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## **Development of a Selective COX-2 Inhibitor: From Synthesis to Enhanced Efficacy via Nano-Formulation**

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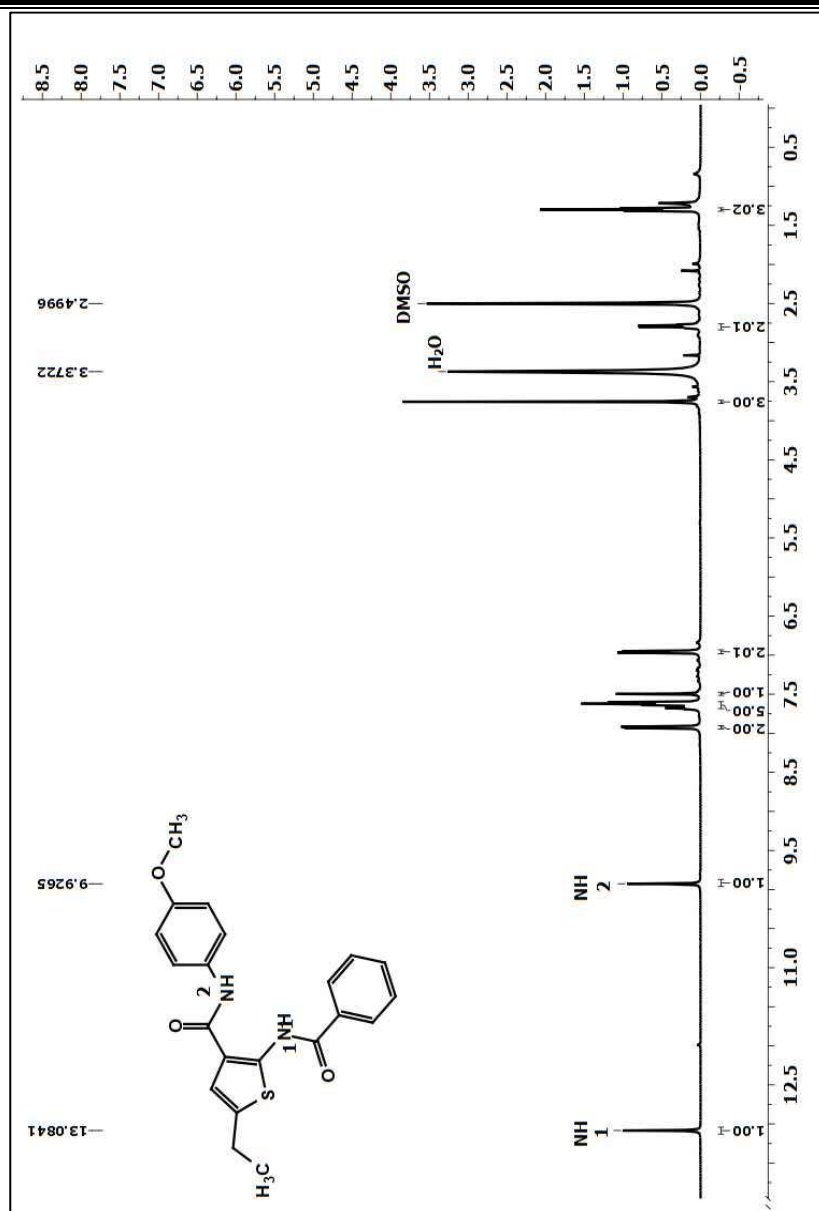
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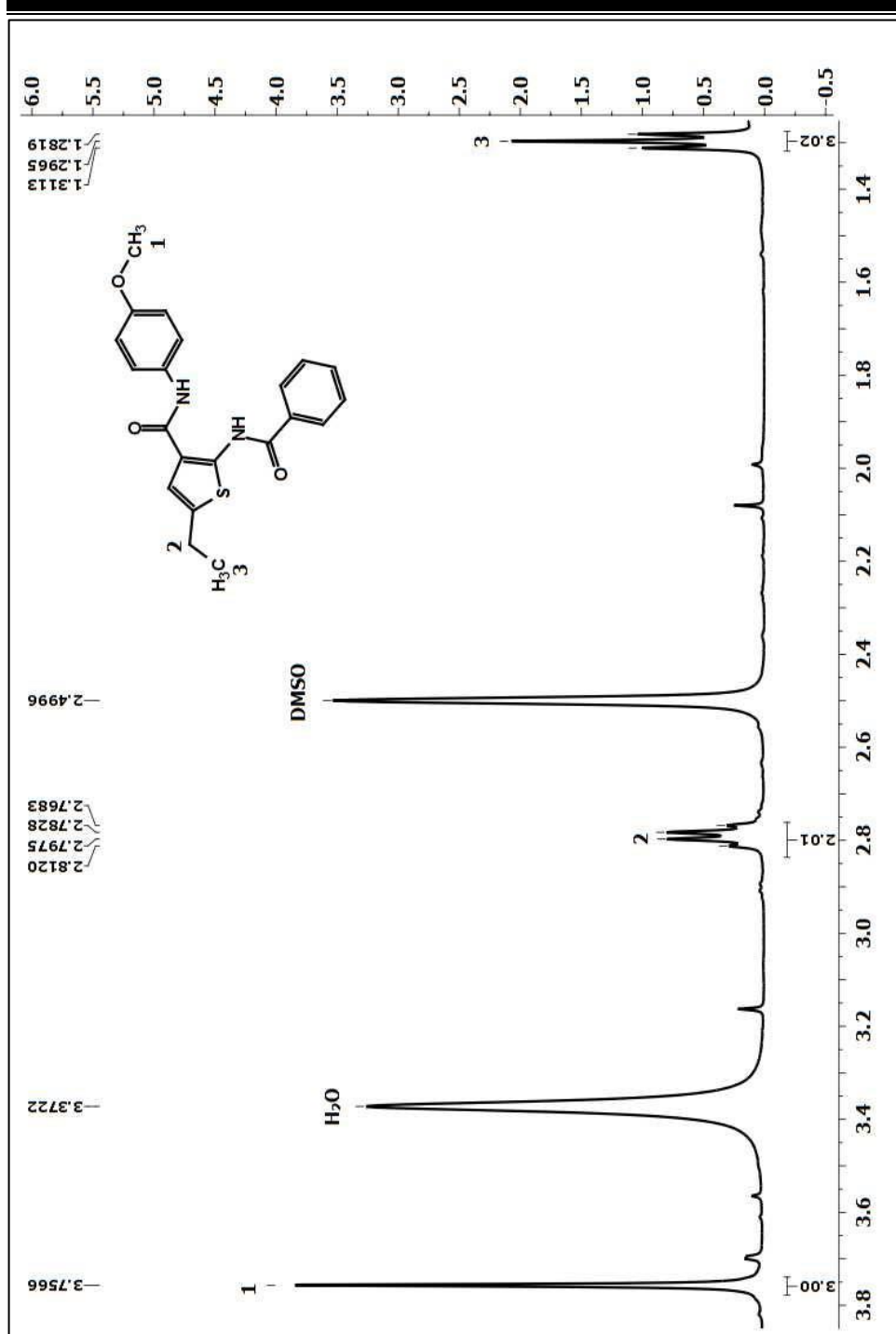
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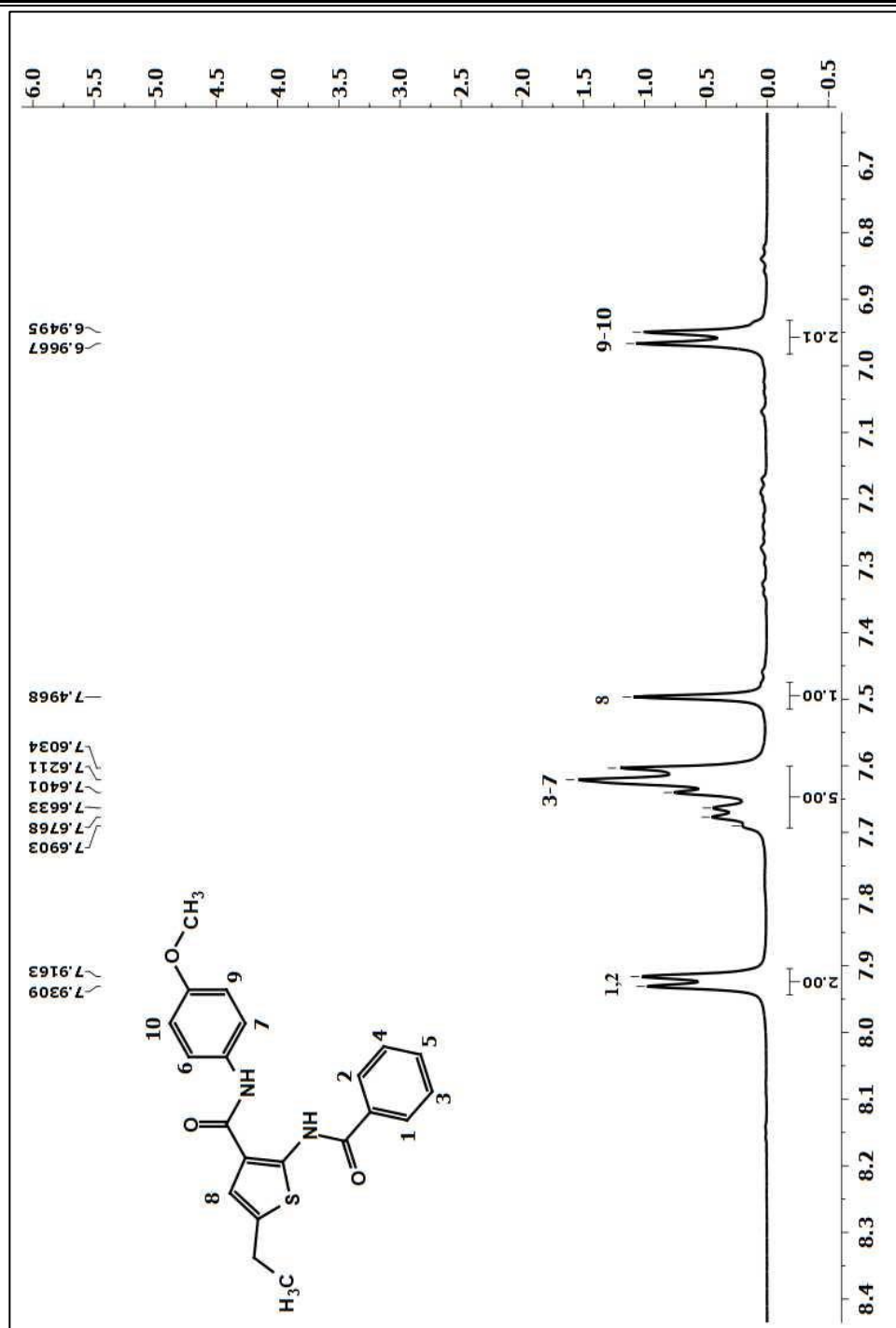
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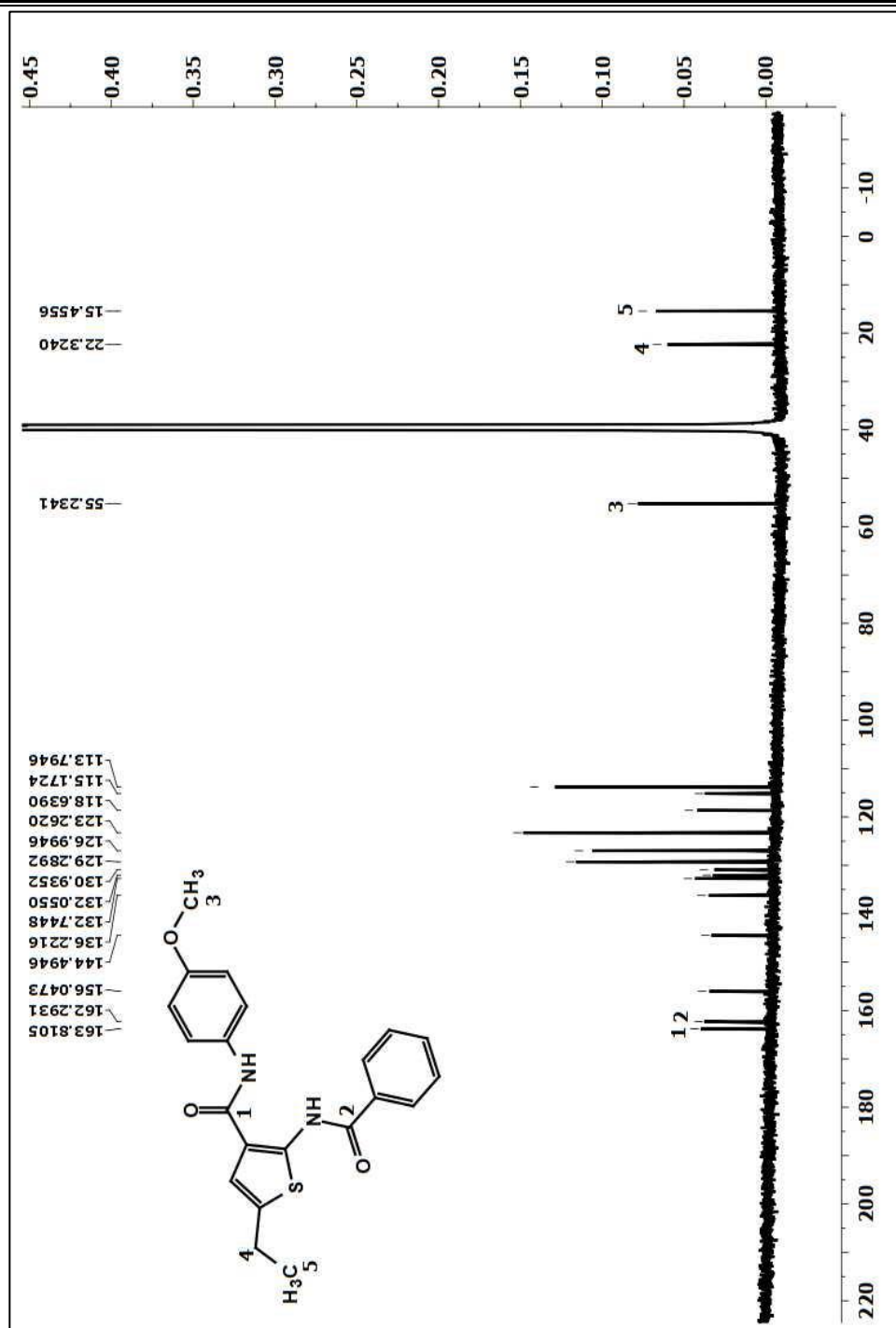
**Spectrum 1:**  $^1\text{H-NMR}$  spectrum (1) of 2-benzamido-5-ethyl-N-(4-methoxyphenyl)thiophene-3-carboxamide (**Va**)

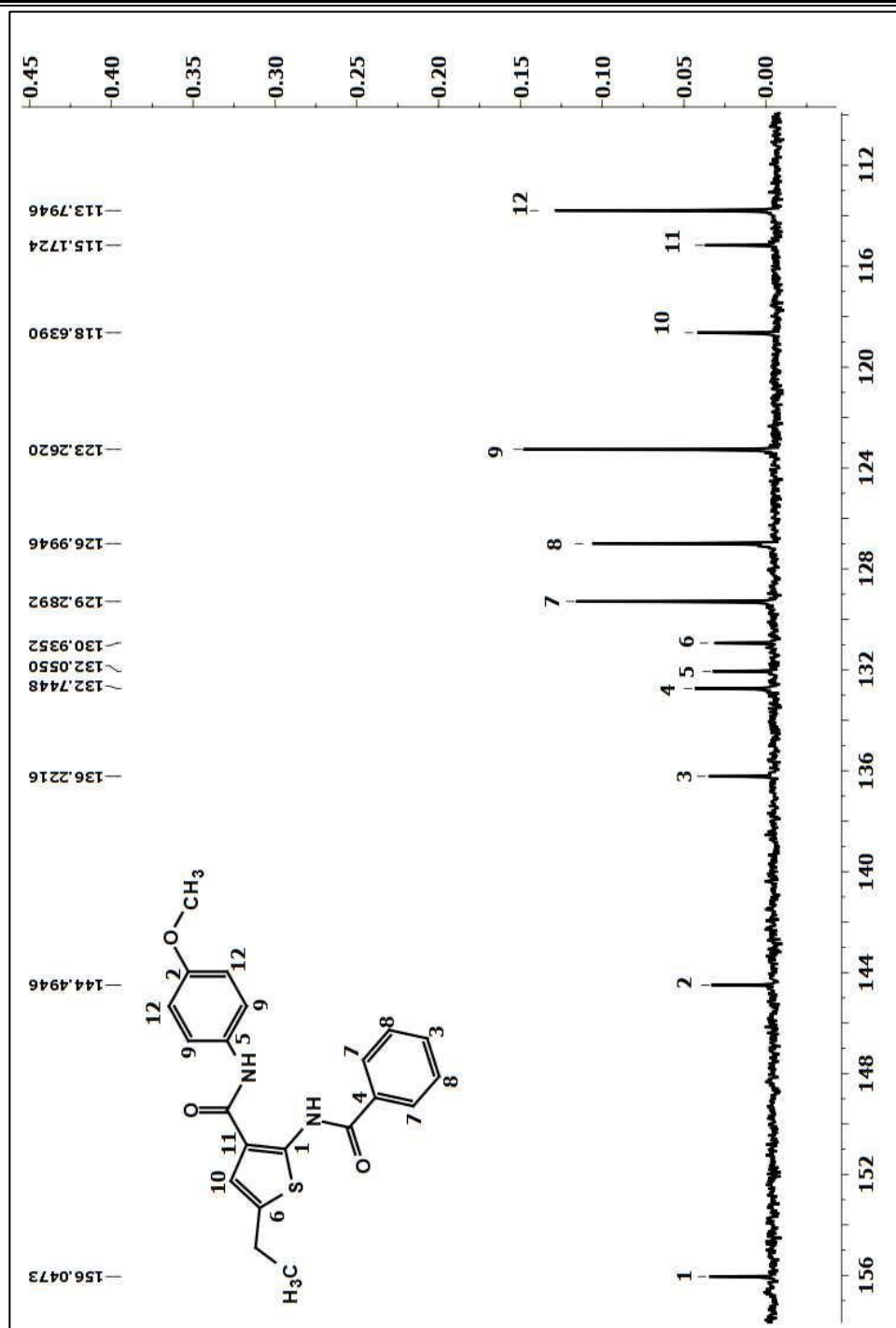


**Spectrum 2:** <sup>1</sup>H-NMR spectrum (2) of 2-benzamido-5-ethyl-N-(4-methoxyphenyl)thiophene-3-carboxamide (**Va**)

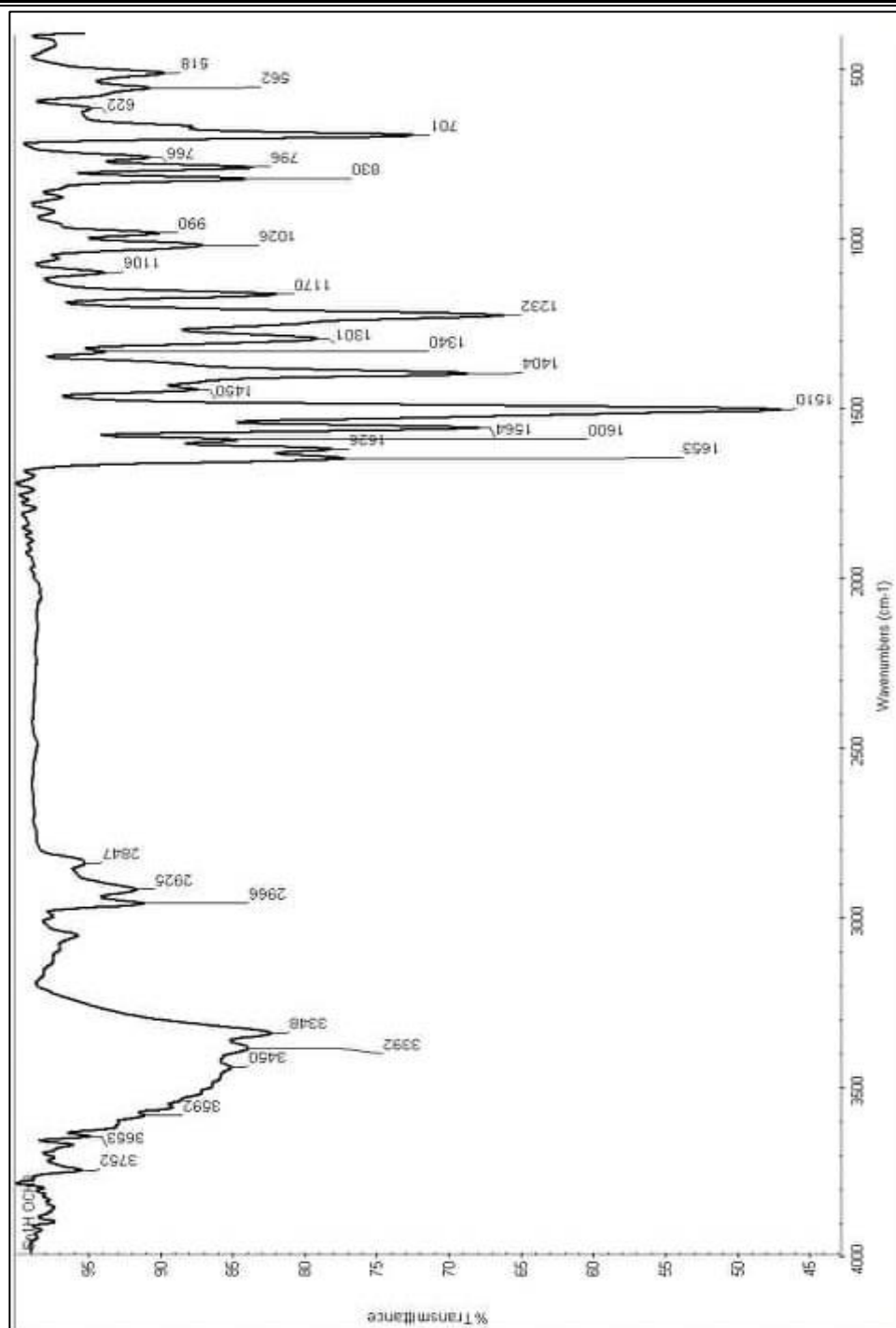


**Spectrum 3:** <sup>1</sup>H-NMR spectrum (3) of 2-benzamido-5-ethyl-N-(4-methoxyphenyl)thiophene-3-carboxamide (**Va**)

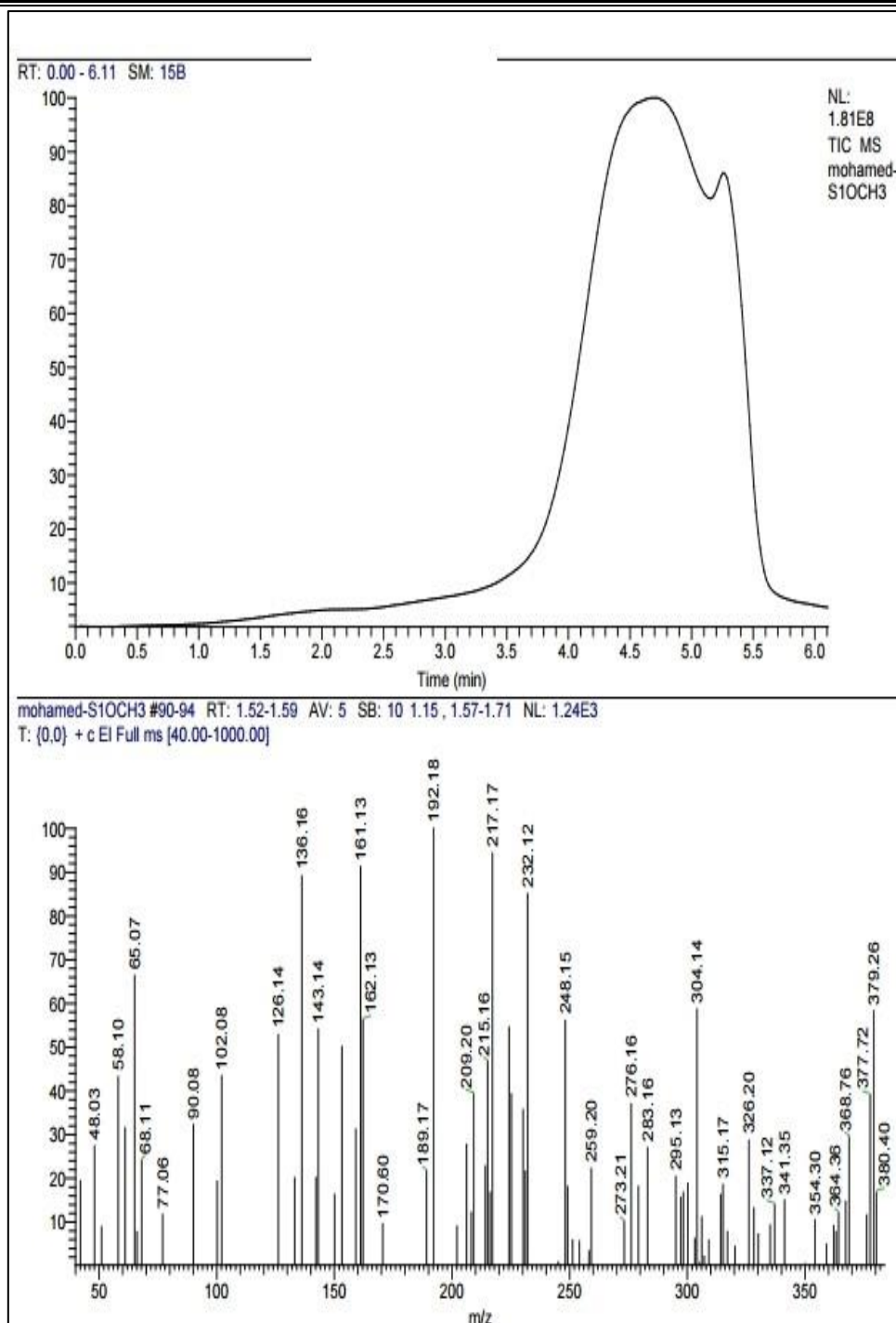




**Spectrum 5:**  $^{13}\text{C}$ -NMR spectrum (2) of 2-benzamido-5-ethyl-N-(4-methoxyphenyl)thiophene-3-carboxamide (**Va**)

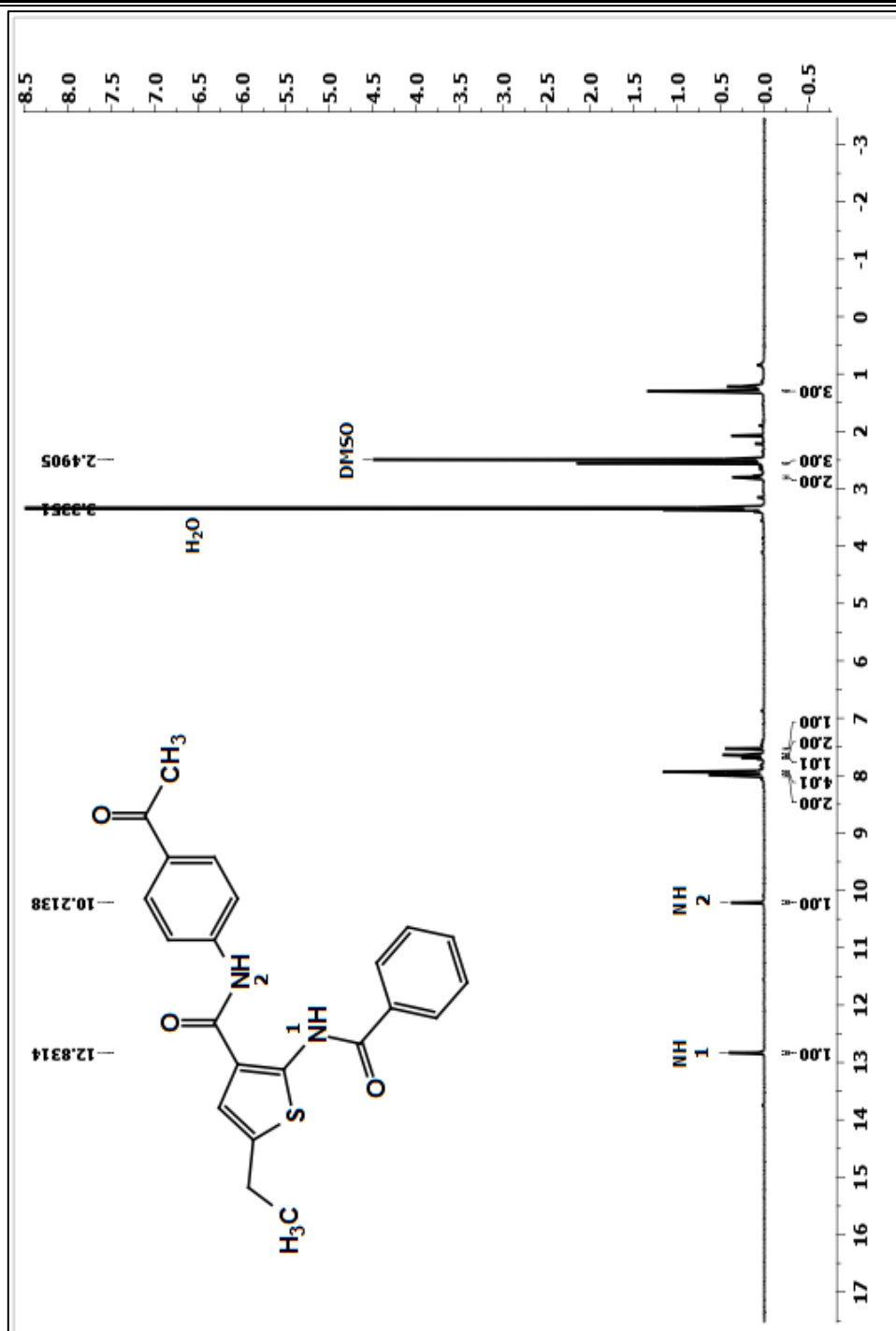


**Spectrum 6:** IR spectrum of 2-benzamido-5-ethyl-N-(4-methoxyphenyl)thiophene-3-carboxamide (**Va**)

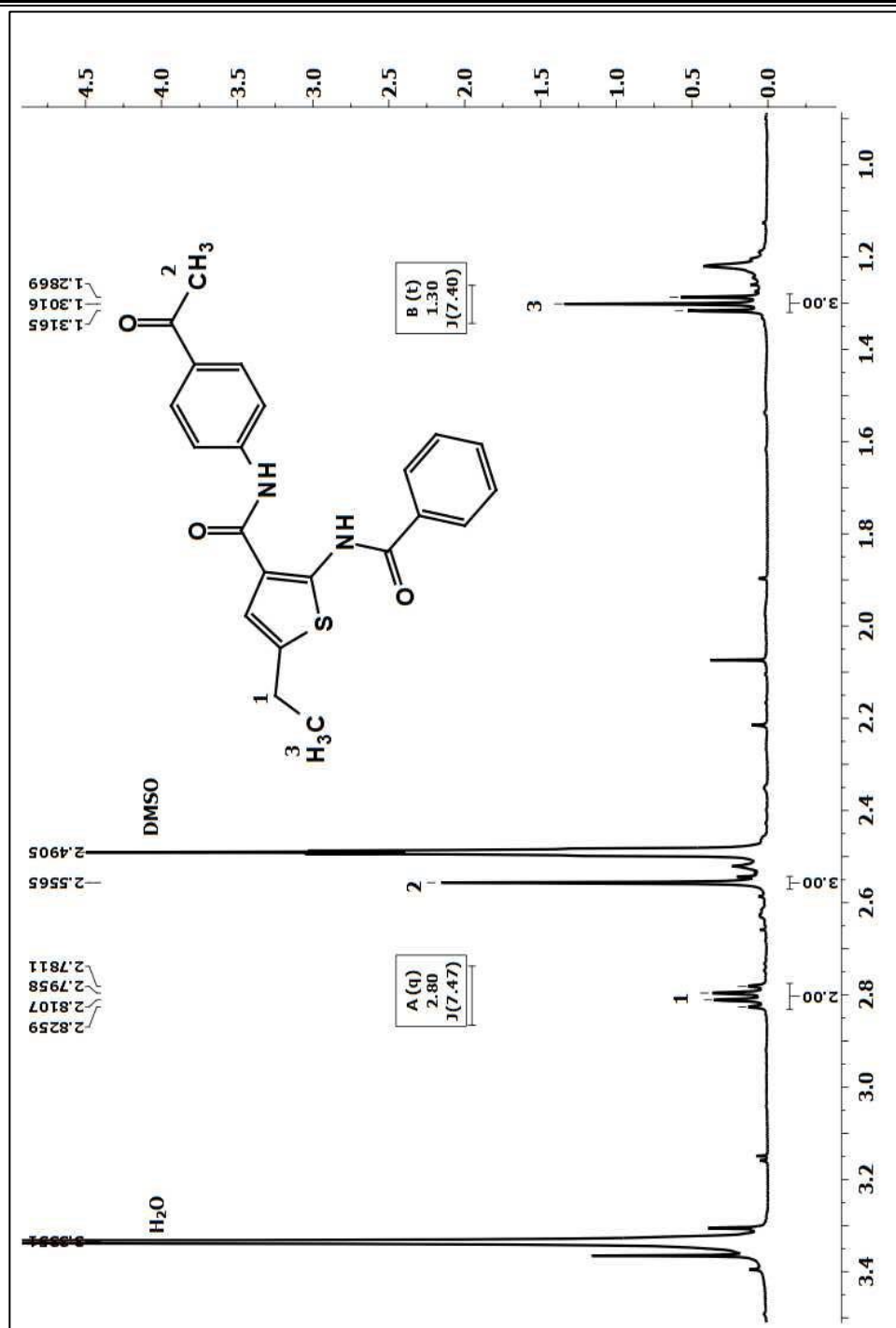


**Spectrum 7:** Mass spectrum of 2-benzamido-5-ethyl-N-(4-methoxyphenyl)thiophene-3-carboxamide (**Va**)

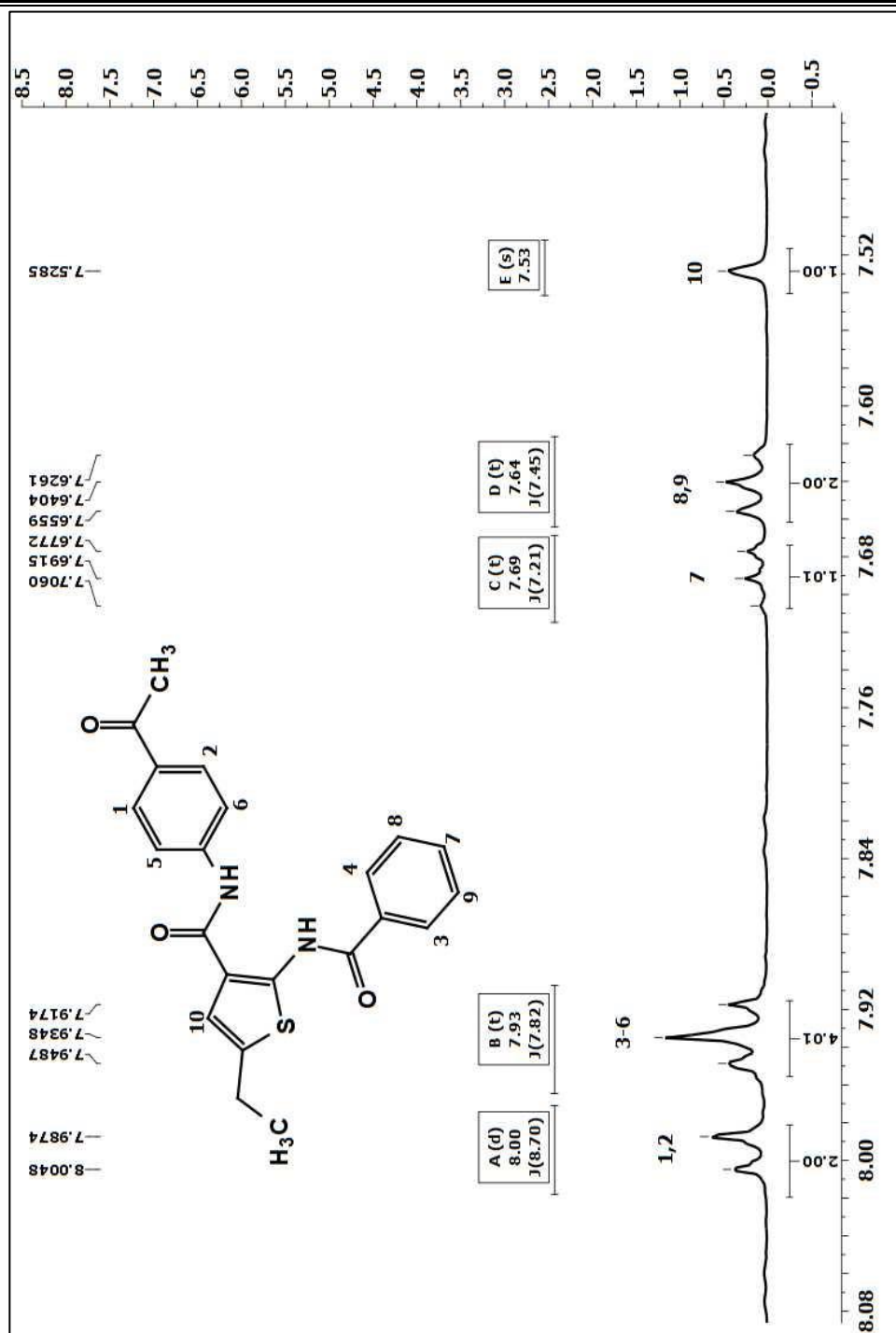




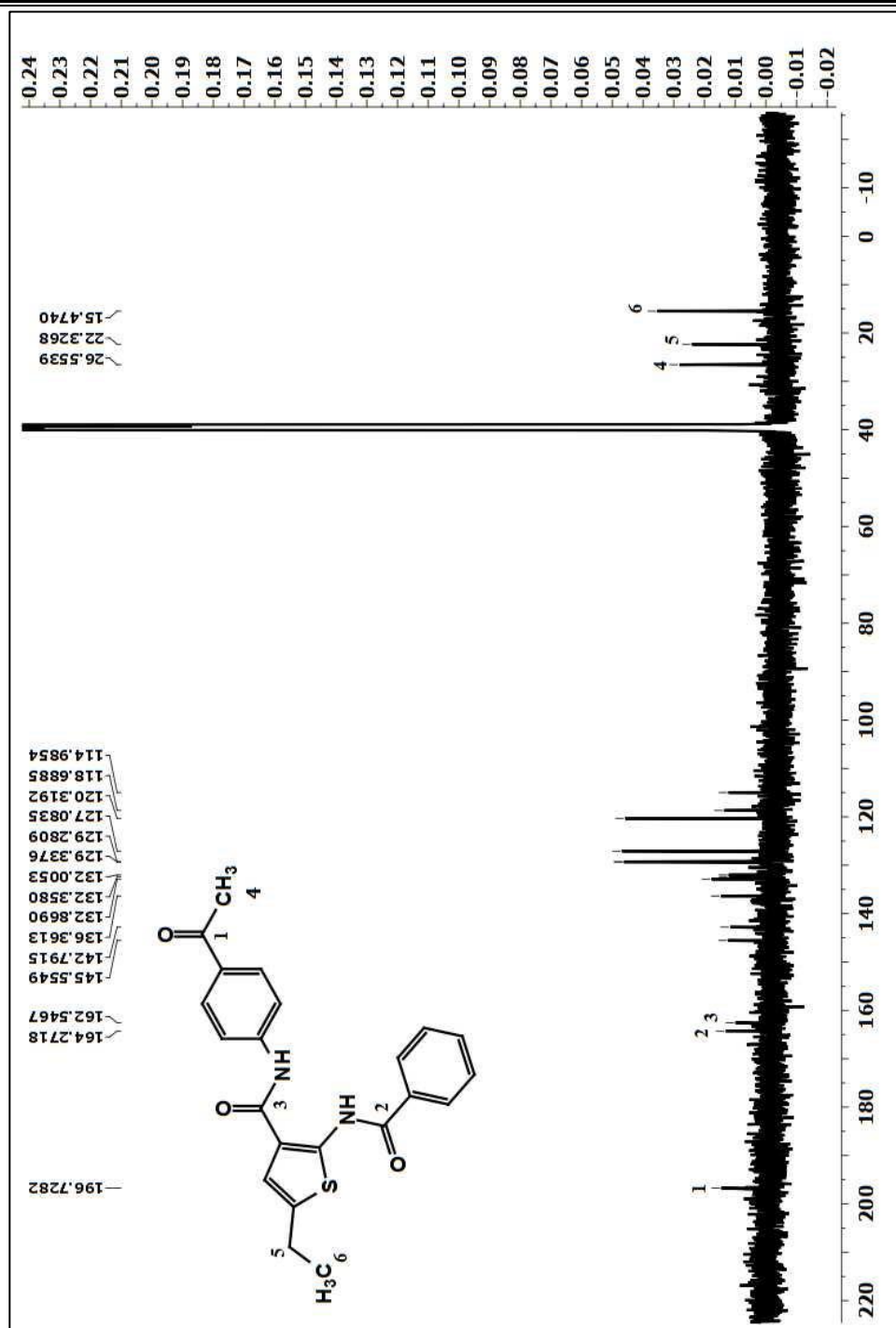
**Spectrum 8:** <sup>1</sup>H-NMR spectrum (1) of N-(4-acetylphenyl)-2-benzamido-5-ethylthiophene-3-carboxamide (VIa)



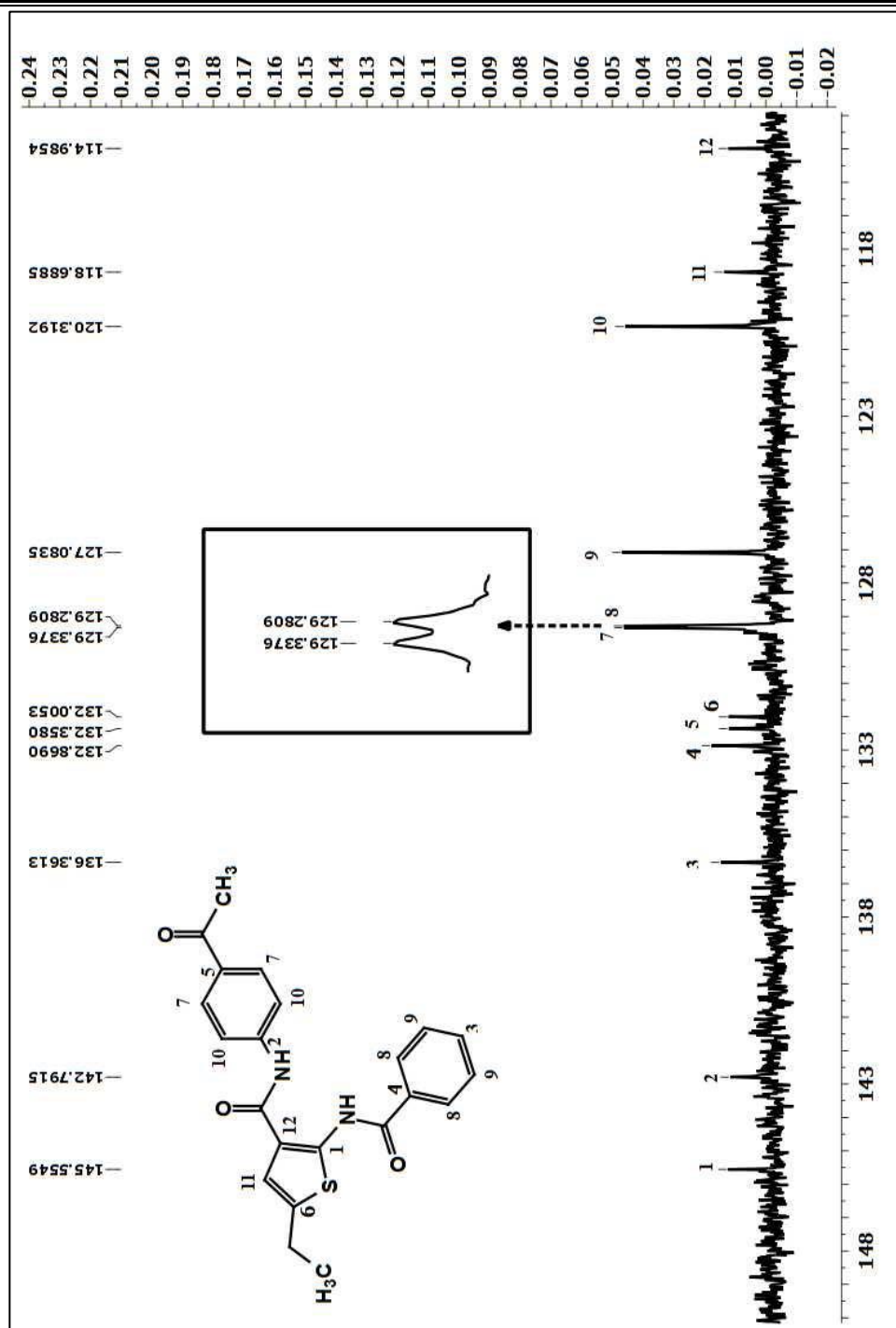
**Spectrum 9:**  $^1\text{H-NMR}$  spectrum (2) of N-(4-acetylphenyl)-2-benzamido-5-ethylthiophene-3-carboxamide (VIa)



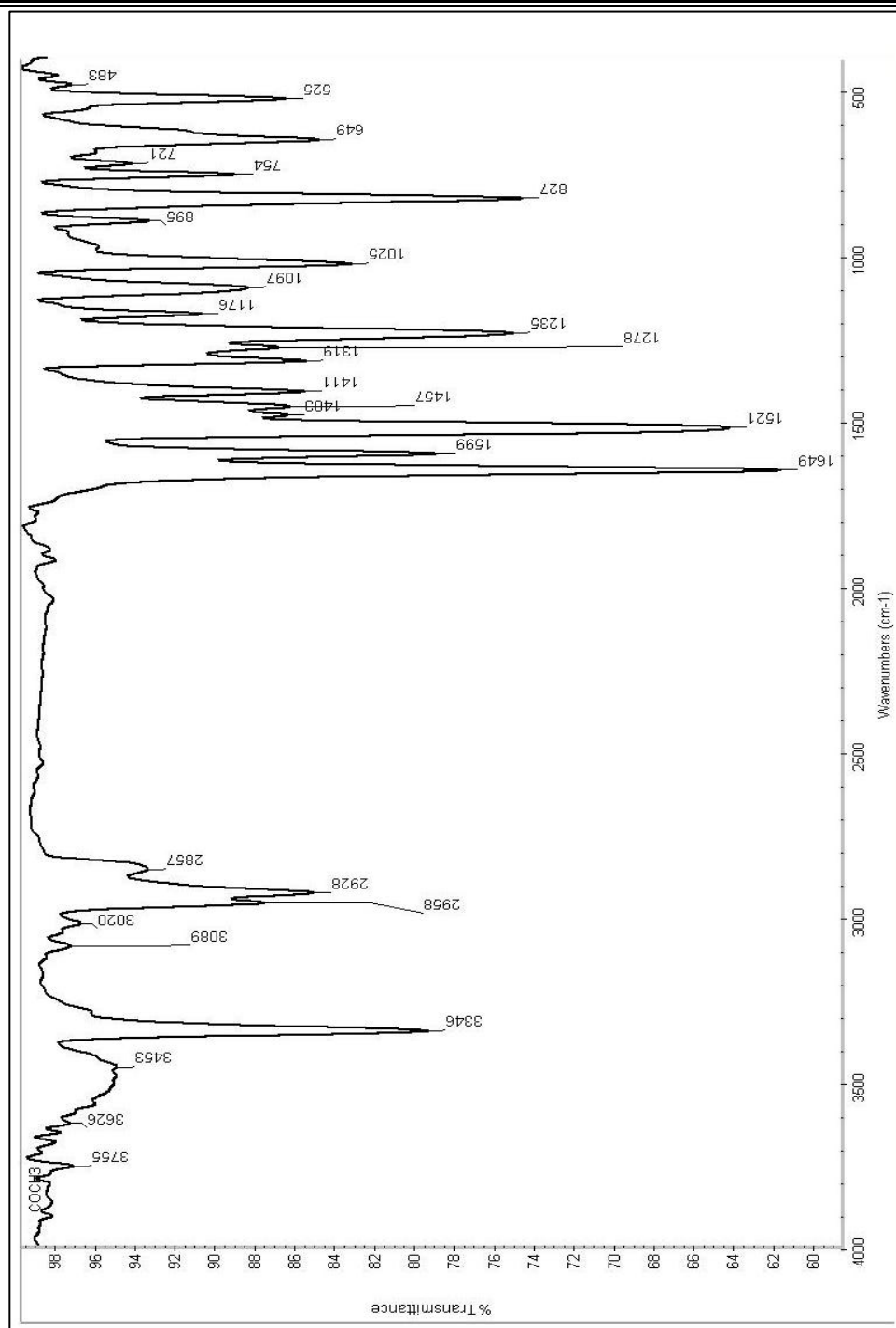
**Spectrum 10:** <sup>1</sup>H-NMR spectrum (3) of N-(4-acetylphenyl)-2-benzamido-5-ethylthiophene-3-carboxamide (VIa)



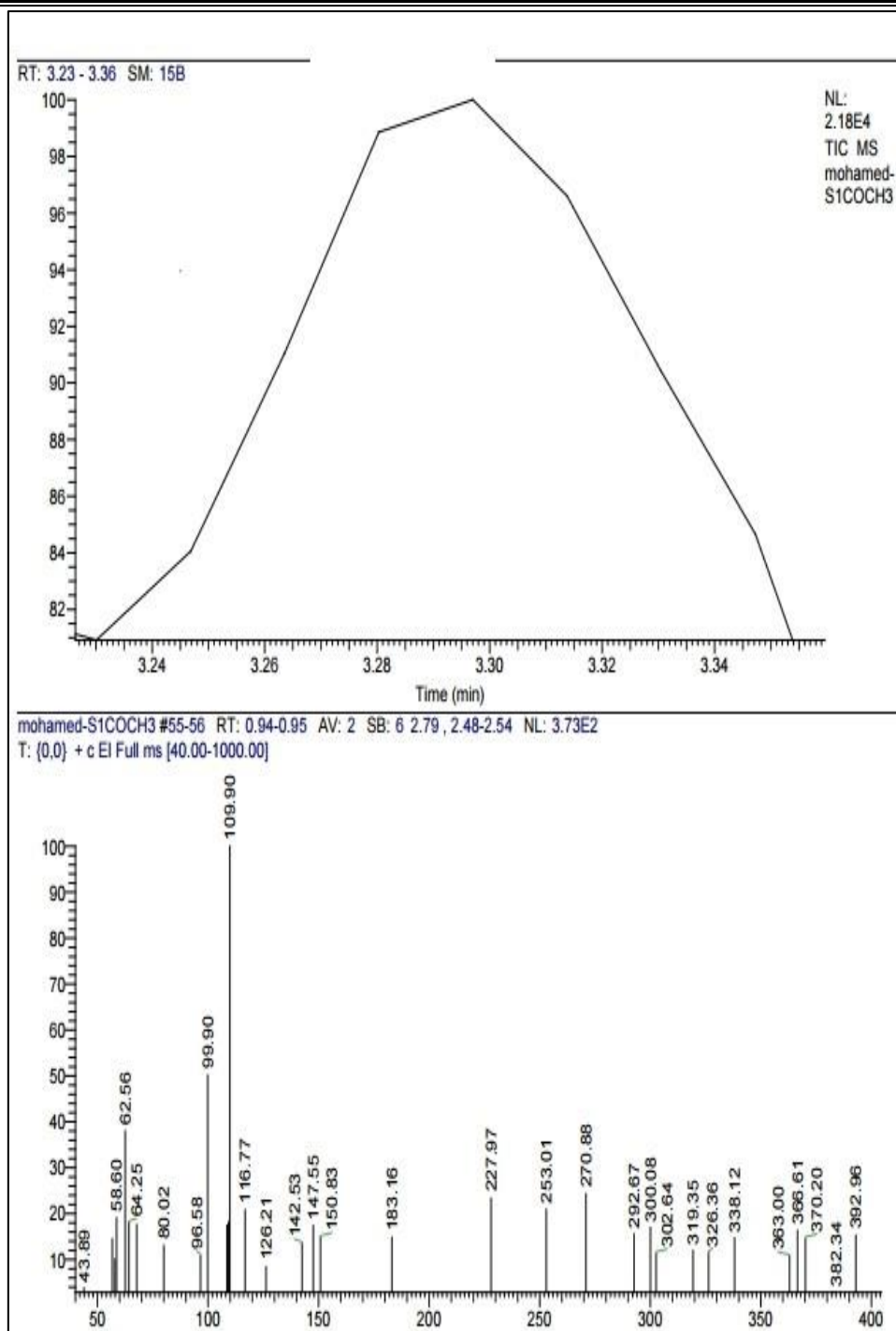
**Spectrum 11:**  $^{13}\text{C}$ -NMR spectrum (1) of N-(4-acetylphenyl)-2-benzamido-5-ethylthiophene-3-carboxamide (VIa)



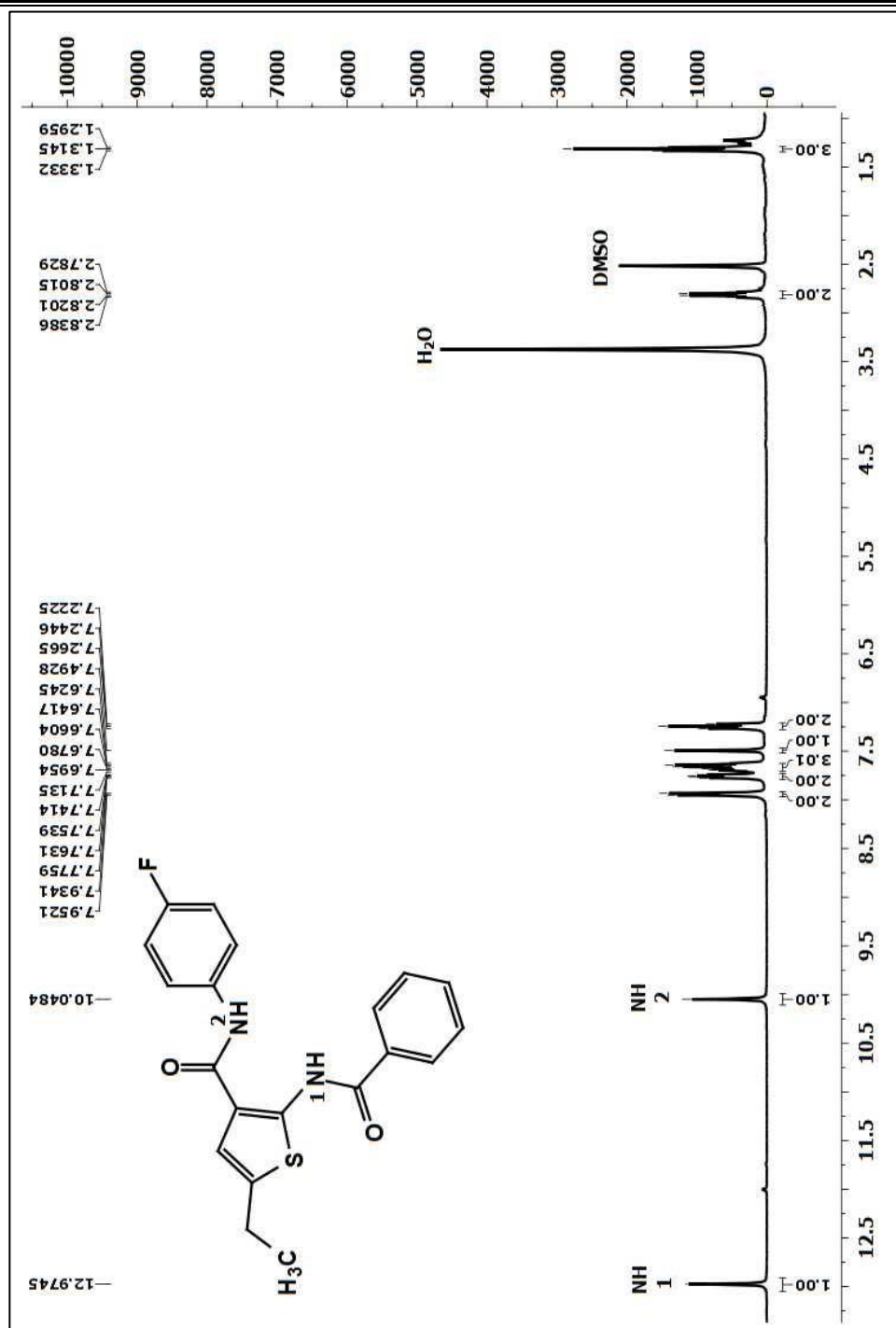
**Spectrum 12:**  $^{13}\text{C}$ -NMR spectrum (2) of N-(4-acetylphenyl)-2-benzamido-5-ethylthiophene-3-carboxamide (VIa)



**Spectrum 13:** IR spectrum of N-(4-acetylphenyl)-2-benzamido-5-ethylthiophene-3-carboxamide (**VIa**)

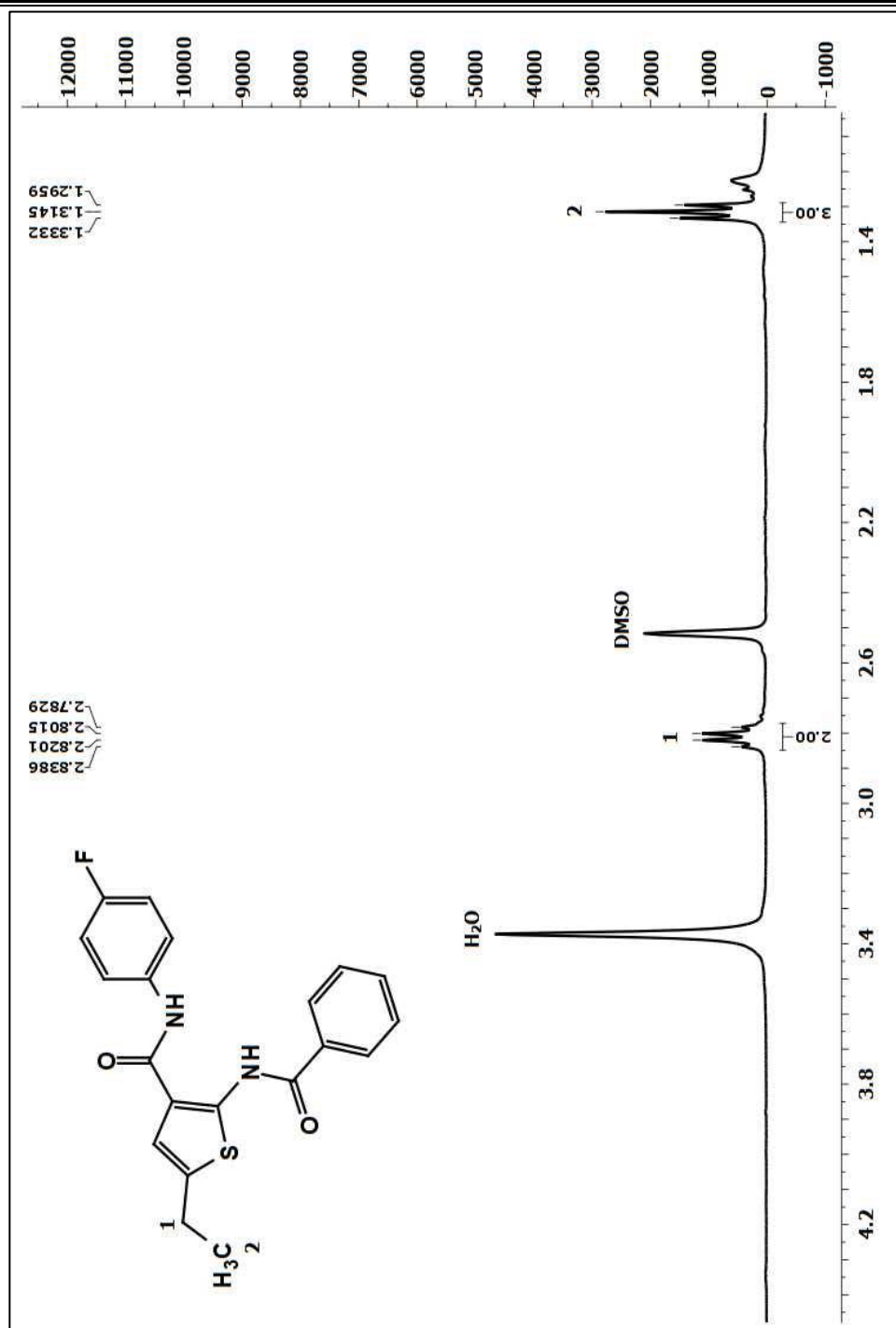


**Spectrum 14:** Mass spectrum of N-(4-acetylphenyl)-2-benzamido-5-ethylthiophene-3-carboxamide (**VIa**)

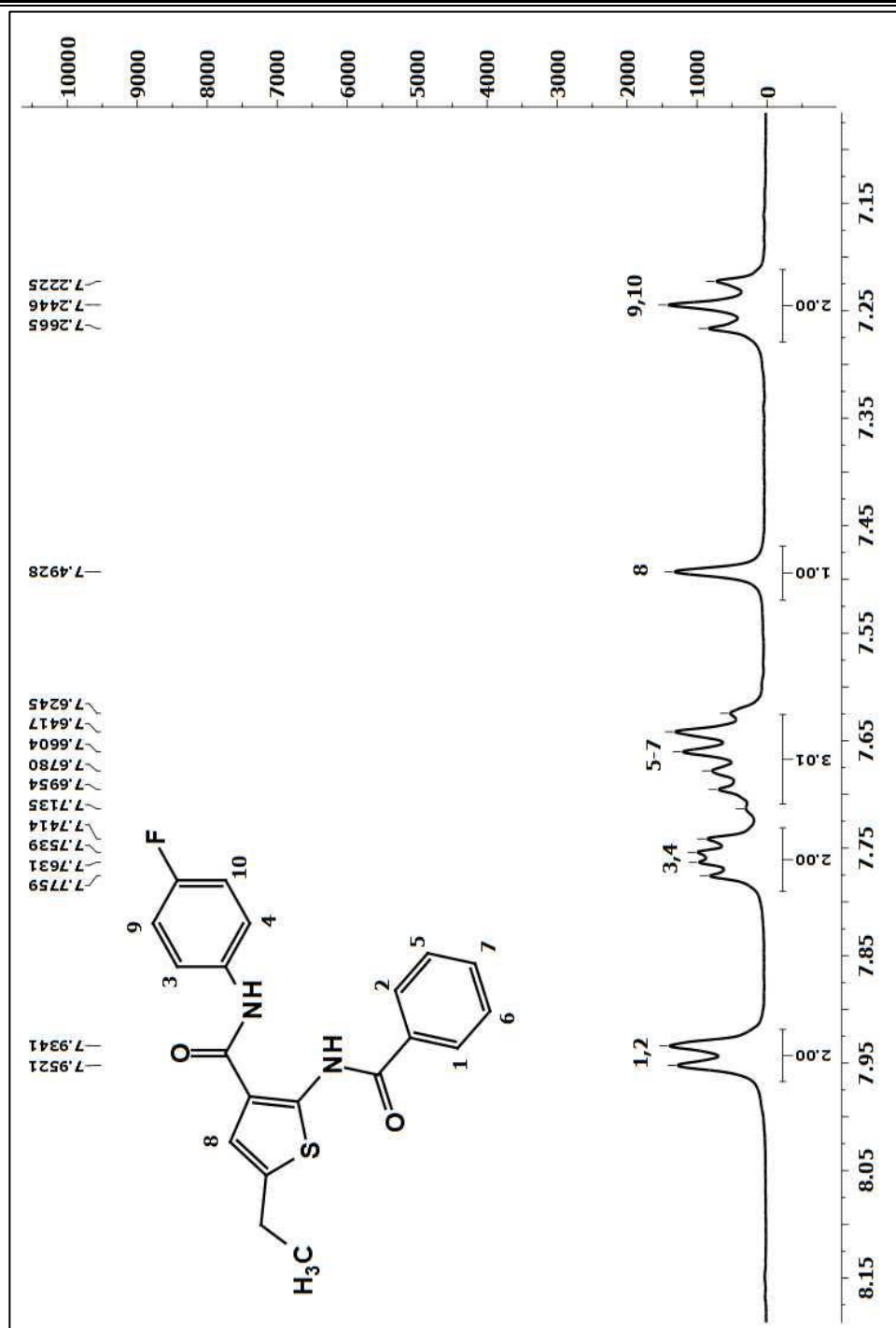


**Spectrum 15:**  $^1\text{H-NMR}$  spectrum (1) of 2-benzamido-5-ethyl-N-(4-fluorophenyl)thiophene-3-carboxamide (VIIa)

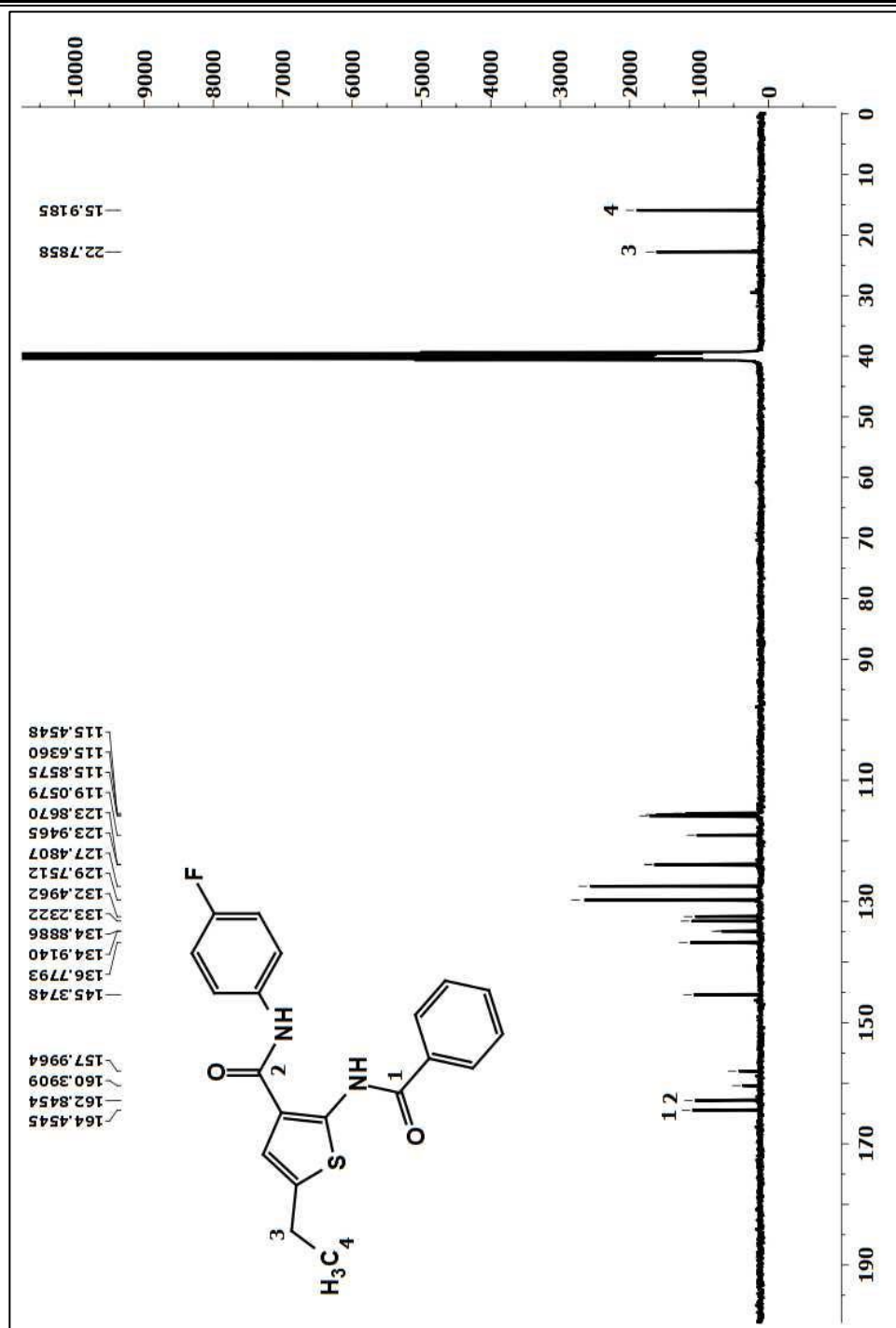




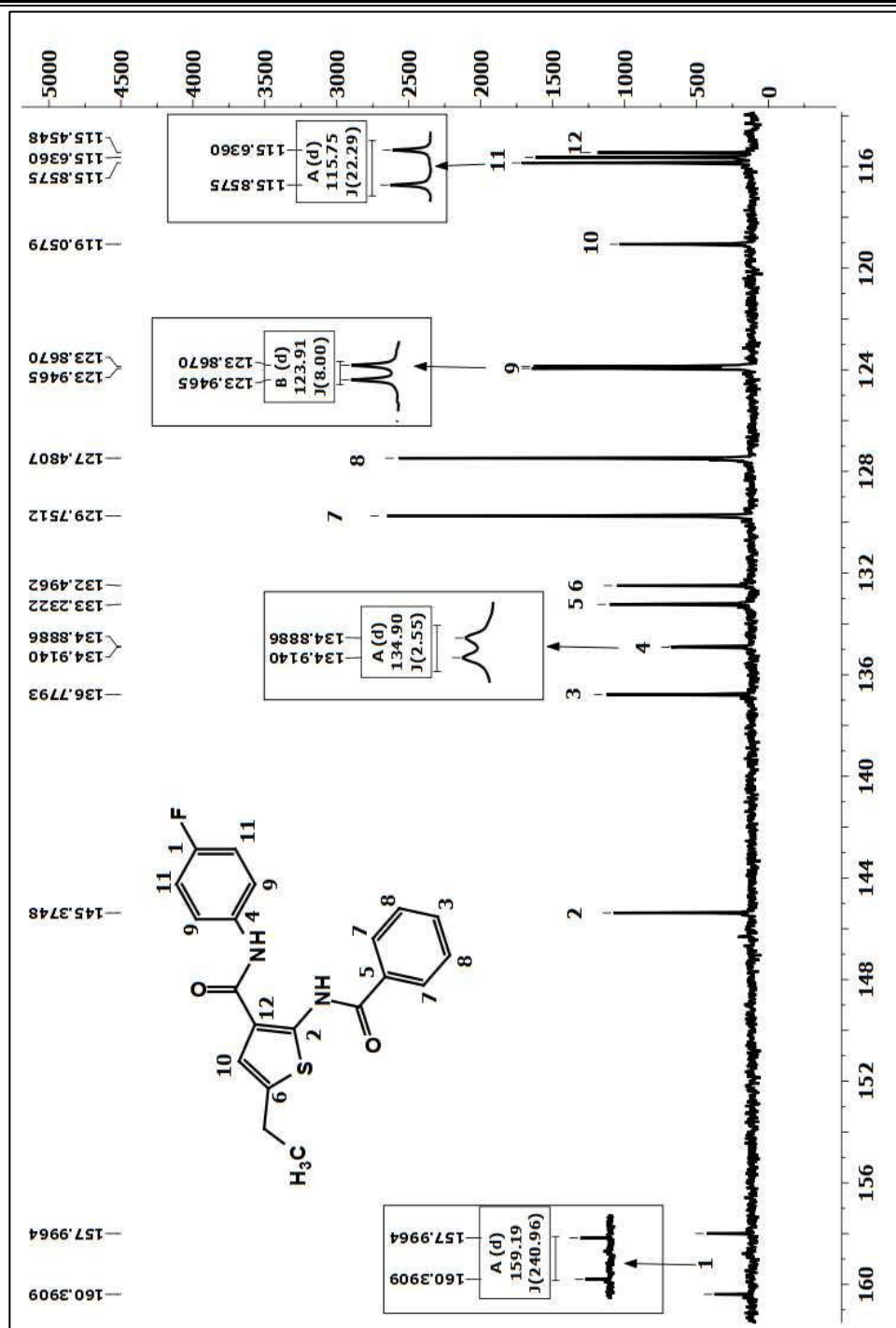
**Spectrum 16:**  $^1\text{H-NMR}$  spectrum (2) of 2-benzamido-5-ethyl-N-(4-fluorophenyl)thiophene-3-carboxamide (**VIIa**)



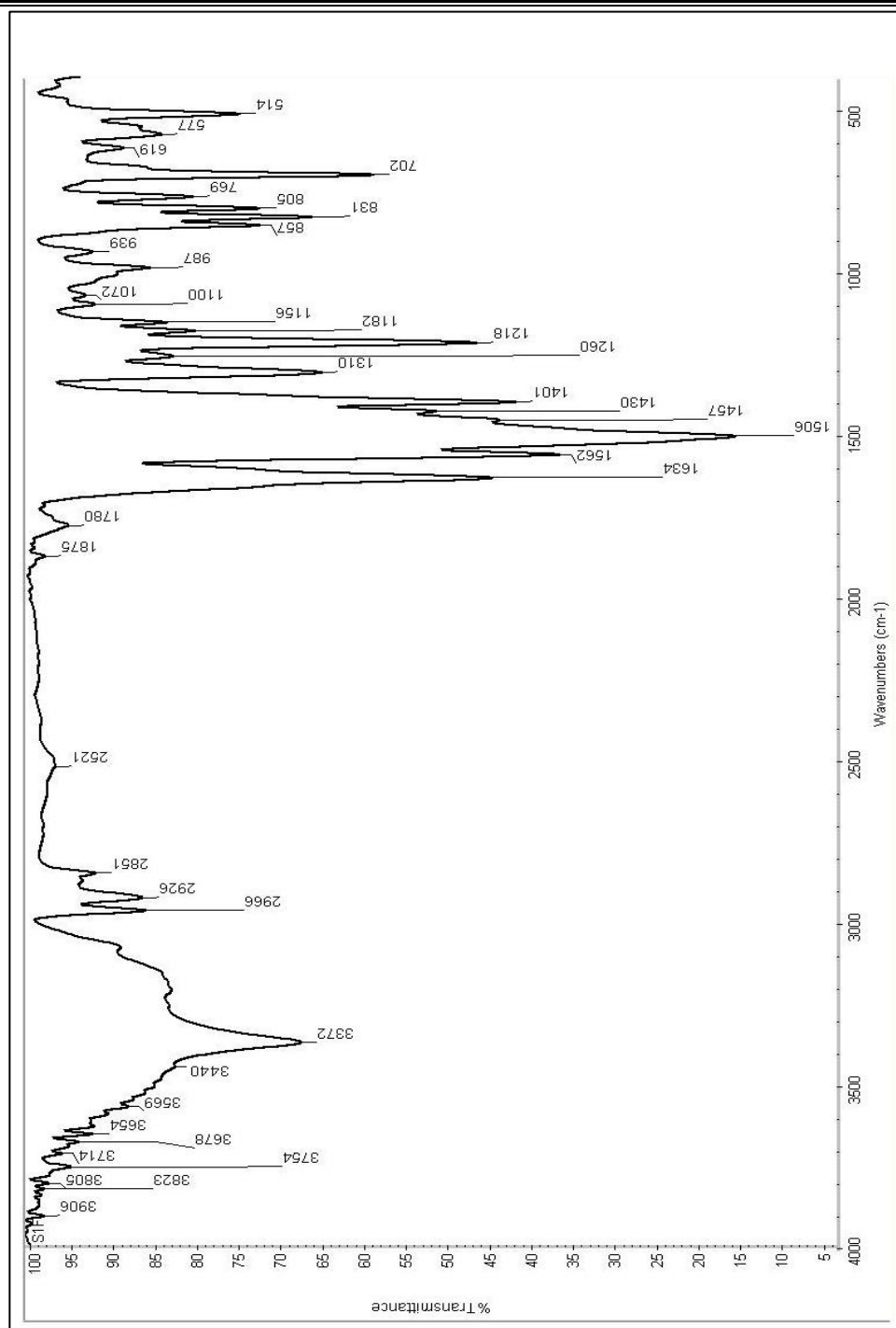
**Spectrum 17:**  $^1\text{H-NMR}$  spectrum (3) of 2-benzamido-5-ethyl-N-(4-fluorophenyl)thiophene-3-carboxamide (VIIa)



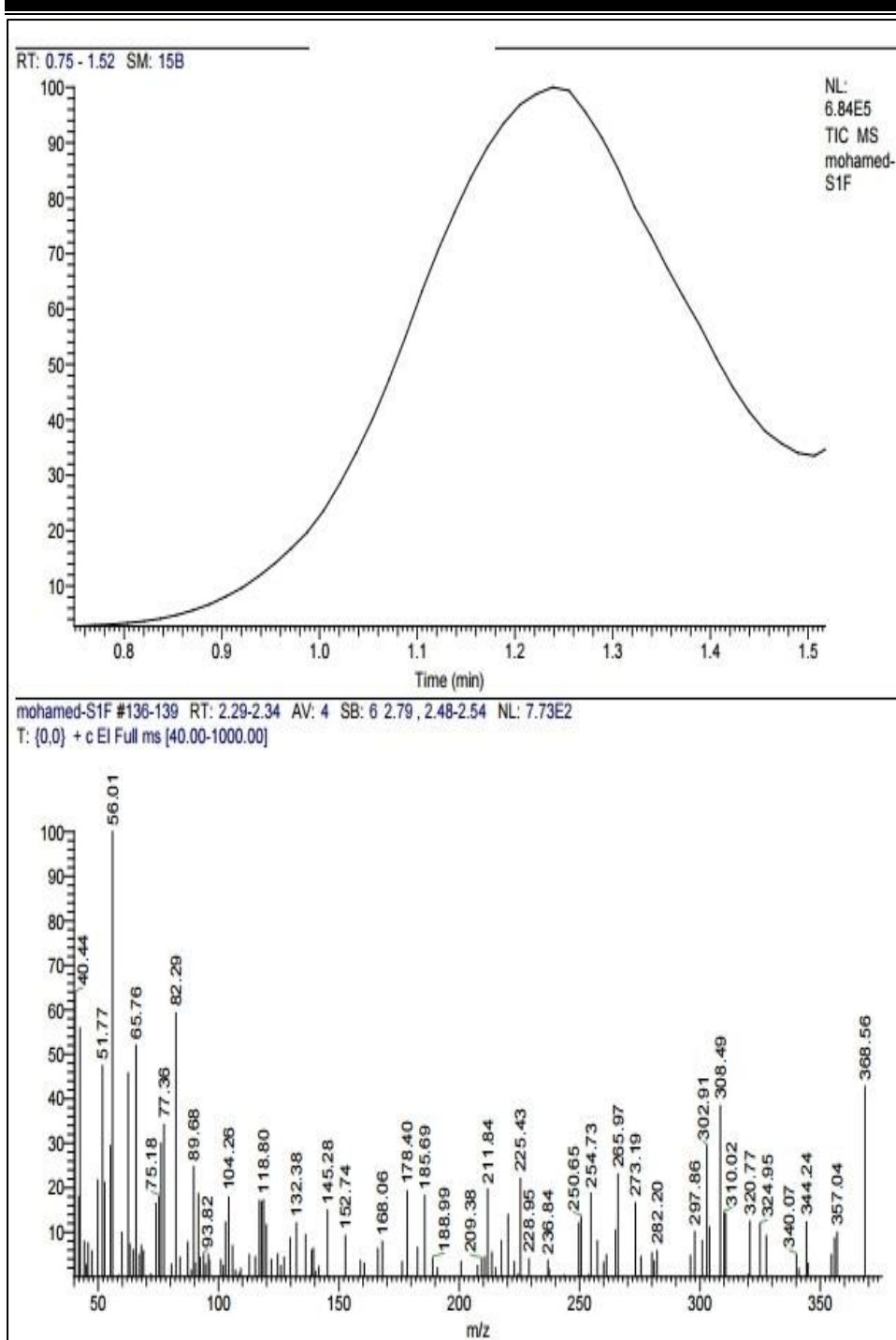
**Spectrum 18:**  $^{13}\text{C}$ -NMR spectrum (1) of 2-benzamido-5-ethyl-N-(4-fluorophenyl)thiophene-3-carboxamide (VIIa)



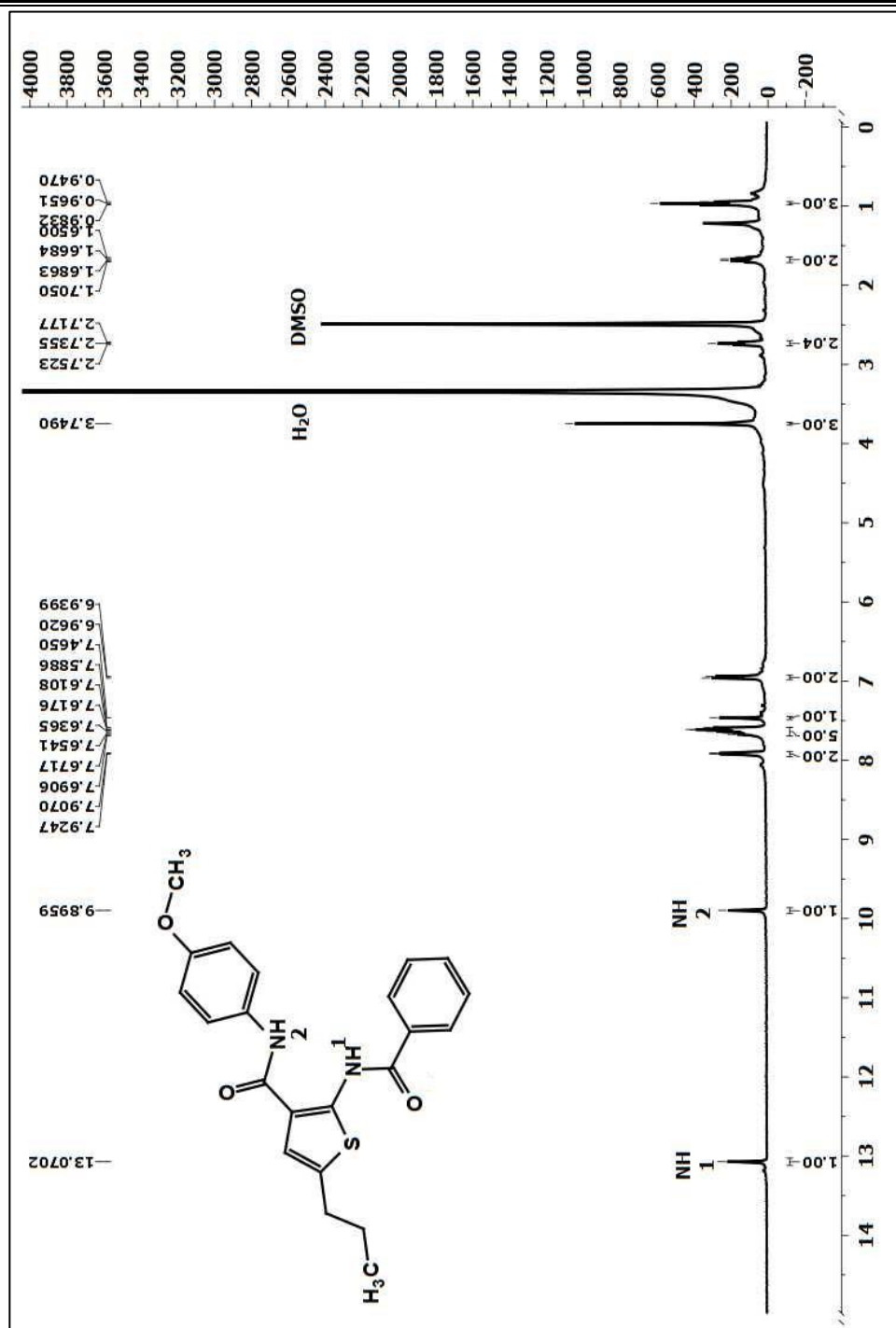
**Spectrum 19:**  $^{13}\text{C}$ -NMR spectrum (2) of 2-benzamido-5-ethyl-N-(4-fluorophenyl)thiophene-3-carboxamide (VIIa)



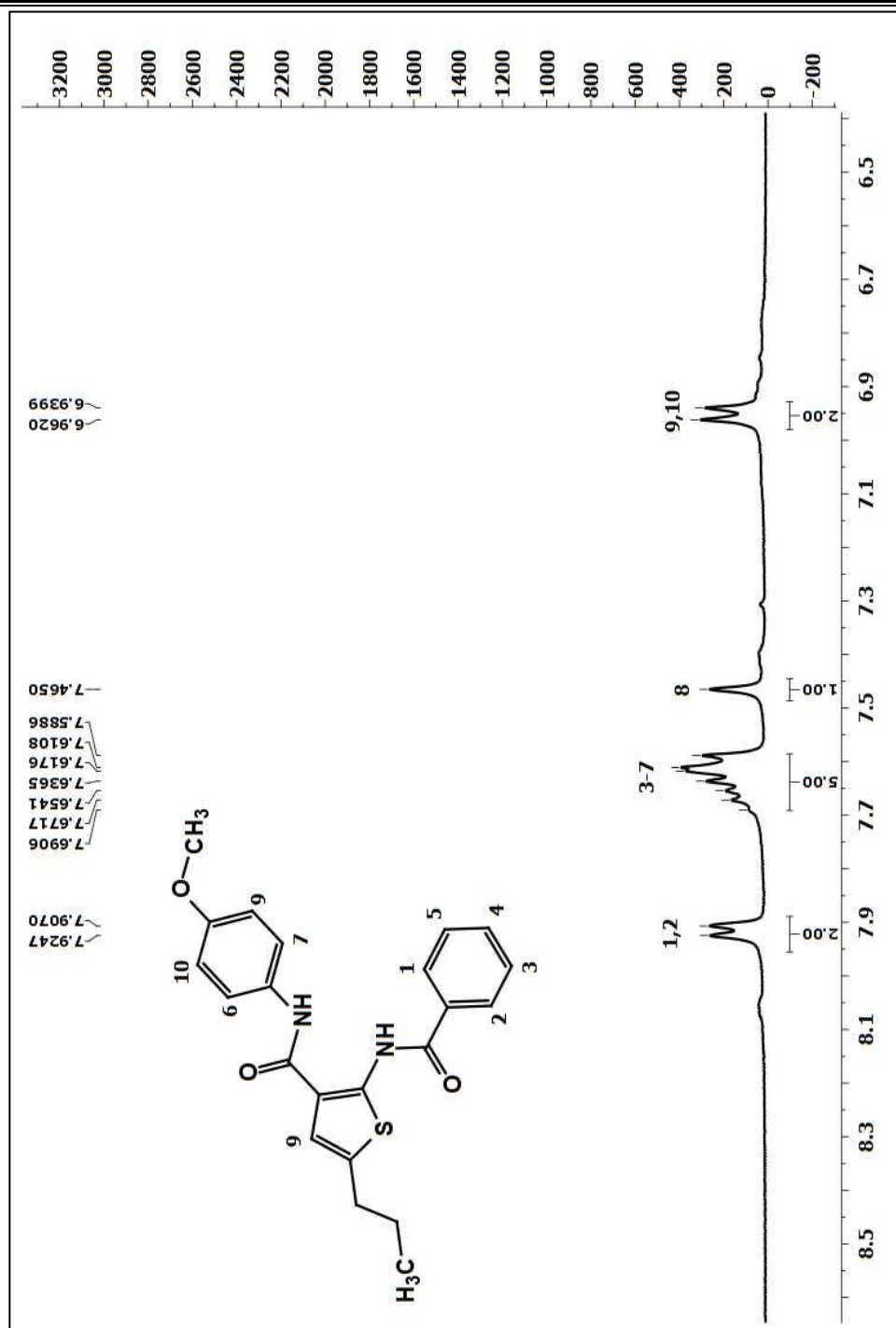
**Spectrum 20:** IR spectrum of 2-benzamido-5-ethyl-N-(4-fluorophenyl)thiophene-3-carboxamide (**VIIa**)



**Spectrum 21:** Mass spectrum of 2-benzamido-5-ethyl-N-(4-fluorophenyl)thiophene-3-carboxamide (**VIIa**)

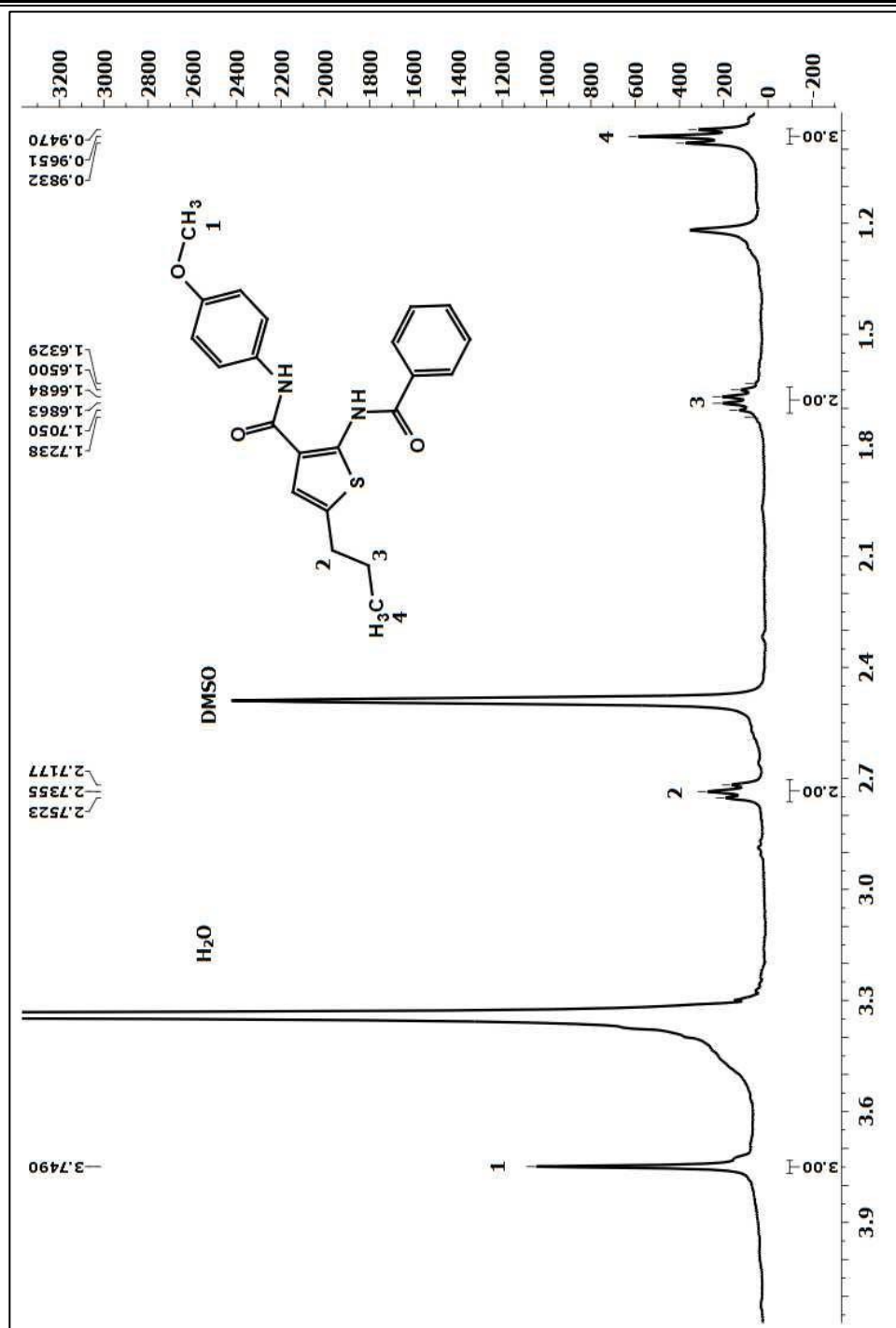


**Spectrum 22:** <sup>1</sup>H-NMR spectrum (1) of 2-benzamido-N-(4-methoxyphenyl)-5-propylthiophene-3-carboxamide (**Vb**)

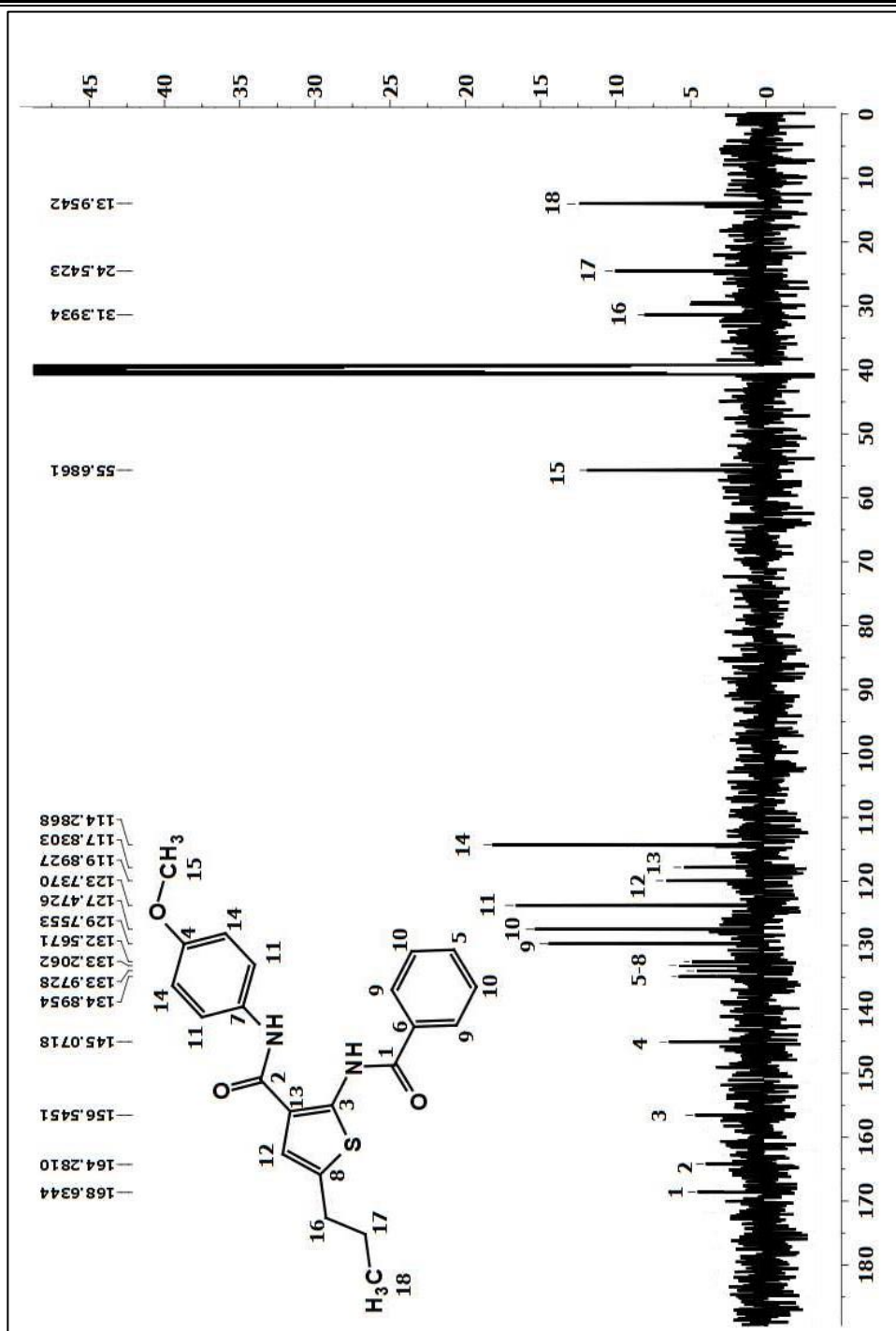


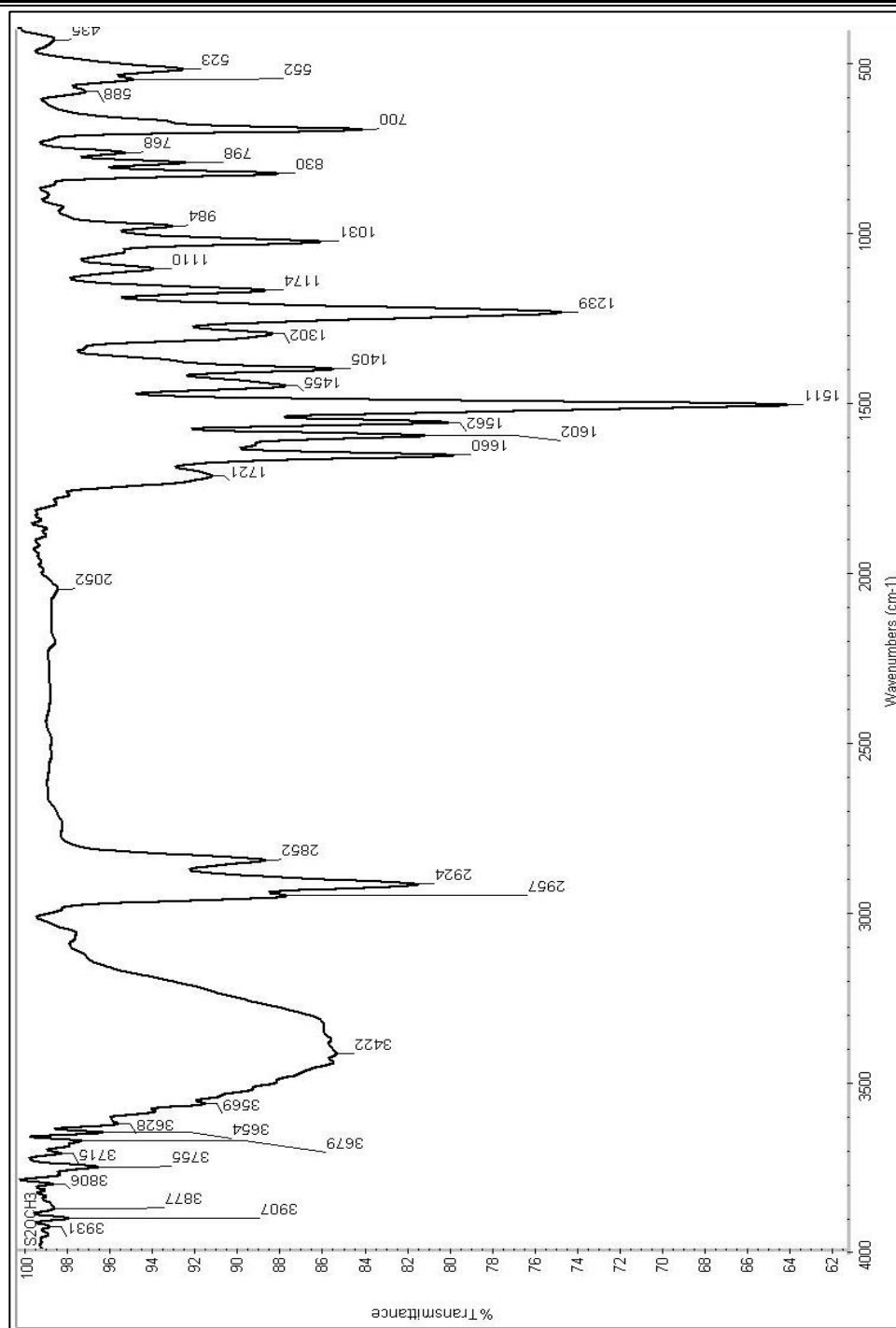
**Spectrum 23:** <sup>1</sup>H-NMR spectrum (2) of 2-benzamido-N-(4-methoxyphenyl)-5-propylthiophene-3-carboxamide (**Vb**)



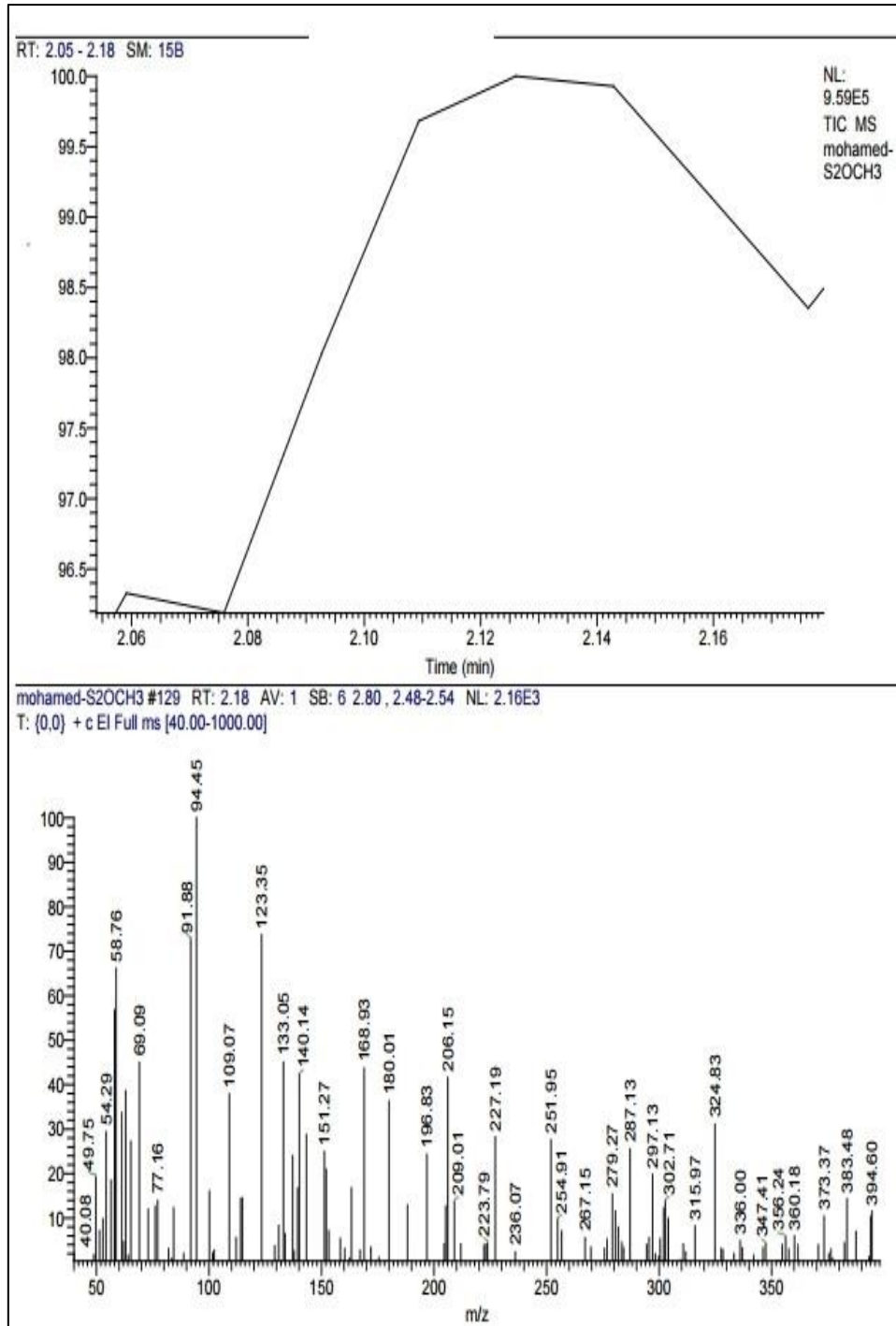


**Spectrum 24:**  $^1\text{H-NMR}$  spectrum (3) of 2-benzamido-N-(4-methoxyphenyl)-5-propylthiophene-3-carboxamide (**Vb**)

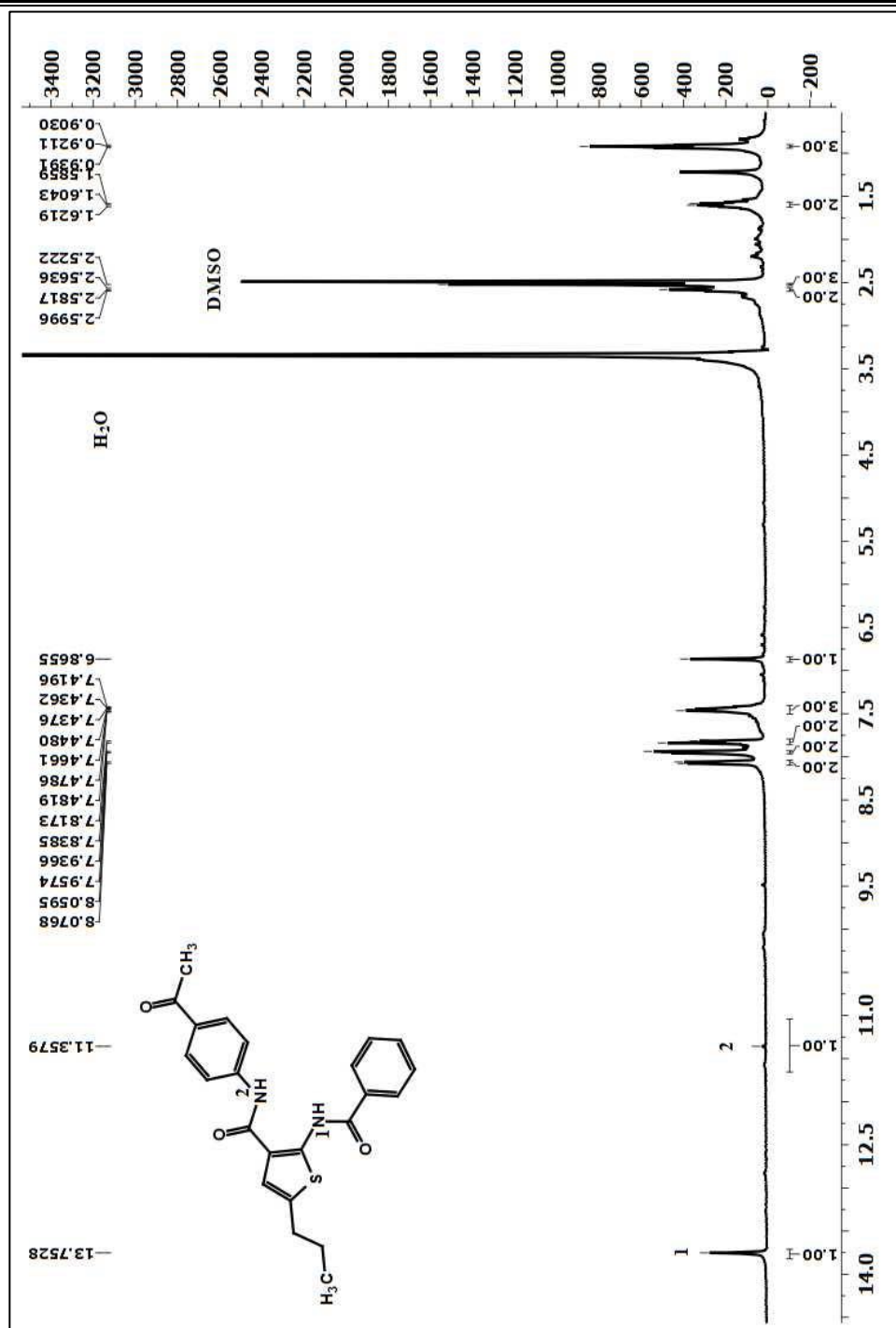


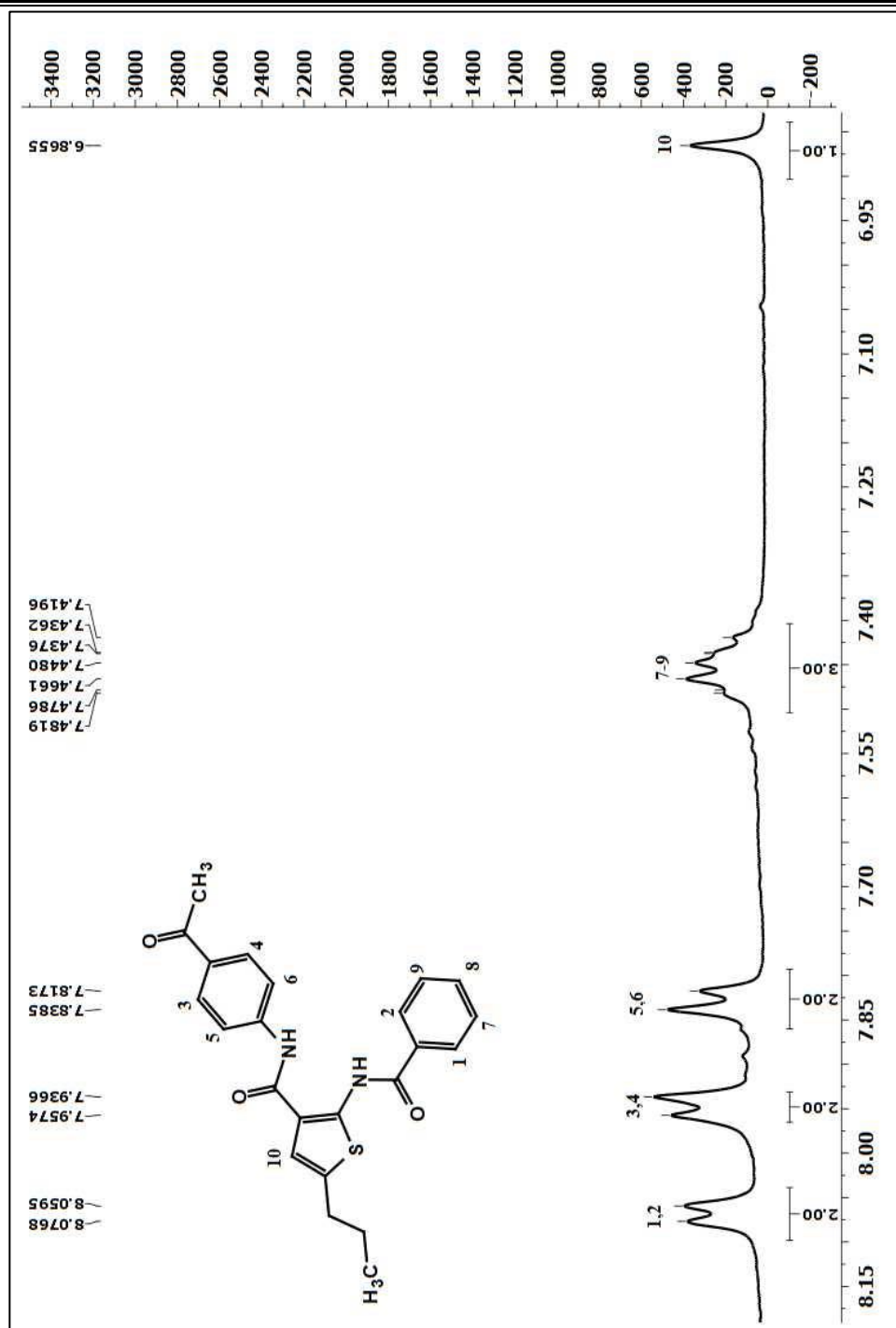


**Spectrum 26:** IR spectrum of 2-benzamido-N-(4-methoxyphenyl)-5-propylthiophene-3-carboxamide (**Vb**)

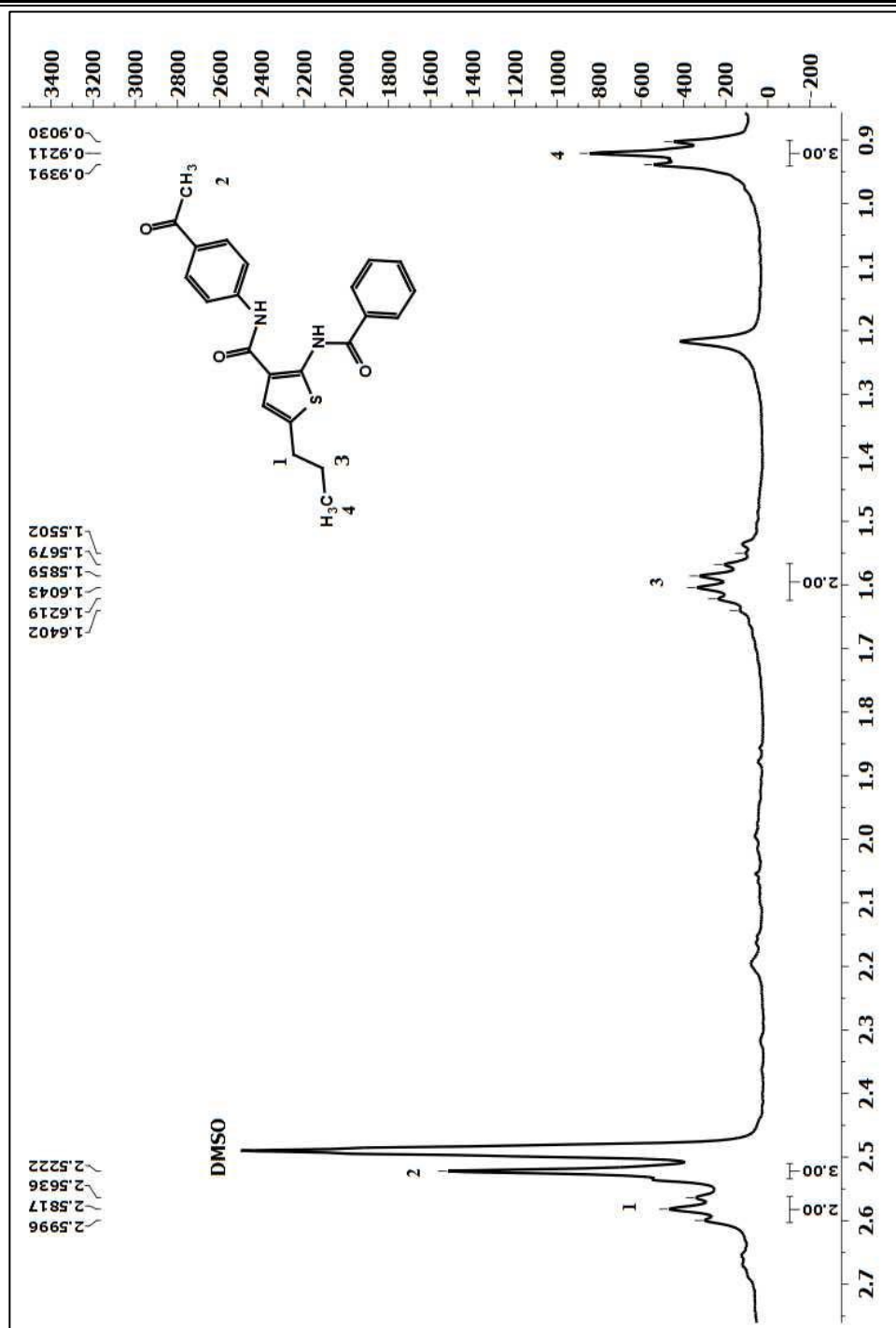


**Spectrum 27:** Mass spectrum of 2-benzamido-N-(4-methoxyphenyl)-5-propylthiophene-3-carboxamide (**Vb**)

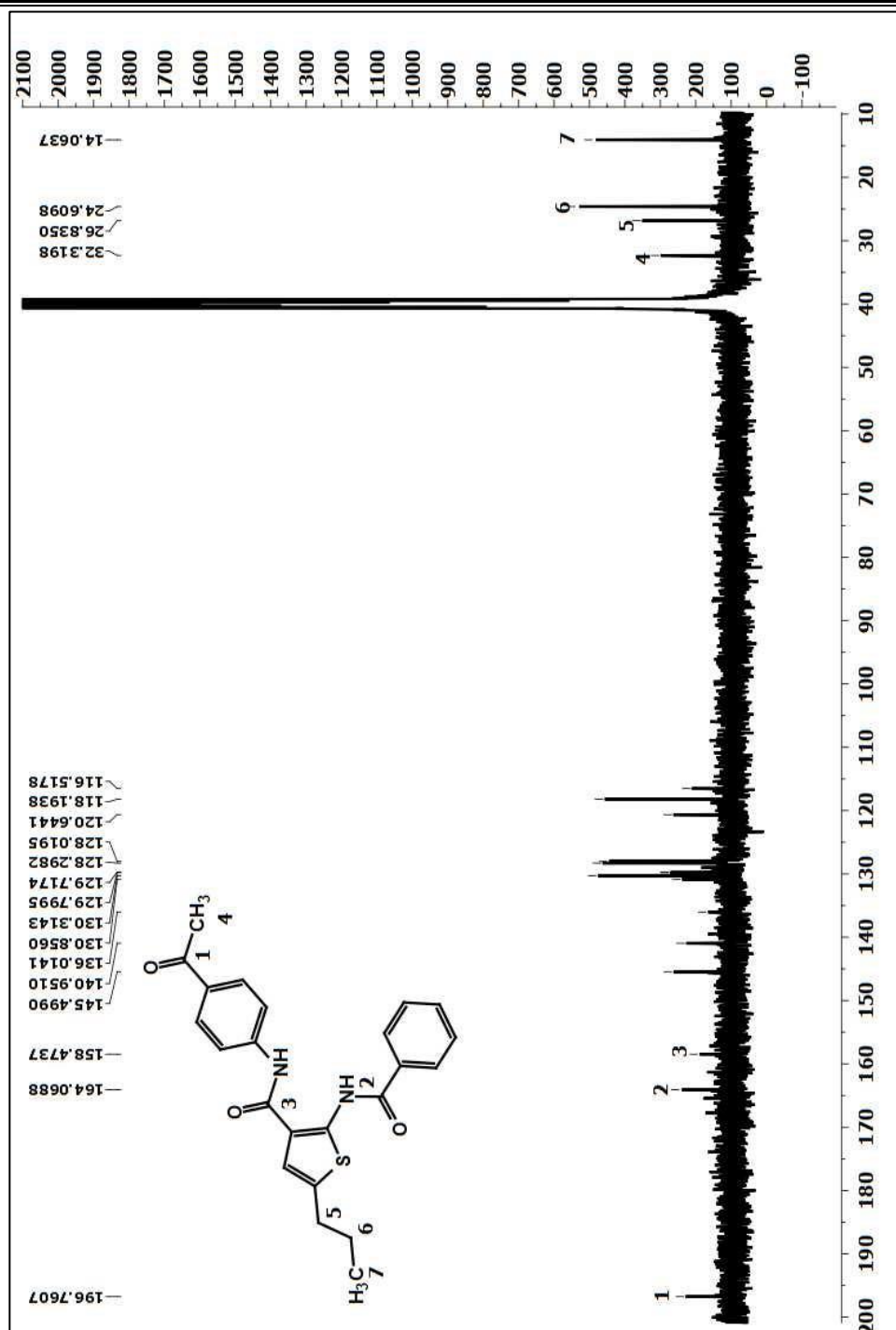




**Spectrum 29:**  $^1\text{H-NMR}$  spectrum (2) of N-(4-acetylphenyl)-2-benzamido-5-propylthiophene-3-carboxamide (VIb)

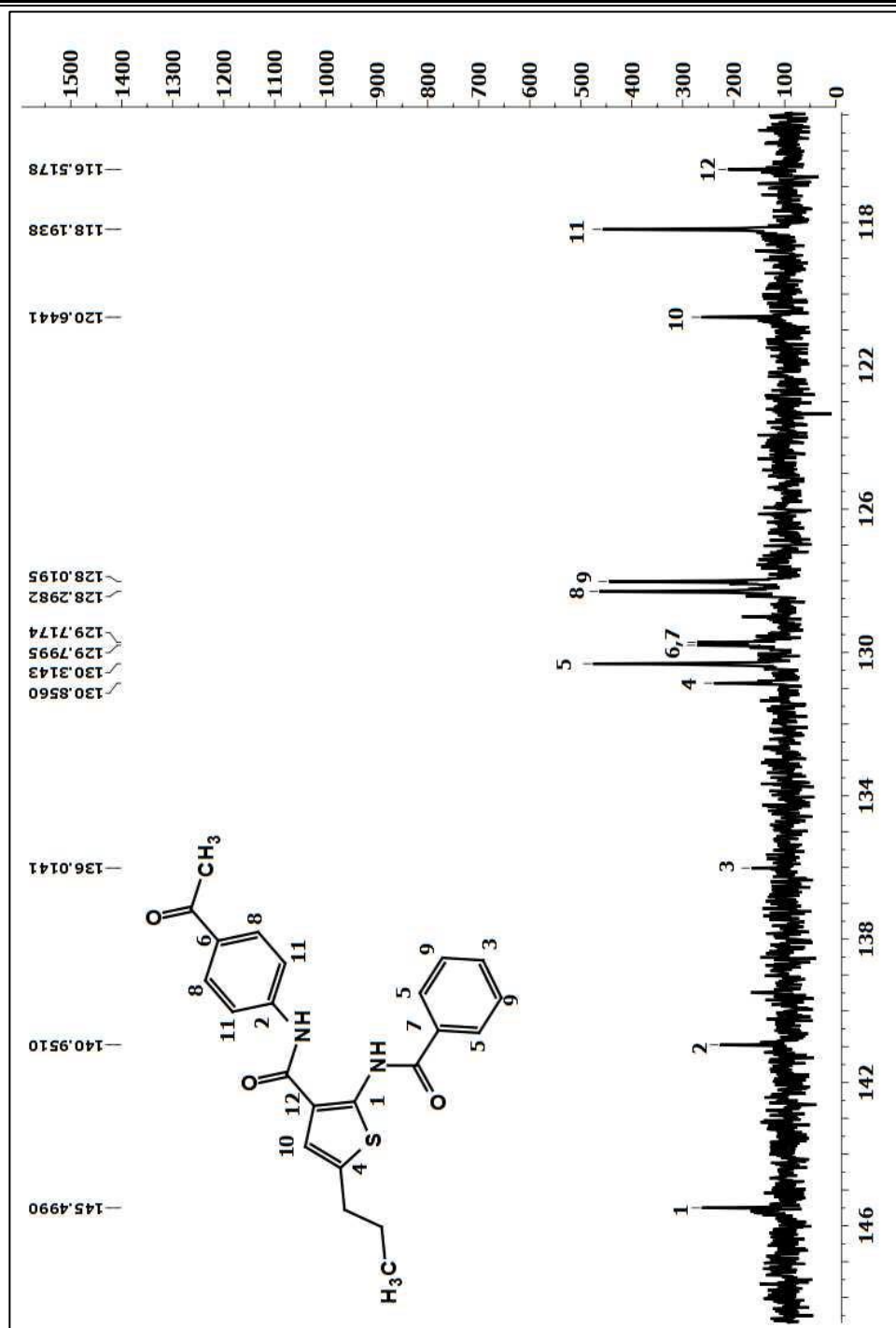


**Spectrum 30:** <sup>1</sup>H-NMR spectrum (3) of N-(4-acetylphenyl)-2-benzamido-5-propylthiophene-3-carboxamide (VIb)

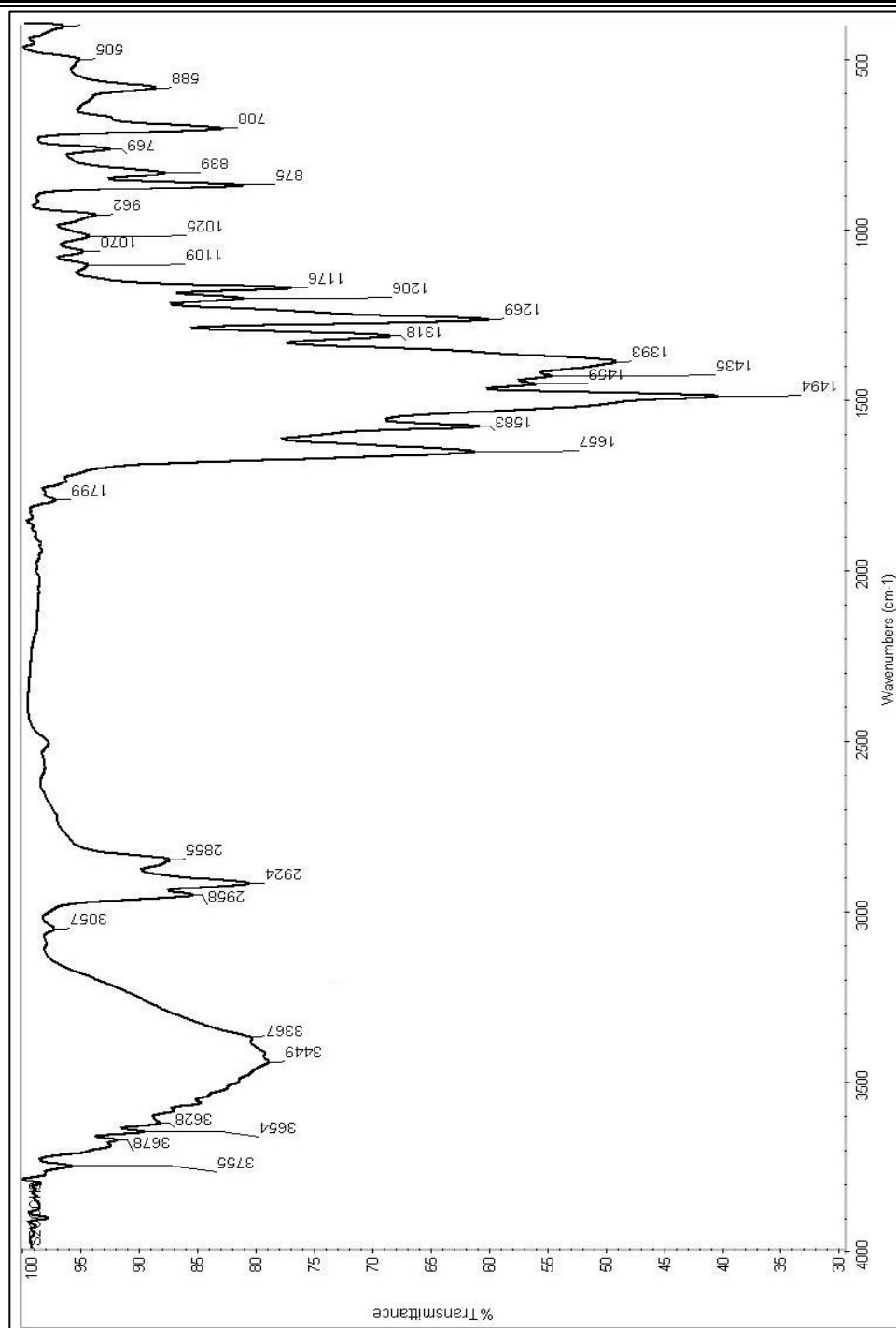


**Spectrum 31:**  $^{13}\text{C}$ -NMR spectrum (1) of N-(4-acetylphenyl)-2-benzamido-5-propylthiophene-3-carboxamide (VIb)

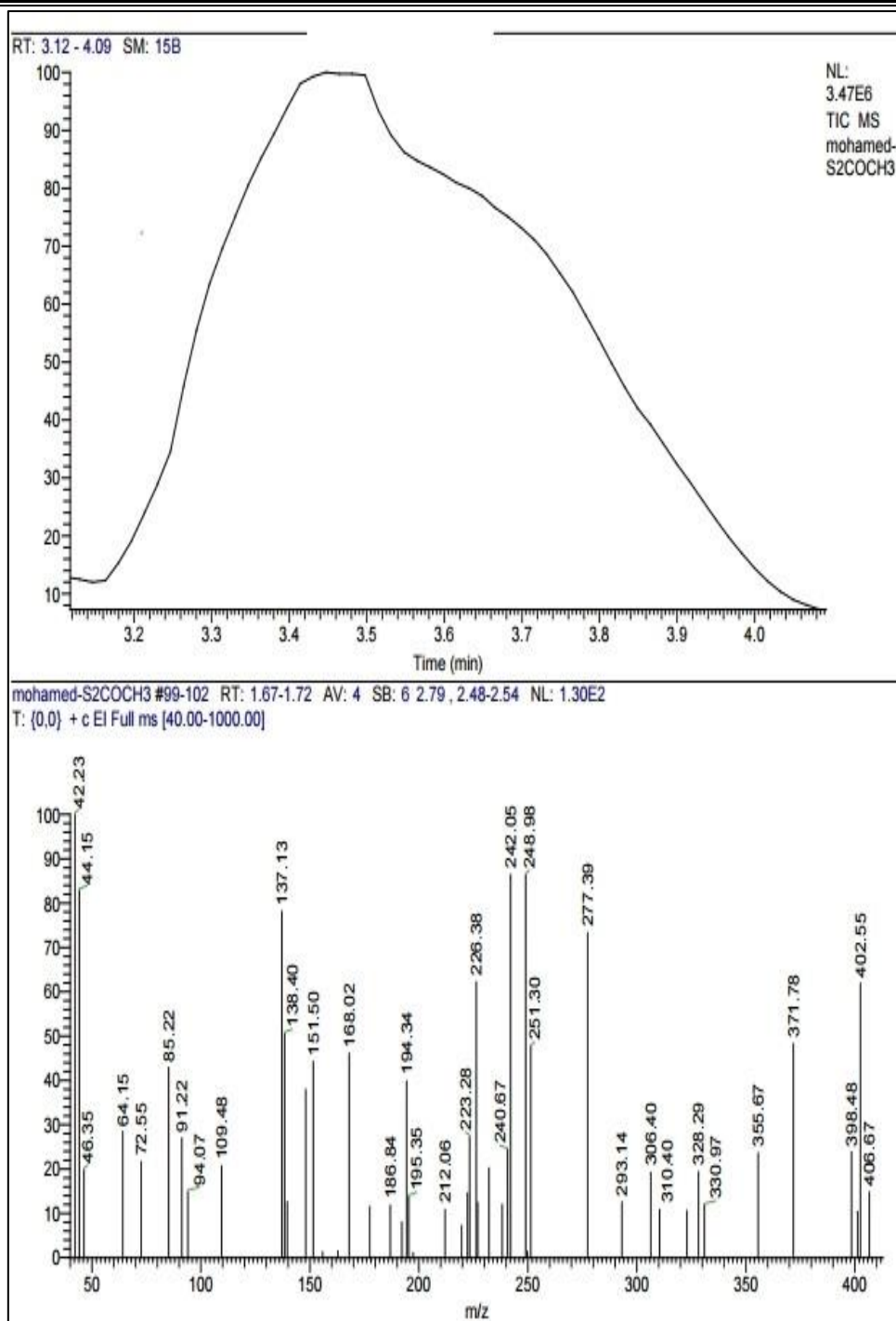




**Spectrum 32:**  $^{13}\text{C}$ -NMR spectrum (2) of N-(4-acetylphenyl)-2-benzamido-5-propylthiophene-3-carboxamide (VIb)

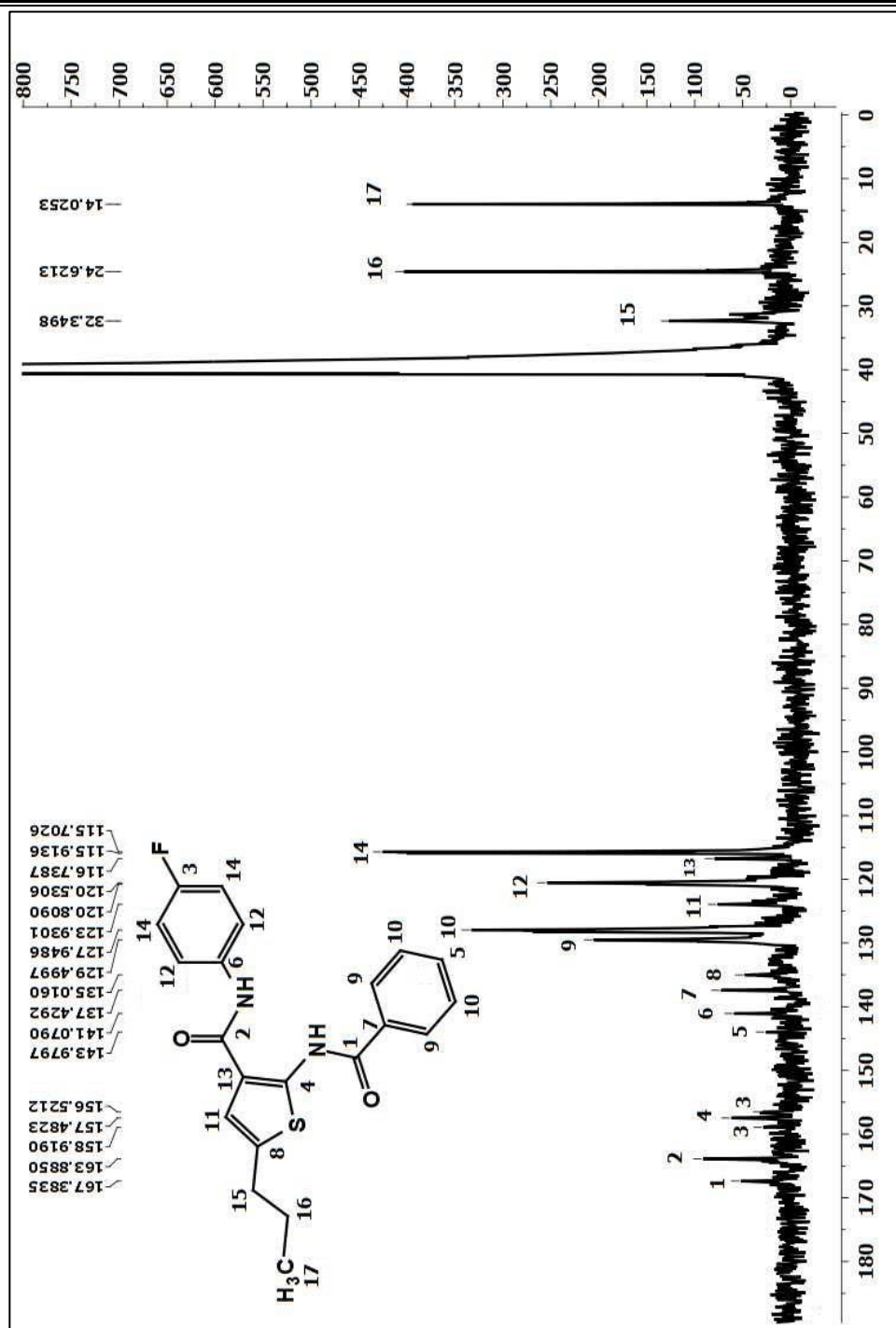


**Spectrum 33:** IR spectrum of N-(4-acetylphenyl)-2-benzamido-5-propylthiophene-3-carboxamide (**VIb**)

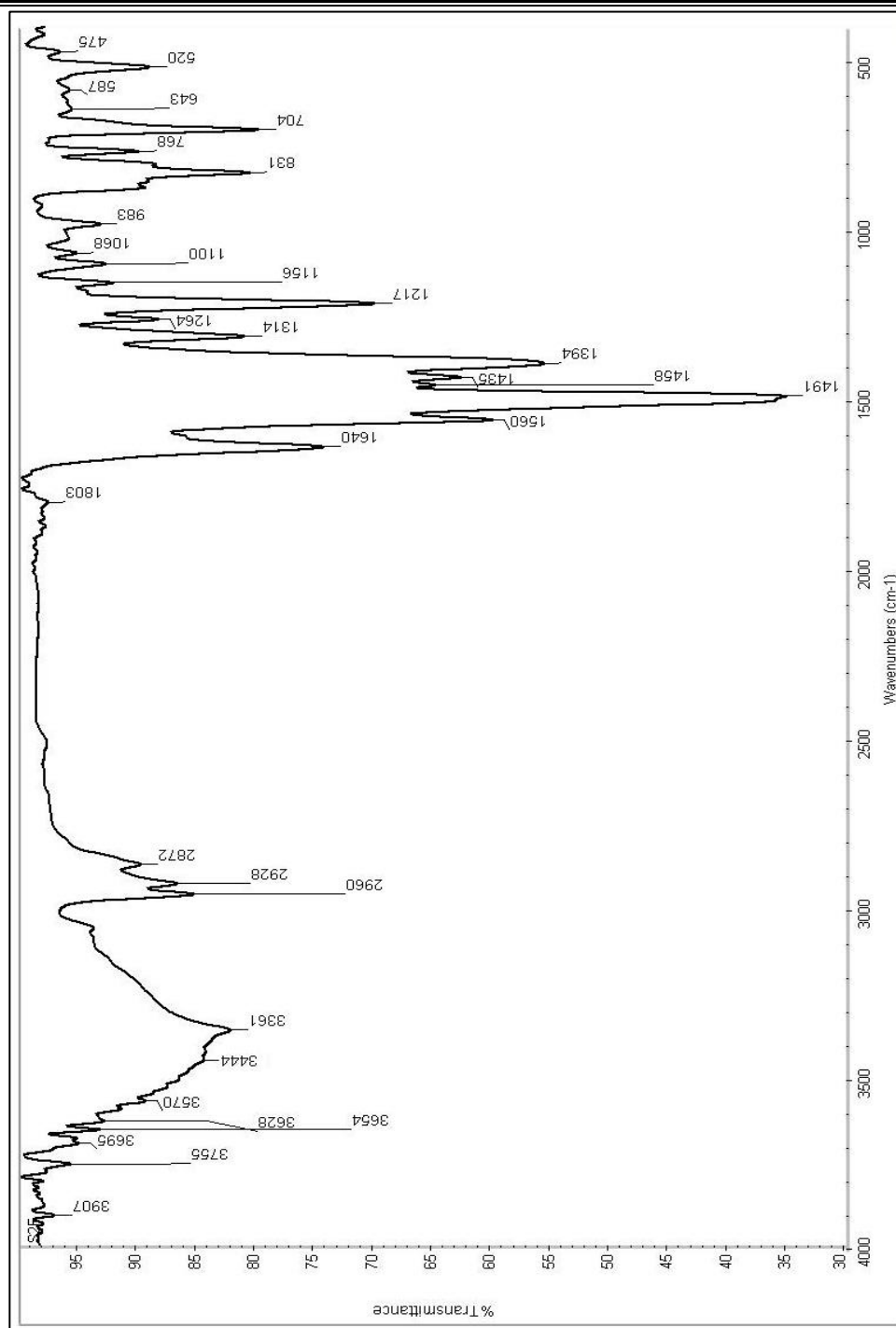


**Spectrum 34:** Mass spectrum of N-(4-acetylphenyl)-2-benzamido-5-propylthiophene-3-carboxamide (**VIb**)

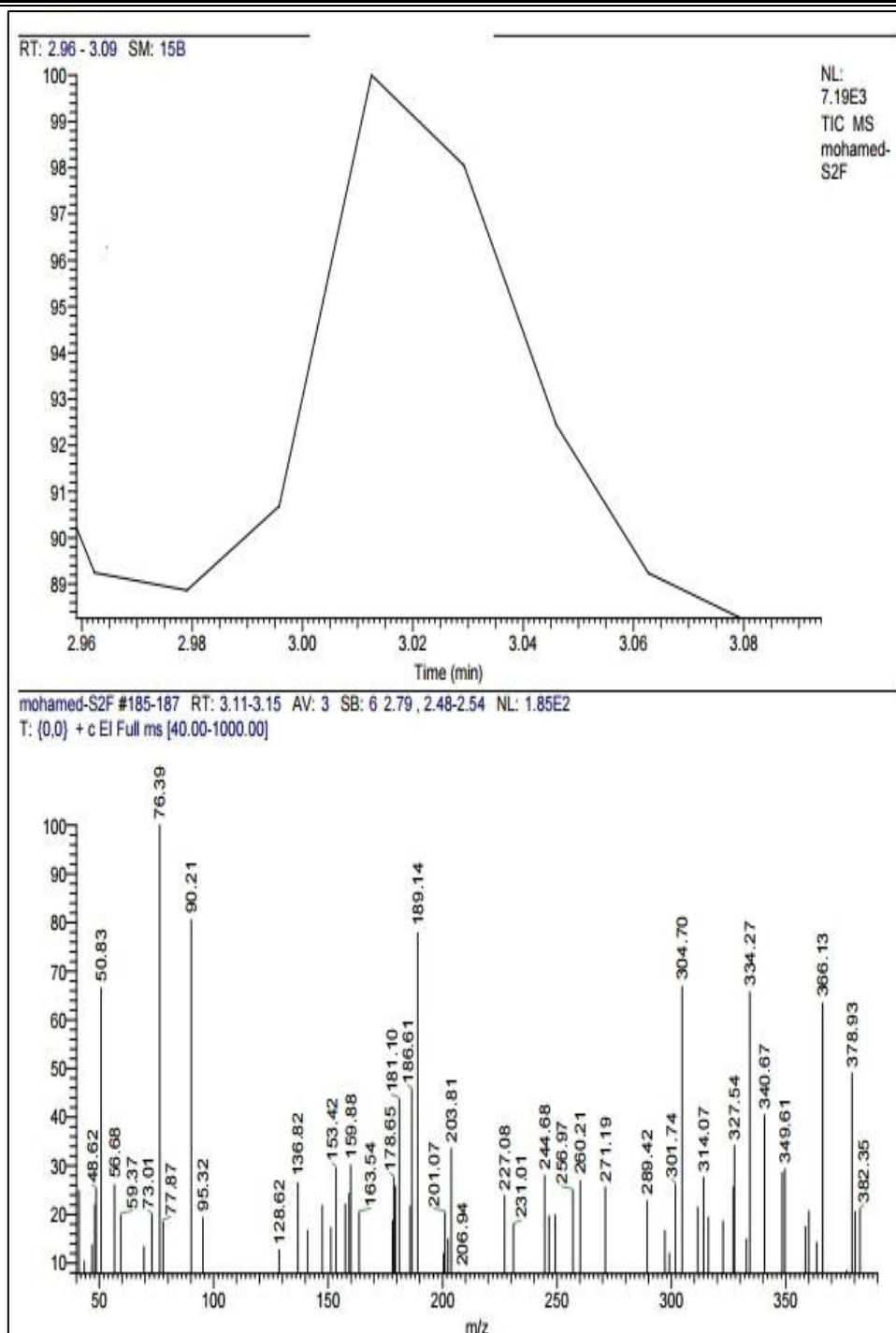




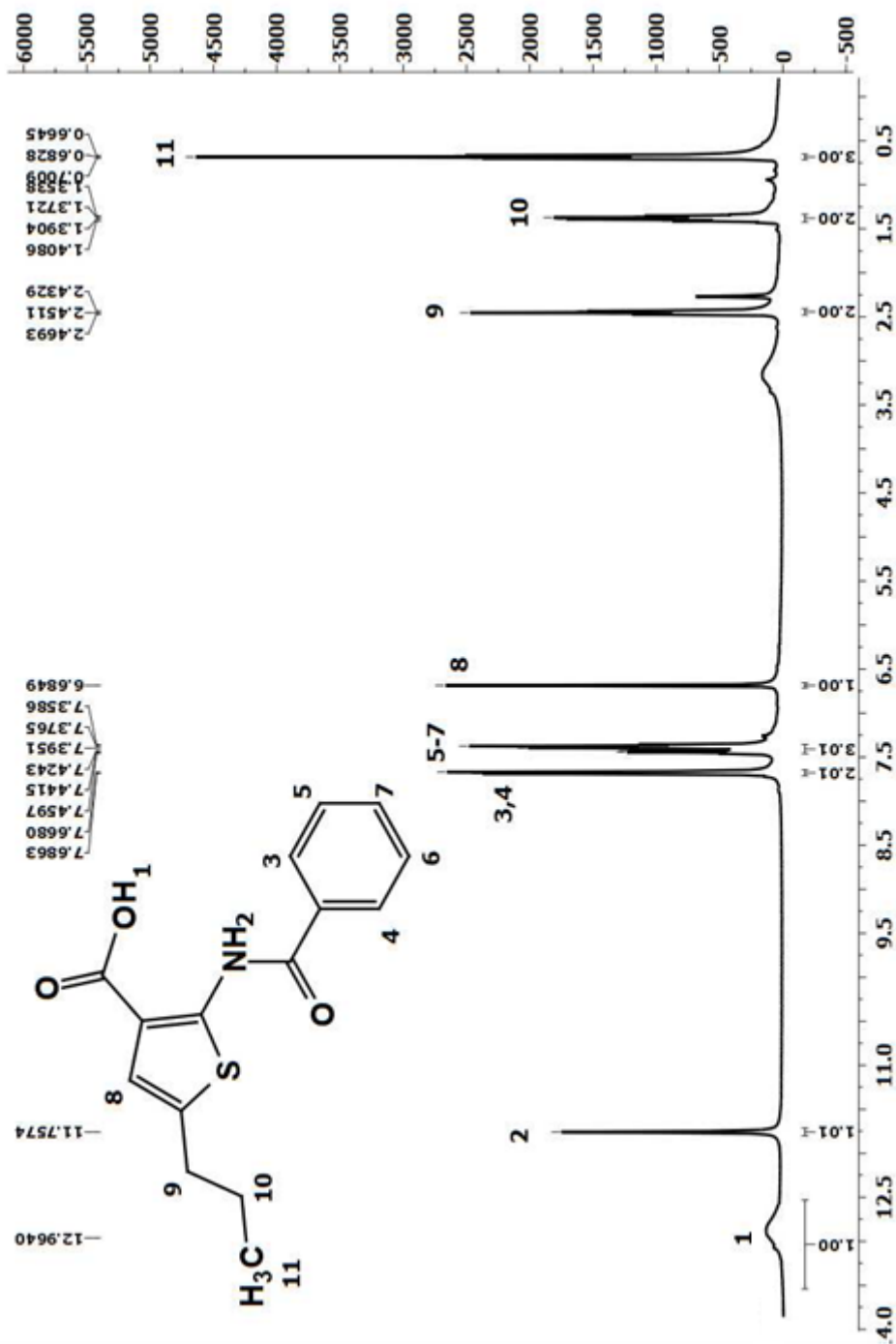
**Spectrum 36:** <sup>13</sup>C-NMR spectrum of 2-benzamido-N-(4-fluorophenyl)-5-propylthiophene-3-carboxamide (**VIIIb**)



**Spectrum 37:** IR spectrum of 2-benzamido-N-(4-fluorophenyl)-5-propylthiophene-3-carboxamide (**VIIb**)

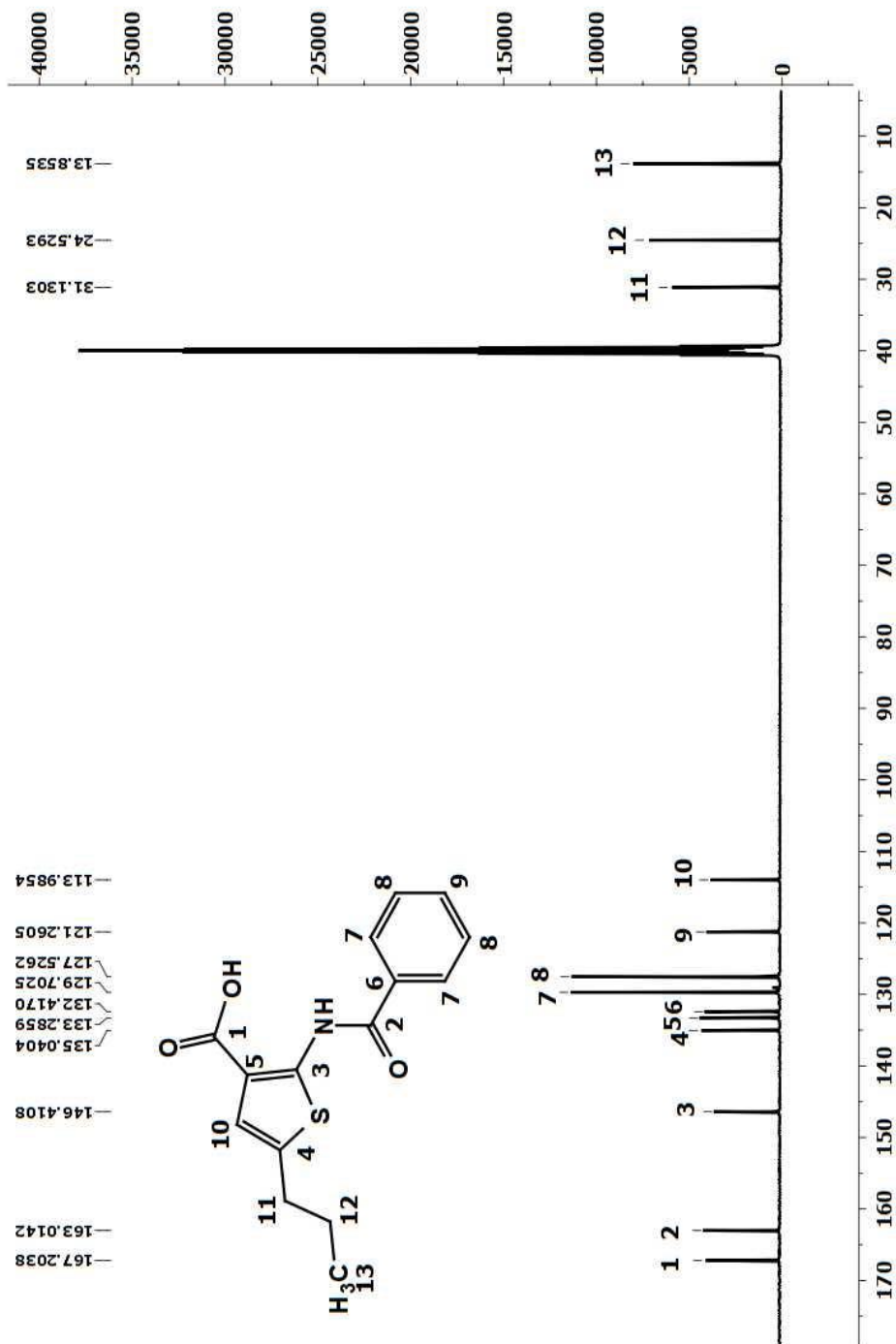


**Spectrum 38:** Mass spectrum of 2-benzamido-N-(4-fluorophenyl)-5-propylthiophene-3-carboxamide (**VIIb**)



Spectrum 39:  $^1\text{H-NMR}$  of 2-benzamido-5-propylthiophene-3-carboxamide (IIIb)





**Spectrum 40:**  $^{13}\text{C}$ -NMR of 2-benzamido-5-propylthiophene-3-carboxamide (**IIIb**)

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## **The in vitro anti-inflammatory Assay using egg albumin**

The reaction mixture consists of our tested compounds with serial dilutions (1000, 100, 10, 1, 0.1  $\mu$ M), phosphate buffered saline (PBS; pH 6.4), and egg albumin. The control used was double distilled water (equal volume). These samples were incubated at 37 °C for 15 min and then heated at 70 °C for 5 min. in a water bath. After attaining ambient temperature, the absorbance of samples was measured spectrophotometrically at 660 nm using vehicle as blank. Celecoxib was taken as the standard drug. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated using the Equation Inhibition (%) of protein denaturation =  $\frac{A_c - A_s}{A_c} \times 100$ , where  $A_c$ : absorbance of control,  $A_s$ : absorbance of sample.

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