

*Supplemental Information*

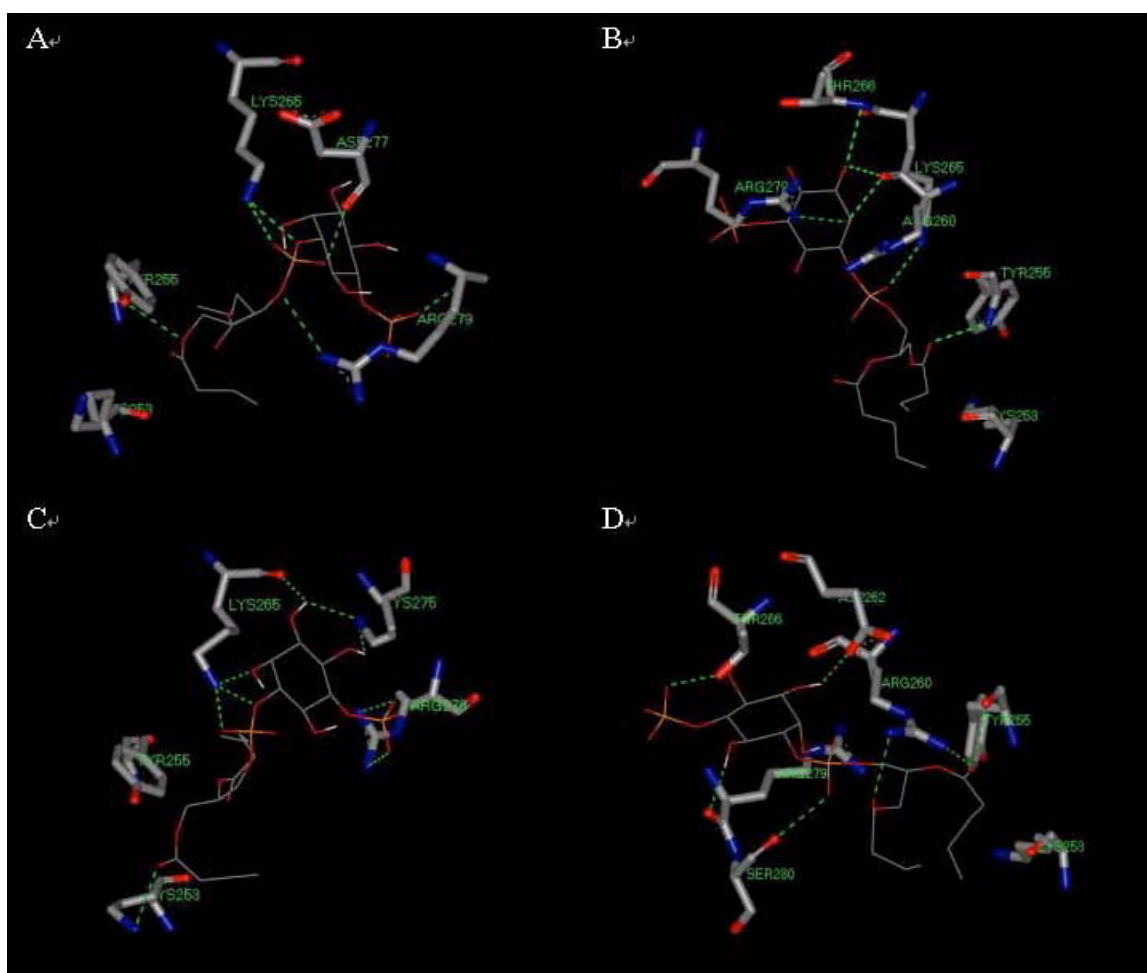
**Stabilized Phosphatidylinositol-5-Phosphate Analogues as Ligands for the Nuclear Protein ING2: Chemistry, Biology and Molecular Modeling**

Wei Huang, Honglu Zhang, Foteini Davrazou, Tatiana G. Kutateladze, Xiaobing Shi, Or Gozani, and Glenn D Prestwich

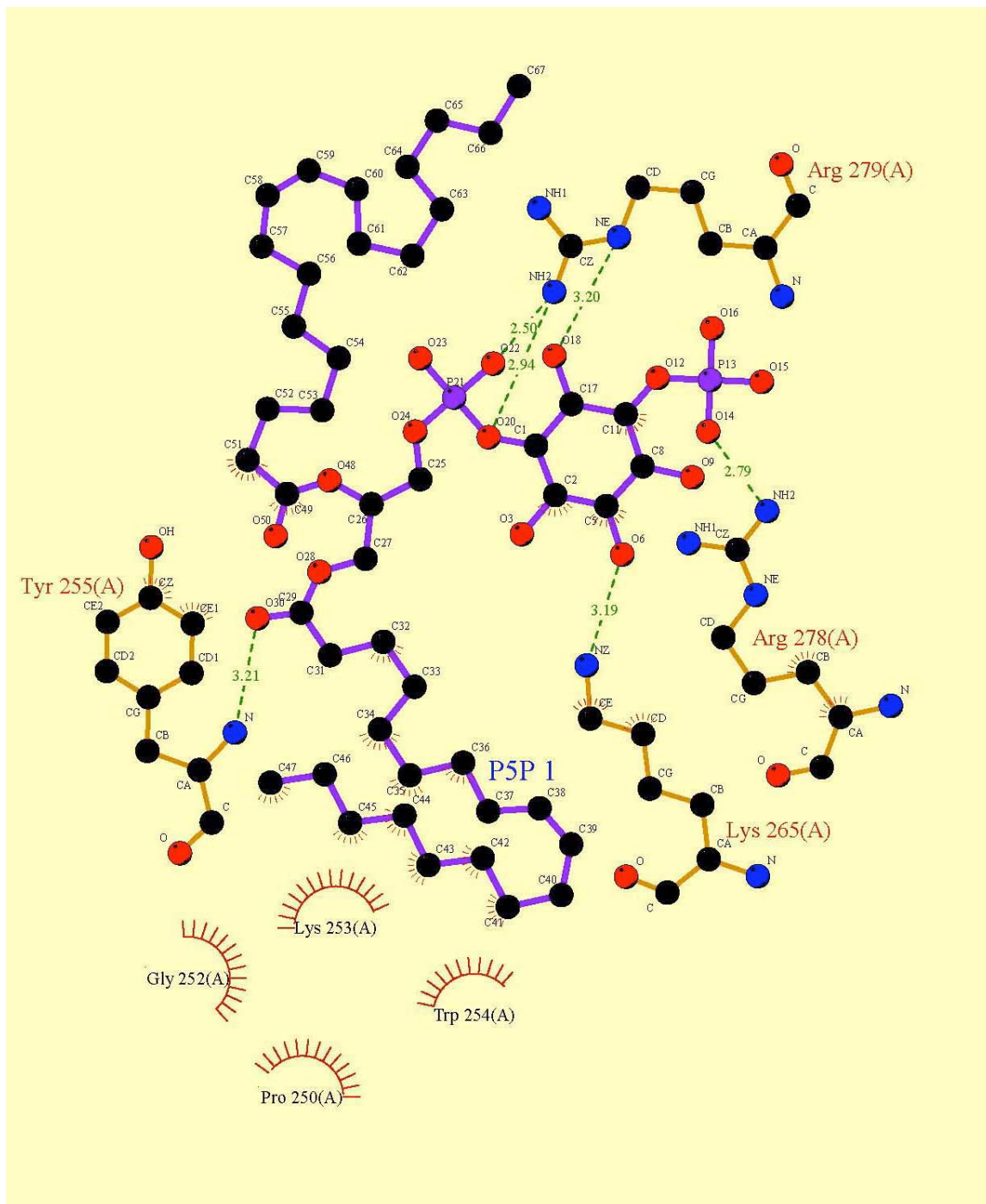
**Supplementary Figure S1. Panel A.** Interactions of diC<sub>4</sub>-PtdIns(5)P with model 2.

**Panel B.** Interactions of diC<sub>4</sub>-PtdIns(5)P with model 7. **Panel C.** Interactions of diC<sub>4</sub>-

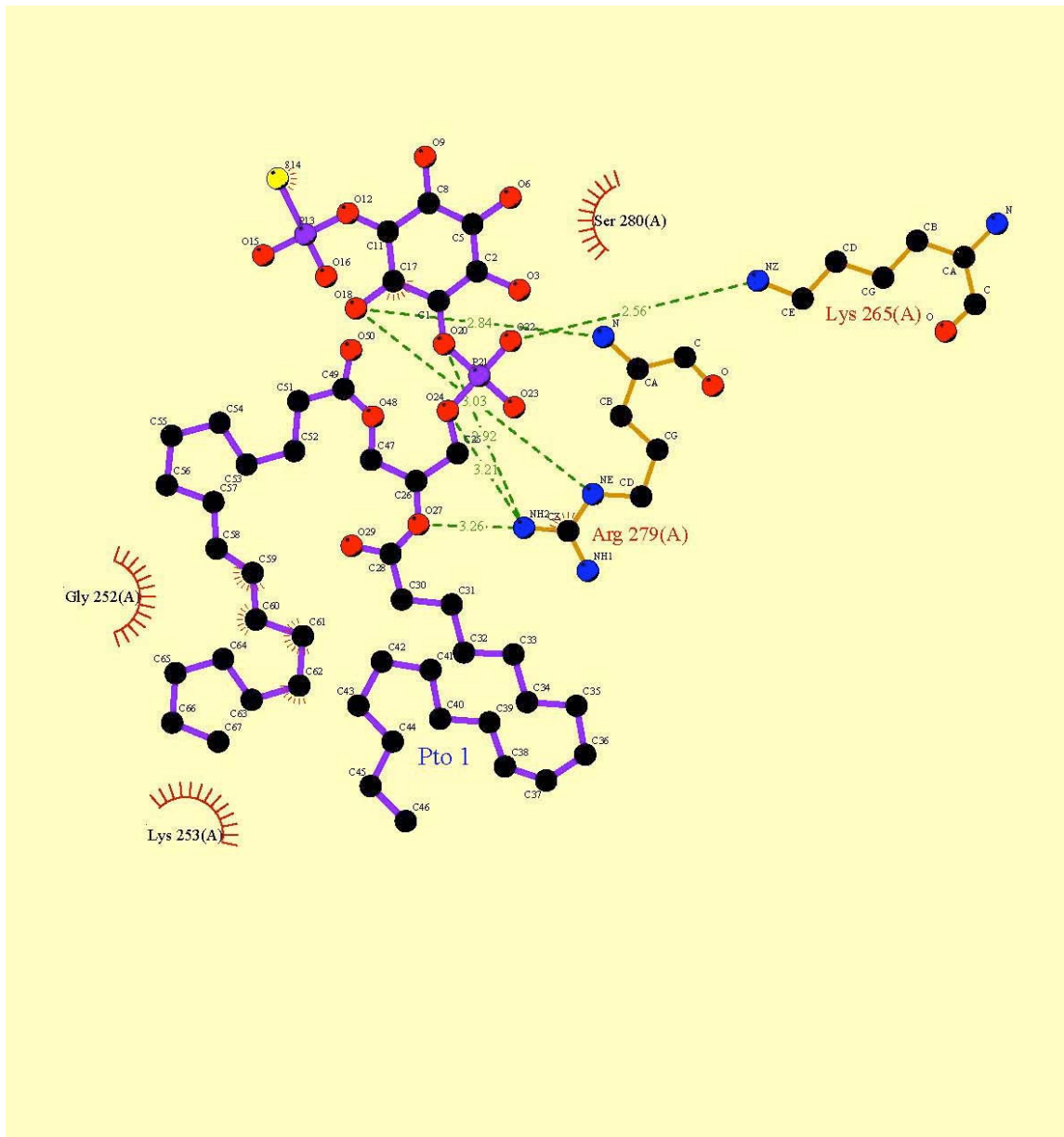
PtdIns(5)P with model 9. **Panel D.** Interactions of diC<sub>4</sub>-PtdIns(5)P with model 10.



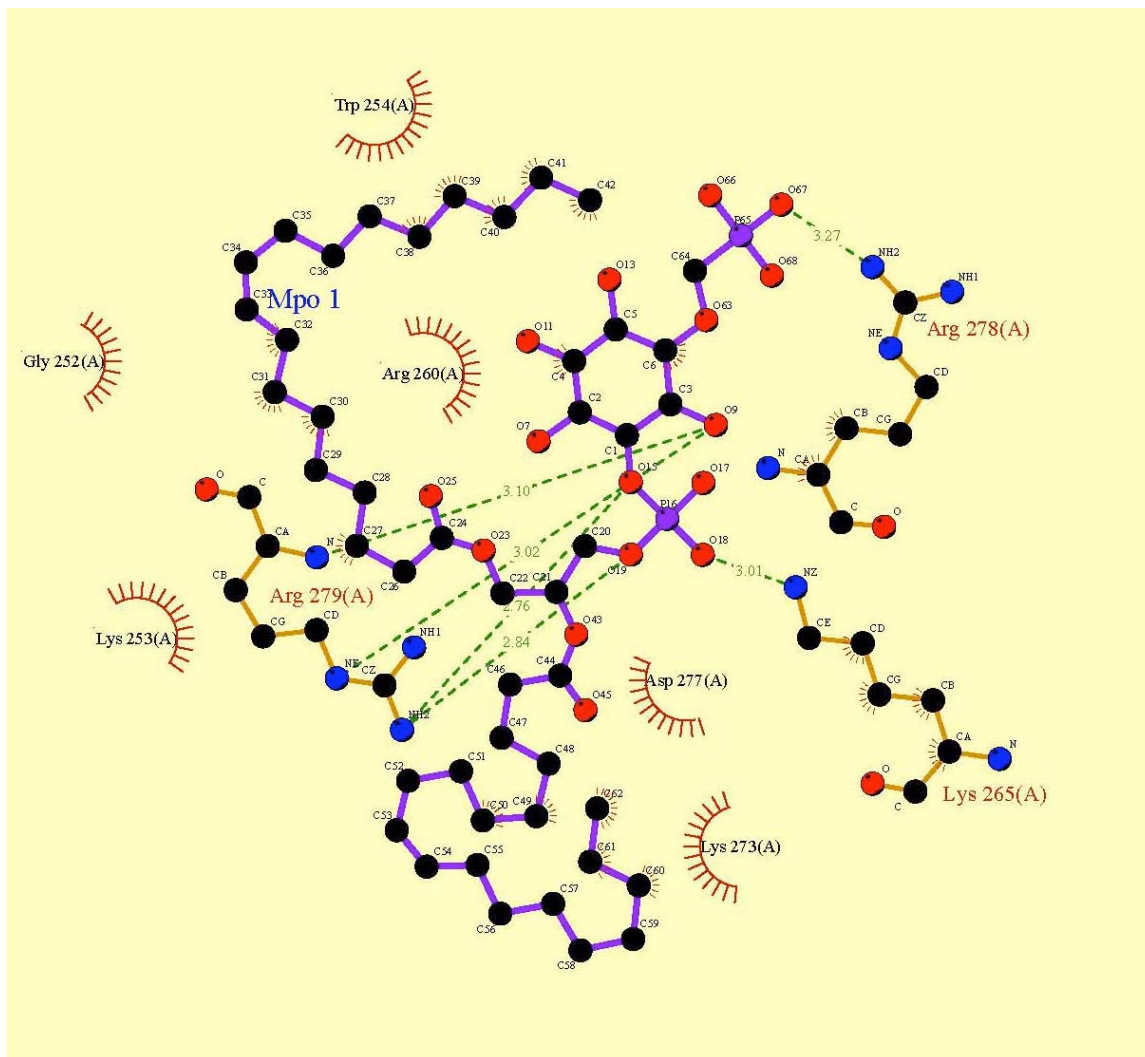
**Supplementary Figure S2.** Hydrogen bonds (green line with distance) and hydrophobic contacts (eyelash sketch) of diC<sub>18:1</sub>-PtdIns(5)P with ING2.



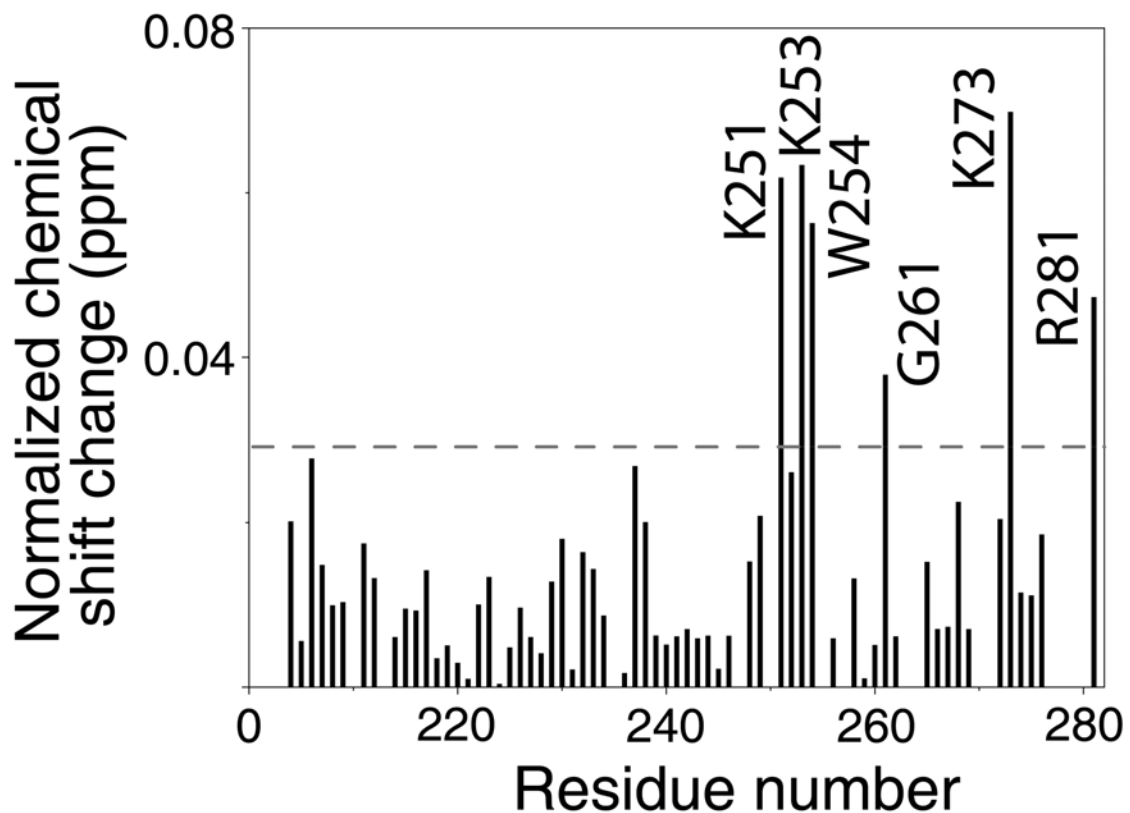
**Supplementary Figure S3.** Hydrogen bonds (green line with distance) and hydrophobic contacts (eyelash sketch) of PT-diC<sub>18:1</sub>-PtdIns(5)P (**4**) with ING2.



**Supplementary Figure 4.** Hydrogen bonds (green line with distance) and hydrophobic contacts (eyelash sketch) of diC<sub>18:1</sub>-MP-PtdIns(5)P (**2**) with ING2.

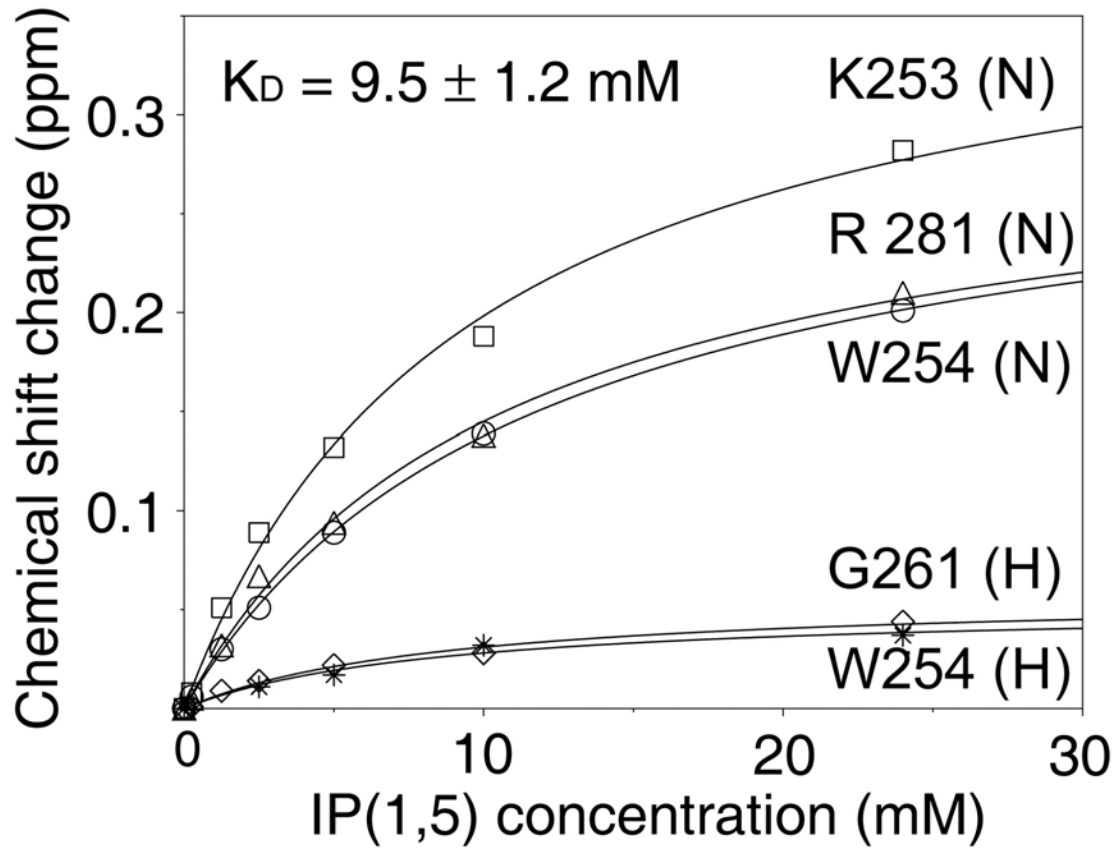


**Supplementary Figure S5.** Interaction of the ING2 C-terminus with inositol 1,5-bisphosphate (IP1,5). The histogram shows backbone amide chemical shift changes observed in  $^1\text{H},^{15}\text{N}$  HSQC spectra of the C-terminal region of ING2 (0.2 mM) upon addition of IP1,5 (24 mM). The normalized<sup>1</sup> chemical shift change was calculated using equation  $[(\Delta\delta\text{H})^2 + (\Delta\delta\text{N}/5)^2]^{0.5}$ , where is the chemical shift in parts per million (ppm). Residues that exhibit significant resonance perturbations are labeled.

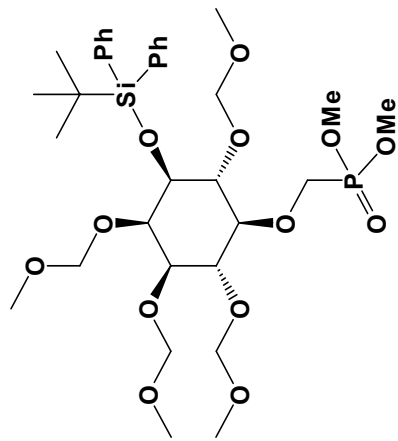


1. Grzesiek, S., Stahl, S. J., Wingfield, P. T. & Bax, A. The CD4 determinant for downregulation by HIV-1 Nef directly binds to Nef. Mapping of the Nef binding surface by NMR. *Biochemistry* **35**, 10256-61 (1996).

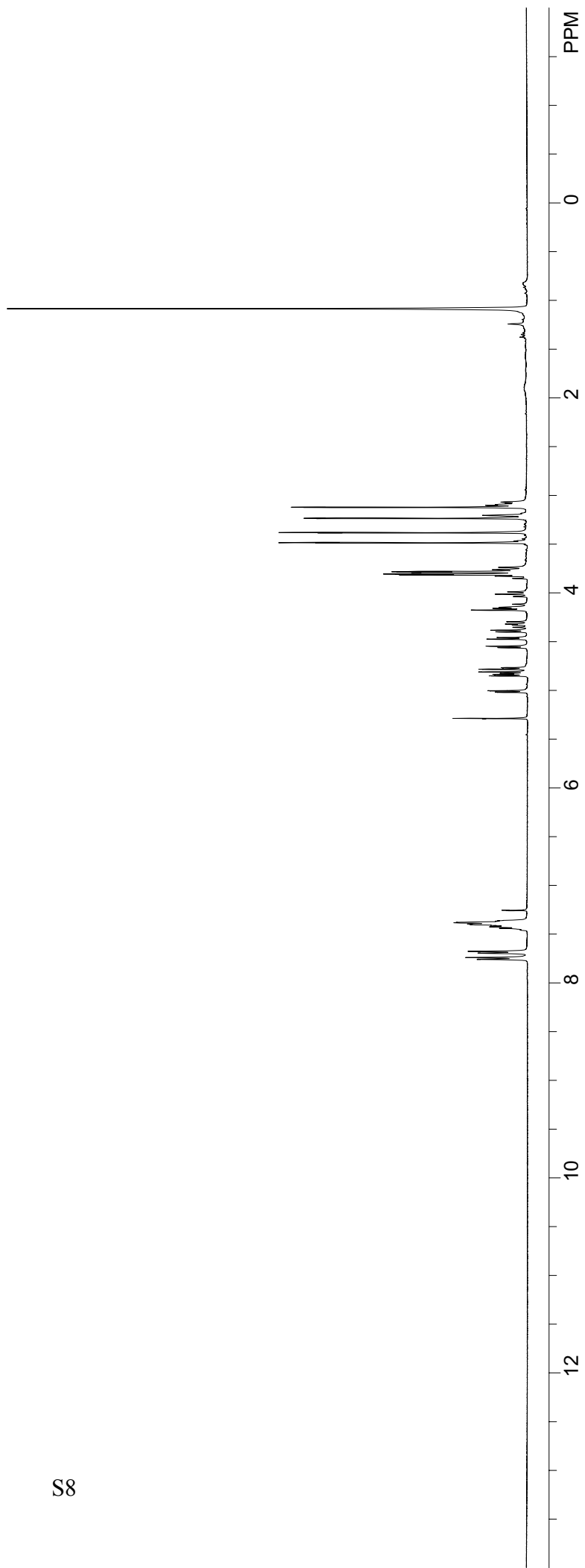
**Supplementary Figure S6.** Inositol 1,5-bisphosphate binding affinity of the ING2 C-terminus determined by resonance perturbations in  $^1\text{H},^{15}\text{N}$  HSQC spectra of  $^{15}\text{N}$ -labeled protein as IP1,5 was titrated in.



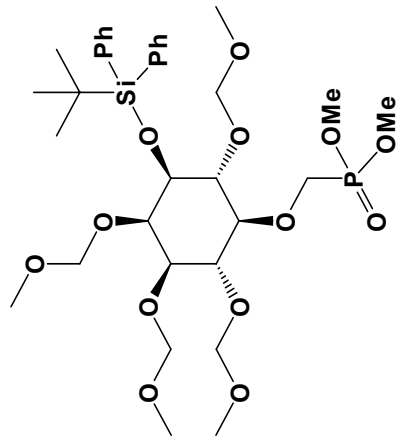
**NMR spectra for Intermediates and Products.** Spectra were obtained using a Varian INOVA 400 at 400 MHz ( $^1\text{H}$ ), 101 MHz ( $^{13}\text{C}$ ), 162 MHz ( $^{31}\text{P}$ ) and 376 MHz ( $^{19}\text{F}$ ) at 25 °C. Chemical shifts are reported in ppm with TMS as internal standard ( $\delta = 0.00$ );  $^{31}\text{P}$ , 85%  $\text{H}_3\text{PO}_4$  ( $\delta = 0.00$ );  $^{19}\text{F}$ ,  $\text{CFCl}_3$  ( $\delta = 0.00$ ).



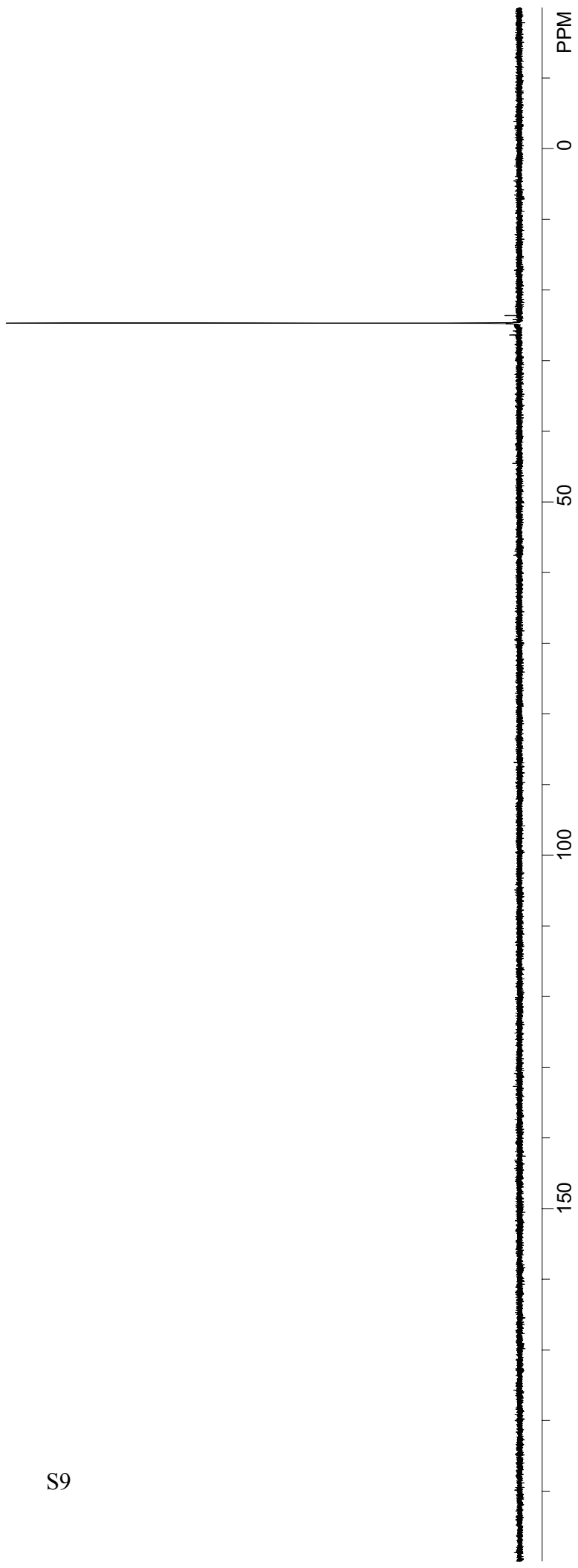
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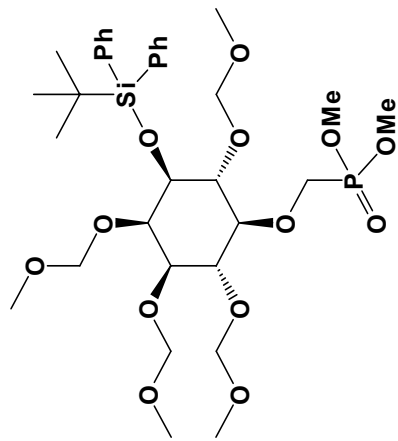




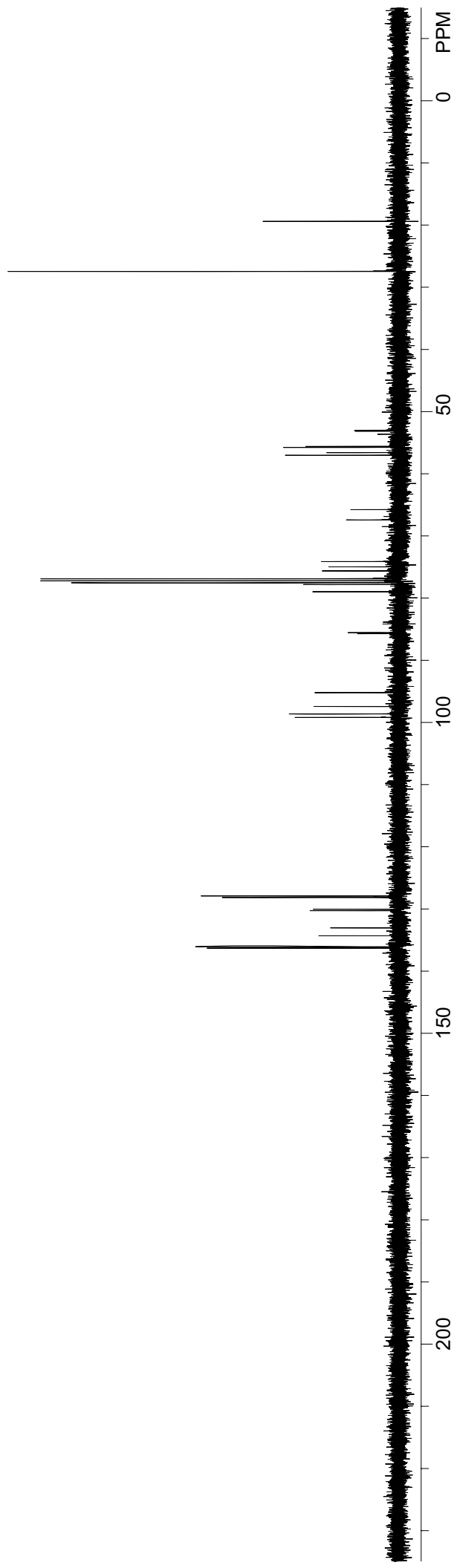


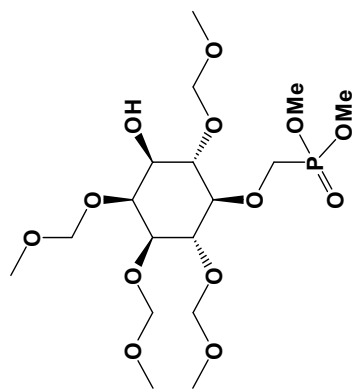
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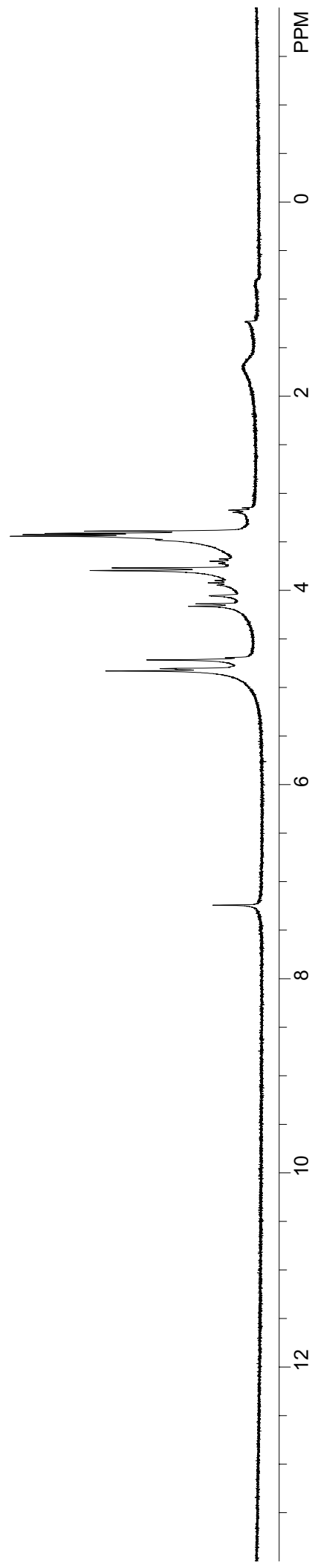


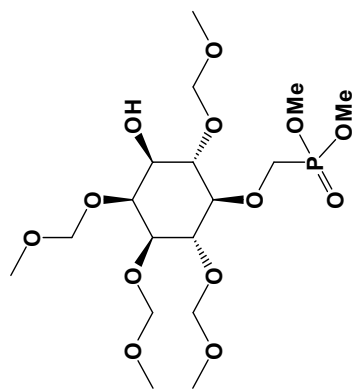
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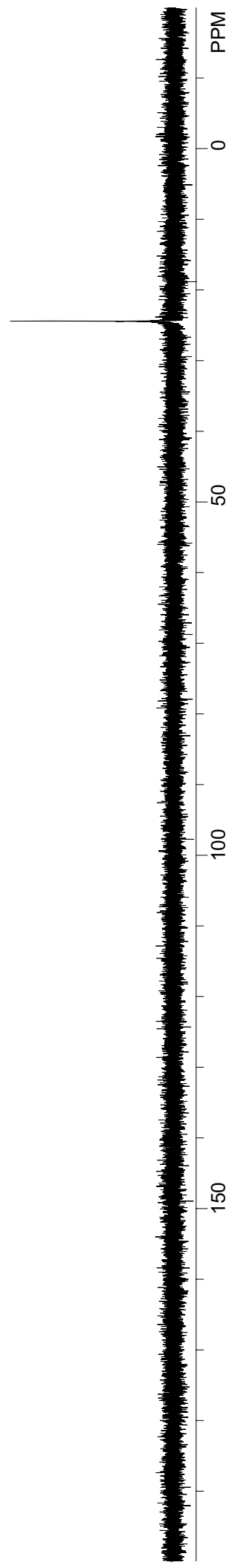


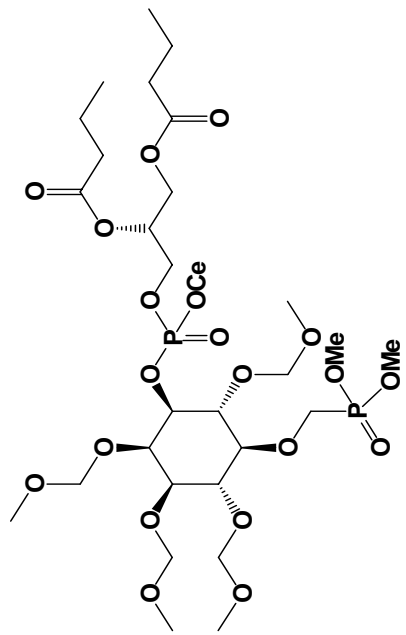
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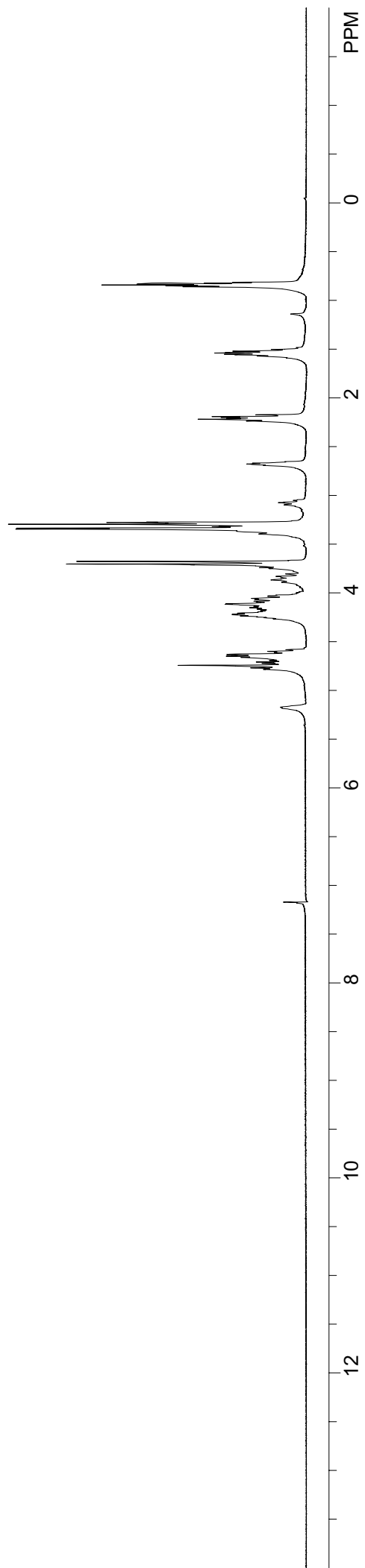


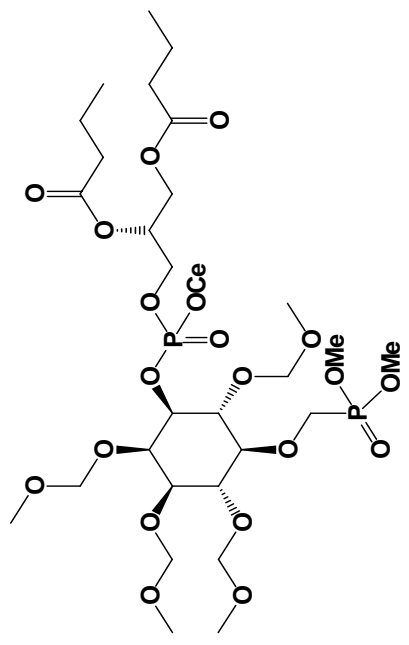
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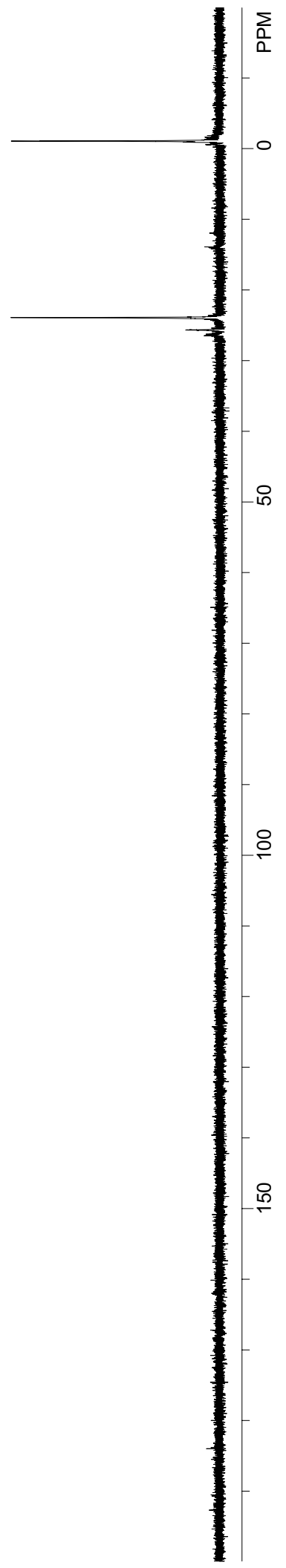


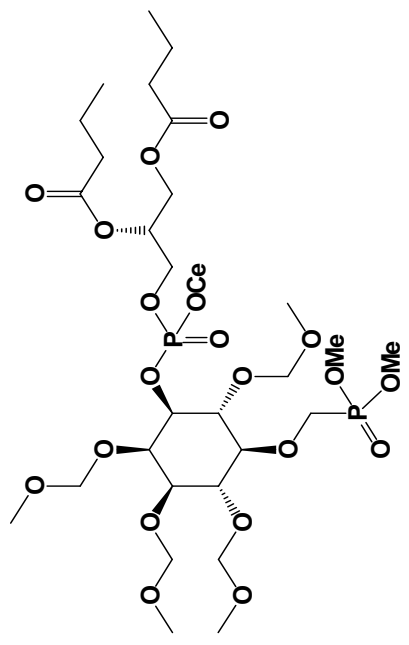
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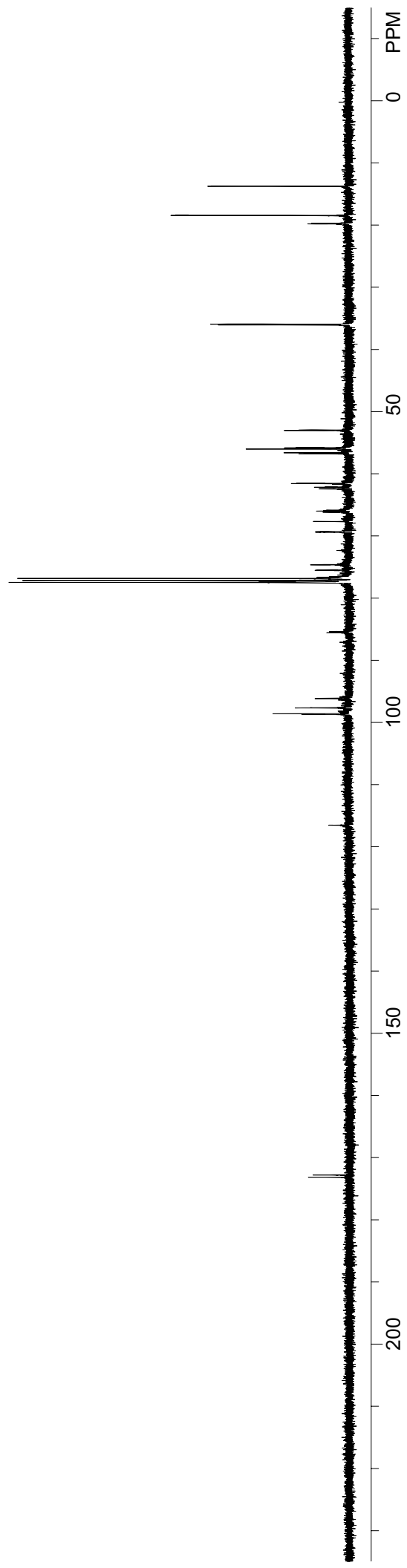


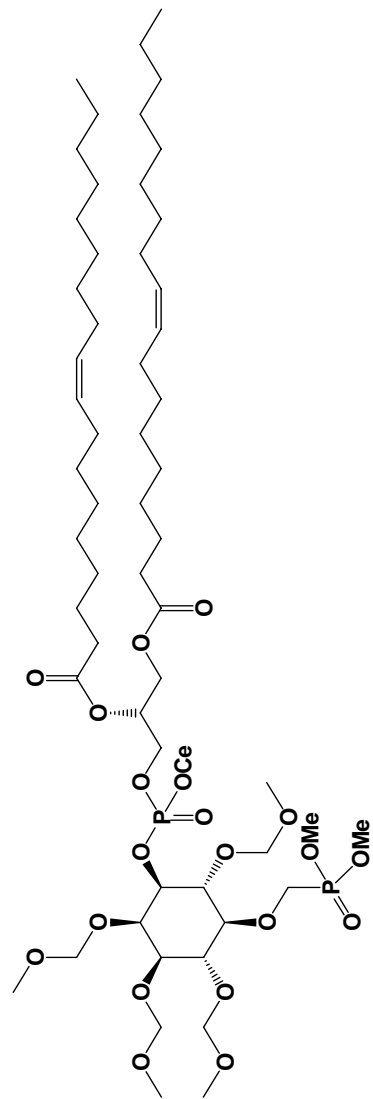
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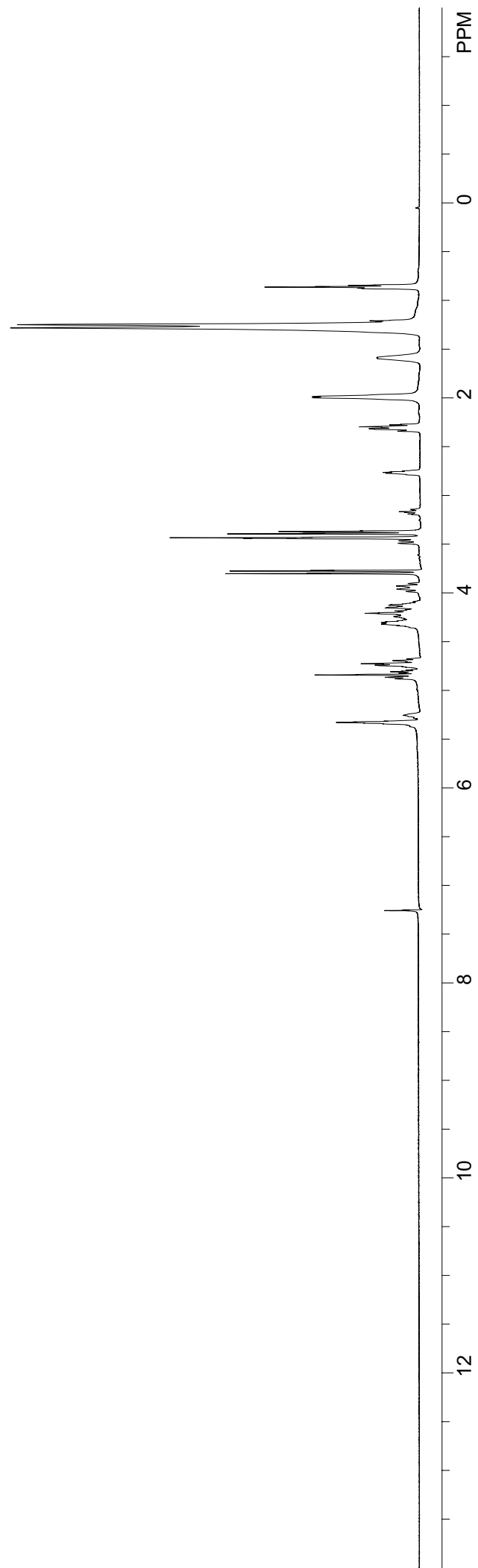


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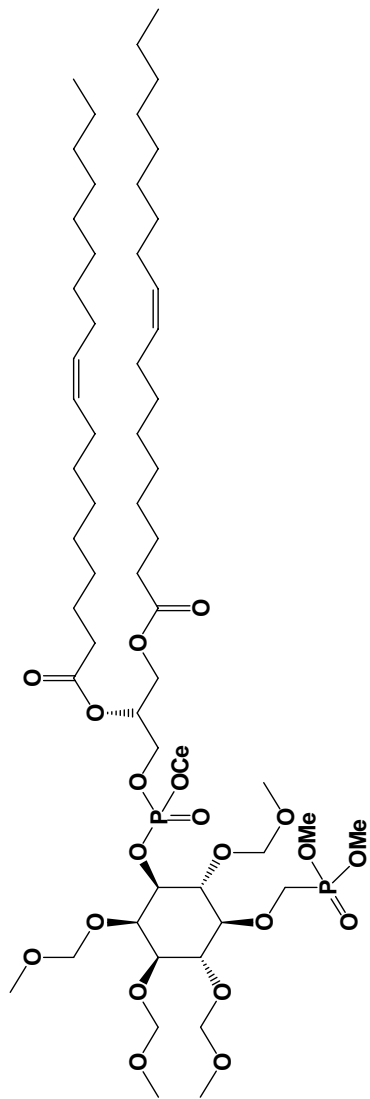




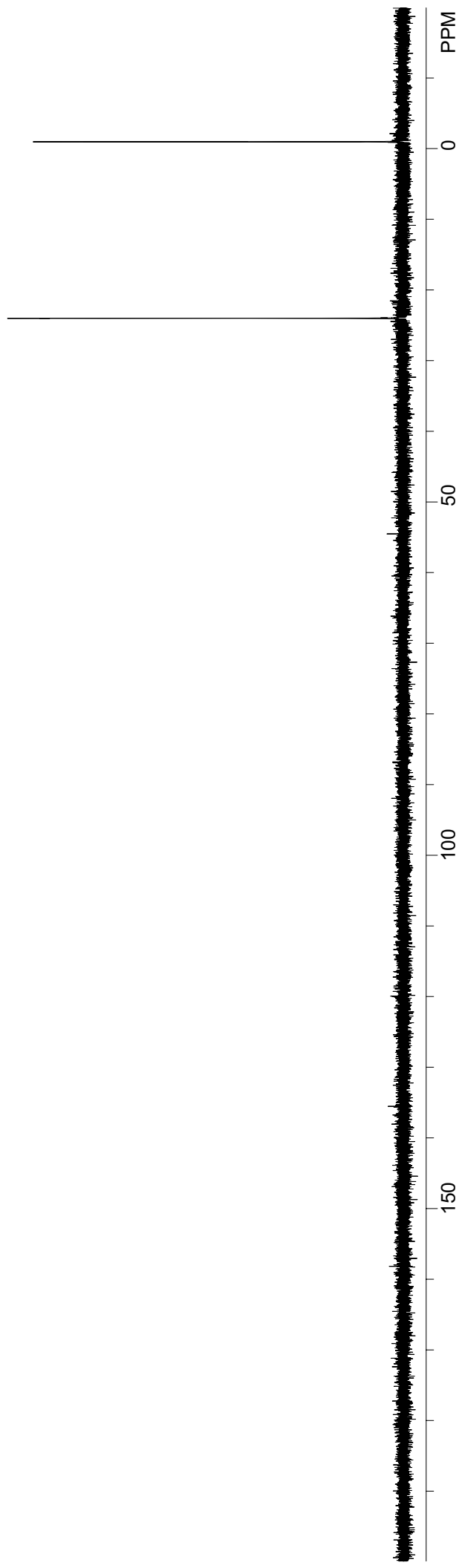
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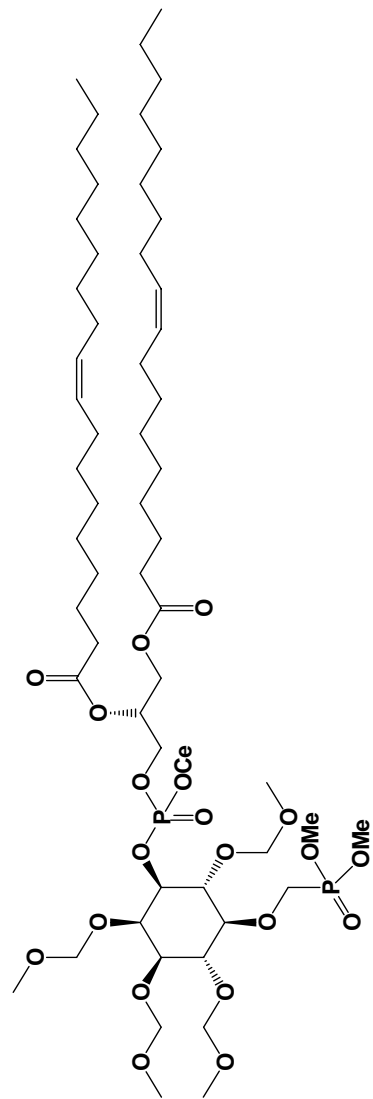




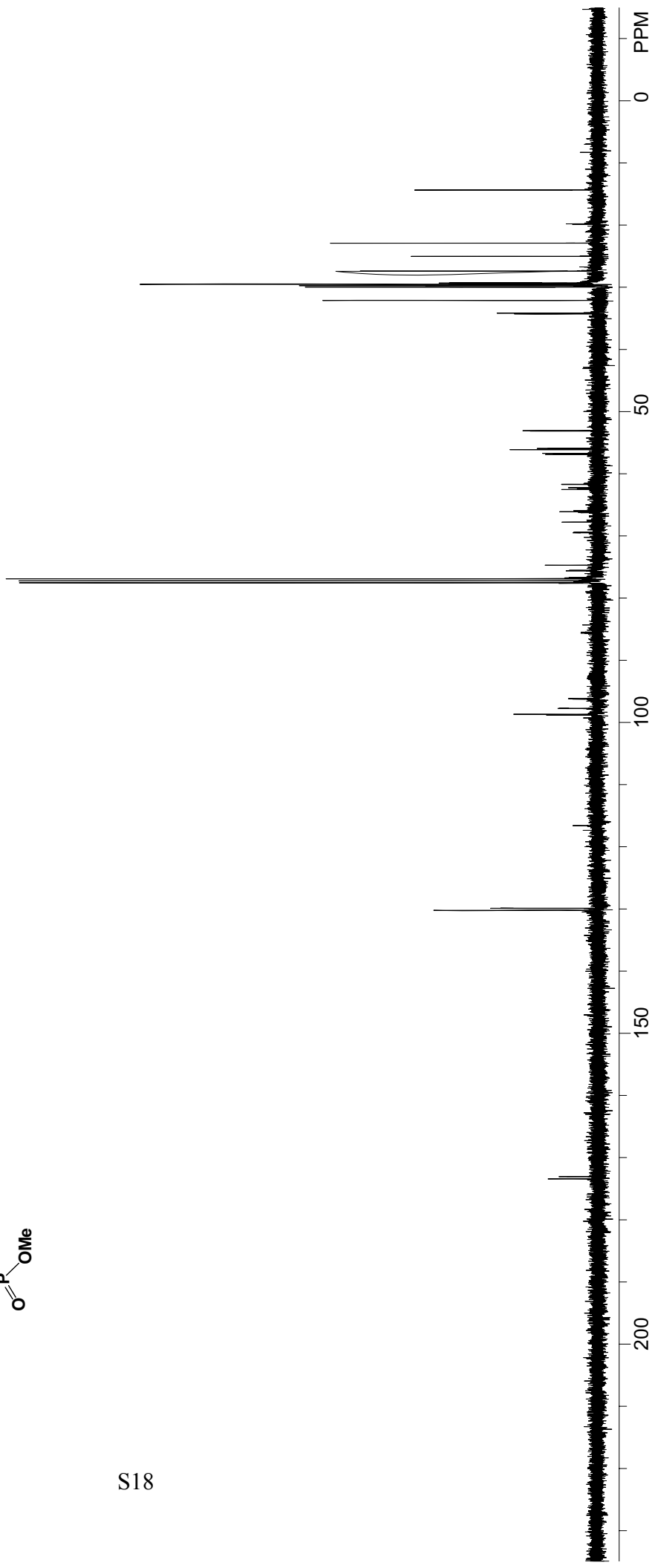


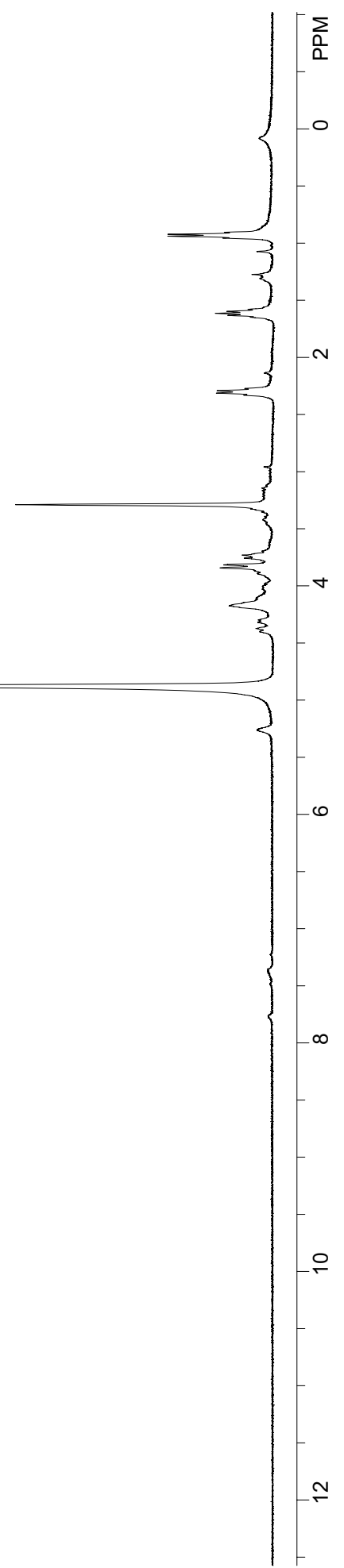
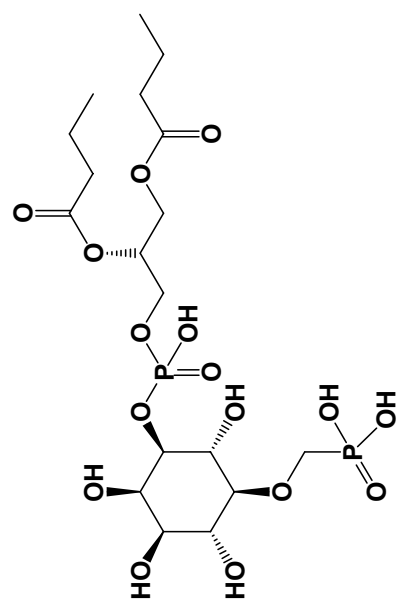
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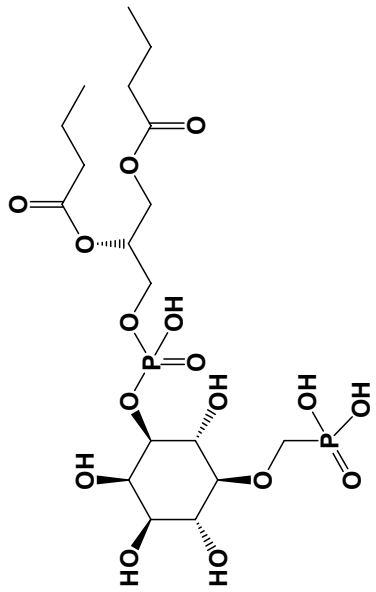




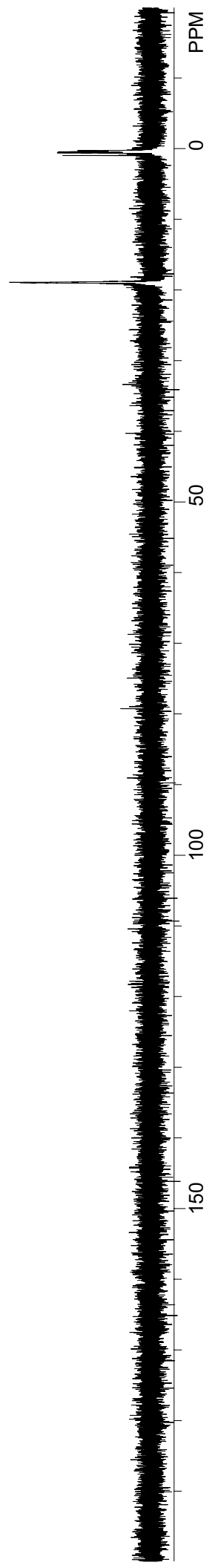
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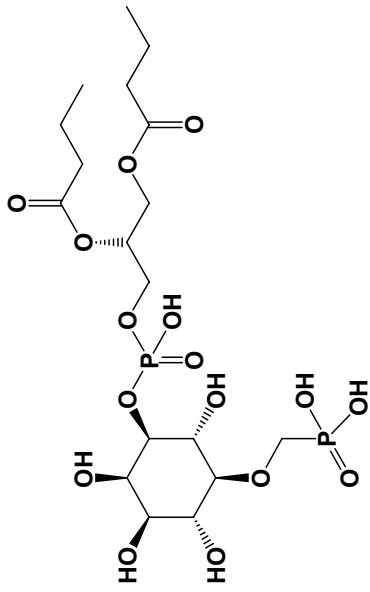




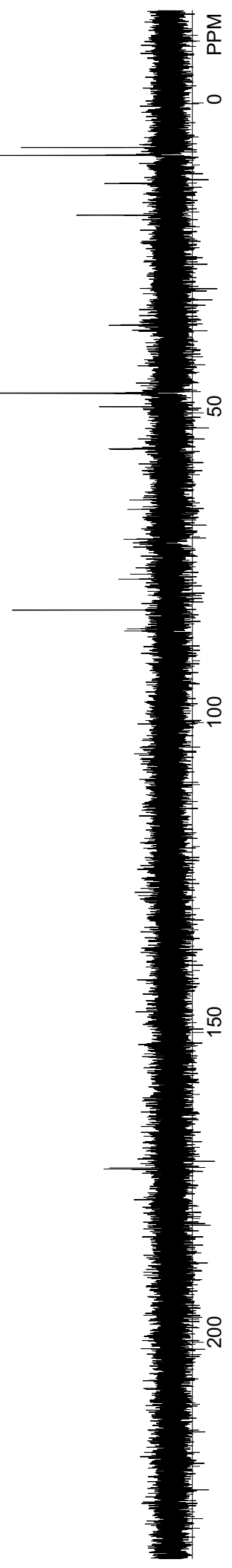


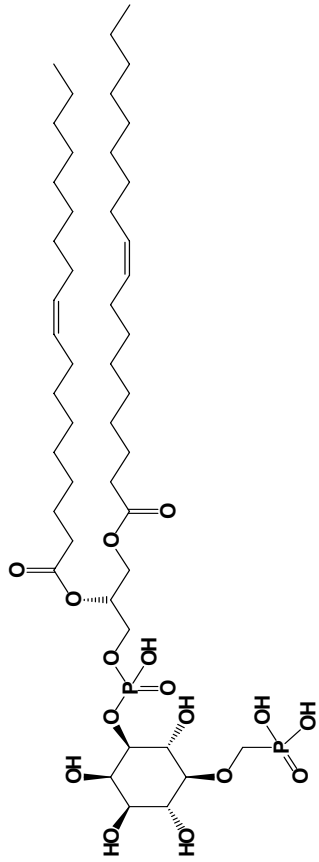
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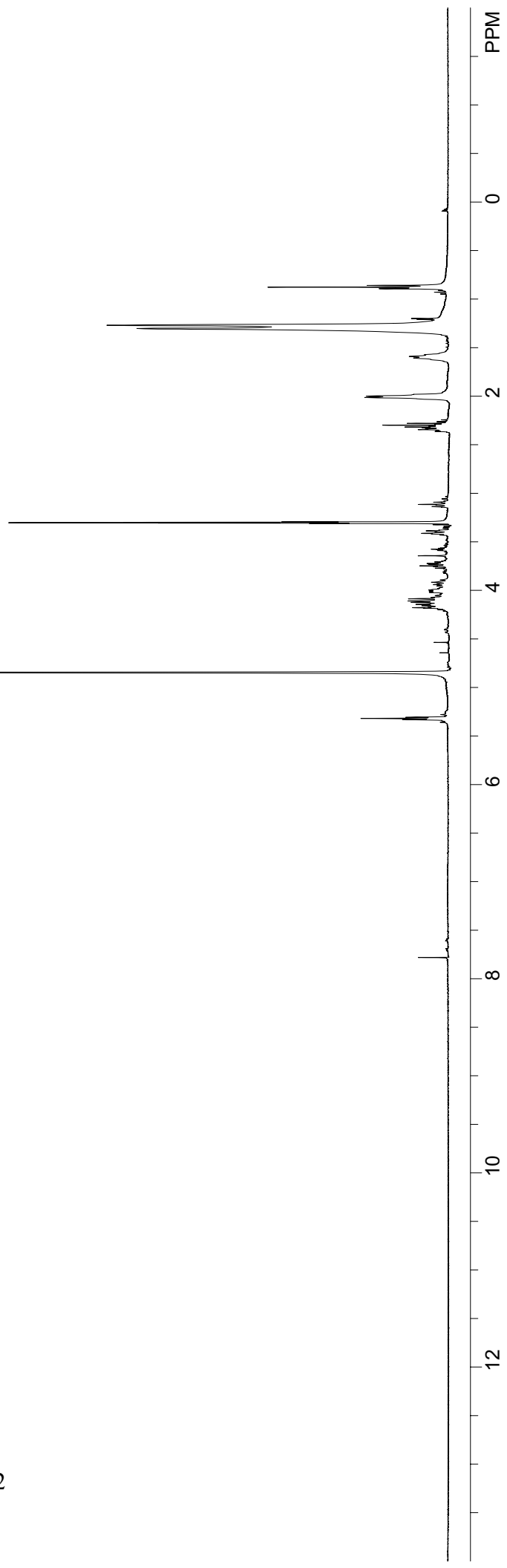


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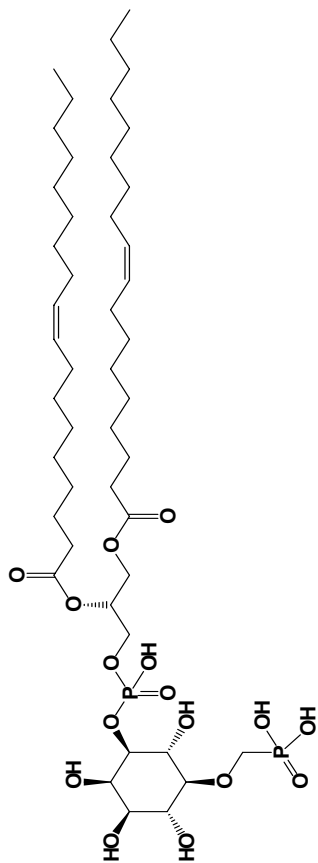




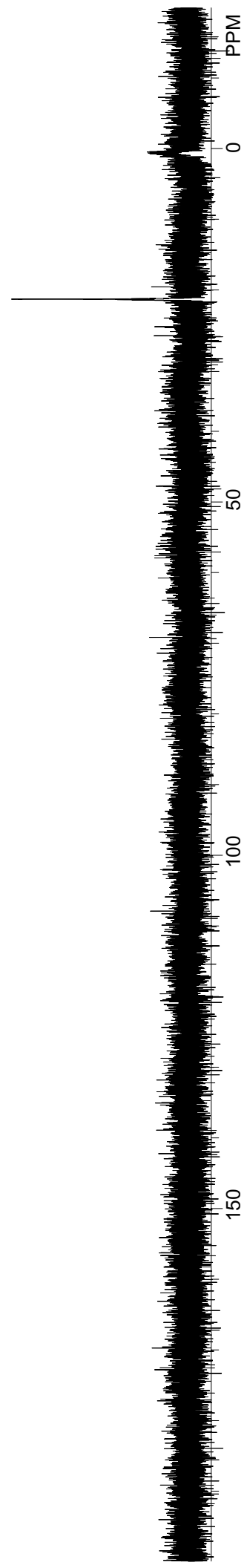
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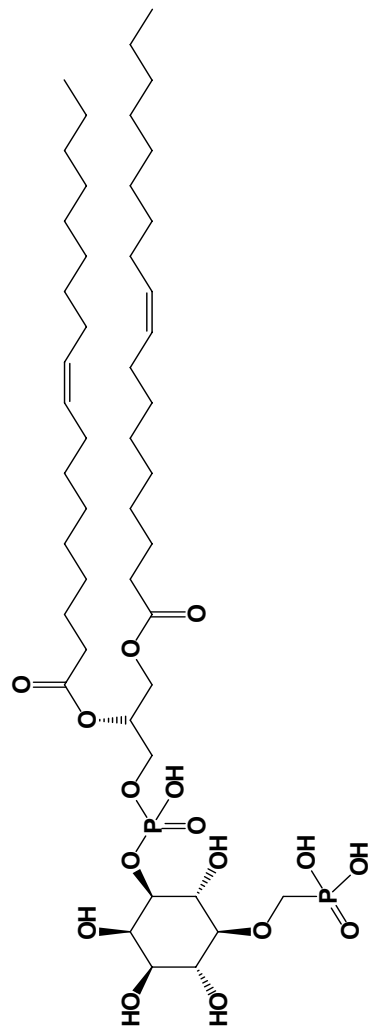


2, 31P NMR, CD3OD-CDCl3

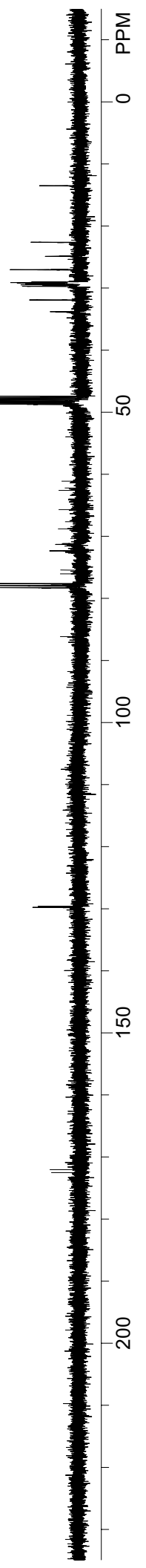


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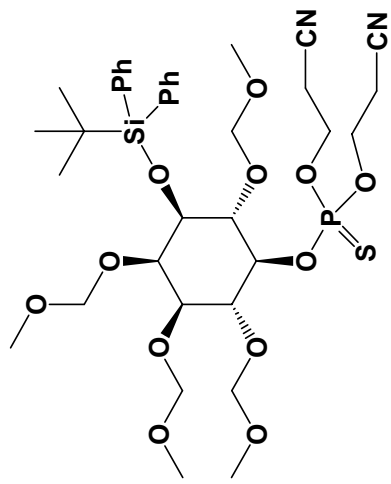




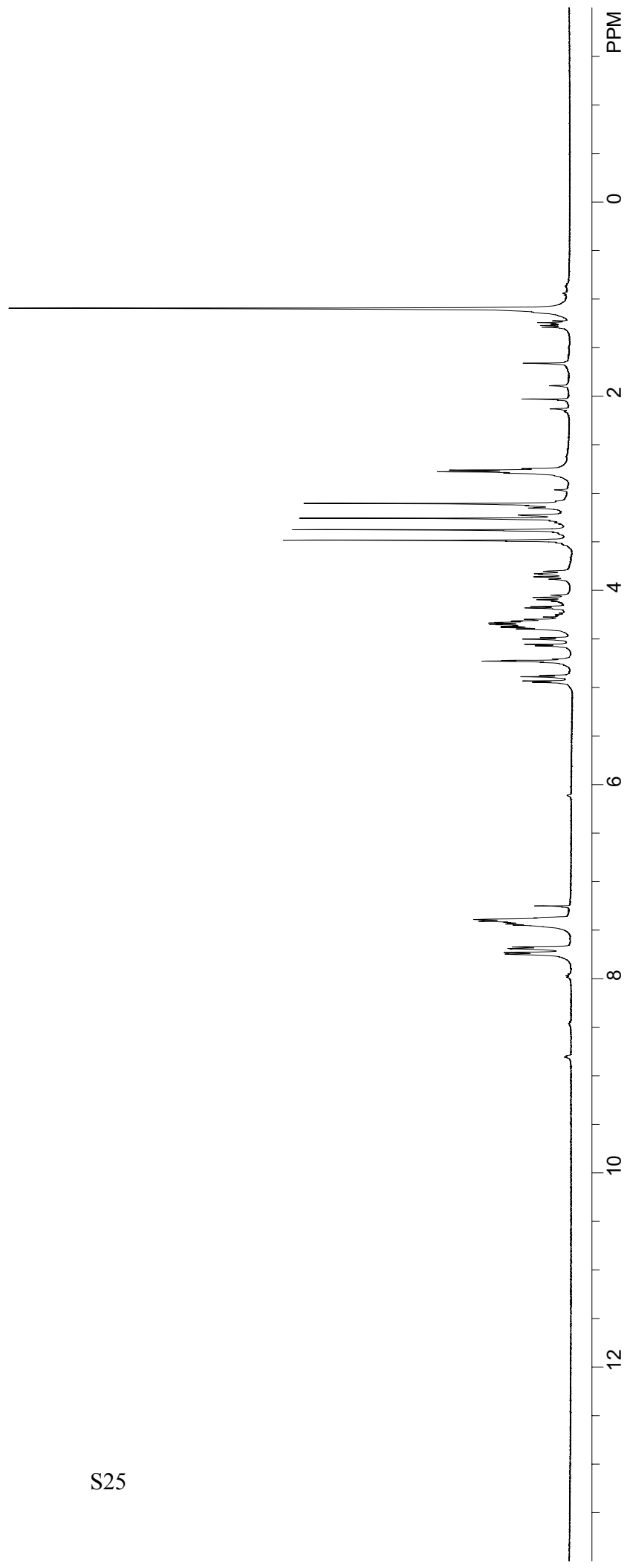
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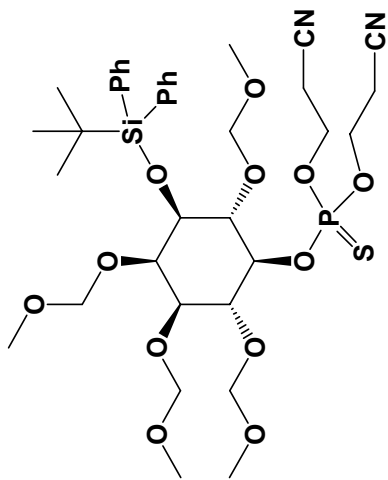




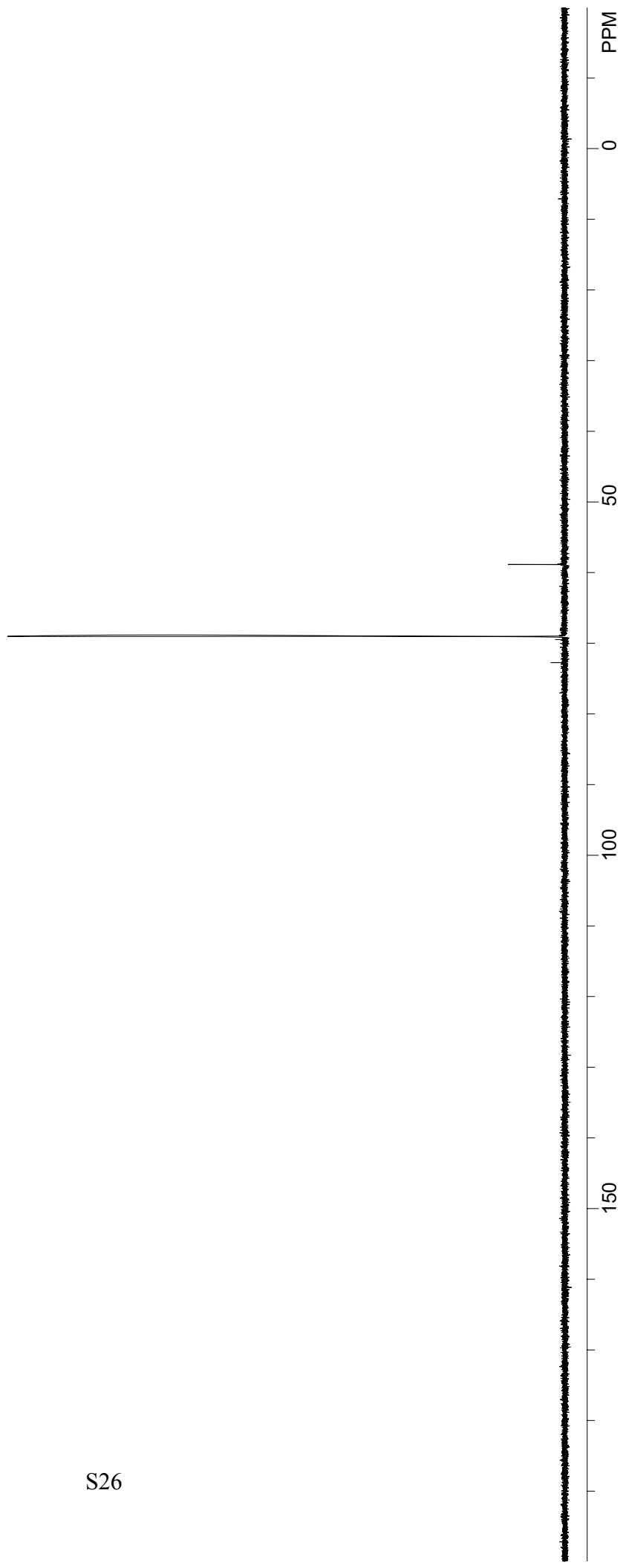


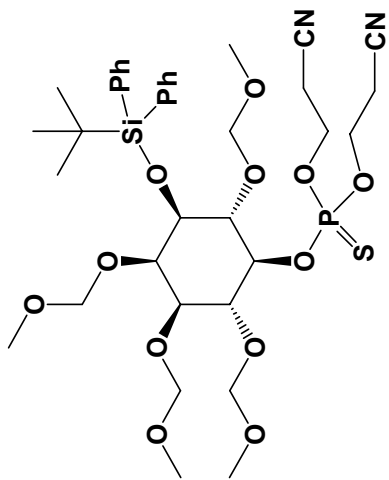
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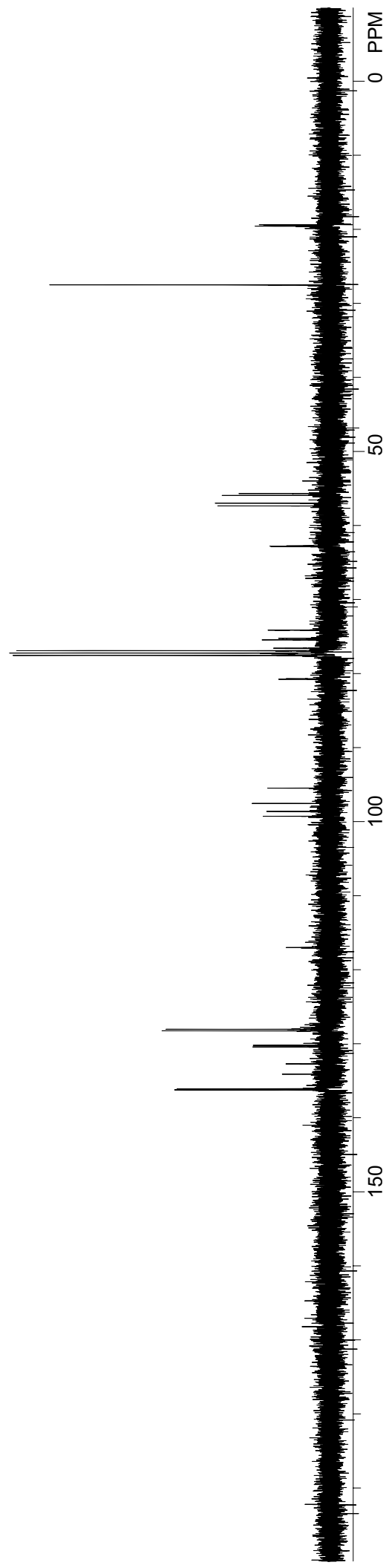


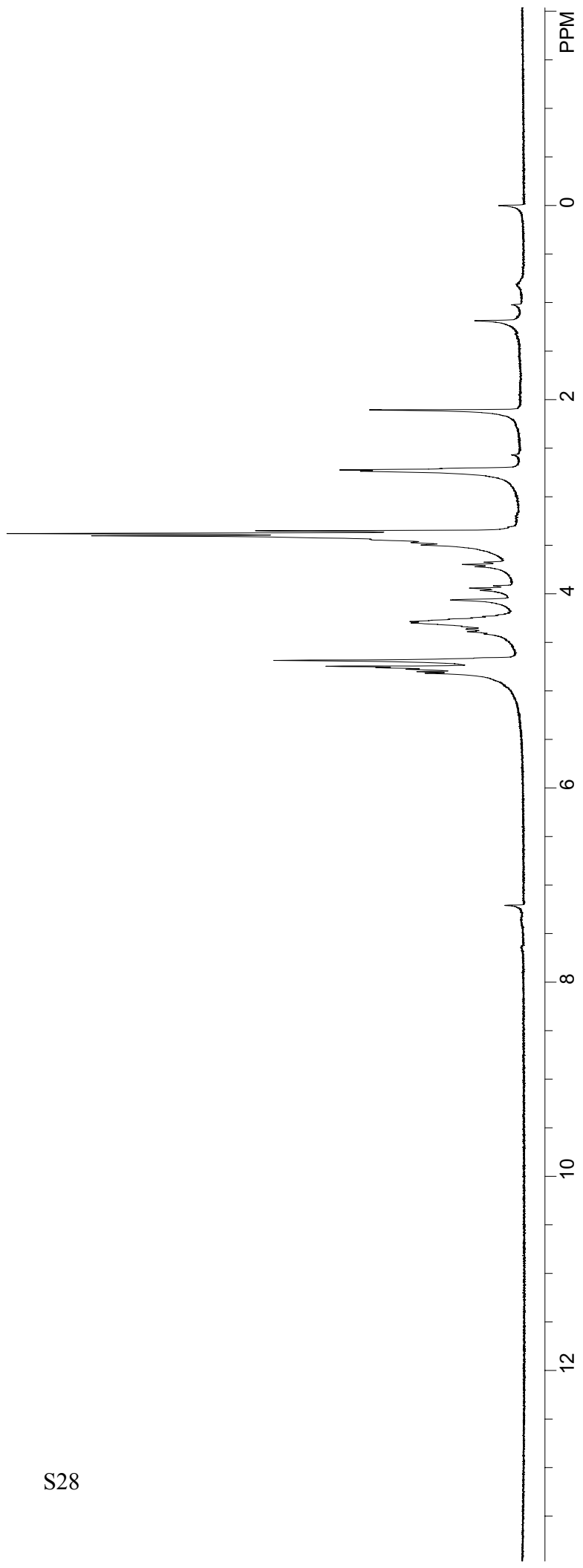
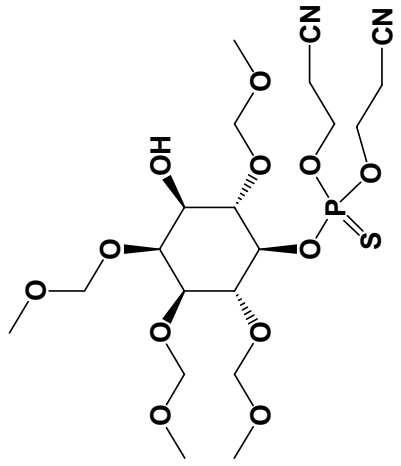
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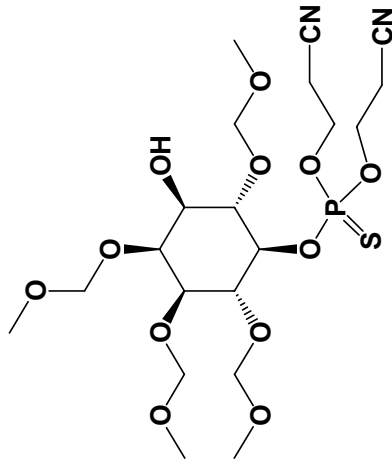




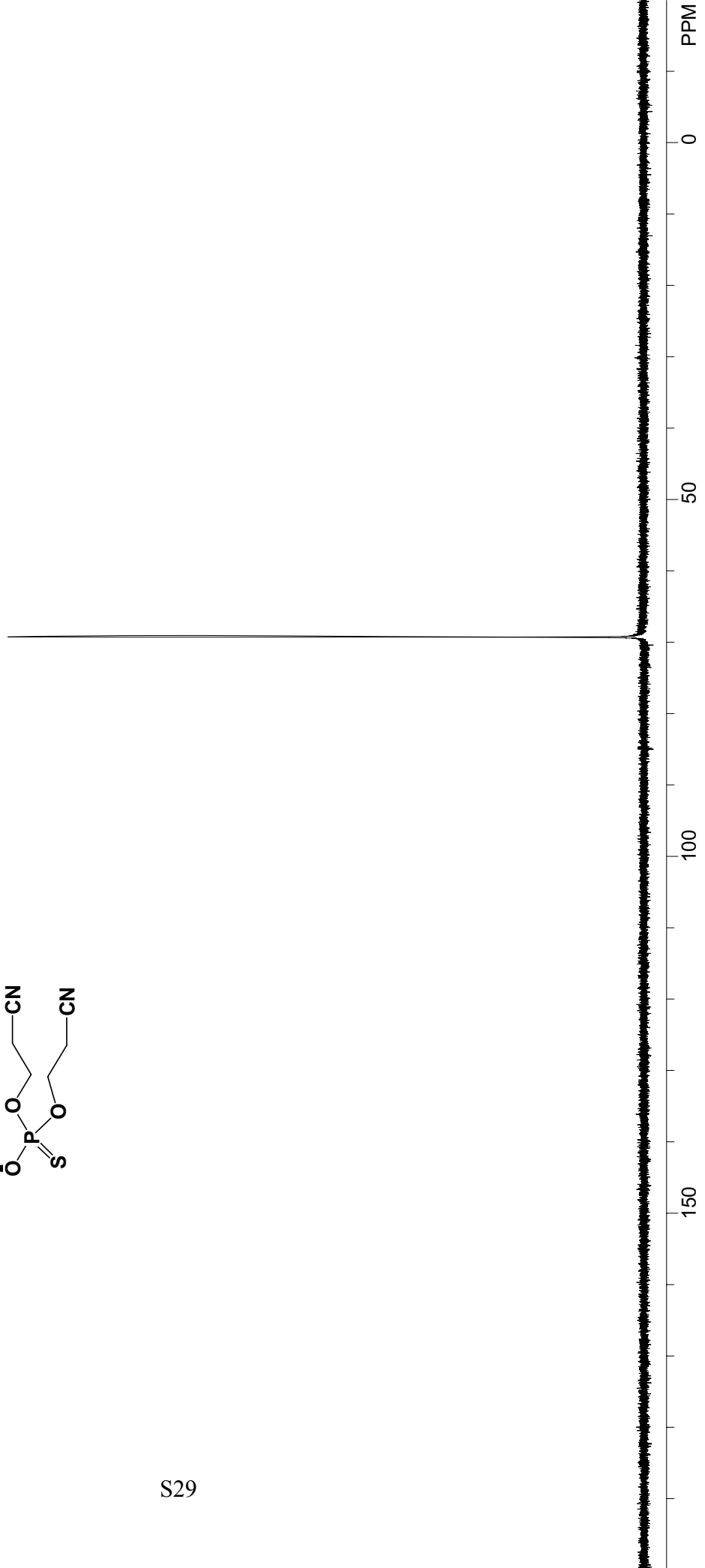
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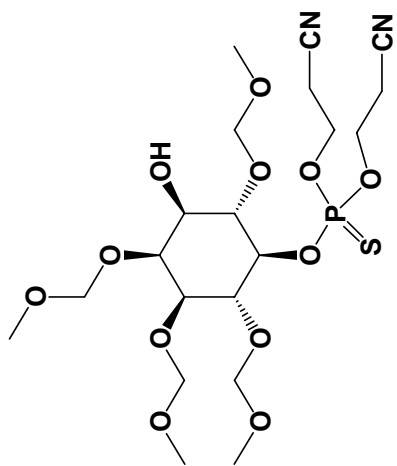




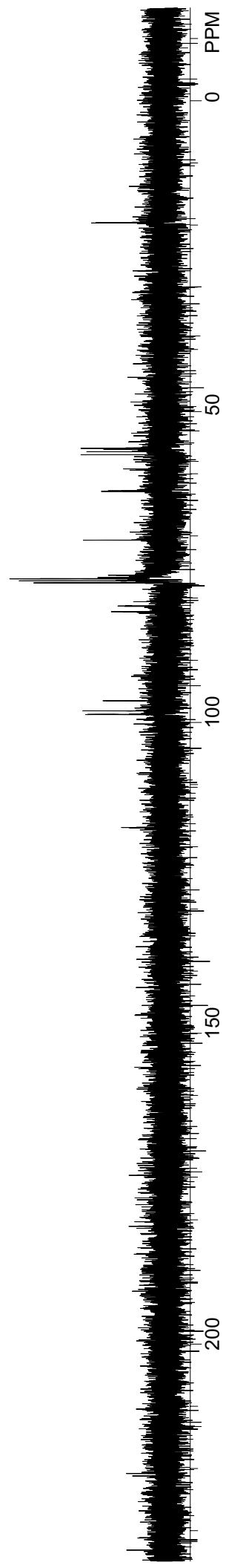


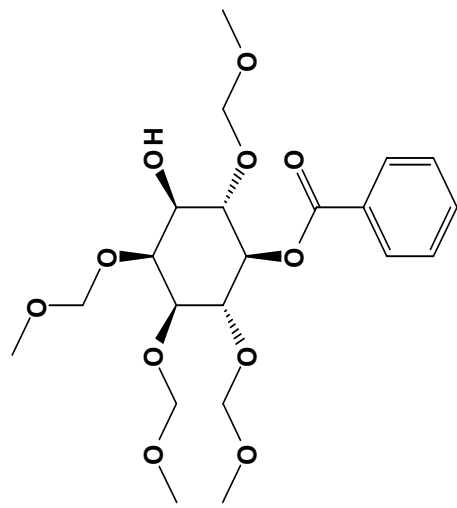
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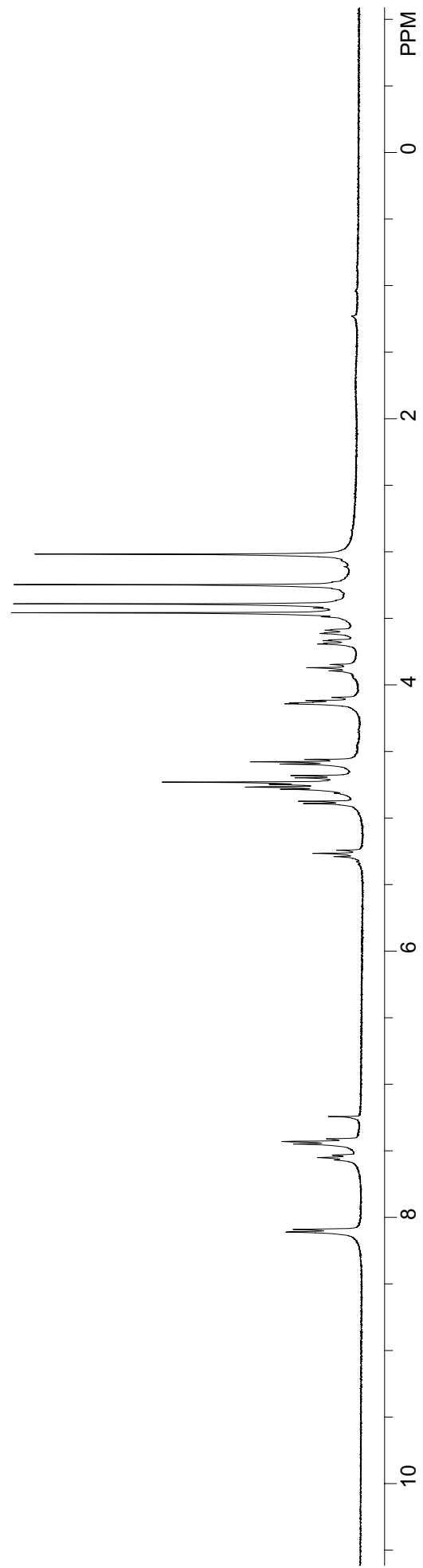


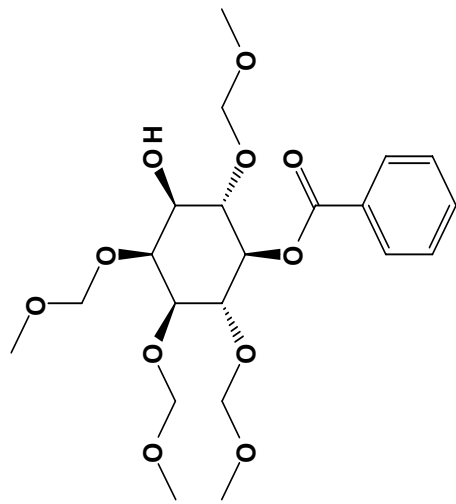
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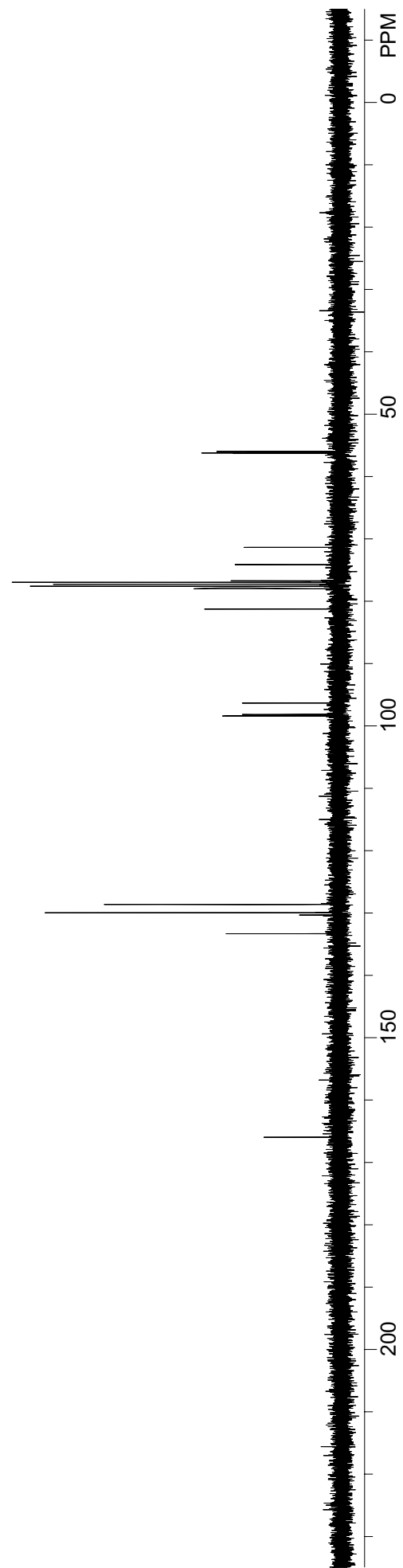


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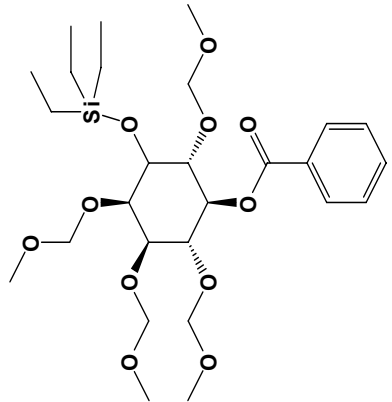




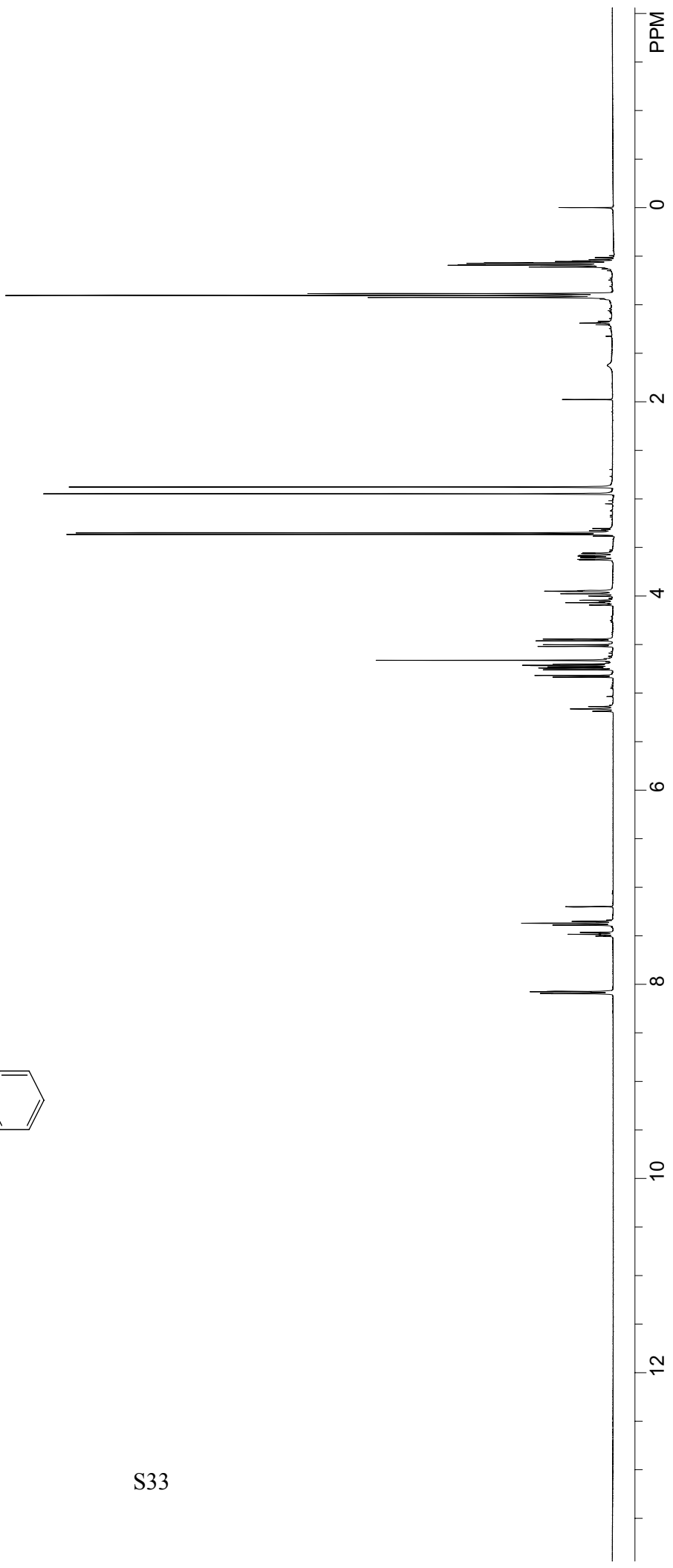
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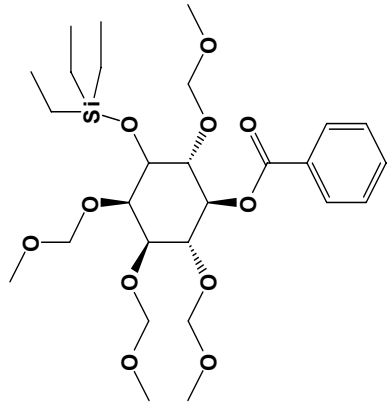




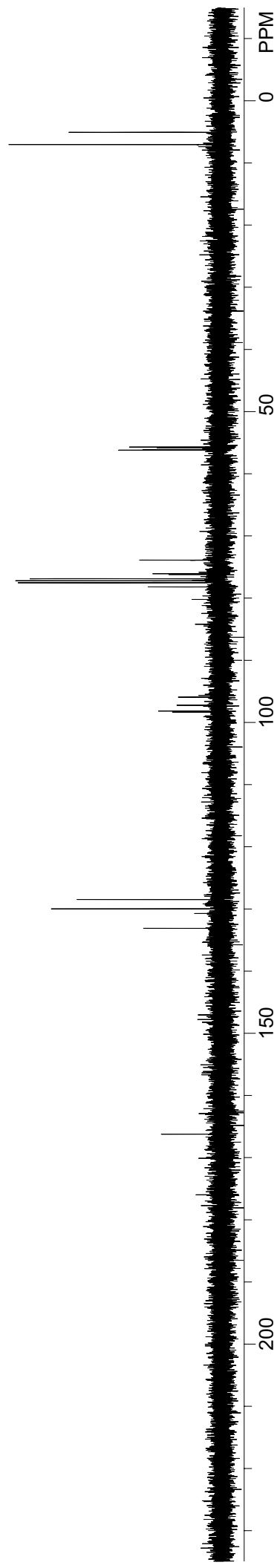


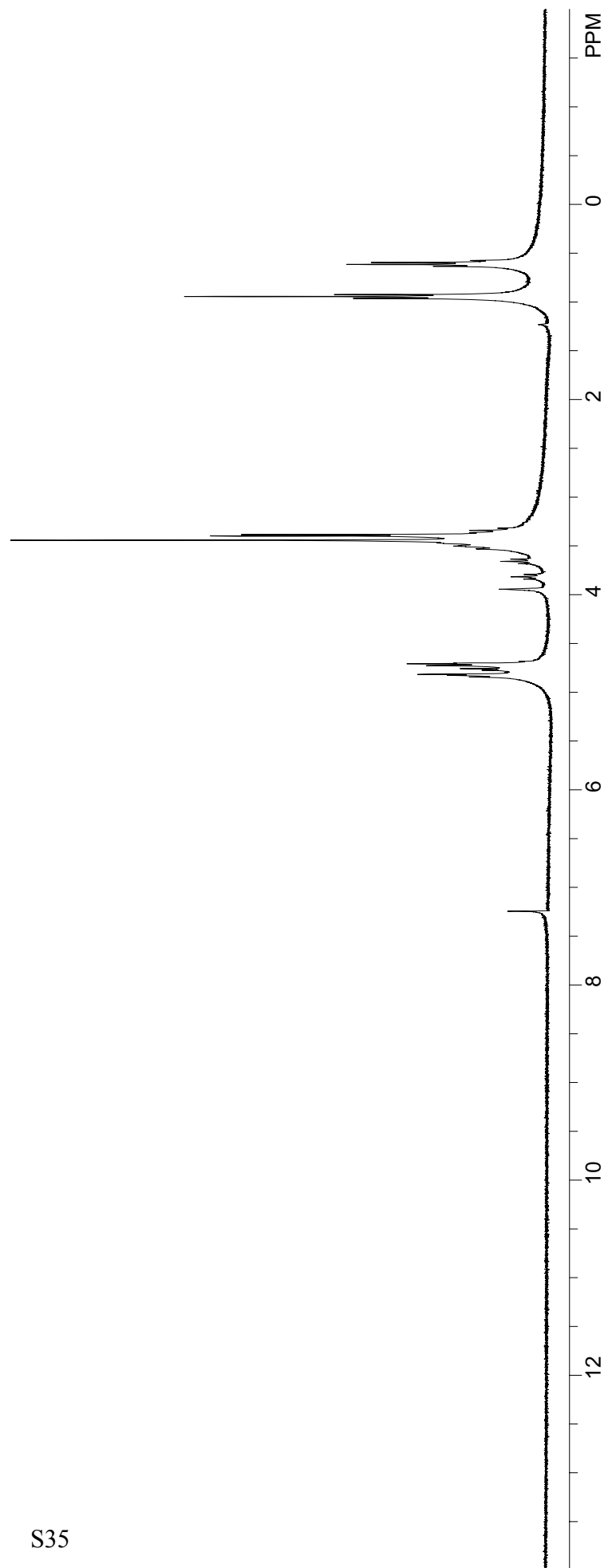
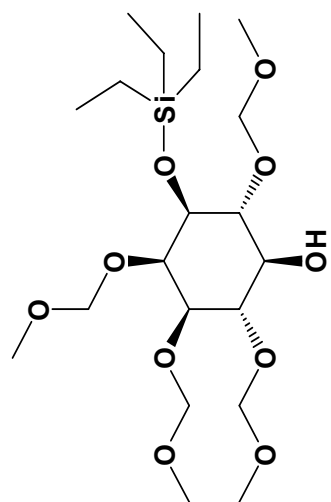
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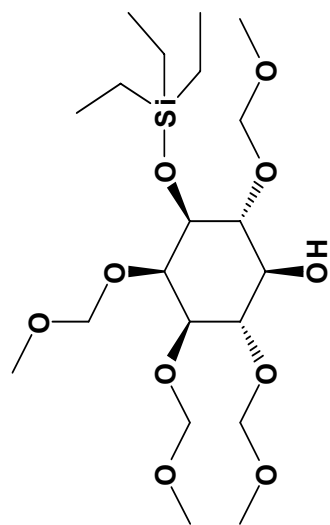


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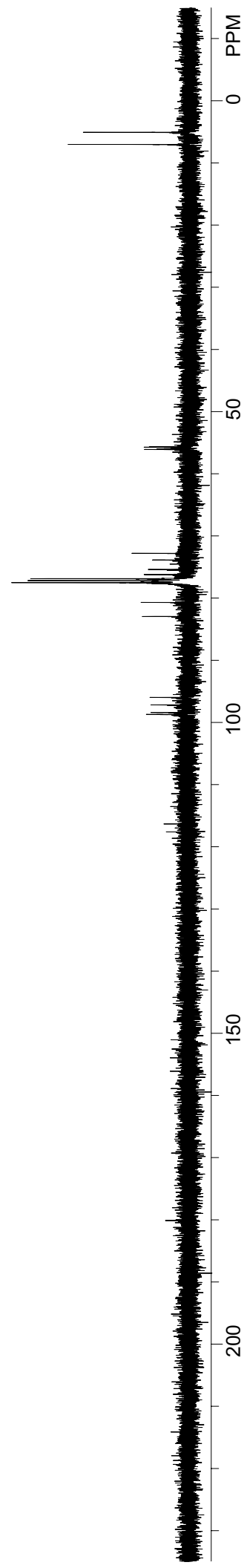




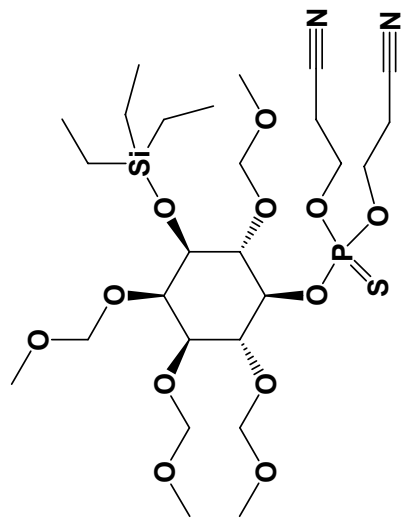
15, <sup>13</sup>C NMR, CDCl<sub>3</sub>



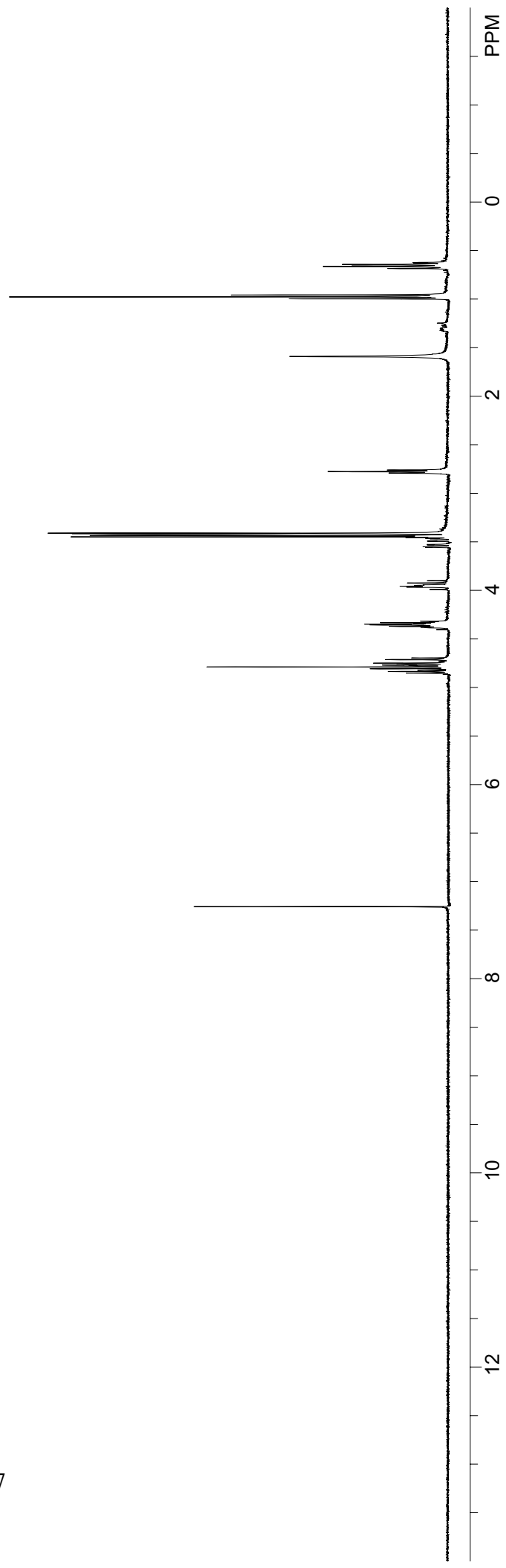
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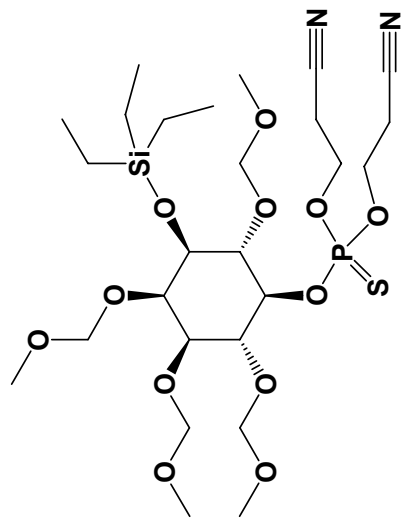


16, 1H NMR, CDCl3

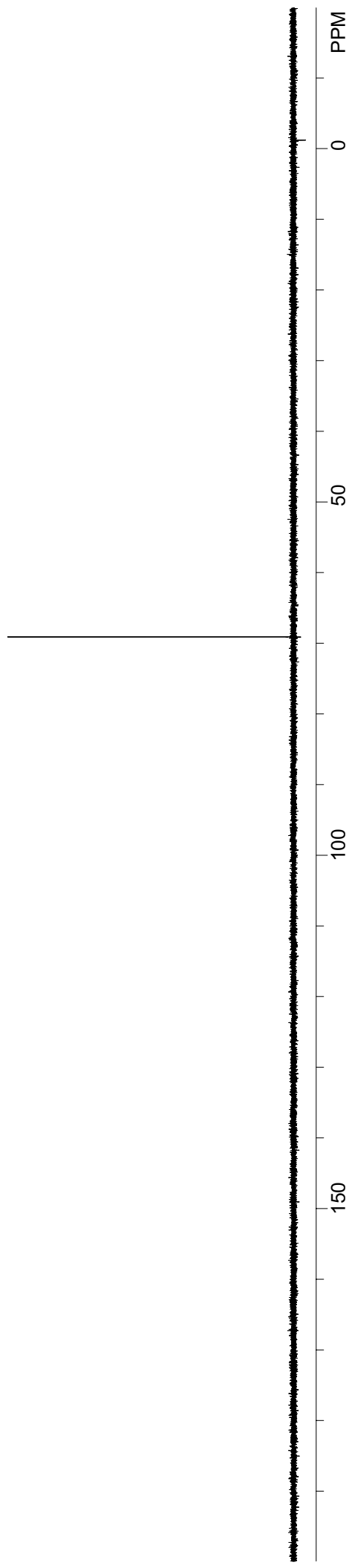


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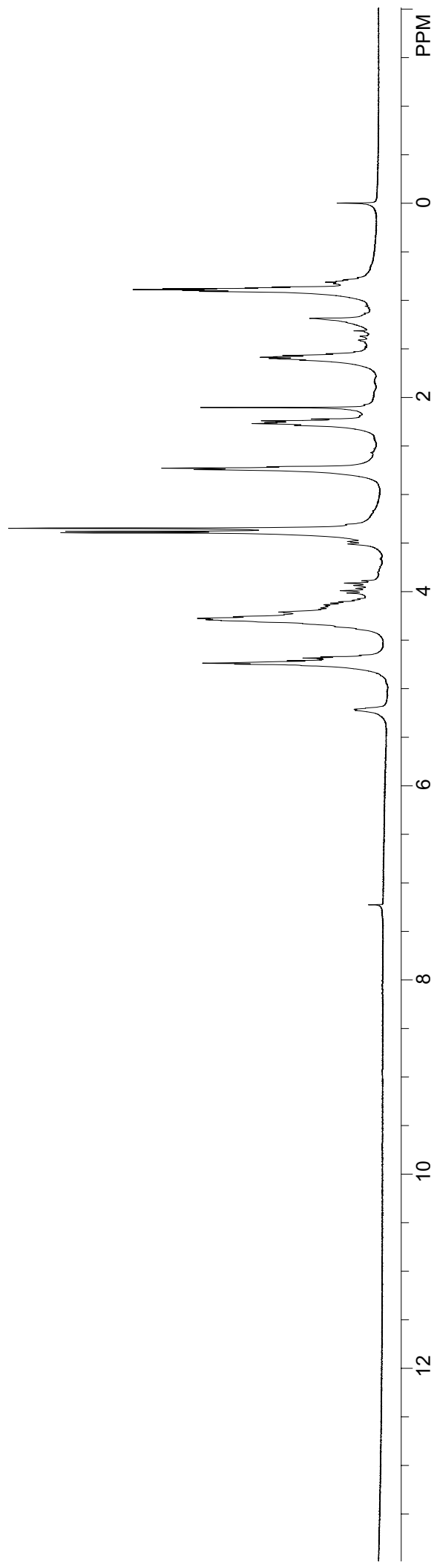
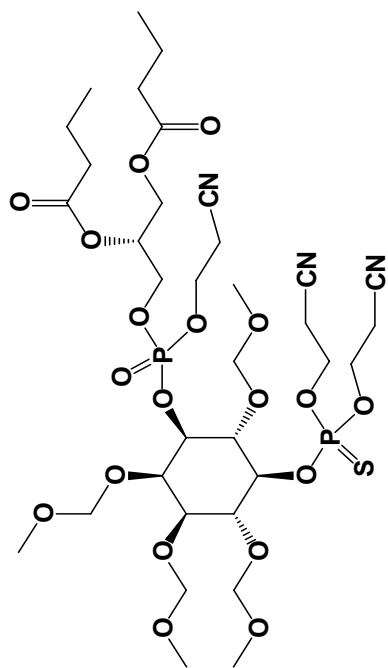


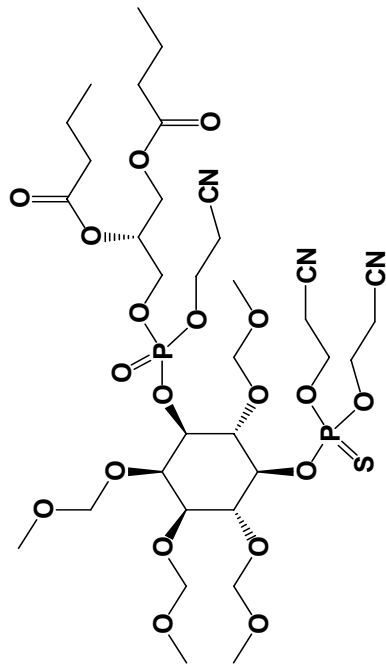


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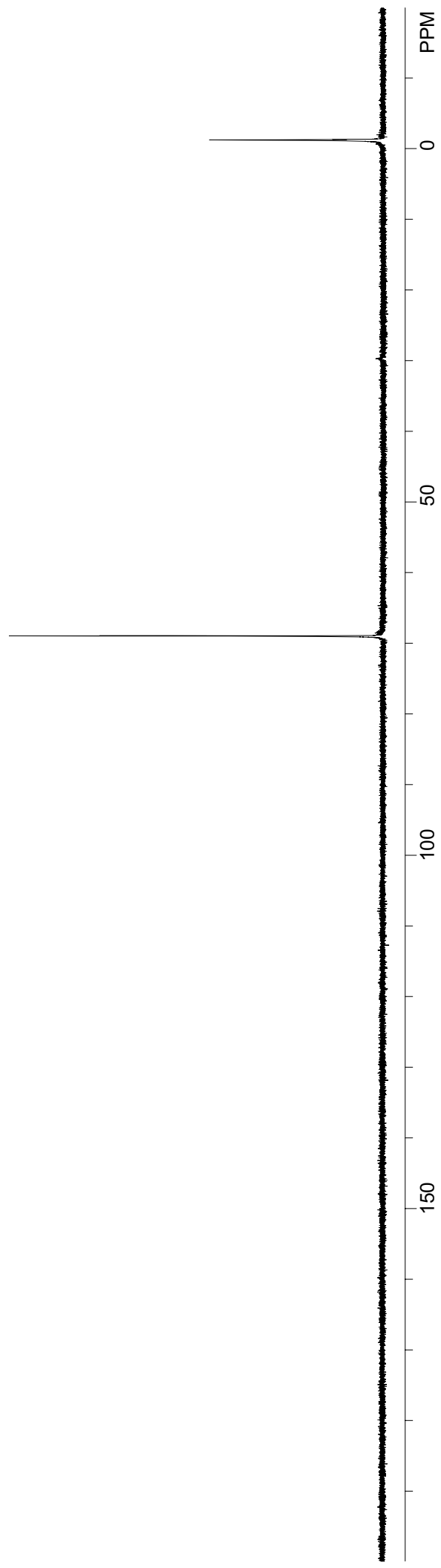


17a, 1H NMR, CDCl3

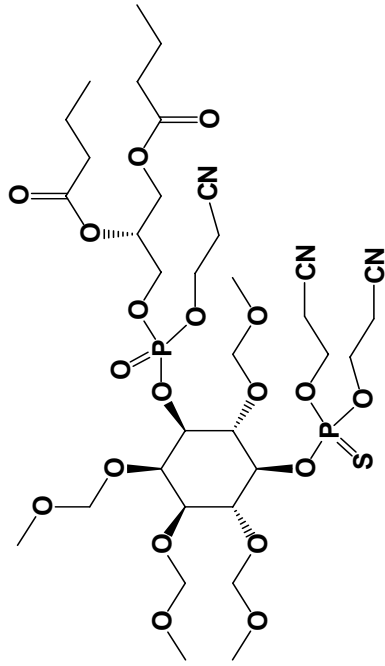




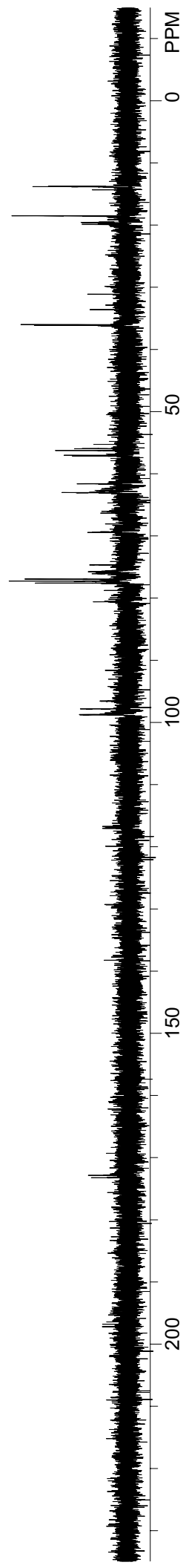
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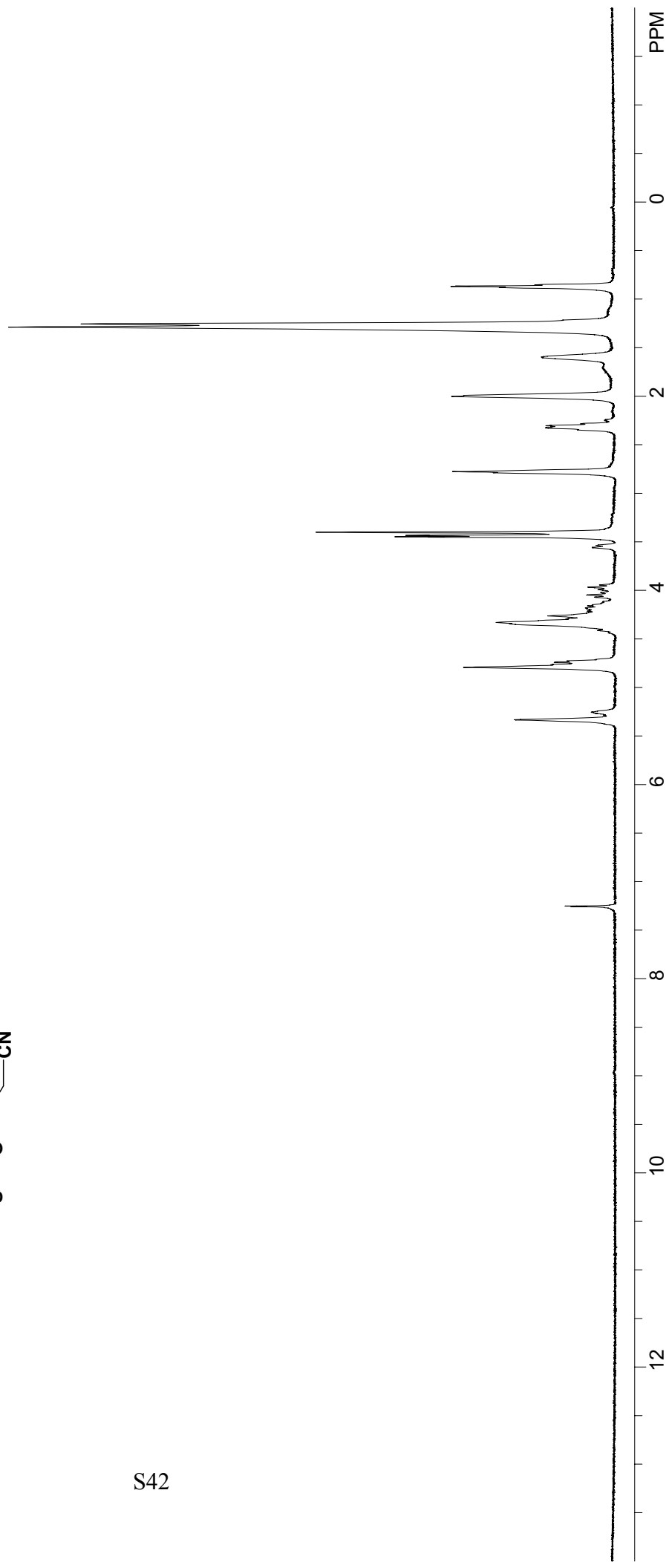
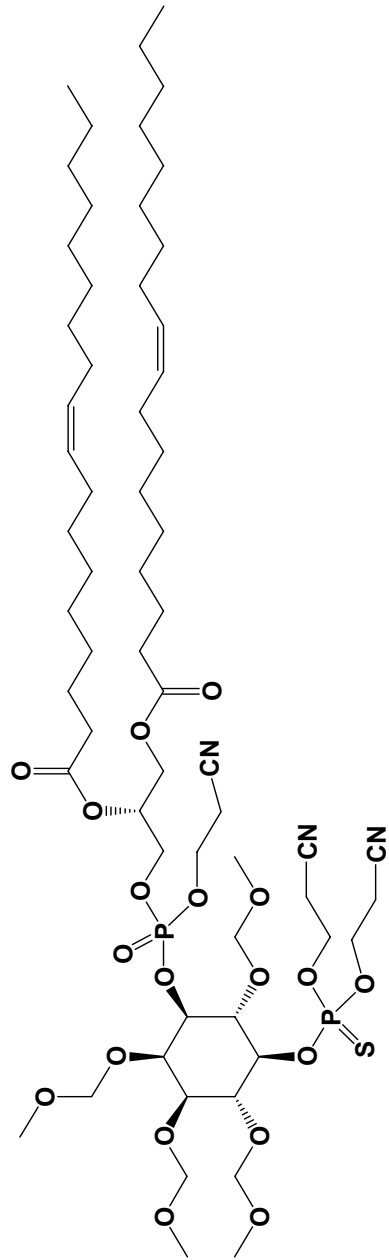


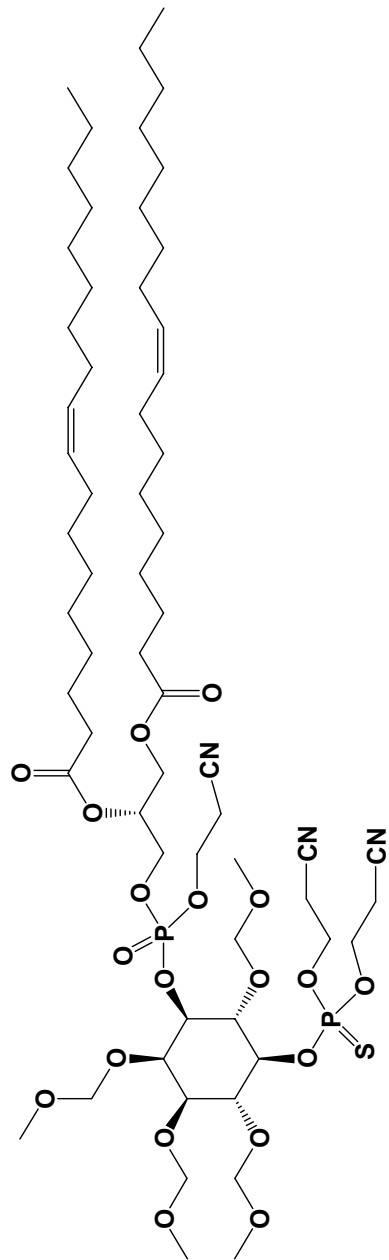




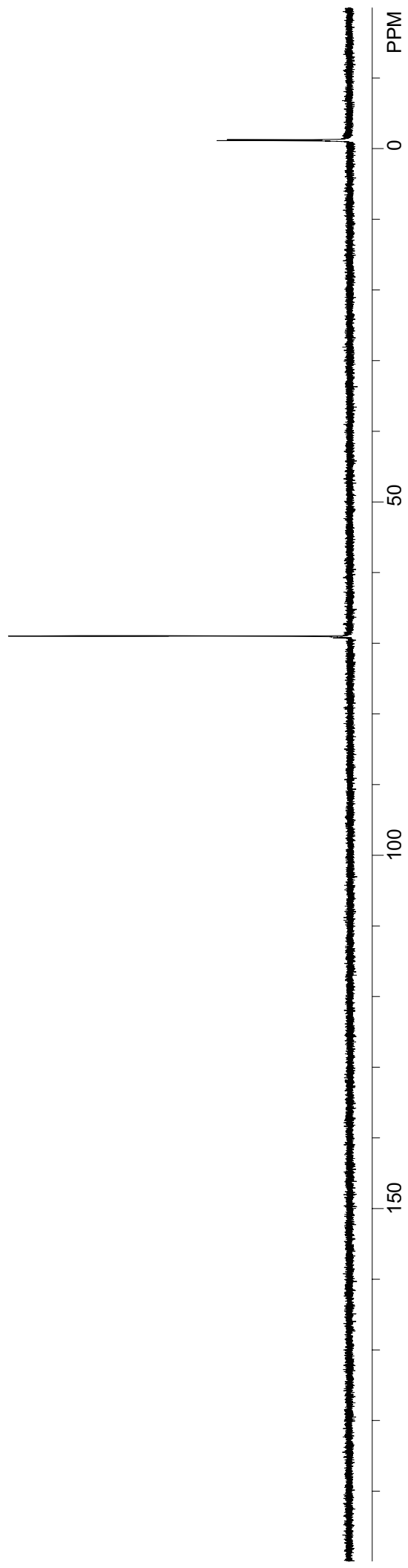
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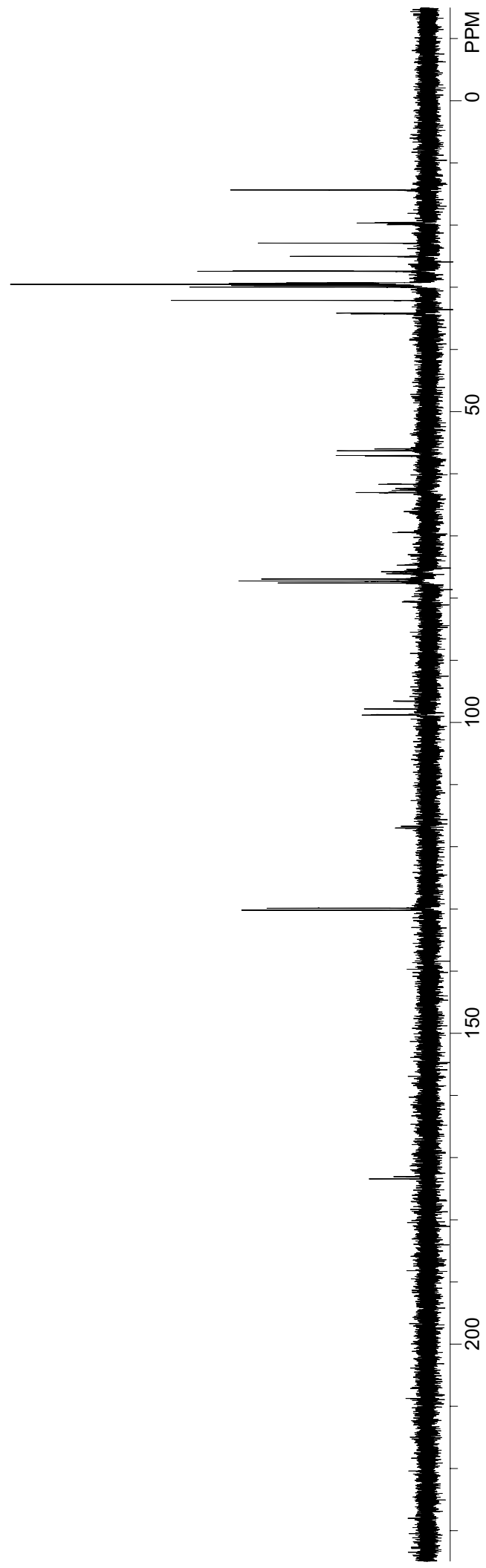
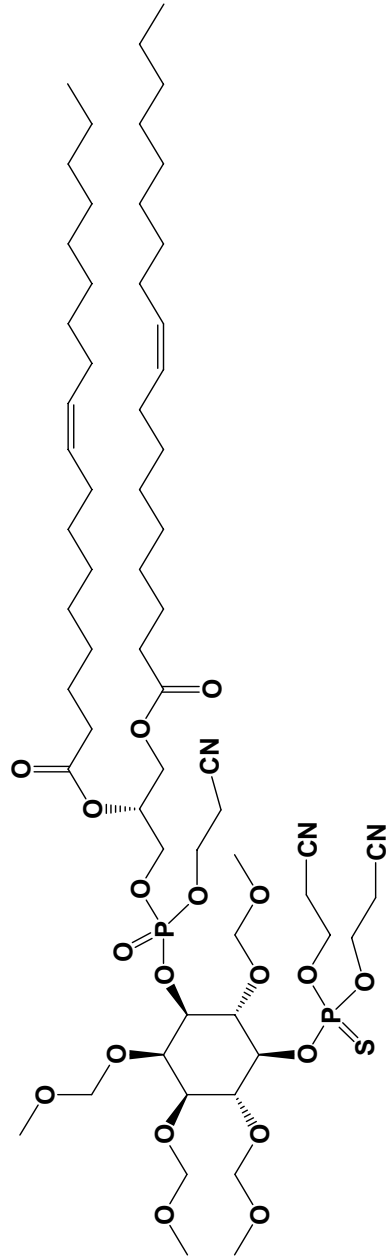


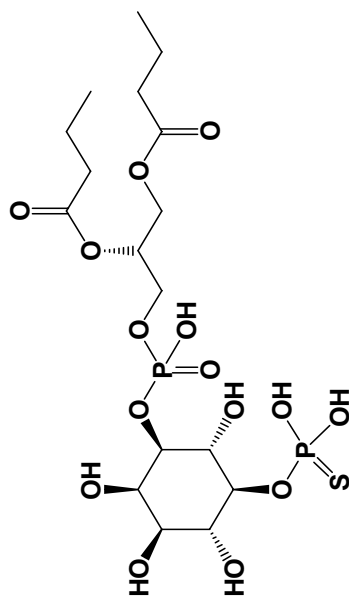




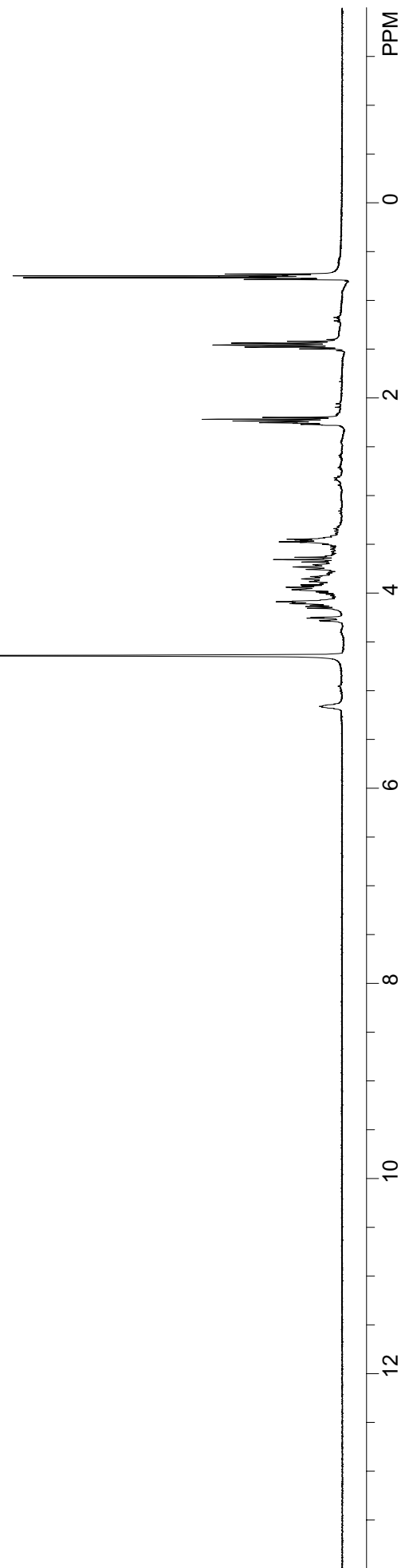
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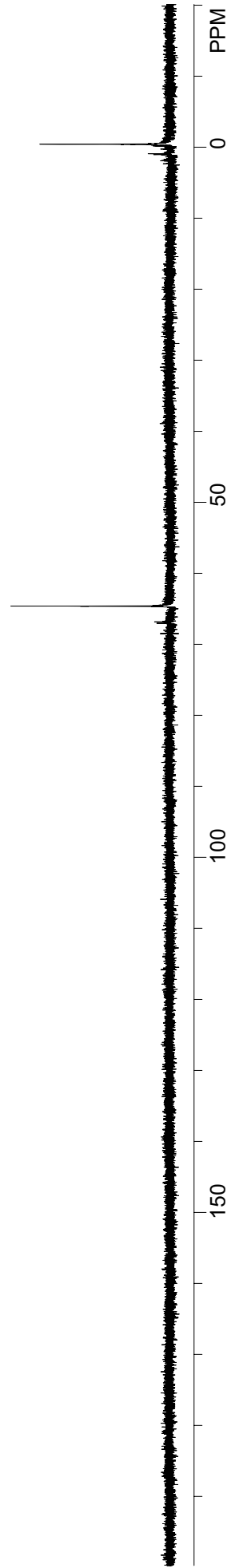
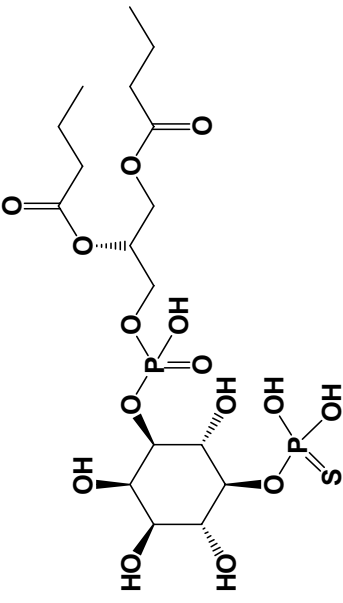




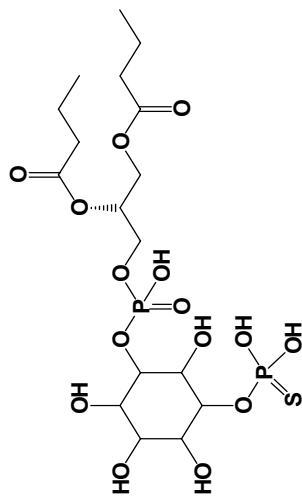


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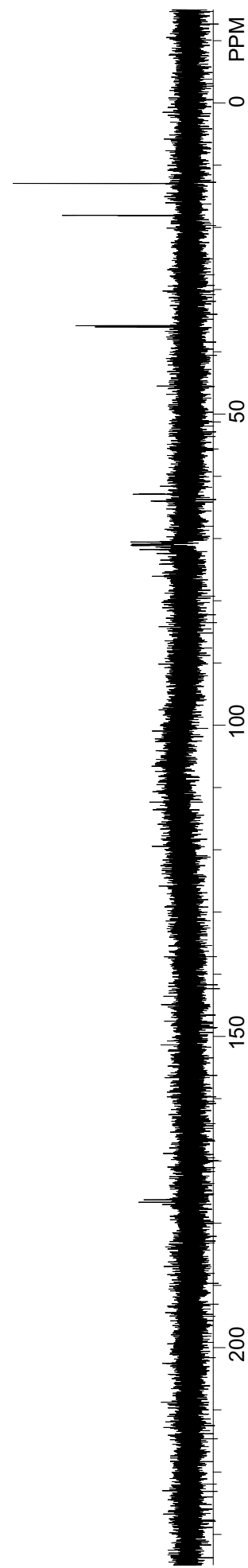




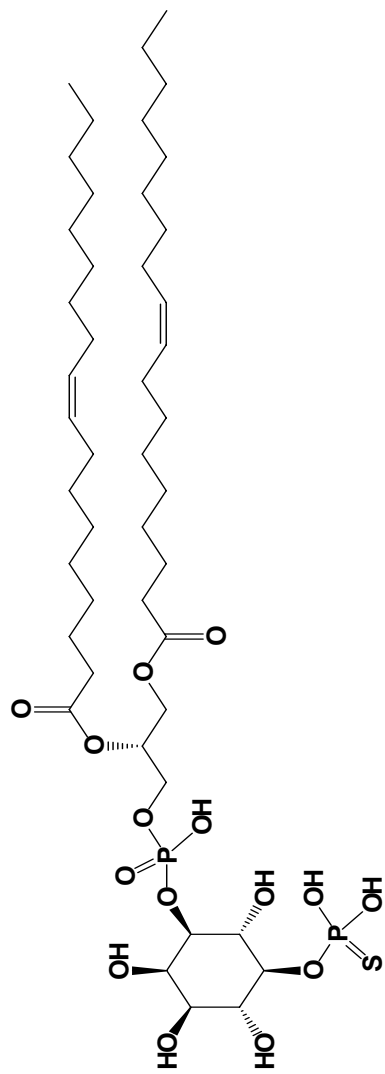
3, 13C NMR, D2O



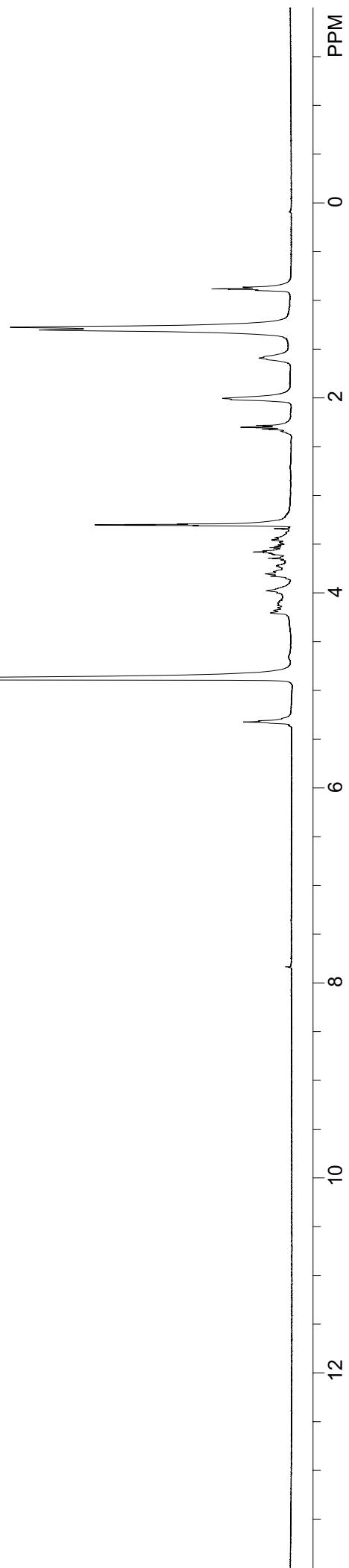
S47



4, 1H NMR, CD3OD

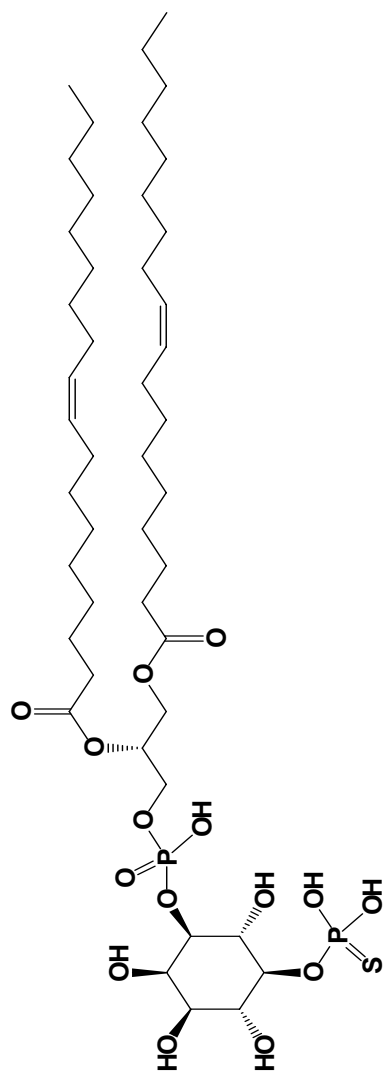


S48

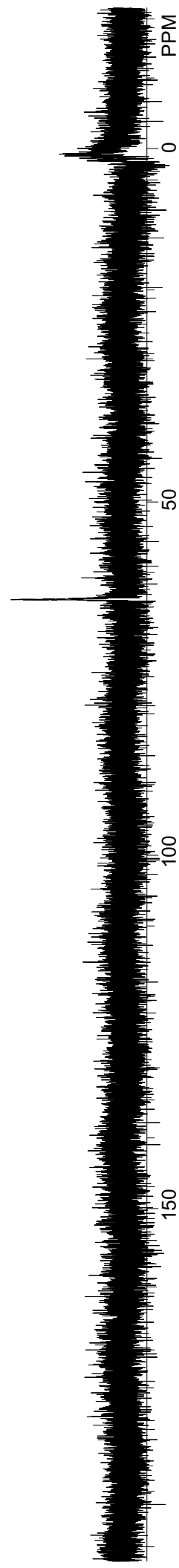


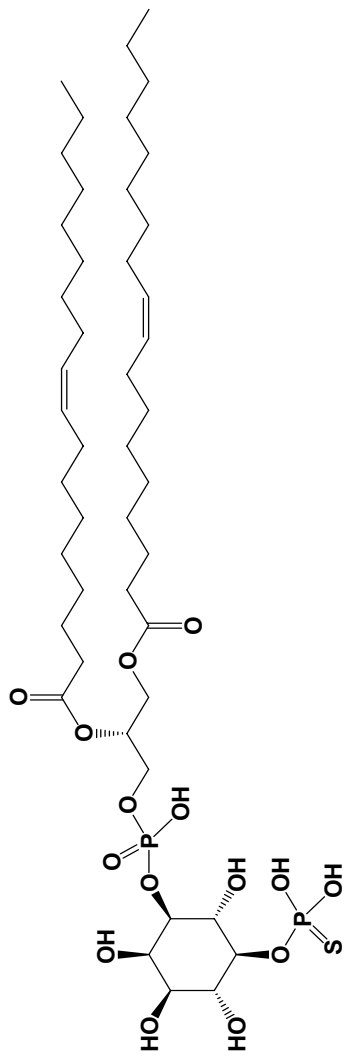


4, <sup>31</sup>P NMR, CD3OD



S49





S50

