

## **Regioselective Synthesis of Water Soluble Monophosphate Derivatives of Combretastatin A-1**

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## Table of Contents

1) Tubulin polymerization, Cell lines, and Sulforhodamine B (SRB) assay	Pages S3'-S4'
2) $^1\text{H}$ NMR Spectra for compound <b>2a</b> , <b>2b</b> in $\text{CDCl}_3$	Pages S5'-S6'
3) $^1\text{H}$ NMR Spectra for compounds <b>3a</b> , <b>3b</b> , <b>4a</b> , <b>4b</b> in $\text{D}_2\text{O}$	Pages S7'-S10'
4) $^1\text{H}$ NMR Spectra for compound <b>6a</b> , <b>6b</b> in $\text{CDCl}_3$	Pages S11'-S12'
5) $^1\text{H}$ NMR Spectra for compounds <b>7a</b> , <b>7b</b> , <b>8a</b> , <b>8b</b> in $\text{D}_2\text{O}$	Pages S13'-S16'
6) $^{13}\text{C}$ NMR Spectra for compound <b>2a</b> , <b>2b</b> in $\text{CDCl}_3$	Pages S17'-S18'
7) $^{13}\text{C}$ NMR Spectra for compounds <b>3a</b> , <b>3b</b> , <b>4a</b> , <b>4b</b> in $\text{D}_2\text{O}$	Pages S19'-S22'
8) $^{13}\text{C}$ NMR Spectra for compound <b>6a</b> , <b>6b</b> in $\text{CDCl}_3$	Pages S23'-S24'
9) $^{13}\text{C}$ NMR Spectra for compounds <b>7a</b> , <b>7b</b> , <b>8a</b> , <b>8b</b> in $\text{D}_2\text{O}$	Pages S25'-S28'
10) $^{31}\text{P}$ NMR Spectrum for compounds <b>2b</b> in $\text{CDCl}_3$	Page S29'
11) $^{31}\text{P}$ NMR Spectra for compounds <b>3a</b> , <b>3b</b> , <b>4a</b> , <b>4b</b> in $\text{D}_2\text{O}$	Pages S30'-S33'
12) $^{31}\text{P}$ NMR Spectrum for compounds <b>6b</b> in $\text{D}_2\text{O}$	Page S34'
13) $^{31}\text{P}$ NMR Spectra for compounds <b>7b</b> , <b>8a</b> , <b>8b</b> in $\text{D}_2\text{O}$	Pages S35'-S37'
14) HSQC Spectra for compounds <b>2a</b> , <b>2b</b> in $\text{CDCl}_3$	Pages S38'-S39'
15) HSQC Spectra for compounds <b>3a</b> , <b>4a</b> , <b>4b</b> in $\text{D}_2\text{O}$	Pages S40'-S42'
16) HSQC Spectra for compounds <b>6a</b> , <b>6b</b> in $\text{CDCl}_3$	Pages S43'-S44'
17) HSQC Spectra for compounds <b>7a</b> , <b>7b</b> , <b>8a</b> , <b>8b</b> in $\text{D}_2\text{O}$	Pages S45'-S48'
18) HMBC Spectra for compounds <b>2a</b> , <b>2b</b> in $\text{CDCl}_3$	Pages S49'-S50'
19) HMBC Spectra for compounds <b>3a</b> , <b>3b</b> , <b>4a</b> , <b>4b</b> in $\text{D}_2\text{O}$	Pages S51'-S54'
20) HMBC Spectra for compounds <b>6a</b> , <b>6b</b> in $\text{CDCl}_3$	Pages S55'-S56'

21) HMBC Spectra for compounds **7a,7b, 8a, 8b** in D<sub>2</sub>O      Pages S57'-S60'

22) Tables **S1** and **S2** for NMR Spectroscopic data for      Page S61'  
compounds **4a, 4b, 8a, and 8b**.

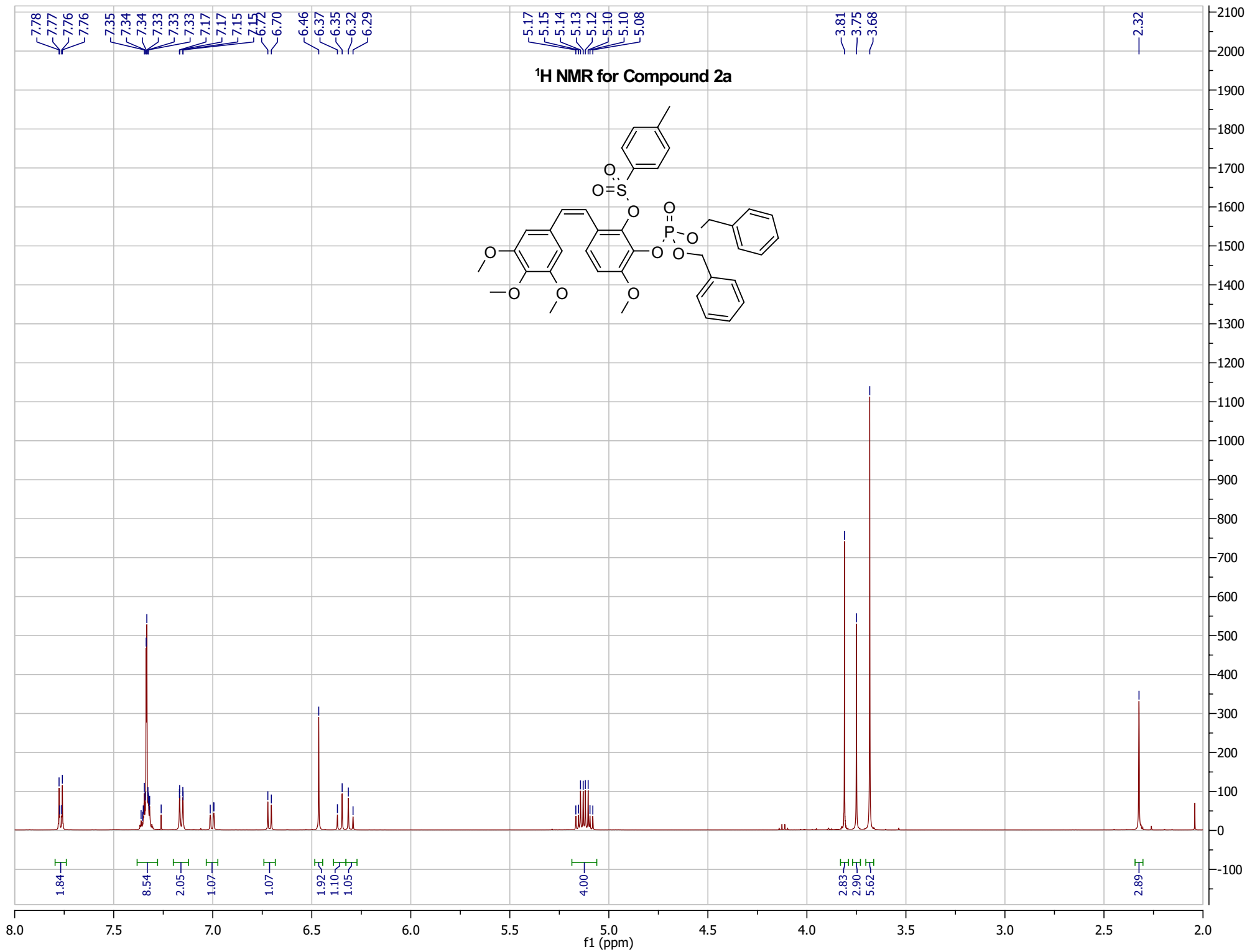
**Biology. Effects on Tubulin Polymerization.** Bovine brain tubulin was purified as described previously.<sup>S1</sup> To evaluate the effect of the compounds on tubulin assembly in vitro, an appropriate series of concentrations were preincubated with 10  $\mu$ M tubulin (1.0 mg/mL) in glutamate buffer at 30 °C and then cooled to 0 °C. After the addition of GTP, the mixtures were transferred to 0 °C cuvettes in a recording spectrophotometer and warmed to 30 °C, and the assembly of tubulin was observed turbidimetrically.<sup>S2</sup> The IC<sub>50</sub> was defined as the compound concentration that inhibited the extent of assembly by 50% after a 20 min incubation.

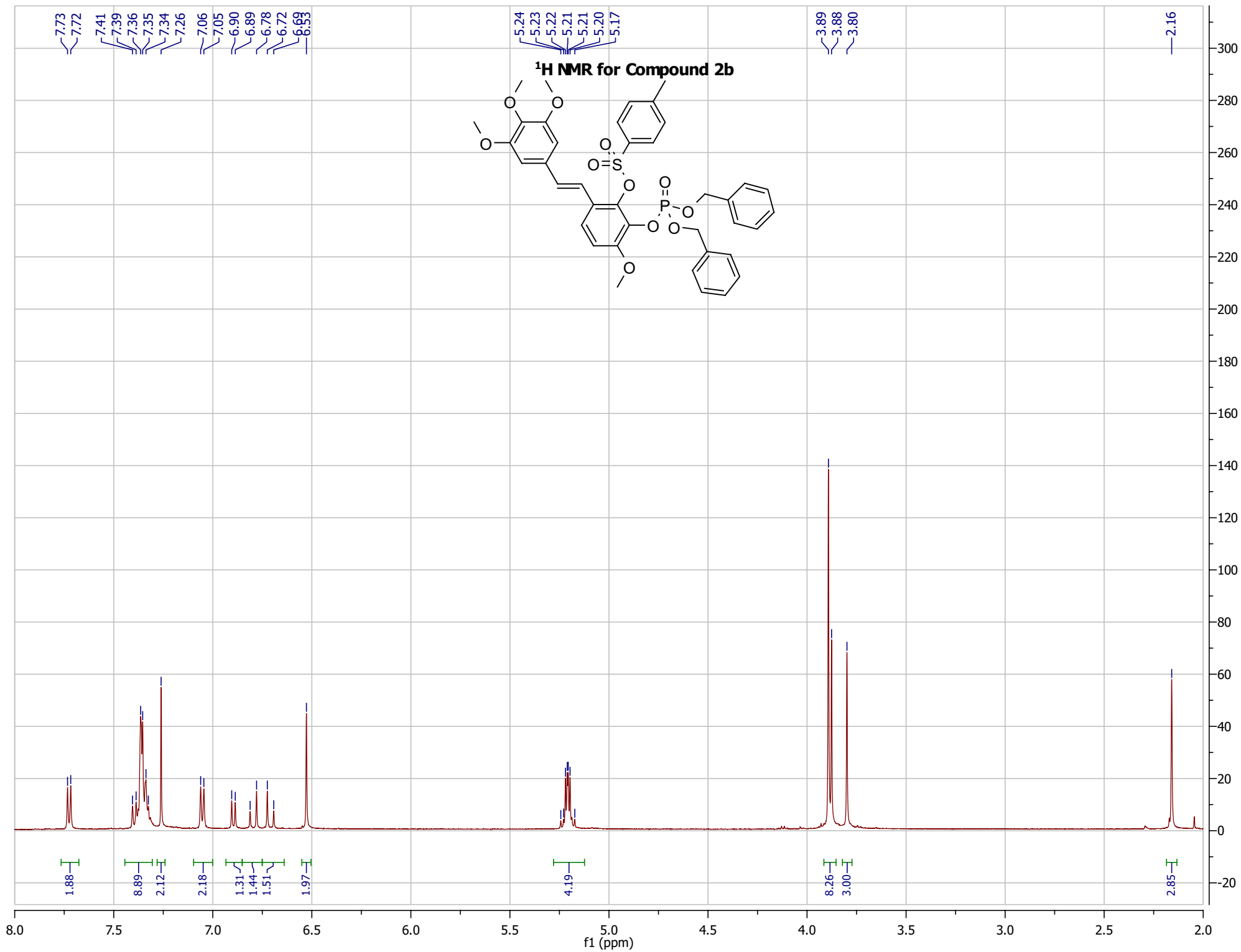
**Cell lines, and Sulforhodamine B (SRB) assay.** DU-145 (prostate), SK-OV-3 (ovarian), and NCI-H460 (lung) cancer cell lines were obtained from ATCC and maintained according to recommended conditions. Gentamicin and amphotericin were used as appropriate antibiotic and antifungal agents. Inhibition of human cancer cell line growth was assessed using the National Cancer Institute's standard sulforhodamine B (SRB) assay, as previously described.<sup>S3-S5</sup> A growth inhibition of 50% in comparison to untreated controls (GI<sub>50</sub> or the drug concentrations causing a 50% reduction in net protein increase) was calculated.<sup>S3,S5</sup> Results reported are averages of at least three separate experiments, each of which was carried out in triplicate.

## References

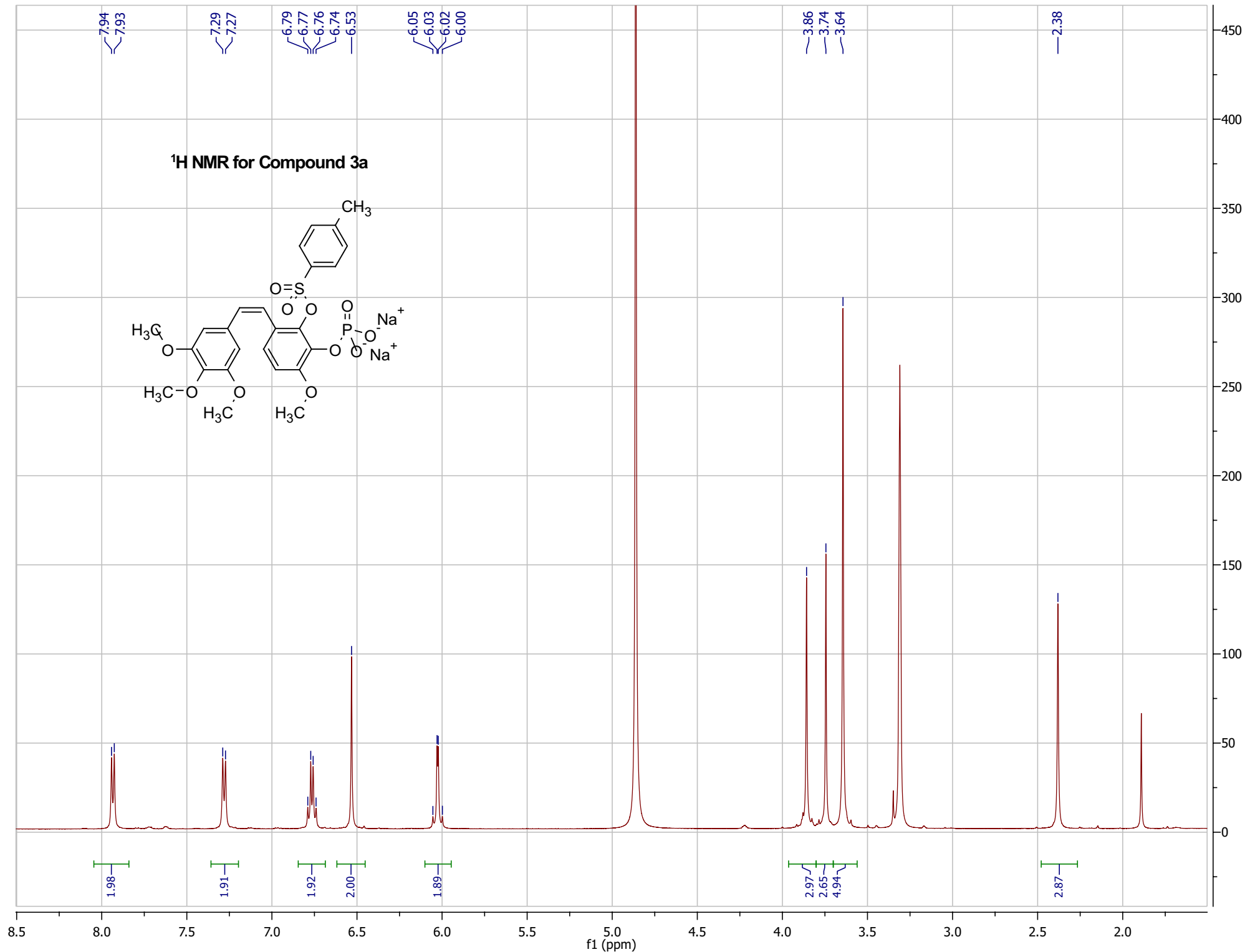
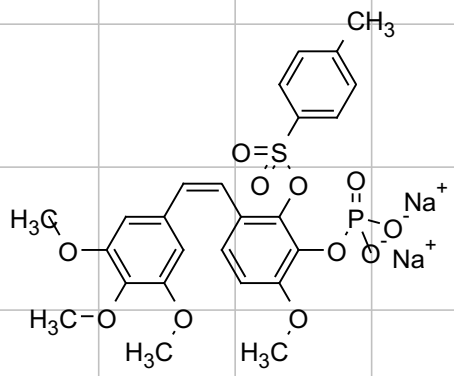
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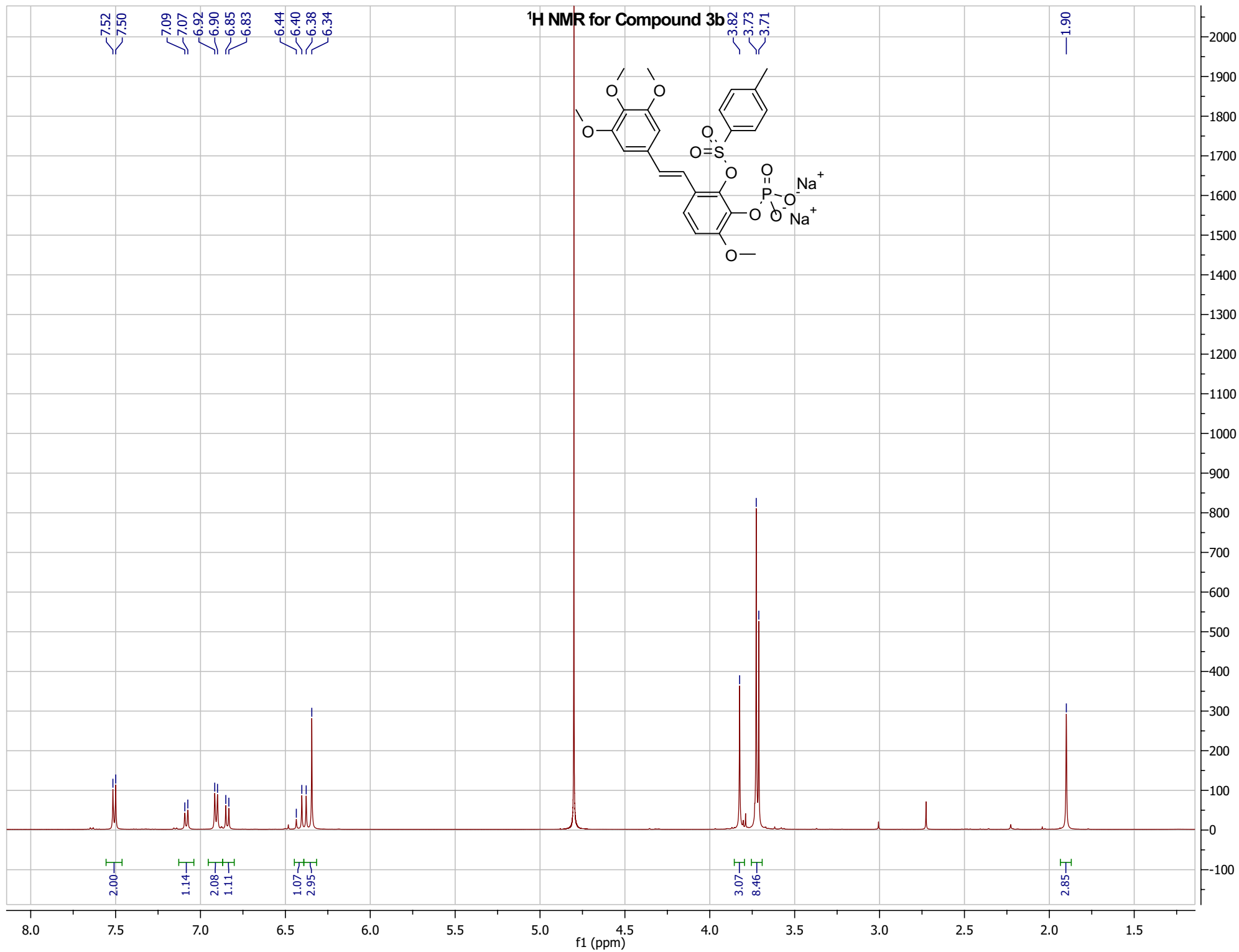




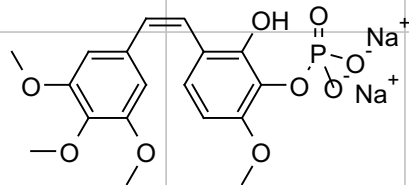


**<sup>1</sup>H NMR for Compound 3a**



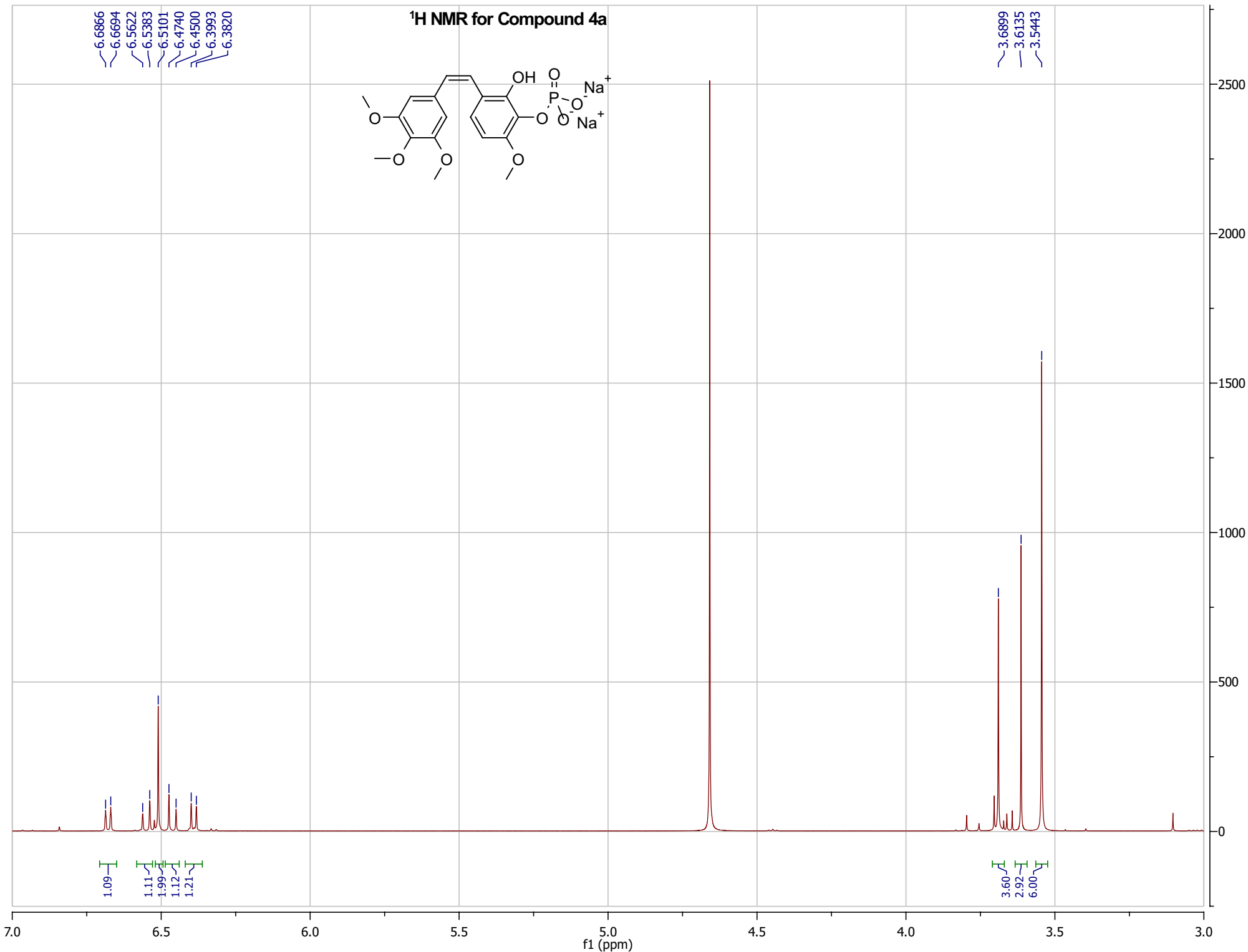


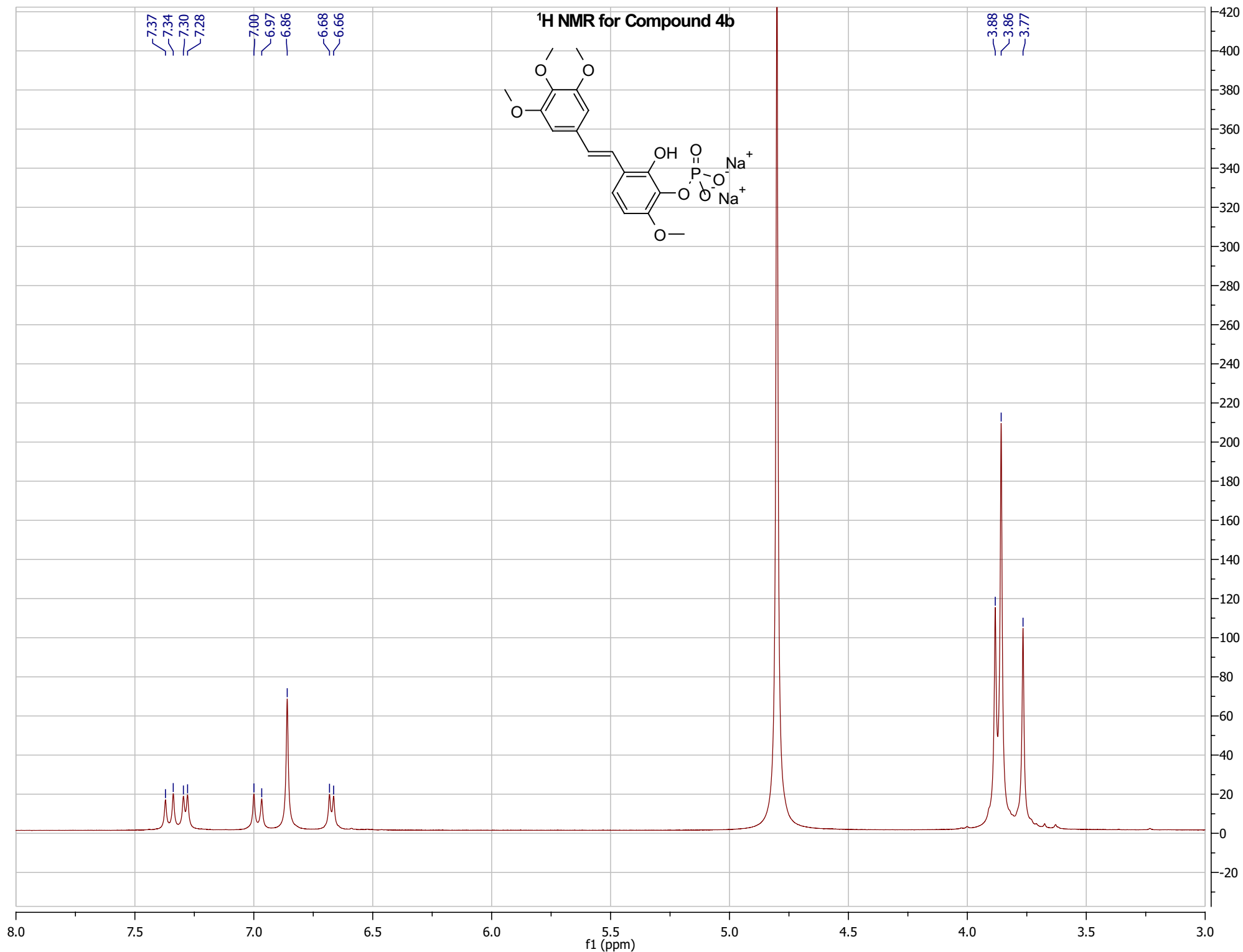
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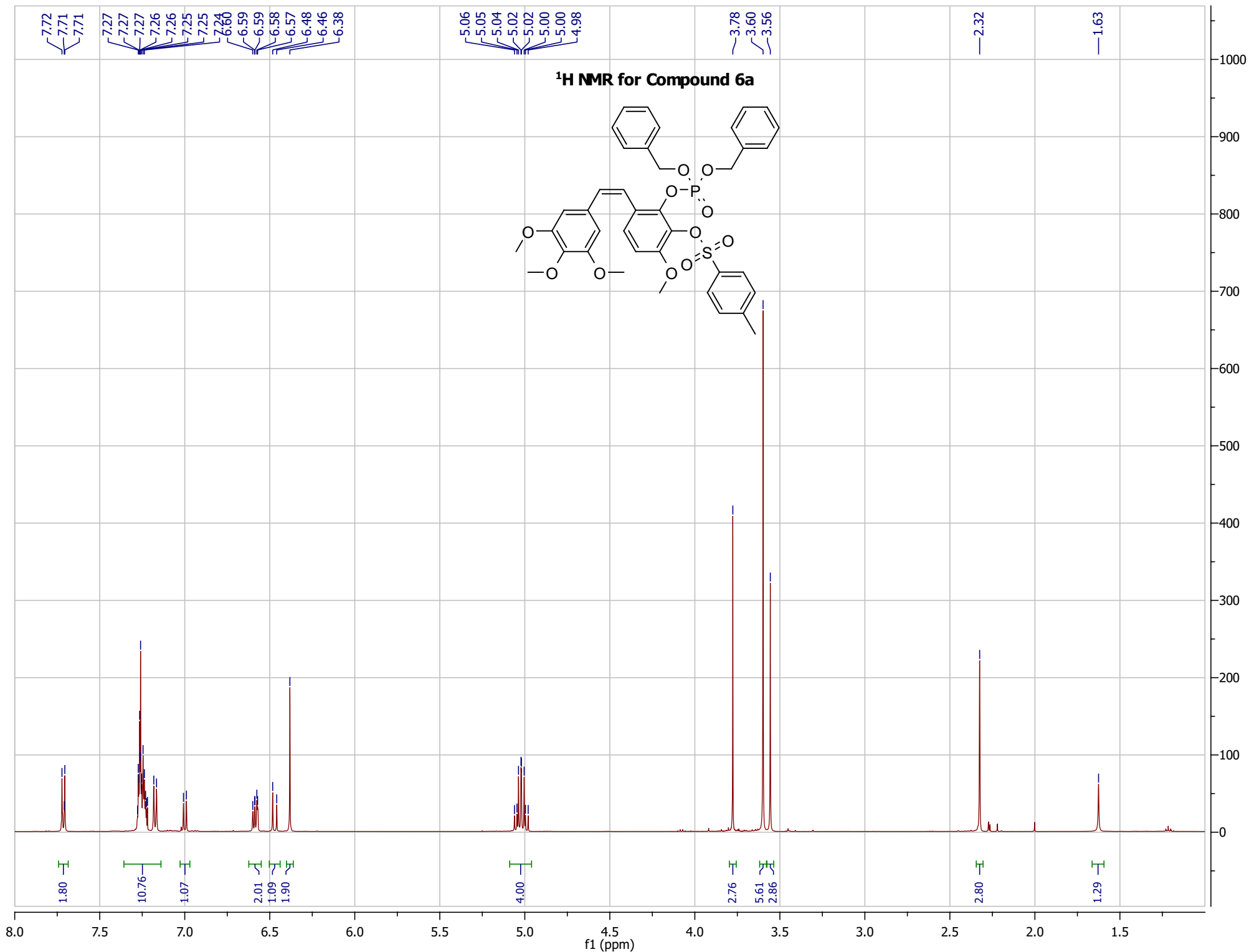


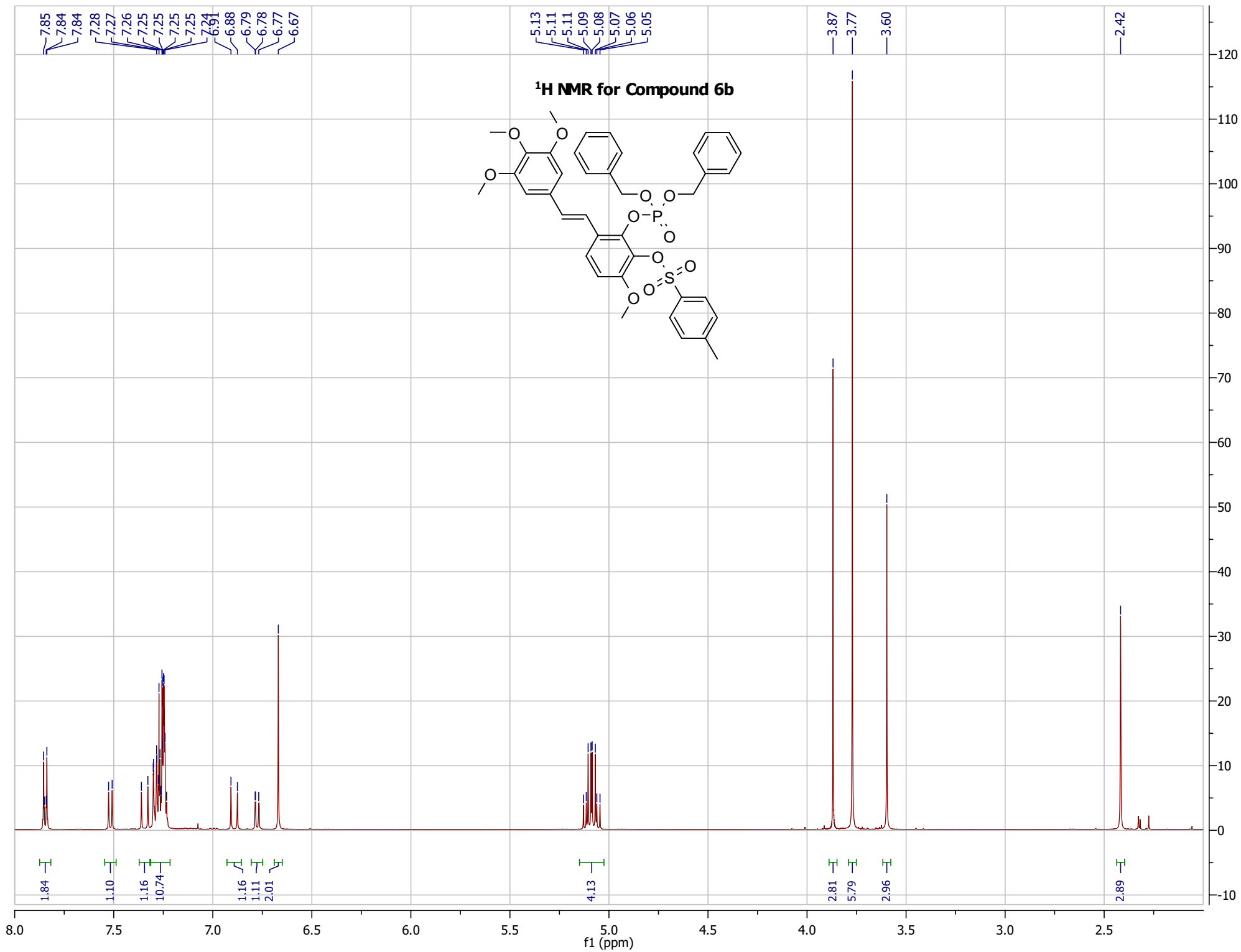
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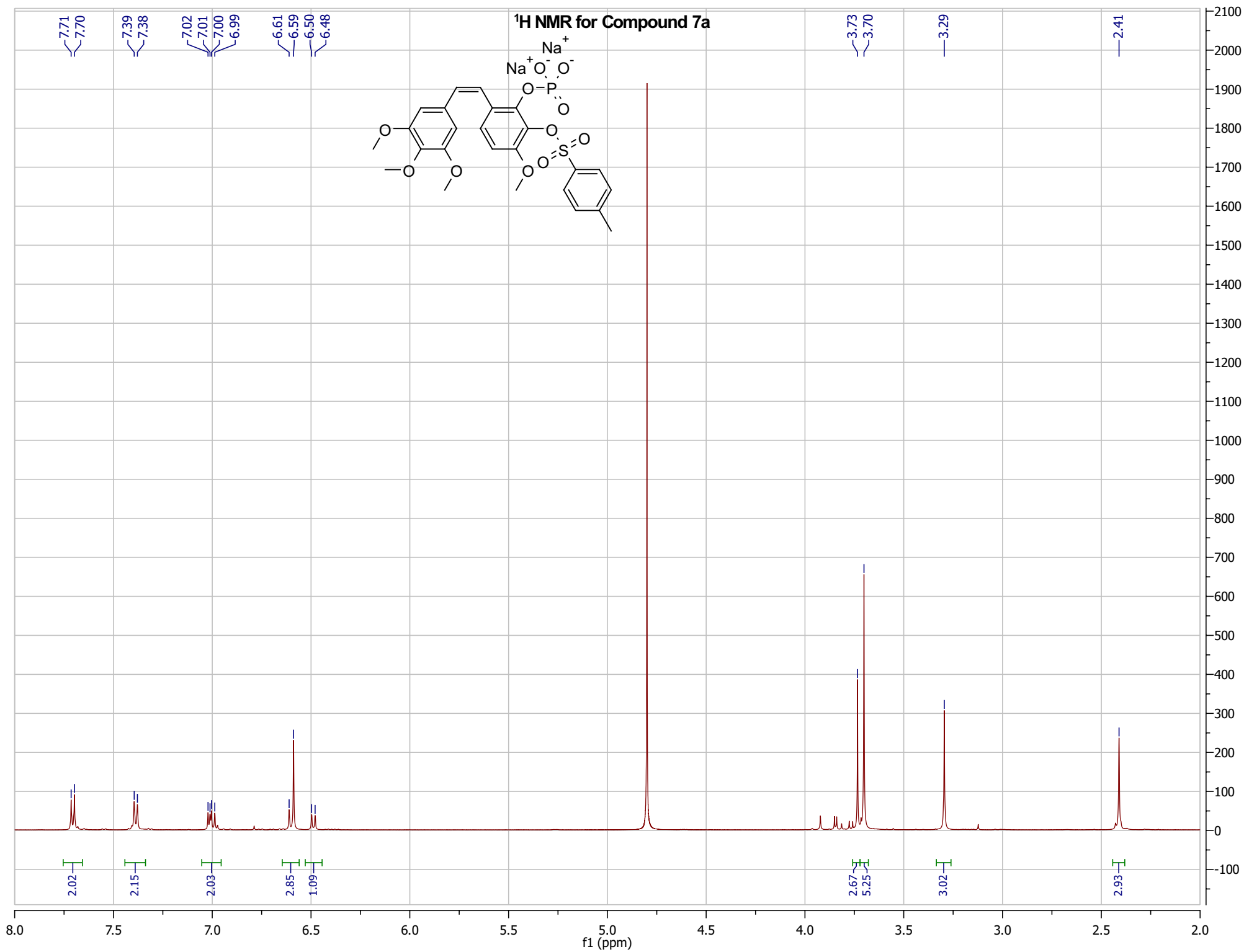


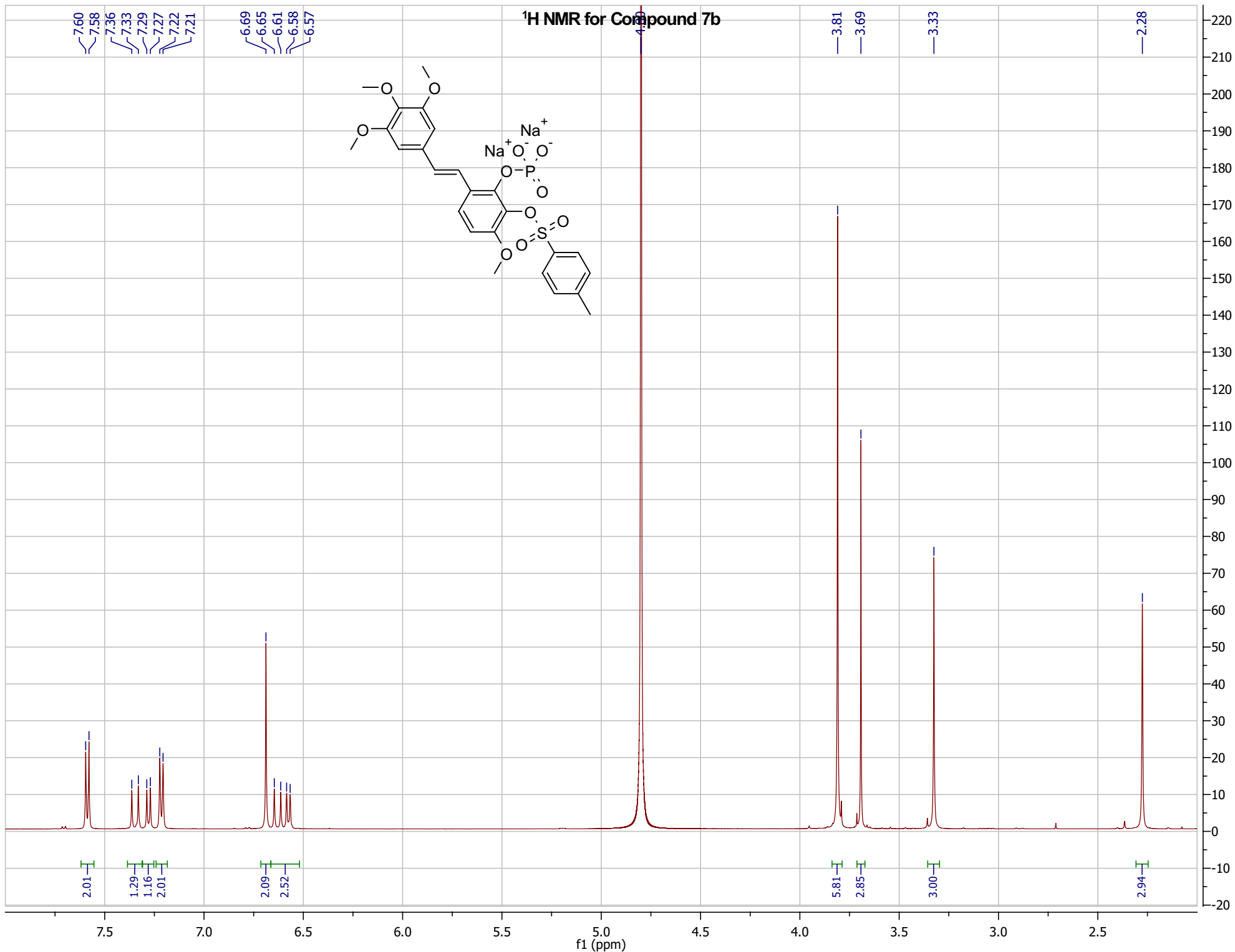




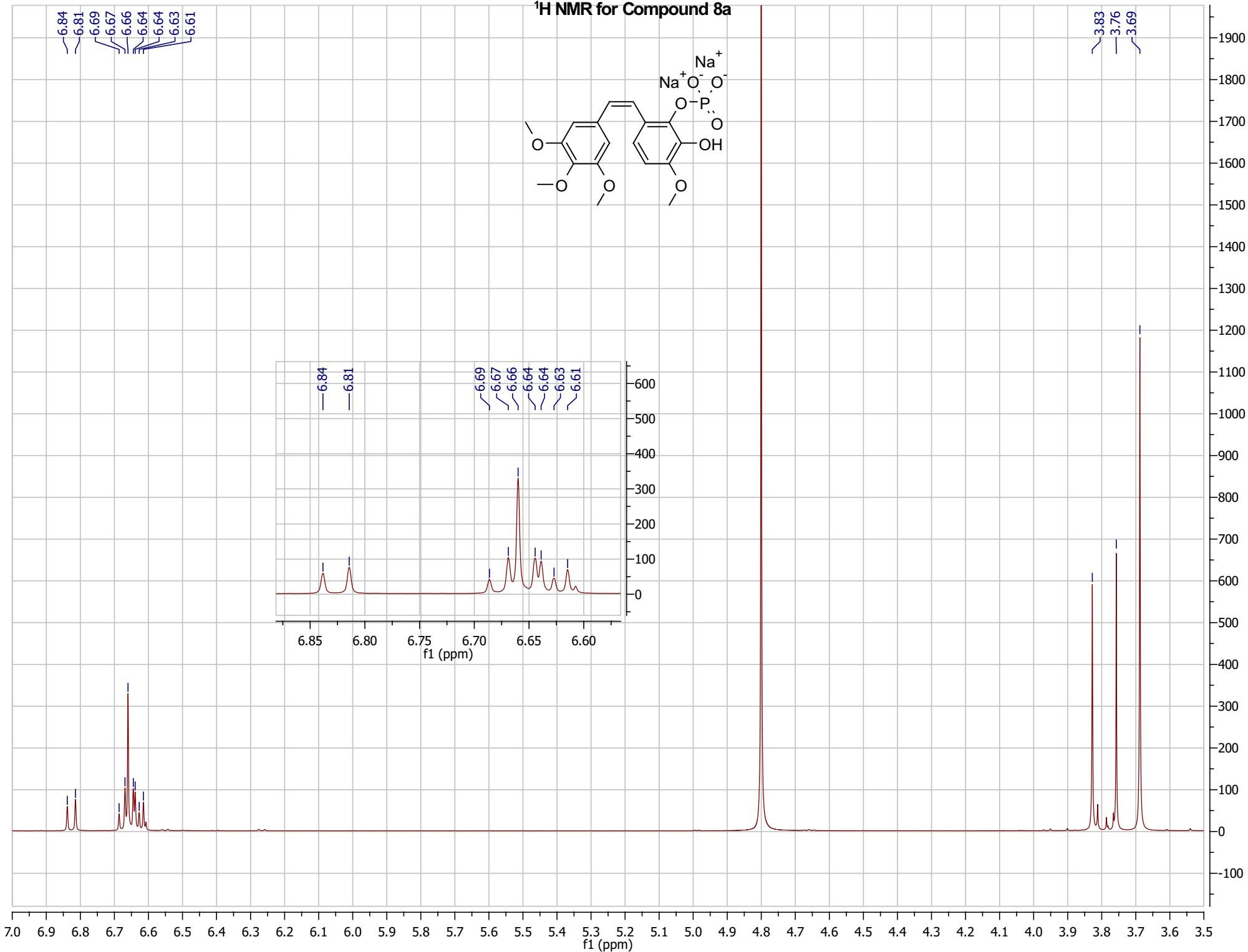
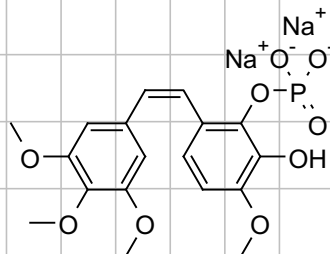




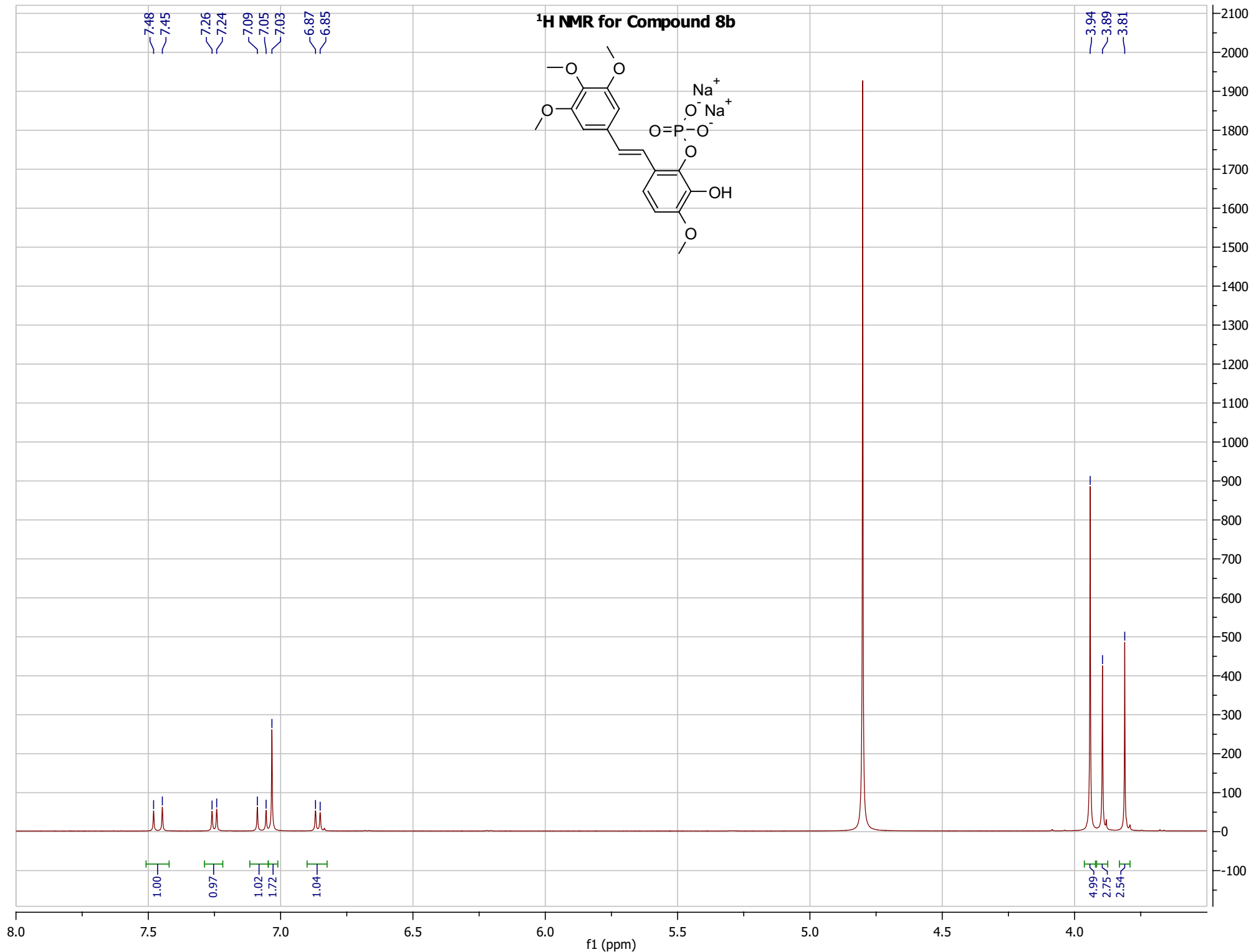
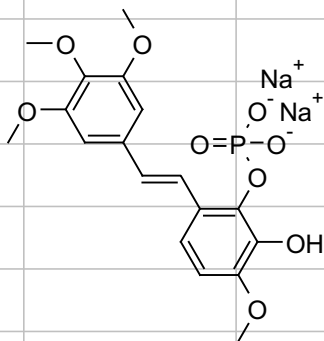




<sup>1</sup>H NMR for Compound 8a



**<sup>1</sup>H NMR for Compound 8b**

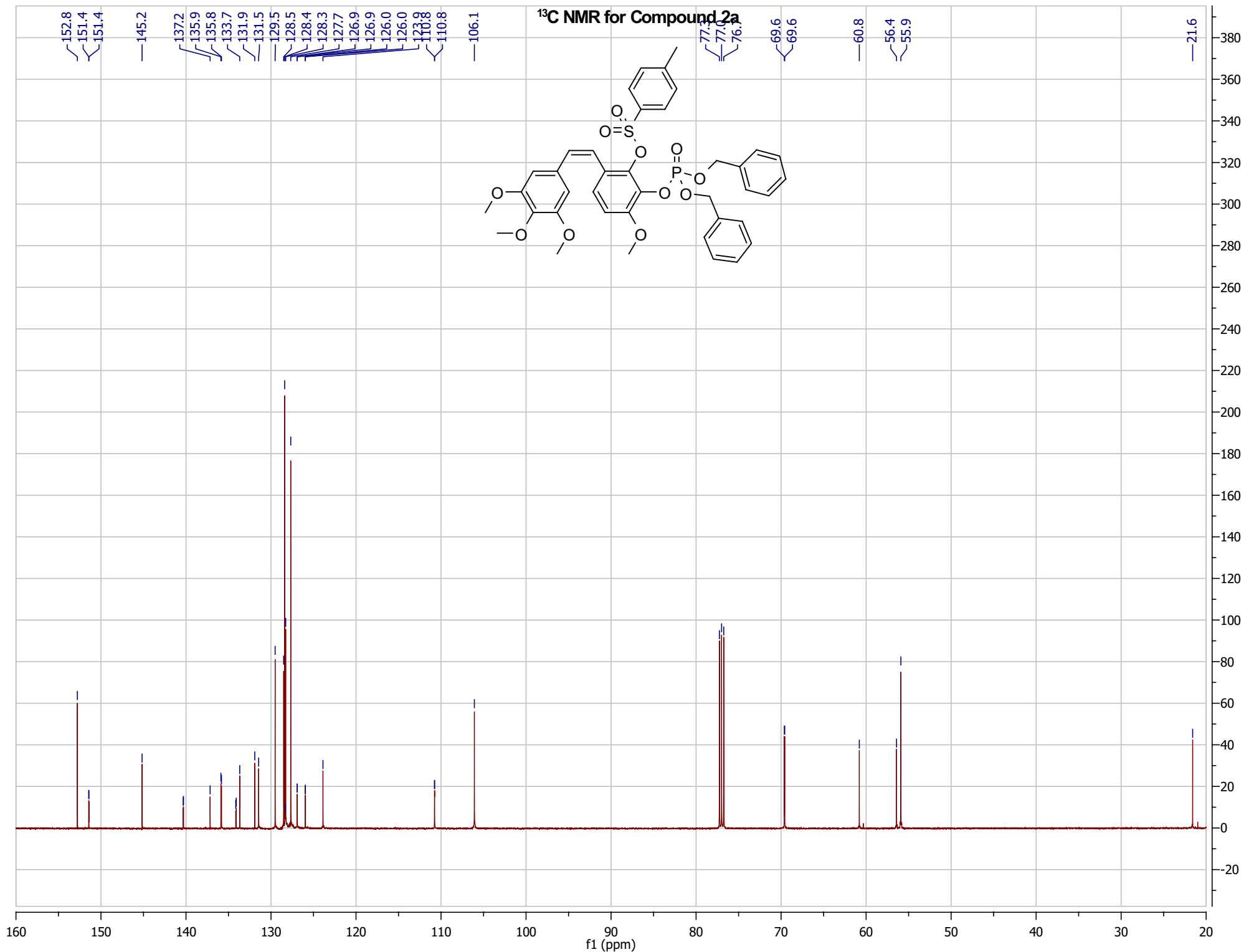


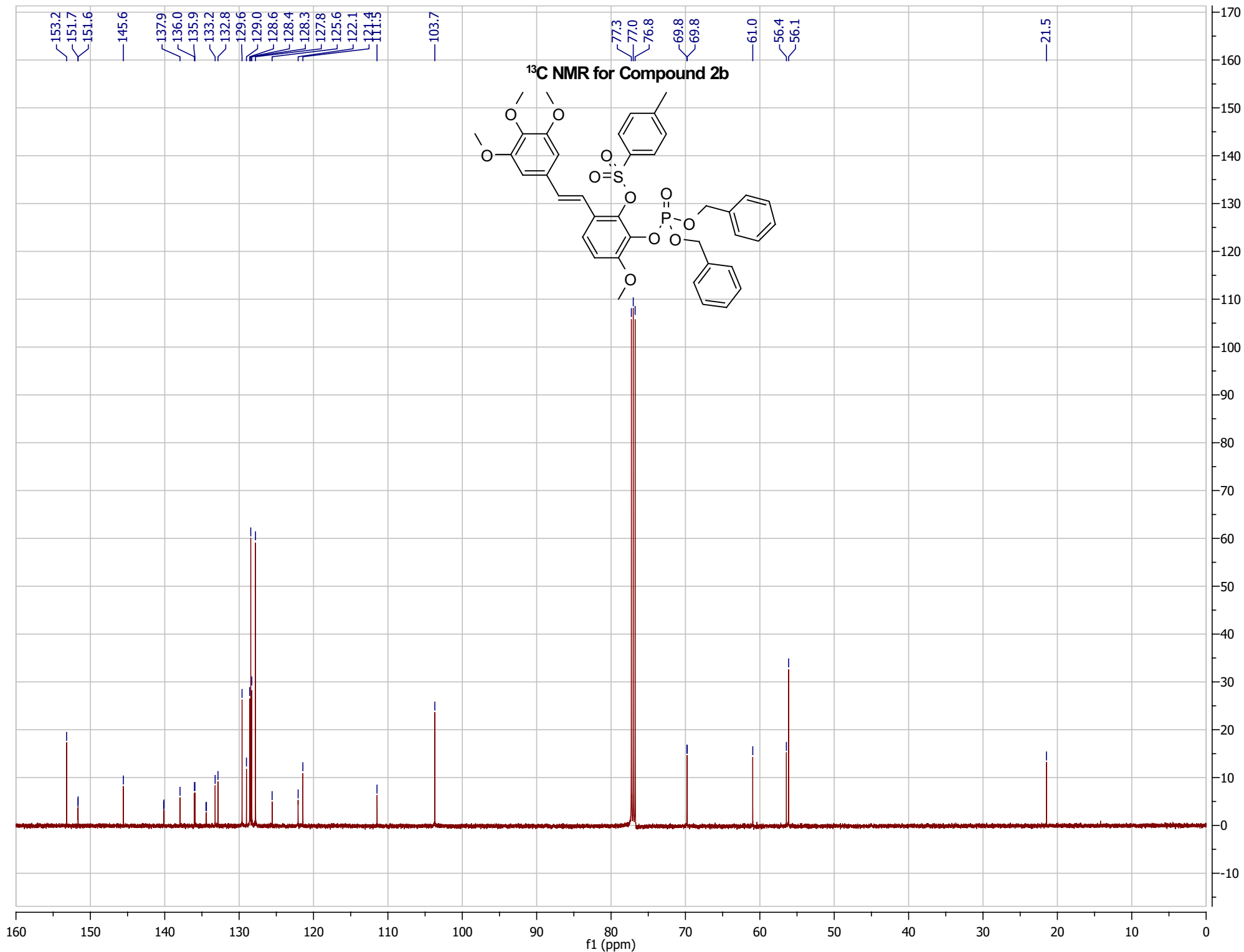
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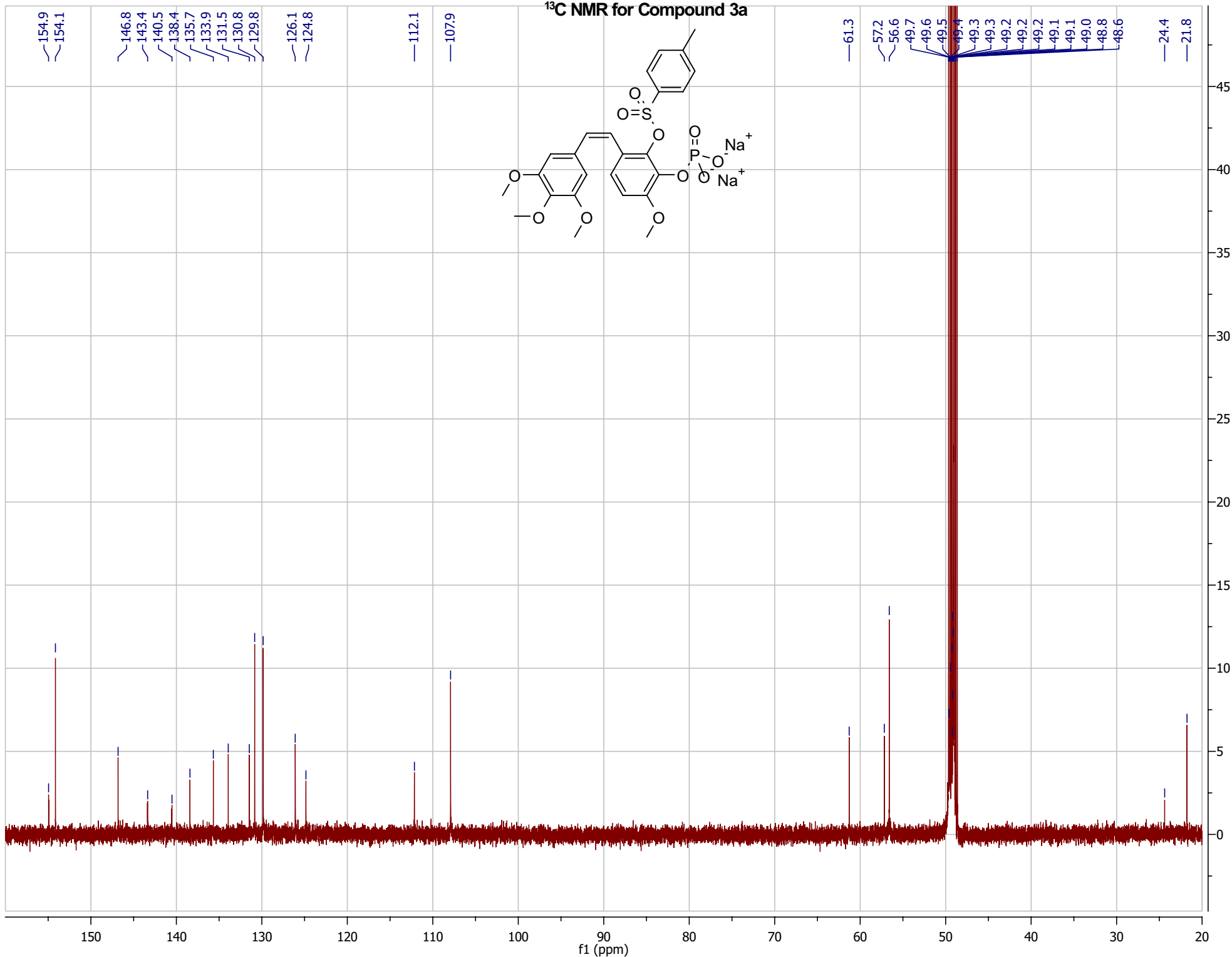
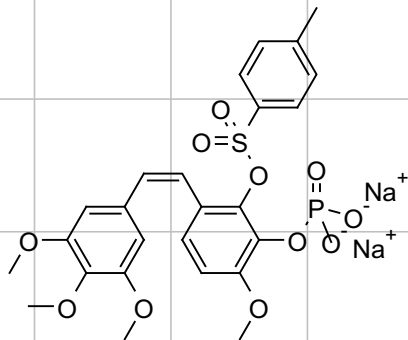
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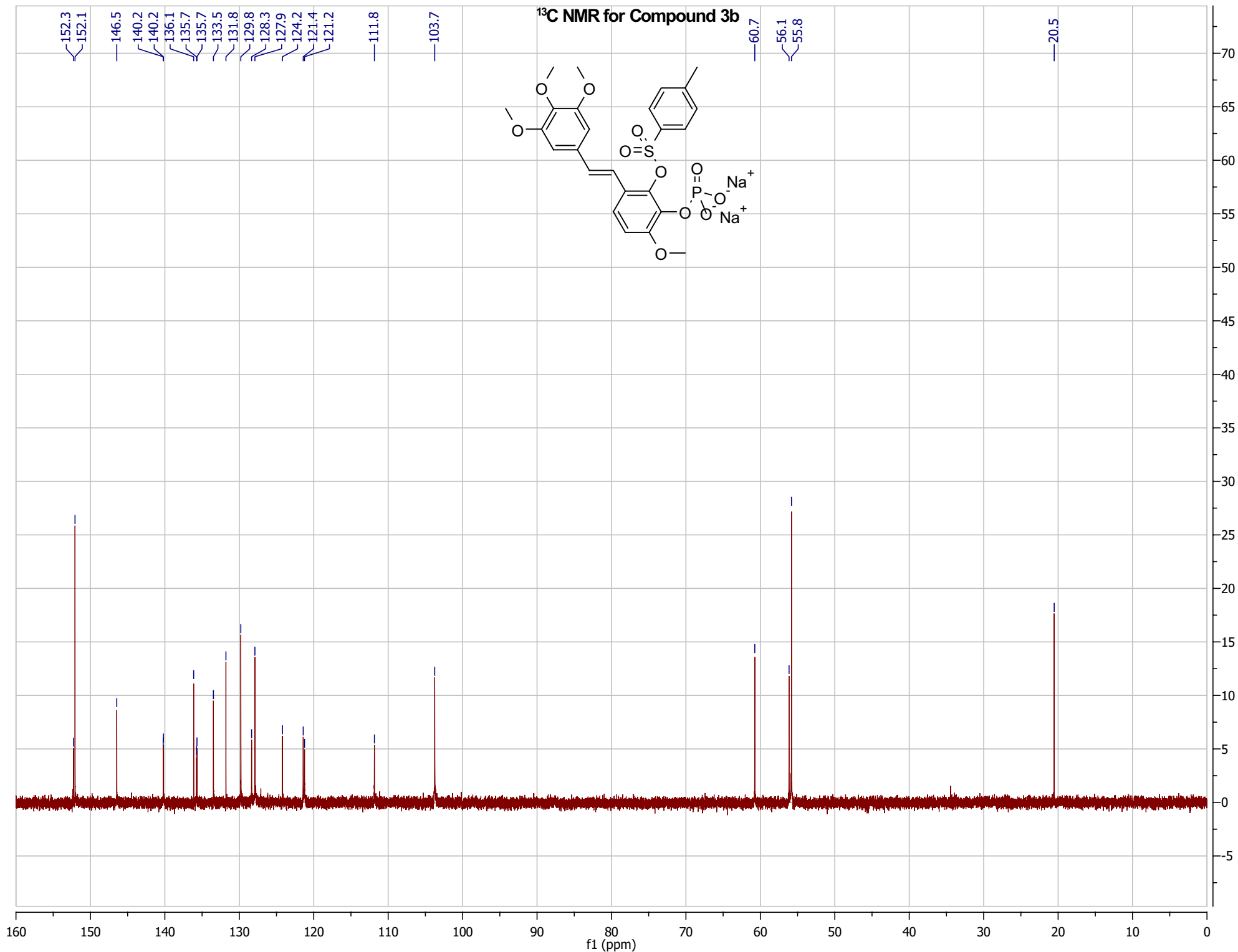
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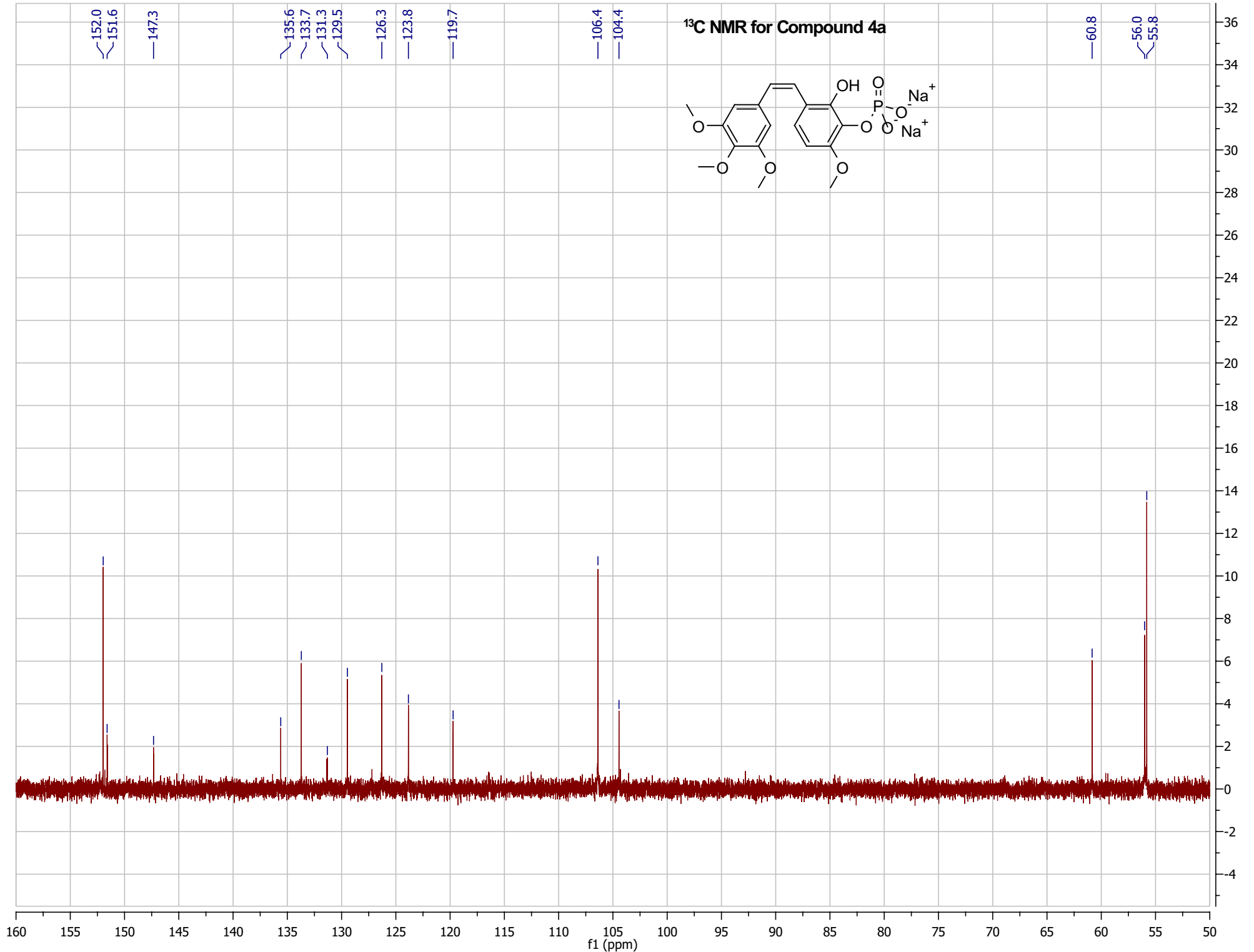
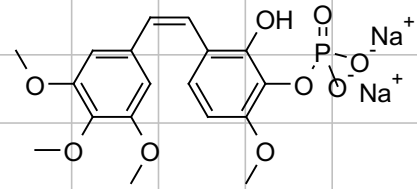
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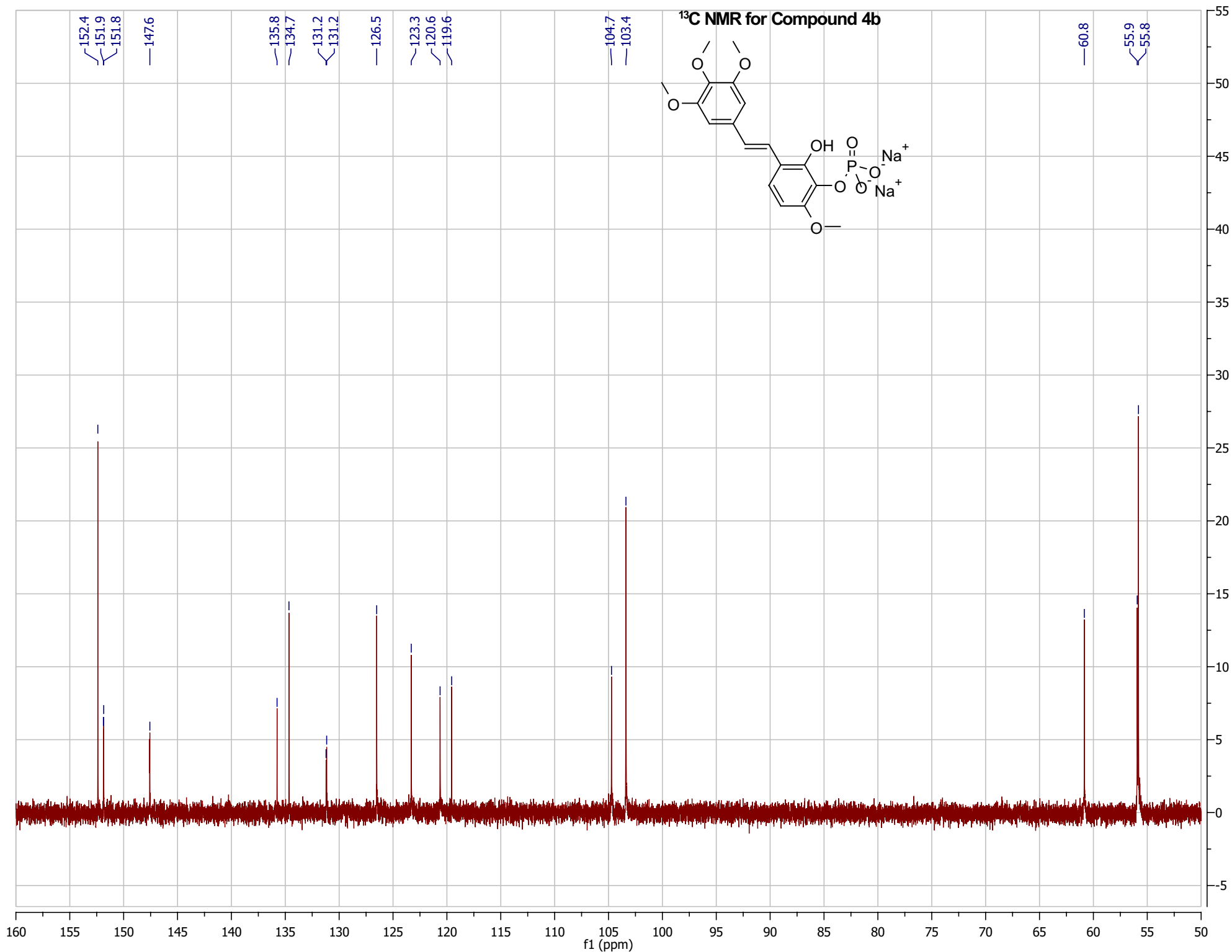


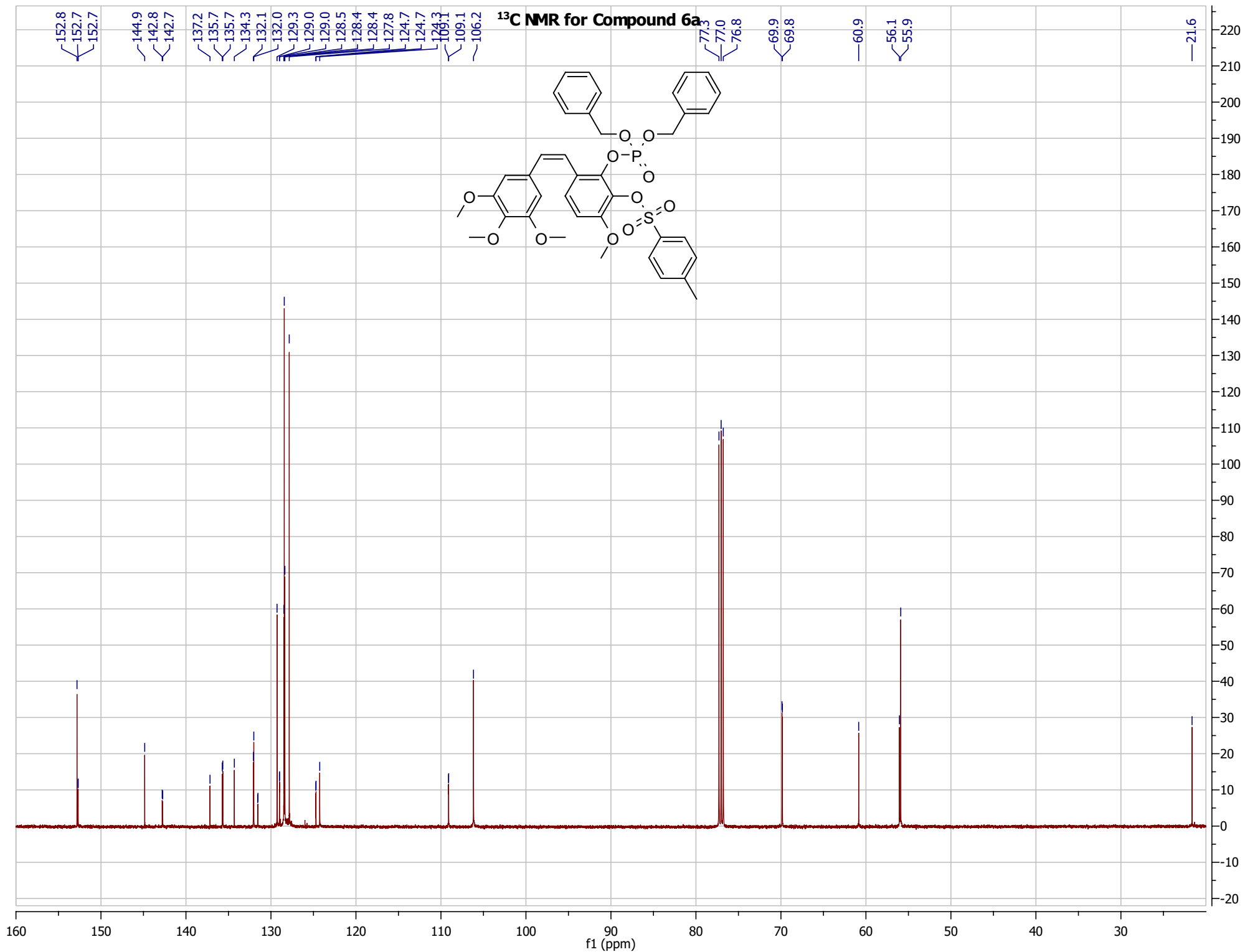


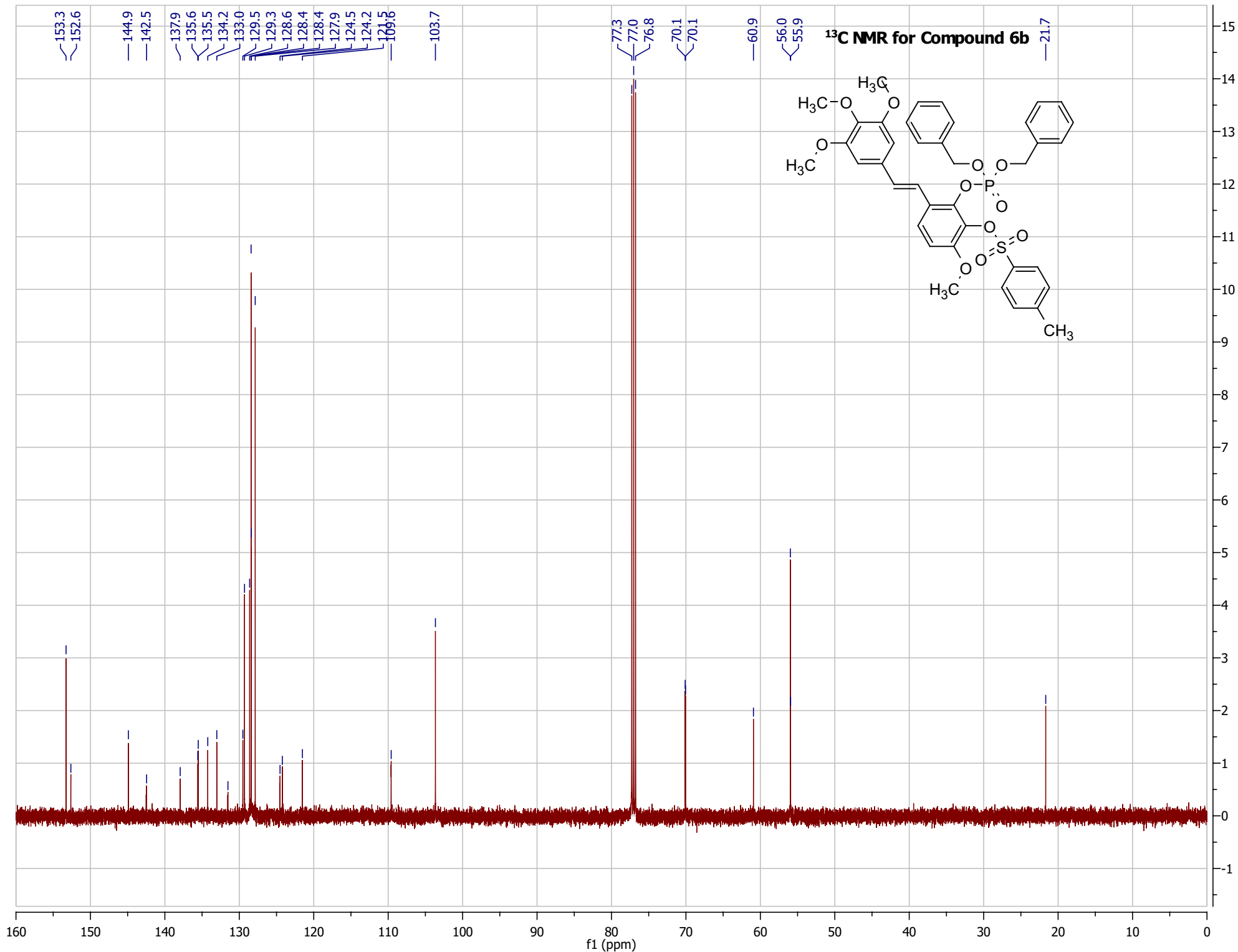


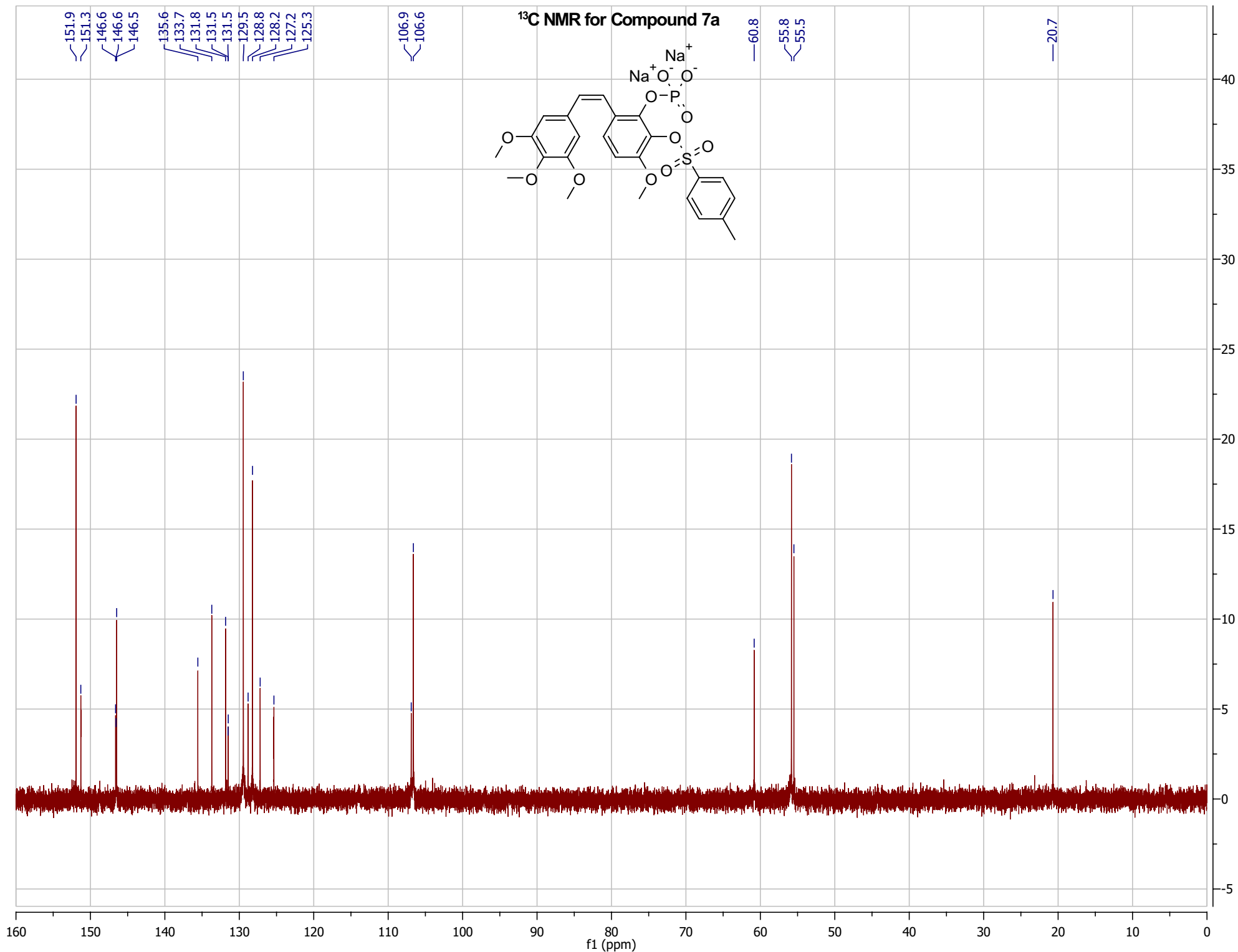
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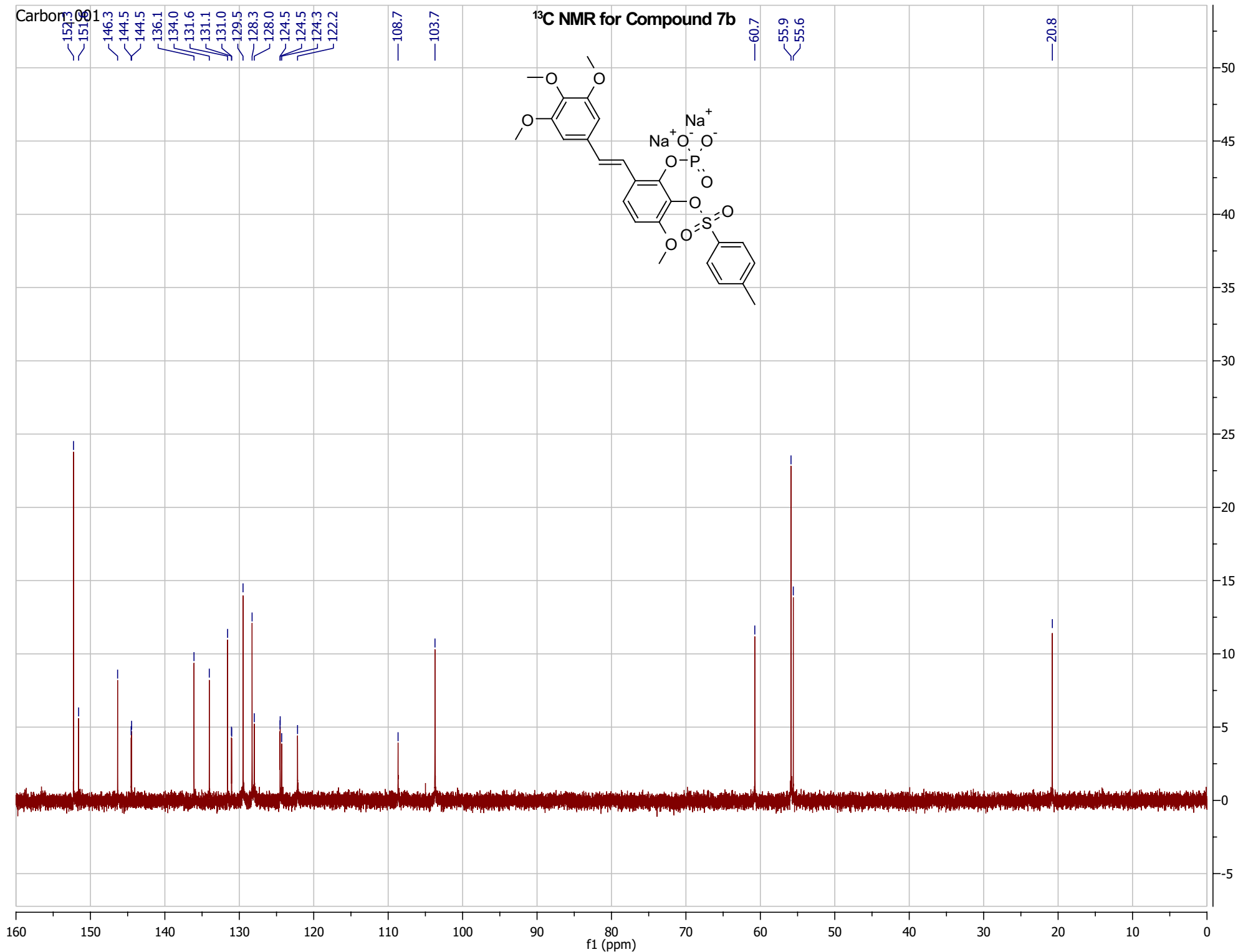


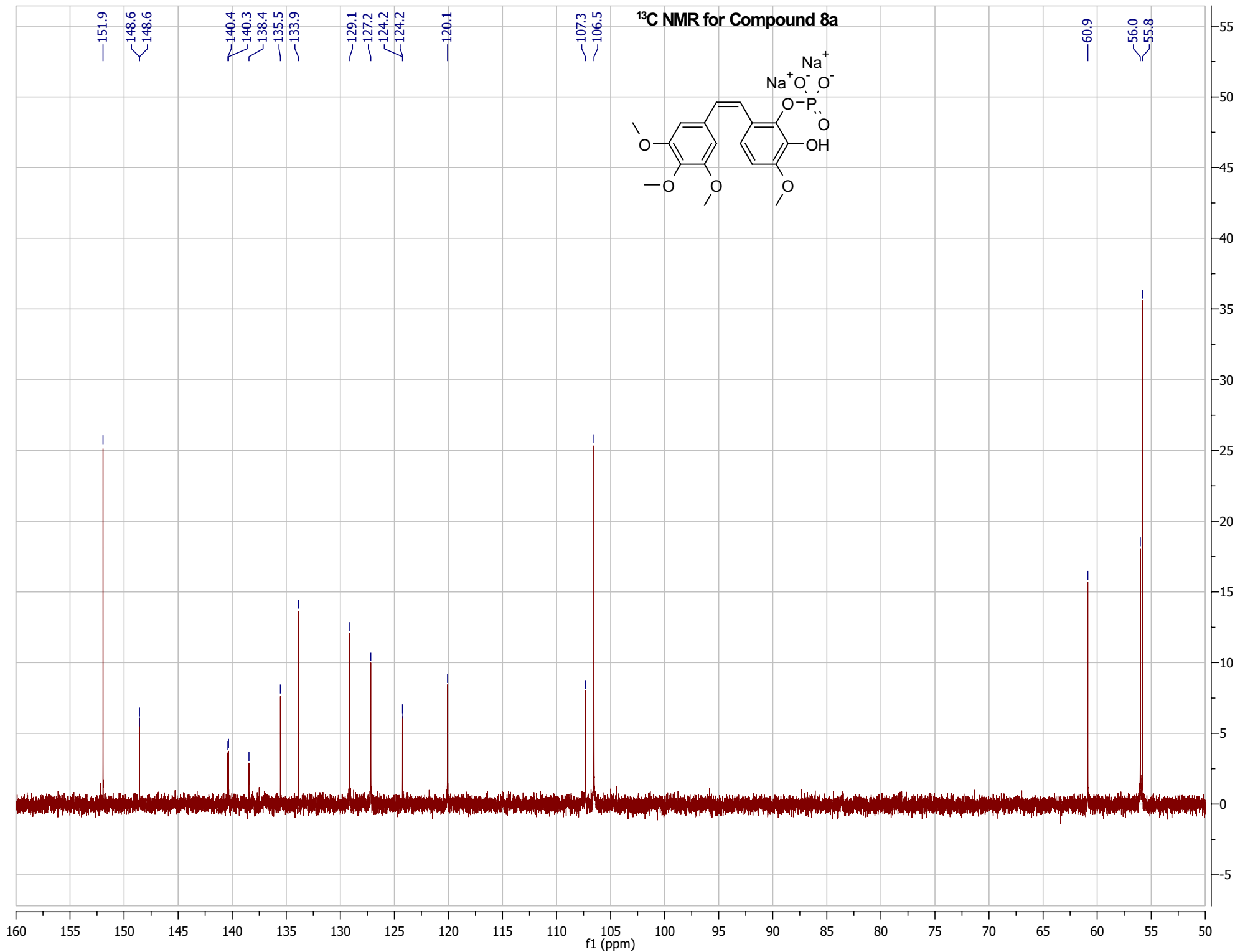


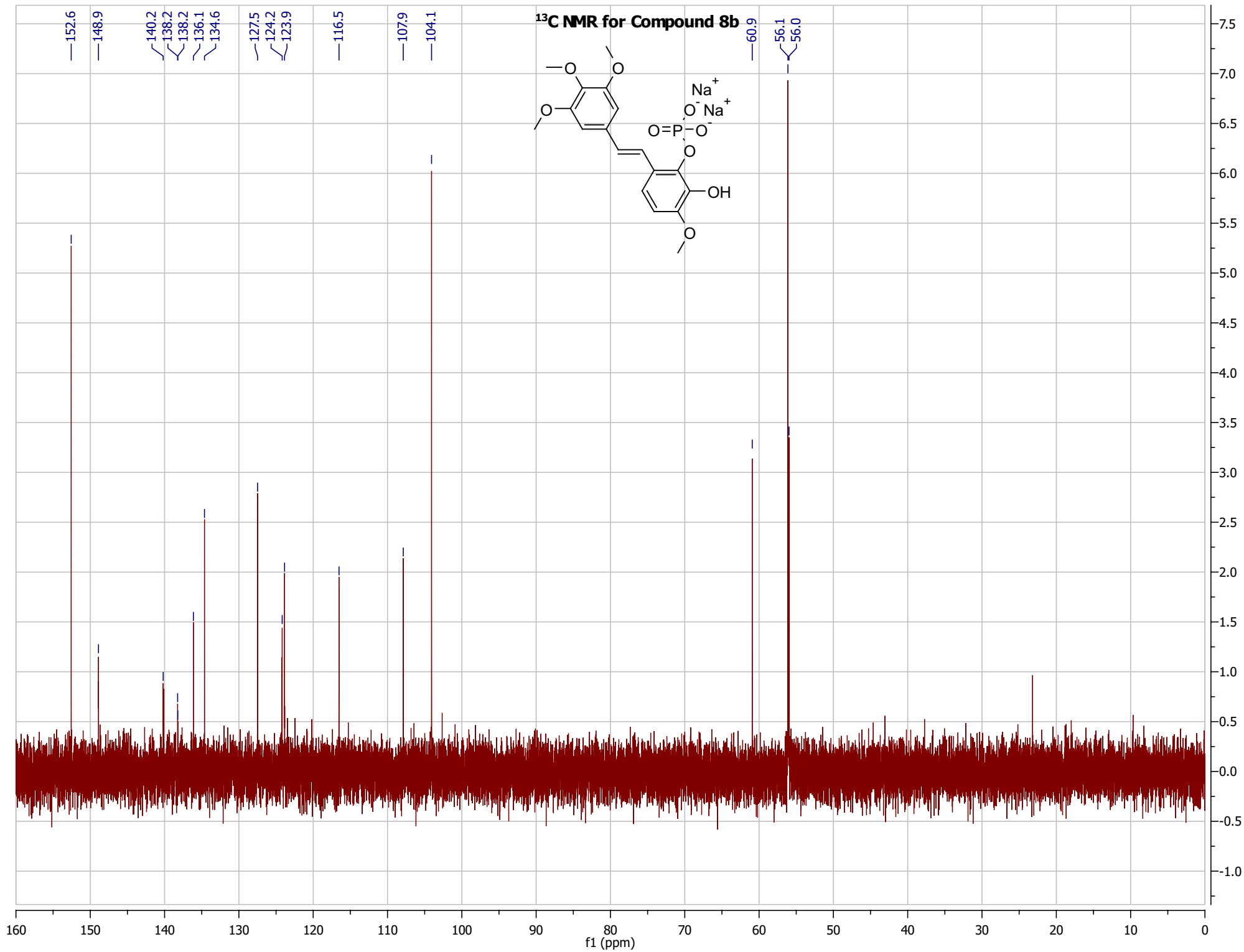


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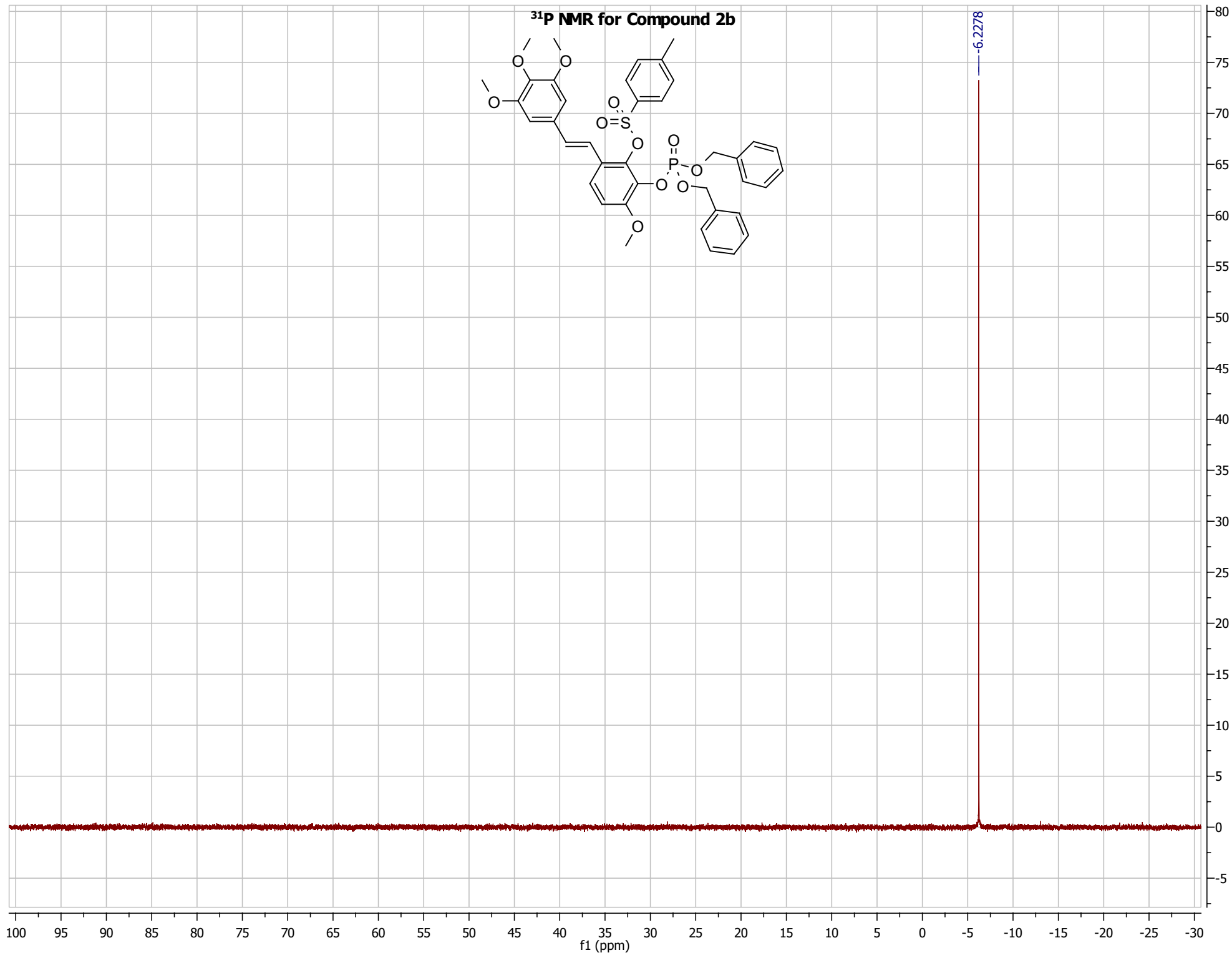
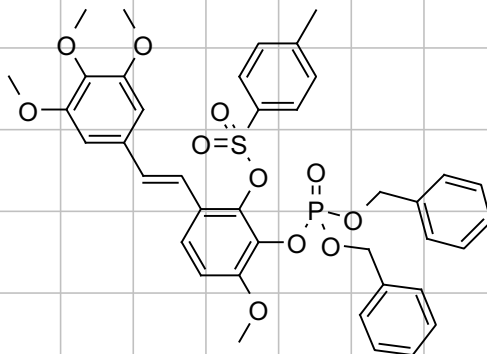




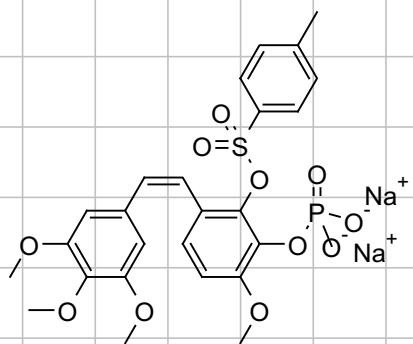




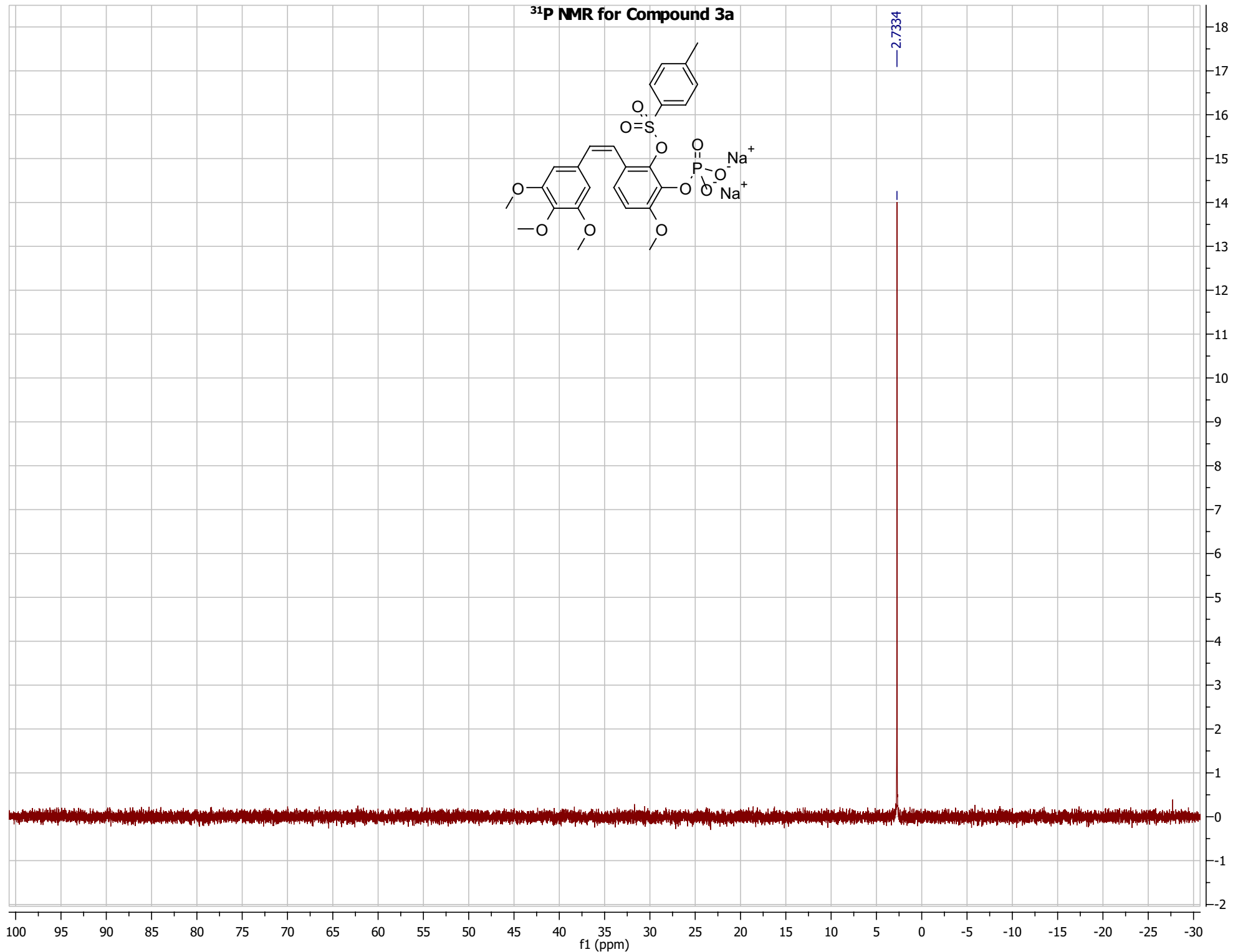
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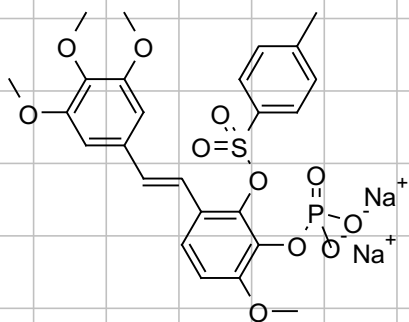
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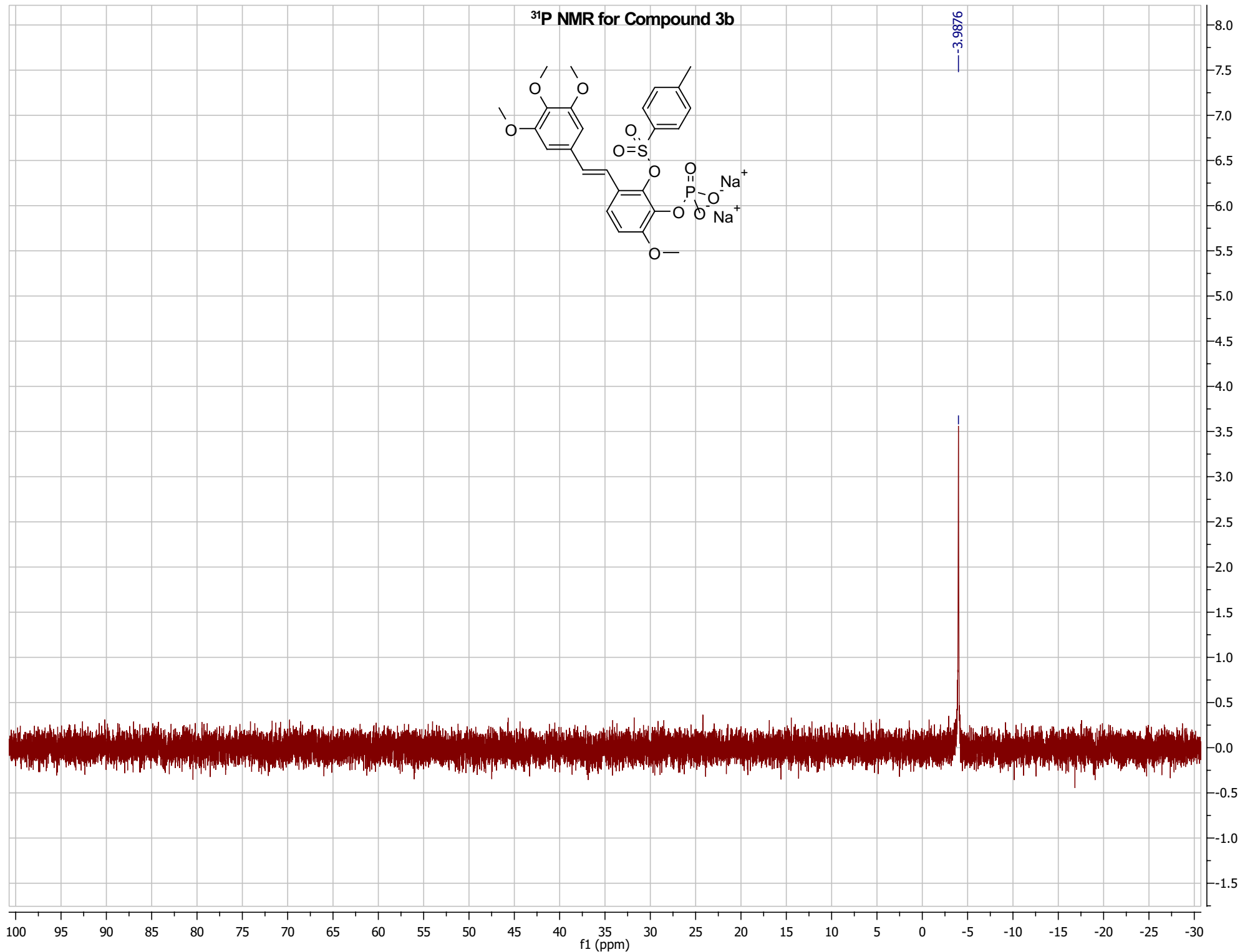
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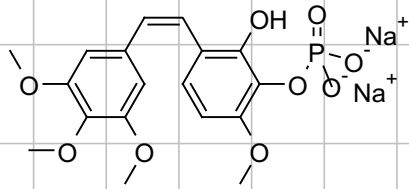
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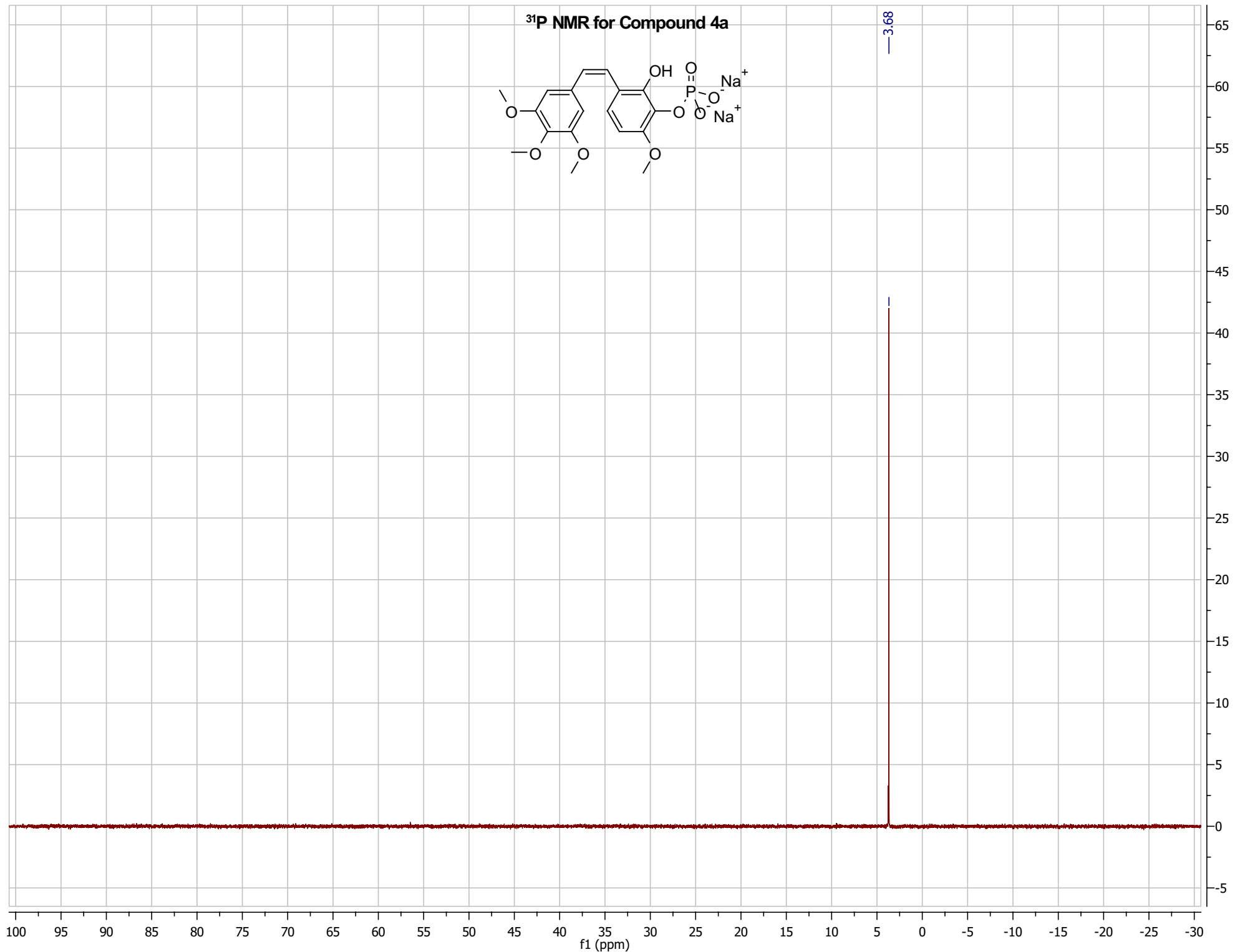
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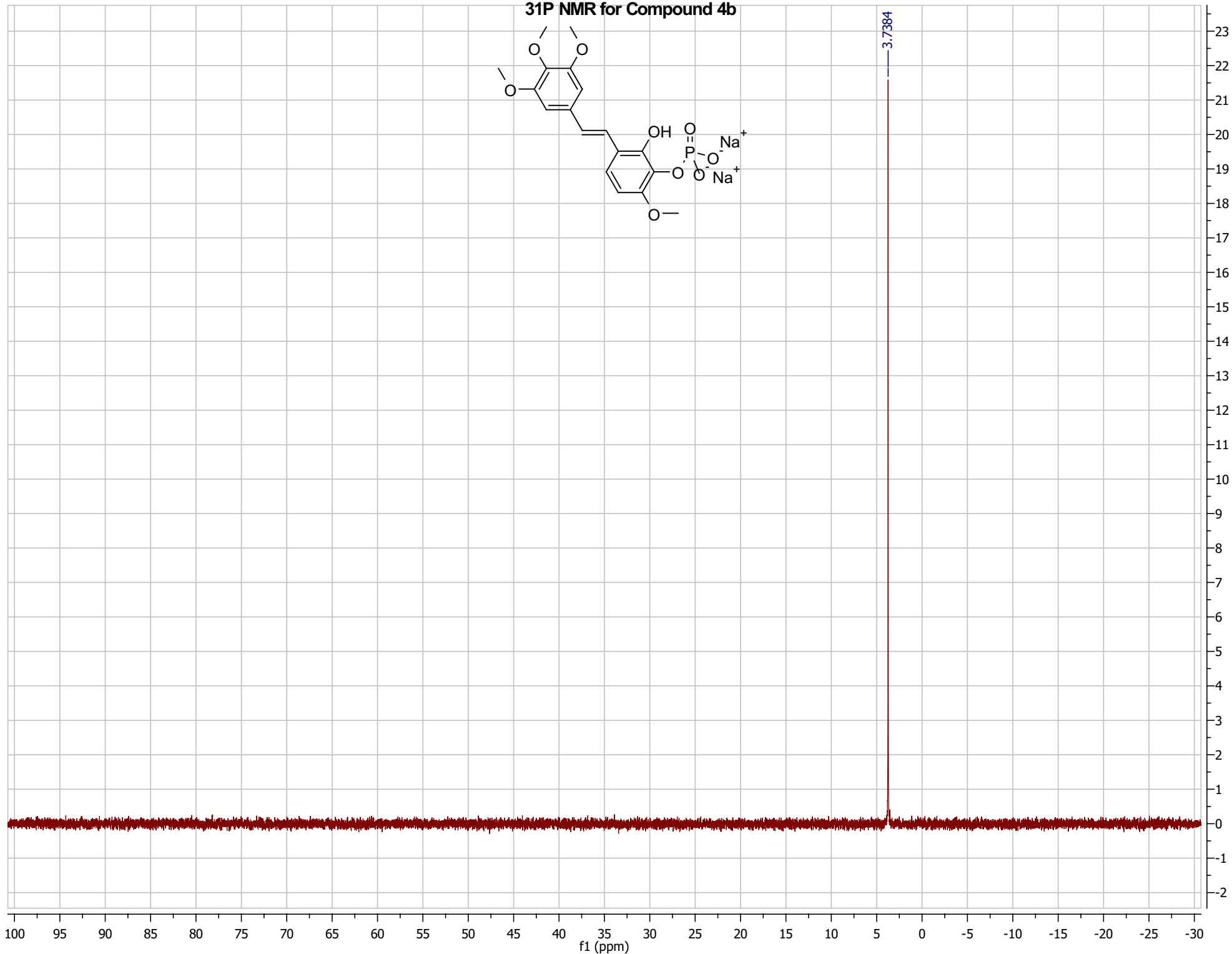
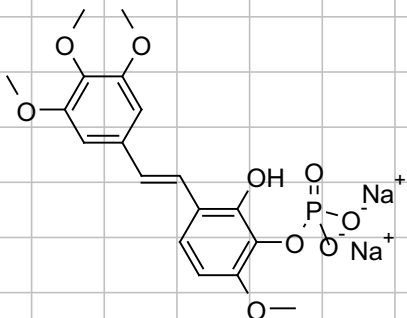
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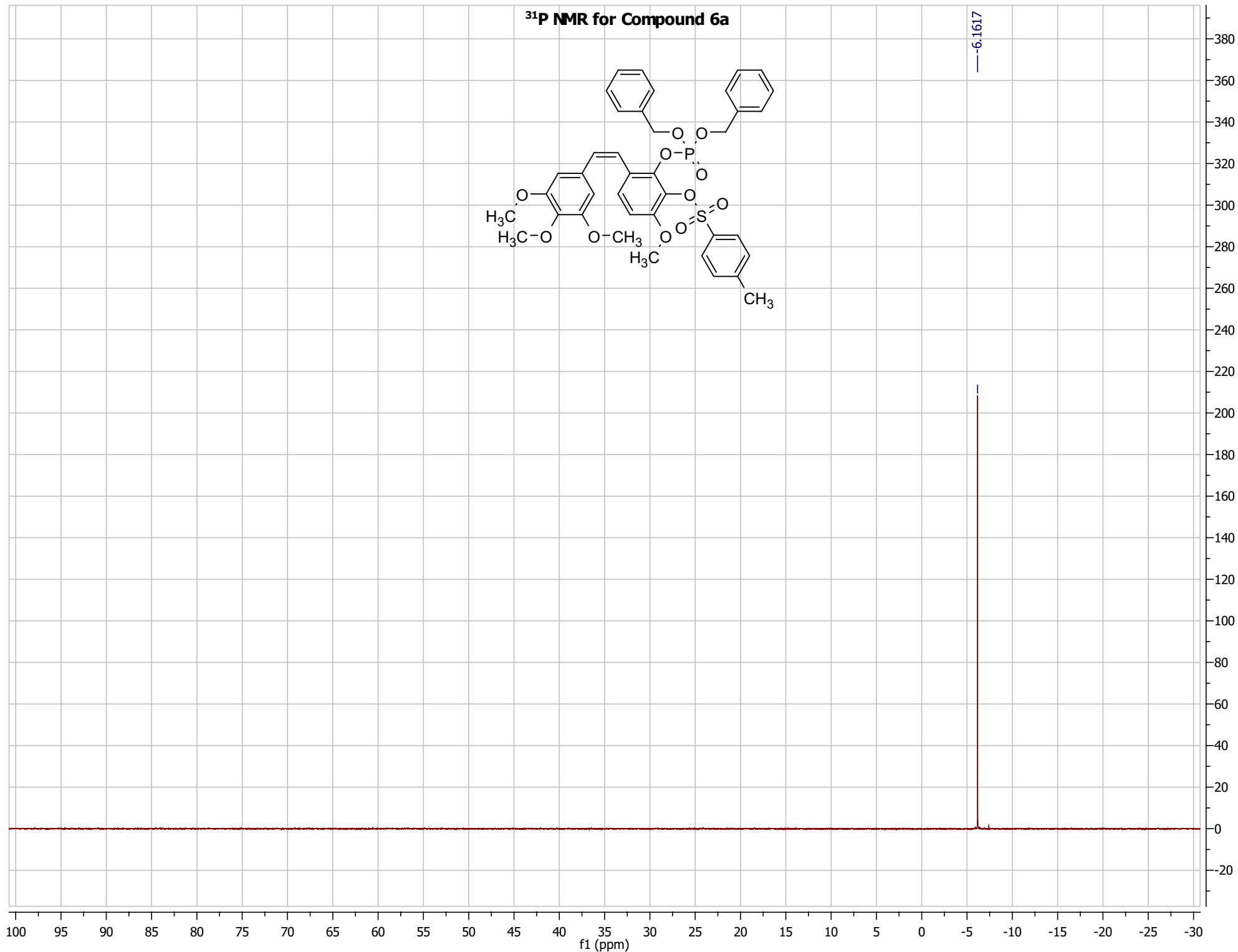
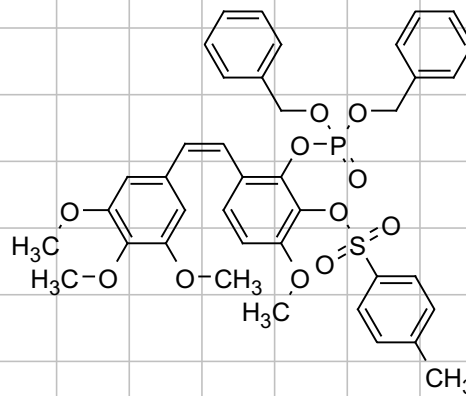
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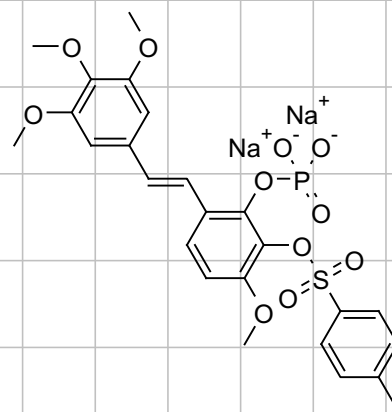
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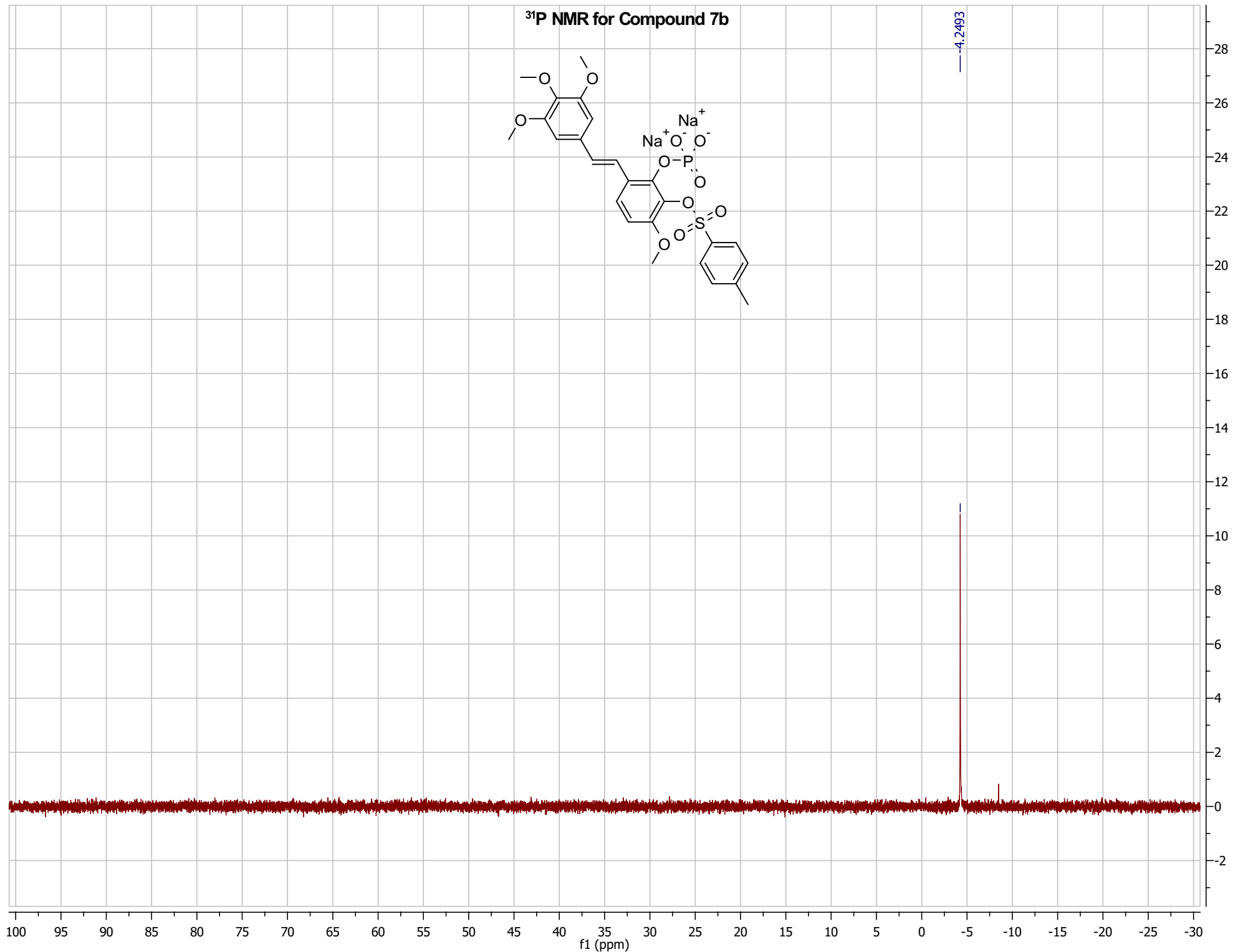
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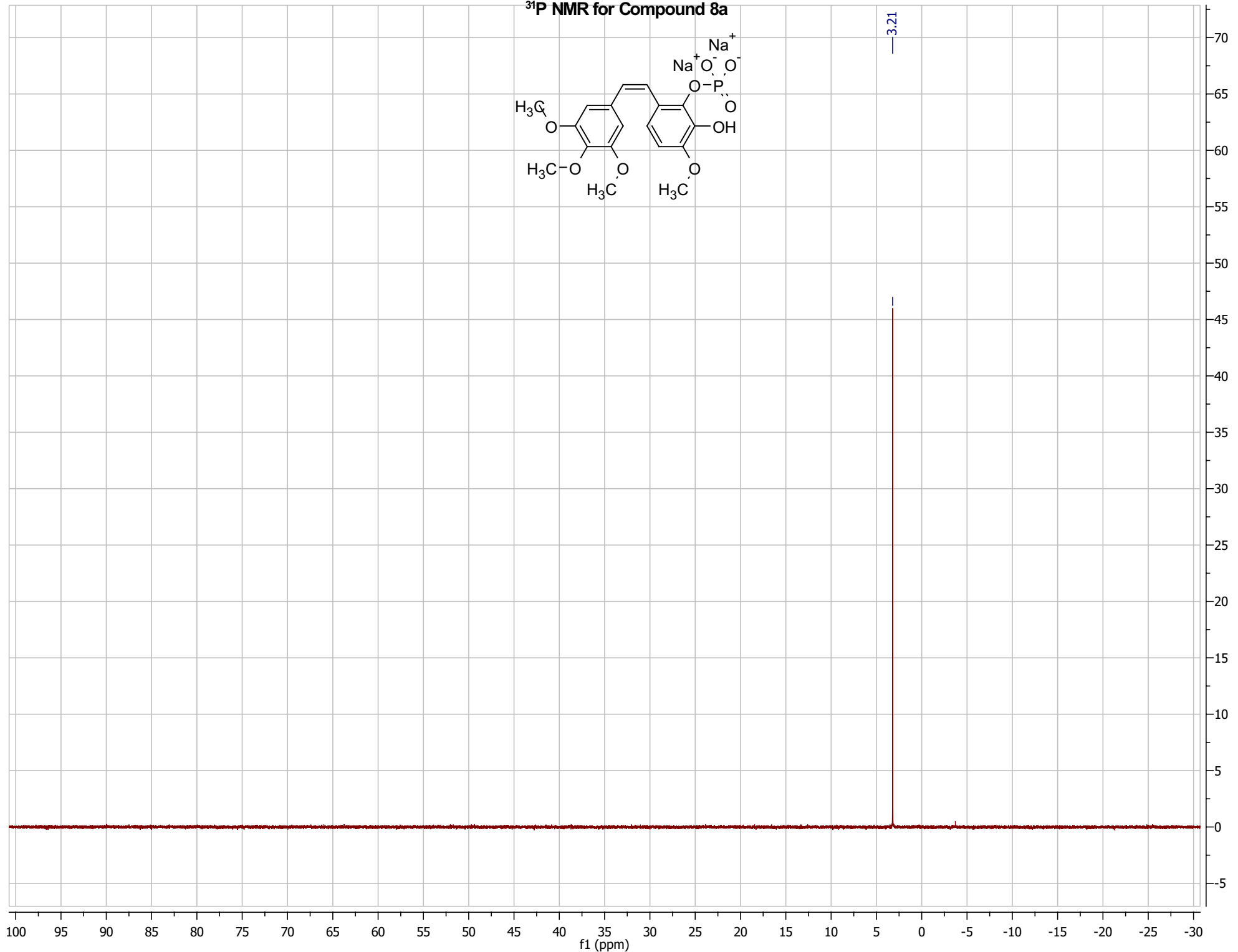
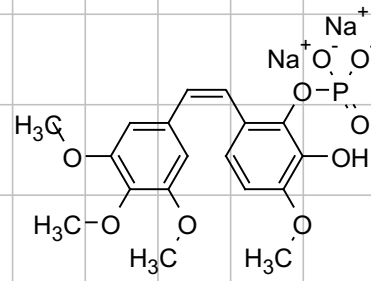
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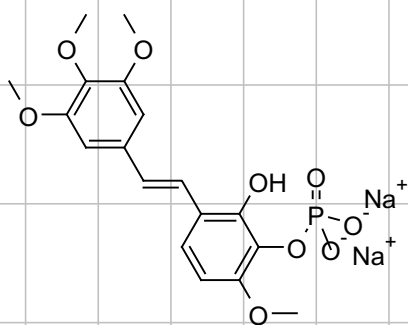


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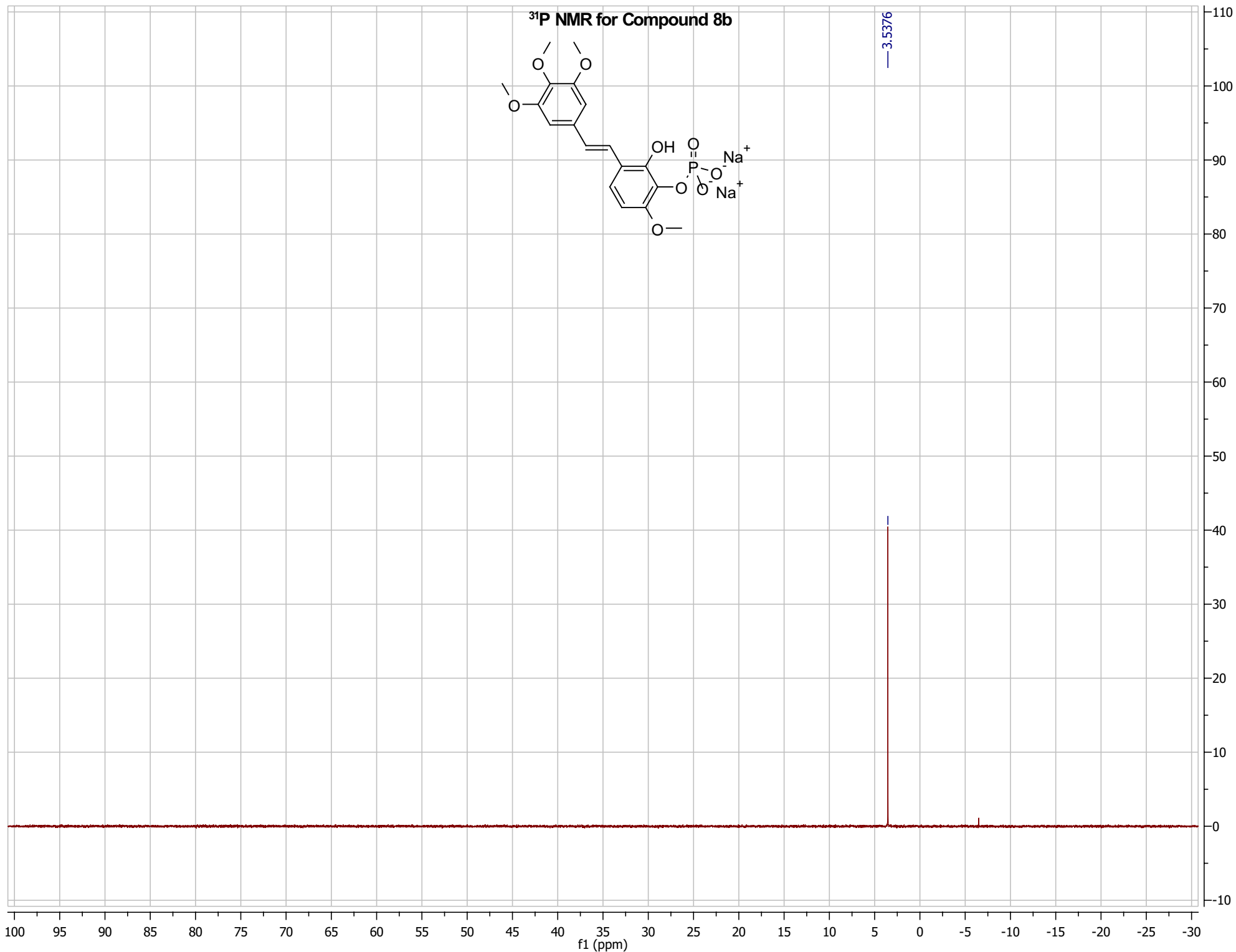




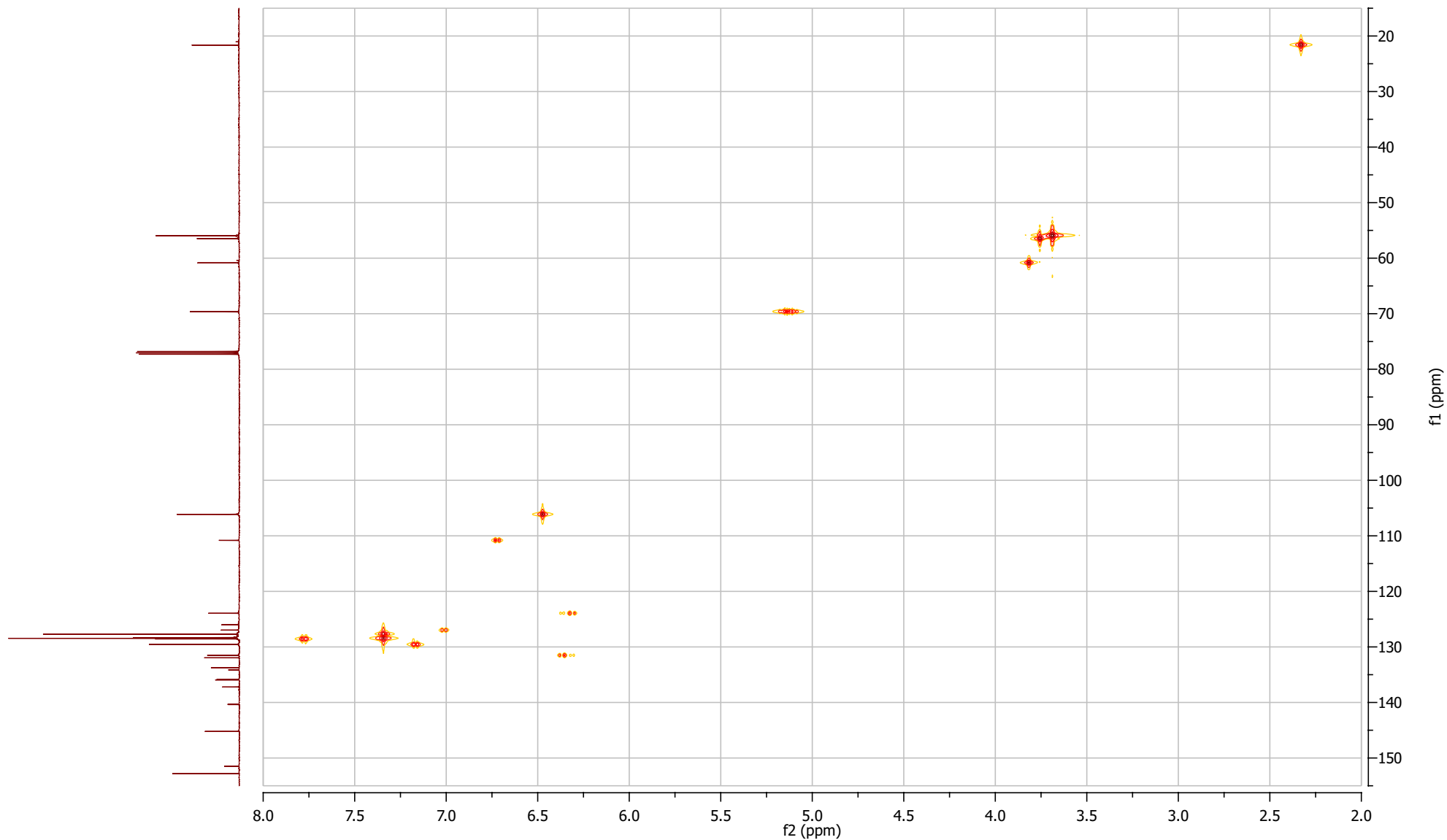
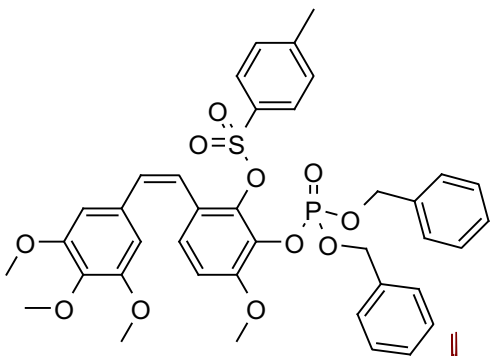
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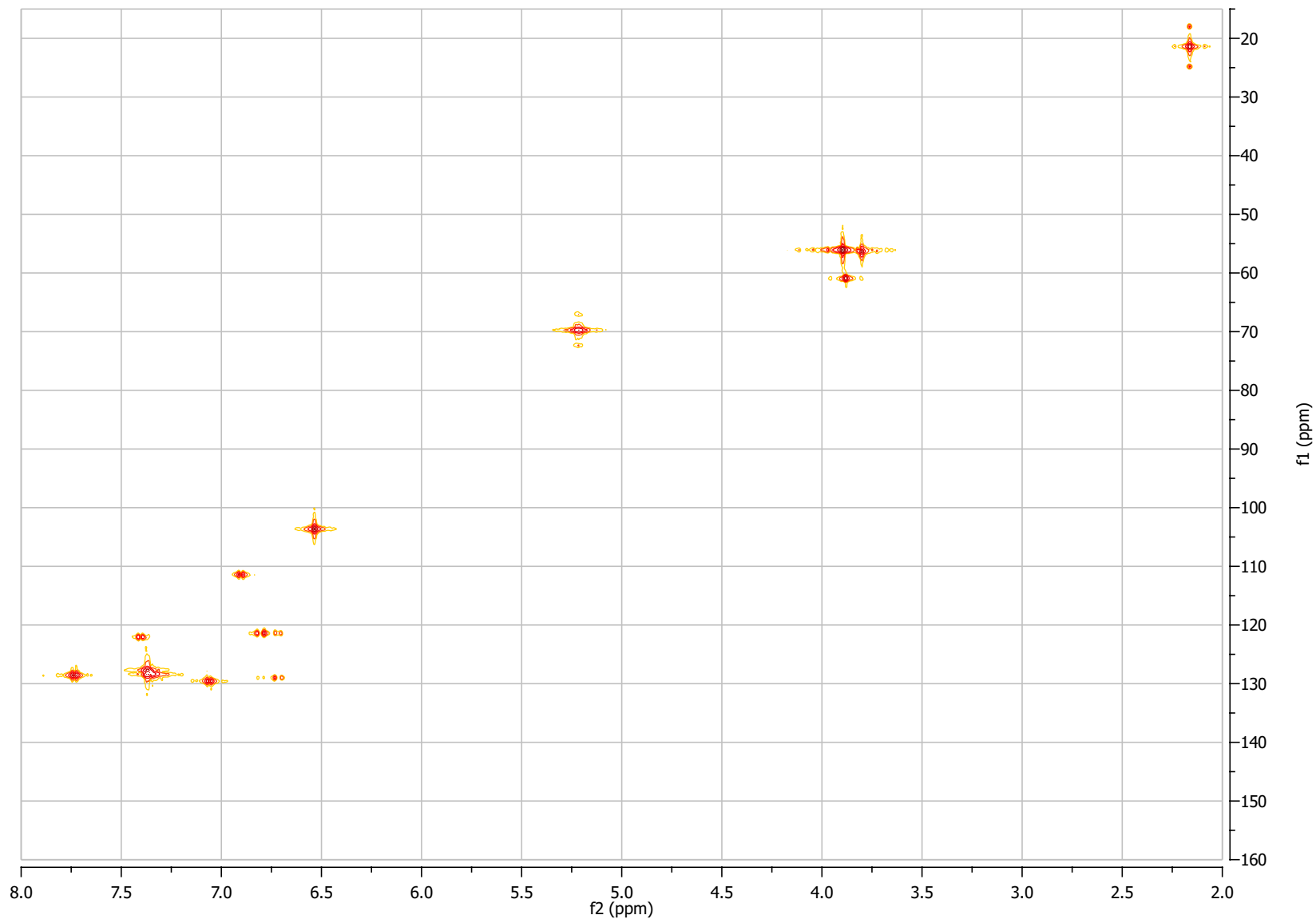
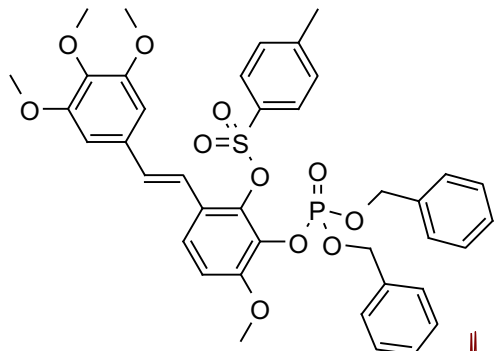
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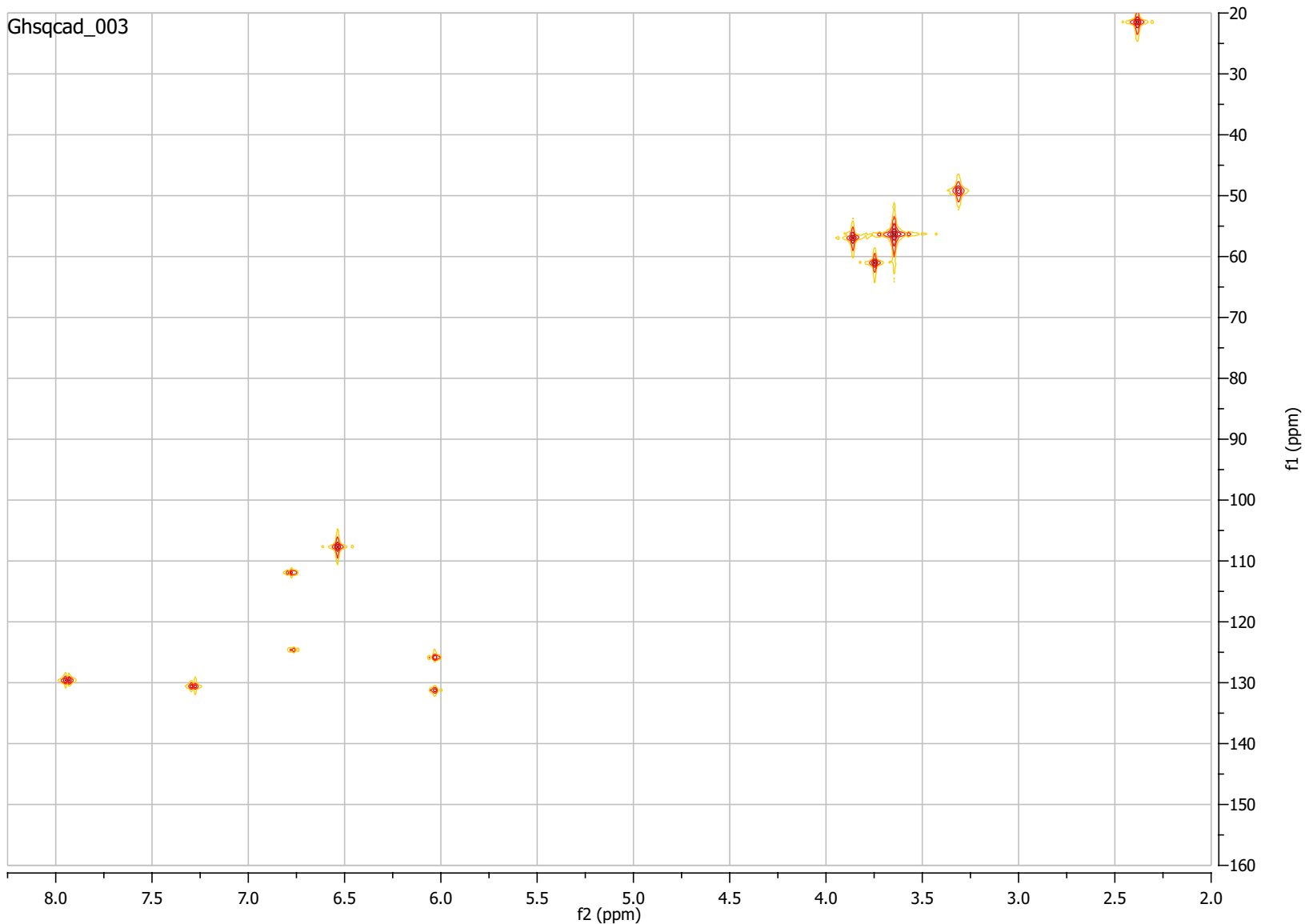
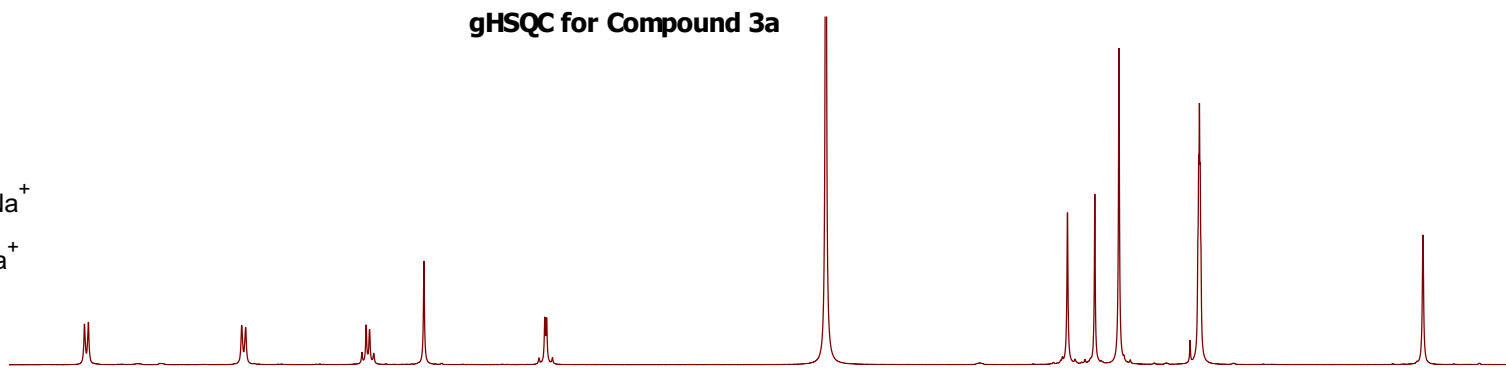
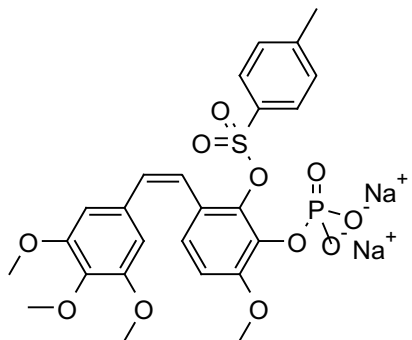
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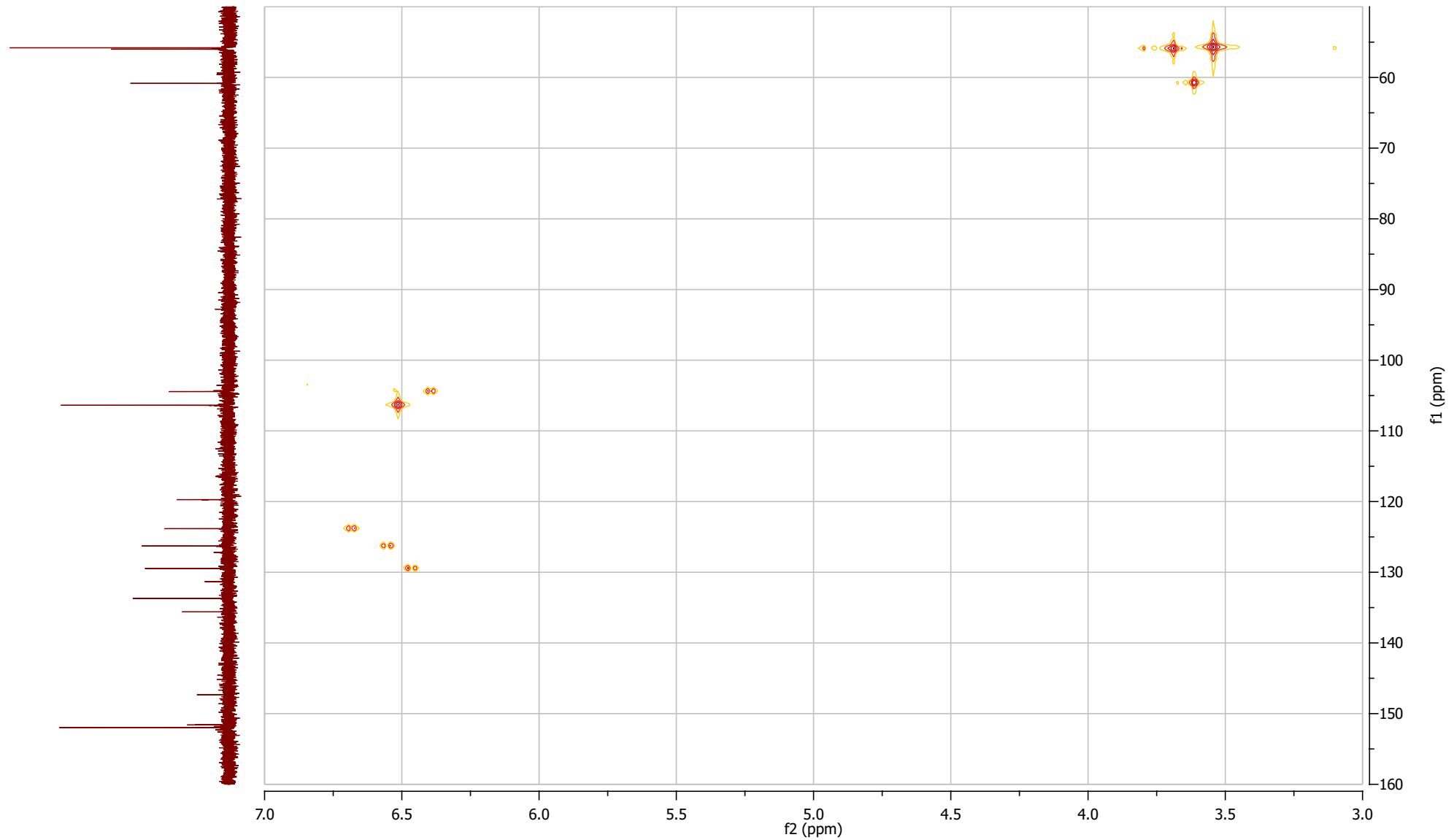
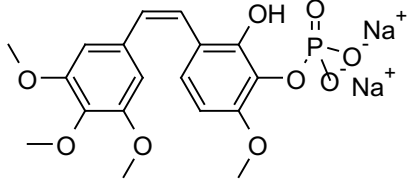
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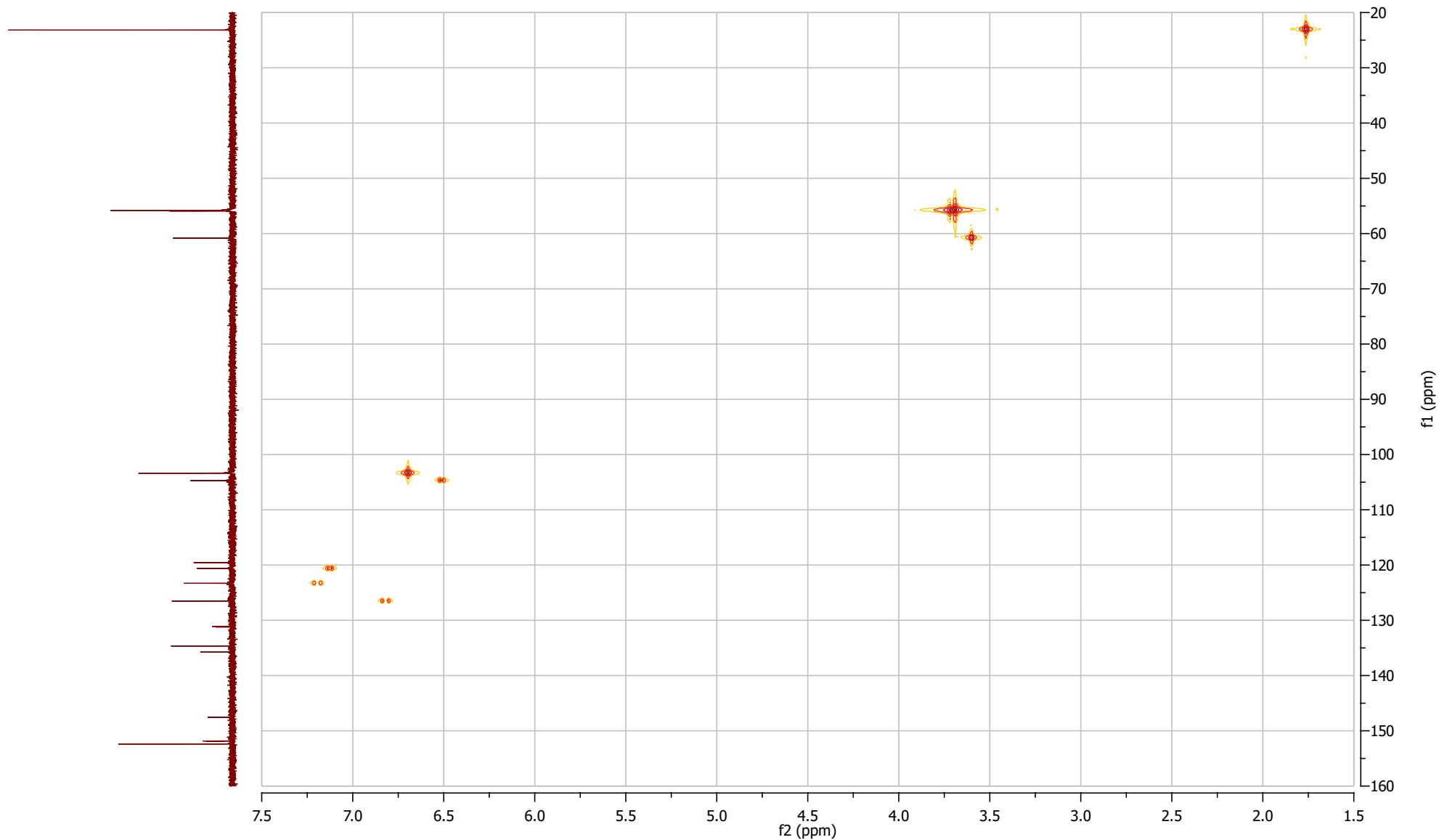
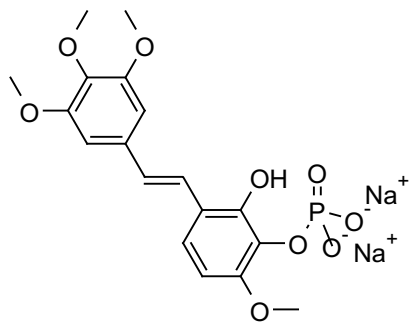
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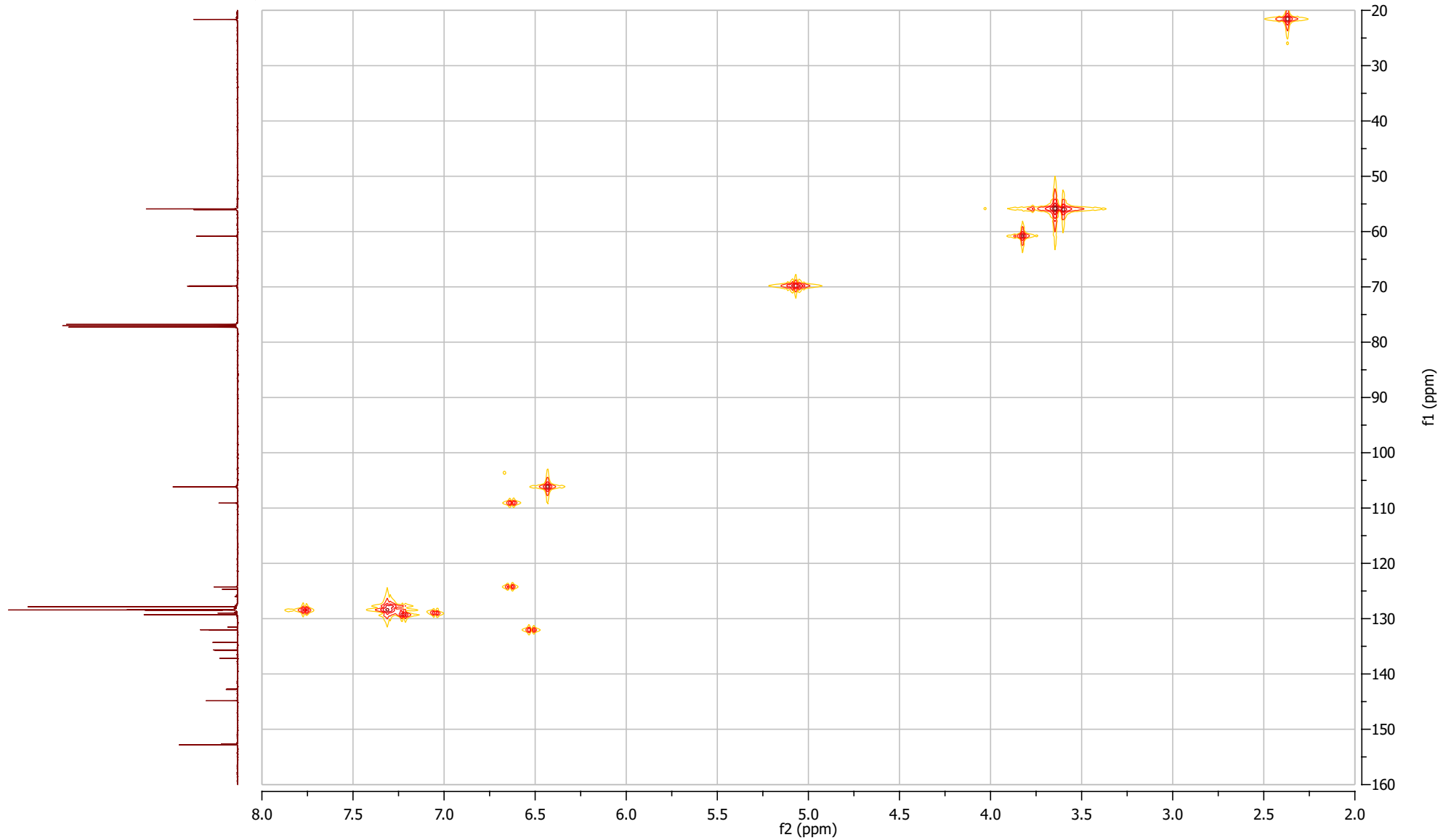
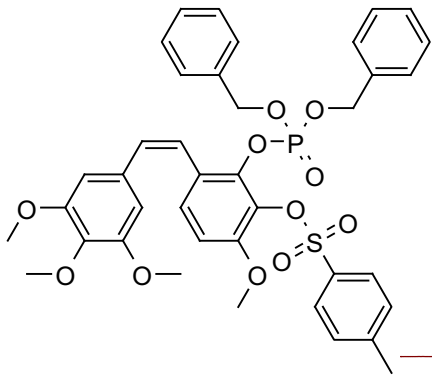
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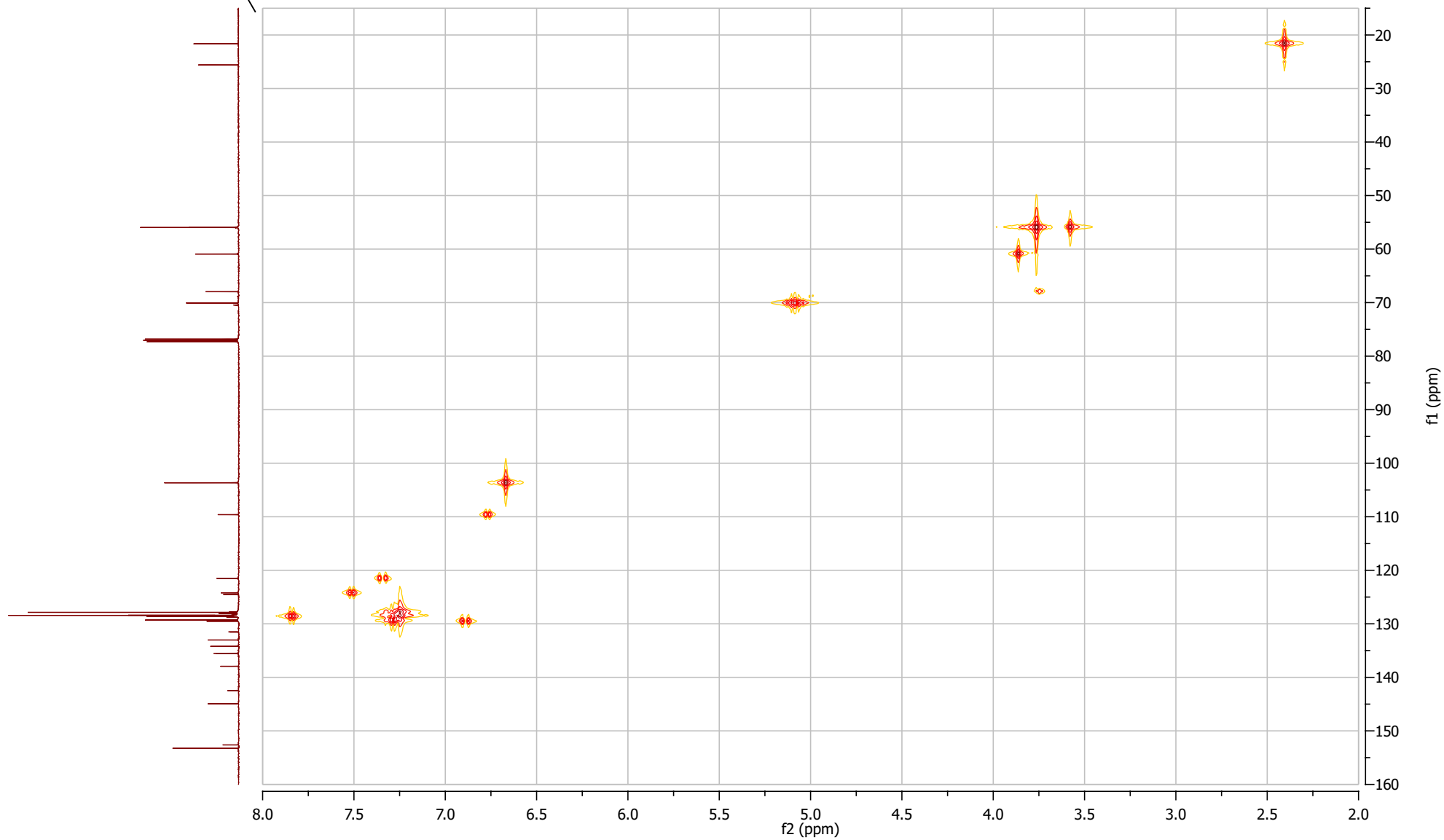
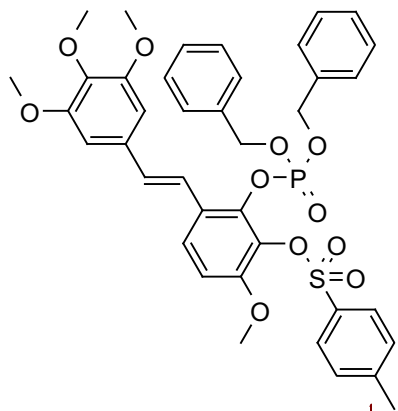
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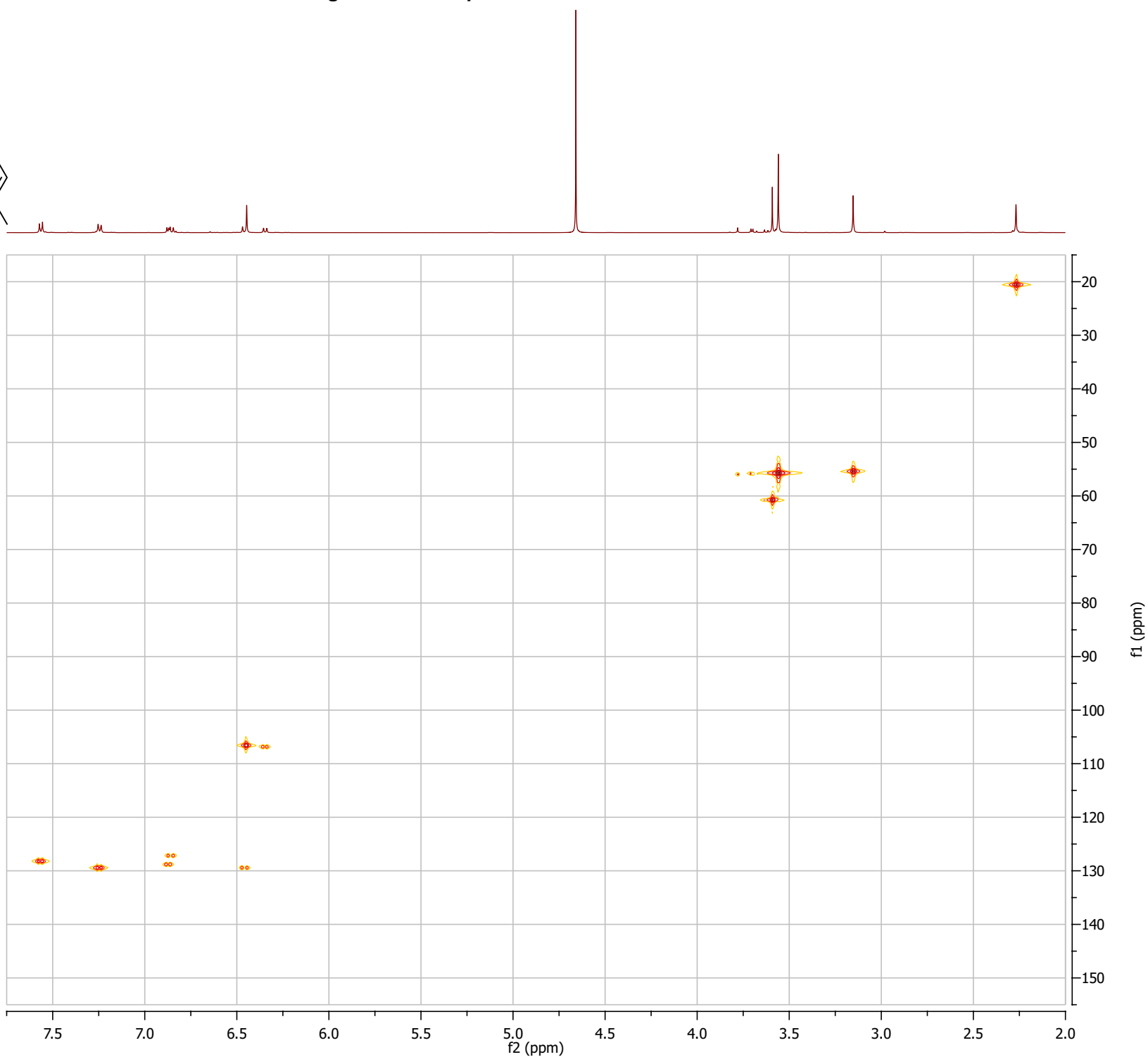
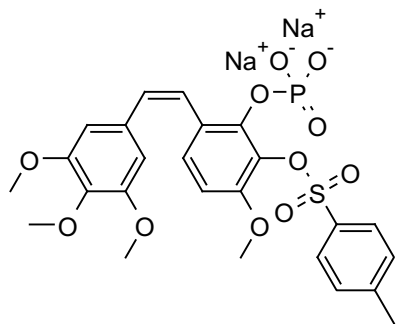


gHSQC for Compound 6b

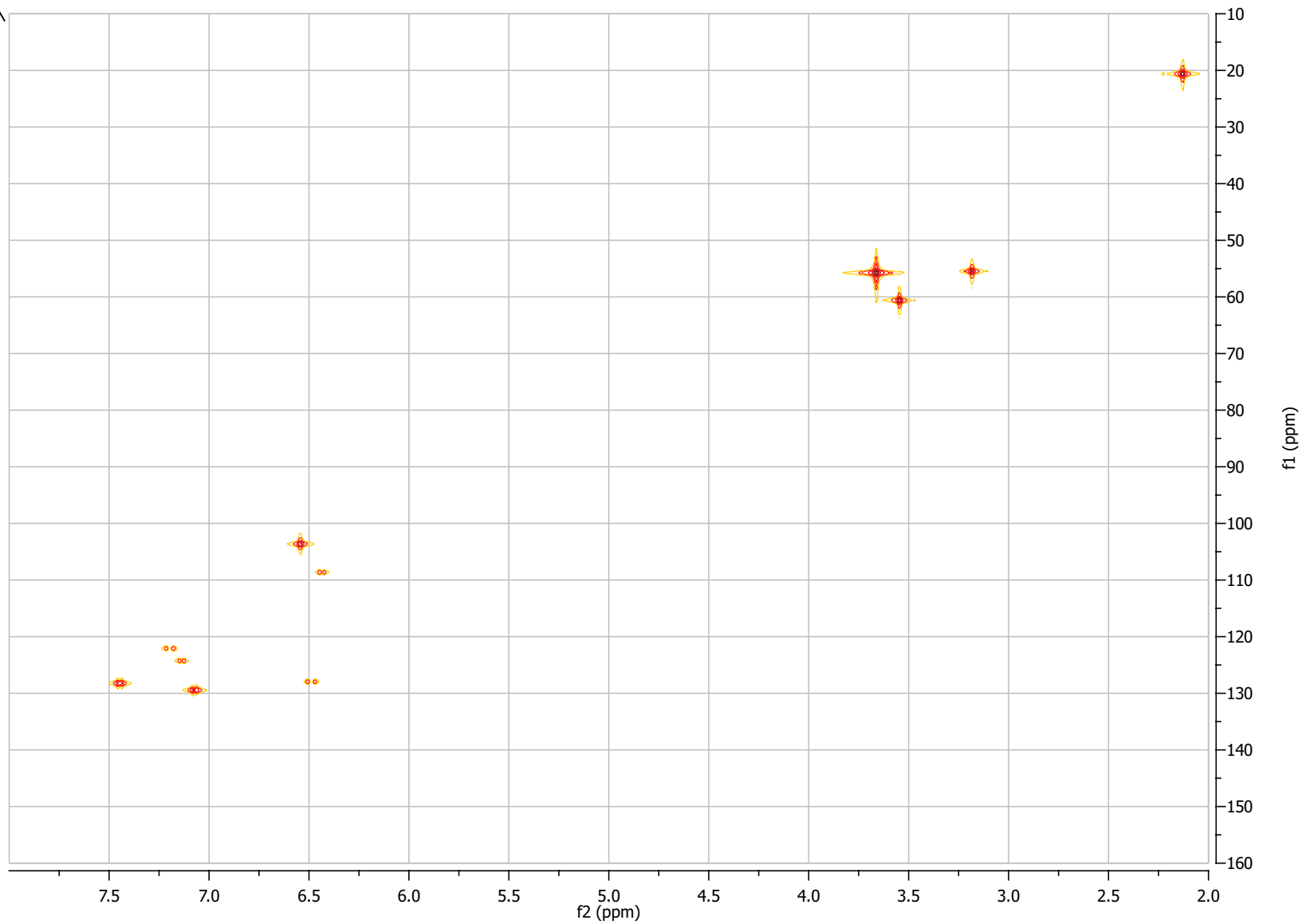
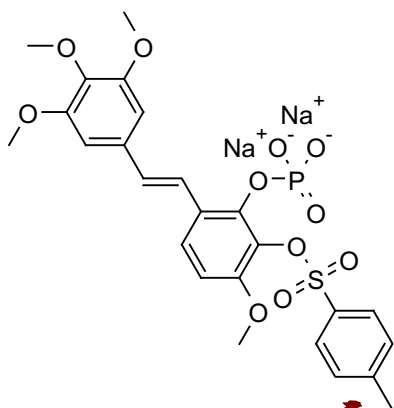




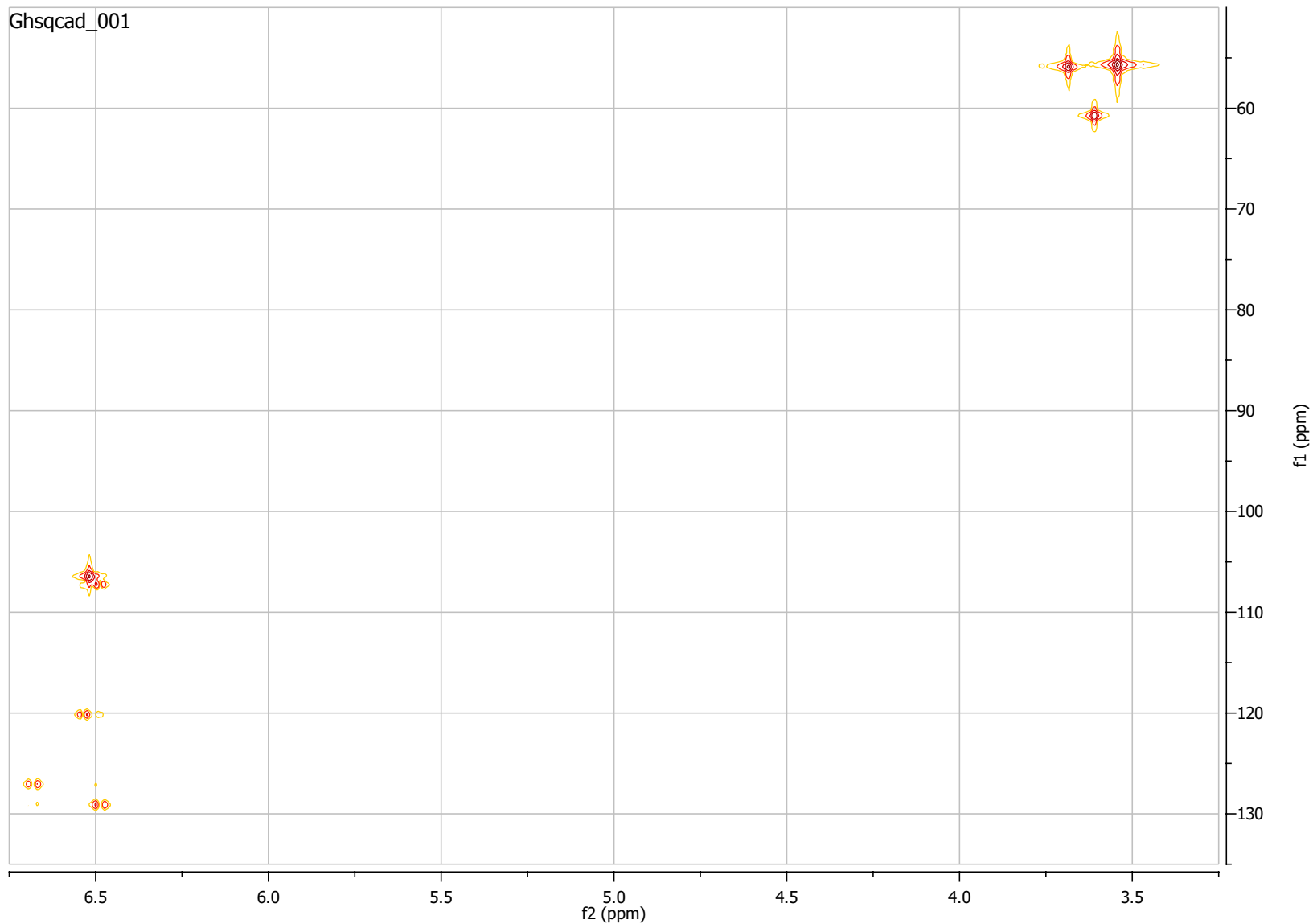
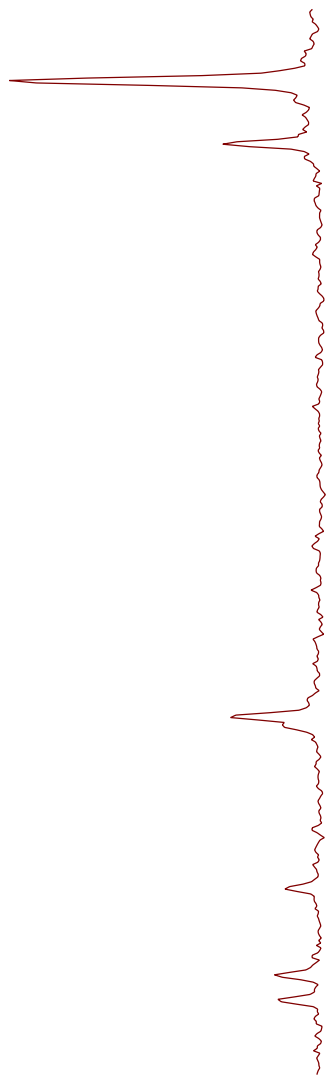
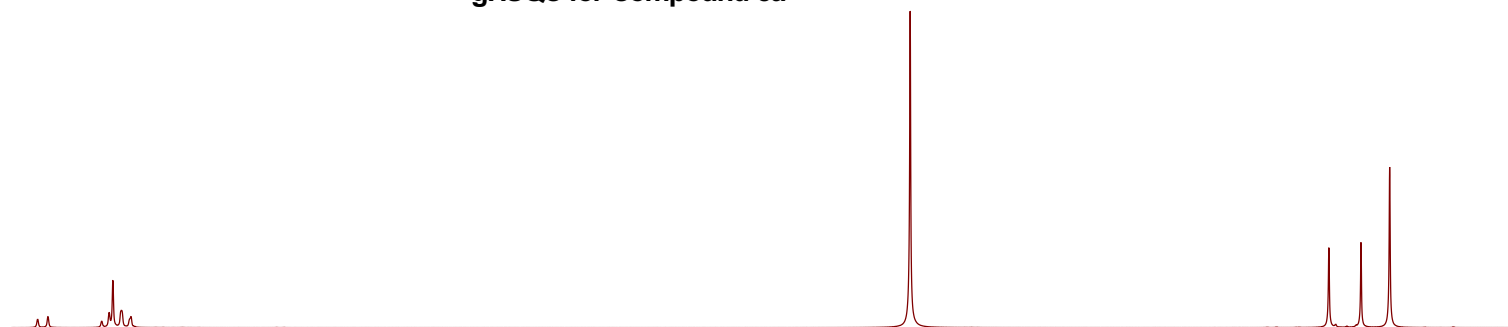
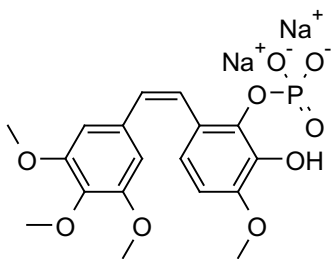
gHSQC for Compound 7a



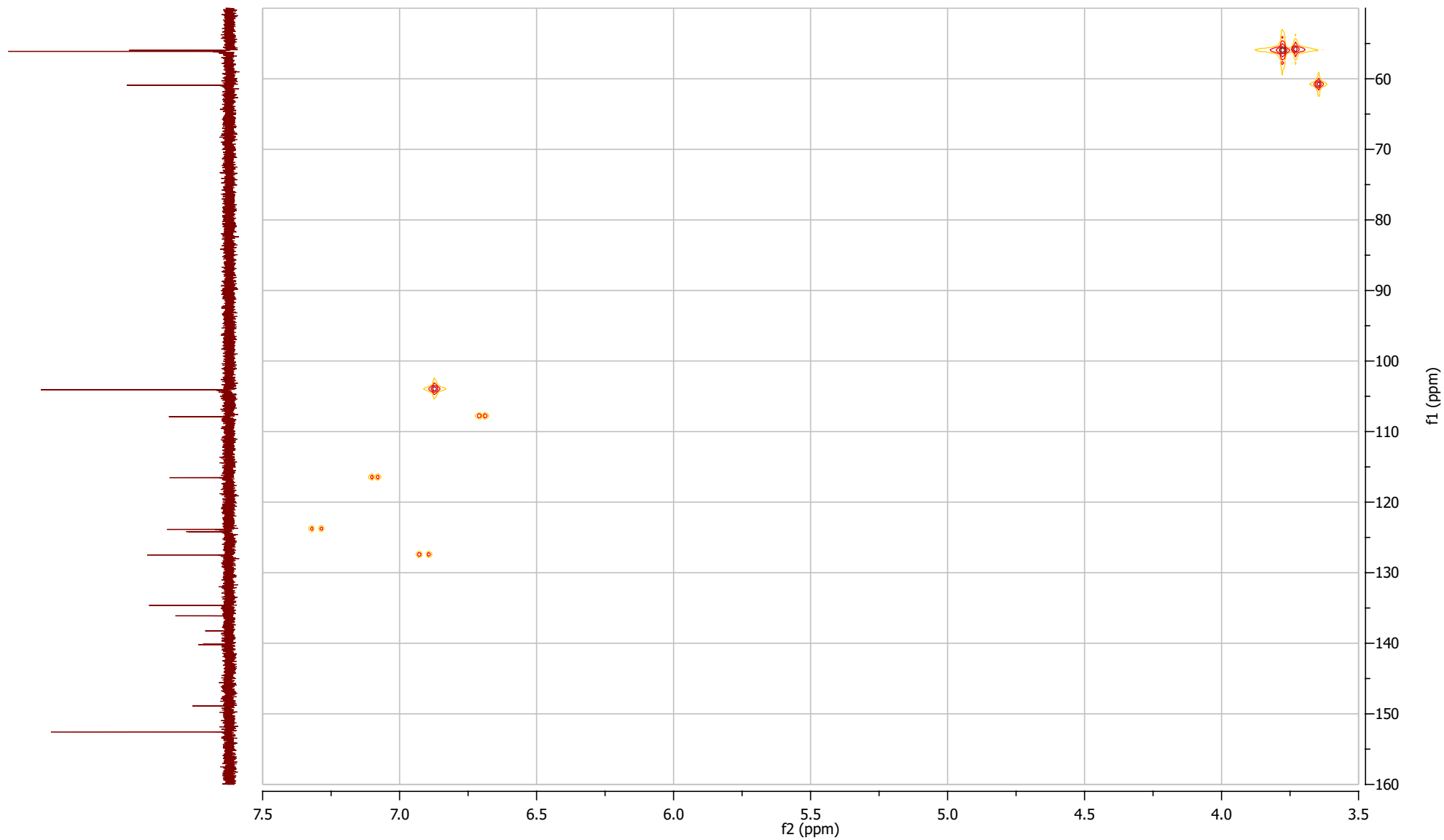
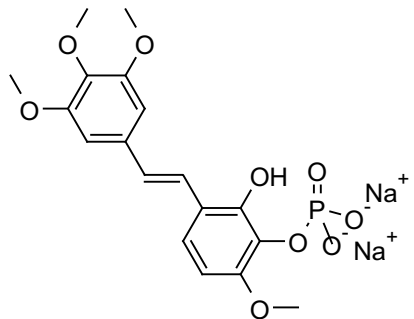
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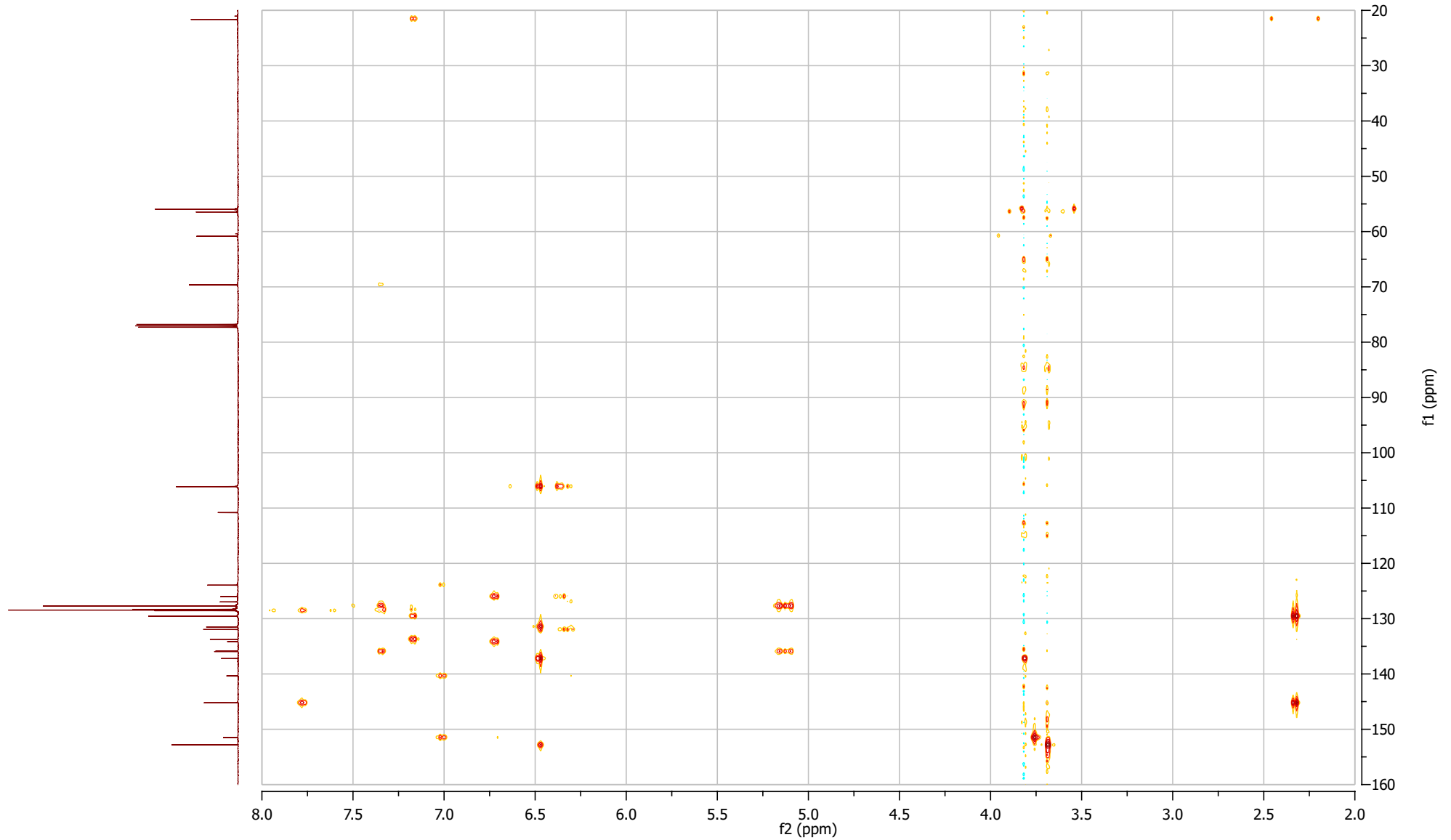
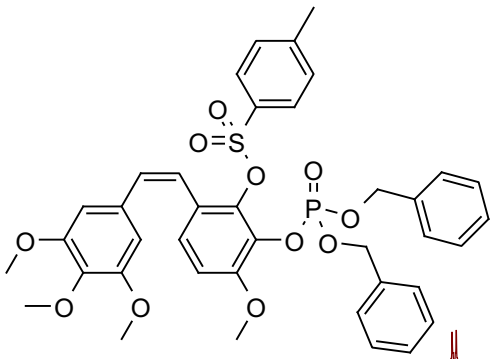
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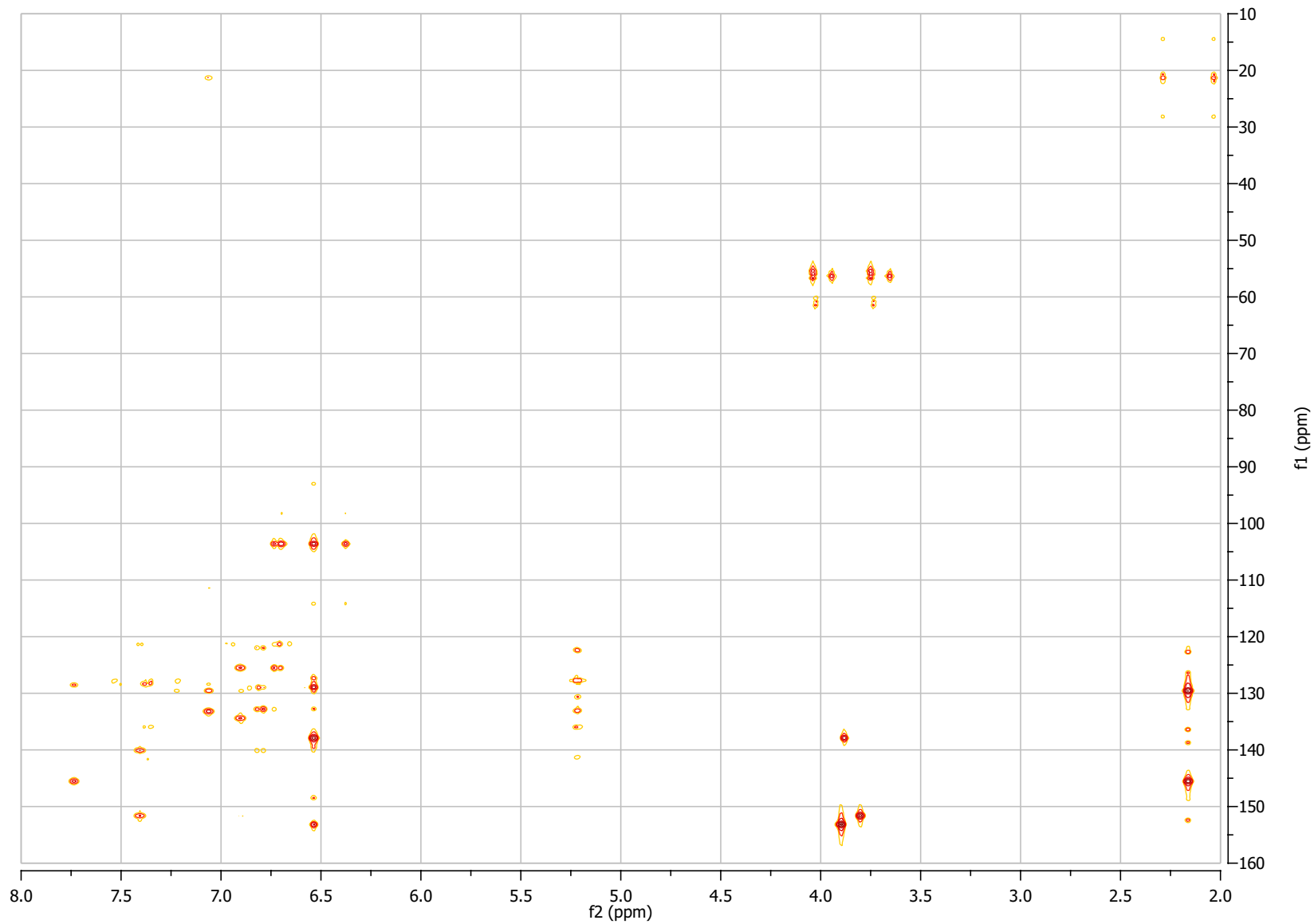
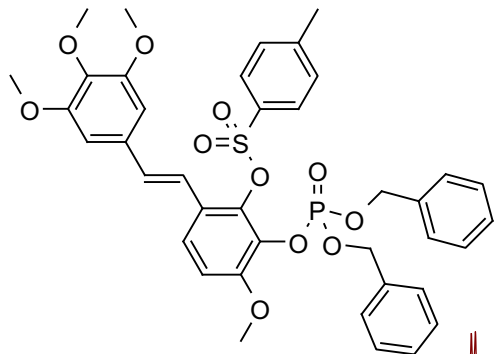
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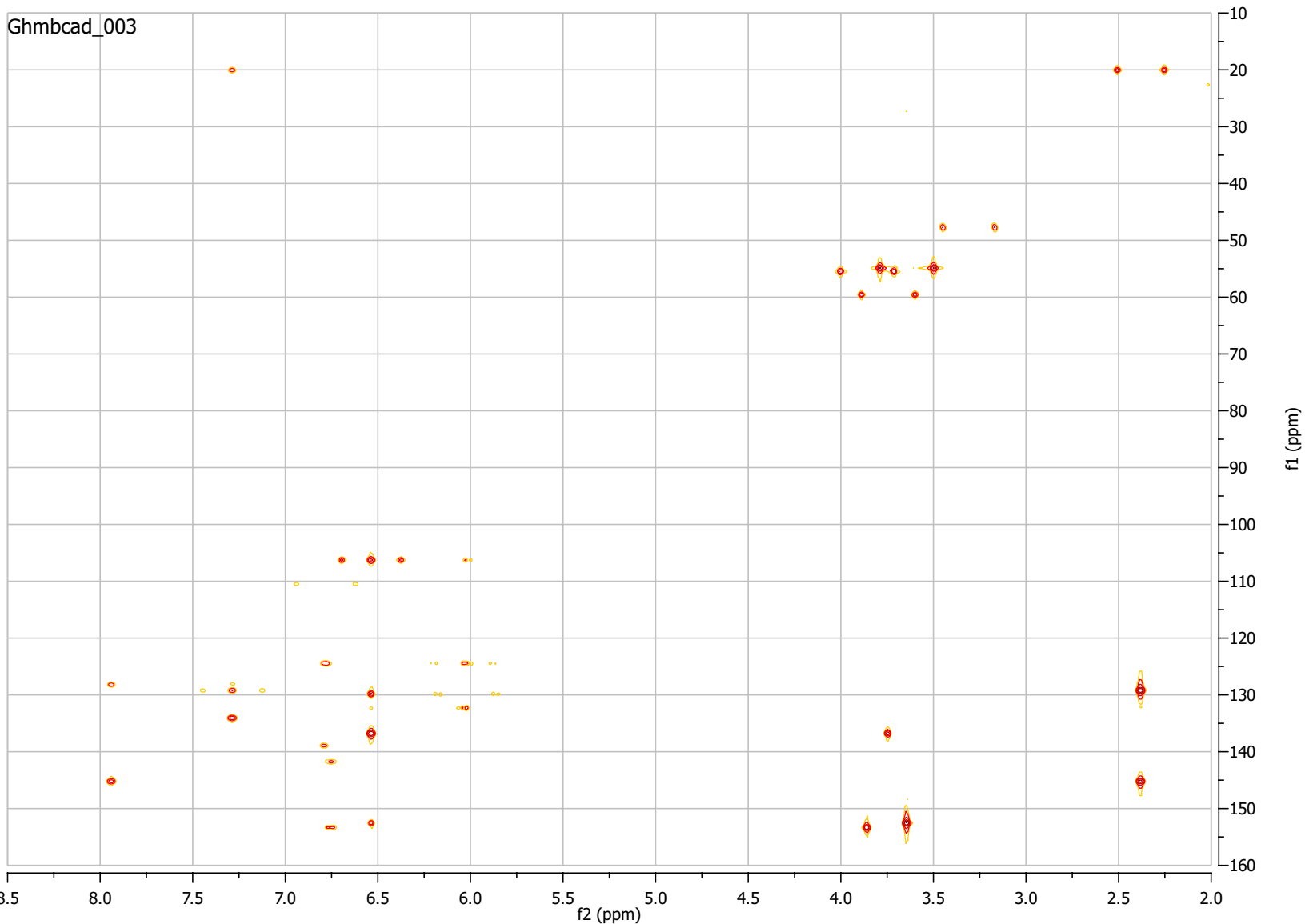
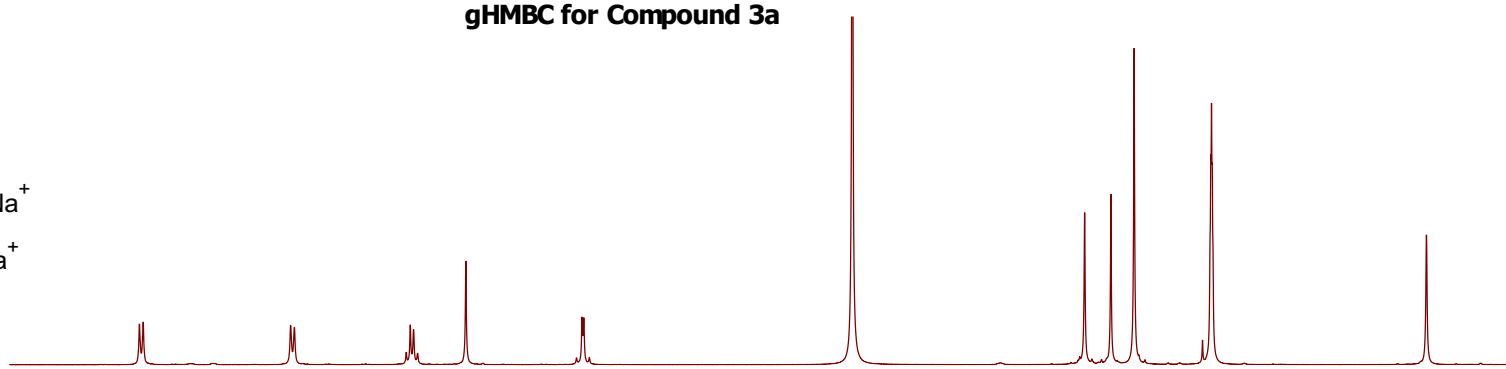
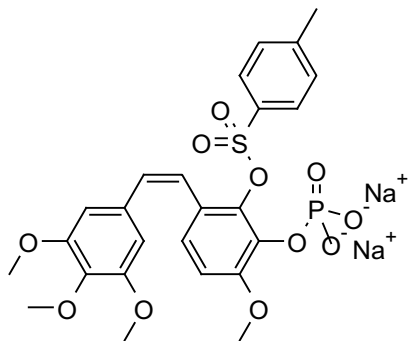
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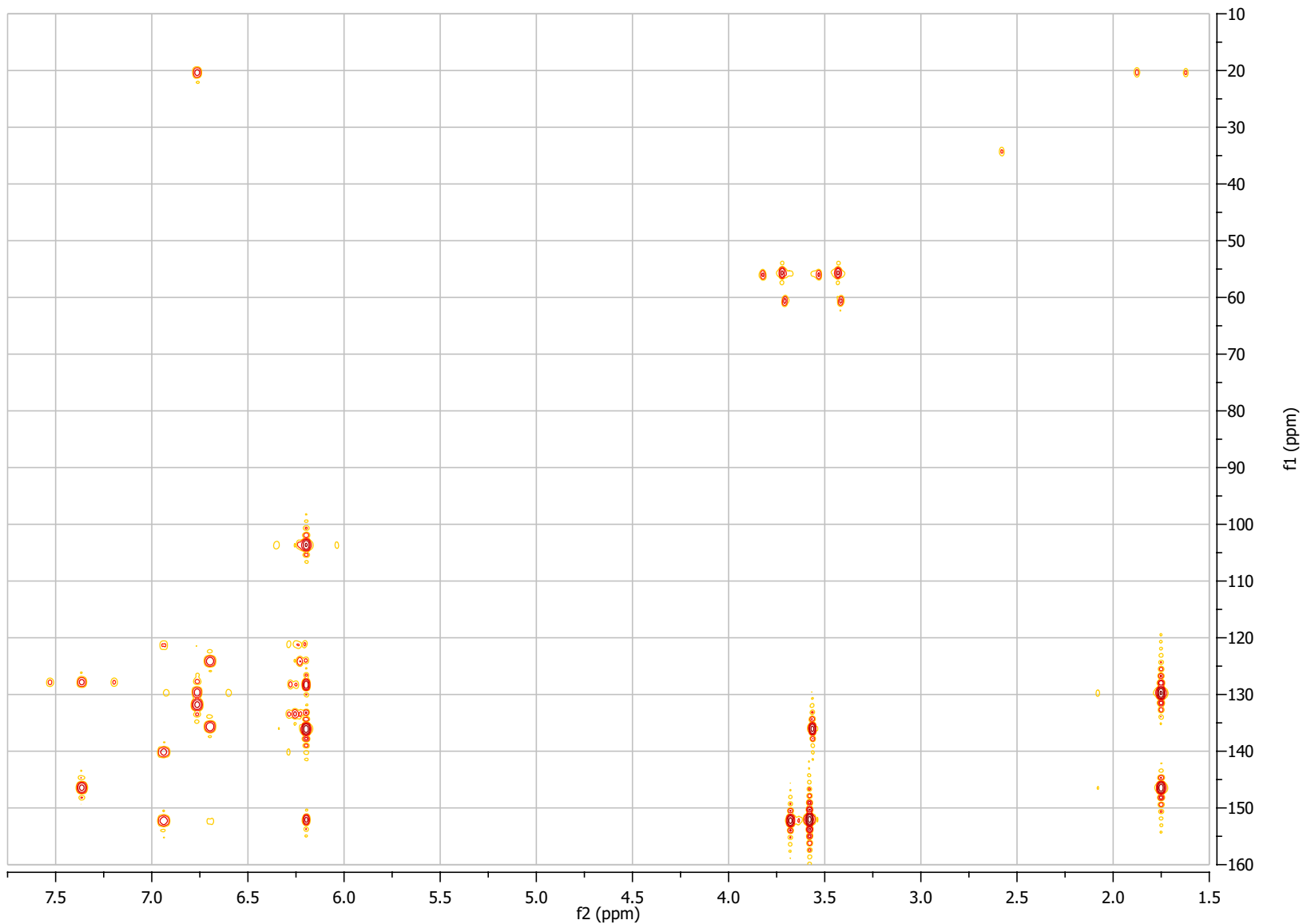
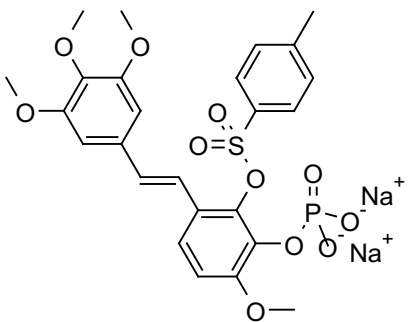
gHMBC for Compound 2b



gHMBC for Compound 3a

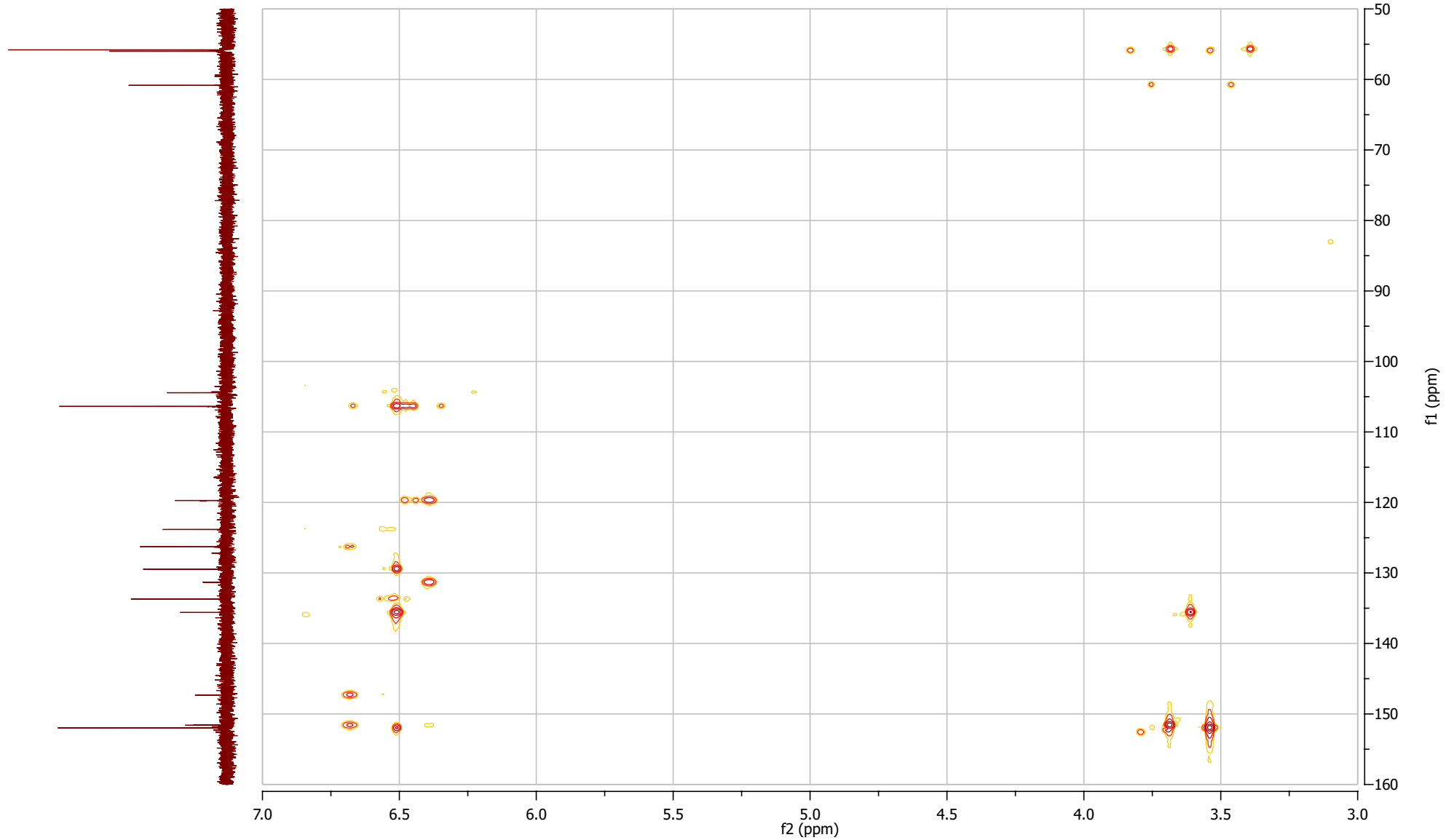
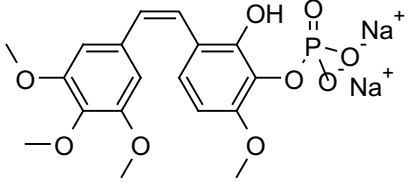


gHMBC for Compound 3b

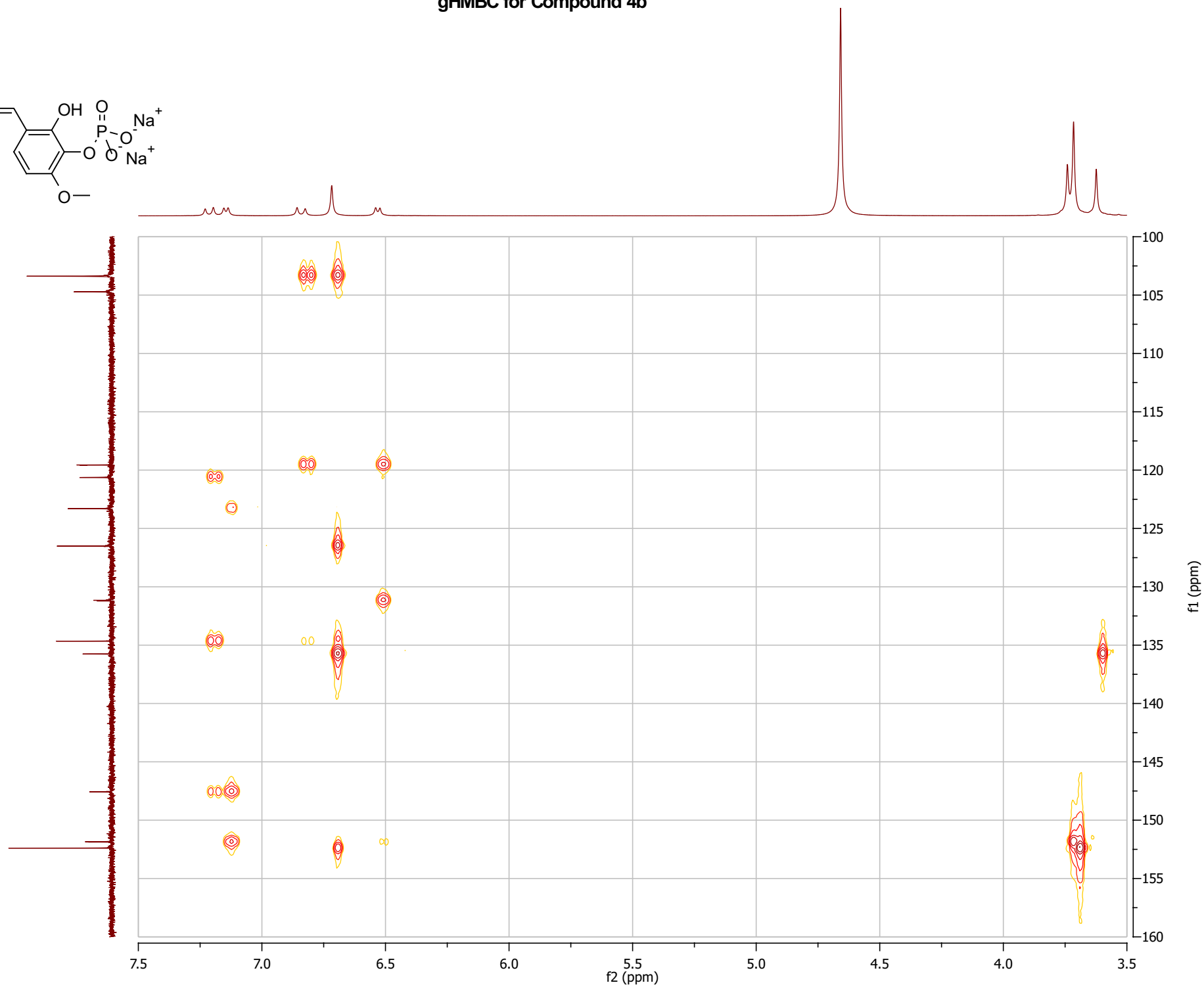
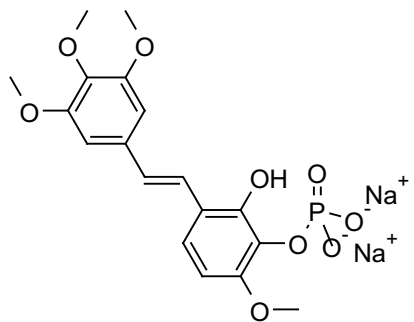




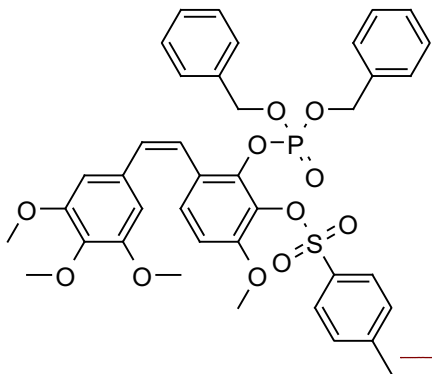
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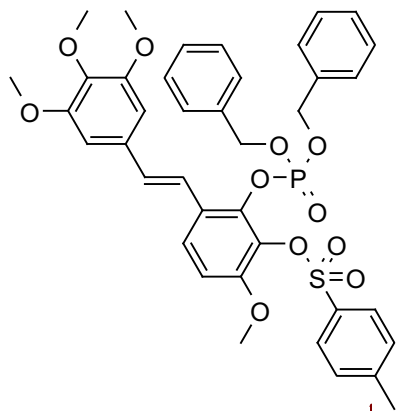
gHMBC for Compound 4b



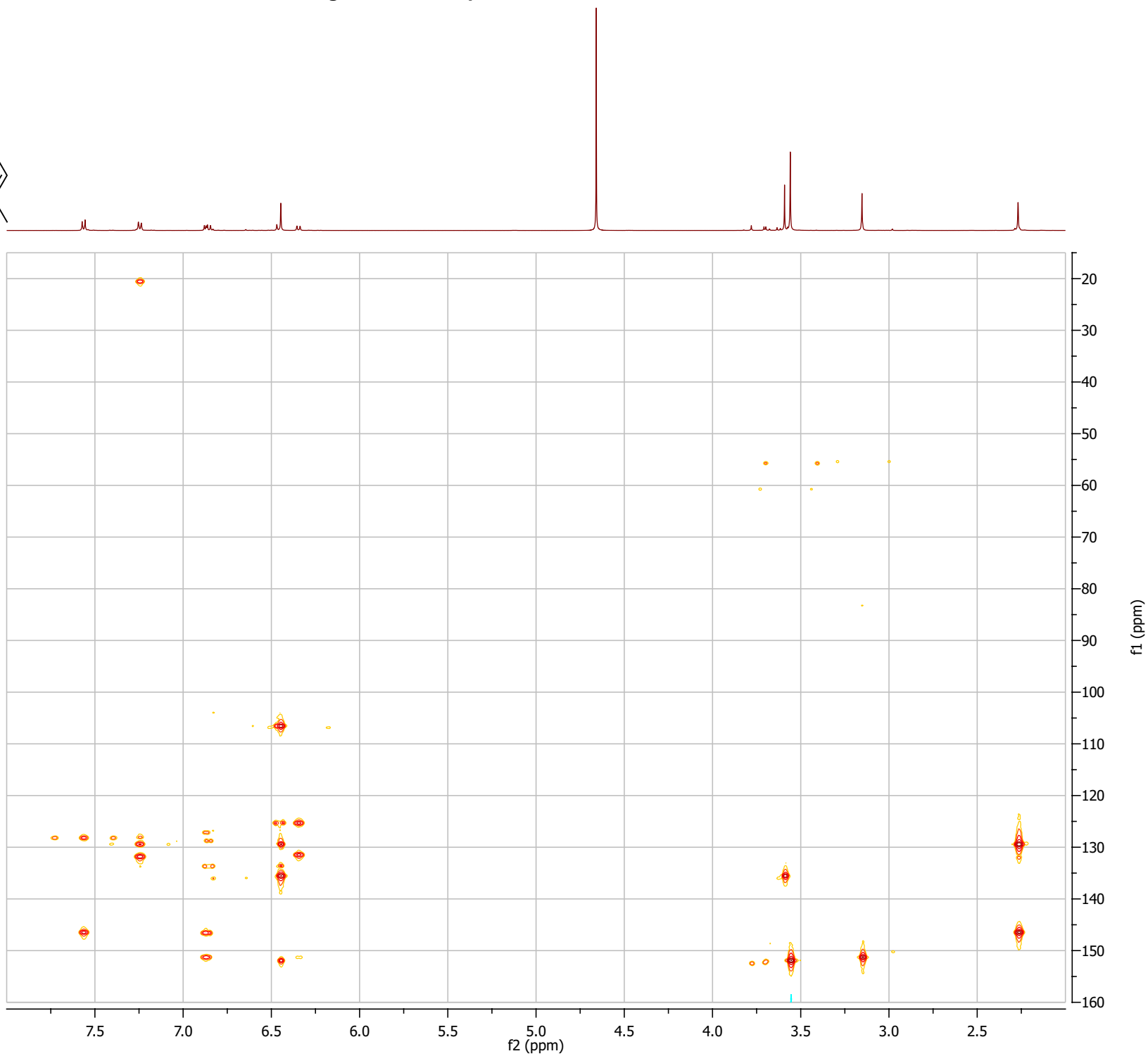
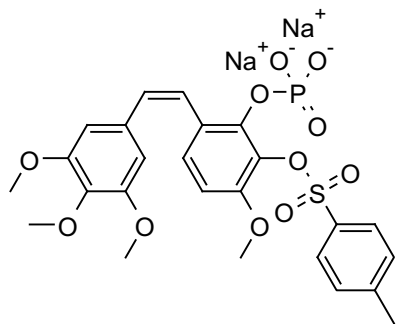
gHMBC for Compound 6a



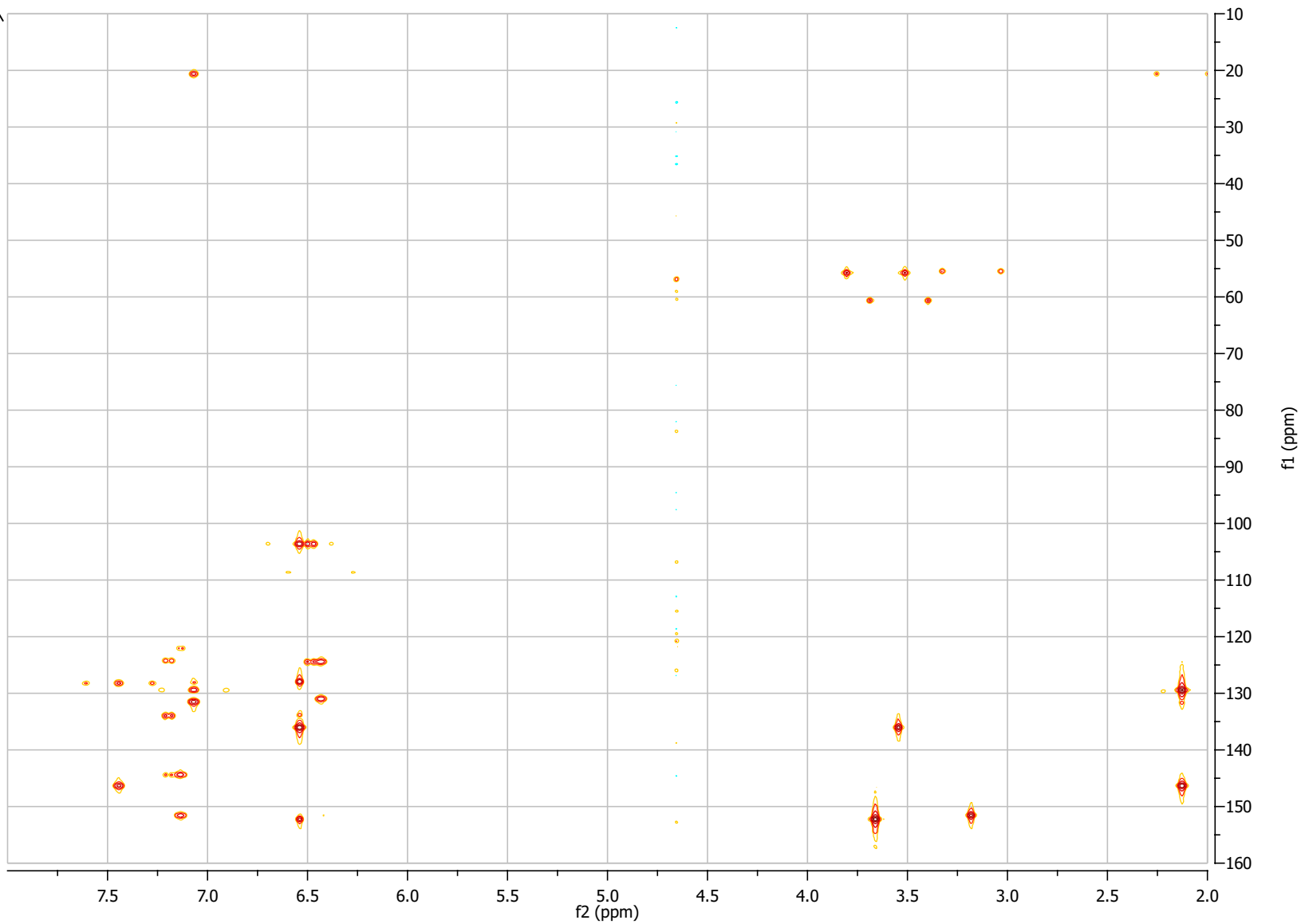
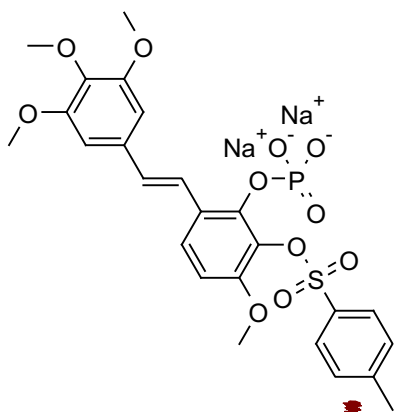
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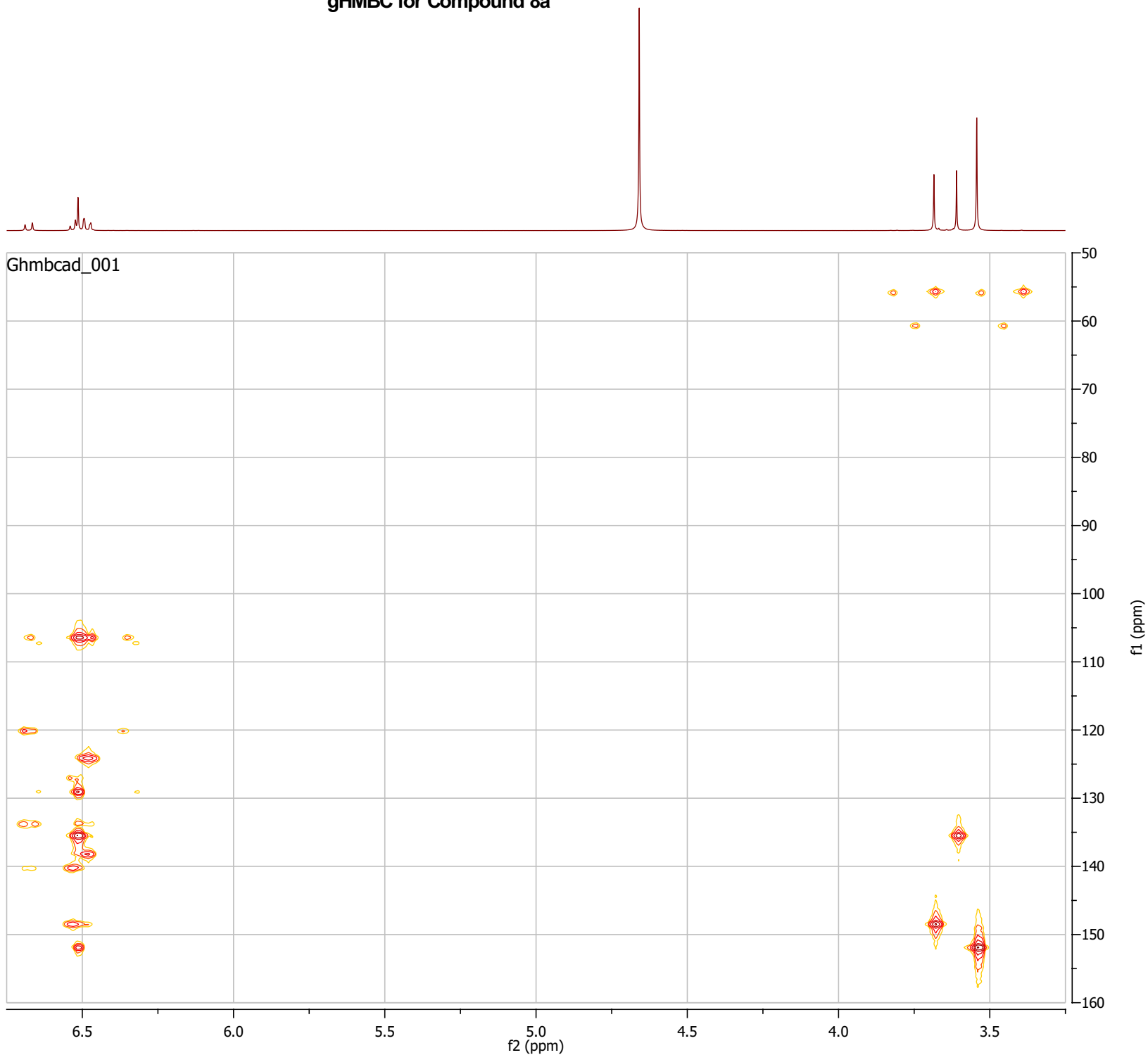
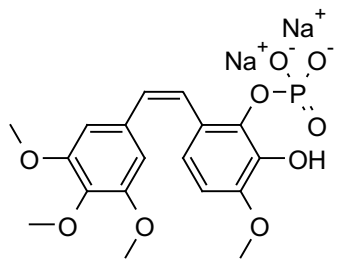
gHMBC for Compound 7a



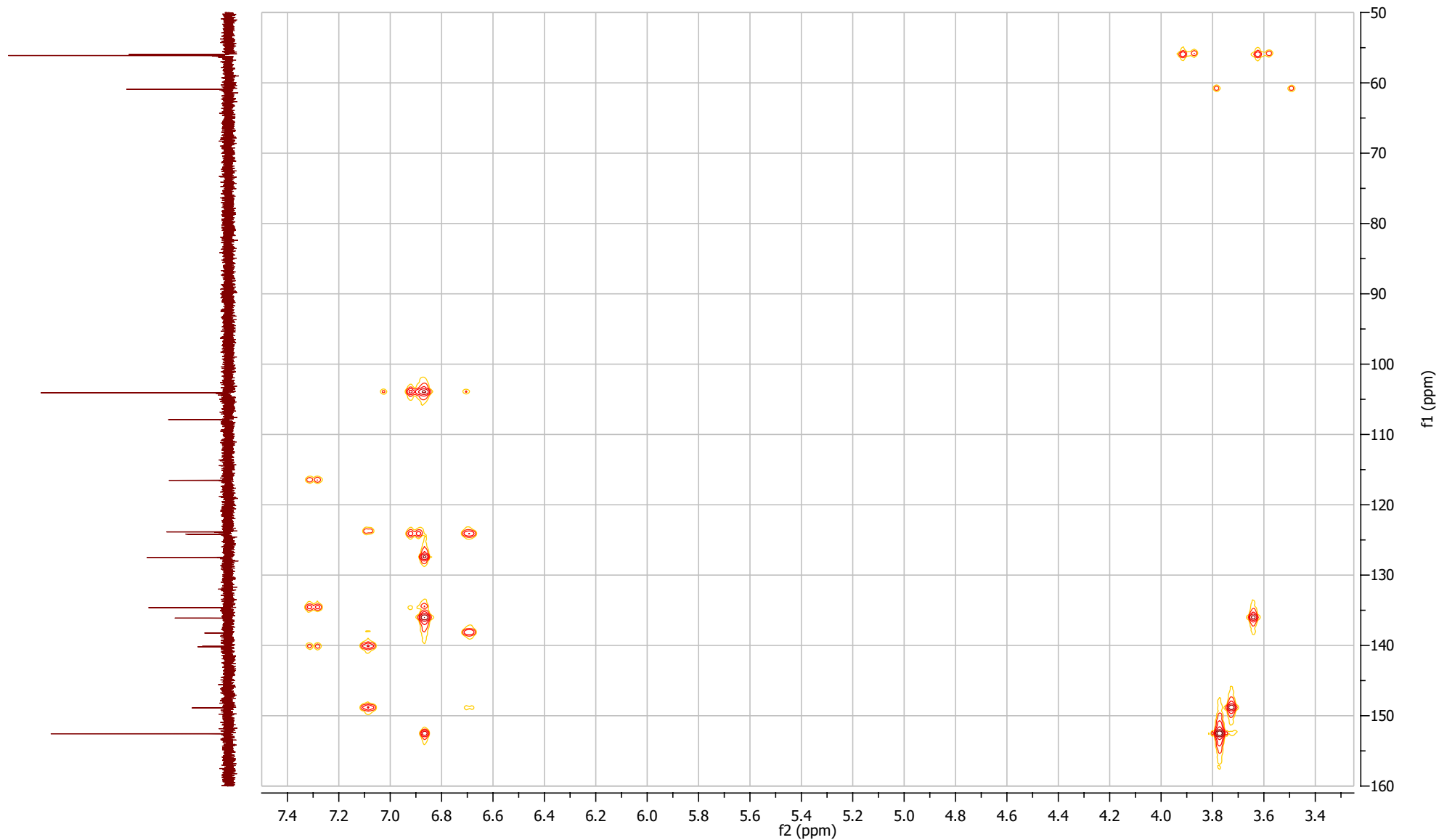
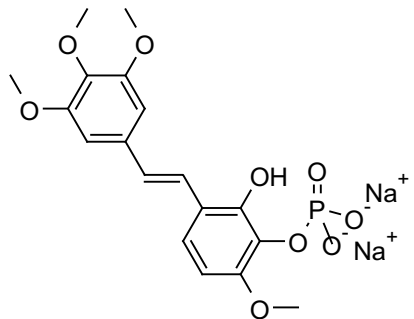
gHSQC for Compound 7b



gHMBC for Compound 8a



gHMBC for Compound 8b





**Table S1.** NMR Spectroscopic Data (500 MHz, D<sub>2</sub>O) for (*Z*) Combretastatin A1 monophosphates **4a** and **8a**.

Position	<i>(Z)</i> -CA1-monophosphate A ( <b>4a</b> )		<i>(Z)</i> -CA1-monophosphate B ( <b>8a</b> )	
	$\delta_C$ , type	$\delta_H$ , ( <i>J</i> in Hz)	$\delta_C$ , type	$\delta_H$ , ( <i>J</i> in Hz)
1	133.7, C		133.9, C	
2	106.4, CH	6.65, s	106.5, CH	6.66, s
3	152.0, C		151.9, C	
4	135.6, C		135.5, C	
5	152.0, C		151.9, C	
6	106.4, CH	6.65, s	106.5, CH	6.66, s
1a	129.5, CH	6.60, d (12.0)	129.1, CH	6.62, d (12.0)
1'a	126.3, CH	6.69, d (12.0)	127.2, CH	6.82, d (12.0)
1'	119.7, C		124.2, C	
2'	147.3, C		140.4, C	
3'	131.3, C		138.5, C	
4'	151.6, C		148.6, C	
5'	104.4, CH	6.53, d (8.6)	107.3, CH	6.64, d (8.7)
6'	123.8, CH	6.82, d (8.6)	120.2, CH	6.67, d (8.7)
3-OCH <sub>3</sub>	55.8, CH <sub>3</sub>	3.69, s	55.8, CH <sub>3</sub>	3.69, s
4-OCH <sub>3</sub>	60.8, CH <sub>3</sub>	3.75, s	60.9, CH <sub>3</sub>	3.76, s
5-OCH <sub>3</sub>	55.8, CH <sub>3</sub>	3.69, s	55.8, CH <sub>3</sub>	3.69, s
4'-OCH <sub>3</sub>	56.0, CH <sub>3</sub>	3.83, s	56.0, CH <sub>3</sub>	3.83, s

**Table S2.** NMR Spectroscopic Data (500 MHz, D<sub>2</sub>O) for (*E*) Combretastatin A1 monophosphates **4b** and **8b**.

Position	<i>(E)</i> -CA1-monophosphate A ( <b>4b</b> )		<i>(E)</i> -CA1-monophosphate B ( <b>8b</b> )	
	$\delta_C$ , type	$\delta_H$ , ( <i>J</i> in Hz)	$\delta_C$ , type	$\delta_H$ , ( <i>J</i> in Hz)
1	134.7, C		134.6, C	
2	103.4, CH	6.86, s	104.1, CH	7.03, s
3	152.4, C		152.6, C	
4	135.8, C		136.1, C	
5	152.4, C		152.6, C	
6	103.4, CH	6.86, s	104.1, CH	7.03, s
1a	126.5, CH	6.98, d (16.4)	127.5, CH	7.07, d (16.6)
1'a	123.3, CH	7.36, d (16.4)	123.9, CH	7.46, d (16.6)
1'	119.6, C		124.2, C	
2'	147.6, C		140.2, C	
3'	131.3, C		138.3, C	
4'	151.9, C		148.9, C	
5'	104.7, CH	6.67, d (8.7)	107.9, CH	6.86, d (8.8)
6'	120.6, CH	7.29, d (8.7)	116.5, CH	7.25, d (8.8)
3-OCH <sub>3</sub>	55.8, CH <sub>3</sub>	3.86, s	56.1, CH <sub>3</sub>	3.94, s
4-OCH <sub>3</sub>	60.8, CH <sub>3</sub>	3.77, s	60.9, CH <sub>3</sub>	3.81, s
5-OCH <sub>3</sub>	55.8, CH <sub>3</sub>	3.86, s	56.1, CH <sub>3</sub>	3.94, s
4'-OCH <sub>3</sub>	55.9, CH <sub>3</sub>	3.88, s	56.0, CH <sub>3</sub>	3.89, s