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# Post-translational site-selective protein backbone $\alpha$ -deuteration

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## SUPPORTING INFORMATION

### Post-Translational Site-selective Protein Backbone $\alpha$ -Deuteration

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**Supplementary Tables**

Protein	Conditions <sup>a</sup>	Calculated Mass (Da) <sup>b</sup>	%D
H3C10	1	15254.6407±0.0566	0
H3dC10	2	15254.9409±0.0283	30±6
H3dC10	3	15255.1812±0.0284	54±6
H3dC10	4	15255.5405±0.1416	90±15
H3dC10	5	15255.5405±0.0284	90±6
H3C26	1	15185.5605 <sup>c</sup>	0
H3dC26	6	15186.1611 <sup>c</sup>	60
H3dC26	5	15186.5205 <sup>c</sup>	90

<sup>a</sup> See table S2; <sup>b</sup> The duplicated mass values for each sample were averaged and the error was calculated as the standard variation; <sup>c</sup> the mass was measured once.

Supplementary Table 1. Determination of deuterium incorporation in H3C10 and H3C26 under different conditions

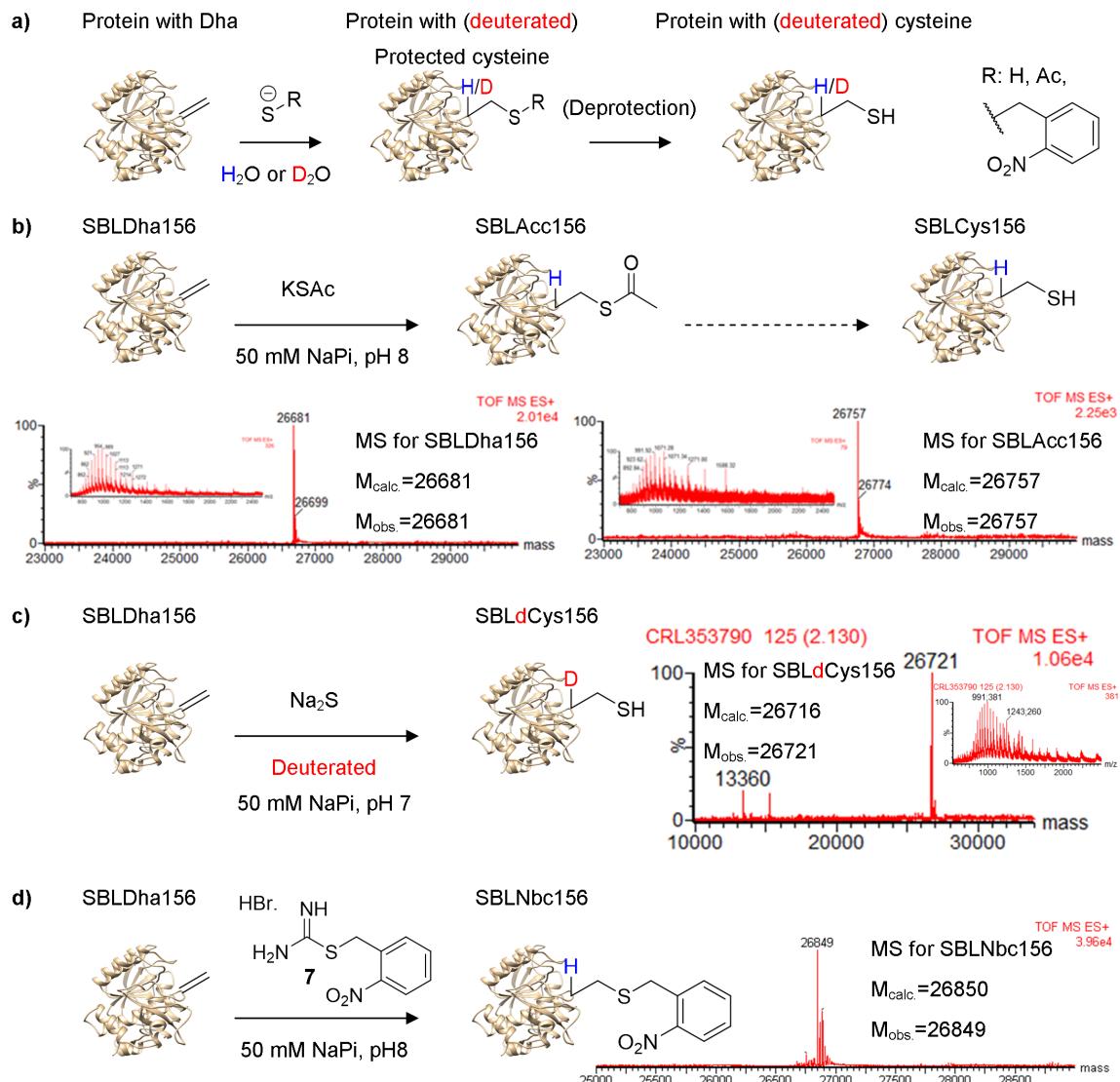
Conditions	Protein	Na <sub>3</sub> SPO <sub>3</sub>	Buffer
1	lyophilised	Hydrate	50 mM Tris, 5 M GdnCl, pH8
2	lyophilised	Hydrate	50 mM Tris, 5 M GdnCl, pH 8 (70% deuterated) <sup>b</sup>
3	lyophilised	Hydrate	50 mM Tris, 5 M GdnCl, pH 8 deuterated <sup>a</sup>
4	lyophilised	Anhydrous <sup>a</sup>	50 mM Tris, 5 M GdnCl, pH 8 deuterated <sup>a</sup>
5	lyophilised in D <sub>2</sub> O <sup>a</sup>	Anhydrous <sup>a</sup>	50 mM Tris, 5 M GdnCl, pH 8 deuterated <sup>a</sup>
6	lyophilised in D <sub>2</sub> O <sup>a</sup>	Hydrate	50 mM Tris, 5 M GdnCl, pH 8 deuterated <sup>a</sup>

<sup>a</sup> See Online Methods; <sup>b</sup> Buffer components directly added to D<sub>2</sub>O.

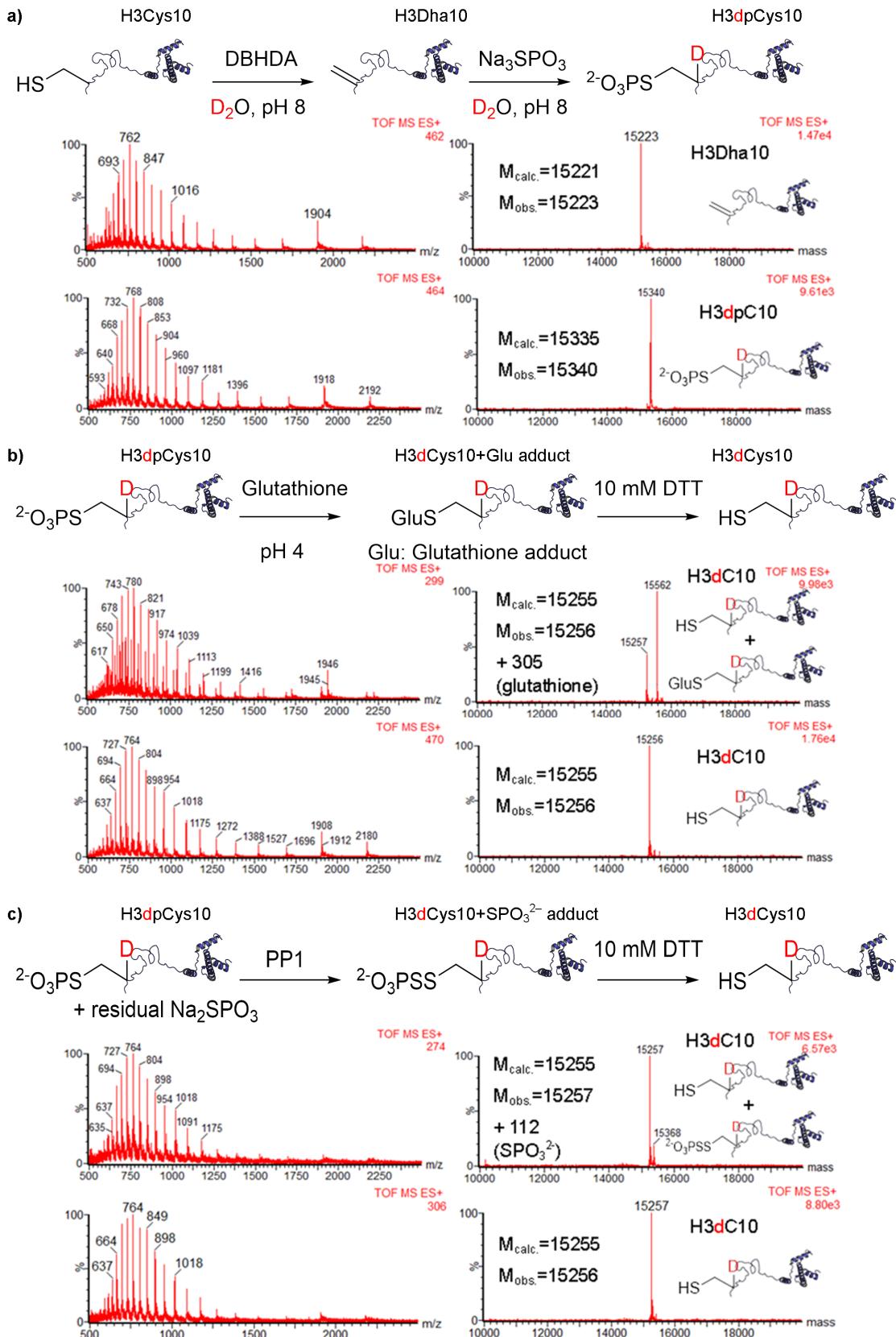
Supplementary Table 2. Conditions used for site-selective deuteration of cysteine residues on protein

Structure	E/au	ZPE/au	H/au	Qh-G/au	Imaginary Freq/cm <sup>-1</sup>
<b>Intramolecular pathway:</b>					
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	-
<b>E2-elimination pathway:</b>					
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	-

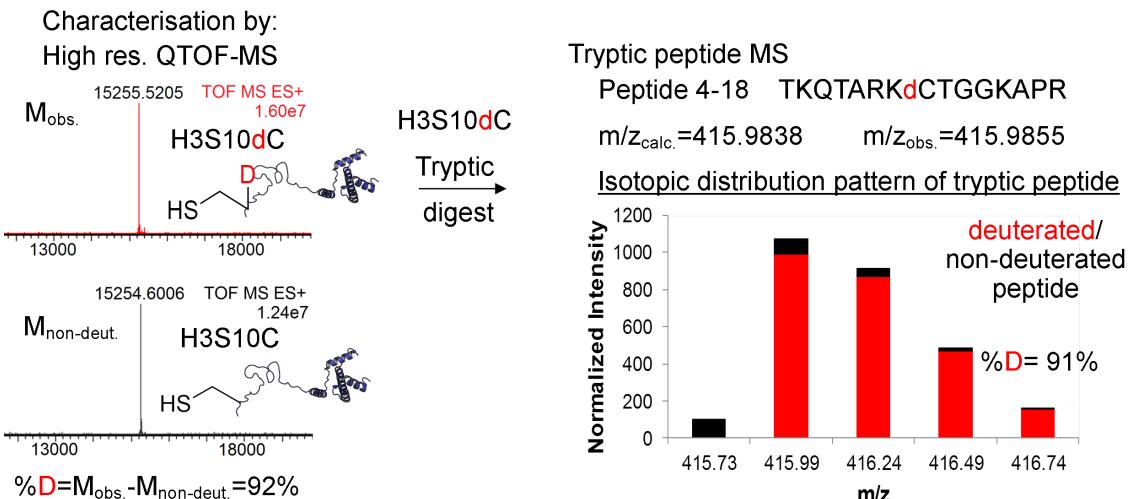
Supplementary Table 3. Absolute values (Hartrees) for SCF energy, zero-point vibrational energy (ZPE), enthalpy and free energy (at 298K) for the lowest energy structures.

**Supplementary Figures***Development of Site-Selective Protein Deuteration Method*

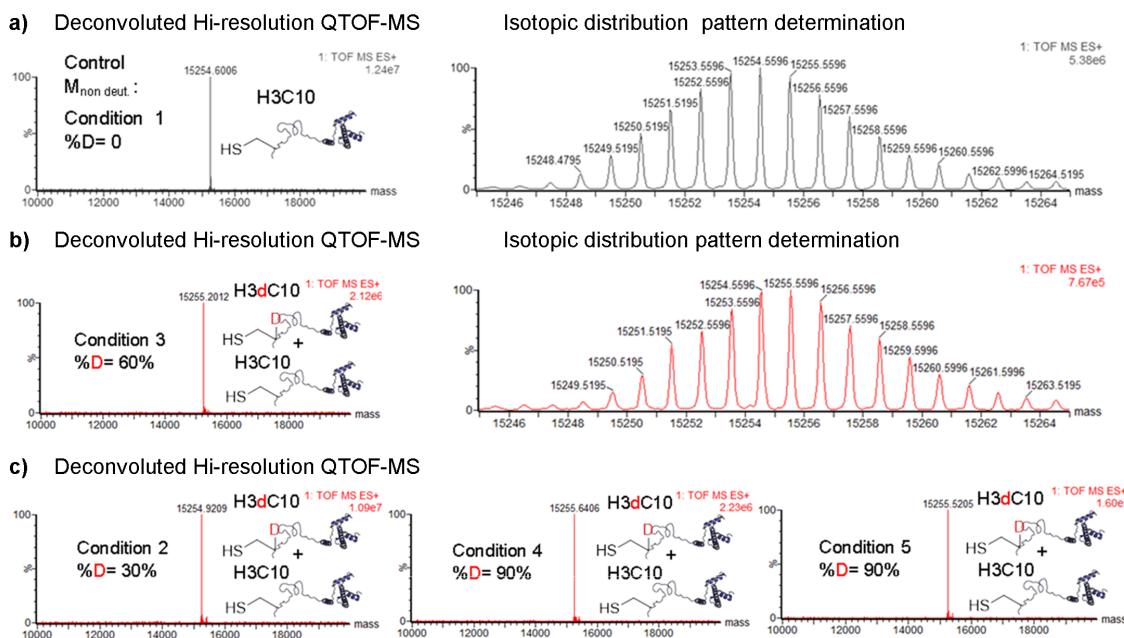
Supplementary Figure 1. **(a)** General scheme towards formation of deuterated protein using various sulfur nucleophiles. **(b)** Exploratory Reactions with Sodium Thioacetate. **(c)** Exploratory Reactions with Sodium Sulfide. **(d)** Exploratory Reactions towards protein containing photocaged cysteine residue. Experiments (b), (c) and (d) were repeated independently twice with similar results.



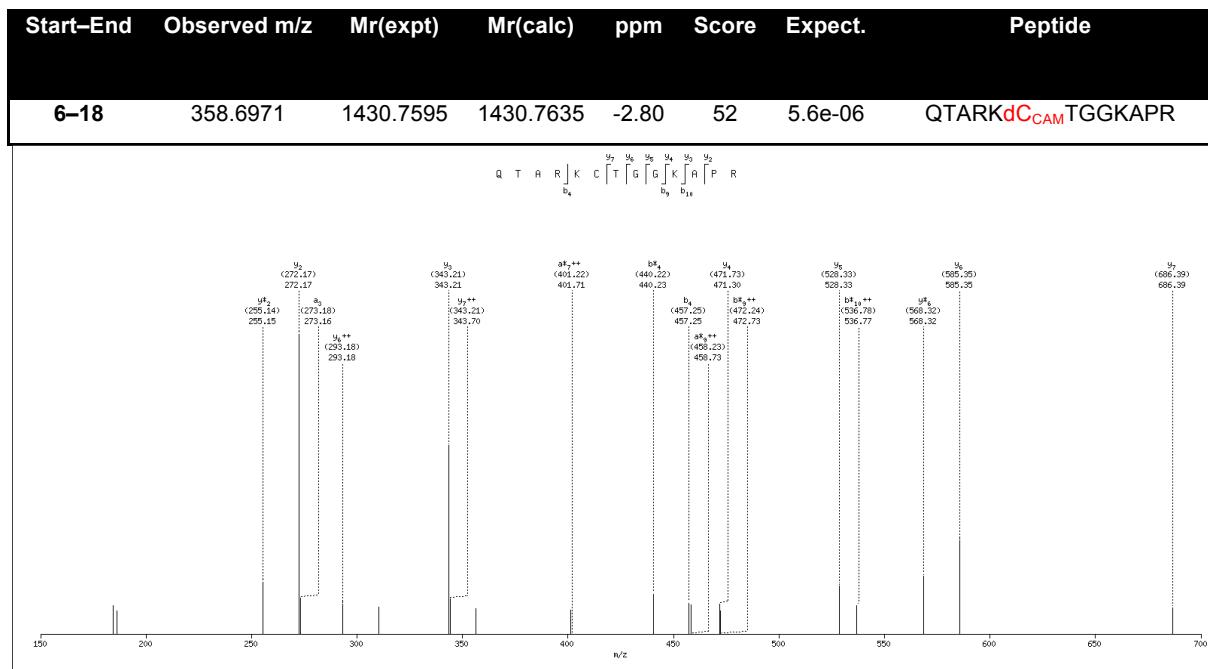
Supplementary Figure 2. **(a)** Formation of H3dpC10 monitored by LCMS, observed differences in mass during synthesis were also due to slow exchangeable deuterons inside the protein. **(b)** Formation of H3dC10, Method A. **(c)** Formation of H3dC10, Method B. The experiments were repeated independently three times with similar results. Experiments (a), (b) and (c) were repeated independently 4 times with similar results.



Supplementary Figure 3. Summary of Characterization of H3dC10. High resolution QTOF-MS and subsequent tryptic digest-MS analyses (as acetamide d<sub>CCAM</sub>) confirmed D incorporation >90%; shown here for H3-dC10 (see below for further details). These experiments were repeated independently twice with similar results.



Supplementary Figure 4. (a) Control deconvoluted spectra and isotopic pattern determination of non-deuterated H3C10. (b) Deconvoluted spectra and isotopic pattern determination of H3C10 with 60% deuterium incorporation. (c) Examples of deconvoluted spectra with various level of deuterium incorporation. See supplementary Table 1 for summary and supplementary Table 2 for conditions. These experiments were repeated independently twice with similar results.

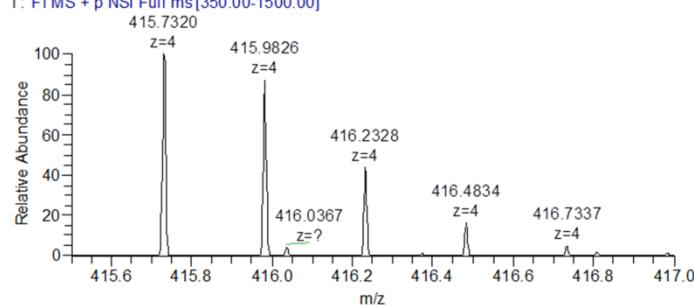


Supplementary Figure 5. Validation of deuterium incorporation levels and conformation of location of deuterium by LCMSMS of tryptic peptide for H3dC10. This experiments was repeated independently twice with similar results.

H3C10 peptide 4-18 TKQTARKC<sub>CAM</sub>TGGKAPR m/z<sub>calc.</sub>=415.7322, m/z<sub>obs.</sub>=415.7320, z=4

#### Condition 1 MS1 spectra (Isotopic pattern)

QEX01\_RR\_160105\_SG\_H3S10C\_01 #2239-2854 RT: 13.29-16.45 AV: 483 NL: 4.35E5  
T: FTMS + p NSI Full ms[350.00-1500.00]



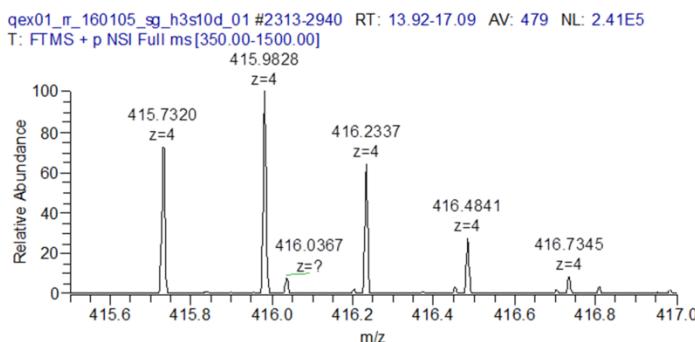
#### Relative intensities

m/z	Intensity	Relative (%)	Charge
415.7320	444257.5	100	4
415.9826	379098.8	85.33	4
416.2328	192332.2	43.29	4
416.4834	70806.5	15.94	4
416.7337	20483.4	4.61	4

Supplementary Figure 6. MS1 analysis of the H3C10 peptide 4-18 TKQTARKCCAMTGGKAPR showing its isotopic pattern.

H3dC10 peptide 4-18 TKQTARK**dC<sub>CAM</sub>**TGGKAPR m/z<sub>calc.</sub>=415.9838, z=4

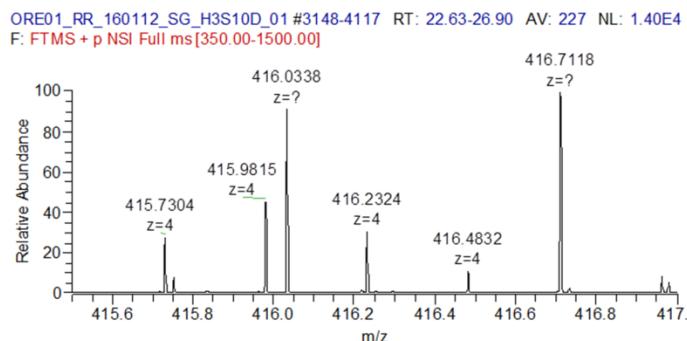
Condition 2 MS1 spectra (Isotopic pattern)



Relative intensities

m/z	Intensity	Relative (%)	Charge
415.7320	181181.2	74.71	4
415.9828	242499.2	100	4
416.2337	155601.2	64.17	4
416.4841	66534.4	27.44	4
416.7345	21275.1	4.61	4

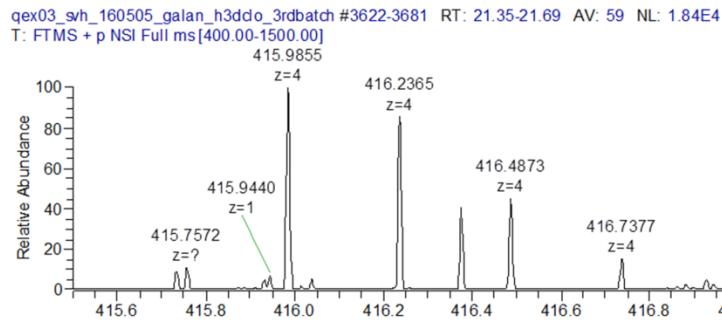
Condition 3 MS1 spectra (Isotopic pattern)



Relative intensities

m/z	Intensity	Relative (%)	Charge
415.7304	3970.6	28.03	4
415.9815	6540.2	46.18	4
416.2324	4326.6	30.55	4
416.4832	1538.5	10.86	4
416.7333	412.1	2.91	4

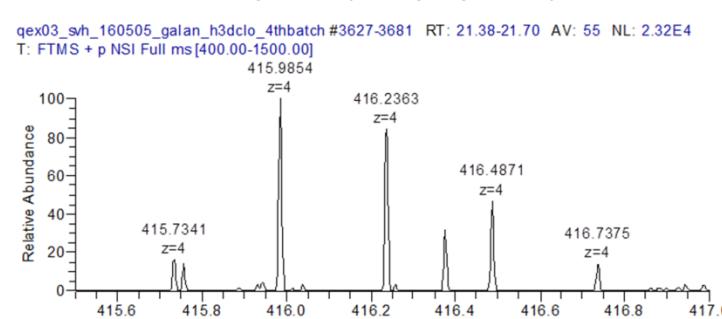
Condition 4 MS1 spectra (Isotopic pattern)



Relative intensities

m/z	Intensity	Relative (%)	Charge
415.7340	1628.2	8.61	4
415.9855	18908.1	100	4
416.2328	16045.7	84.86	4
416.4834	8440.8	44.64	4
416.7337	2930.9	15.50	4

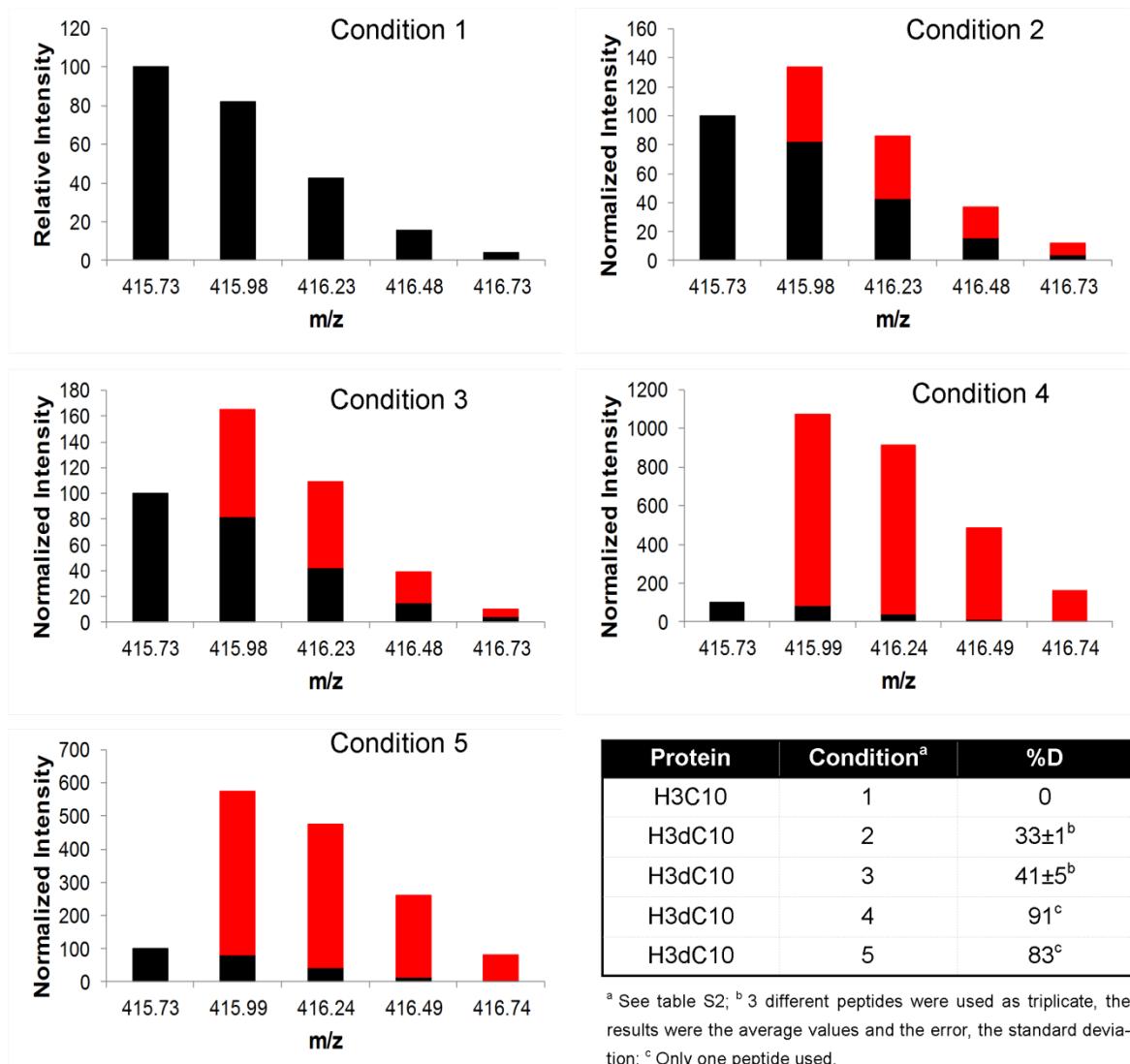
Condition 5 MS1 spectra (Isotopic pattern)



Relative intensities

m/z	Intensity	Relative (%)	Charge
415.7341	3949.4	16.57	4
415.9854	23838.4	100	4
416.2363	19878.4	83.39	4
416.4871	11007.4	46.18	4
416.7375	3368.7	4.61	4

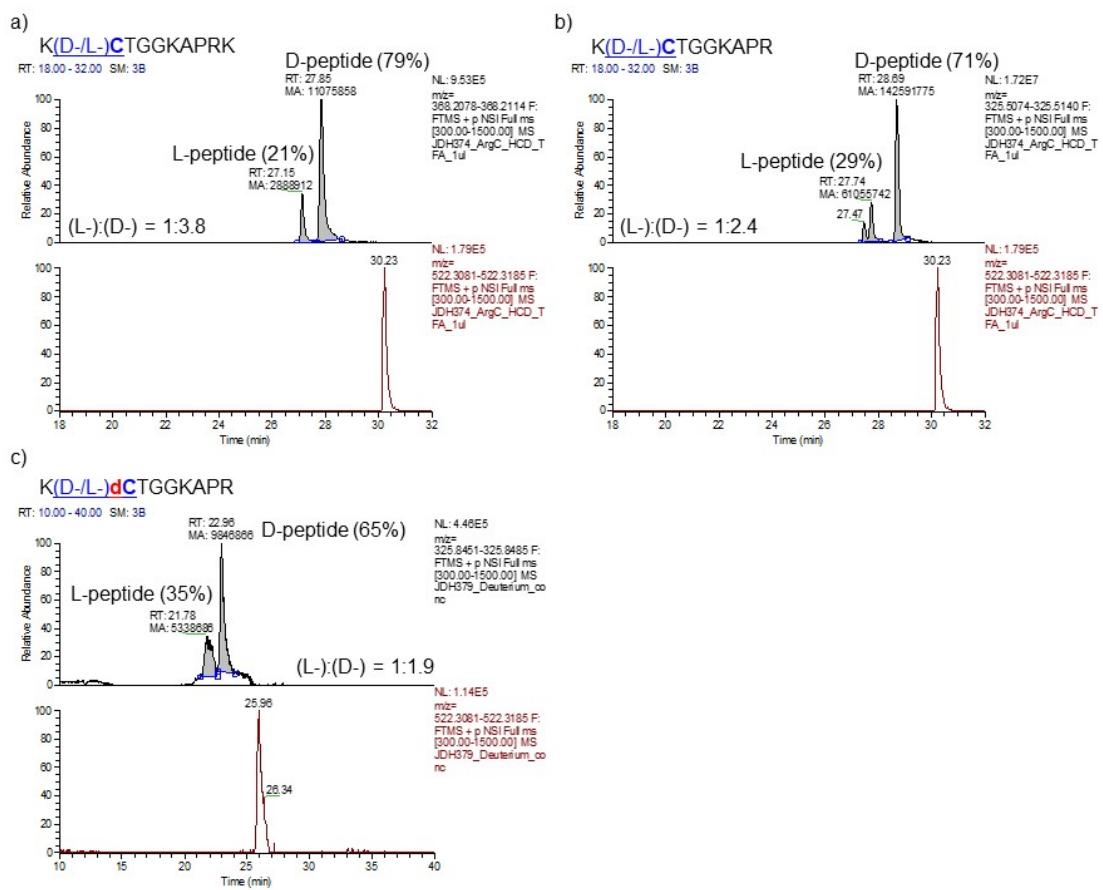
Supplementary Figure 7. MS1 analysis of the H3dC10 peptides 4-18 TKQTARKdCCAMTGGKAPR prepared with different conditions and their isotopic pattern



Protein	Condition <sup>a</sup>	%D
H3C10	1	0
H3dC10	2	33±1 <sup>b</sup>
H3dC10	3	41±5 <sup>b</sup>
H3dC10	4	91 <sup>c</sup>
H3dC10	5	83 <sup>c</sup>

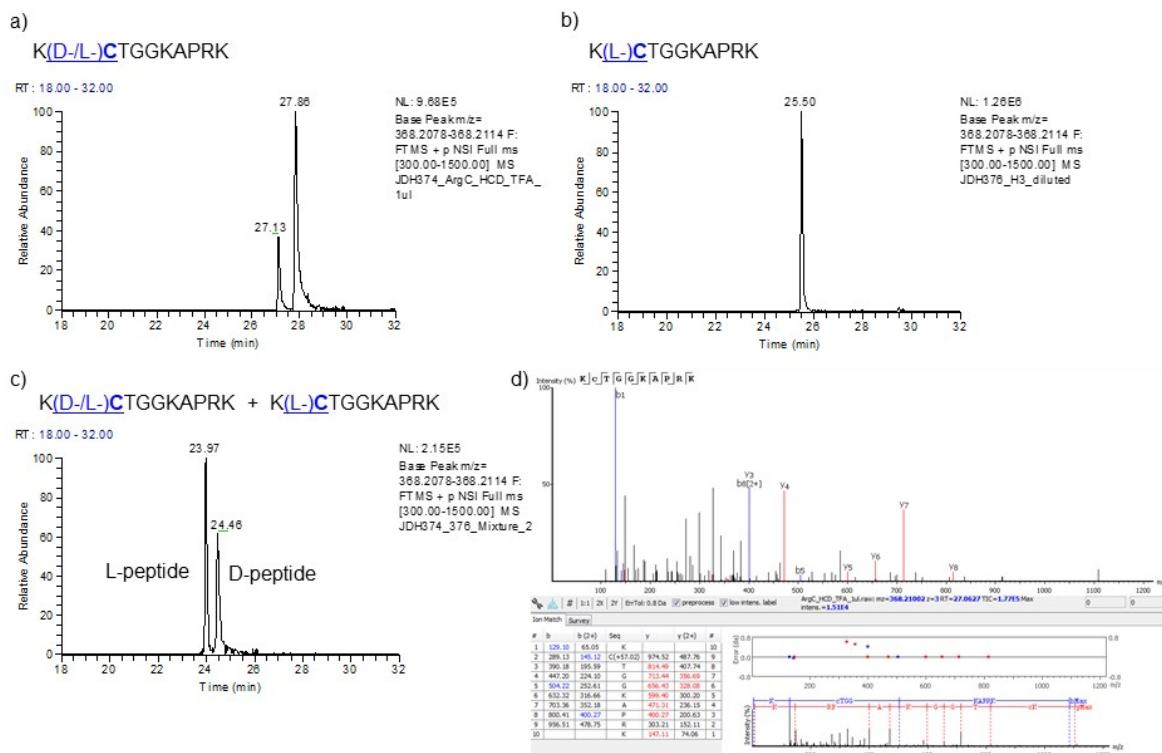
<sup>a</sup> See table S2; <sup>b</sup> 3 different peptides were used as triplicate, the results were the average values and the error, the standard deviation; <sup>c</sup> Only one peptide used.

Supplementary Figure 8. Representation of MS1 spectra showing isotopic pattern of mixtures of deuterated and non-deuterated tryptic peptides (4-18) depending on reaction conditions and %D estimation.

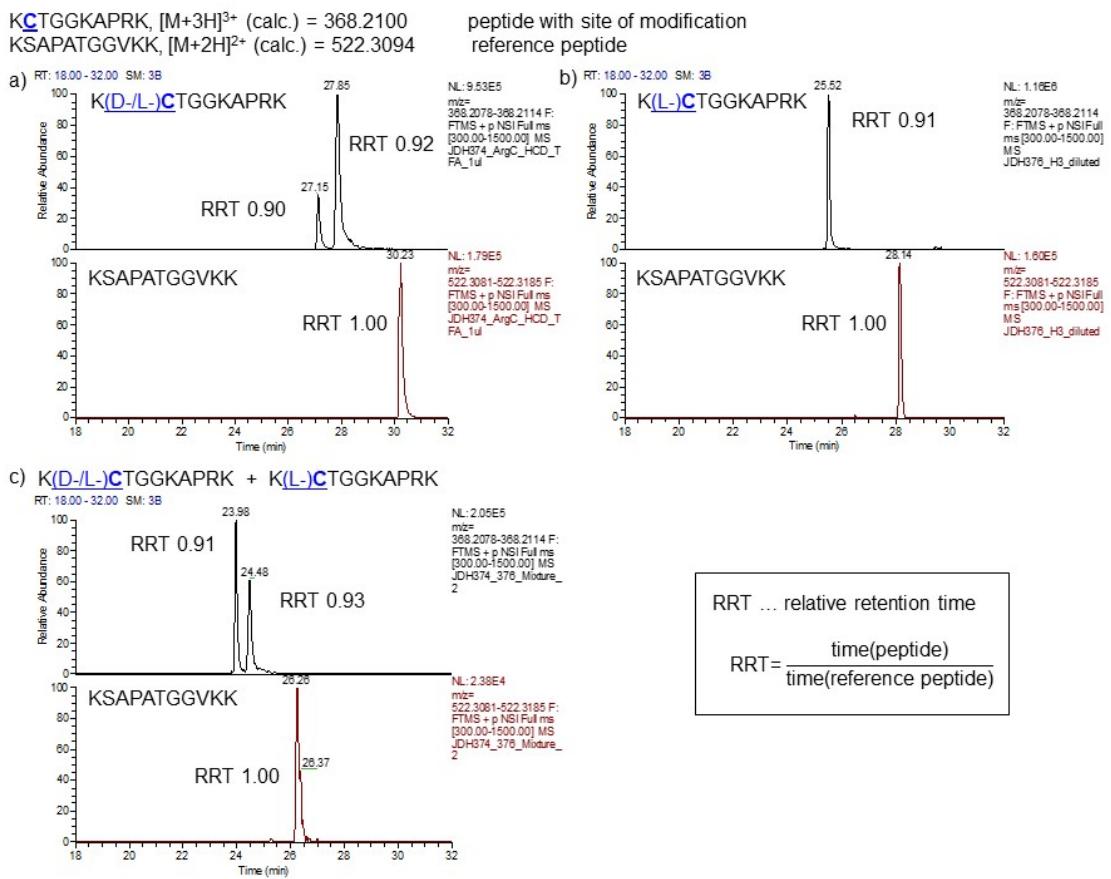


Supplementary Figure 9. LC/MSMS analysis of histone H3-C10 digested with ArgC. Integrated extracted ion chromatograms indicating ratios of D-/L- peptides containing a) (D-/L-)C10 (peptide K9-K18), b) (D-/L-)C10 (peptide K9-R17), c) (D-/L-)dC10 (peptide K9-R17). See below (Supplementary figures 10 to 17) for more details. The experiments were repeated independently twice with similar results.

**K<sub>C</sub>TGGKAPRK, [M+3H]<sup>3+</sup> (calc.) = 368.2100**

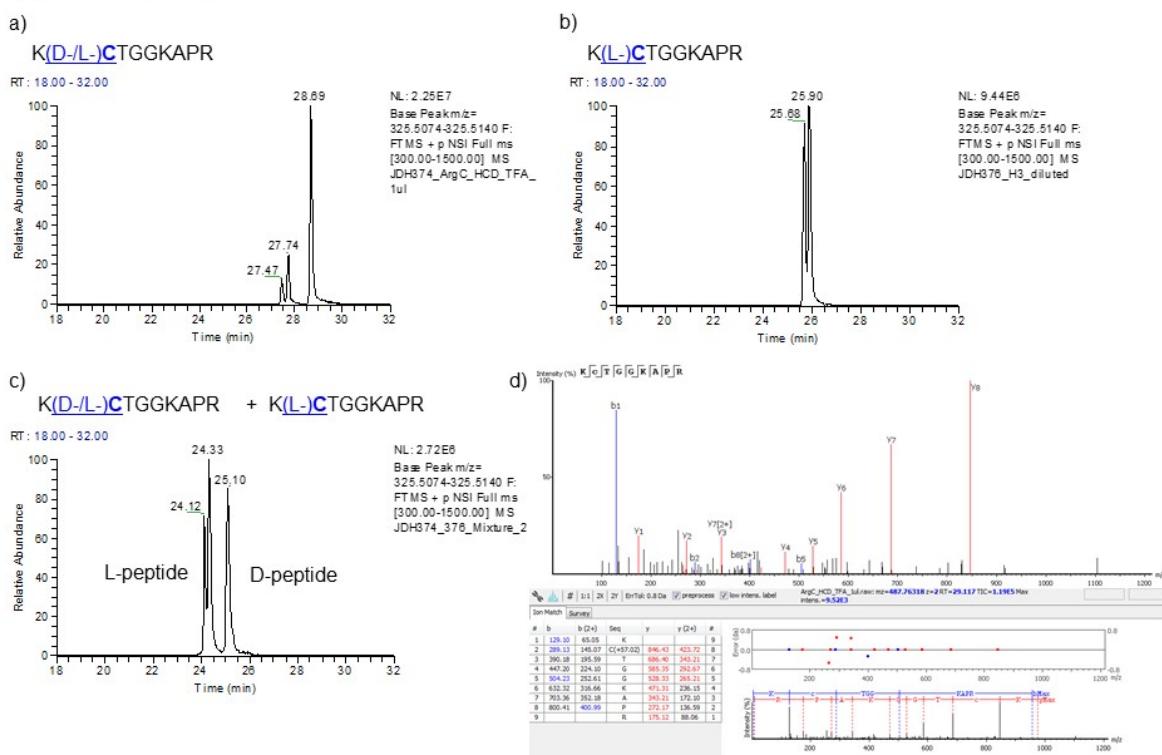


Supplementary Figure 10. LC/MSMS analysis of histone H3-C10 digested with ArgC. a-c) Extracted ion chromatograms of peptide K9-K18 ( $M_{\text{calc.}} = 1101.6063$ ) obtained from a) H3-(D-L-)C10 following reaction sequence, b) natural H3 (L-)C10, c) mixture of a) and b). d) MSMS spectra of peptide K9-K18. The experiments were repeated independently twice with similar results.

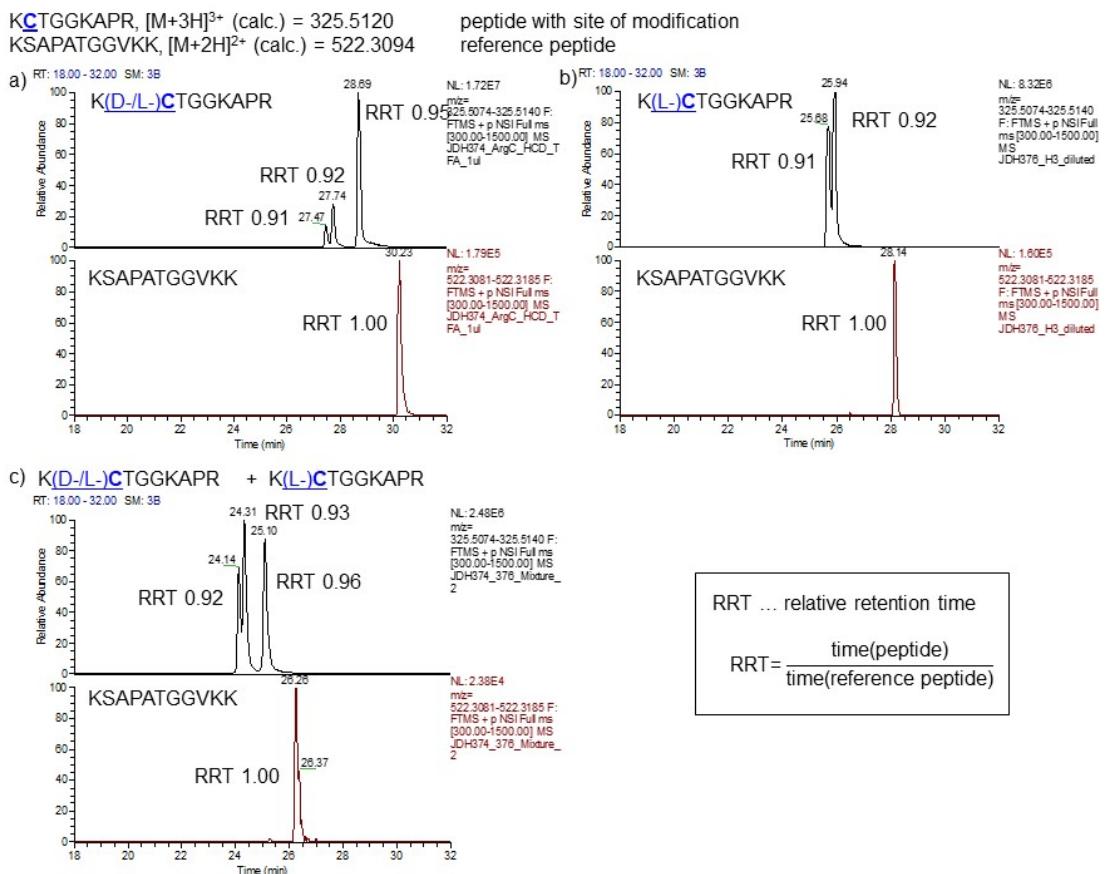


Supplementary Figure 11. LC/MS/MS analysis of histone H3-C10 digested with ArgC. Extracted ion chromatograms showing relative retention times (RRT) of peptide K9-K18 ( $M_{\text{calc.}} = 1101.6063$ ) to reference peptide K27-K37 ( $M_{\text{calc.}} = 1042.6030$ ) in a) H3-(D/L)-C10, b) natural H3-(L)-C10, c) mixture of a) and b). The experiments were repeated independently twice with similar results.

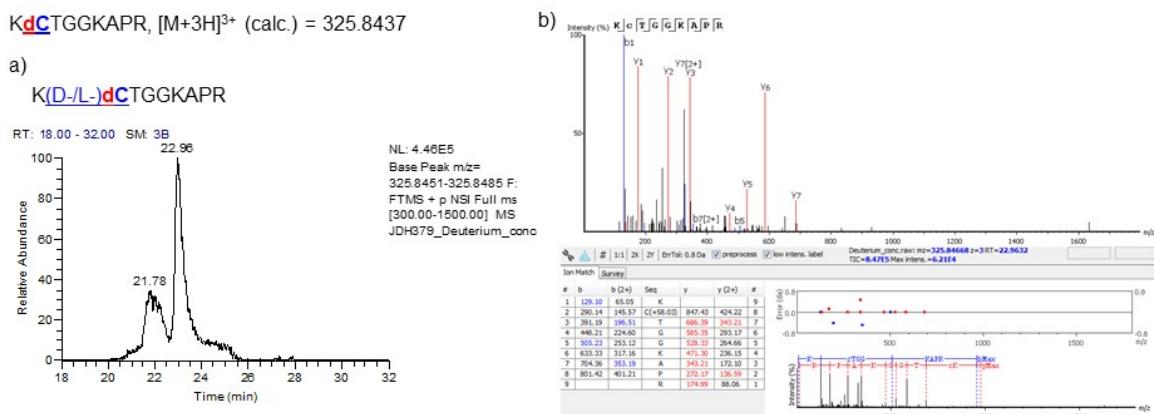
KLTGGKAPR, [M+3H]<sup>3+</sup> (calc.) = 325.5120



Supplementary Figure 12. LC/MSMS analysis of histone H3 C10 digested with ArgC. a-c) Extracted ion chromatograms of peptide K9-R17 ( $M_{\text{calc.}} = 973.5123$ ) obtained from a) H3-(D-L)-C10 following reaction sequence, b) natural H3-(L)-C10, c) mixture of a) and b). d) MSMS spectra of peptide K9-R17. The experiments were repeated independently twice with similar results.

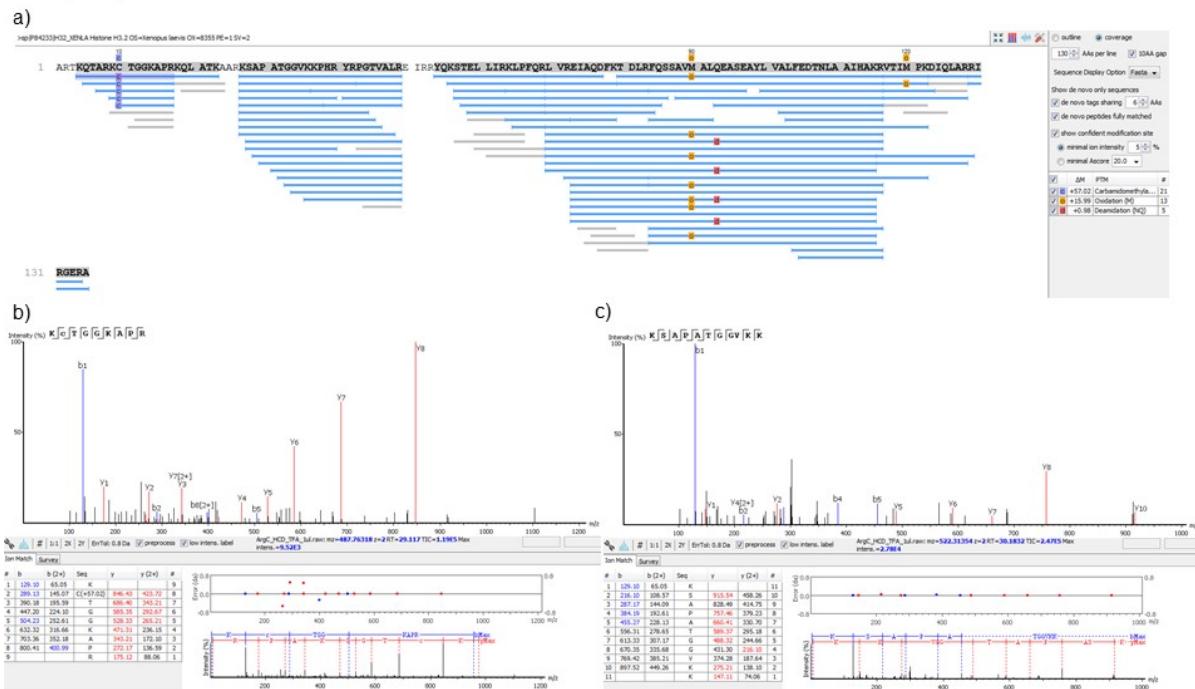


Supplementary Figure 13. LC/MSMS analysis of histone H3-C10 digested with ArgC. Extracted ion chromatograms showing relative retention times (RRT) of peptide K9-R17 ( $M_{\text{calc.}} = 973.5123$ ) to reference peptide K27-K37 ( $M_{\text{calc.}} = 1042.6030$ ) in a) H3 (D-/L-)C10, b) natural H3-(L-)C10, c) mixture of a) and b). The experiments were repeated independently twice with similar results.



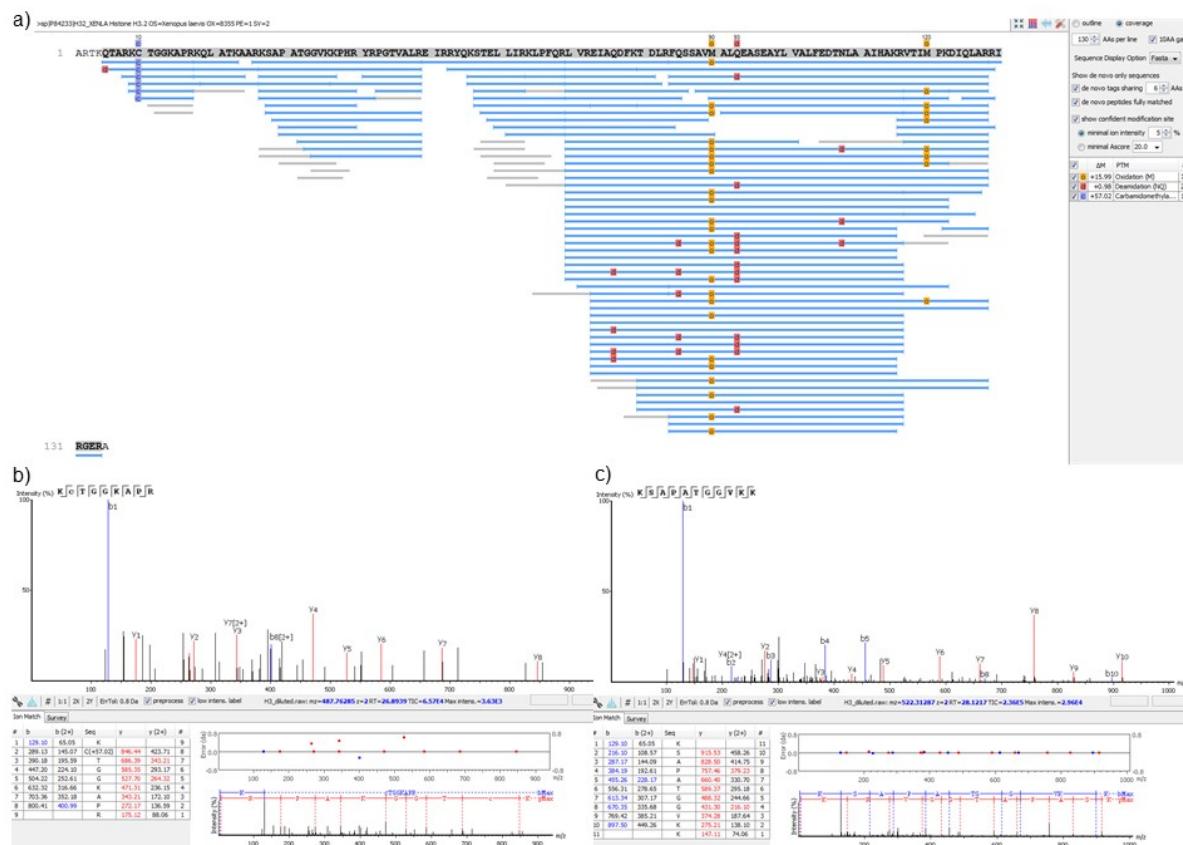
Supplementary Figure 14. LC/MSMS analysis of H3-(D-/L-)dC10 digested with ArgC (>90% incorporation). a) Extracted ion chromatogram of deuterated peptide K9-R17 ( $M_{\text{calc.}} = 974.5074$ ). b) MSMS spectra of deuterated peptide K9-R17. The experiments were repeated independently twice with similar results.

## H3 (D-L)-C10



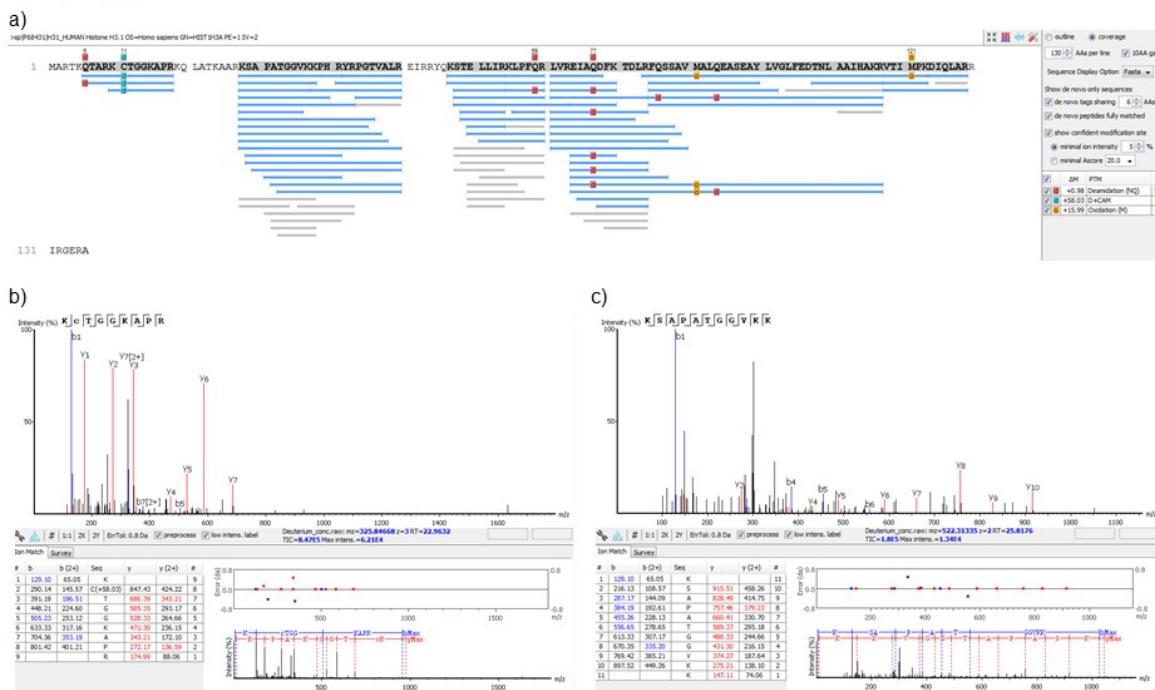
Supplementary Figure 15. LC/MSMS analysis of histone H3 (D-L)-C10 digested with ArgC. a) Sequence coverage, b) MSMS spectrum of peptide K9-R17 containing site of modification, c) MSMS spectrum of reference peptide K27-K37. The experiments were repeated independently twice with similar results.

## H3 C10

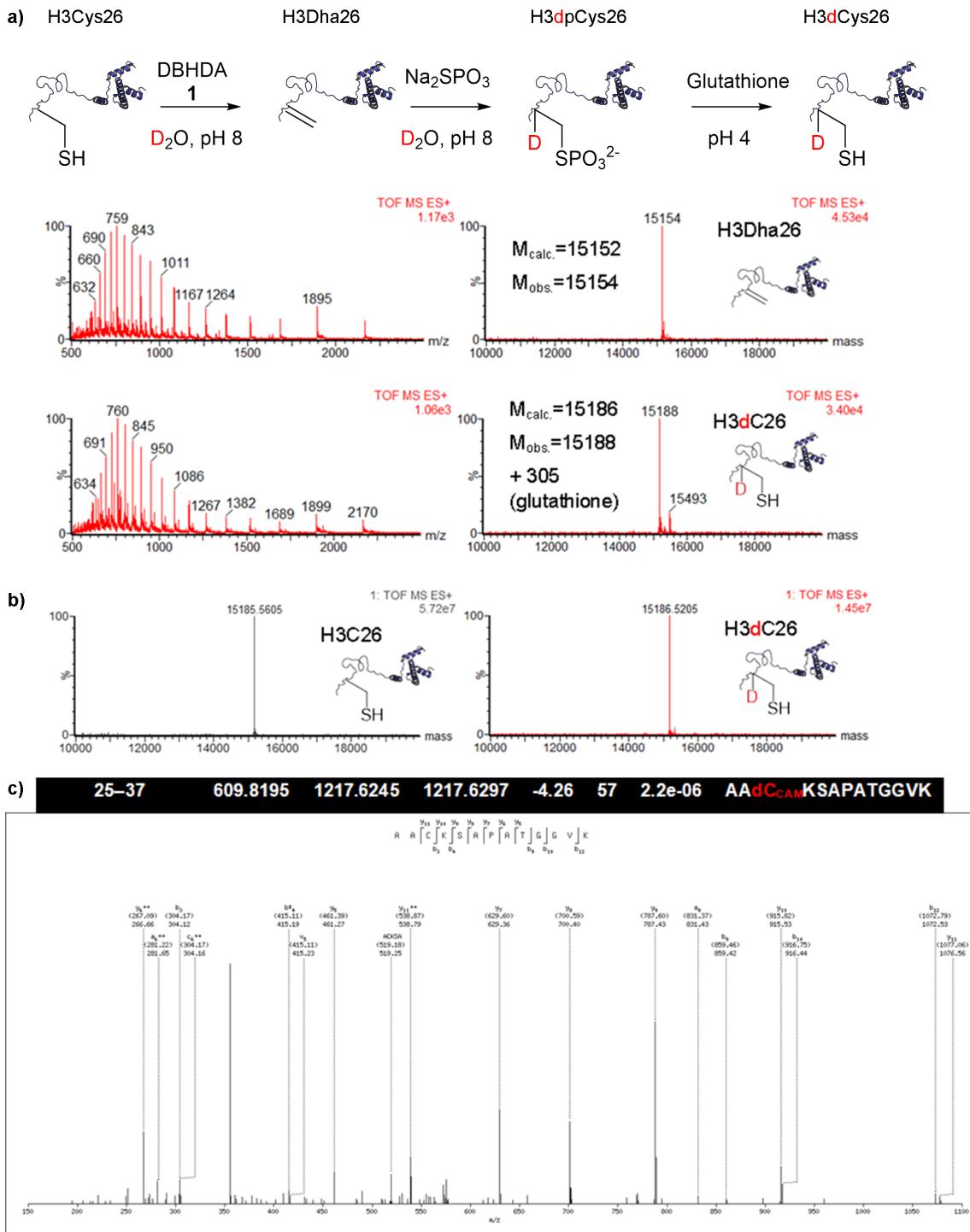


Supplementary Figure 16. LC/MSMS analysis of natural histone H3-(L)-C10 digested with ArgC. a) Sequence coverage, b) MSMS spectrum of peptide K9-R17 containing site of modification, c) MSMS spectrum of reference peptide K27-K37. The experiments were repeated independently twice with similar results.

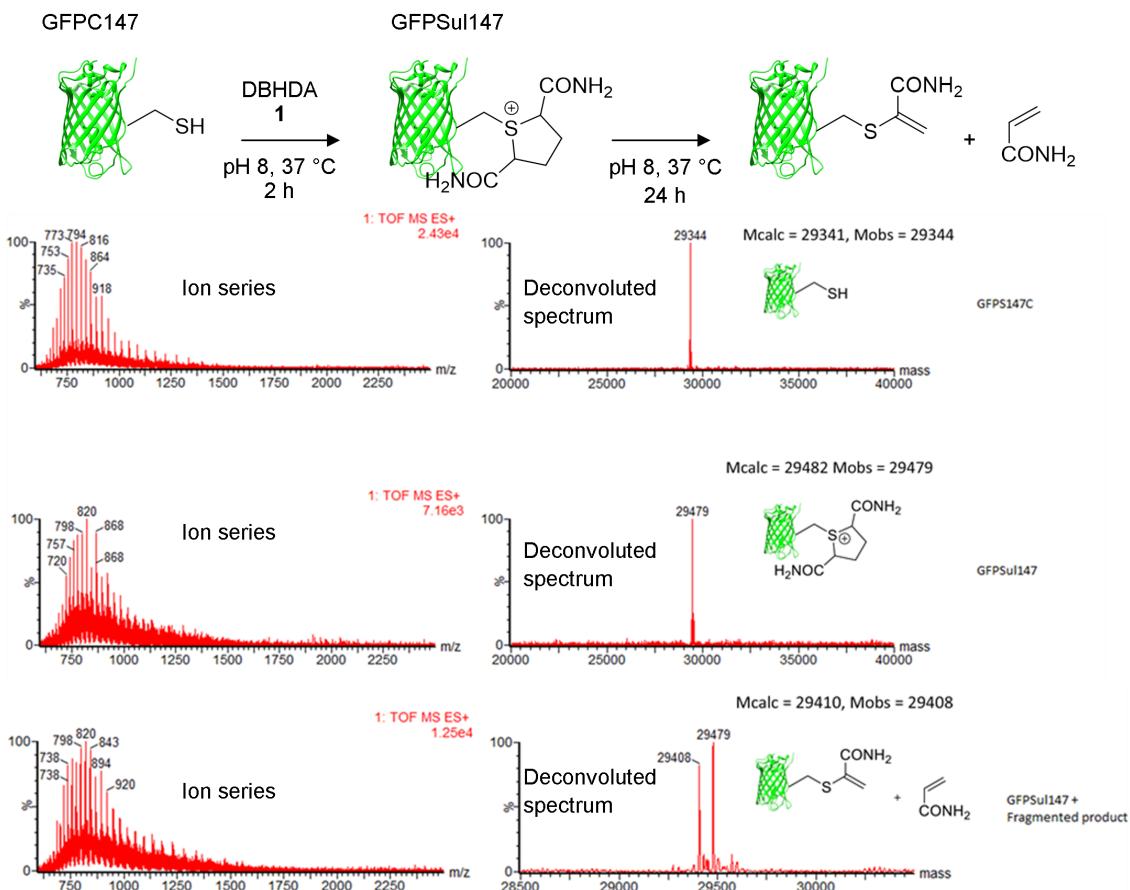
## H3 (D-L)-dC10



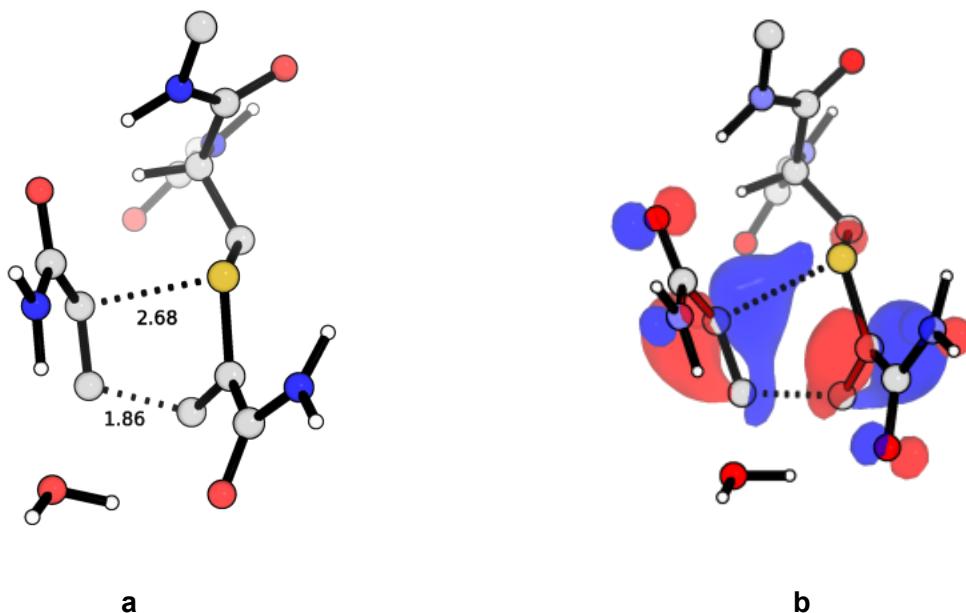
Supplementary Figure 17. LC/MSMS analysis of deuterated histone H3-(D-L)-dC10 digested with ArgC. a) Sequence coverage, b) MSMS spectrum of peptide K9-R17 containing site of modification, c) MSMS spectrum of reference peptide K27-K37. The experiments were repeated independently twice with similar results.



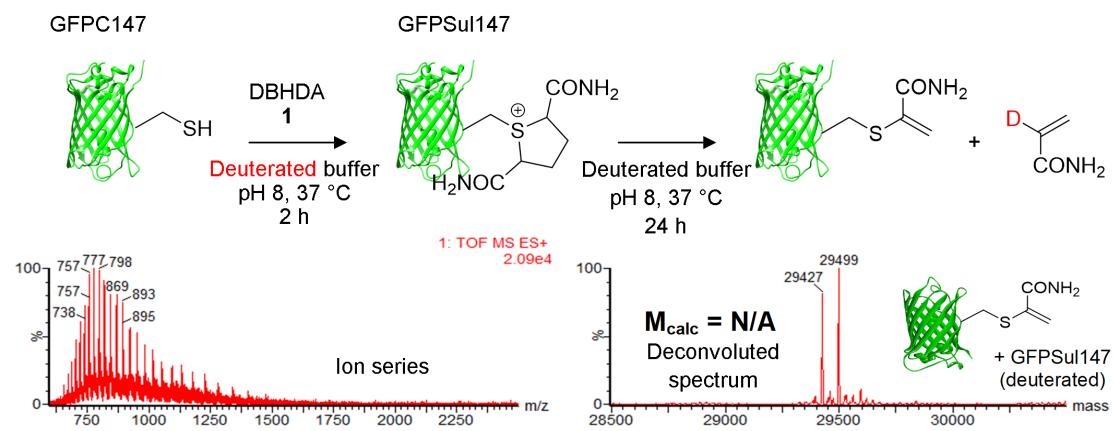
Supplementary Figure 18. **(a)** Formation of H3dC26 monitored by LCMS. **(b)** Comparison of high resolution QTOF-MS deconvoluted spectra of H3C26 and H3dC26. **(c)** Validation of deuterium incorporation levels and conformation of location of deuterium by LCMSMS of tryptic peptide for H3dC26. This experiments was conducted once.



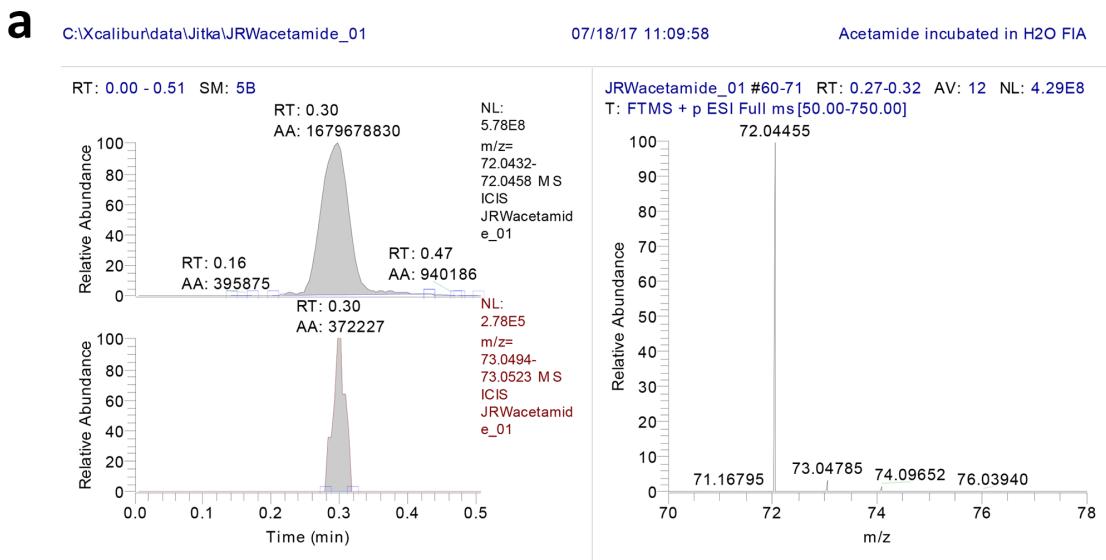
Supplementary Figure 19. Formation of GFPSul147 monitored by LCMS and Identification of Fragmentation Products. The experiments were repeated independently three times with similar results.



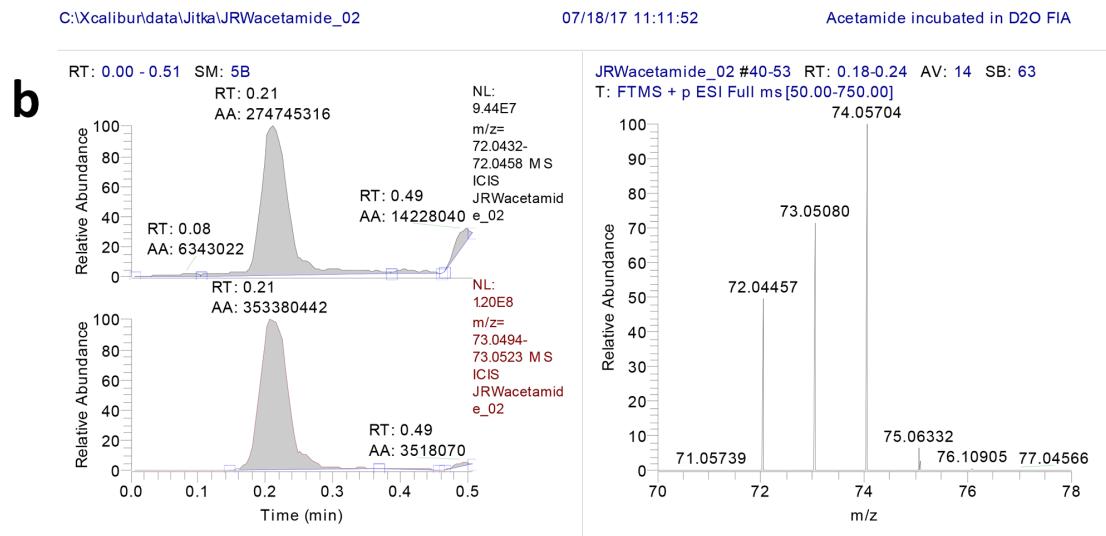
Supplementary Figure 20 (a) Left: Lowest energy TS for sulfonium ylid decomposition via concerted [3+2] cycloreversion. Bond distances are given in Å. (b) Right: The corresponding HOMO (at an isovalue of 0.05) for this lowest energy TS, indicating a concerted electron movement, with dominant electron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



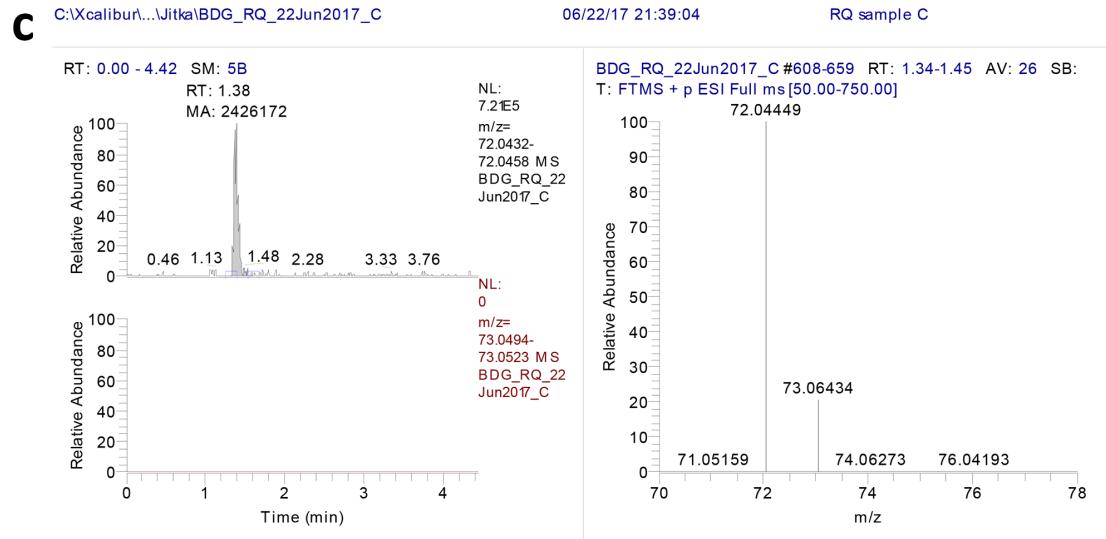
Supplementary Figure 21. Formation and fragmentation of GFPSul147 under deuterating “wash-in” conditions.  
The experiments were repeated independently twice with similar results.



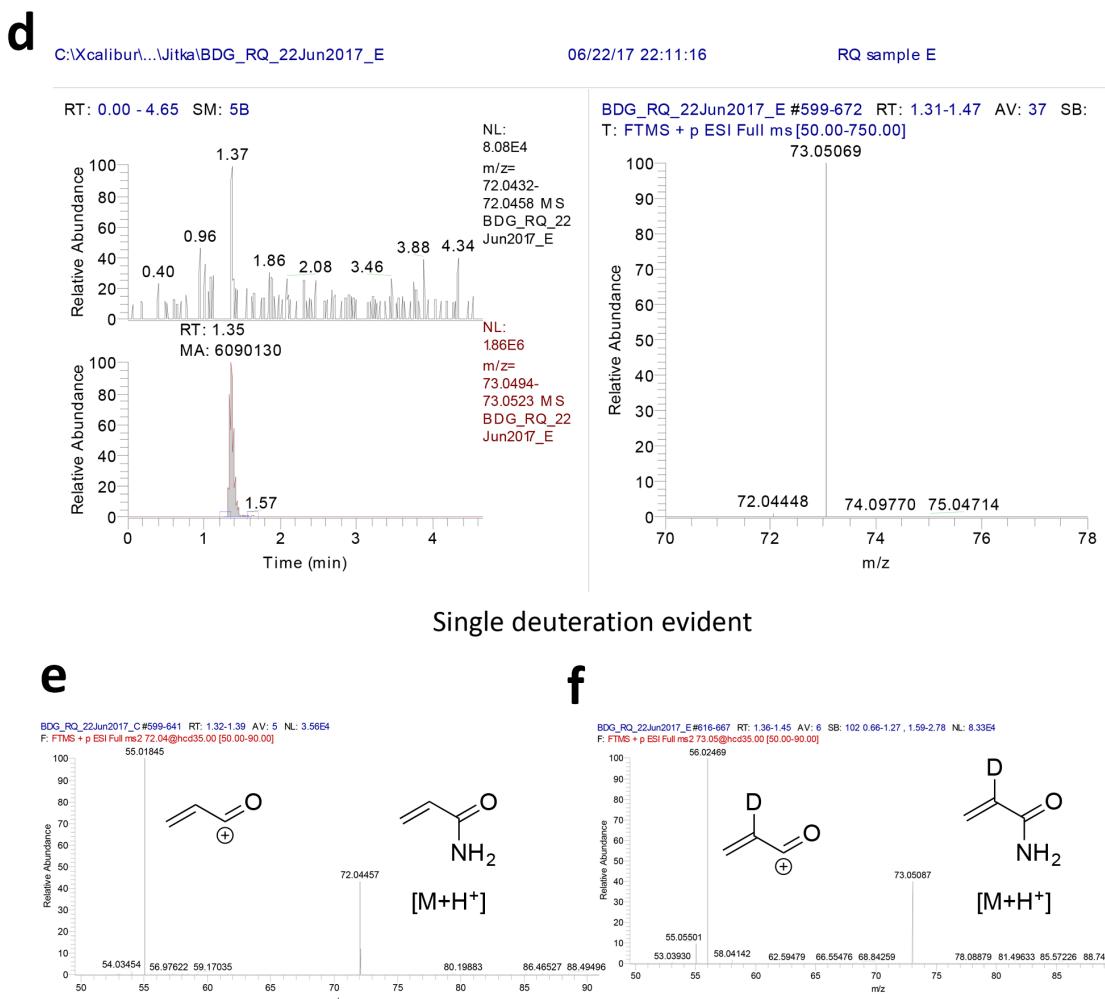
Very little deuterium present ≈ 0.022% (natural abundance = 0.015%, experimental error)



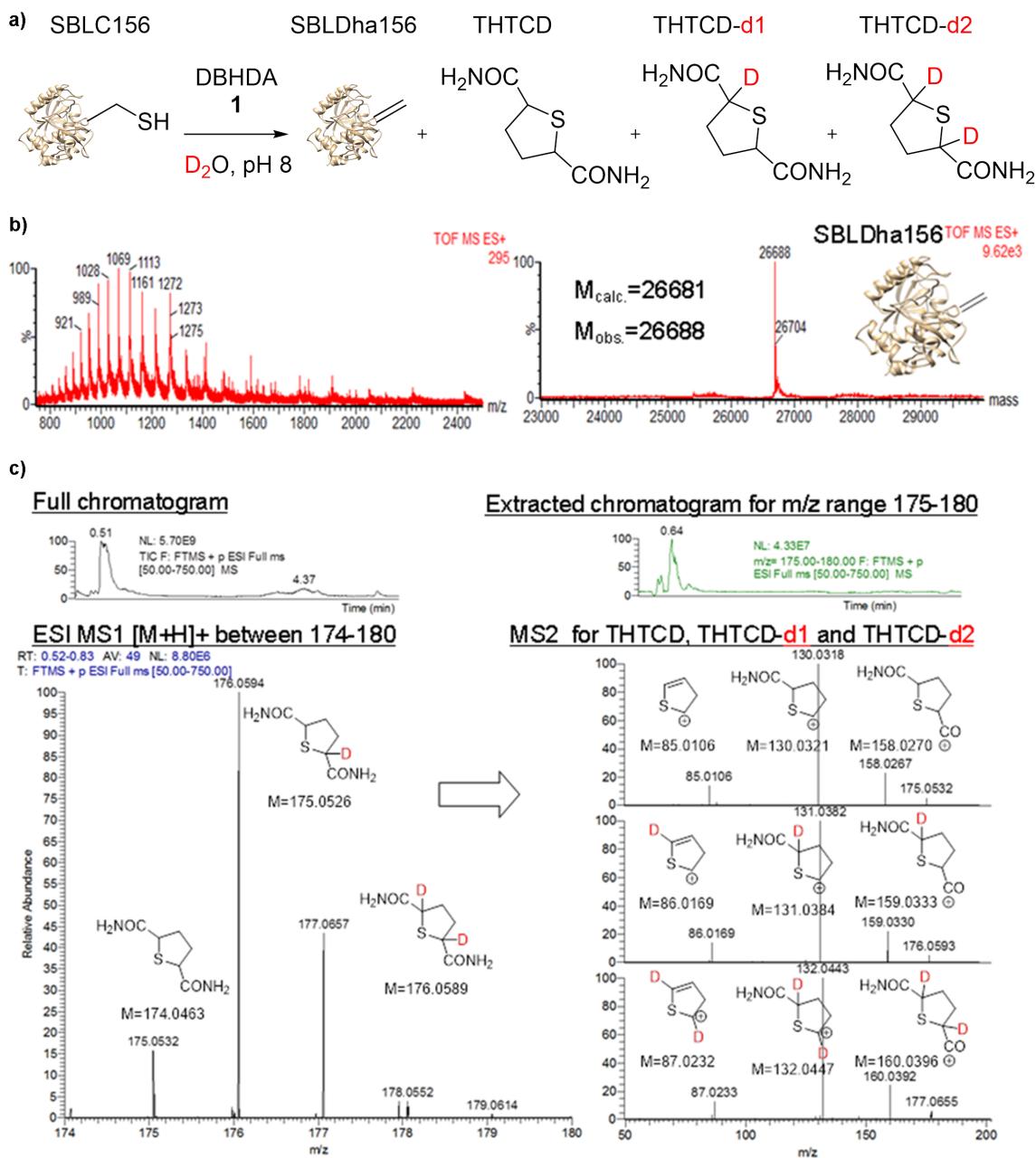
Variety of deuteration, further evidence of exchangeables



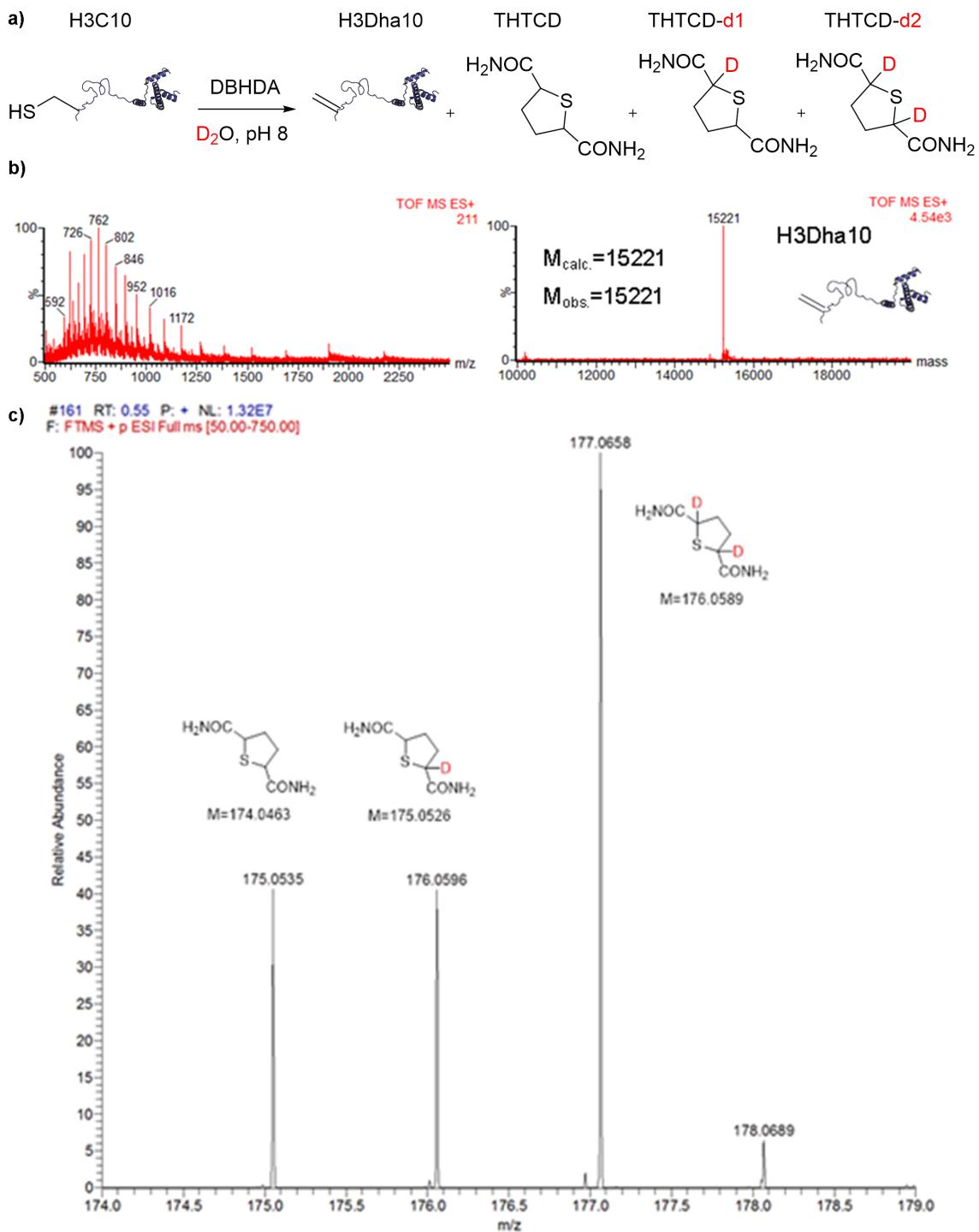
No deuteration evident



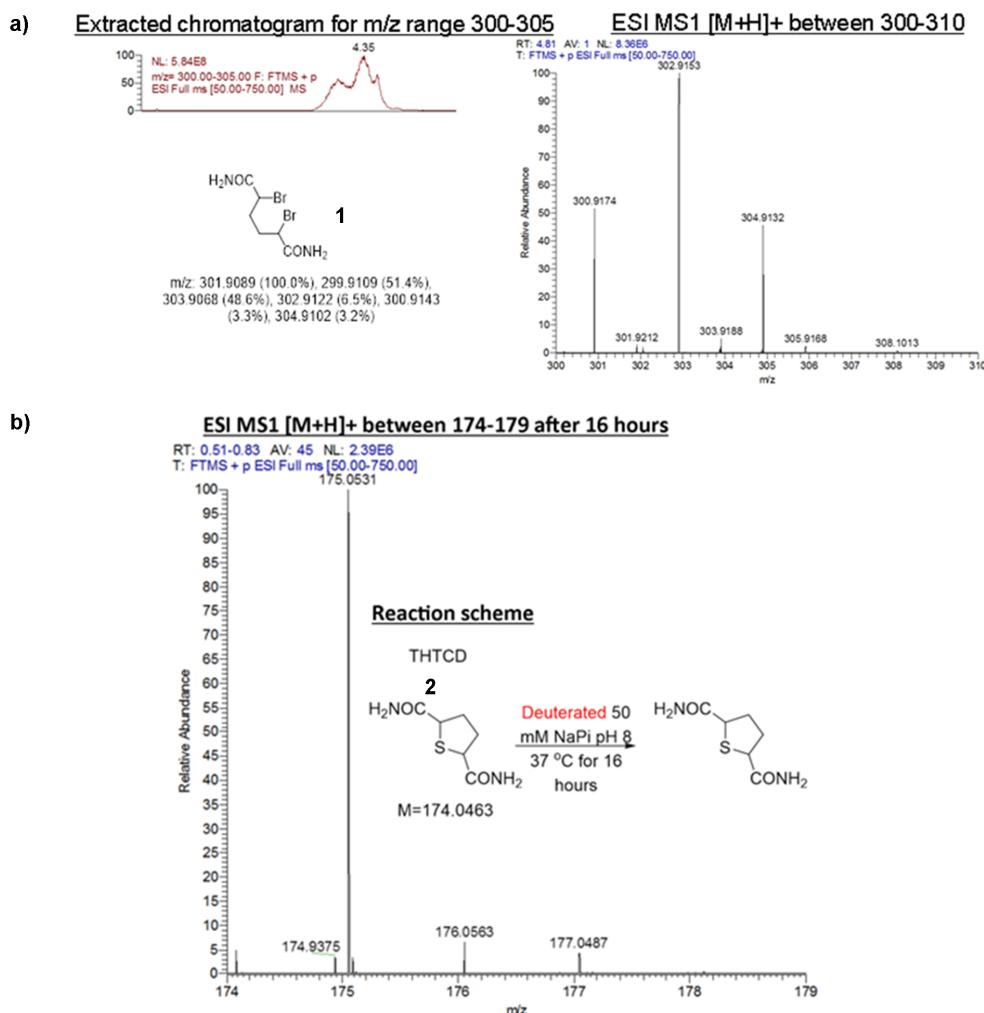
Supplementary Figure 22. Small Molecule Fragment Analyses from GFPSul147 under deuterating “wash-in” conditions. (a) acrylamide standard in water; (b) acrylamide standard incubated in D<sub>2</sub>O; (c) fragment isolated from GFPSul147 fragmentation in water; (d) fragment isolated from GFPSul147 fragmentation in D<sub>2</sub>O and equilibrated in water; (e) MSMS from (c); (f) MSMS from (d). The experiments were repeated independently twice with similar results.



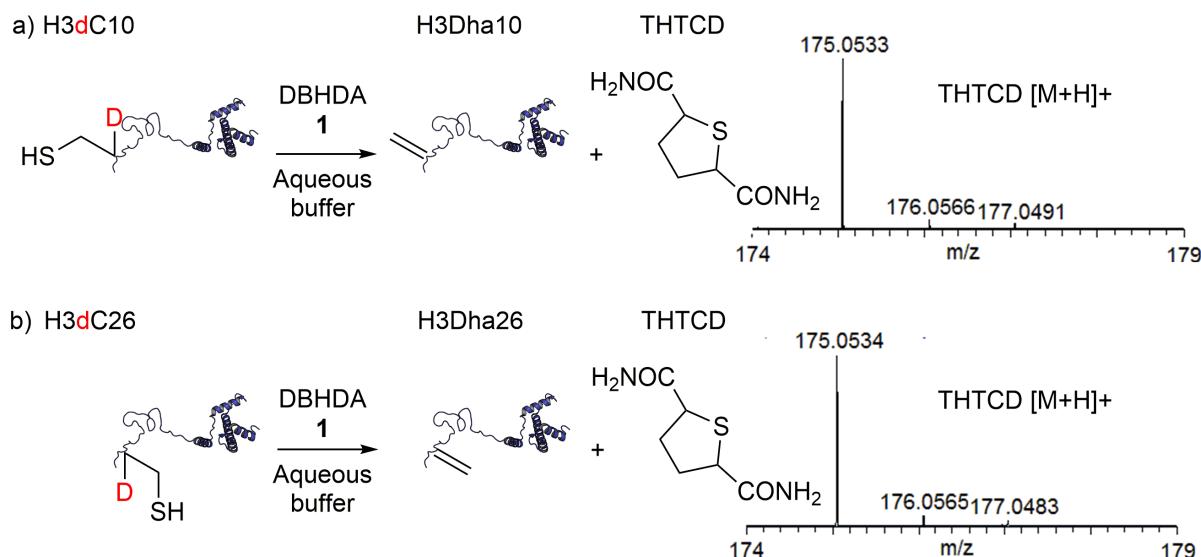
Supplementary Figure 23. **(a)** Formation of SBLDha156 in deuterated buffer, **(b)** monitored by LC-MS and, **(c)** LC-MSMS. This experiment was repeated independently twice with similar results.



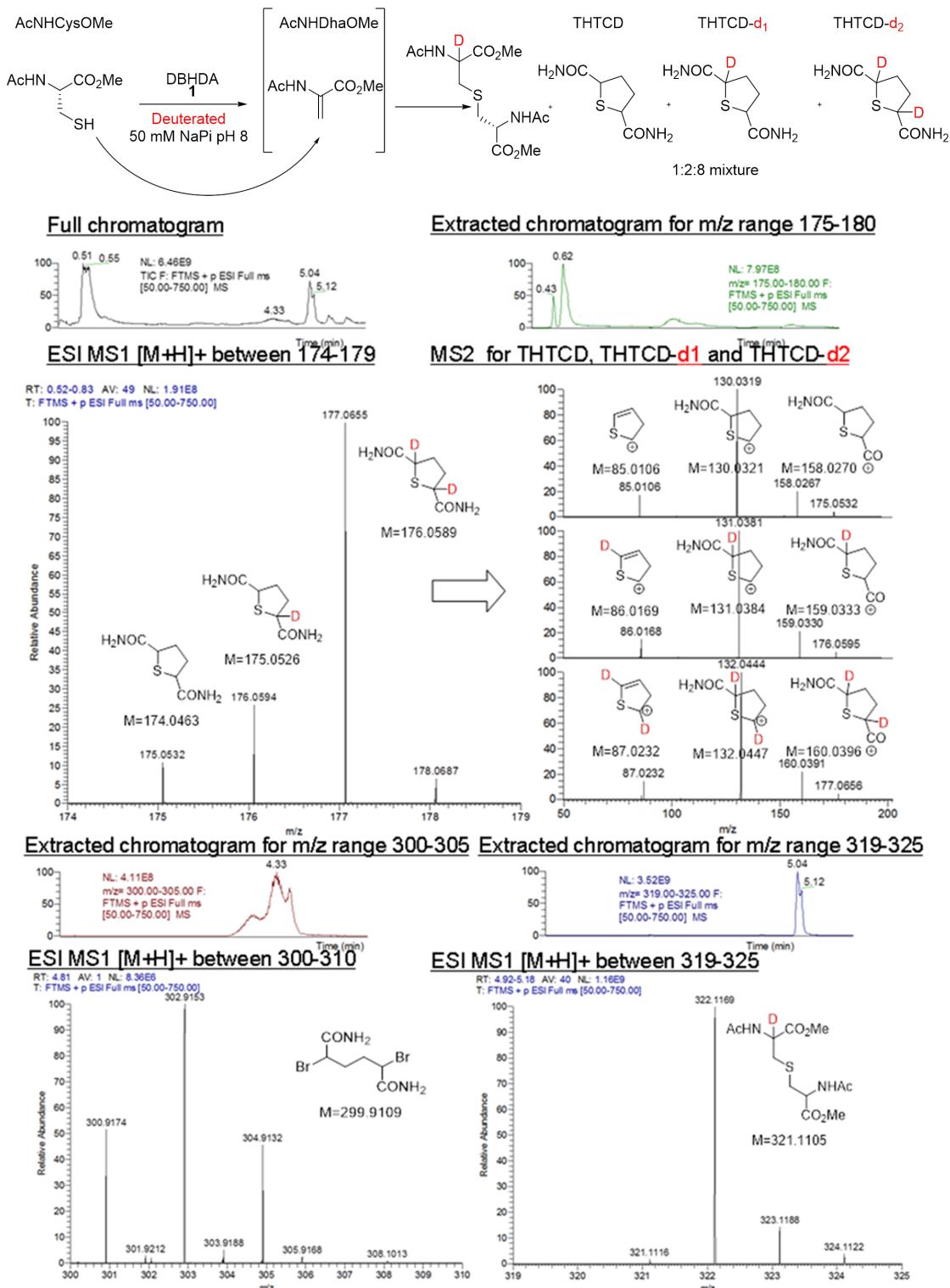
Supplementary Figure 24. (a) Formation of H3Dha10 in deuterated buffer, (b) monitored by LC-MS and,(c) LC-MSMS. This experiments was repeated independently twice with similar results.



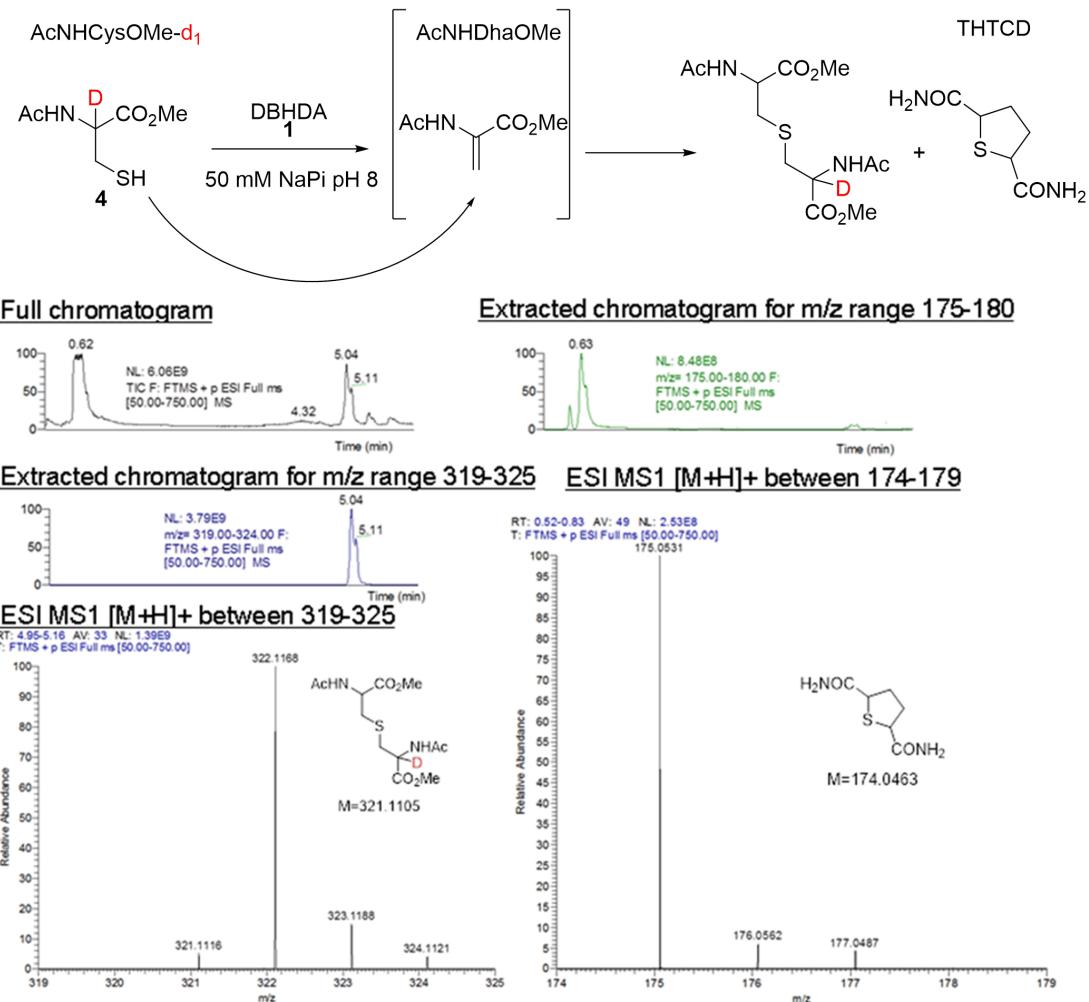
Supplementary Figure 25. Control experiments of (a) DBHDA **1** and (b) THTCD **2** in deuterated buffer.  
Experiments (a) was repeated independently 6 times with similar results, (b) 3 times.



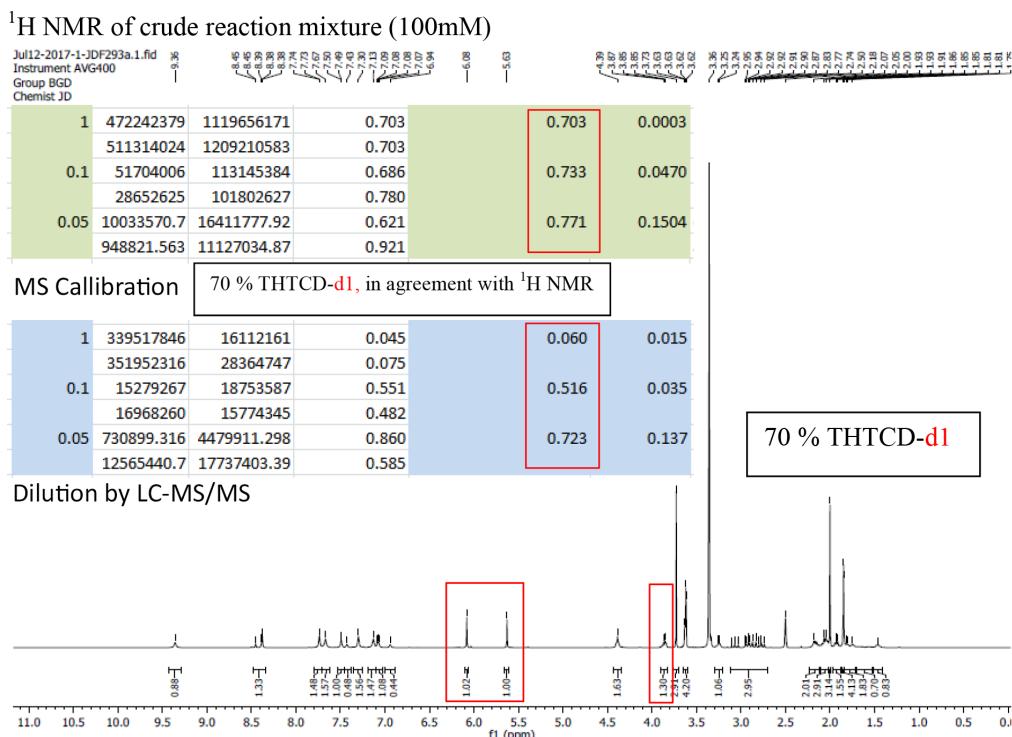
Supplementary Figure 26. Reaction of (a) H3dC10 and (b) H3dC26 with DBHDA **1** in aqueous buffer. These experiments were repeated independently twice with similar results.



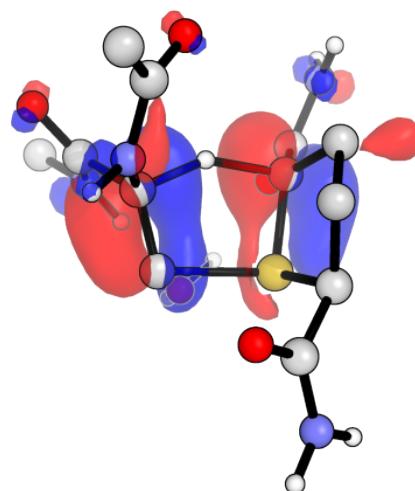
Supplementary Figure 27. Typical reaction of AcNHDhaOMe with DBHDA 1 in deuterated buffer analysed by LC-MSMS. AcNHCysOMe (6 mg, 0.03 mmol) in deuterated 50 mM sodium phosphate buffer pH 8 (1 mL) was treated with a solution of DBHDA (0.40 mmol) in N,N-dimethylformamide (100  $\mu$ L). The reaction mixture was shaken at 600 rpm at room temperature for 30 minutes, followed by shaking at 37 °C for 3 hours. The reactions were duplicated. The reaction mixtures were analysed by LC-MS-MS. MS-MS results confirmed that deuterium atom(s) of THTCD was/were located at the THT core but not the amide moiety. This experiment was repeated independently twice with similar results.



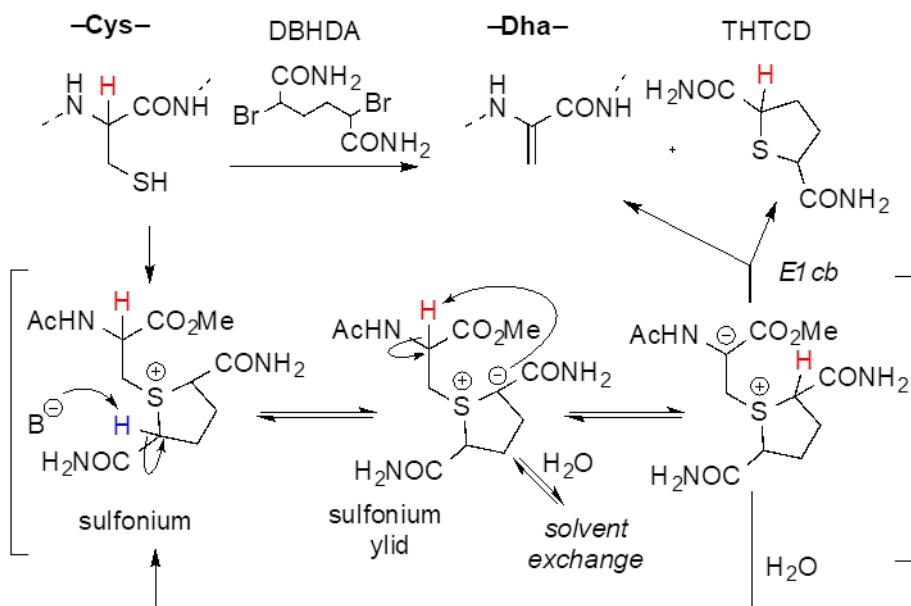
Supplementary Figure 28. Typical reaction of AcNHDhaOMe-d<sub>1</sub> **4** with DBHDA **1** in aqueous buffer analysed by LC-MSMS. AcNHCysOMe-d<sub>1</sub> (6 mg, 0.03 mmol) in 50 mM sodium phosphate buffer pH 8 (1 mL) was treated with a solution of DBHDA (0.40 mmol) in N,N-dimethylformamide (100  $\mu$ L). The reaction mixture was shaken at 600 rpm at room temperature for 30 minutes, followed by shaking at 37 °C for 3 hours. The reactions were duplicated. The reaction mixtures were analysed by LC-MS-MS. MS-MS results confirmed that deuterium atom(s) of THTCD was/were located at the THT core but not the amide moiety. This experiments was repeated independently twice with the same result.



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  $\mu$ L of each reaction mixture) in DMSO-d6 were prepared immediately. All reactions were incubated at 25 °C and 300 rpm for 1 hour and then at 37 °C and 300 rpm for 5 hours. Formation of AcNHDhaOMe and THTCD-d1 was confirmed by <sup>1</sup>H NMR of crude reaction mixture (100mM). The ratio of THTCD-d1 and THTCD was determined by LC/MS-MS analysis. <sup>1</sup>H 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