

## Supplementary Materials

# New Conjugates of Polyhydroxysteroids with Long-Chain Fatty Acids from the Deep-Water Far Eastern Starfish *Ceramaster patagonicus* and Their Anticancer Activity

Timofey V. Malyarenko<sup>1,2\*</sup>, Alla A. Kicha<sup>1</sup>, Olesya S. Malyarenko<sup>1</sup>, Viktor M. Zakharenko<sup>2</sup>, Ivan P. Kotlyarov<sup>2</sup>, Anatoly I. Kalinovskiy<sup>1</sup>, Roman S. Popov<sup>1</sup>, Vasily I. Svetashev<sup>3</sup>, and Natalia V. Ivanchina<sup>1</sup>

<sup>1</sup> G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Far Eastern Branch of the Russian Academy of Sciences, Pr. 100-let Vladivostoku 159, 690022, Vladivostok, Russia; kicha@piboc.dvo.ru (A.A.K.); malyarenko.os@gmail.com (O.S.M.); kaaniw@piboc.dvo.ru (A.I.K.); prs\_90@mail.ru (R.S.P.); ivanchina@piboc.dvo.ru (N.V.I.).

<sup>2</sup> Department of Bioorganic Chemistry and Biotechnology, School of Natural Sciences, Far Eastern Federal University, Sukhanova str. 8, 690000, Vladivostok, Russia; rarf247@gmail.com (V.M.Z.); ivan\_1999\_19@icloud.com (I.P.K.).

<sup>3</sup> A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences, 17 Palchevsky st., 690041, Vladivostok, Russia; vsvetashev@mail.ru (V.I.S.).

**List**

**Figure S1.** (+)-HRESIMS spectrum of compound **1**.

**Figure S2.** (-)-HRESIMS spectrum of compound **1**.

**Figure S3.** (-)-ESIMS/MS spectrum of compound **1**.

**Figure S4.**  $^1\text{H}$ -NMR spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S5.** Enlarged-1  $^1\text{H}$ -NMR spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S6.** Enlarged-2  $^1\text{H}$ -NMR spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S7.** Enlarged-3  $^1\text{H}$ -NMR spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S8.** Enlarged-4  $^1\text{H}$ -NMR spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S9.**  $^{13}\text{C}$ -NMR spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S10.** Enlarged-1  $^{13}\text{C}$ -NMR spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S11.** Enlarged-2  $^{13}\text{C}$ -NMR spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S12.** Enlarged-3  $^{13}\text{C}$ -NMR spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S13.**  $^1\text{H}$ - $^1\text{H}$ -COSY spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S14.** Enlarged  $^1\text{H}$ - $^1\text{H}$ -COSY spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S15.** HSQC spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S16.** Enlarged of HSQC spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S17.** HMBC spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S18.** Enlarged of HMBC spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S19.** ROESY spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S20.** Enlarged of ROESY spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S21.** (+)-HRESIMS spectrum of compound **2**.

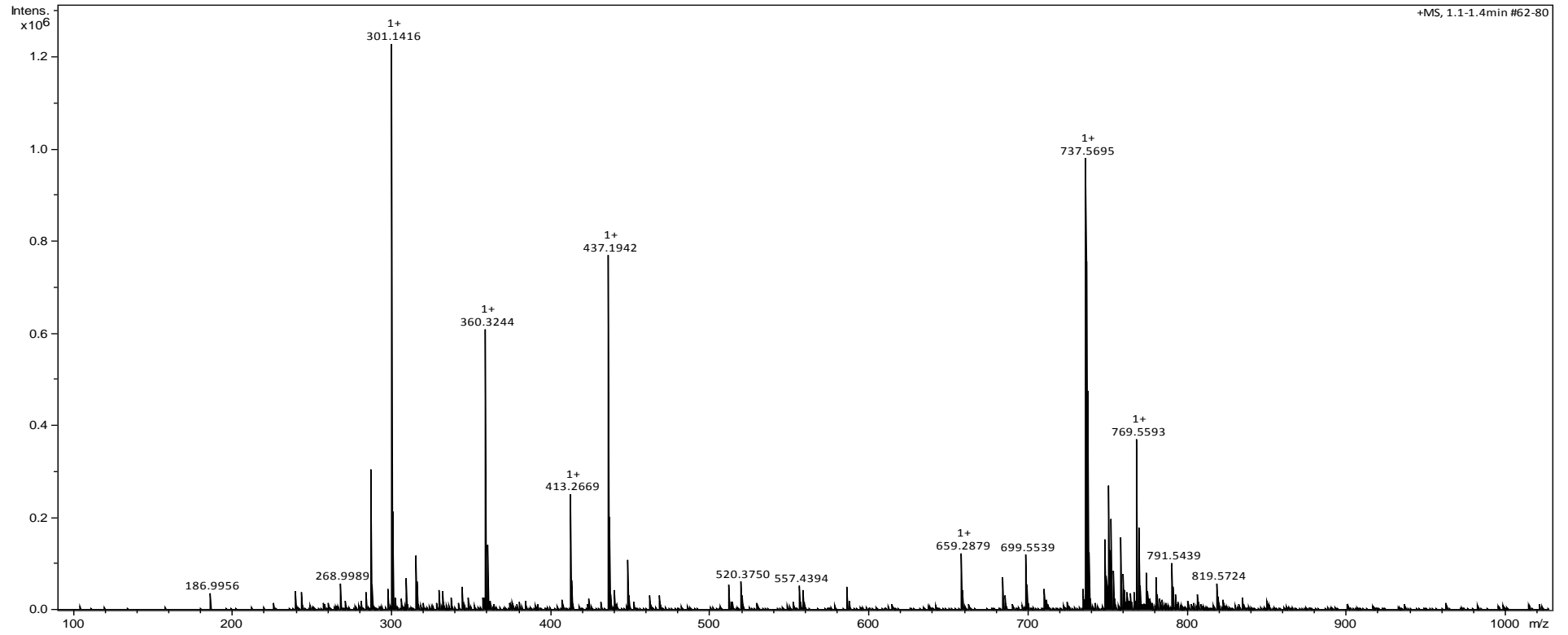
**Figure S22.** (-)-HRESIMS spectrum of compound **2**.

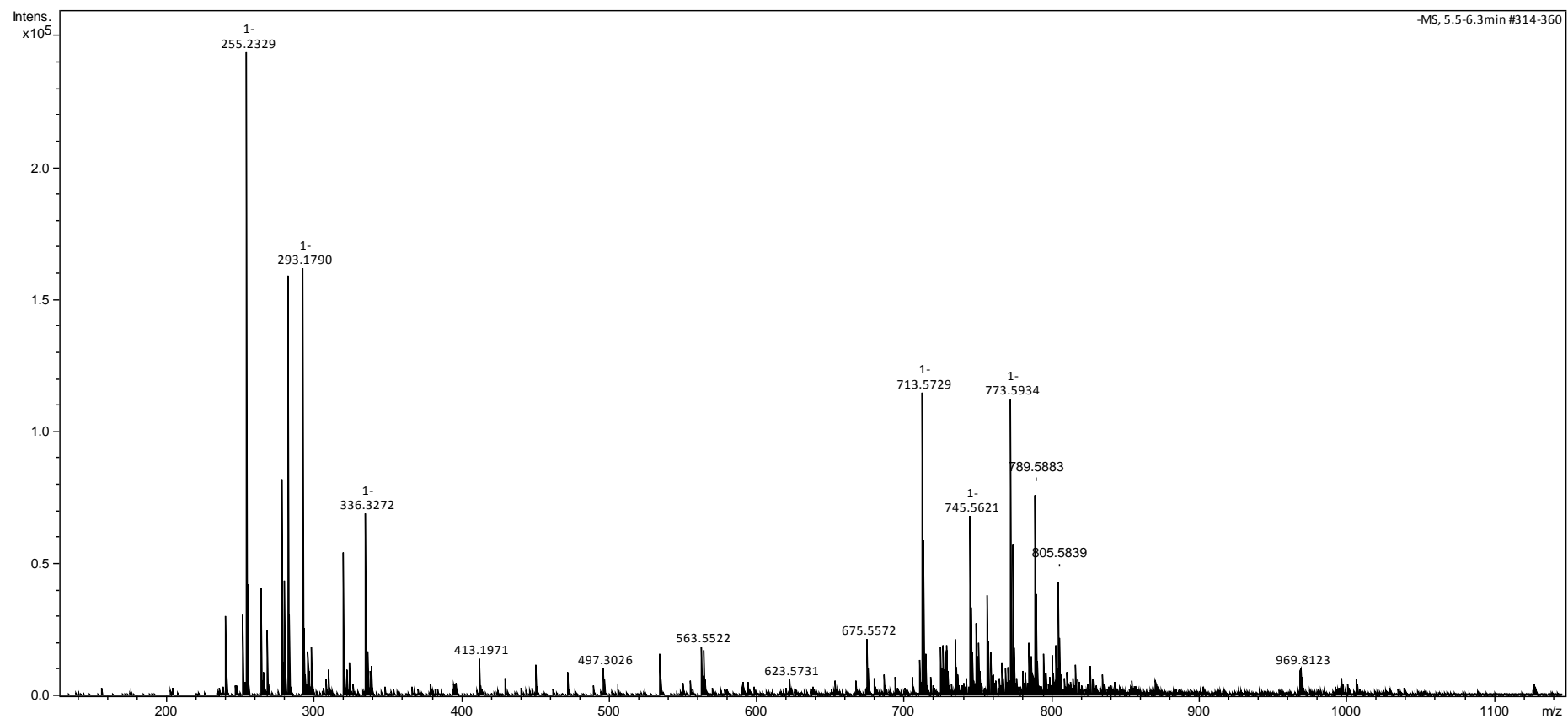
**Figure S23.** (-)-ESIMS/MS spectrum of compound **2**.

**Figure S24.**  $^1\text{H}$ -NMR spectrum of compound **2** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S25.**  $^{13}\text{C}$ -NMR spectrum of compound **2** in  $\text{C}_5\text{D}_5\text{N}$ .

- Figure S26.**  $^1\text{H}$ - $^1\text{H}$ -COSY spectrum of compound **2** in  $\text{C}_5\text{D}_5\text{N}$ .
- Figure S27.** HSQC spectrum of compound **2** in  $\text{C}_5\text{D}_5\text{N}$ .
- Figure S28.** HMBC spectrum of compound **2** in  $\text{C}_5\text{D}_5\text{N}$ .
- Figure S29.** ROESY spectrum of compound **2** in  $\text{C}_5\text{D}_5\text{N}$ .
- Figure S30.** (+)-HRESIMS spectrum of compound **3**.
- Figure S31.** (-)-HRESIMS spectrum of compound **3**.
- Figure S32.** (-)-ESIMS/MS spectrum of compound **3**.
- Figure S33.**  $^1\text{H}$ -NMR spectrum of compound **3** in  $\text{C}_5\text{D}_5\text{N}$ .
- Figure S34.**  $^{13}\text{C}$ -NMR spectrum of compound **3** in  $\text{C}_5\text{D}_5\text{N}$ .
- Figure S35.**  $^1\text{H}$ - $^1\text{H}$ -COSY spectrum of compound **3** in  $\text{C}_5\text{D}_5\text{N}$ .
- Figure S36.** HSQC spectrum of compound **3** in  $\text{C}_5\text{D}_5\text{N}$ .
- Figure S37.** HMBC spectrum of compound **3** in  $\text{C}_5\text{D}_5\text{N}$ .
- Figure S38.** ROESY spectrum of compound **3** in  $\text{C}_5\text{D}_5\text{N}$ .
- Figure S39.** (+)-HRESIMS spectrum of compound **4**.
- Figure S40.** (-)-HRESIMS spectrum of compound **4**.
- Figure S41.** (-)-ESIMS/MS spectrum of compound **4**.
- Figure S42.**  $^1\text{H}$ -NMR spectrum of compound **4** in  $\text{C}_5\text{D}_5\text{N}$ .
- Figure S43.**  $^{13}\text{C}$ -NMR spectrum of compound **4** in  $\text{C}_5\text{D}_5\text{N}$ .
- Figure S44.**  $^1\text{H}$ - $^1\text{H}$ -COSY spectrum of compound **4** in  $\text{C}_5\text{D}_5\text{N}$ .
- Figure S45.** HSQC spectrum of compound **4** in  $\text{C}_5\text{D}_5\text{N}$ .
- Figure S46.** HMBC spectrum of compound **4** in  $\text{C}_5\text{D}_5\text{N}$ .
- Figure S47.** ROESY spectrum of compound **4** in  $\text{C}_5\text{D}_5\text{N}$ .
- Figure S48.** Cytotoxicity compounds **1–4** after 24 h.

**Figure S1.** (+)-HRESIMS spectrum of compound **1**.

**Figure S2.** (-)-HRESIMS spectrum of compound **1**.

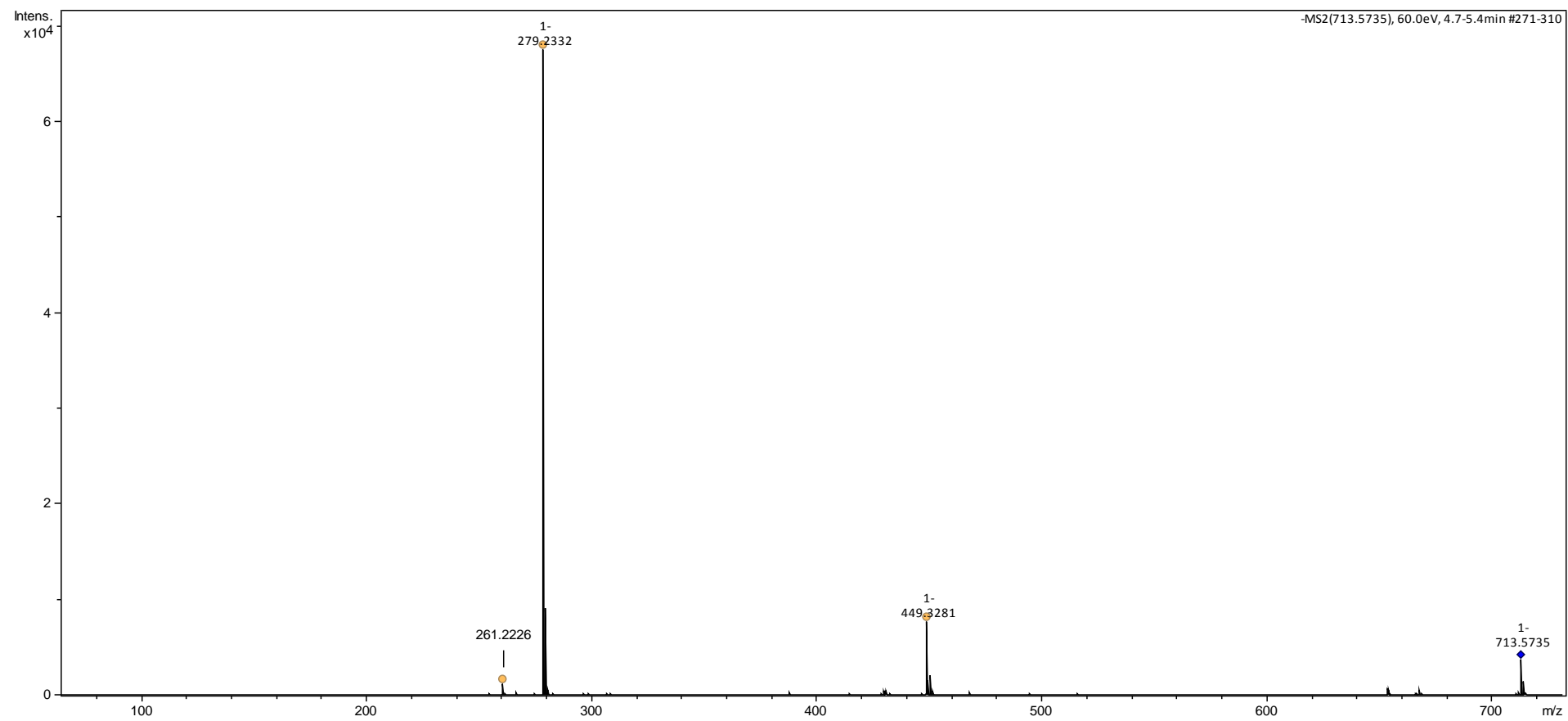
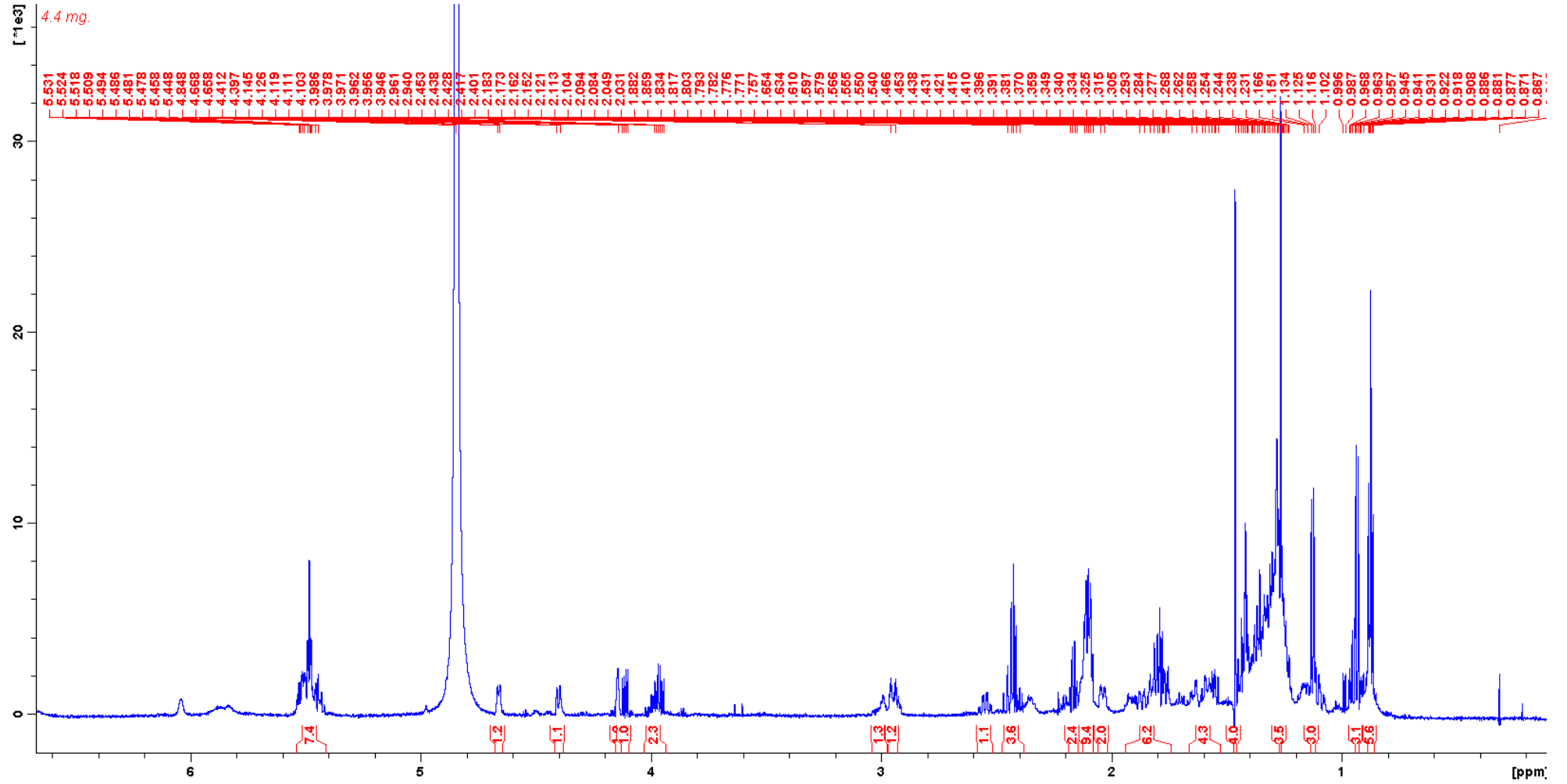
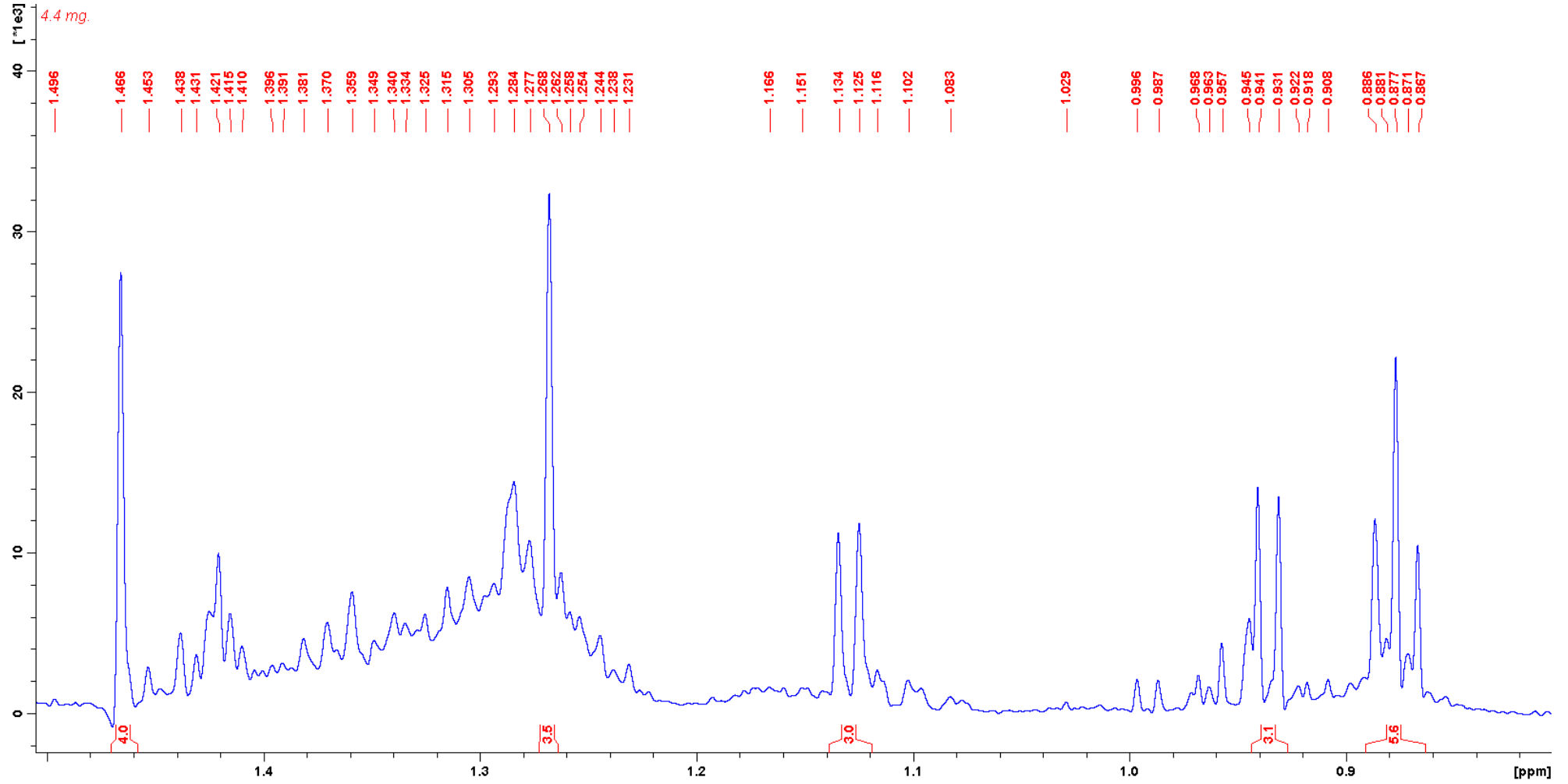
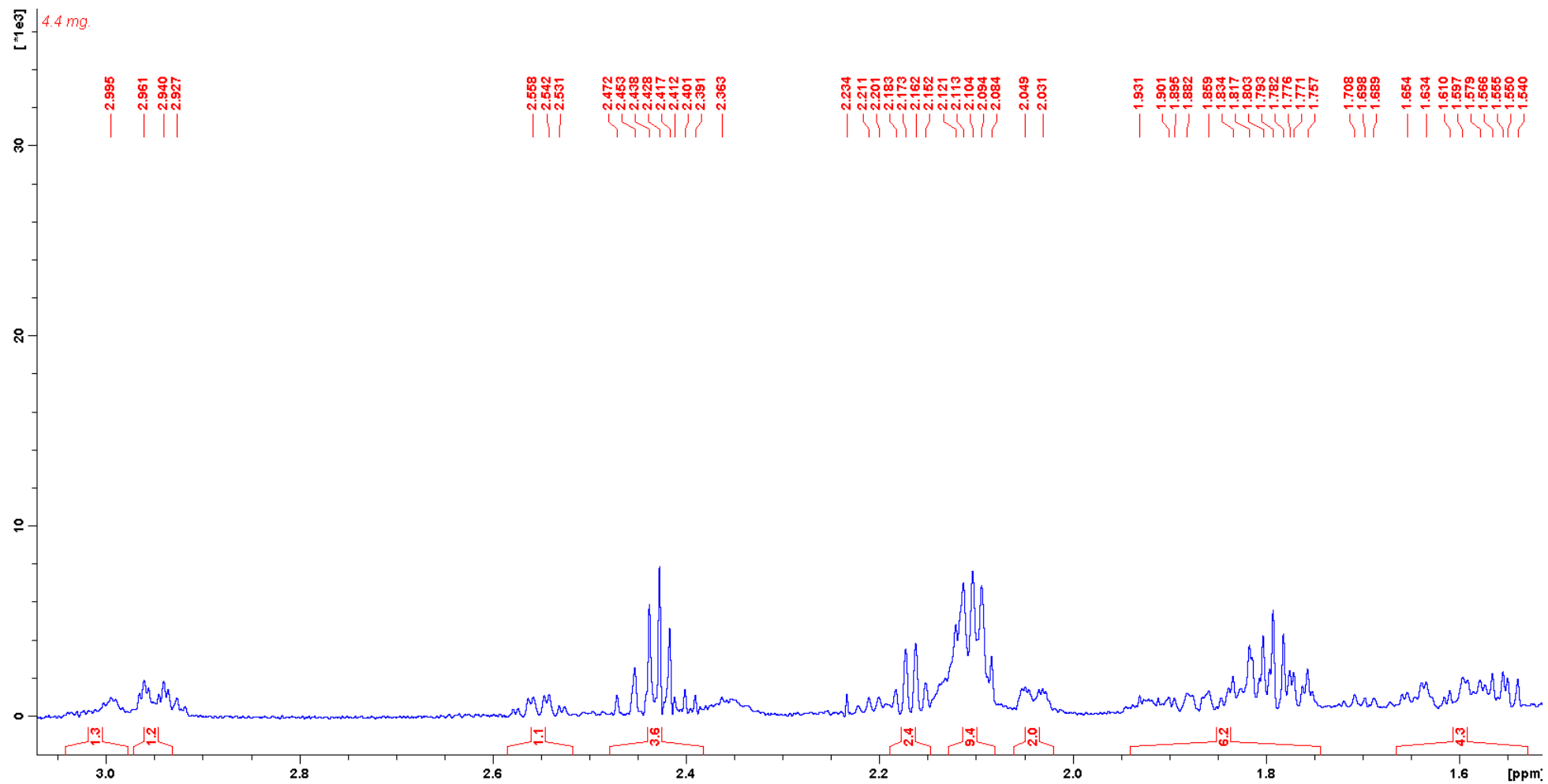
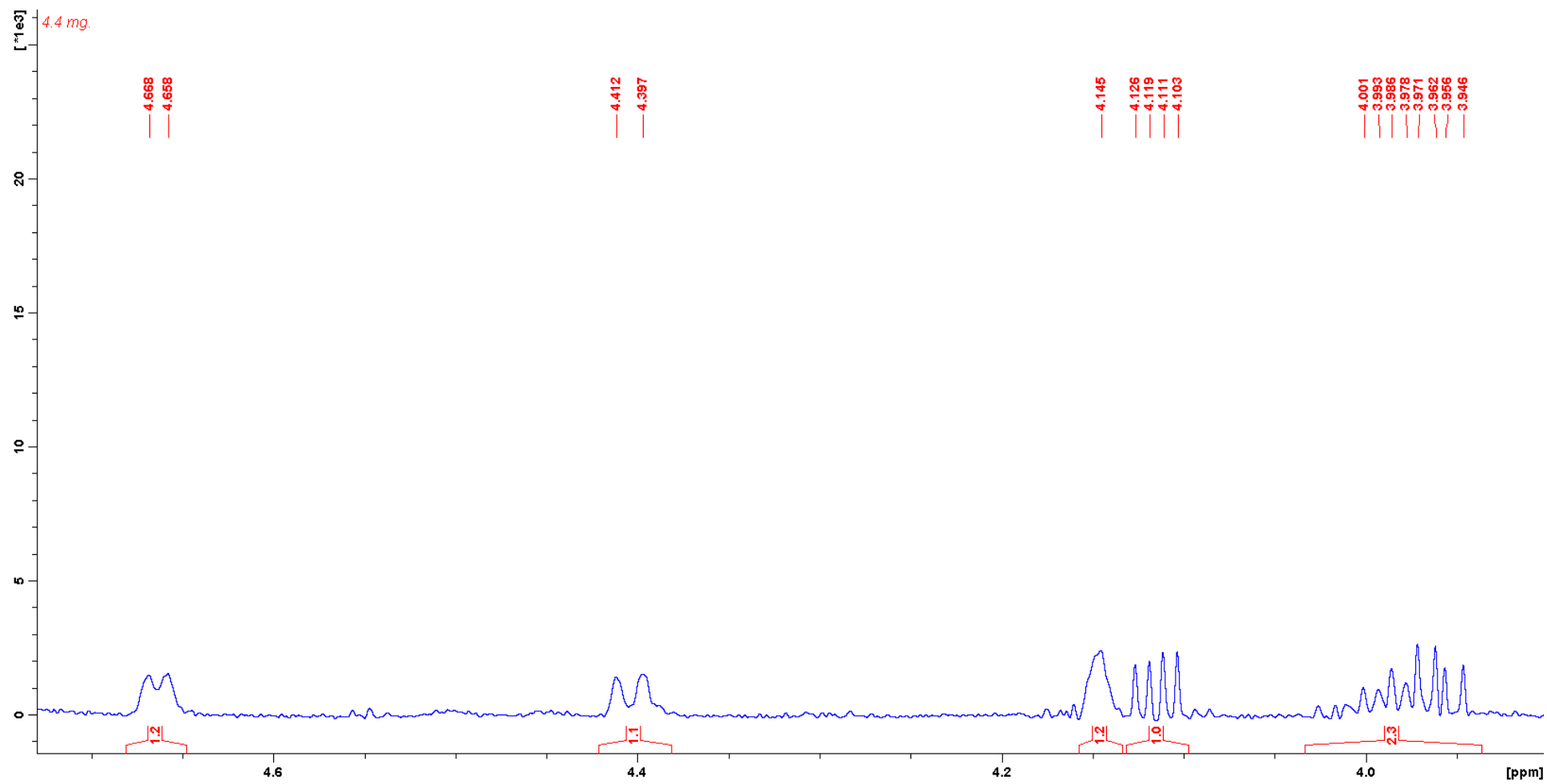
**Figure S3.** (-)-ESIMS/MS spectrum of compound **1**.

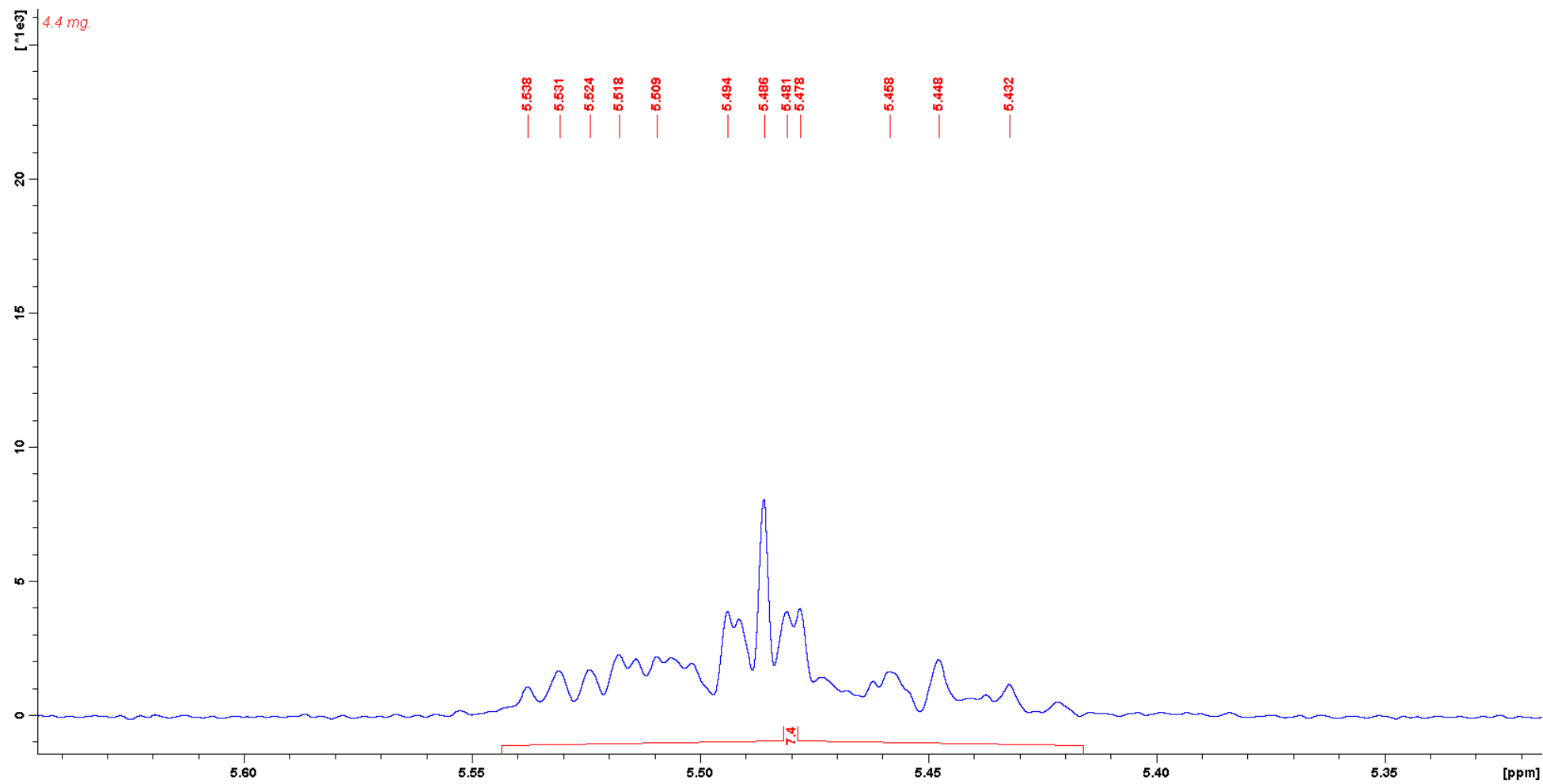
Figure S4.  $^1\text{H-NMR}$  spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

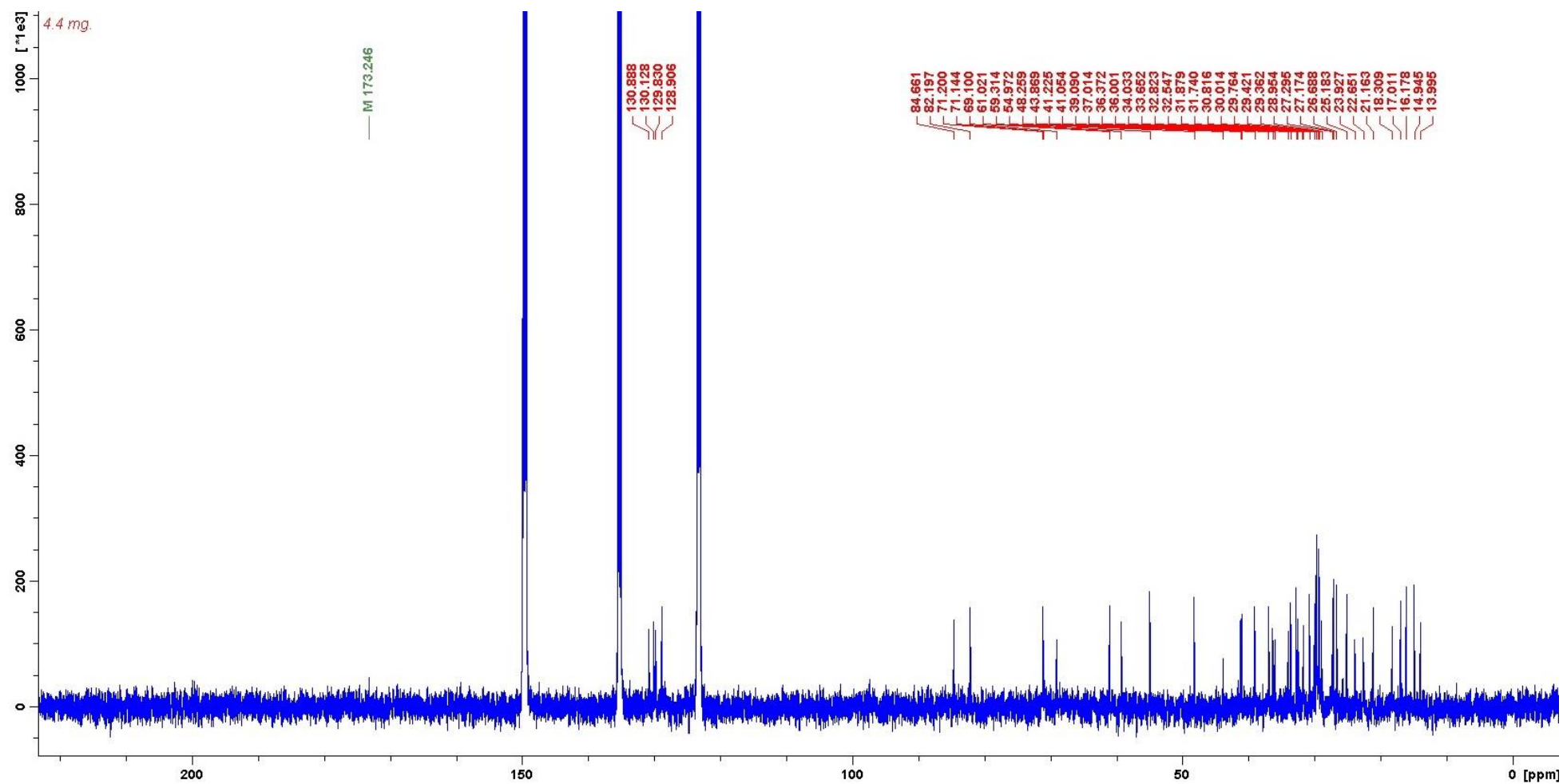
**Figure S5.** Enlarged-1  $^1\text{H-NMR}$  spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

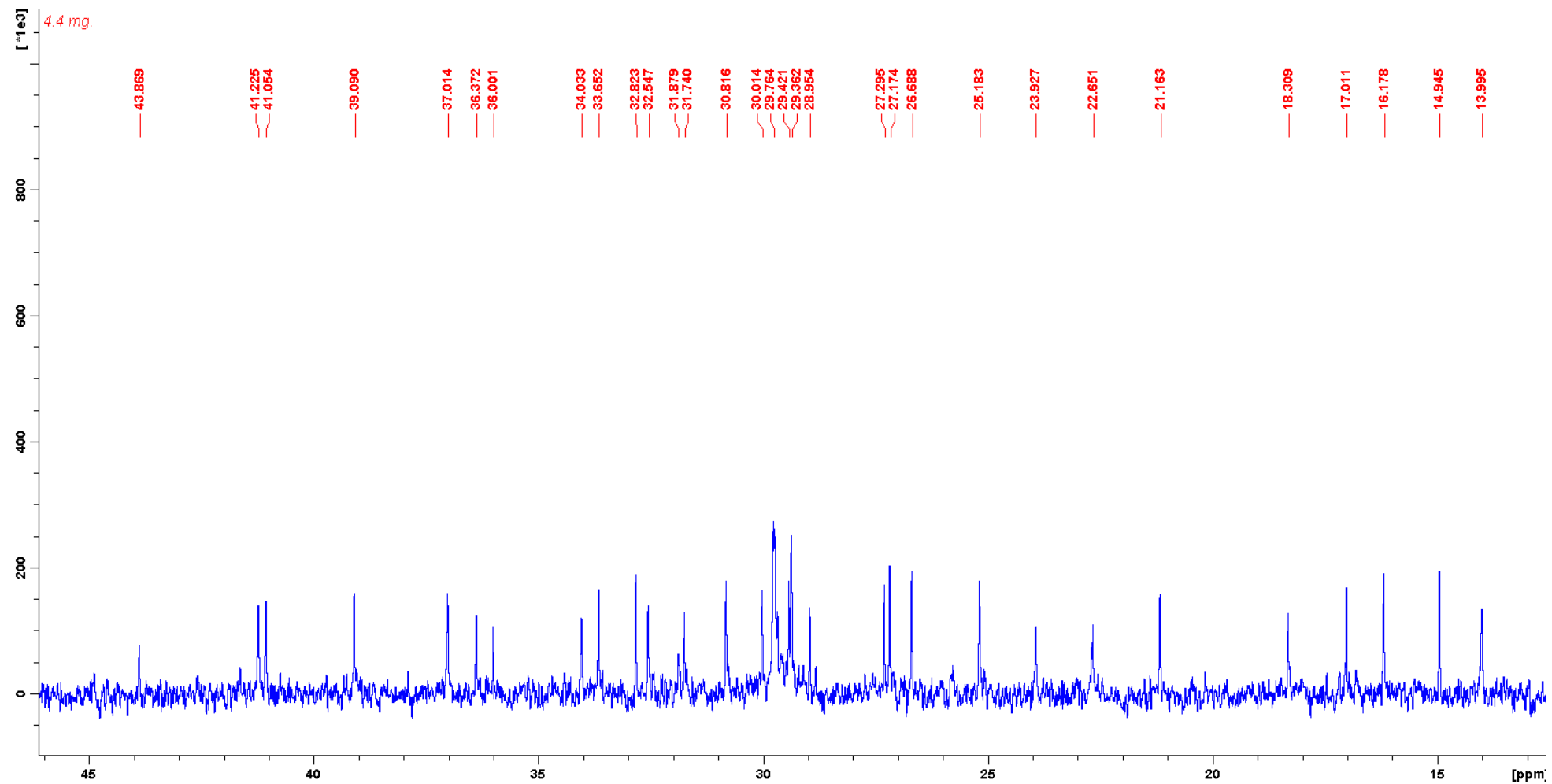


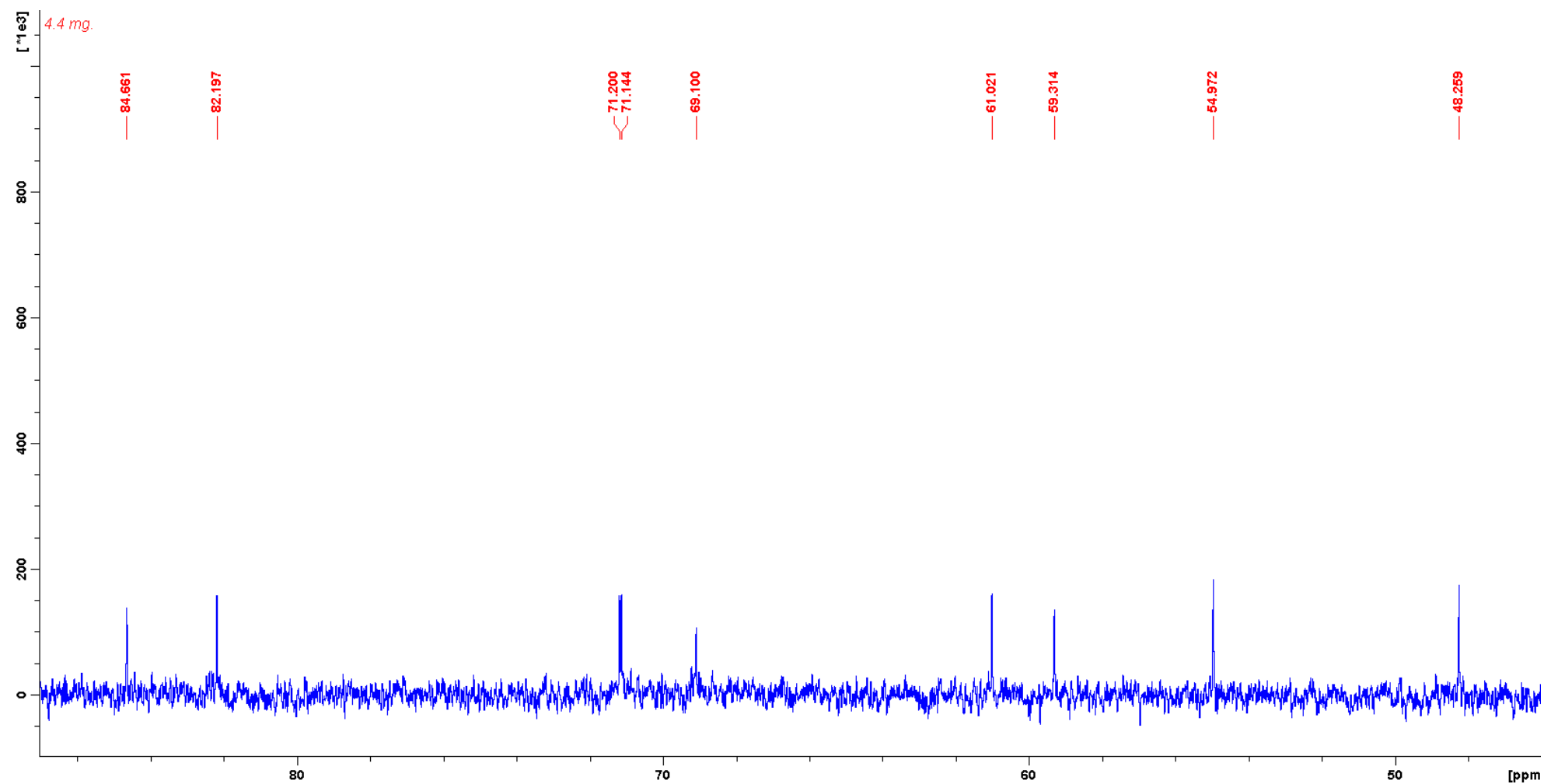
**Figure S6.** Enlarged-2  $^1\text{H}$ -NMR spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

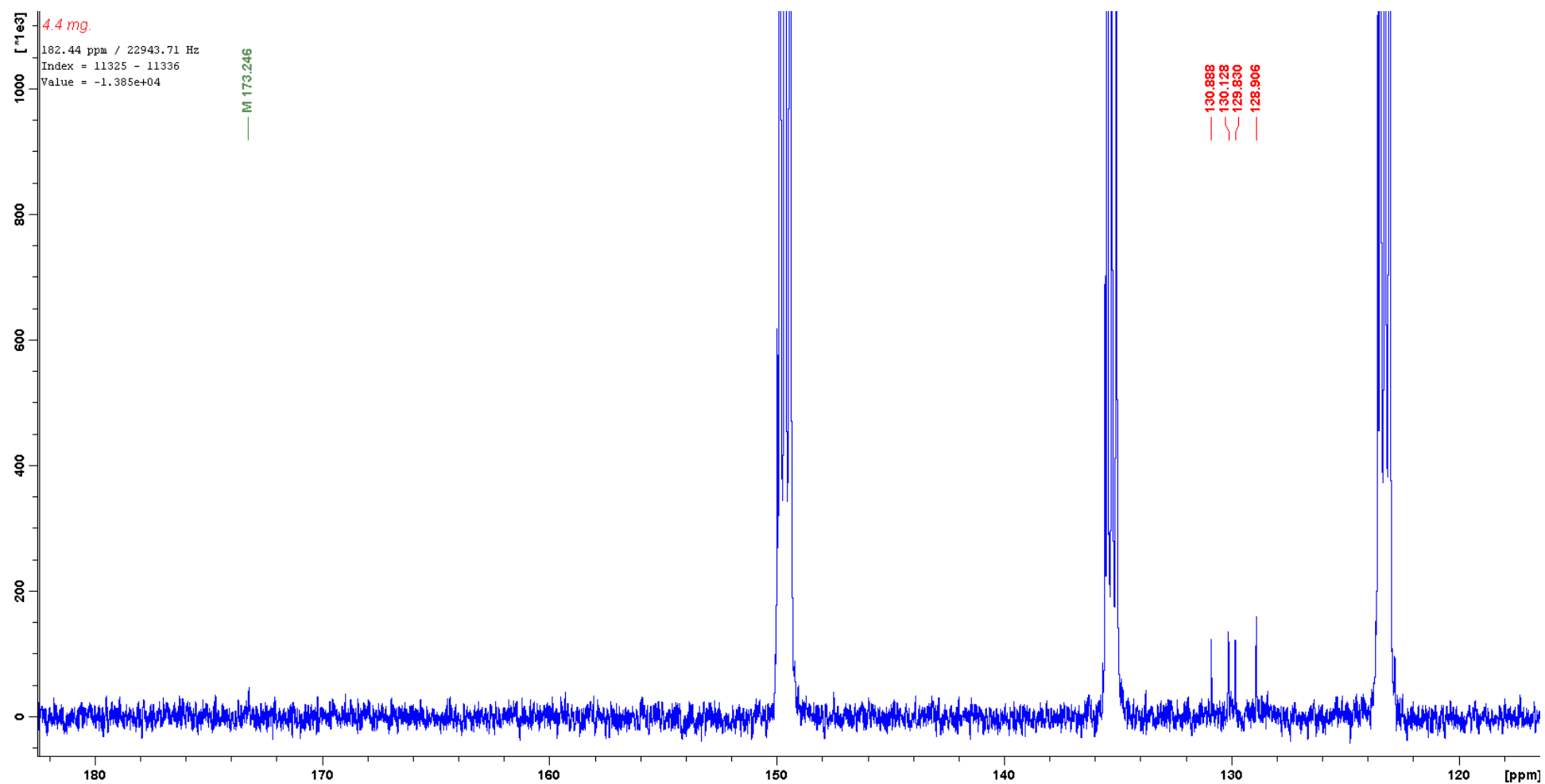
**Figure S7.** Enlarged-3  $^1\text{H}$ -NMR spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

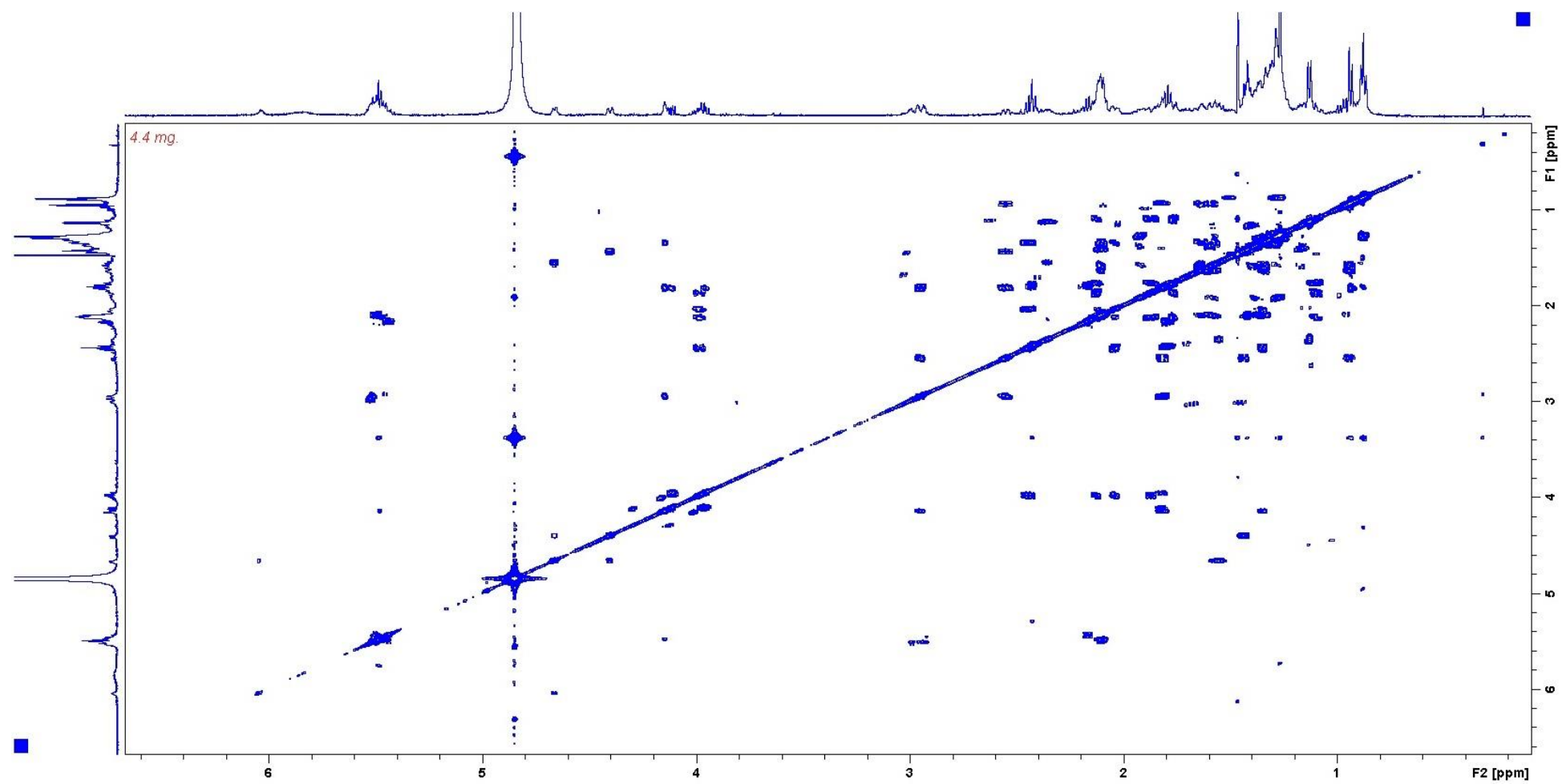
**Figure S8.** Enlarged-4  $^1\text{H}$ -NMR spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S9.**  $^{13}\text{C}$ -NMR spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S10.** Enlarged-1  $^{13}\text{C}$ -NMR spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S11.** Enlarged-2  $^{13}\text{C}$ -NMR spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S12.** Enlarged-3  $^{13}\text{C}$ -NMR spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S13.**  $^1\text{H}$ - $^1\text{H}$ -COSY spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .



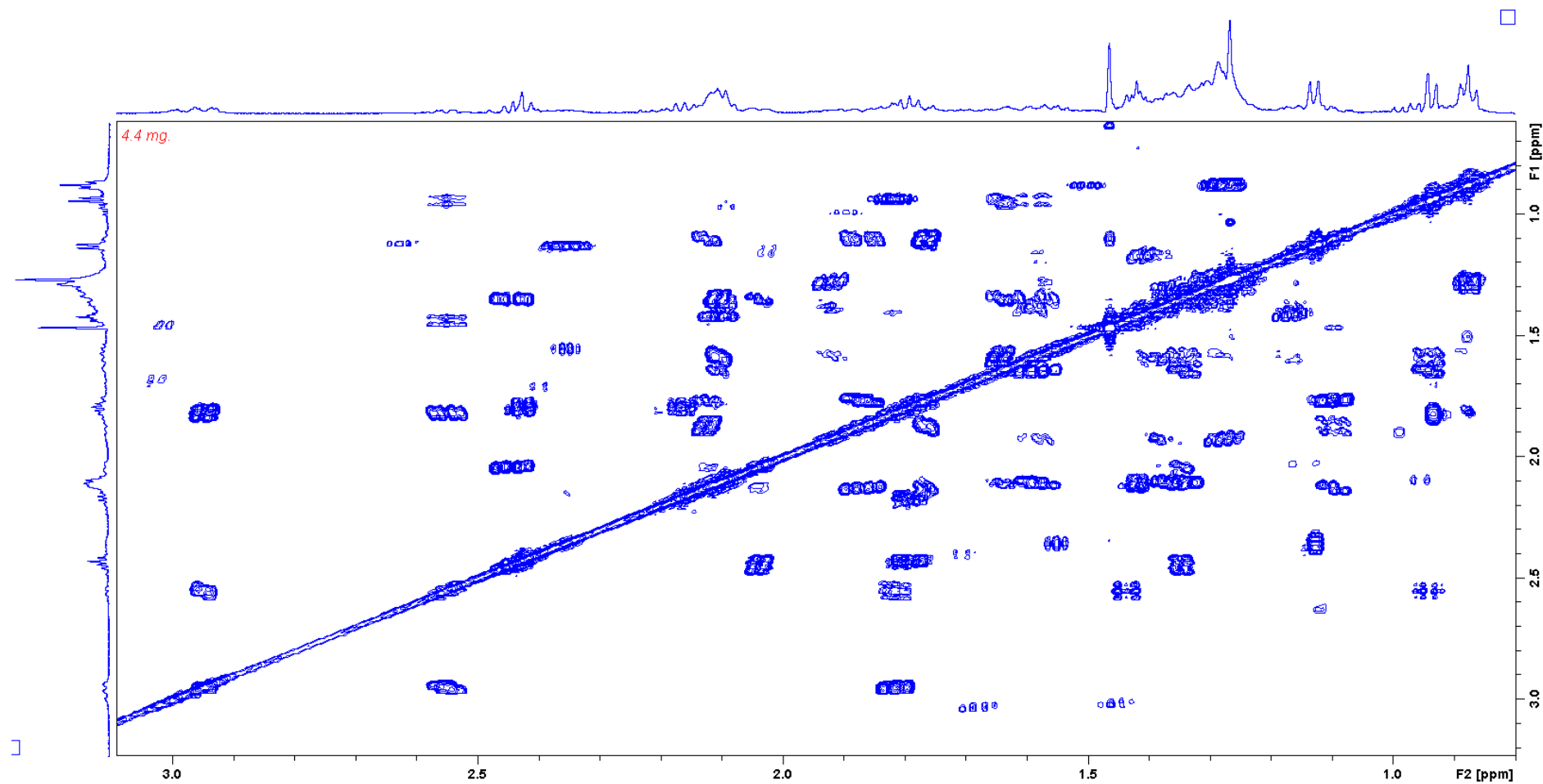
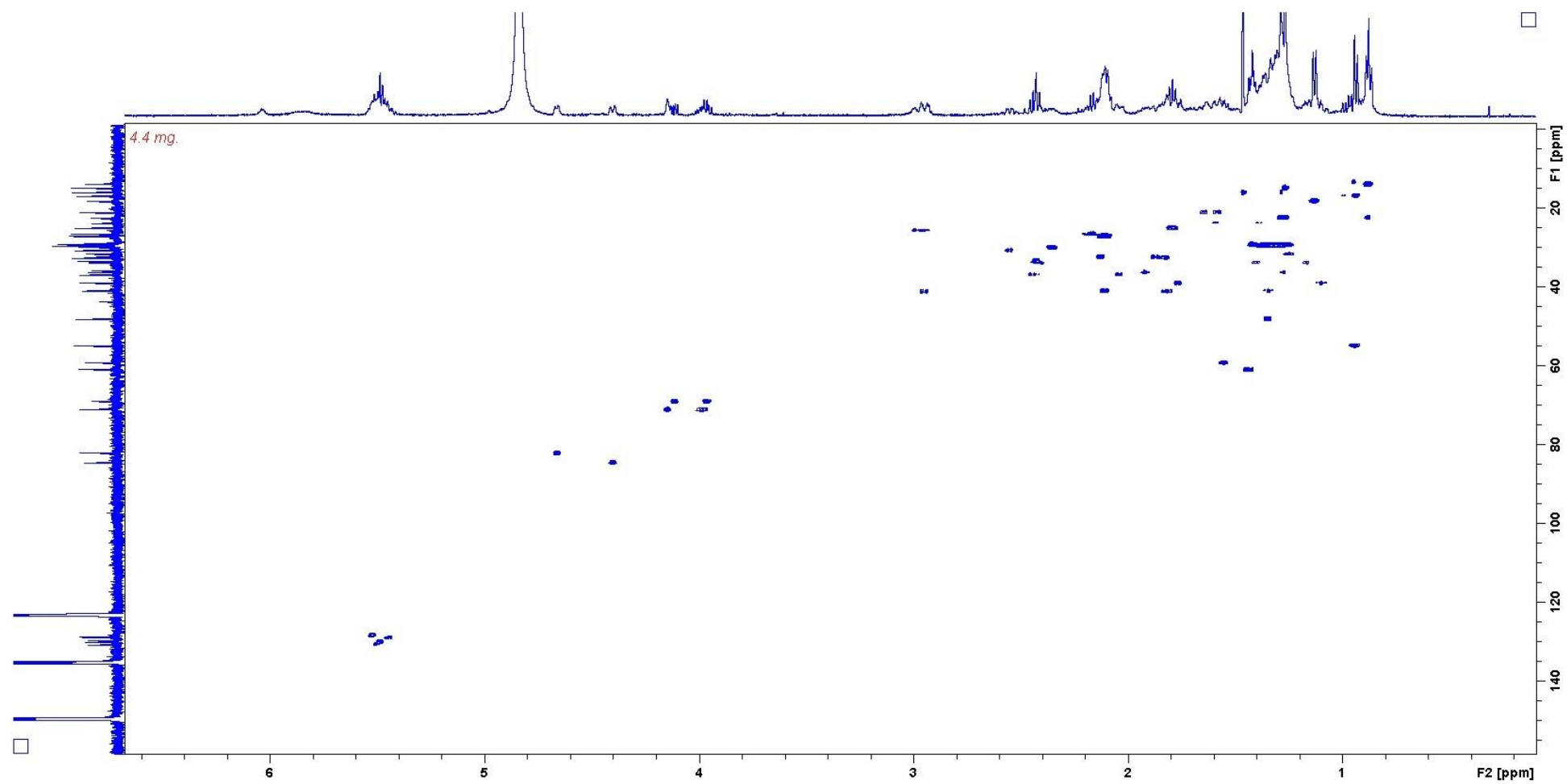
**Figure S14.** Enlarged  $^1\text{H}$ - $^1\text{H}$ -COSY spectrum of compound **1** in  $\text{C}_5\text{D}_5\text{N}$ .

Figure S15. HSQC spectrum of compound **1** in  $C_5D_5N$ .

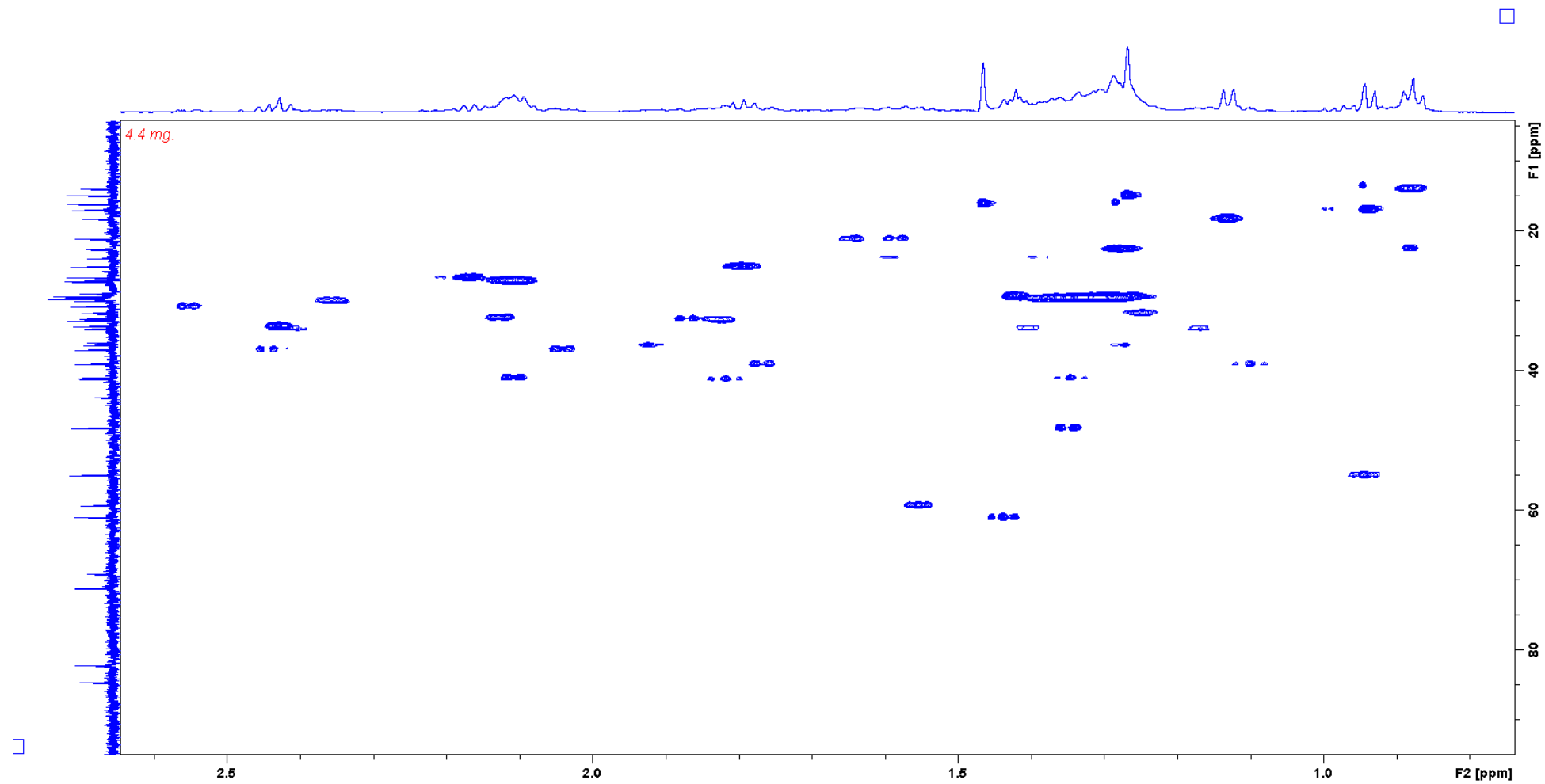
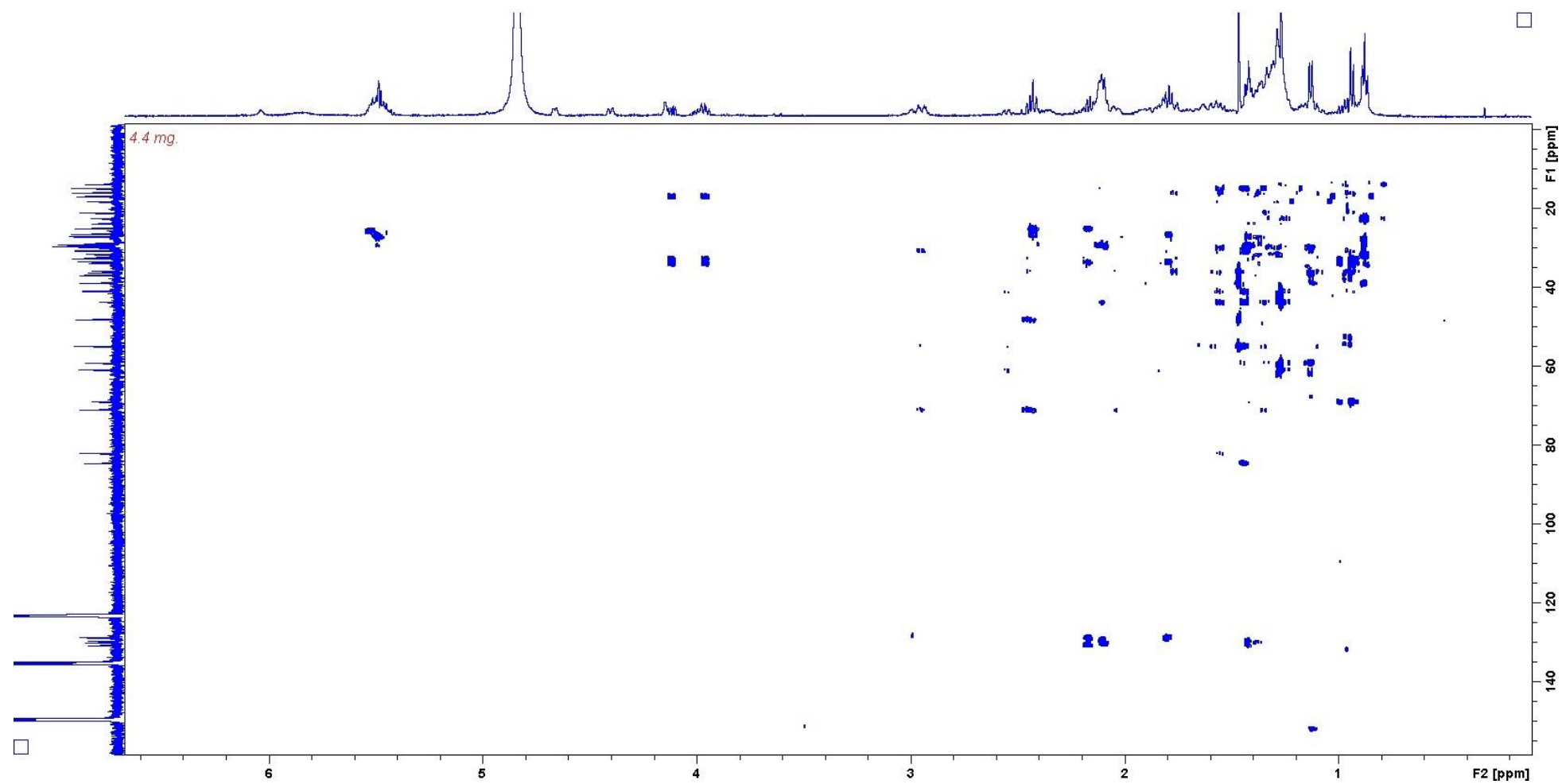
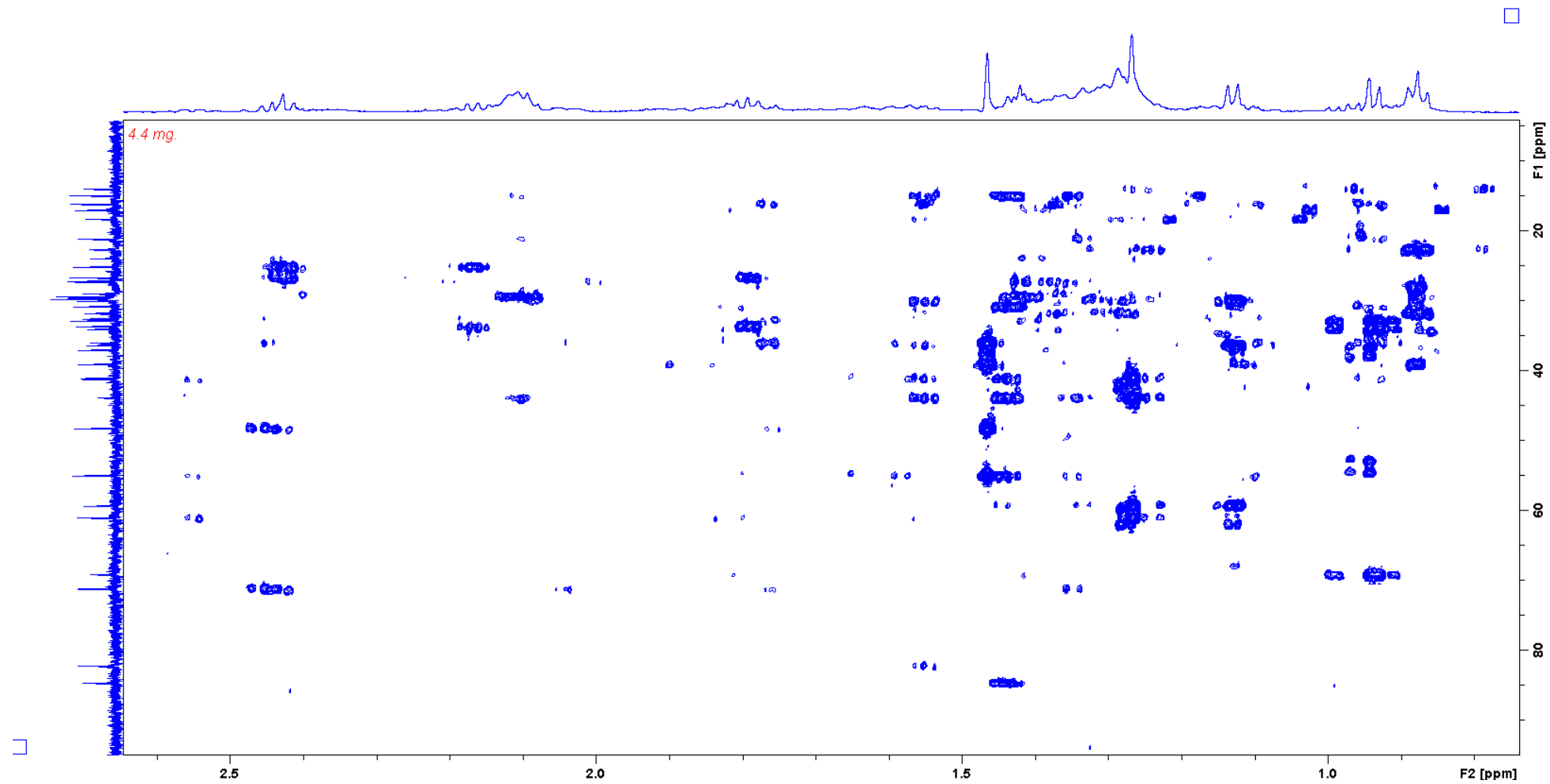
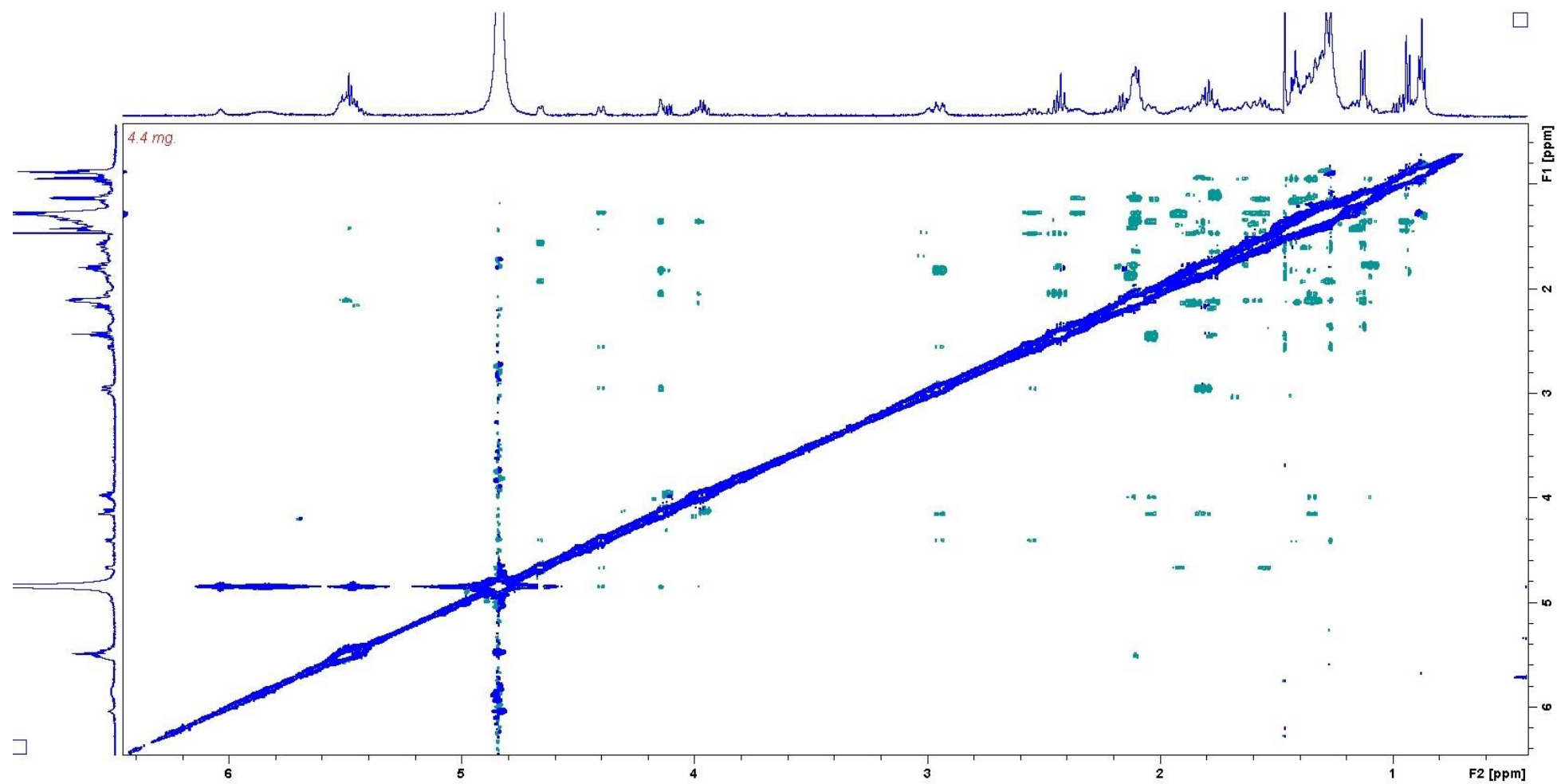
**Figure S16.** Enlarged HSQC spectrum of compound **1** in  $C_5D_5N$ .

Figure S17. HMBC spectrum of compound **1** in C<sub>5</sub>D<sub>5</sub>N.

**Figure S18.** Enlarged HMBC spectrum of compound **1** in  $C_5D_5N$ .

**Figure S19.** ROESY spectrum of compound **1** in  $C_5D_5N$ .

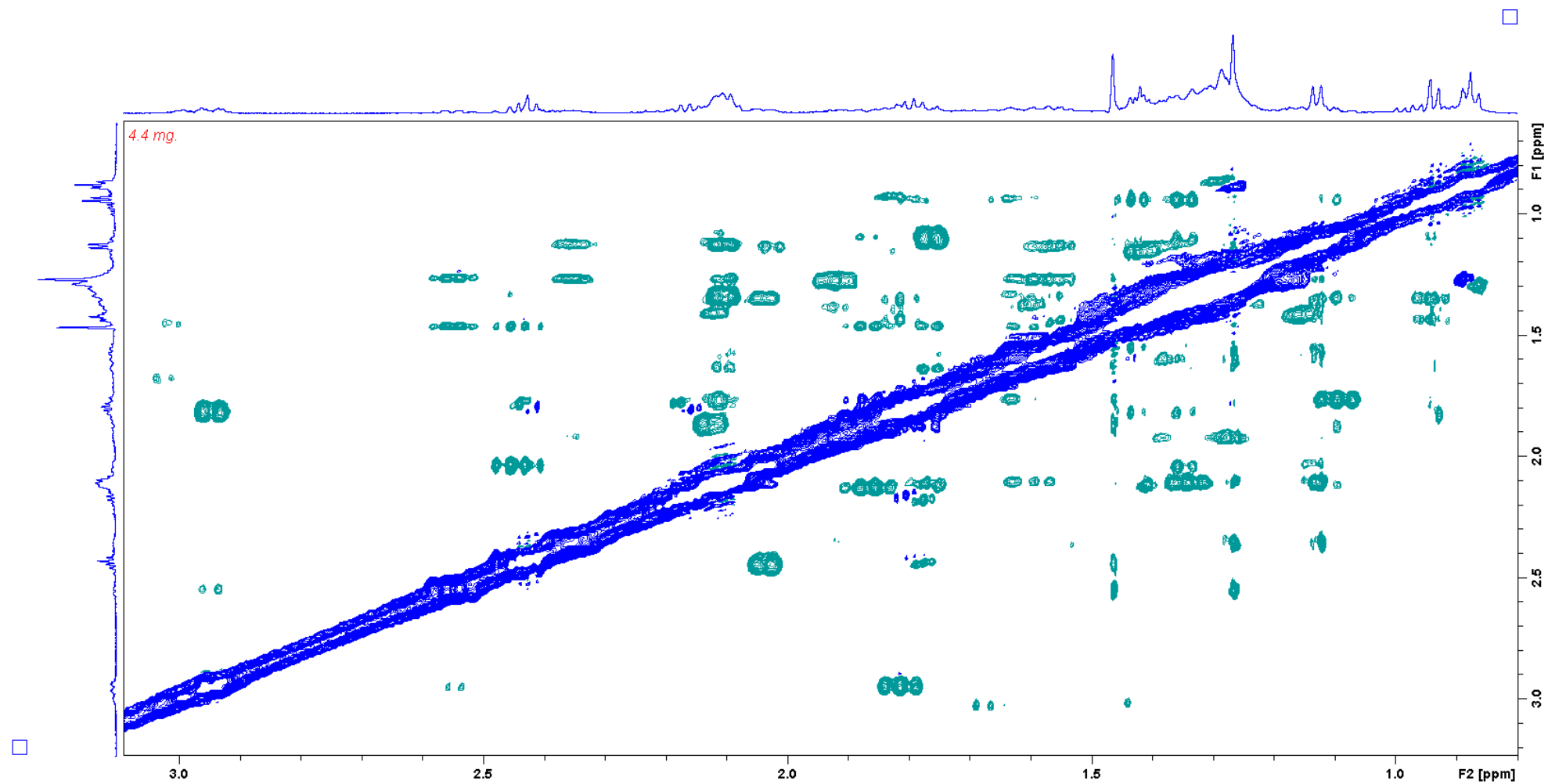
**Figure S20.** Enlarged ROESY spectrum of compound **1** in  $C_5D_5N$ .

Figure S21. (+)-HRESIMS spectrum of compound 2.

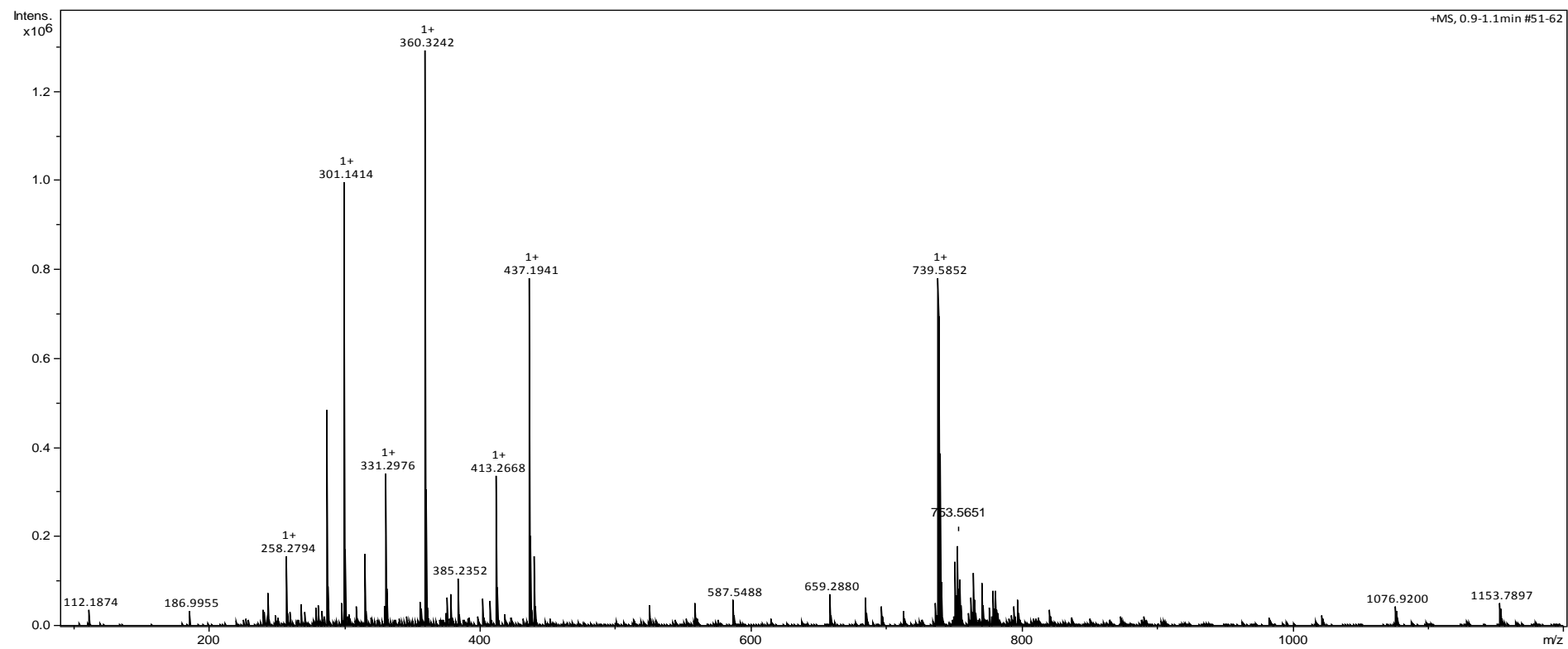




Figure S22. (-)-HRESIMS spectrum of compound 2.

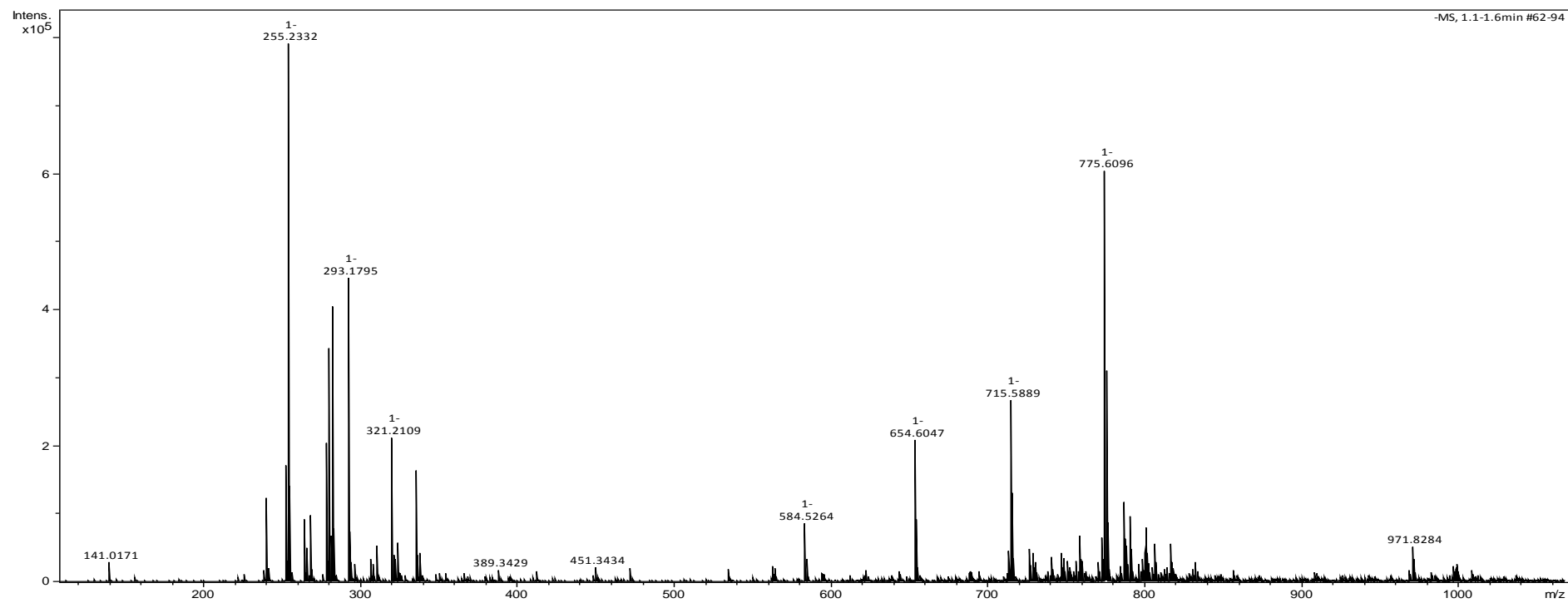


Figure S23. (-)-ESIMS/MS spectrum of compound 2.

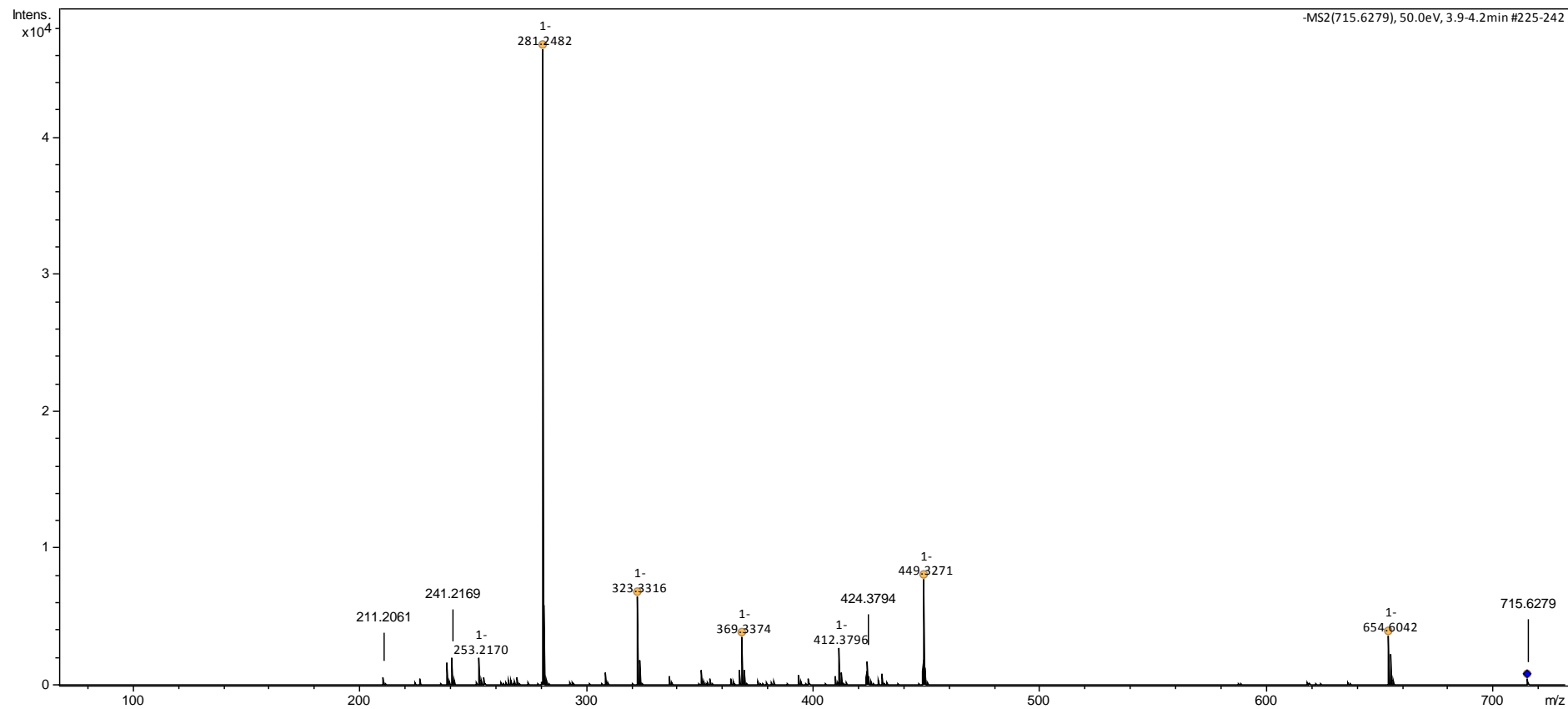


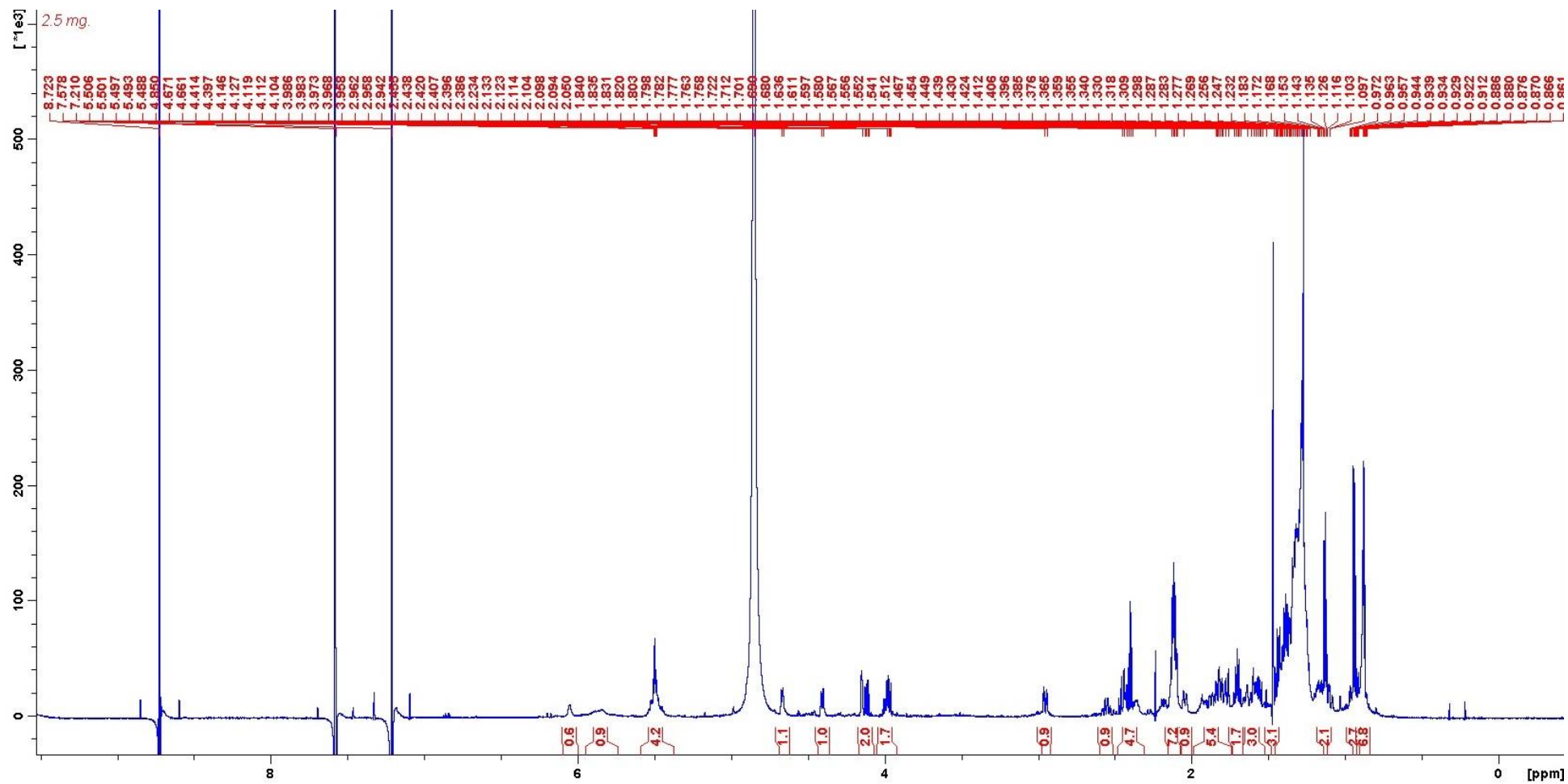
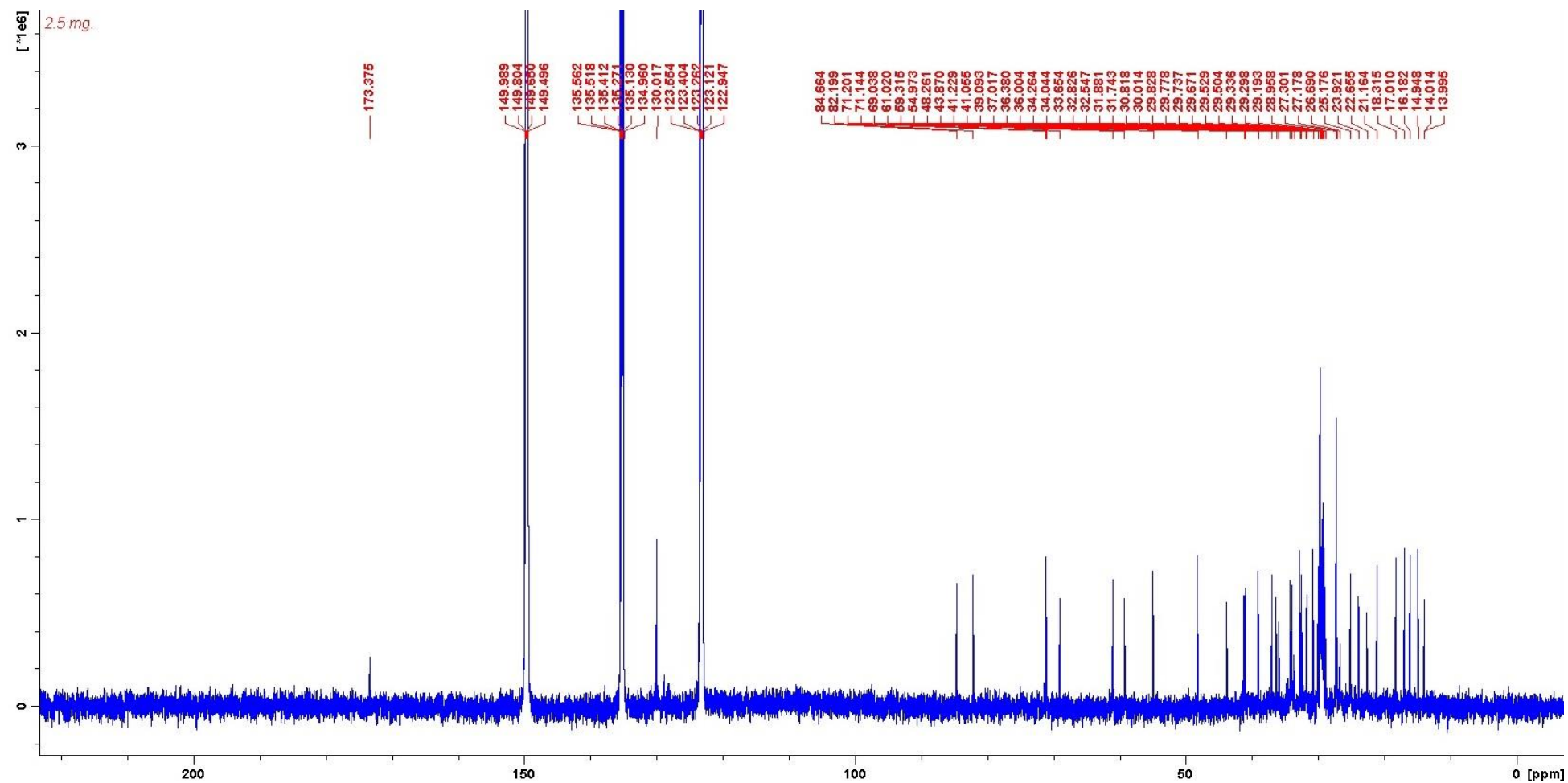
Figure S24.  $^1\text{H-NMR}$  spectrum of compound **2** in  $\text{C}_5\text{D}_5\text{N}$ .

Figure S25.  $^{13}\text{C}$ -NMR spectrum of compound **2** in  $\text{C}_5\text{D}_5\text{N}$ .

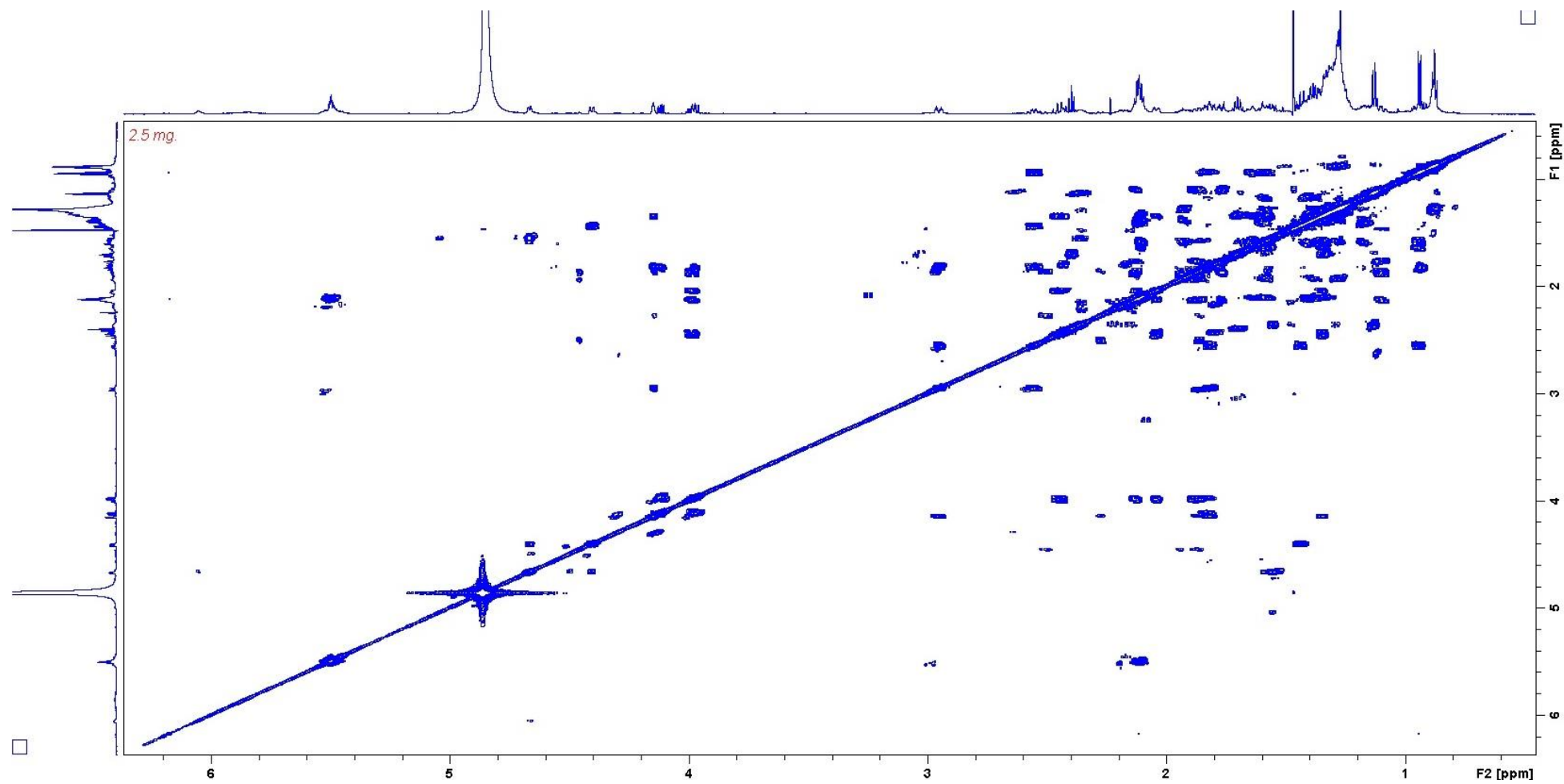
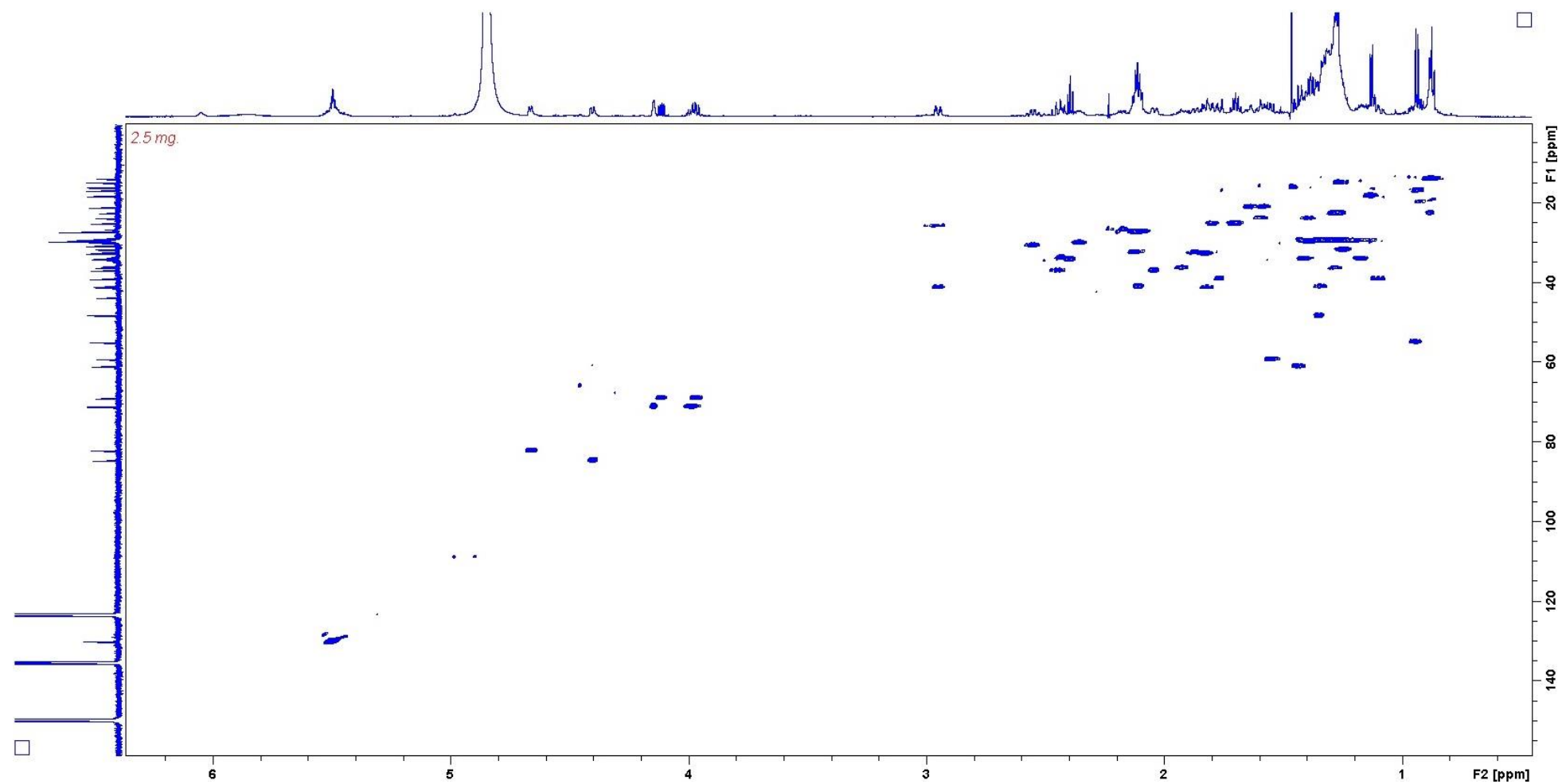
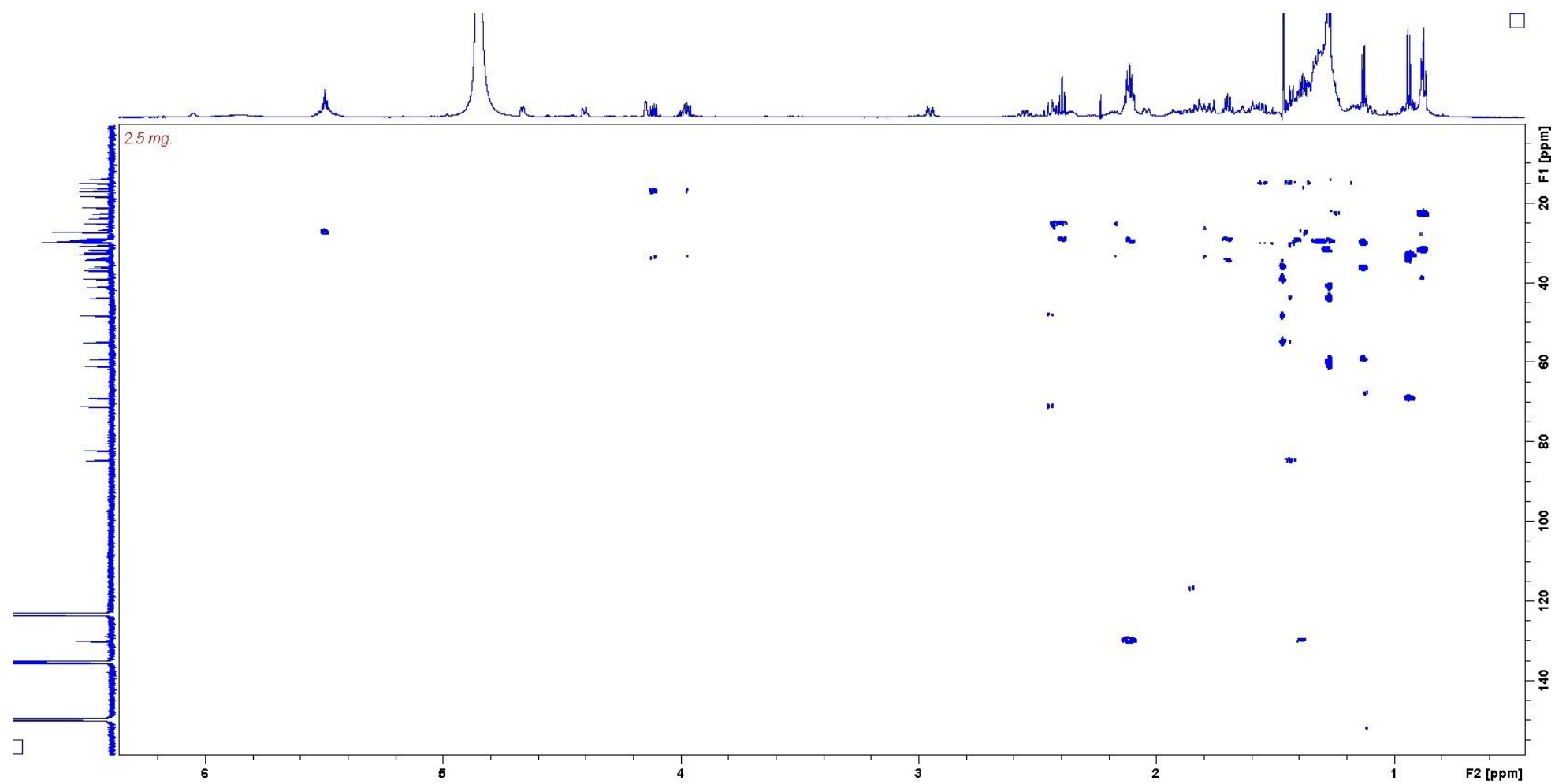
**Figure S26.**  $^1\text{H}$ - $^1\text{H}$ -COSY spectrum of compound **2** in  $\text{C}_5\text{D}_5\text{N}$ .

Figure S27. HSQC spectrum of compound 2 in  $C_5D_5N$ .

**Figure S28.** HMBC spectrum of compound **2** in  $C_5D_5N$ .

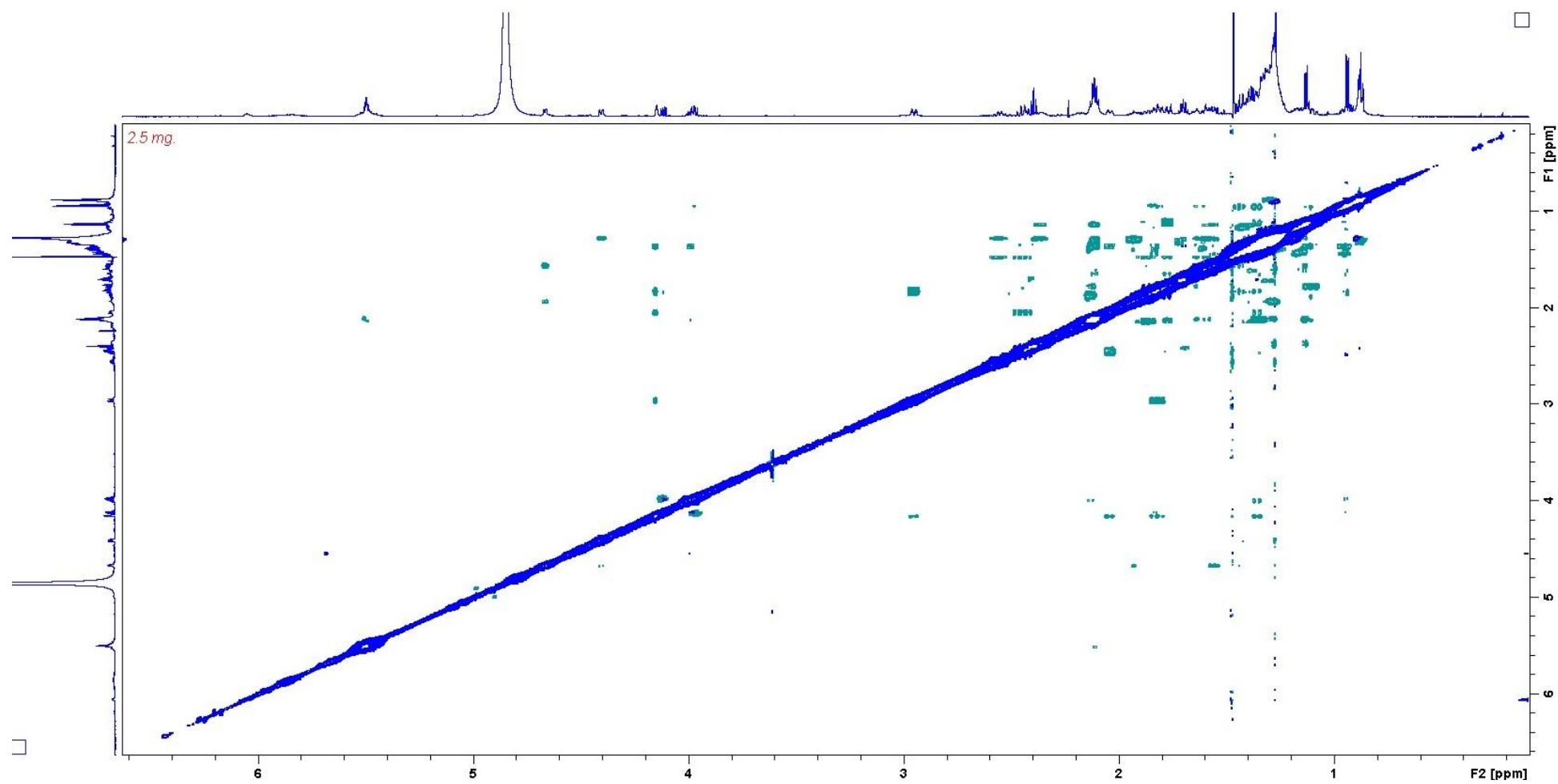
**Figure S29.** ROESY spectrum of compound **2** in  $C_5D_5N$ .



Figure S30. (+)-HRESIMS spectrum of compound 3.

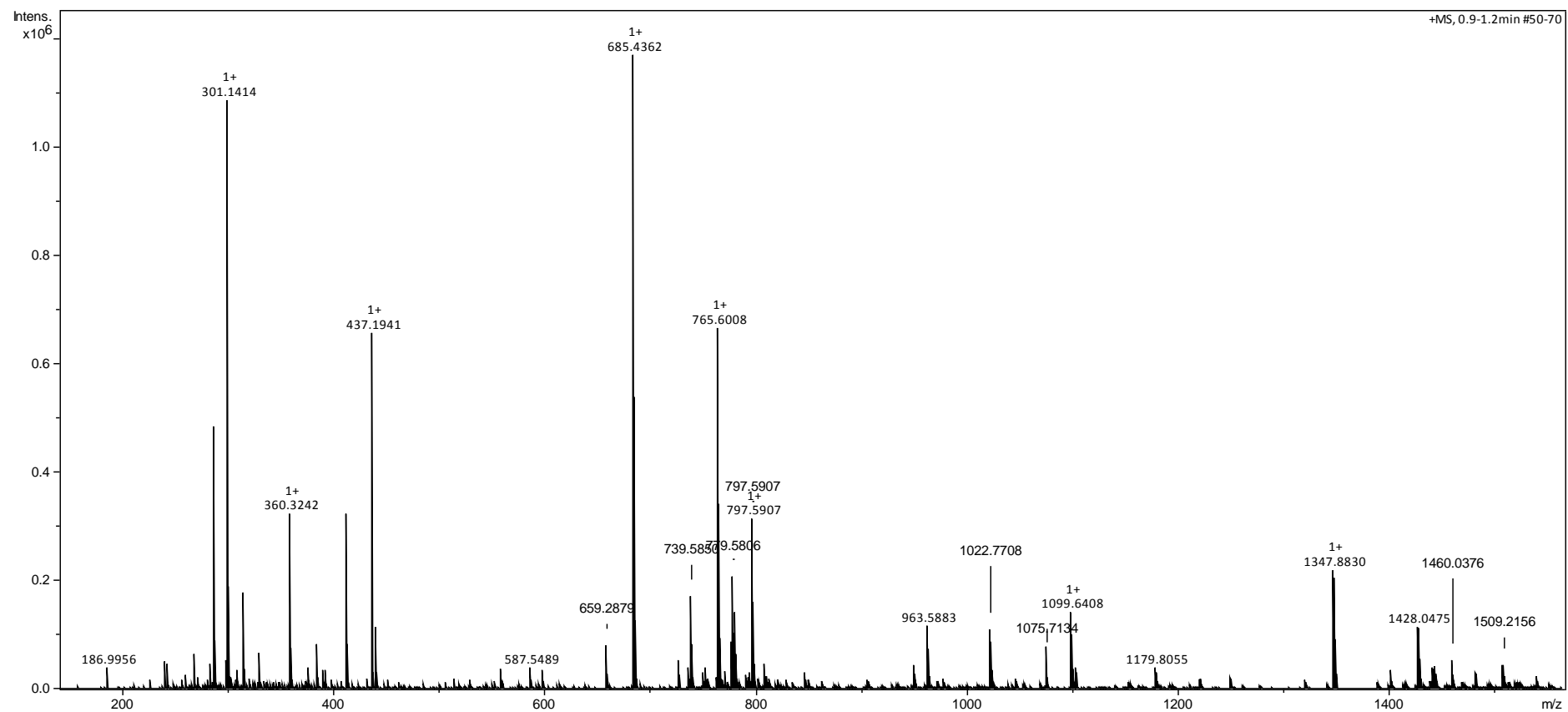
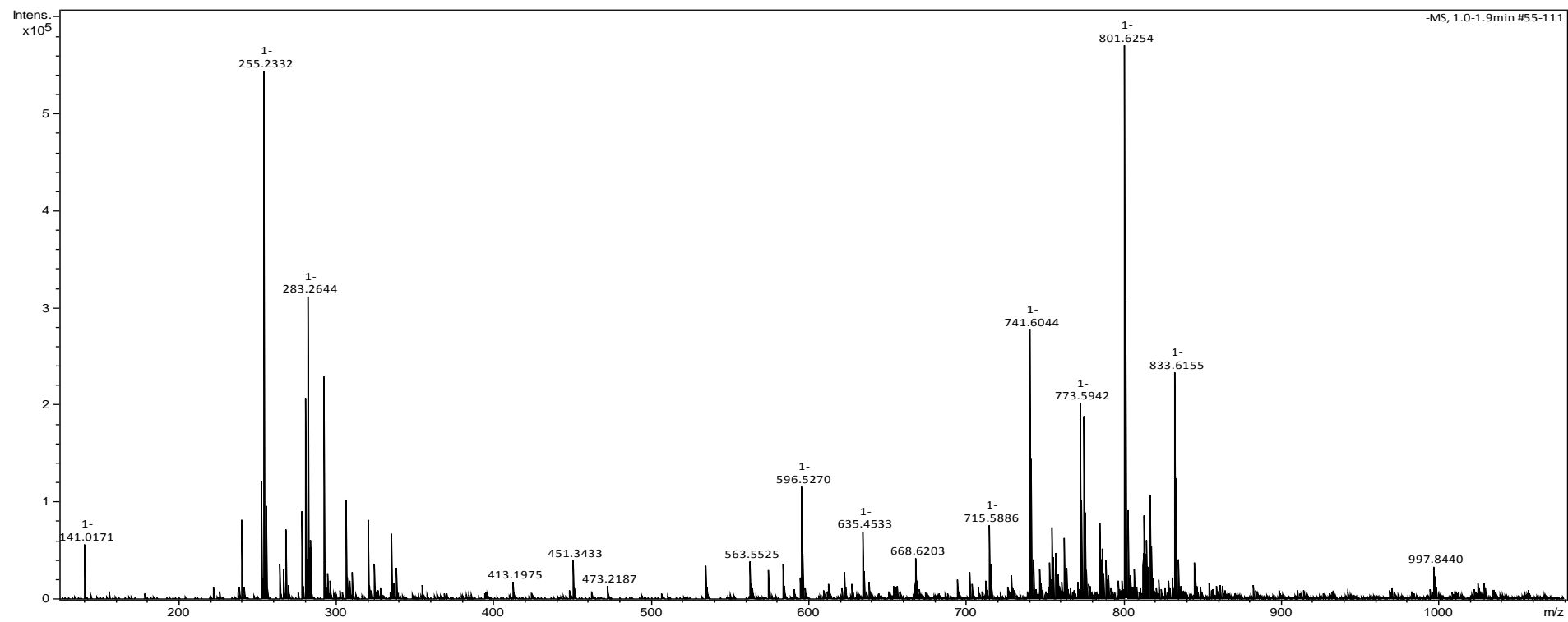


Figure S31. (-)-HRESIMS spectrum of compound 3.



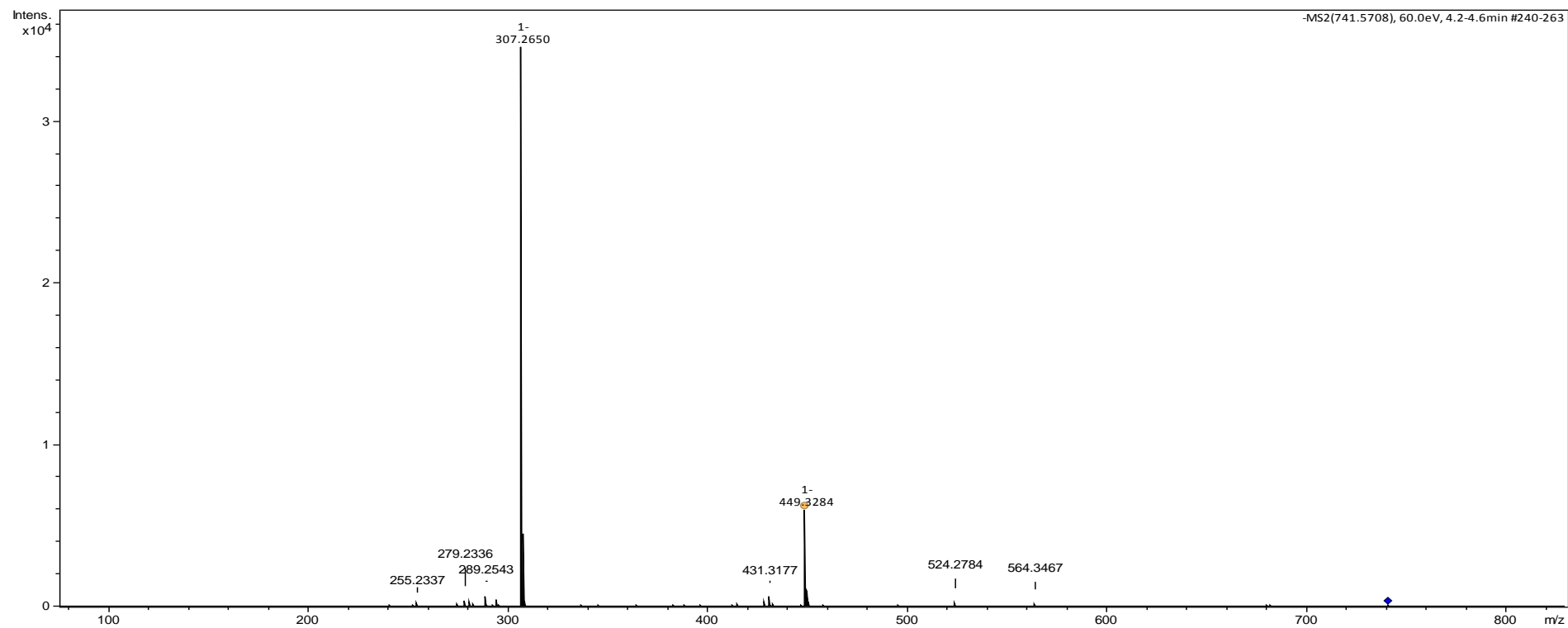
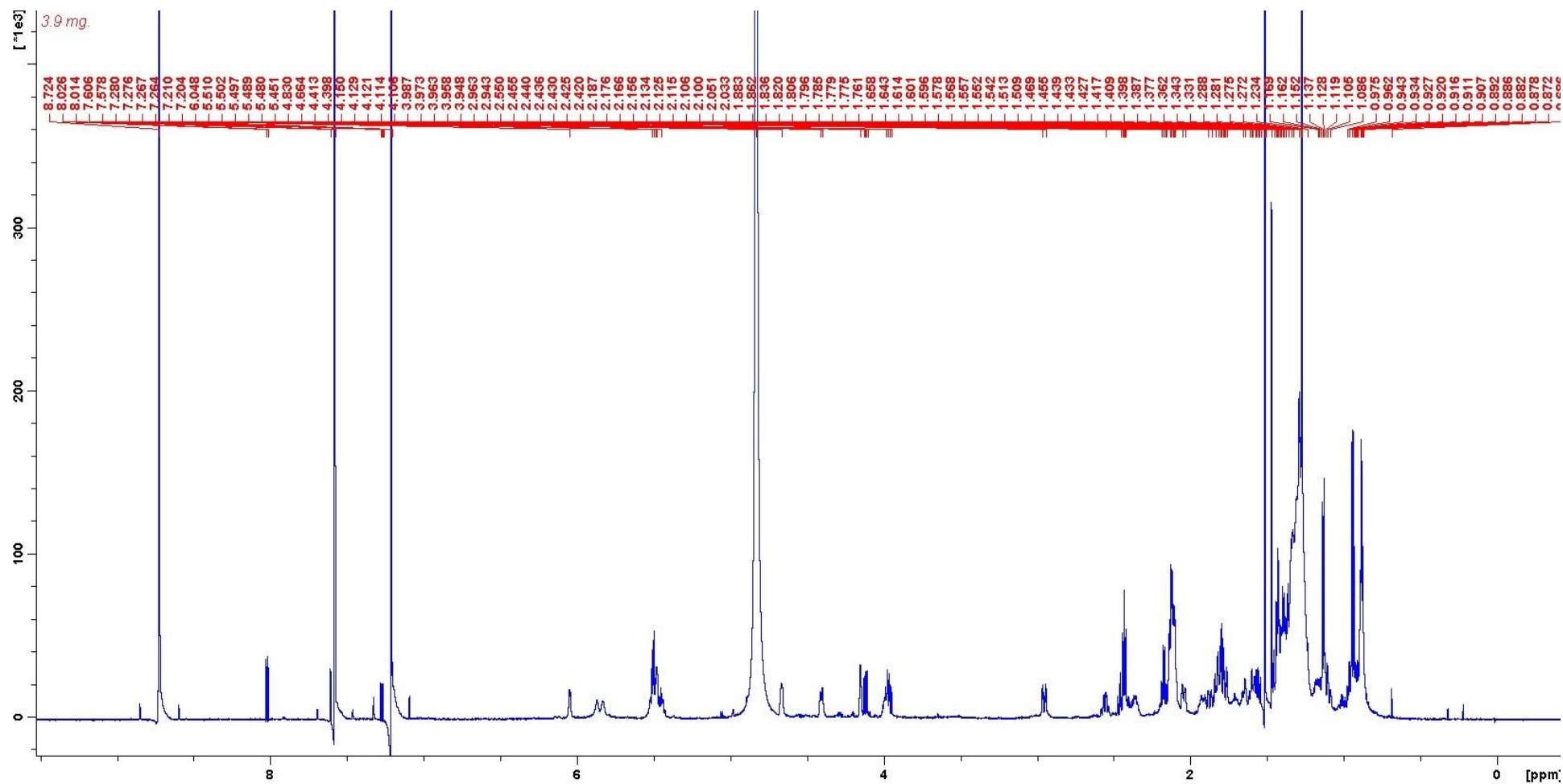
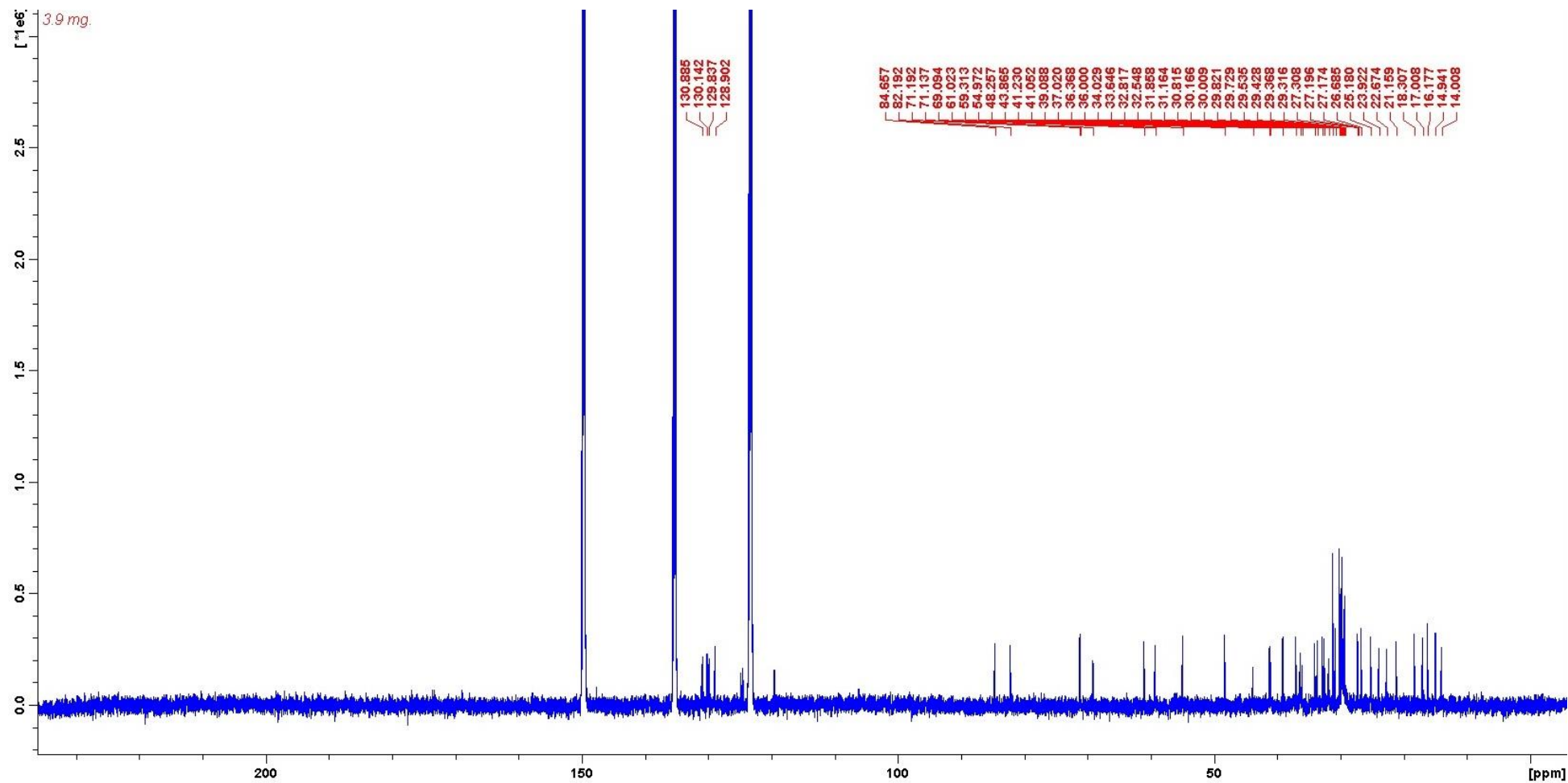
**Figure S32.** (-)-ESIMS/MS spectrum of compound **3**.

Figure S33.  $^1\text{H-NMR}$  spectrum of compound **3** in  $\text{C}_5\text{D}_5\text{N}$ .

**Figure S34.**  $^{13}\text{C}$ -NMR spectrum of compound **3** in  $\text{C}_5\text{D}_5\text{N}$ .

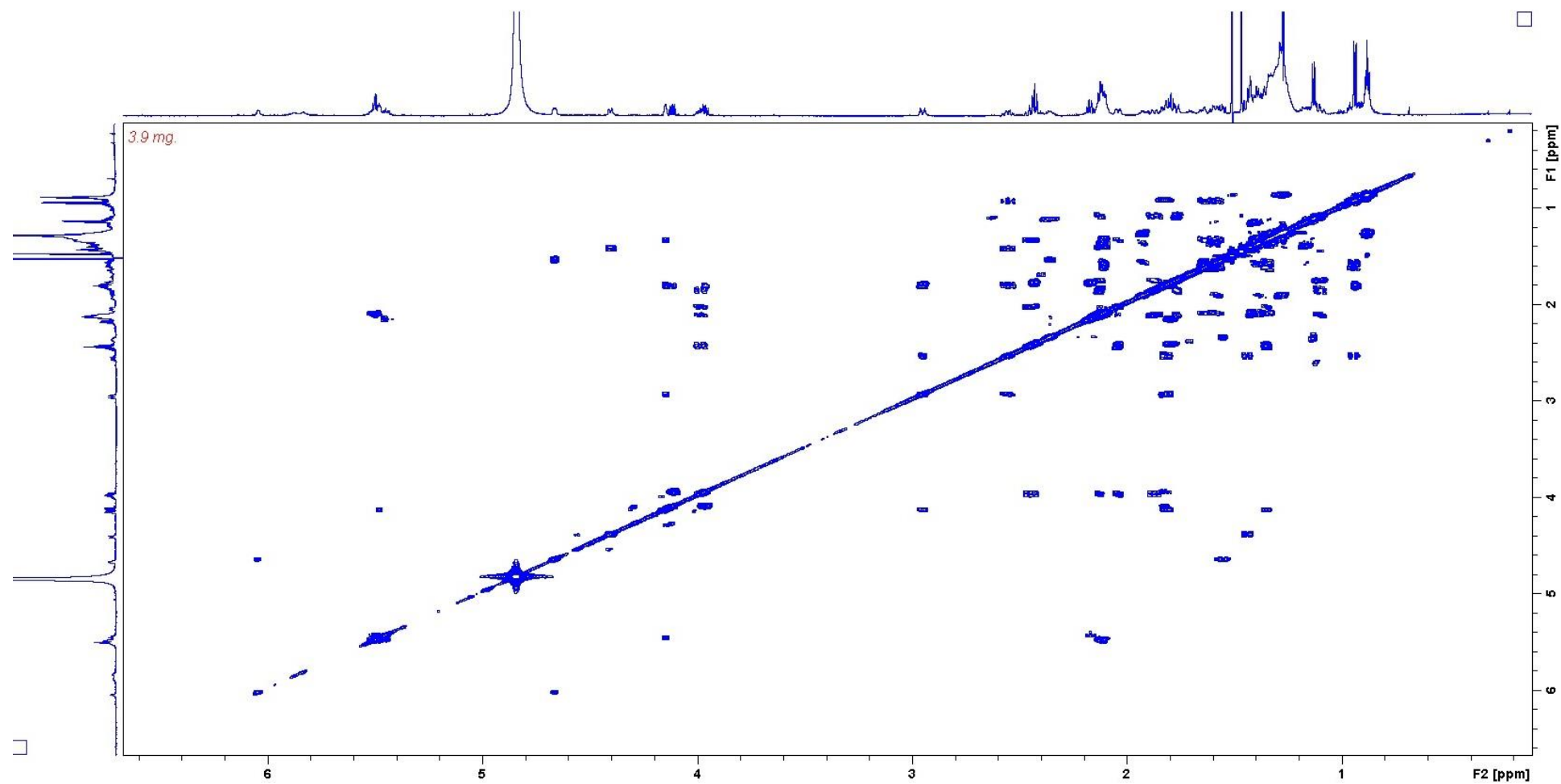
**Figure S35.**  $^1\text{H}$ - $^1\text{H}$ -COSY spectrum of compound **3** in  $\text{C}_5\text{D}_5\text{N}$ .

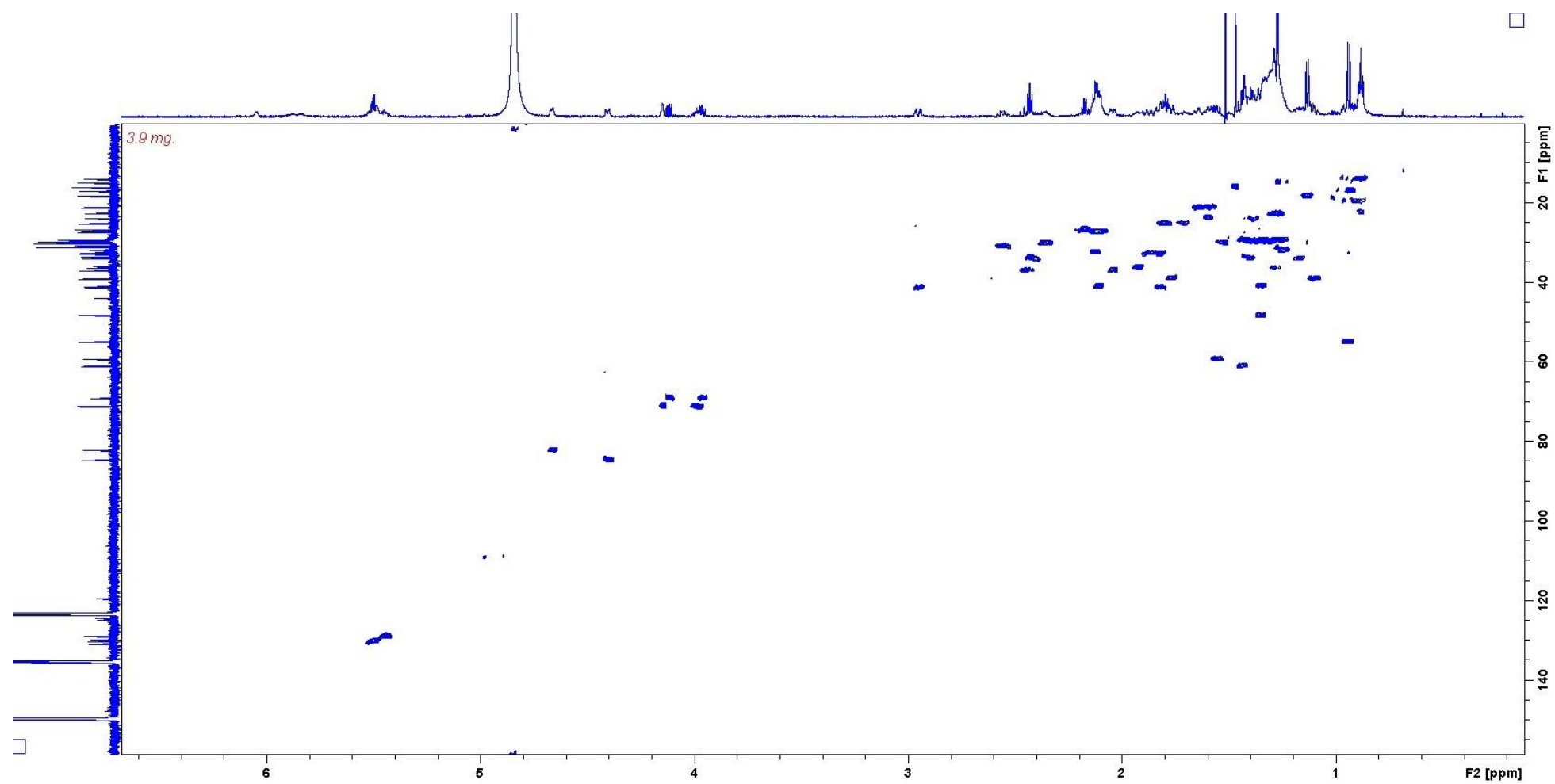
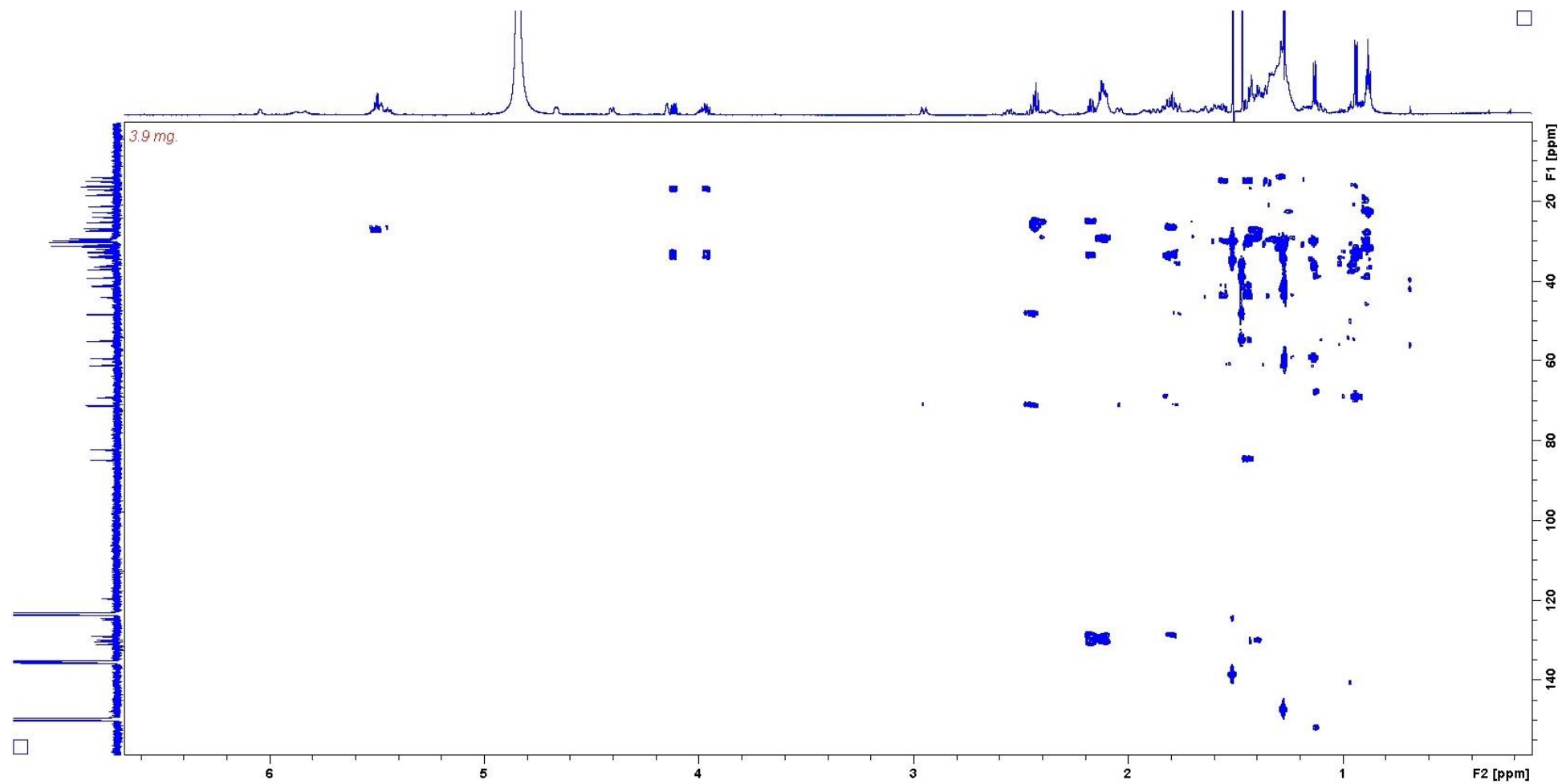
Figure S36. HSQC spectrum of compound **3** in  $C_5D_5N$ .

Figure S37. HMBC spectrum of compound **3** in C<sub>5</sub>D<sub>5</sub>N.



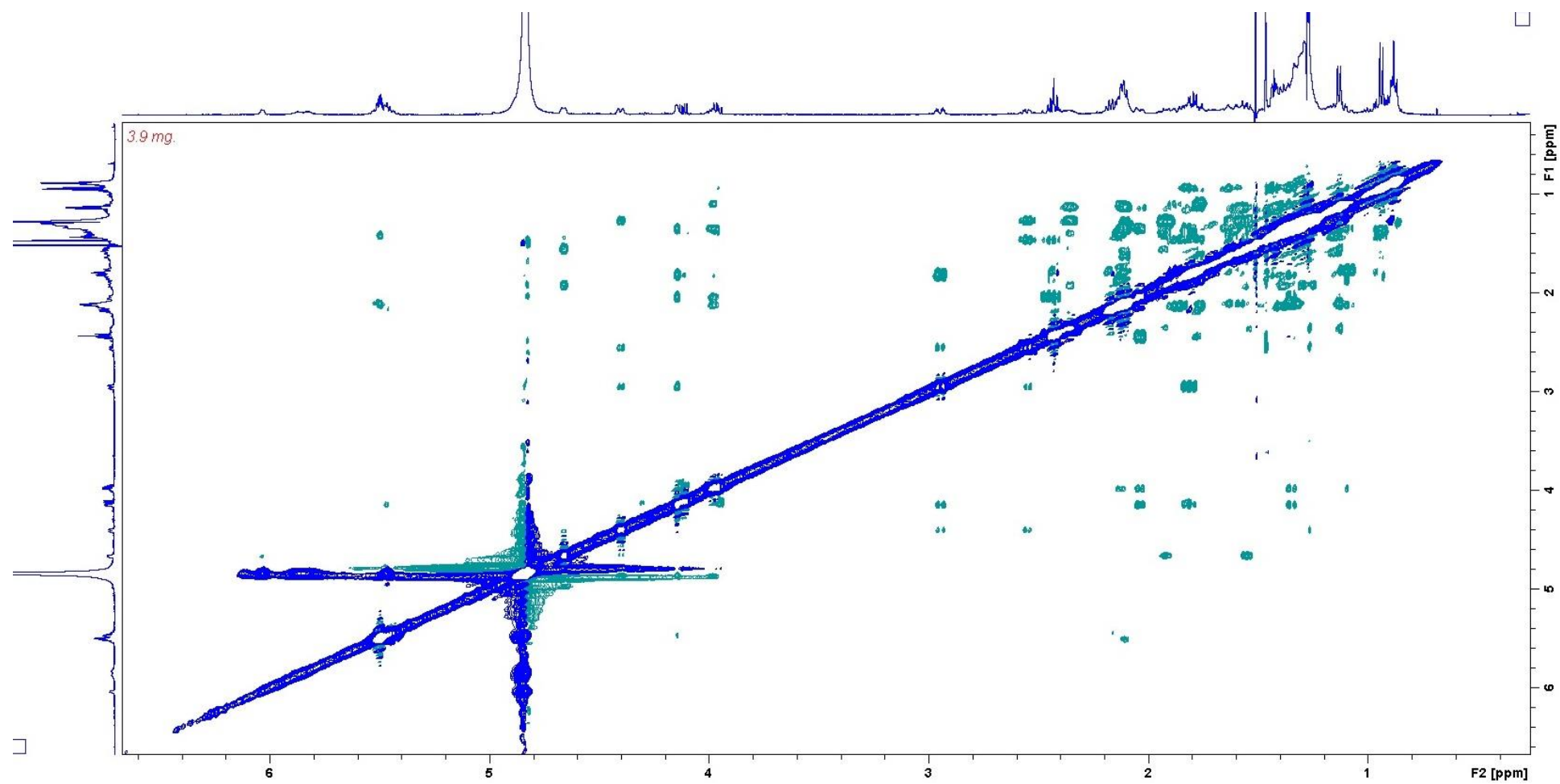
**Figure S38.** ROESY spectrum of compound **3** in  $C_5D_5N$ .

Figure S39. (+)-HRESIMS spectrum of compound 4.

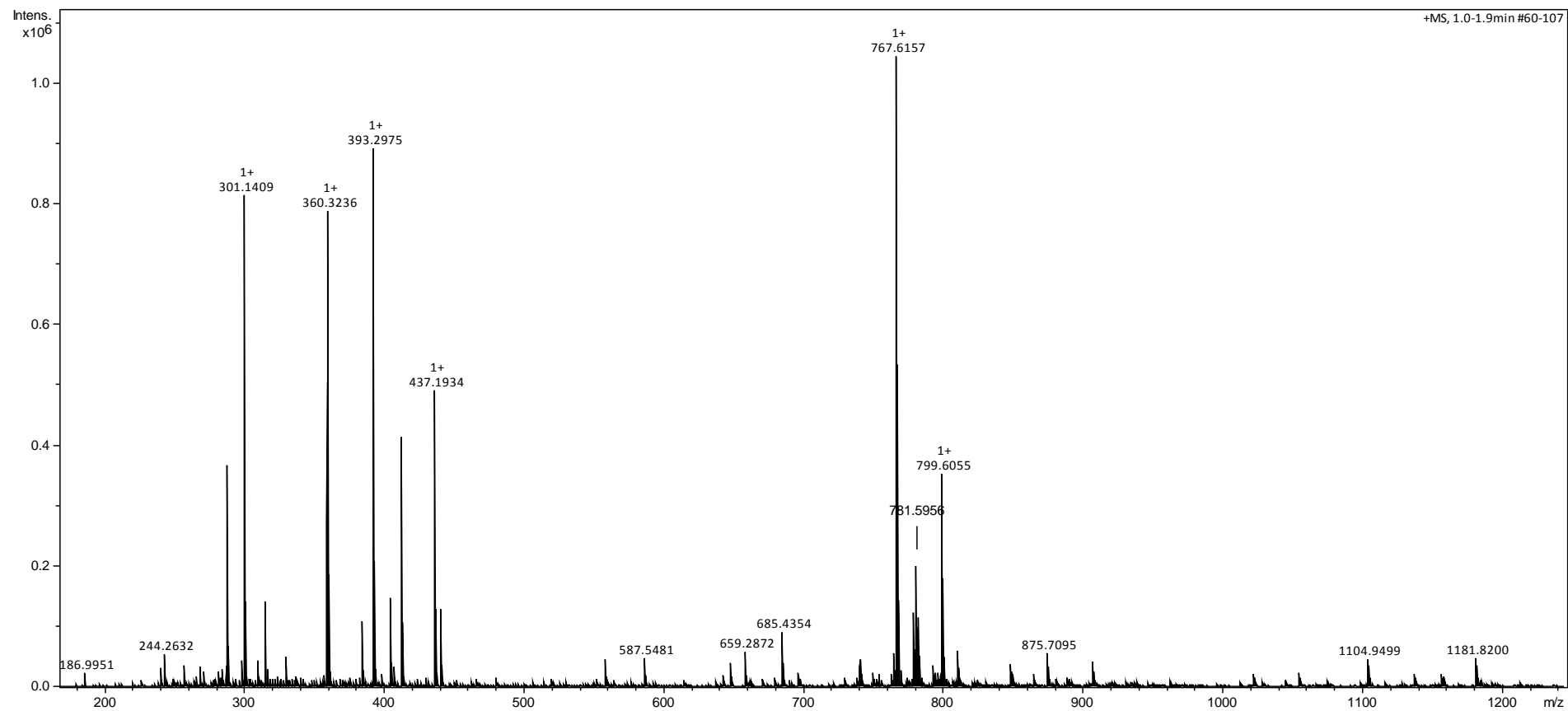
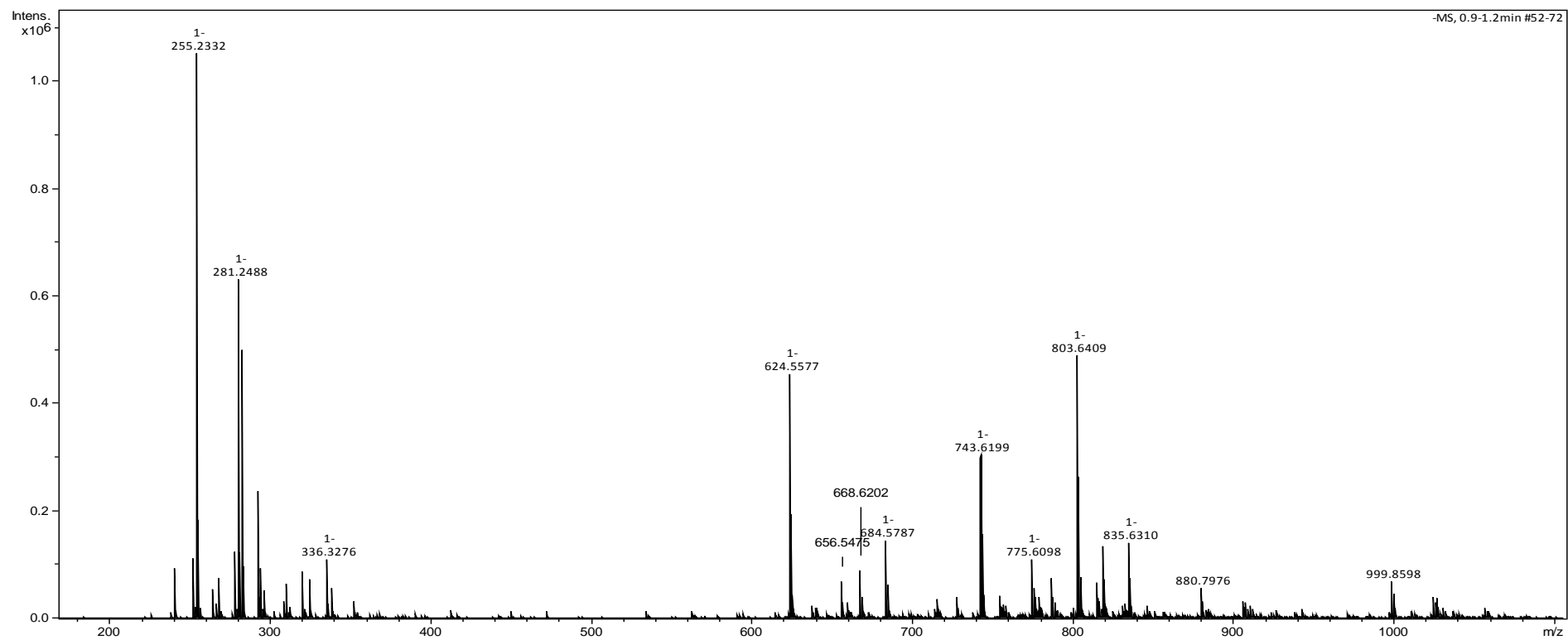


Figure S40. (-)-HRESIMS spectrum of compound 4.



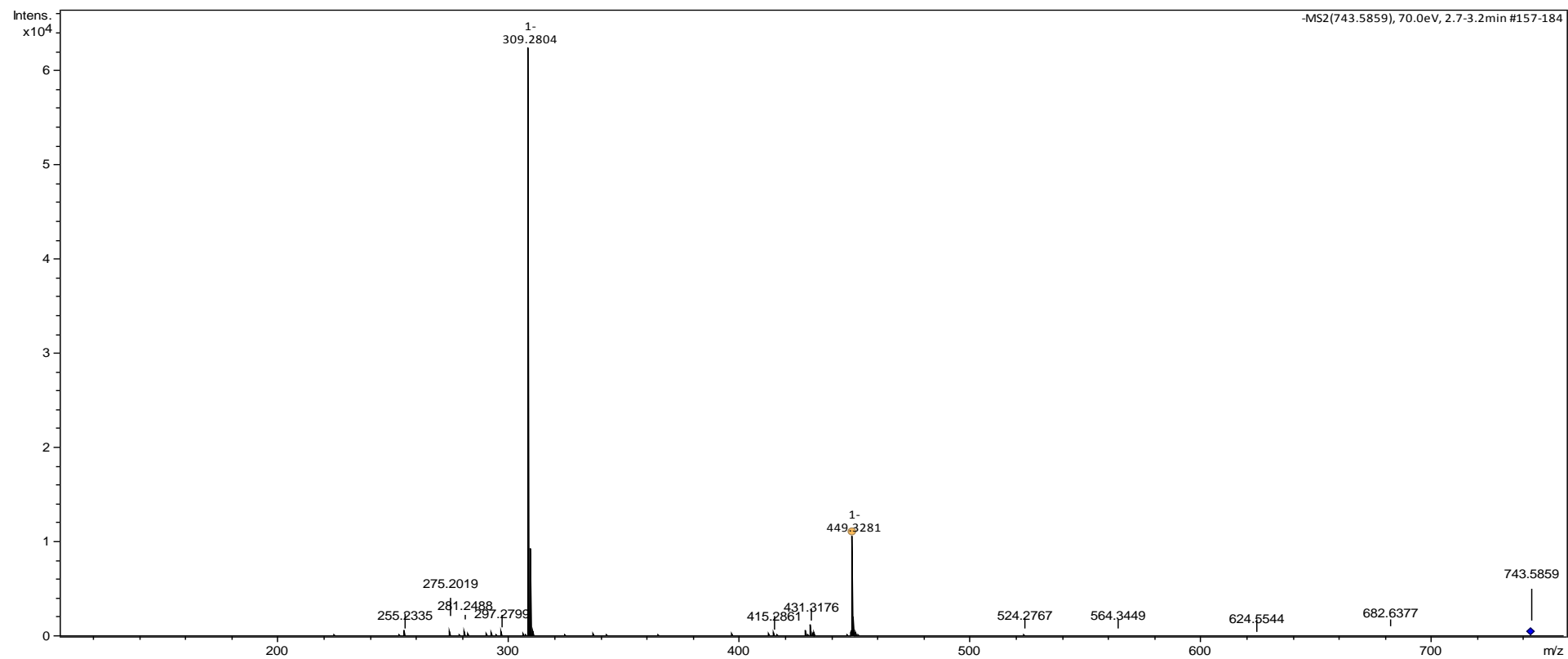
**Figure S41.** (-)-ESIMS/MS spectrum of compound **4**.

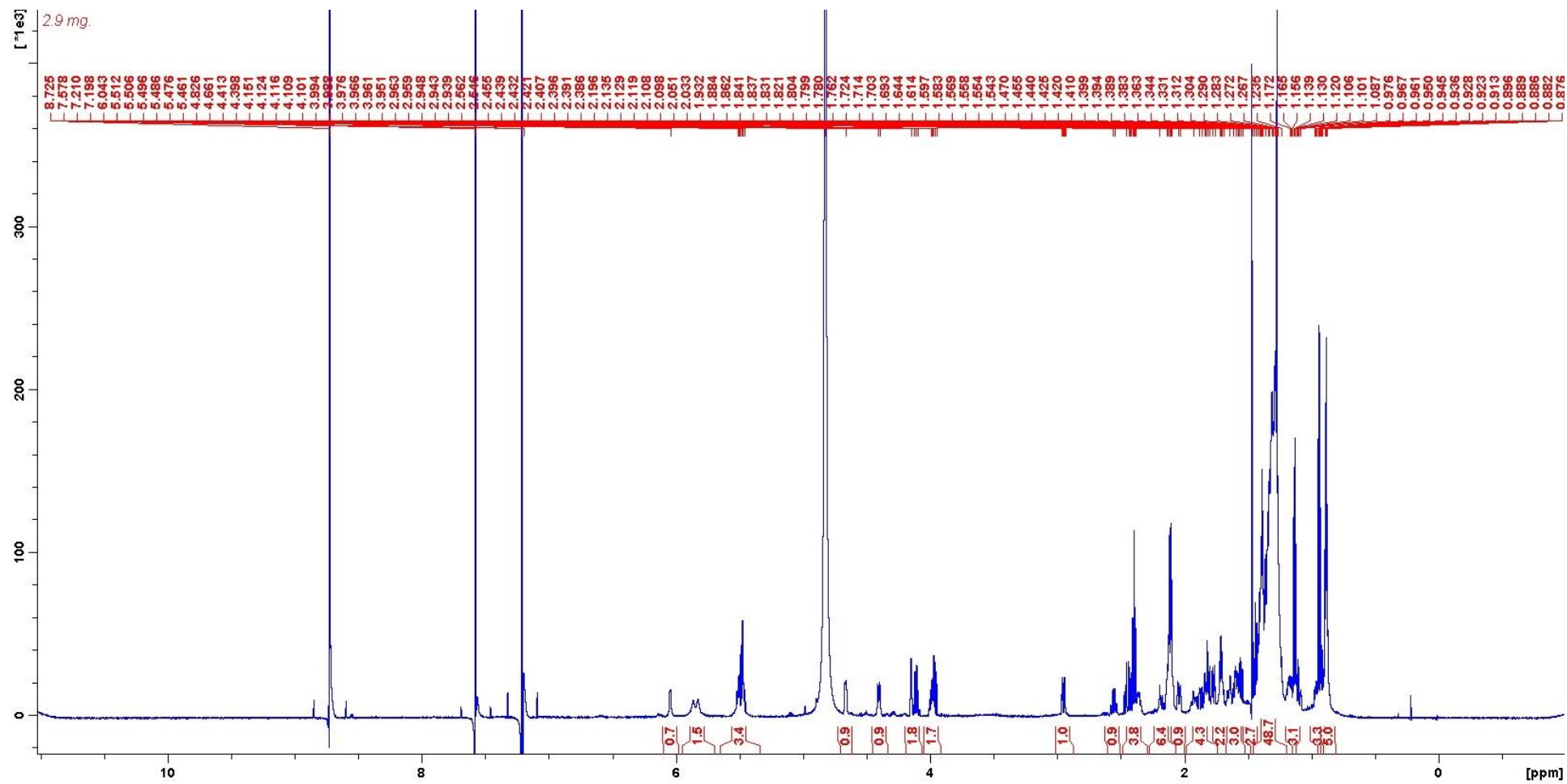
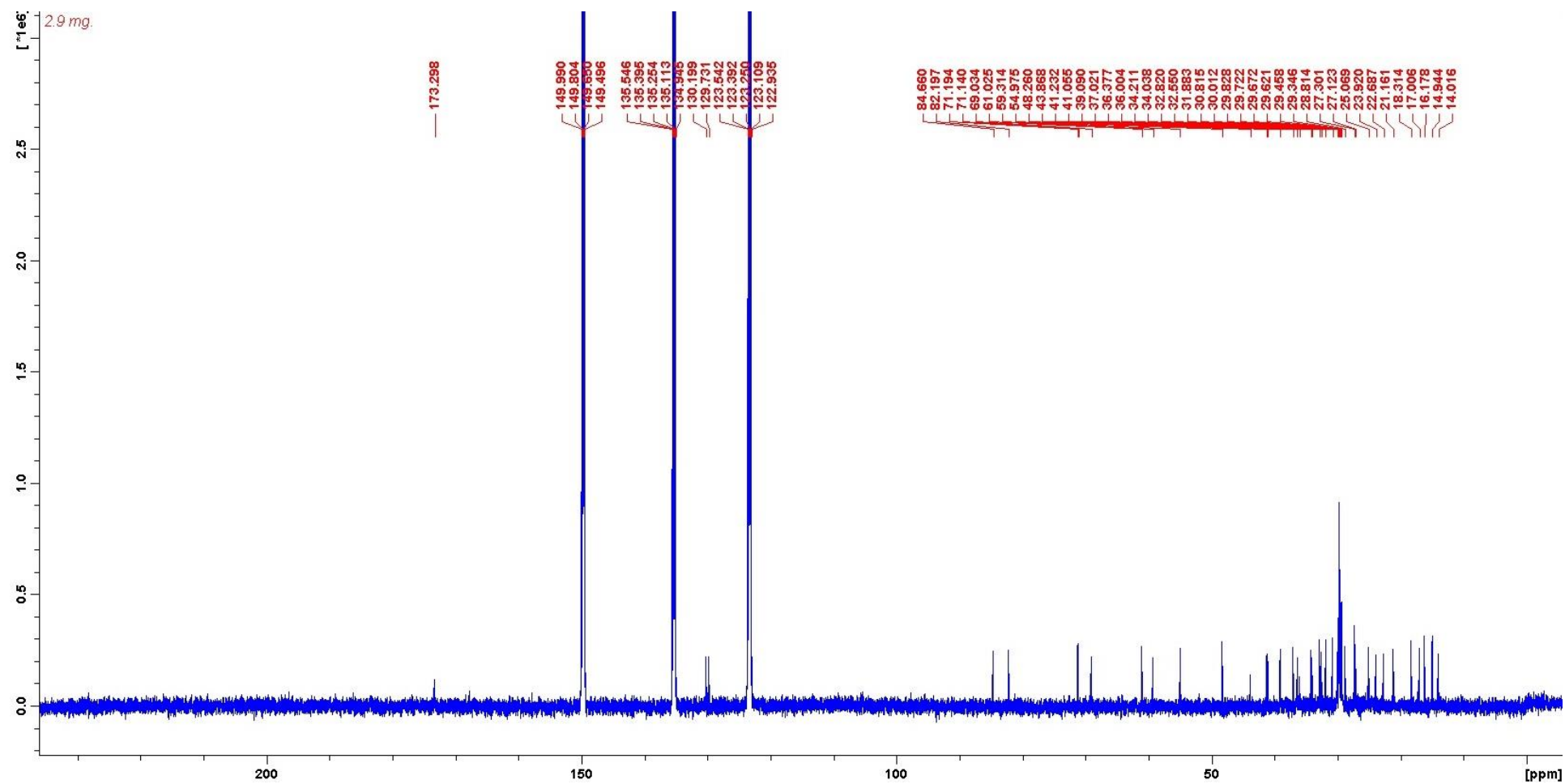
Figure S42.  $^1\text{H}$ -NMR spectrum of compound **4** in  $\text{C}_5\text{D}_5\text{N}$ .

Figure S43.  $^{13}\text{C}$ -NMR spectrum of compound **4** in  $\text{C}_5\text{D}_5\text{N}$ .

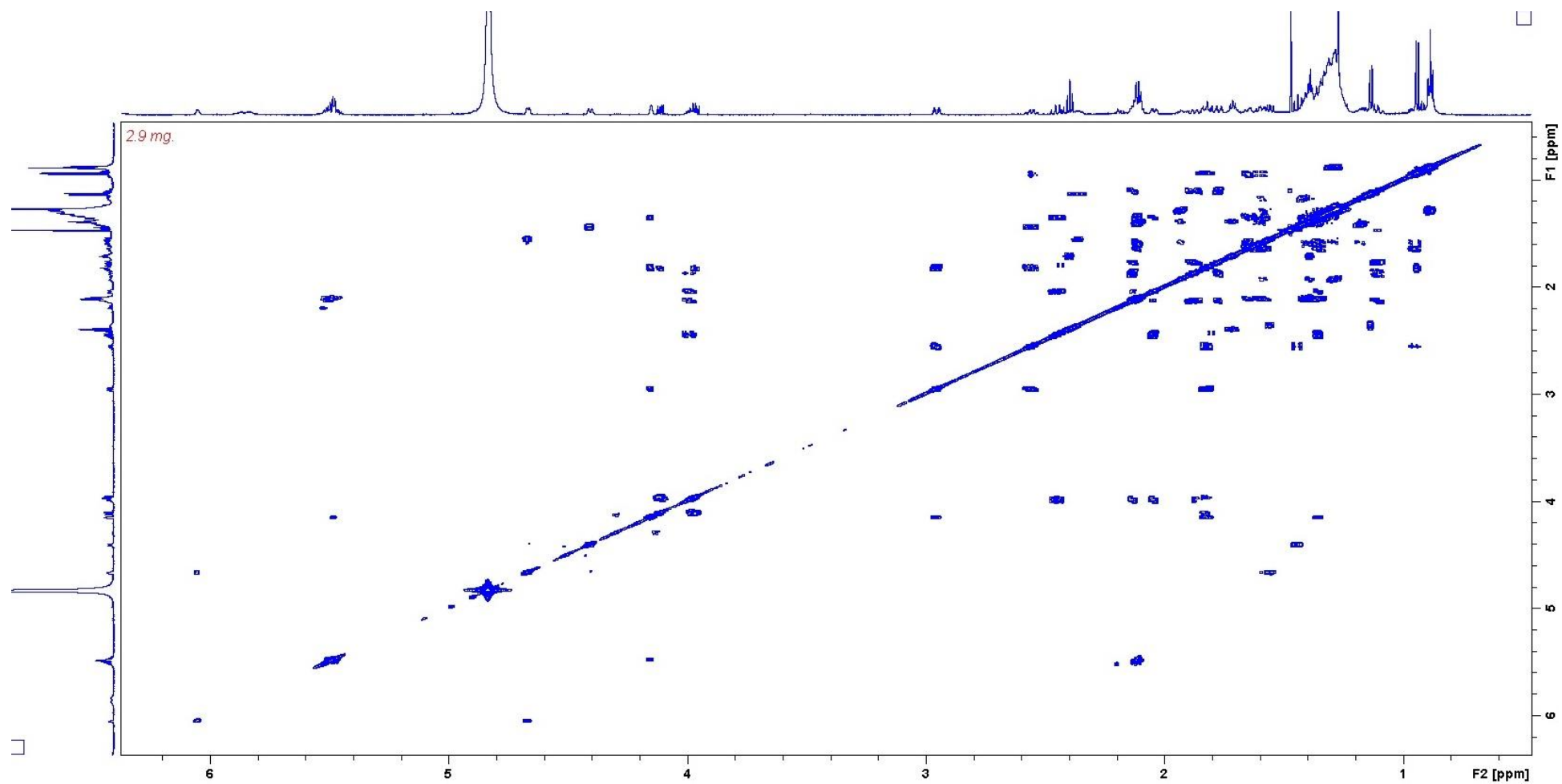
**Figure S44.**  $^1\text{H}$ - $^1\text{H}$ -COSY spectrum of compound **4** in  $\text{C}_5\text{D}_5\text{N}$ .

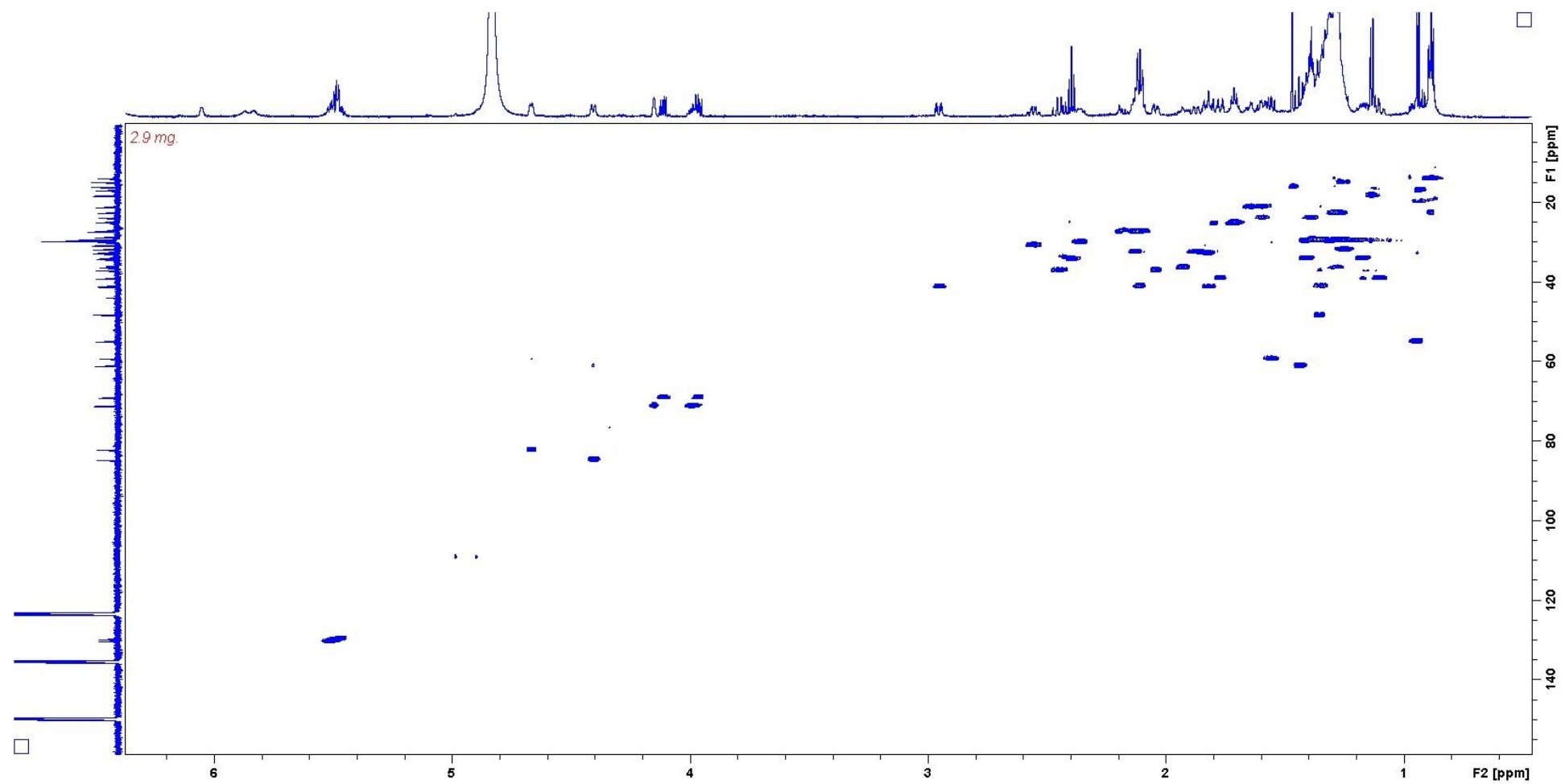
Figure S45. HSQC spectrum of compound **4** in  $C_5D_5N$ .



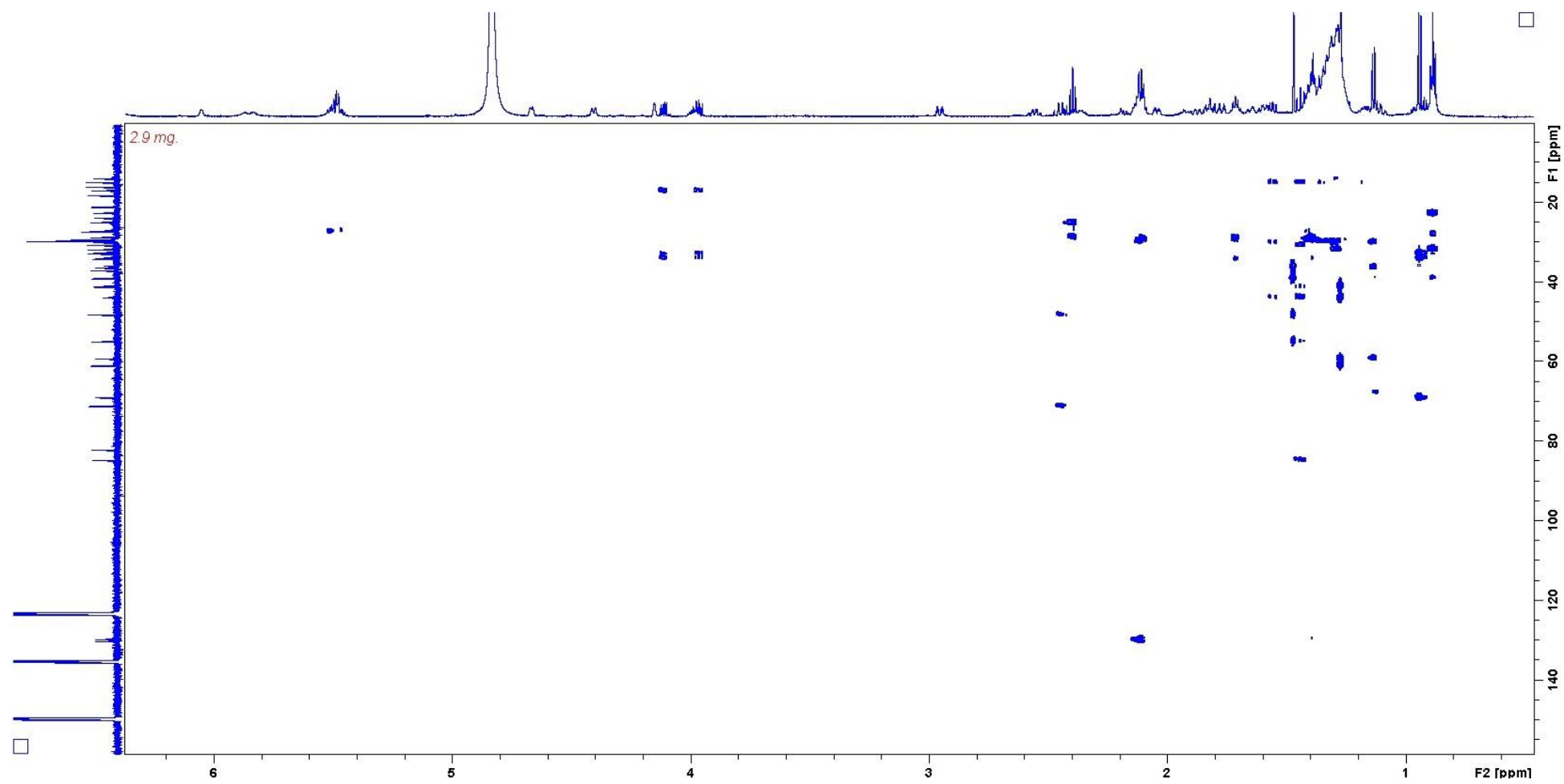
Figure S46. HMBC spectrum of compound **4** in C<sub>5</sub>D<sub>5</sub>N.

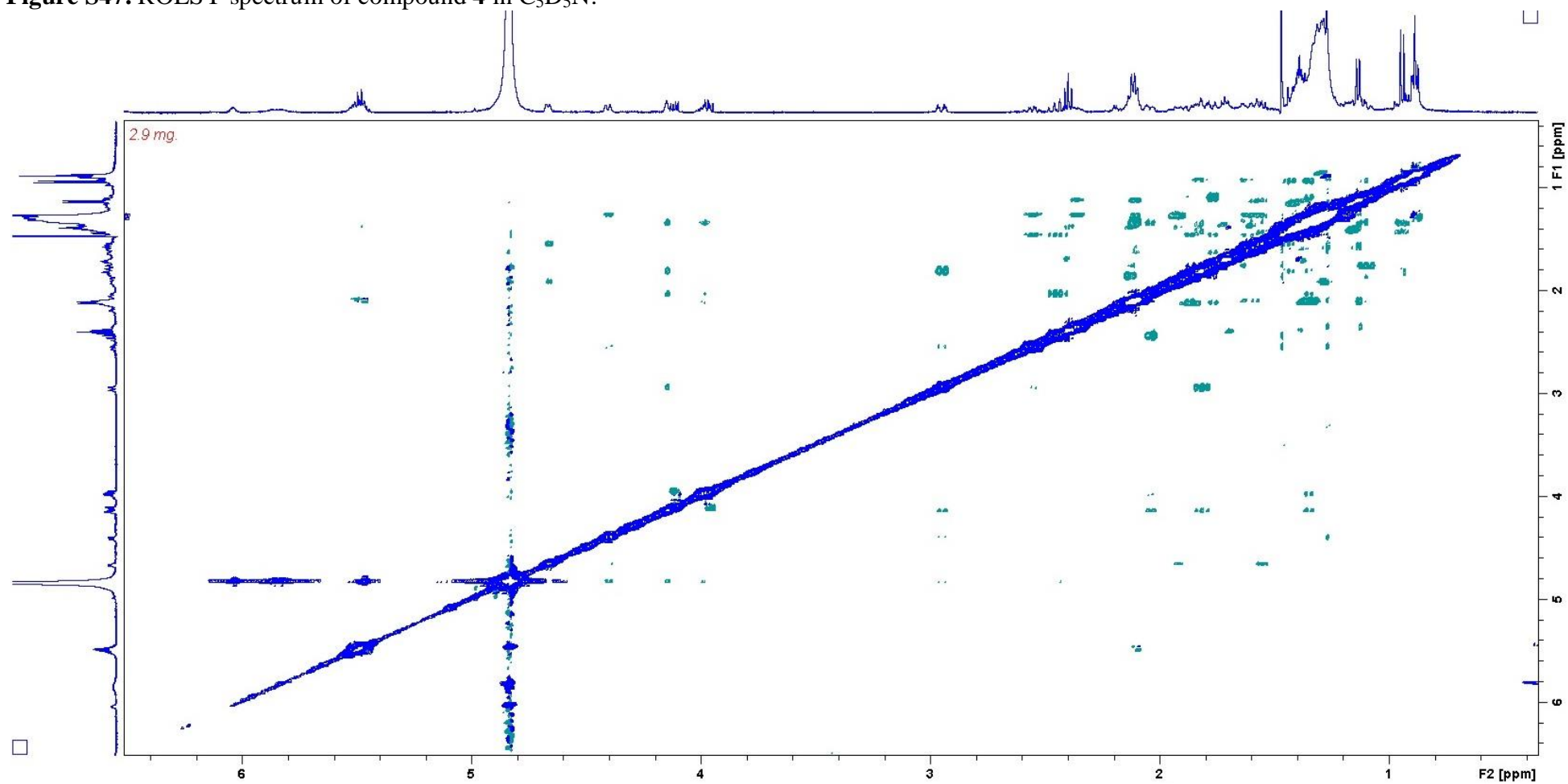
Figure S47. ROESY spectrum of compound 4 in  $C_5D_5N$ .

Figure S48. Cytotoxicity compounds 1–4 after 24 h.

