

**Multiple *N*-methylation of MT-II backbone amide bonds leads to melanocortin receptor subtype hMC1R selectivity; pharmacological and conformational studies.**

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## Supporting information

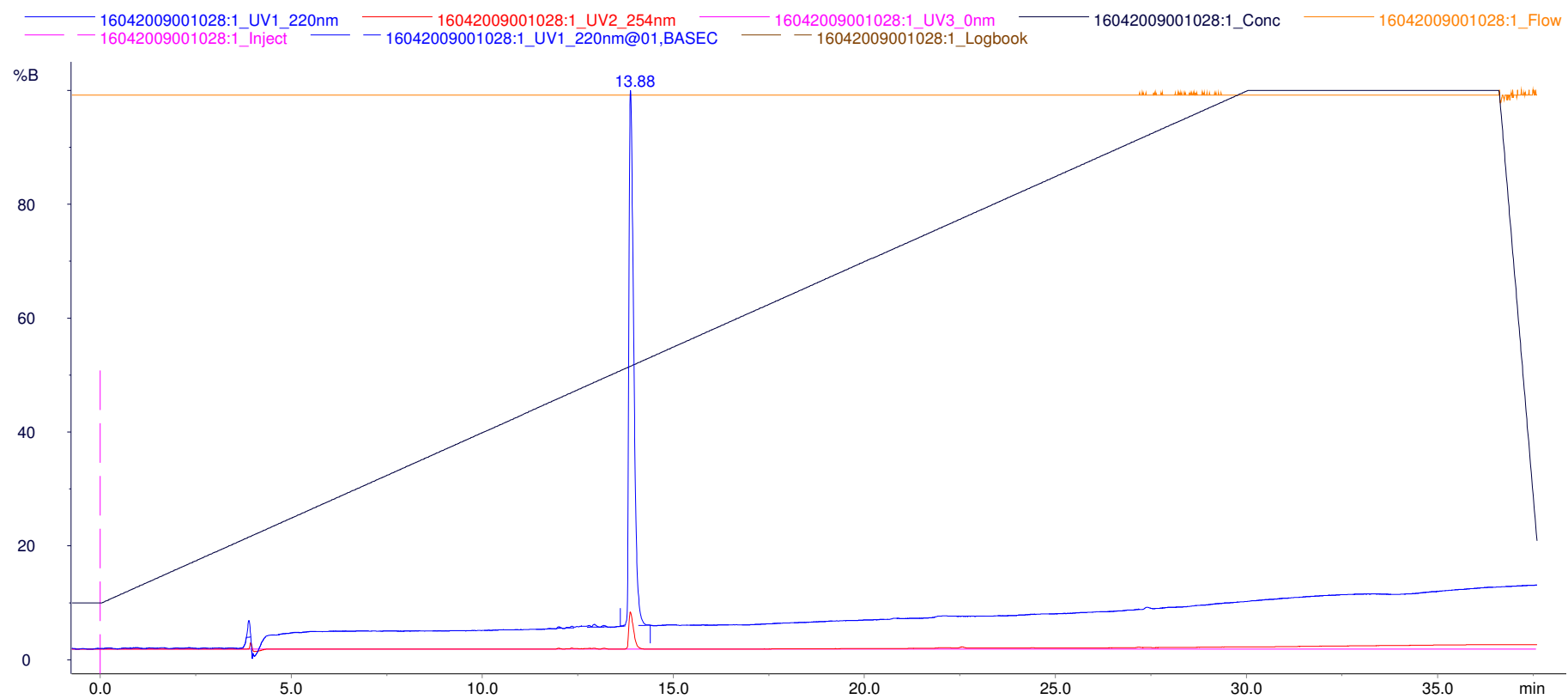
**Table 1.** Sequences and Physicochemical Properties of the *N*-methylated MT-II Analogs

No.	sequence	HPLC [t <sub>R</sub> ] (min) <sup>a</sup>	HR-ESI-MS [M+H] <sup>+</sup>
1	Nle D H f R W K	13.88	1024.5440
2	Nle D H f R W K $\alpha$	14.39	1038.5593
3	Nle D H f R W K	13.29	1038.5592
4	Nle D H f R W K	12.64	1038.5592
5	Nle D H f R W K	12.45	1038.5593
6	Nle D H f R W K	14.15	1038.5592
7	Nle D H f R W K $\alpha$	14.44	1052.5751
8	Nle D H f R W K $\alpha$	12.76	1052.5750
9	Nle D H f R W K $\alpha$	13.02	1052.5750
10	Nle D H f R W K $\alpha$	14.19	1052.5749
11	Nle D H f R W K	12.06	1052.5750
12	Nle D H f R W K	12.24	1052.5749
13	Nle D H f R W K	13.79	1052.5749
14	Nle D H f R W K	12.54	1052.5749
15	Nle D H f R W K	12.26	1052.5749
16	Nle D H f R W K	13.51	1052.5749
17	Nle D H f R W K $\alpha$	12.03	1066.5904
18	Nle D H f R W K $\alpha$	13.38	1066.5905
19	Nle D H f R W K $\alpha$	14.22	1066.5905
20	Nle D H f R W K $\alpha$	12.61	1066.5906
21	Nle D H f R W K $\alpha$	12.74	1066.5908
22	Nle D H f R W K $\alpha$	12.97	1066.5905
23	Nle D H f R W K	12.78	1066.5903
24	Nle D H f R W K	12.20	1066.5905
25	Nle D H f R W K	13.77	1066.5906
26	Nle D H f R W K	12.49	1066.5905
27	Nle D H f R W K $\alpha$	13.11	1080.6061
28	Nle D H f R W K $\alpha$	11.89	1080.6062
29	Nle D H f R W K $\alpha$	13.92	1080.6060
30	Nle D H f R W K $\alpha$	12.90	1080.6060
31	Nle D H f R W K	12.47	1080.6059
32	Nle D H f R W K $\alpha$	12.98	1094.6210
33	Nle D H f R W K $\beta$	22.16 <sup>b</sup>	1038.5593

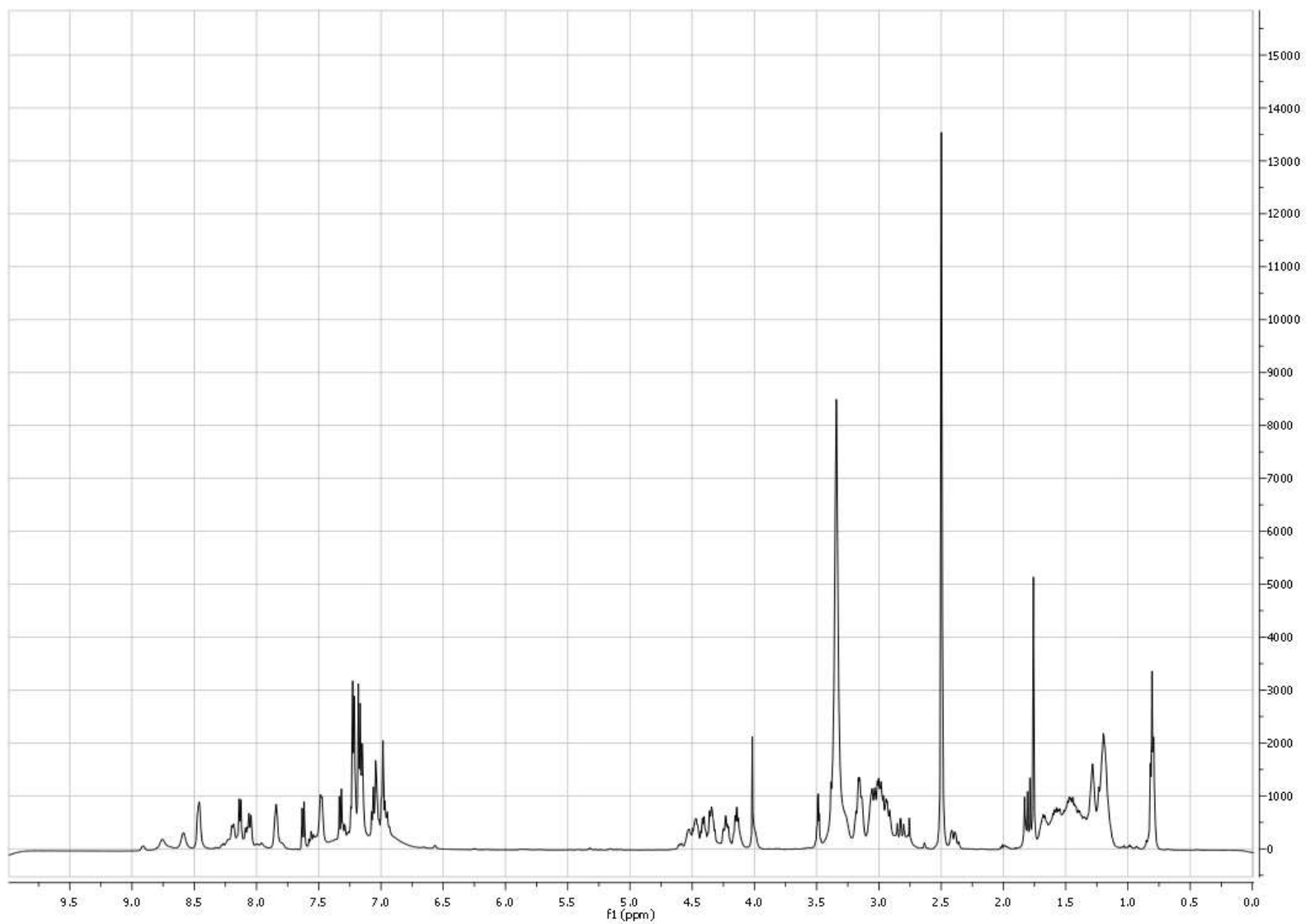
*N*-methylated amino acids are highlighted in grey.

<sup>a</sup> RP-HPLC on C18 column, MeCN 10 to 100% in 30 min.

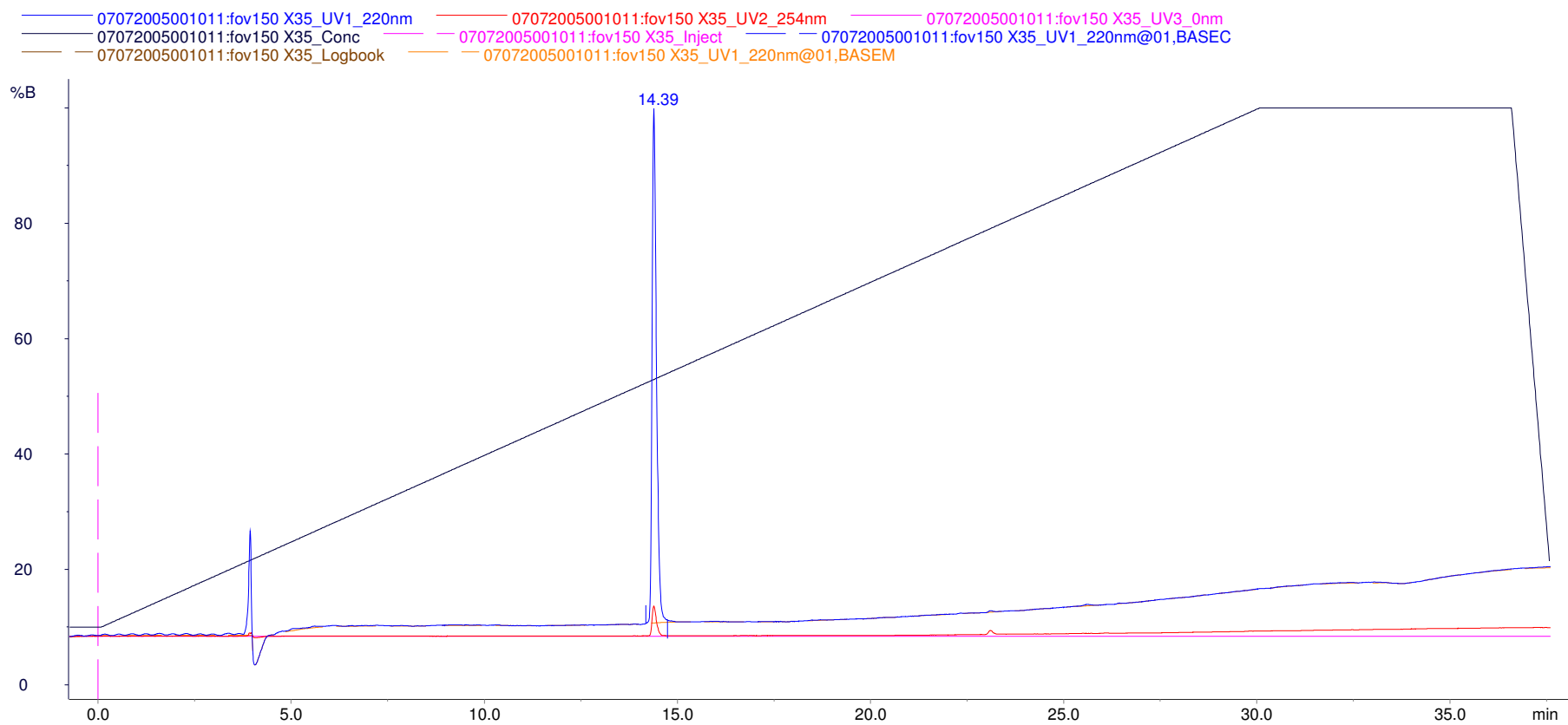
<sup>b</sup> RP-HPLC on C18 column, MeCN 10 to 50% in 30 min.



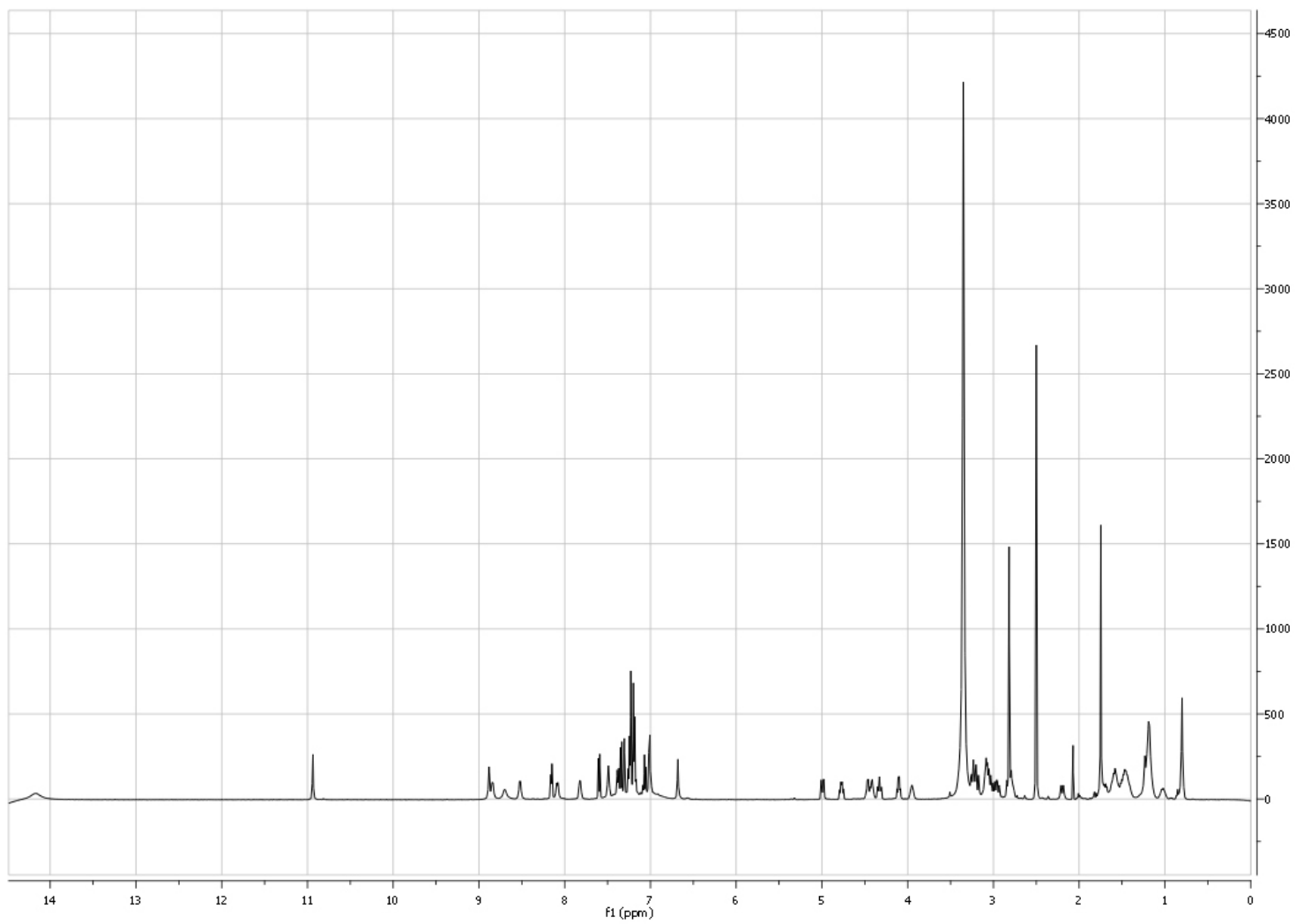
**Figure 1A:** HPLC chromatogram of compound 1 (MT-II).



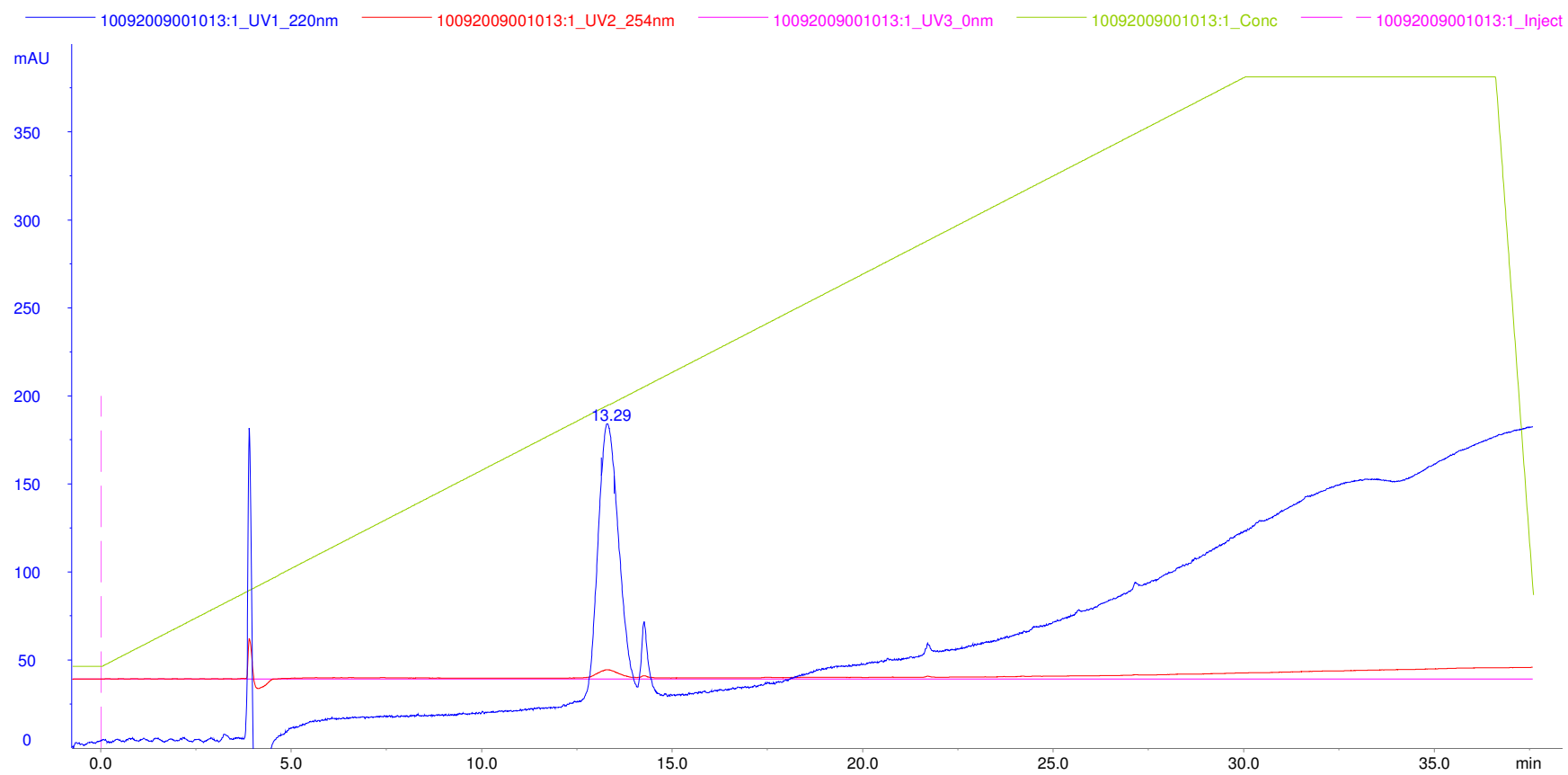
**Figure 1B:** <sup>1</sup>H-NMR spectrum of compound 1 (MT-II).



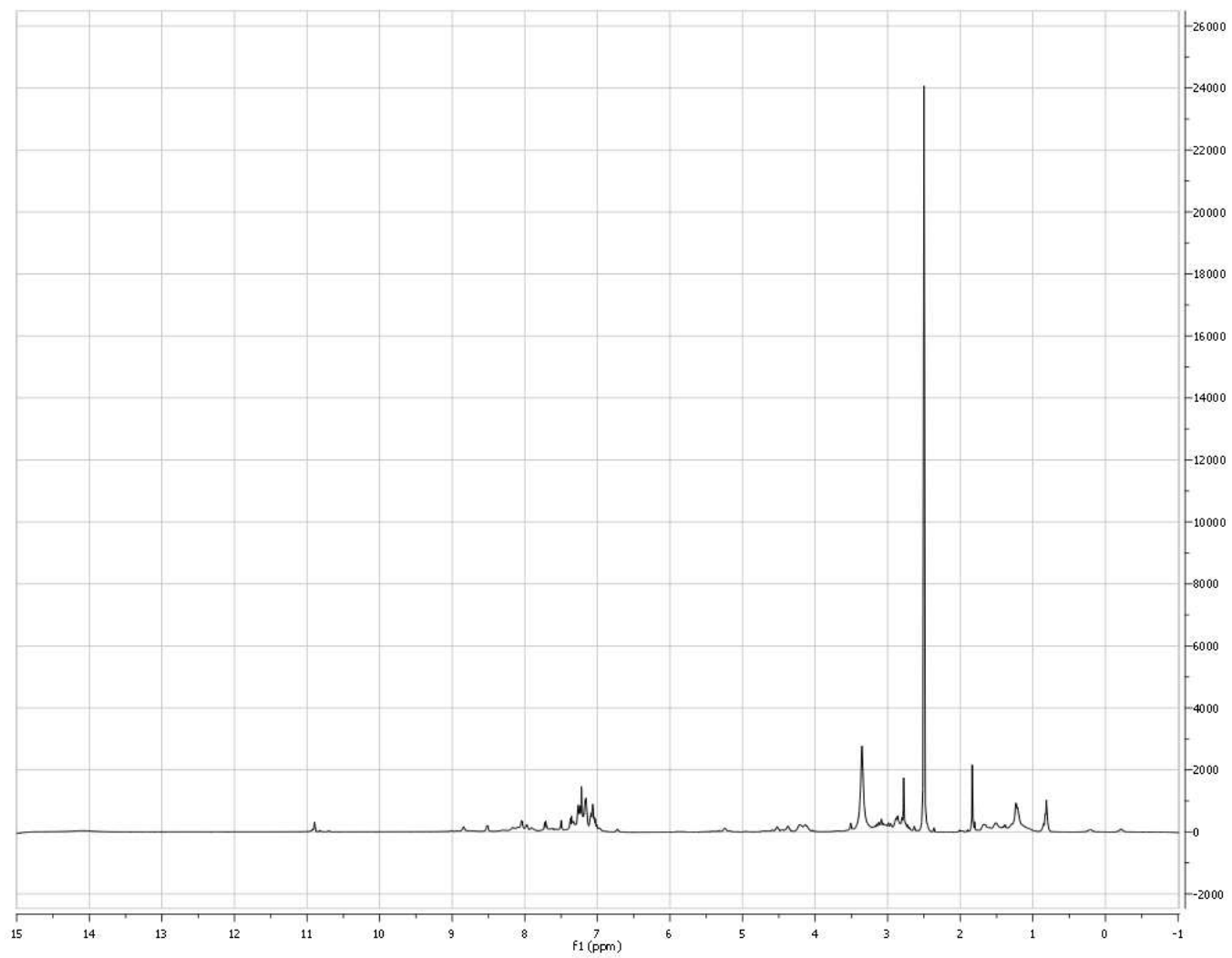
**Figure 2A:** HPLC chromatogram of compound 2.



**Figure 2B:**  $^1\text{H-NMR}$  spectrum of compound 2.

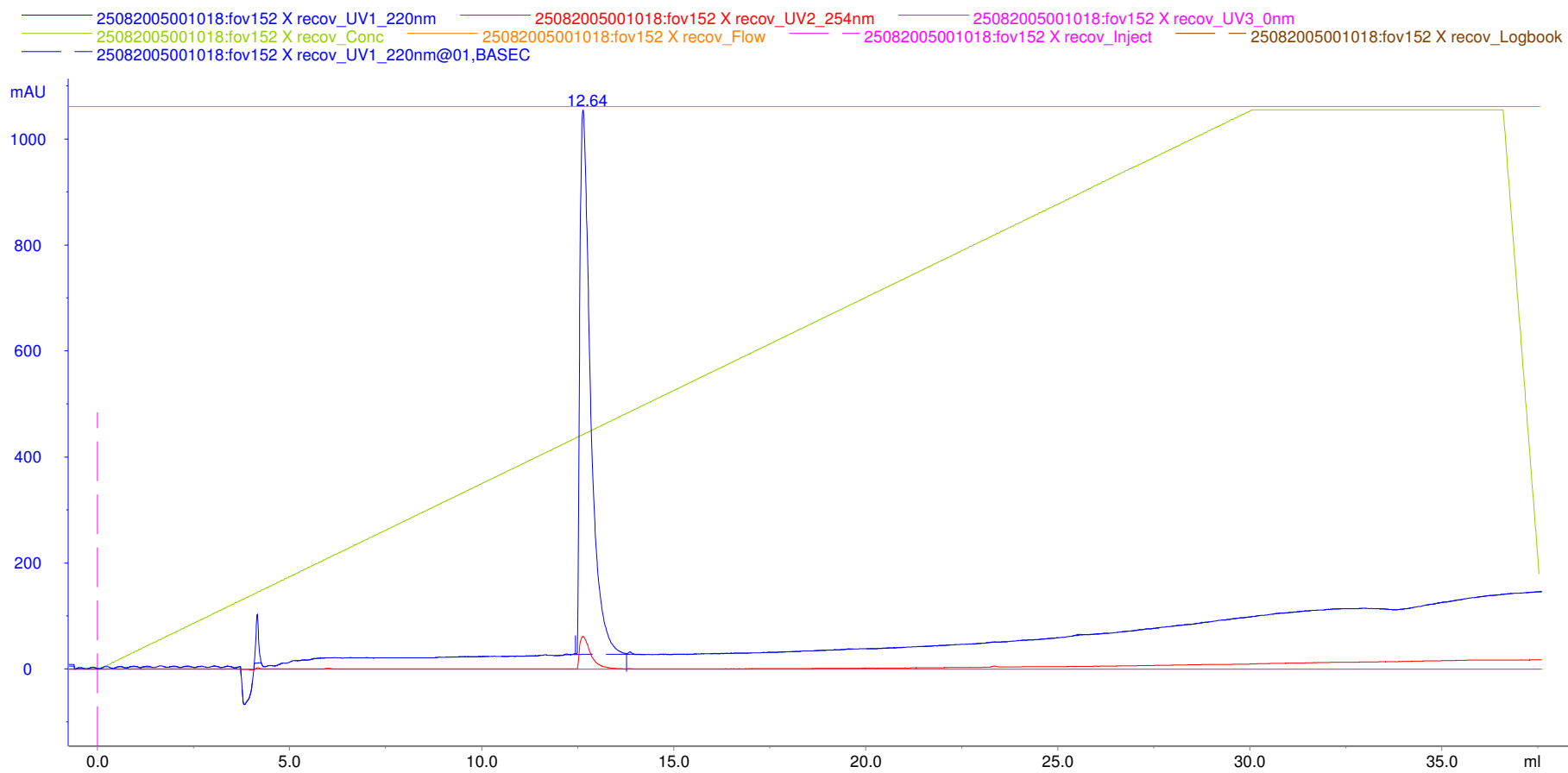


**Figure 3A:** HPLC chromatogram of compound 3.

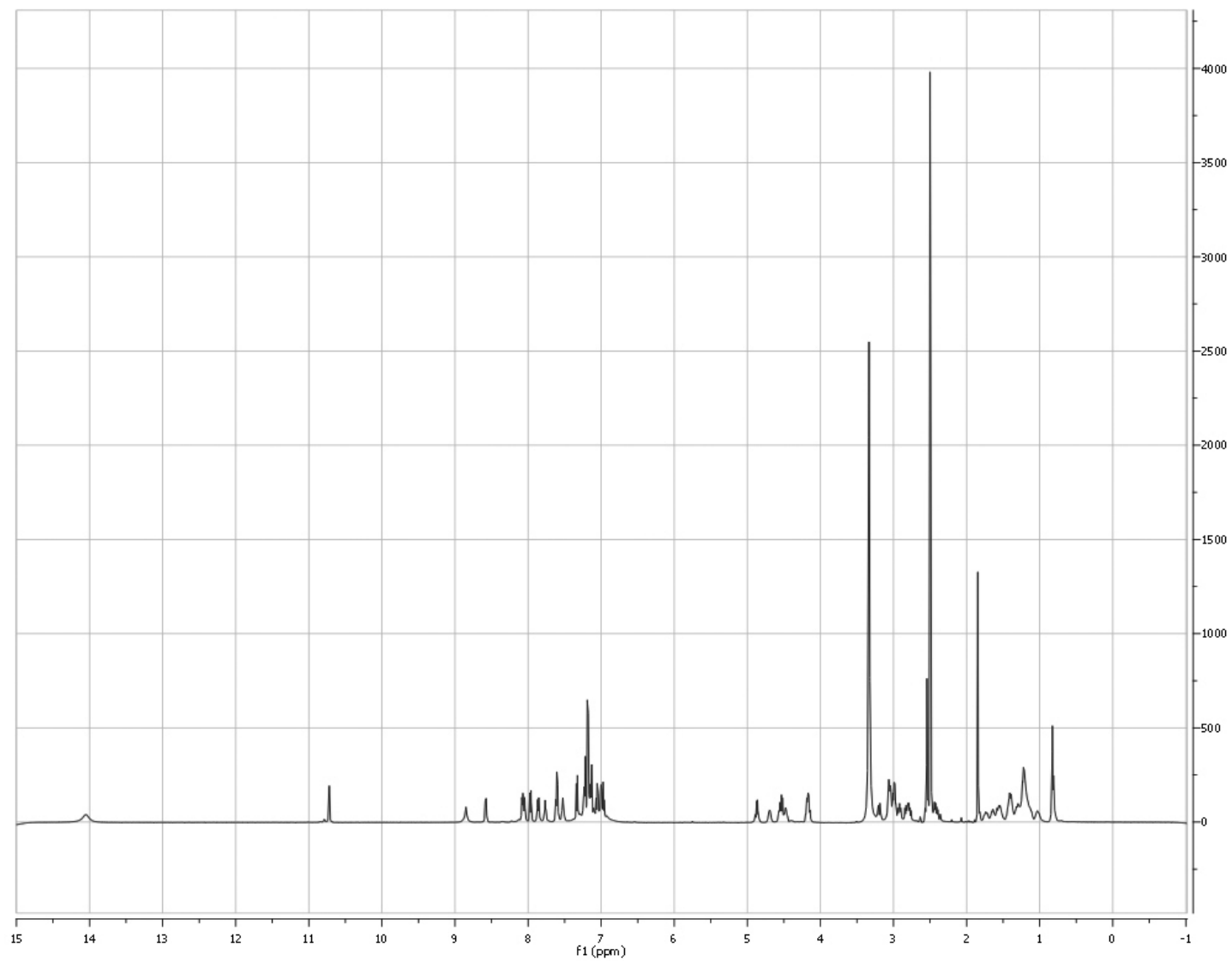


**Figure 3B:**  $^1\text{H-NMR}$  spectrum of compound 3.

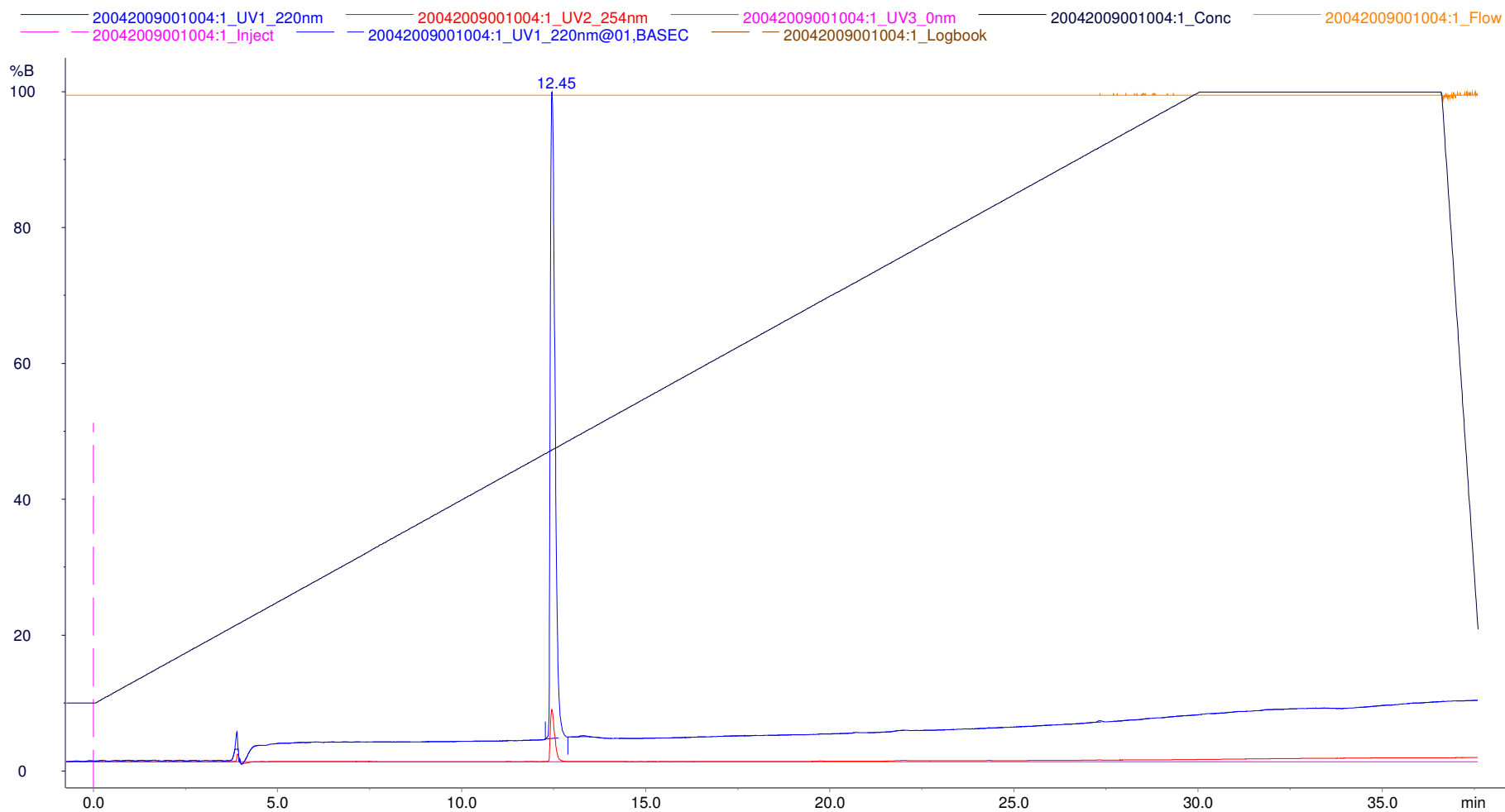




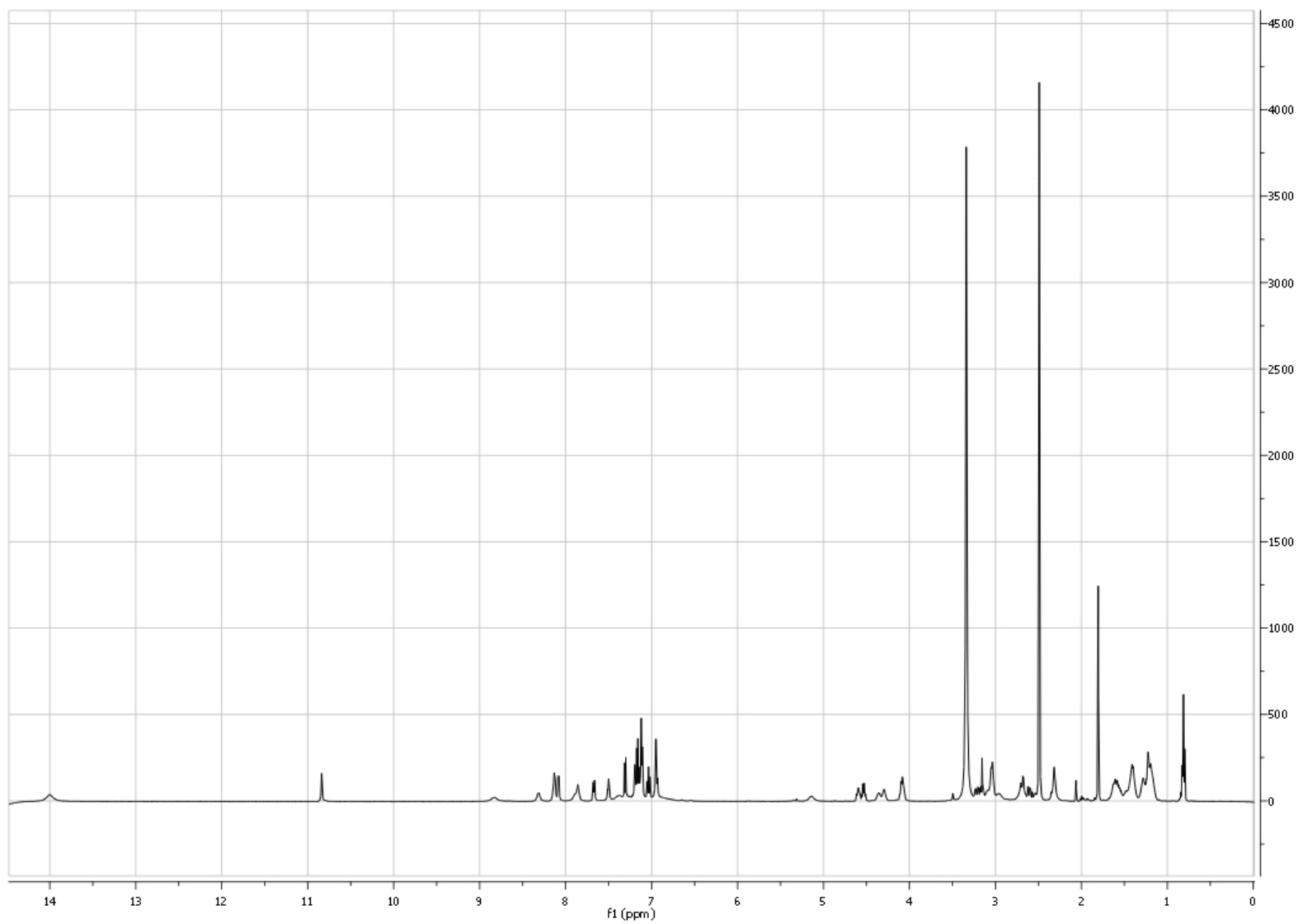
**Figure 4A:** HPLC chromatogram of compound 4.



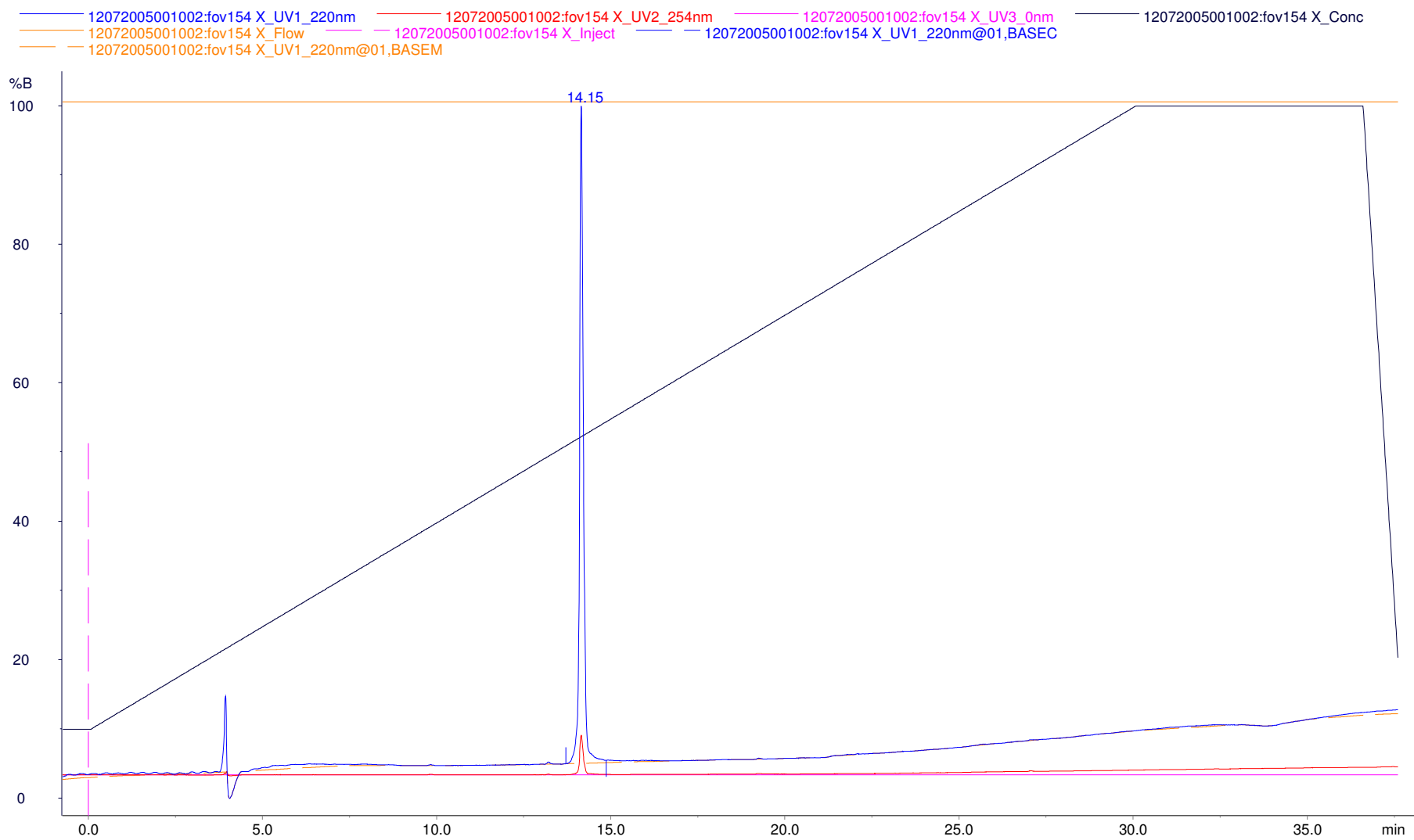
**Figure 4B:**  $^1\text{H-NMR}$  spectrum of compound 4.



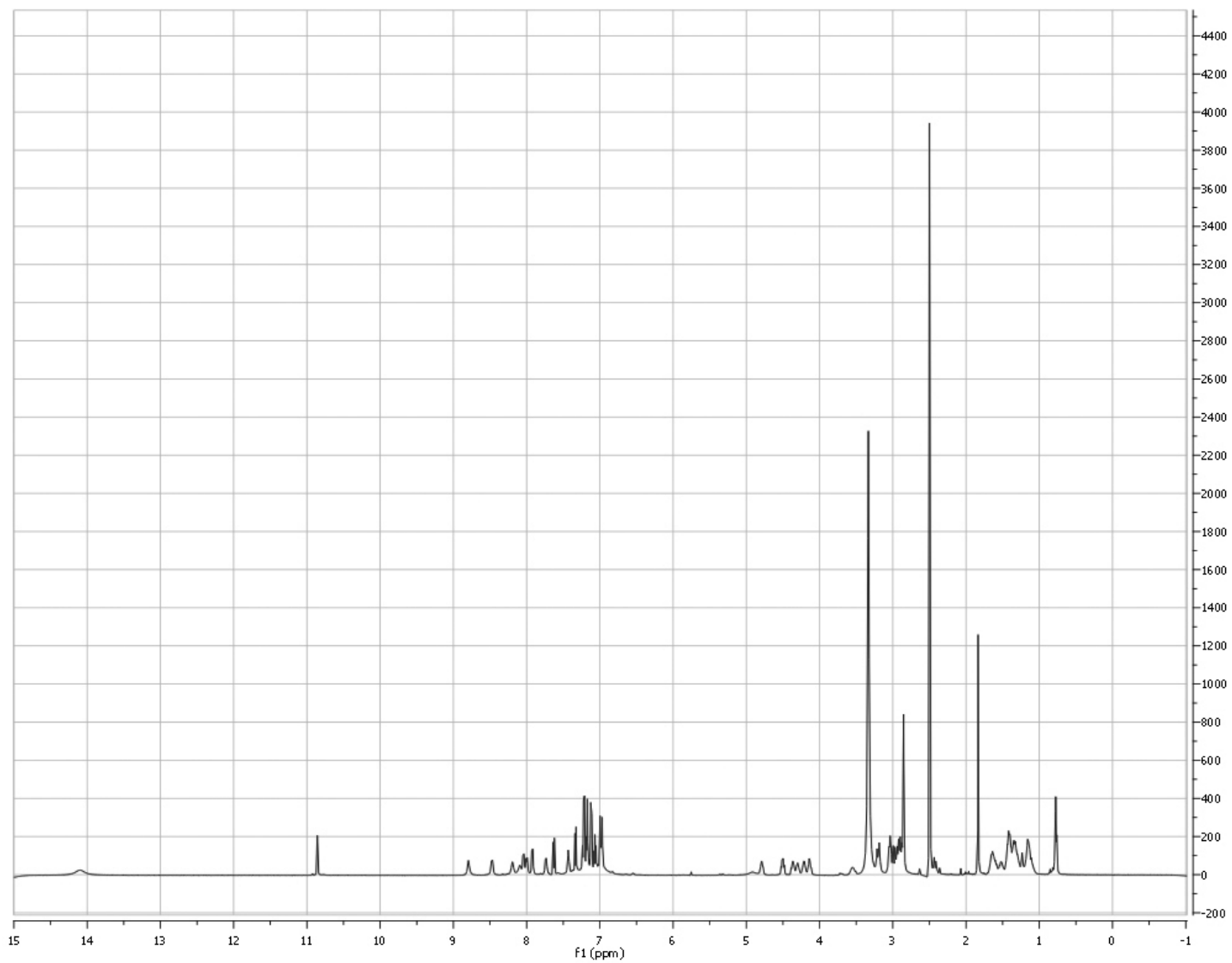
**Figure 5A:** HPLC chromatogram of compound 5.



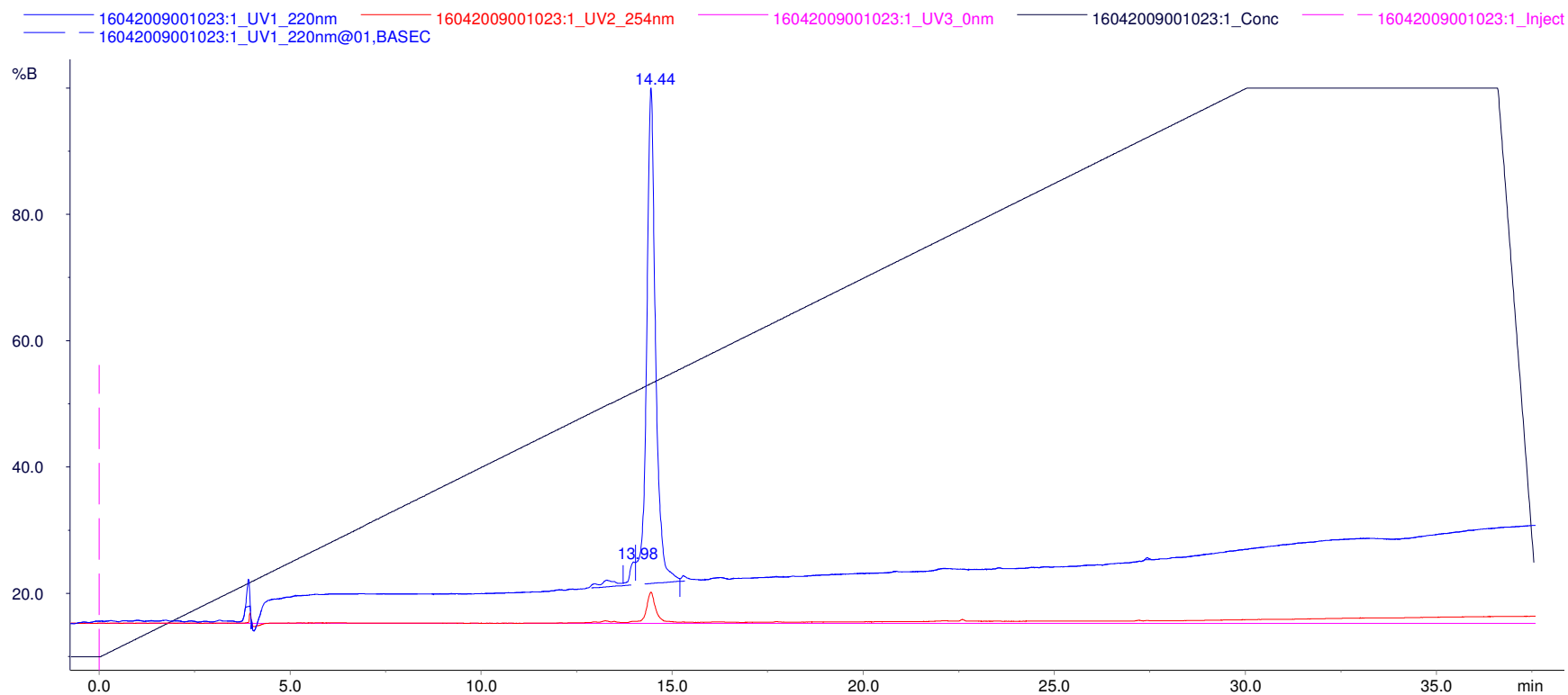
**Figure 5B:**  $^1\text{H-NMR}$  spectrum of compound 5.



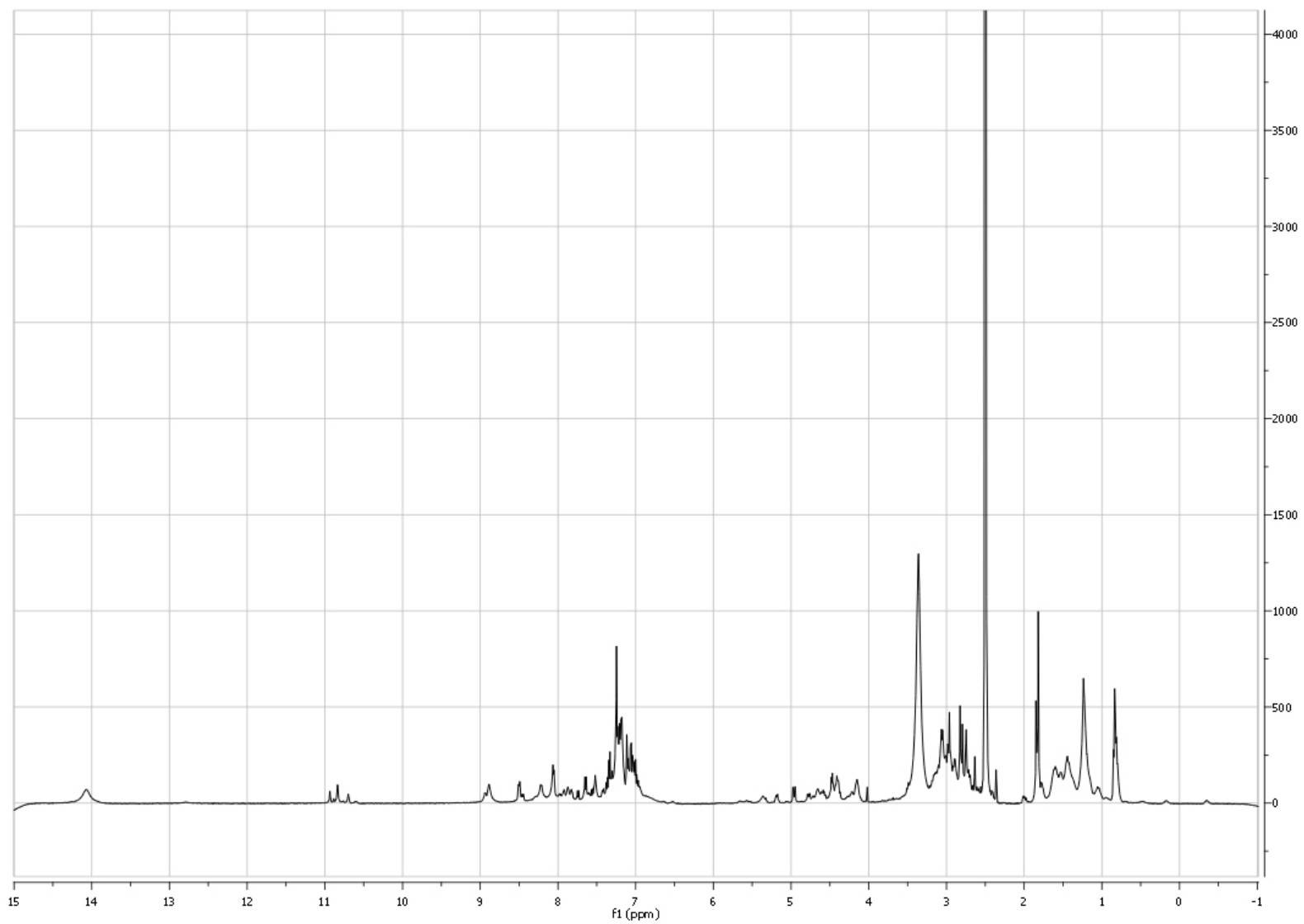
**Figure 6A:** HPLC chromatogram of compound 6.



**Figure 6B:** <sup>1</sup>H-NMR spectrum of compound 6.

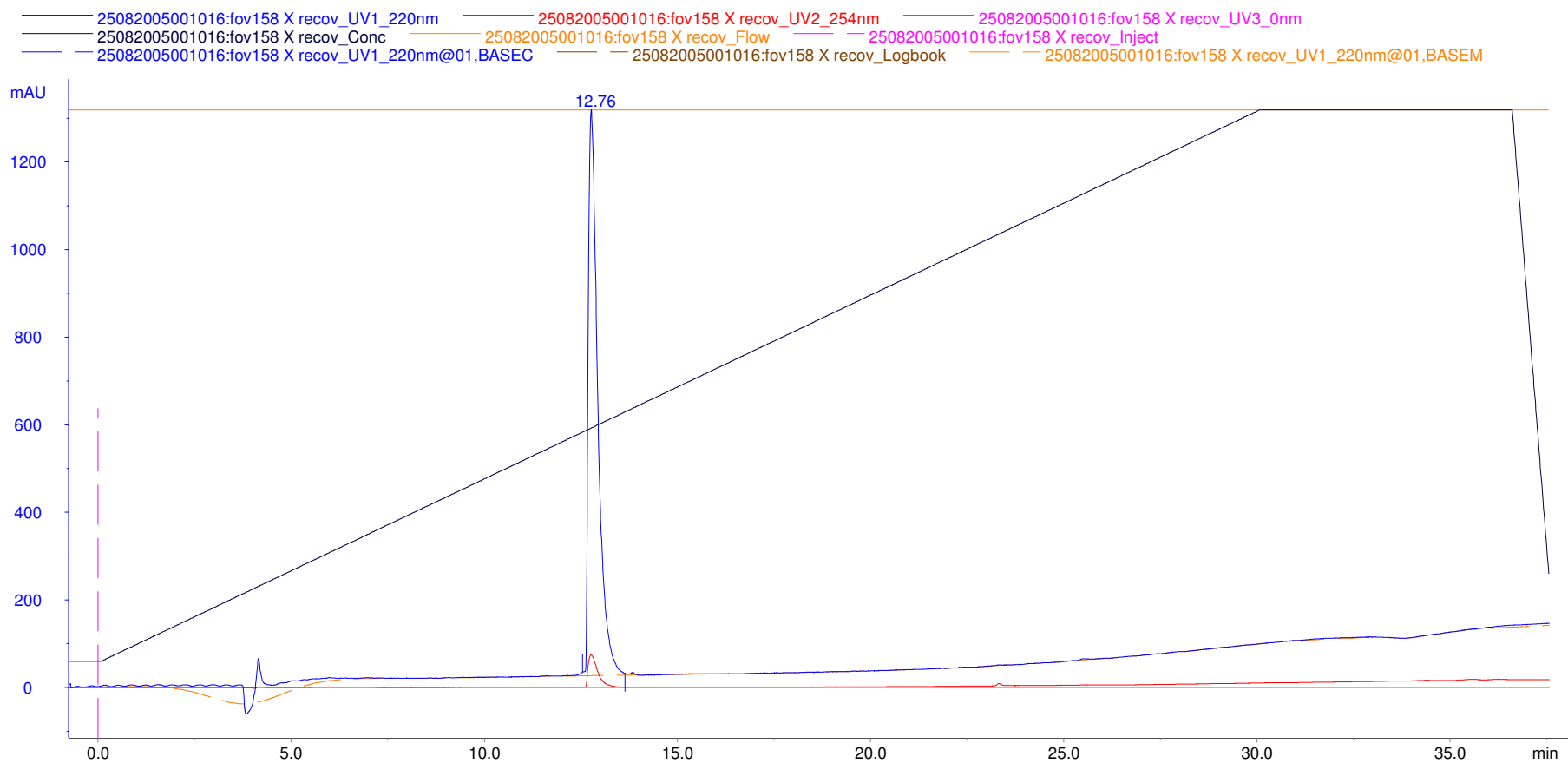


**Figure 7A:** HPLC chromatogram of compound 7.

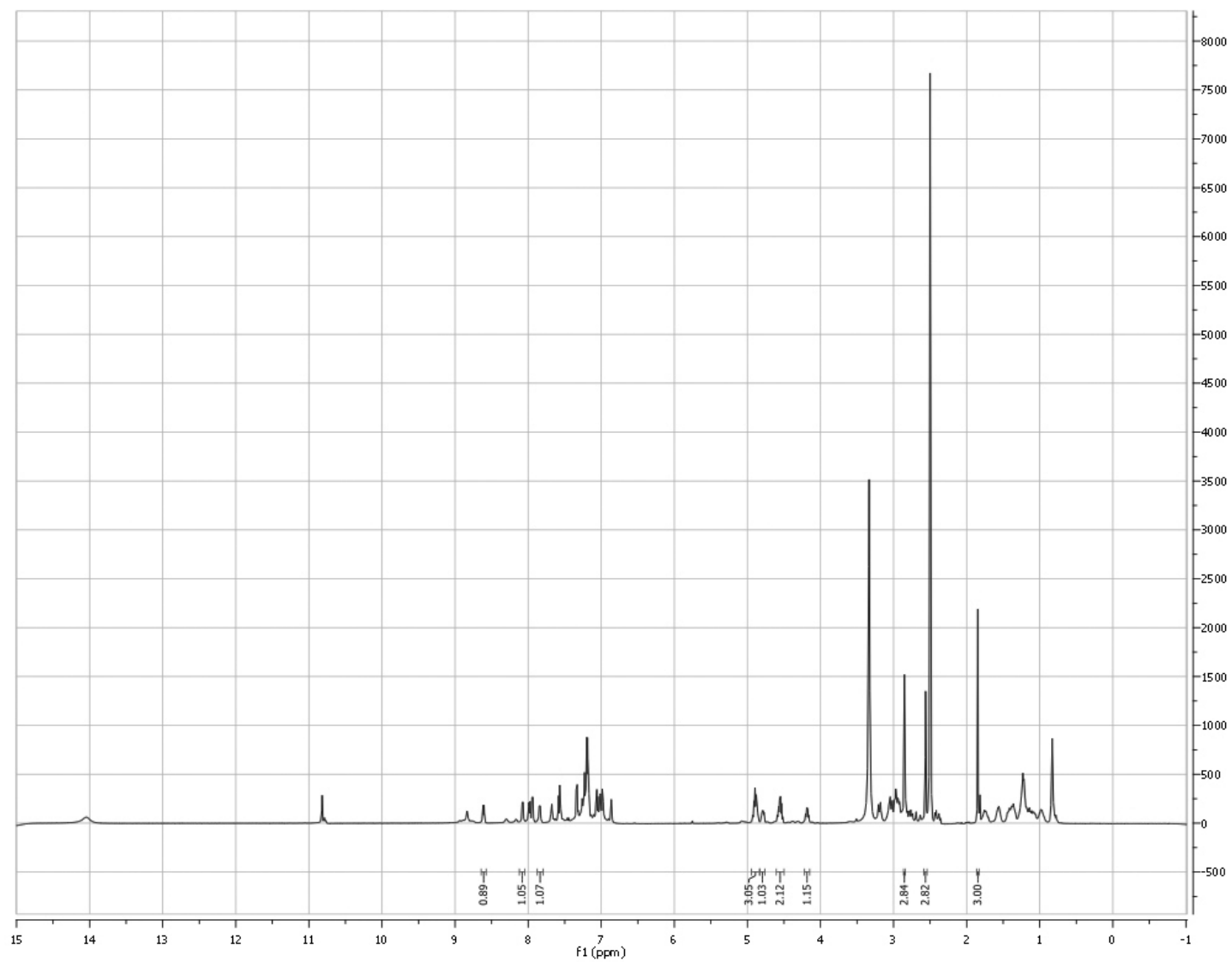


**Figure 7B:** <sup>1</sup>H-NMR spectrum of compound 7.

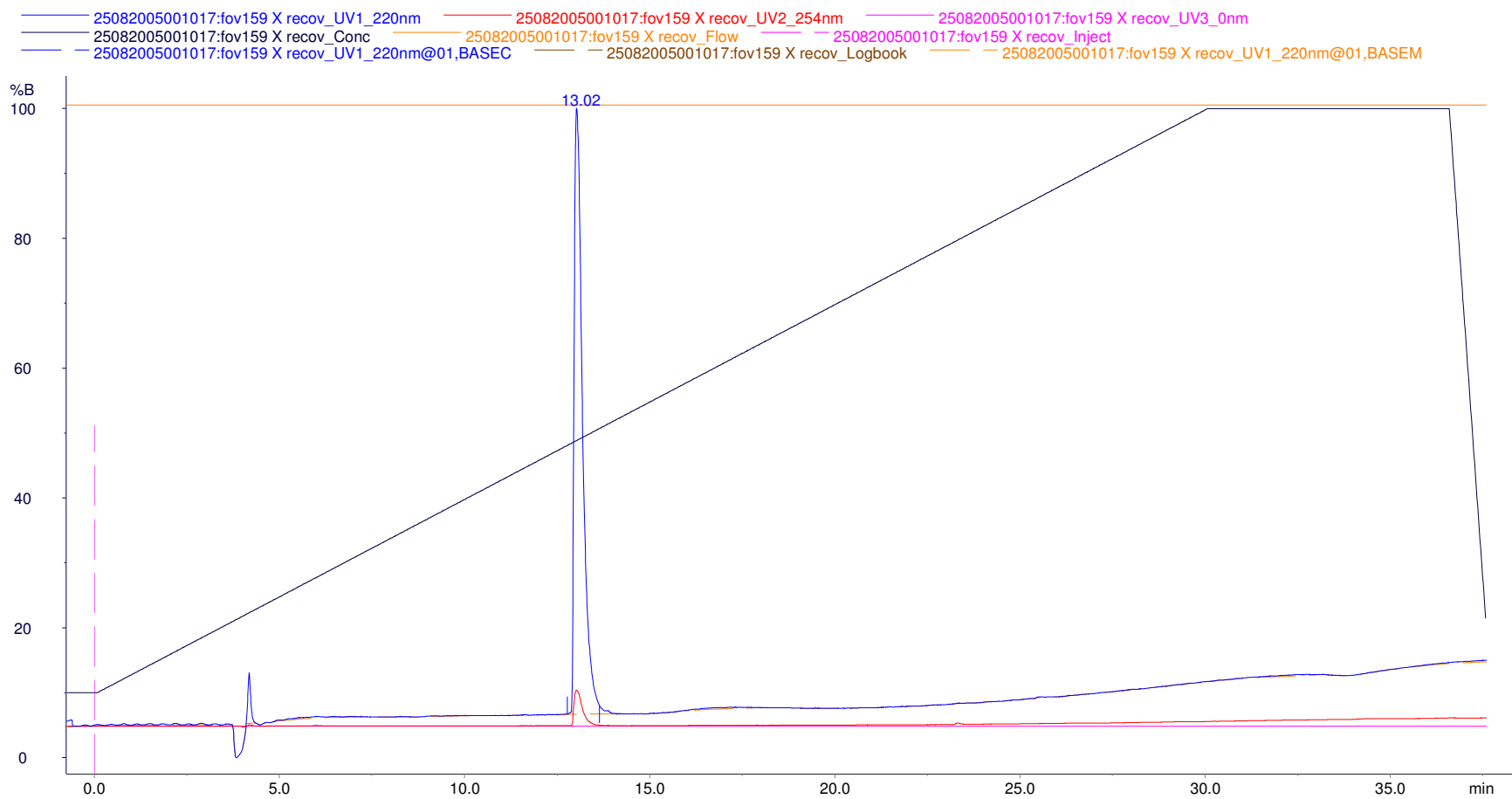




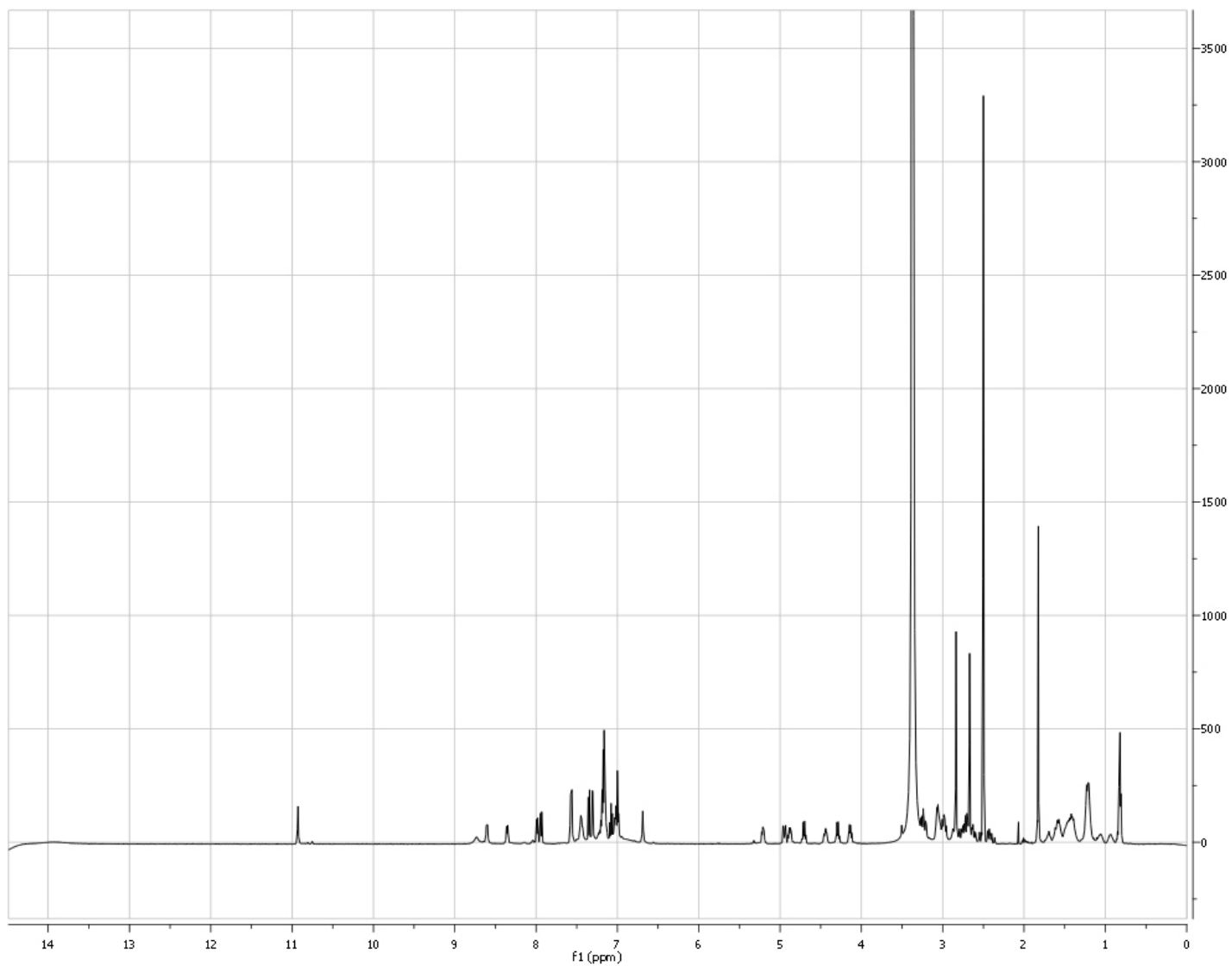
**Figure 8A:** HPLC chromatogram of compound 8.



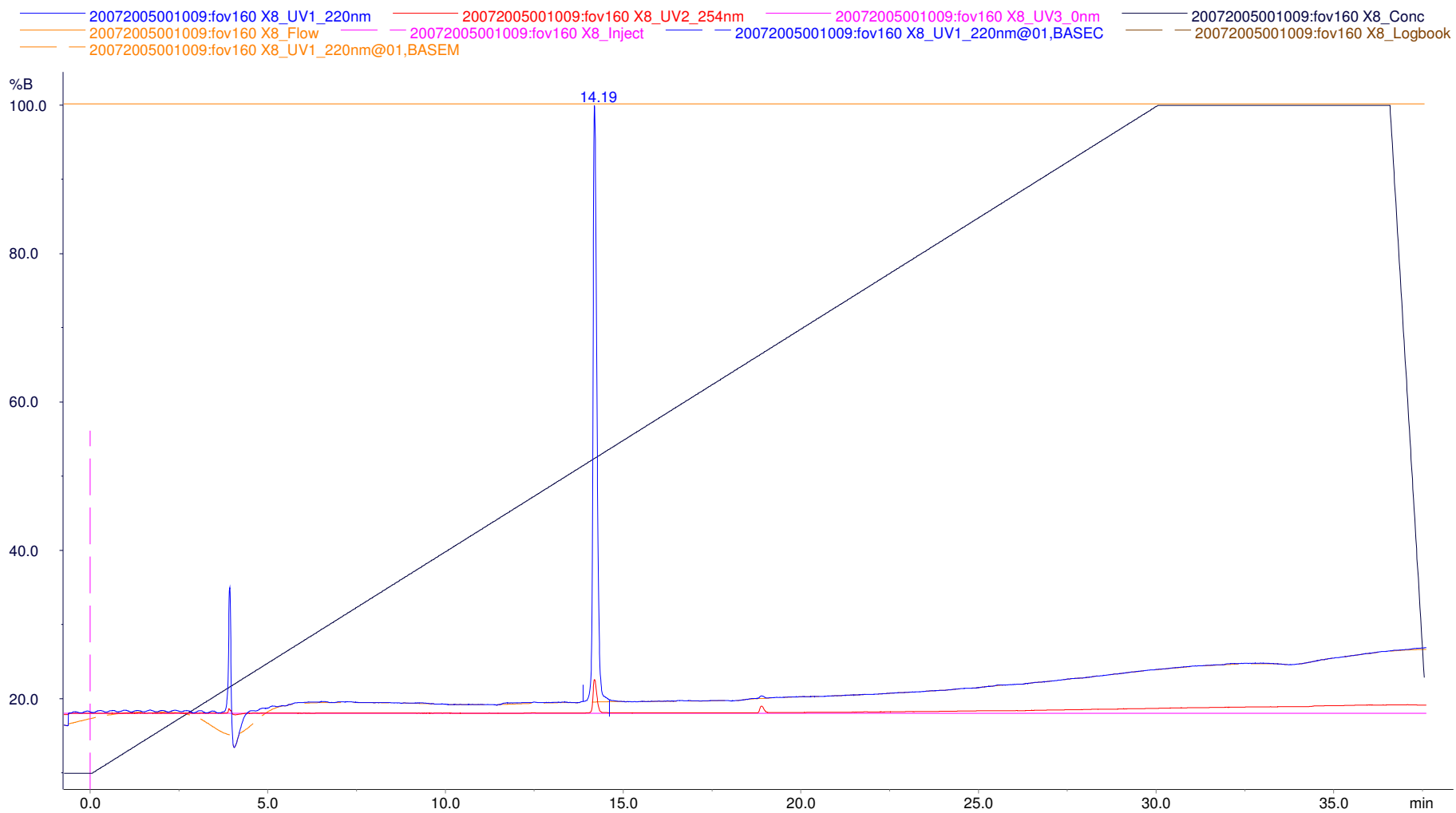
**Figure 8B:** <sup>1</sup>H-NMR spectrum of compound 8.



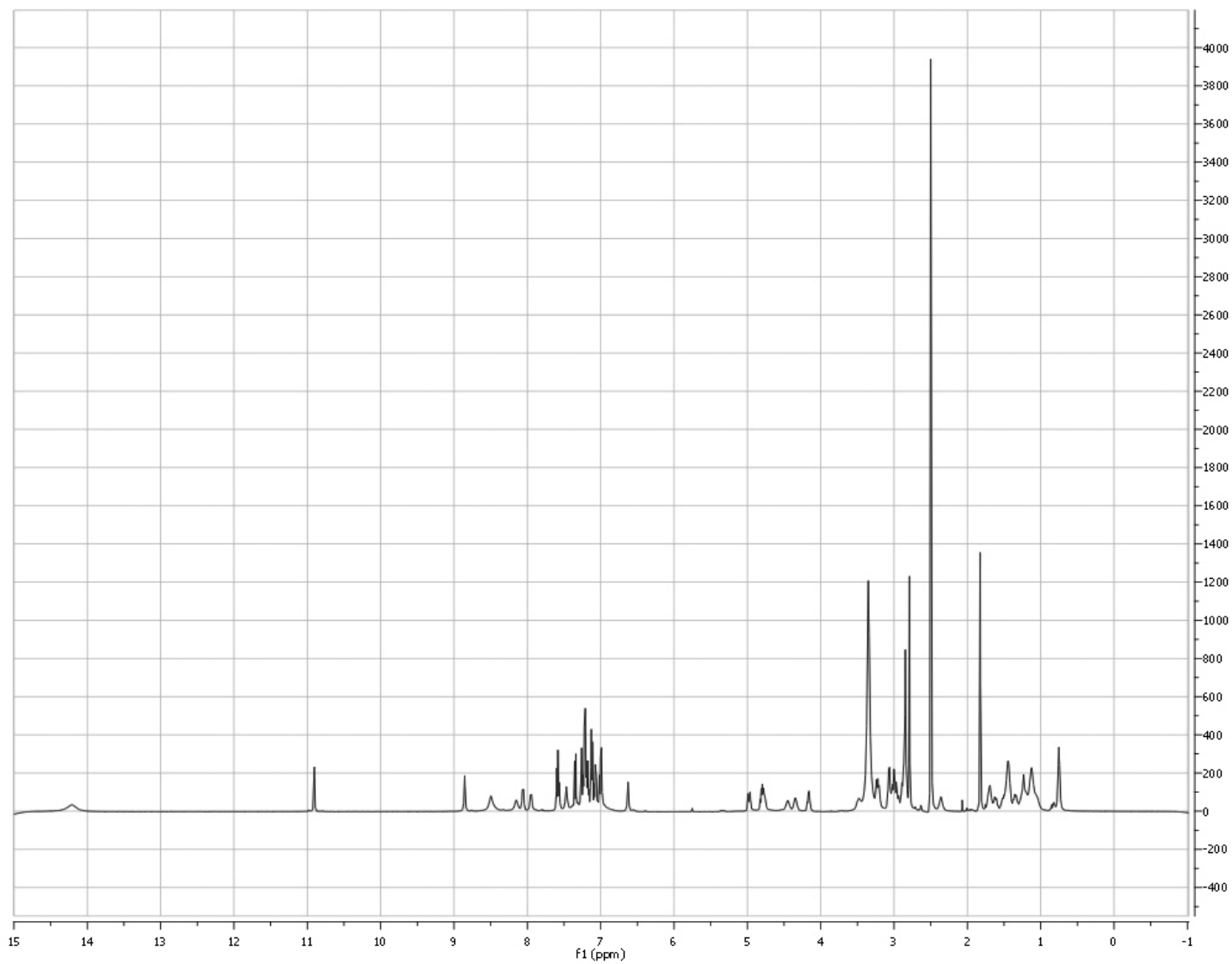
**Figure 9A:** HPLC chromatogram of compound 9.



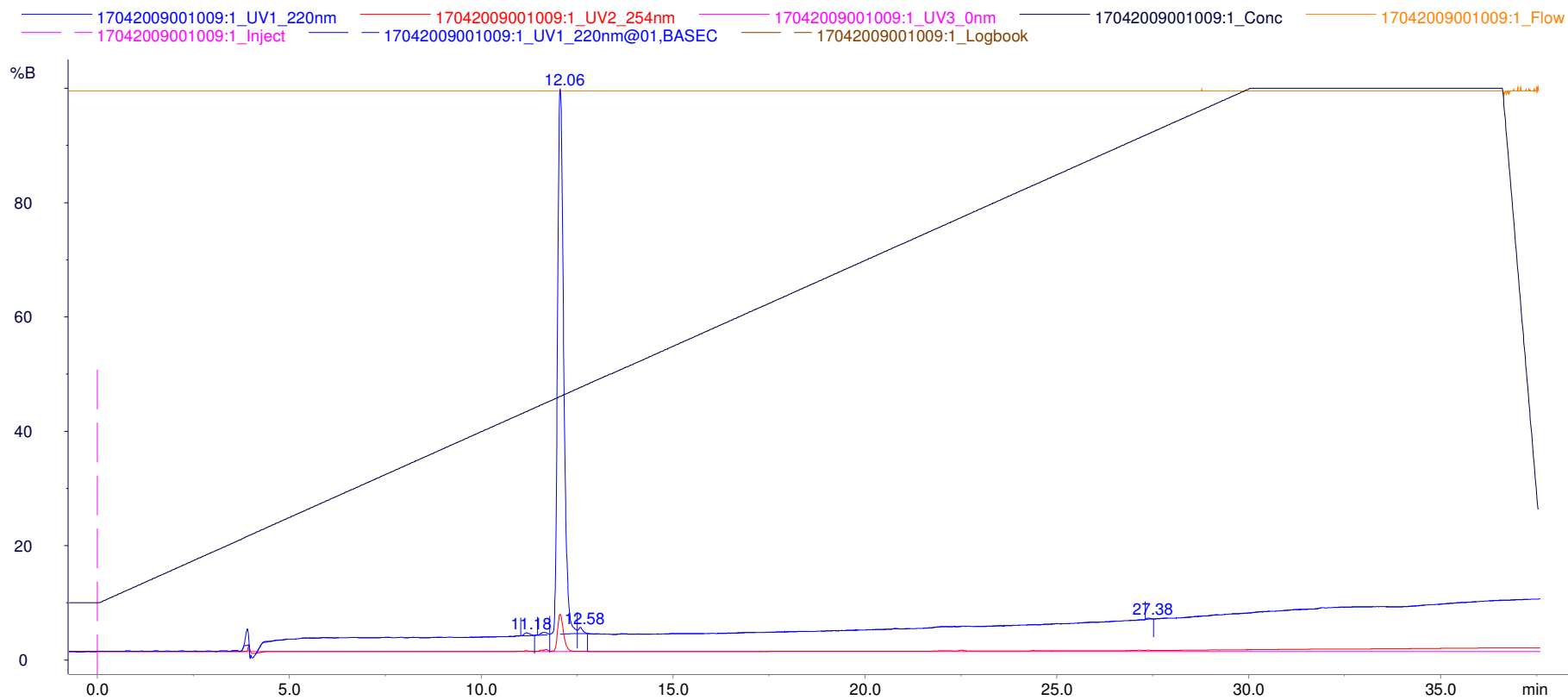
**Figure 9B:**  $^1\text{H-NMR}$  spectrum of compound 9.



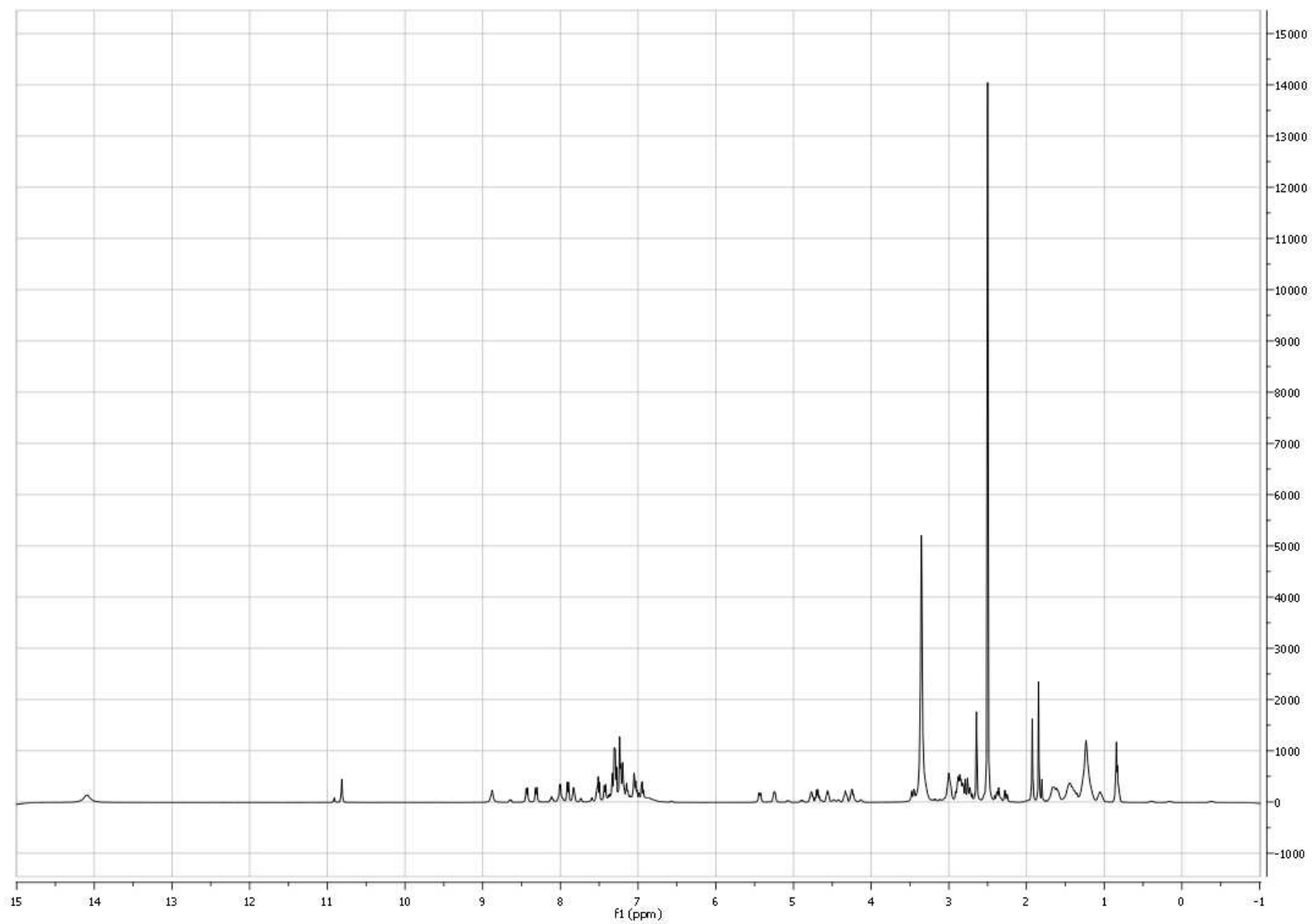
**Figure 10A:** HPLC chromatogram of compound 10.



**Figure 10B:**  $^1\text{H-NMR}$  spectrum of compound 10.

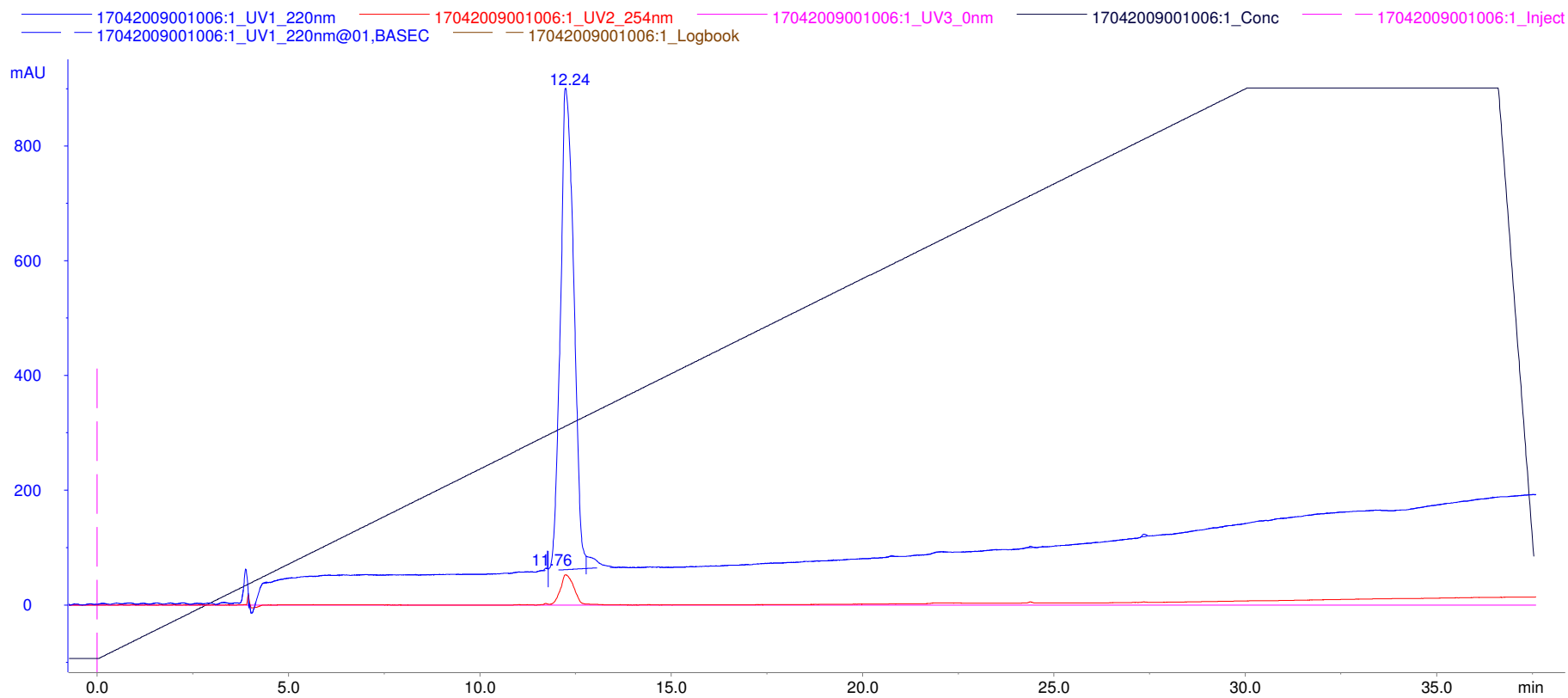


**Figure 11A:** HPLC chromatogram of compound 11.

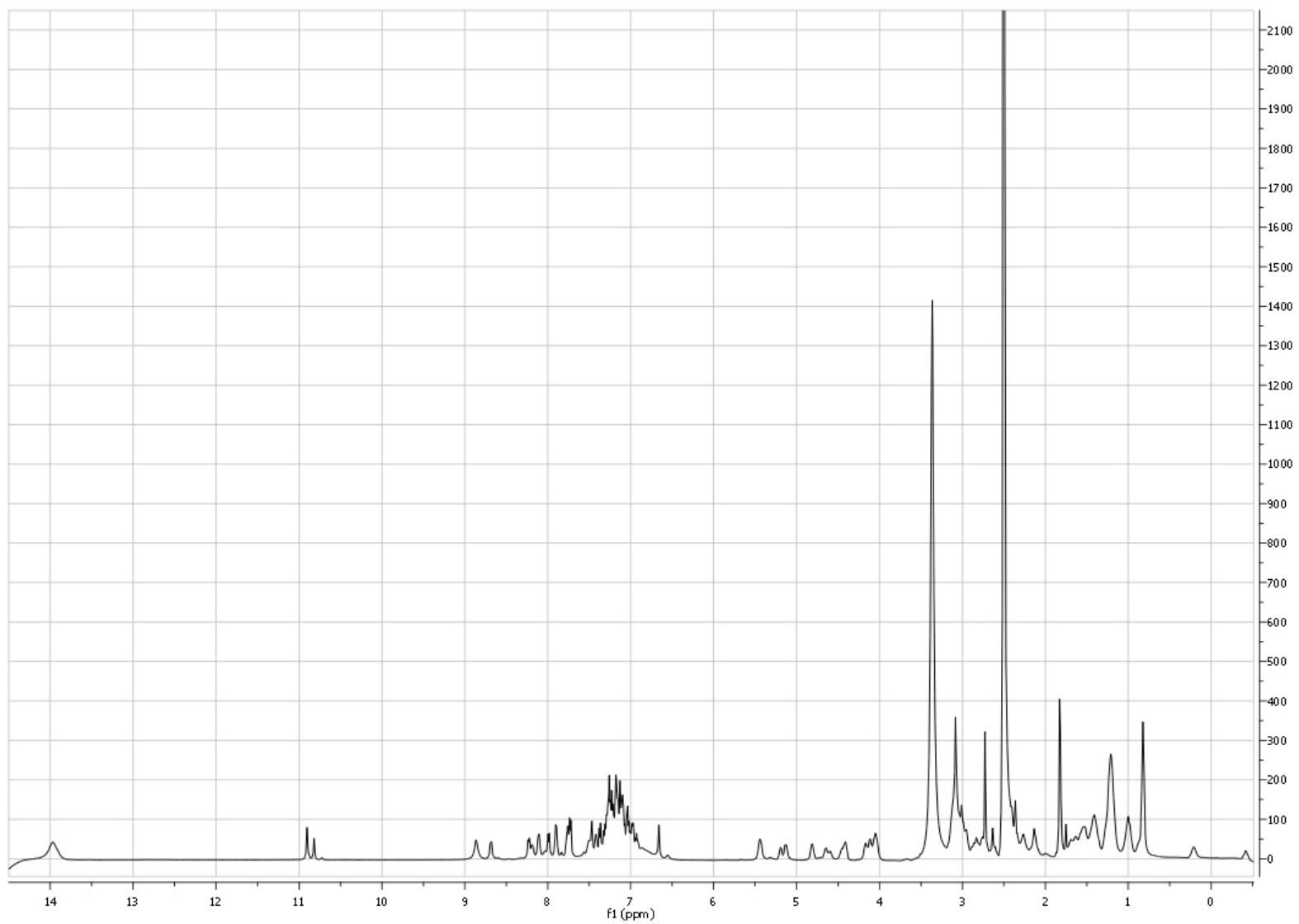


**Figure 11B:**  $^1\text{H-NMR}$  spectrum of compound 11.

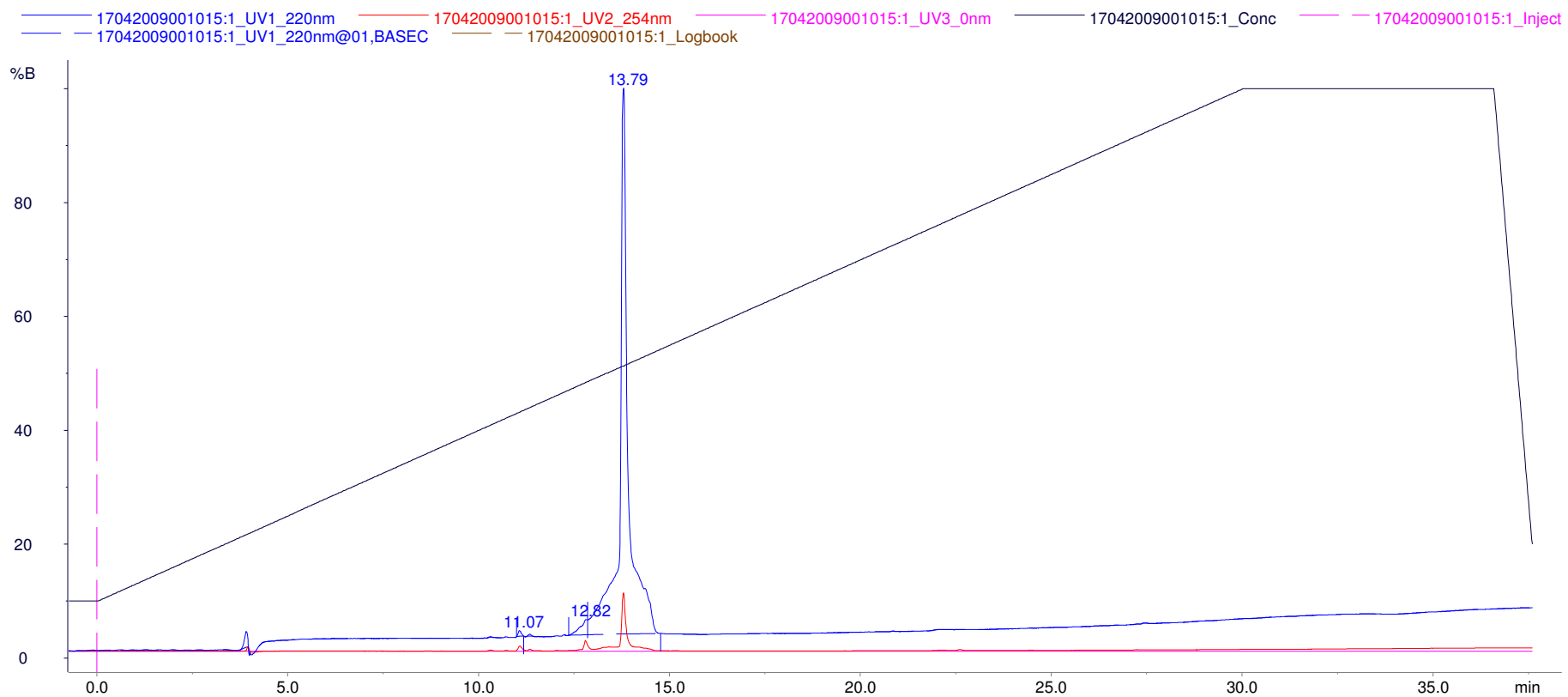




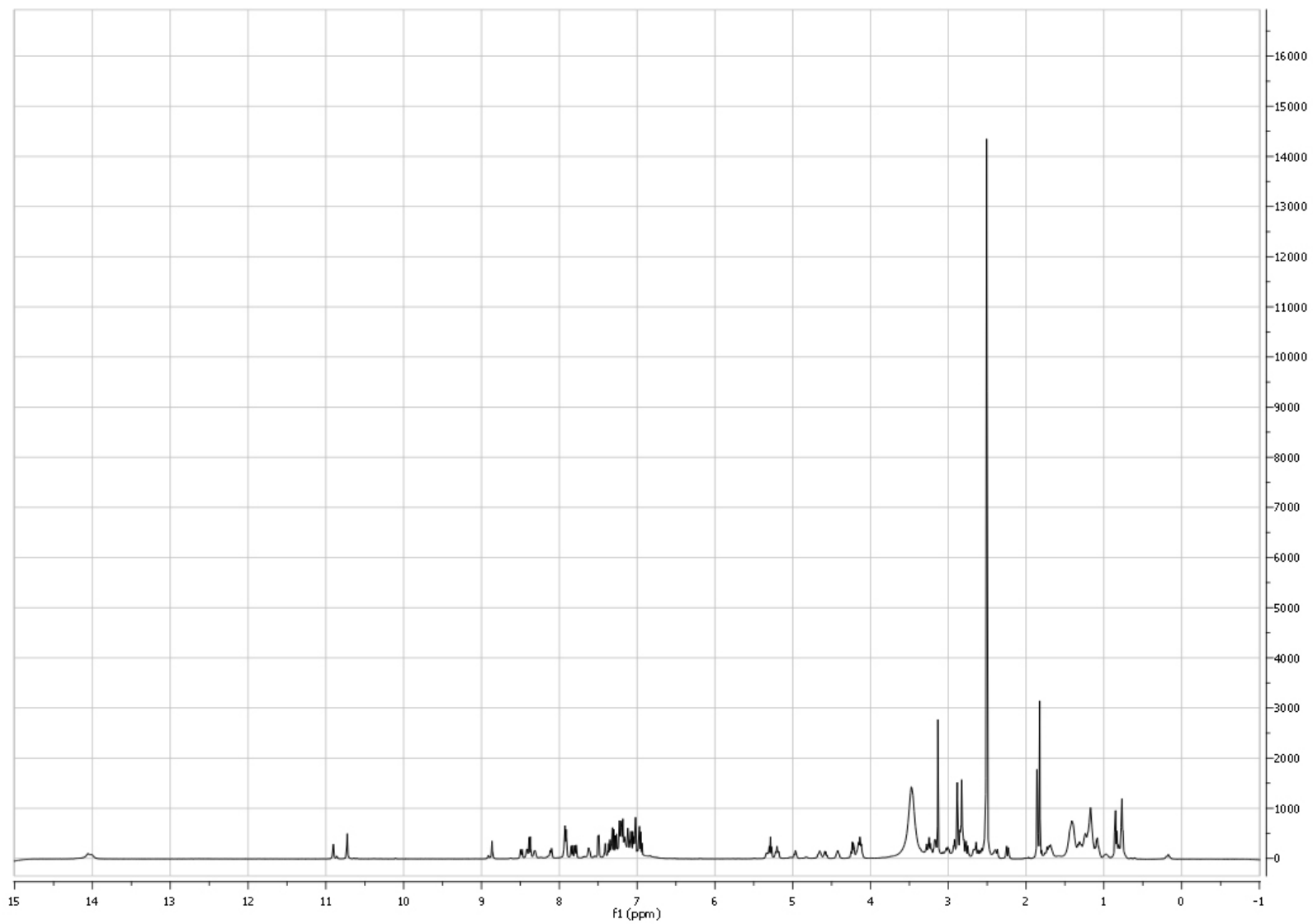
**Figure 12A:** HPLC chromatogram of compound 12.



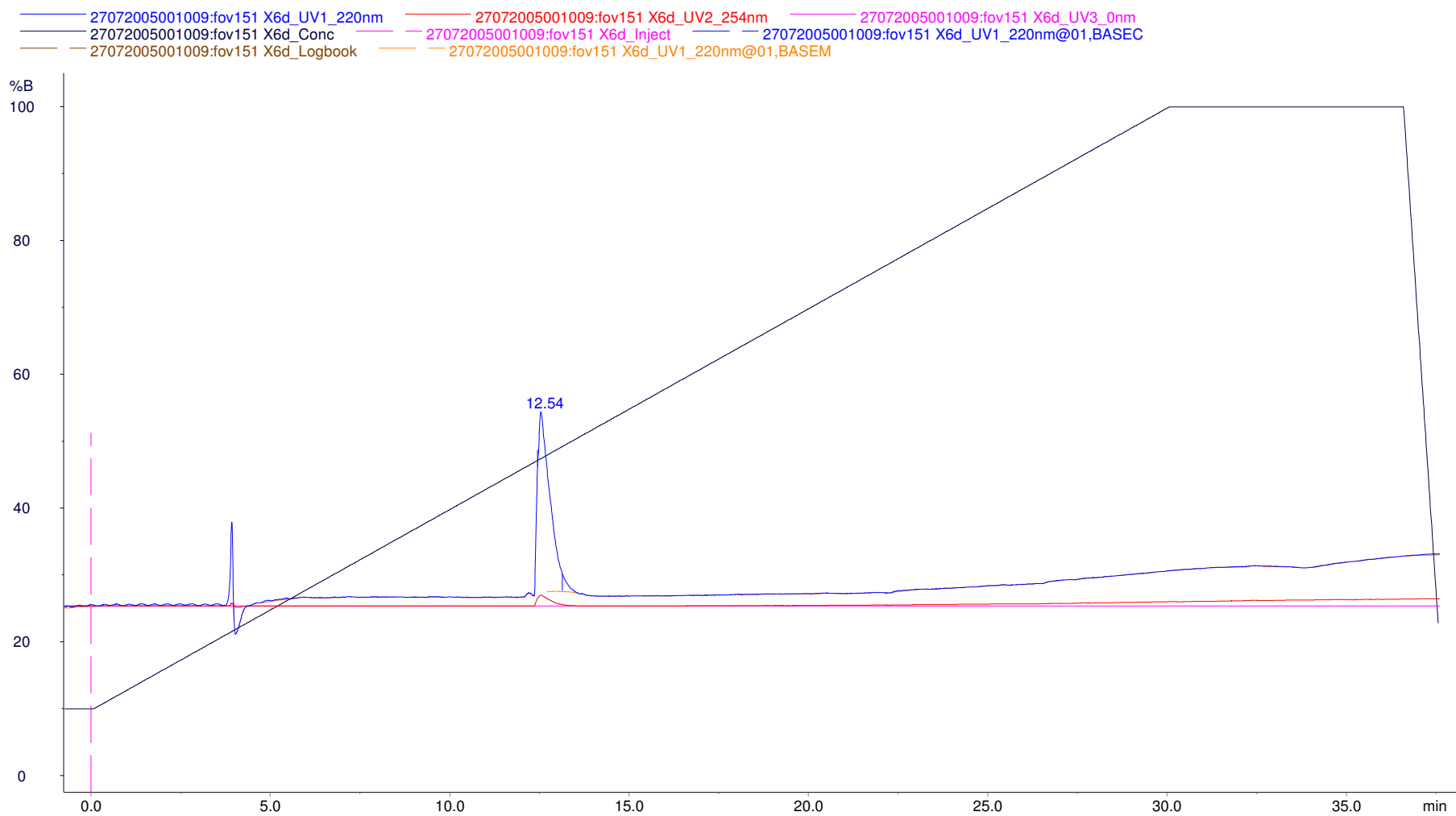
**Figure 12B:** <sup>1</sup>H-NMR spectrum of compound 12.



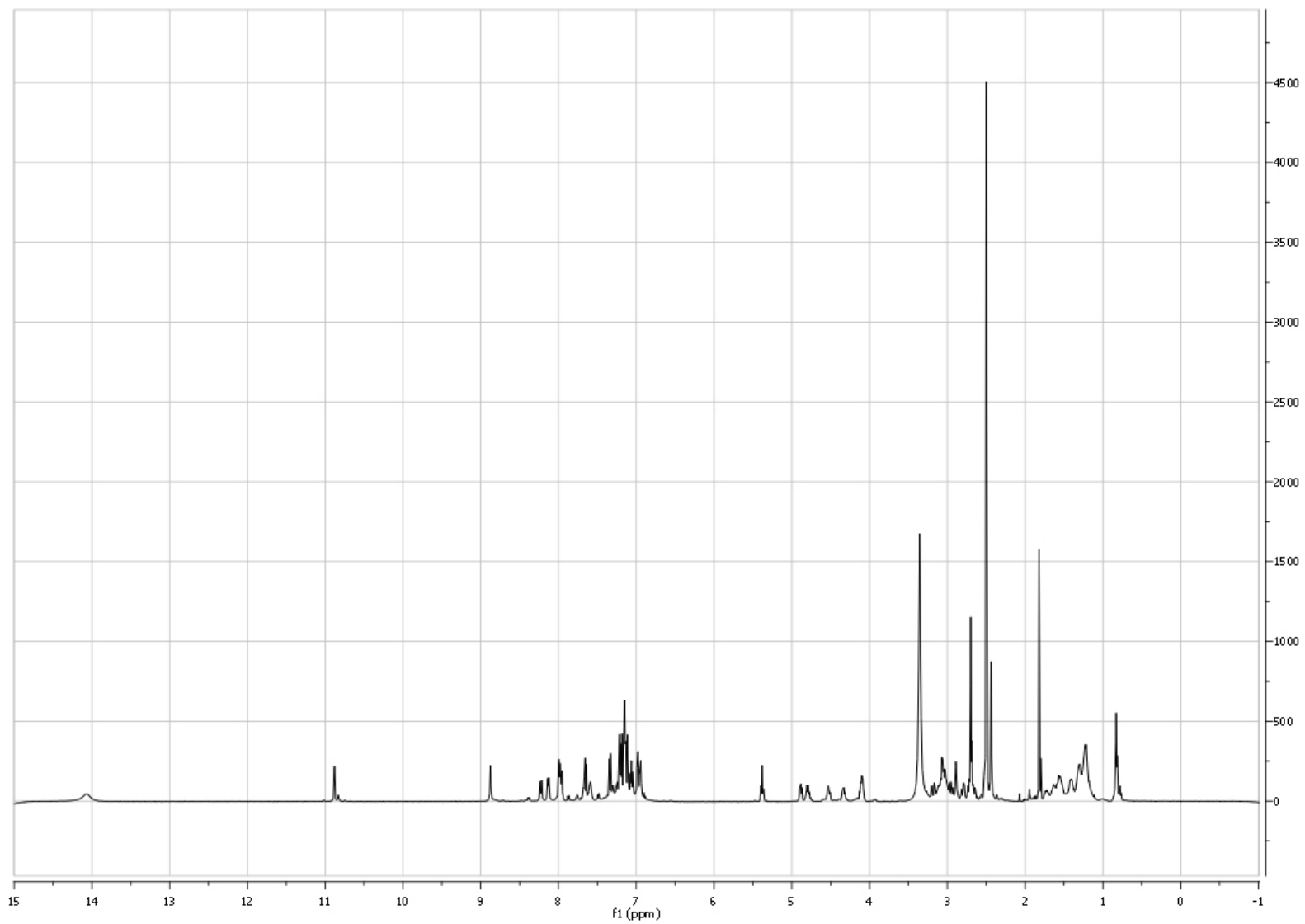
**Figure 13A:** HPLC chromatogram of compound 13.



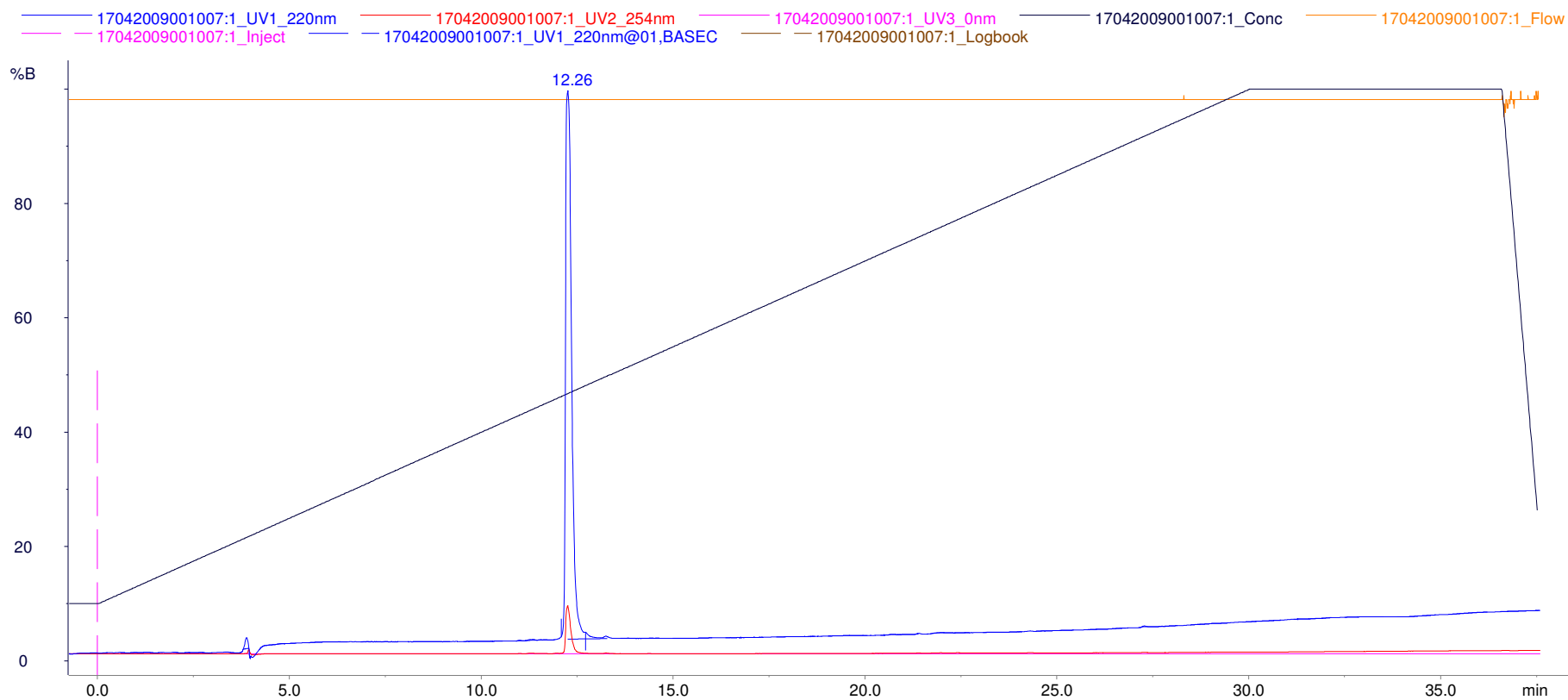
**Figure 13B:**  $^1\text{H-NMR}$  spectrum of compound 13.



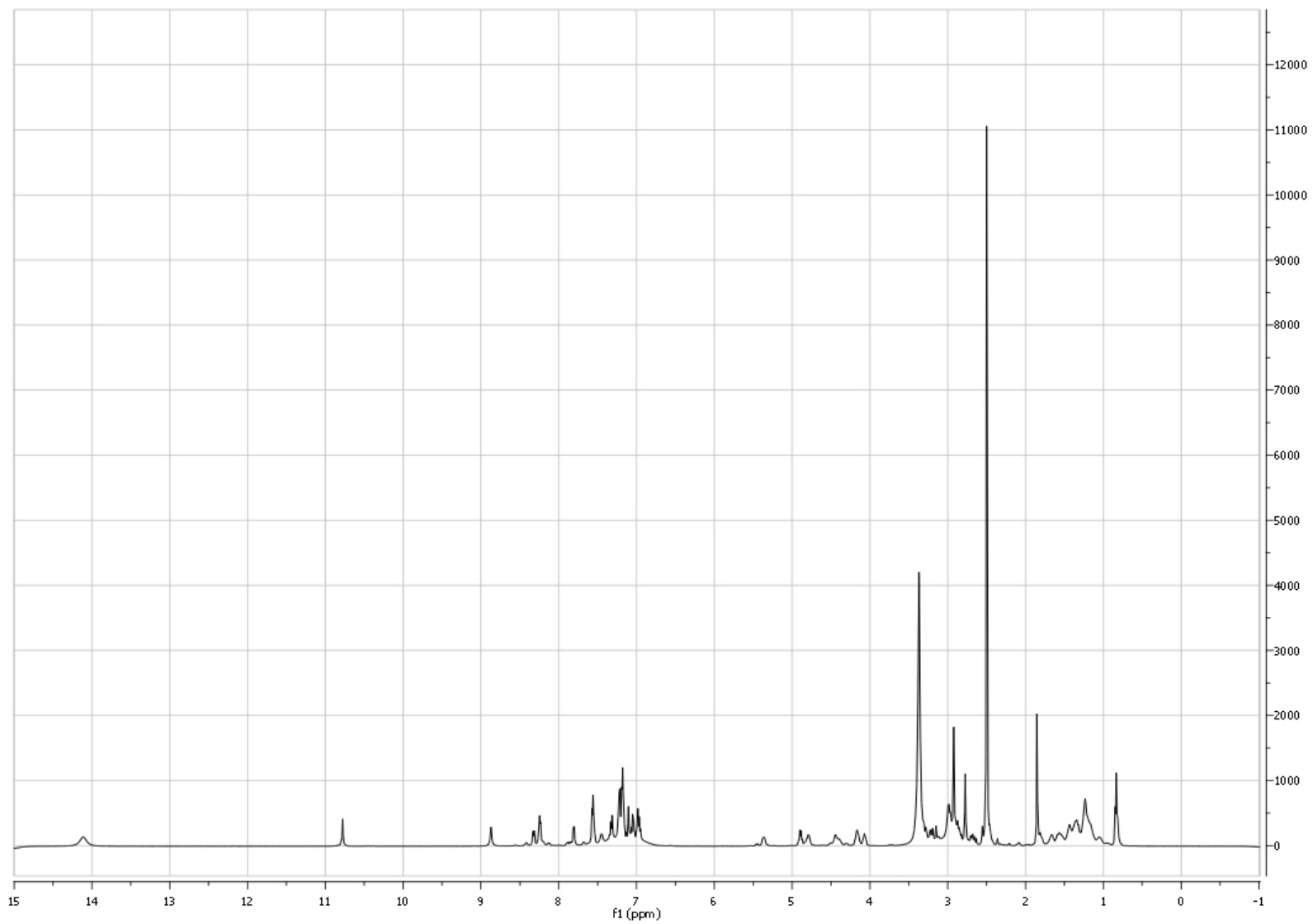
**Figure 14A:** HPLC chromatogram of compound 14.



**Figure 14B:**  $^1\text{H-NMR}$  spectrum of compound 14.



**Figure 15A:** HPLC chromatogram of compound 15.



**Figure 15B:** <sup>1</sup>H-NMR spectrum of compound 15.



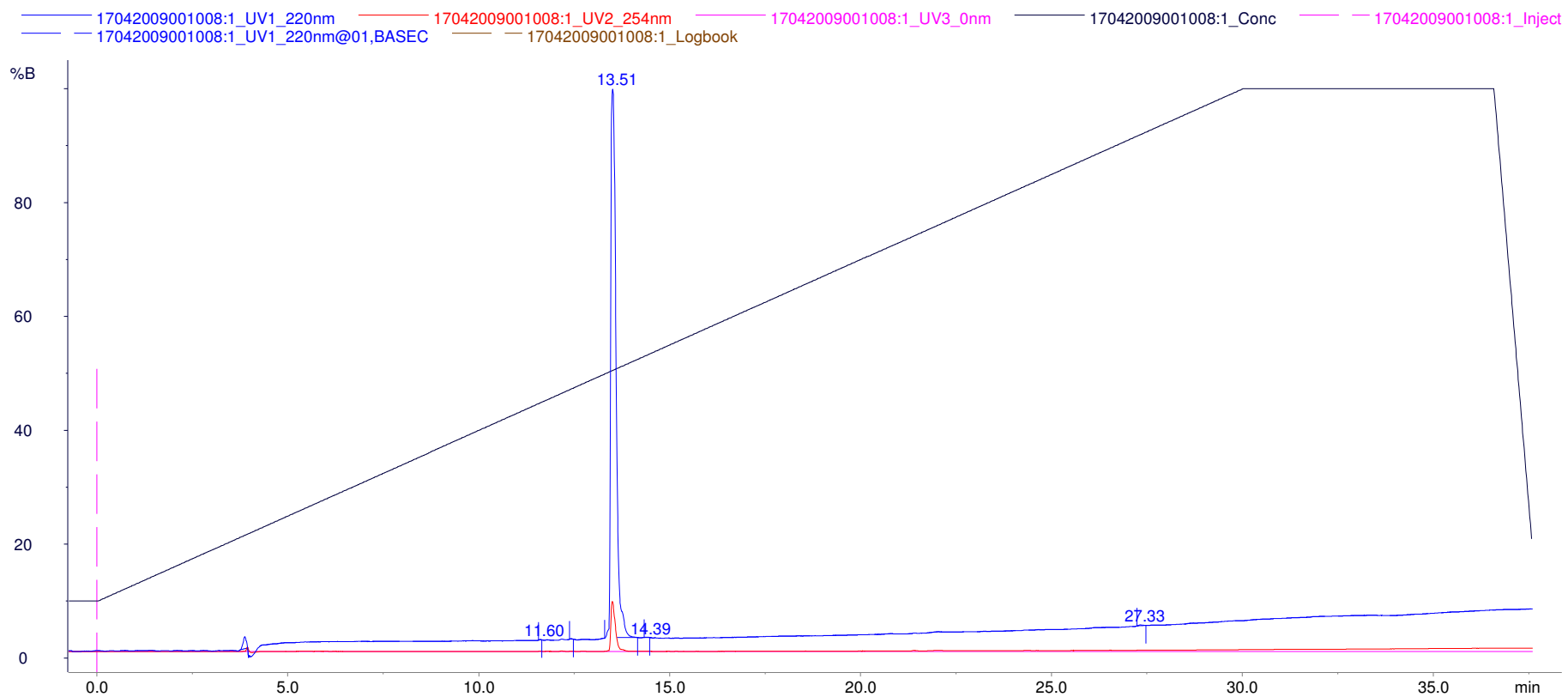
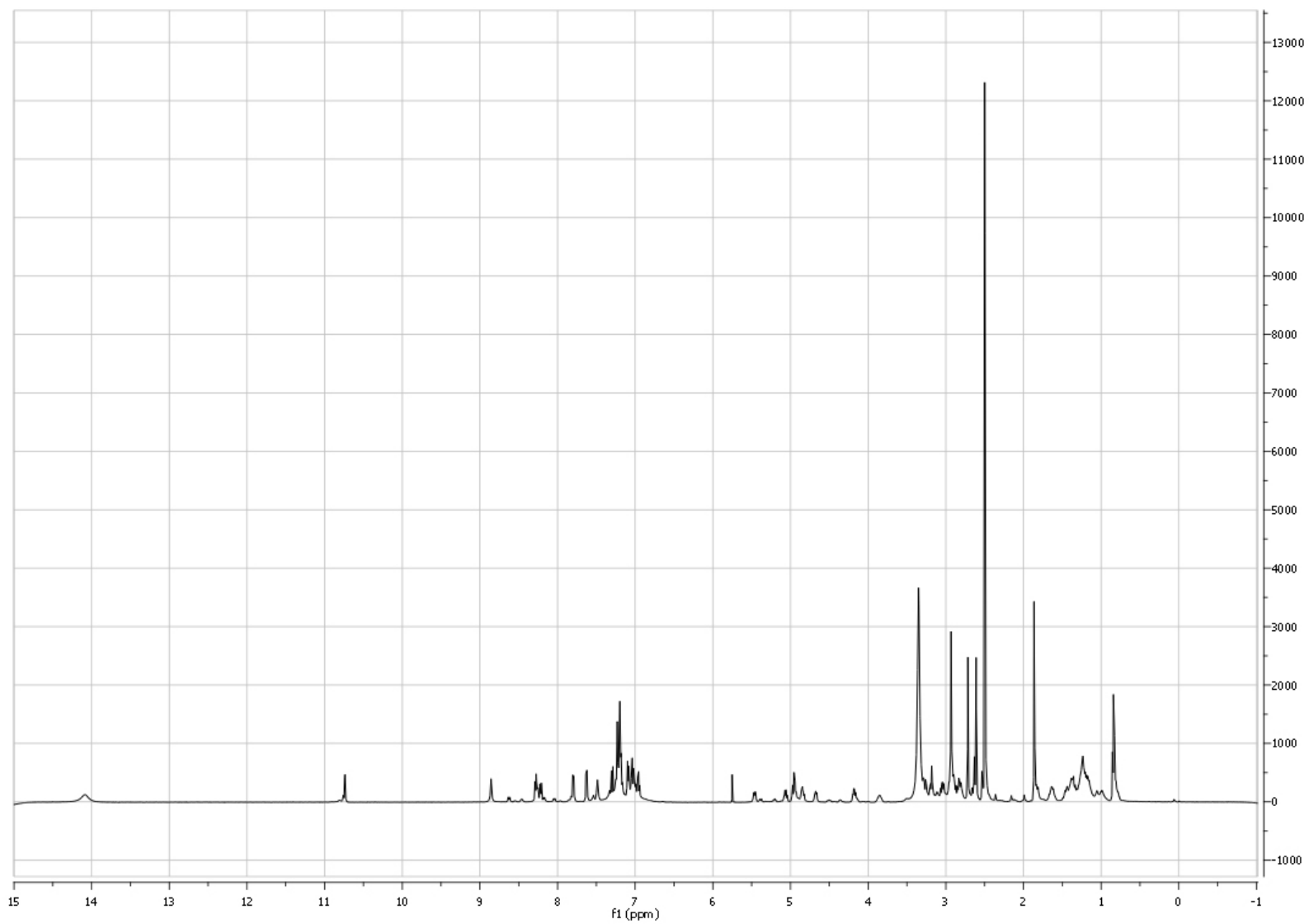
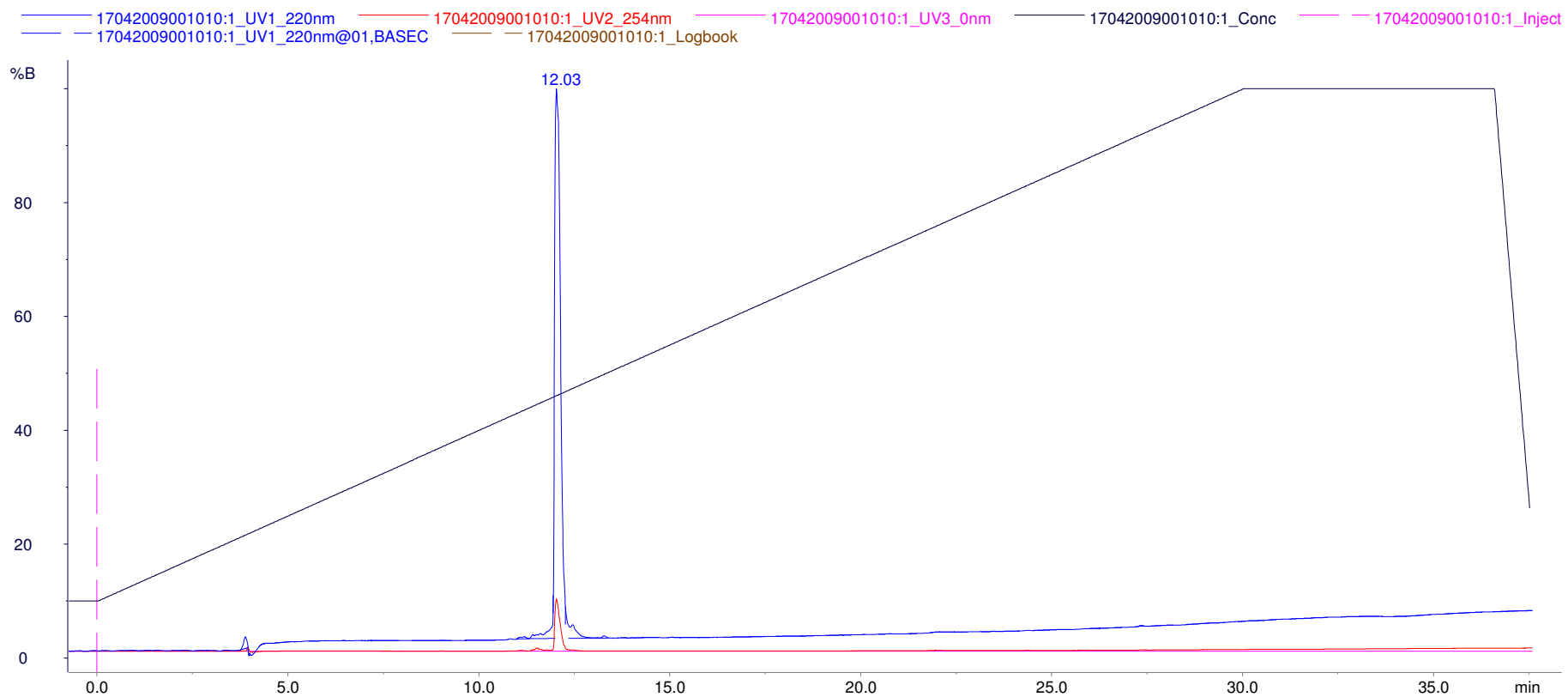


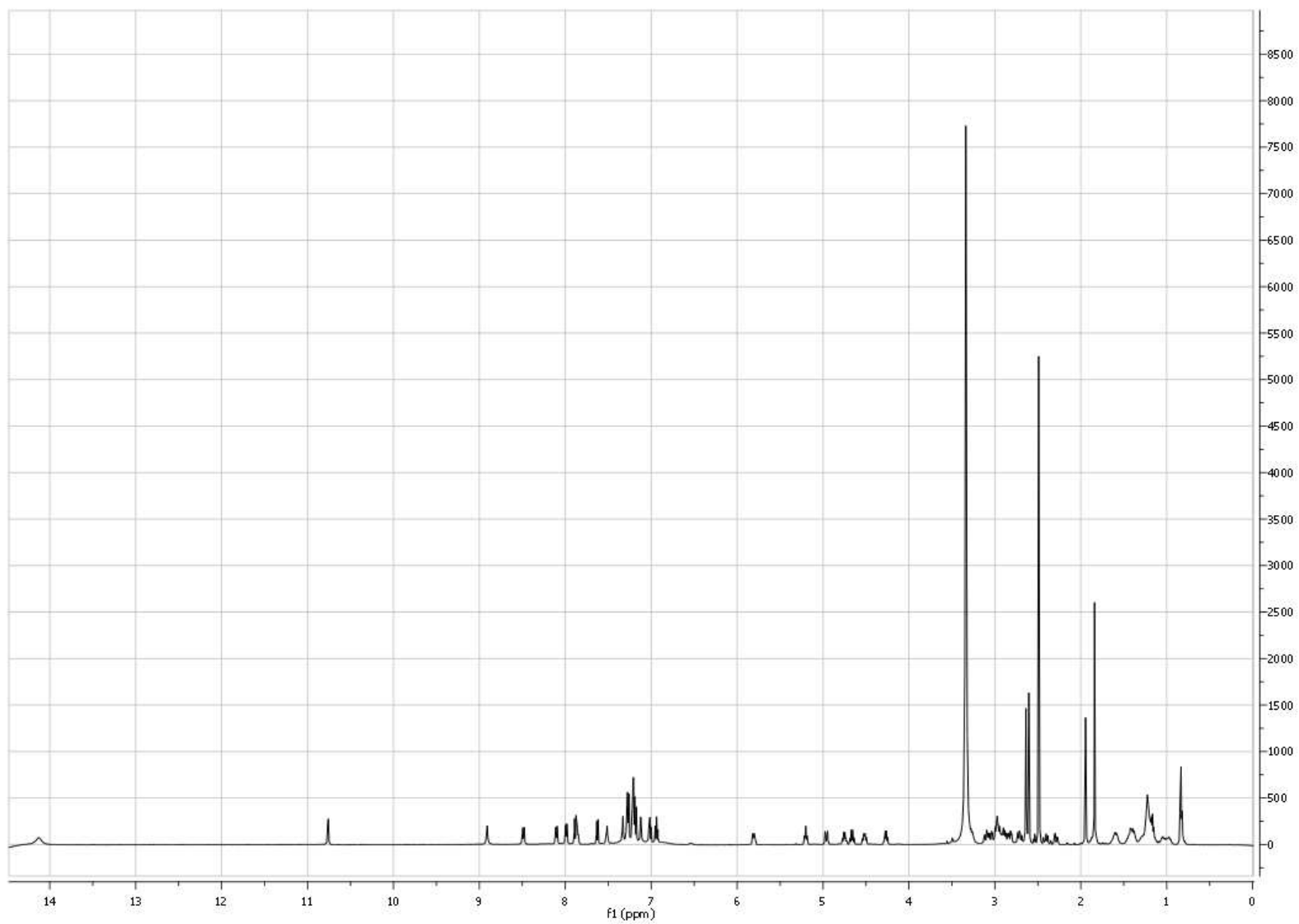
Figure 16A: HPLC chromatogram of compound 16.



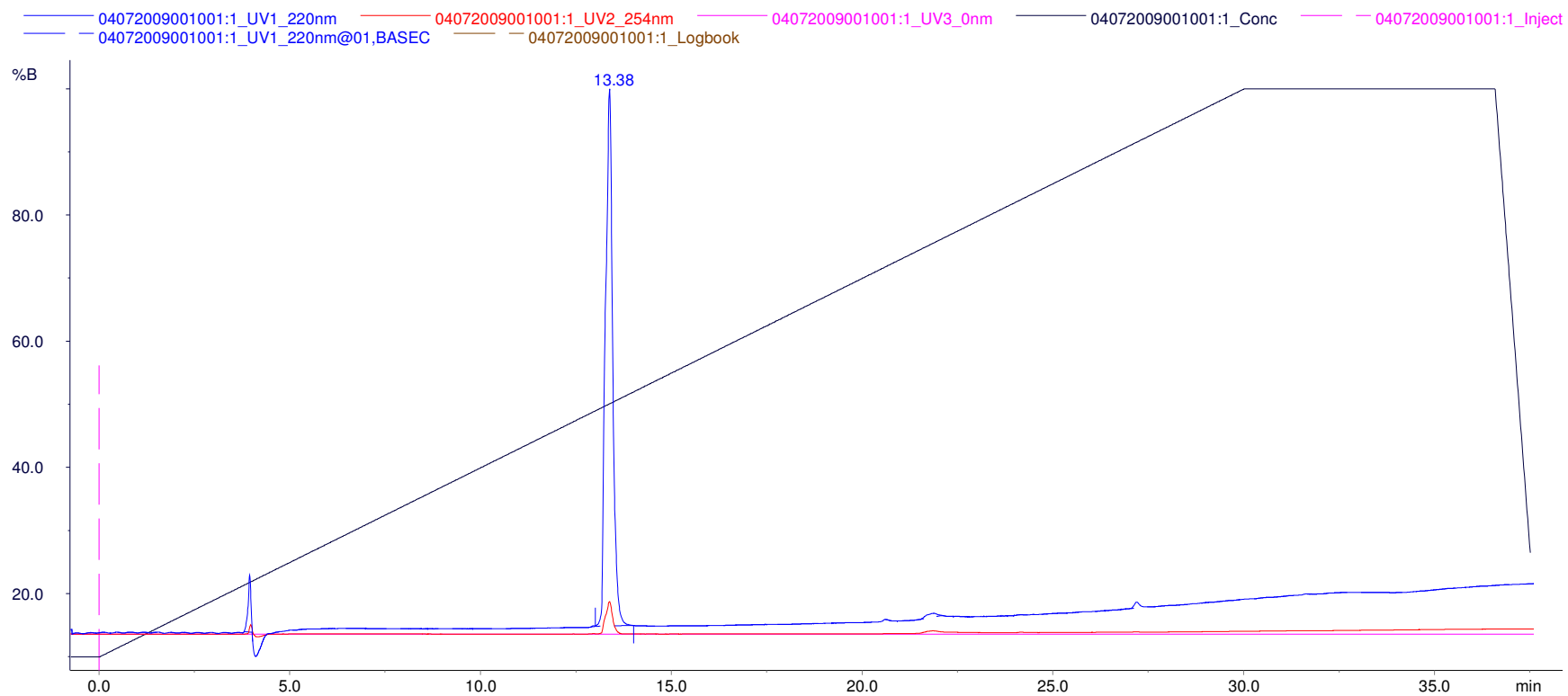
**Figure 16B:**  $^1\text{H}$ -NMR spectrum of compound 16.



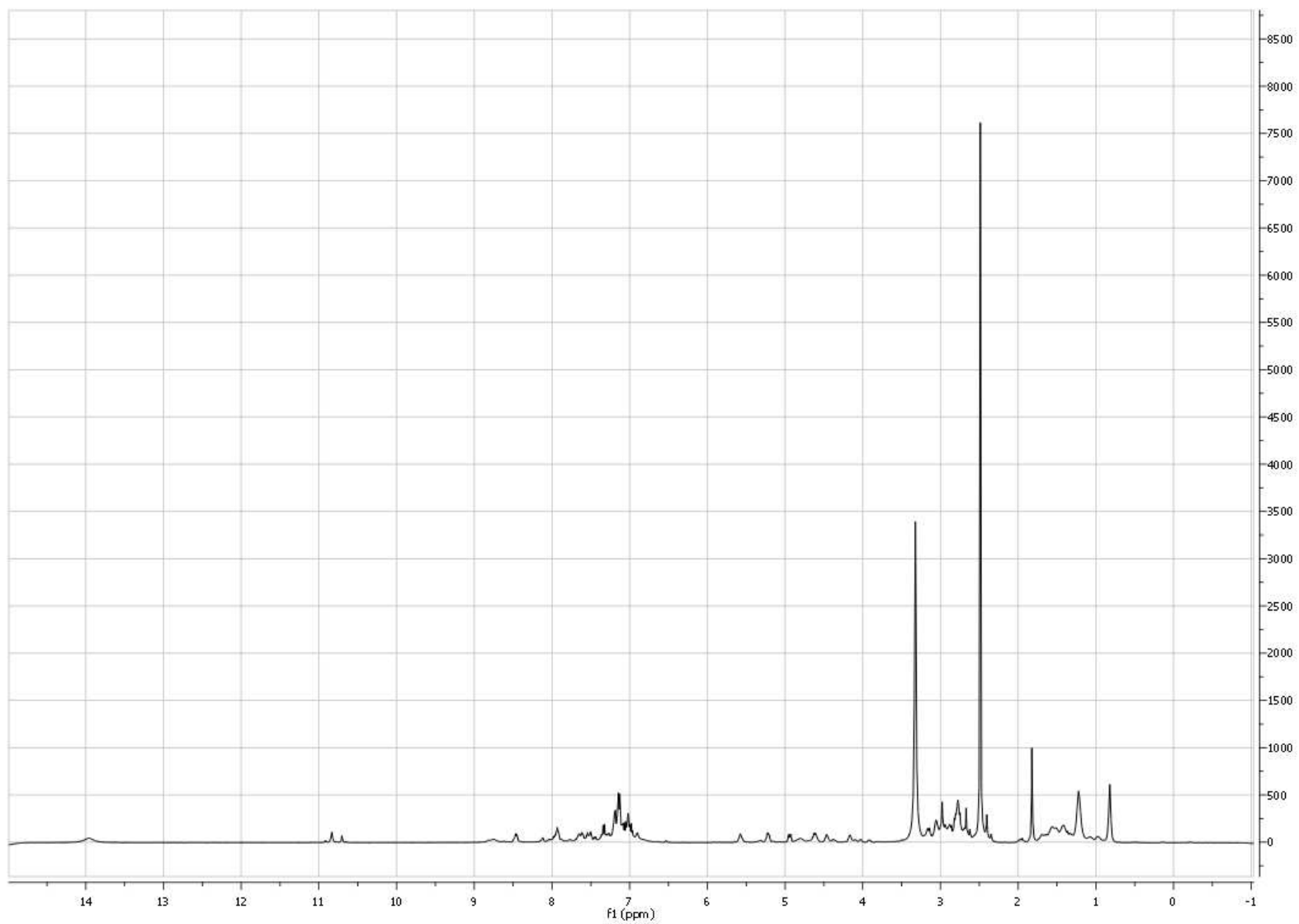
**Figure 17A:** HPLC chromatogram of compound 17.



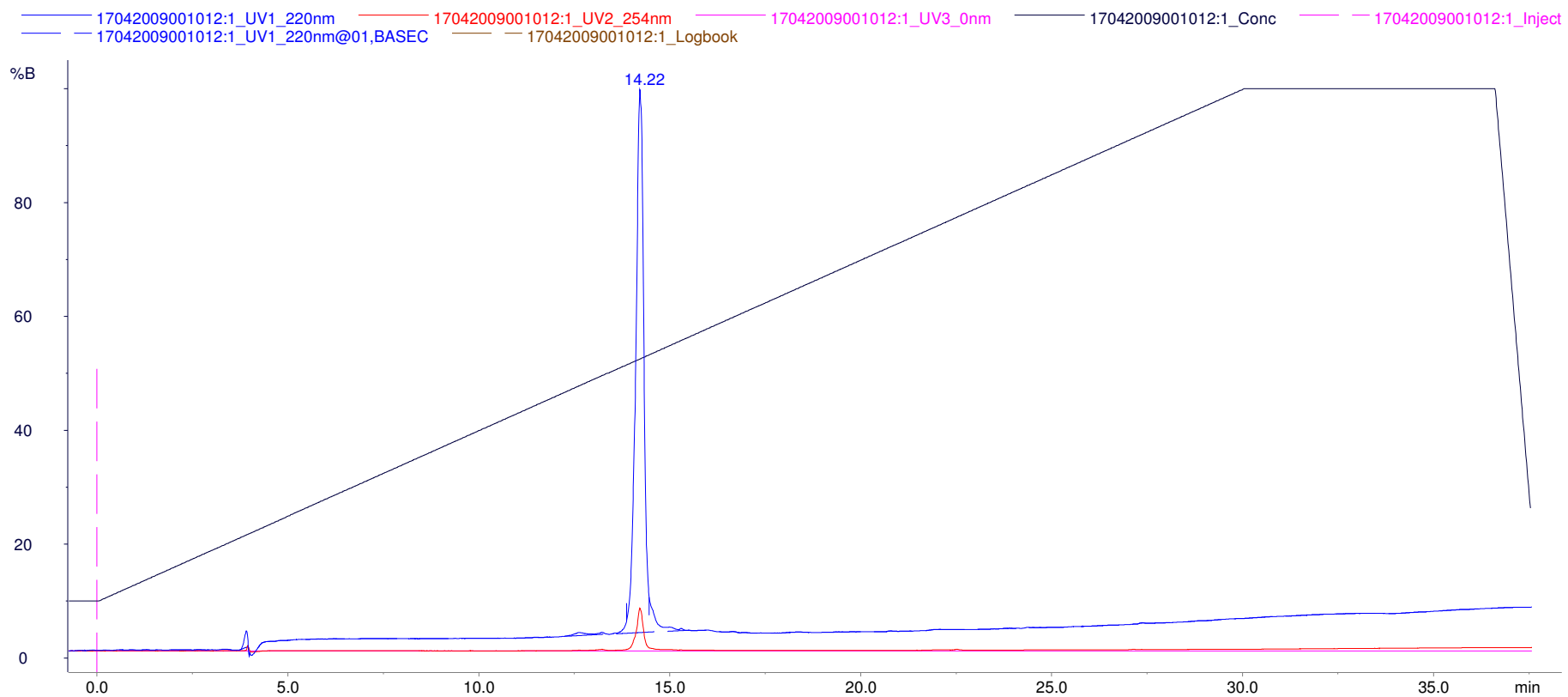
**Figure 17B:** <sup>1</sup>H-NMR spectrum of compound 17.



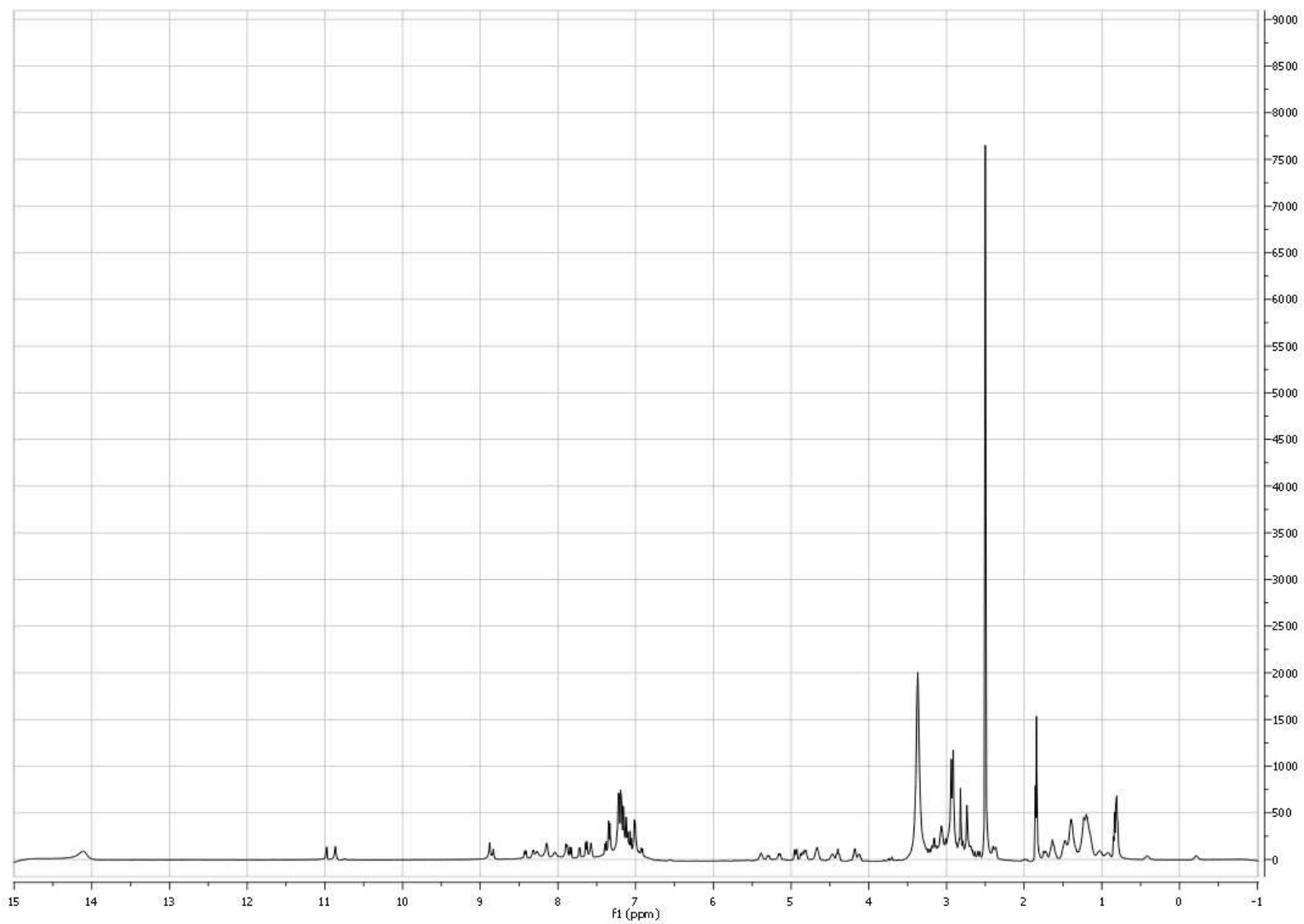
**Figure 18A:** HPLC chromatogram of compound 18.



**Figure 18B:** <sup>1</sup>H-NMR spectrum of compound 18.

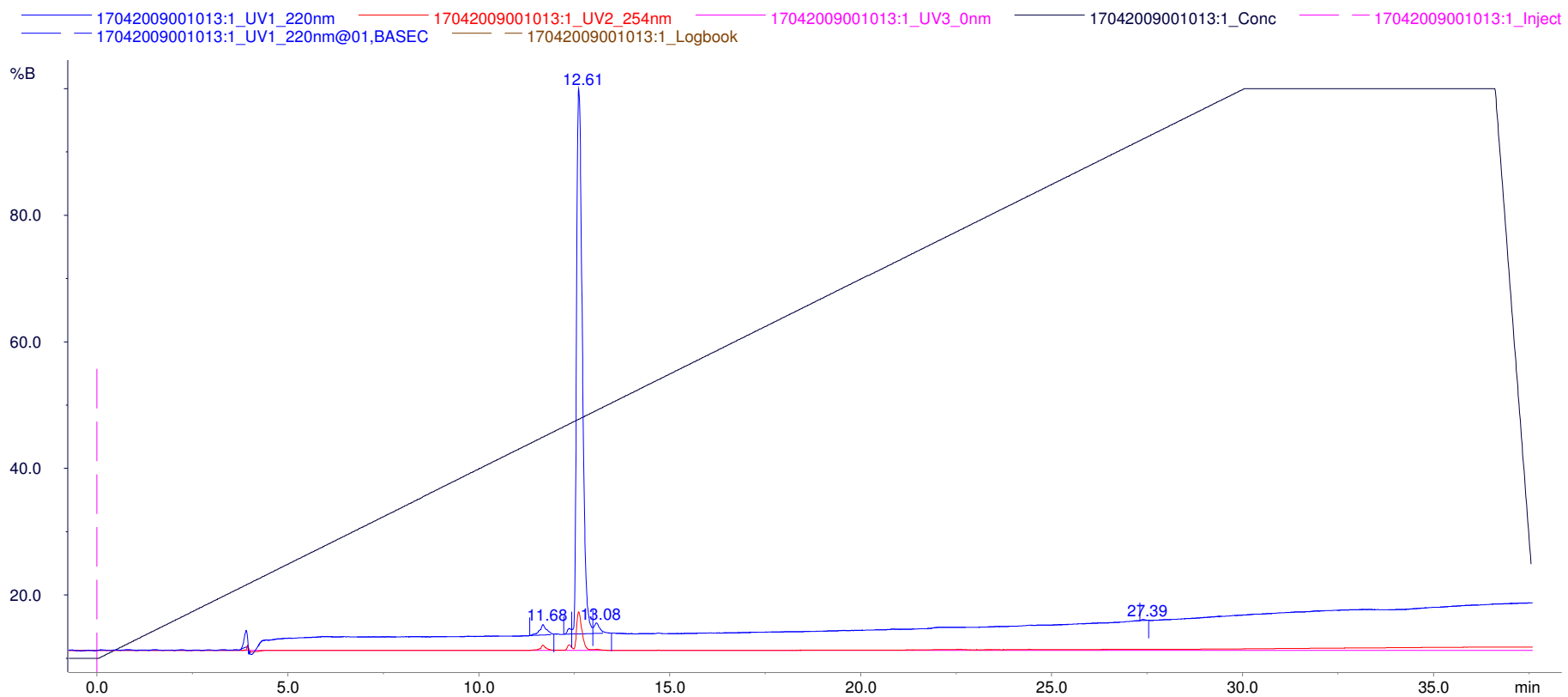


**Figure 19A:** HPLC chromatogram of compound 19.

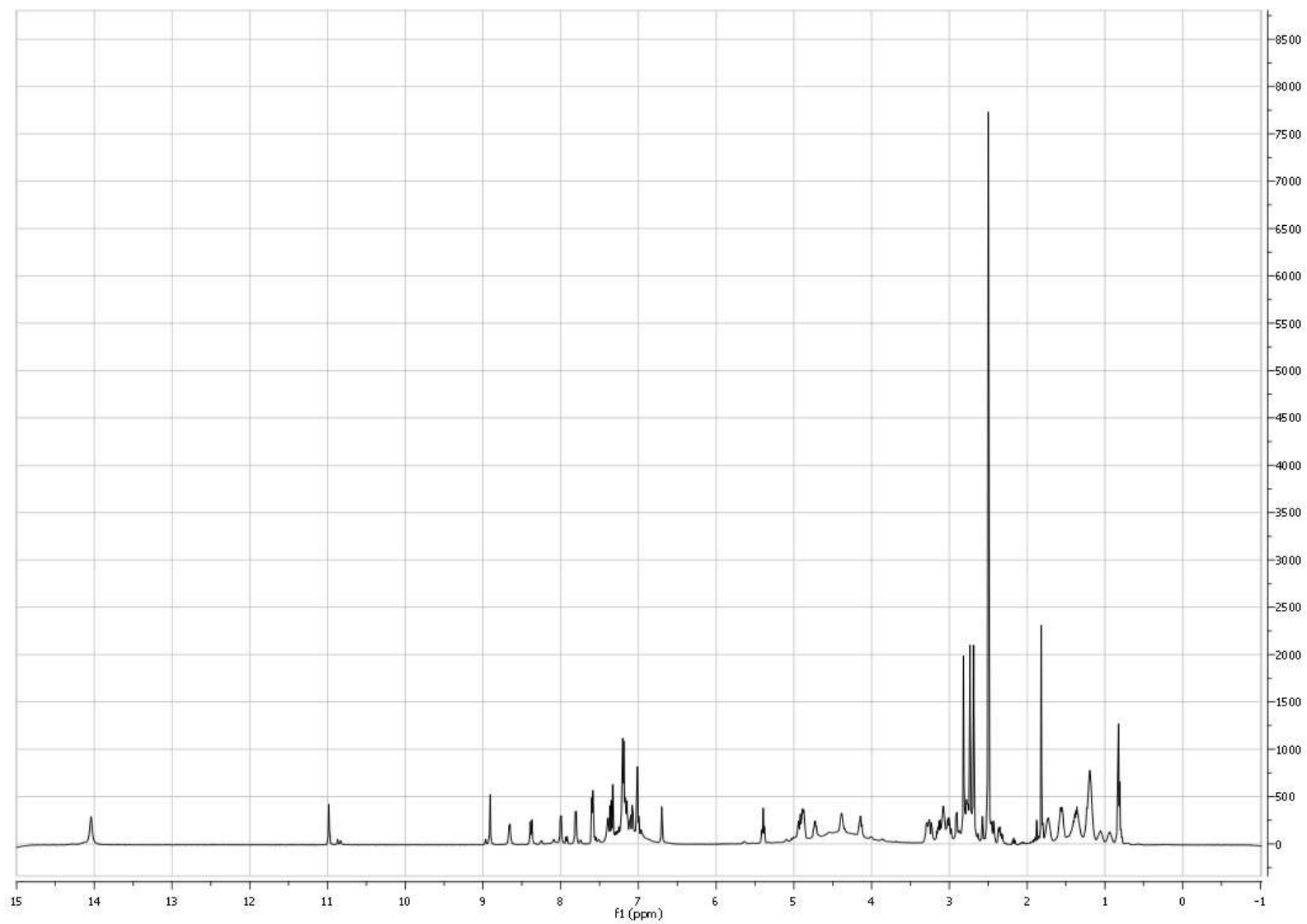


**Figure 19B:** <sup>1</sup>H-NMR spectrum of compound 19.

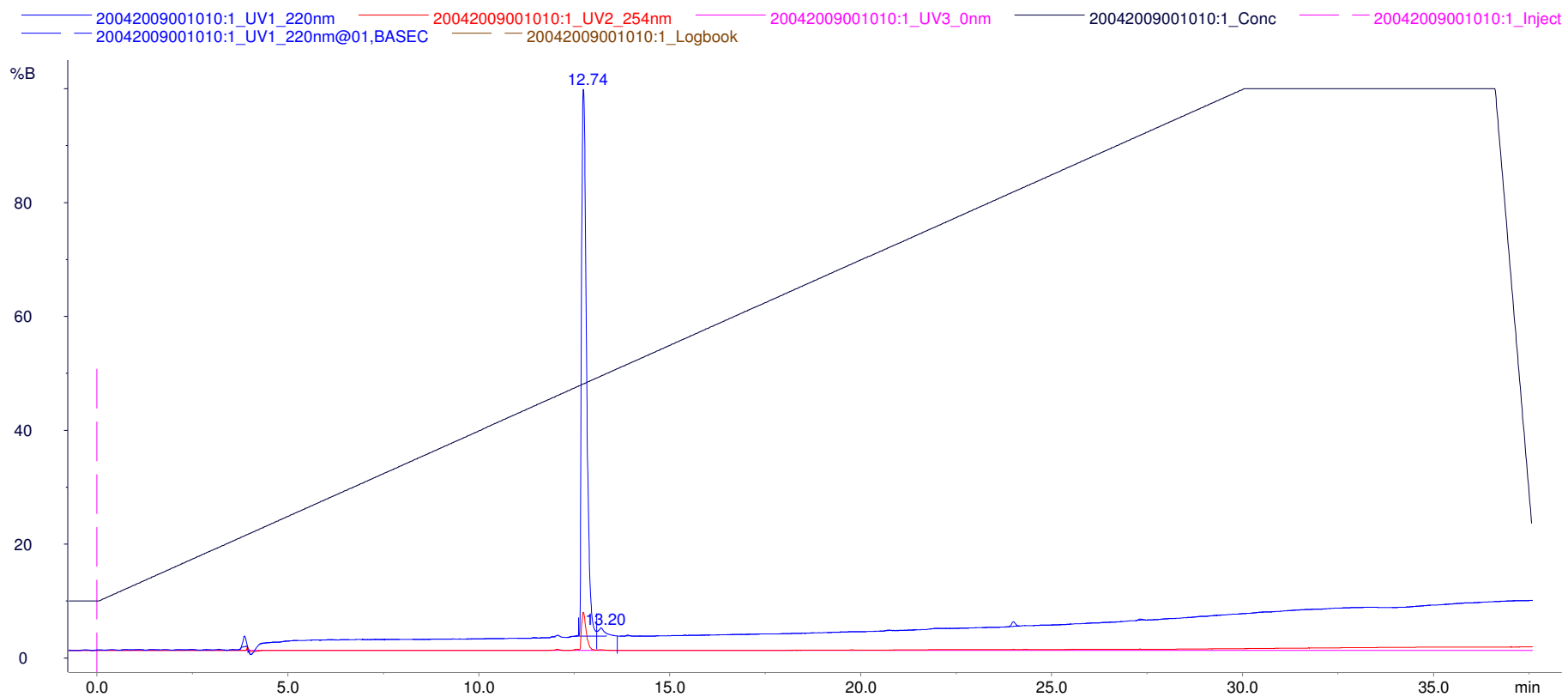




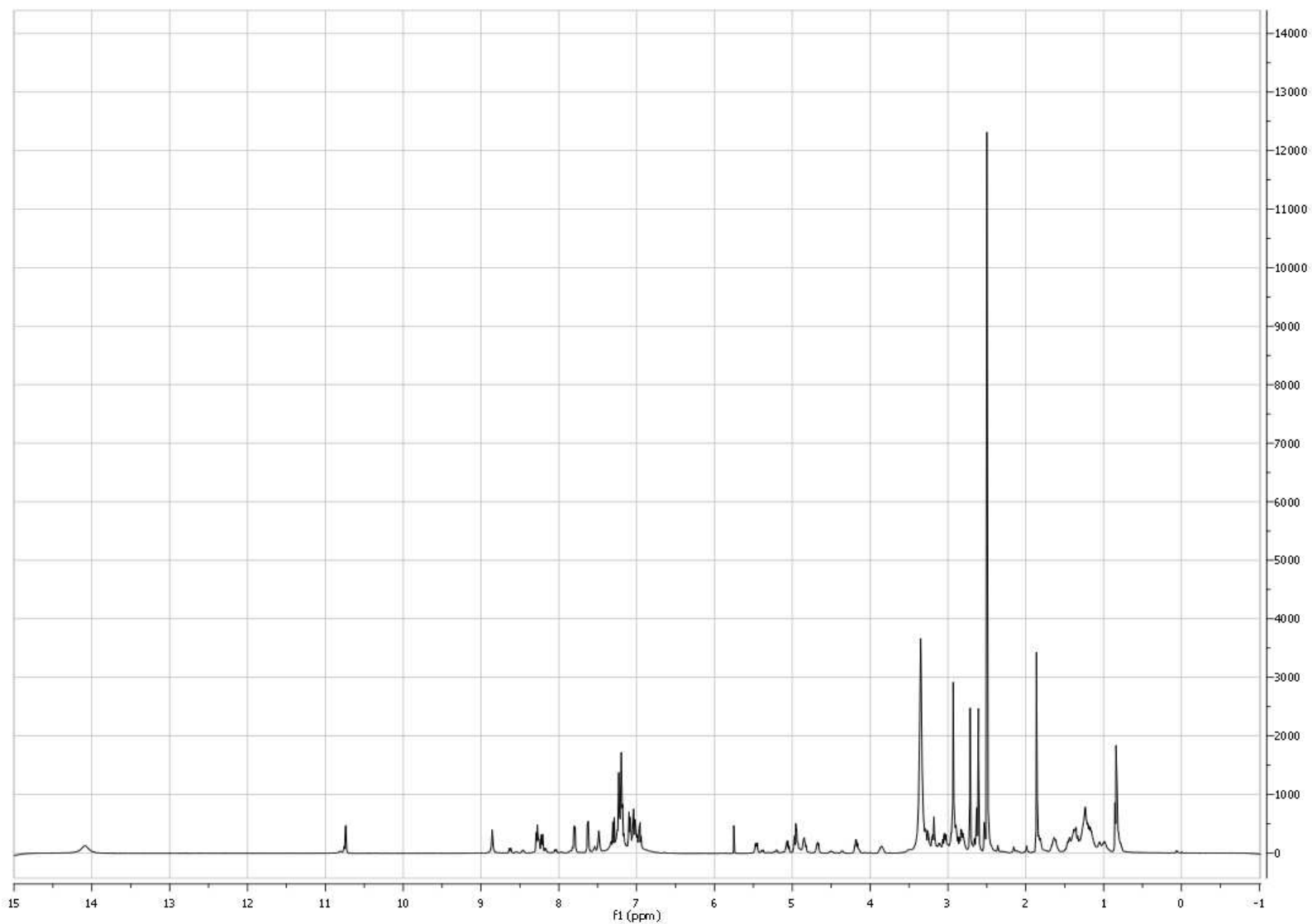
**Figure 20A:** HPLC chromatogram of compound 20.



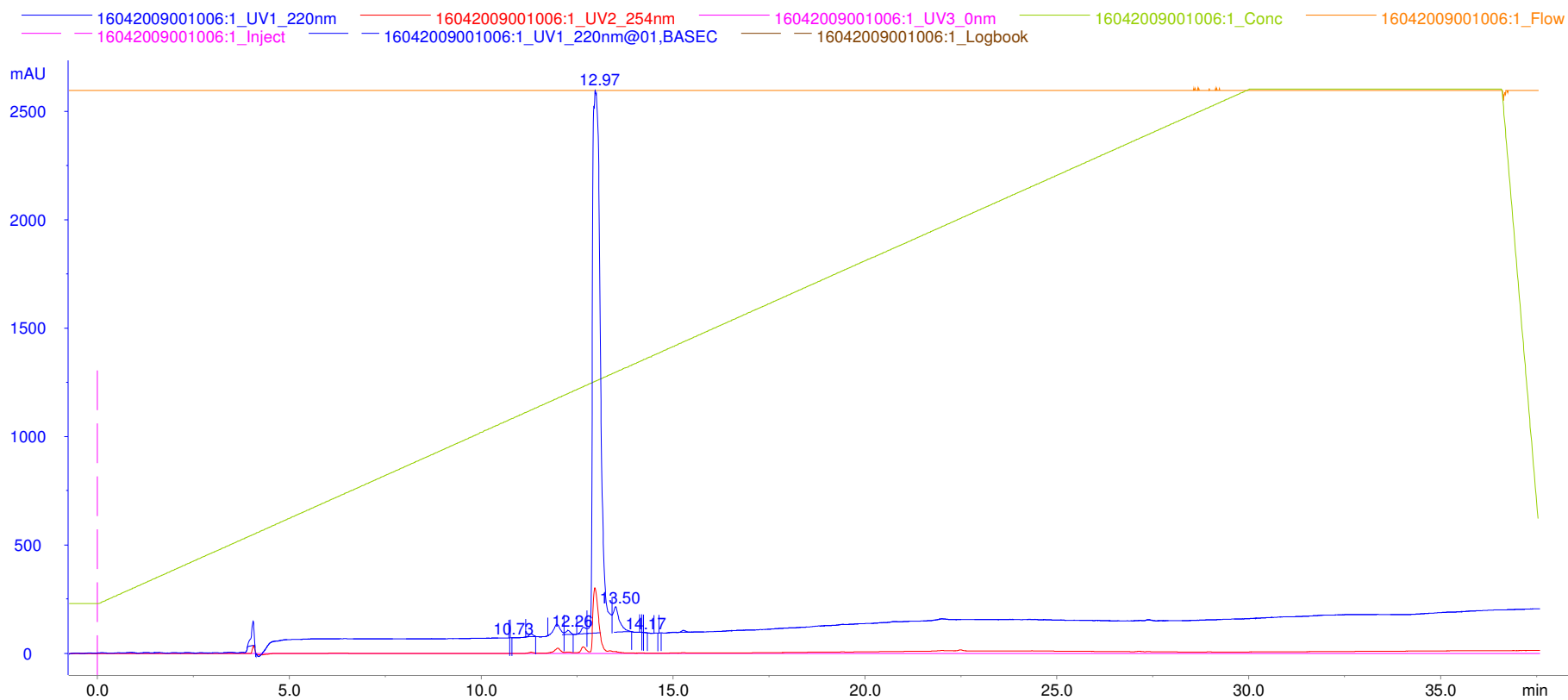
**Figure 20B:** <sup>1</sup>H-NMR spectrum of compound 20.



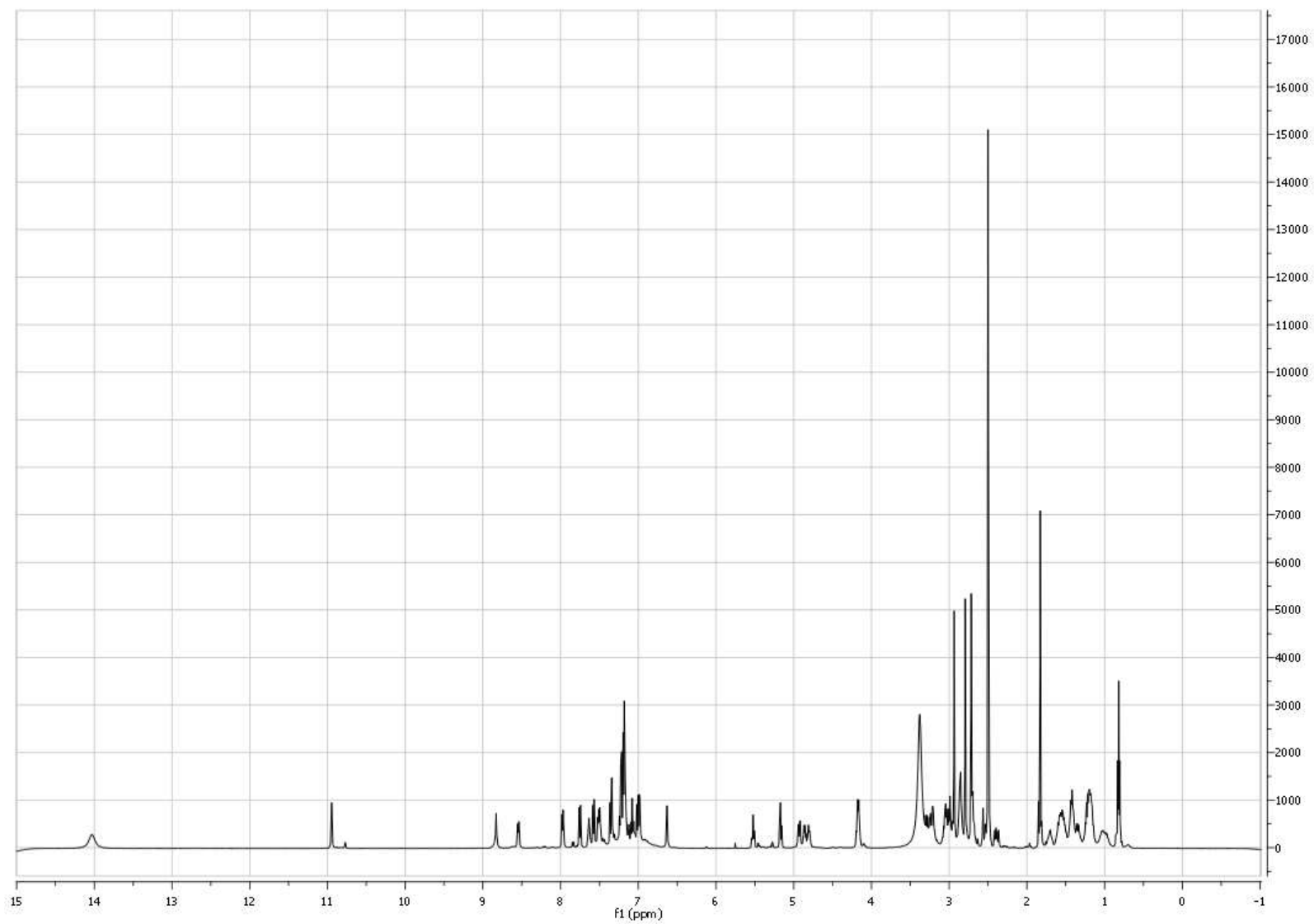
**Figure 21A:** HPLC chromatogram of compound 21.



**Figure 21B:**  $^1\text{H-NMR}$  spectrum of compound 21.



**Figure 22A:** HPLC chromatogram of compound 22.



**Figure 22B:**  $^1\text{H-NMR}$  spectrum of compound 22.

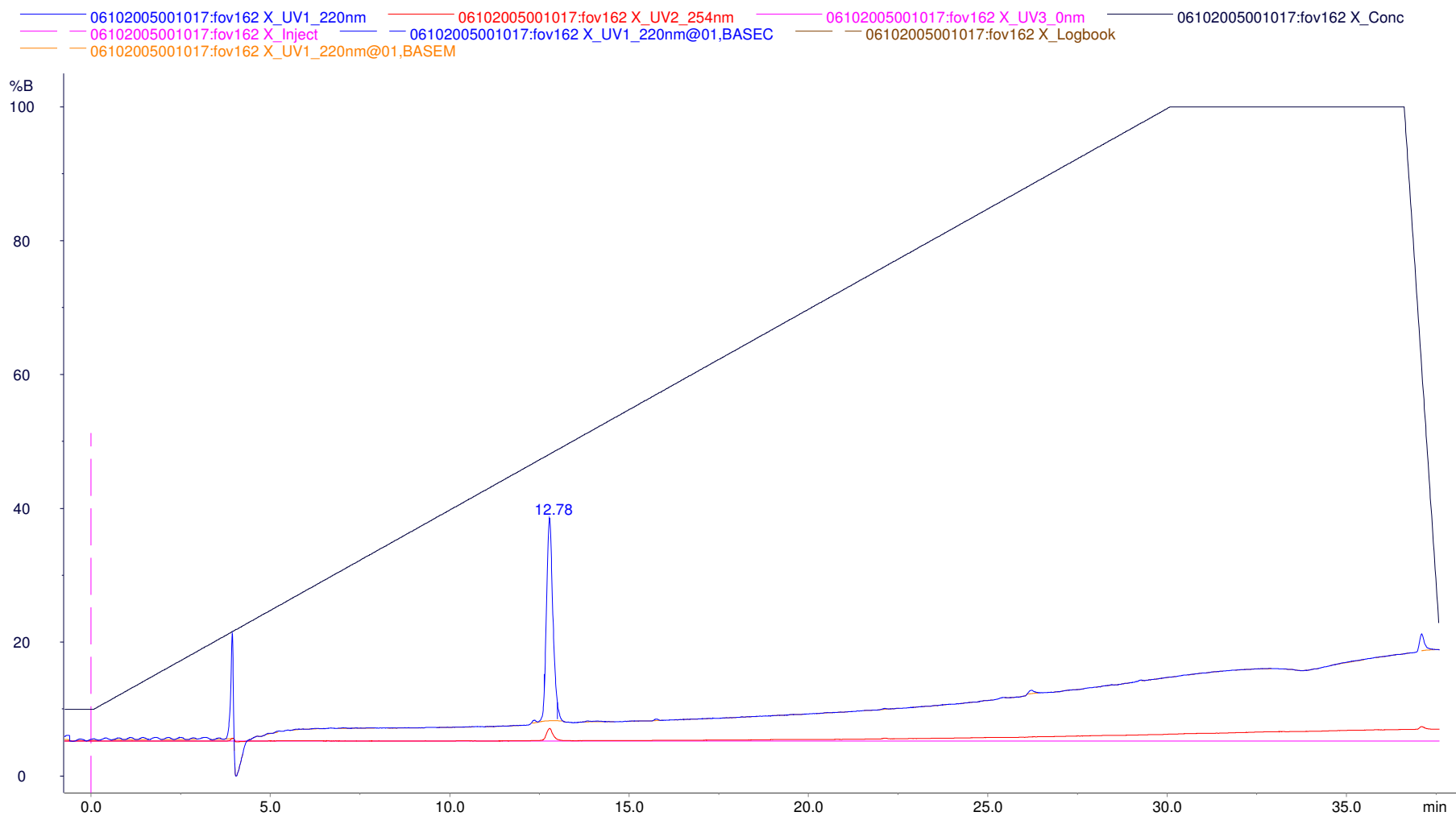
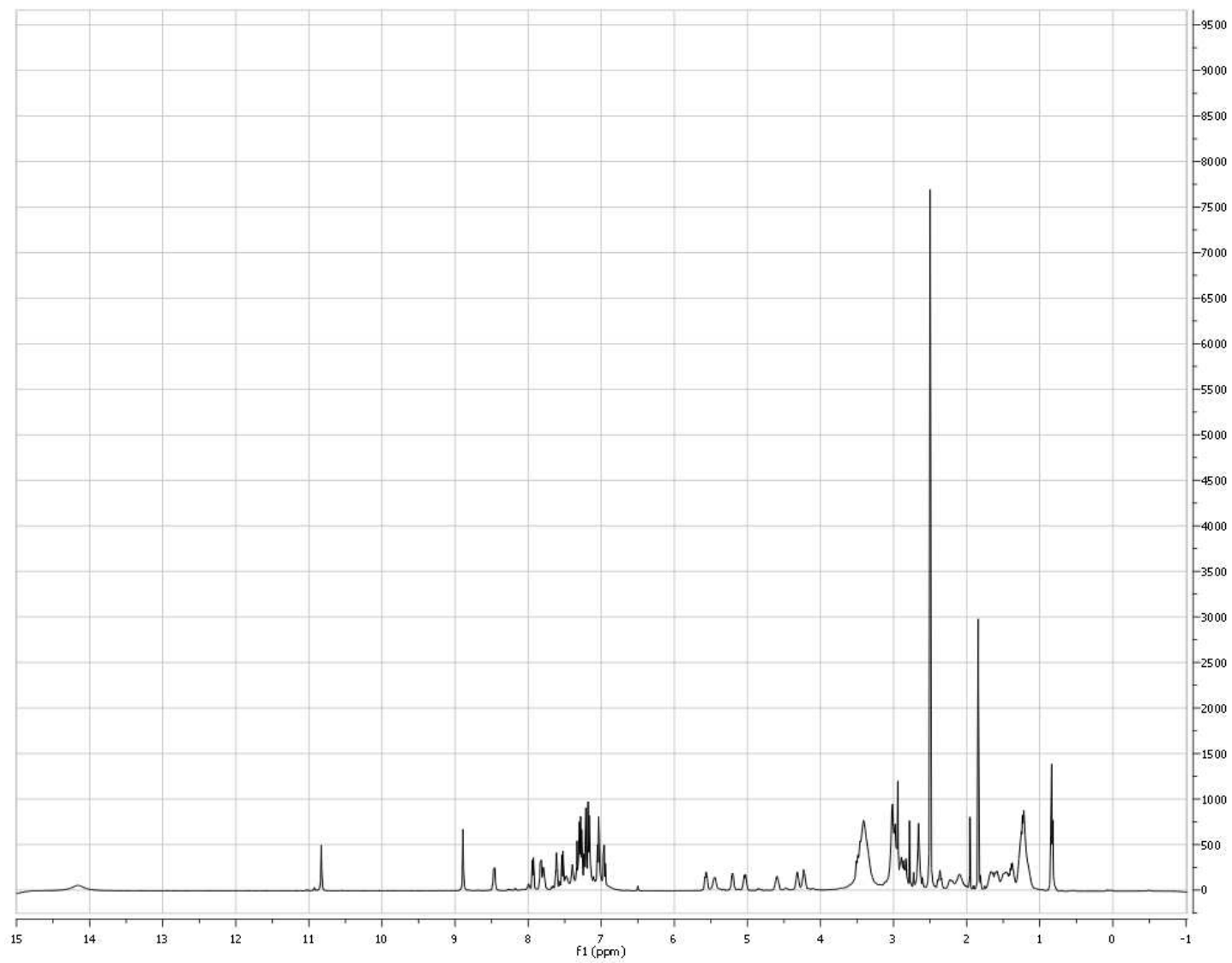


Figure 23A: HPLC chromatogram of compound 23.



**Figure 23B:**  $^1\text{H-NMR}$  spectrum of compound 23.



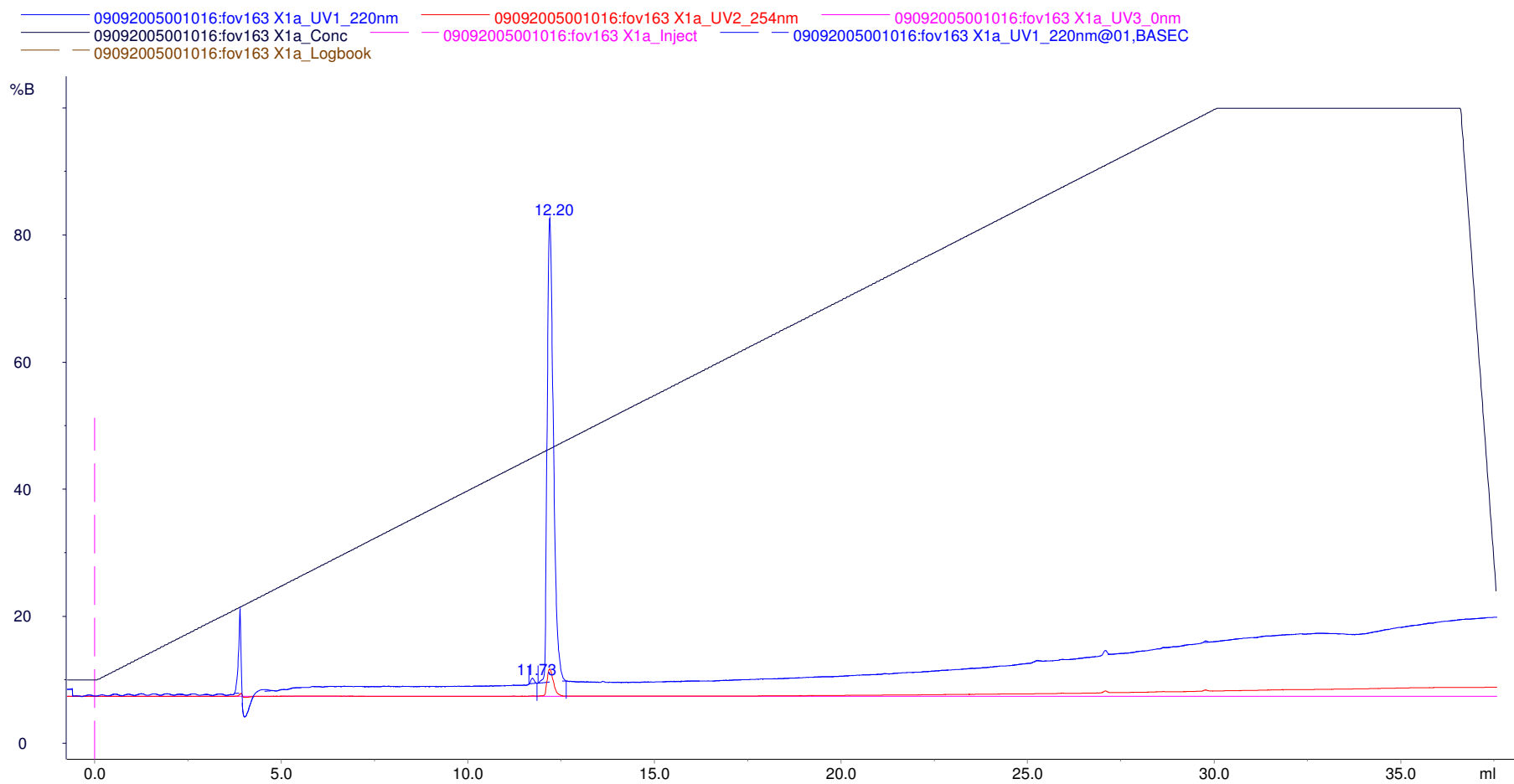
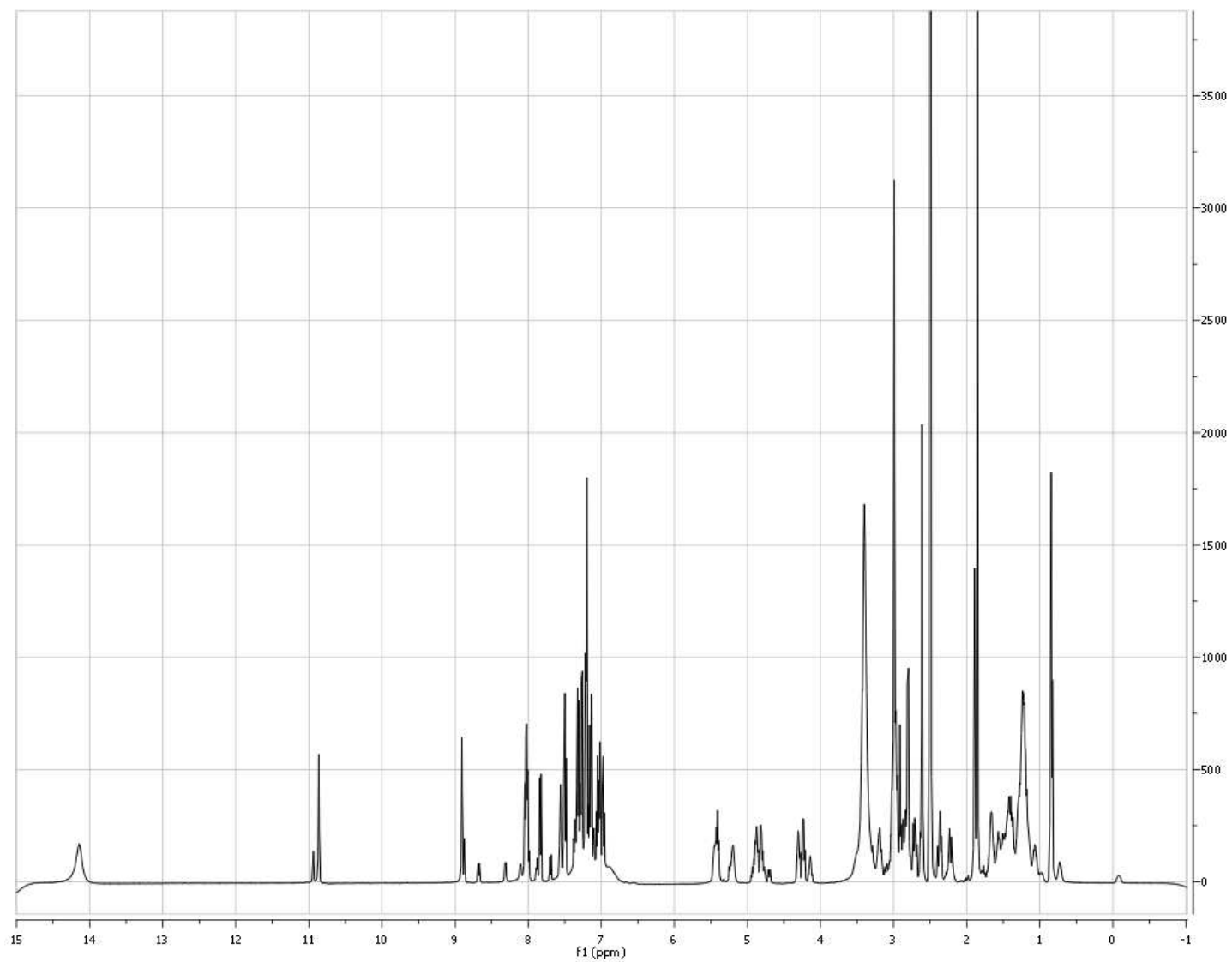


Figure 24A: HPLC chromatogram of compound 24.



**Figure 24B:**  $^1\text{H-NMR}$  spectrum of compound 24.

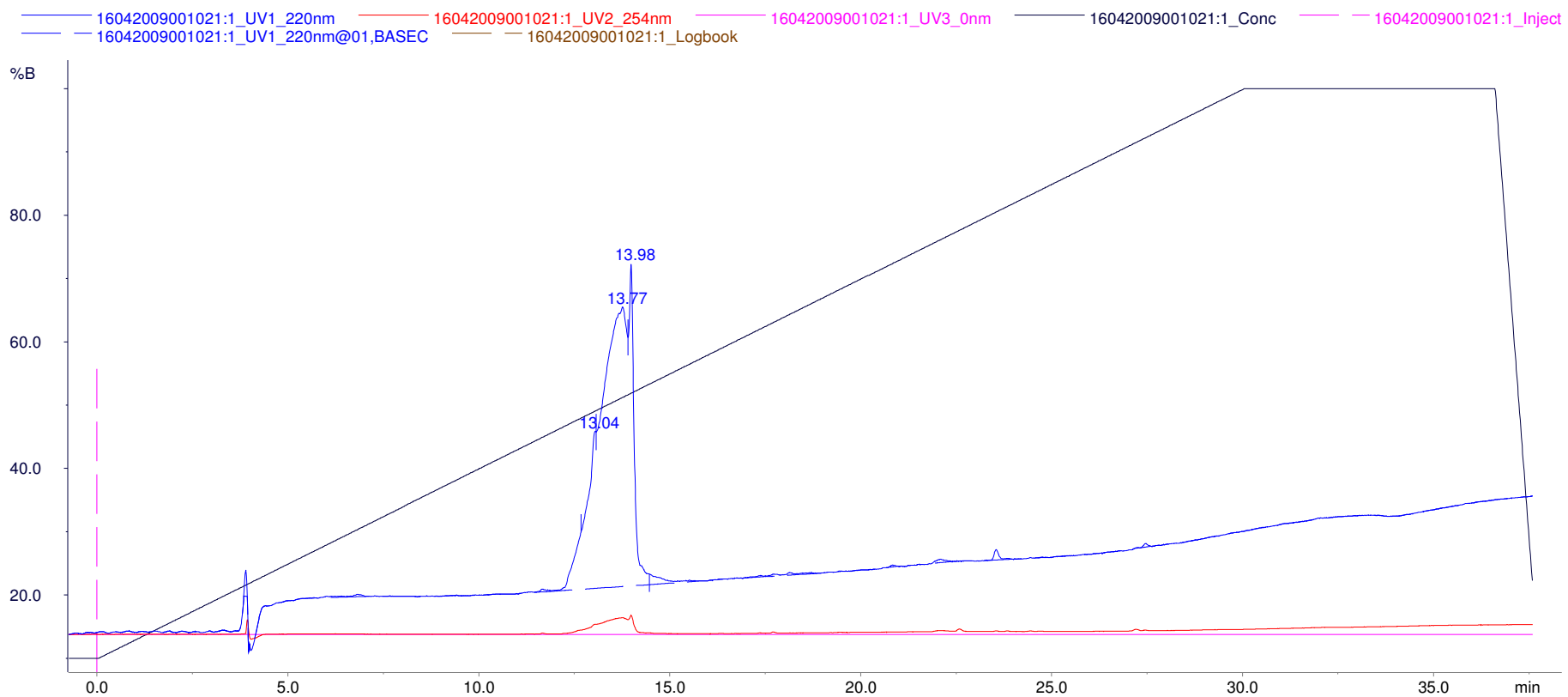
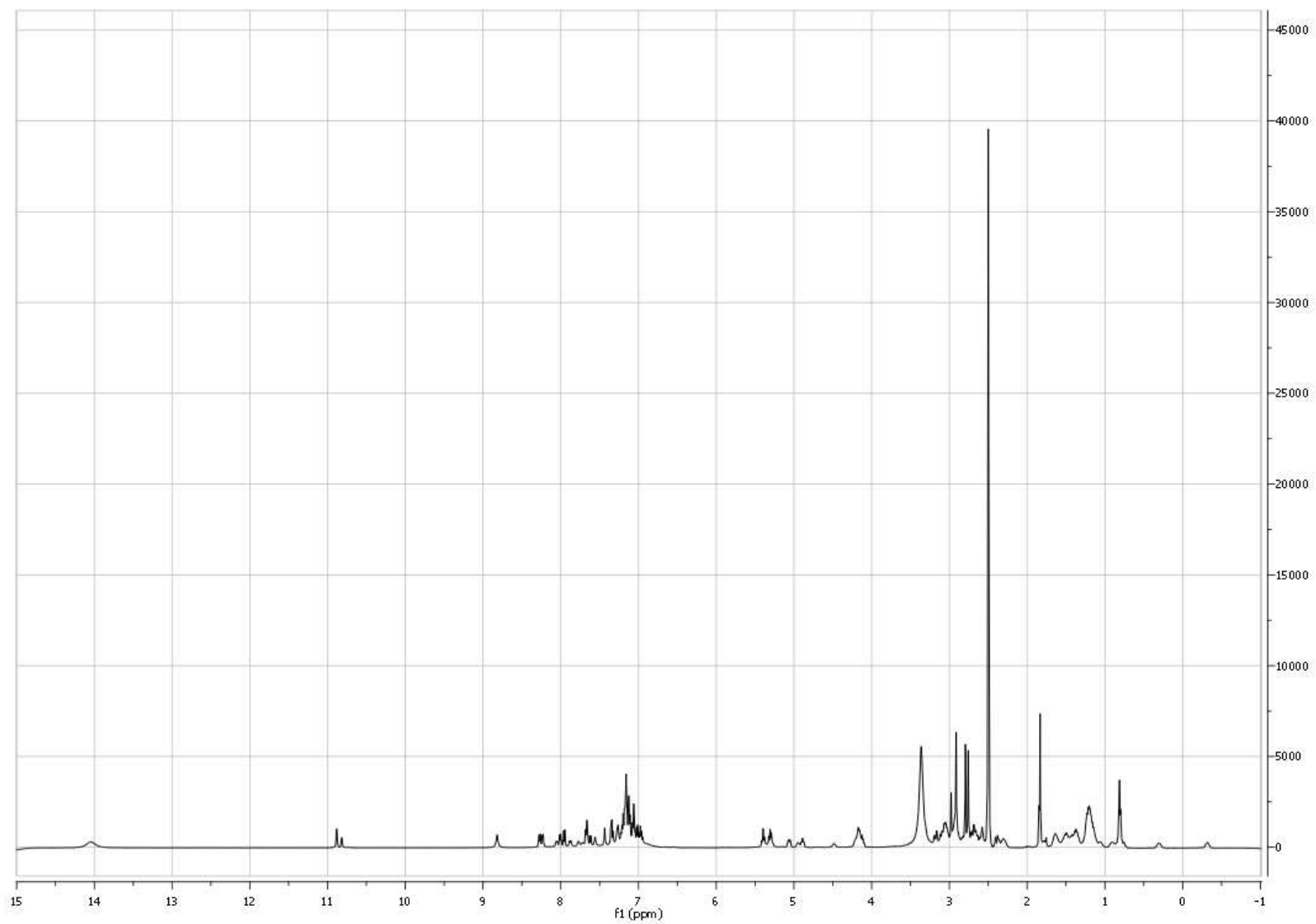
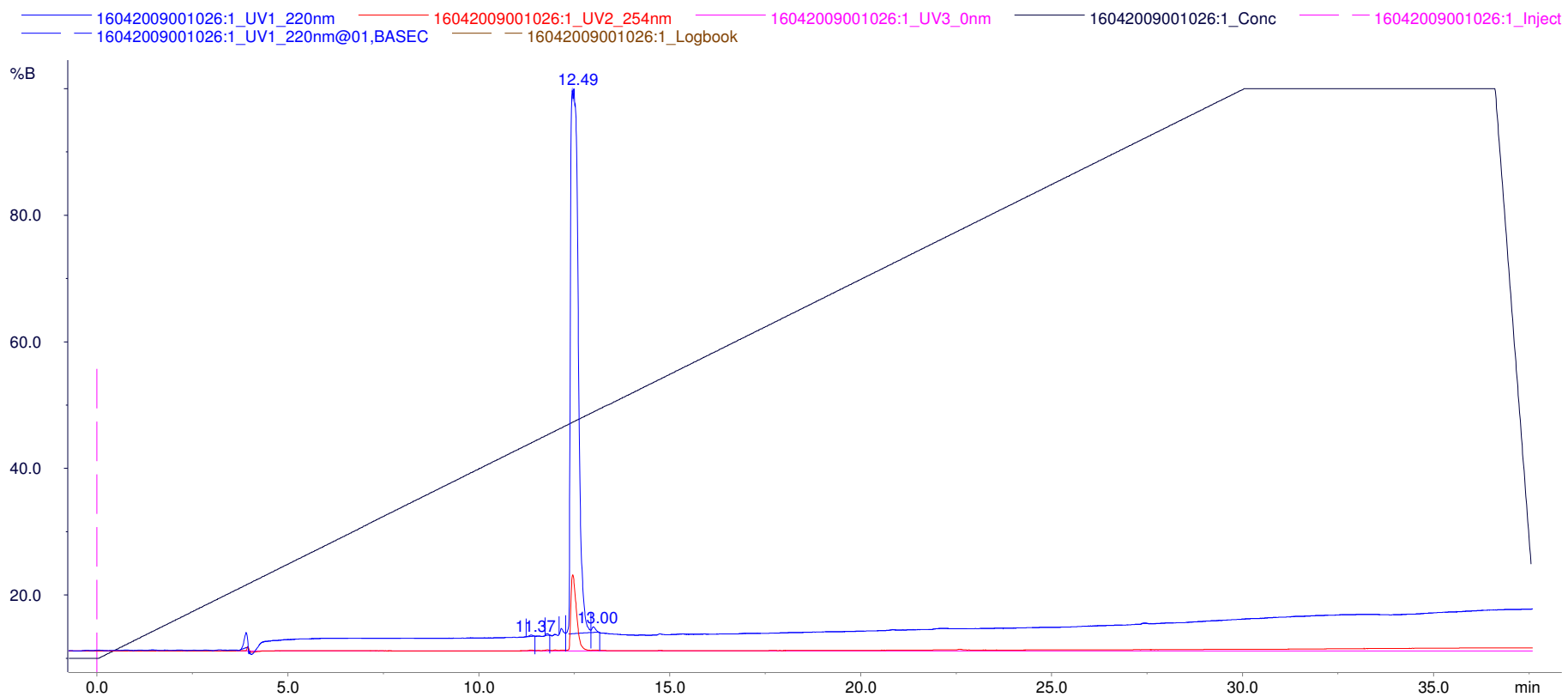


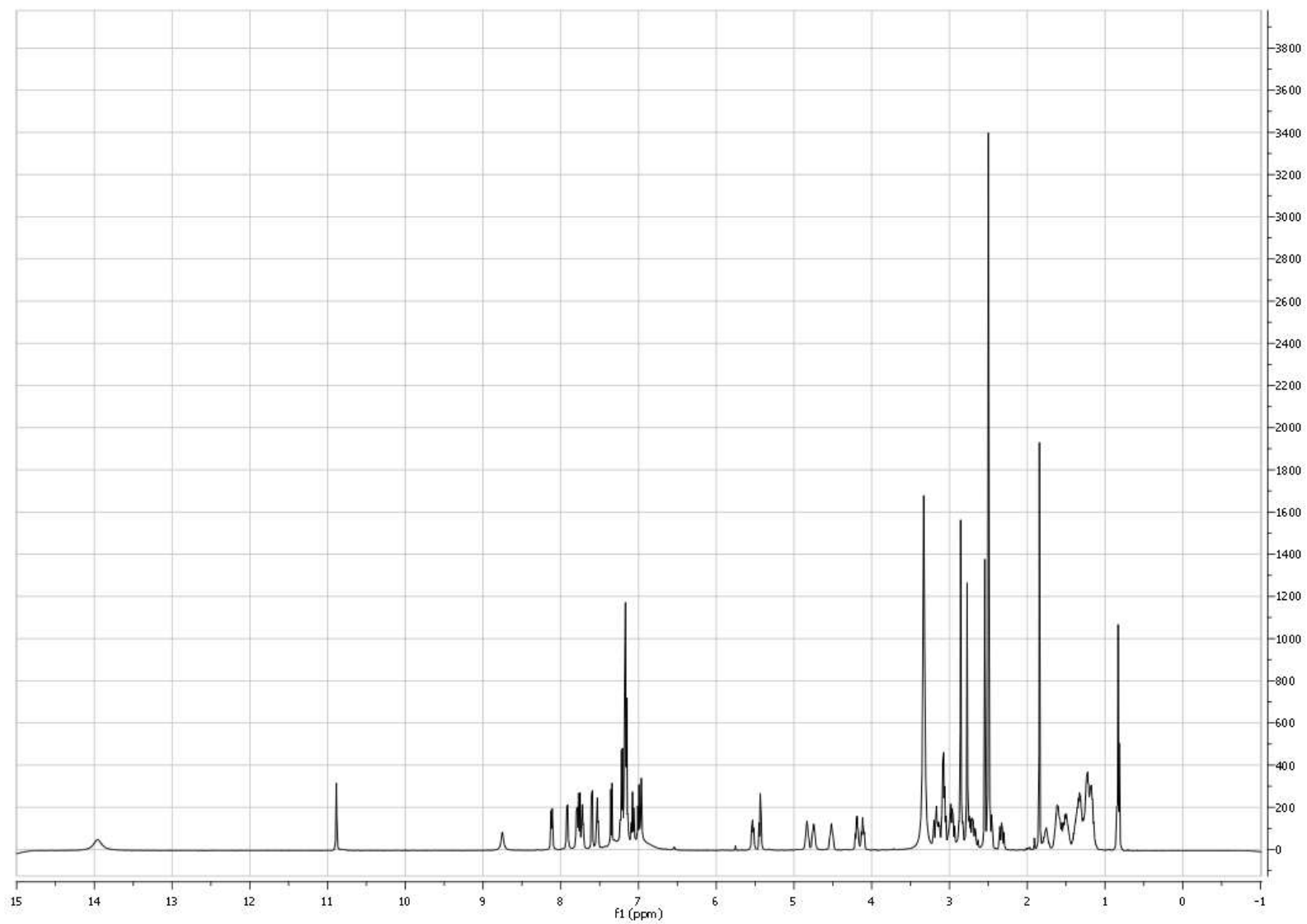
Figure 25A: HPLC chromatogram of compound 25.



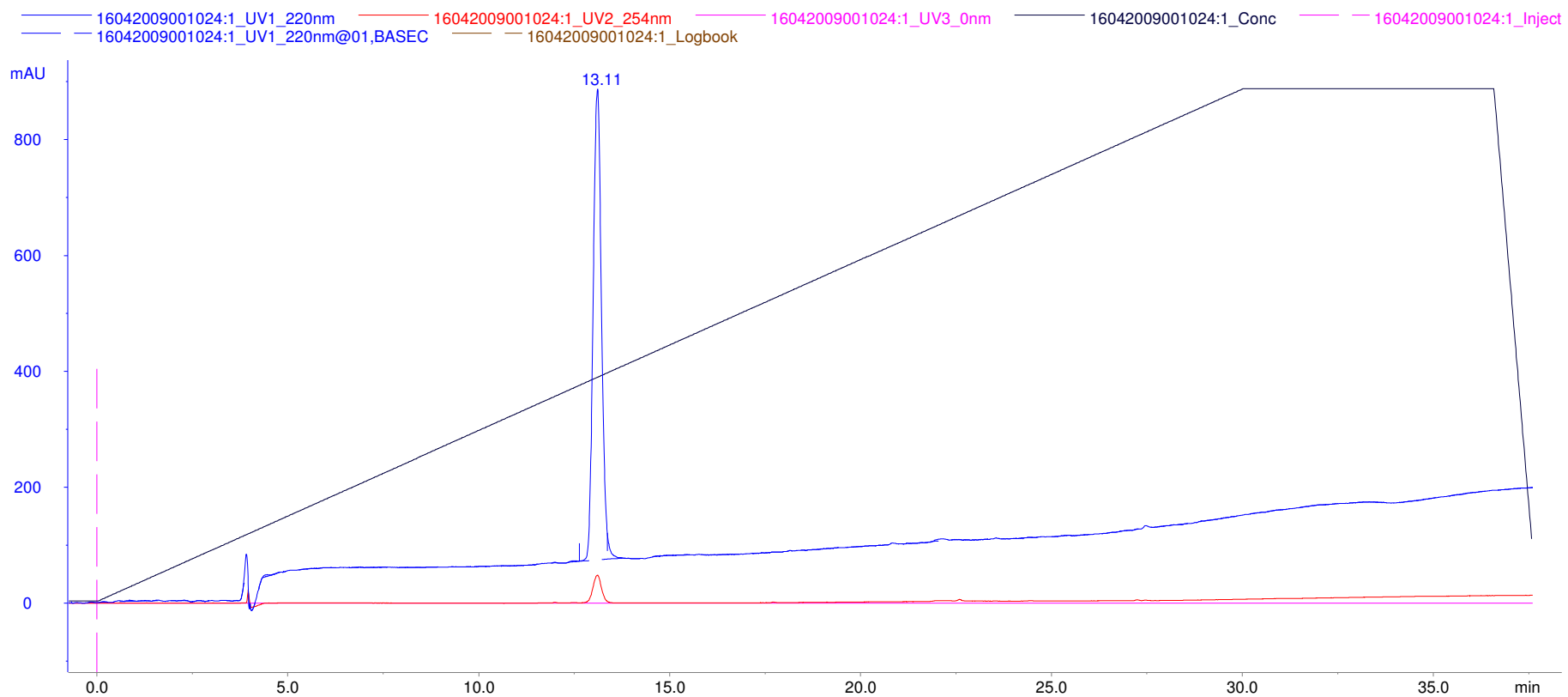
**Figure 25B:**  $^1\text{H-NMR}$  spectrum of compound 25.



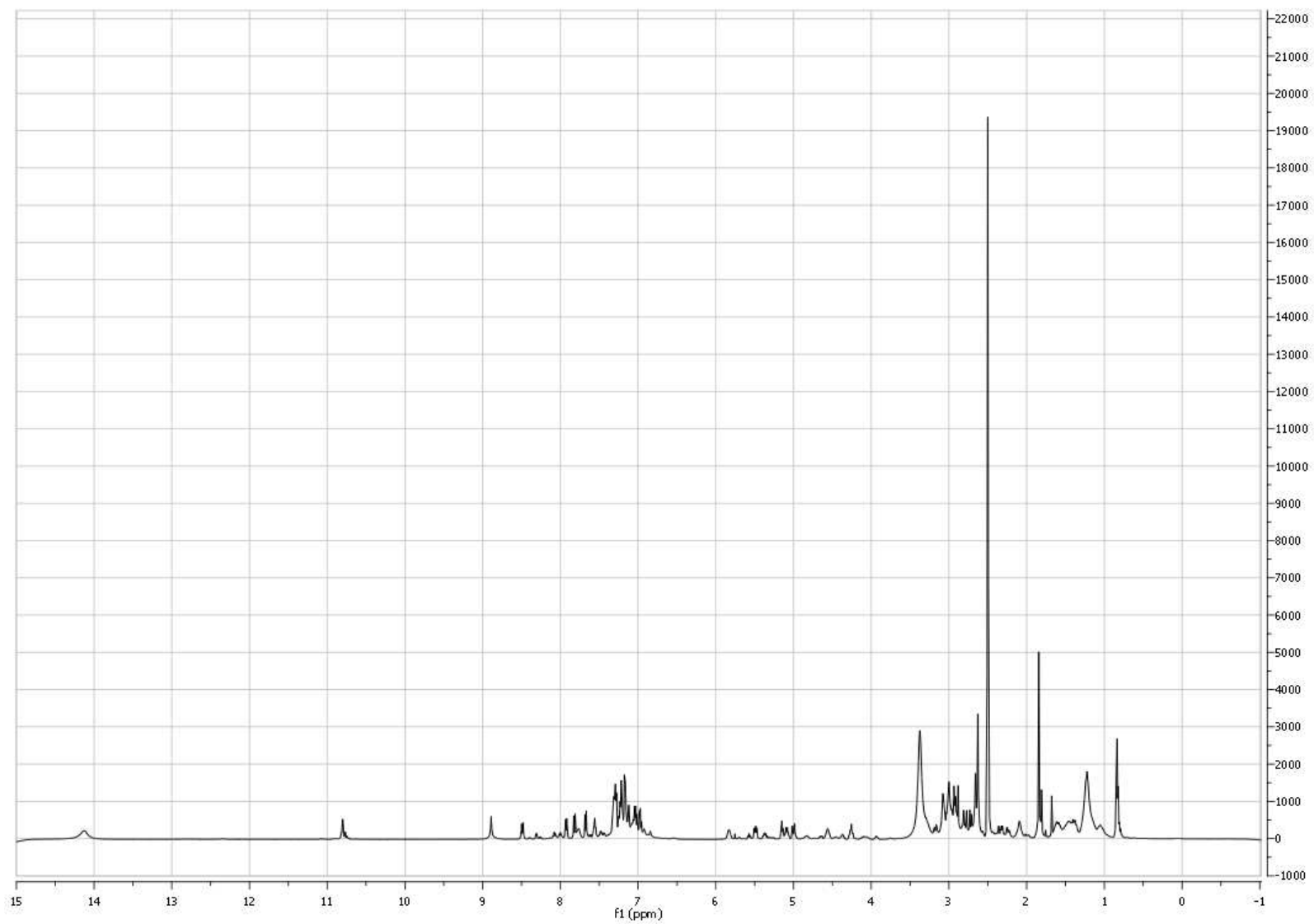
**Figure 26A:** HPLC chromatogram of compound 26.



**Figure 26B:**  $^1\text{H-NMR}$  spectrum of compound 26.

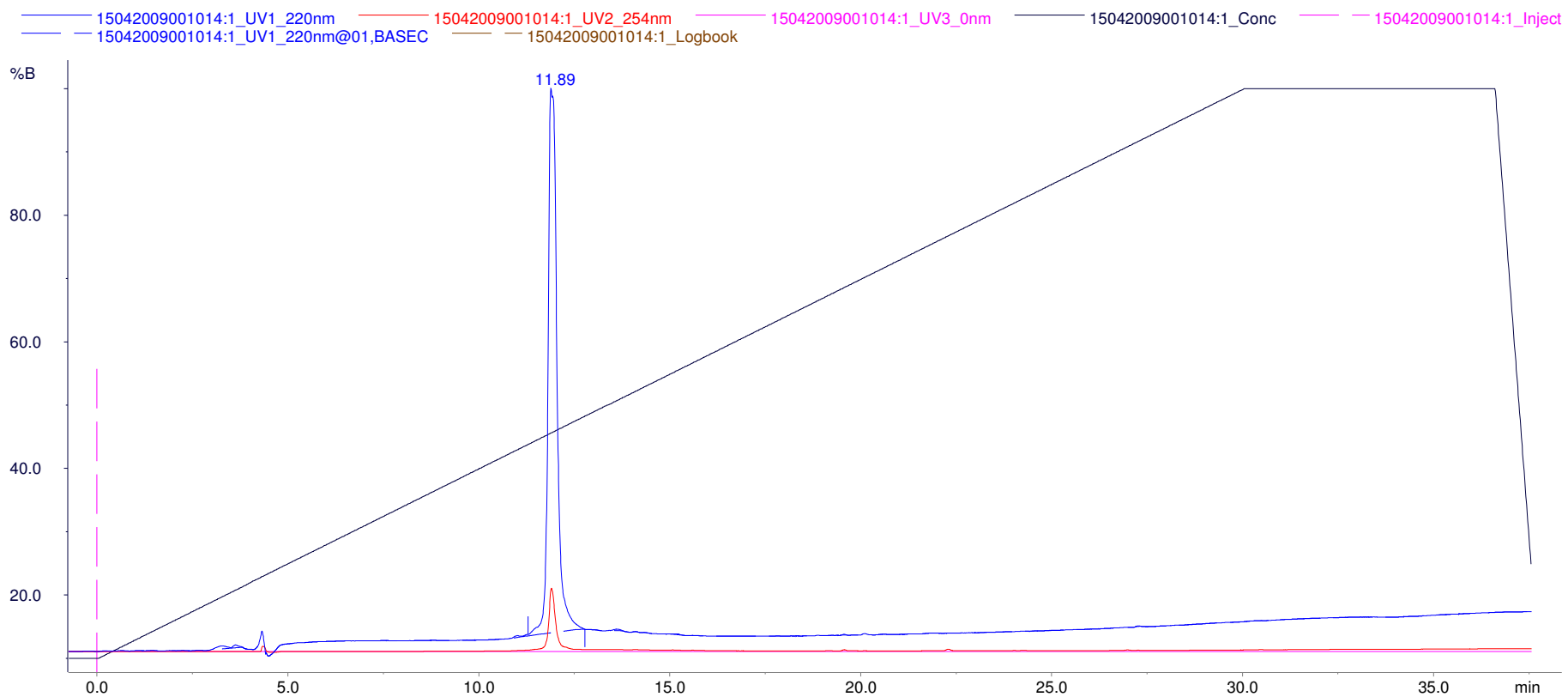


**Figure 27A:** HPLC chromatogram of compound 27.

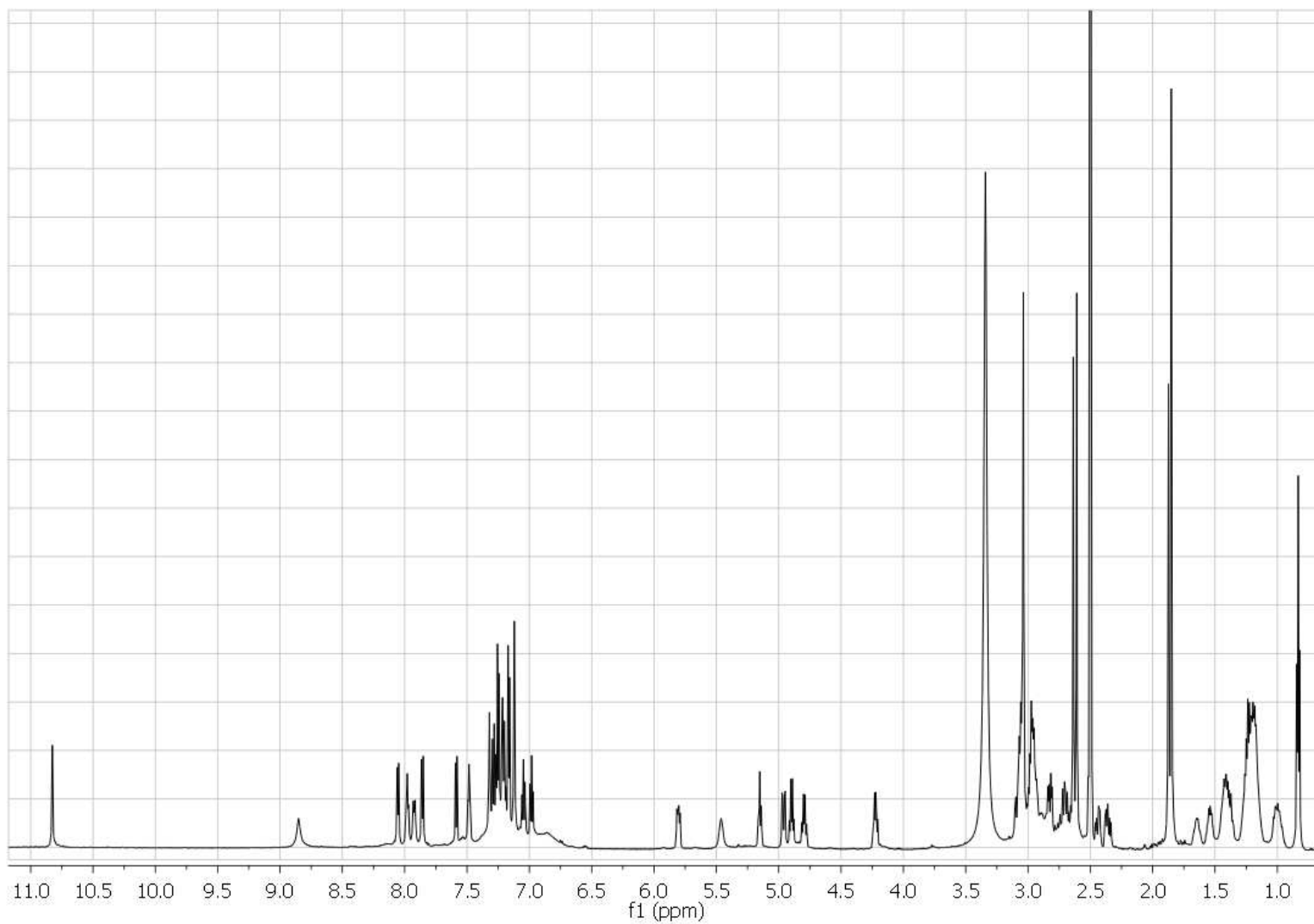


**Figure 27B:** <sup>1</sup>H-NMR spectrum of compound 27.

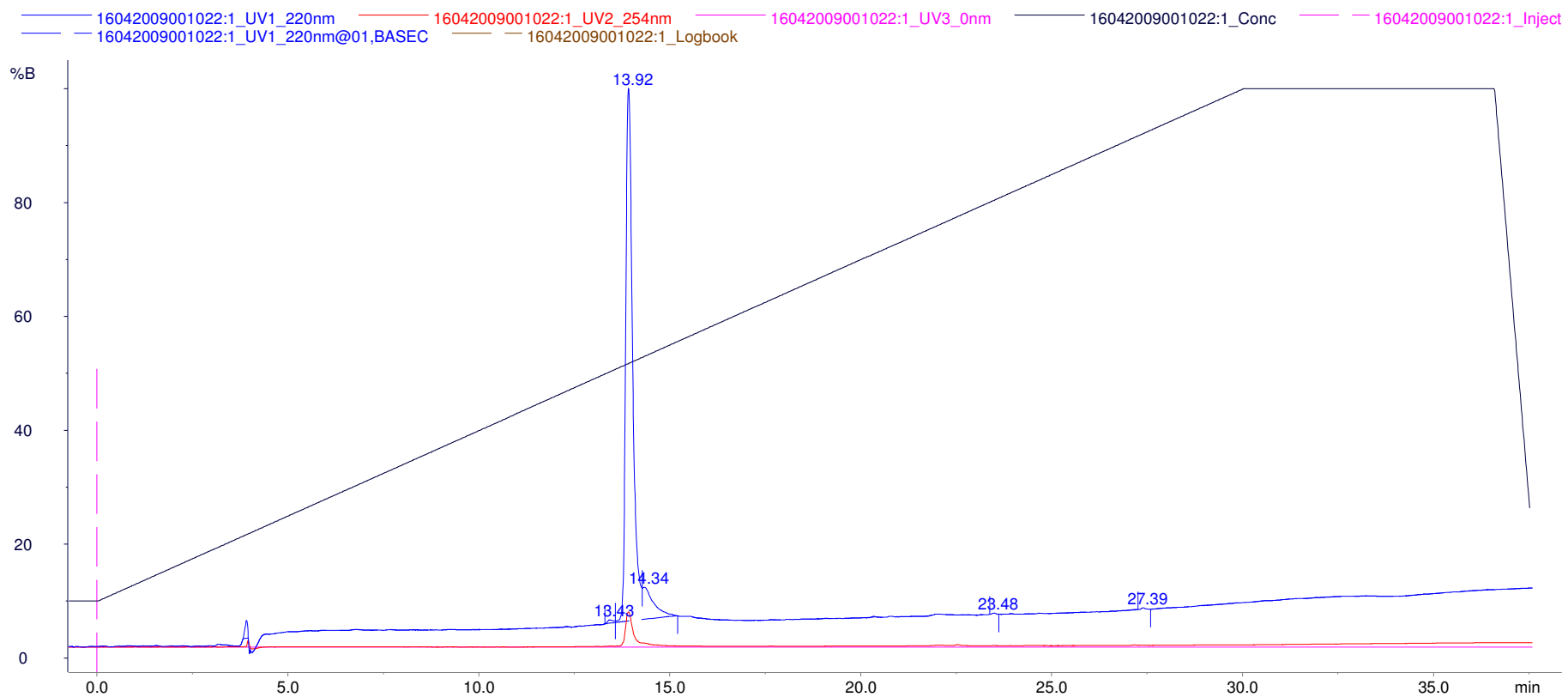




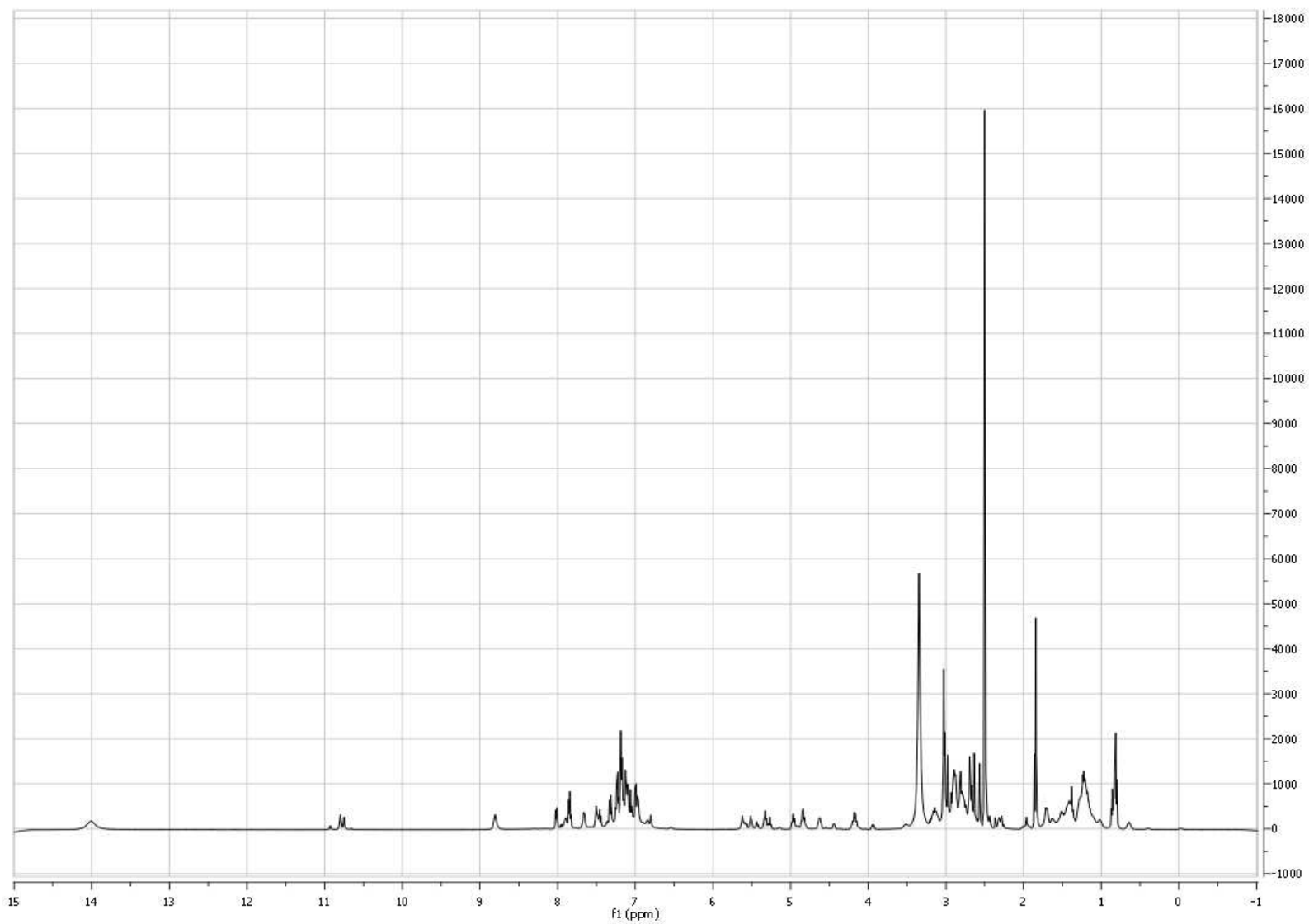
**Figure 28A:** HPLC chromatogram of compound 28.



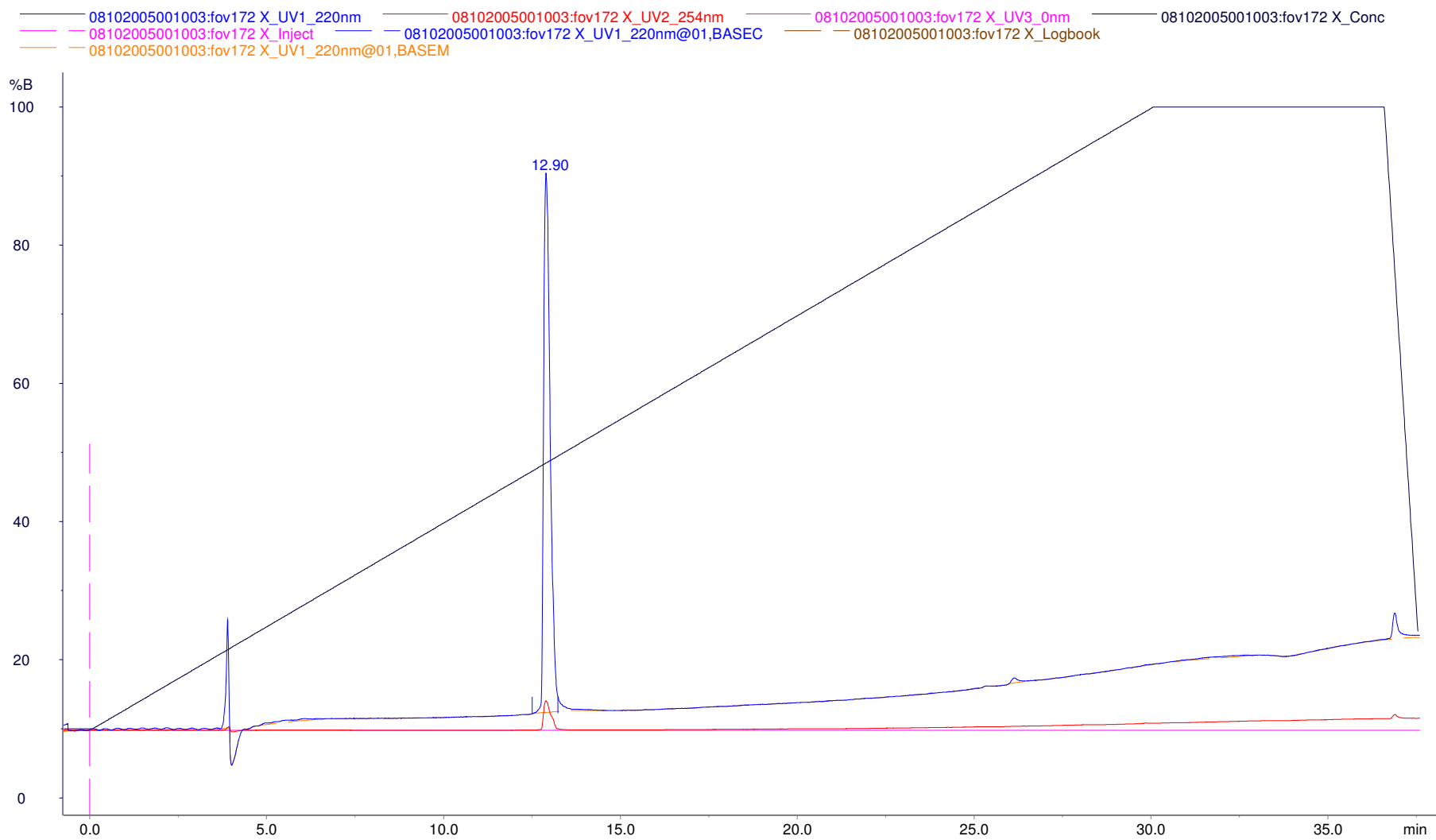
**Figure 28B:** <sup>1</sup>H-NMR spectrum of compound 28.



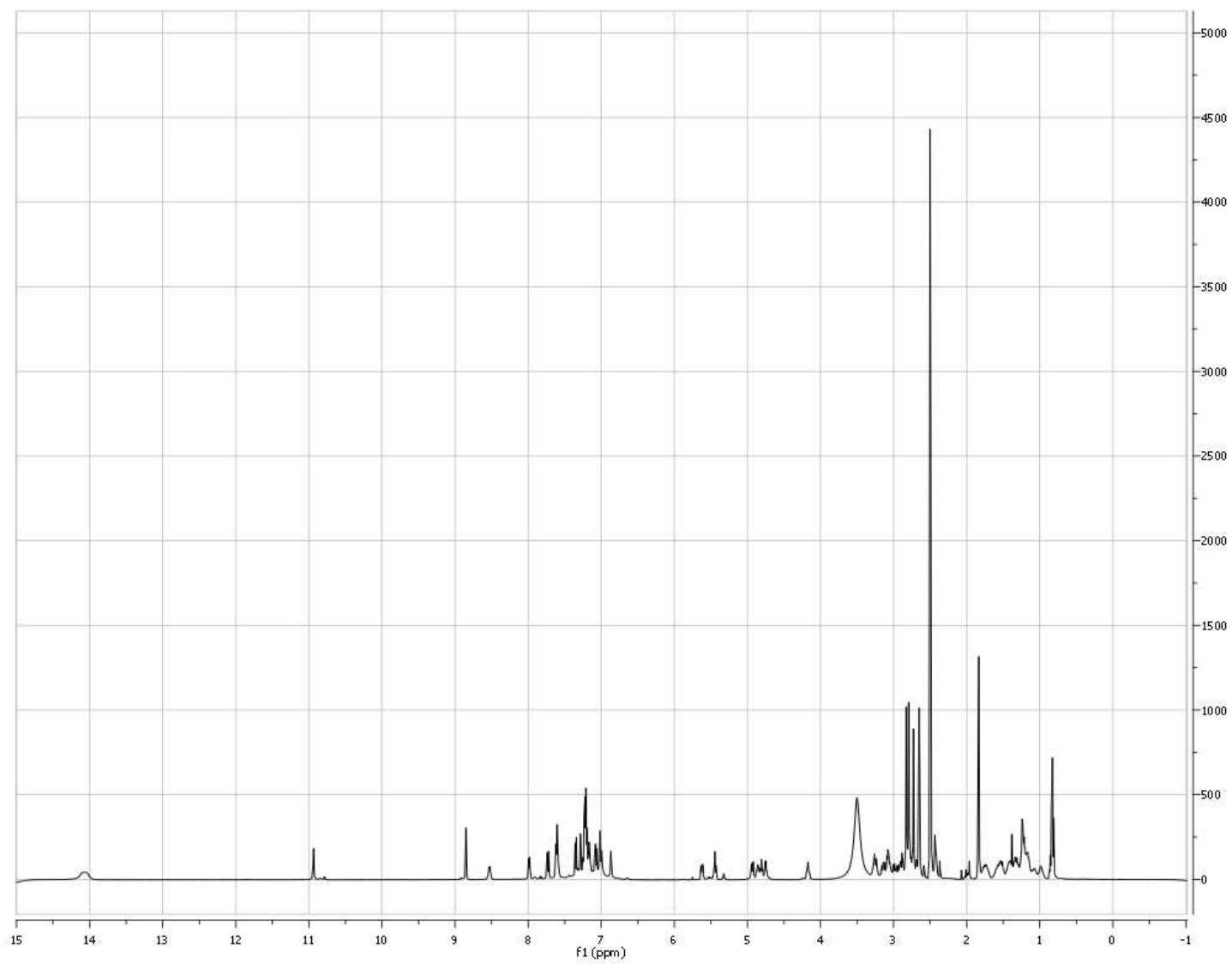
**Figure 29A:** HPLC chromatogram of compound 29.



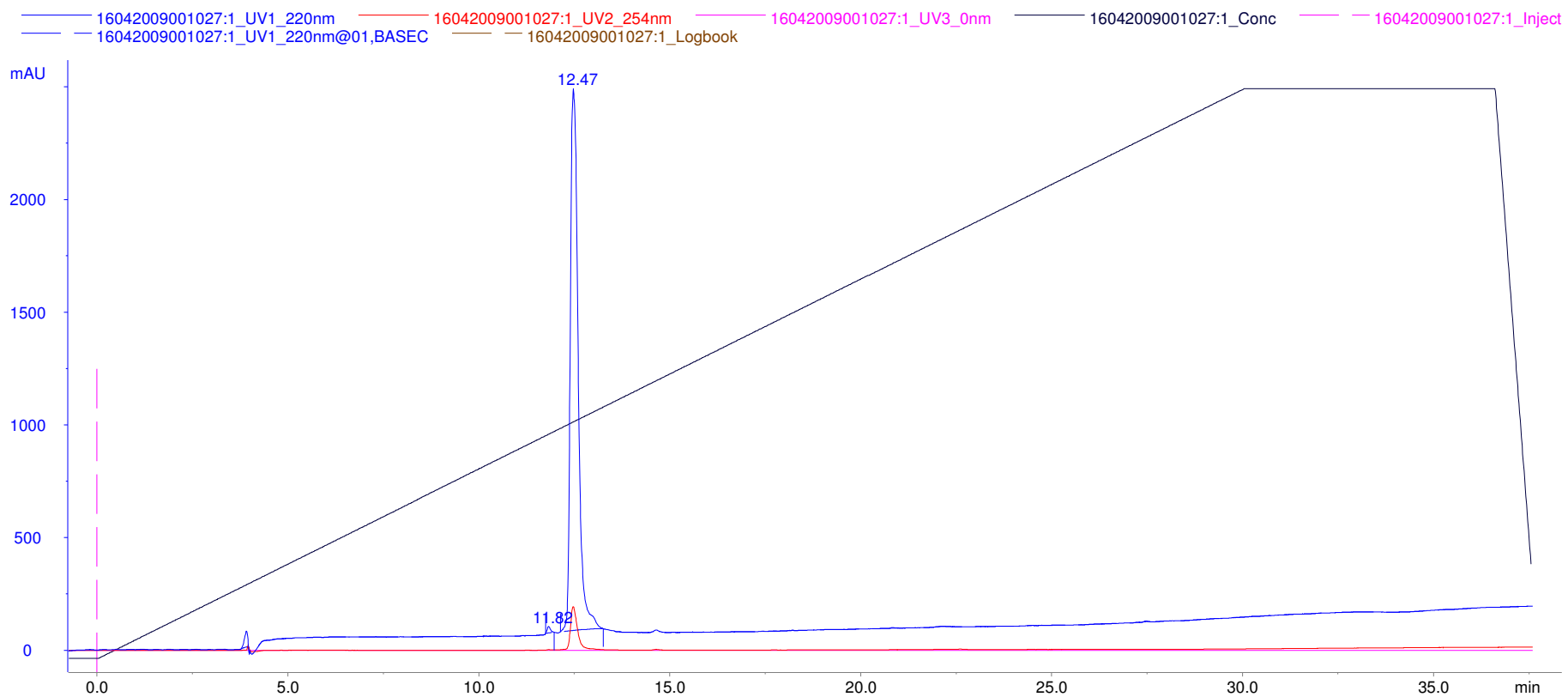
**Figure 29B:** <sup>1</sup>H-NMR spectrum of compound 29.



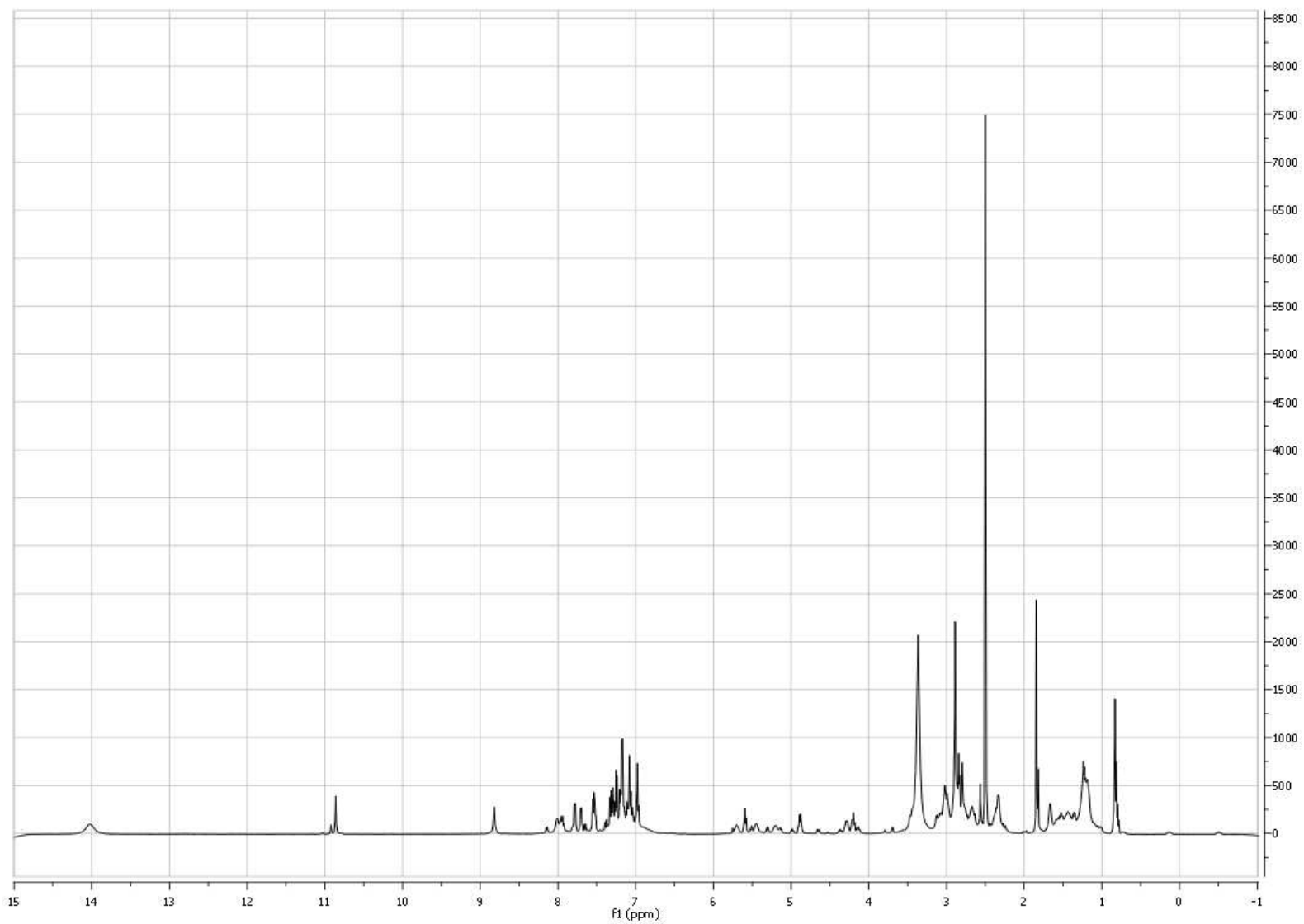
**Figure 30A:** HPLC chromatogram of compound 30.



**Figure 30B:**  $^1\text{H-NMR}$  spectrum of compound 30.

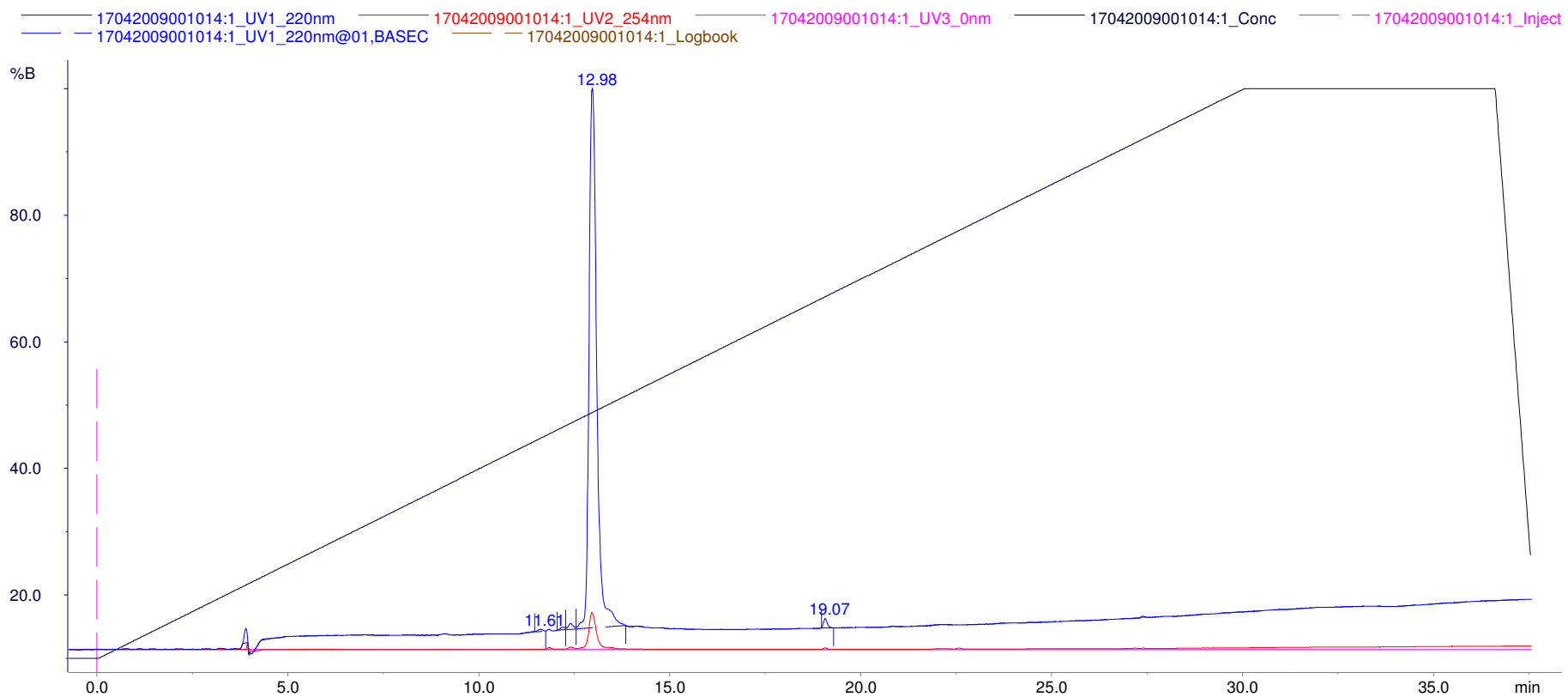


**Figure 31A:** HPLC chromatogram of compound 31.

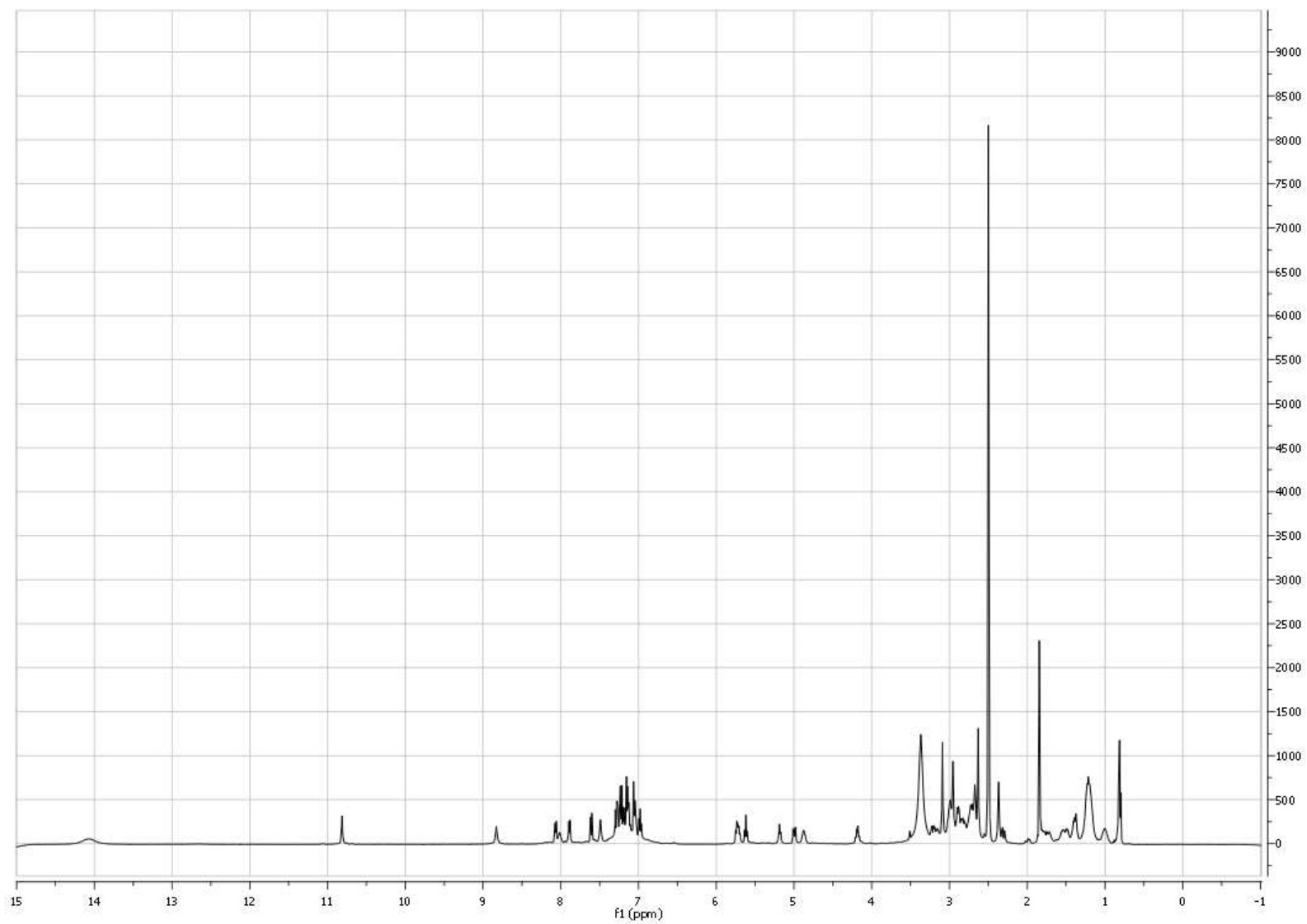


**Figure 31B:**  $^1\text{H}$ -NMR spectrum of compound 31.

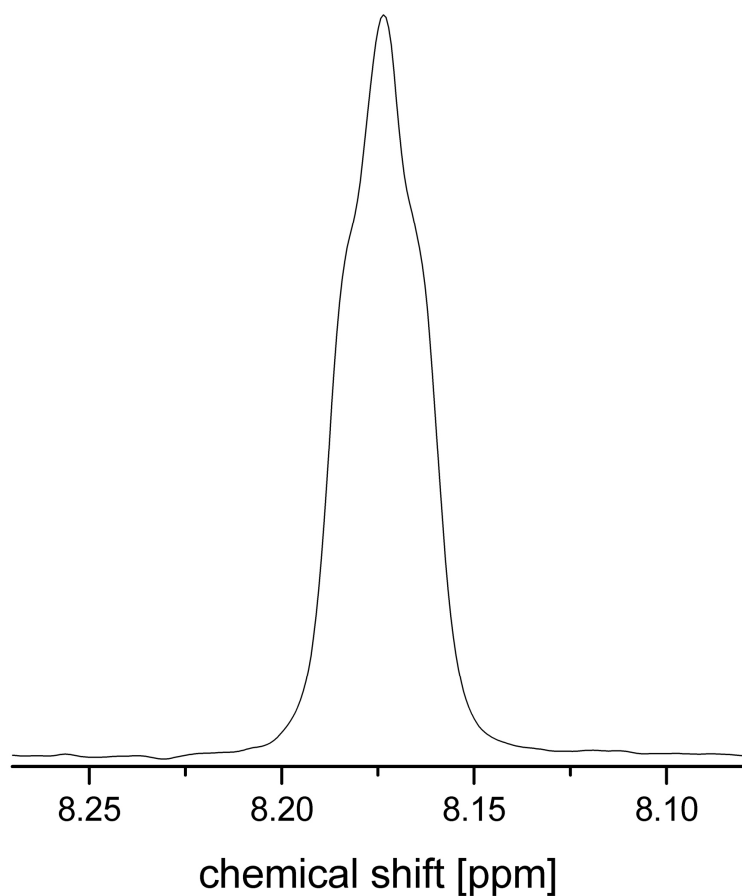




**Figure 32A:** HPLC chromatogram of compound 32.



**Figure 32B:**  $^1\text{H-NMR}$  spectrum of compound 32.



**Figure 33.** f2 slice of the ROESY crosspeak between *N*Me-Lys<sup>10</sup> H<sup>ζ</sup> and Asp<sup>5</sup> H<sup>β</sup><sub>proS</sub> detected with 8192 complex datapoints in f2, processed by multiplication with a squared cosine window function on 16384 datapoints in f2.