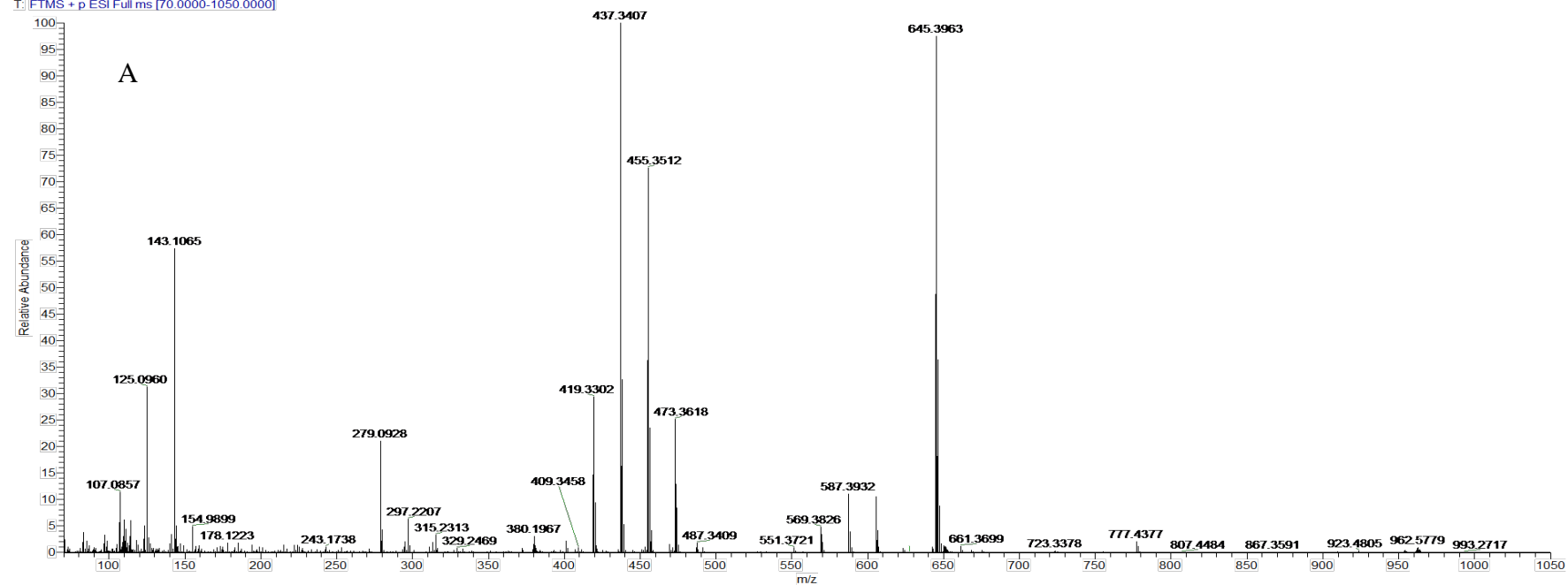
**Table S3** Anti-inflammatory of compound **14** activities against LPS-induced lymphocyte cells

| NO.                | Cell survival (%) |
|--------------------|-------------------|
| DMSO Control       | 100.00            |
| LPS Control        | 61.35             |
| Compound <b>14</b> |                   |
| 10 nM              | 67.54             |
| 100 nM             | 79.46             |
| 1 $\mu$ M          | 89.15             |

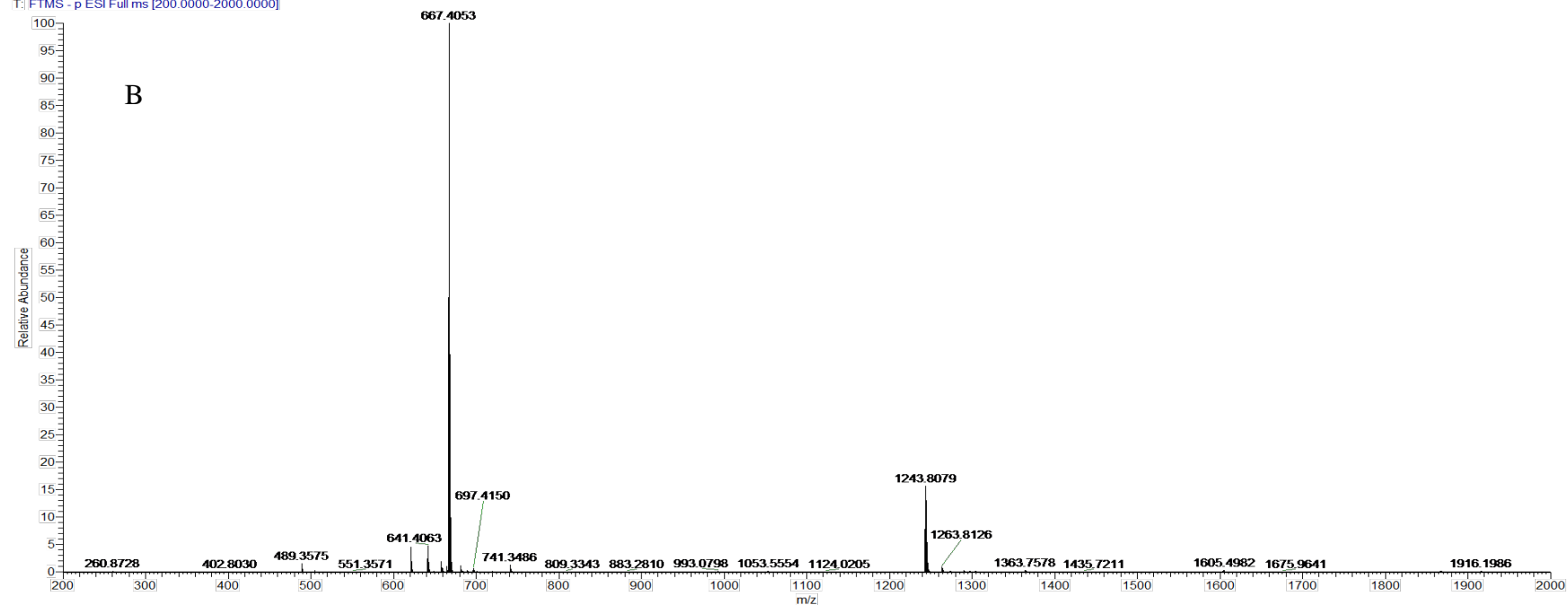
**Table S4** Anti-inflammatory of compound **9** and **14** activities against CSE-induced MLE-12 cells

| NO.                |            | CD69+Cell survival (%) |
|--------------------|------------|------------------------|
| DMSO Control       |            | 13.15                  |
| CSE Control        |            | 59.03                  |
| Compound <b>9</b>  |            |                        |
|                    | 3 $\mu$ M  | 57.96                  |
|                    | 10 $\mu$ M | 56.17                  |
|                    | 30 $\mu$ M | 49.61                  |
| Compound <b>14</b> |            |                        |
|                    | 3 $\mu$ M  | 60.14                  |
|                    | 10 $\mu$ M | 59.03                  |
|                    | 30 $\mu$ M | 50.40                  |

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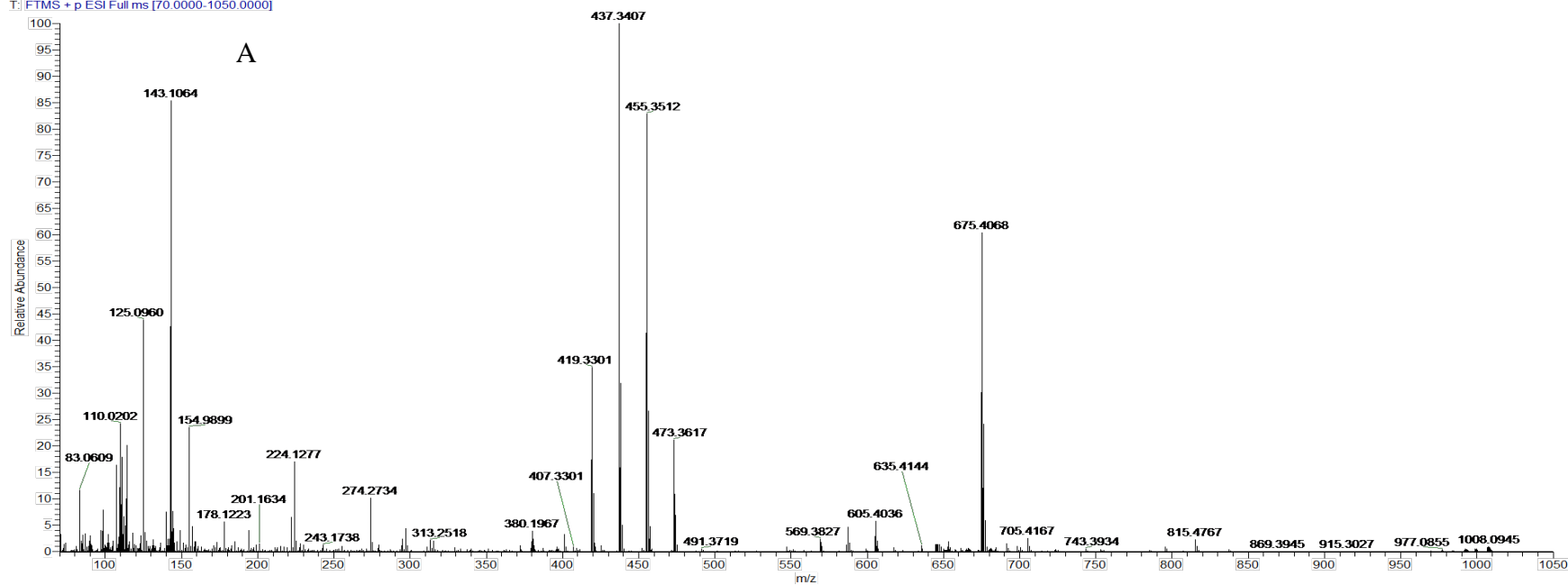


15-24-1 (2) #3598 RT: 9.69 AV: 1 NL: 6.47E8  
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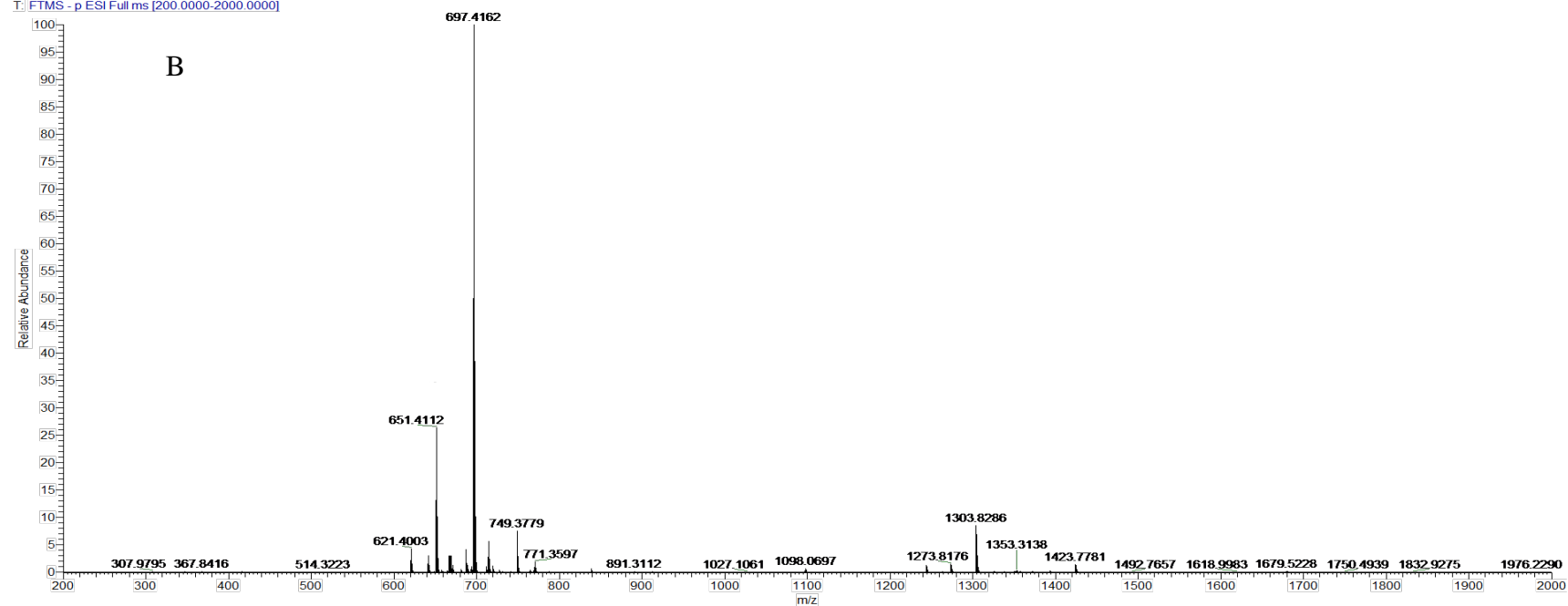


**Figure S24** Positive ions (A) and negative ions (B) ESI-MS/MS spectra of compound **1**

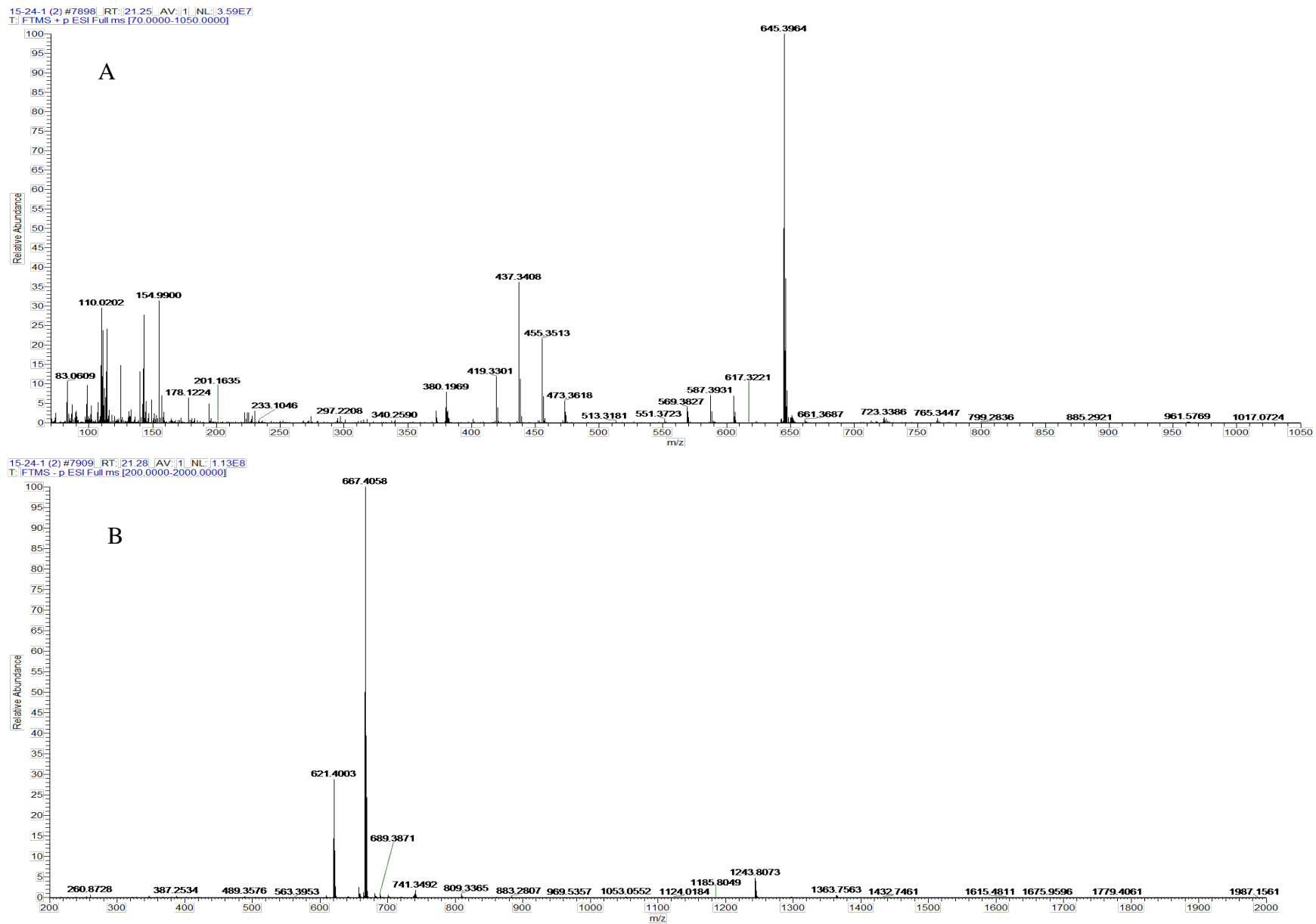
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T: FTMS + p.ESI Full ms [70.0000-1050.0000]



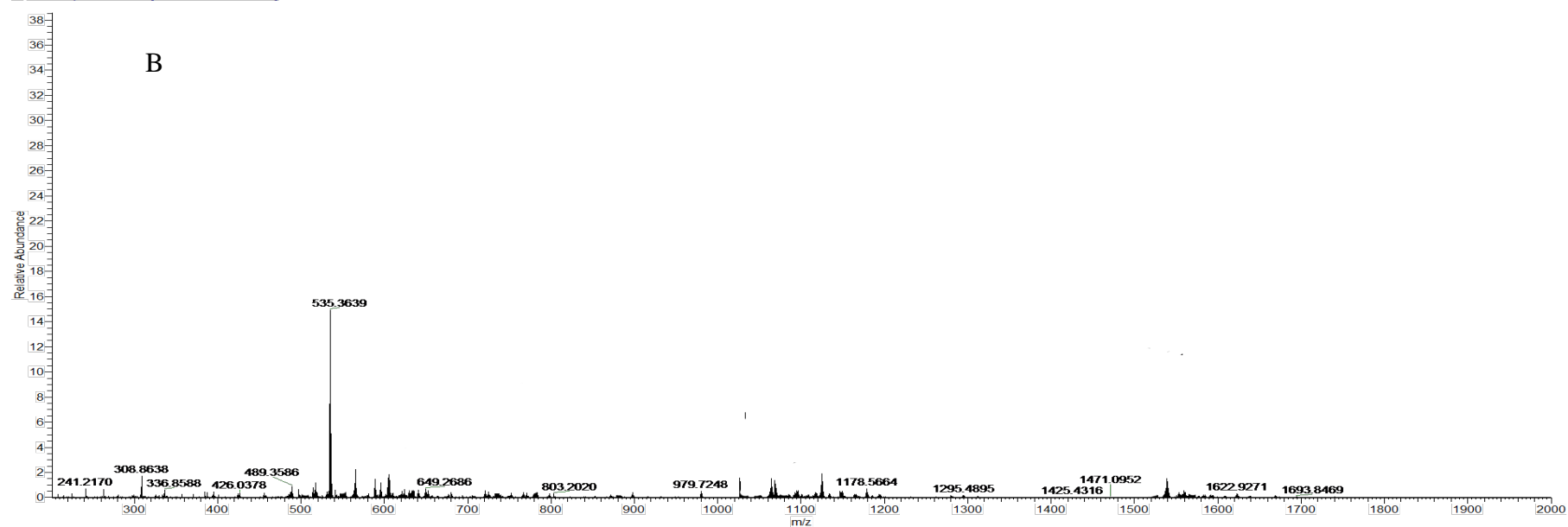
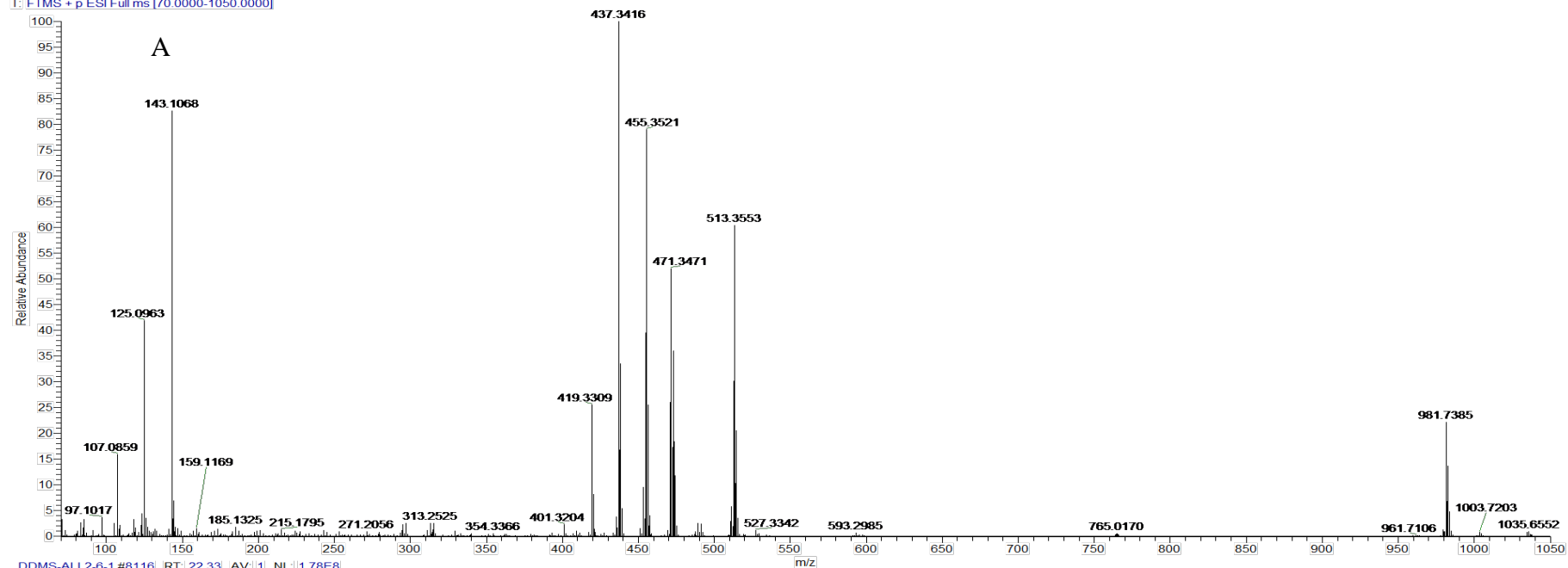
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**Figure S25** Positive ions (A) and negative ions(B) ESI-MS/MS spectra of compound 2



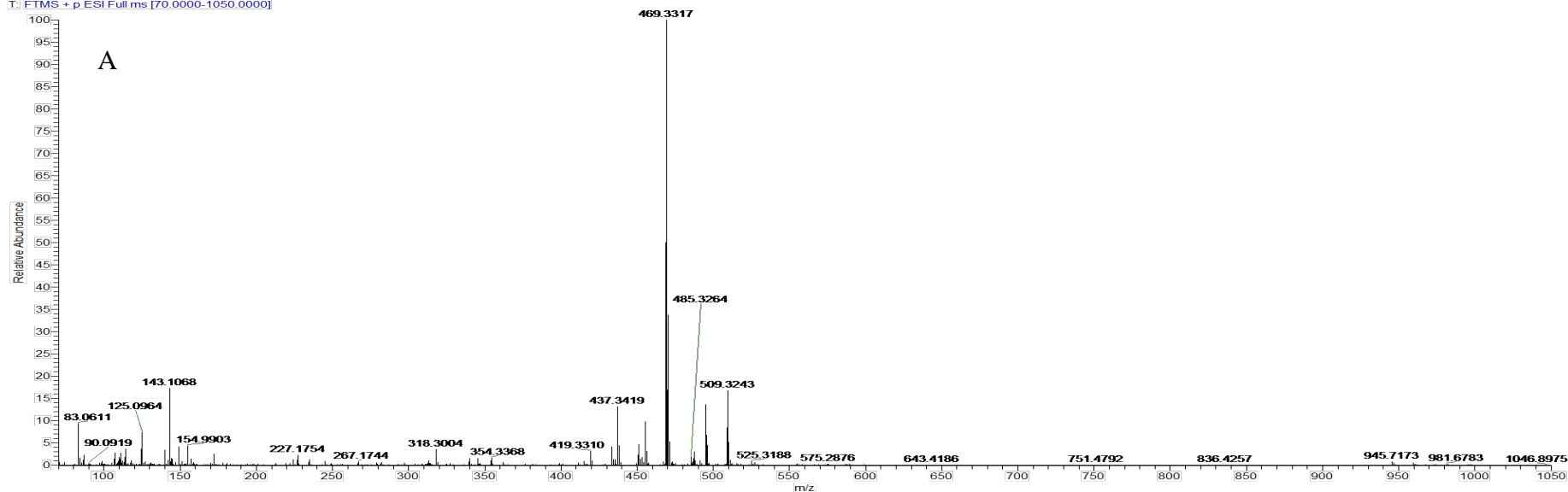
**Figure S26** Positive ions (A) and negative ions (B) ESI-MS/MS spectra of compound **3**



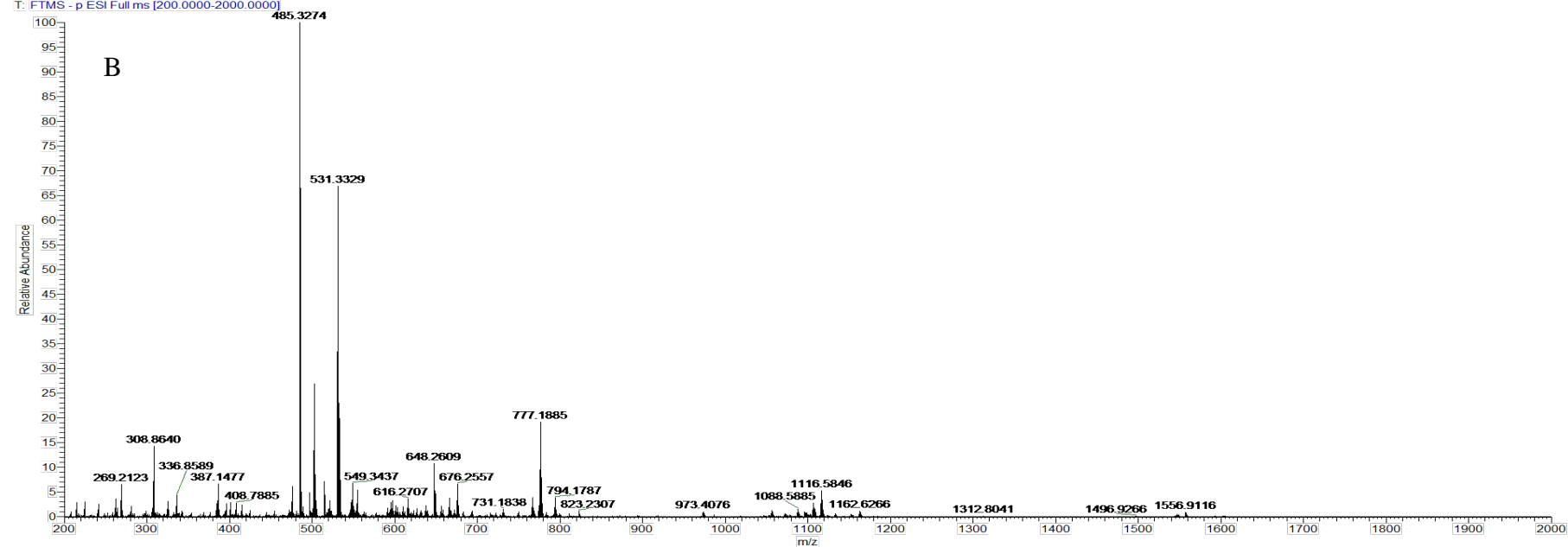
**Figure S27** Positive ions (A) and negative ions (B) ESI-MS/MS spectra of compound **4**



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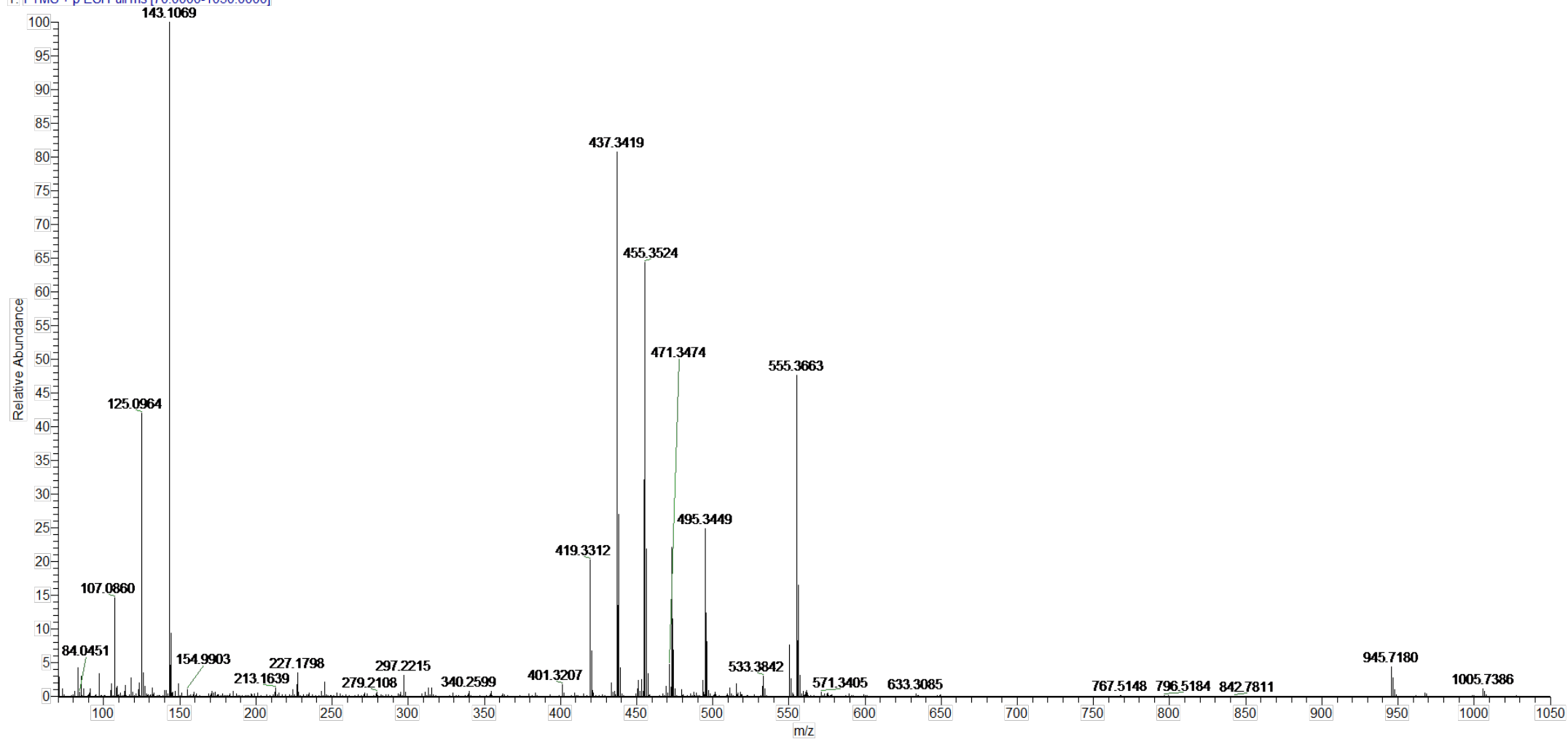


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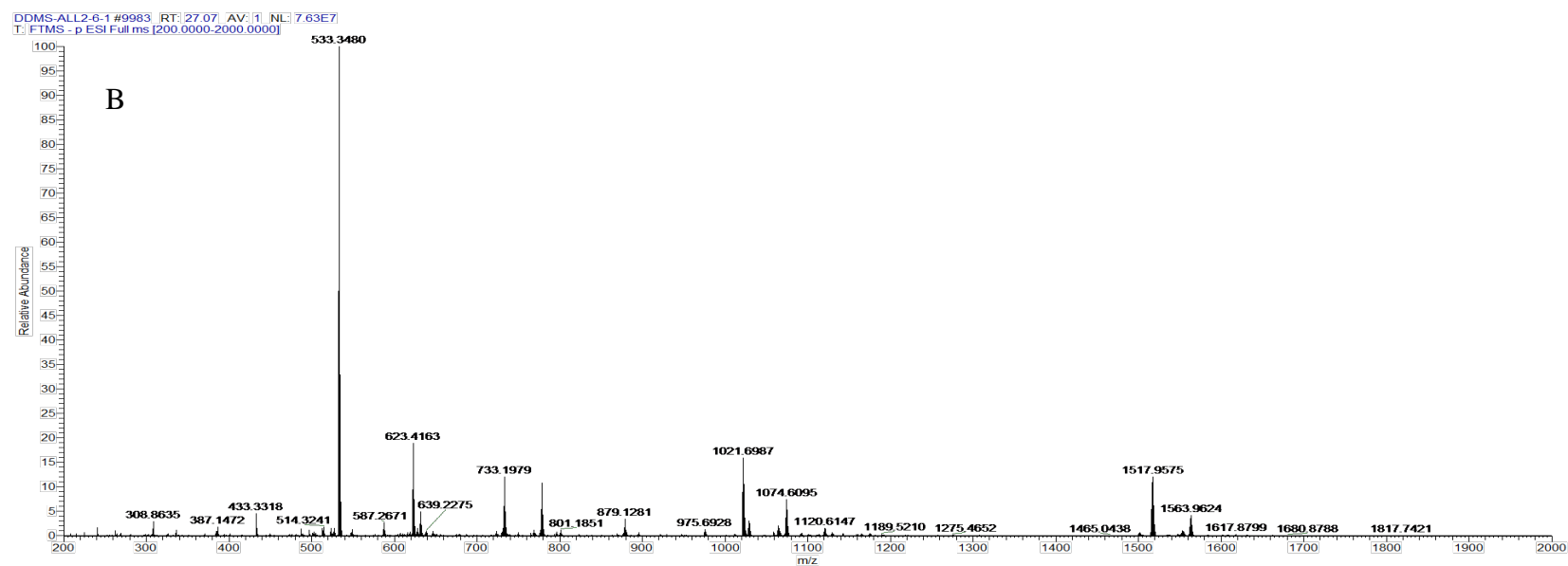
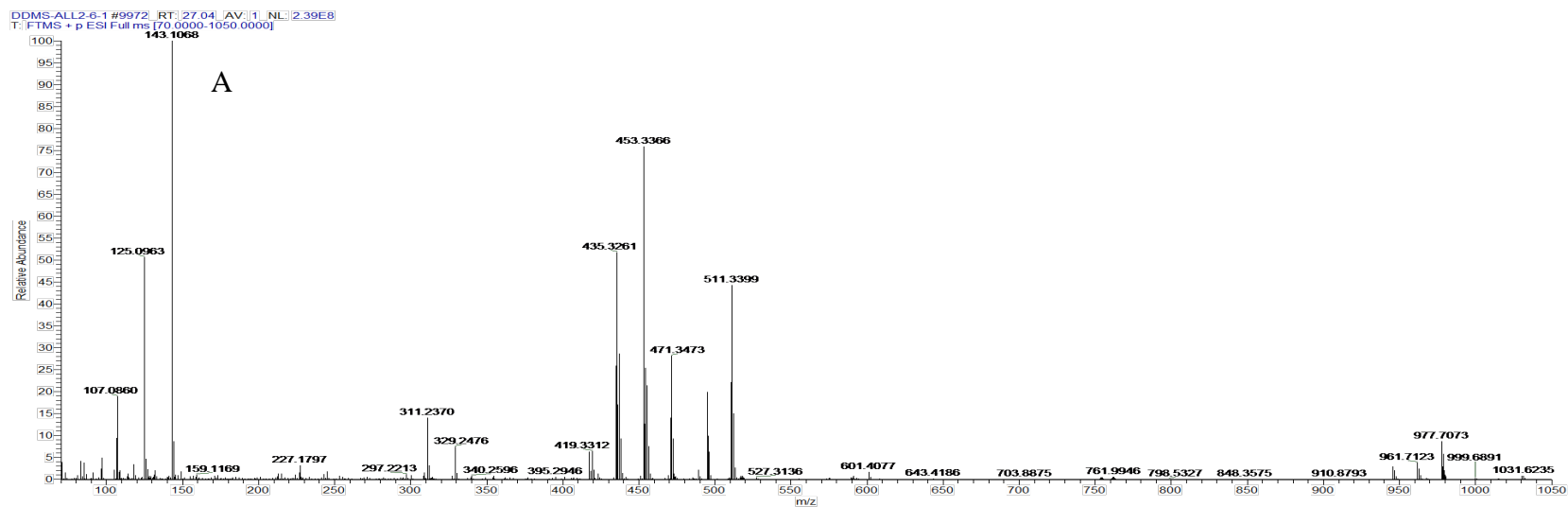


**Figure S28** Positive ions (A) and negative ions (B) ESI-MS/MS spectra of compound **5**

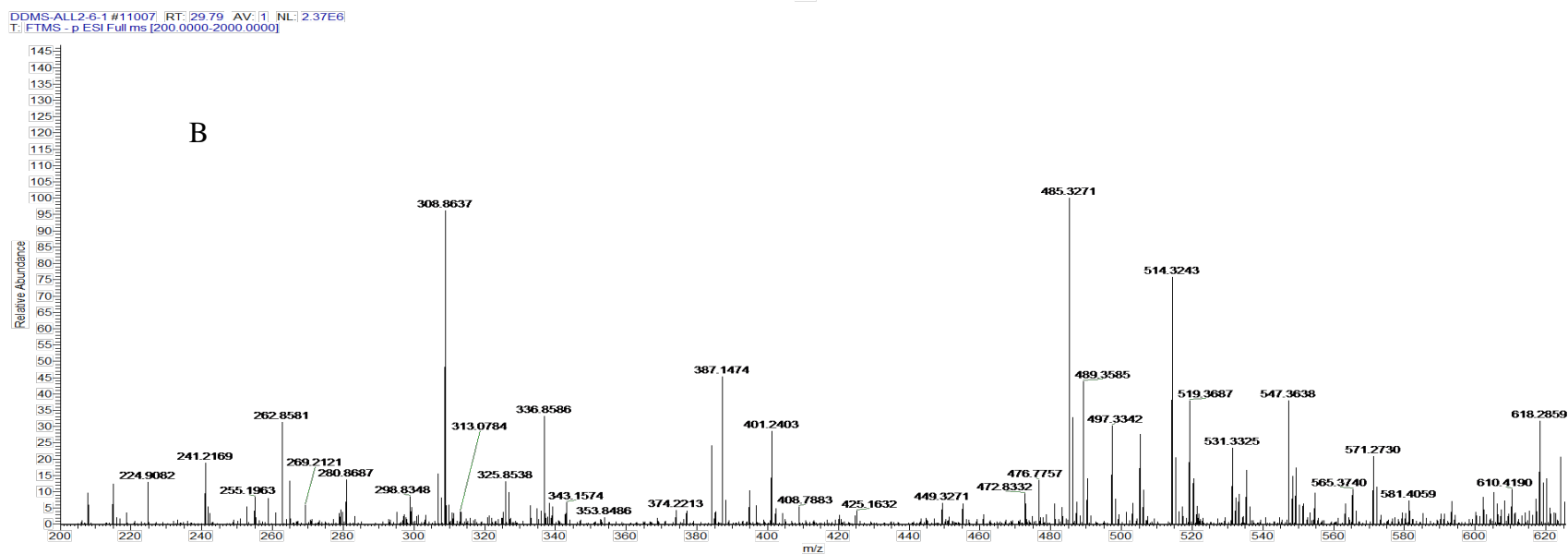
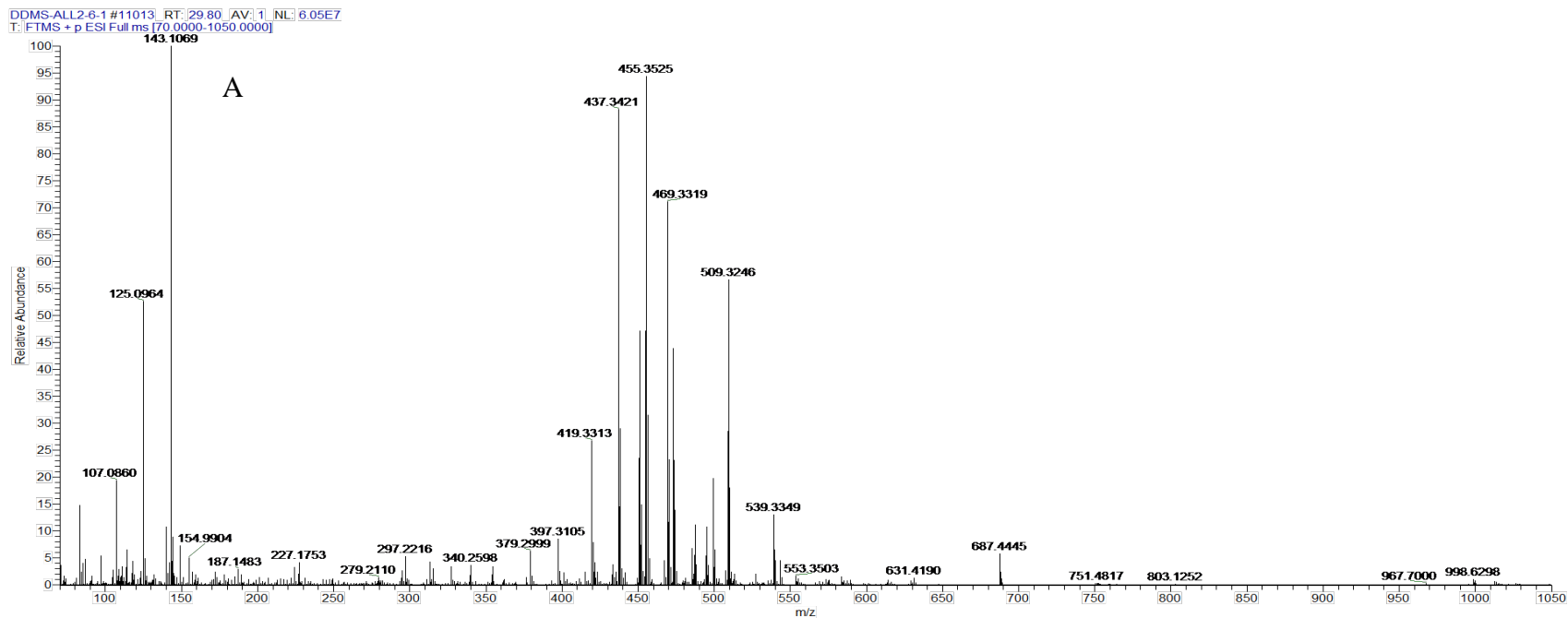
DDMS-ALL2-6-1 #9751 RT: 26.49 AV: 1 NL: 1.99E8  
T: FTMS + p ESI Full ms [70.0000-1050.0000]



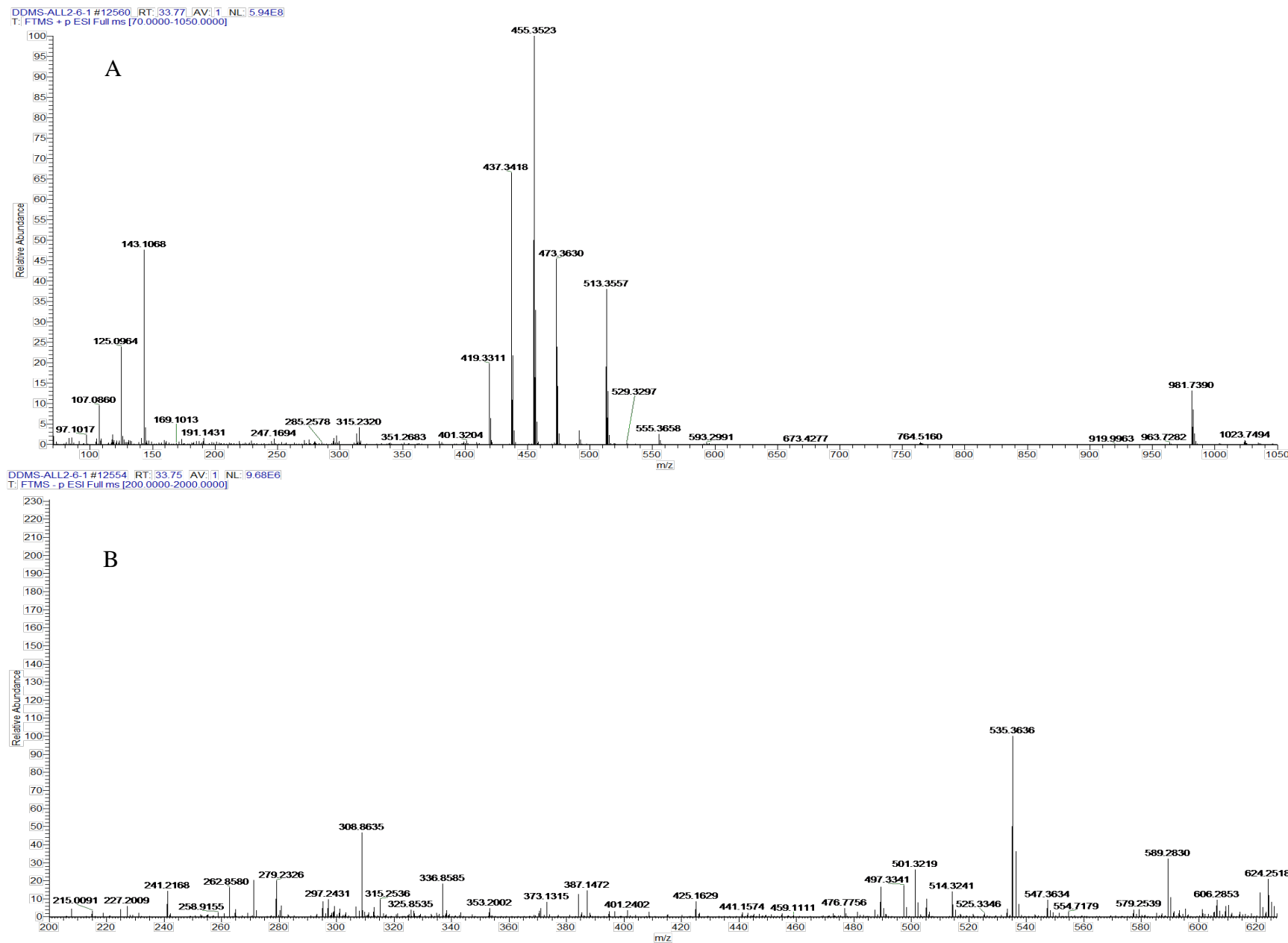
**Figure S29** Positive ions ESI-MS/MS spectra of compound **6**



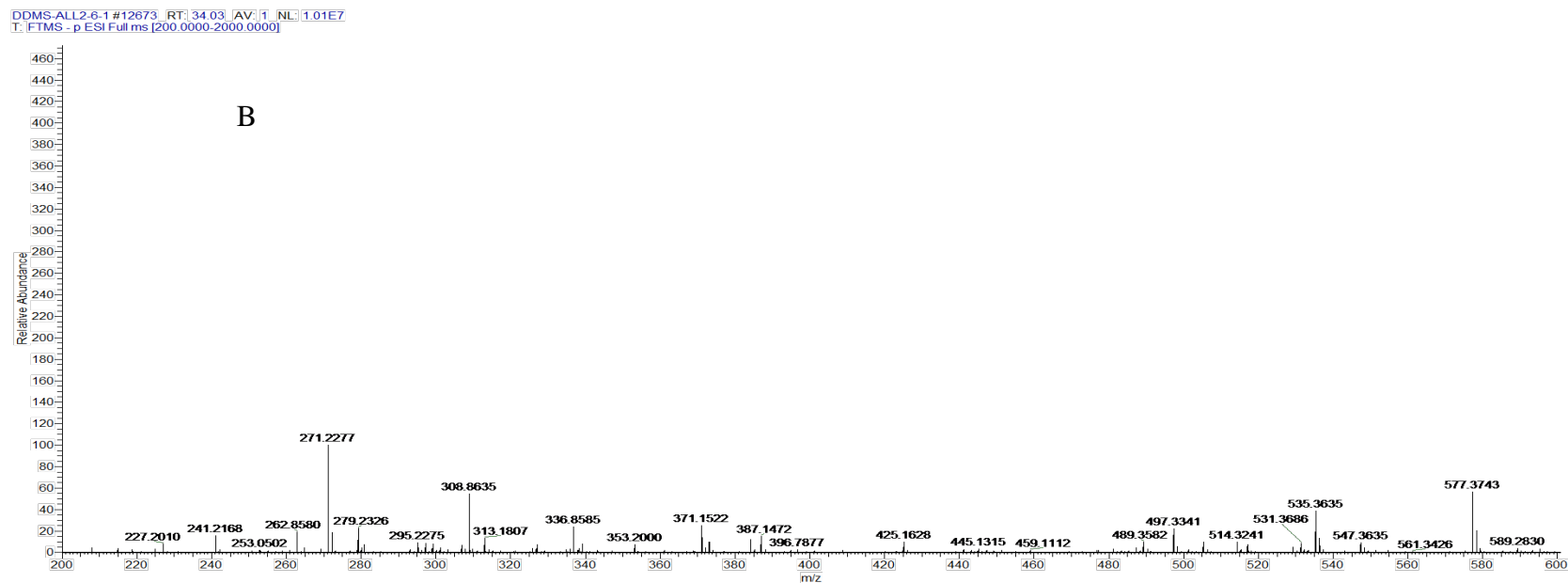
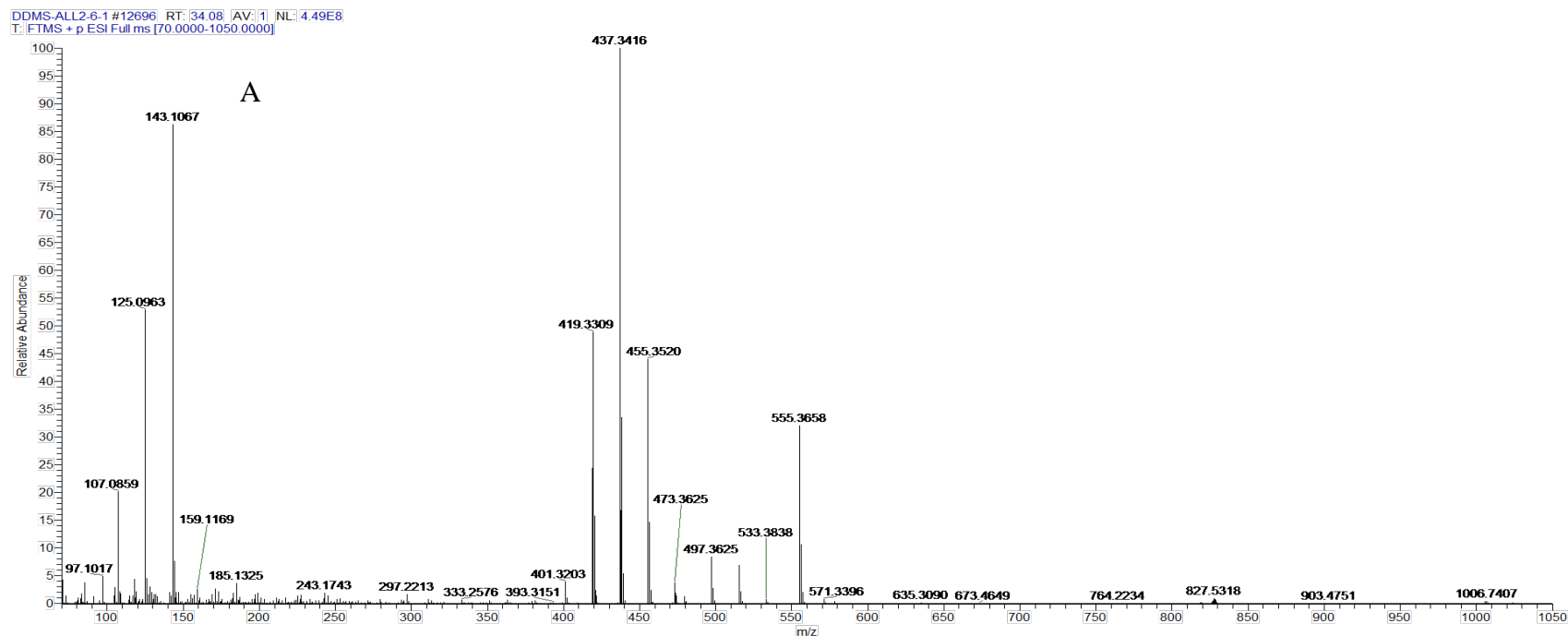
**Figure S30** Positive ions (A) and negative ions (B) ESI-MS/MS spectra of compound **7**



**Figure S31** Positive ions (A) and negative ions (B) ESI-MS/MS spectra of compound **8**

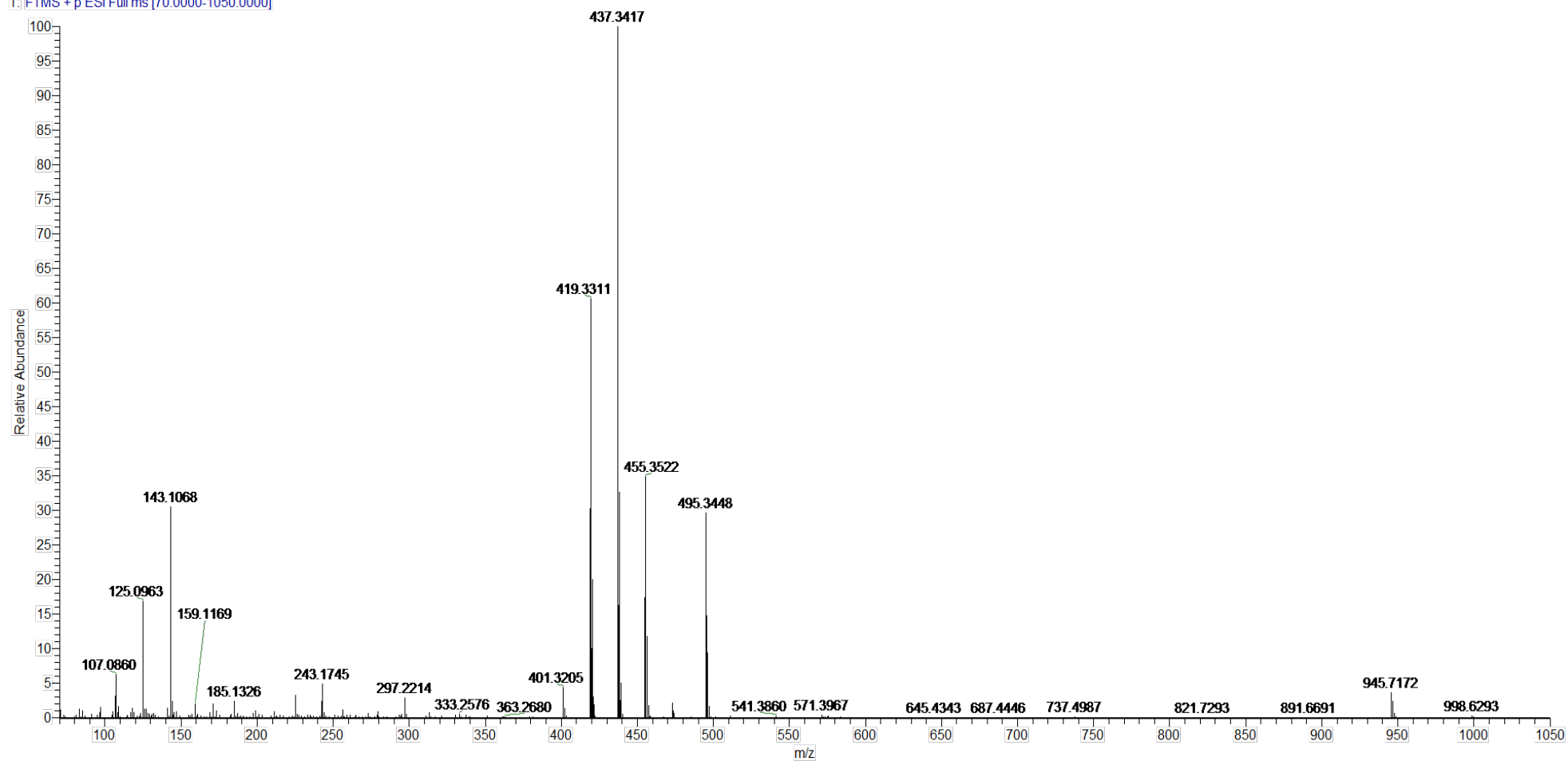


**Figure S32** Positive ions (A) and negative ions (B) ESI-MS/MS spectra of compound **9**



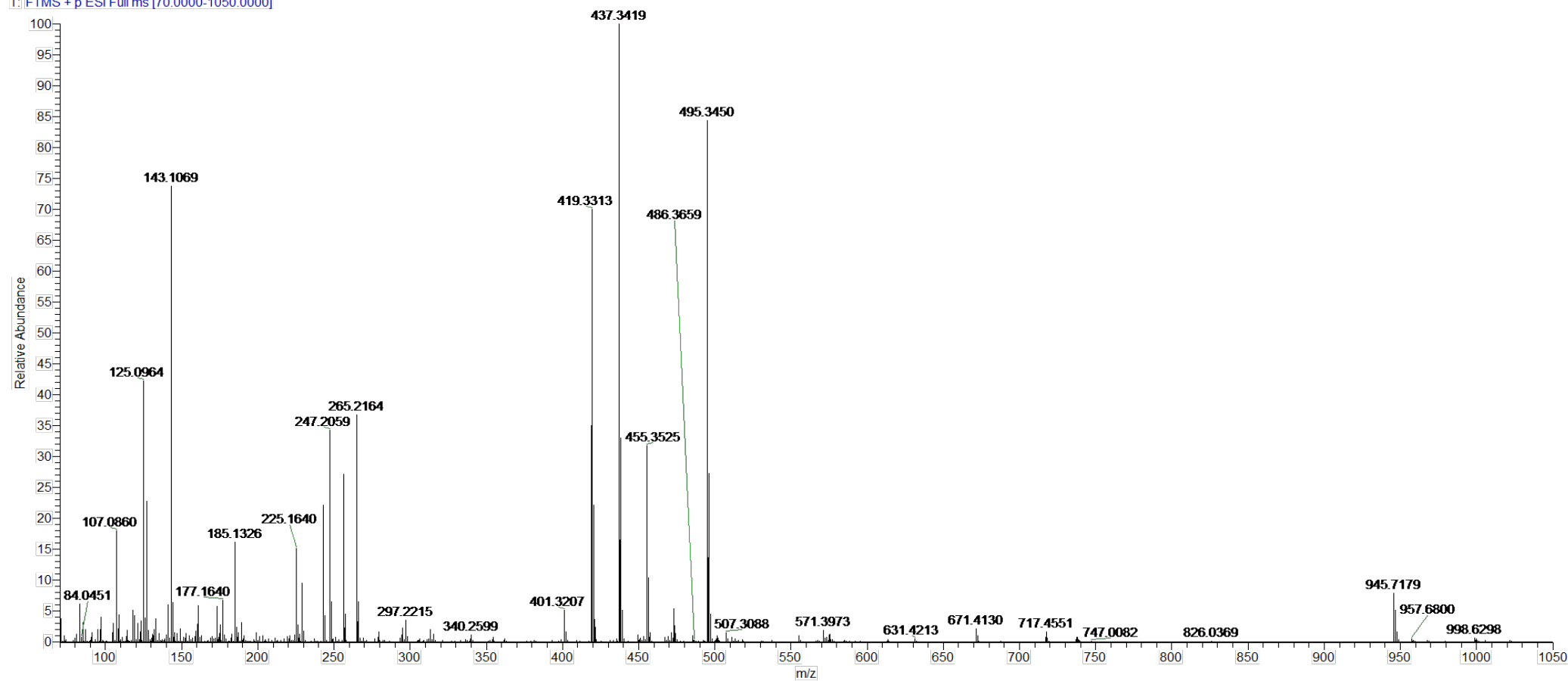
**Figure S33** Positive ions (A) and negative ions (B) ESI-MS/MS spectra of compound **10**

DDMS-ALL2-6-1 #13989 RT: 37.38 AV: 1 NL: 8.91E8  
T: FTMS + p ESI Full ms [70.0000-1050.0000]



**Figure S34** Positive ions ESI-MS/MS spectra of compound **11**

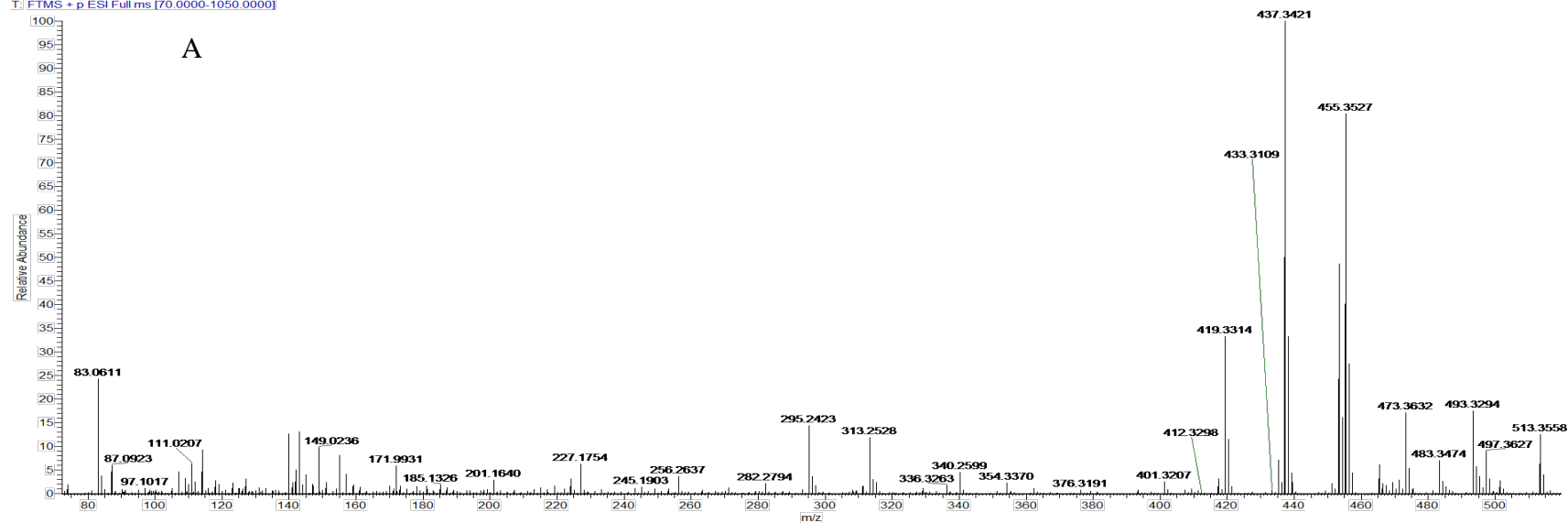
DDMS-ALL2-6-1 #14414 RT: 38.43 AV: 1 NL: 1.74E8  
T: FTMS + p ESI Full ms [70.0000-1050.0000]



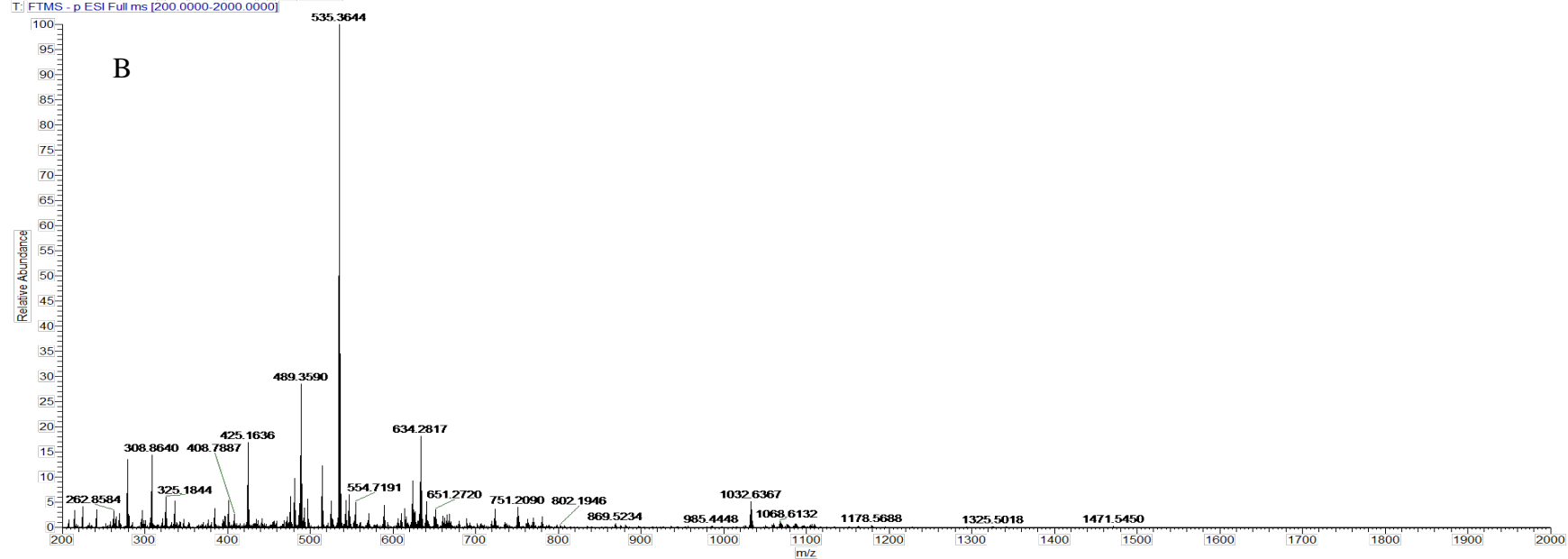
**Figure S35** Positive ions ESI-MS/MS spectra of compound **12**



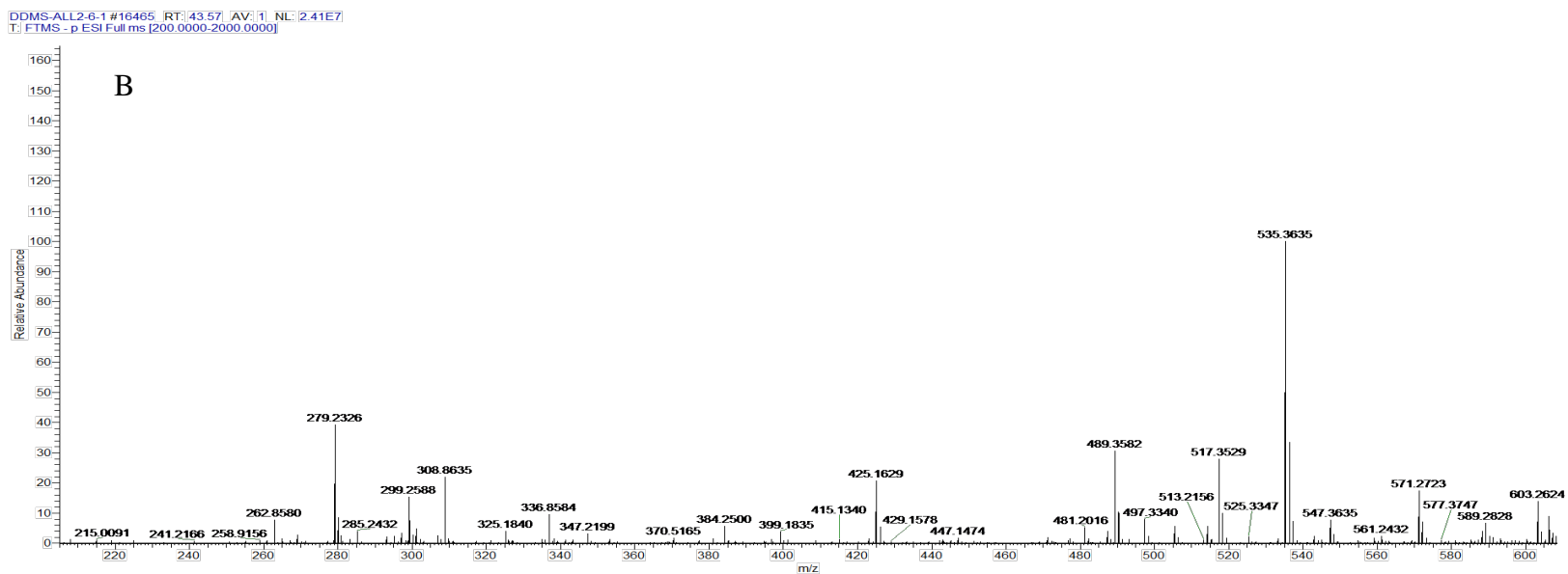
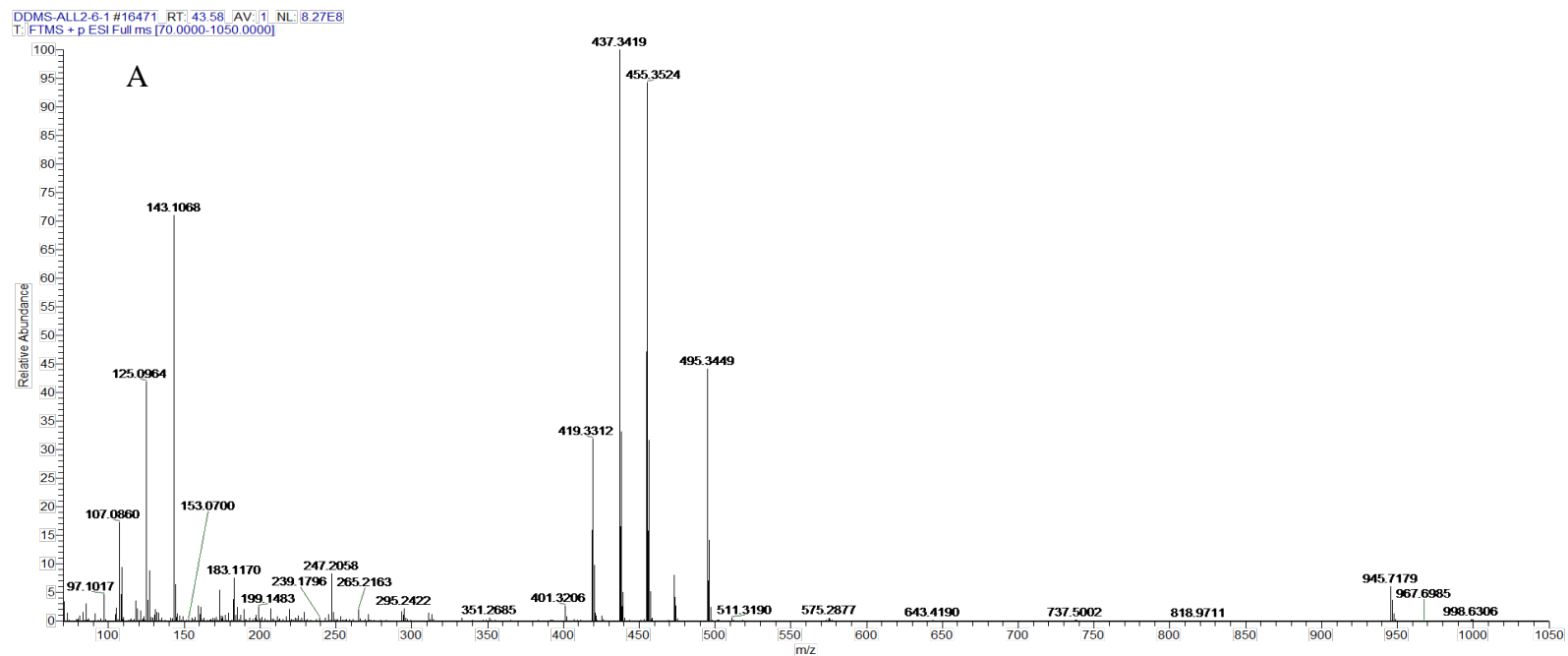
DDMS-ALL2-6-1 #15672 RT: 41.60 AV: 1 NL: 4.23E7  
T: FTMS + p ESI Full ms [70.0000-1050.0000]



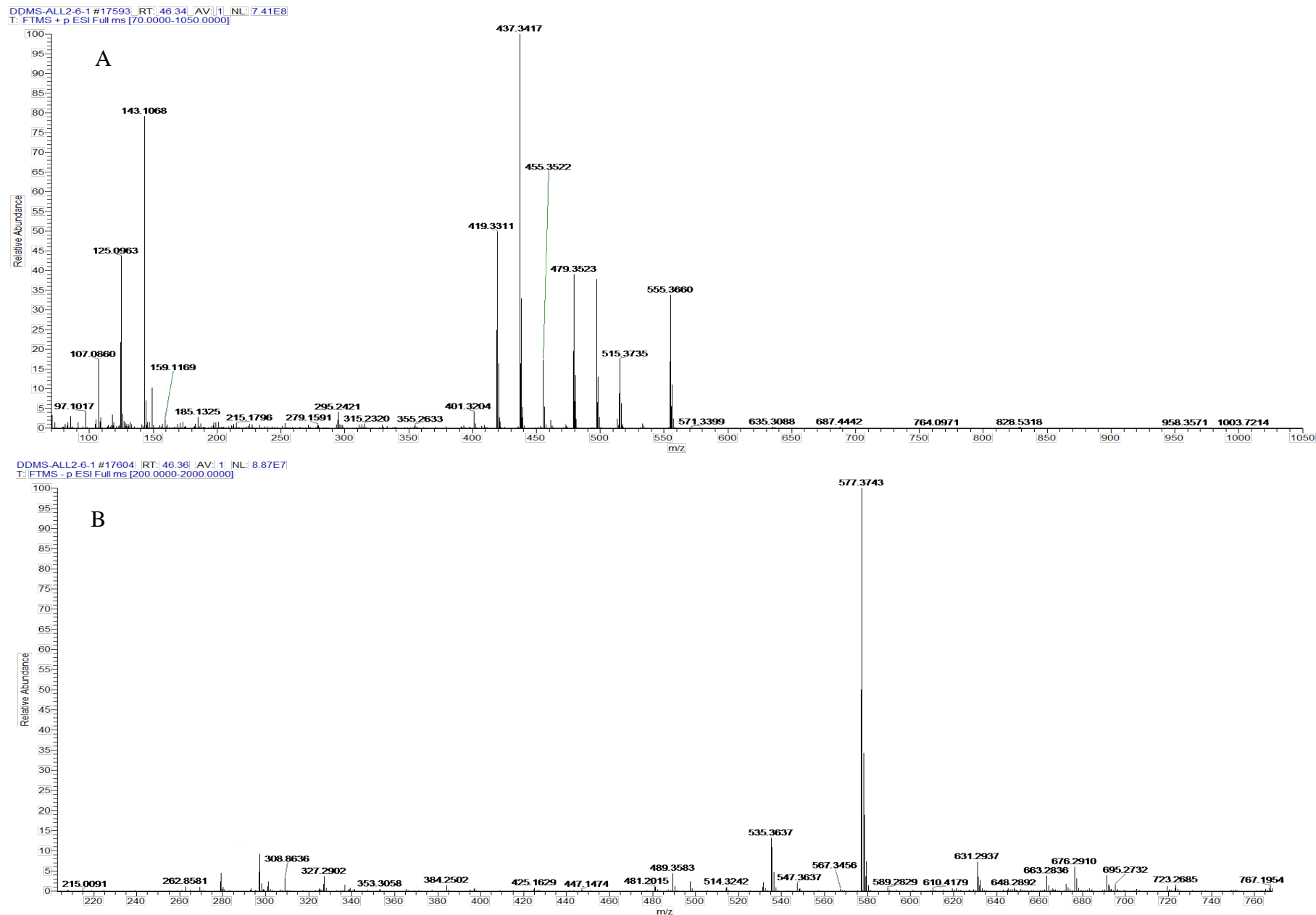
DDMS-ALL2-6-1 #15564 RT: 41.32 AV: 1 NL: 6.47E6  
T: FTMS - p ESI Full ms [200.0000-2000.0000]



**Figure S36** Positive ions (A) and negative ions (B) ESI-MS/MS spectra of compound **13**



**Figure S37** Positive ions (A) and negative ions (B) ESI-MS/MS spectra of compound **14**



**Figure S38** Positive ions (A) and negative ions (B) ESI-MS/MS spectra of compound **15**