

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, F₂₅₄ 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. ¹H NMR spectral data were collected at, 500 MHz (JEOL), ¹³C NMR were recorded at 125 MHz, ³¹P NMR were recorded at 202 MHz, and ¹⁹F NMR spectra were recorded at 471 MHz. ¹H 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. ¹³C NMR, ³¹P NMR, and ¹⁹F 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.

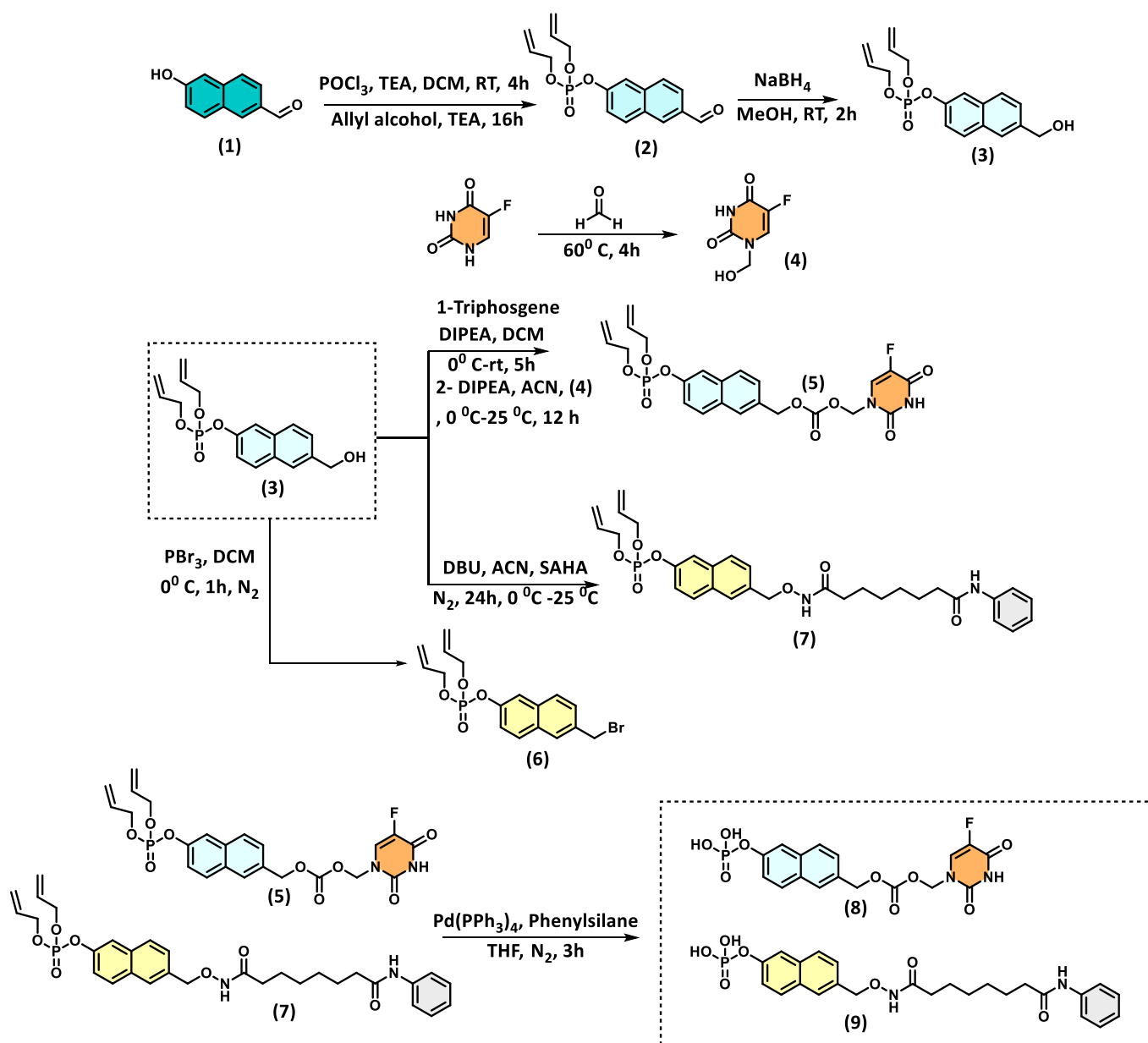


Fig. S1 Stepwise synthetic route for prodrug synthesis

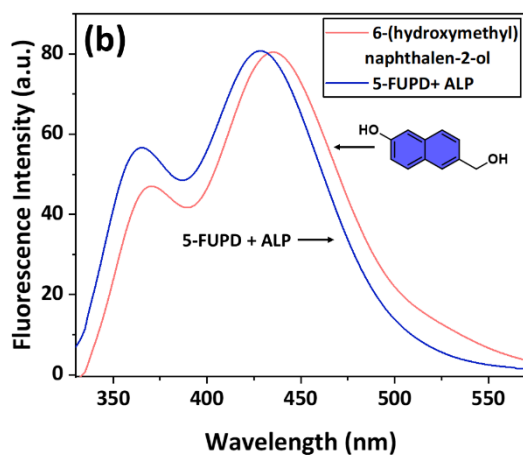
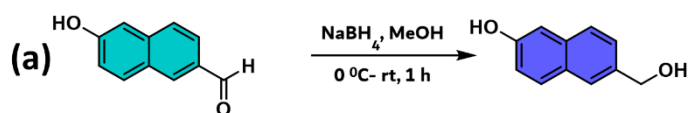


Fig. S2: (a) Reduction of 6-hydroxy-2-naphthaldehyde into of 6-(hydroxymethyl)naphthalen-2-ol; (b) Fluorescence spectrum of 6-(hydroxymethyl)naphthalen-2-ol and fluorophore generated from ALP triggered hydrolysis of 5-FUPD.

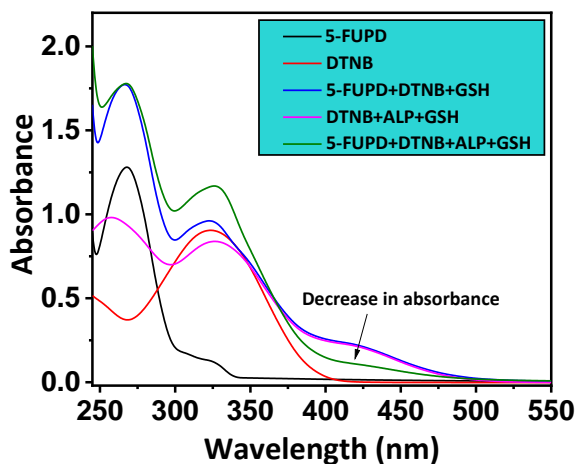


Fig. S3: Assessment of GSH depletion by treatment with prodrugs using DTNB assay. GSH, prodrug, and ALP solutions were incubated at 37 °C for 20 minutes and DTNB reagent was added. Depletion in GSH levels was observed by taking absorption spectrum using UV-Vis. spectrophotometer.

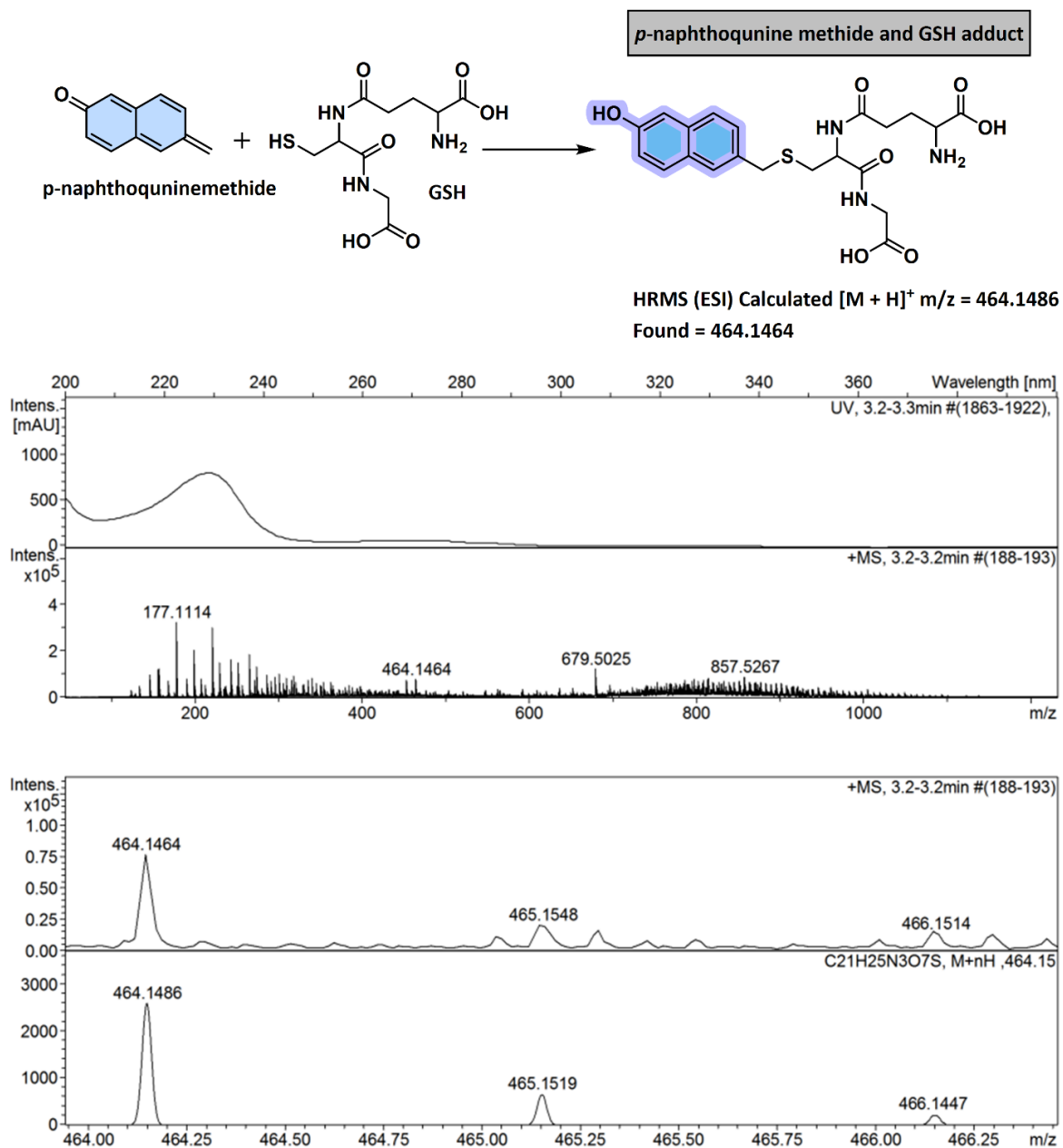


Fig. S4: HRMS spectrum of captured *p*-naphthoquinone methide and GSH adduct.

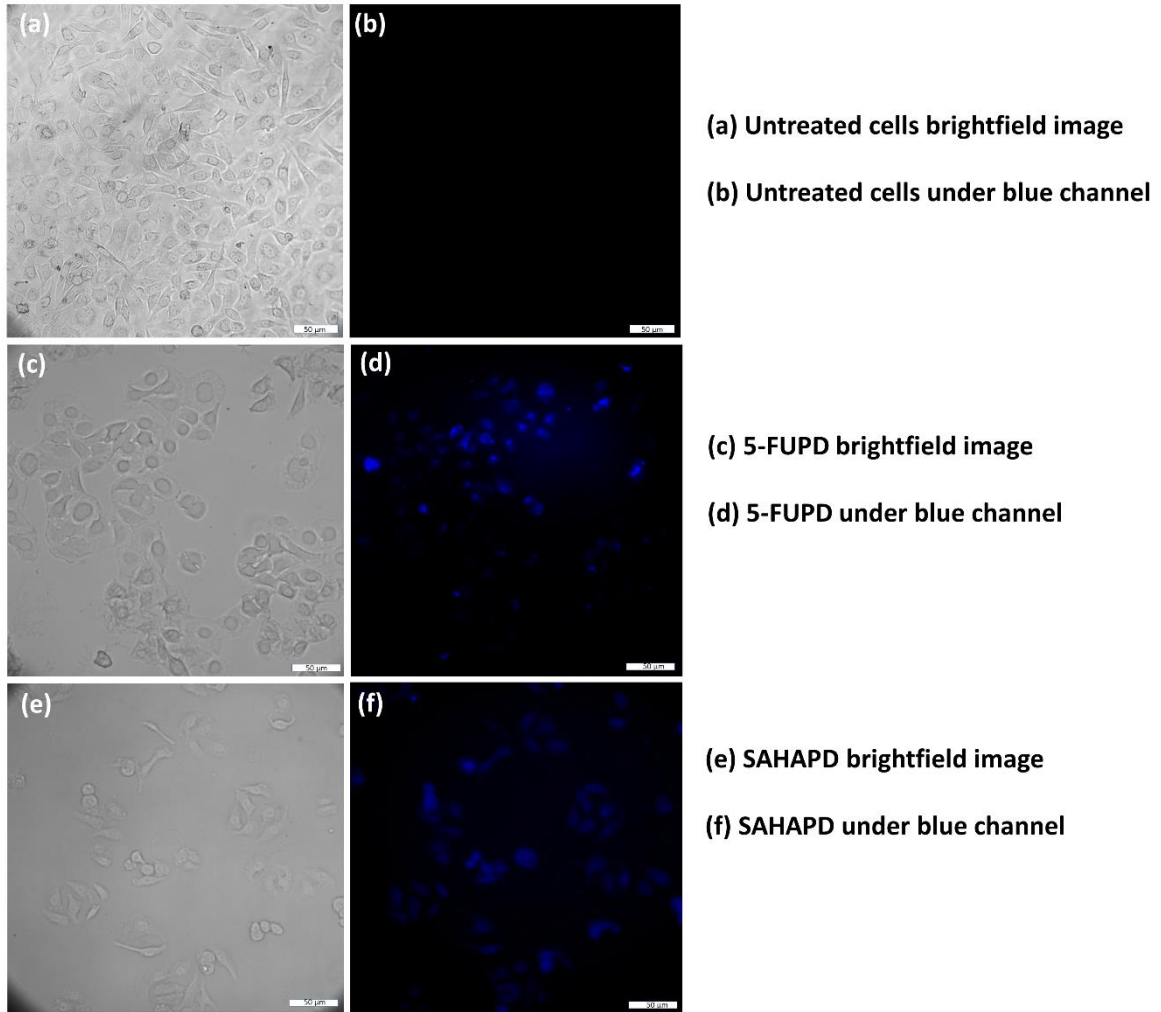
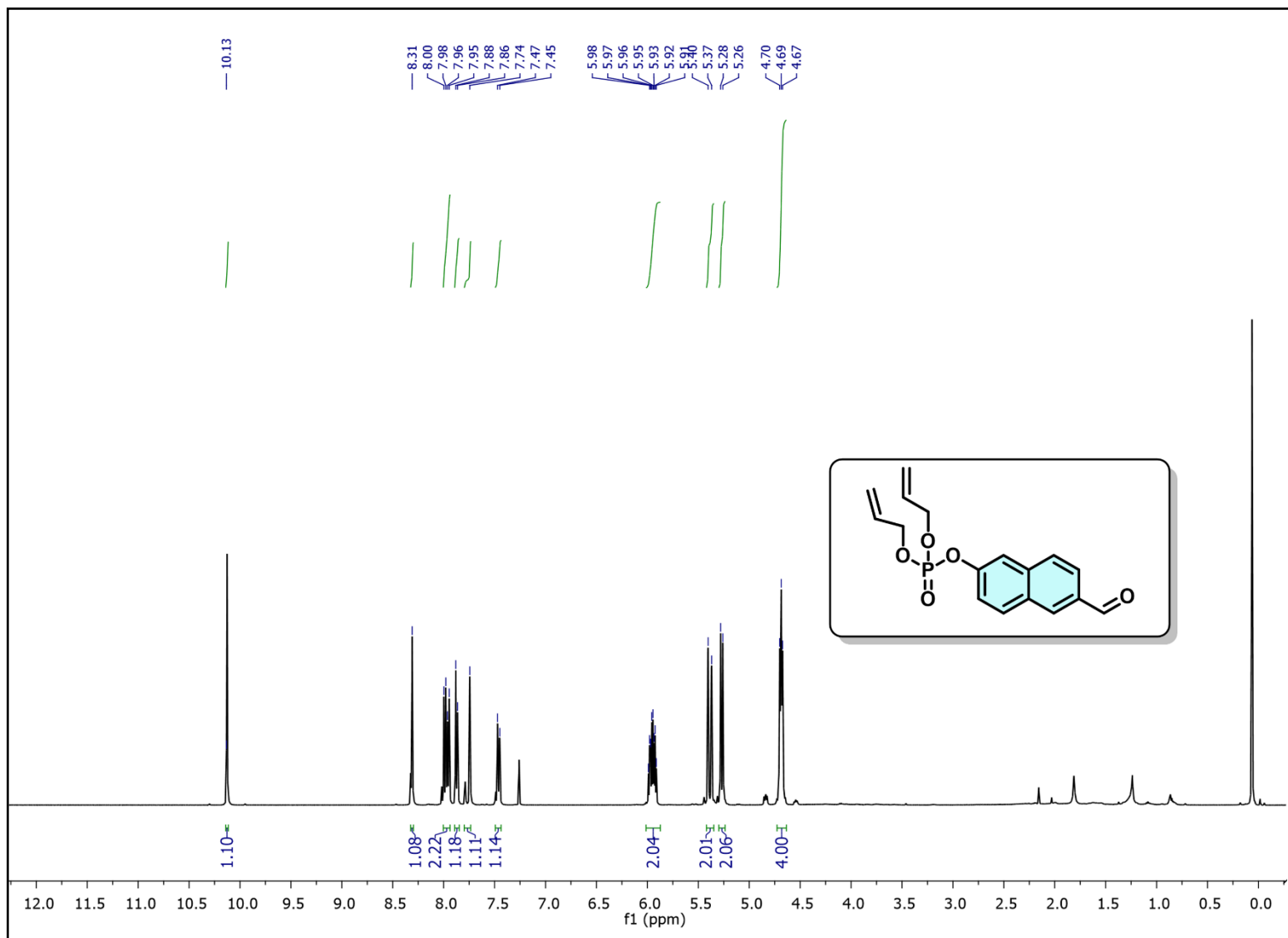
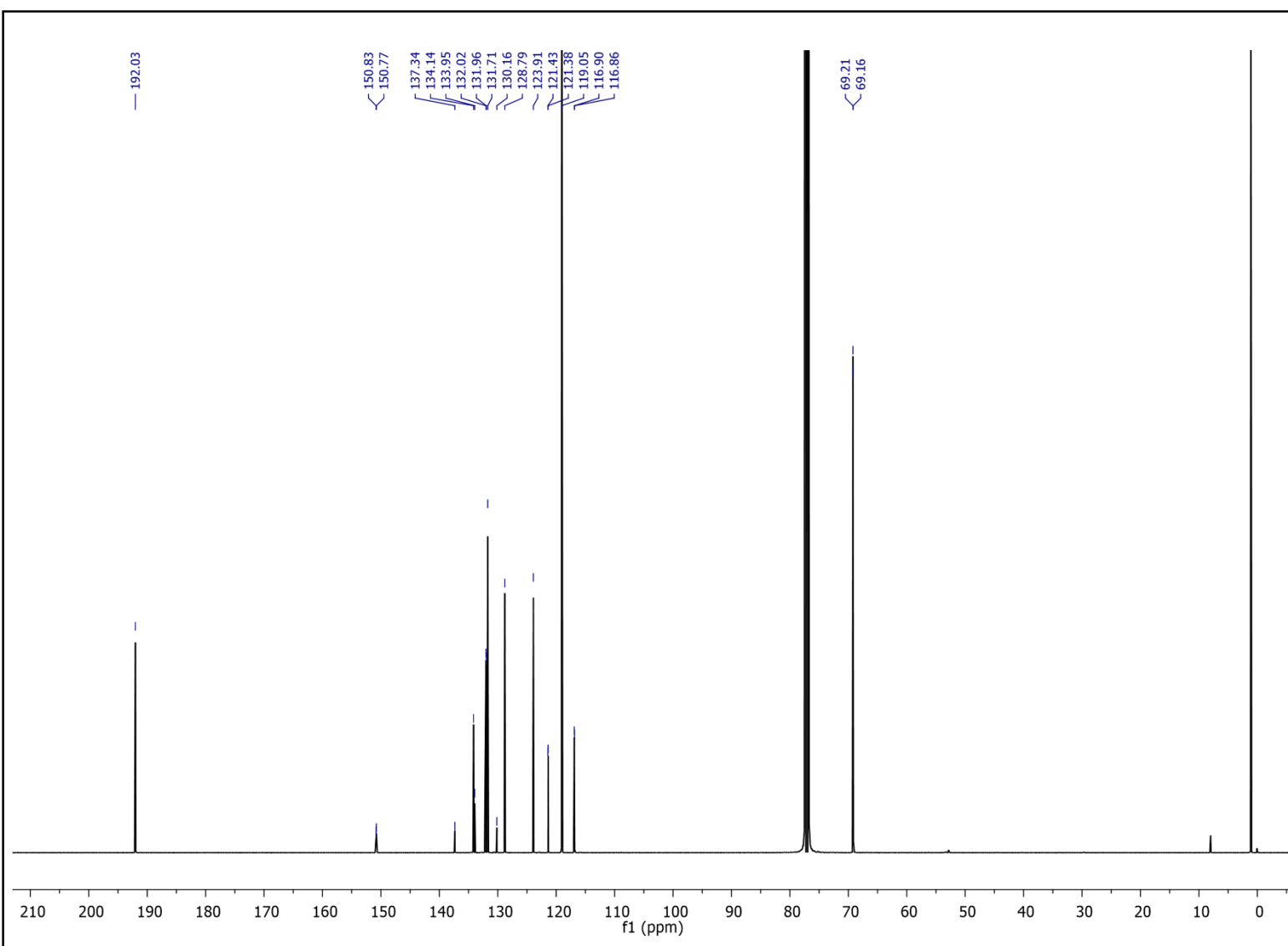


Fig. S5: Fluorescence images (a, b) untreated cells brightfield image and untreated cells under blue channel; (c, d) brightfield and fluorescence images of 5-FUPD under blue channel; (e, f) brightfield and fluorescence images of SAHAPD under blue channel.

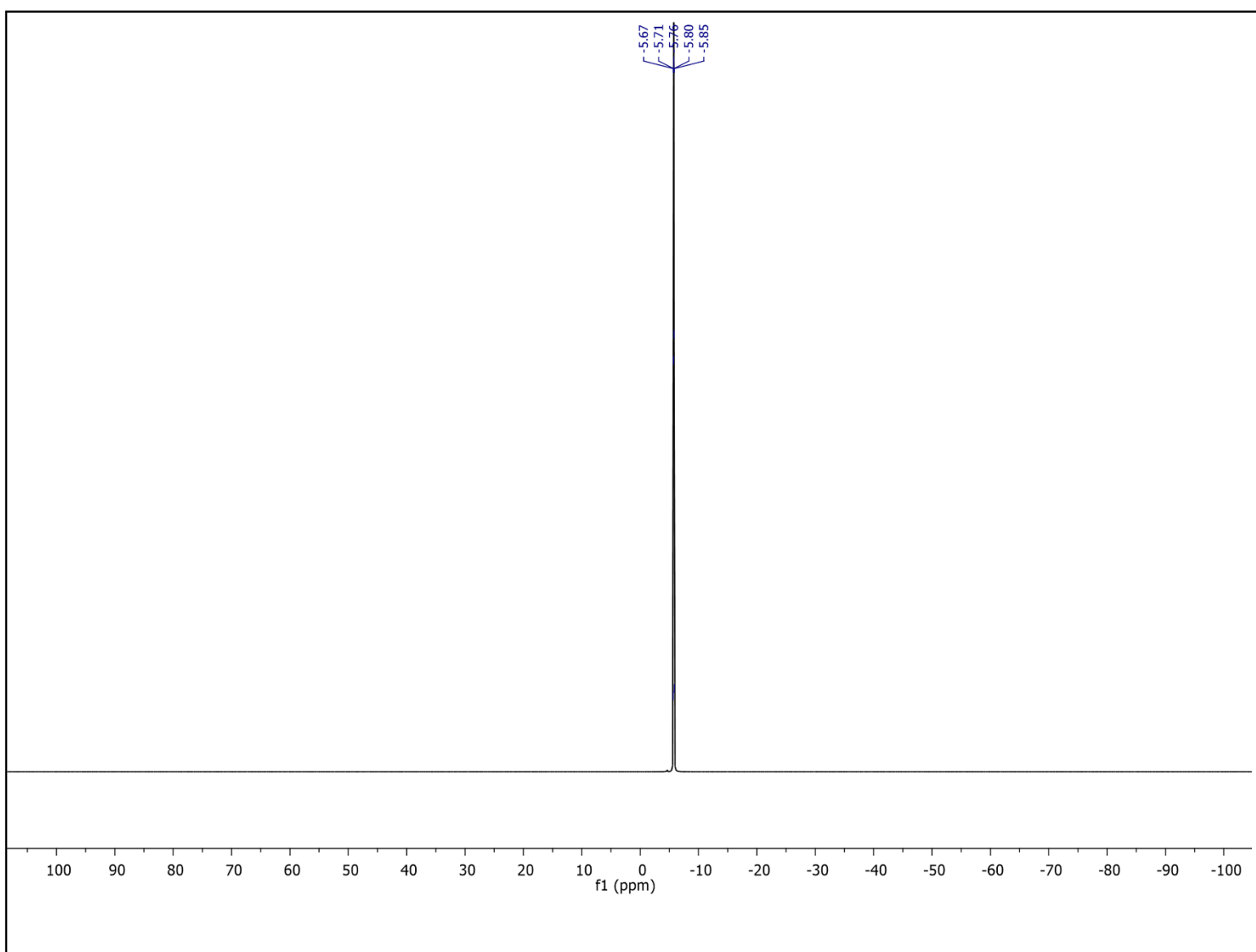
¹H NMR of compound 2 (CDCl₃)



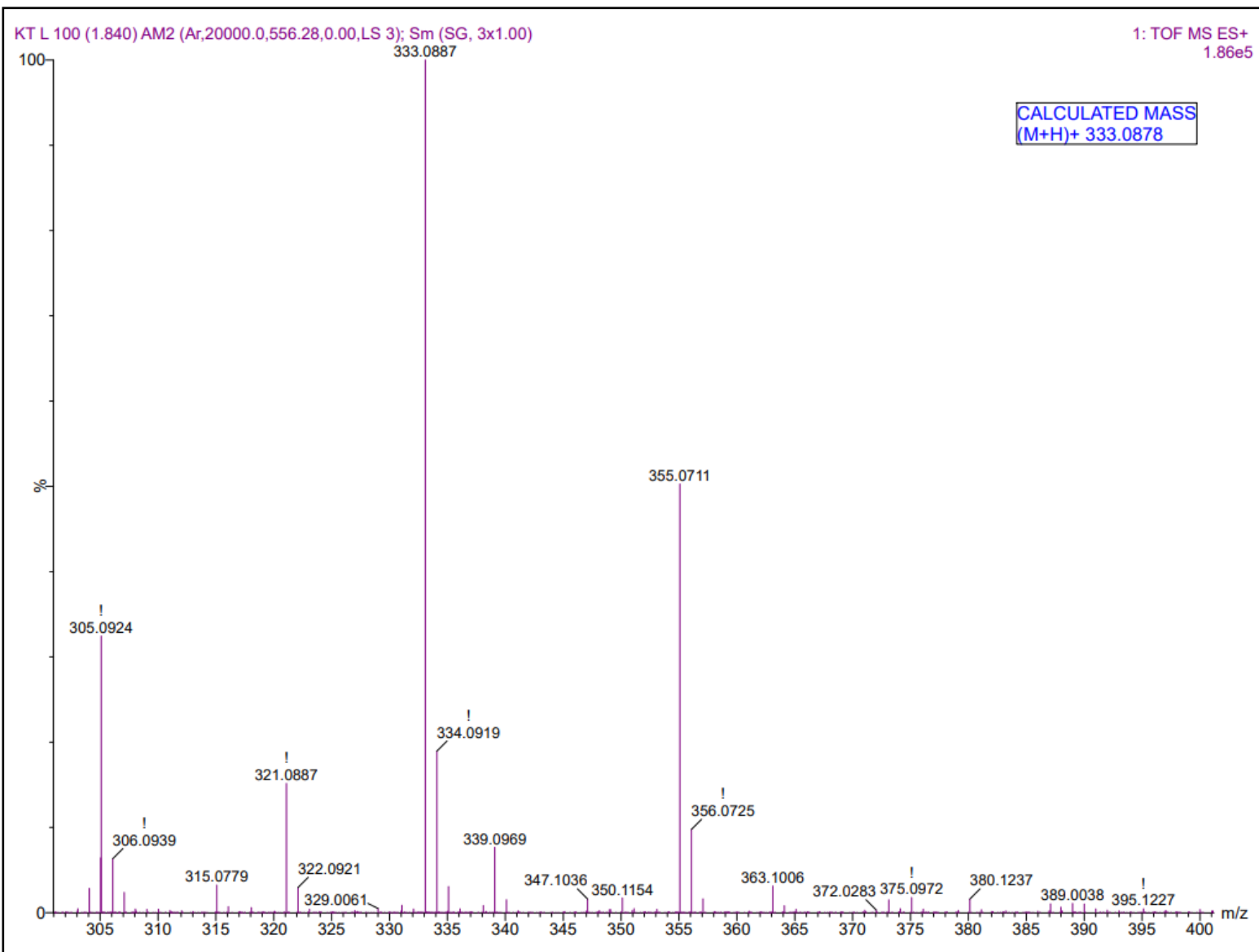
^{13}C NMR of compound 2 (CDCl_3)



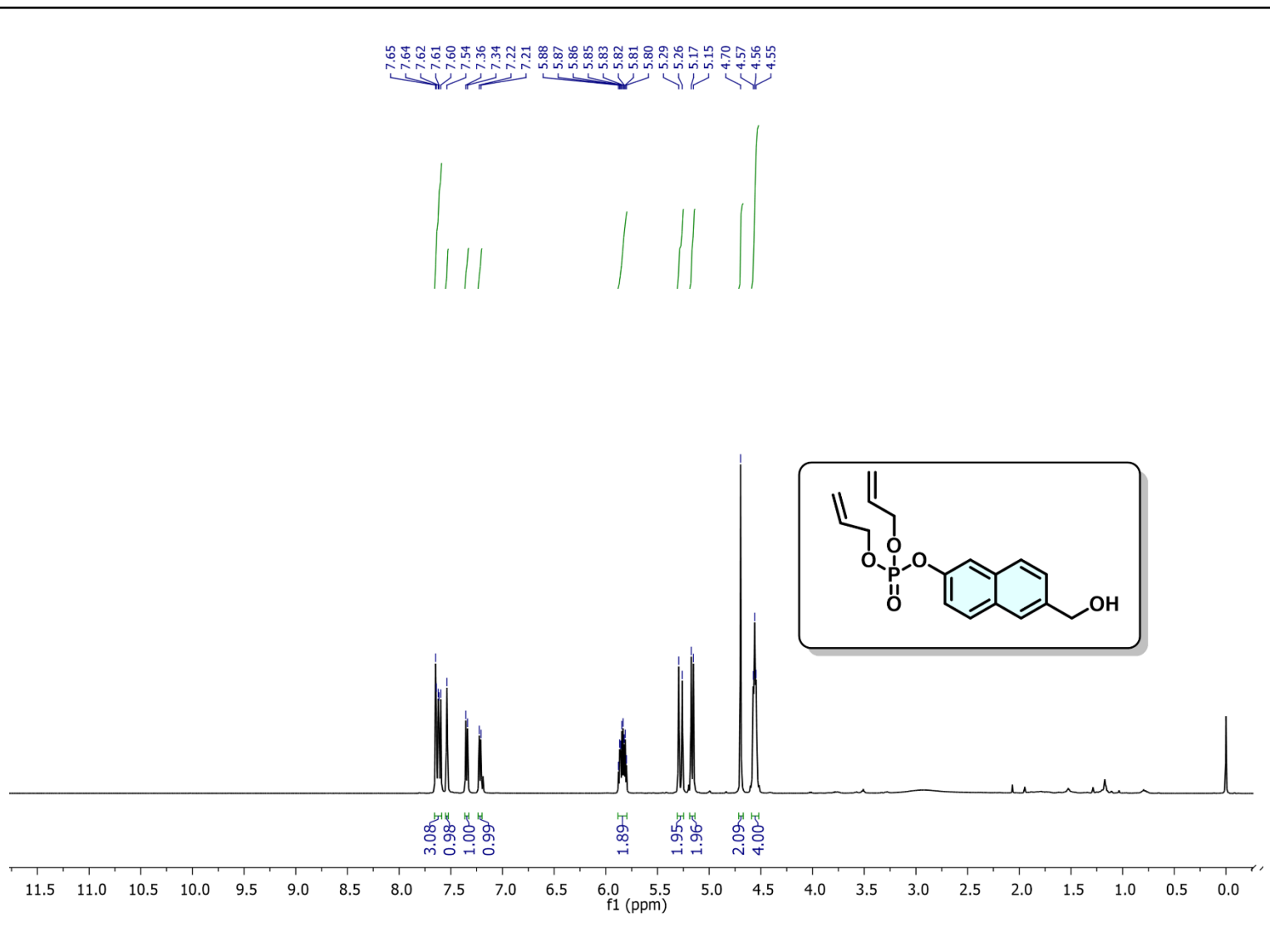
³¹P NMR of compound 2 (CDCl₃)



HRMS of compound 2

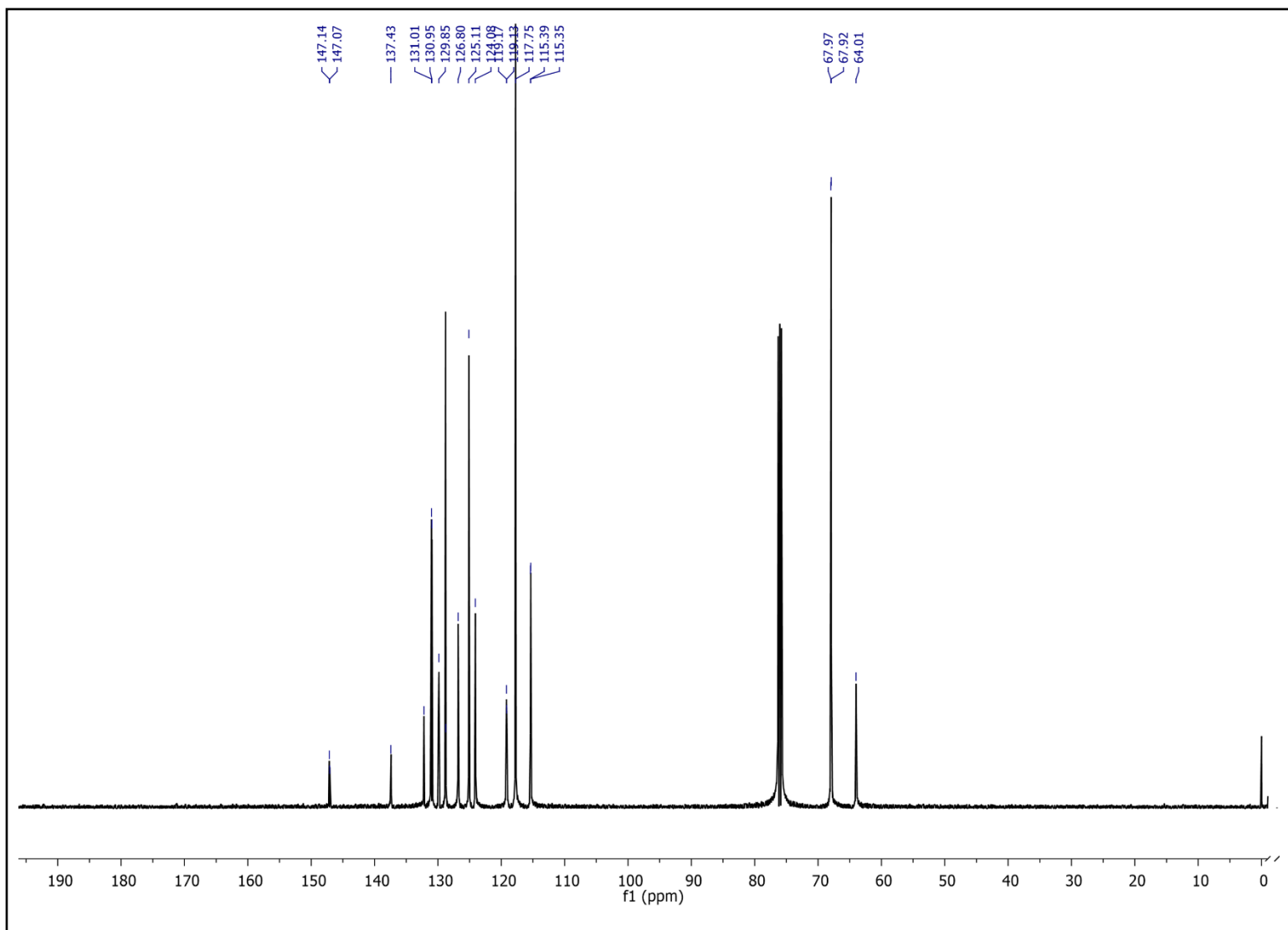


¹H NMR of compound 3 (CDCl₃)



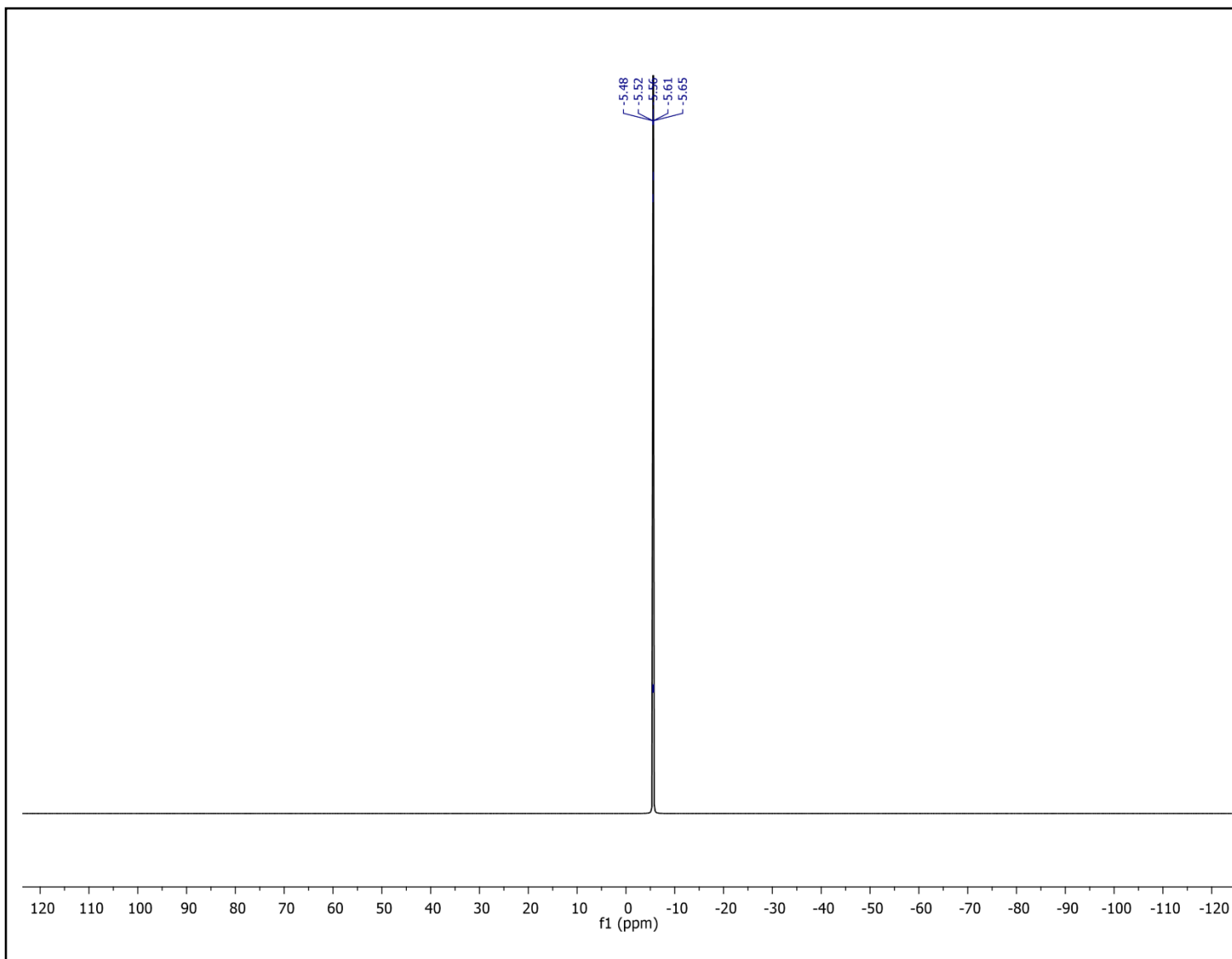
S10

^{13}C NMR of compound 3 (CDCl_3)



S11

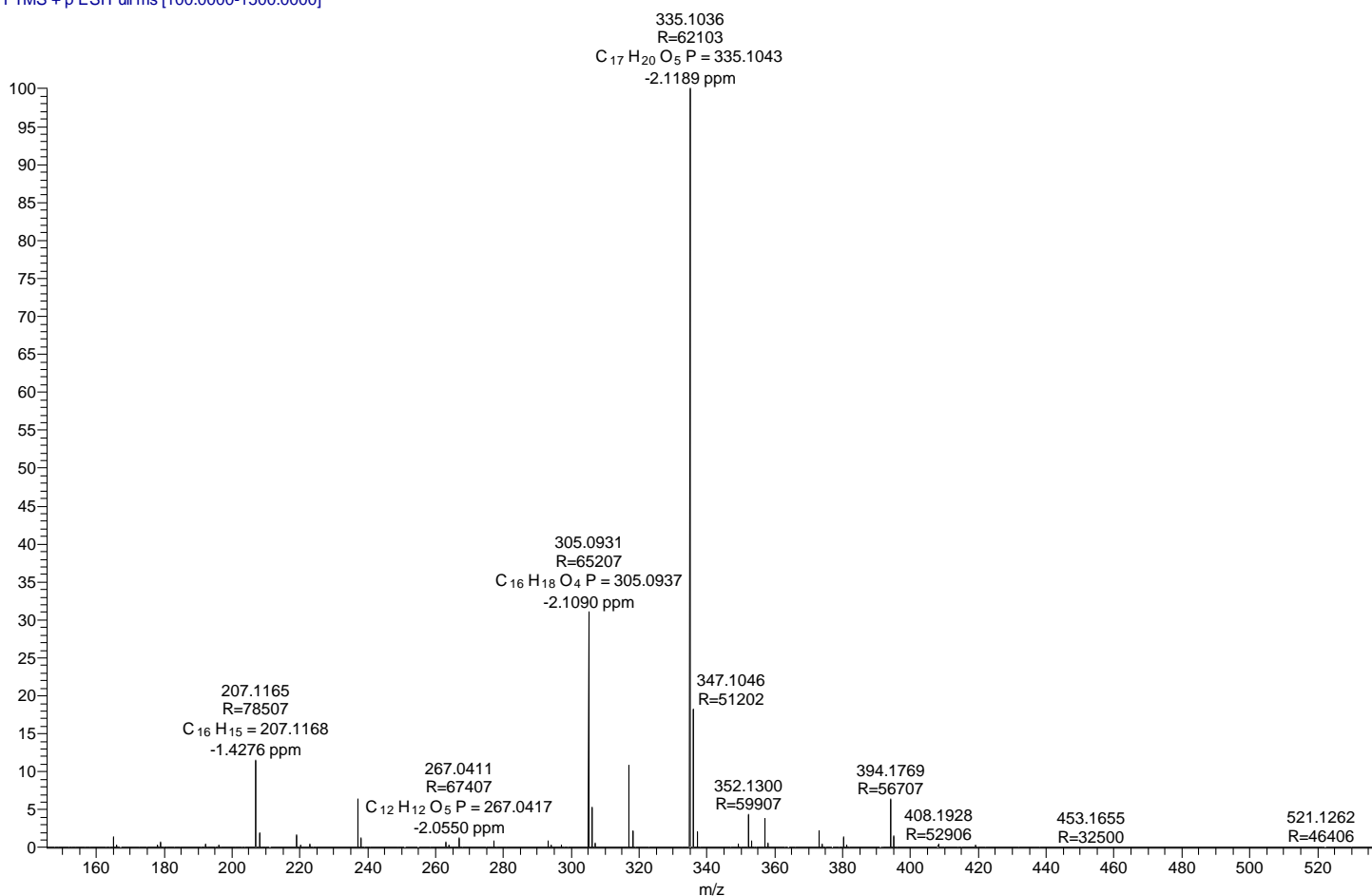
^{31}P NMR of compound 3 (CDCl_3)



S12

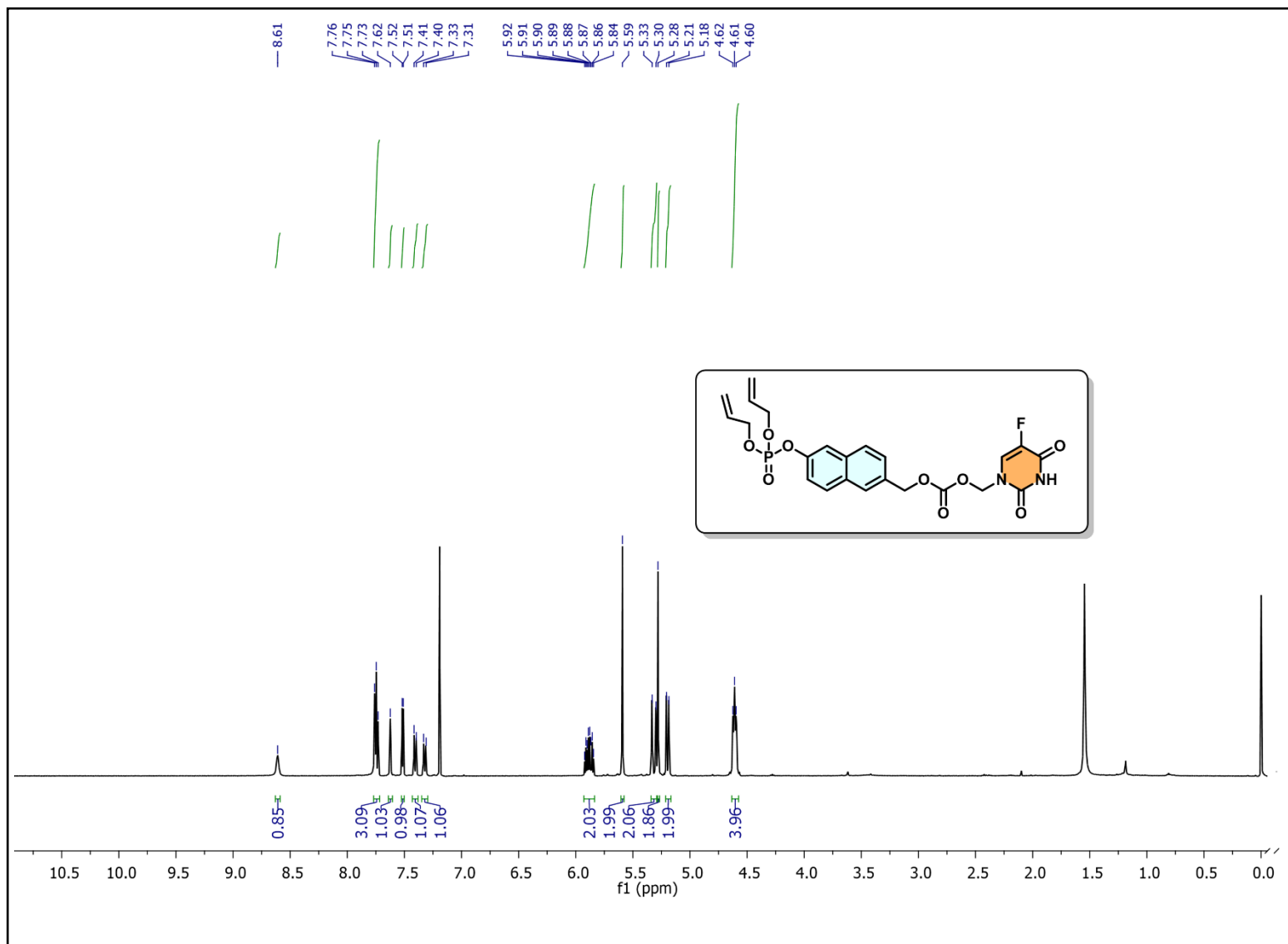
HRMS of compound 3

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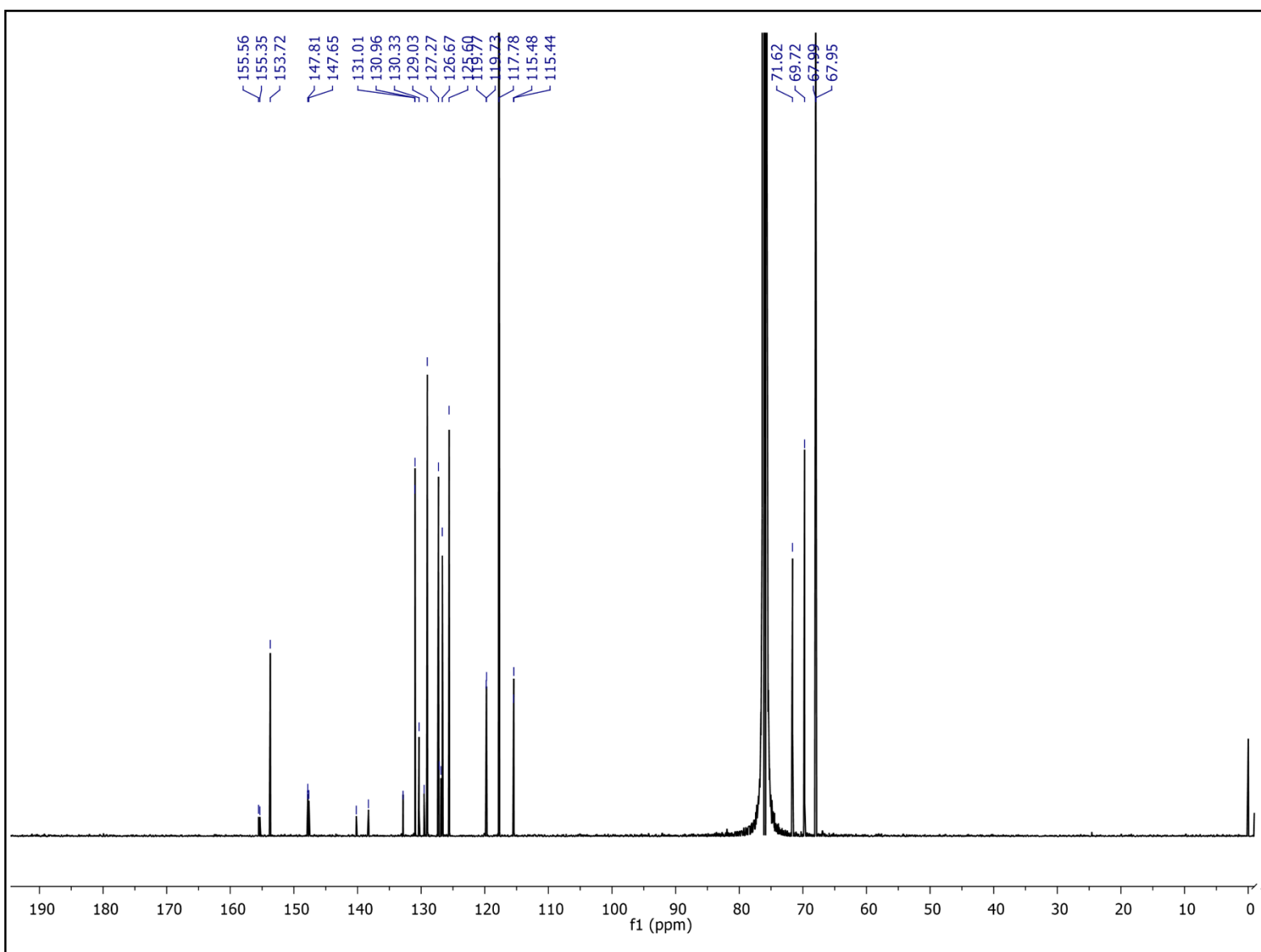
S13

¹H NMR of compound 5 (CDCl₃)



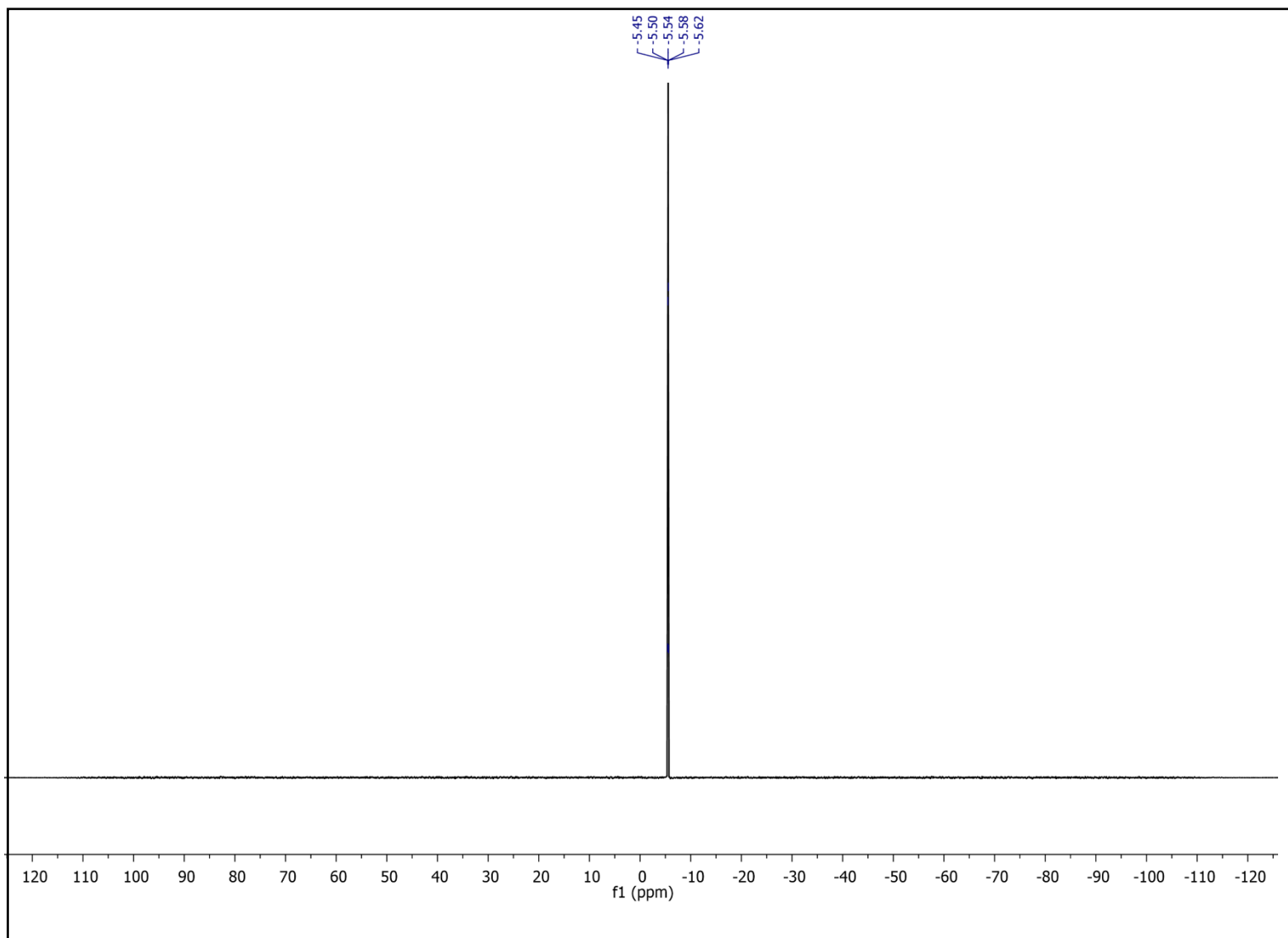
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¹³C NMR of compound 5 (CDCl₃)



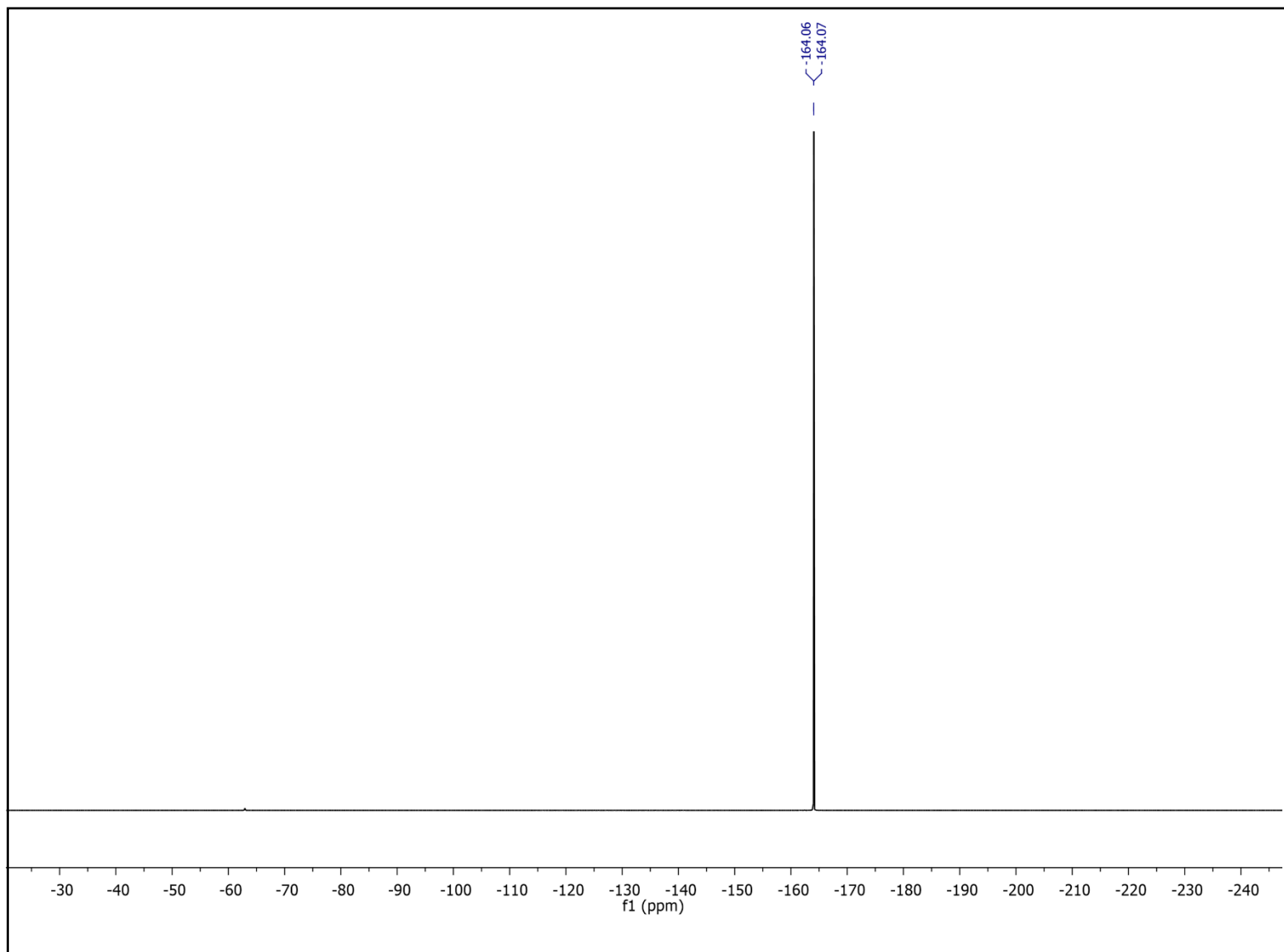
S15

^{31}P NMR of compound 5 (CDCl_3)



S16

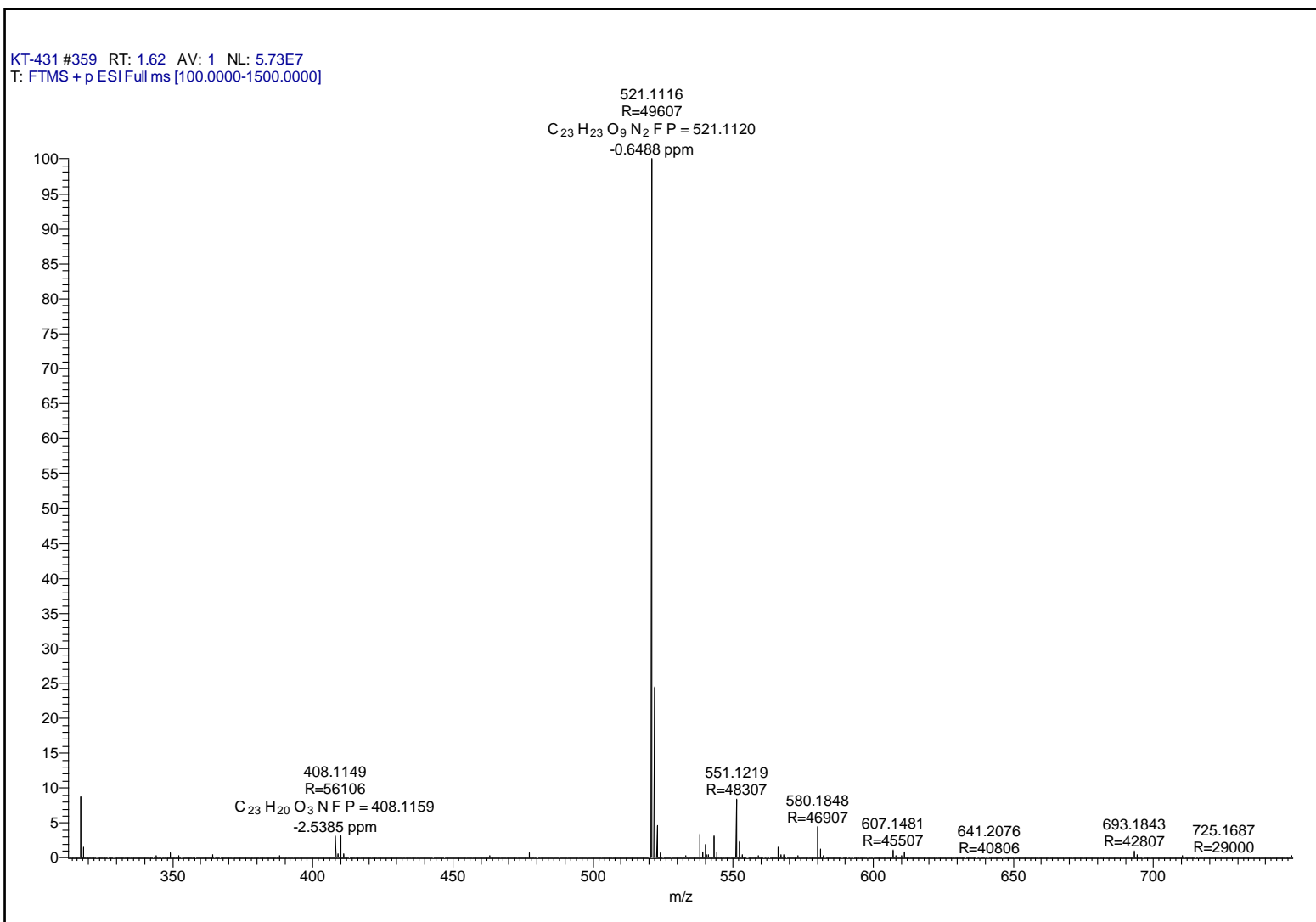
^{19}F NMR of compound 5 (CDCl_3)



S17

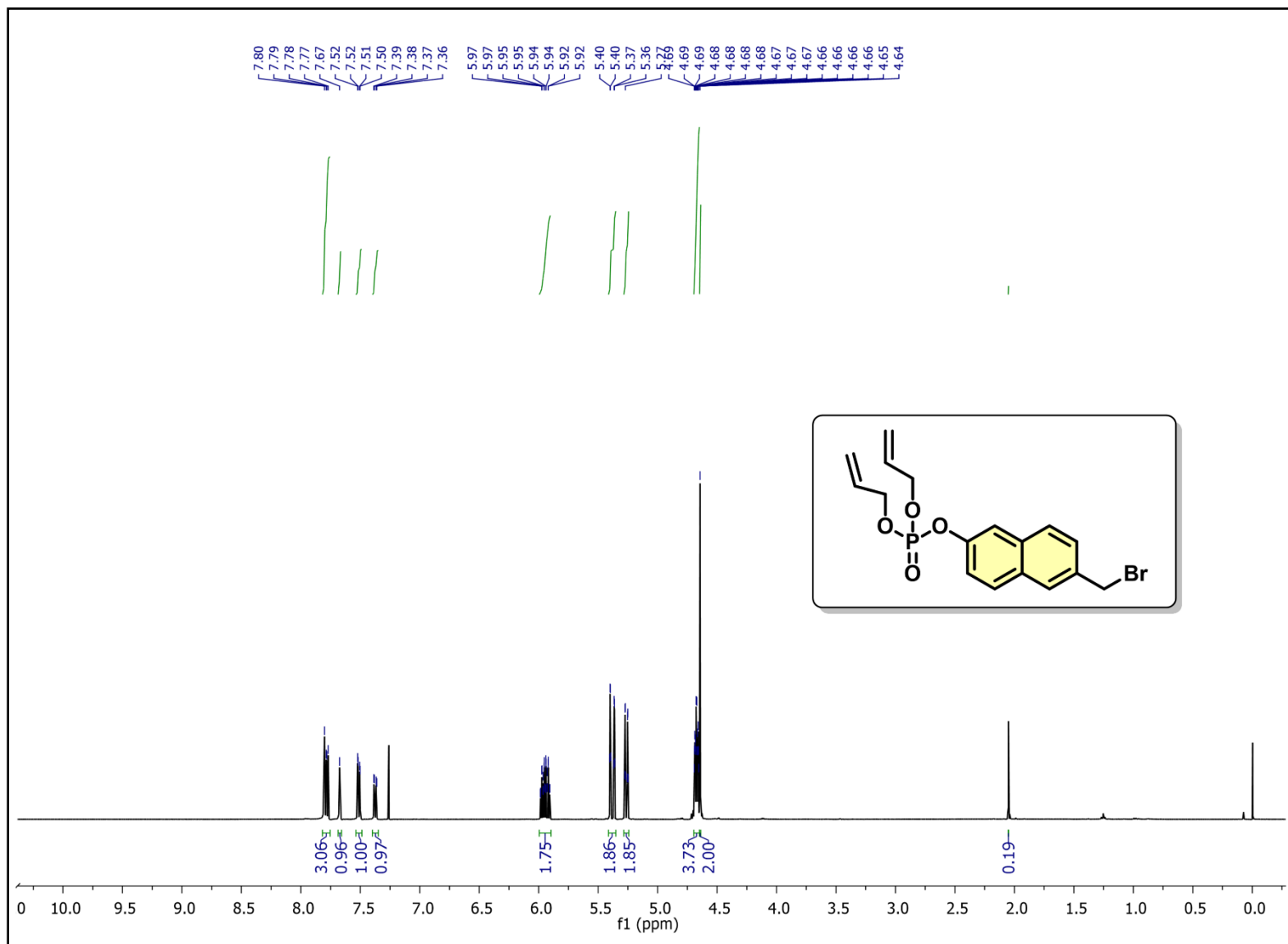
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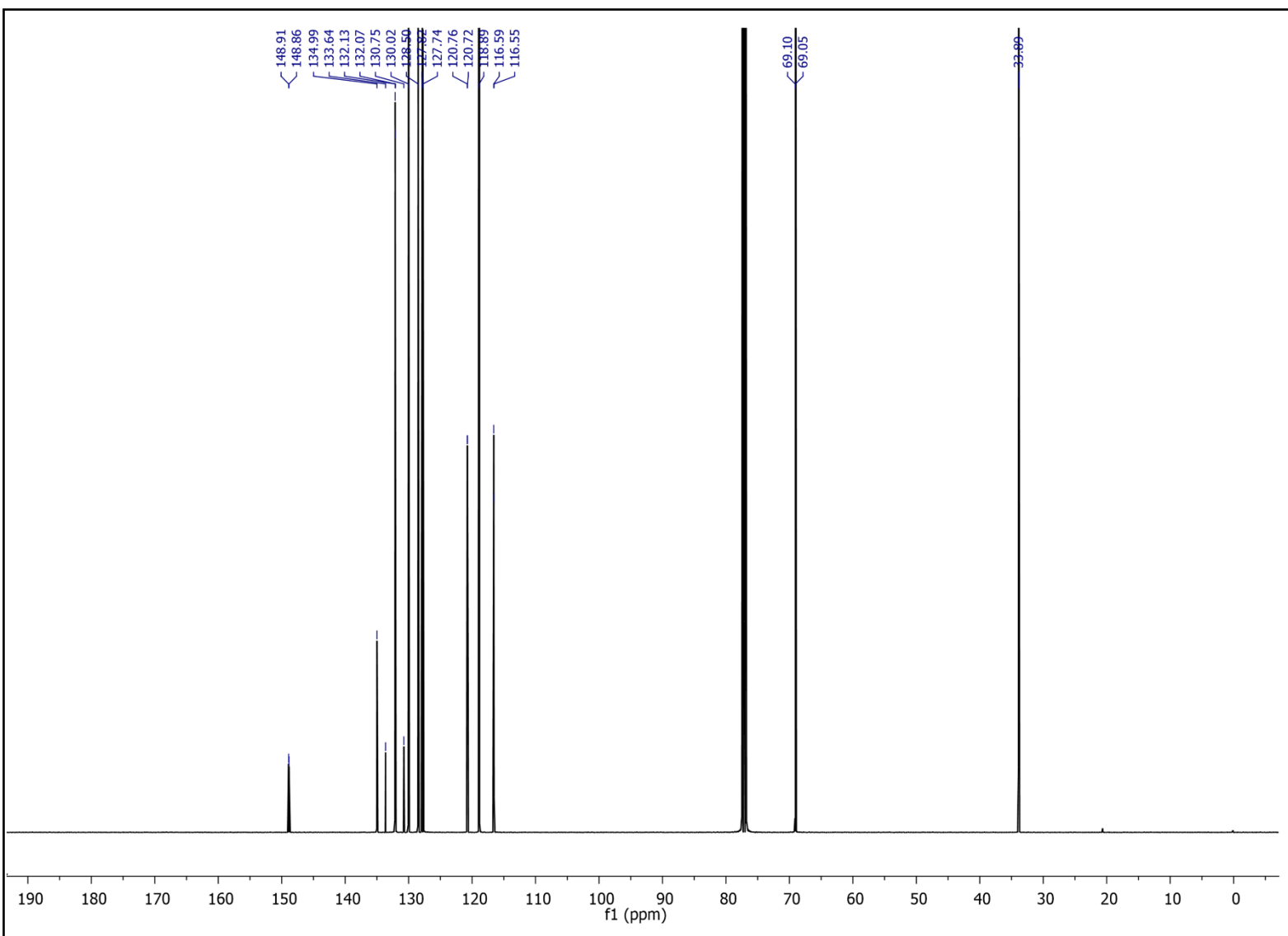
S18

¹H NMR of compound 6 (CDCl₃)



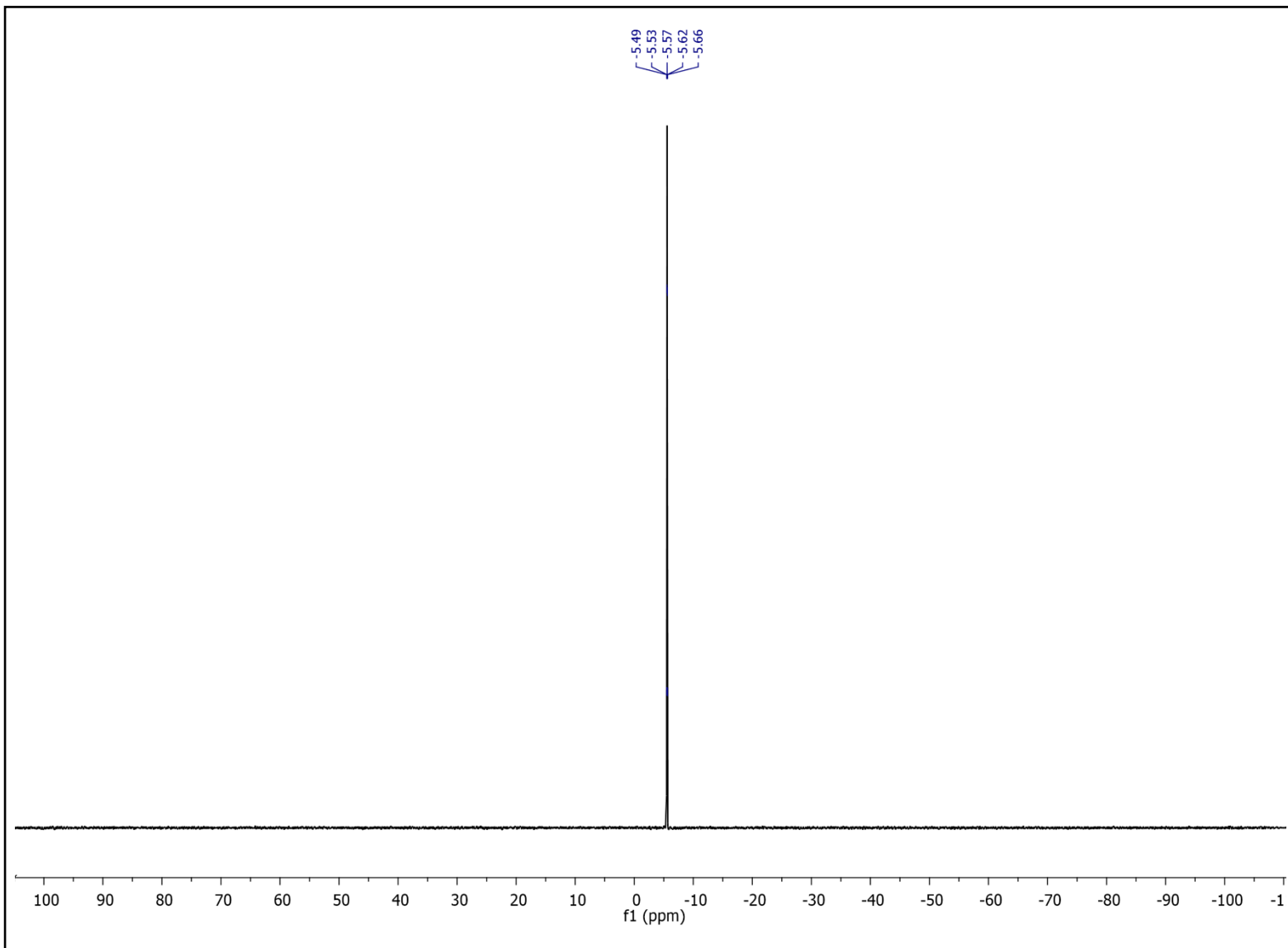
S19

^{13}C NMR of compound 6 (CDCl_3)



S20

^{31}P NMR of compound 6 (CDCl_3)

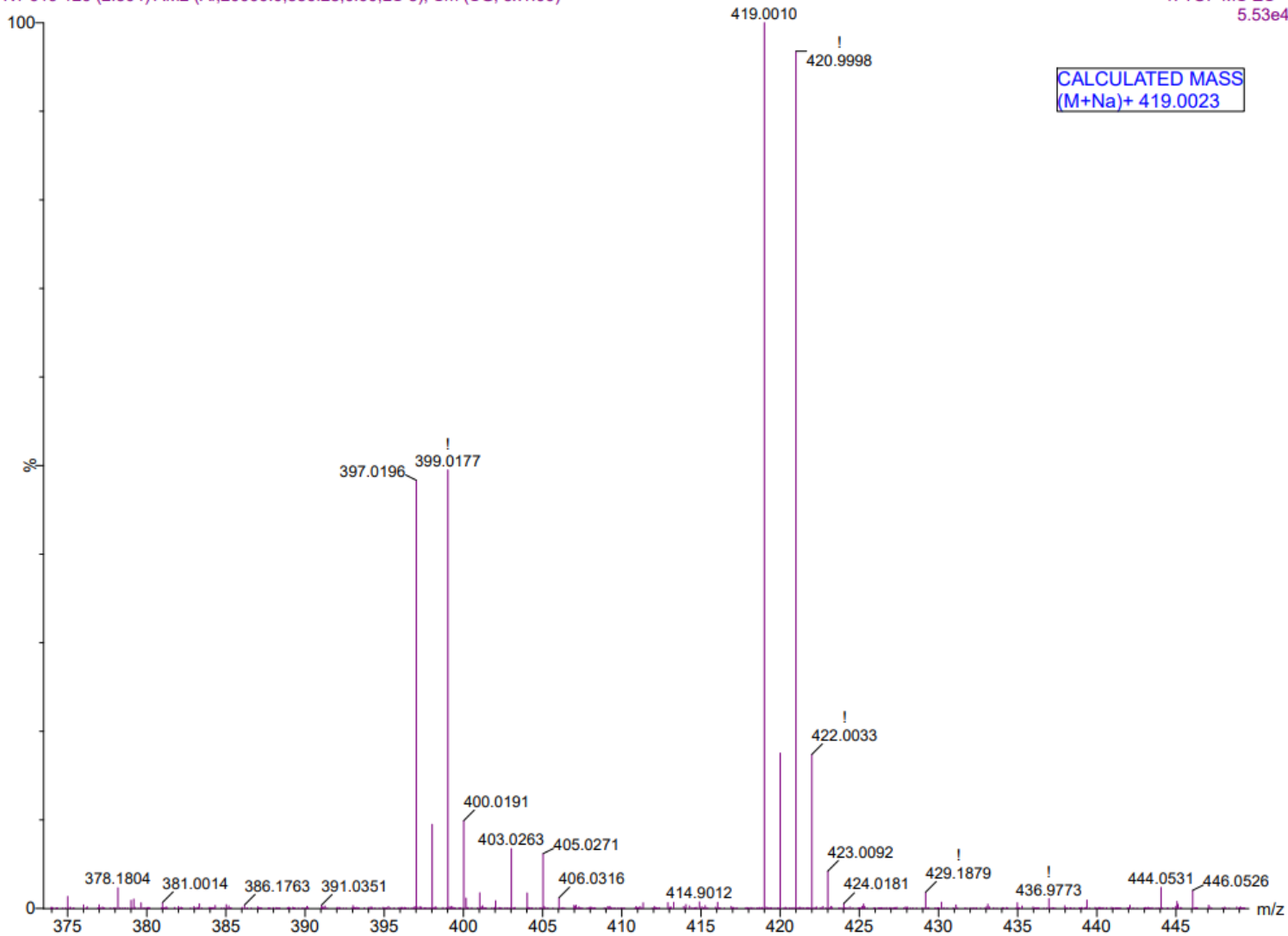


S21

HRMS of compound 6

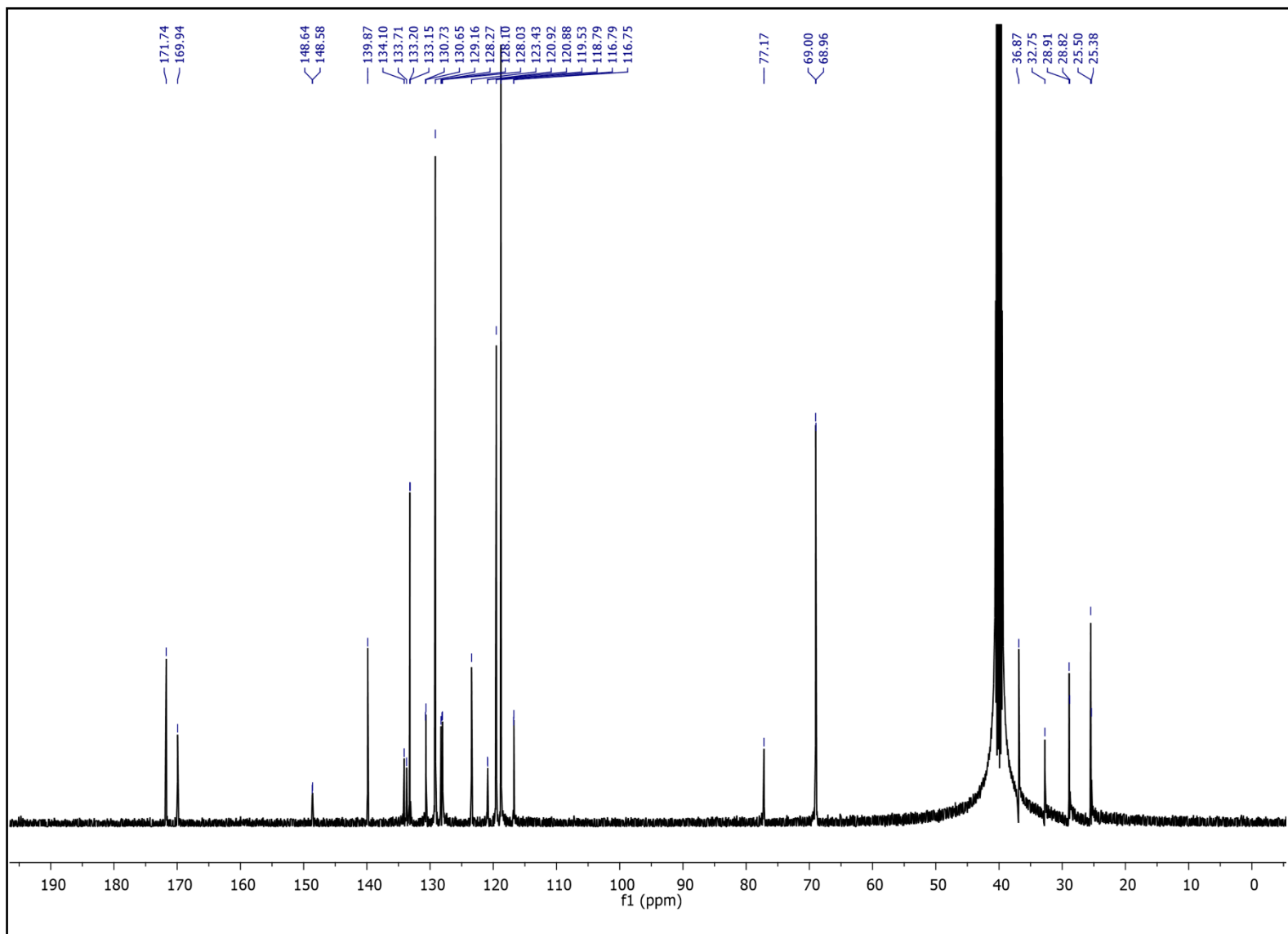
KT 619 126 (2.304) AM2 (Ar,20000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)

1: TOF MS ES+
5.53e4



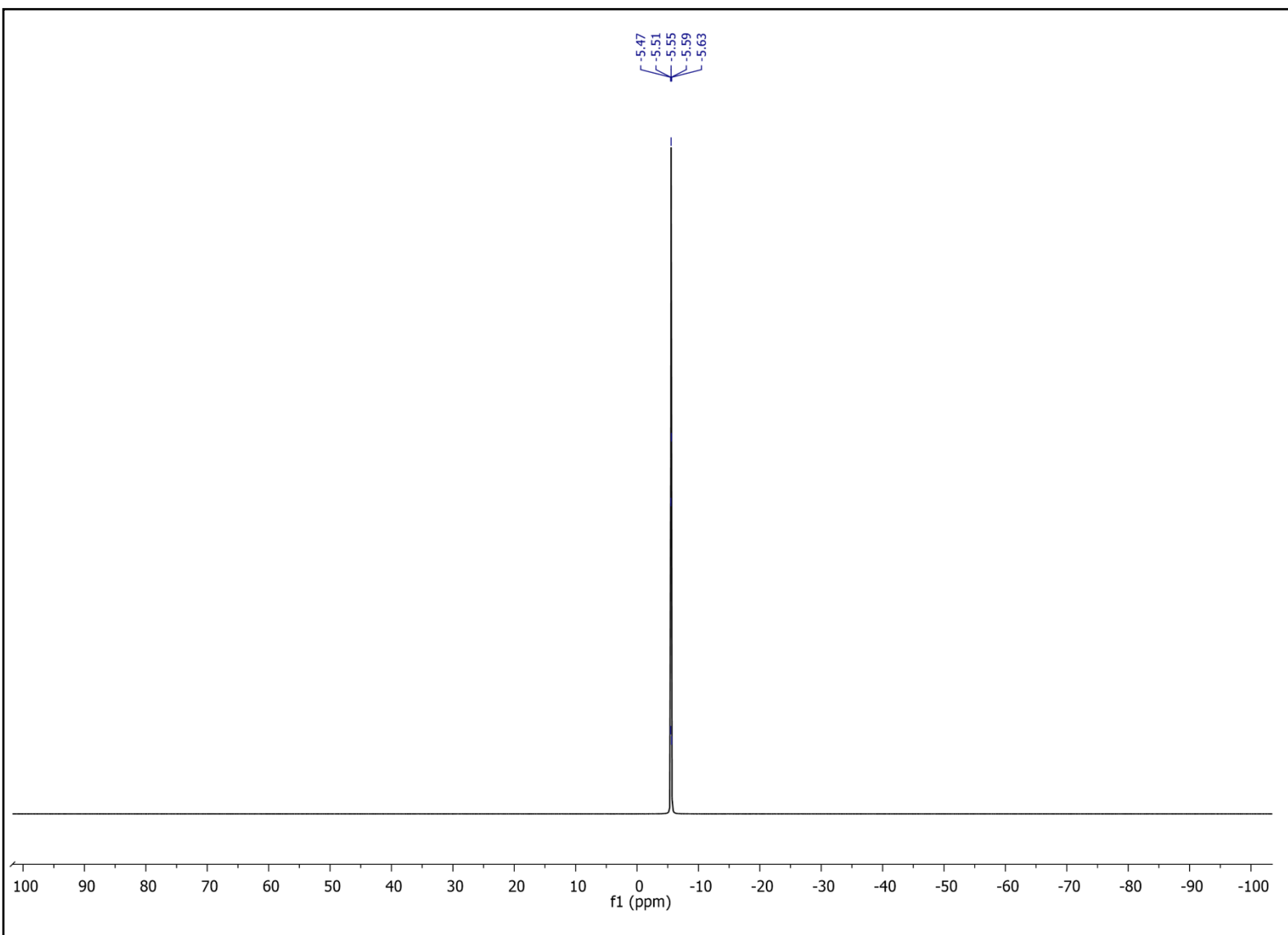
S22

¹³C NMR of compound 7 (DMSO-d₆)



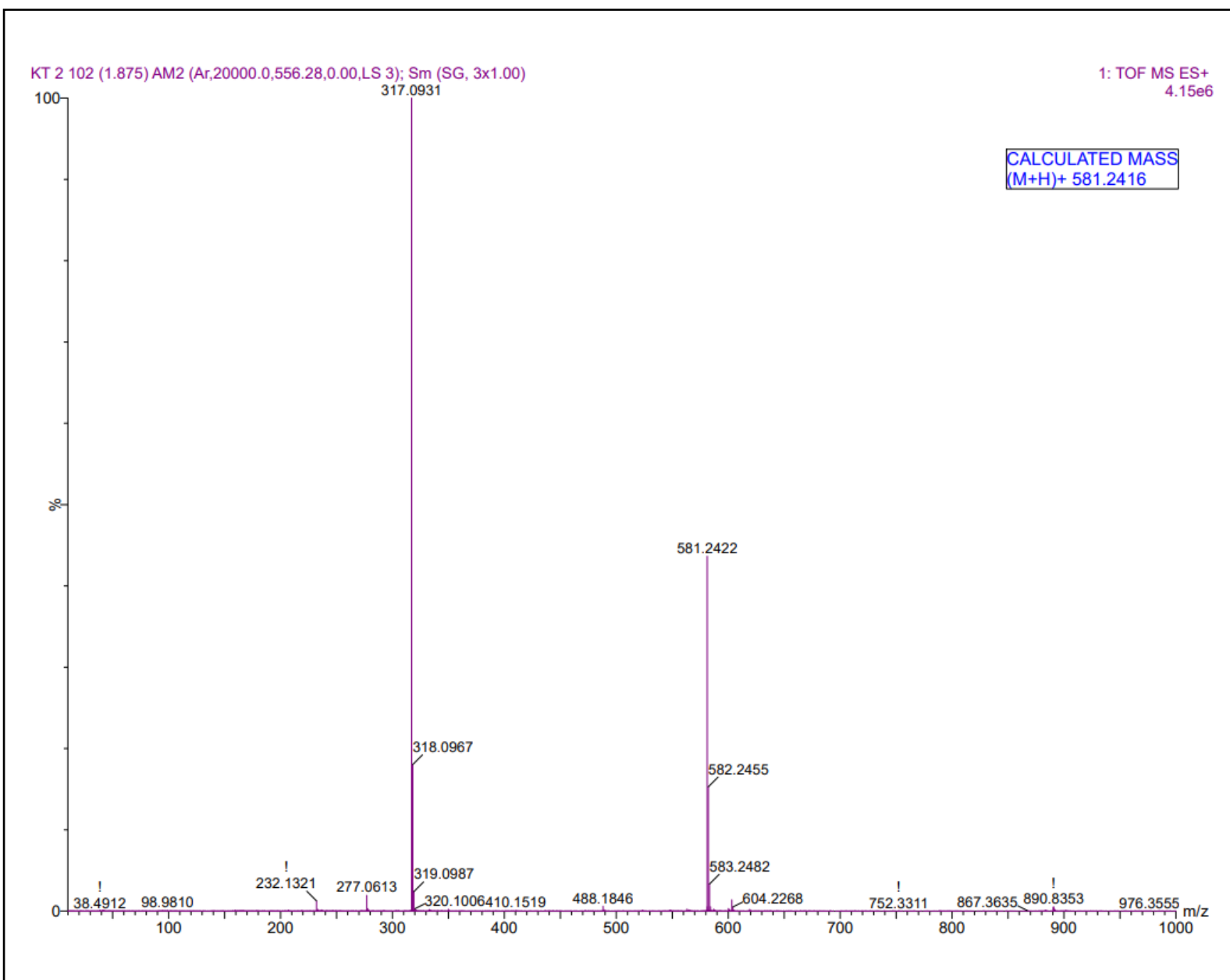
S24

³¹P NMR of compound 7 (DMSO-d₆)



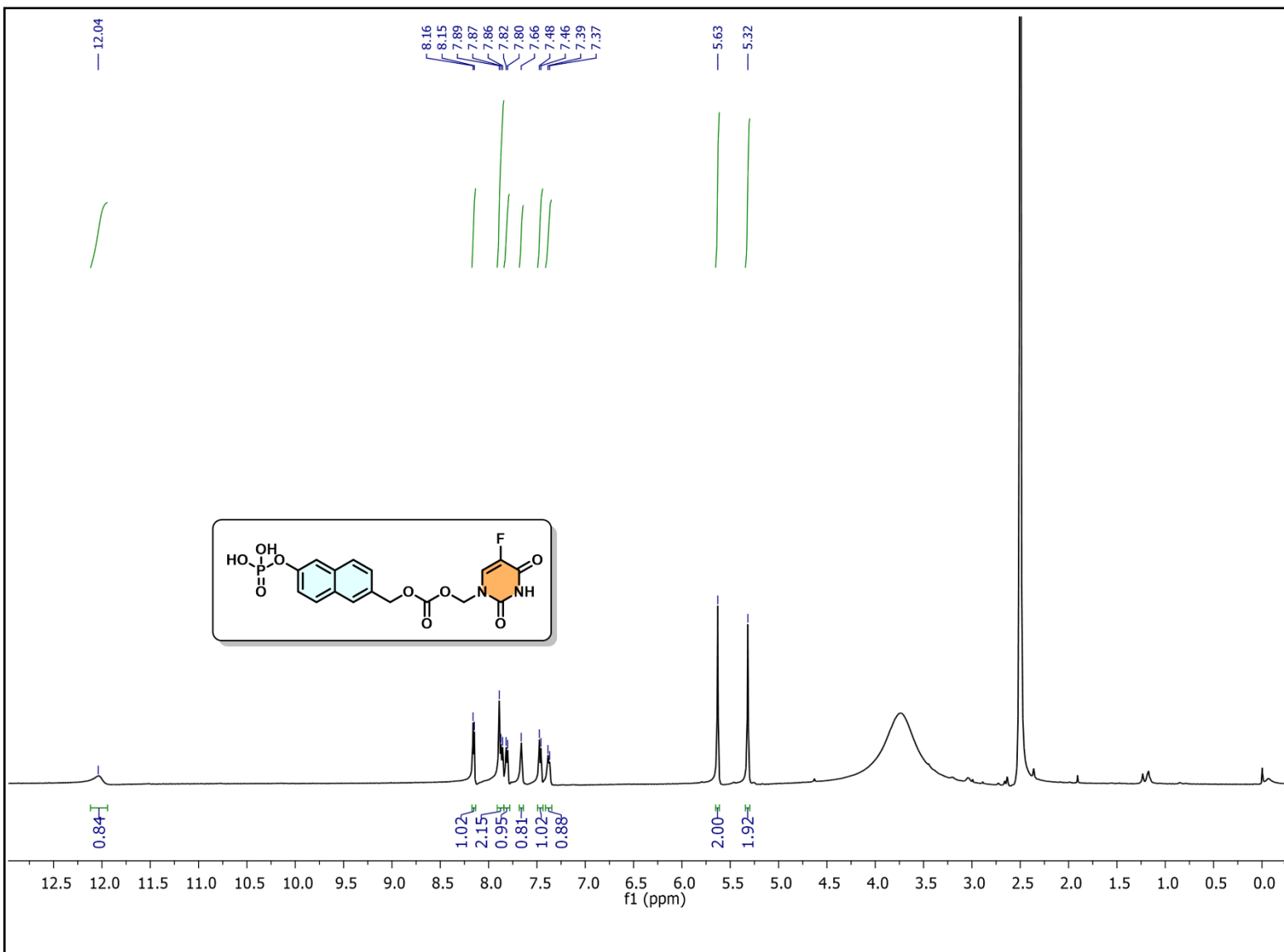
S25

HRMS of compound 7



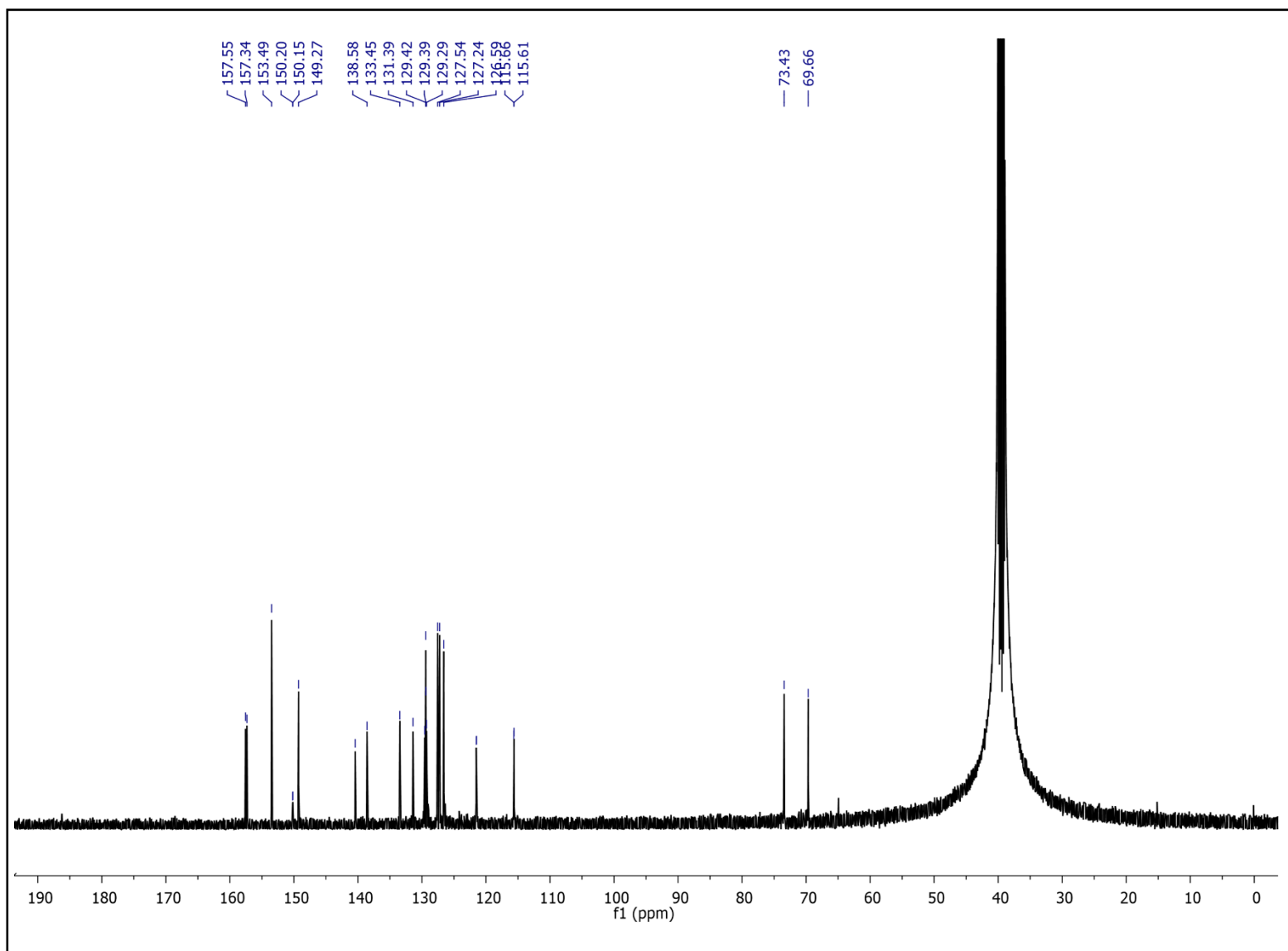
S26

¹H NMR of compound 8 (DMSO-d₆)



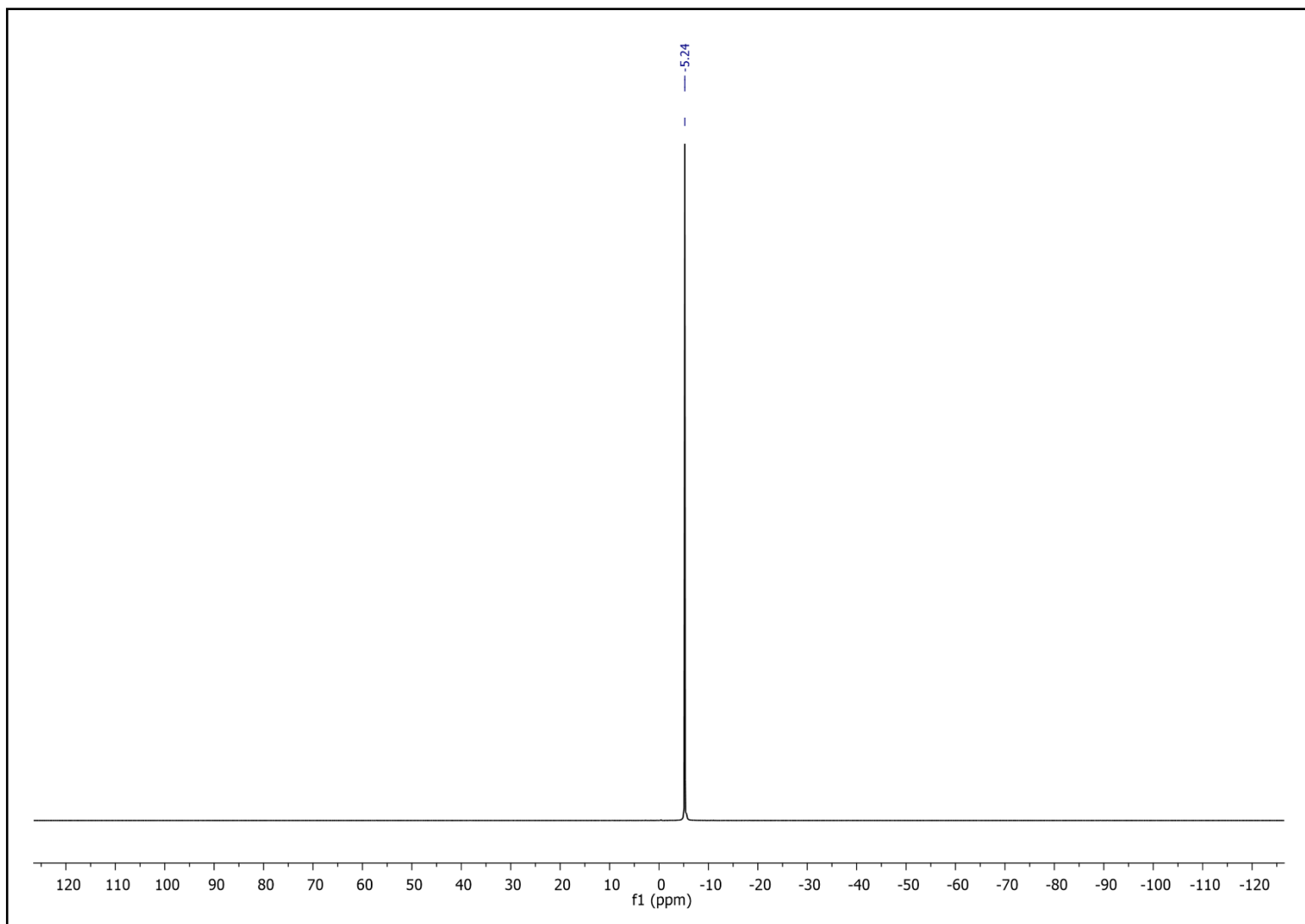
S27

¹³C NMR of compound 8 (DMSO-d₆)



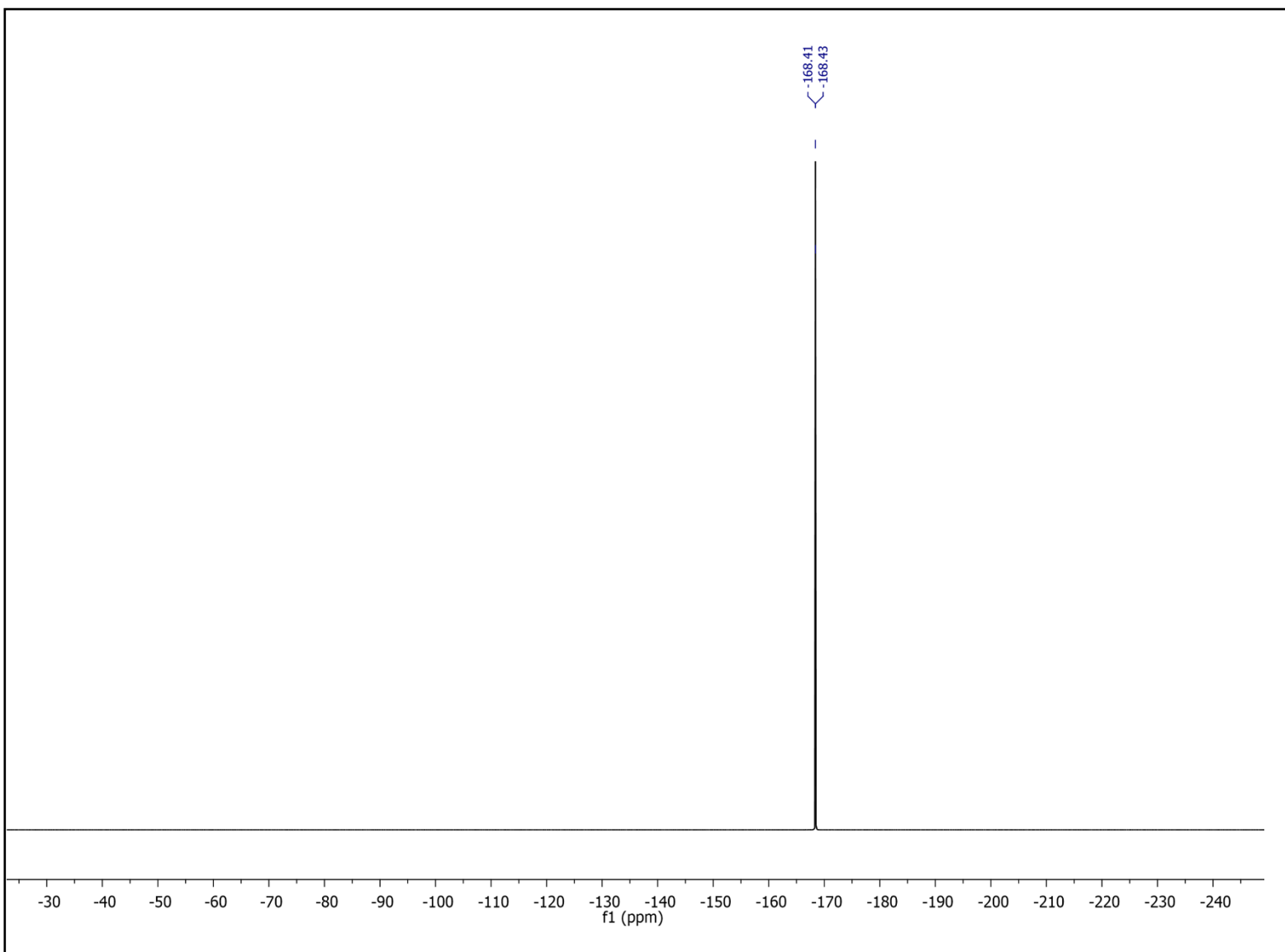
S28

^{31}P NMR of compound 8 (DMSO- d_6)



S29

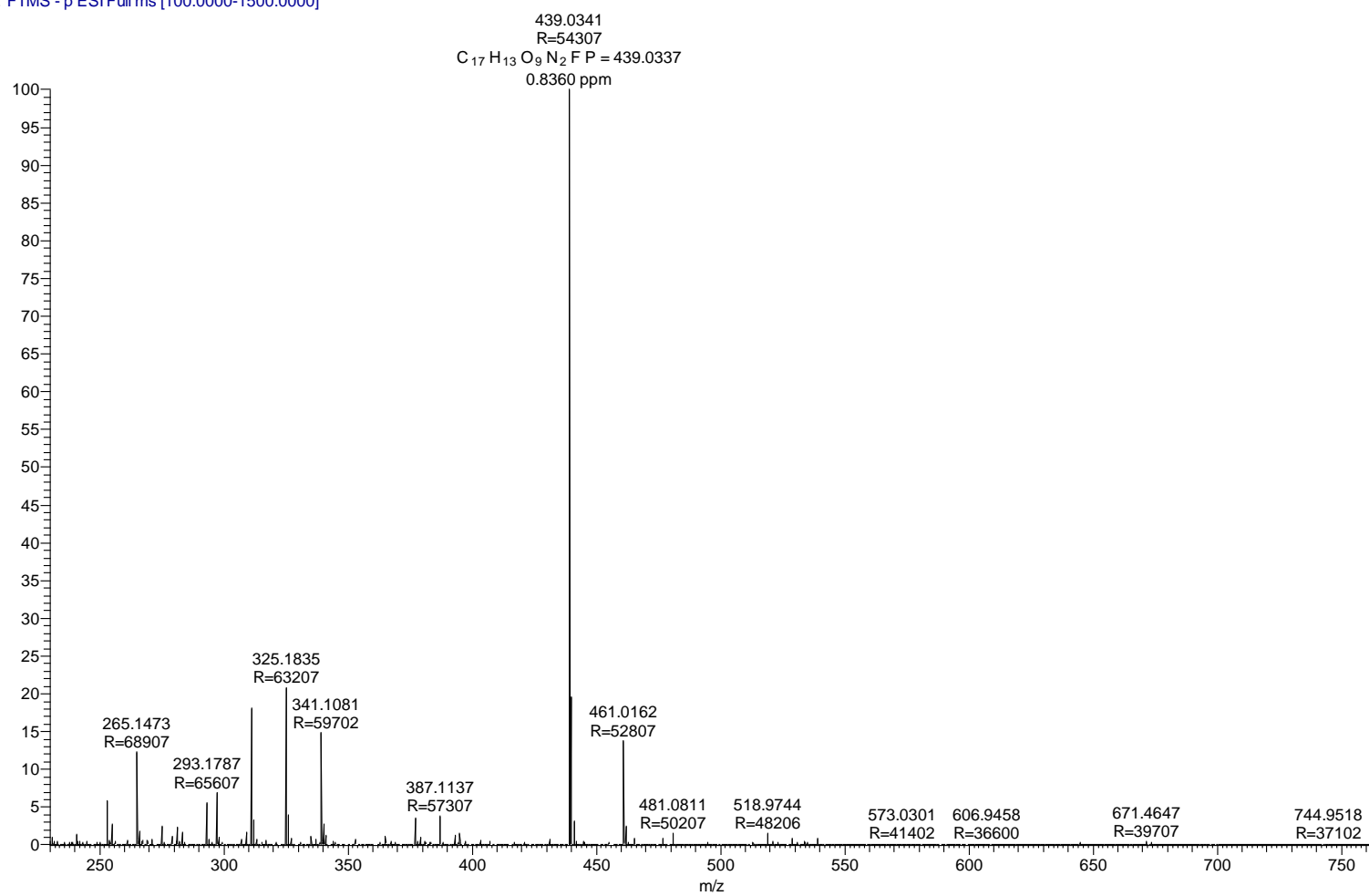
^{19}F NMR of compound 8 (DMSO- d_6)



S30

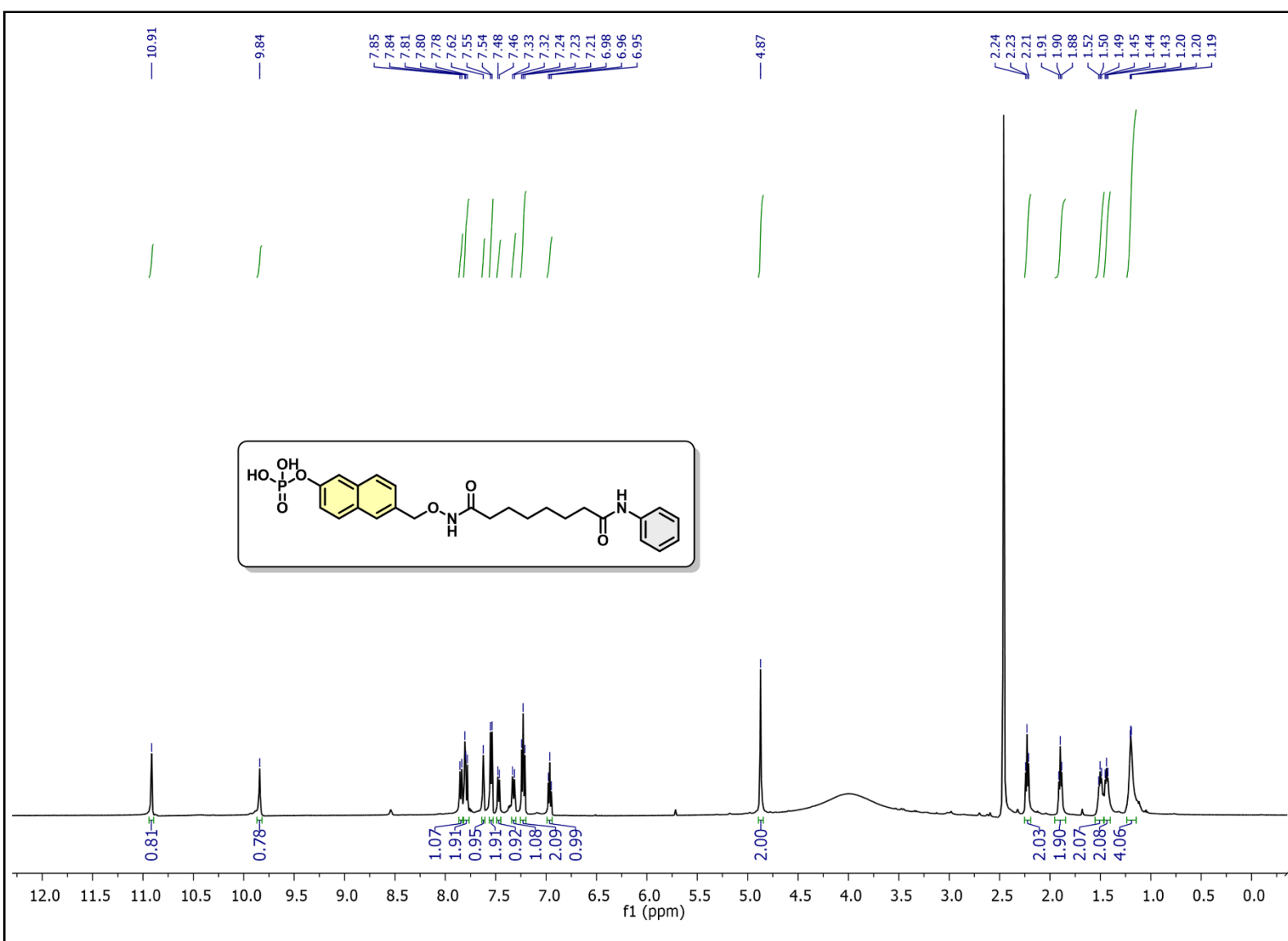
HRMS of compound 8

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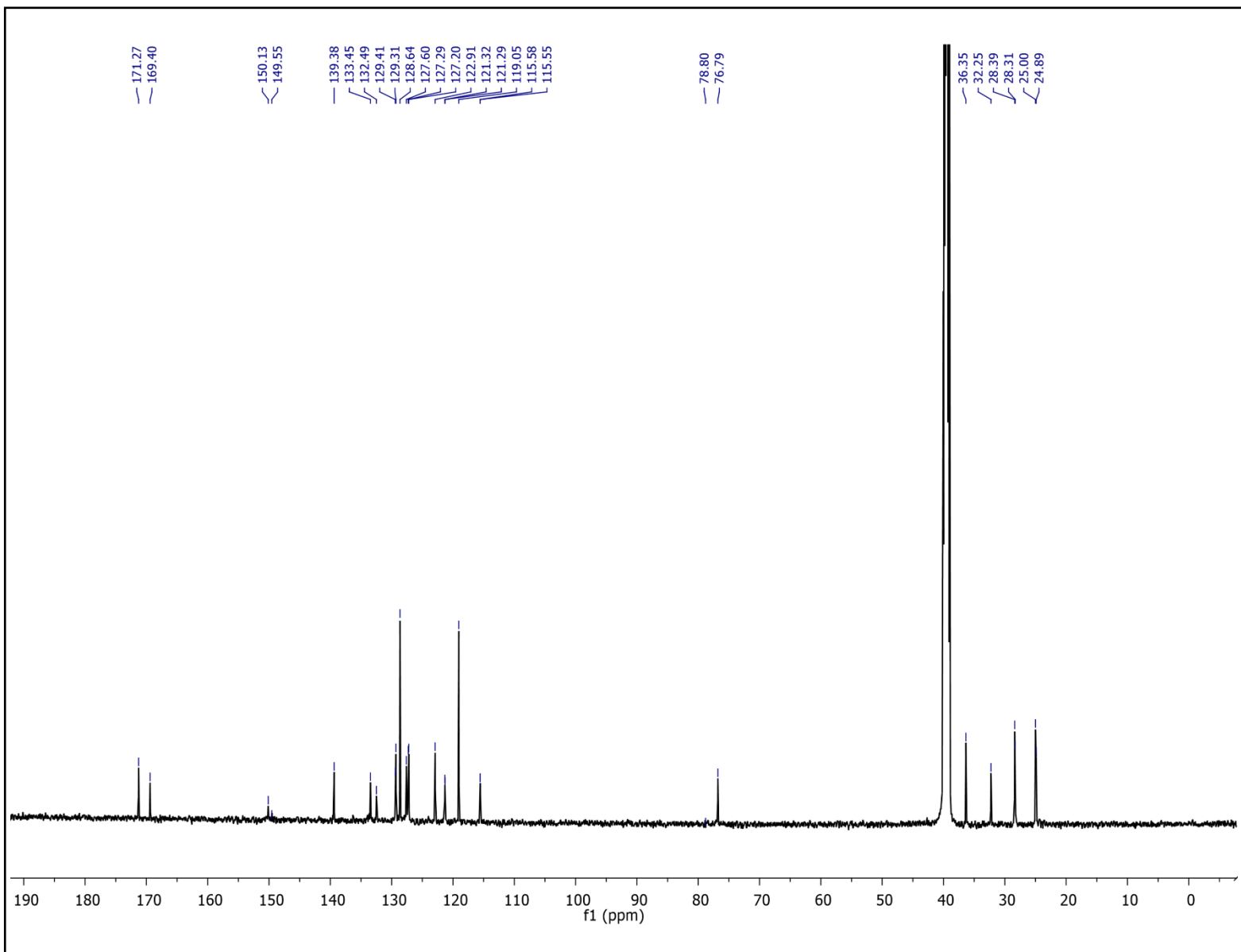
S31

¹H NMR of compound 9 (DMSO-d₆)



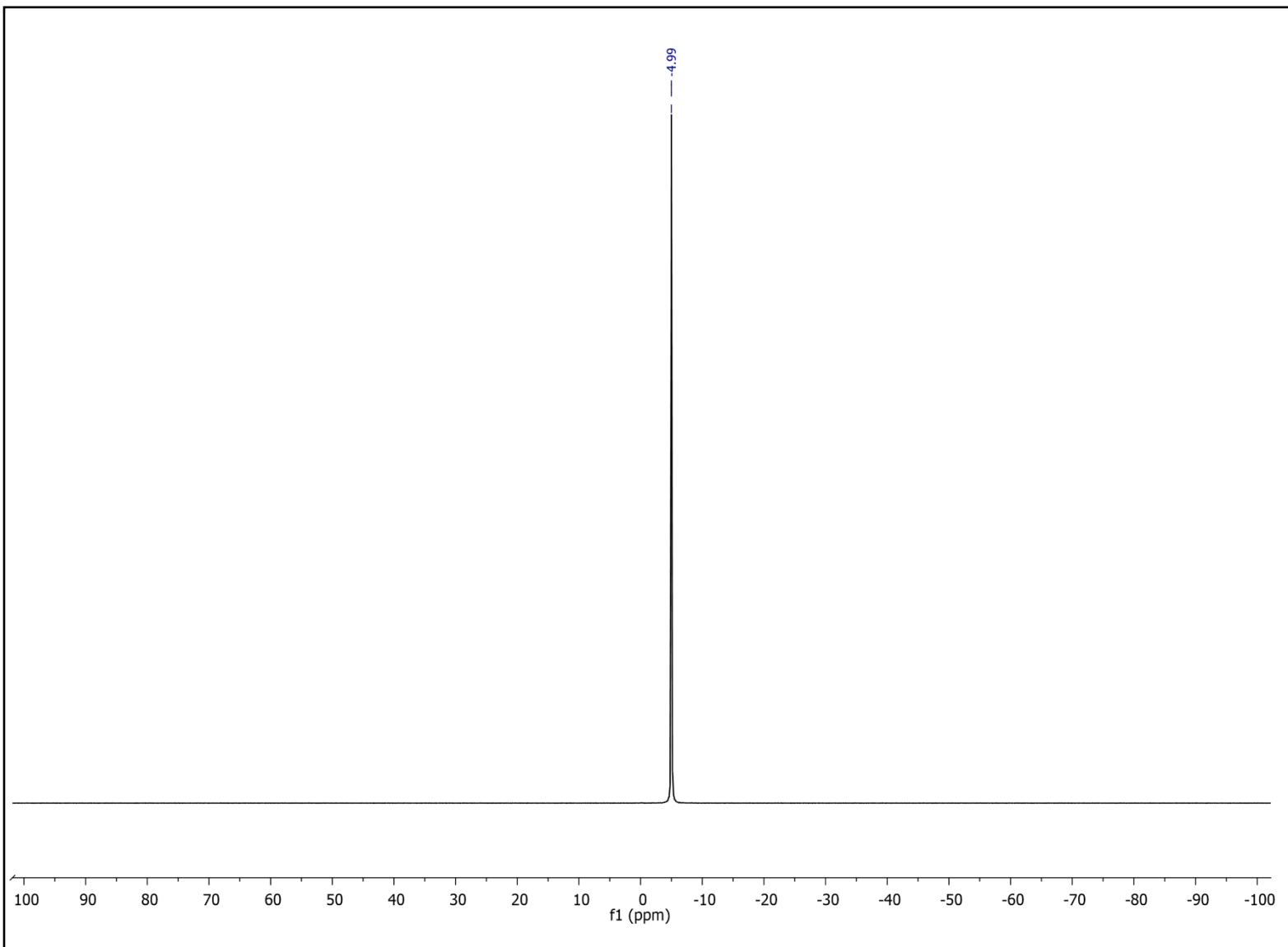
S32

¹³C NMR of compound 9 (DMSO-d₆)



S33

³¹P NMR of compound 9 (DMSO-d₆)



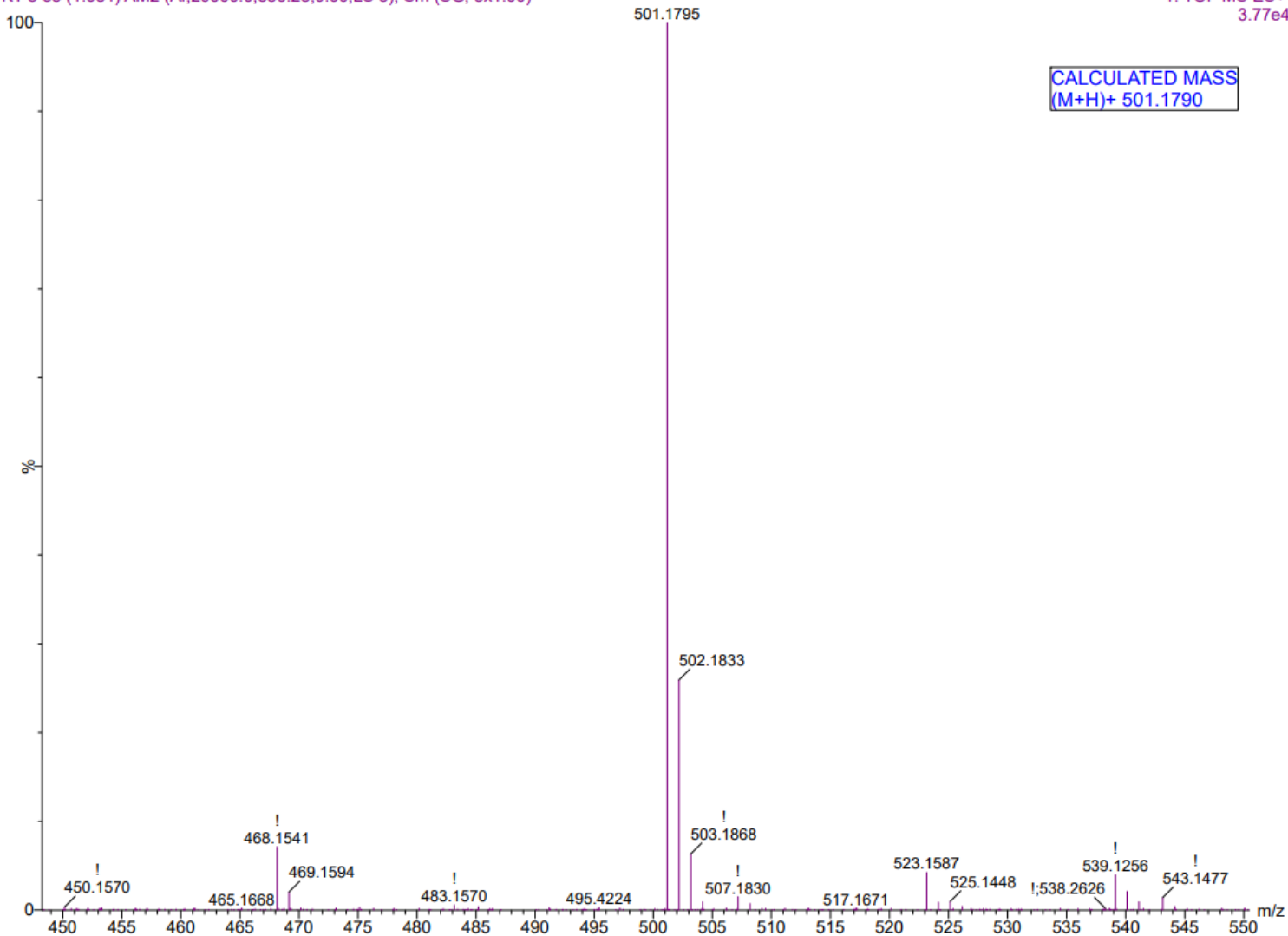
S34

HRMS of compound 9

KT 3 58 (1.084) AM2 (Ar,20000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)

1: TOF MS ES+
3.77e4

CALCULATED MASS
(M+H)+ 501.1790



S35