Supporting Information

NMR Analyses of Acetylated H2AZ Isoforms Identify Differential Binding Interactions with the Bromodomain of the NURF Nucleosome Remodeling Complex

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Methods: Protein Purification. To the cell pellet was added 40 mL of lysis buffer (50 mM phosphate, 300 mM NaCl, pH 7.4) and 20 mg phenylmethanesulfonyl fluoride (PMSF) and the mixture was allowed to thaw at room temperature for 30 minutes. Cells were put on ice and sonicated in 30 second intervals followed by 60 seconds of cooling for a total of 12 minutes sonication time. The lysed cells were centrifuged at 10,000 g for 30 minutes. The supernatant was decanted from the pelleted cell debris and filtered using Whatman filters. Ni affinity purification was done using a Ni HisTrap FF 5 mL column (GE Healthcare) on an AKTA Fast Protein Liquid Chromatography (FPLC) system by monitoring the absorbance at 280 nm. Proteins were eluted with a 0-100% gradient of wash buffer (50 mM phosphate, 100 mM NaCl, 40 mM imidazole, pH 7.4) and elution buffer (50 mM phosphate, 100 mM NaCl, 400 mM imidazole, pH 7.4) across 20 column volumes. Purified protein was then buffer exchanged into storage buffer (50 mM Tris, 100 mM NaCl, pH 7.4 or 50 mM HEPES, 100 mM NaCl, pH 7.4) using a HiPrep desalting column (GE Healthcare) equilibrated with 1 column volume of buffer. The hexahistidine tag was removed by adding Tobacco Etch Virus (TEV) protease and incubating for 4-16 hours at 4°C. Nickel NTA affinity resin was added, incubated for 2-24 hours at 4°C, then filtered to remove the TEV. Protein purity was assessed using SDS-polyacrylamide gel electrophoresis (12% Bis-Tris, 1.0 mM gels.

Running conditions: 120 V, 90 minutes in MES buffer). Protein was concentrated to ~50 μM using Amicon Ultra-15 (Millipore) centrifugal filters with a 3 kDa molecular weight cut off (MWCO = 3000 Da), flash frozen and stored at -20°C. Quadrupole Time-of-Flight (Q-TOF) LC/MS was used to confirm the identity of the protein and determine percent fluorine incorporation using the following equation.

$$\%\ Incorporation = \frac{(0\ F\ protein*0)+(1F\ protein*1)+\cdots(nF\ protein*n)}{(0F\ protein*n)+(1F\ protein*n)+\cdots(nF\ protein*n)} * 100$$

Protein masses and fluorine incorporation are shown in Table S2.

Methods: His_6-BPTF Protein Purification for Crystallography. Protein purification was performed at 4 °C by FPLC using columns and chromatography resins from GE Healthcare. Cell pellets were re-suspended in 50 mM Na/K Phosphate buffer (pH 7.4) containing 100 mM NaCl, 20 mM imidazole, 0.01% w/v lysozyme, 0.01% v/v Triton X-100 and 1mM DTT. Cells were lysed using a homogenizer, the lysate was clarified by centrifugation and subjected to purification on immobilized Ni^{2+}-affinity chromatography (Qiagen) using a linear gradient of 20 – 500 mM imidazole. Fractions containing BPTF were pooled and incubated overnight with TEV protease at 4 ºC. Cleaved BPTF was subjected to a second Ni^{2+}-affinity chromatography run to remove His-TEV and the cleaved His-tag. The flow-through containing BPTF was concentrated and purified to homogeneity by size exclusion chromatography using a Superdex Hiload 26/60 column. Protein was eluted using 50 mM Tris/HCl (pH 8.0) containing 100 mM NaCl and 1 mM DTT. Peak fractions were combined, concentrated to 5 mg/mL, flash-frozen in liquid N_2 and stored at -80 °C.

Methods: Crystallization and Structure Determination. Crystallization screening campaigns were performed at 18 °C with precipitant solutions from Hampton Research using a Mosquito liquid handler (TTP Labtech). Robust crystallization conditions were established using 25% PEG

3,350, 0.2 M lithium sulfate monohydrate, 0.1 M Bis-Tris pH 6.5 mixed with an equal volume of protein in hanging droplets. H2A.Z peptides were co-crystallized with BPTF at 1 mM final concentration. Crystals were cryoprotected by addition of 20% ethylene glycol in the precipitant and flash-frozen in liquid N_2. During data collection, crystals were maintained under a constant stream of N_2 gas. X-ray diffraction data were recorded at beamlines 22-ID and 22-BM hosted by Ser-Cat of Argonne National Laboratories. Data were indexed and scaled with XDS[1]. Phasing and refinement was performed using PHENIX[2] and model building with Coot[3]. PDB entry 3UV2 served as the search model for molecular replacement. Initial models for small molecule ligands were generated through MarvinSketch (ChemAxon, Cambridge, MA) and ligands restraints through eLBOW of the PHENIX suite. All structures have been validated by MolProbity. Figures were prepared using PyMOL (Schrödinger, LLC).

Sequence Alignment of H2A.Z I, H2A.Z I II, and H2A

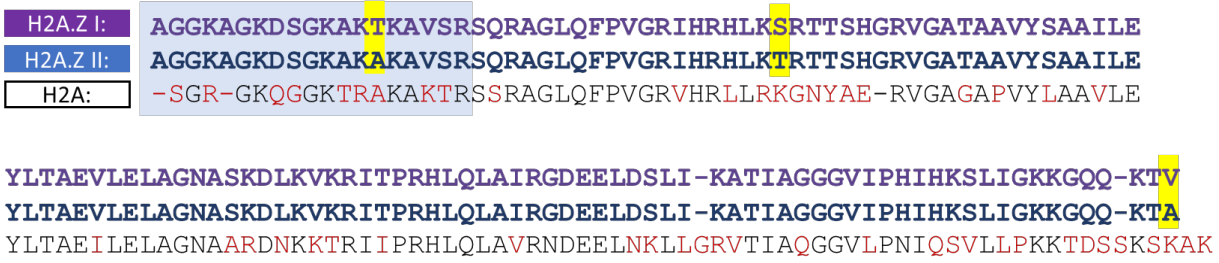


Figure S1: Sequence alignment of H2A.Z isoforms with canonical H2A. Divergent amino acids between the two isoforms are highlighted in yellow (T14/A14, S38/T38, and V127/A127). Divergent amino acids in the H2A sequence are shown in red. The N-terminal region corresponding to the synthetic peptide sequence used in these experiments is highlighted in blue.

Representative MALDI-TOF MS Spectra of H2A.Z II K7ac, K13ac

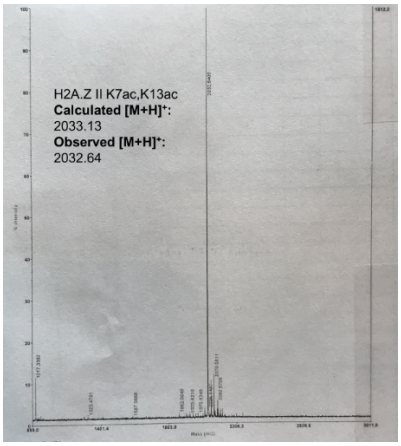


Figure S2. Representative MALDI-TOF MS spectra of H2A.Z II K7ac,K13ac showing major peak corresponding to expected $[M+H]^+$ of the peptide. The masses of each peptide synthesized for these experiments were confirmed using this method.

Table S1: Peptide theoretical and observed masses using MALDI-TOF MS.

Peptide	**Sequence**	**Calculated $[M+H]^+$**	**Observed $[M+H]^+$**
H4 K16ac	H_2N-YSGRGKGGKGLGKGGAKacRHRK-$C(O)NH_2$	2196.27	2196.01
H2A.Z II unacetylated	H_2N-YAGGKAGKDSGKAKAKAVSR-$C(O)NH_2$	1949.11	1949.17
H2A.Z II K4ac	H_2N-YAGGKacAGKDSGKAKAKAVSR-$C(O)NH_2$	1991.12	1991.56
H2A.Z II K7ac	H_2N-YAGGKAGKacDSGKAKAKAVSR-$C(O)NH_2$	1991.12	1991.41
H2A.Z II K11ac	H_2N-YAGGKAGKDSGKacAKAKAVSR-$C(O)NH_2$	1991.12	1991.63
H2A.Z II K13ac	H_2N-YAGGKAGKDSGKAKacAKAVSR-$C(O)NH_2$	1991.12	1991.80
H2A.Z II K15ac	H_2N-YAGGKAGKDSGKAKAKacAVSR-$C(O)NH_2$	1991.12	1991.40
H2A.Z II K4ac,K7ac	H_2N-YAGGKacAGKacDSGKAKAKAVSR-$C(O)NH_2$	2033.13	2033.25
H2A.Z II K4ac,11ac	H_2N-YAGGKacAGKDSGKacAKAKAVSR-$C(O)NH_2$	2033.13	2032.86

H2A.Z II K4ac,K13ac	H_2N-YAGGKacAGKDSGKAKacAKAVSR-$C(O)NH_2$	2033.13	2033.31
H2A.Z II K4ac,K15ac	H_2N-YAGGKacAGKDSGKAKAKacAVSR-$C(O)NH_2$	2033.13	2032.91
H2A.Z II K7ac,11ac	H_2N-YAGGKAGKacDSGKAKacAKAVSR-$C(O)NH_2$	2033.13	2033.13
H2A.Z II K7ac,K13ac	H_2N-YAGGKAGKacDSGKAKacAKAVSR-$C(O)NH_2$	2033.13	2032.64
H2A.Z II K7ac-d_3,K13ac	H_2N-YAGGKAGKac(d_3)DSGKAKacAKAVSR-$C(O)NH_2$	2036.13	2036.37
H2A.Z II K7ac,K15ac	H_2N-YAGGKAGKacDSGKAKAKacAVSR-$C(O)NH_2$	2033.13	2033.51
H2A.Z II K11ac,K13ac	H_2N-YAGGKAGKDSGKacAKacAKAVSR-$C(O)NH_2$	2033.13	2032.46
H2A.Z II K11ac,K15ac	H_2N-YAGGKAGKDSGKacAKAKacAVSR-$C(O)NH_2$	2033.13	2032.49
H2A.Z II K13ac,K15ac	H_2N-YAGGKAGKDSGKAKacAKacAVSR-$C(O)NH_2$	2033.13	2032.71
H2A.Z II K4ac,K7ac,K11ac	H_2N-YAGGKacAGKacDSGKacAKAKAVSR-$C(O)NH_2$	2075.14	2074.71
H2A.Z II K7ac,K13ac,K15ac	H_2N-YAGGKAGKacDSGKAKacAKacAVSR-$C(O)NH_2$	2075.14	2075.62
H2A.Z II K4me,K7ac,K13ac	H_2N-YAGGKmeAGKacDSGKAKacAKAVSR-$C(O)NH_2$	2048.13	2047.22
H2A.Z I K4ac,K11ac	H_2N-YAGGKacAGKDSGKacAKTKAVSR-$C(O)NH_2$	2063.14	2063.13
H2A.Z I K4ac,K13ac	H_2N-YAGGKacAGKDSGKAKacTKAVSR-$C(O)NH_2$	2063.14	2062.96
H2A.Z I K4ac,K15ac	H_2N-YAGGKacAGKDSGKAKTKacAVSR-$C(O)NH_2$	2063.14	2063.65
H2A.Z I K7ac,K13ac	H_2N-YAGGKAGKacDSGKAKacTKAVSR-$C(O)NH_2$	2063.14	2063.02

H2A.Z I K7ac,K15ac	H_2N-YAGGKAGKacDSGKAKTKacAVSR-C(O)NH_2	2063.14	2062.82
H2A.Z I K11ac,K13ac	H_2N-YAGGKAGKDSGKacAKacTKAVSR-C(O)NH_2	2063.14	2062.70
H2A.Z I K11ac,K15ac	H_2N-YAGGKAGKDSGKacAKTKacAVSR-C(O)NH_2	2063.14	2062.65
H2A.Z I K13ac,K15ac	H_2N-YAGGKAGKDSGKAKacTKacAVSR-C(O)NH_2	2063.14	2062.62
H2A.Z I K7ac,K13ac,K15ac	H_2N-YAGGKAGKacDSGKAKacTKacAVSR-C(O)NH_2	2105.15	2105.47

Representative LC/MS Spectra of 5FW-BPTF

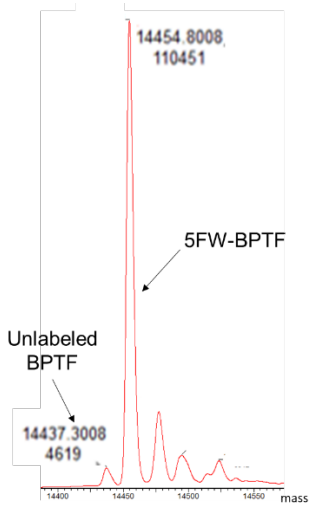


Figure S3. Representative LC/MS spectra of purified 5FW-BPTF showing peaks for unlabeled and 5FW BPTF. The intensities of these peaks were used to determine percent fluorine incorporation. All fluorinated proteins expressed and used in these experiments were characterized in this manner.

Table S2: LC/MS Characterization of Proteins.

Protein	Calculated m/z (Da)	Observed m/z (Da)	% Fluorine Incorporation
Unlabeled BPTF	14437	14437	N.A.
5FW-BPTF	14455	14453	95
5FW-CECR2	13852	13853	93
5FW-PCAF	17182	17182	86
5FW-BRD2(1)	14951	14951	96
5FW-BRD2(2)	13369	13369	99
5FW-BRD4(1)	15137	15135	95-98
5FW-BRD4(2)	15054	15054	95
5FW-BRDT(1)	14184	14183	93

Assignment of BRD2(1) Resonances

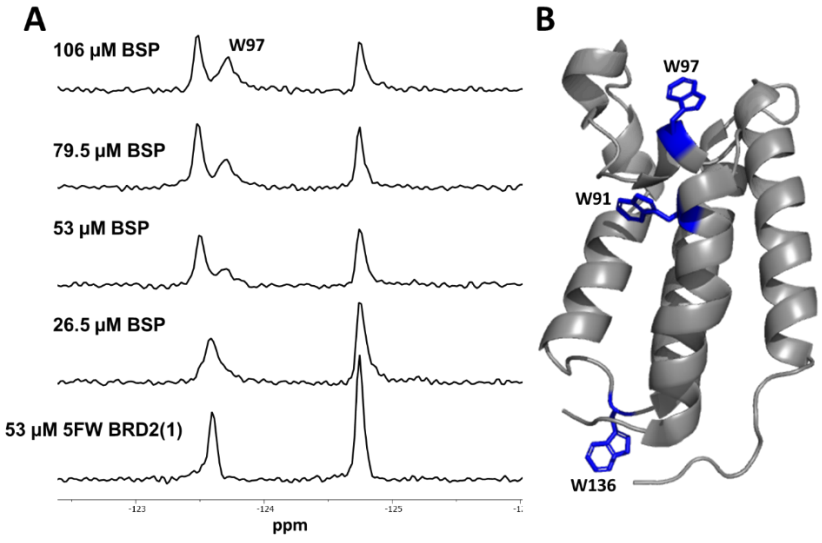


Figure S4. A) PrOF NMR titration of 5FW-BRD2(1) with Bromosporine (BSP) to assign WPF shelf 5FW resonance. The ^{19}F NMR spectrum of 5FW-BRD2(D2) shows only two resonances although there are three tryptophan residues in this protein. Upon the addition of BSP, the resonance at -124.75 ppm decreases in intensity and a resonance at -123.70 appears. This allows us to tentatively assign this resonance to W97, which lies in the WPF shelf. B) X-ray crystal structure of BRD2(1) (PDB: 2DVS) showing its three tryptophan residues.

SPR with GST-BPTF and H2A.Z II K7ac,K13ac.

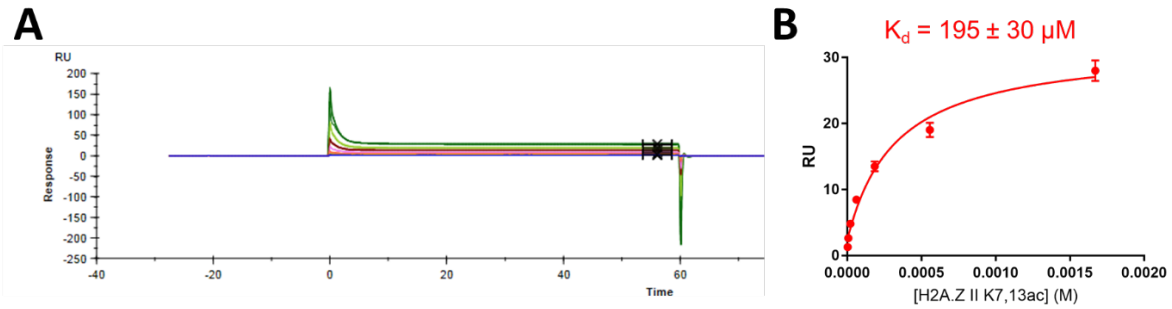


Figure S5. A) Sensorgram of SPR titration with H2A.Z II K7ac,K13ac. B) Binding isotherm of H2A.Z II K7ac,K13ac fitted with GraphPad Prism 5 software.

AlphaScreen competition with His_9 BPTF.

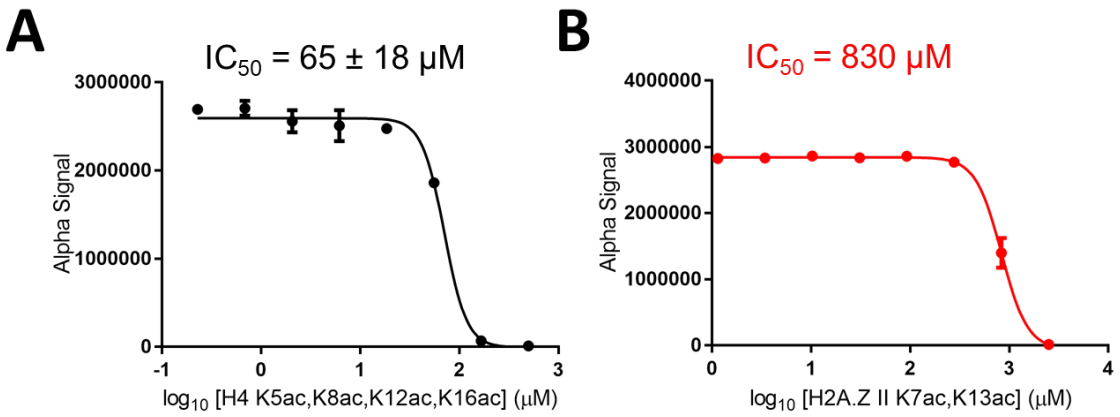


Figure S6. Alpha Screen titration with His_9 BPTF and A) positive control H4 K5ac,K8ac,K12ac,K16ac peptide giving an IC_{50} of 65 ± 18 μM across three technical replicates. The K_d determined by PrOF NMR, 70 μM is in close agreement, validating the robustness of this competition-based assay. B) AlphaScreen titration of H2A.Z II K7ac,K13ac yielded average IC_{50} of 830 μm from two technical replicates.

X-ray Crystallography

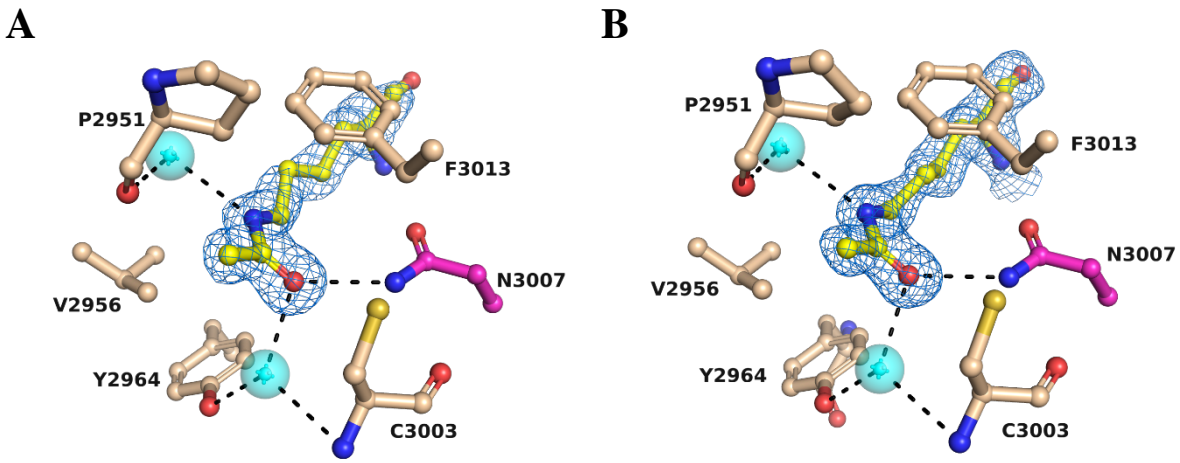


Figure S7. Co-crystal structures of BPTF BRD with acetylated peptides. A) View of the acetylated lysine of peptide H2A.Z I (K4ac,K11ac) in the Kac site. The blue mesh is the 2Fo-Fc density contoured at 1σ and determined at 1.51 Å resolution. Water molecules are shown as cyan spheres, H-bonding interactions as black dotted lines. B) Same as A) for peptide H2A.Z II (K7ac,K13ac) determined at 1.22 Å resolution.

Table S3: Crystallography Statistics.

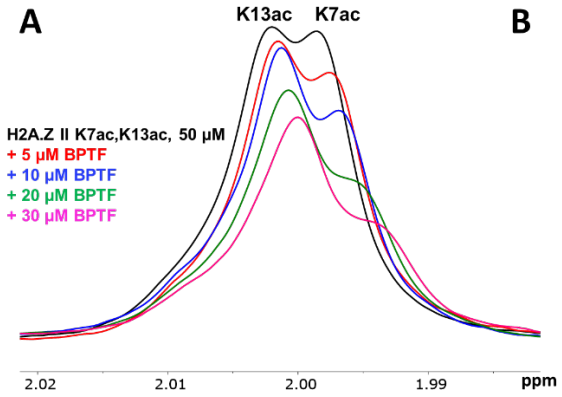
Inhibitor		Hac_RS2016 (H2A.Z I K4ac,K11ac)	NO2021 (H2A.Z II K7ac,K13ac)
Data collection			
Space group		C 1 2 1	C 1 2 1
Unit cell dimensions	a	111.71	111.32
	b	27.15	27.16
	c	38.01	38.03
	α	90	90
	β	97	96
	γ	90	90
Resolution range (Å)		37.73 - 1.51 (1.564 - 1.51)	37.79 - 1.22 (1.264 - 1.22)
Unique reflections		18040 (1745)	31161 (2748)
Rmeas (%)		5.4 (31.7)	5.9 (68.4)
Completeness (%)		99.64 (99.94)	91.51 (81.71)
I/σI		24.71 (8.0)	14.06 (3.1)
Structure refinement			
Rwork (%)		16.65 (15.98)	19.19 (34.14)
Rfree[a] (%)		19.50 (17.96)	22.31 (32.54)
Wilson B (Å²)		13.3	12.2
Average B (Å²)	all	19.40	18.1
	protein	18.77	17.4
	ligand	34.80	41.0
	solvent	25.46	24.6
rmsd[b] bond lengths (Å)		0.005	0.005
rmsd angles (deg)		0.80	0.79
Ramachandran	favored (%)	100.00	100.00
	allowed (%)	0.00	0.00
	outliers (%)	0	0

Values in parenthesis are for the highest resolution bins.

[a] Rfree is Rcryst calculated for randomly chosen unique reflections

[b] rmsd = root-mean-square deviation from ideal values, which were excluded from the refinement.

^{1}H CPMG with BPTF and H2A.Z I K7ac, K13ac increasing protein concentration.



B

Percent Drop in Signal Intensity		
	K13ac	K7ac
+ 5 µM BPTF	4.6	13.3
+ 10 µM BPTF	6.6	25.7
+ 20 µM BPTF	20.2	74.2
+ 30 µM BPTF	28.8	83.1

Figure S8. Ligand-observed ^{1}H NMR CPMG competition experiment evaluating H2A.Z II K7,K13ac for BPTF binding site engagement. A) The experimental spectrum for H2A.Z II K7,K13ac peptide alone (black), with the addition of 5 μM BPTF (red), 10 μM BPTF (blue), 20 μM BPTF (green), and 30 μM BPTF (magenta). The upfield resonance that has been assigned to K7ac decreases significantly more than the downfield resonance (K13ac) in a protein dependent manner. B) Percent drop in acetyl methyl resonance intensity for K13ac and K7ac upon the addition of BPTF.

^{1}H CPMG competition with BPTF and H2A.Z I K7ac-d_3, K13ac

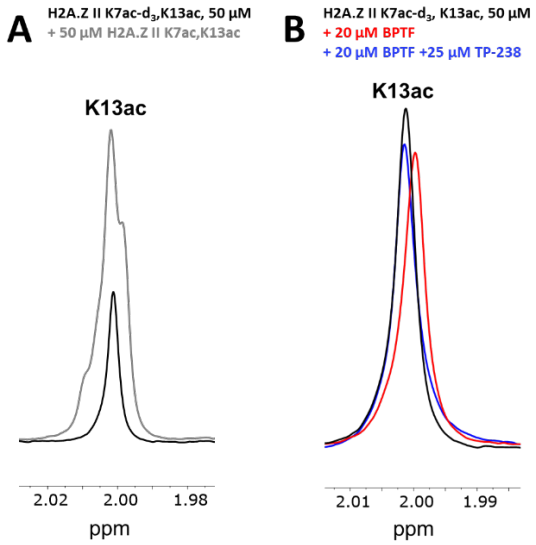


Figure S9. A) Overlaid spectra of H2A.Z II K7ac-d3,K13ac peptide alone (black) and with the addition of H2A.Z II K7,K13 peptide (grey). (B) Experimental spectra for H2A.Z II K7ac-d3,K13ac peptide alone (red), with the addition of BPTF (red), and with addition of competitor TP-238 (blue) are overlaid. A significant drop in resonance intensity is not observed, with no restoration upon competitor addition. This confirms the preferential engagement of K7ac on H2A.Z II by BPTF.

^{1}H CPMG with CECR2 and H2A.Z II K7ac, K13ac.

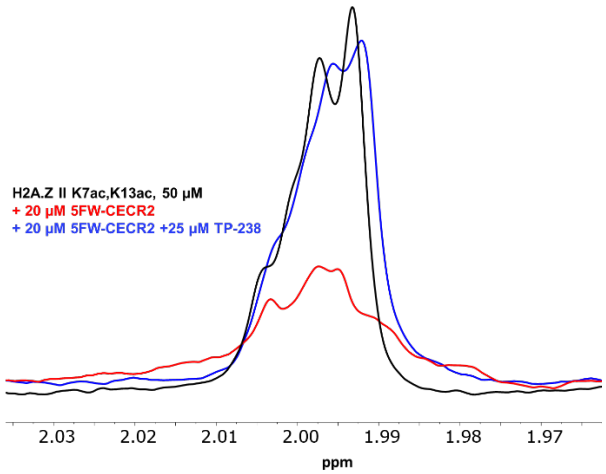
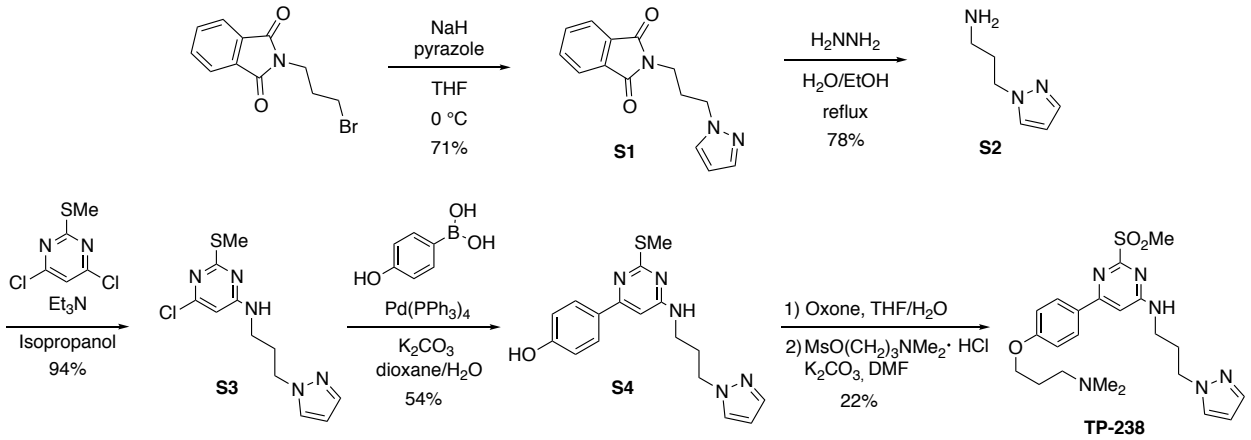


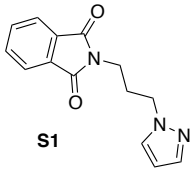
Figure S10. Ligand-observed ^{1}H NMR CPMG competition experiment evaluating H2A.Z II K7ac,K13ac for CECR2 binding site engagement. Experimental spectra for peptide alone (red), with the addition of 5FW-CECR2 (red), and with addition of competitor TP-238 (blue) are overlaid.

Synthesis of TP-238

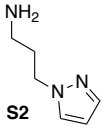


Scheme 1: Synthesis of TP-238

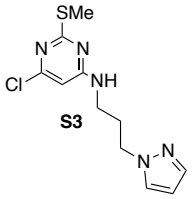
Experimental Procedures:



2-(3-bromopropyl)isoindoline-1,3-dione: To a suspension of NaH (60%) (881 mg, 22.0 mmol) in THF (50 mL) at 0 °C was added pyrazole (1.50 g, 22.0 mmol) dropwise at 0 °C. The resultant solution stirred at 0 °C for 1.5 h before *N*-(3-Bromopropyl)phthalimide (5.37 g, 20.0 mmol) was added and the mixture was slowly warmed to room temperature and stirred overnight. It was then concentrated and purified by Combiflash Rf system (hexanes/ EtOAc, 0-100% EtOAc) to yield 3.64 g (71%) of **S1** as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (dd, J = 5.4, 3.1 Hz, 2H), 7.75 – 7.68 (m, 2H), 7.52 – 7.45 (m, 2H), 6.21 (t, J = 2.1 Hz, 1H), 4.19 (t, J = 6.8 Hz, 2H), 3.72 (t, J = 6.7 Hz, 2H), 2.28 (p, J = 6.8 Hz, 2H).^{13}C NMR (126 MHz, $CDCl_3$) δ 168.5, 139.6, 134.2, 132.2, 129.6, 123.4, 123.1, 116.3, 105.5, 105.3, 49.7, 35.5, 29.5. HRMS (ESI): calc'd for $C_{14}H_{14}N_3O_2[M+H]^+$: 278.0900; found 278.0882.

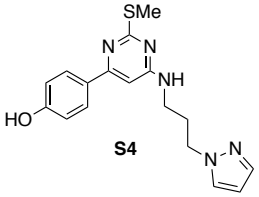


3-(1*H*-pyrazol-1-yl)propan-1-amine: To a solution of **S1** (2.88 g, 11.3 mmol) in EtOH (30 mL) and H_2O (10 mL) was added hydrazine hydrate (1.21 mL, 24.8 mmol). The solution was stirred and heated to reflux for 18 h at which point concentrated HCl (7.72 mL) was added and the solution refluxed for an additional 6 h. It was then cooled to room temperature, filtered and concentrated partially *in vacuo* before washing with DCM (2 x 10 mL). The aqueous layer was then adjusted to pH *ca.* 12 with NaOH pellets before extraction with DCM (2x 10 mL). The extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo* to yield 1.41 g (78%) of amine **S2** as a yellow oil and was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, J = 2.0 Hz, 1H), 7.34 (d, J = 2.3 Hz, 1H), 6.18 (t, J = 2.1 Hz, 1H), 4.17 (t, J = 6.9 Hz, 2H), 2.63 (t, J = 6.8 Hz, 2H), 1.93 (p, J = 6.8 Hz, 2H), 1.68 (s, 2H).^{13}C NMR (126 MHz, $CDCl_3$) δ 139.1, 129.0, 105.3, 49.5, 39.0, 33.9. HRMS (ESI): calc'd for $C_6H_{12}N_3[M+H]^+$: 126.1026; found 126.1039.



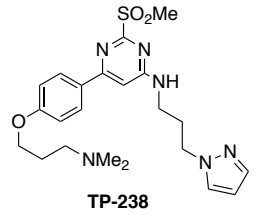
S3

***N*-(3-(1*H*-pyrazol-1-yl)propyl)-6-chloro-2-(methylthio)pyrimidin-4-amine:** To a solution of the sulfide (162 mg, 0.833 mmol) in isopropanol (3 mL) was added amine **S2** (125 mg, 0.999 mmol) and Et_3N (116 uL, 0.833 mmol) at room temperature. The solution was then warmed to reflux and stirred for 18 h at which point it was concentrated *in vacuo* and purified by Combiflash Rf system (hexanes/ EtOAc, 0-100% EtOAc) to yield 222 mg (94%) of sulfide **S3** as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (d, J = 1.9 Hz, 1H), 7.39 (d, J = 2.3 Hz, 1H), 6.28 (t, J = 2.1 Hz, 1H), 5.98 (s, 1H), 5.52 (t, J = 6.2 Hz, 1H), 4.30 – 4.19 (m, 2H), 3.36 (s, 2H), 2.47 (s, 3H), 2.13 (p, J = 6.4 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.4, 162.7, 139.7, 129.5, 106.1, 49.4, 38.6, 29.8, 14.2.[1] HRMS (ESI): calc'd for $C_{14}H_{14}N_3O_2[M+H]^+$: 284.0731; found 284.0746.



S4

4-(6-((3-(1*H*-pyrazol-1-yl)propyl)amino)-2-(methylsulfonyl)pyrimidin-4-yl)phenol: A solution of (4-hydroxyphenyl)boronic acid (66 mg, 0.479 mmol), sulfide **S3** and K_2CO_3 (110 mg, 0.798 mmol) in dioxanes (1.5 mL) and H_2O (0.5 mL) was degassed prior to the addition of $Pd(PPh_3)_4$ (46 mg, 0.40 mmol). The solution was warmed to reflux and stirred under N_2 for 18 h, at which point it was quenched with H_2O (2 mL), extracted with EtOAc (3 x 3 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purified by Combiflash Rf system (hexanes/ EtOAc, 0-100% EtOAc) to yield 80 mg (59%) of phenol **S4** as a white solid. ^{1}H NMR (500 MHz, DMSO) δ 9.86 (s, 1H), 7.84 (s, 1H), 7.74 (d, J = 2.2 Hz, 1H), 7.44 (d, J = 1.8 Hz, 2H), 6.88 – 6.81 (m, 2H), 6.51 (s, 1H), 6.23 (t, J = 2.0 Hz, 1H), 4.18 (t, J = 6.9 Hz, 2H), 2.45 (s, 3H), 2.03 (p, J = 6.9 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 169.9, 162.6, 159.5, 138.6, 129.9, 127.9, 127.6, 115.4, 104.9, 48.8, 37.5, 29.8, 13.3. HRMS (ESI): calc'd for $C_{17}H_{20}N_5OS$ $[M+H]^+$: 342.1383; found 342.1427.



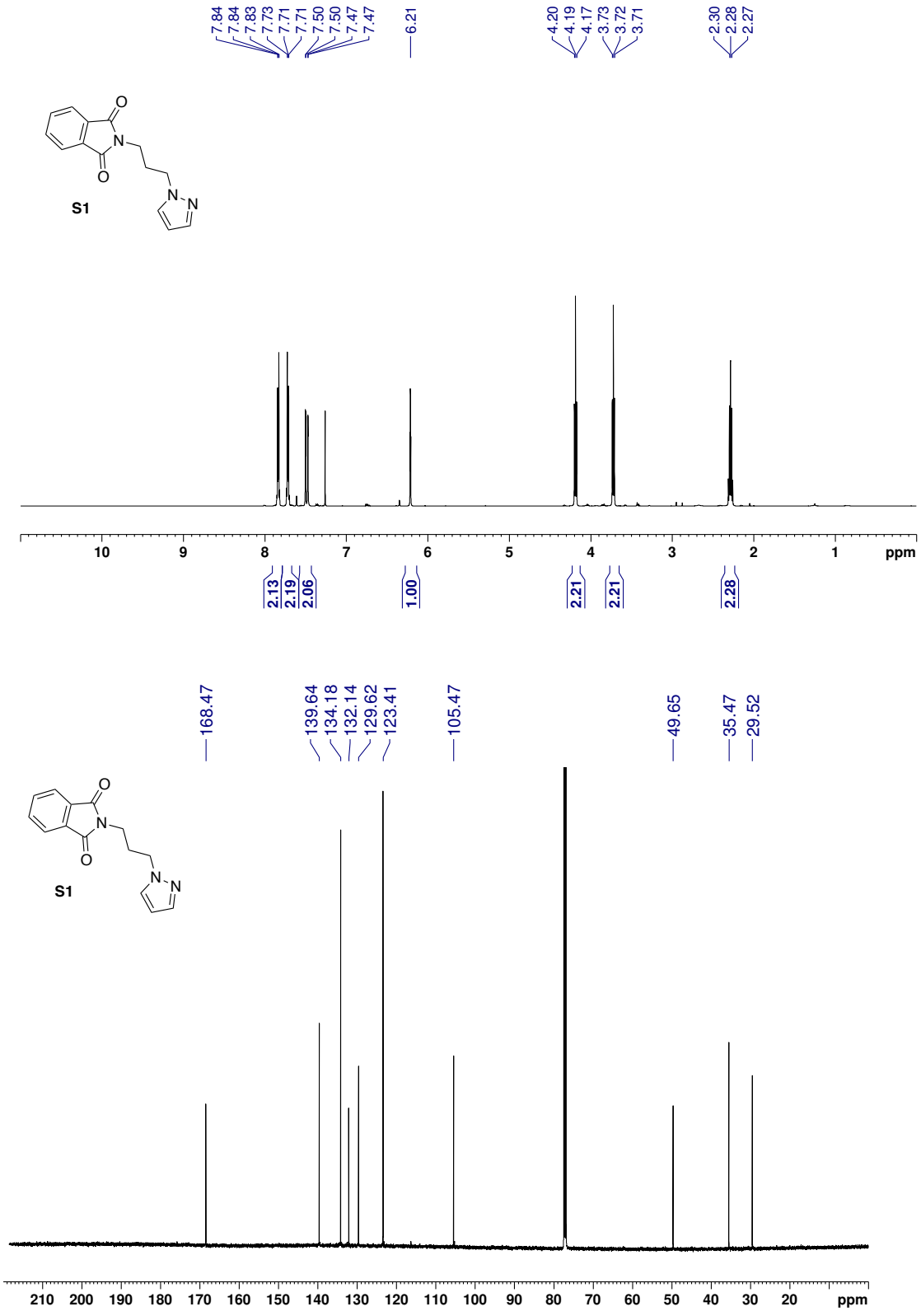
TP-238

***N*-(3-(1*H*-pyrazol-1-yl)propyl)-6-(4-(3-(dimethylamino)propoxy)phenyl)-2-(methylsulfonyl)pyrimidin-4-amine:** To a solution of sulfide **S4** (31 mg, 0.091 mmol) in THF (1.5 mL) at room temperature was added Oxone (140 mg, 0.227 mmol) in H_2O (0.5 mL) dropwise. The solution stirred for 18 h at room temperature before the addition of H_2O (2.0 mL). The product was extracted from the aqueous layer with EtOAc (3 x 3 mL) and the organic extracts were combined, dried ($MgSO_4$) filtered and concentrated *in vacuo*. The crude sulfone was then dissolved in DMF (2.0 mL) before the addition of K_2CO_3 (38 mg) and 3-(dimethylamino)propyl methanesulfonate hydrochloride[2] (59 mg, 0.272 mmol). The solution was heated to 80°C for 18 h. The reaction was then quenched with H_2O (2 mL) and extracted with EtOAc (3 x 3 mL). Organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purified via CombiFlash Rf system (hexanes/ EtOac, 0-100% EtOAc, then DCM/MeOH, 0-30% MeOH). Isolated 9 mg, (22%) TP-238 as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (s, 1H), 7.42 (s, 1H), 7.04 – 6.88 (m, 2H), 6.68 (d, J = 2.7 Hz, 1H), 6.27 (t, J = 2.2 Hz, 1H), 6.00 (s, 1H), 4.27 (d, J = 6.9 Hz, 2H), 4.07 (dt, J = 6.6, 4.2 Hz, 2H), 3.54 – 3.39 (m, 2H), 3.34 (d, J = 2.5 Hz, 3H), 2.48 (td, J = 7.7, 2.4 Hz, 2H), 2.27 (d, J = 2.6 Hz, 6H), 2.17 (t, J = 6.8 Hz, 2H), 2.04 – 1.91 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.8, 163.9, 161.7, 139.7, 129.7, 128.7, 128.2, 114.8, 106.0, 66.5, 56.4, 45.6, 38.9, 29.7, 27.6. HRMS (ESI): calc'd for $C_{22}H_{30}N_6NaO_3S$ $[M+Na]^+$: 481.1992; found 481.2622.

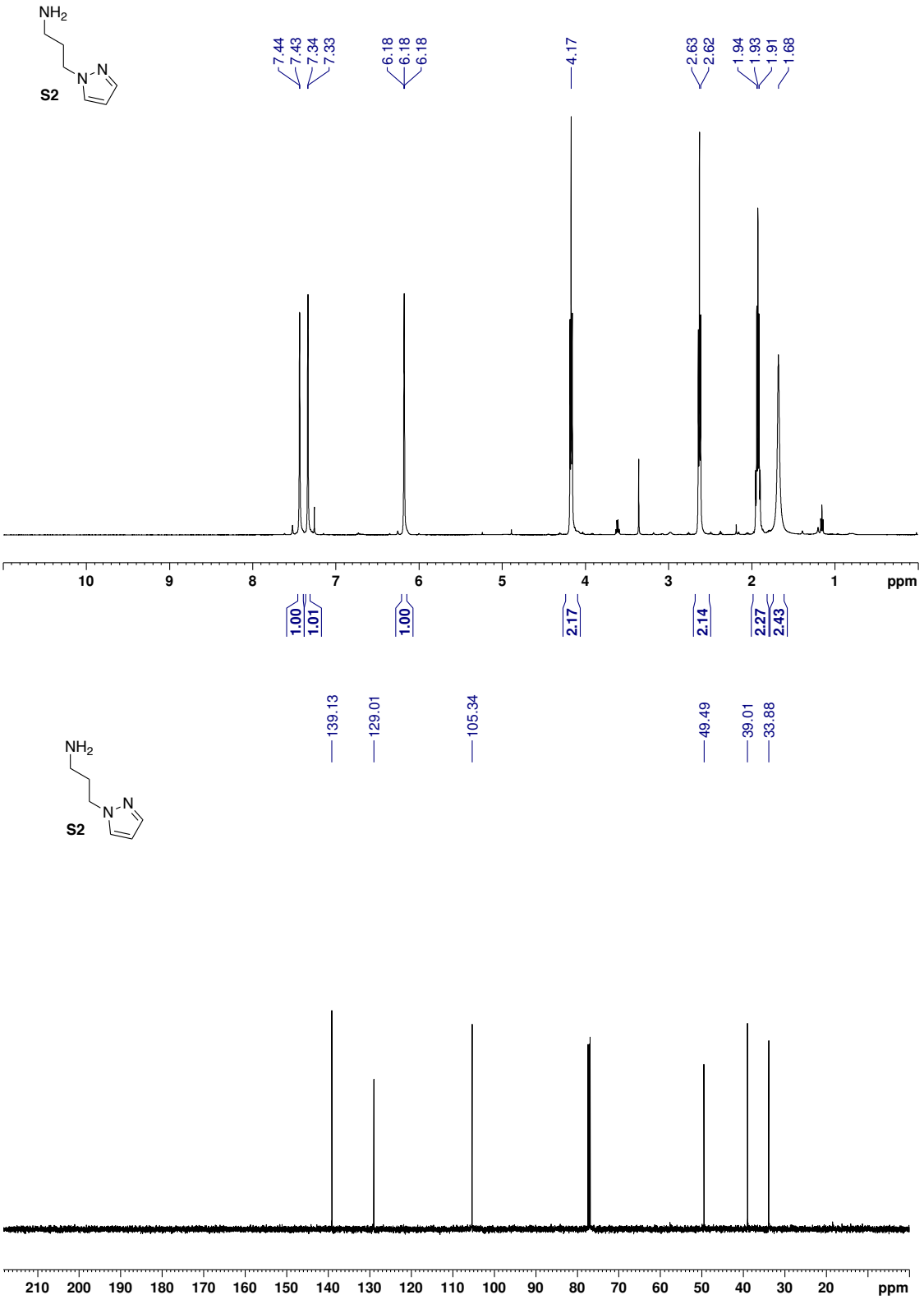
Notes:

1. We did not observe two shifts from the aromatic region that correspond to two carbons on the substituted pyrimidine ring for **S3**, **S4** or **TP-238**. However, the supporting ^{1}H NMR and HRMS data unequivocally confirm the indicated structures.

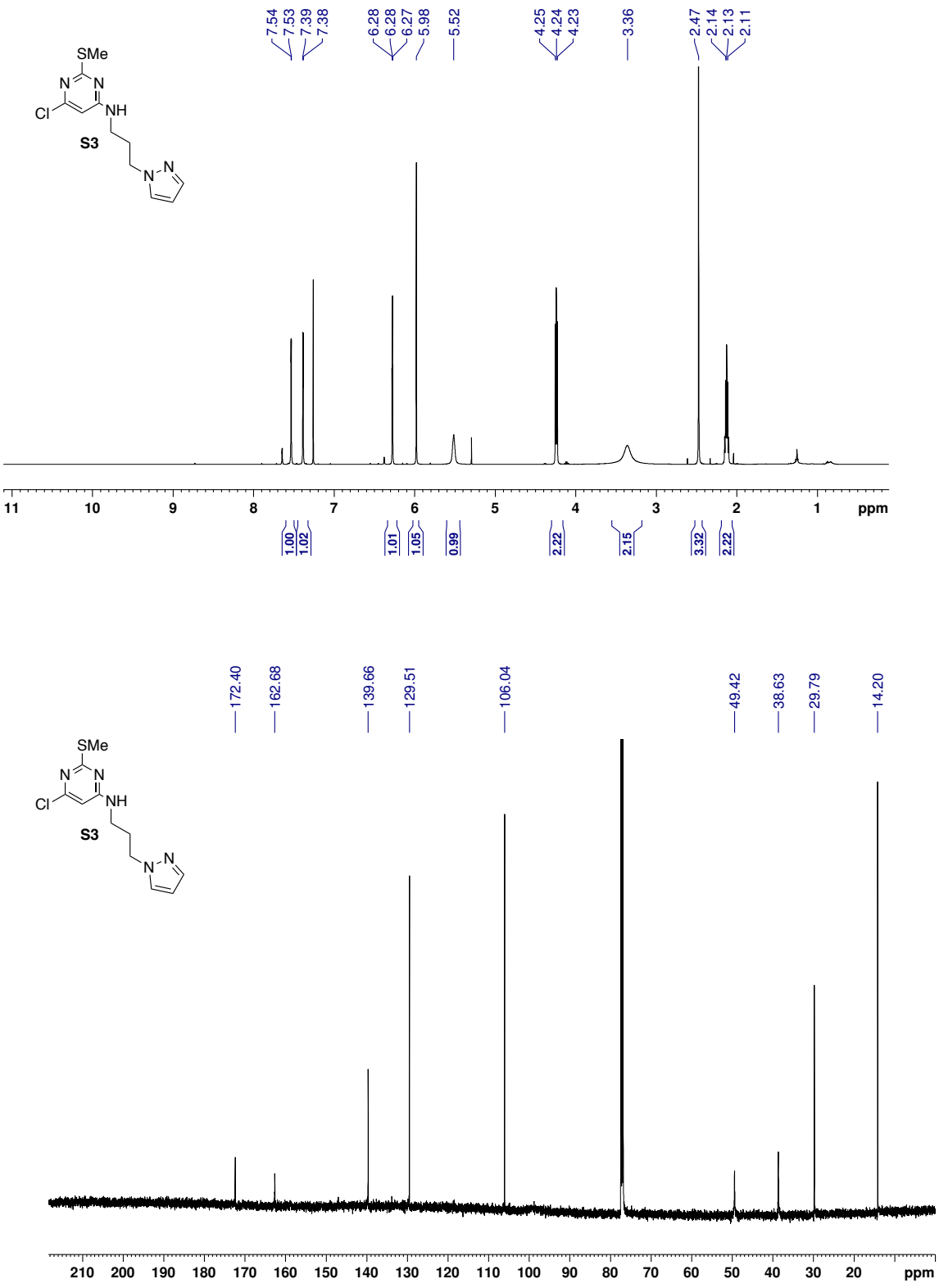
2. 3-(dimethylamino)propyl methanesulfonate hydrochloride was prepared according to literature procedure from *J. Am. Chem. Soc.* **1999,** *121,* 1452-1459.



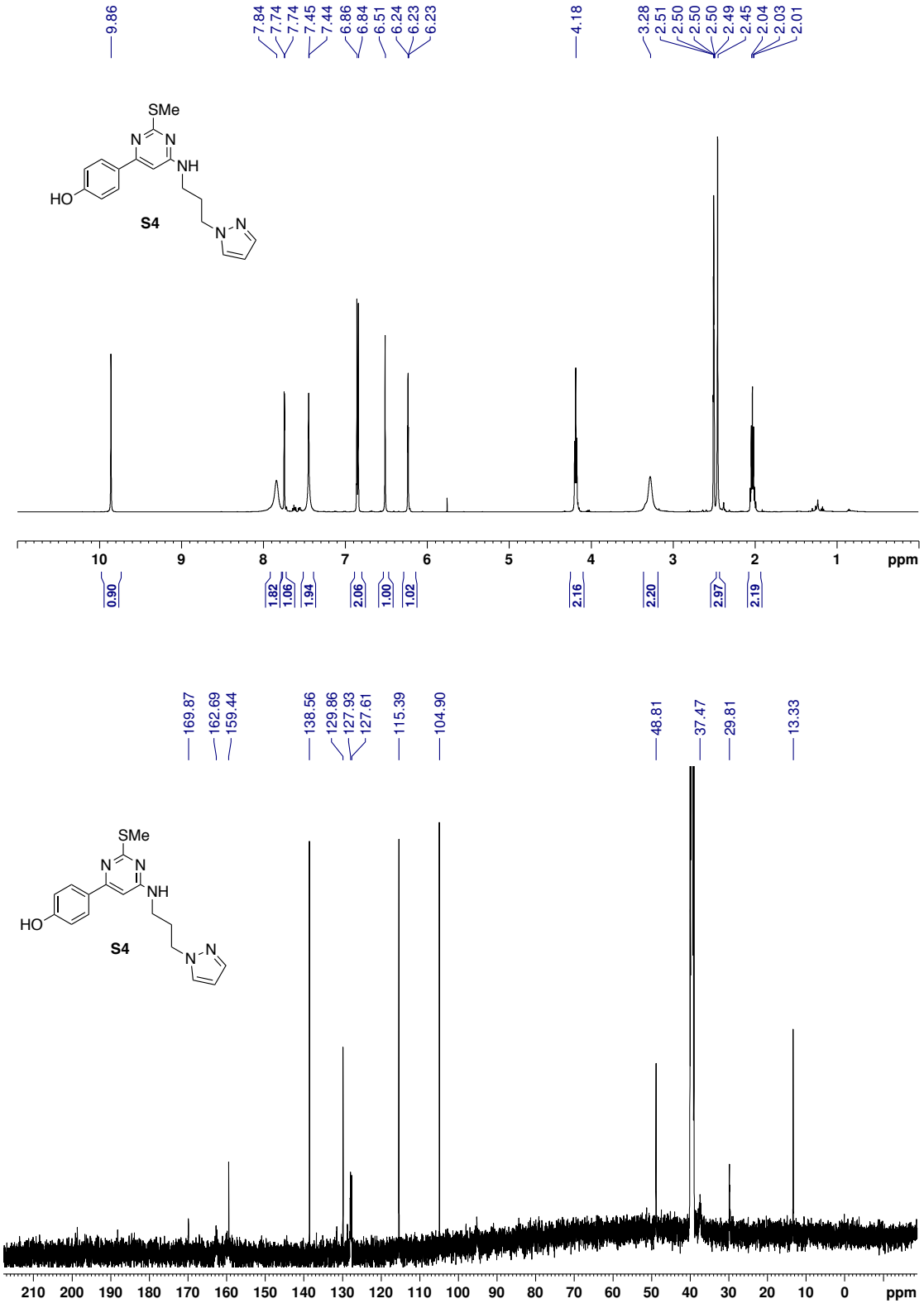
^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}CNMR (126 MHz) of **S1**



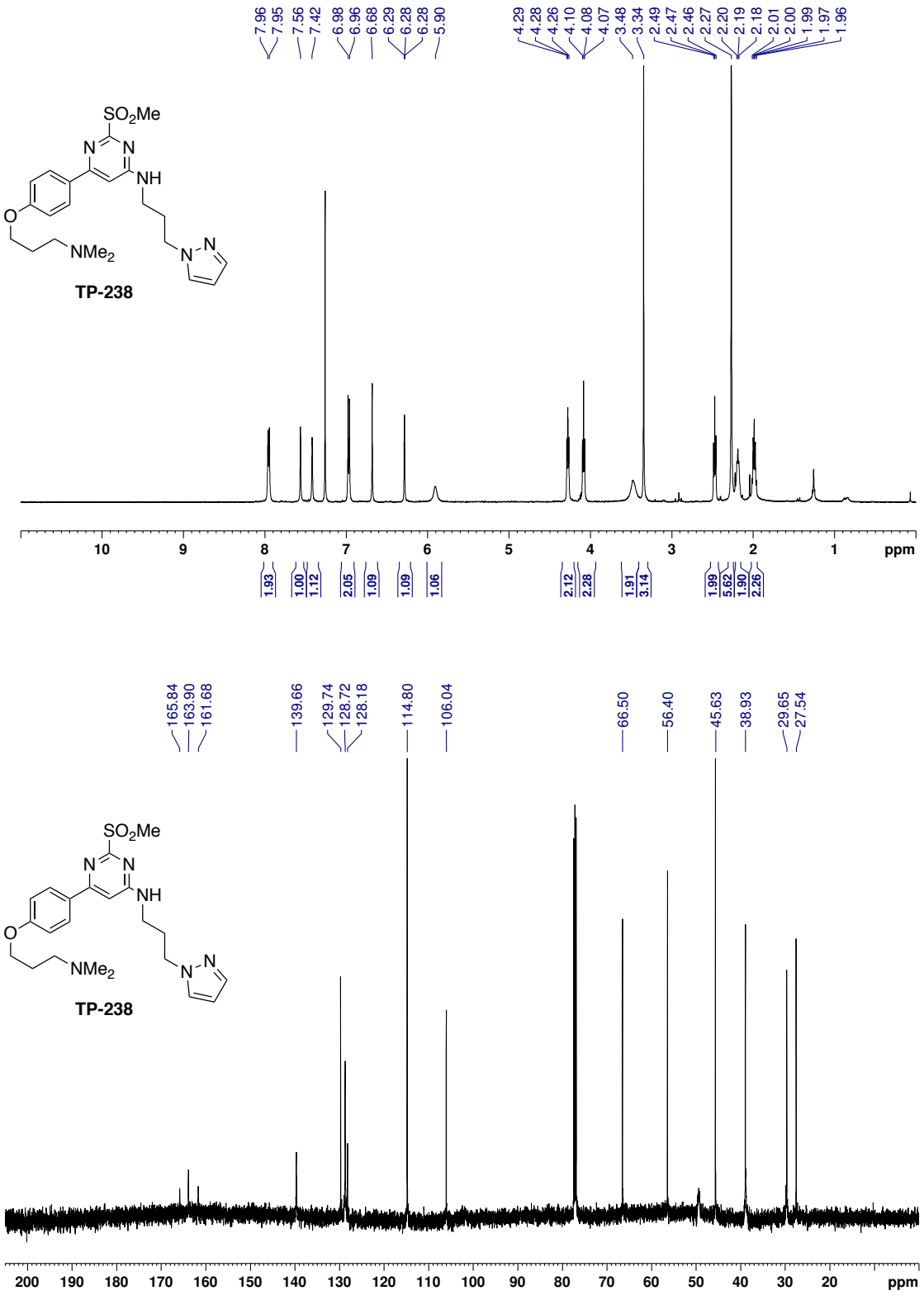
^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}CNMR (126 MHz) of **S2**



^{1}HNMR (500 MHz, $CDCl_3$) and ^{13}CNMR (126 MHz) of **S3**

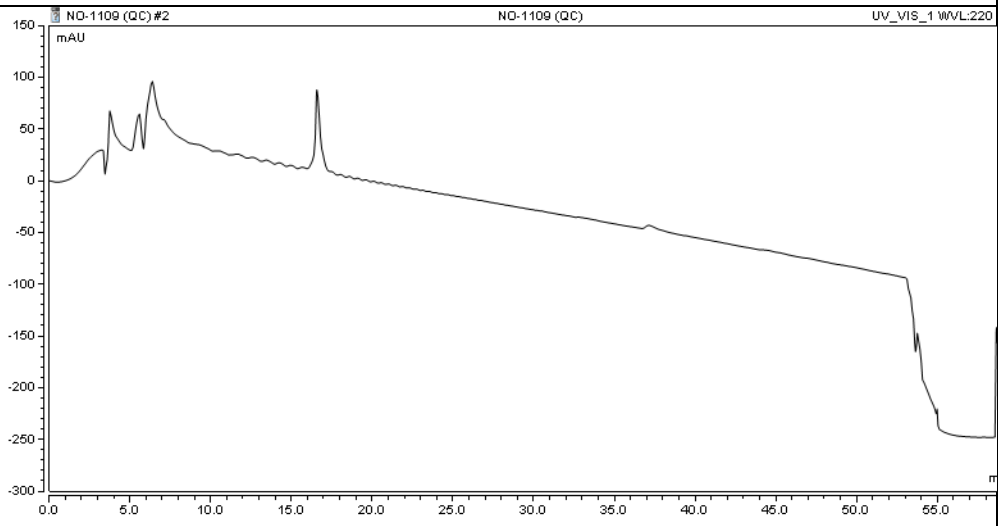
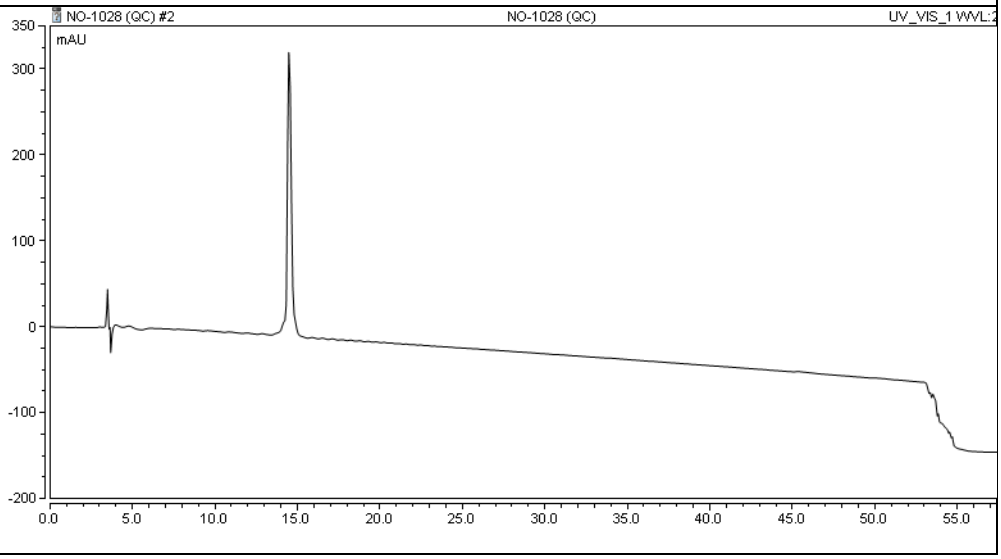


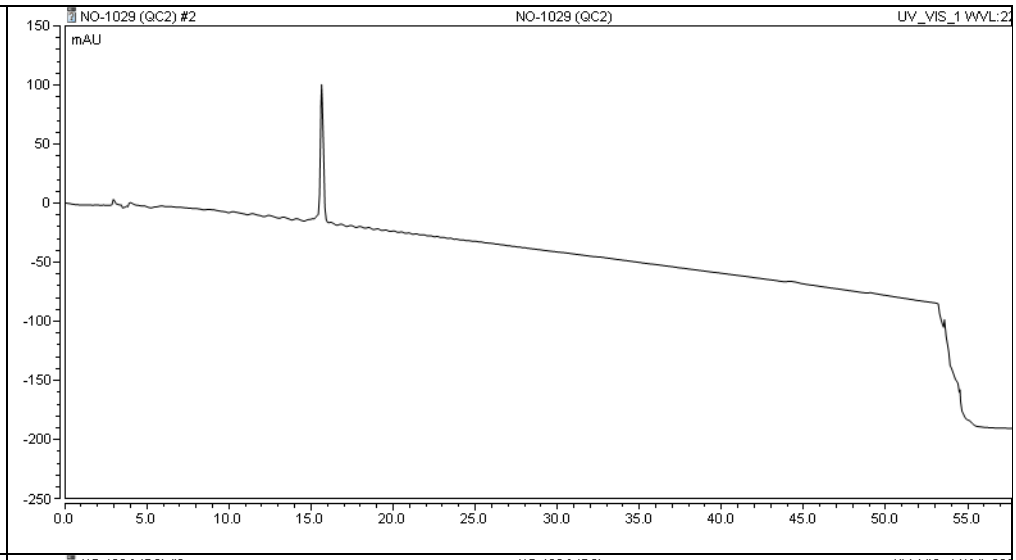
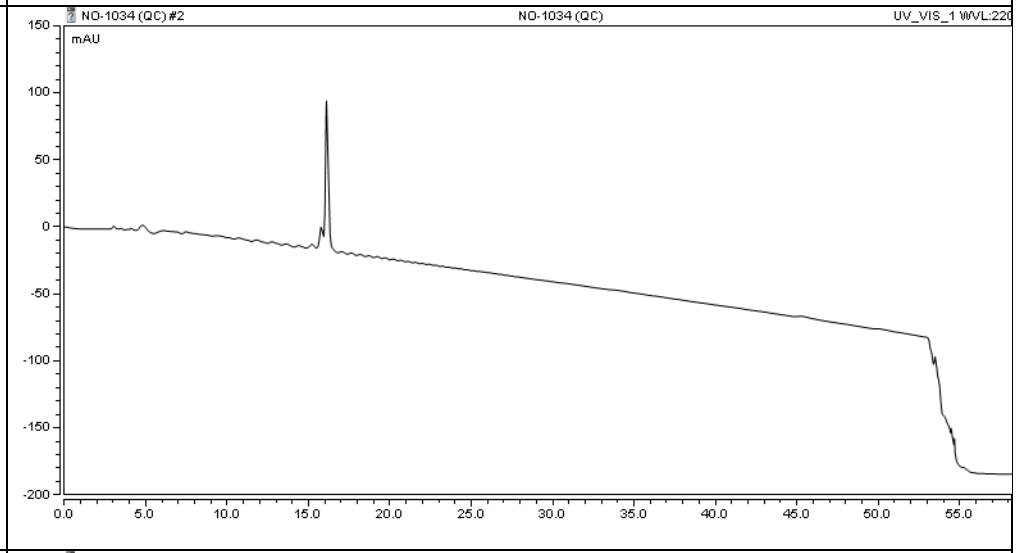
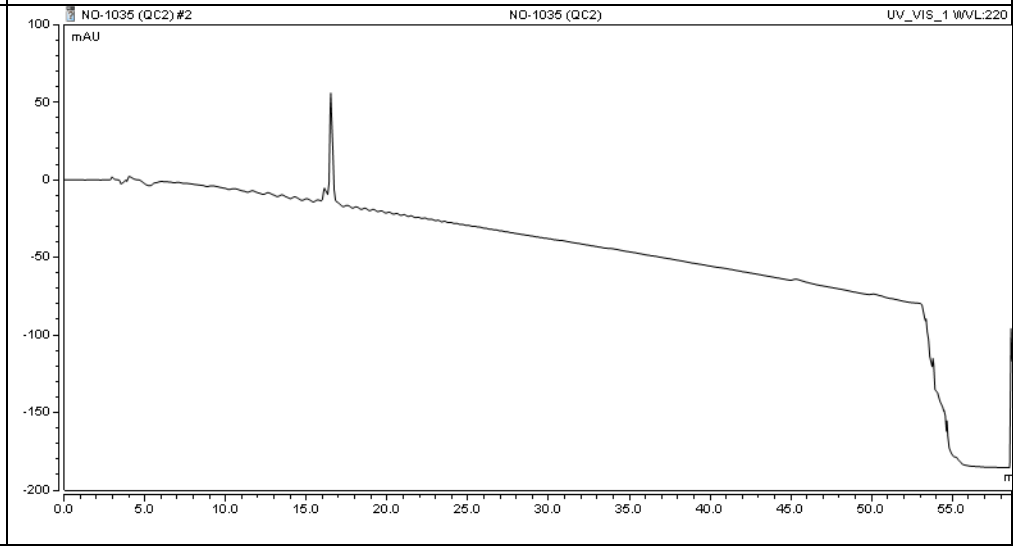
^{1}H NMR (500 MHz, d_6-DMSO) and ^{13}CNMR (126 MHz, d_6-DMSO) of **S4**

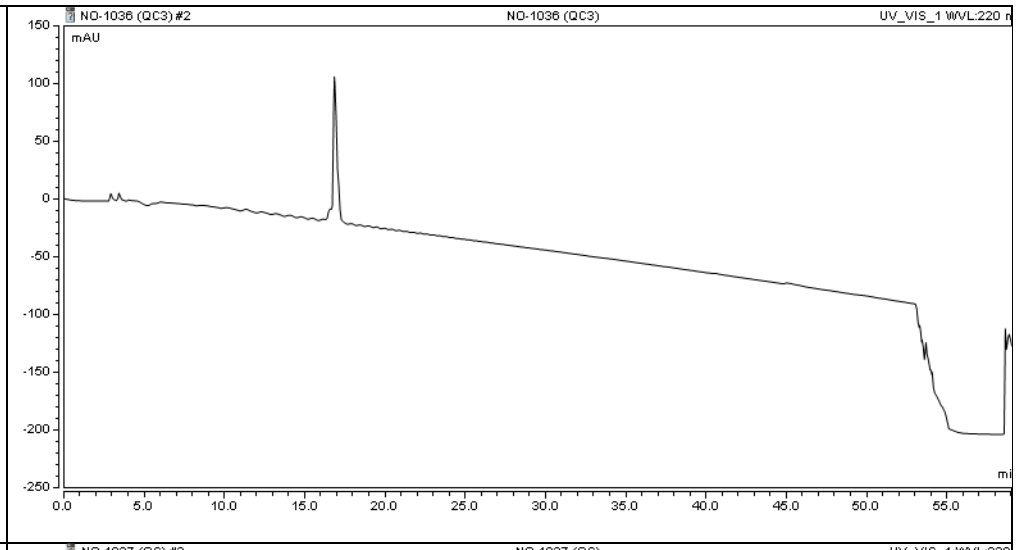
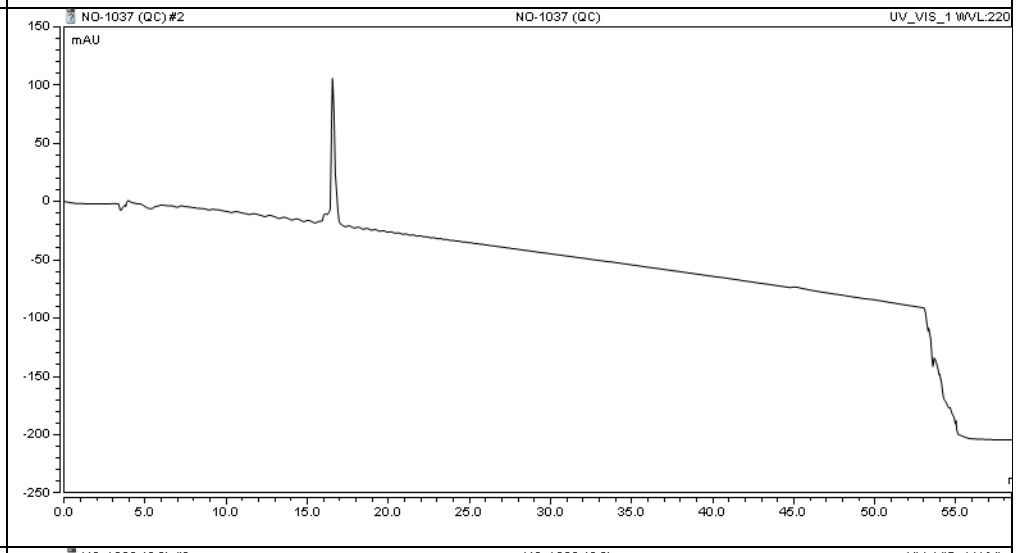
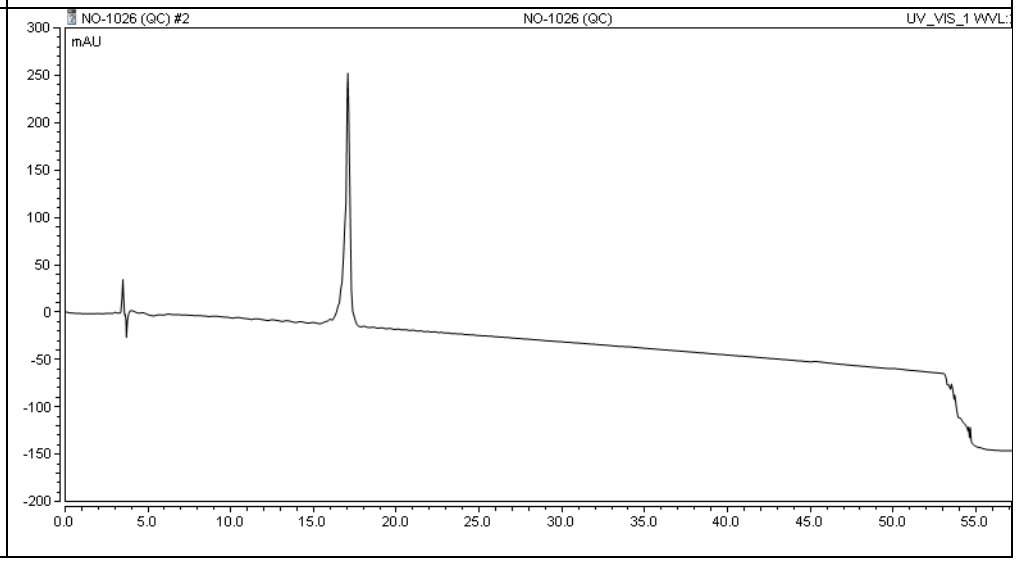


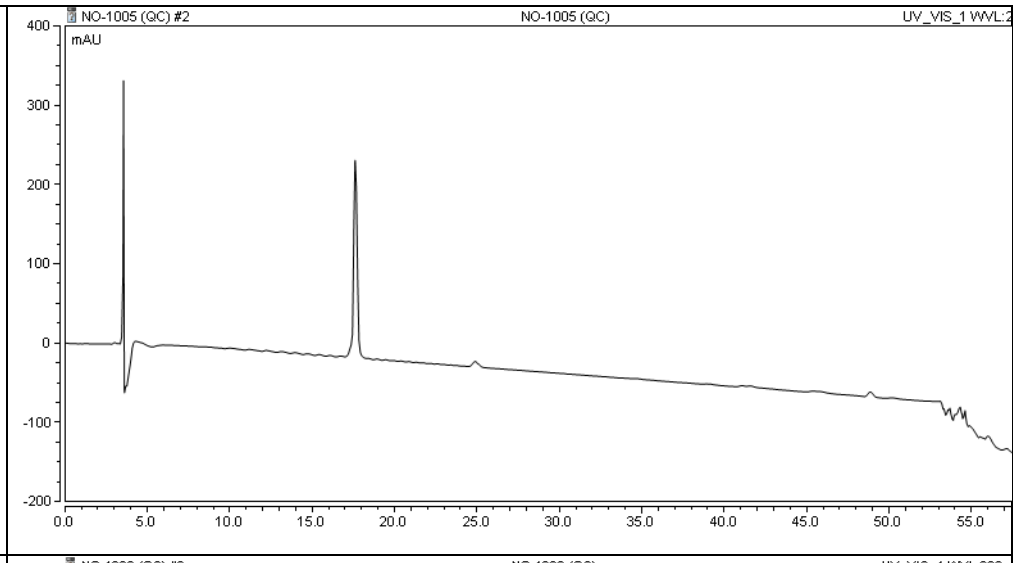
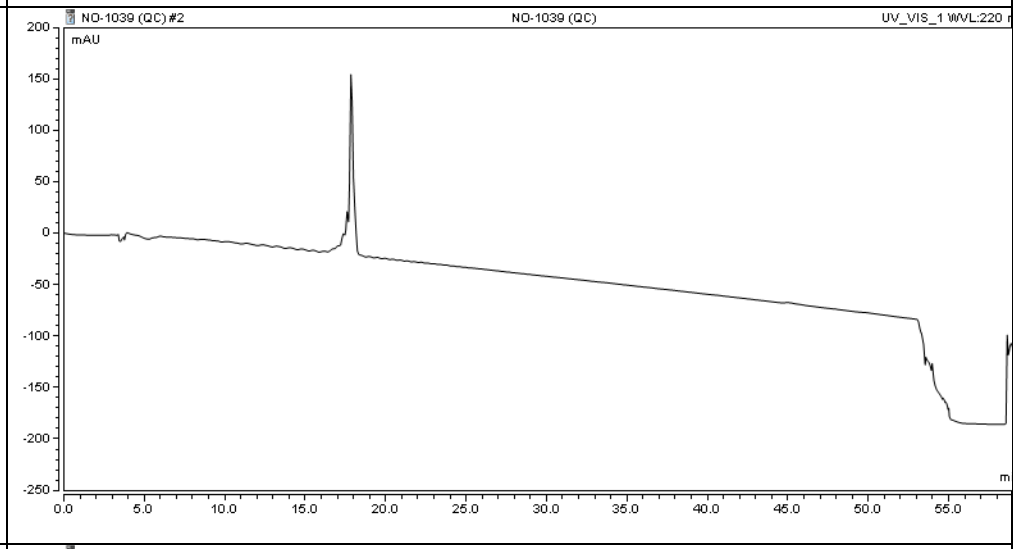
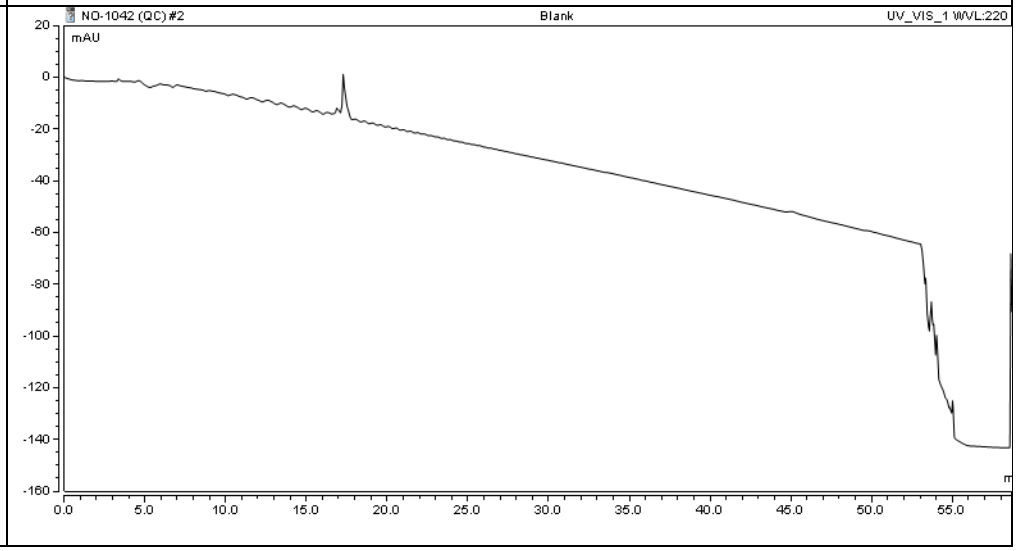
^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}CNMR (126 MHz, $CDCl_3$) of **TP-238**

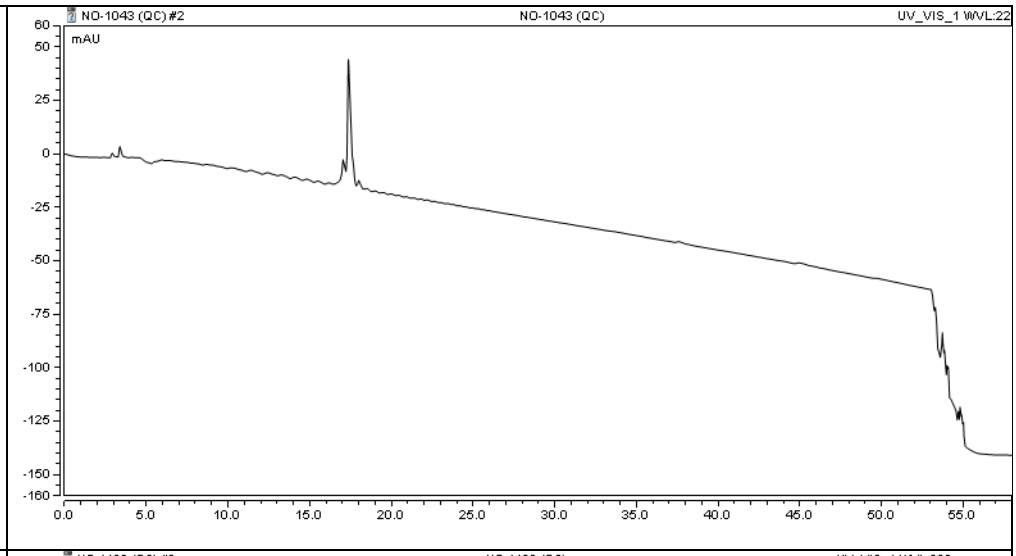
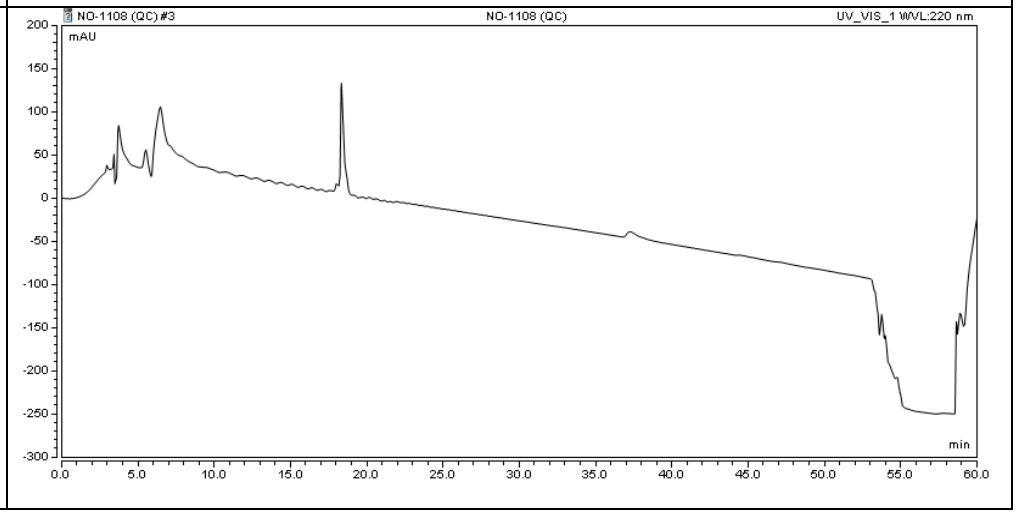
HPLC analytical purity traces for synthesized peptides. Peptide purity was assessed with a Dionex Ultimate RP-HPLC system using a Vydac C-18 column and a 0-50% CH_3CH gradient over 50 minutes. Traces are shown below.

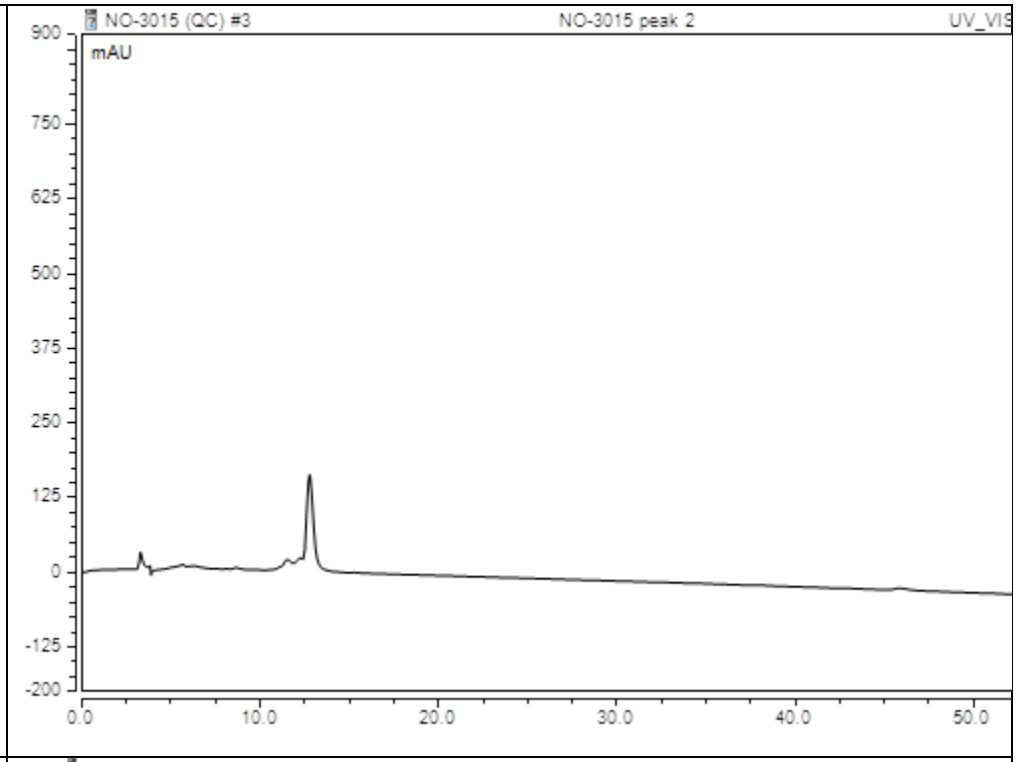
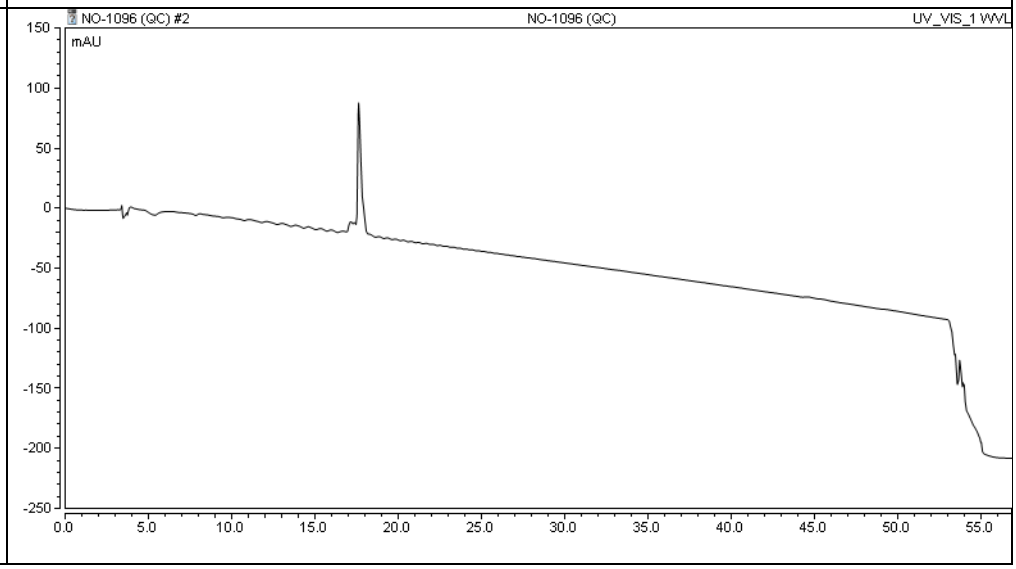
Peptide	
H4 K16ac	
H2A.Z II unacetylated	

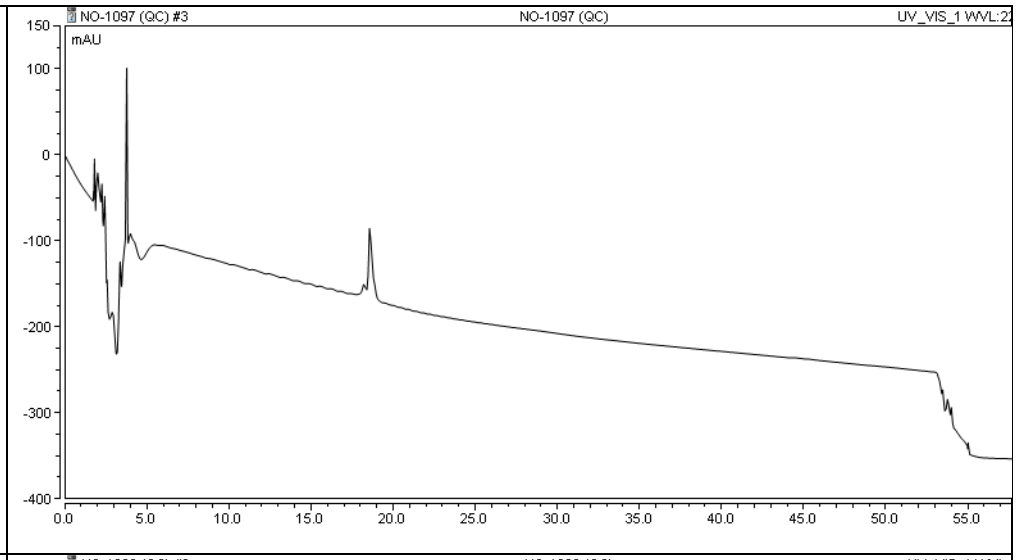
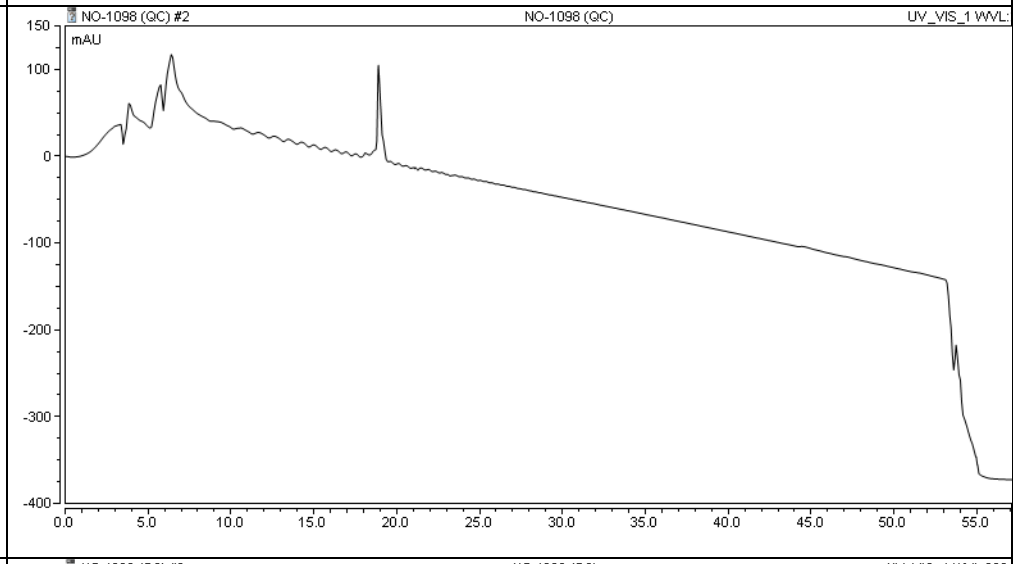
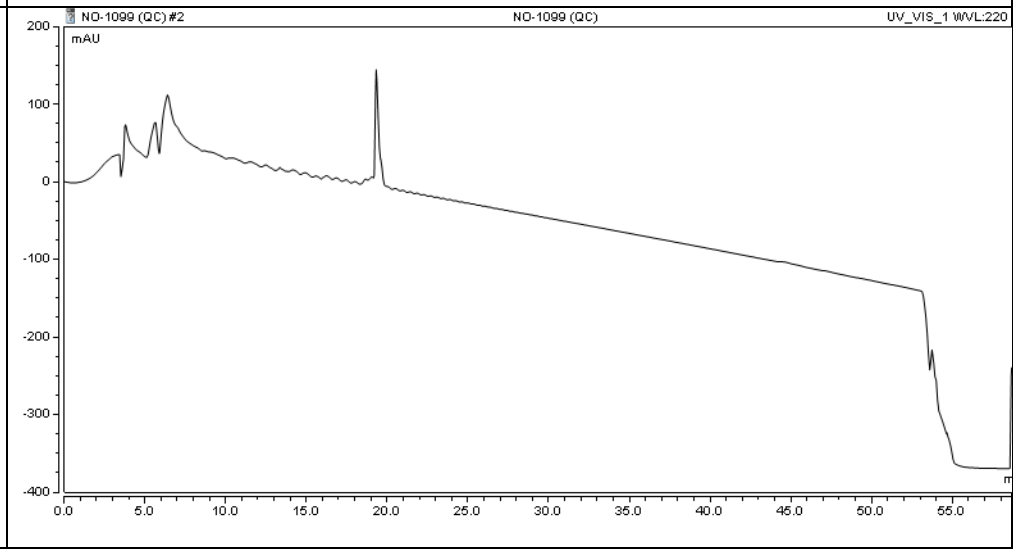
H2A.Z II K4ac	
H2A.Z II K7ac	
H2A.Z II K11ac	

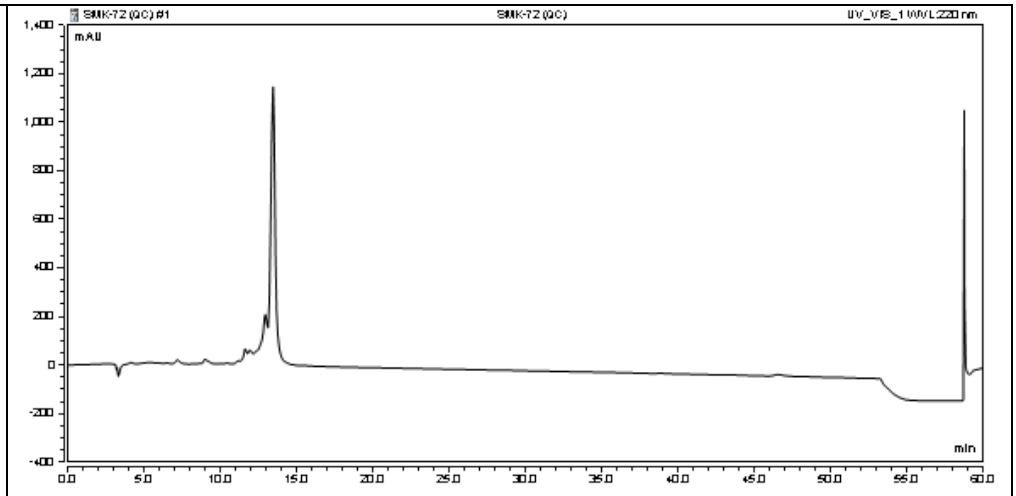
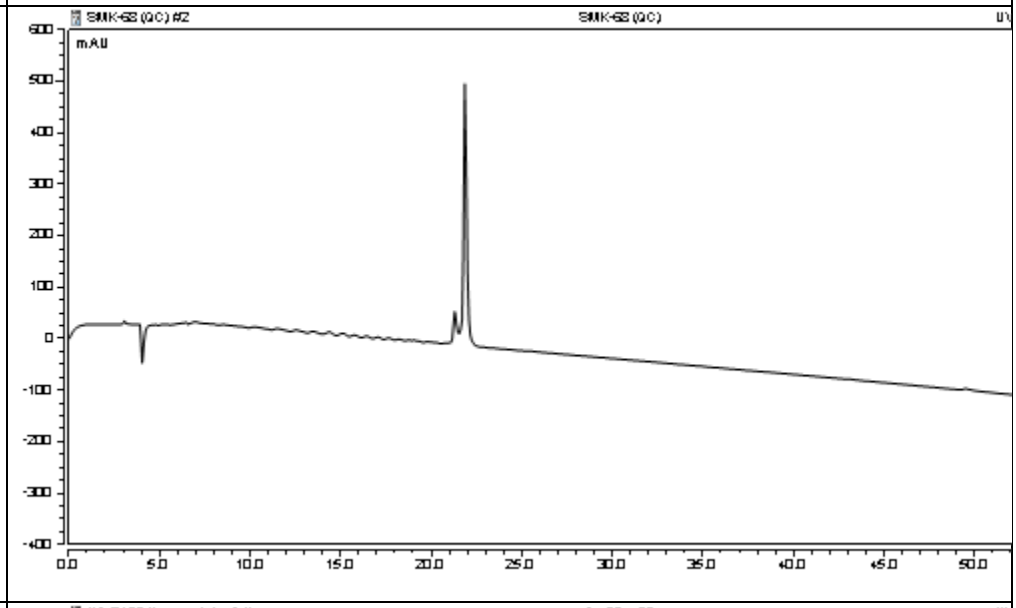
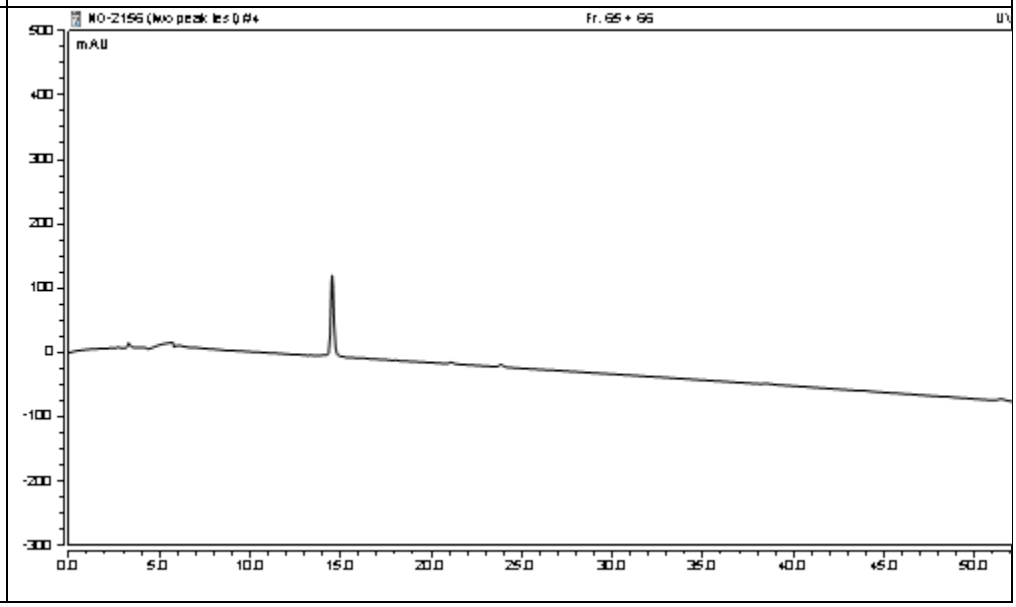
H2A.Z II K13ac	
H2A.Z II K15ac	
H2A.Z II K4ac,K7ac	

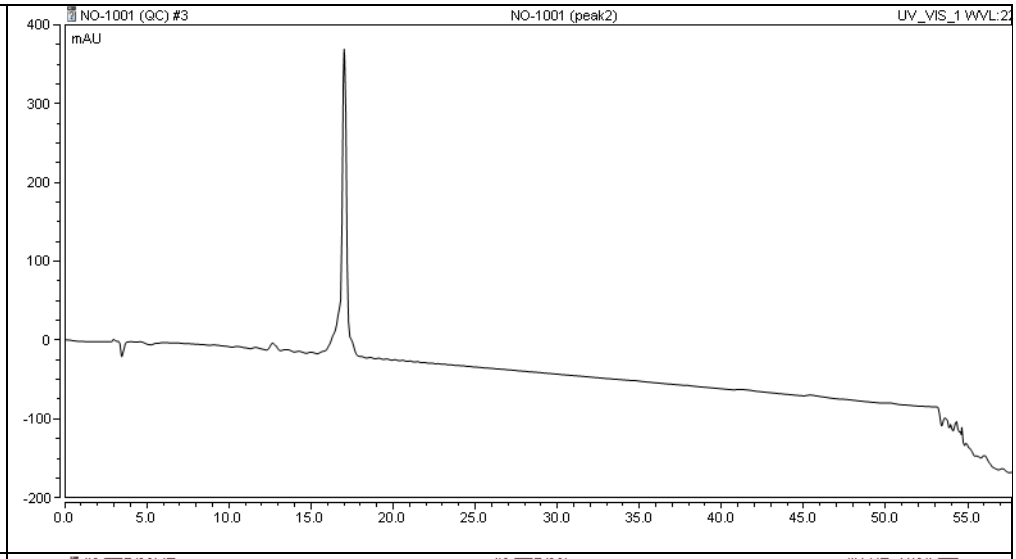
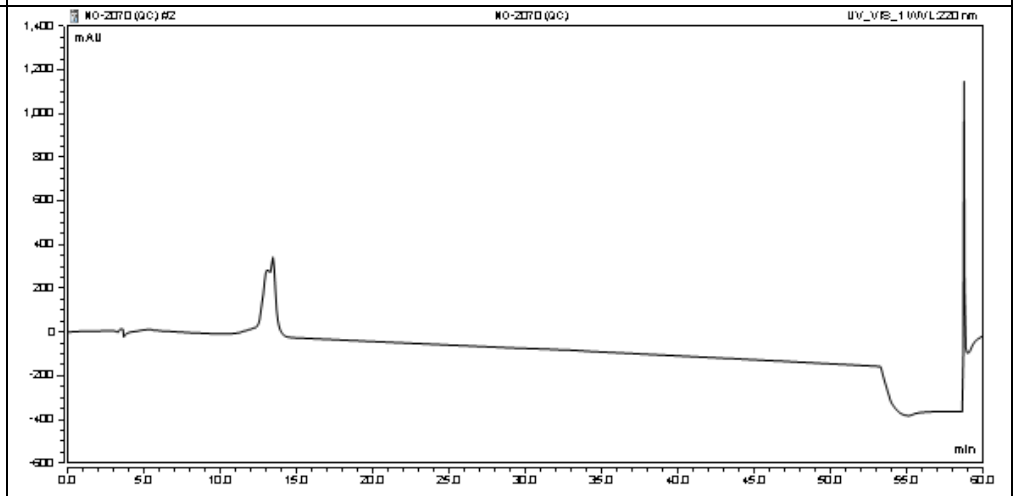
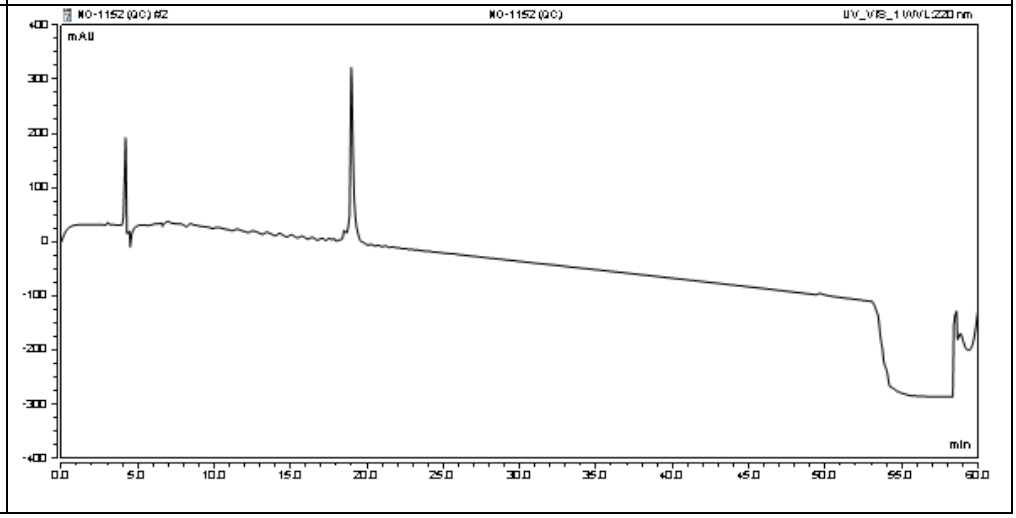
H2A.Z II K4ac,11ac	
H2A.Z II K4ac,K13ac	
H2A.Z II K4ac,K15ac	

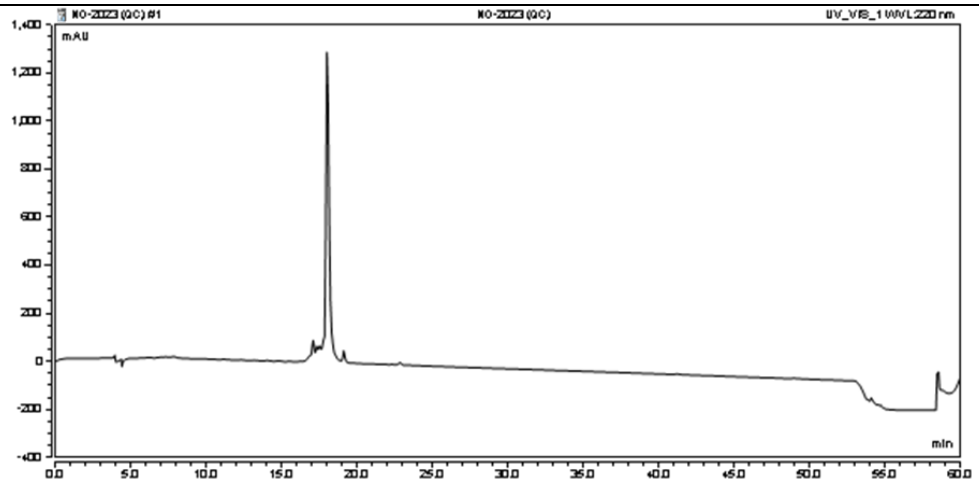
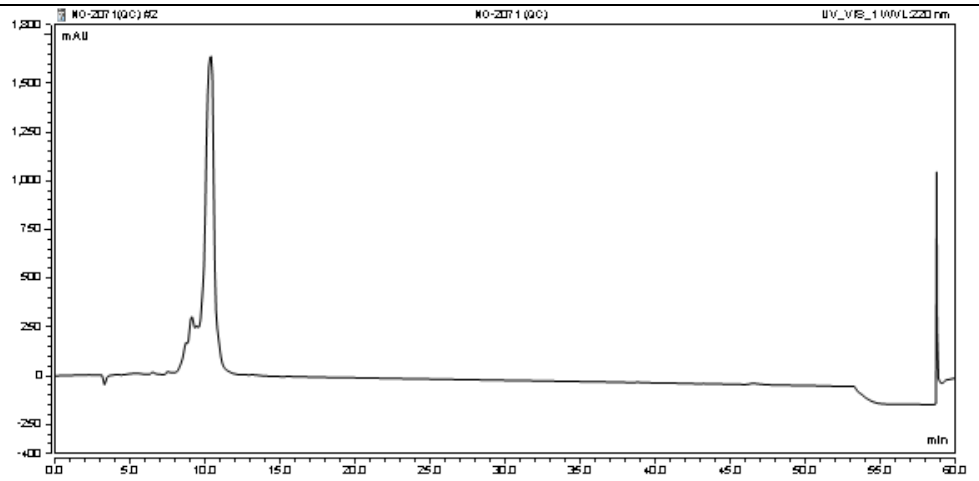
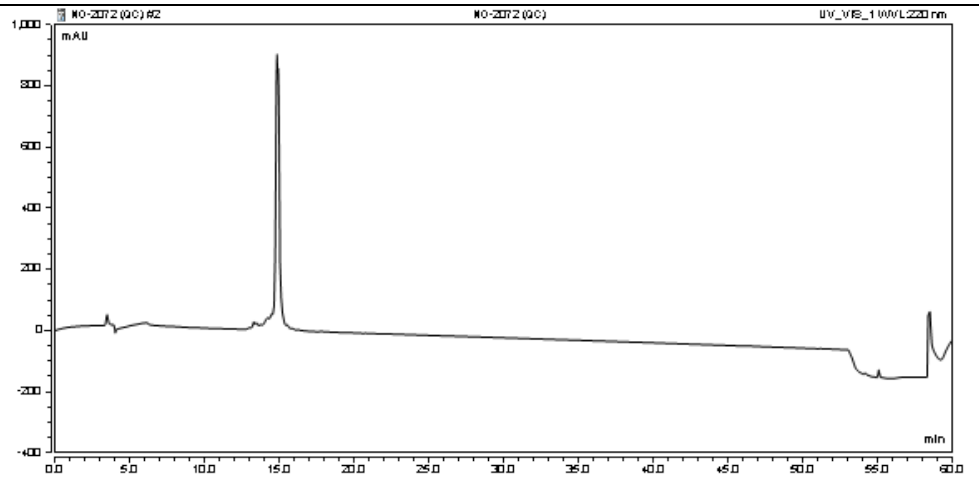
H2A.Z II K7ac,11ac	
H2A.Z II K7ac,K13ac	

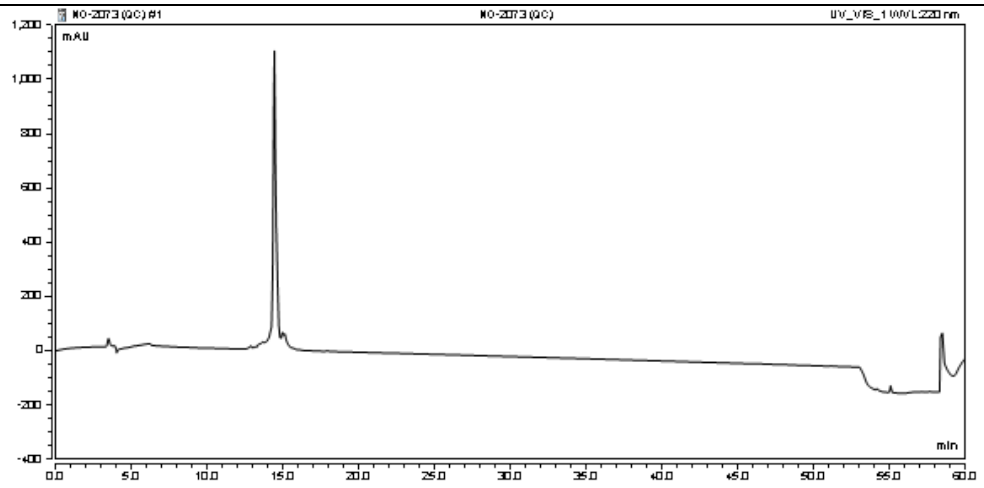
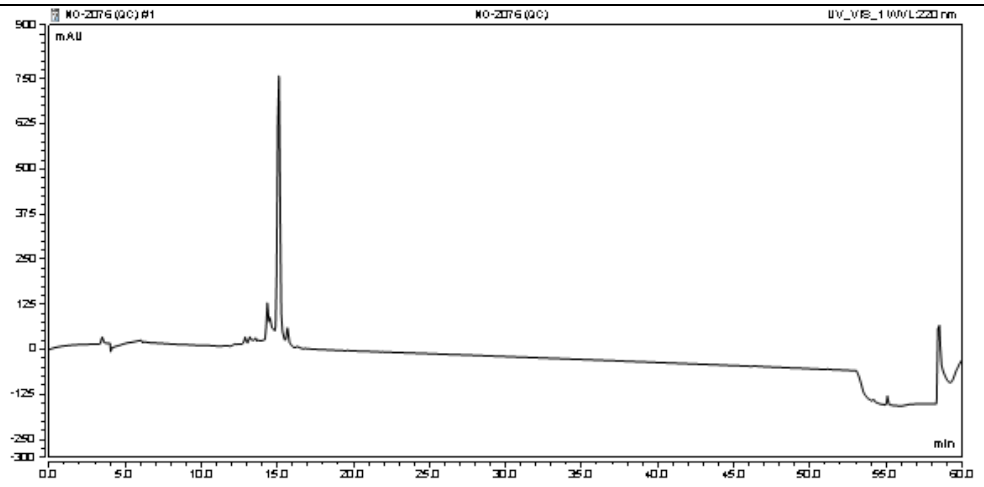
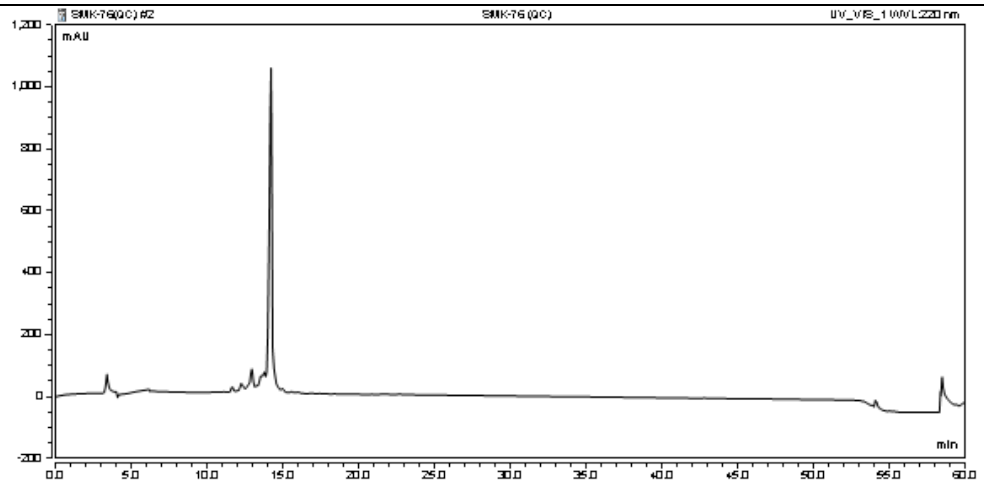
H2A.Z II K7ac-d_3,K13ac	
H2A.Z II K7ac,K15ac	

H2A.Z II K11ac,K13ac	
H2A.Z II K11ac,K15ac	
H2A.Z II K13ac,K15ac	

H2A.Z II K4ac,K7ac,K11ac	
H2A.Z II K7ac,K13ac,K15ac	
H2A.Z II K4me,K7ac,K13ac	

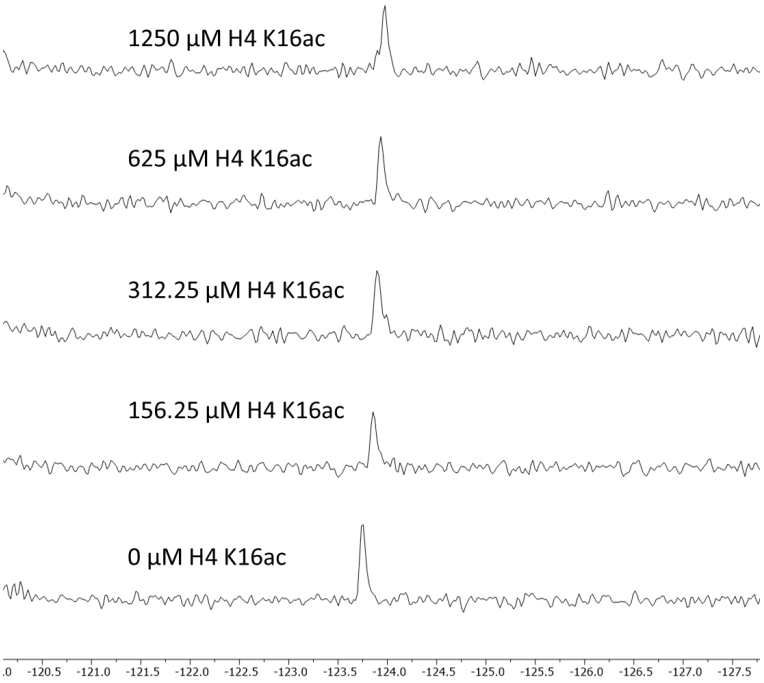
H2A.Z I K4ac,K11ac	
H2A.Z I K4ac,K13ac	
H2A.Z I K4ac,K15ac	

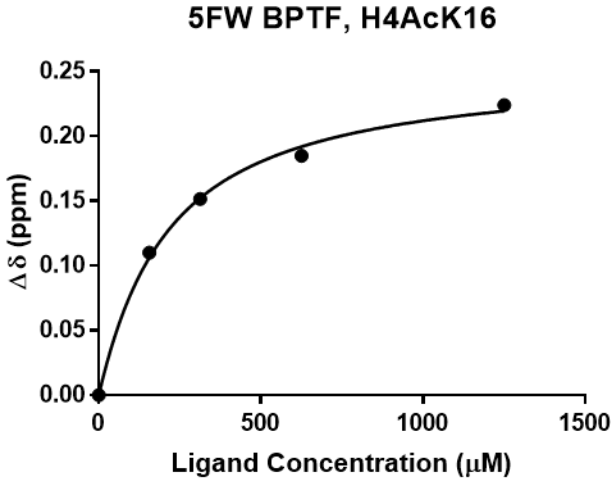
H2A.Z I K7ac,K13ac	
H2A.Z I K7ac,K15ac	
H2A.Z I K11ac,K13ac	

H2A.Z I K11ac,K15ac	
H2A.Z I K13ac,K15ac	
H2A.Z I K7ac,K13ac,K15ac	

PrOF NMR Titrations with 5FW-BPTF.

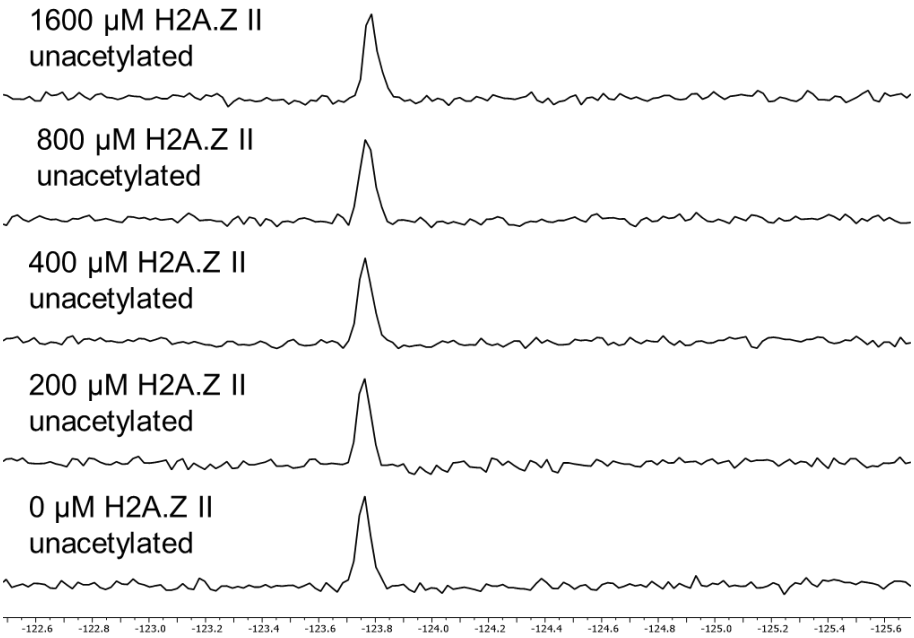
PrOF NMR titration with 5FW-BPTF and H4 K16ac peptide.

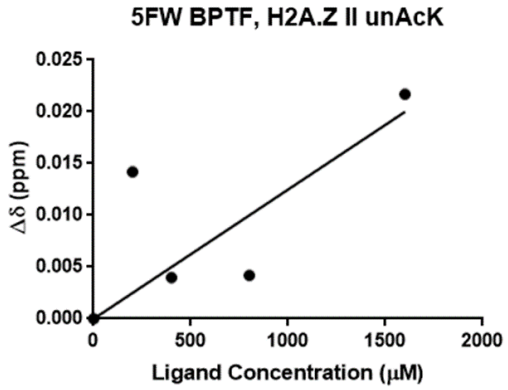




[Ligand]	5FW 2950 (ppm)	Δδ
0 µM	-123.7473	
156.25 µM	-123.8571	-0.1098
312.5 µM	-123.8989	-0.1516
625 µM	-123.9329	-0.1856
1250 µM	-123.972	-0.2247

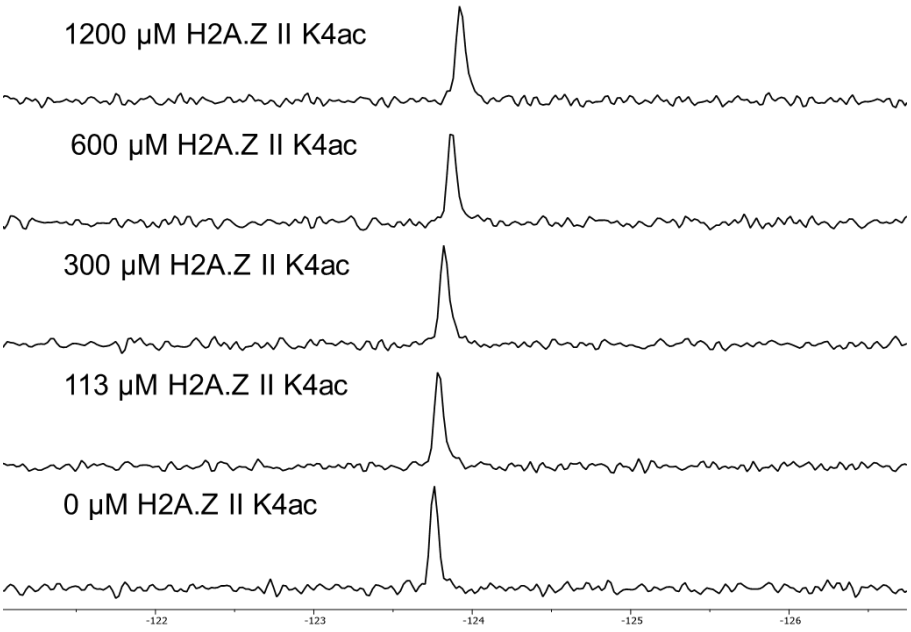
PrOF NMR titration with 5FW-BPTF and H2A.Z II unacetylated peptide.

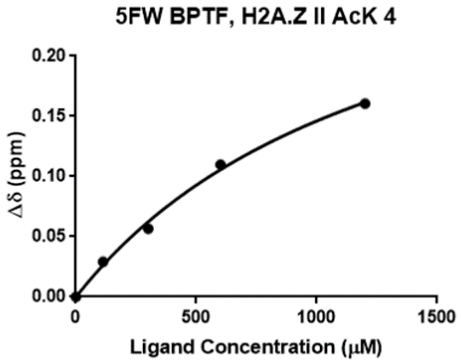




[Ligand]	5FW 2950 (ppm)	Δδ
0 μM	-123.7598	
200 μM	-123.7591	0.0007
400 μM	-123.7629	-0.0031
800 μM	-123.7692	-0.0094
1600 μM	-123.7815	-0.0217

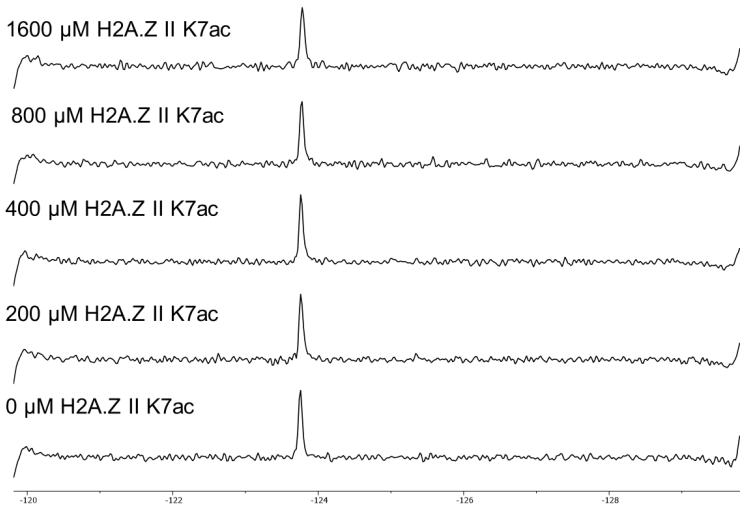
PrOF NMR titration with 5FW-BPTF and H2A.Z II K4ac peptide.

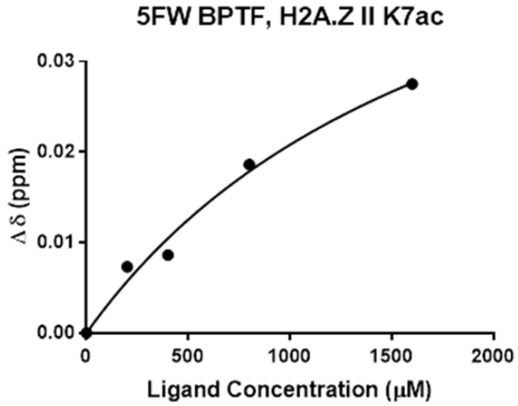




[Ligand]	5FW 2950 (ppm)	Δδ
0 μM	-123.7588	
113 μM	-123.7868	-0.028
300 μM	-123.8219	-0.0631
600 μM	-123.8688	-0.11
1200 μM	-123.9238	-0.165

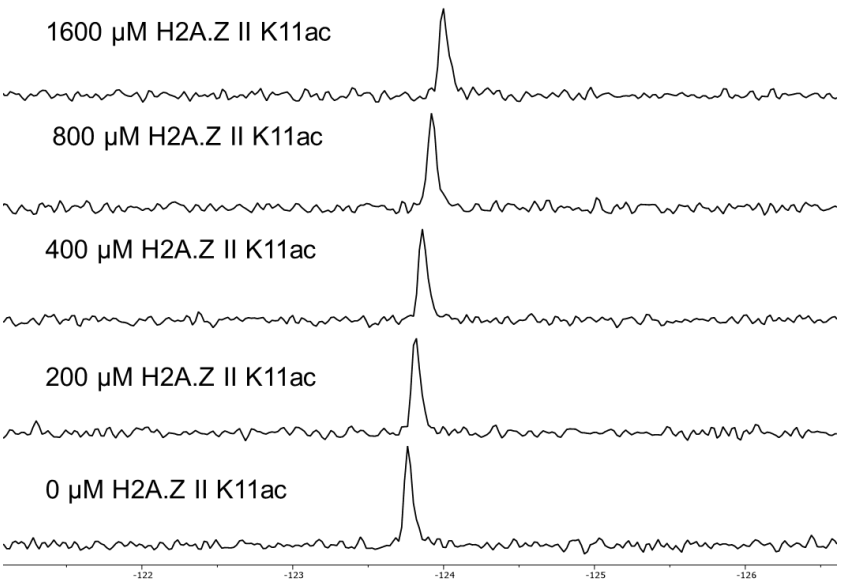
PrOF NMR titration with 5FW-BPTF and H2A.Z II K7ac peptide.

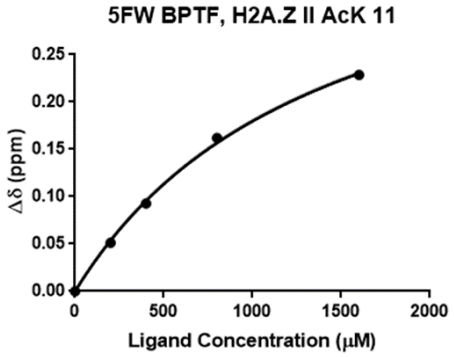




[Ligand]	5FW 2950 (ppm)	Δδ
0 μM	-123.7546	
200 μM	-123.7619	-0.0073
400 μM	-123.7632	-0.0086
800 μM	-123.7732	-0.0186
1600 μM	-123.7821	-0.0275

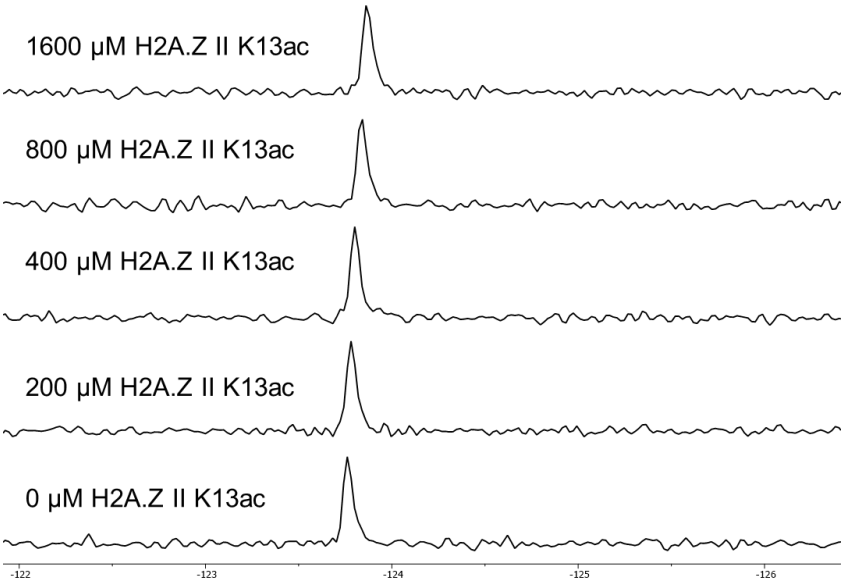
PrOF NMR titration with 5FW-BPTF and H2A.Z II K11ac peptide.

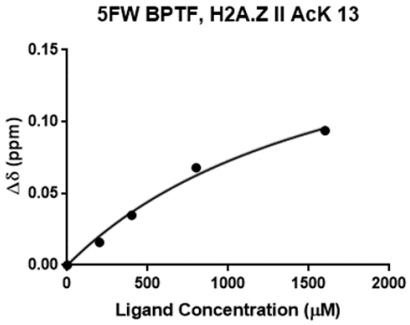




[Ligand]	5FW 2950 (ppm)	Δδ
0 μM	-123.7635	
200 μM	-123.8136	-0.0501
400 μM	-123.8591	-0.0956
800 μM	-123.9223	-0.1588
1600 μM	-123.996	-0.2325

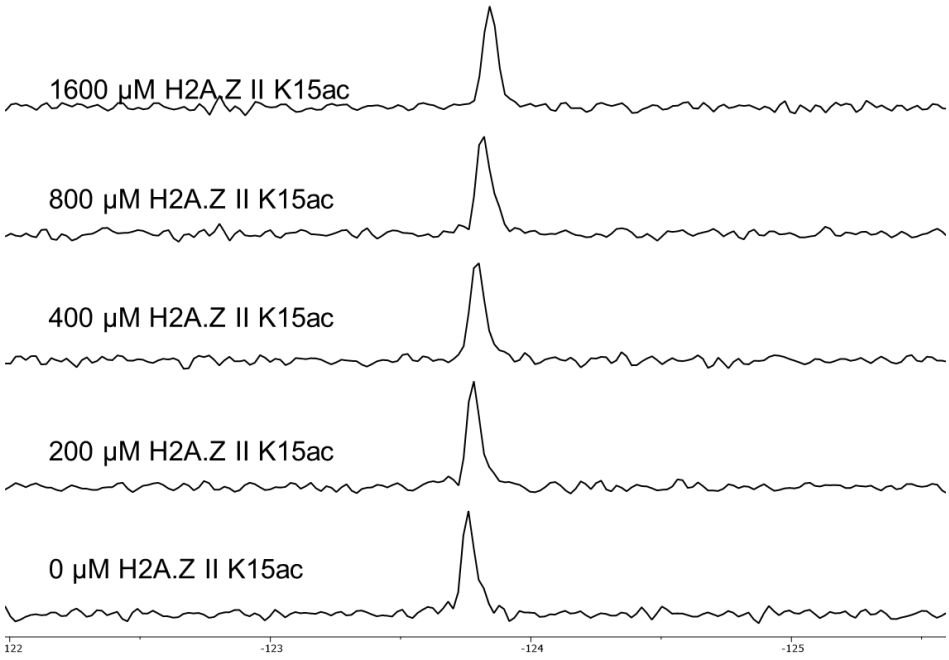
PrOF NMR titration with 5FW-BPTF and H2A.Z II K13ac peptide.

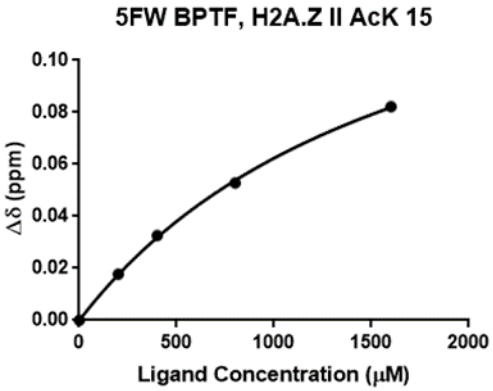




[Ligand]	5FW 2950 (ppm)	Δδ
0 μM	-123.7621	
200 μM	-123.7818	-0.0197
400 μM	-123.8017	-0.0396
800 μM	-123.8377	-0.0756
1600 μM	-123.8658	-0.1037

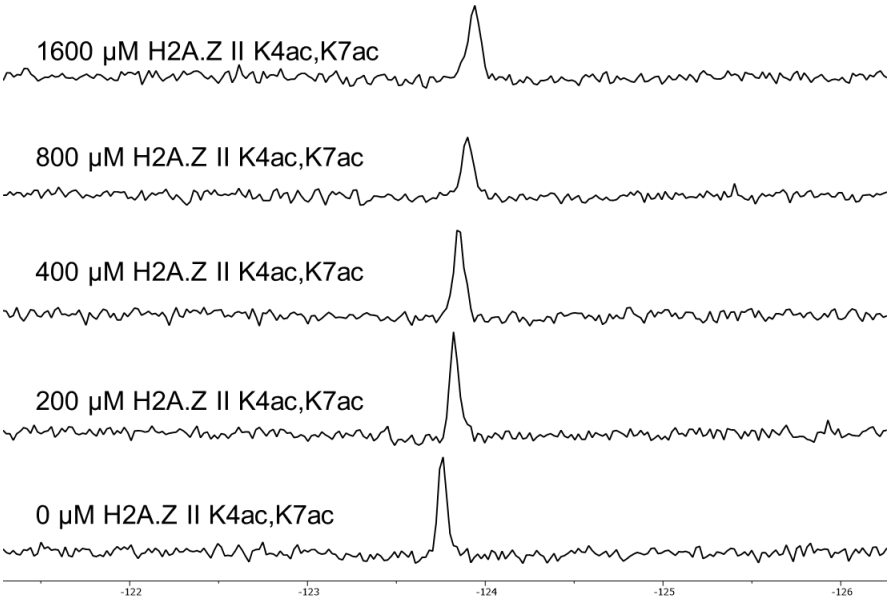
PrOF NMR titration with 5FW-BPTF and H2A.Z II K15ac peptide.

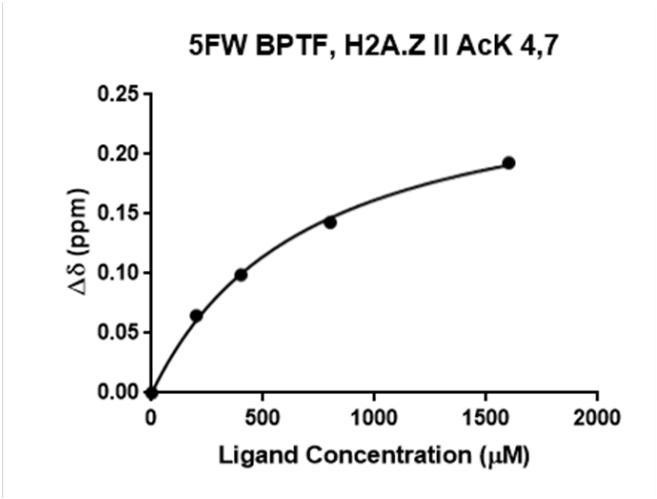




[Ligand]	5FW 2950 (ppm)	Δδ
0 µM	-123.7594	
200 µM	-123.7785	-0.0191
400 µM	-123.7932	-0.0338
800 µM	-123.8152	-0.0558
1600 µM	-123.843	-0.0836

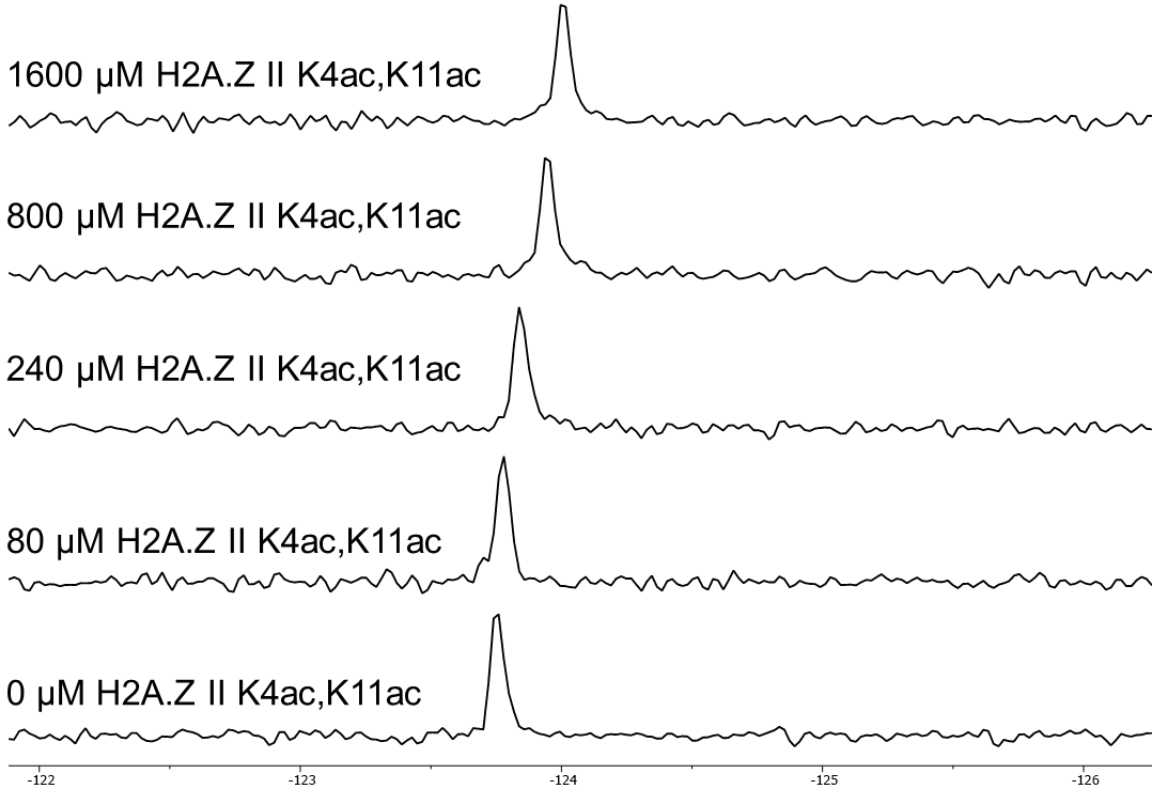
PrOF NMR titration with 5FW-BPTF and H2A.Z II K4ac,K7ac peptide.

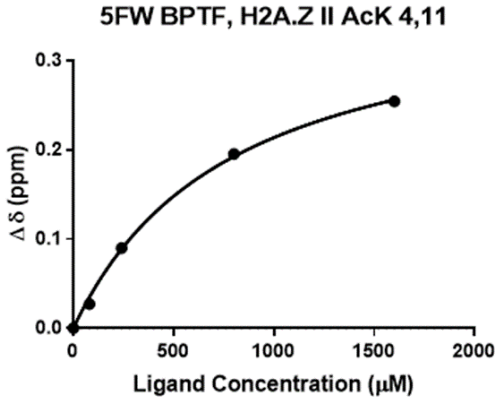




[Ligand]	5FW 2950 (ppm)	Δδ
0 μM	-123.7577	
200 μM	-123.8234	-0.0657
400 μM	-123.8497	-0.092
800 μM	-123.8985	-0.1408
1600 μM	-123.9389	-0.1812

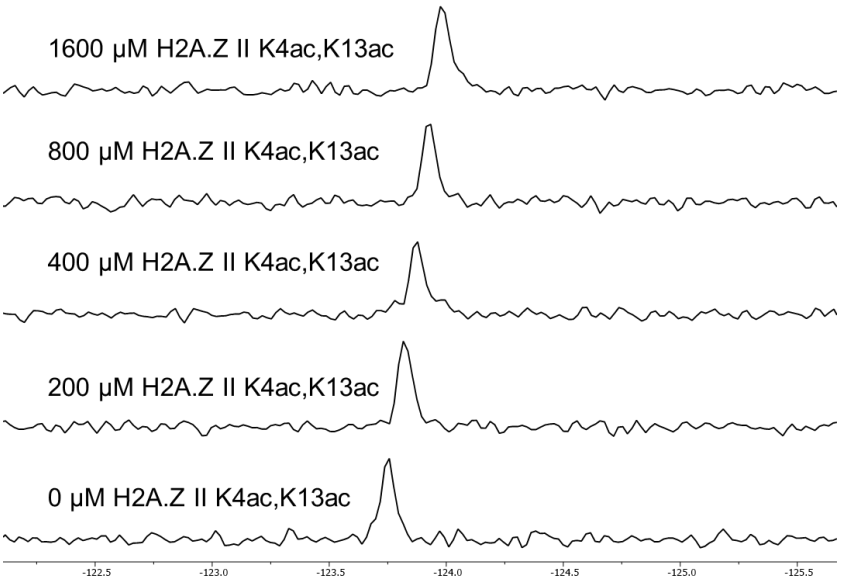
PrOF NMR titration with 5FW-BPTF and H2A.Z II K4ac,K11ac peptide.

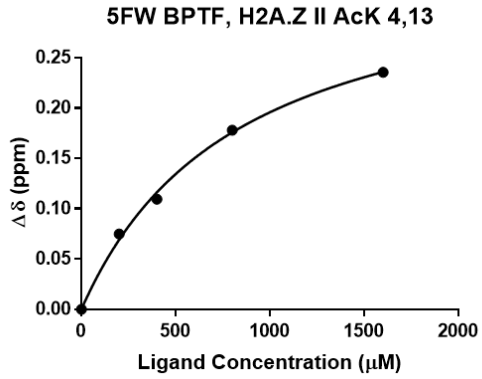




[Ligand]	5FW 2950 (ppm)	Δδ
0 µM	-123.7515	
80 µM	-123.7769	-0.0254
240 µM	-123.8401	-0.0886
800 µM	-123.9451	-0.1936
1600 µM	-124.0058	-0.2543

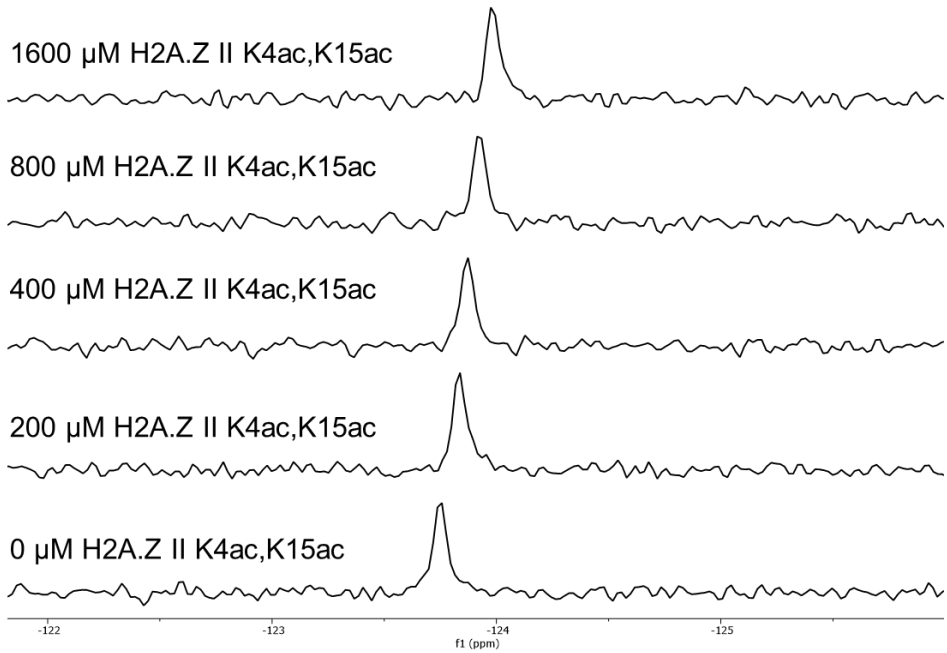
PrOF NMR titration with 5FW-BPTF and H2A.Z II K4ac,K13ac peptide.

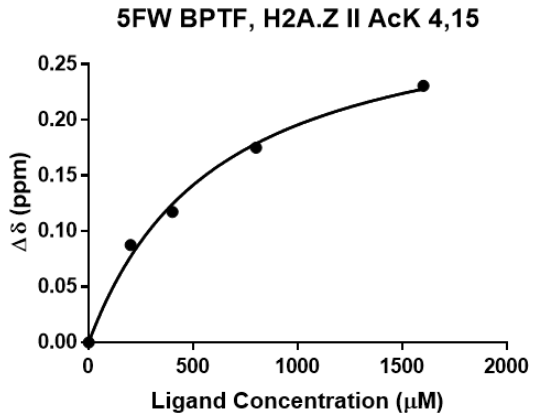




[Ligand]	5FW 2950 (ppm)	Δδ
0 μM	-123.7515	
200 μM	-123.8204	-0.0689
400 μM	-123.8724	-0.1209
800 μM	-123.9254	-0.1739
1600 μM	-123.9803	-0.2288

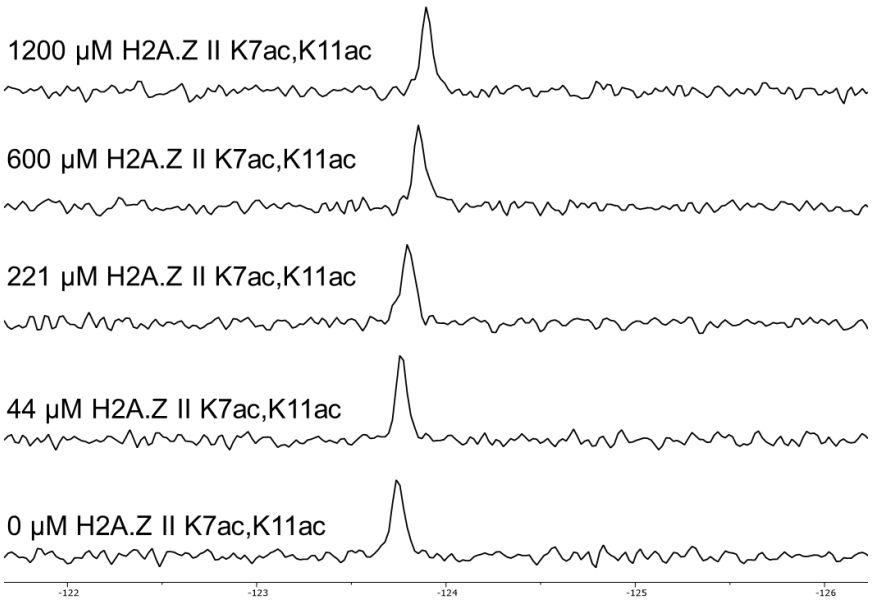
PrOF NMR titration with 5FW-BPTF and H2A.Z II K4ac,K15ac peptide.

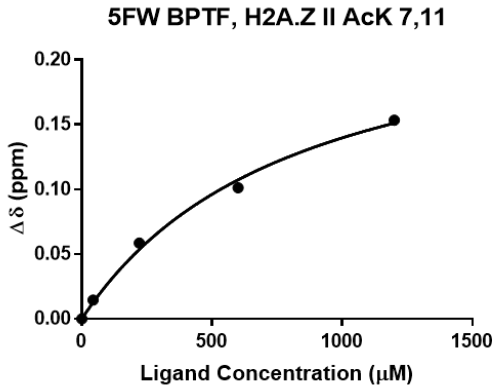




[Ligand]	5FW 2950 (ppm)	Δδ
0 μM	-123.7474	
200 μM	-123.8335	-0.0861
400 μM	-123.8707	-0.1233
800 μM	-123.9226	-0.1752
1600 μM	-123.9807	-0.2333

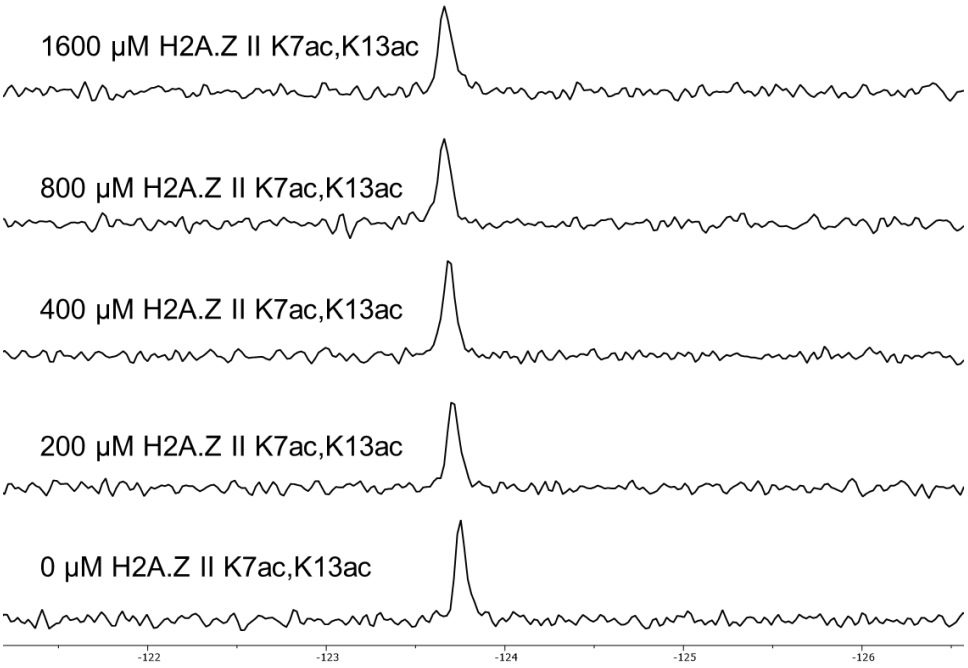
PrOF NMR titration with 5FW-BPTF and H2A.Z II K7ac,K11ac peptide.

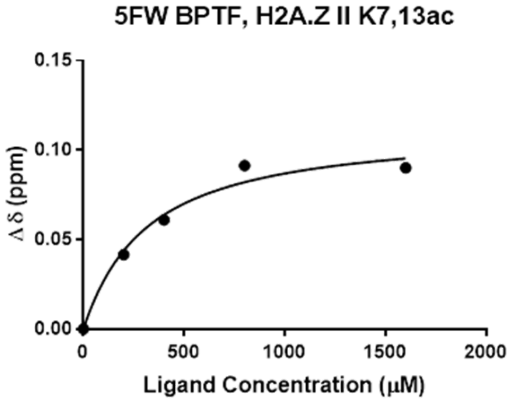




[Ligand]	5FW 2950 (ppm)	Δδ
0 µM	-123.743	
44 µM	-123.7629	-0.0199
221 µM	-123.7979	-0.0549
600 µM	-123.8546	-0.1116
1200 µM	-123.8964	-0.1534

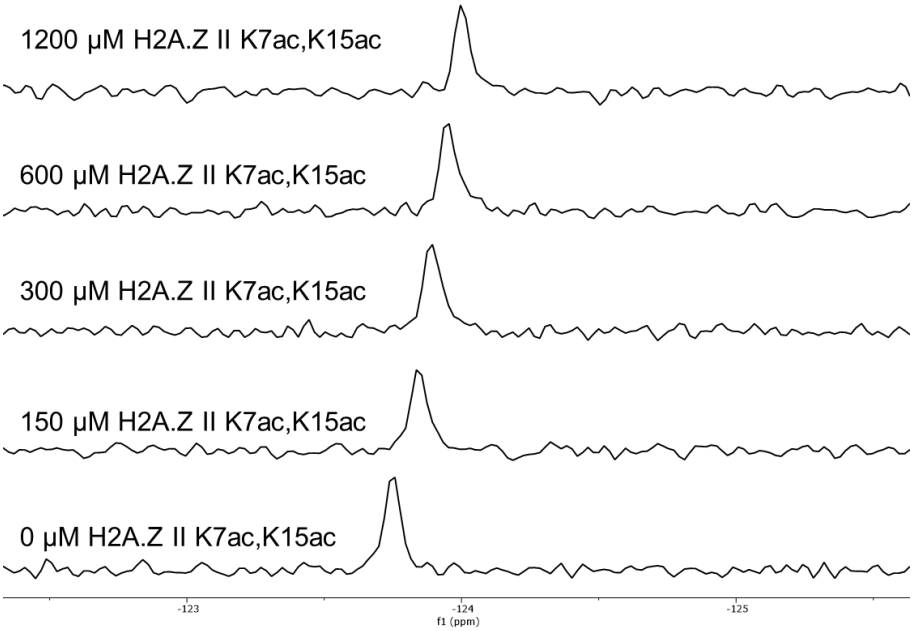
PrOF NMR titration with 5FW-BPTF and H2A.Z II K7ac,K13ac peptide.

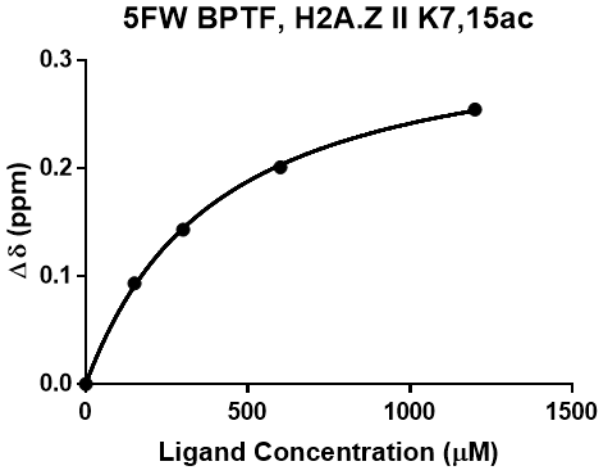




[Ligand]	5FW 2950 (ppm)	Δδ
0 uM	-123.7488	
200 uM	-123.7072	0.0416
400 uM	-123.6878	0.061
800 uM	-123.6575	0.0913
1600 uM	-123.6587	0.0901

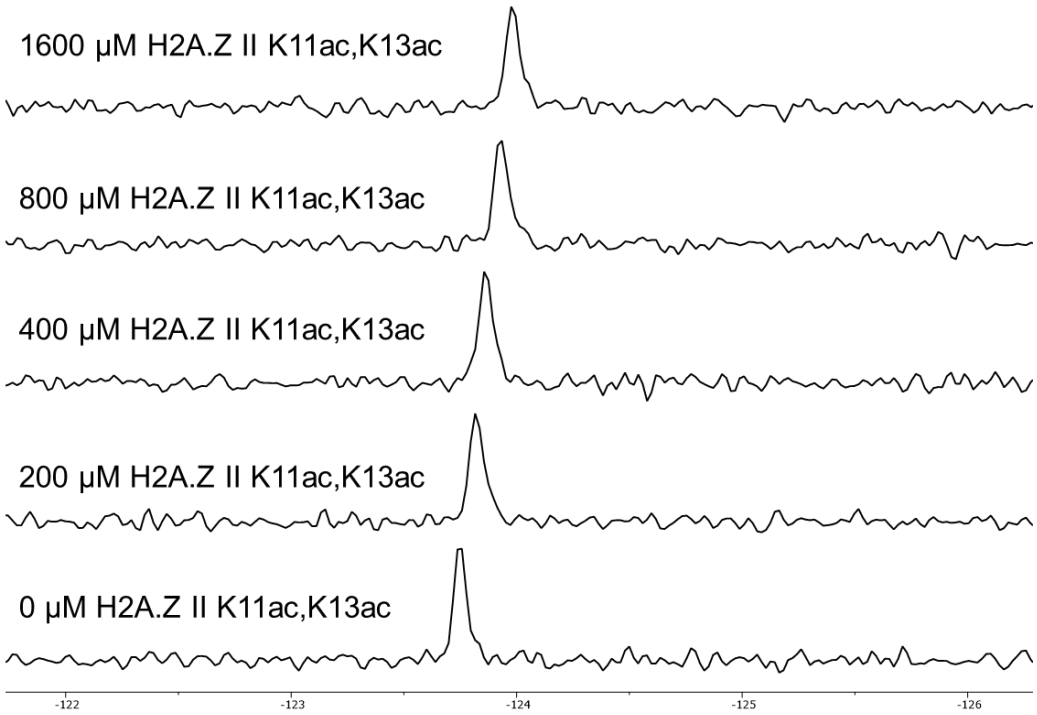
PrOF NMR titration with 5FW-BPTF and H2A.Z II K7ac,K15ac peptide.

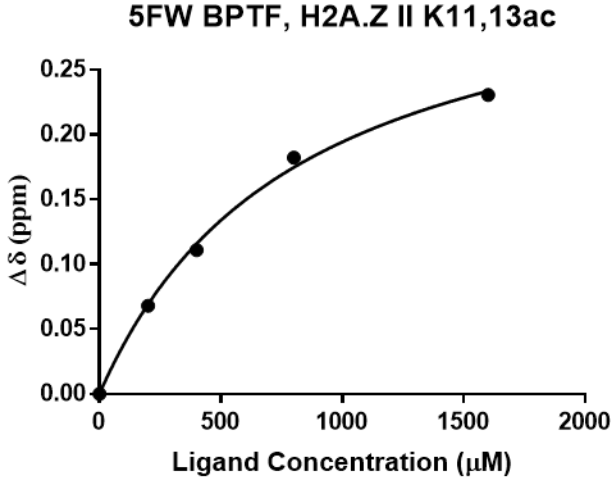




[Ligand]	5FW 2950 (ppm)	Δδ
0 μM	-123.7497	
150 μM	-123.8424	-0.0927
300 μM	-123.8893	-0.1396
600 μM	-123.9475	-0.1978
1200 μM	-123.9994	-0.2497

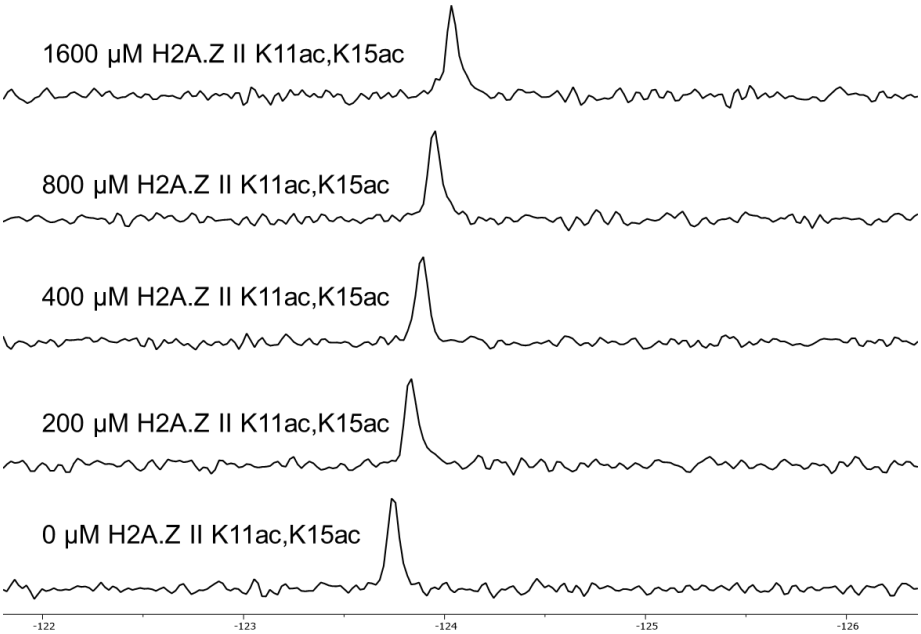
PrOF NMR titration with 5FW-BPTF and H2A.Z II K11ac,K13ac peptide.

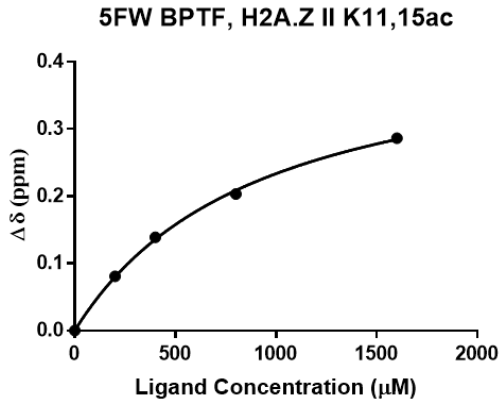




[Ligand]	5FW 2950 (ppm)	Δδ
0 μM	-123.7475	
200 μM	-123.8188	-0.0713
400 μM	-123.8614	-0.1139
800 μM	-123.9279	-0.1804
1600 μM	-123.9809	-0.2334

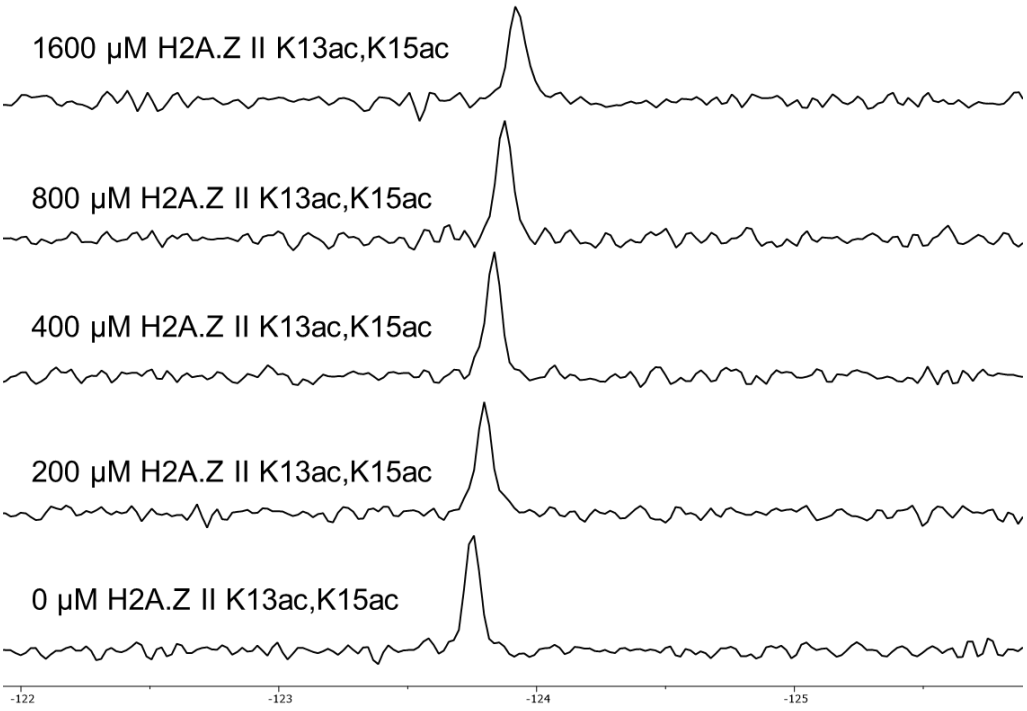
PrOF NMR titration with 5FW-BPTF and H2A.Z II K11ac,K15ac peptide.

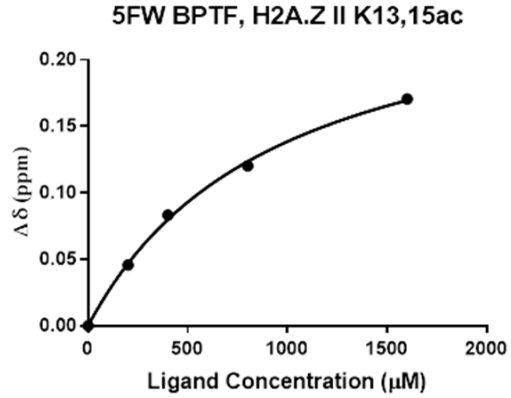




[Ligand]	5FW 2950 (ppm)	Δδ
0 μM	-123.7439	
200 μM	-123.8302	-0.0863
400 μM	-123.8887	-0.1448
800 μM	-123.9482	-0.2043
1600 μM	-124.0354	-0.2915

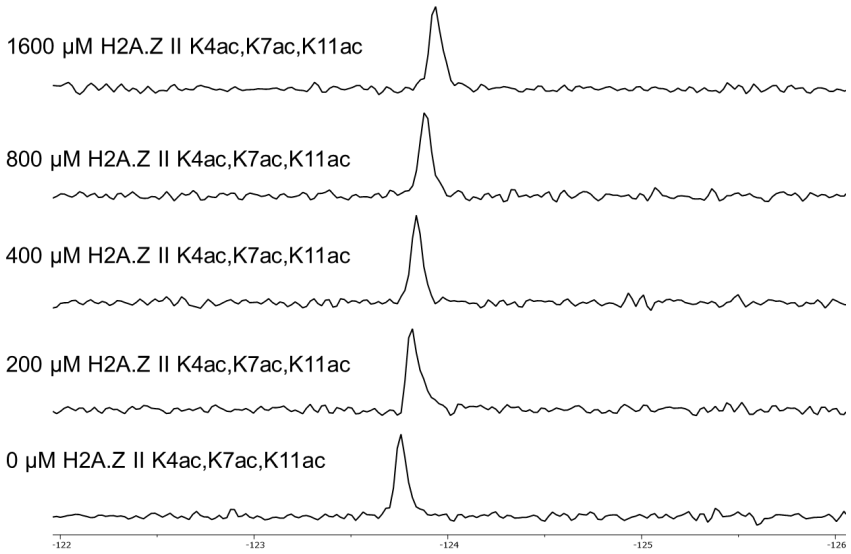
PrOF NMR titration with 5FW-BPTF and H2A.Z II K13ac,K15ac peptide.

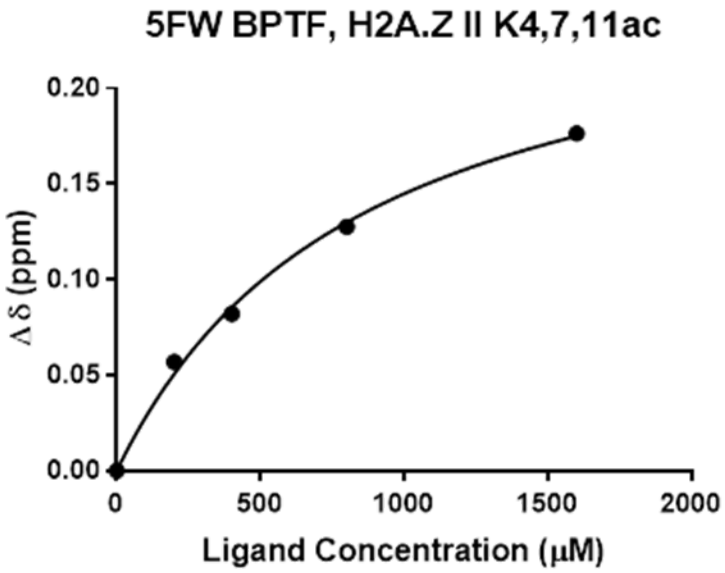




[Ligand]	5FW 2950 (ppm)	Δδ
0 μM	-123.751	
200 μM	-123.7971	-0.0461
400 μM	-123.8344	-0.0834
800 μM	-123.8746	-0.1236
1600 μM	-123.9208	-0.1698

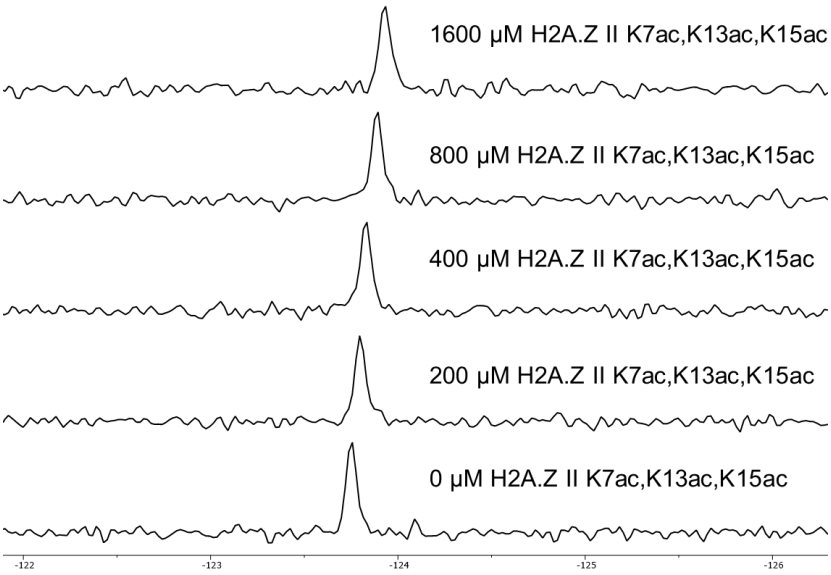
PrOF NMR titration with 5FW-BPTF and H2A.Z II K4ac,K7ac,K11ac peptide.

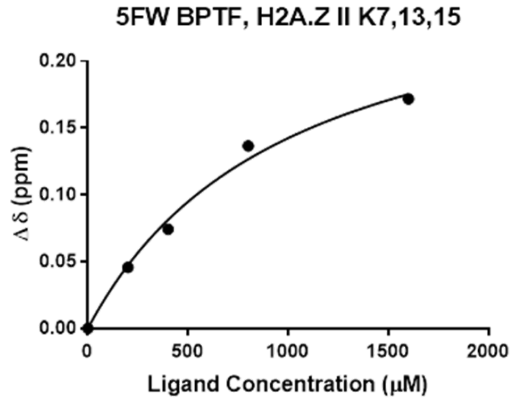




[Ligand]	5FW 2950 (ppm)	Δδ
0 uM	-123.7561	
200 uM	-123.813	-0.0569
400 uM	-123.8382	-0.0821
800 uM	-123.8837	-0.1276
1600 uM	-123.9326	-0.1765

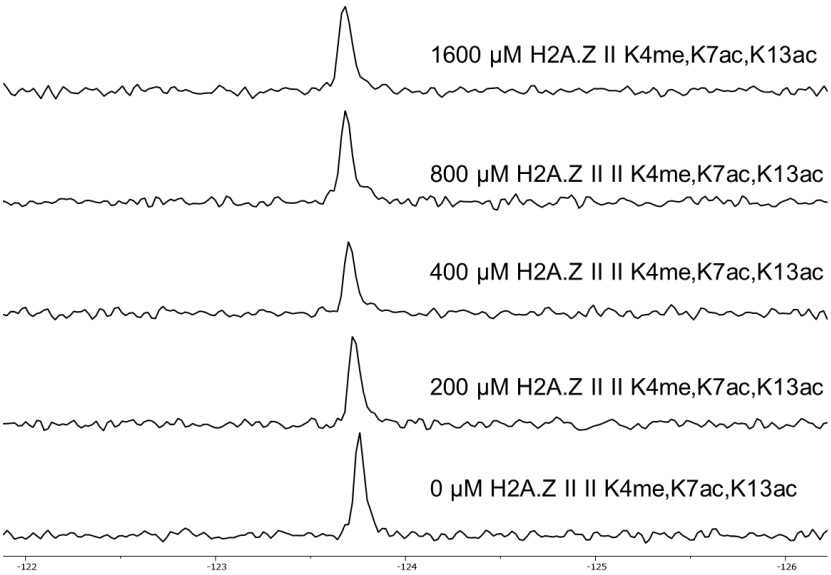
PrOF NMR titration with 5FW-BPTF and H2A.Z II K7ac,K13ac,K15ac peptide.

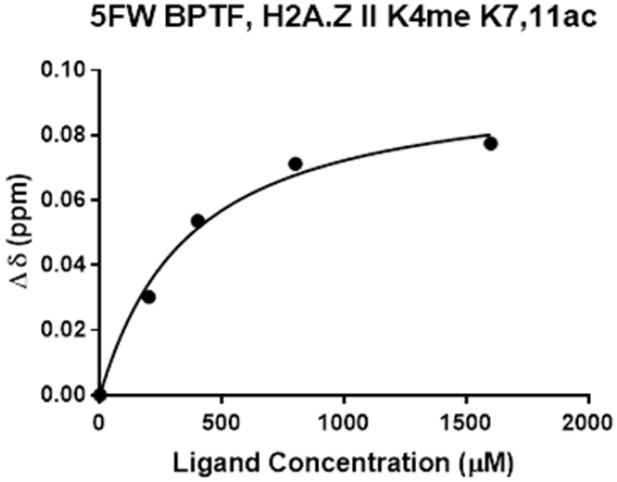




[Ligand]	5FW 2950 (ppm)	Δδ
0 μM	-123.7516	
200 μM	-123.7976	-0.046
400 μM	-123.8286	-0.077
800 μM	-123.8885	-0.1369
1600 μM	-123.929	-0.1774

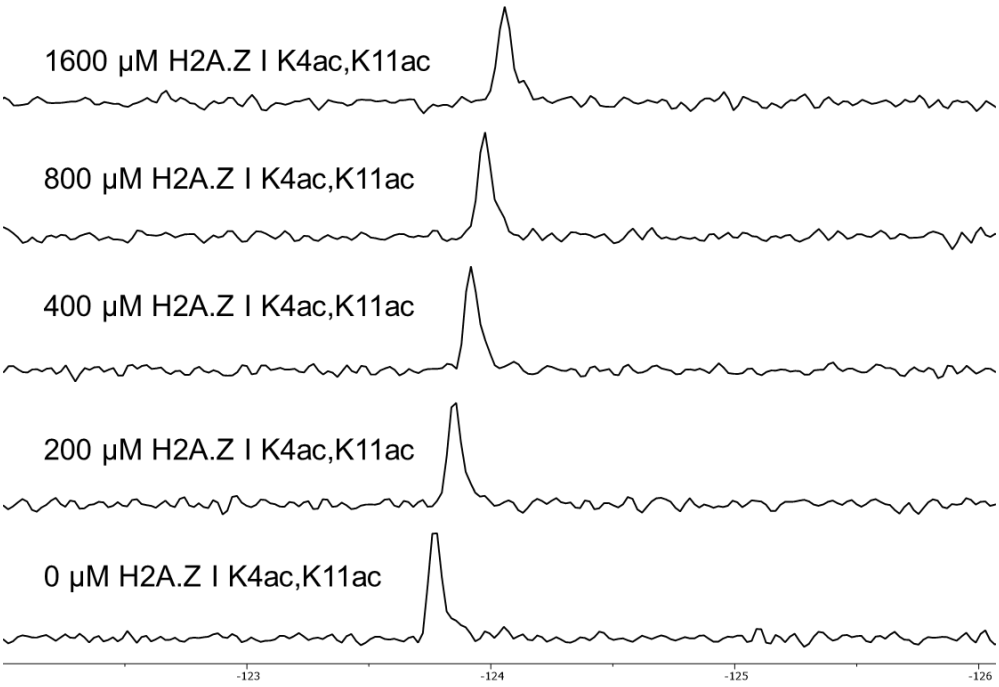
PrOF NMR titration with 5FW-BPTF and H2A.Z II K4me,K7ac,K13ac peptide.

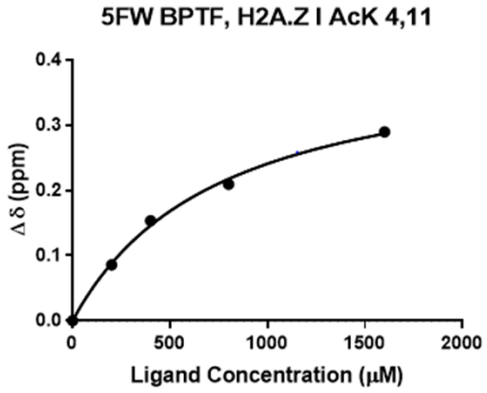




[Ligand]	5FW 2950 (ppm)	Δδ
0 uM	-123.7566	
200 uM	-123.7264	0.0302
400 uM	-123.703	0.0536
800 uM	-123.6854	0.0712
1600 uM	-123.6791	0.0775

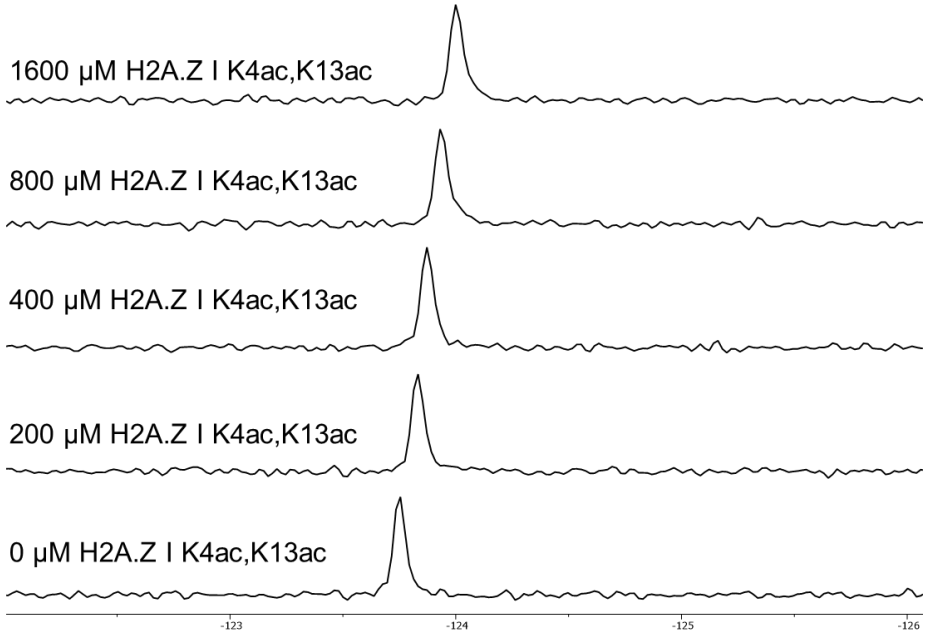
PrOF NMR titration with 5FW-BPTF and H2A.Z I K4ac,K11ac peptide.

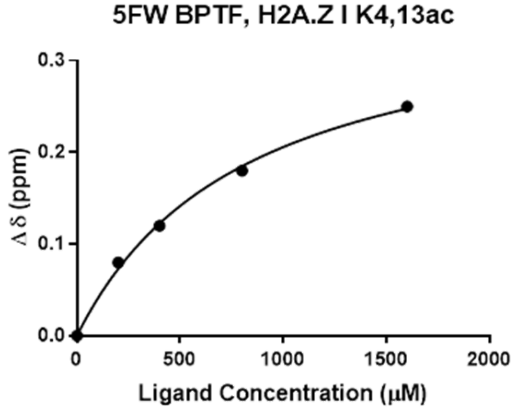




[Ligand]	5FW 2950 (ppm)	Δδ
0 μM	-123.763	
200 μM	-123.848	-0.085
400 μM	-123.916	-0.153
800 μM	-123.972	-0.209
1600 μM	-124.052	-0.289

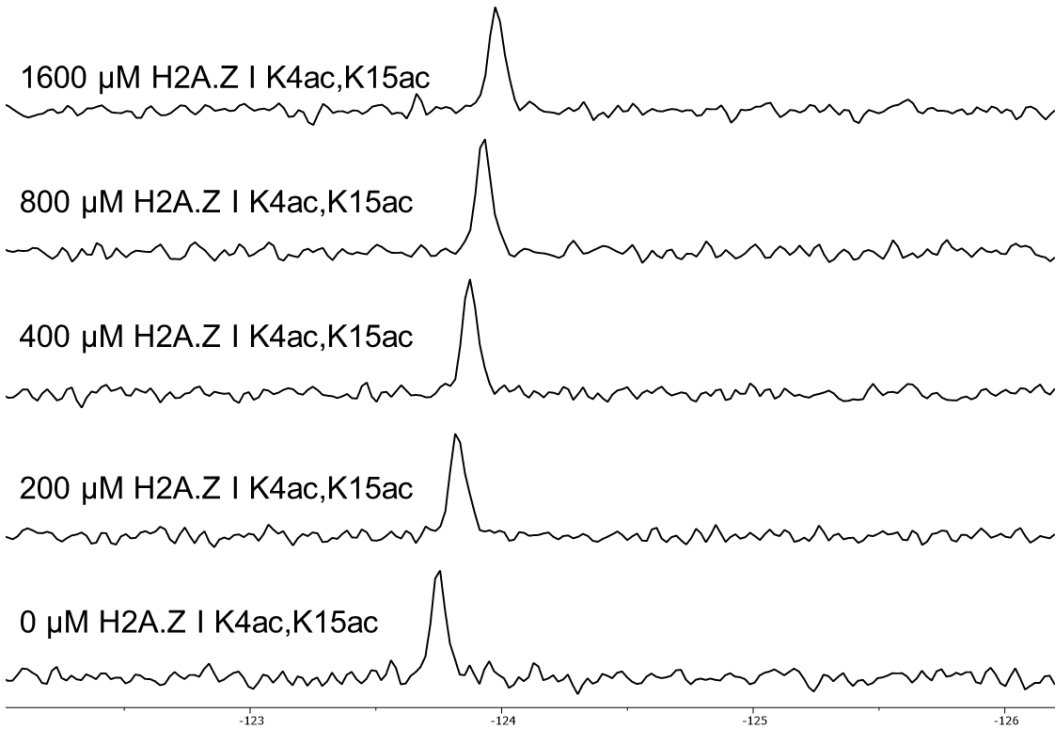
PrOF NMR titration with 5FW-BPTF and H2A.Z I K4ac,K13ac peptide.

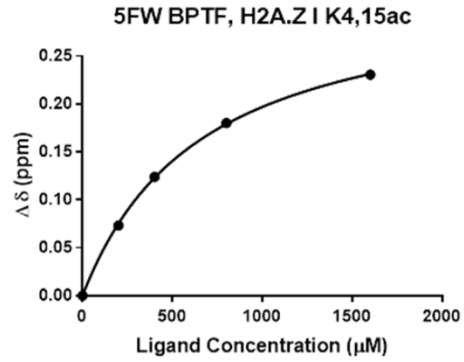




[Ligand]	5FW 2950 (ppm)	Δδ
0 uM	-123.75	
200 uM	-123.83	-0.08
400 uM	-123.87	-0.12
800 uM	-123.93	-0.18
1600 uM	-124	-0.25

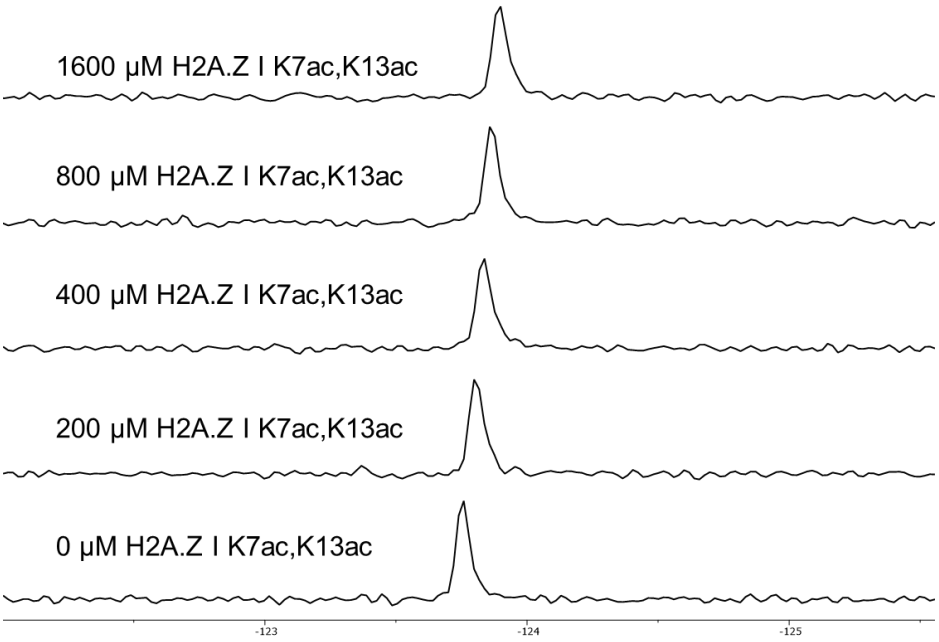
PrOF NMR titration with 5FW-BPTF and H2A.Z I K4ac,K15ac peptide.

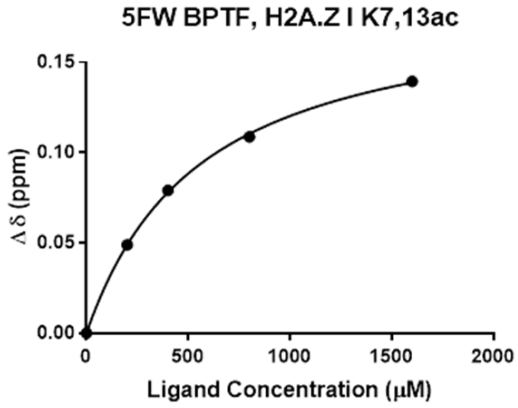




[Ligand]	5FW 2950 (ppm)	Δδ
0 µM	-123.7488	
200 µM	-123.8212	-0.0724
400 µM	-123.8725	-0.1237
800 µM	-123.9277	-0.1789
1600 µM	-123.9772	-0.2284

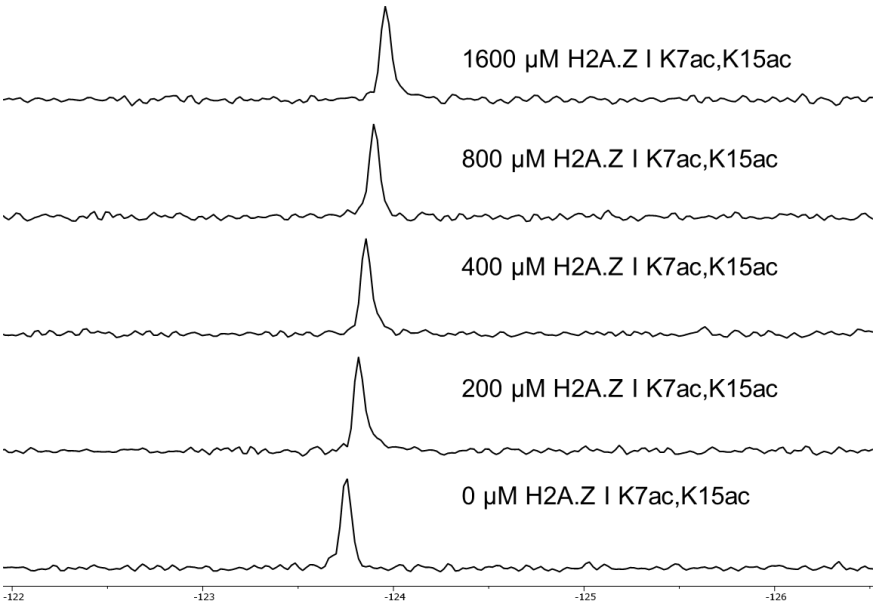
PrOF NMR titration with 5FW-BPTF and H2A.Z I K7ac,K13ac peptide.

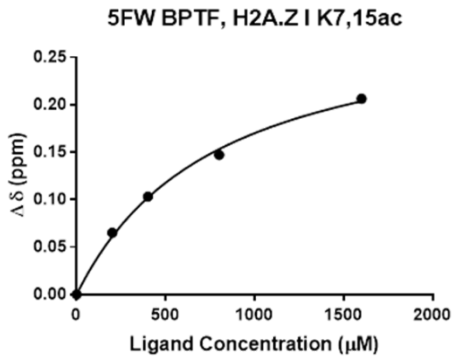




[Ligand]	5FW 2950 (ppm)	Δδ
0 uM	-123.7546	
200 uM	-123.8035	-0.0489
400 uM	-123.8337	-0.0791
800 uM	-123.8633	-0.1087
1600 uM	-123.894	-0.1394

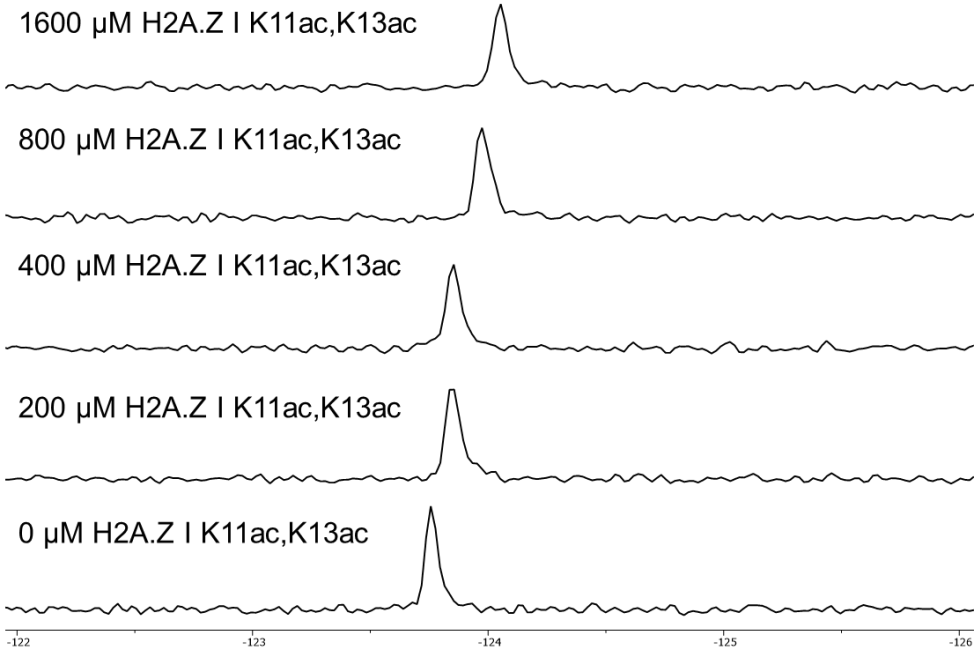
PrOF NMR titration with 5FW-BPTF and H2A.Z I K7ac,K15ac peptide.

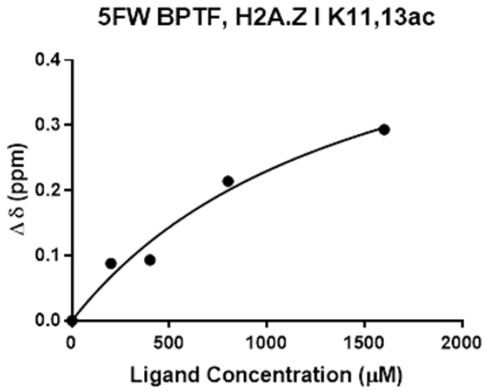




[Ligand]	5FW 2950 (ppm)	Δδ
0 uM	-123.7521	
200 uM	-123.8173	-0.0652
400 uM	-123.8553	-0.1032
800 uM	-123.8992	-0.1471
1600 uM	-123.9584	-0.2063

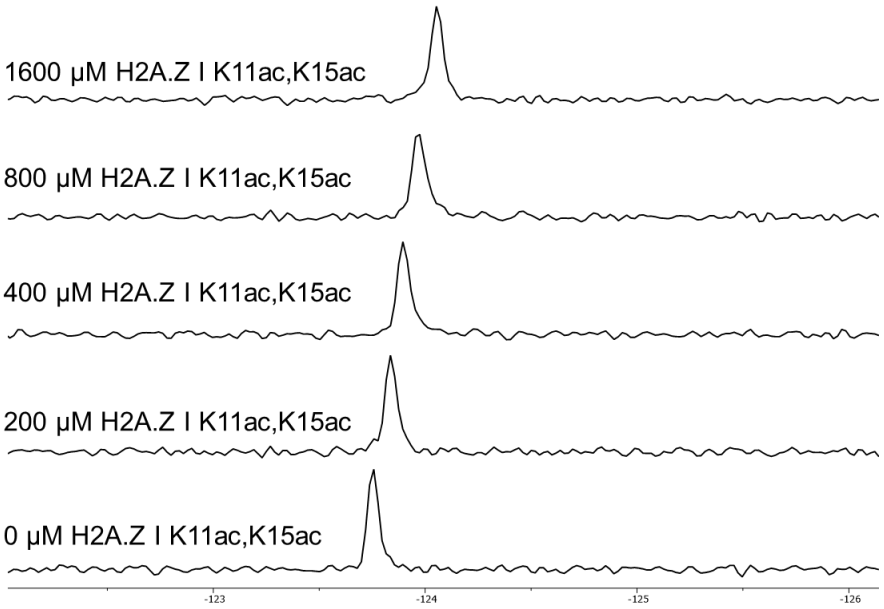
PrOF NMR titration with 5FW-BPTF and H2A.Z I K11ac,K13ac peptide.

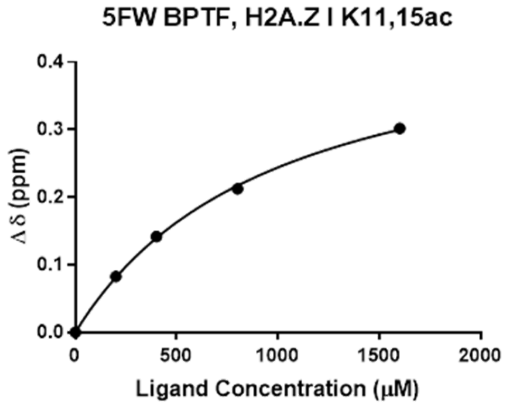




[Ligand]	5FW 2950 (ppm)	Δδ
0 uM	-123.7581	
200 uM	-123.846	-0.0879
400 uM	-123.8513	-0.0932
800 uM	-123.9722	-0.2141
1600 uM	-124.0517	-0.2936

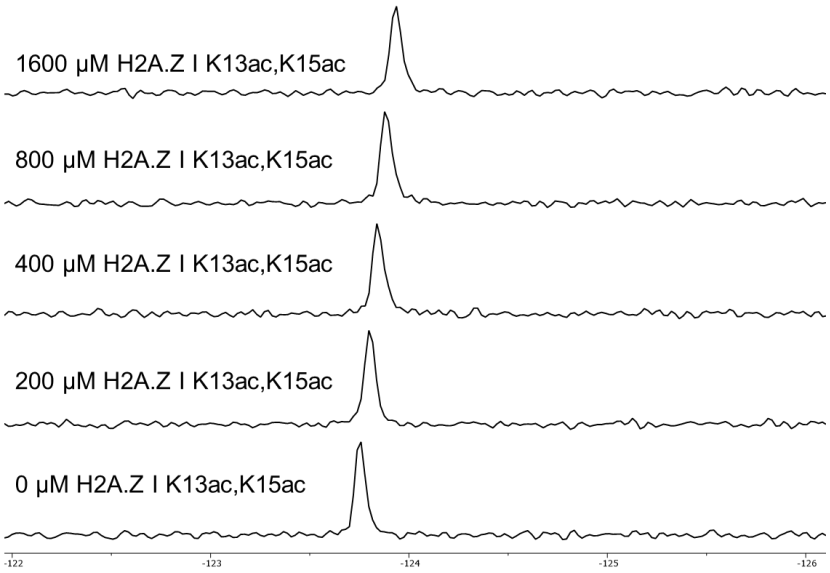
PrOF NMR titration with 5FW-BPTF and H2A.Z I K11ac,K15ac peptide.

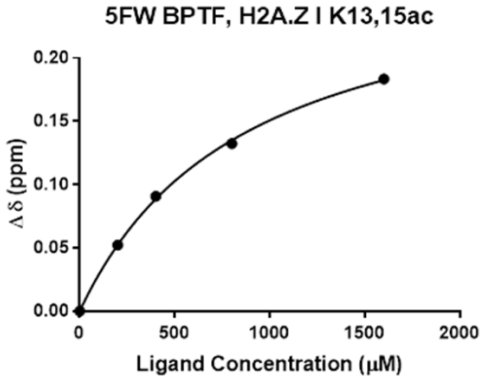




[Ligand]	5FW 2950 (ppm)	Δδ
0 uM	-123.754	
200 uM	-123.8369	-0.0829
400 uM	-123.896	-0.142
800 uM	-123.9665	-0.2125
1600 uM	-124.0557	-0.3017

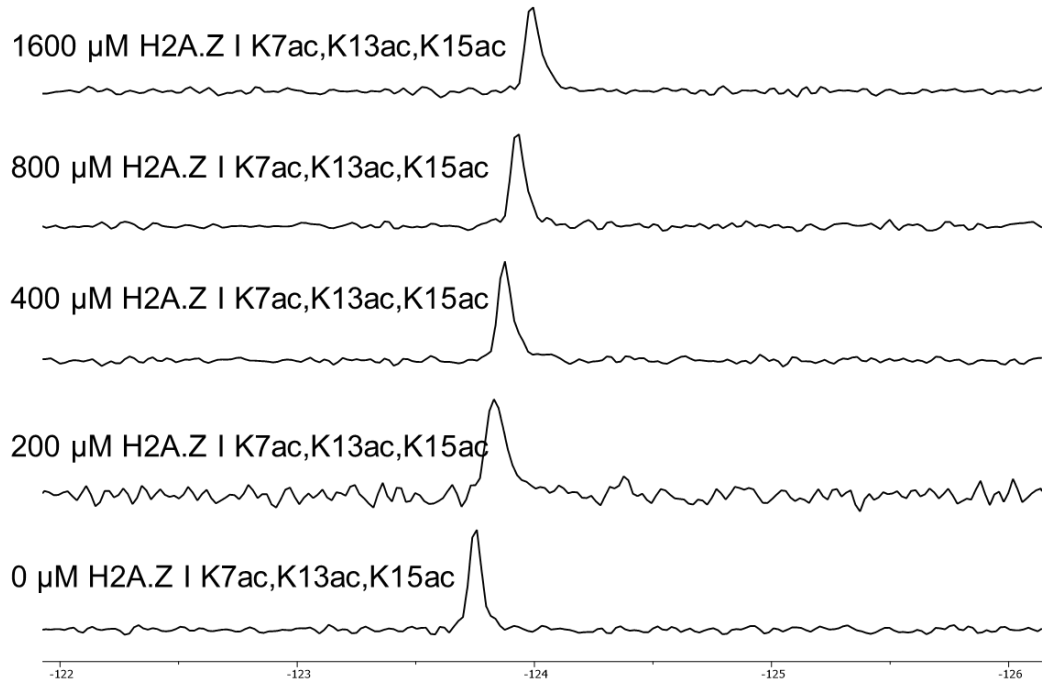
PrOF NMR titration with 5FW-BPTF and H2A.Z I K13ac,K15ac peptide.

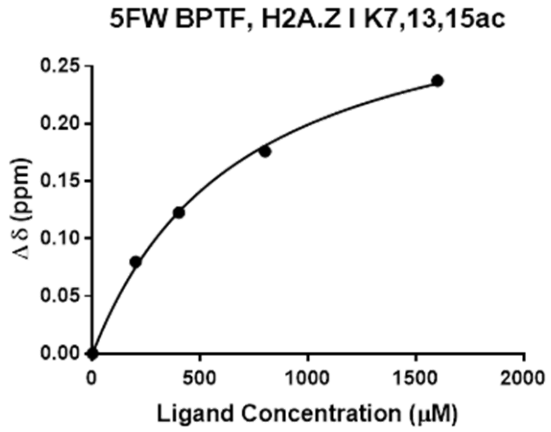




[Ligand]	5FW 2950 (ppm)	Δδ
0 uM	-123.75	
200 uM	-123.8022	-0.0522
400 uM	-123.8407	-0.0907
800 uM	-123.8824	-0.1324
1600 uM	-123.9334	-0.1834

PrOF NMR titration with 5FW-BPTF and H2A.Z I K7ac,K13ac,K15ac peptide.

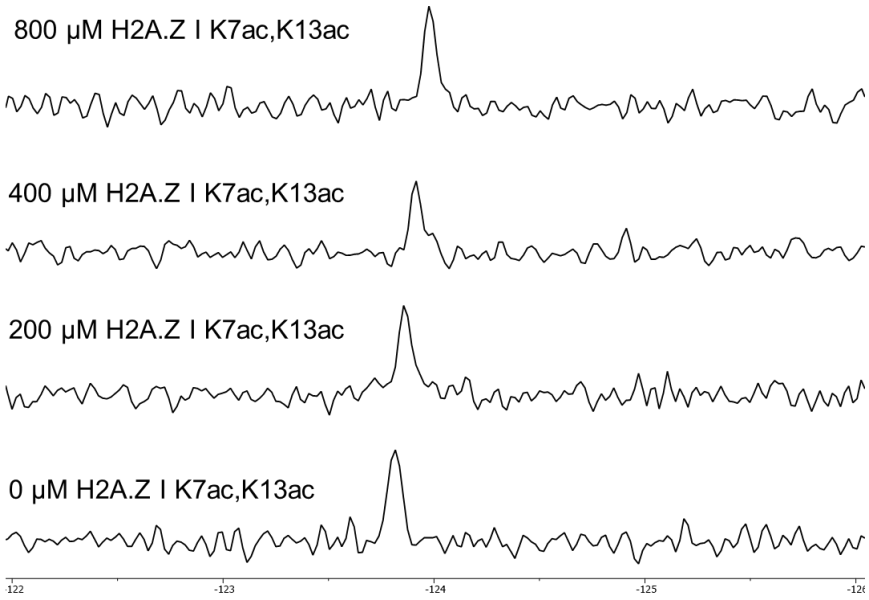


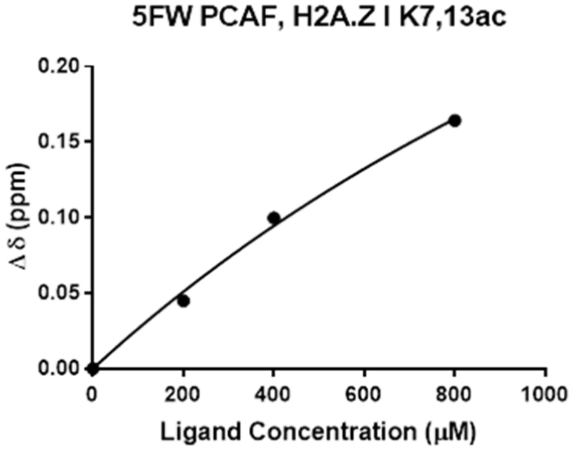


[Ligand]	5FW 2950 (ppm)	Δδ
0 uM	-123.7509	
200 uM	-123.8308	-0.0799
400 uM	-123.8734	-0.1225
800 uM	-123.9269	-0.176
1600 uM	-123.9882	-0.2373

Bromodomain Scope PrOF NMR Titrations.

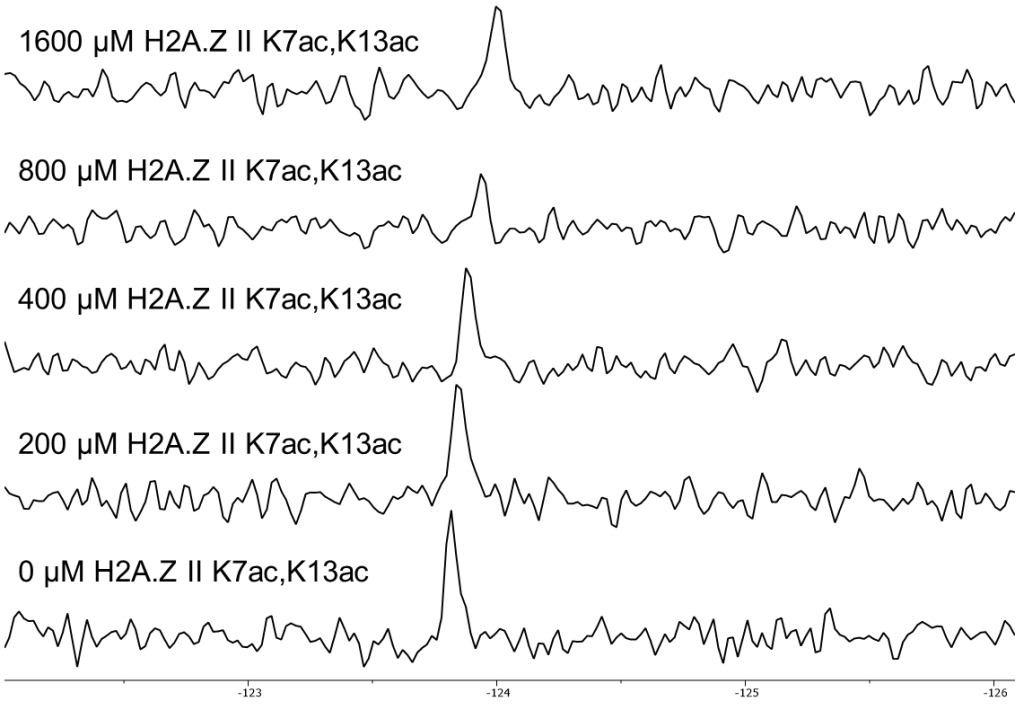
PrOF NMR titration with 5FW PCAF and H2A.Z I K7ac,K13ac peptide.

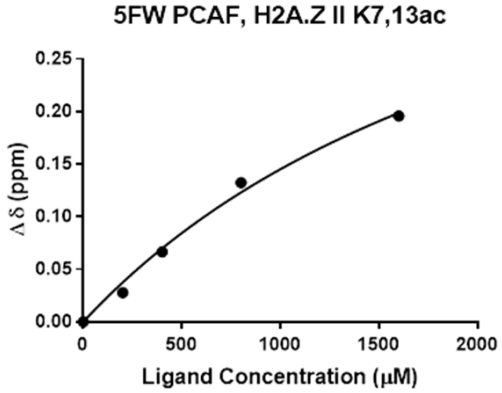




[Ligand]	5FW 746 (ppm)	Δδ
0 μM	-123.8146	
200 μM	-123.8598	-0.0452
400 μM	-123.9129	-0.0983
800 μM	-123.9782	-0.1636

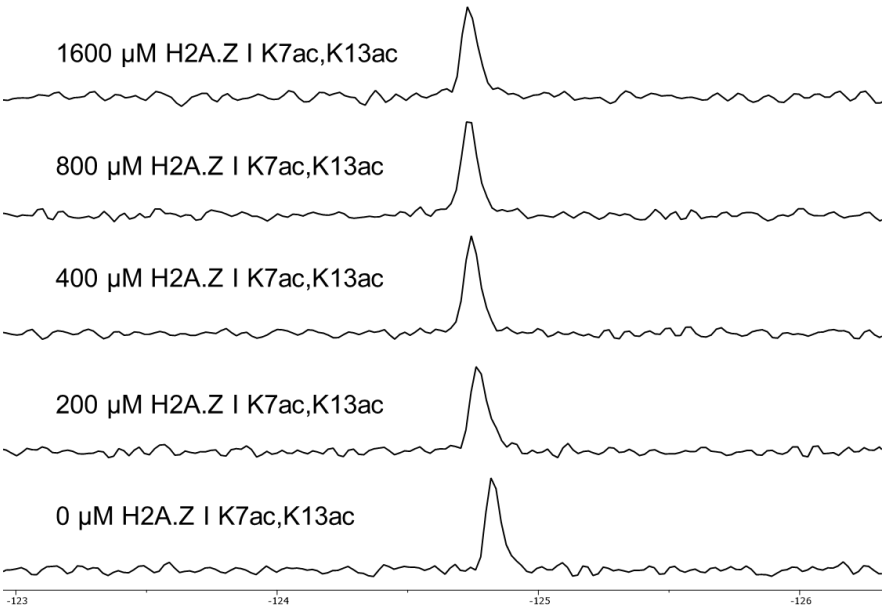
PrOF NMR titration with 5FW PCAF and H2A.Z II K7ac,K13ac peptide.

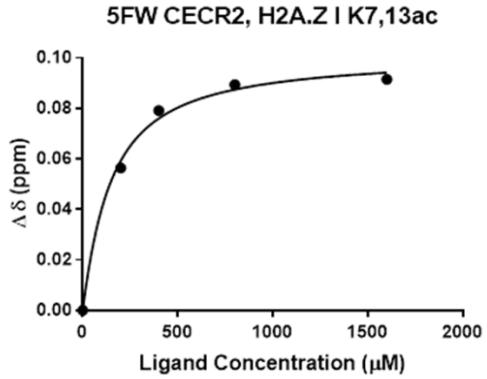




[Ligand]	5FW 746 (ppm)	Δδ
0 μM	-123.8154	
200 μM	-123.8449	-0.0295
400 μM	-123.8821	-0.0667
800 μM	-123.9398	-0.1244
1600 μM	-124.0036	-0.1882

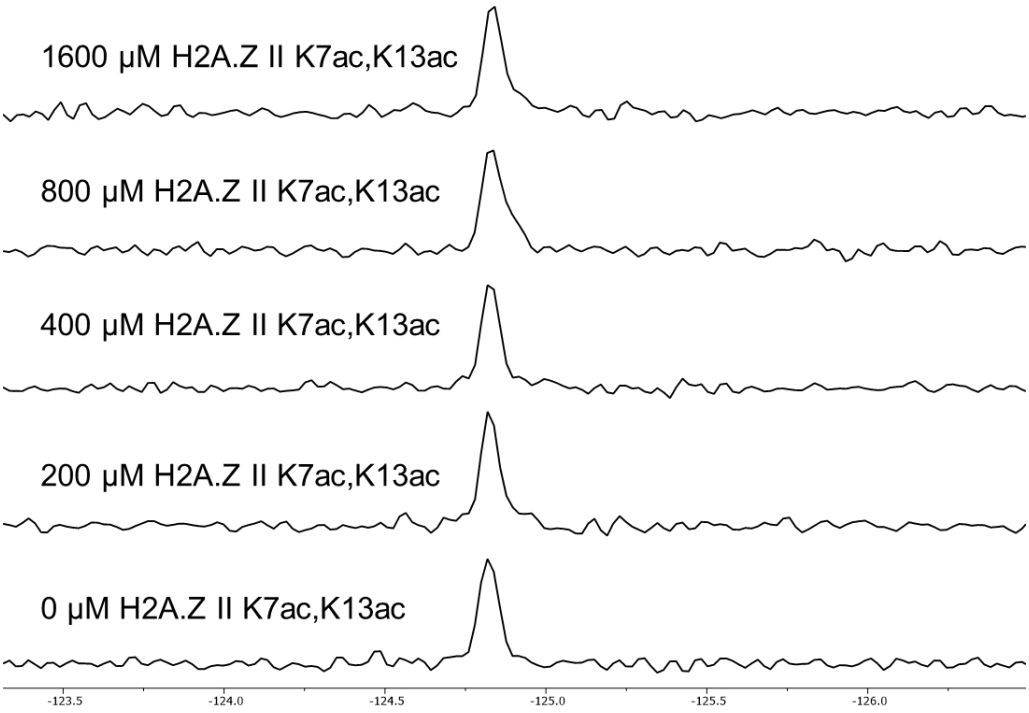
PrOF NMR titration with 5FW CECR2 and H2A.Z I K7ac,K13ac peptide.

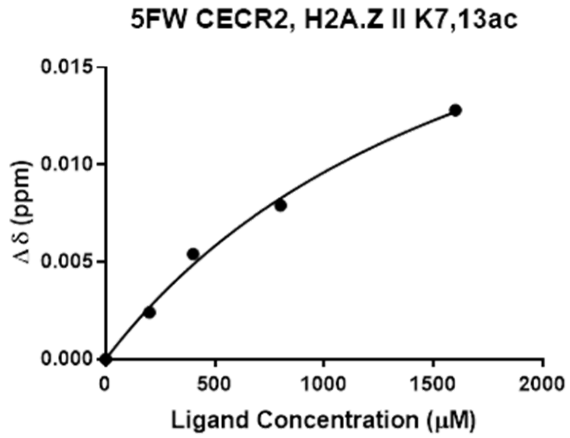




[Ligand]	5FW 457 (ppm)	Δδ
0 uM	-124.8233	
200 uM	-124.7669	0.0564
400 uM	-124.7442	0.0791
800 uM	-124.7339	0.0894
1600 uM	-124.7318	0.0915

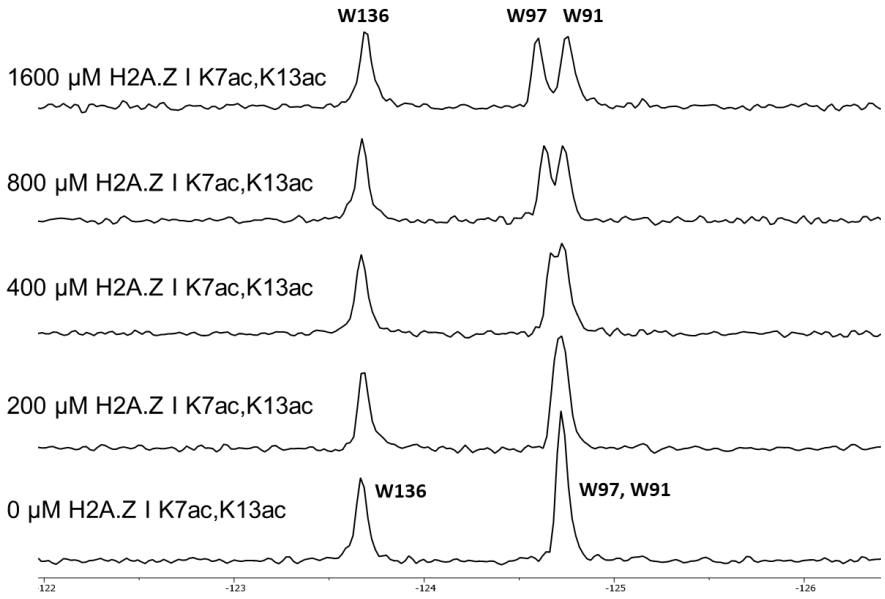
PrOF NMR titration with 5FW CECR2 and H2A.Z II K7ac,K13ac peptide.

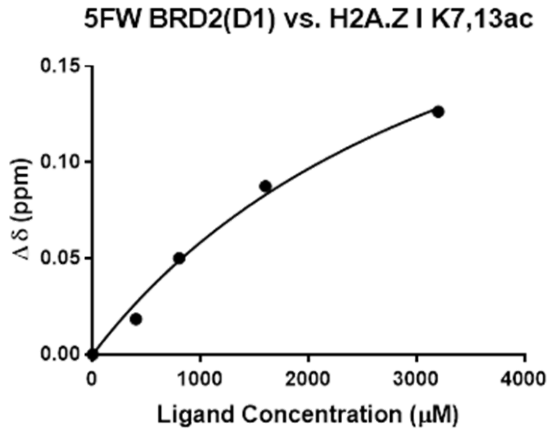




[Ligand]	5FW 457 (ppm)	Δδ
0 uM	-124.8211	
200 uM	-124.8235	-0.0024
400 uM	-124.8265	-0.0054
800 uM	-124.829	-0.0079
1600 uM	-124.8339	-0.0128

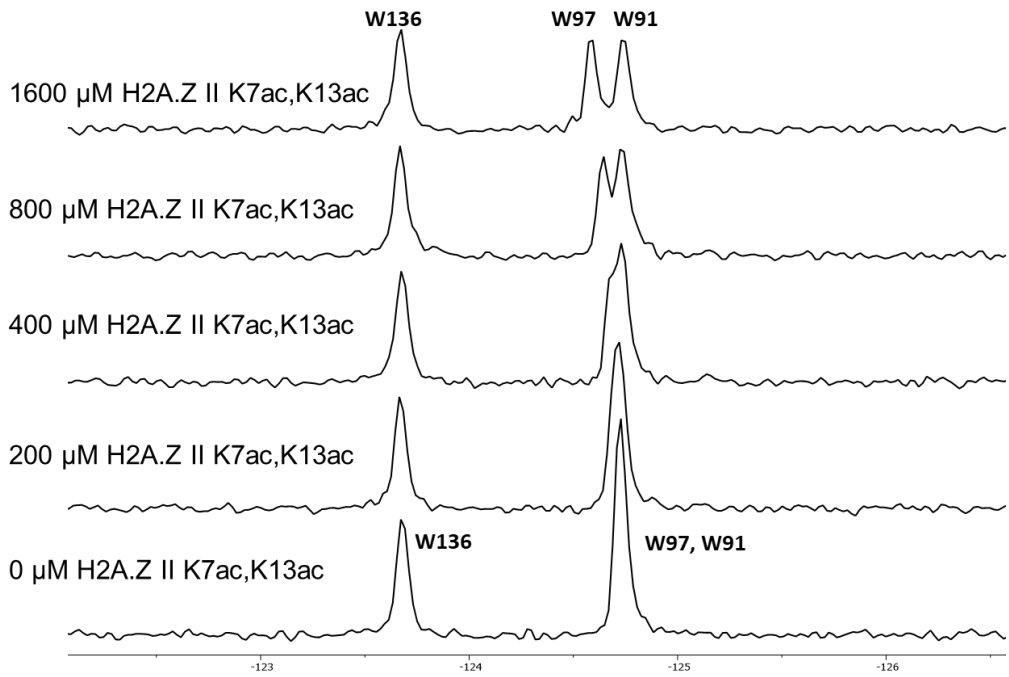
PrOF NMR titration with 5FW BRD2(1) and H2A.Z I K7ac,K13ac peptide.

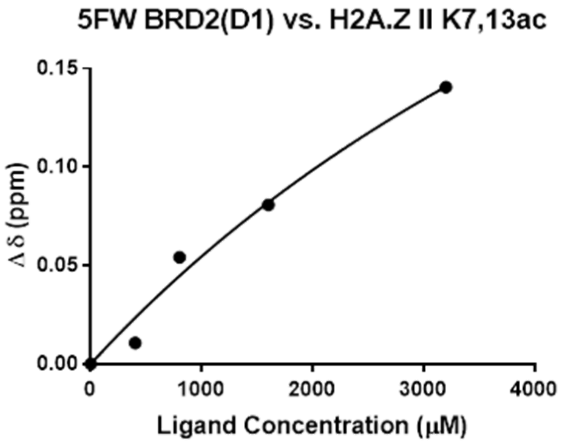




[Ligand]	5FW 97 (ppm)	Δδ
0 uM	-124.7226	
400 uM	-124.7041	0.0185
800 uM	-124.6725	0.0501
1600 uM	-124.635	0.0876
3200 uM	-124.5961	0.1265

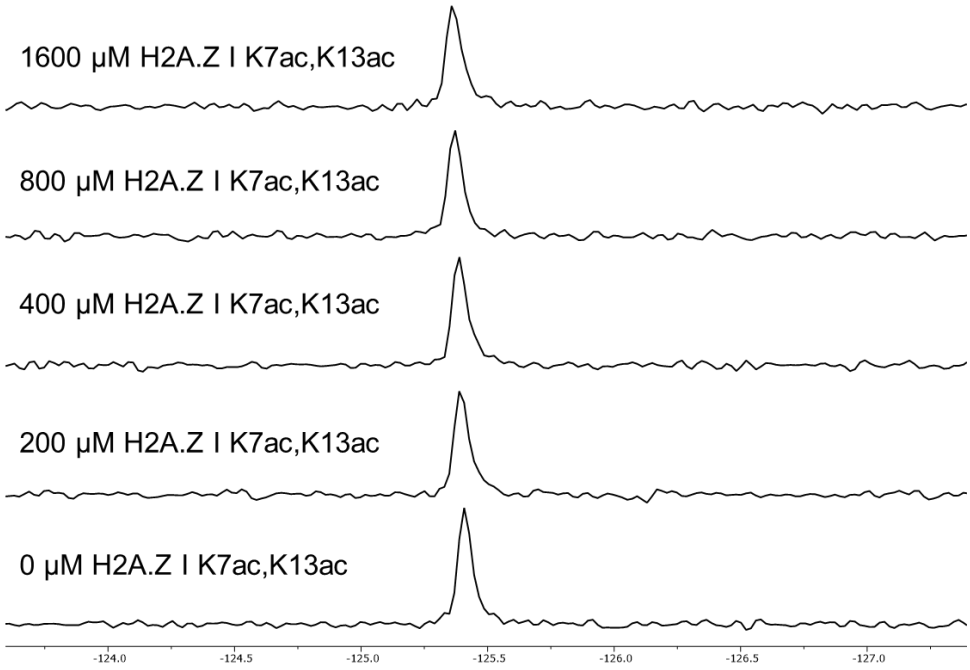
PrOF NMR titration with 5FW BRD2(1) and H2A.Z II K7ac,K13ac peptide.

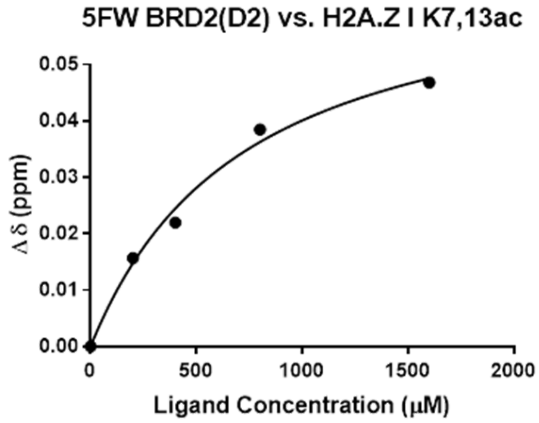




[Ligand]	5FW 97 (ppm)	Δδ
0 uM	-124.7249	
400 uM	-124.7142	0.0107
800 uM	-124.6707	0.0542
1600 uM	-124.6441	0.0808
3200 uM	-124.5843	0.1406

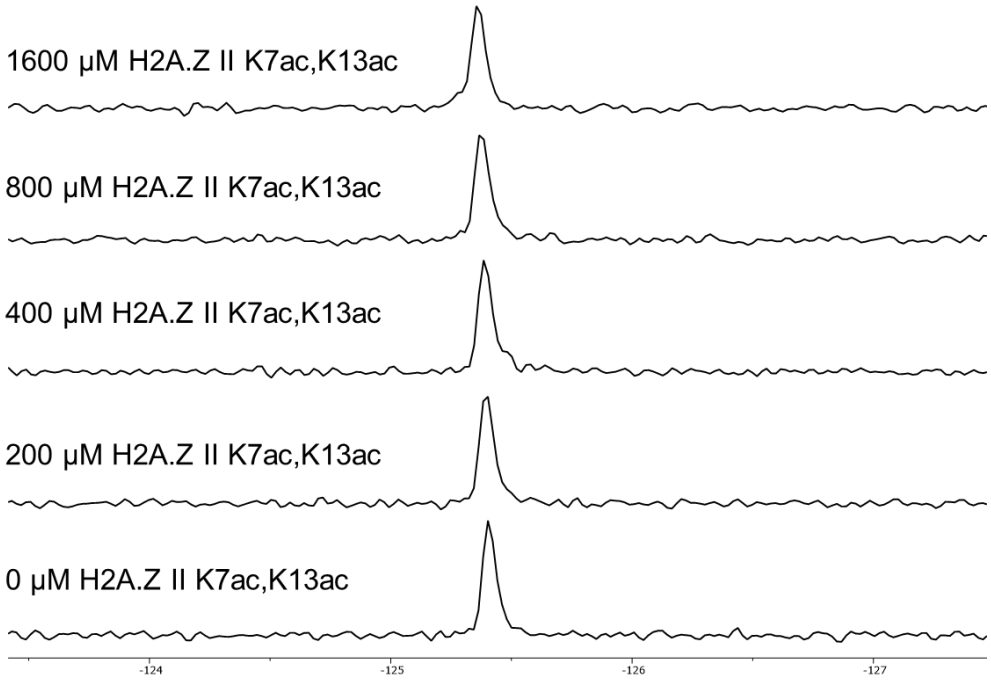
PrOF NMR titration with 5FW BRD2(D2) and H2A.Z I K7ac,K13ac peptide.

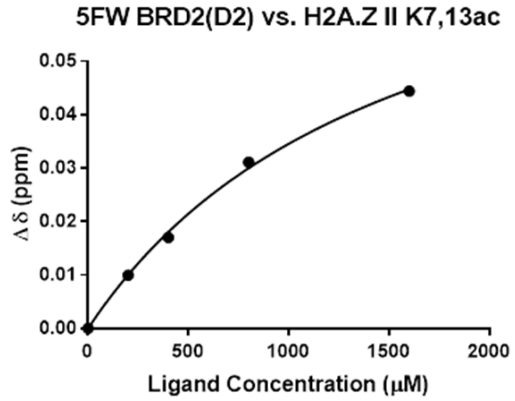




[Ligand]	5FW 370 (ppm)	Δδ
0 uM	-125.4084	
200 uM	-125.3927	0.0157
400 uM	-125.3864	0.022
800 uM	-125.3699	0.0385
1600 uM	-125.3616	0.0468

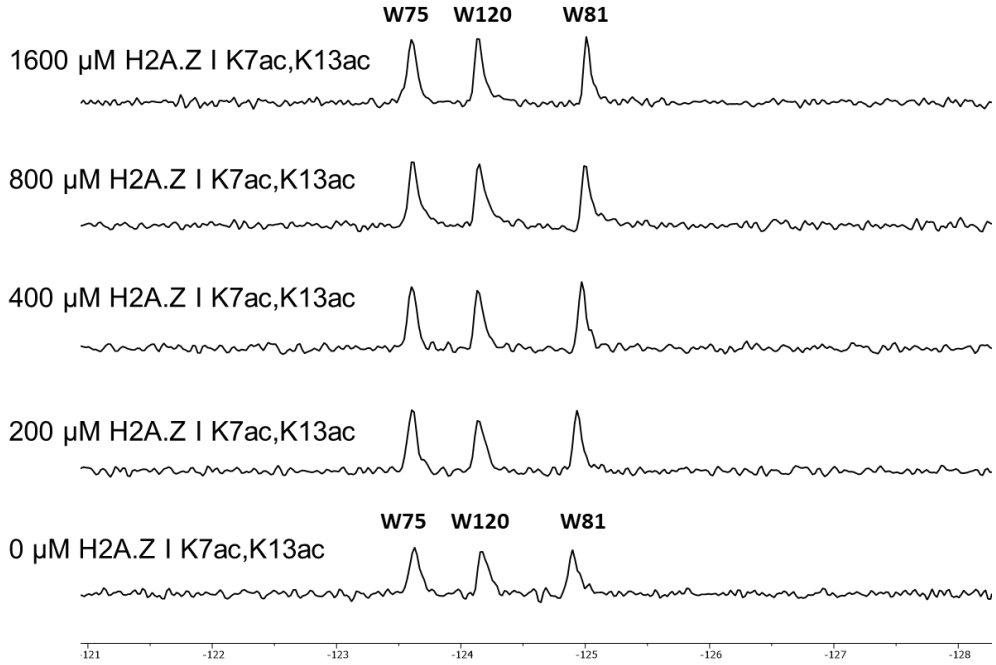
PrOF NMR titration with 5FW BRD2(D2) and H2A.Z II K7ac,K13ac peptide.

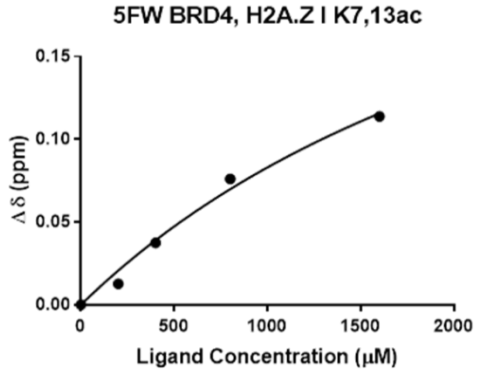




[Ligand]	5FW 370 (ppm)	Δδ
0 uM	-125.4053	
200 uM	-125.3953	0.01
400 uM	-125.3883	0.017
800 uM	-125.3742	0.0311
1600 uM	-125.3609	0.0444

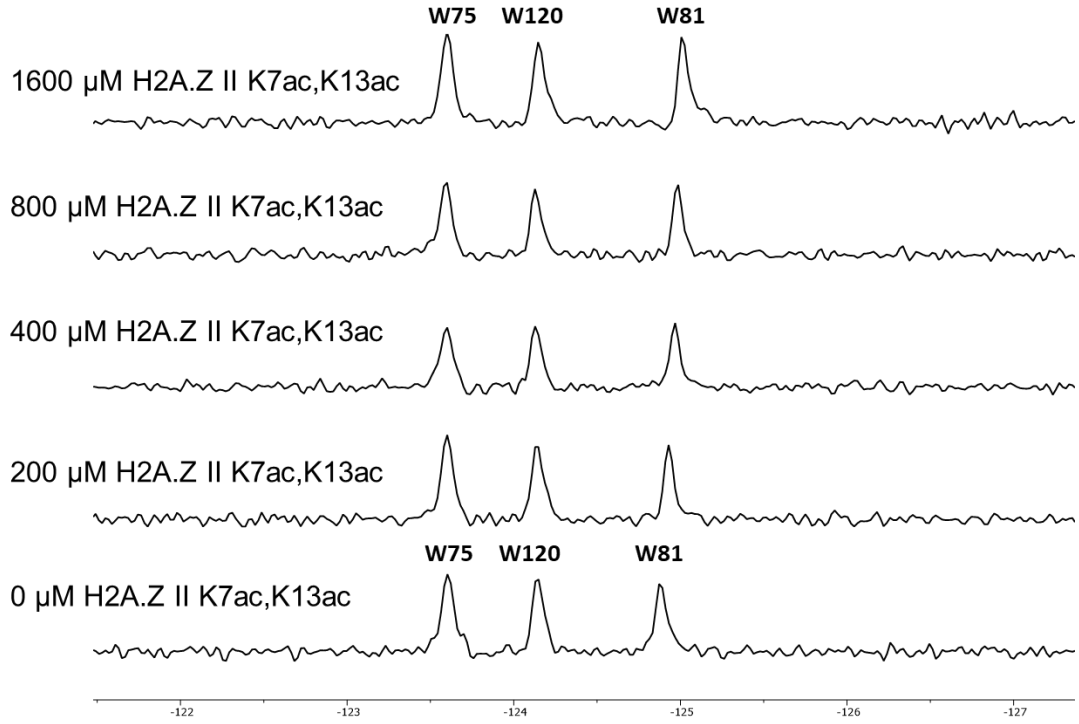
PrOF NMR titration with 5FW BRD4(1) and H2A.Z I K7ac,K13ac peptide.

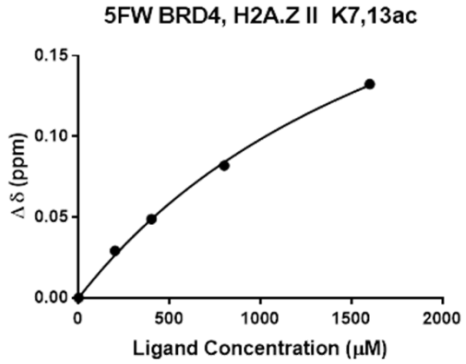




[Ligand]	5FW 81 (ppm)	Δδ
0 uM	-125.0113	
200 uM	-124.9986	0.0127
400 uM	-124.9738	0.0375
800 uM	-124.9352	0.0761
1600 uM	-124.8975	0.1138

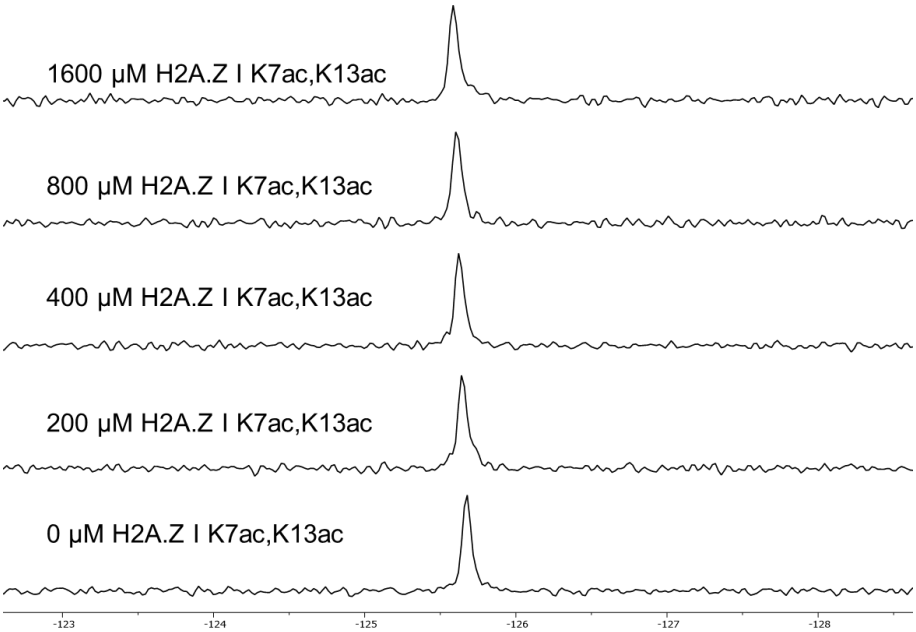
PrOF NMR titration with 5FW BRD4(1) and H2A.Z II K7ac,K13ac peptide.

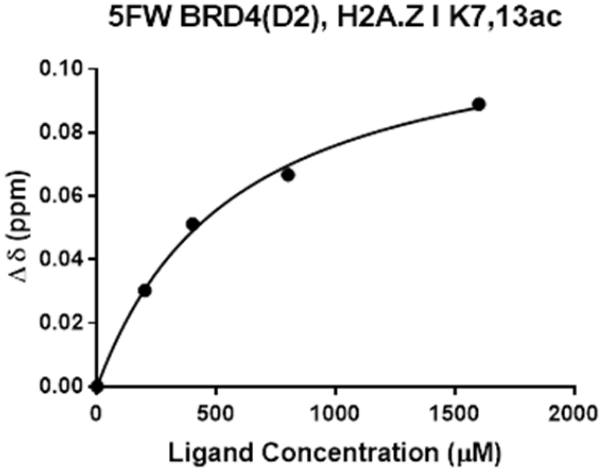




[Ligand]	5FW 81 (ppm)	Δδ
0 uM	-125.0116	
200 uM	-124.9824	0.0292
400 uM	-124.9668	0.0448
800 uM	-124.9296	0.082
1600 uM	-124.8792	0.1324

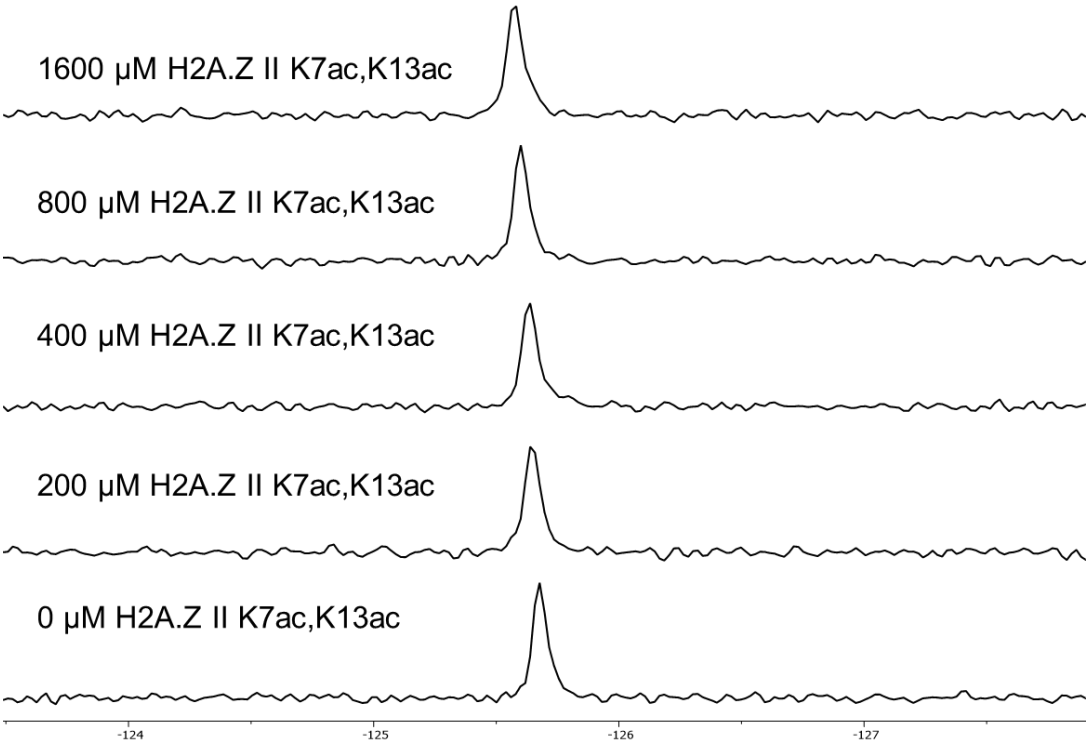
PrOF NMR titration with 5FW BRD4(D2) and H2A.Z I K7ac,K13ac peptide.

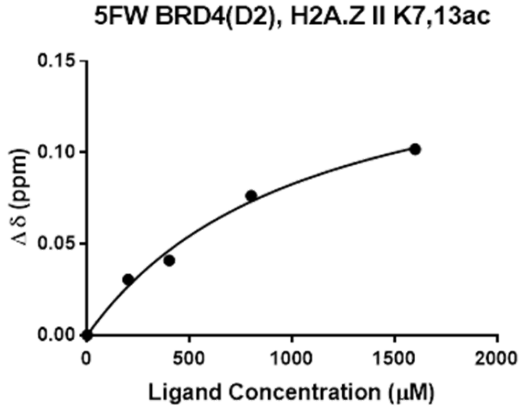




[Ligand]	5FW 374 (ppm)	Δδ
0 uM	-125.6744	
200 uM	-125.6441	0.0303
400 uM	-125.6232	0.0512
800 uM	-125.6077	0.0667
1600 uM	-125.5854	0.089

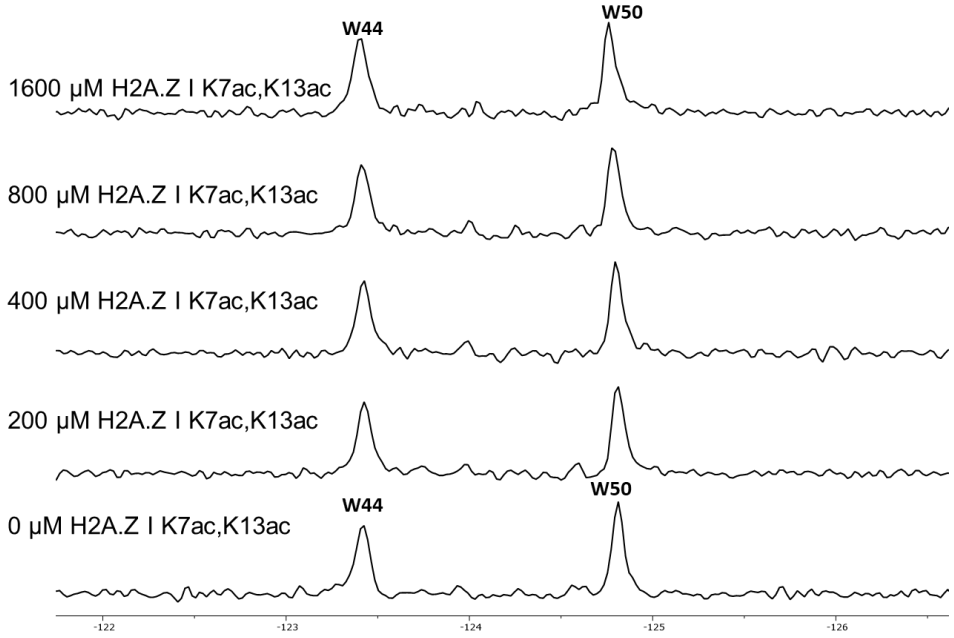
PrOF NMR titration with 5FW BRD4(D2) and H2A.Z II K7ac,K13ac peptide.

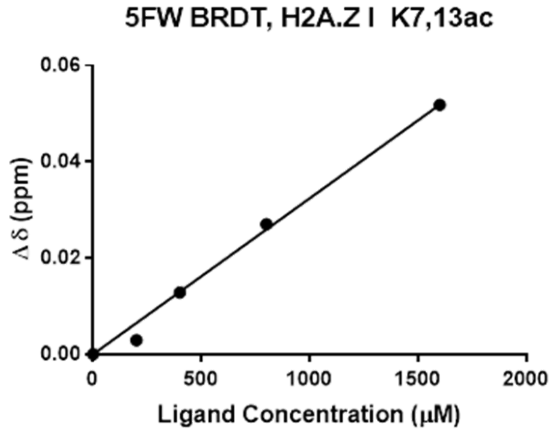




[Ligand]	5FW 374 (ppm)	Δδ
0 uM	-125.6753	
200 uM	-125.6449	0.0304
400 uM	-125.6343	0.041
800 uM	-125.599	0.0763
1600 uM	-125.5735	0.1018

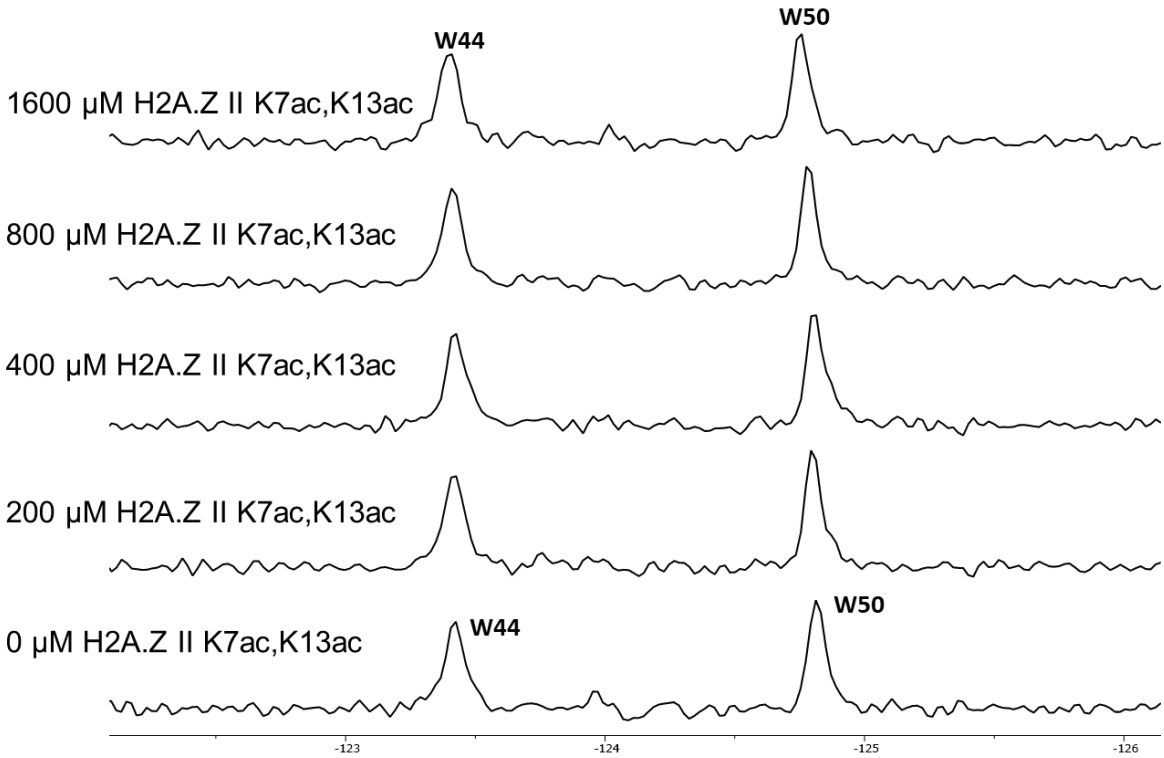
PrOF NMR titration with 5FW BRDT(1) and H2A.Z I K7ac,K13ac peptide.

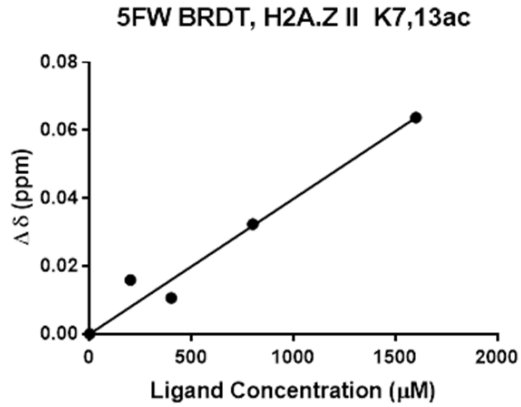




[Ligand]	5FW 50 (ppm)	Δδ
0 uM	-124.8112	
200 uM	-124.8083	0.0029
400 uM	-124.7984	0.0128
800 uM	-124.7842	0.027
1600 uM	-124.7594	0.0518

PrOF NMR titration with 5FW BRDT(1) and H2A.Z II K7ac,K13ac peptide.





[Ligand]	5FW 50 (ppm)	Δδ
0 uM	-124.8152	
200 uM	-124.7993	0.0159
400 uM	-124.8046	0.0106
800 uM	-124.7828	0.0324
1600 uM	-124.7514	0.0638

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