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

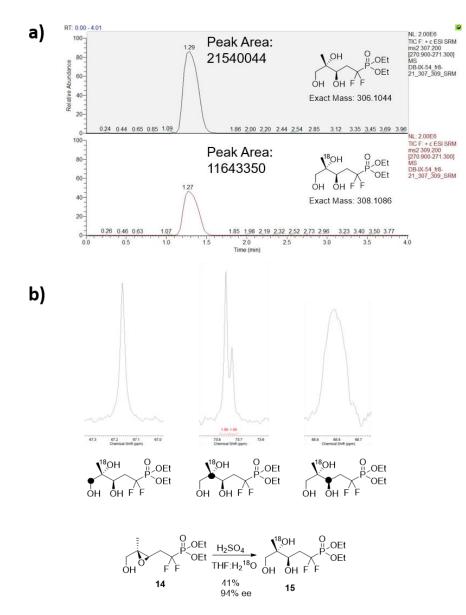
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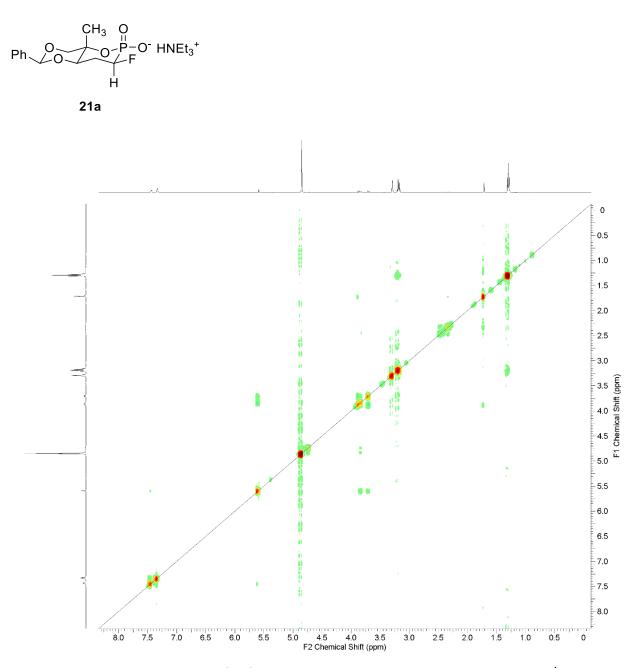
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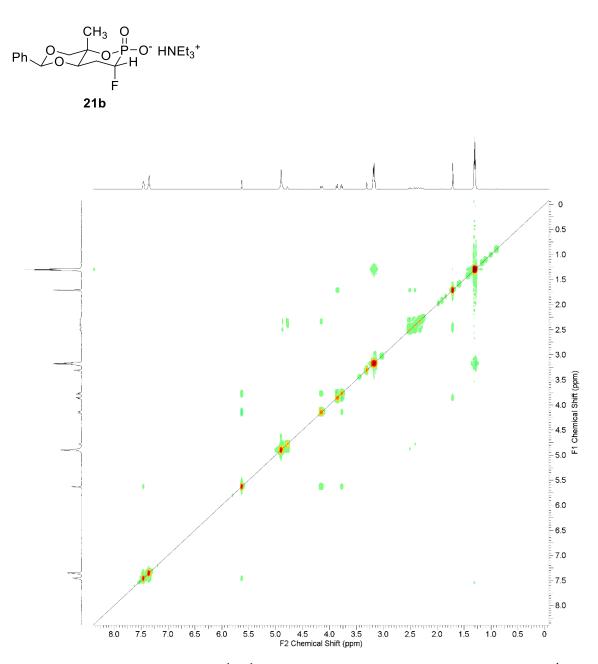
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**Figure S1. Regioselectivity of the acid-catalyzed ring-opening of epoxide 14.** Epoxide **14** was treated with H<sub>2</sub>SO<sub>4</sub> solution (5M in H<sub>2</sub>O, ~98% <sup>18</sup>O atom). a) The total <sup>18</sup>O incorporation was determined by LC-MS/MS to be 34%. The diminished incorporation is likely due to <sup>16</sup>O/<sup>18</sup>O exchange between the H<sub>2</sub><sup>18</sup>O and the H<sub>2</sub>S<sup>16</sup>O<sub>4</sub>. b) The regioselectivity of <sup>18</sup>O was determined by <sup>13</sup>C NMR. The <sup>18</sup>O induces an upfield  $\Delta\delta$  of 30 ppb revealing 33% <sup>18</sup>O incorporation at C<sub>2</sub> and no discernible incorporation at either C<sub>1</sub> or C<sub>3</sub>. The regioselectivity was determined to be 97% (94% ee) by taking the ratio of the <sup>18</sup>O incorporation at C<sub>2</sub> and the total <sup>18</sup>O incorporation into **15**.



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



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

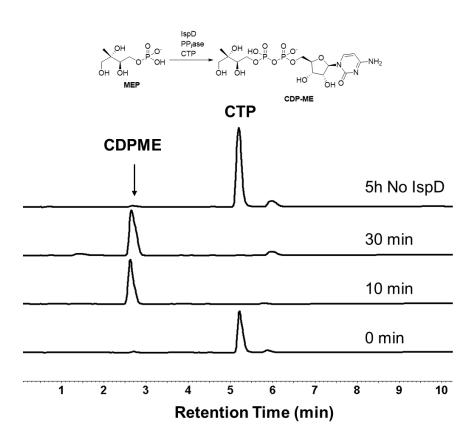


Figure S4. HPLC analysis of MEP turnover by E. coli IspD

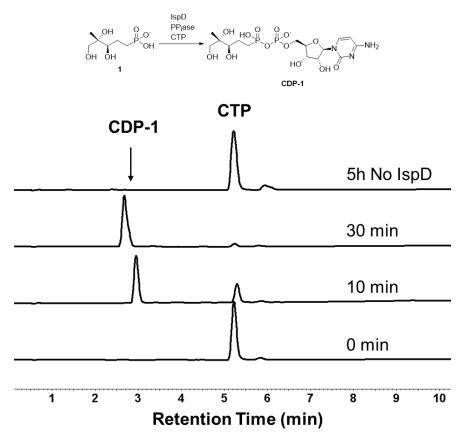


Figure S5. HPLC analysis of 1 turnover by E. coli IspD

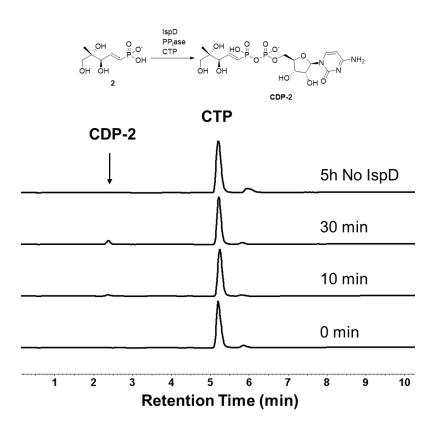


Figure S6. HPLC analysis of 2 turnover by E. coli IspD

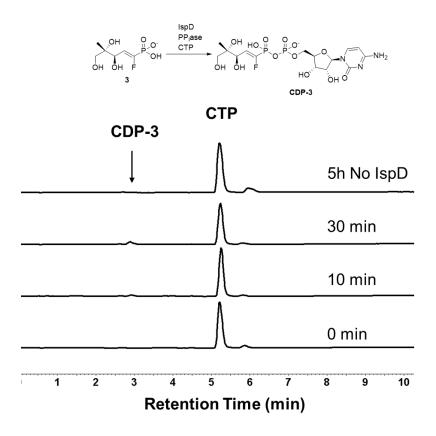


Figure S7. HPLC analysis of 3 turnover by E. coli IspD

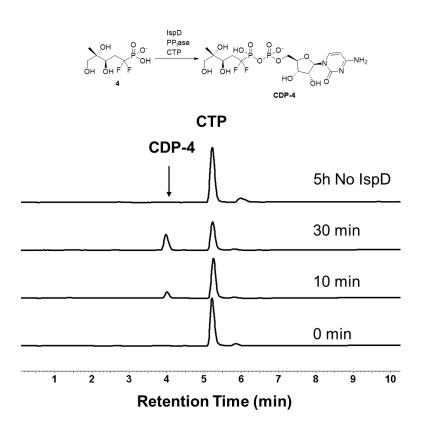


Figure S8. HPLC analysis of 4 turnover by E. coli IspD

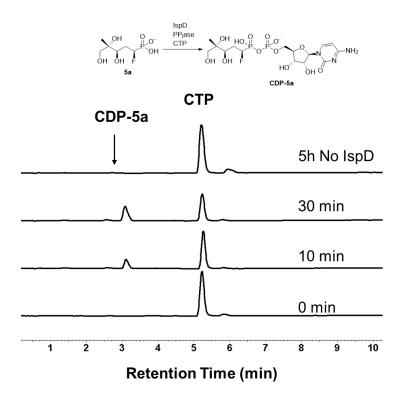


Figure S9. HPLC analysis of 5a turnover by E. coli IspD

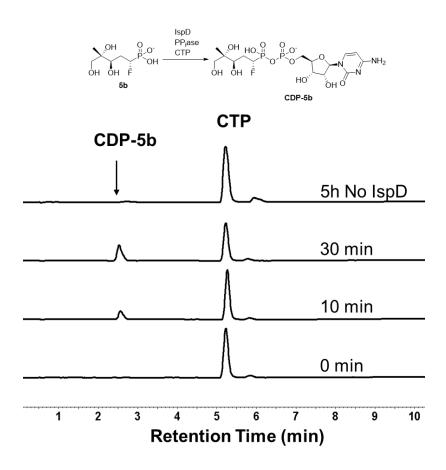
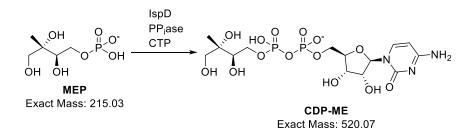
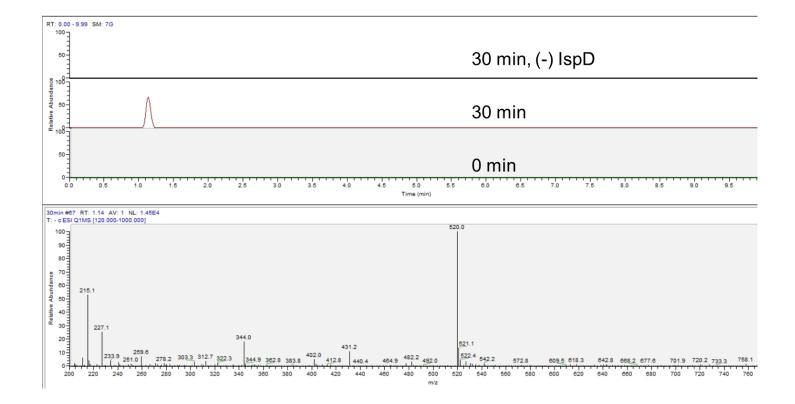
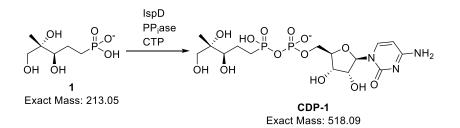


Figure S10. HPLC analysis of 5b turnover by E. coli IspD





**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.



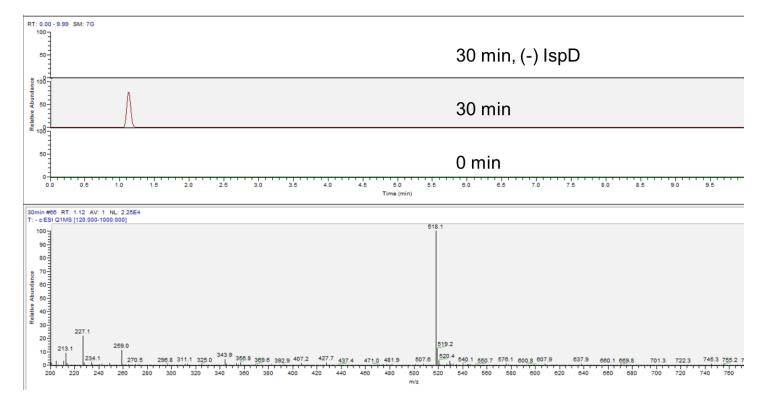
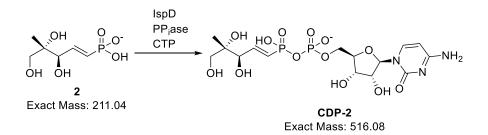
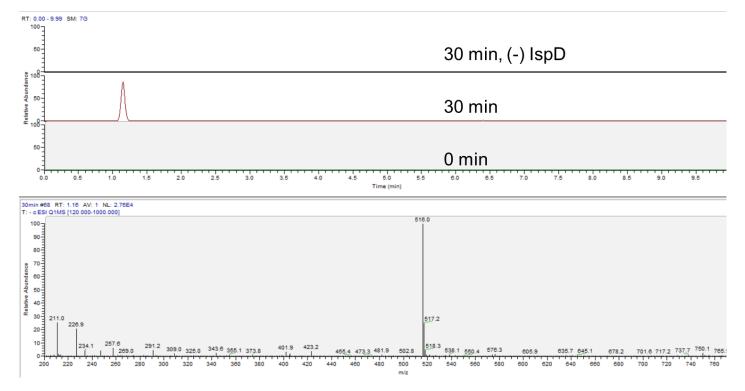
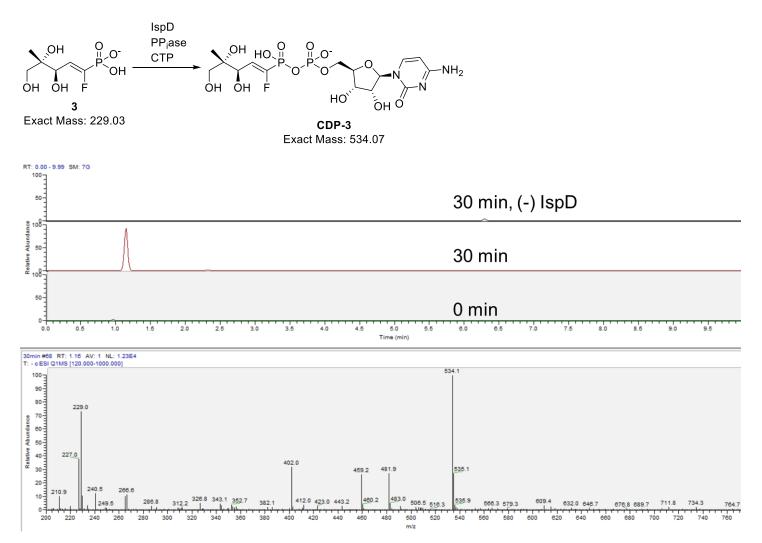


Figure S12. LC-MS analysis of 1 turnover. Chromatograms display the total ion count (TIC) for scans containing an ion with an m/z of 518 corresponding to the CDP-1 product. The mass spectrum is the scan at the center of the peak in the 30 min sample.

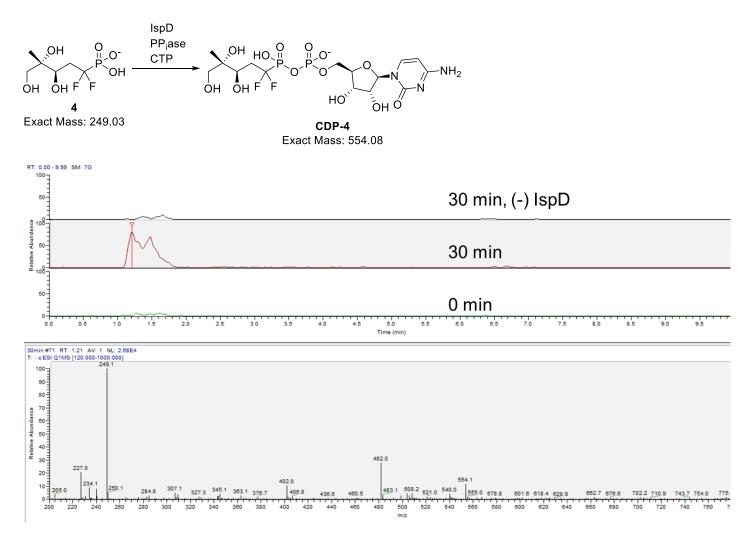




**Figure S13. LC-MS analysis of 2 turnover.** Chromatograms display the total ion count (TIC) for scans containing an ion with an m/z of 516 corresponding to the **CDP-2** product. The mass spectrum is the scan at the center of the peak in the 30 min sample.



**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.

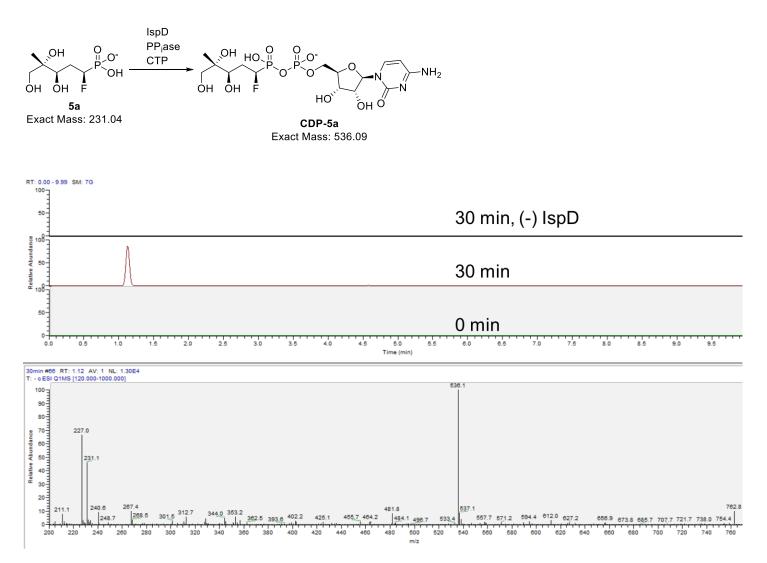
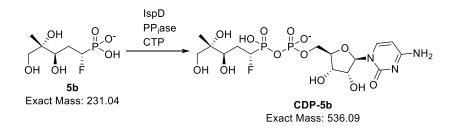
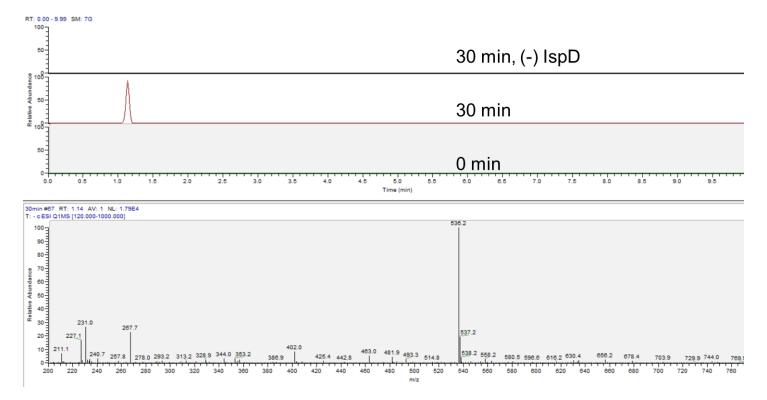


Figure S16. LC-MS analysis of 5a turnover. Chromatograms display the total ion count (TIC) for scans containing an ion with an m/z of 536 corresponding to the **CDP-5a** product. The mass spectrum is the scan at the center of the peak in the 30 min sample.





**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.

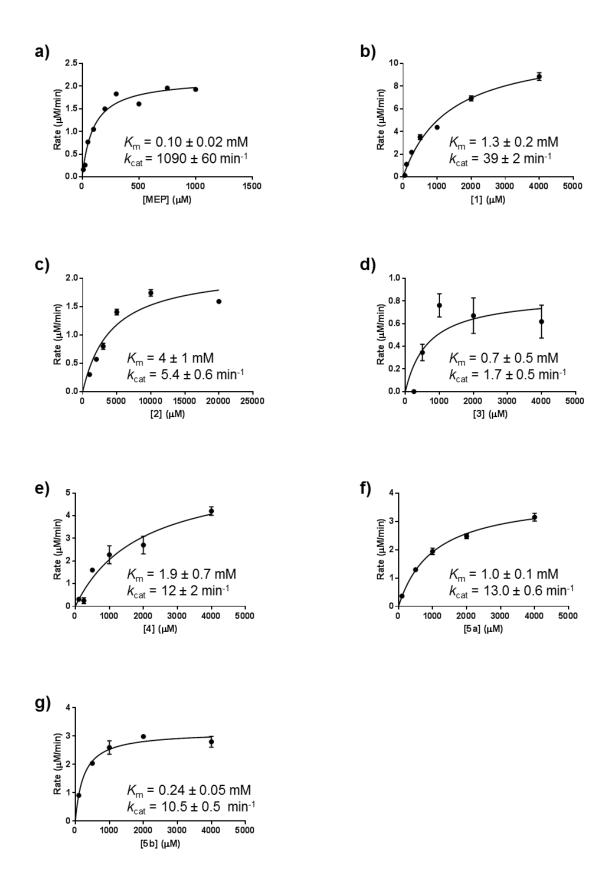
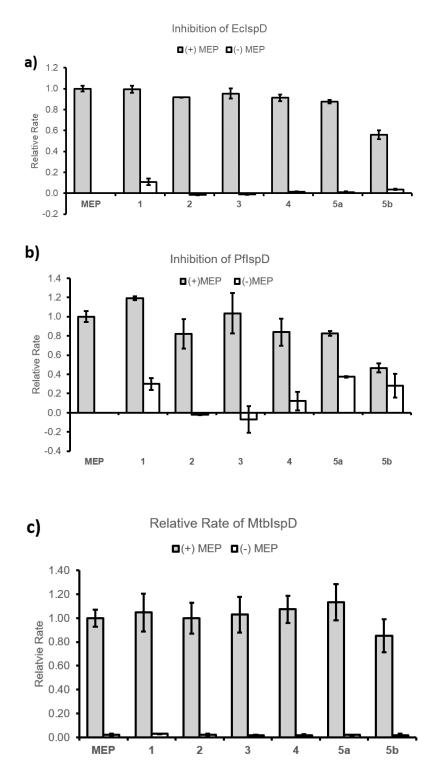


Figure S18. Michaelis-Menten plots for MEP and analogs 1 – 5a,b with E. coli IspD.



**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 were 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).

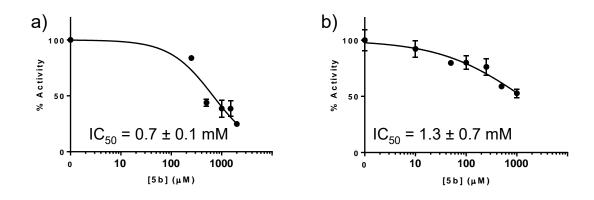
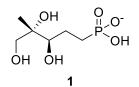


Figure S20. IC50 plots for analog 5b against a) E. coli IspD and b) P. falciparum IspD.



Exact Mass: 213.0533

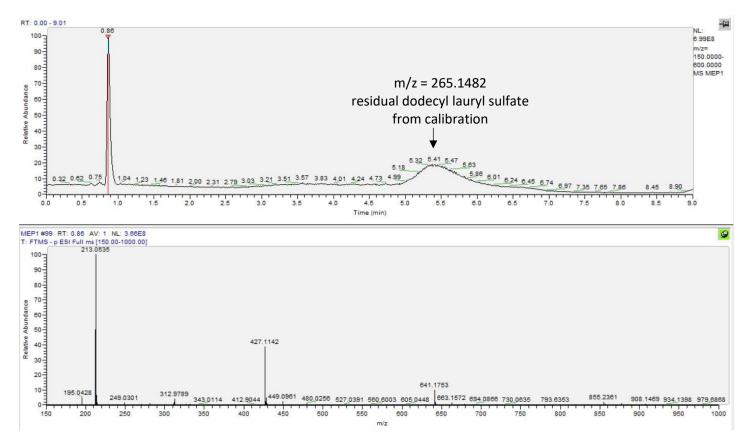
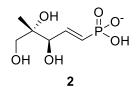


Figure S21. LC-HRMS analysis of analog 1 demonstrating >95% purity.



Exact Mass: 211.0377

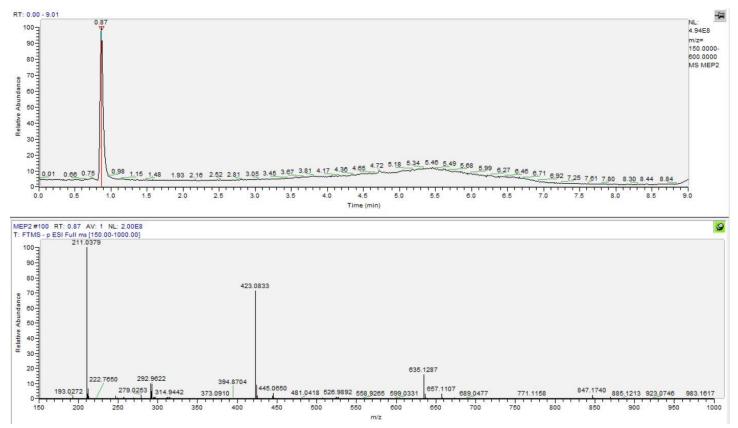


Figure S22. LC-HRMS analysis of analog 2 demonstrating >95% purity.

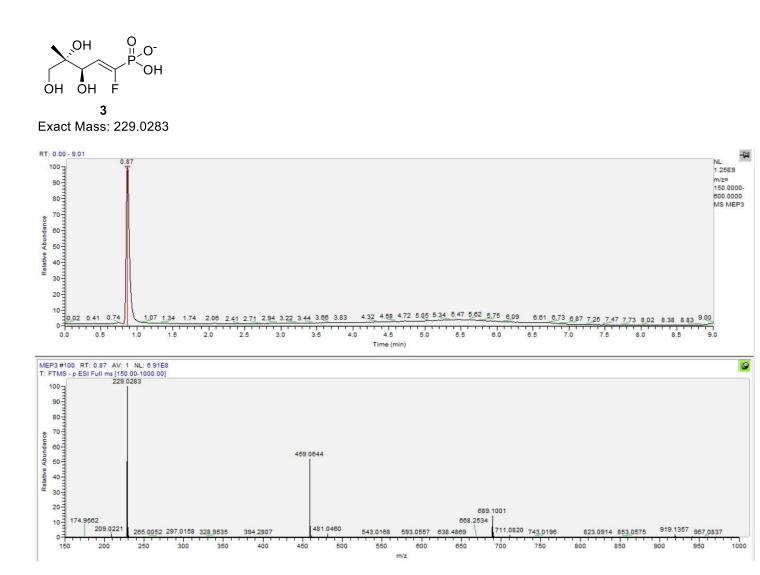
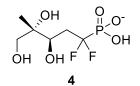


Figure S23. LC-HRMS analysis of analog 3 demonstrating >95% purity.



Exact Mass: 249.0345

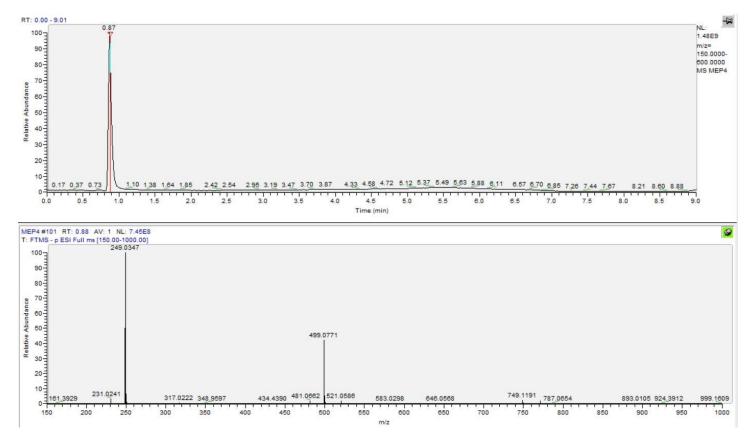
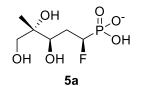
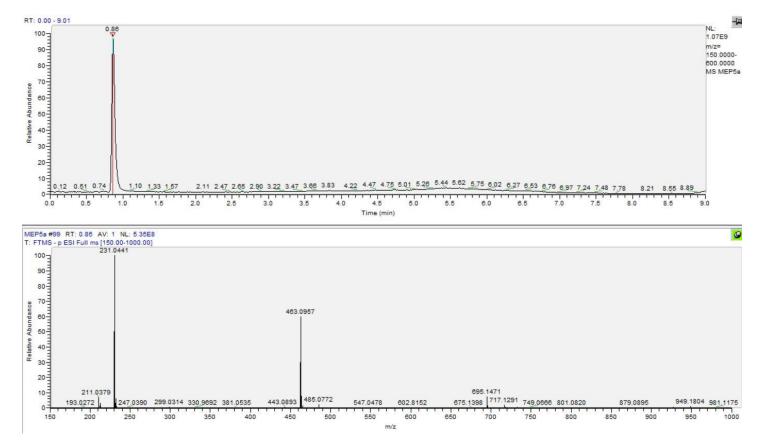


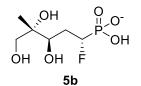
Figure S24. LC-HRMS analysis of analog 4 demonstrating >95% purity.



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 <sup>1</sup>H NMR spectrum (below).



Exact Mass: 231.0439

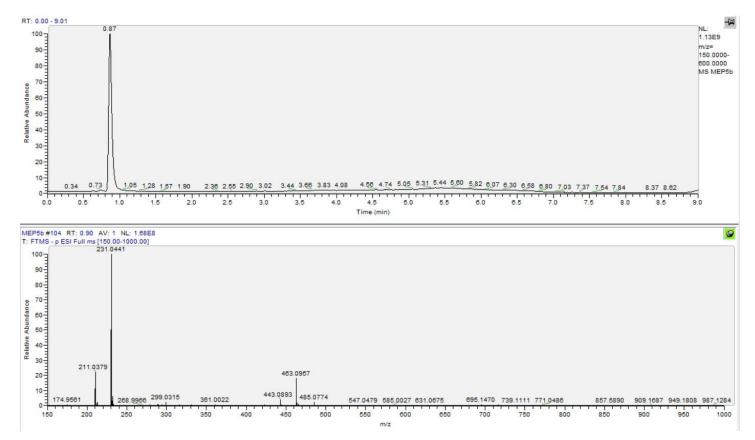


Figure S26. LC-HRMS analysis of analog 5b demonstrating >95% purity. The mass observed at 211.0379 corresponds to the fluoride elimination product, vinyl phosphonate 2. This product appears to be an artifact of the ionization process as there are no observable peaks corresponding to 2 in the <sup>1</sup>H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

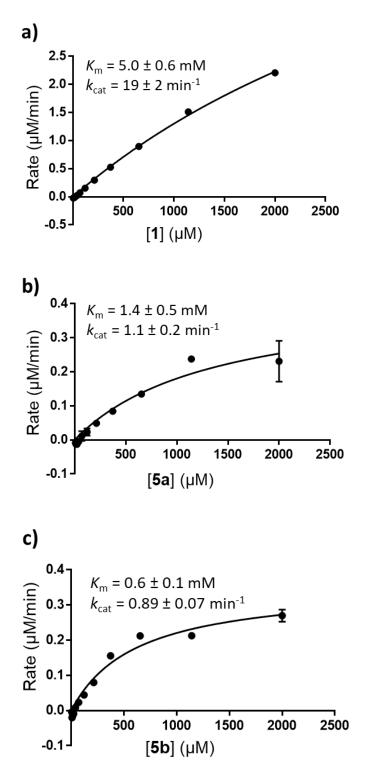


Figure S27. Michaelis-Menten plots for MEP and analogs 1 and 5a,b with *P. falciparum* IspD. The relatively high  $K_m$  values determined by these experiments demonstrate that the non-negligible (-) MEP control rates observed in Figure S19 are very unlikely to be masking potent inhibition.

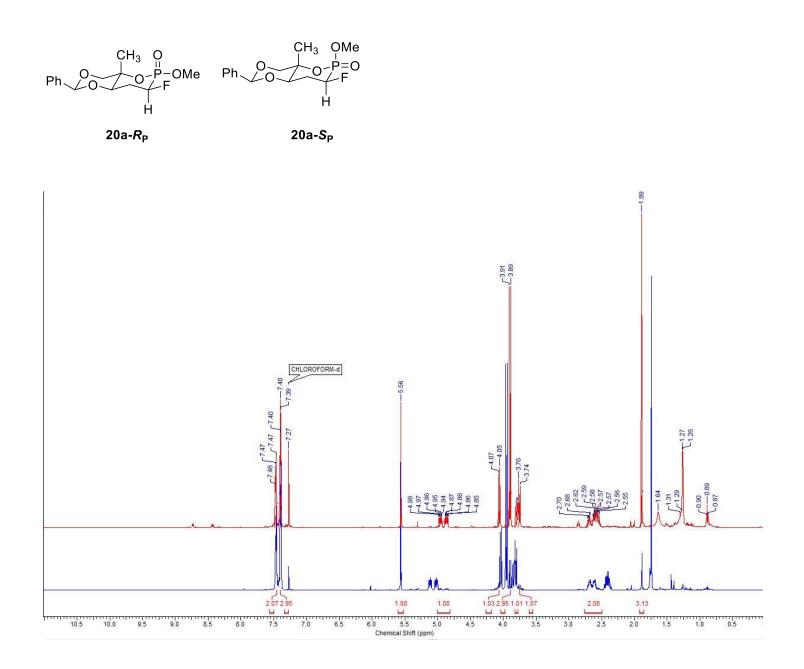


Figure S28. Overlay of <sup>1</sup>H spectra for diastereomers of phosphonolactone 20a. The top and bottom spectra are assigned to be the *R*- and *S*- configurations at the phosphonate, respectively.

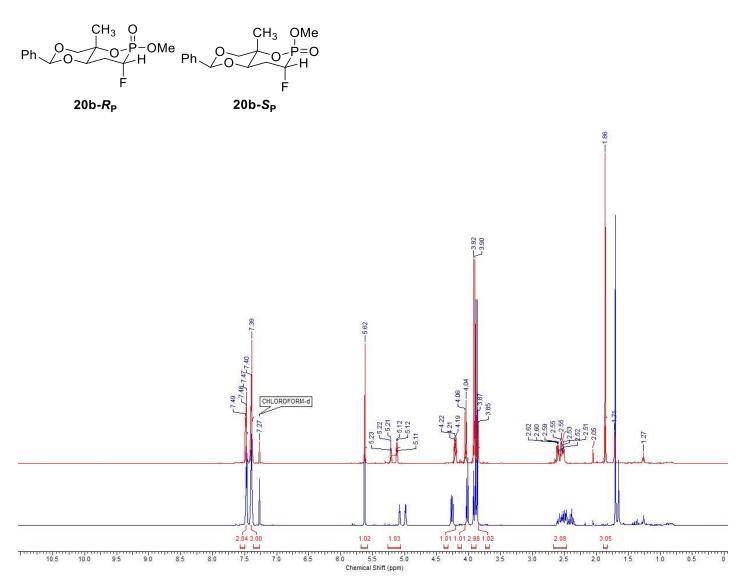
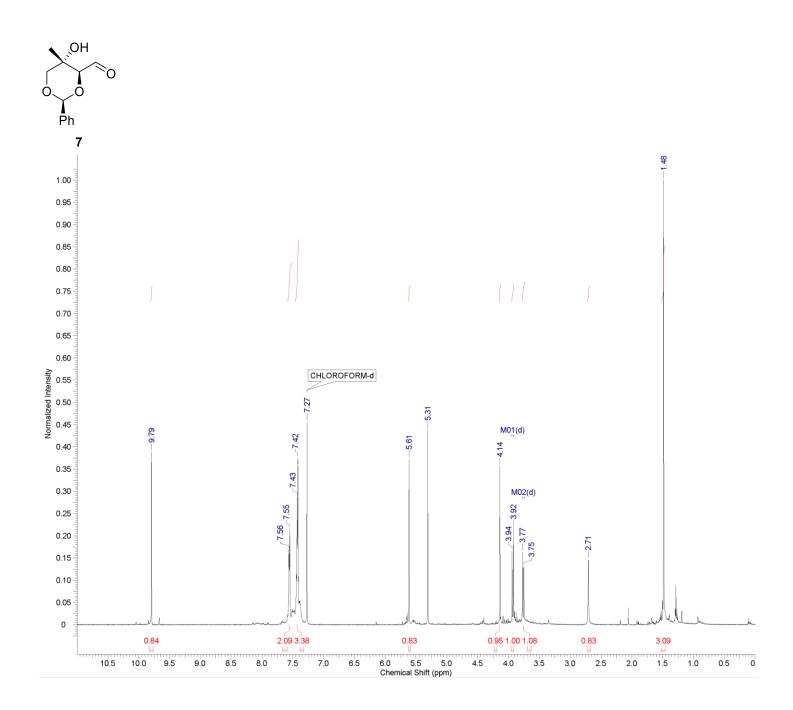
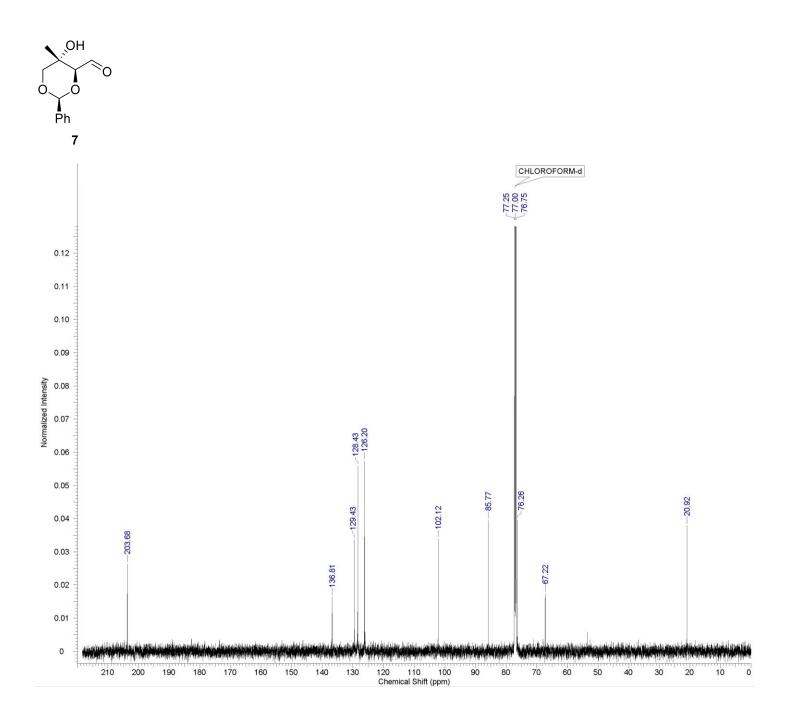
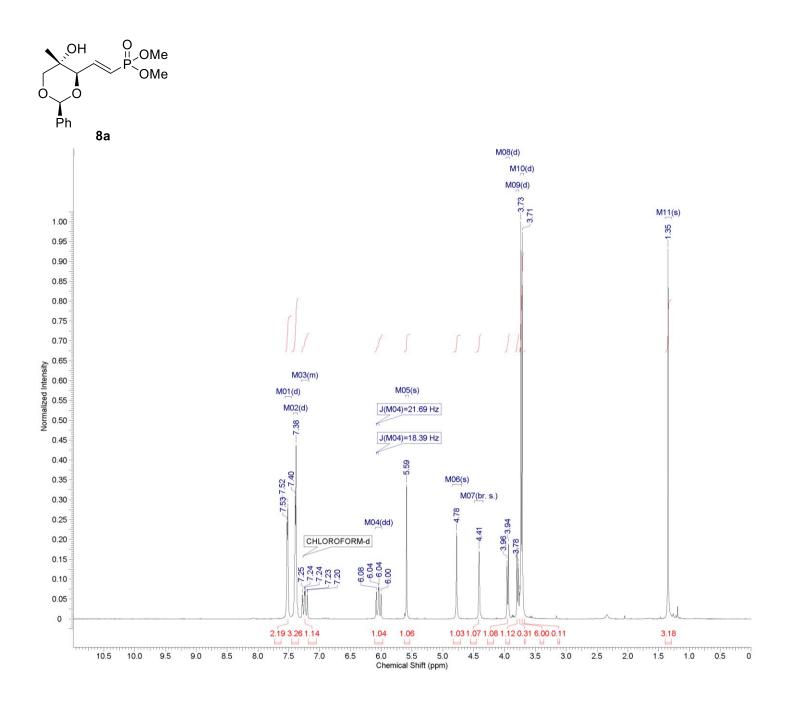
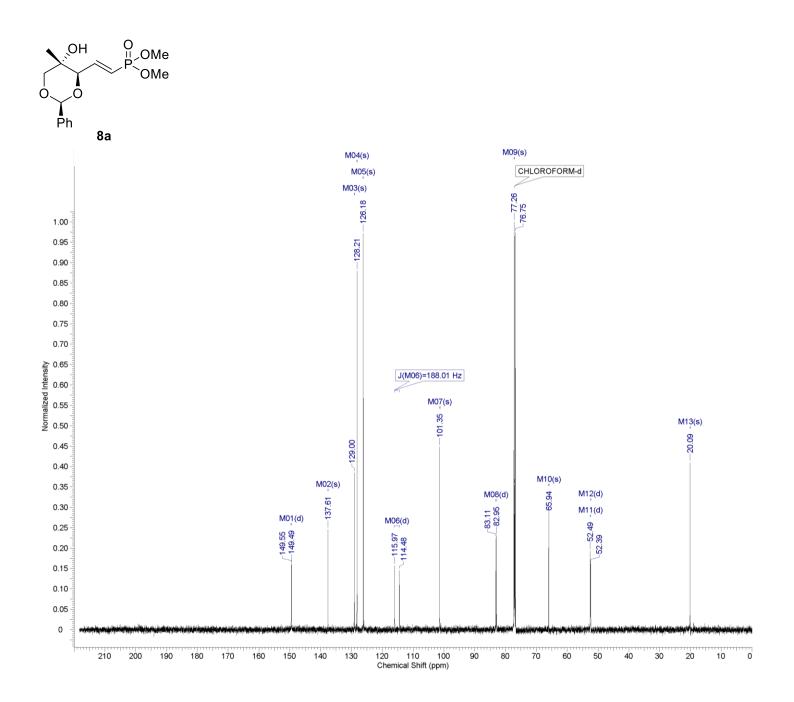


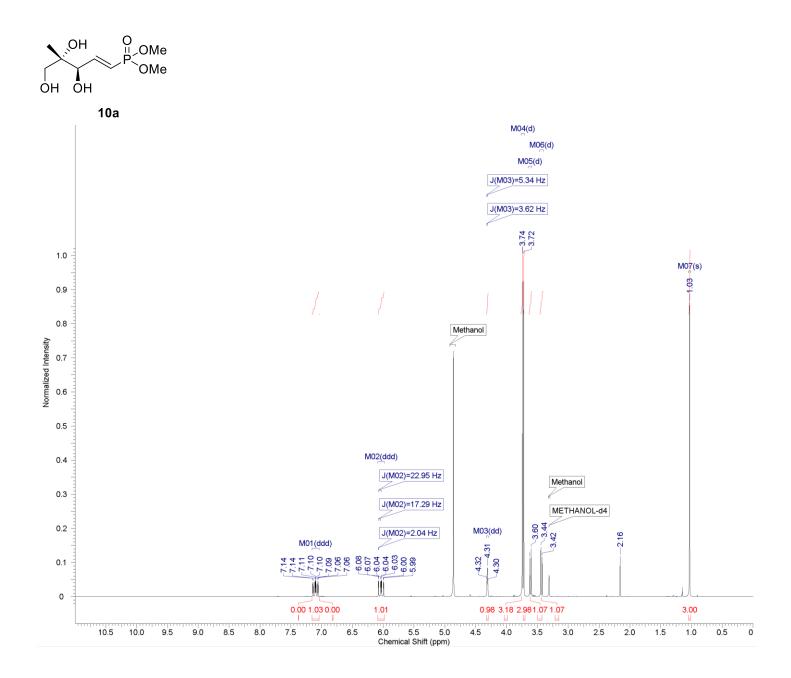
Figure S29. Overlay of <sup>1</sup>H spectra for diastereomers of phosphonolactone 20b. The top and bottom spectra are assigned to be the *R*- and *S*- configurations at the phosphonate, respectively.

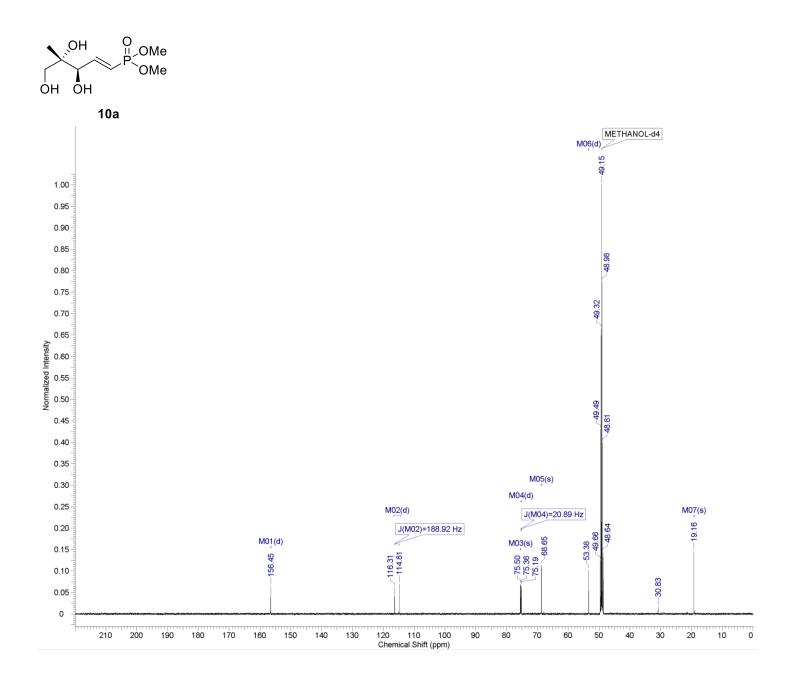


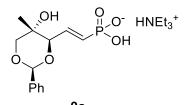


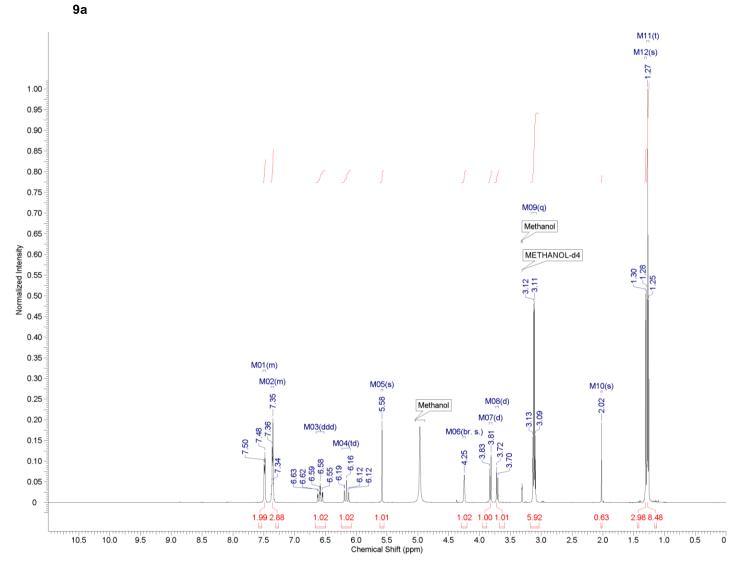


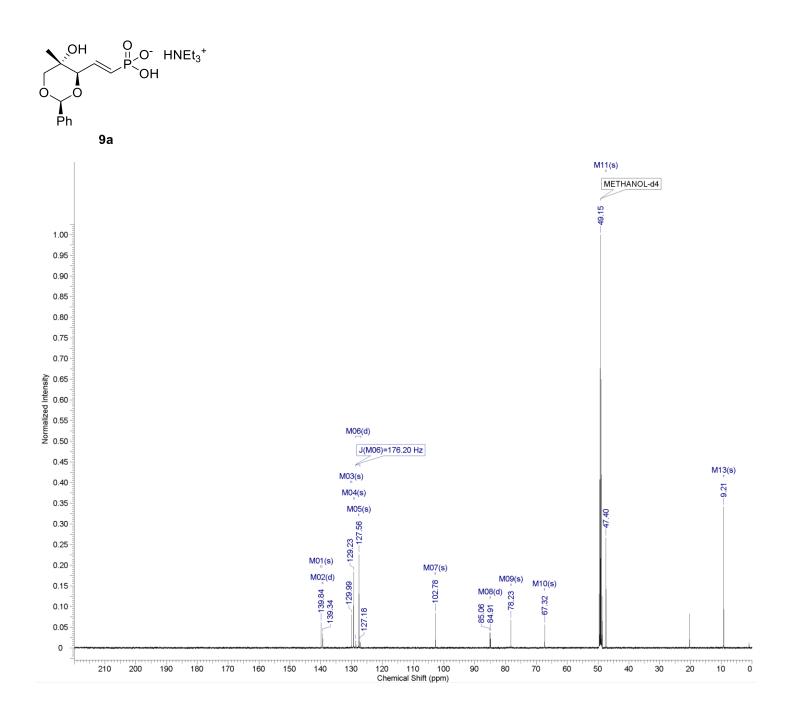


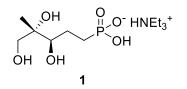


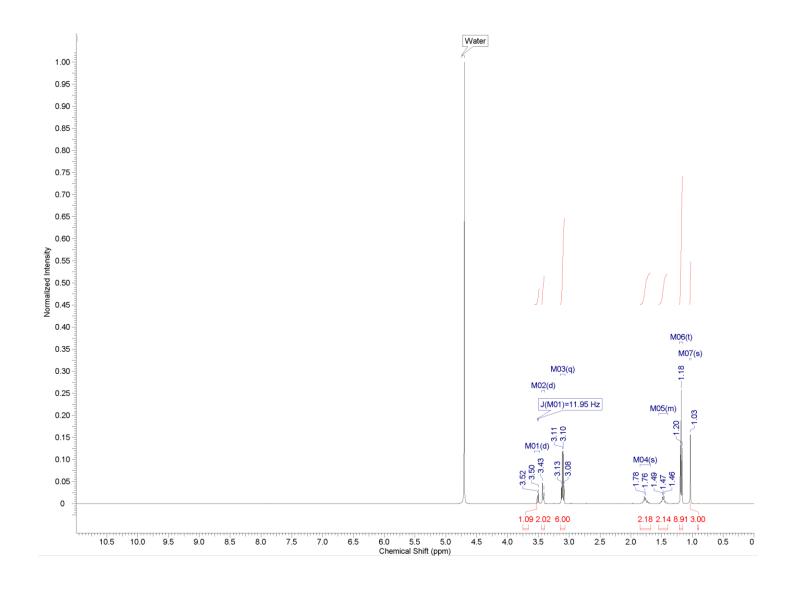


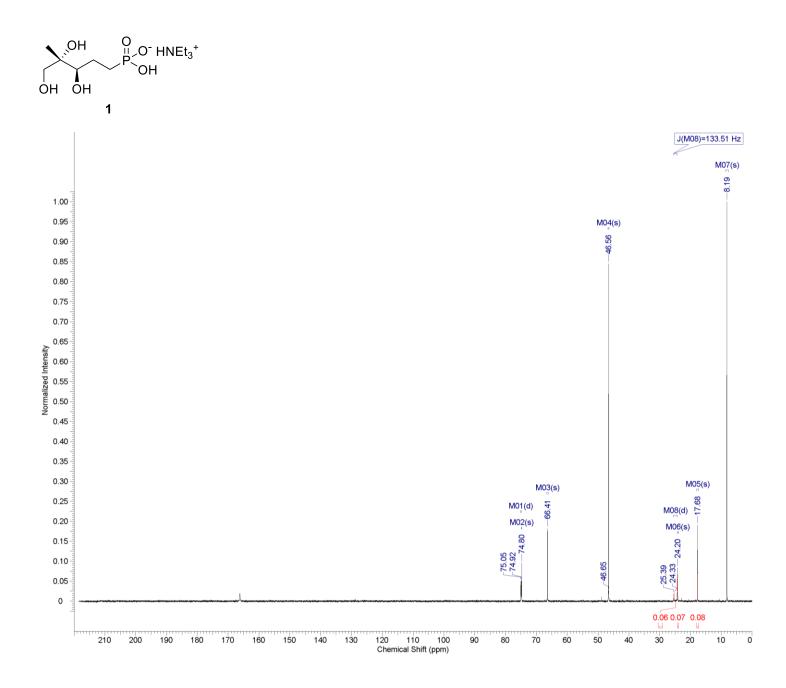


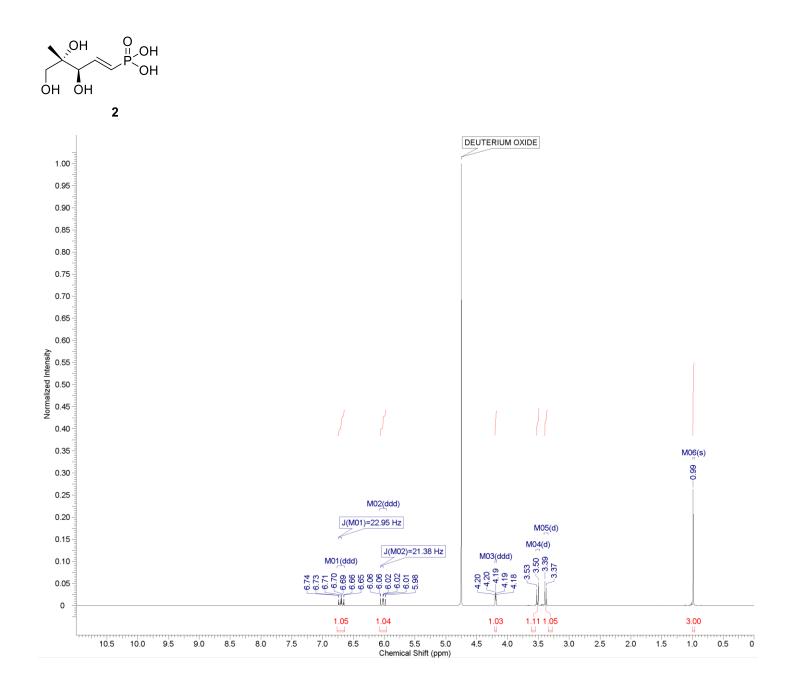


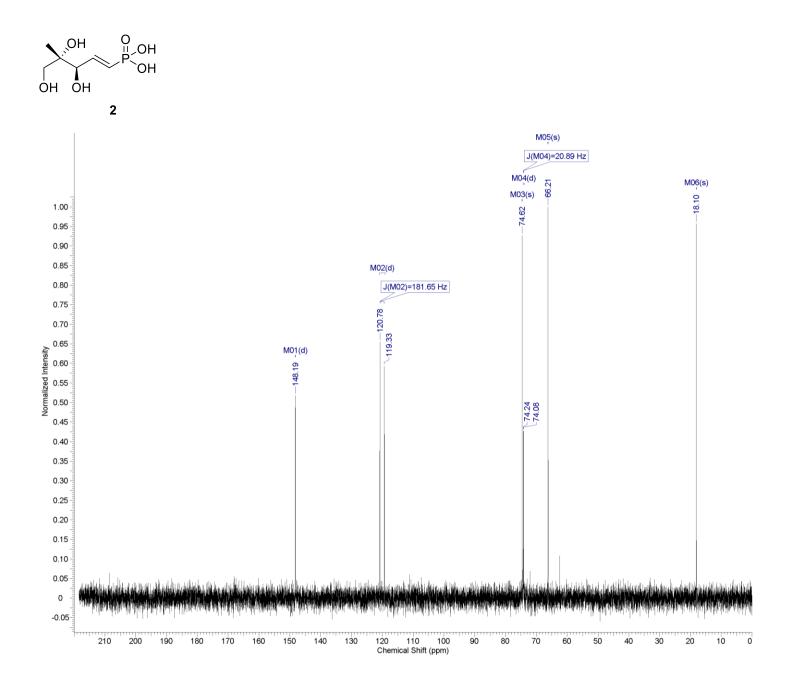


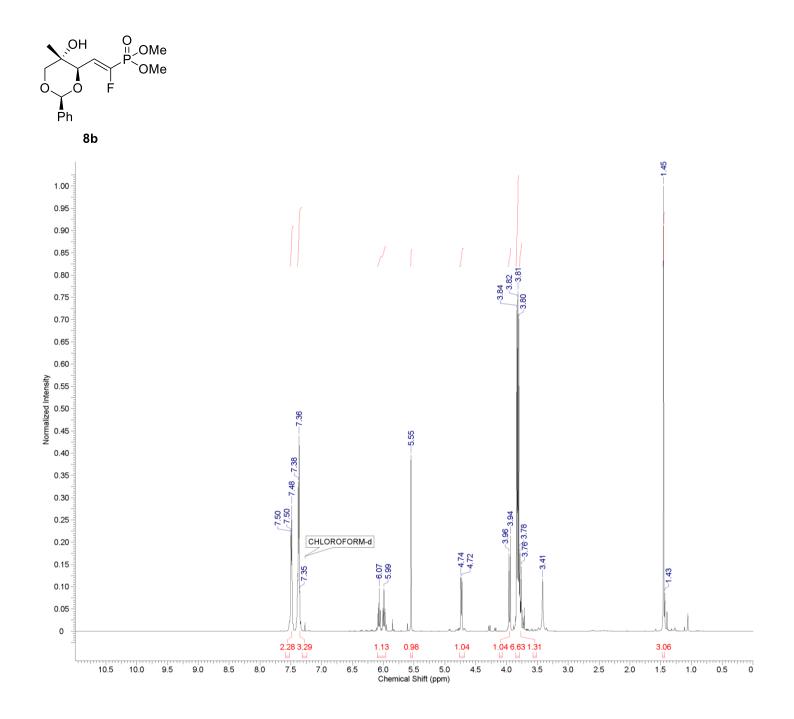


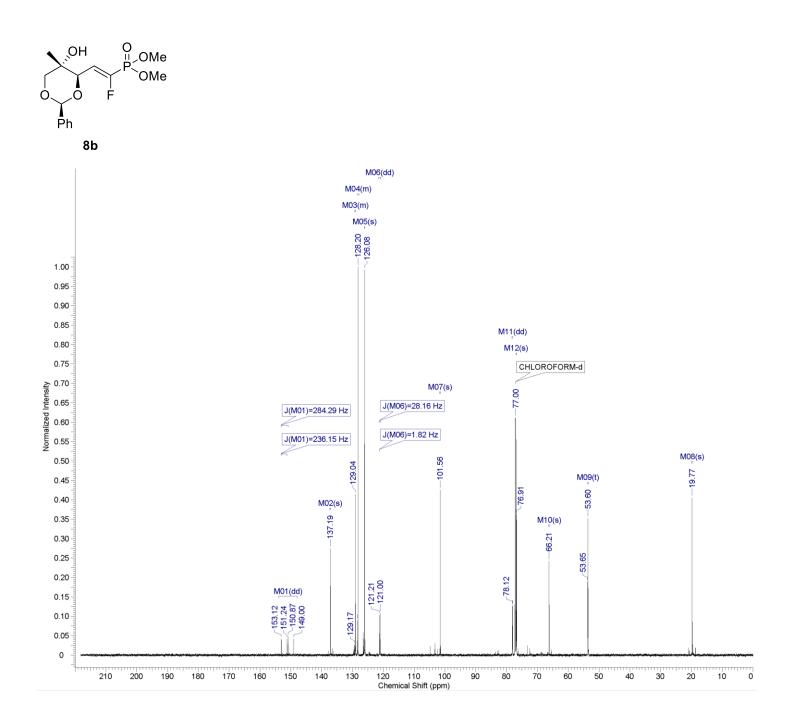


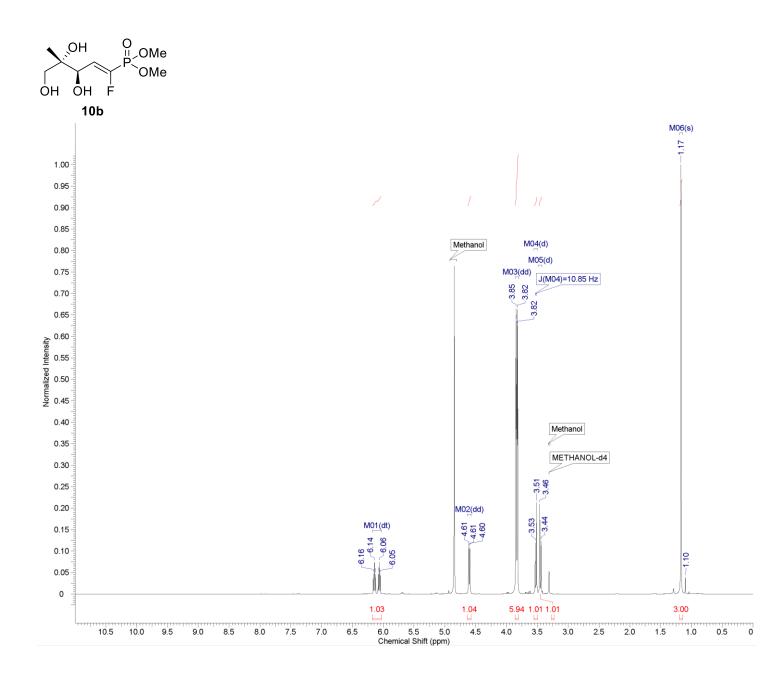


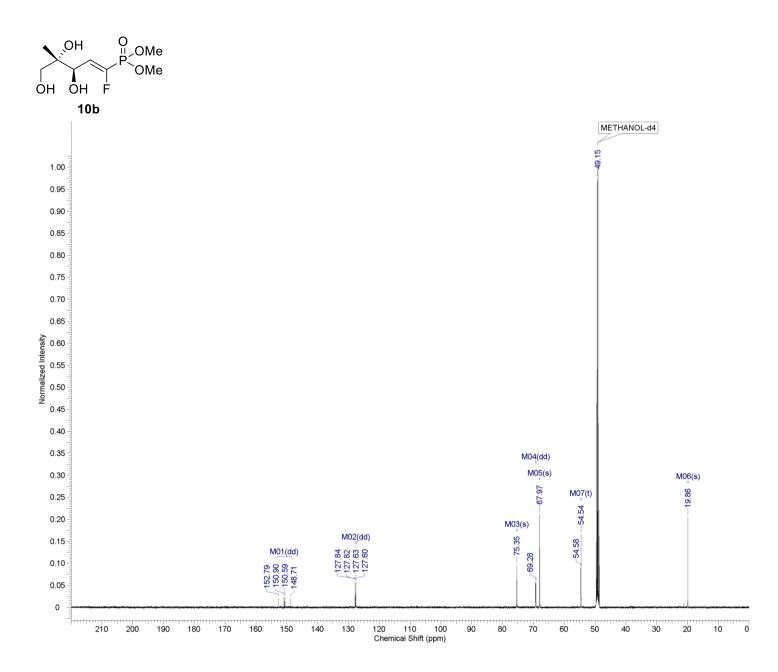


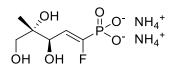


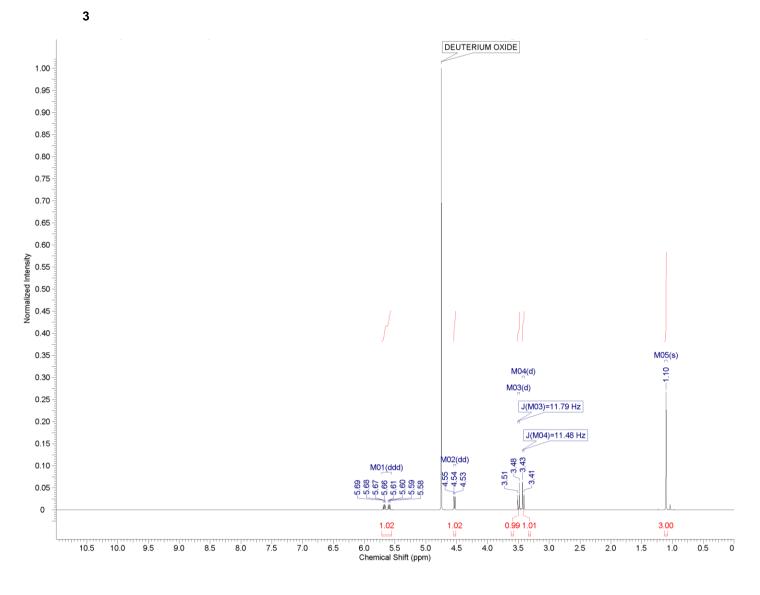


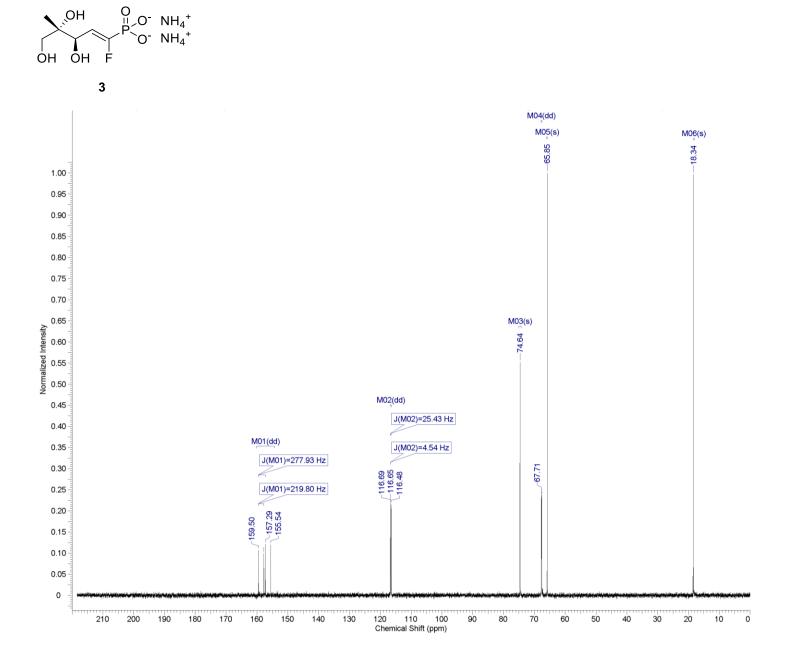


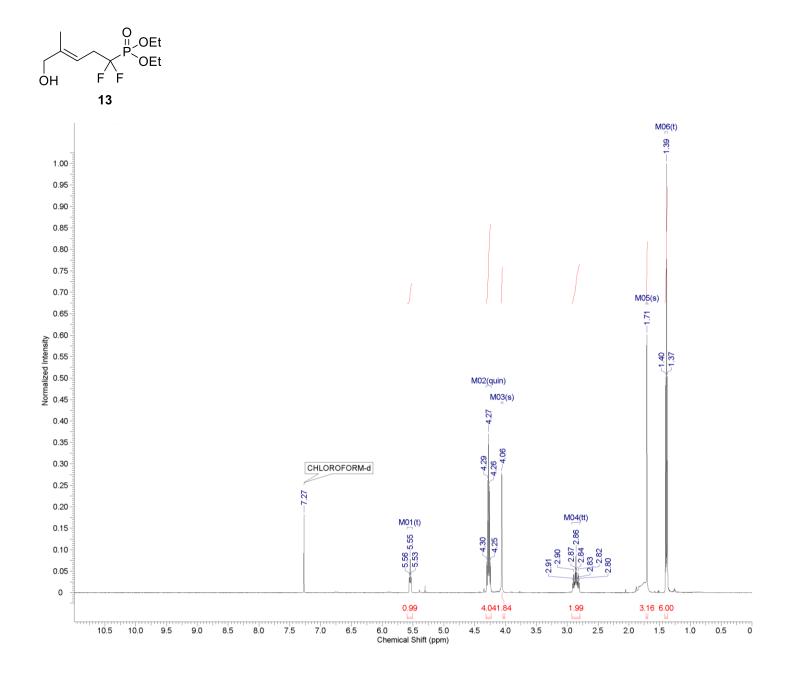


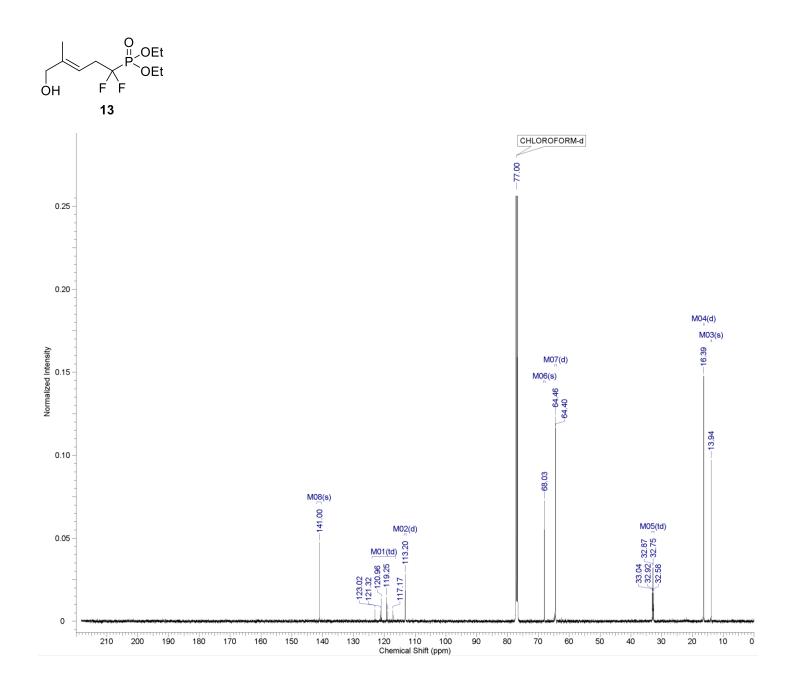


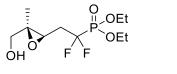




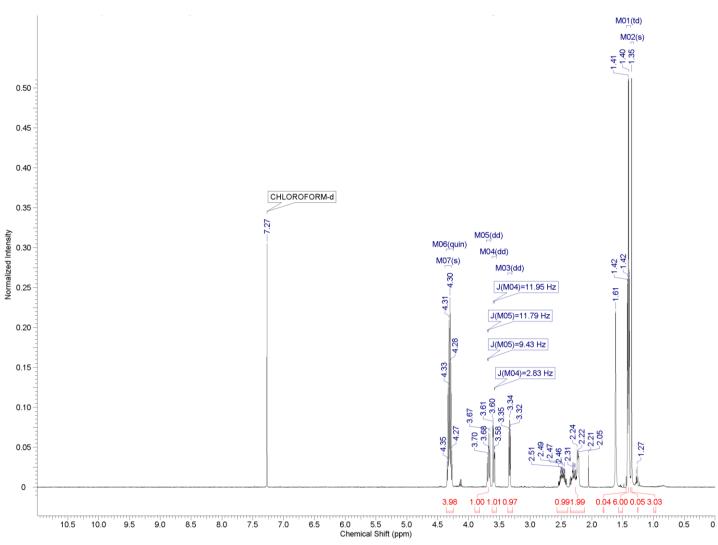


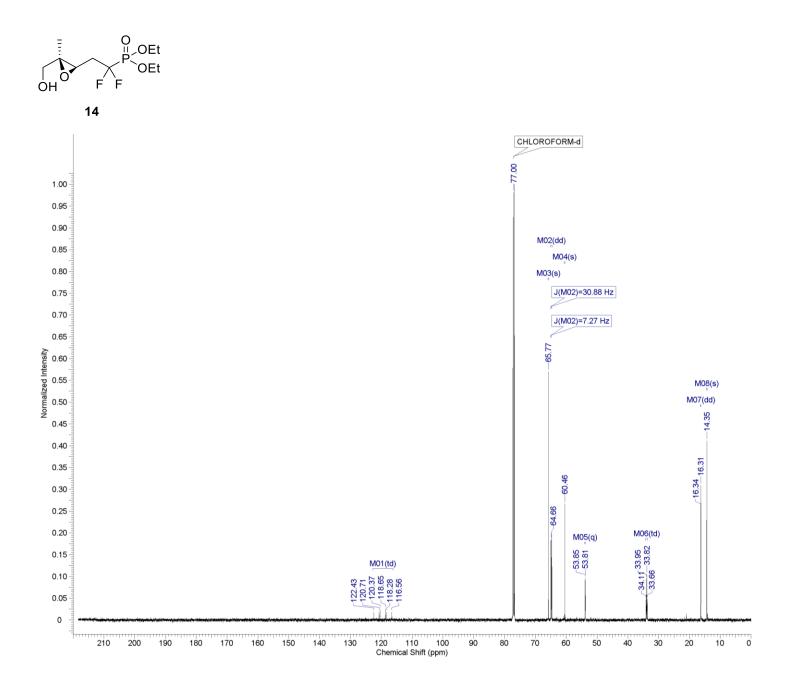


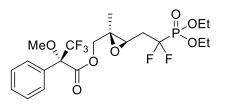






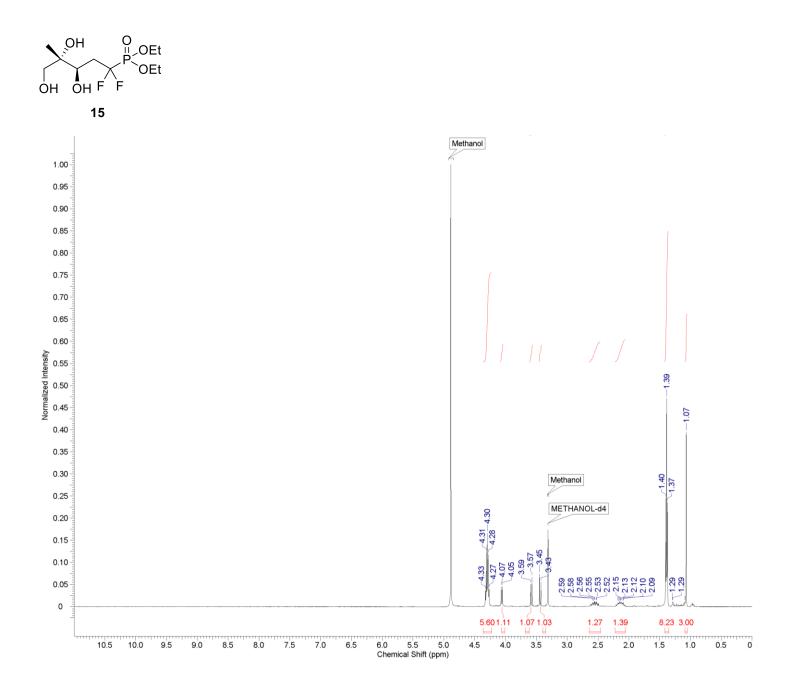


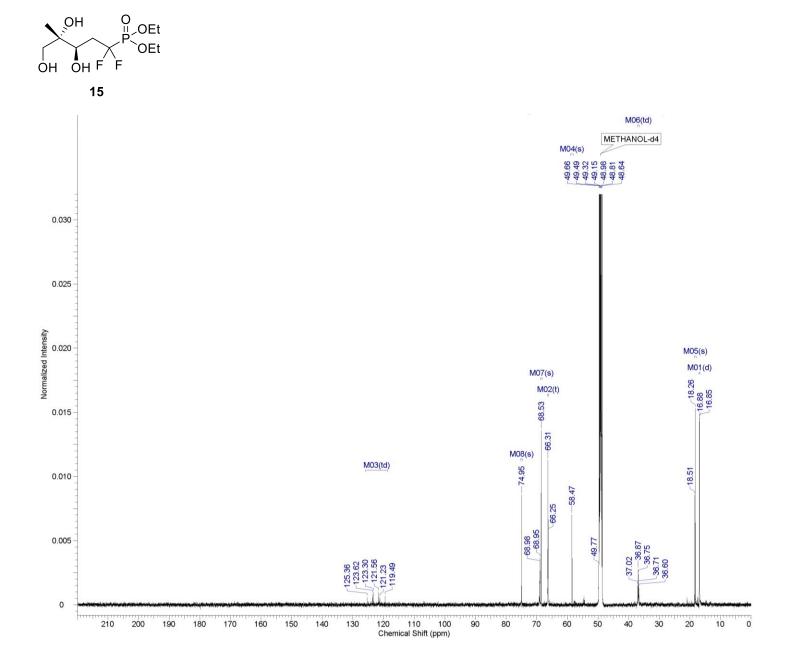


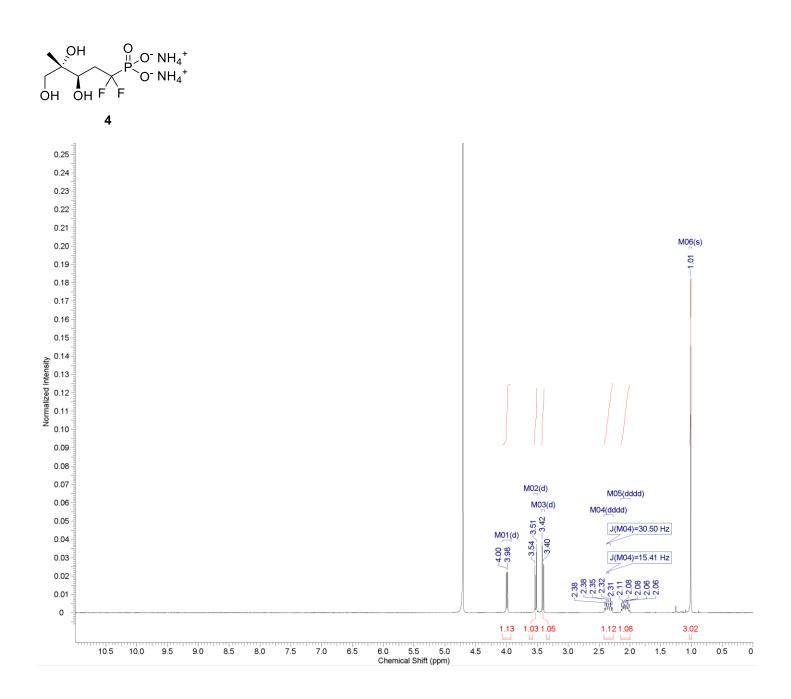


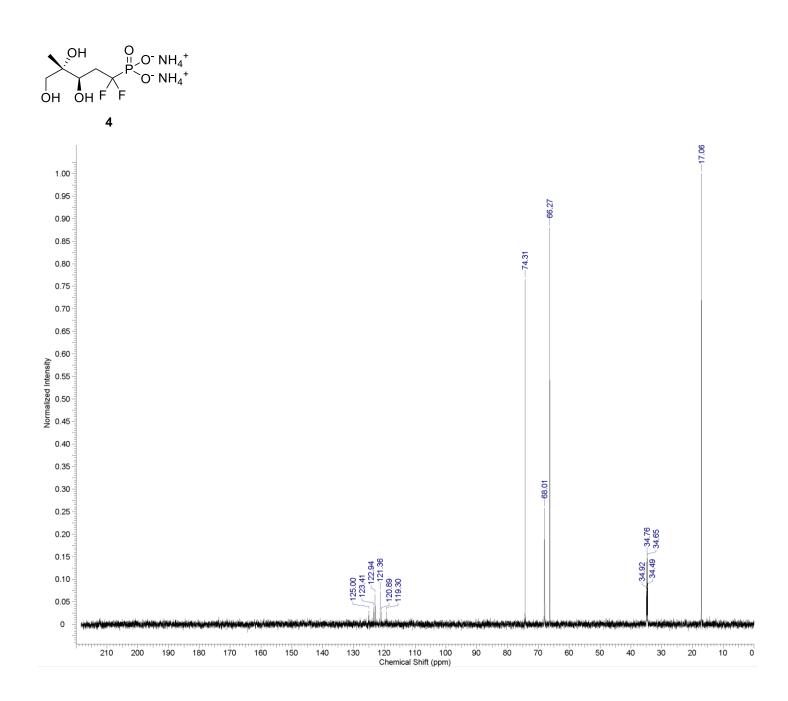
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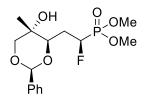
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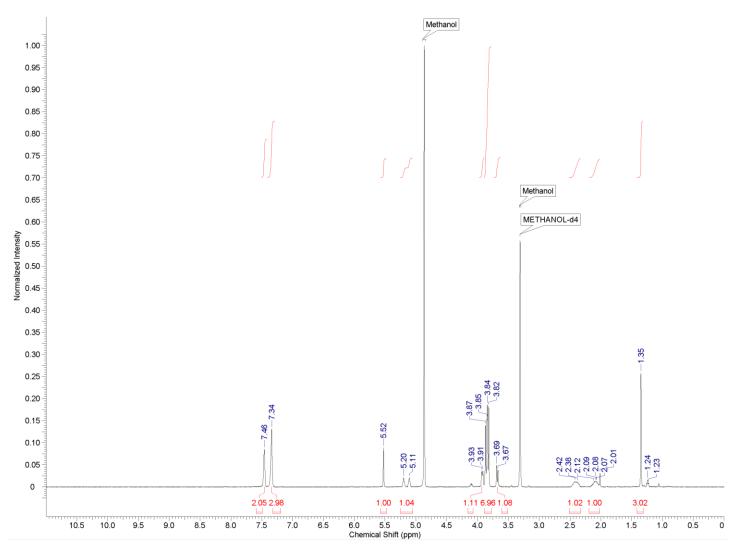


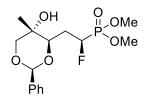




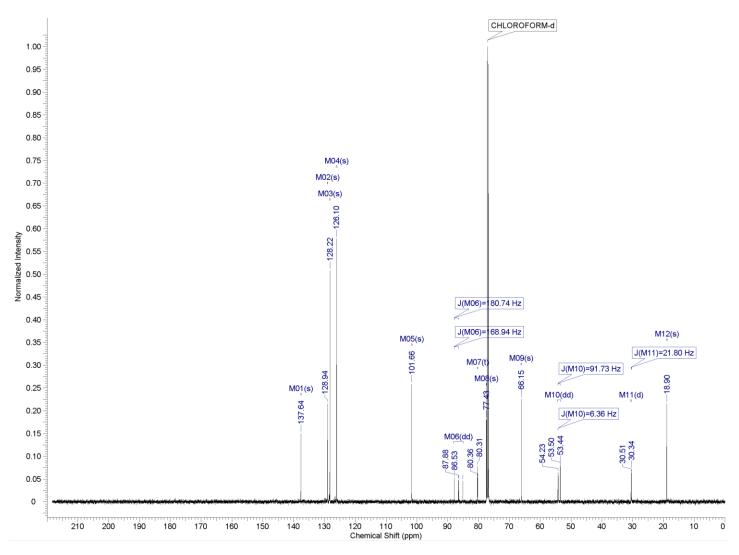


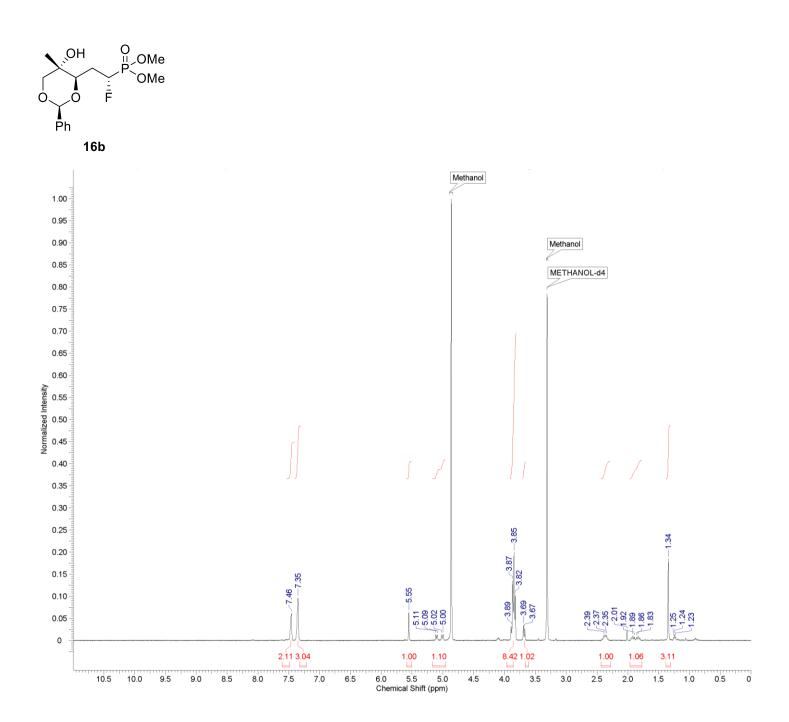


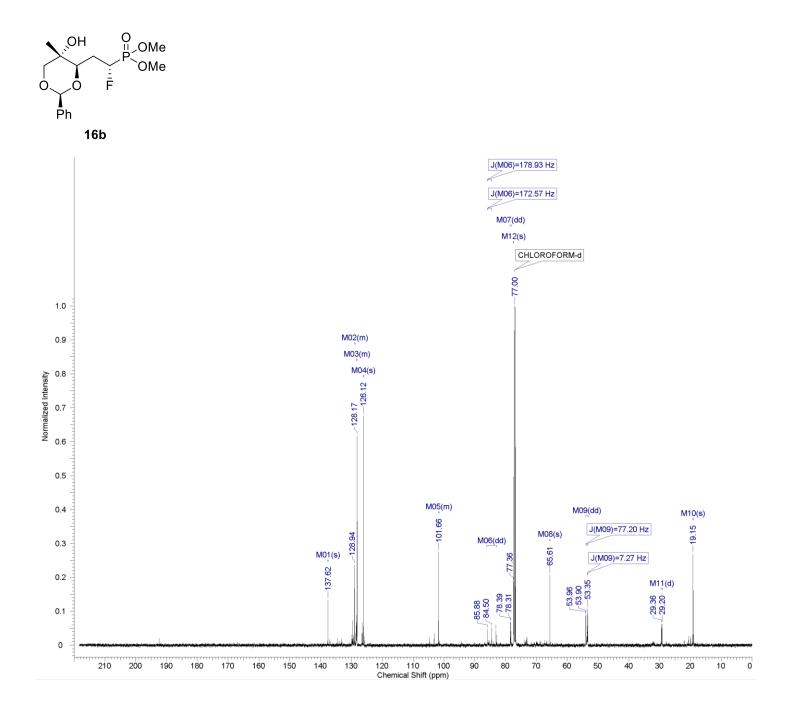


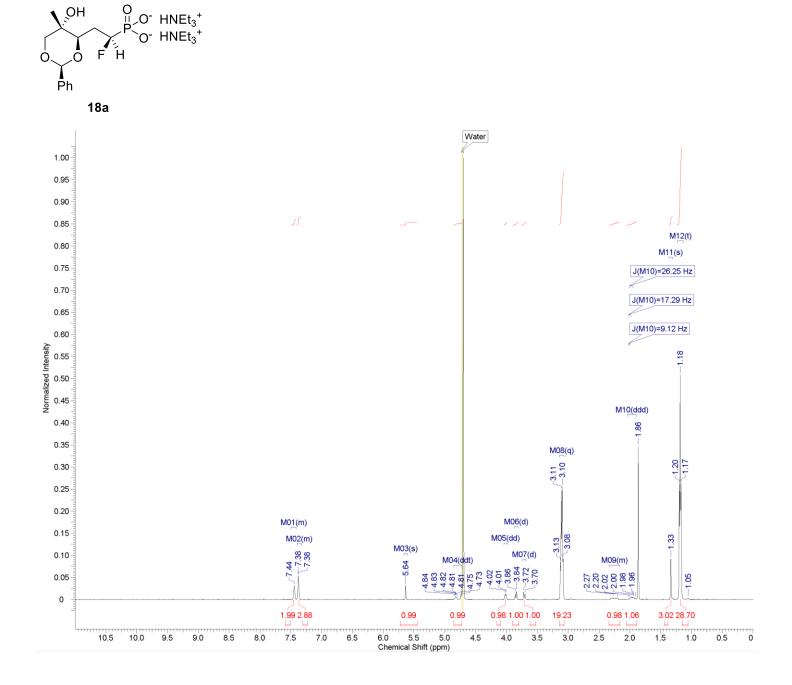


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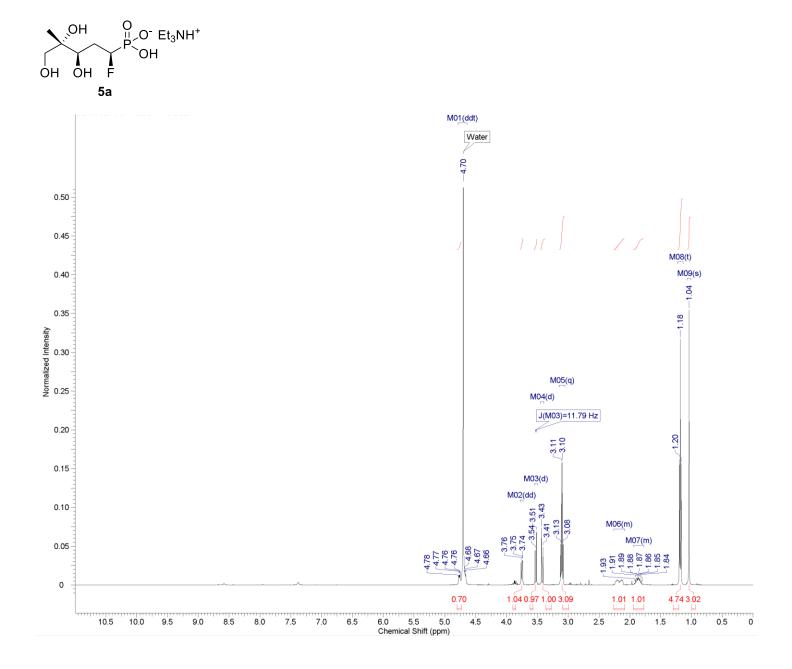


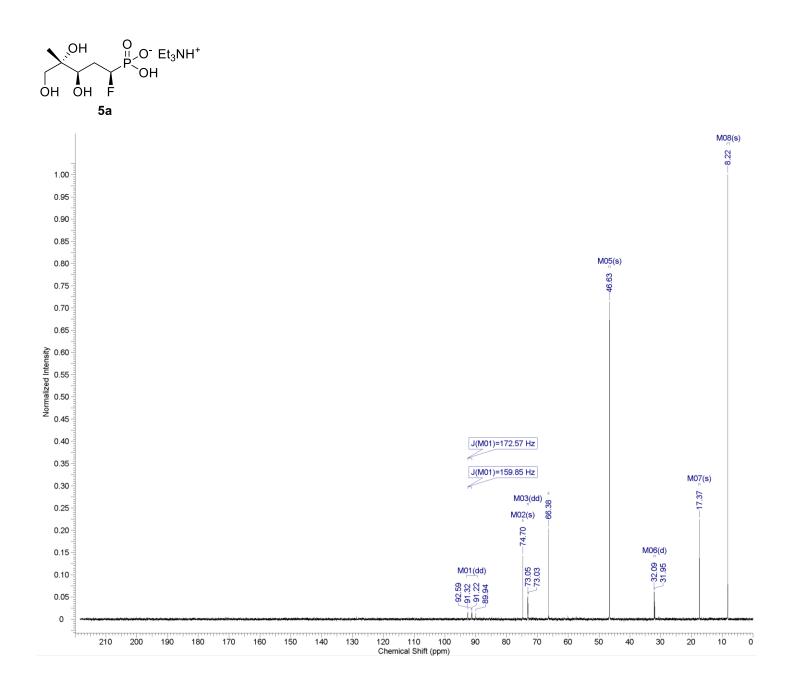


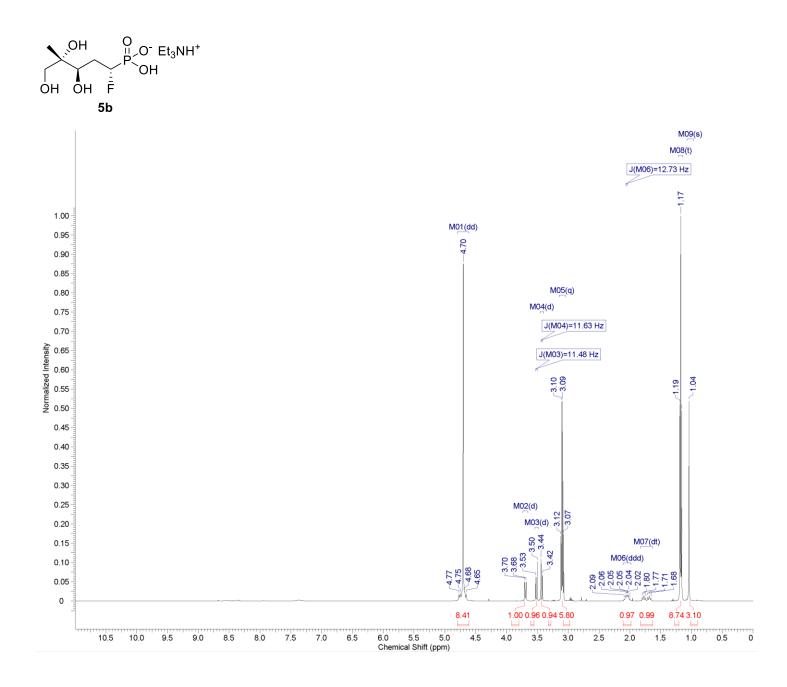


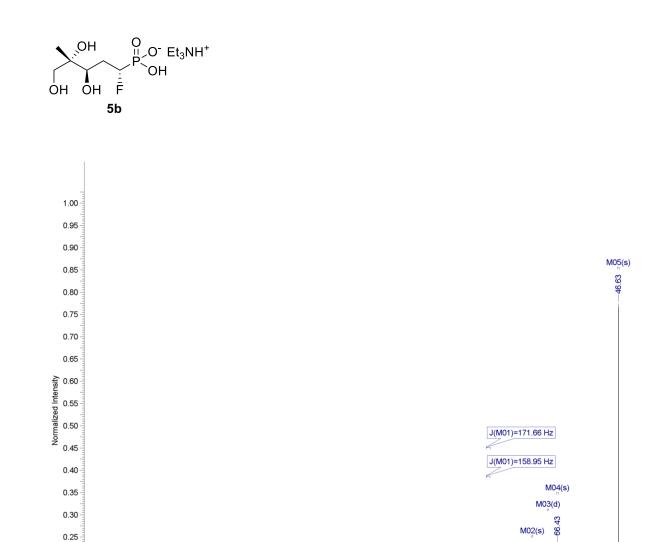


 $P^{O^{-}} HNEt_{3}^{+}$ H F Ō 0 ∎ Ph 18b M04(dd) Water 0.50 0.45 M12(t) 0.40 M11(s) M<u>10(</u>dt) 1.18 M09(m) 0.35 8 Normalized Intensity 0.30 0.25 M08(q) 1.20 0.20 3.11 3.10 0.15 M02(m) 0.10 M07(d) -1.32 -3.13 -3.09 M01(m) M05(d) 7.47 7.39 7.39 M03(s) M06(d) 0.05 -5.66 -3.97 -3.95 -3.88 -3.86 -3.73 -3.71 4.79 -4.76 4.67 2.22 2.19 2.15 2.15 2.15 -1.87 -1.83 -1.81 0 1.91 0.02 2.79 0.01 0.93 30.98 0.94 0.96 0.97 16.67 0.92 11.00 2.89 24.90 4.5 7.5 7.0 6.0 5.5 5.0 Chemical Shift (ppm) 10.5 4.0 0.5 0 1.0 6.5 3.5 3.0 10.0 2.5 2.0 1.5 9.5 9.0 8.5 8.0









0.20

0.15

0.10

0.05

210

200

190

180

170

160

150

140

130

M06(s)

M07(s)

-17.64

0

10

M08(d)

30

20

31.80 31.65

-74.54

M01(dd)

80

89.57 -88.31 -88.21 -86.92

90

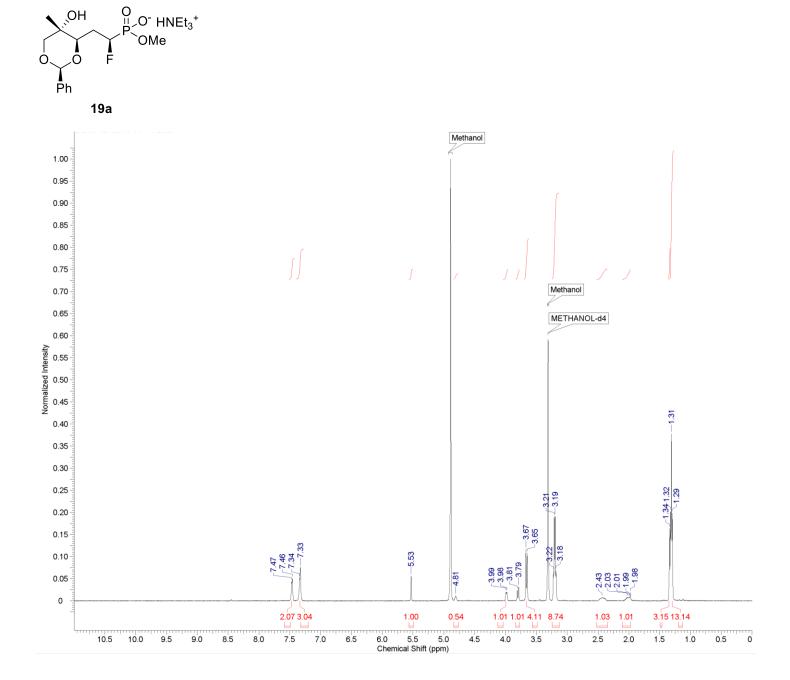
120 110 100 Chemical Shift (ppm) 69.55 69.46

70

50

60

40



19a

Ē

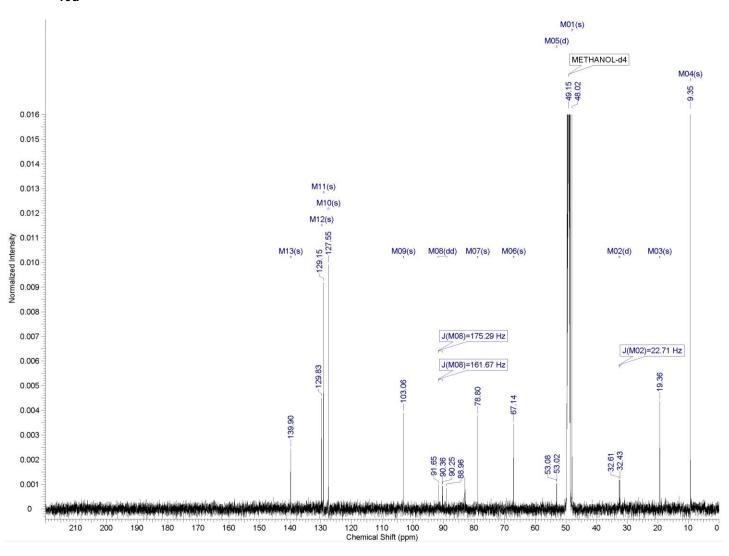
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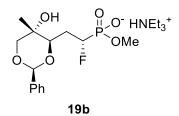
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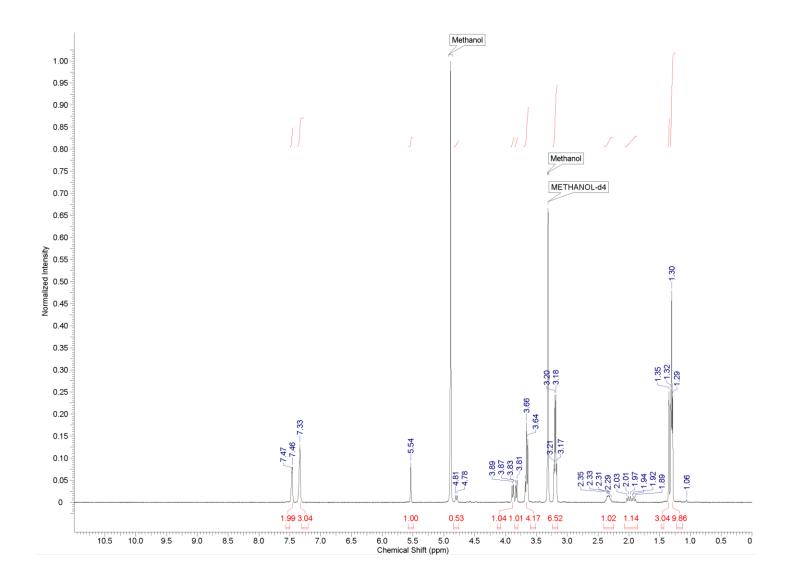
Ph

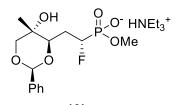
О.

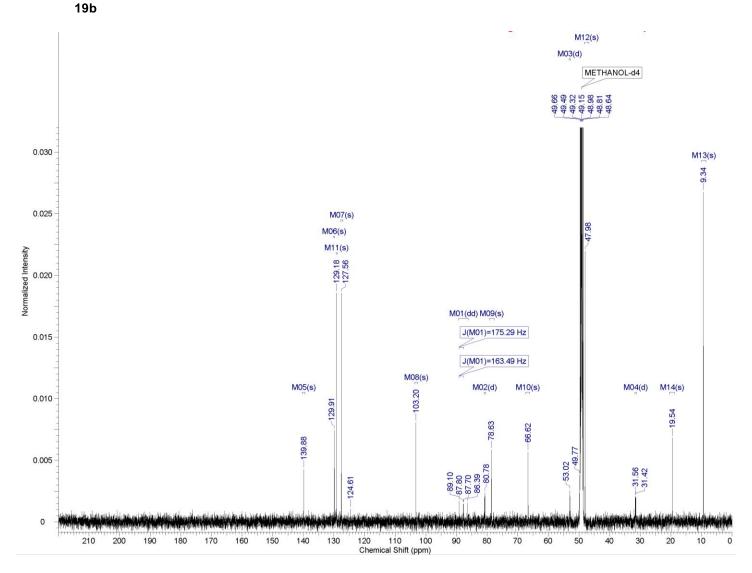
O II\_O<sup>-</sup> HNEt₃<sup>+</sup> OMe

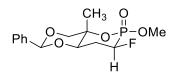




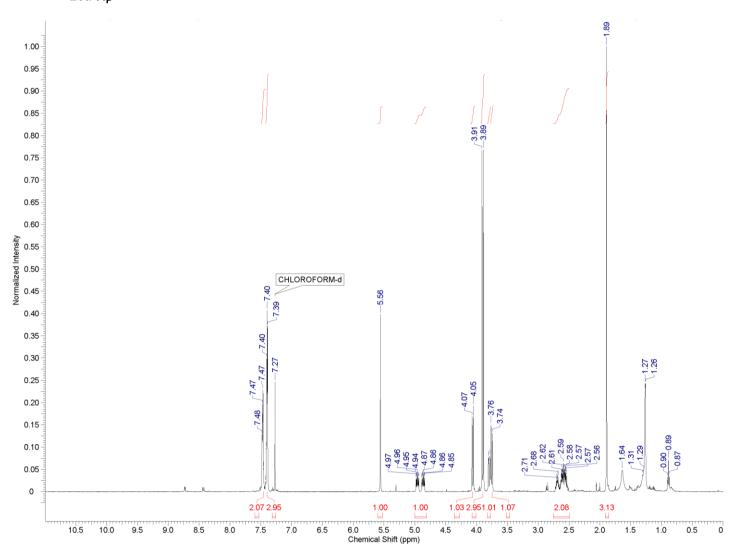


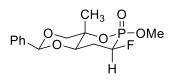




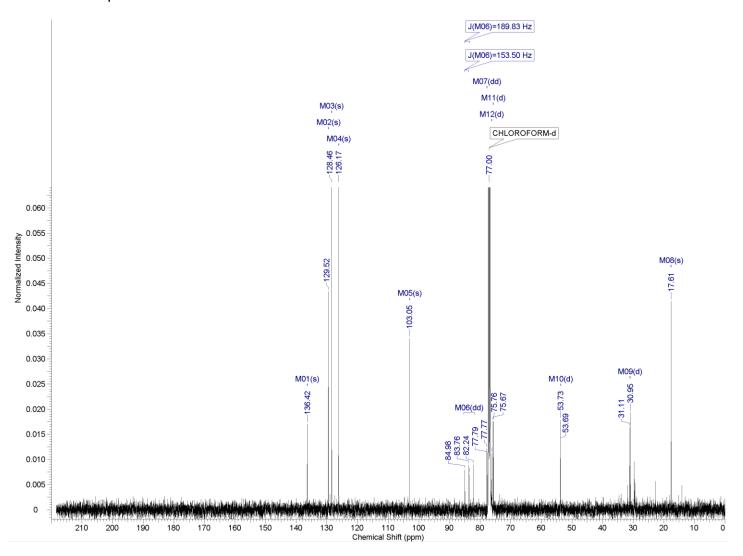


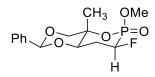
20a-*R*P



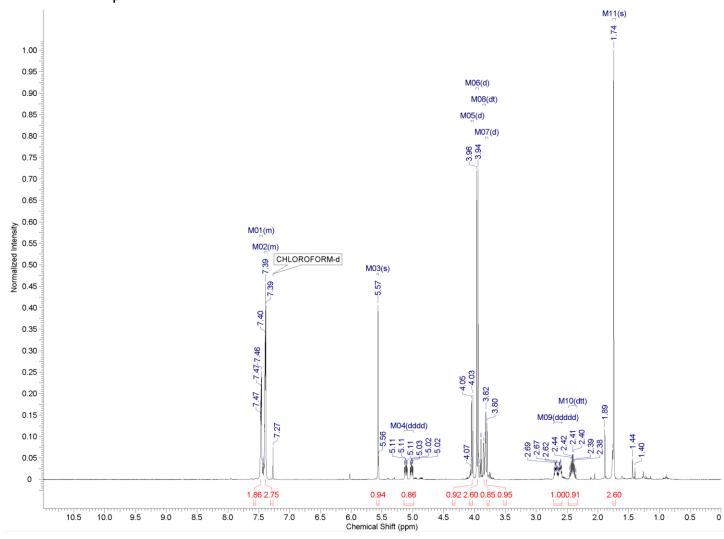


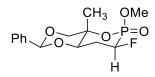






20a-S<sub>P</sub>





20a-S<sub>P</sub>

