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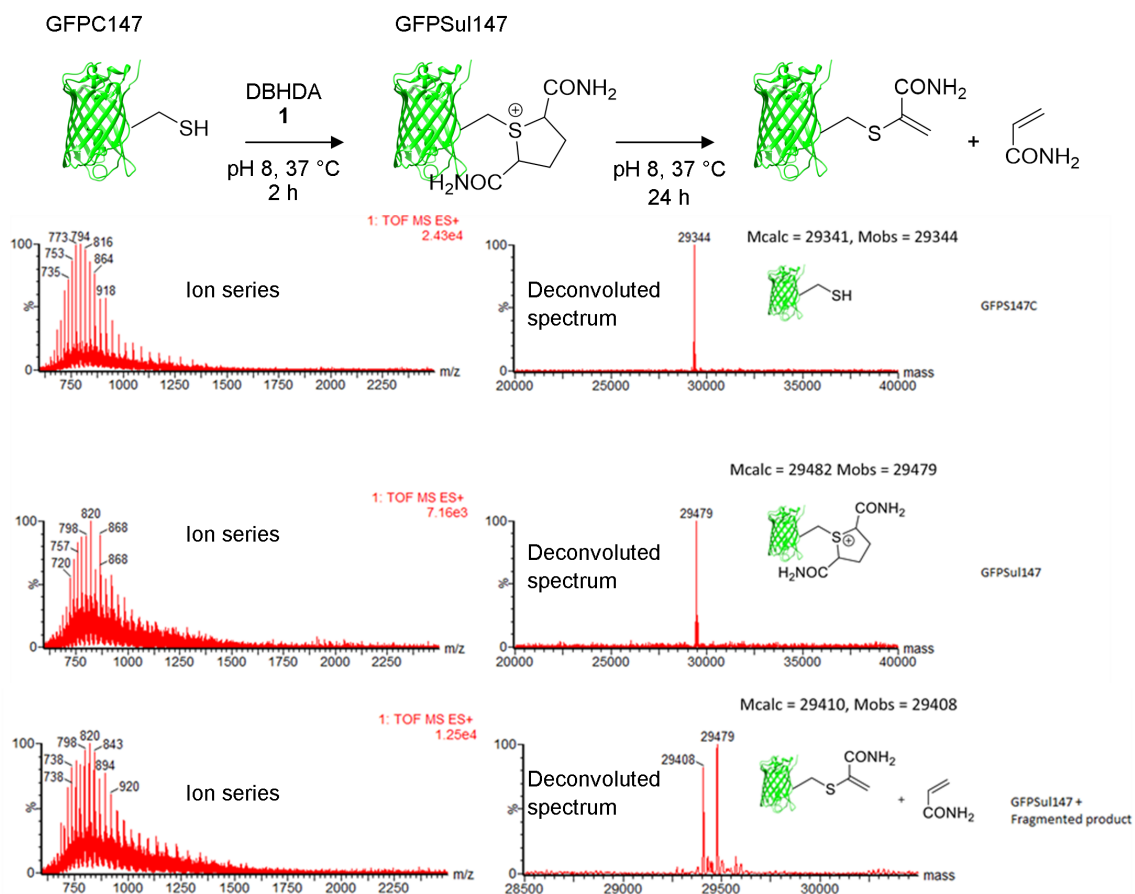
Post-translational site-selective protein backbone α -deuteration

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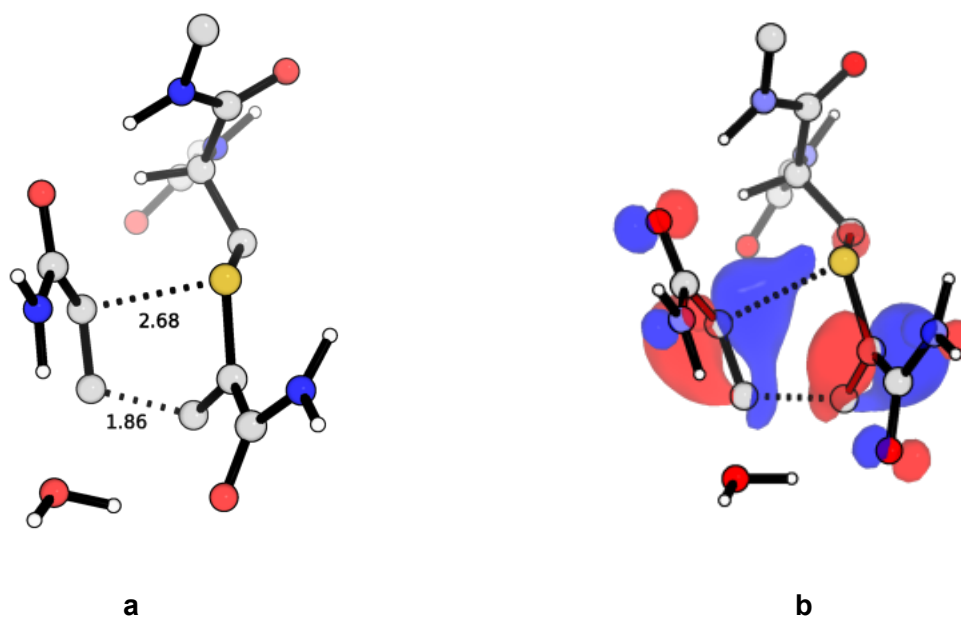
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Structure	E/au	ZPE/au	H/au	Qh-G/au	Imaginary Freq/cm ⁻¹
Intramolecular pathway:					
Starting complex	-1463.804556	0.361782	-1463.417309	-1463.493081	-
Deprotonation TS	-1463.774236	0.355911	-1463.392496	-1463.468716	-932.184
Sulfonium ylid	-1463.796078	0.361477	-1463.408186	-1463.486067	-
Intramolecular deprot. TS	-1463.769112	0.354586	-1463.387347	-1463.466589	-1167.357
Product	-1463.837479	0.359888	-1463.449591	-1463.531014	-
E2-elimination pathway:					
Starting complex	-1463.79543	0.361028	-1463.408127	-1463.485822	-
E2-elimination TS	-1463.763324	0.355009	-1463.381935	-1463.459659	-1262.9813
Product	-1463.844519	0.360551	-1463.456635	-1463.536136	-

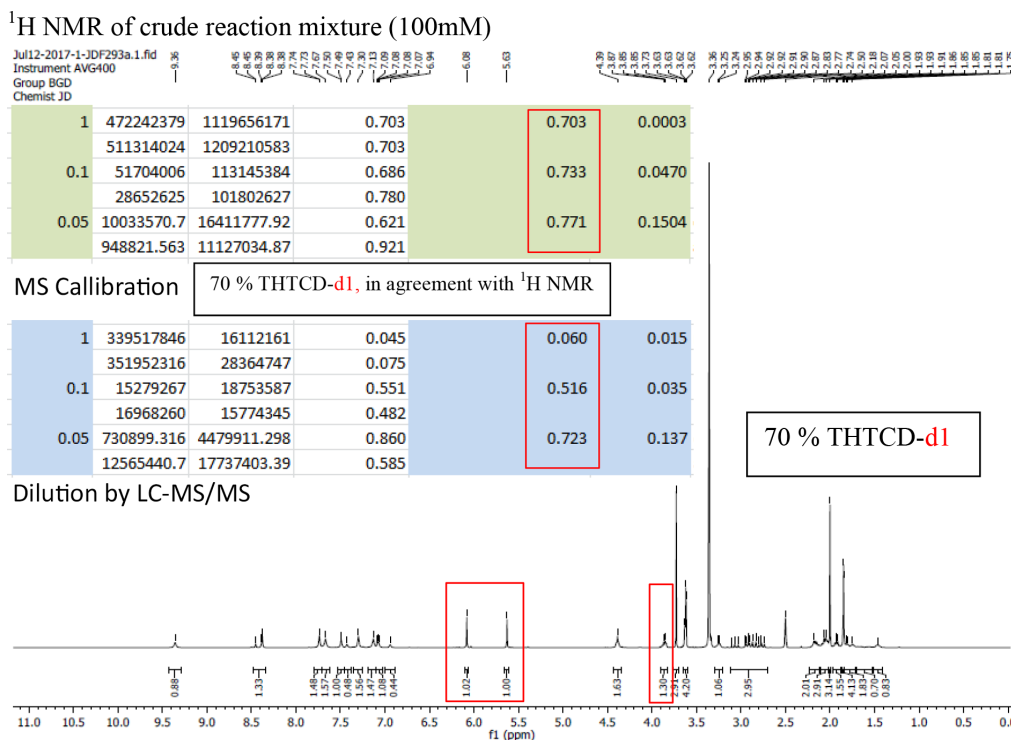
Supplementary Table 3. Absolute values (Hartrees) for SCF energy, zero-point vibrational energy (ZPE), enthalpy and free energy (at 298K) for the lowest energy structures.



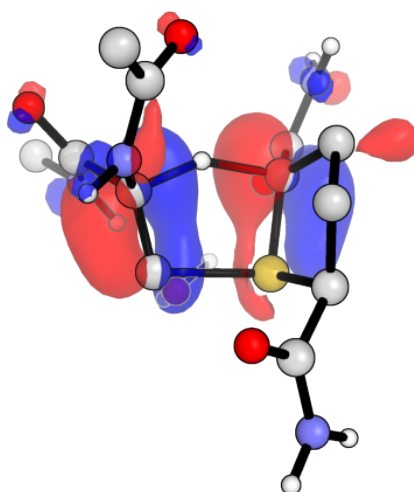
Supplementary Figure 19. Formation of GFPSul147 monitored by LCMS and Identification of Fragmentation Products. The experiments were repeated independently three times with similar results.



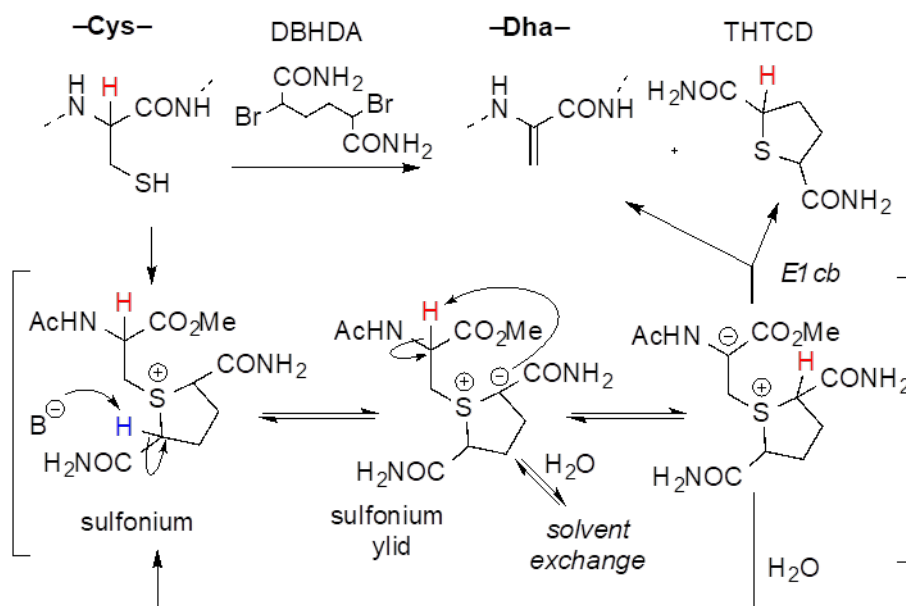
Supplementary Figure 20 (a) Left: Lowest energy TS for sulfonium ylid decomposition via concerted [3+2] cycloreversion. Bond distances are given in Å. (b) Right: The corresponding HOMO (at an isovalue of 0.05) for this lowest energy TS, indicating a concerted electron movement, with dominant electron density on the ylid carbon and the α -carbon of the acylamide fragment, as the cycloreversion occurs.



Supplementary Figure 29. Dilution experiments were also performed to explore the effect of concentration upon incorporation as a proxy for relative rate of intra- vs inter- molecular alpha-deuteron abstraction. D-AcNHCysOMe-d1 **4** (17.7 mg, 0.1 mmol), DBHDA (30.2 mg, 0.1 mmol) and potassium carbonate (41 mg, 0.3 mmol) were dissolved in DMSO-d6 (1 mL) to give 100mM AcCysOMe-d1 and DBHDA stock solution. A series of sequential dilutions (1mM, 0.1mM, 0.05mM, 0.01mM; final volume 200 μ L of each reaction mixture) in DMSO-d6 were prepared immediately. All reactions were incubated at 25 $^{\circ}$ C and 300 rpm for 1 hour and then at 37 $^{\circ}$ C and 300 rpm for 5 hours. Formation of AcNHDhaOMe and THTCD-d1 was confirmed by ¹H NMR of crude reaction mixture (100mM). The ratio of THTCD-d1 and THTCD was determined by LC/MS-MS analysis. ¹H NMR was also used to confirm incorporation correlations and estimate associated precision. The experiments were repeated independently twice with similar results.



Supplementary Figure 30. HOMO (at an isovalue of 0.05) for the lowest energy intramolecular deprotonation TS, indicating a concerted electron movement as the deprotonation step occurs.



Supplementary Figure 31. Summary of Proposed Mechanism