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Supplementary information

Posttranslational, site-directed photochemical fluorine editing of protein sidechains to probe residue oxidation state via ^{19}F-nuclear magnetic resonance

In the format provided by the authors and unedited

Supplementary Methods for:

Light-driven post-translational installation of sidechains into proteins reveals γCF_2 as a sensitive, 'zero-size-zero-background' reporter of proteinogenic residue state via ^{19}F-NMR

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Supplementary Methods

General Experimental Procedures

Unless otherwise stated, chemical reagents, media, and *Escherichia coli* cell stocks were obtained from commercial suppliers (Sigma-Aldrich, Fluorochem, Carbosynth, VWR, Alfa Aesar, Fisher Scientific) and used without further purification. Sonication was performed using a Fisher Scientific Model 505 Sonic Dismembrator. Proteins were purified using an Äkta FPLC System UPC-900 (GE Healthcare, UK). Gel electrophoresis was performed using Invitrogen NuPAGE 12% Bis-Tris gels, Novex MiniCell tanks, and a BioRad PowerPac controller. Thin layer chromatography was performed using Silica Gel 60 F254 plates (Merck). Nuclear magnetic resonance spectra for small molecule were recorded on a Bruker AVIII HD 400 nanobay (400 MHz) spectrometer and analyzed on MestReNova11. Carbon nuclear magnetic resonance spectra were recorded on a Bruker DQX 400 (100 MHz) spectrometer. All ^{1}H-NMR chemical shifts are quoted in ppm using residual solvent as the internal standard relative to TMS (d6-acetone: 2.09 ppm). All ^{13}C NMR chemical shifts are quoted in ppm using the central solvent peak as the internal standard relative to TMS (d6-DMSO 39.3 ppm). Coupling constants (J) are reported in Hertz (Hz). Infrared (IR) spectra were recorded on a Bruker Tensor 27 Fourier-Transform spectrophotometer. High-resolution small molecule mass spectra were recorded on a Micromass LCT (resolution = 5000 RWHM) using a lock-spray source. Protein crystal structures were analyzed and displayed using MacPyMOL v. 1.3 (Schrodinger, Inc.). RCSB-PDB reference 1KX5 was used to generate histone H3 images.

Mass Spectrometry General Methods

Intact protein mass spectrometry was performed on a Waters Xevo G2-XS QTof coupled to Water Acquity UPLC. Separation was achieved using a Thermo Proswift (250 mm x 4.6 mm x 5 µm) column with water + 0.1% formic acid (solvent A) and acetonitrile + 0.1% formic acid (solvent B) as the eluent system over a 10-min linear gradient. Nitrogen was used as the desolvation gas (600 L/h) for positive electrospray ionization. Voltages used were capillary: 3000 V, cone: 160 V. Lock-spray analysis ensured continual calibration against a leucine enkephalin standard solution. Raw spectra containing multiple charged ion series were deconvoluted using MassLynx (Waters) and its maximum entropy (MaxEnt1) deconvolution algorithm (Resolution: 1.00 Da/channel, Width at half height: 0.400 Da for Histone eH3.1, Minimun intensity ratios: 33% Left and Right). Spectra were deconvoluted between 10000 and 25000 Da for Histone eH3.1. Any reaction conversions were calculated from relative peak intensities in the deconvoluted spectra. For Histones, ~10% baseline methionine oxidation often occurred during production, storage, and use, and these “+16 Da adducts” were combined into this total sum for starting material/product conversion calculations.

Protein NMR General Methods

^{19}F NMR spectra were recorded at 25°C, using a Bruker AVANCE NEO 600 MHz NMR spectrometer equipped with a CPRHe-QR-1H/19F/13C/15N-5mm-Z helium cooled cryoprobe. Samples were stored in a Bruker SampleJet sample changer while not in the magnet, at 4 °C.

Spectra were recorded with a sweep width of 131578.95 Hz, 131072 complex points (TD), transmitter offset of -100ppm, 28000 scans and 4 dummy scans. A relaxation delay (D1) of 1 s yielded a total acquisition time of just under 12 hours. Where 1H decoupling was applied, waltz16 CPD was used, centred at 4ppm.

In the case of the mixture sample a 1D ^{19}F spectrum with ^{1}H decoupling was acquired. In this case, the average linewidth of γF_2-Met species was 35.57 Hz. 1D ^{19}F spectra of the three individual γF_2-Met oxidation states were acquired without ^{1}H decoupling. Whilst we did note some increase linewidth (average of γF_2-Met species was 39.79 Hz) due to lack of refocussing unresolved long range $^{n}J_{HF}$ our fitting method still performed excellently, demonstrating that this method can be used without access to an NMR spectrometer able to record $^{19}F\{^{1}H\}$ spectra.

Details of the Fitting Methodology

The NMR spectra to be fitted consist of N_s singlets and N_q AB quartets. The normalised intensity in an NMR spectrum at frequency ν_0 is described by a normalised Lorentzian function centre on frequency, ν_j with linewidth parameter, R_2.

$$L(\nu, R_2) = \frac{1}{\pi} \frac{R_2}{{R_2}^2 + (\nu - \nu_0)^2}$$

The mathematical form of an AB quartet comprises four signals, k, whose positions and relative intensities, S_i, vary as a function of the two original chemical shifts, $\Omega_{1,i}$ and $\Omega_{2,i}$ and the coupling constant J_i that links them.

k	S_i Observed signal intensity	$\nu_{i,k}$ Observed frequency
1	(1-(sin (J_i/$\Delta\Omega_i$)))/4	+ 0.5 (J_i + Y_i + Z_i)
2	(1+ (sin (J_i/$\Delta\Omega_i$)))/4	+ 0.5 (J_i - Y_i - Z_i)
3	(1+(sin (J_i/$\Delta\Omega_i$)))/4	- 0.5 (J_i - Y_i - Z_i)
4	(1-(sin (J_i/$\Delta\Omega_i$)))/4	- 0.5 (J_i + Y_i + Z_i)

In which Z_i, Y_i and $\Delta\Omega_i$ are defined as:

$$Z_i = \sqrt{{J_i}^2 + {\Delta\Omega_i}^2}$$

$$Y_i = \Omega_{1,i} + \Omega_{2,i}$$

$$\Delta\Omega_i = \Omega_{1,i} - \Omega_{2,i}$$

Note that in the case $J_i \gg \Delta\Omega_i$, $Z = J$, and only one resonance is observed at Y_i/2, whilst in the case $J_i \ll \Delta\Omega_i$ we observe four resonances at $\Omega_{1,i} + \frac{J_i}{2}, \Omega_{1,i} - \frac{J_i}{2}, \Omega_{2,i} + \frac{J_i}{2}, \Omega_{2,i} - \frac{J_i}{2}$, each of equal intensity. In all cases, the intensity at frequency ν_0 contributed by each of the four multiplets, k, will be a given by $S_{i,k}L(\nu_{i,k}, R_{2,i})$, of the AB quartet i.

The simulated spectra comprise N_q AB quartets and N_s singlets, where each species is characterised by a linewidth parameter (R2i) and overall intensity (Ii) such that the overall simulated spectrum can be described as:

$$S = \sum_{i=1}^{N_q} \sum_{k=1}^{4} \mathrm{I}_i \, S_{i,k} L\left(\nu_{i,k}, R_{2,i}\right) + \sum_{j=1}^{N_s} I_j L\left(\nu_j, R_{2,j}\right)$$

The factor of four used in calculation of S_i balances the relative intensities such that the overall contribution to the spectrum from singlets or AB quartets will be equal given equal intensity parameters, I_i and I_j. Initial guesses for all parameters need to be provided by the user before the simulated spectrum S is fitted to the experimentally determined spectrum. There will be $5N_q$+$3N_s$ fitting parameters, 5 (two frequencies, a linewidth, a coupling constant and an intensity) per AB quartet and 3 (one frequency, a linewidth and an intensity) per singlet. Parameters are then optimised to fit *S* to the experimental data by minimising their residual least square using standard gradient based methods (LMDR, as implemented in leastsq in python).

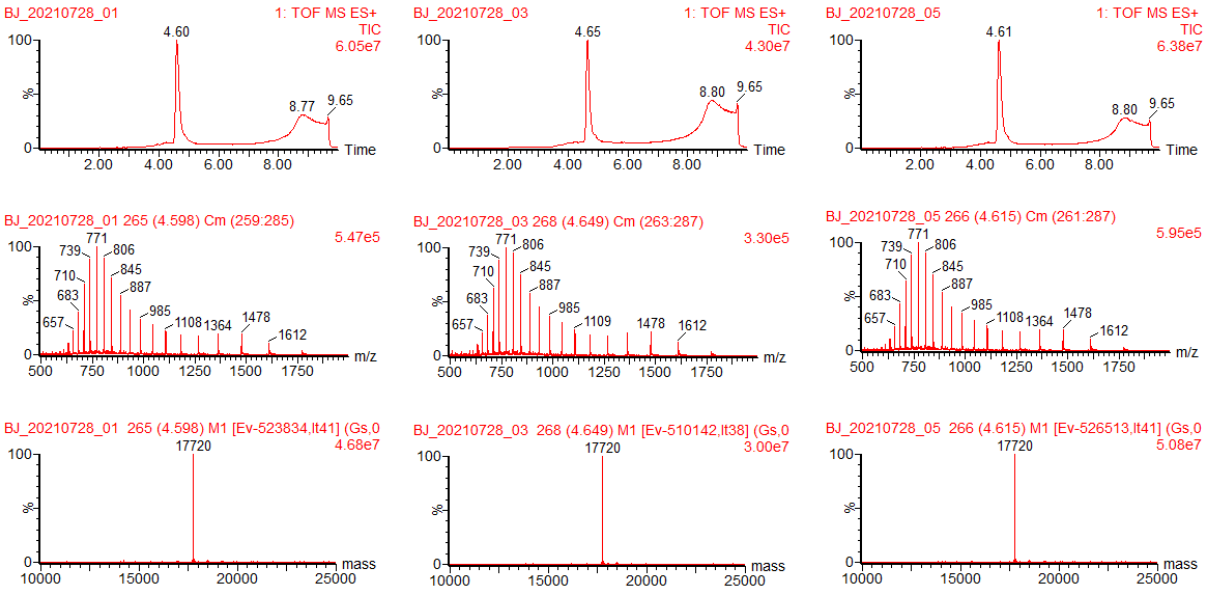
Protein Production

The model protein used for this paper, Human Histone variant eH3.1 (e stands for epitope in this case, representing the C-terminal FLAG-HA dual epitope tag, native residues Cys96 and Cys110 are also mutated to Ala), was expressed and purified as previously described[1,2]. Briefly, the *Escherichia coli* strain BL21(DE3)pLysS was transformed as appropriate and selected with chloramphenicol and ampicillin. Single colonies were picked to inoculate 20 mL starter cultures in LB broth with the same antibiotics. Flasks containing 0.5 L 2xYT media were inoculated with 1% v/v of starter cultures and grown at 37 °C until OD_{600} = 0.4-0.8. Histone expression was induced with addition of IPTA to 0.5 mM and allowed to proceed for 2 h (250 rpm, 37 °C) before harvesting and resuspension into a 5-fold volume/weight "wash buffer" (50 mM Tris, pH 7.5, 100 mM NaCl with a cOmplete™, EDTA-free Protease Inhibitor Cocktail, Roche, cat. no. COEDTAF-RO). Suspensions were flash-frozen and stored at -80 °C until lysis. Lysis proceeded via sonication in the presence of 1 mg DNase for 5 x 30 s bursts at 40% amplitude. The sonicate was centrifuged for 20 min at 20k rpm at 4 °C. The supernatant was discarded and the pellet resuspended in 40 mL "wash buffer" + 1% Triton-X detergent. Sonication was repeated once at 40% amplitude, 30 s, and the suspension centrifuged at 20k rpm for 10 min. The pellet was washed twice more in this fashion, then once with the non-Triton containing "wash buffer." 1 mL of DMSO was added to the pellet and crudely mixed with a spatula to aid histone desolvation for 10 min. 10 mL "unfolding buffer" (7 M Gdn·HCl, 20 mM Tris, pH 7.5, 10 mM DTT) was added and shaken for 1 h at RT, then the mixture was centrifuged for 10 min at 20k rpm at room temperature. The supernatant was loaded onto an S200 size exclusion column (GE Healthcare) pre-equilibrated with "SAU-100" buffer (7 M urea, 20 mM NaOAc, pH 5.2, 100 mM NaCl, 1 mM EDTA, 10 mM DTT, 1 mM benzamidine). Protein was eluted with SAU-100, analyzed by SDS-PAGE, and histone fractions were pooled and concentrated to 1-4 mL. Cation exchange chromatography was used to further purify histones (HiTrap SP 5 mL) using a linear gradient of 0-100% SAU-1000 buffer ("SAU-100" with 1000 mM NaCl final concentration). Pure fractions were pooled, dialyzed thrice against water (2 h each, with 2 mM β-mercaptoethanol) and lyophilized.

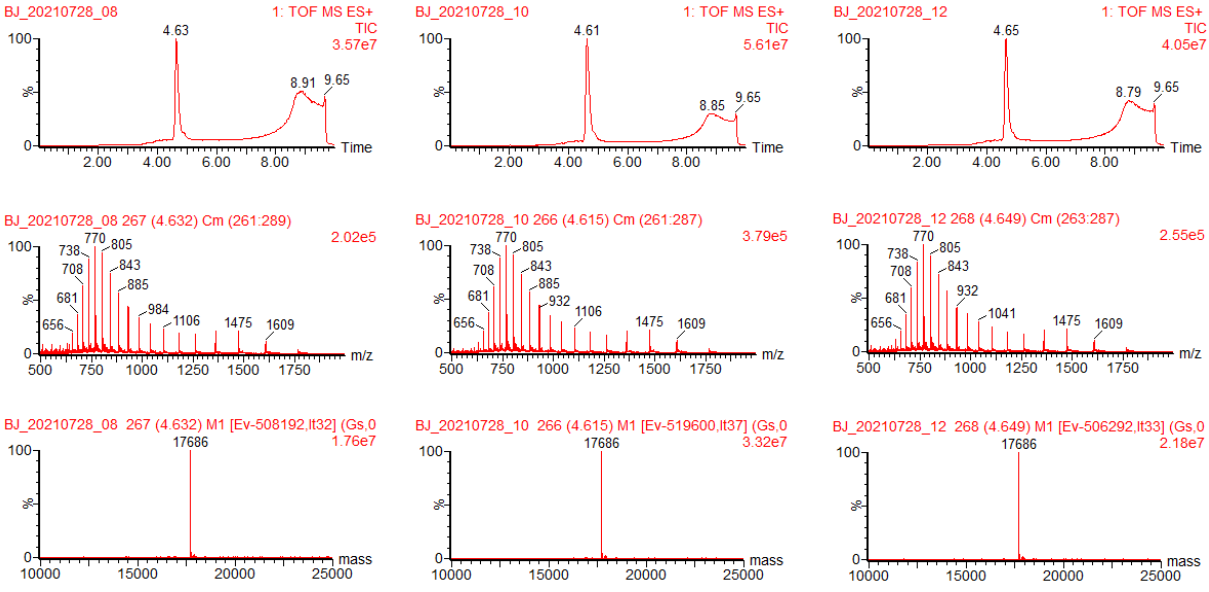
Protein Reactions

Protein reactions described in the main text were monitored by intact protein LC-MS (see General methods for details). Below are the total ion count chromatograms (top), ion series (middle), and deconvoluted spectra (bottom) measured in triplicate (three columns) for the starting material human Histone eH3.1-Cys4, as well as modified Histone eH3.1-Dha4, Histone eH3.1-γF_2Met4, Histone eH3.1-γF_2Met(O)4, and Histone eH3.1-γF_2Met(O_2)4.

Histone eH3.1-Cys4 (Calc mass: 17720 Da, Obs mass: 17720 Da)



Histone eH3.1-Dha4 (Calc mass: 17686 Da, Obs mass: 17686 Da)



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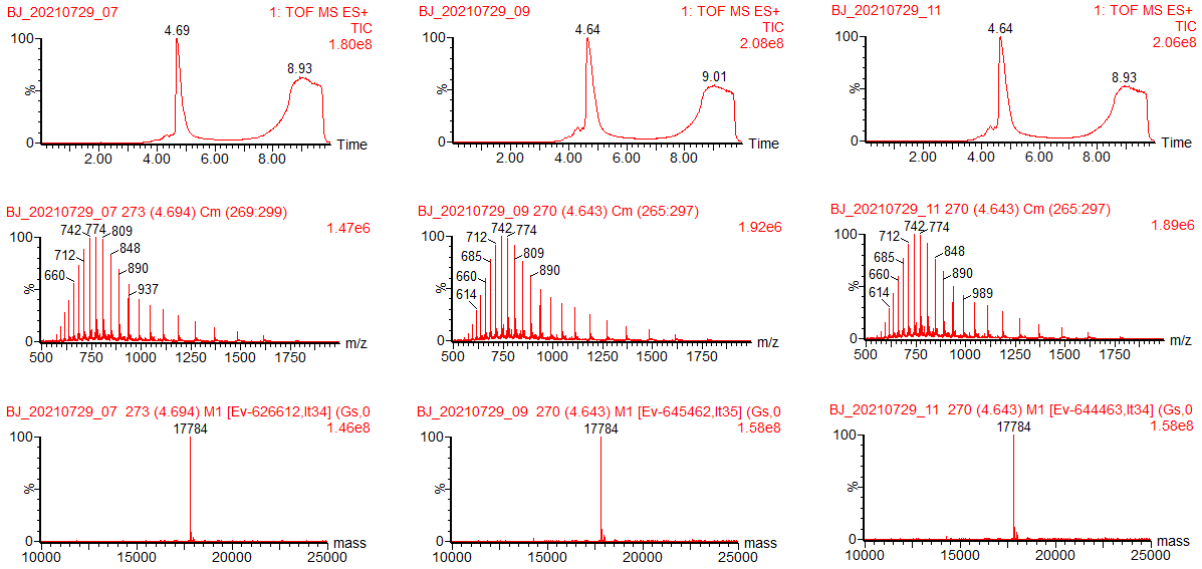
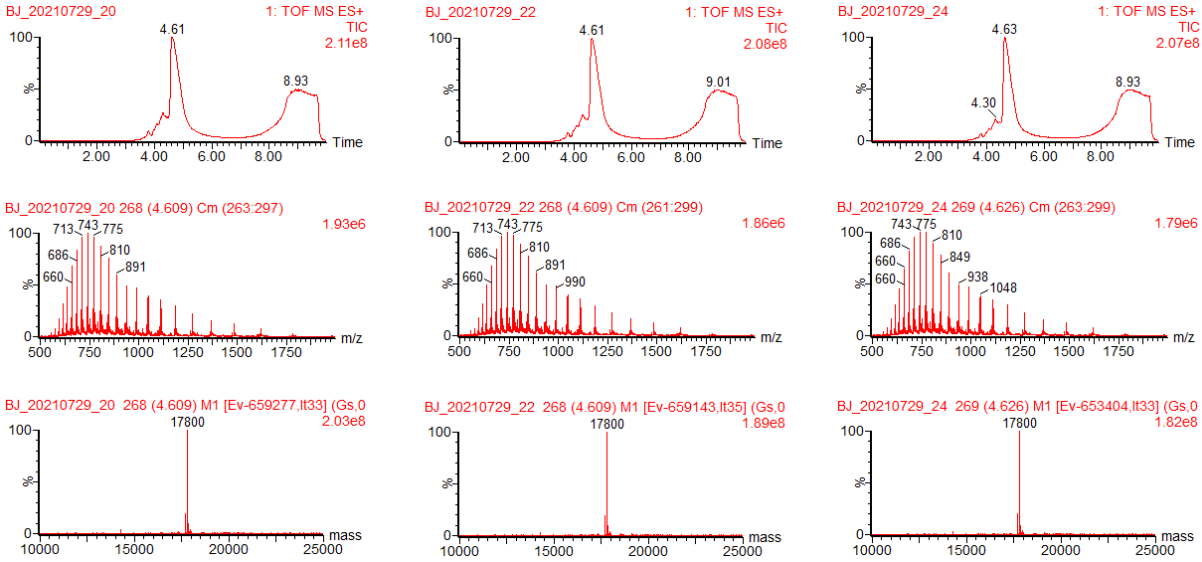
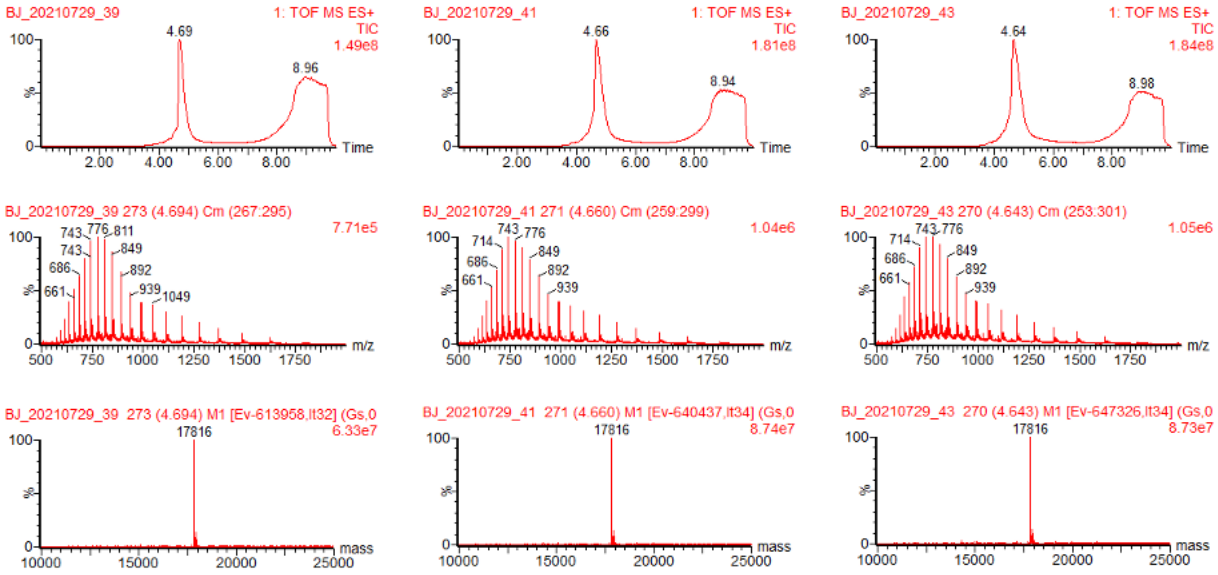


Histone eH3.1-γF_2Met(O)4 (Calc mass: 17800 Da, Obs mass: 17800 Da)



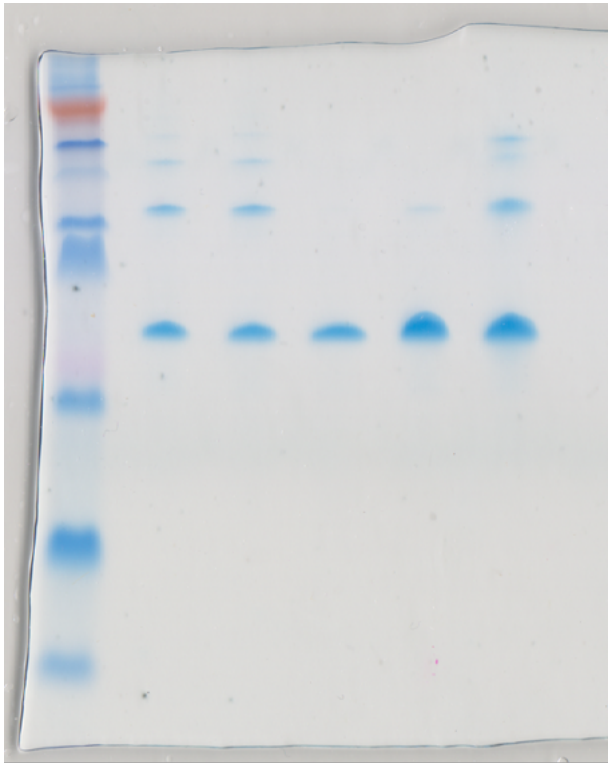
Histone eH3.1-γF_2Met(O_2)4 (Calc mass: 17816 Da, Obs mass: 17816 Da)


BJ_20210729_39
1: TOF MS ES+
TIC
1.49e8
4.69
8.96
Time
BJ_20210729_41
1: TOF MS ES+
TIC
1.81e8
4.66
8.94
Time
BJ_20210729_43
1: TOF MS ES+
TIC
1.84e8
4.64
8.98
Time
BJ_20210729_39 273 (4.694) Cm (267:295)
7.71e5
743
776
811
743
849
686
892
661
939
1049
m/z
BJ_20210729_41 271 (4.660) Cm (259:299)
1.04e6
743
776
714
849
686
892
661
939
m/z
BJ_20210729_43 270 (4.643) Cm (253:301)
1.05e6
743
776
714
849
686
892
661
939
m/z
BJ_20210729_39 273 (4.694) M1 [Ev-613958,It32] (Gs,0
6.33e7
17816
mass
BJ_20210729_41 271 (4.660) M1 [Ev-640437,It34] (Gs,0
8.74e7
17816
mass
BJ_20210729_43 270 (4.643) M1 [Ev-647326,It34] (Gs,0
8.73e7
17816
mass

Gel characterization

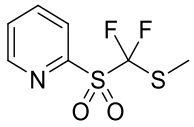
SDS-PAGE analysis was conducted on expressed Histone eH3.1-Cys4 as well as Dha, γF_2Met, γF_2Met(O), and γF_2Met(O_2) modified variants to check the presence of intact protein at the expected size of ~ 17.7 kDa. Protein (1 µg) boiled for 10 min in NuPAGE LDS Sample Buffer (Thermofischer, cat. No. NP0007, with added reducing agent) was loaded into NuPAGE 12% Bis-Tris gels along with a protein ladder (5 µL, SeeBlue Plus2 pre-stained protein standard, Thermofischer, cat. no. LC5925). Gels were ran in a Novex MiniCell tank (40 min, 200 V, 4 °C) with a BioRad PowerPac controller. After electrophoresis, gels were removed from the tank and stained (1 h) with InstantBlue Ultrafast Protein Stain (Sigma, cat. no. ISB1L), followed by destaining overnight in milliQ water. Gels were then scanned to obtain the gel image. Often, faint higher molecular weight bands would appear as histone dimers or multimers.

Complete, unedited gel image from Figure 4



Reagent Synthesis

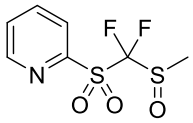
2-((Difluoro(methylthio)methyl)sulfonyl)pyridine



Under nitrogen atmosphere a heat-gun dried two-neck flask was charged with difluoromethyl 2-pyridyl sulfone (500 mg, 2.59 mmol), THF (10 mL), DMI (1 mL) and *S*-methyl methanethiosulfonate (488 mg, 368 µL, 3.90 mmol). Then, the reaction mixture was cooled to −78 °C in an iso-propanol/dry ice mixture followed by dropwise addition of LiHMDS (1M in THF, 3.2 mL, 3.20 mmol) and after complete addition the mixture was stirred for 30 minutes at −78 °C. After quenching with aqueous ammonium chloride solution (sat., 10 mL) the resulting aqueous solution was extracted with ethyl acetate (3 x 25 mL). The combined organic layers were washed with aqueous LiCl solution (sat. 25 mL), brine (25 mL), dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by using a CombiFlash R_f flash chromatography system equipped with an 12 g RediSep R_f silica gold column (gradient: 2 min 100% $CHCl_3$/heptane (1:1) then linear gradient to 100% $CHCl_3$/heptane/EtOAc (3:3:1) over 14 min) to afford the product (500 mg, 2.10 mmol, 81%) as a white solid.

$C_7H_7F_2NO_2S_2$ (239.3 g/mol):
TLC: R_f = 0.49 (SiO_2, petroleum ether:EtOAc (2:1)); **MP:** 35-36 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ/ppm = 8.88 (ddd, $^3J_{HH}$ = 4.6 Hz, $^4J_{HH}$ = 1.7 Hz, $^5J_{HH}$ = 0.9 Hz, 1H), 8.18 (d, $^3J_{HH}$ = 7.9 Hz, 1H), 8.03 (td, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.7 Hz, 1H), 7.67 (ddd, $^3J_{HH}$ = 7.7 Hz, $^3J_{HH}$ = 4.7 Hz, $^5J_{HH}$ = 1.1 Hz, 1H), 2.57 (t, $^4J_{HF}$ = 1.3 Hz, 3H); **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$) δ/ppm = 152.4, 151.2, 138.4, 129.9 (t, $^1J_{CF}$ = 323.7 Hz), 128.9, 126.6, 13.3 (t, $^3J_{CF}$ = 4.6 Hz); **^{19}F{^{1}H} NMR** (376 MHz, $CDCl_3$) δ/ppm = –81.6 (q, $^4J_{FH}$ = 1.3 Hz); **IR** (ATR): $\tilde{\nu}$/cm^{-1} = 3659 (w), 2980 (s), 2888 (m), 1578 (w), 1451 (w9; 1427 (m), 1382 (w), 1346 (s), 1322 (s), 1259 (m), 1173 (s), 1120 (s), 1105 (s), 1078 (s), 989 (s), 909 (s), 785 (s), 732 (s), 706 (w), 640 (s), 616 (w); **HRMS** (ESI (+), MeOH): (*m/z*) calc. for $C_6H_4F_2INO_2S$: 261.97785 $[M+Na]^+$; found: 261.97794.

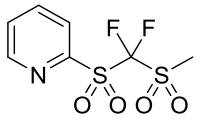
2-((Difluoro(methylsulfinyl)methyl)sulfonyl)pyridine



Under nitrogen atmosphere a heat-gun dried round-bottomed neck flask was charged with 2-((difluoro(methylthio)methyl)sulfonyl)pyridine (180 mg, 0.75 mmol) and CH_2Cl_2 (3 mL). Then, the reaction mixture was cooled to 0 °C in an ice/water mixture followed by dropwise addition of 3-chloroperbenzoic acid (≤77%, 186 mg, 0.82 mmol) in CH_2Cl_2 (1 mL) and after complete addition the mixture was stirred for 16 hours at room temperature. The crude mixture was concentrated under vacuum, dissolved in EtOAc (30 mL) and the organic layer was were washed with aqueous $NaHCO_3$ solution (sat., 2 x 30 mL), water (30 mL), brine (30 mL), dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by using a CombiFlash R_f flash chromatography system equipped with an 12 g RediSep R_f silica gold column (gradient: 2 min 100% hexane then linear gradient to 100% petroleum ether/EtOAc (4:5) over 14 min) to afford the product (110 mg, 0.43 mmol, 56%) as a colorless liquid.

$C_7H_7F_2NO_3S_2$ (255.3 g/mol):
TLC: R_f = 0.18 (SiO_2, petroleum ether:EtOAc (5:4)); **^{1}H NMR** (400 MHz, $CDCl_3$) δ/ppm = 8.88 (ddd, $^3J_{HH}$ = 4.6 Hz, $^4J_{HH}$ = 1.7 Hz, $^5J_{HH}$ = 0.8 Hz, 1H), 8.22 (dt, $^3J_{HH}$ = 7.9 Hz, $^5J_{HH}$ = 1.0 Hz, 1H), 8.09 (td, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.7 Hz, 1H), 7.73 (ddd, $^3J_{HH}$ = 7.7 Hz, $^3J_{HH}$ = 4.7 Hz, $^4J_{HH}$ = 1.2 Hz, 1H), 3.07 (t, $^4J_{HF}$ = 1.5 Hz, 3H); **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$) δ/ppm = 152.2, 151.4, 138.9, 129.7, 126.5, 125.0 (dd, $^1J_{CF}$ = 350.4 Hz, $^1J_{CF}$ = 333.7 Hz), 36.2 (dd, $^3J_{CF}$ = 7.1 Hz, $^3J_{CF}$ = 3.2 Hz); **^{19}F{^{1}H} NMR** (376 MHz, $CDCl_3$) δ/ppm = –103.8 (dd, $^2J_{FF}$ = 75.2 Hz, $^4J_{FH}$ = 1.6 Hz), –111.8 (dd, $^2J_{FF}$ = 75.2 Hz, $^4J_{FH}$ = 1.6 Hz); **IR** (ATR): $\tilde{\nu}$/cm^{-1} = 3094 (w), 3009 w), 2359 (w), 1455 (w), 1426 (w), 1349 (s), 1305 (w), 1261 (w), 1187 (m), 1146 (s), 1117 (m), 1094 (s), 1079 (s), 1044 (m), 987 (m), 963 (m), 951 (m), 889 (w), 782 (s), 738 (s), 726 (m), 631 (s), 614 (w); **HRMS** (ESI (+), MeOH): (*m/z*) calc. for $C_7H_7F_2NO_3S_2$: 277.97276 $[M+Na]^+$; found: 277.97269.

2-((Difluoro(methylsulfonyl)methyl)sulfonyl)pyridine



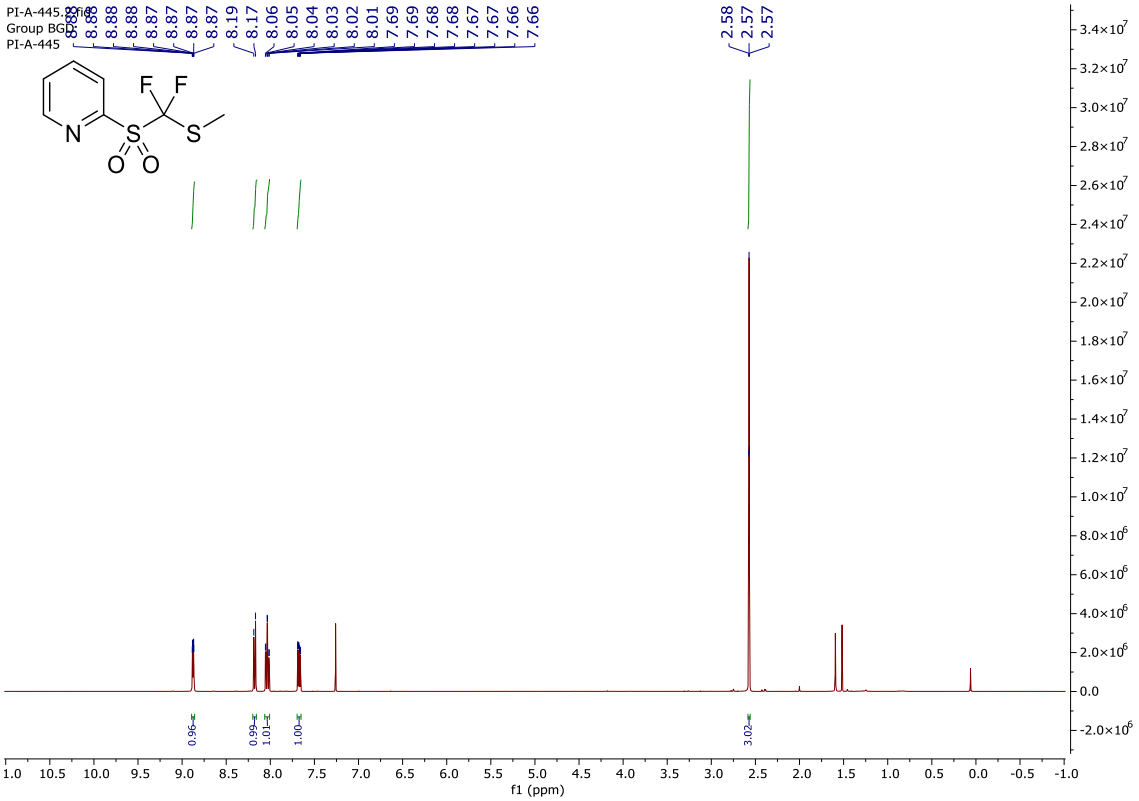
Under nitrogen atmosphere a round-bottom flask was charged with 2-((difluoro(methylthio)methyl)sulfonyl)pyridine (100 mg, 0.42 mmol), MeCN (2 mL), CH_2Cl_2 (1 mL) and water (3mL). Then, the reaction mixture was cooled to 0 °C in an ice-water mixture followed by addition of sodium periodate (411 mg, 1.93 mmol) and $RuCl_3xH_2O$ (1 mg) and the mixture was stirred for 16 hours. After dilution with water (30 mL) the resulting aqueous solution was extracted with ethyl acetate (3 x 30 mL). The combined organic layers were washed with water (25 mL), brine (25 mL), dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by using a CombiFlash R_f flash chromatography system equipped with an 4 g RediSep R_f silica gold column (gradient: 2 min 100% petrol ether then linear gradient to 100% petrol ether/EtOAc (4:3) over 12 min) to afford the product (108 mg, 0.40 mmol, 95%) as a white solid.

$C_7H_7F_2NO_4S_2$ (271.3 g/mol):

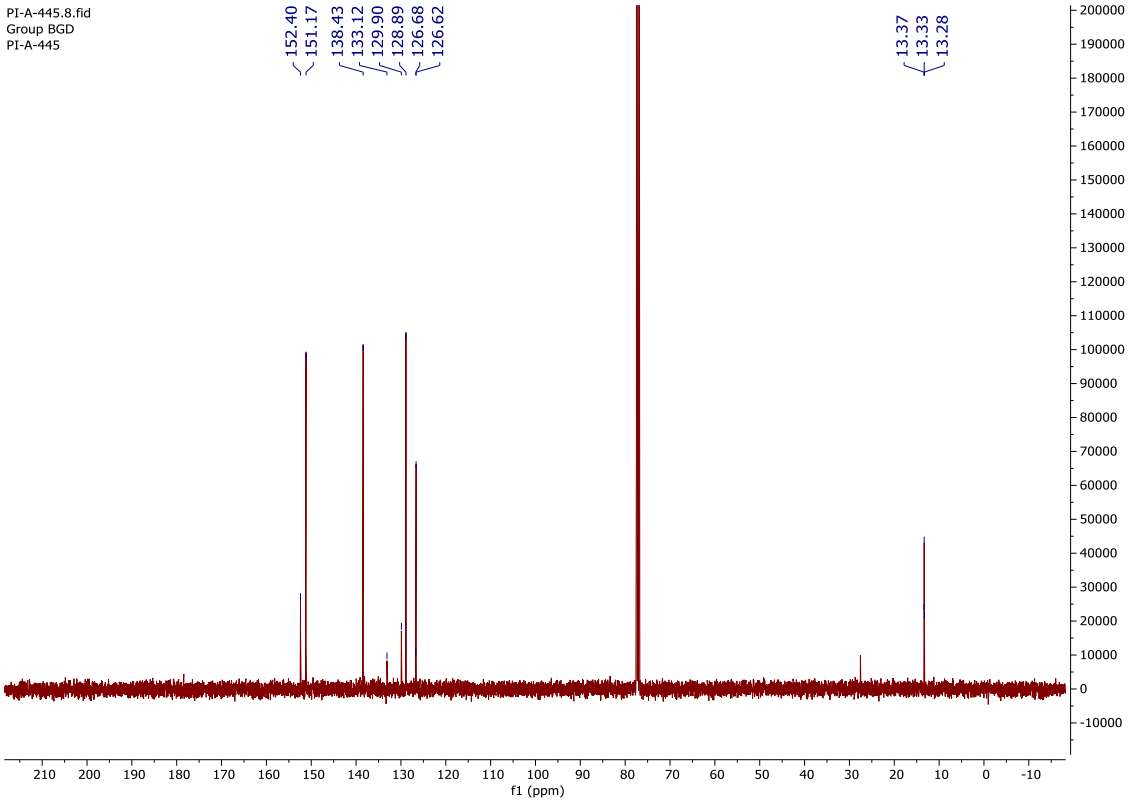
TLC: R_f = 0.40 (SiO_2, petroleum ether:EtOAc (4:3)); **MP:** 83-84 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ/ppm = 8.90 (ddd, $^3J_{HH}$ = 4.6, $^4J_{HH}$ = 1.7 Hz, $^4J_{HH}$ = 0.9 Hz, 1H), 8.25 (dt, $^3J_{HH}$ = 7.9 Hz, $^5J_{HH}$ = 1.0 Hz, 1H), 8.10 (td, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.7 Hz, 1H), 7.75 (ddd, $^3J_{HH}$ = 7.7 Hz, $^3J_{HH}$ = 4.6 Hz, $^4J_{HH}$ = 1.1 Hz, 1H), 3.36 (t, $^4J_{HF}$ = 1.6 Hz, 3H); **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$) δ/ppm = 151.9, 151.4, 138.8, 129.9, 127.2, 118.8 (t, $^1J_{CF}$ = 336.7 Hz); **^{19}F{^{1}H} NMR** (376 MHz, $CDCl_3$) δ/ppm = –106.1 (q, $^4J_{FH}$ = 1.6 Hz); **IR** (ATR): $\tilde{\nu}$/cm^{-1} = 2980 (s), 1577 (w), 1561 (w), 1454 (w), 1429 (w), 1417 (w), 1365 (s), 1338 (s), 1314 (m), 1262 (m), 1195 (s), 1167 (s), 1151 (s), 1121 (s), 1099 (s), 1079 (s), 1042 (s), 1023 (m), 990 (m), 977 (m), 955 (m), 912 (w), 830 (m), 303 (m), 789 (s), 7676 (s), 734 (s), 683 (w), 666 (w), 639 (w), 616 (w); **HRMS** (ESI (+), MeOH): (*m/z*) calc. for C $C_7H_7F_2NO_4S_2$ 271.98573 $[M+H]^+$; found: 271.98575.

NMR Spectra of pySOOF Reagents

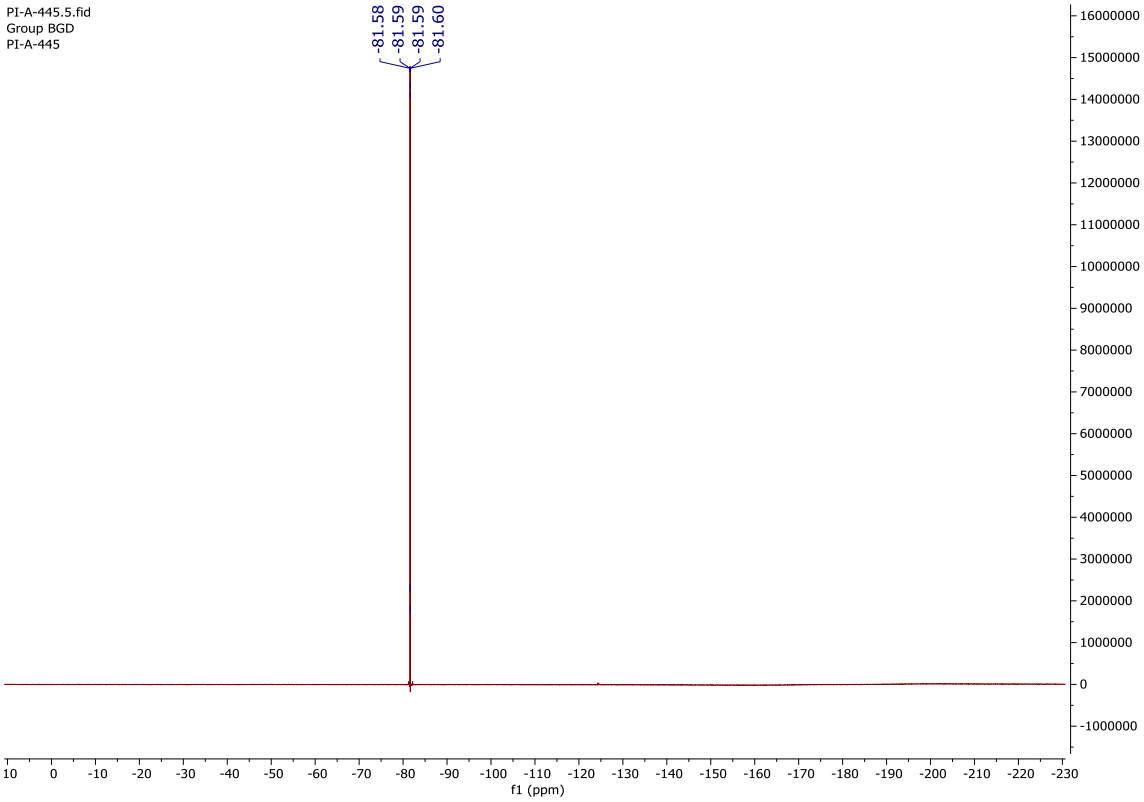
^{1}H NMR Spectrum of 2-((Difluoro(methylthio)methyl)sulfonyl)pyridine



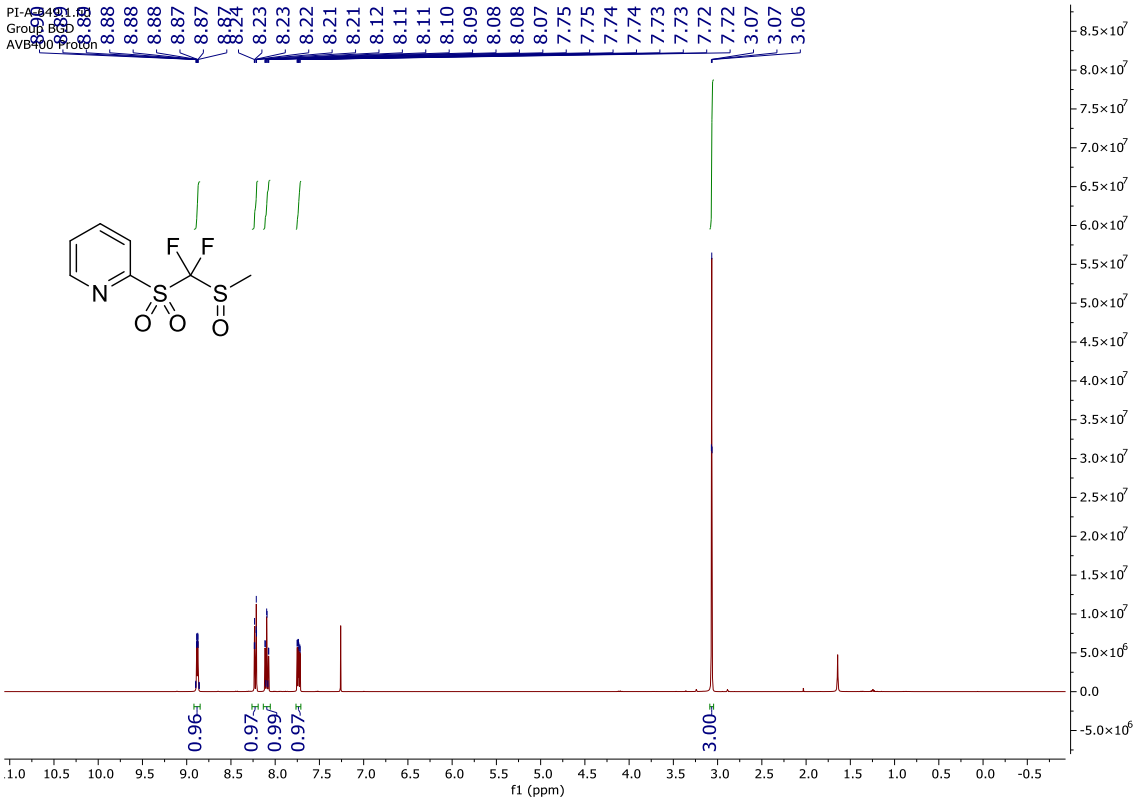
^{13}C NMR Spectrum of 2-((Difluoro(methylthio)methyl)sulfonyl)pyridine



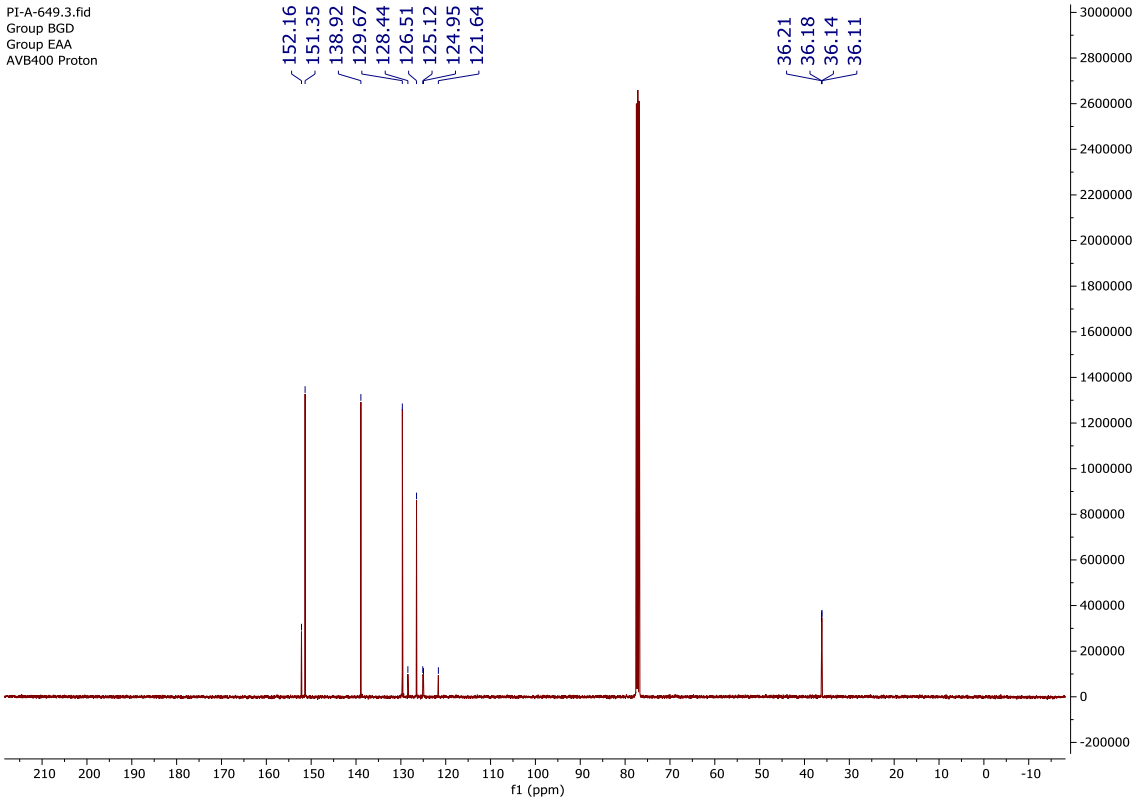
^{19}F NMR Spectrum of 2-((Difluoro(methylthio)methyl)sulfonyl)pyridine



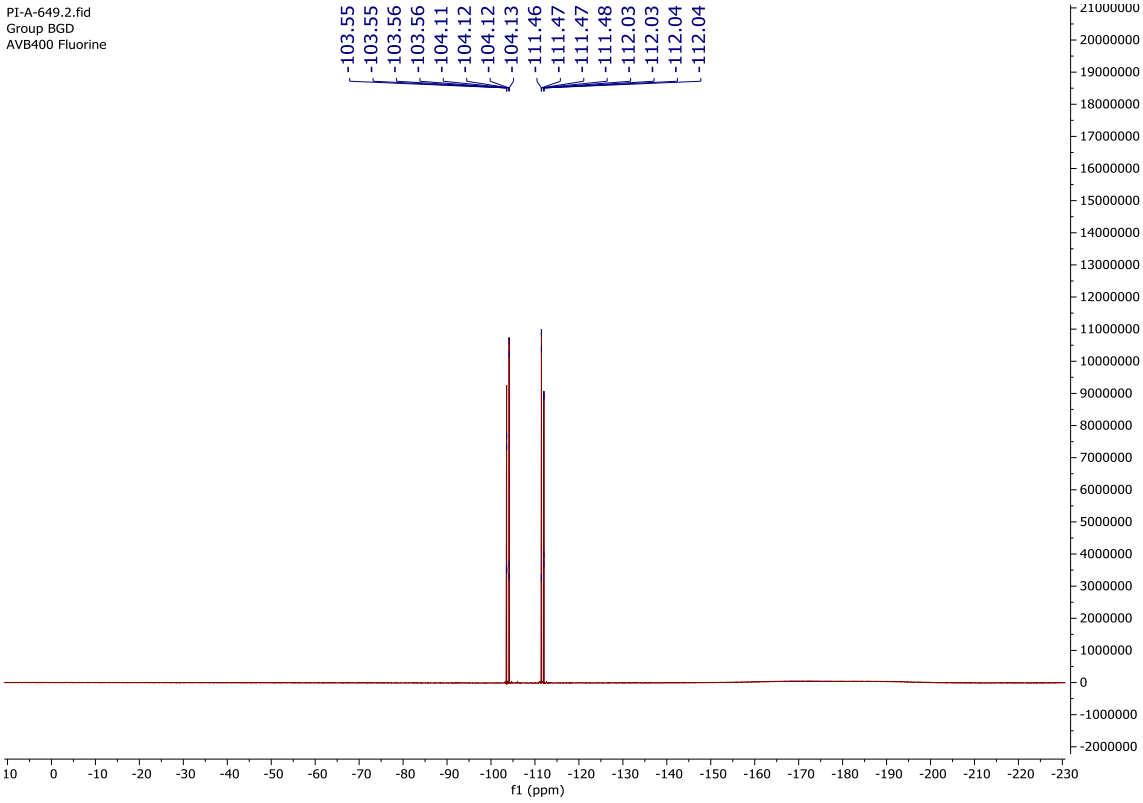
^{1}H NMR Spectrum of 2-((Difluoro(methylsulfinyl)methyl)sulfonyl)pyridine



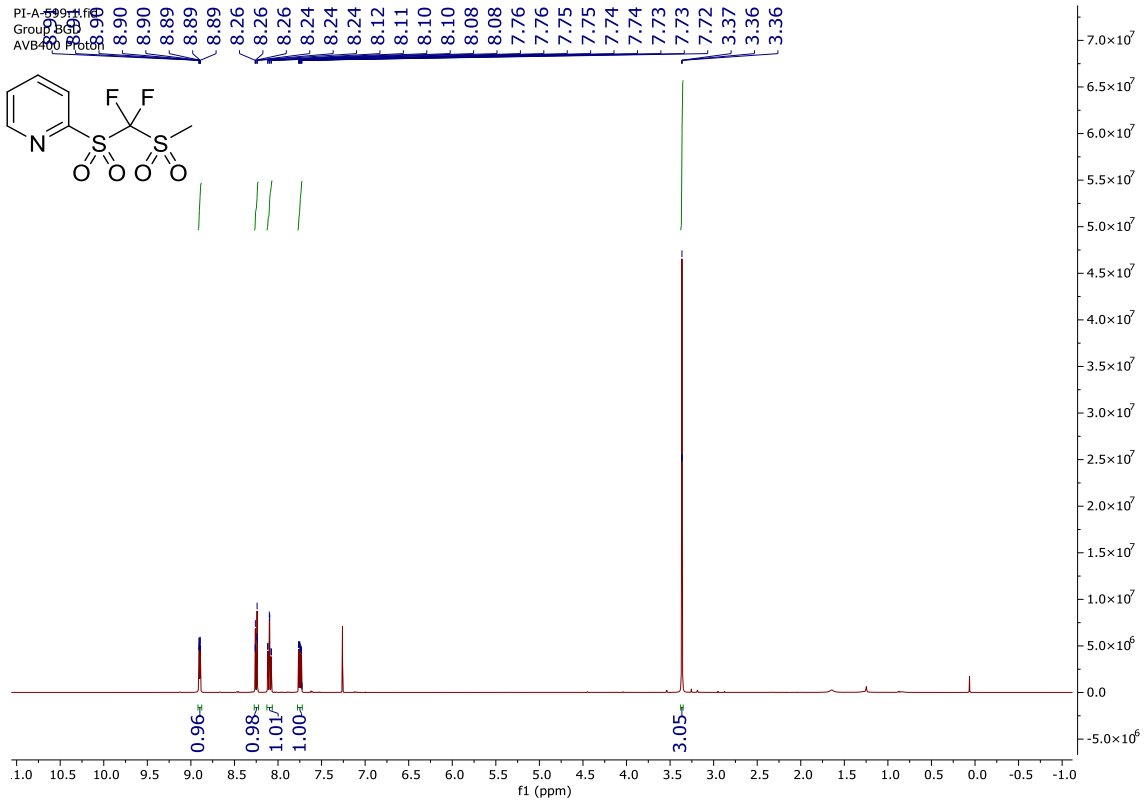
^{13}C NMR Spectrum of 2-((Difluoro(methylsulfinyl)methyl)sulfonyl)pyridine



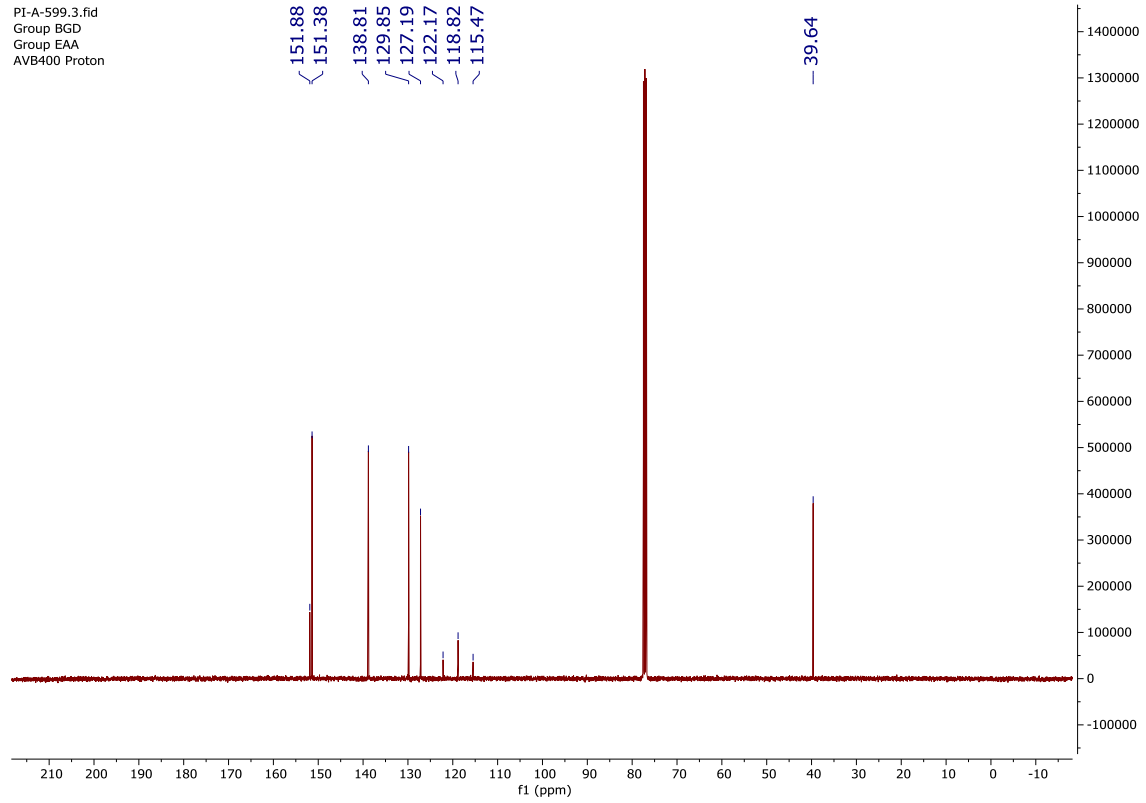
^{19}F NMR Spectrum of 2-((Difluoro(methylsulfinyl)methyl)sulfonyl)pyridine



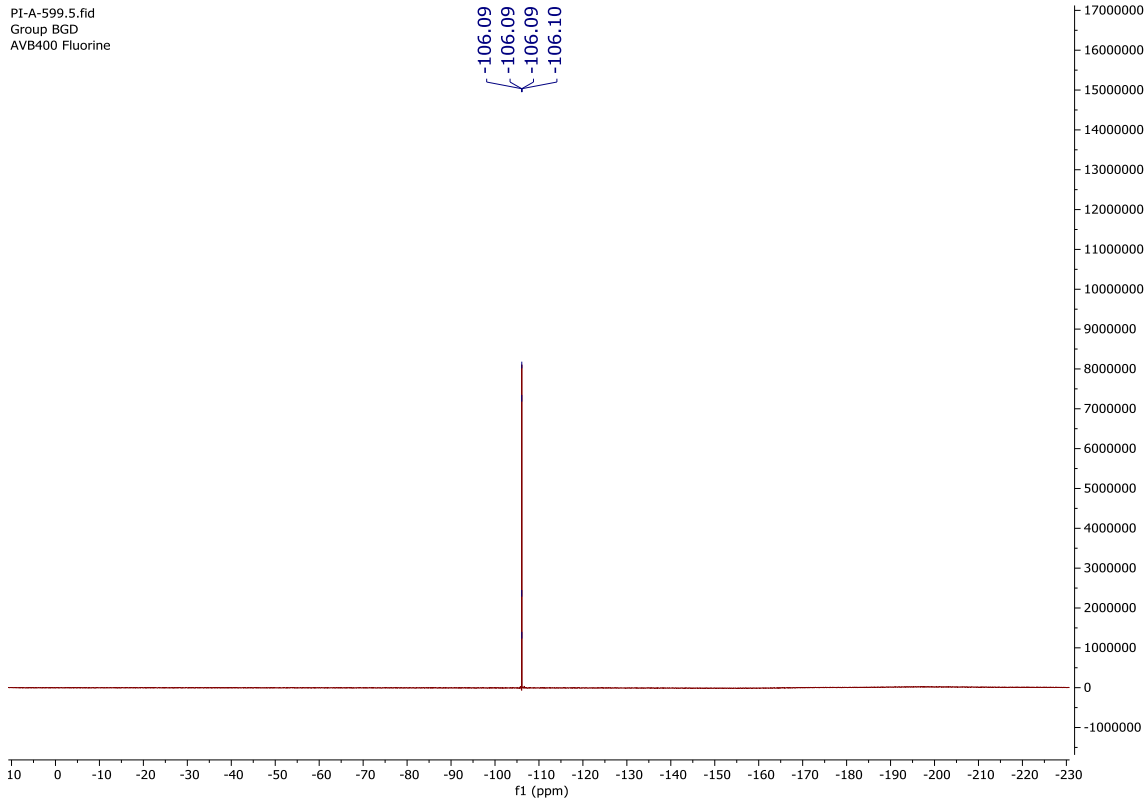
^{1}H NMR Spectrum of 2-((Difluoro(methylsulfonyl)methyl)sulfonyl)pyridine



^{13}C NMR Spectrum of 2-((Difluoro(methylsulfonyl)methyl)sulfonyl)pyridine



^{19}F NMR Spectrum of 2-((Difluoro(methylsulfonyl)methyl)sulfonyl)pyridine



Supplemental References

1 Josephson, B. *et al.* Light-driven post-translational installation of reactive protein side chains. *Nature* **585**, 530-537, doi:10.1038/s41586-020-2733-7 (2020).

2 Wright, T. H. *et al.* Posttranslational mutagenesis: A chemical strategy for exploring protein side-chain diversity. *Science*, aag1465, doi:10.1126/science.aag1465 (2016).