α-Azido bisphosphonates: synthesis and nucleotide analogues

*Brian T. Chamberlain, Thomas G. Upton, Boris A. Kashemirov and Charles E. McKenna**

Department of Chemistry, University of Southern California, Los Angeles, CA 90089-0744 USA

*mckenna@usc.edu

Supporting Information

Table of Contents

Materials and Methods

Proton spectra were referenced to residual $CHCl_3$ (δ 7.24) in $CDCl_3$ or to HDO (δ 4.79). ^{13}C spectra were referenced to internal $CDCl_3$ (δ 77.03) or internal carbonate (δ 168.88). ^{31}P NMR spectra were referenced to external 85 % H_3PO_4 (capillary, δ 0.00). pH values are reported without a deuterium isotope correction. IR samples were prepared for **1** and **2** as films on NaCl disks, and for **3** and **4** as solid KBr pellets.

Preparation of azido transfer reagents

The desired sulfonyl chloride (1 equiv.) was dissolved in acetone and mixed with a solution of 3.5 equiv. of sodium azide in water, then stirred overnight at room temperature. The sulfonyl azide was precipitated by the addition of excess water, extracted into hexanes, dried over $MgSO_4$, and concentrated under reduced pressure. The sulfonyl azide reagents were used without further purification.

Preparation of tert-butyl hypochlorite, *t*-BuOCl

To ice cold Clorox® (250 mL), a solution of 18.5 mL *t*-BuOH and 12.25 mL glacial acetic acid was added quickly. The mixture was stirred and poured into a separatory funnel where it was washed with 25 mL 10 % Na_2CO_3.[1] The organic layer was dried and stored over $CaCl_2$ and used without further purification.

Figure S1. ^{1}H NMR spectrum ($CDCl_3$; 399.8 MHz) of **1**.

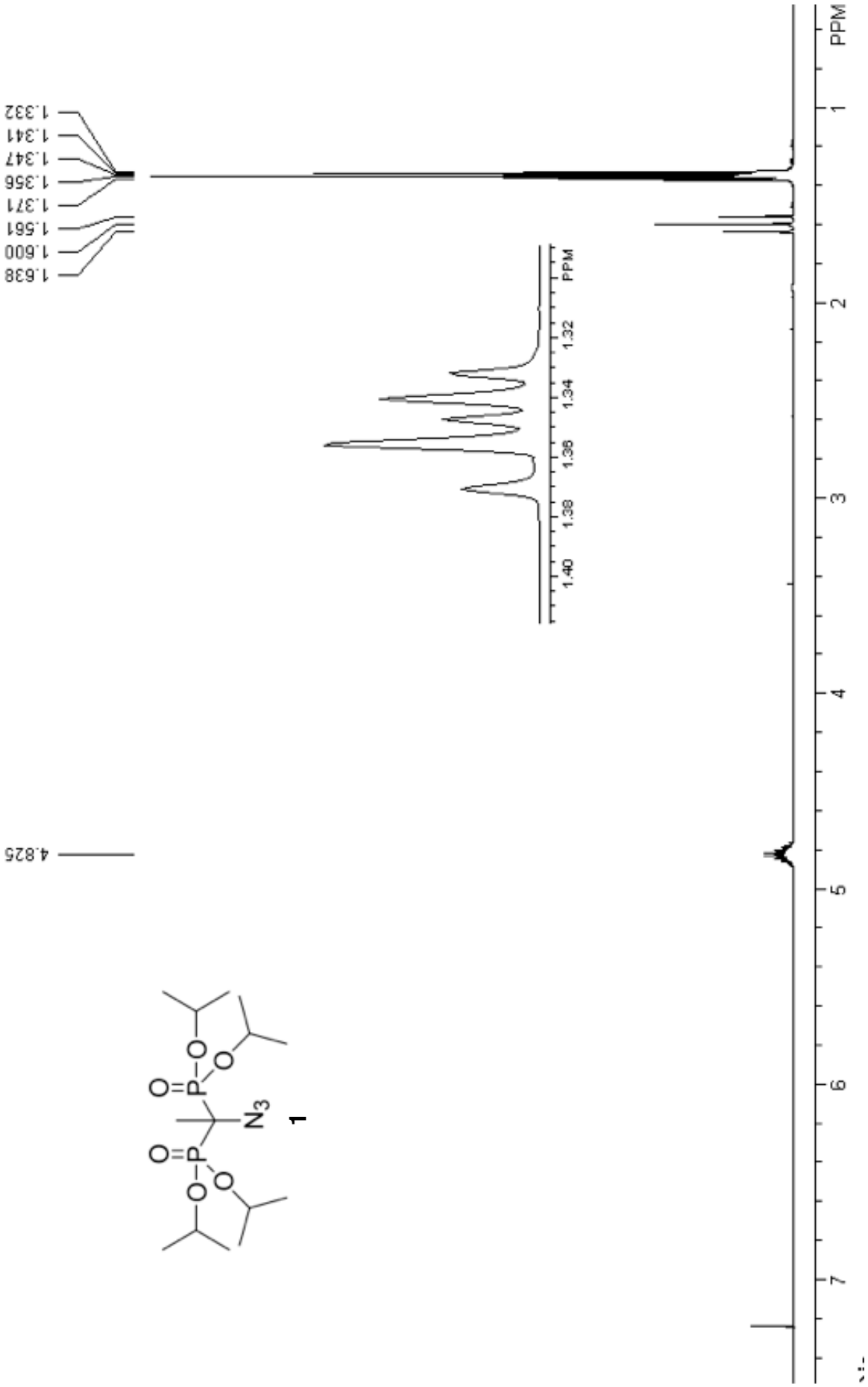


Figure S2. gCOSY NMR spectrum ($CDCl_3$; 400.2 MHz) of **1**.

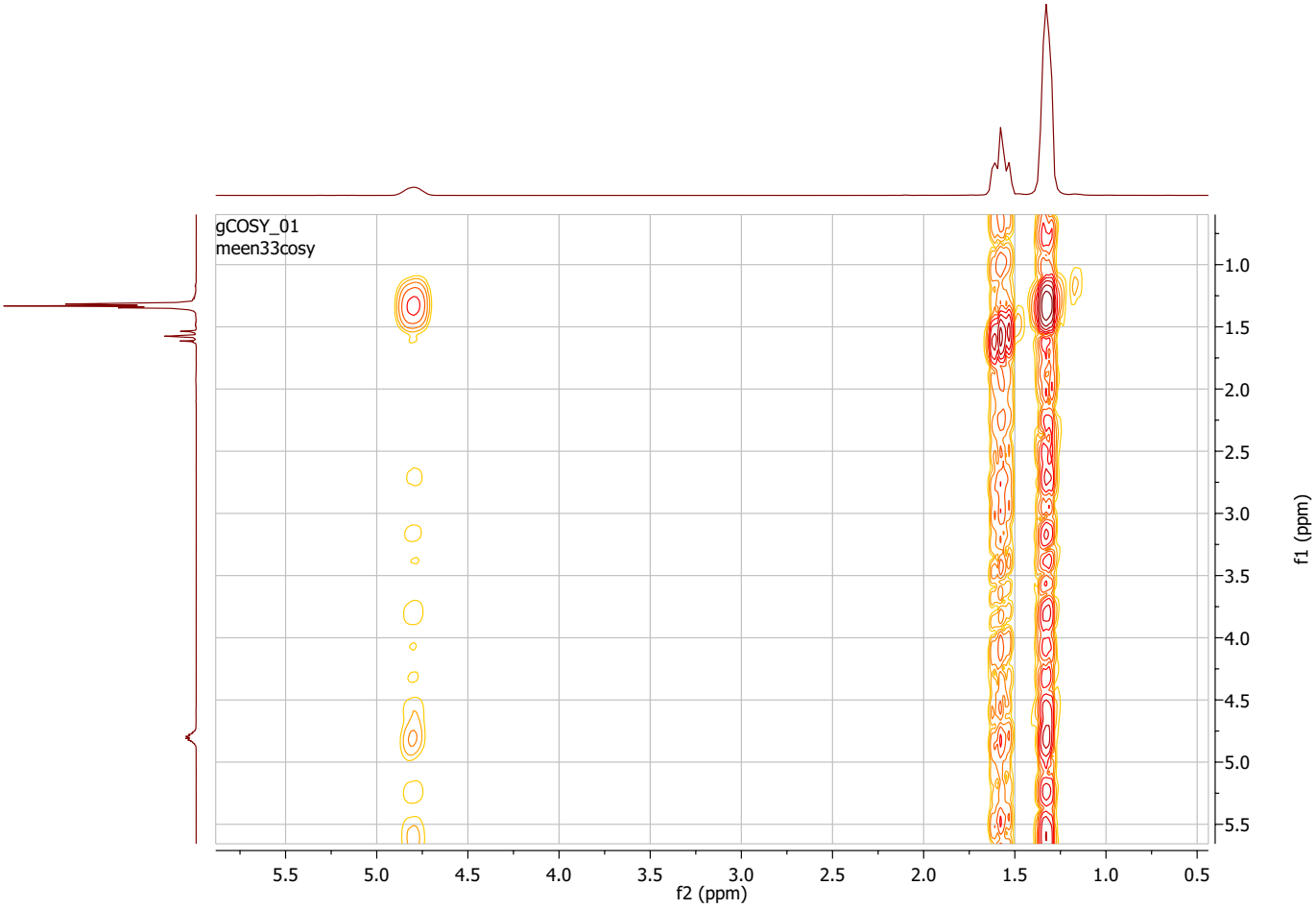


Figure S3. ^{13}C {^{1}H} NMR spectrum ($CDCl_3$; 100.5 MHz) of **1**.

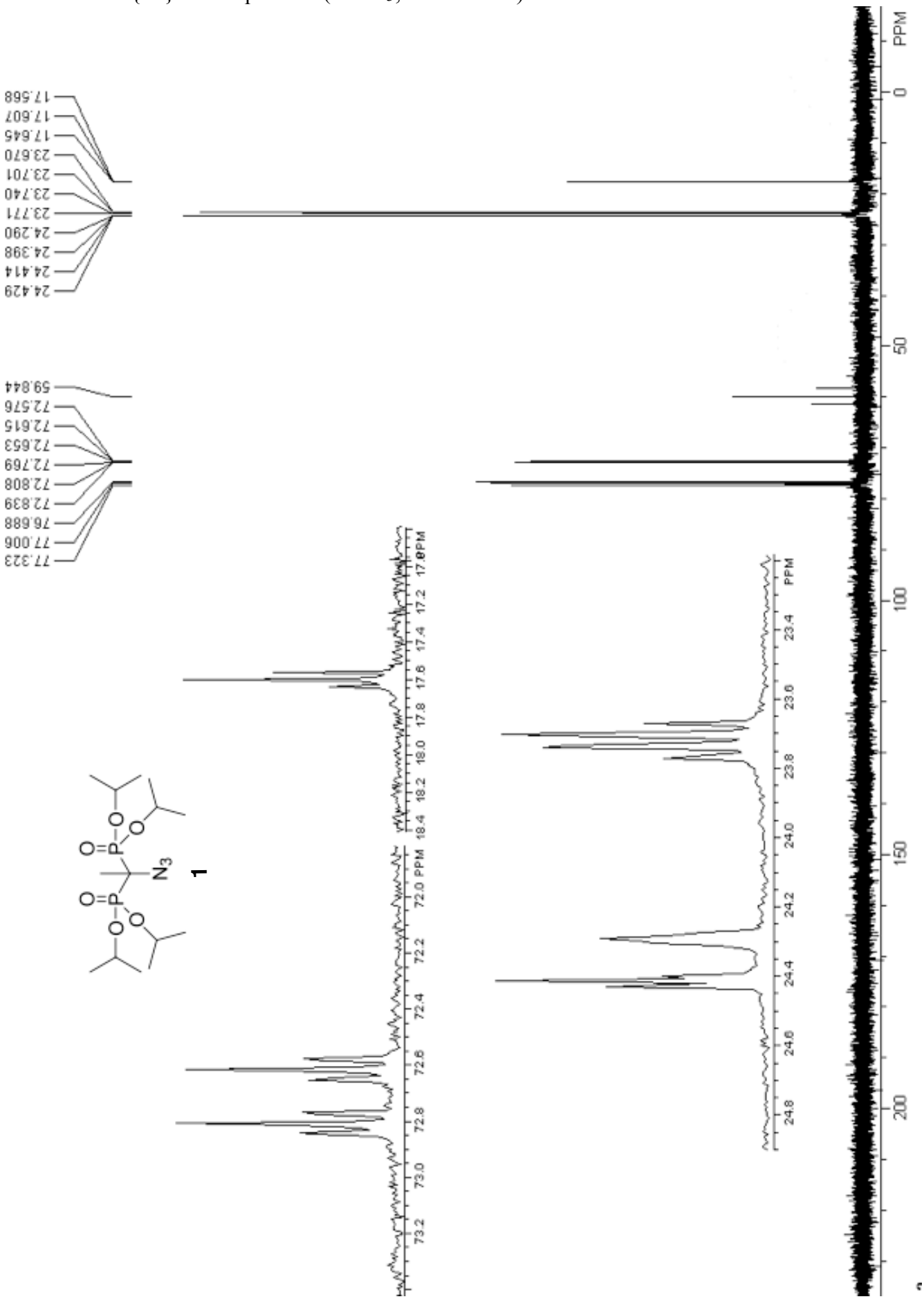


Figure S4. ^{31}P NMR spectra ($CDCl_3$; 161.9 MHz) of **1**.

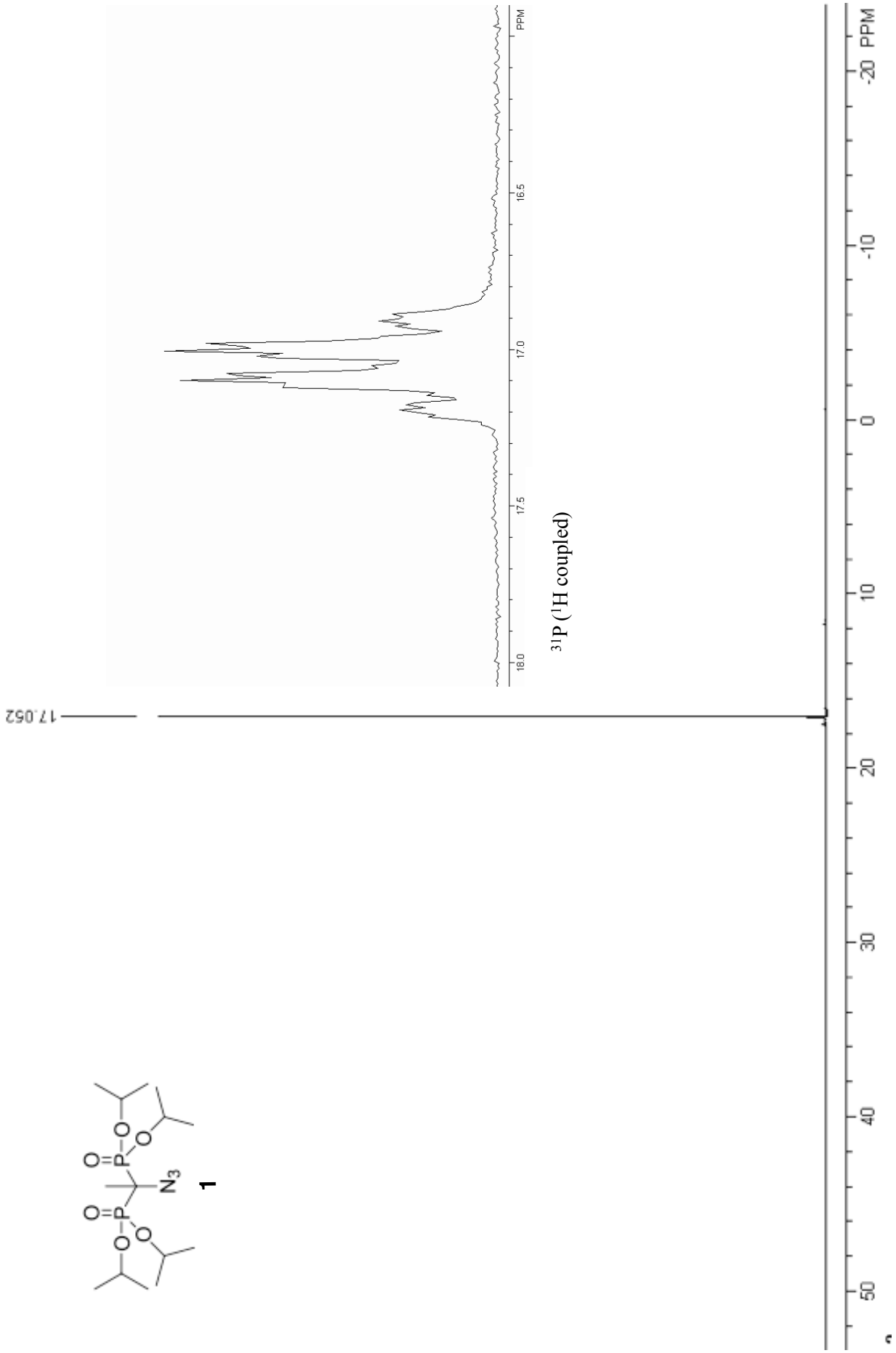


Figure S5. IR spectrum (film, NaCl plate) of **1**.

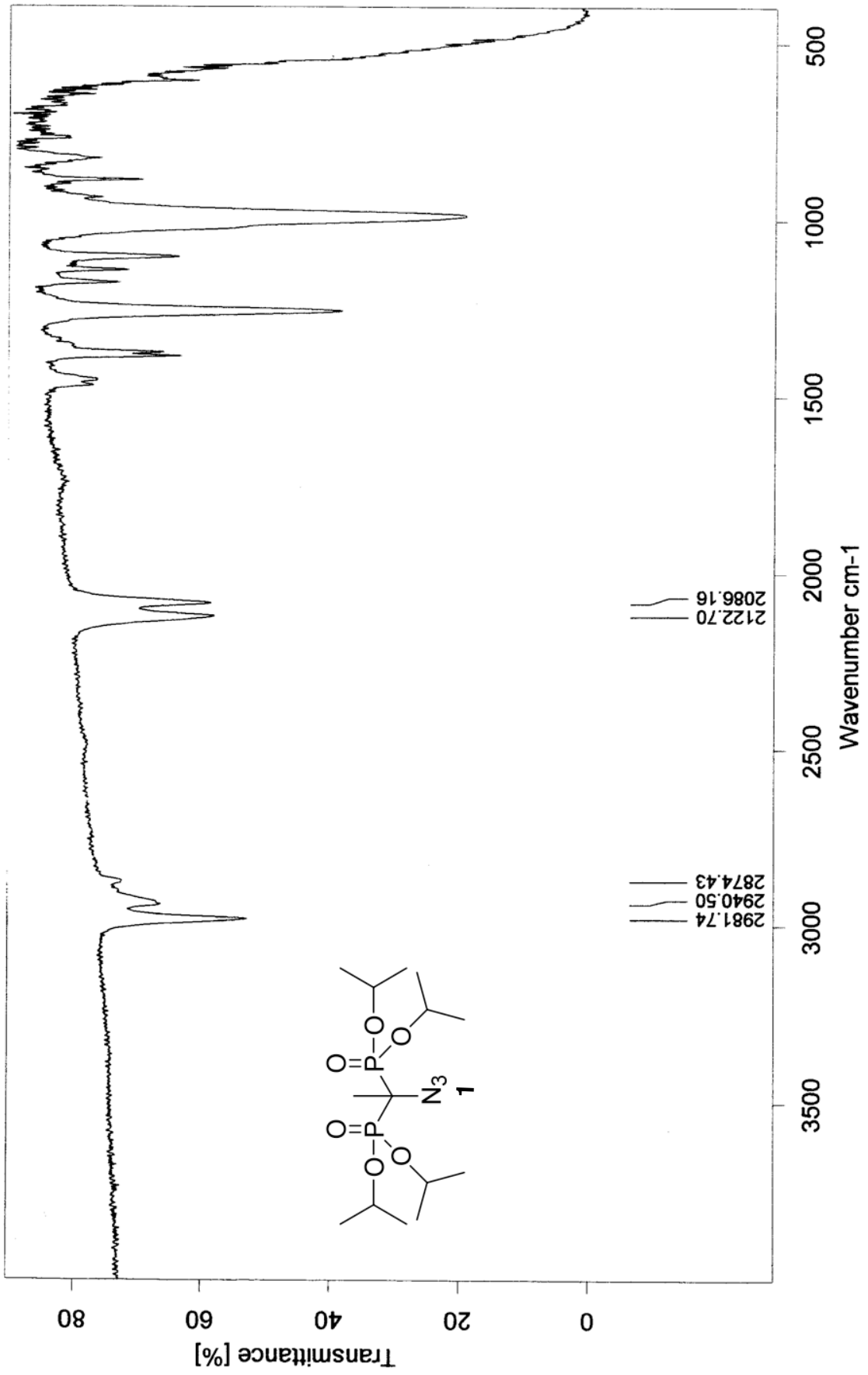


Figure S6. ^{1}H NMR spectrum ($CDCl_3$; 399.8 MHz) of **2**.

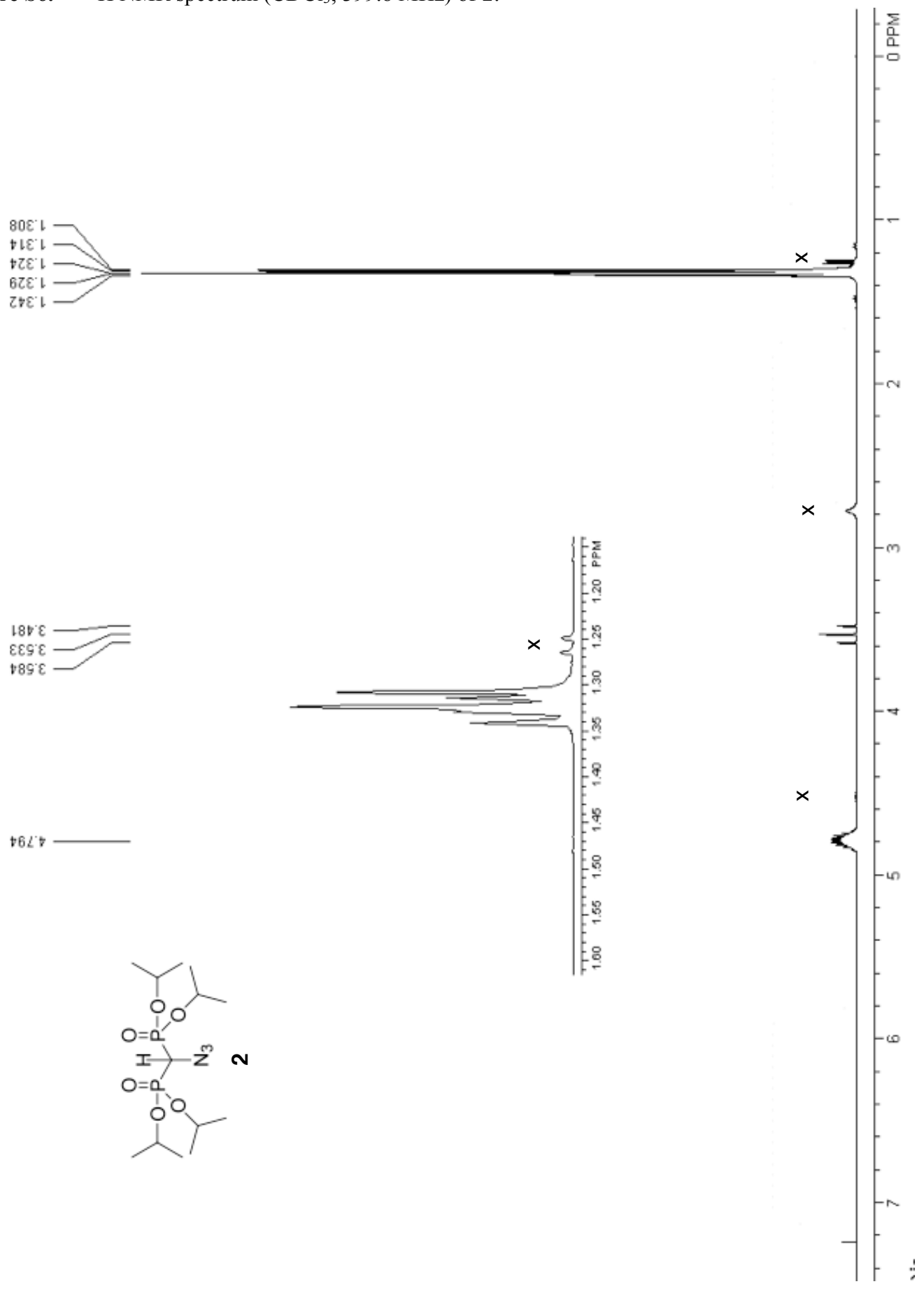


Figure S7. gCOSY NMR spectrum ($CDCl_3$; 400.2 MHz) of **2**.

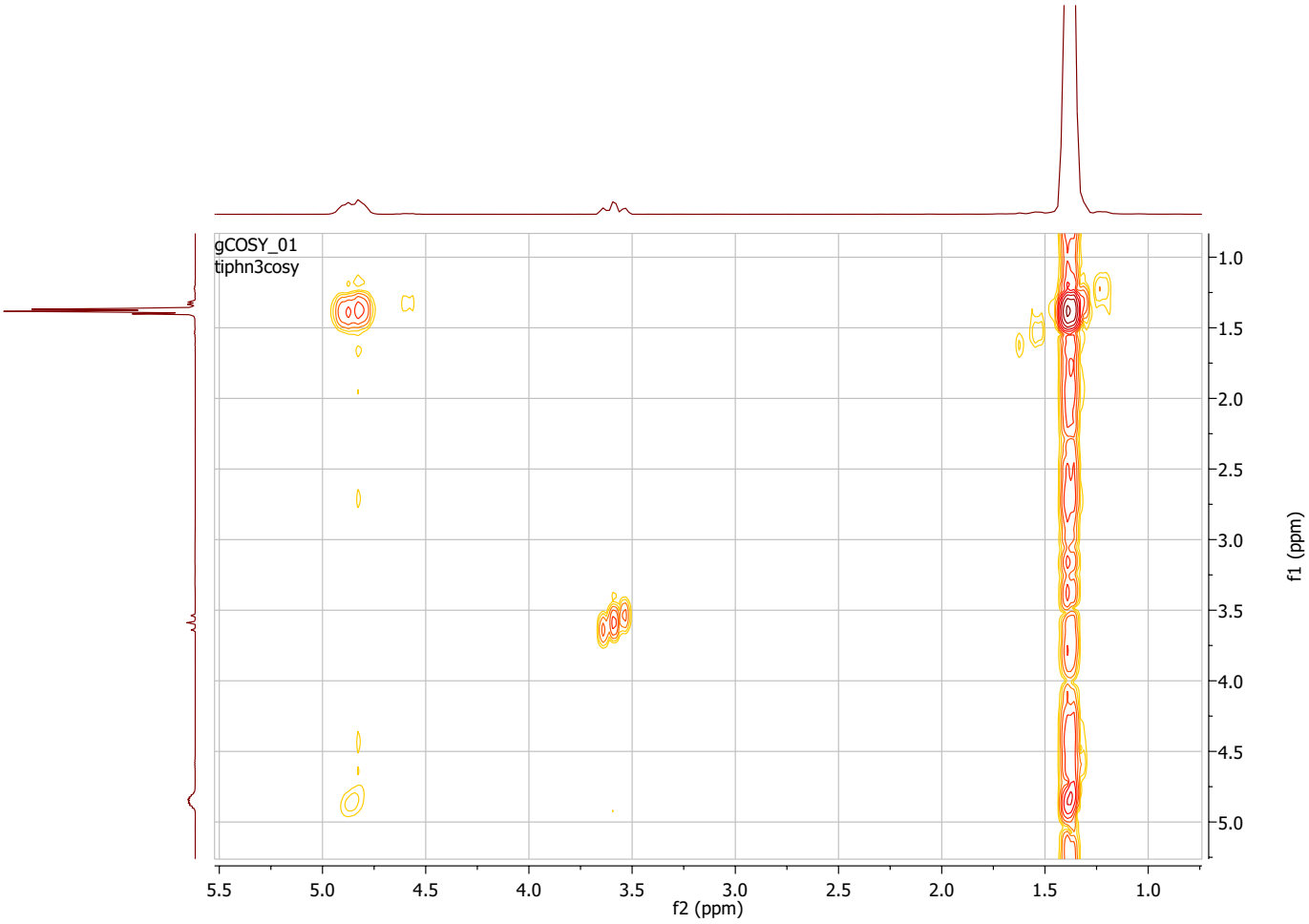


Figure S8. ^{13}C {^{1}H} NMR spectrum ($CDCl_3$; 100.5 MHz) of **2**.

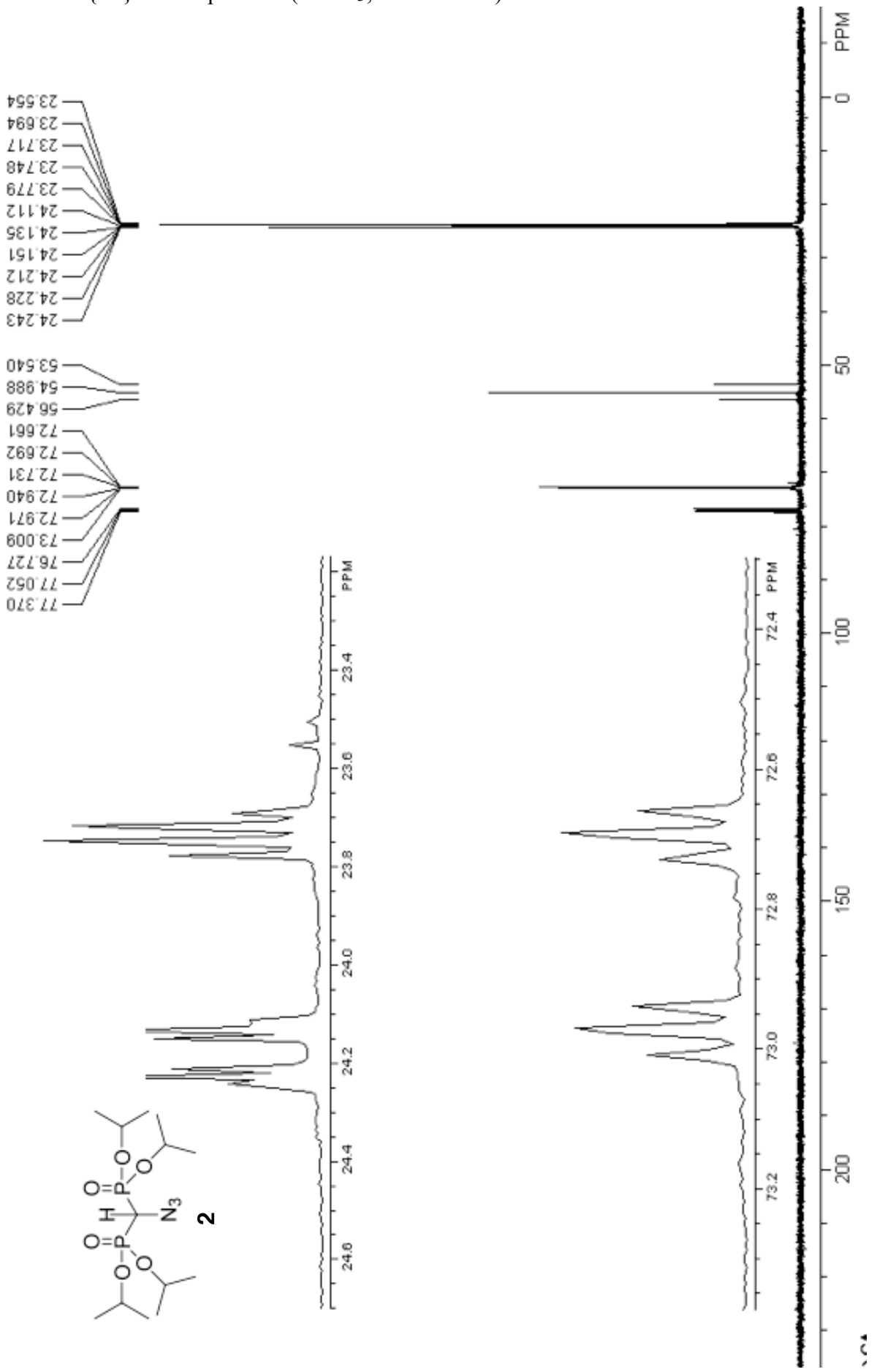


Figure S9. ^{31}P NMR spectra ($CDCl_3$; 161.9 MHz) of **2**.

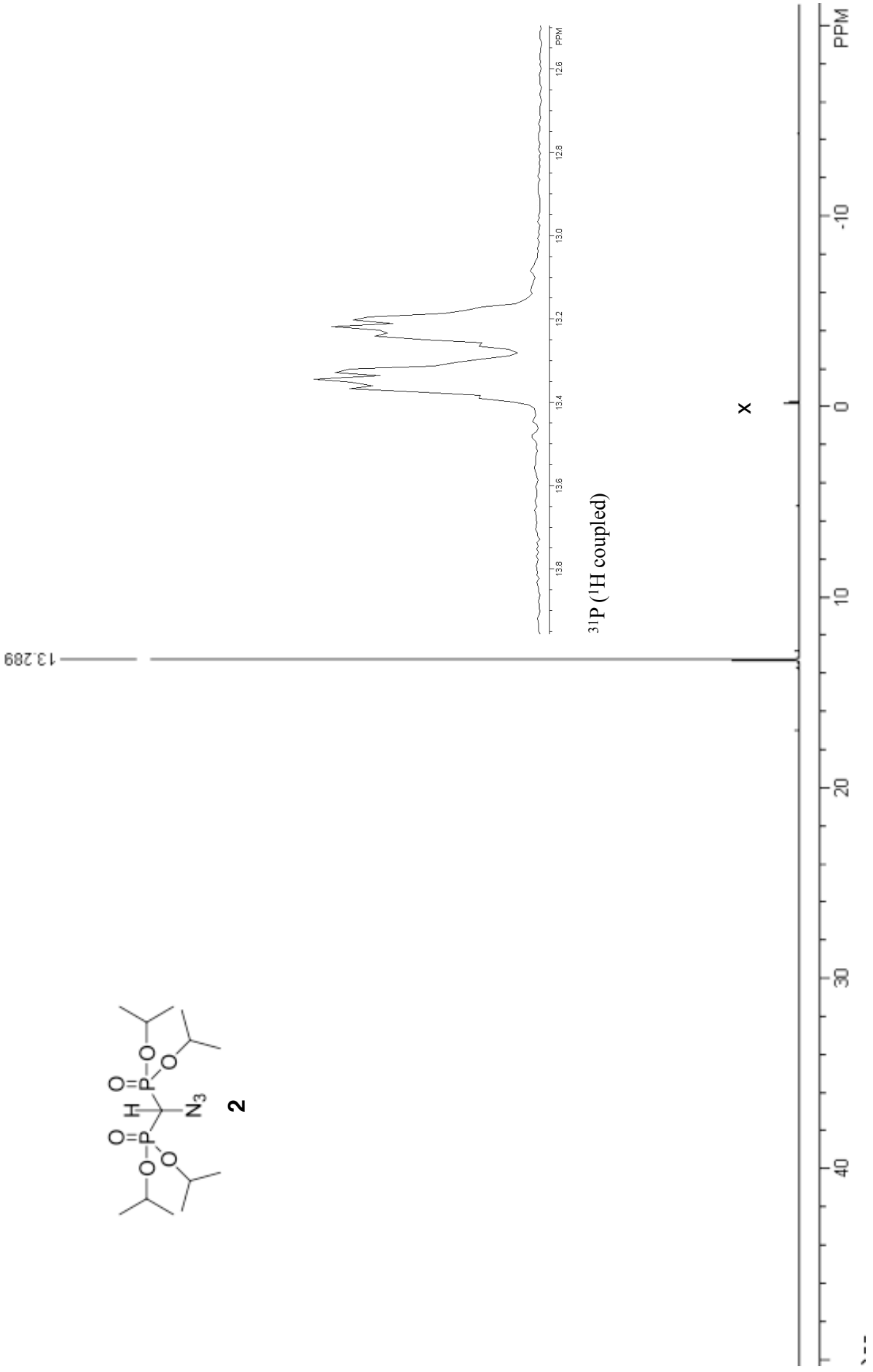


Figure S10. IR spectrum (film, NaCl plate) of **2**.

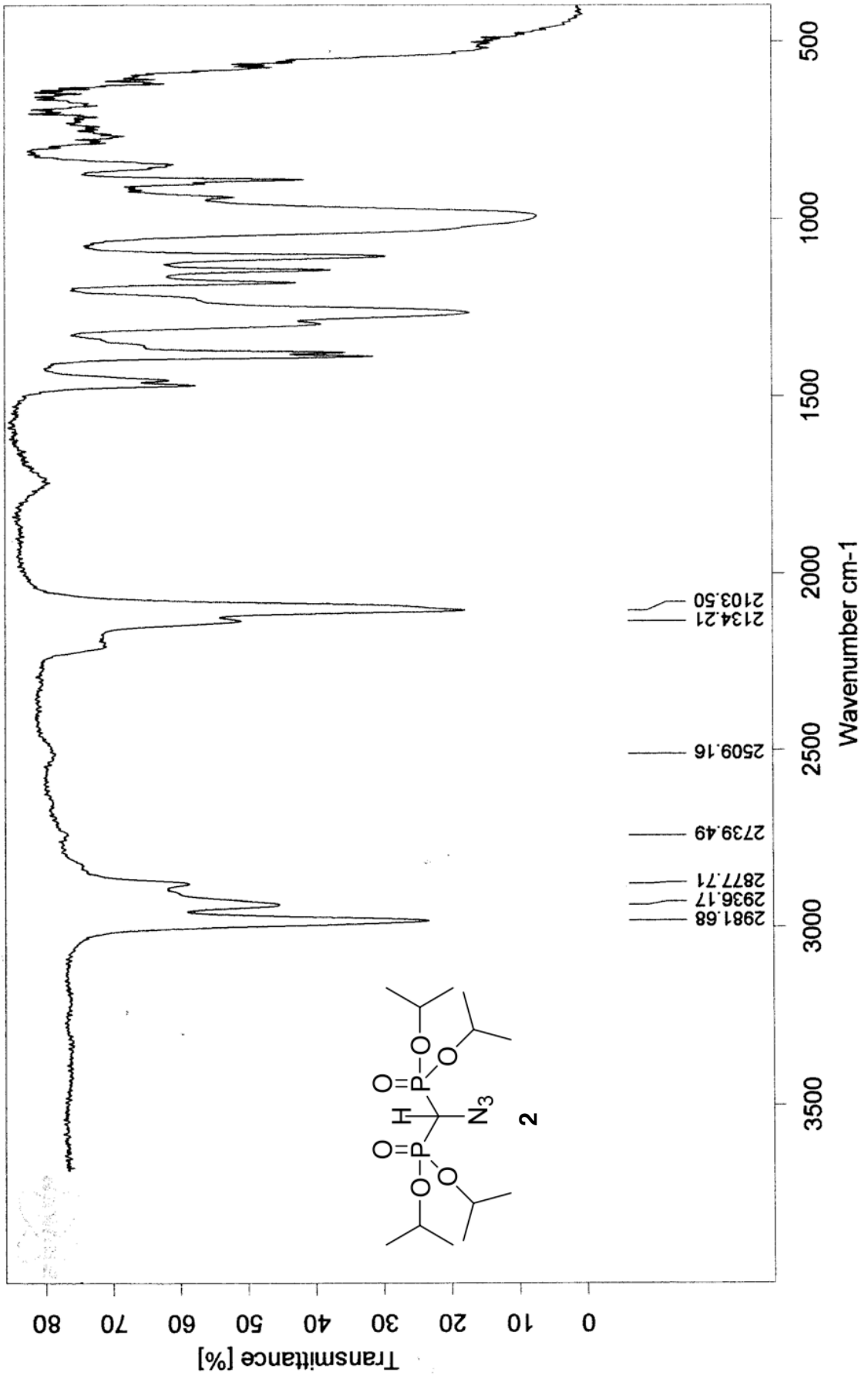


Figure S11. ^{1}H NMR spectrum (D_2O, pH 10.88; 399.8 MHz) of **3**.

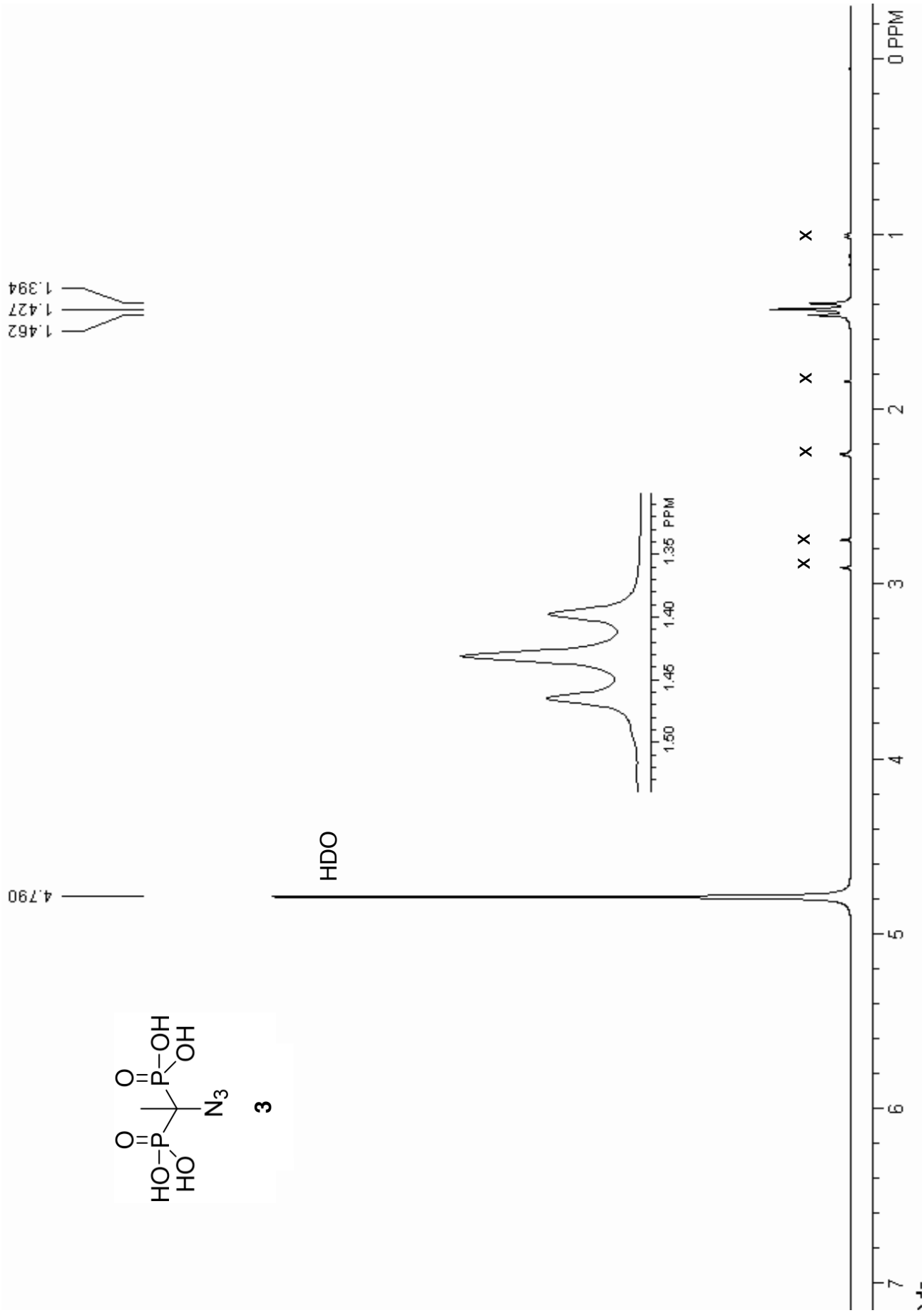


Figure S12. ^{13}C {^{1}H} NMR spectrum (D_2O, pH 10.88; 100.5 MHz) of **3**.

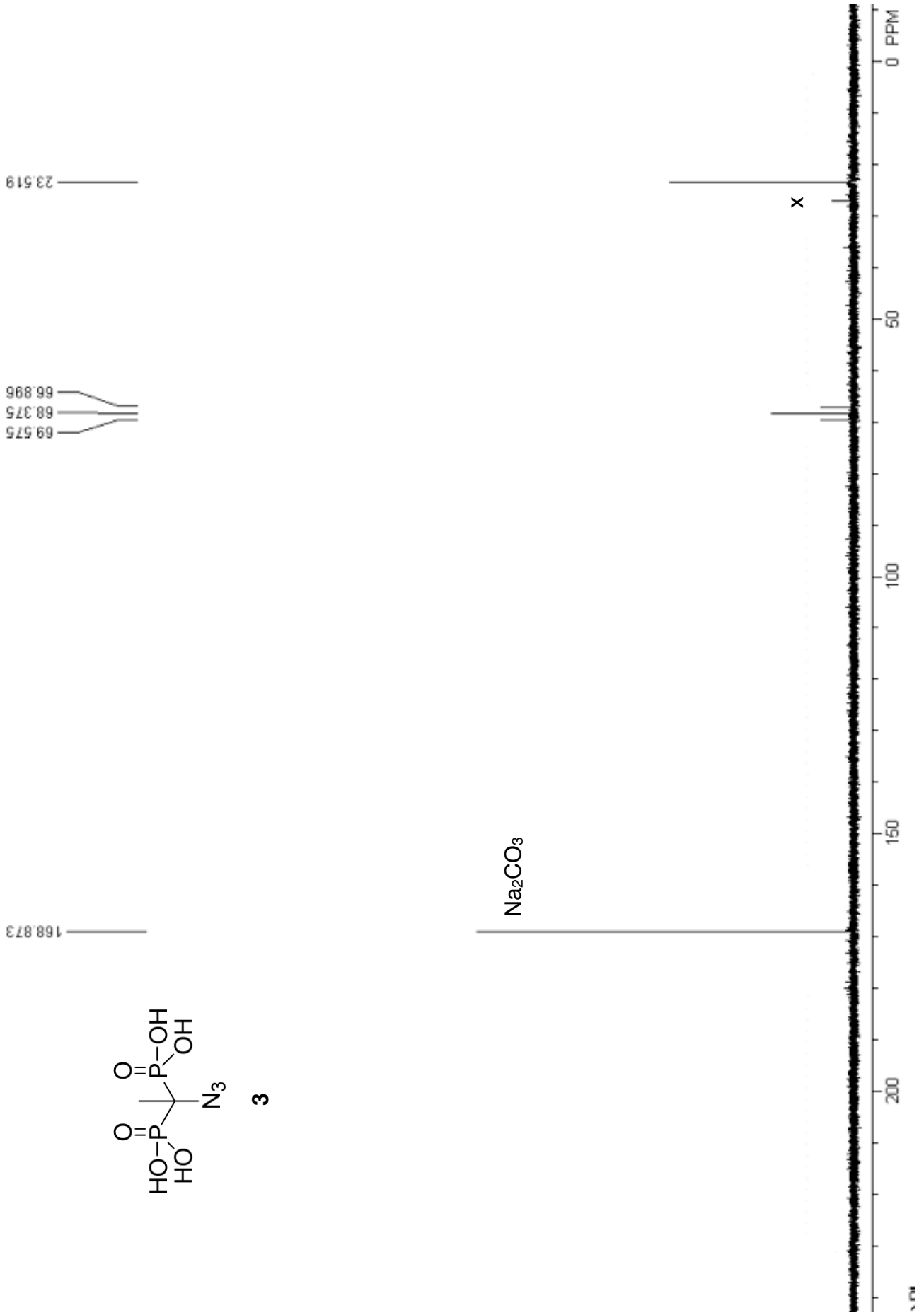


Figure S13. ^{31}P NMR spectra (D_2O, pH 10.88; 161.9 MHz) of **3**.

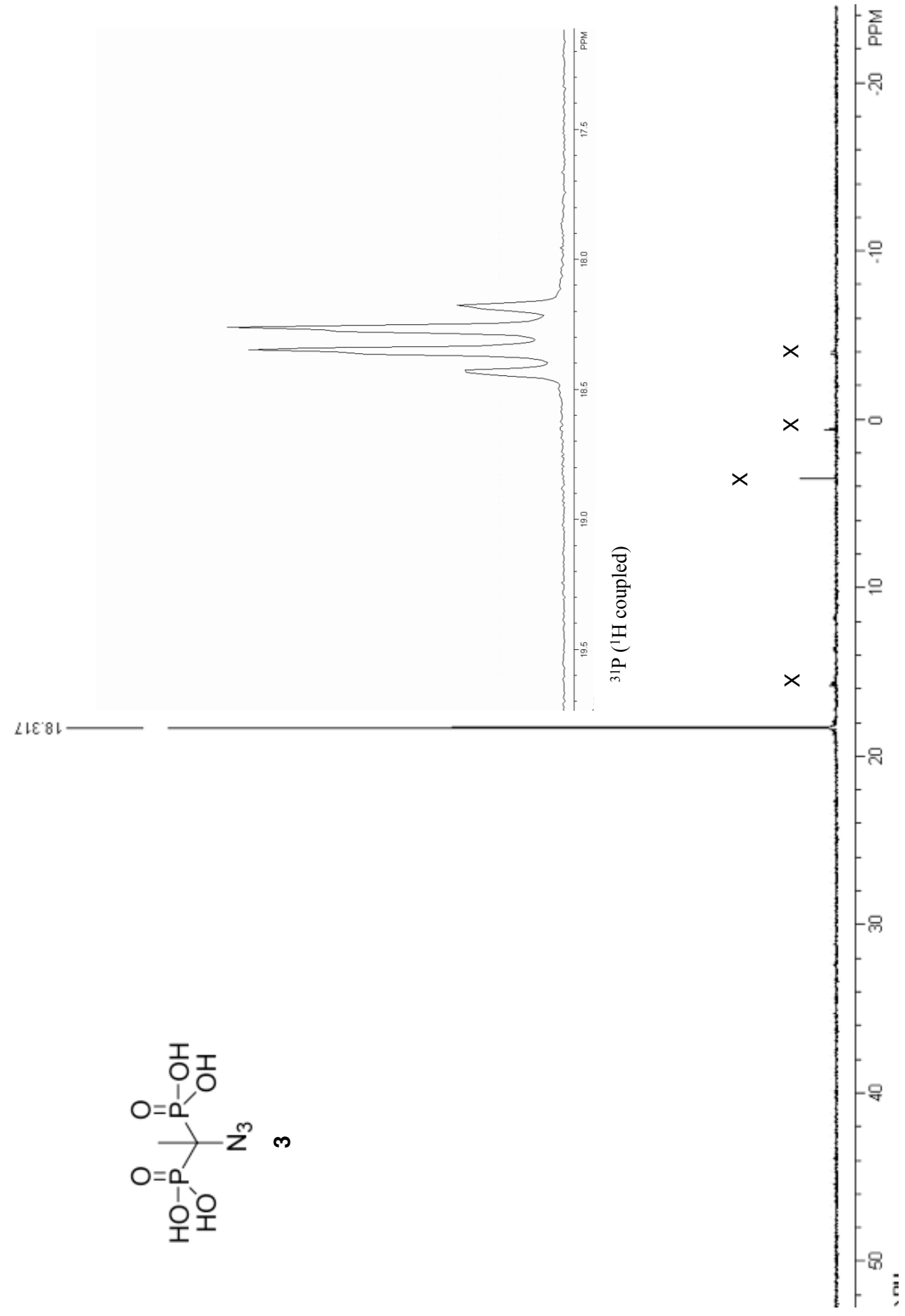


Figure S14. IR spectrum (KBr pellet) of **3**.

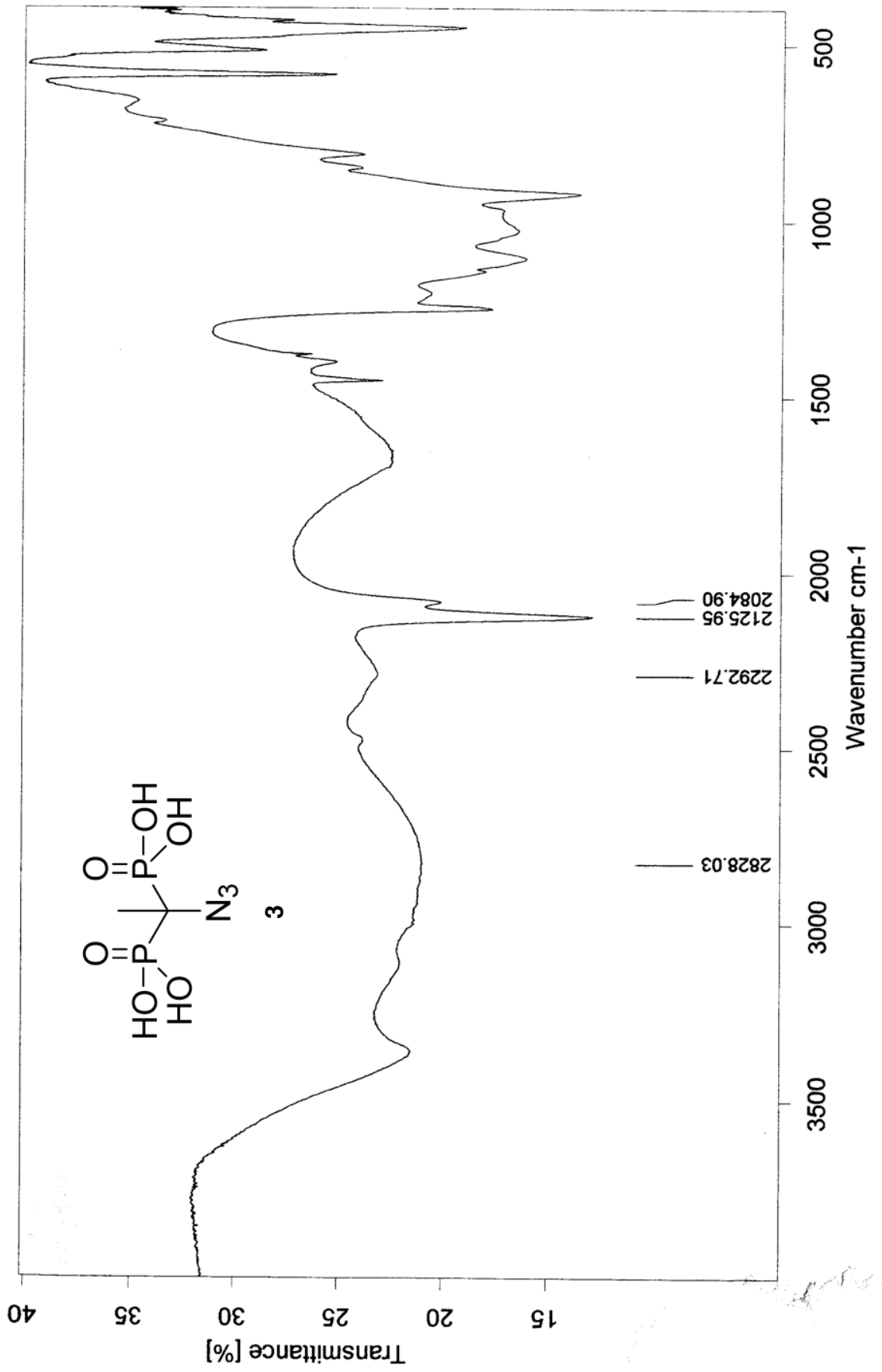
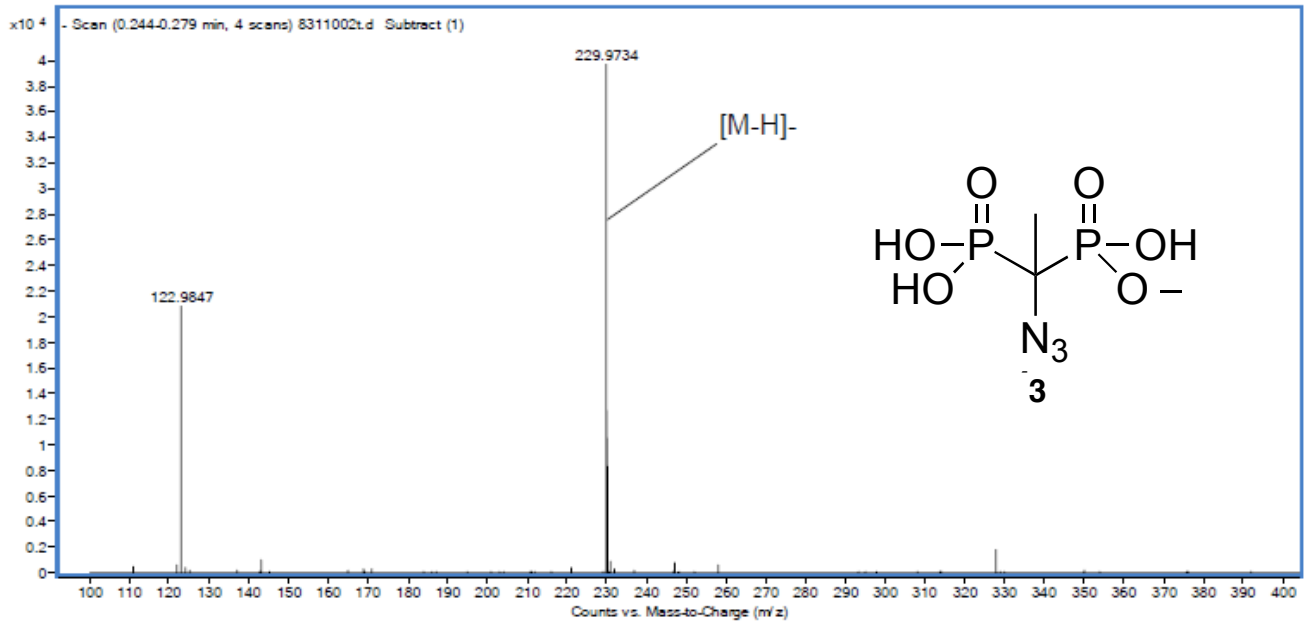


Figure S15. HRMS (ESI/ACPI) of **3**.



3

Measured Mass	229.9734	
Element	Low Limit	High Limit
C	0	5
H	0	15
N	0	5
O	4	8
P	0	2

Formula	Calculated Mass	mDaError	ppmError	RDB
C2 H6 N3 O6 P2	229.9737	-0.3	-1.5	2.5
C4 H N5 O5 P	229.9721	1.3	5.7	7.5
C4 H8 O7 P2	229.9751	-1.7	-7.3	2

Figure S16. ^{1}H NMR spectrum (D_2O, pH 10.88; 399.8 MHz) of **4**.

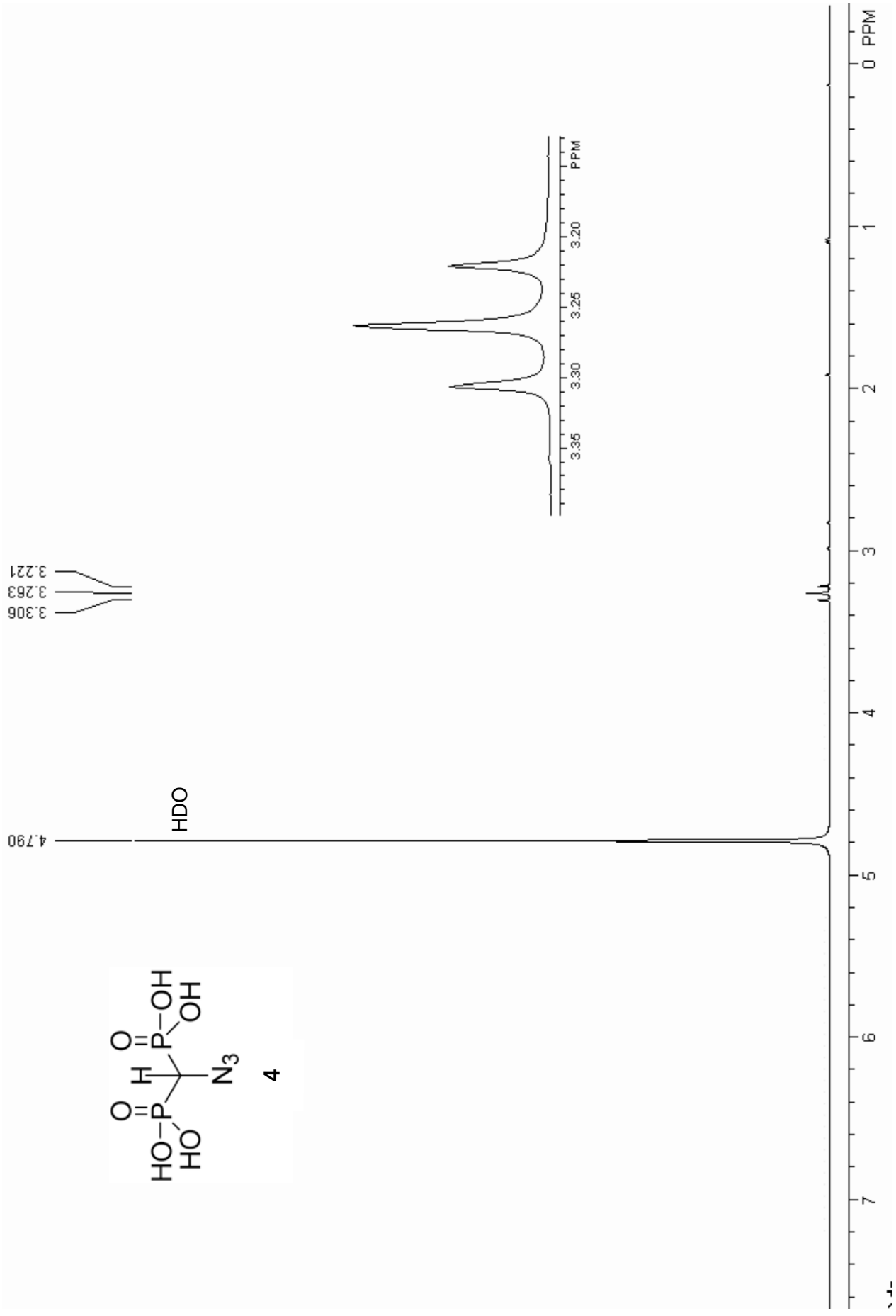


Figure S17. ^{13}C {^{1}H} NMR spectrum (D_2O, pH 10.88; 100.5 MHz) of **4**.

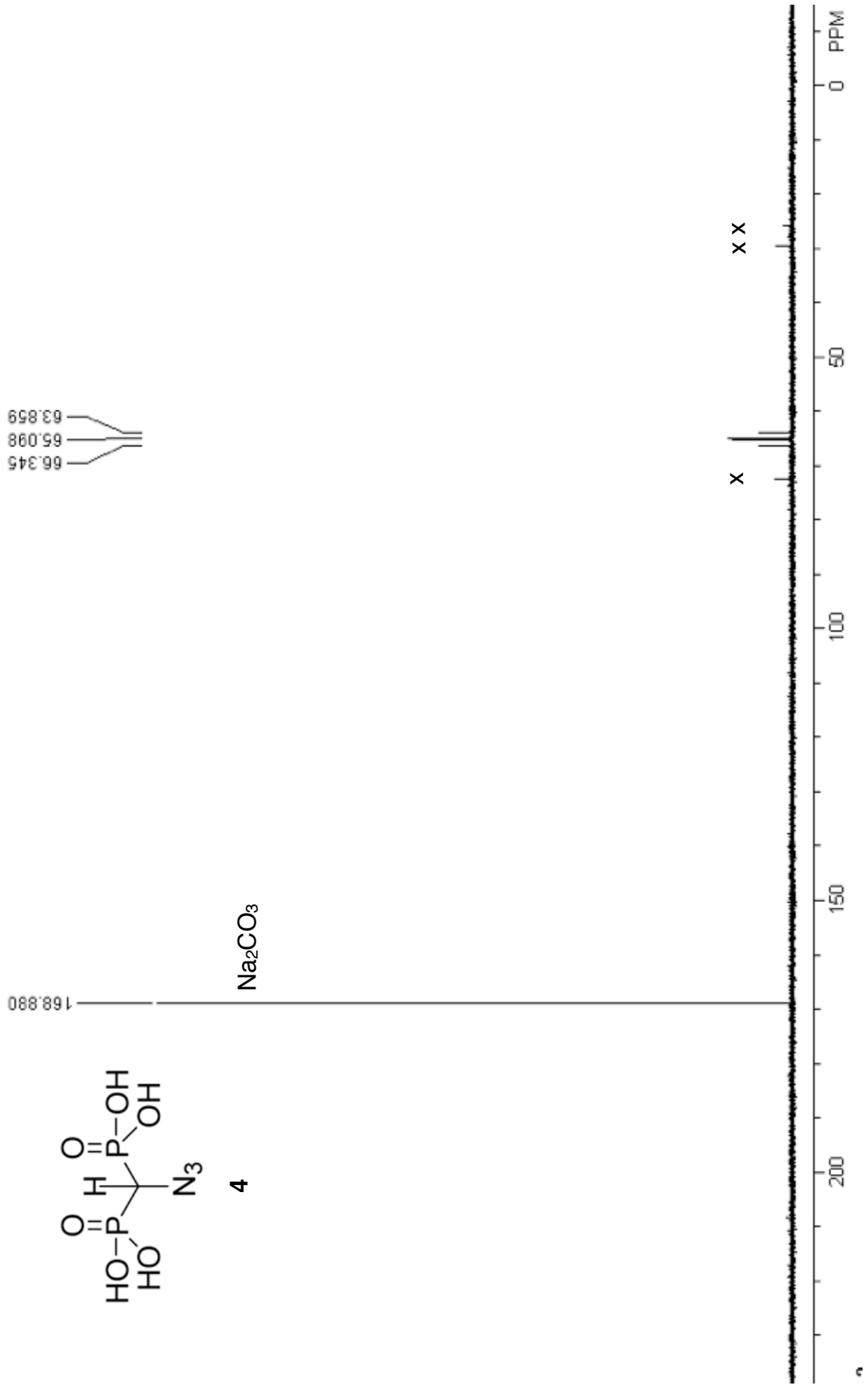
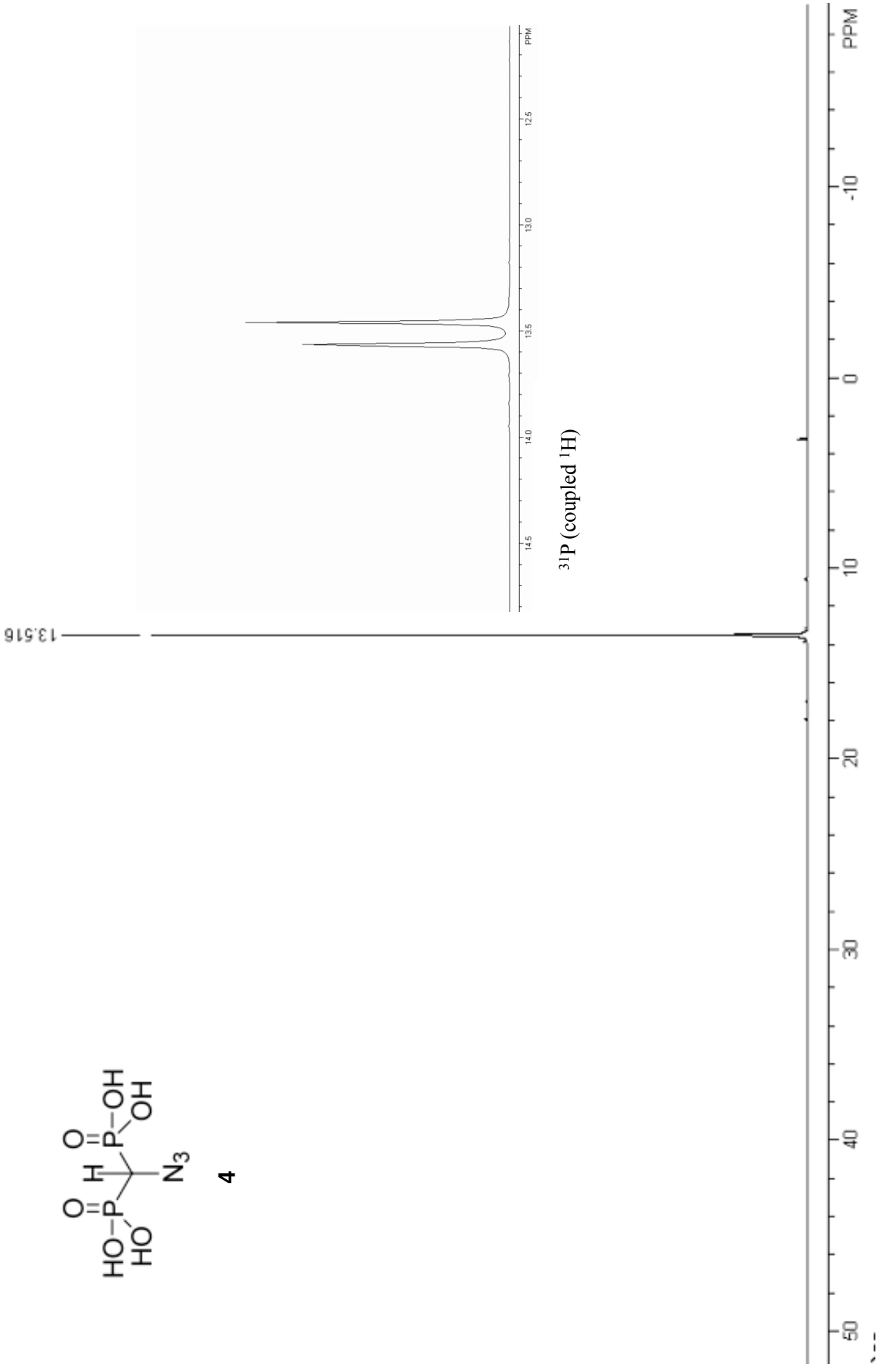


Figure S18. ^{31}P NMR spectra (D_2O, pH 10.88; 161.9 MHz) of **4**.



4

Figure S19. IR spectrum (KBr pellet) of **4**.

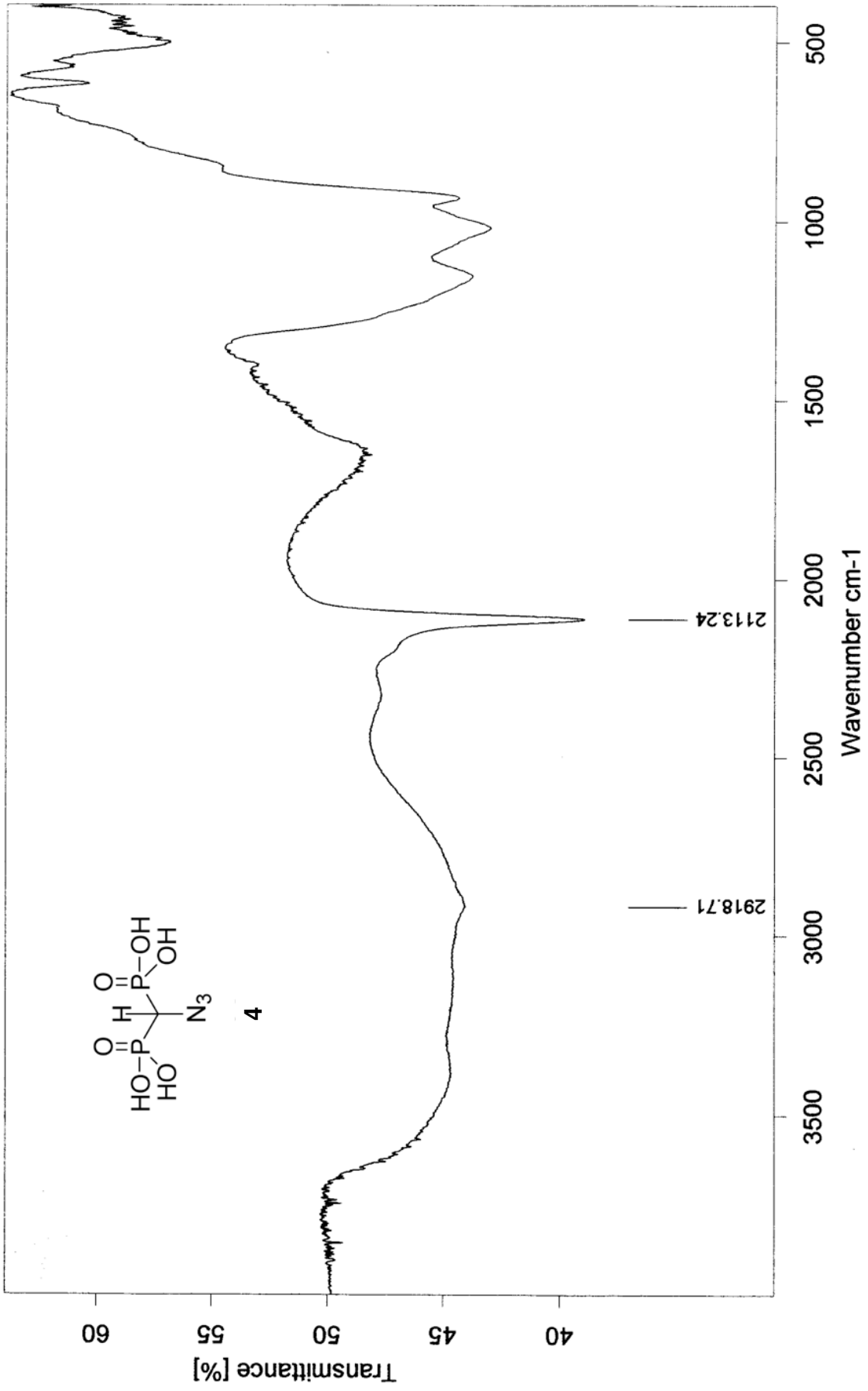
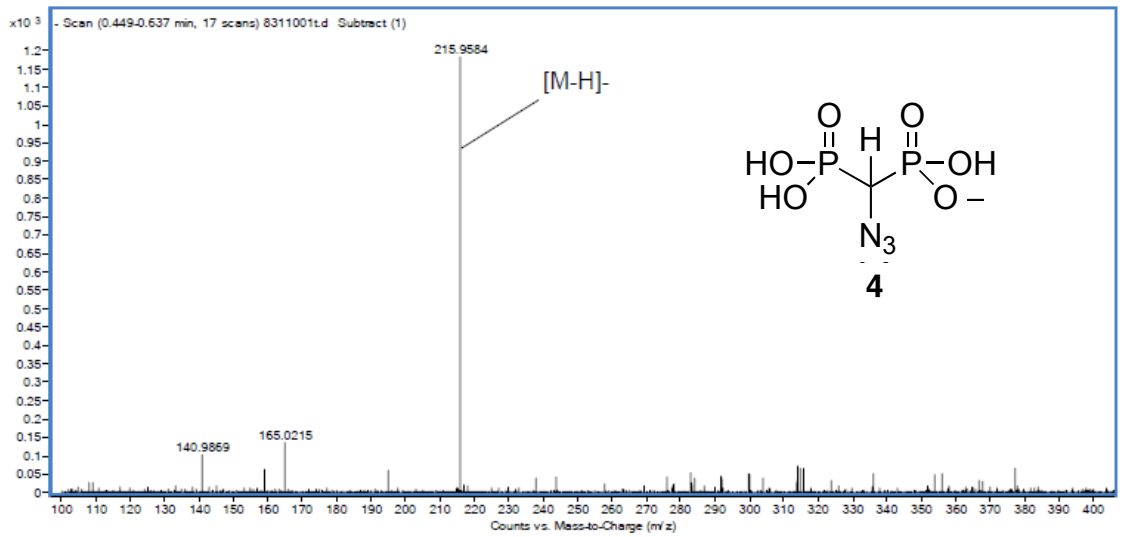


Figure S20. HRMS (ESI/ACPI) of **4**.



Measured Mass 215.9584

Element	Low Limit	High Limit
C	0	5
H	0	15
N	0	5
O	4	8
P	0	2

Formula	Calculated Mass	mDaError	ppmError	RDB
C H4 N3 O6 P2	215.9581	0.3	1.5	2.5
C5 H N2 O6 P	215.9578	0.6	2.9	7
C3 H6 O7 P2	215.9594	-1.0	-4.8	2

Figure S21. ^{1}H NMR spectrum (D_2O, pH 10.88; 399.8 MHz) of **5a/b**.

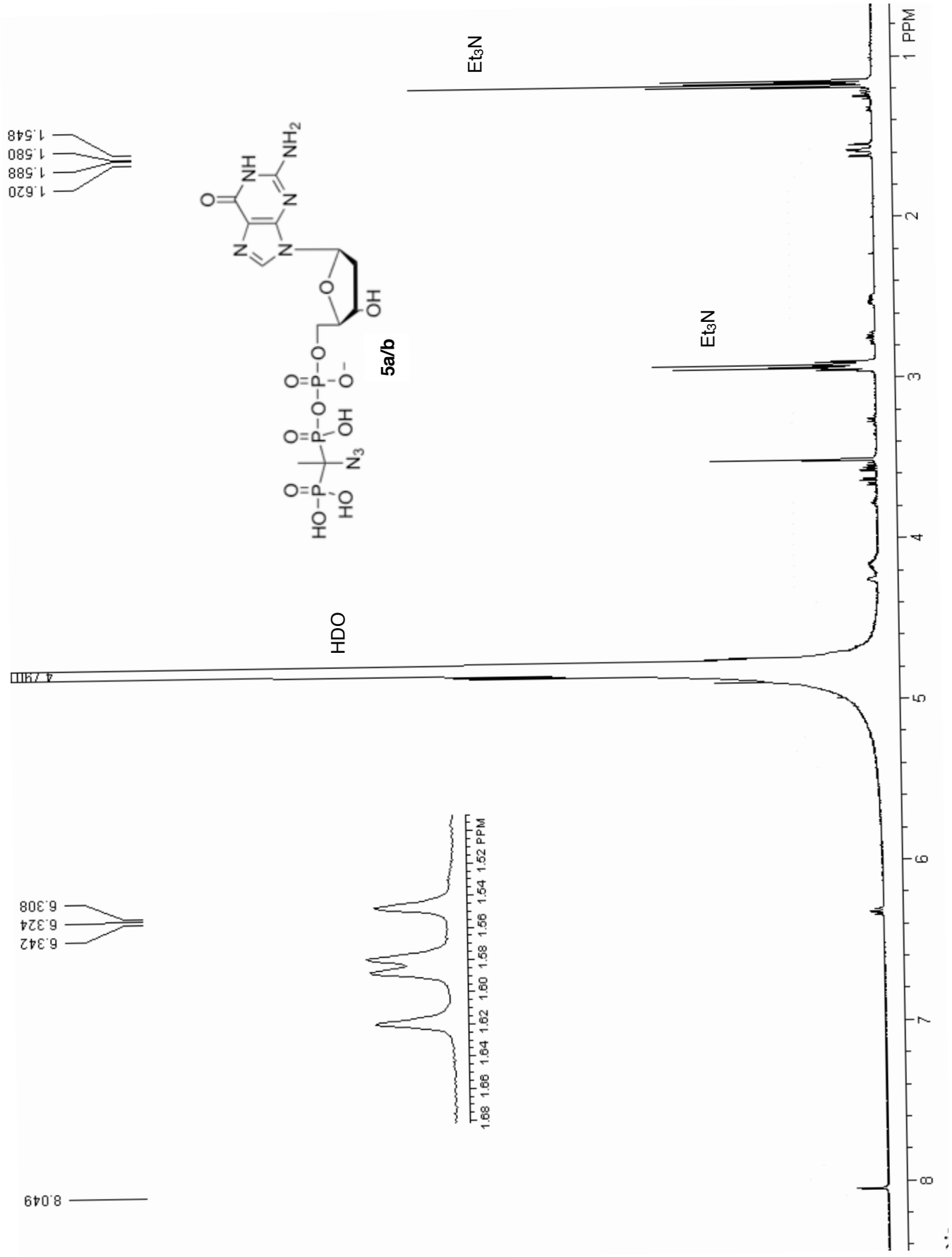


Figure S22. ^{31}P {^{1}H} NMR spectrum (D_2O, pH 10.88; 161.9 MHz) of **5a/b**.

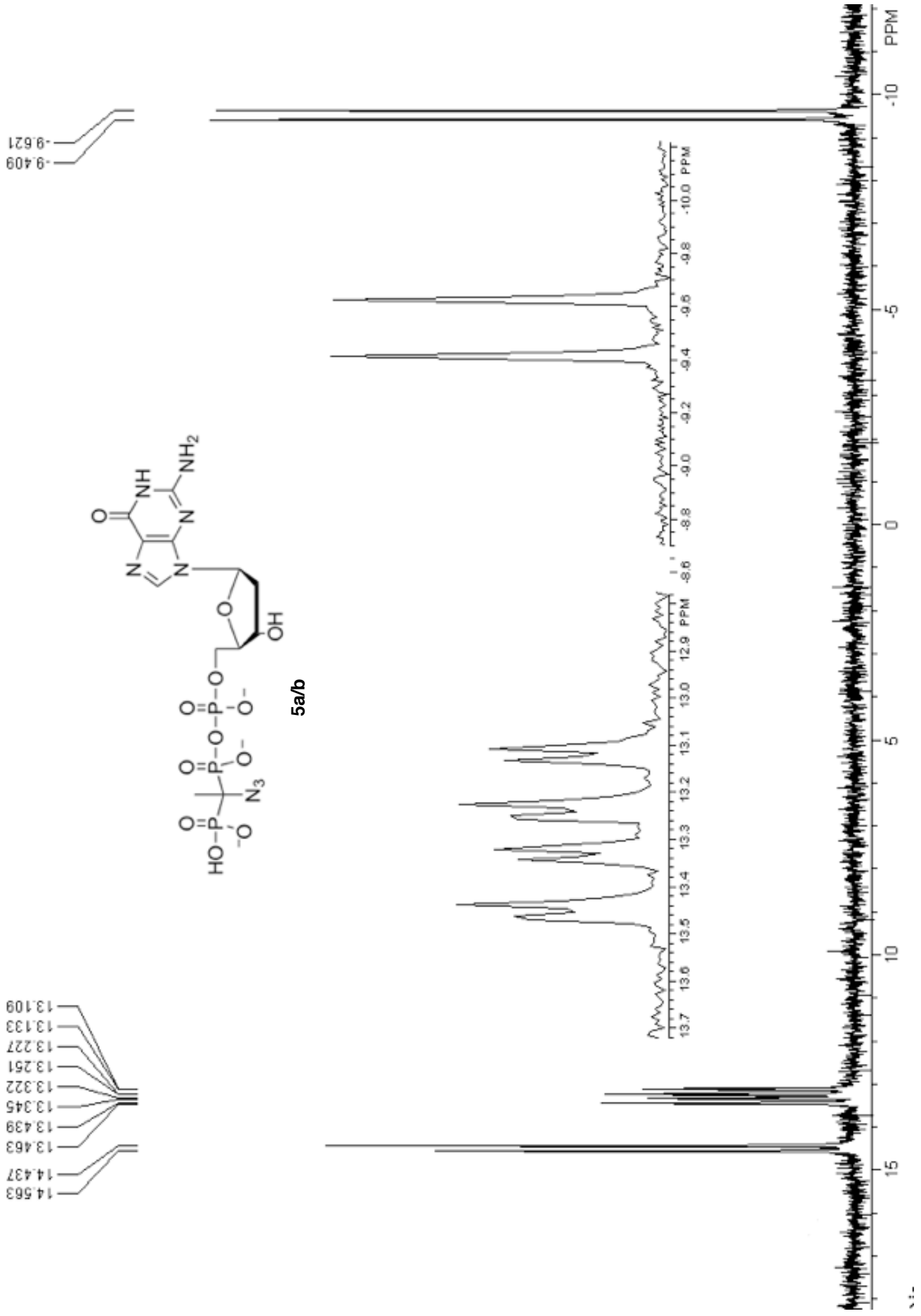


Figure S23. ^{31}P $\{^{1}H\}$ NMR of **5a/b**.
P_β at 202.5 MHz (left) and 161.9 MHz (right), pH 10.88 confirming δ vs. *J* assignments. The chemical shifts in the spectrum were normalized to Figure S20.

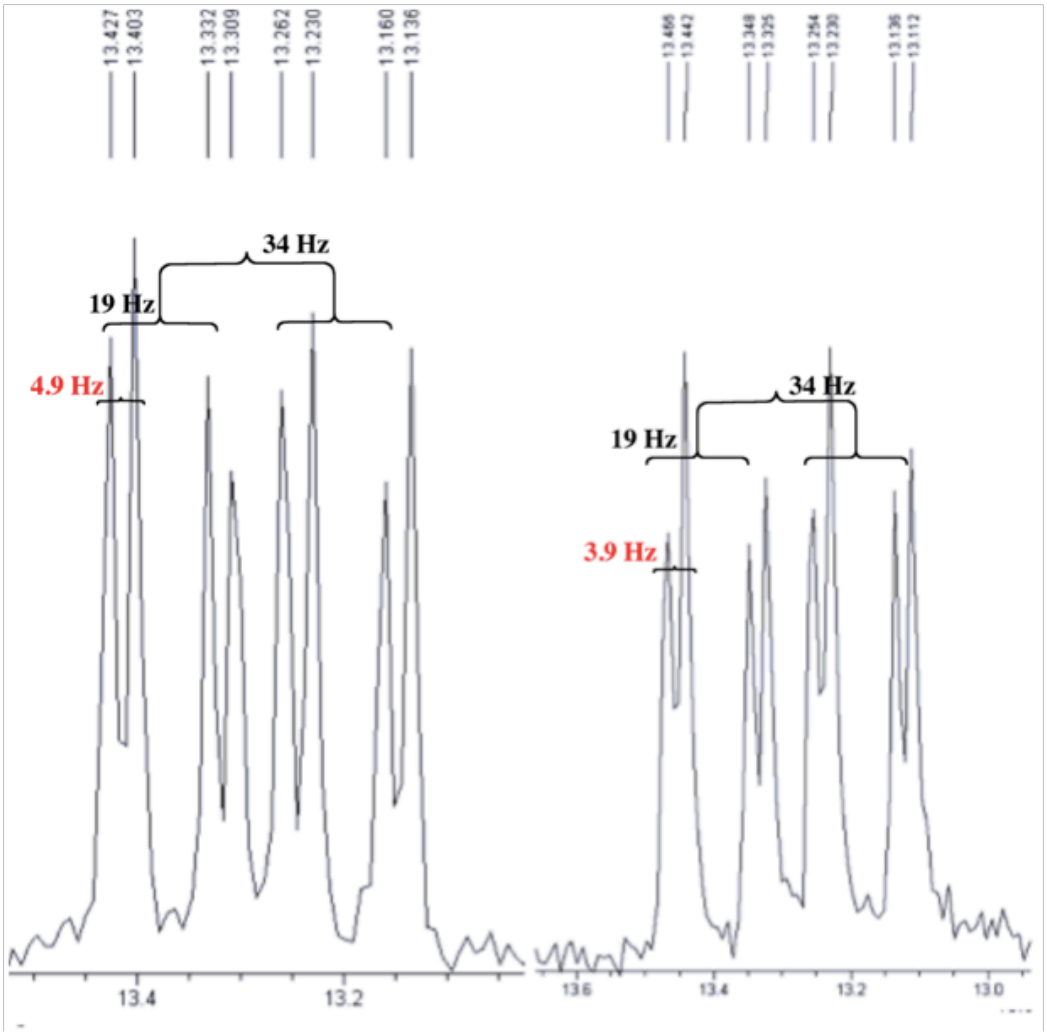
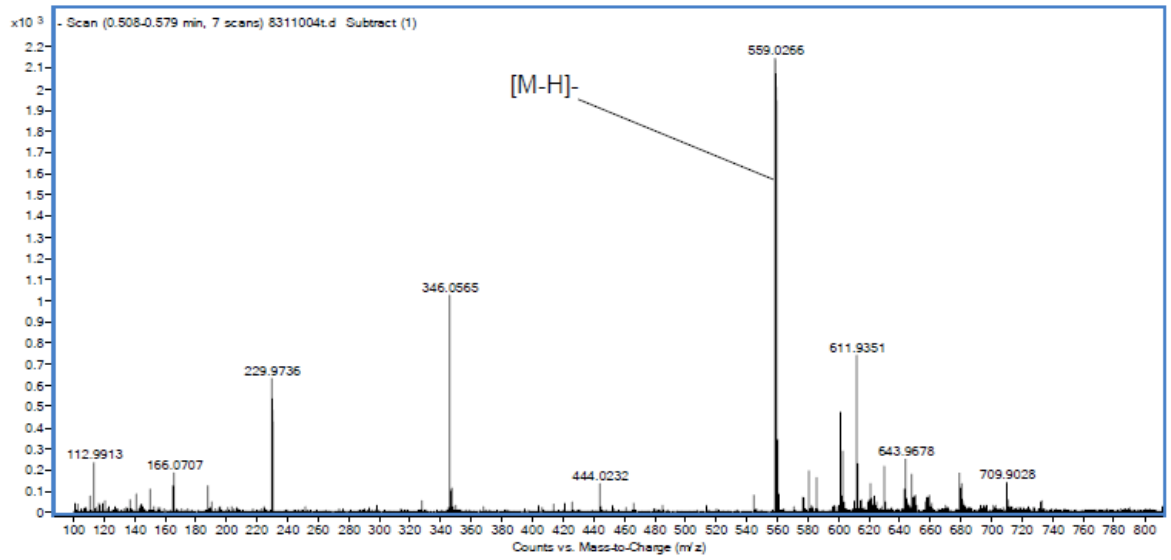


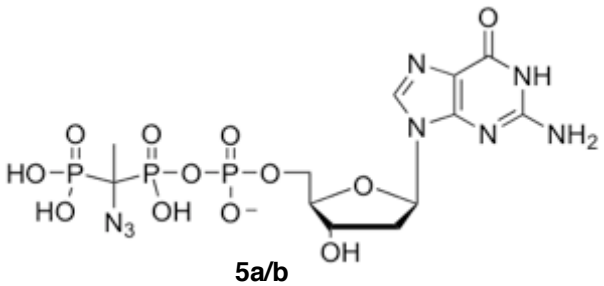
Figure S24. HRMS (ESI/ACPI) of **5a/b**.



Measured Mass 559.0266

Element	Low Limit	High Limit
C	6	16
H	5	25
N	6	10
O	10	14
P	0	3

Formula	Calculated Mass	mDaError	ppmError	RDB
C12 H18 N8 O12 P3	559.0263	0.3	0.6	9.5
C16 H15 N7 O12 P2	559.0259	0.7	1.2	14
C14 H13 N10 O11 P2	559.0246	2.0	3.6	14.5
C7 H18 N10 O14 P3	559.0222	4.4	7.8	5.5
C11 H15 N9 O14 P2	559.0219	4.7	8.4	10
C15 H12 N8 O14 P	559.0216	5.0	8.9	14.5



5a/b

Figure S25. ^{1}H NMR spectrum (D_2O, pH 10.88; 399.8 MHz) of **6a/b**.

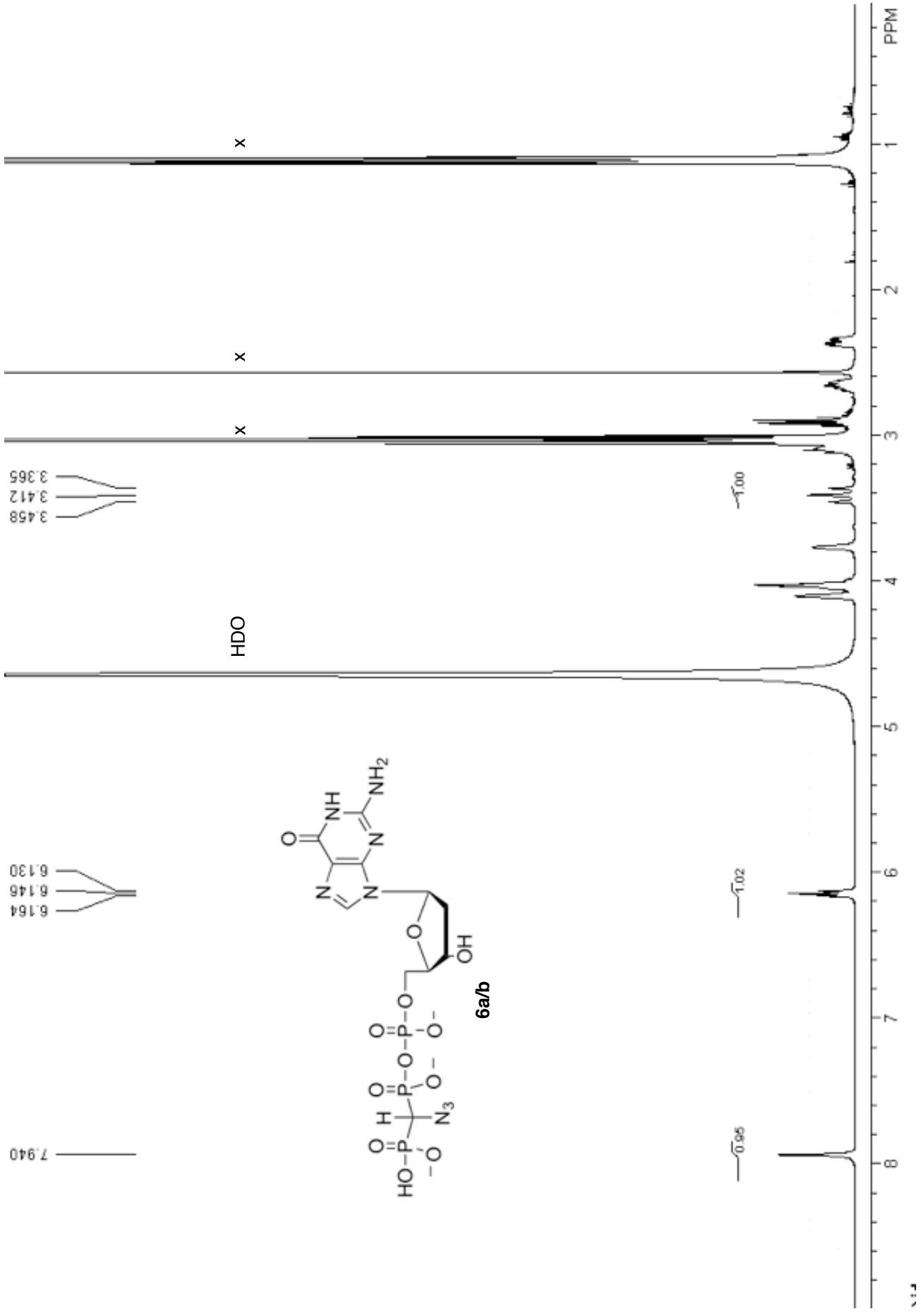


Figure S26. ^{31}P $\{^{1}H\}$ NMR spectrum (D_2O, pH 10.88; 161.9 MHz) of **6a/b**.

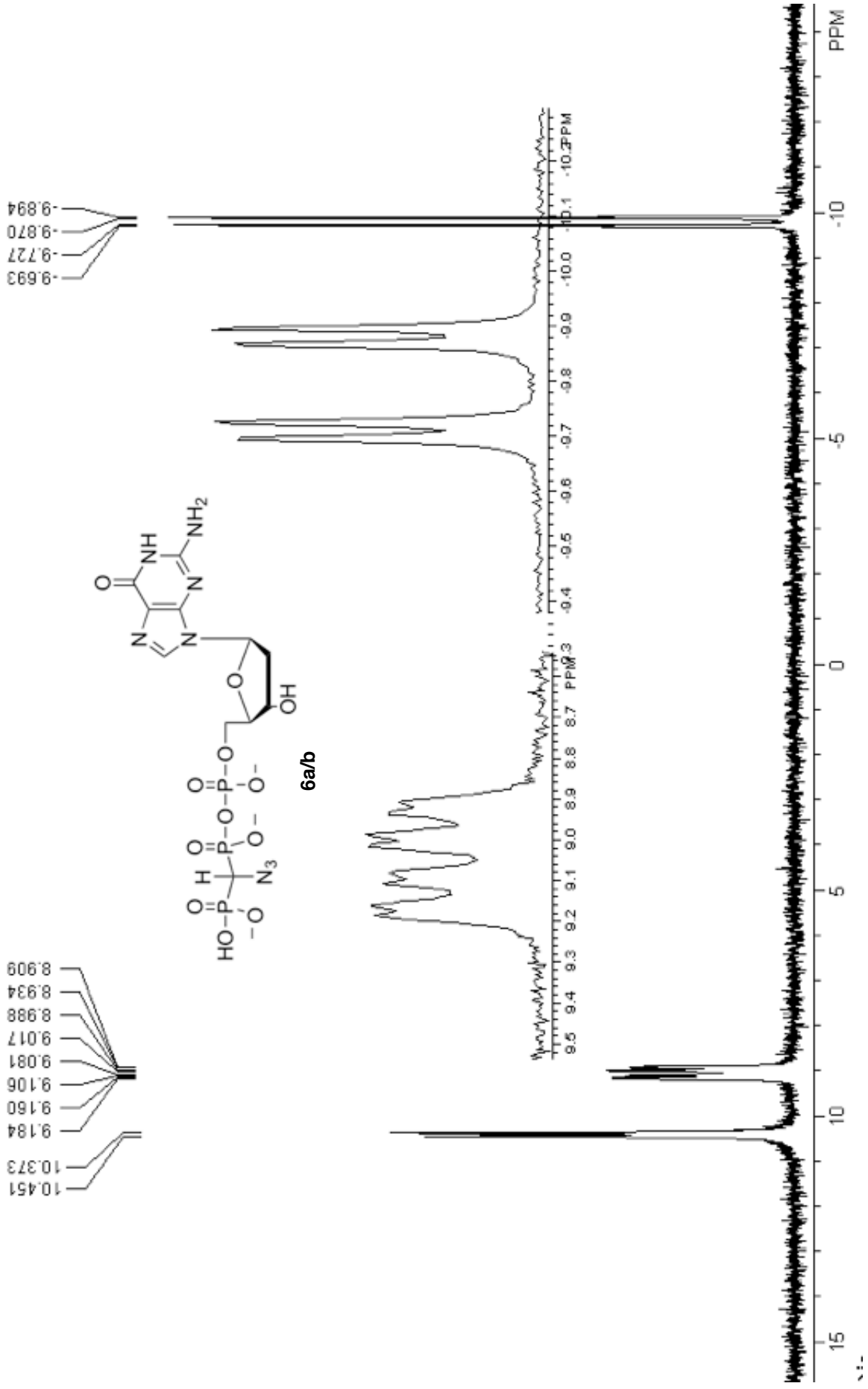
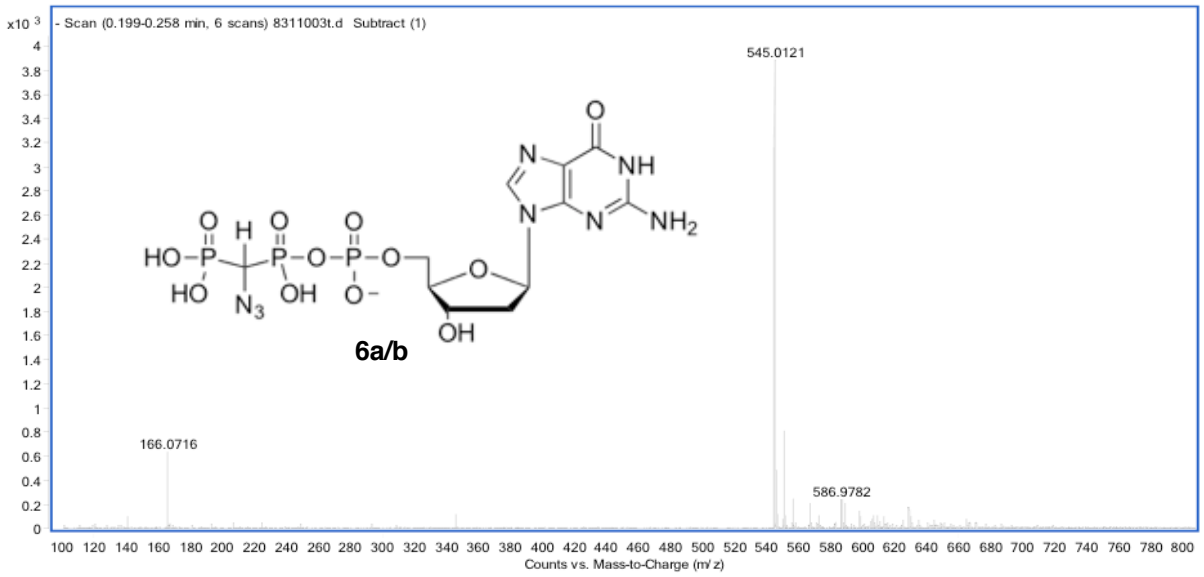


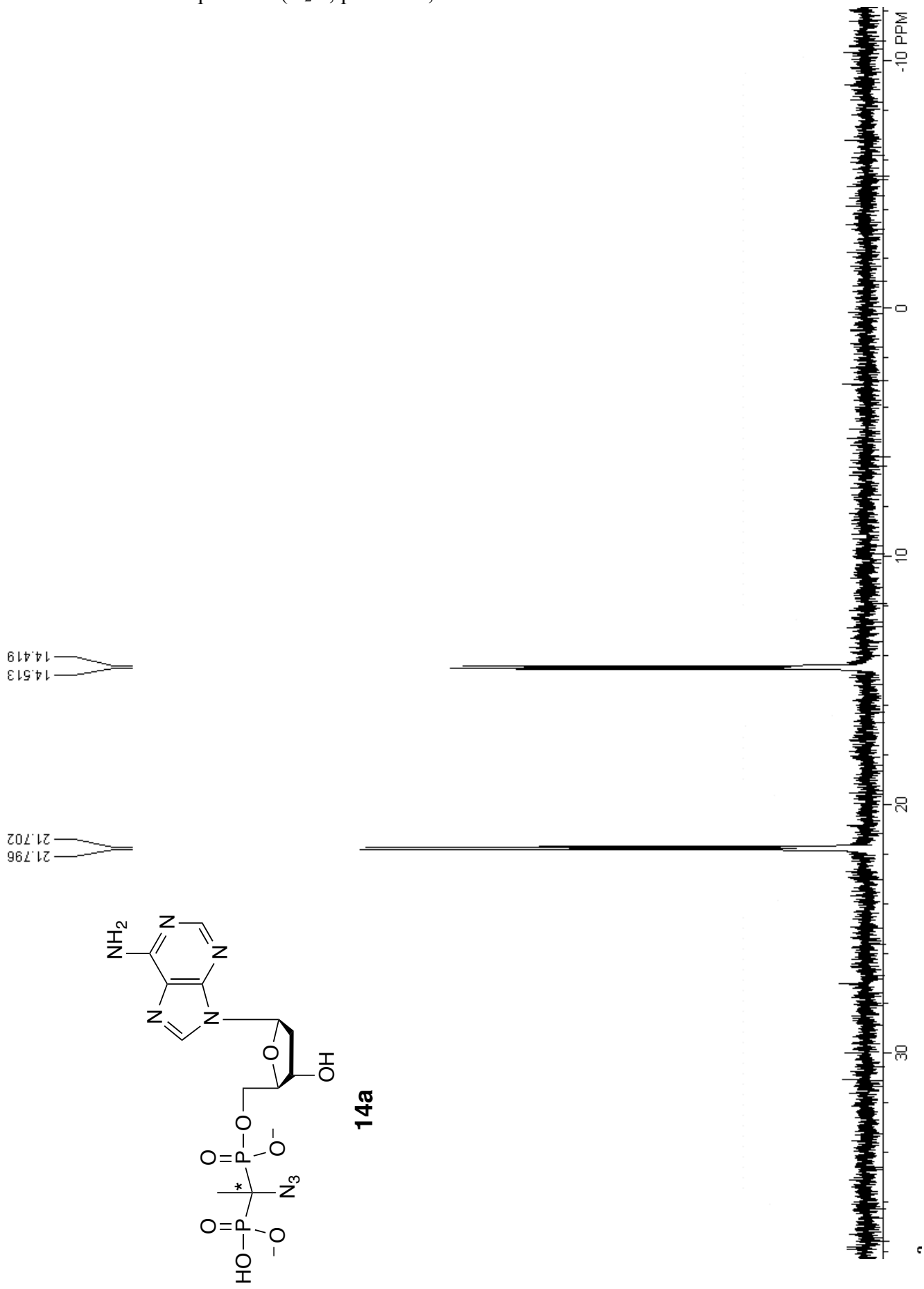
Figure S27. HRMS (ESI/ACPI) of **6a/b**.



Measured Mass	545.0121	
Element	**Low Limit**	**High Limit**
C	6	16
H	5	25
N	6	10
O	10	14
P	0	3

Formula	Calculated Mass	mDaError	ppmError	RDB
C11 H16 N8 O12 P3	545.0106	1.5	2.7	9.5
C15 H13 N7 O12 P2	545.0103	1.8	3.3	14
C16 H16 N6 O10 P3	545.0146	-2.5	-4.6	13.5
C13 H11 N10 O11 P2	545.0090	3.1	5.8	14.5
C13 H10 N10 O13 P	545.0172	-5.1	-9.3	14.5

Figure S28. ^{31}P NMR spectrum (D_2O, pH 10.88; 161.9 MHz of **14a.**



14a

Figure S29. ^{31}P NMR specturm (D_2O, pH 10.88; 161.9 MHz) of **14b.**

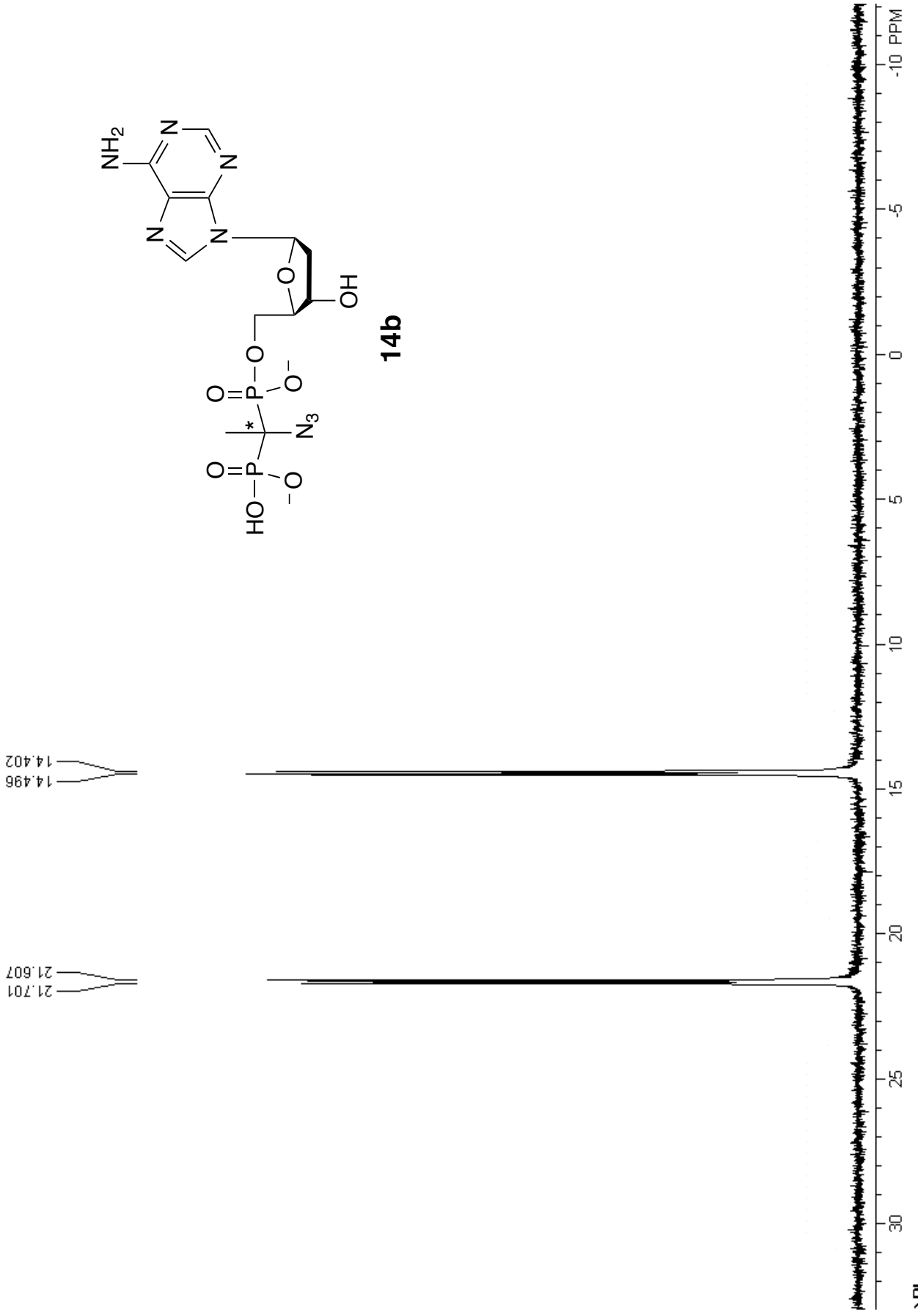


Figure S30. ^{1}H NMR spectrum (D_2O, pH 10.88; 399.8 MHz) of **14a**.

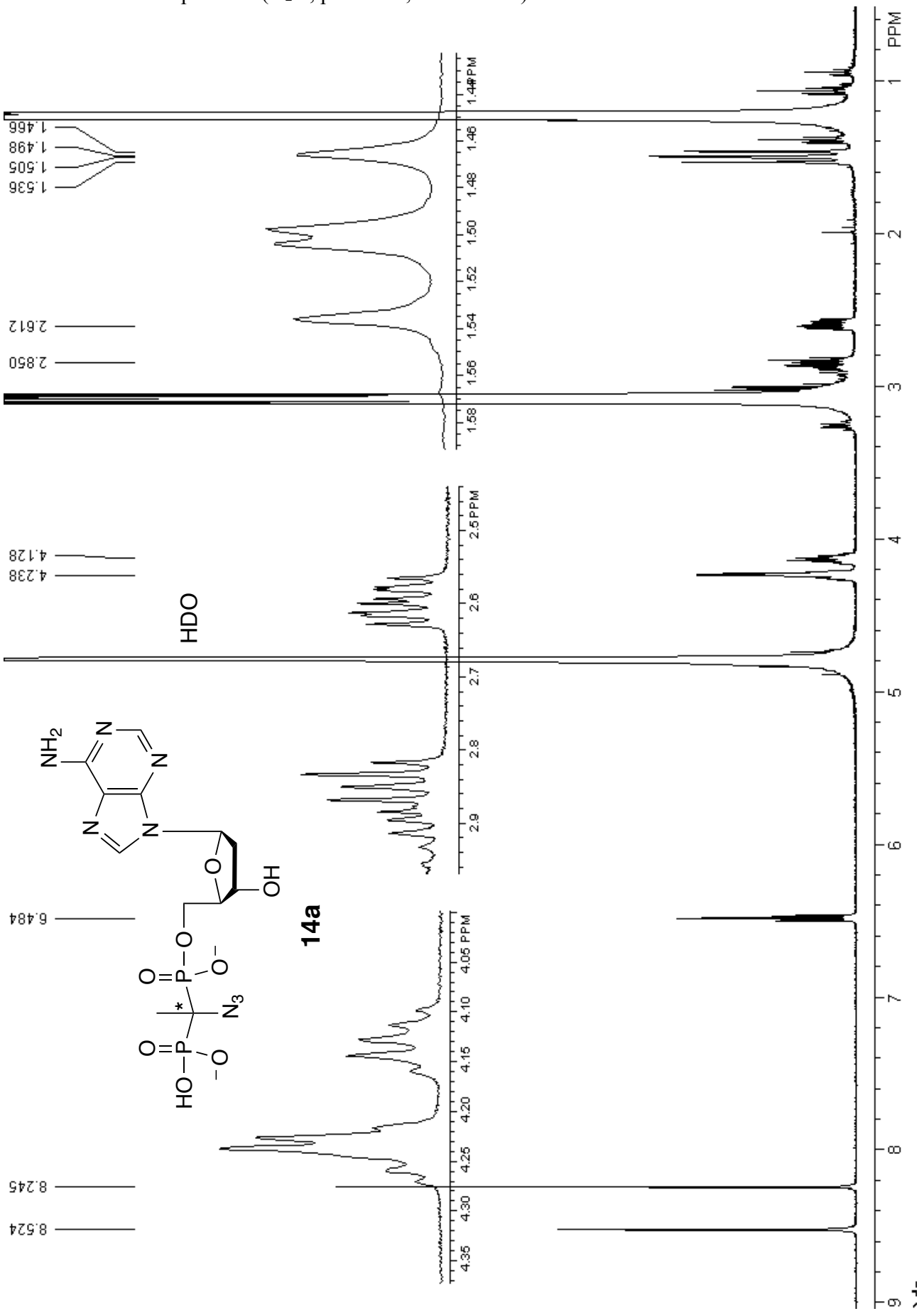


Figure S31. ^{1}H NMR spectrum (D_2O, pH 10.88; 399.8 MHz) of **14b**.

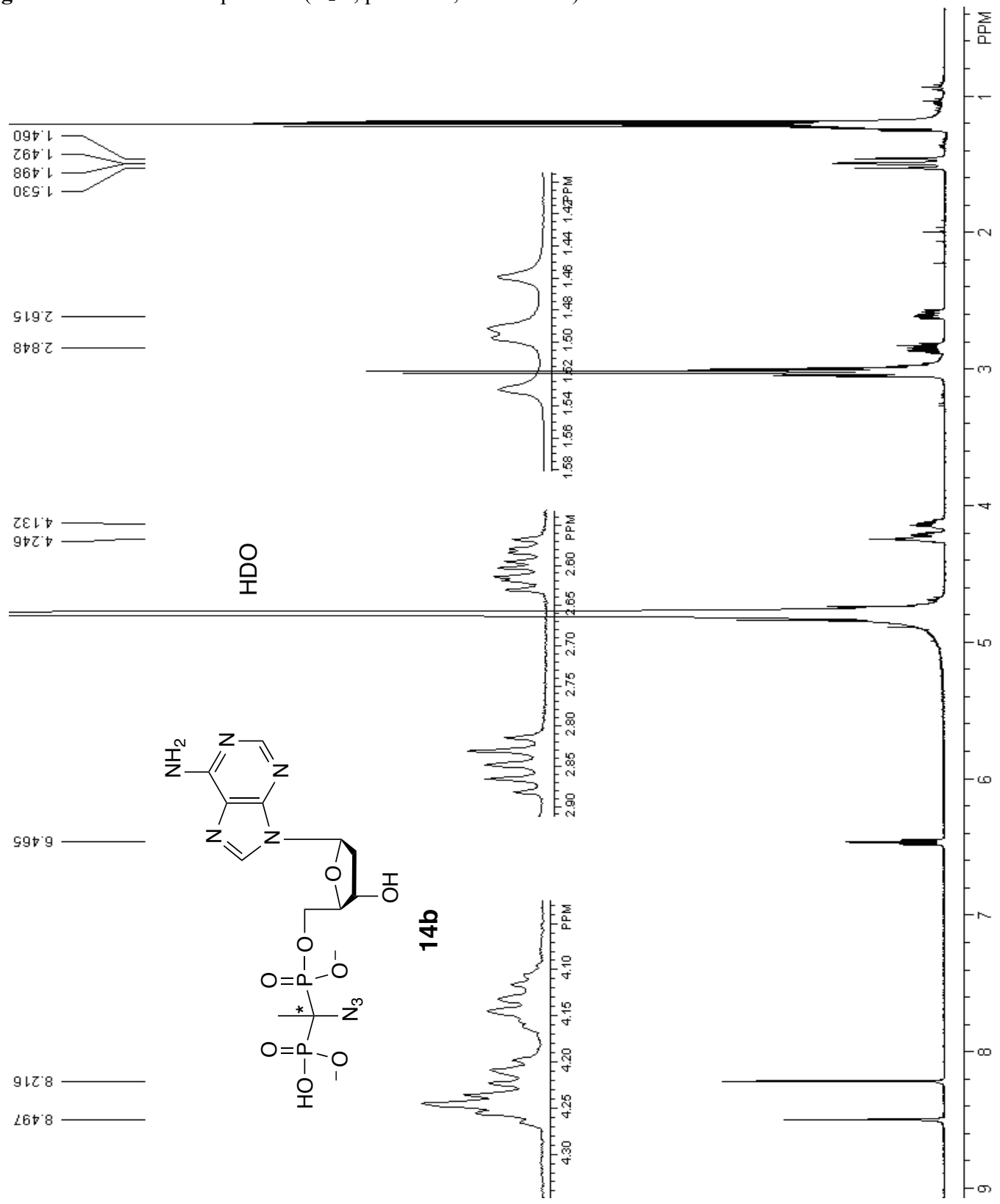


Figure S32. ^{1}H NMR spectrum (D_2O, pH 10.88; 499.7 MHz) of **7a**.

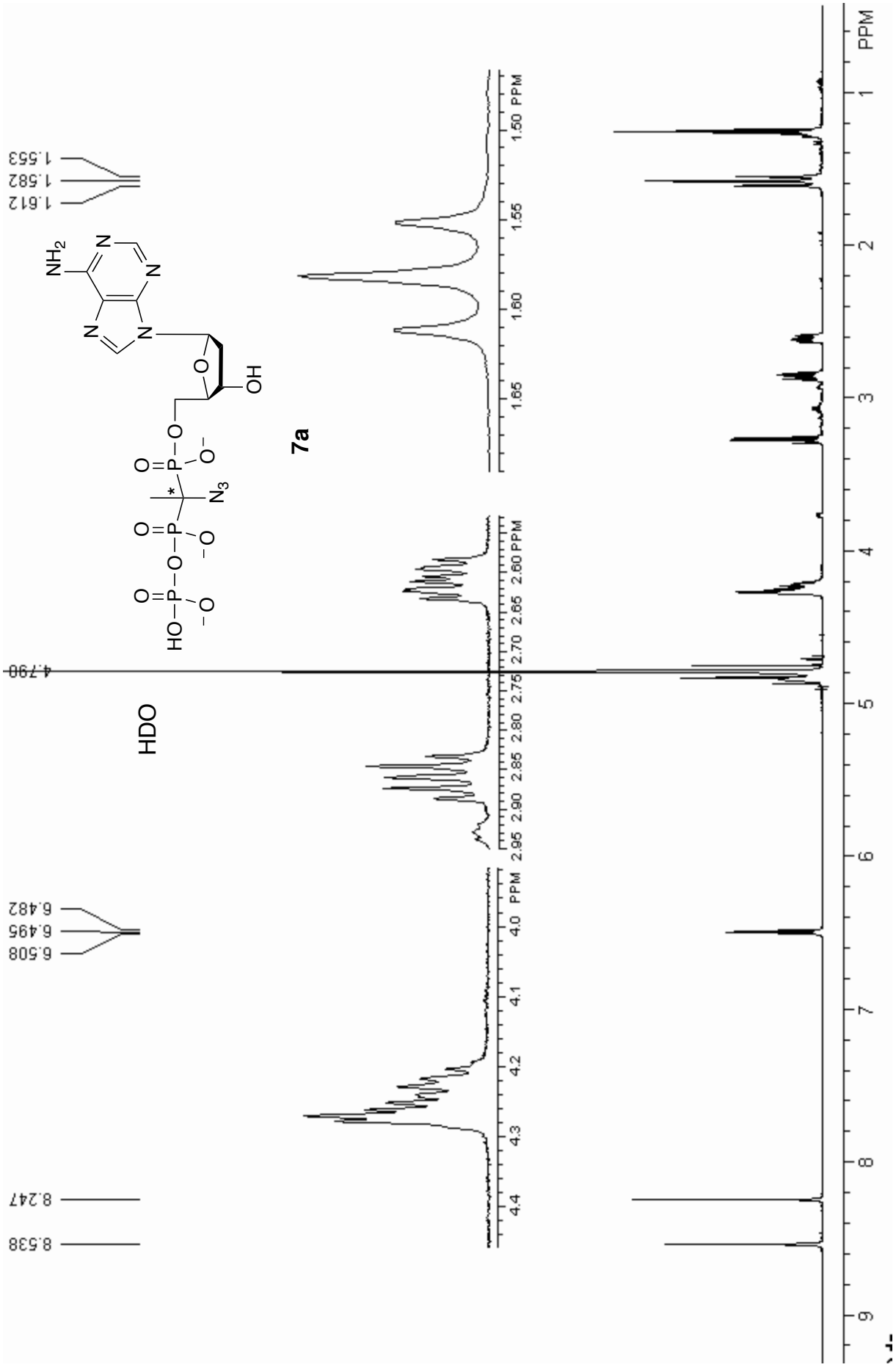


Figure S33. ^{31}P {^{1}H} NMR spectrum (D_2O, pH 10.88; 202.5 MHz) of **7a**.

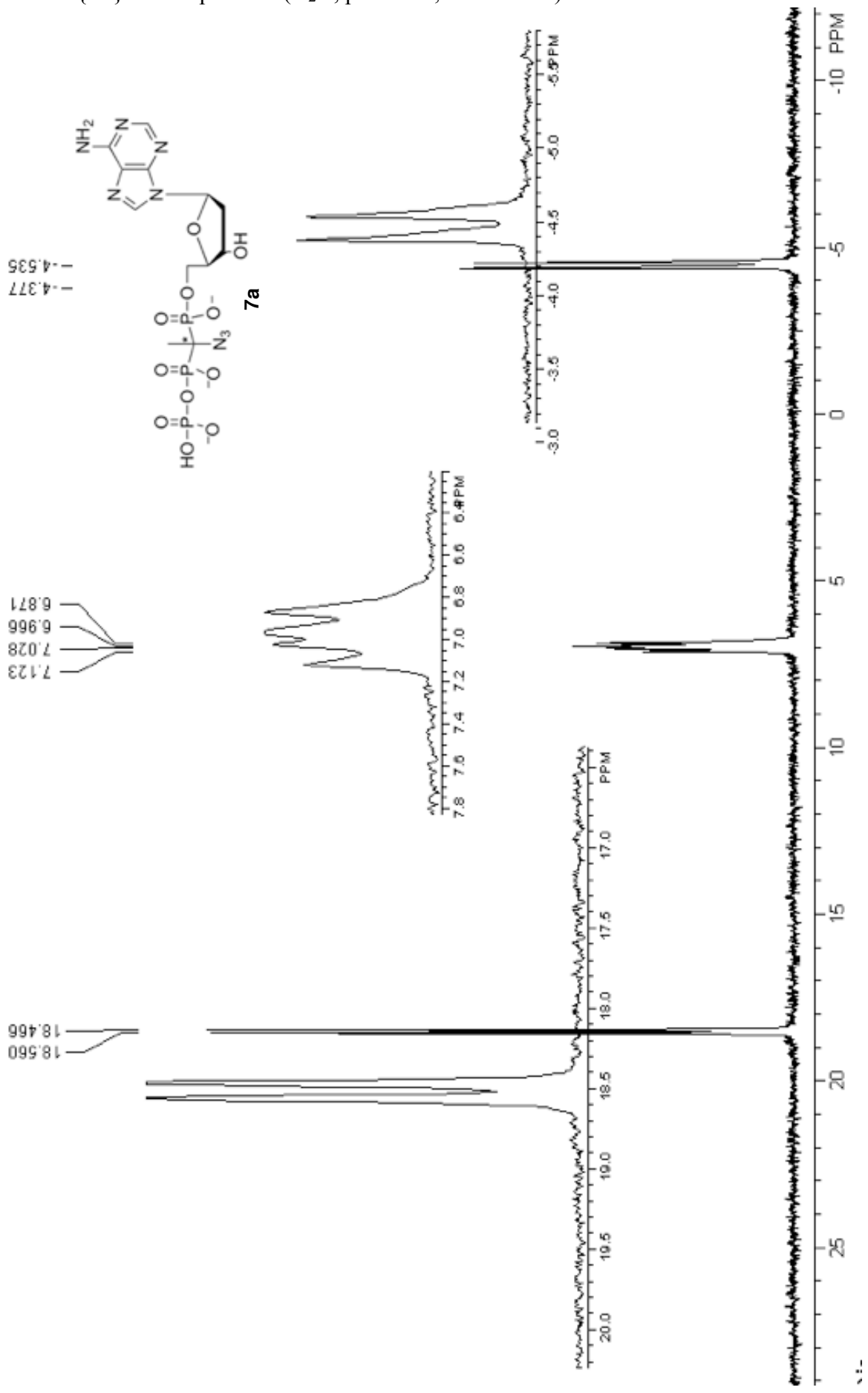


Figure S34. ^{1}H NMR spectrum (D_2O, pH 10.88; 499.7 MHz) of **7b**.

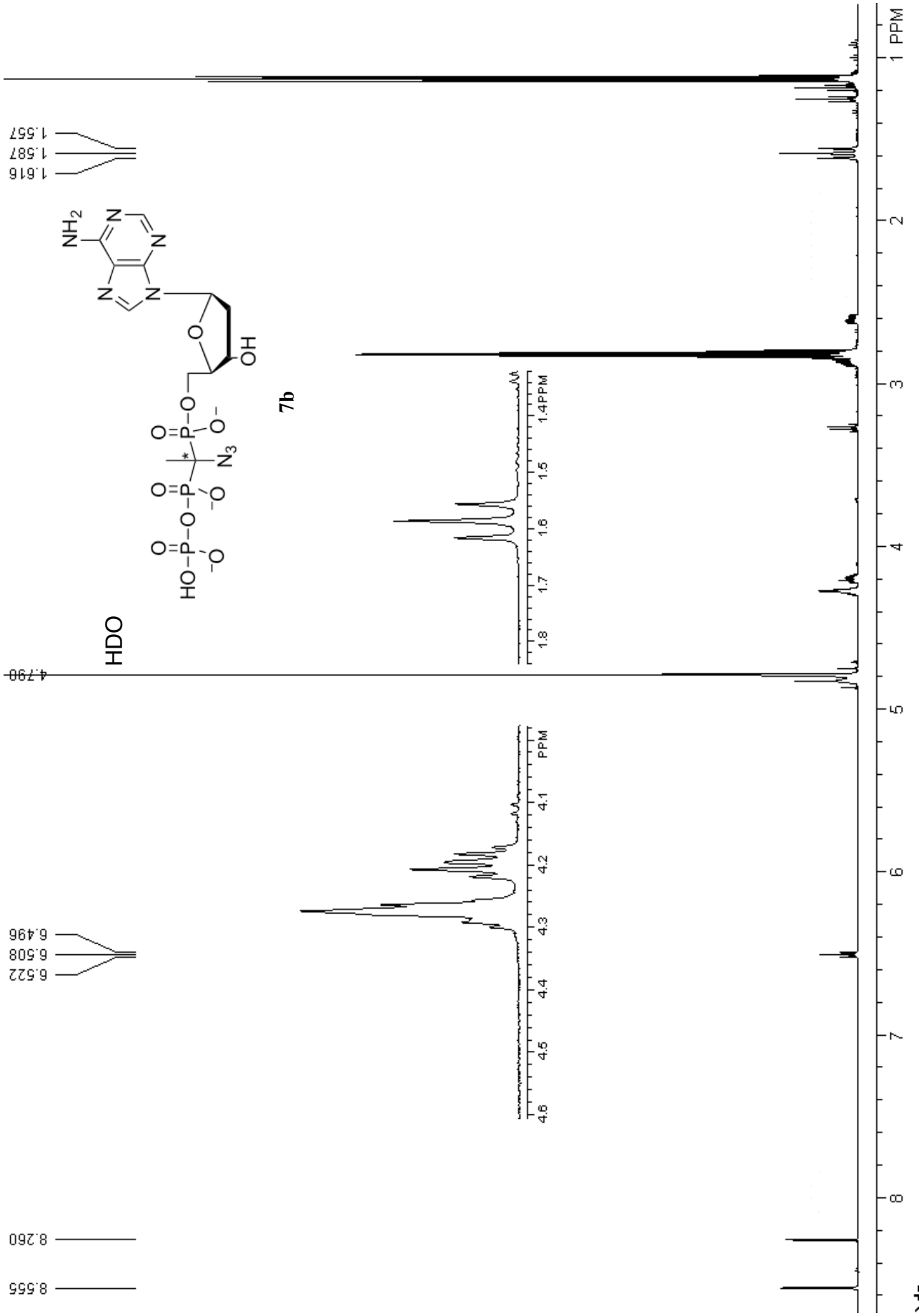


Figure S35. ^{31}P {^{1}H} NMR spectrum (D_2O, pH 10.88; 202.3 MHz) of **7b**.

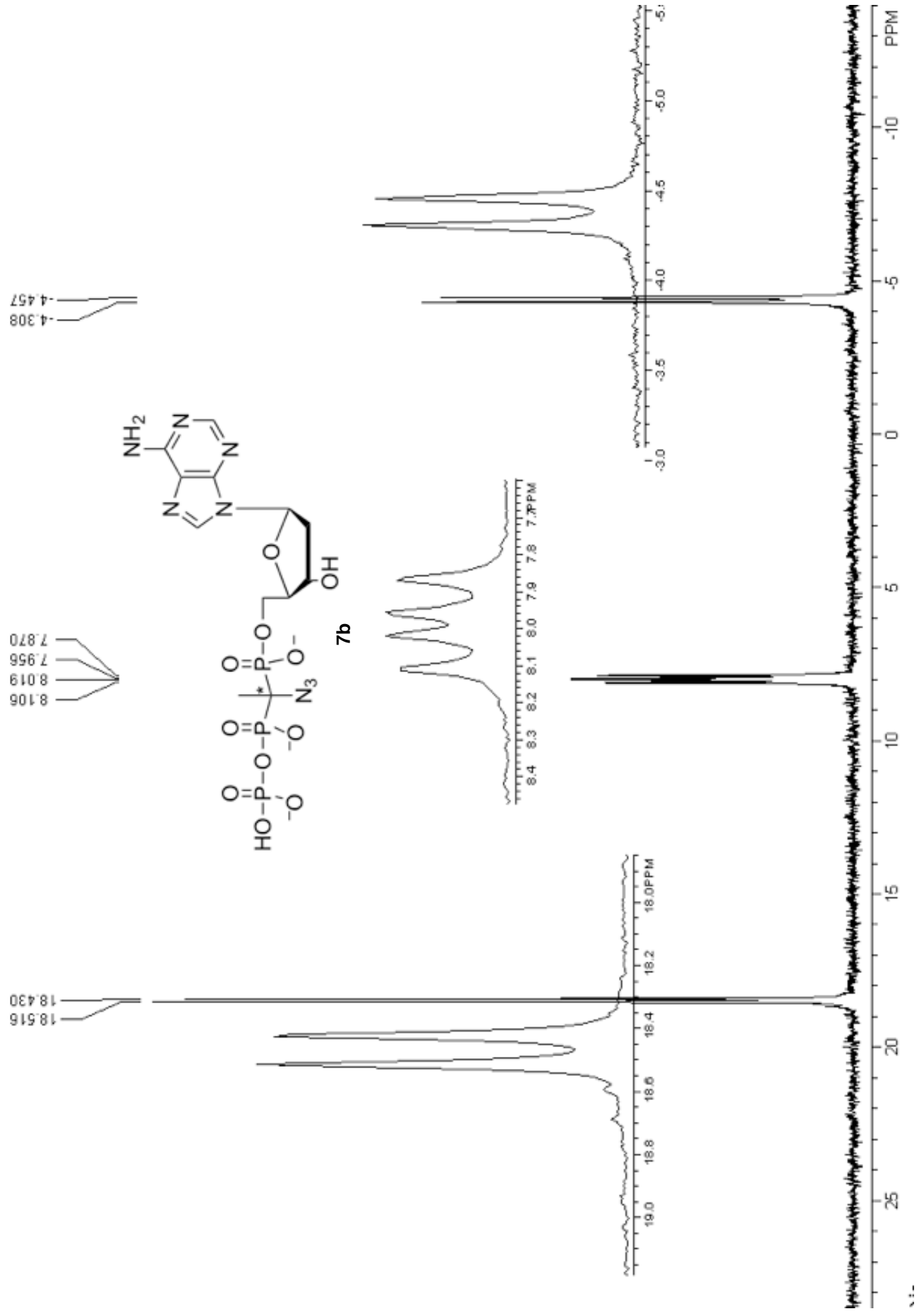


Figure S36. UV/VIS spectrum of **7a** in H_2O. pH 8.
Est. conc. ~0.05mM (E=15300).[2]

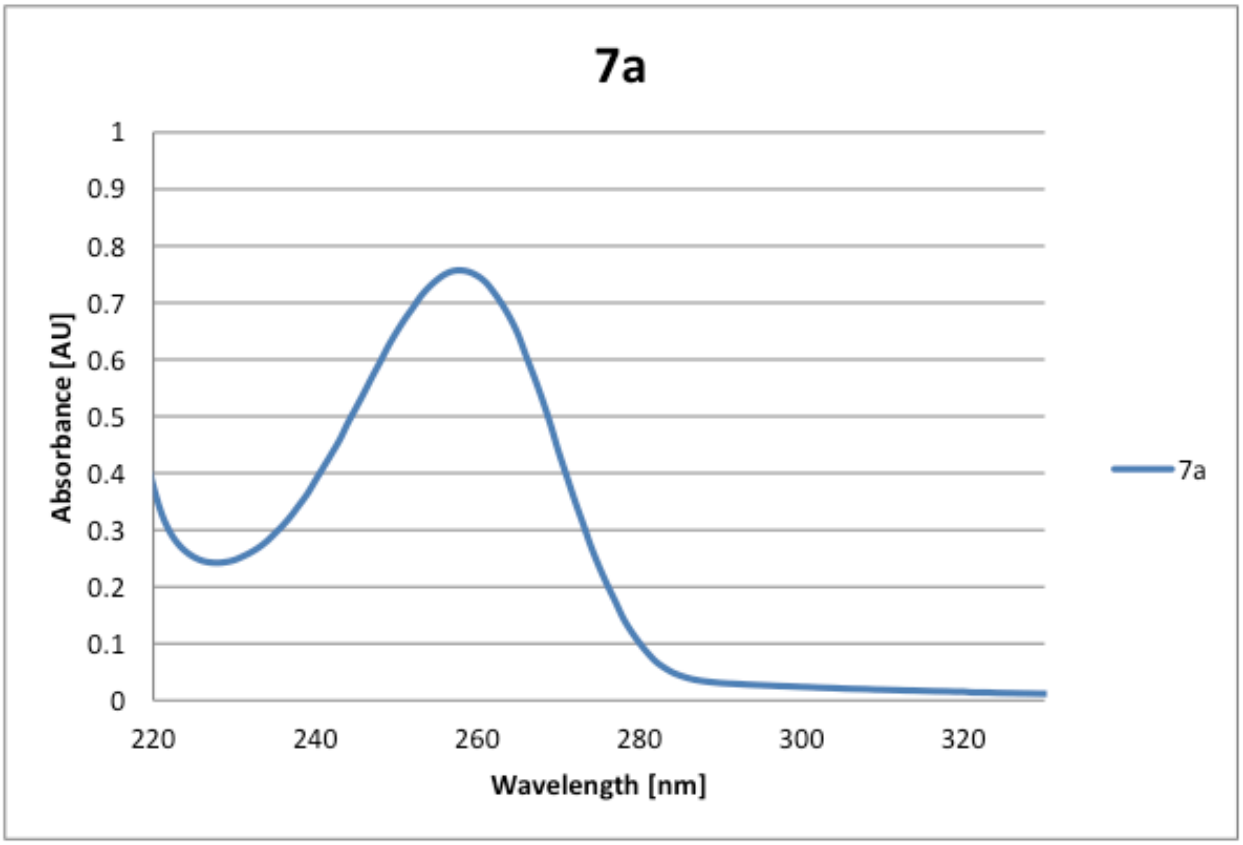


Figure S37. UV/VIS spectrum of **7b** in H_2O, pH 8.
Est. conc. ~0.05 mM (E=15300).[2]

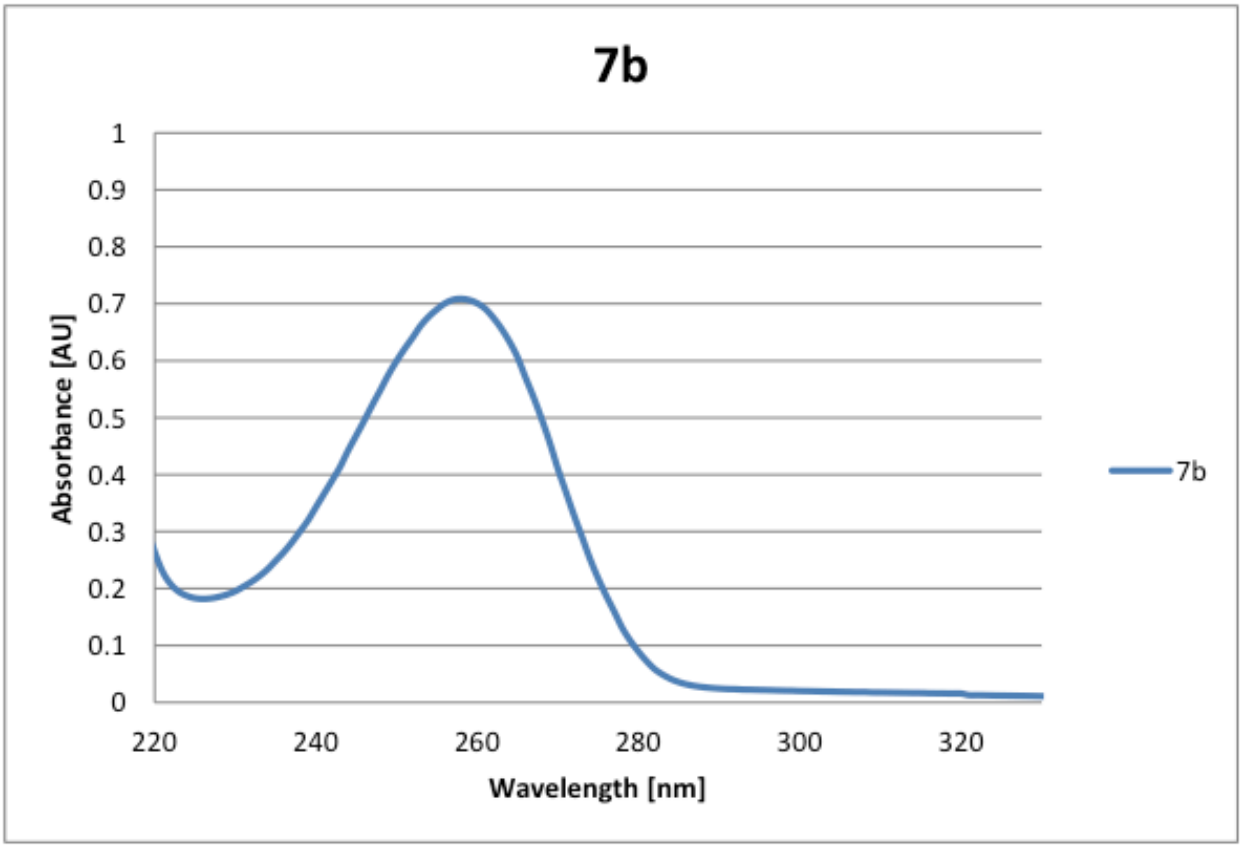


Figure S38. CD spectra of **7a** and **7b** (25° C, 0.05 mM) in H_2O, pH 8.

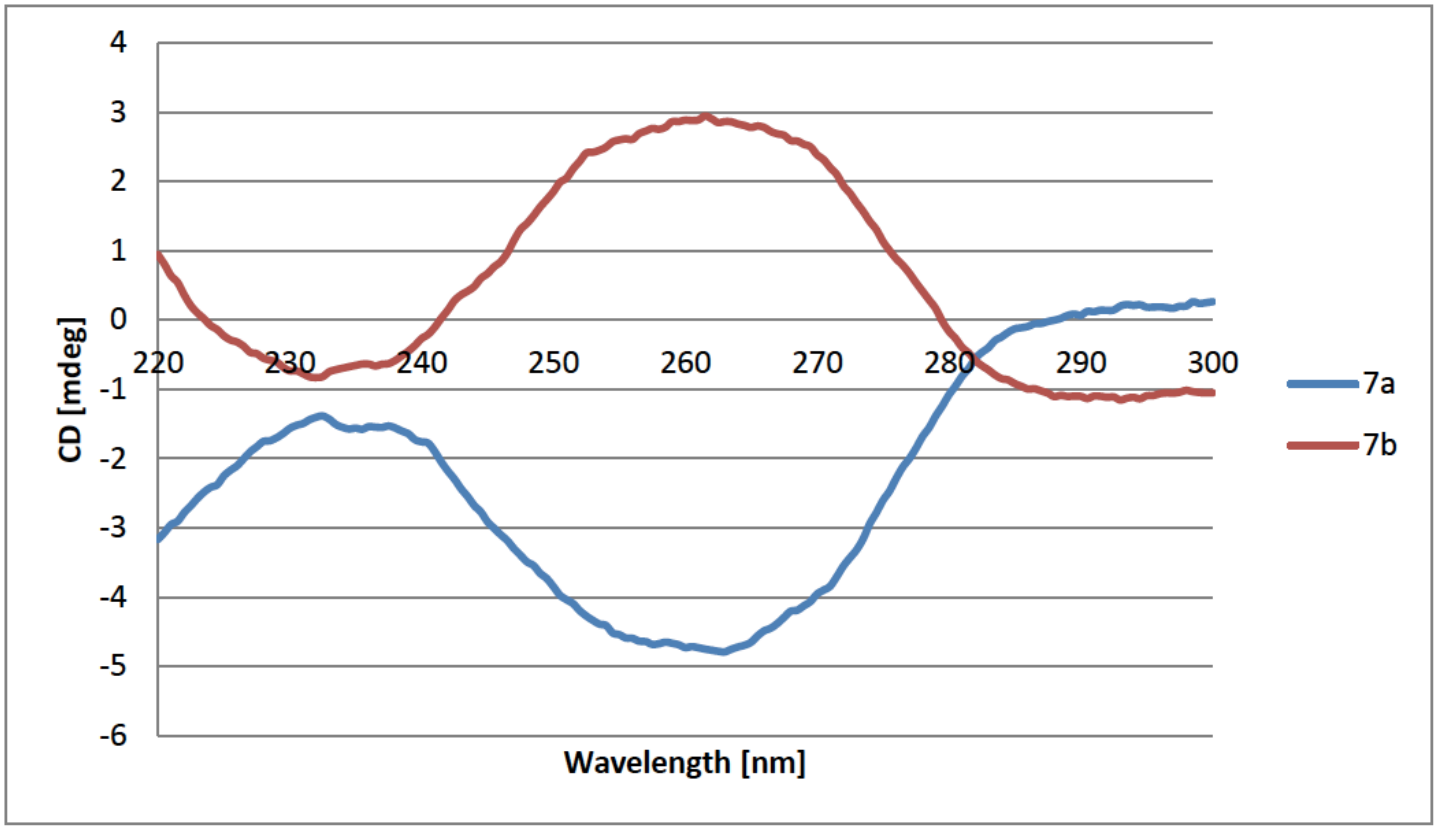


Figure S39. ^{31}P spectrum (D_2O, pH 10.88; 242.8 MHz) of **15a/b**.

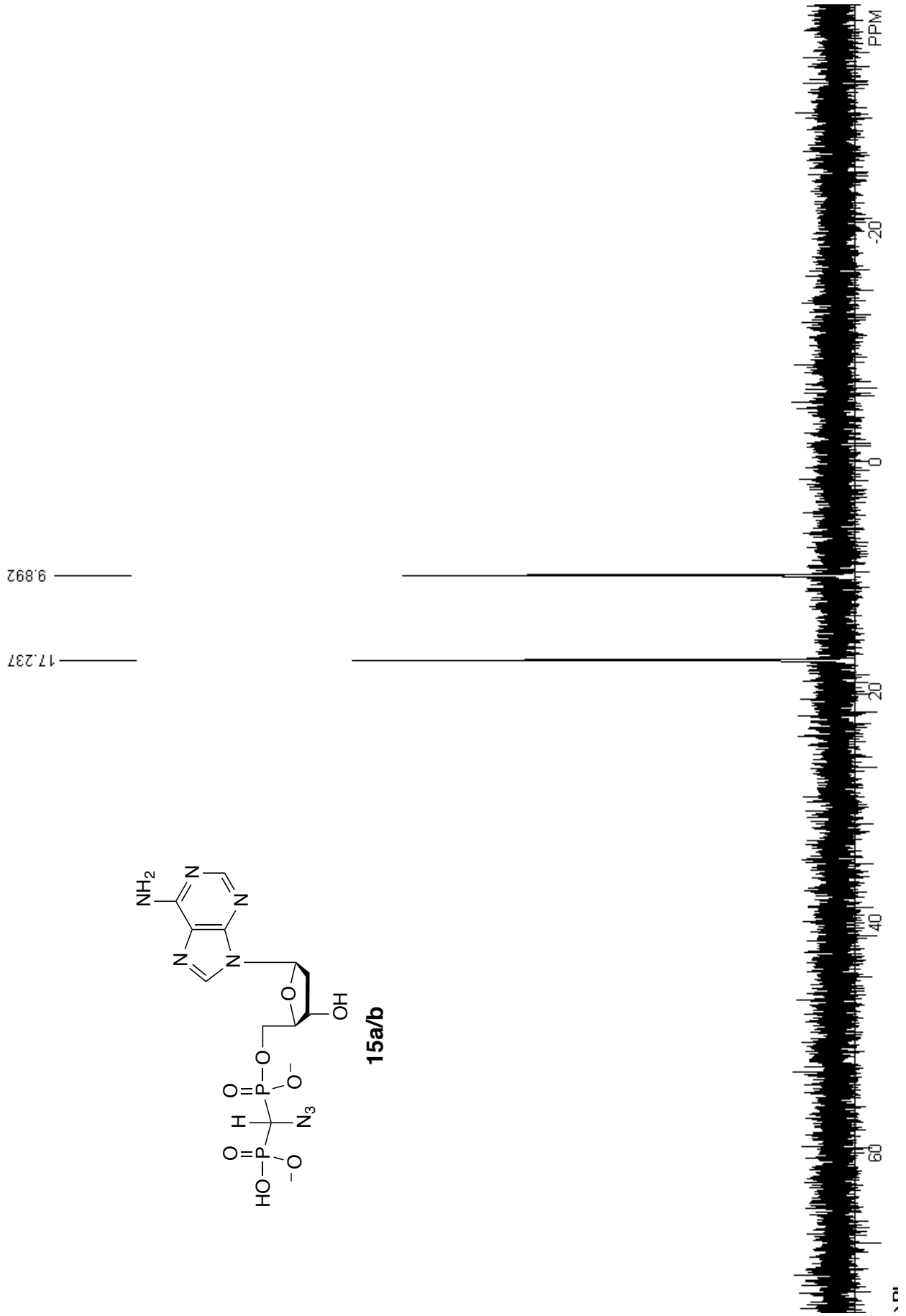


Figure S40. ^{1}H NMR spectrum (D_2O, pH 10.88; 400.2 MHz) of **8a/b**.

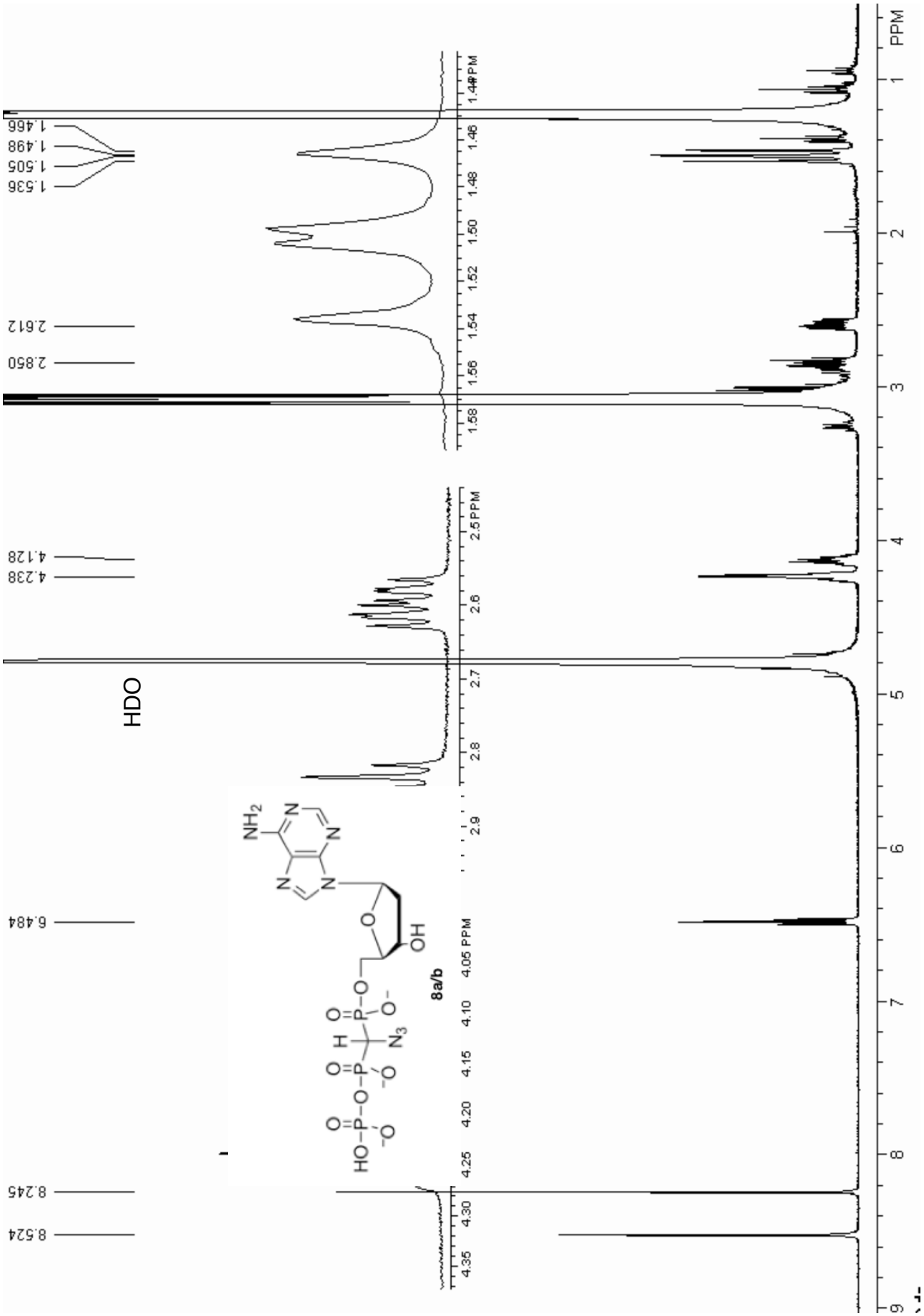


Figure S41. ^{31}P {^{1}H} NMR spectrum (D_2O, pH 10.88; 202.3 MHz) of **8a/b**.

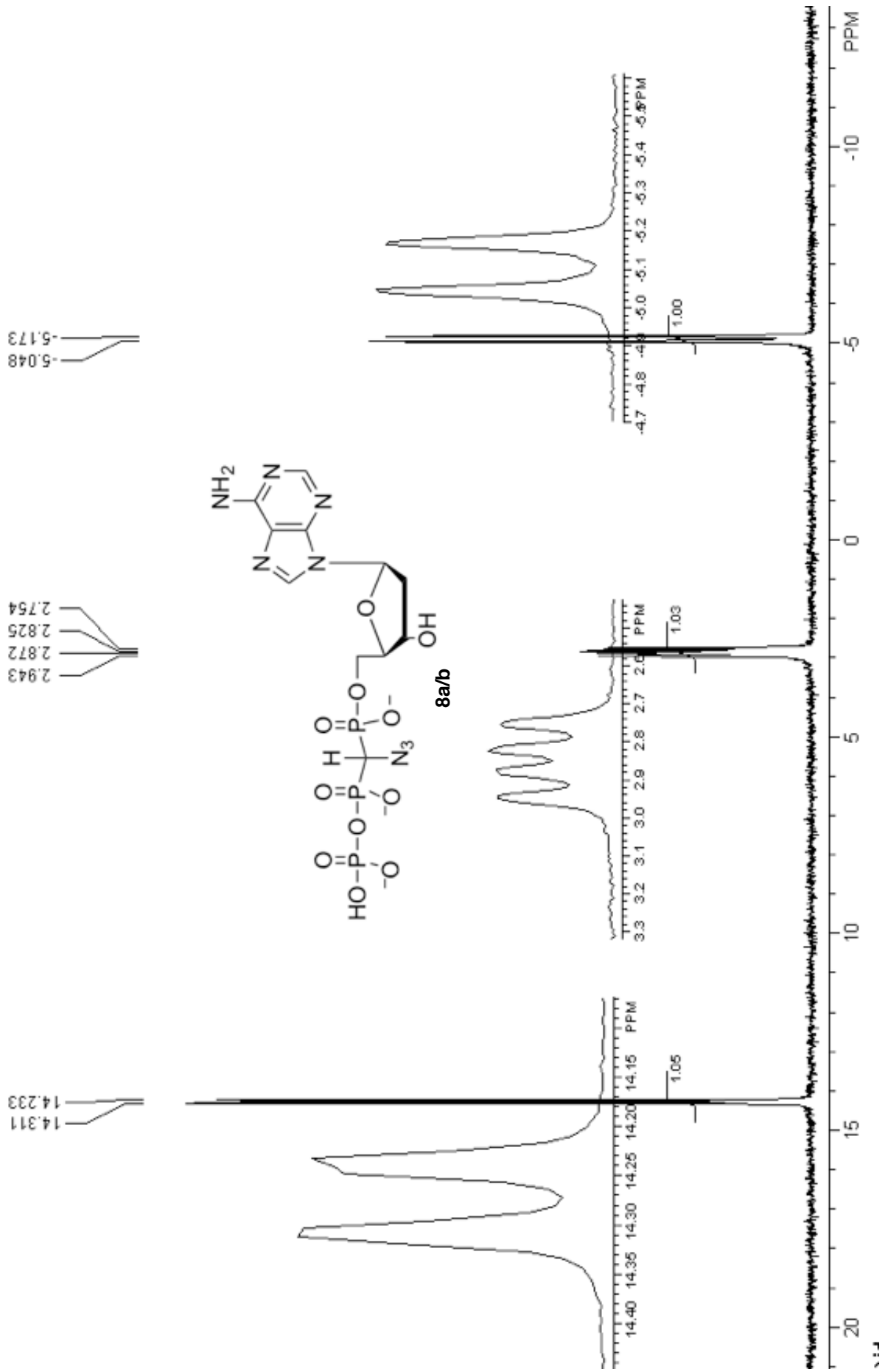


Table S1. Summary of HPLC (SAX) retention data for dNTP analogues.

Compound	RT (min)
5a/b	9.6
6a/b	10.3
7a	9.5
7b	9.5
8a/b	10.4

Figure S42. Representative analytical HPLC (SAX) analysis.
Shown, **5a/b**, conditions described in Materials and Methods.

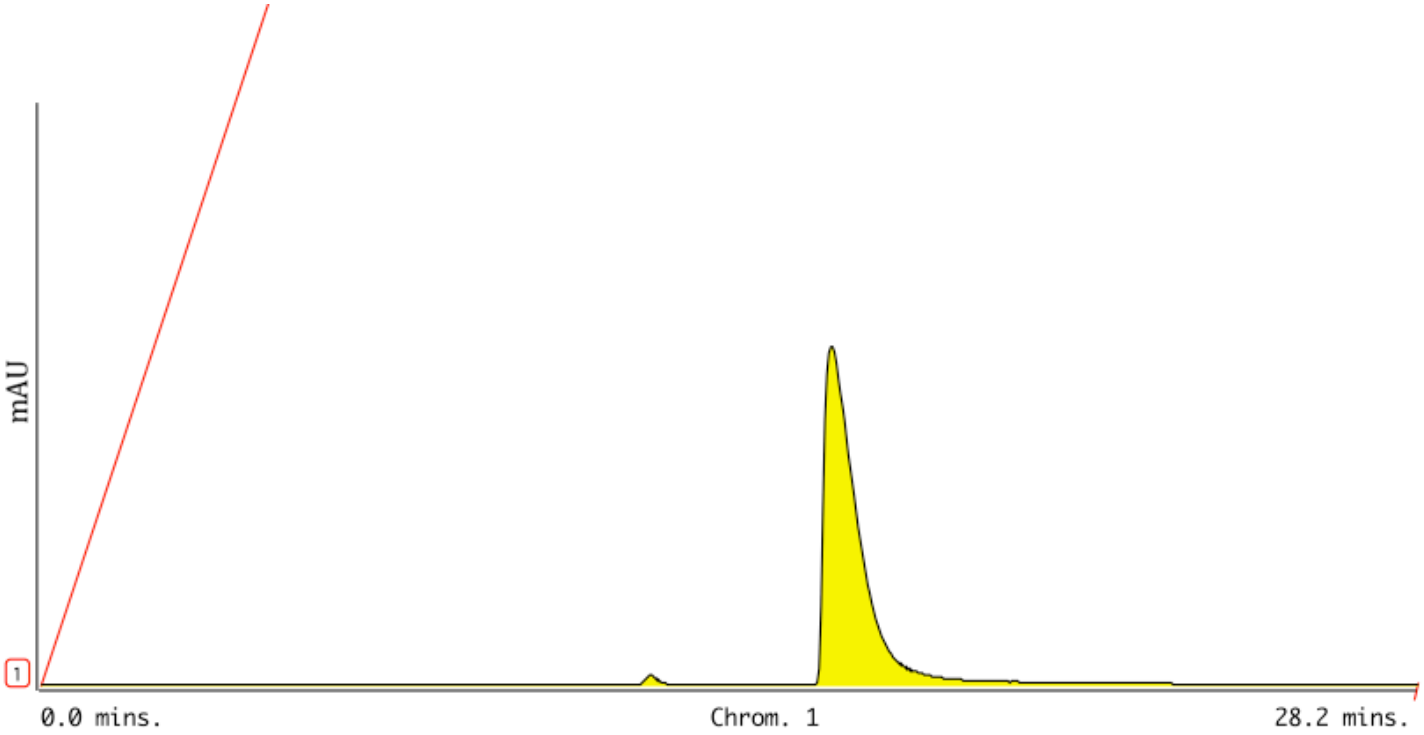


Figure S43. ^{31}P $\{^{1}H\}$ of *t*-BuOK-induced decomposition of **2**.
In cold THF ($CDCl_3$; 202.5 MHz), to **12** (literature value: ^{31}P -23.1)[3] and **13**.[4] Diisopropyl phosphate (δ 1.313) probably arises from hydrolysis of **12**. **13** assignment based on ^{31}P NMR and MS (not shown).

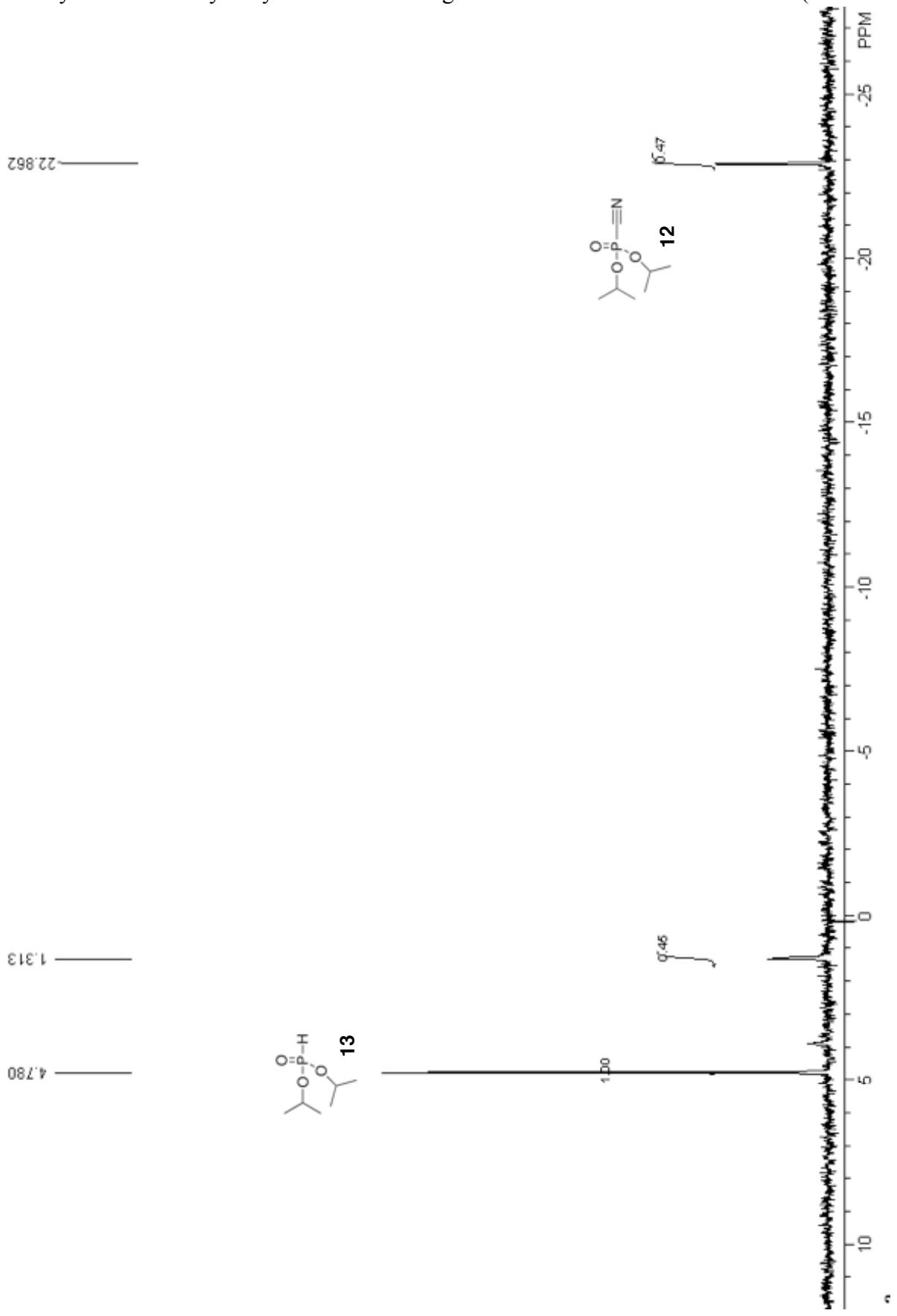


Figure S44. ^{31}P NMR spectrum (DMF; 202.5 MHz) of reaction mixture obtained by procedure for synthesis of "azido" product ref. 5.

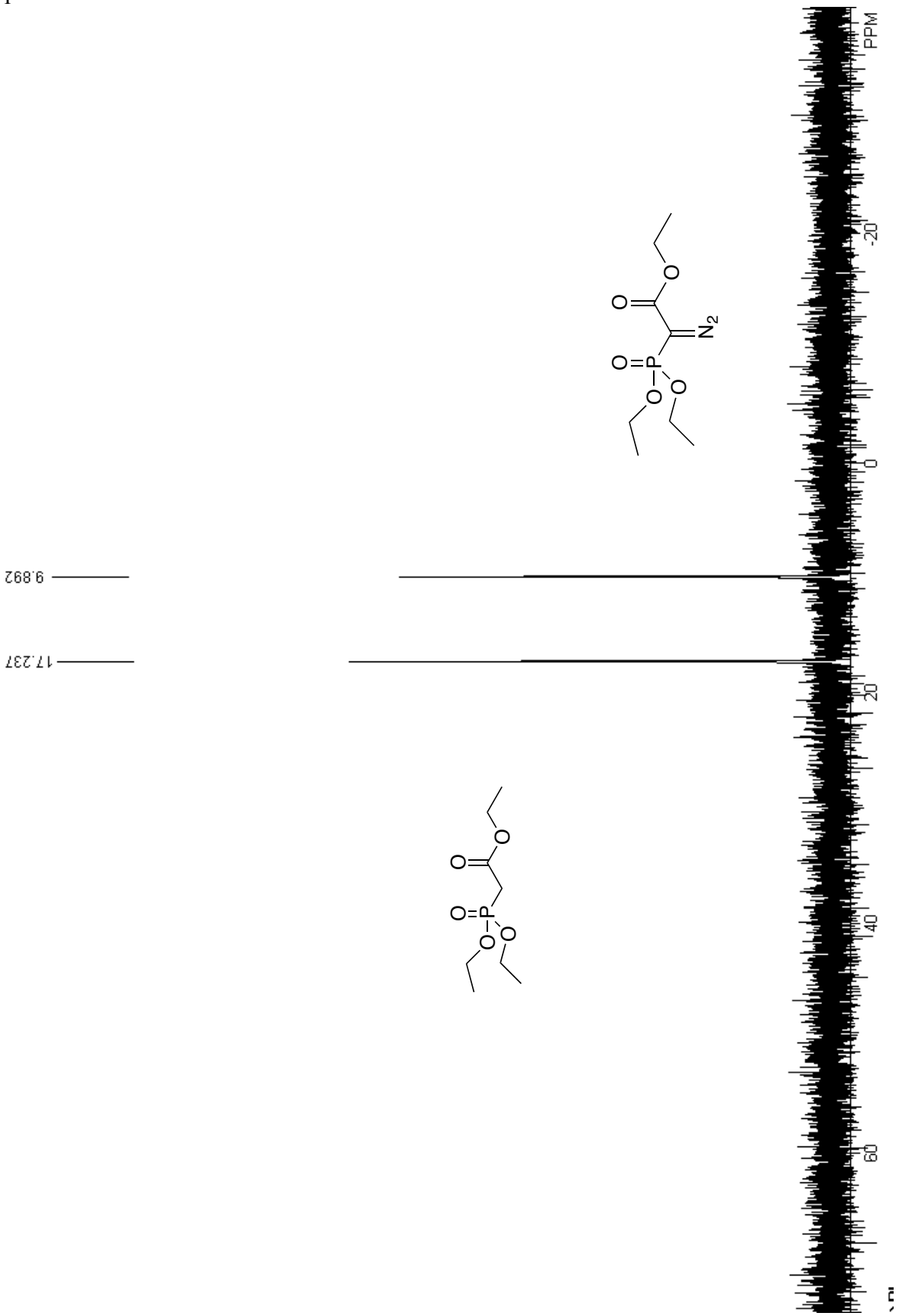


Figure S45. ^{31}P NMR spectrum ($CDCl_3$; 202.5 MHz) of triethyl diazophosphonoacetate obtained using procedure from ref. 5.

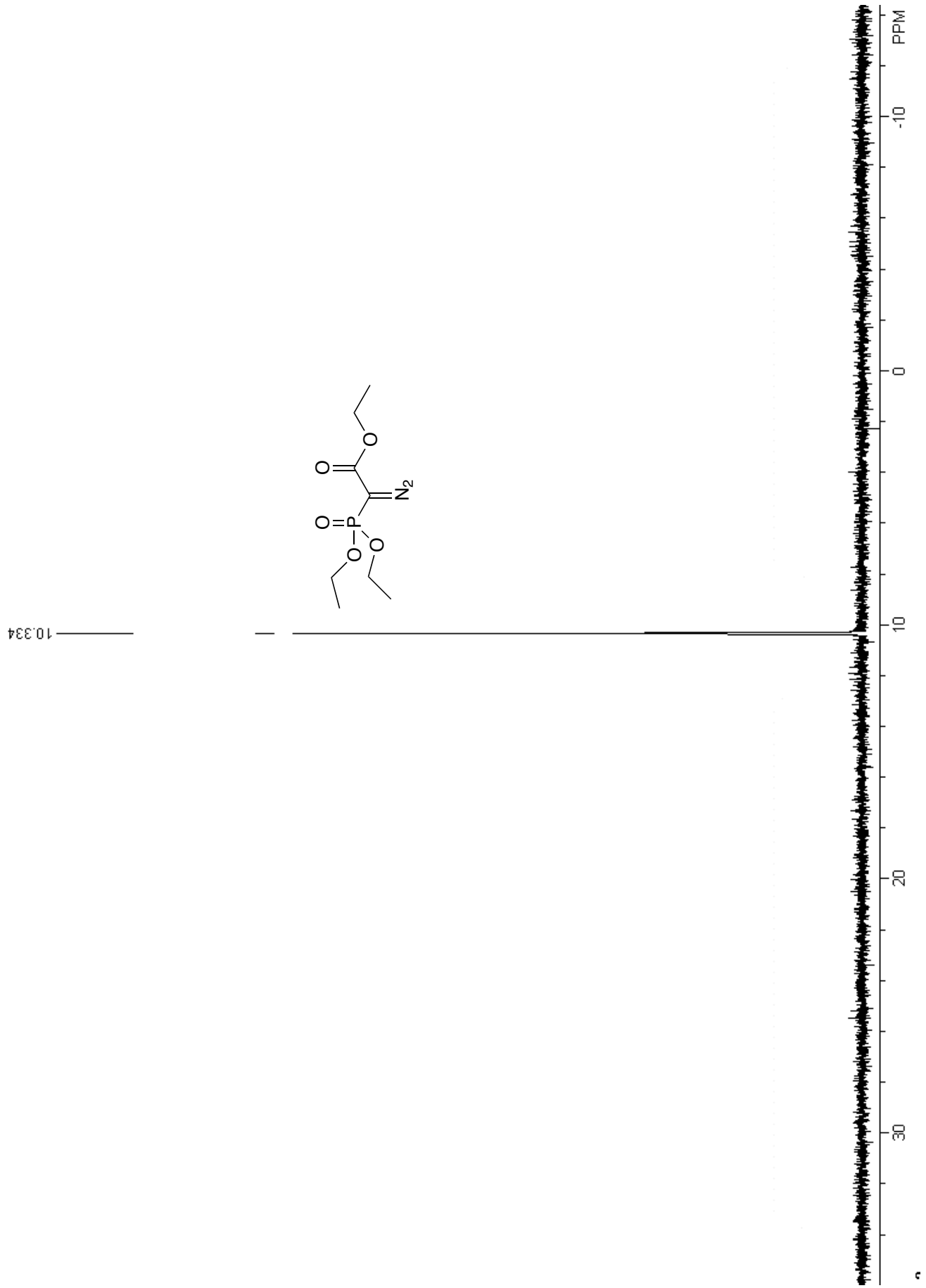


Figure S46. ^{1}H NMR spectrum ($CDCl_3$; 400.2 MHz) of triethyl diazophosphonoacetate obtained using procedure from ref. 5.

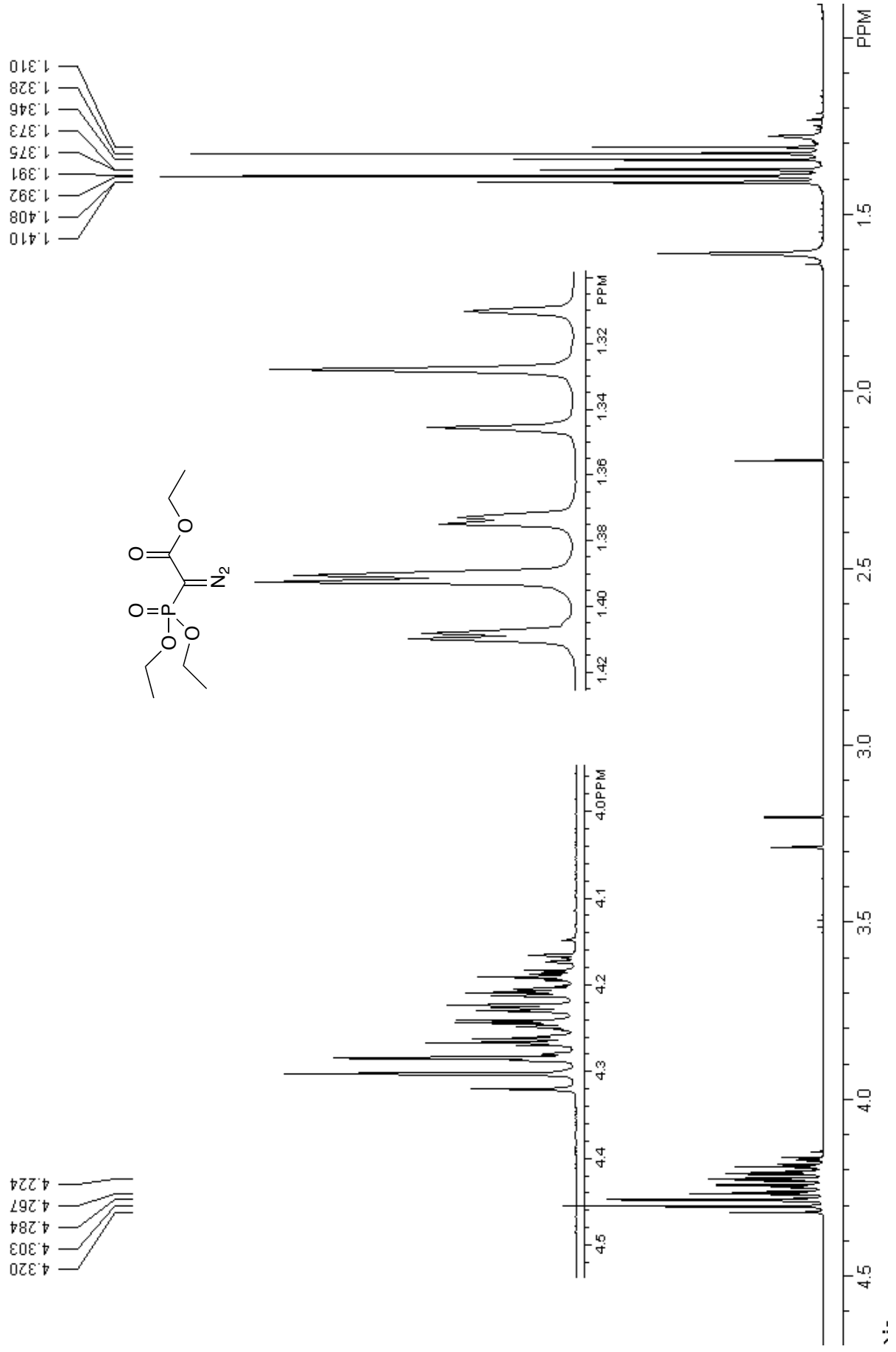


Figure S47. MS (ACPi) $[M+1]^+$ spectrum of reaction mixture obtained using procedure from ref. 5.

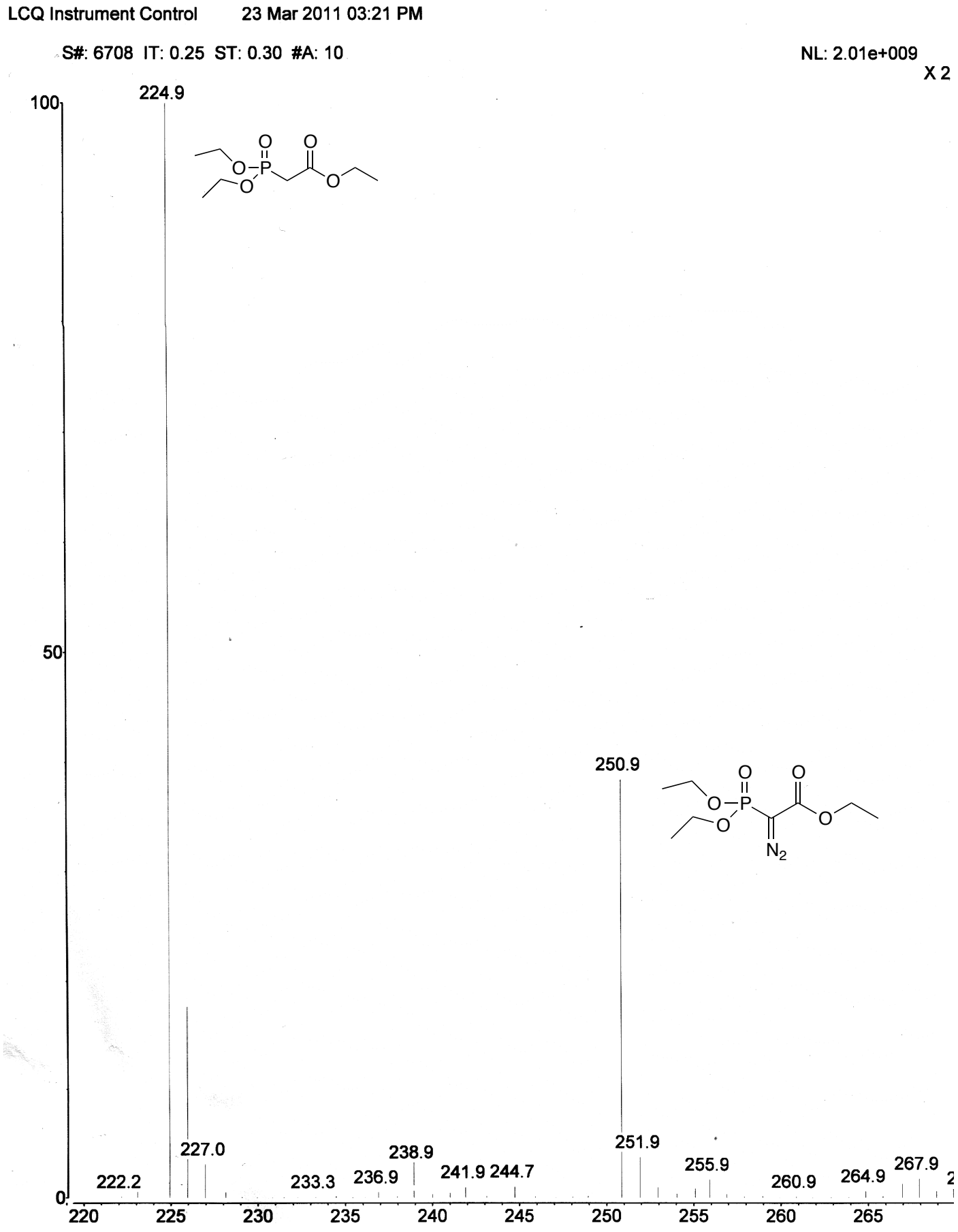
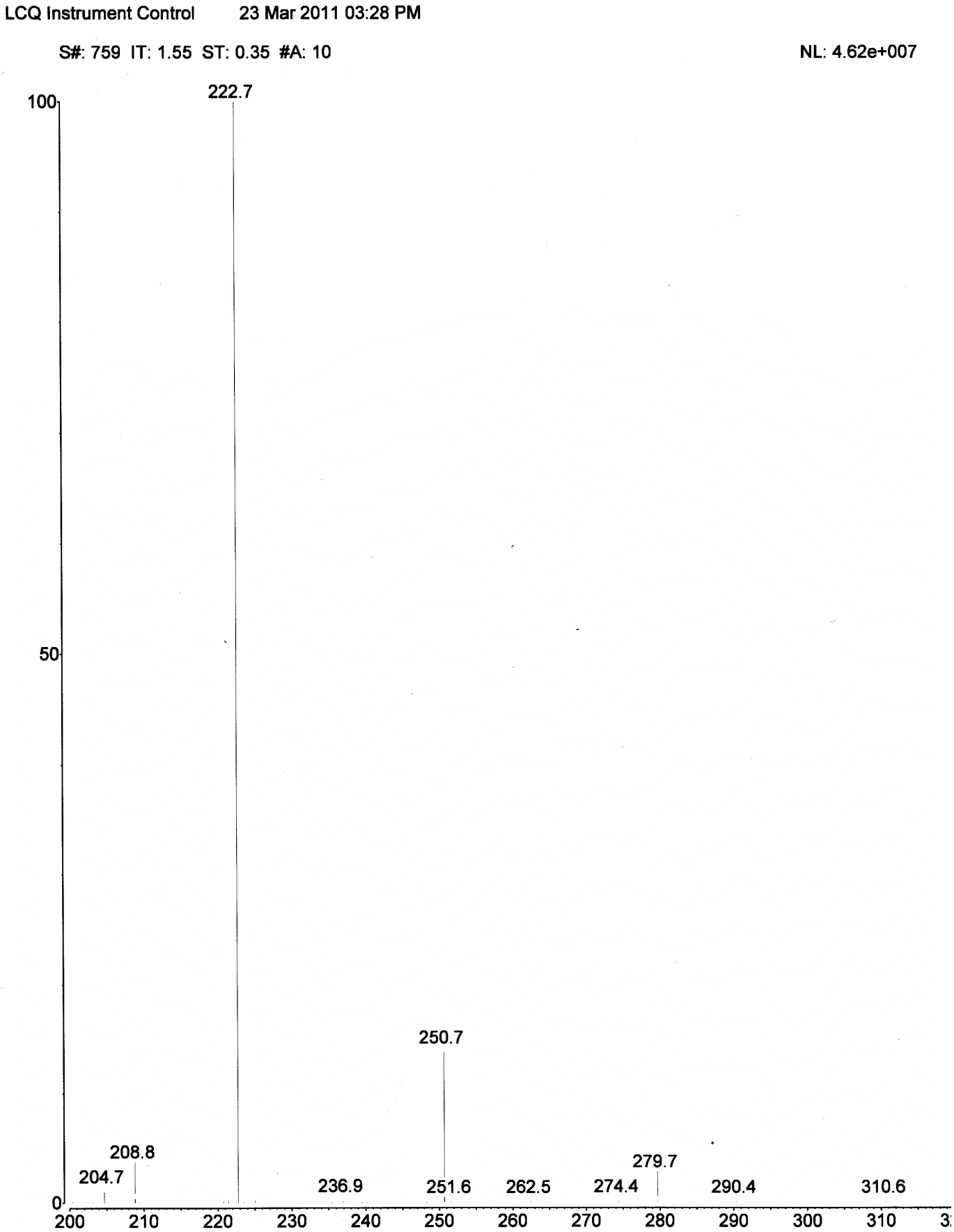


Figure S48. MS/MS spectrum of m/z 251.



References

1. Mintz, M.J., Walding, C.; *Org. Synth.*, **1969**, 49, 9.
2. Dawson, R.M.C.; *Data for Biochemical Research, 3rd ed.* Oxford University Press, **1986**.
3. Sun, D., et al.; *Phosphorus Sulfur Silicon., Relat. Elem.*, **2005**, 180, 2155-2161.
4. Chen, Y., Zhao, Y.-F., Yin, Y.-W., Yang, X.-Q.; *Phosphorus Sulfur Silicon., Relat. Elem.,* **1991**, 61, 31-39.
5. Hakimelahi, G. H.; Just, G. Synth. Commun. **1980**, 10, 429.