Electronic Supplementary Information

Alkaline Phosphatase (ALP) Activatable Small Molecule-based Prodrugs for Cancer Theranostics

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General Experimental Details: All solvents and reagents were used, as received from the suppliers. TLC was performed on Merck Kiesel gel 60, F_{254} plates with the layer thickness of 0.25 mm. Column chromatography was performed on silica gel (100-200 mesh) using a gradient of ethyl acetate and hexane as mobile phase. ^{1}H NMR spectral data were collected at, 500 MHz (JEOL), ^{13}C NMR were recorded at 125 MHz, ^{31}P NMR were recorded at 202 MHz, and ^{19}F NMR spectra were recorded at 471 MHz. ^{1}H NMR spectral data are given as chemical shifts in ppm followed by multiplicity (s- singlet; d- doublet; t- triplet; q- quartet; m- multiplet), number of protons and coupling constants. ^{13}C NMR, ^{31}P NMR, and ^{19}F NMR chemical shifts are expressed in ppm. Fluorescence spectra was recorded using Horiba Fluoromax. Results obtained from MTT assay, GSH depletion assay, and DCFH-DA assays were recorded by Biotek synergy H1 plate reader. All the biological analysis were completed using GraphPad Prism software.

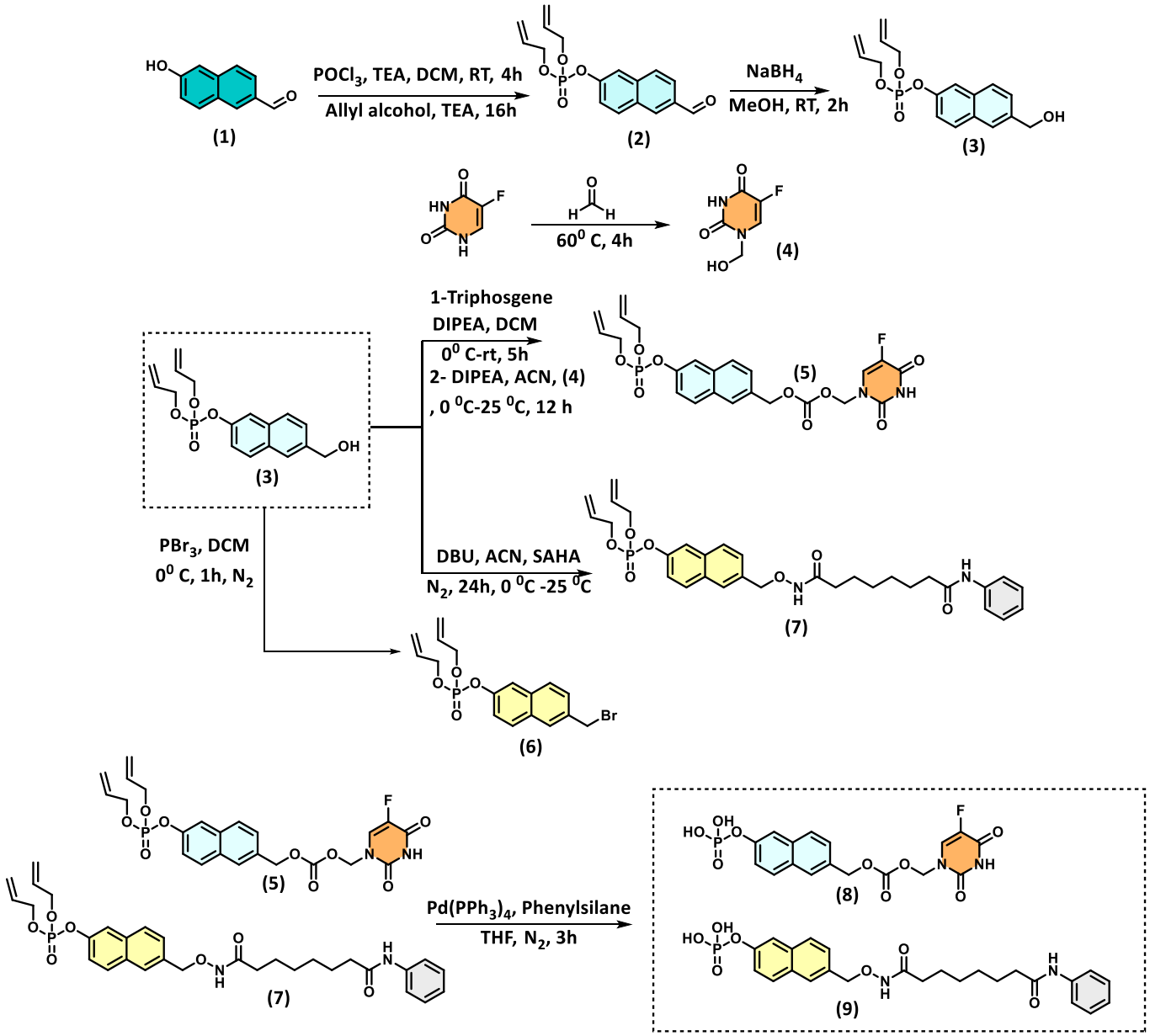


Fig. S1 Stepwise synthetic route for prodrug synthesis

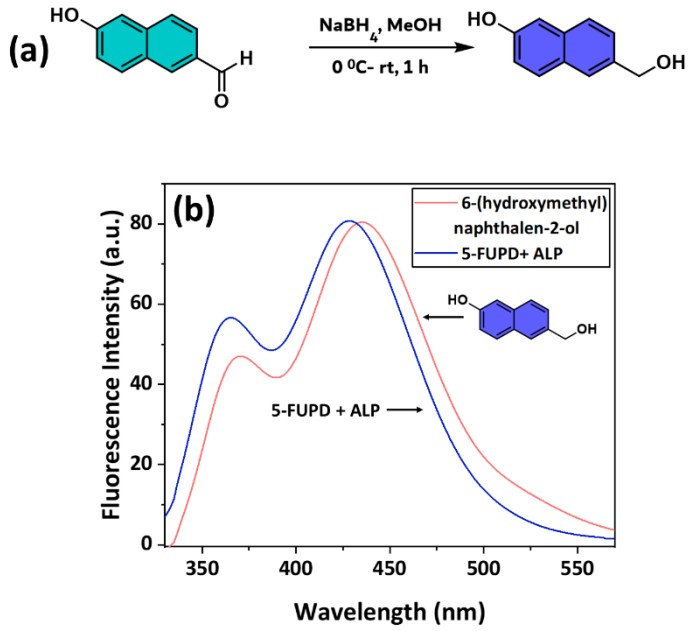


Fig. S2: (a) Reduction of 6-hydroxy-2-naphthaldehyde into of 6-(hydroxymethyl)naphthalen-2-ol; (b) Fluorescence spectrum of 6-(hydroxymethyl)naphthalen-2-ol and fluorophore generated from ALP triggered hydrolysis of 5-FUPD.

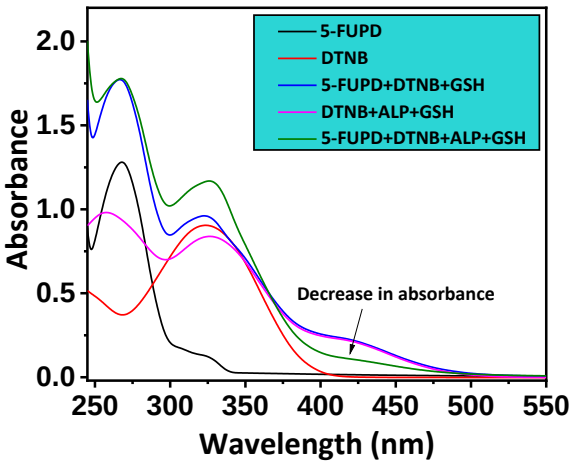


Fig. S3: Assessment of GSH depletion by treatment with prodrugs using DTNB assay. GSH, prodrug, and ALP solutions were incubated at 37 ^{0}C for 20 minutes and DTNB reagent was added. Depletion in GSH levels was observed by taking absorption spectrum using UV-Vis. spectrophotometer.

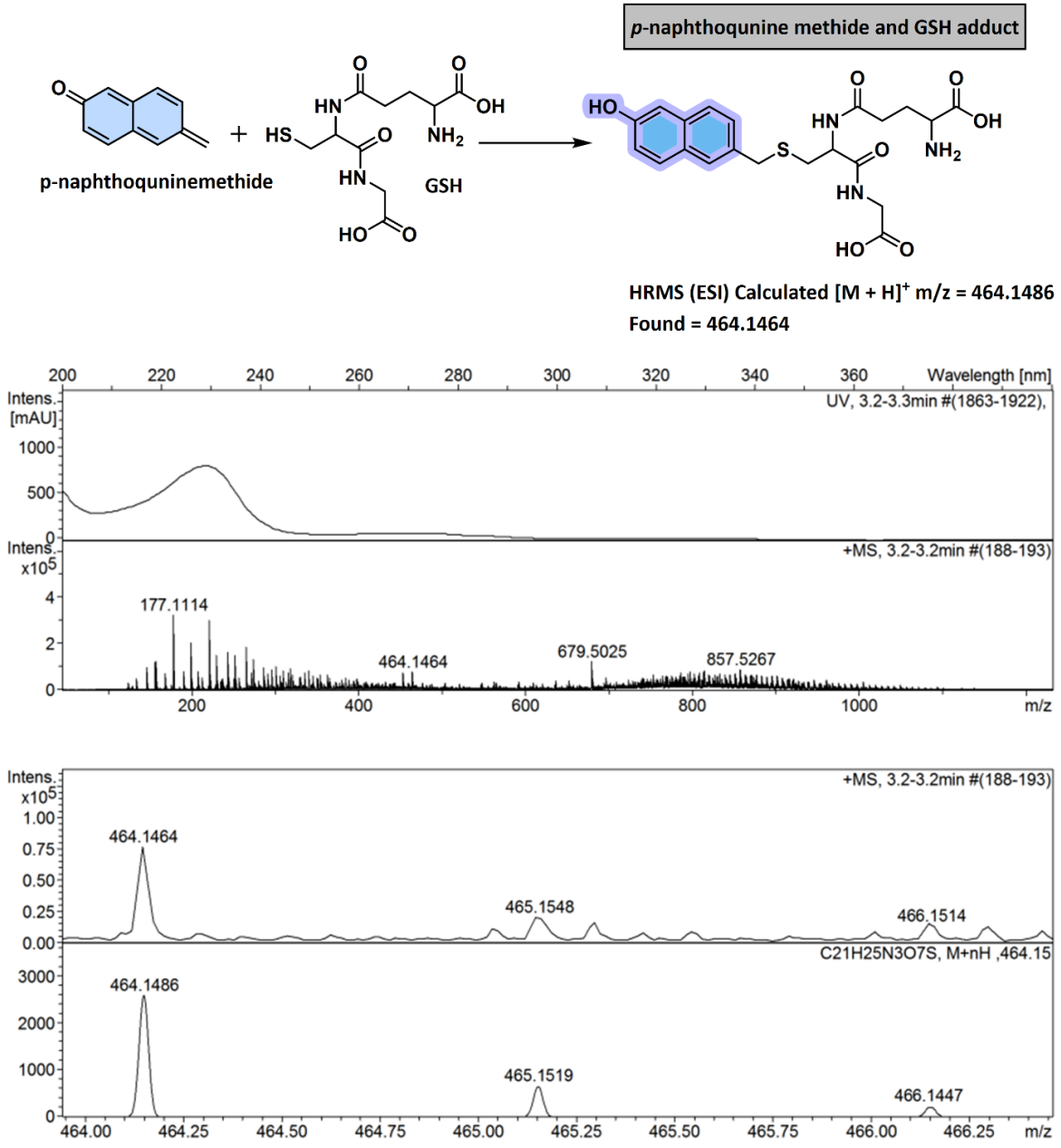


Fig. S4: HRMS spectrum of captured *p*-naphthoquinone methide and GSH adduct.

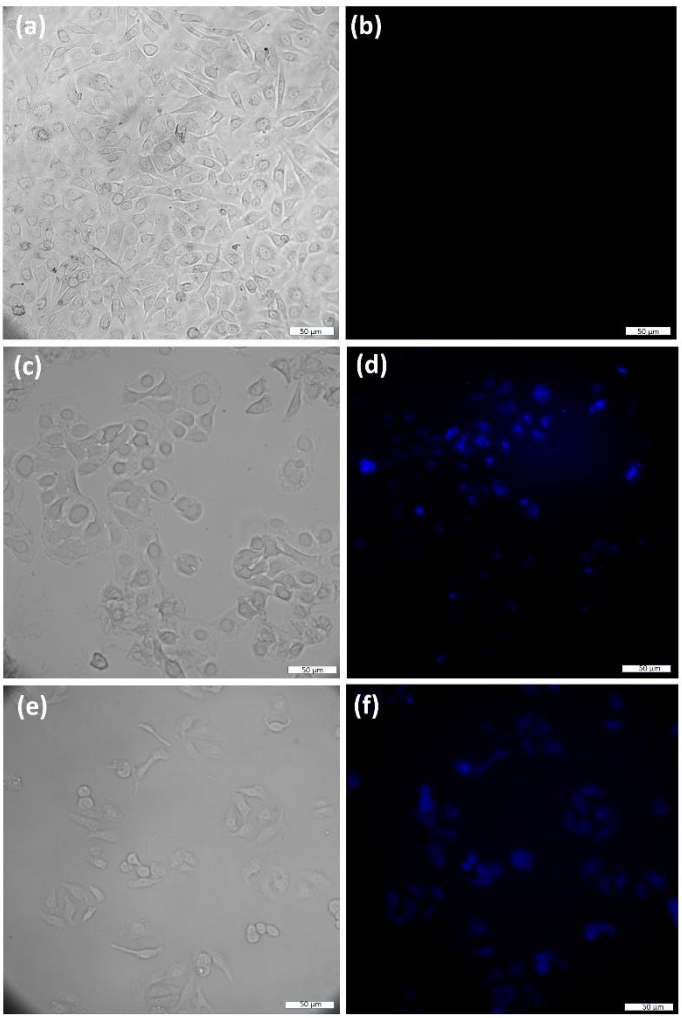
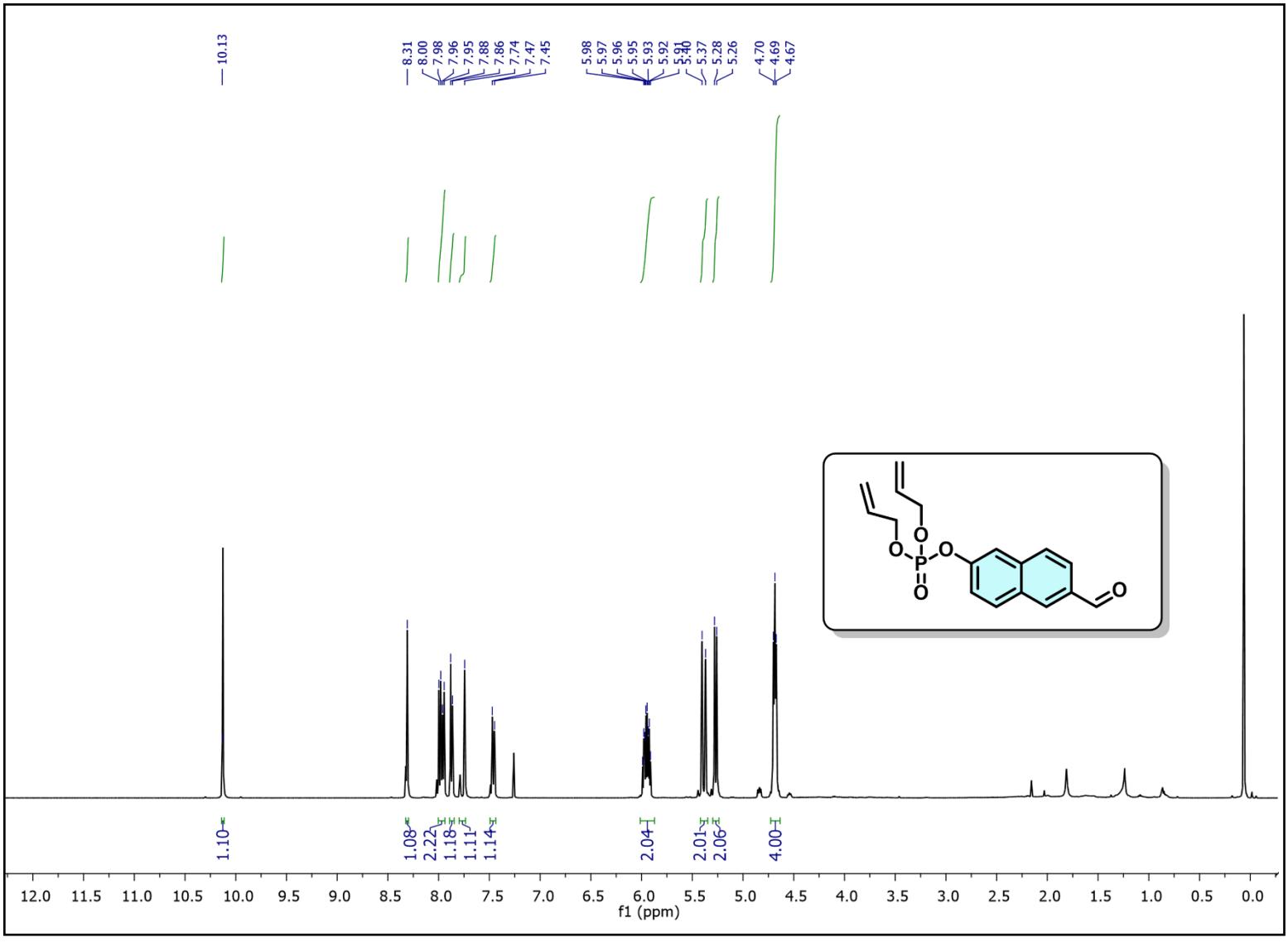
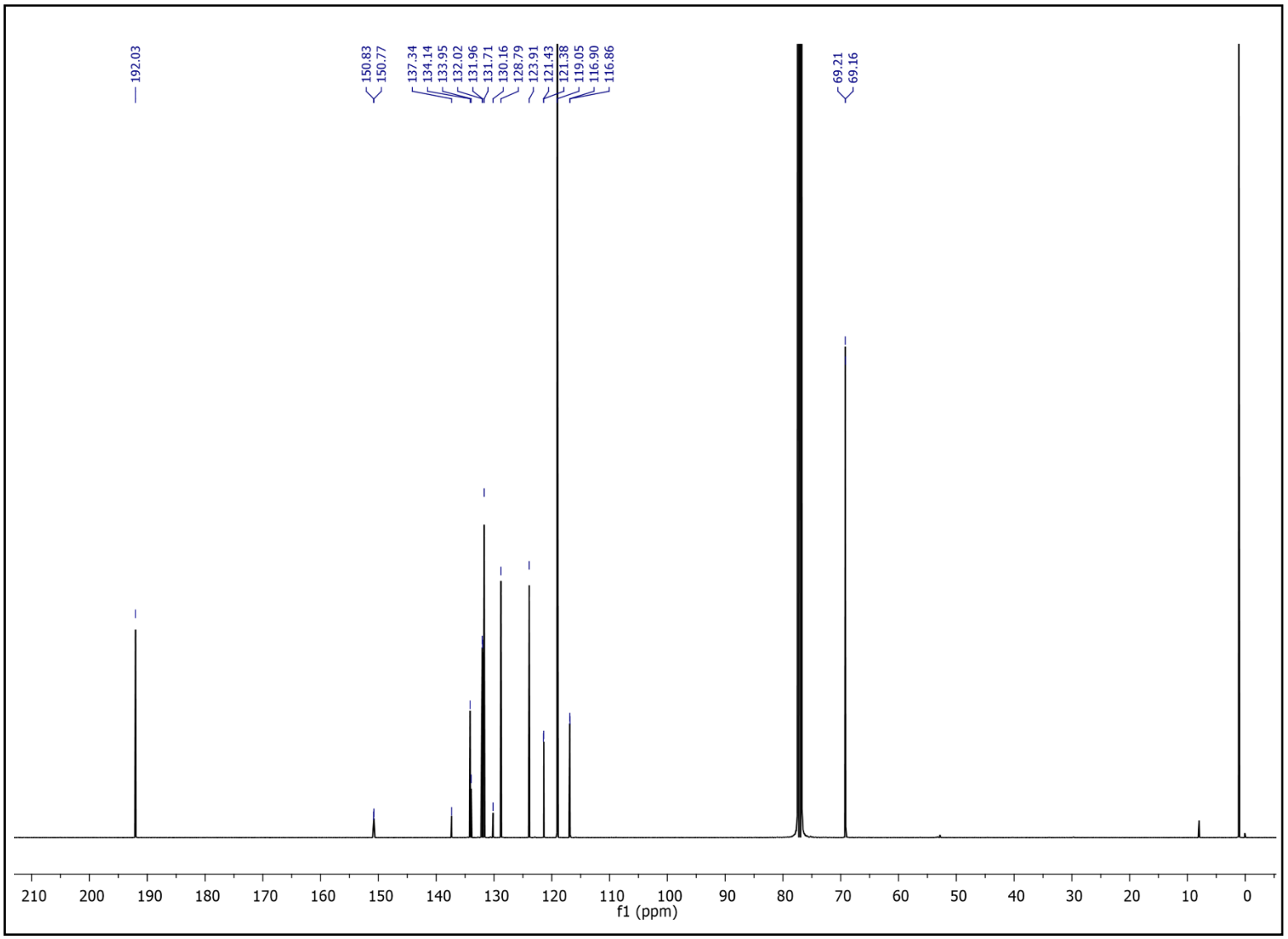


Fig. S5: Fluorescence images (a, b) untreated cells brightfield image and untreated cells under blue channel; (c, d) brightfield and fluorescence images of 5-FUPD under blue channel; (e, f) brightfield and fluorescence images of SAHAPD under blue channel.

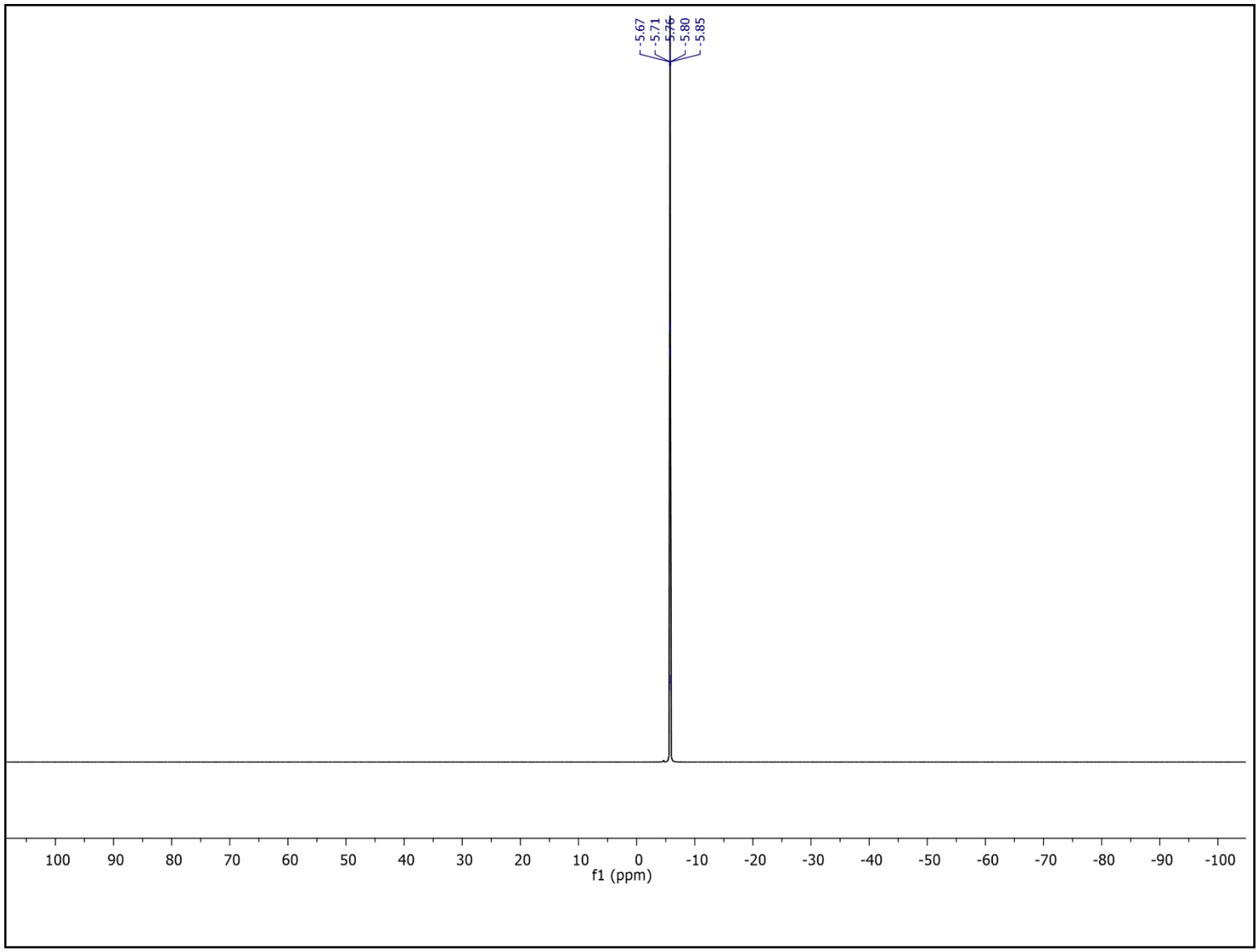
^{1}H NMR of compound 2 ($CDCl_3$)



^{13}C NMR of compound 2 ($CDCl_3$)



^{31}P NMR of compound 2 ($CDCl_3$)



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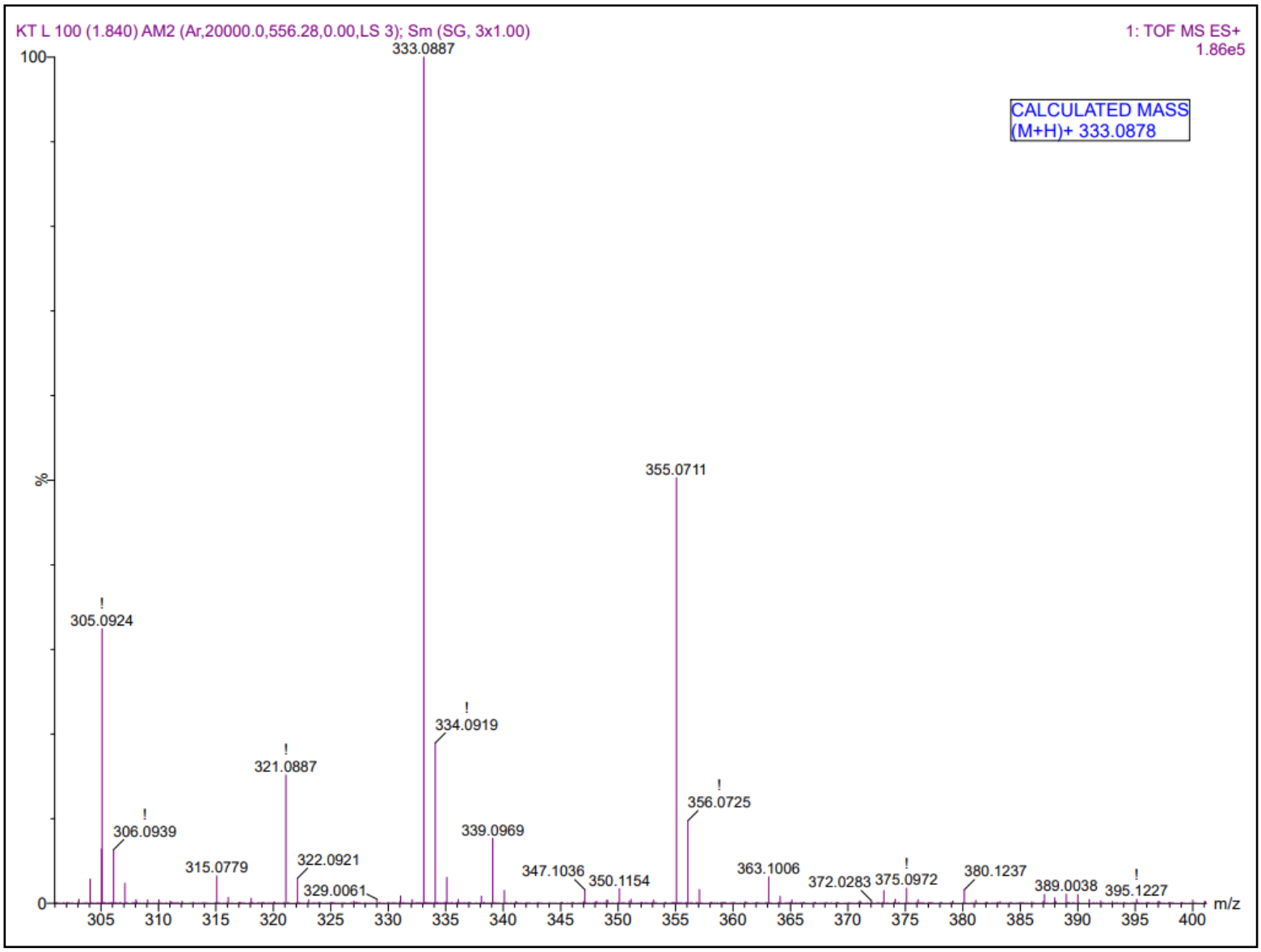

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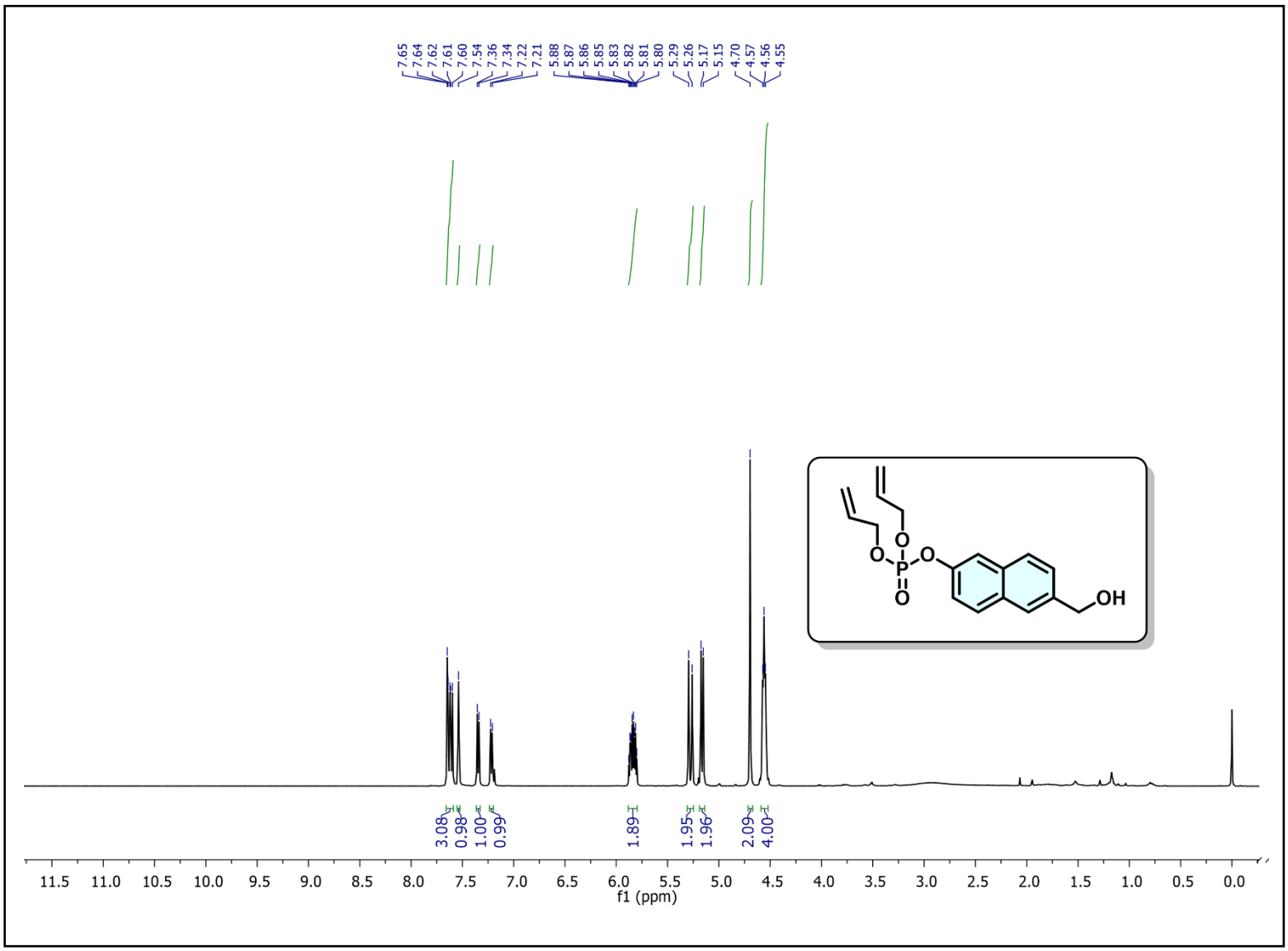

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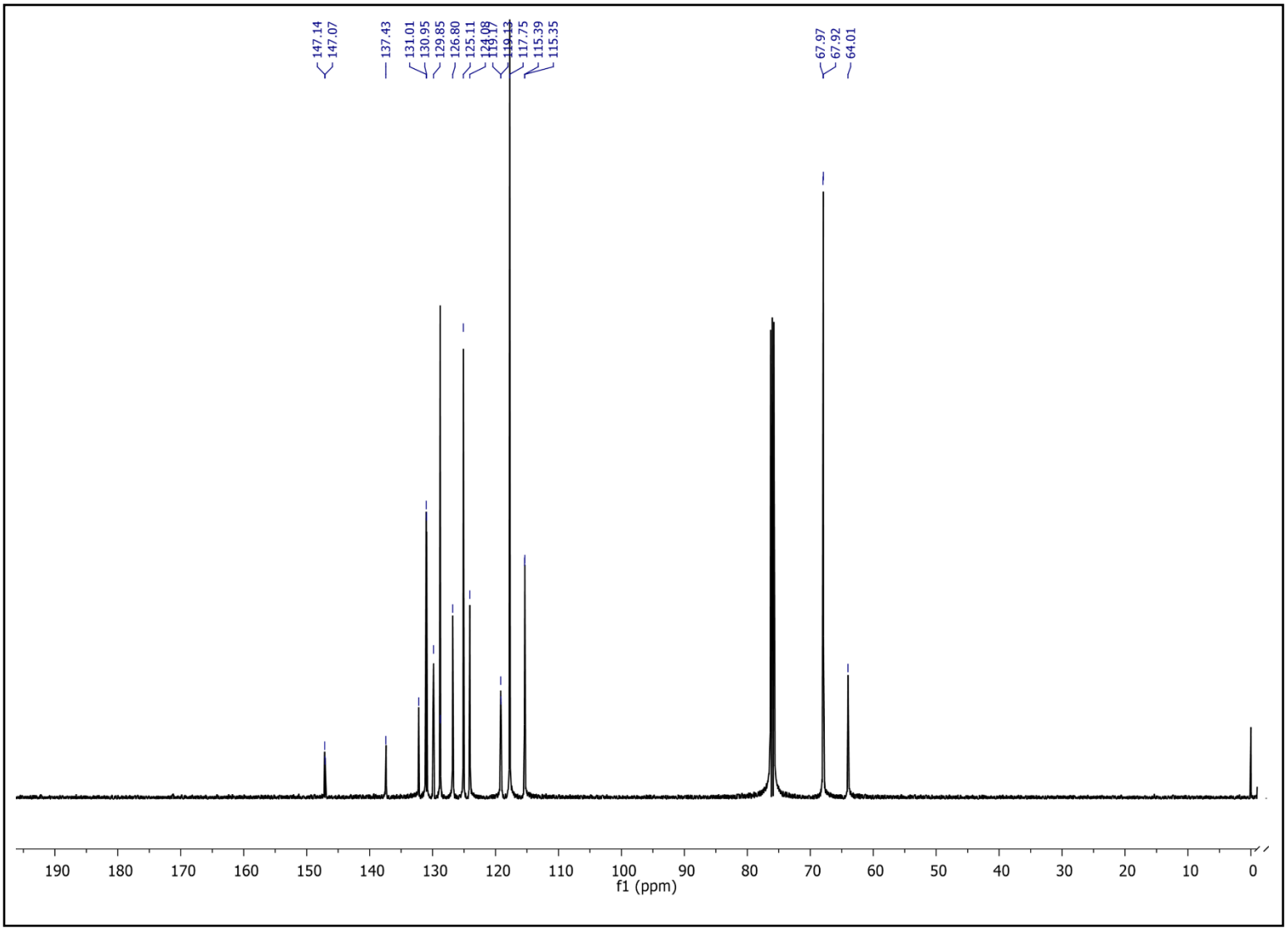

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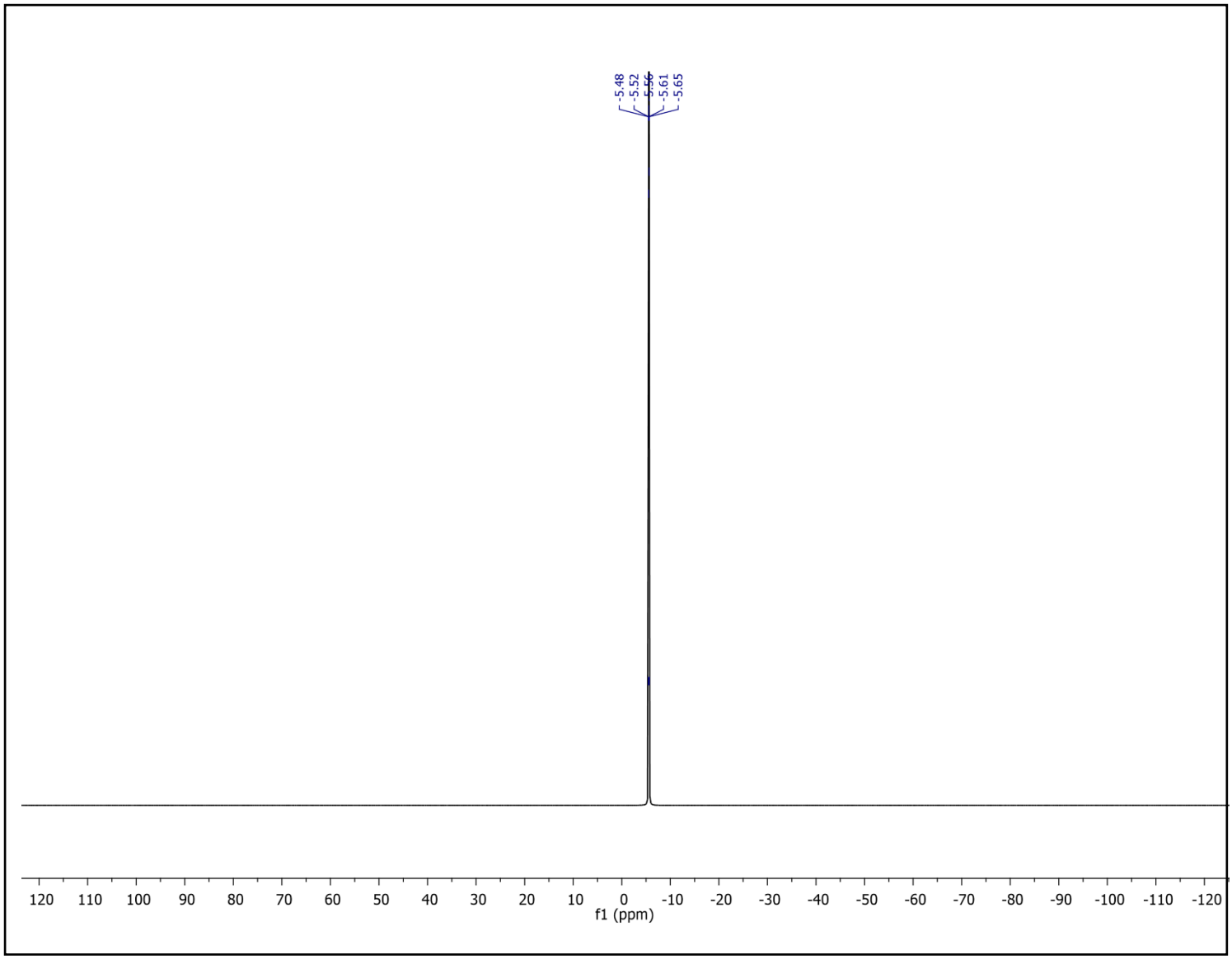

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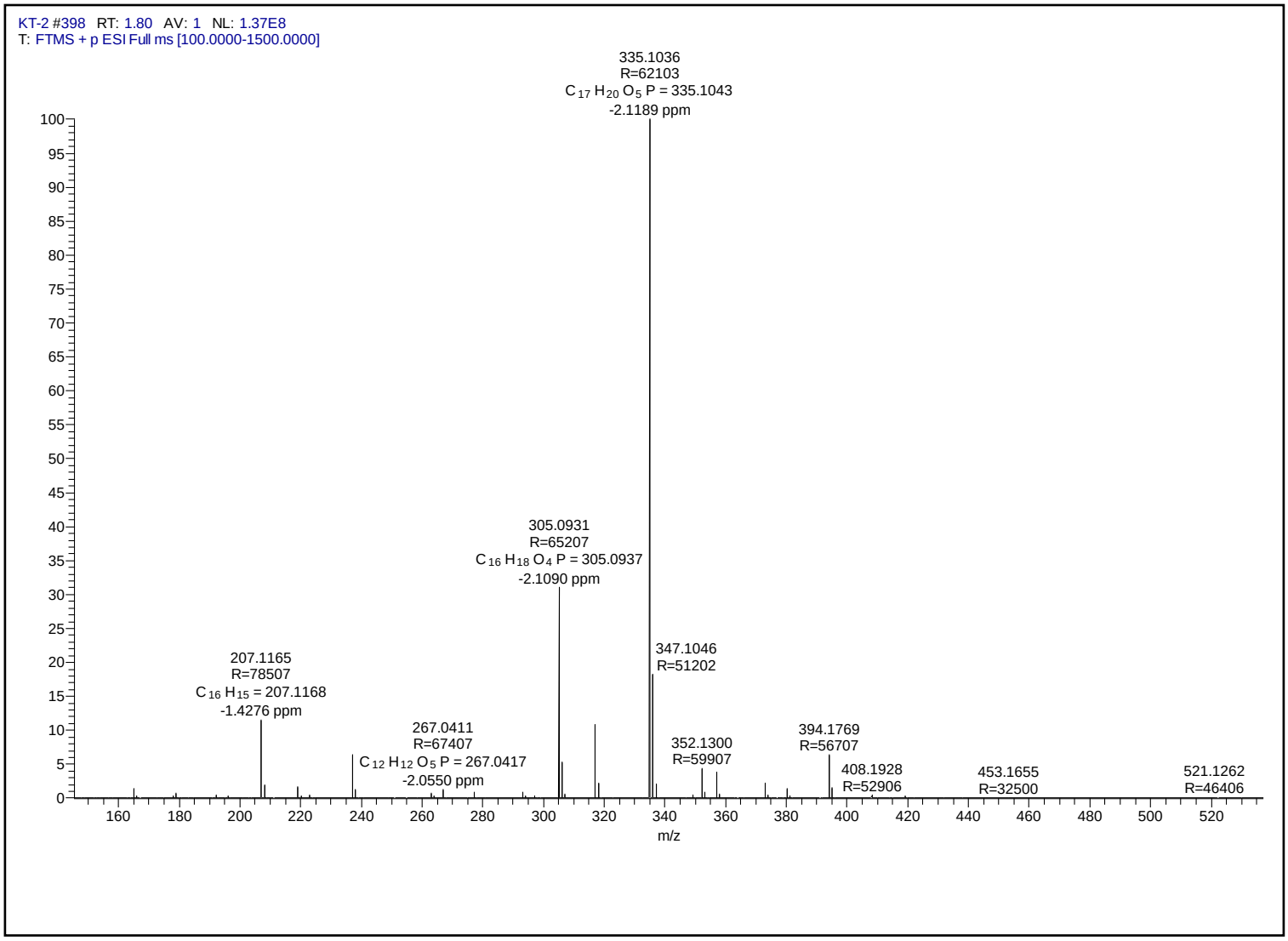

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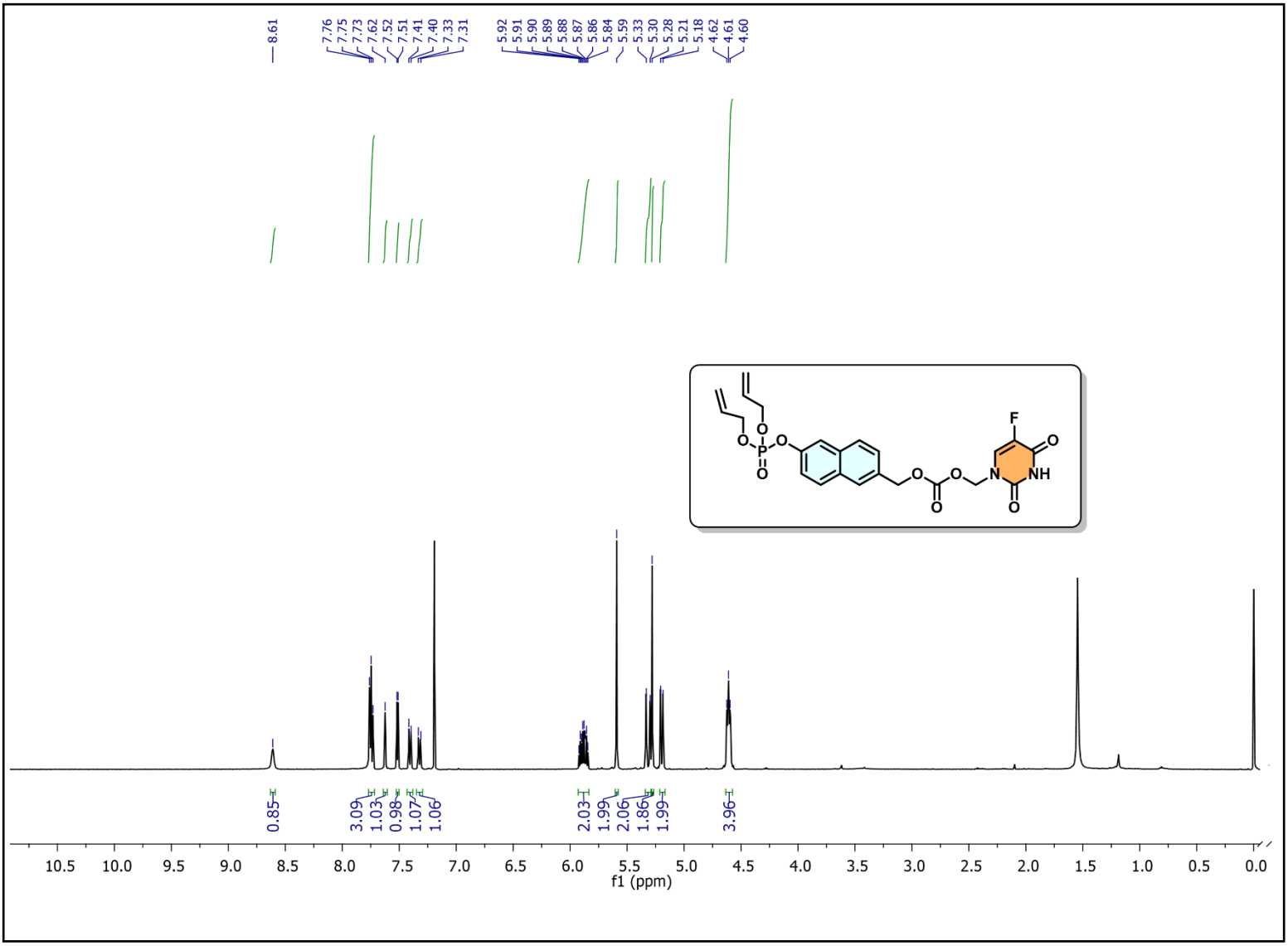

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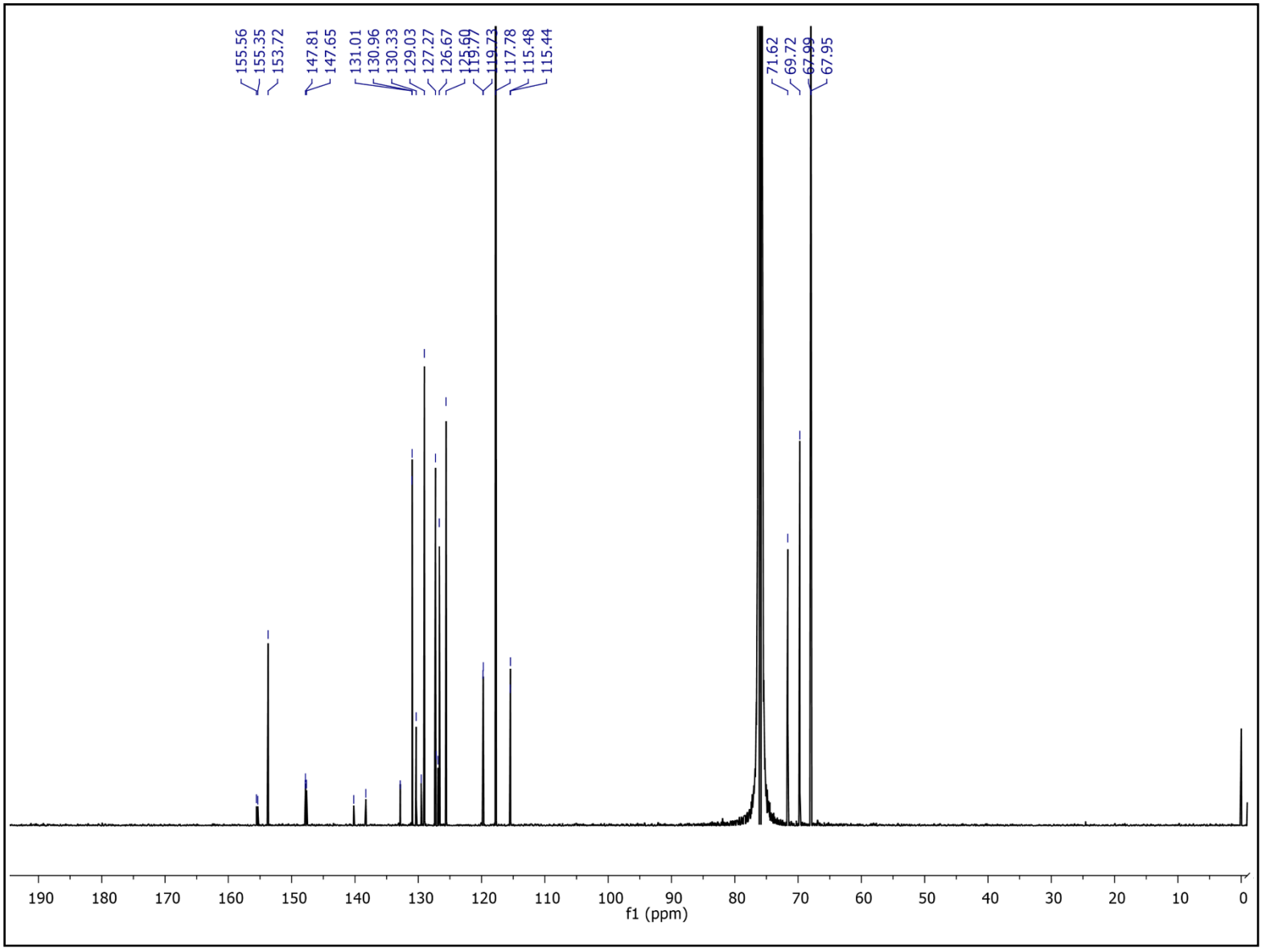

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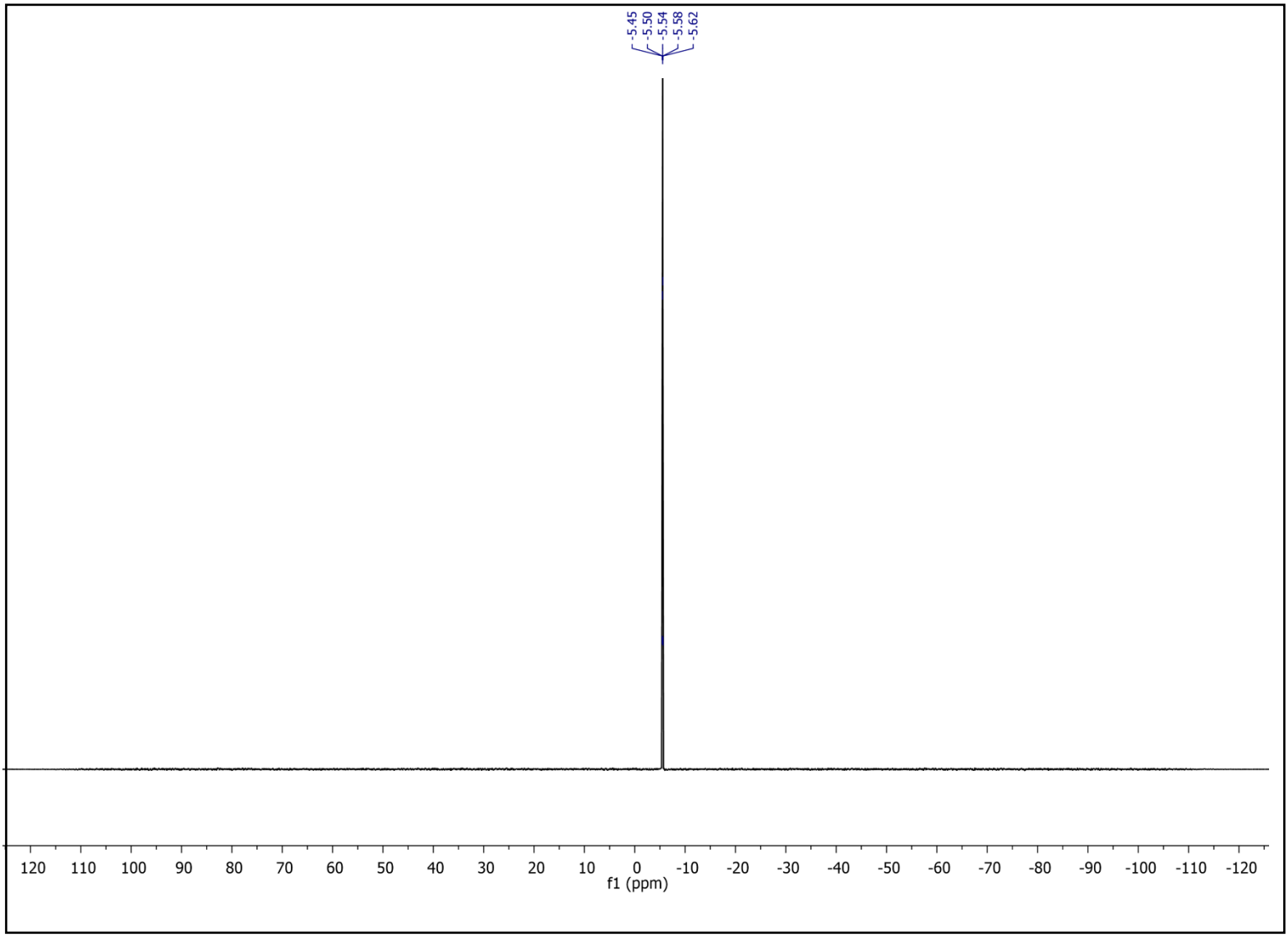

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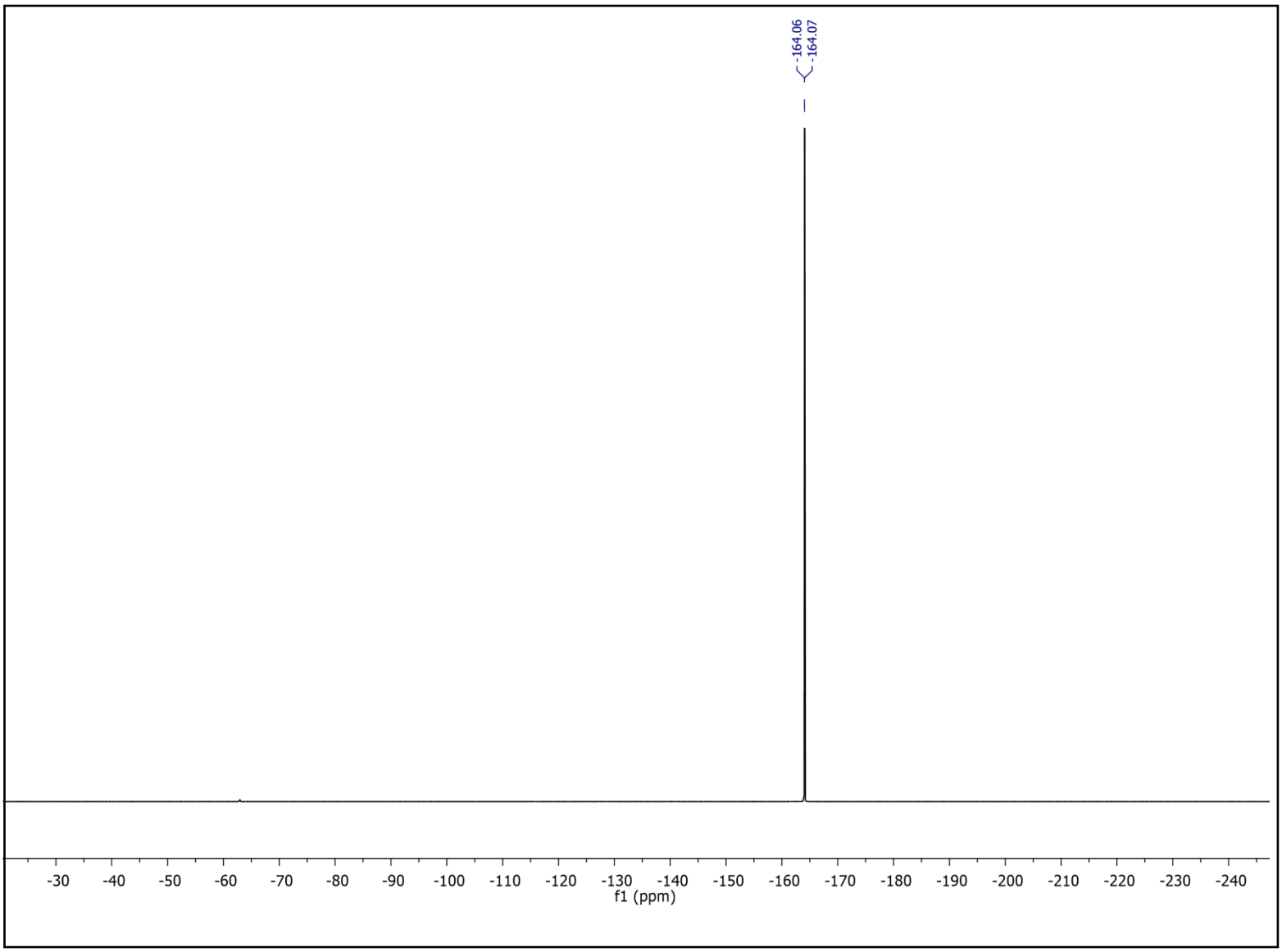
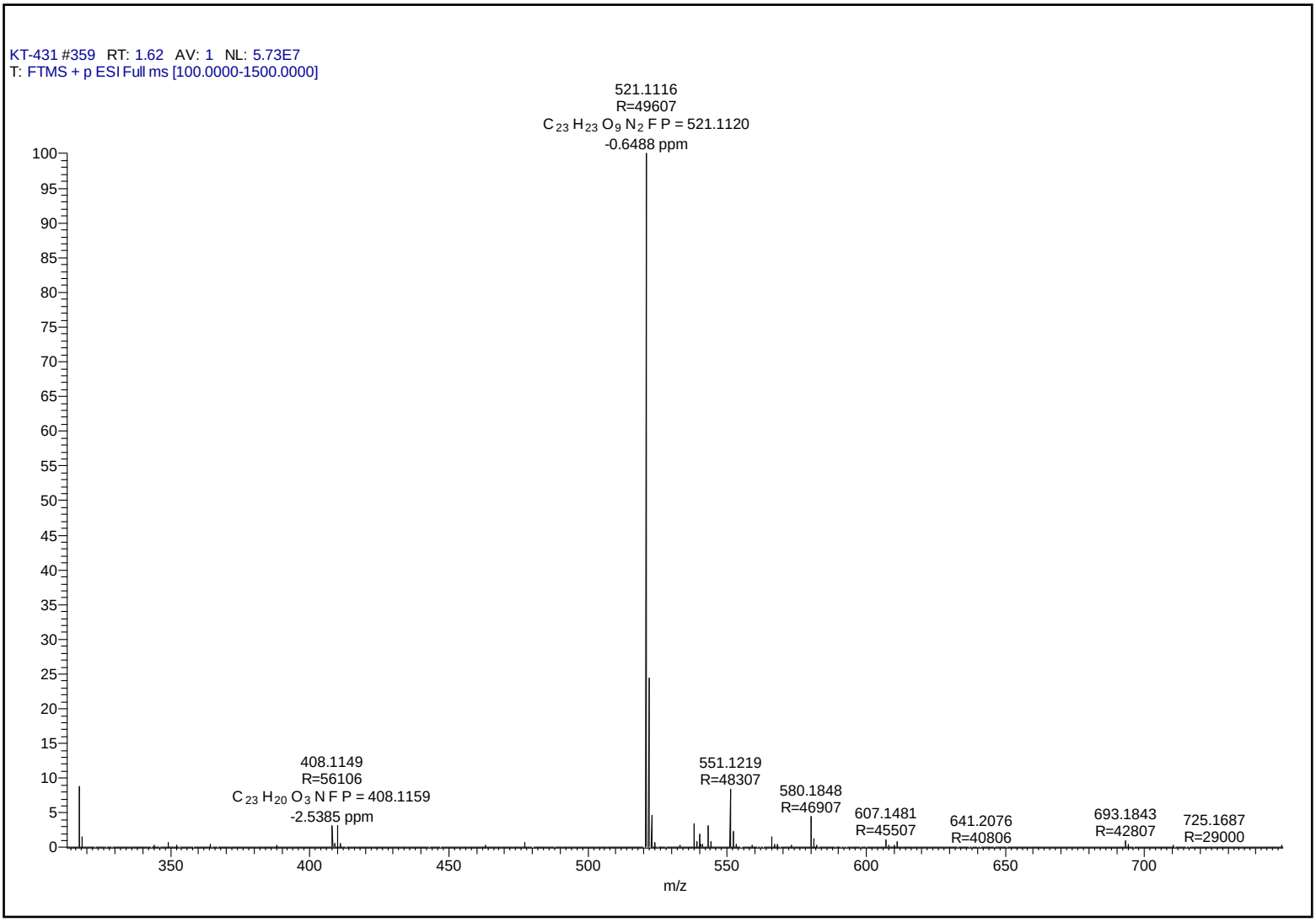


S17

HRMS of compound 5



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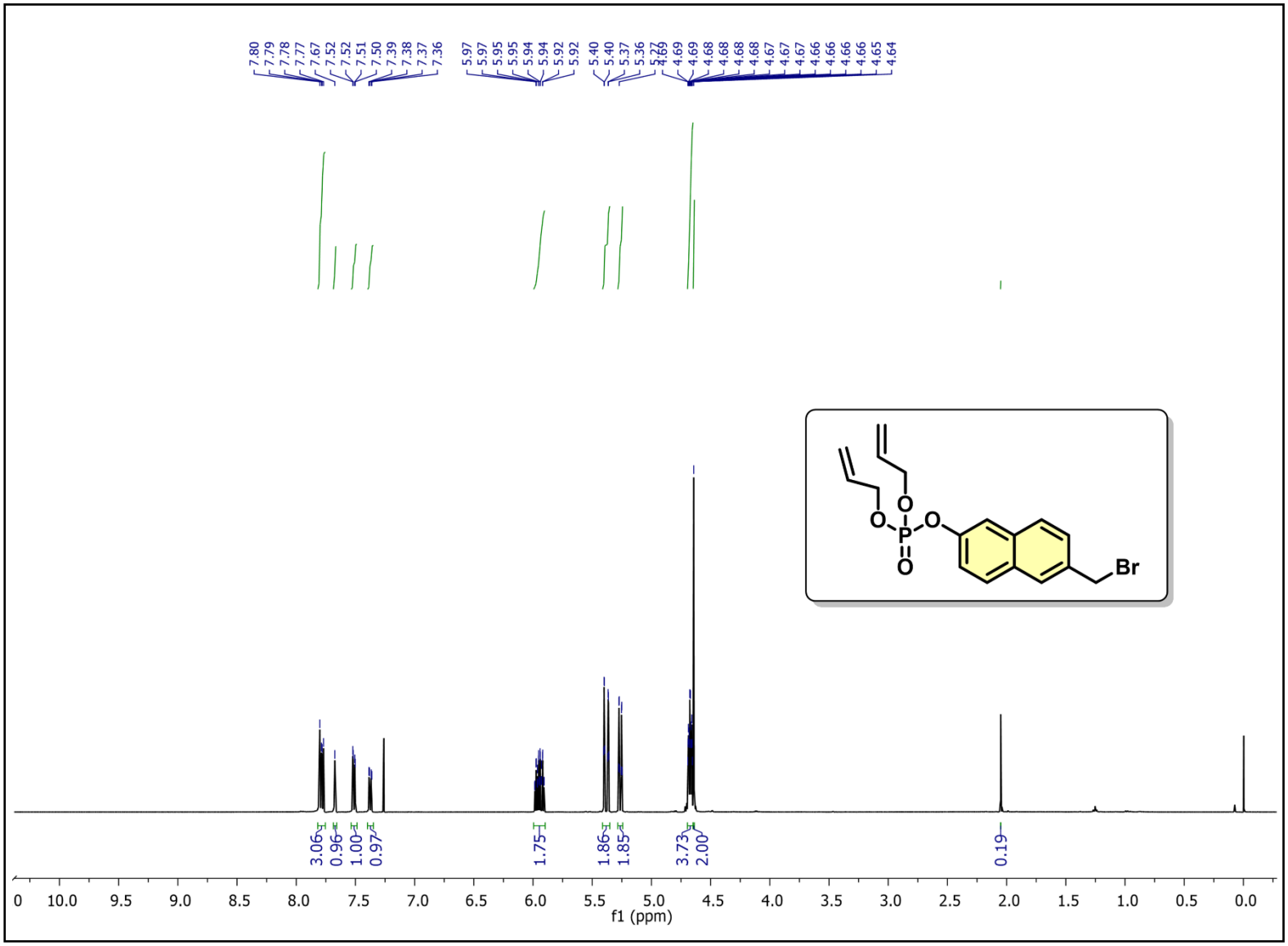

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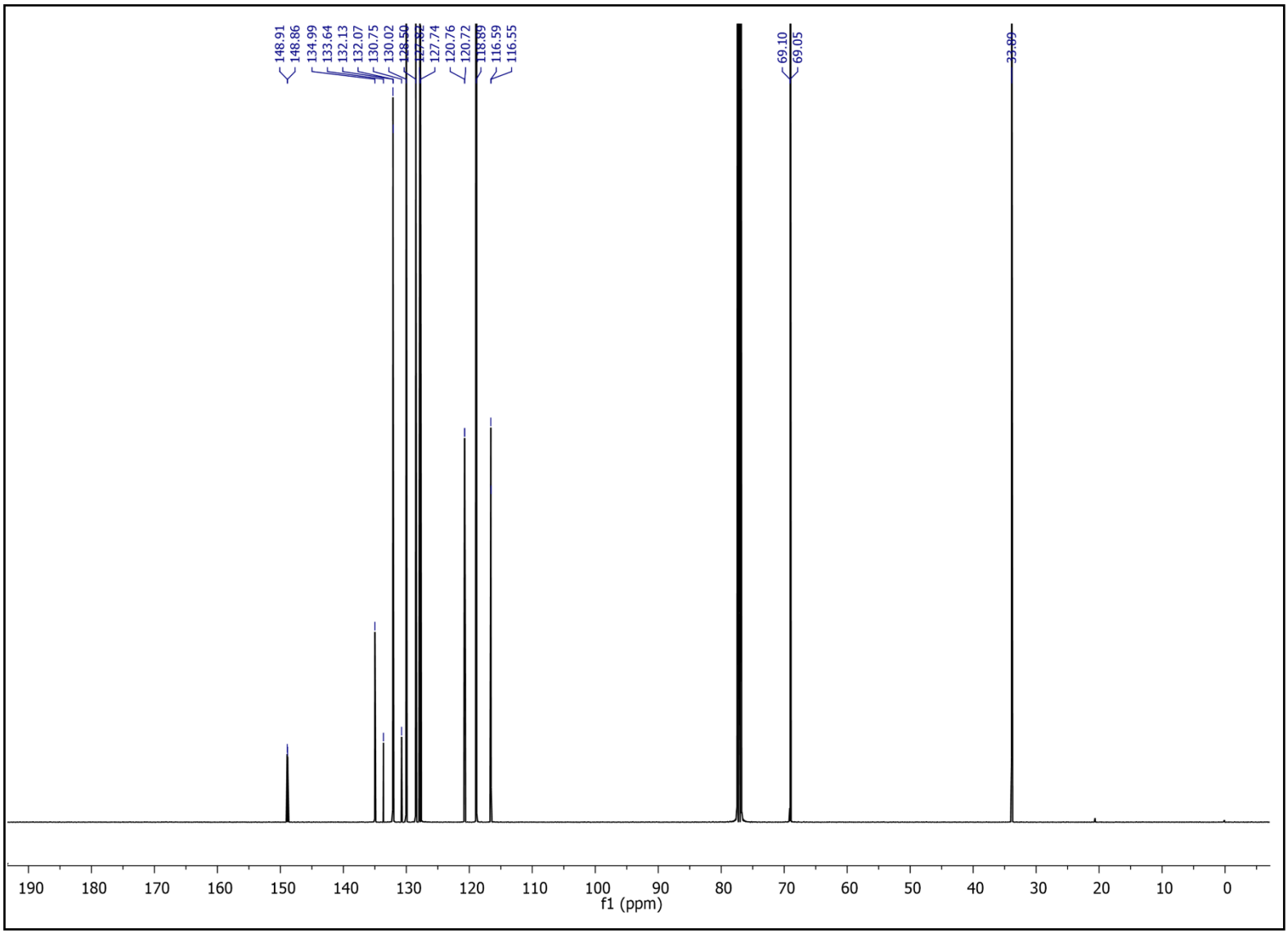

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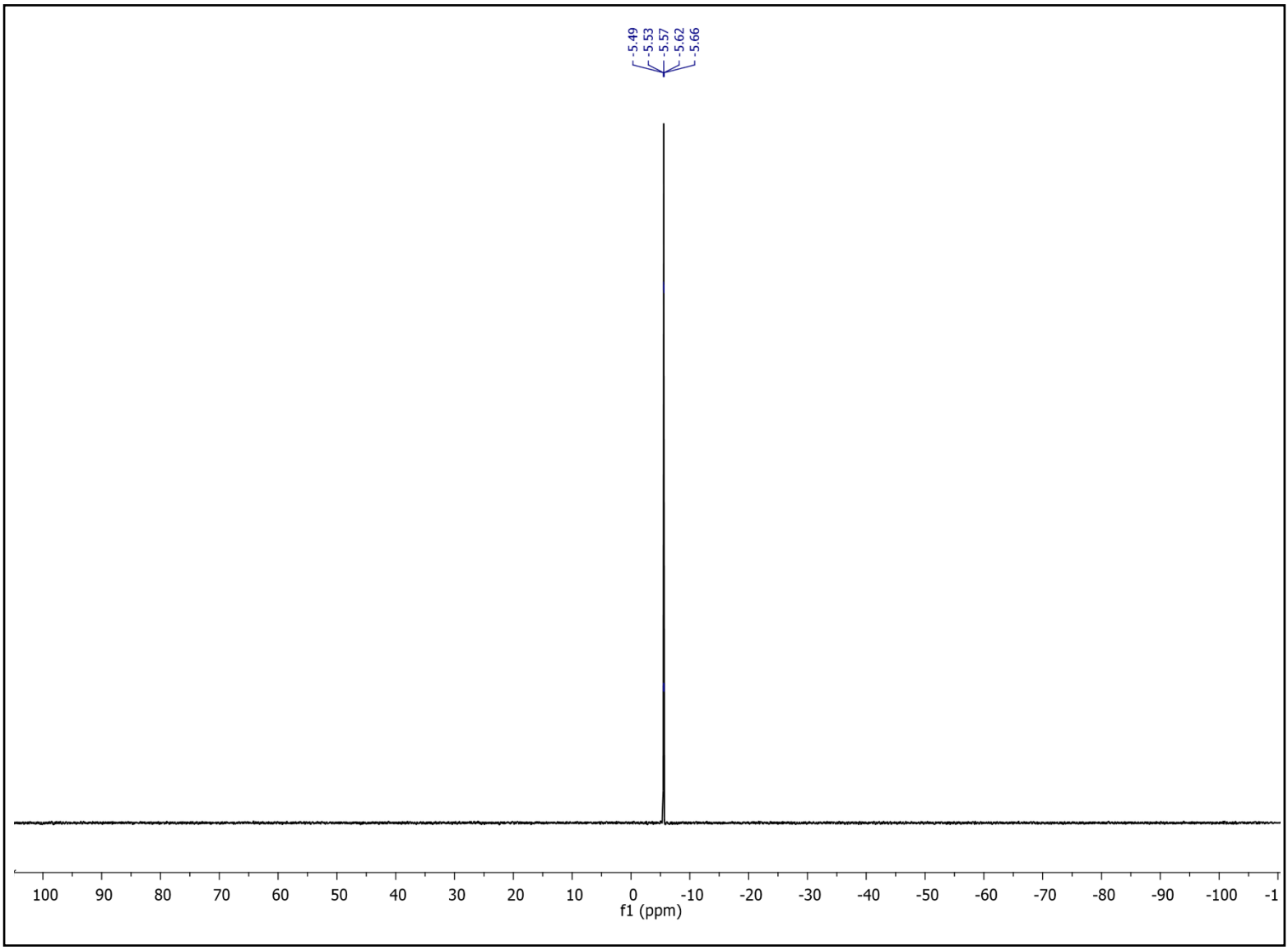

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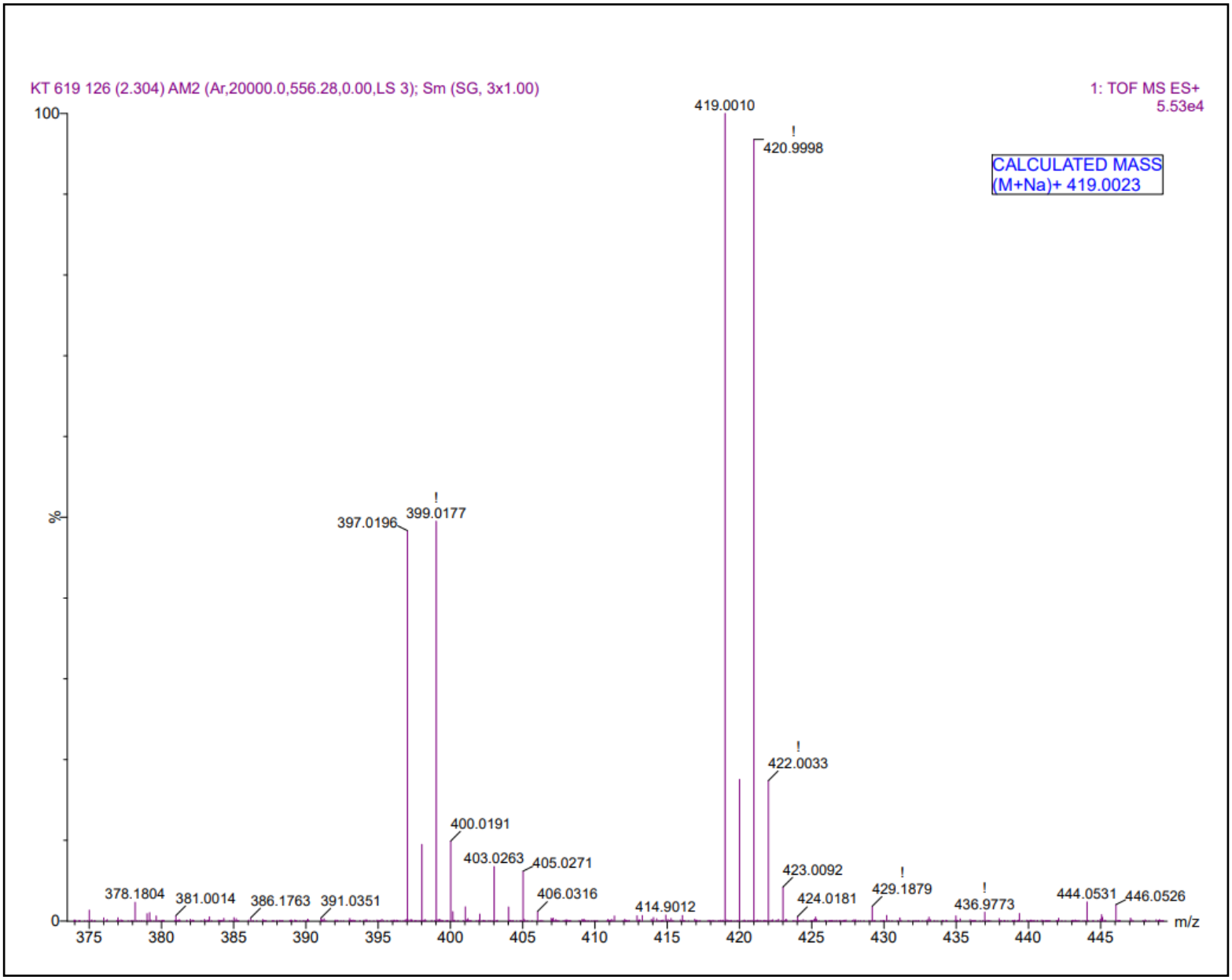

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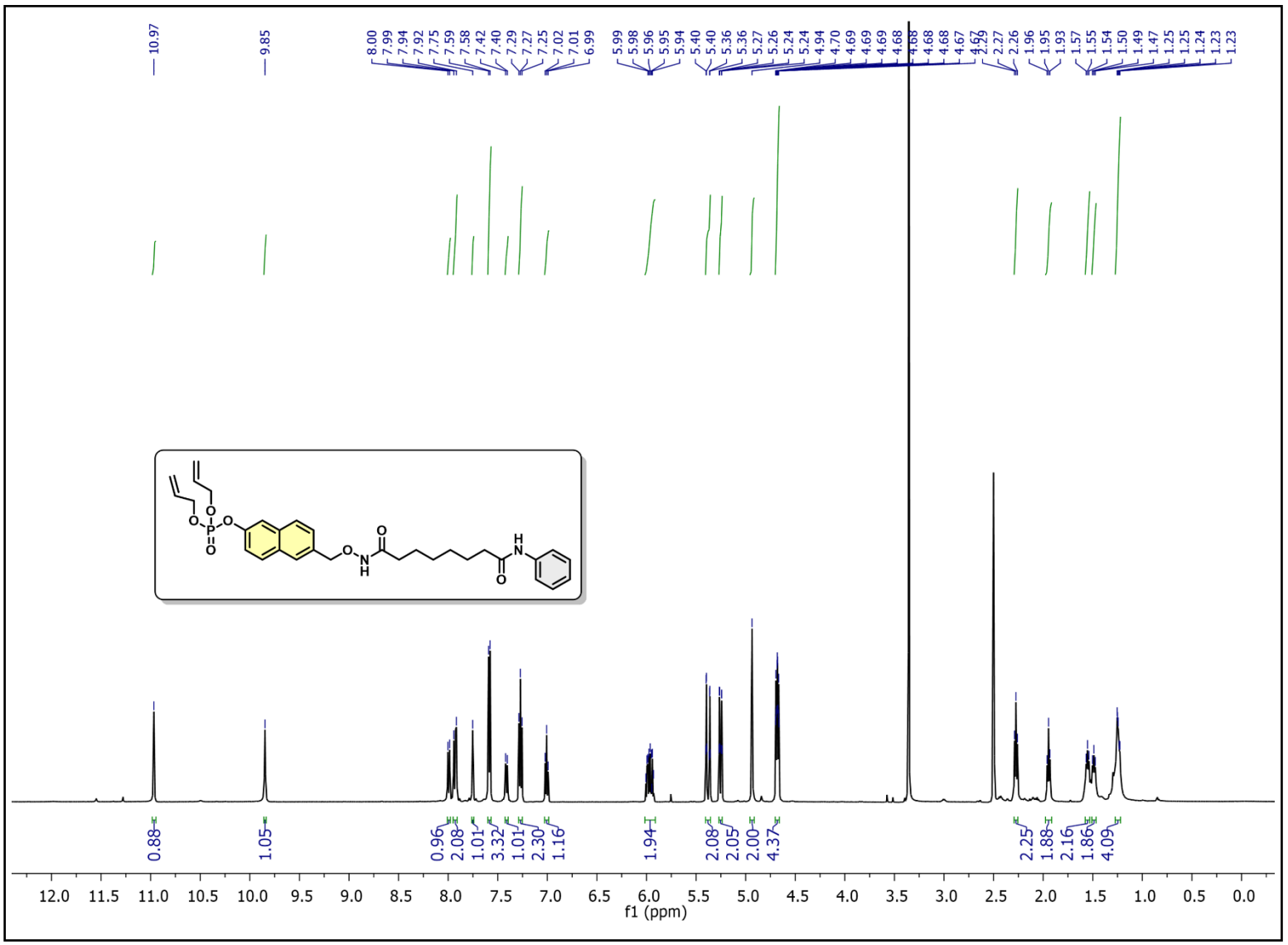

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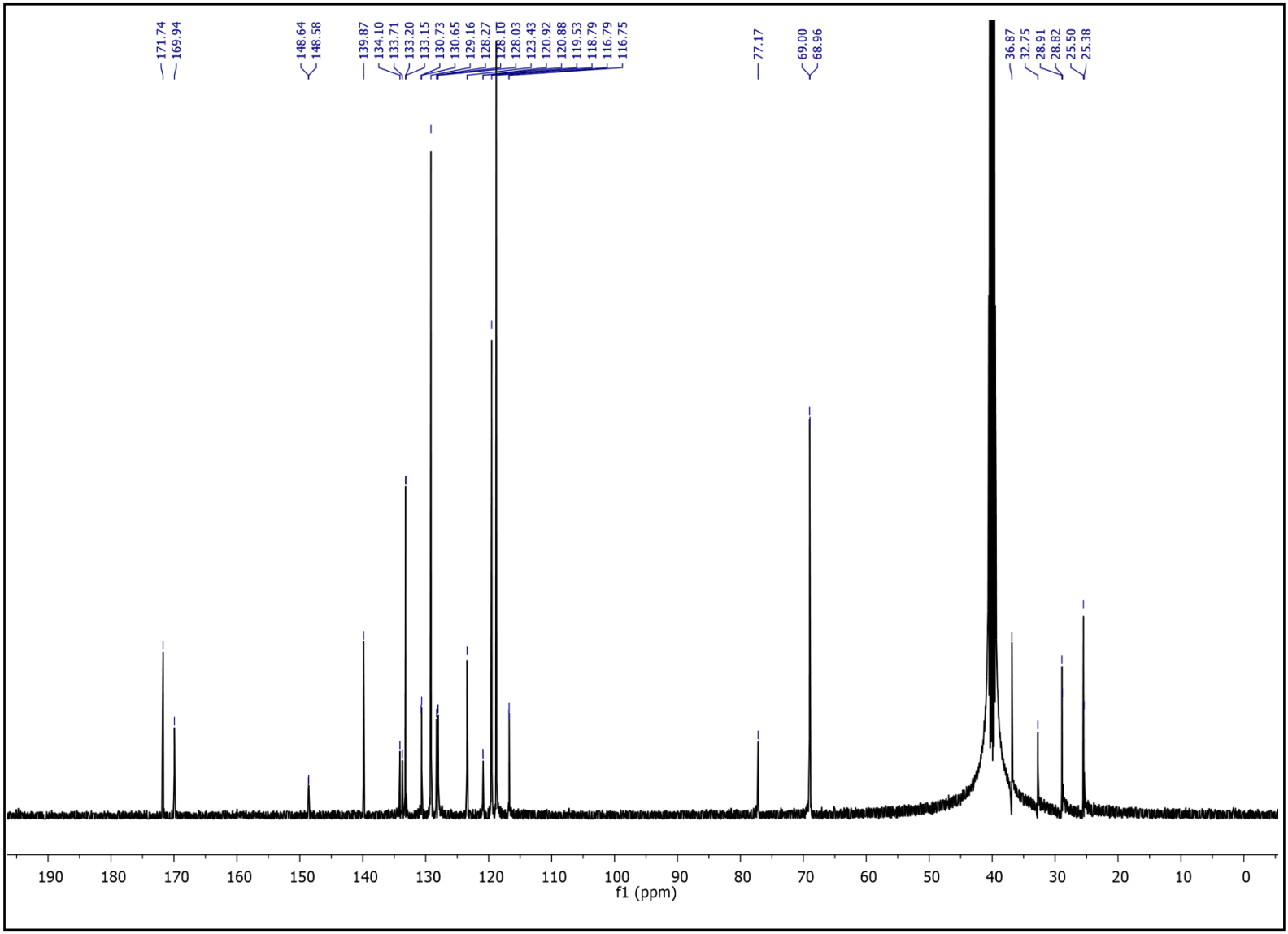

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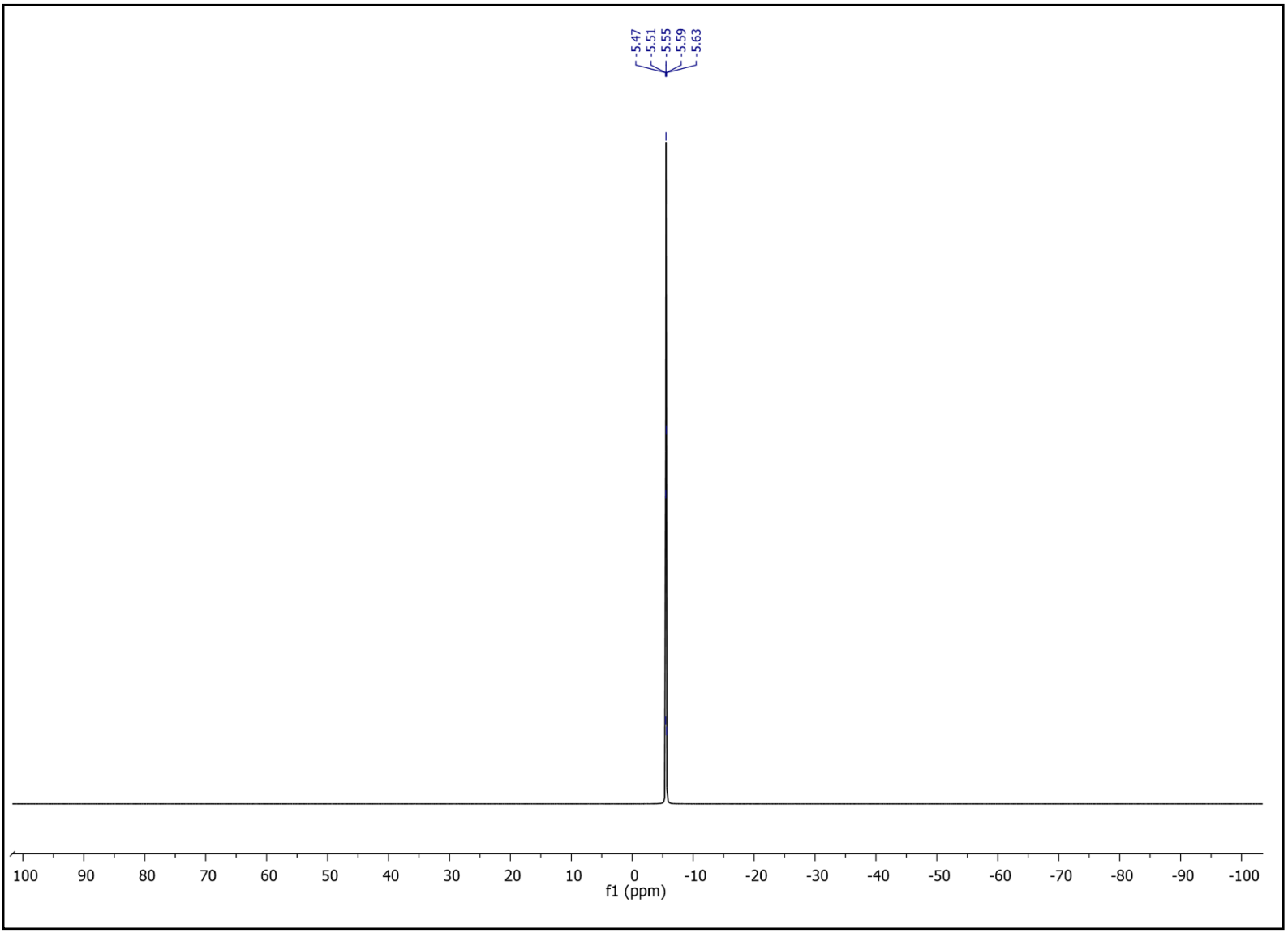

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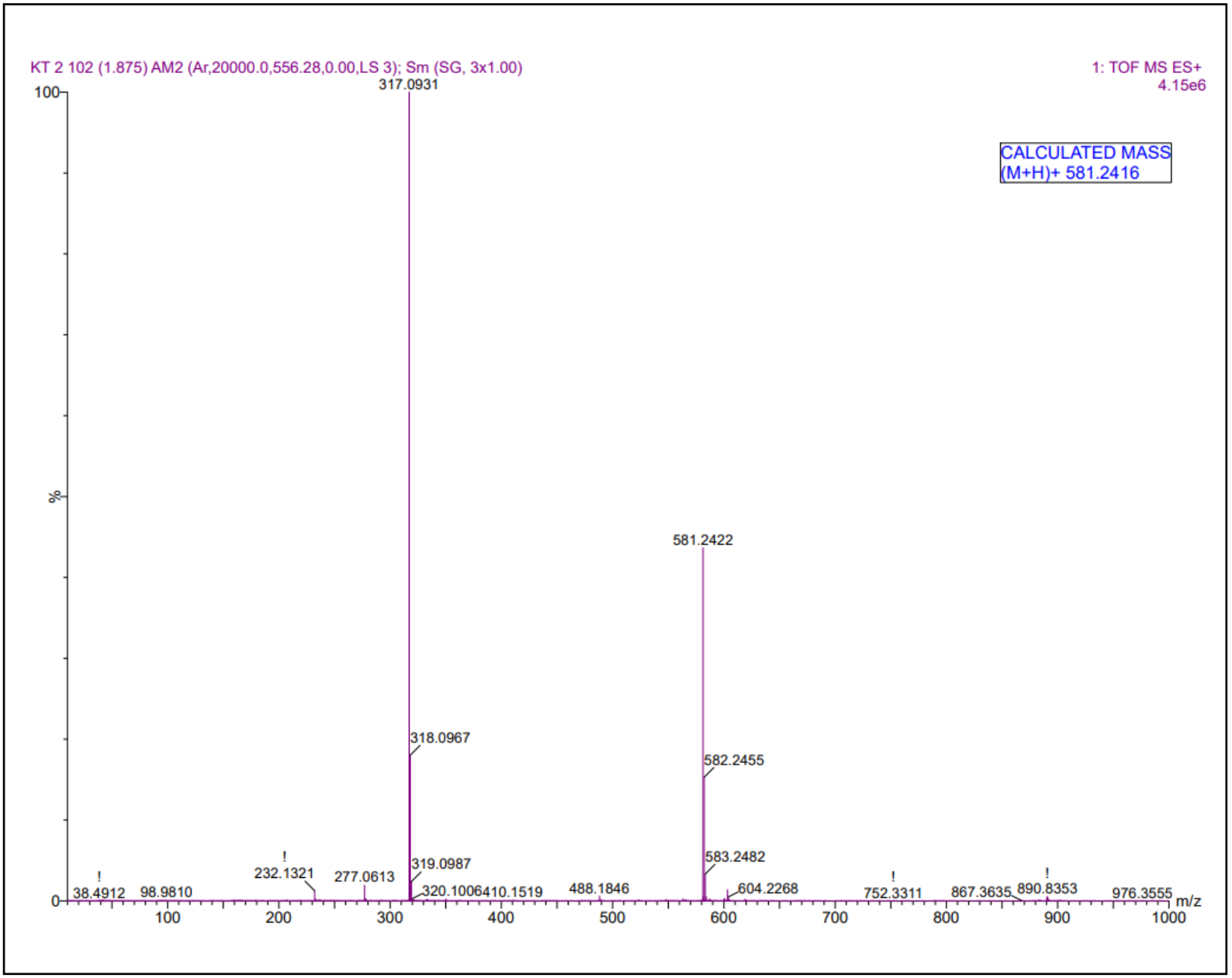

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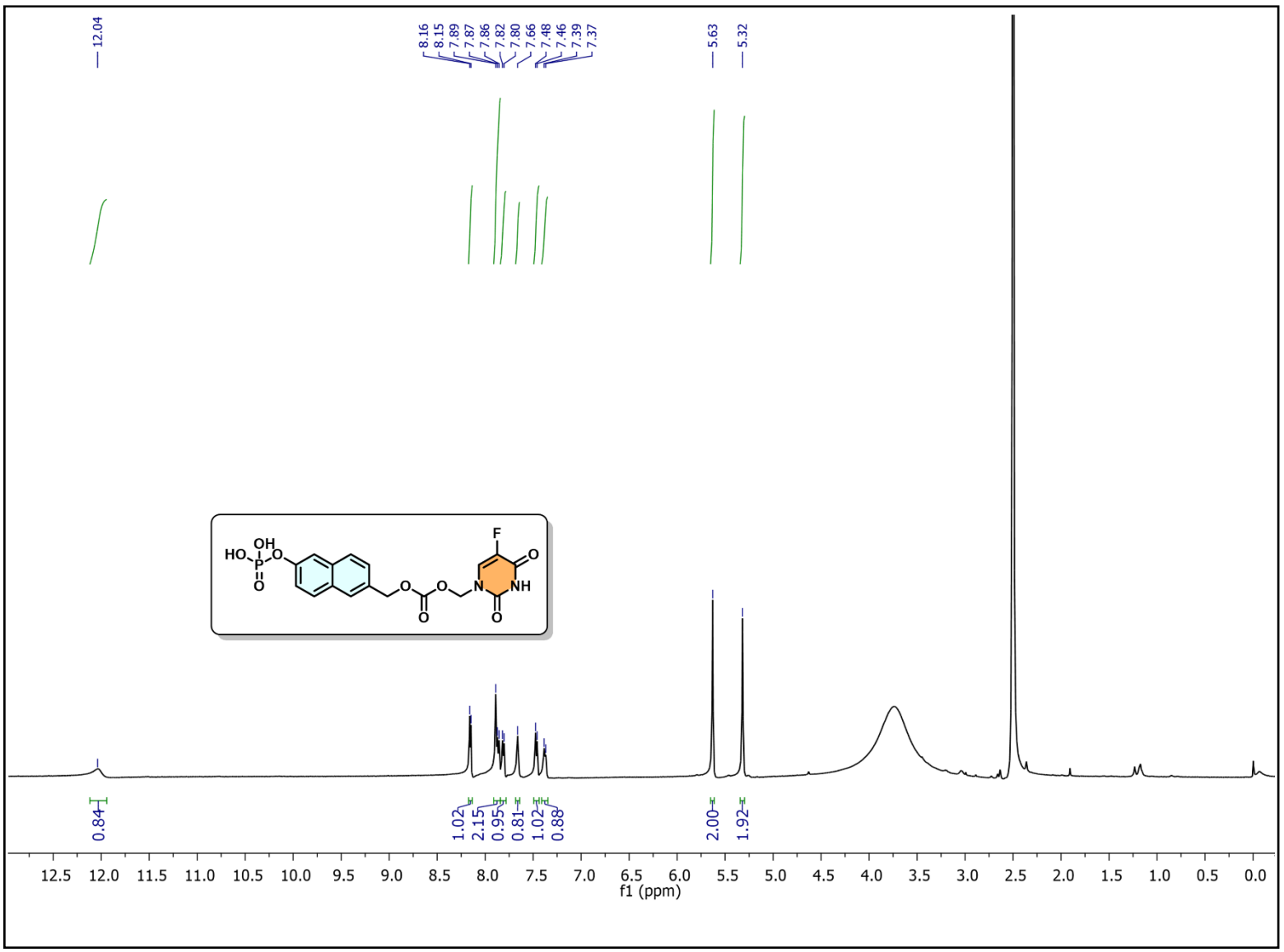

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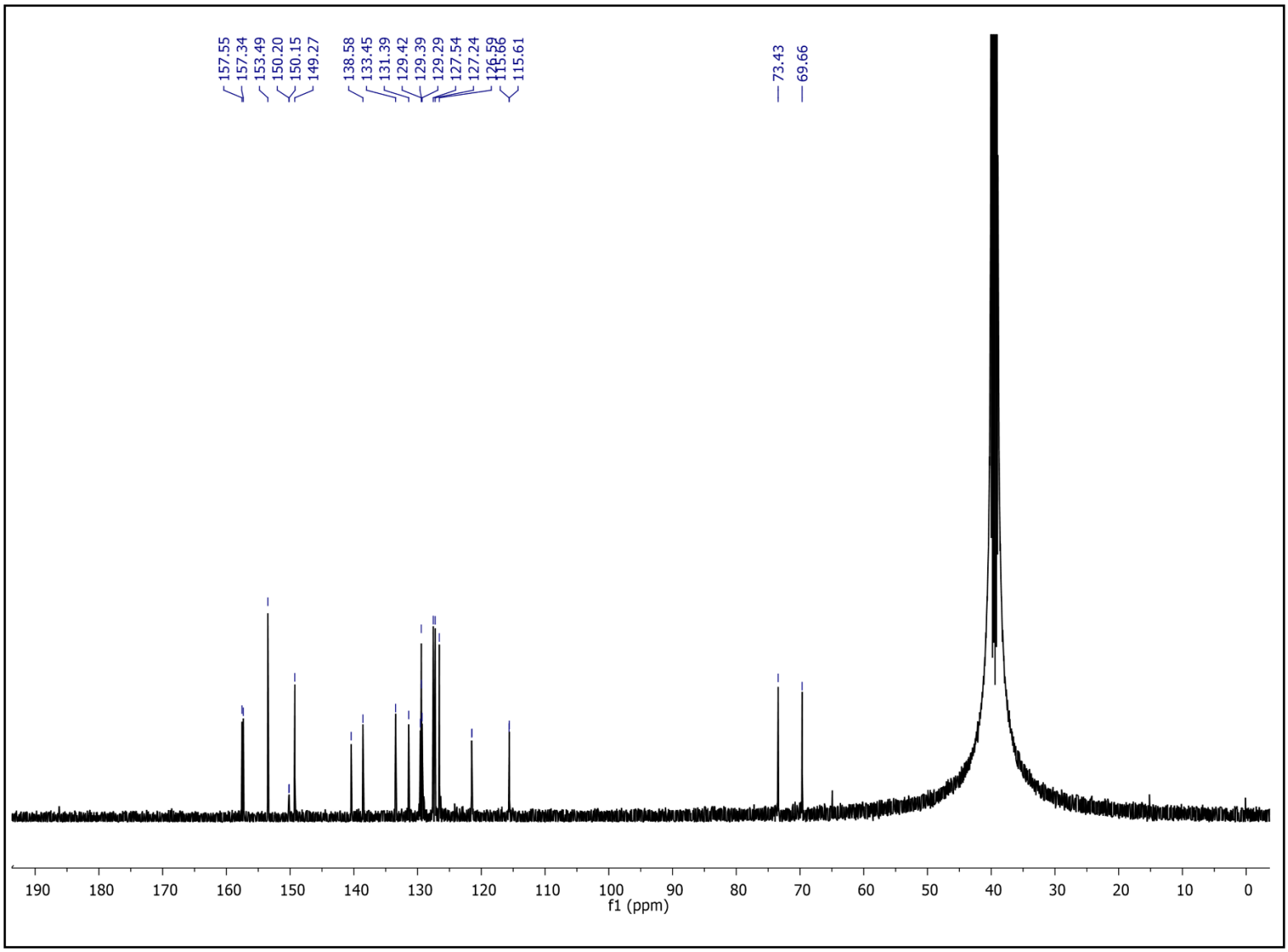

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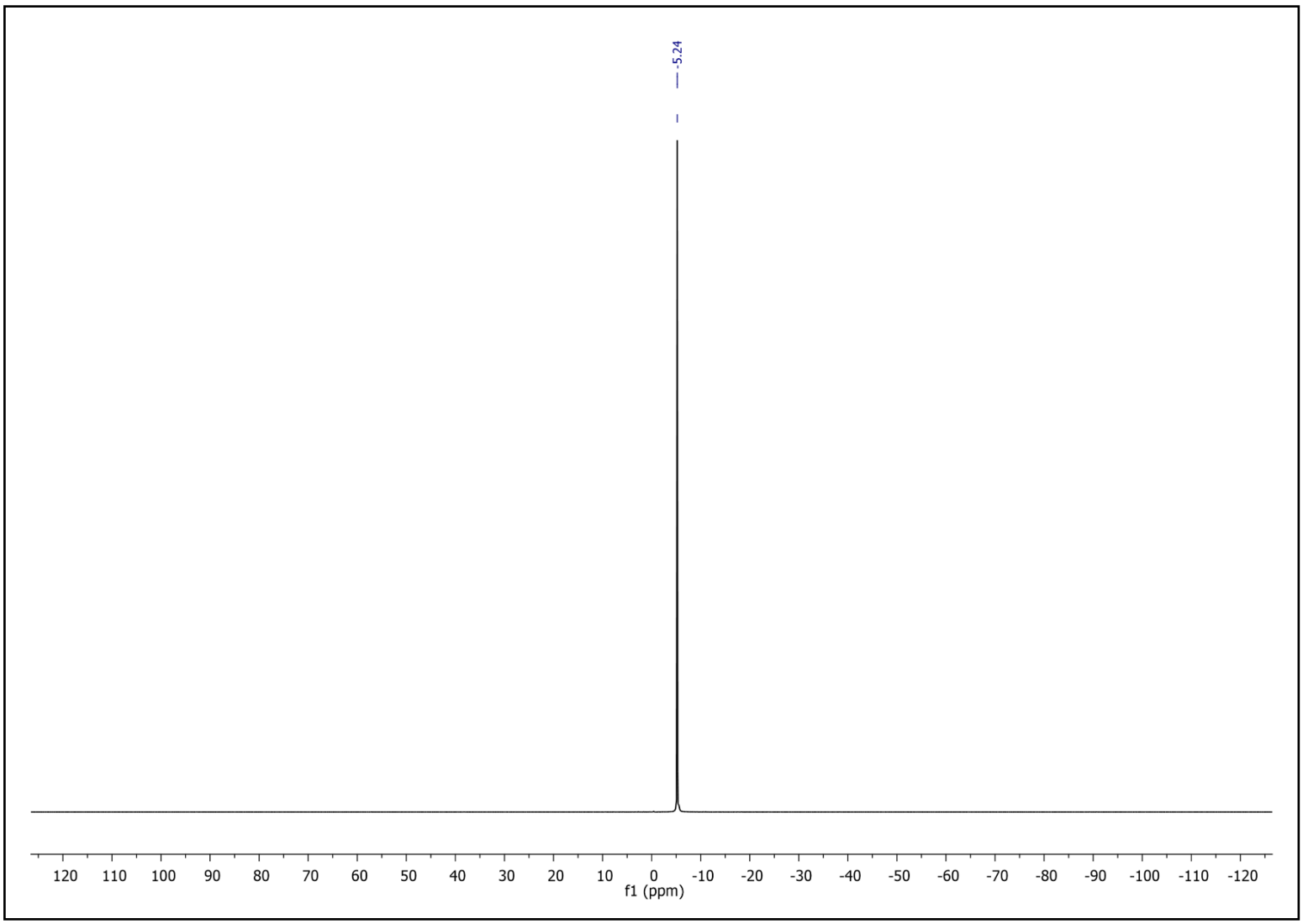

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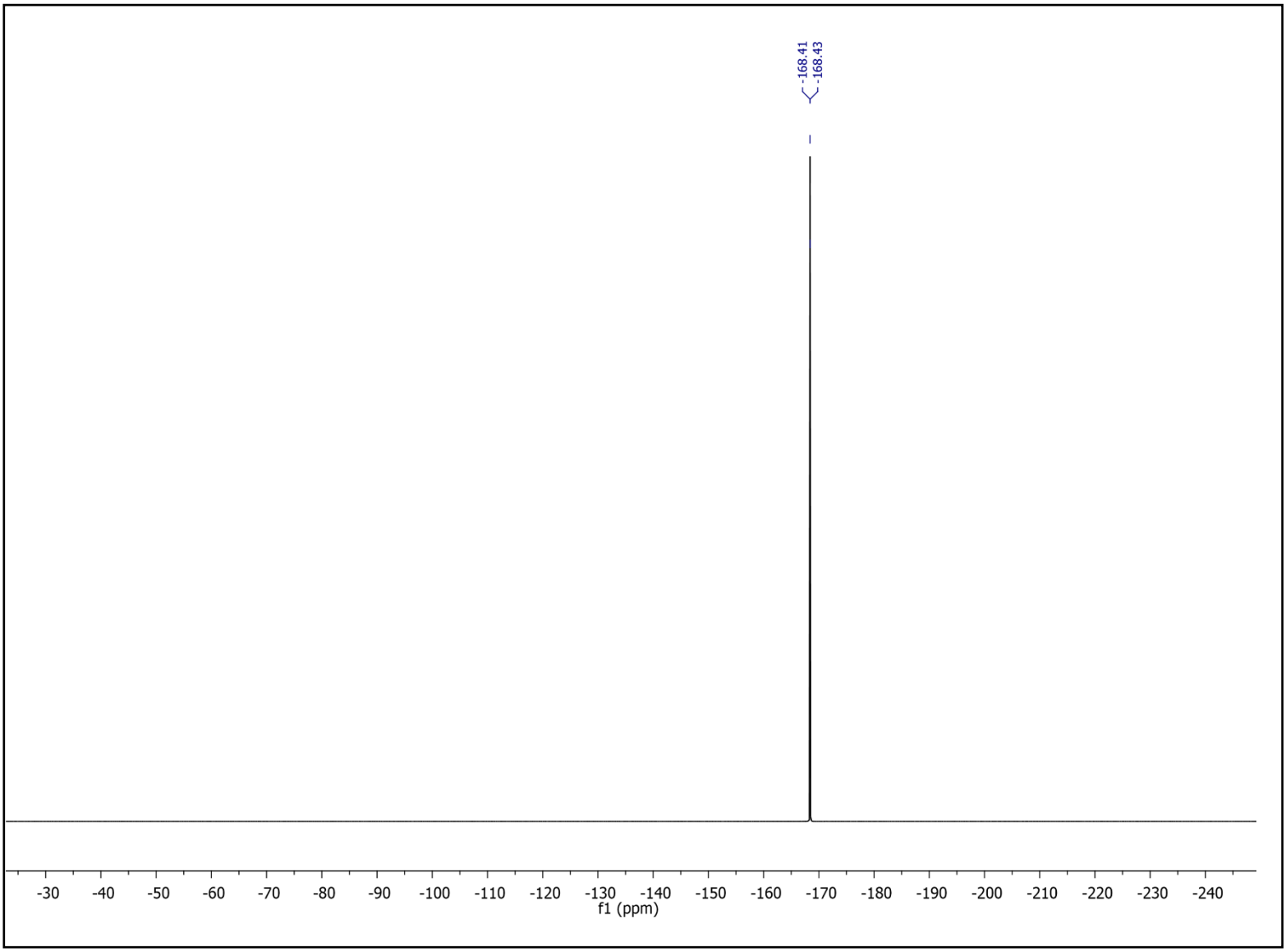

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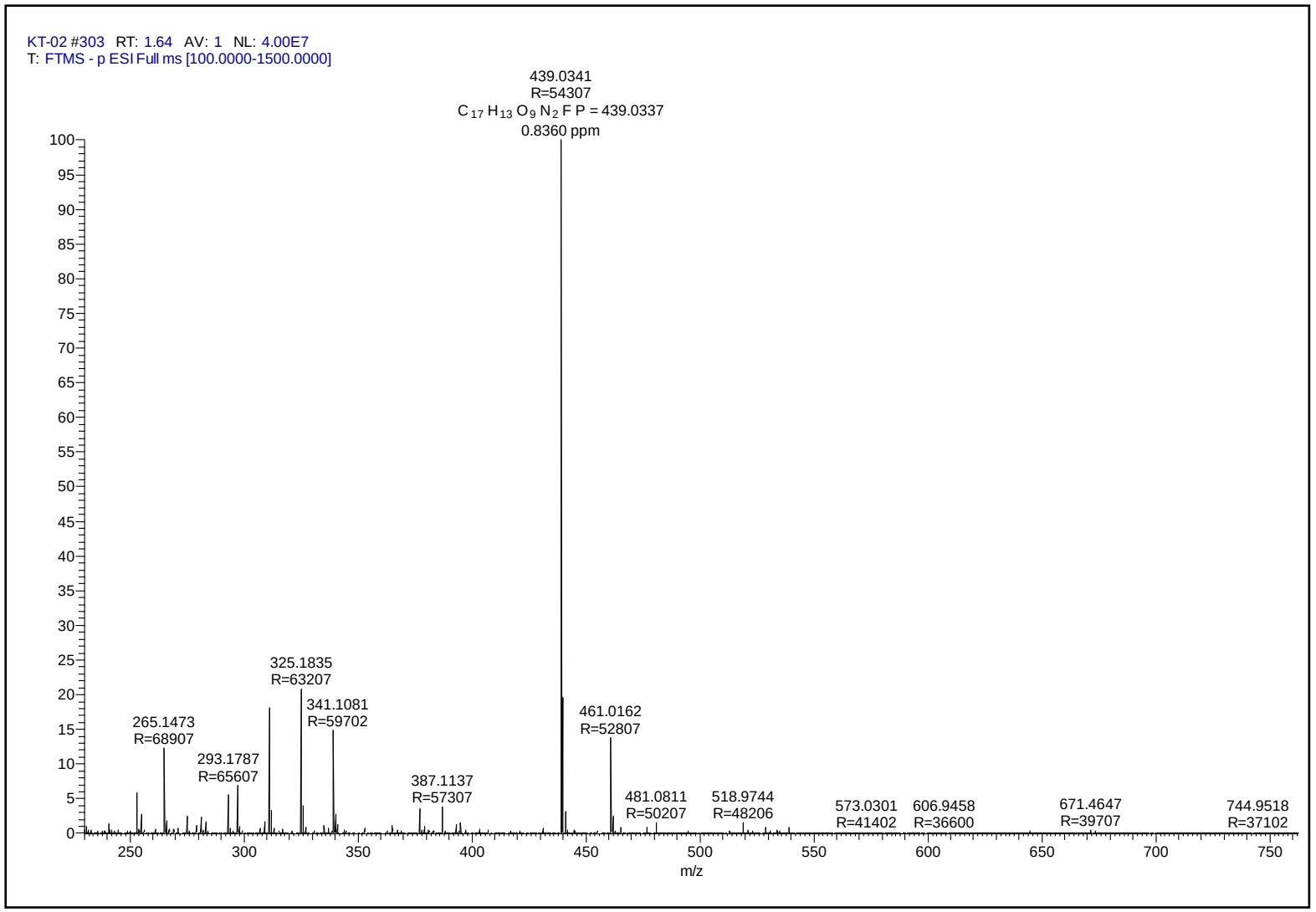

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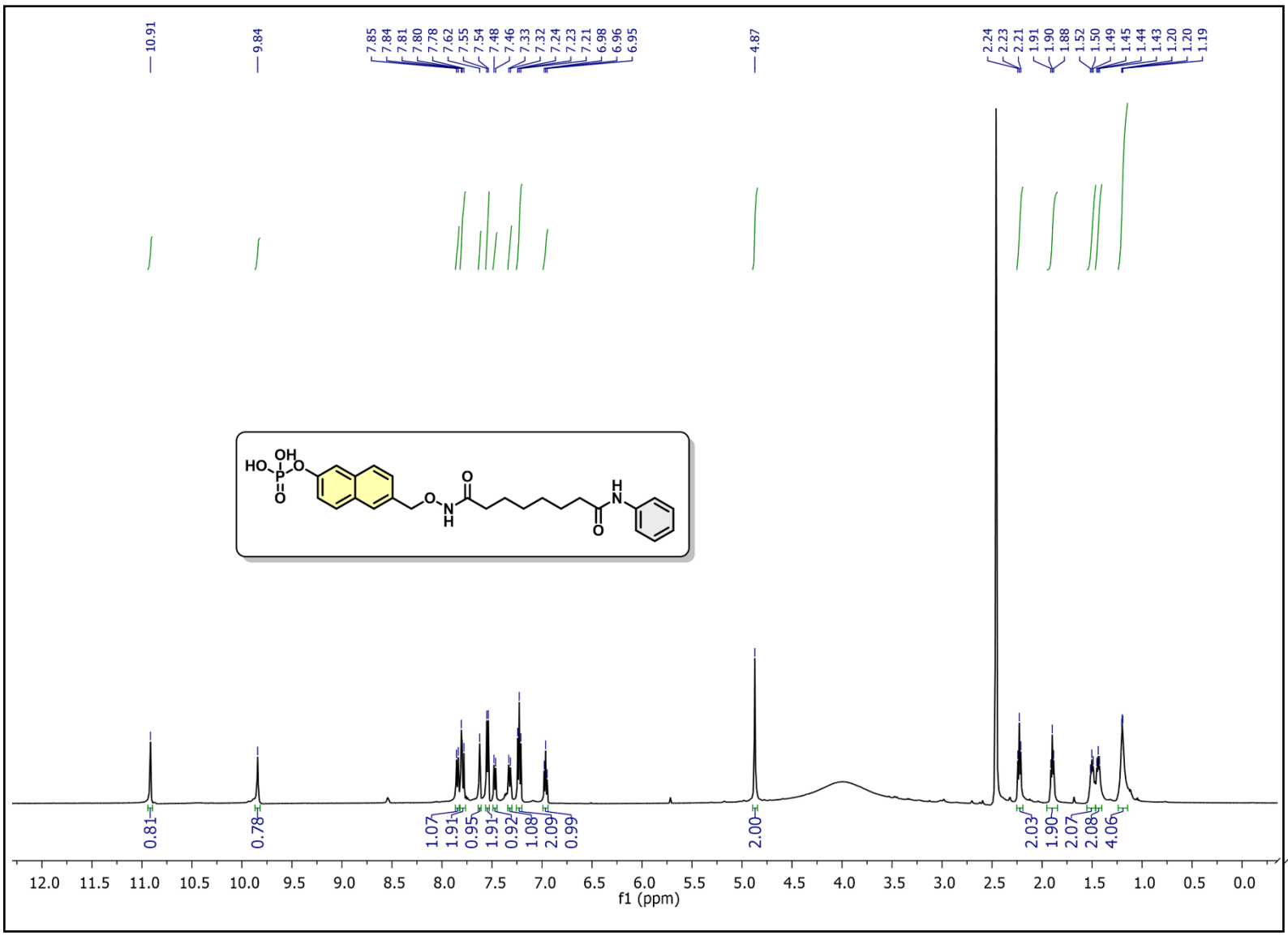

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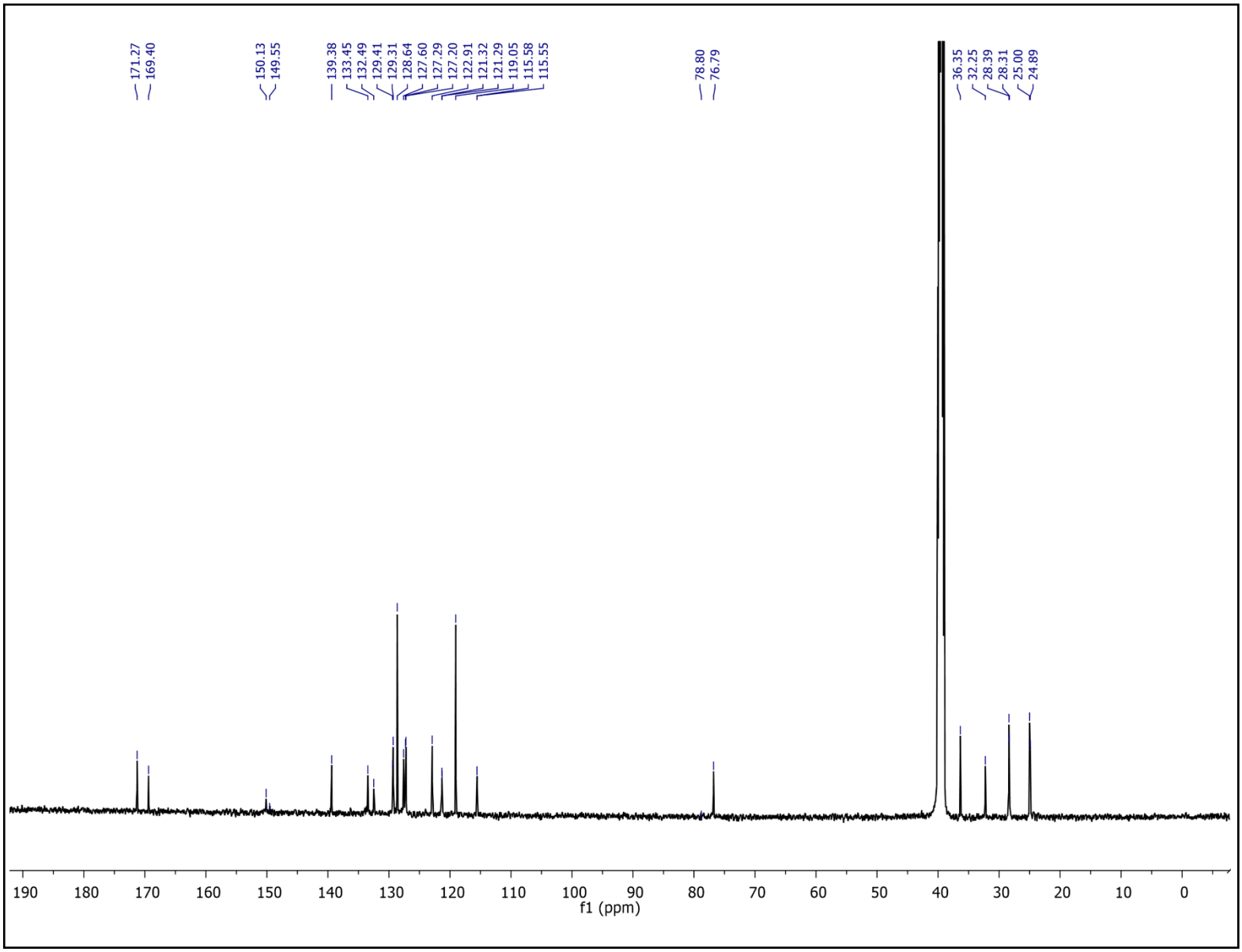

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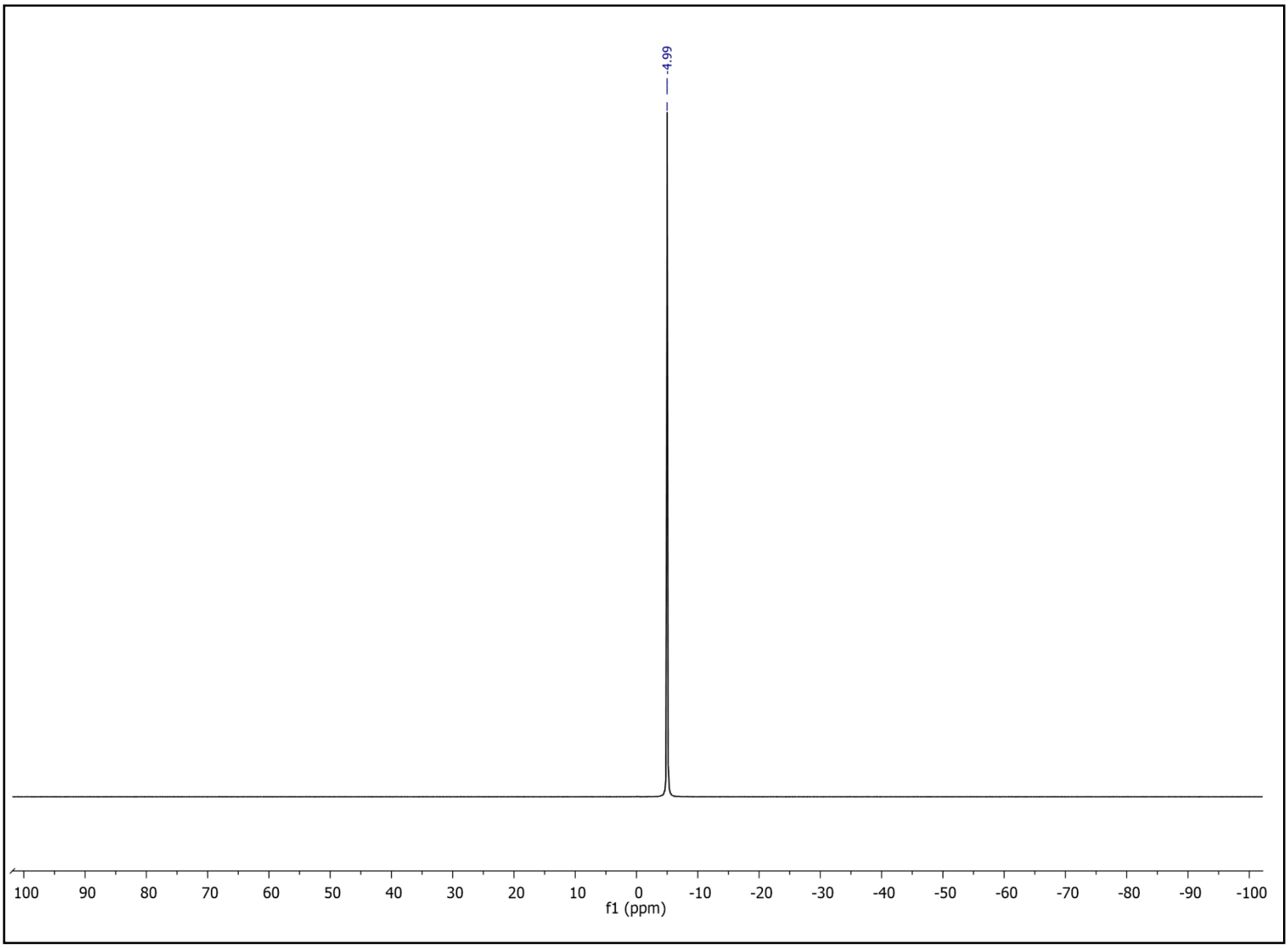

HRMS of compound 9