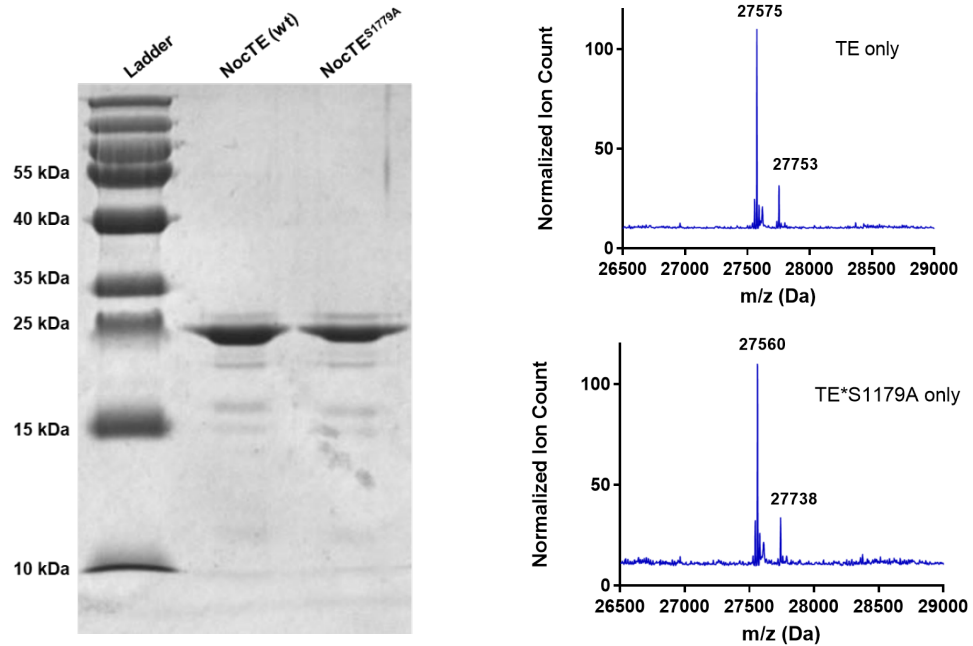


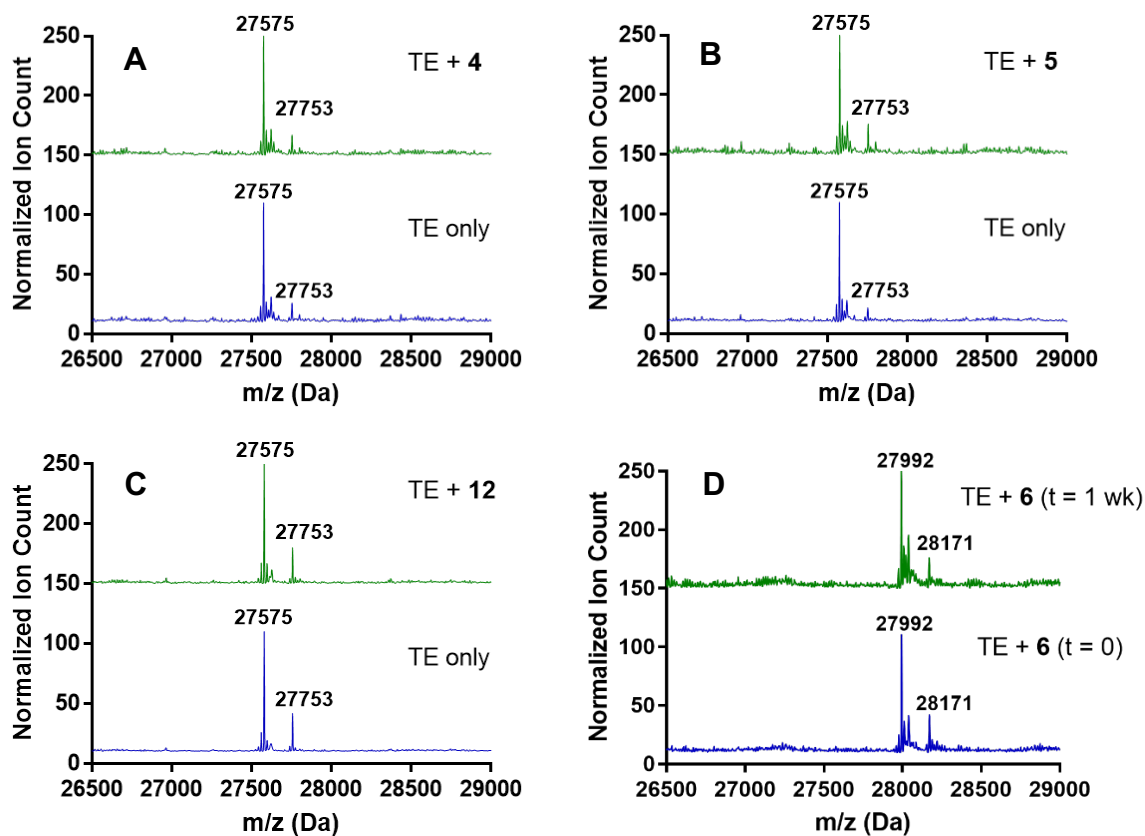
## Supplementary Information

### Protein Expression and Purification



**Figure S1. Related to Figure 3.** (Left) 12% SDS-PAGE gel of affinity-purified wild-type NocTE and NocTE<sup>S1179A</sup> mutant used in covalent modification experiments. (Right) UPLC-HRMS of purified wild type NocTE (MW = 27575 Da) and NocTE<sup>S1179A</sup> (MW = 27560 Da). Masses at 27753 Da and 27738 Da correspond to *N*-glucuronidated masses, respectively.

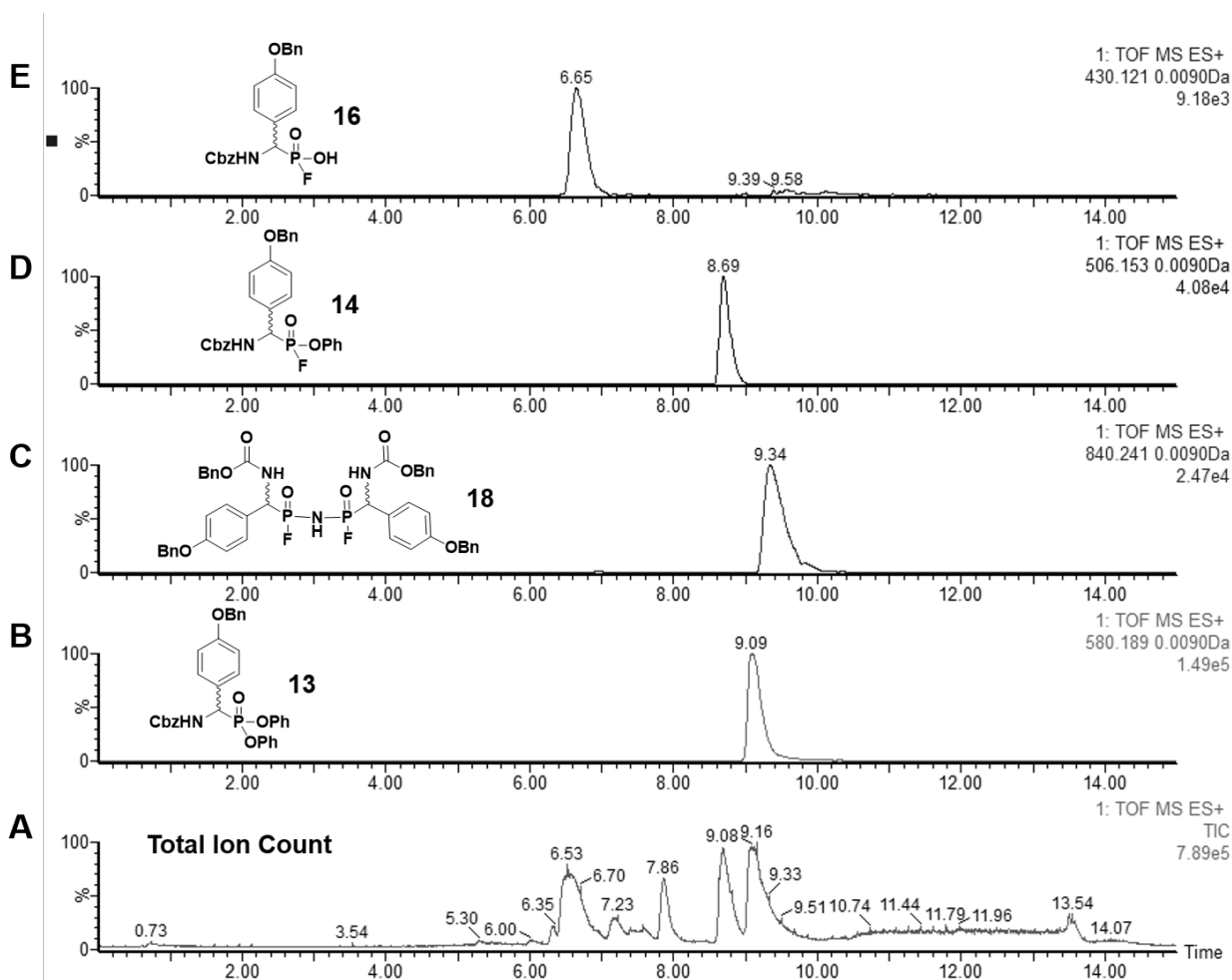
## Biochemical Experiments



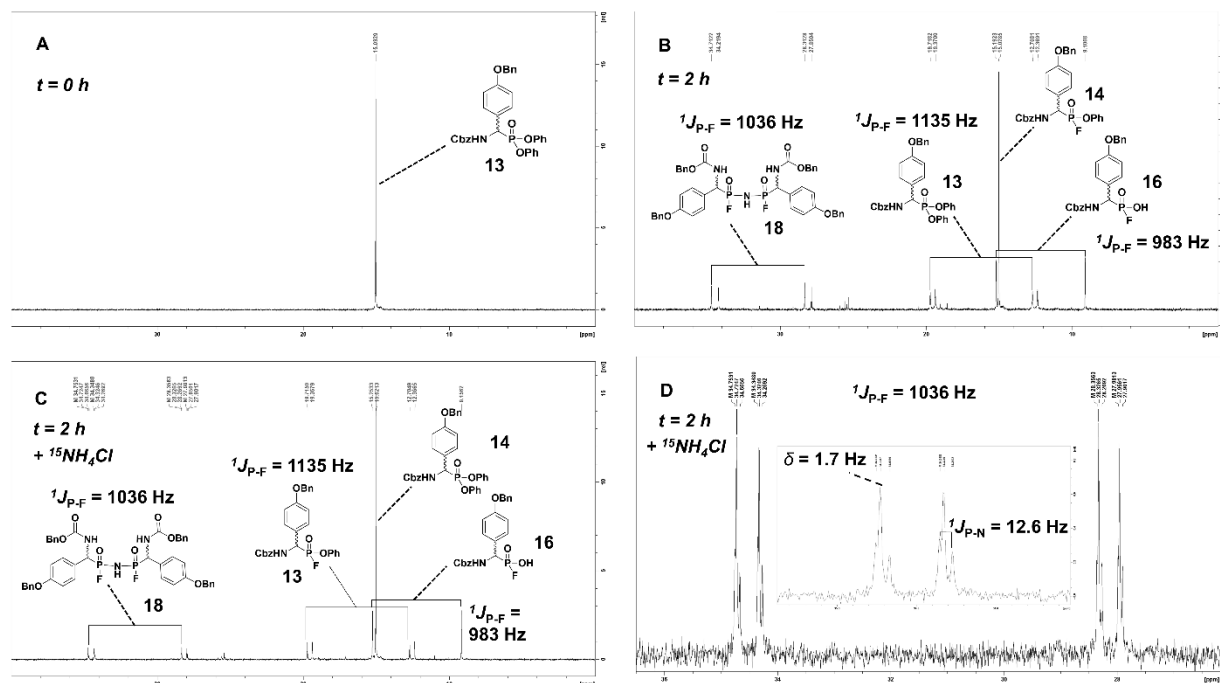
**Figure S2. Related to Scheme 1 and Figure 3.** (A) Incubation of NocTE with DPP 4. No evidence of a covalent adduct was detected by UPLC-HRMS analysis. (B) Incubation of NocTE with DPP 5. No evidence of a covalent adduct was detected by UPLC-HRMS analysis. (C) Incubation of NocTE with 12. No evidence of a covalent adduct was detected by UPLC-HRMS analysis. (D). Analysis of NocTE-6 complex stability over time. No evidence of hydrolysis or loss of the 417 Da covalent adduct was detected by UPLC-HRMS after 1 week in buffer.

## Mechanism Studies of Fluorinative Hydrolysis

### <sup>31</sup>P NMR and UPLC-HRMS Monitoring of the Fluorinative Hydrolysis Reaction



**Figure S3. Related to Figure 2.** UPLC-HRMS analysis of the fluorinative hydrolysis of **13** reaction mixture after 1 h. (A) Total Ion Count (TIC) of the crude reaction mixture after 1 h. (B) Extracted Ion Chromatogram (EIC) of **13**,  $[M+1]^+$   $m/z = 580.1884$ . (C) Extracted Ion Chromatogram (EIC) of **18**,  $[M+1]^+$   $m/z = 840.2410$ . (D) Extracted Ion Chromatogram (EIC) of **14**,  $[M+1]^+$   $m/z = 506.1527$ . (E) Extracted Ion Chromatogram (EIC) of **16**,  $[M+1]^+$   $m/z = 430.1214$ .



**Figure S4. Related to Figure 2.**  $^{31}\text{P}$  NMR monitoring of the fluorinative hydrolysis of **13**. (A)  $^{31}\text{P}$  NMR of the reaction mixture at  $t = 0 \text{ h}$  indicating the exclusive presence of starting material. (B)  $^{31}\text{P}$  NMR of the reaction mixture at  $t = 2 \text{ h}$ , indicating detected intermediates (corroborated by UPLC-HRMS, see Figure S6) and their respective  $^{31}\text{P}$ - $^{19}\text{F}$  coupling constants. (C)  $^{31}\text{P}$  NMR of the reaction mixture supplemented with  $^{15}\text{NH}_4\text{Cl}$  at  $t = 2 \text{ h}$ . The two sets of doublets corresponding to **18** have been further split due to  $^{31}\text{P}$ - $^{15}\text{N}$  coupling, supporting incorporation of  $^{15}\text{N}$  at the P-N-P bridging position. (D) Enhanced view of the  $^{31}\text{P}$ -NMR signals belonging to **18** from (C) highlighting additional splitting caused by  $^{15}\text{N}$  incorporation.  $^1J_{P,N}$  was found to be 12.6 Hz, with an upfield heavy atom chemical shift effect of 1.7 Hz.