Synthesis of furo[3,2-c]coumarin derivatives using visible-light-promoted radical alkyne insertion with bromocoumarins

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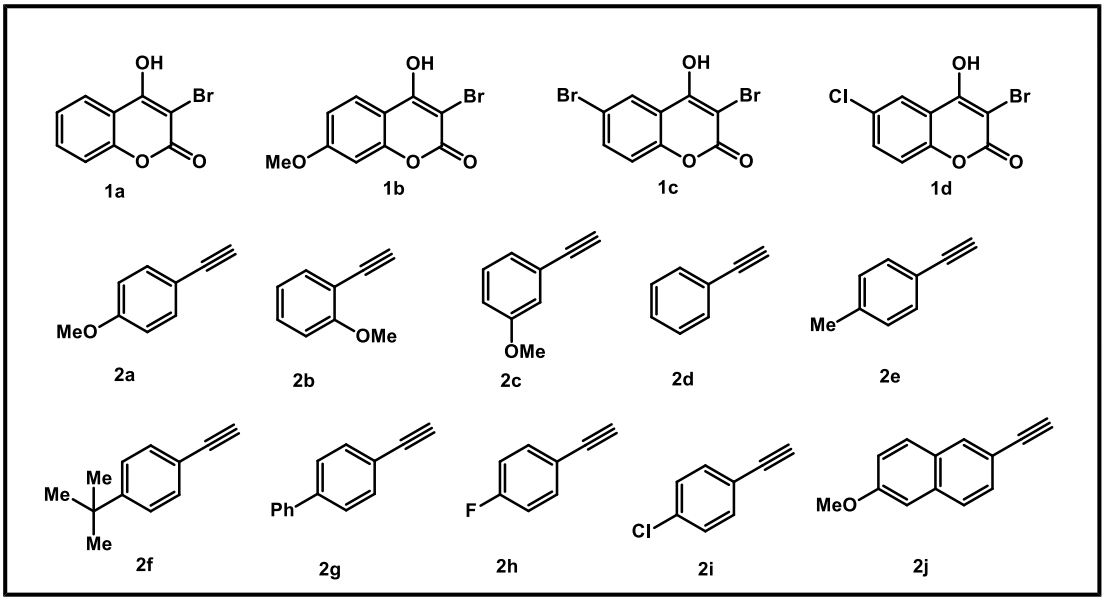
1. List of the substrates

3-Bromo-4-hydroxycoumarin could be generated by treatment of commercially available 4-hydroxycoumarin with NBS using $Mg(ClO_4)_2$ as a Lewis acid.[1] All alkynes were obtained from commercial suppliers and used without further purification.



2. Biological evaluation

(1) Cell Culture

Cells were maintained in cell culture dishes in Dulbecco's modified Eagle's medium (DMEM, WISENT), supplemented with 10% Fetal bovine serum (FBS, Gibco) 100 U/mL penicillin (Sangon Biotech, Shanghai, China), and 100 μg/mL streptomycin (Sangon Biotech, Shanghai, China). The cells were grown in a carbon dioxide incubator (37 °C, 5% CO_2).

(2) Cytotoxicity

The cytotoxicity was tested on the three cell lines (human lung carcinoma A549, human promyelocytic leukemia HL-60 and human colon carcinoma SW480) by MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide] (Sigma-Aldrich) assay as described.[2] Briefly, the test cell at the exponential growth phase were collected and transferred into 96-well plates. After incubated for 24 h, compound dilutions were dispensed to the established culture plates, and doxorubicin

(Sigma-Aldrich) used as a positive control. Two days (48 h) later, the MTT solution (0.1 mg per well) was then added to each well. After further incubation for 4 h, the supernatant was removed, the crystals were fully dissolved in DMSO (150 mL), and the absorbance of each well was read at 570 nm (Sunrise, Tecan). The IC_{50} value was determined as the concentration, at which a half of the test cell growth was inhibited. The experiment was performed in triplicate, and the data expressed as means ±SD.[3]

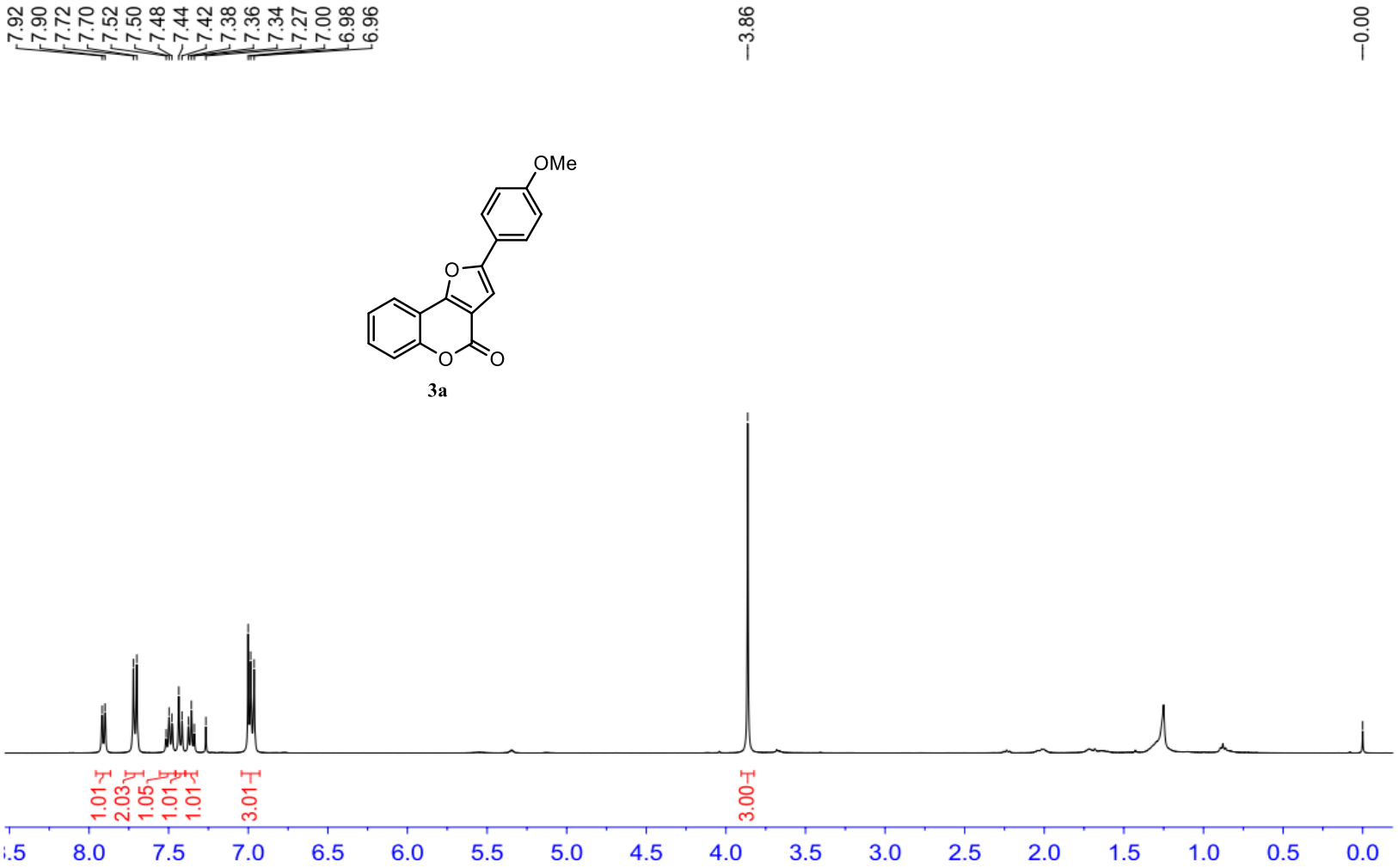
(3) Acetylcholinesterase inhibitory activity assay

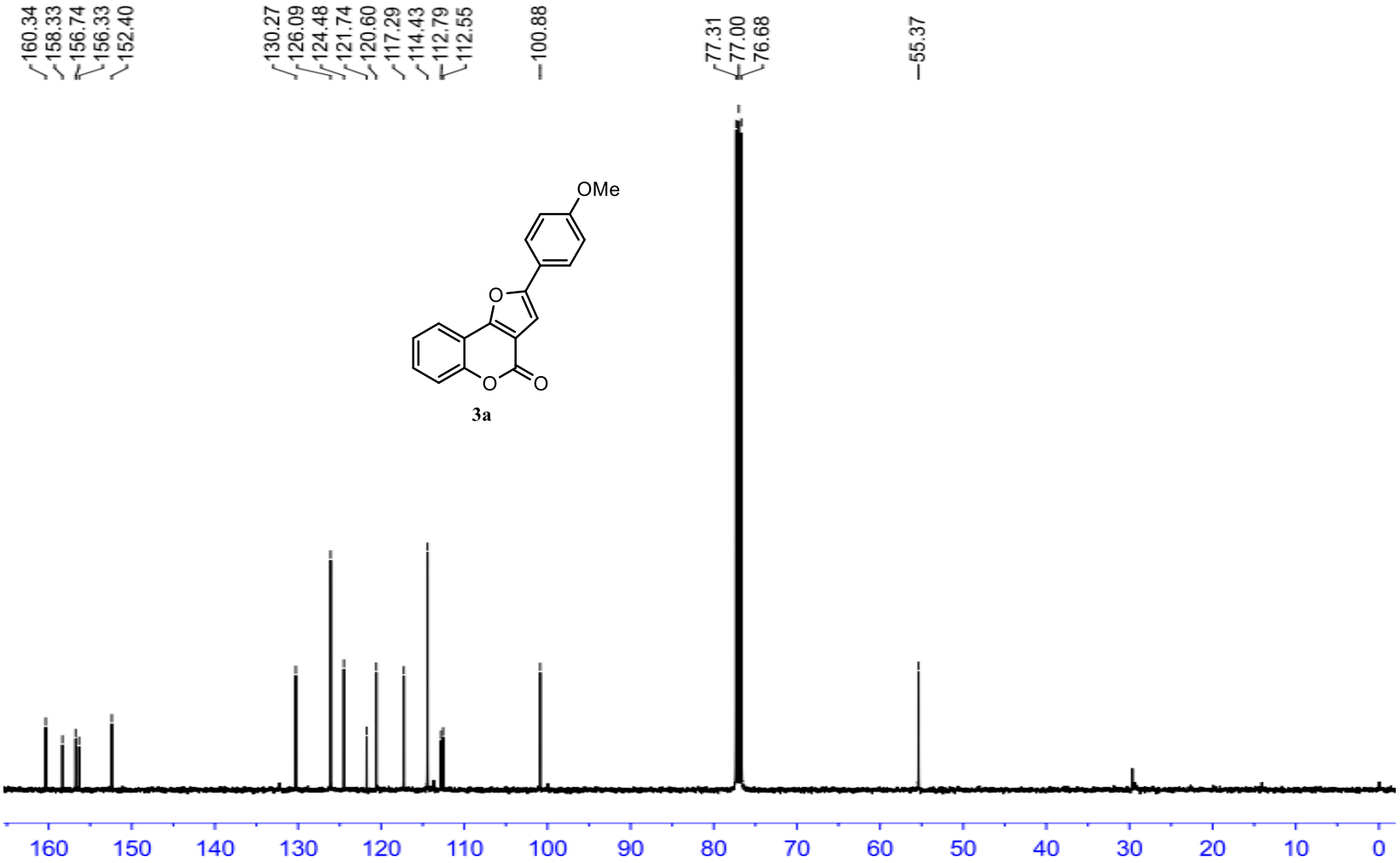
The AchE inhibitory activities were measured by a spectrophotometric method developed by Ellman et al.[4] The reaction was run at 25°C in a final volume of 200 μL of a 0.1M phosphate buffer (pH = 8.0), 333 μM 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB, Sigma-Aldrich), 0.035 U/mL AchE (Sigma-Aldrich), and 530 μM of acetylthiocholine iodide (Sigma-Aldrich) in 96-well microplates. Test compounds were added to the assay solution and followed at 412 nm for 5 min with a plate reader (Sunrise, Tecan). Seven different concentrations of each compound were used in order to measure the inhibition of AChE activity. IC_{50} (concentration of the drug producing 50% of enzyme–activity inhibition) values were determined graphically from log concentration–inhibition curves.[5]

3. Reference

1. (a) L. Zhang, T. Meng, R. Fan and J. Wu, *J. Org. Chem.*, 2007, **72,** 7279; (b) Z. Wang, B. Wang, and J. Wu, *J. Comb. Chem.*, 2007, **9,** 811.

4. NMR Spectra for All Compounds

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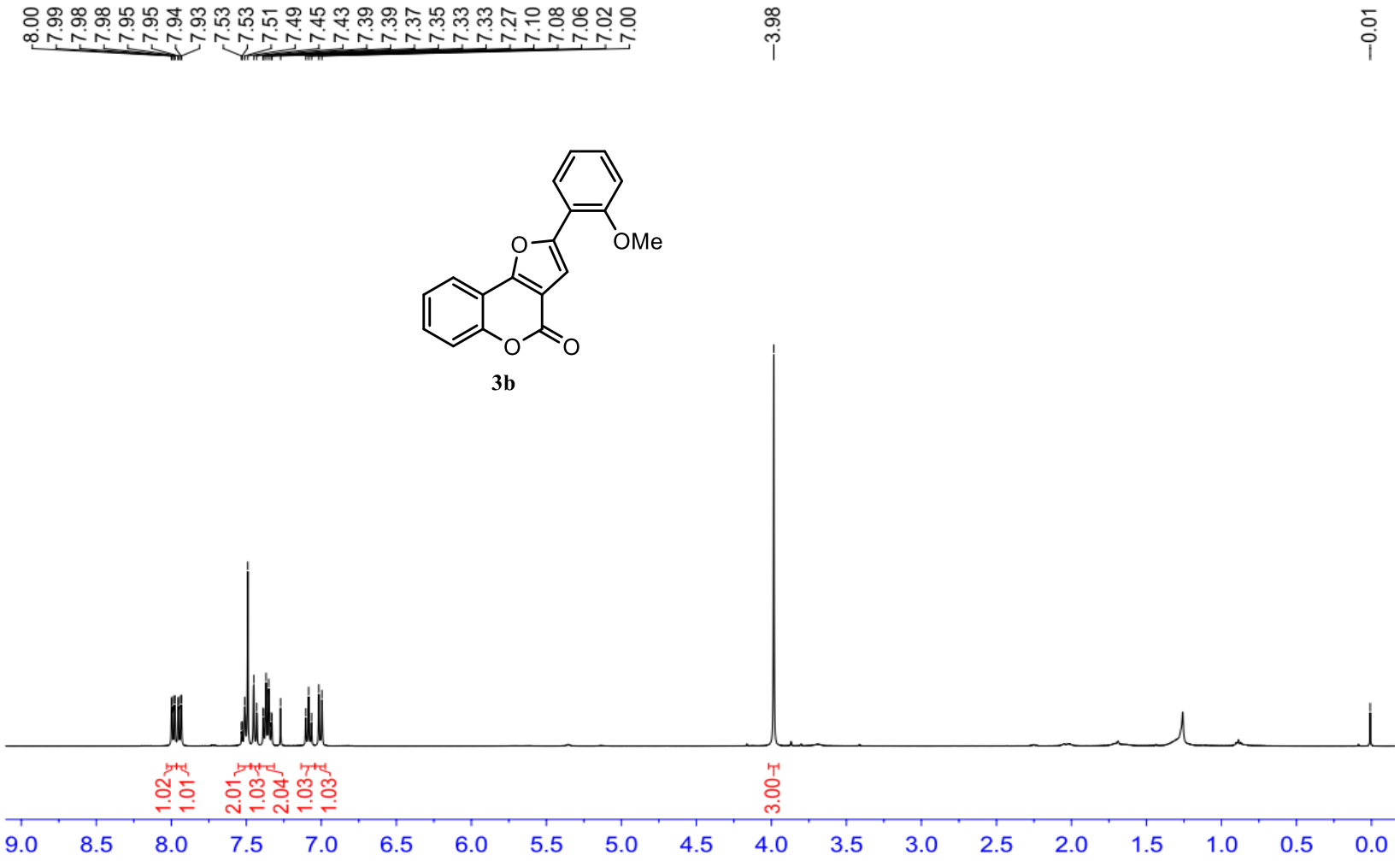
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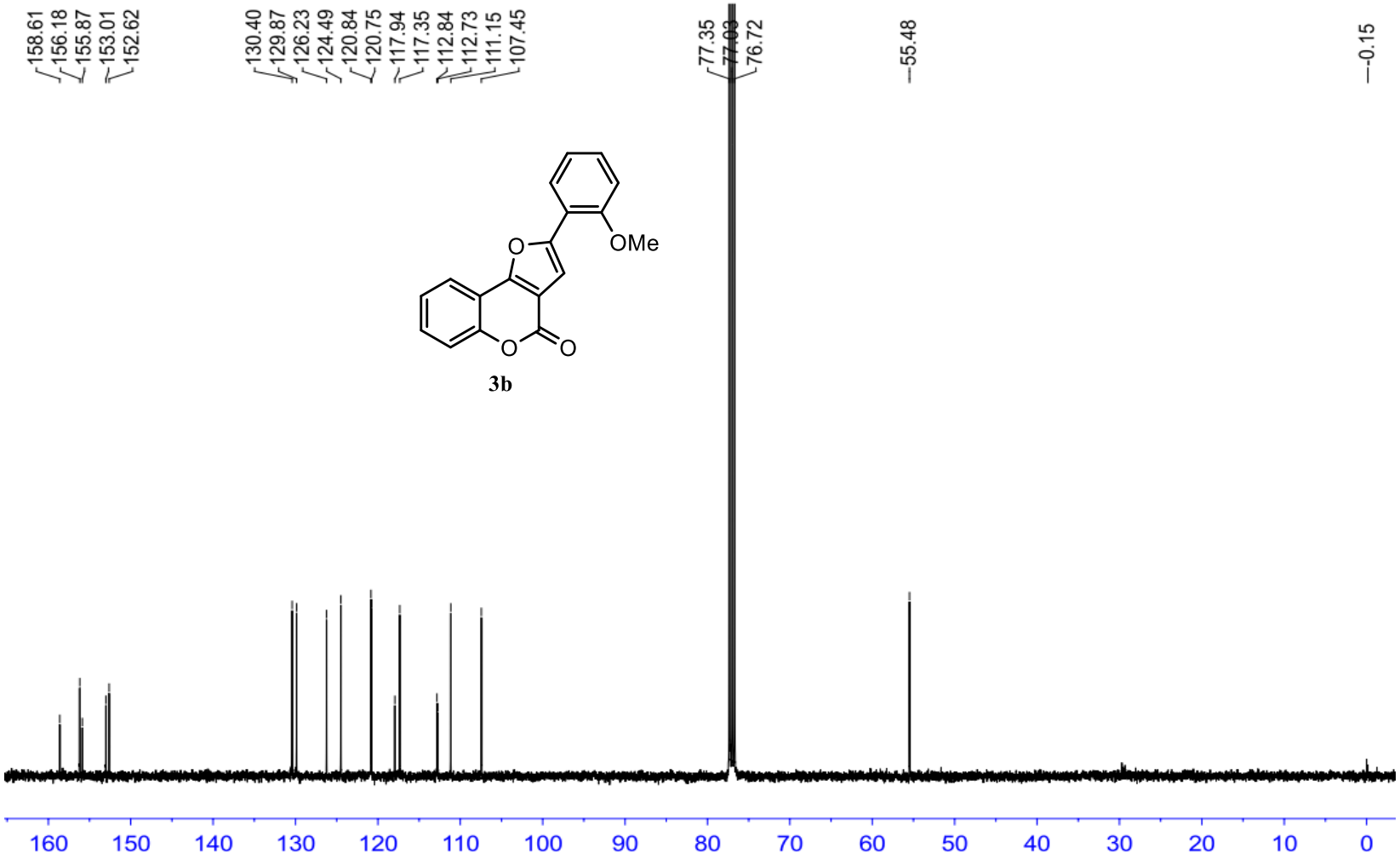
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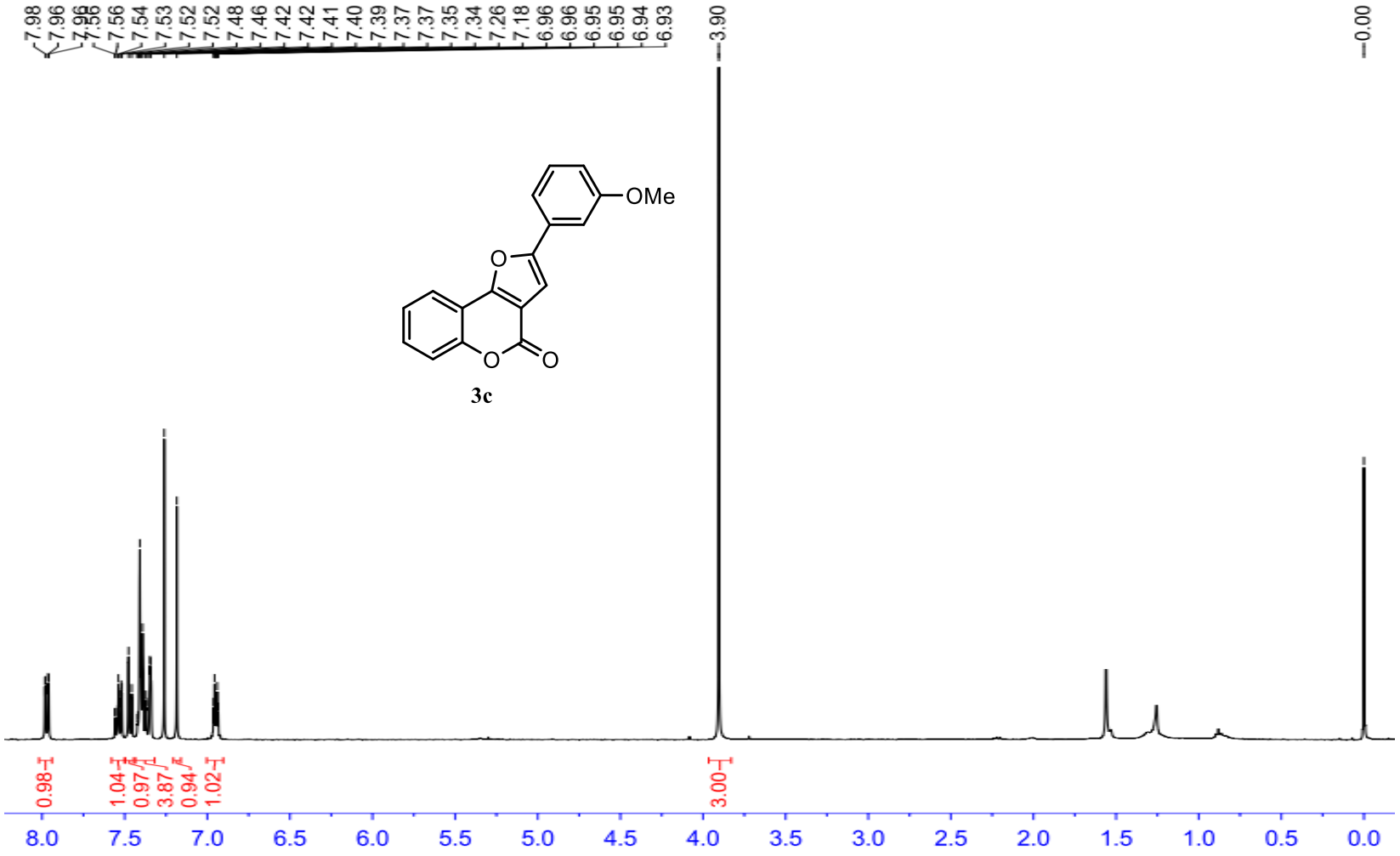
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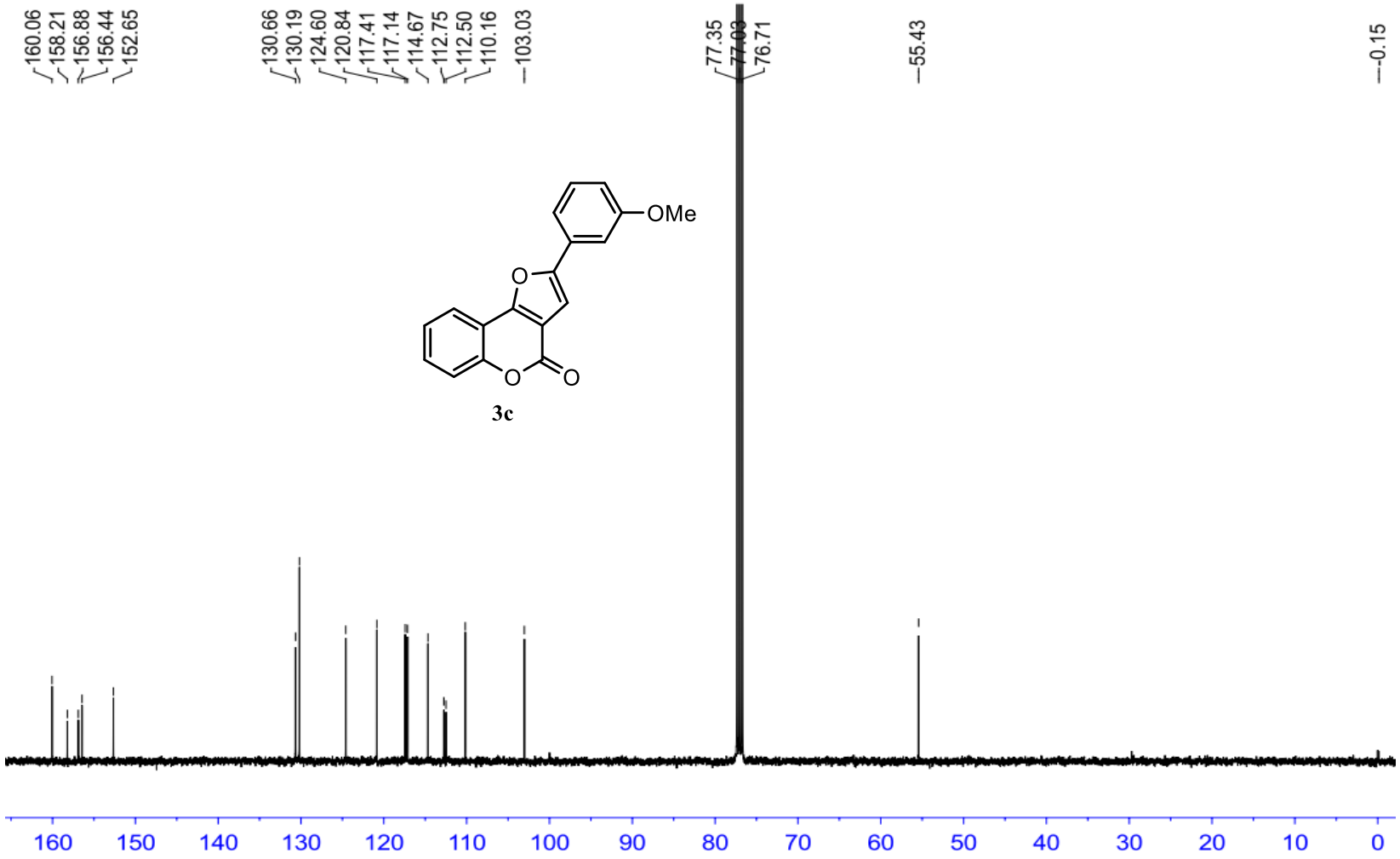
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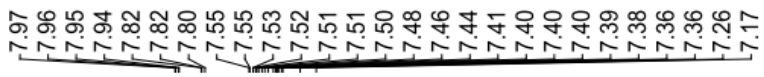
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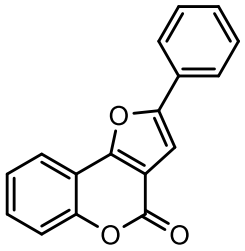
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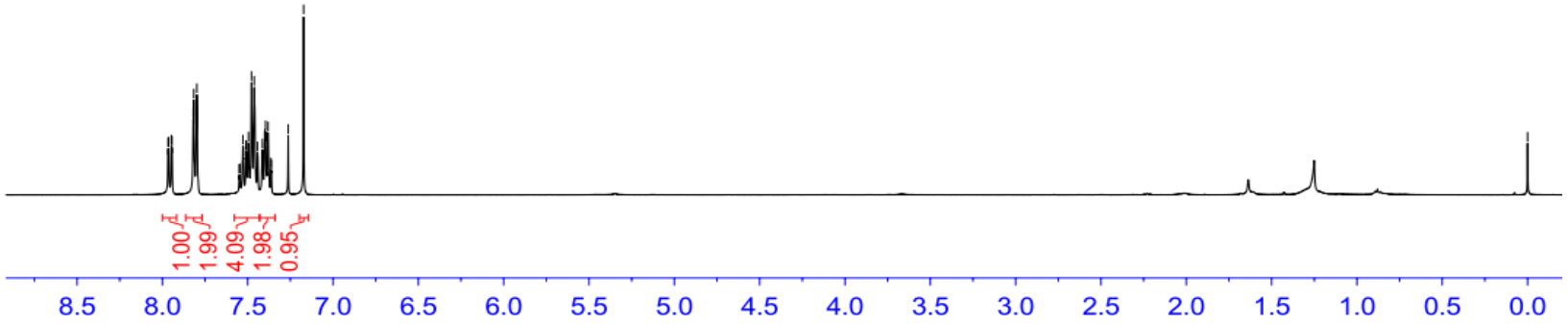
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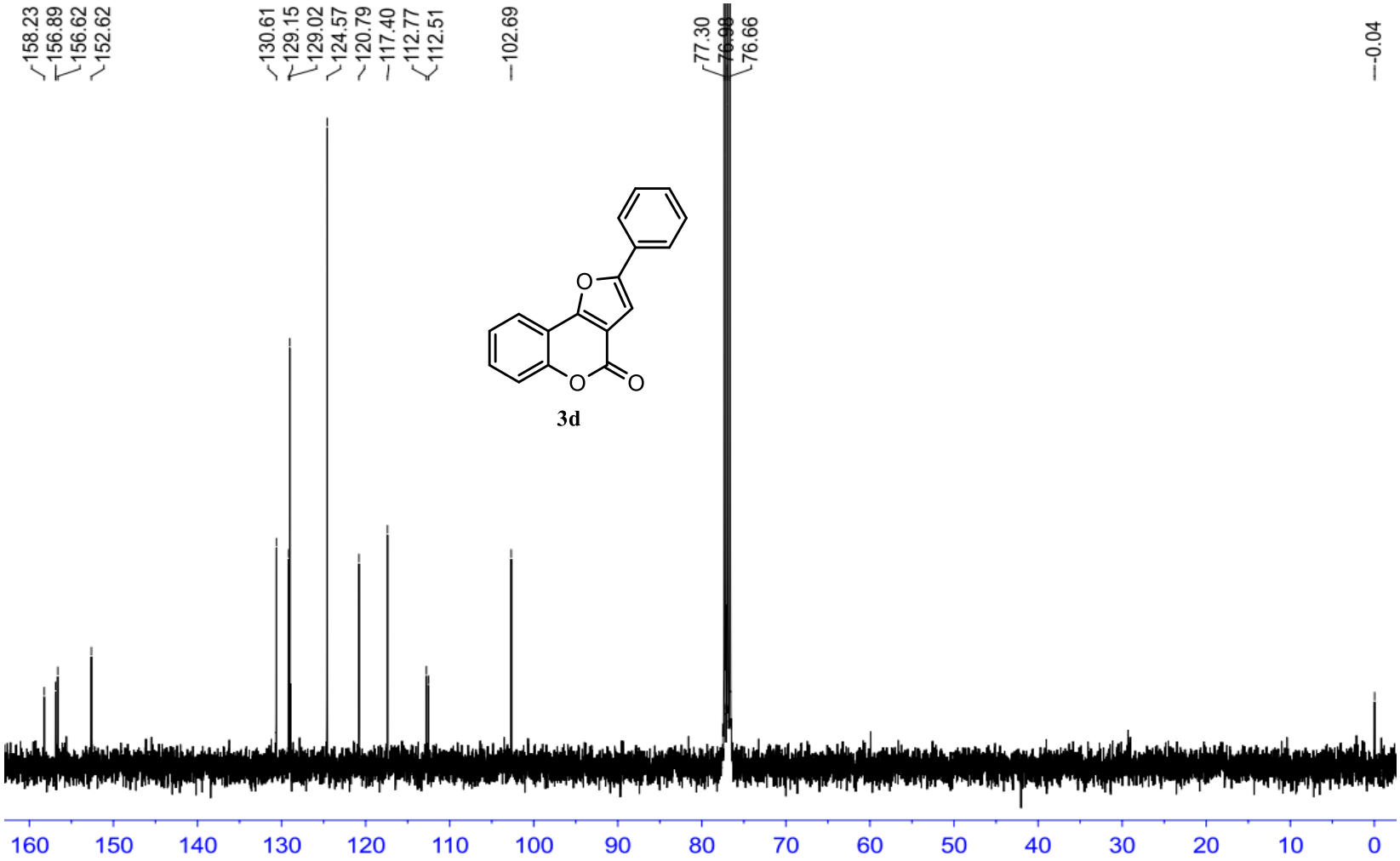
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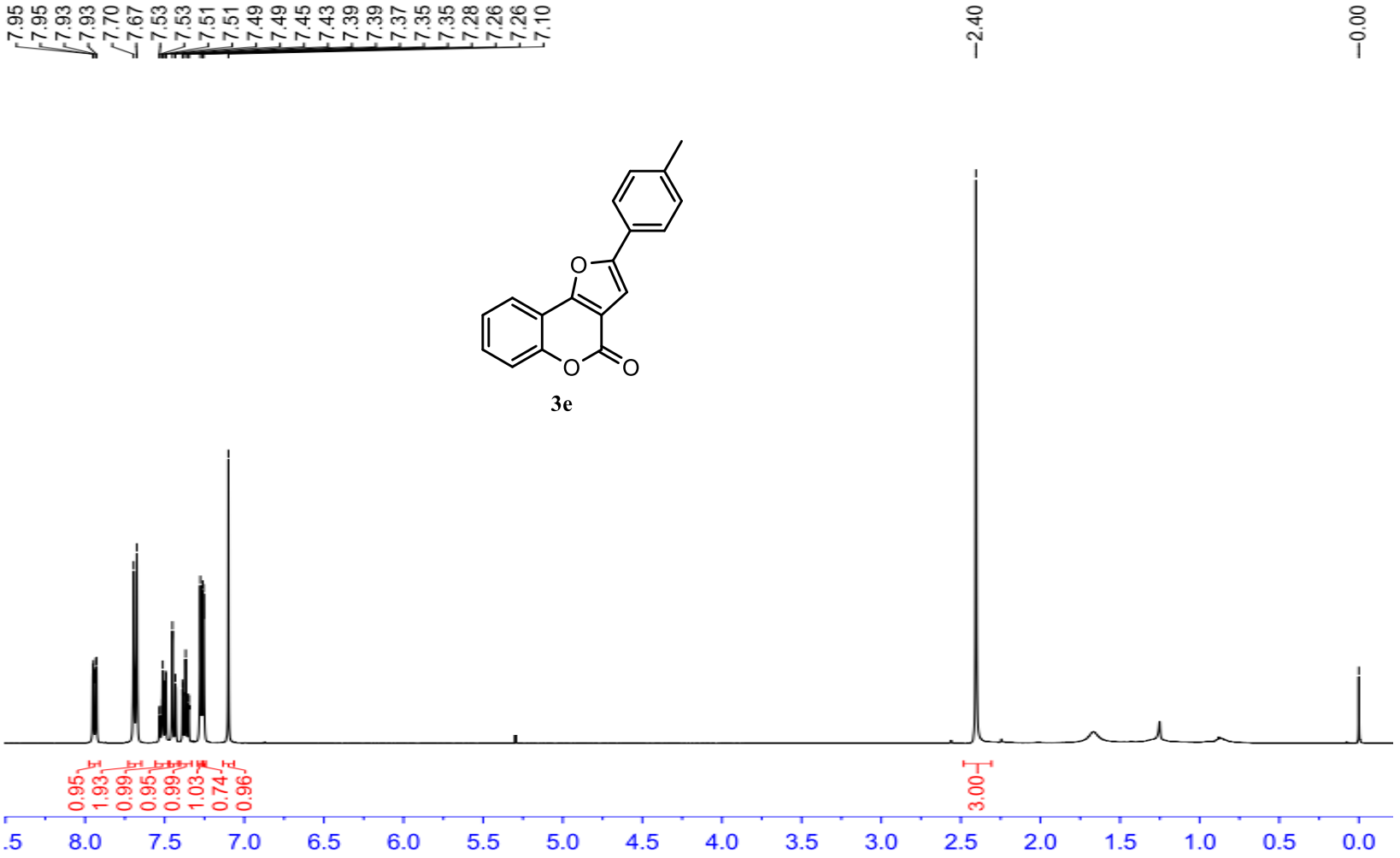
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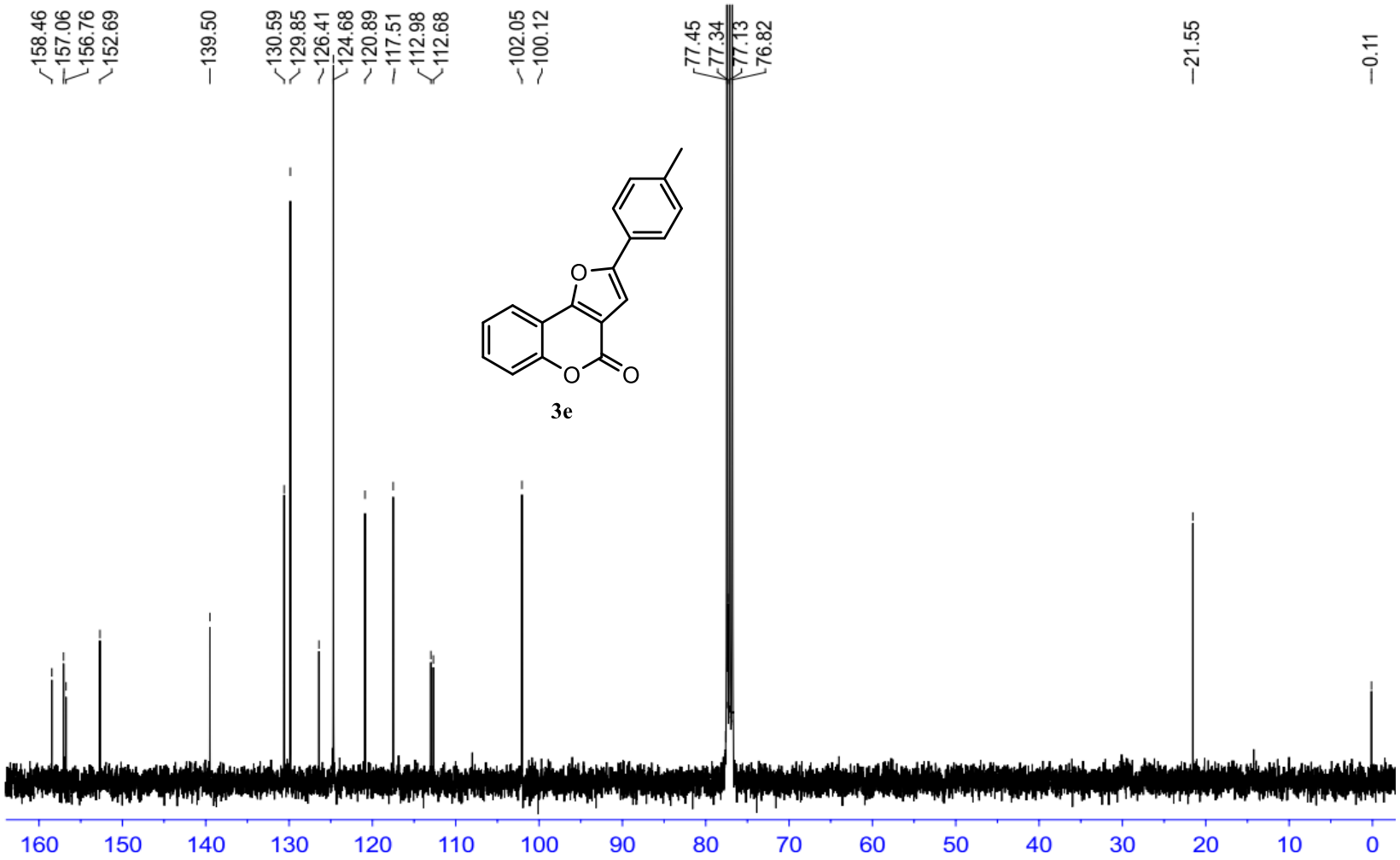
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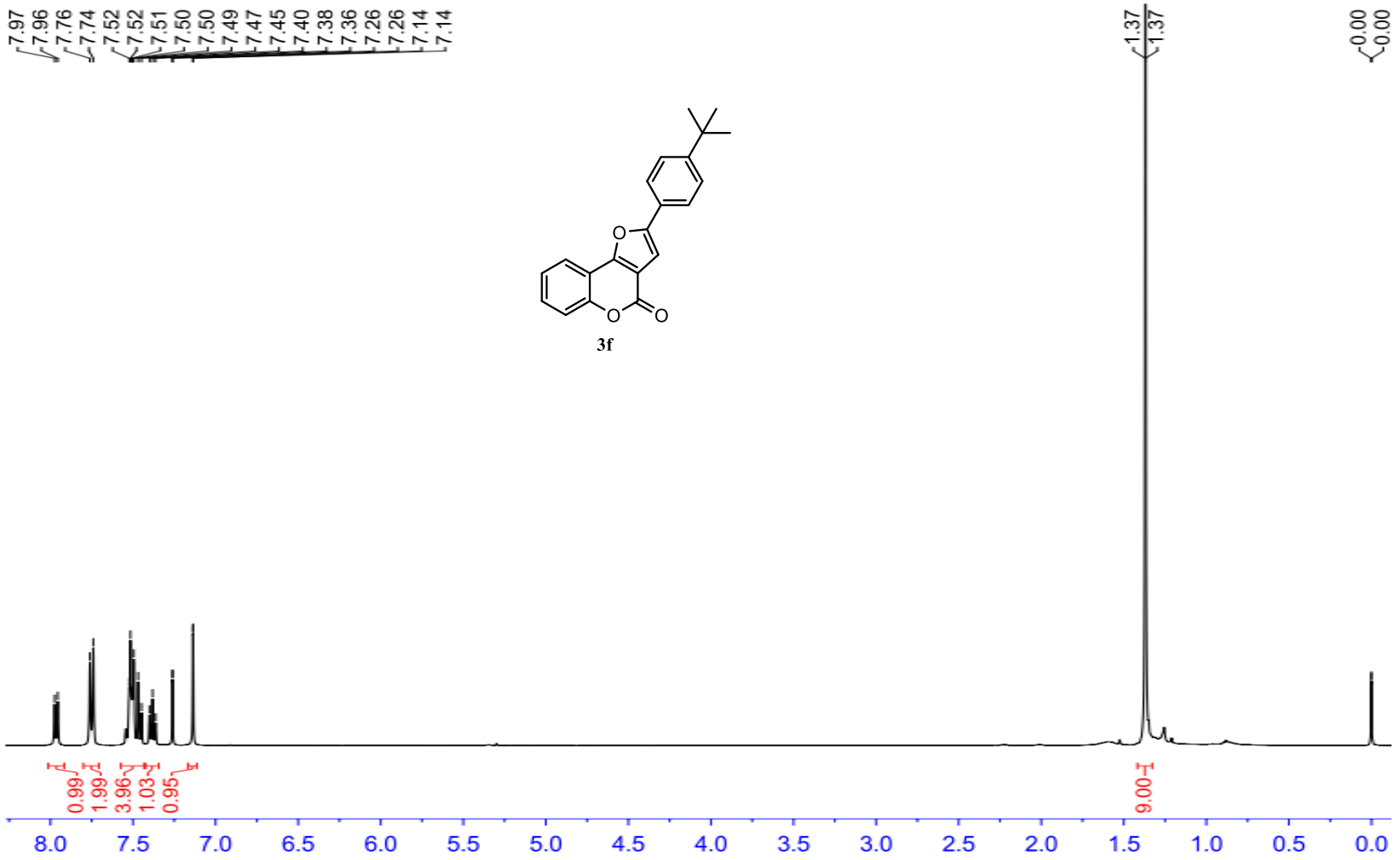
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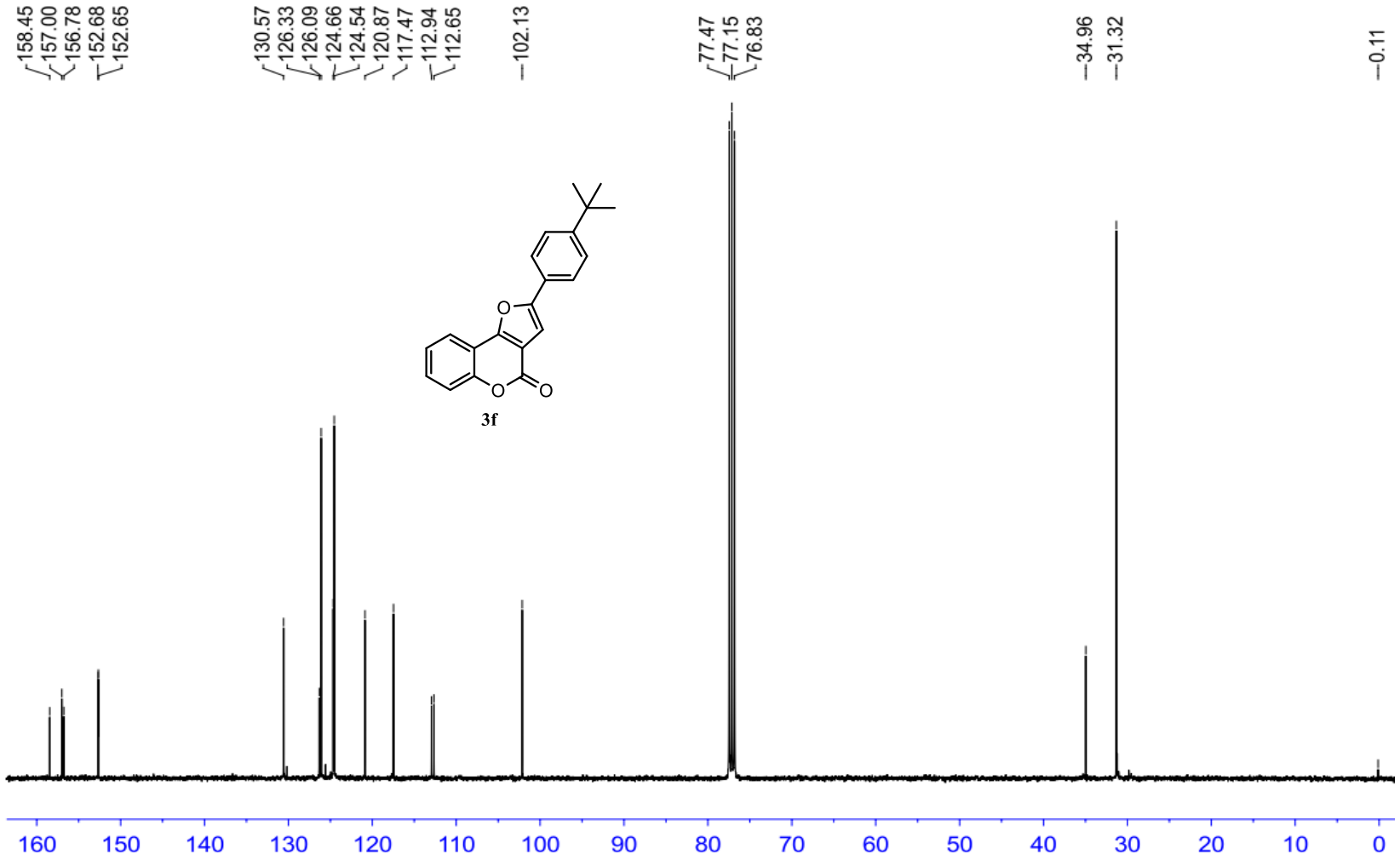
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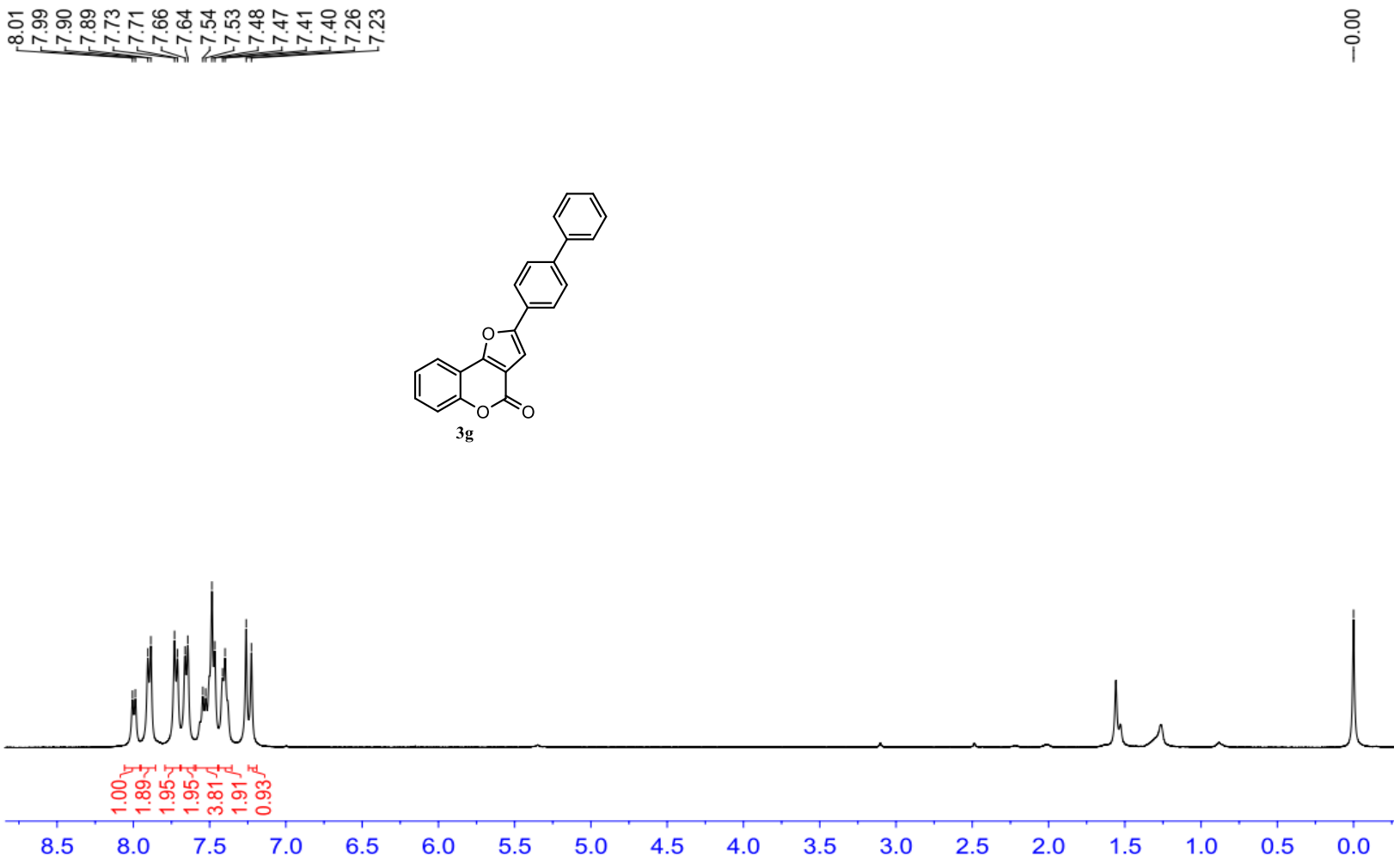
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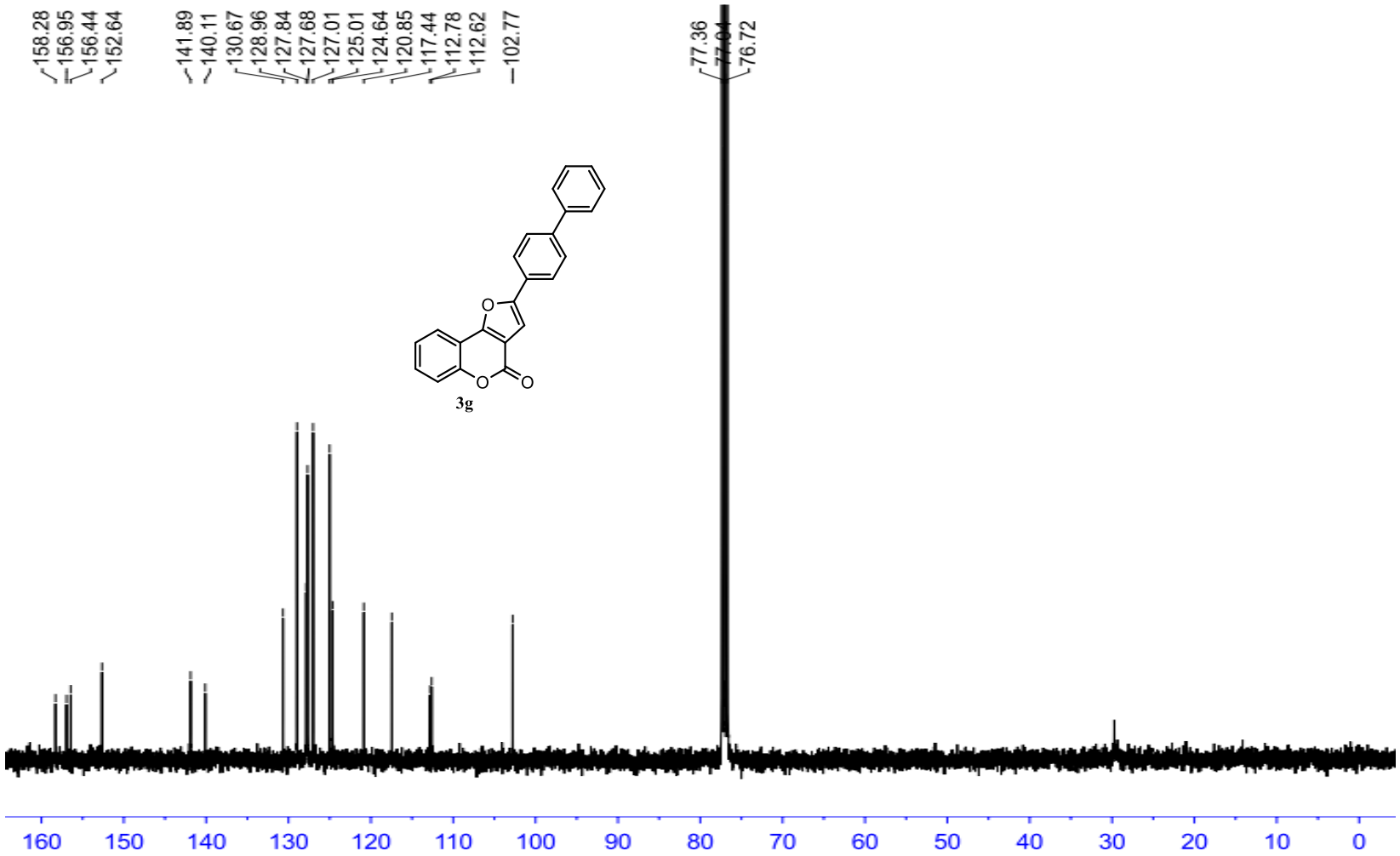
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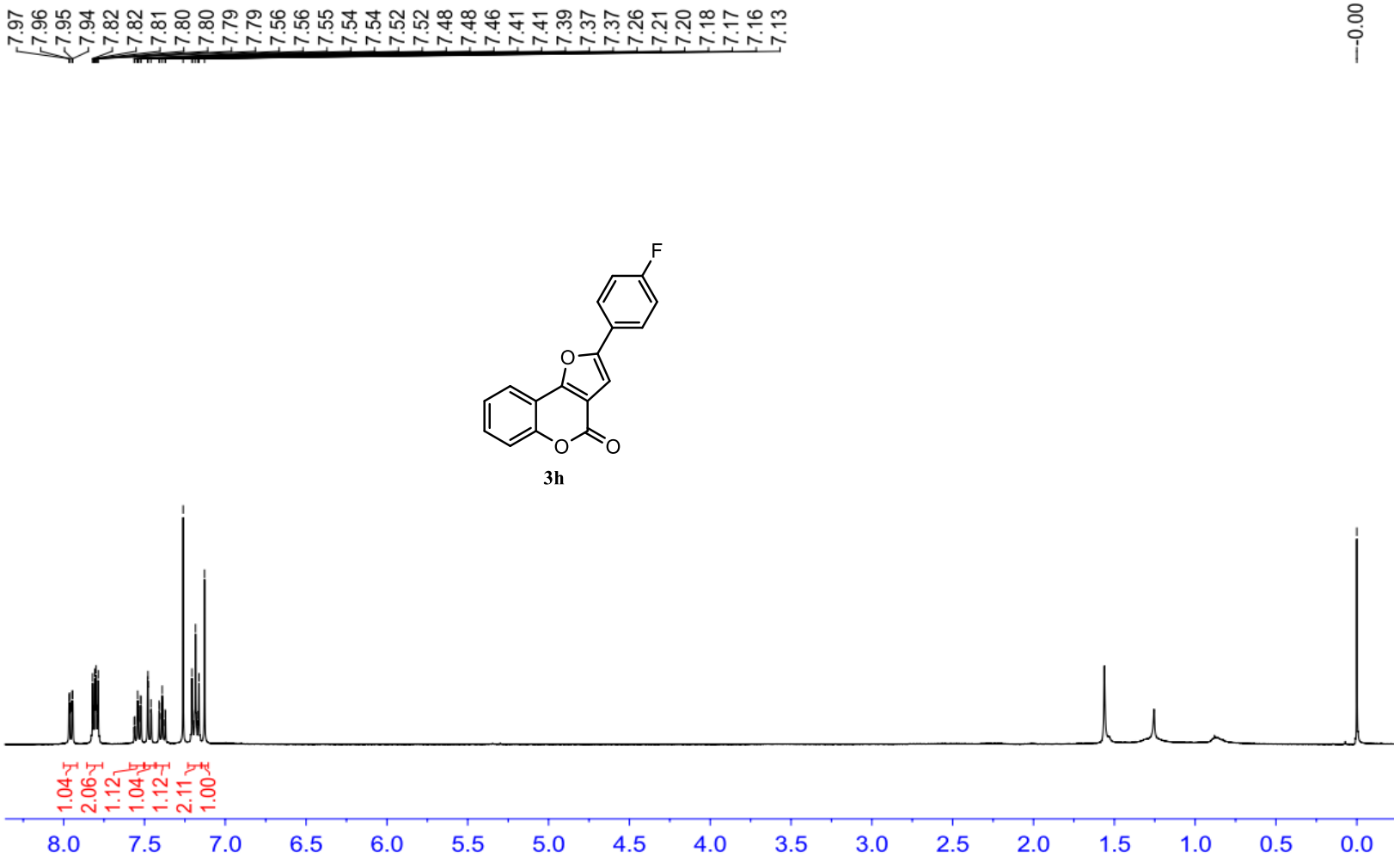
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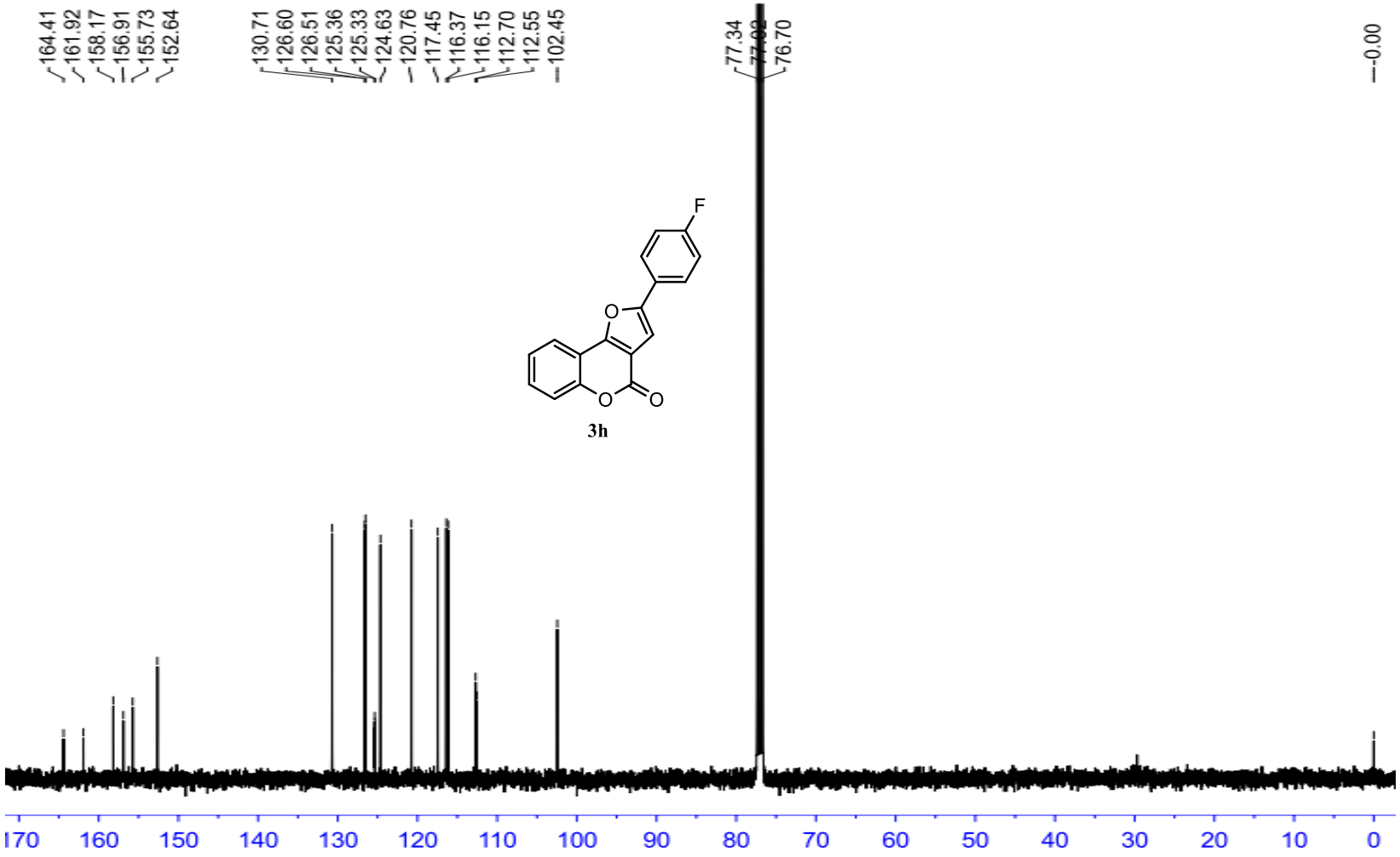
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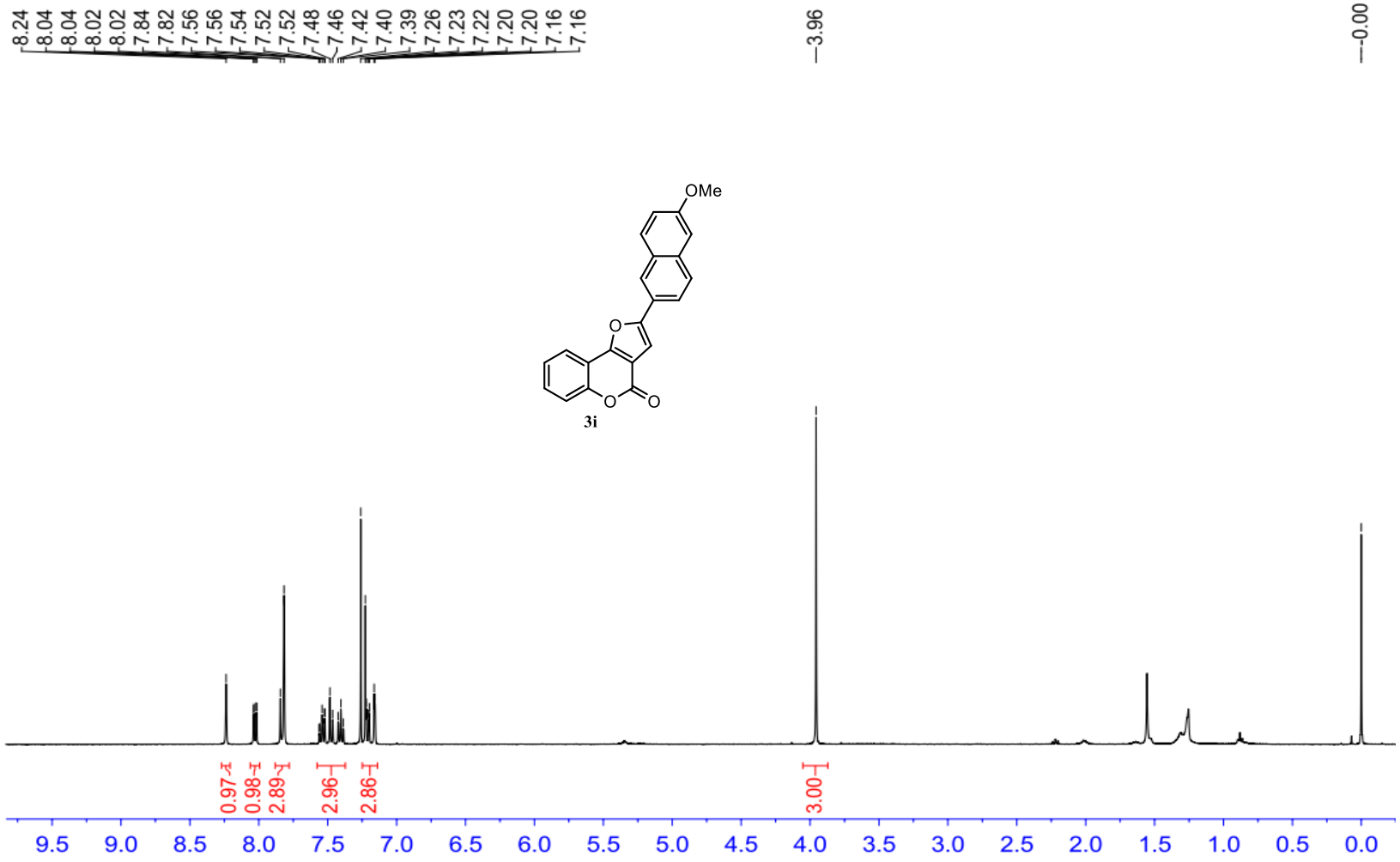
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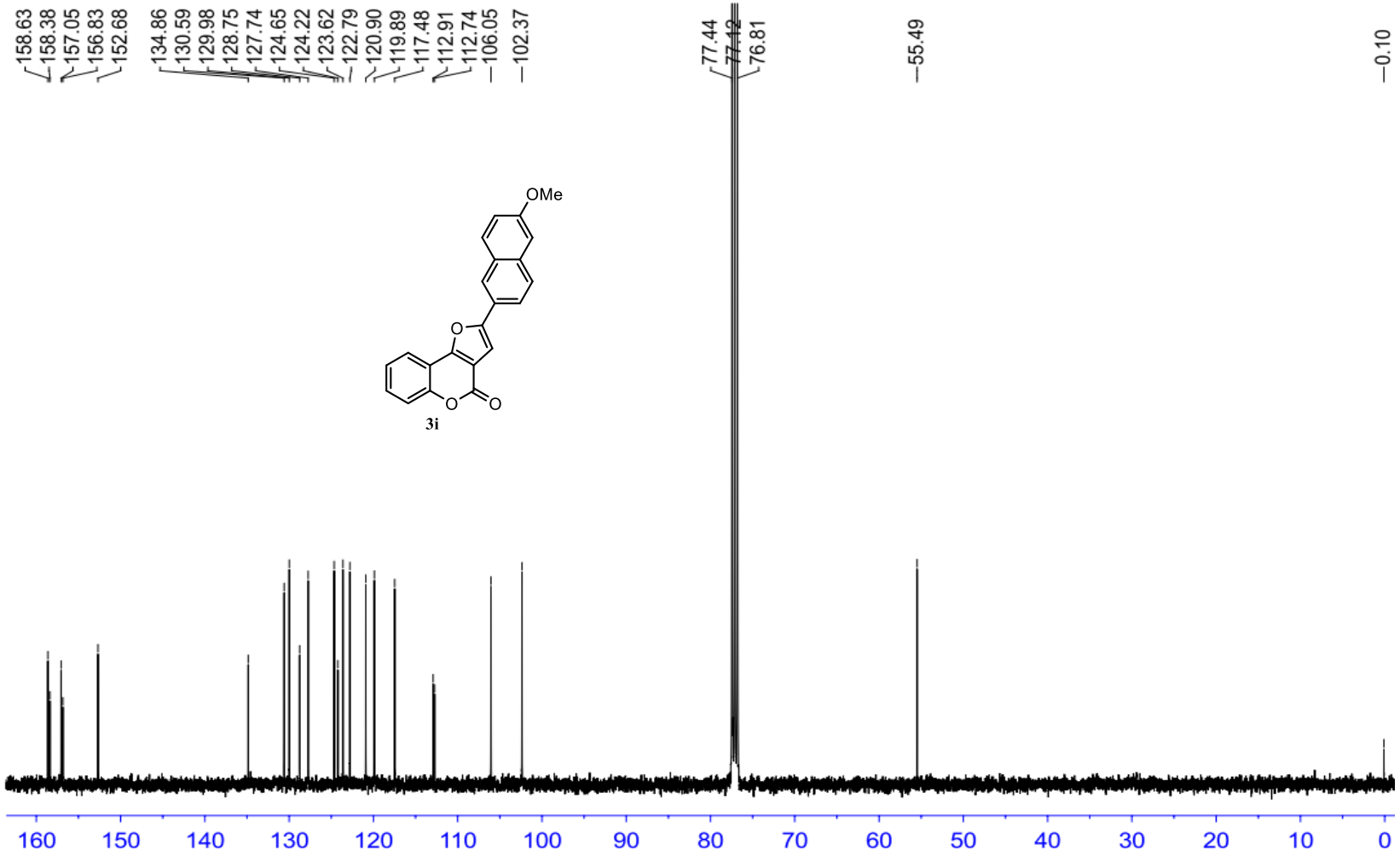
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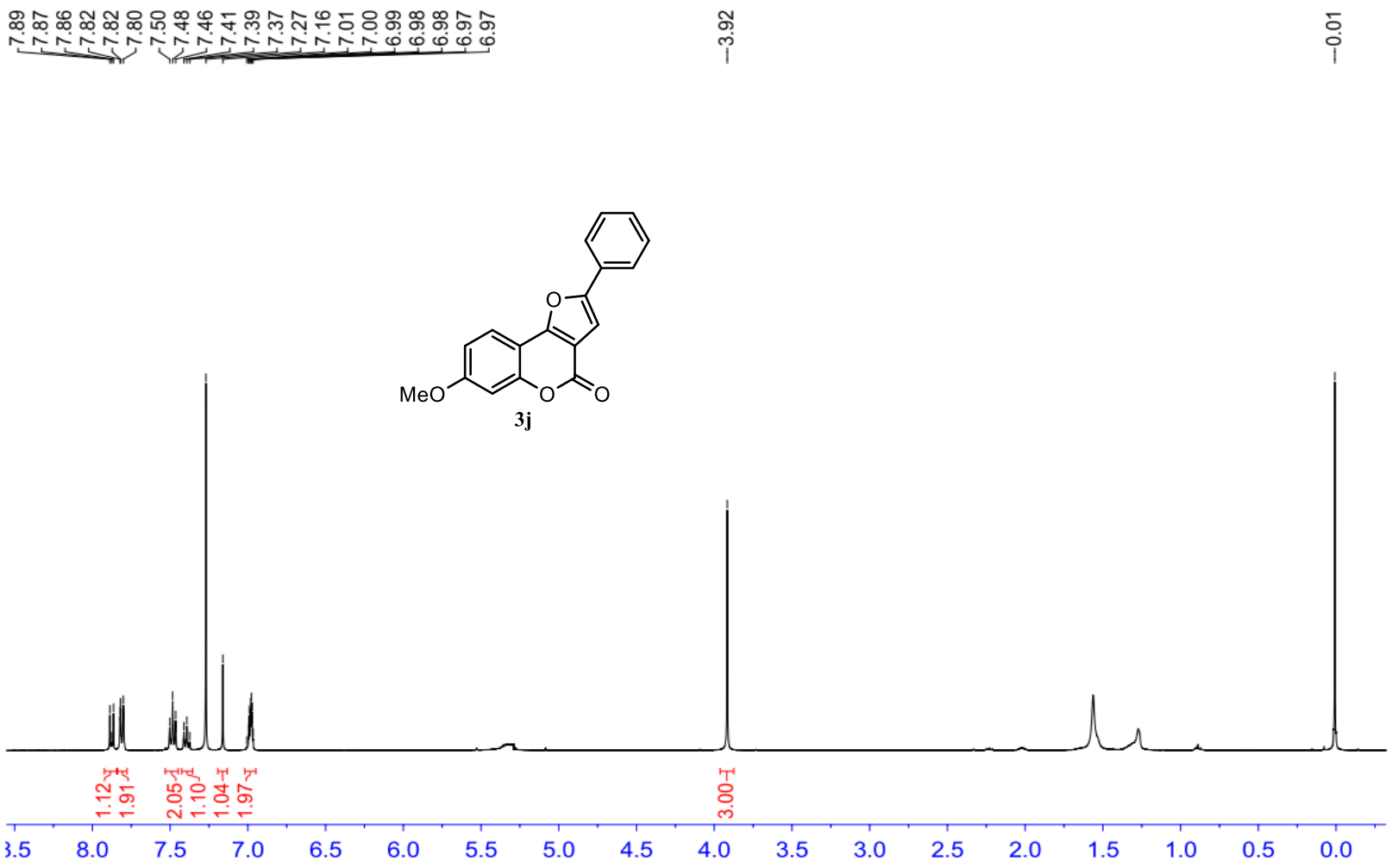
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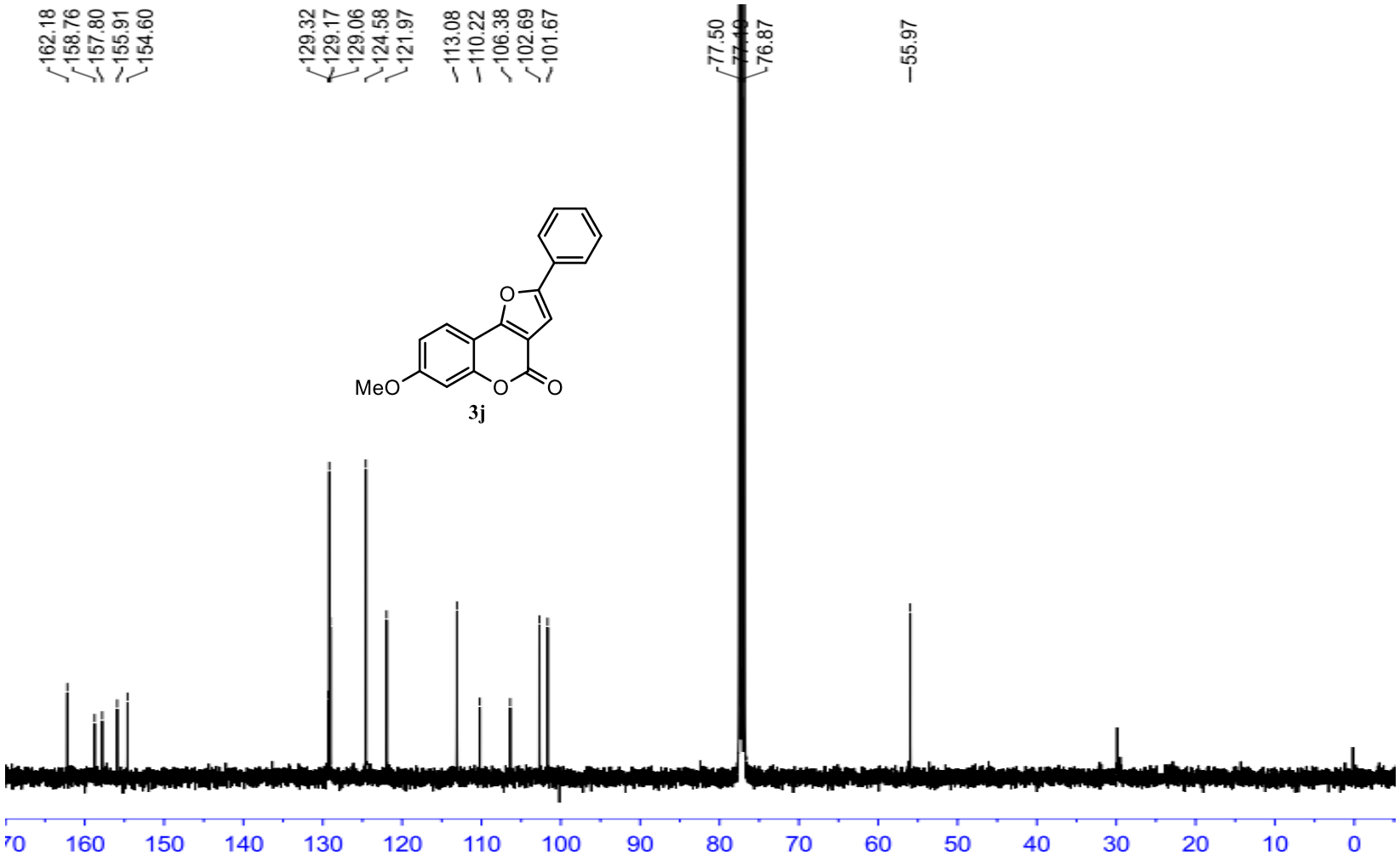
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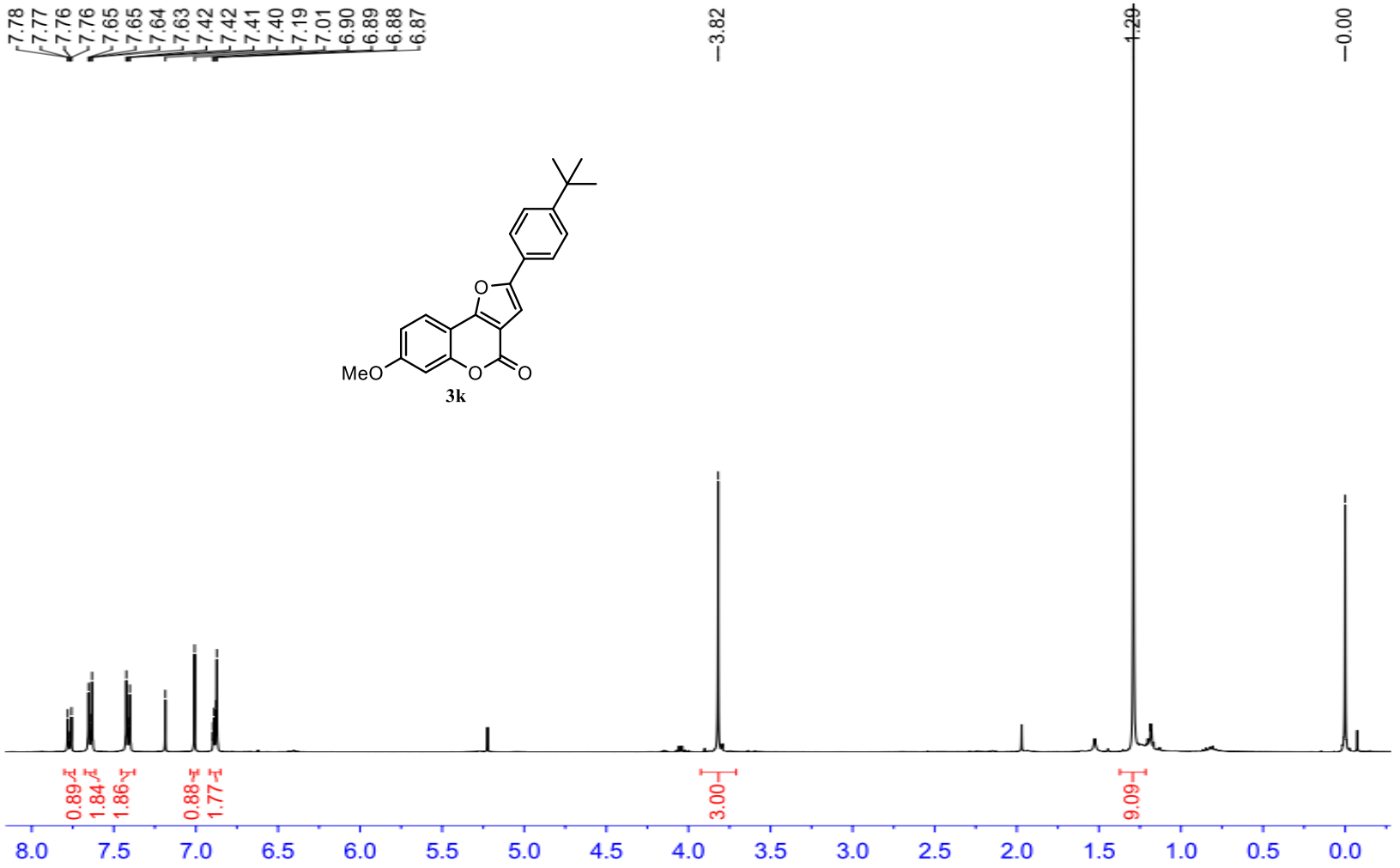
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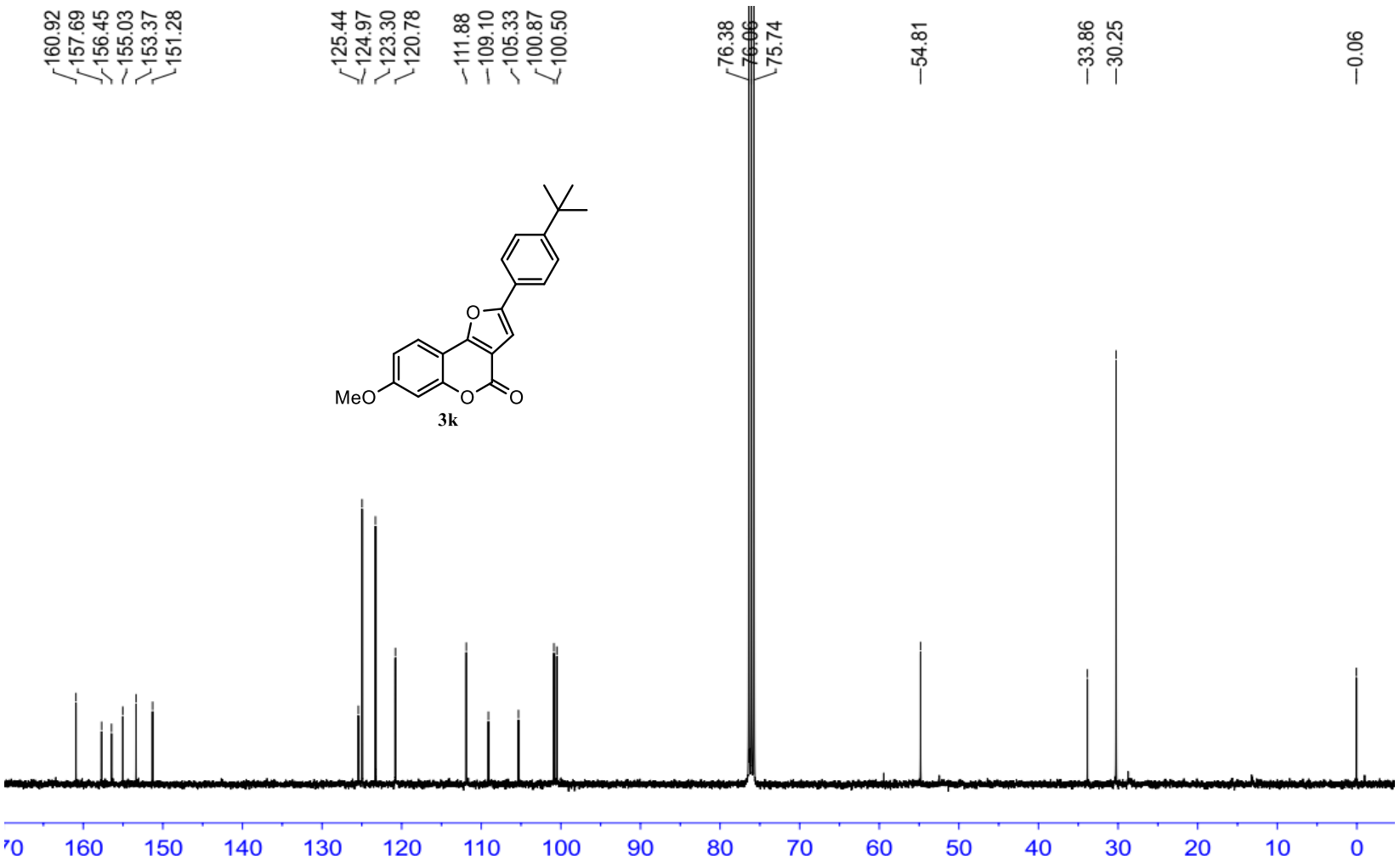
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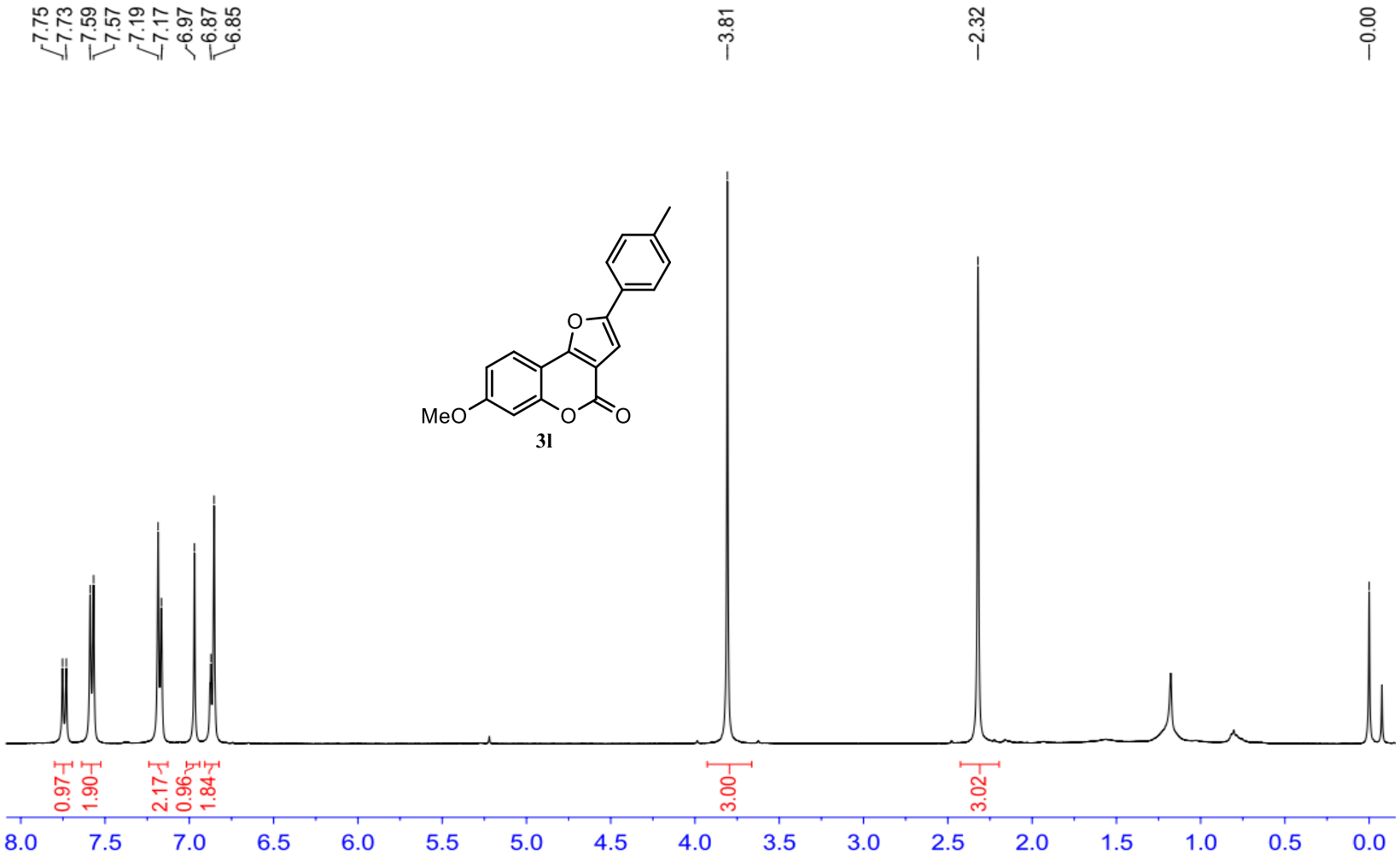
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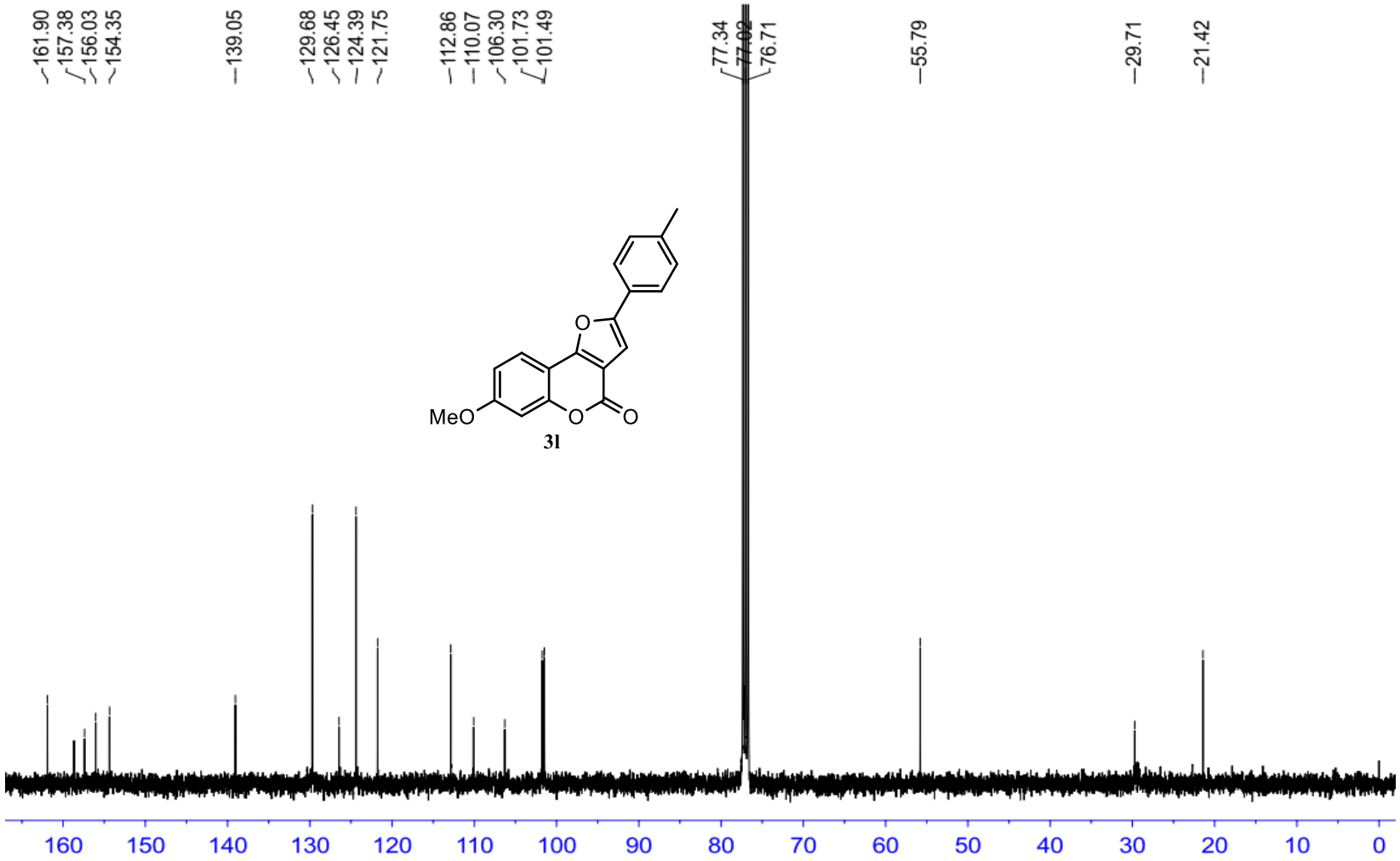
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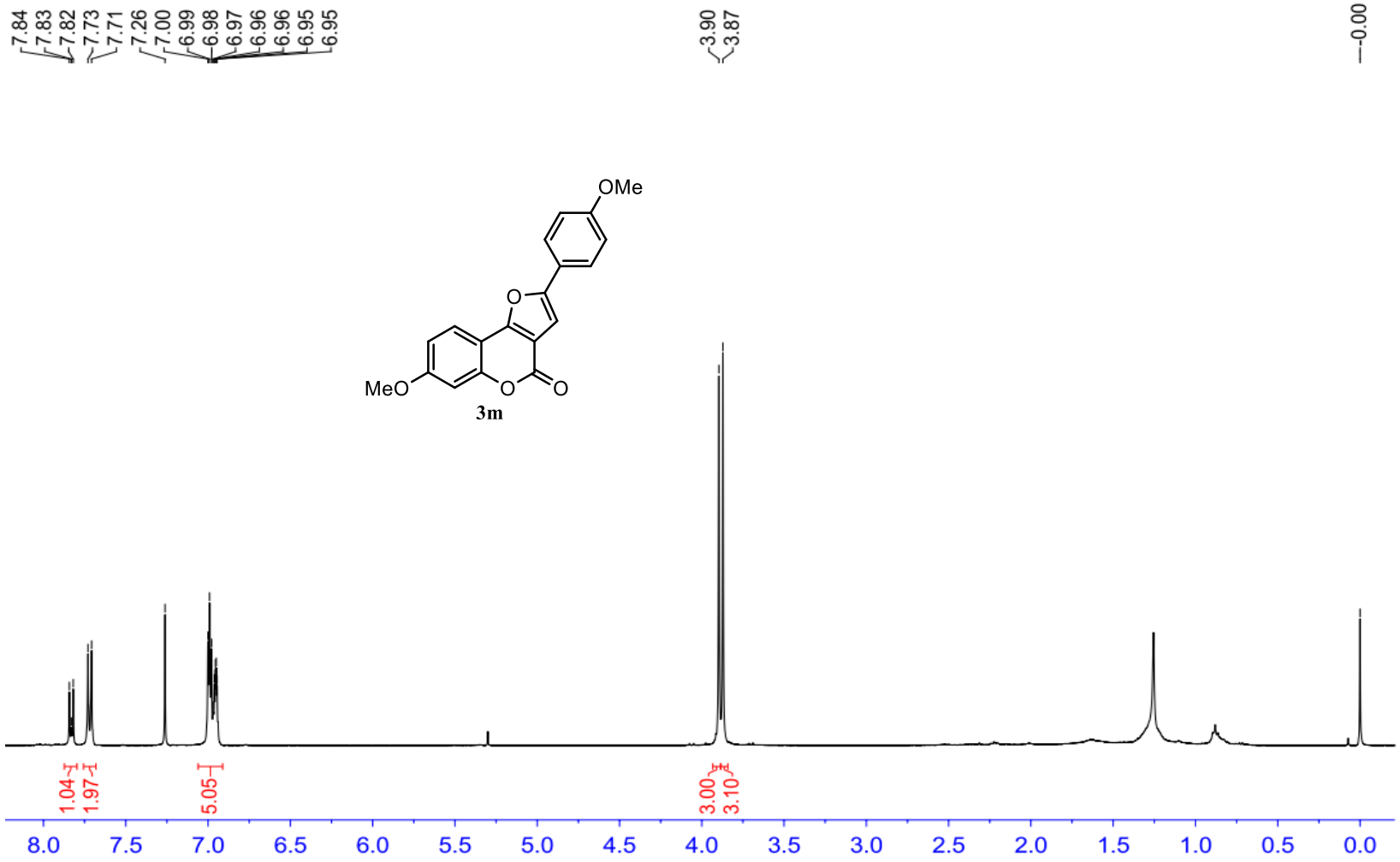
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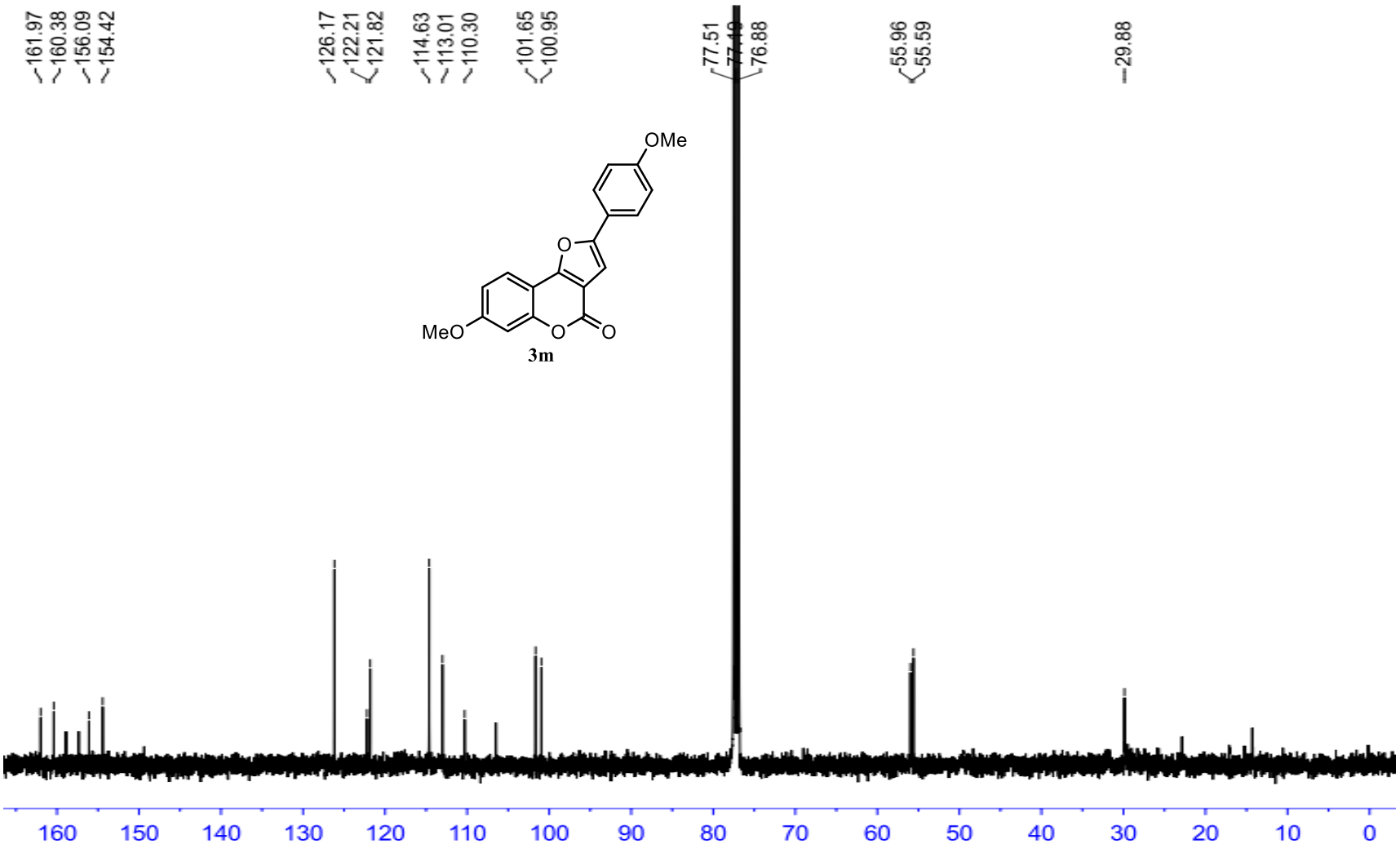
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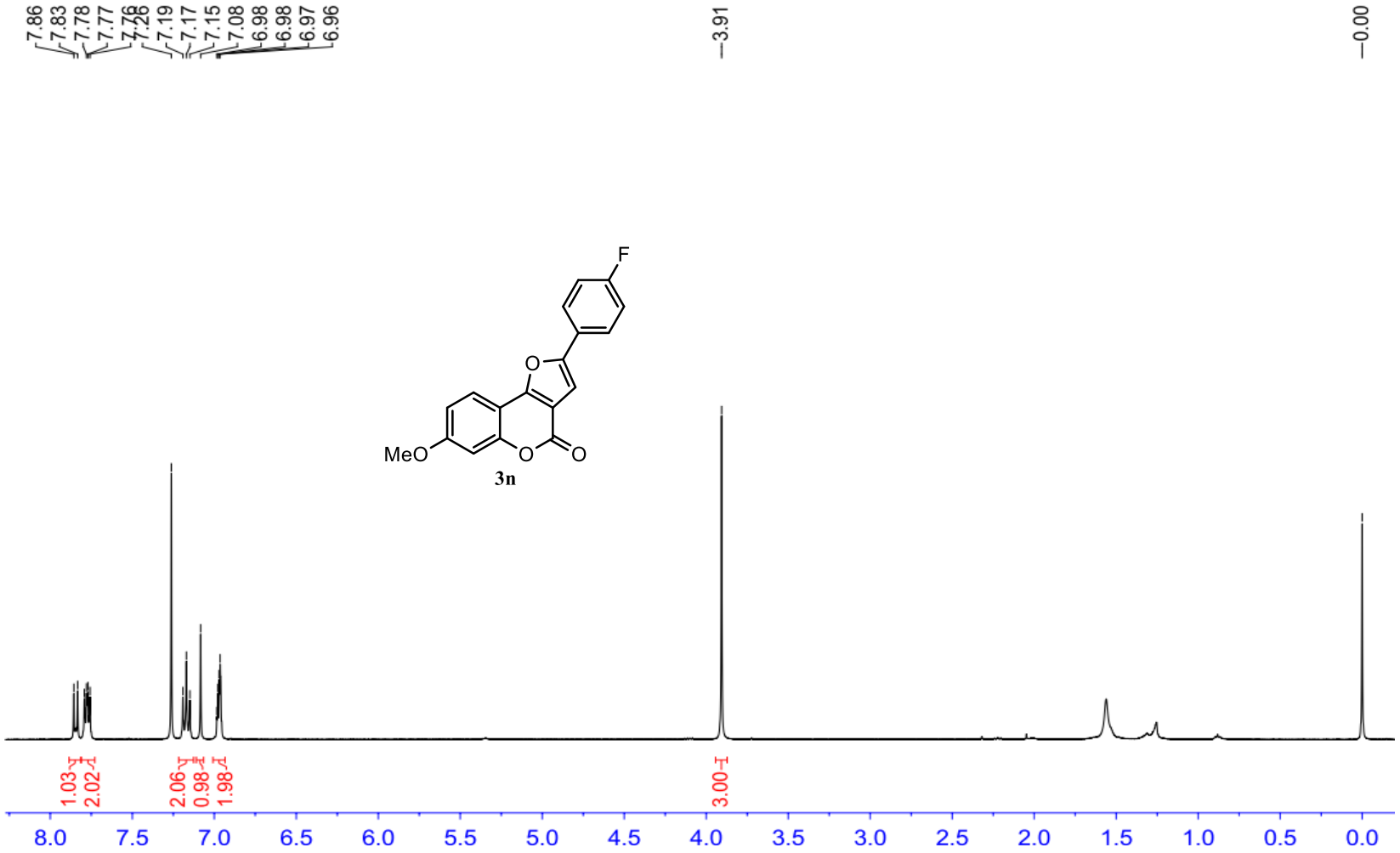
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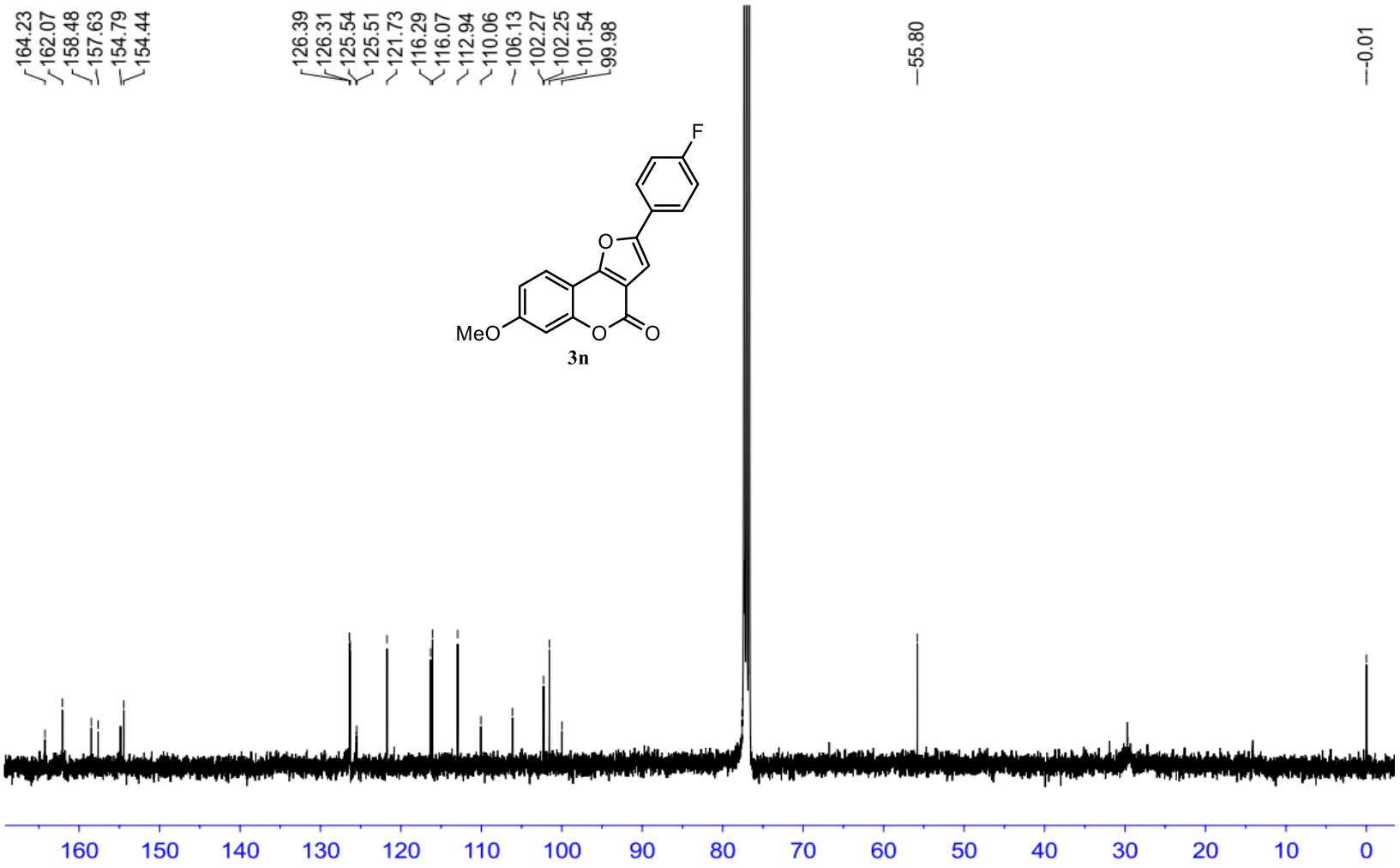
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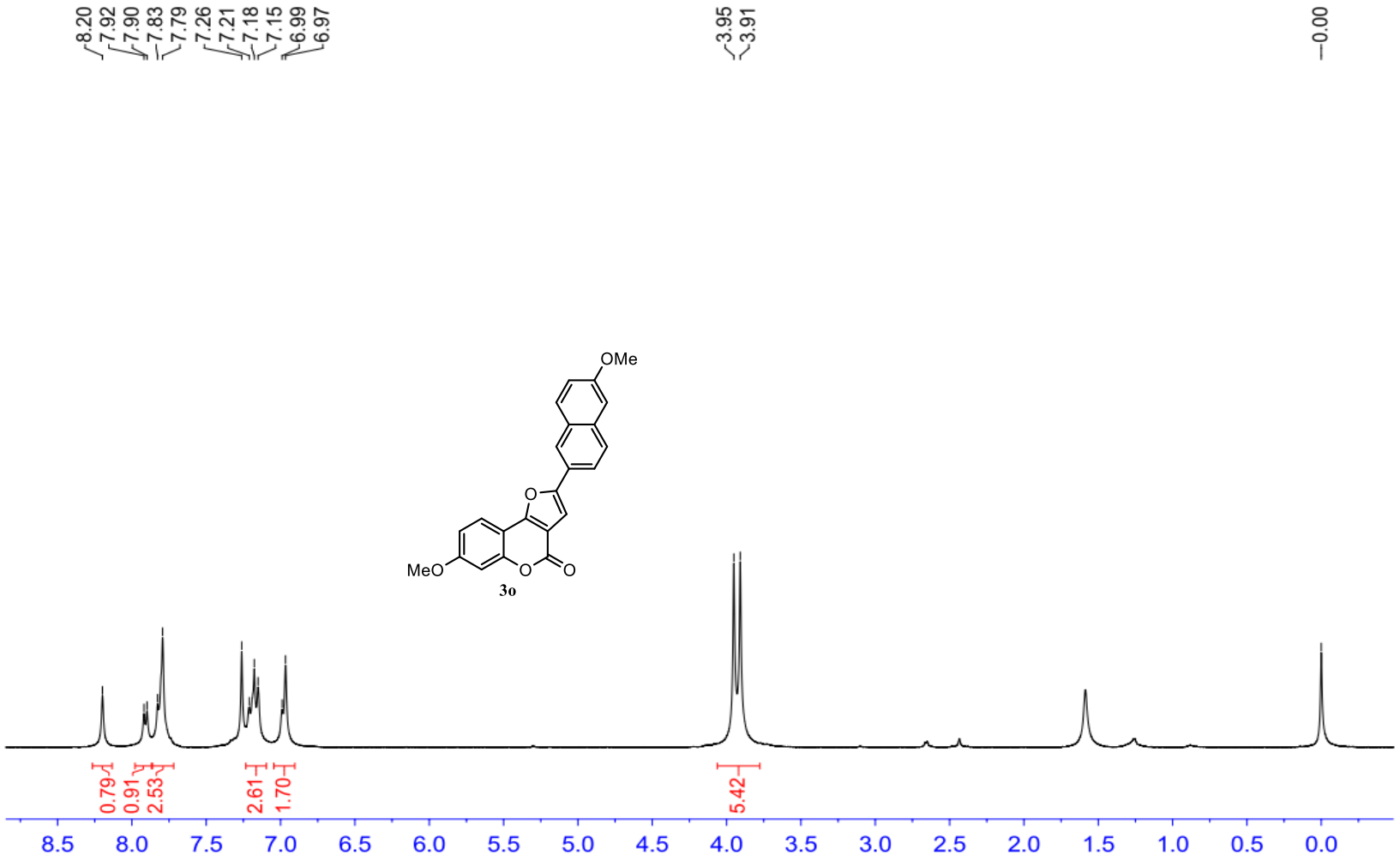
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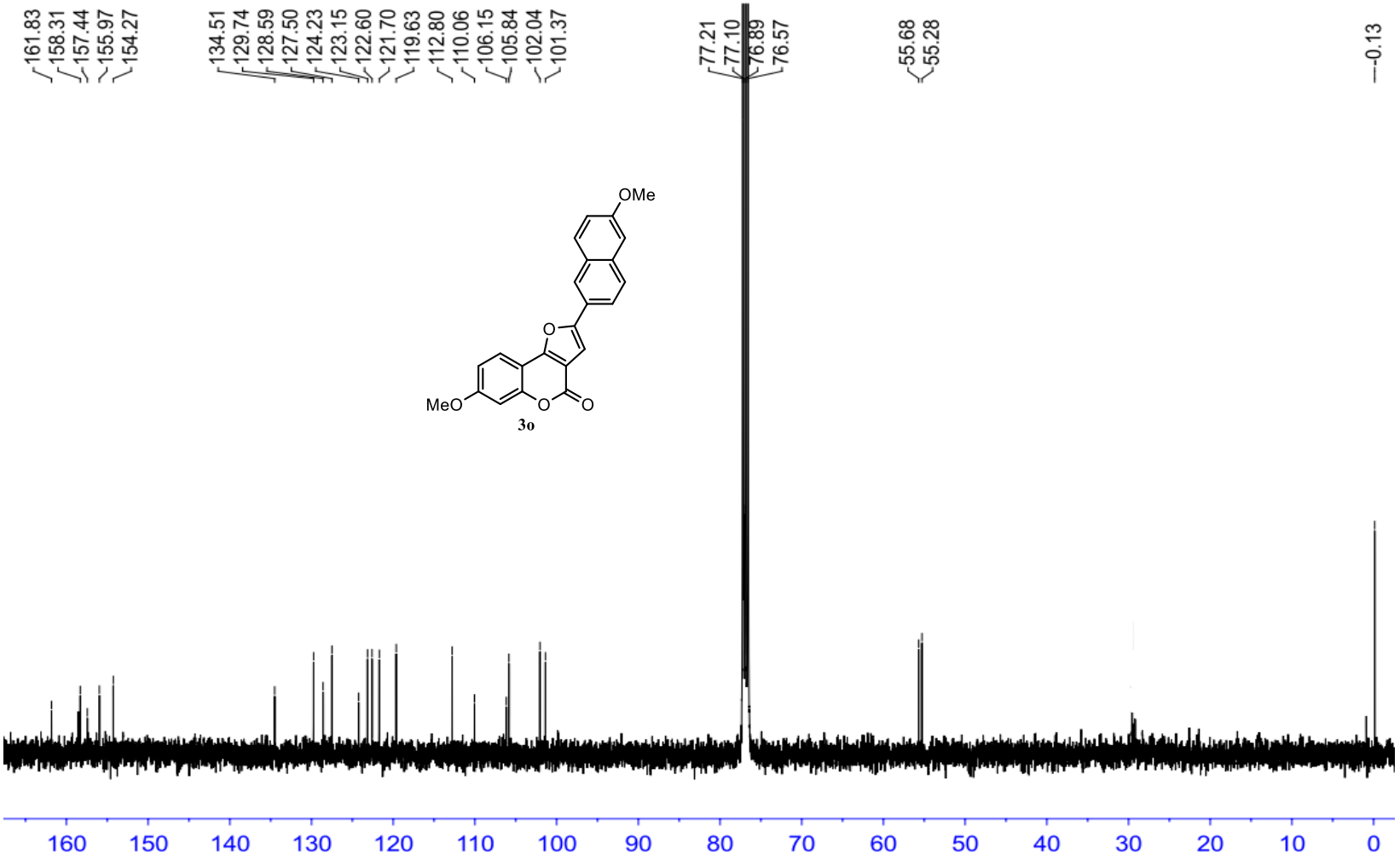
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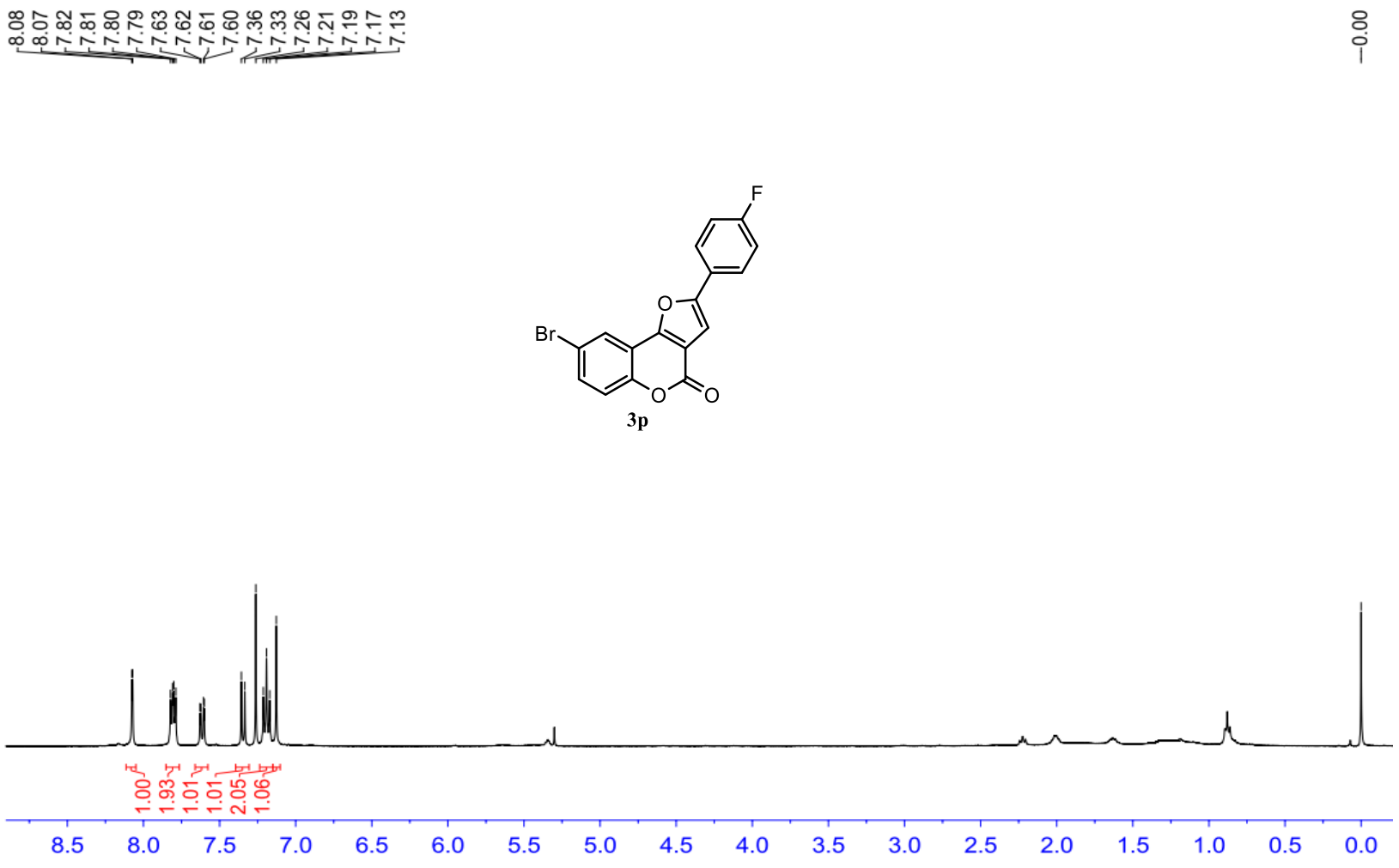
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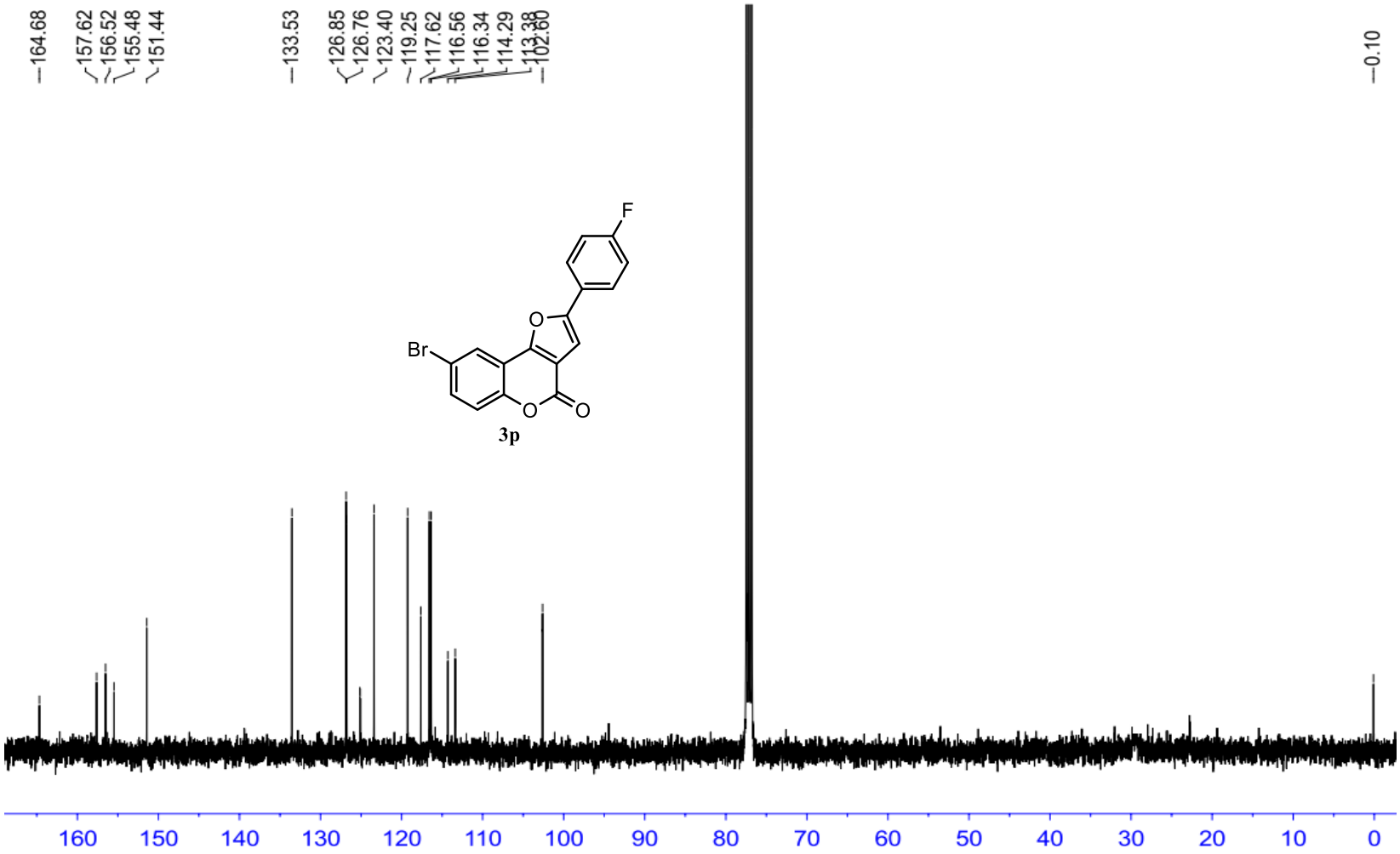
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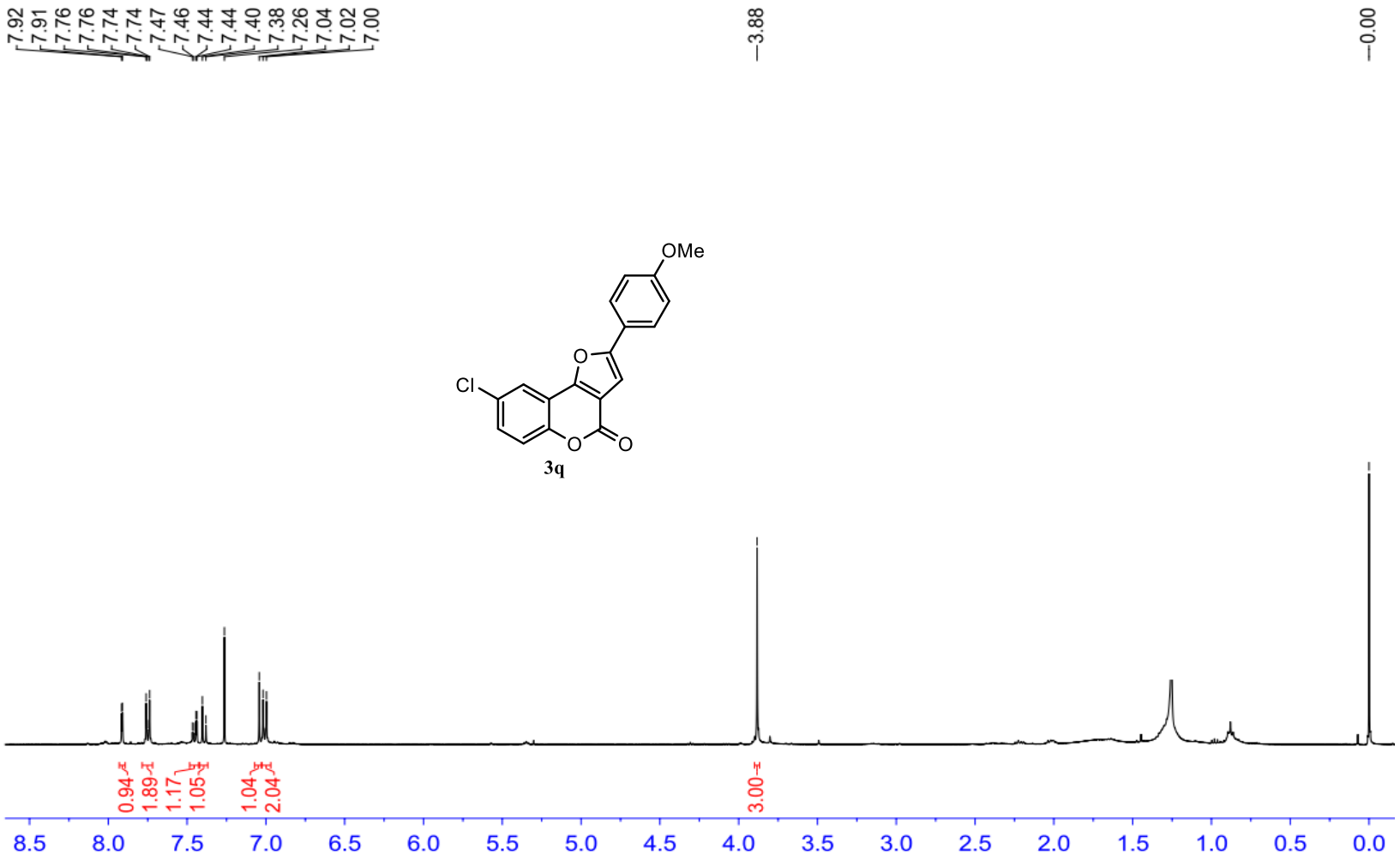
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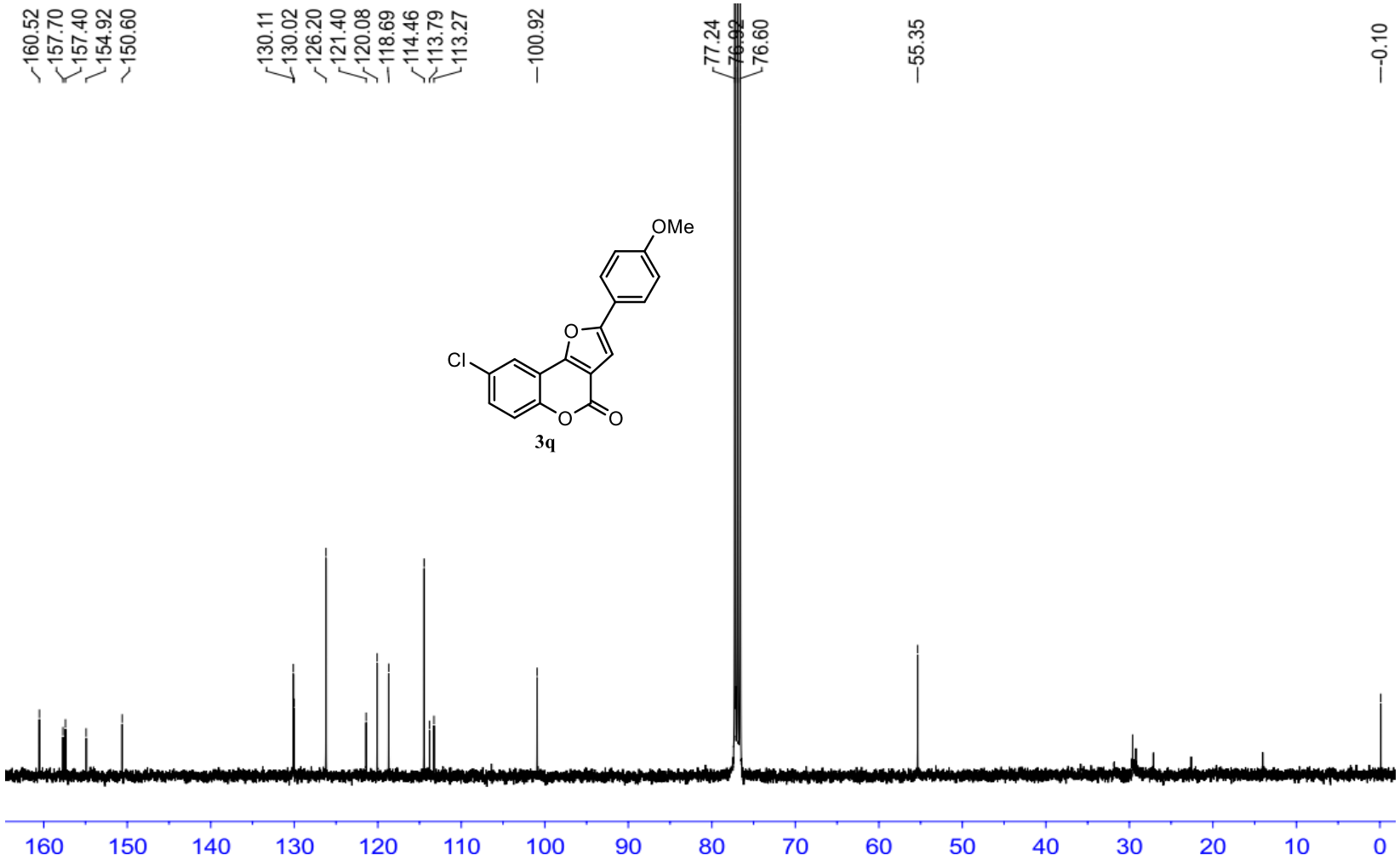
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