

## Supplementary Material

### Design and synthesis of *N*-benzimidazol-2-yl-*N'*-sulfonyl acetamidines

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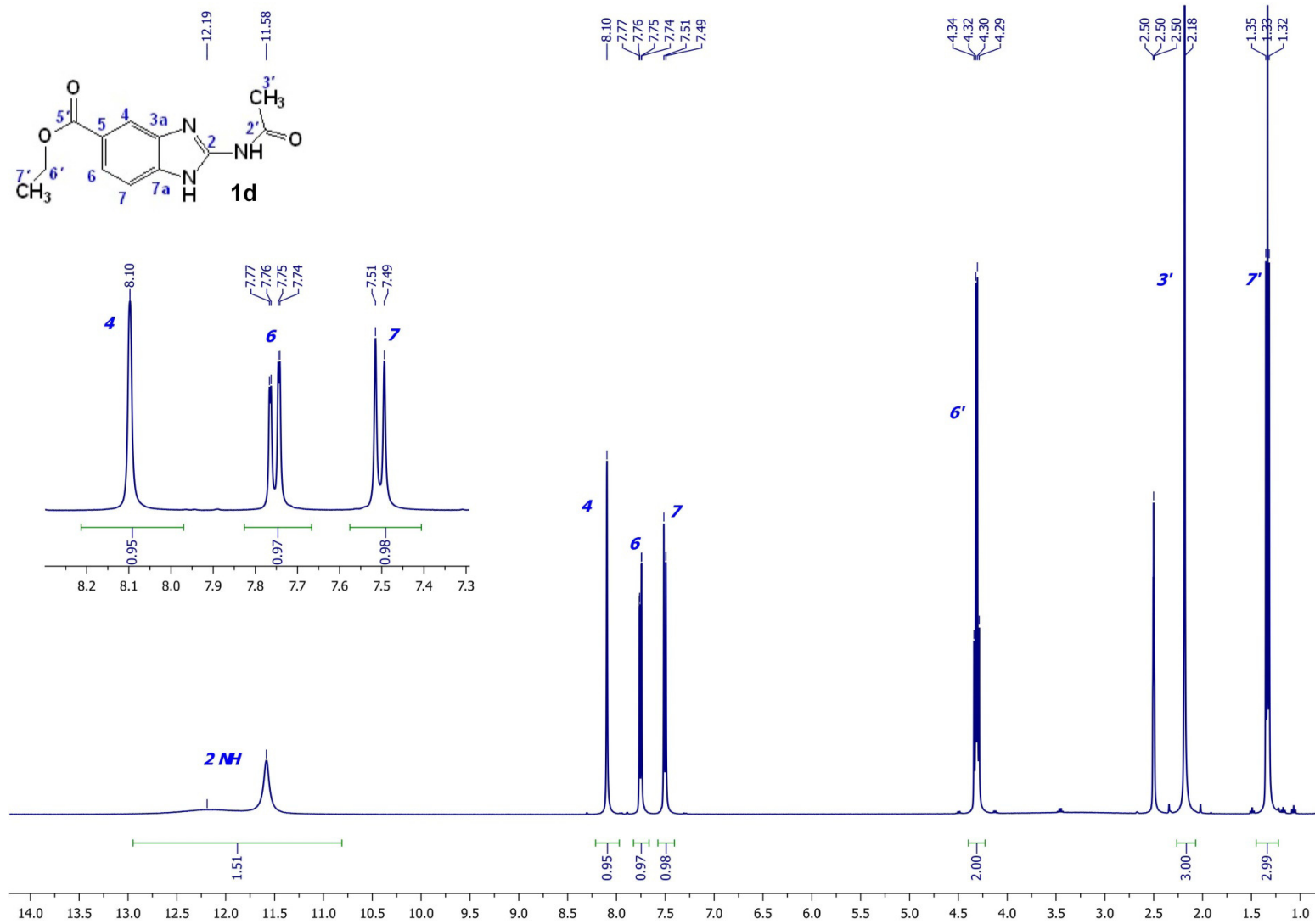
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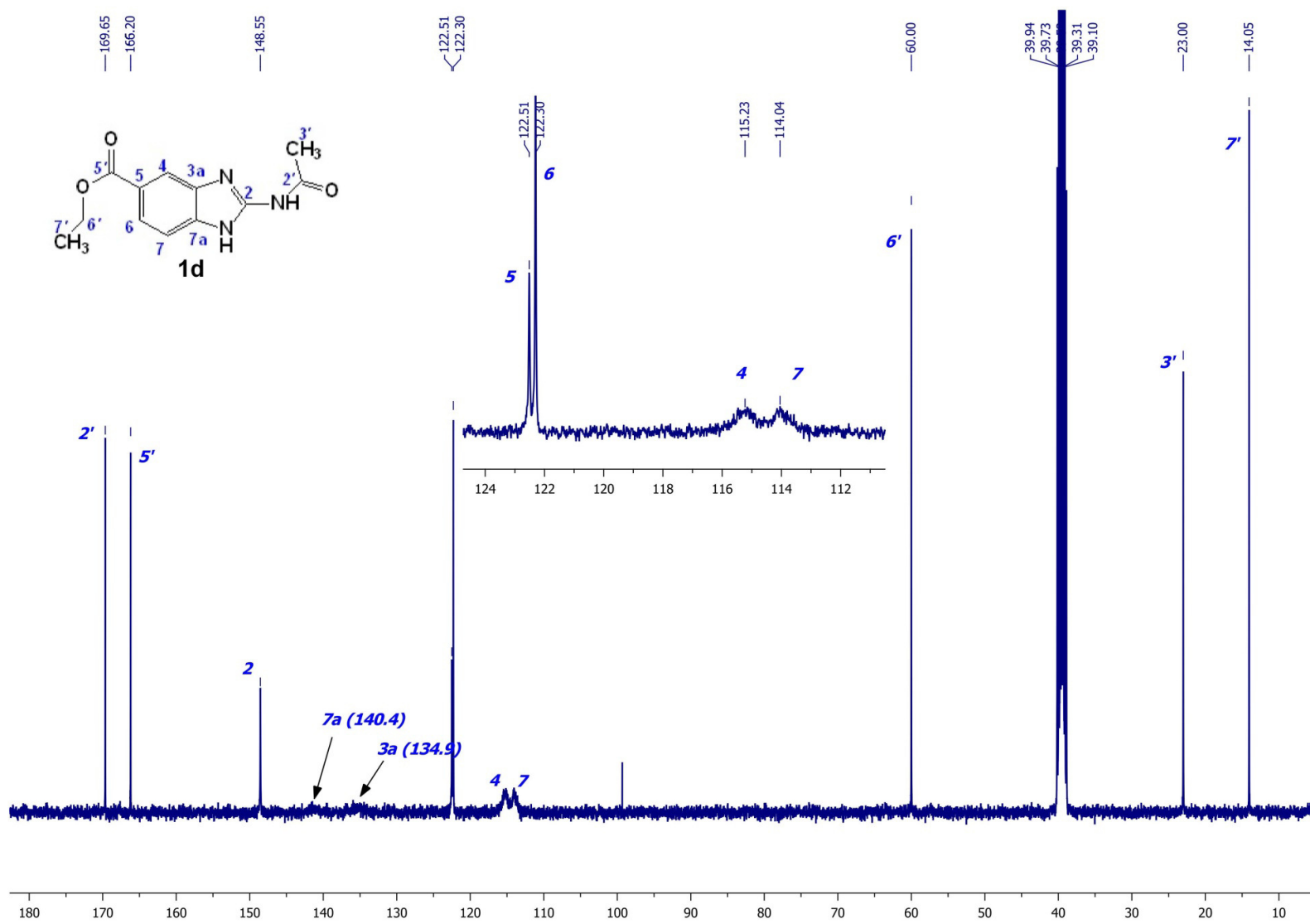
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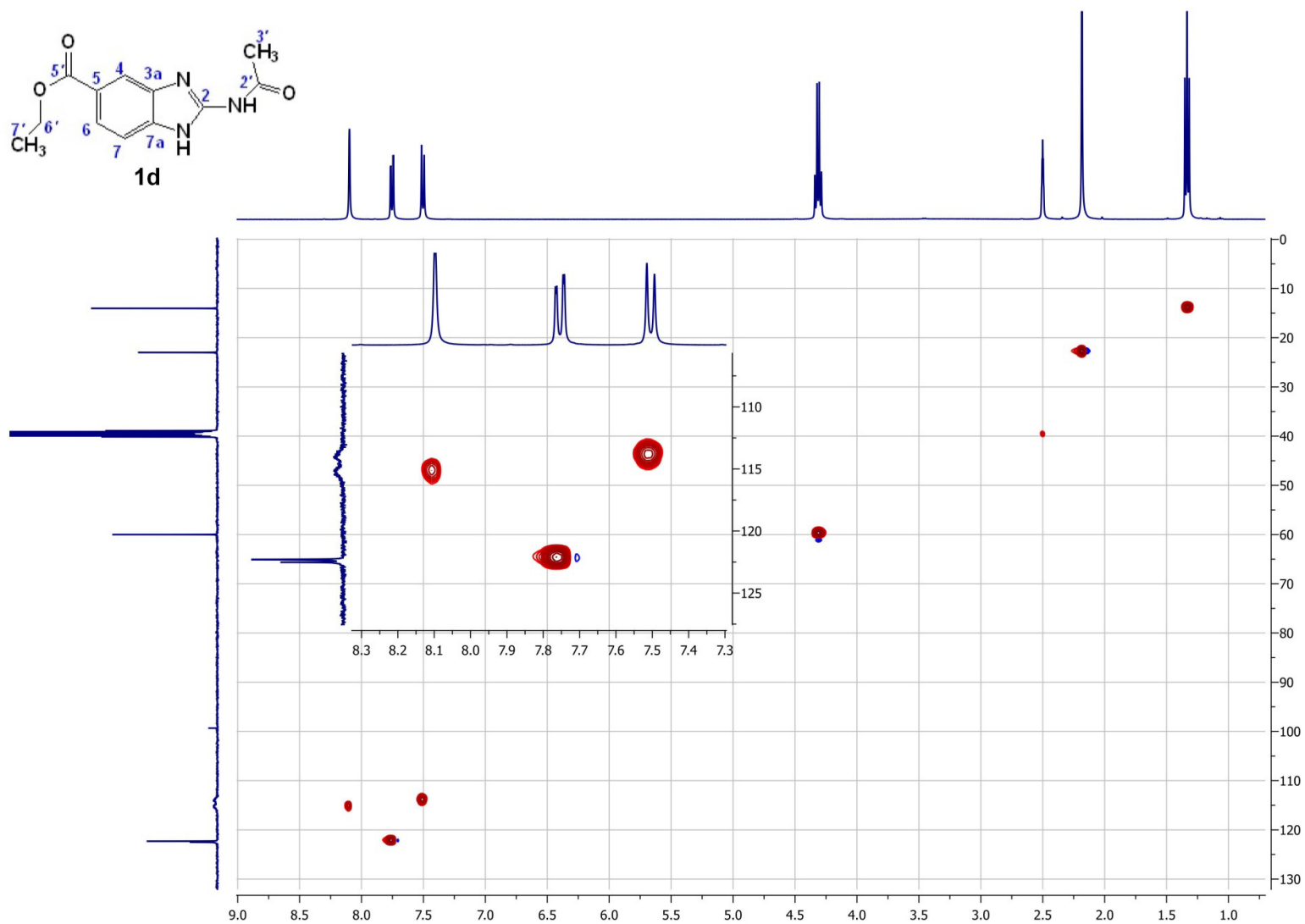
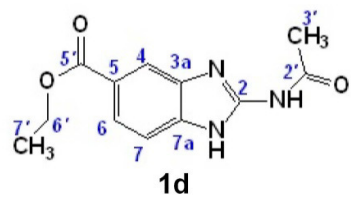
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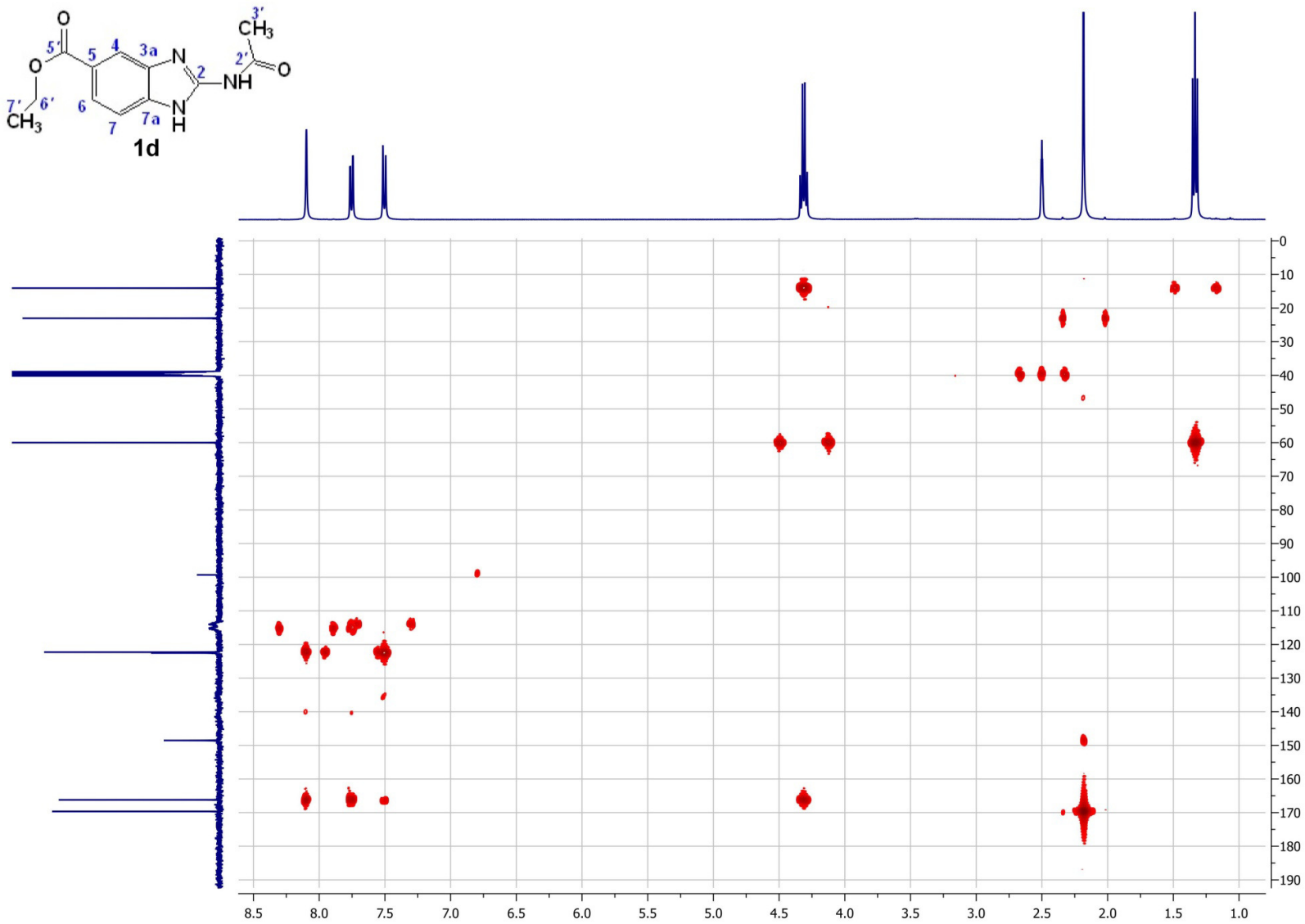
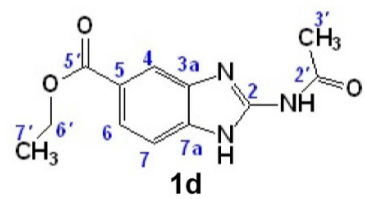
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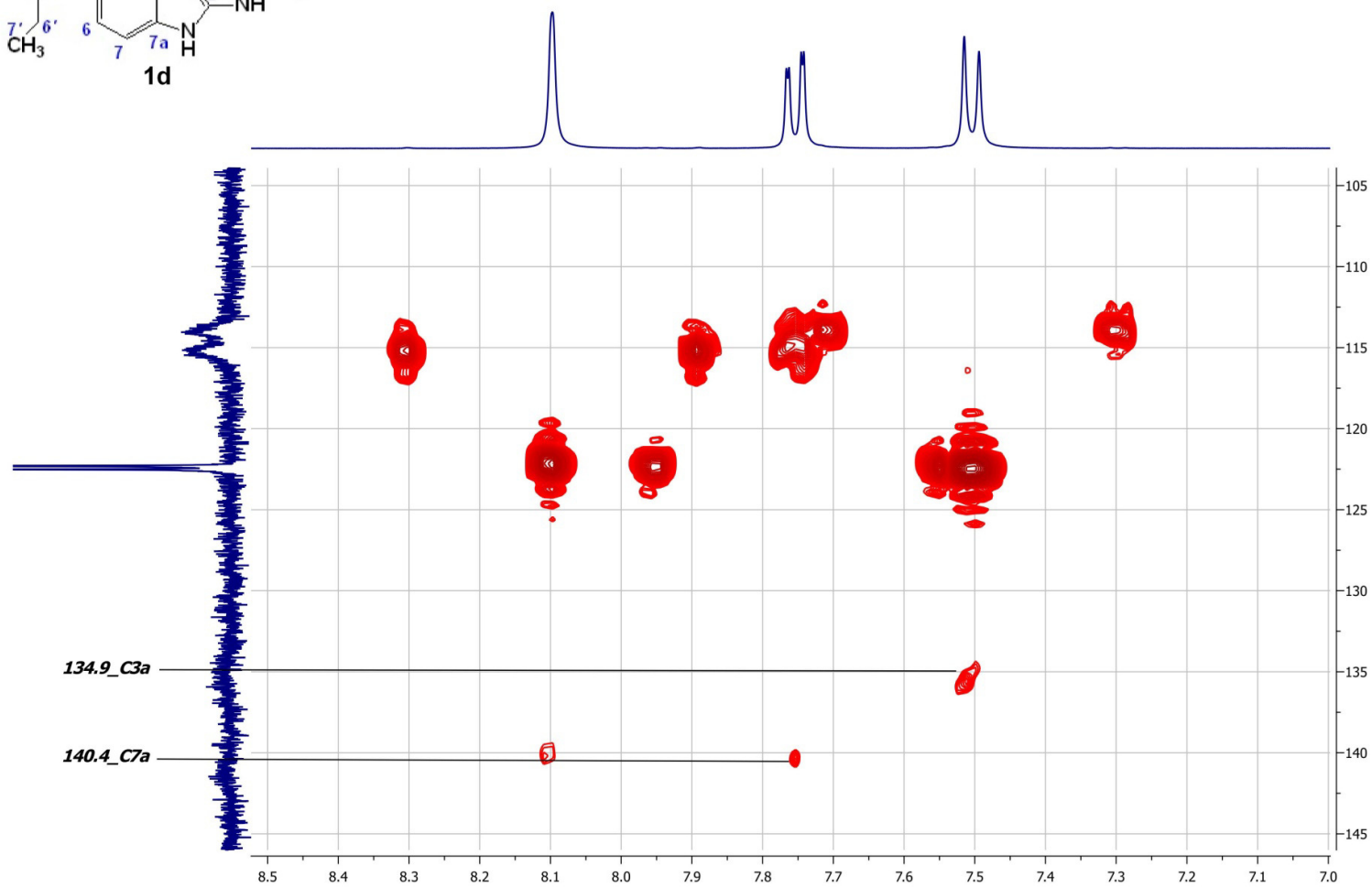
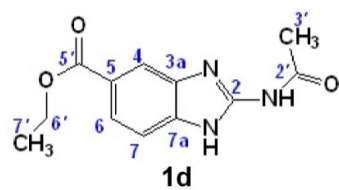
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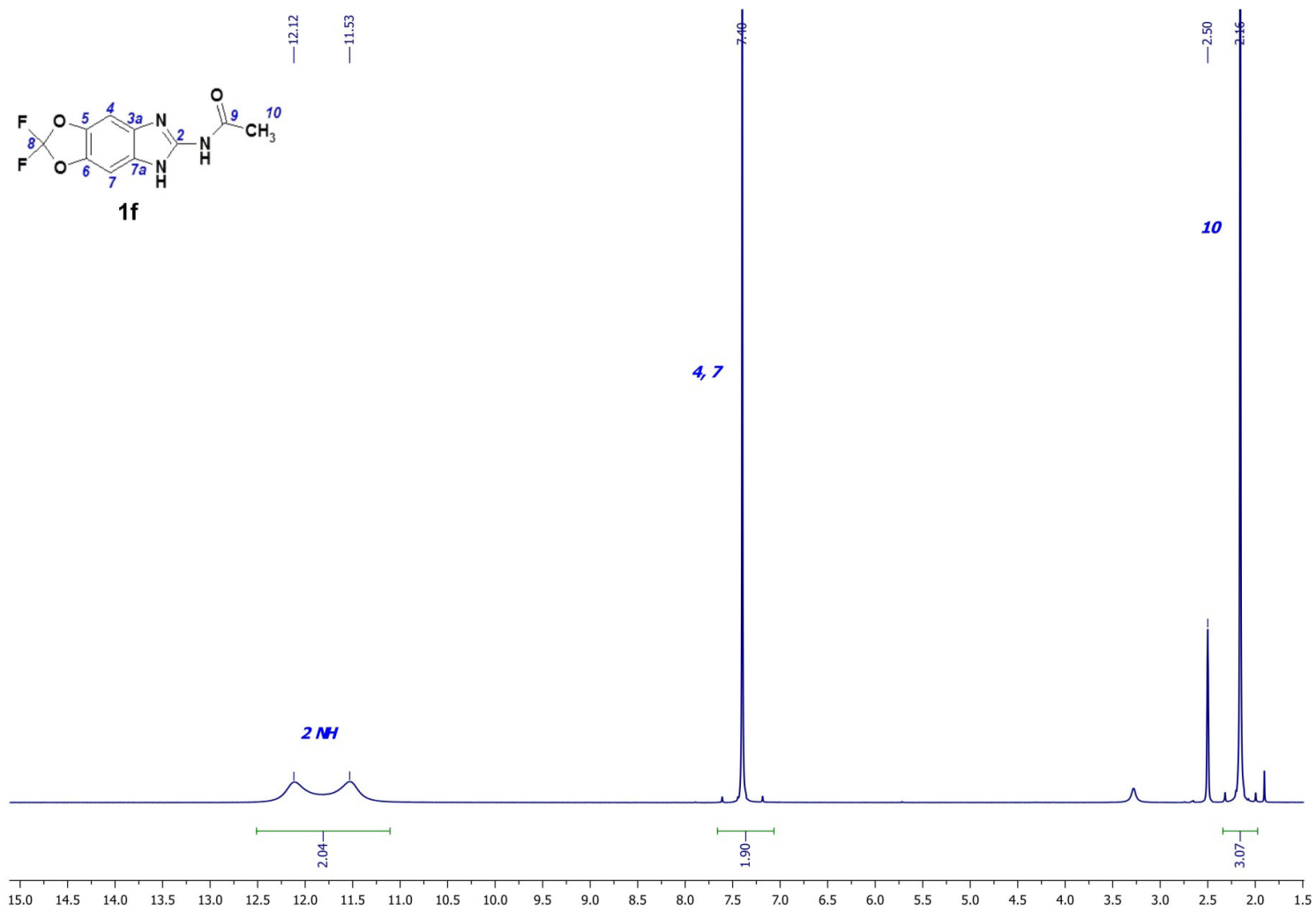




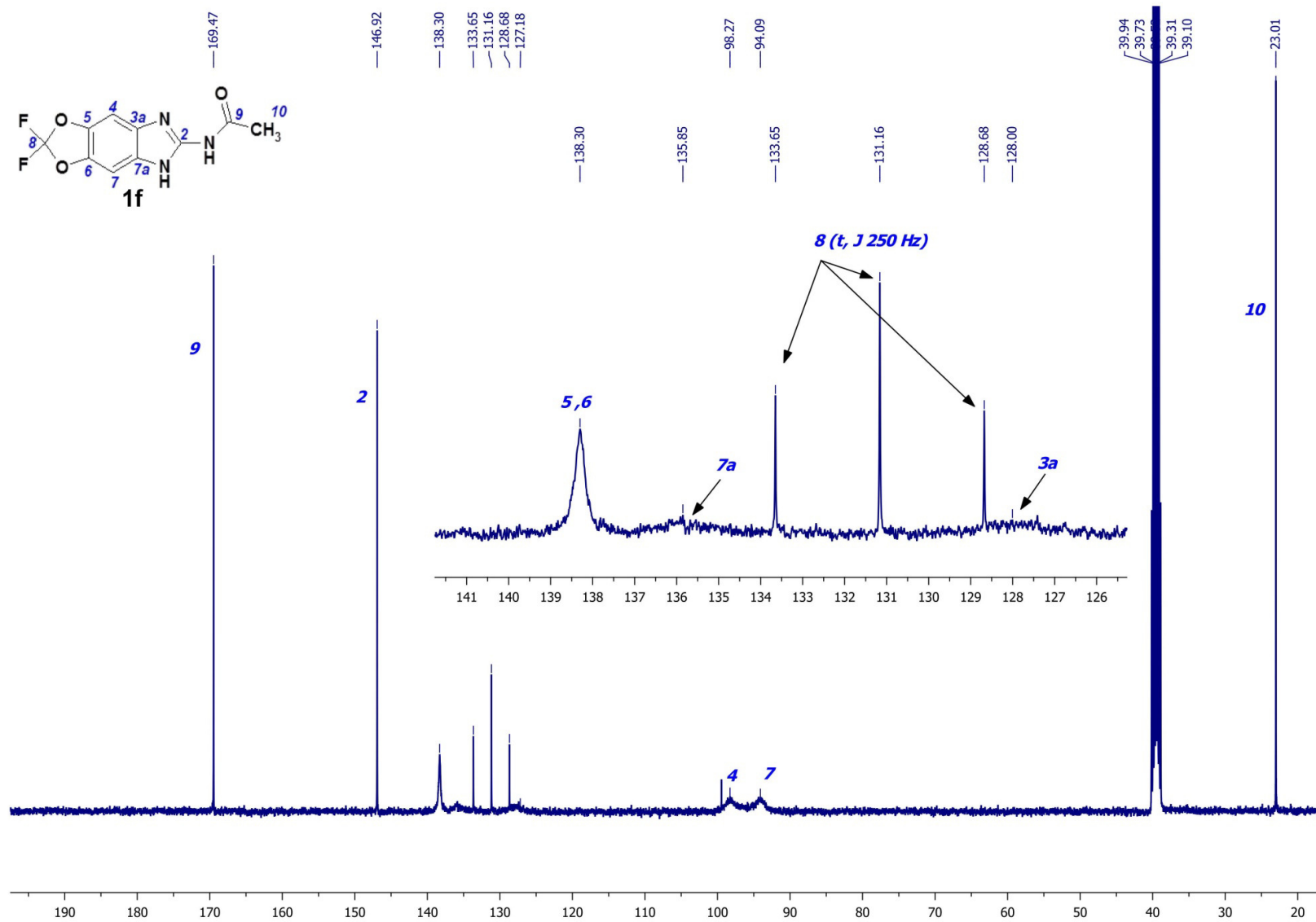


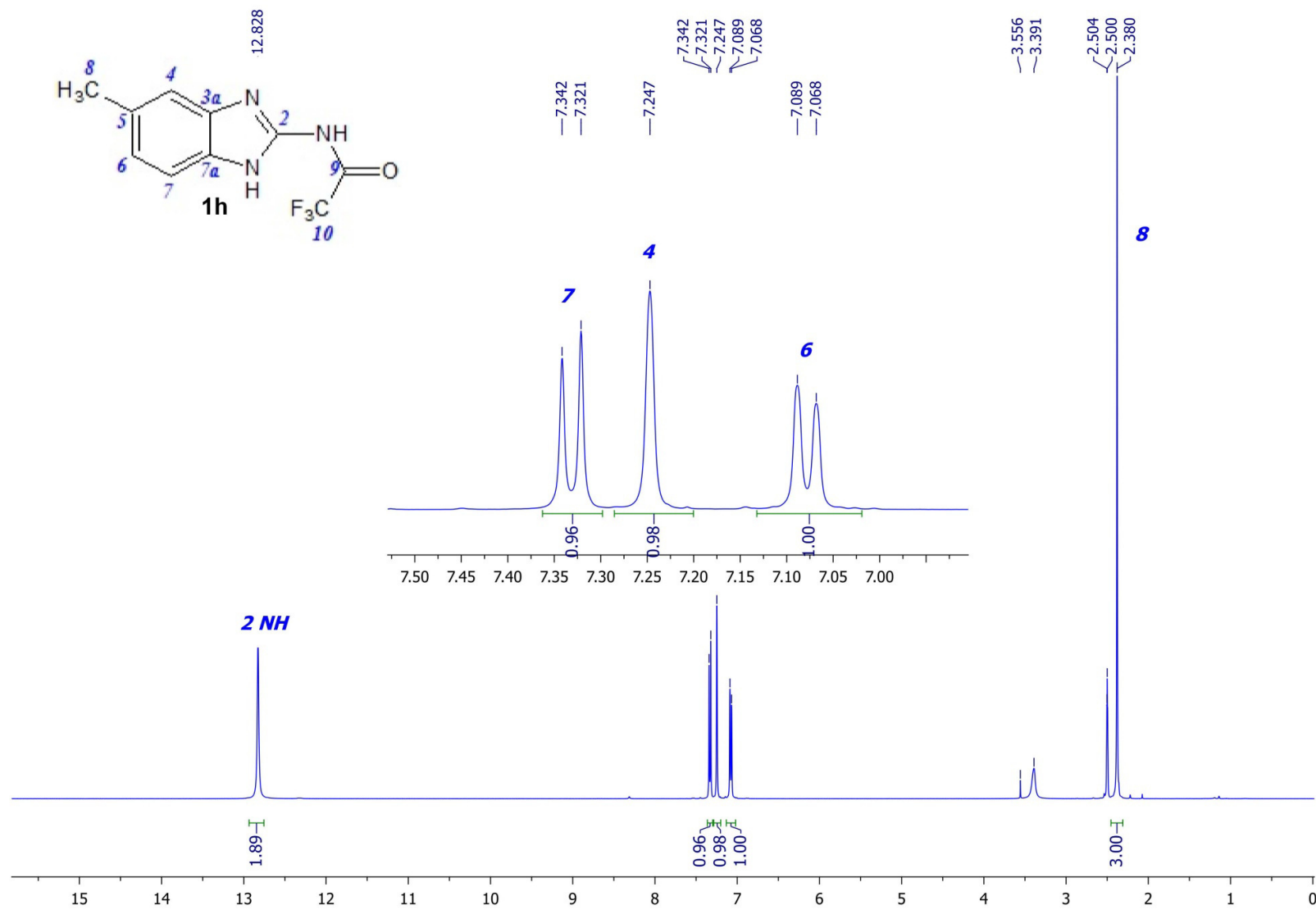


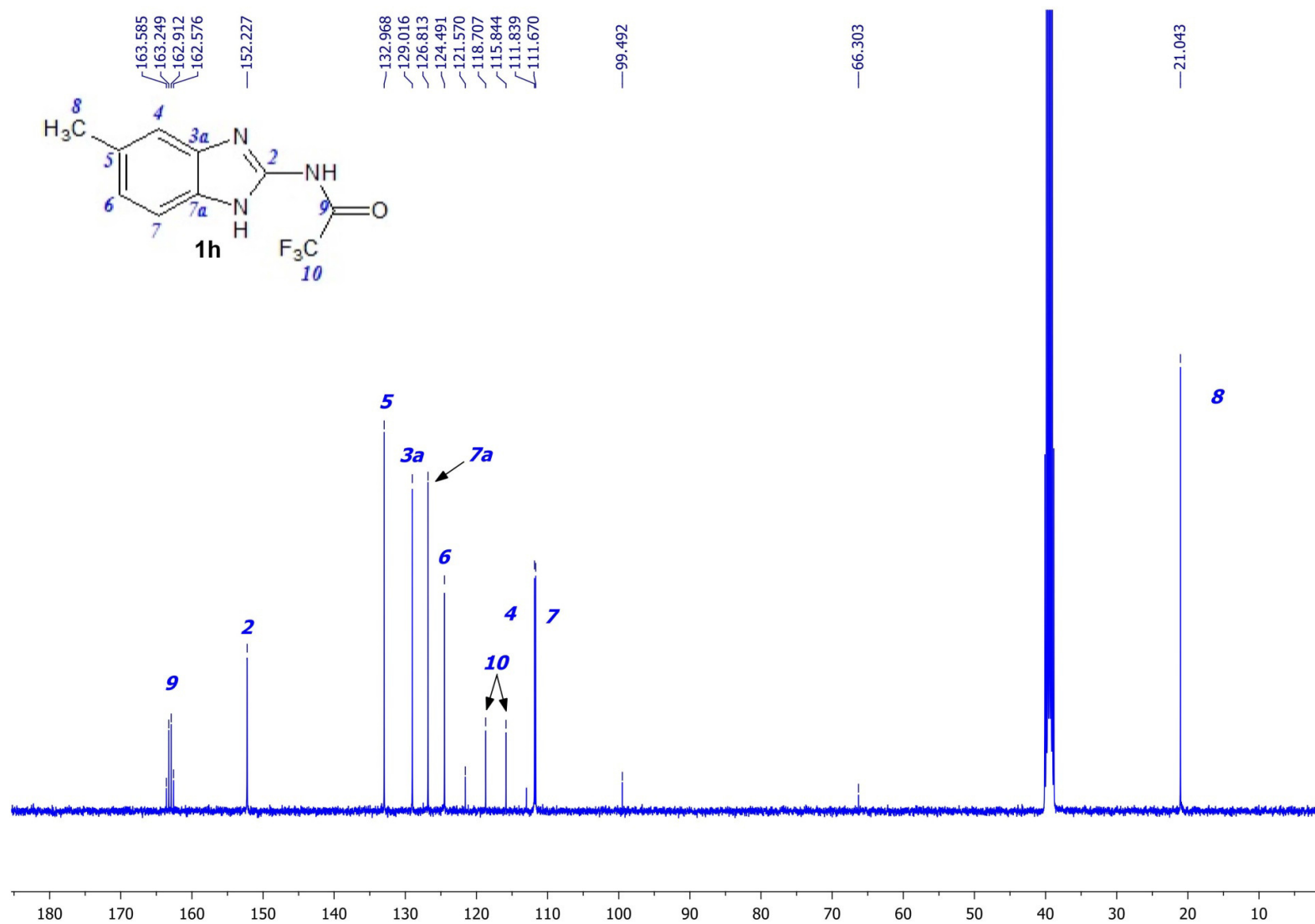


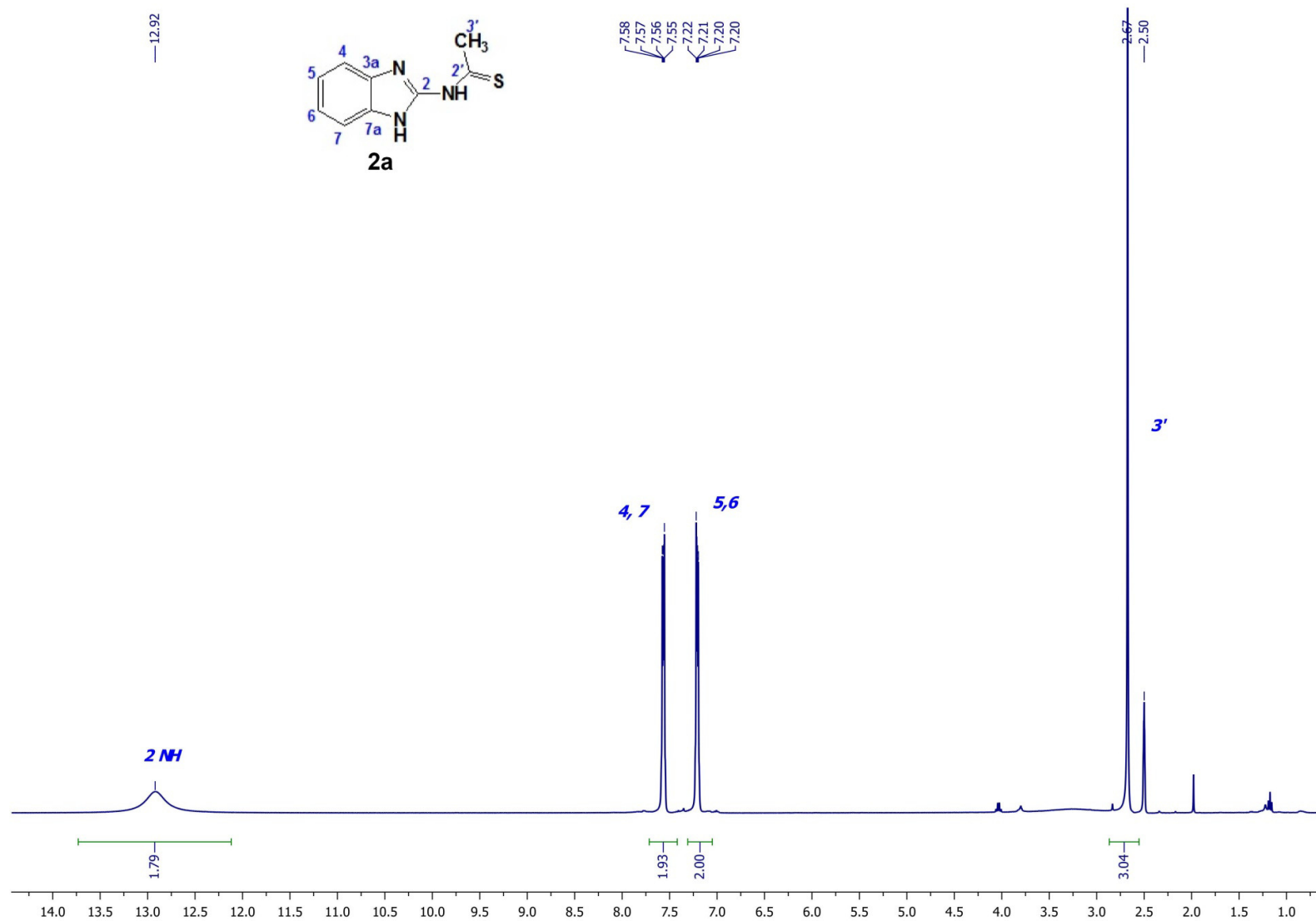


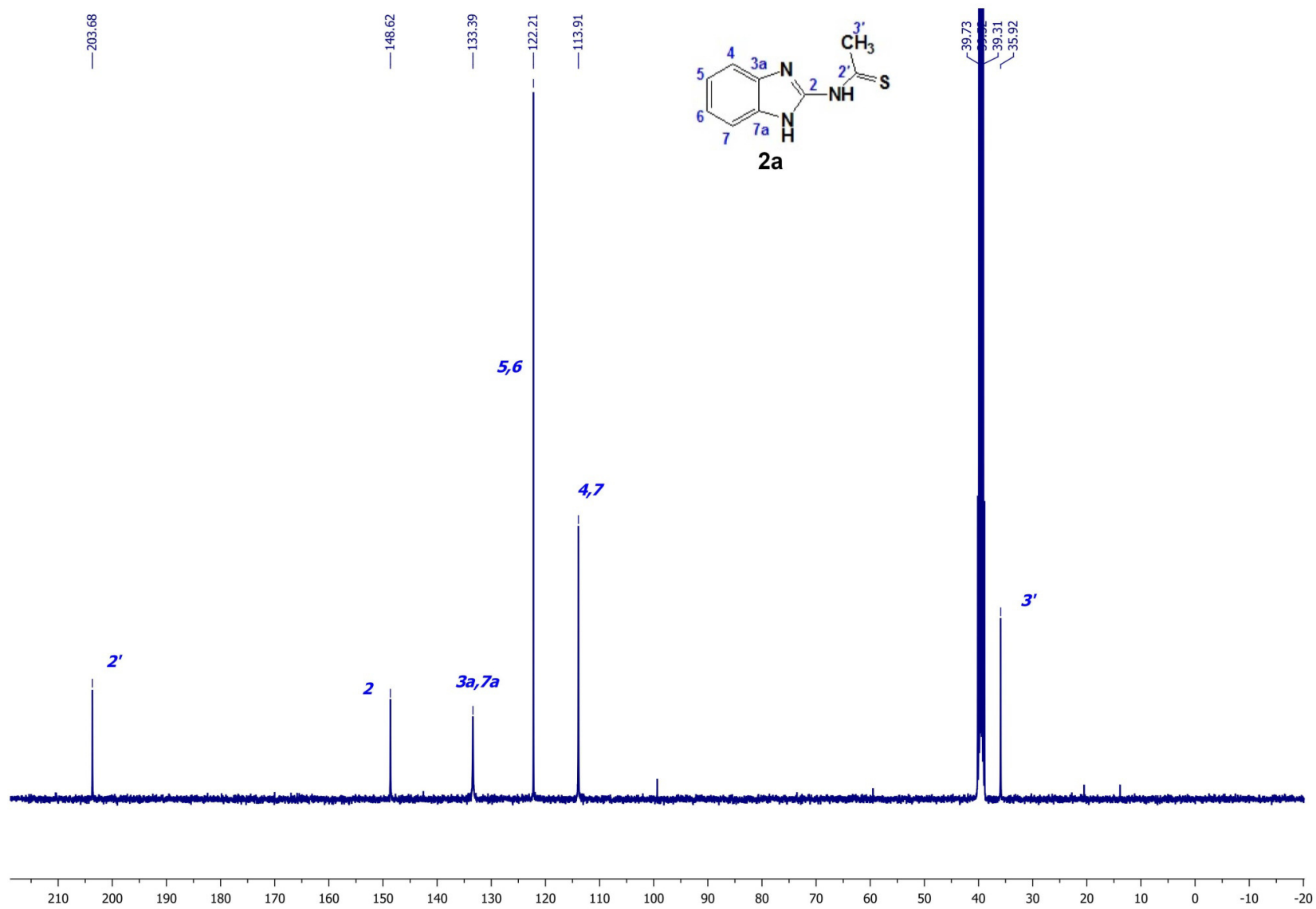


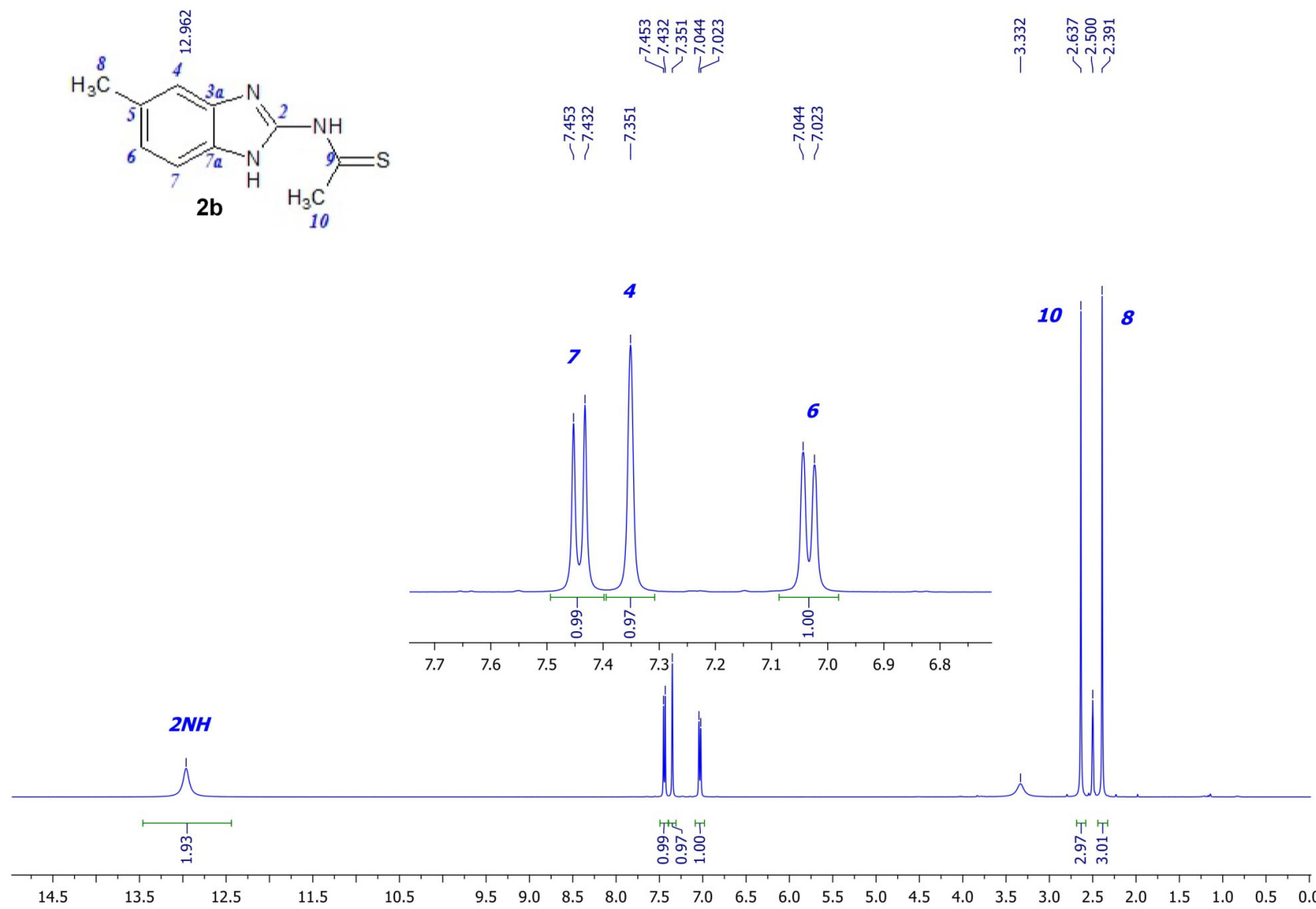


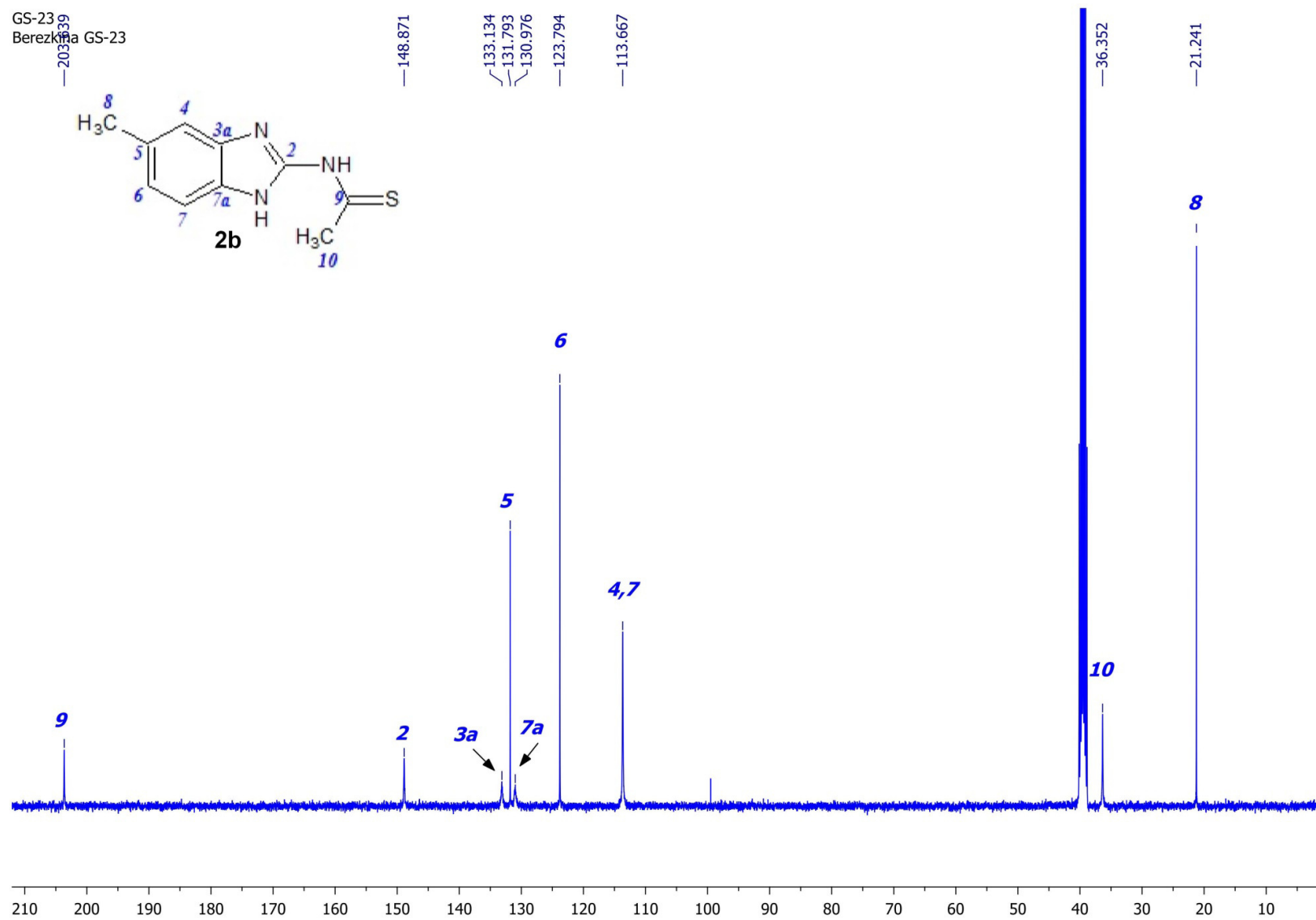


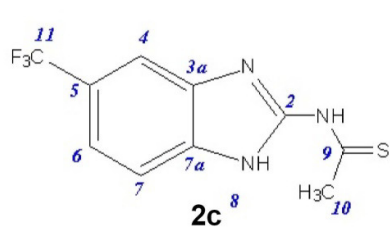




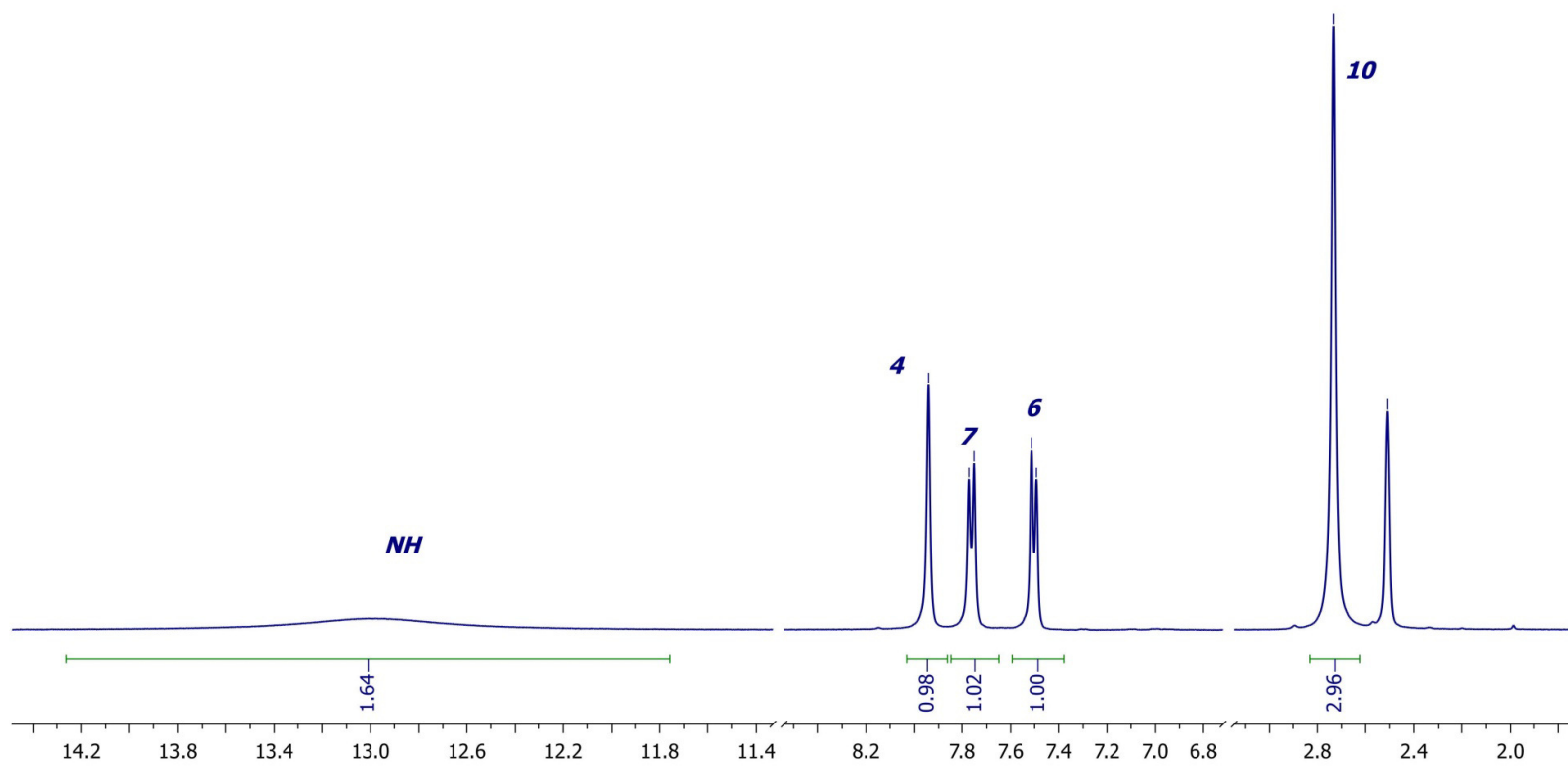




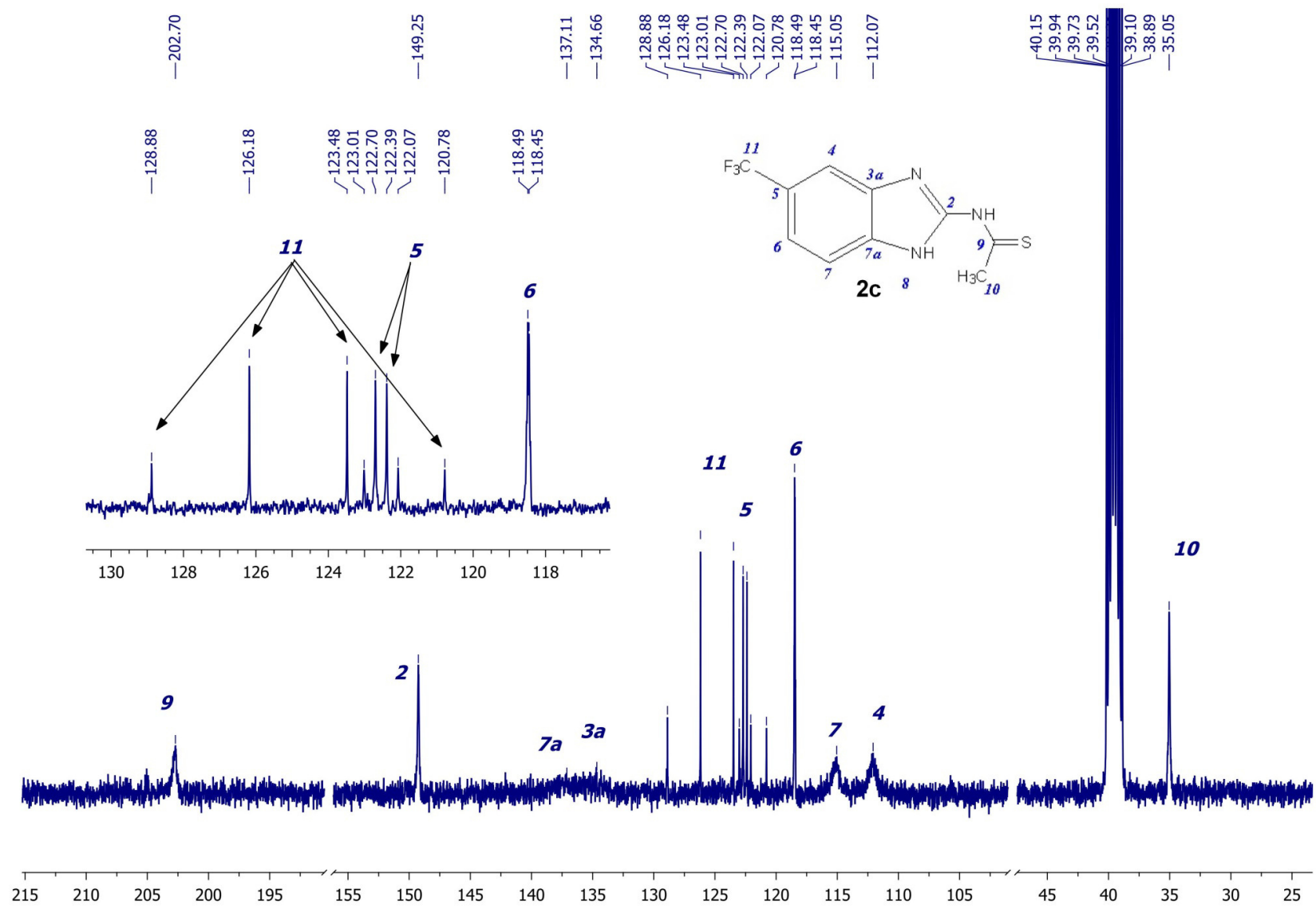


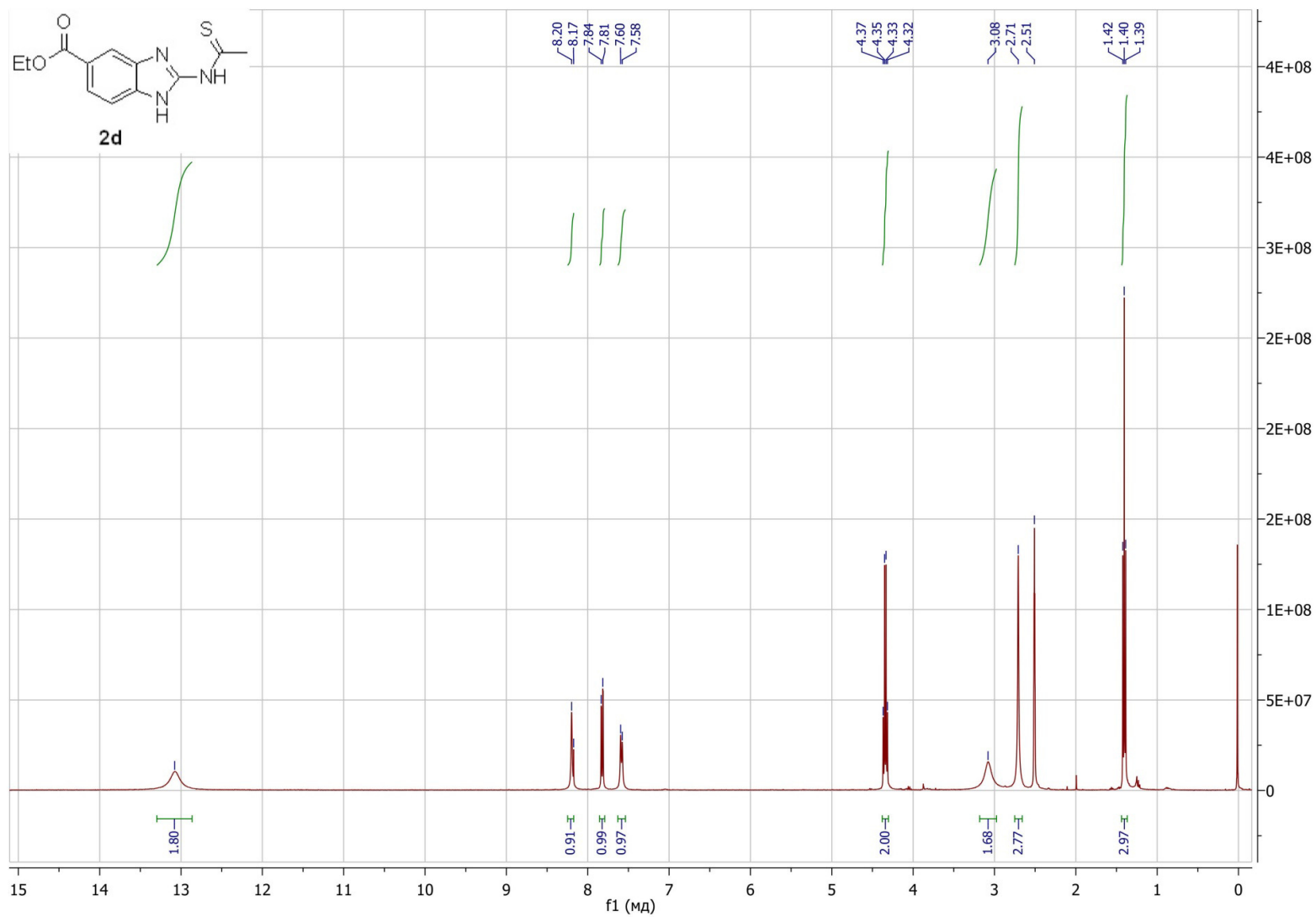


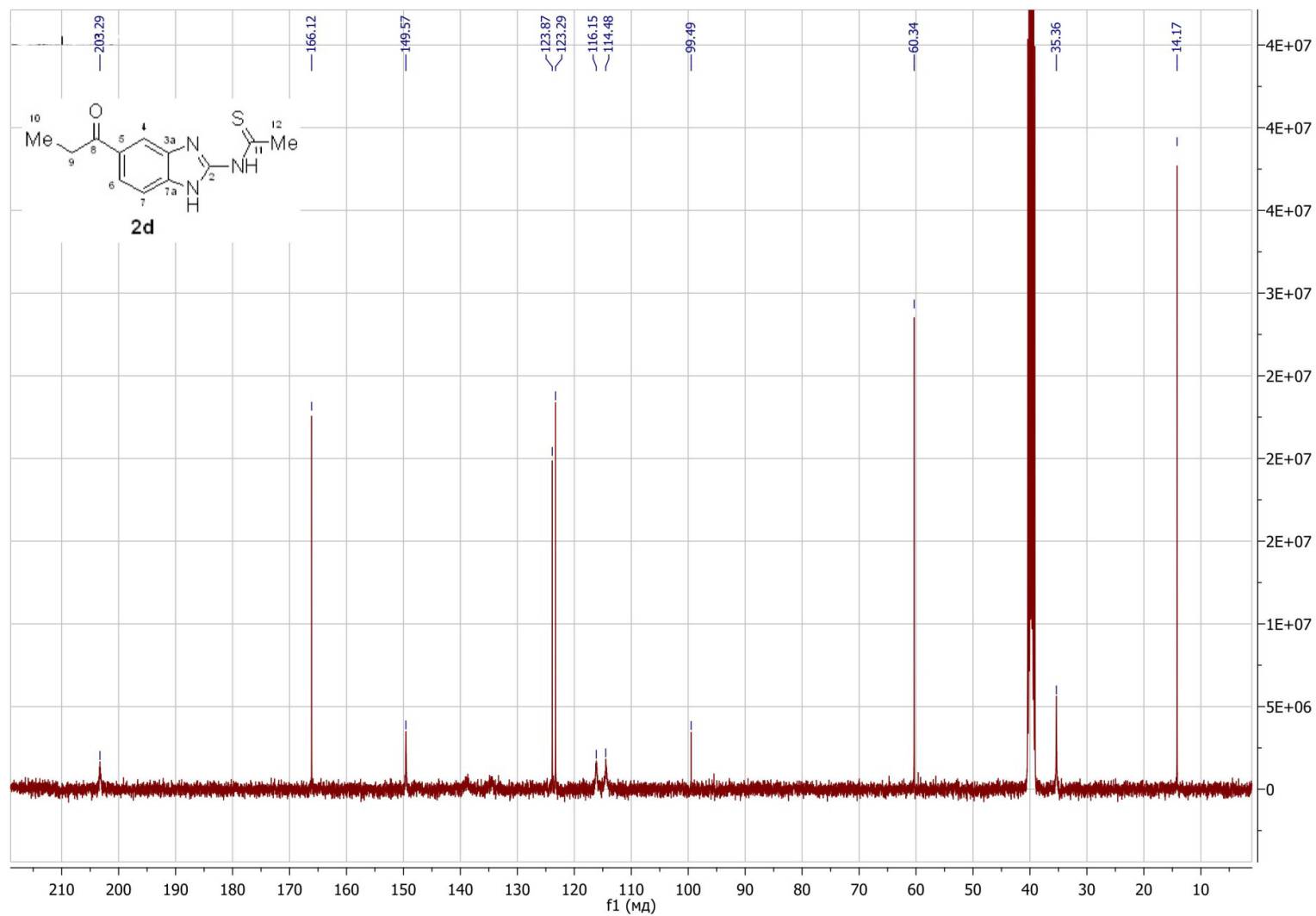
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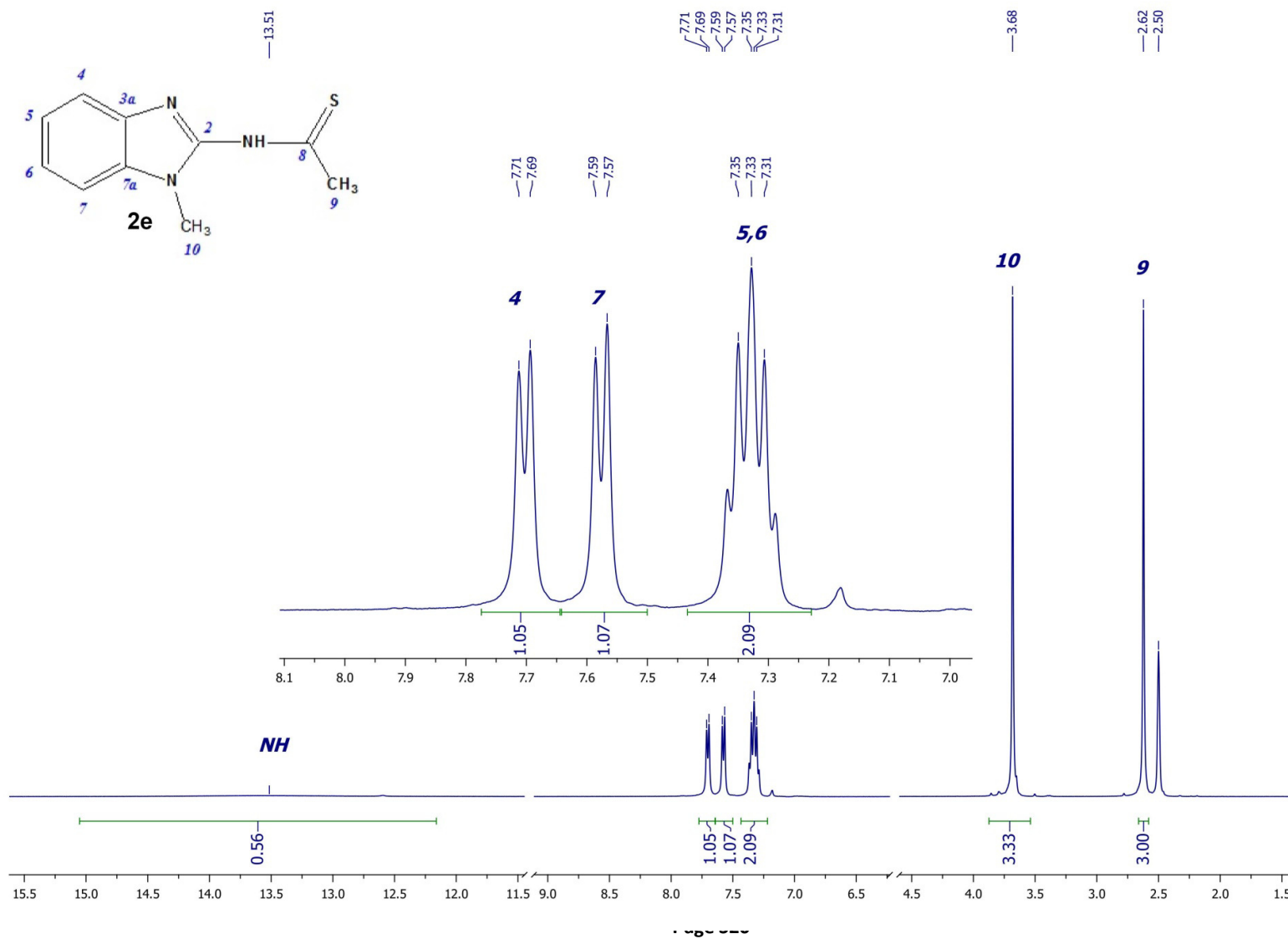


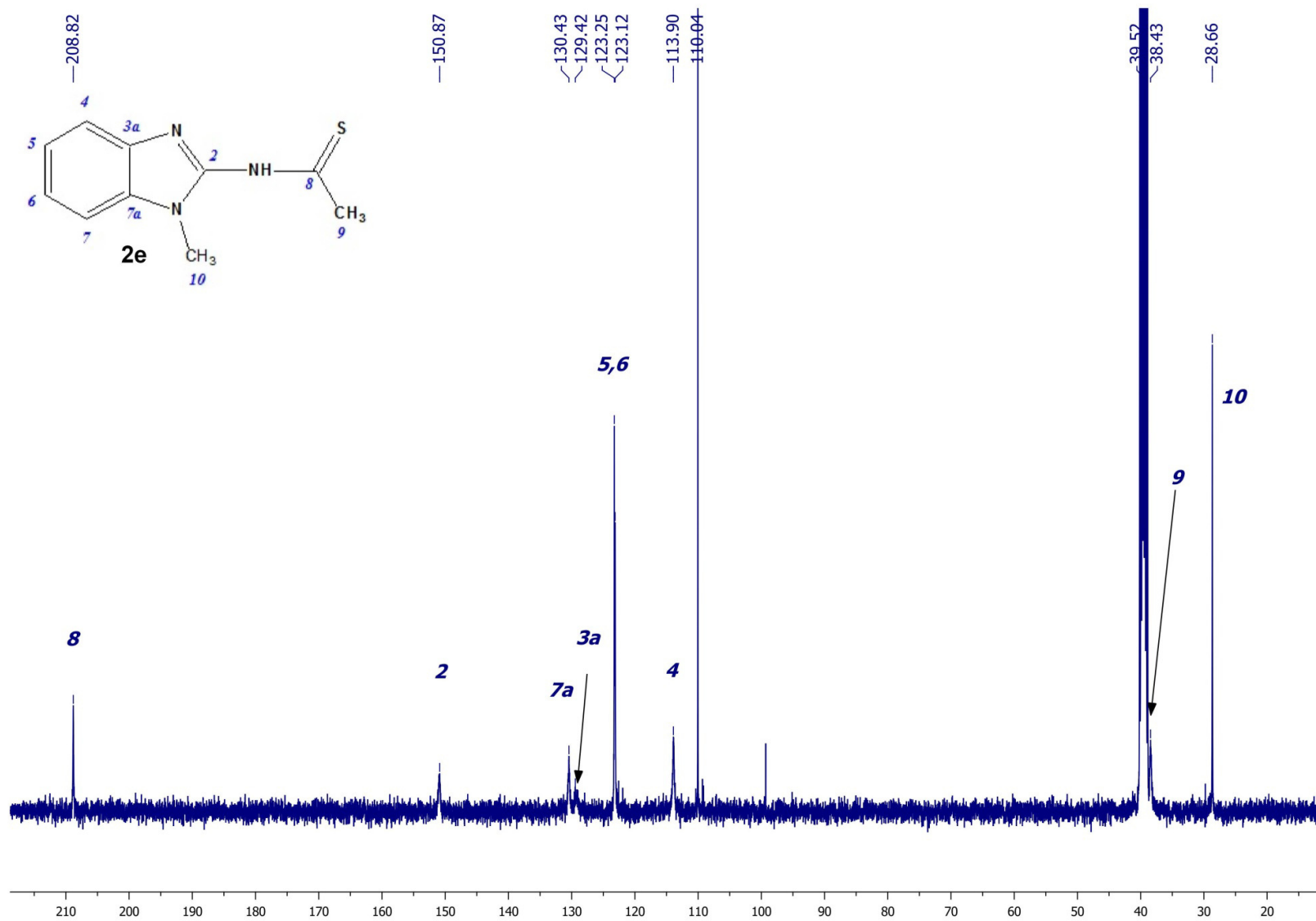


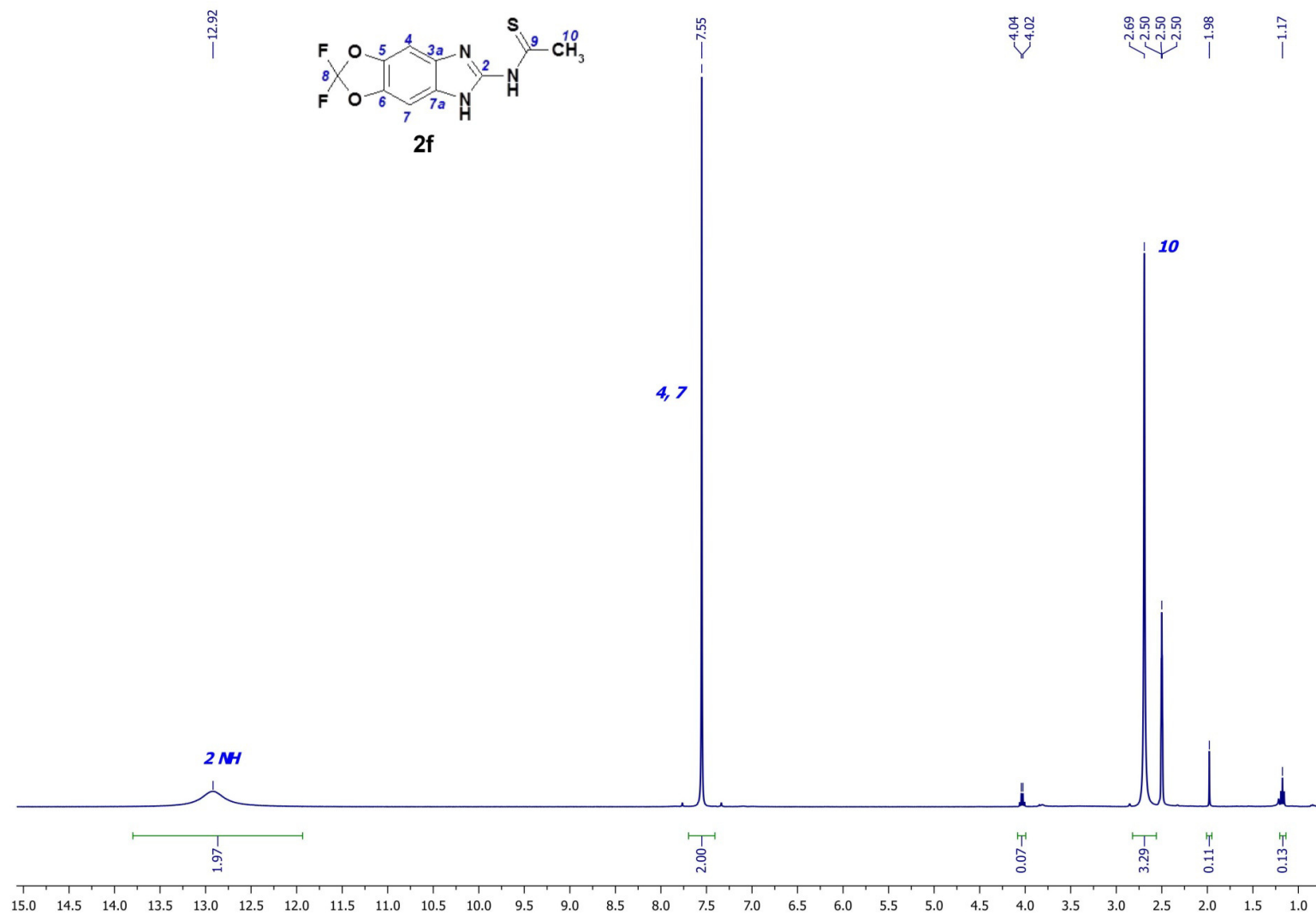


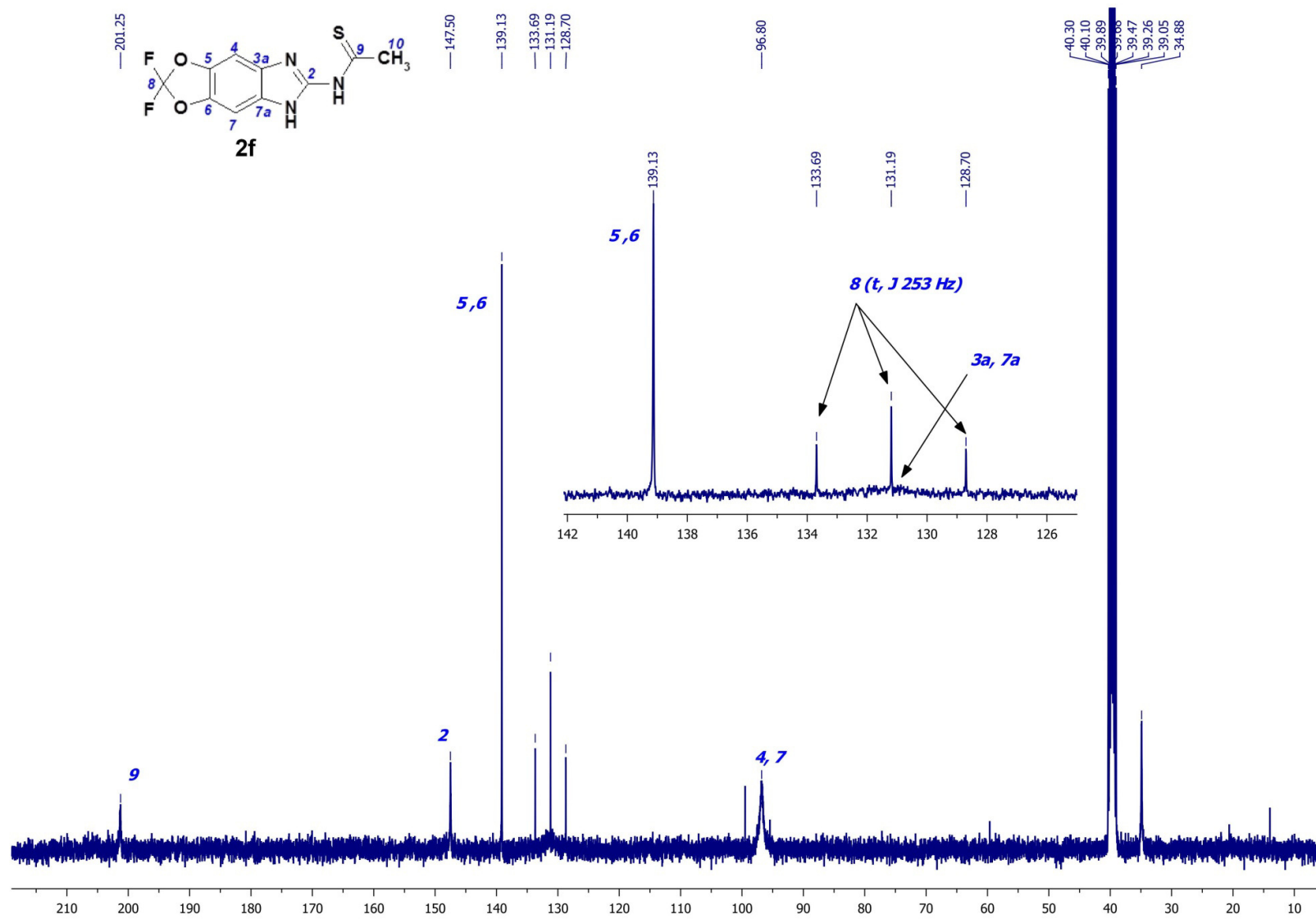


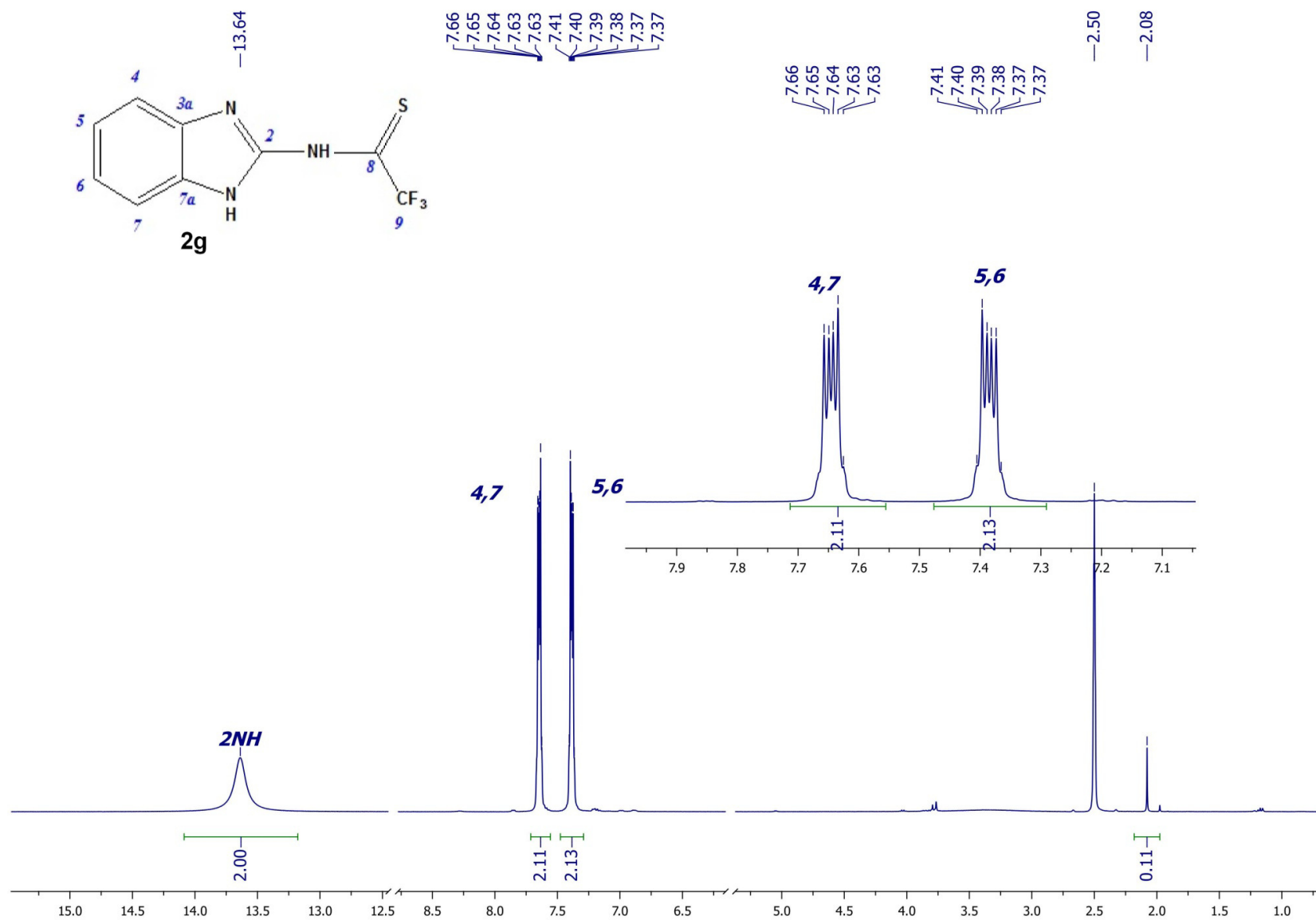




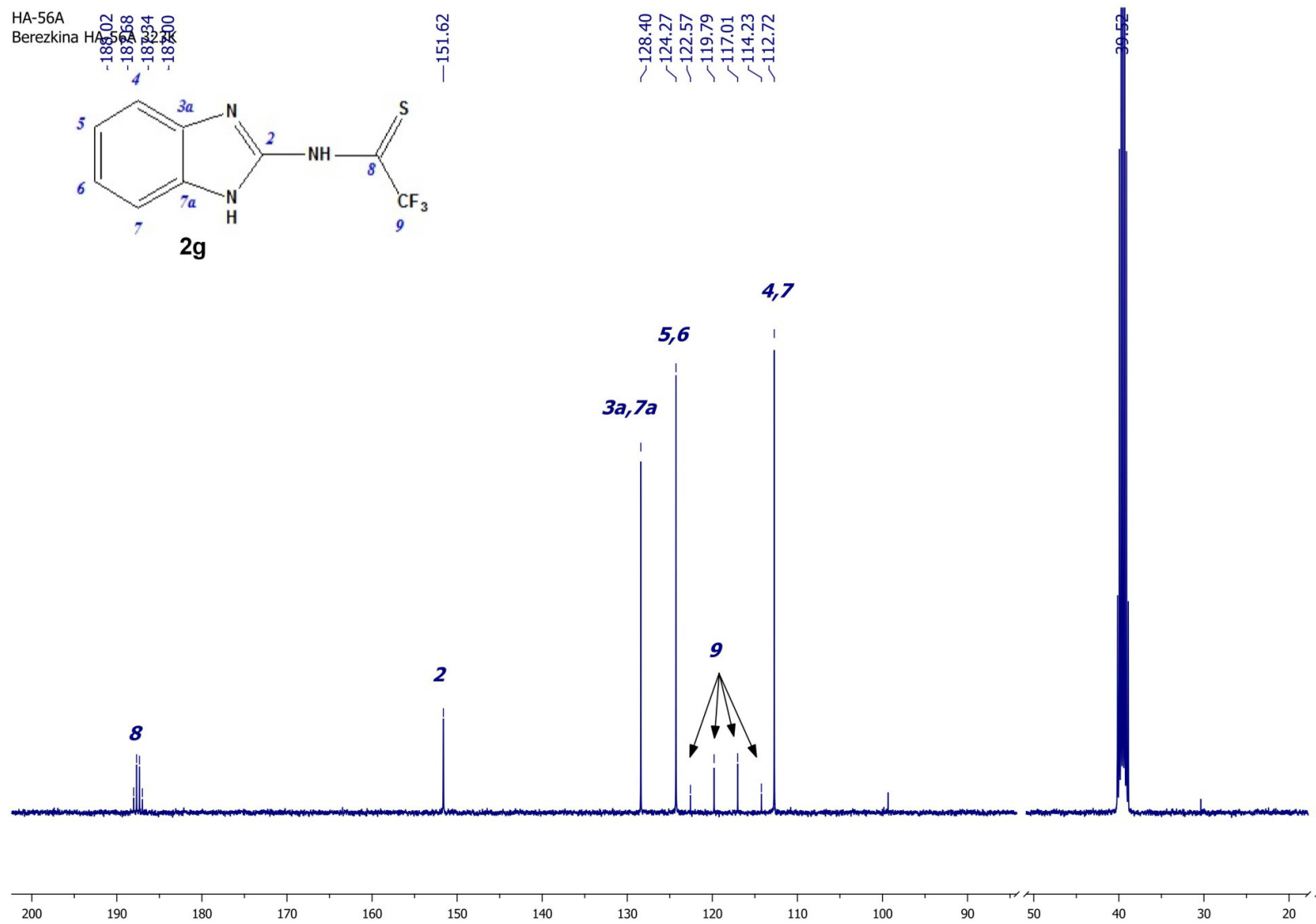


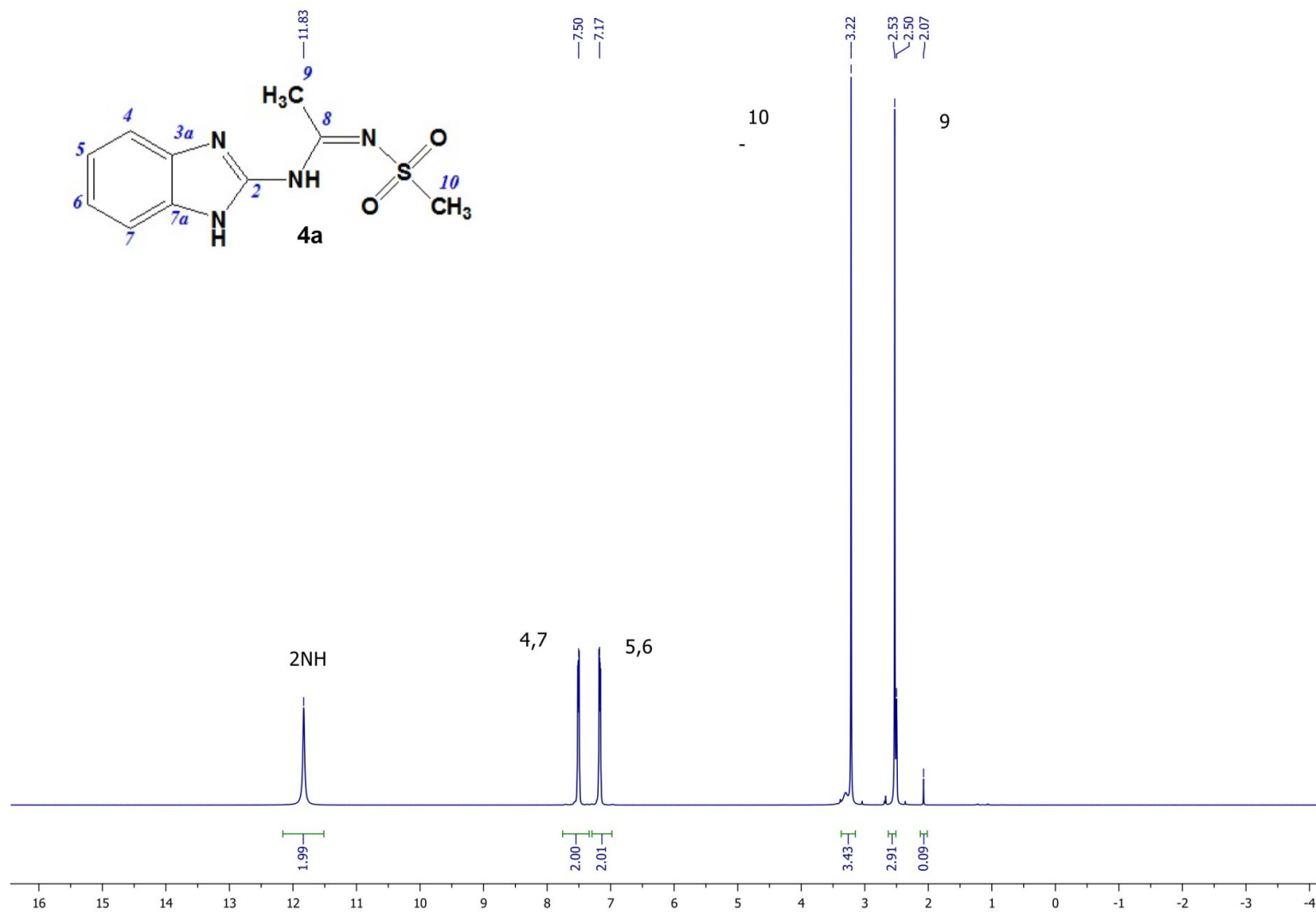


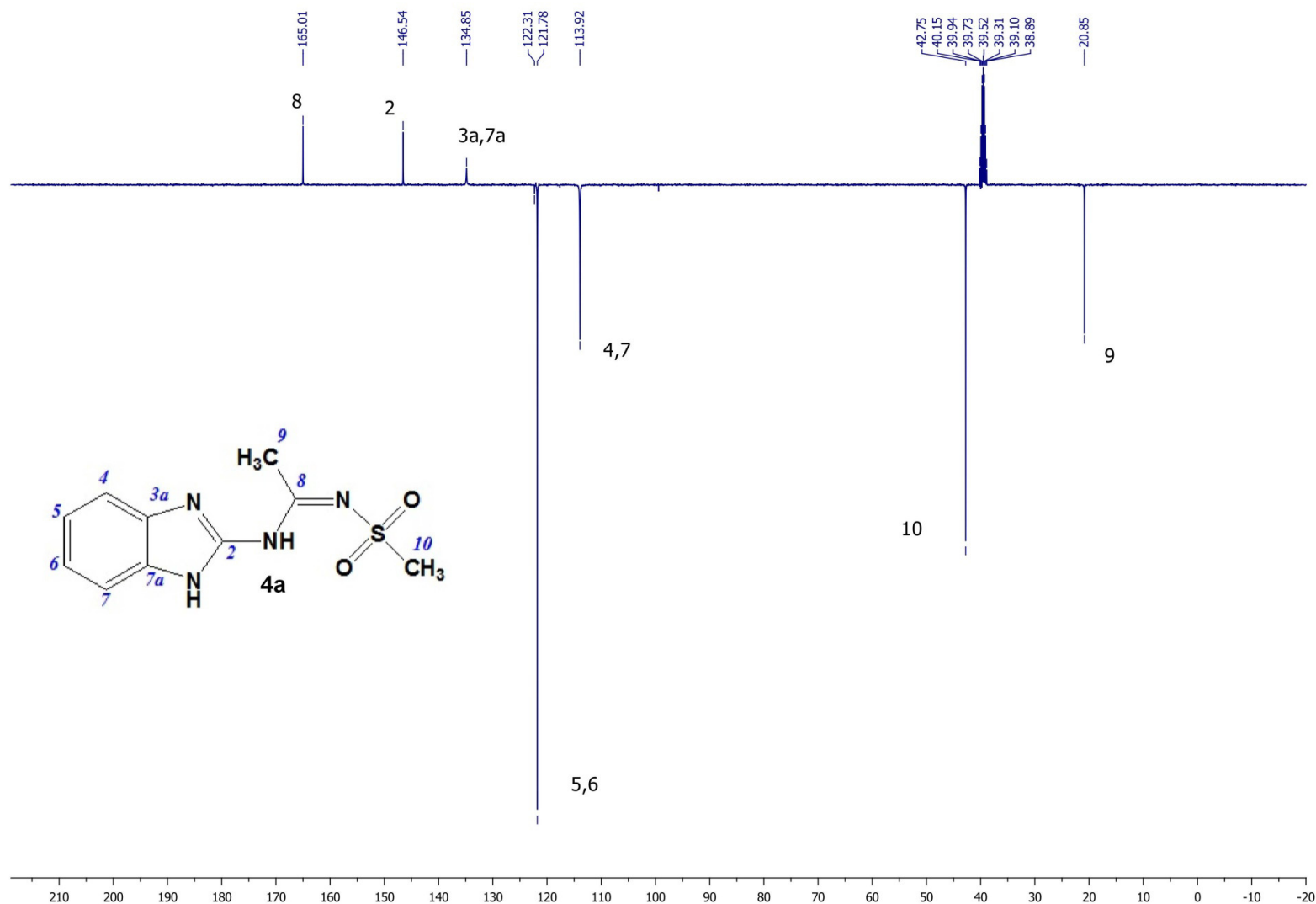


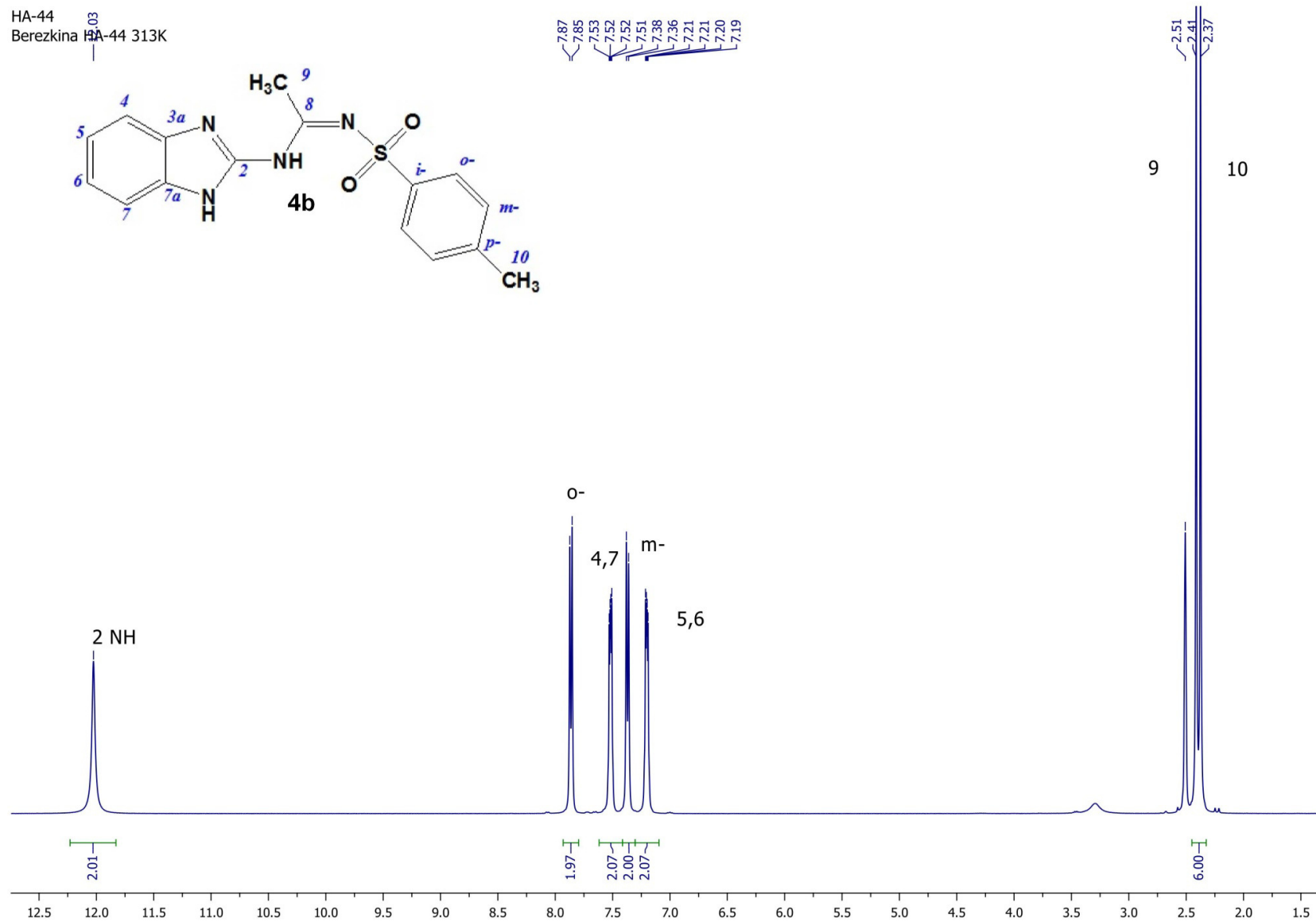


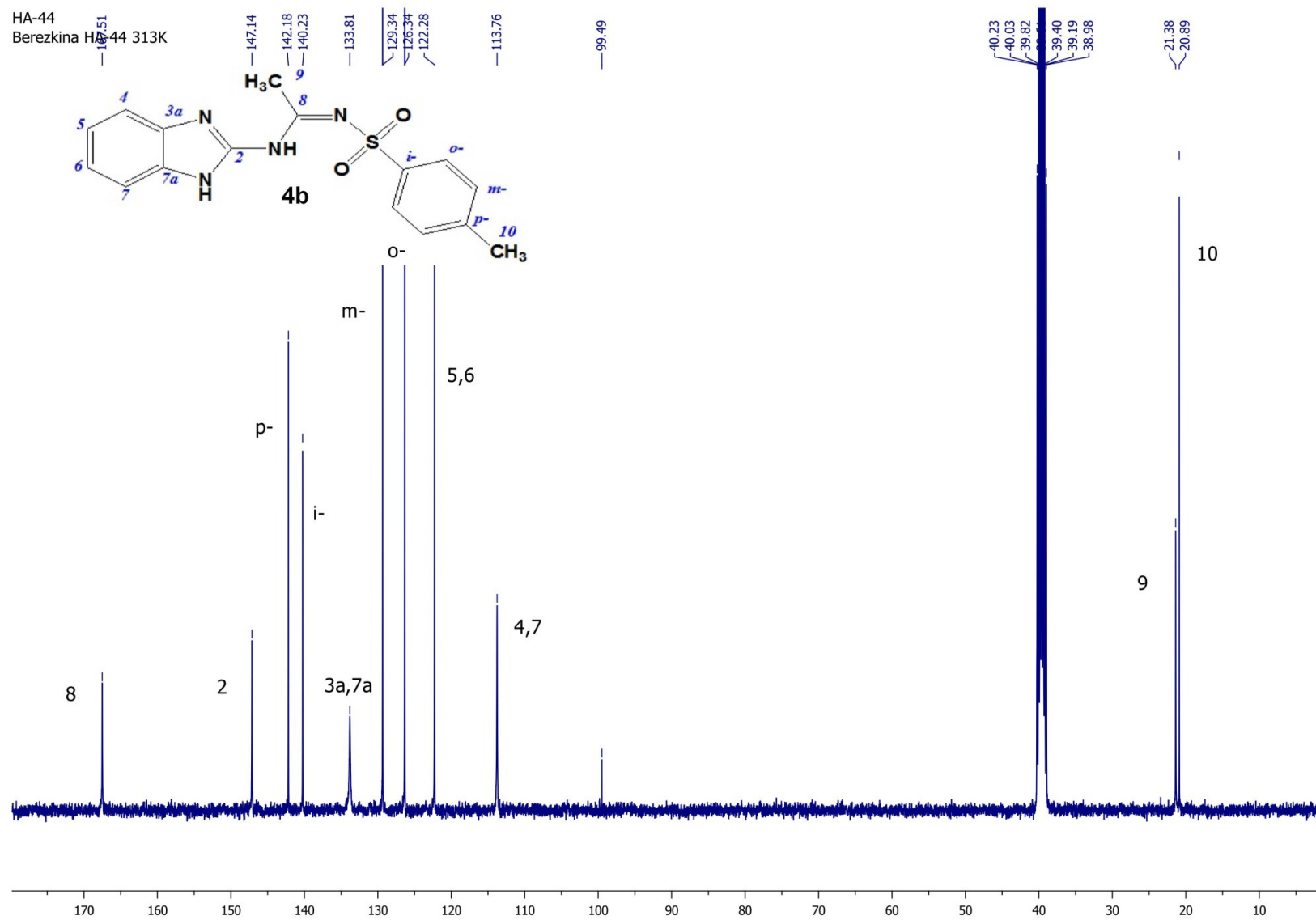




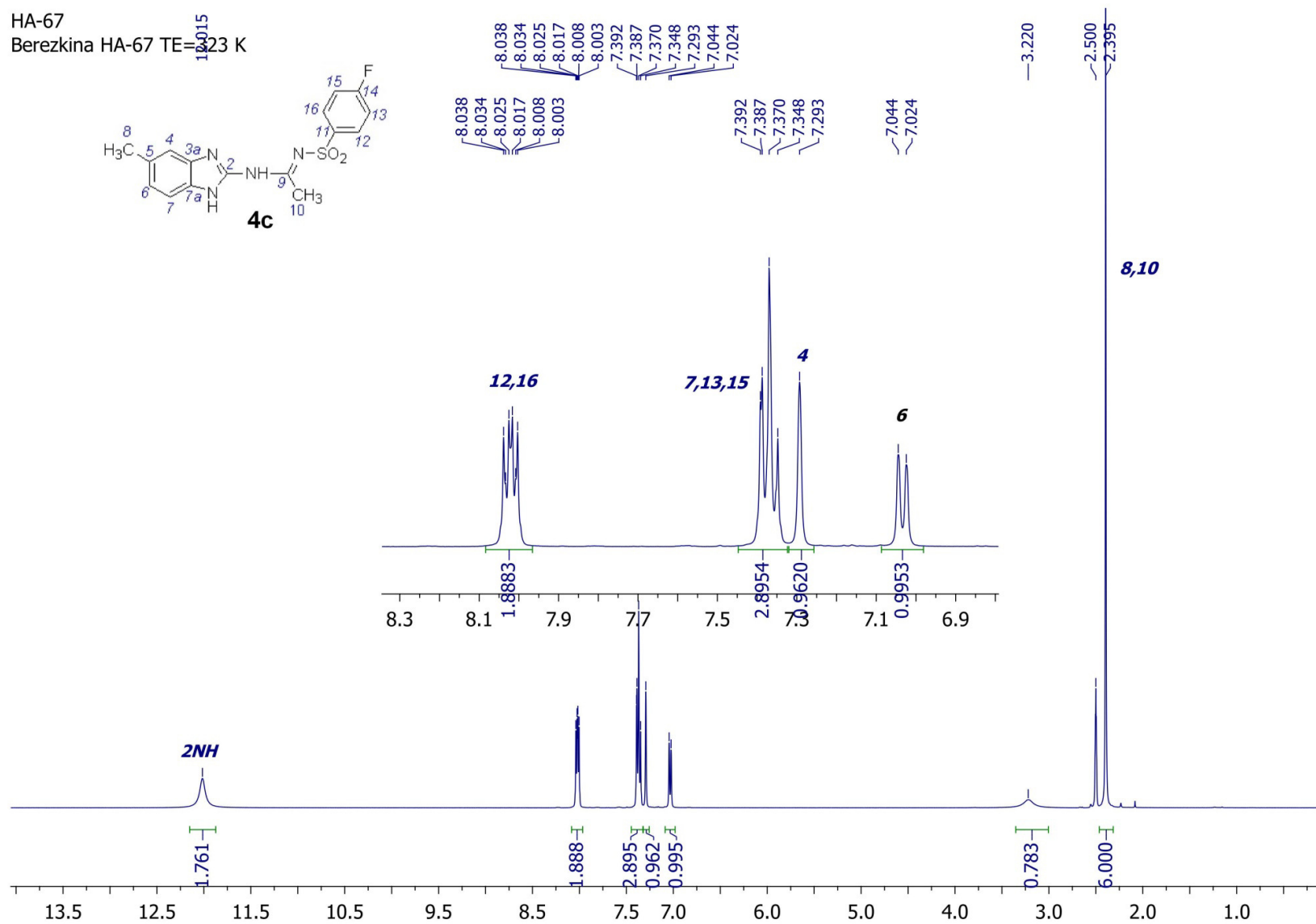
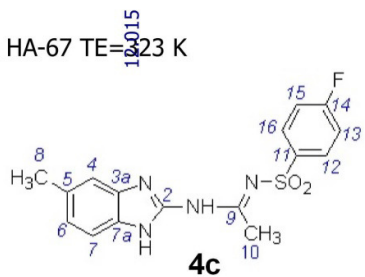


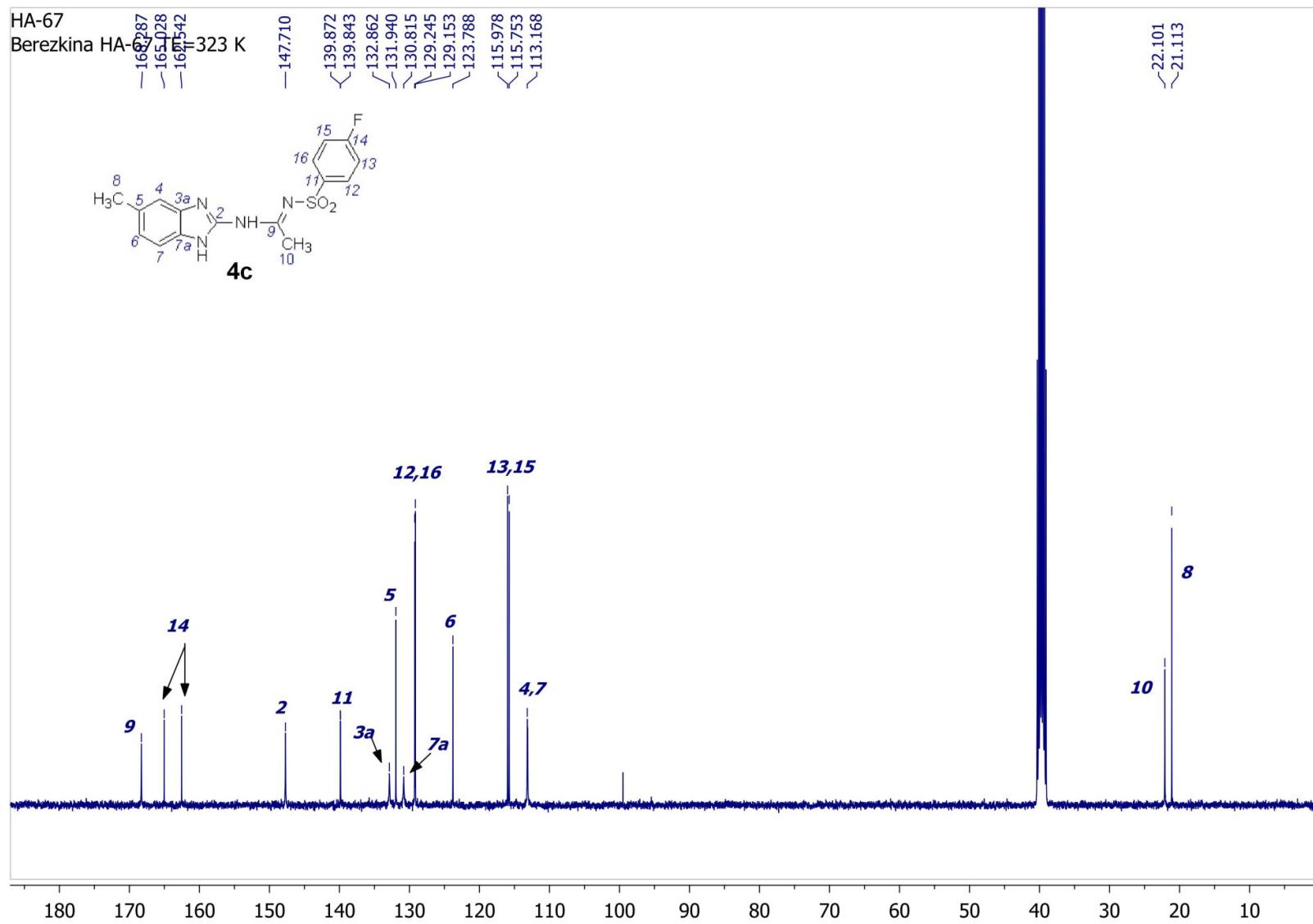


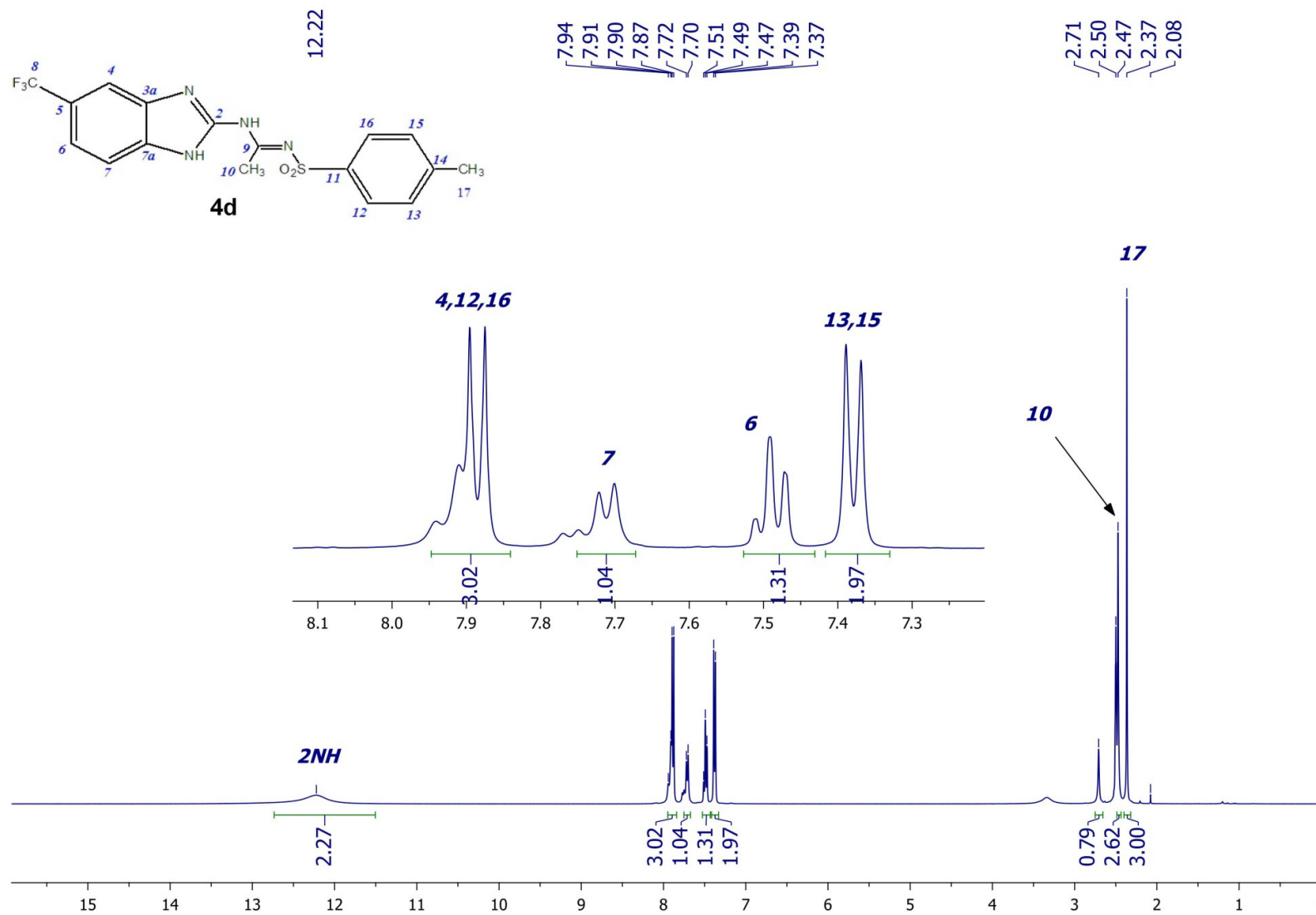




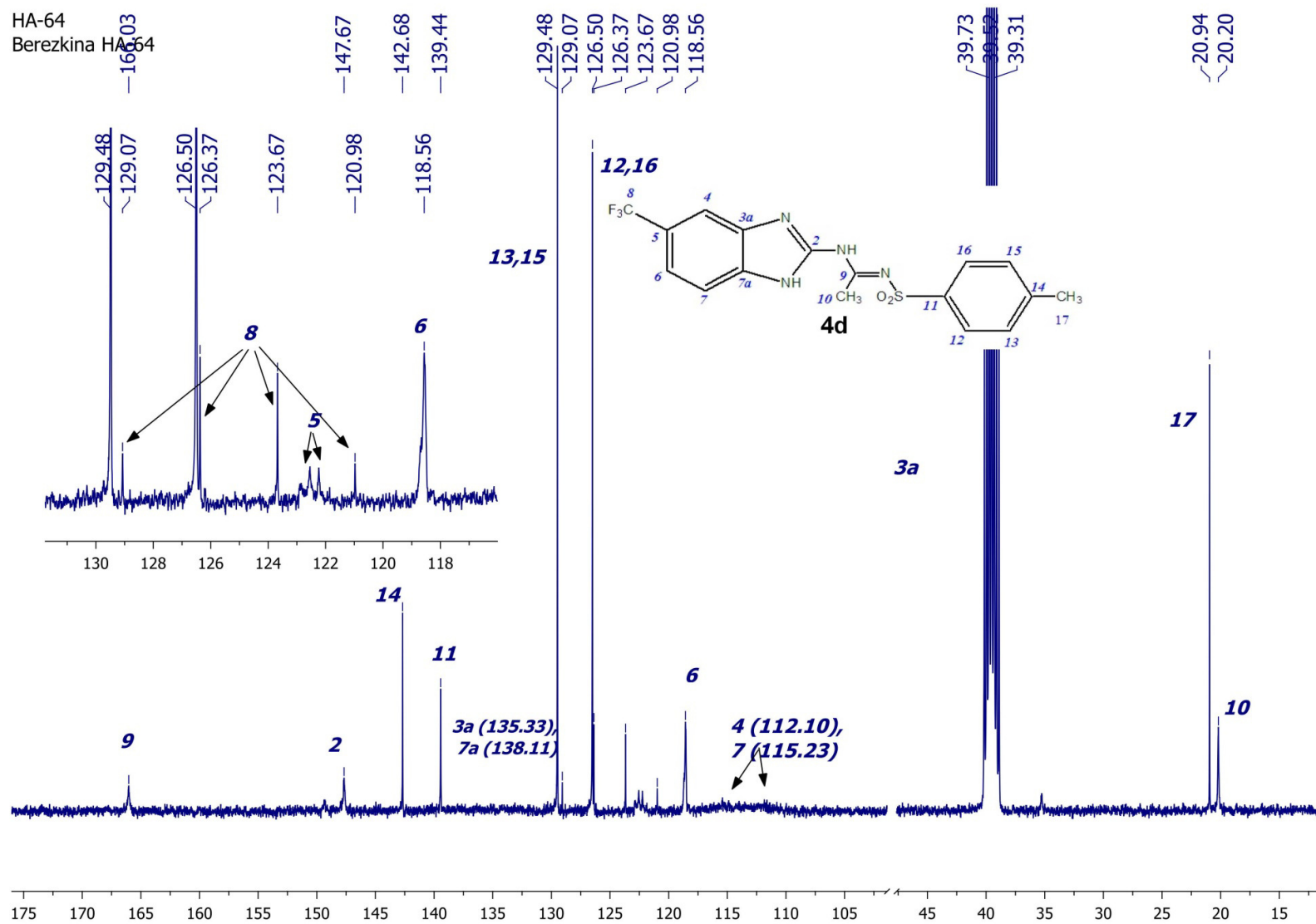
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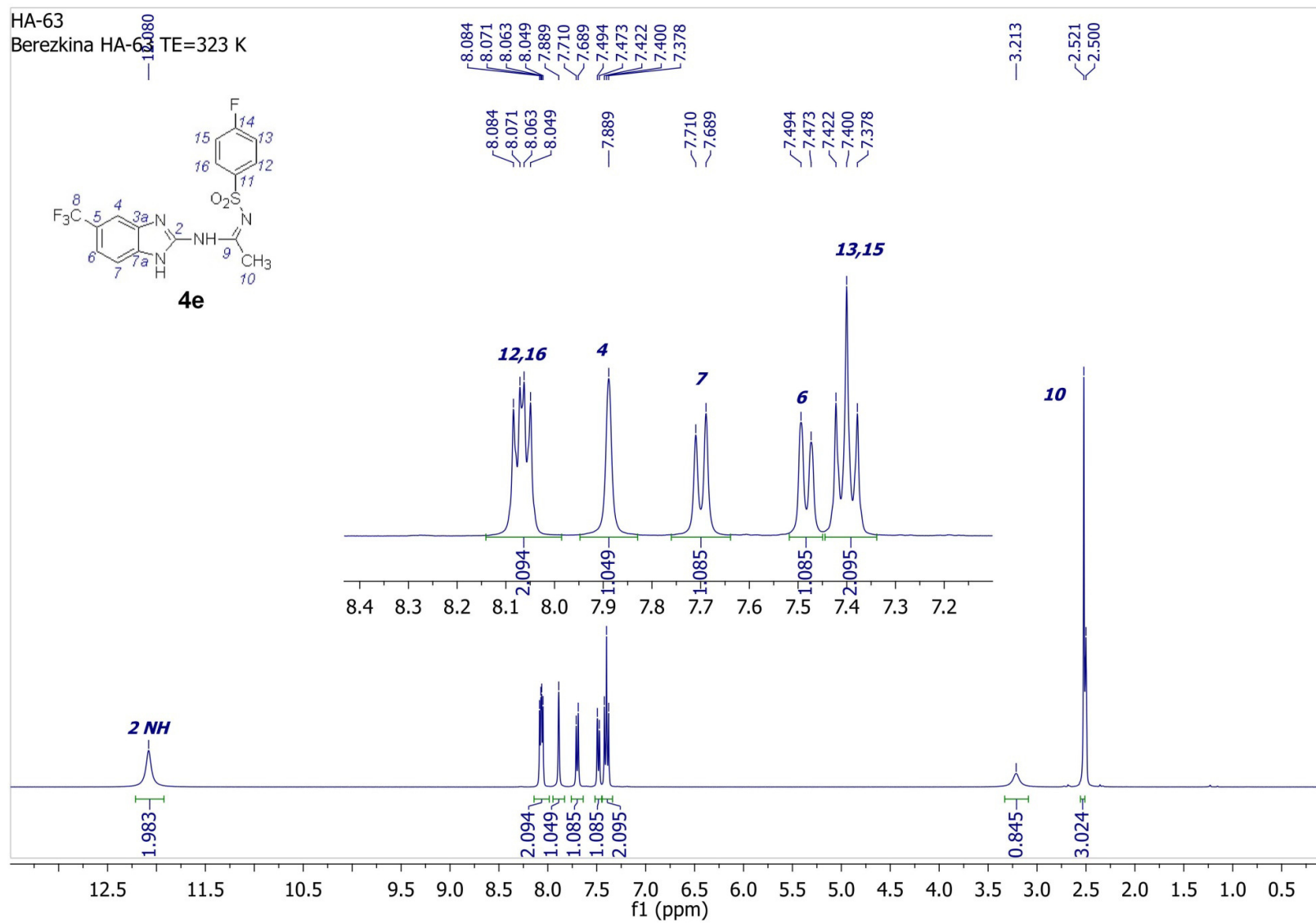


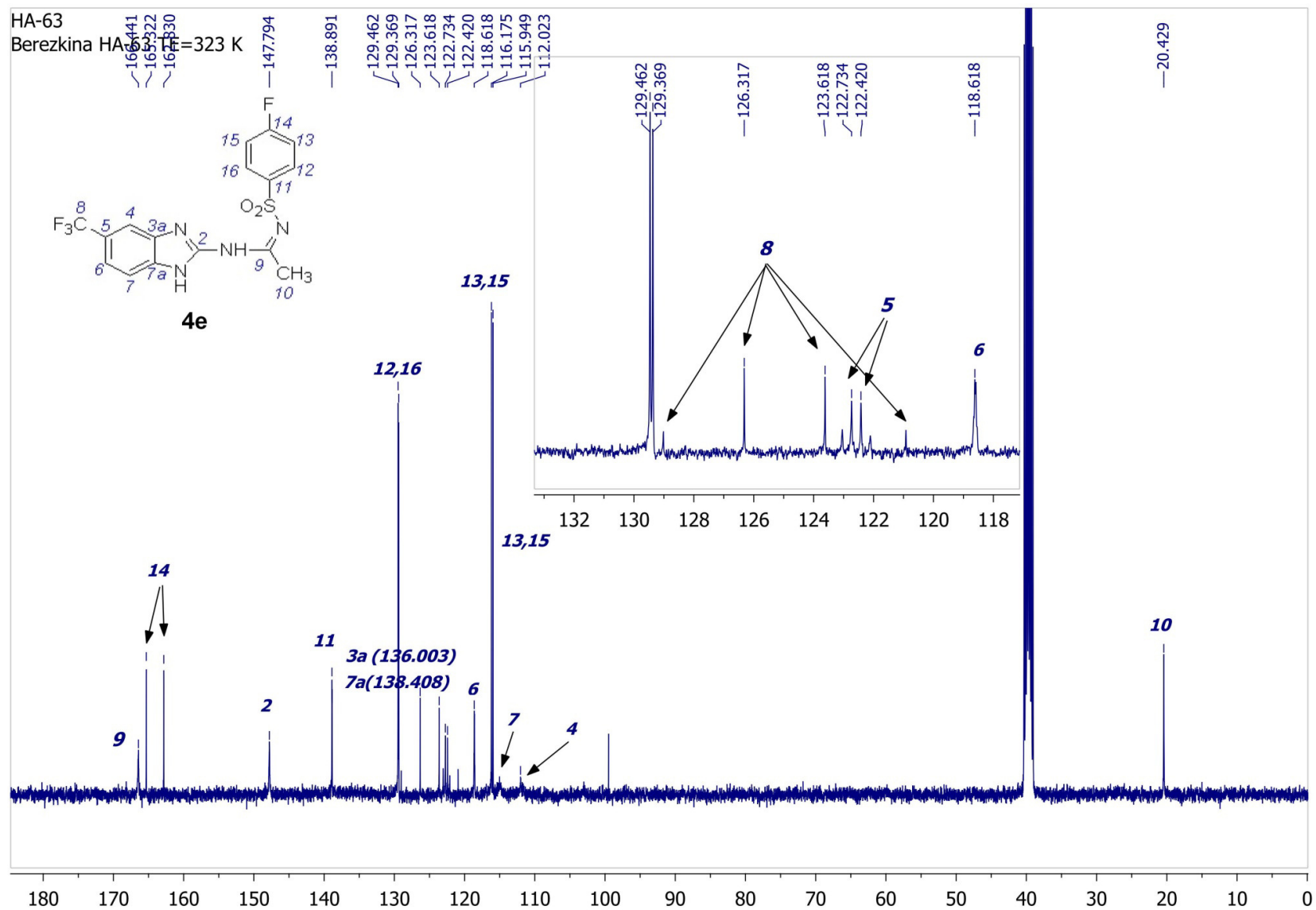


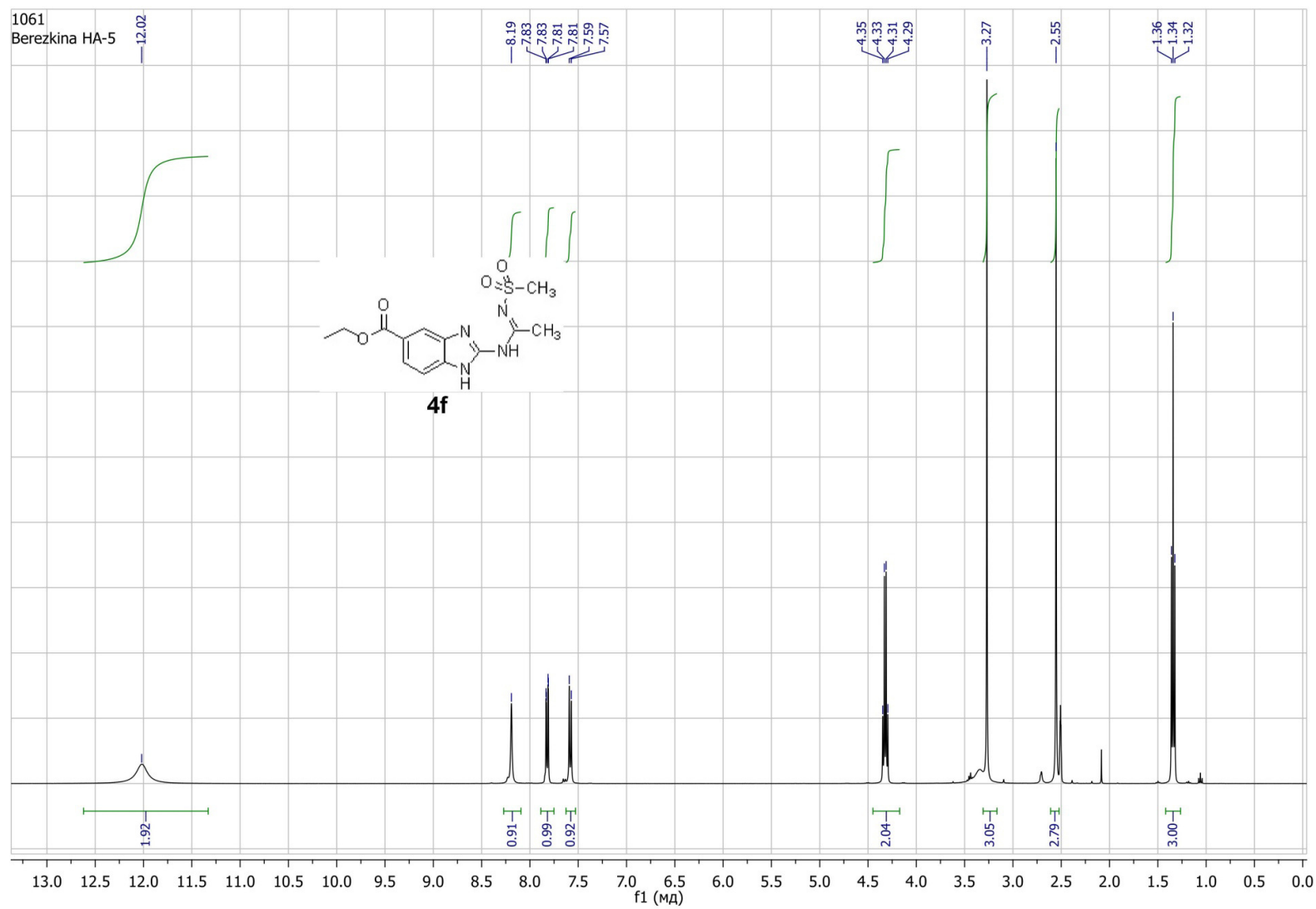


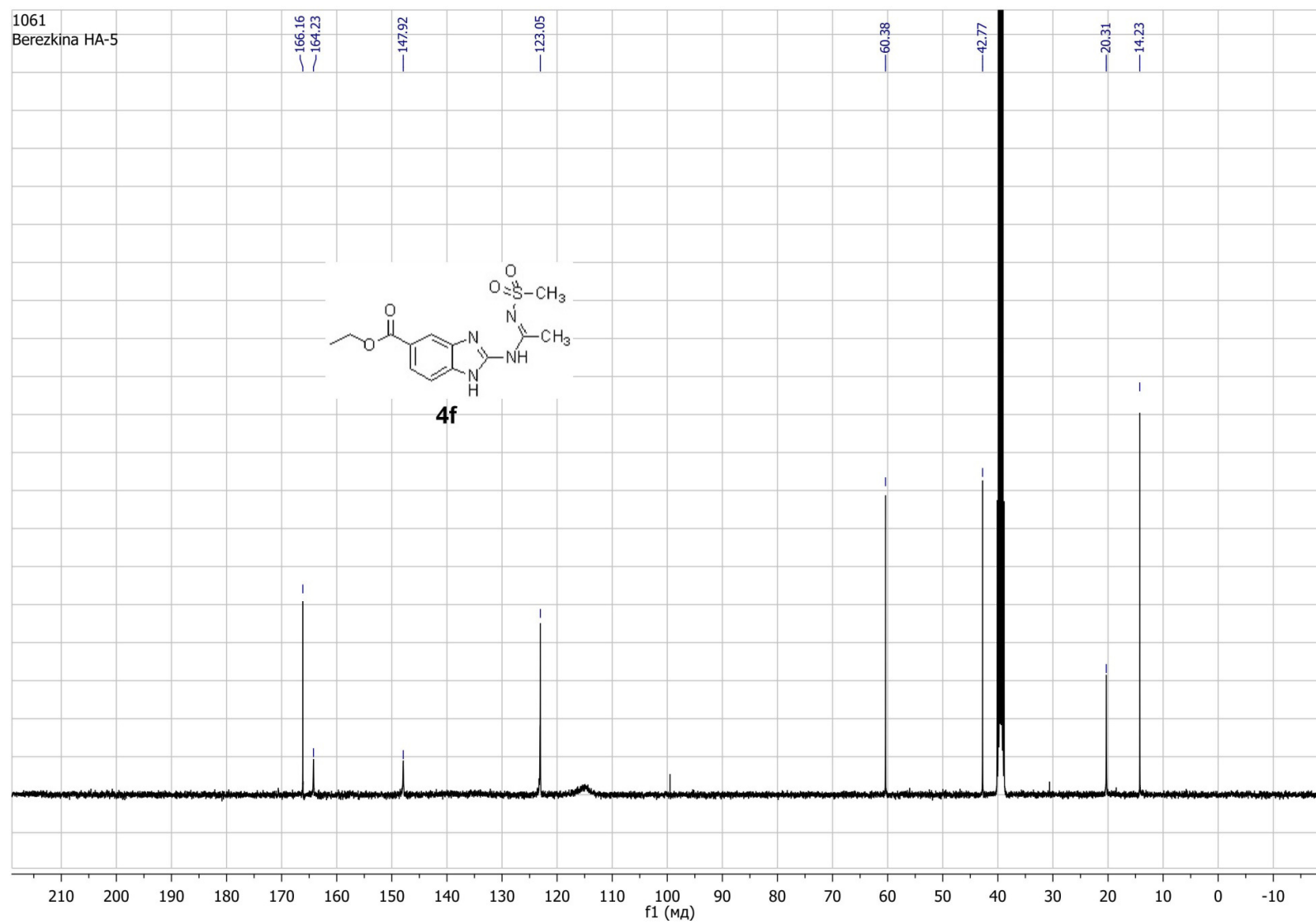


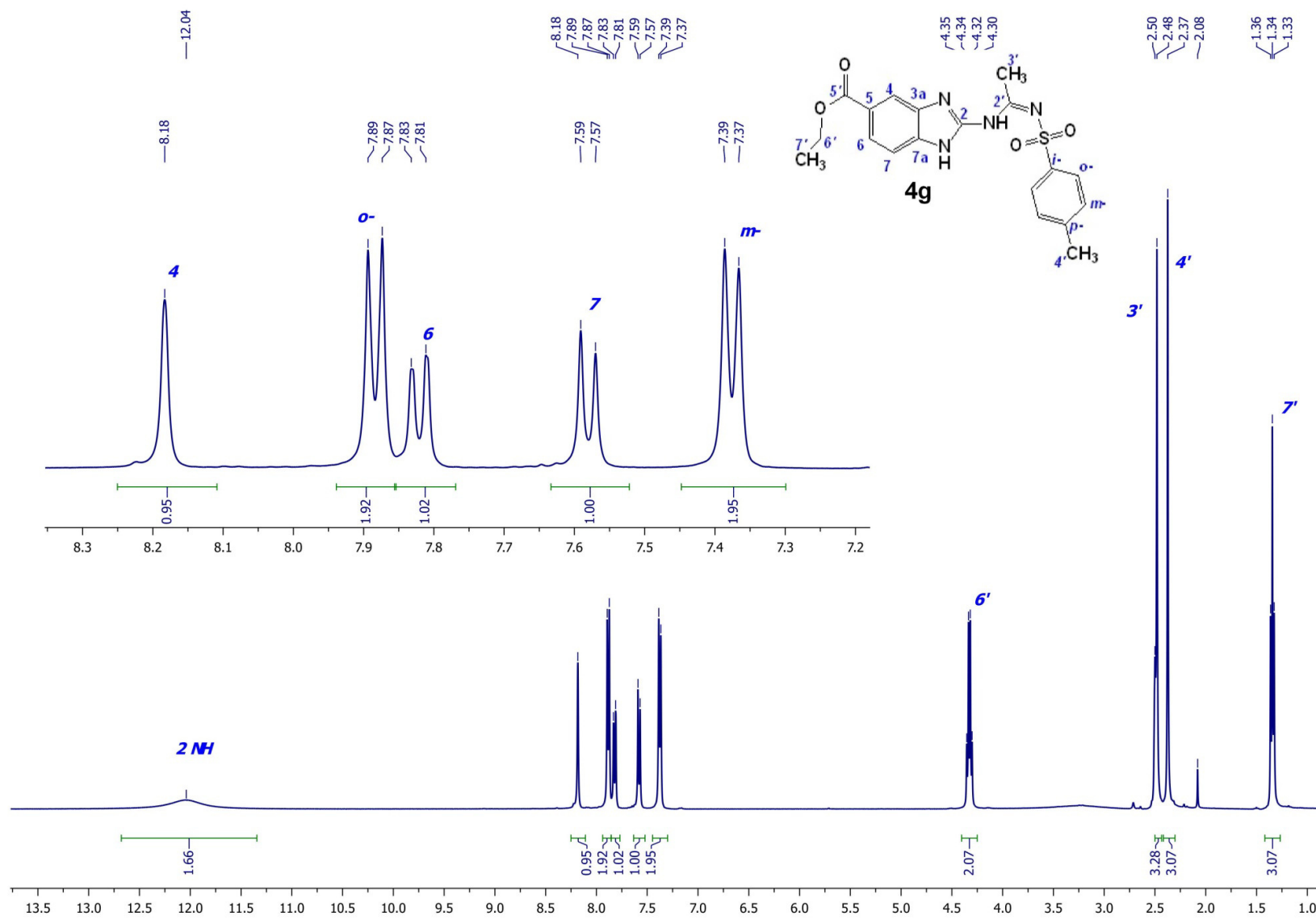


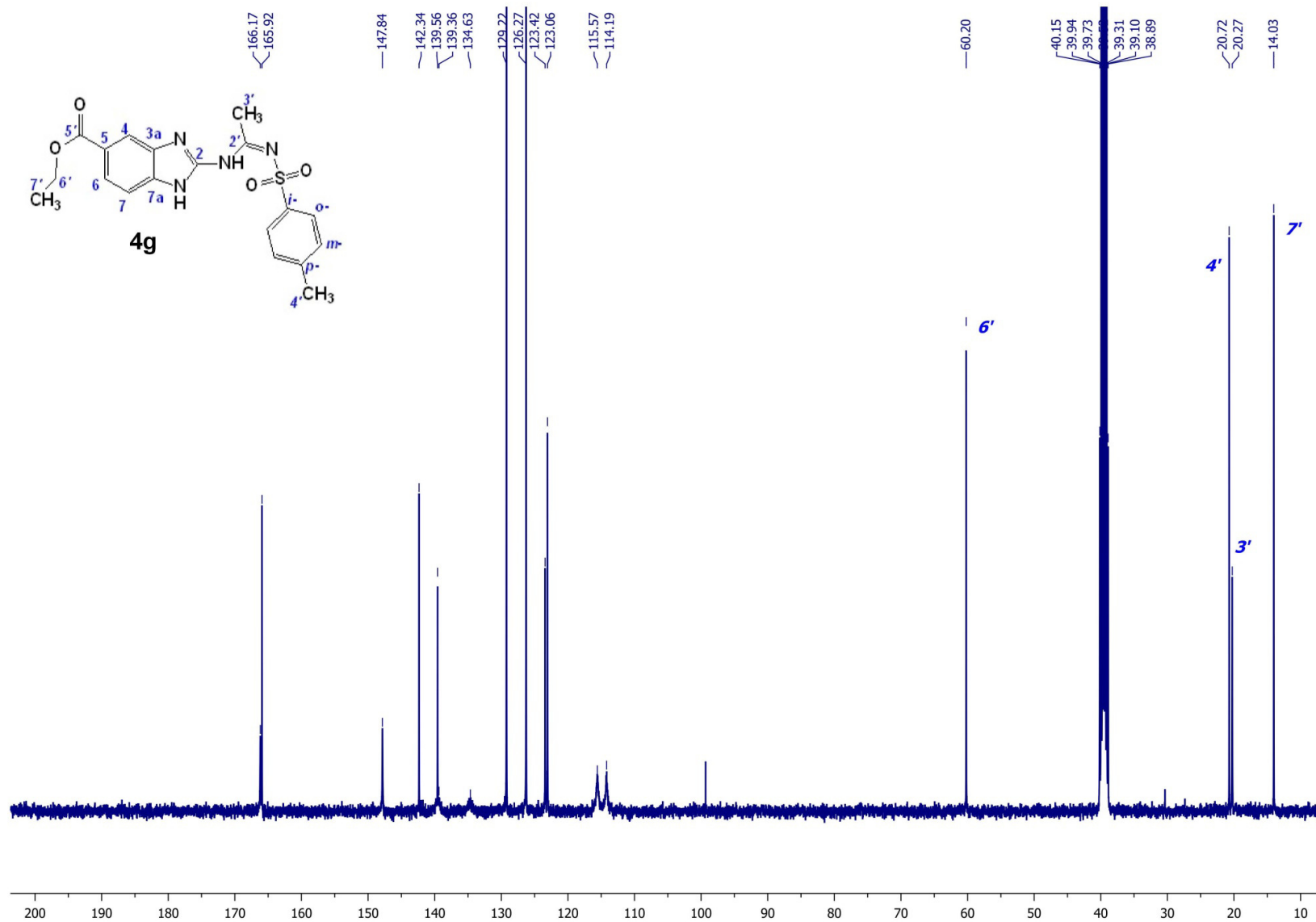


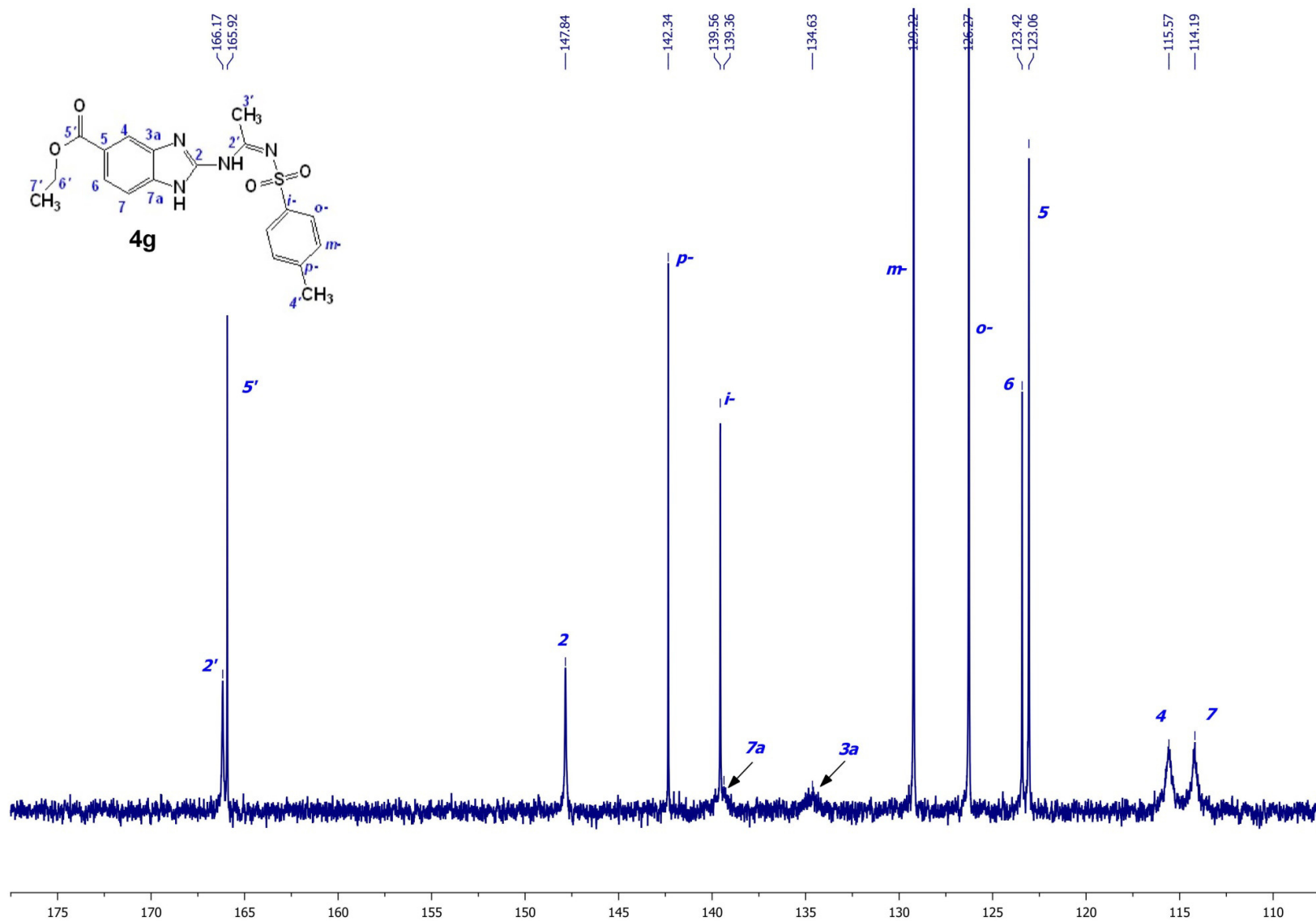




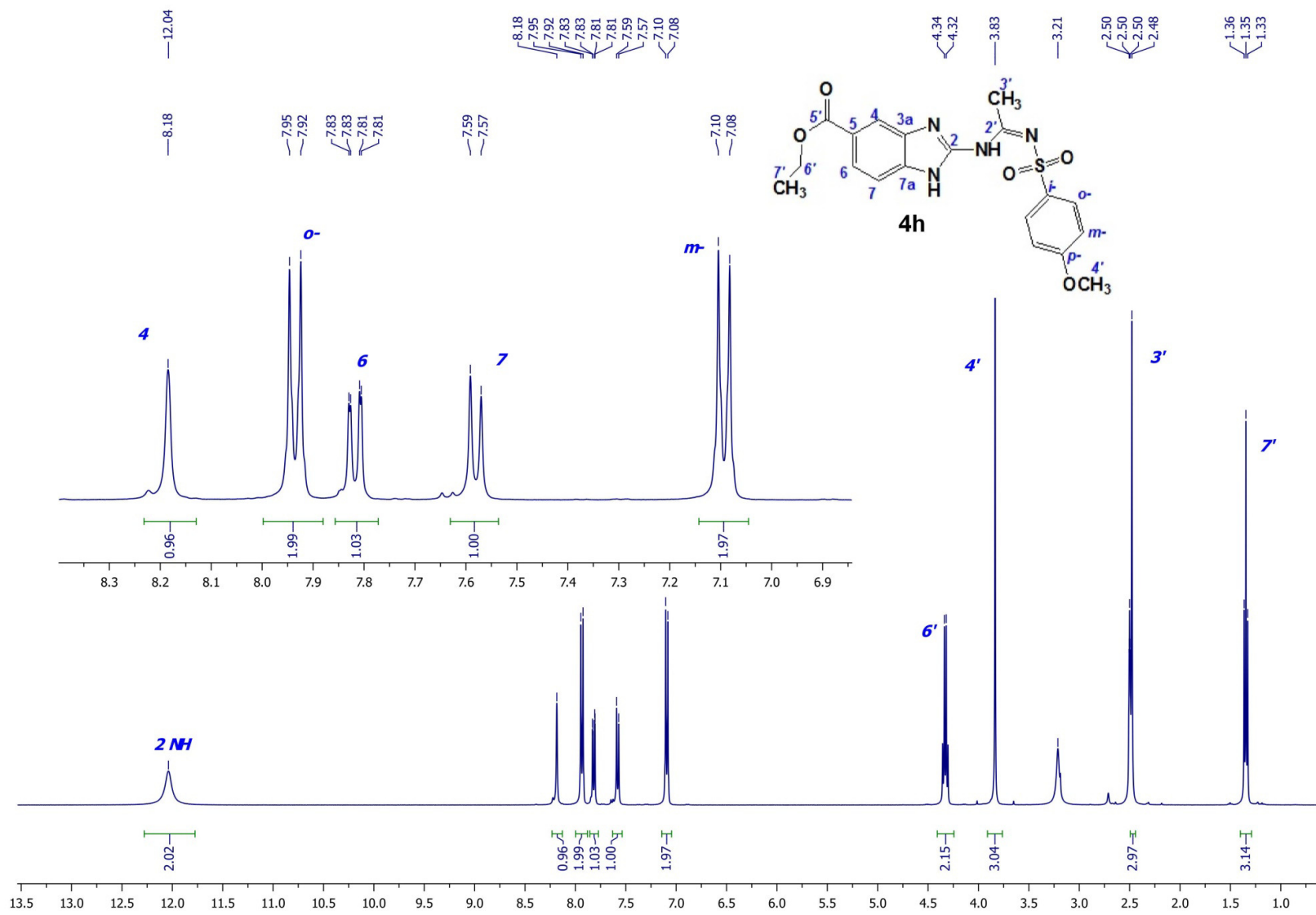












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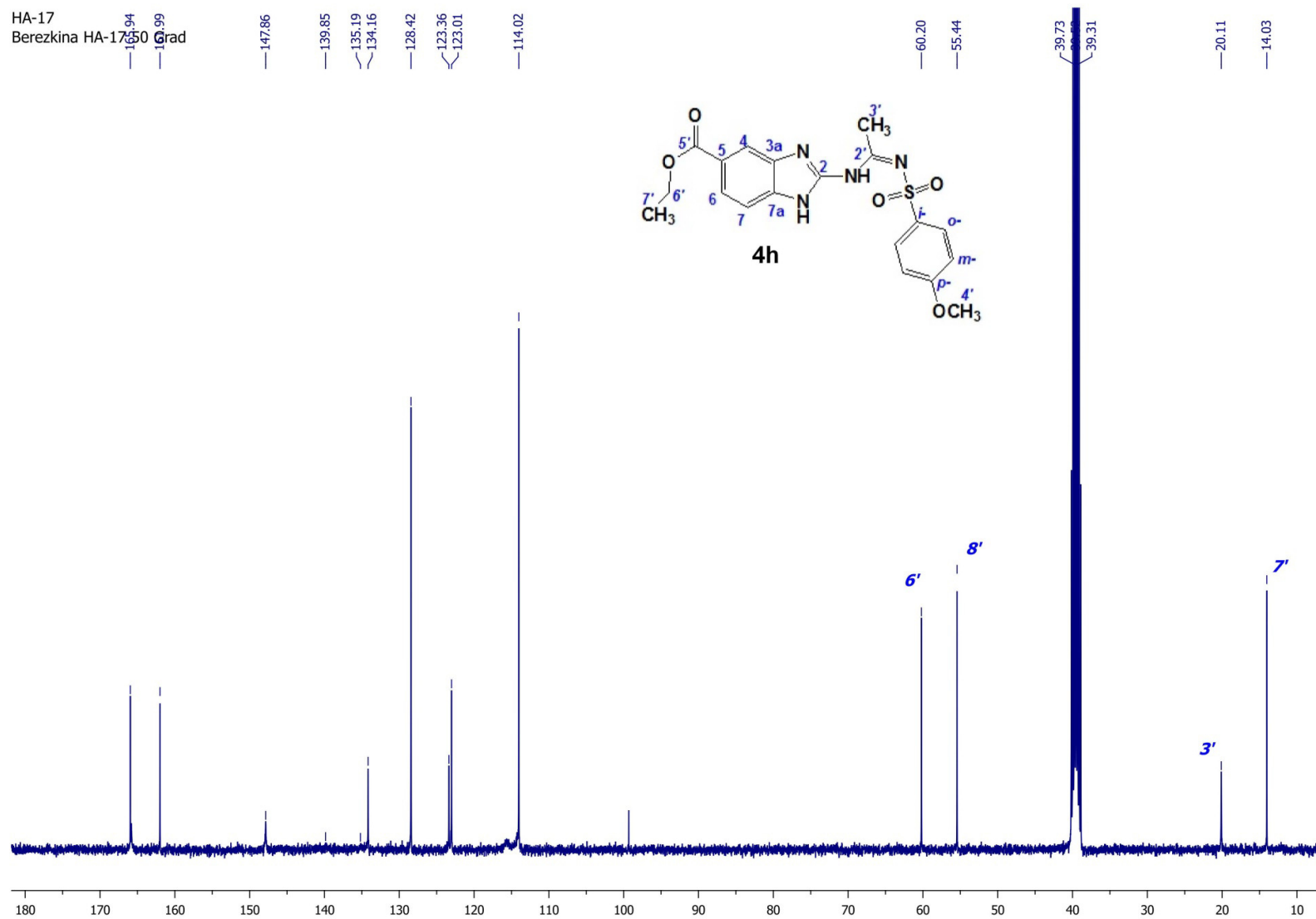
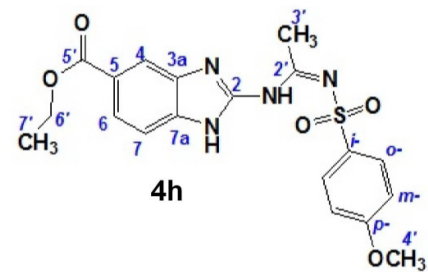
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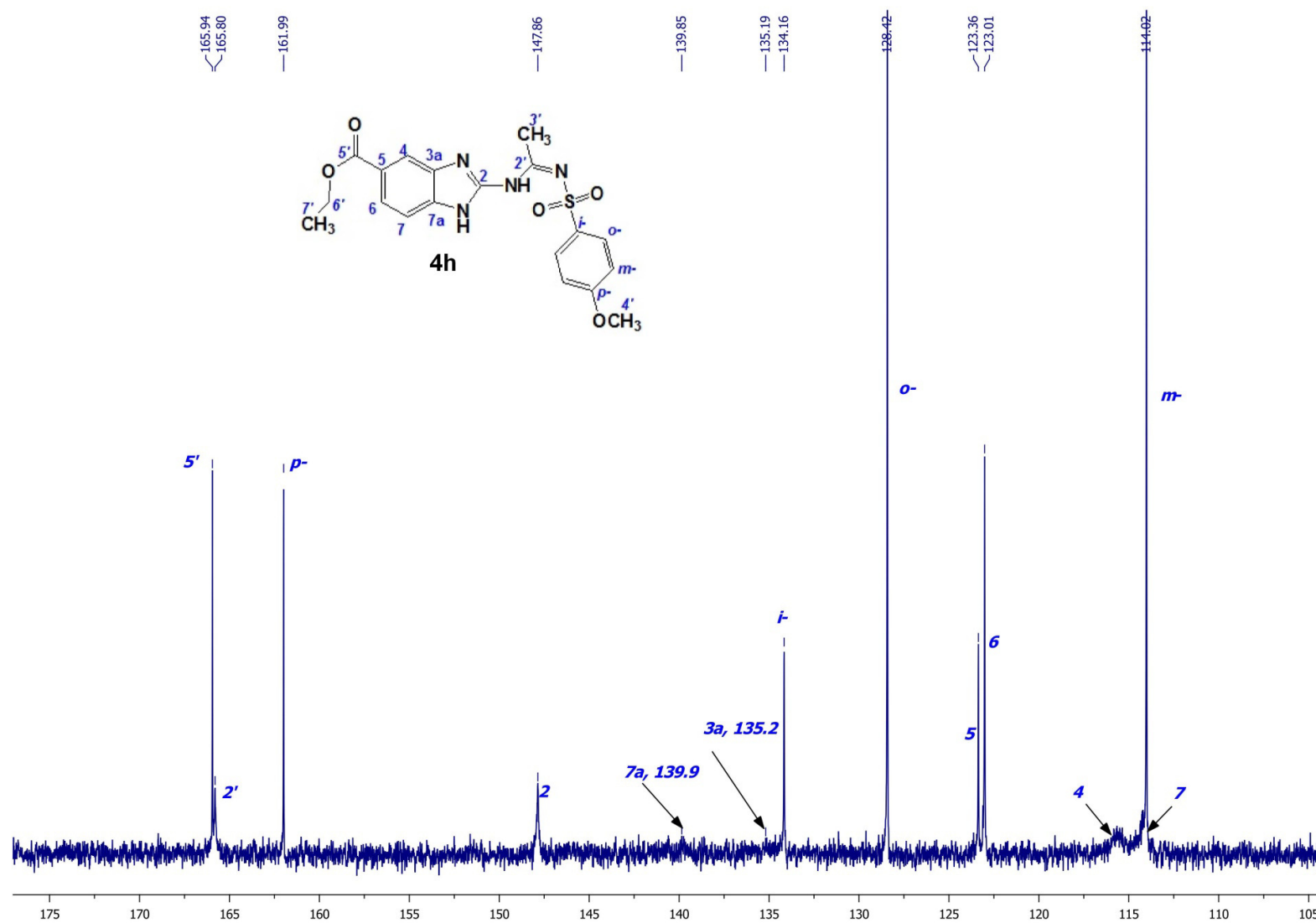
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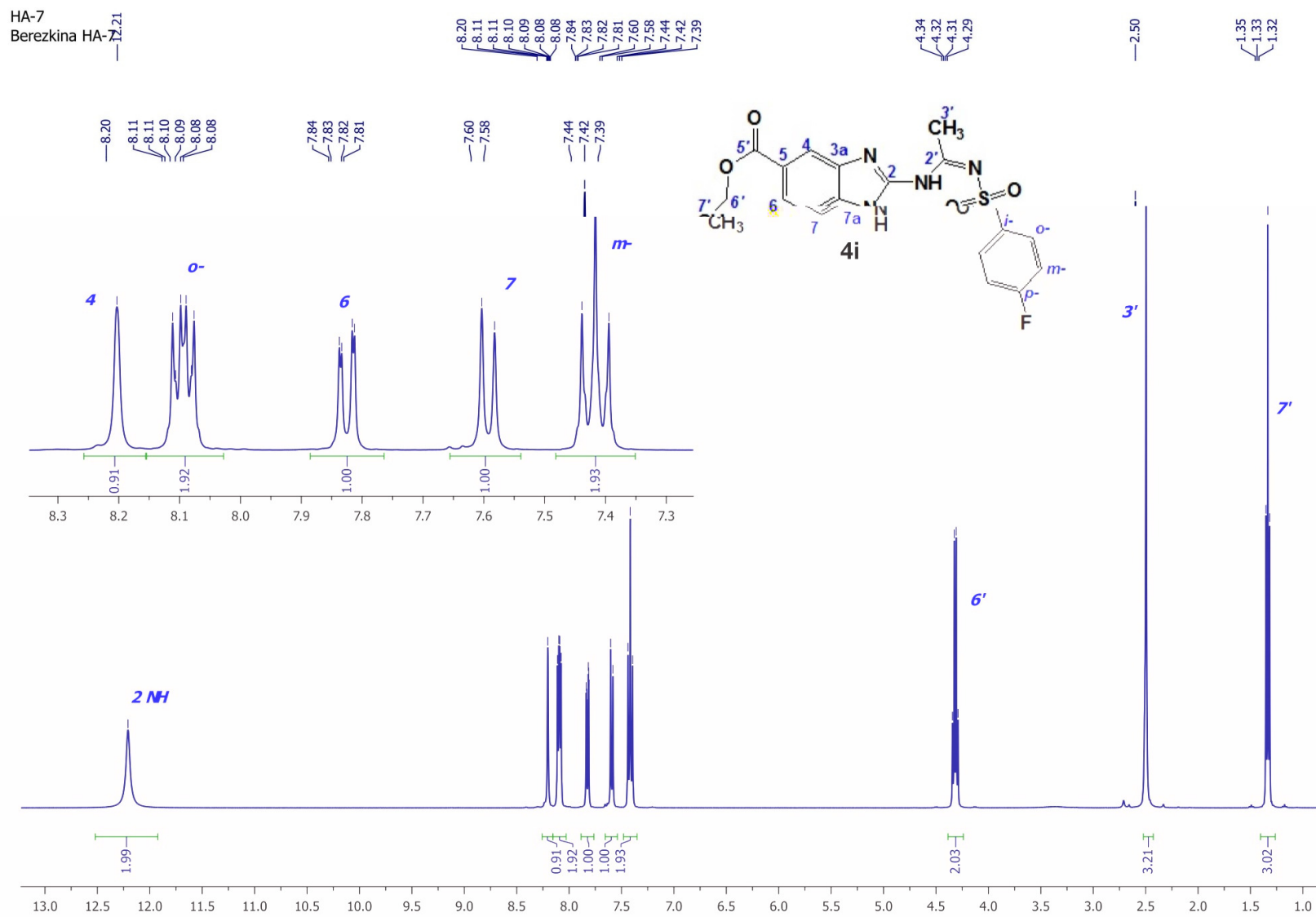
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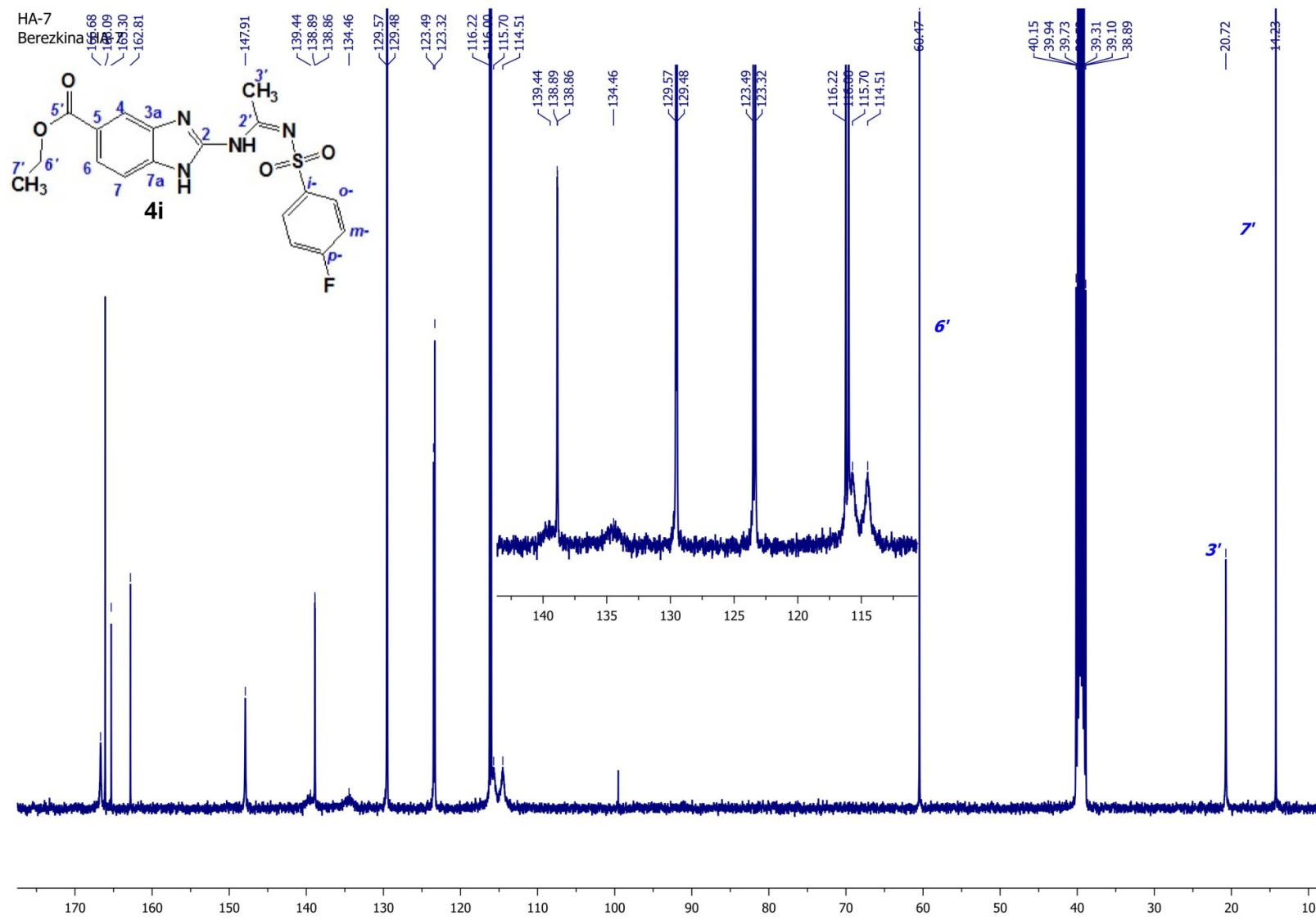
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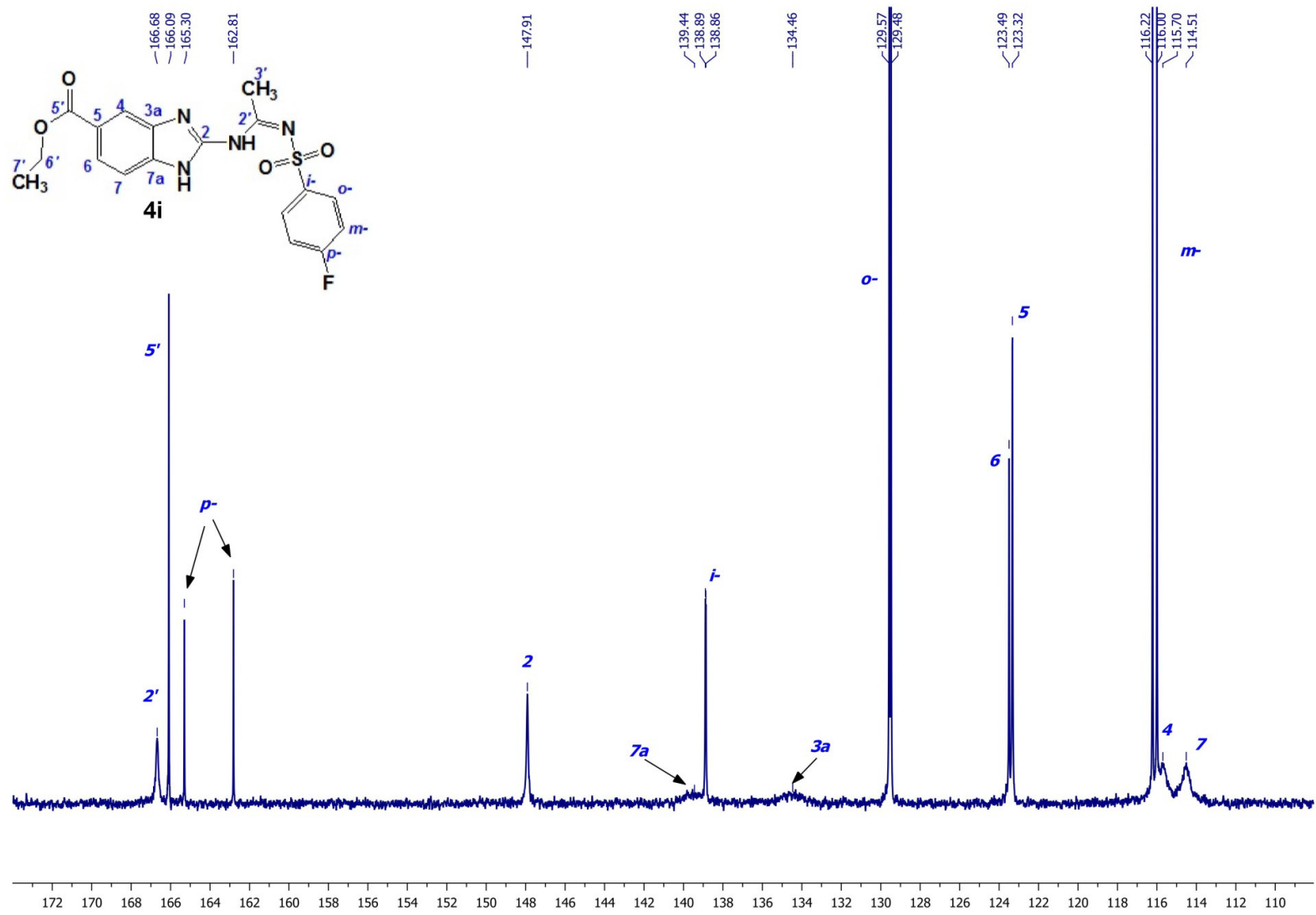
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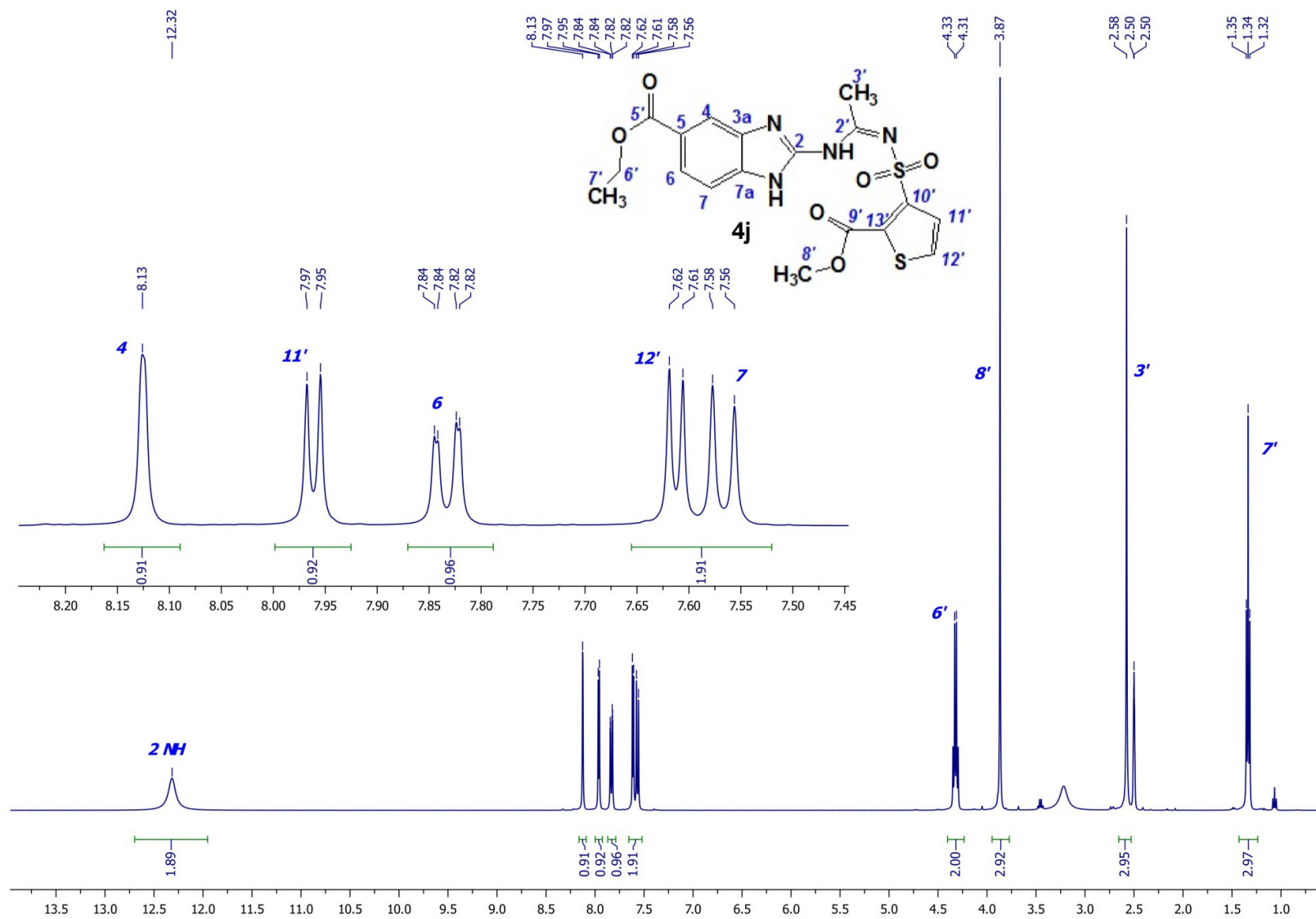


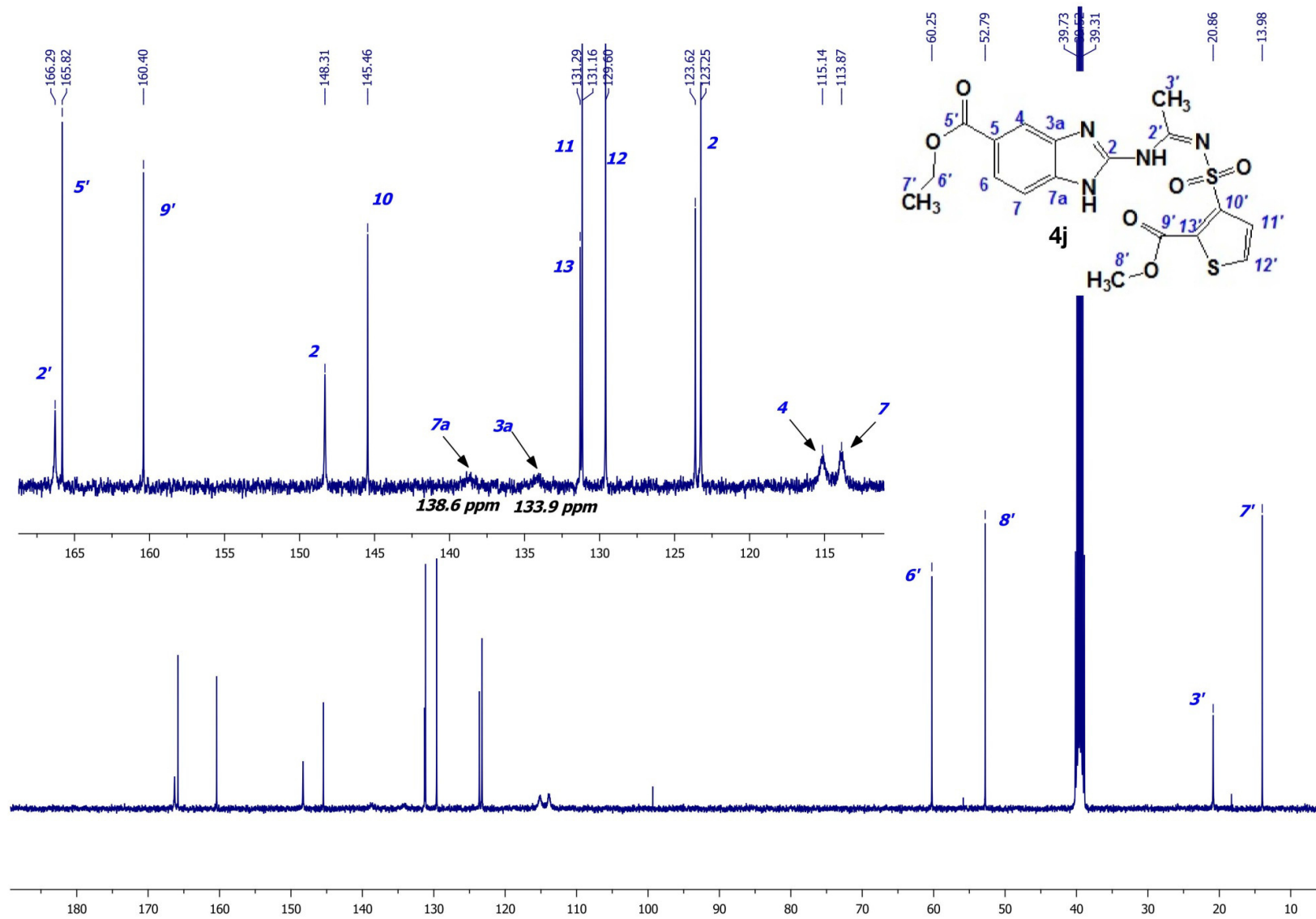




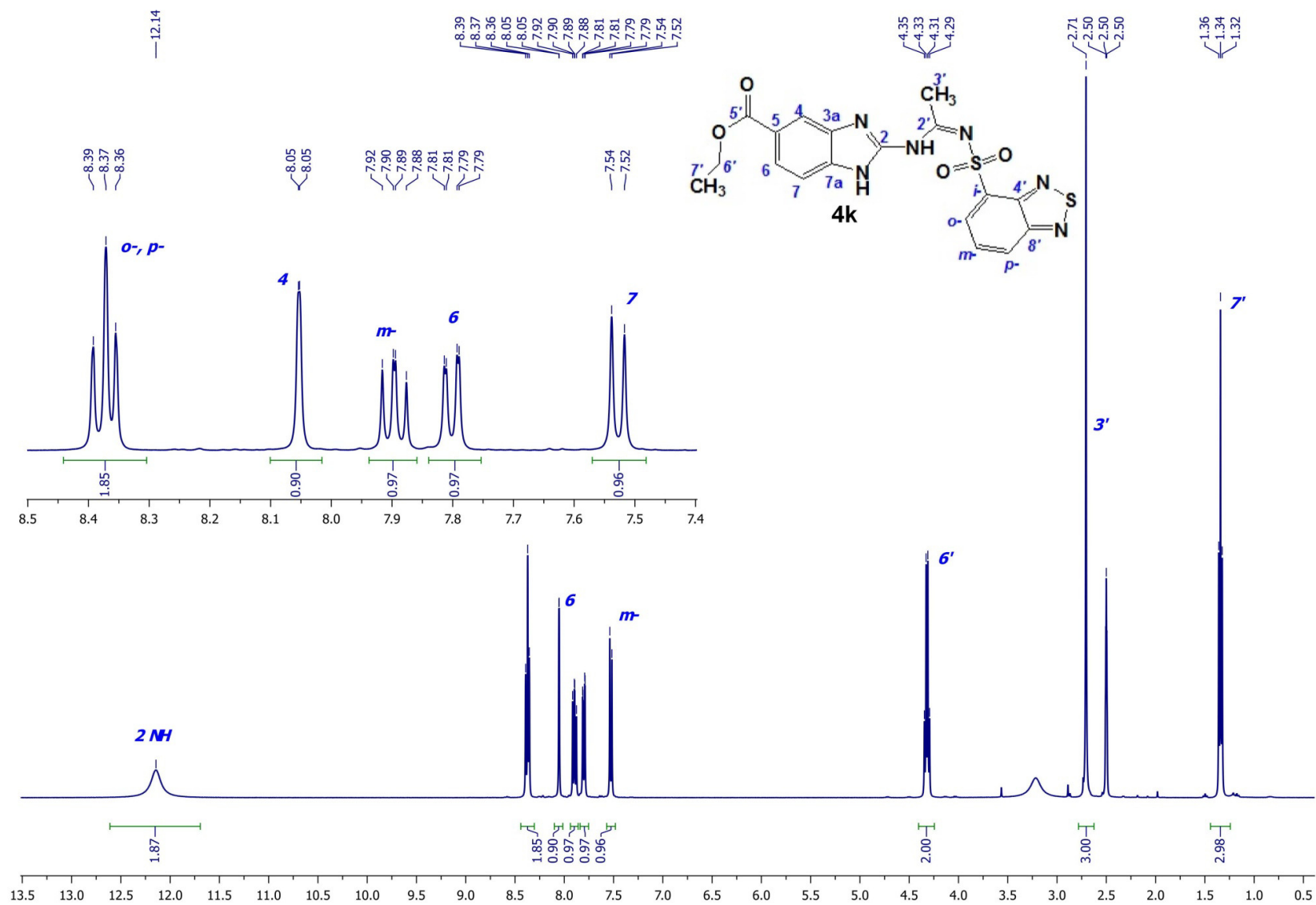


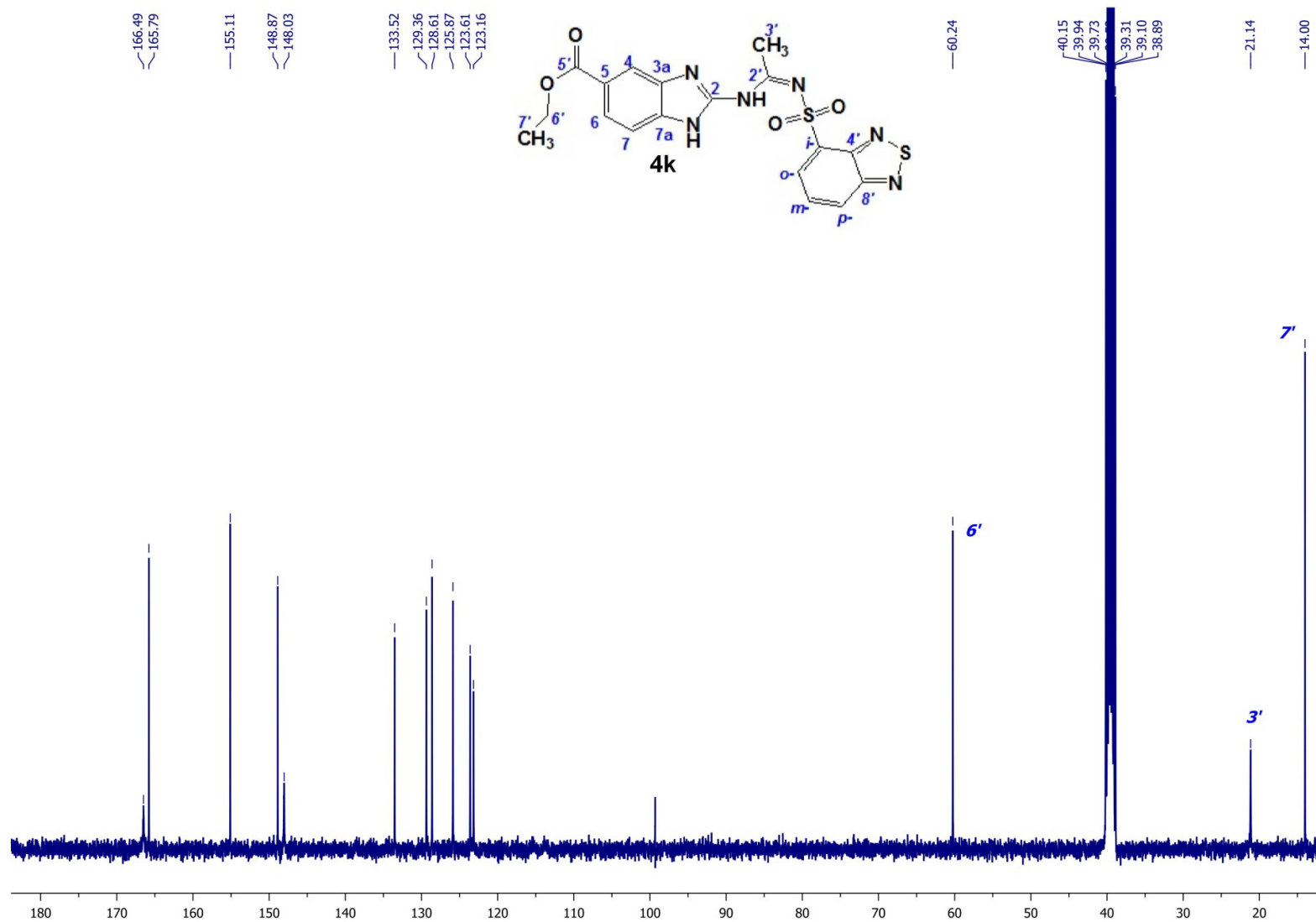


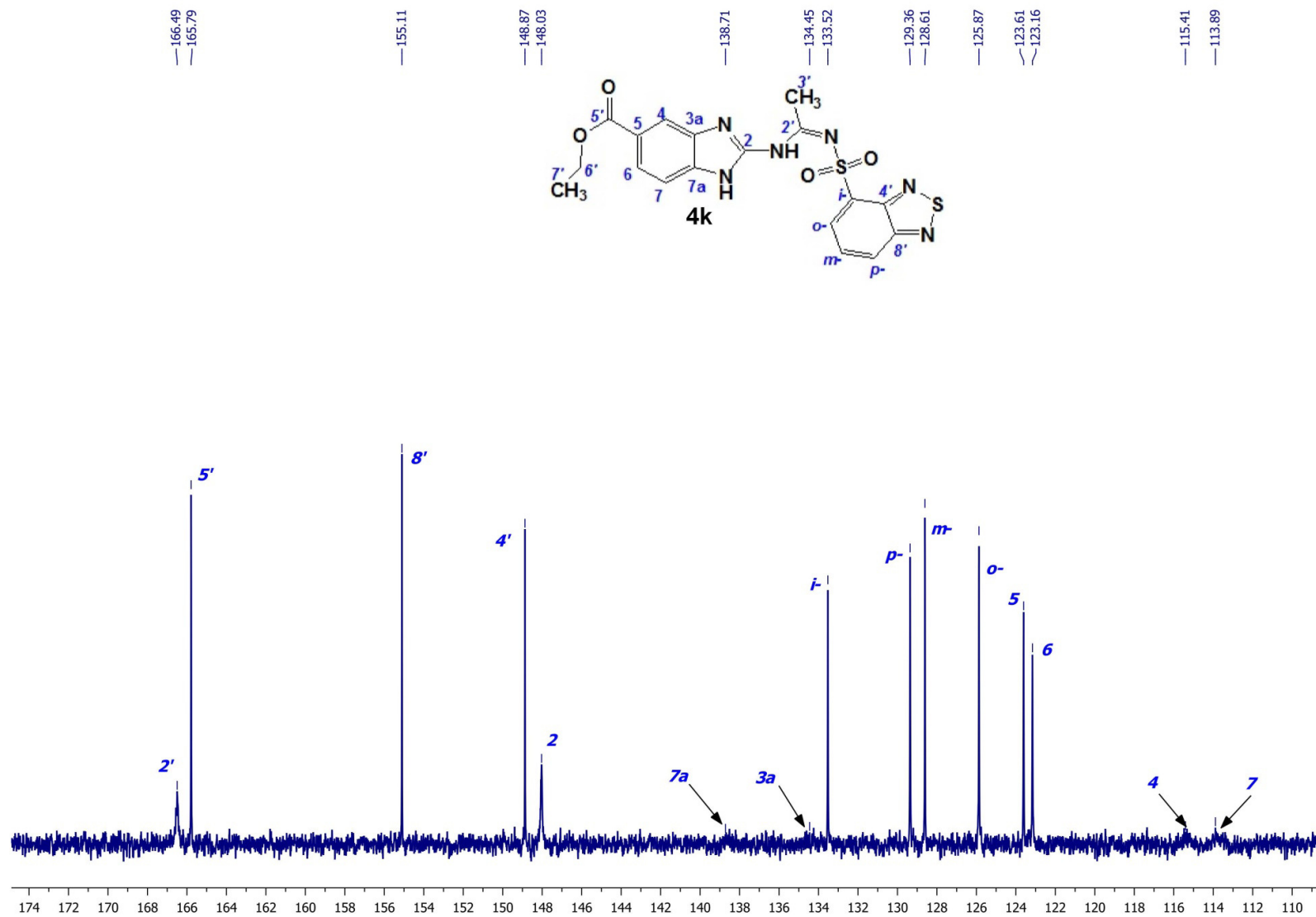


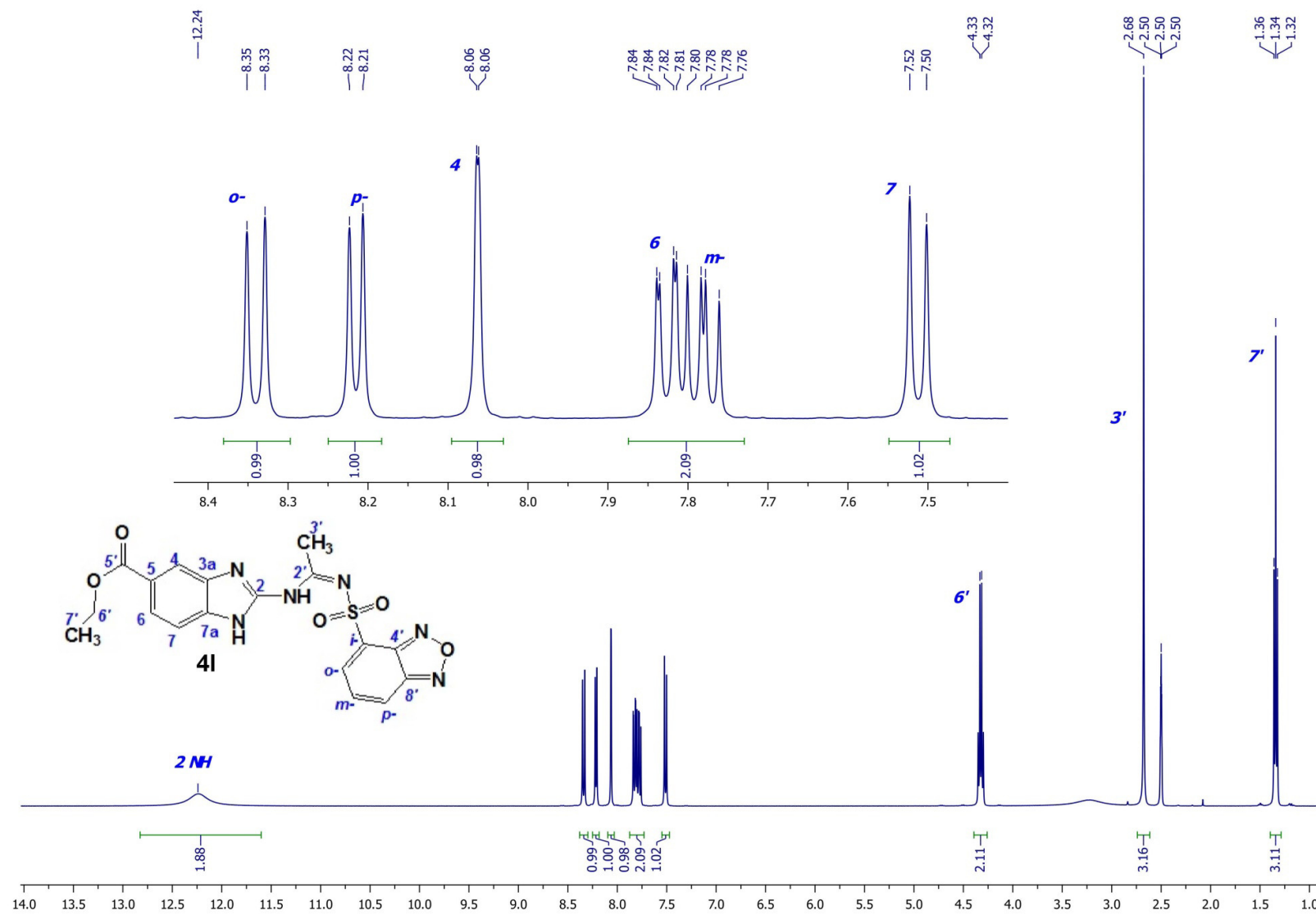


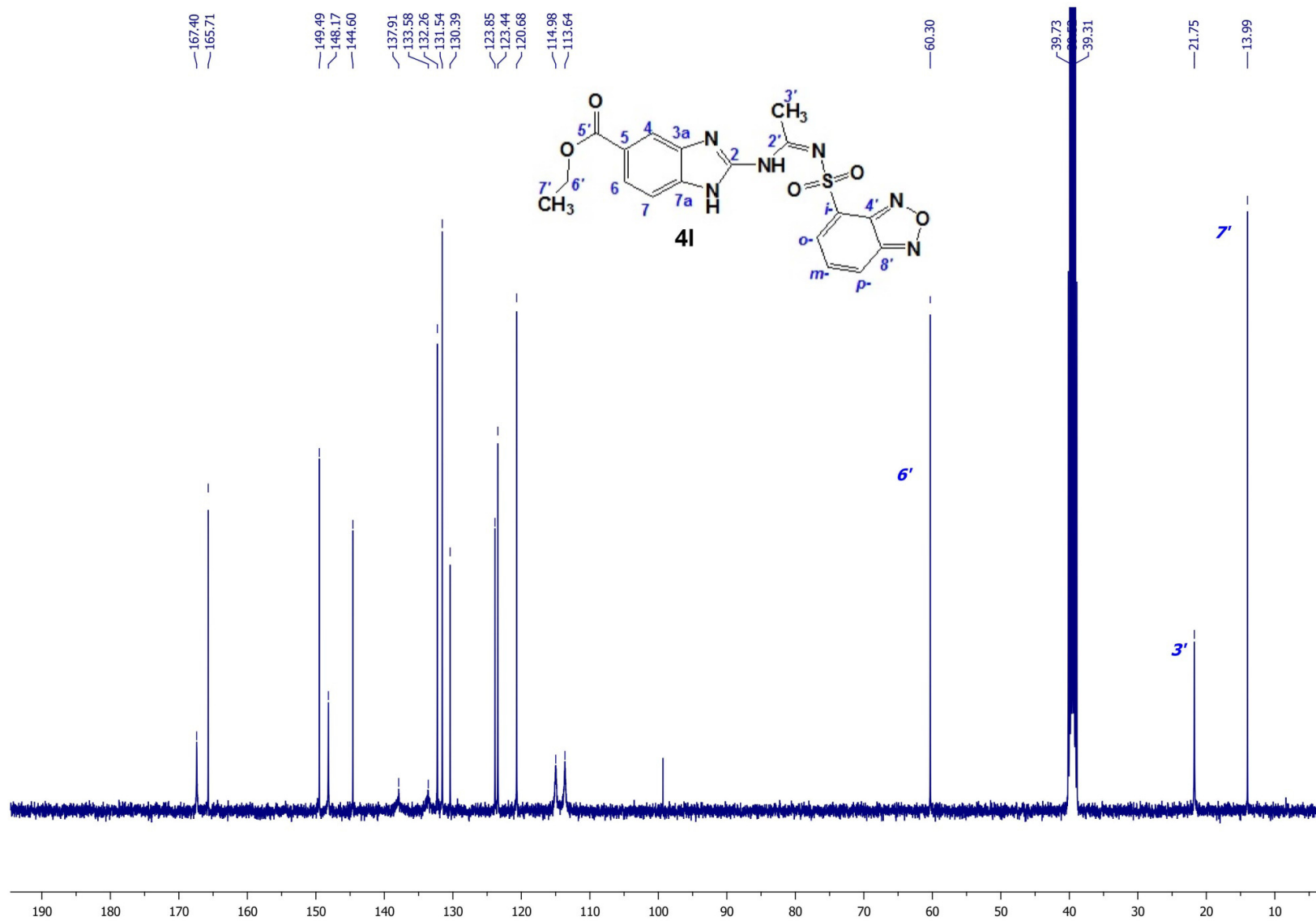


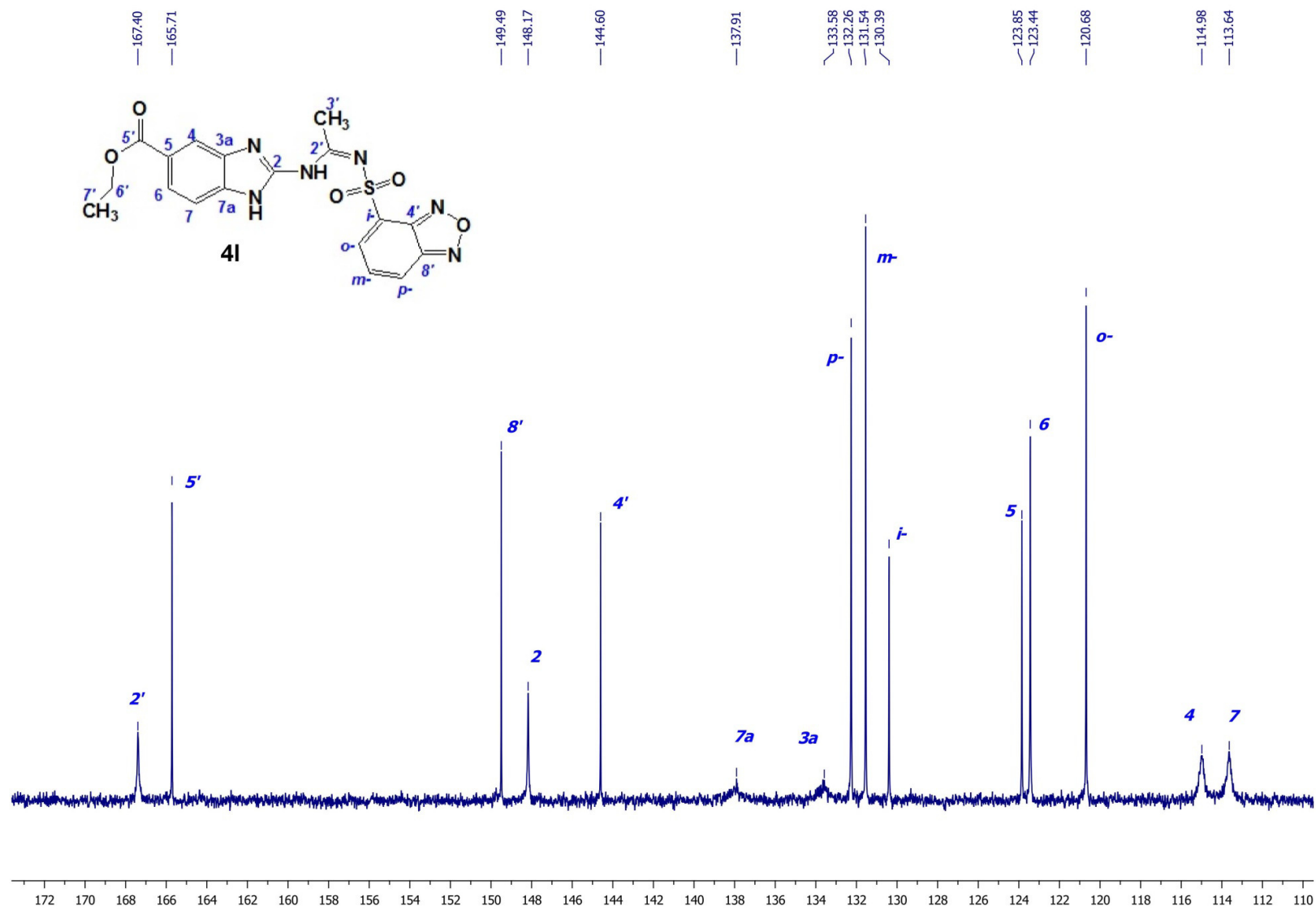


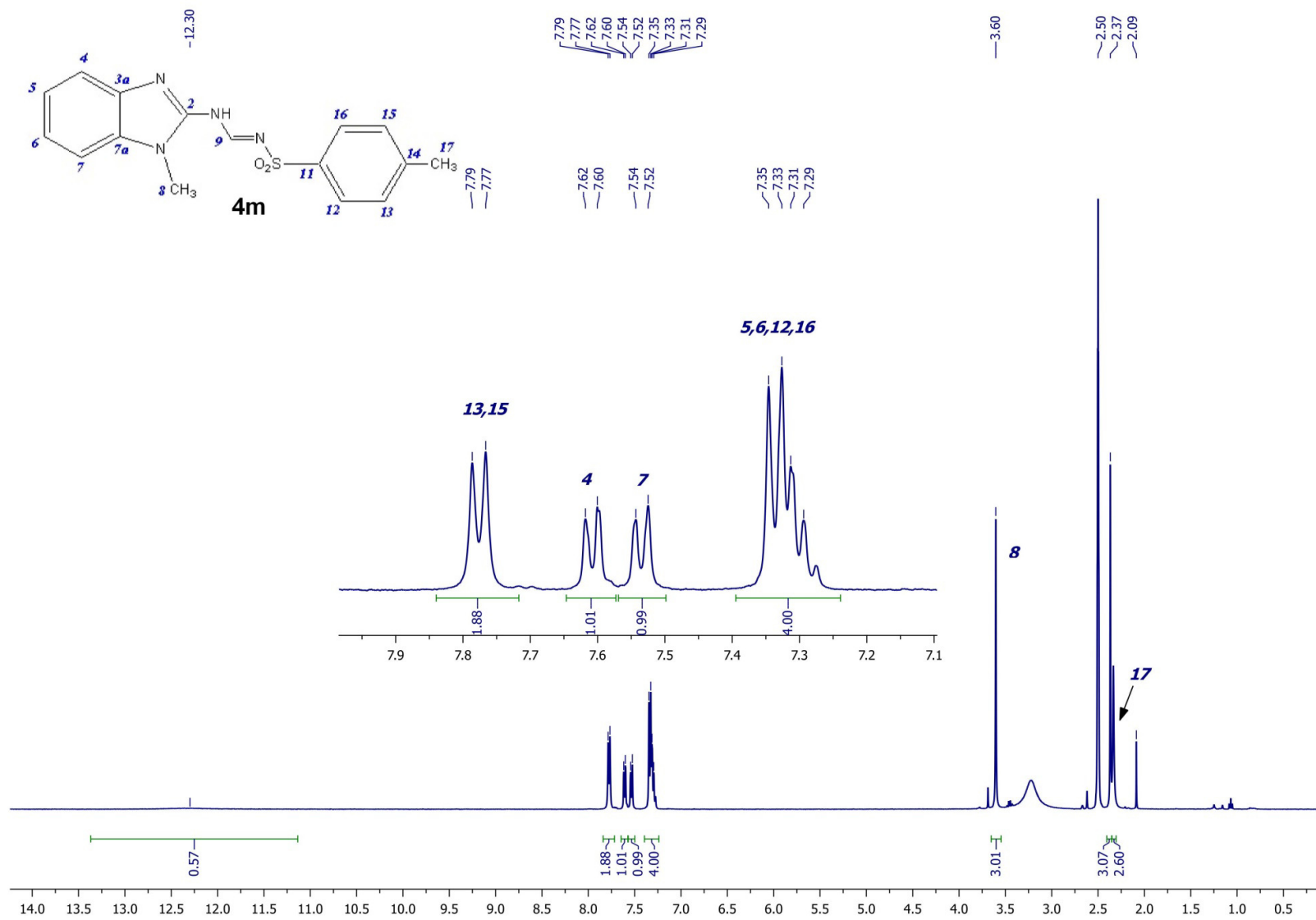


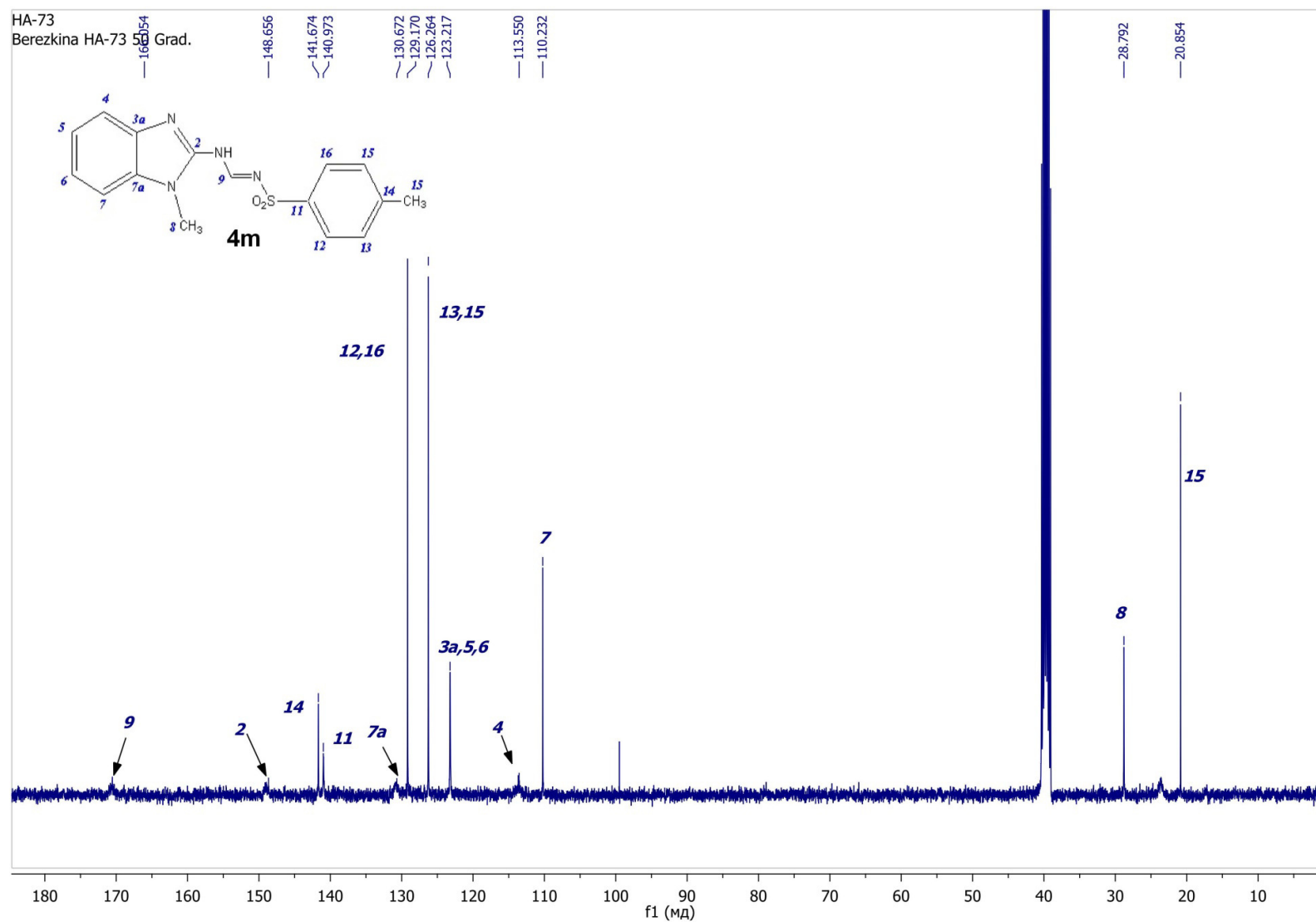




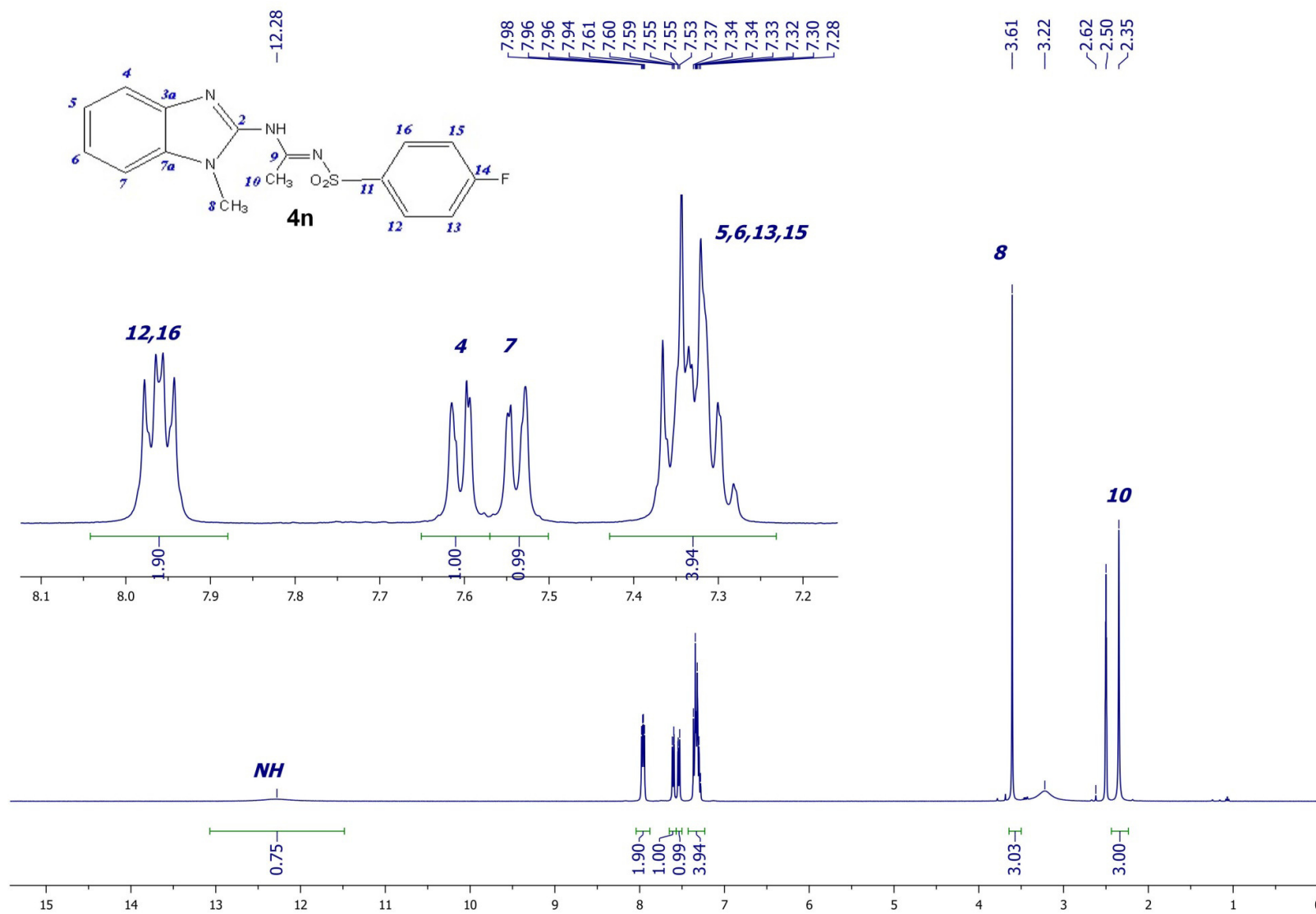


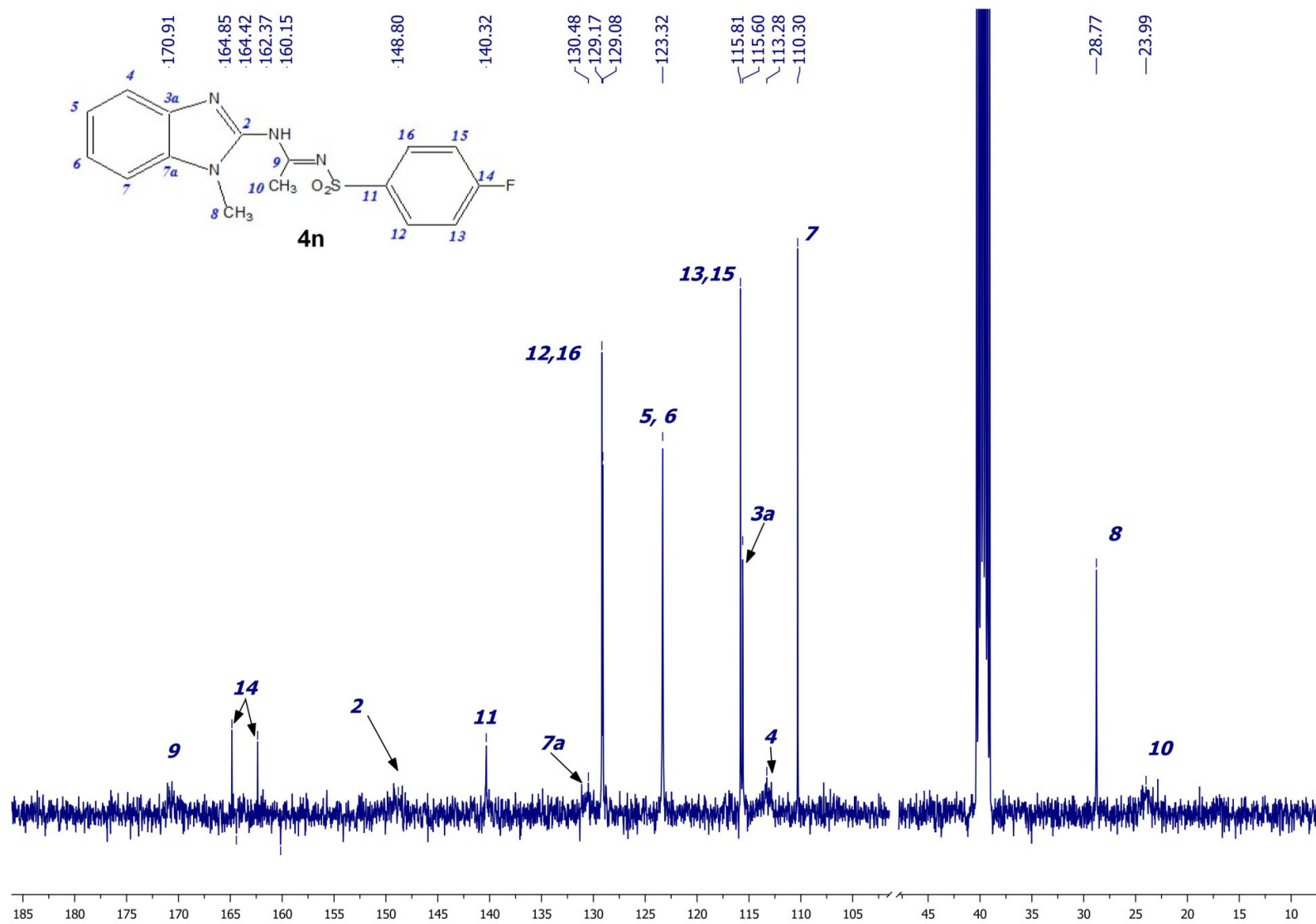


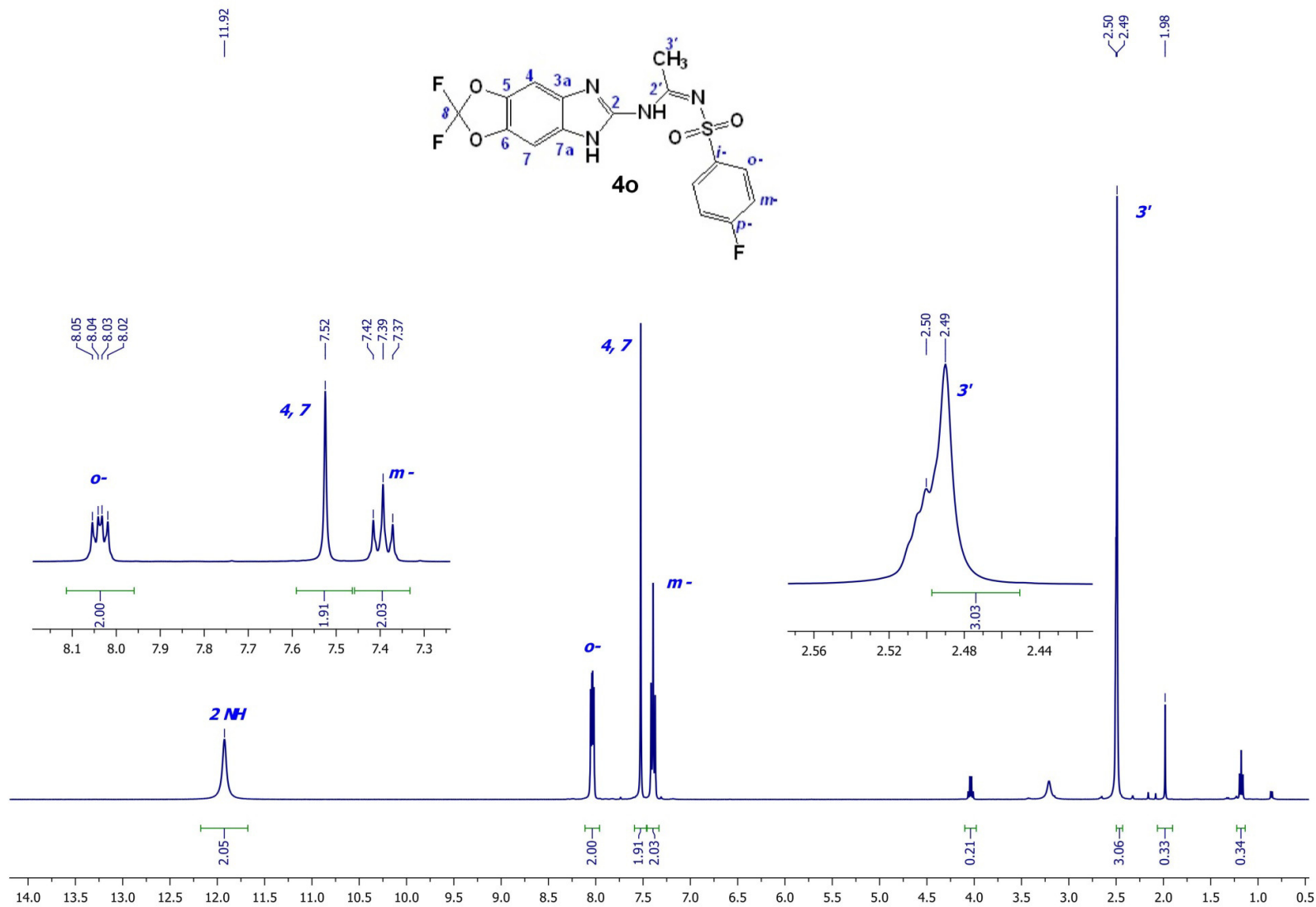


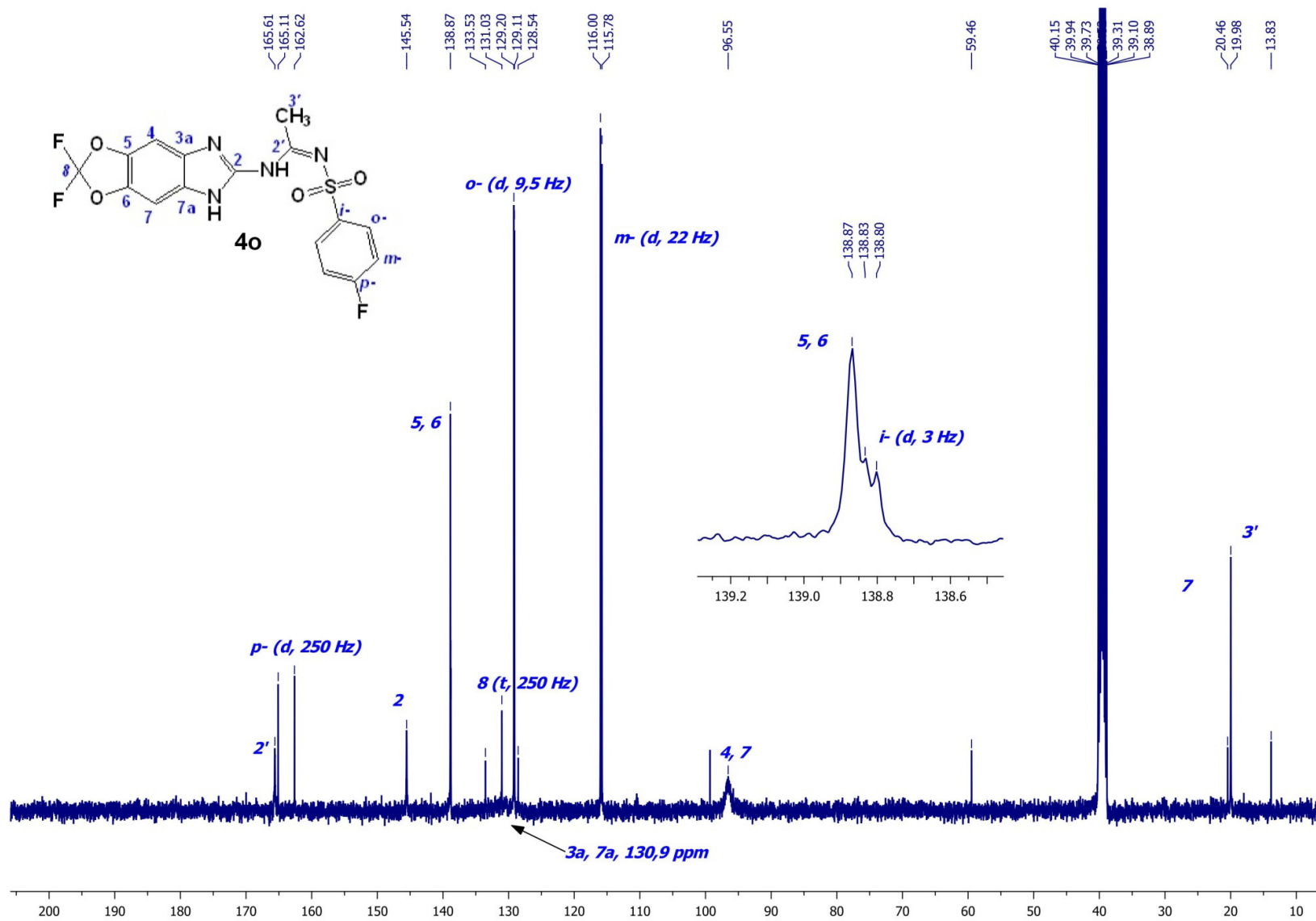


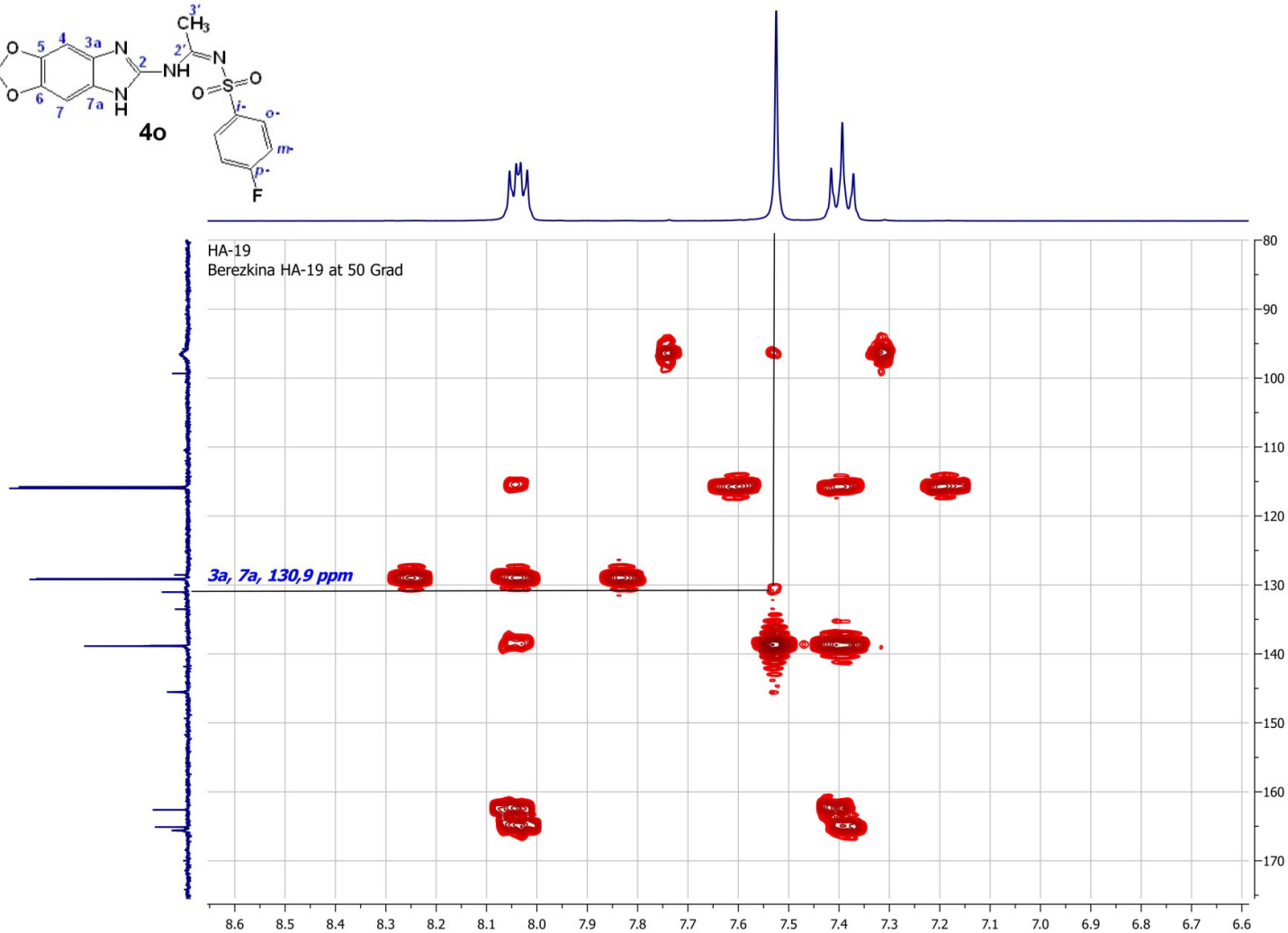
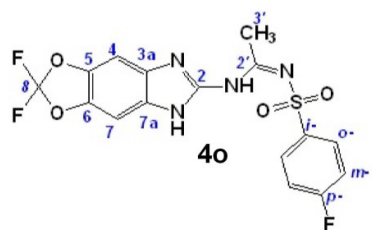




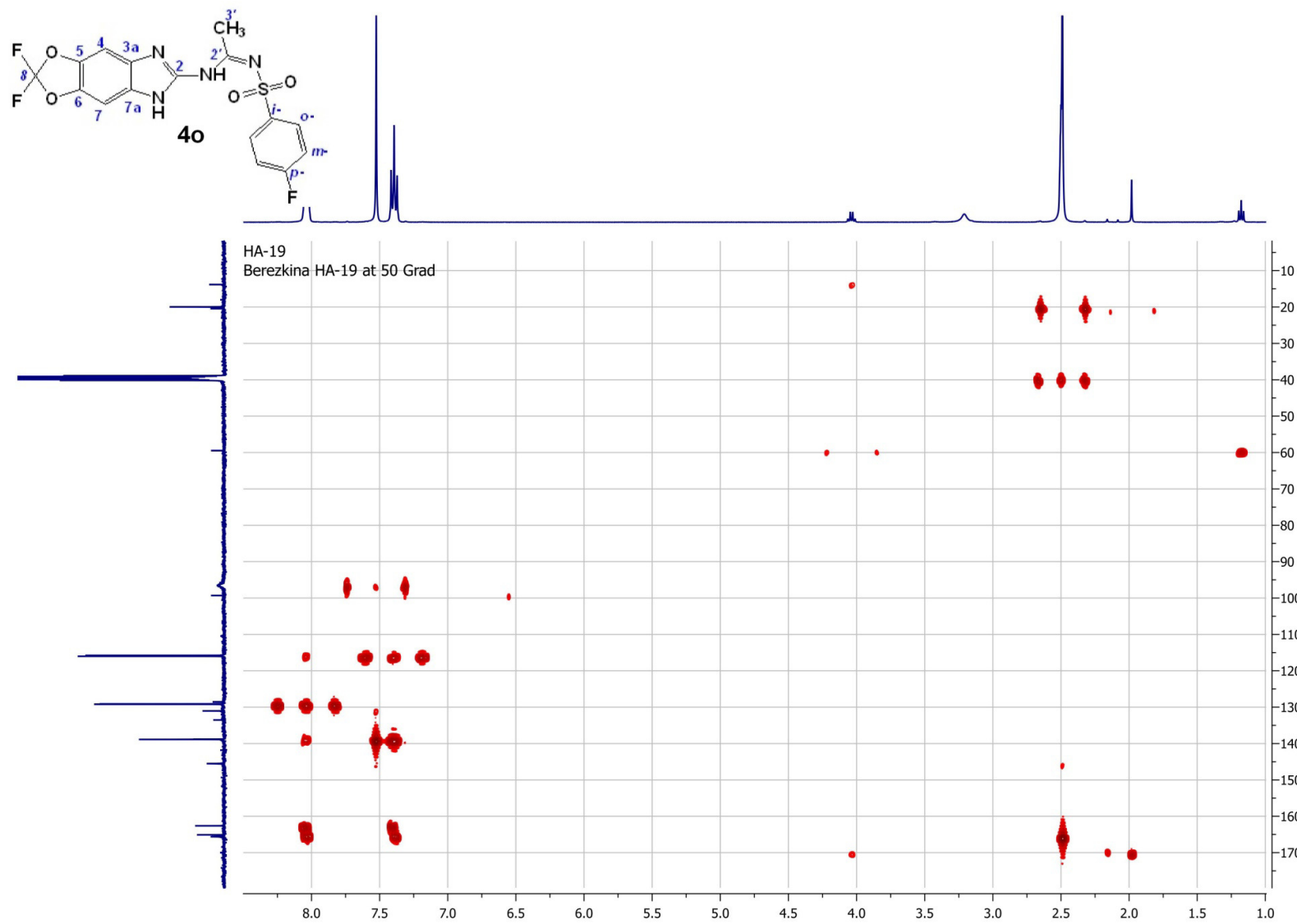


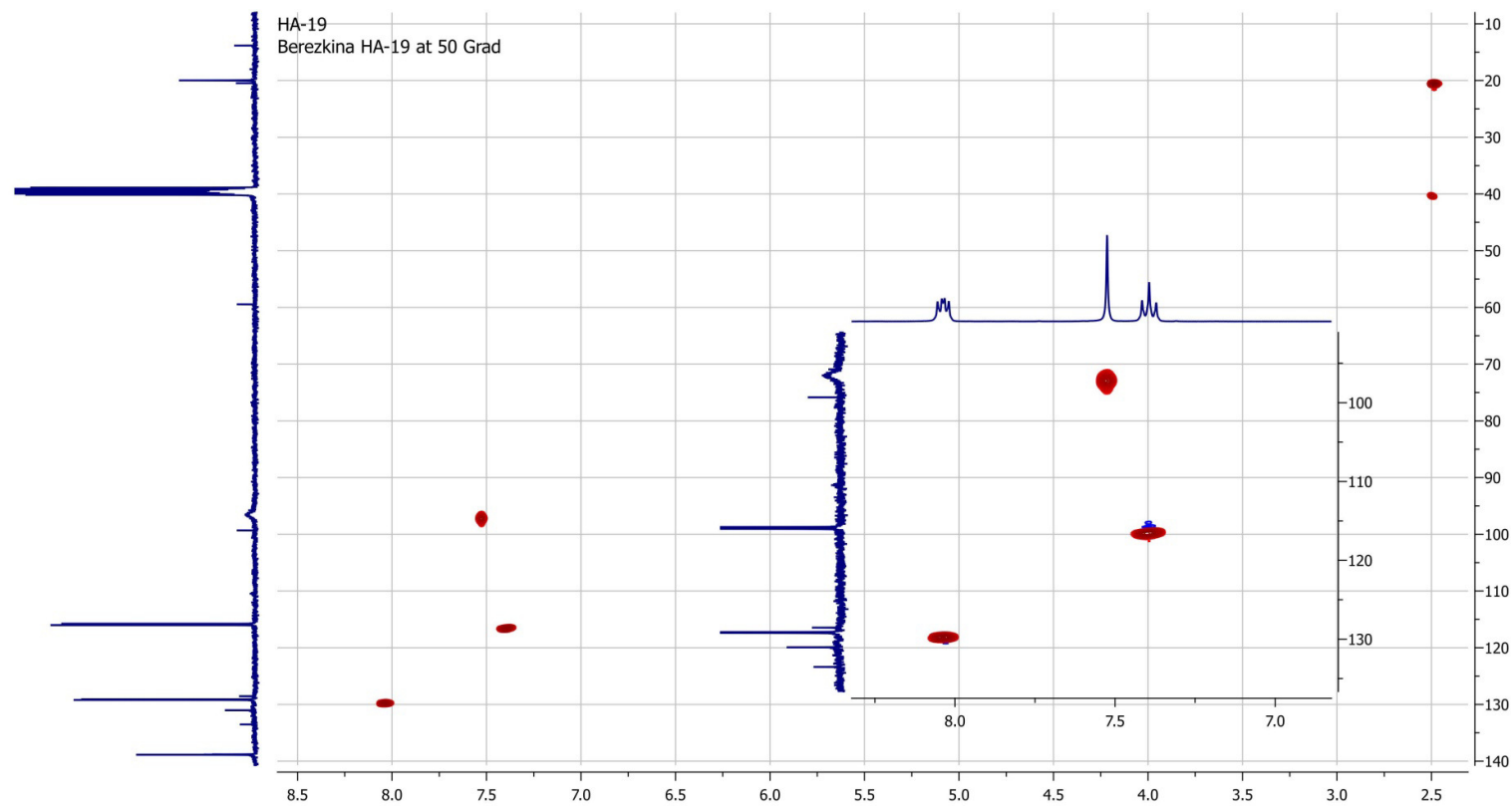








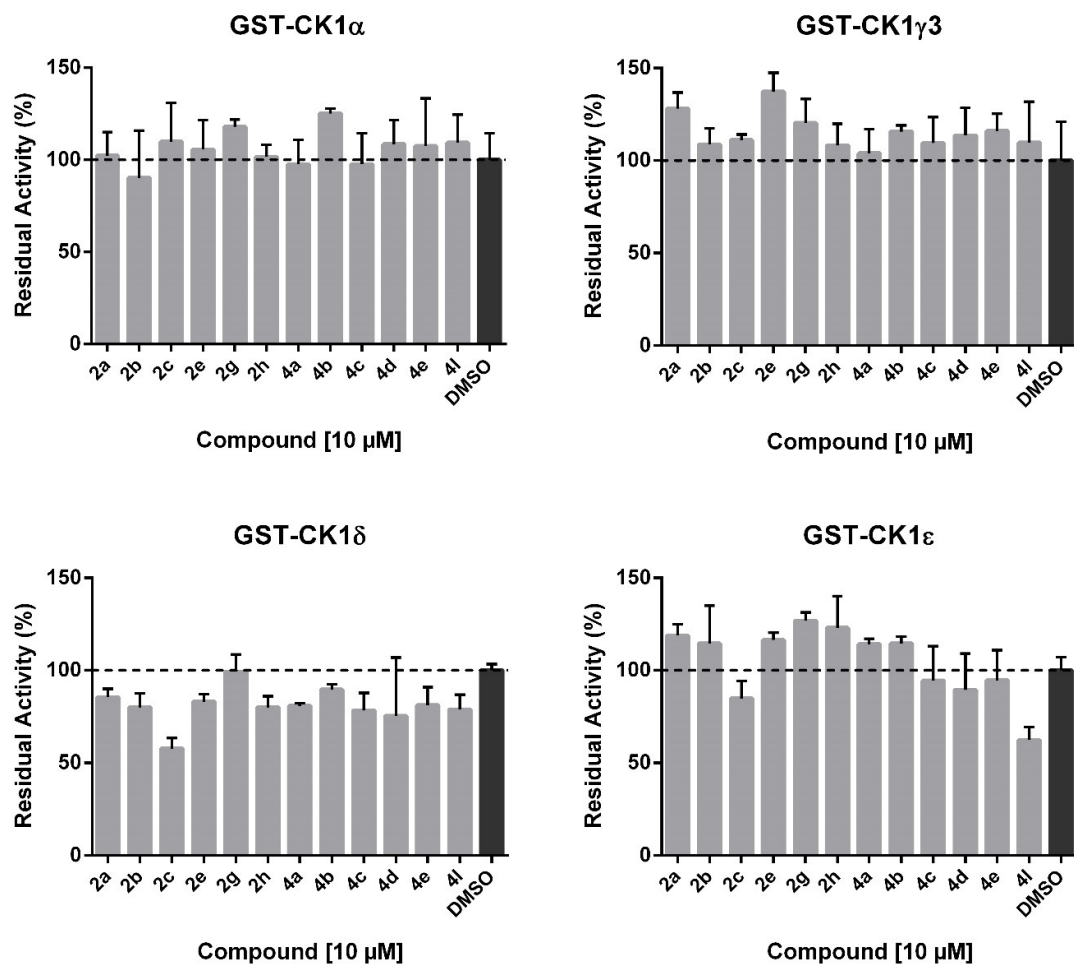




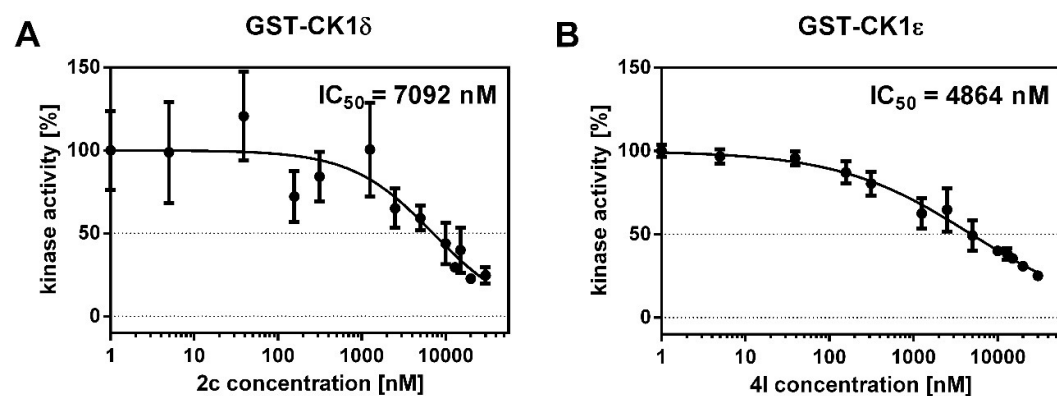


**Inhibitory effects of selected compounds on the kinase activity of CK1 isoforms**

Selected compounds were initially screened for their activity against different CK1 isoforms against a concentration of 10  $\mu\text{M}$  ATP (Figure S11). Most compounds had a weak effect on CK1 $\delta$  (Figure S11C), with **2c** being the most potent inhibitor of this series. CK1 $\epsilon$  was inhibited only moderately by compound **4l** (Figure S11D). All compounds did not inhibit CK1 $\alpha$  and CK1 $\gamma$ 3 at all (Figure S11A and S11B). We next calculated  $\text{IC}_{50}$  values for CK1 $\delta$  and CK1 $\epsilon$  regarding compounds **2c** and **4l** by assaying different concentrations in a range of 0.005  $\mu\text{M}$  to 30  $\mu\text{M}$  (Figure S12). Hit compound **2c** showed an  $\text{IC}_{50}$  value of approximately 7  $\mu\text{M}$  for CK1 $\delta$  and **4l** an  $\text{IC}_{50}$  of 4.86  $\mu\text{M}$  for CK1 $\epsilon$ . Although these values are significantly weaker than  $\text{IC}_{50}$  values of known highly potent CK1-specific inhibitors, our results suggest that our scaffolds might be improved for further development of potent CK1 isoform kinase inhibitors.



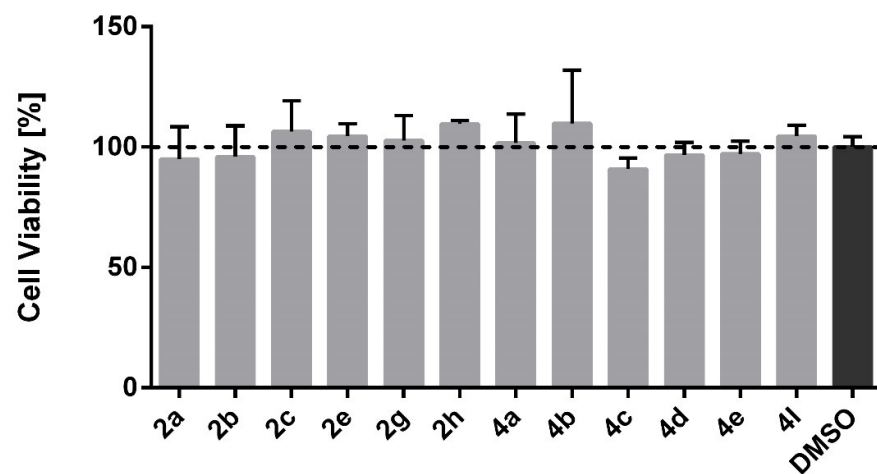
**Figure S11.** Inhibitory effects of selected compounds on the kinase activity of CK1 isoforms.



**Figure S12.** IC<sub>50</sub> curves of relevant compounds against CK1 $\delta$  and CK1 $\epsilon$ . Curve **A**: **2c**; curve **B**: **4l**.

Selected compounds were further evaluated with regard to their efficacy in cell culture by MTT viability assay using the glioblastoma cell line DK-MG (Figure S13). The cells were exposed to 10  $\mu$ M of each compound with a periodic standard dosing scheme of 48 h. Compared to vehicle treatment the cell viability was not significantly diminished by the end of an exposure period of 7 days. The lack of biological activity of these compounds in this particular cellular system probably indicates suboptimal physicochemical properties.

Glioblastoma cell line DK-MG cultures were exposed to 10  $\mu$ M of selected synthesized compounds and analyzed for their effects on cell viability using MTT assay. Results are shown as normalized bar graphs using DMSO as a control for 100% cell viability (dotted line), error bars represent the standard deviation (SD) (DMSO: dimethyl sulfoxide) (Figure S13)



**Figure S13.** Effect on cell viability of selected synthesized compounds.