

Supplementary Information

Synthesis and Evaluation of Fluoroalkyl Phosphonyl Analogs of 2-C-Methylethanol Phosphate as Substrates and Inhibitors of IspD from Human Pathogens

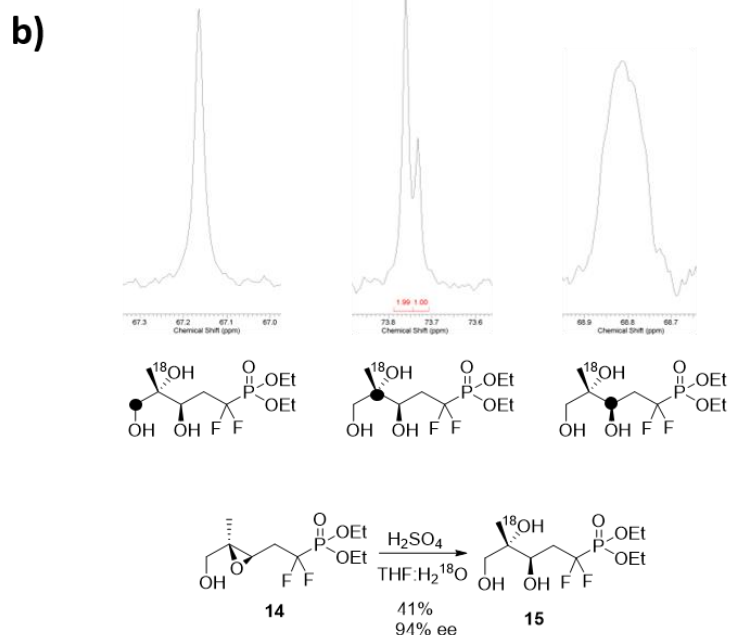
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S2

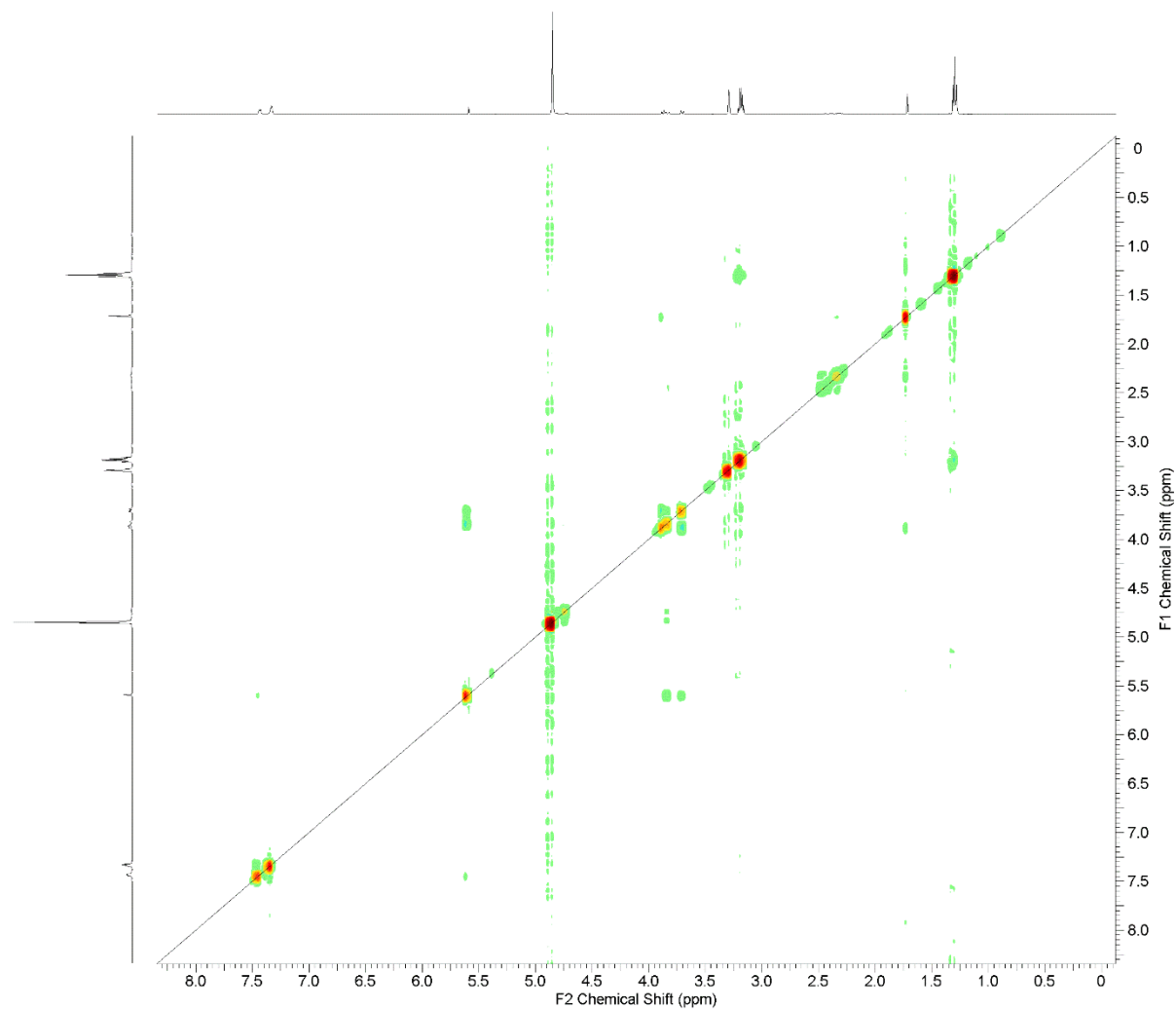
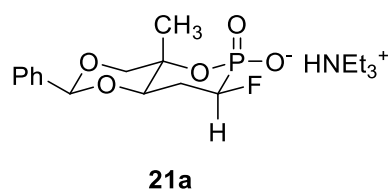


Figure S2. Full spectrum of the ^1H - ^1H NOE NMR experiment with 21a. The full ^1H NMR spectrum of 21a can be found on page S76.

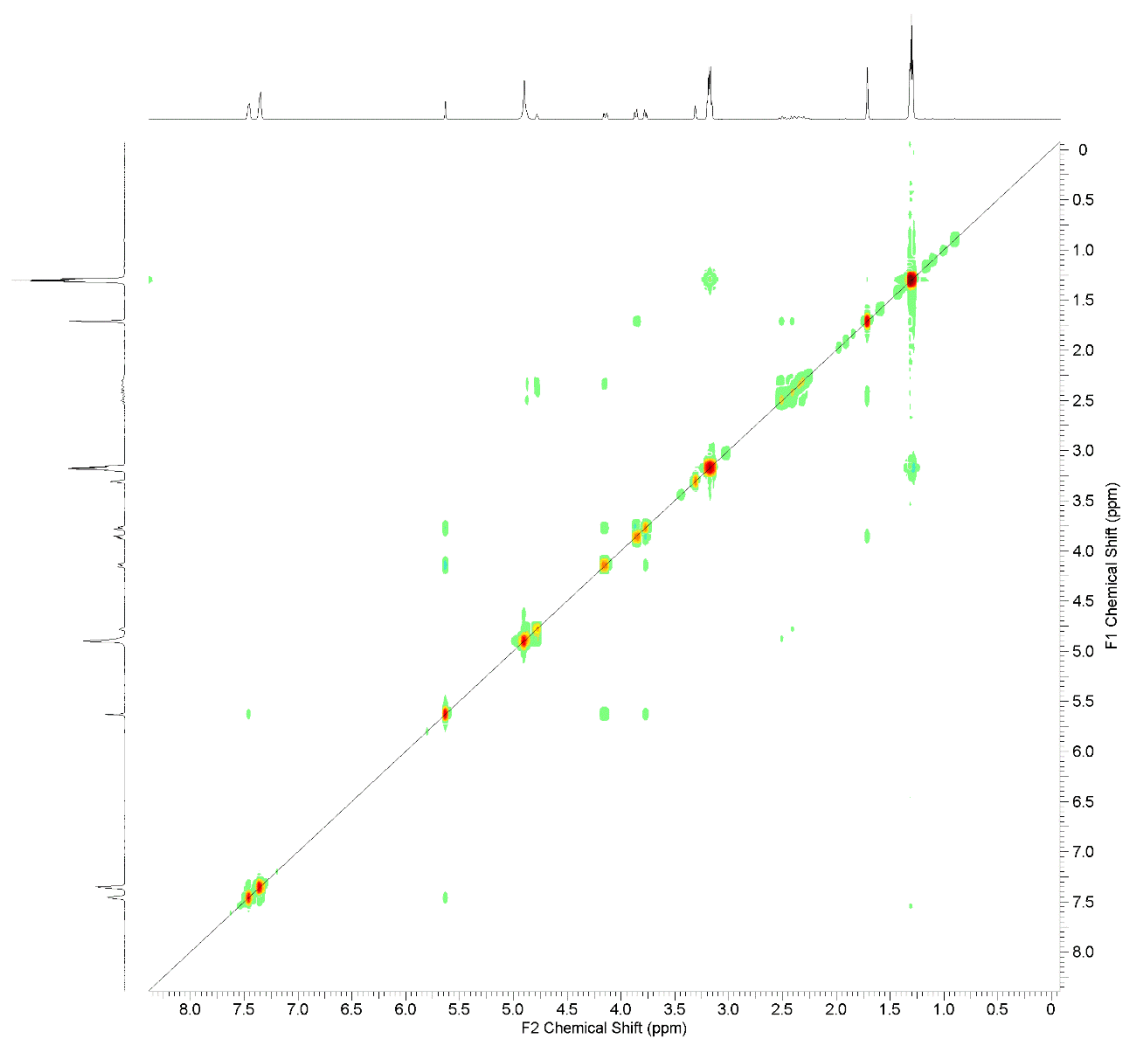
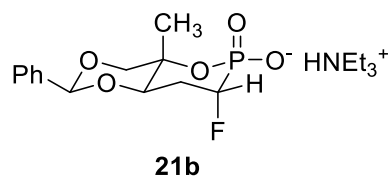


Figure S3. Full spectrum of the ^1H - ^1H NOE NMR experiment with 21b. The full ^1H NMR spectrum of 21b can be found on page S78.

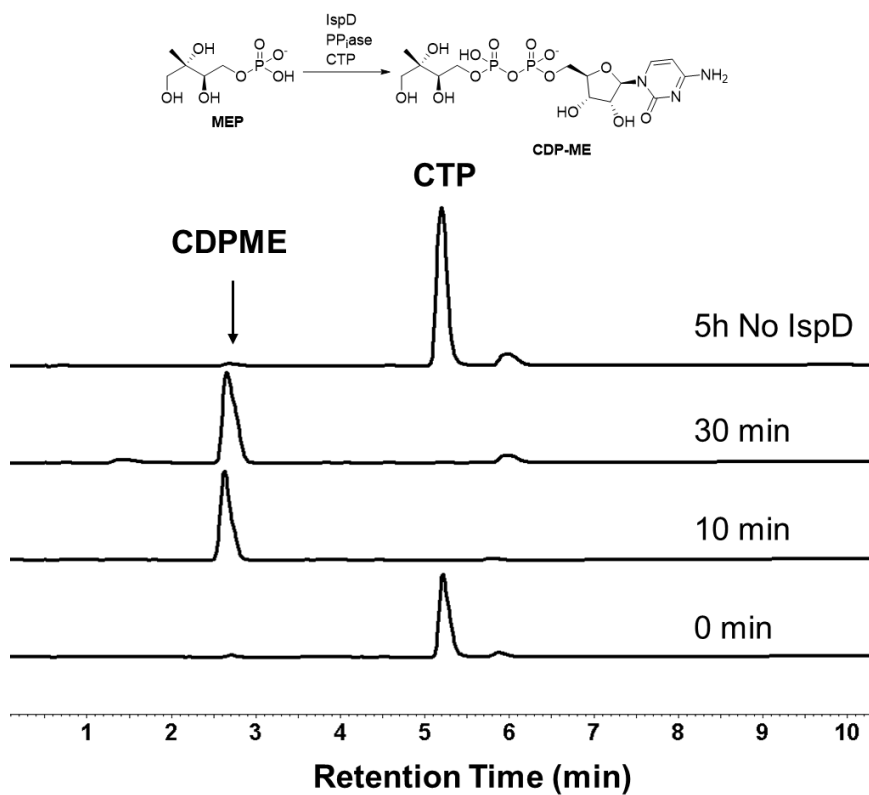


Figure S4. HPLC analysis of MEP turnover by *E. coli* IspD

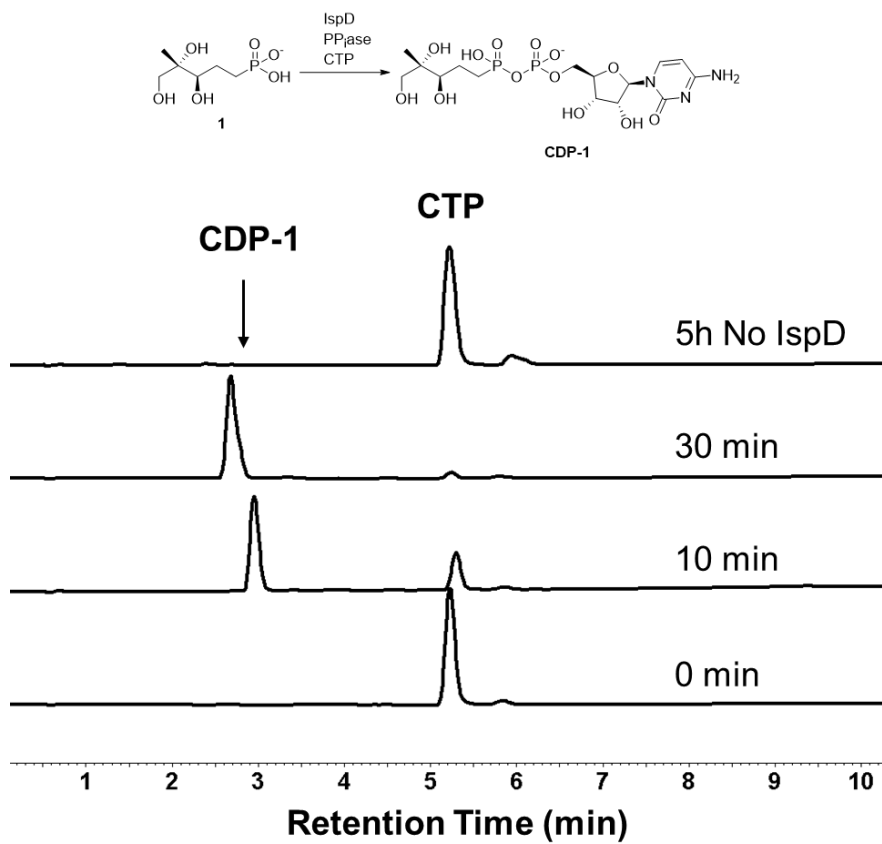


Figure S5. HPLC analysis of 1 turnover by *E. coli* IspD

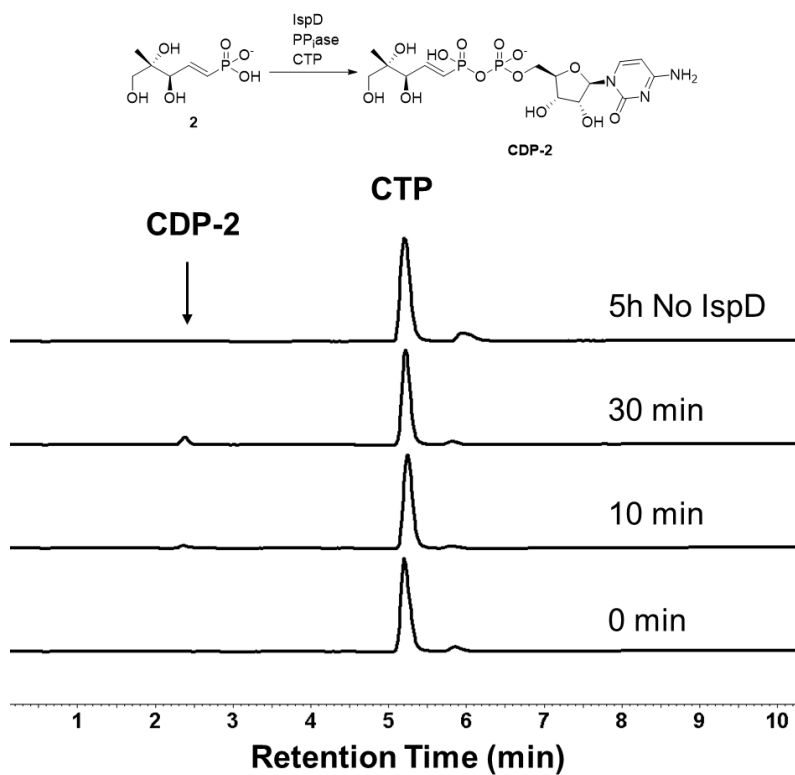


Figure S6. HPLC analysis of **2 turnover by *E. coli* IspD**

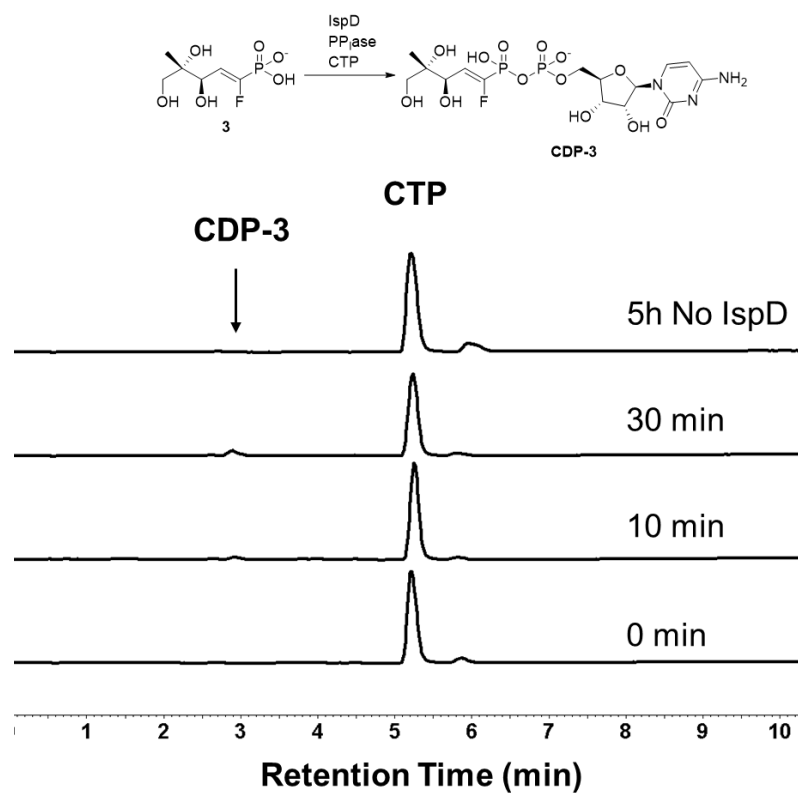


Figure S7. HPLC analysis of **3 turnover by *E. coli* IspD**

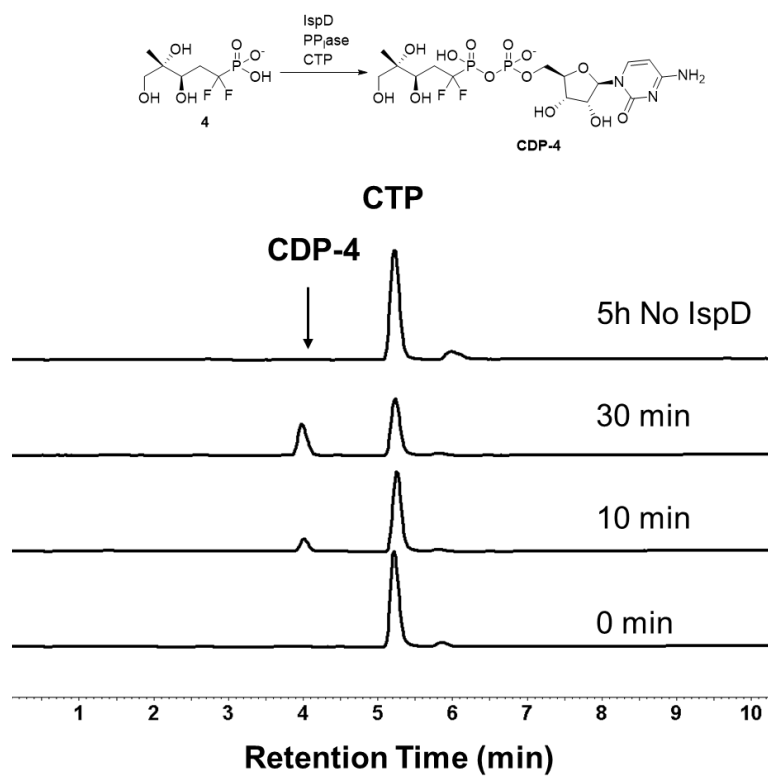


Figure S8. HPLC analysis of **4 turnover by *E. coli* IspD**

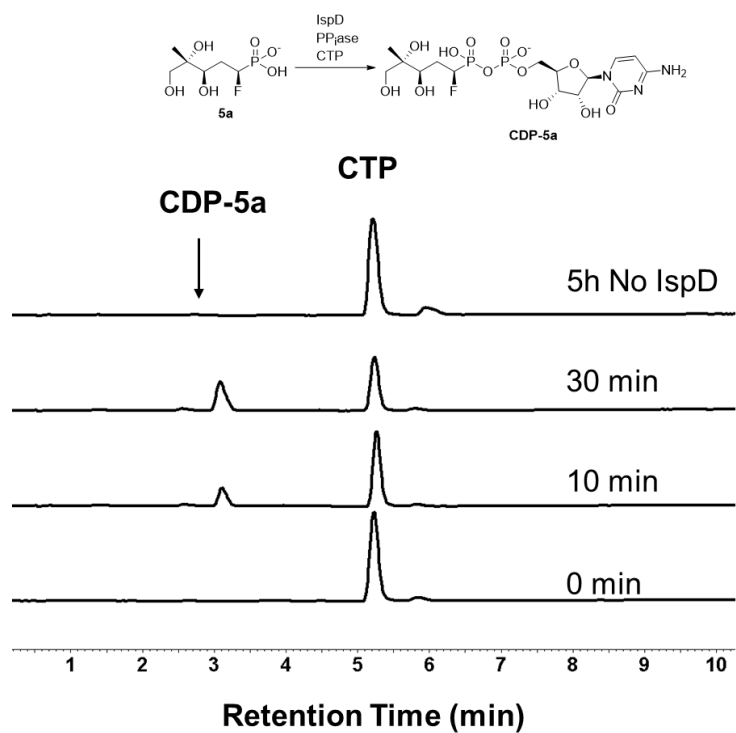


Figure S9. HPLC analysis of **5a turnover by *E. coli* IspD**

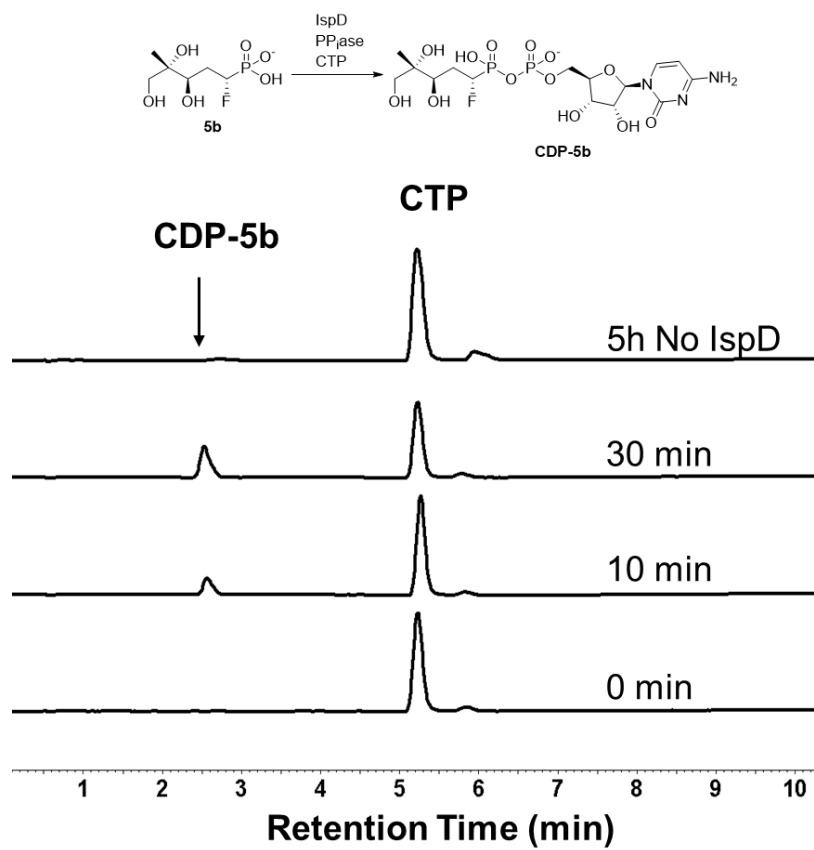


Figure S10. HPLC analysis of 5b turnover by *E. coli* IspD

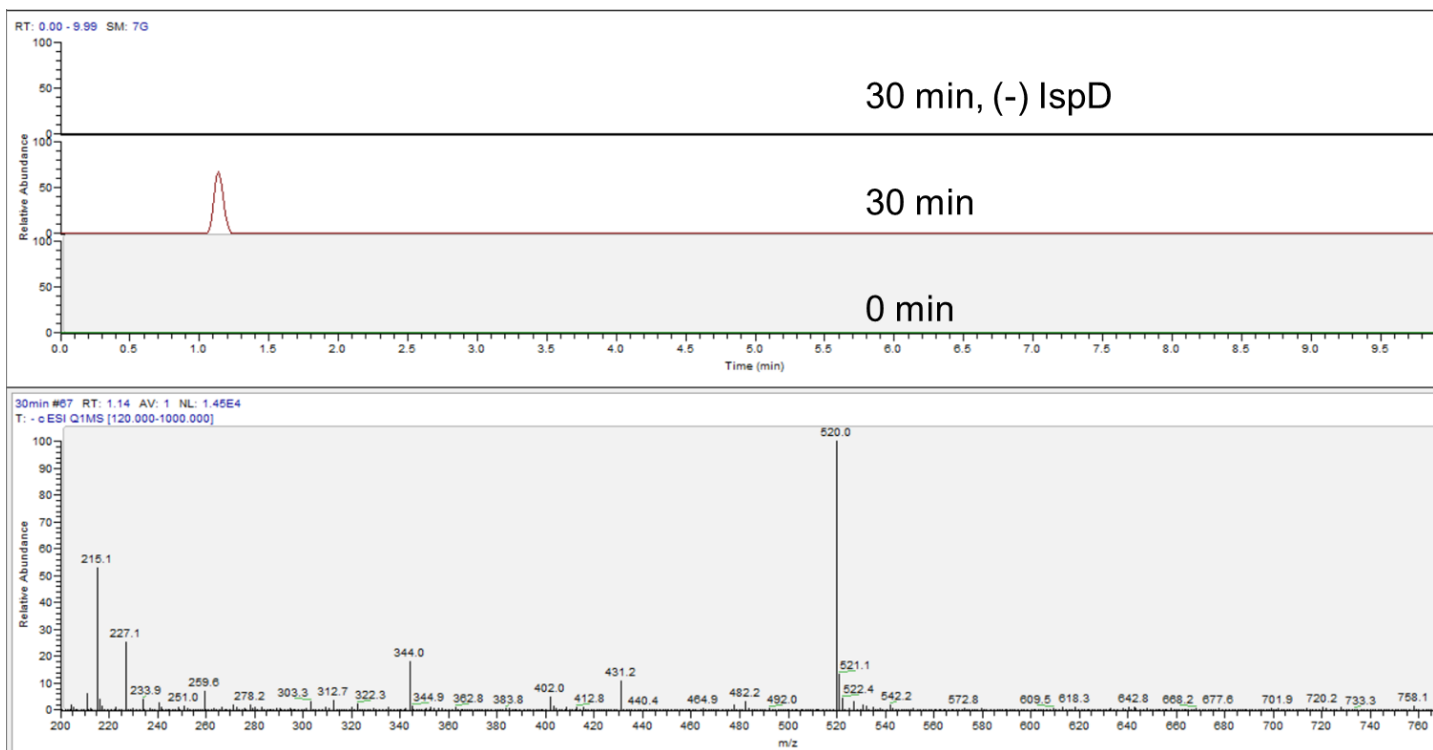
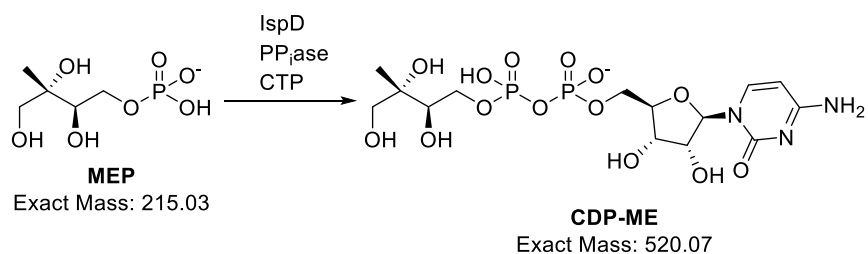


Figure S11. LC-MS analysis of MEP turnover. Chromatograms display the total ion count (TIC) for scans containing an ion with an m/z of 520 corresponding to **CDPME**. The mass spectrum is the scan at the center of the peak in the 30 min sample.

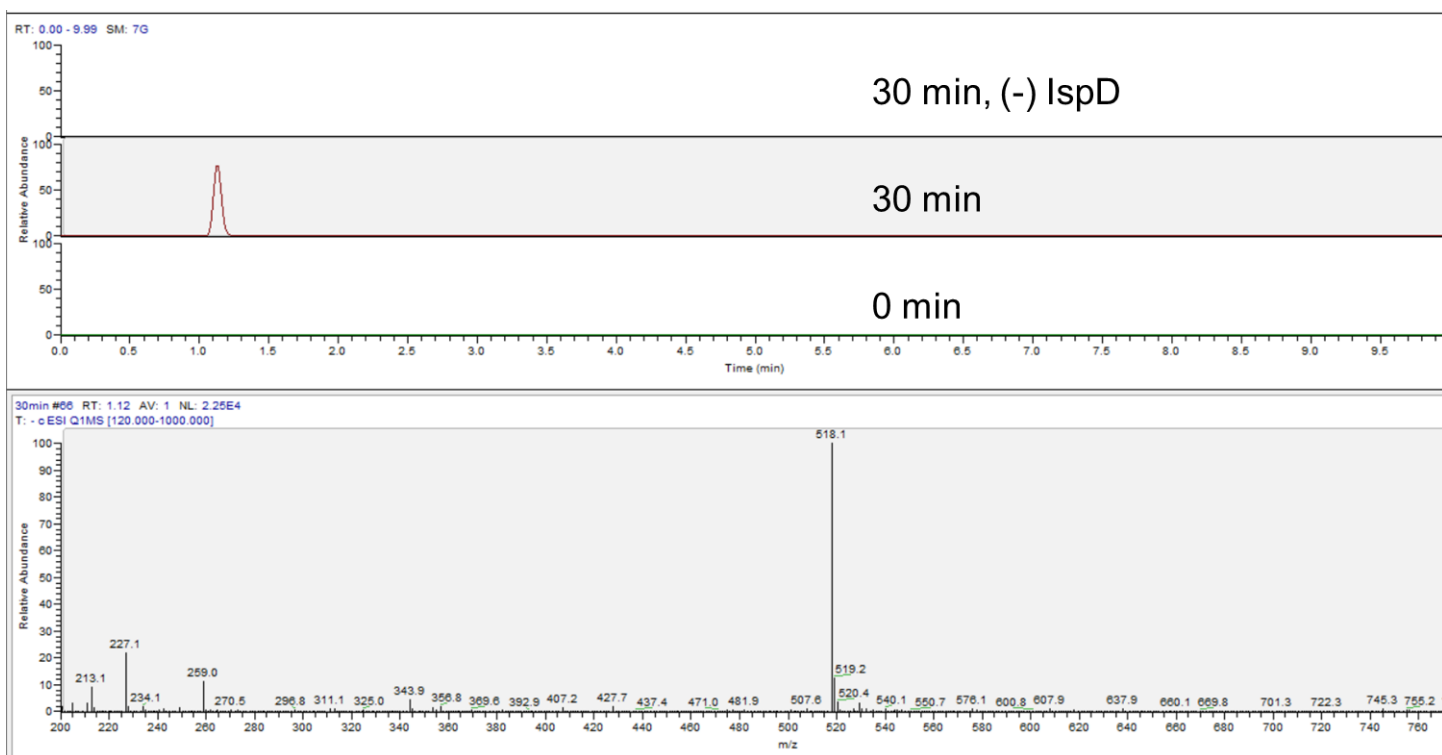
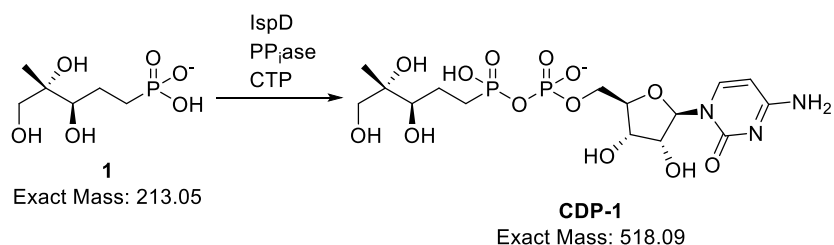


Figure S12. LC-MS analysis of 1 turnover. Chromatograms display the total ion count (TIC) for scans containing an ion with an m/z of 518 corresponding to the **CDP-1** product. The mass spectrum is the scan at the center of the peak in the 30 min sample.

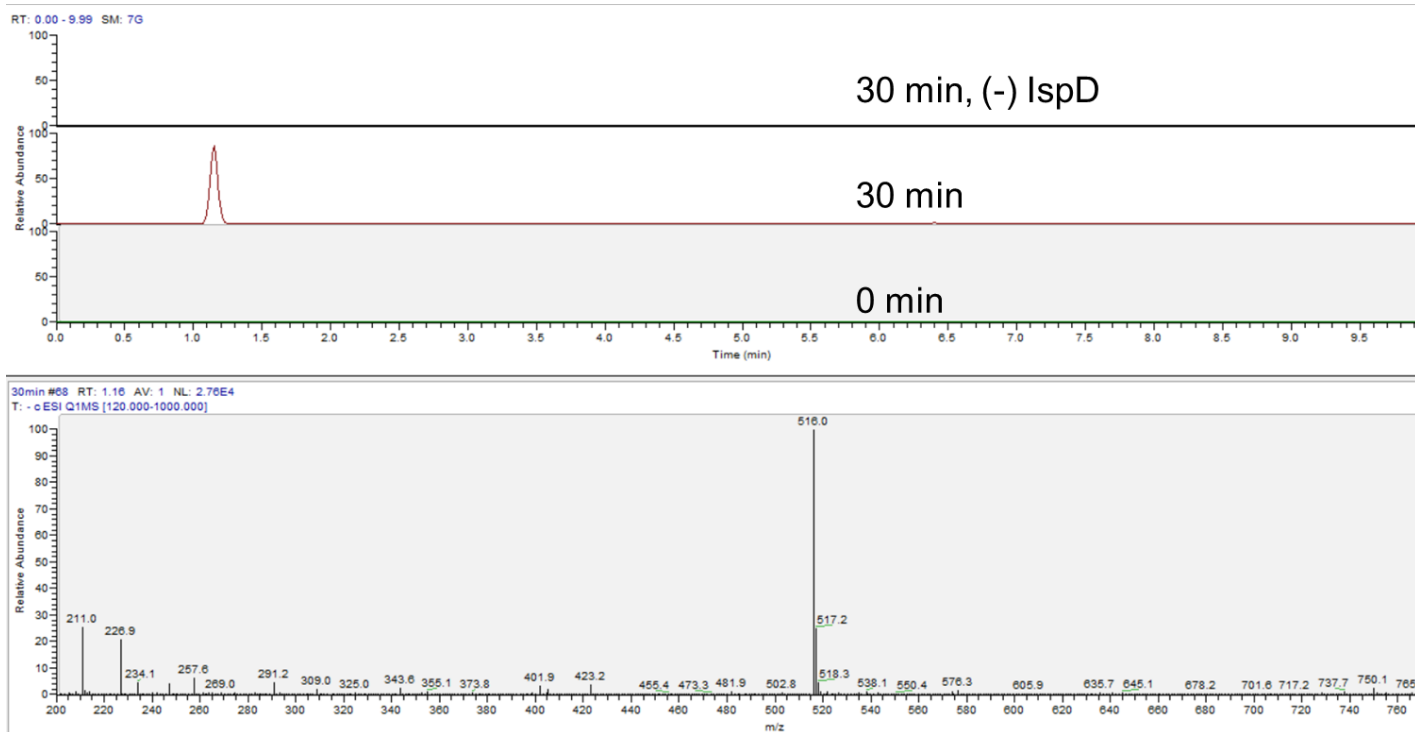
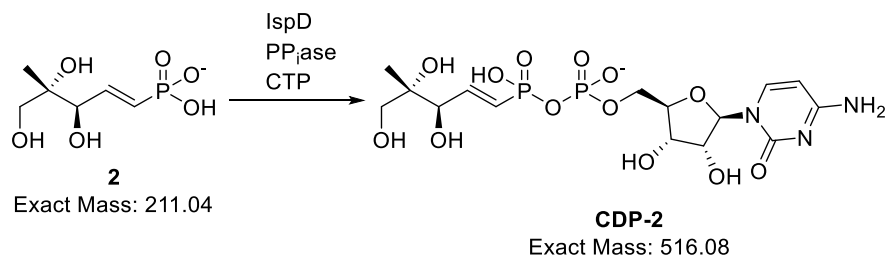


Figure S13. LC-MS analysis of 2 turnover. Chromatograms display the total ion count (TIC) for scans containing an ion with an m/z of 516 corresponding to the **CDP-2** product. The mass spectrum is the scan at the center of the peak in the 30 min sample.

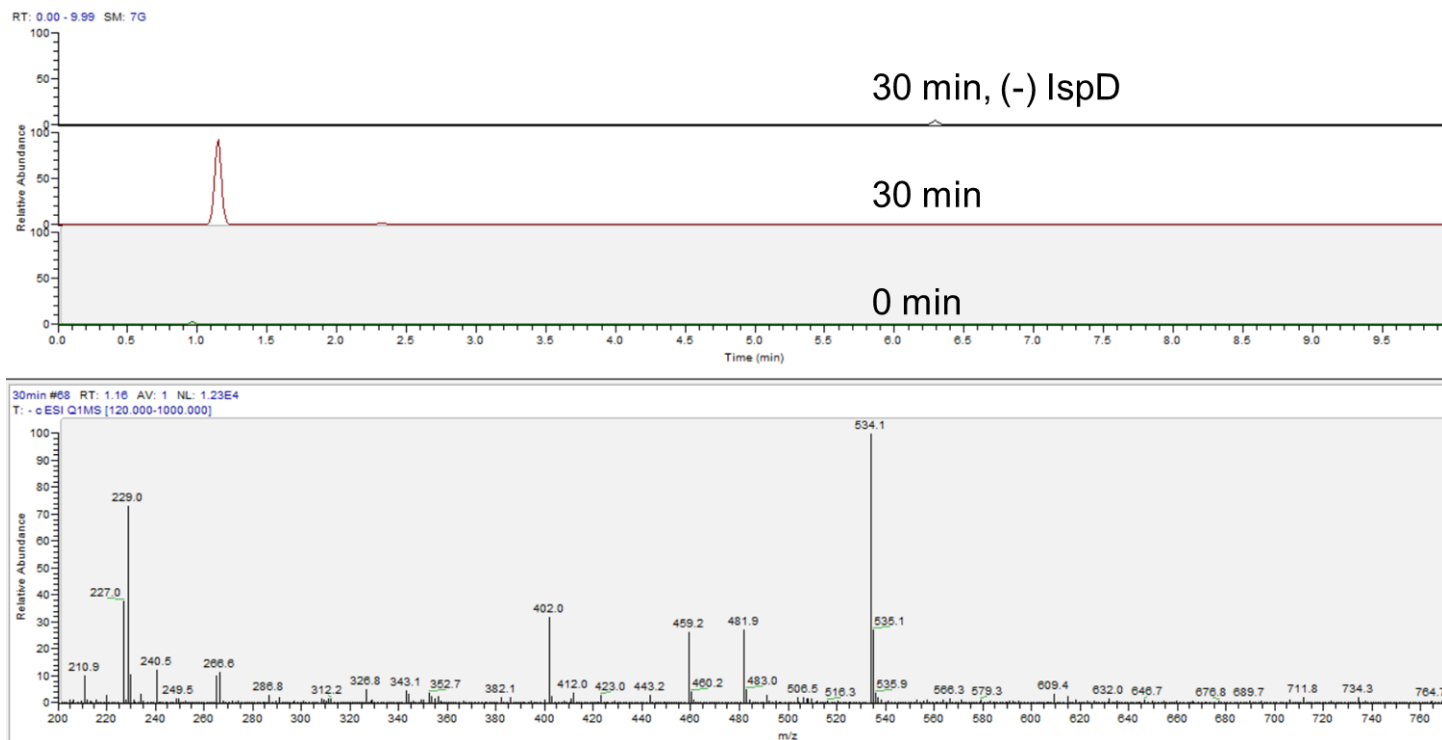
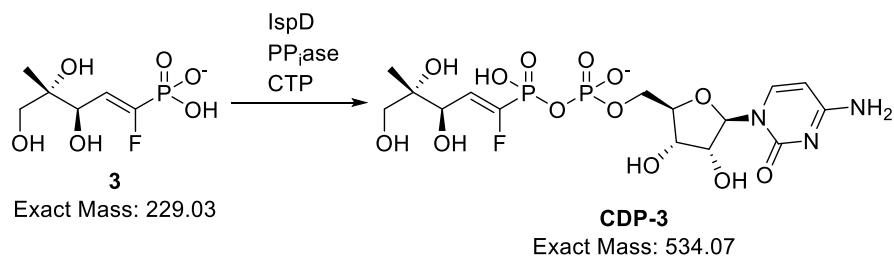


Figure S14. LC-MS analysis of 1 turnover. Chromatograms display the total ion count (TIC) for scans containing an ion with an m/z of 534 corresponding to the **CDP-3** product. The mass spectrum is the scan at the center of the peak in the 30 min sample.

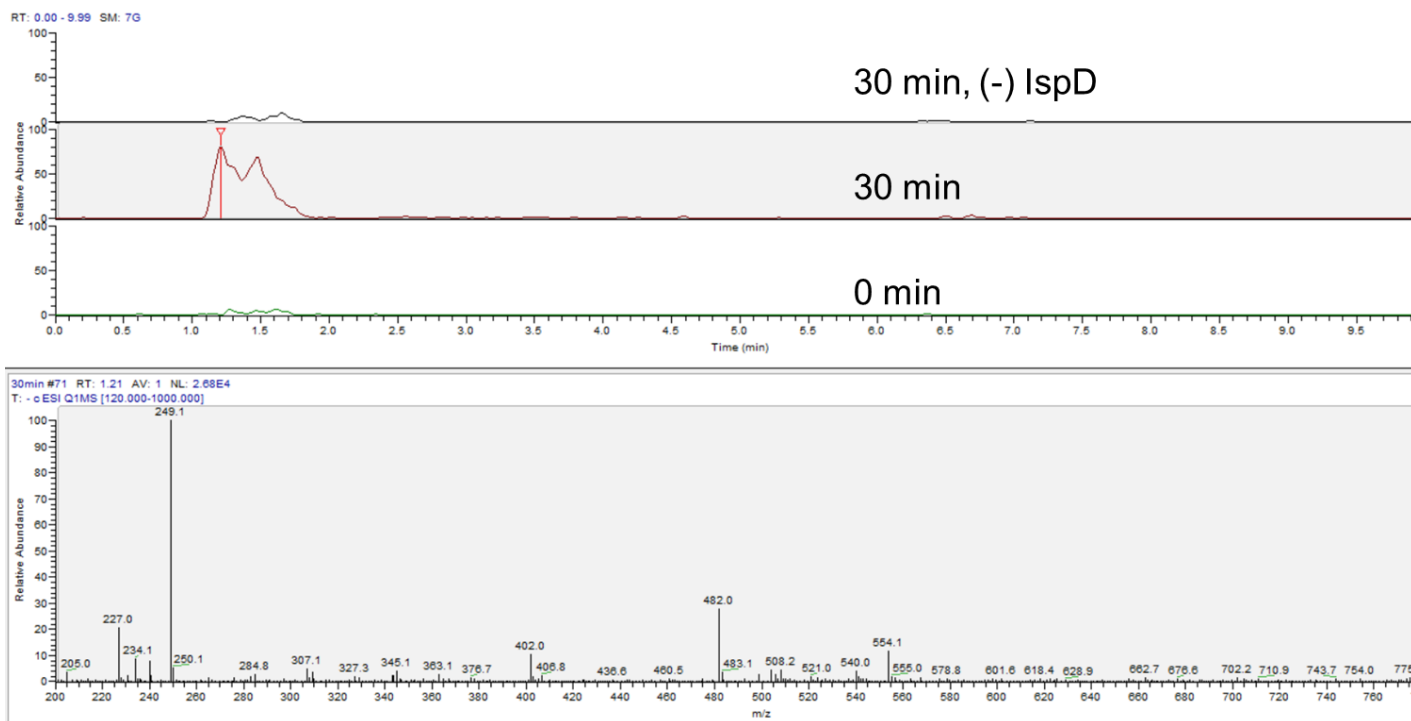
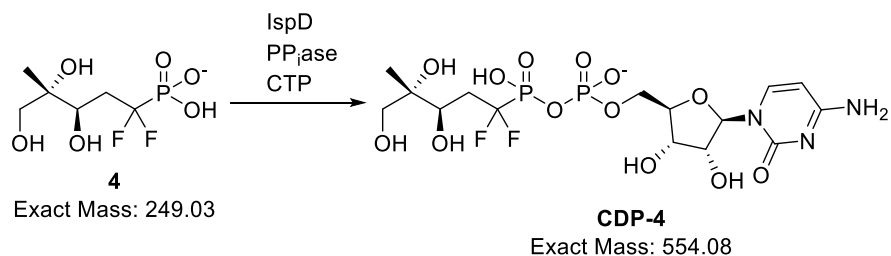


Figure S15. LC-MS analysis of 4 turnover. Chromatograms display the total ion count (TIC) for scans containing an ion with an m/z of 554 corresponding to the **CDP-4** product. The mass spectrum is the scan at 1.21 min (indicated by the red line) in the 30 min sample.

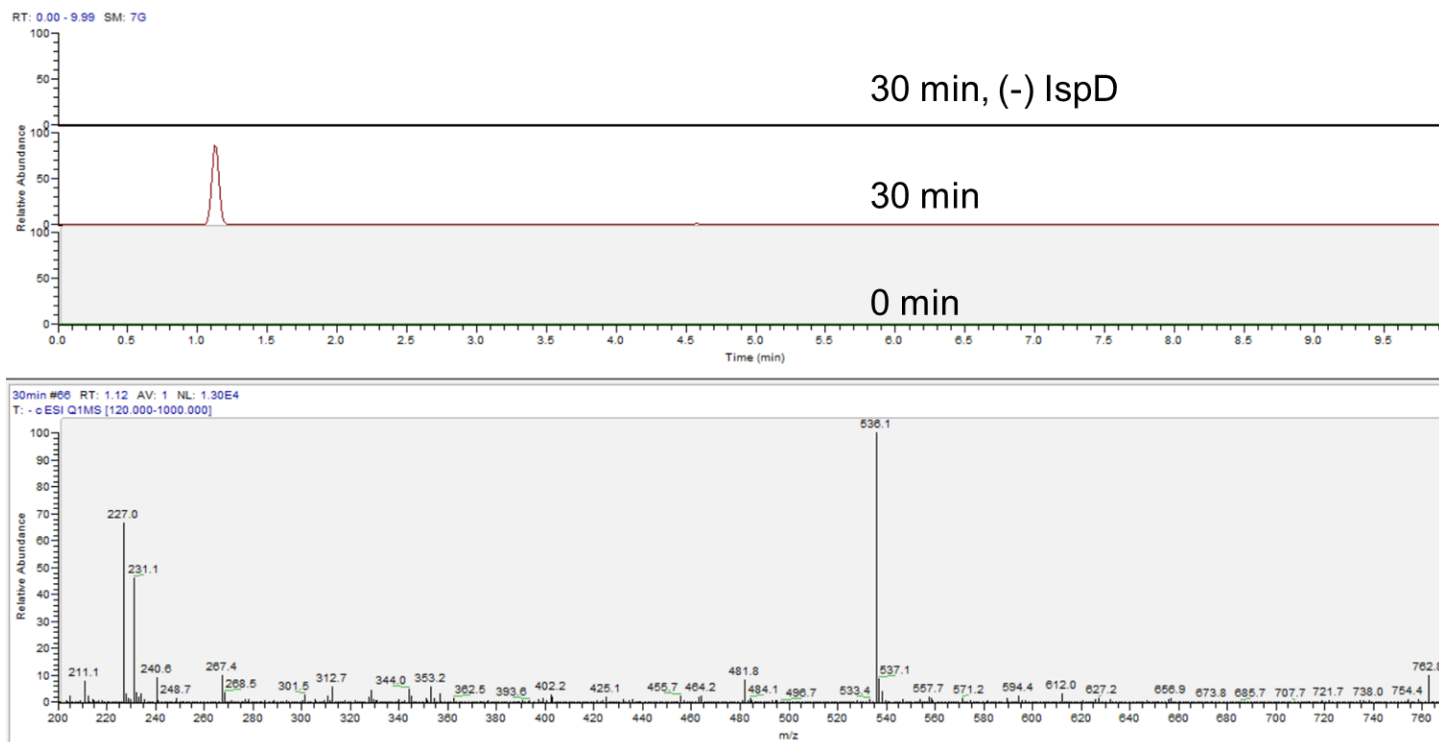
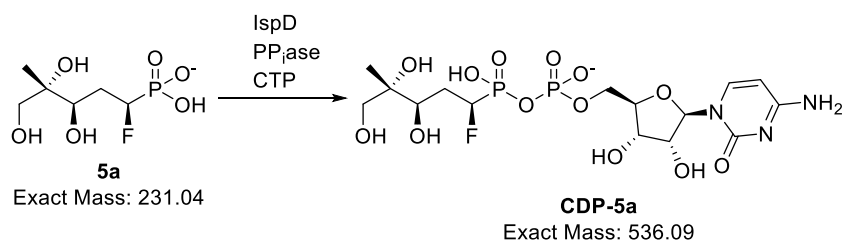


Figure S16. LC-MS analysis of 5a turnover. Chromatograms display the total ion count (TIC) for scans containing an ion with an m/z of 536 corresponding to the **CDP-5a** product. The mass spectrum is the scan at the center of the peak in the 30 min sample.

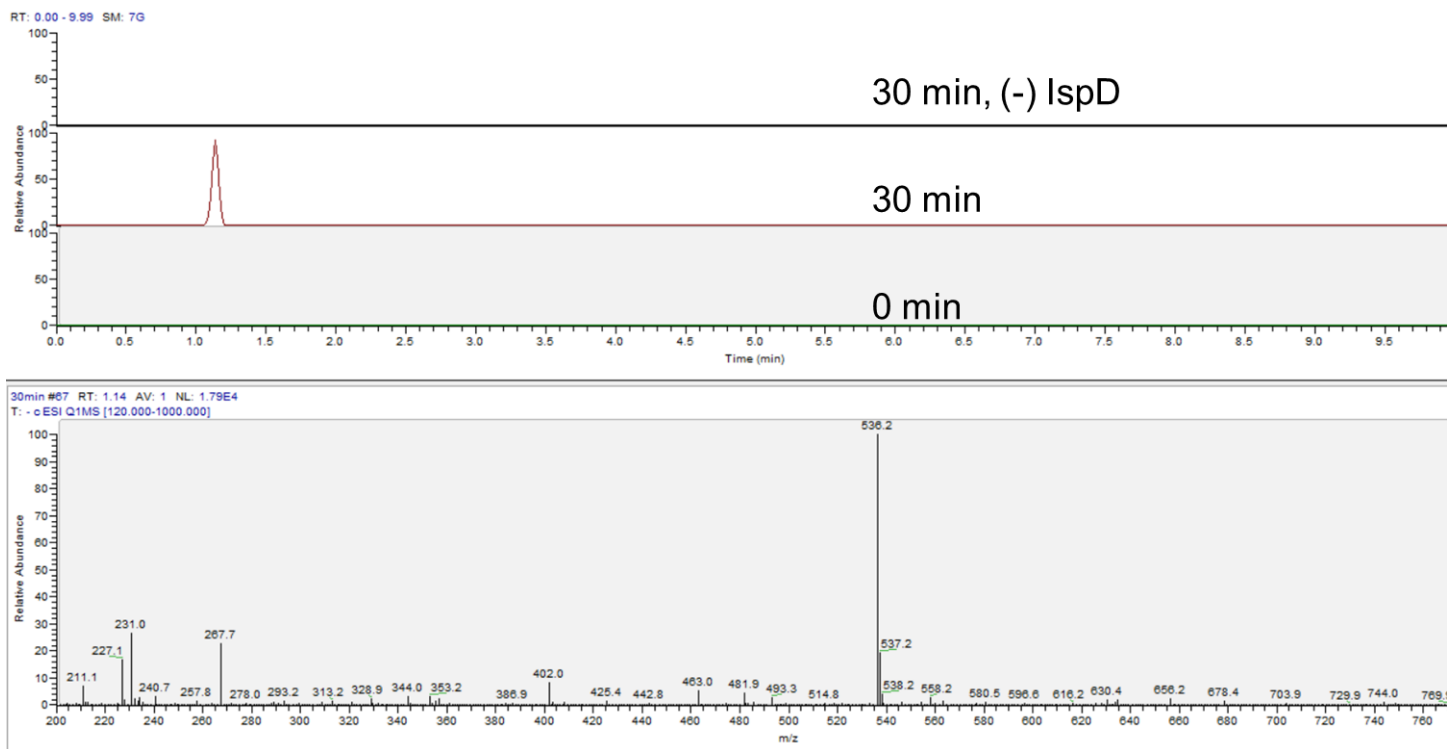
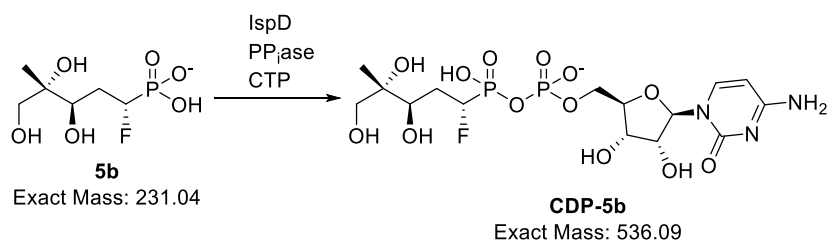


Figure S17. LC-MS analysis of 5a turnover. Chromatograms display the total ion count (TIC) for scans containing an ion with an m/z of 536 corresponding to the **CDP-5b** product. The mass spectrum is the scan at the center of the peak in the 30 min sample.

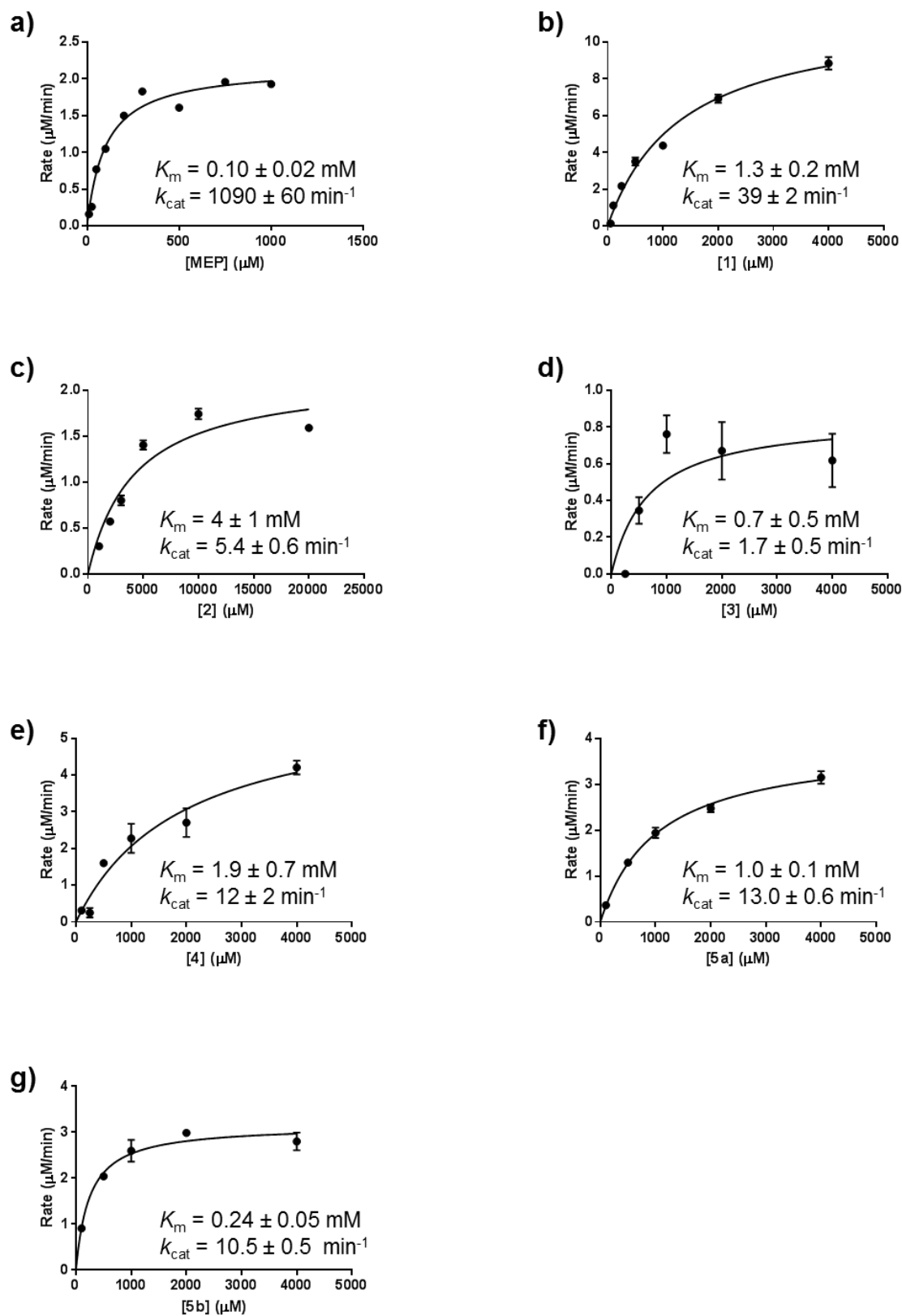


Figure S18. Michaelis-Menten plots for MEP and analogs 1 – 5a,b with *E. coli* IspD.

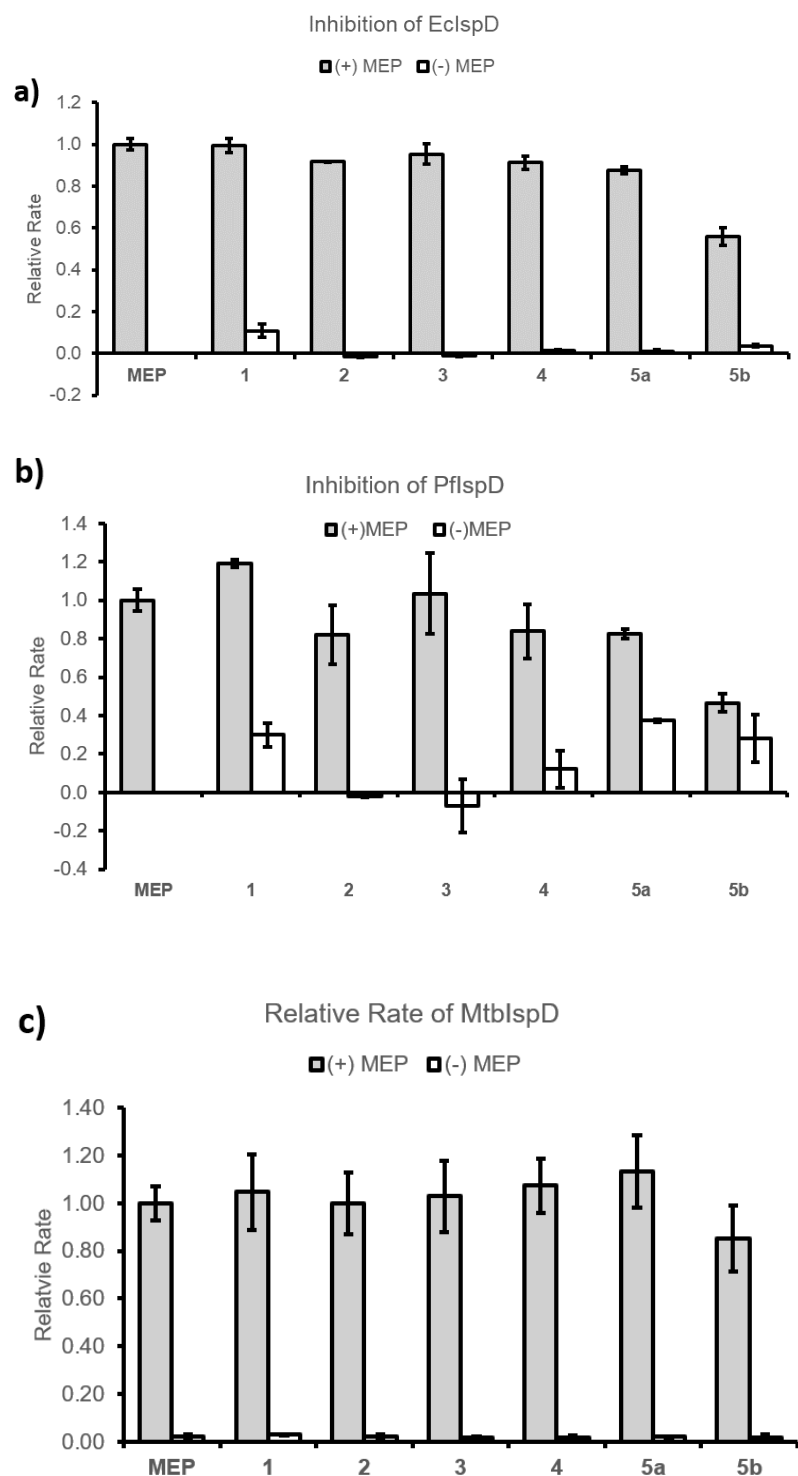


Figure S19. Evaluation of inhibitory activity of analogs 1 – 5a,b against a) *E. coli*, b) *P. falciparum*, and c) *M. tuberculosis* IspD. The white (-) MEP bars indicate control experiments where MEP was excluded from the reaction mixture in order to determine if the observed rates were being significantly influenced by analog turnover. In the *P. falciparum* experiments testing analogs **1**, **5a** and **5b**, analog turnover appeared to be non-negligible prompting the evaluation of these analogs as substrates (see below, figure S27).

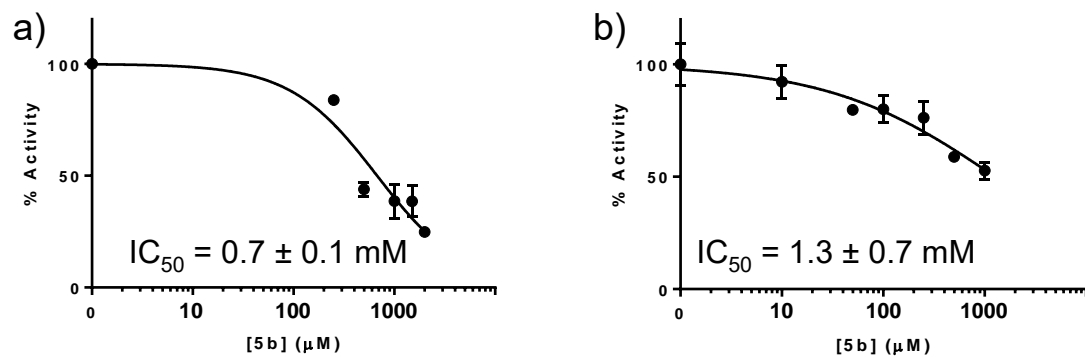
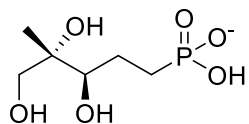


Figure S20. IC₅₀ plots for analog 5b against a) *E. coli* IspD and b) *P. falciparum* IspD.



1

Exact Mass: 213.0533

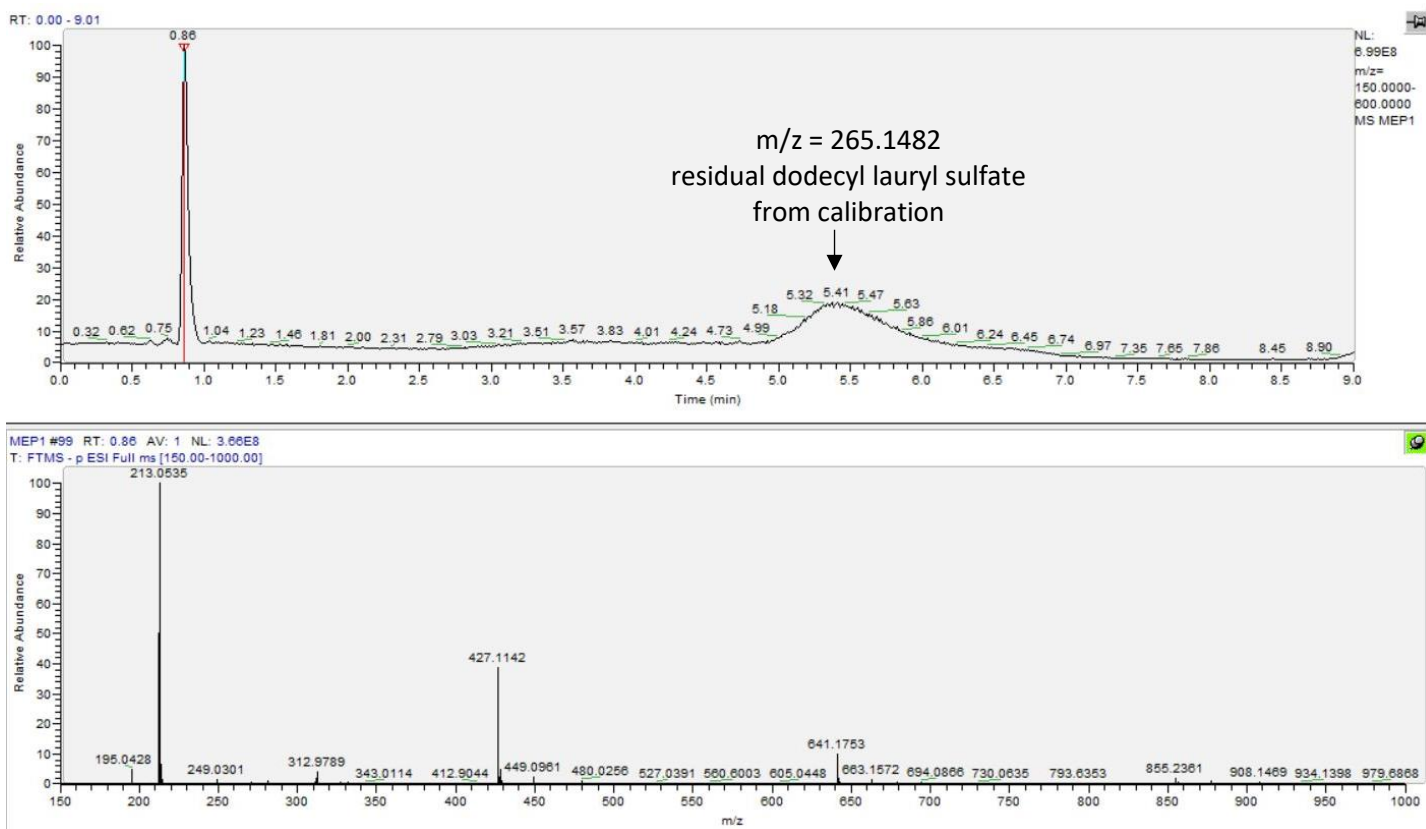
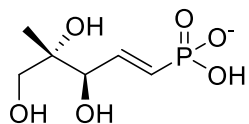


Figure S21. LC-HRMS analysis of analog 1 demonstrating >95% purity.



2

Exact Mass: 211.0377

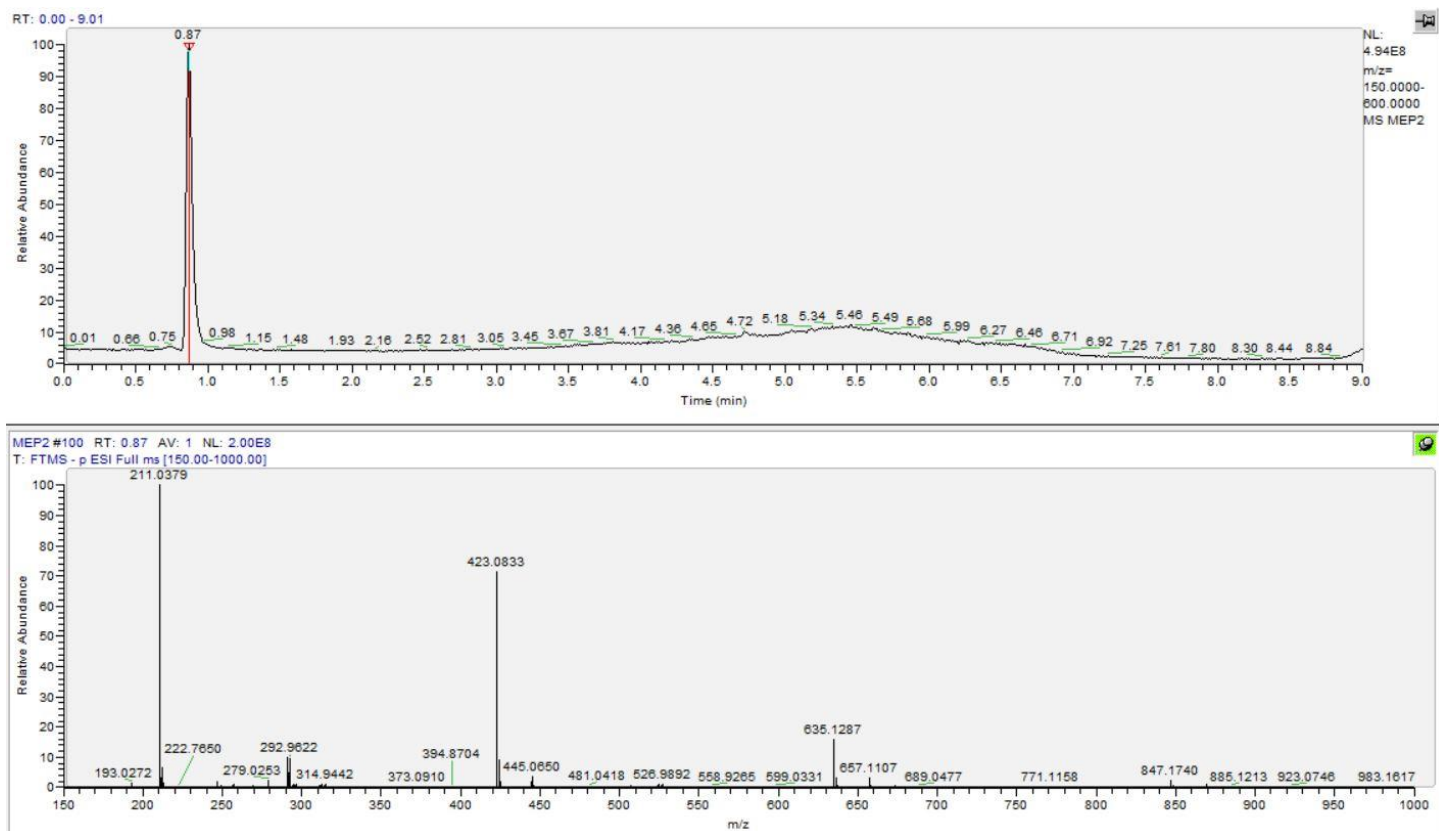
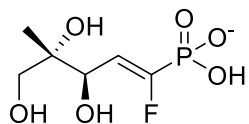


Figure S22. LC-HRMS analysis of analog 2 demonstrating >95% purity.



3

Exact Mass: 229.0283

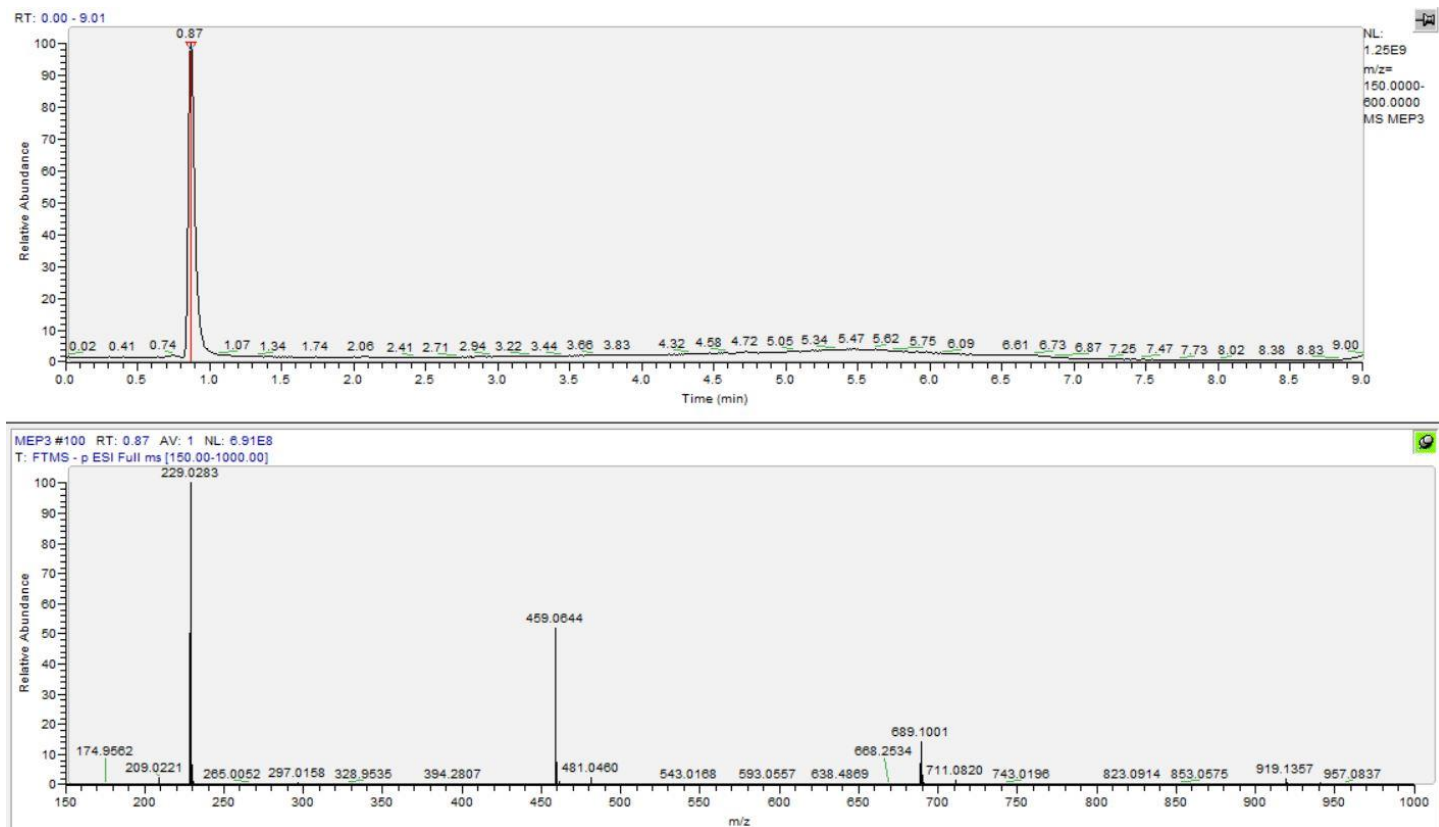
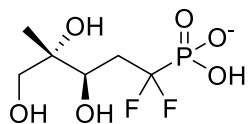


Figure S23. LC-HRMS analysis of analog 3 demonstrating >95% purity.



4

Exact Mass: 249.0345

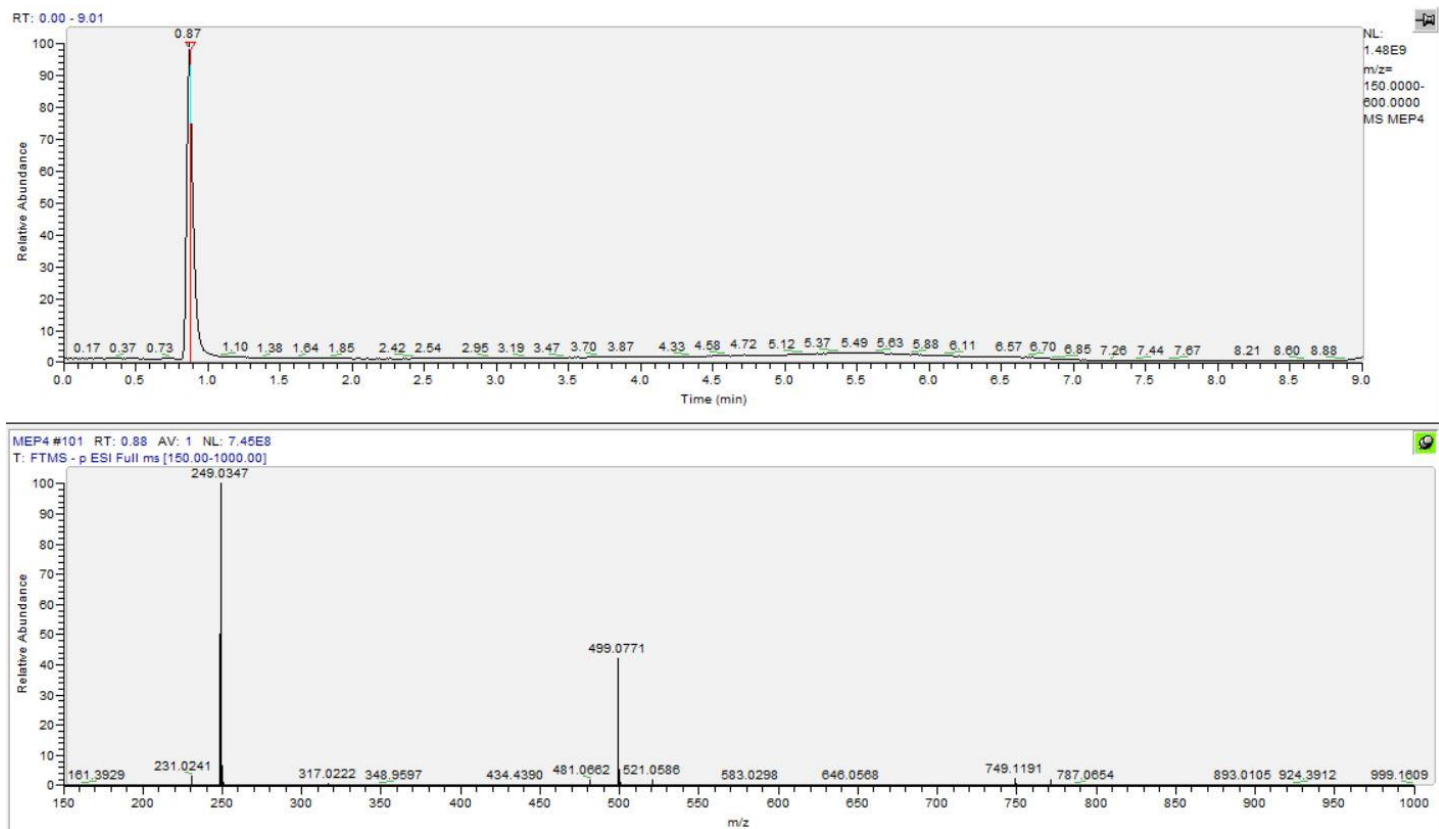
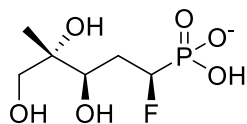


Figure S24. LC-HRMS analysis of analog 4 demonstrating >95% purity.



5a

Exact Mass: 231.0439

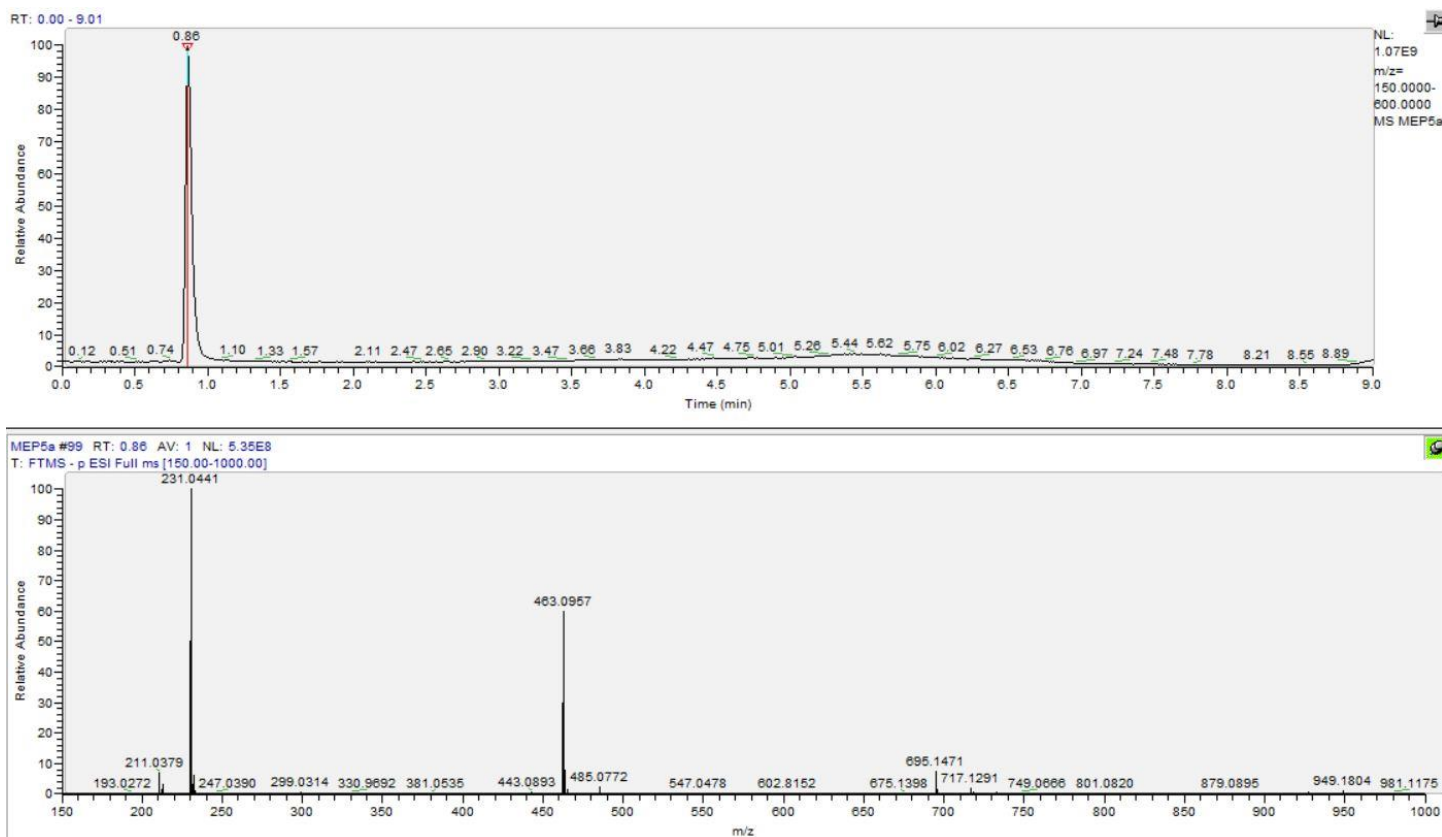
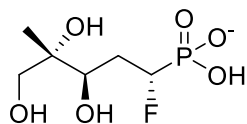


Figure S25. LC-HRMS analysis of analog 5a demonstrating >95% purity. The mass observed at 211.0379 corresponds to the fluoride elimination product, vinyl phosphonate **2**. This product appears to be an artifact of the ionization process as there are no observable peaks corresponding to **2** in the ^1H NMR spectrum (below).



5b

Exact Mass: 231.0439

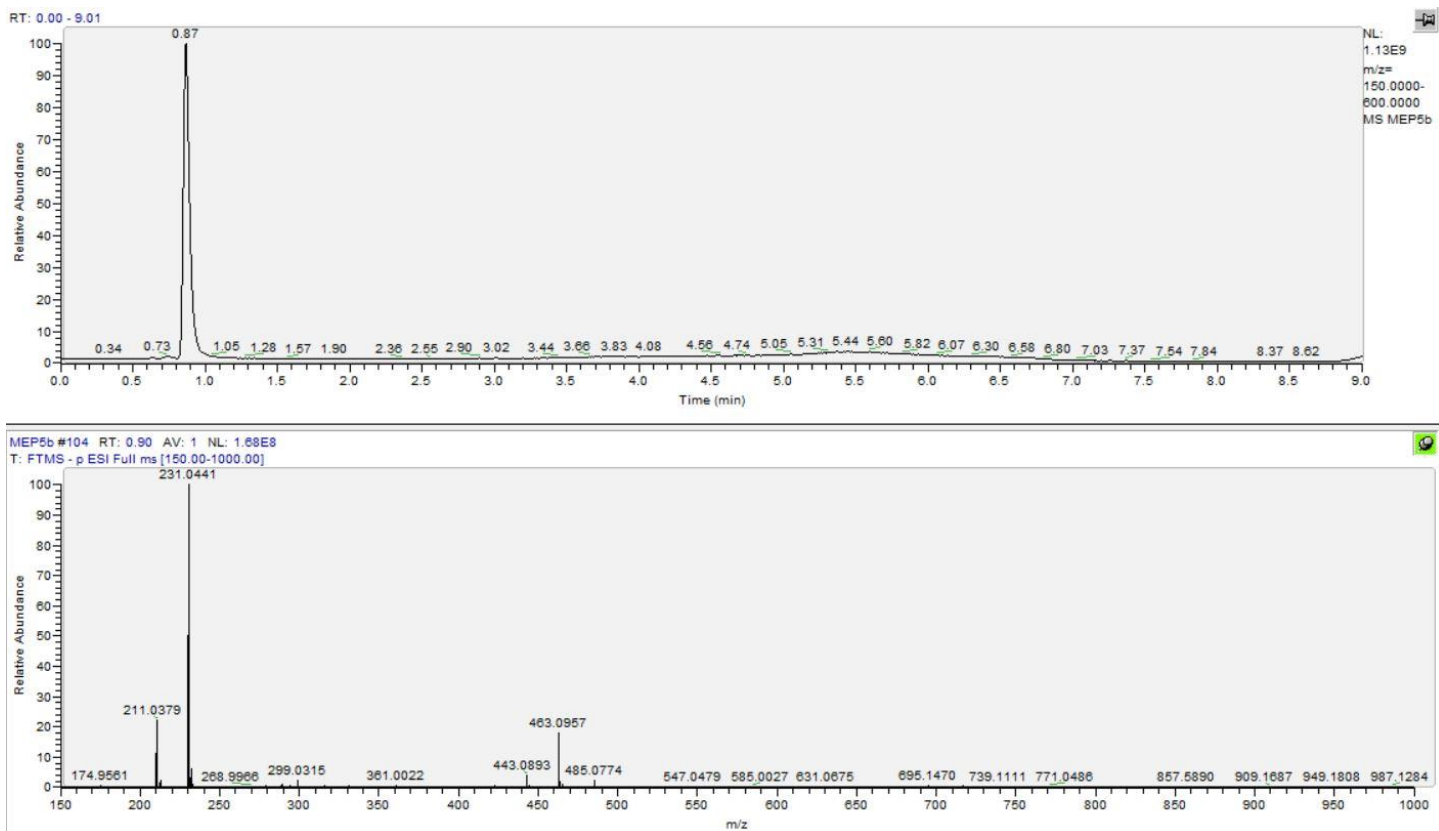


Figure S26. LC-HRMS analysis of analog 5b demonstrating >95% purity. The mass observed at 211.0379 corresponds to the fluoride elimination product, vinyl phosphonate **2**. This product appears to be an artifact of the ionization process as there are no observable peaks corresponding to **2** in the ^1H NMR spectrum (below).

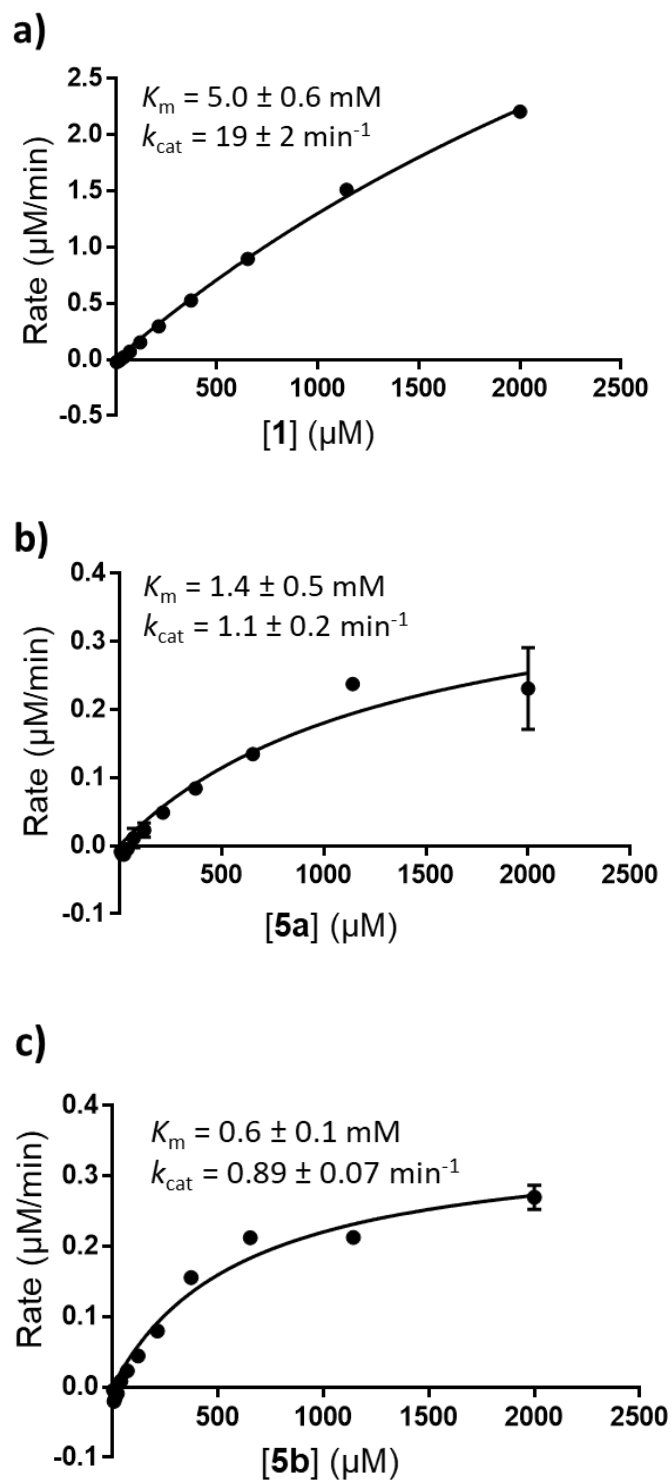


Figure S27. Michaelis-Menten plots for MEP and analogs 1 and 5a,b with *P. falciparum* IspD. The relatively high K_m values determined by these experiments demonstrate that the non-negligible (-) MEP control rates observed in Figure S19 are very unlikely to be masking potent inhibition.

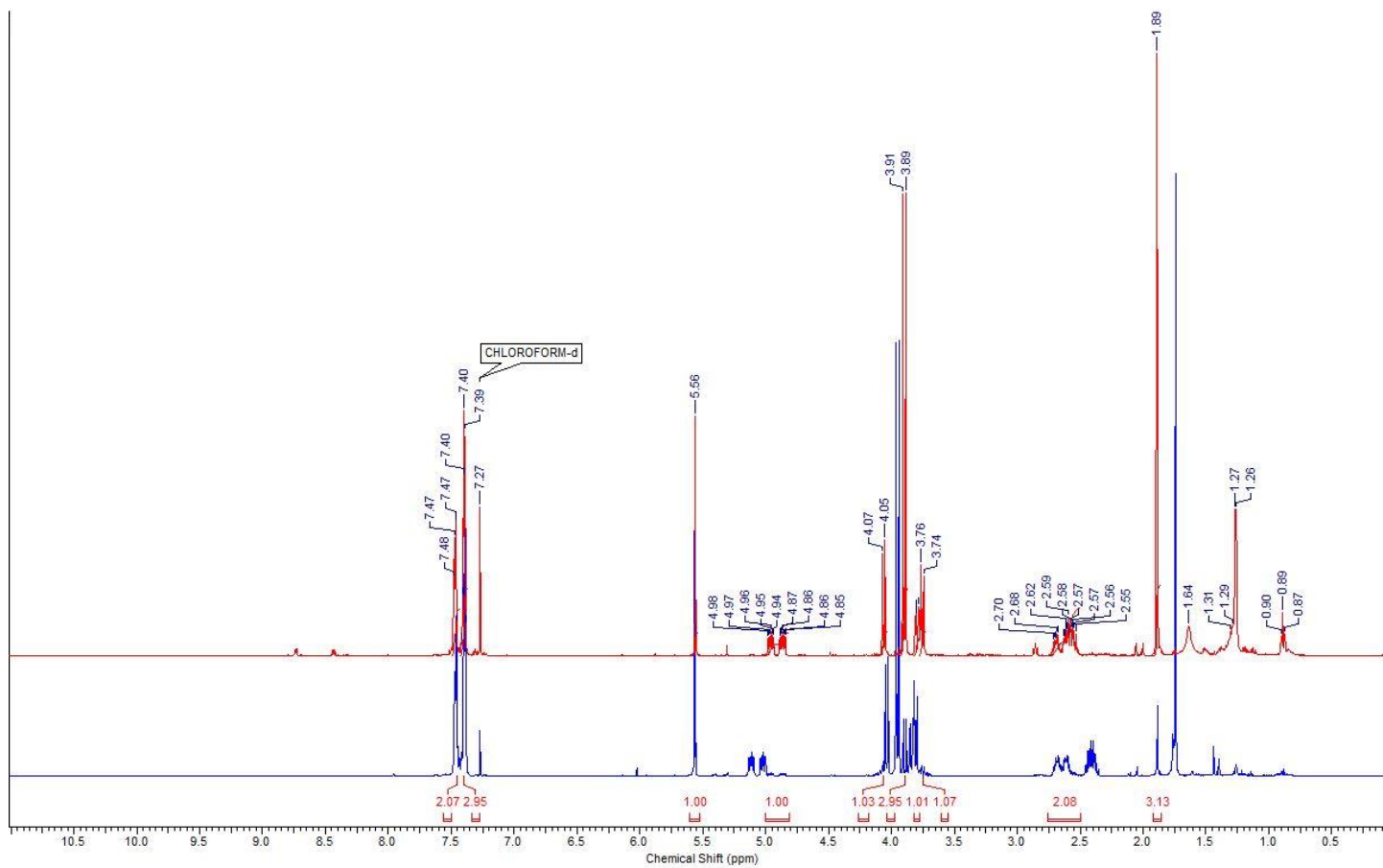
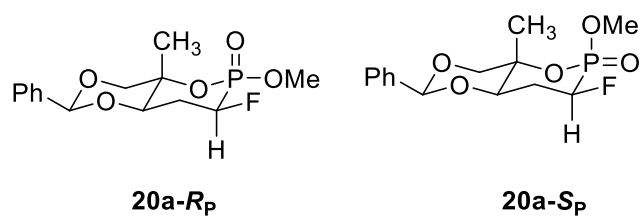


Figure S28. Overlay of ^1H spectra for diastereomers of phosphonolactone 20a. The top and bottom spectra are assigned to be the *R*- and *S*- configurations at the phosphonate, respectively.

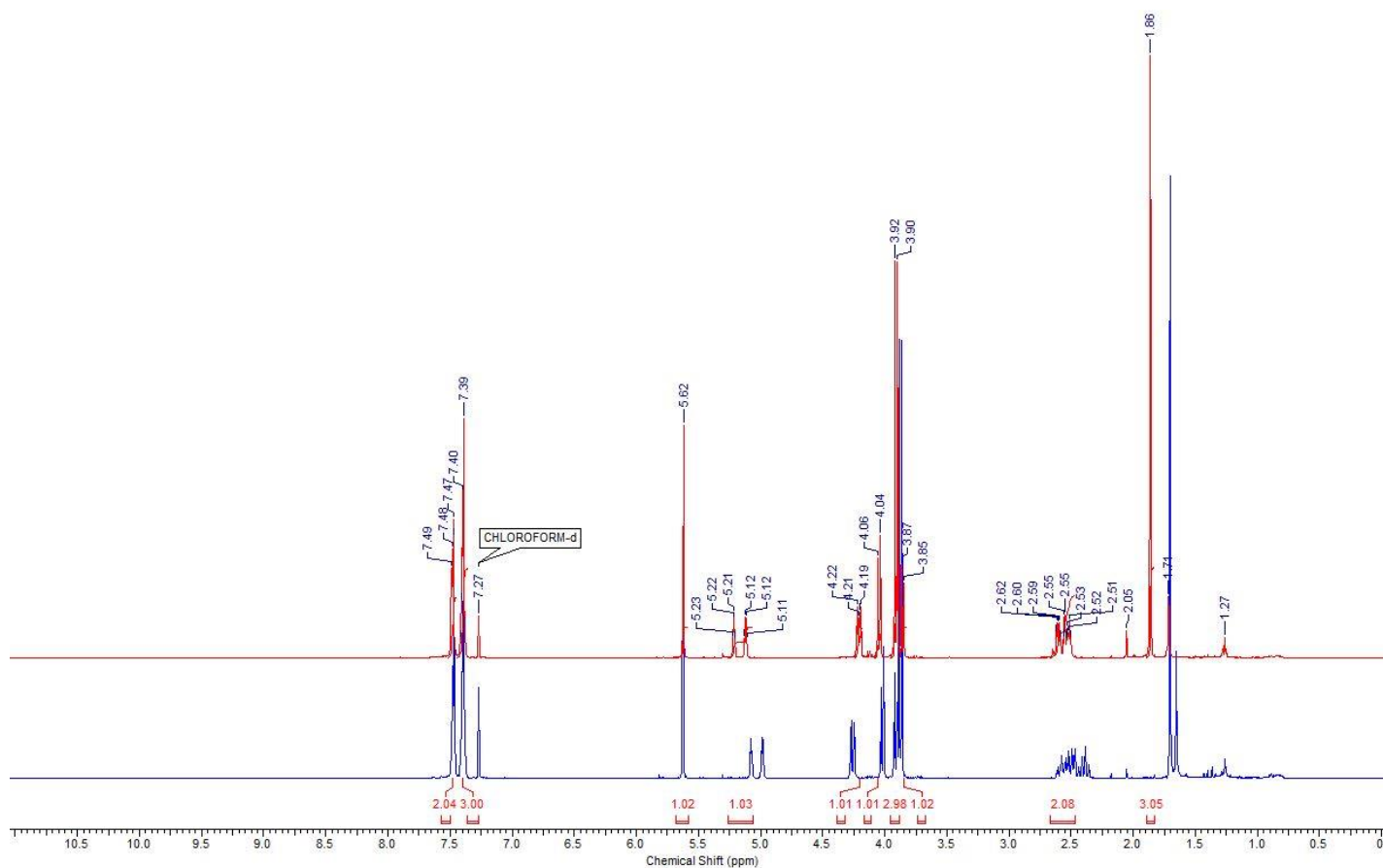
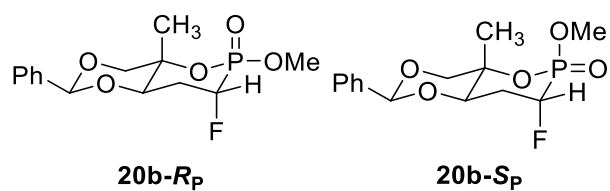
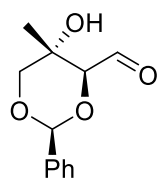
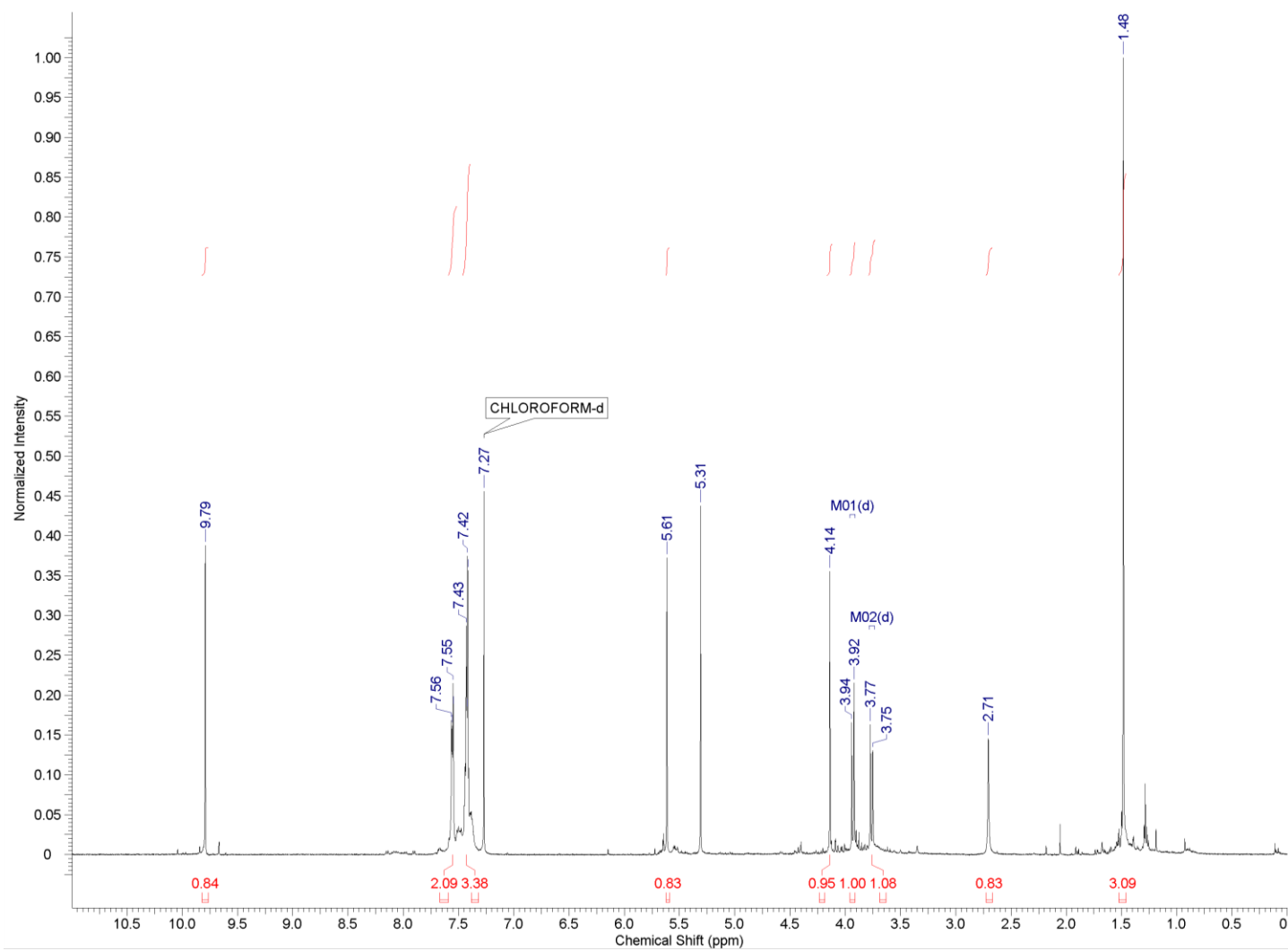
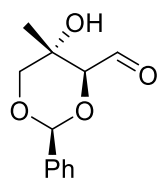


Figure S29. Overlay of ^1H spectra for diastereomers of phosphonolactone 20b. The top and bottom spectra are assigned to be the *R*- and *S*- configurations at the phosphonate, respectively.

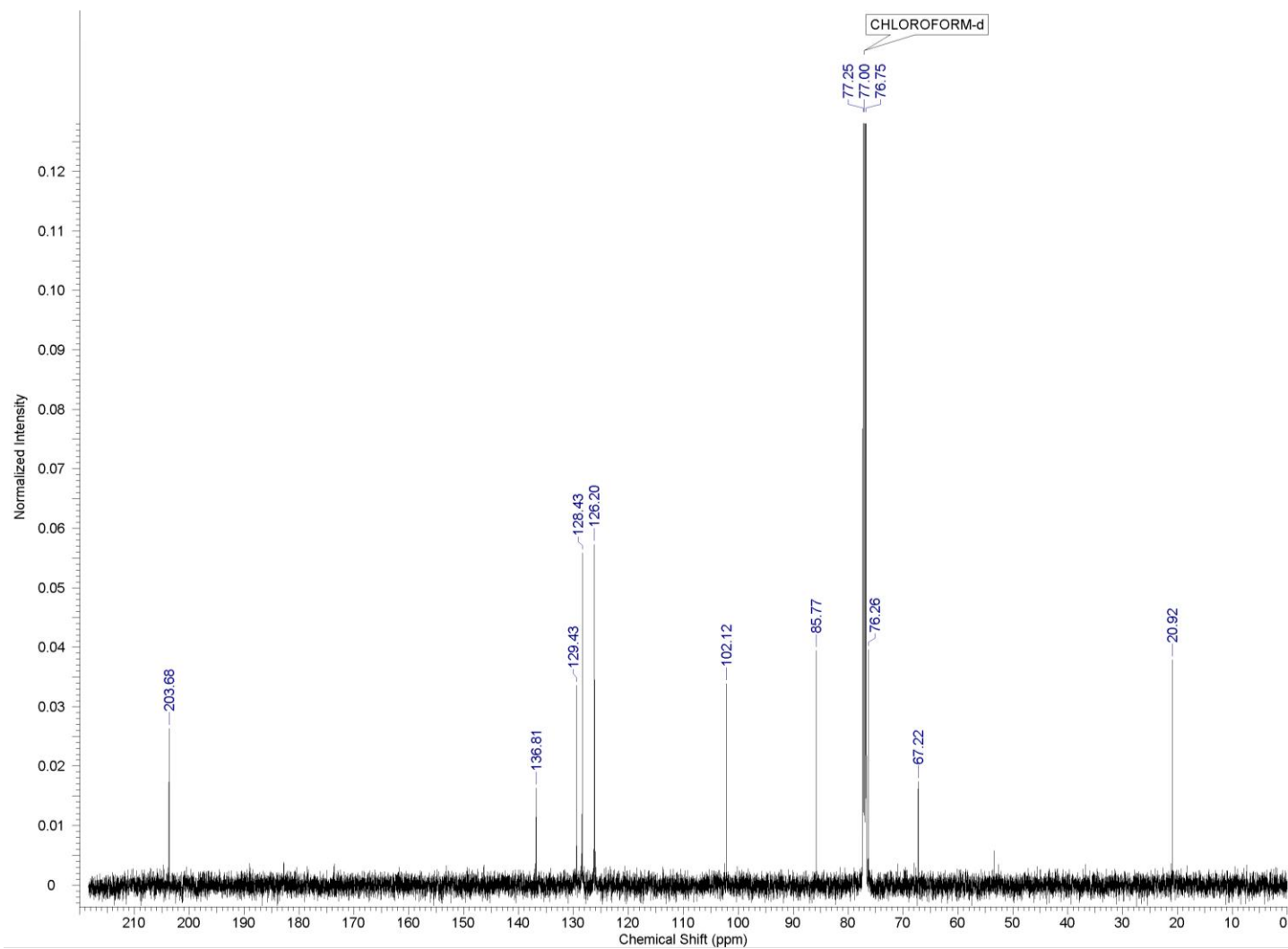


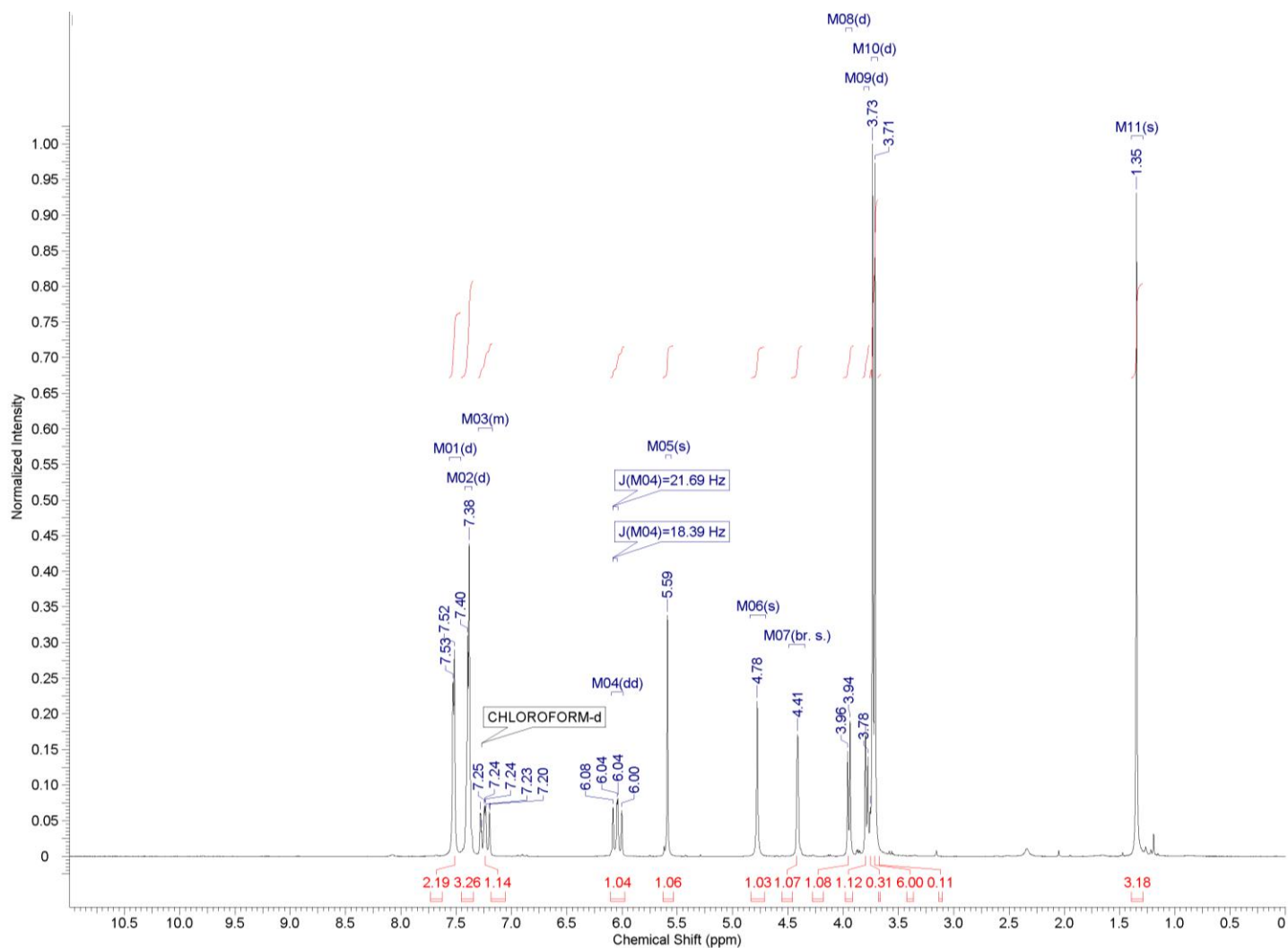
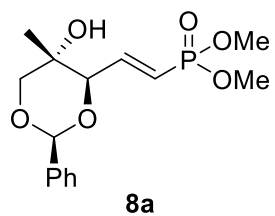
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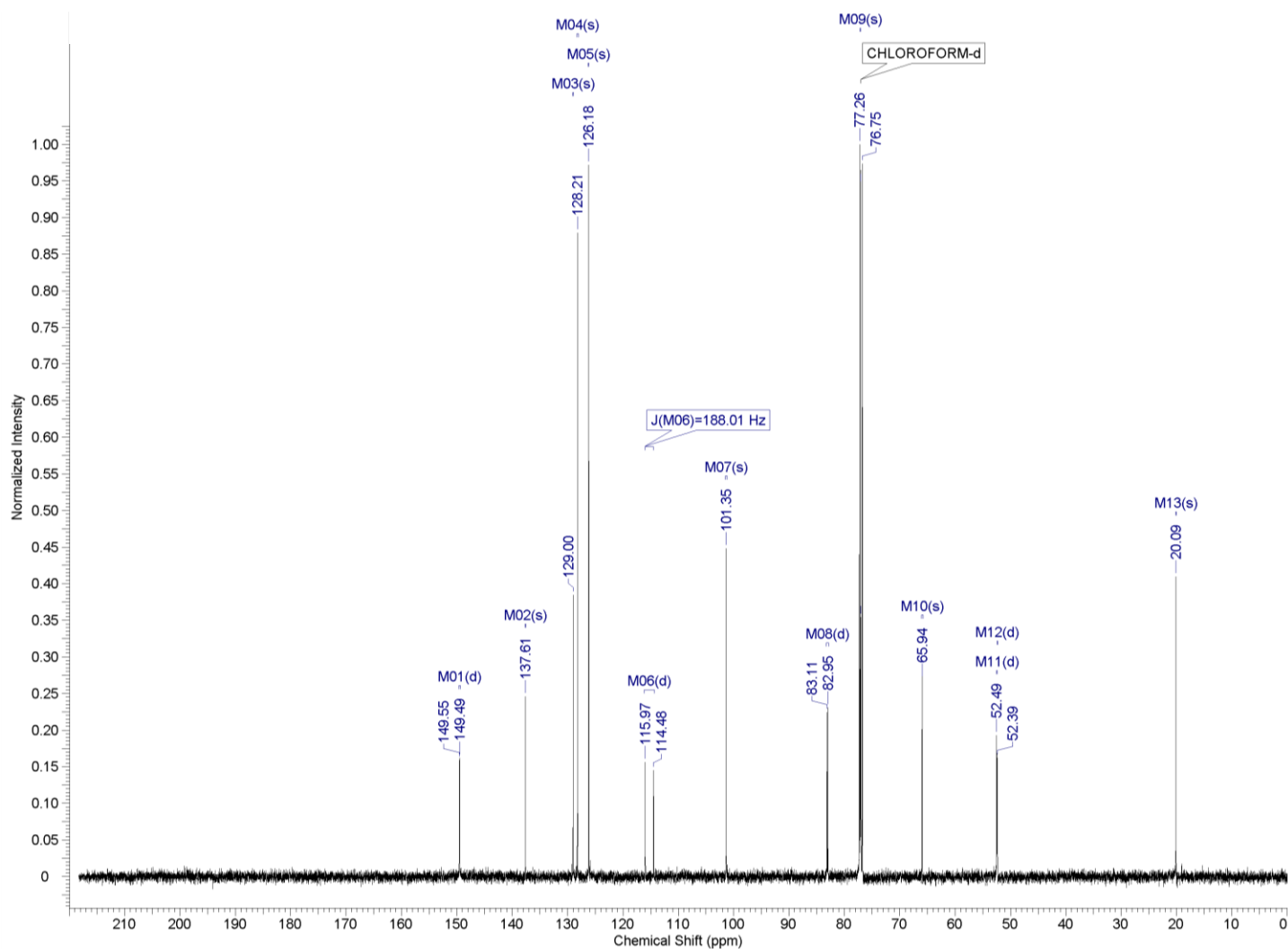
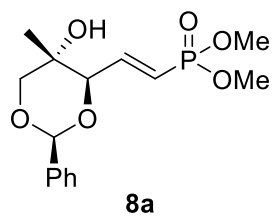


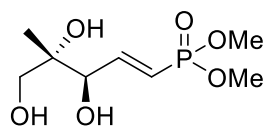


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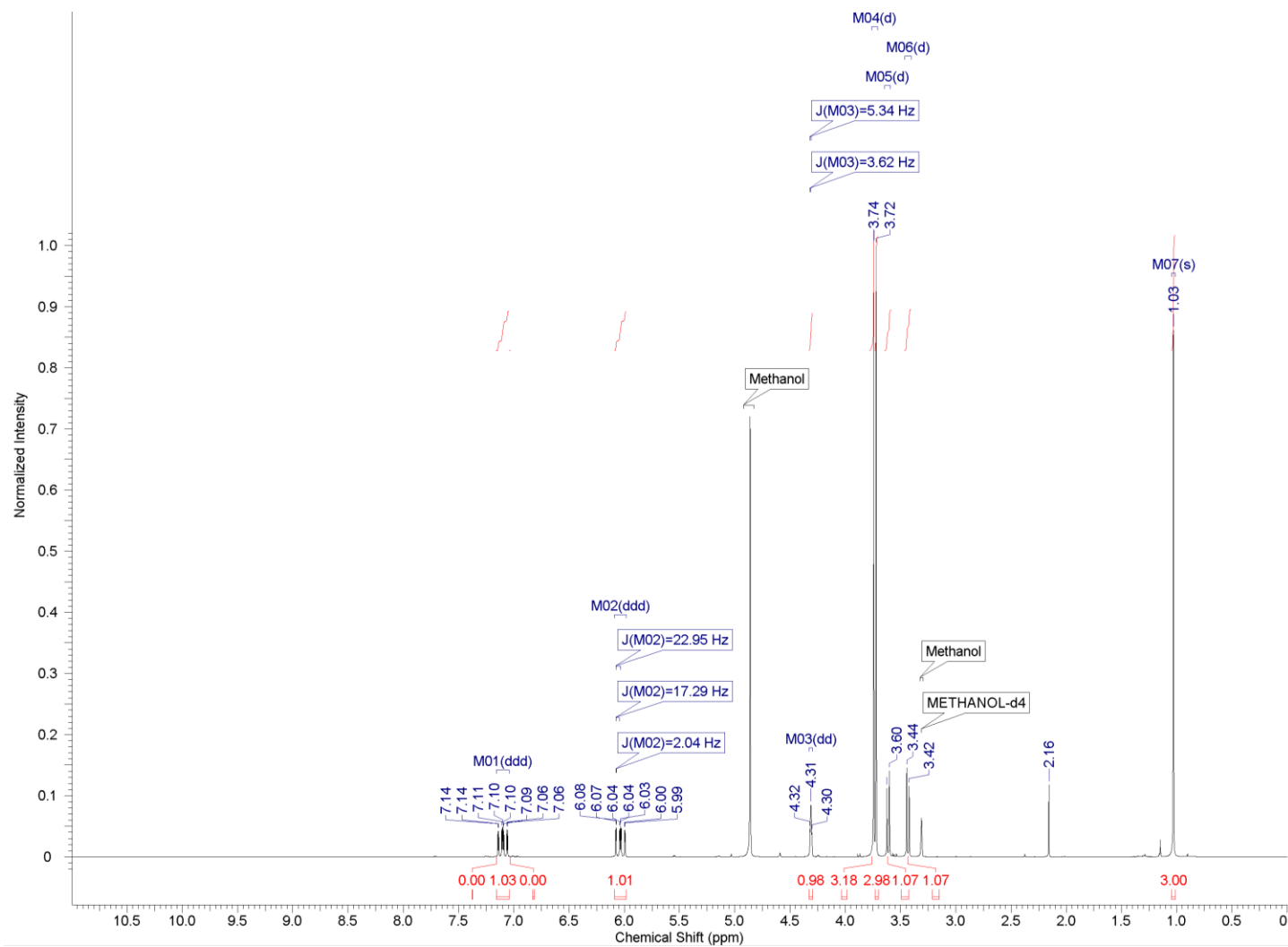


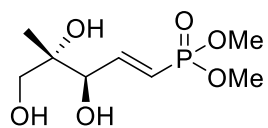




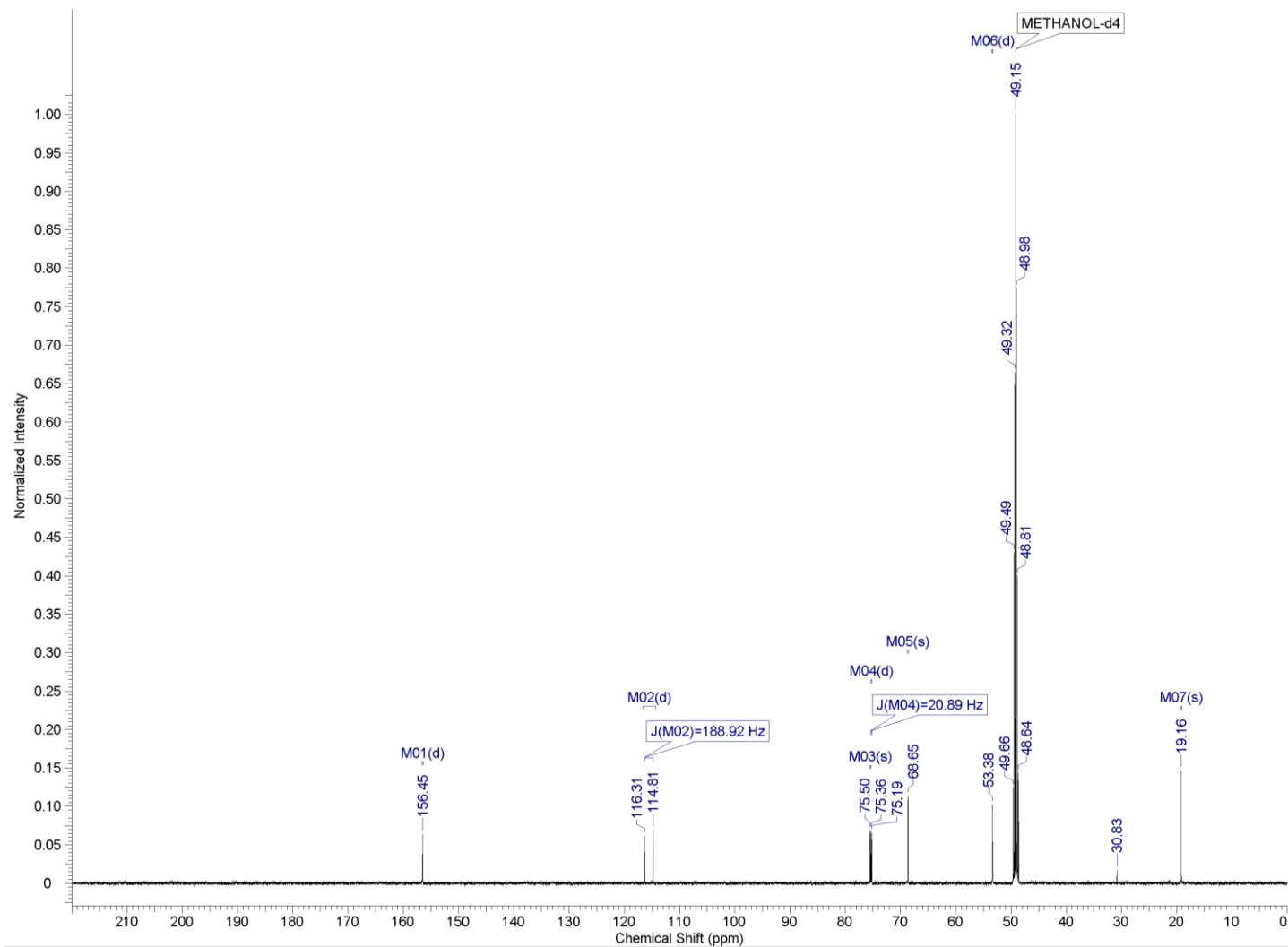


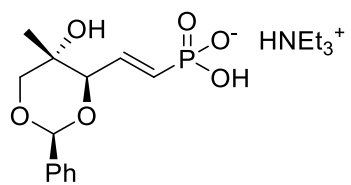
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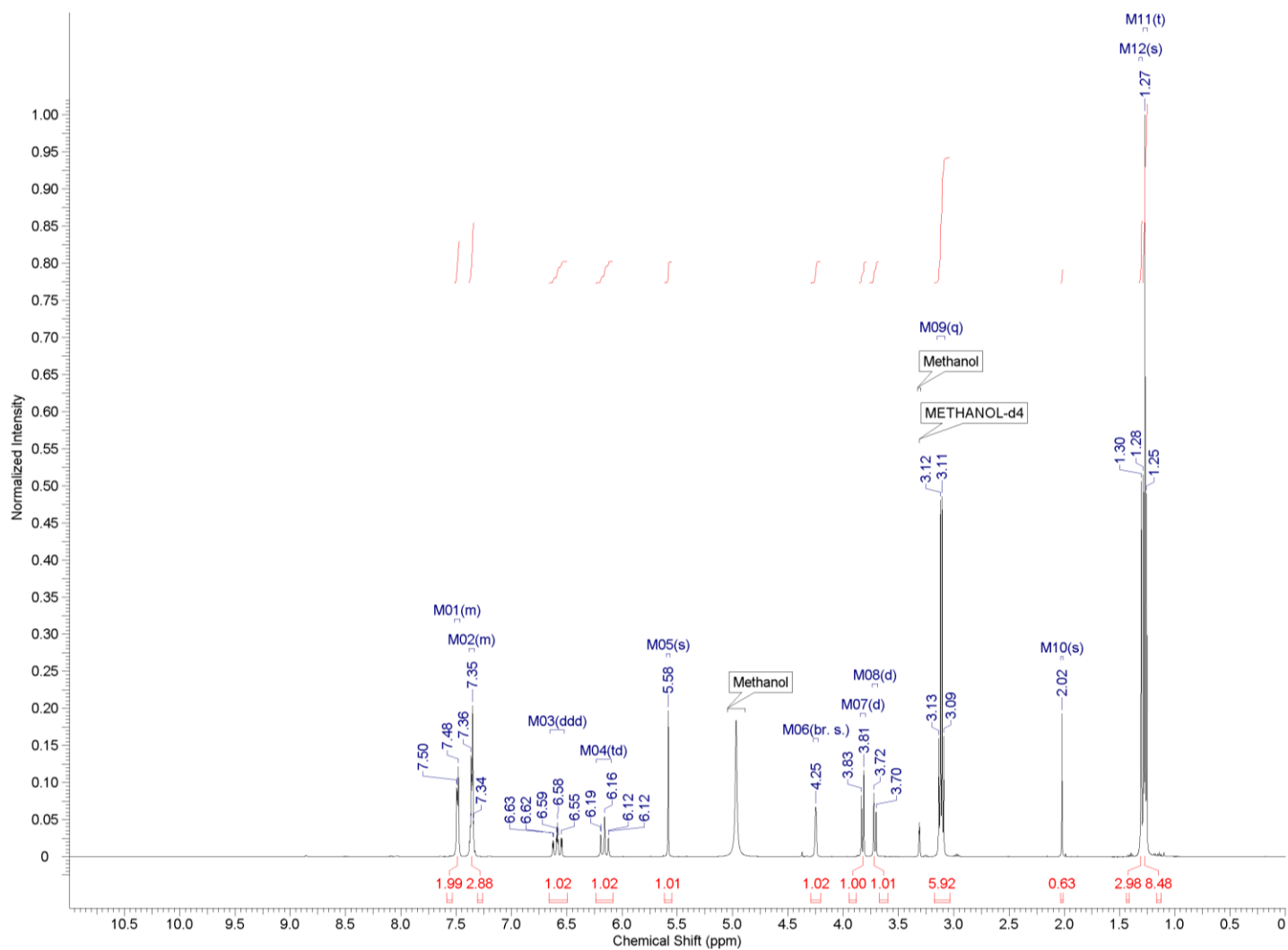


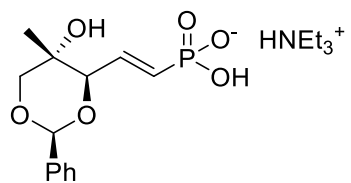
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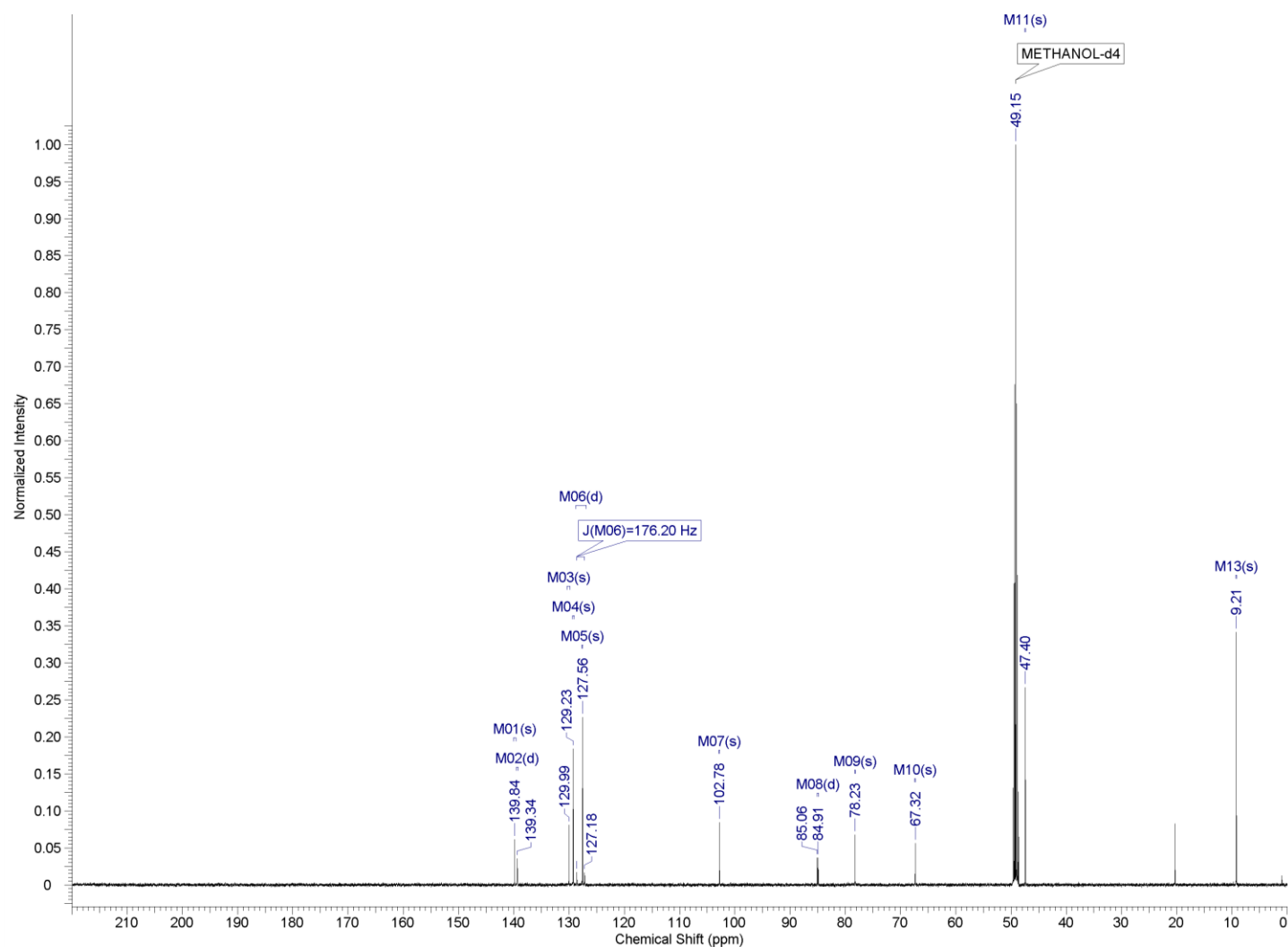


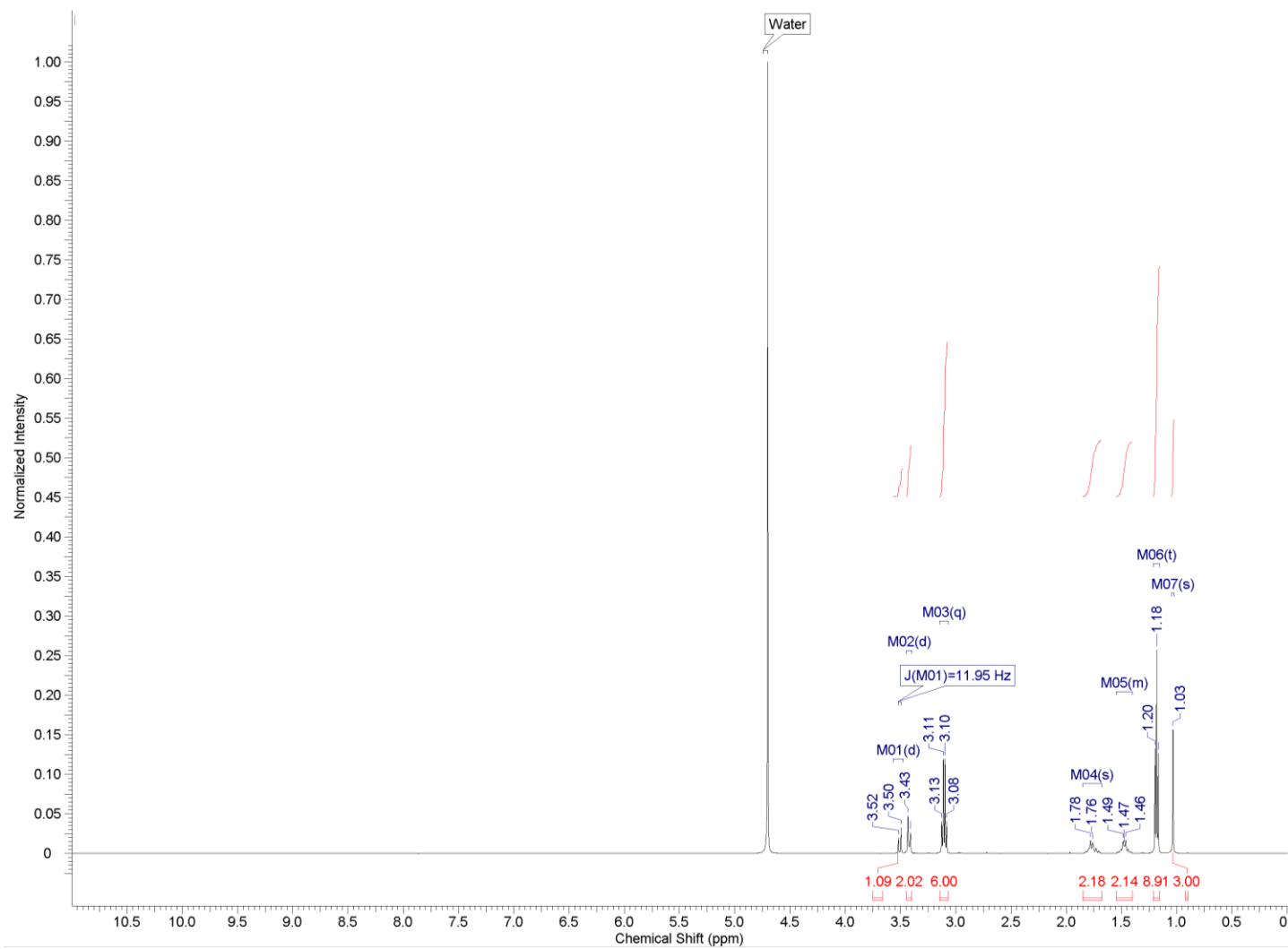
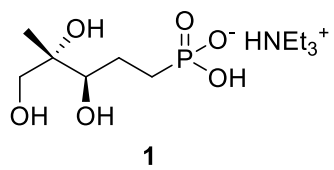
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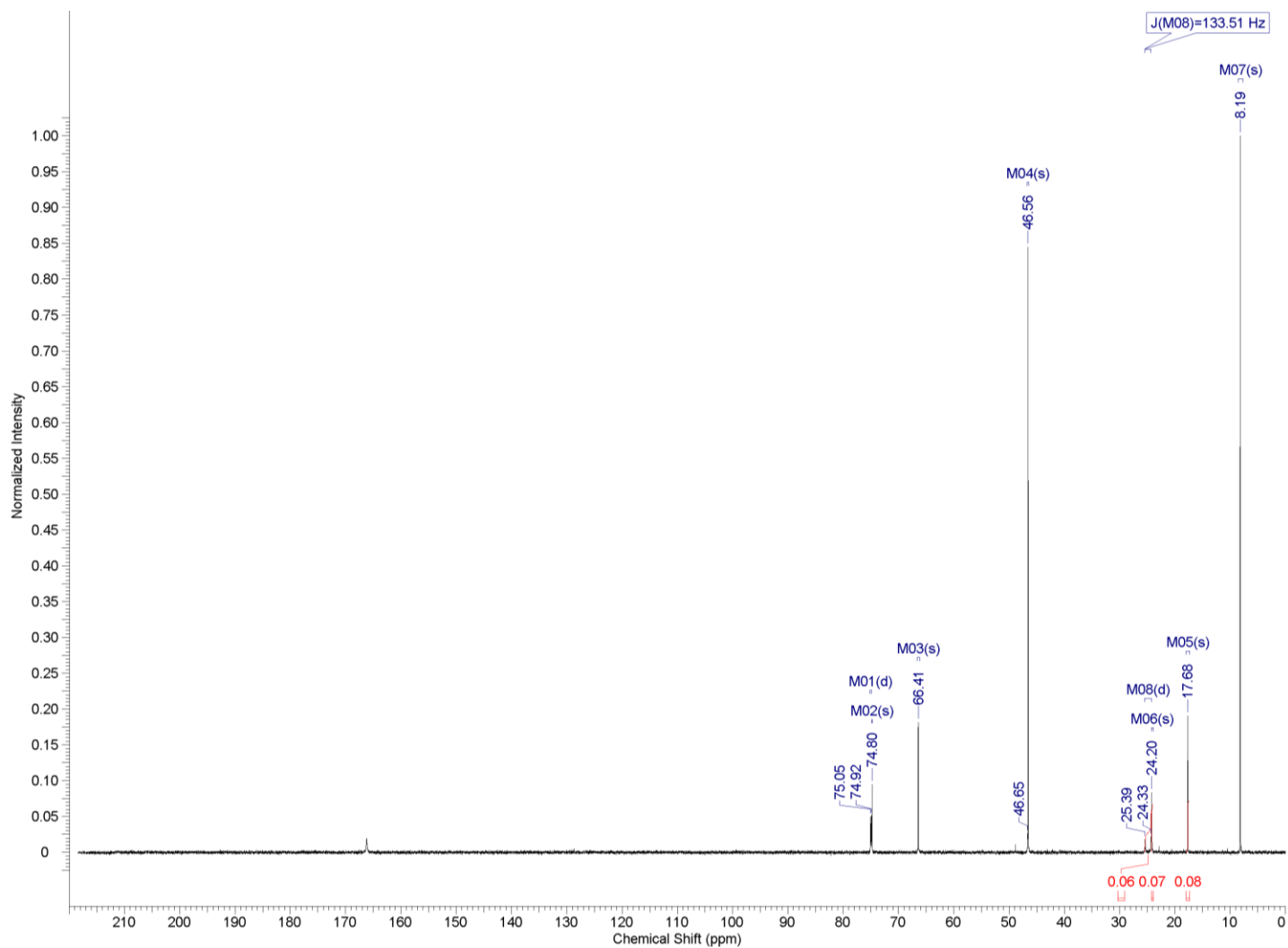
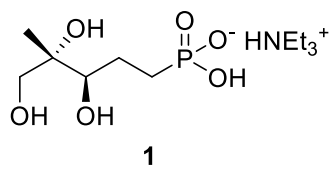


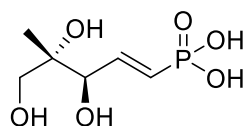


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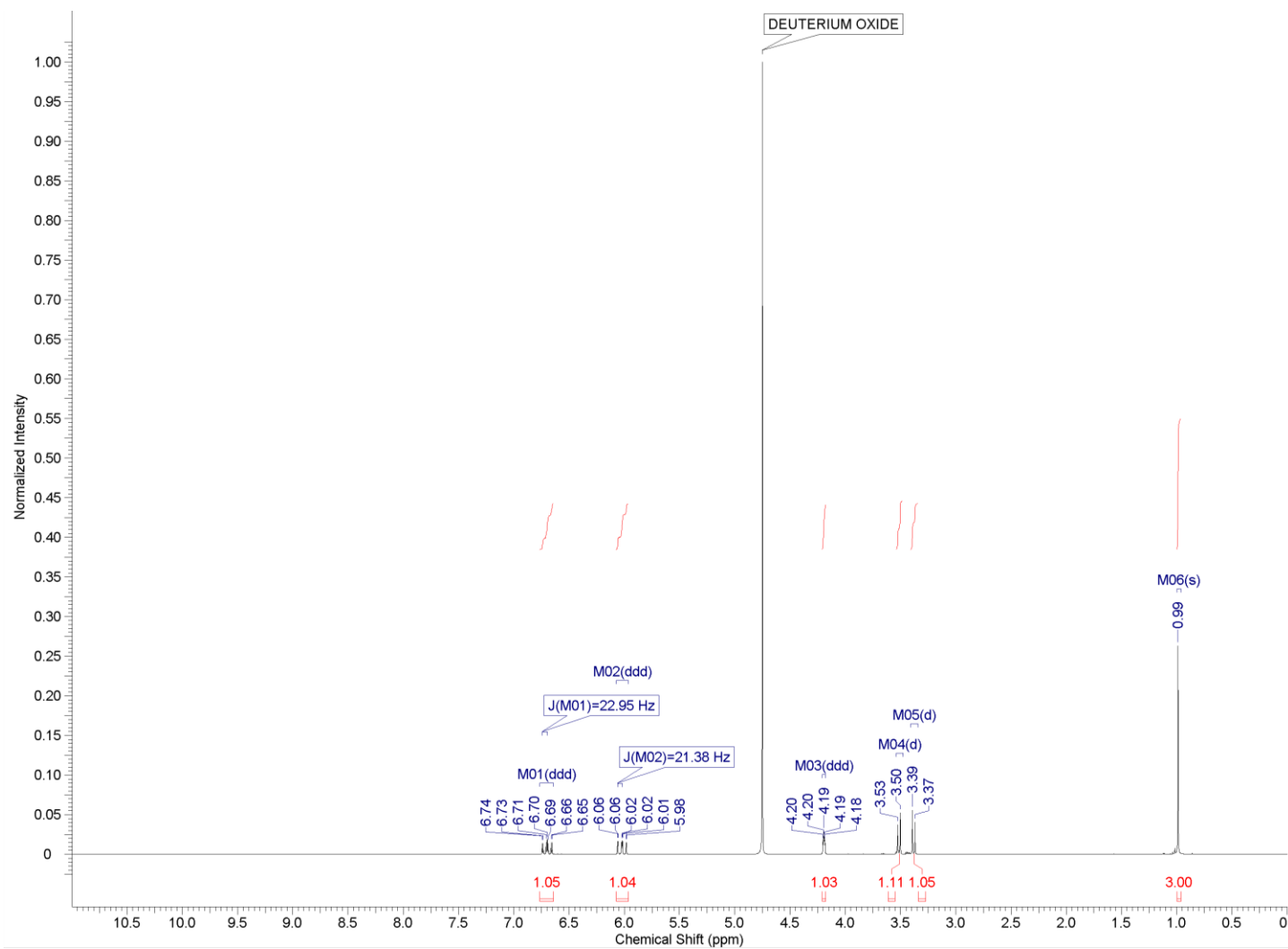


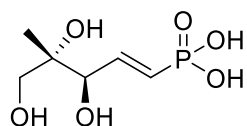




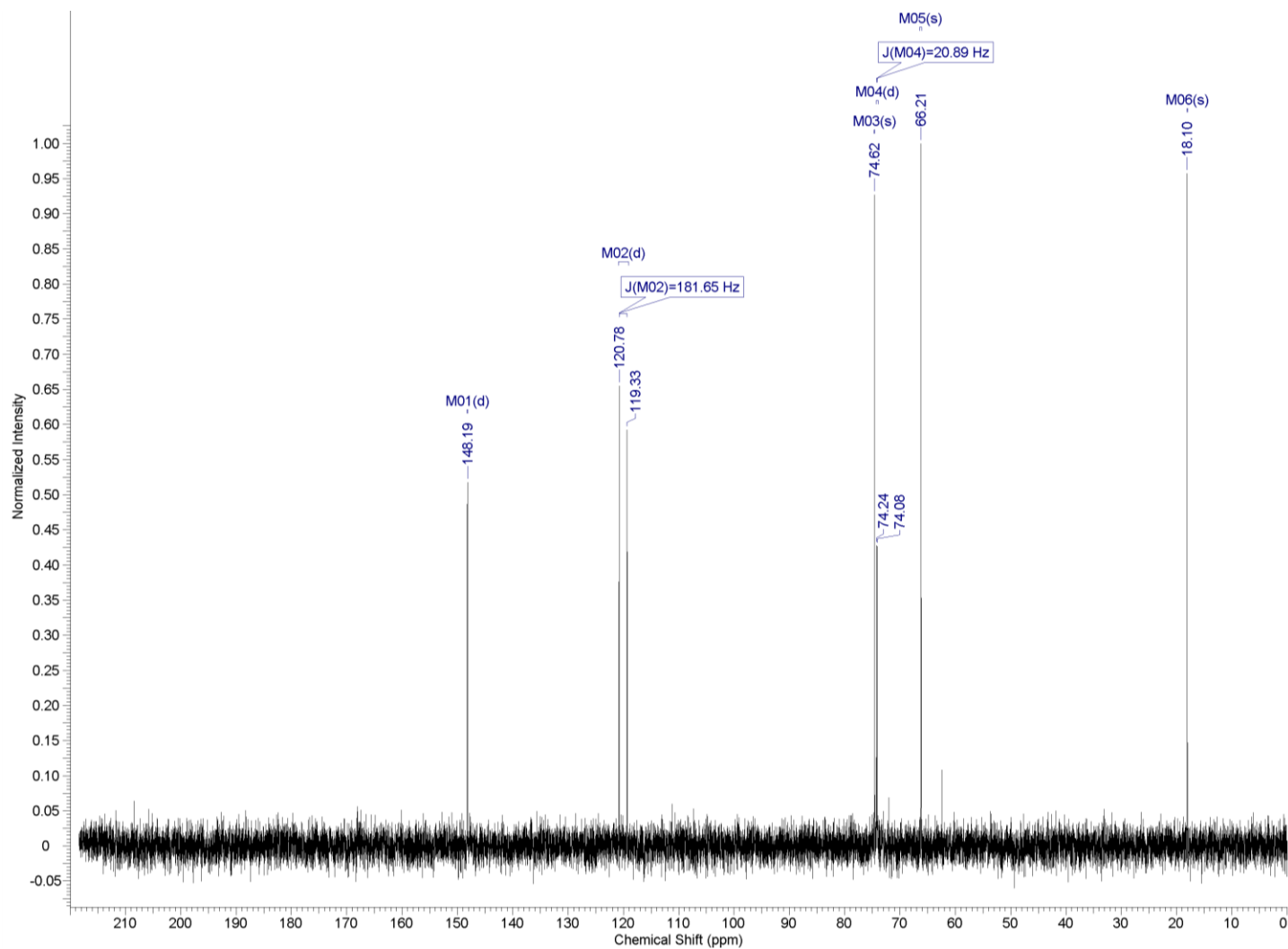


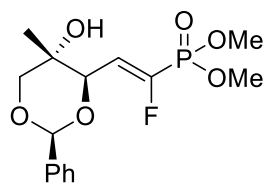
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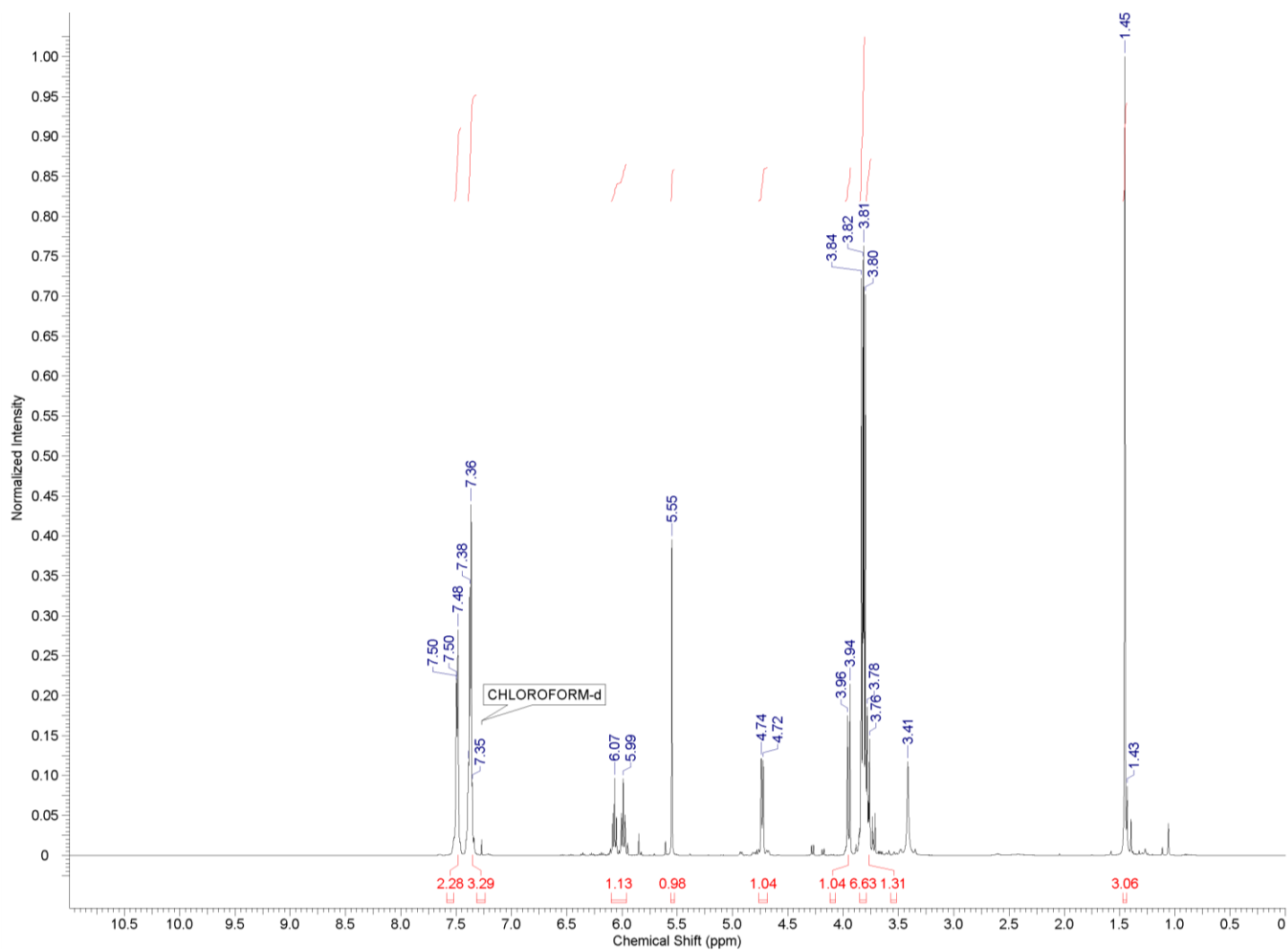


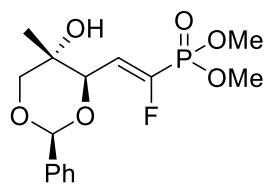
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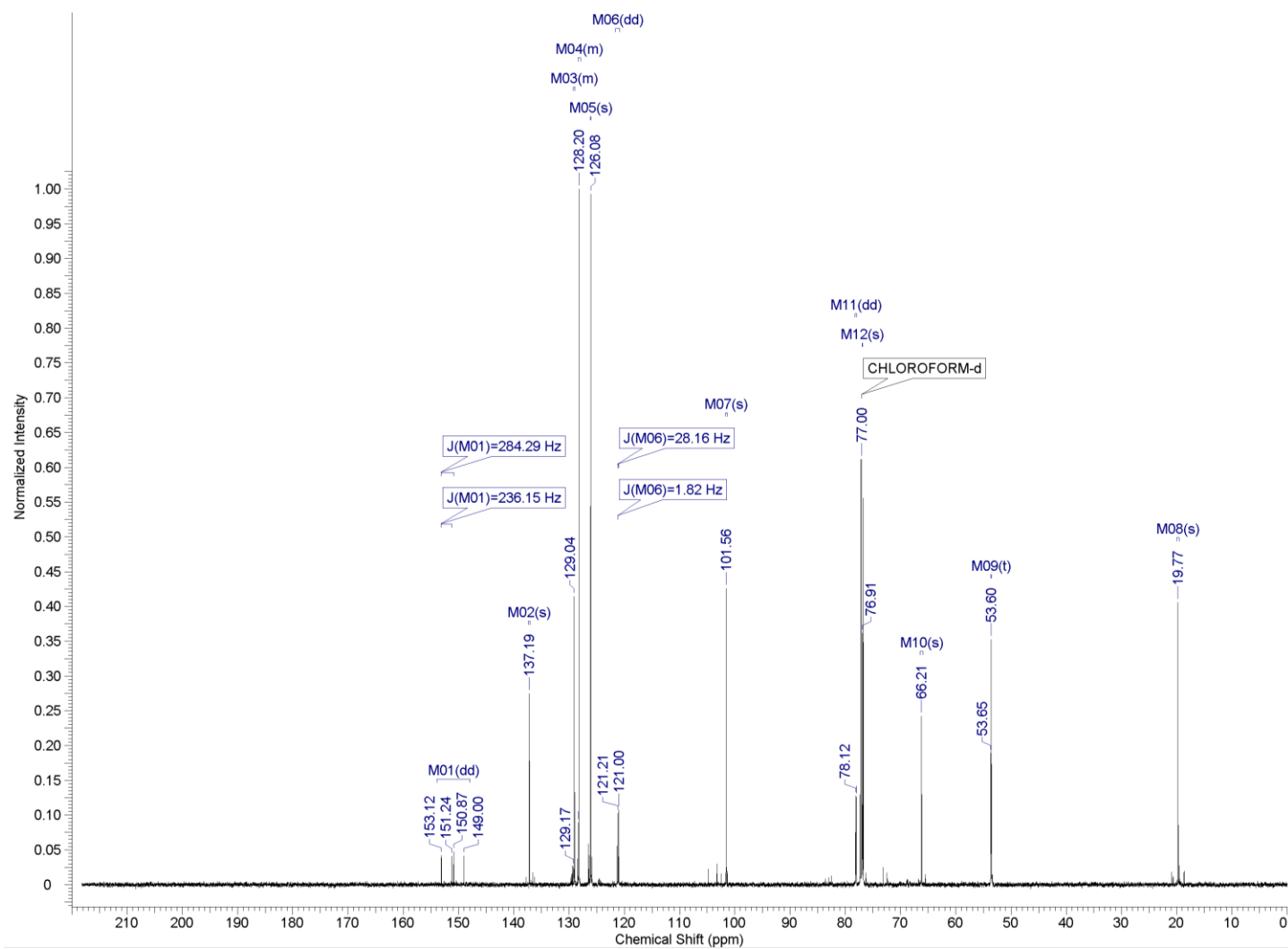


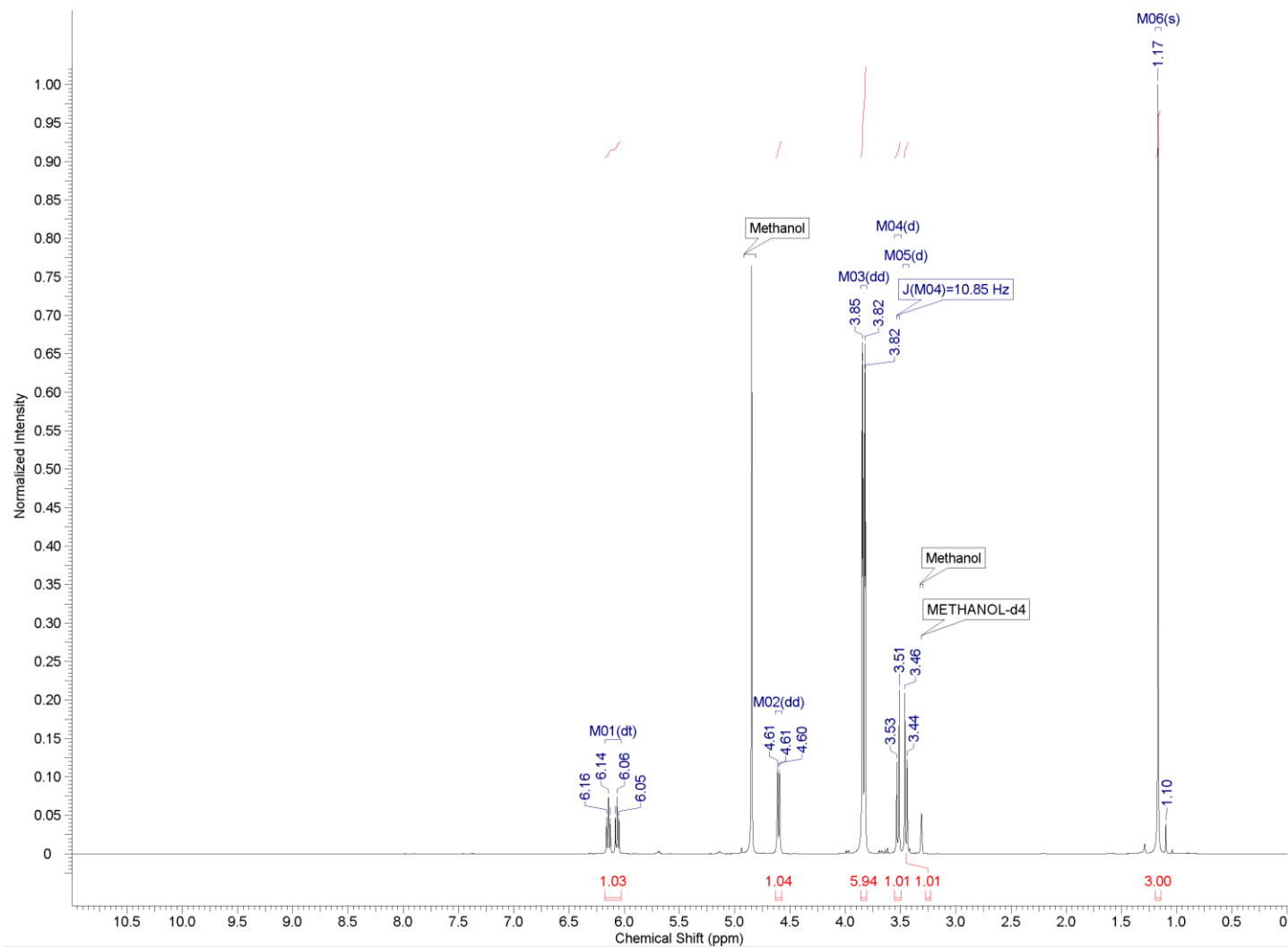
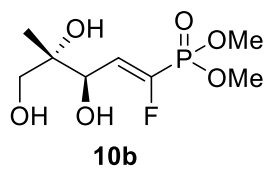
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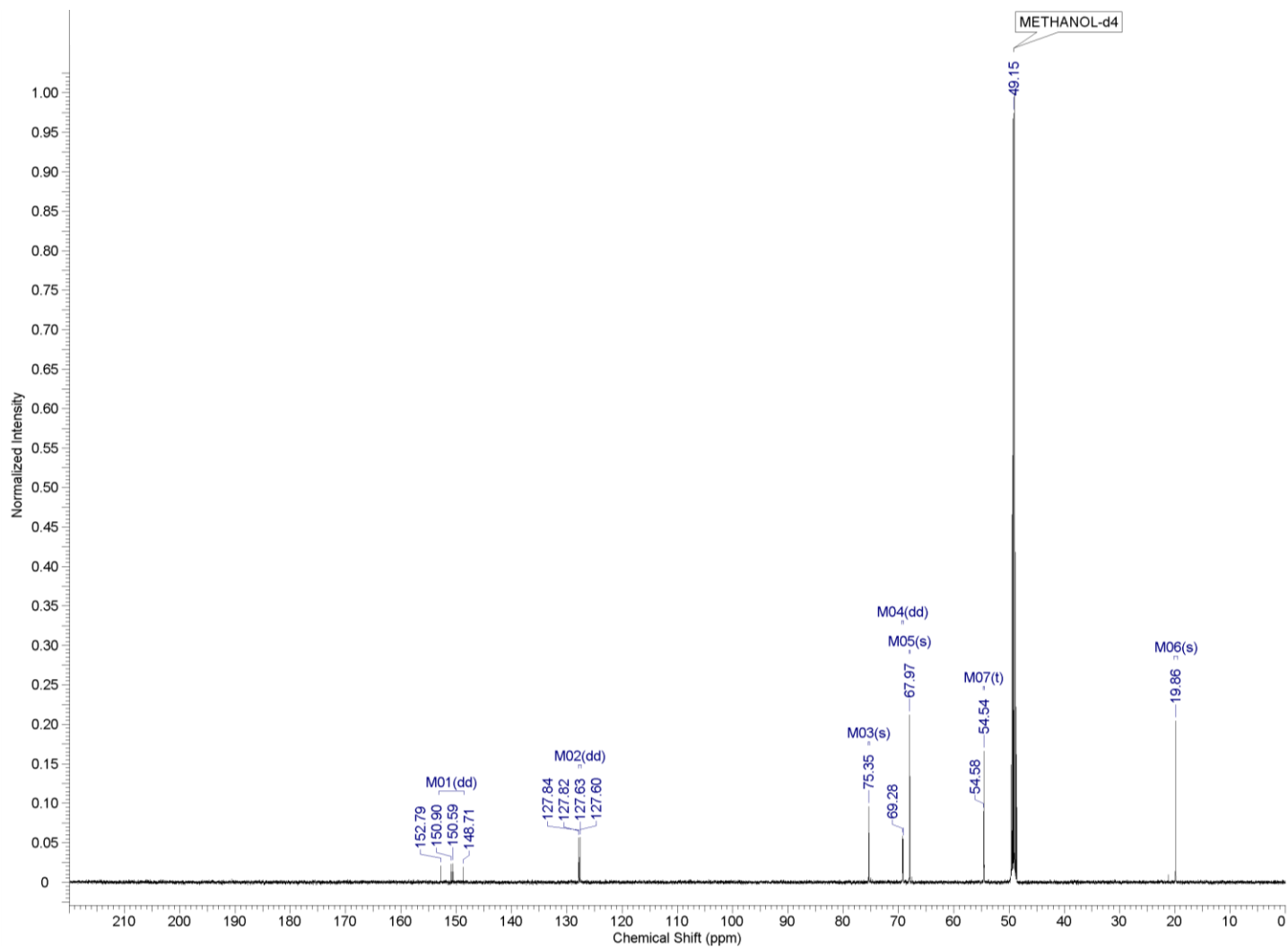
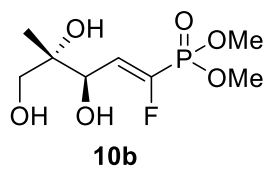


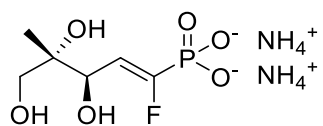


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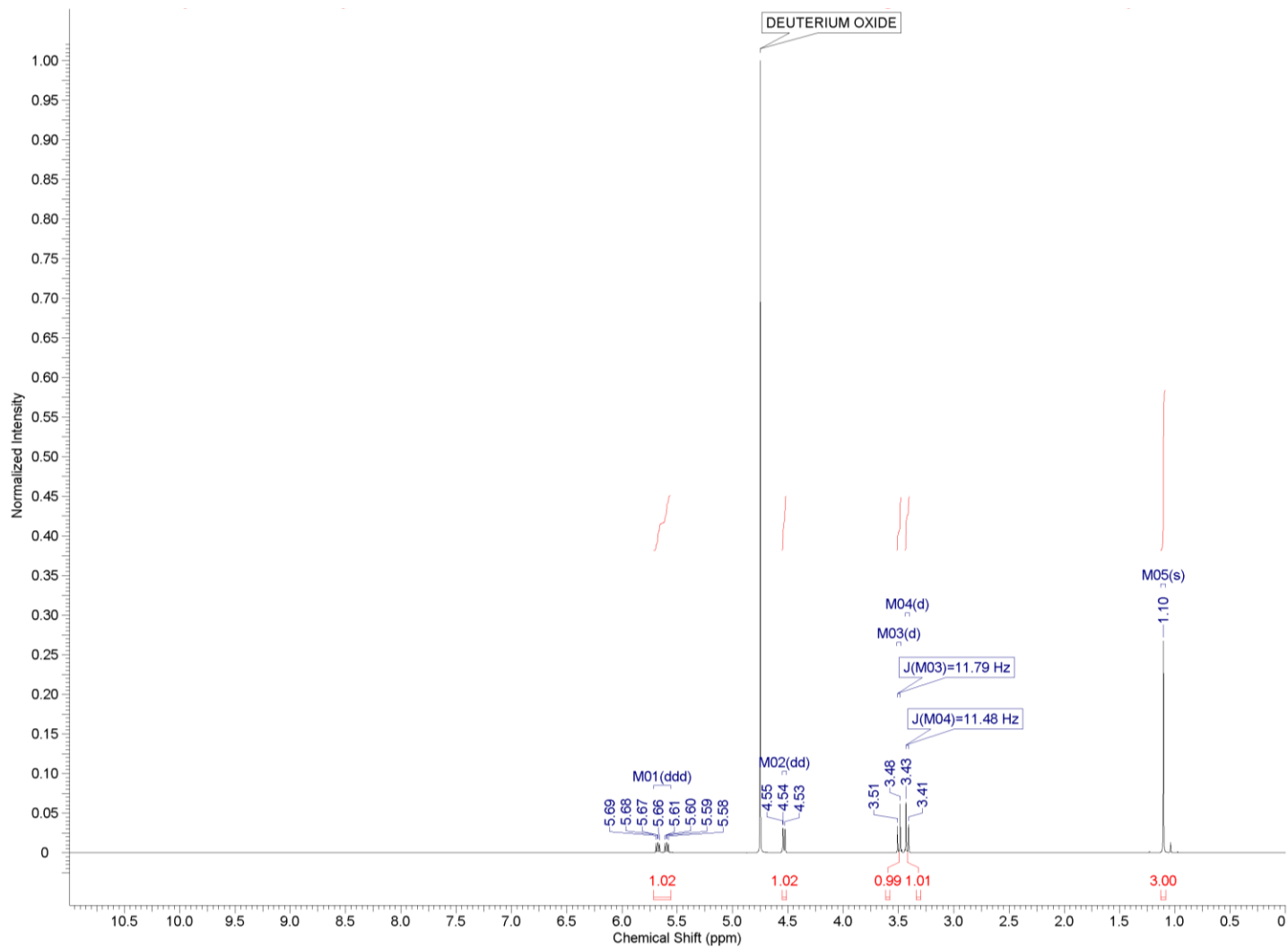


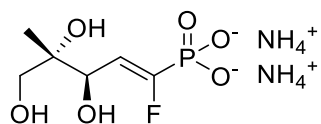




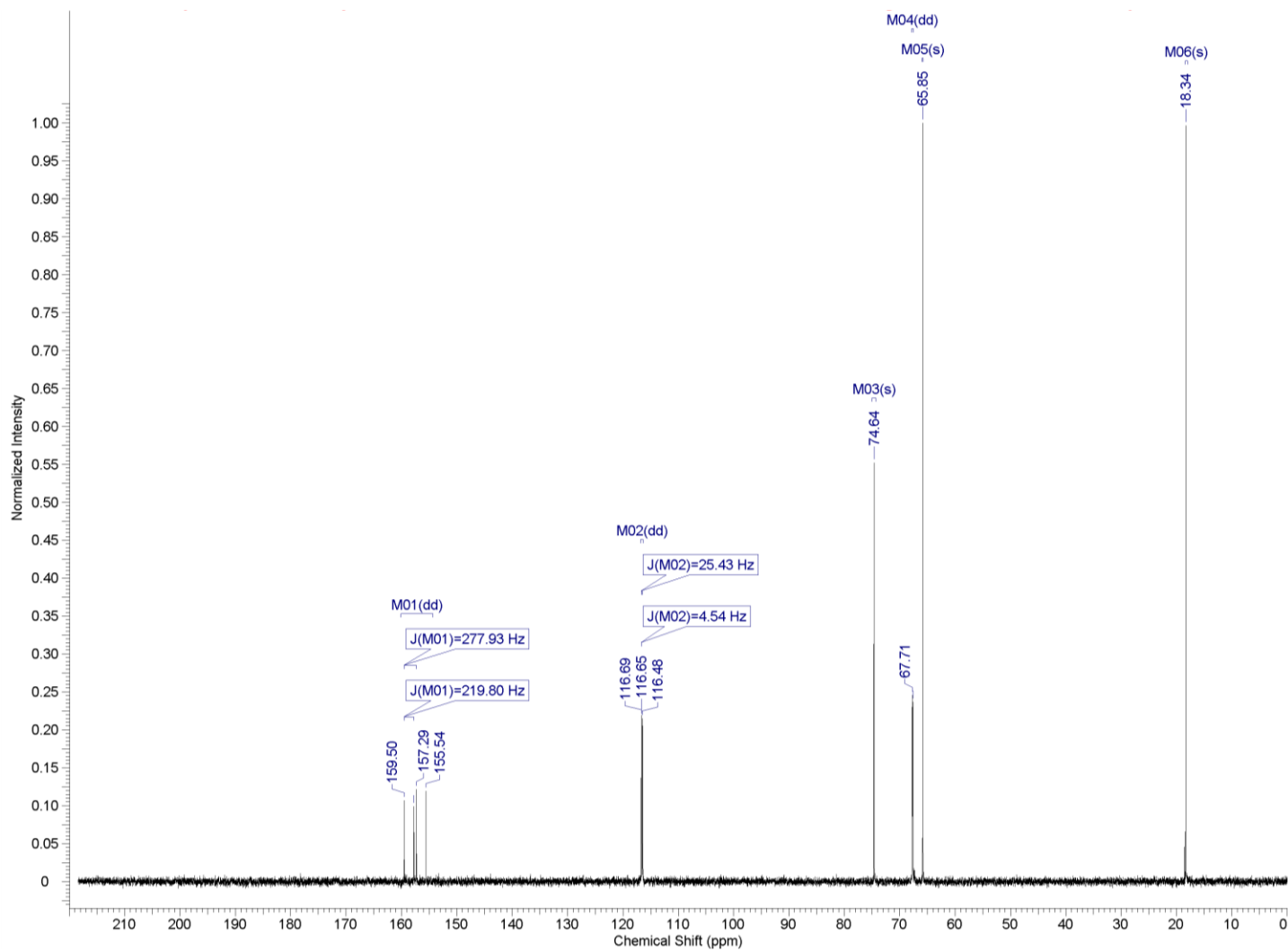


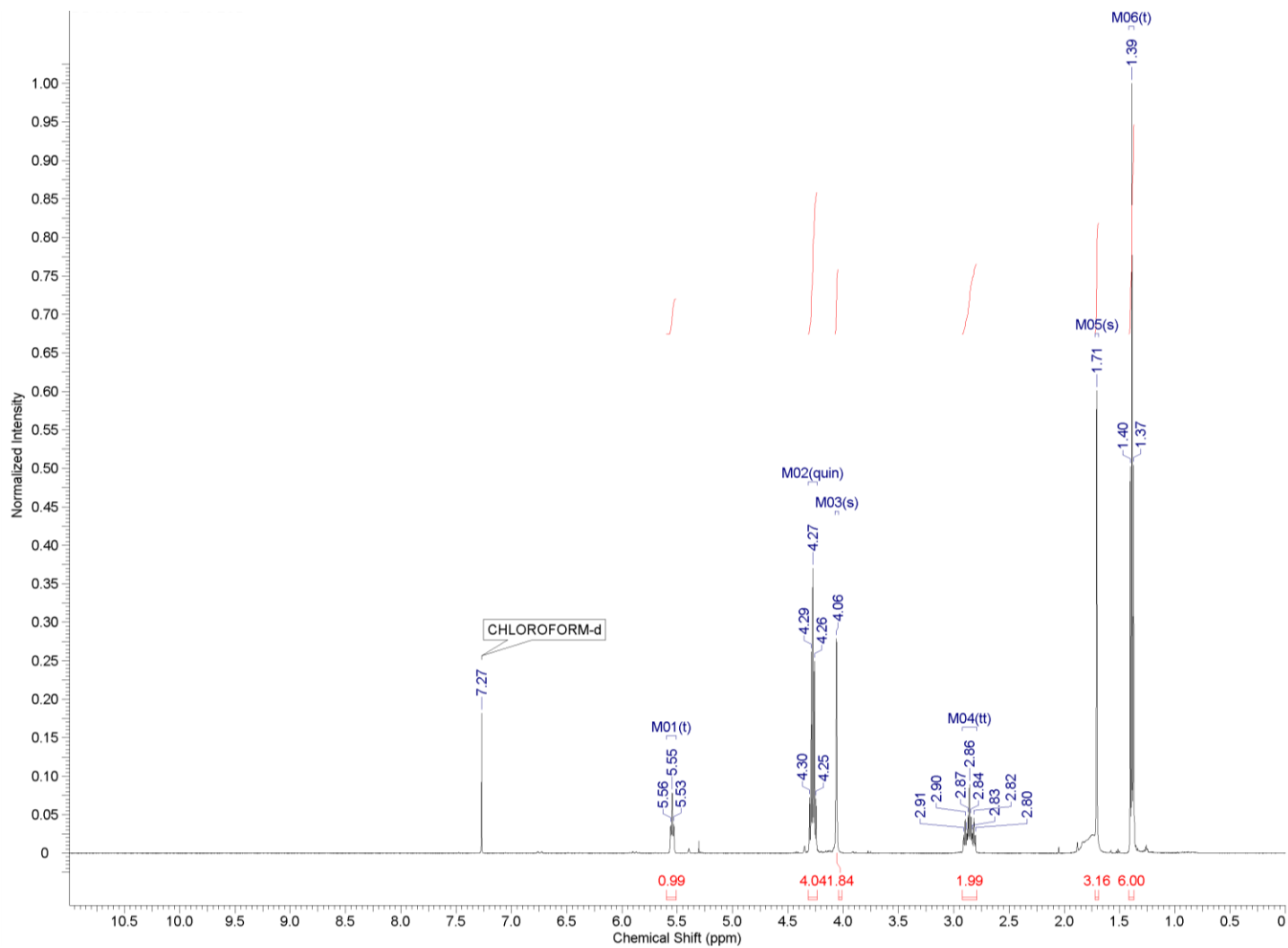
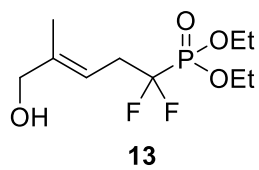
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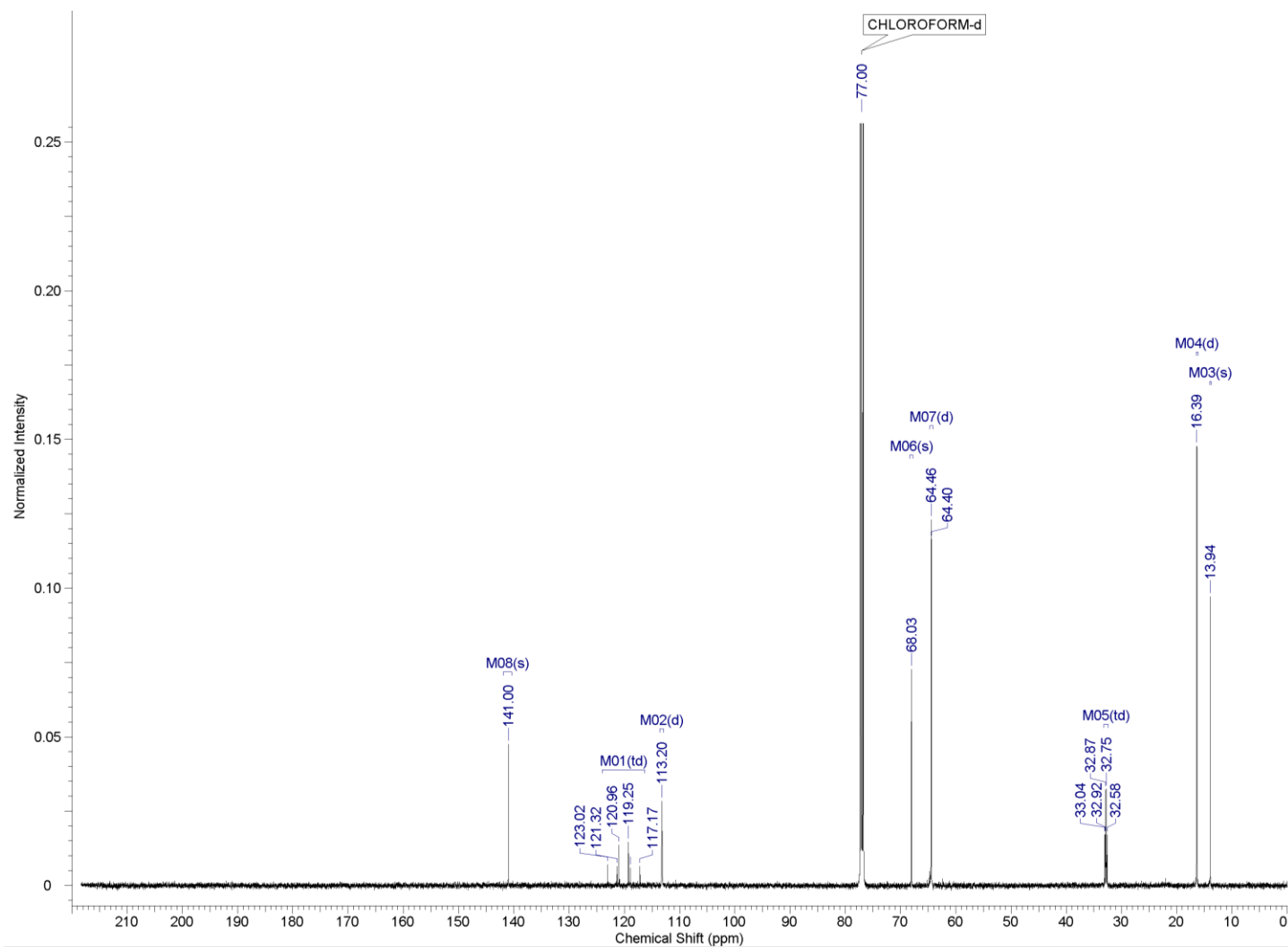
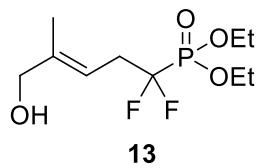


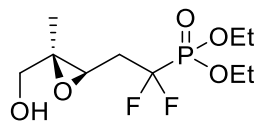


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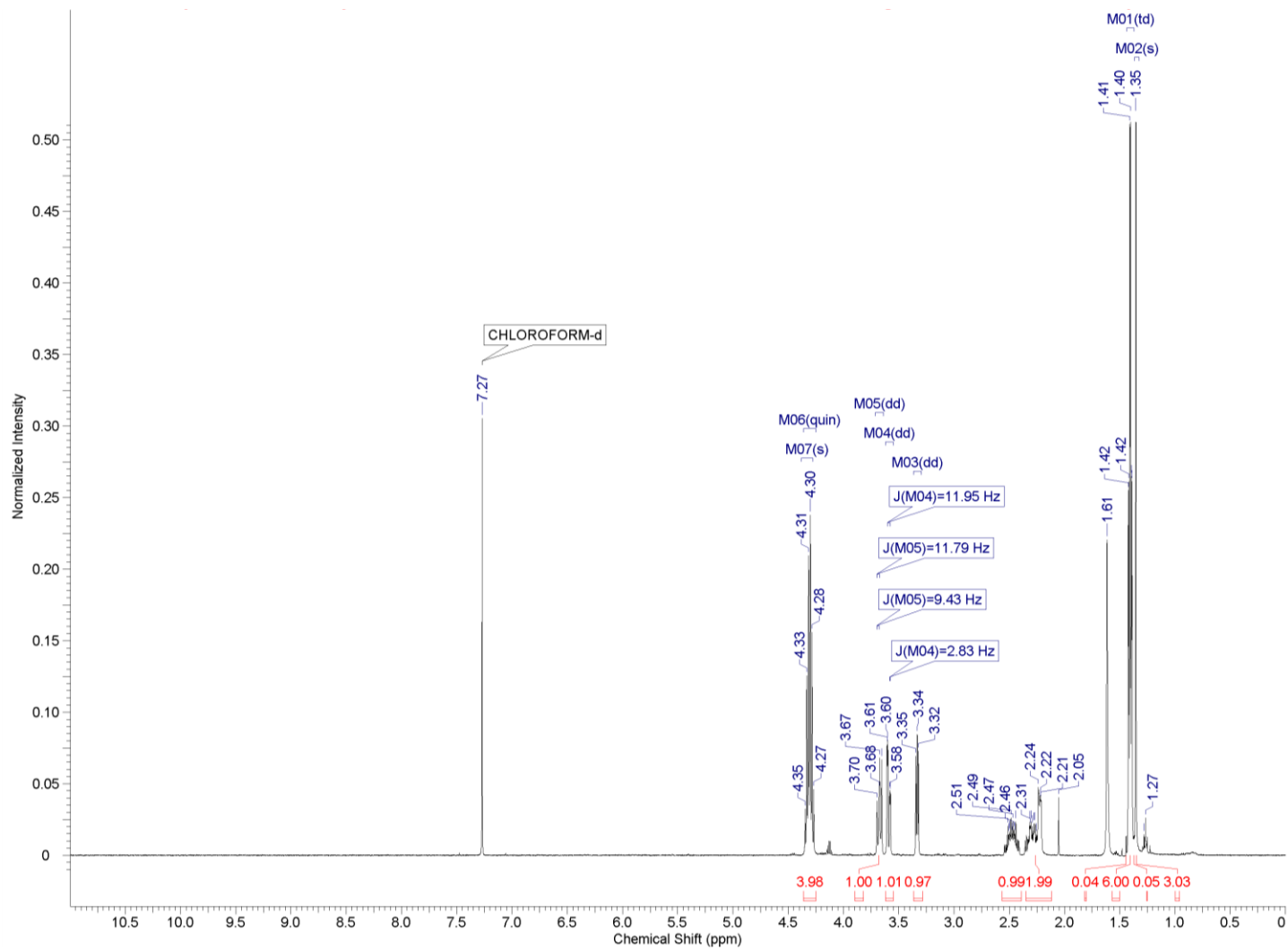


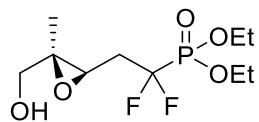




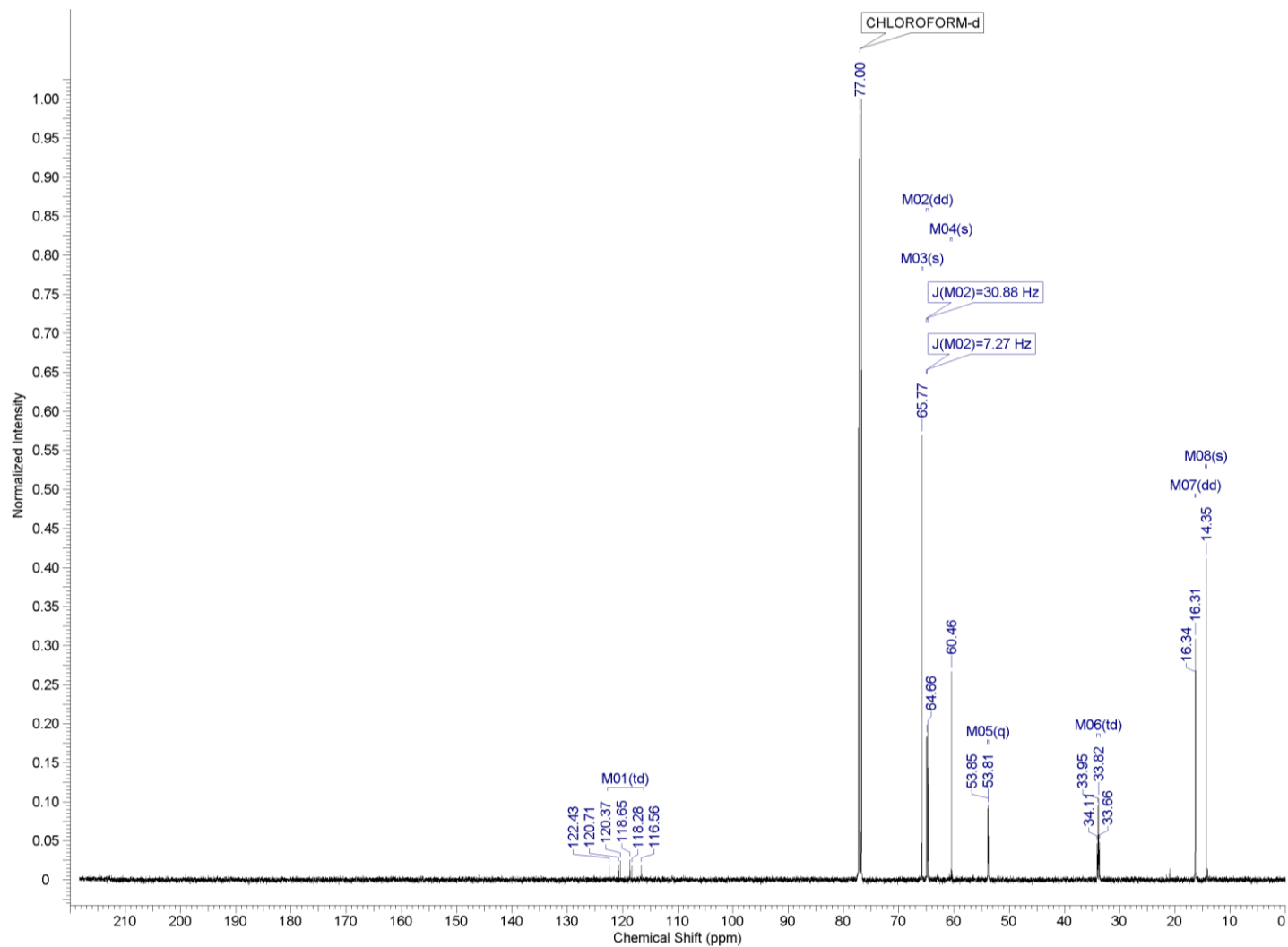


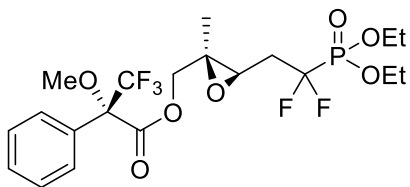
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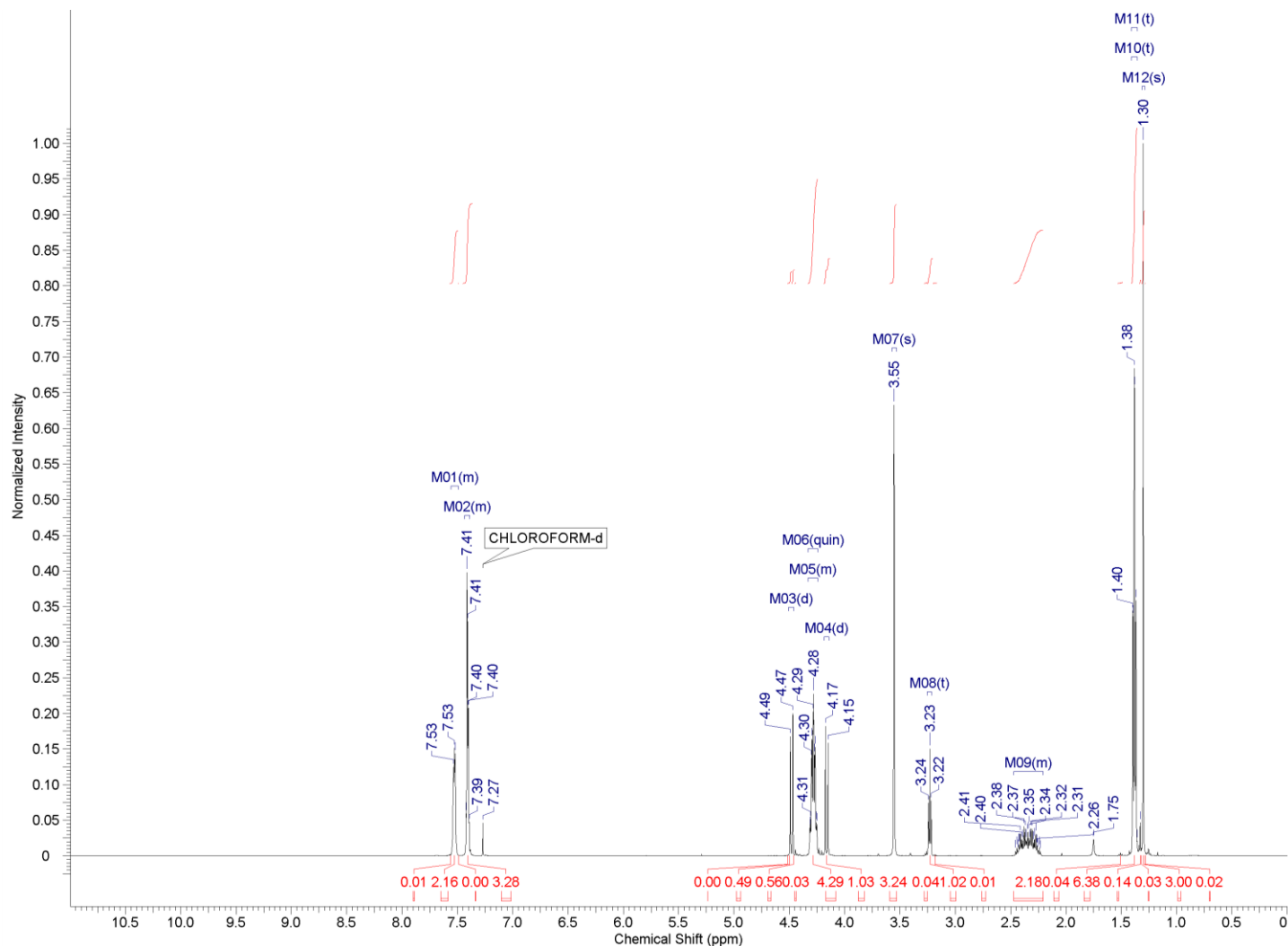


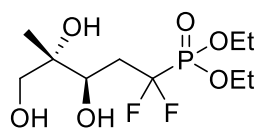
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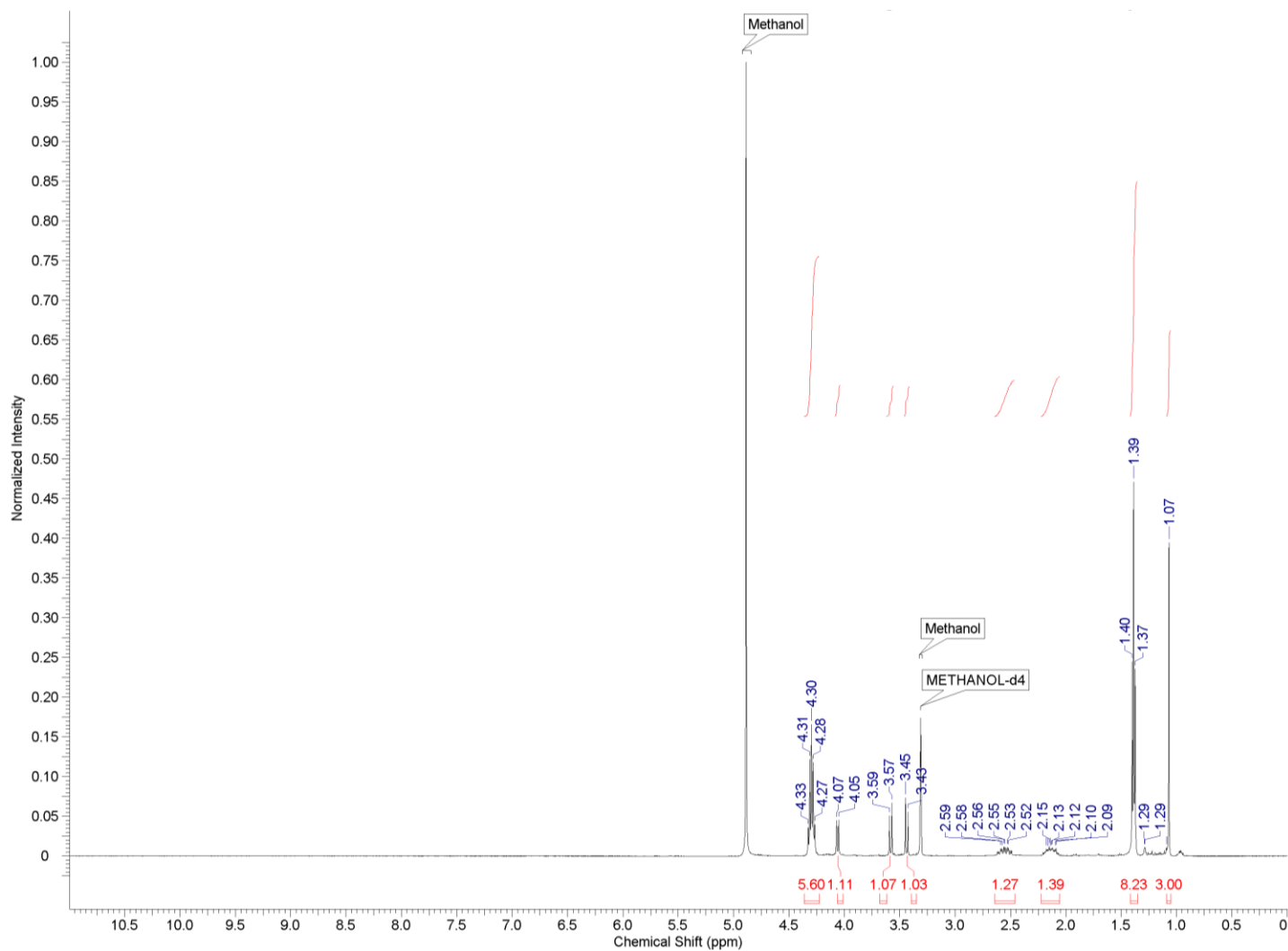


Mosher's Acid Derivative of 14

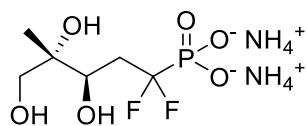




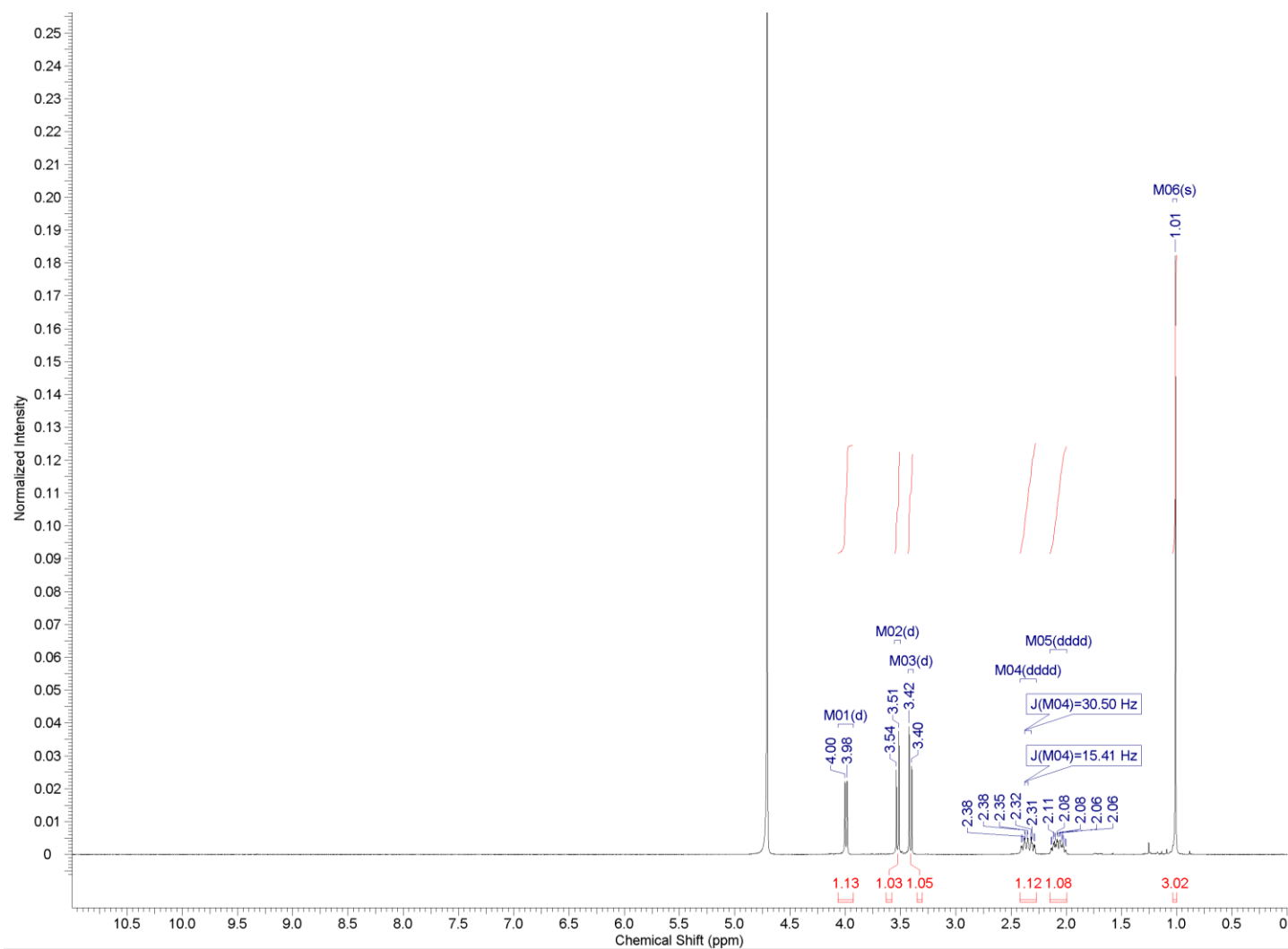
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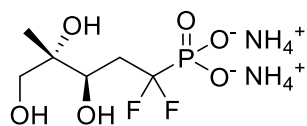




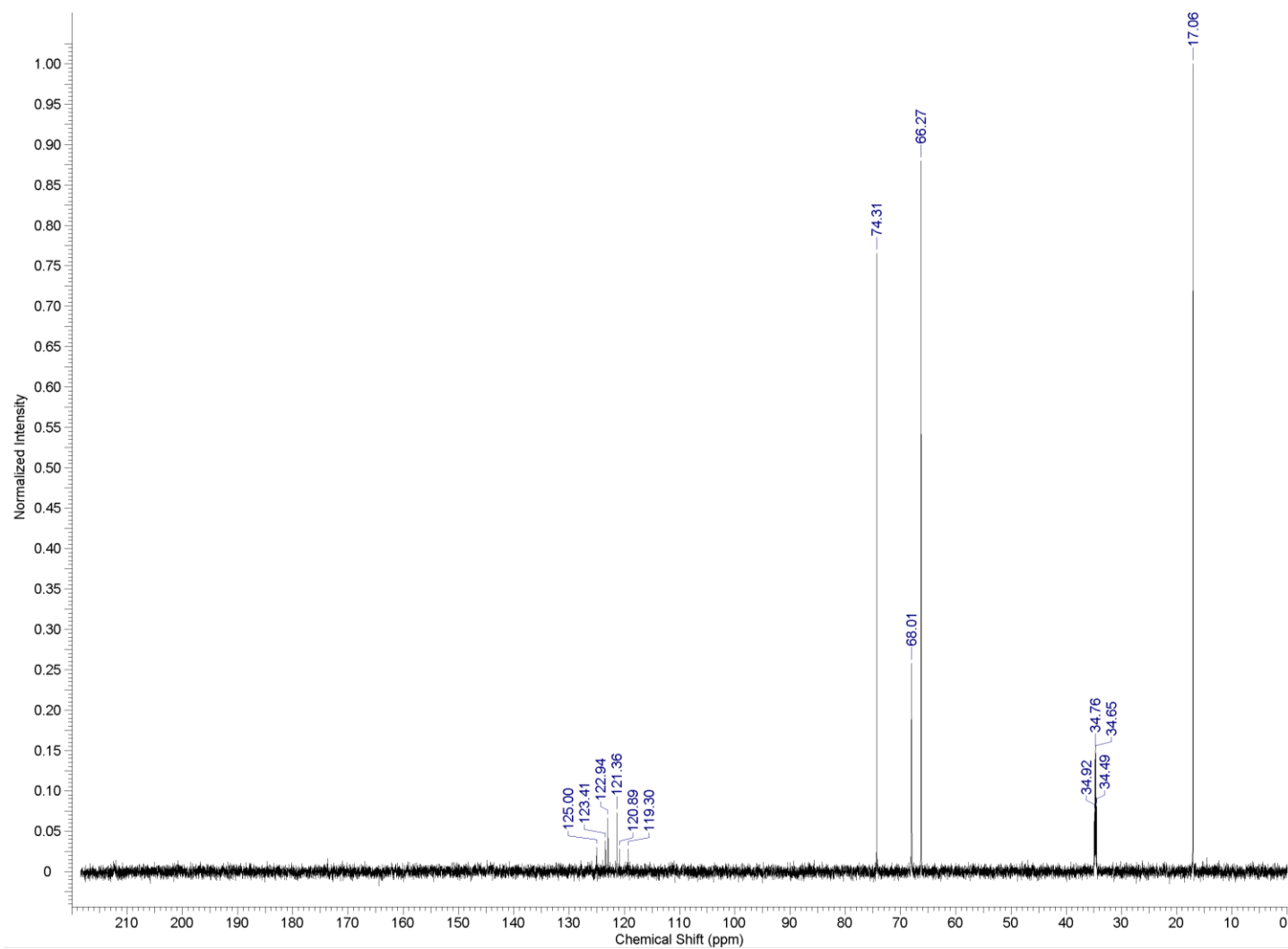


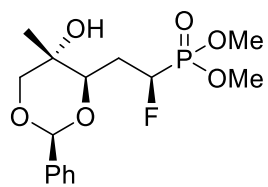
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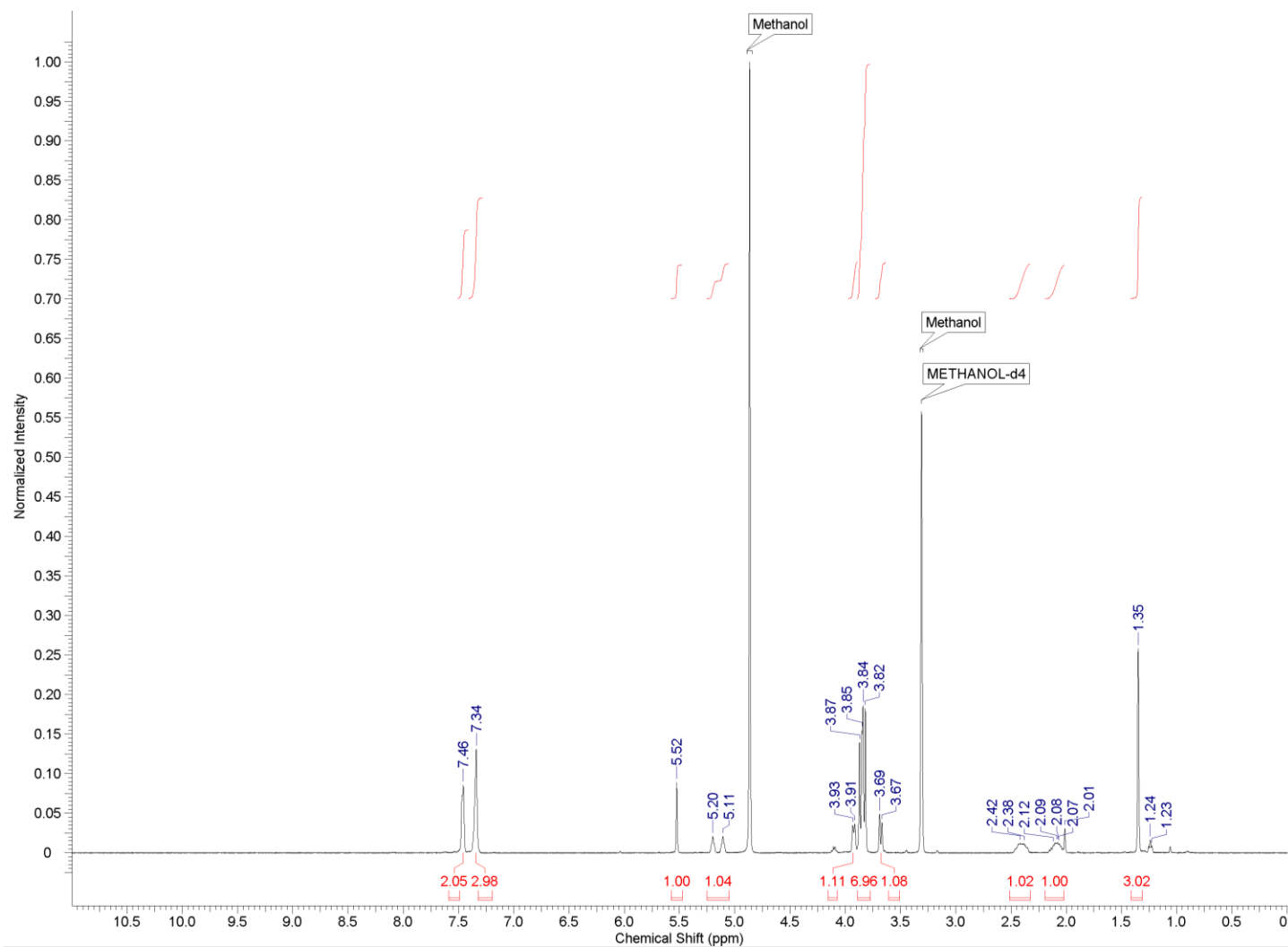


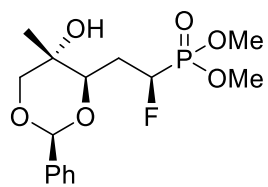
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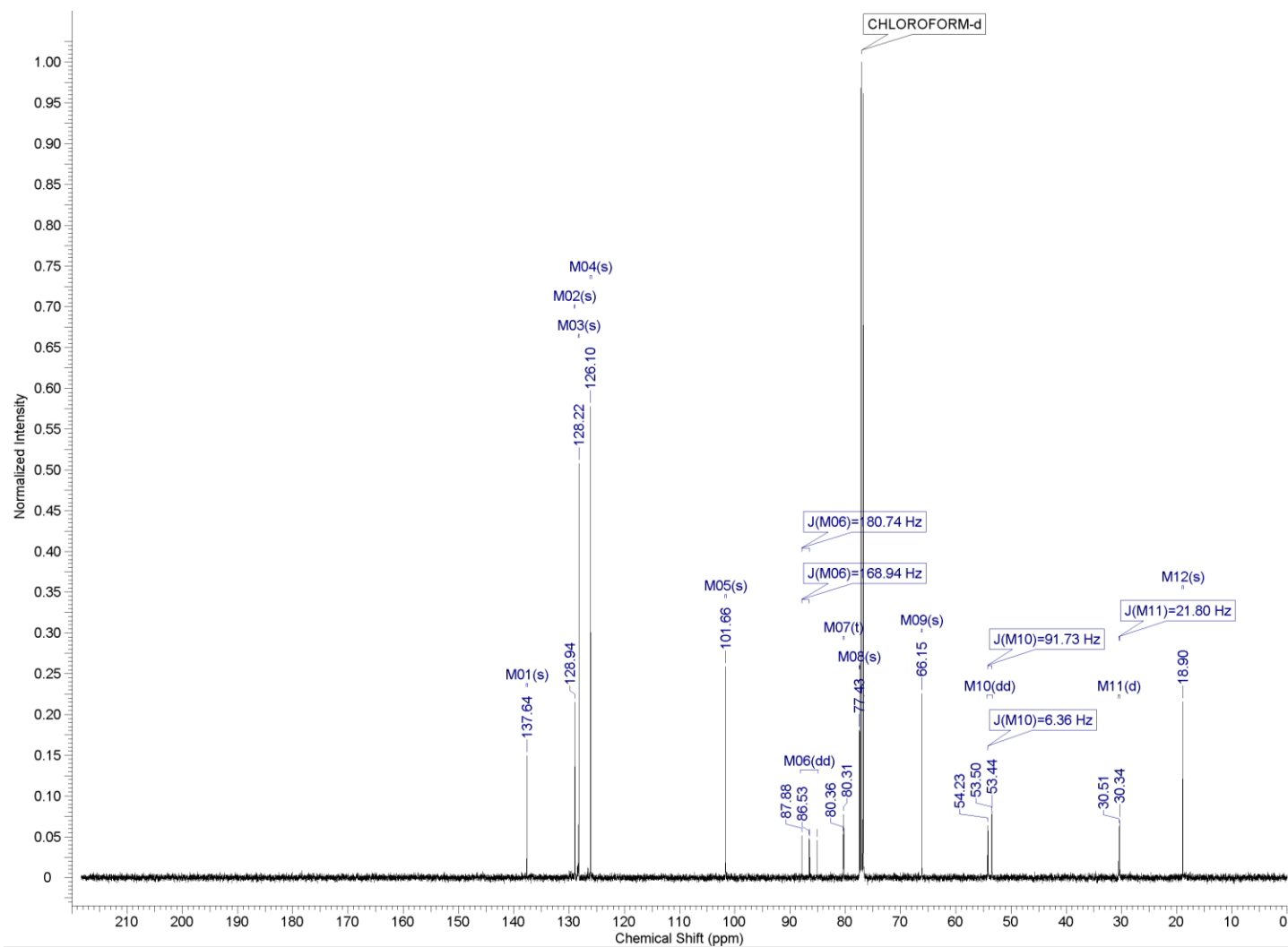


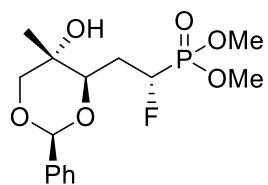
16a



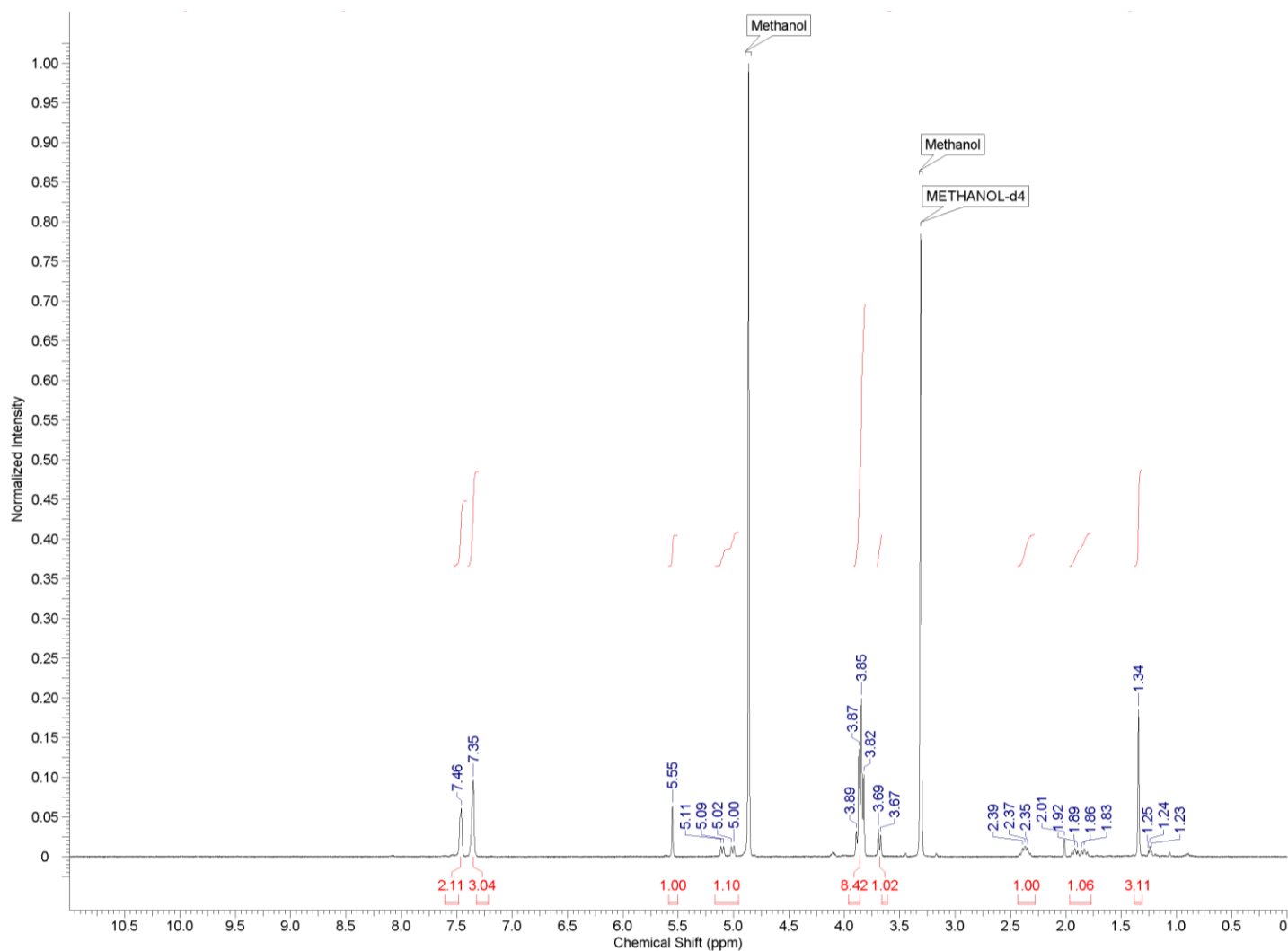


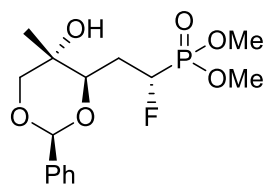
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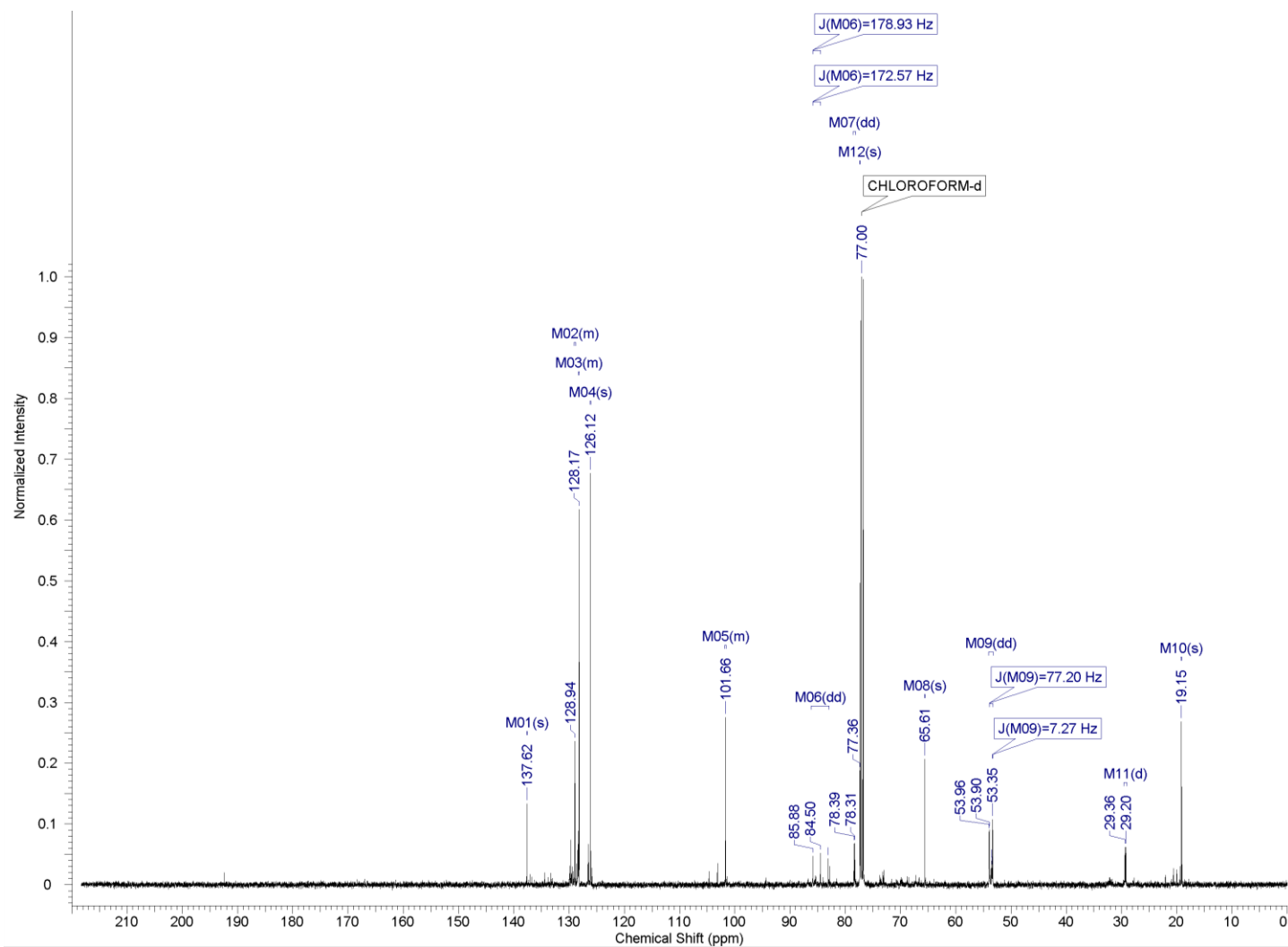


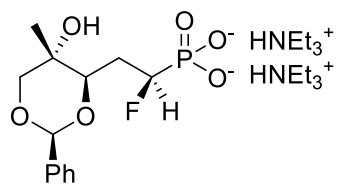
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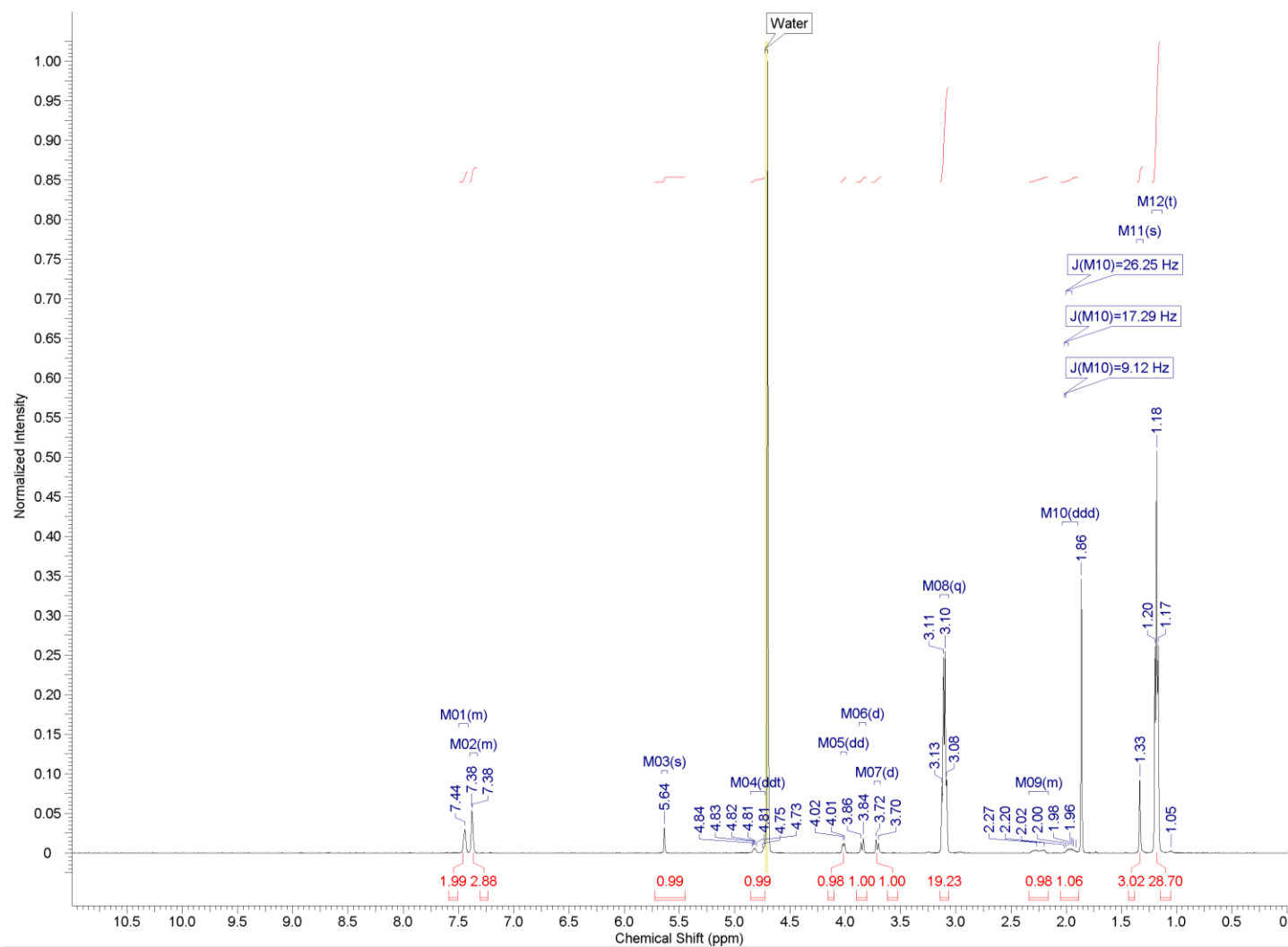


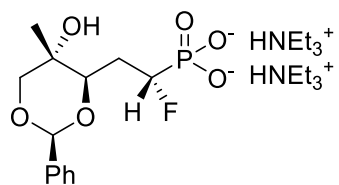
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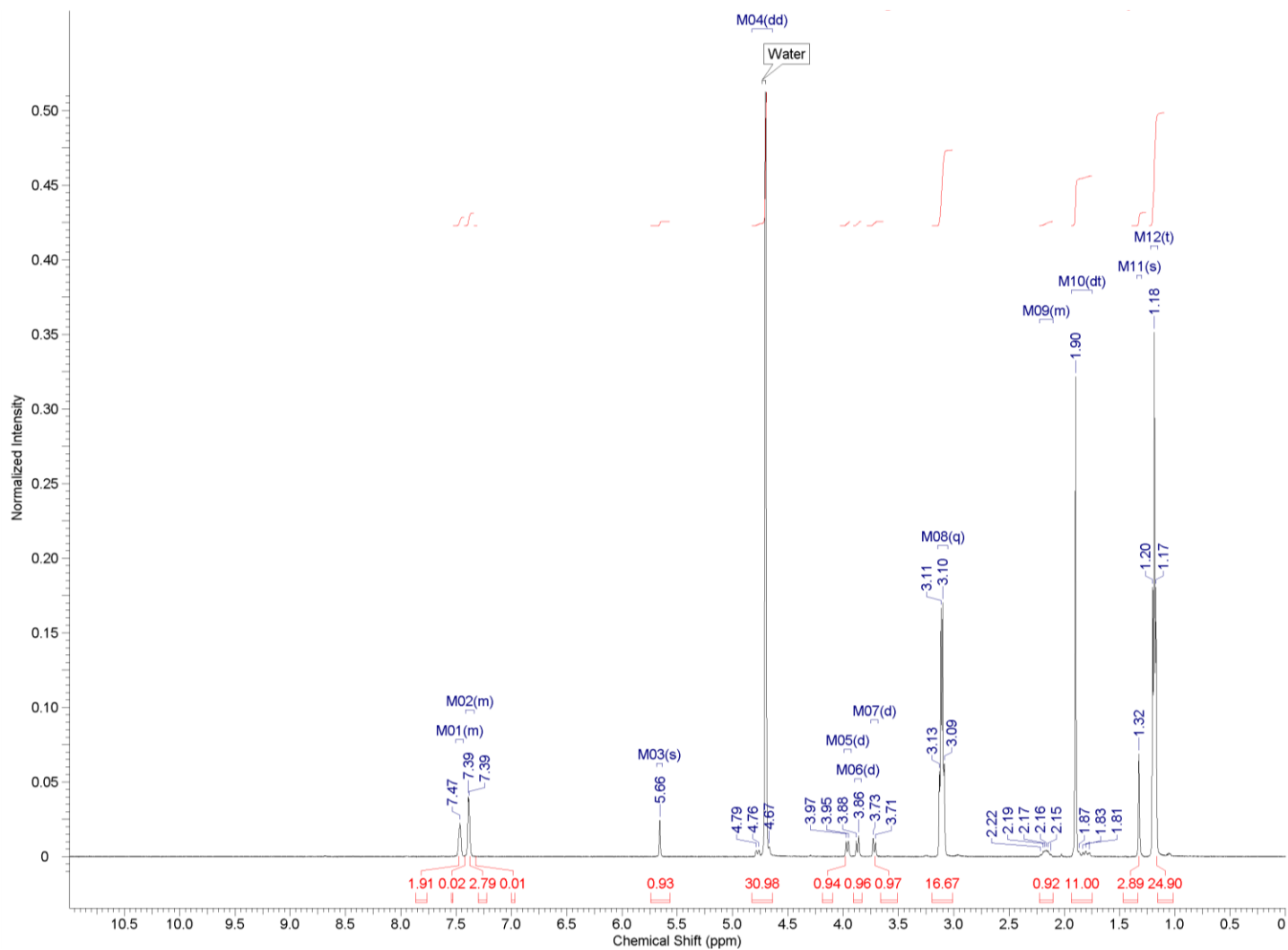


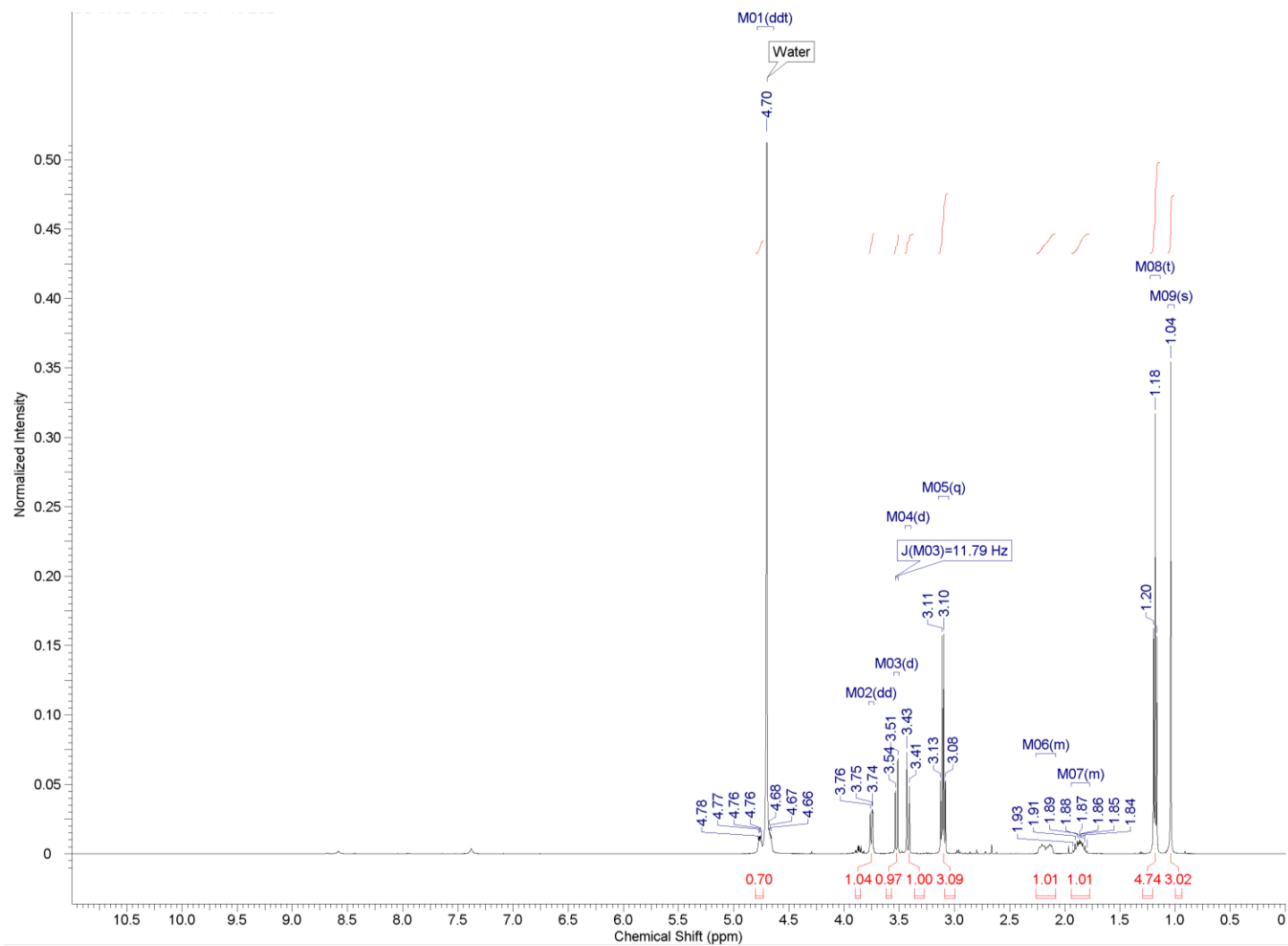
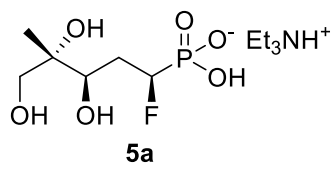
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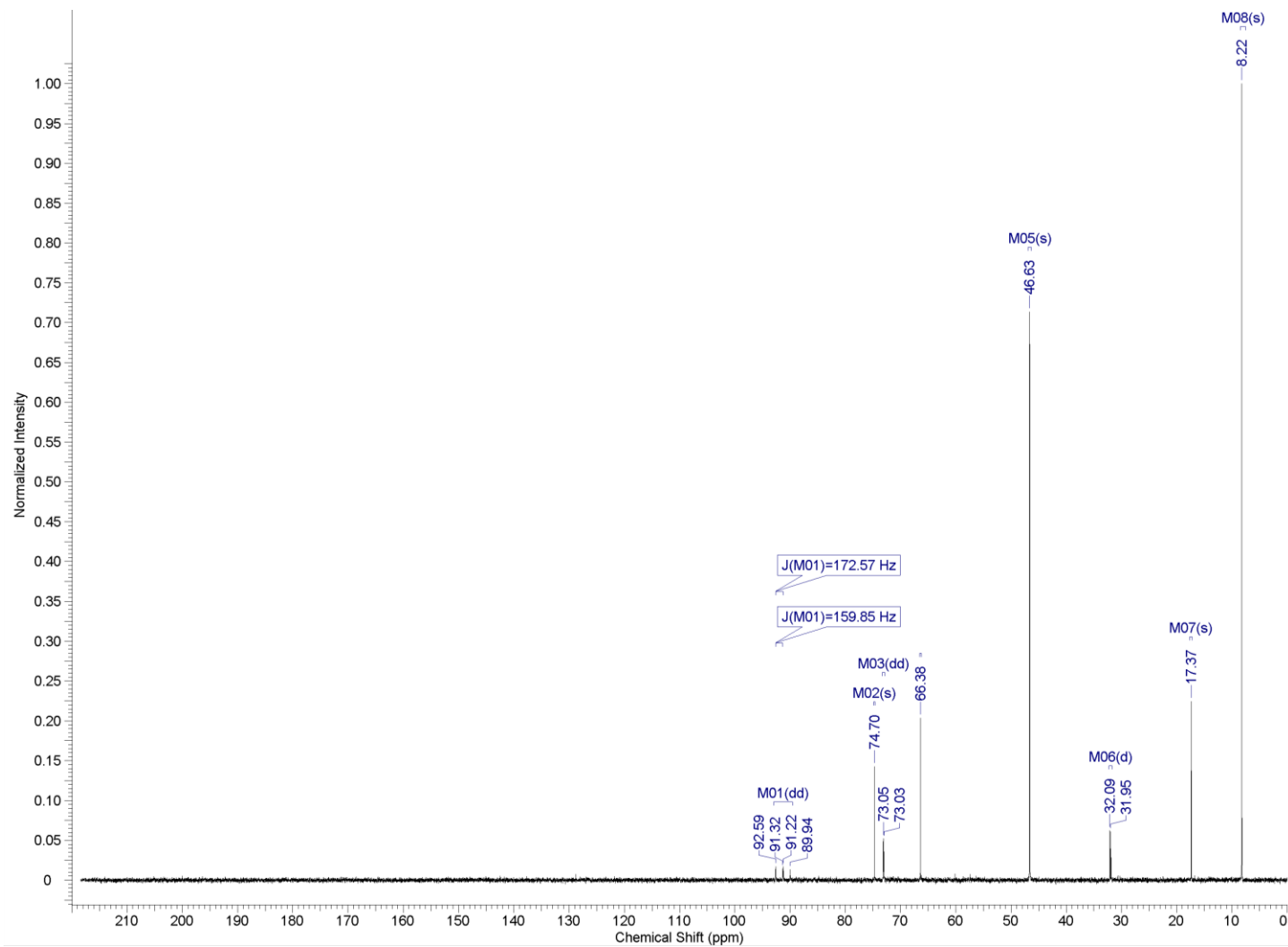
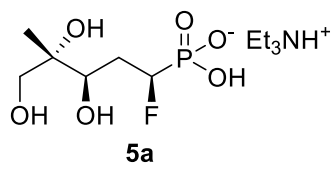


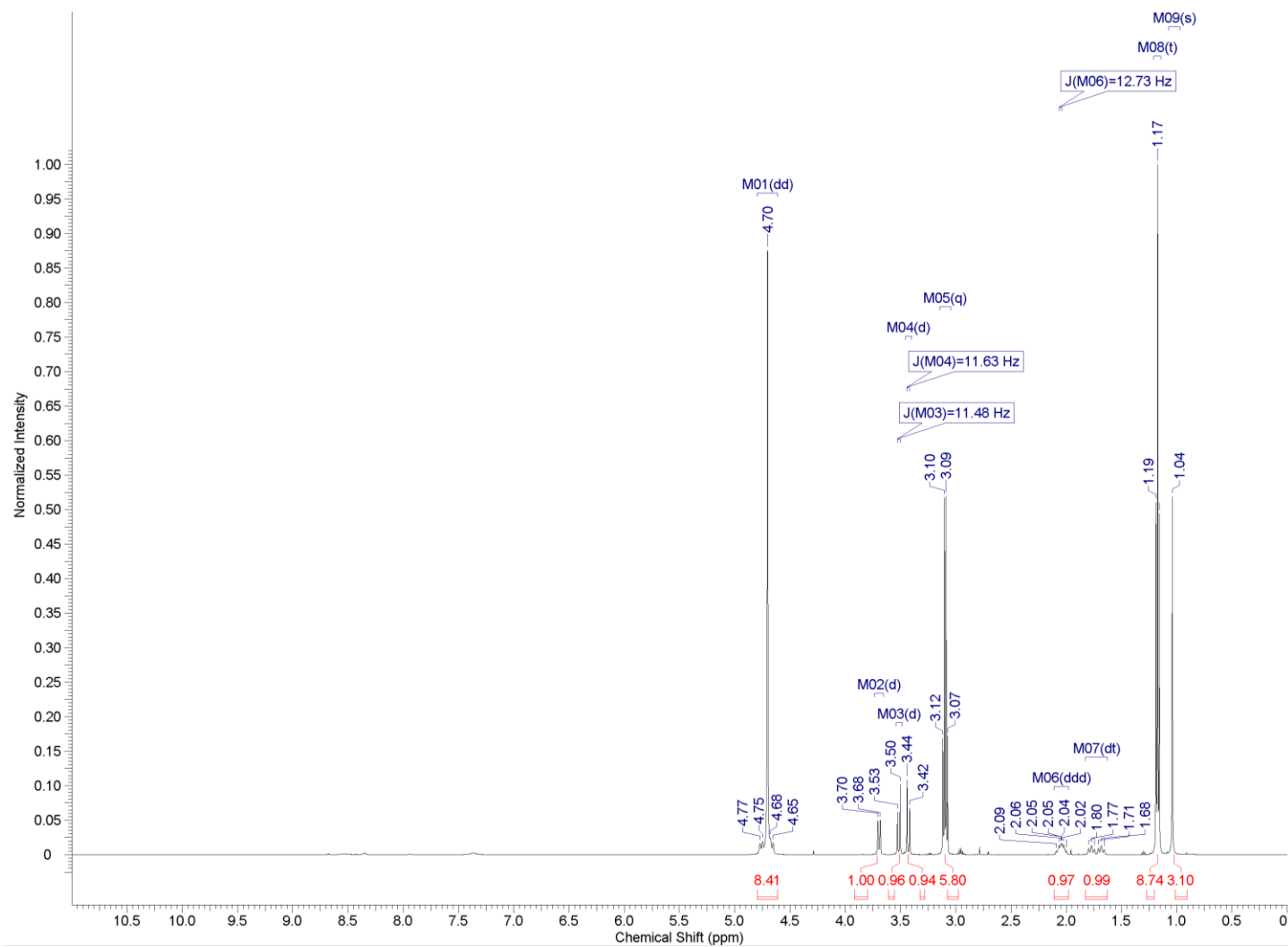
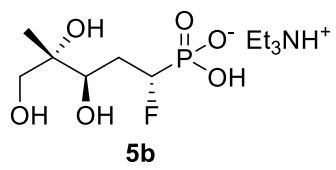


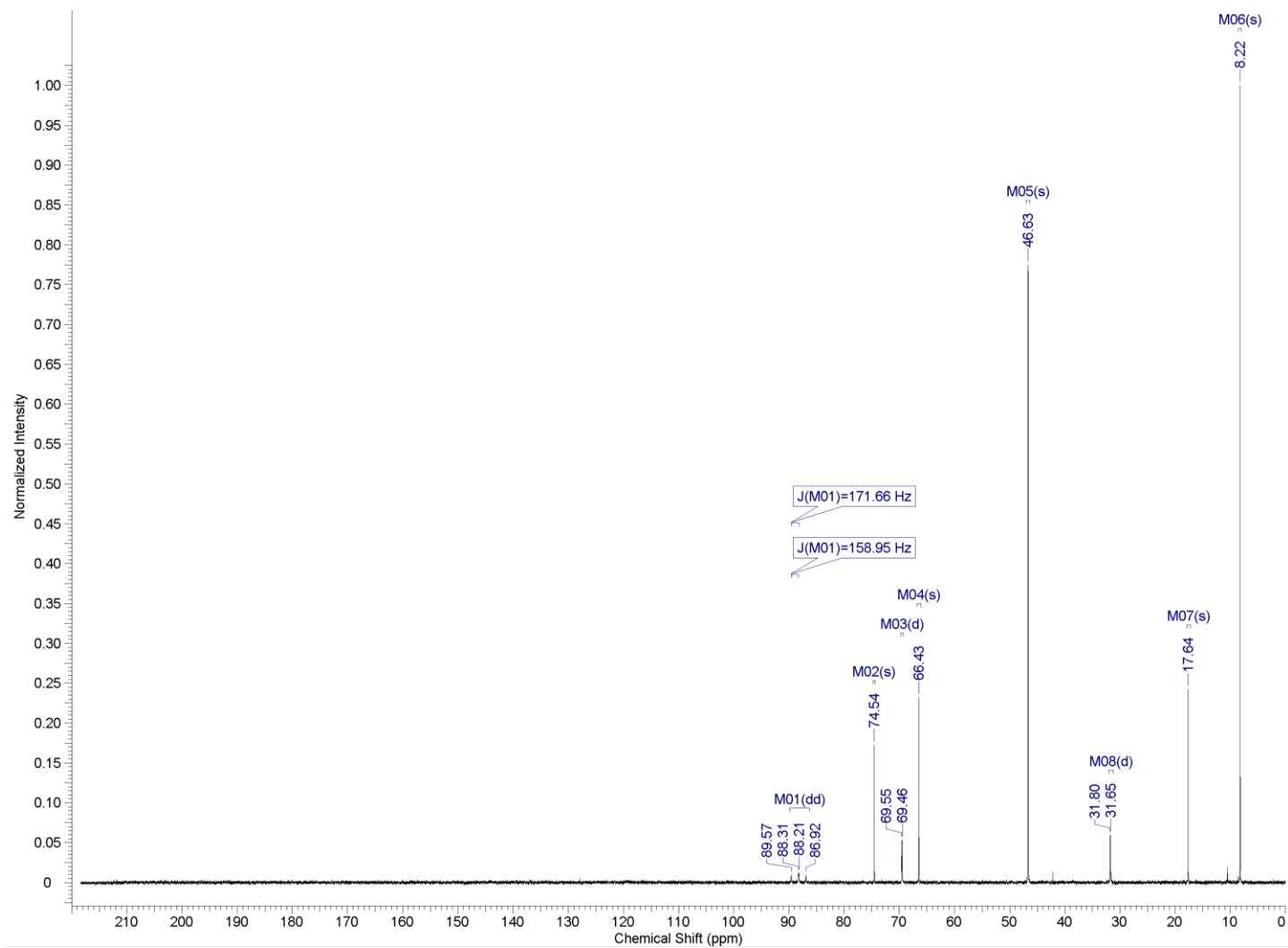
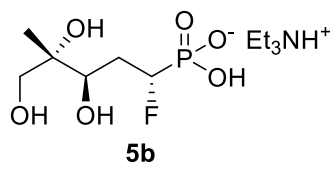
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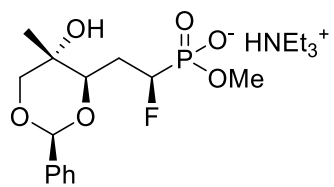




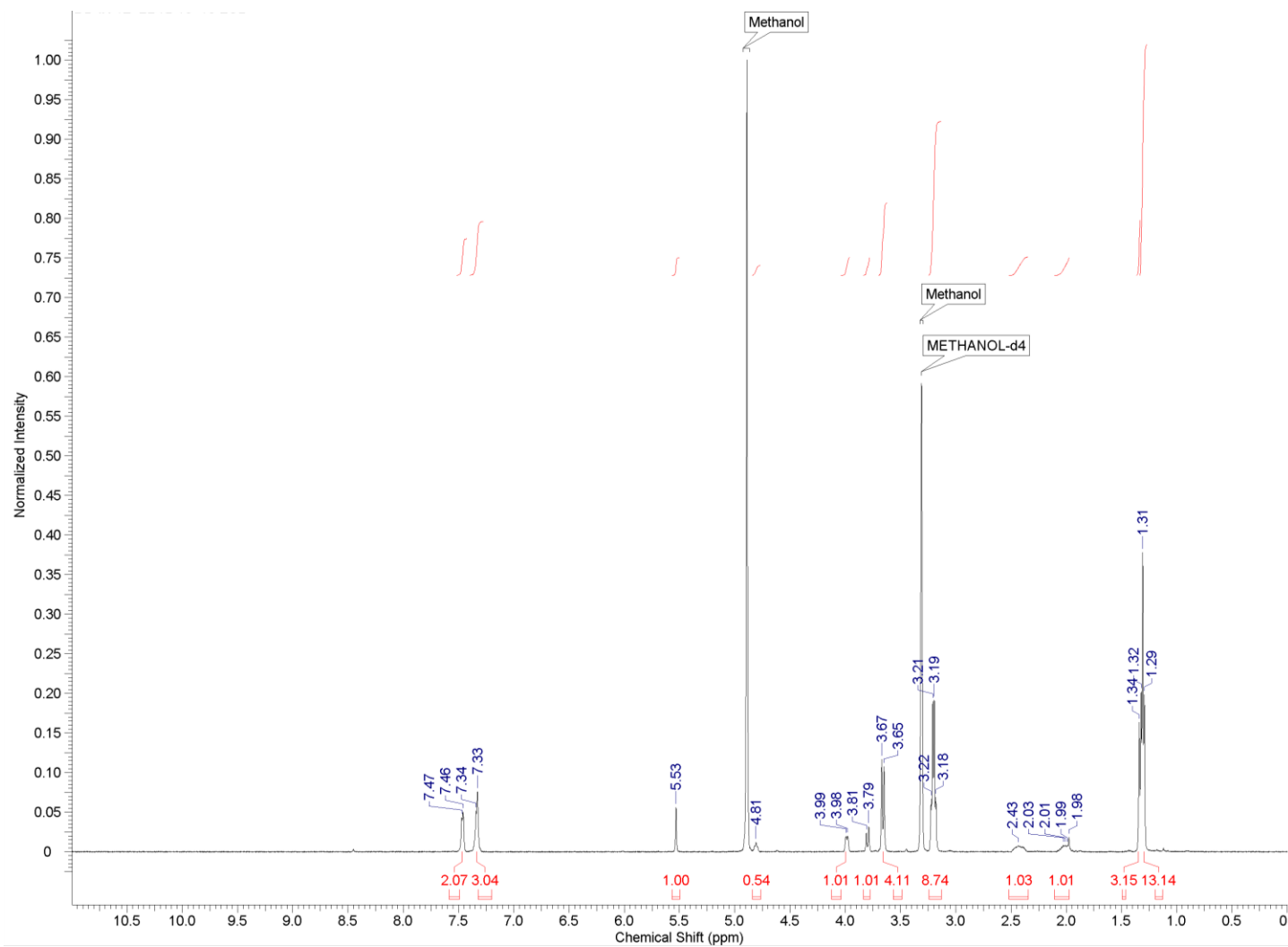


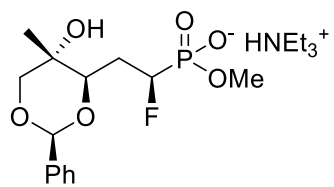




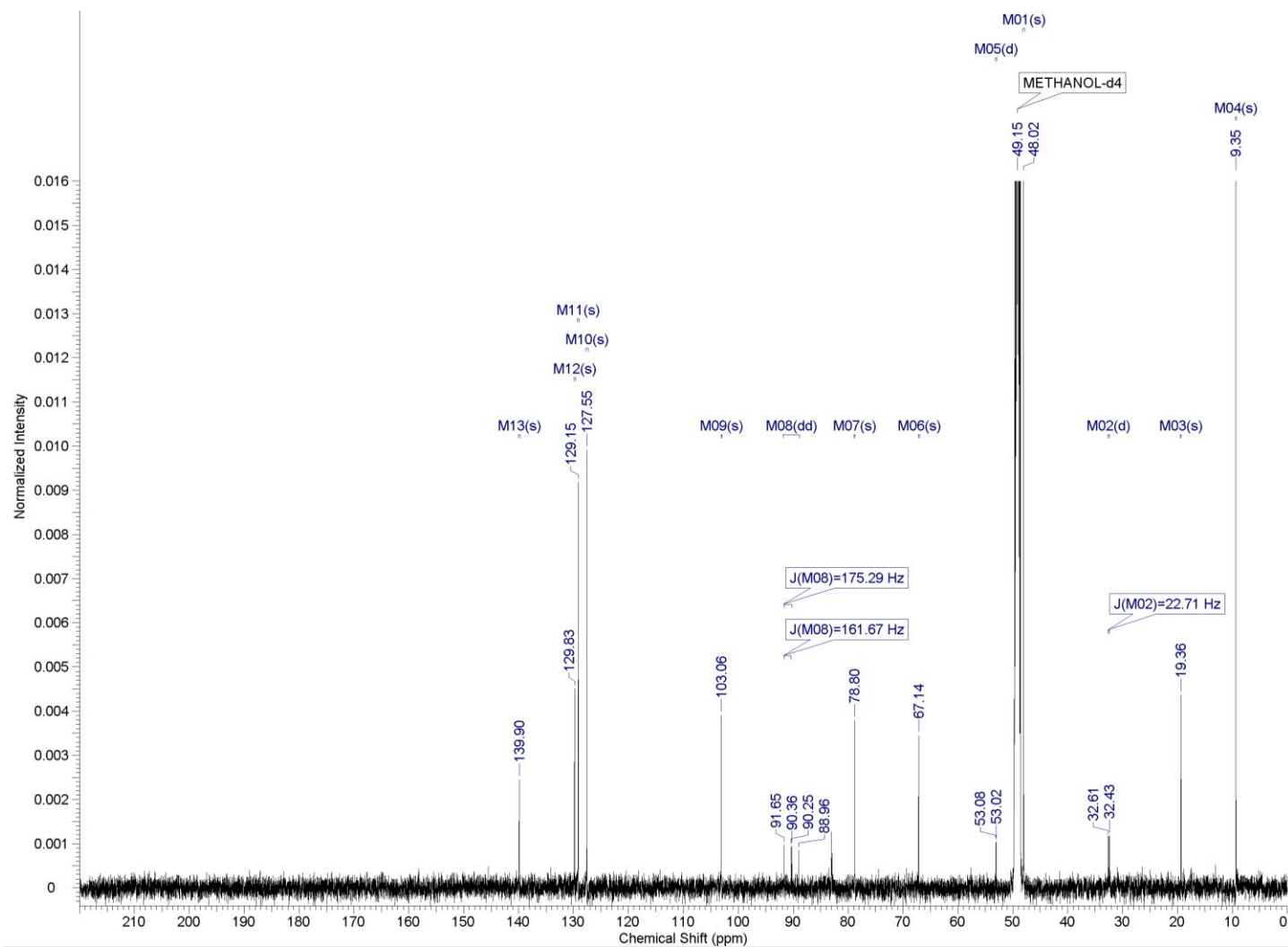


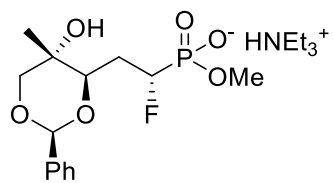
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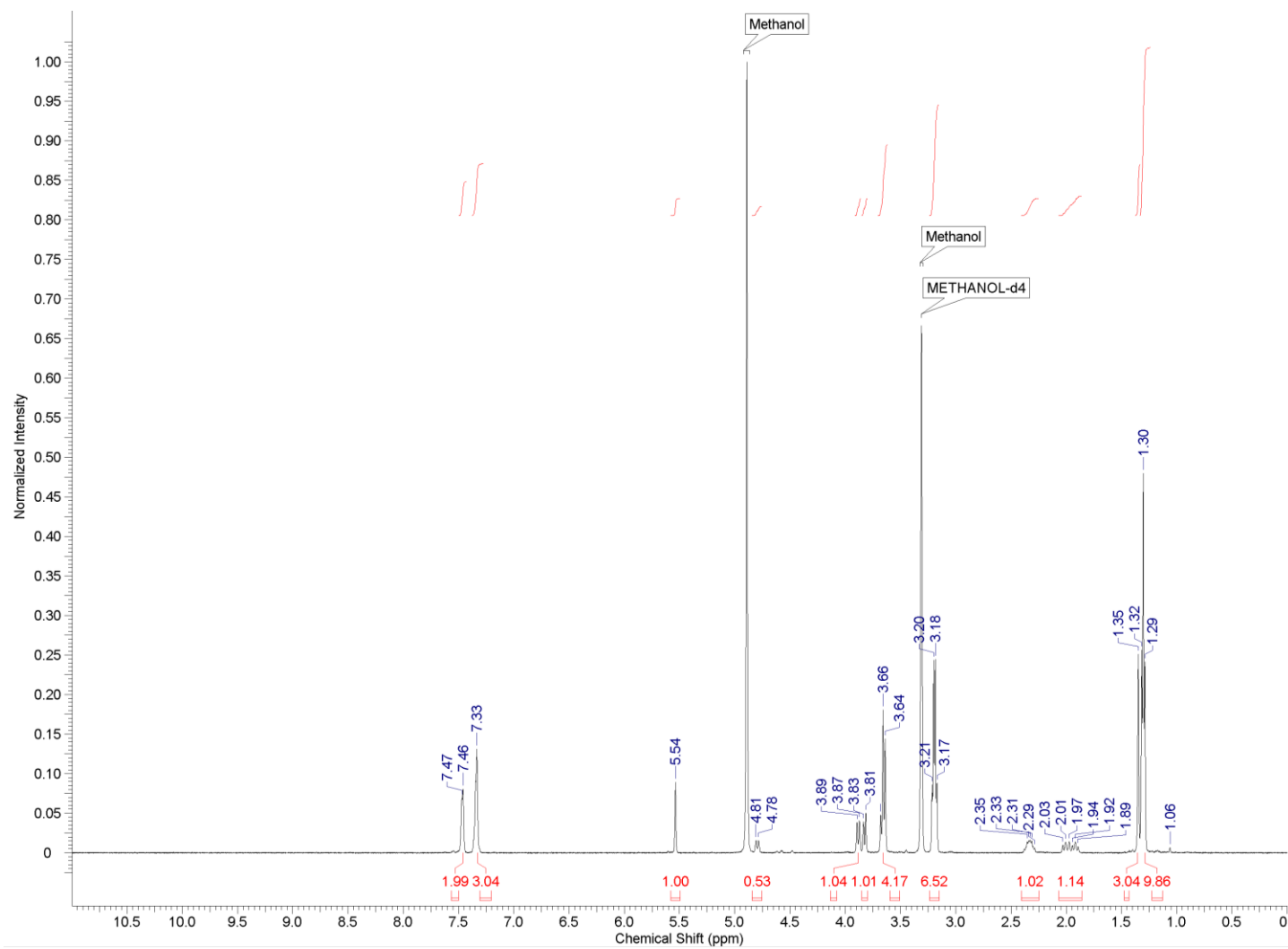


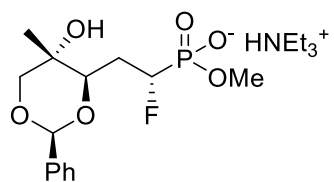
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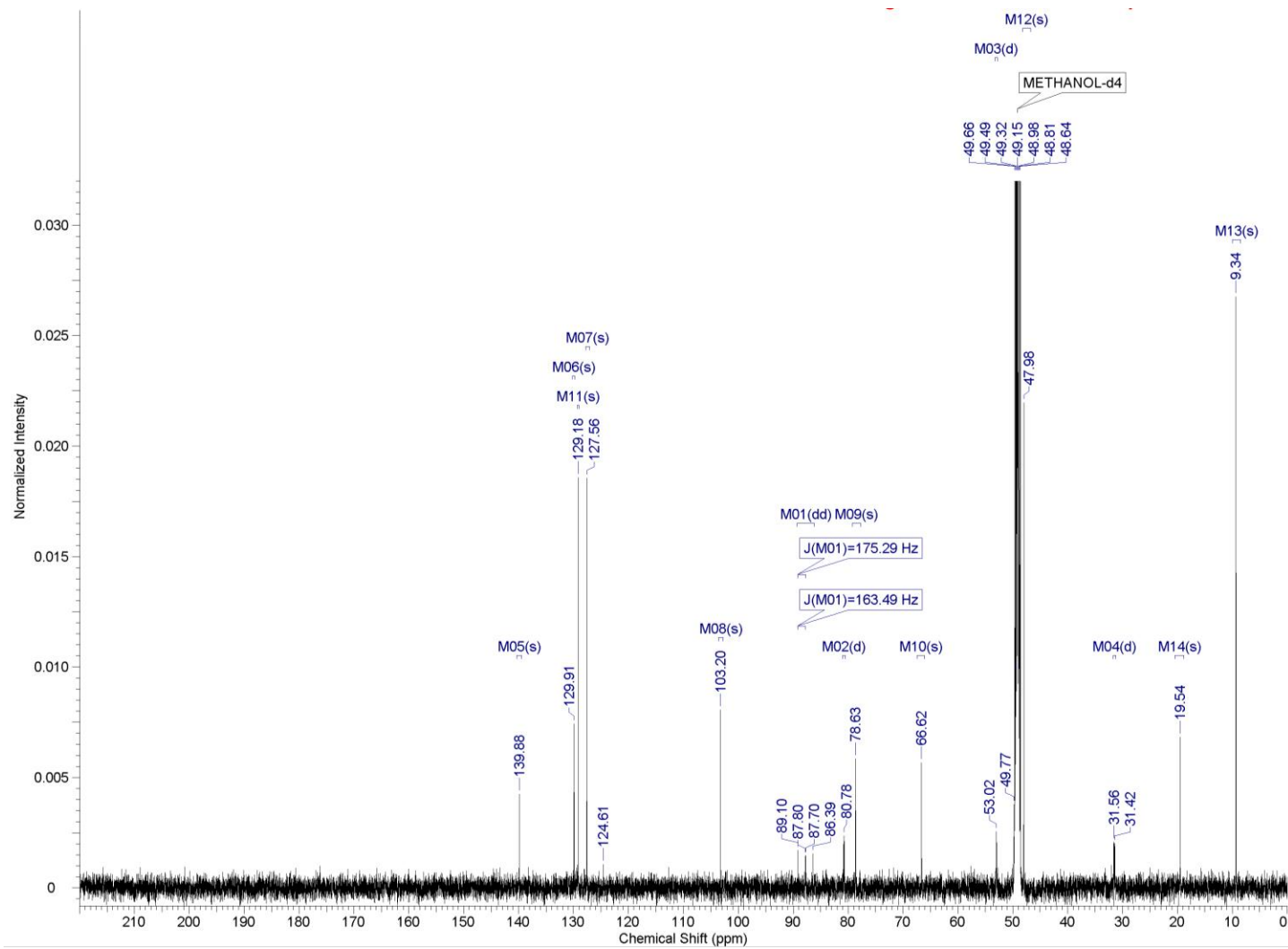


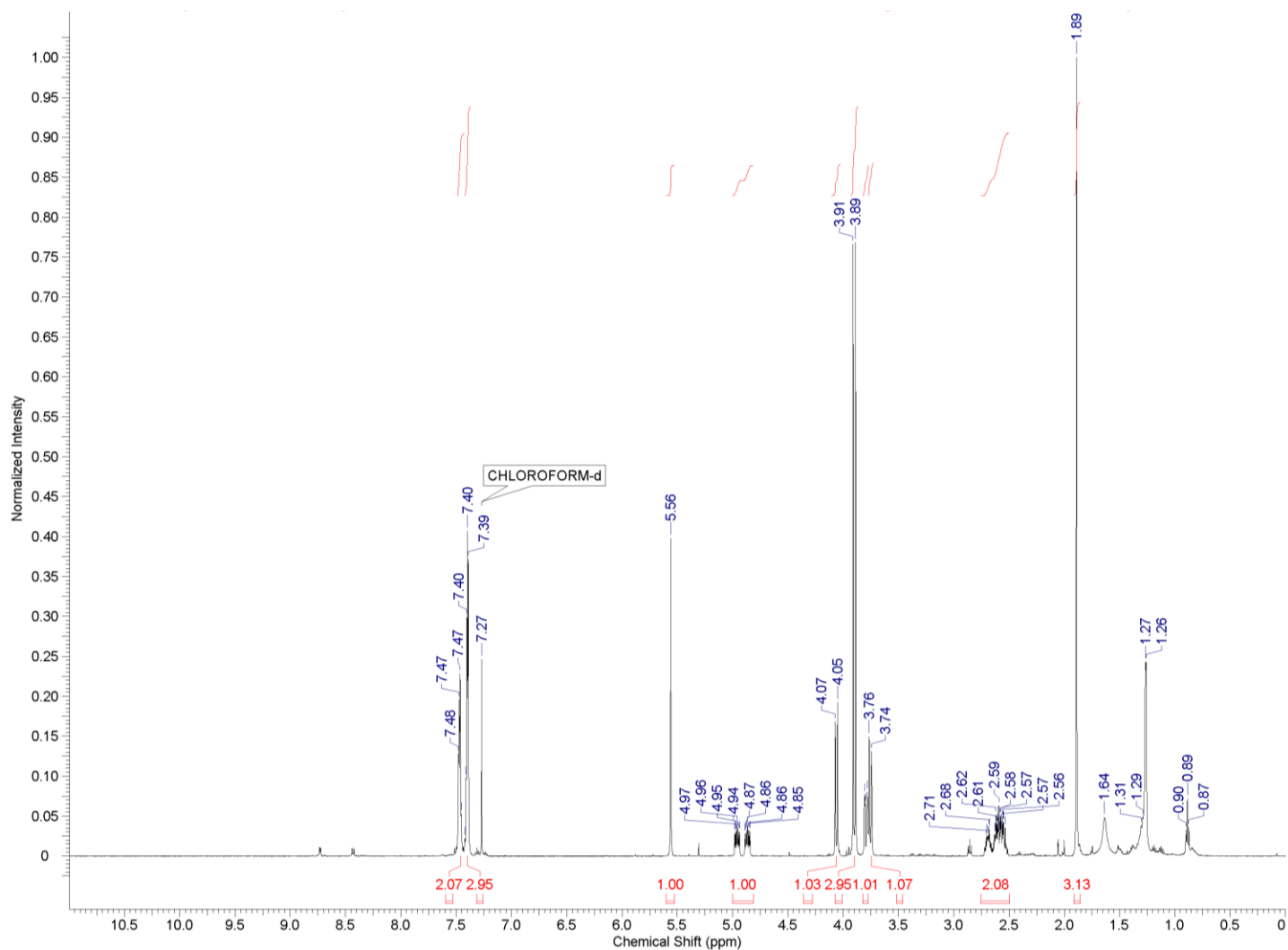
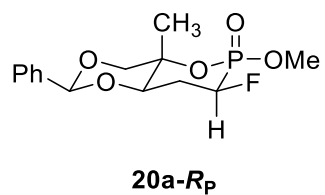
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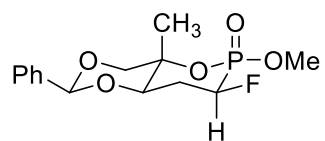




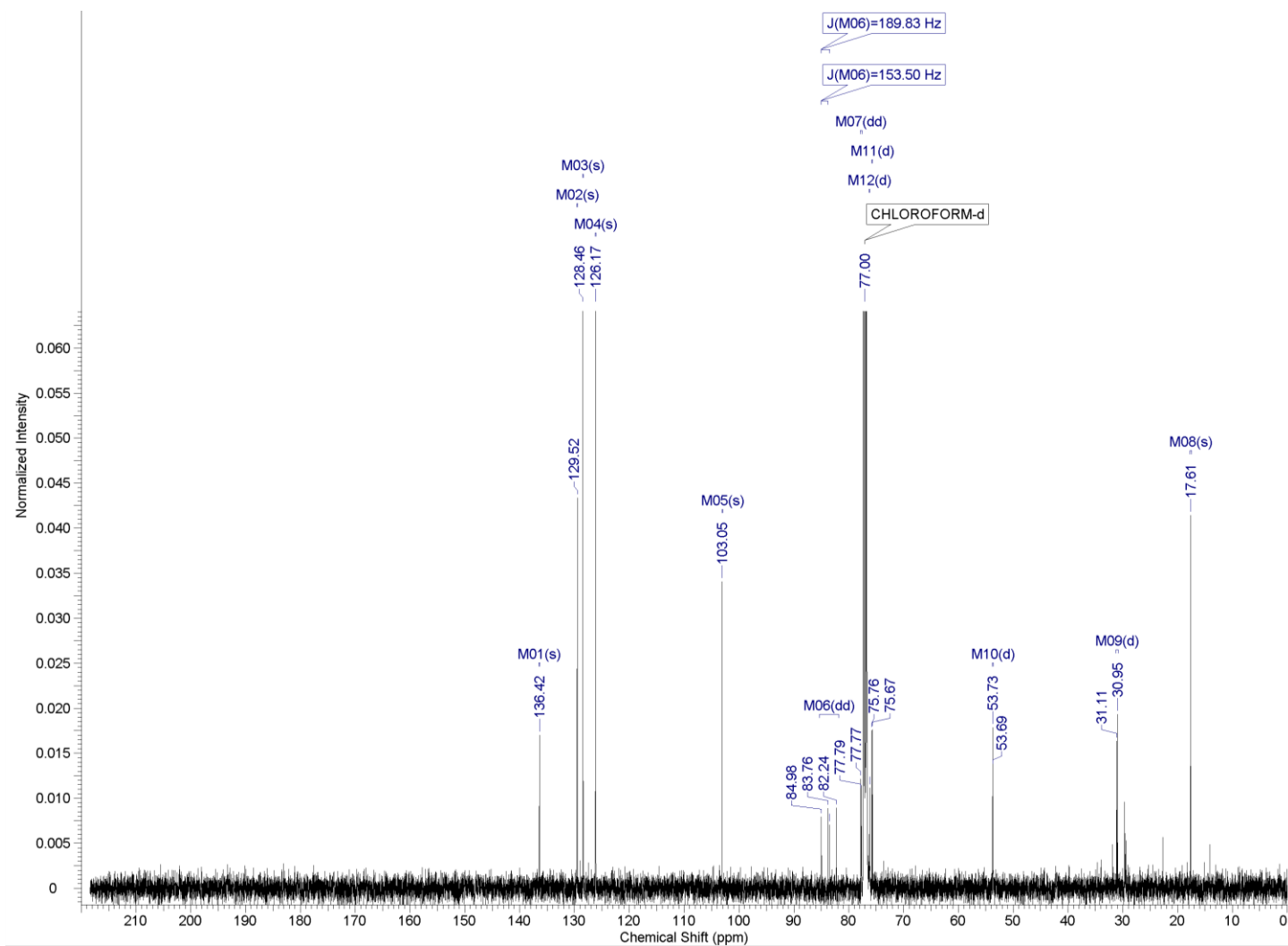
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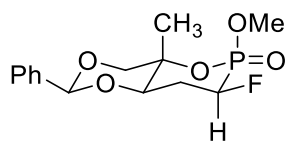




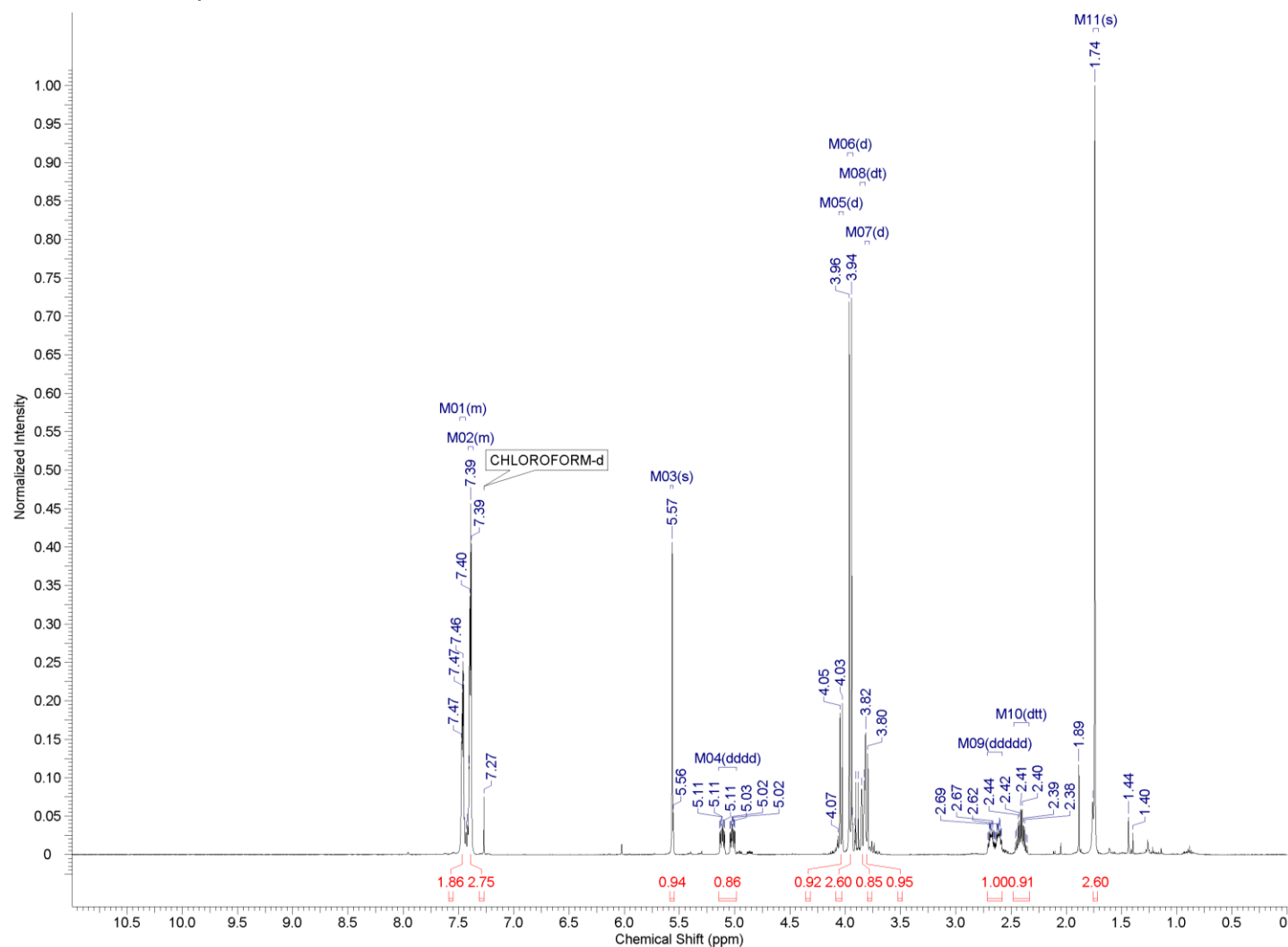


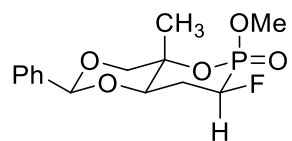
20a-*R_p*



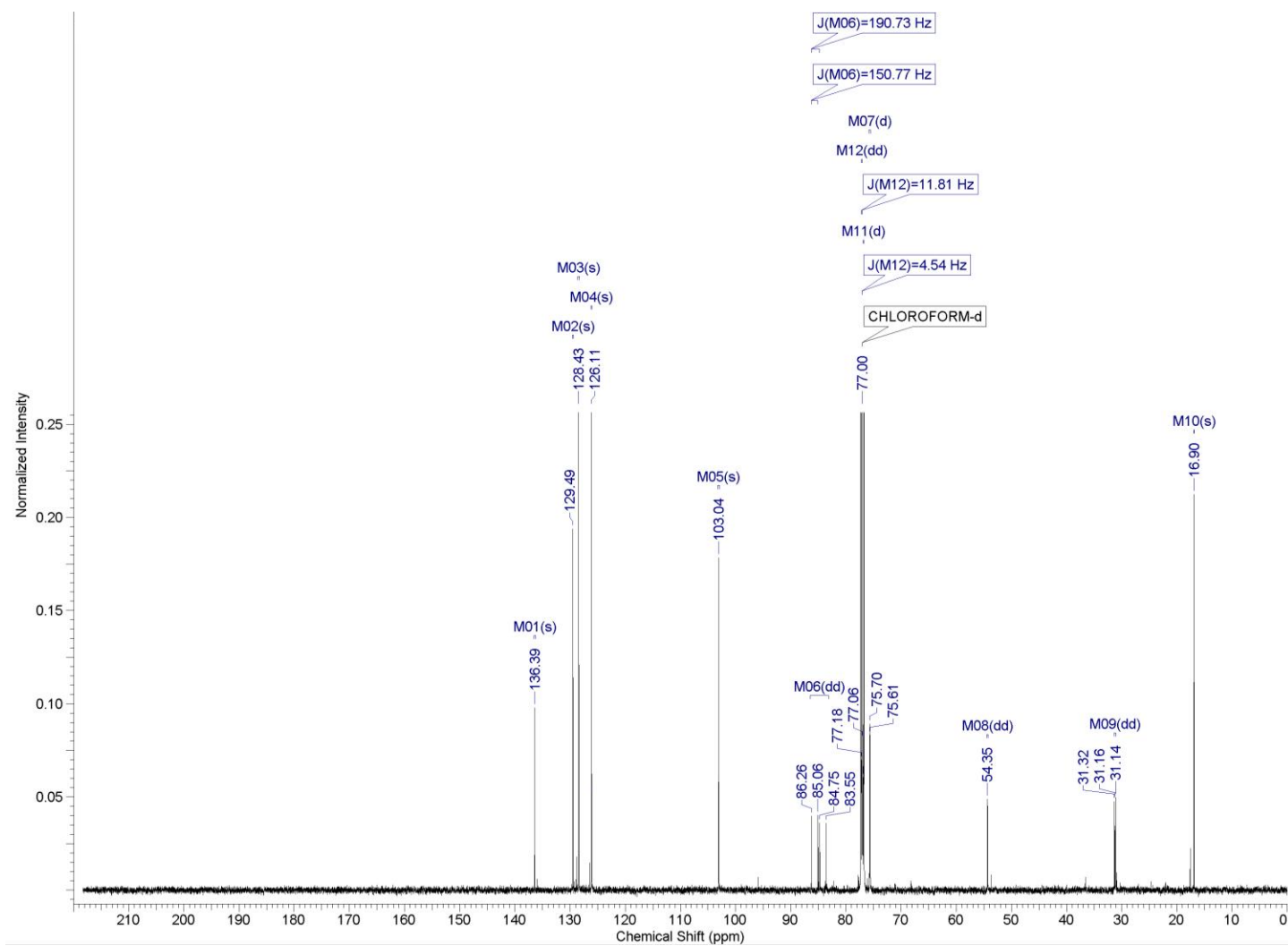


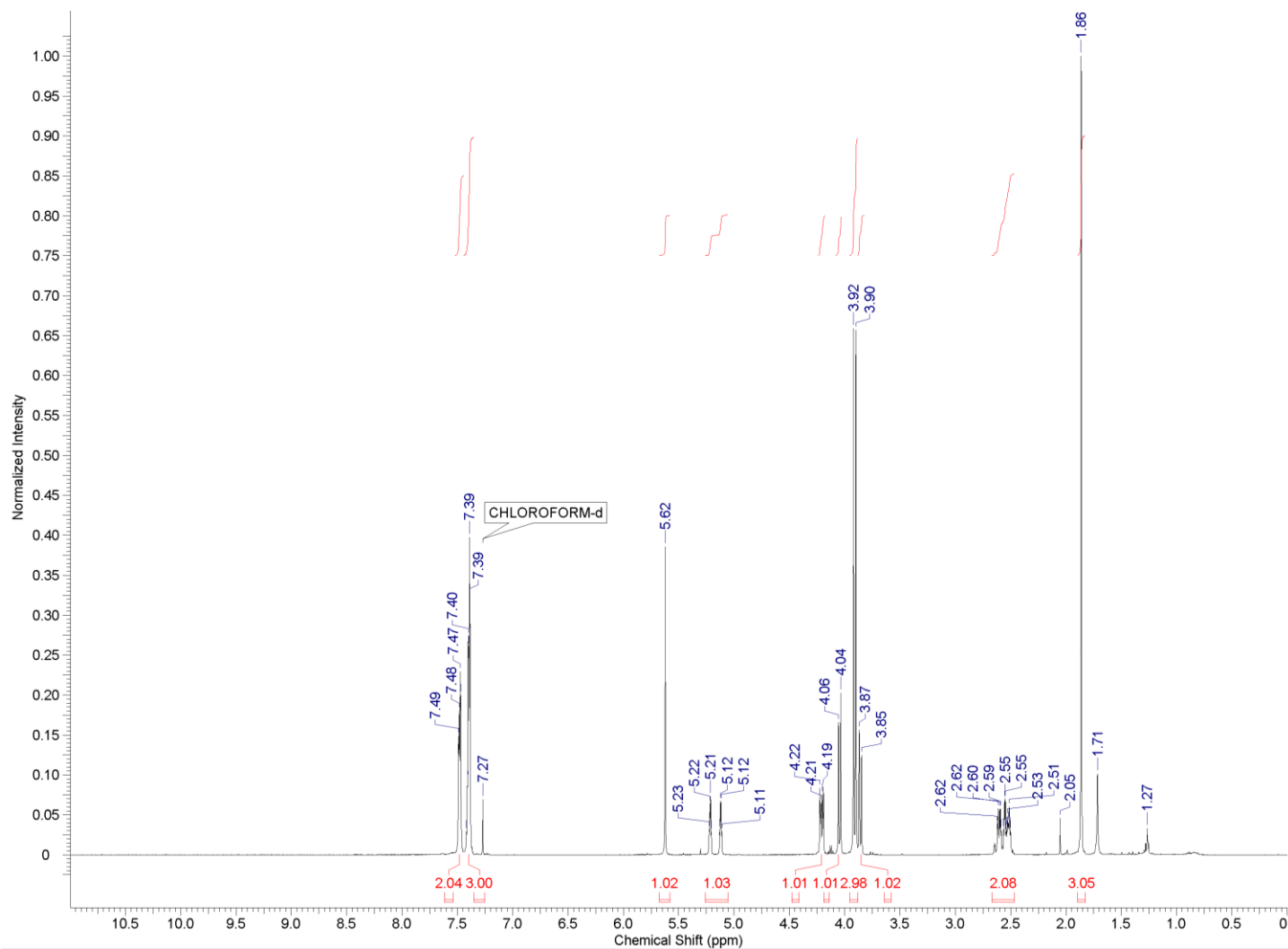
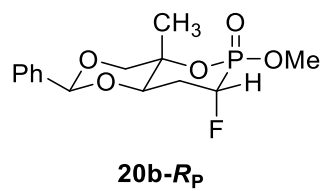
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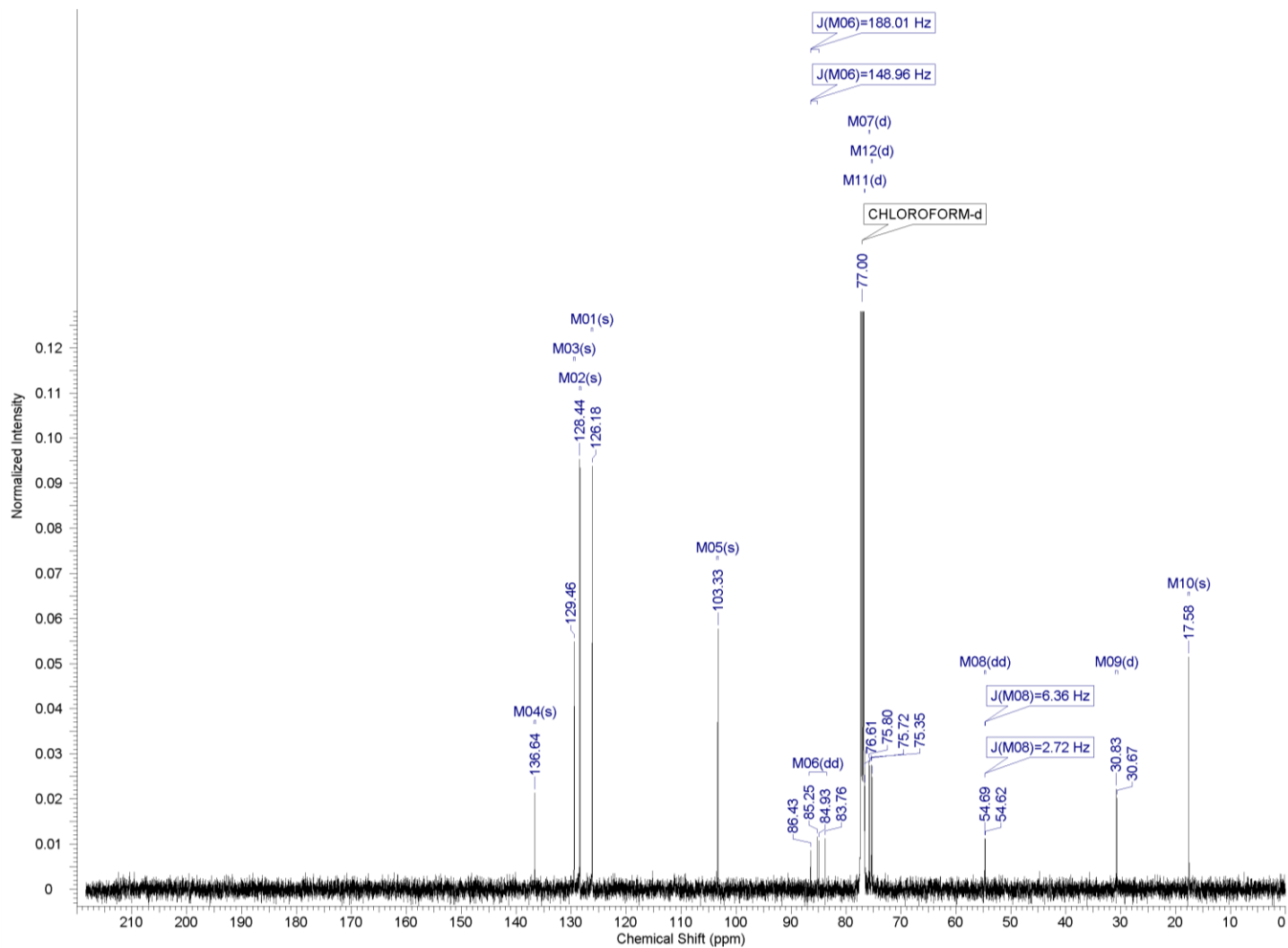
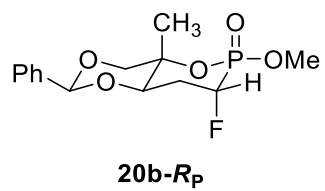


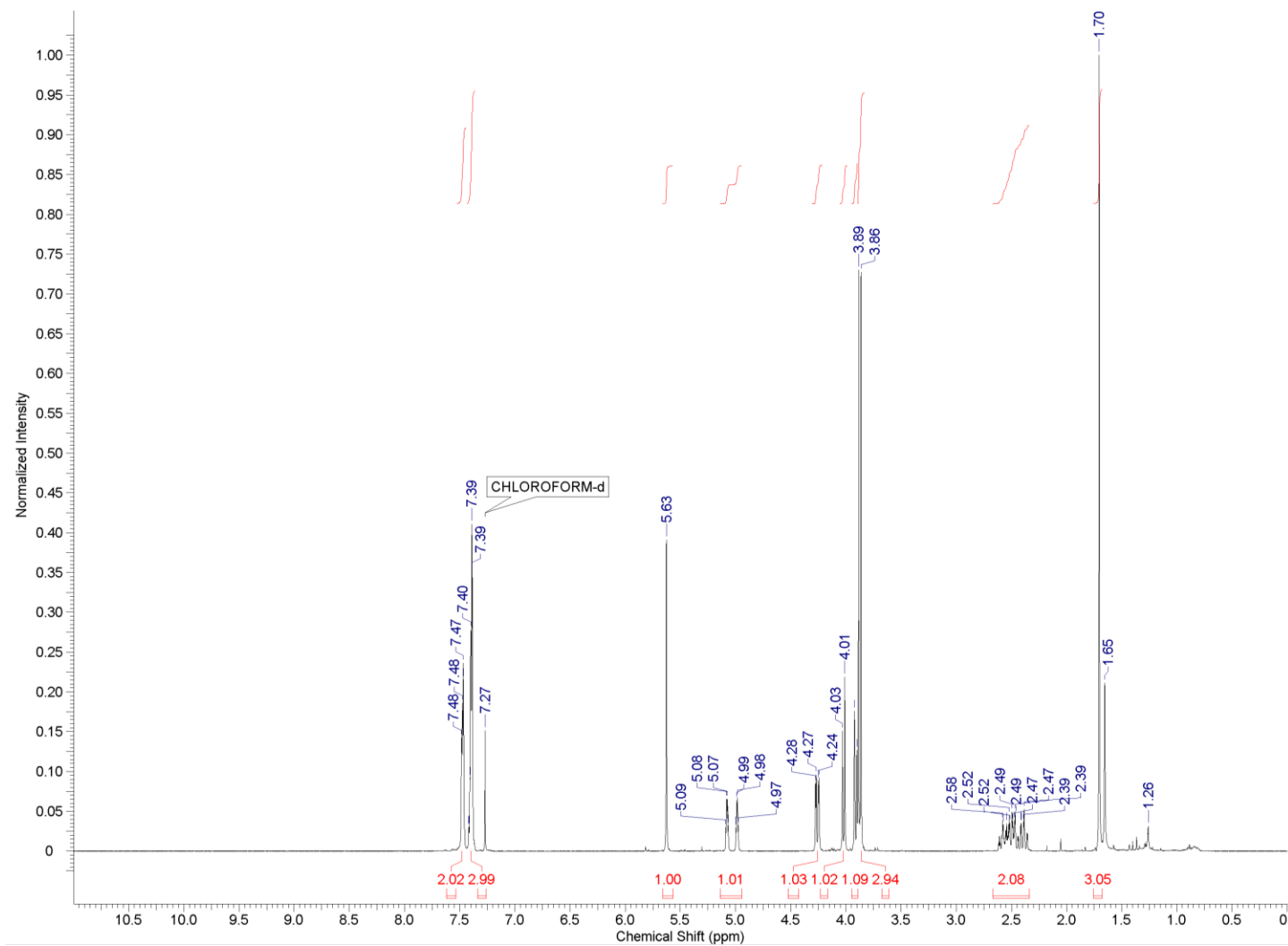
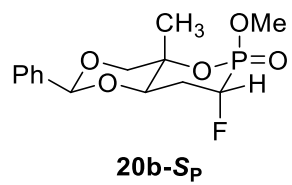


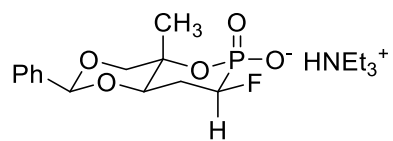
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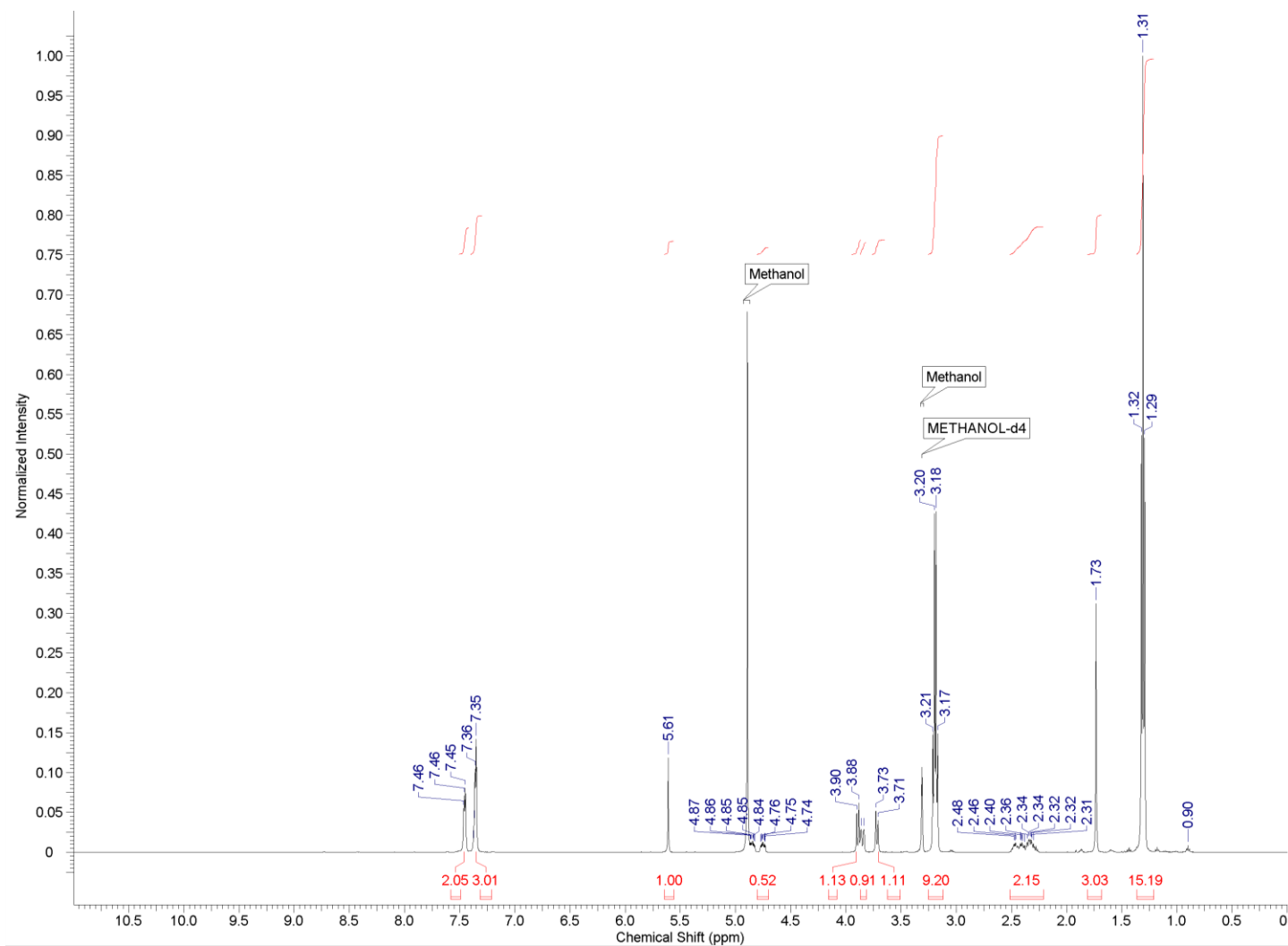


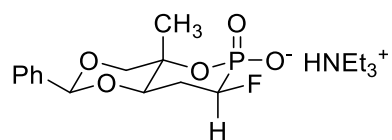






21a





21a

