

Supporting Information for

A Cyanohydrin Phosphonate Natural Product from *Streptomyces regensis*

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## Supplementary Experimental Procedures

### *Disk Diffusion Bioassays against an E. coli phosphonate uptake strain*

Sensitivity of the IPTG-inducible phosphonate uptake strain to the compounds reported in this study was tested using a disk diffusion assay. Plates containing 20 mL of 1.5% agar (M9 or LB) were overlaid with 10 mL of 0.75% top agar (M9 or LB) that was warmed to 50°C and inoculated with 100  $\mu$ L ( $OD_{600}=0.5$ ) of an *E. coli* strain (WM6242) engineered with an IPTG-inducible phosphonate uptake system (*phnCDE*). 1mM IPTG was added for induction of phosphonate uptake or withheld for repression of uptake machinery. After the seeded overlay solidified, 6 mm paper disks were spotted with 50  $\mu$ g of compound and applied to the plates that were then incubated at 37°C overnight. Sensitivity to a compound was visualized by a clear zone of inhibition surrounding the respective disk. Phosphonate-specific activity was queried by preparing plates with and without 1mM IPTG.



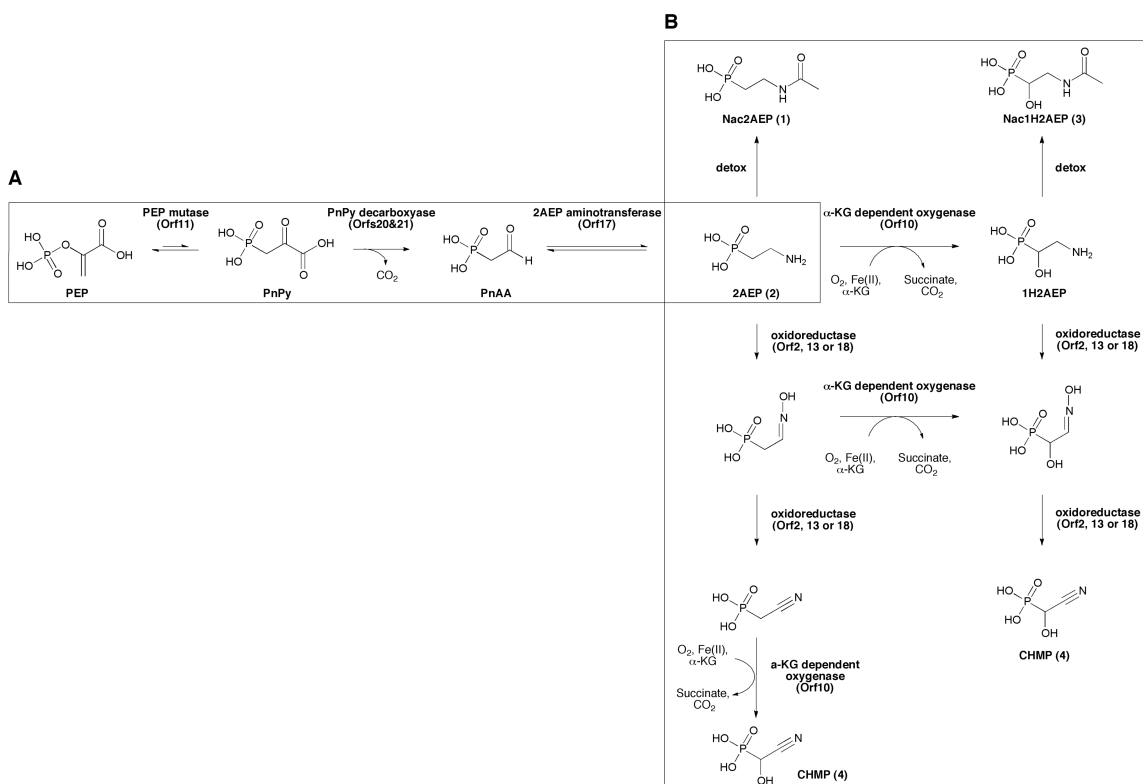


Figure S1. Proposed Biosynthetic Pathway for Compounds 1-4 (A) First three proposed steps in the *Streptomyces regensis* WC-3744 phosphonate biosynthetic pathway are based on the presence of genes in the *S. regensis* gene cluster with homology to characterized enzymes from known phosphonate biosynthetic pathways. *S. regensis* homologs are in parentheses. The pathway begins with the conversion of phosphoenolpyruvate (PEP) to phosphonopyruvate (PnPy) catalyzed by PEP mutase, followed by decarboxylation of PnPy to phosphonoacetaldehyde (PnAA) catalyzed by PnPy decarboxylase. The PLP dependent 2AEP aminotransferase would then transfer an amino group to PnAA to afford compound 2, (2-aminoethyl)phosphonic acid (2AEP). (B) Possible routes to compound 1, (2-acetamidoethyl)phosphonic acid (Ac2AEP); compound 3, (2-acetamido-1-hydroxyethyl)phosphonic acid (NAc1H2AEP); and compound 4, (cyano(hydroxy)methyl)phosphonic acid via a 2AEP intermediate. Candidate Orfs from the *S. regensis* gene cluster for each conversion are in parentheses.

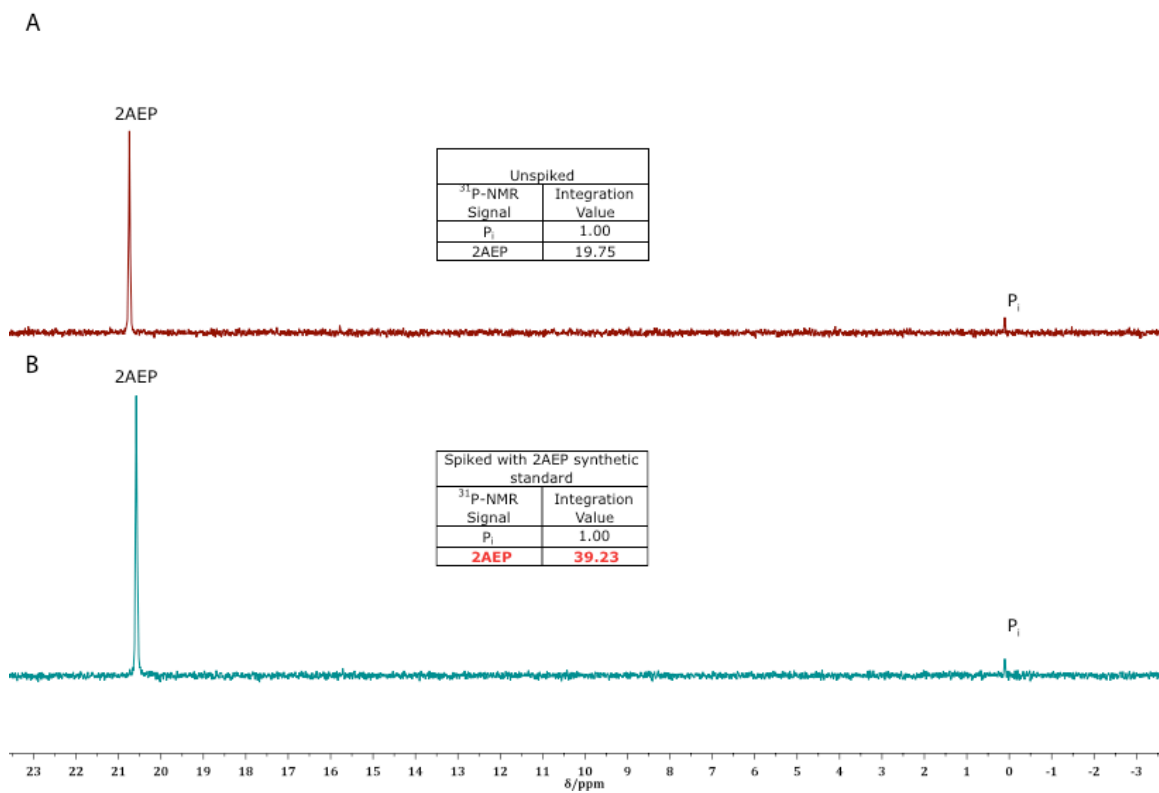


Figure S2. <sup>31</sup>P NMR Spectra of a 2AEP-enriched Fraction from *S. regensis* Spiked With Synthetic 2AEP. Fraction of *S. regensis* extract retained on AG 50W-X8 cation exchange resin. (A) <sup>31</sup>P NMR spectrum prior to spiking and (B) post spiking with synthetic 2AEP. The table insert for each shows calculated integration values after normalizing the integration of P<sub>i</sub> to 1.00.

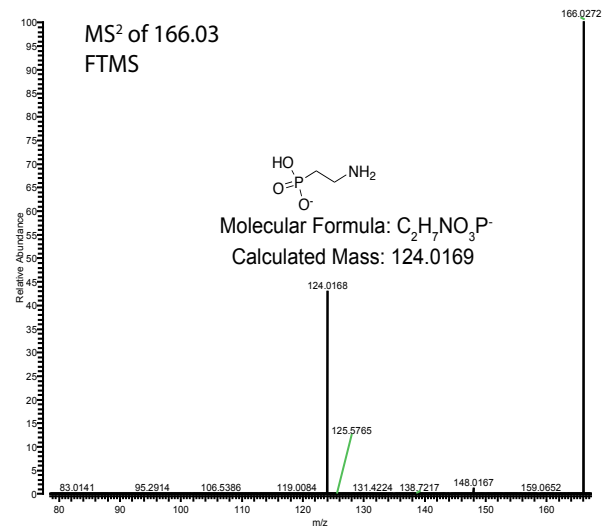
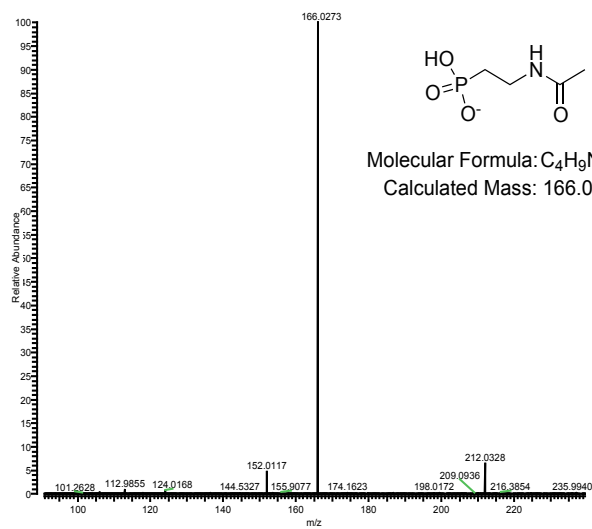


Figure S3. FT-ICR MS (left) and MS2 (right) Spectra of Isolated Compound 1.

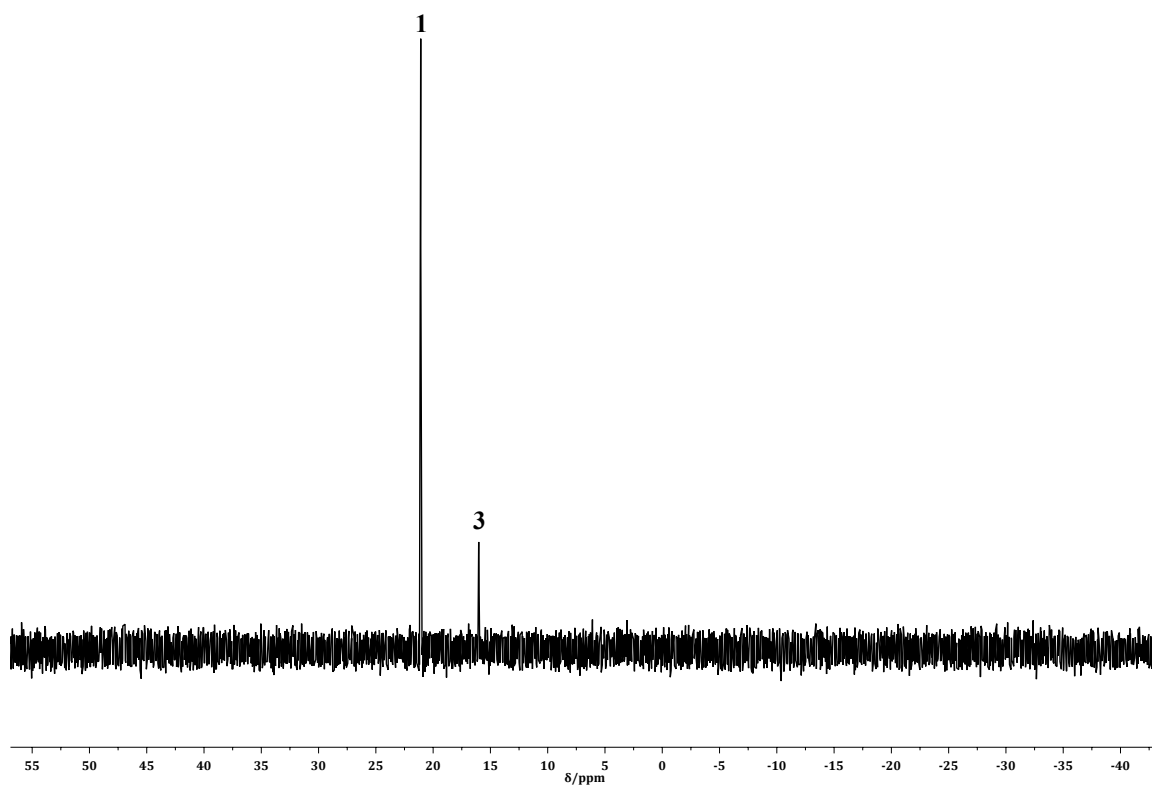


Figure S4.  $^{31}\text{P}$  NMR Spectrum (242 MHz,  $\text{D}_2\text{O}$ ) of Co-Purified Compounds 1 and 3.

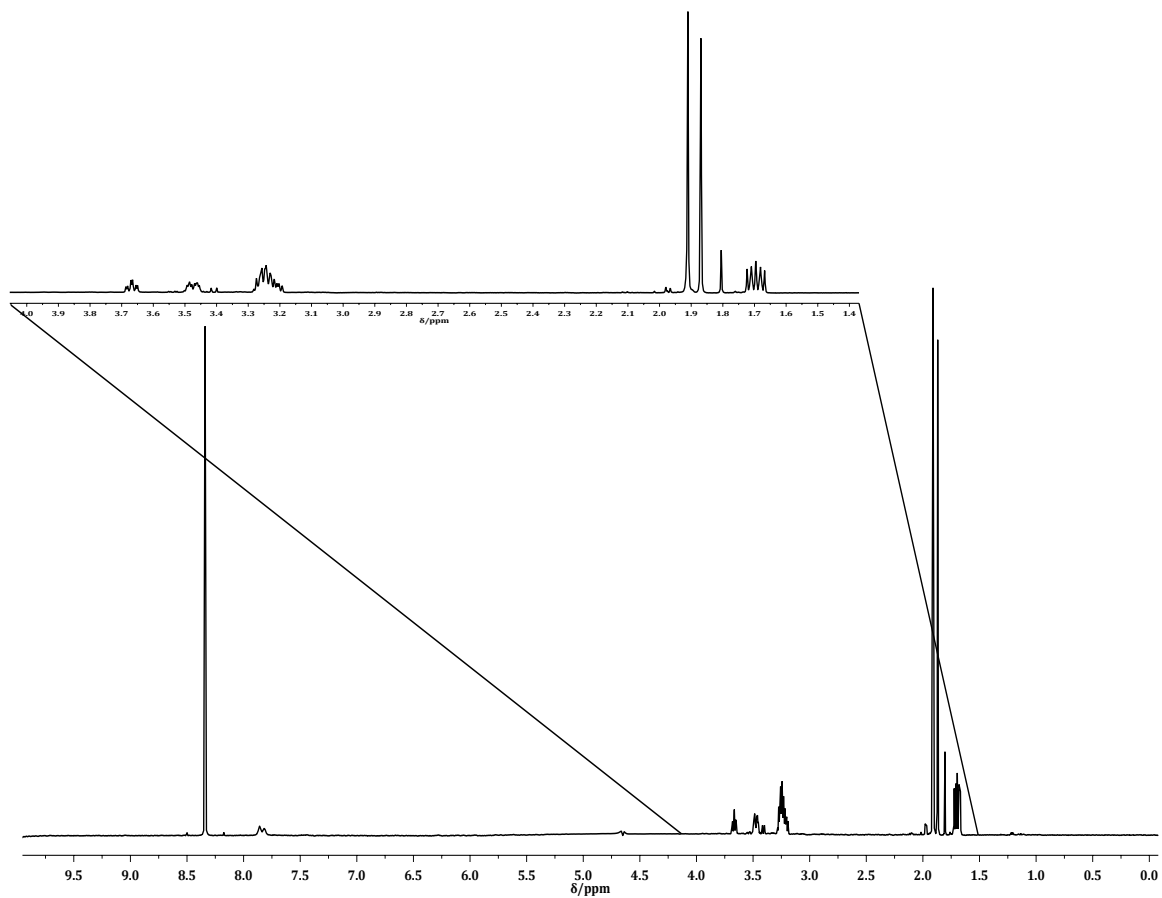


Figure S5.  $^1\text{H}$  NMR Spectrum (600 MHz,  $\text{D}_2\text{O}$ ) of Co-Purified Compounds 1 and 3.

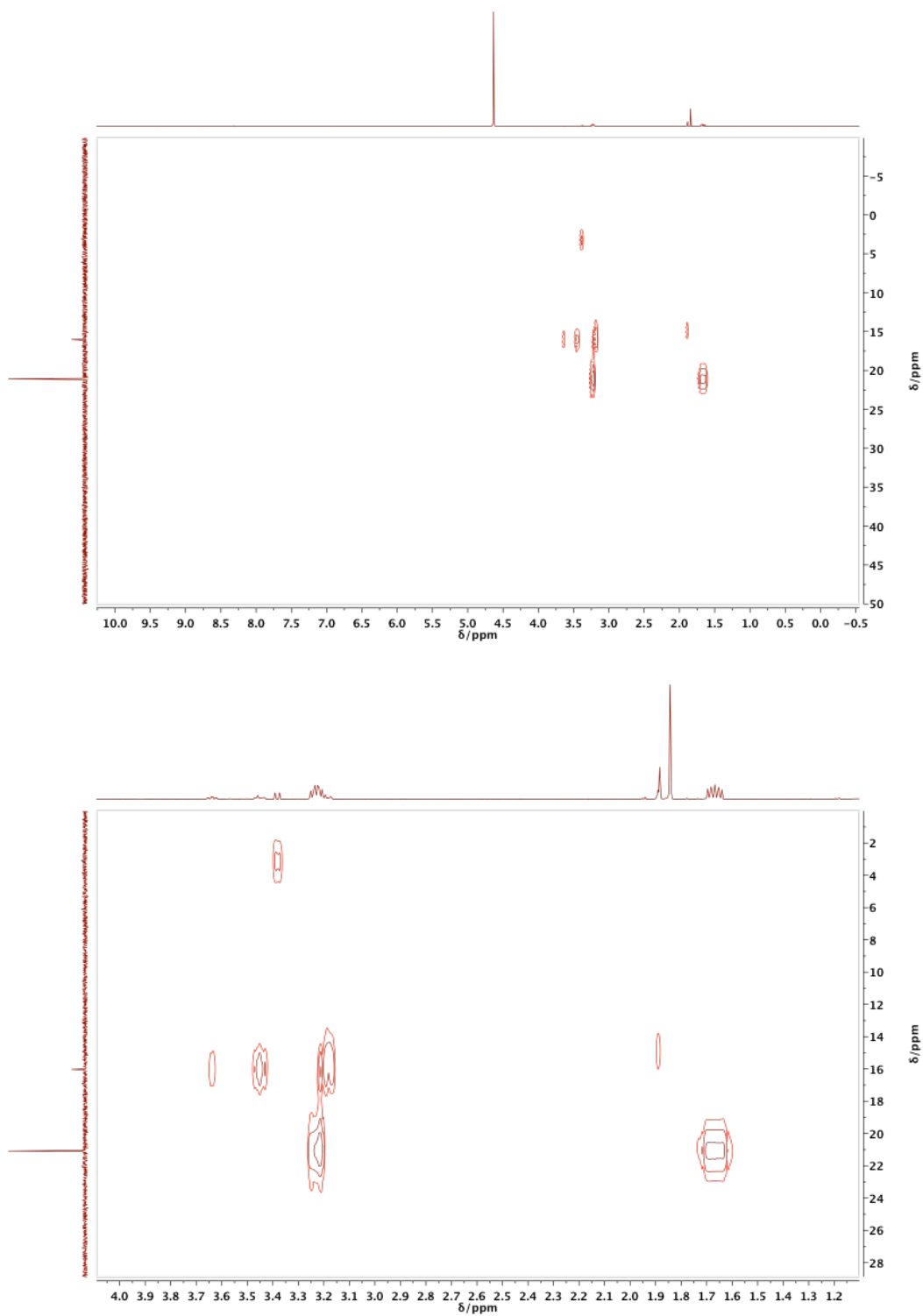


Figure S6.  $^1\text{H}$ - $^{31}\text{P}$  HMBC NMR Spectrum of Co-Purified Compounds 1 and 3 in  $\text{D}_2\text{O}$ . Top panel shows entire spectrum. Bottom panel is zoomed in on region of interest.

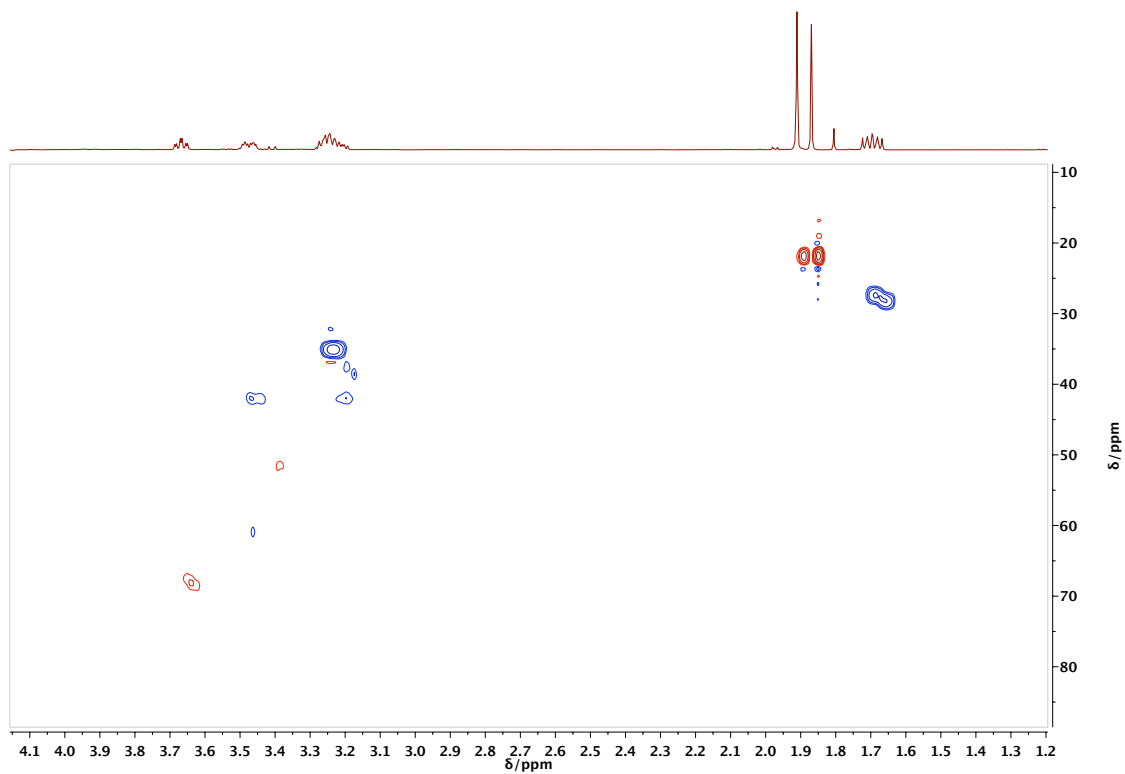
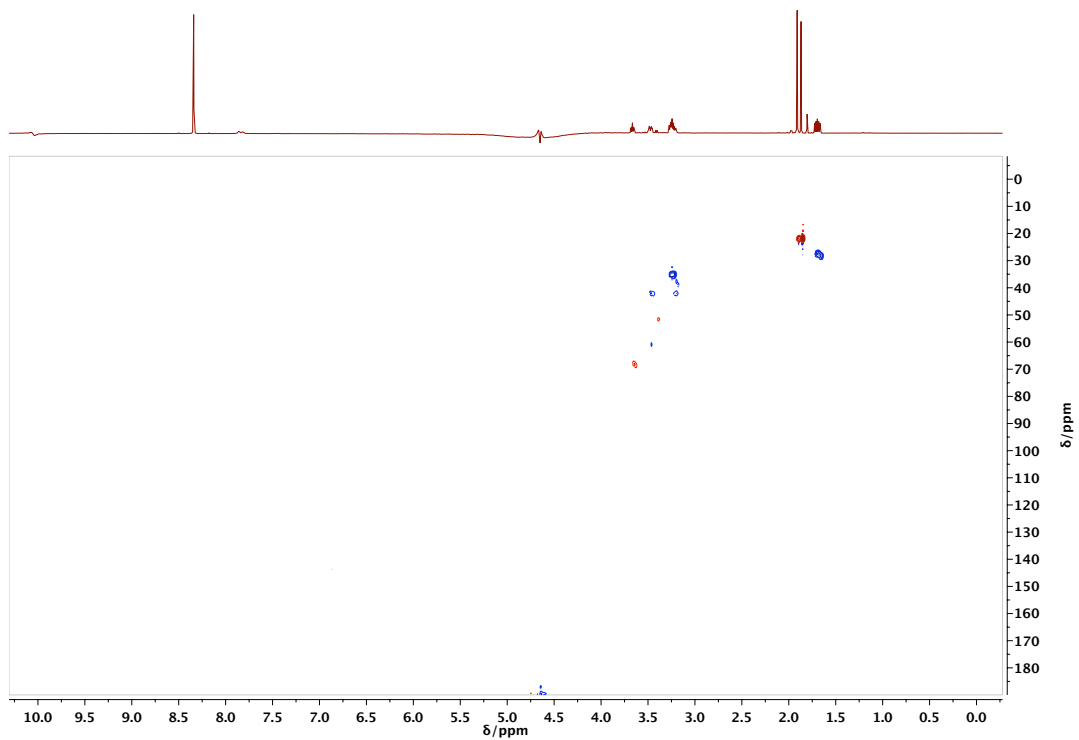


Figure S7.  $^1\text{H}$ - $^{13}\text{C}$  HSQC NMR Spectrum of Co-Purified compounds 1 and 3 in  $\text{D}_2\text{O}$ . Top panel shows entire spectrum. Bottom panel is zoomed in on region of interest.

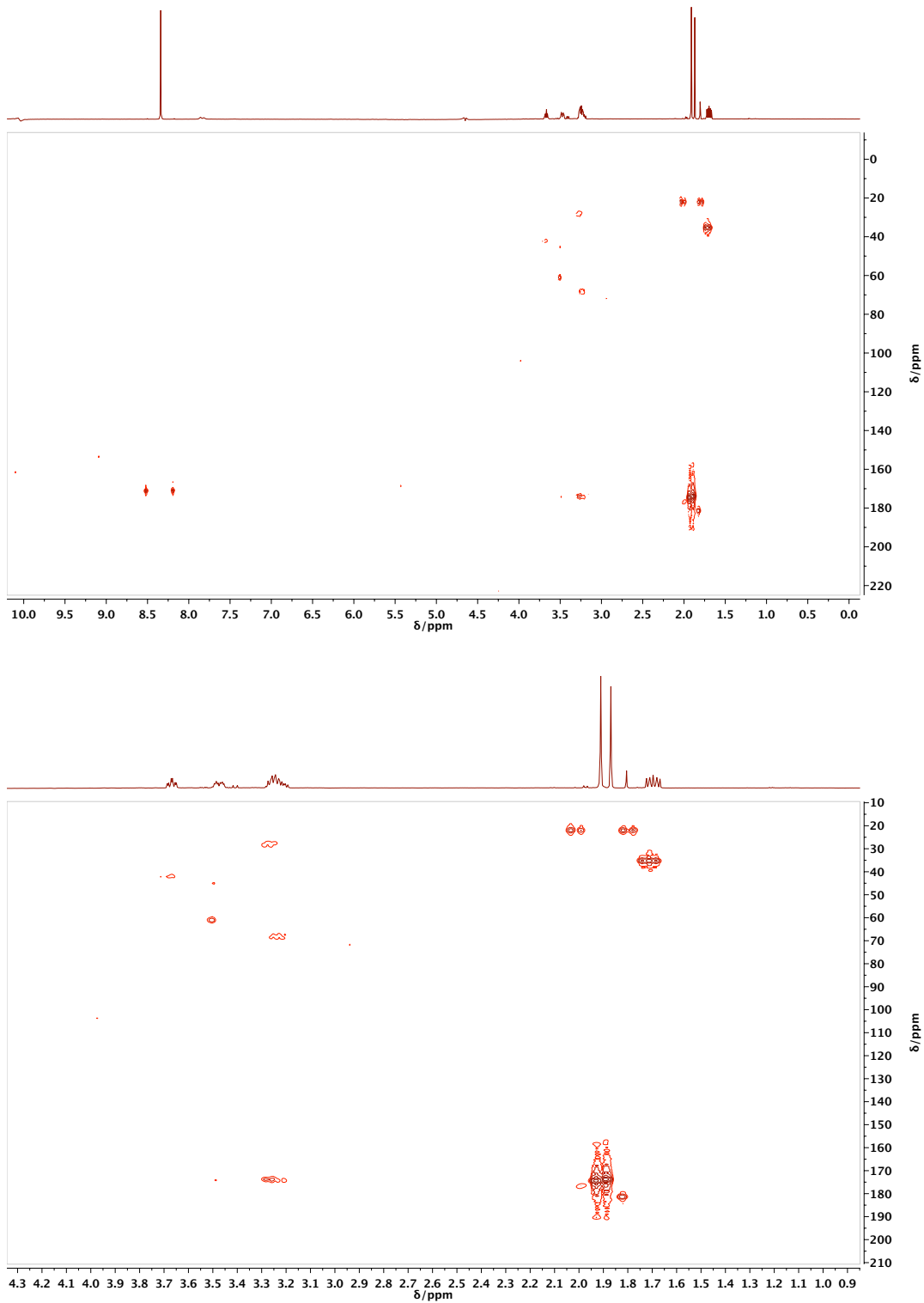


Figure S8.  $^1\text{H}$ - $^{13}\text{C}$  HMBC NMR Spectrum of Co-Purified compounds 1 and 3 in  $\text{D}_2\text{O}$ . Top panel shows entire spectrum. Bottom panel is zoomed in on region of interest.



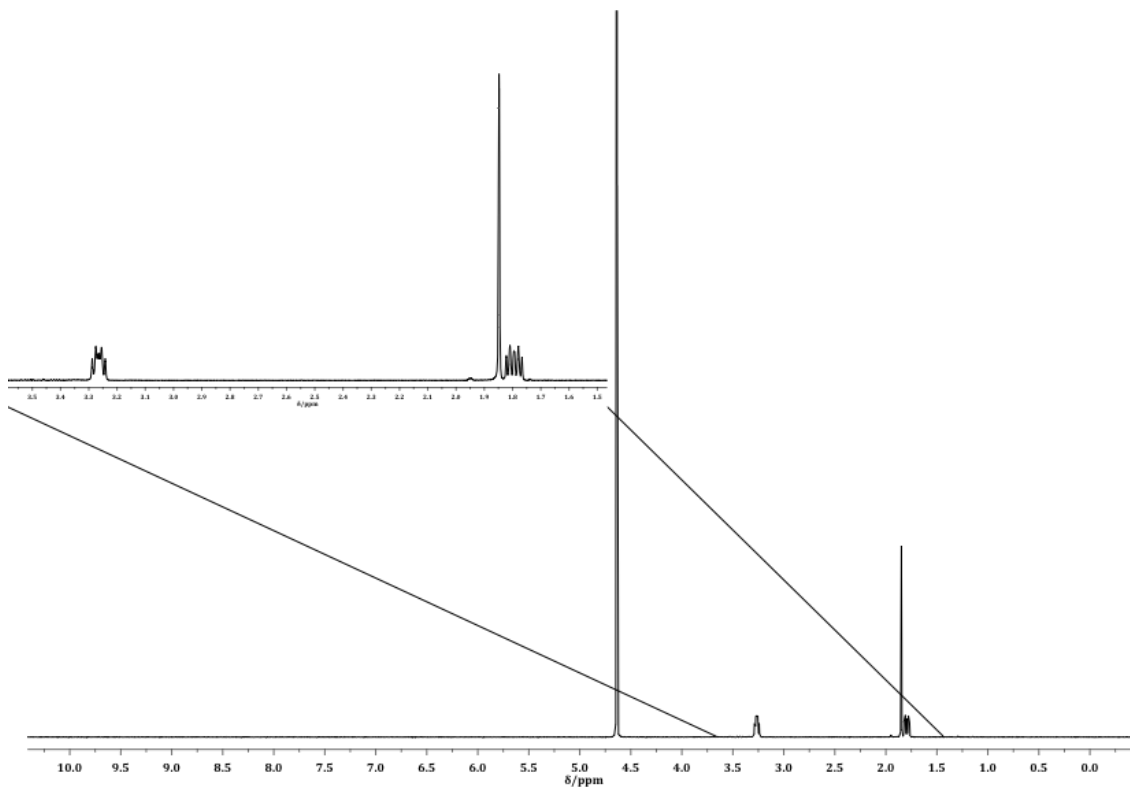


Figure S9  $^1\text{H}$  NMR Spectrum (600 MHz,  $\text{D}_2\text{O}$ ) of Synthetic Compound 1.

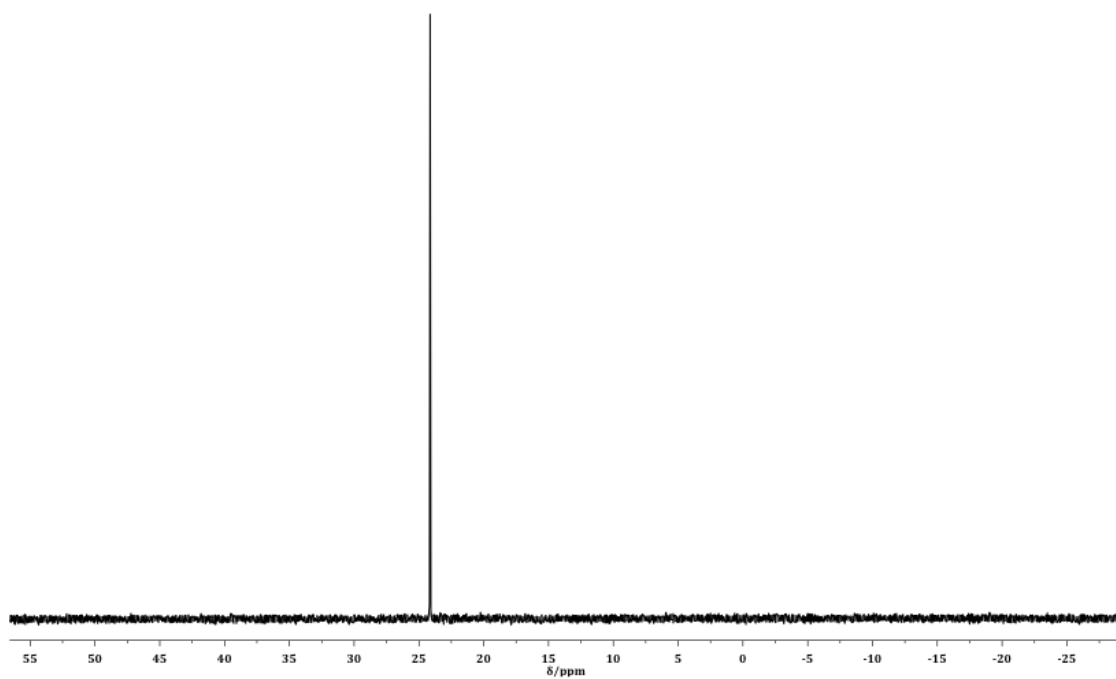


Figure S10.  $^{31}\text{P}$  NMR Spectrum (242 MHz,  $\text{D}_2\text{O}$ ) of synthetic compound 1.

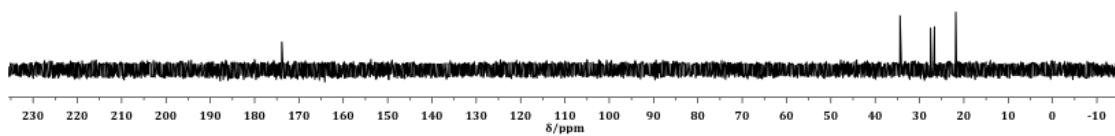


Figure S11.  $^{13}\text{C}$  NMR Spectrum (150 MHz,  $\text{D}_2\text{O}$ ) of Synthetic Compound 1.

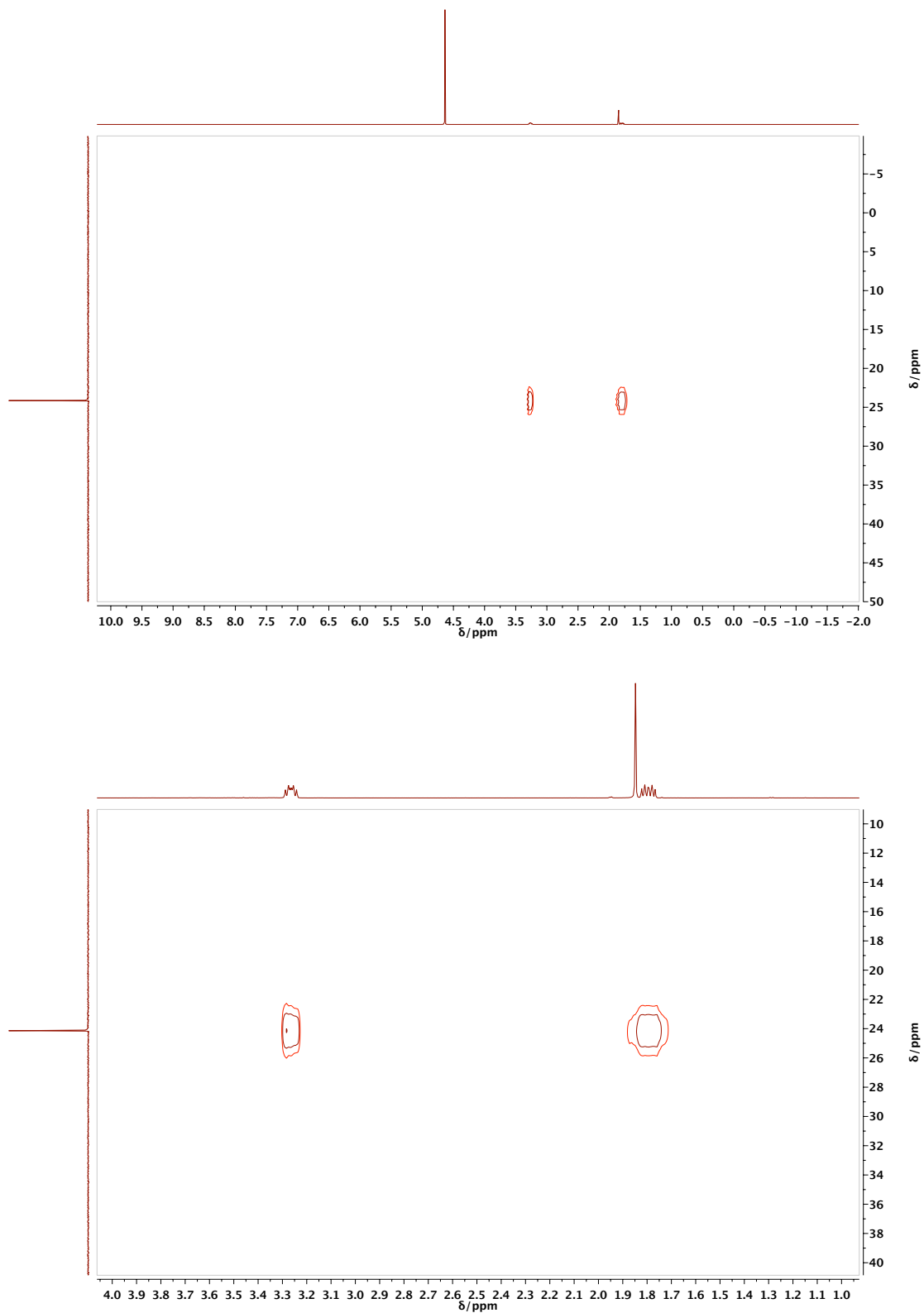


Figure S12.  $^1\text{H}$ - $^{31}\text{P}$  HMBC NMR Spectrum of Synthetic Compound 1 in  $\text{D}_2\text{O}$ . Top panel shows entire spectrum. Bottom panel is zoomed in on region of interest.

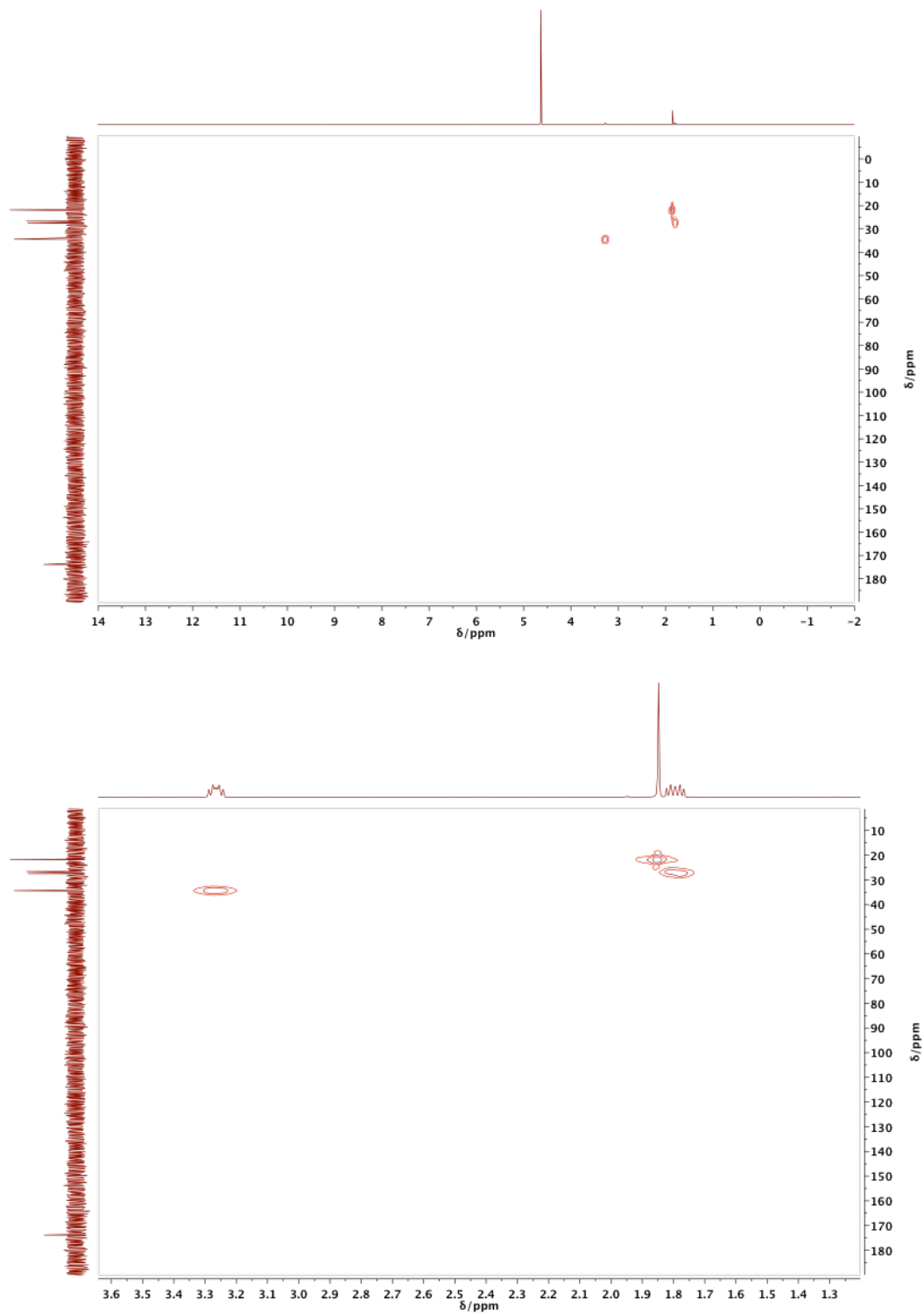


Figure S13.  $^1\text{H}$ - $^{13}\text{C}$  HSQC NMR Spectrum of Synthetic Compound 1 in  $\text{D}_2\text{O}$ . Top panel shows entire spectrum. Bottom panel is zoomed in on region of interest.

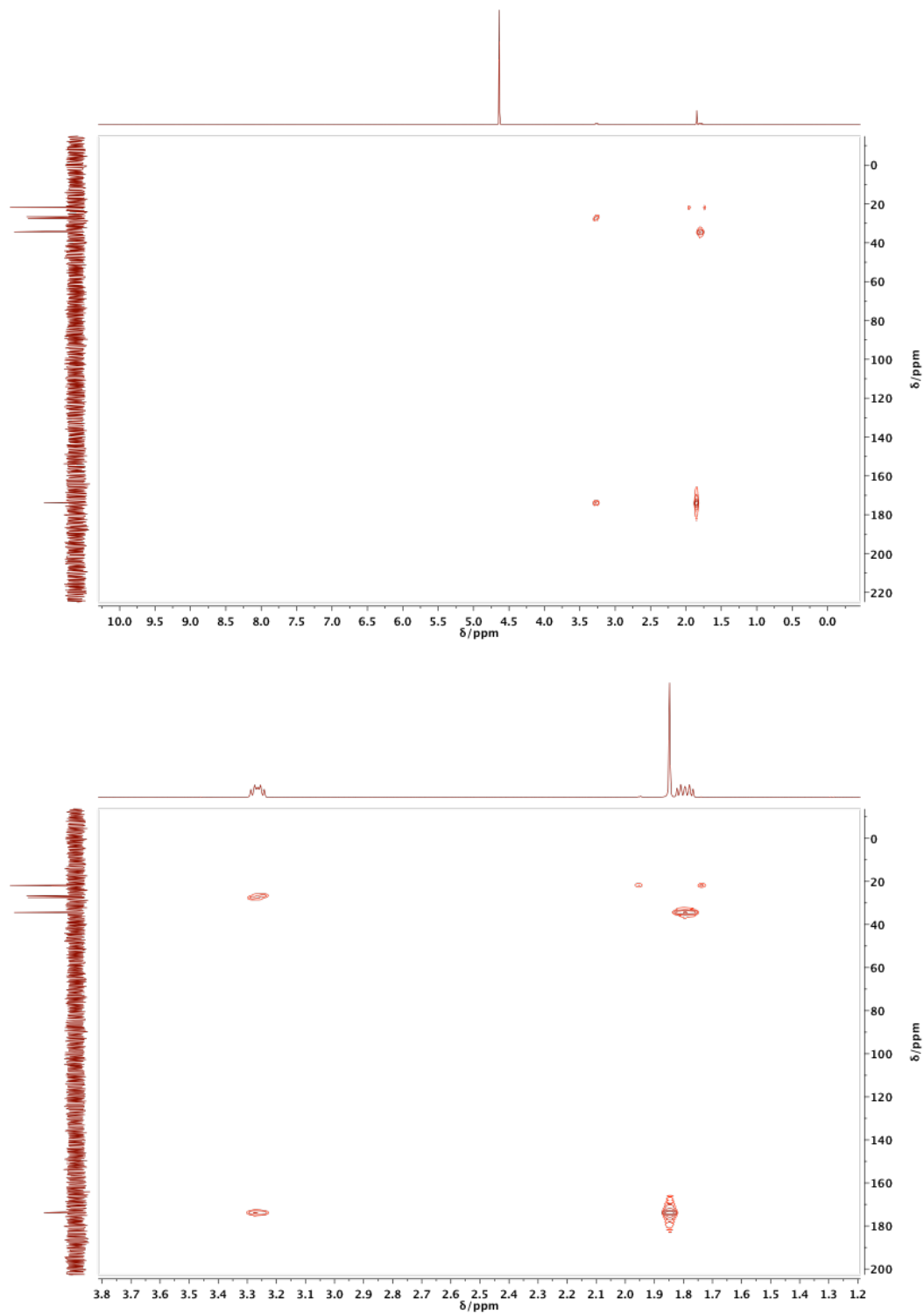


Figure S14.  $^1\text{H}$ - $^{13}\text{C}$  HMBC NMR Spectrum of Synthetic Compound 1 in  $\text{D}_2\text{O}$ . Top panel shows entire spectrum. Bottom panel is zoomed in on region of interest.

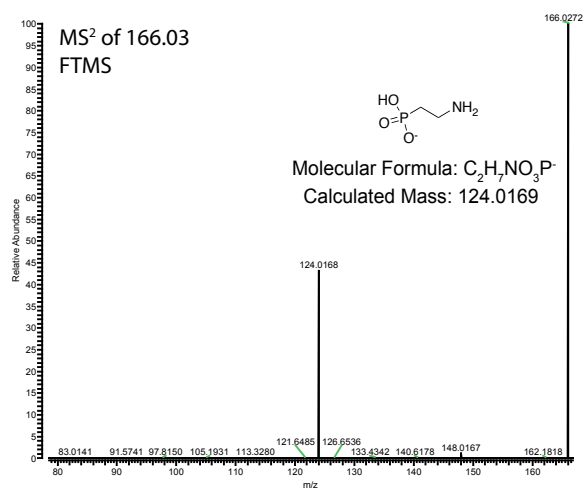
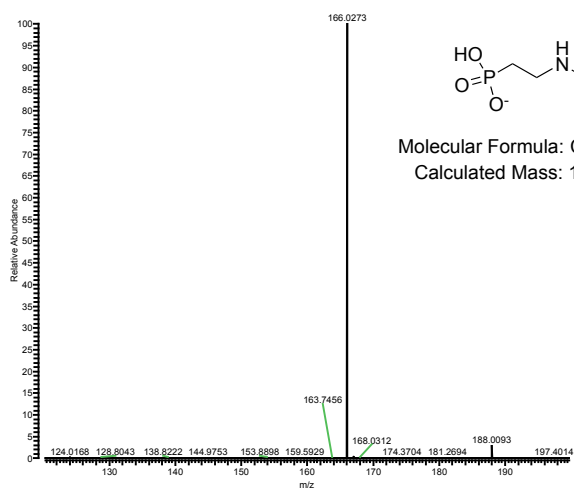


Figure S15. FT-ICR MS (left) and MS<sup>2</sup> (right) Spectra of Synthetic Compound 1.

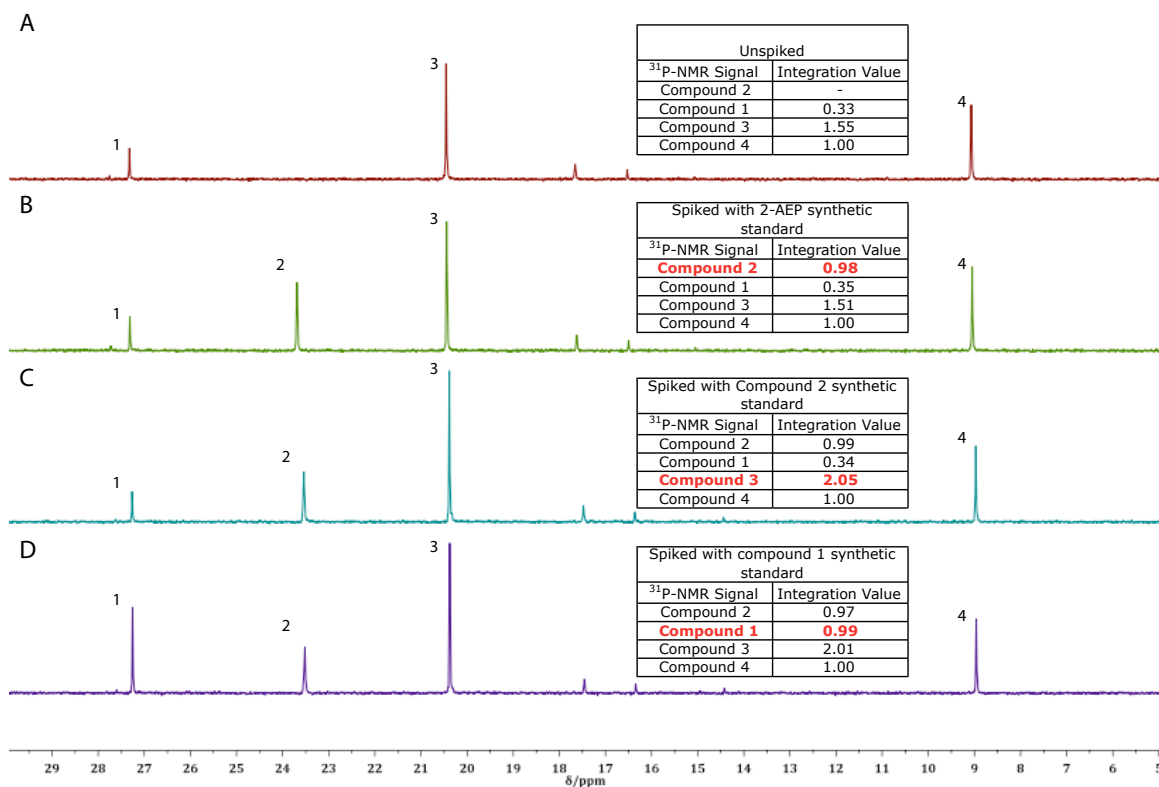


Figure S16. <sup>31</sup>P NMR Spectra of a Phosphonate-Enriched Extract from *S. regensis* Spiked With Synthetic Compounds 1-3. Signals are labeled with corresponding compound numbers. Compound 1: (2-acetamidoethyl)phosphonic acid (Ac2AEP); Compound 2: (2-aminoethyl)phosphonic acid (2AEP); Compound 3: (2-acetamido-1-hydroxyethyl)phosphonic acid (NAc1H2AEP); Compound 4: (cyano(hydroxy)methyl)phosphonic acid. The table inserts show calculated integration values for each signal after normalizing compound 4 signal to 1.00 (A) *S. regensis* extract prior to spiking with synthetic standards, (B) post spiking with synthetic 2, (C) synthetic compound 3, and (D) synthetic compound 1.



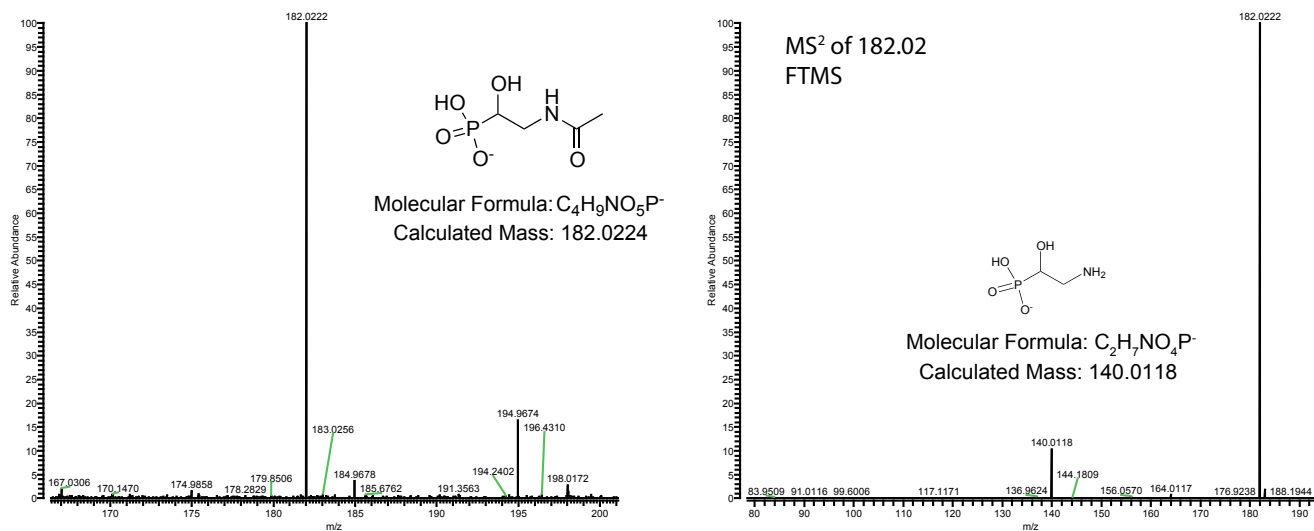


Figure S17. FT-ICR MS (left) and MS<sup>2</sup> (right) Spectra of Isolated Compound 3.

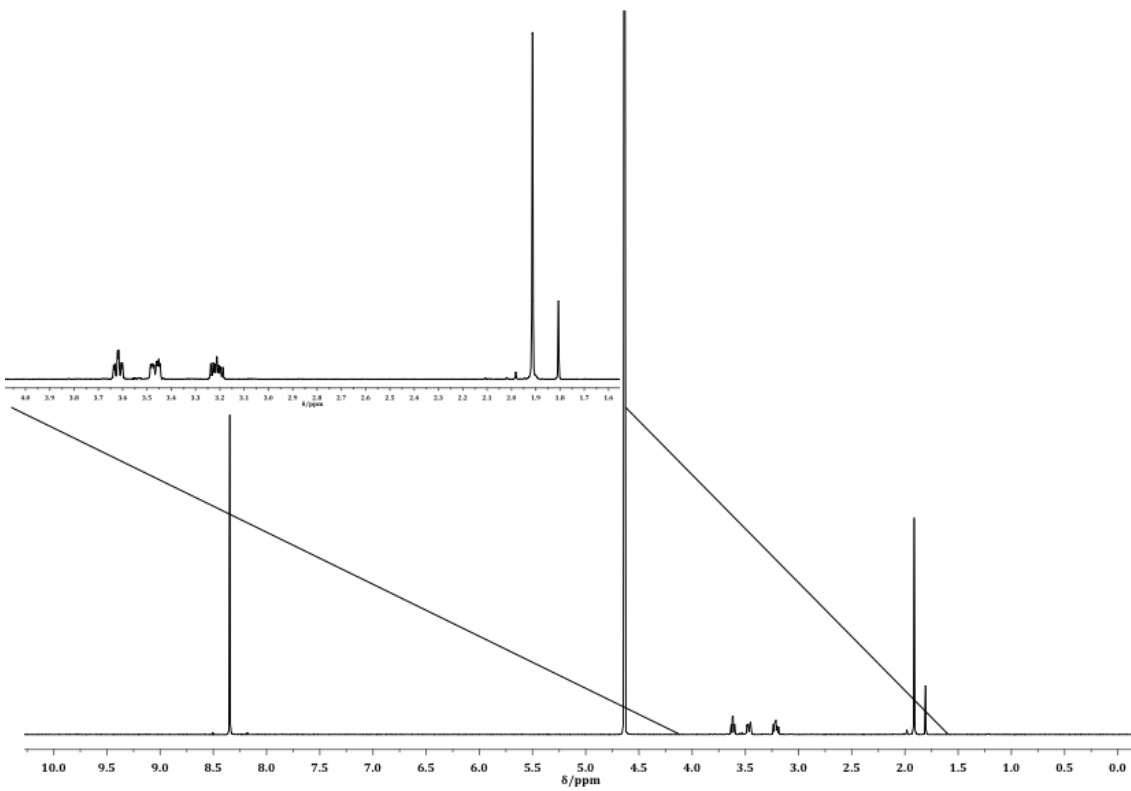


Figure S18.  $^1\text{H}$  NMR Spectrum (600 MHz,  $\text{D}_2\text{O}$ ) of Synthetic Compound 3.

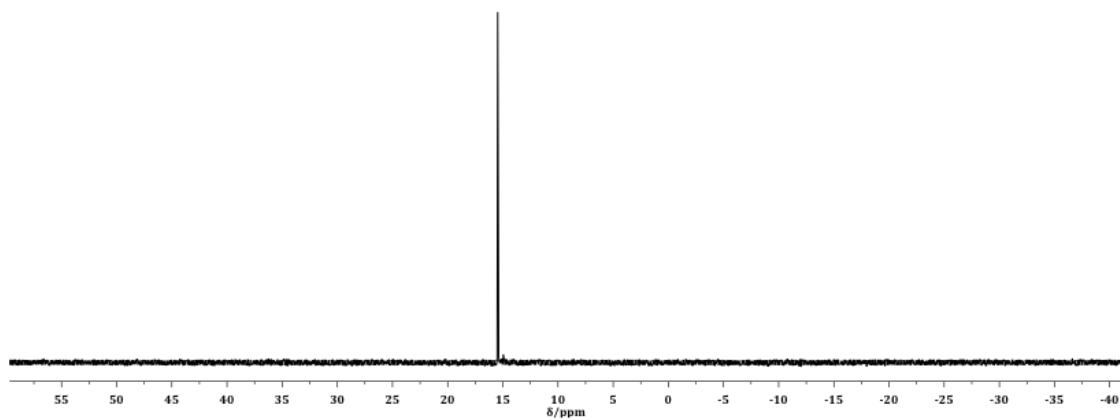


Figure S19.  $^{31}\text{P}$  NMR Spectrum (242 MHz,  $\text{D}_2\text{O}$ ) of synthetic compound 3.

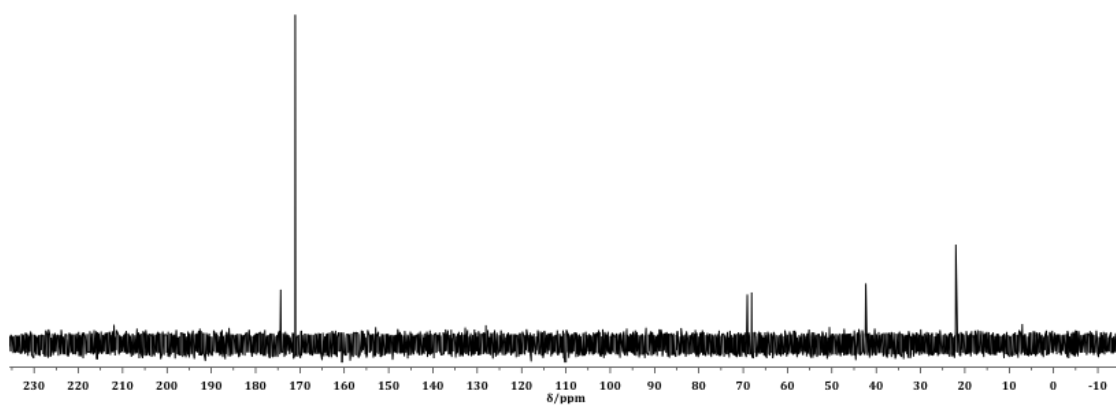


Figure S20.  $^{13}\text{C}$  NMR Spectrum (150 MHz,  $\text{D}_2\text{O}$ ) of Synthetic Compound 3.

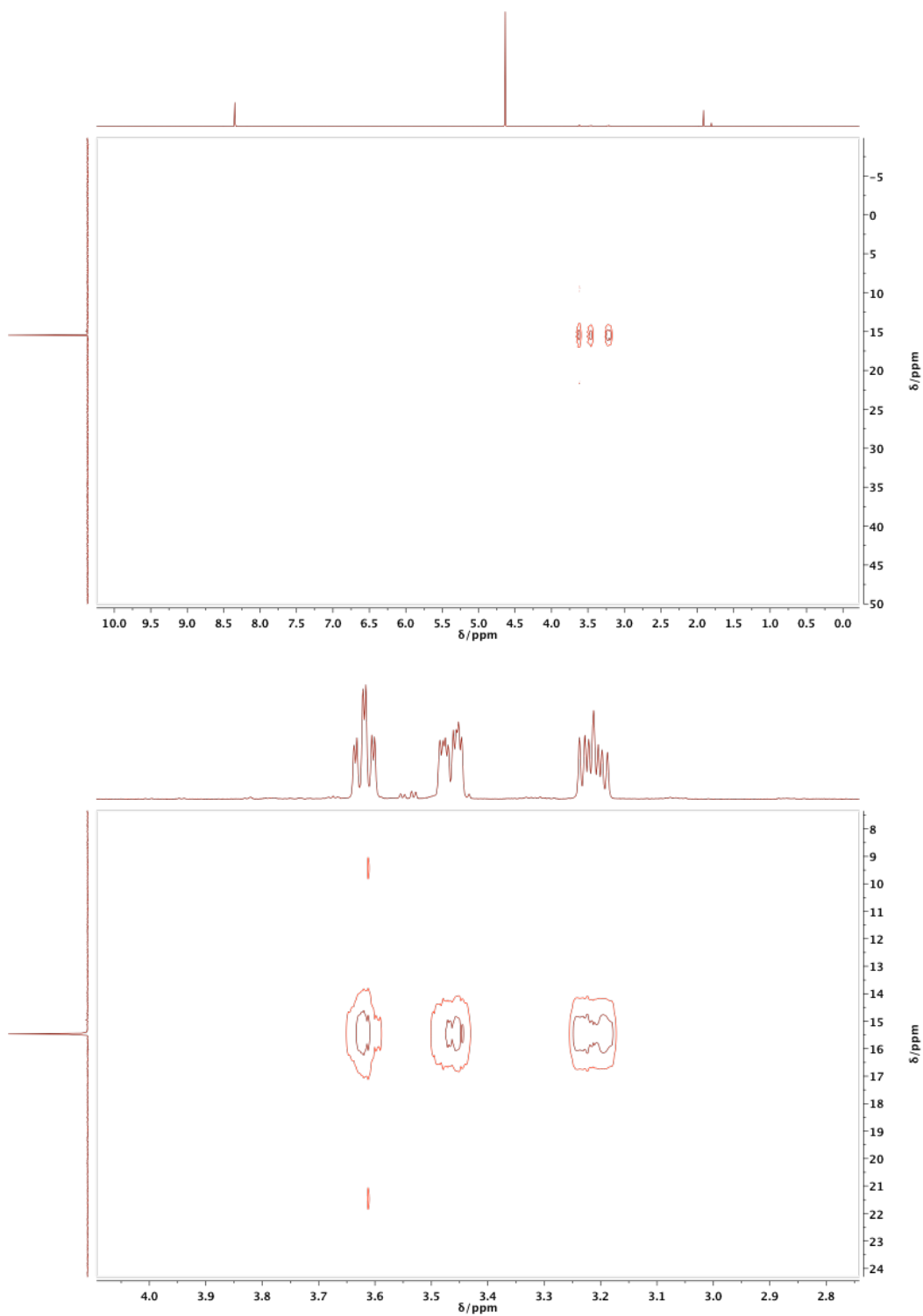


Figure S21.  $^1\text{H}$ - $^{31}\text{P}$  HMBC NMR Spectrum of Synthetic Compound 3 in  $\text{D}_2\text{O}$ . Top panel shows entire spectrum. Bottom panel is zoomed in on region of interest.

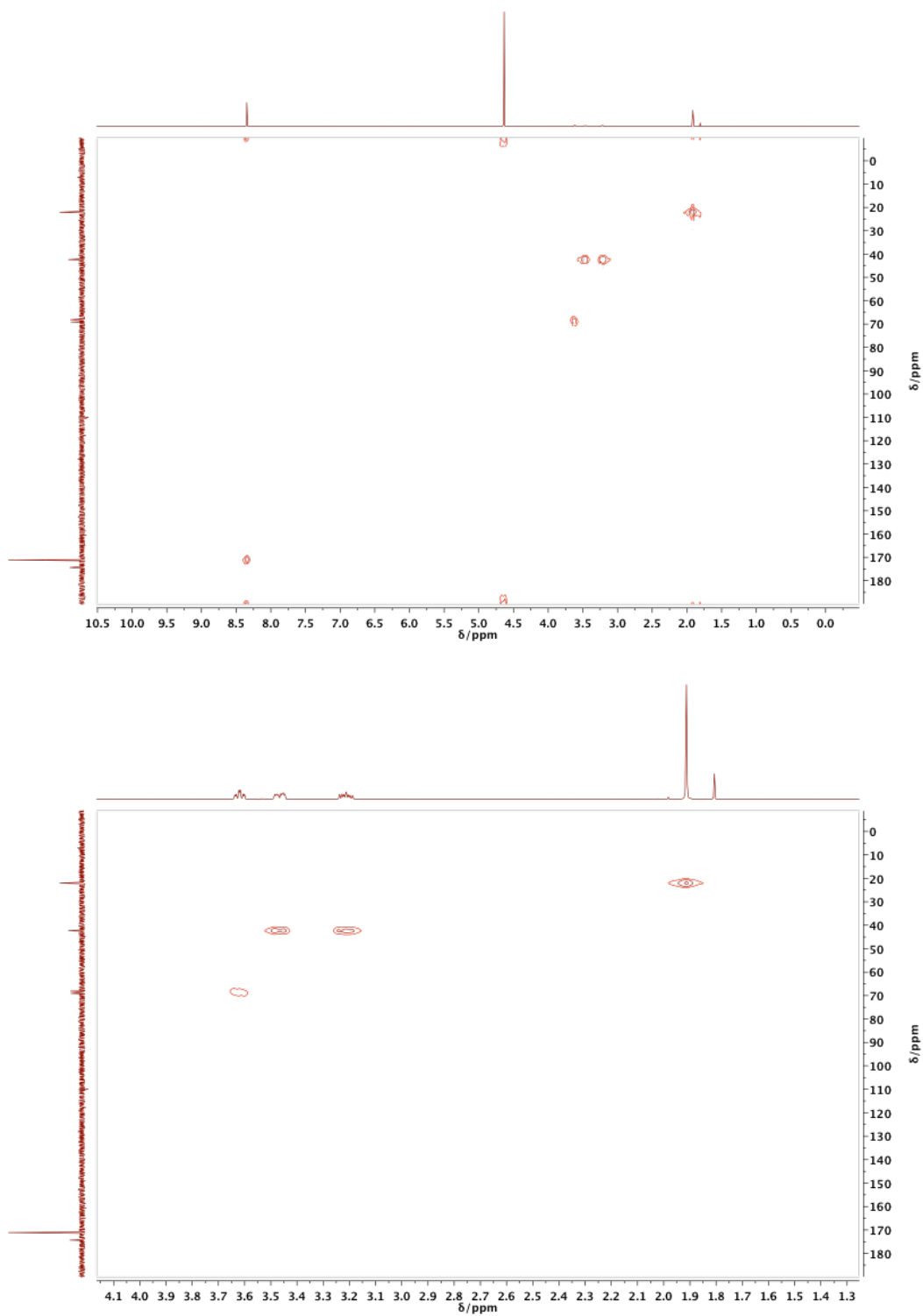


Figure S22.  $^1\text{H}$ - $^{13}\text{C}$  HSQC NMR Spectrum of Synthetic Compound 3 in  $\text{D}_2\text{O}$ . Top panel shows entire spectrum. Bottom panel is zoomed in on region of interest.

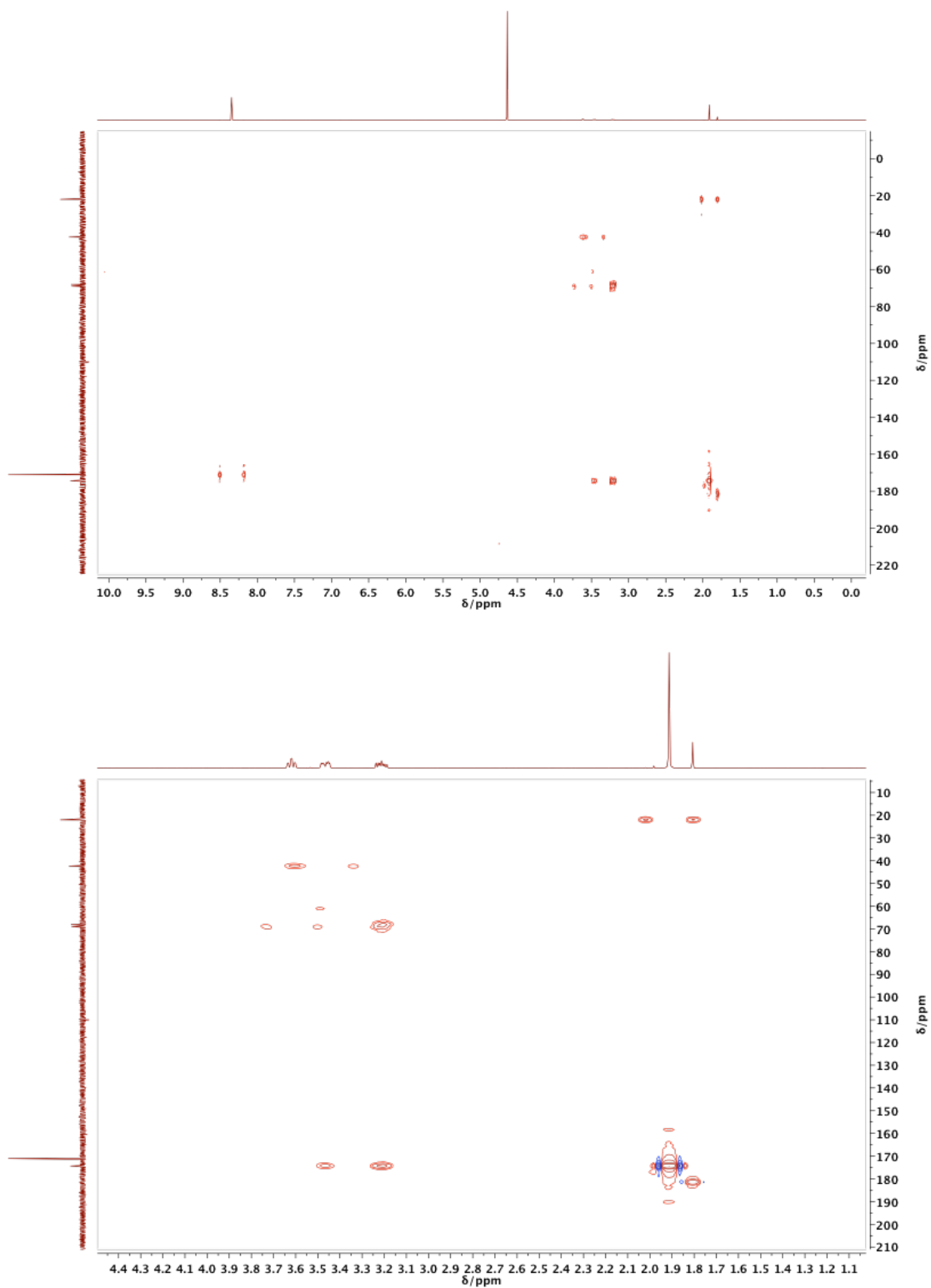


Figure S23.  $^1\text{H}$ - $^{13}\text{C}$  HMBC NMR Spectrum of Synthetic Compound 3 in  $\text{D}_2\text{O}$ . Top panel shows entire spectrum. Bottom panel is zoomed in on region of interest.

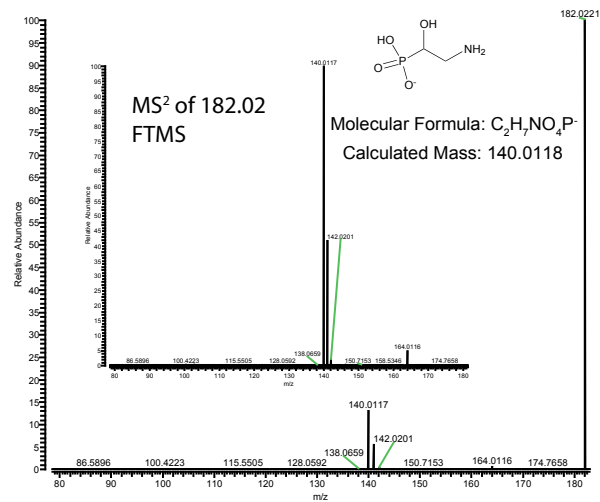
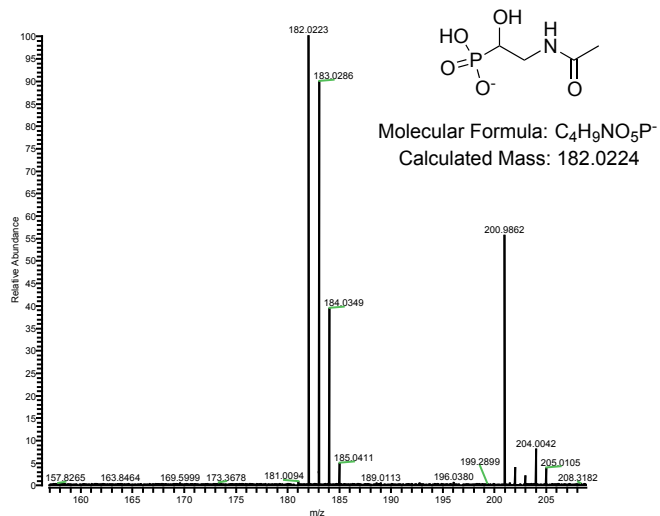


Figure S24. FT-ICR MS (left) and MS<sup>2</sup> (right) Spectra of Synthetic Compound 3.



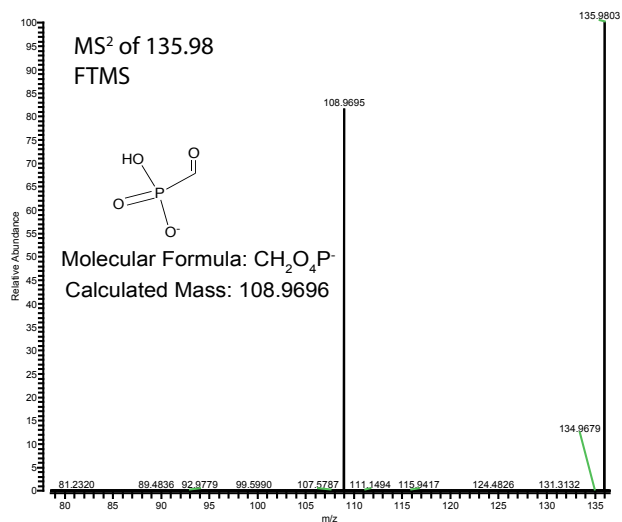
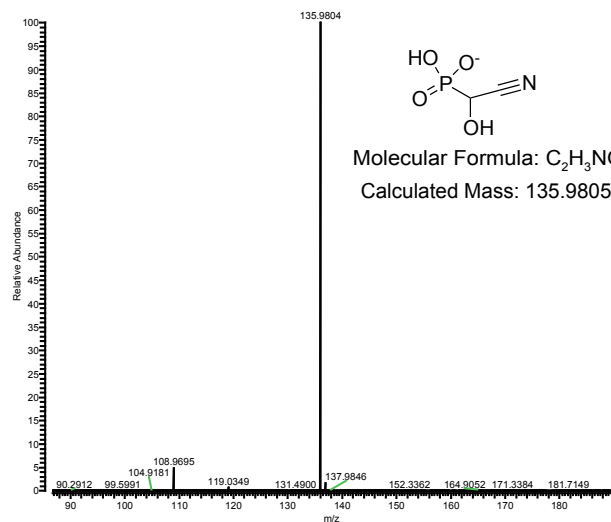


Figure S25. FT-ICR MS (left) and MS<sup>2</sup> (right) of Isolated Compound 4.

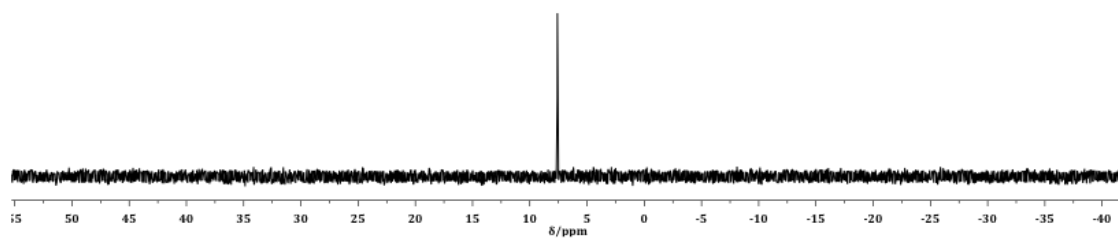


Figure S26.  $^{31}\text{P}$  NMR Spectrum (242 MHz,  $\text{D}_2\text{O}$ ) of Isolated Compound 4.

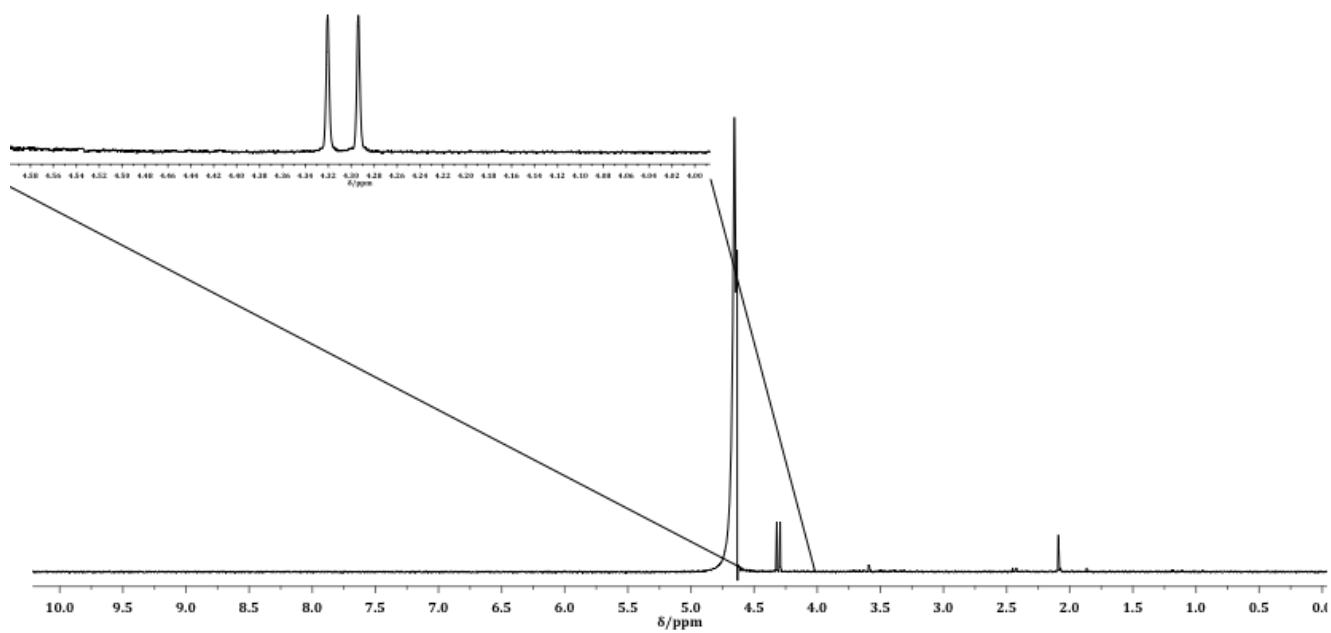


Figure S27.  $^1\text{H}$  NMR Spectrum (600 MHz,  $\text{D}_2\text{O}$ ) of Isolated Compound 4.

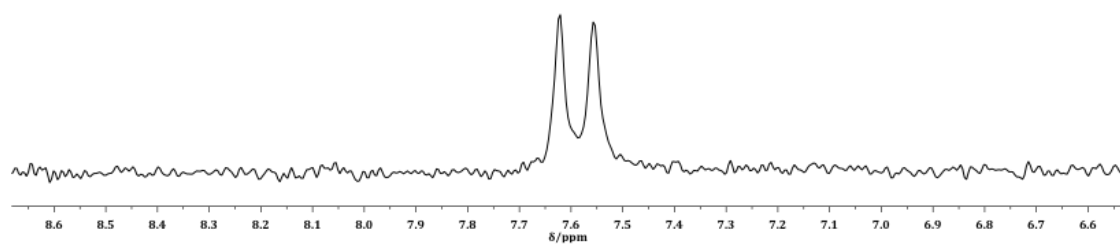


Figure S28.  $^1\text{H}$ -Coupled  $^{31}\text{P}$  NMR Spectrum (242 MHz,  $\text{D}_2\text{O}$ ) of Isolated Compound 4.  $J_{\text{PH}}=16.0$  Hz.

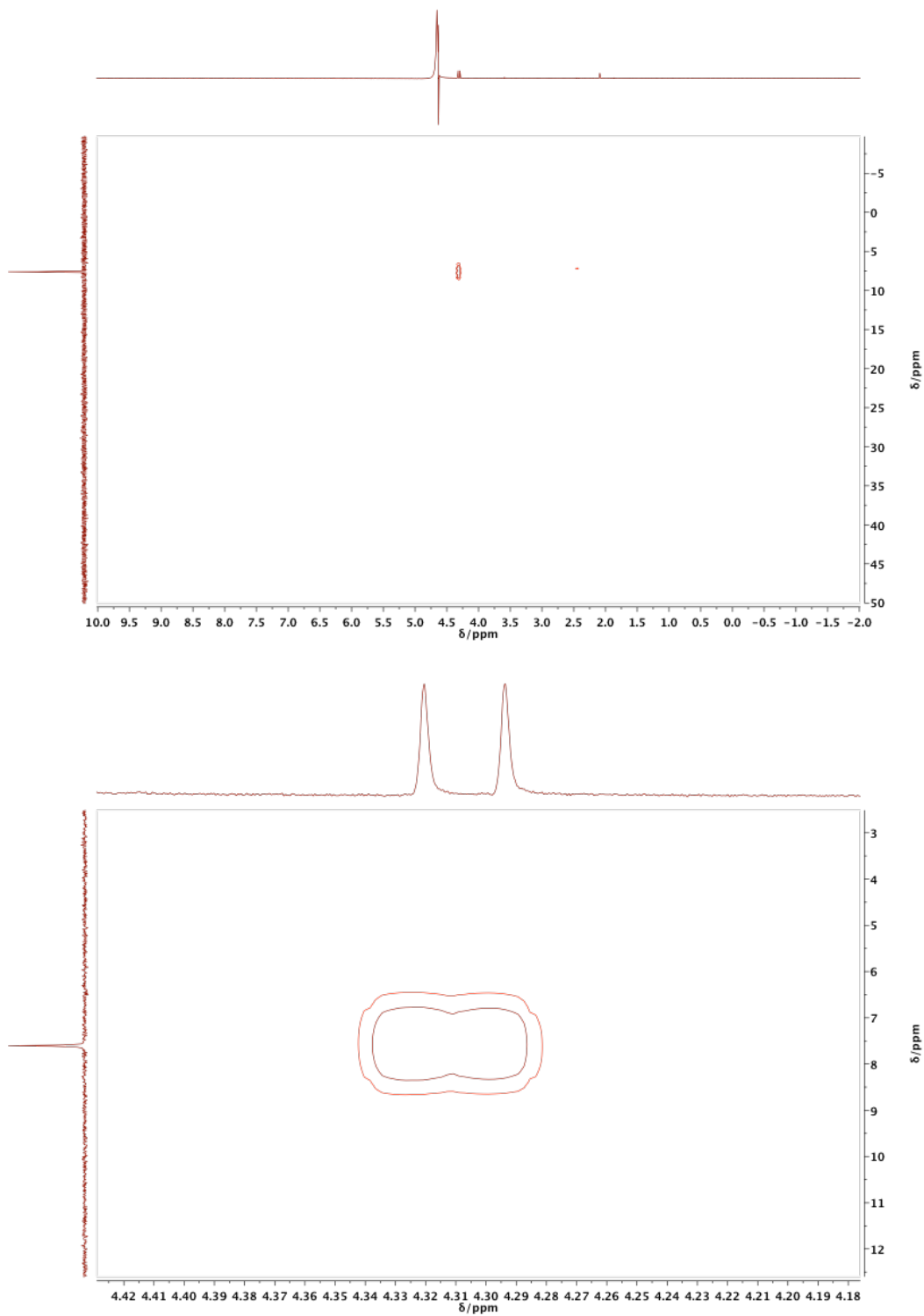


Figure S29.  $^1\text{H}$ - $^{31}\text{P}$  HMBC NMR Spectrum of Isolated Compound 4 in  $\text{D}_2\text{O}$ . Top panel shows entire spectrum. Bottom panel is zoomed in on region of interest.

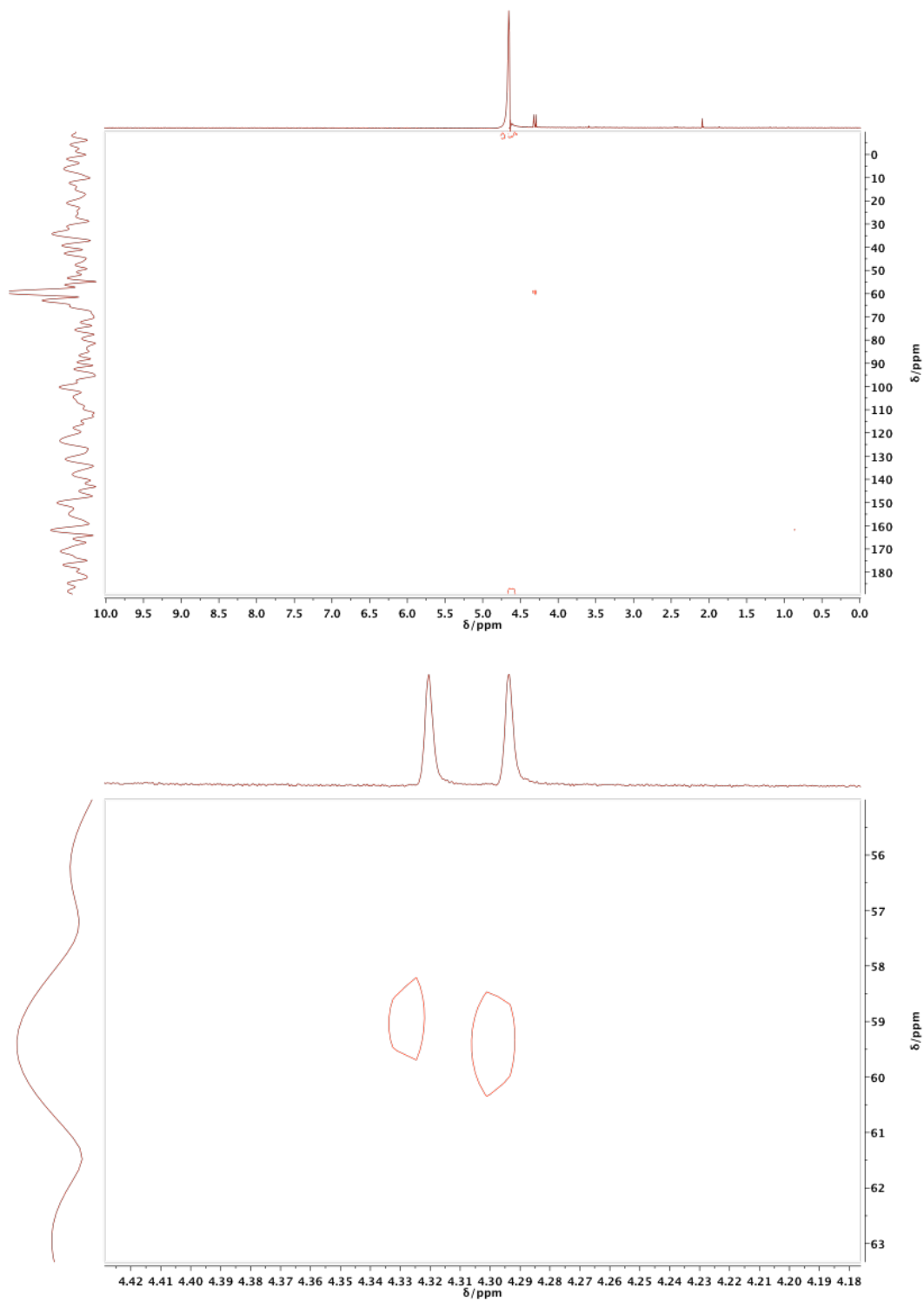


Figure S30.  $^1\text{H}$ - $^{13}\text{C}$  HSQC NMR Spectrum of Isolated Compound 4 in  $\text{D}_2\text{O}$ . Top panel shows entire spectrum. Bottom panel is zoomed in on region of interest.

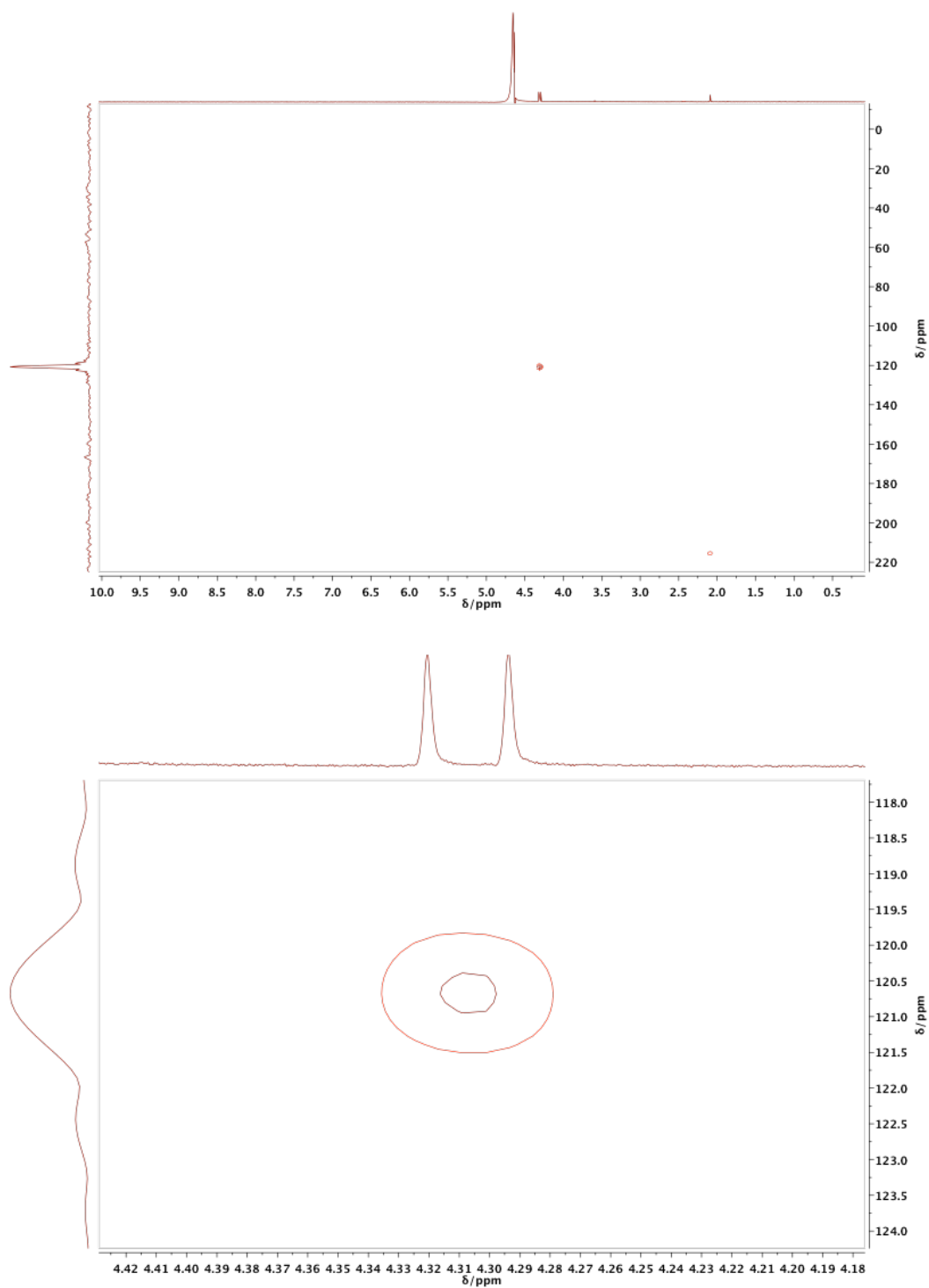


Figure S31.  $^1\text{H}$ - $^{13}\text{C}$  HMBC NMR Spectrum of Isolated Compound 4 in  $\text{D}_2\text{O}$ . Top panel shows entire spectrum. Bottom panel is zoomed in on region of interest.

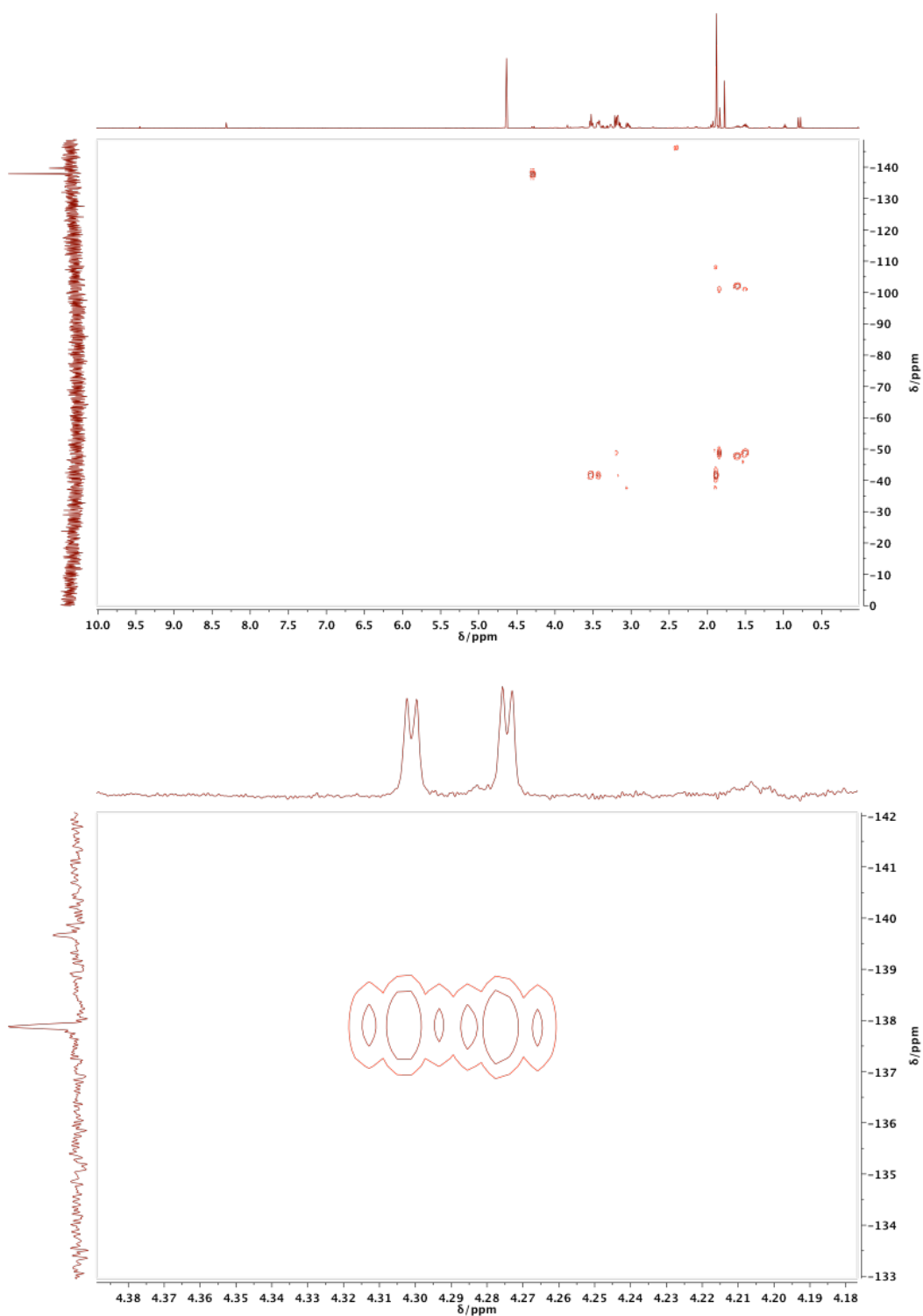


Figure S32.  $^1\text{H}$ - $^{15}\text{N}$  HMBC NMR Spectrum of Compound 4 in  $\text{D}_2\text{O}$ . A  $^{15}\text{N}$  labeled extract from *S. regensis* enriched for phosphonate compounds. Top panel shows entire spectrum. Bottom panel is zoomed in on region of interest.



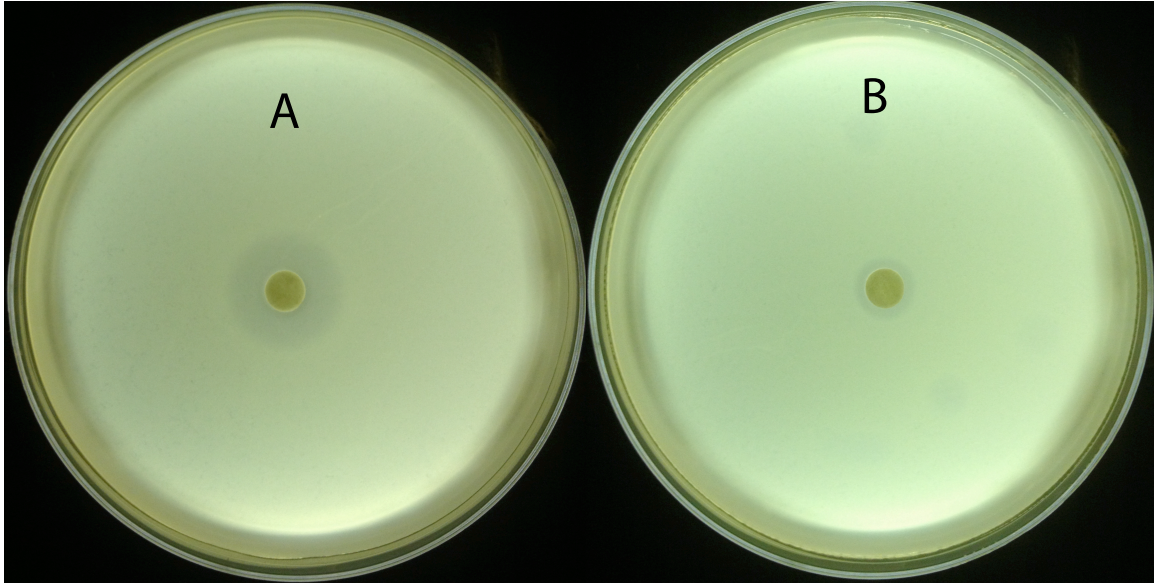


Figure S33. Inhibition of *E. coli* phosphonate uptake strain WM6242 by 1H2AEP in a disk diffusion bioassay on LB agar. (A) Addition of 1mM IPTG for induction of the phosphonate uptake system (*phnCDE*) and (B) no IPTG.