

Water-Soluble Phosphinothiols for Traceless Staudinger Ligation and Integration with Expressed Protein Ligation

Annie Tam, Matthew B. Soellner, and Ronald T. Raines*

*Contribution from the Departments of Chemistry and Biochemistry,
University of Wisconsin–Madison, Madison, WI 53706*

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S38	¹³ C NMR Spectrum of Compound 15		

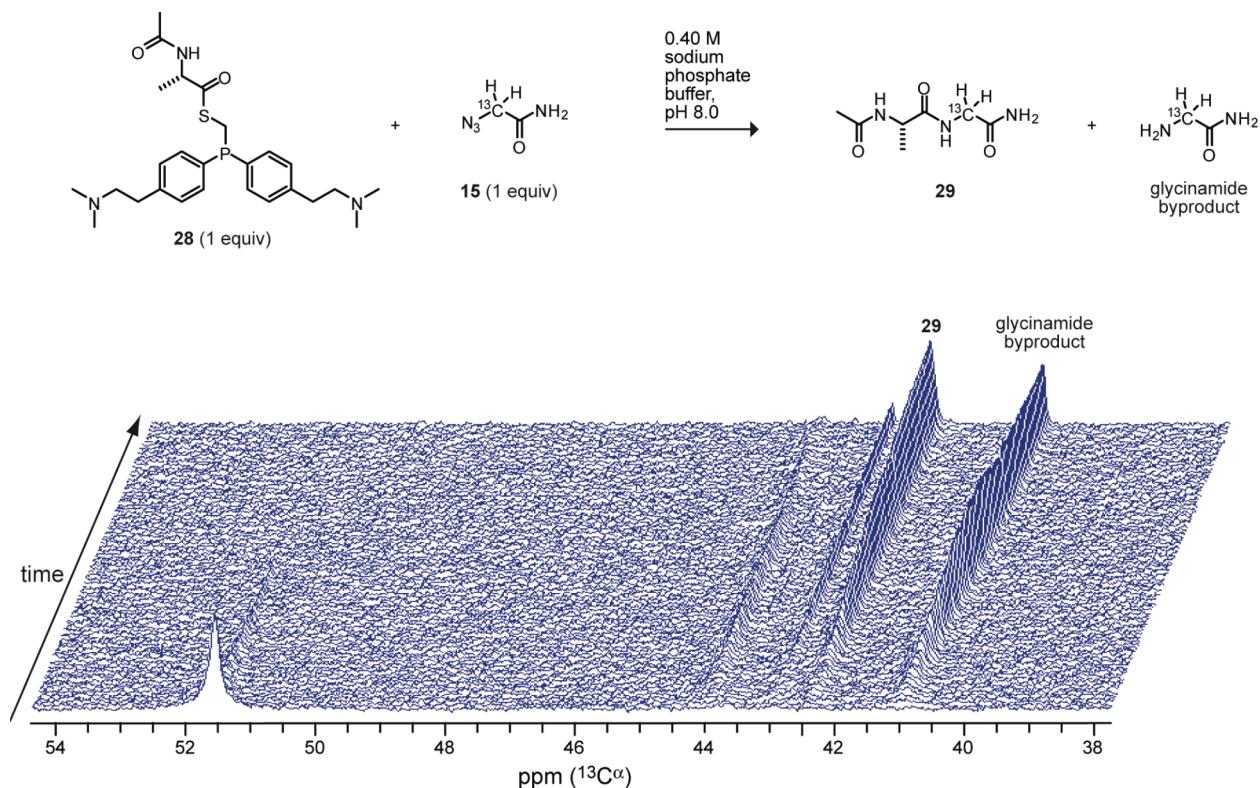


Figure S1. ^{13}C NMR assay of a traceless Staudinger ligation mediated by phosphinothiol **17**. The reaction between phosphinothioester **28** and ^{13}C -labeled azide **15** was performed at room temperature with reactant concentrations of 0.16 M in 0.40 M sodium phosphate buffer at pH 8.0. Ninety ^{13}C NMR spectra were acquired over a 16-h time course. Yields were determined from peak integrations on the final spectrum. The second-order rate constant for the appearance of amide **29** was $k_2 = 7.4 \times 10^{-3} \text{ M}^{-1}\text{s}^{-1}$.

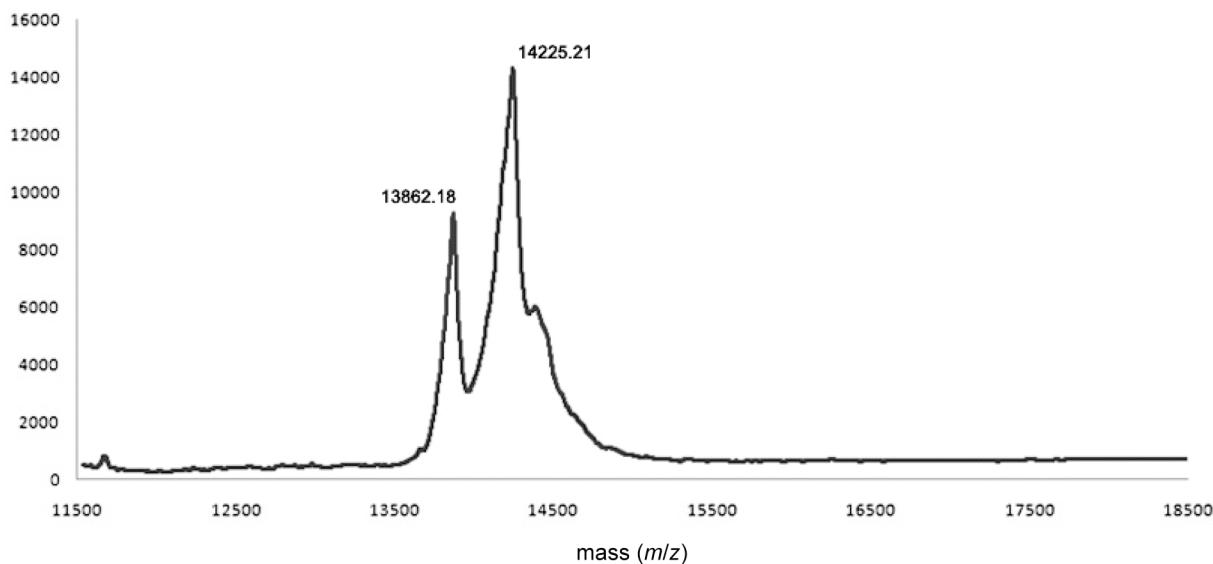
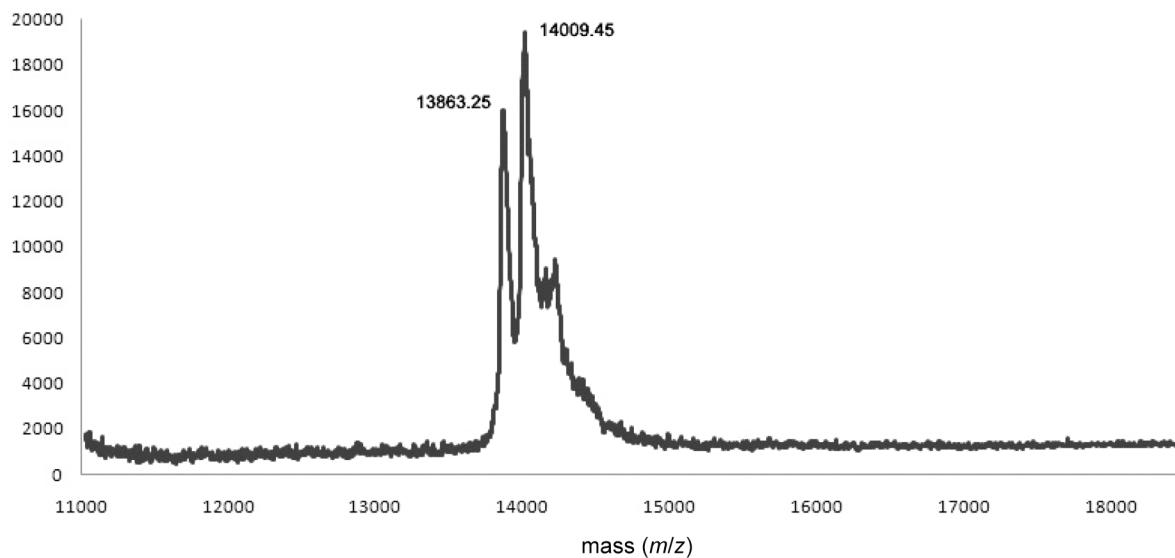
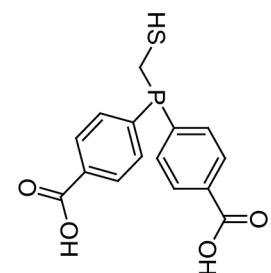
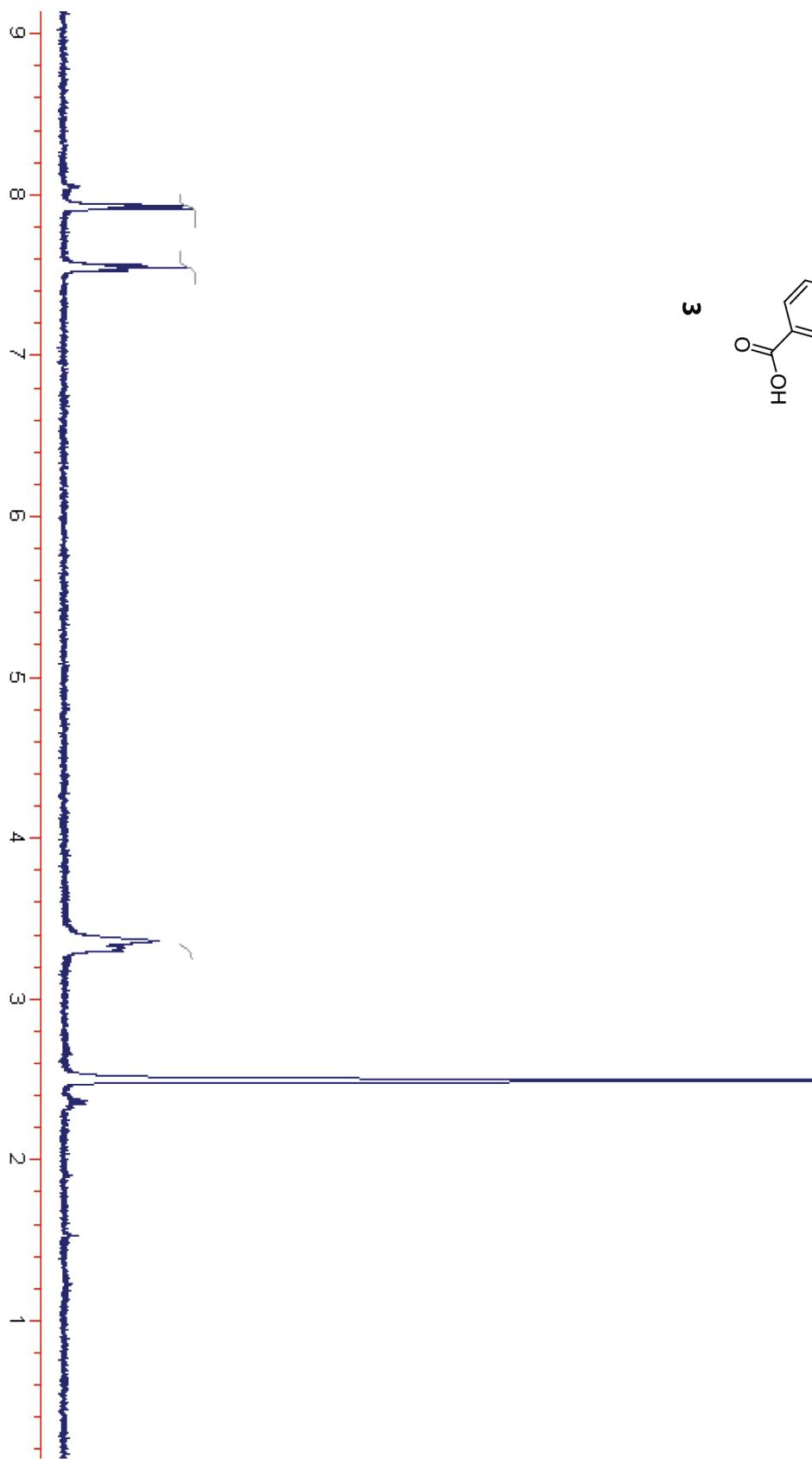
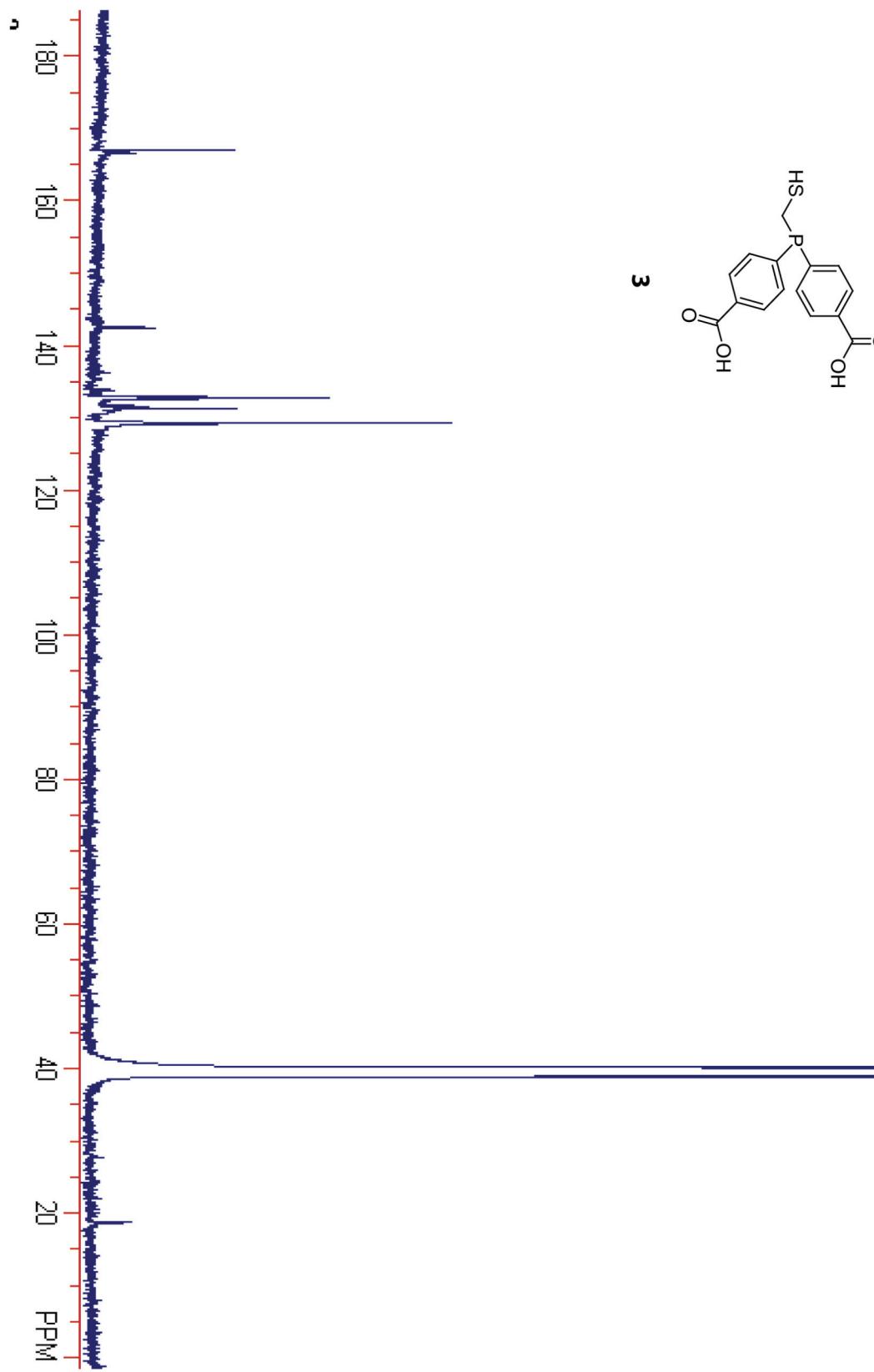
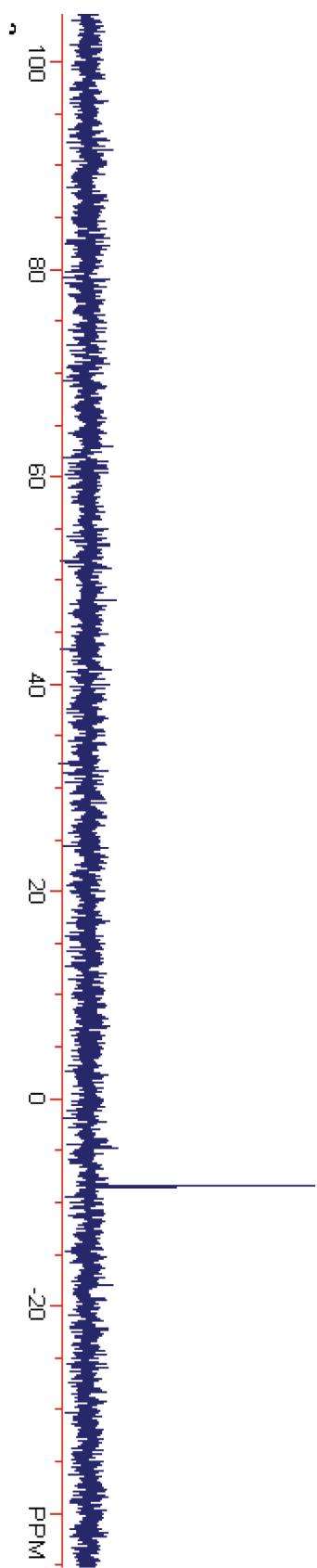
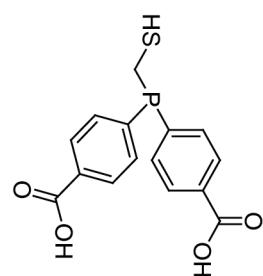
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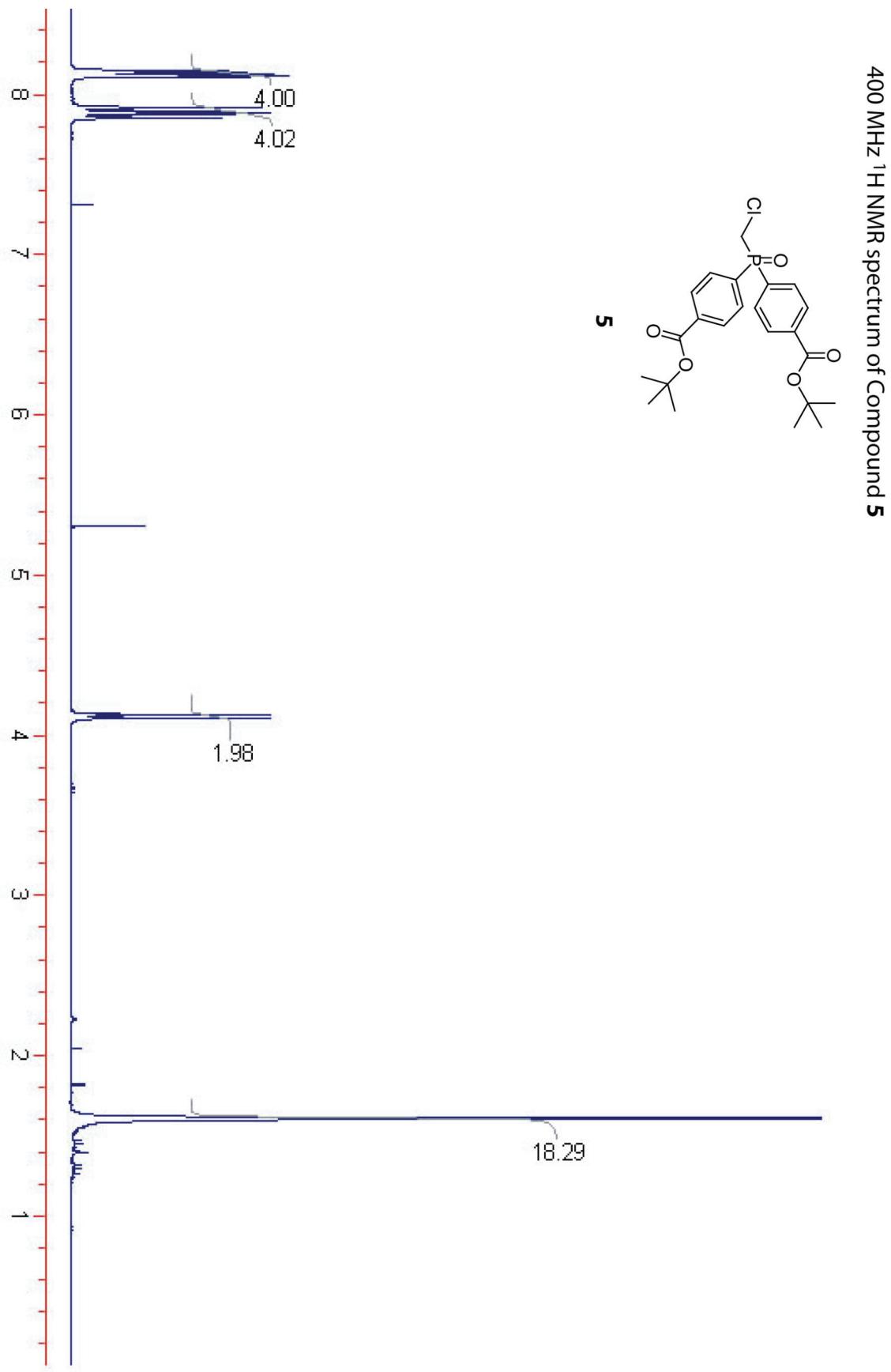
Figure S2. MALDI-TOF mass spectra of protein thioesters. (A) Protein phosphinothioester **32** (*m/z* 14225, expected for Met(-1)RNase A–Gly–SCH₂(P(C₆H₄-*p*-CH₂CH₂NMe₂)₂), C₆₀₃H₉₅₀N₁₇₅O₁₉₄PS₁₄, 14224). (B) Control: protein phosphinothioester with 2-mercaptopethanesulfonic acid (MESNA; *m/z* 14009, expected for Met(-1)RNase A–Gly–SCH₂CH₂SO₃H, C₅₈₄H₉₂₅N₁₇₃O₁₉₇S₁₅, 14002). The yield of protein phosphinothioester **32** was 70% that of the MESNA control, as determined by ultraviolet absorbance of the two samples.

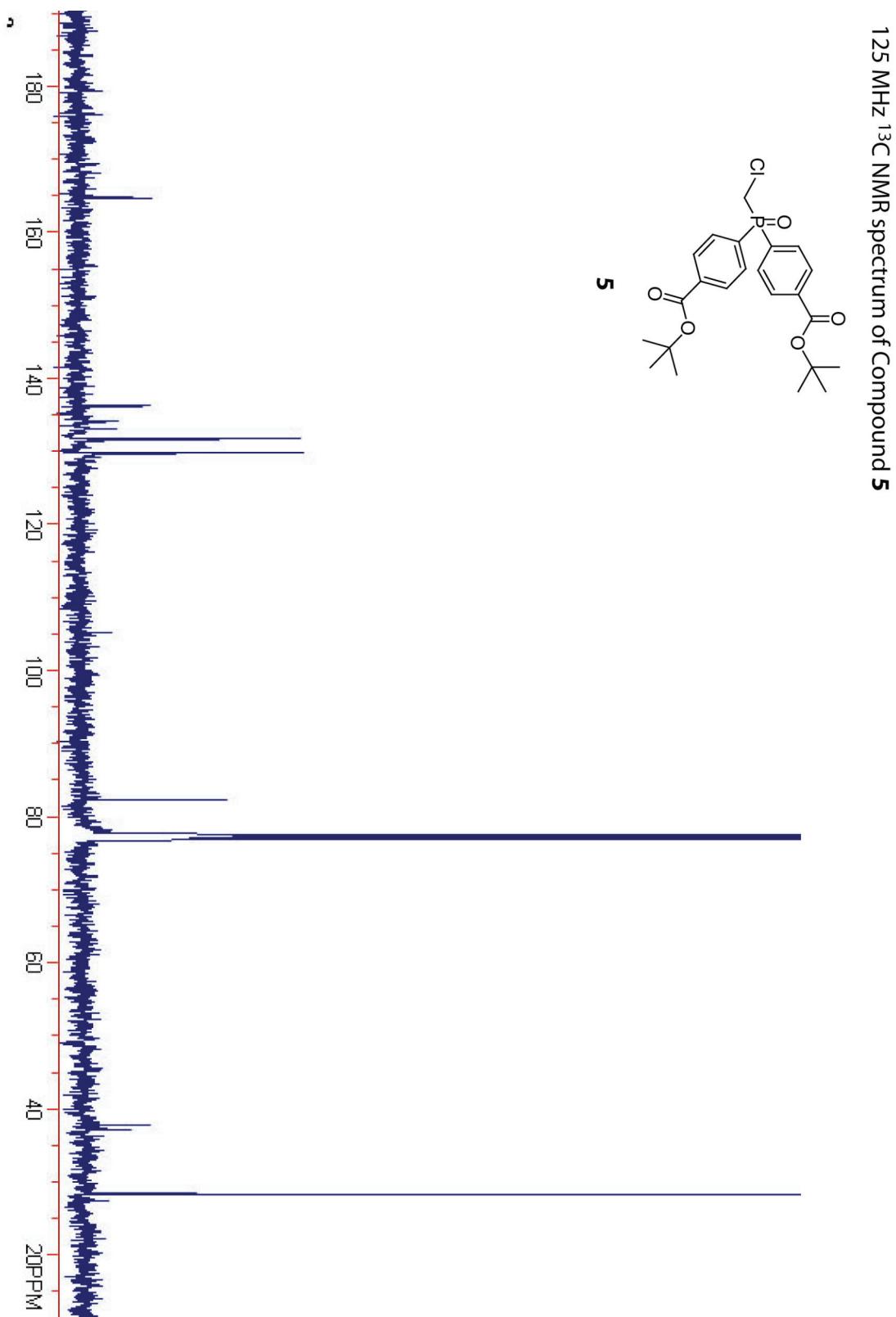
400 MHz ^1H NMR spectrum of Compound 3

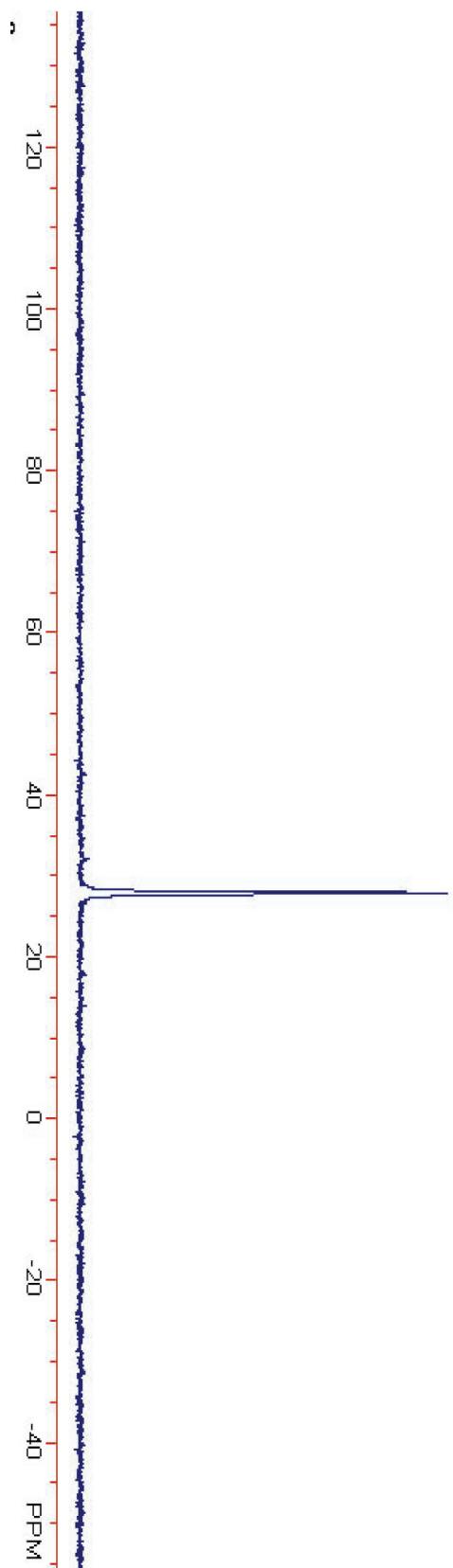
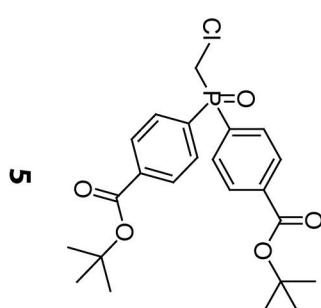


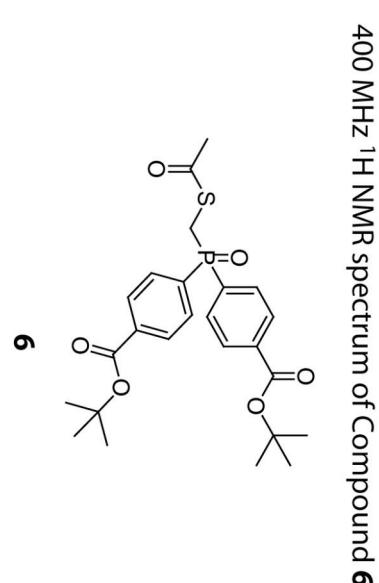
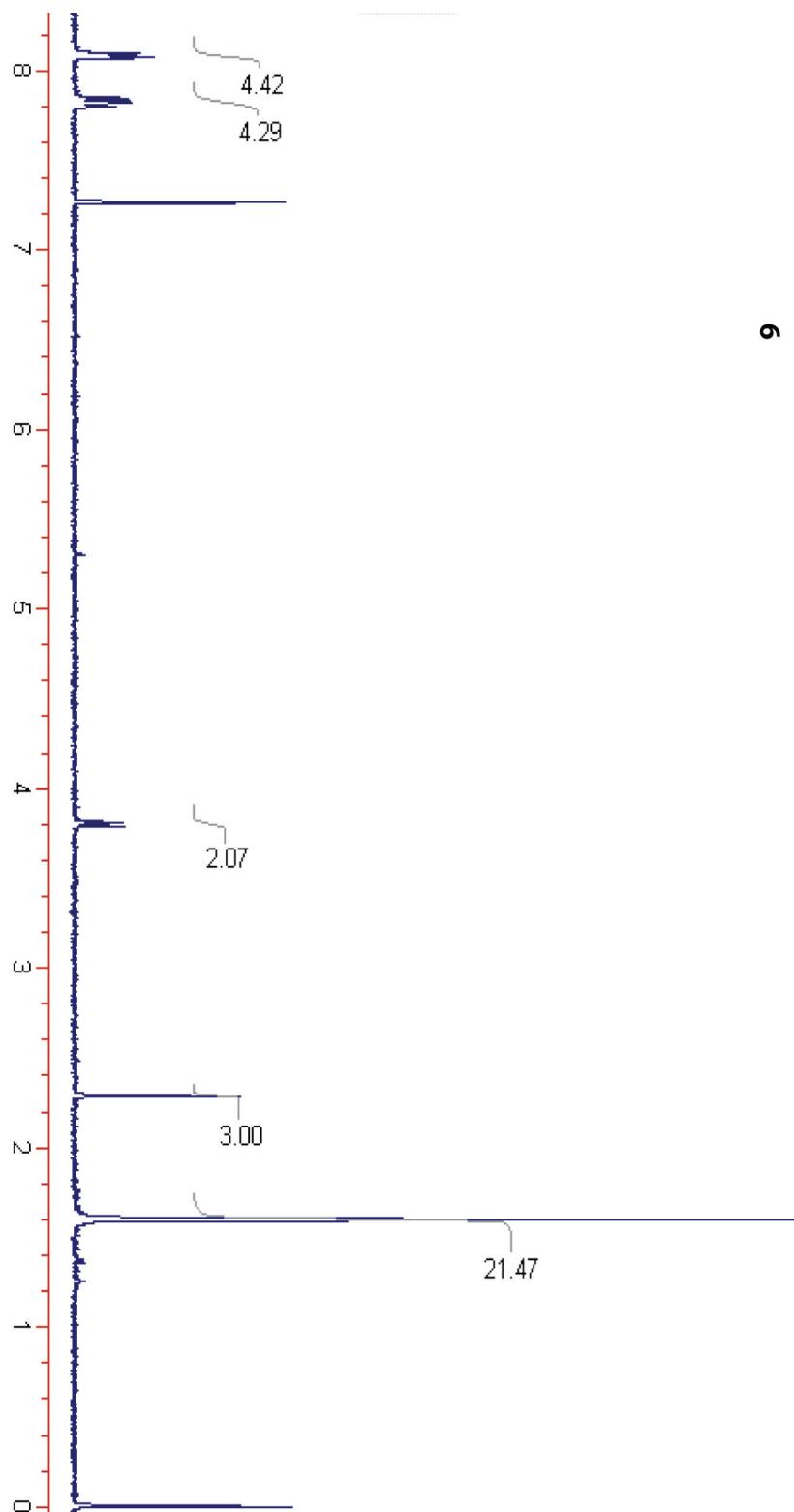
161 MHz ^{31}P NMR spectrum of Compound 3

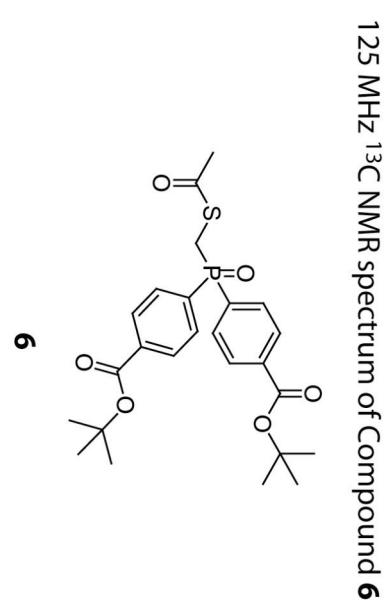
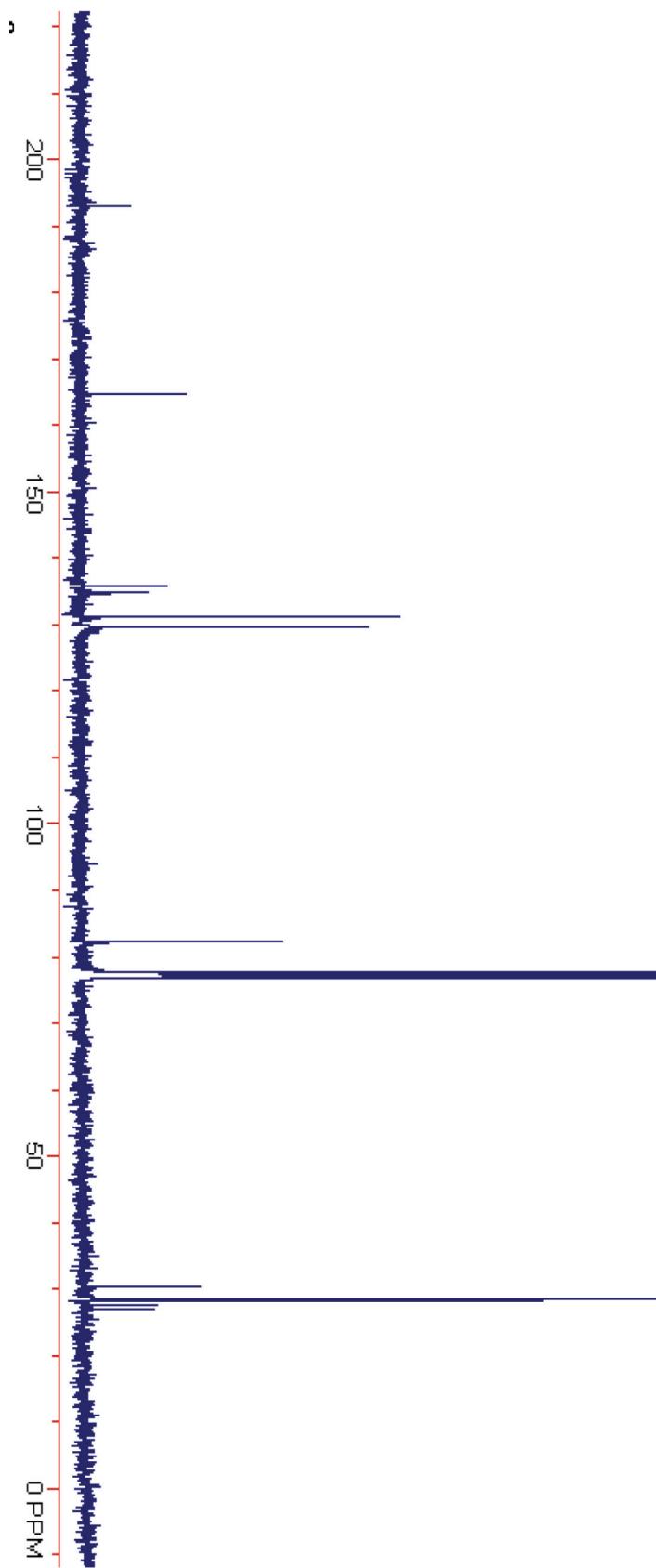


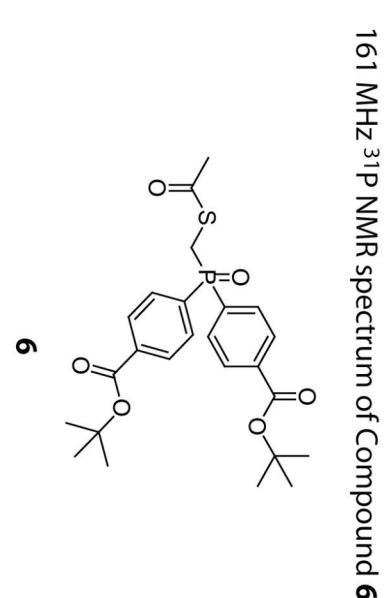
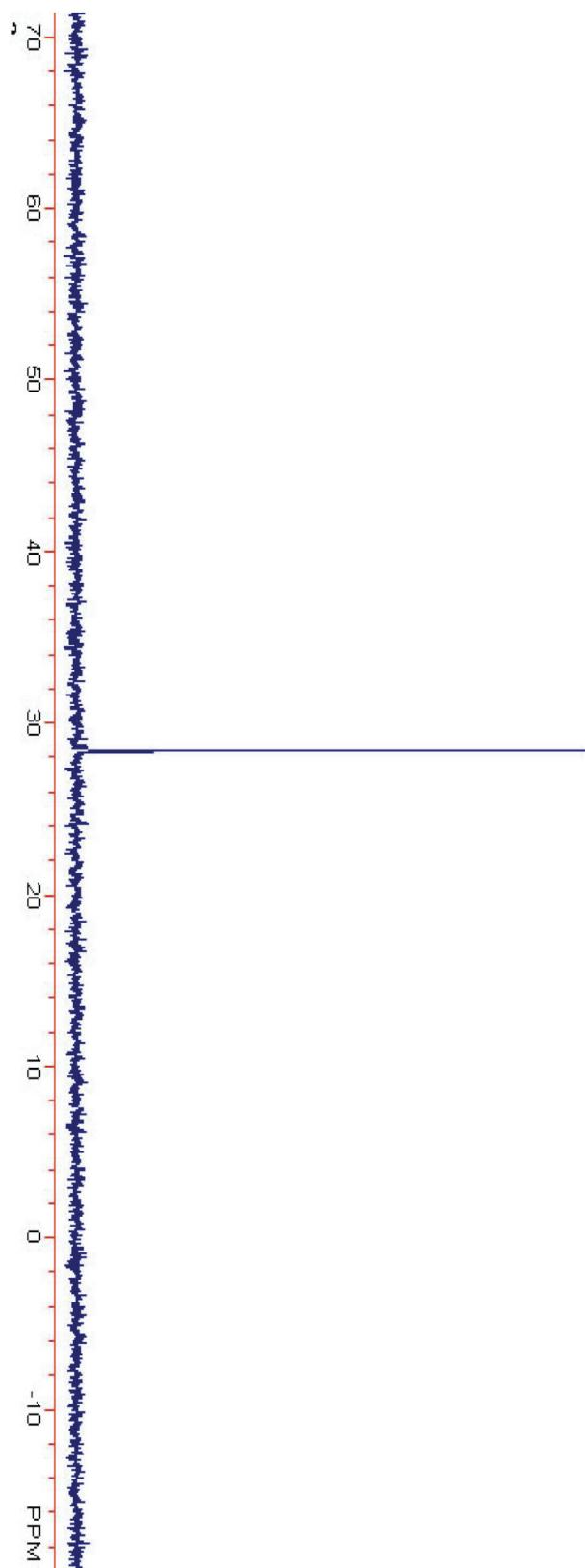


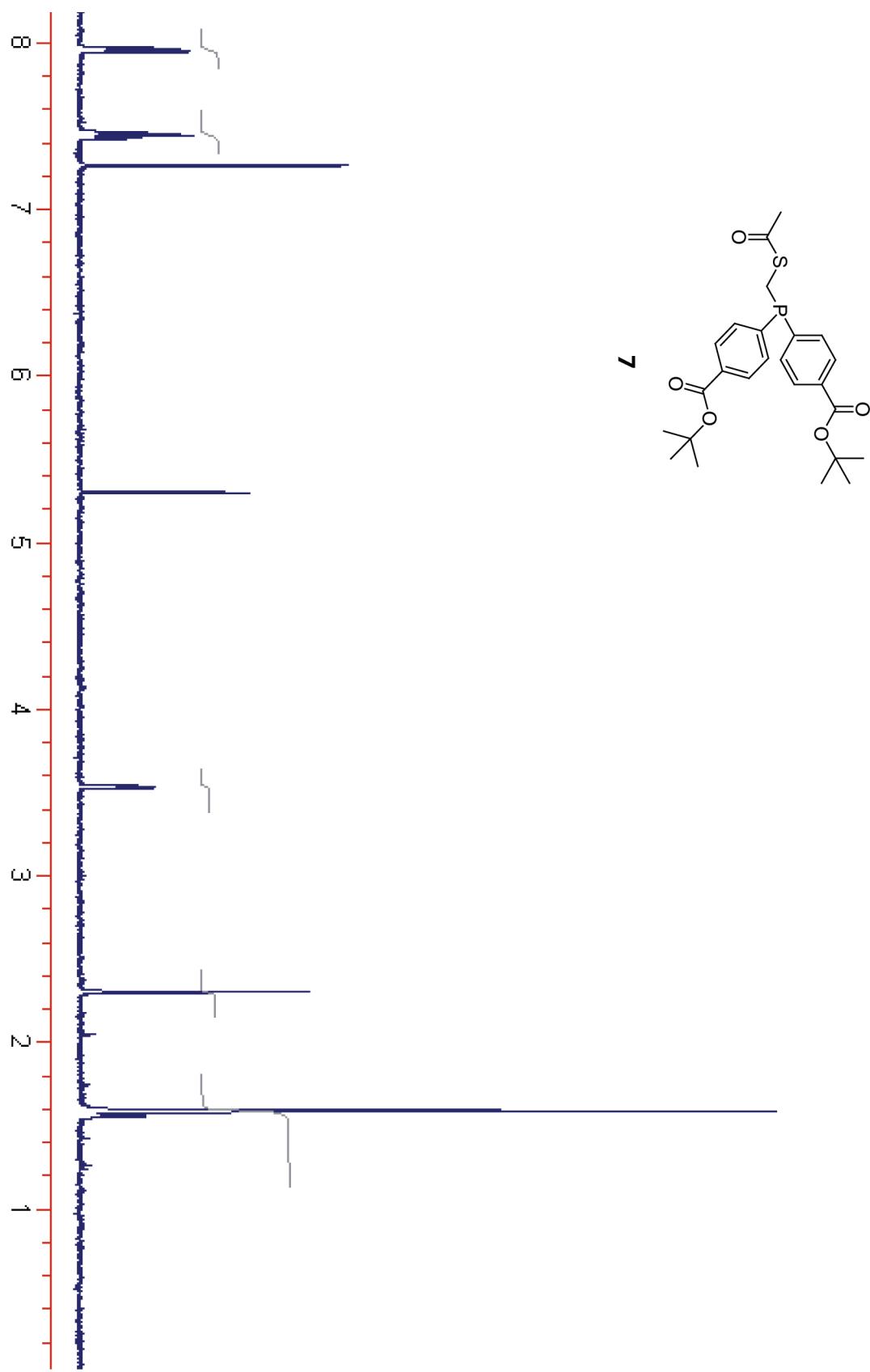


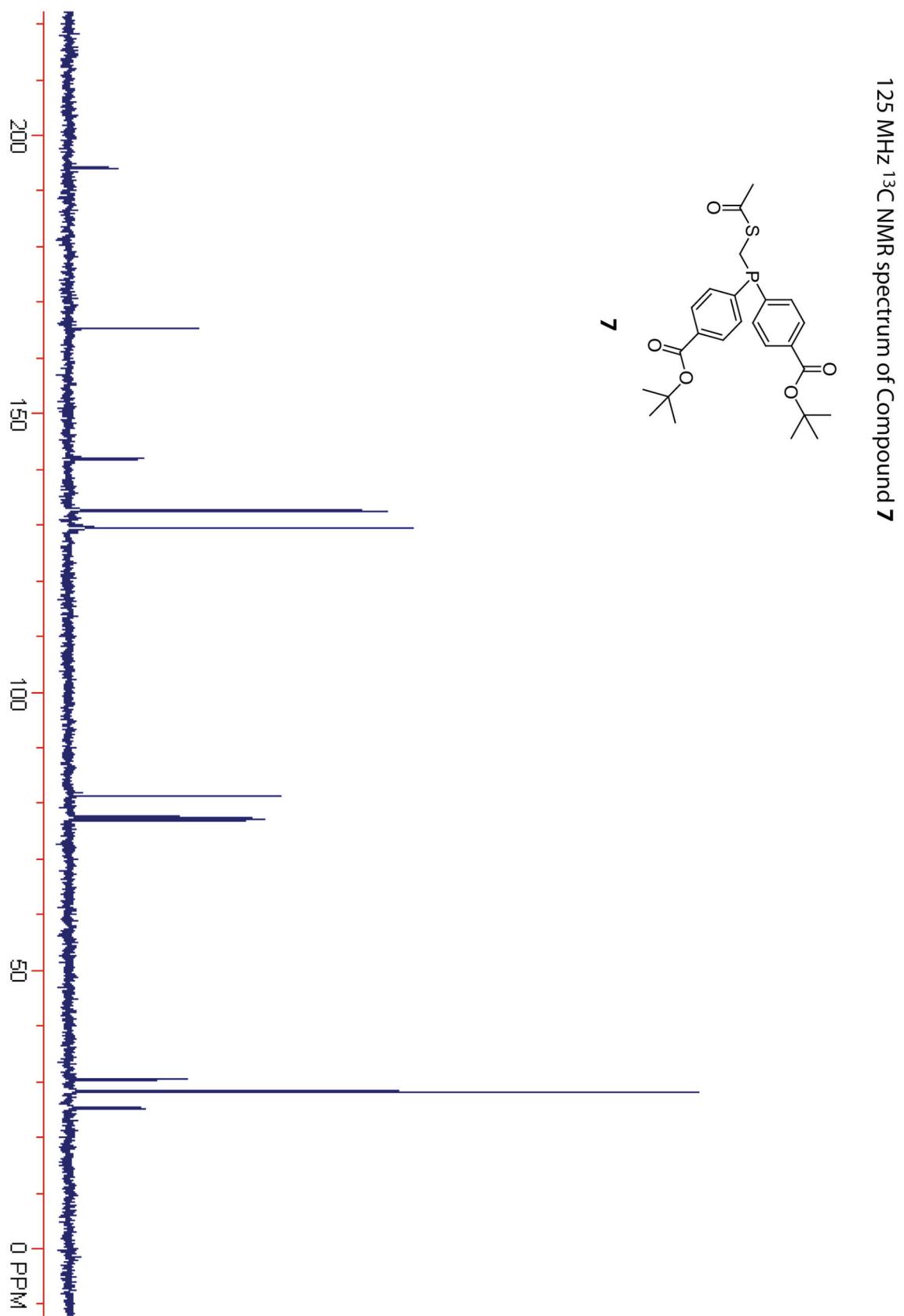
161 MHz ^{31}P NMR spectrum of Compound 5

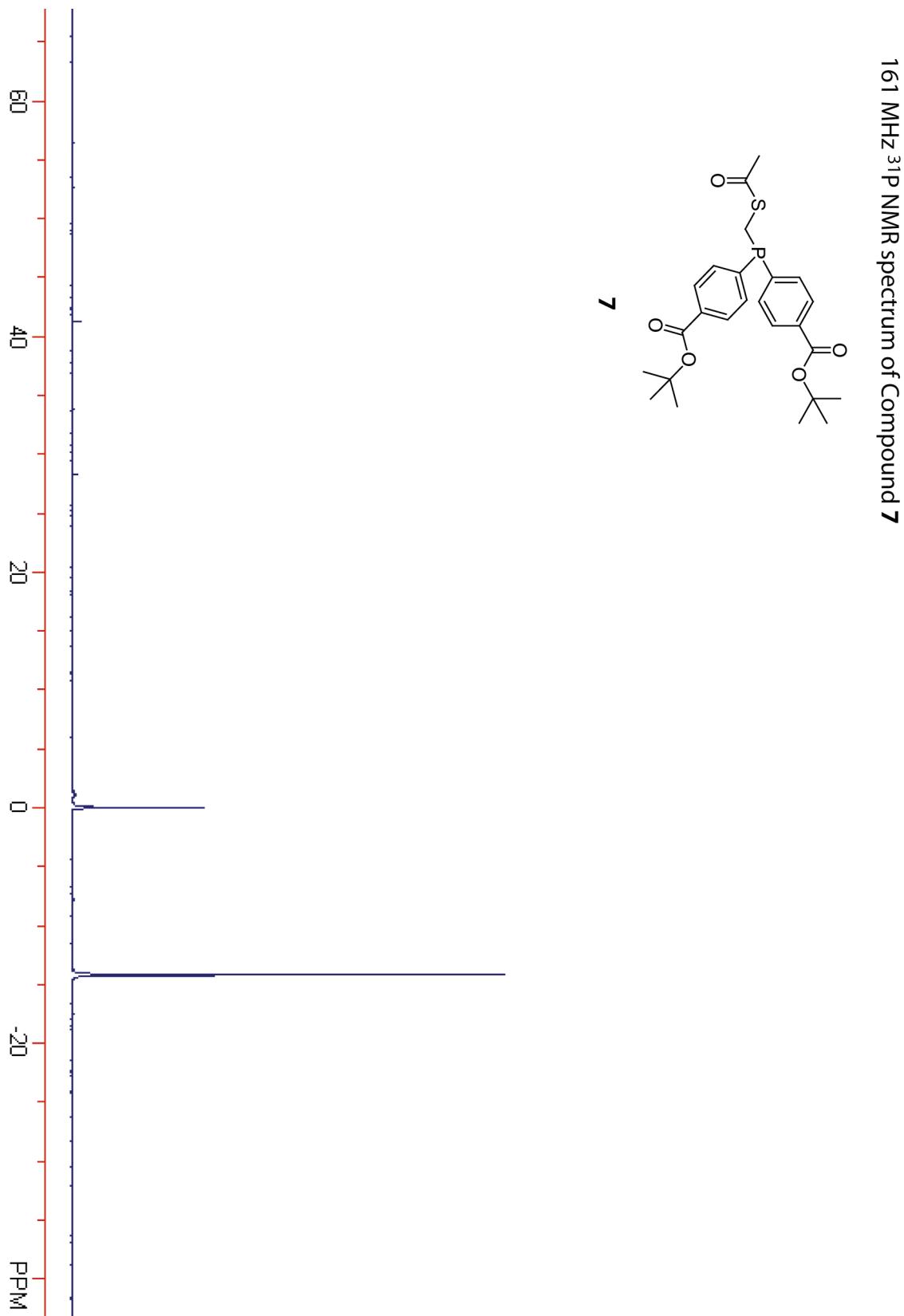


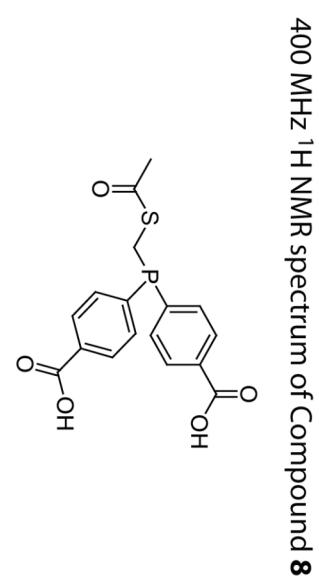
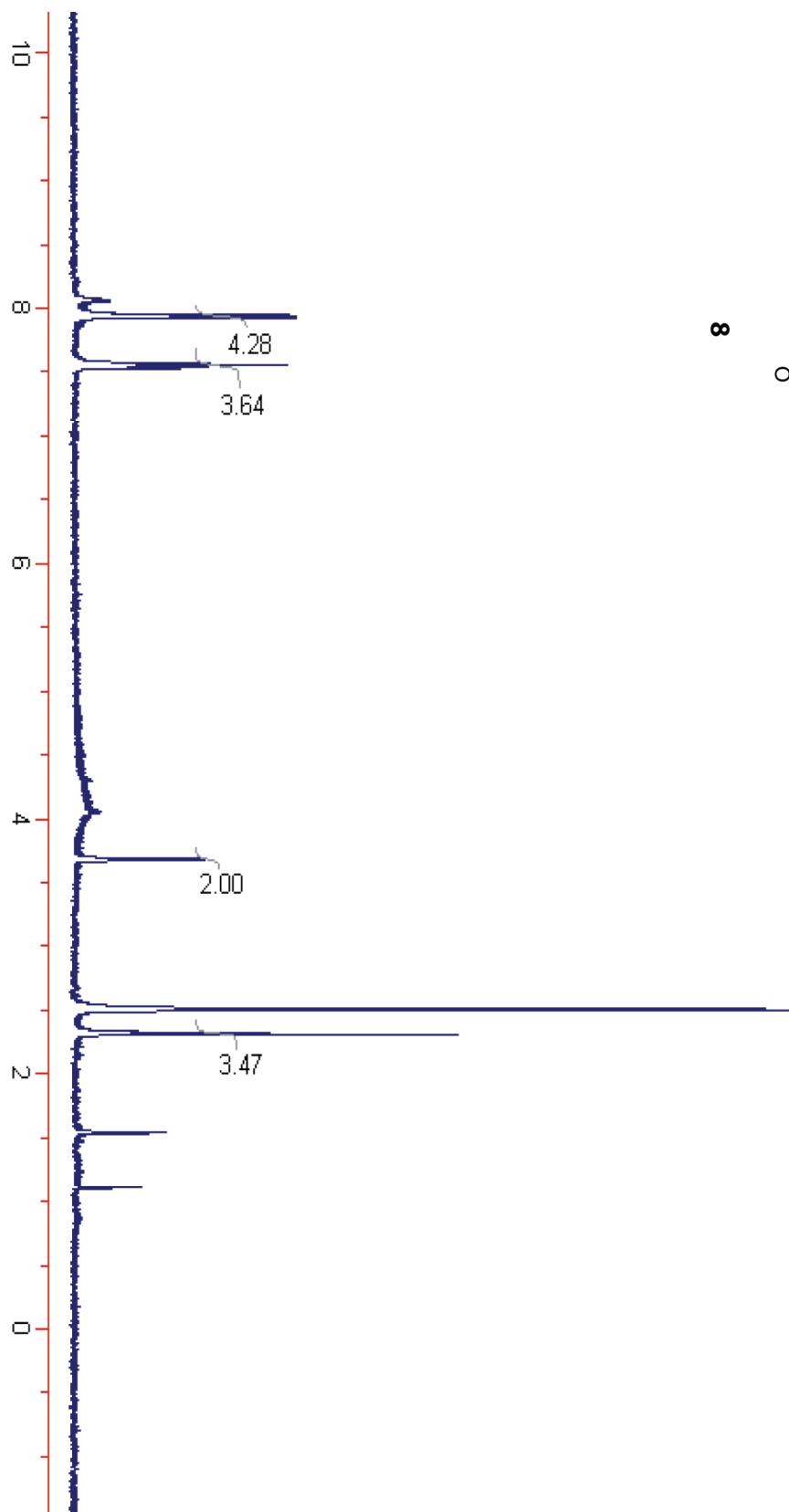


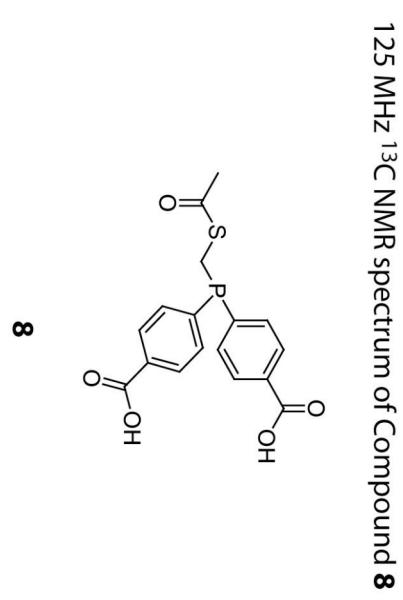
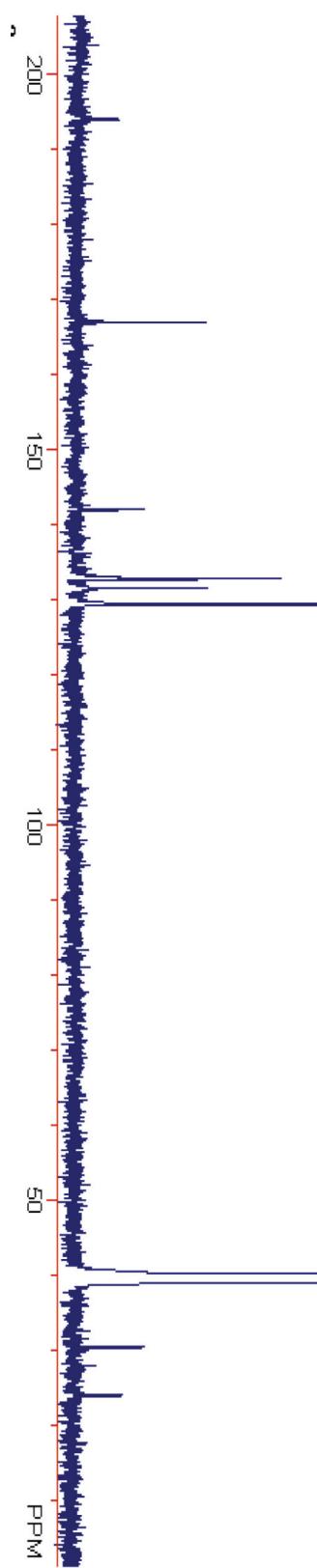


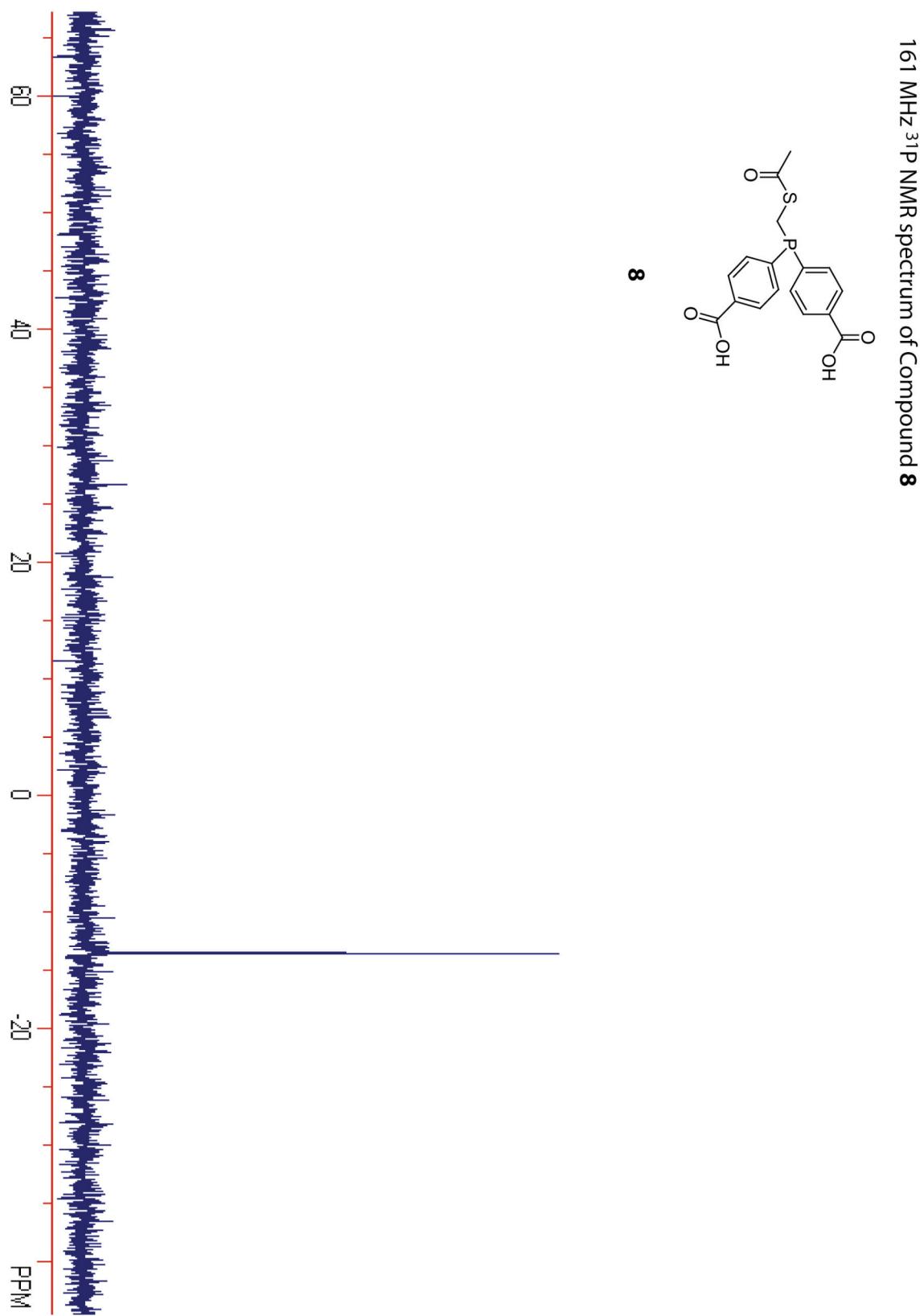
400 MHz ^1H NMR spectrum of Compound 7

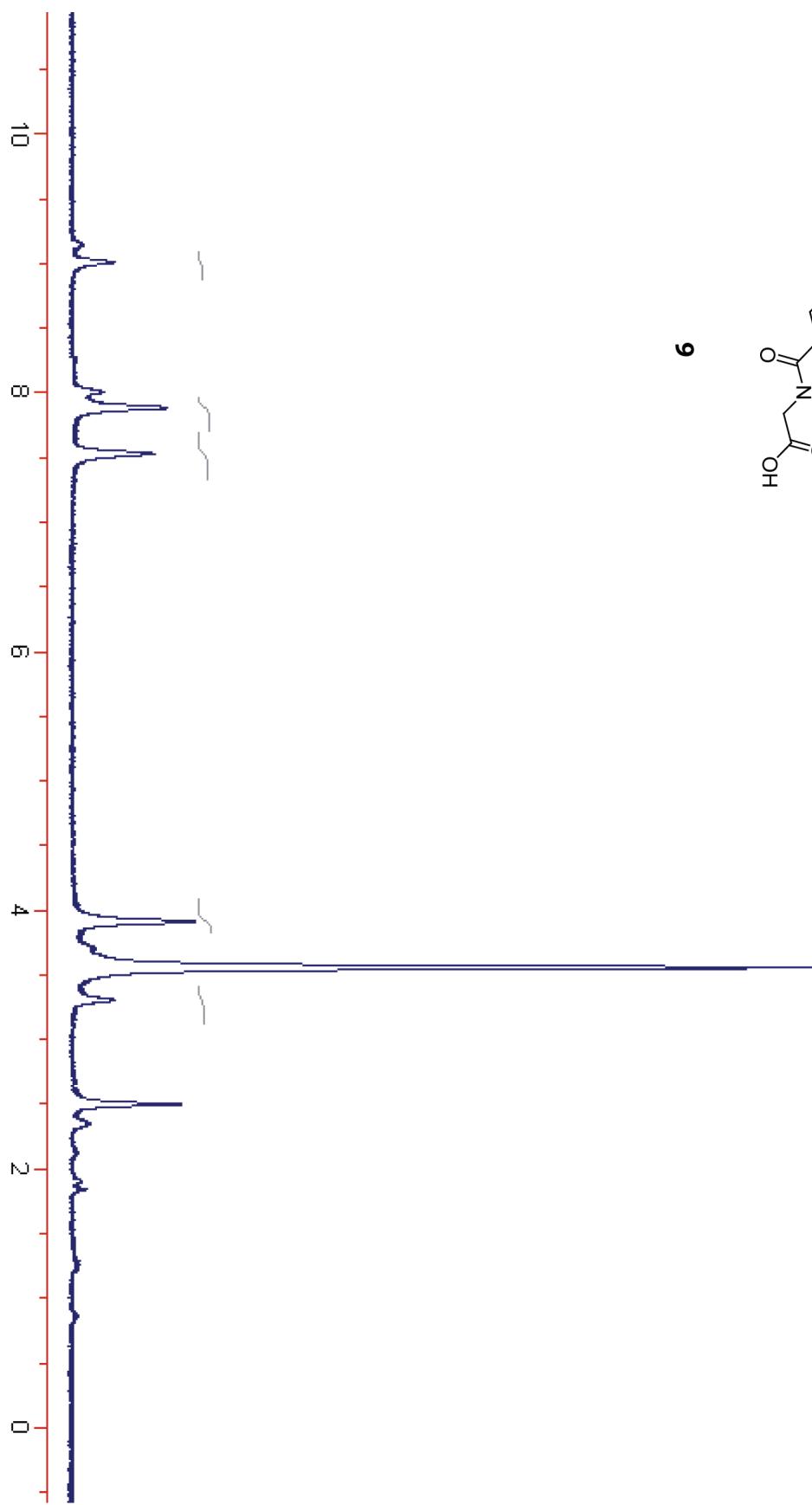


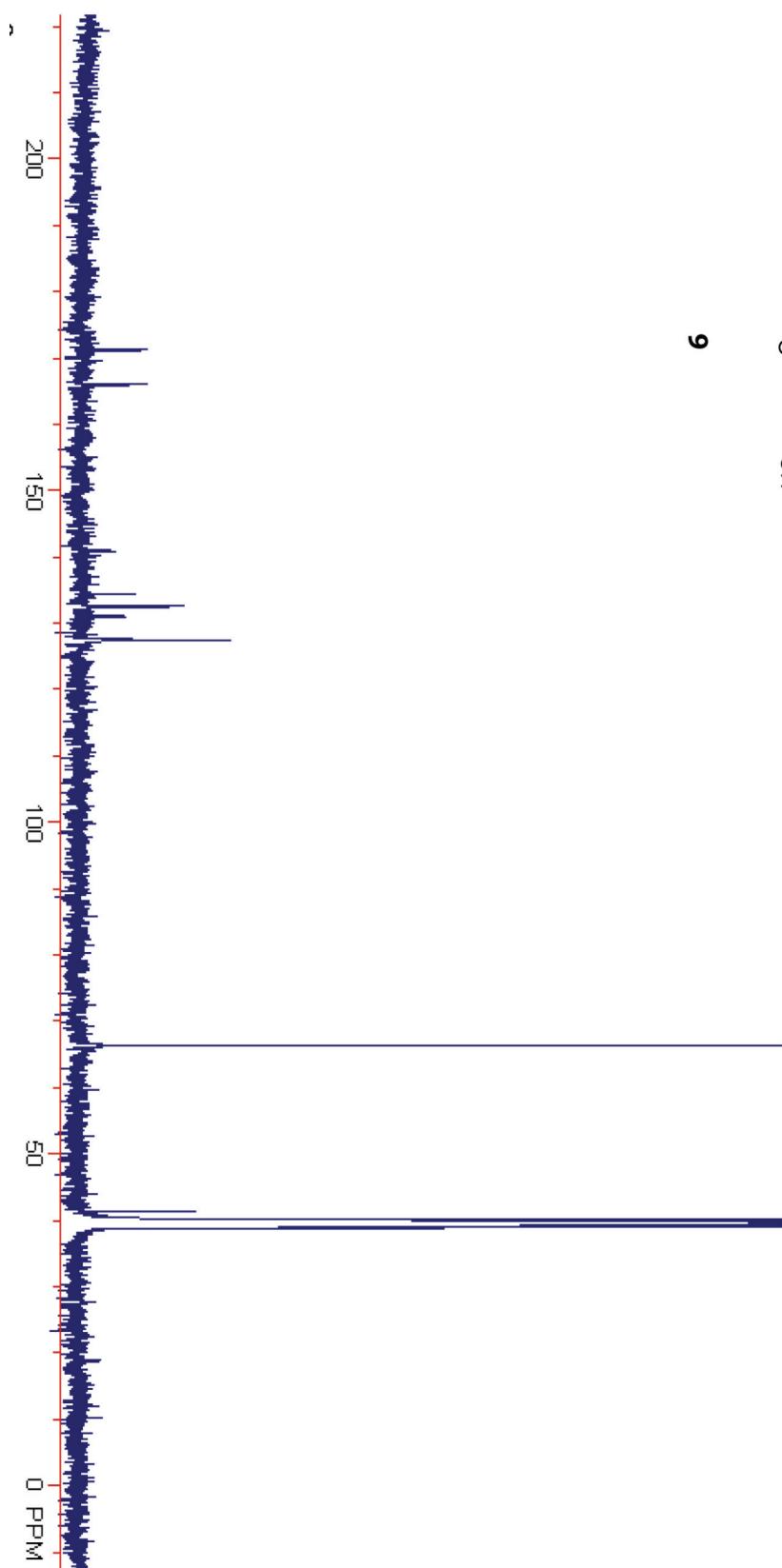
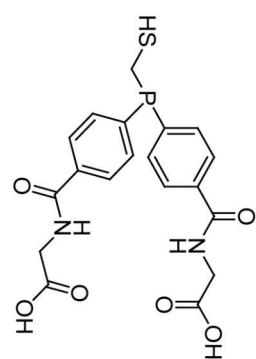




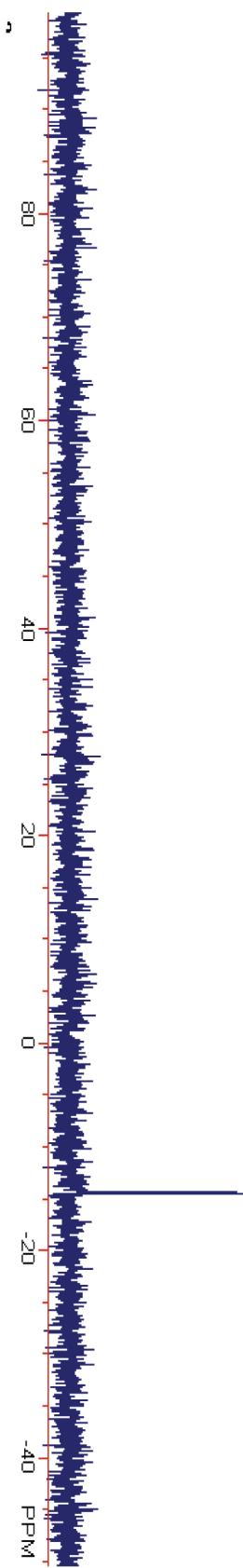
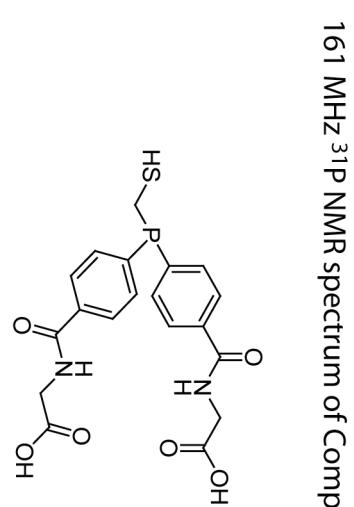




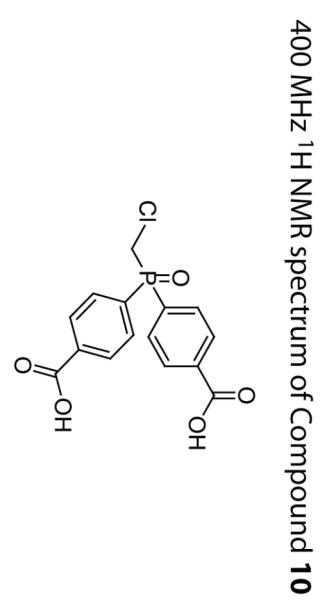
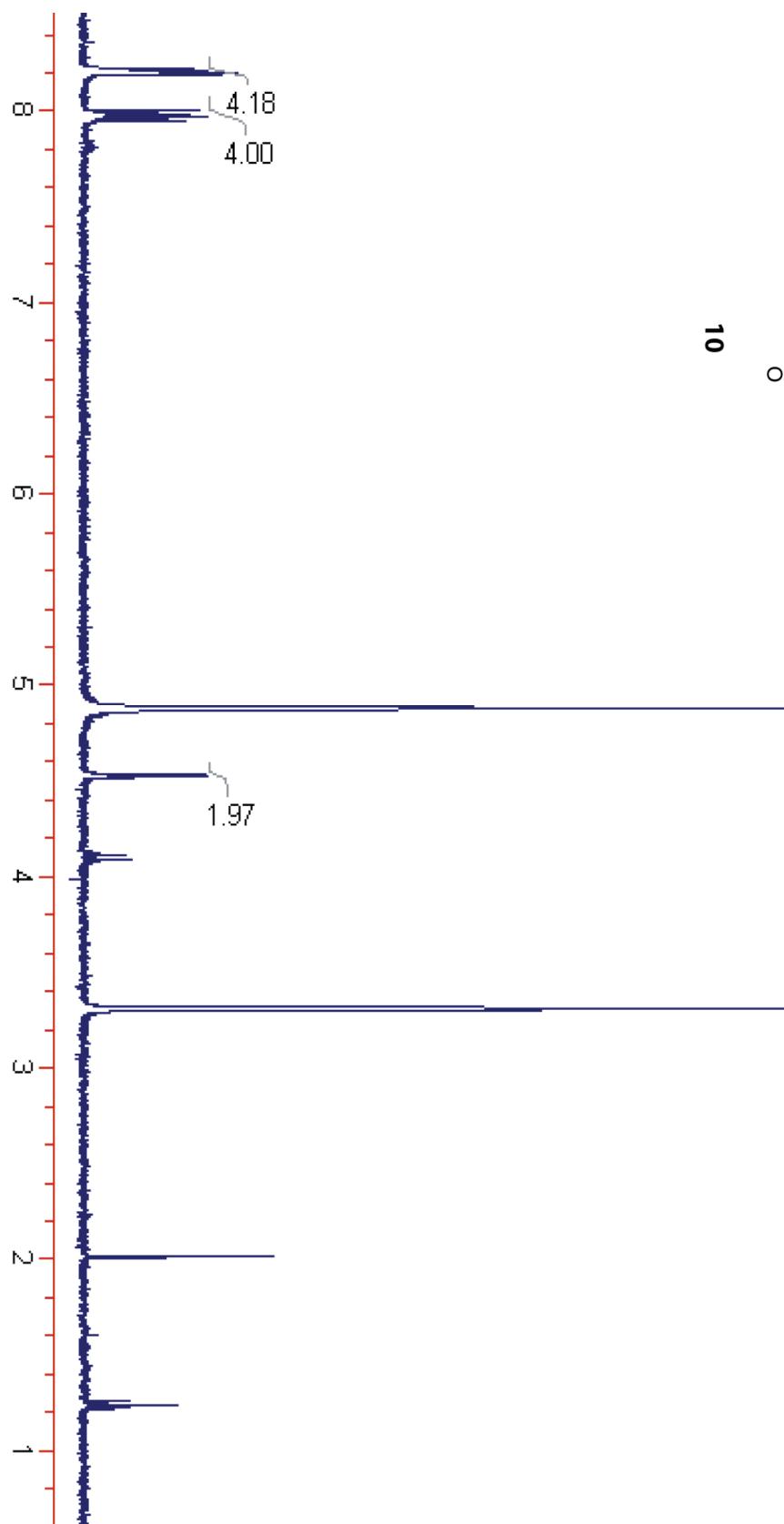
400 MHz ^1H NMR spectrum of Compound 9

**9**

125 MHz ^{13}C NMR spectrum of Compound **9**

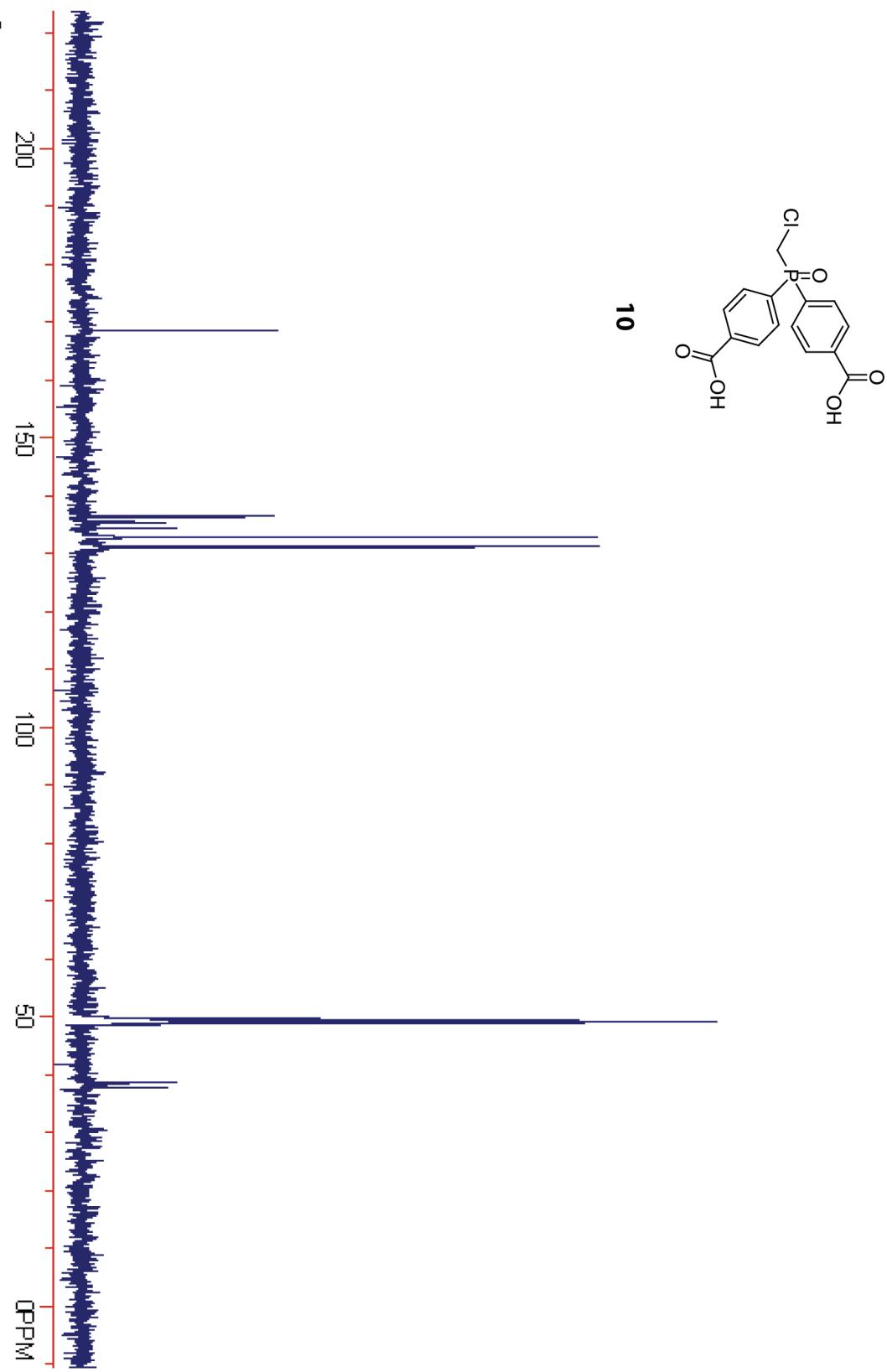
**9**

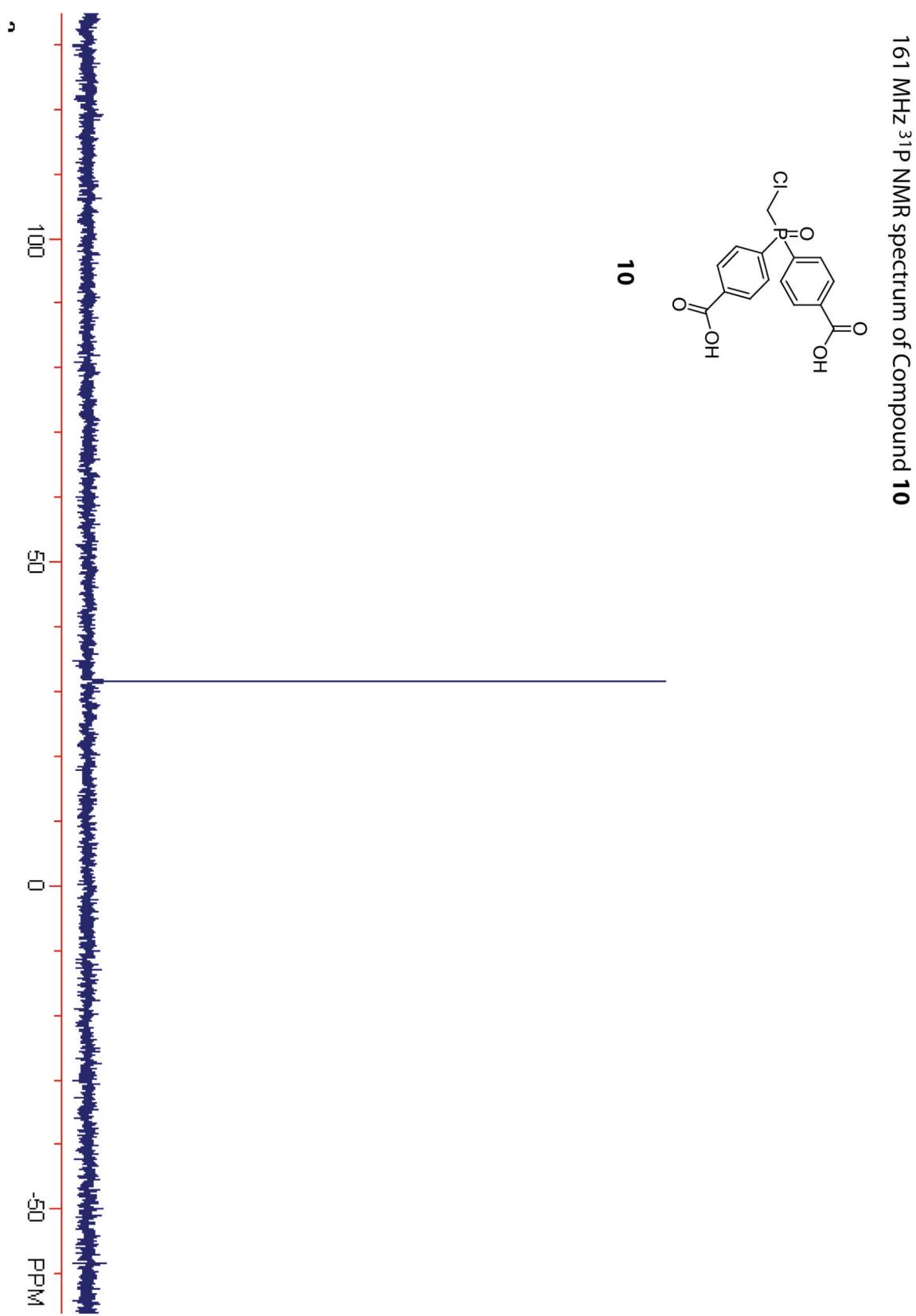
161 MHz ^{31}P NMR spectrum of Compound **9**

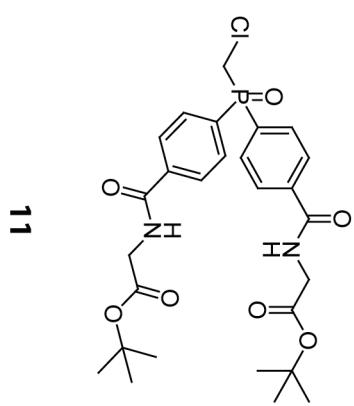
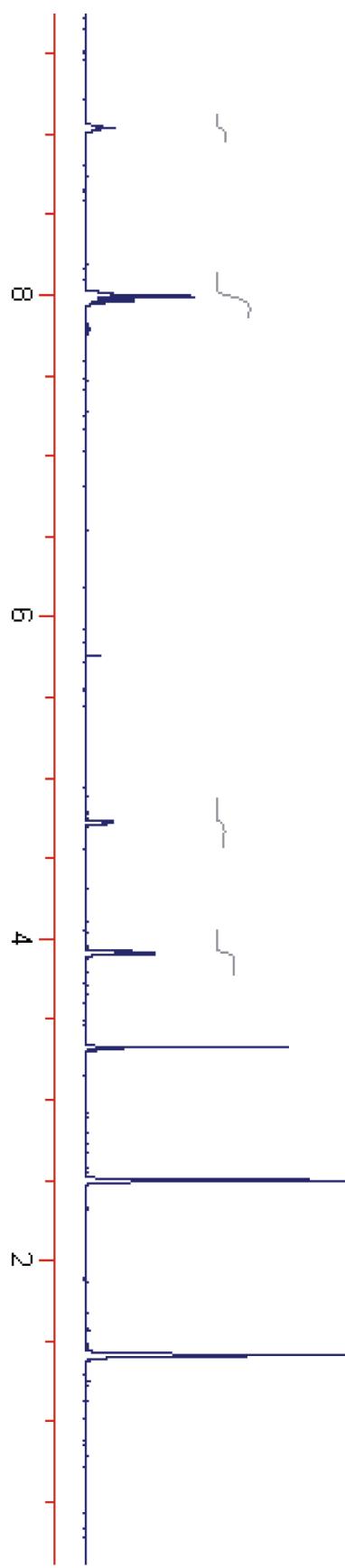


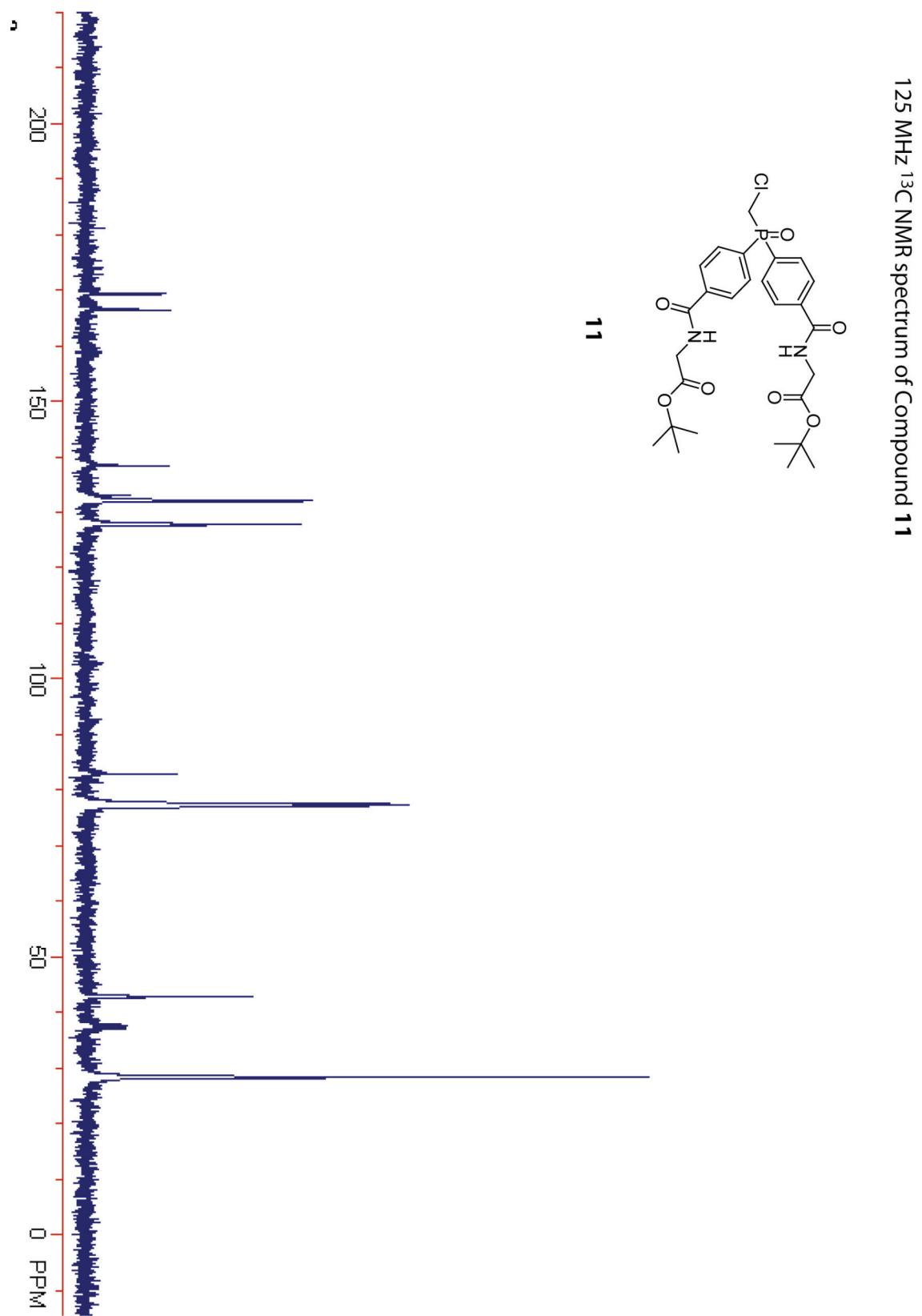
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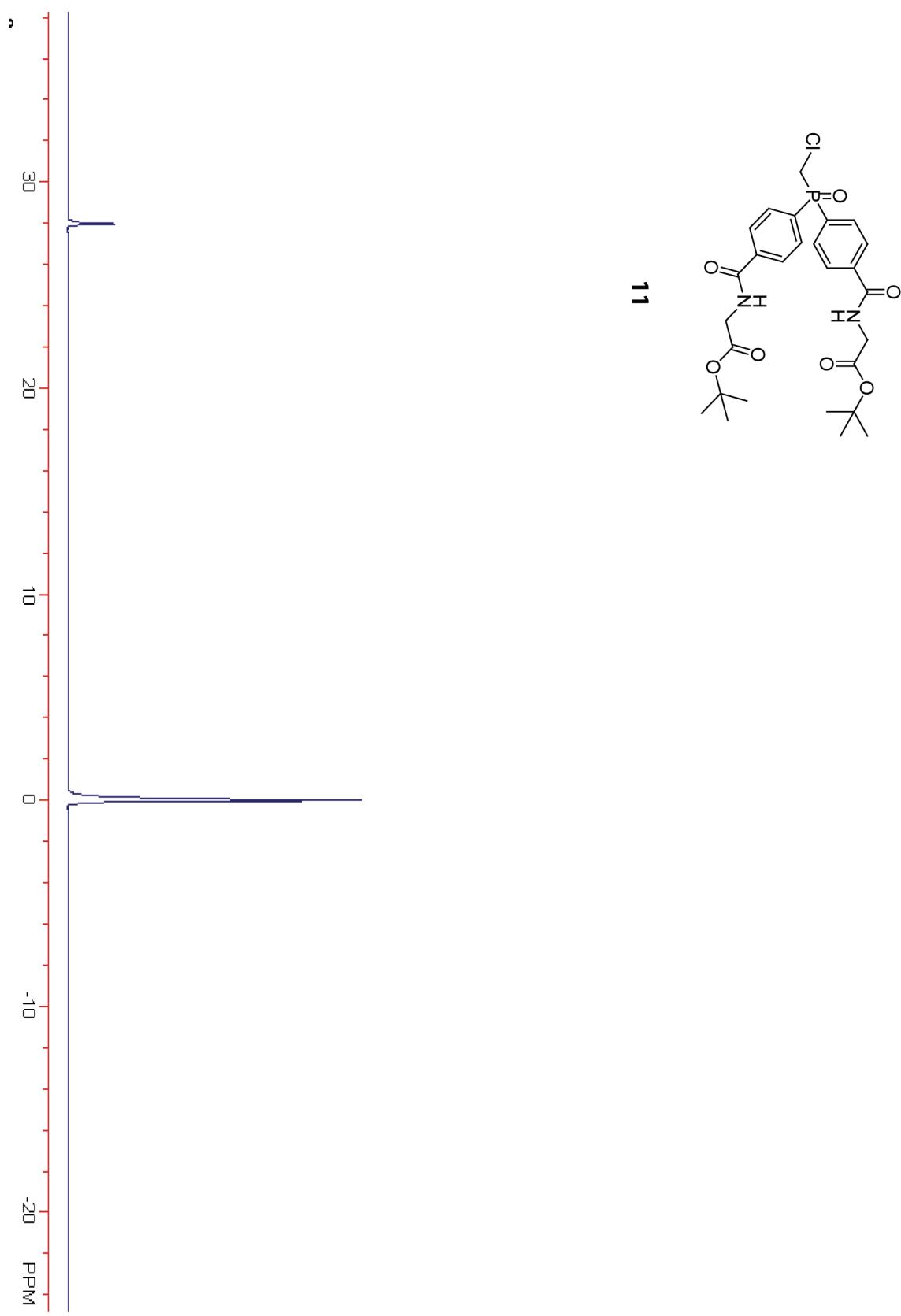
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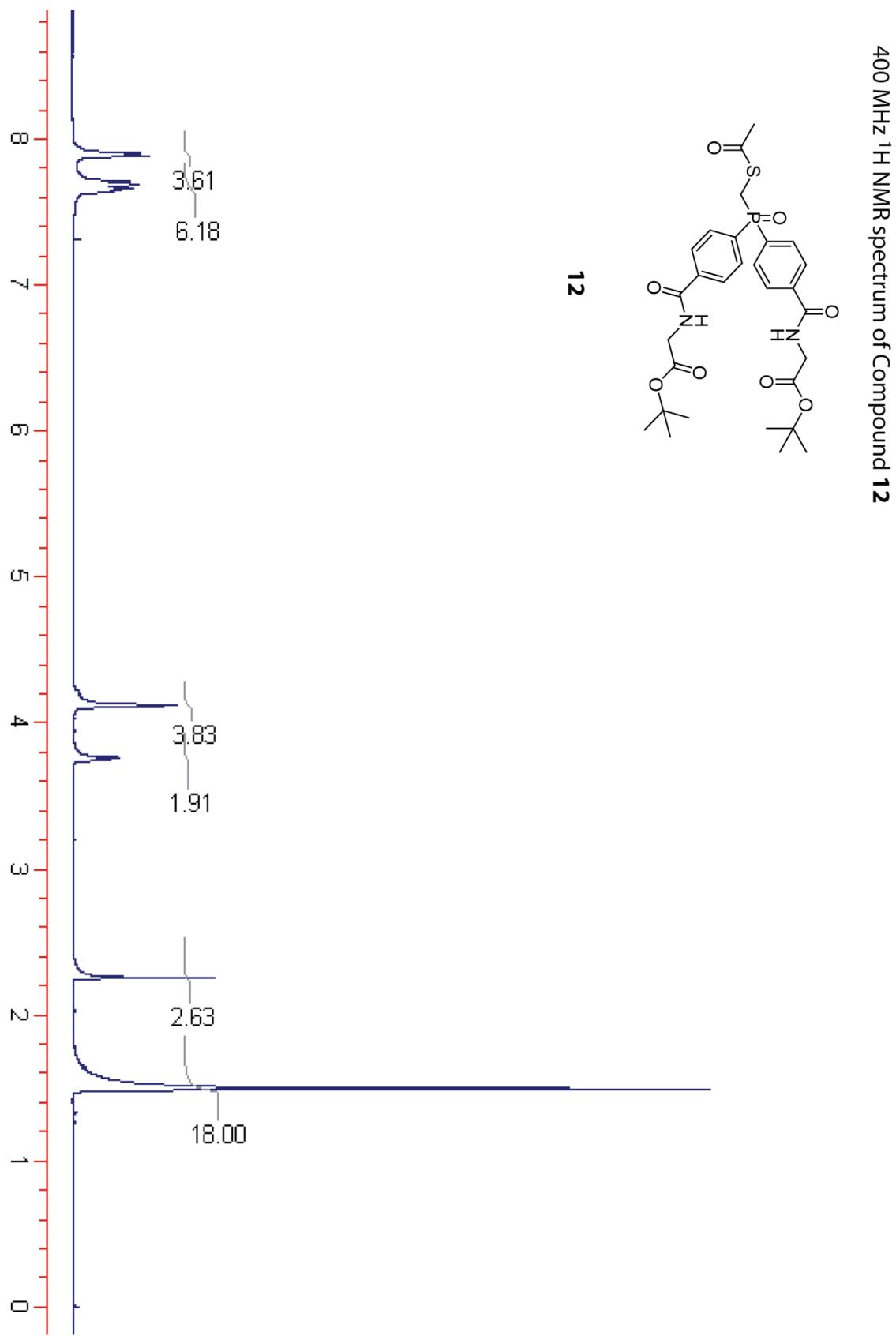


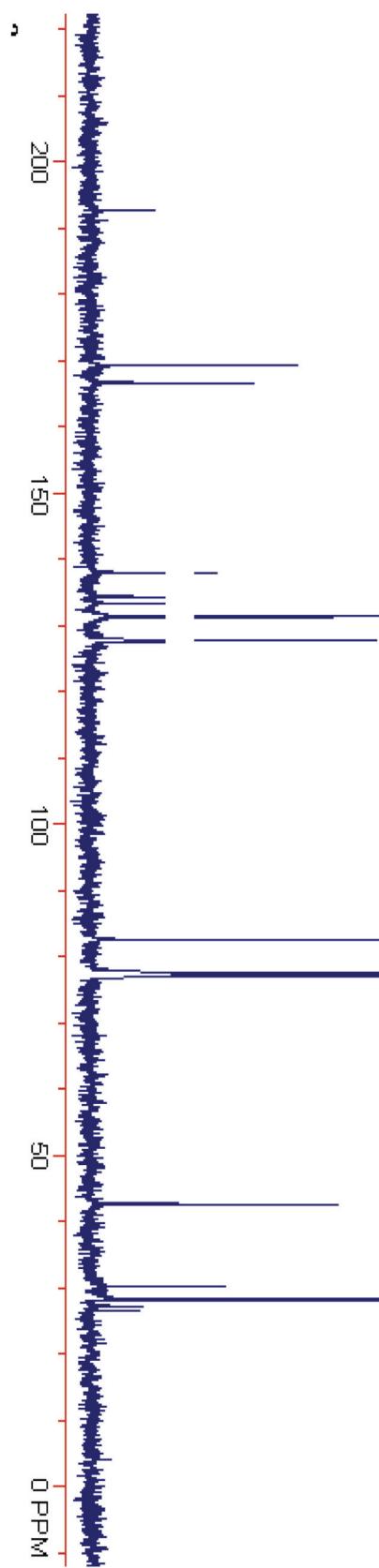
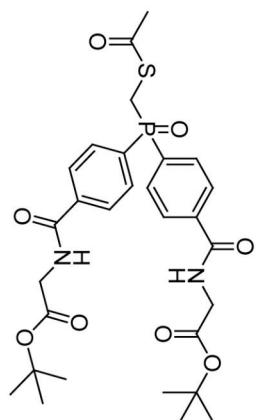


400 MHz ^1H NMR spectrum of Compound **11**

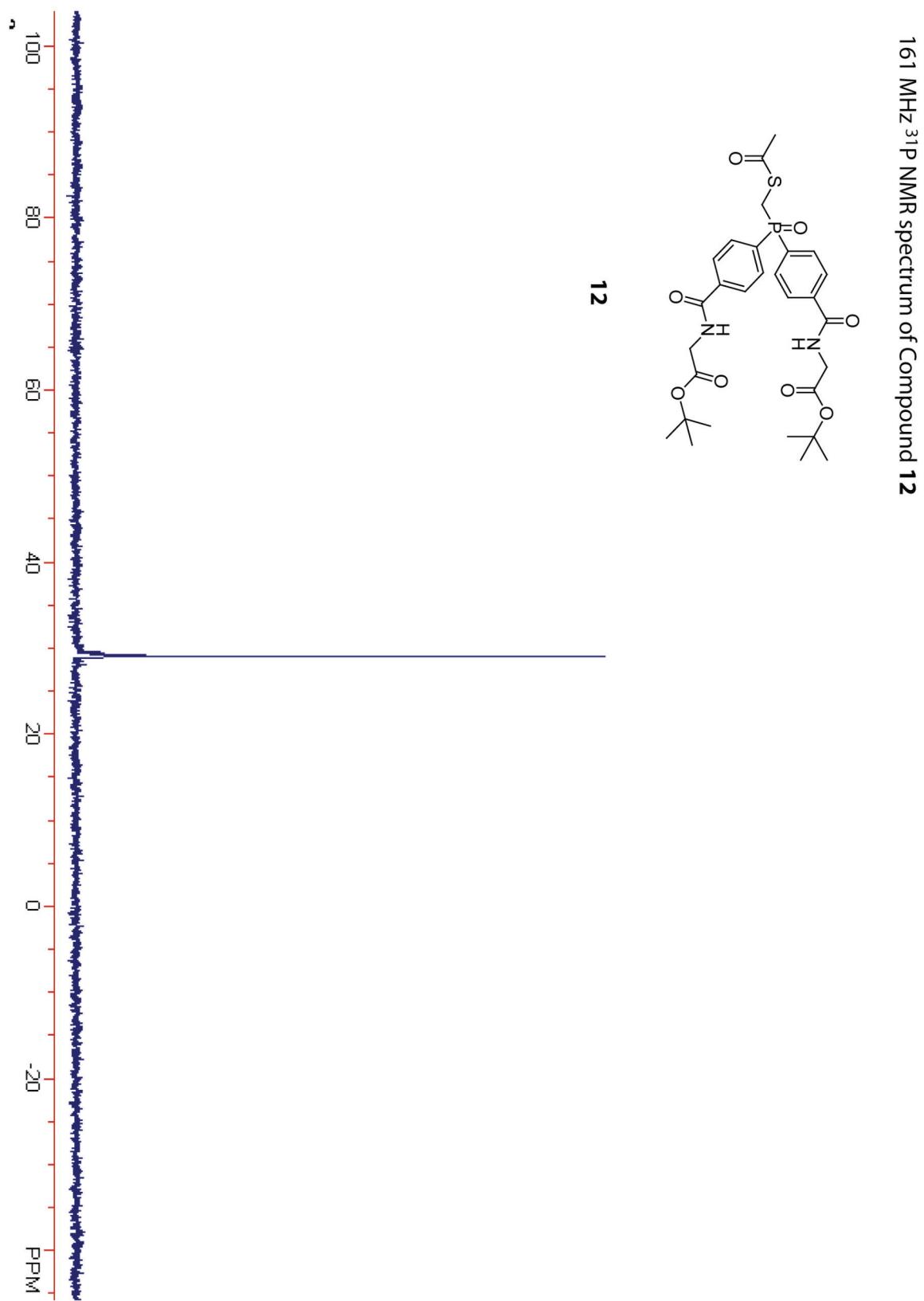


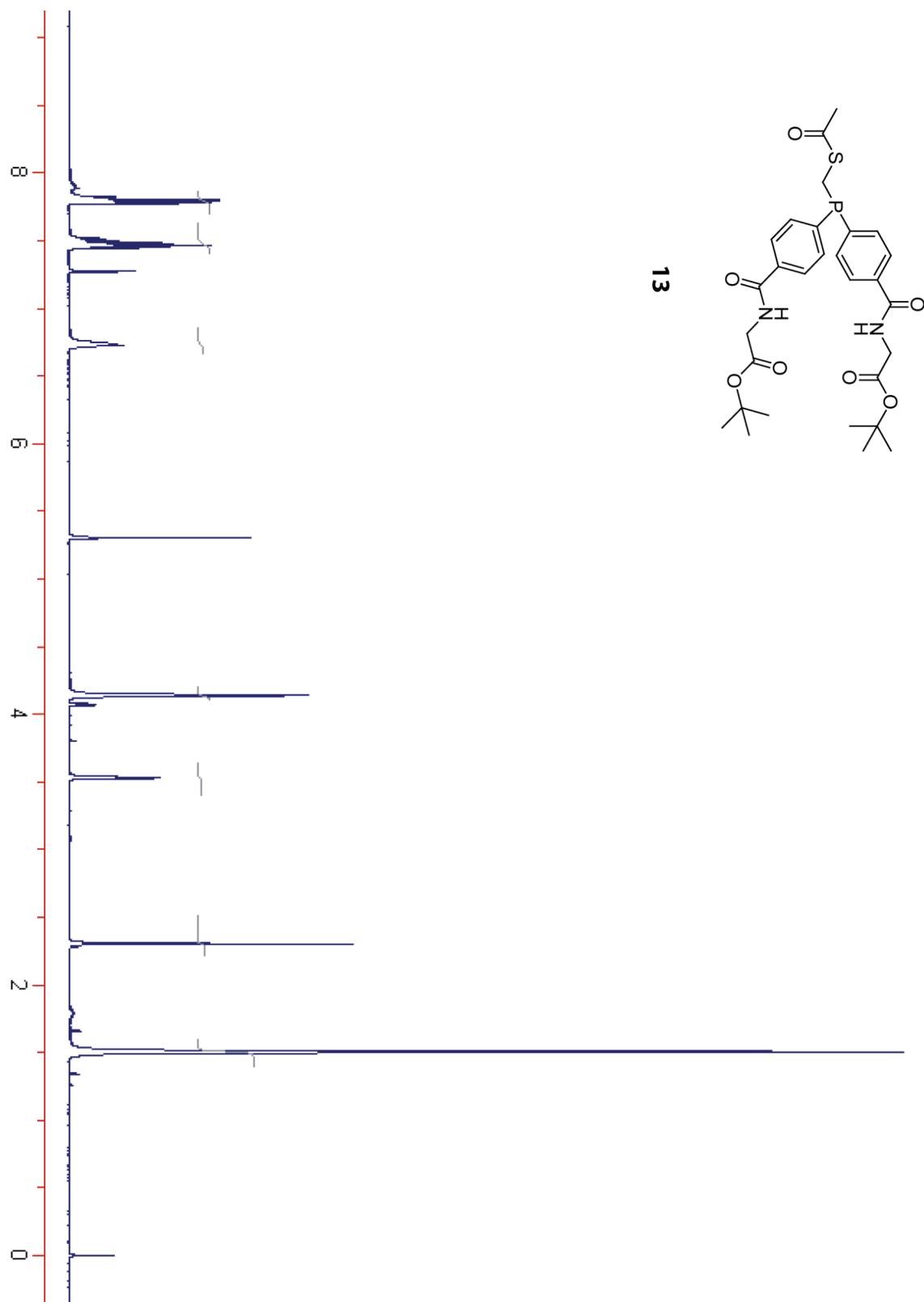
161 MHz ^{31}P NMR spectrum of Compound 11

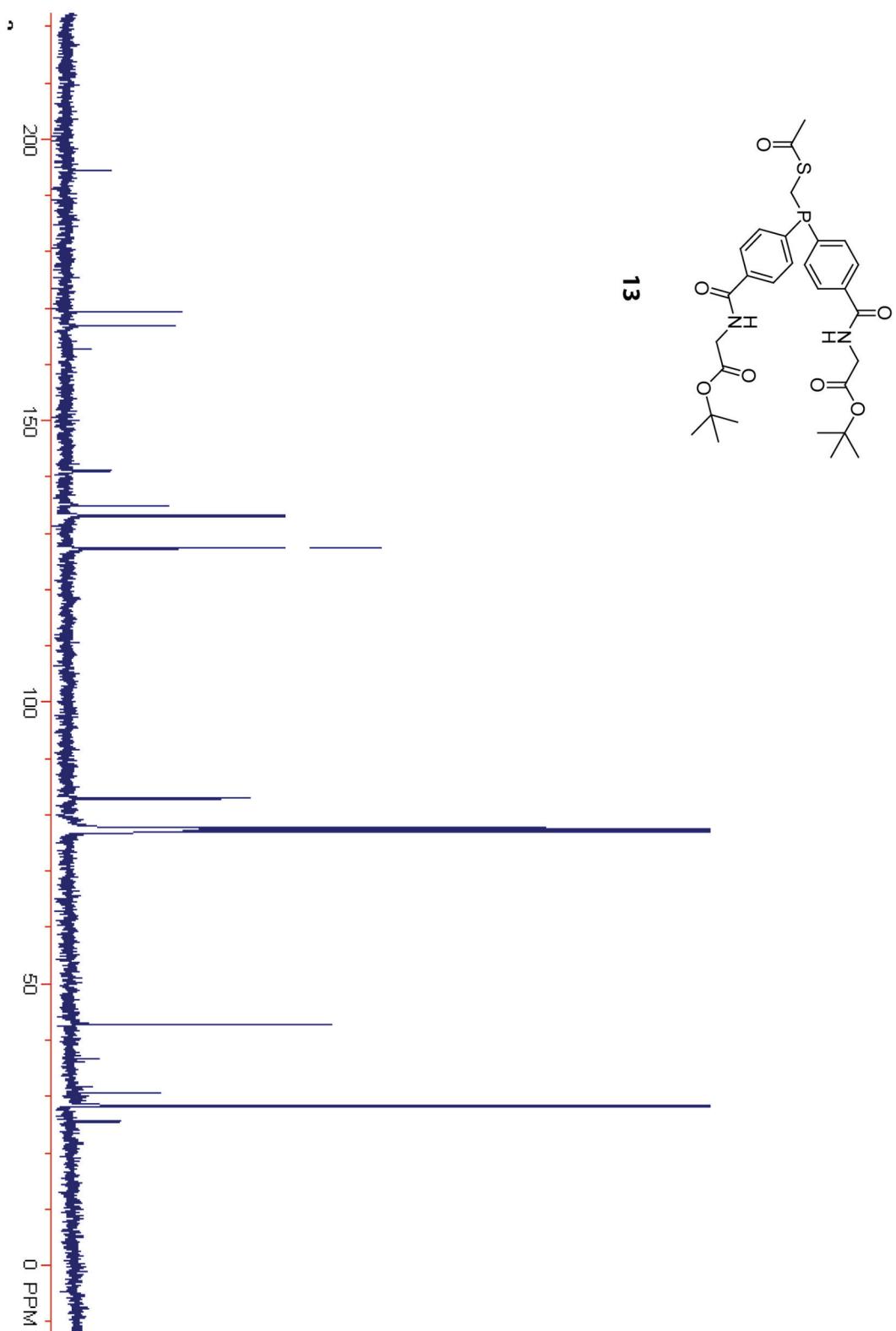


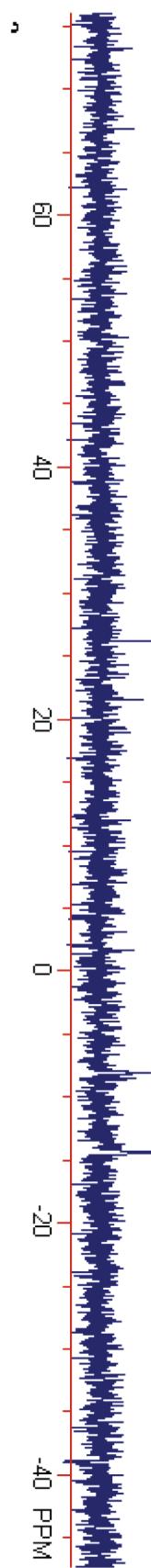
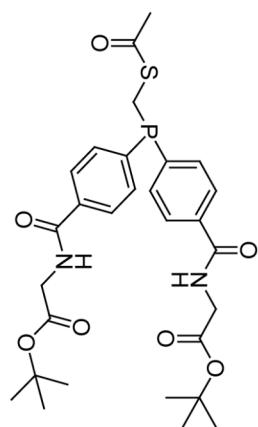
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125 MHz ^{13}C NMR spectrum of Compound **12**

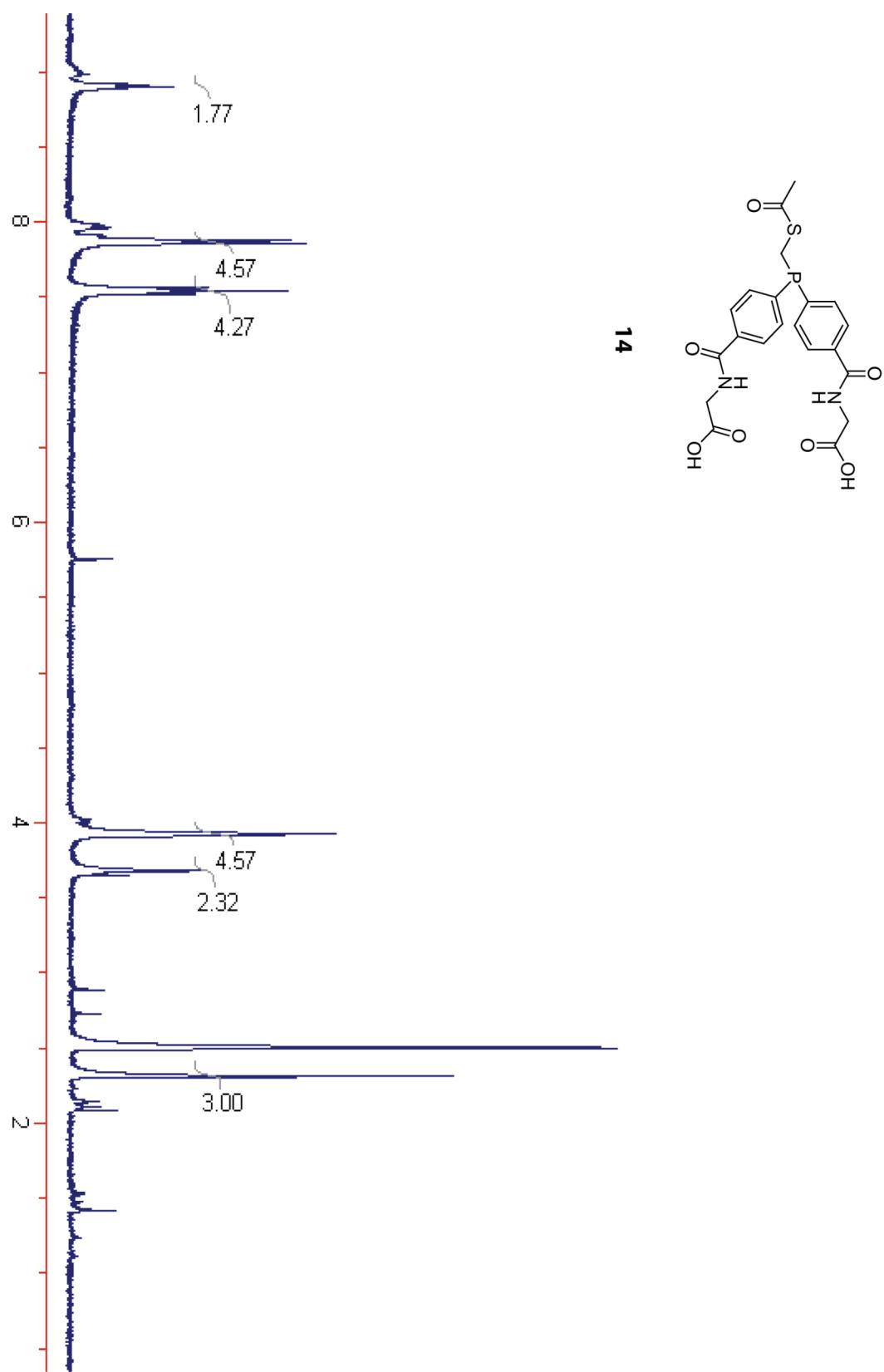


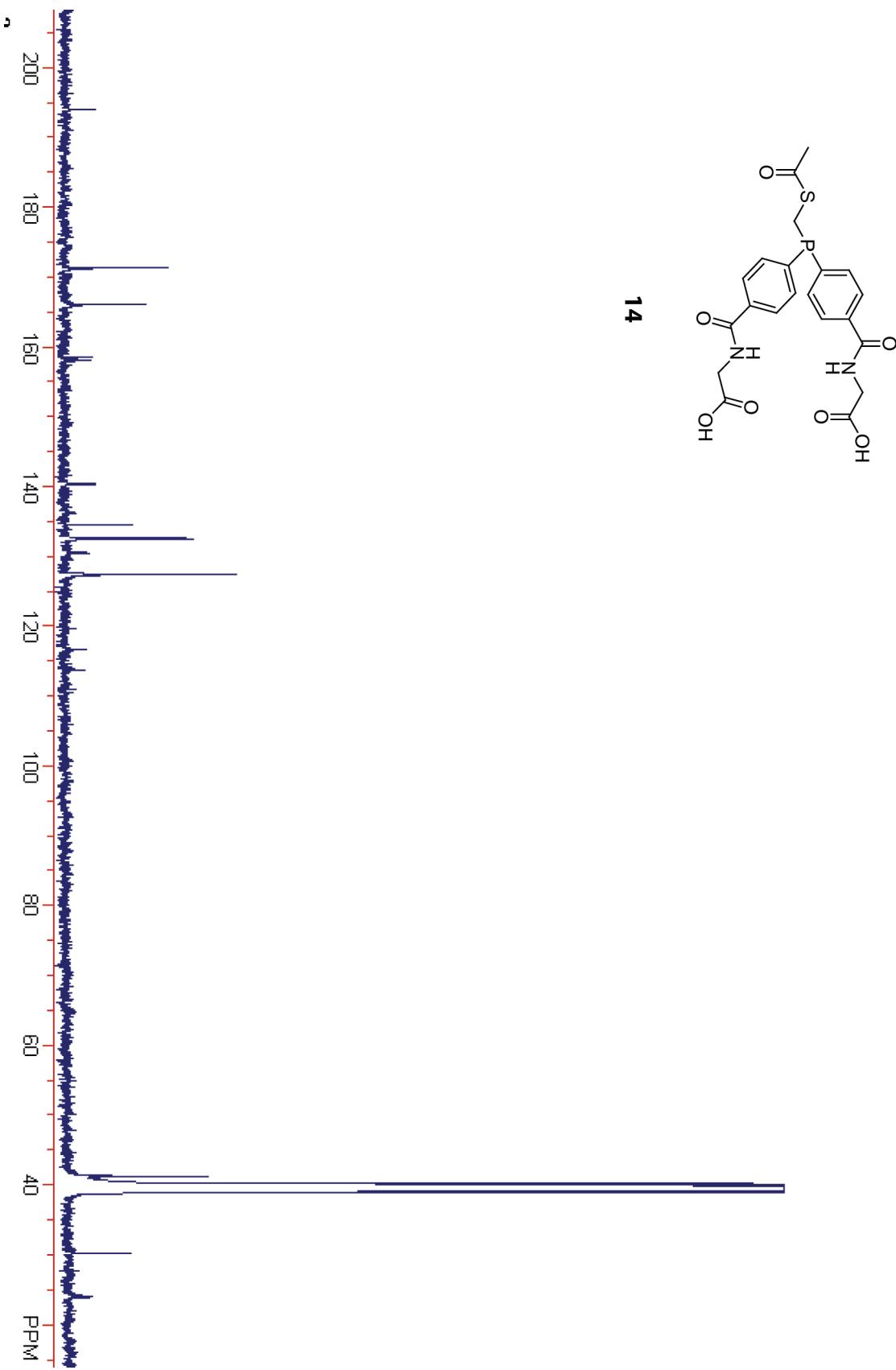
400 MHz ^1H NMR spectrum of Compound 13

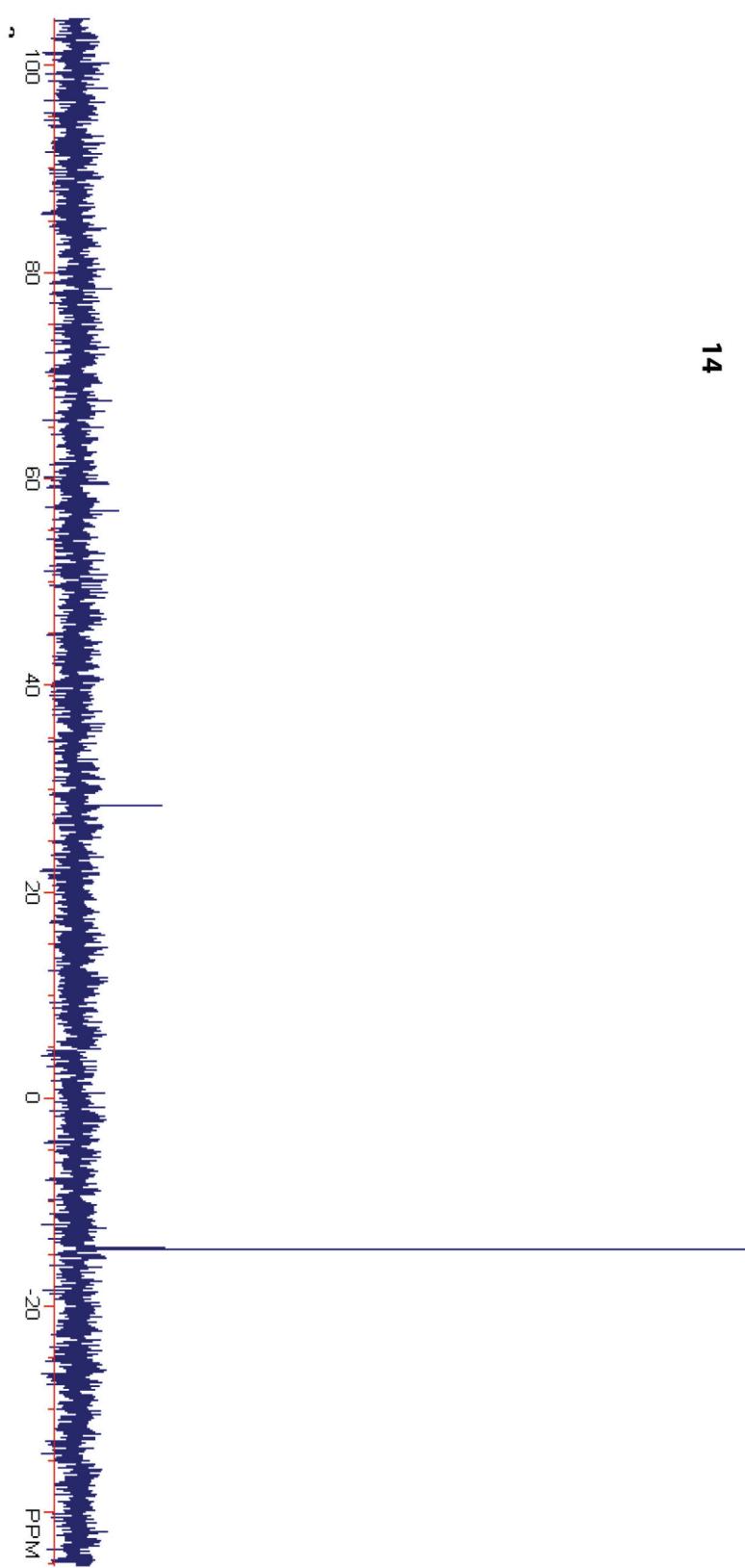
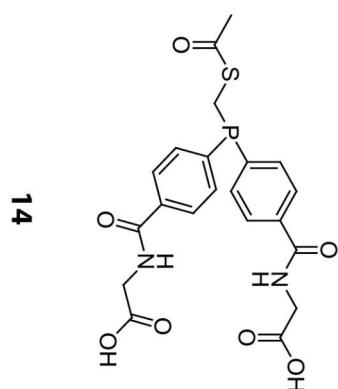


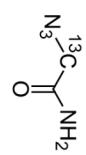
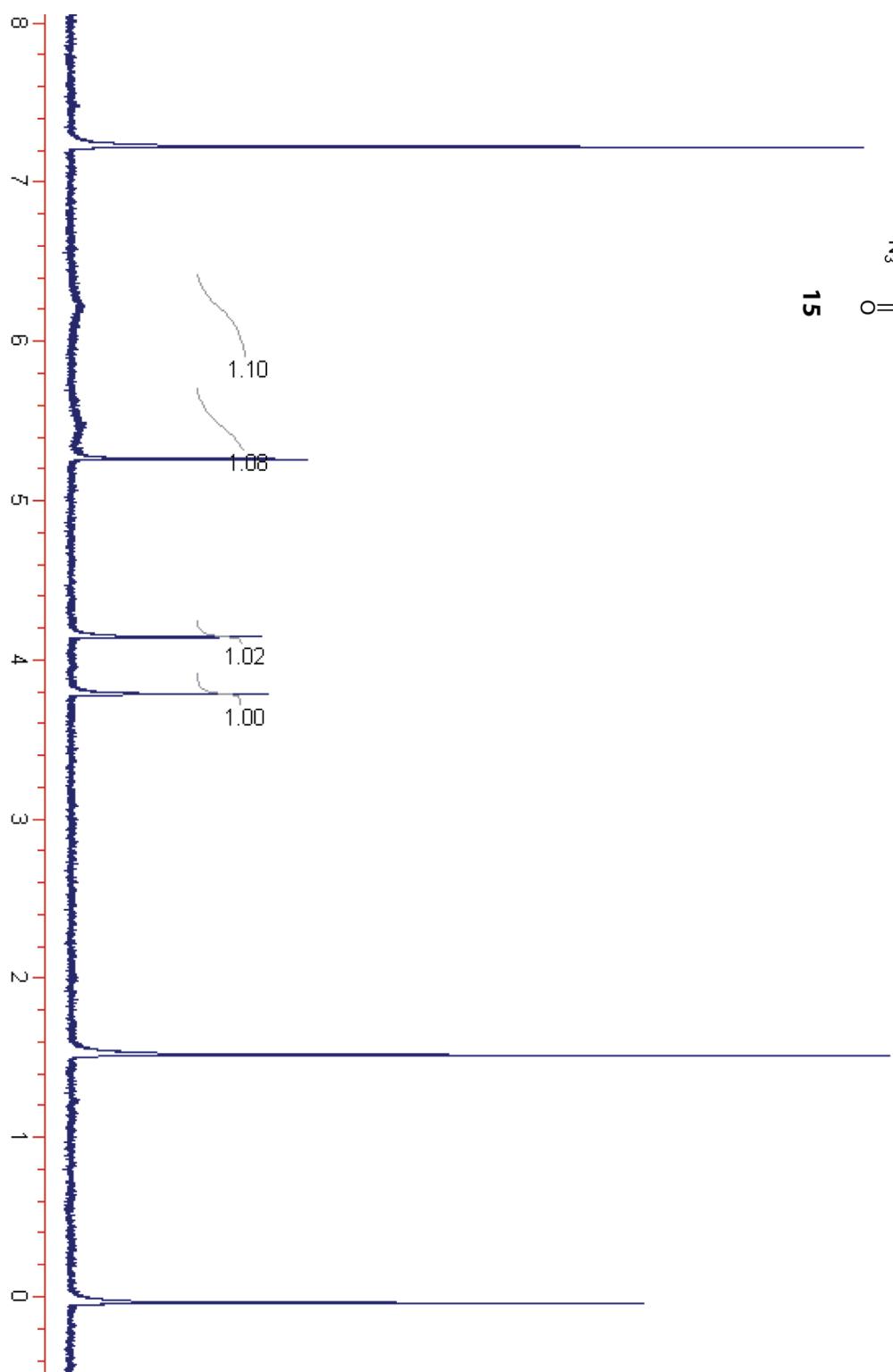
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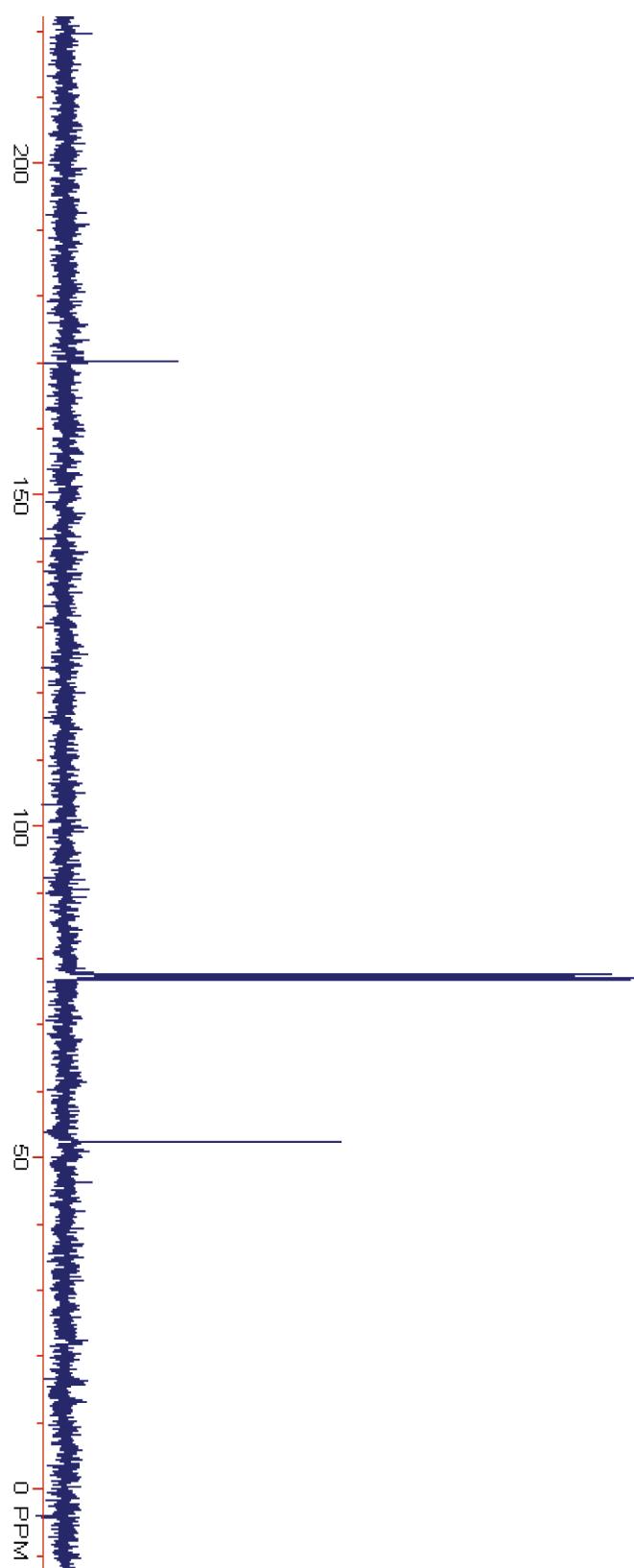
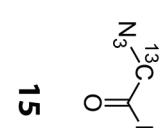
161 MHz ^{31}P NMR spectrum of Compound **13**

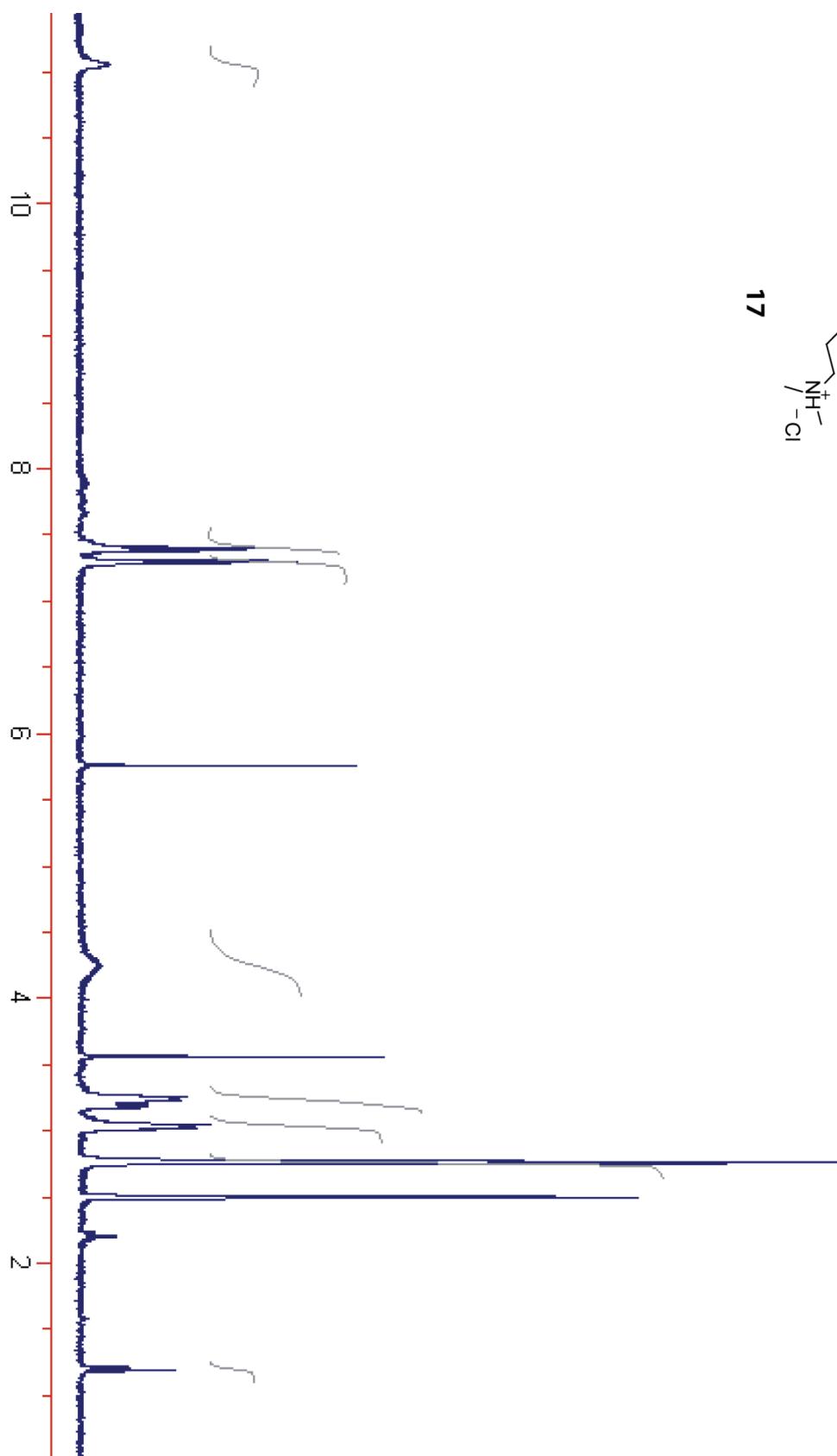
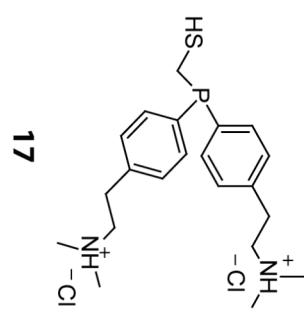


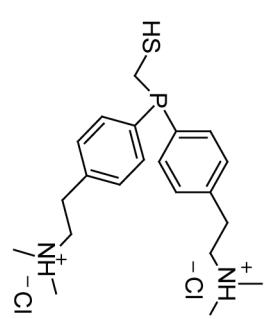
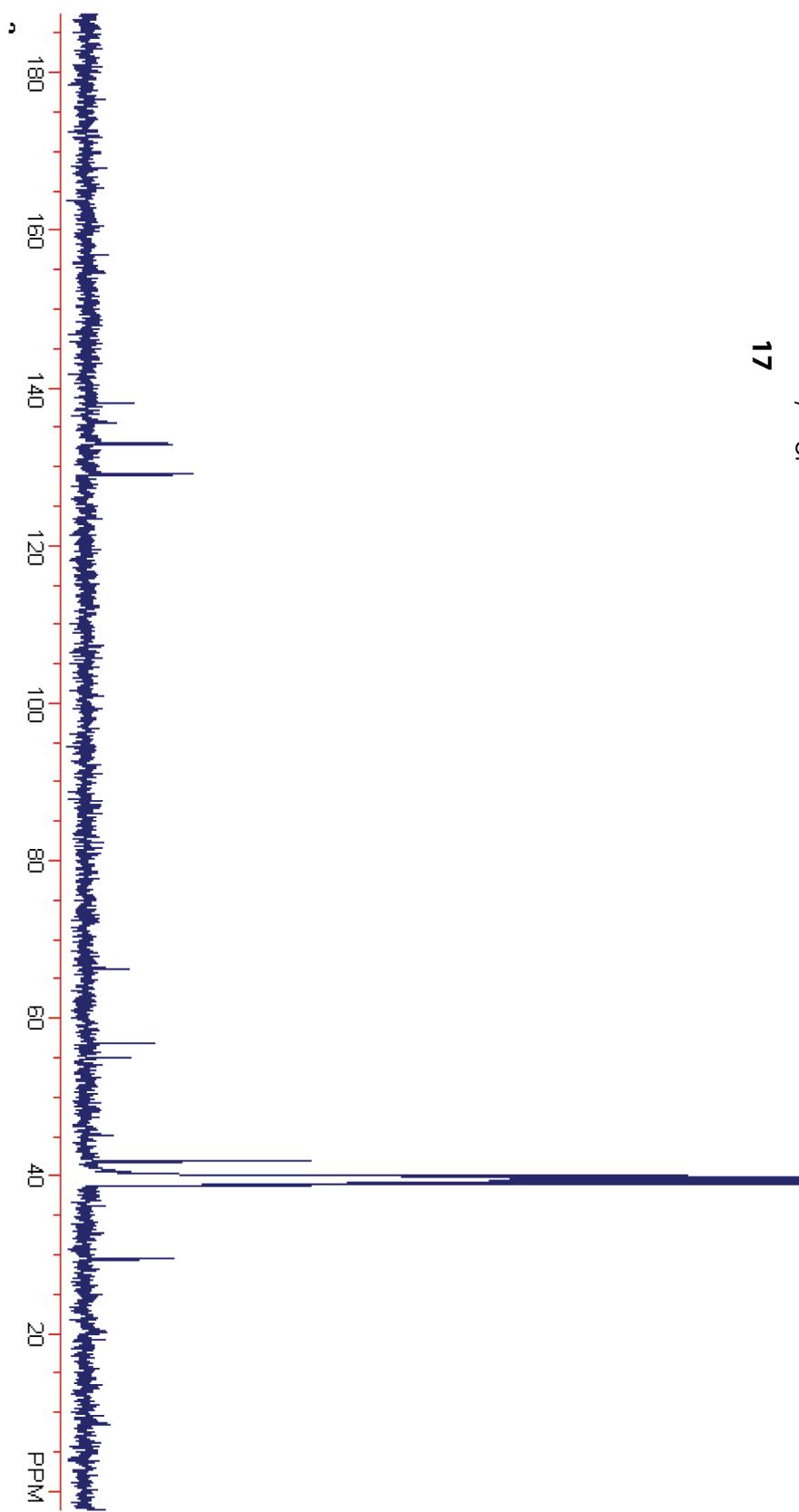
125 MHz ^{13}C NMR spectrum of Compound **14**

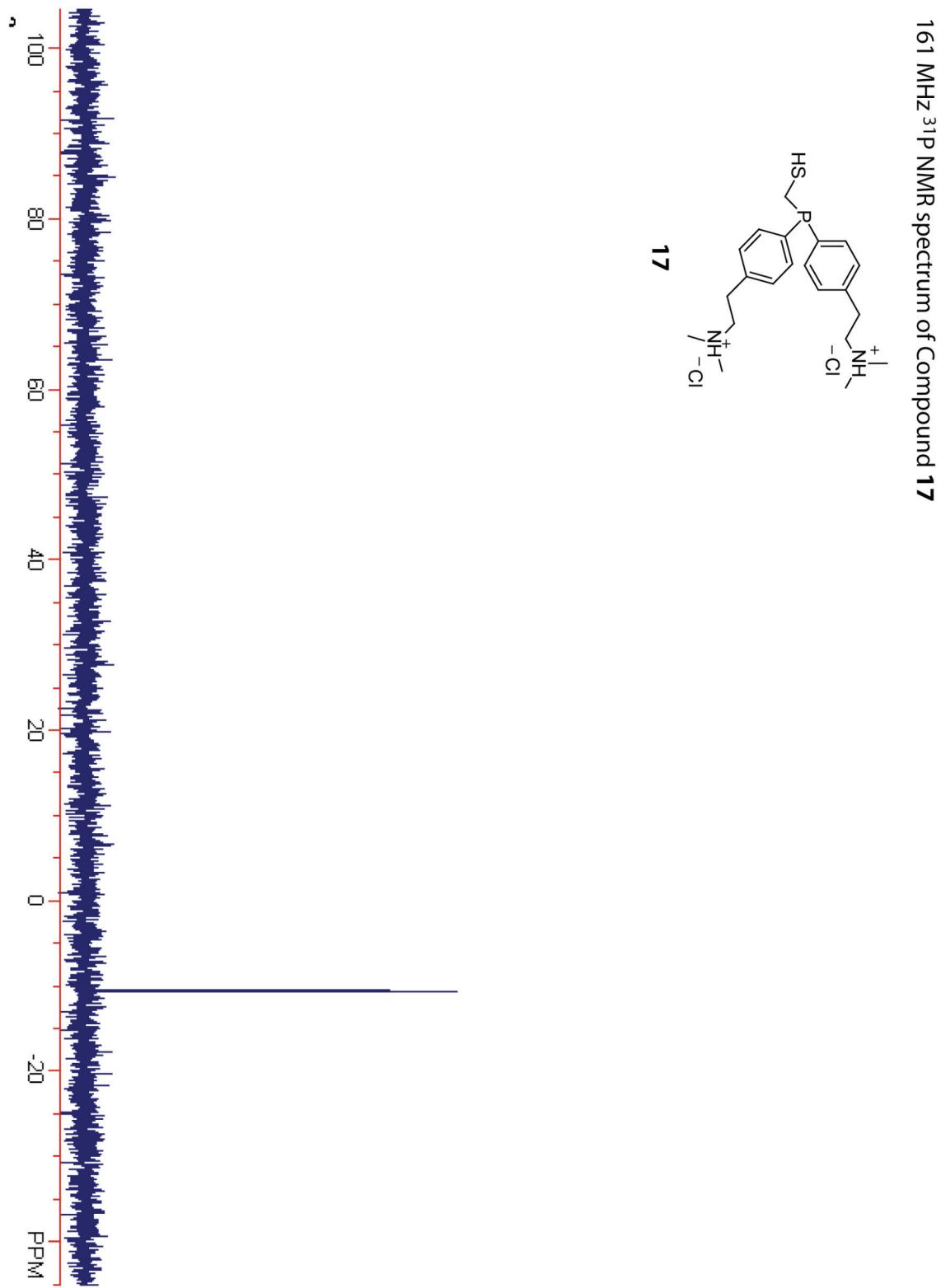
161 MHz ^{31}P NMR spectrum of Compound 14

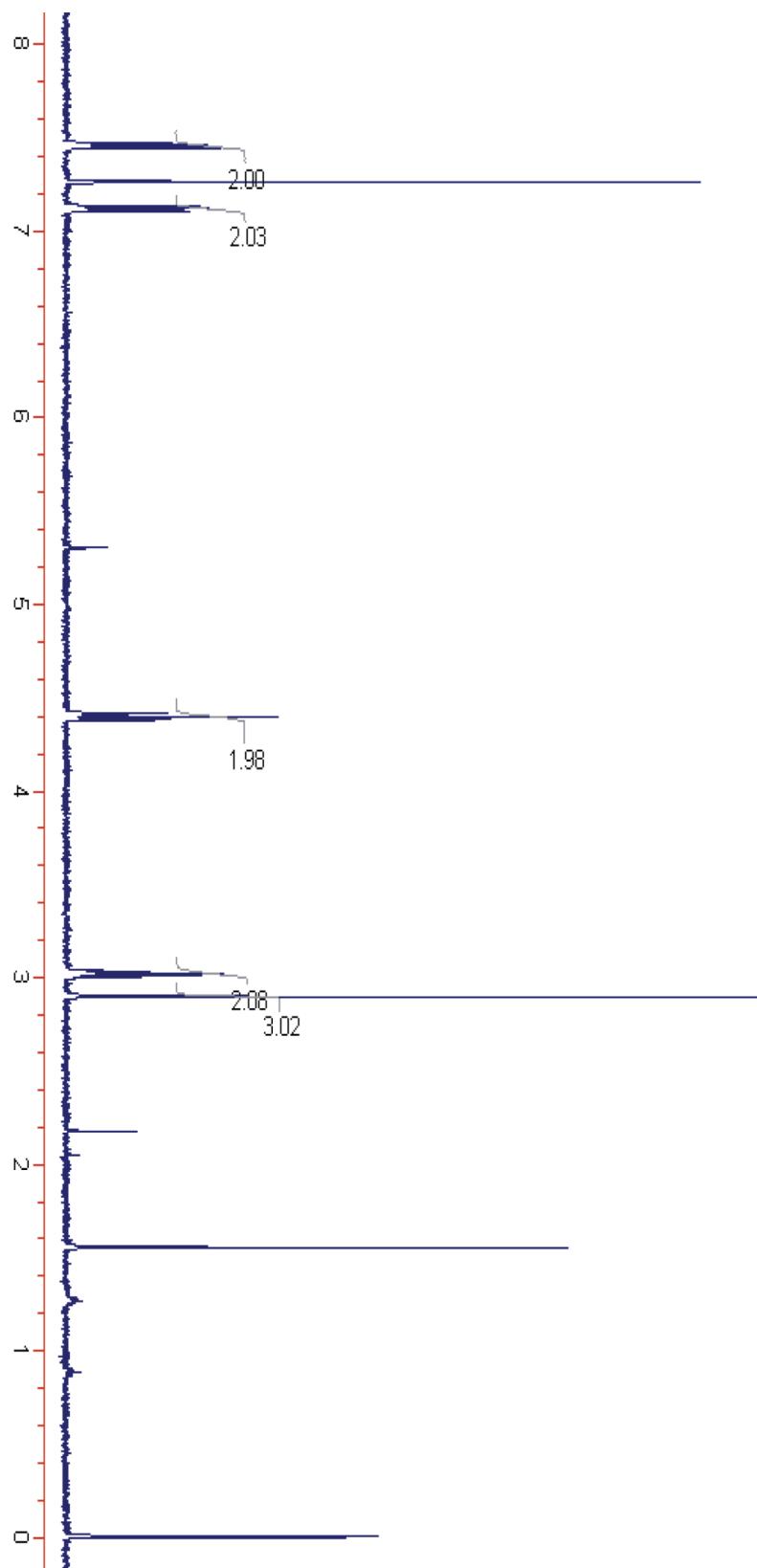
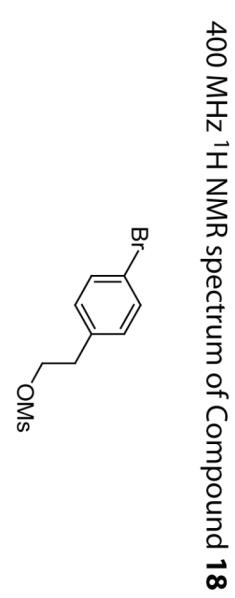
400 MHz ^1H NMR spectrum of Compound **15****15**

125 MHz ^{13}C NMR spectrum of Compound 15

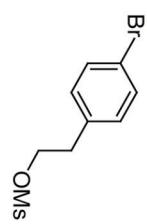
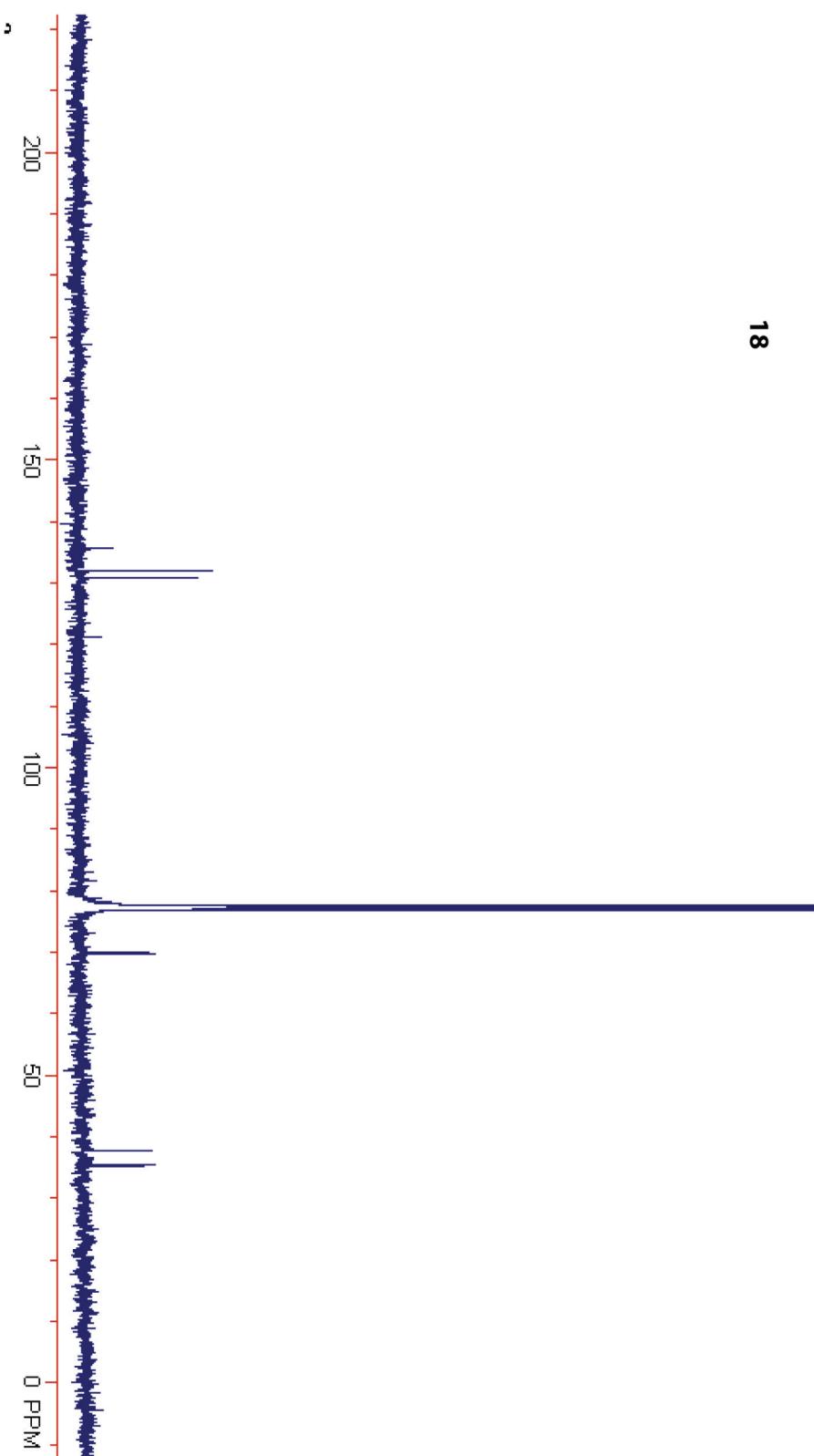
400 MHz ^1H NMR spectrum of Compound 17

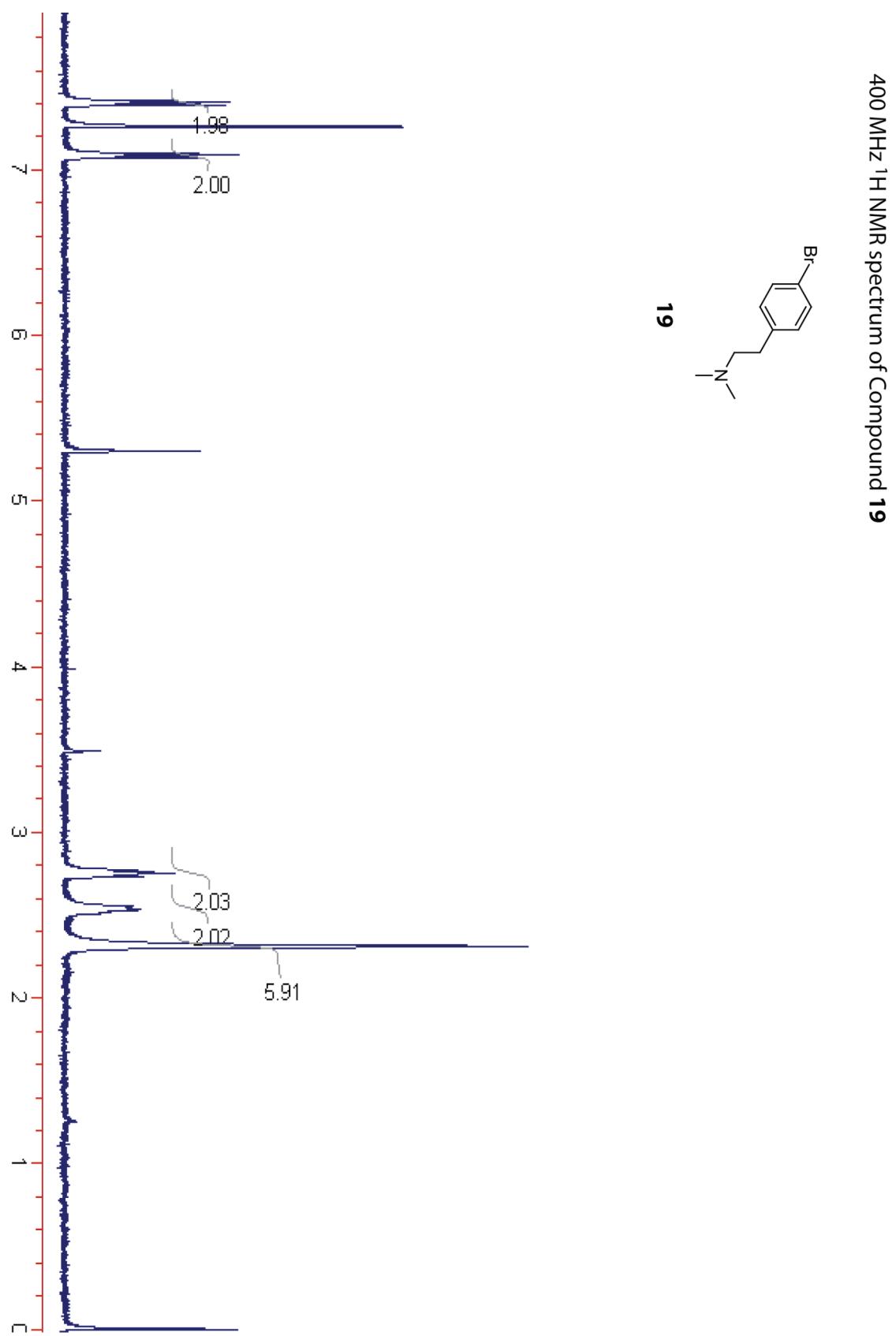
125 MHz ^{13}C NMR spectrum of Compound 17**17**

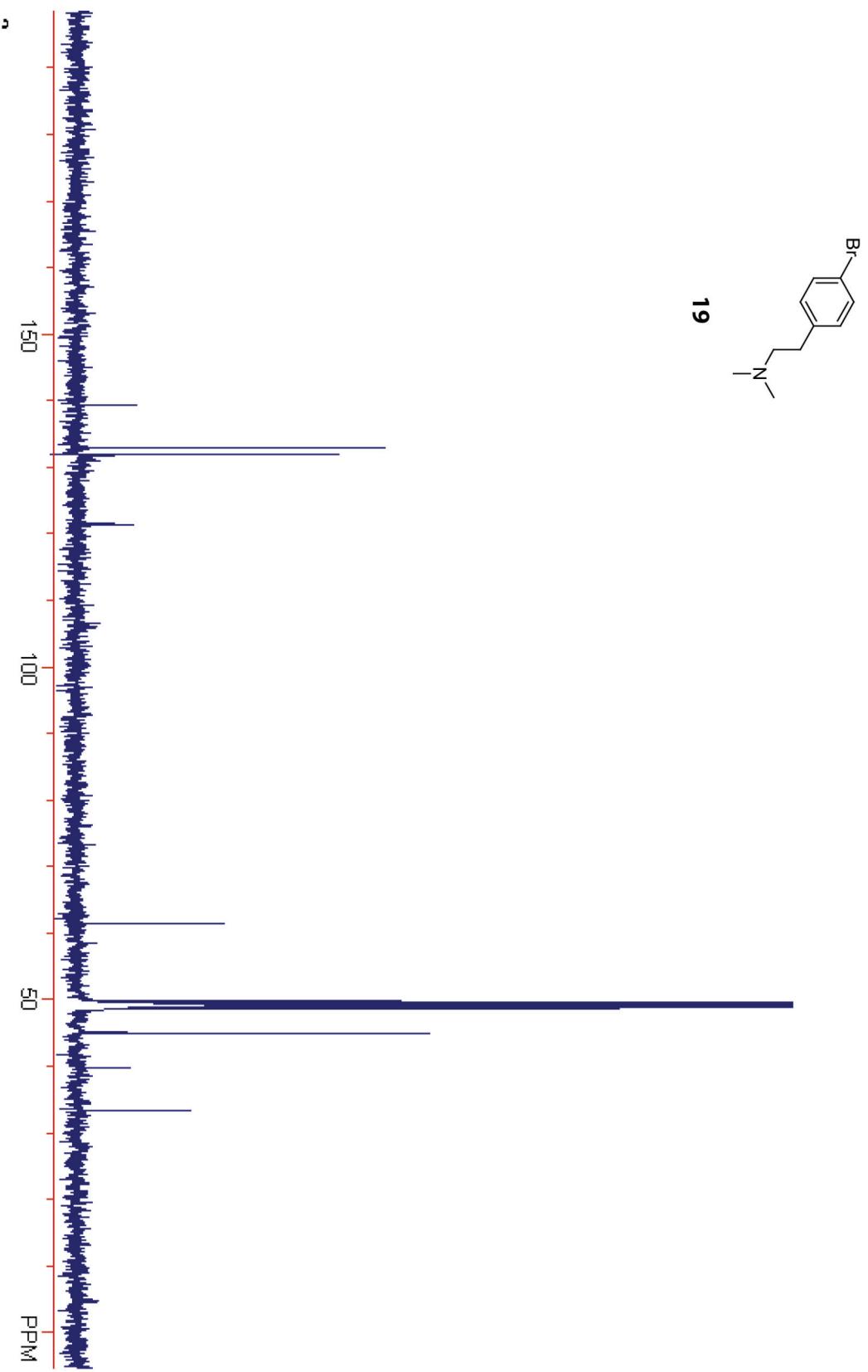


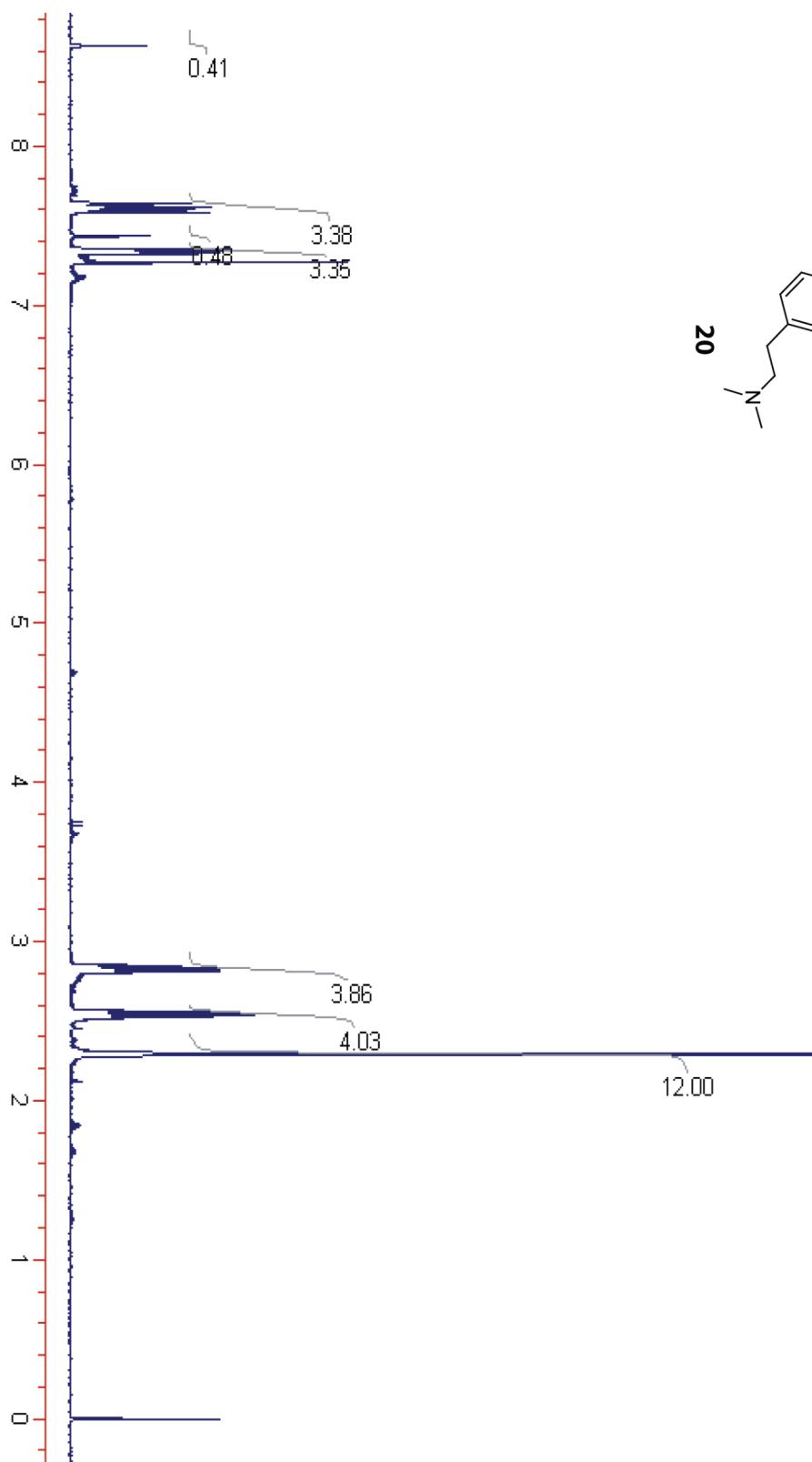
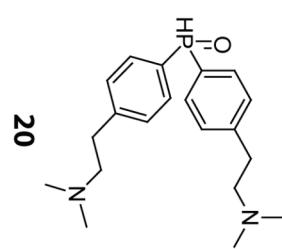
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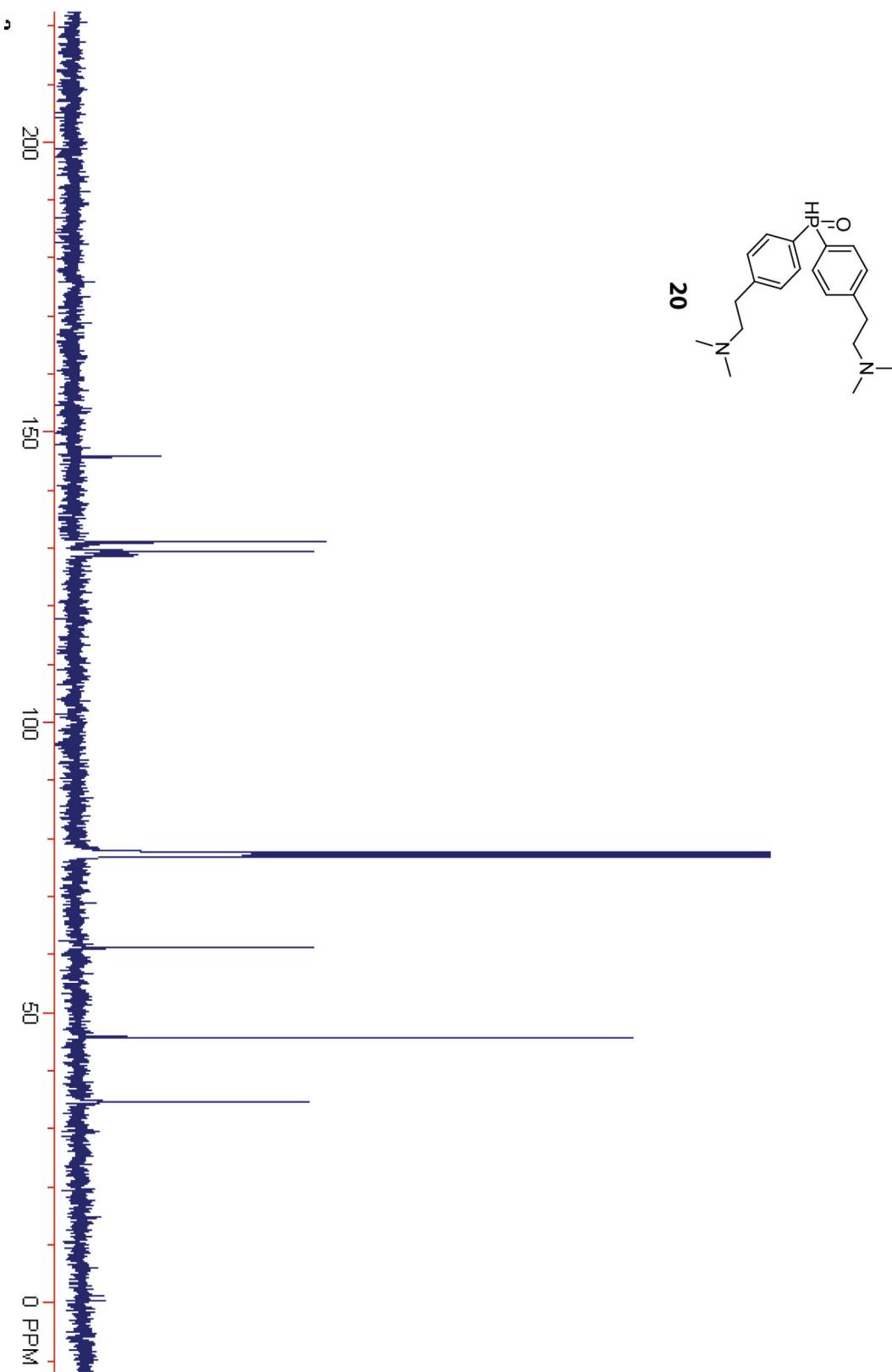
400 MHz ^1H NMR spectrum of Compound **18**

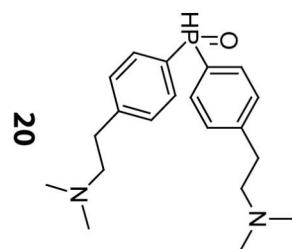
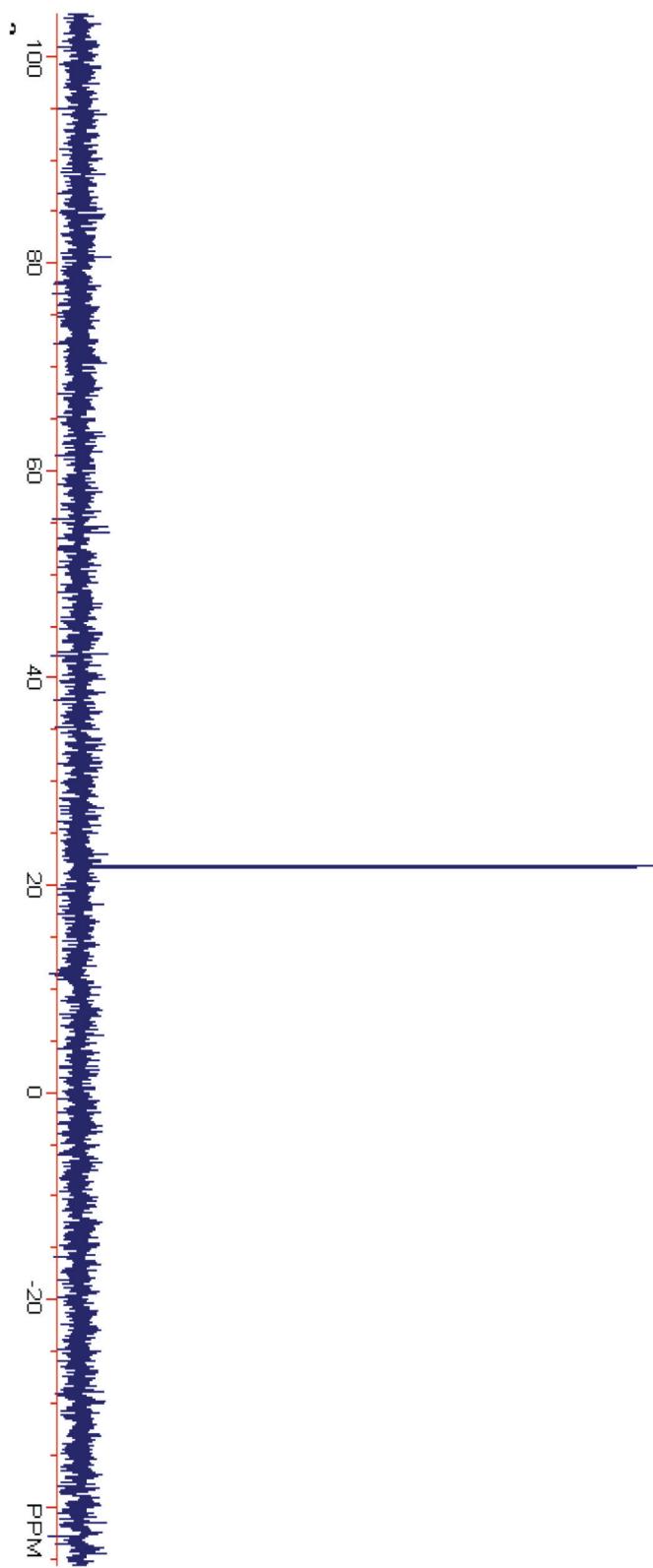
125 MHz ^{13}C NMR spectrum of Compound **18****18**

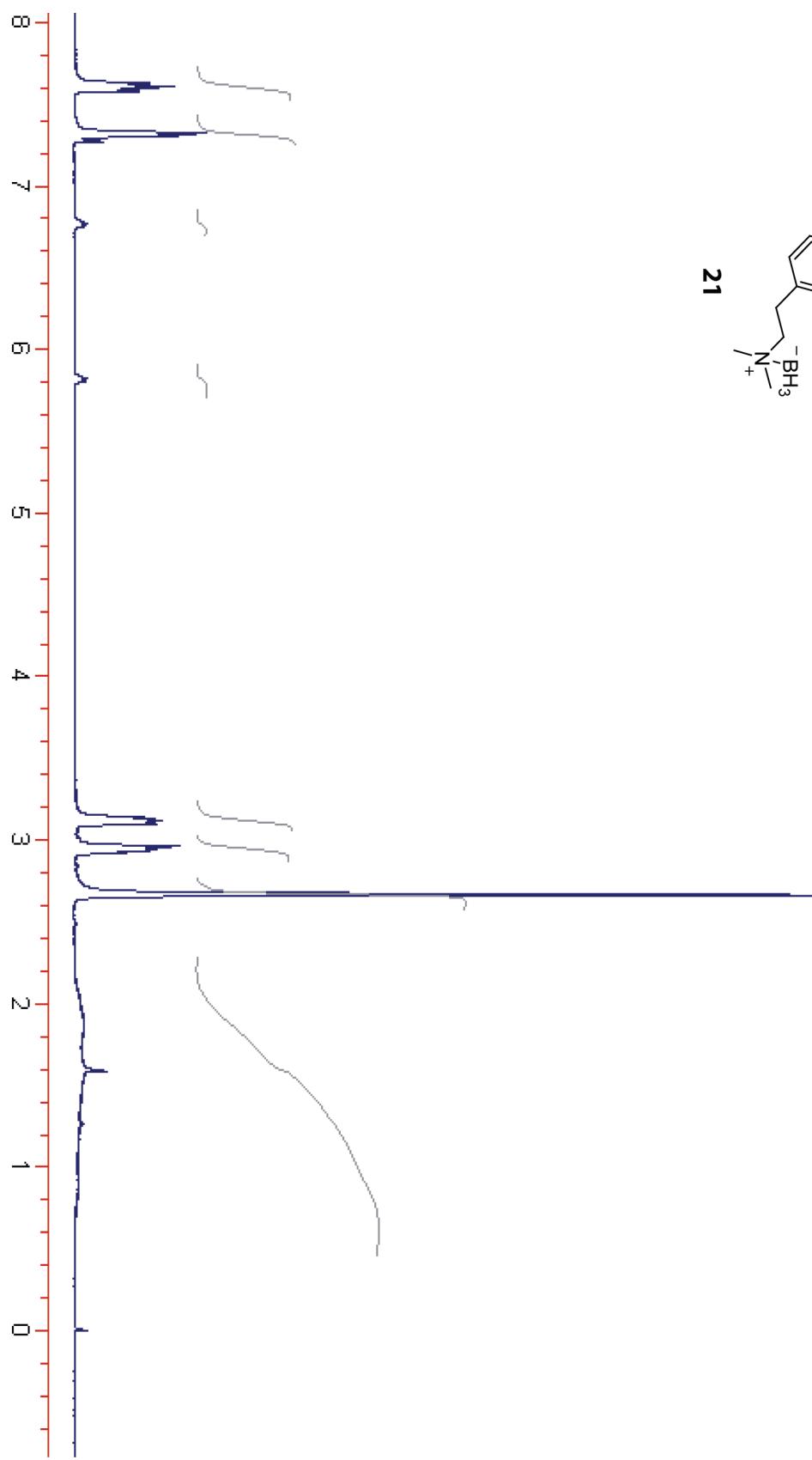
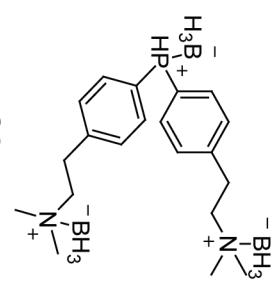


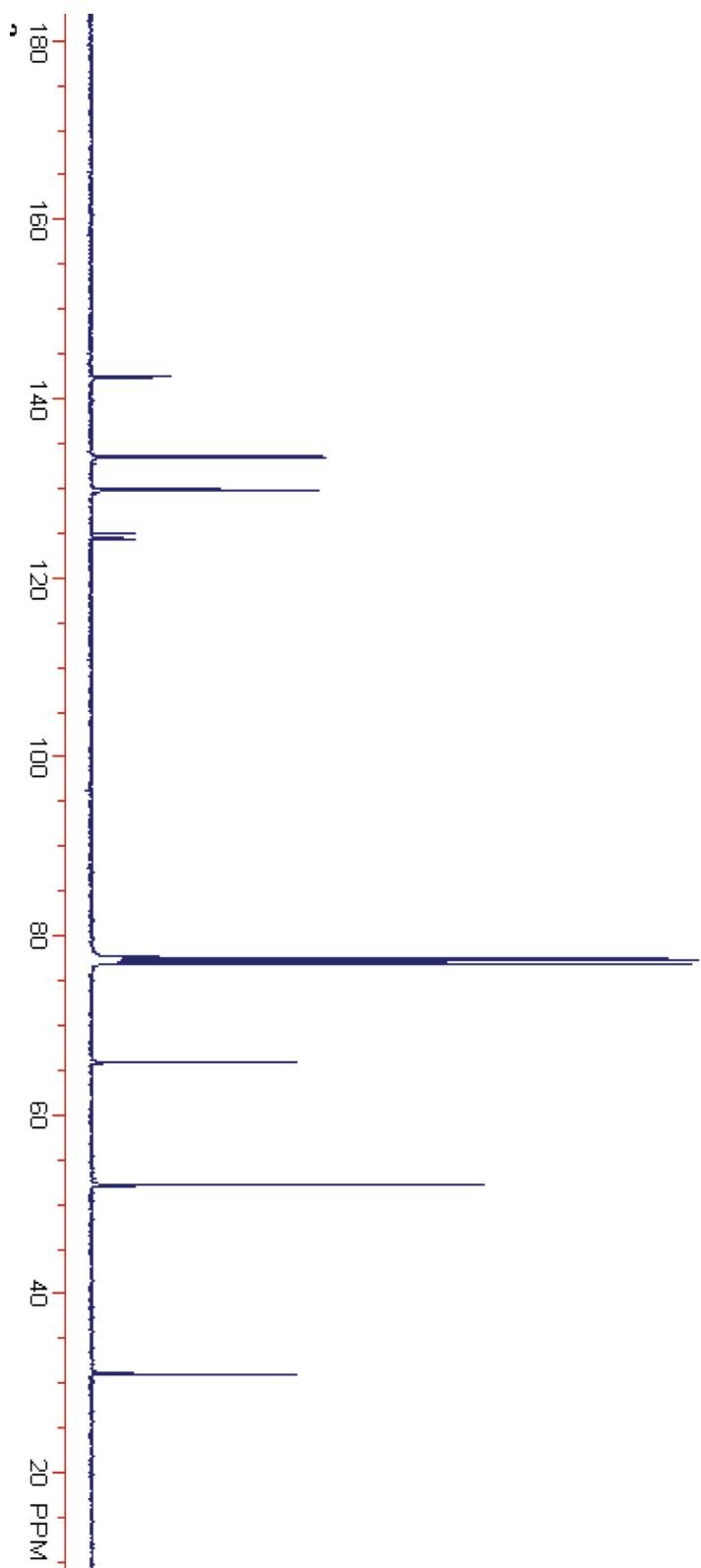
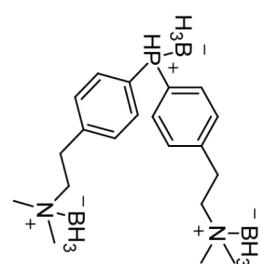


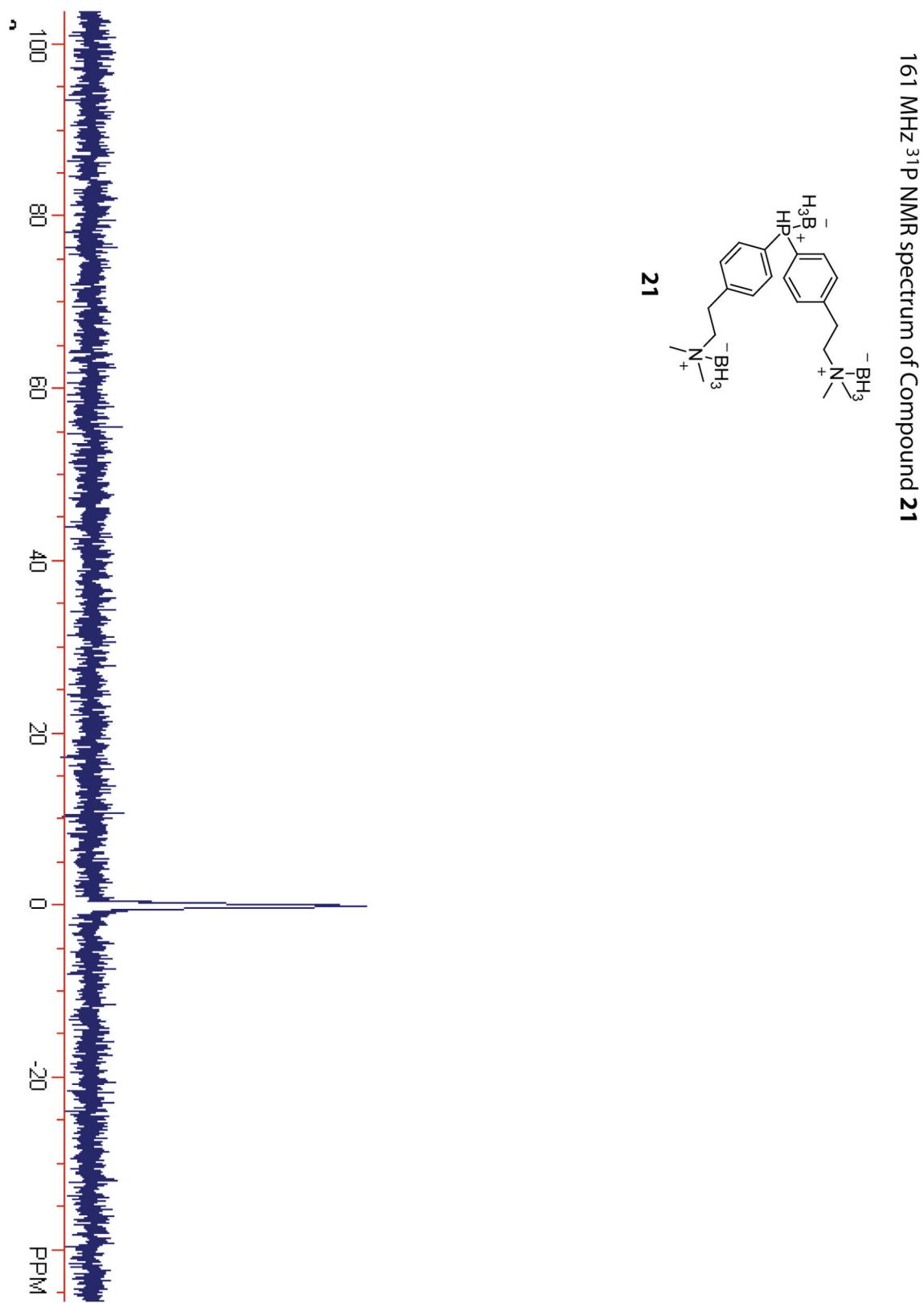
400 MHz ¹H NMR spectrum of Compound 20

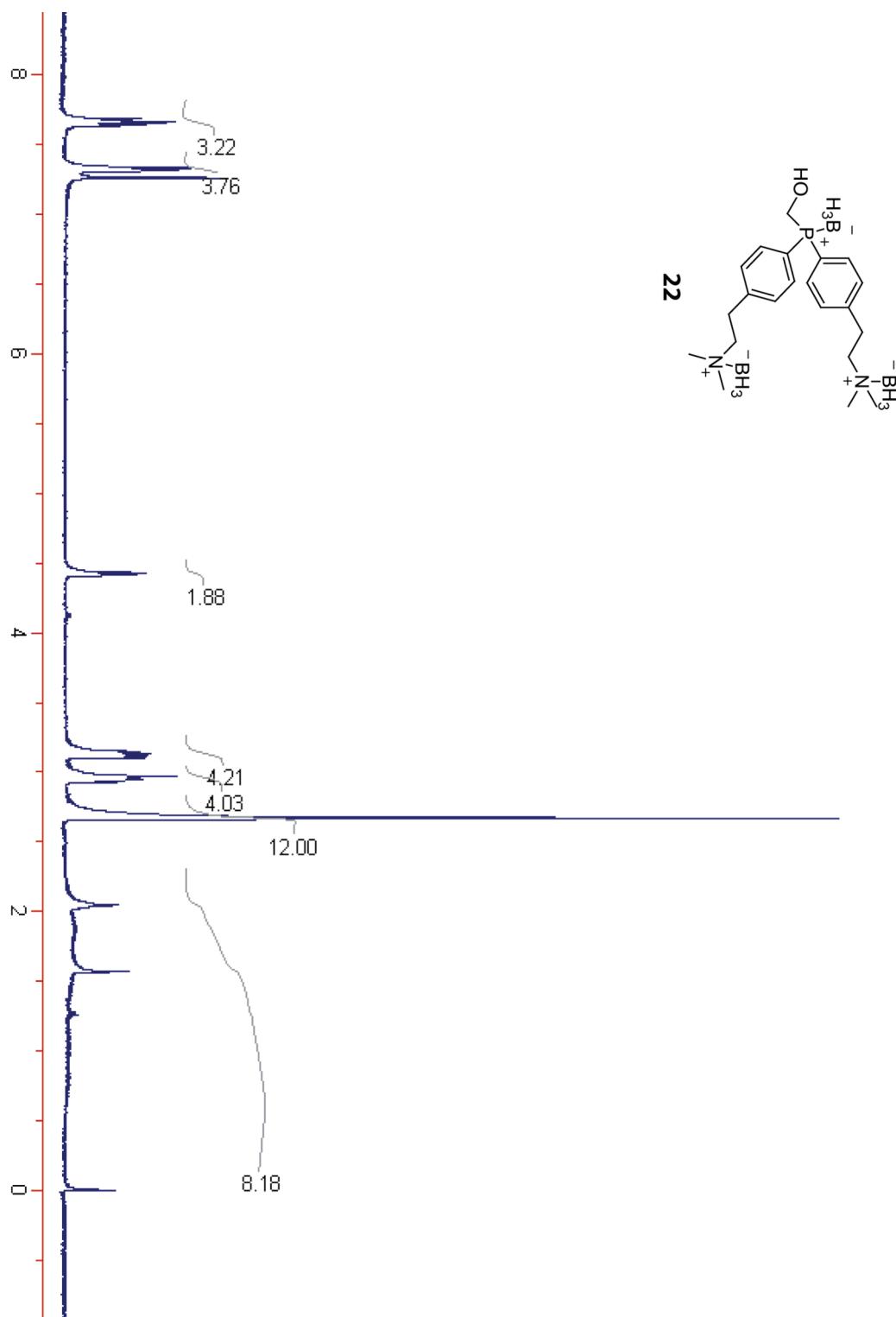
125 MHz ^{13}C NMR spectrum of Compound 20

161 MHz ^{31}P NMR spectrum of Compound 20

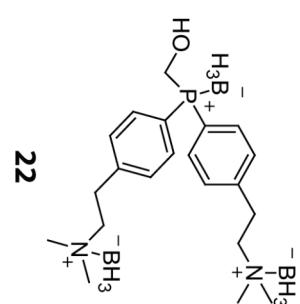
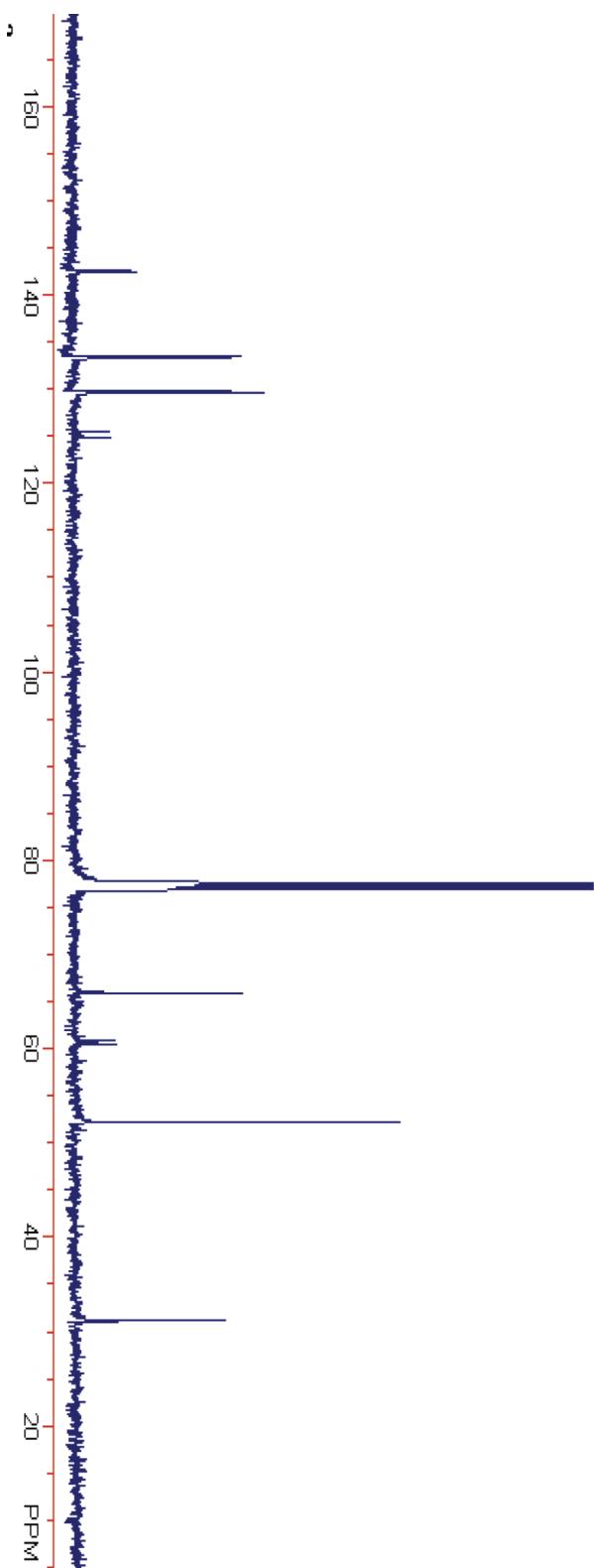
400 MHz ^1H NMR spectrum of Compound 21

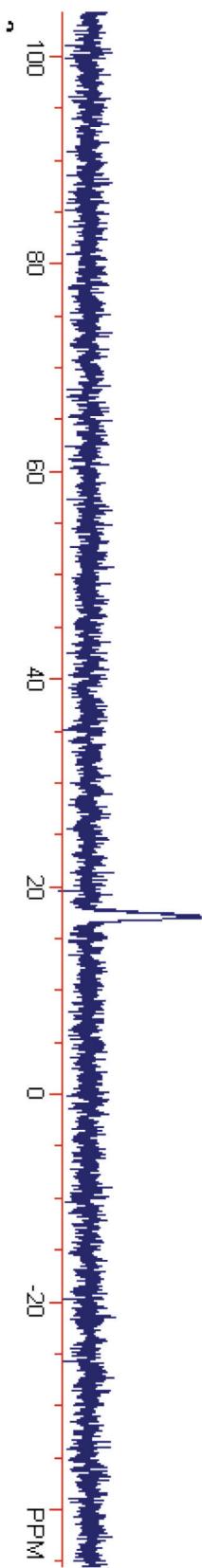
125 MHz ^{13}C NMR spectrum of Compound 21



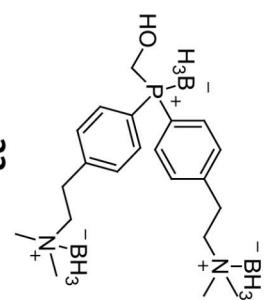
400 MHz ^1H NMR spectrum of Compound 22

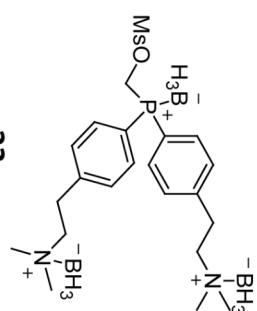
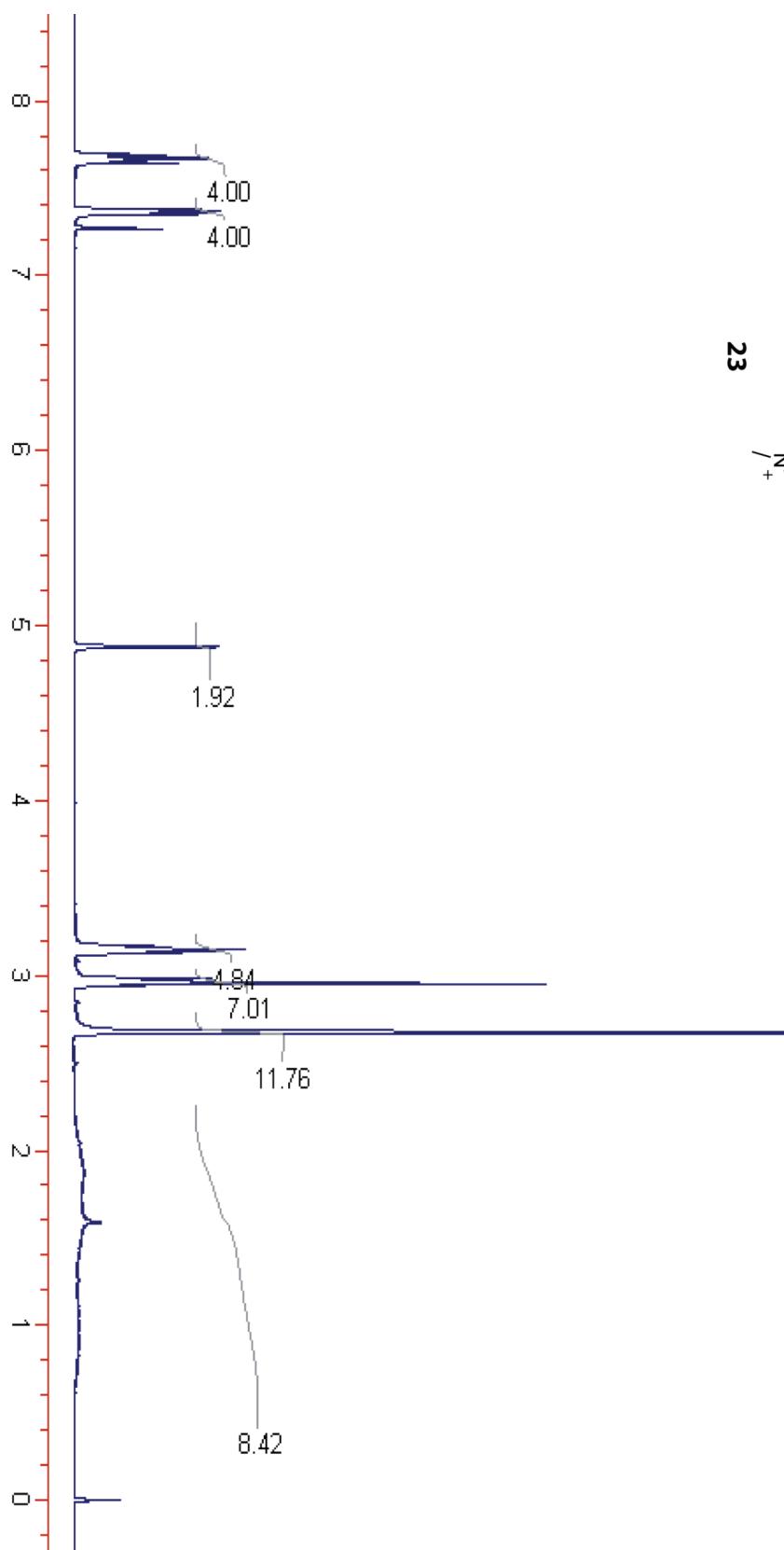
125 MHz ^{13}C NMR spectrum of Compound 22

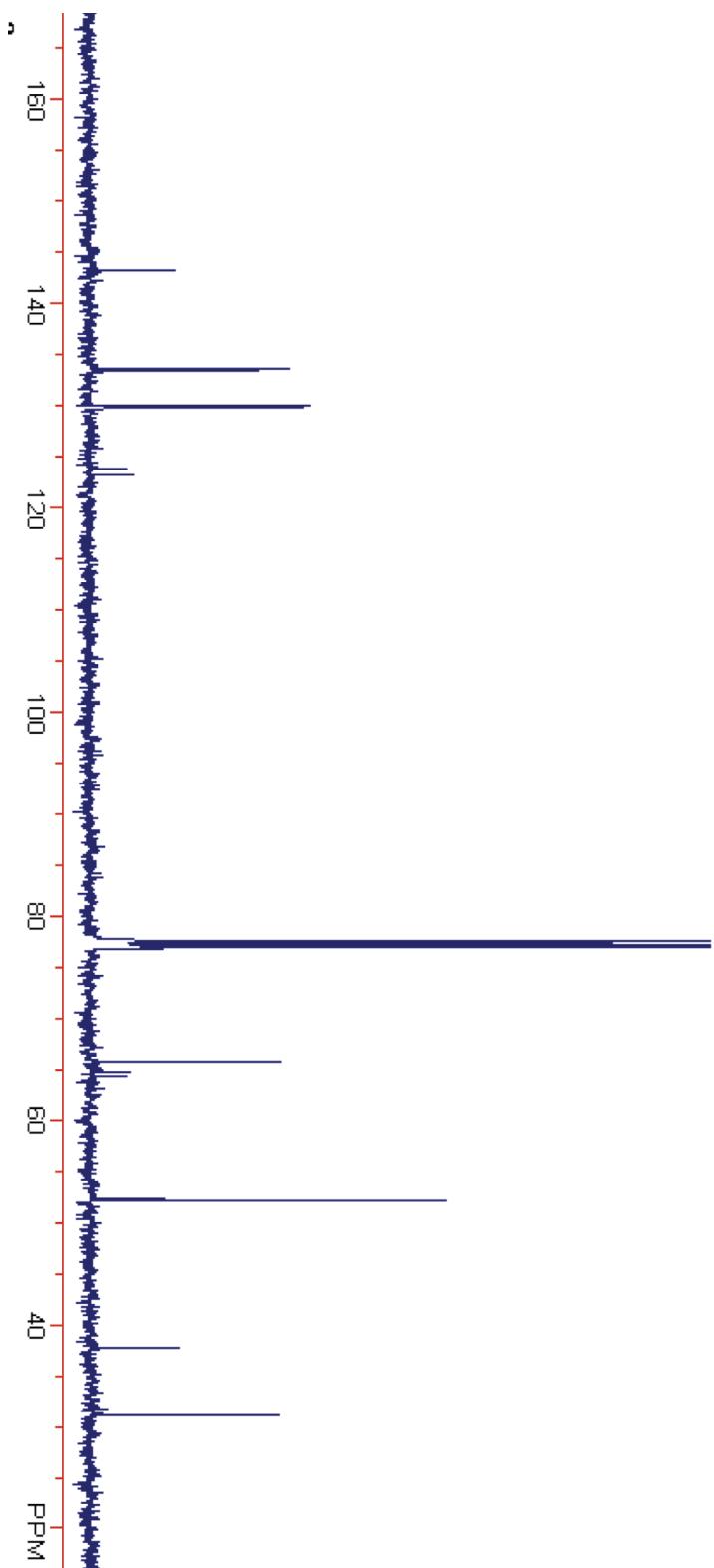
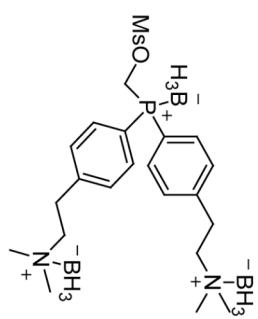


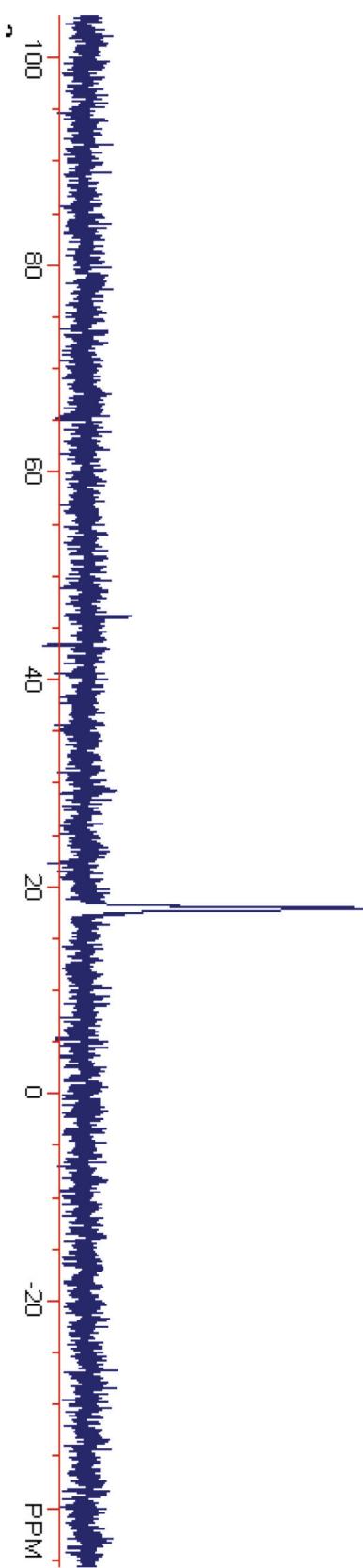
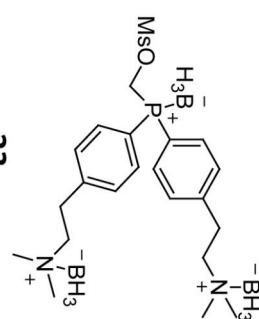


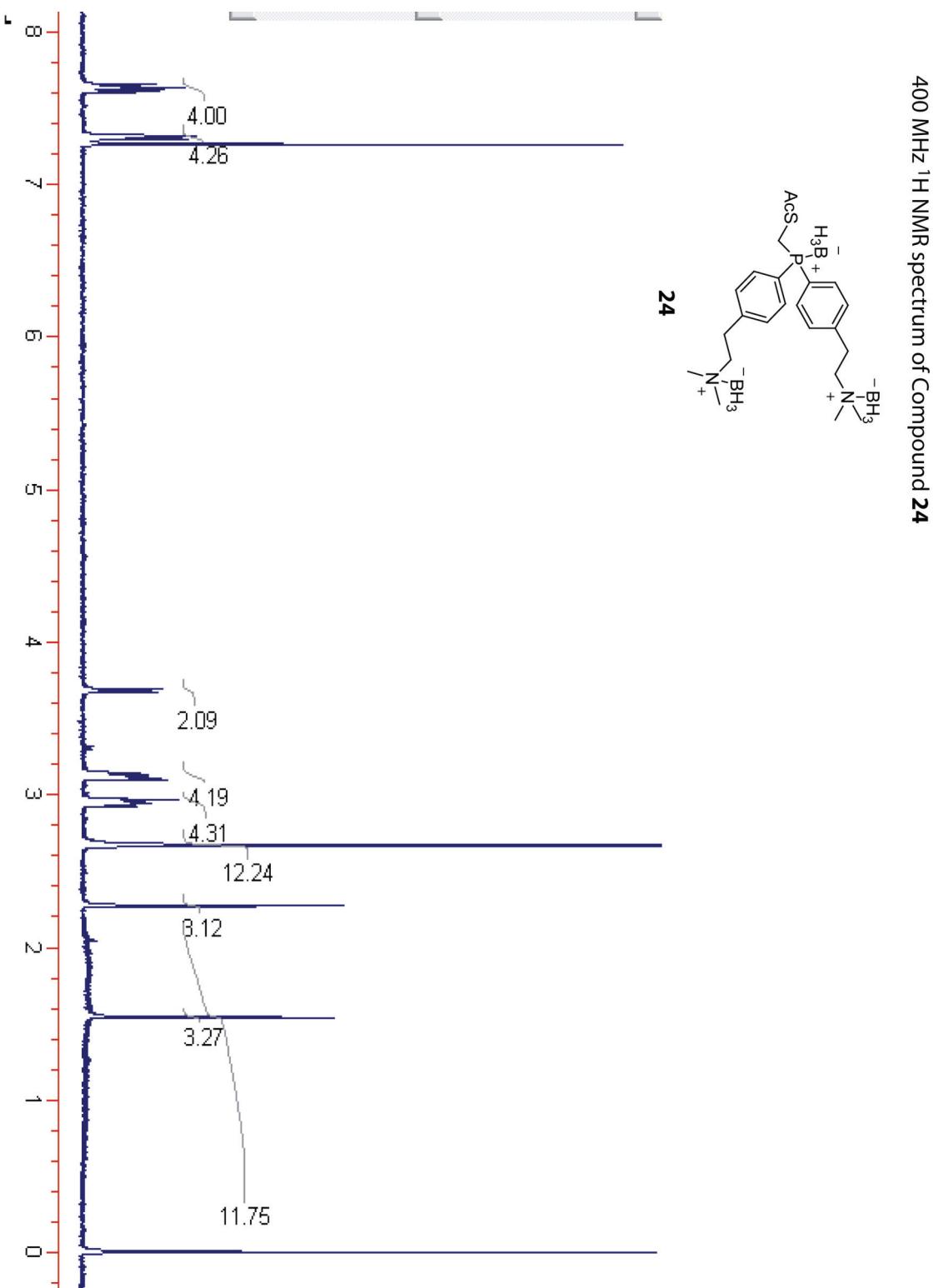
161 MHz ^{31}P NMR spectrum of Compound 22

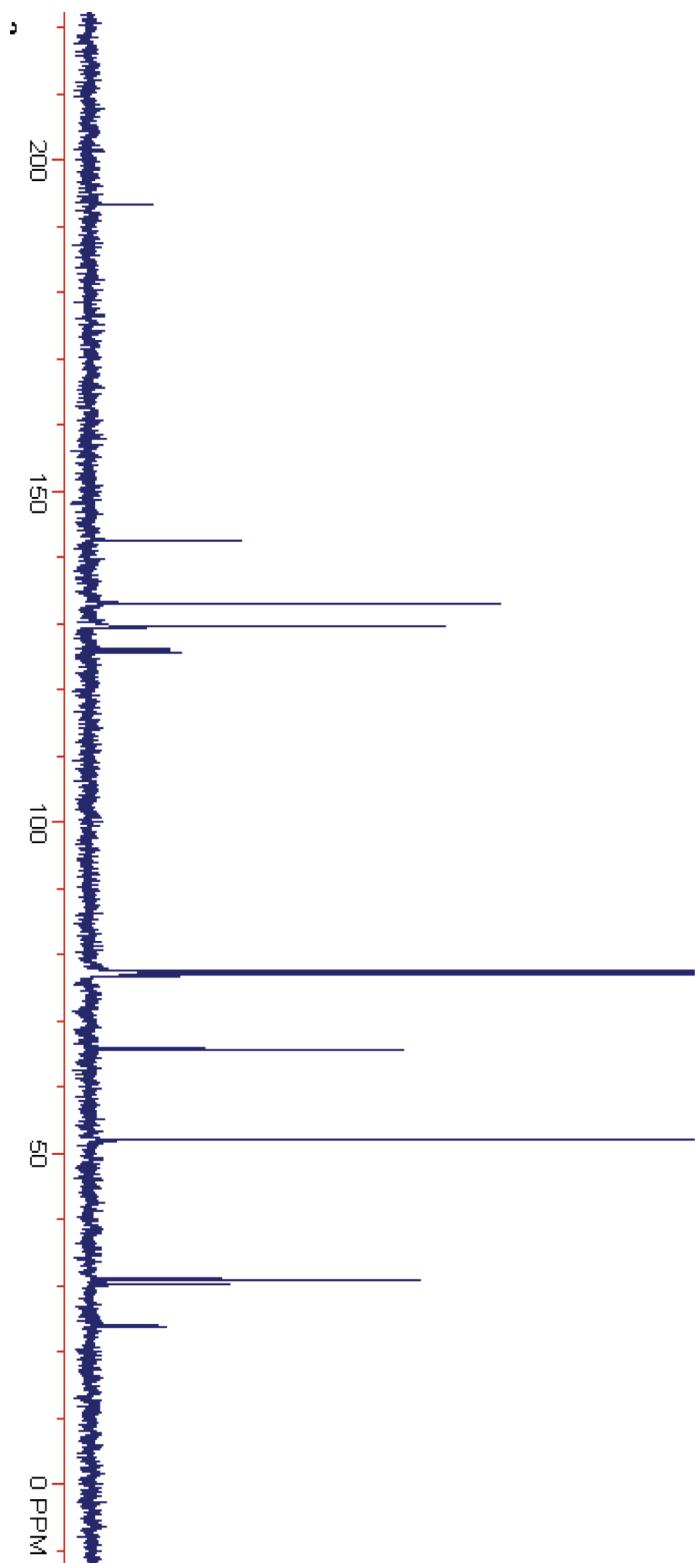
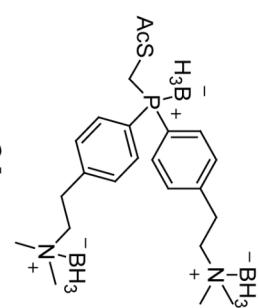


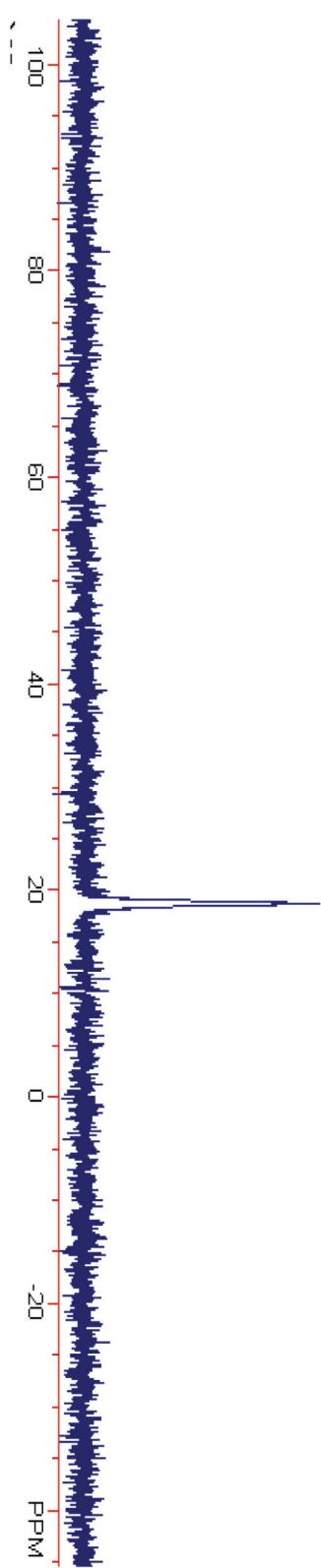
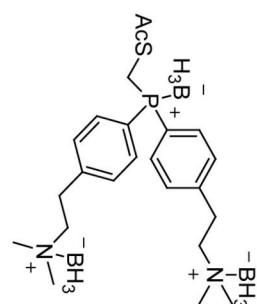
400 MHz ^1H NMR spectrum of Compound **23**

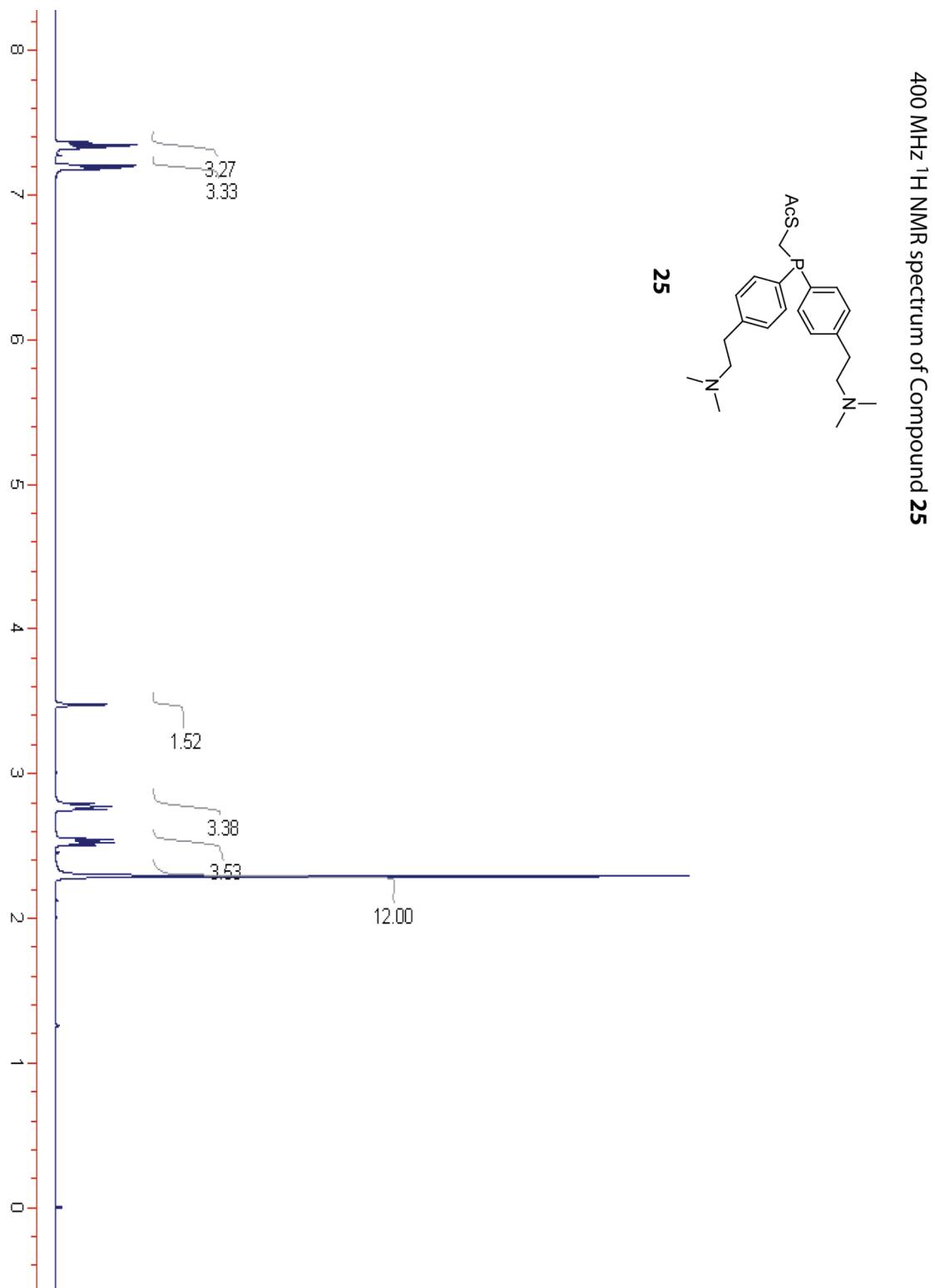
125 MHz ^{13}C NMR spectrum of Compound 23

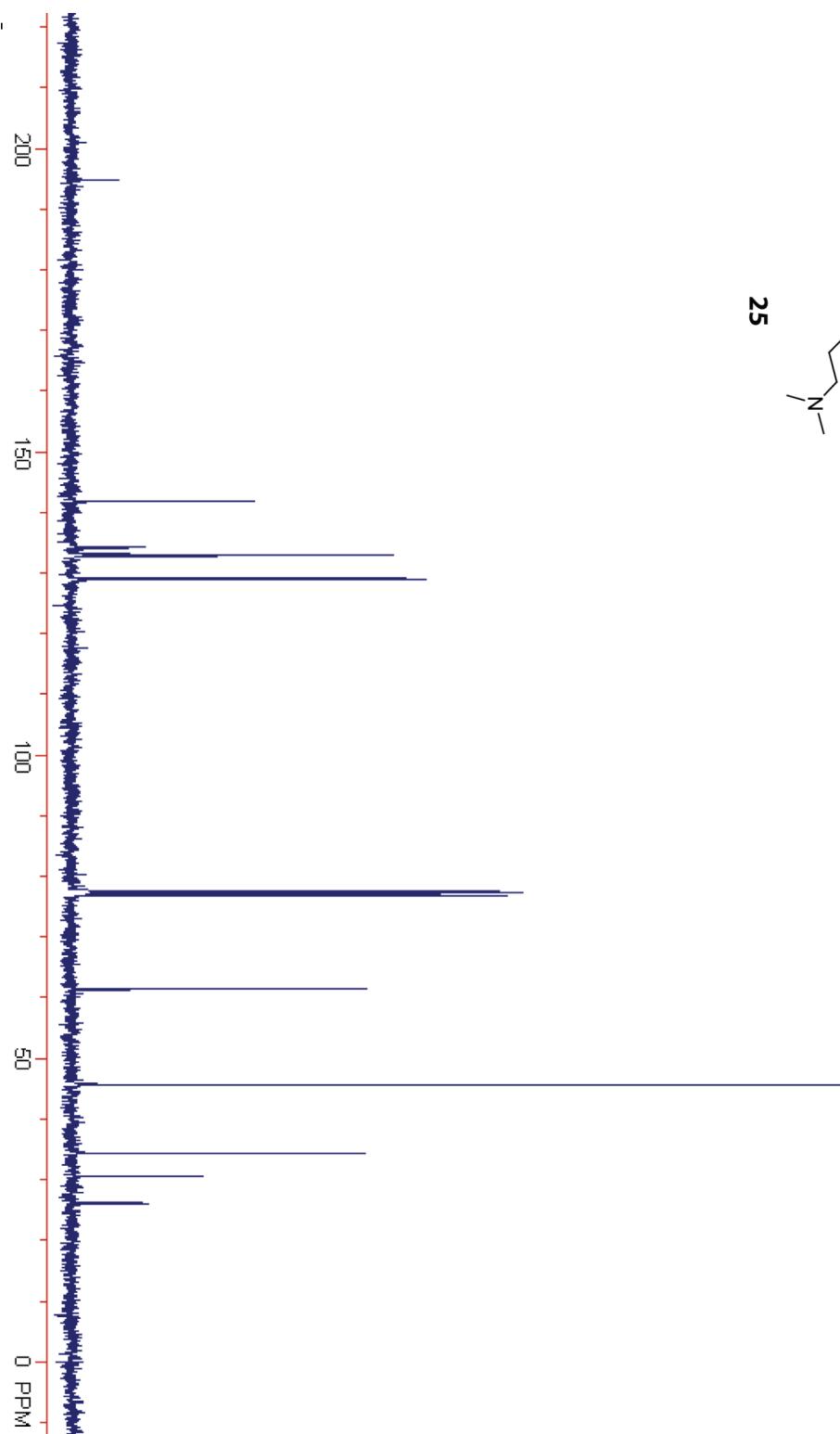
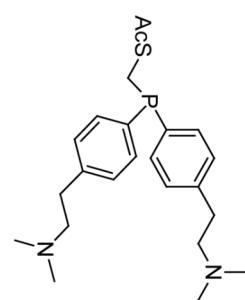
161 MHz ^{31}P NMR spectrum of Compound 23



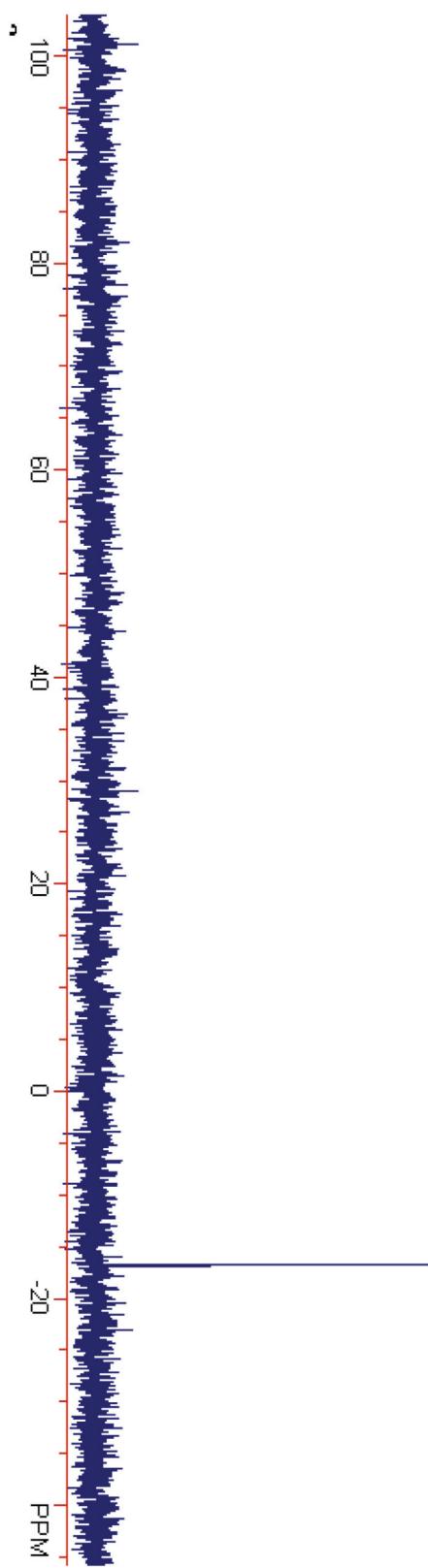
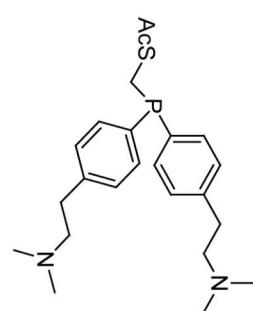
125 MHz ^{13}C NMR spectrum of Compound 24

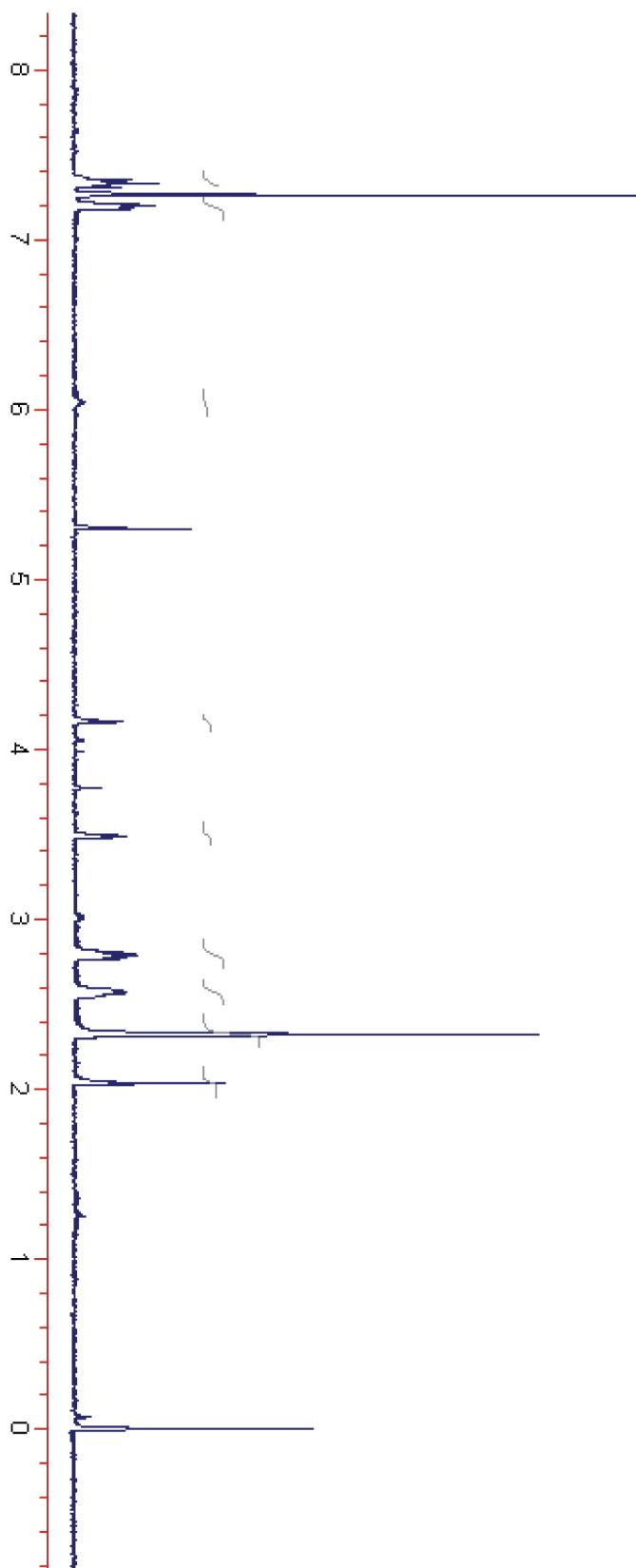
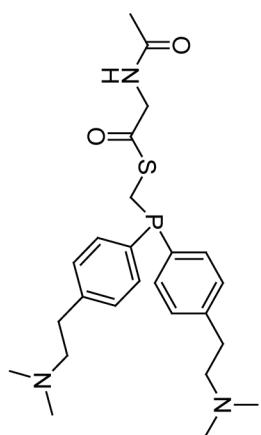
161 MHz ^{31}P NMR spectrum of Compound 24

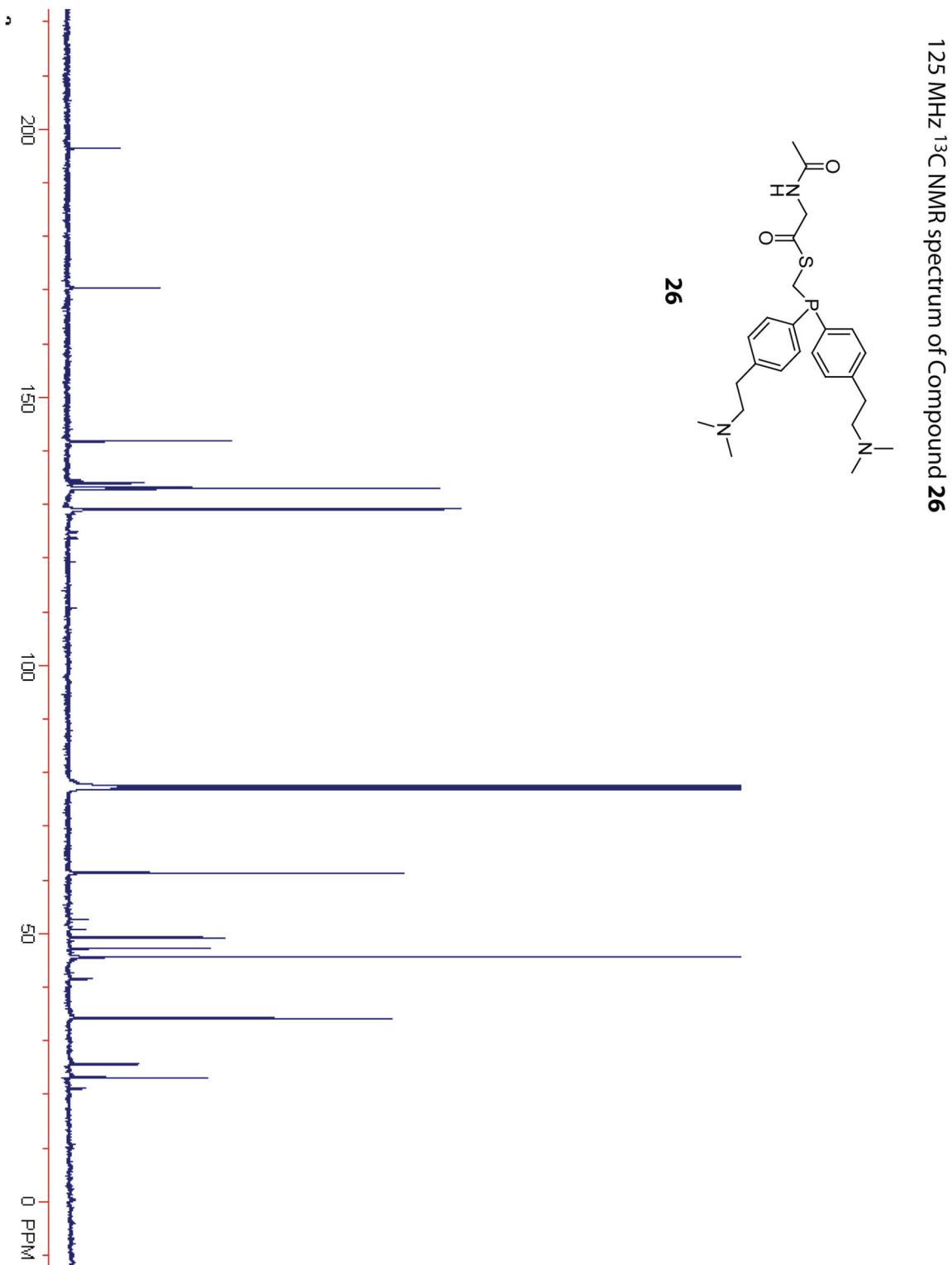


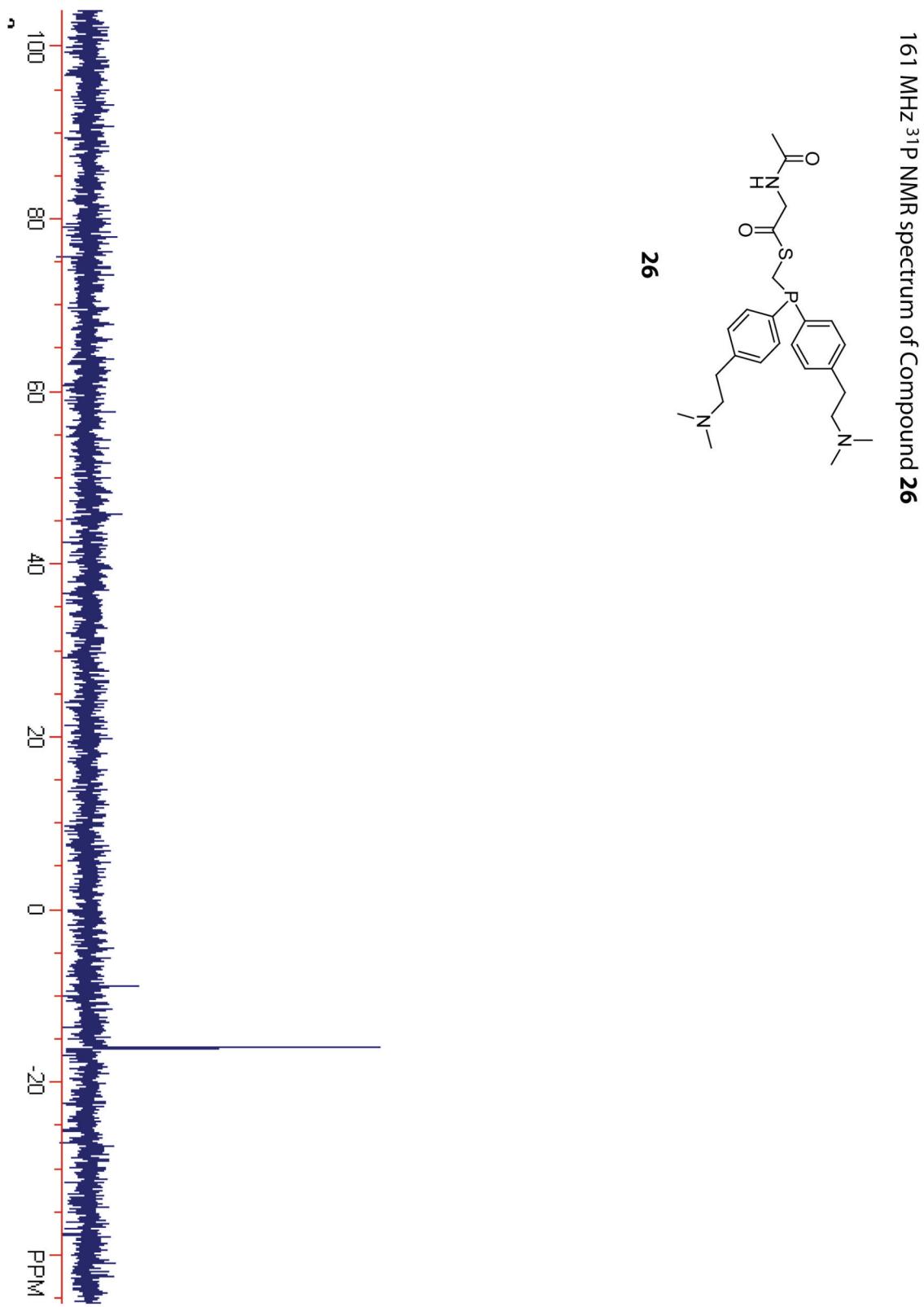
125 MHz ^{13}C NMR spectrum of Compound 25

161 MHz ^{31}P NMR spectrum of Compound 25

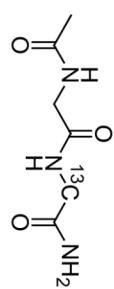


400 MHz ^1H NMR spectrum of Compound 26**26**

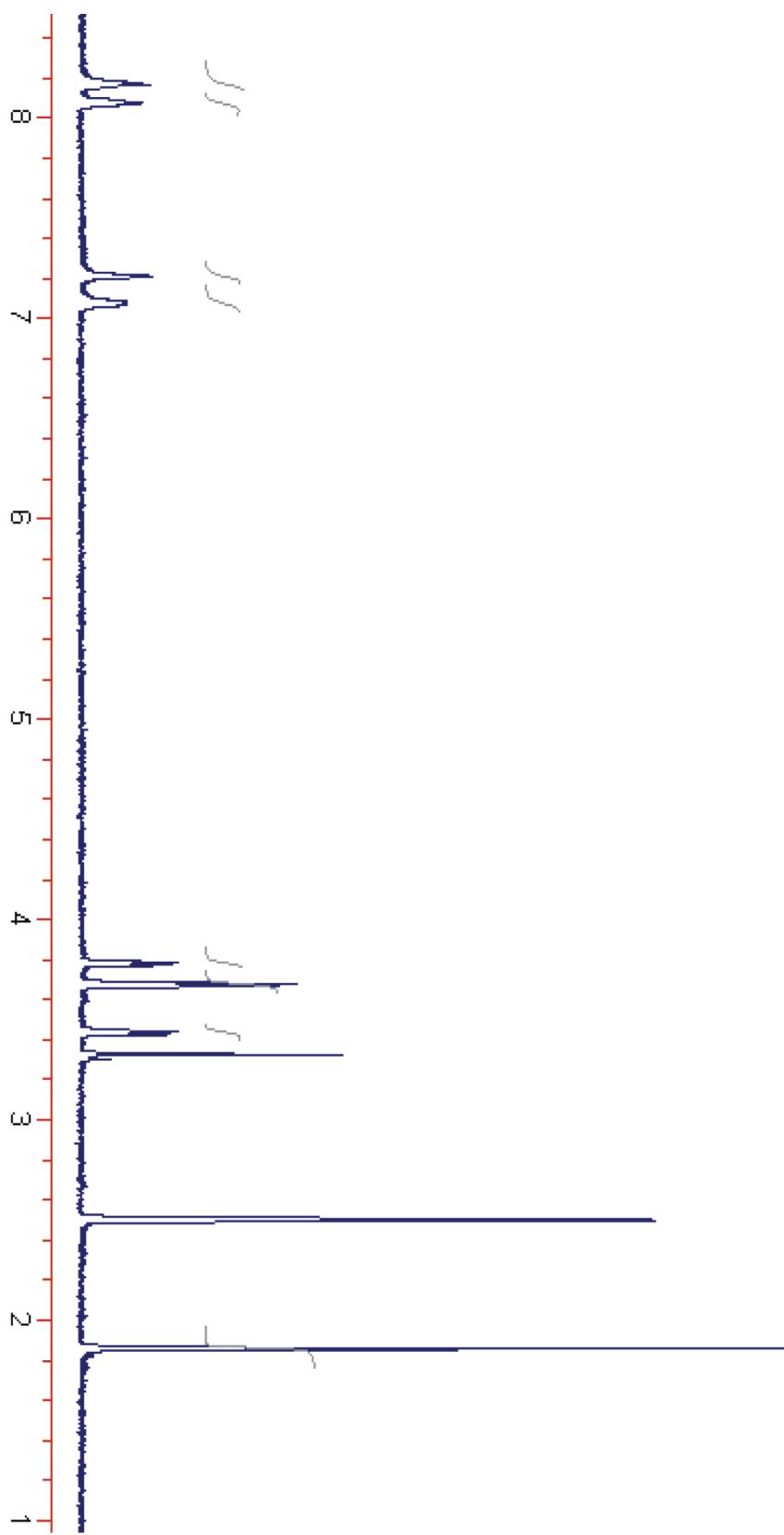




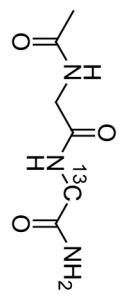
400 MHz ^1H NMR spectrum of Compound 27



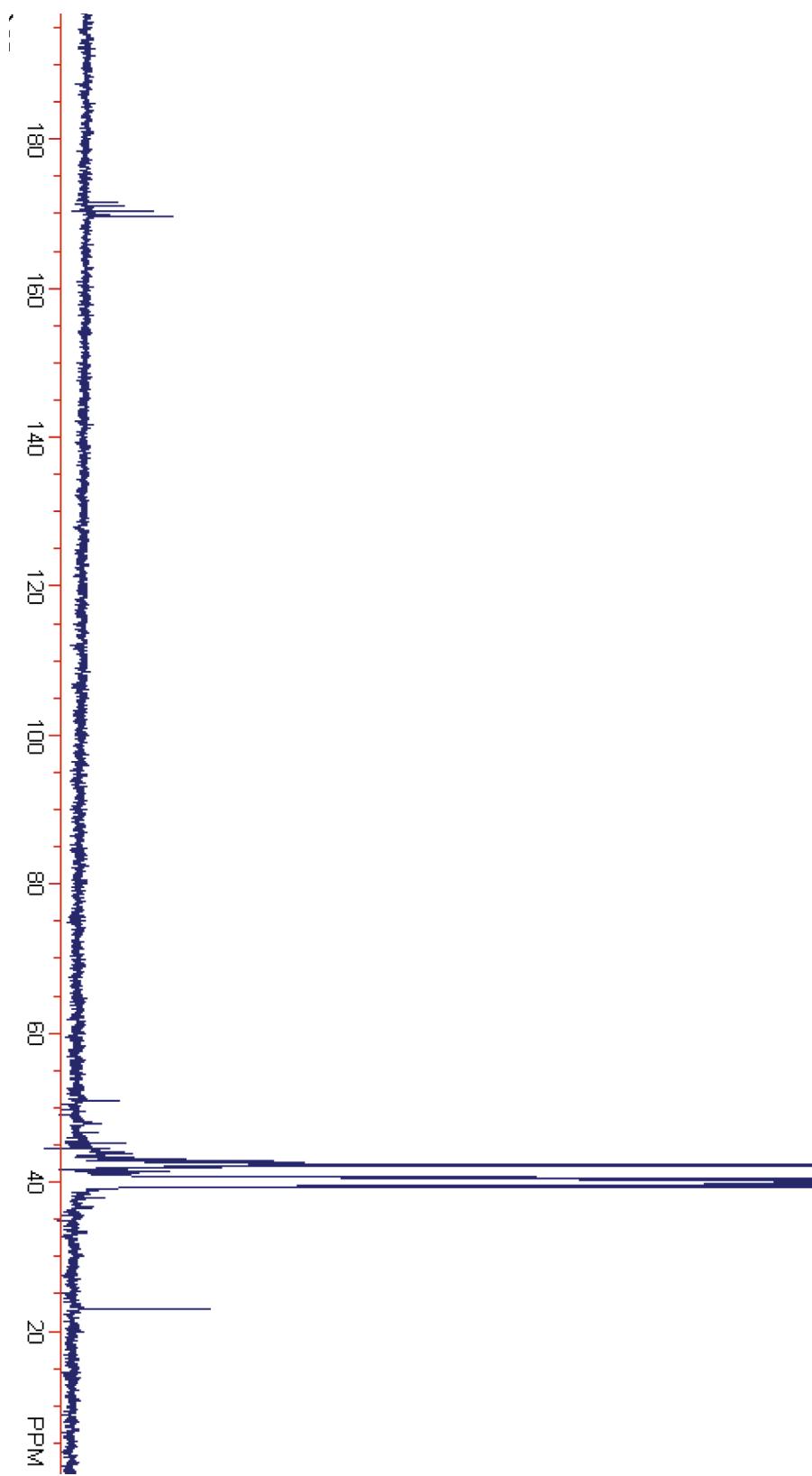
27

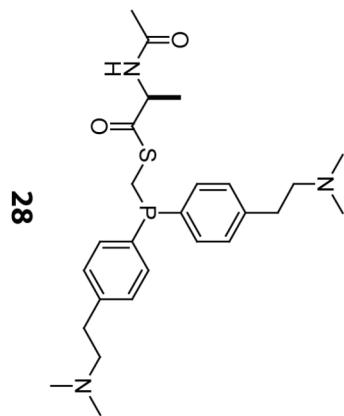
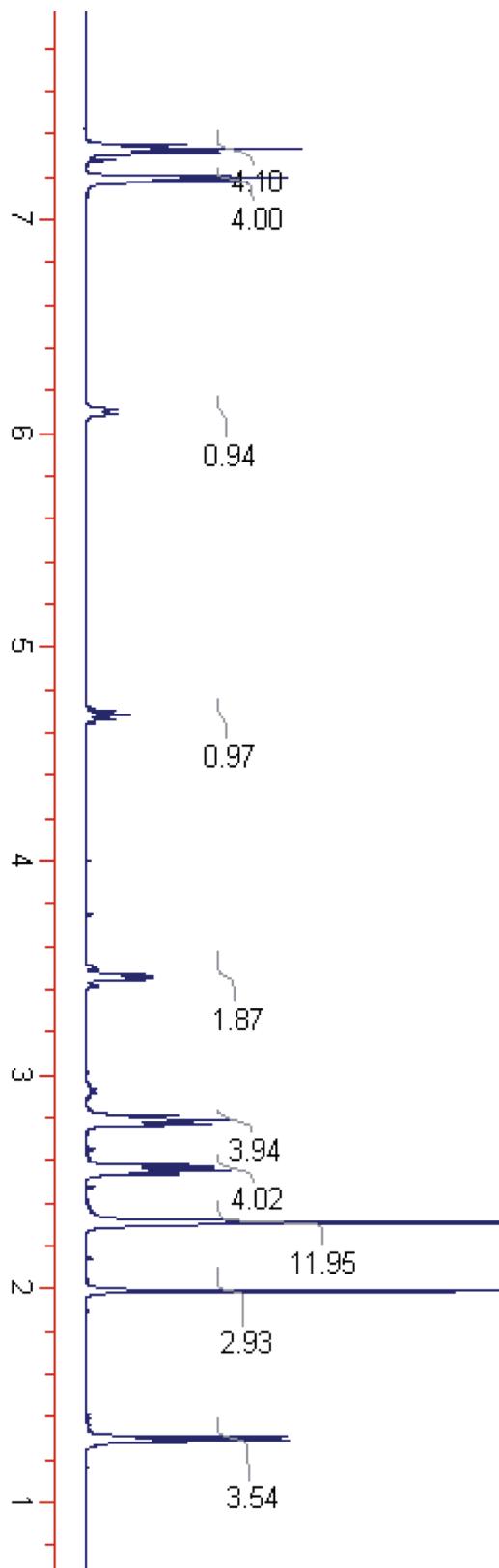


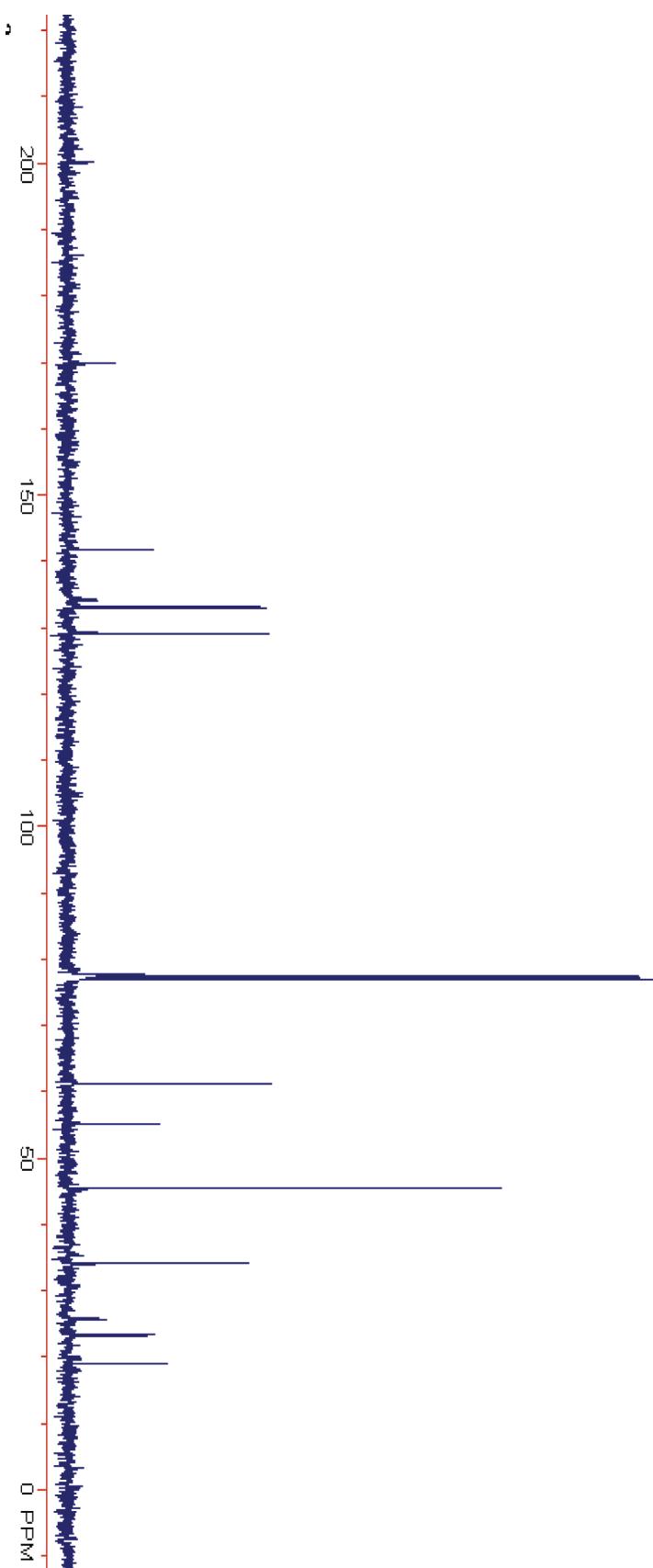
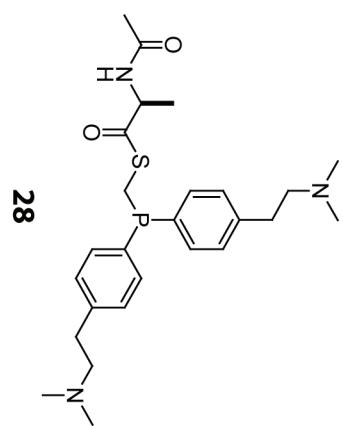
125 MHz ^{13}C NMR spectrum of Compound 27

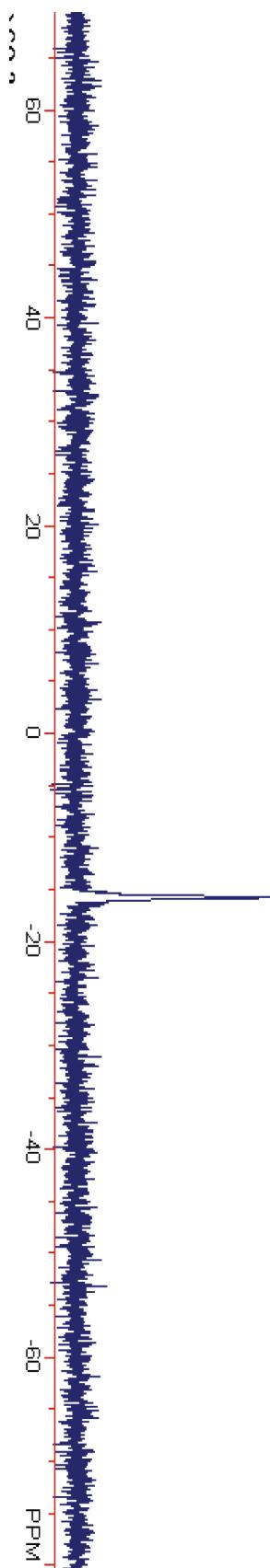
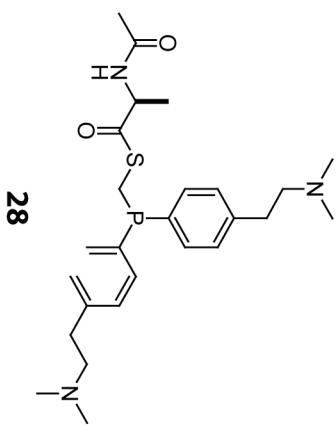


27

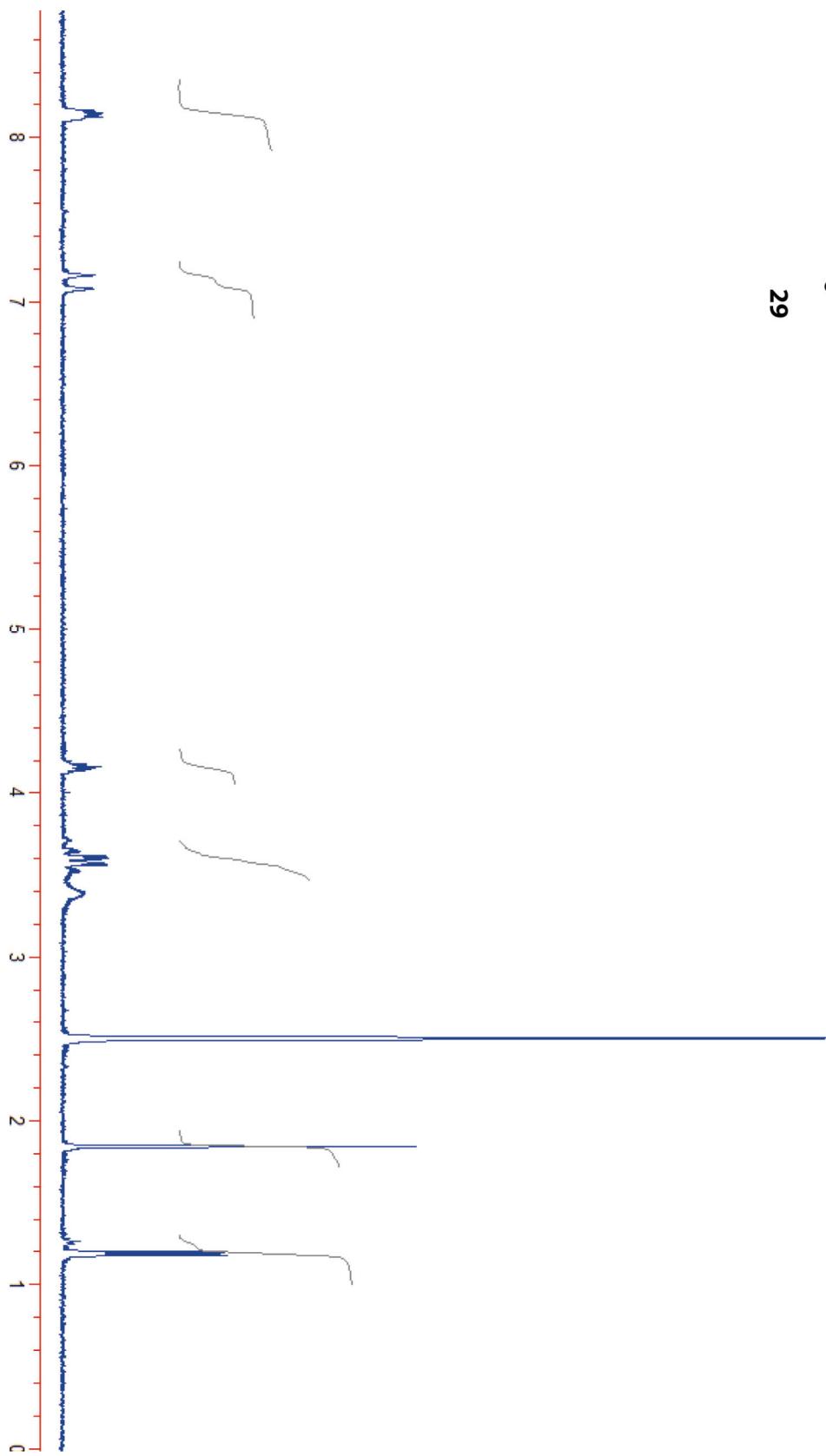
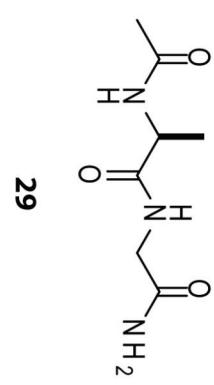


400 MHz ^1H NMR spectrum of Compound **28**

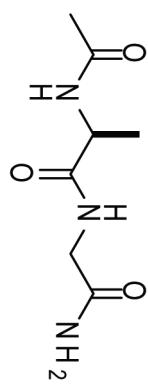
125 MHz ^{13}C NMR spectrum of Compound 28

161 MHz ^{31}P NMR spectrum of Compound **28**

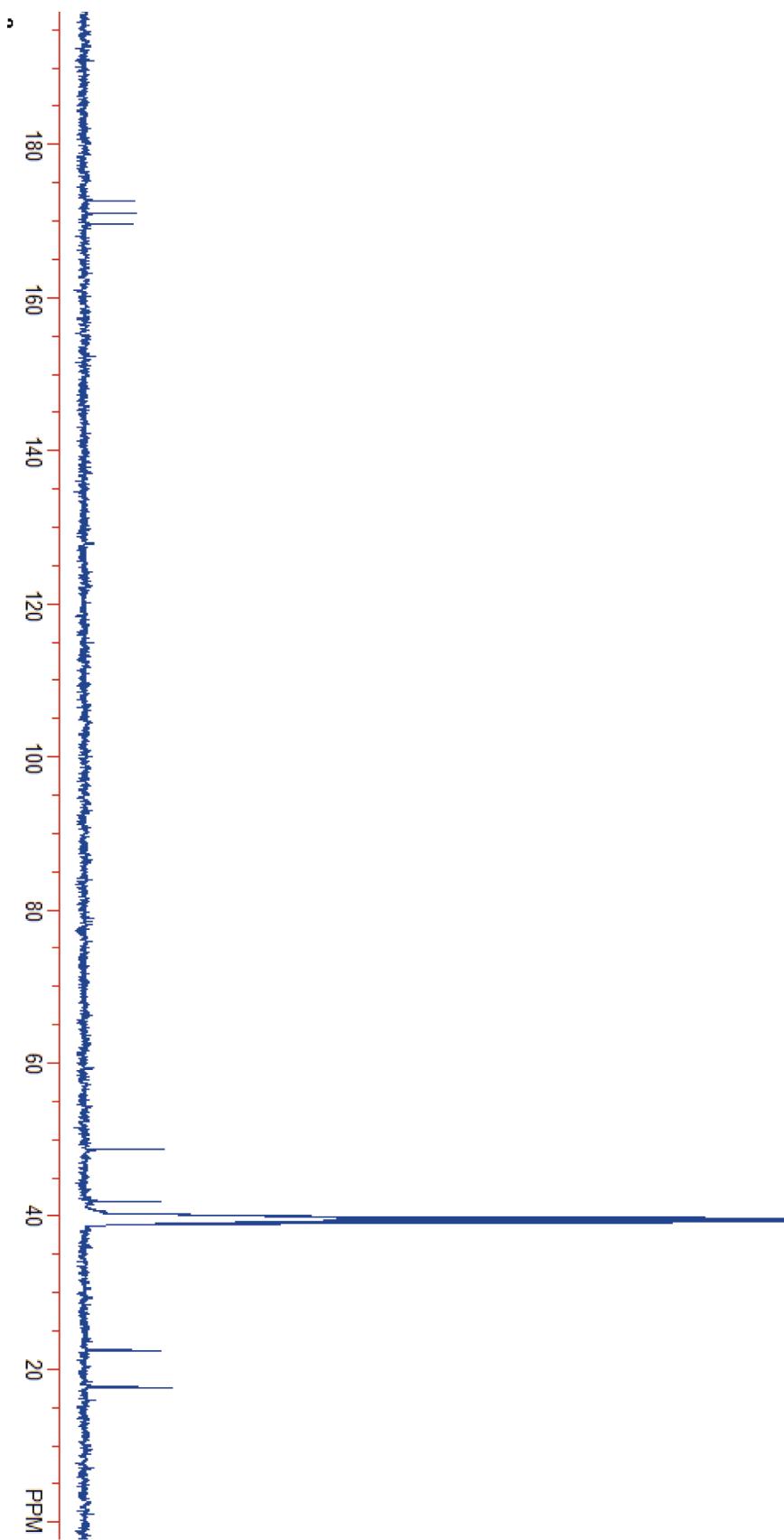
400 MHz ^1H NMR spectrum of Compound **29**

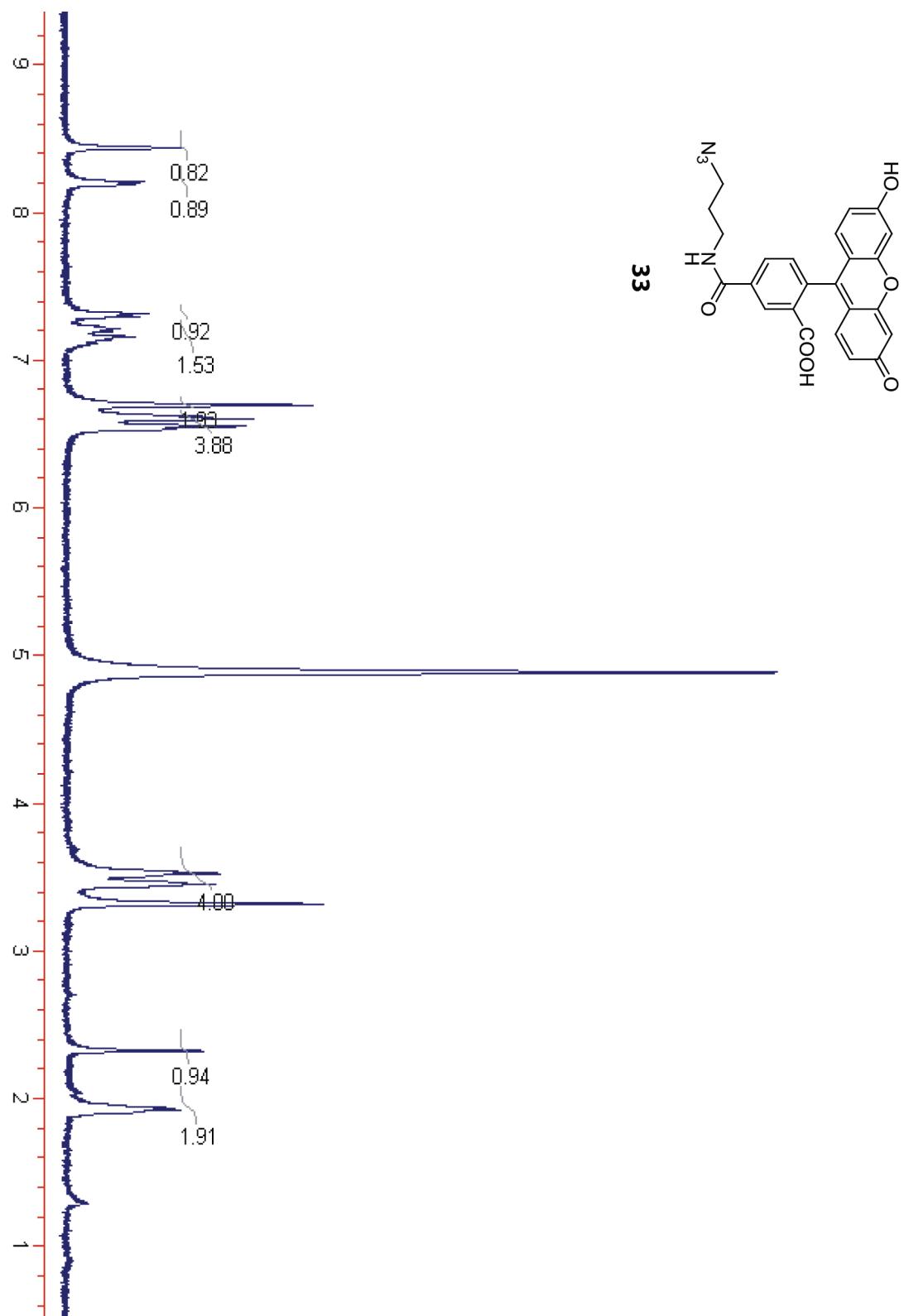


125 MHz ^{13}C NMR spectrum of Compound **29**



29



400 MHz ^1H NMR spectrum of Compound 33

125 MHz ^{13}C NMR spectrum of Compound 33