Electronic Supplementary Information

Synthesis of new unnatural N^{α}-Fmoc pyrimidin-4-one amino acids: use of p-benzyloxybenzyloxy group as a pyrimidinone masking group

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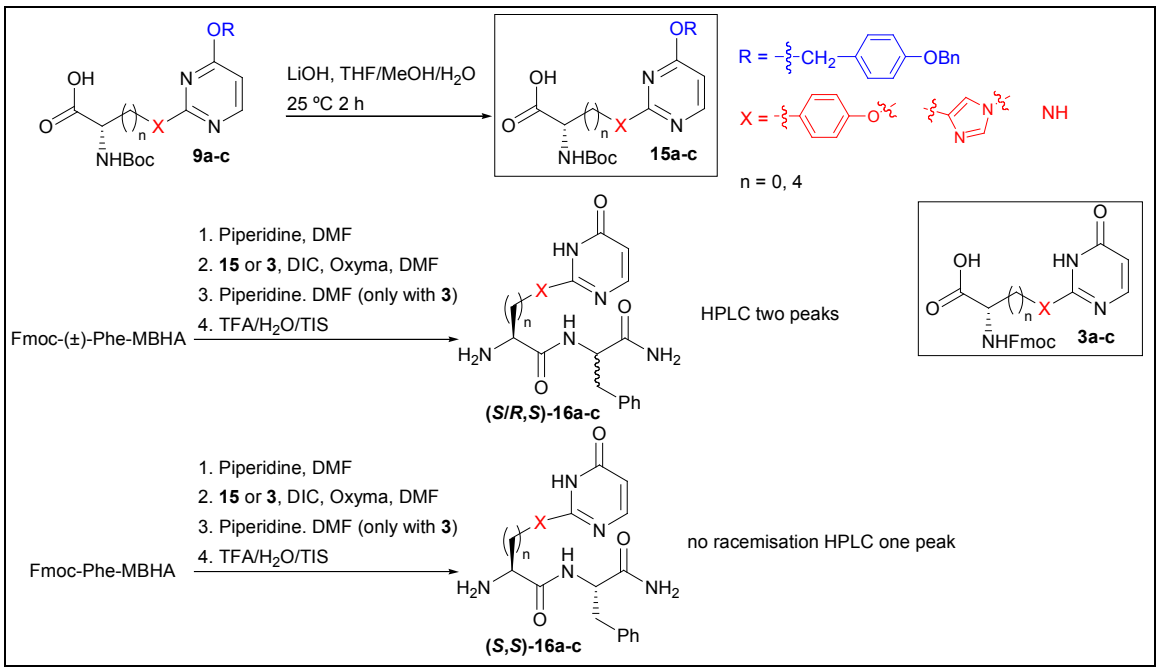
Instruments

High performance liquid chromatography (HPLC) was performed on a Dionex liquid chromatography instrument. Detection was carried out at 220 nm. Analysis was performed using a Kromasil 100 C_{18} (40 mm x 4.6 mm, 3.5 µm particle size) reverse-phase column with a 2-100% B linear gradient over 17 min at a flow rate of 1 mL min^{-1}. Solvent A was 0.1% aqueous TFA, and solvent B was 0.1% TFA in CH_3CN. ESI-MS analyses were performed with an Esquire 6000 ESI ion Trap LC/MS (Bruker Daltonics) instrument equipped with an electrospray ion source. The instrument was operated in the positive ESI(+) ion mode.

Analysis of optical purity of N^{α}-Boc-pyrimidin-4-one amino esters 9 and amino acids 3

The optical purity of compounds **9** was verified by coupling with both racemic phenylalanine and L-phenylalanine in order to measure the degree of racemisation by HPLC. Thus, samples of N^{α}-Boc amino esters **9** (1 equivalent) were deprotected using lithium hydroxide (2.5 equivalents) to give amino acids **15** in near quantitative yield. These compounds were first coupled to a resin bound racemic phenylalanine using standard protocols for solid-phase peptide synthesis following Fmoc/*tert*-butyl strategy. After cleavage from the resin, the HPLC analyses of the resulting dipeptides **(*S*/*R*,*S*)-16** showed the formation of two diastereoisomers. Dipeptides **16** were then synthesized equally by coupling N^{α}-Boc amino acids **15** to a resin bound to L-phenylalanine. When no reasonable racemisation occurred during the synthesis of **9**, HPLC analysis of **(*S*,*S*)-16** showed the formation of one single diastereoisomer (Scheme 1). Analogously, the optical purity of N^{α}-Fmoc amino acids **3** were confirmed by coupling with both racemic phenylalanine and L-phenylalanine. In this case, Fmoc-deprotection step, by treatment with piperidine, was required before the final cleavage of the peptide from the resin (Scheme 1).



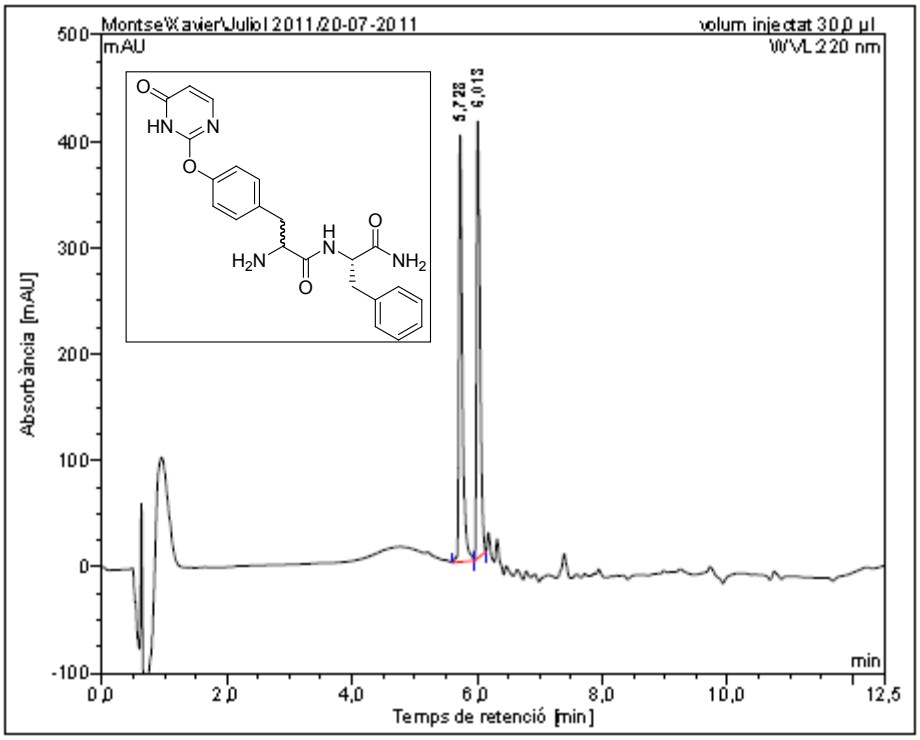
Scheme 1. Solid-phase synthesis of dipeptides **16a-c**.

Synthesis of Dipeptides 16. General procedure

Dipeptides **16** were prepared manually by solid-phase method using Fmoc-Rink-MBHA resin (0.56 mmol/g) as solid support following standard Fmoc-strategy. Fmoc group was removed with 30% piperidine in DMF (2 min + 10 min). Coupling of amino acids were mediated by *N*,*N*'-diisopropylcarbodiimide DIC (4 equiv) and 2-cyano-2-(hydroxyimino)acetate (Oxyma Pure) (4 equiv) in DMF at room temperature for 1-3 h. The completion of the coupling reactions was checked by the Kaiser test.[1] After each coupling and deprotection step, the resin was washed with DMF (6 x 1 min), and CH_2Cl_2 (3 x 1 min), and air dried. The Fmoc-Rink-MBHA resin (10 mg) was placed into a plastic syringe fitted with a polypropylene frit, and the Fmoc protecting group was removed followed by coupling with Fmoc-Phe-OH. Then, the Fmoc protecting group was removed again and the resin was treated with pyrimidinyl amino acids **15** or N^{α}-Fmoc amino acids **3** under coupling conditions. In the case of amino acids **3** the Fmoc group was removed before the final cleavage of the peptide. The resulting dipeptides were cleaved from resin by treatment with TFA/H_2O/TIS (95:2.5:2.5) for 2 h. Then, the solvents were evaporated to dryness and the crude dipeptides **16** were dissolved in H_2O, lyophilized, analysed by HPLC, and characterized by mass spectrometry.

[1] Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595-598

HPLC of dipeptide (*S*/*R*,*S*)-16a



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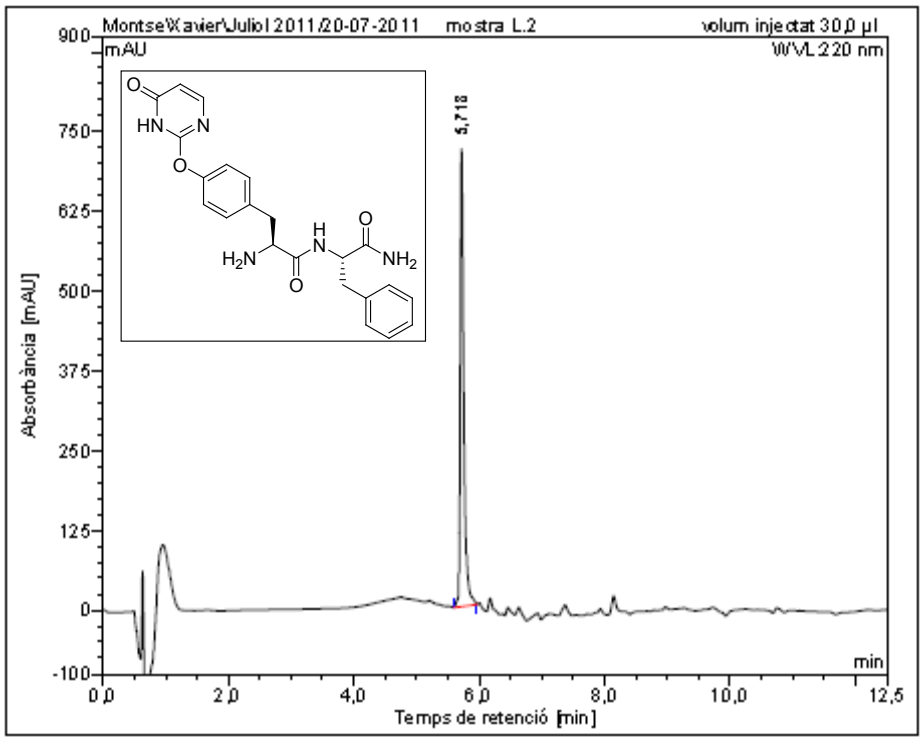

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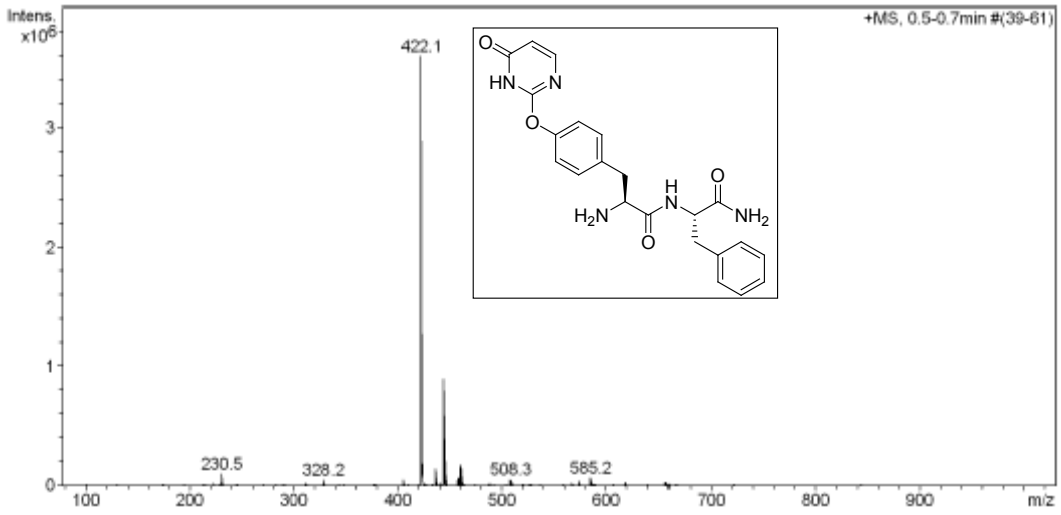

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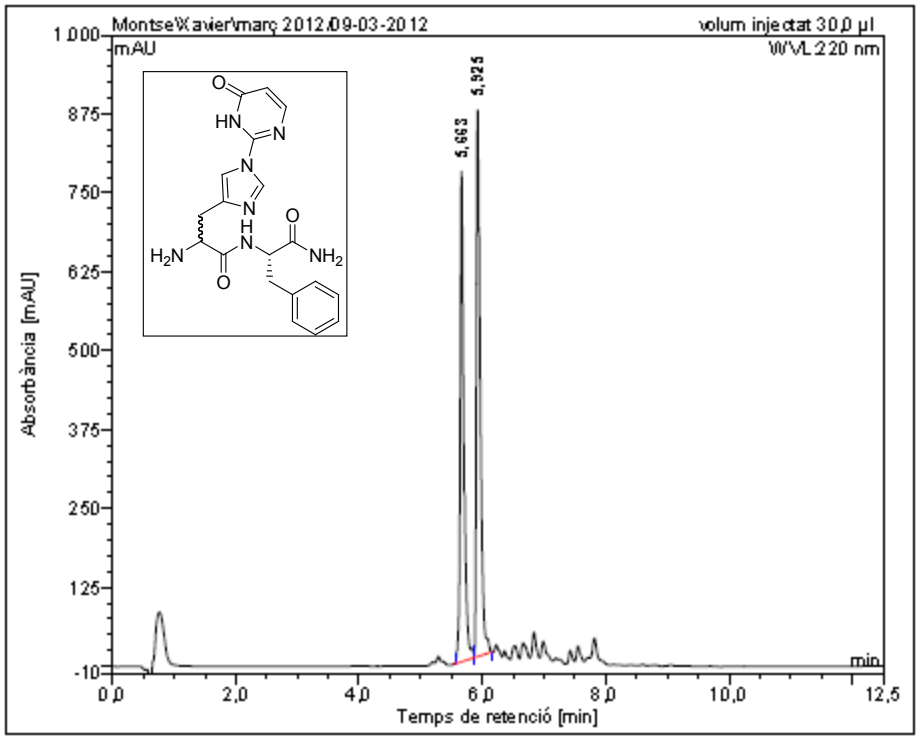

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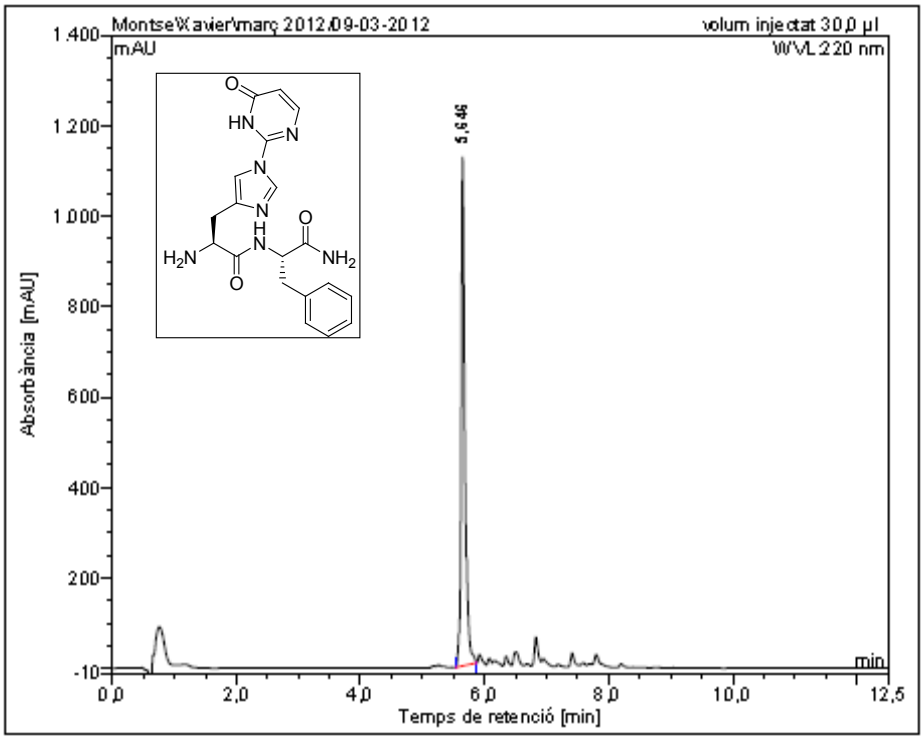

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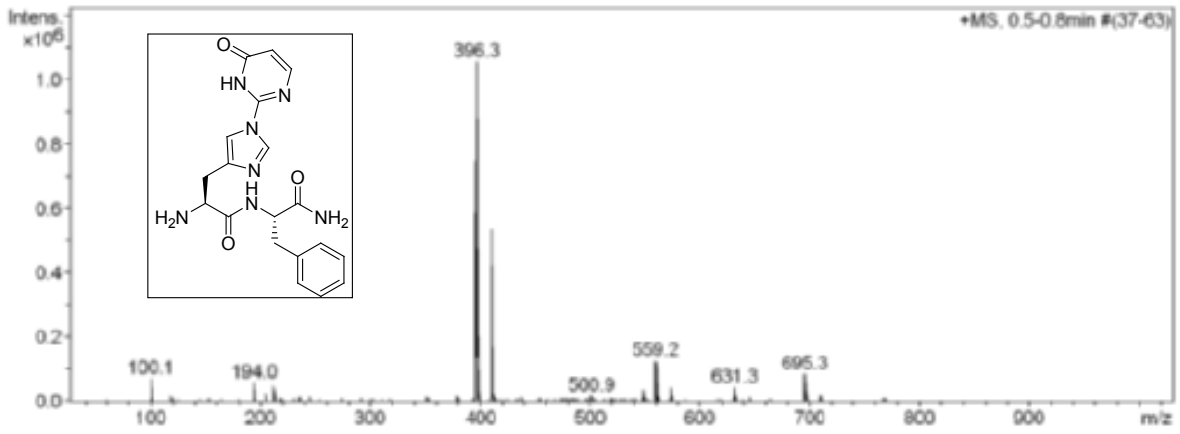

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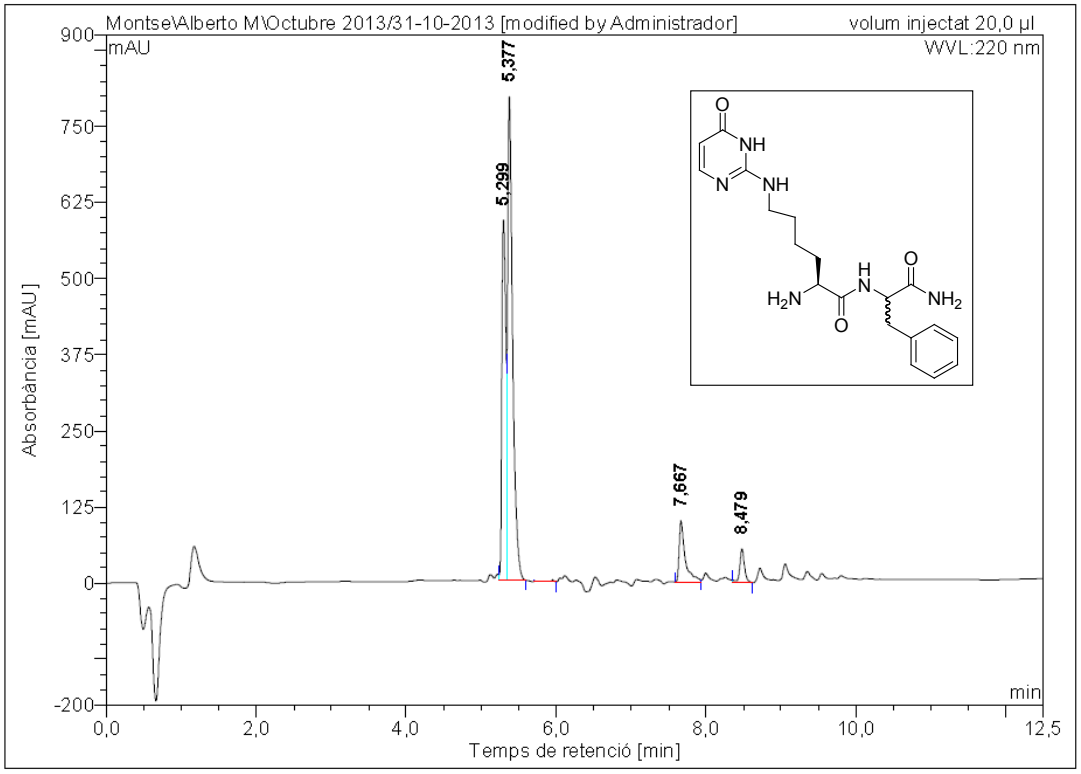

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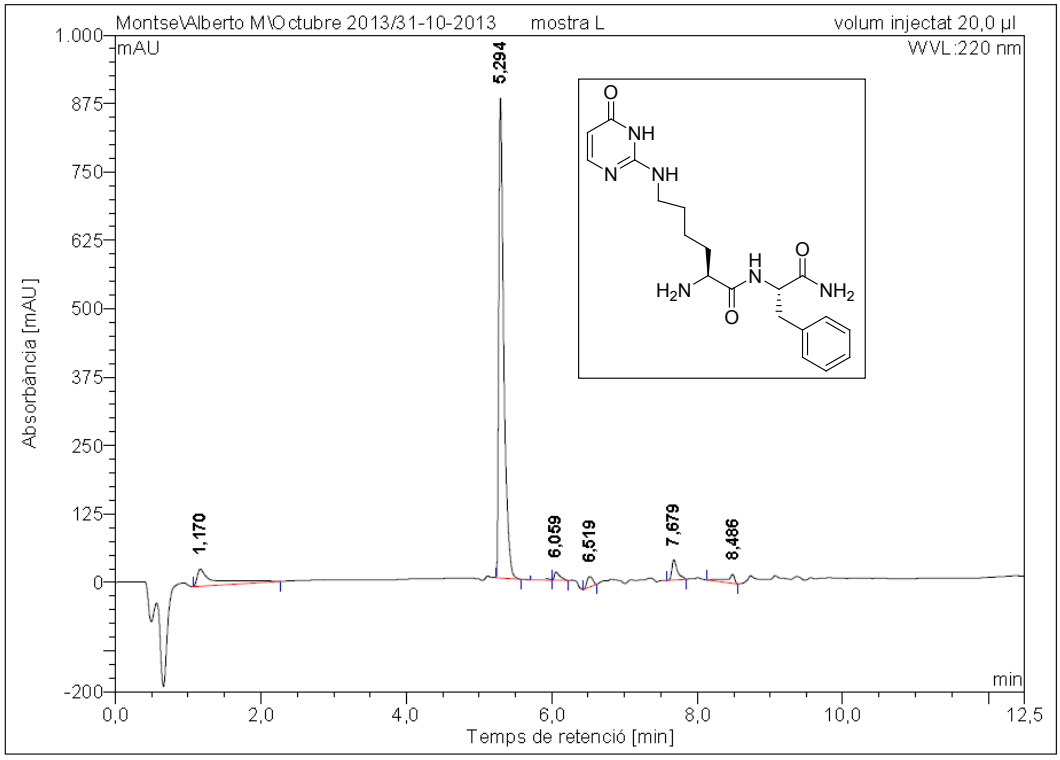

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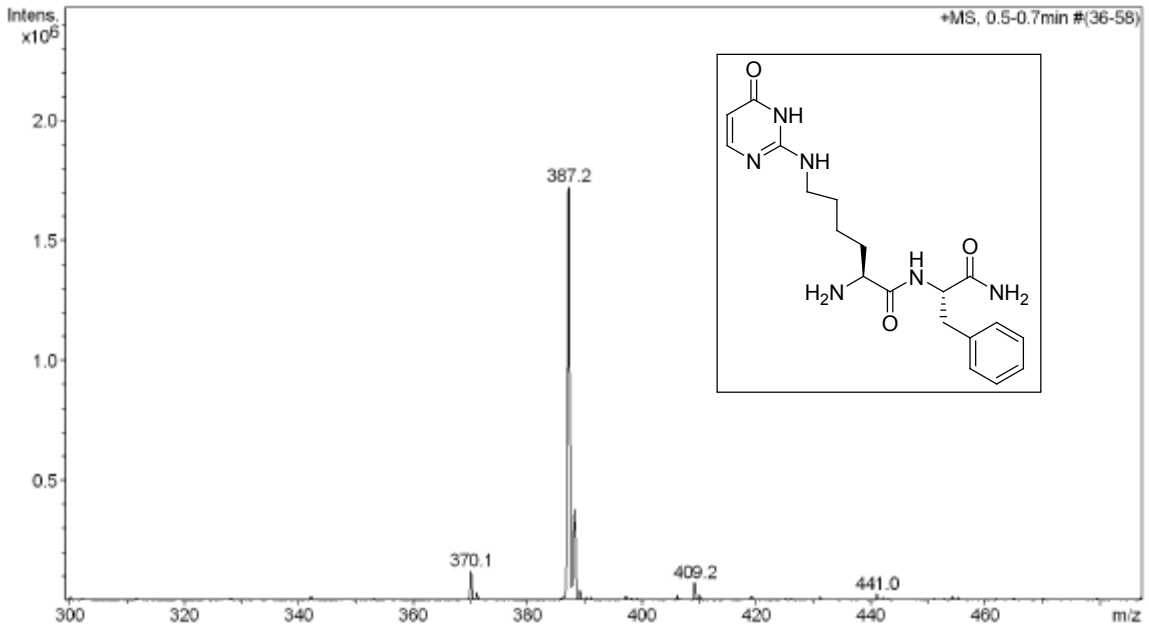

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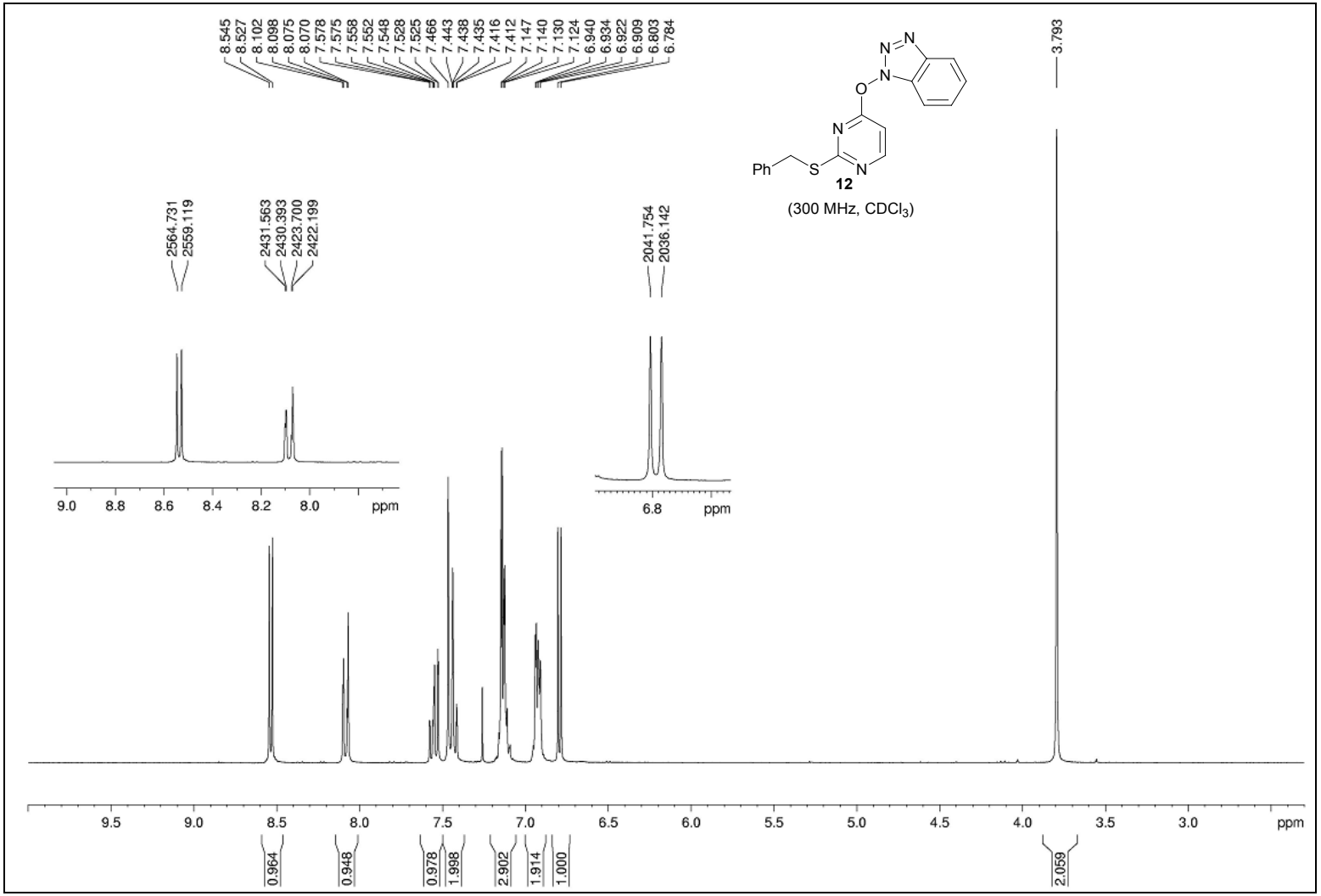
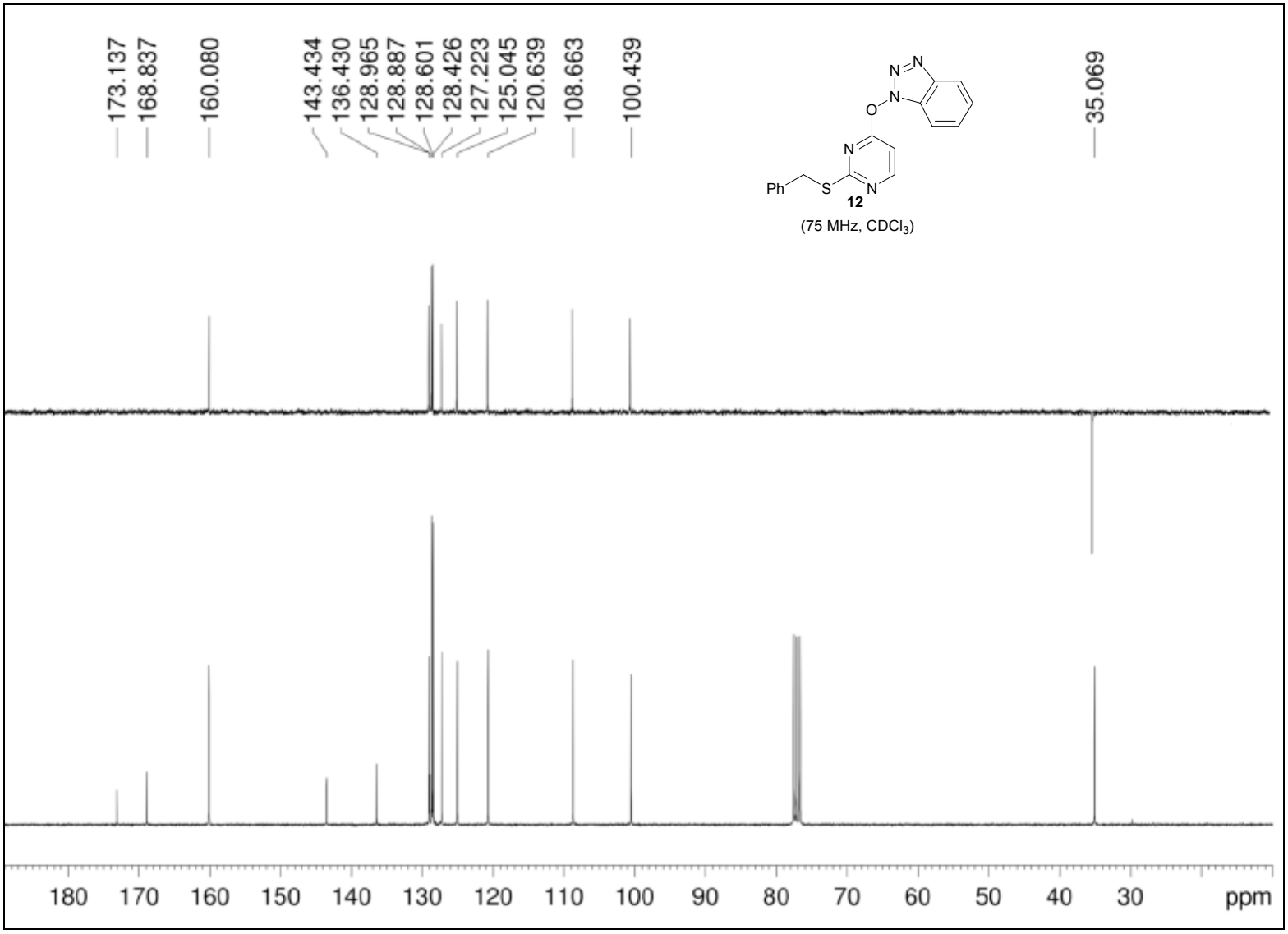


^{13}C-NMR and DEPT experiment of compound 12



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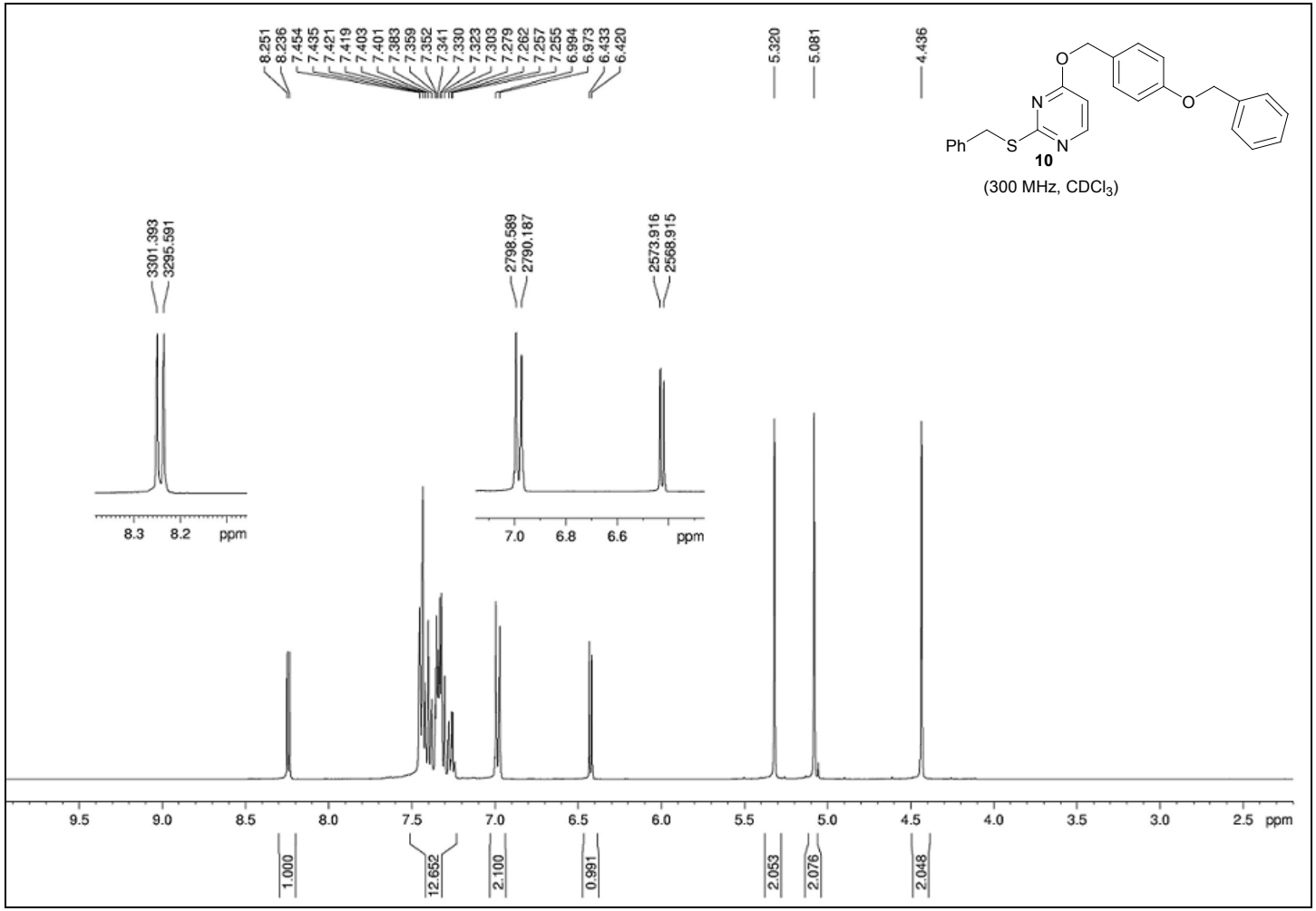

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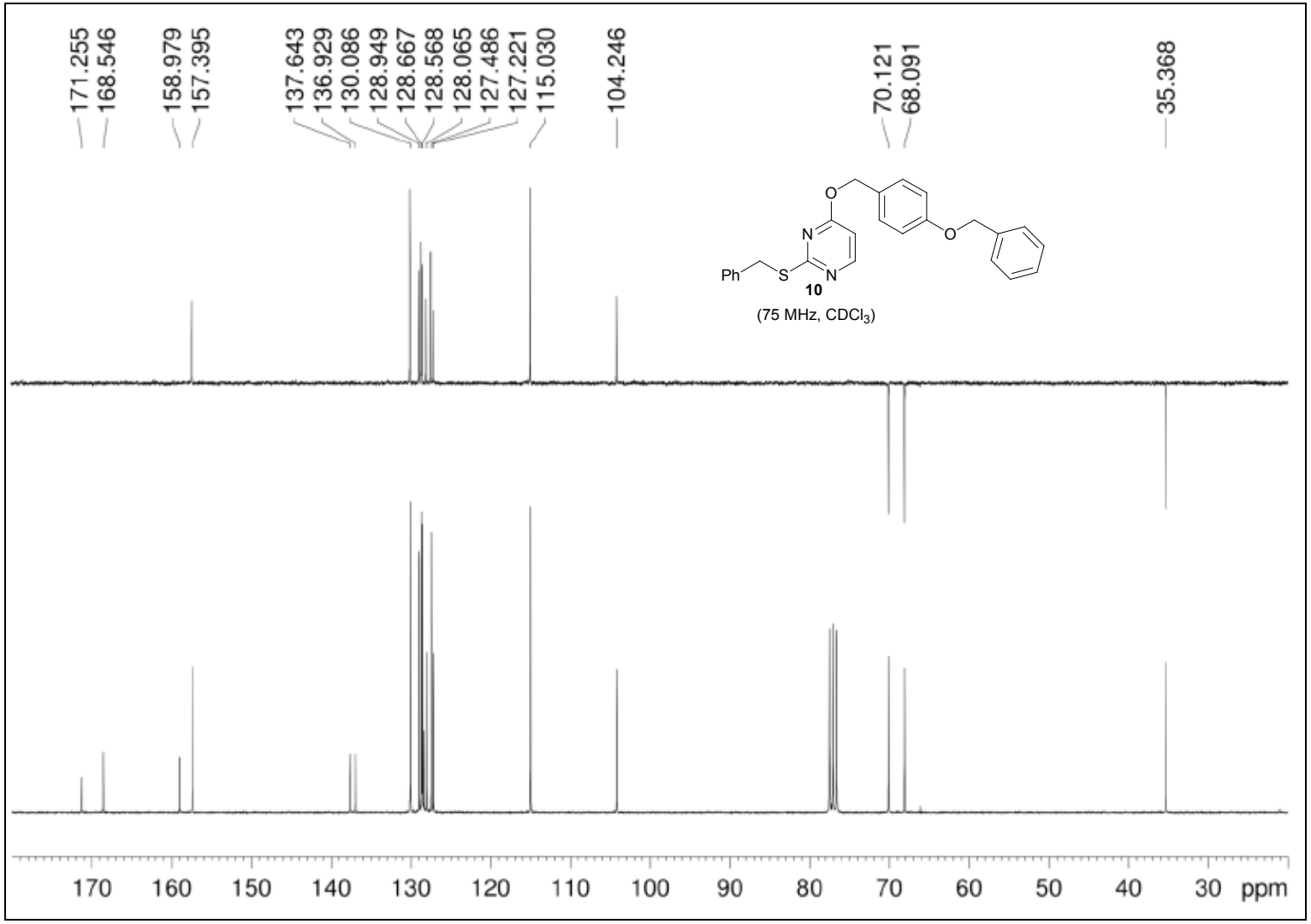

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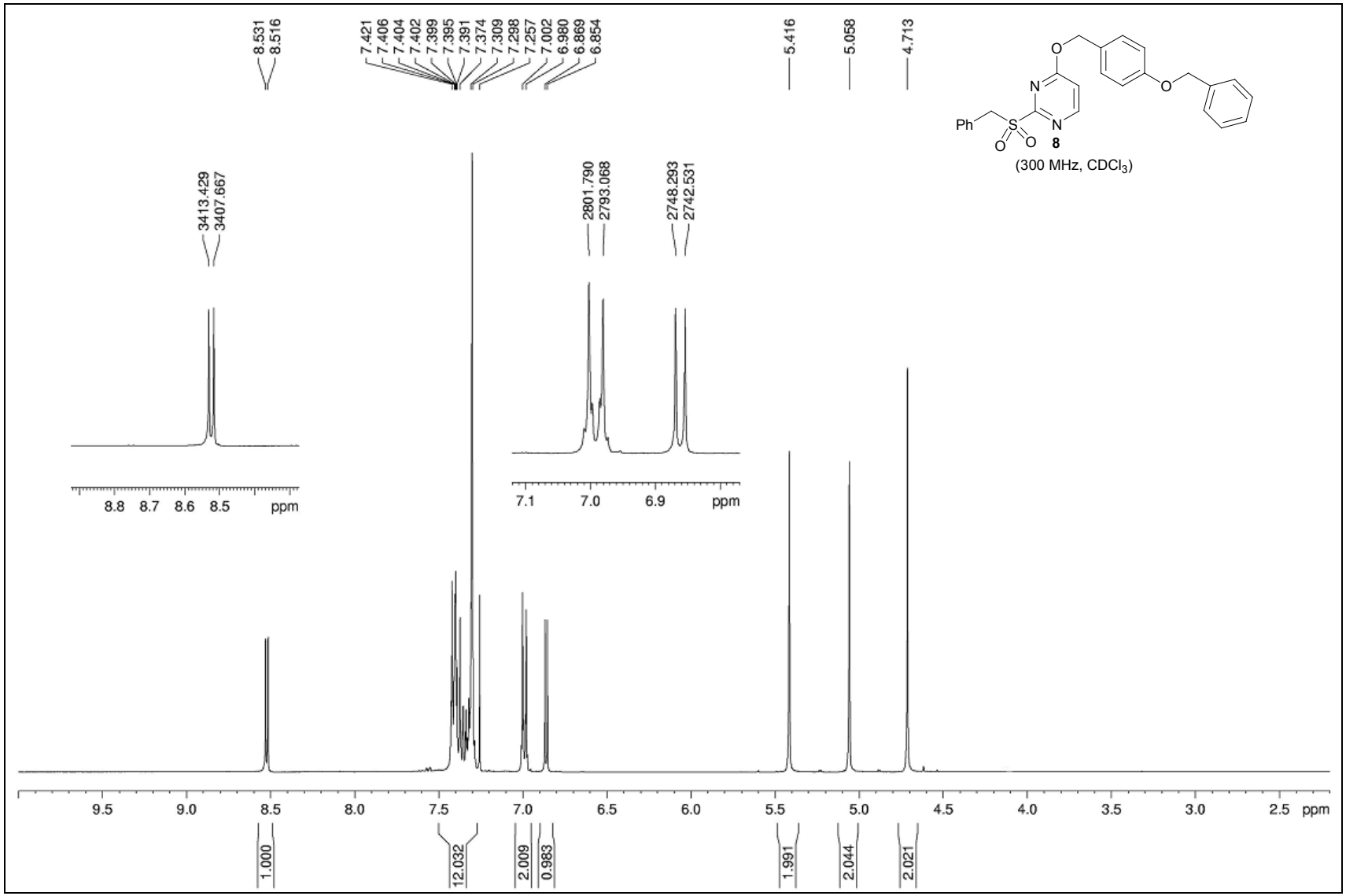

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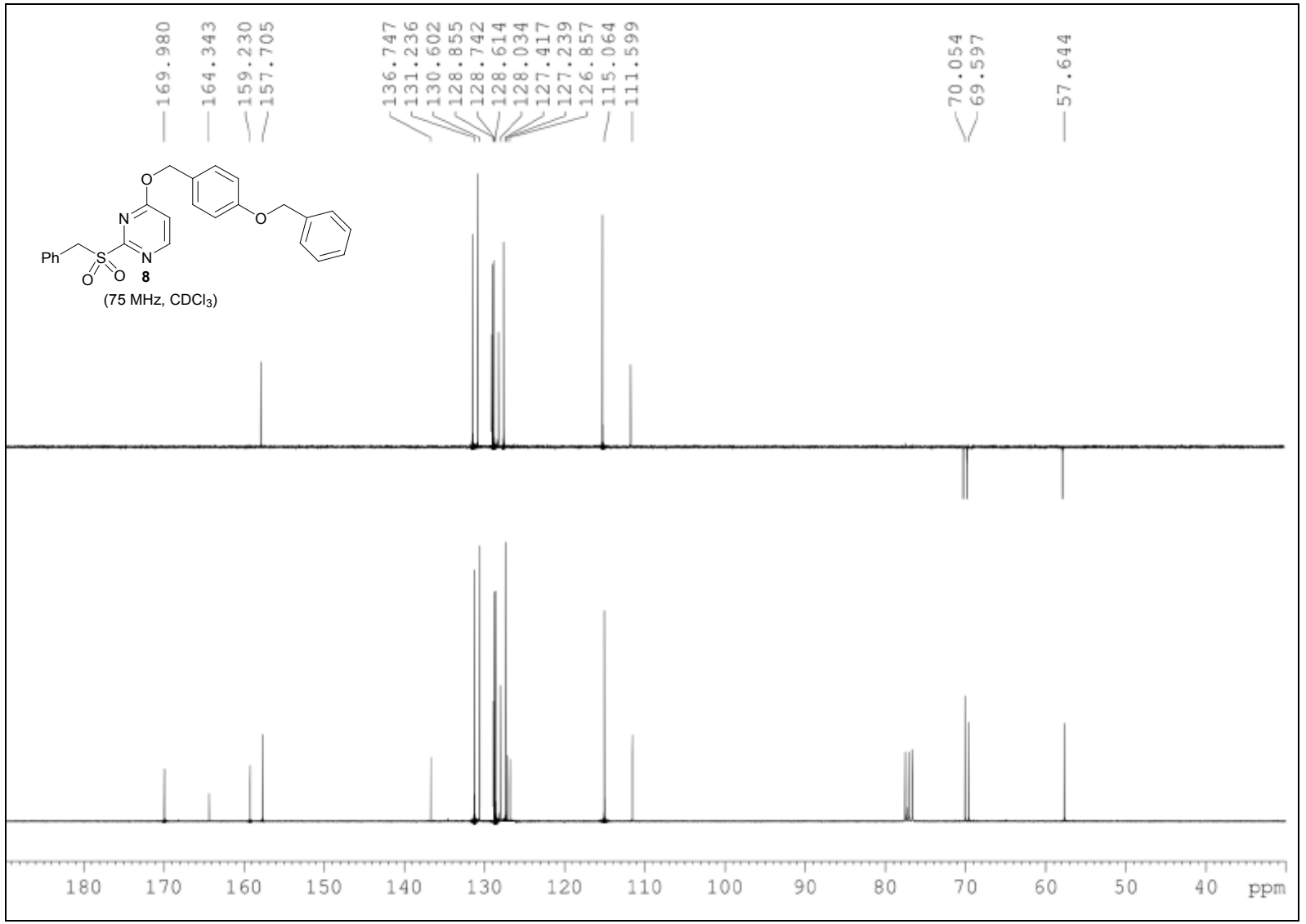

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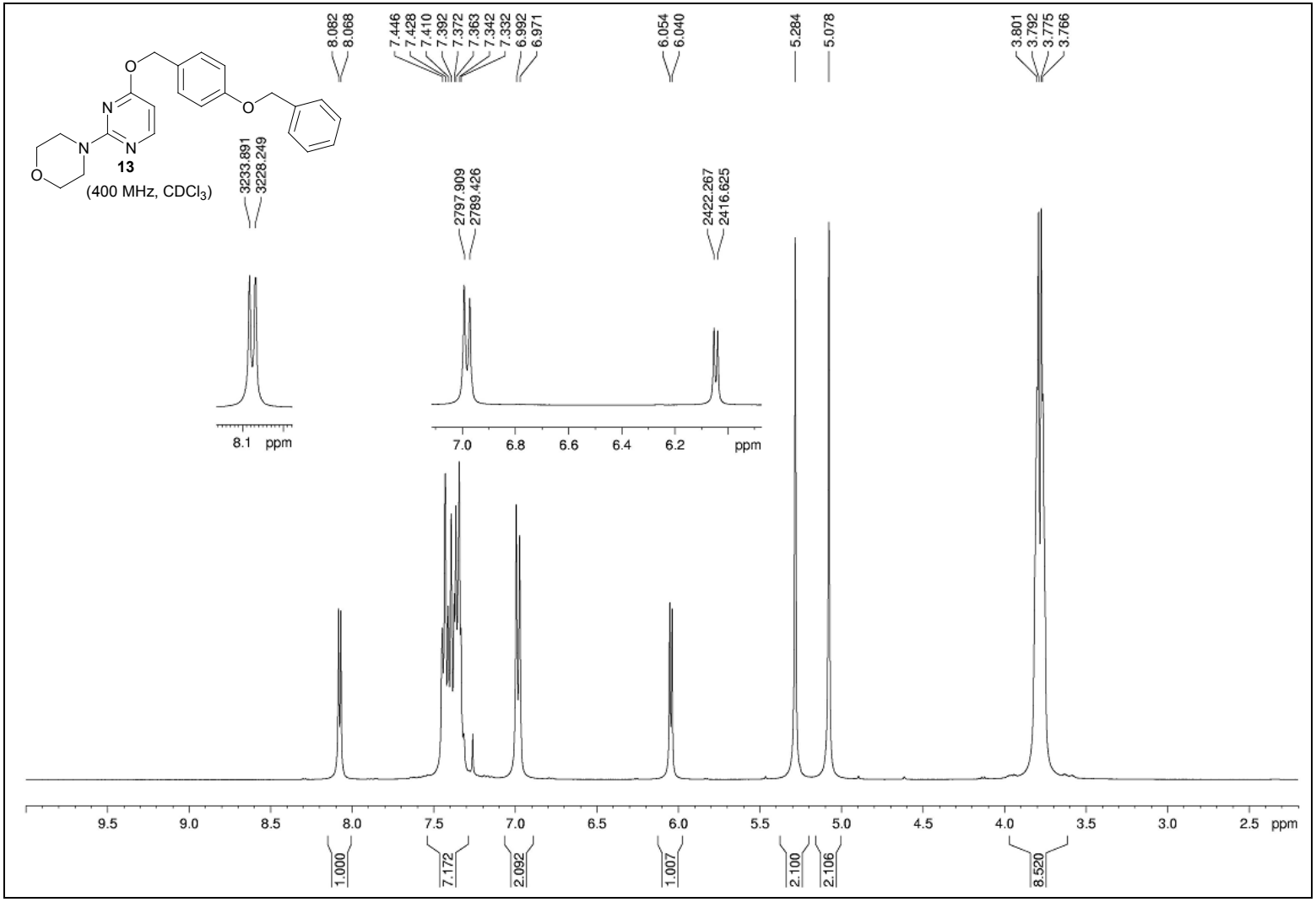

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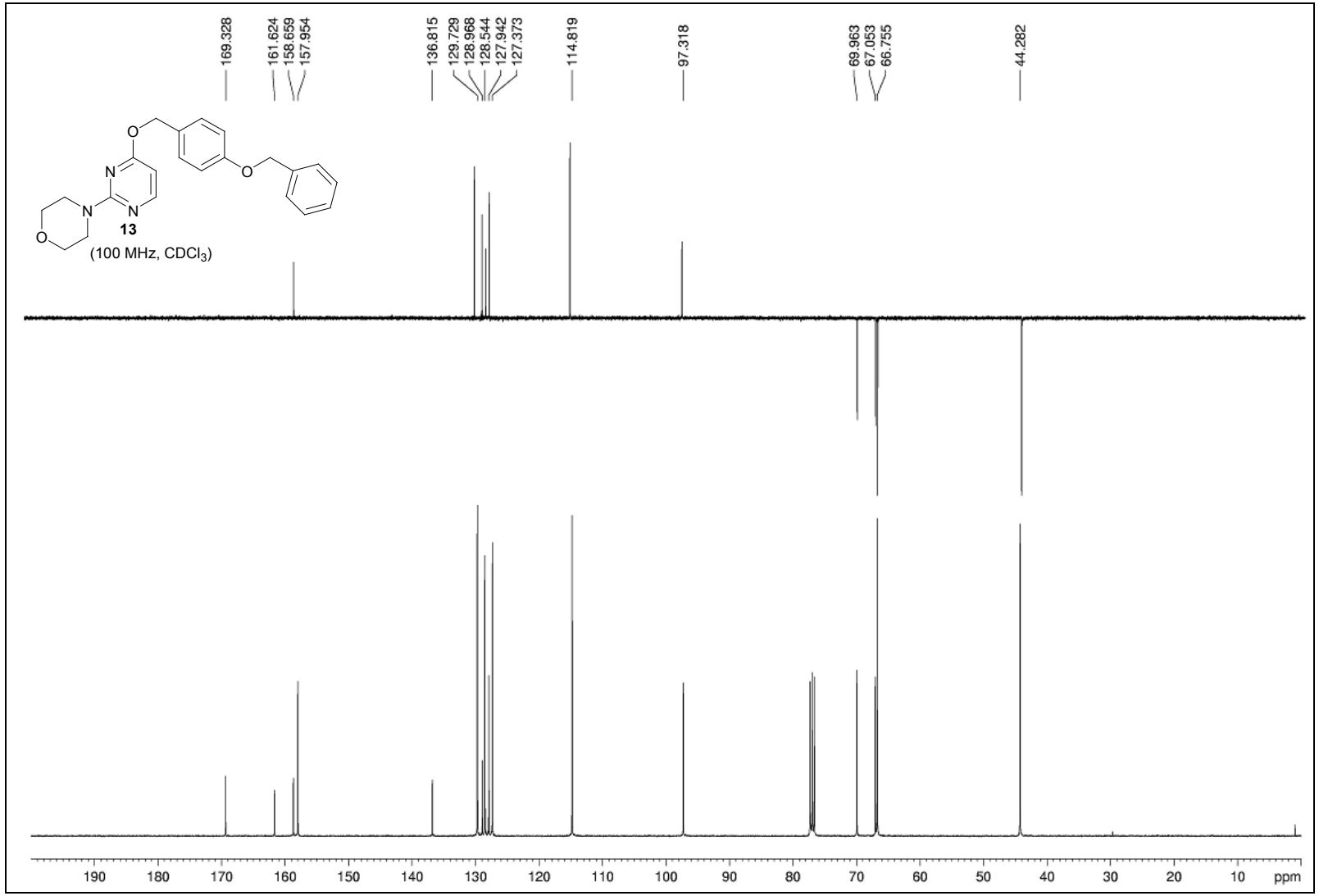

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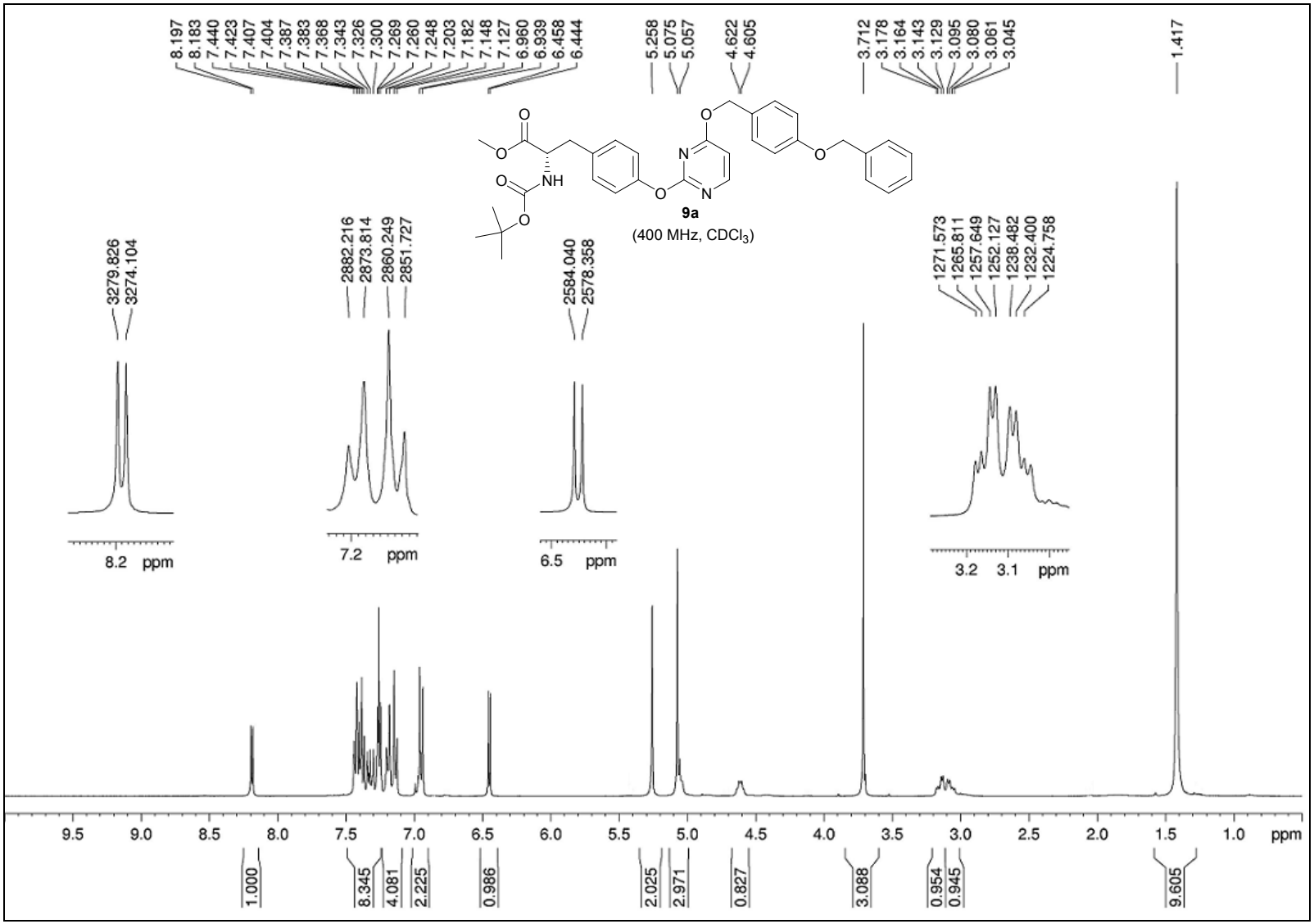

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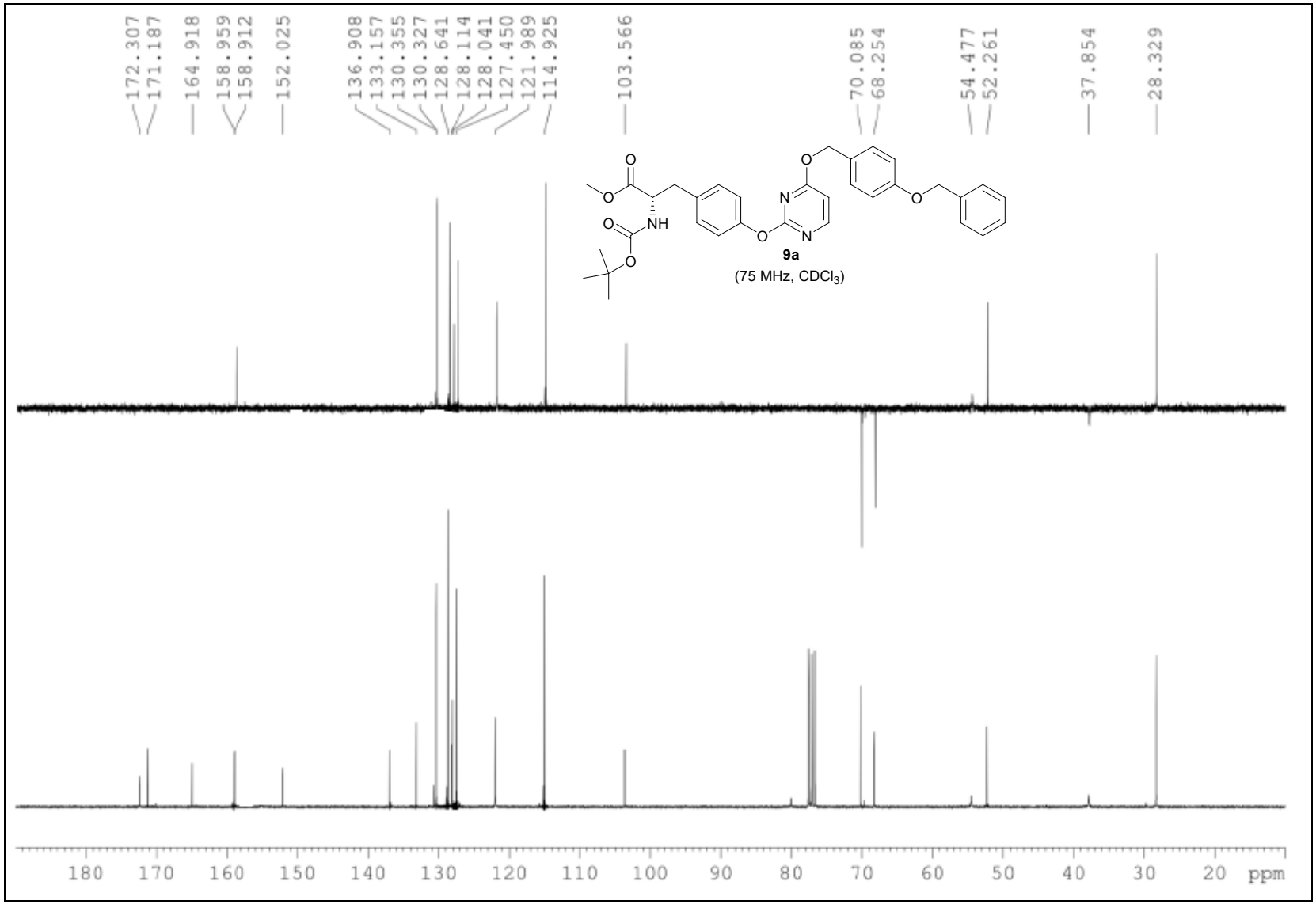

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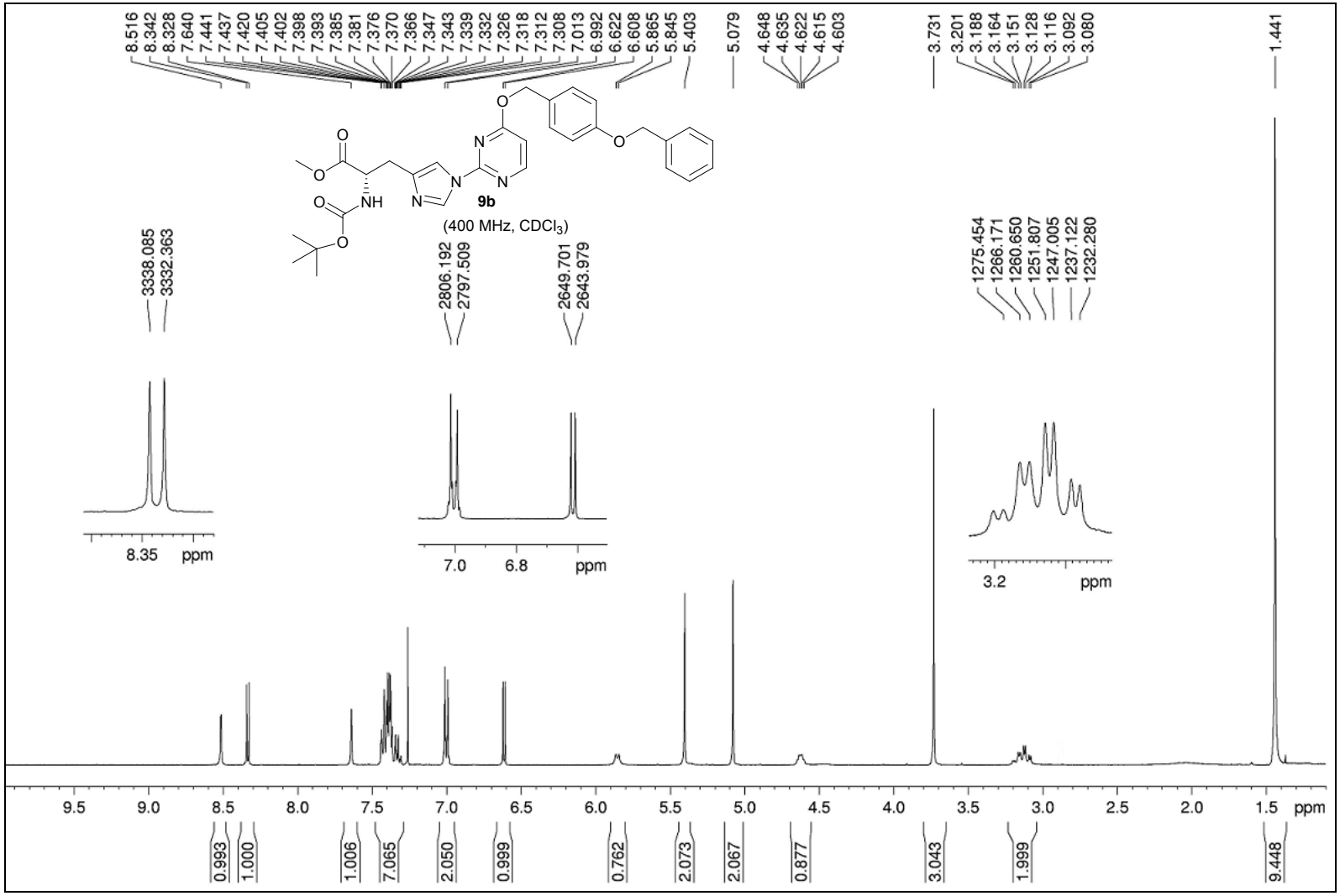

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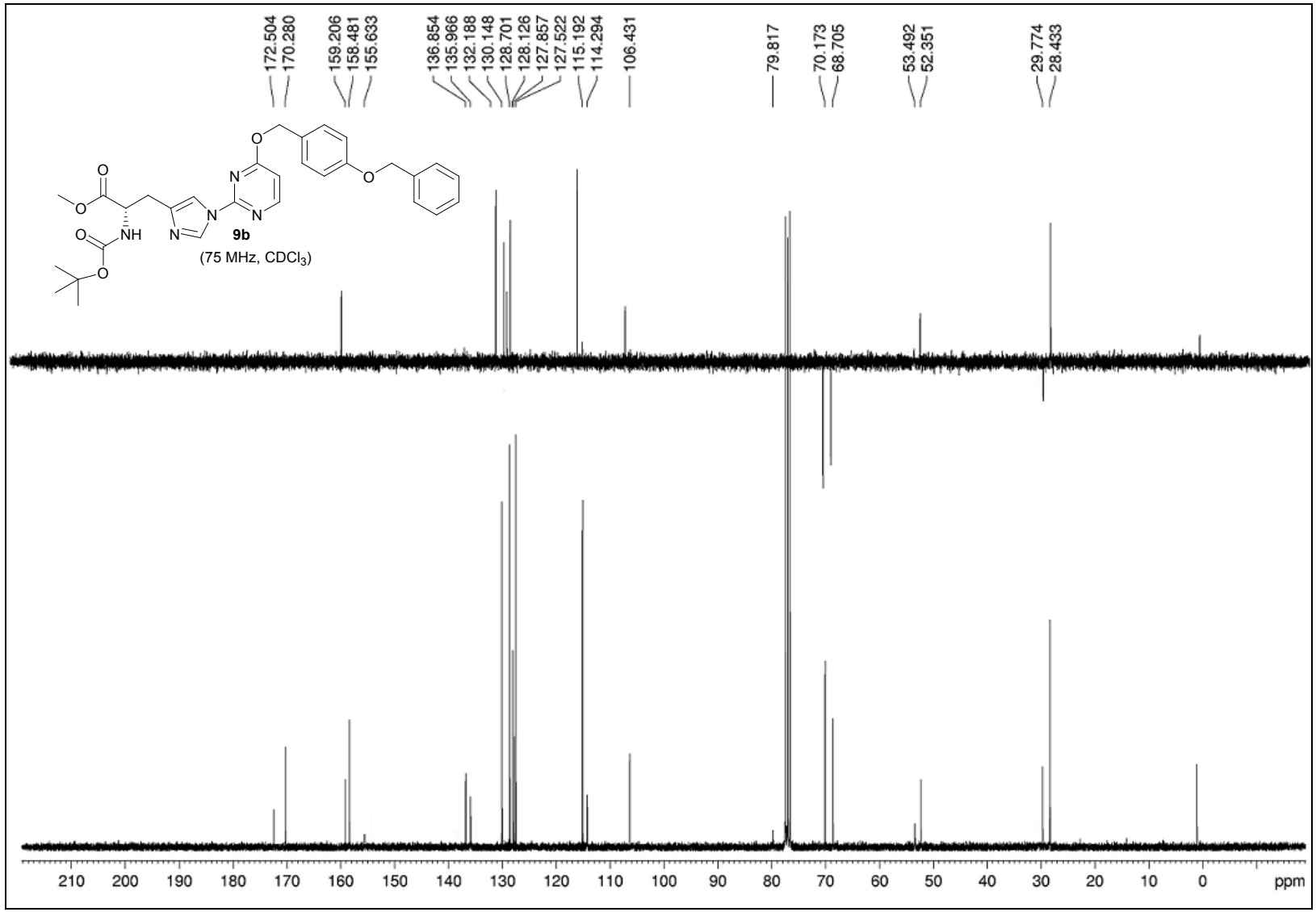

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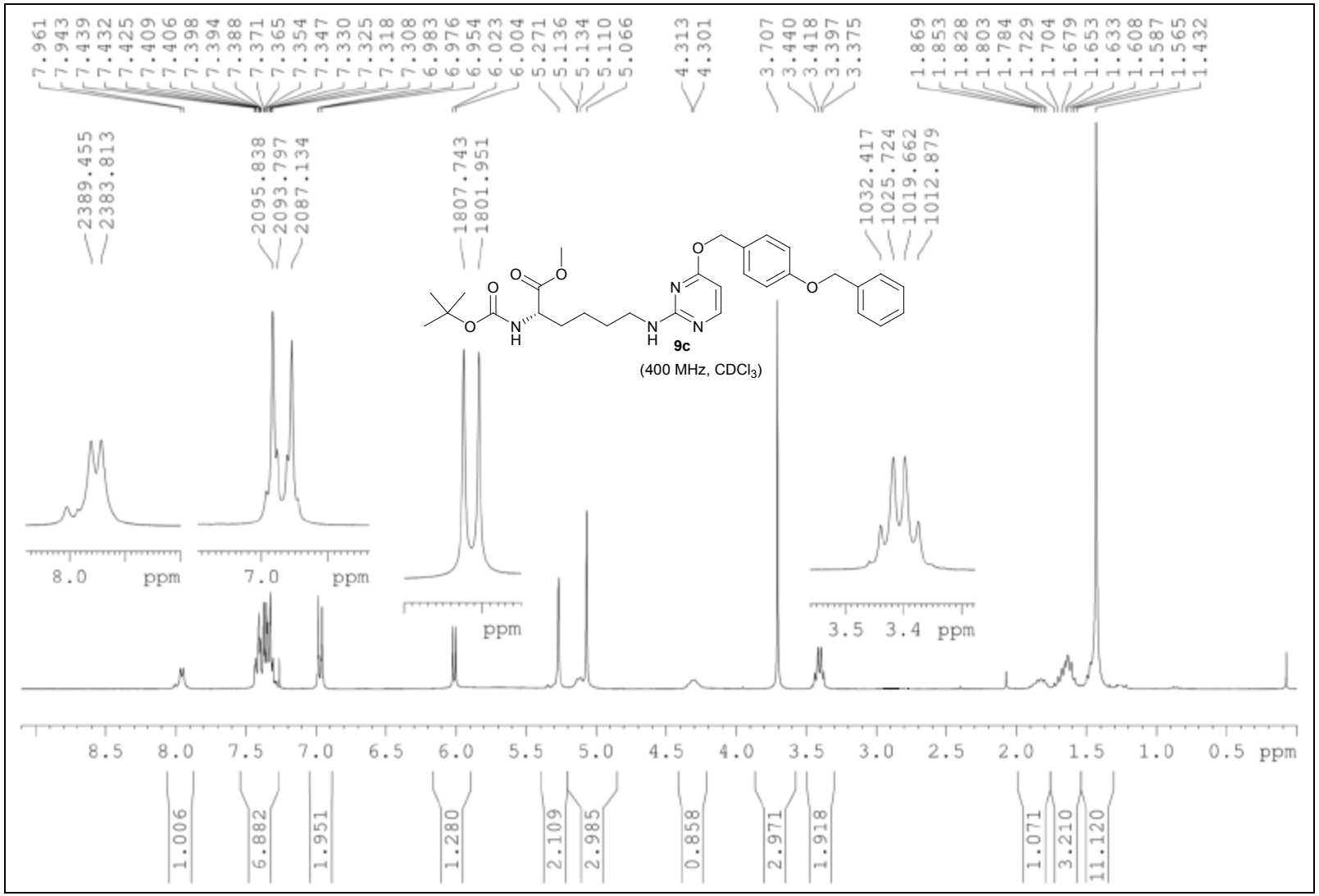
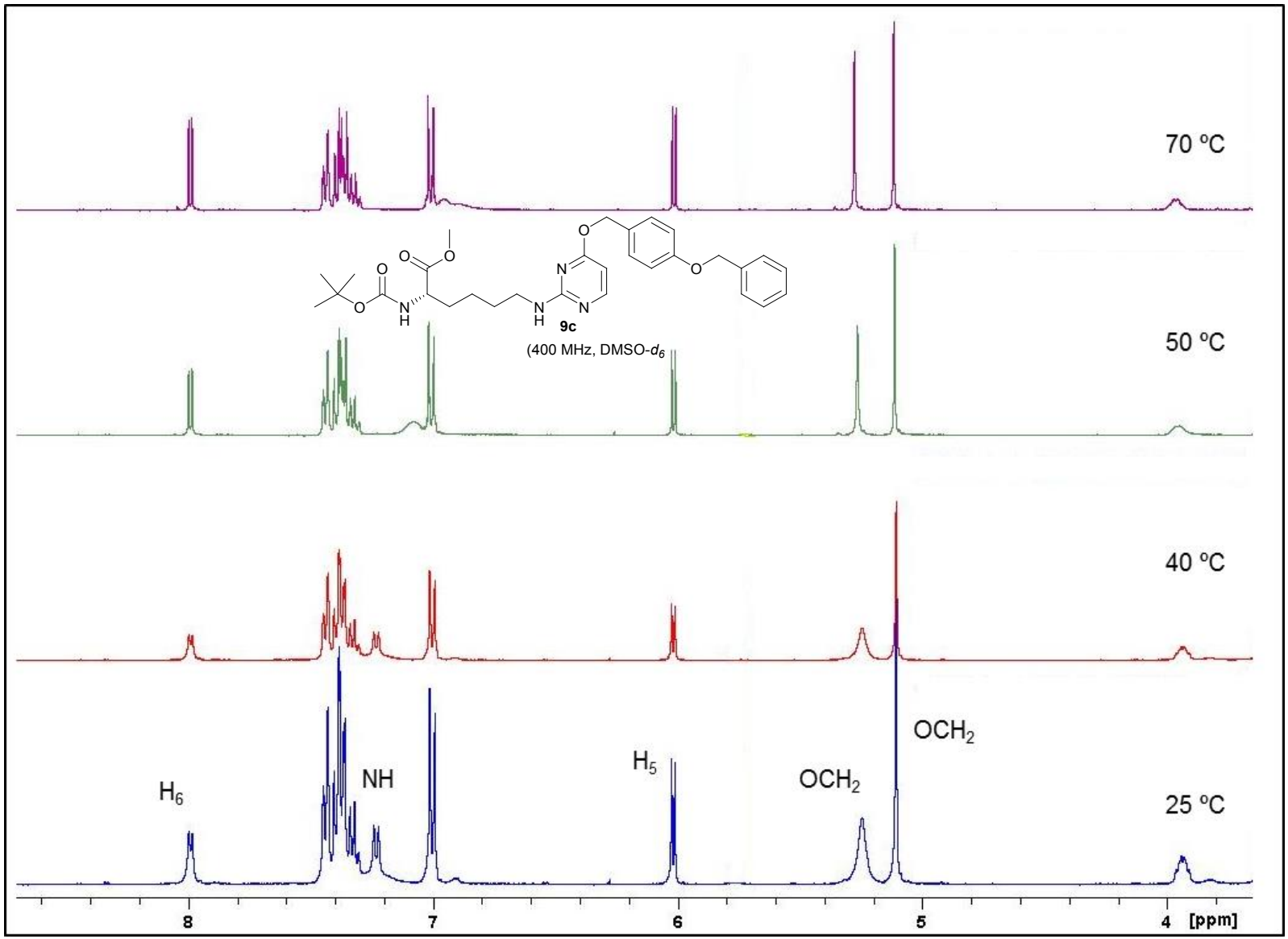
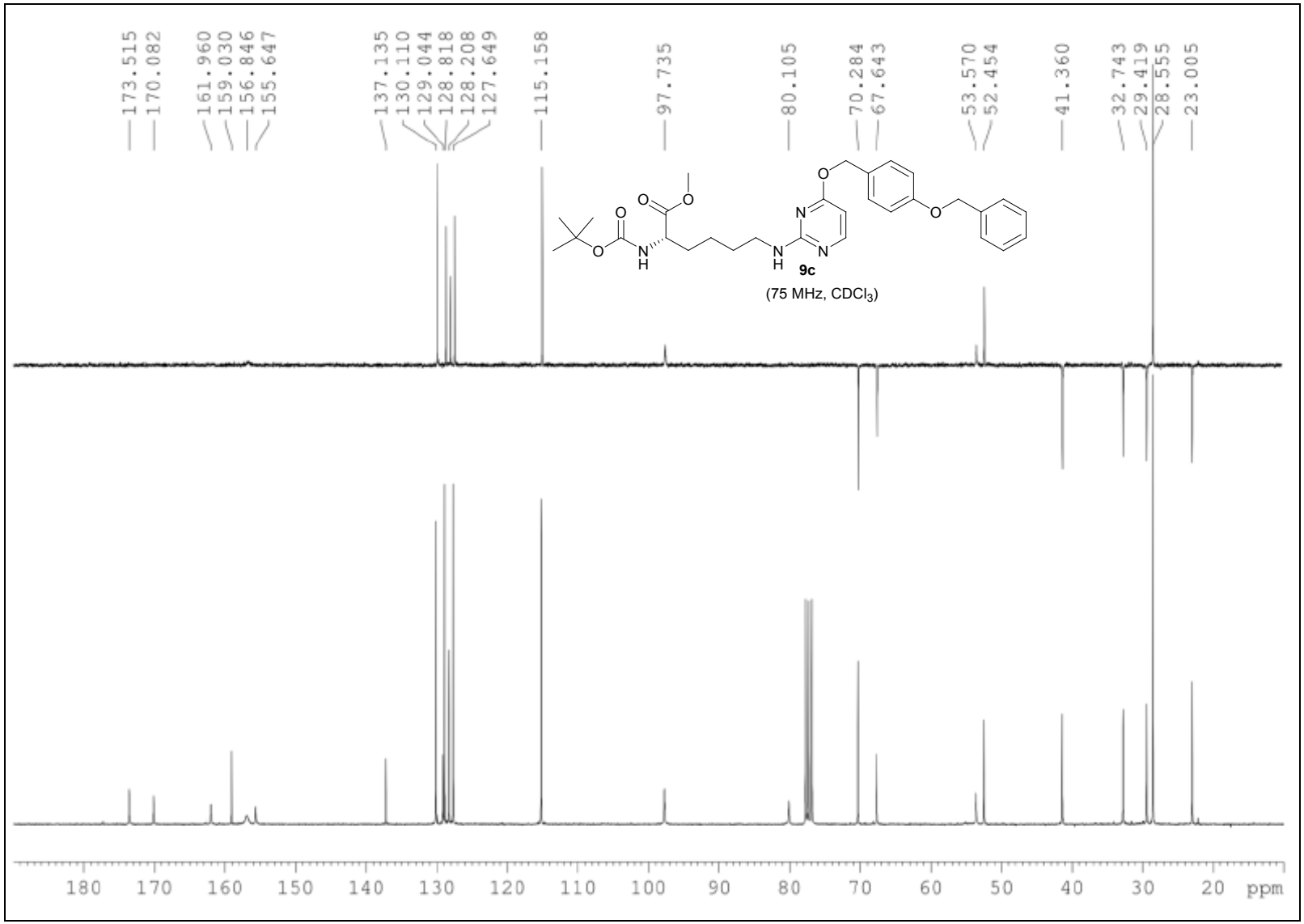


Variable temperature experiment ^{1}H-NMR of compound 9c



^{13}C-NMR and DEPT experiment of compound 9c



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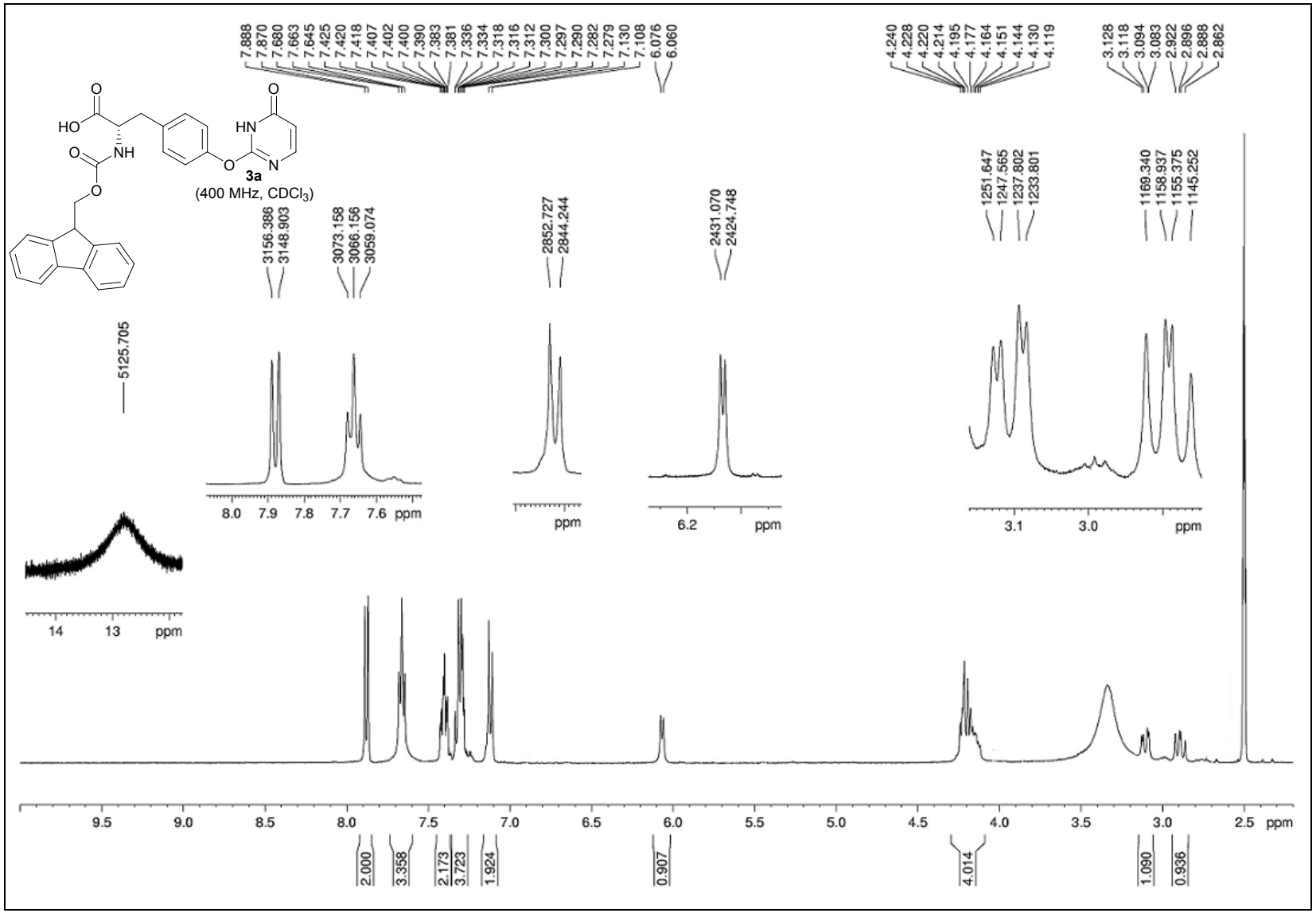

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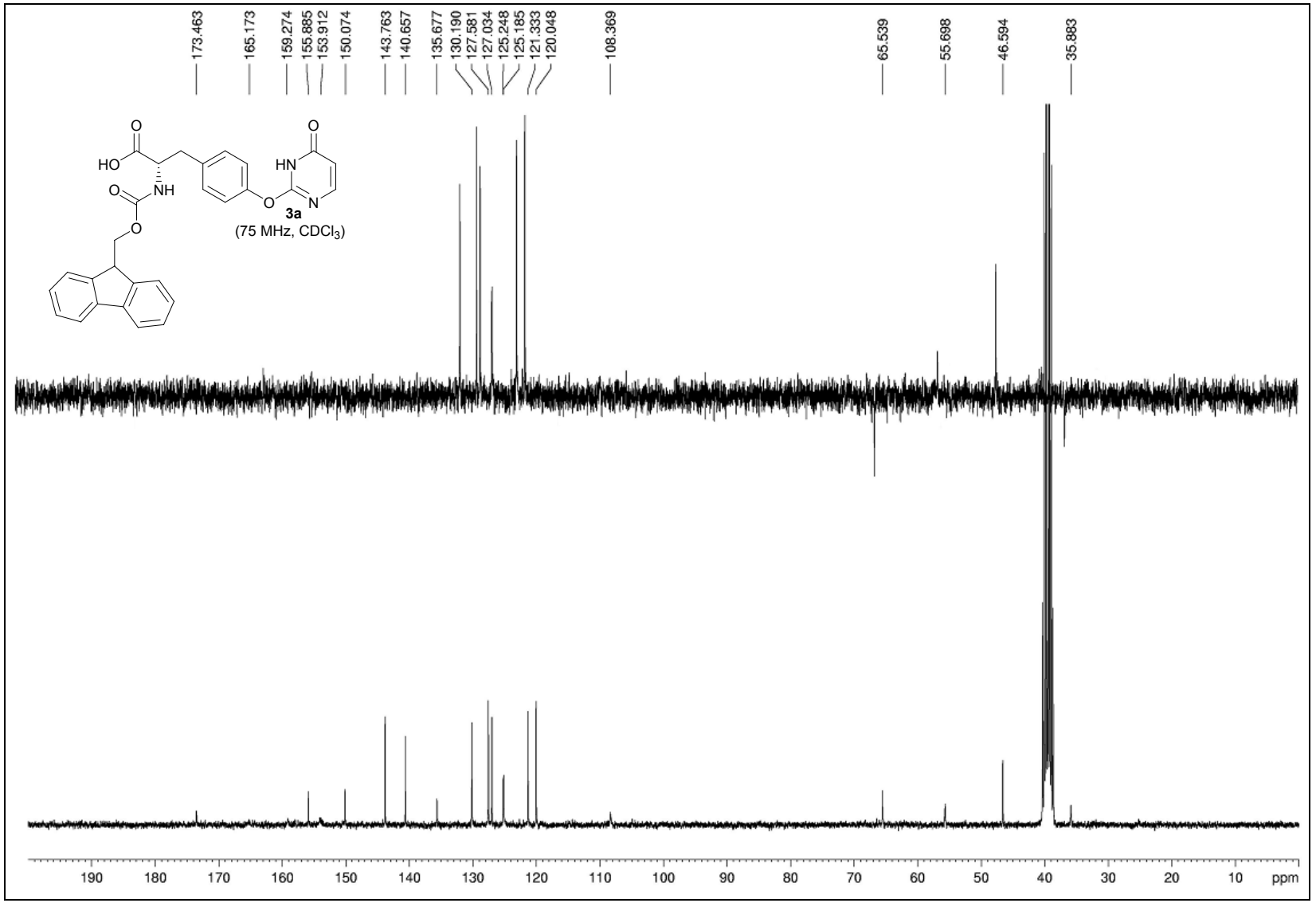

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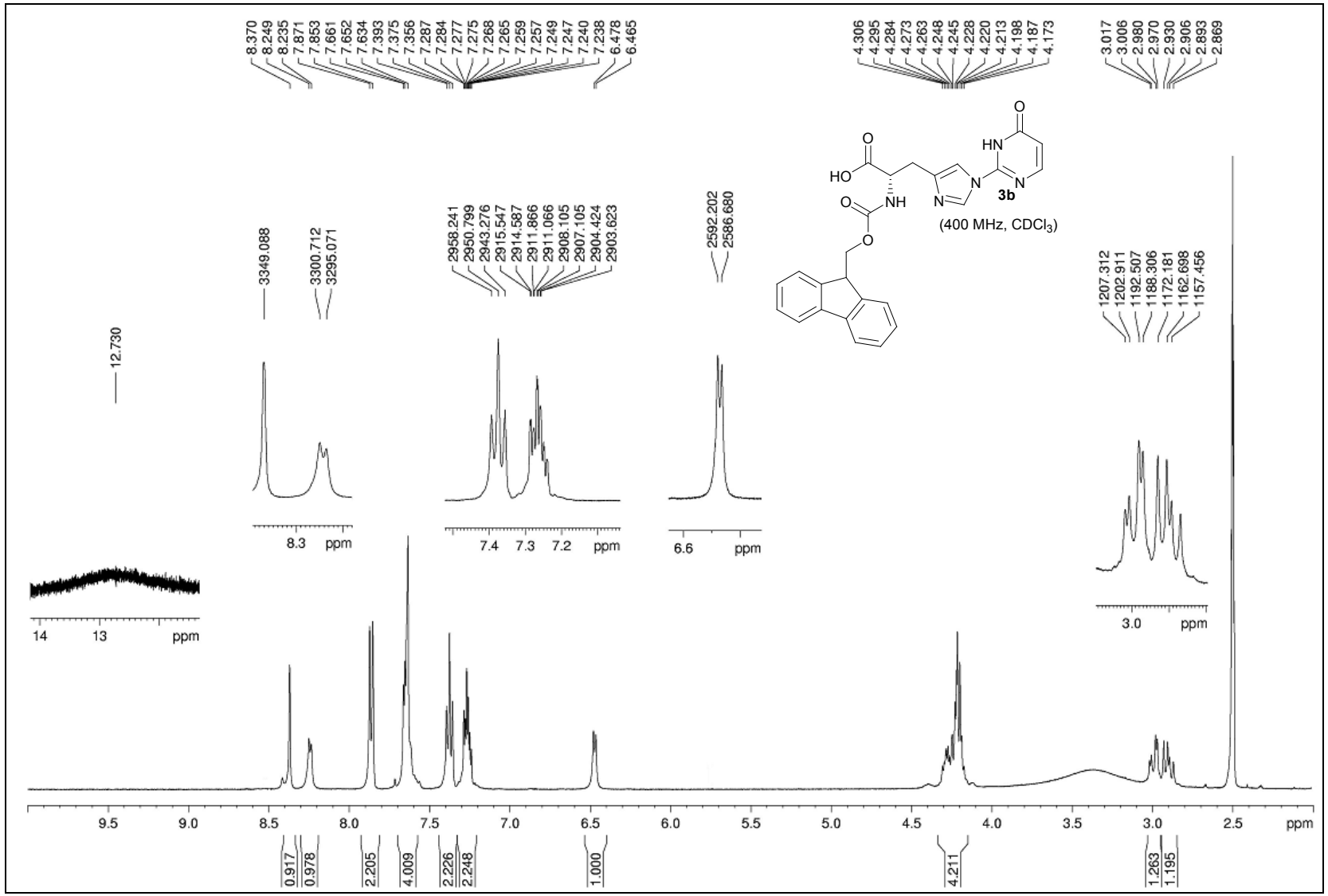

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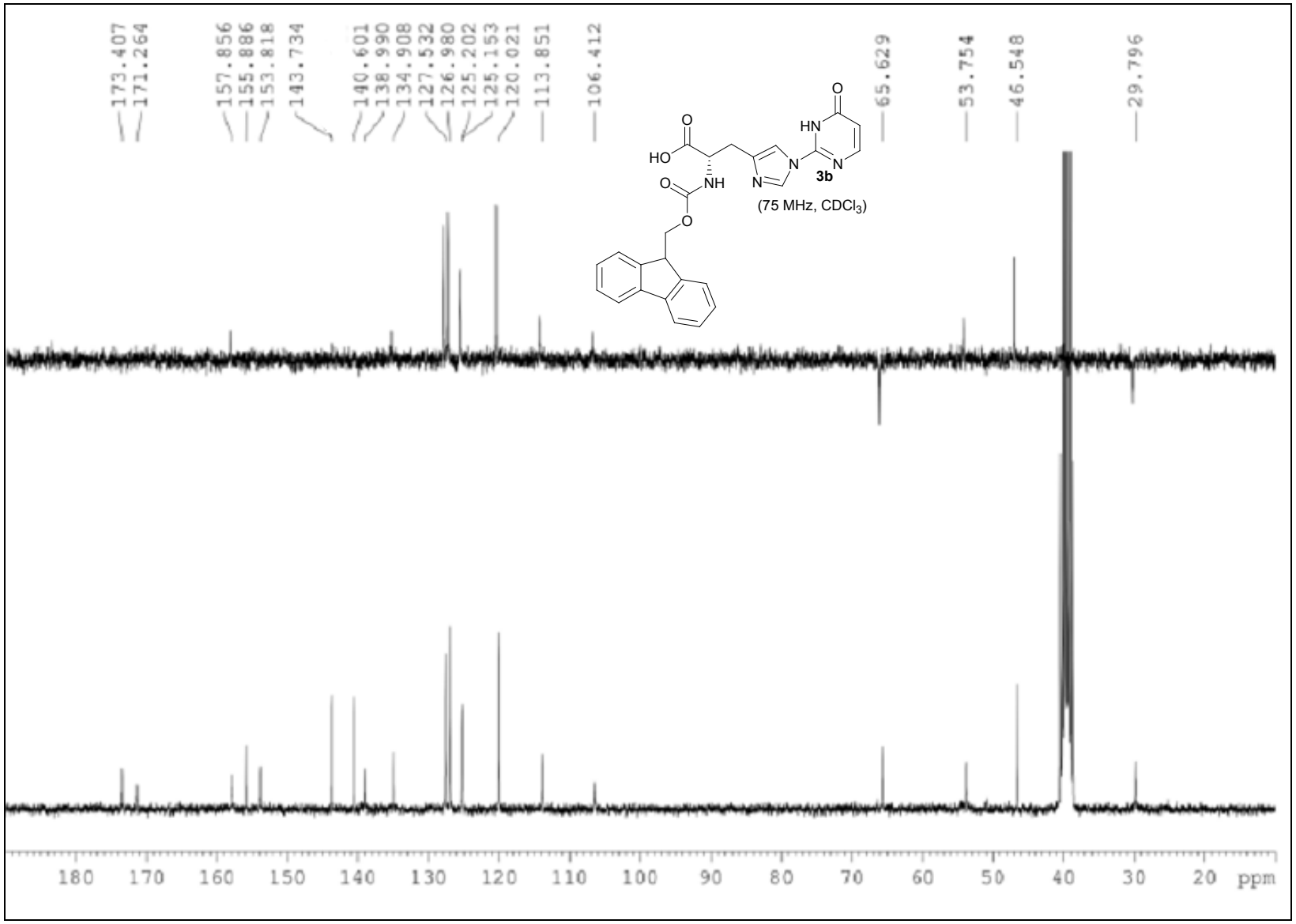

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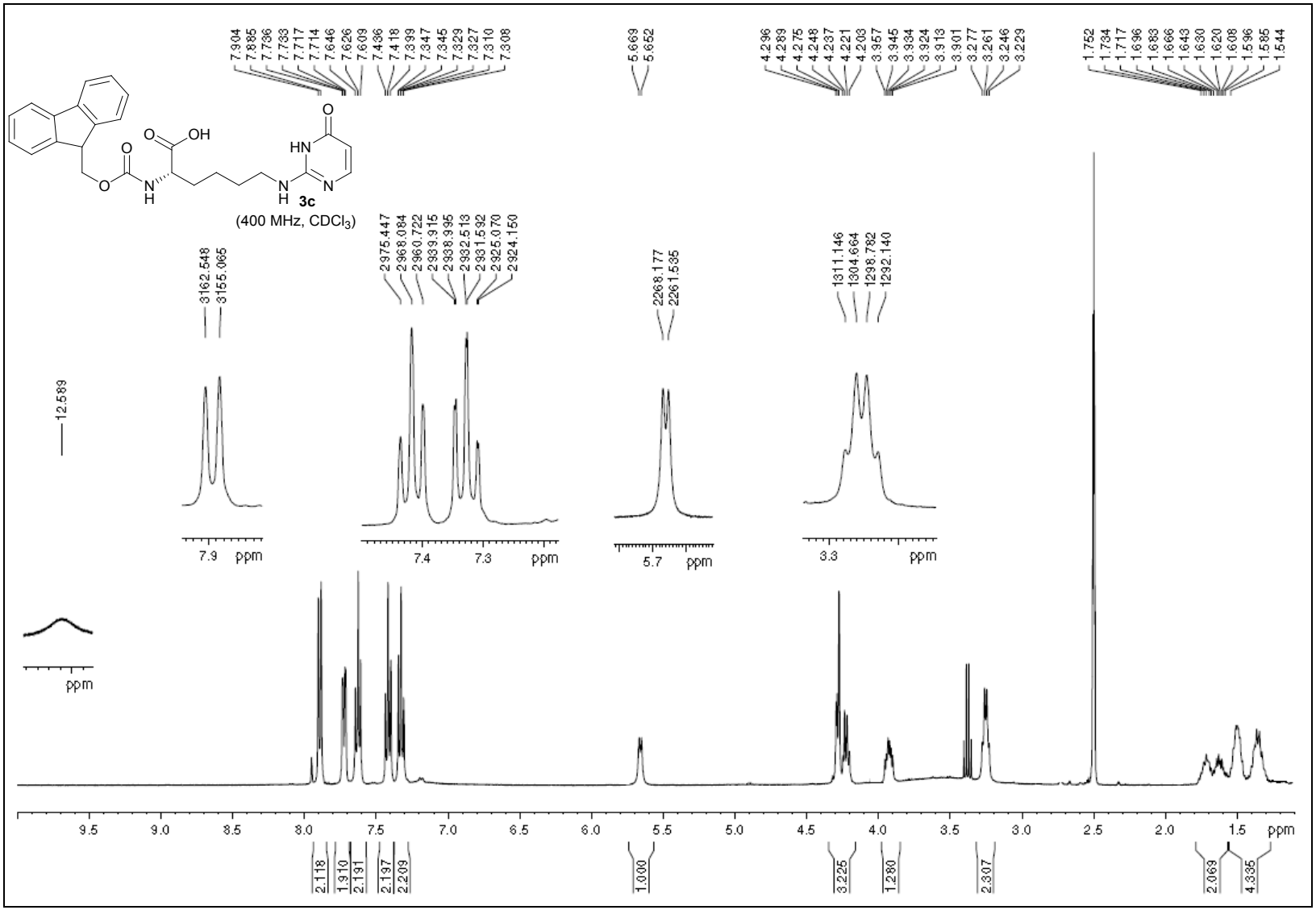

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