

## Supplementary Material

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

Nadezhda A. Rupakova,<sup>a</sup> Vasiliy A. Bakulev,<sup>a</sup> Uwe Knippschild,<sup>b</sup> Balbina García-Reyes,<sup>b</sup> Oleg S. Eltsov,<sup>a</sup> Grigoriy P. Slesarev,<sup>a</sup> Nikolai Beliaev,<sup>a</sup> Pavel A. Slepukhin,<sup>c</sup> Lydia Witt,<sup>d</sup> Christian Peifer,<sup>d</sup> and Tetyana V. Beryozkina<sup>a\*</sup>

<sup>a</sup>Ural Federal University named after the first President of Russia B. N. Yeltsin, 19 Mira st., Yekaterinburg 620002, Russia

<sup>b</sup>Klinik für Allgemein- und Viszeralchirurgie Zentrum für Chirurgie Universitätsklinikum Ulm, 23 Albert-Einstein-Allee, 89081 Ulm, Germany

<sup>c</sup>I. Ya. Postovsky Institute of Organic Synthesis, Ural Branch of Russian Academy of Sciences, 20 S. Kovalevskaya st., Yekaterinburg 620990, Russia

<sup>d</sup>Institute of Pharmacy, Christian-Albrechts-University of Kiel, Gutenbergstraße 76, D- 24118 Kiel, Germany

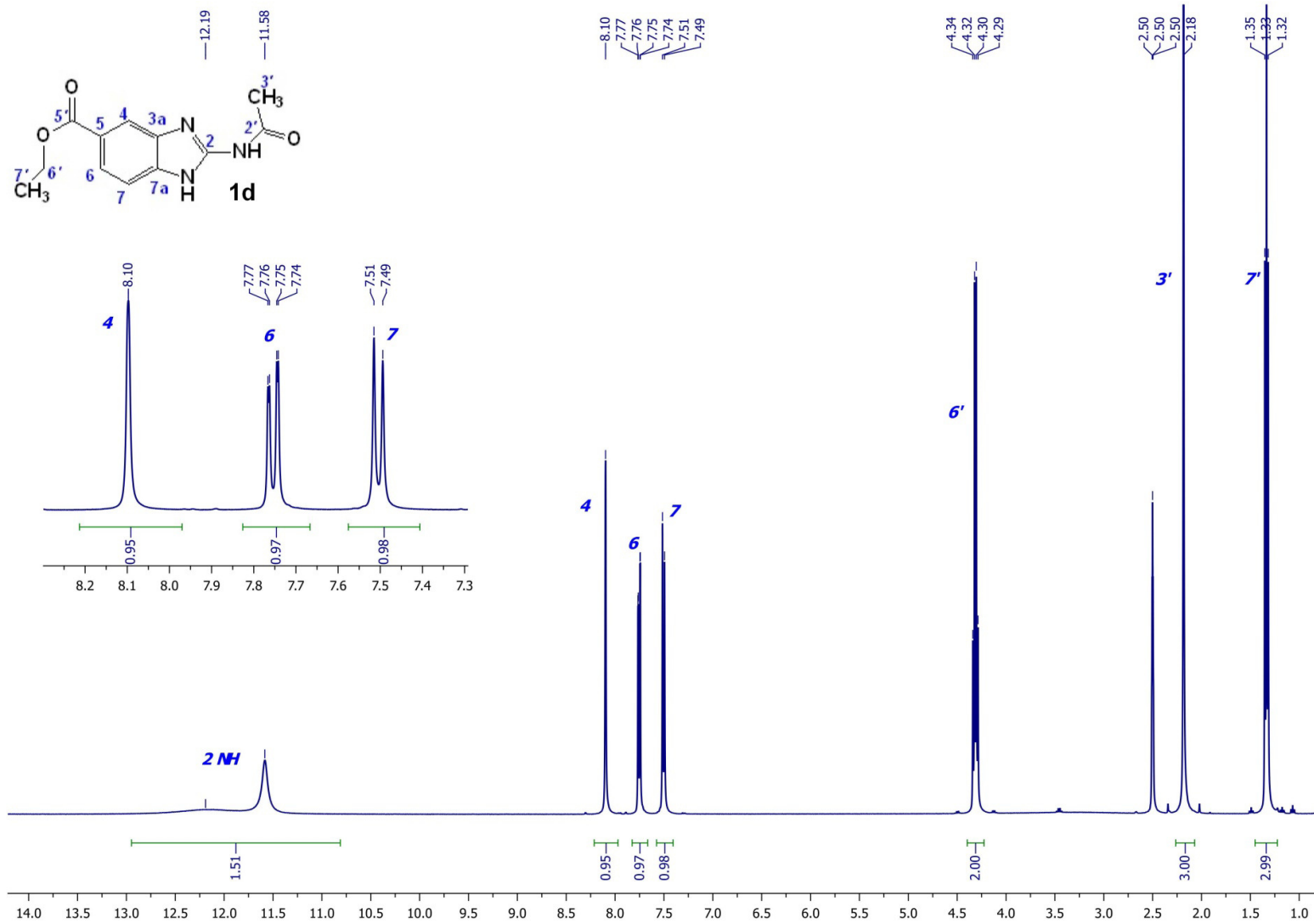
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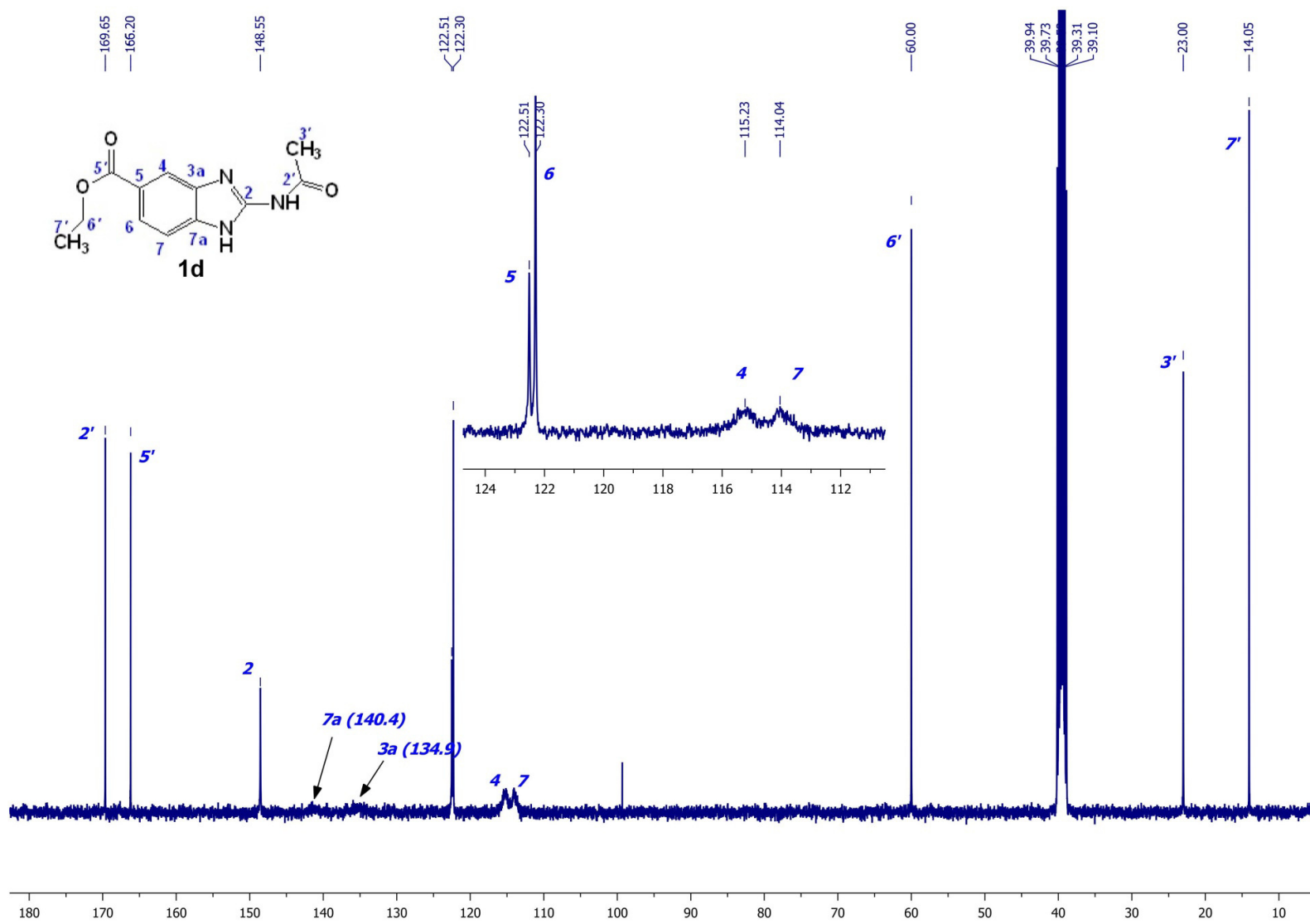
### Table of Contents

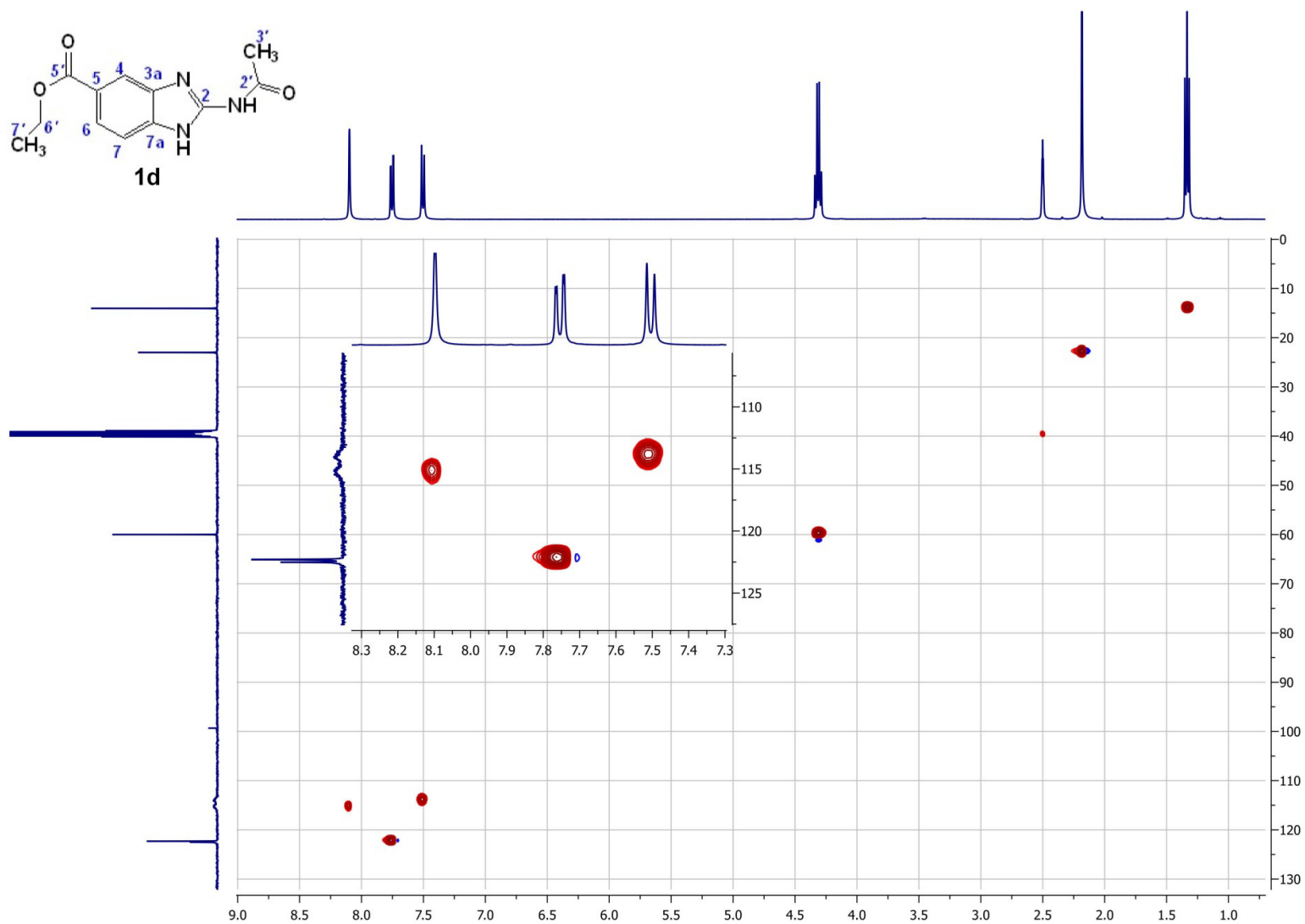
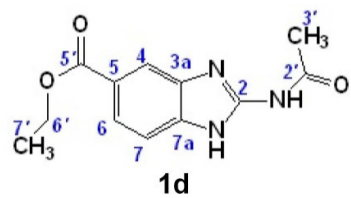
NMR spectra of <b>1d</b>	3
NMR spectra of <b>1f</b>	7
NMR spectra of <b>1h</b>	9
NMR spectra of <b>2a</b>	11
NMR spectra of <b>2b</b>	13
NMR spectra of <b>2c</b>	15
NMR spectra of <b>2d</b>	18
NMR spectra of <b>2e</b>	20
NMR spectra of <b>2f</b>	22
NMR spectra of <b>2g</b>	24
NMR spectra of <b>4a</b>	26

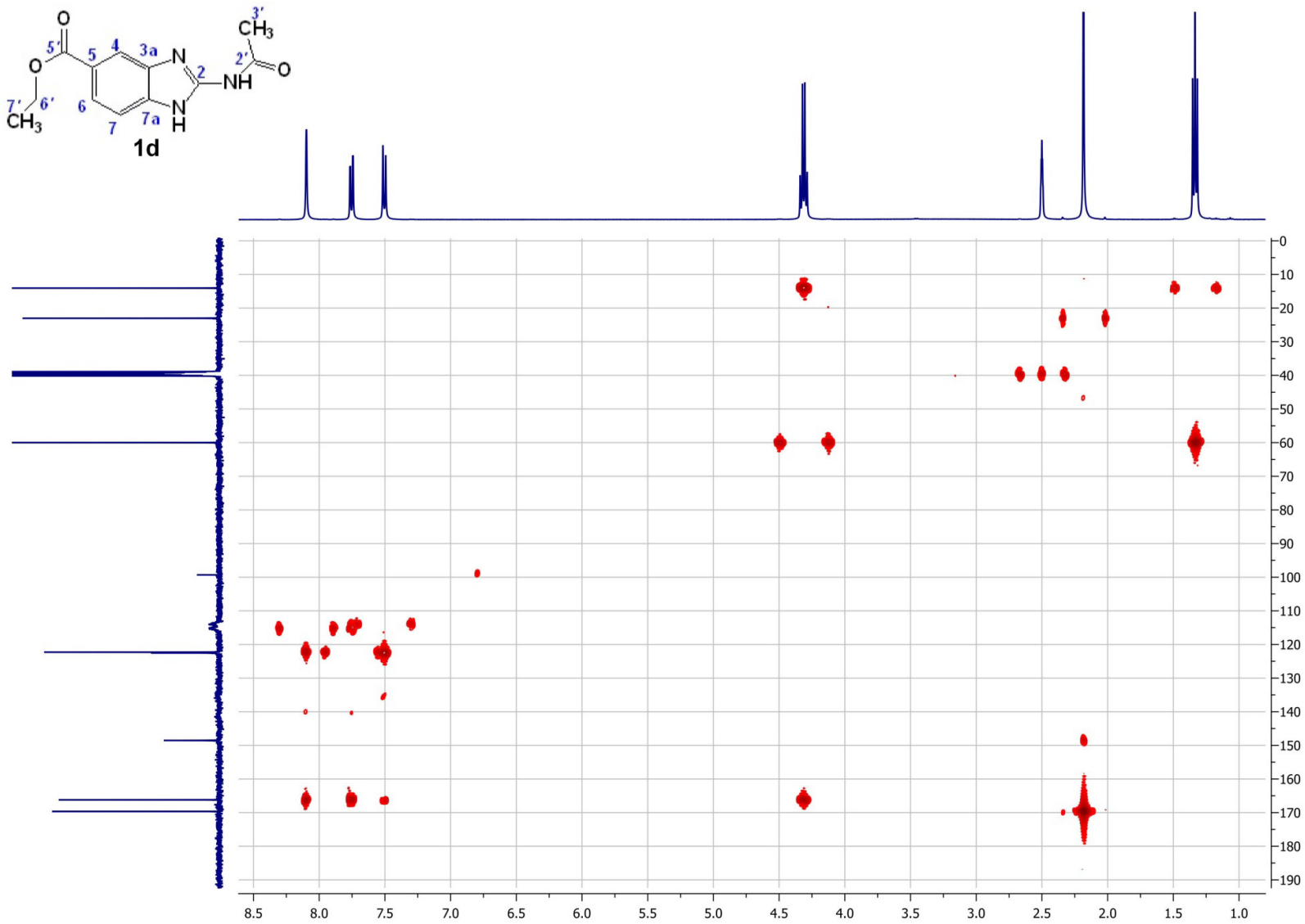
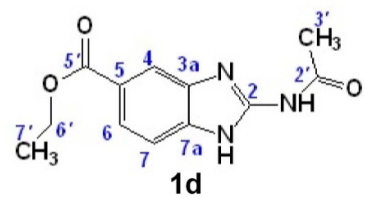
NMR spectra of <b>4b</b>	28
NMR spectra of <b>4c</b>	30
NMR spectra of <b>4d</b>	32
NMR spectra of <b>4e</b>	34
NMR spectra of <b>4f</b>	36
NMR spectra of <b>4g</b>	38
NMR spectra of <b>4h</b>	41
NMR spectra of <b>4i</b>	44
NMR spectra of <b>4j</b>	47
NMR spectra of <b>4k</b>	49
NMR spectra of <b>4l</b>	52
NMR spectra of <b>4m</b>	55
NMR spectra of <b>4n</b>	57
NMR spectra of <b>4o</b>	59
Inhibitory effects of selected compounds on the kinase activity of CK1 isoforms	64
Effect on cell viability of selected synthesized compounds	65

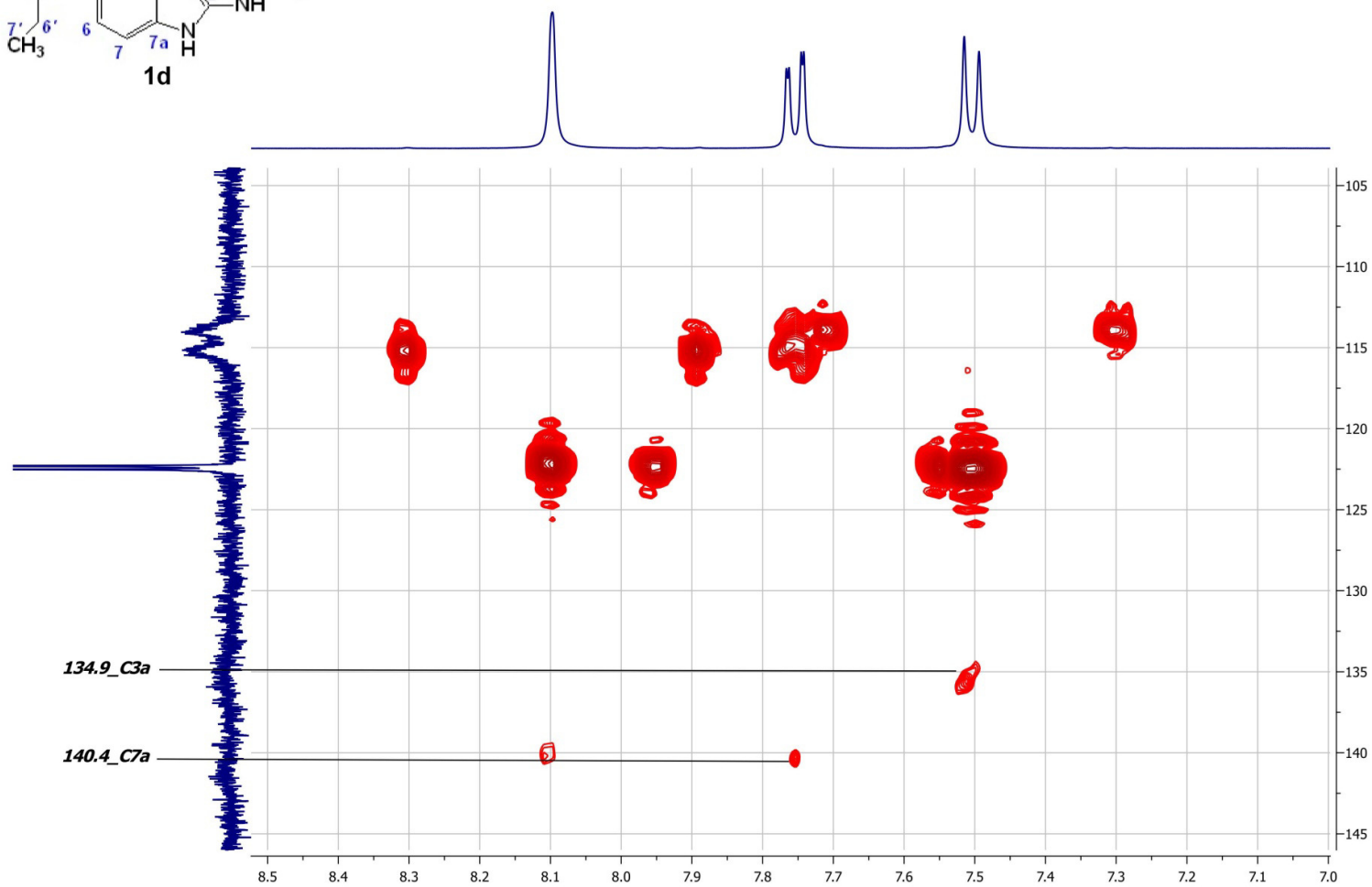
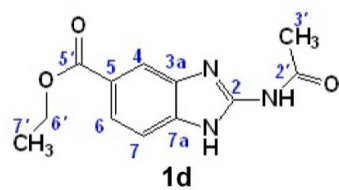


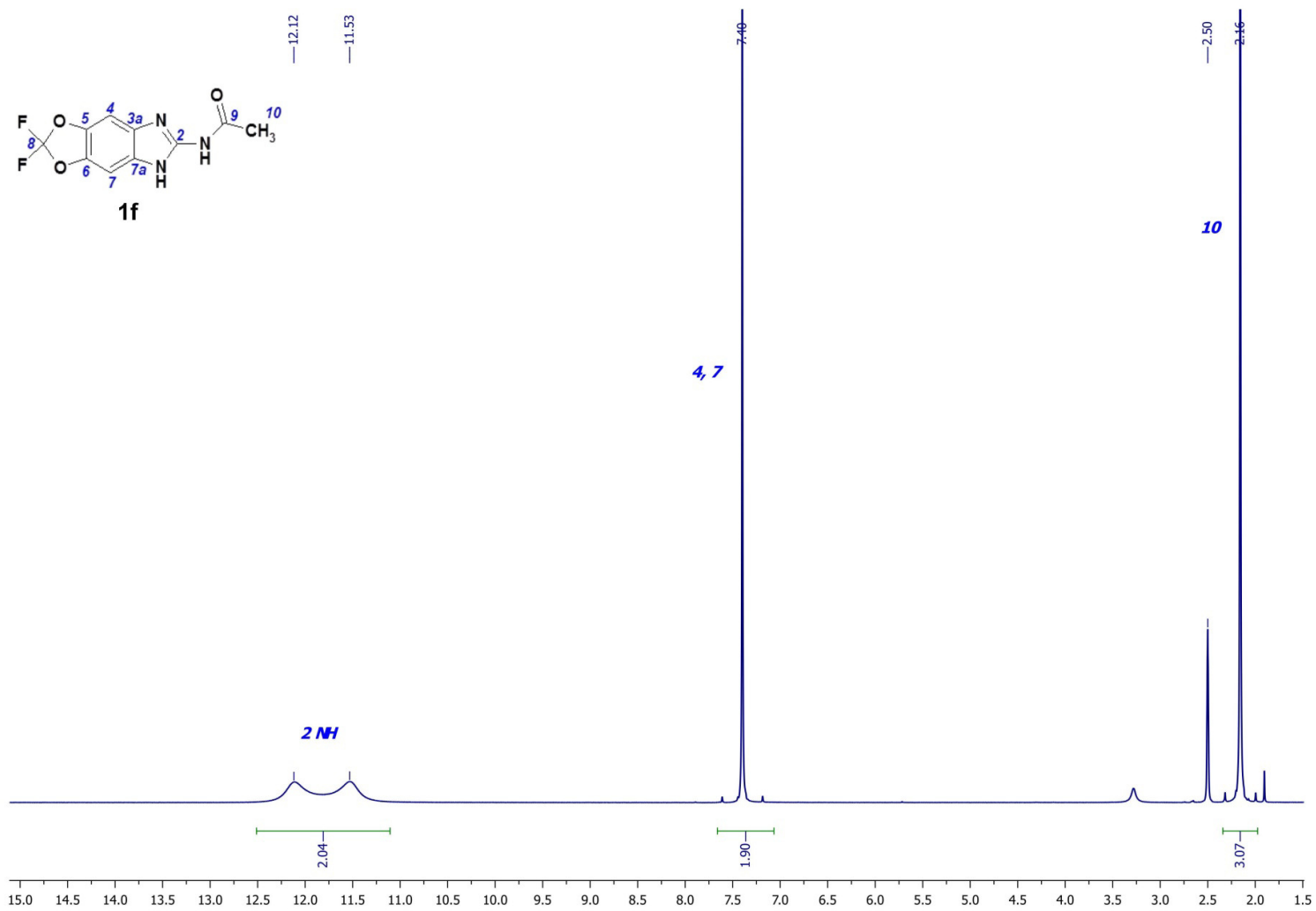


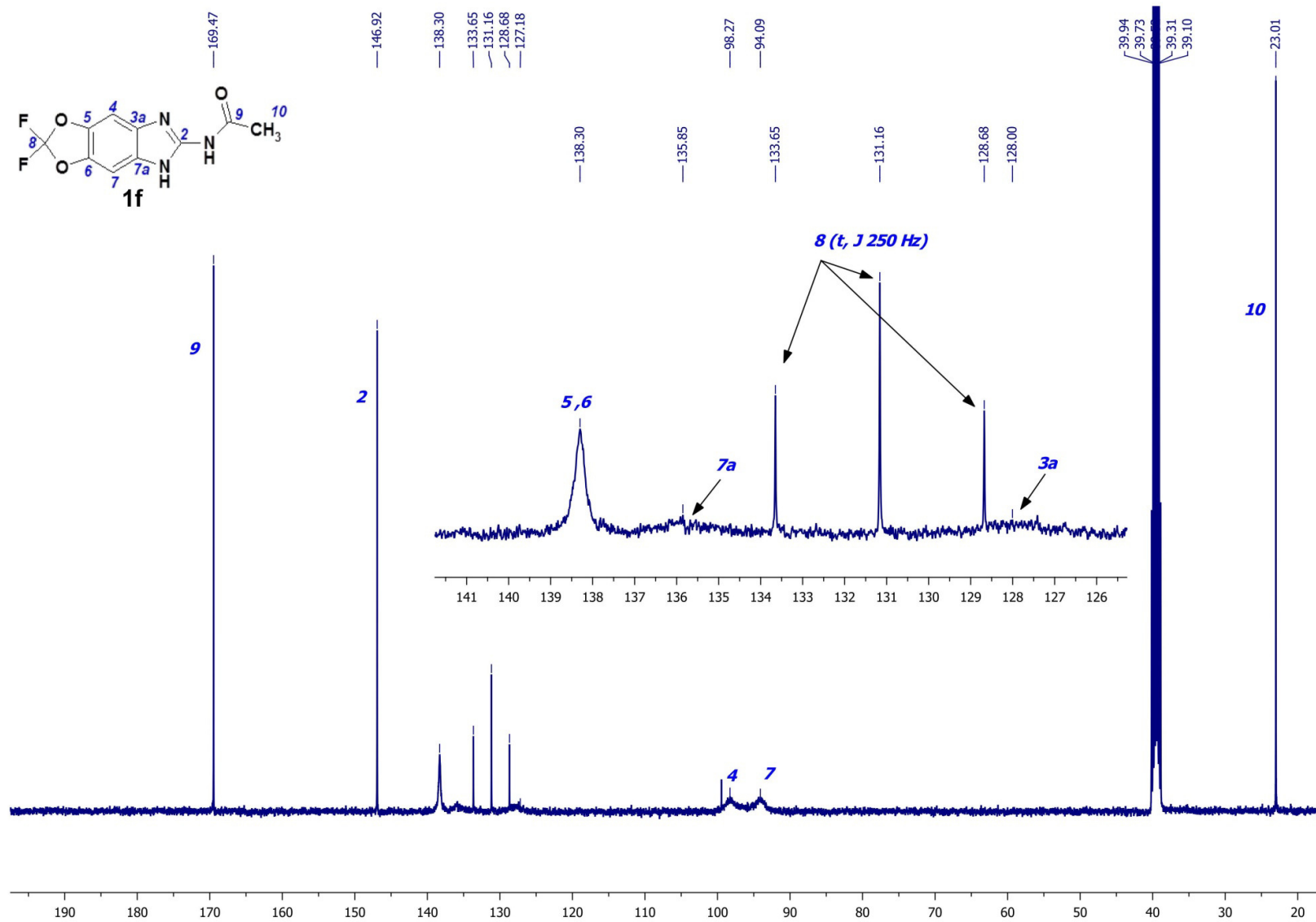


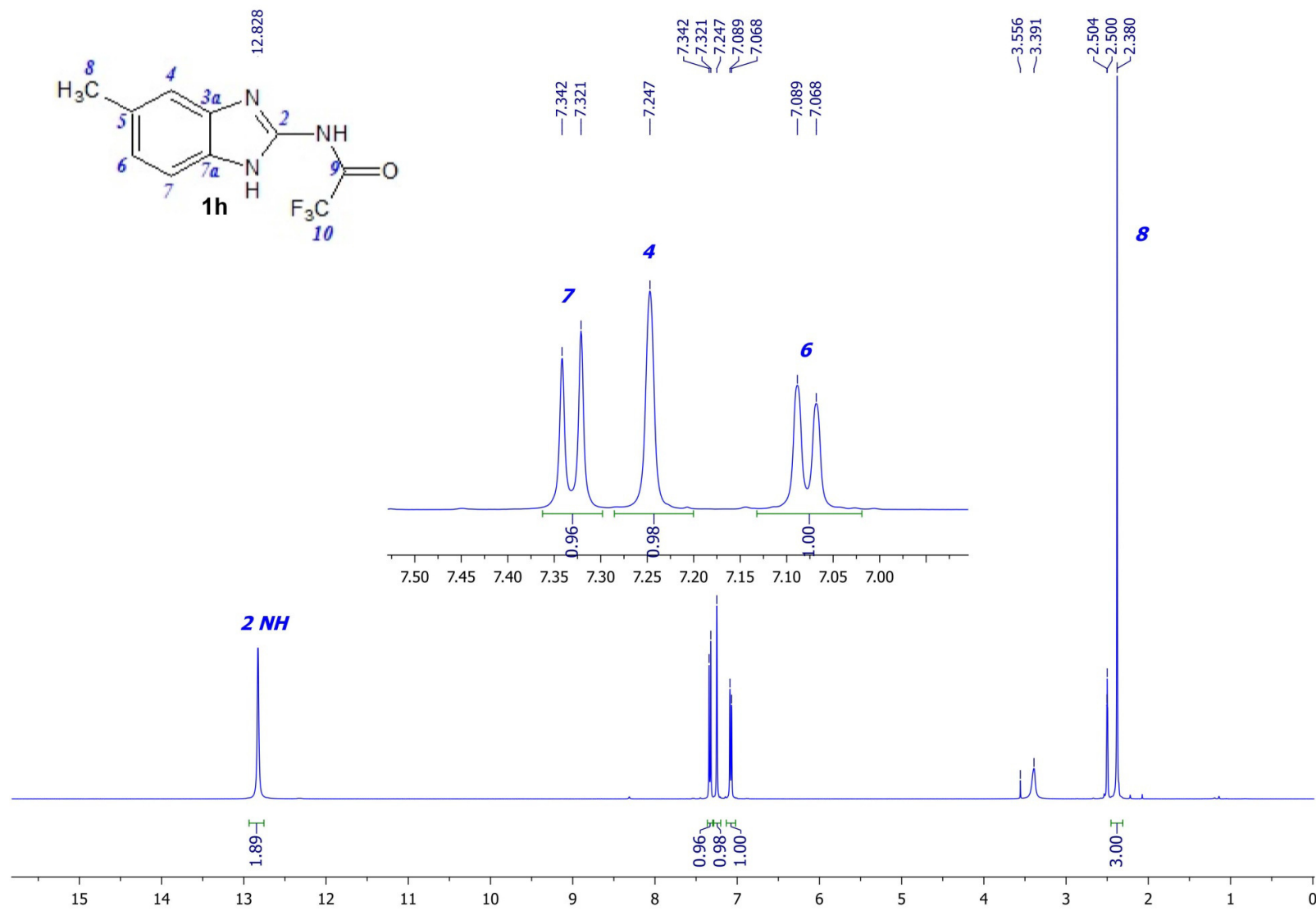




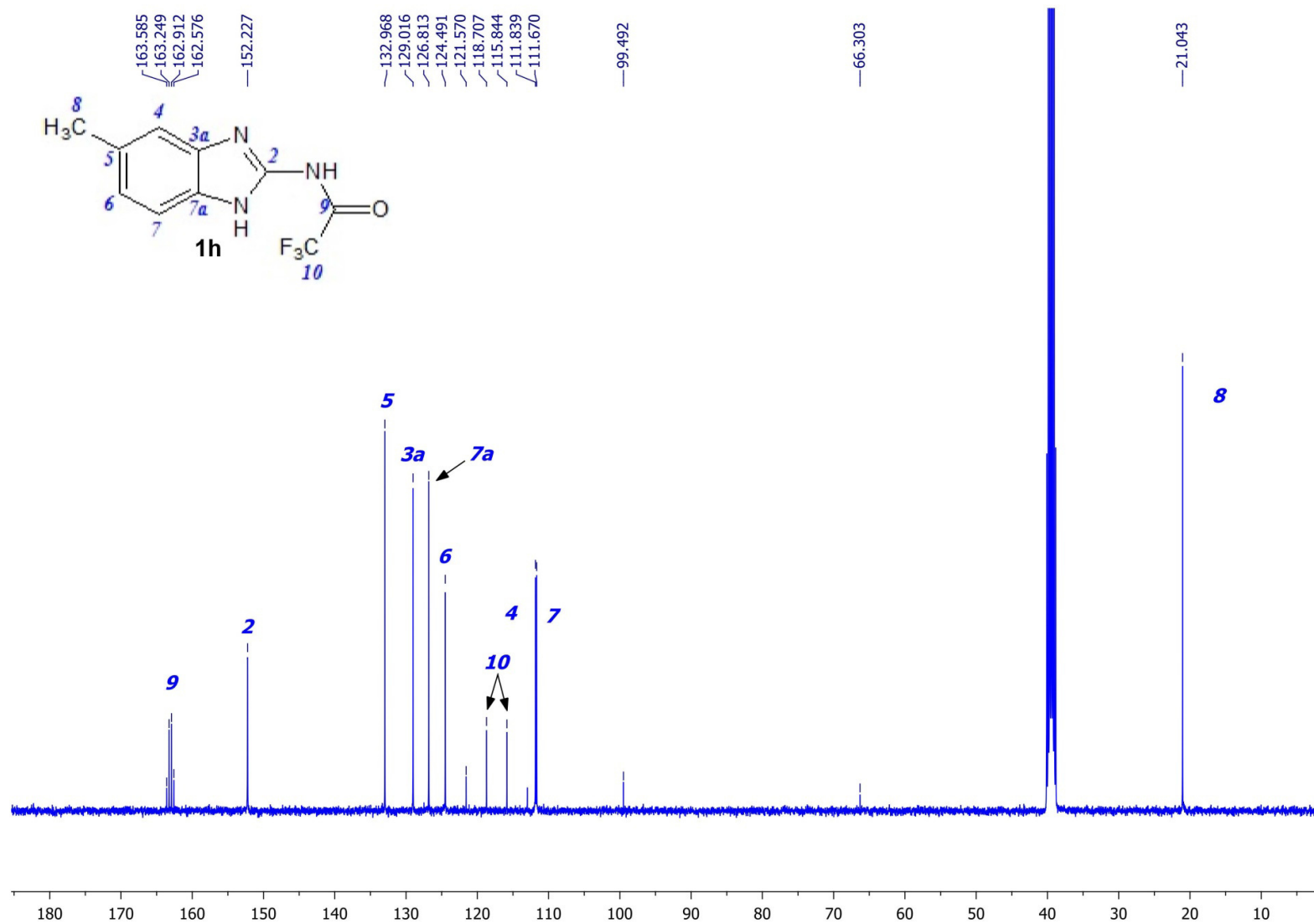


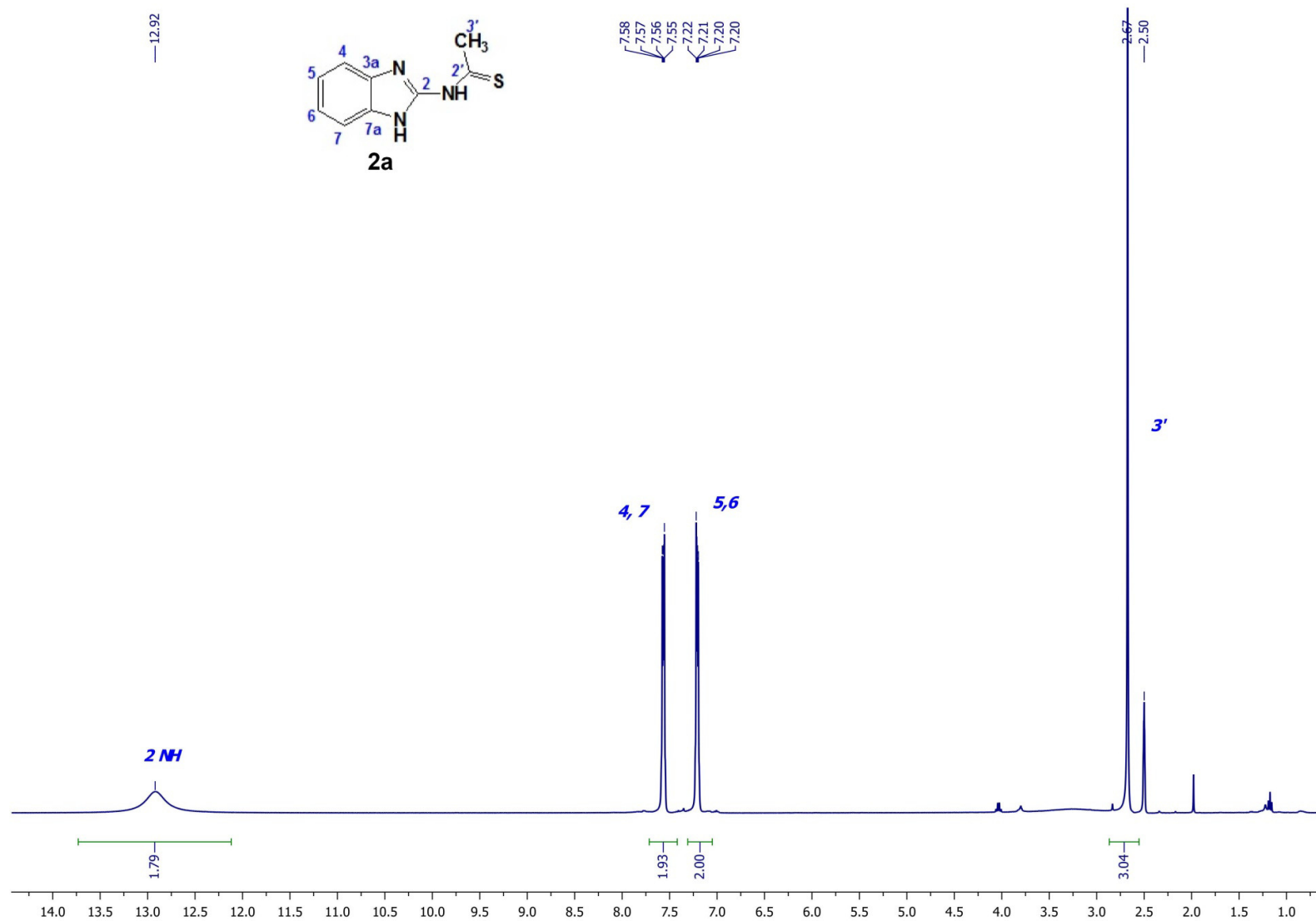


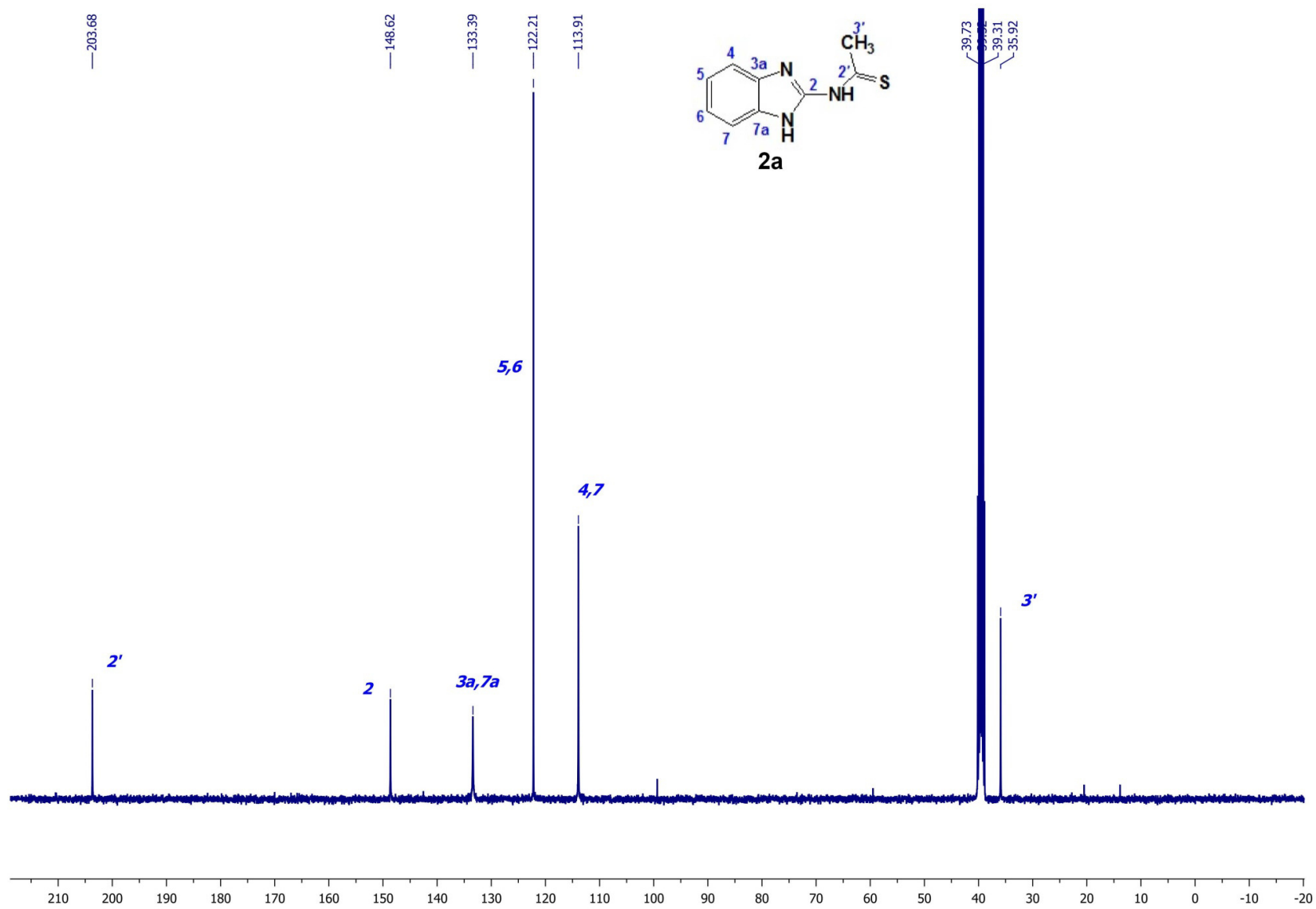


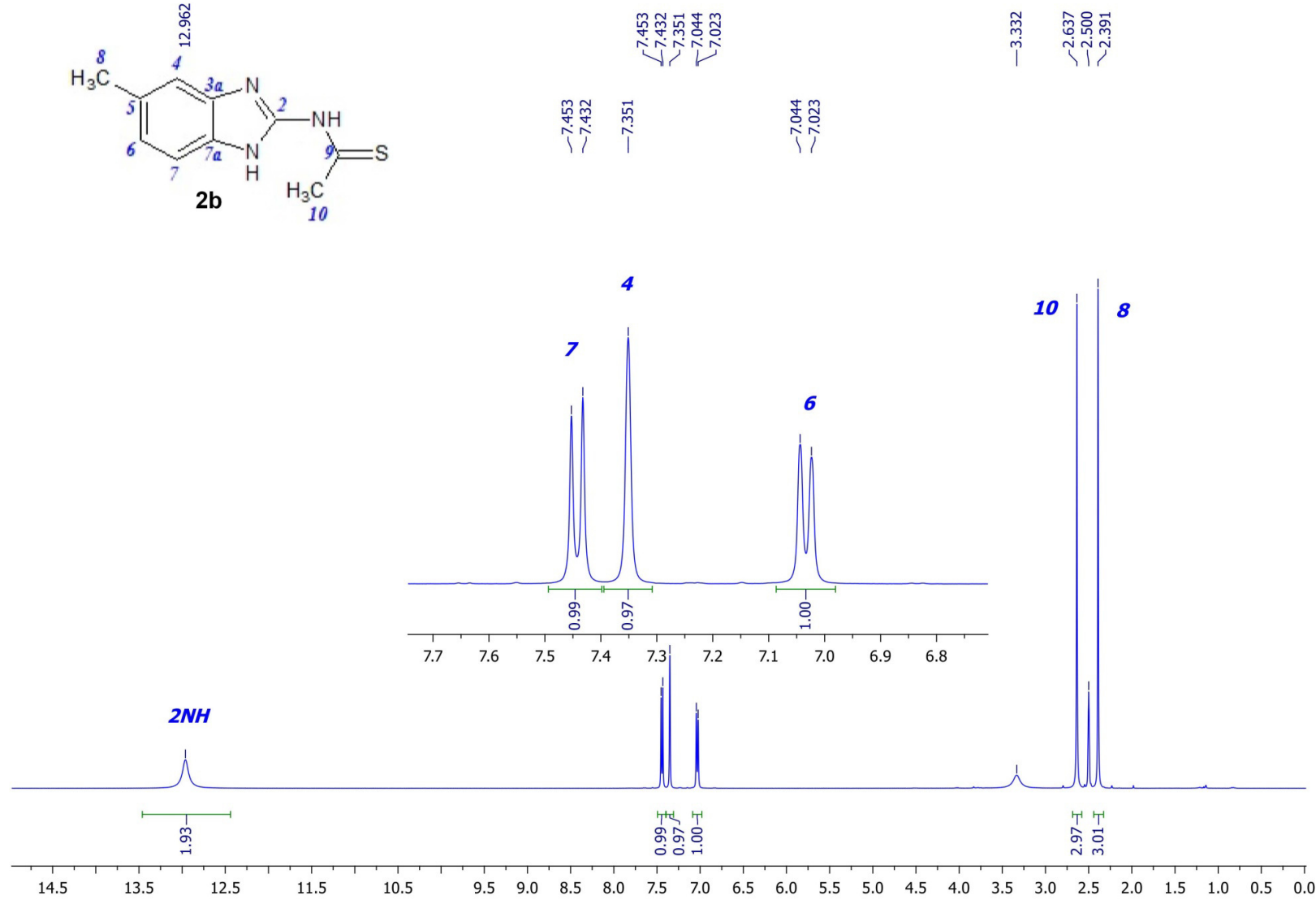
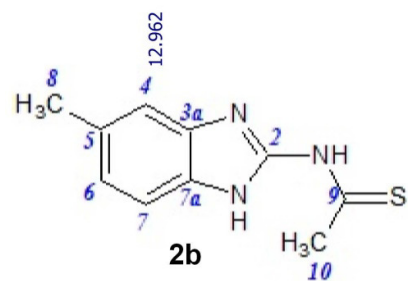


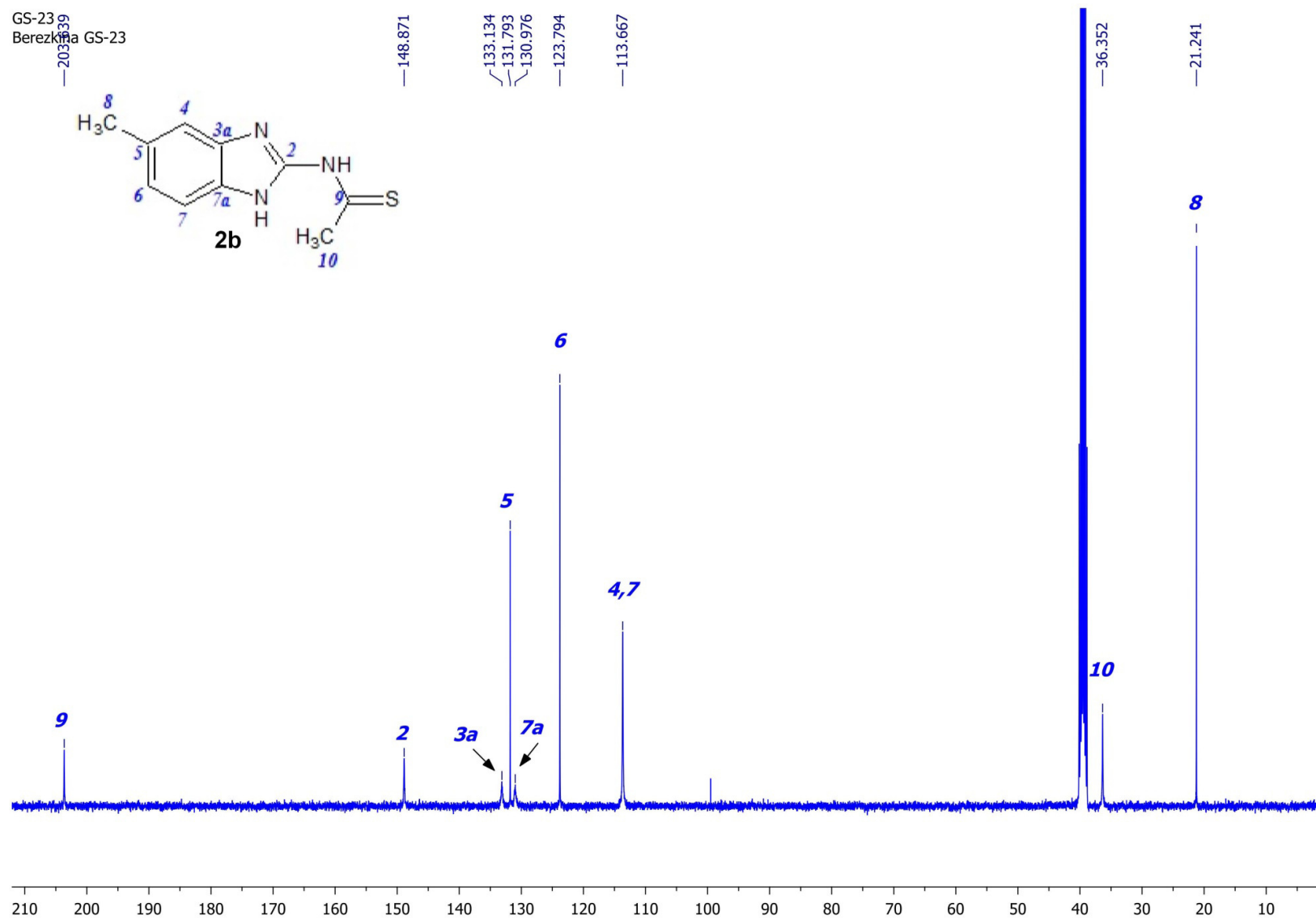


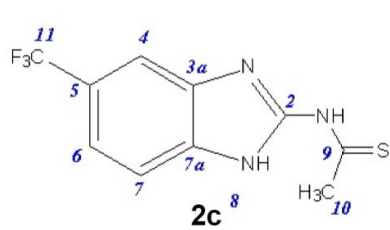




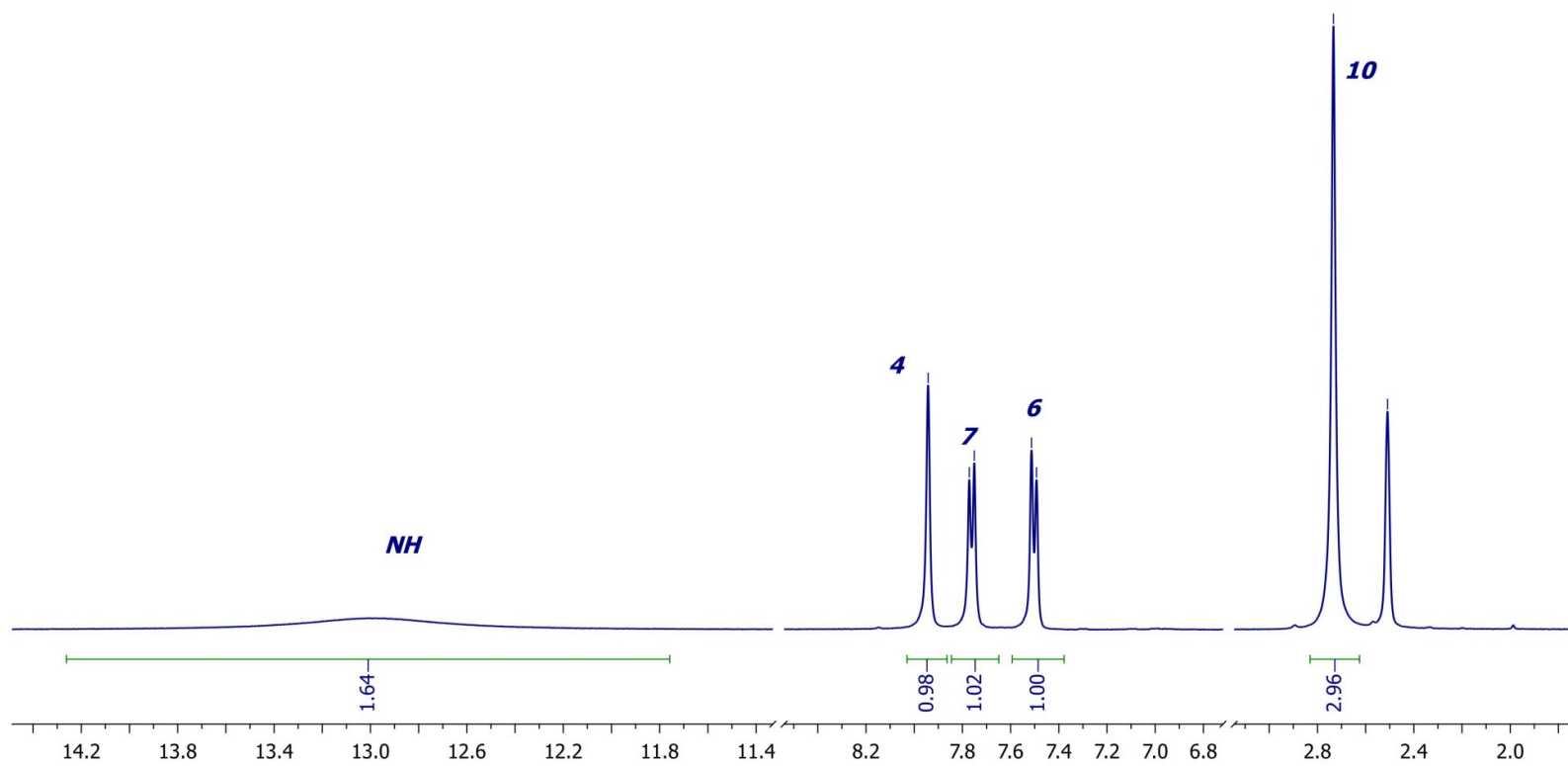


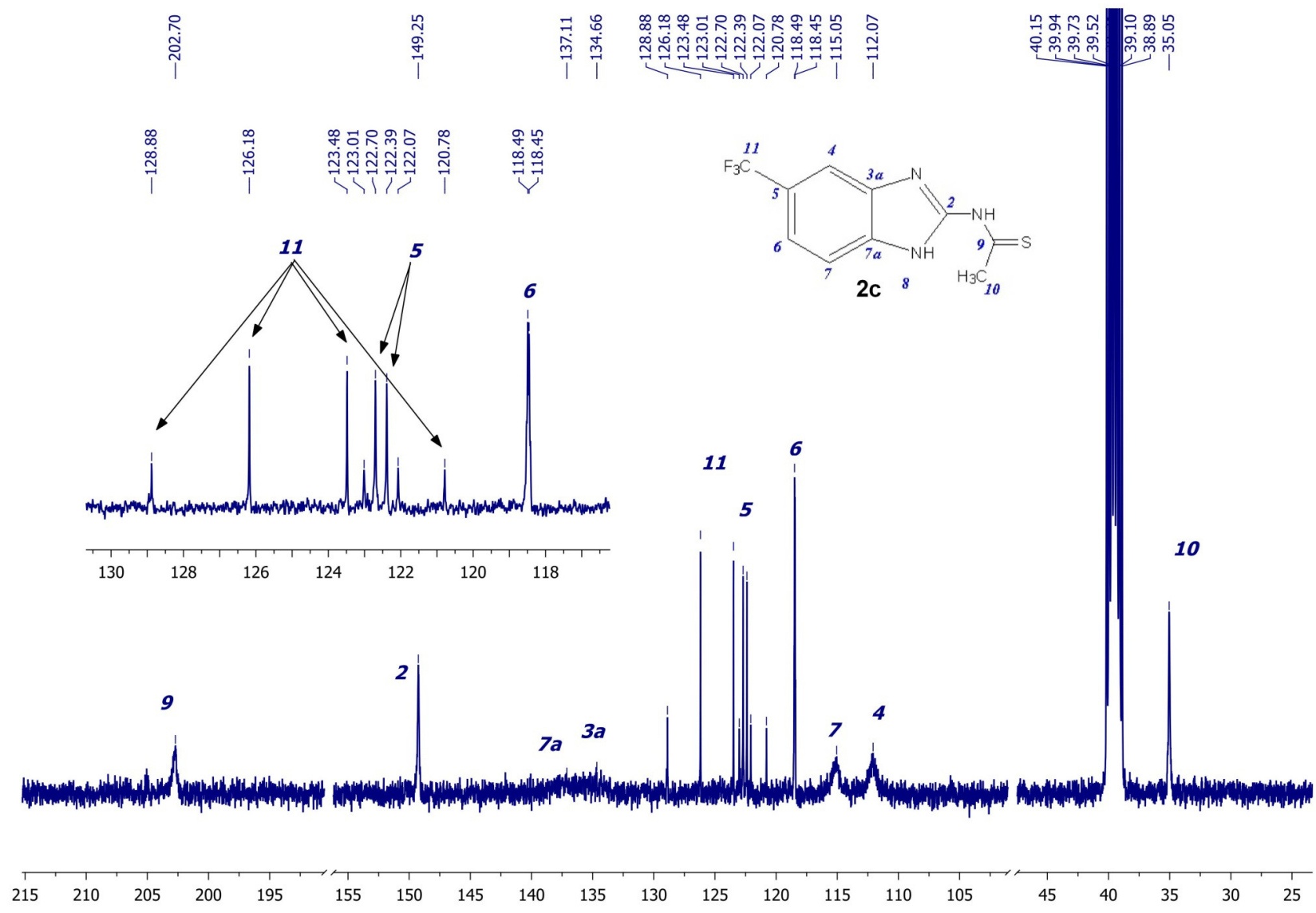


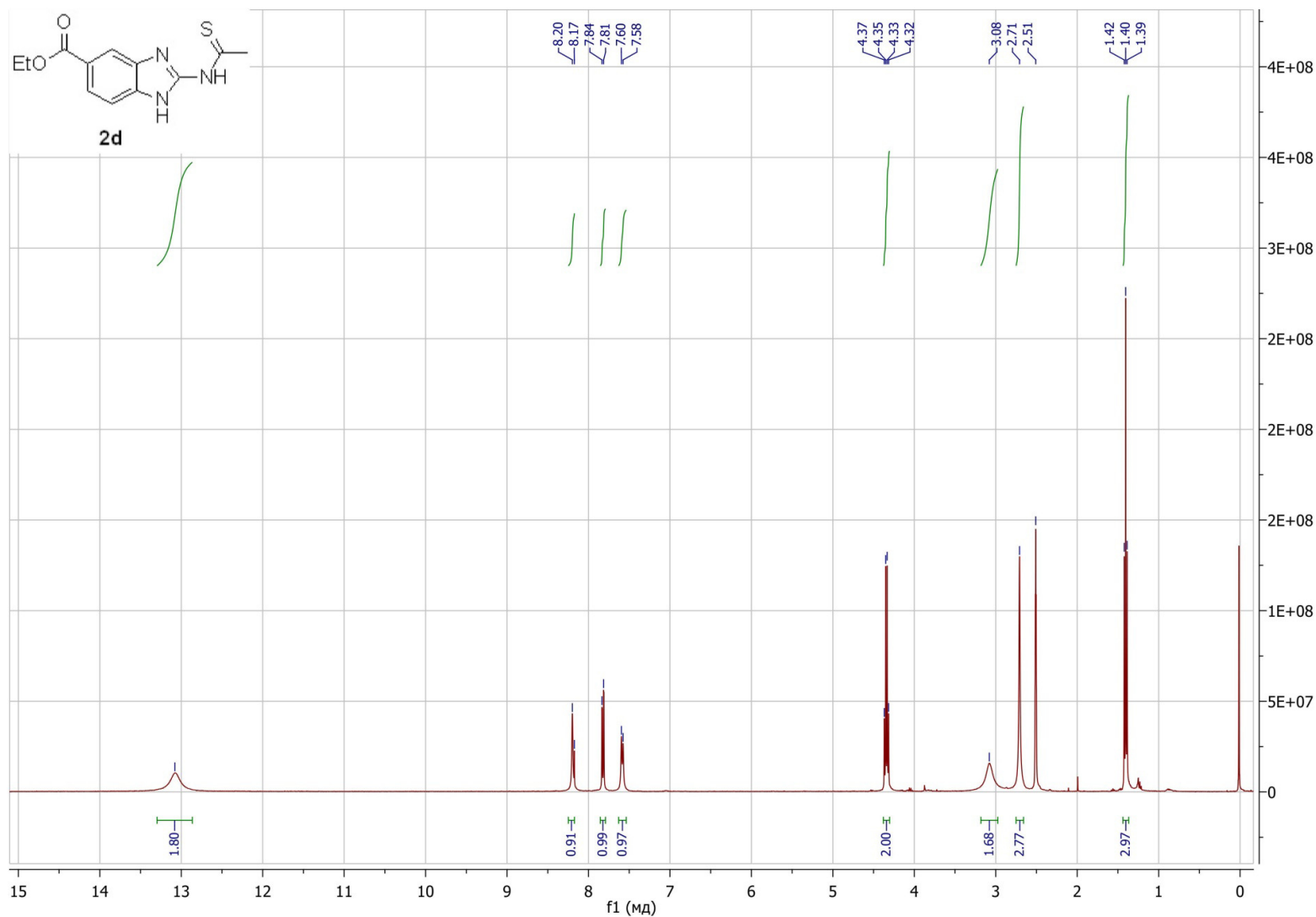




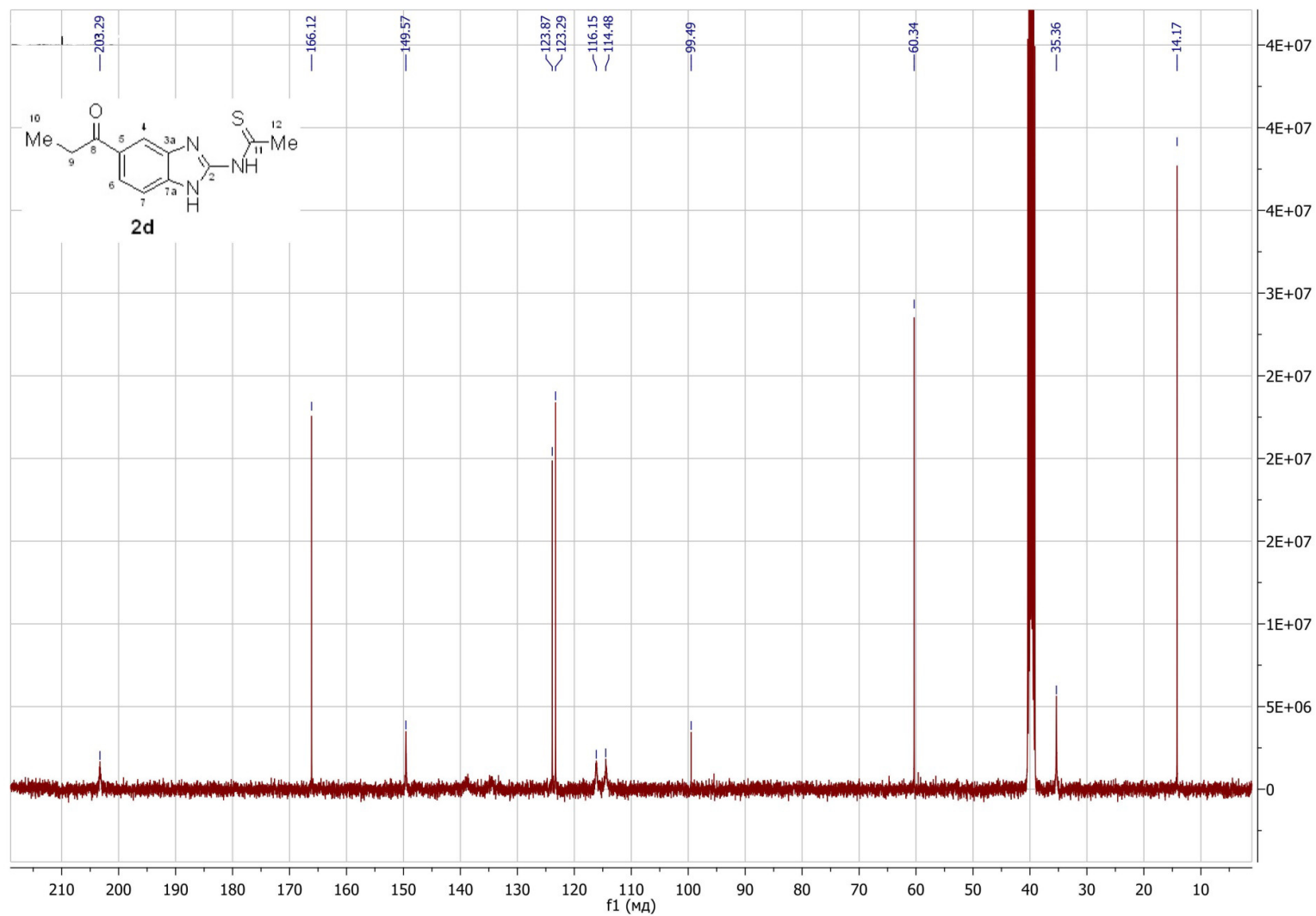
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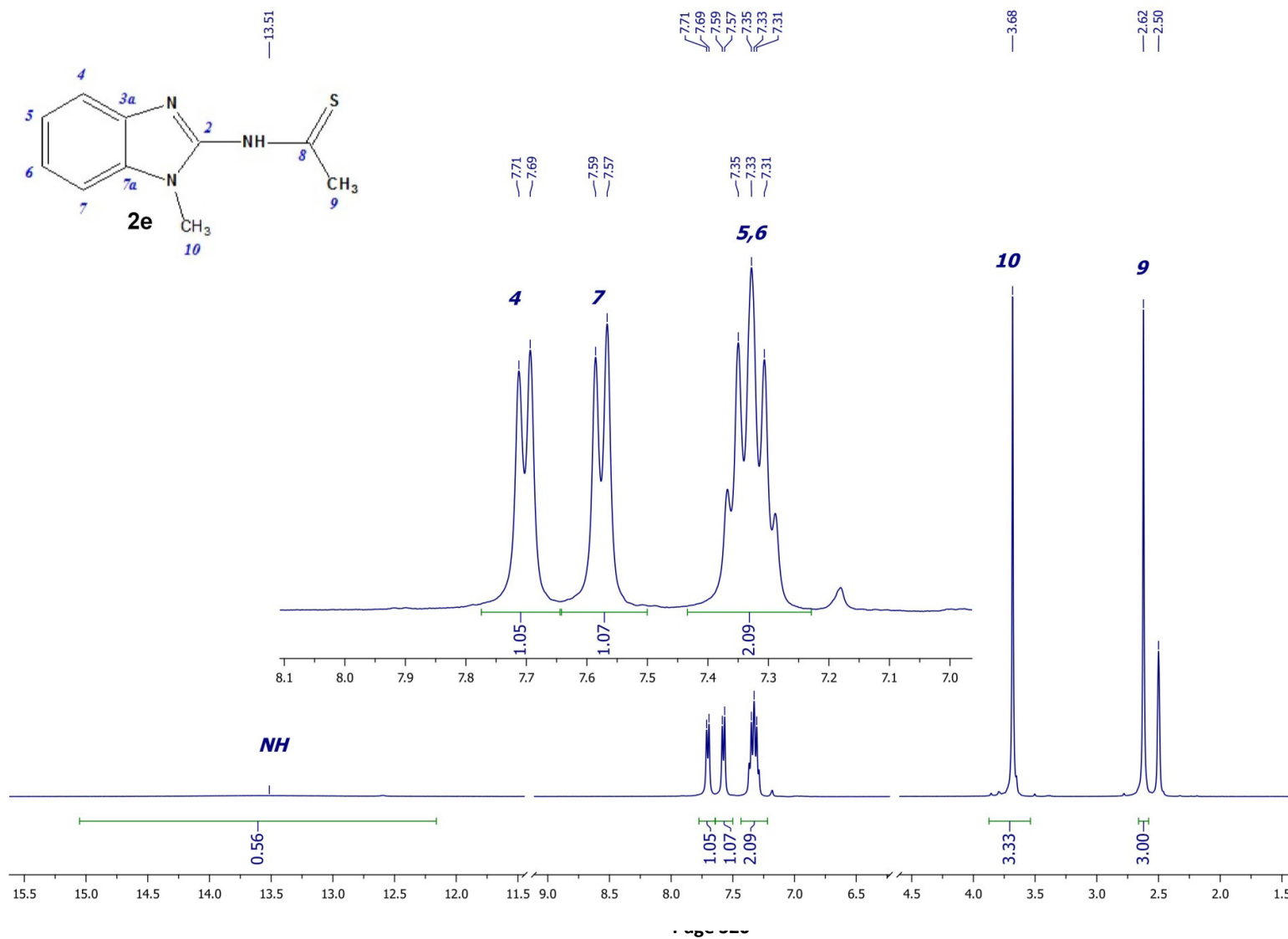


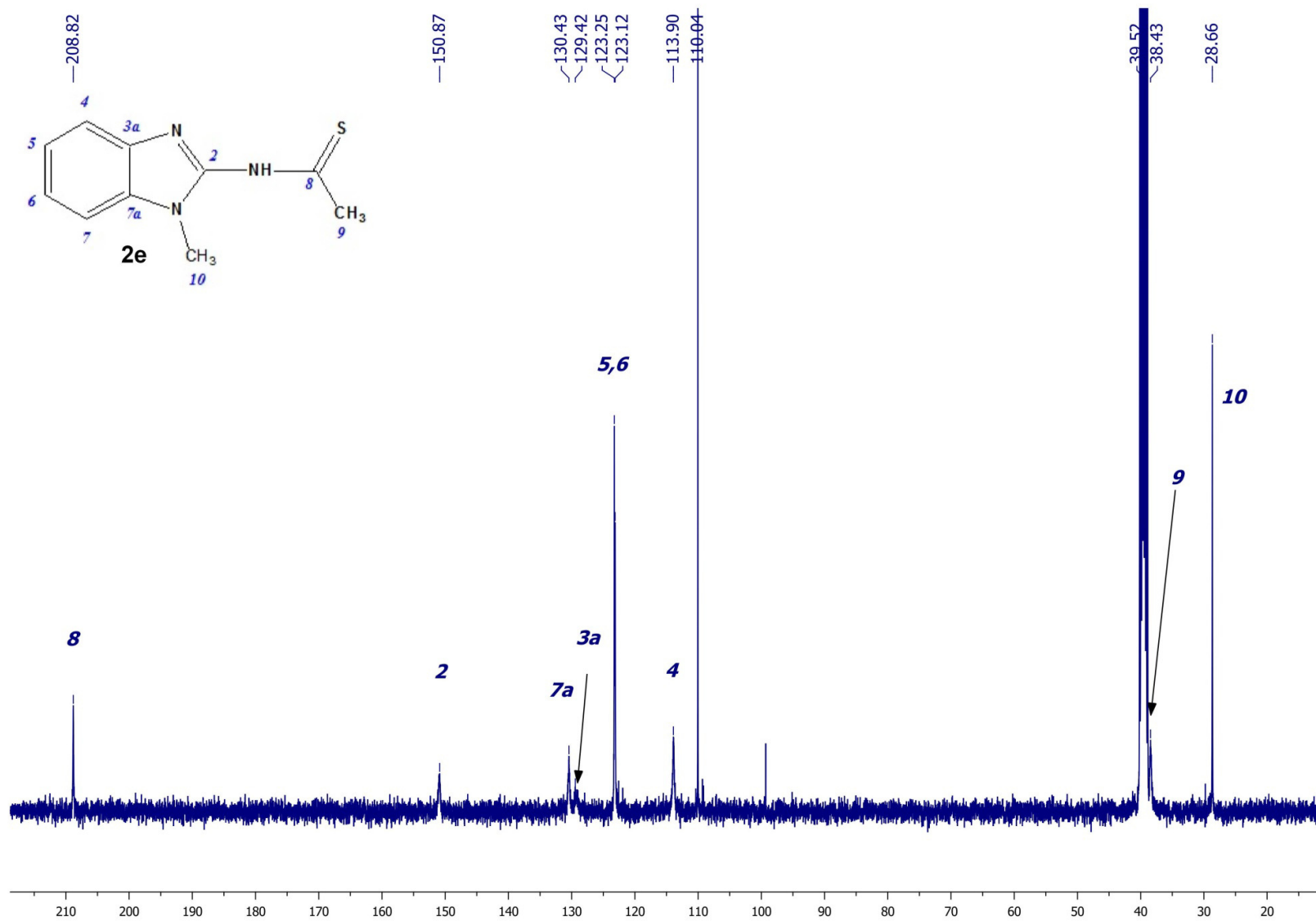


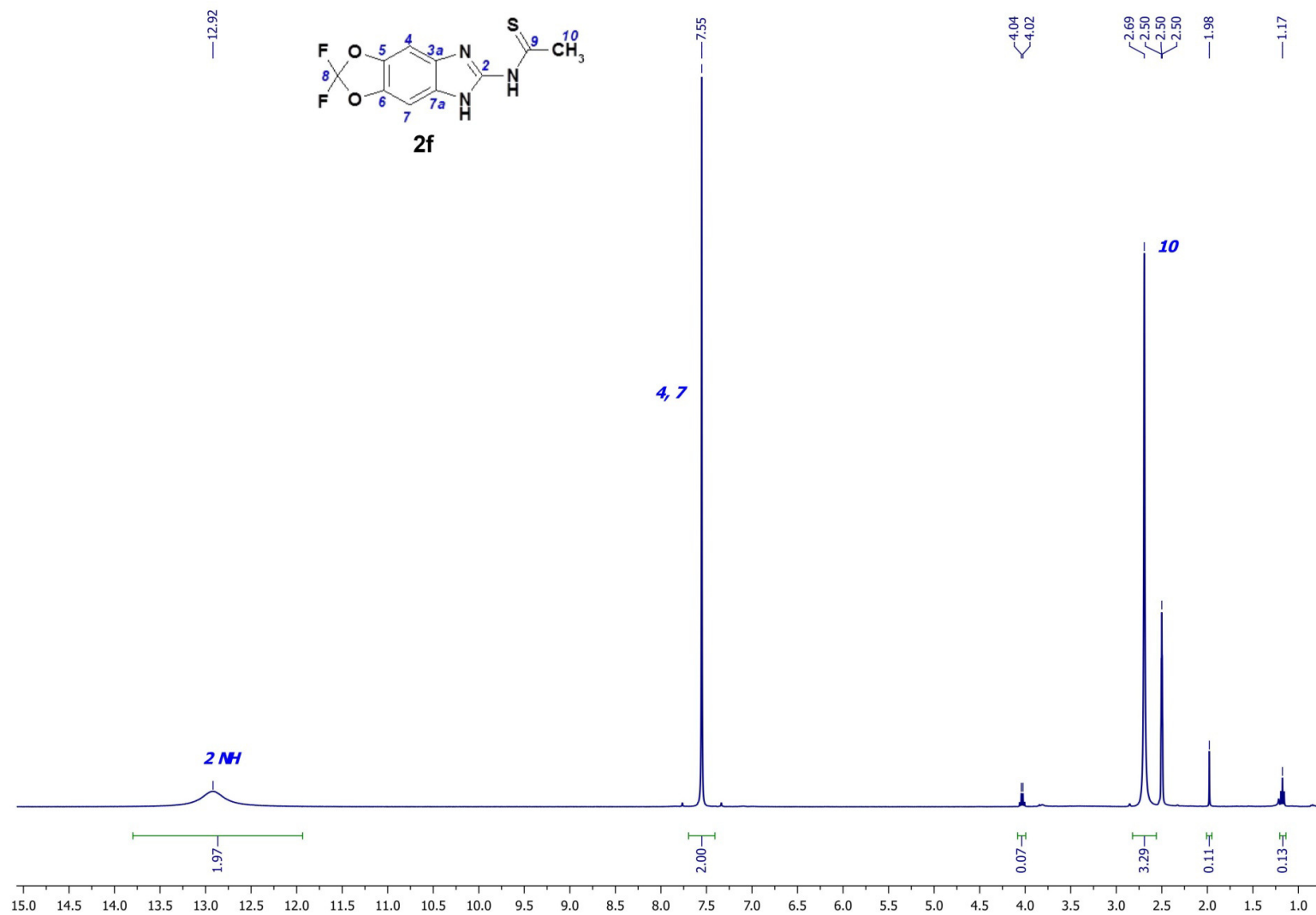


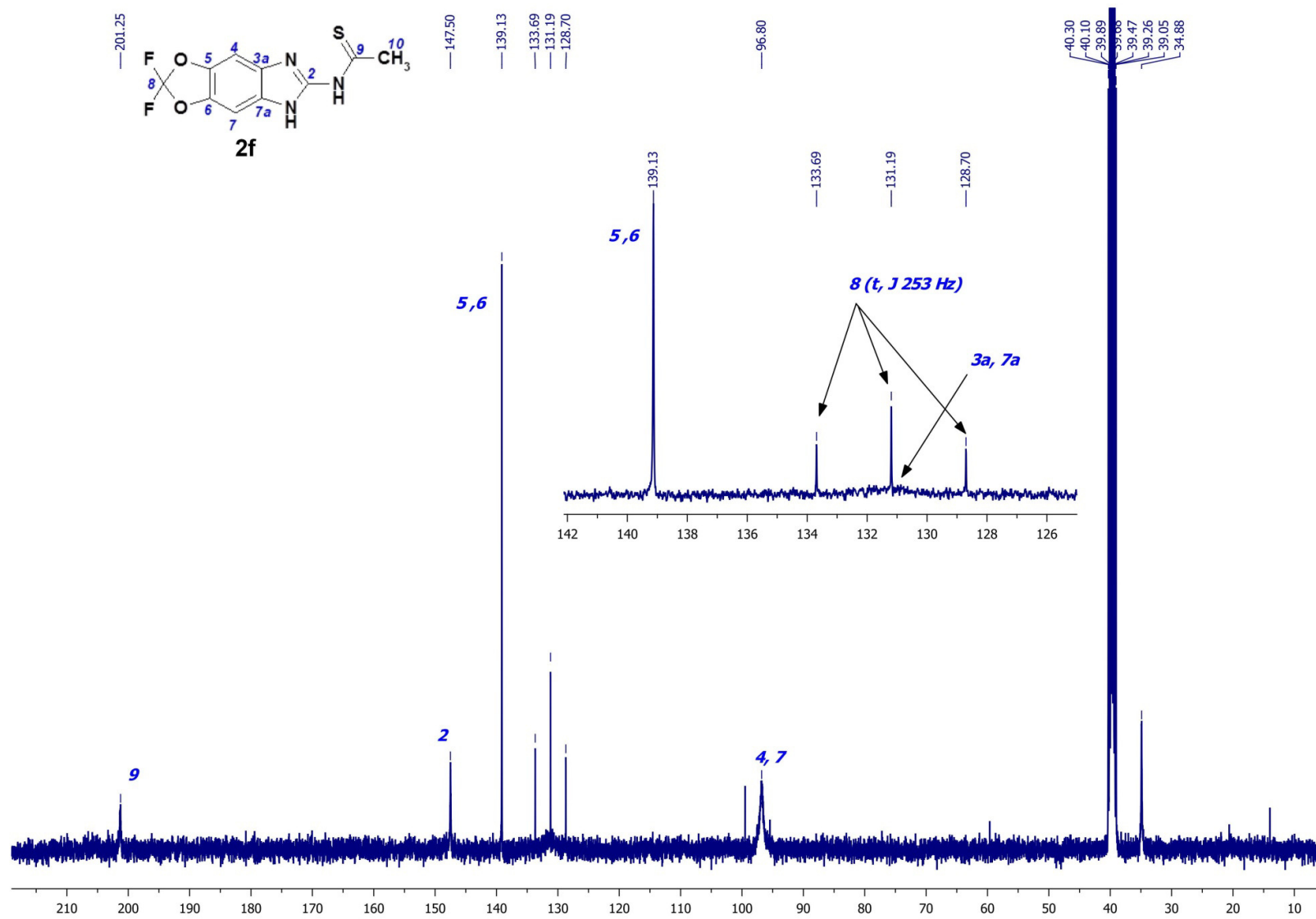


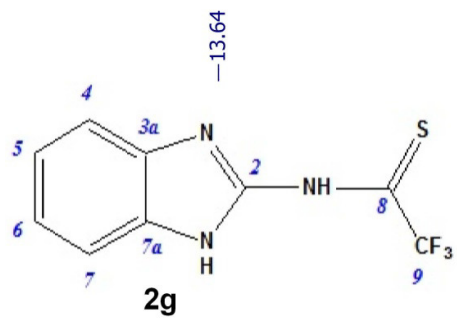










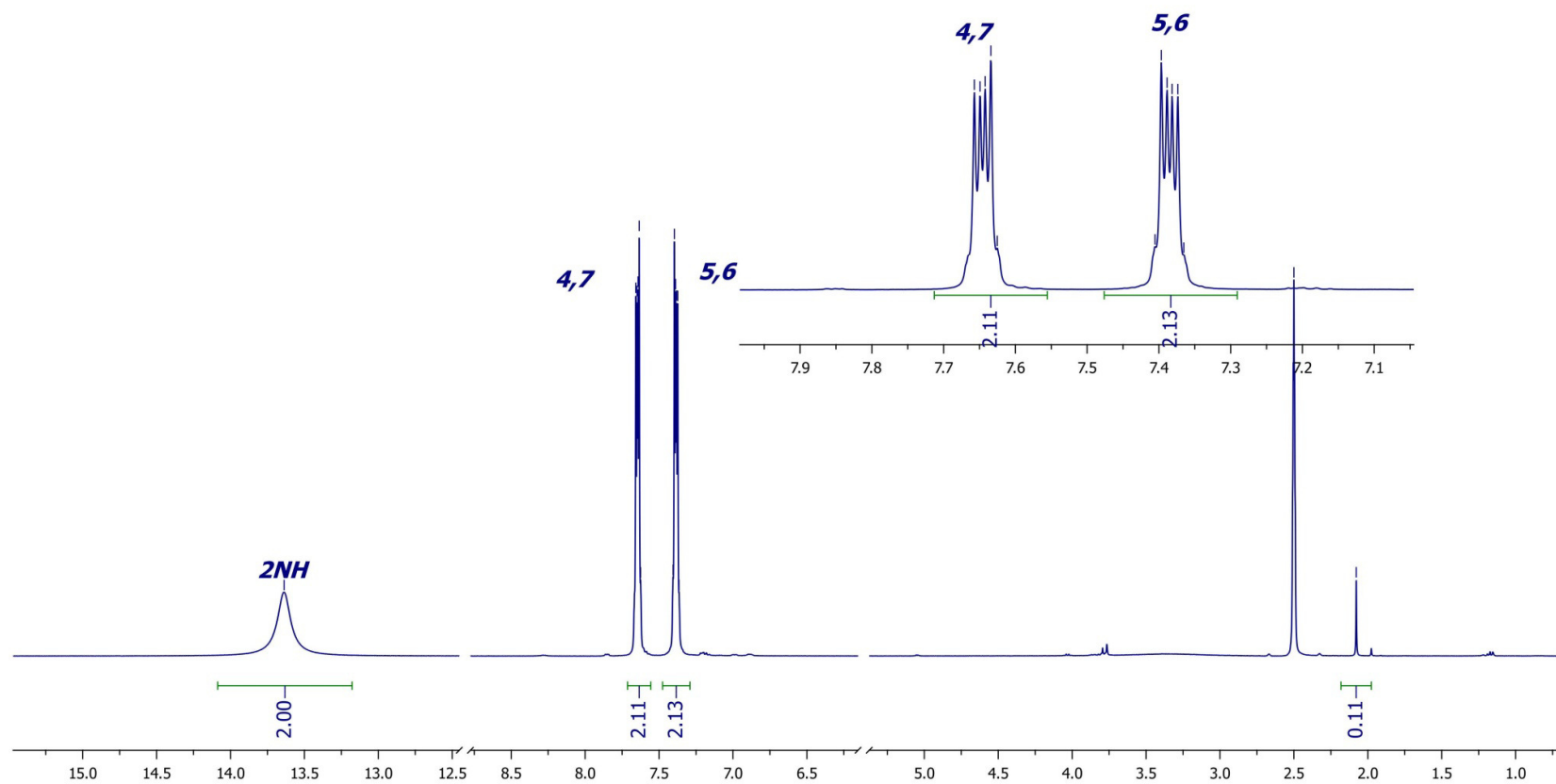


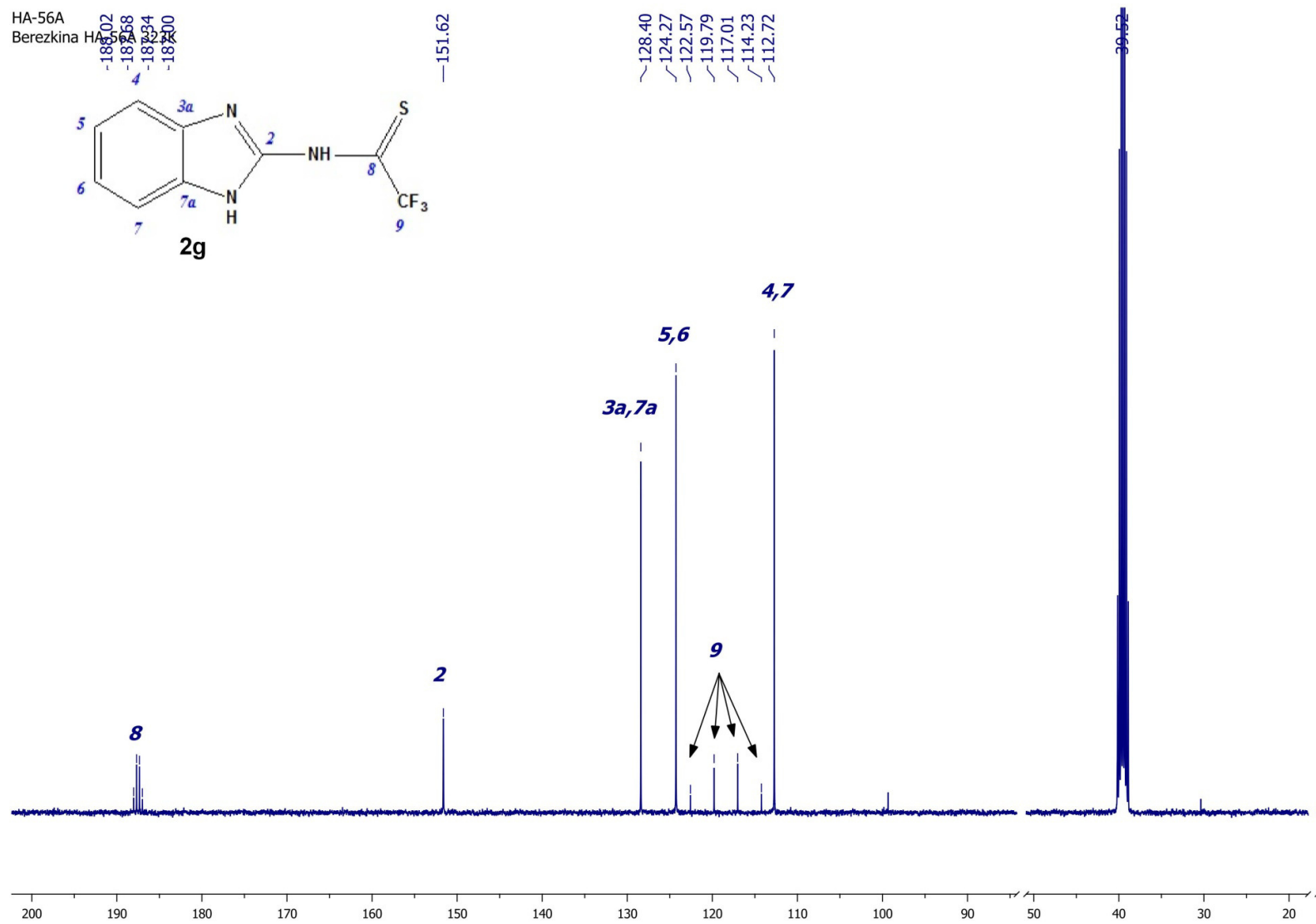
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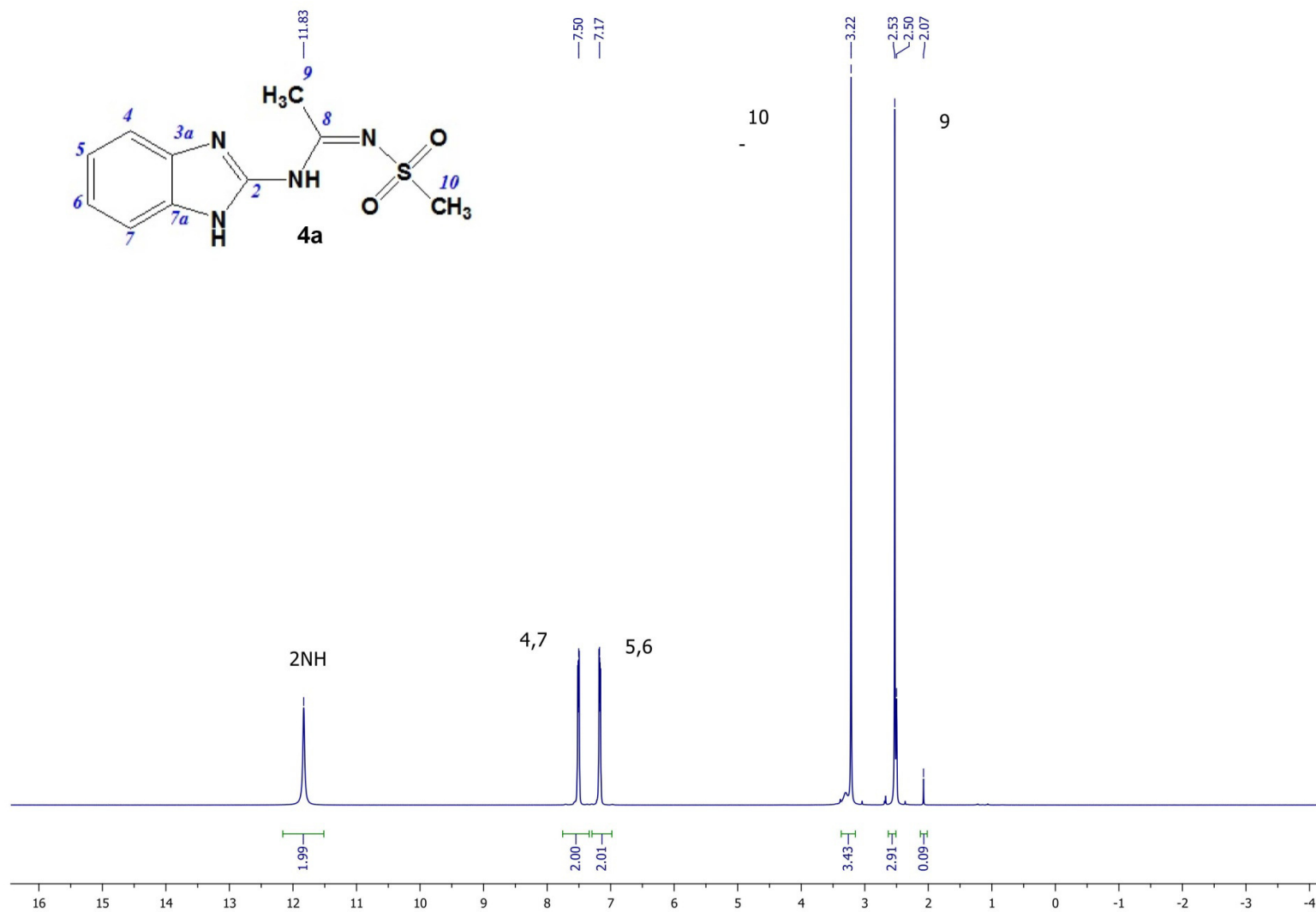
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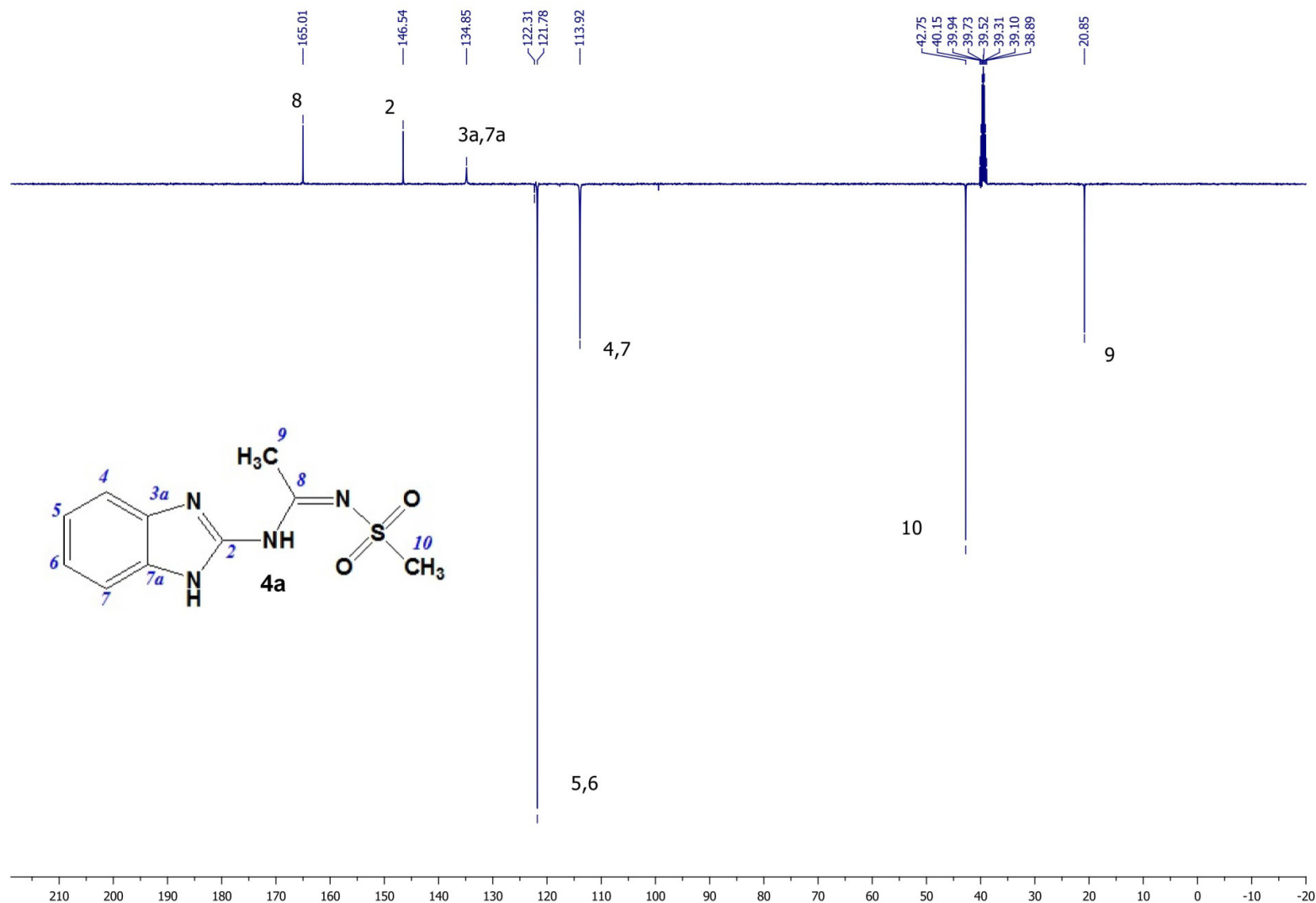
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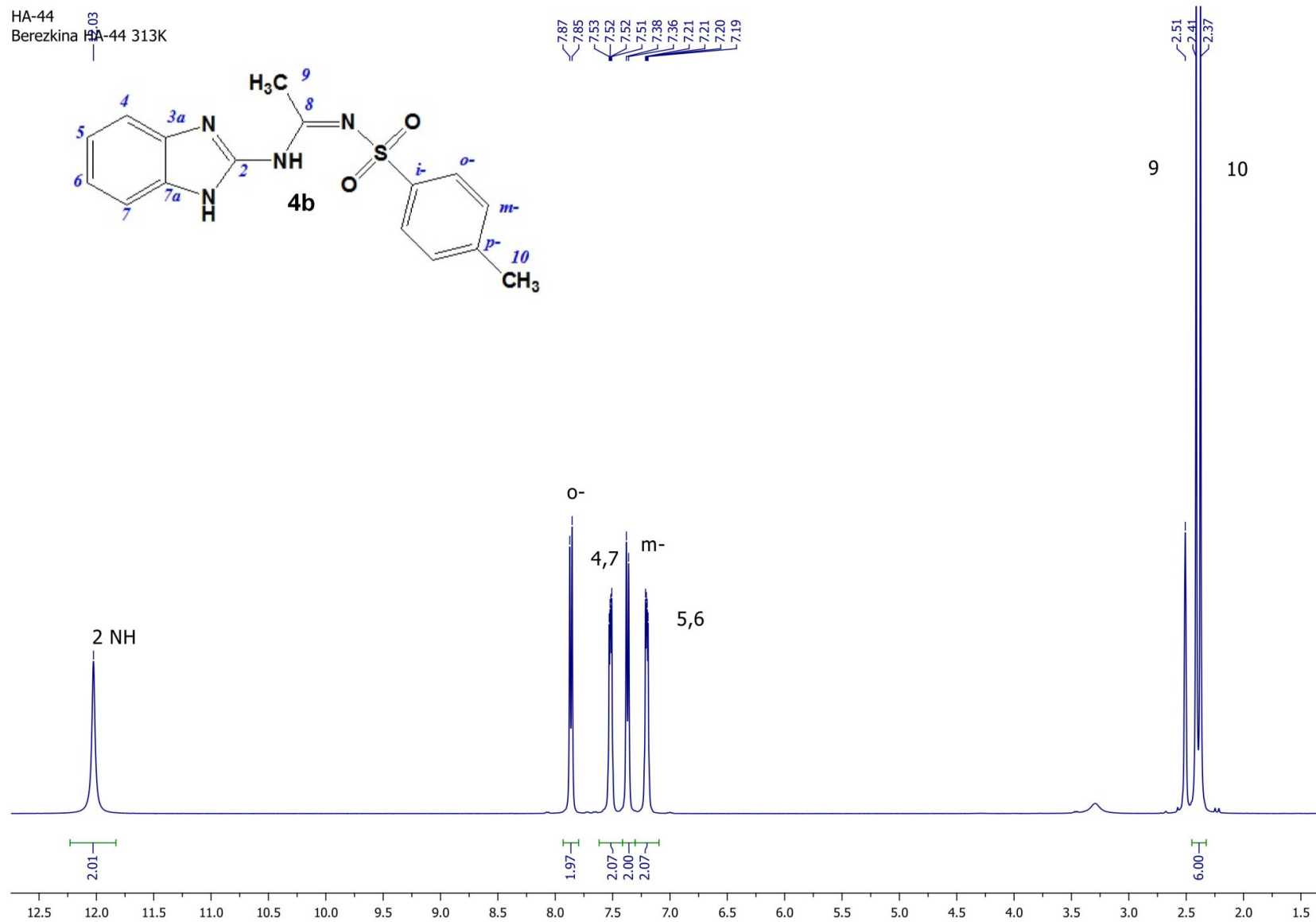


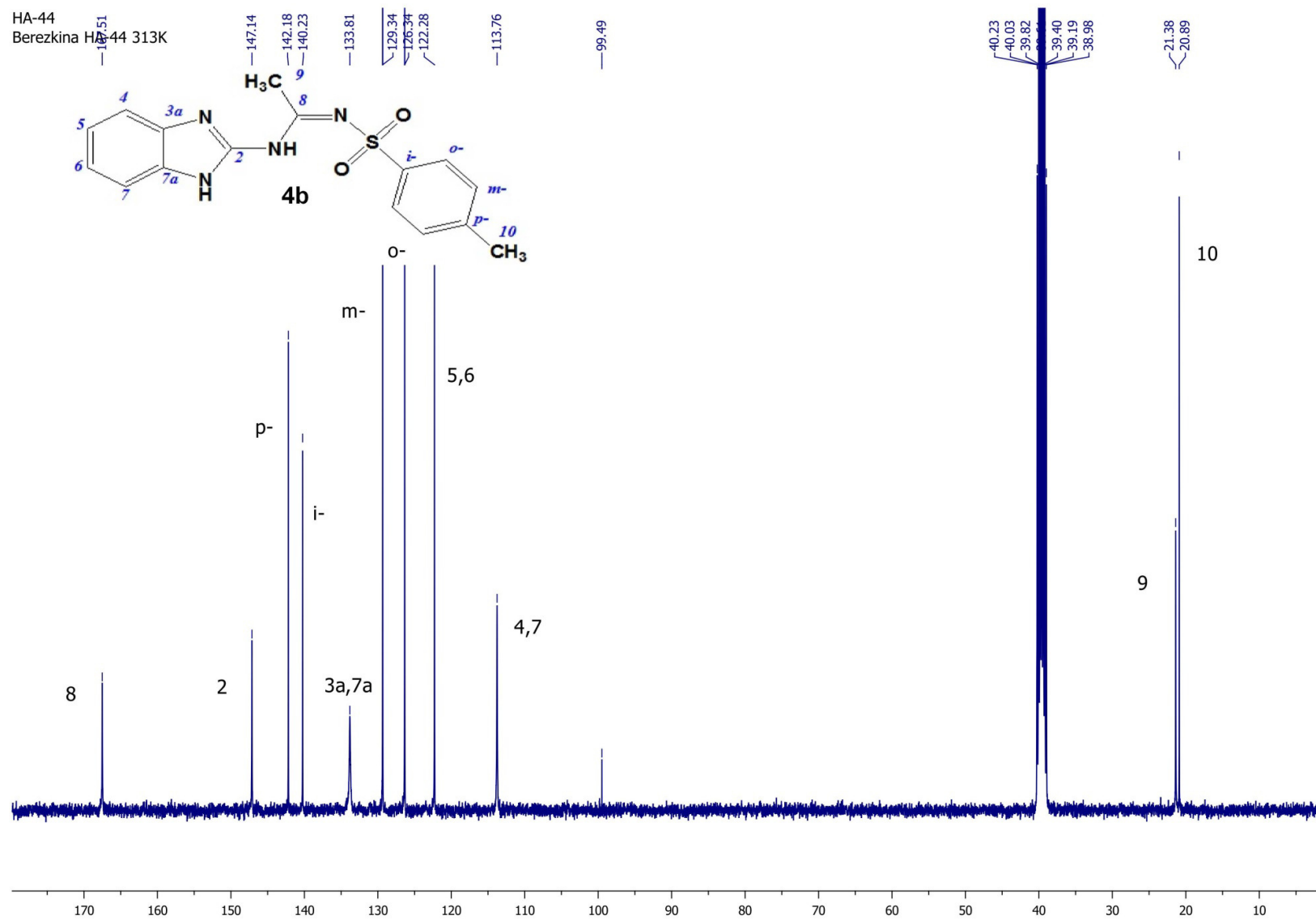




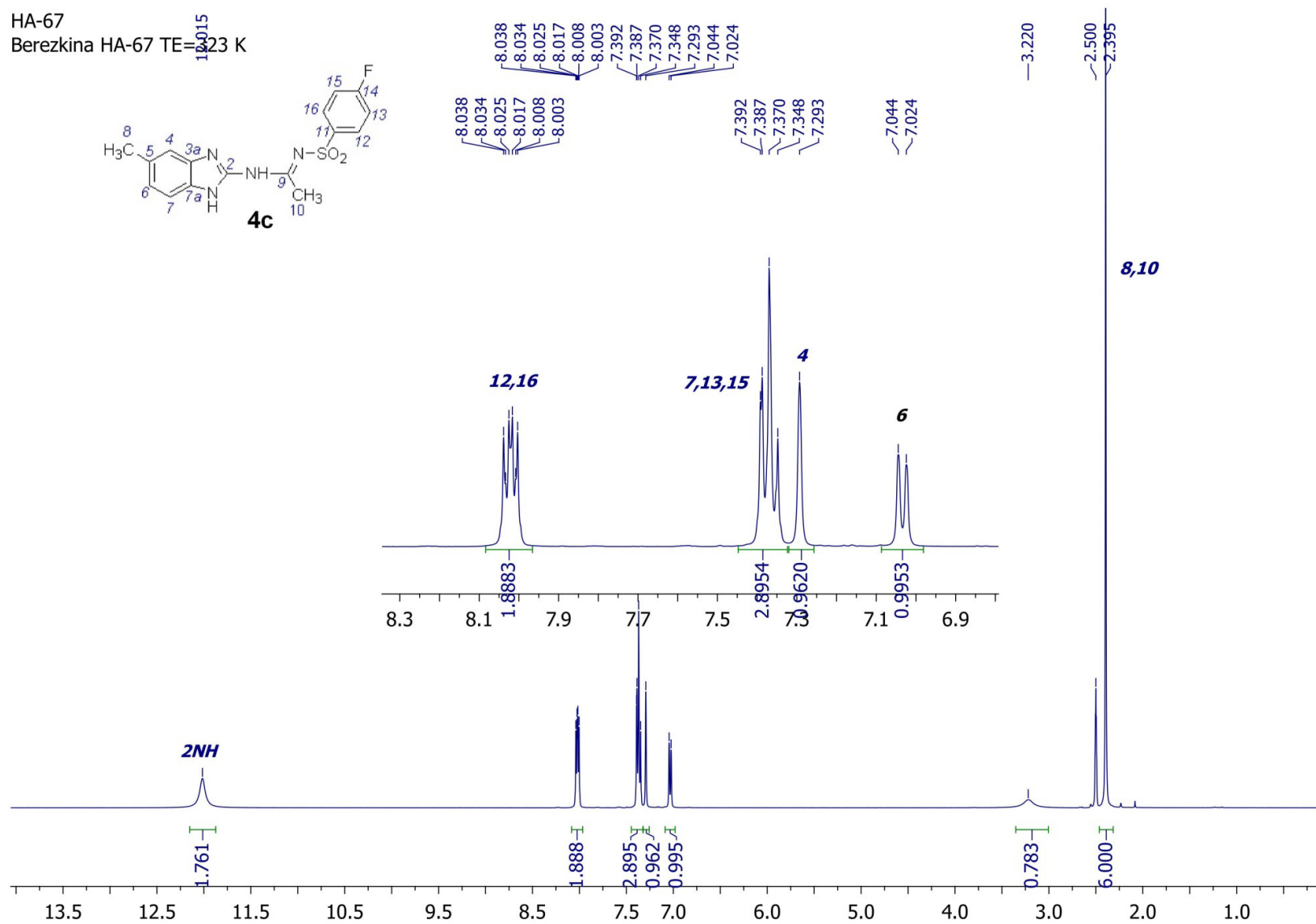
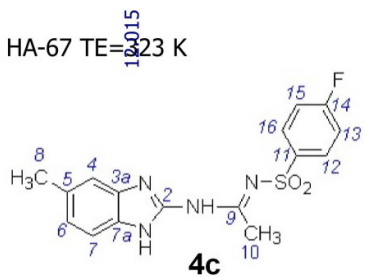


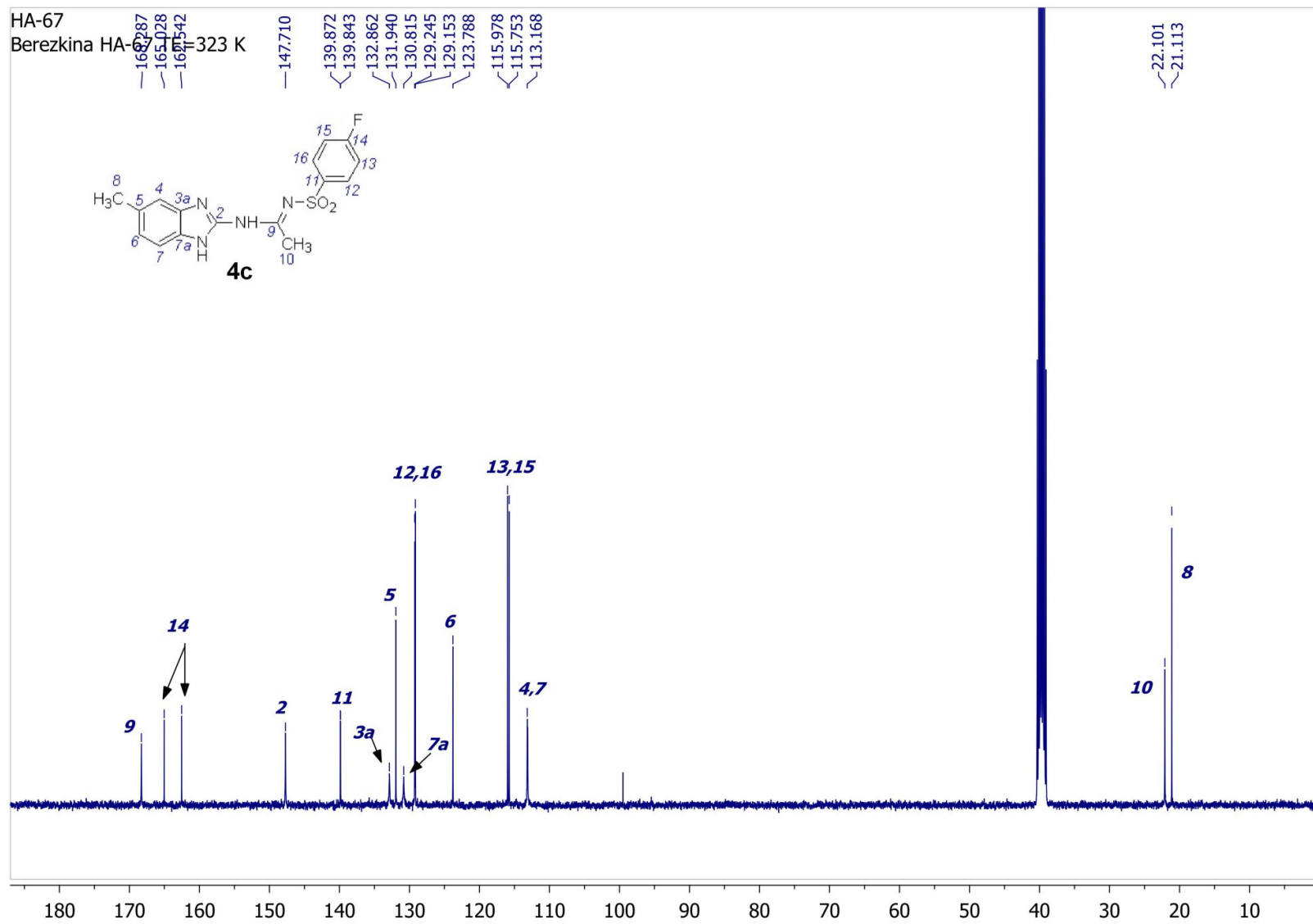


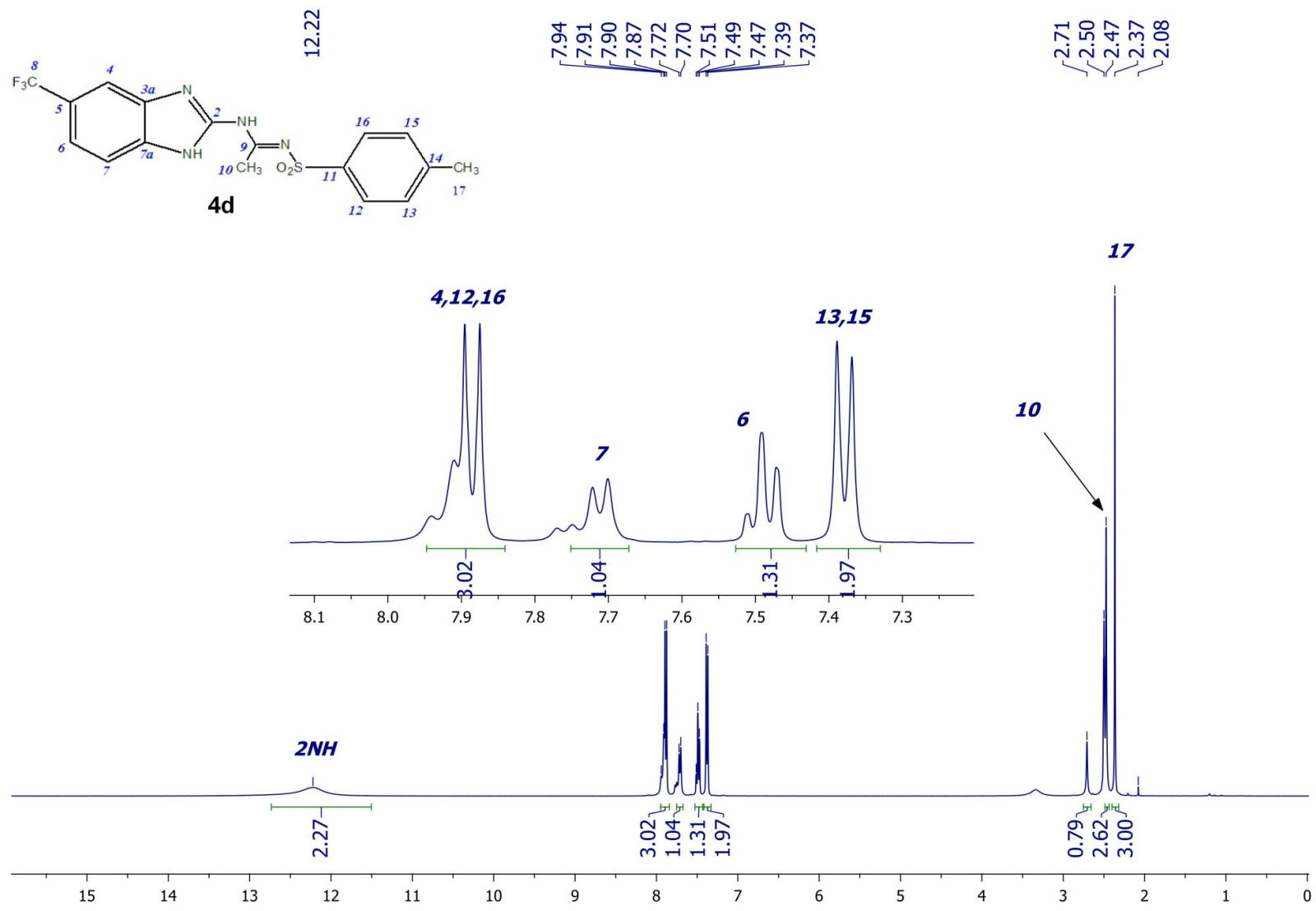


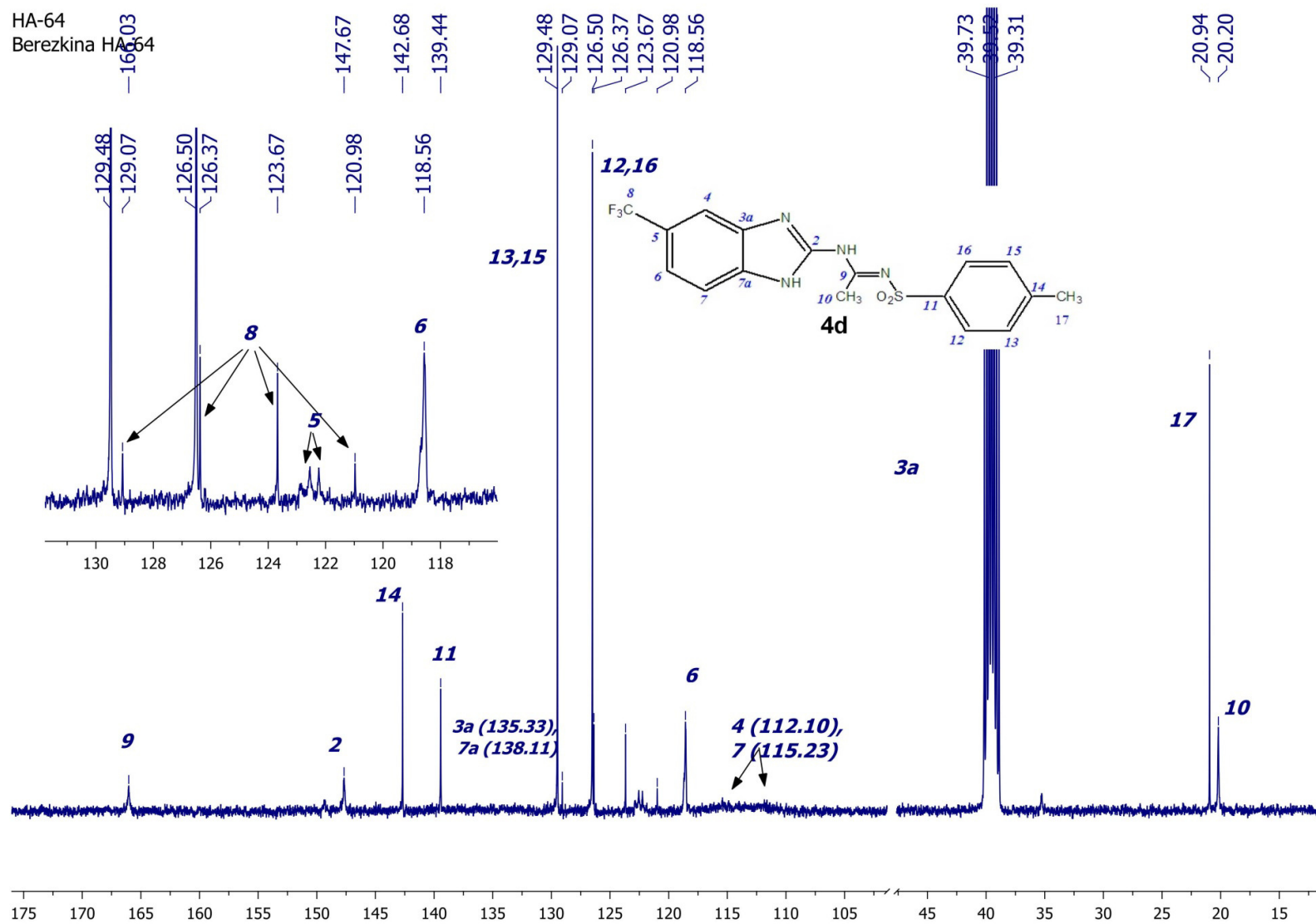


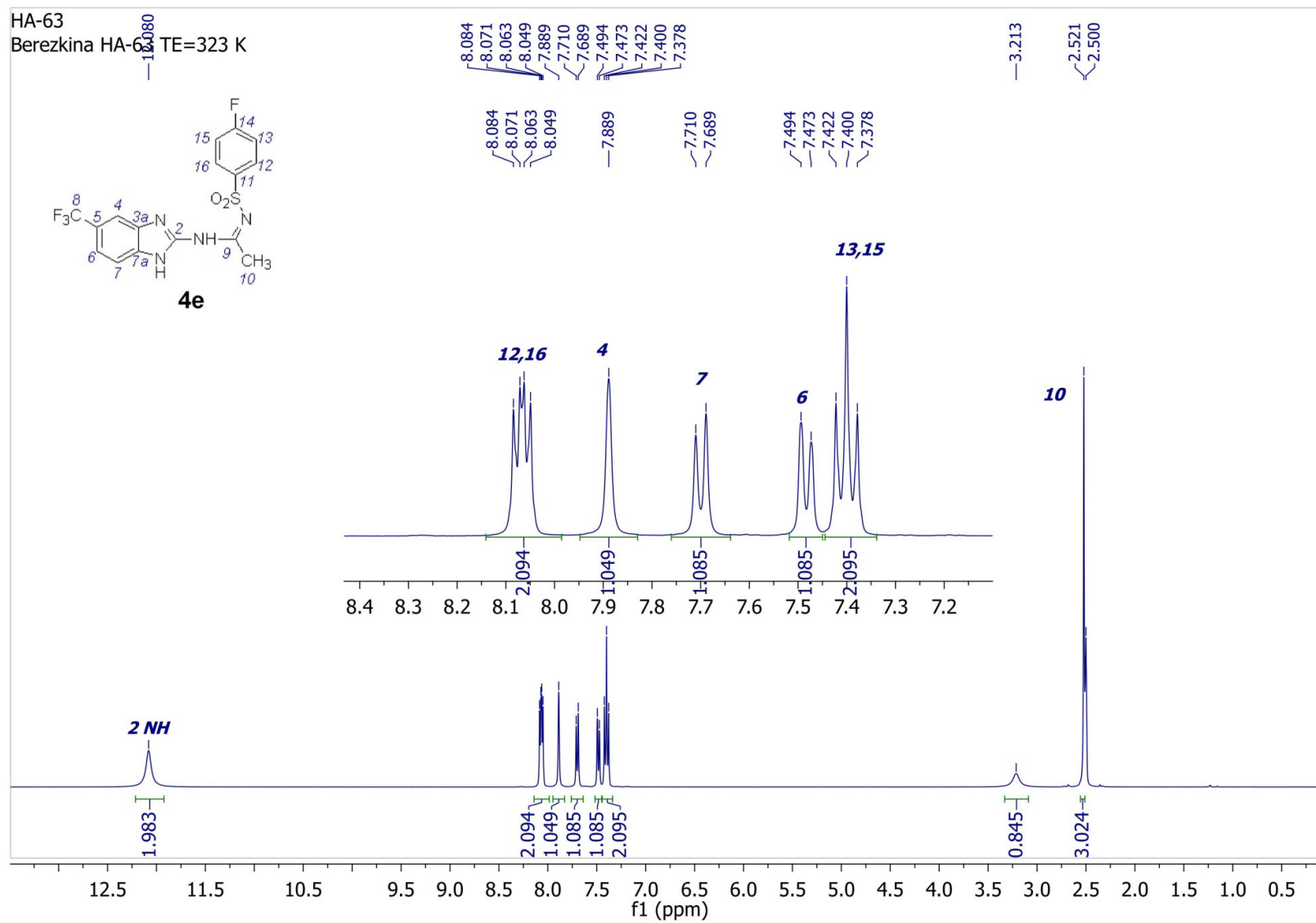
HA-67  
Berezkina HA-67 TE=323 K



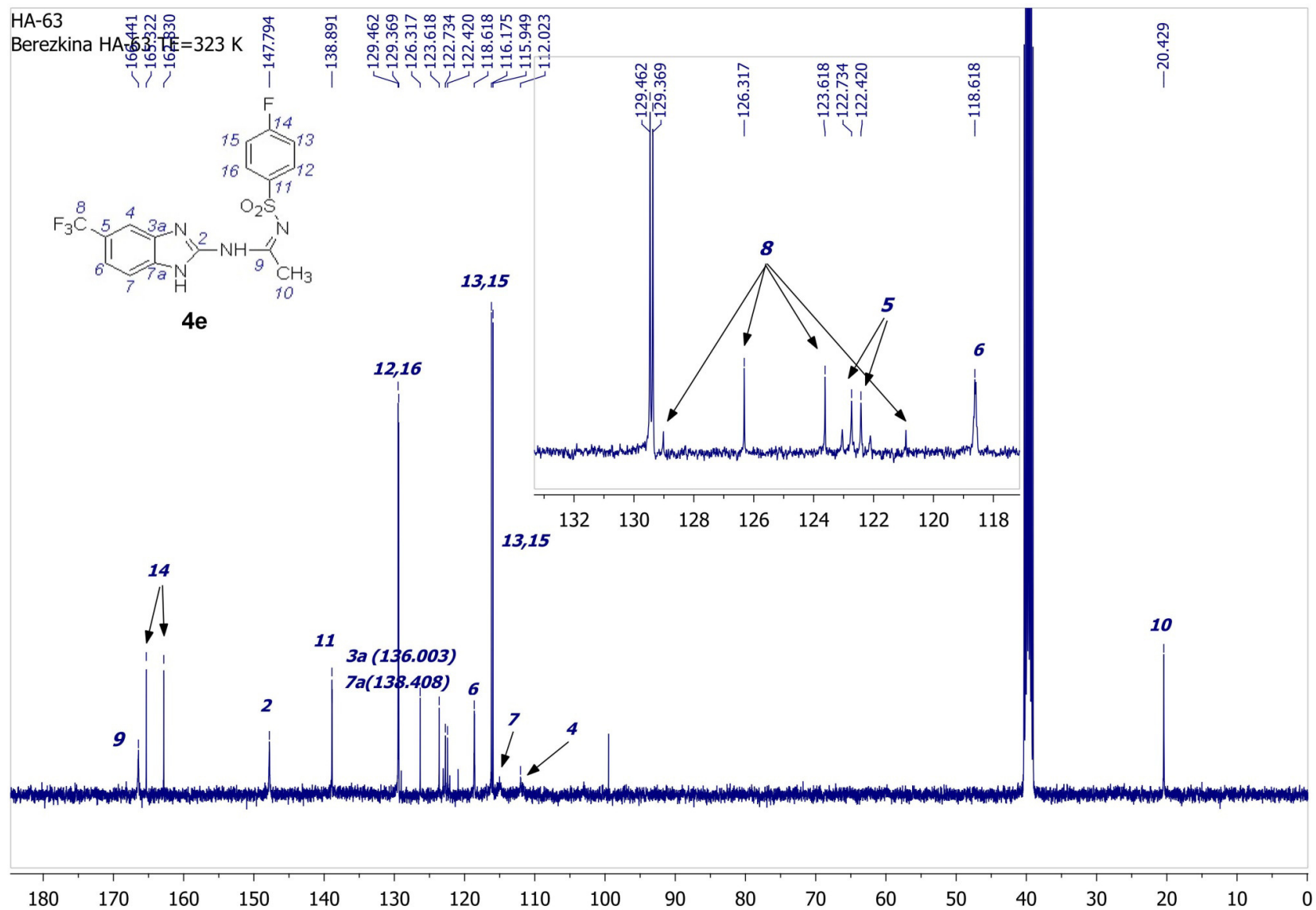


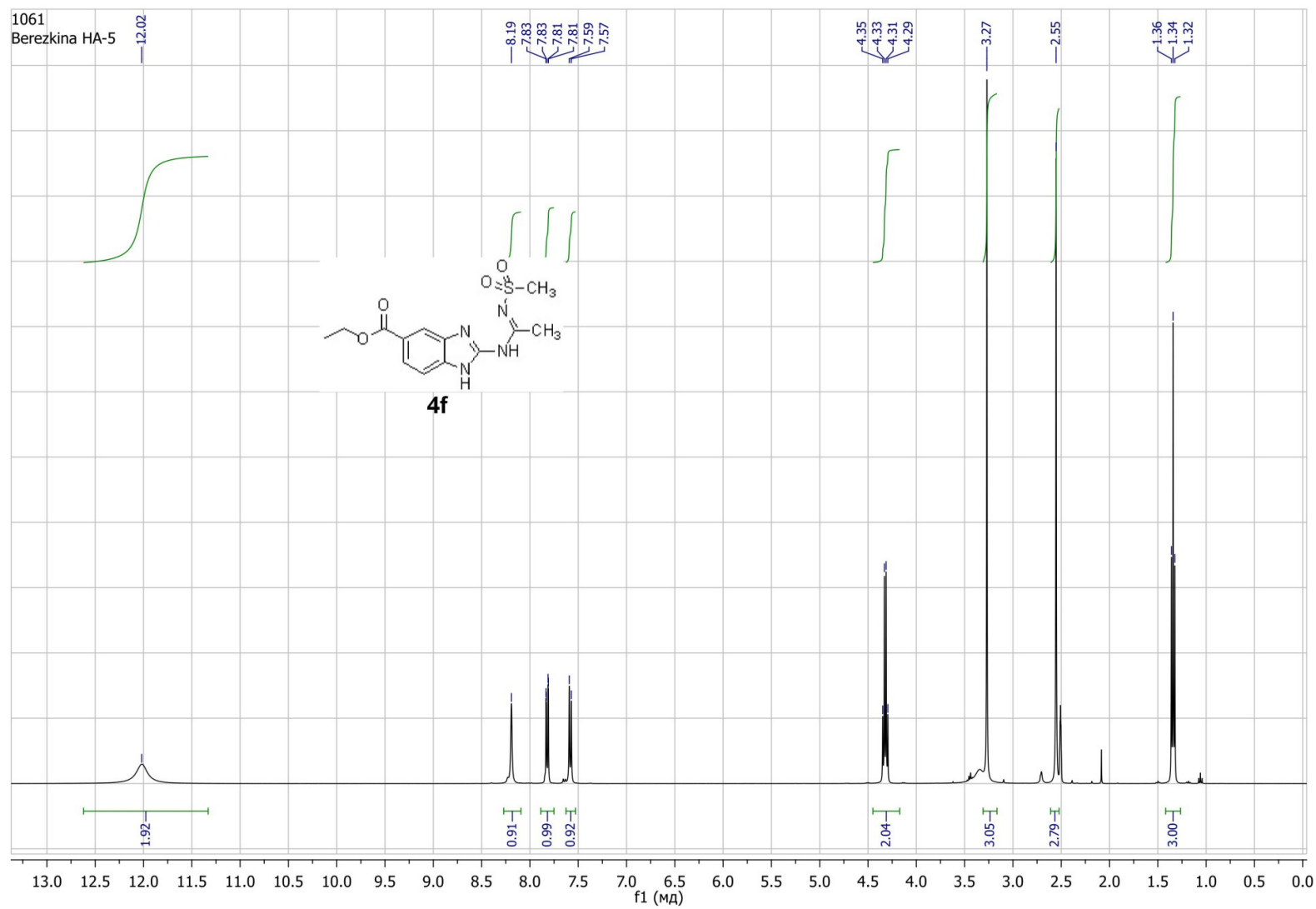


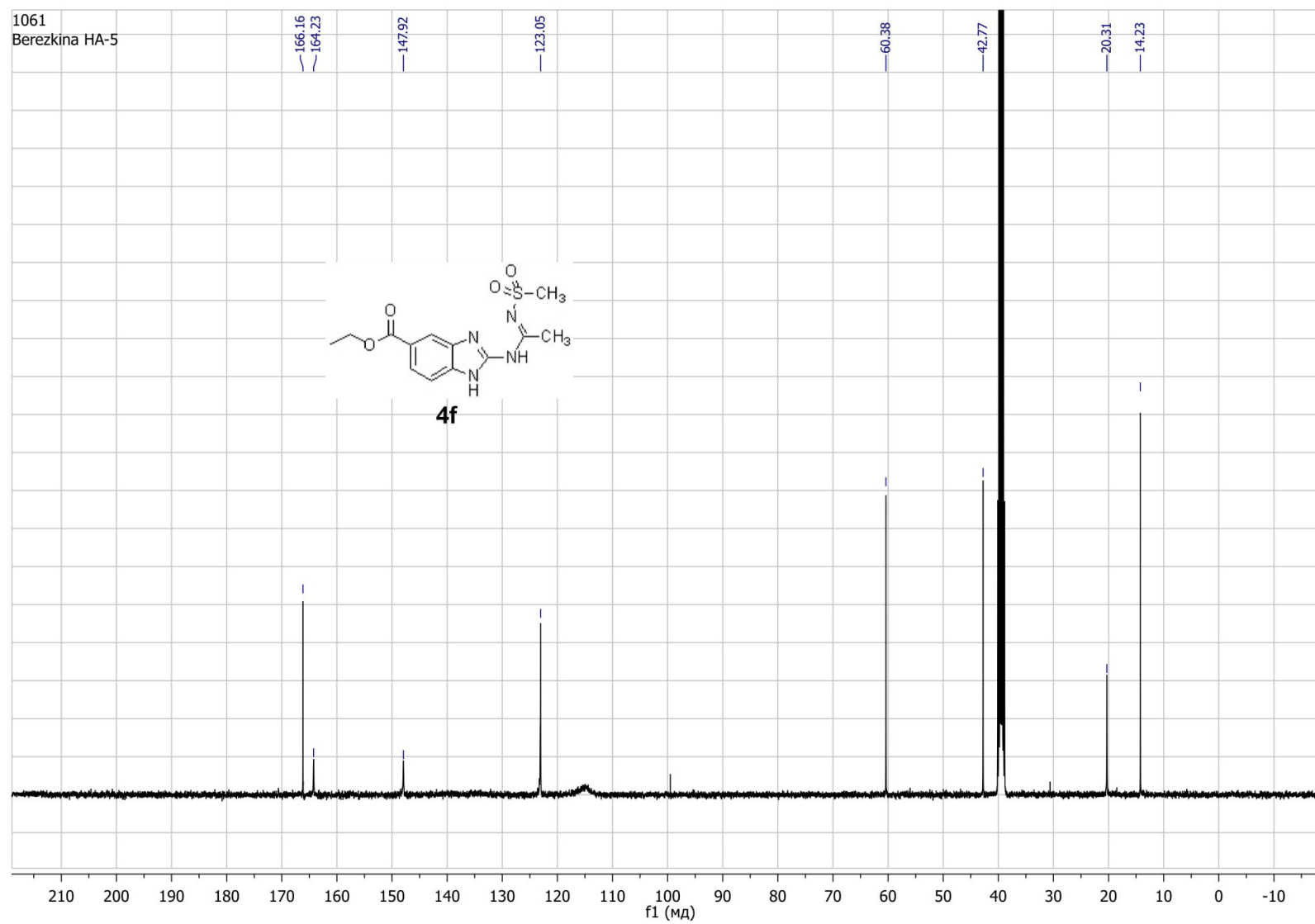


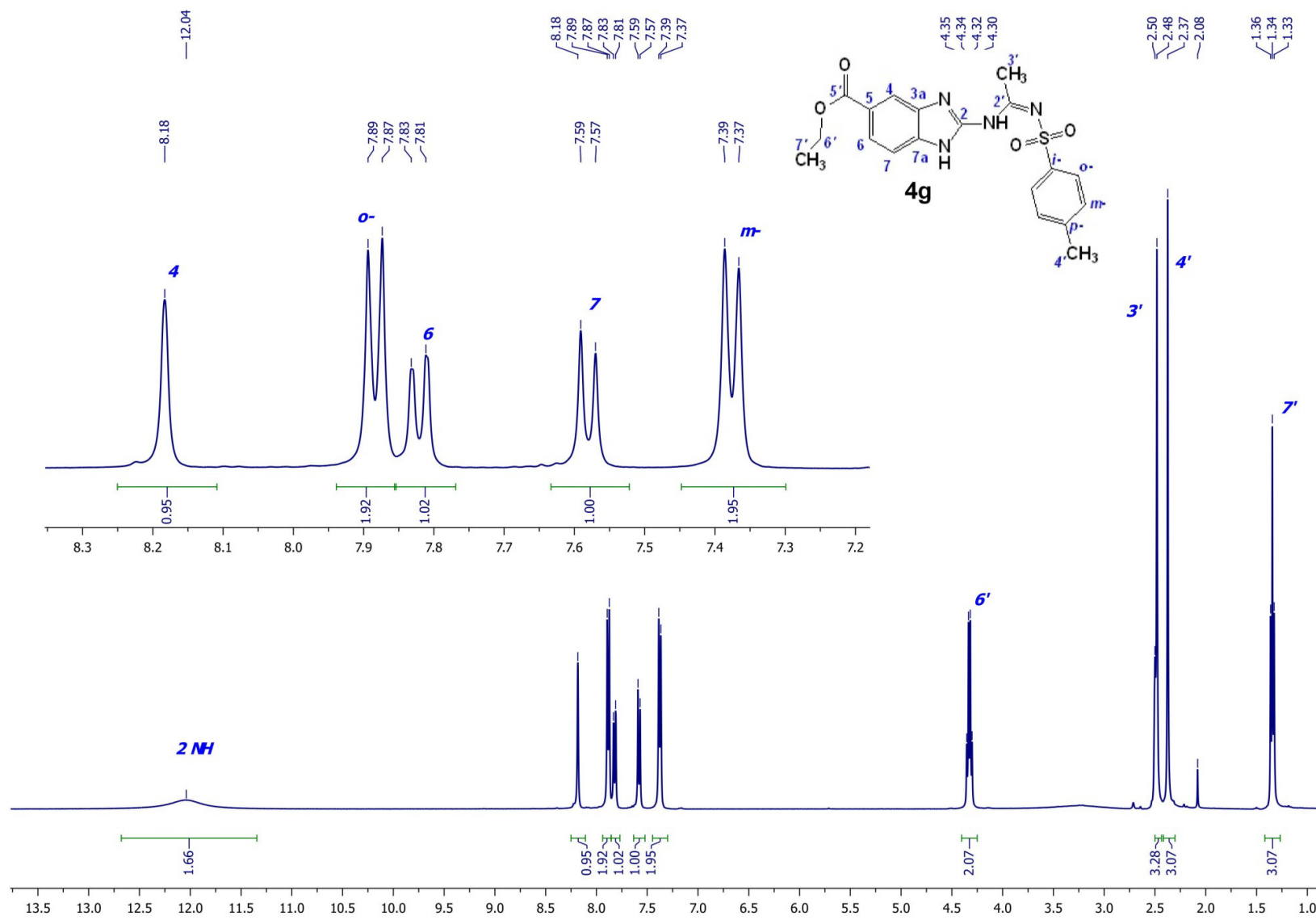


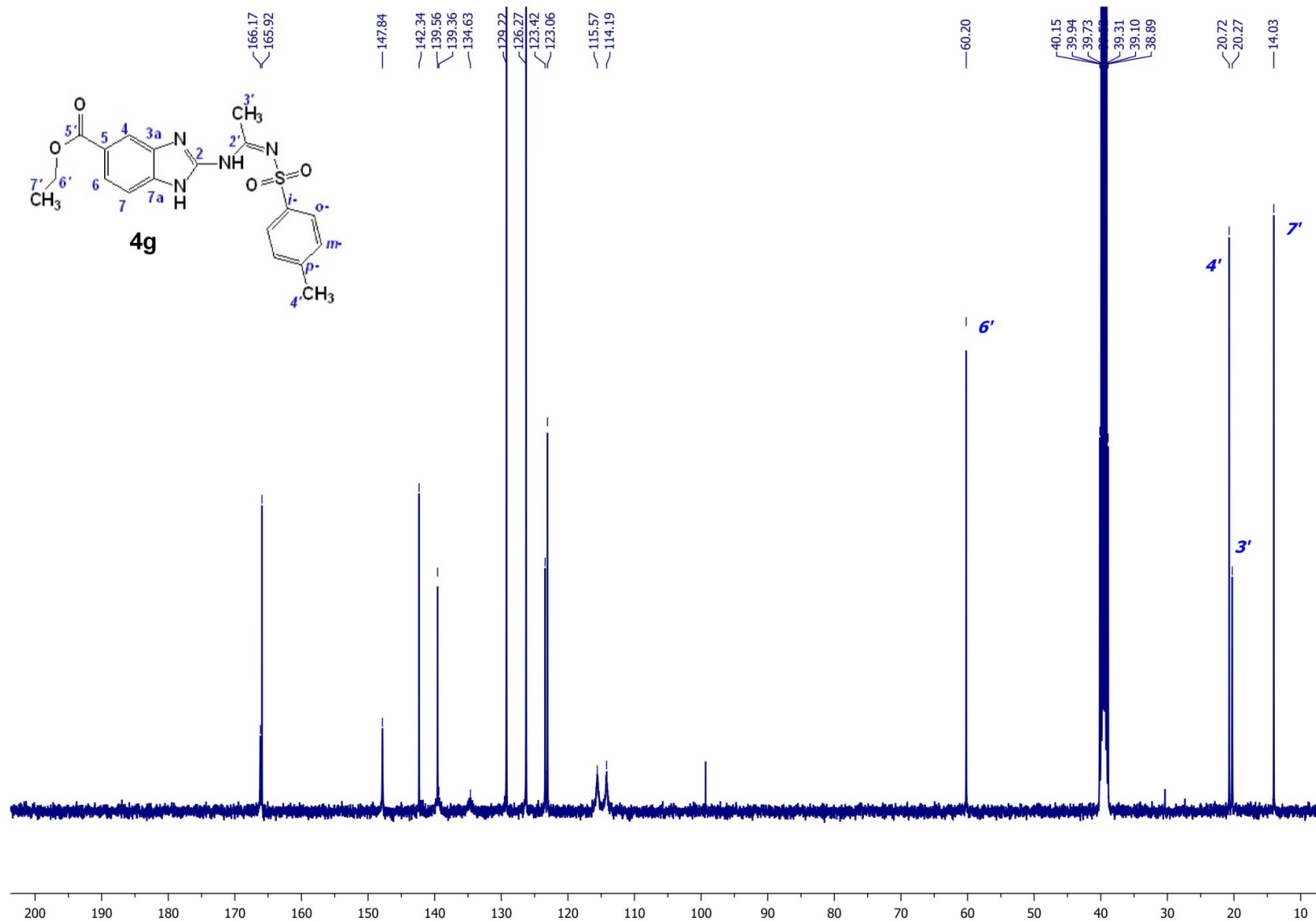


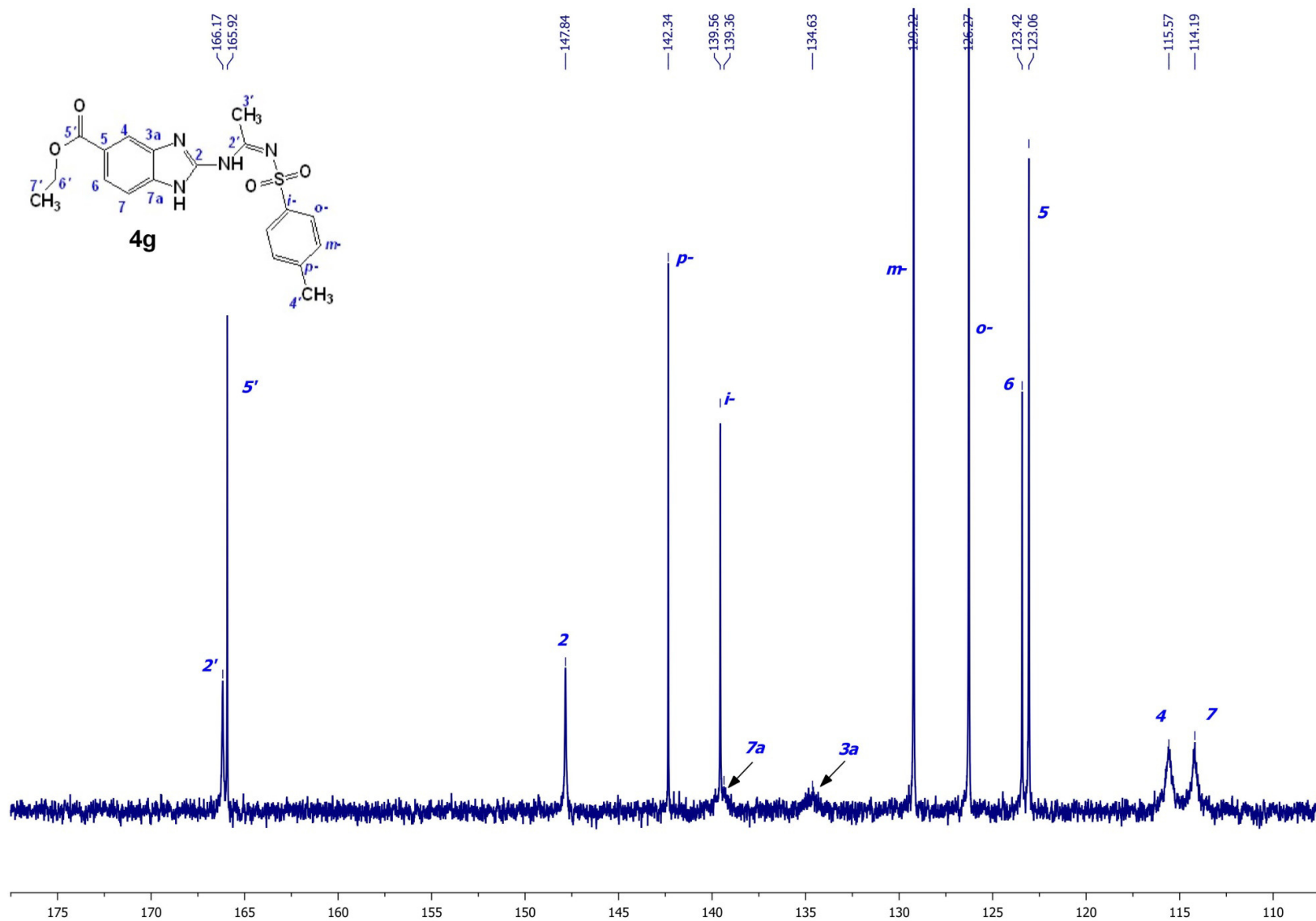


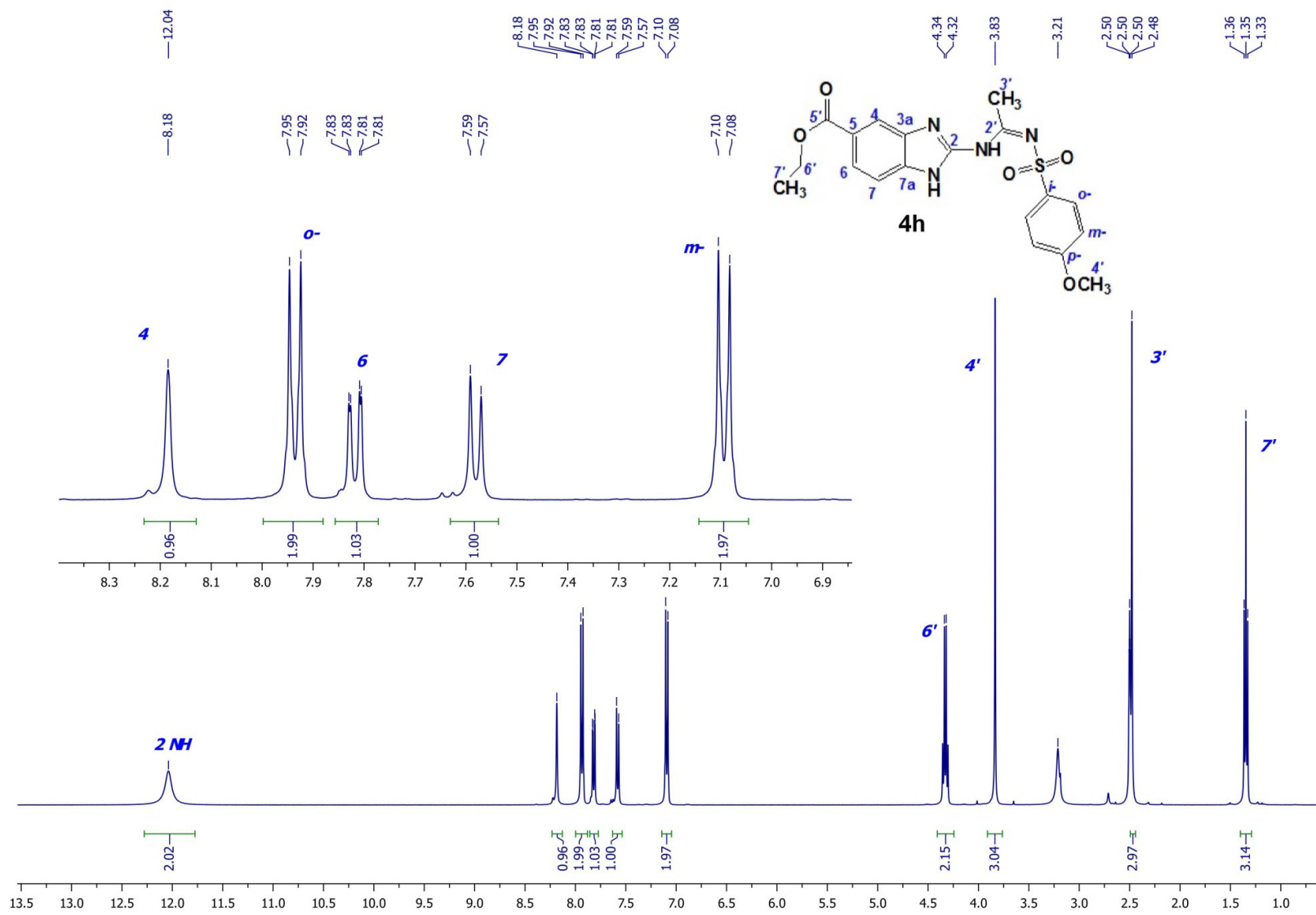












HA-17  
Berezkina HA-17

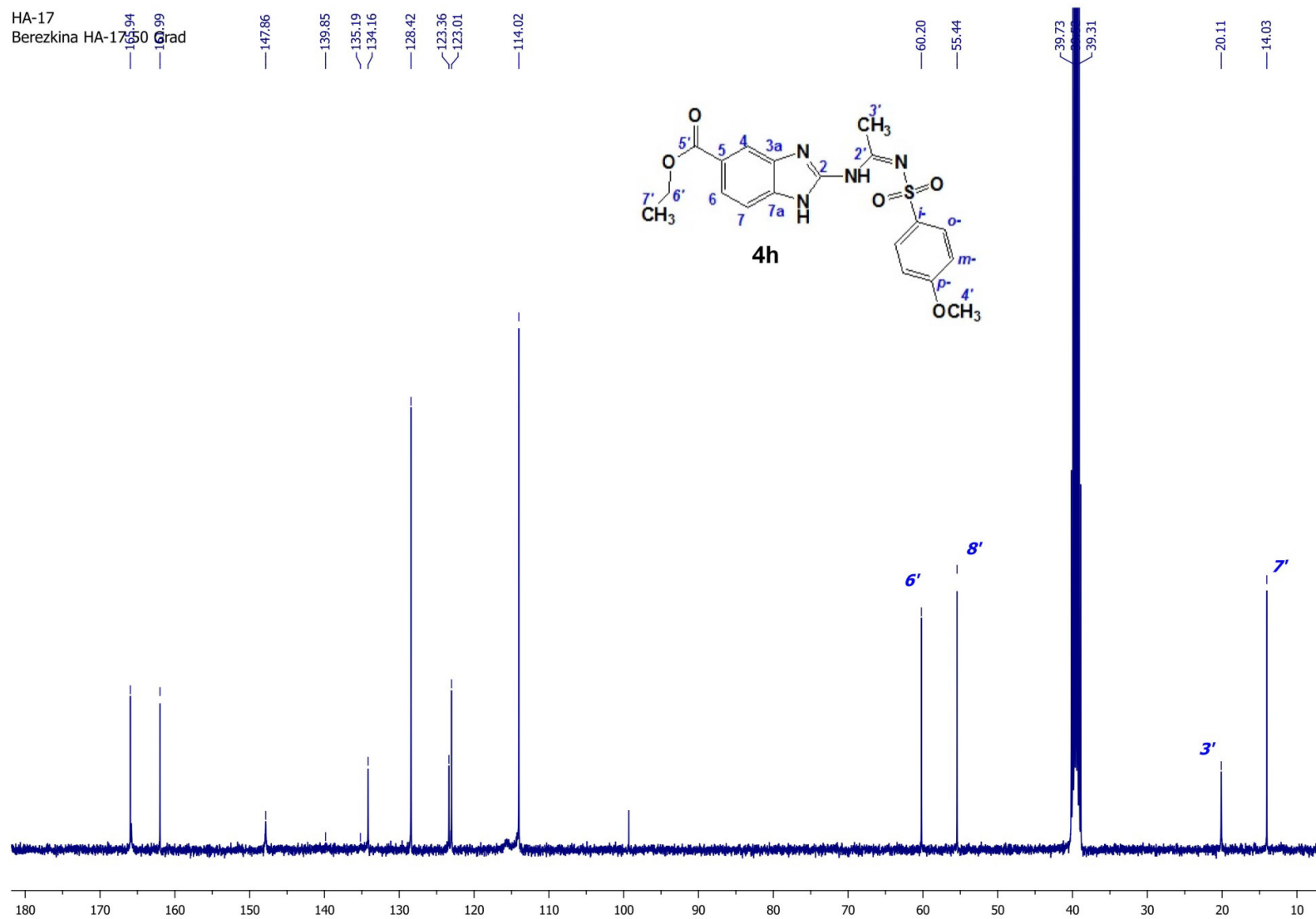
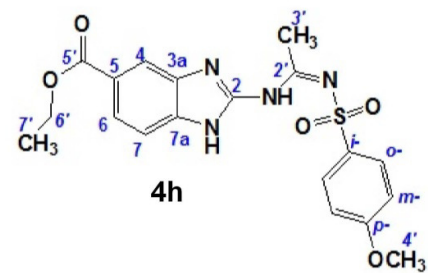
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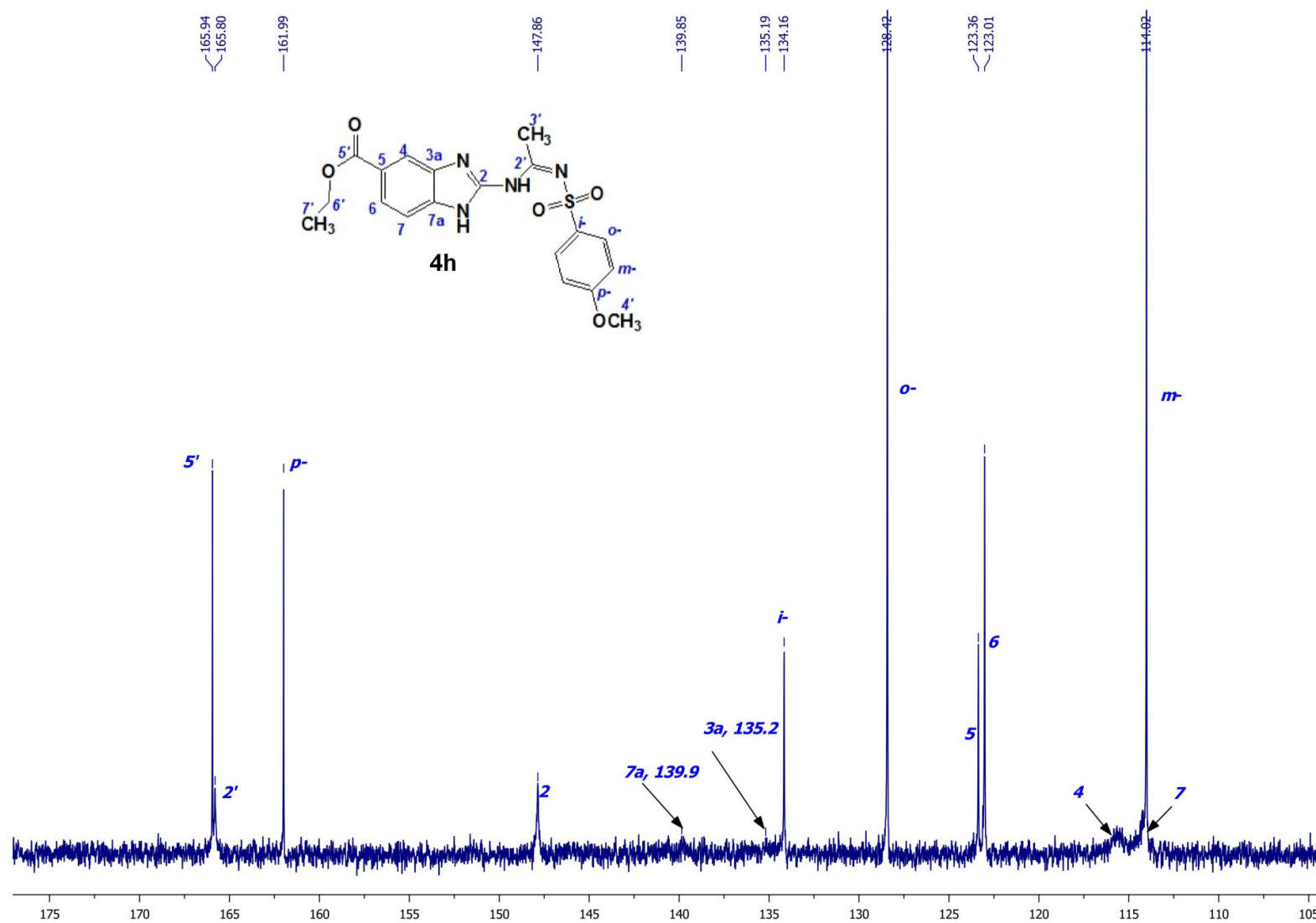
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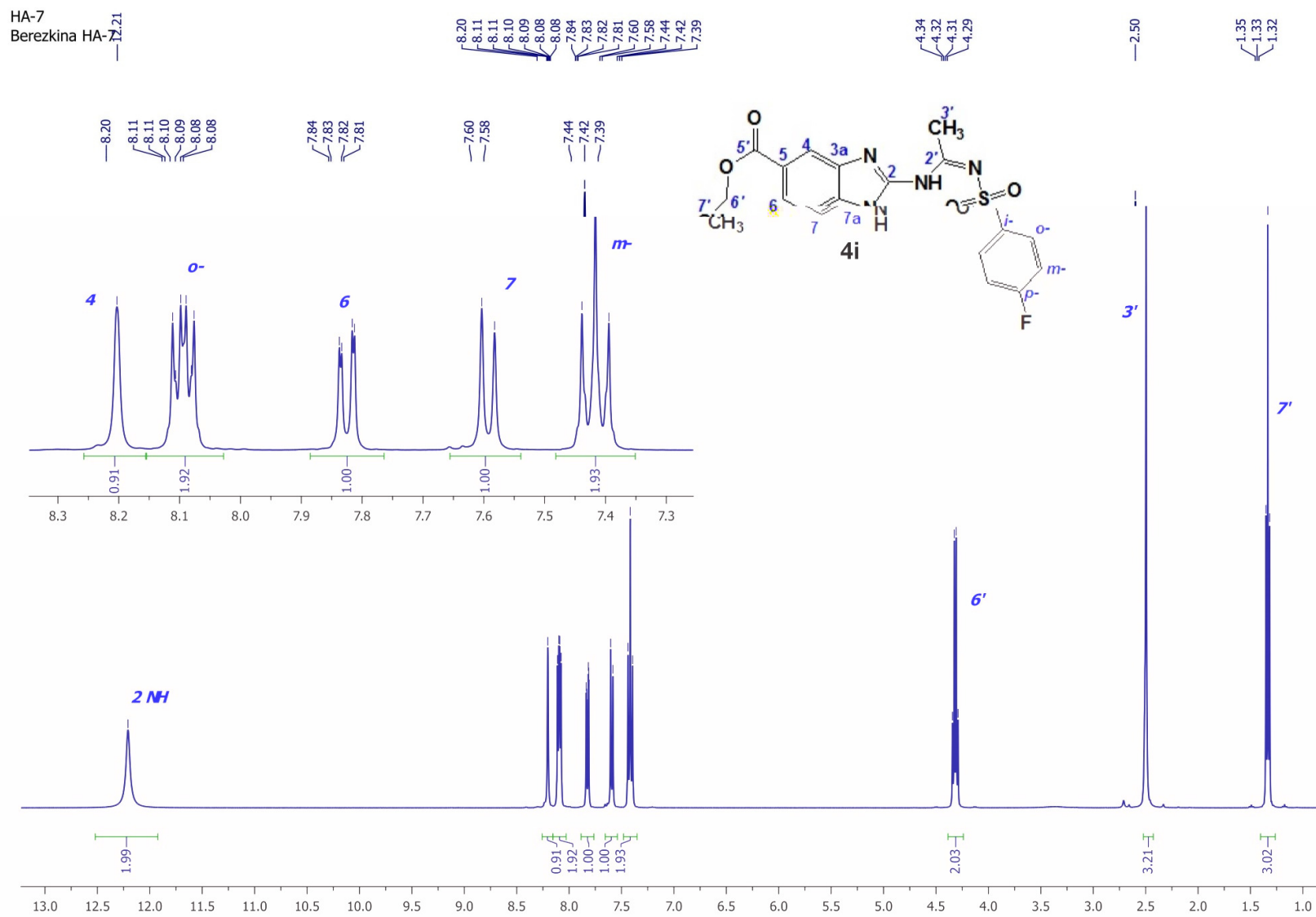
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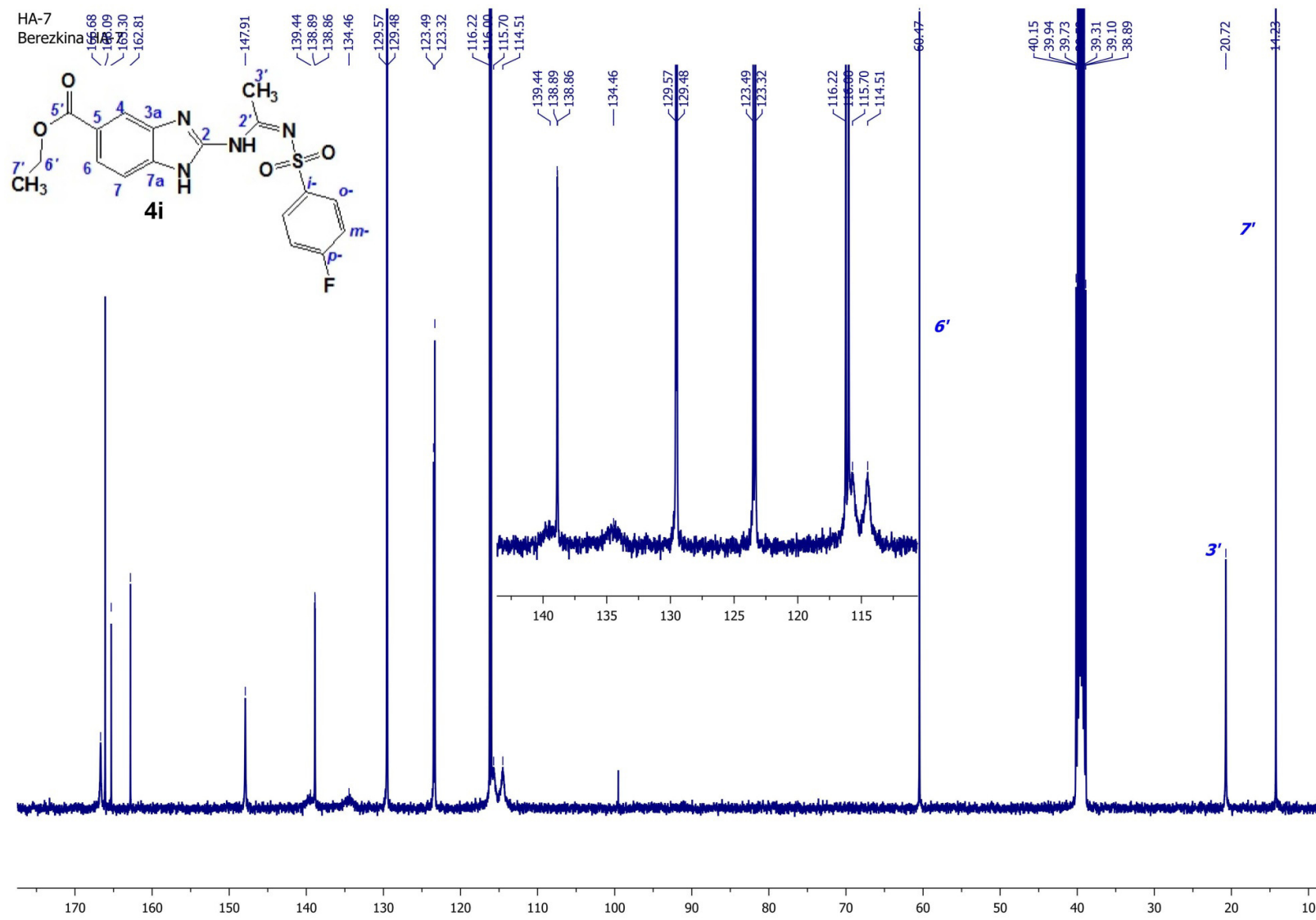
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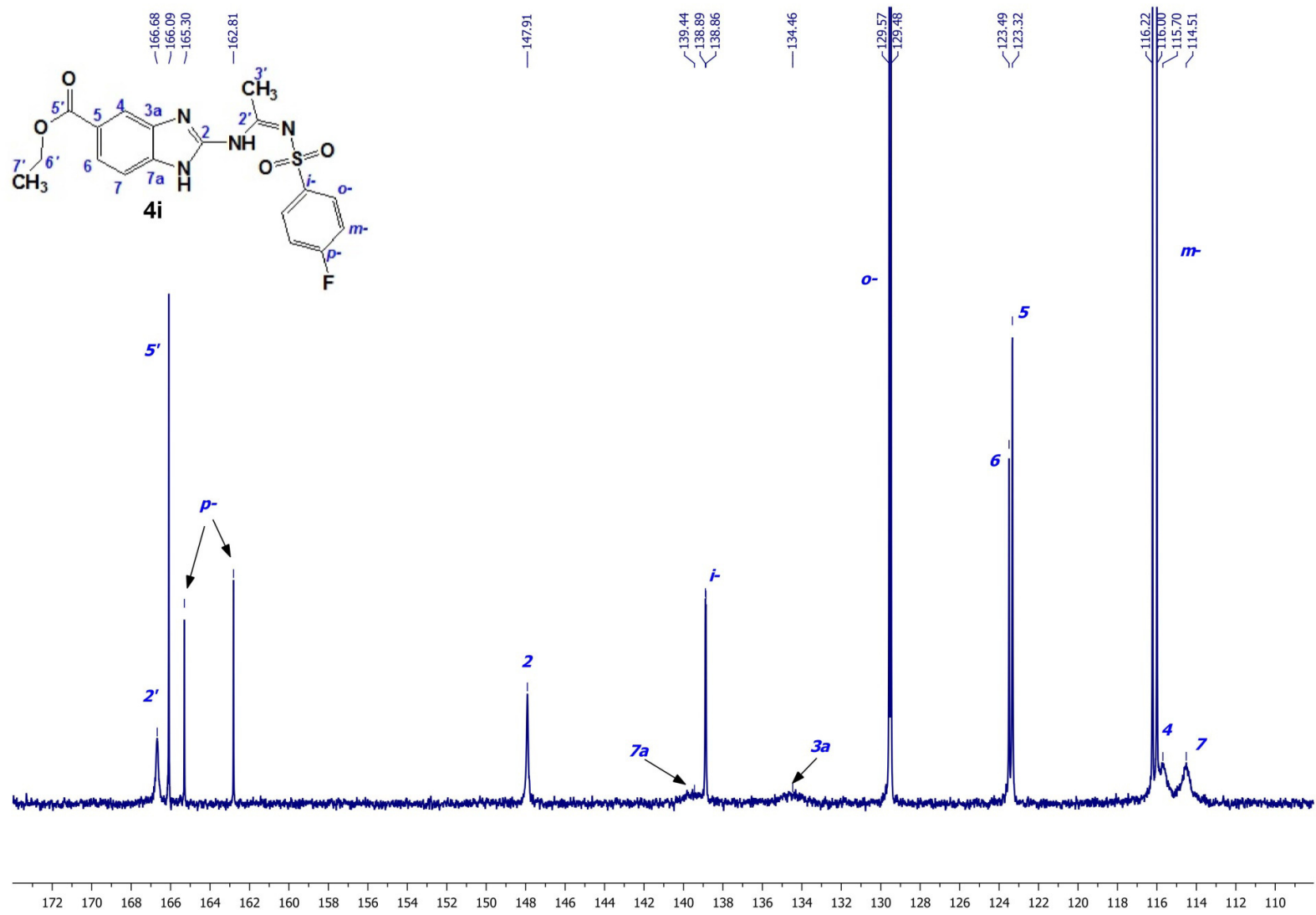


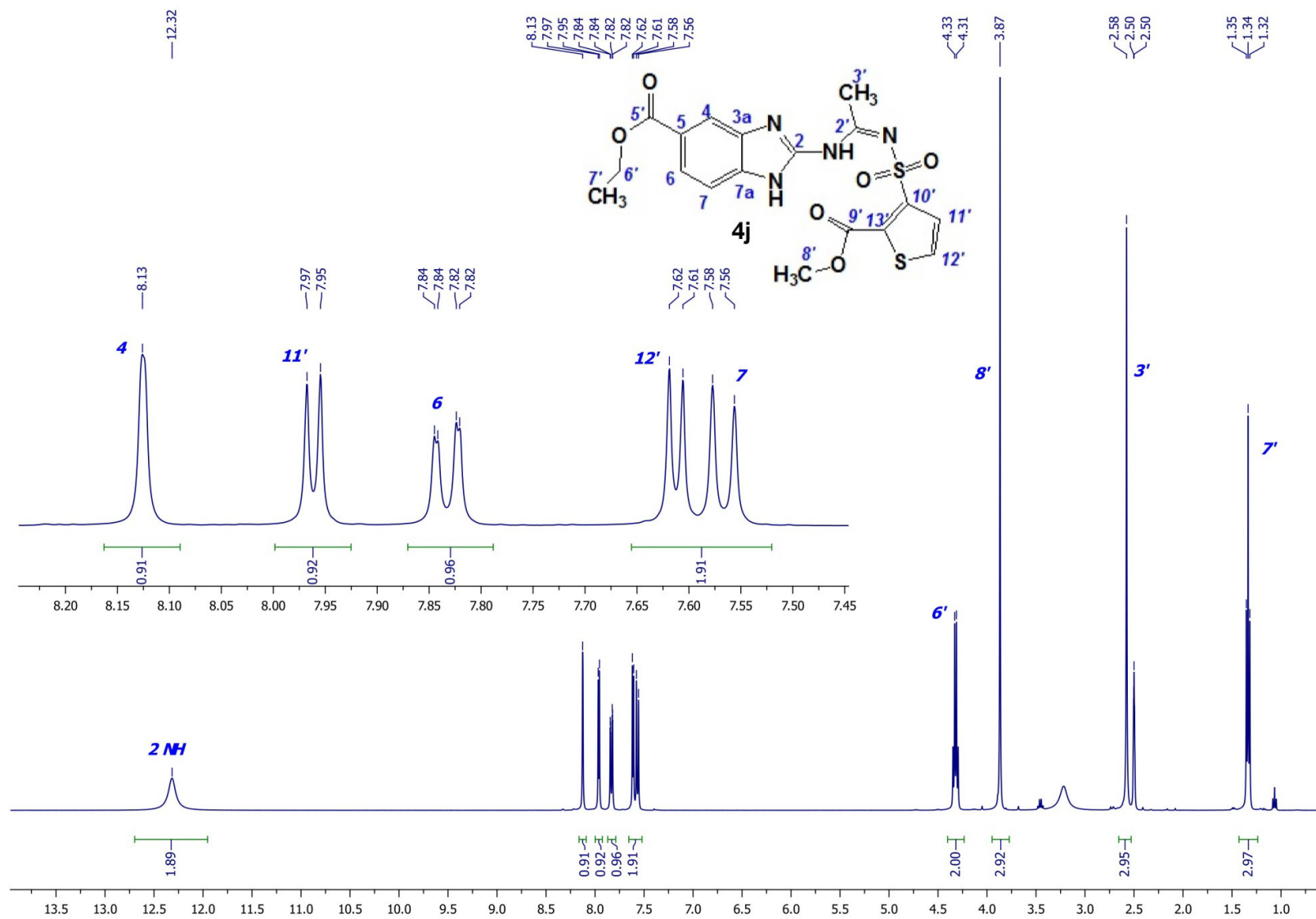


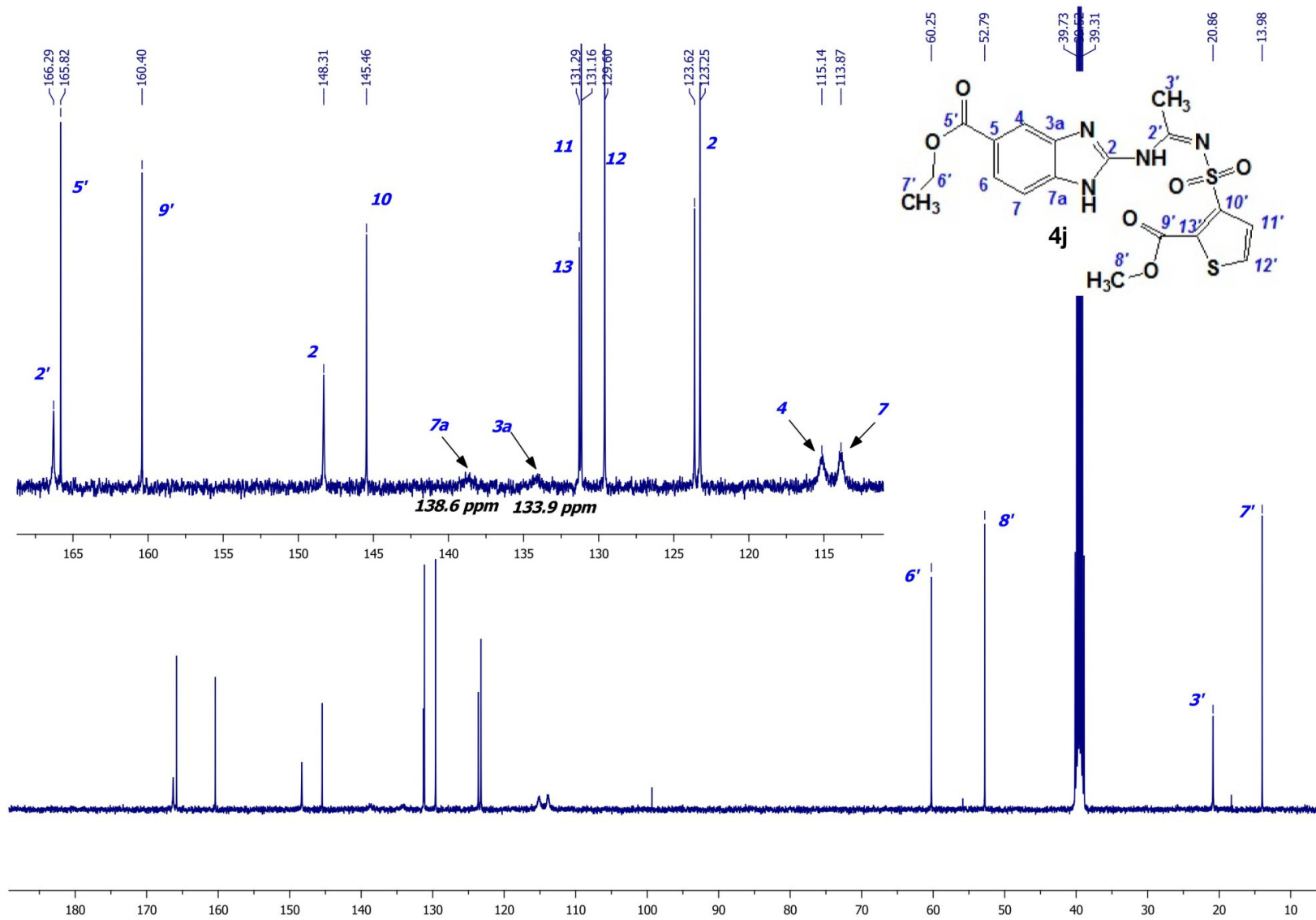


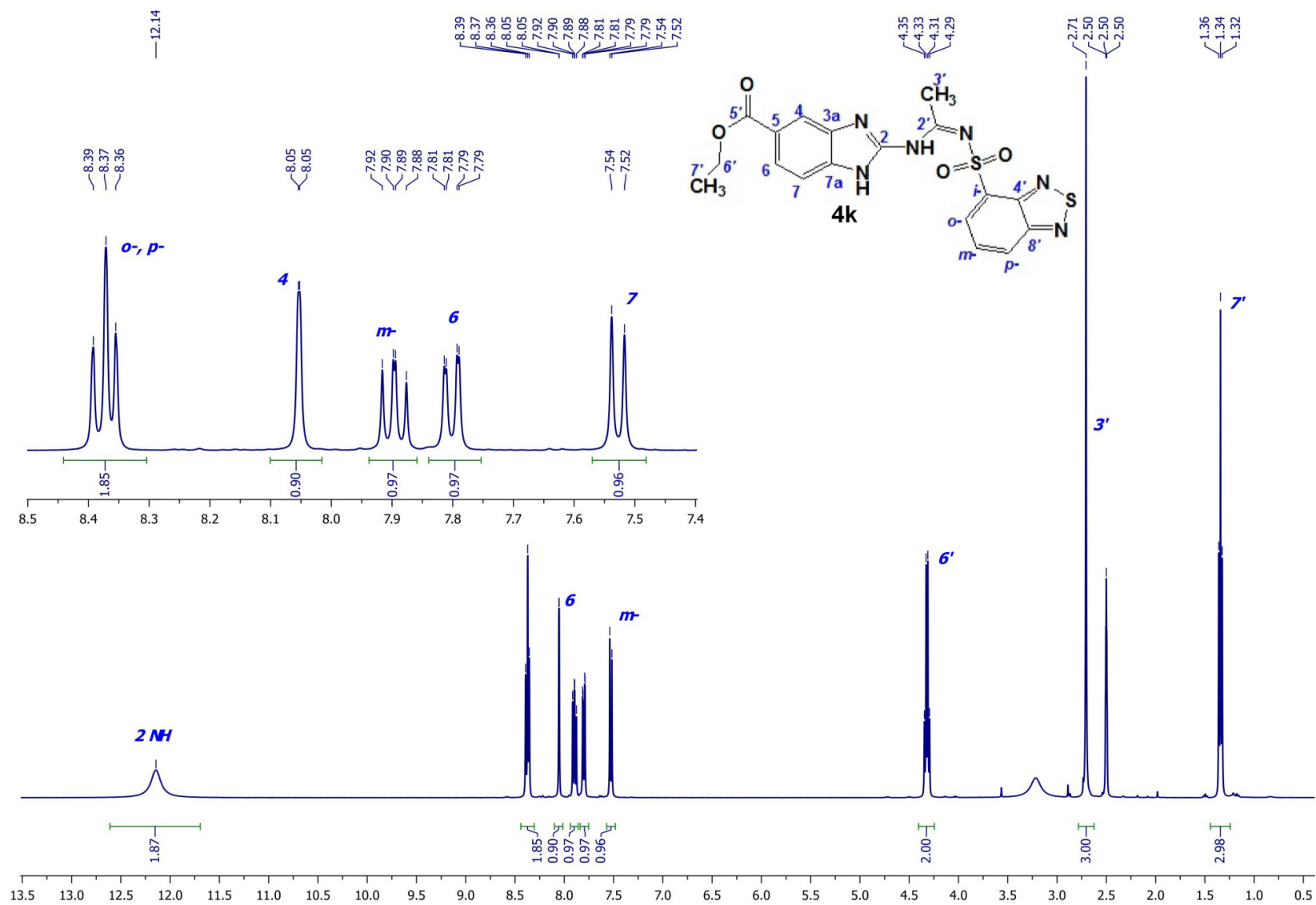


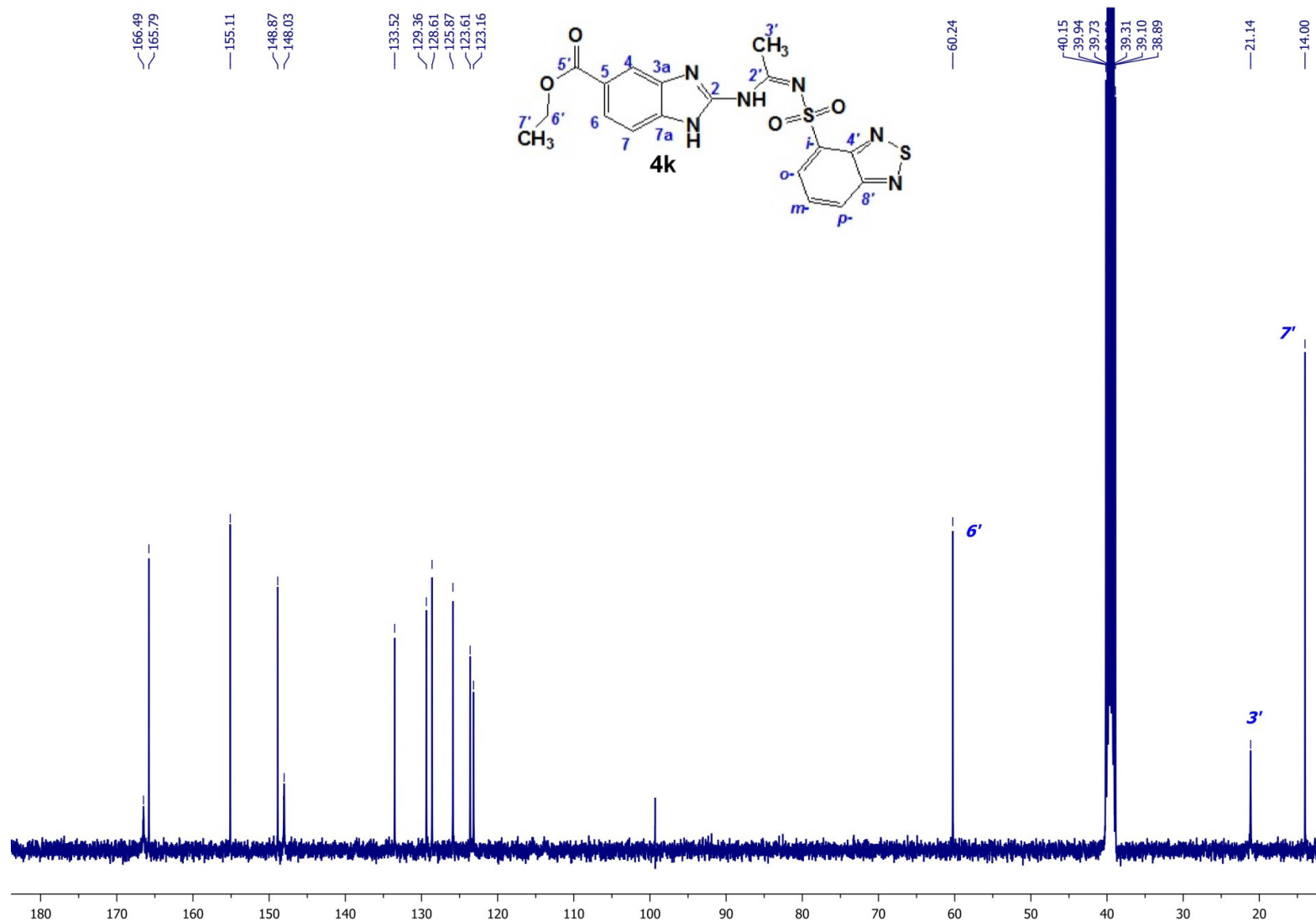




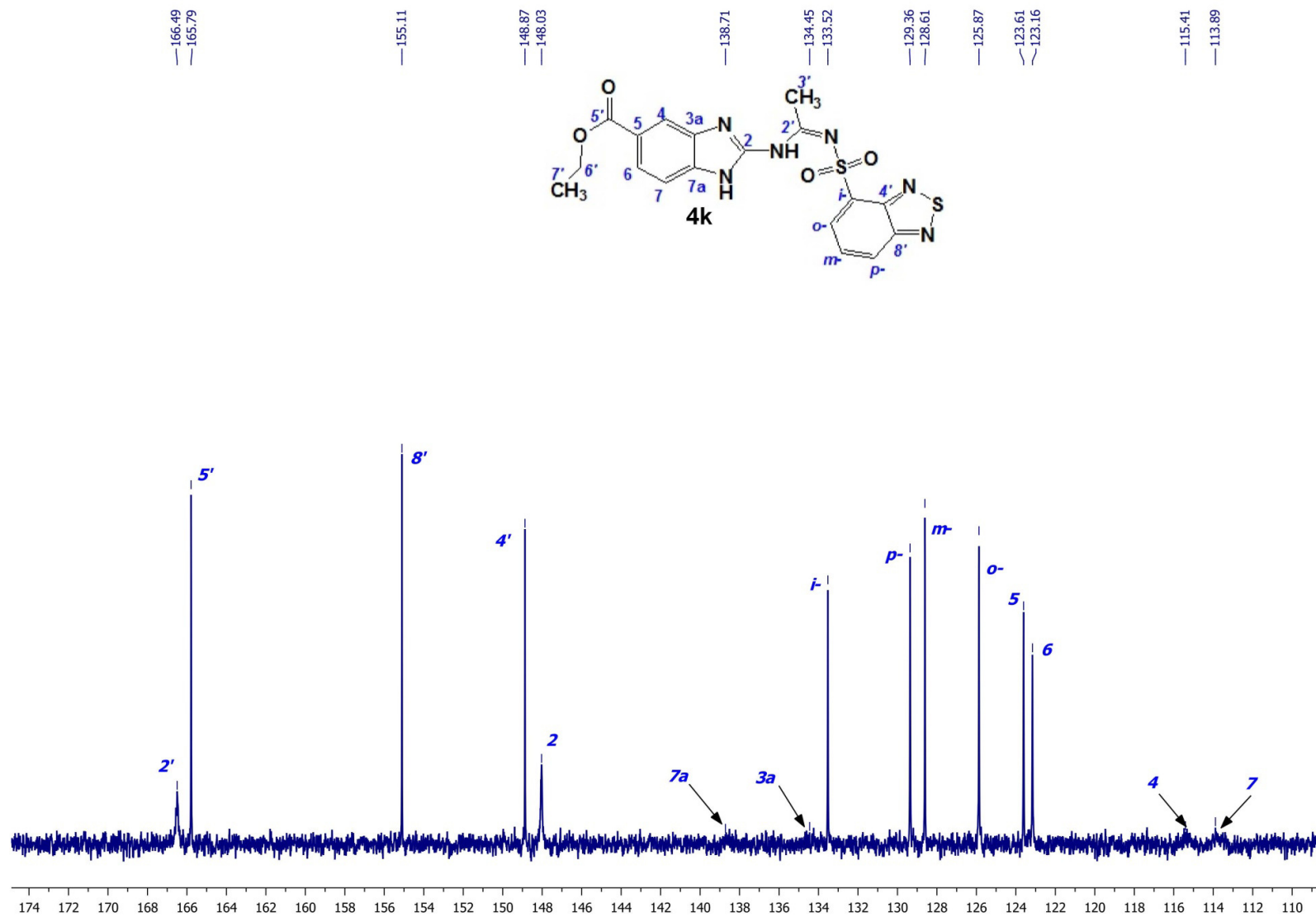


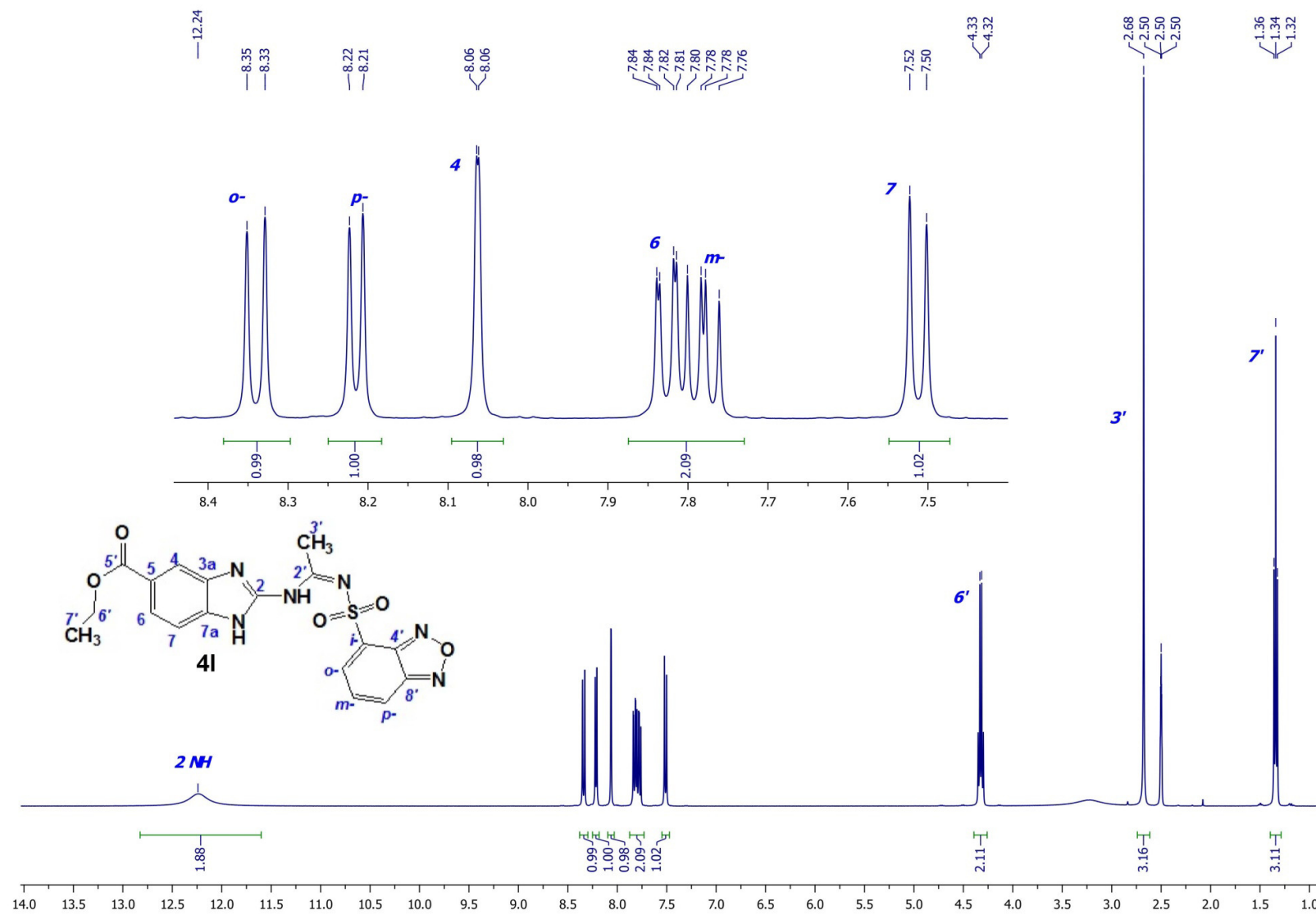


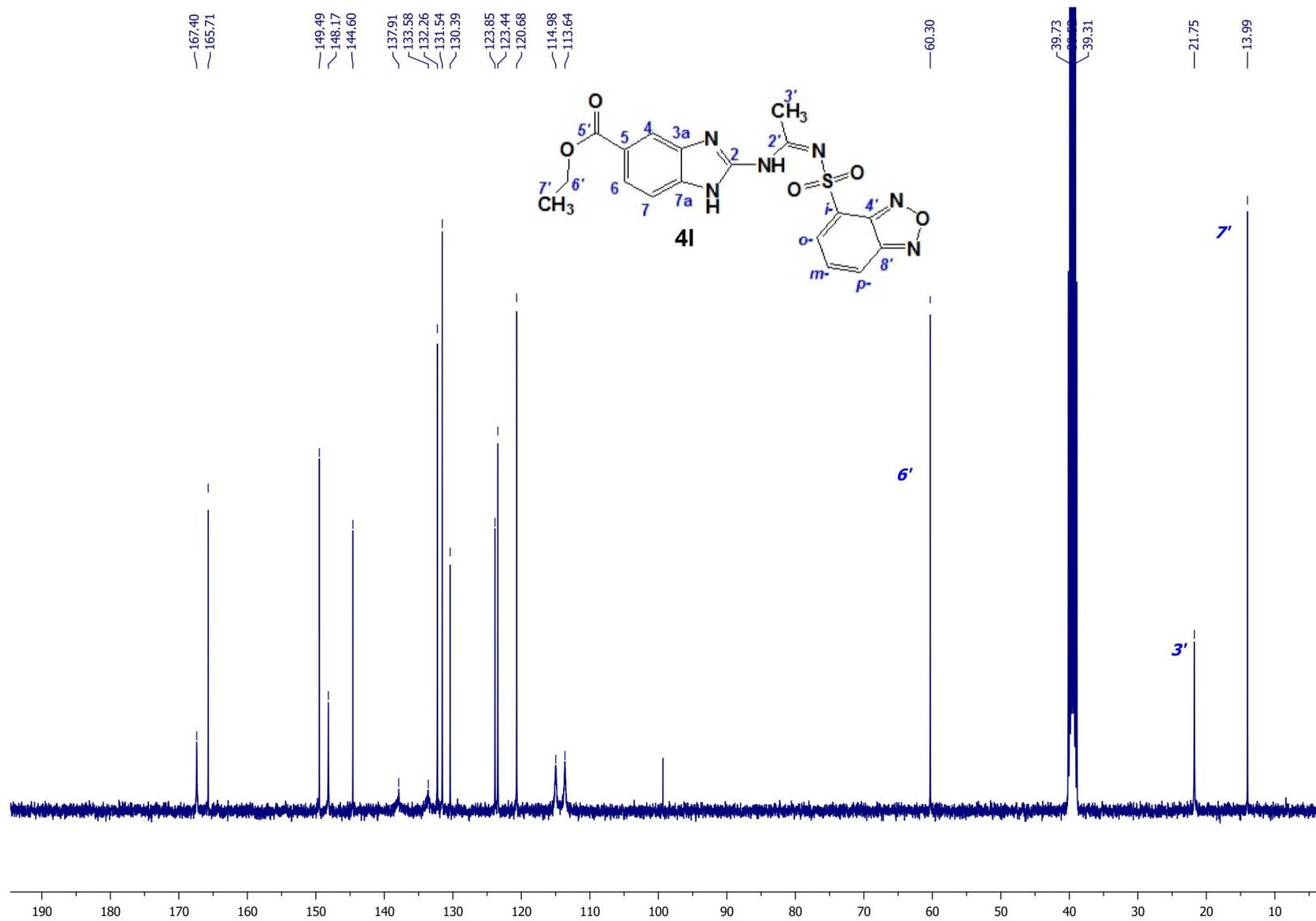


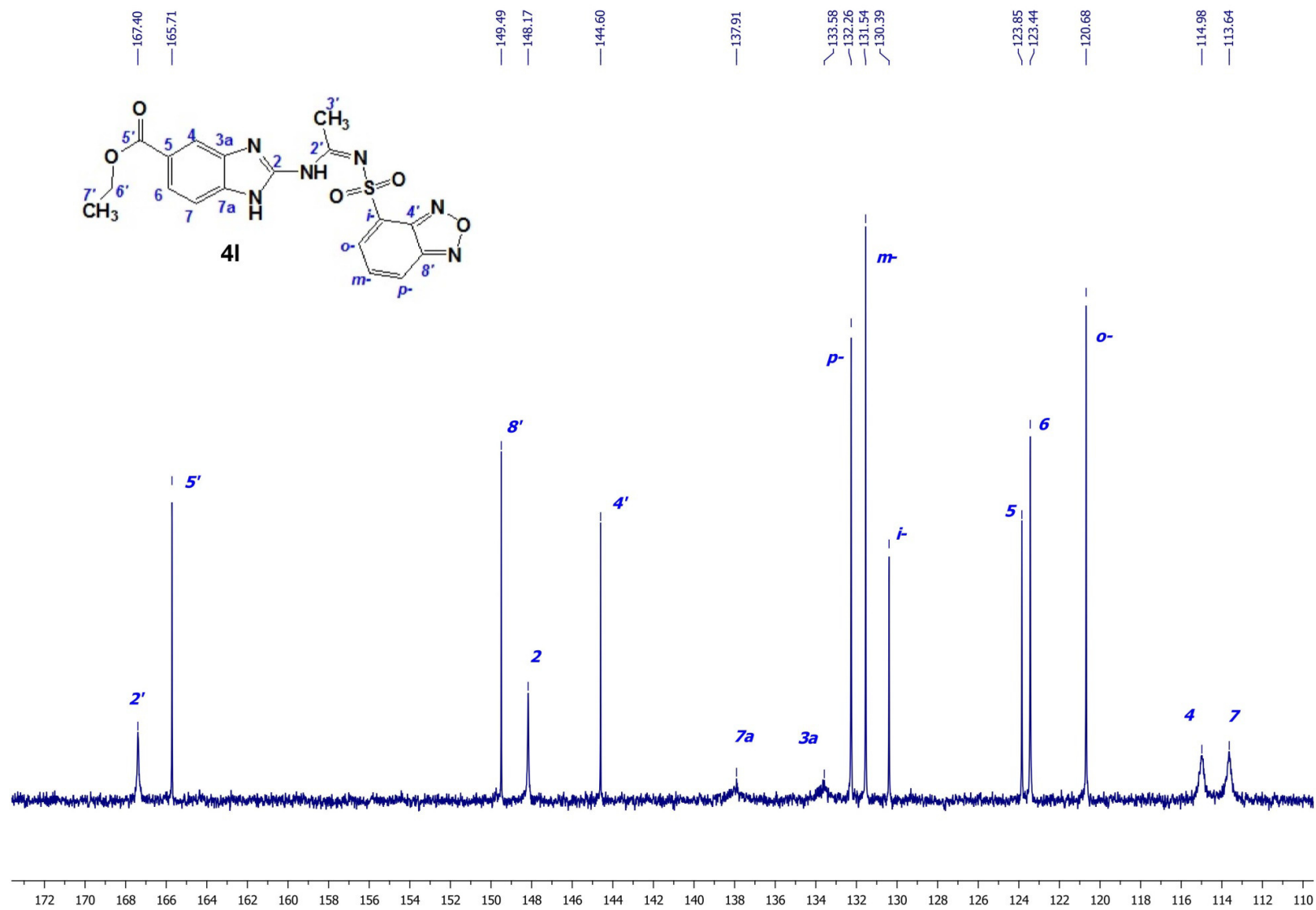


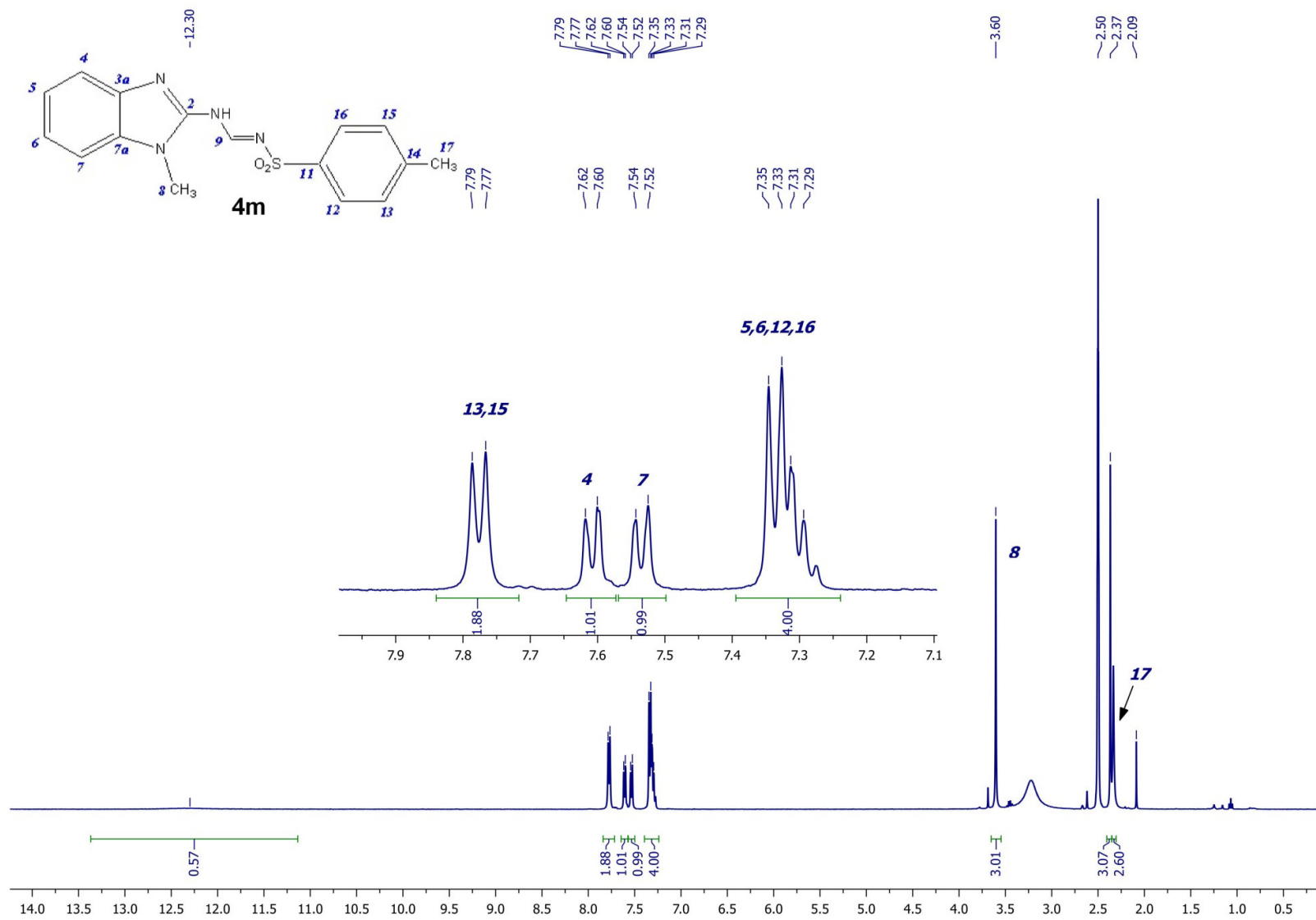


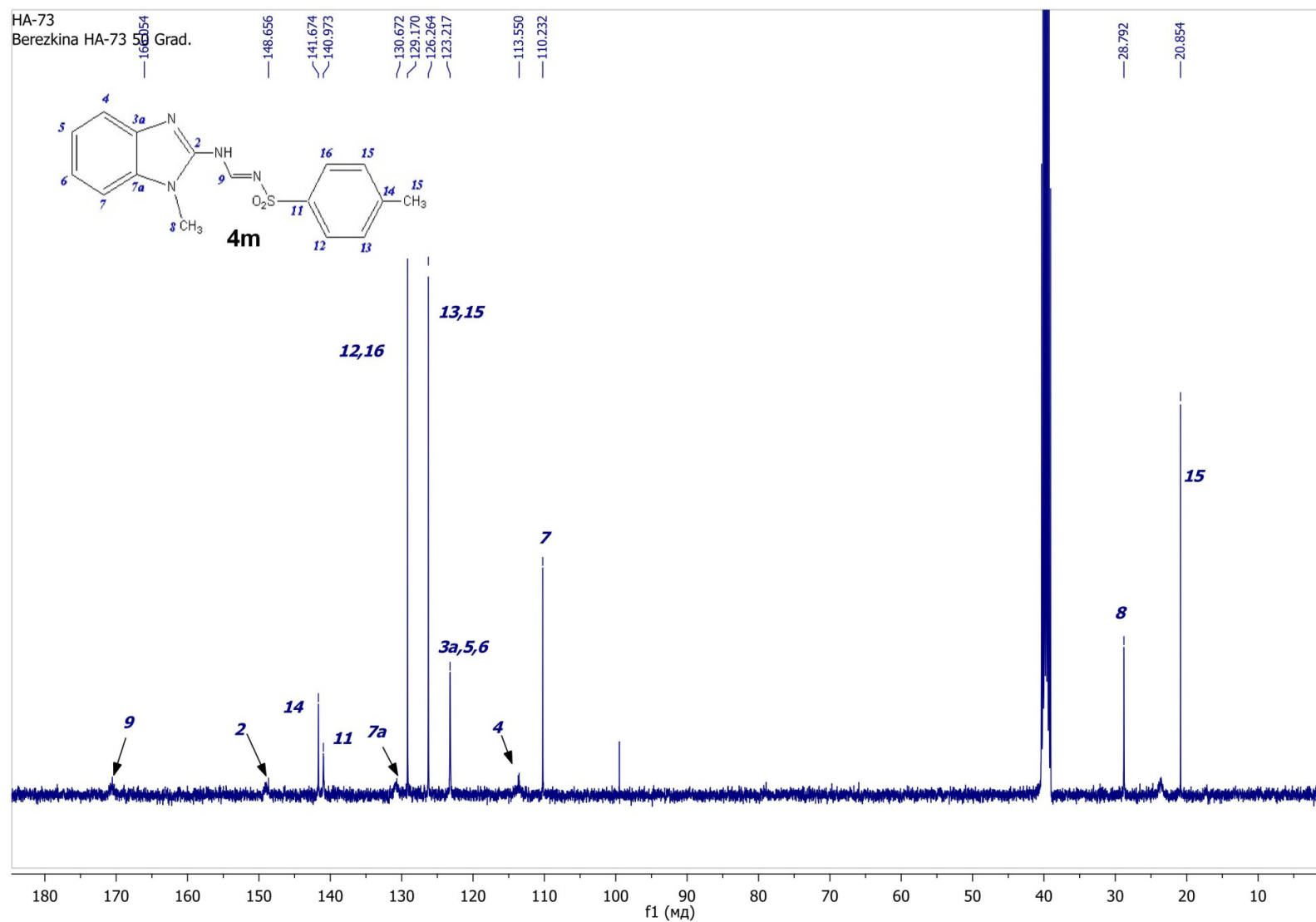


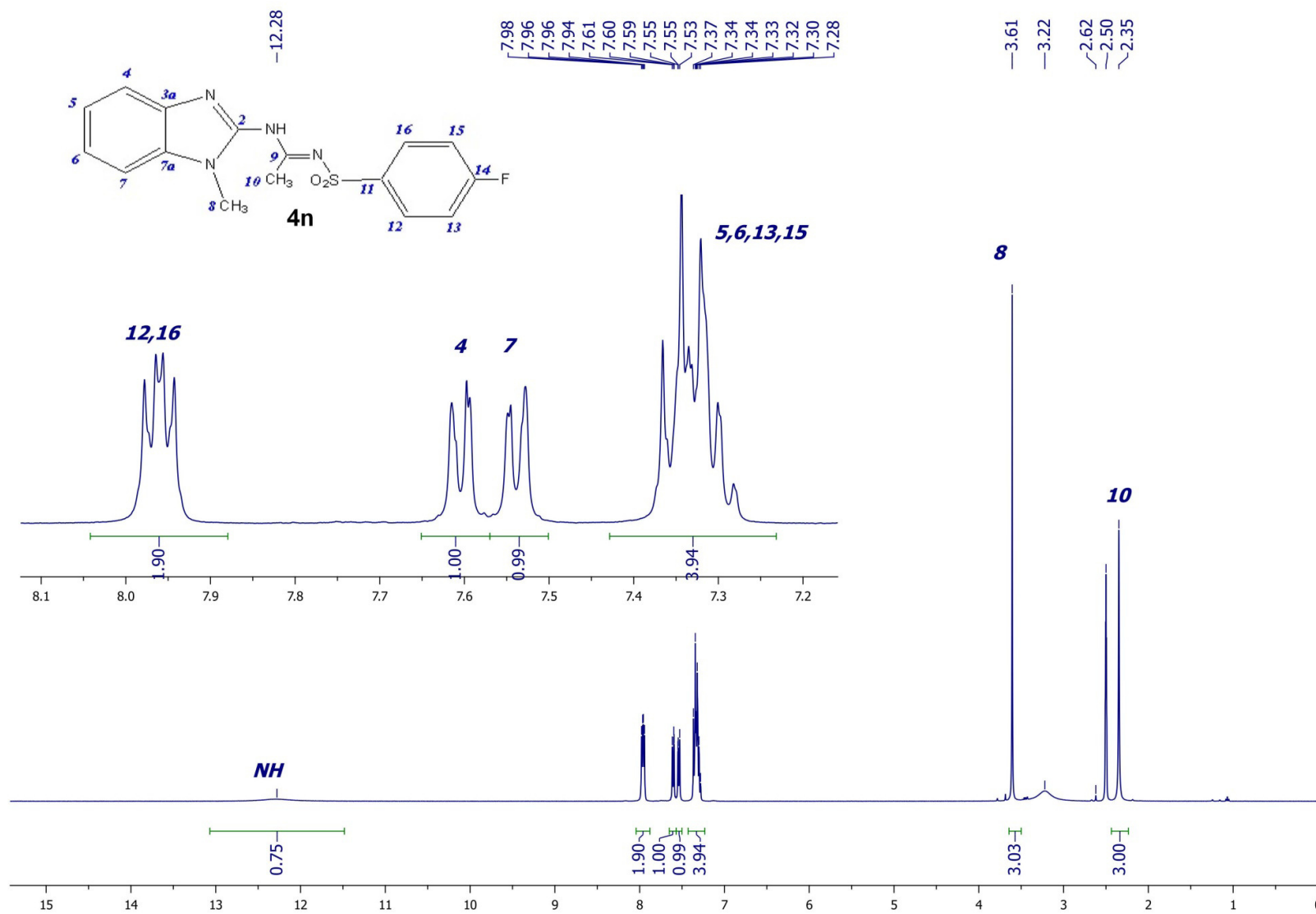


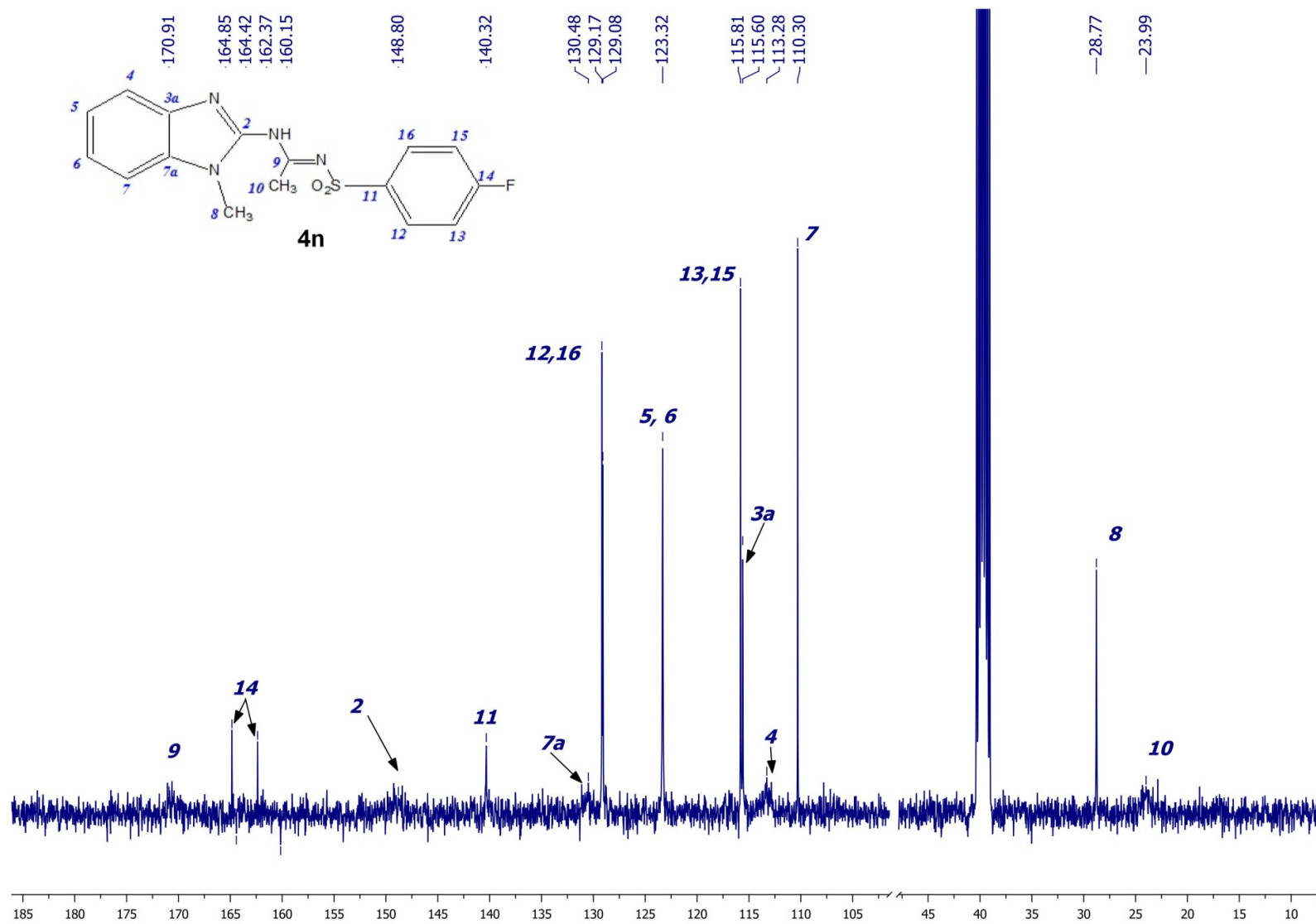




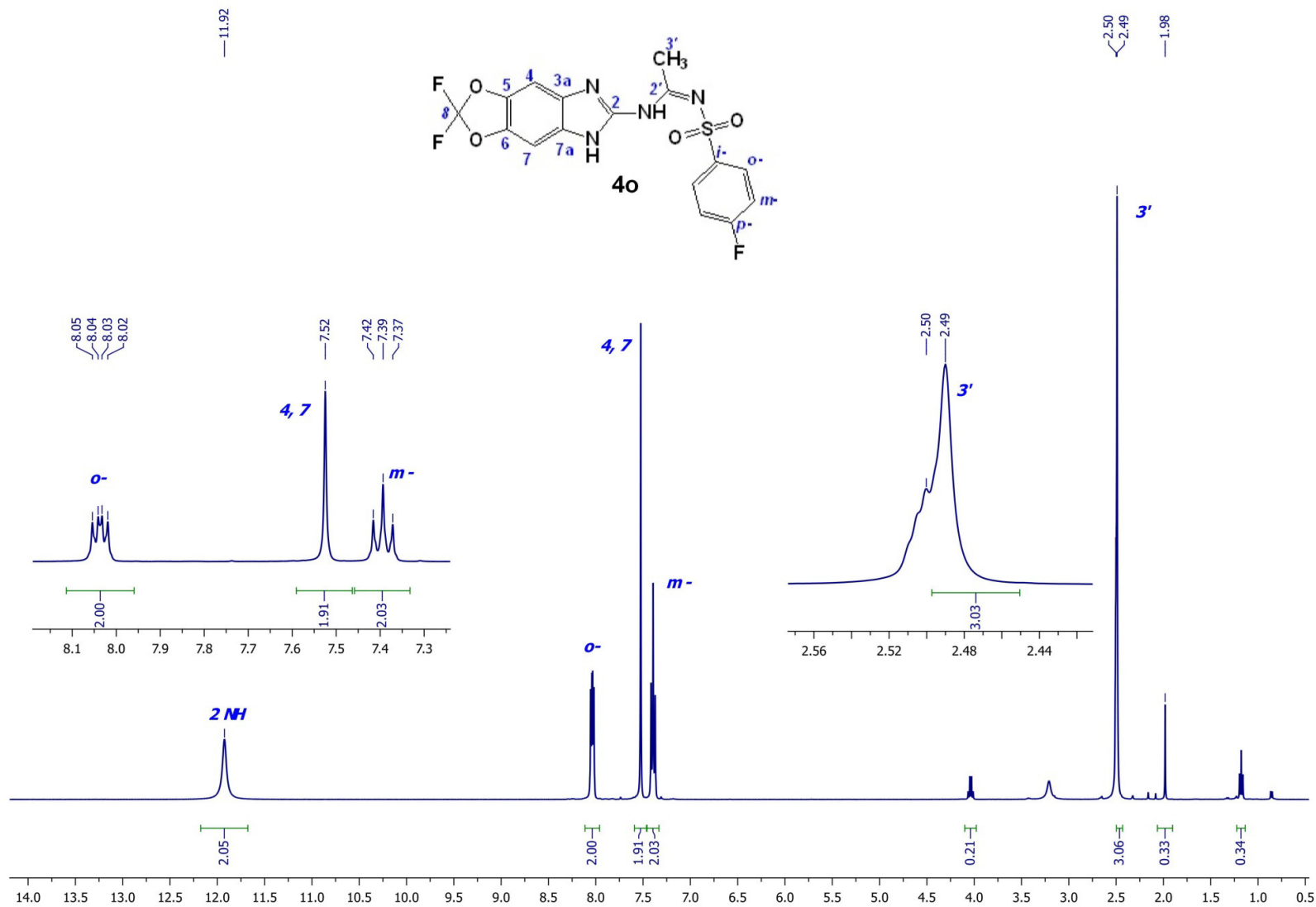


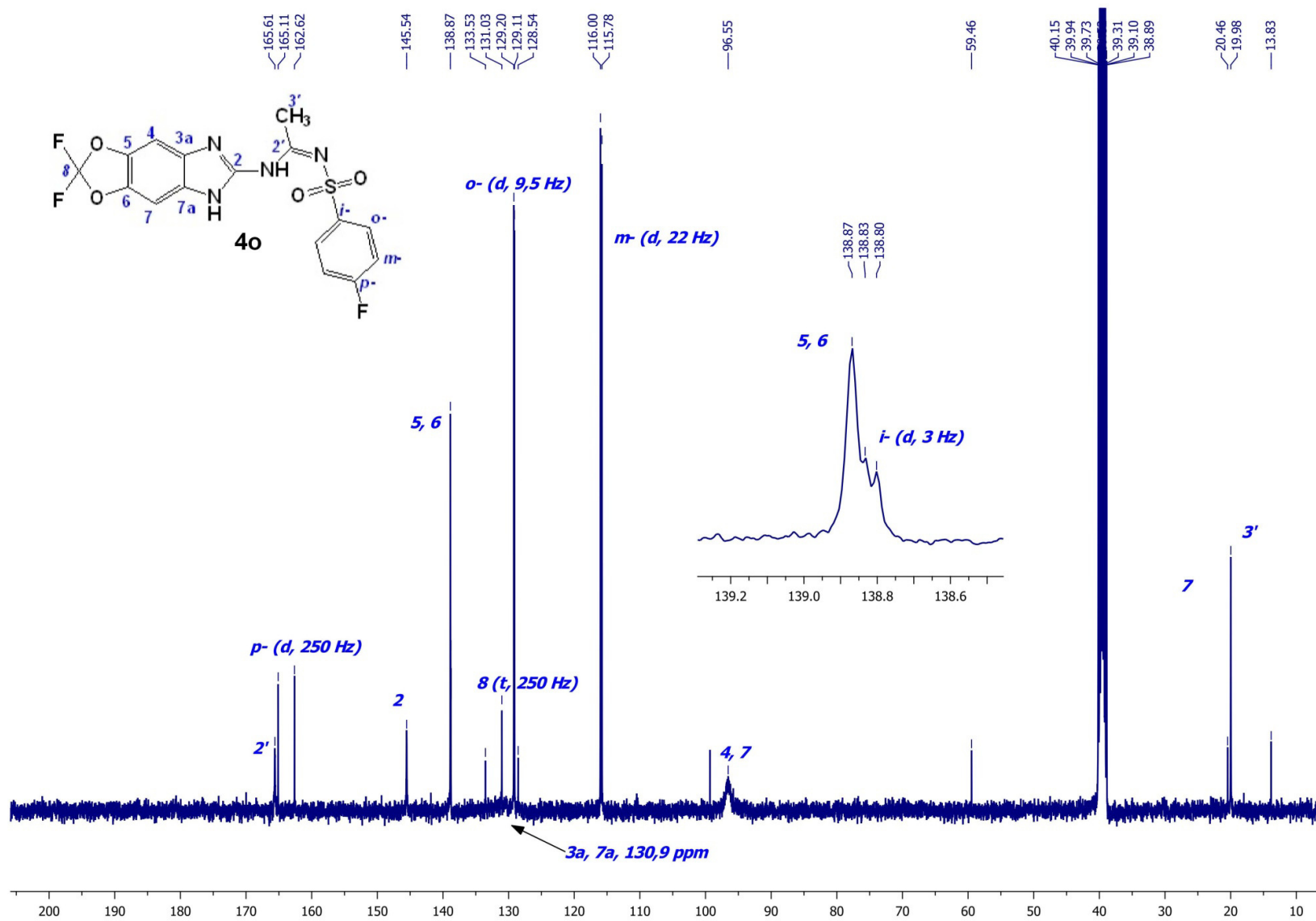


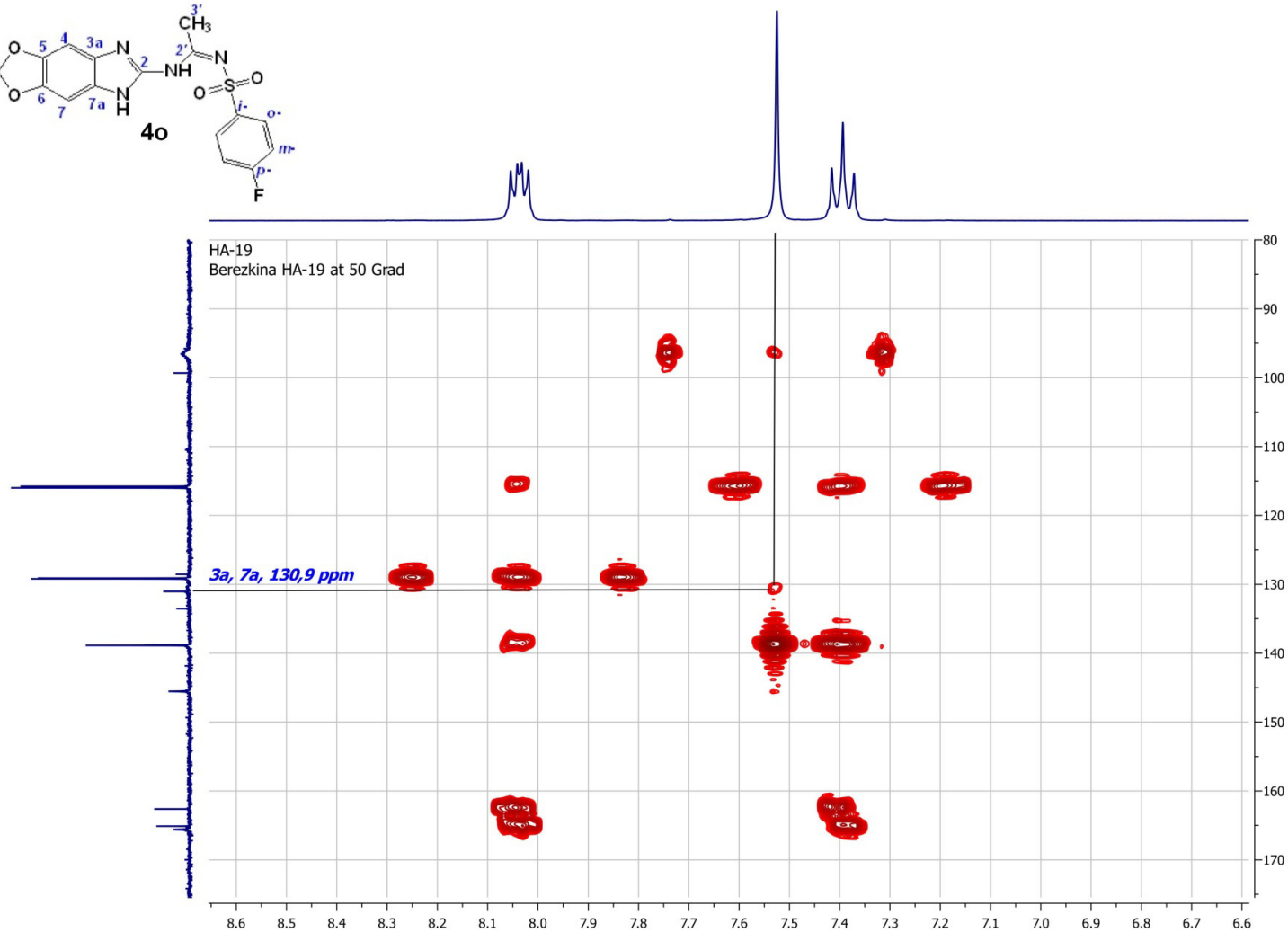
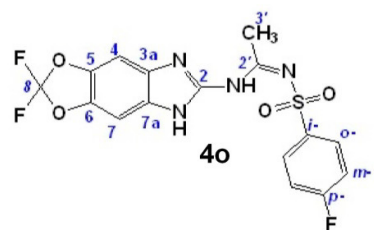




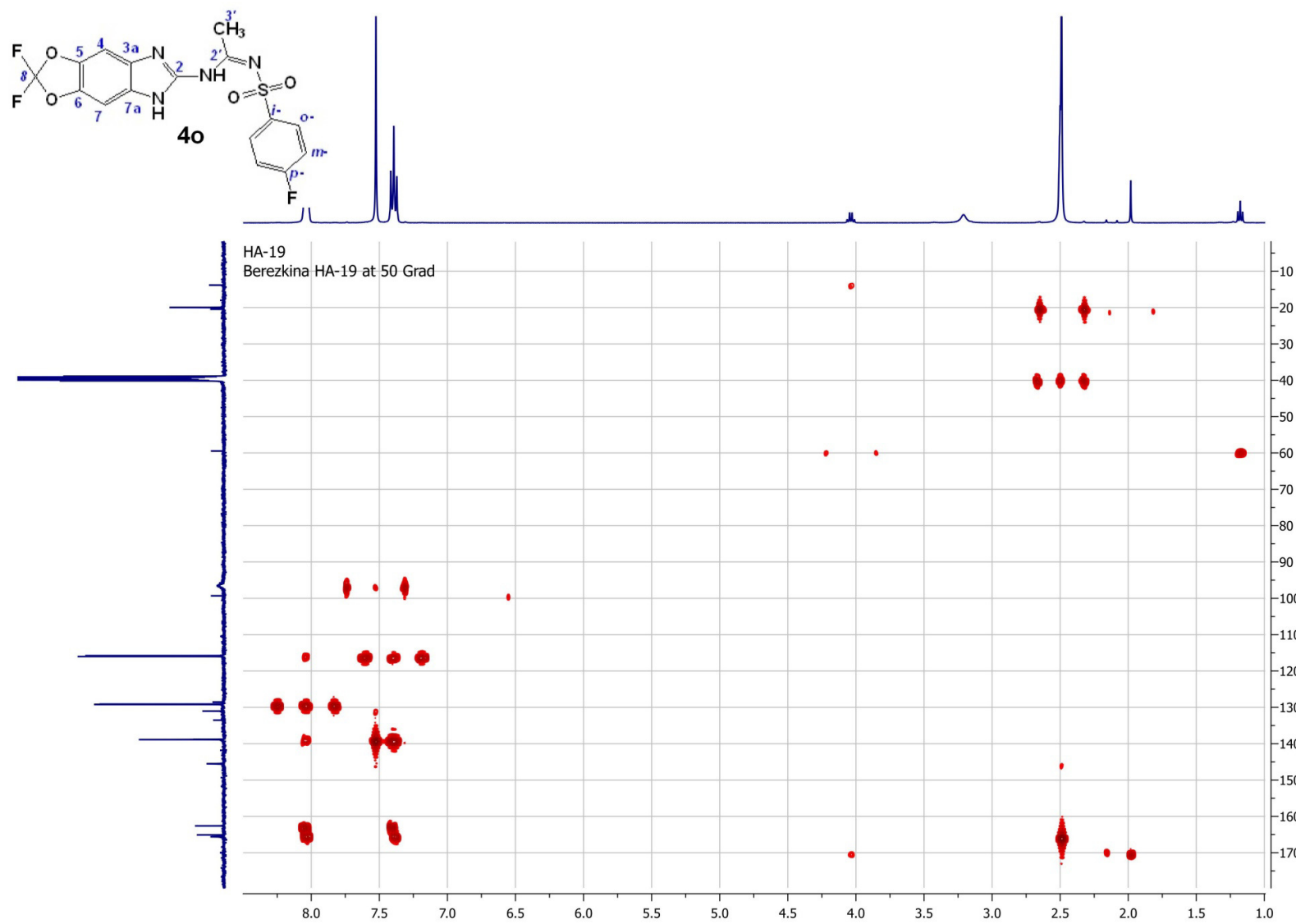


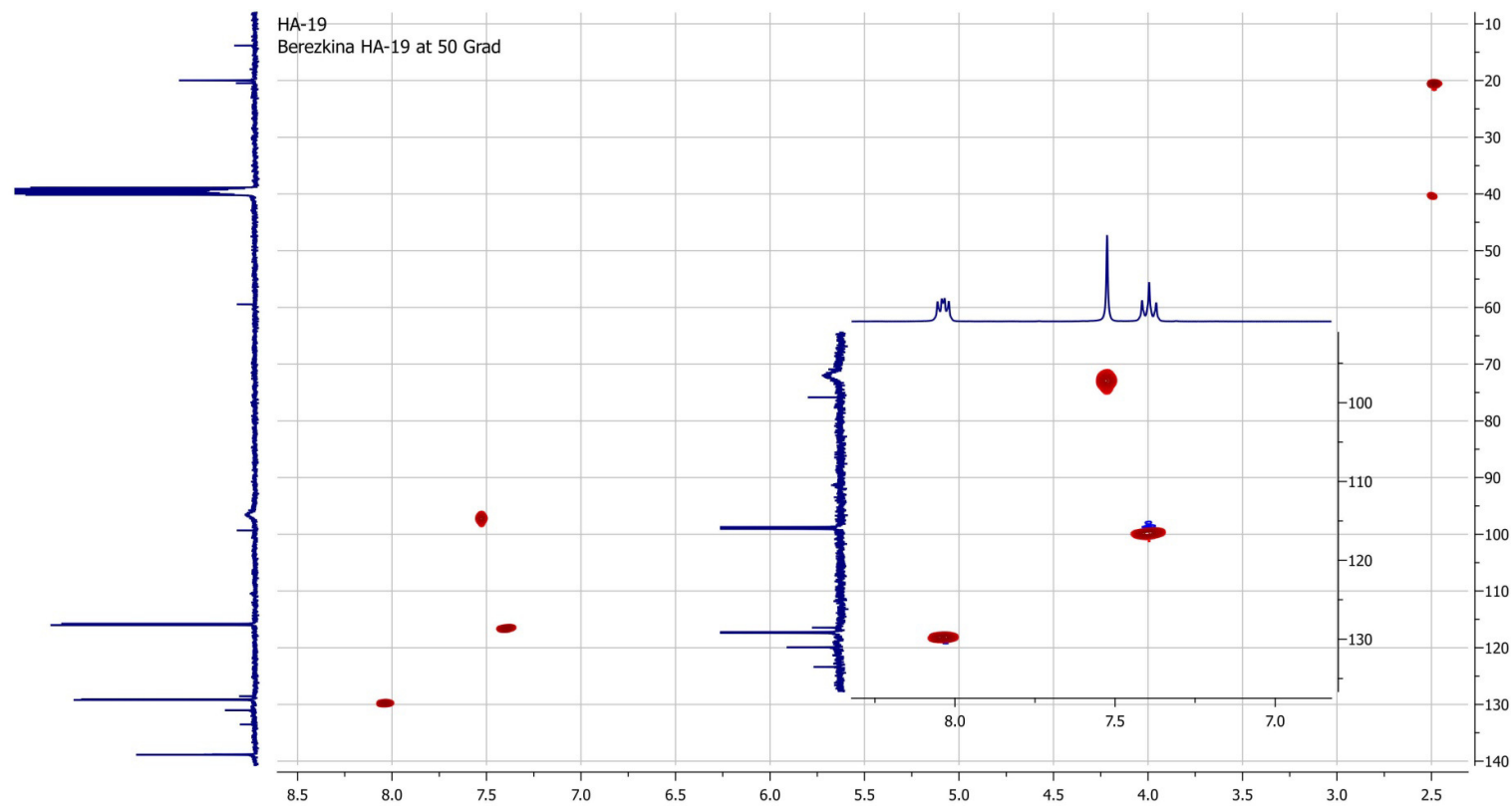






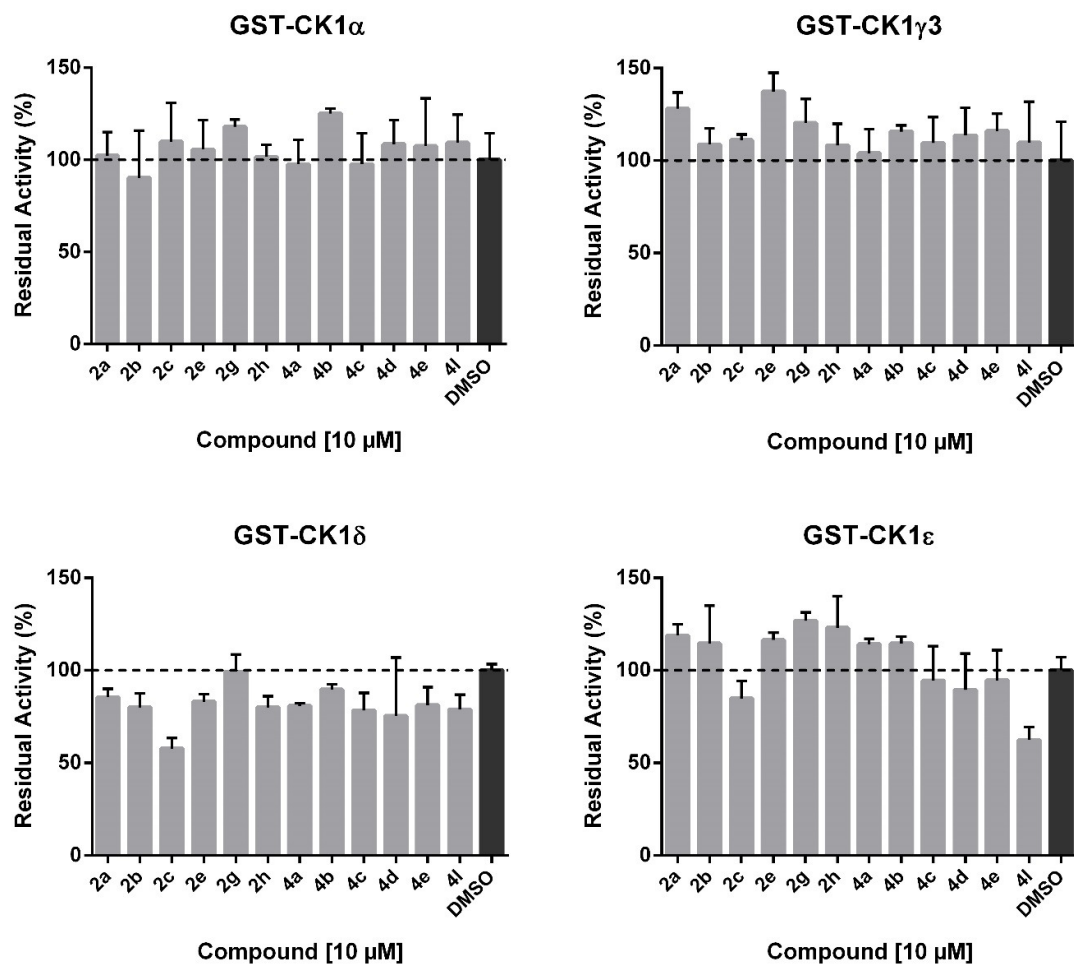






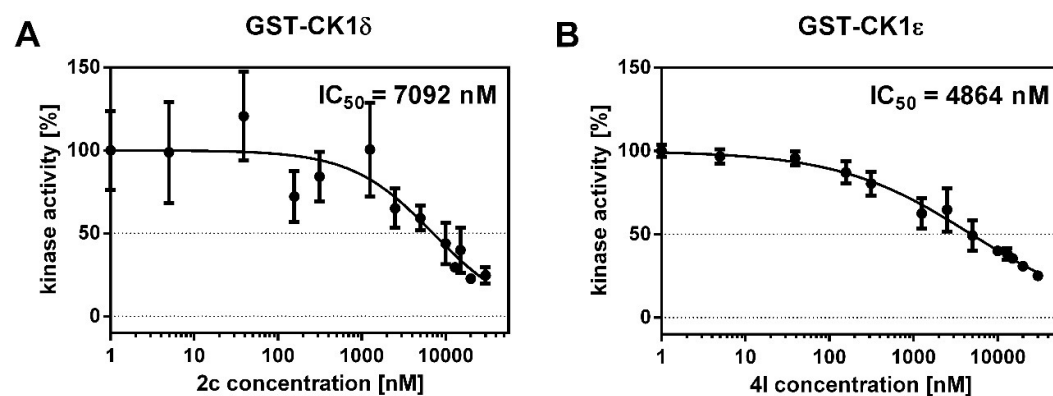
**Inhibitory effects of selected compounds on the kinase activity of CK1 isoforms**

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.

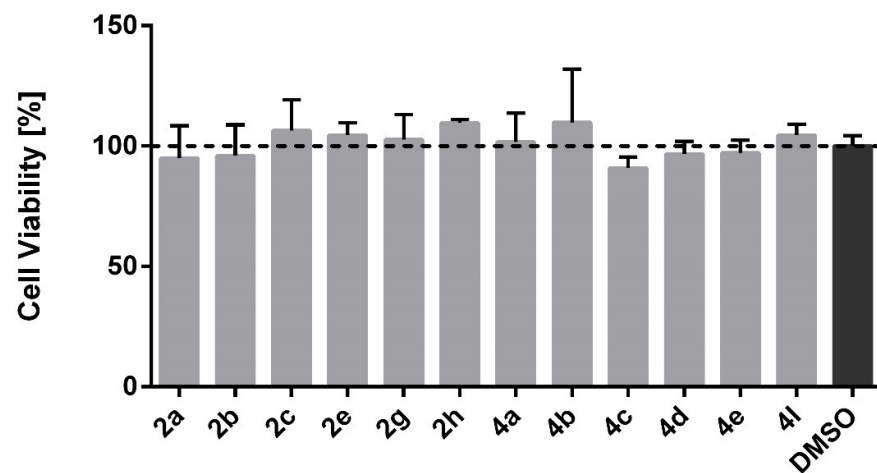




**Figure S12.** IC<sub>50</sub> curves of relevant compounds against CK1 $\delta$  and CK1 $\epsilon$ . Curve **A**: **2c**; curve **B**: **4l**.

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.