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Supplementary Information

γ-Difluorolysine as a ^{19}F NMR probe for histone lysine methyltransferases and acetyltransferases

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Table of Contents

1. Experimental section

All commercially available reagents were purchased and used without further purification. Reactinos were magnetically stirred, and monitoring by thin layer chromatography (TLC) was performed on glass backed silica sheets (Merck Silica Gel 60 F254) and plates were visualized by UV fluoresence (254 nm) and/or spraying with potassium permanganate ($KMnO_4$) or ninhydrin.

NMR Spectroscopic Characterization of Starting Materials and Intermediates

^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker Avance III 400 MHz. ^{1}H NMR chemical shift values are reported as δ in units of parts per million (ppm) relative to the internal standard tetramethylsilane (TMS, δ = 0 ppm). ^{13}C NMR shifts are reported as δ in units of parts per million (ppm) and the spectra were internally referenced to the residual solvent signal ($CHCl_3$ δ = 77.0 ppm). Coupling constant are reported as *J* values in Hertz (Hz). The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad.

1D and 2D NMR Spectroscopic Assignment of Pre- and Postfunctionalized Peptides

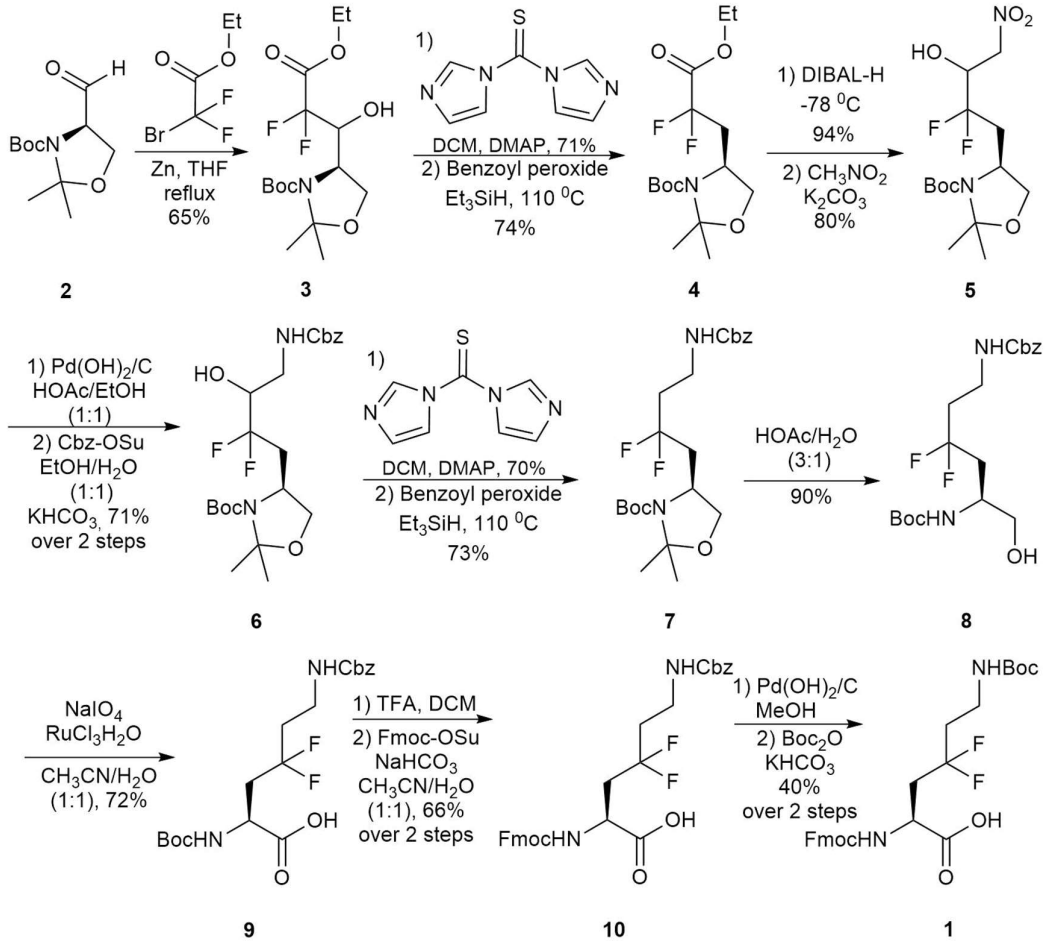
All ^{1}H, ^{1}H-^{13}C edited HSQC and ^{1}H-^{13}C HMBC spectra were recorded at room temperature on a Bruker 500 MHz Avance III spectrometer equipped with a Prodigy BB cryoprobe. ^{1}H spectra were acquired with composite-pulse presaturation water suppression, 16 scans, 3 seconds of saturation/relaxation and an acquisition time of 3.27 s. HSQC spectra were acquired using 25% NUS with 32 scans per increment, 512 increments (128 discretely sampled), a relaxation delay of 1.5 s and $^{1}J_{CH}$ = 145 Hz. HMBC spectra were acquired using 25% NUS with 128 scans per increment, 512 increments (128 discretely sampled), a relaxation delay of 1.5 s, a double low-pass 1-bond filter using $^{1}J_{CH}$ = 120 Hz and 170 Hz, and $^{n}J_{CH}$ = 8 Hz. The HSQC and HMBC data were processed out to 1024 x 1024 points with 90° shifted sine-bell squared (HSQC) and 0° shifted sine-bell squared (HMBC) apodization in both dimensions. All $^{19}F\{^{1}H\}$ and ^{1}H-^{19}F HMBC spectra were acquired at room

temperature on a Bruker 400 MHz Avance III HD nanobay spectrometer equipped with a BBFO probe. The inverse-gated $^{19}F\{^{1}H\}$ spectra were acquired with 128 -2048 scans, 2.7 s acquisition time, and a relaxation delay of 1 s. ^{1}H-^{19}F HMBC spectra were acquired using 25% NUS, 128 scans per increment, 160 increments (40 discretely sampled), a low-pass ^{1}J filter of 250 Hz and $^{n}J_{HF}$ = 5 Hz. The HMBC were process to 1024 x 512 with 0° shifted sine-bell squared apodization in both dimensions. All data were processed using MestreNova 14.

Peptide Purification and MALDI-TOF Instrumentation

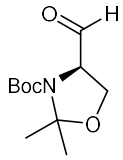
Histone peptides were purified on a preparative HPLC employing a Phenomenex Gemini-NX 3u C18 110A reversed-phase column (150 × 21.2 mm) with a flow rate of 4 mL/min. Analytical traces were monitored at 215 nm on a Phenomenex Gemini 5 µm C18 110 Å LC column at a flow rate of 1 mL/min. MALDI-TOF MS enzymatic analysis was performed by mixing aliquotes of the reaction mixture with the α-Cyano-4-hydroxycinnamic acid (CHCCA) matrix in 1:1 MQ and ACN (0.1% TFA) and MALDI-TOF MS spectra were recorded on a UltrafleXtreme-II tandem mass spectrometer (Bruker, Billerica, MA, USA) employing a MTP 384 polished steel target.

2. Synthetic procedures



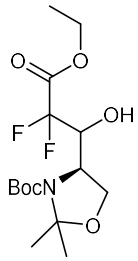
Scheme S1. Synthetic route to Fmoc/Boc-protected γ-difluorolysine **1.**

***tert*-butyl (*R*)-4-formyl-2,2-dimethyloxazolidine-3-carboxylate (2)**



To a stirred solution of 3-(*tert*-butyl) 4-methyl (*R*)-2,2-dimethyloxazolidine-3,4-dicarboxylate (58 mmol, 15 g) in 150 mL of DCM at -78 °C was added DIBAL-H in toluene (1.0 M, 90 mmol, 90 mL, 1.6 eq) while maintaining the temperature below -65 °C. After stirring the reaction for 8 h, 10 mL of EtOH was added to the reaction, followed by 150 mL of a Rochelle salt solution. The reaction mixture was stirred overnight. Once the emulsion had dissipated, the layers were separated. The aqueous layer was extracted with EtOAc (2 × 150 mL). The combined organic layers were washed with a saturated brine solution (2 × 250 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The sample was subjected to silica gel chromatography (PE/EtOAc, 5 : 1) to give quantitatively **2** as a colorless liquid (12.4 g, 94%). δ_H (400 MHz, $CDCl_3$) 1.43 (9 H, br s), 1.52 (6 H, s), 4.03–4.13 (2 H, m), 4.17–4.21 (1 H, m), 9.55 (1 H, d, J = 2.4 Hz).

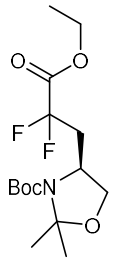
***tert*-butyl (4*R*)-4-(3-ethoxy-2,2-difluoro-1-hydroxy-3-oxopropyl)-2,2-dimethyl oxazolidine-3-carboxylate (3)**



Aldehyde **2** (3.7 g, 16 mmol) in dry THF (30 mL) was added dropwise to a refluxing suspension solution of ethyl bromodifluoroacetate (4.9 g, 24.0 mmol, 1.5 eq) and zinc powder (2.1 g, 32 mmol, 2.0 eq) in dry THF (70 mL). After complete addition the mixture was reflux for another 2 h before being cooled to room temperature. The reaction mixture was diluted with EtOAc (100 mL) then treated with 1 M $NaHSO_3$ (100 mL). After separation, extracted the aqueous layer with EtOAc (2 × 100 mL). The combined EtOAc layer was washed with brine, dried over Na_2SO_4, filtered and concentrated under vacuum. Compound **3** (3.7 g, 65%) was obtained as a colorless liquid after purified by silica gel chromatography (PE/EtOAc, 4 : 1). δ_H (400 MHz, $CDCl_3$) 1.37 (3 H, t, J = 7.2 Hz), 1.49 (12 H, br s), 1.58 (3 H, s), 3.96–4.06 (1 H,

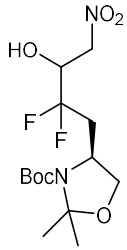
m), 4.21–4.52 (5 H, m). δ_F (376 MHz, $CDCl_3$) -111.9 (d, J = 88 Hz), -112.6 (d, J = 88 Hz). HRMS: calcd for $C_{15}H_{25}F_2NNaO_6^+$ 376.1542, found 376.1556.

***tert*-butyl (*S*)-4-(3-ethoxy-2,2-difluoro-3-oxopropyl)-2,2-dimethyloxazolidine-3-carboxylate (4)**



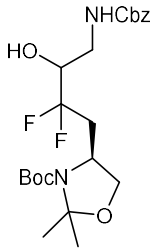
To a stirred solution of **3** (1.76 g, 5.0 mmol) in 10 mL was added 1,1-thiocarbonyldiimidazole (1.78 g, 10 mmol, 2.0 eq) followed by DMAP (60 mg, 0.5 mmol, 0.1 eq). After 2 h, the reaction was concentrated under vacuum and purified by silica gel (Petro ether/EtOAc, 3 : 1) to yield the desired intermediate (1.58g, 69%) as a yellow oil. δ_H (400 MHz, $CDCl_3$) 1.26 (3 H, t, J = 7.2 Hz), 1.30 (3 H, s), 1.43 (3 H, s), 1.48 (3 H, s), 1.54 (6 H, s), 4.07–4.14 (1H, m), 4.23–4.37 (3 H, m), 4.44–4.51 (1 H, m), 6.69–6.86 (1 H, m), 7.06 (1 H, s), 7.63 (1 H, s), 8.35 (1 H, s). HRMS: calcd for $C_{19}H_{28}F_2N_3O_6S^+$ 464.1661, found 464.1671. The product of the desired intermediate (1.58 g, 3.4 mmol) was dissolved in 30 mL of dioxane : Et_3SiH (1 : 1), the solution was heated to reflux. Benzoyl peroxide (0.82 g, 3.4 mmol, 1.0 eq) in 4 mL of dioxane in two portions was added to this solution. After refluxing for 2 h, the reaction mixture was cooled to room temperature diluted with EtOAc (30 mL). After evaporation, the residue was again diluted with EtOAc (3 × 30 mL) and evaporated. The residue was purified by silica gel chromatography (PE/EtOAc, 15 : 1) to yield **4** (0.85 g, 74%) as a colorless liquid. δ_H (400 MHz, $CDCl_3$) 1.49 (15 H, br s), 1.61 (3 H, s), 1.90–2.02 (2 H, m), 4.05–4.29 (4 H, m), 4.73 (1 H, d, J = 6.8 Hz). δ_F (376 MHz, $CDCl_3$) -105.8 (AB quartet). HRMS: calcd for $C_{15}H_{25}F_2NNaO_5^+$ 360.1593, found 360.1580.

***tert*-butyl (4*S*)-4-(2,2-difluoro-3-hydroxy-4-nitrobutyl)-2,2-dimethyloxazolidine-3-carboxylate (5)**



The aldehyde (1.49 g, 78%) was obtained as a colorless solid after purification by flash column chromatography (PE/EtOAc, 2:1), which was synthesized according to the preparation method for **2**, starting from compound **4** (1.9 g, 5.6 mmol), DIBAL-H in toluene (1.0 M, 9.0 mmol, 9 mL, 1.6 eq). To an ice bath cooled stirred suspension of K_2CO_3 (1.0 mmol, 0.14 g) in 20 mL of THF was added dropwise a solution of the aldehyde (1.0 g, 2.9 mmol) and nitromethane (0.72 g, 11.8 mmol, 4 eq). After stirring for 2 d at room temperature, 20 mL of Et_2O was added to the reaction. The organic layer was washed with 1 M $KHSO_4$ (20 mL), saturated brine (20 mL), water (20 mL). The organic layer was washed with brine, dried over Na_2SO_4. filtered and concentrated under vacuum. The residue was subjected to silica gel chromatography (PE/EtOAc, 5 : 1) to give equilibrating diastereomers **5** as colorless liquid (0.82, 80%). The spectra data of one of the isomers are as follows: δ_H (400 MHz, $CDCl_3$) 1.46 (3 H, s), 1.48 (9 H, s), 1.55 (3 H, s), 2.19–2.36 (1 H, m), 2.45–2.61 (1 H, m), 3.82–3.84 (1 H, m), 3.99–4.06 (2 H, m), 4.59–4.65 (m, 2 H), 4.81 (1 H, dhext, J = 4.0, 22.4 Hz), 5.51 (1 H, br d). δ_C (100 MHz, $CDCl_3$) 24.2, 27.4, 28.5, 37.9 (t, J = 92 Hz), 52.3, 68.8, 75.1, 82.7, 93.7, 127.2, 128.7, 153.1. δ_F (376 MHz, $CDCl_3$) -108.4 (AB quartet). HRMS: calcd for $C_{14}H_{24}F_2N_2NaO_6^+$ 377.1495, found 377.1489.

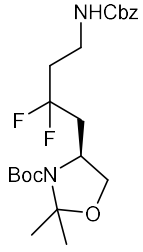
***tert*-butyl (4*S*)-4-(4-(((benzyloxy)carbonyl)amino)-2,2-difluoro-3-hydroxybutyl)-2,2-dimethyloxazolidine-3-carboxylate (6)**



Reduction of the nitro group of **5 (**1.2 g, 3.4 mmol**)** was achieved under catalytic hydrogenation conditions at 5 psi, 20 mL EtOH : HOAc (1 : 1) and ambient temperature with 20% $Pd(OH)_2/C$ (200 mg). The mixture was stirring for 2 d, filtered, the filtrate was evaporated under vacuum to quantitatively yield the free ε-amine crude

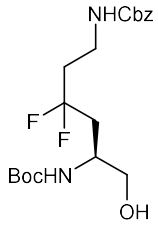
(1.3 g). To a stirred ice bath cooled solution of ε-amine crude (1.3 g) in 20 mL of acetone : H_2O (1 : 1) was added $KHCO_3$ (1.36 g, 13.6 mmol, 4.0 eq) and benzyloxycarbonylsuccinimde (1.02 g, 4.1 mmol, 1.2 eq). The reaction was allowed to warm to room temperature and was stirred for 1 h. After concentrating the reaction under vacuum, EtOAc : Et_2O (1 : 1, 20 mL) was added to the reaction mixture. The aqueous layer was extracted with EtOAc : Et_2O (1 : 1, 2 × 10 mL). The combined organic layer was washed with 1 M $KHSO_4$ (30 mL), saturated solution of $KHCO_3$ (30 mL), and brine (30 mL). And then the organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified using flash chromatography (PE/EtOAc, 5 : 1) conditions yielding **6** (1.1 g, 71% for 2 steps) as a colorless liquid. δ_H (400 MHz,$CDCl_3$) 1.42 (9 H, s), 2.06–2.20 (4 H, m), 3.39–3.42 (2 H, m), 3.63–3.64 (2 H, d, J = 4.0 Hz), 3.86–3.93 (1 H, m), 5.08 (2 H, br s), 5.23–5.26 (1 H, t, J = 5.6 Hz), 7.34 (5 H, br s). δ_C (100 MHz, $CDCl_3$) 24.2, 27.3, 28.3, 35.0, 36.7 (t, J = 92 Hz), 37.8 (t, J = 92 Hz), 48.0, 52.3, 66.9, 68.4, 81.6, 128.0, 128.1, 128.5, 136.4, 152.7, 156.9. δ_F (376 MHz, $CDCl_3$) -109.7, -109.9. HRMS: calcd for $C_{22}H_{32}F_2N_2NaO_6^+$ 481.2121, found 481.2134.

***tert*-butyl (*S*)-4-(4-(((benzyloxy)carbonyl)amino)-2,2-difluorobutyl)-2,2-dimethyl oxazolidine-3-carboxylate (7)**



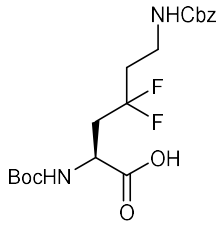
Compound **7** was synthesised starting from **6** (1.06 g, 2.3 mmol) and benzoyl peroxide (0.38 g, 1.6 mmol, 1.0 eq) following the procedure described for **4**, the pure product **7** was obtained (PE/EtOAc, 15 : 1) as a colorless liquid (0.52 g, 51% over two steps). δ_H (400 MHz, $CDCl_3$) 1.46 (12 H, s), 1.53 (3 H, s), 2.03–2.23 (4 H, m), 3.43–3.47 (2 H, m), 3.88–3.99 (2 H, m), 4.08–4.16 (1 H, m), 5.10 (2 H, s), 7.35 (5 H, br s). δ_F (376 MHz, $CDCl_3$) -95.4 (AB quartet). HRMS: calcd for $C_{22}H_{32}F_2N_2NaO_5^+$ 465.2171, found 465.2168.

benzyl *tert*-butyl (3,3-difluoro-6-hydroxyhexane-1,5-diyl)(*S*)-dicarbamate (8)



A solution of **7** (0.58 g, 1.3 mmol) in 8 mL HOAc : H_2O (3 : 1) was stirred for 2 d at room temperature. After the reaction completed, EtOAc (5 mL) and water (5 mL) was added to the mixture. The aqueous layer was extracted with EtOAc (10 mL) for two times. The organic layer was combined and washed with saturated $NaHCO_3$ (20 mL), saturated brine (20 mL) and H_2O (20 mL). The organic layer was dried over Na_2SO_4 anhydrous, filtered, and concentrated under vacuum to give the crude. The product was purified using flash chromatography (PE/EtOAc, 1 : 1) conditions yielding compound **8** (0.48 g, 90%) as a colorless solid. δ_H (400 MHz, $CDCl_3$) 1.42 (9 H, s), 2.02–2.23 (4 H, m), 3.09 (1 H, br s), 3.39–3.42 (2 H, m), 3.63–3.64 (2 H, d, J = 4.0 Hz), 3.85–3.94 (1 H, m), 5.07 (2 H, s), 5.08–5.13 (1 H, m), 5.24 (1 H, t, J = 5.6 Hz), 7.33 (5 H, br s). δ_C (100 MHz, $CDCl_3$) 28.5, 35.1, 36.8 (t, J = 96 Hz), 37.9 (t, J = 96 Hz), 48.0, 65.0, 66.9, 80.0, 128.0, 128.2, 128.6, 136.5, 156.0, 156.6. δ_F (376 MHz, $CDCl_3$) -95.7 (AB quartet). HRMS: calcd for $C_{19}H_{28}F_2N_2NaO_5^+$ 425.1858, found 425.1842.

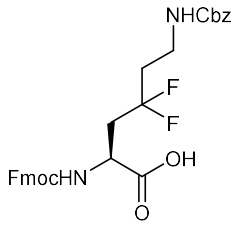
(*S*)-6-(((benzyloxy)carbonyl)amino)-2-((*tert*-butoxycarbonyl)amino)-4,4-difluorohexanoic acid (9)



To a solution of **8** (0.37, 0.93 mmol) in 30 mL of CH_3CN : H_2O (1 : 1) was added Ru(III)Cl_3 H_2O (cat). Then $NaIO_4$ (0.78 g, 3.7 mmol) was added to the stirred greenish brown solution. The stirring was stopped after the addition was complete. After 20 min, a white precipitate had formed. After 3 h, EtOAc : Et_2O (1 : 1, 30 mL) and 10% $NaHSO_3$ solution (30 mL) were added to the reaction. The organic layer was washed with additional 10% $NaHSO_3$ (30 mL), saturated brine (30 mL) and H_2O (30 mL). The organic layer was dried over Na_2SO_4 anhydrous, filtered, and concentrated under vacuum to give the crude. The crude product was purified using flash chromatography (CH_2Cl_2/EtOH, 10 : 1) conditions

yielding **9** (0.27 g, 72%) as a colorless liquid. δ_H (400 MHz, $CDCl_3$) 1.46 (9 H, s), 2.02–2.20 (2 H, m), 2.28–2.57 (2 H, m), 3.35–3.46 (2 H, m), 4.49–4.57 (1 H, m), 5.09 (2 H, s), 5.11–5.21 (1 H, m), 5.37 (1 H, d, J = 7.6 Hz), 7.33 (5 H, br s), 8.53 (1 H, br s). δ_C (100 MHz, $CDCl_3$) 28.4, 34.9, 36.9 (t, J = 96 Hz), 38.2 (t, J = 96 Hz), 49.2, 67.1, 80.8, 128.2, 128.3, 128.7, 136.4, 155.7, 156.7, 174.7. δ_F (376 MHz, $CDCl_3$) -95.9 (AB quartet). HRMS: calcd for $C_{19}H_{26}F_2N_2NaO_6^+$ 439.1651, found 439.1668.

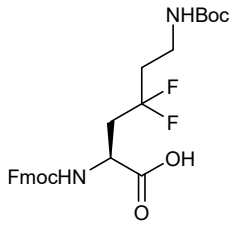
(*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-6-(((benzyloxy)carbonyl)amino) -4,4-difluorohexanoic acid (10)



To a solution of **9** (142 mg, 0.34 mmol) in 6 mL of DCM was added TFA (600 mL). The mixture was stirred overnight. When the reaction complete, evaporated the solvent and TFA under vacuum to give compound the free β-amine (156 mg). The free β-amine (156 mg) was dissolved in 8 mL CH_3CN : H_2O (1 : 1), the solution was cooled to 0 °C, $NaHCO_3$ (57 mg, 0.68 mmol, 2.0 eq) and benzyloxycarbonylsuccinimde (0.11 g, 0.34 mmol). The reaction was warmed to room temperature and stirred for 2 h. After the reaction complete, DCM (10 mL) and water (10 mL) was added to reaction mixture. The organic layer was washed by 1 M $KHSO_4$ (10 mL) and brine (10 mL). Following the washes the organic layer was dried over Na_2SO_4 anhydrous, filtered, and concentrated under vacuum. The crude product was purified using flash chromatography (CH_2Cl_2/EtOH, 10 : 1) conditions yielding **10** (120 mg, 66%, over 2 steps) of a pale yellow solid. δ_H (400 MHz, CD_3OD) 2.03–2.17 (2 H, m, $CH_2CH_2CF_2$), 2.19–2.34 (1 H, m, $CHCH_2CF_2$), 2.34–2.45 (1 H, m, $CHCH_2CF_2$), 3.21–3.31 (2 H, m, $NHCH_2$), 4.06–4.18 (2 H, m, $ArCH_2O$), 4.24–4.34 (2 H, m, NHC*H*, OCH_2CH), 4.96 (2 H, br s, $PhCH_2O$), 7.17–7.32 (9 H, m, Ar-H), 7.55 (2 H, d, J = 7.6 Hz, Ar-H), 7.68 (2 H, d, J = 7.6 Hz, Ar-H). δ_C (100 MHz, CD_3OD) 29.2, 35.8, 37.5 (t, J = 92 Hz, CH_2CF_2), 39.5 (t, J = 92 Hz,

CH_2CF_2), 52.5, 67.5 (Ar*C*O), 68.1 (Ar*C*O), 120.9, 126.2, 126.3, 128.1, 128.8, 128.9, 129.4, 138.1, 142.5, 145.2, 158.4 (*C*=O), 158.7 (*C*=O), 179.04 (*C*OOH). δ_F (376 MHz, CD_3OD) -96.3 (AB quartet). HRMS: calcd for $C_{29}H_{28}F_2N_2NaO_6^+$ 561.1808, found 561.1816.

(*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-6-((*tert*-butoxycarbonyl)amino)-4,4-difluorohexanoic acid (1)



Removal of the benzyloxycarbonyl protecting group of **10** (120 mg, 0.22 mmol) was achieved under catalytic hydrogenation conditions at 5 psi and Pd/C to quantitatively yield the free ε-amine (110 mg). The free ε-amine (110 mg) was dissolved in 8 mL CH_3CN : H_2O (1 : 1), the solution was cooled to 0 °C, then $KHCO_3$ (44.6 mg, 0.44 mmol 2.0 eq) and Boc_2O (48 mg, 0.22 mmol) was added. The reaction was warmed to room temperature and stirred for 2 h. After the reaction complete, DCM (10 mL) and water (10 mL) was added to reaction mixture. The organic layer was washed by brine (10 mL). Following the washes the organic layer was dried over Na_2SO_4 anhydrous, filtered, and concentrated under vacuum. The crude product was purified using flash chromatography (CH_2Cl_2/EtOH, 10 : 1) conditions yielding **1** (45 mg, 40% over 2 steps) of a pale yellow solid. δ_H (400 MHz, CD_3OD) 1.36 (9 H, s, (C*H*$_3$)$_3$C), 1.97–2.16 (2 H, m, $CH_2CH_2CF_2$), 2.18–2.32 (1 H, m, $CHCH_2CF_2$), 2.42–2.59 (1 H, m, $CHCH_2CF_2$), 3.22 (2 H, t, *J* = 7.2 Hz, $NHCH_2$), 4.12–4.24 (2 H, m, $ArCH_2O$), 4.26–4.36 (2 H, m, NHC*H*, OCH_2CH), 7.24 (2 H, t, *J* = 7.6 Hz, Ar-H), 7.33 (2 H, t, *J* = 7.6 Hz, Ar-H), 7.60 (2 H, d, *J* = 7.6 Hz, Ar-H), 7.73 (2 H, d, *J* = 7.6 Hz, Ar-H). δ_C (100 MHz, CD_3OD) 28.8, 35.3, 37.7 (t, *J* = 96 Hz, CH_2CF_2), 39.5 (t, *J* = 96 Hz, CH_2CF_2), 52.3, 68.1 (Ar*C*O), 80.2, 120.9, 125.1,126.2, 126.3, 128.1, 128.7, 142.5, 145.2, 145.3, 158.3 (*C*=O), 158.4 (*C*=O), 178.4 (*C*OOH). δ_F (376 MHz, CD_3OD) -96.9 (AB quartet). HRMS: calcd for $C_{26}H_{30}F_2N_2NaO_6^+$ 527.1964, found 527.1962.

3. Expression and purifcation of histone lysine methyltransferases and acetyltransferases

Human KMTs enzymes were expressed and purified as previously described.[1] The GLP plasmid (residues 951–1235) was transformed into *Escherichia coli* Rosetta BL21 (DE3)pLysS cells. Cultures were grown at 37 °C in LB media containing kanamycin and chloramphenicol. Cells were grown to an OD_{600} of 0.5–0.6 (approximately 2.5–3 h), at which point they induced by isopropyl β-D-1-thiogalactopyranoside (IPTG) and they were transferred to a temperature of 16 °C overnight. After letting the cells grow at this temperature, they were then harvested and lysed by sonication. The lysate was centrifuged at high-speed to remove unbroken cells. The supernatant was then centrifuged to further clean the lysate. Purification of the N-terminally his6-tagged GLP was performed using Ni–NTA affinity chromatography column, which was prewashed with lysis buffer. GLP enzyme was then eluted using a linear gradient concentration of imidazole. The elute was then concentrated with centrifugal concentrators (Millipore). GLP was further purified by gel filtration on a Superdex 75 column (GE Healthcare) on an AKTA system. Purified GLP concentrated employing Amicon Ultra Centrifugal Filter Units (Millipore) with suitable molecular weight cutoffs. Proteins concentrations were determined using the Nanodrop DeNovix DS-11 spectrophotometer and the purity was monitored by SDS-PAGE on a 4–15% gradient polyacrylamide gel (Bio-Rad).

Plasmid carrying recombinant His-tagged Human GCN5 catalytic domain (residues 497–662 in pET28a-LIC vector) was purchased from Addgene (25,482). The protein was expressed and purified as previously described.[2] Briefly, E. coli BL21(DE3) cells enriched with hGCN5 WT plasmid were cultured in TB growth media supplemented with 50 μg/mL kanamycin at 37 °C to an OD_{600} of 0.6, upon which expression was induced by addition of IPTG (0.5 mM final) and followed by incubation at 16 °C overnight. Harvested cells were pelleted and re-suspended into 50 mM Na_2HPO_4 pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM β-ME lysis buffer in presence of protease inhibitor cocktail (Roche, Basel, Switzerland) and lysate by sonication. The supernatant was incubated with Ni–NTA

beads for 2 h at 4 °C. The beads were loaded on a gravity flow column and washed with 20 mM HEPES–NaOH pH 7.5, 500 mM NaCl, 50 mM imidazole, 5% glycerol, 1 mM β-ME. Subsequently, the protein was eluted with 20 mM HEPES–NaOH pH 7.5, 500 mM NaCl, 250 mM imidazole, 5% glycerol, 1 mM β-ME and the buffer was exchanged to 20 mM HEPES–NaOH pH 7.5, 150 mM NaCl, 1 mM βME by concentration with a 10 kDa spinfilter device (AMICON, 10 MWCO). Purity of the eluted protein was assessed with SDS-page, and GCN5 was separated in aliquots, rapidly flash-frozen and stored at -80 °C.

4. Histone peptide synthesis

The H3 13mer peptide (ARTKQTAR**X**^{9}STGG) was chain assembled on Rink amide resin using microwave assisted SPPS on a Liberty Blue peptide synthesizer (CEM corporation, Matthews, NC, USA) up until the underlined K9 position. Amino acid couplings were carried out with the equivalent ratio of [5]:[5]:[7.5] of [Fmoc-protected amino acid]:[DIC]:[Oxyma Pure] at 75 °C for 2 minutes. Then, **(10)** was coupled by manual SPPS with the equivalent ratio of [2]:[2]:[3.5] of [Fmoc-protected amino acid]:[HATU]:[DIPEA] overnight. Full conversion was checked by Kaiser test and further amino acids were coupled manually with the equivalent ratio of [5]:[5]:[7.5] of [Fmoc-protected amino acid]:[HATU]:[DIPEA] for 1 hour. The peptide proceeded to standard cleavage from resin using 0.5% TIPS, 0.5% H_2O in conc. TFA for 3 hours. TFA was blown off using N_2 and the resultant residue suspended in cold Et_2O. After suspension it was subjected to centrifugation for 5 minutes at 5000 rpm in an Eppendorf 5804R centrifuge (Eppendorf, Hamburg, Germany) after which the supernatant was decanted into the waste. The remaining white to yellow solid was washed twice by cold Et_2O and subjected to centrifugation after which the crude peptide was dissolved in a mixture of ACN in H_2O and purified using preparative reverse-phase HPLC (RP-HPLC) using a gradient of H_2O + 0,1% TFA and ACN + 0,1% TFA from 5% ACN to 30% ACN over 30 minutes at 4 ml/min using a Gemini 10µm NX-C18 110Å LC column (Phenomenex, Torrance, CA, USA). Analytical RP-HPLC

was carried out on a Gemini 5µm C18 110Å LC column (Phenomenex) at a flow rate of 1 mL/min. Analytical injections were monitored at 215 nm. The mass of the peptide was confirmed by mixing with a 1:2 with α-Cyano-4-hydroxycinnamic acid (CHCCA) matrix and loaded onto an MTP 384 polished steel target to be analyzed by a MALDI-TOF UltrafleXtreme-II tandem mass spectrometer (Bruker).

5. MALDI-TOF MS enzymatic assays

Histone methyltransferase assays were carried out as described in 30 µL final total volume.[3] In brief, purified GLP (5 µM) was incubated with 100 µM of purified histone peptides in the methyltransferase assay buffer Tris–HCl (pH 8.0) and methyl donor SAM (500 µM) at 37 °C. Histone acetyltransferase assays were carried out by incubating peptides under standard conditions (2 µM GCN5, 100 µM peptide, 300 µM AcCoA) in the reaction buffer (50 mM HEPES, 0.1 mM EDTA, 1 mM DTT, pH=8.0). The reactions were carried out in a final volume of 50 µL. All reactions were shaken in a Thermomixer C at 750 rpm, at 37 °C. All reactions were quenched by the addition of TFA 10% in MilliQ water at different time points. All reactions were aliquoted and mixed 1:2 with a solution of α-Cyano-4-hydroxycinnamic acid (CHCCA) in 1:1 MQ and ACN (0.1% TFA) and loaded onto an MTP 384 polished steel target to be analyzed by a UltrafleXtreme-II tandem mass spectrometer (Bruker, Billerica, MA, USA).

6. MALDI-TOF MS kinetic assays

Histone lysine methyltransferase kinetics studies were performed as described.[3] A solution of histone peptide (0-200 µM), was added to a solution of SAM (5 µM) in assay buffer (50 mM Tris, pH 8.0) at room temperature (final volume of 30 µL). The reaction was then initiated by the addition of GLP (100 nM) and shaken for 5 min. Histone lysine acetyltransferase kinetics evaluation was carried out under steady-state conditions. Histone peptides (0-750 µM) were incubated with AcCoA (100 µM)

and the reactions were started by the addition of the GCN5 (50 nM) in a final volume of 20 µL in kinetic buffer (50 mM HEPES, 0.1 mM EDTA, 0.01% TRITON-X, pH=7.4). Steady state conditions were guaranteed by employing saturating concentrations of AcCoA (>5×Km value). AcCoA and SAM stock solutions in milli-Q water were calibrated with a NanoDrop 2000 spectrophotometer (Thermo Scientifc, Waltham, MA, USA). Reactions were incubated at 37 °C, shaken at 750 rpm and quenched with TFA 10% in milli-Q water at different time points, within linear production of monomethylated or acetylated peptides extrapolated from timecourse plots. All reactions were aliquoted and mixed 1:2 with a solution of α-cyano-4-hydroxycinnamic acid (CHCCA) in 1:1 MQ and ACN (0.1% TFA) and loaded onto MTP 384 polished steel target to be analyzed by UltrafeXtreme-II tandem mass spectrometer (Bruker). The amount of methylated or acetylated peptide was calculated by integration of the product peak area and divided it by the amount of unmodified peptide, taking in account all the ionic species, at any concentration points using the FlexAnalysis sofware. Kinetic values were extrapolated by fitting V0 values (µM of produced peptide per minutes) and histone peptide concentrations to the Michaelis–Menten equation using the GraphPad Prism 5 software. Kinetic experiments were carried out in duplicates and final values are reported as value±SD.

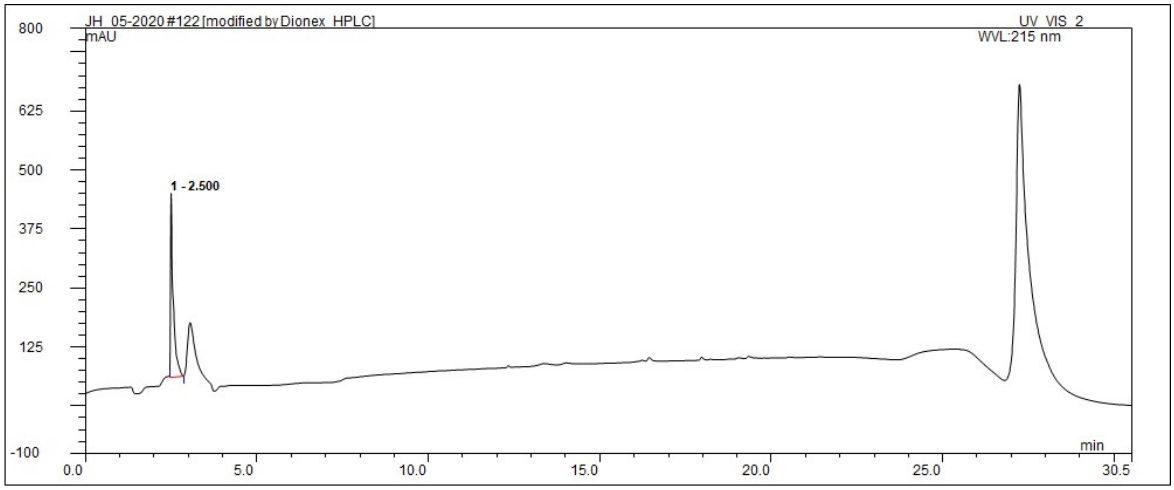
7. NMR assays

Histone lysine methyltransferase NMR studies were carried out in a total volume of 500 µL. GLP (10 µM) was added to a solution of histone peptide (400 µM) and SAM (2 mM) in 50 mM Tris-D_{11} buffer pH 8.0. Histone lysine acetyltransferase NMR studies were carried out in a total volume of 500 µL. GCN5 (10 µM) was added to a solution of histone peptide (400 µM) and AcCoA (2 mM) in 50 mM Tris-D_{11} buffer pH 8.0. After shaking at 750 rpm for 3 hours at 37 °C in Eppendorf, the reaction mixture was transferred to an NMR tube. NMR spectroscopy was conducted on a Bruker Advance III equipped with a 500 MHz magnet and a Prodigy cryoprobe.

Similarly, inhibition of GLP-catalysed methylation was analysed under the same conditions with 30 minutes of preincubation with SAH (2 mM), while inhibition of GCN5-catalysed acetylation was analysed with 10 minutes of preincubation with 300 μM of CoASH.

8. Characterization of histone peptides

A)



B)

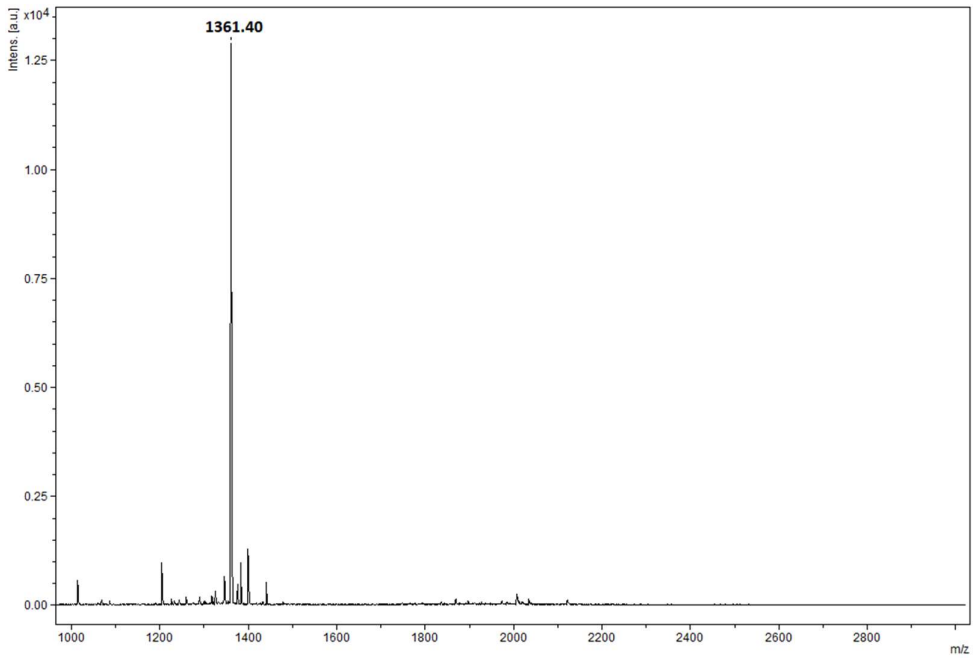
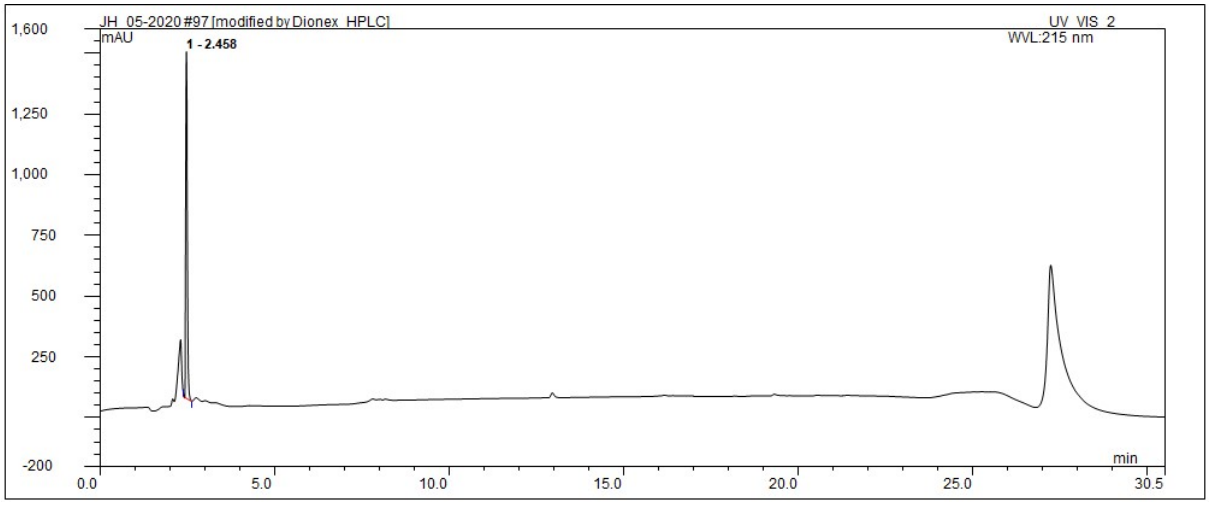


Figure S1. A) Analytical HPLC of the 13-mer H3K9 peptide after RP-HPLC purification. **B)** MALDI-TOF MS spectra of the purified 13-mer H3K9 peptide (Calc. Mass 1361.55, $C_{54}H_{102}N_{23}O_{18}^{+}$).

A)



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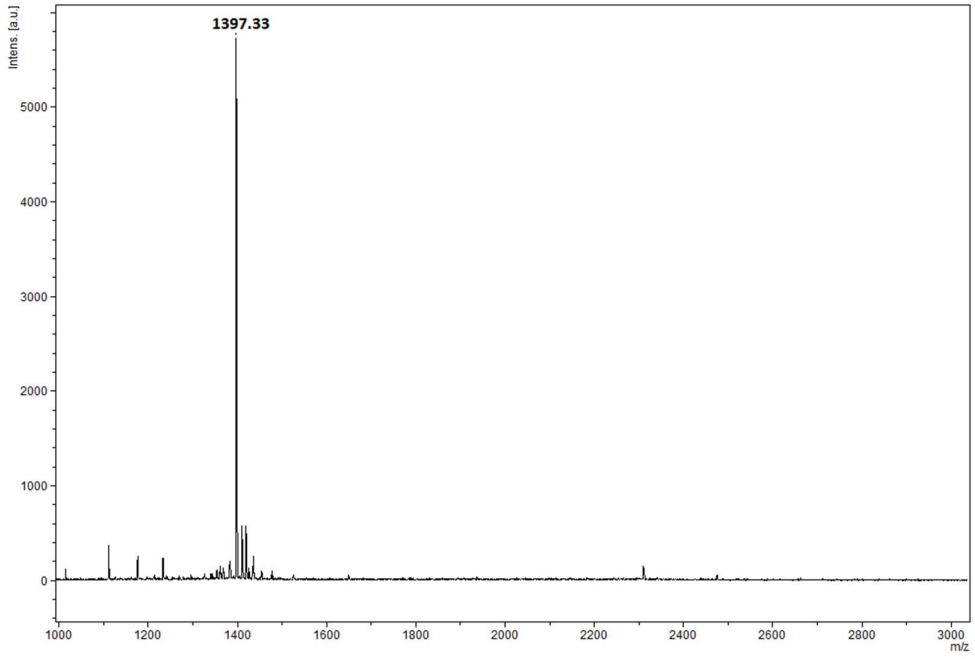


Figure S2. A) Analytical HPLC of the 13-mer H3K$_{diF}$9 peptide after RP-HPLC purification. **B)** MALDI-TOF MS spectra of the purified 13-mer H3K$_{diF}$9 peptide (Calc. Mass 1397.75, $C_{54}H_{100}F_2N_{23}O_{18}^+$).

9. MALDI-TOF MS supporting figures

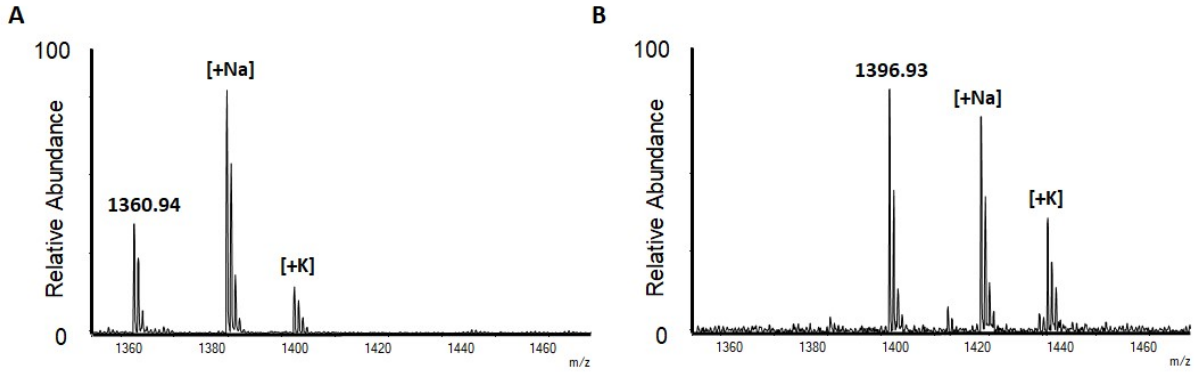


Figure S3 MALDI-TOF MS data showing control reaction of a) H3K9 and b) H3K$_{diF}$9 in the presence of SAM after 3 hours.

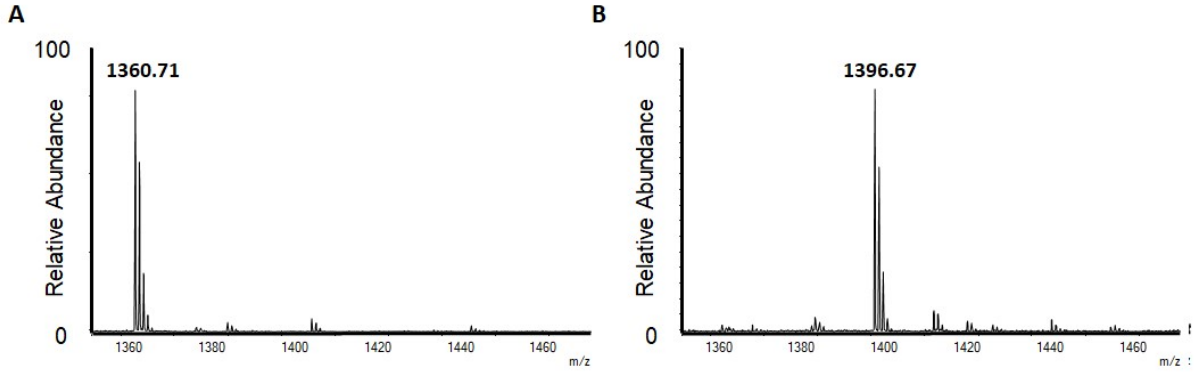


Figure S4 MALDI-TOF MS data showing control reaction of a) H3K9 and b) H3K$_{diF}$9 in the presence of AcCoA after 3 hours.

10. Kinetic analysis supporting figures

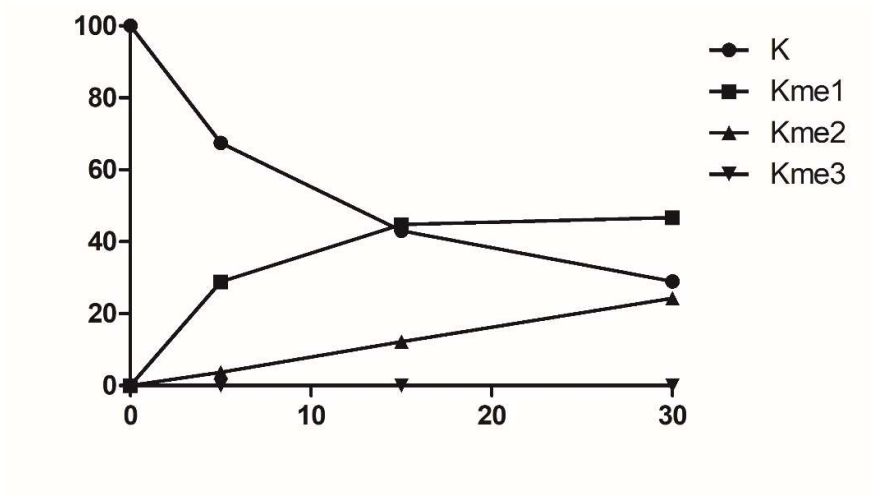


Figure S5. Time-course plot of GLP-catalysed methylation with GLP (100 nM), SAM (5 μM) and H3K9 (10 μM).

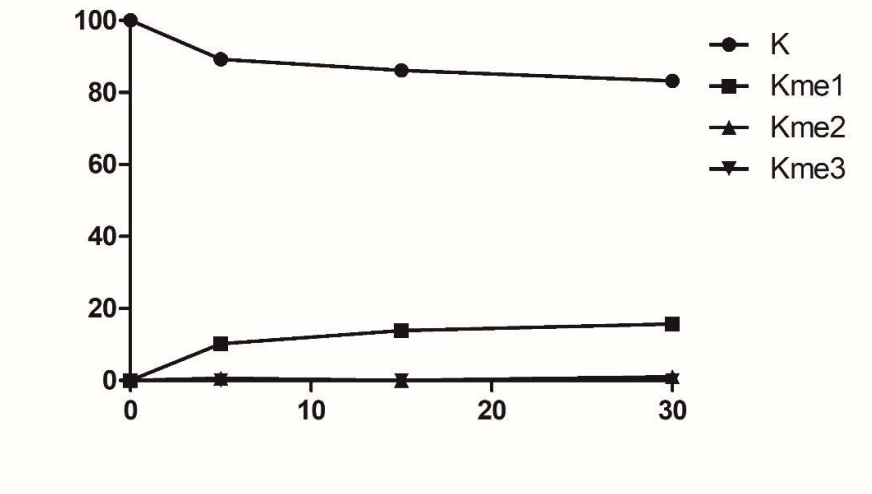


Figure S6. Time-course kinetic plot of GLP-catalysed methylation with GLP (100 nM), SAM (5 μM) and H3K9 (50 μM).

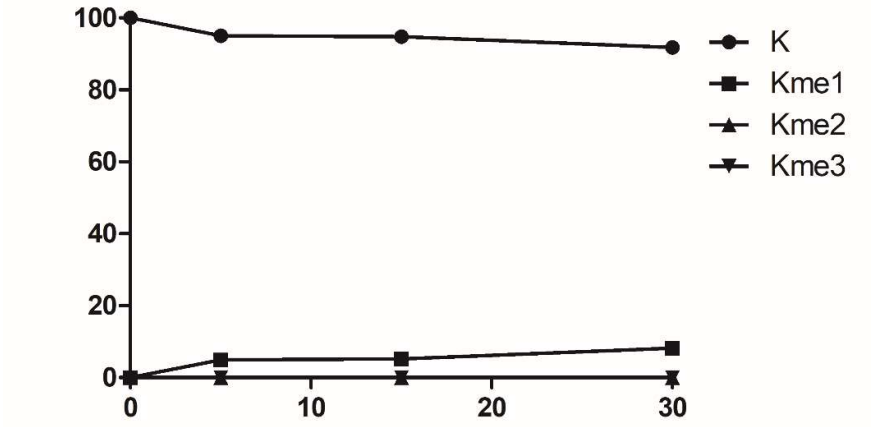


Figure S7. Time-course kinetic plot of GLP-catalysed methylation with GLP (100 nM), SAM (5 μM) and H3K9 (100 μM).

11. NMR enzymatic analysis supporting figures

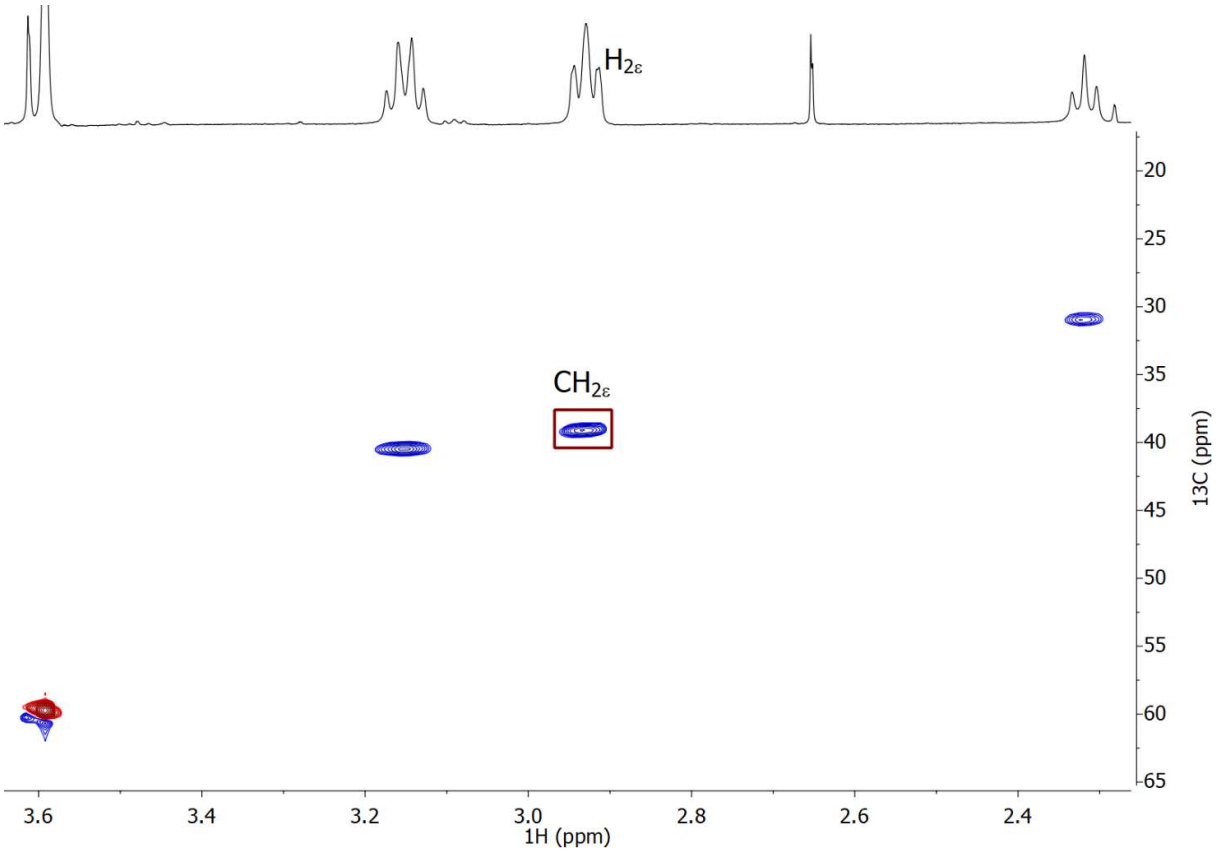


Figure S8. HSQC (red/blue) of H3K9 in 50 mM Tris-D_{11} pH 8.0.

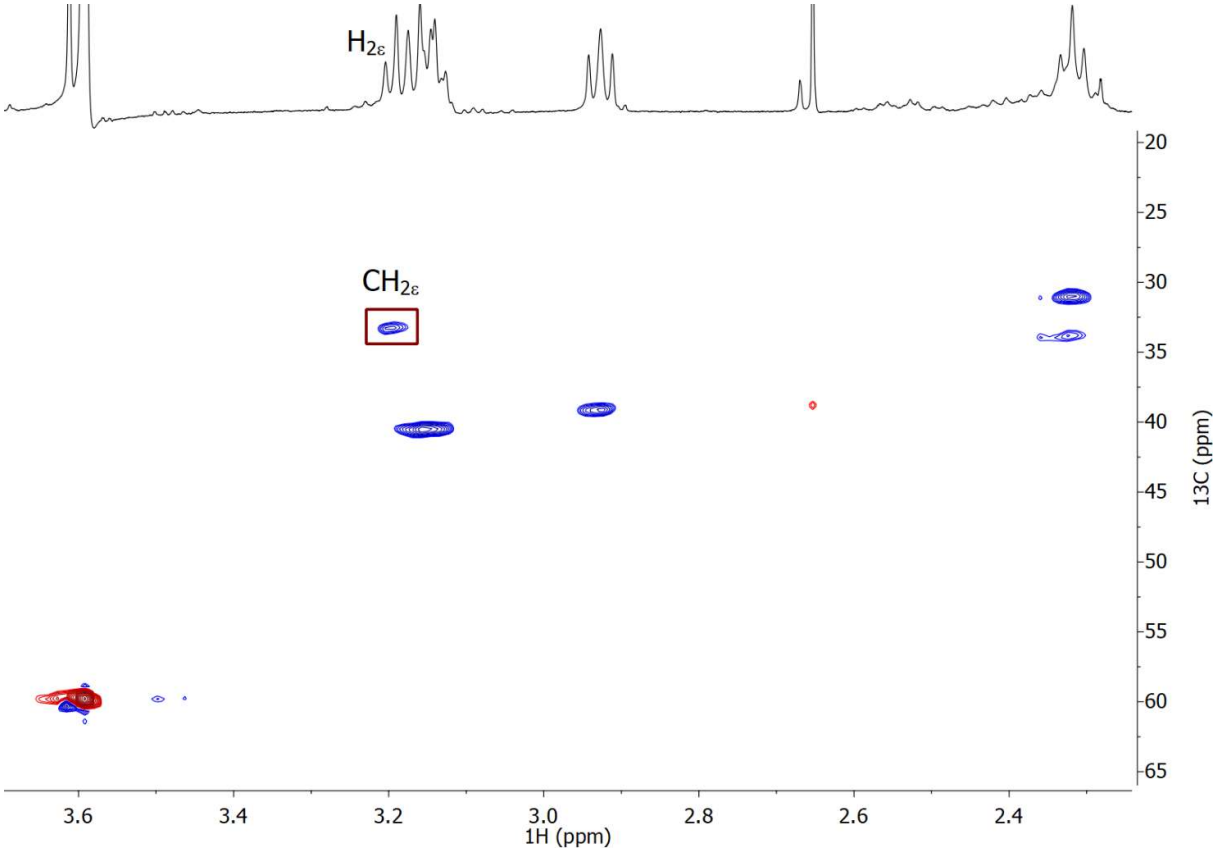


Figure S9. HSQC (red/blue) of H3K_{diF}9 in 50 mM Tris-D_{11} pH 8.0.

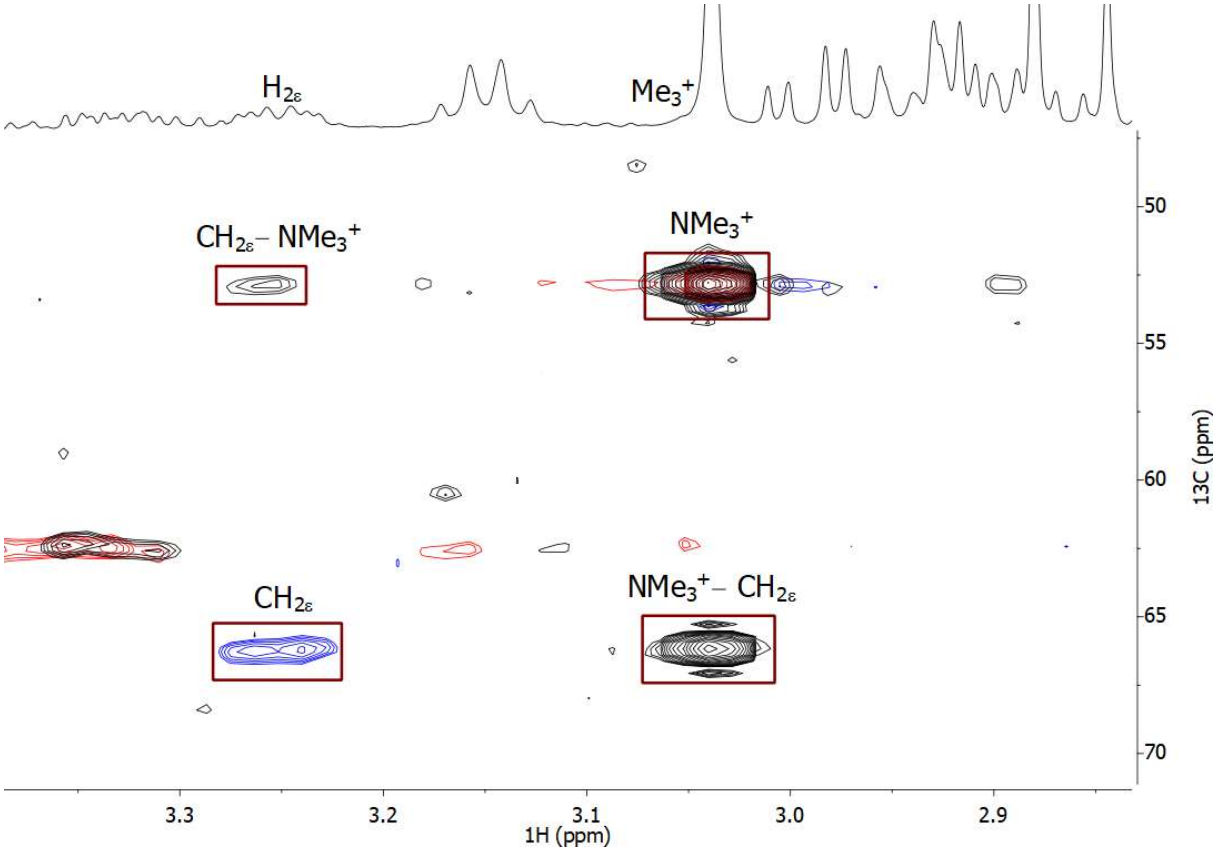


Figure S10. HSQC (red/blue)/HMBC (grey) overlay of H3K9 after incubation with GLP and SAM (400 µM peptide, 10 µM enzyme, 2 mM SAM, 50 mM Tris-D_{11} pH 8.0).

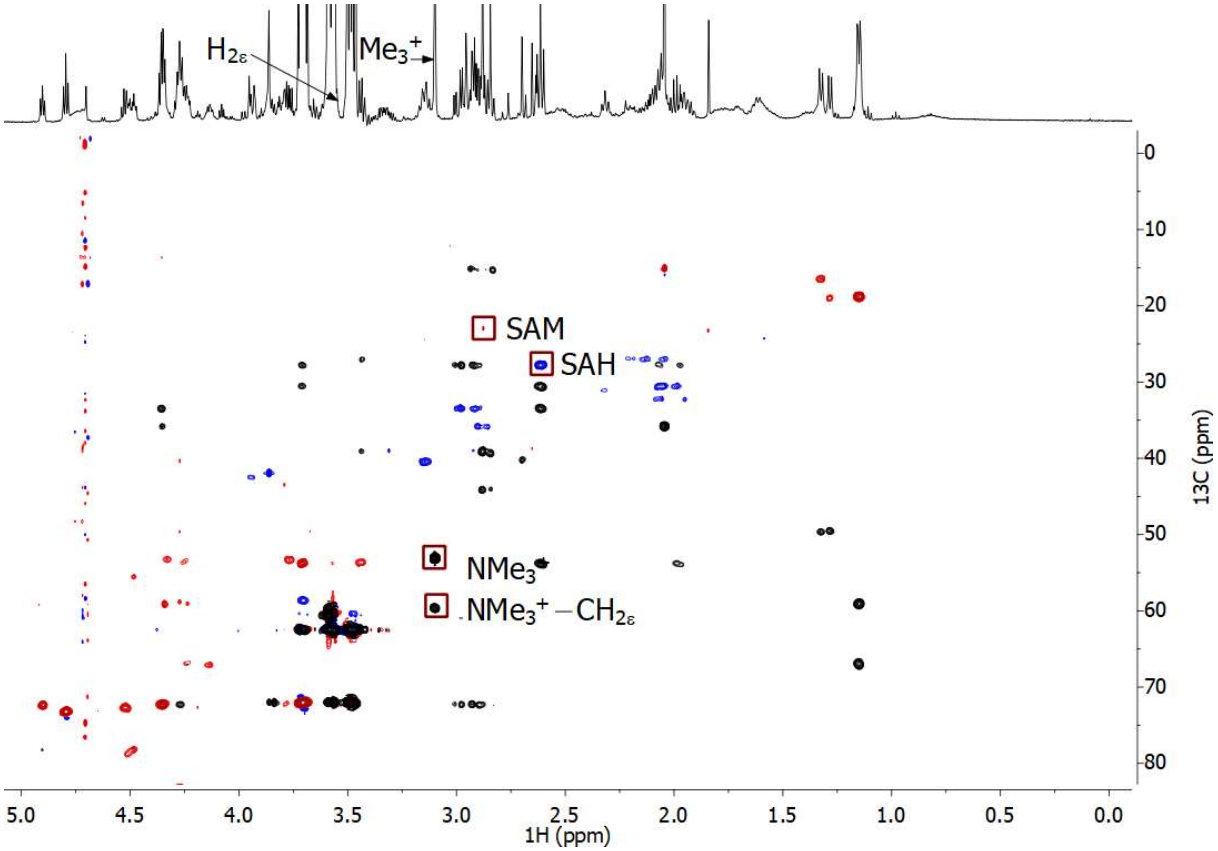


Figure S11. HSQC (red/blue)/HMBC (grey) full spectra overlay of $H3K_{diF}9$ after incubation with GLP and SAM (400 µM peptide, 10 µM enzyme, 2 mM SAM, 50 mM Tris-D_{11} pH 8.0).

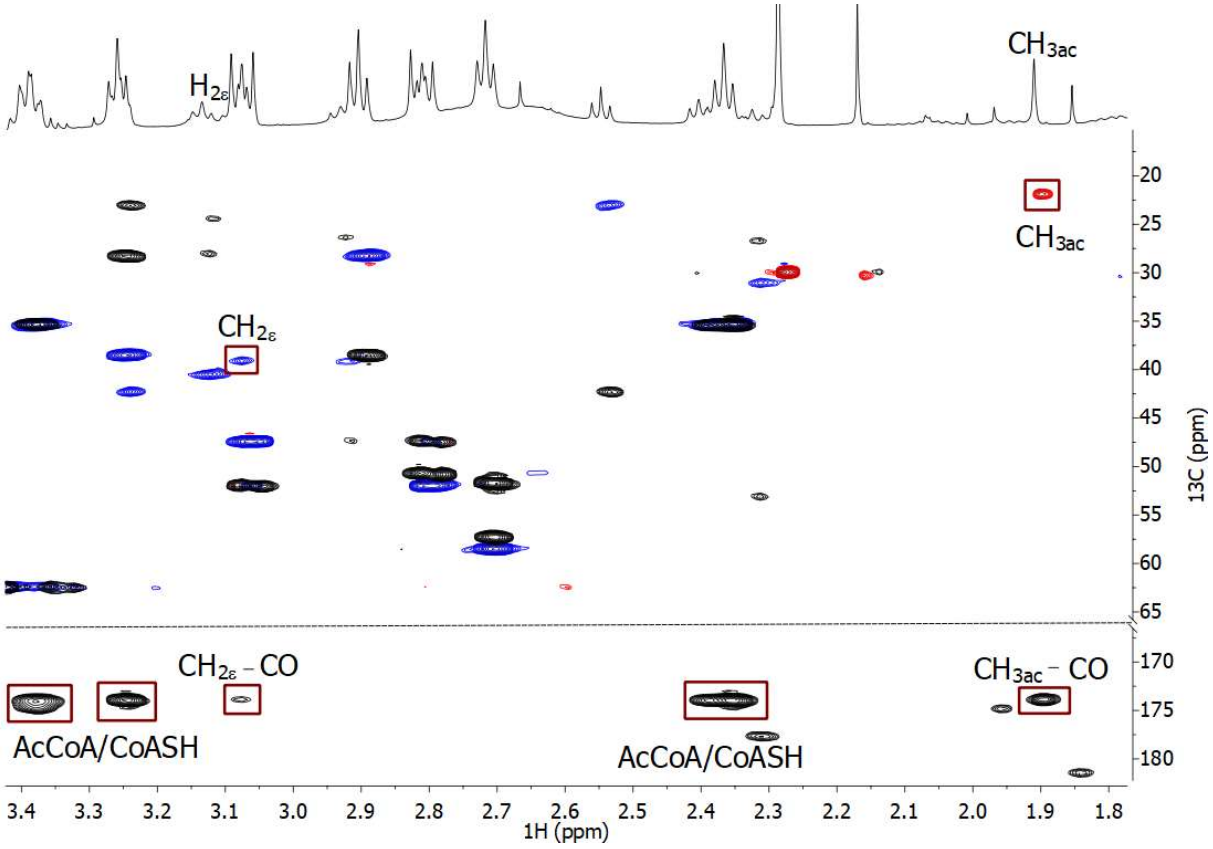


Figure S12. HSQC (red/blue)/HMBC (grey) overlay of H3K9 after incubation with GCN5 and AcCoA (400 µM peptide, 10 µM enzyme, 2 mM AcCoA, 50 mM Tris-D_{11} pH 8.0).

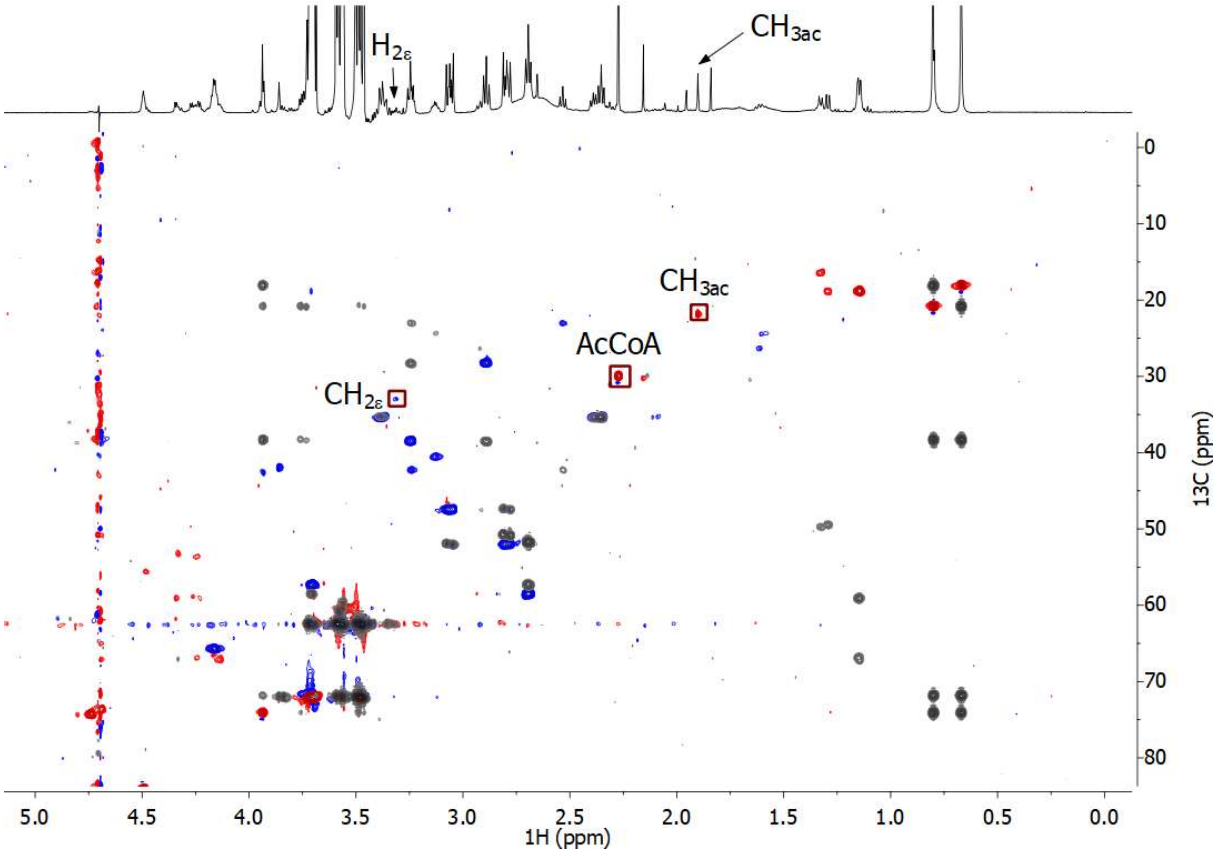


Figure S13. HSQC (red/blue)/HMBC (grey) full spectra overlay of $H3K_{diF}9$ after incubation with GCN5 and AcCoA (400 µM peptide, 10 µM enzyme, 2 mM AcCoA, 50 mM Tris-D_{11} pH 8.0).

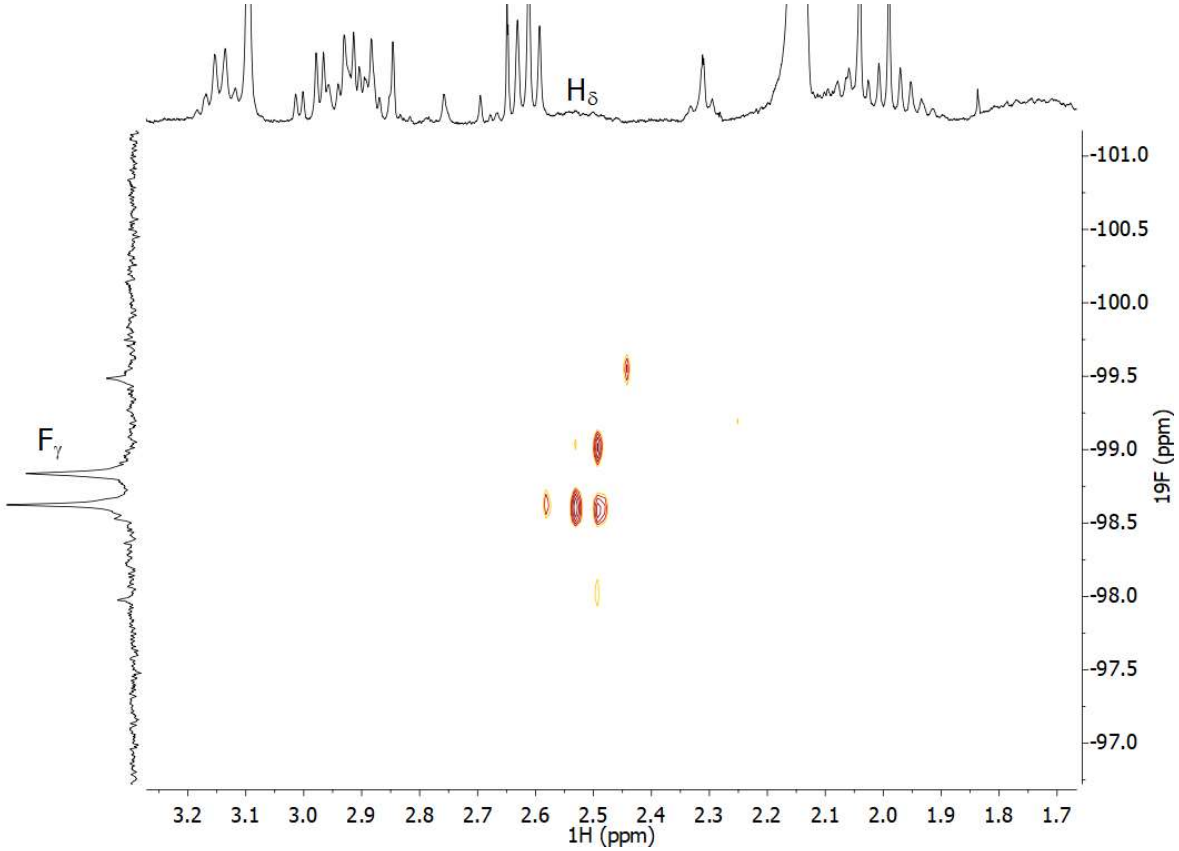


Figure S14. H/F HMBC of H3K_{diF}9 after incubation with GLP and SAM (400 µM peptide, 10 µM enzyme, 2 mM SAM, 50 mM Tris-D_{11} pH 8.0).

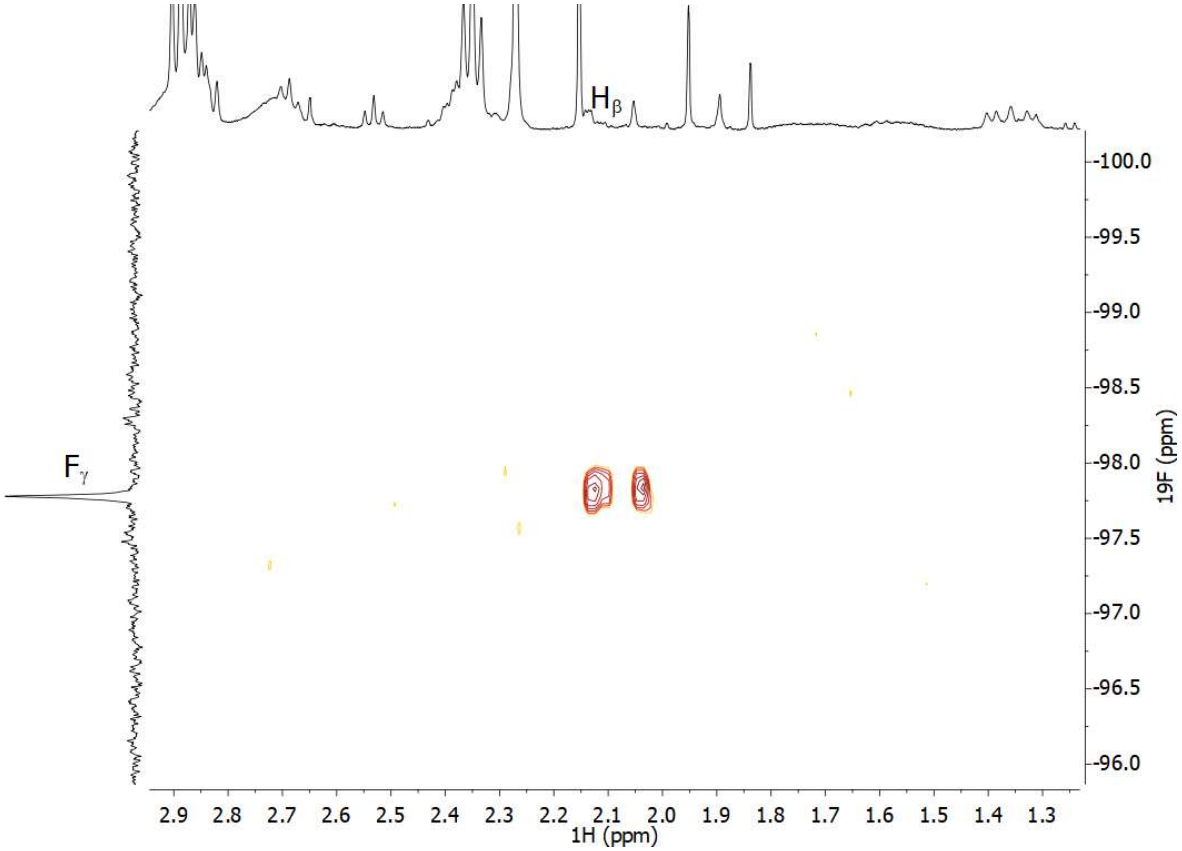


Figure S15. H/F HMBC of H3K_{diF}9 after incubation with GCN5 and AcCoA (400 µM peptide, 10 µM enzyme, 2 mM AcCoA, 50 mM Tris-D_{11} pH 8.0).

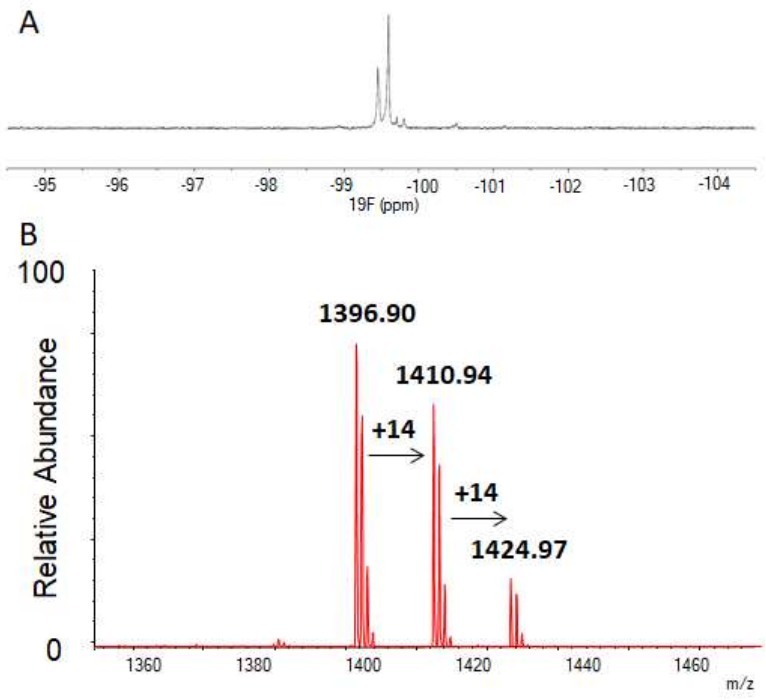


Figure S16. Inhibition of GLP by SAH. a) ^{19}F NMR data showing GLP-catalysed methylation of $H3K_{diF}9$ in the presence of SAM and inhibited by SAH. Sample was referenced to TFA with $H3K_{diF}9me1$ at -99.5 ppm and $H3K_{diF}9$ at -99.6 ppm. b) MALDI-TOF MS data showing GLP-catalysed methylation of $H3K_{diF}9$ inhibited by SAH.

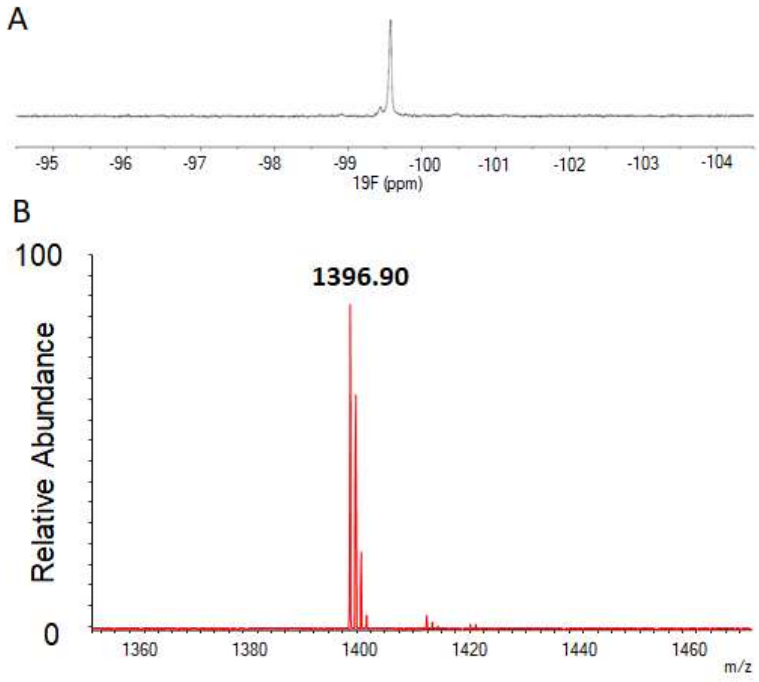
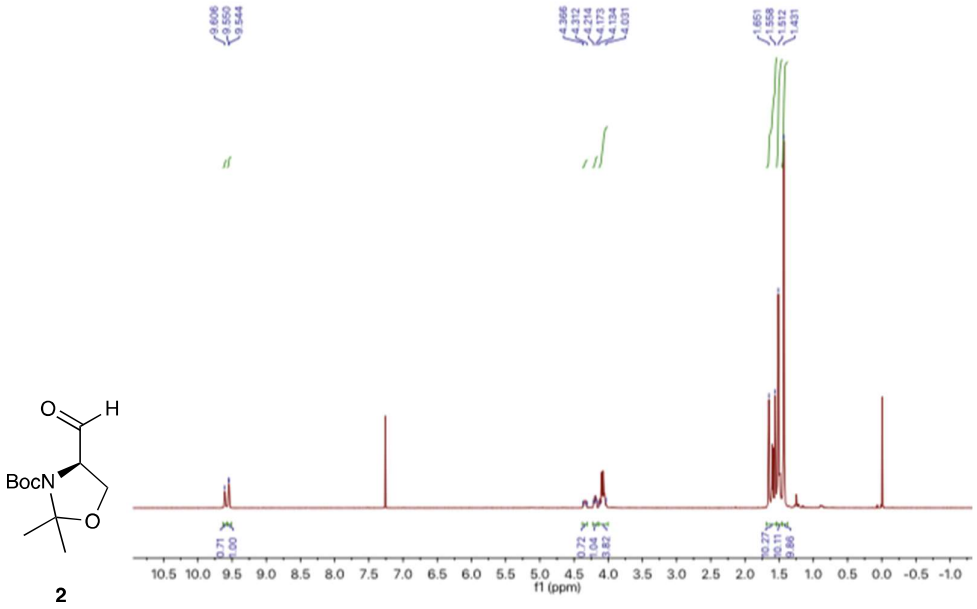
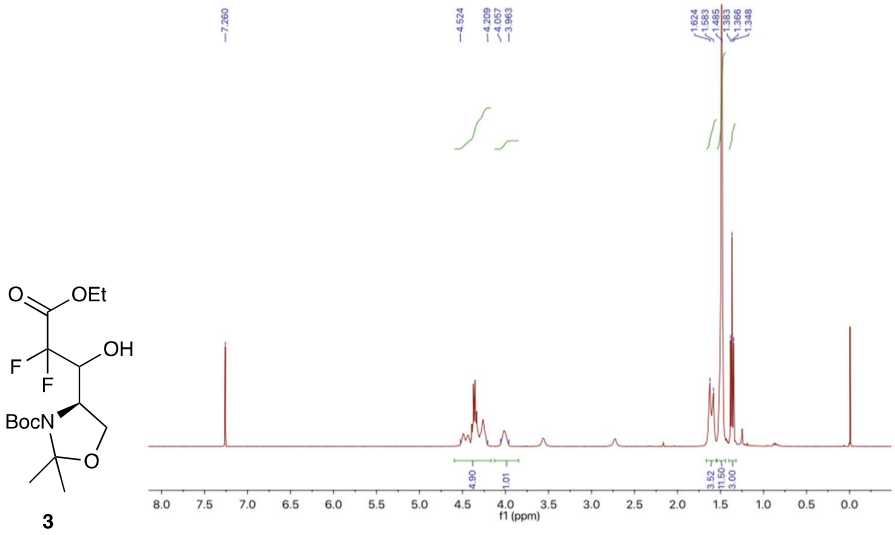
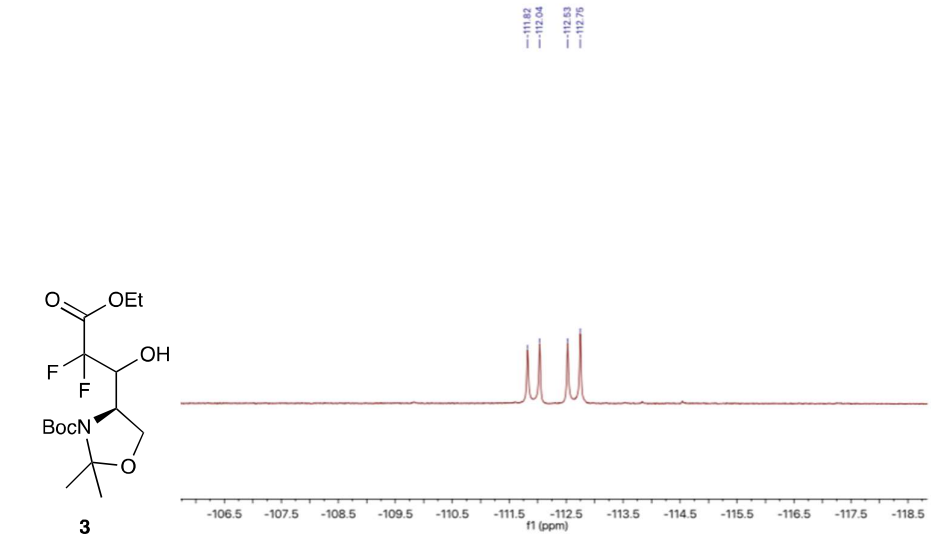


Figure S17. Inhibition of GCN5 by CoASH. a) ^{19}F NMR data showing GCN5-catalysed acetylation of $H3K_{diF}9$ in the presence of AcCoA and inhibited by CoASH. Sample was referenced to TFA with $H3K_{diF}9$ at -99.6 ppm. b) MALDI-TOF MS data showing GCN5-catalysed acetylation of $H3K_{diF}9$ inhibited by CoASH.

12. NMR spectra of synthesised compounds



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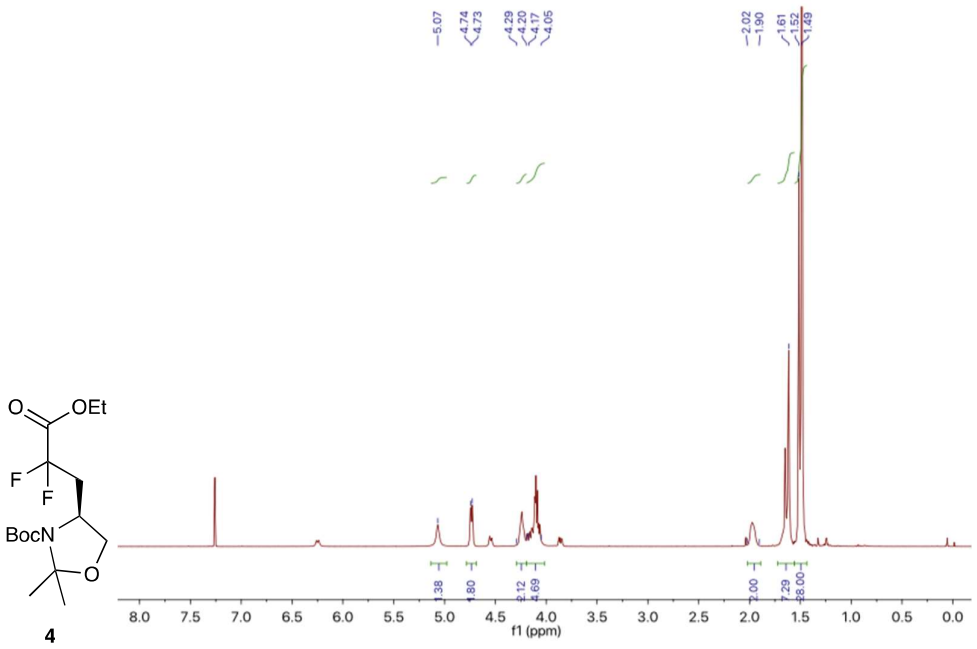
f1 (ppm)

3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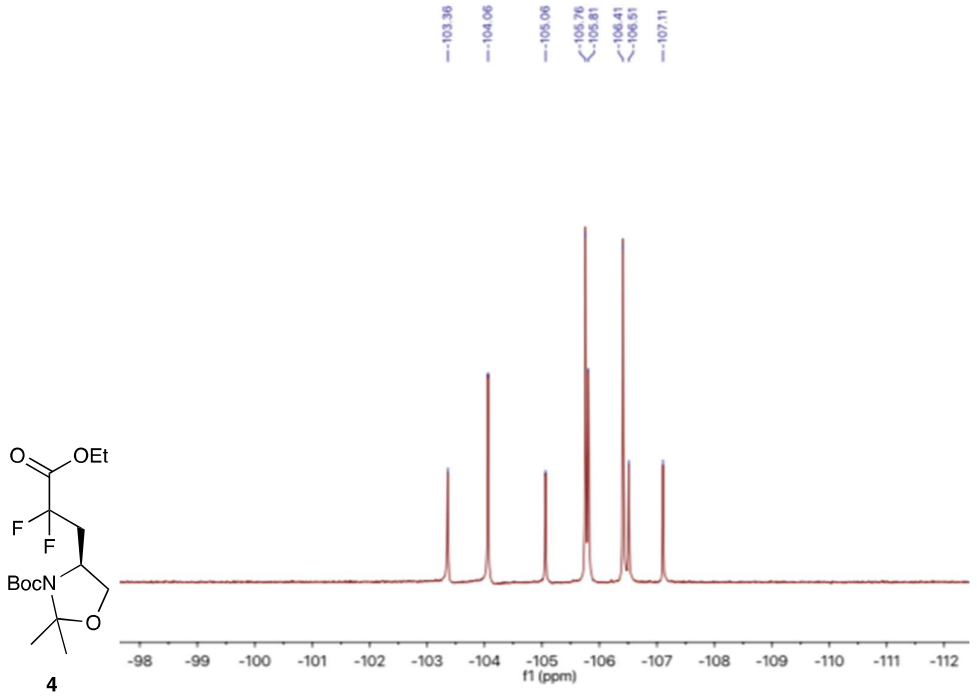
f1 (ppm)

4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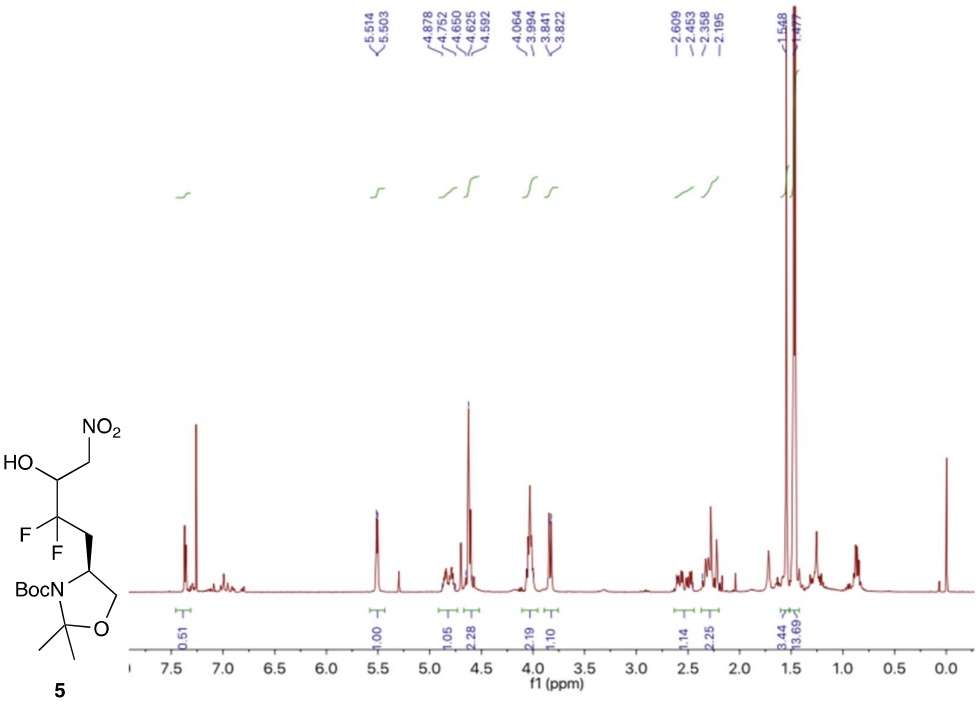
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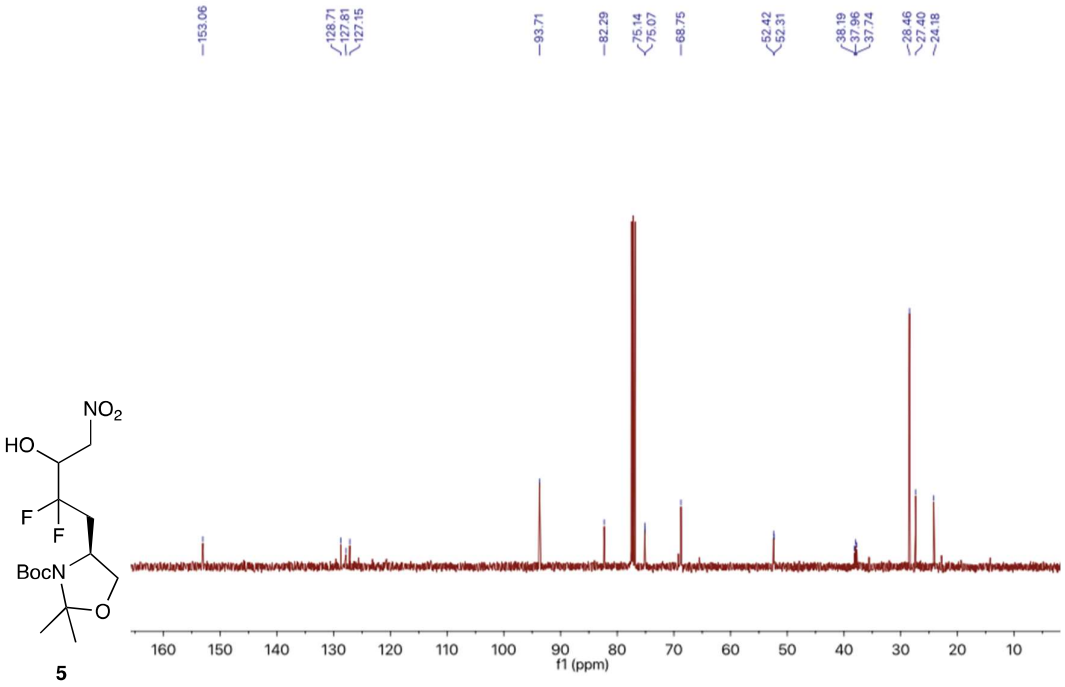
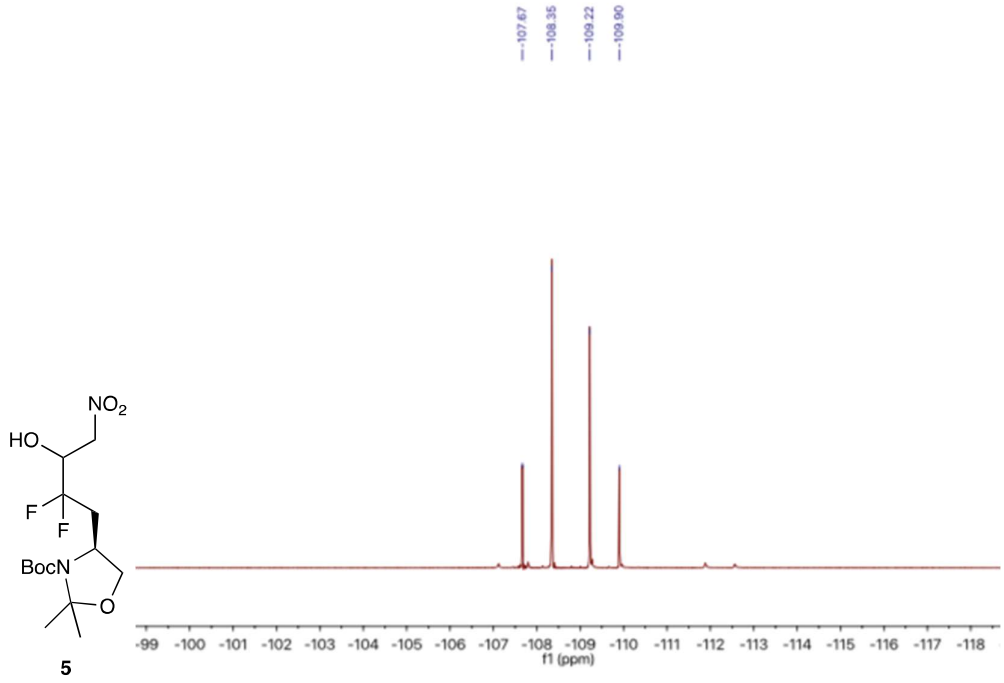
8.44
13.69
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

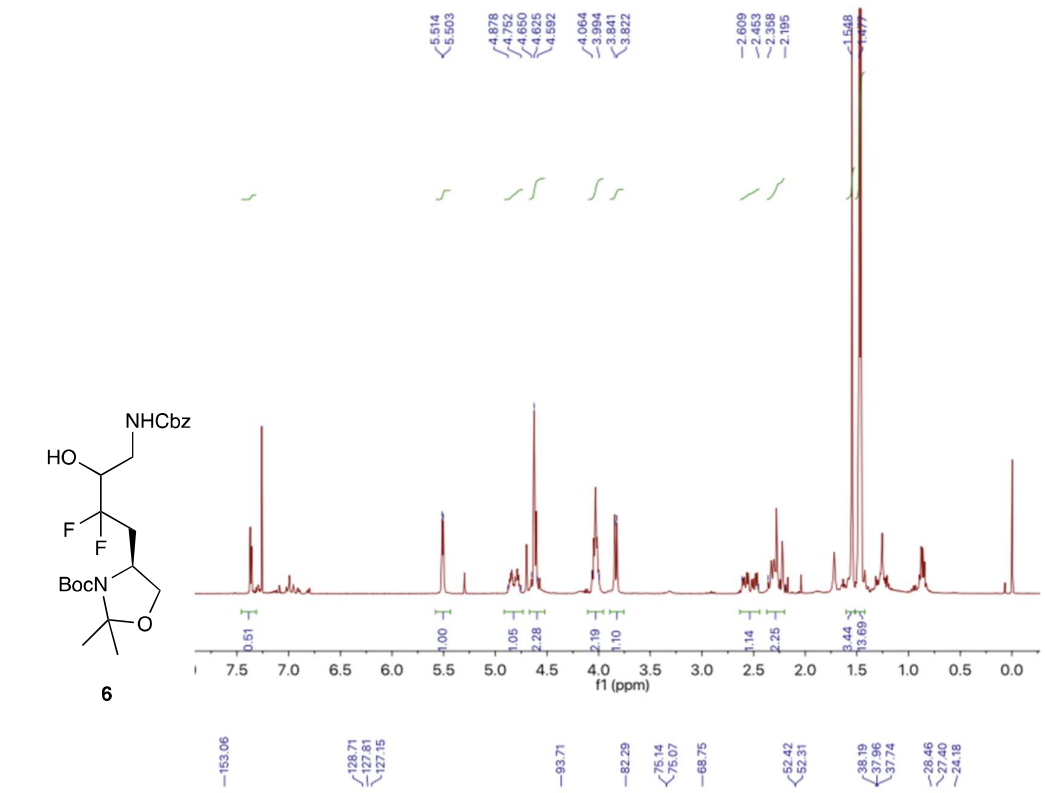
5

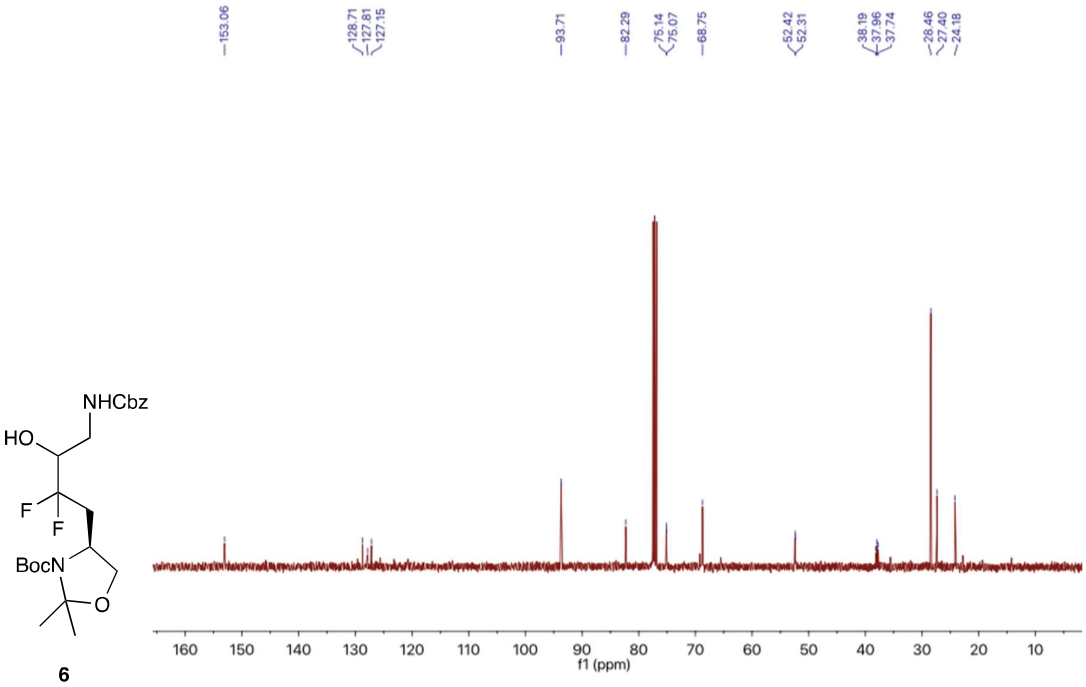

153.06
128.71
127.81
127.15
93.71
82.29
75.14
75.07
68.75
52.42
52.31
38.19
37.96
37.74
28.46
27.40
24.18
NO2
HO
F
F
BocN
O
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

5


-107.67
-108.35
-109.22
-109.90
NO2
HO
F
F
BocN
O
-99 -100 -101 -102 -103 -104 -105 -106 -107 -108 -109 -110 -111 -112 -113 -114 -115 -116 -117 -118
f1 (ppm)

5


NHCbz
HO
F
F
BocN
O
6
5.514
5.503
4.878
4.752
4.650
4.625
4.592
4.064
3.994
3.841
3.822
2.609
2.453
2.358
2.195
1.548
0.51
1.00
1.05
2.28
2.19
1.10
1.14
2.25
3.44
13.69
f1 (ppm)


NHCbz
HO
F
F
BocN
O
6
153.06
128.71
127.81
127.15
93.71
82.29
75.14
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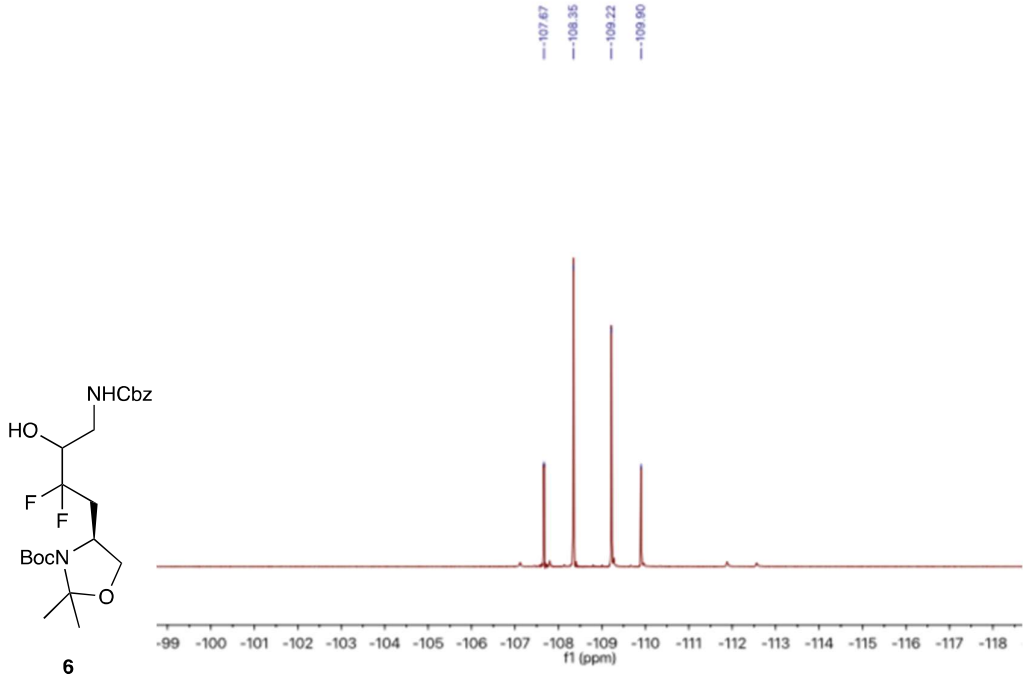
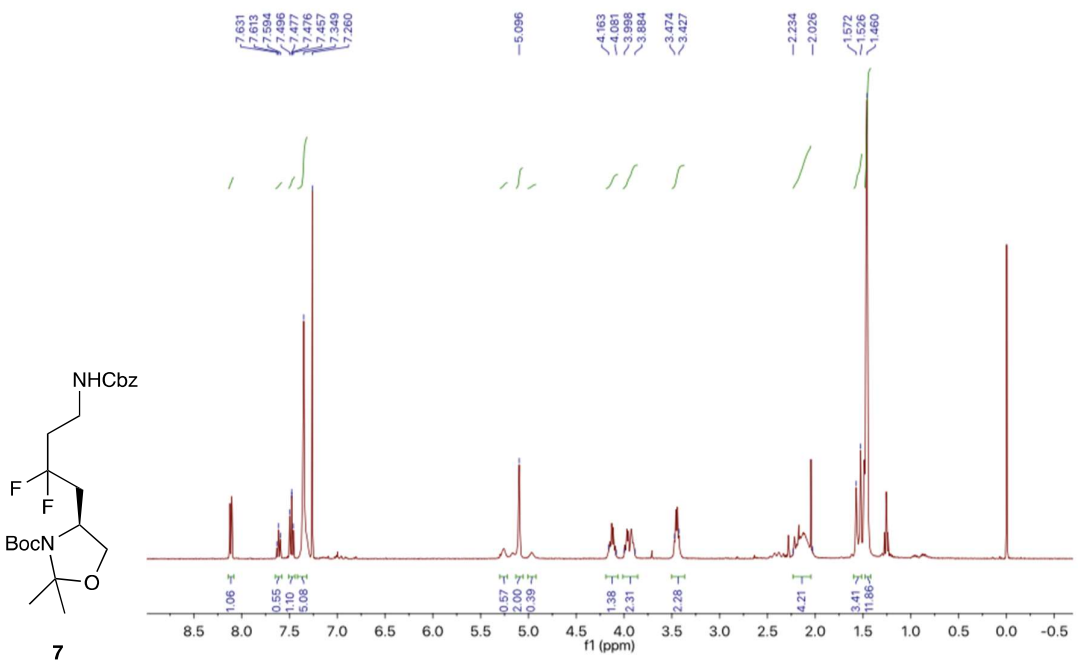
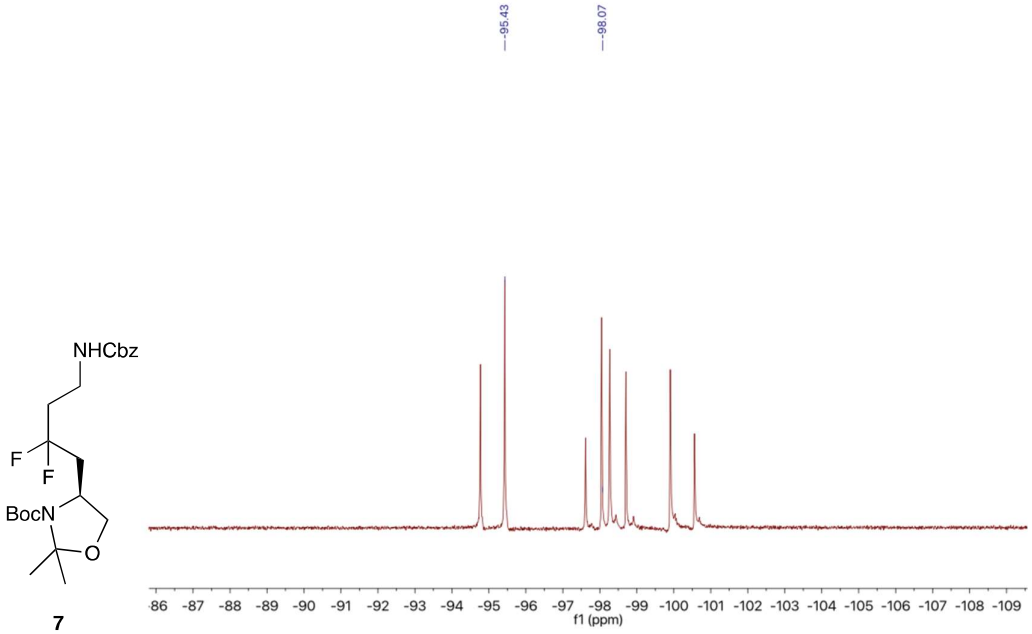
52.31
38.19
37.96
37.74
28.46
27.40
24.18
f1 (ppm)


NHCbz
HO
F
F
BocN
O
-107.67
-108.35
-109.22
-109.90
-99 -100 -101 -102 -103 -104 -105 -106 -107 -108 -109 -110 -111 -112 -113 -114 -115 -116 -117 -118
f1 (ppm)

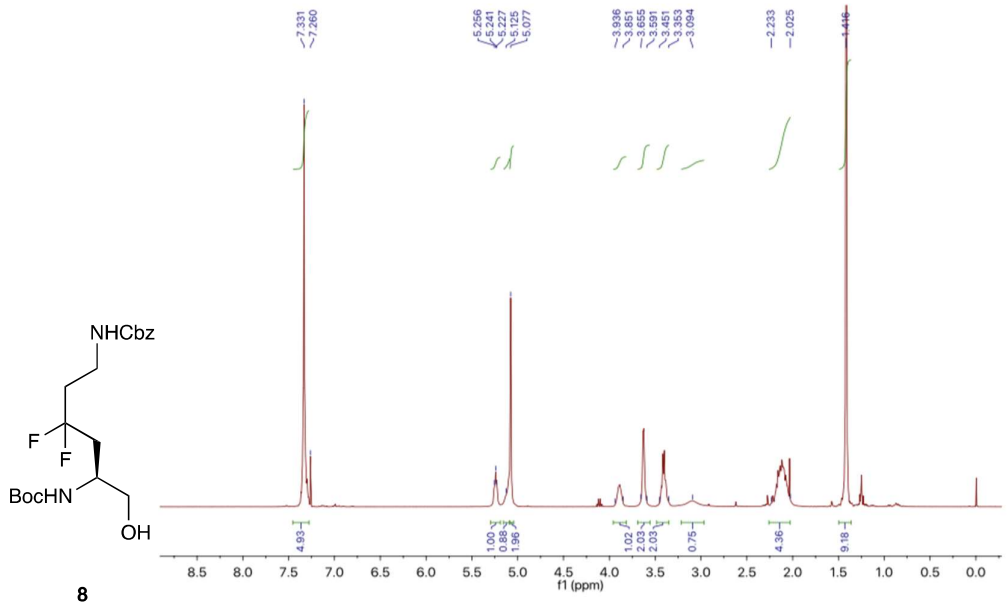
6


NHCbz
F
F
BocN
O
7.631
7.613
7.594
7.496
7.477
7.476
7.457
7.349
7.260
5.096
4.163
4.081
3.998
3.884
3.474
3.427
2.234
2.026
1.572
1.526
1.460
1.06
0.55
1.10
5.08
0.57
2.00
0.39
1.38
2.31
2.28
4.21
3.41
11.86
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

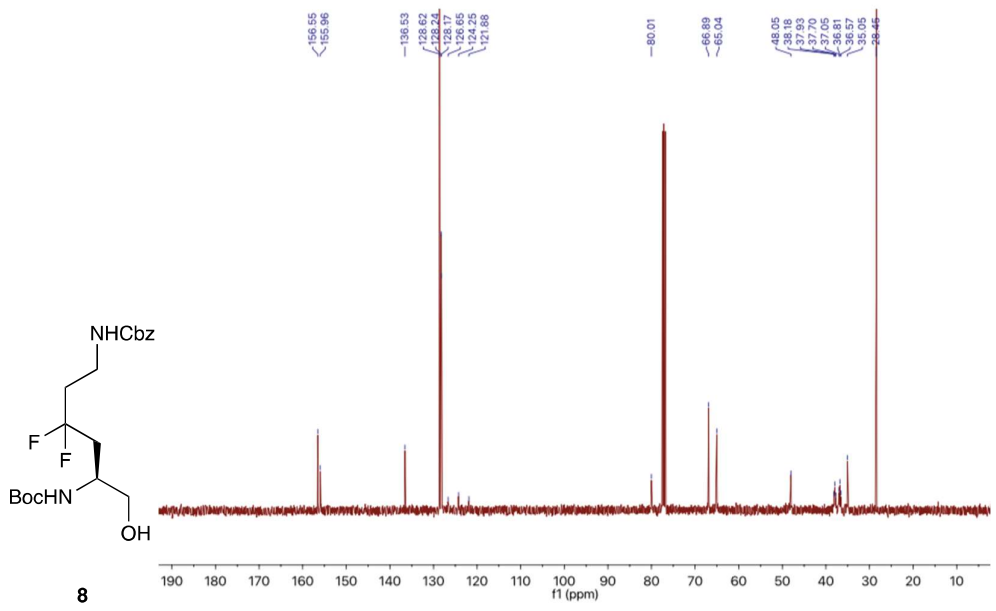
7


NHCbz
F
F
BocN
O
-95.43
-98.07
-86 -87 -88 -89 -90 -91 -92 -93 -94 -95 -96 -97 -98 -99 -100 -101 -102 -103 -104 -105 -106 -107 -108 -109
f1 (ppm)

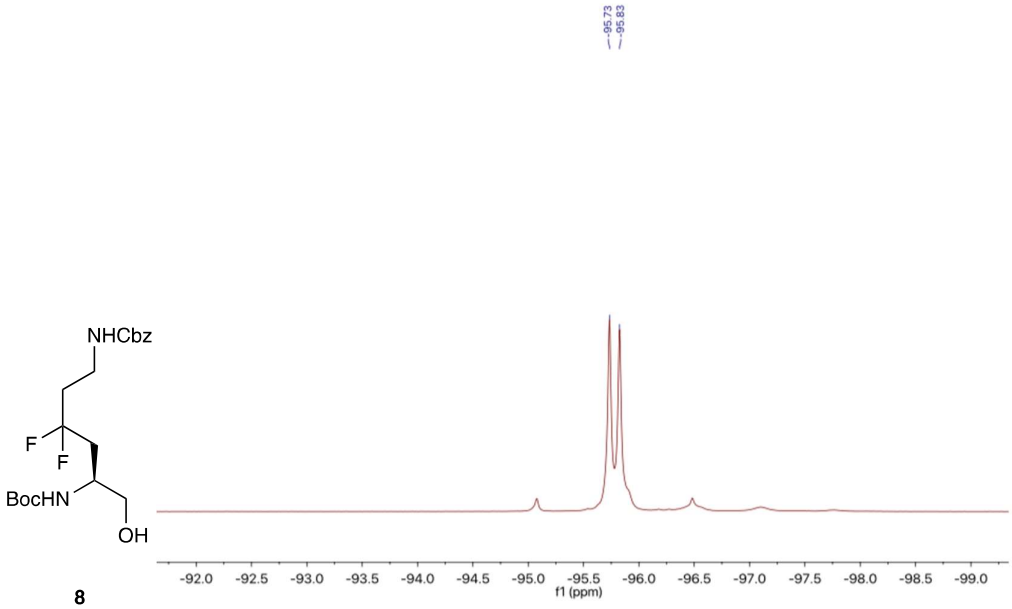
7


NHCbz
F
F
BocHN
OH
7.331
7.260
5.256
5.241
5.227
5.125
5.077
3.936
3.851
3.655
3.591
3.451
3.353
3.094
2.233
2.025
1.416
4.93
1.00
0.88
1.96
1.02
2.03
2.03
0.75
4.36
9.18
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

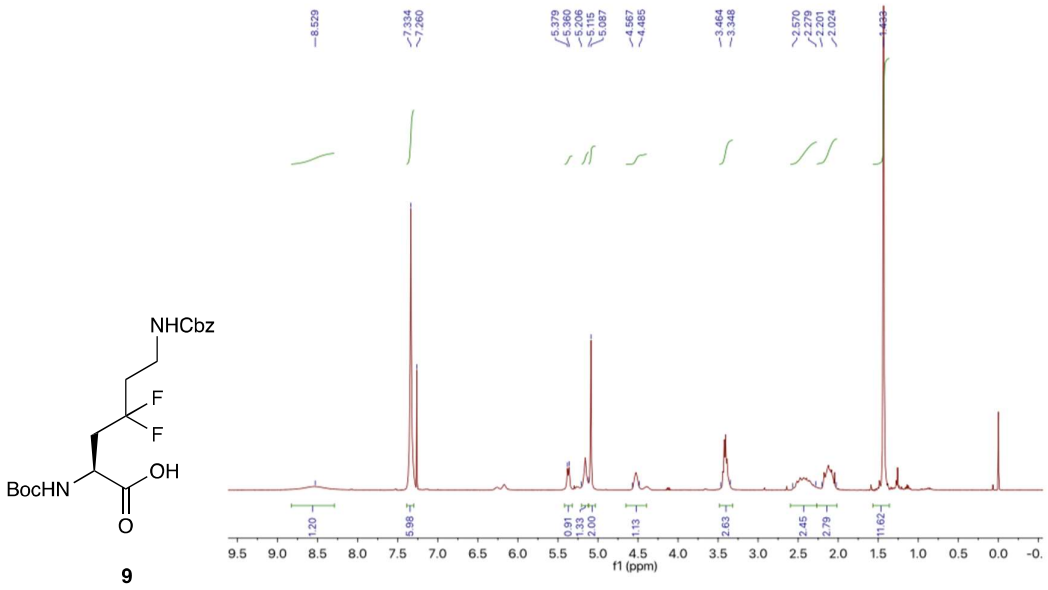
8

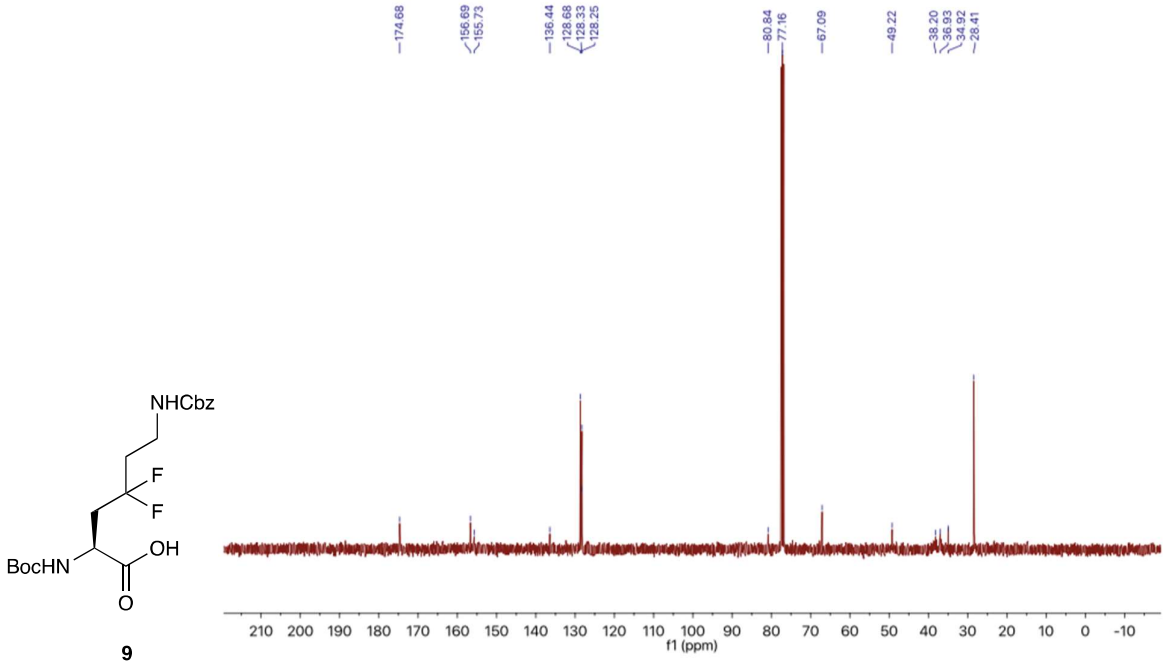

NHCbz
F
F
BocHN
OH
156.55
155.96
136.53
128.62
128.24
128.17
126.65
124.25
121.88
80.01
66.89
65.04
48.05
37.93
37.70
37.05
36.81
36.57
35.05
28.46
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

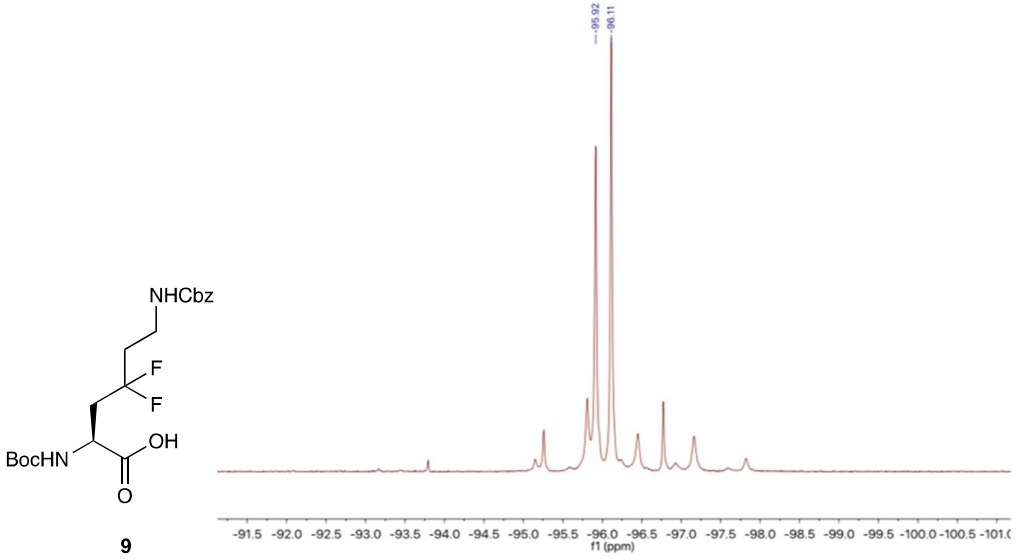
8


-95.73
-95.83
NHCbz
F
F
BocHN
OH
-92.0 -92.5 -93.0 -93.5 -94.0 -94.5 -95.0 -95.5 -96.0 -96.5 -97.0 -97.5 -98.0 -98.5 -99.0
f1 (ppm)

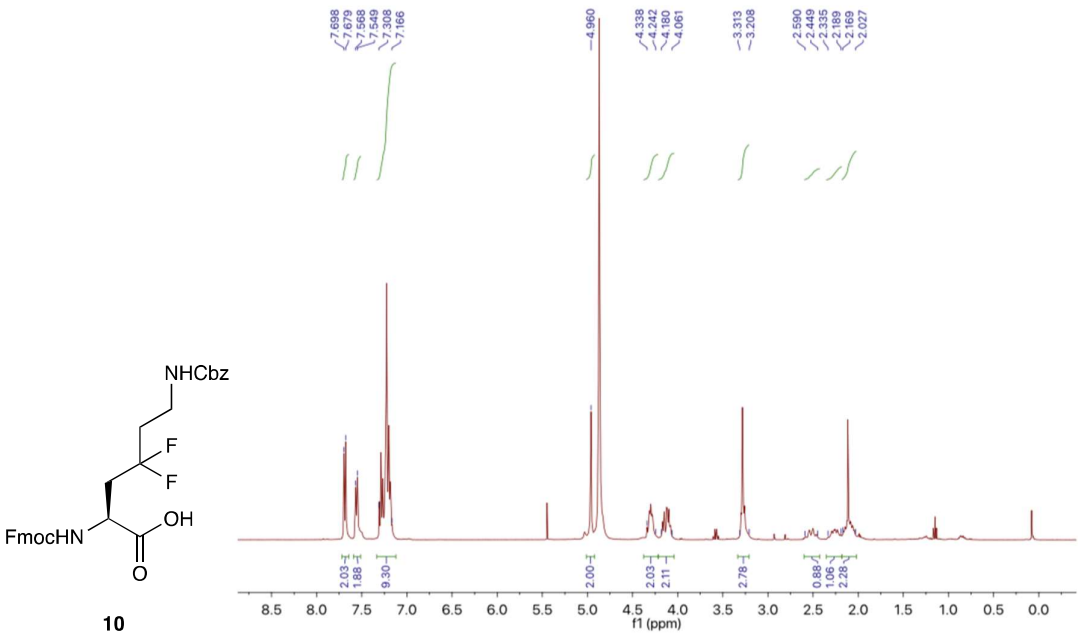
8


NHCbz
F
F
OH
BocHN
O
9
f1 (ppm)

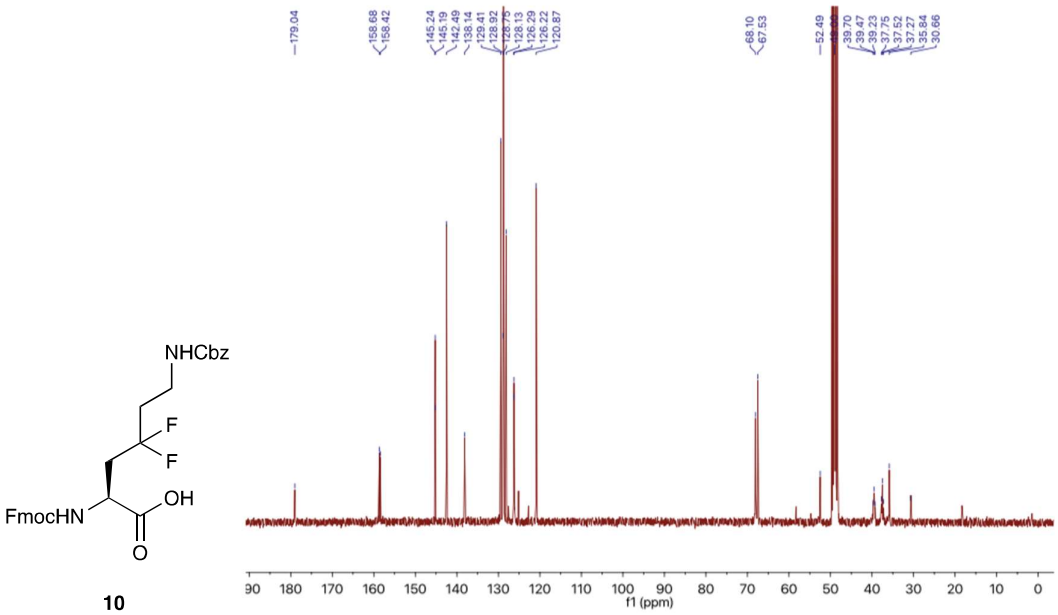

NHCbz
F
F
OH
BocHN
O
9
f1 (ppm)


NHCbz
F
F
OH
BocHN
O
-95.92
-96.11
-91.5 -92.0 -92.5 -93.0 -93.5 -94.0 -94.5 -95.0 -95.5 -96.0 -96.5 -97.0 -97.5 -98.0 -98.5 -99.0 -99.5 -100.0 -100.5 -101.(
f1 (ppm)

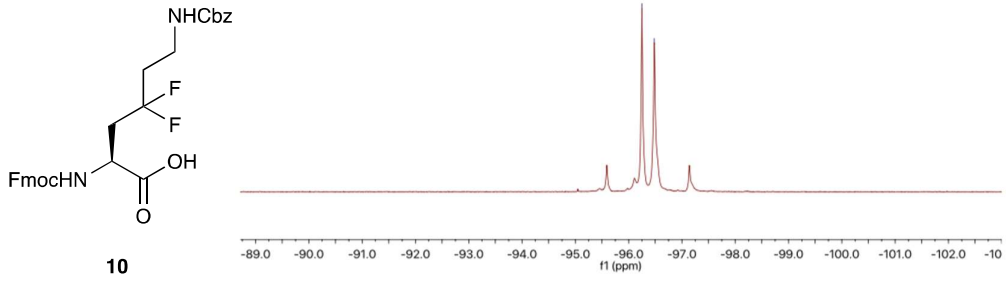
9


NHCbz
F
F
OH
FmocHN
O
7.698
7.679
7.568
7.549
7.308
7.166
4.960
4.338
4.242
4.180
4.061
3.313
3.208
2.590
2.449
2.335
2.189
2.169
2.027
2.03
1.88
9.30
2.00
2.03
2.11
2.78
0.88
1.06
2.28
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

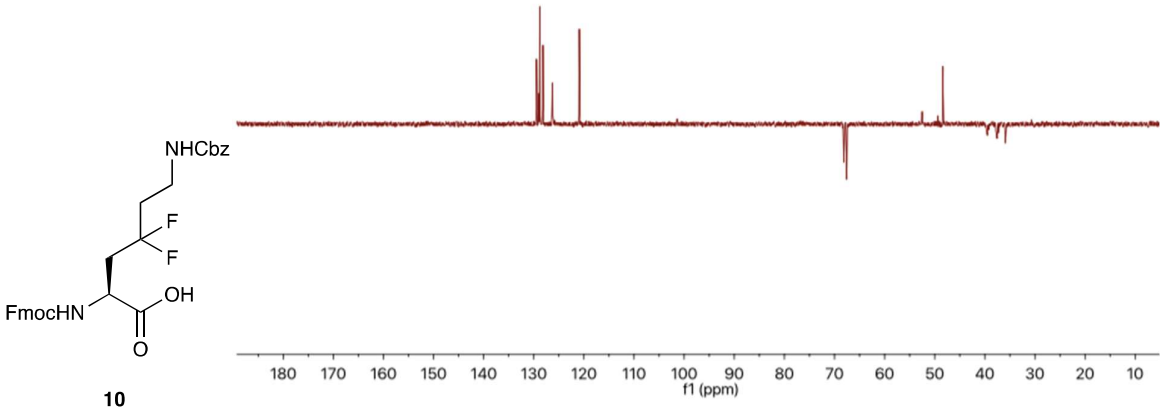
10


NHCbz
F
F
OH
FmocHN
O
179.04
158.68
158.42
145.24
145.19
142.49
139.14
139.41
128.92
128.75
128.13
126.29
126.22
120.87
68.10
67.53
52.49
39.70
39.47
39.23
37.75
37.52
37.27
35.84
30.66
f1 (ppm)

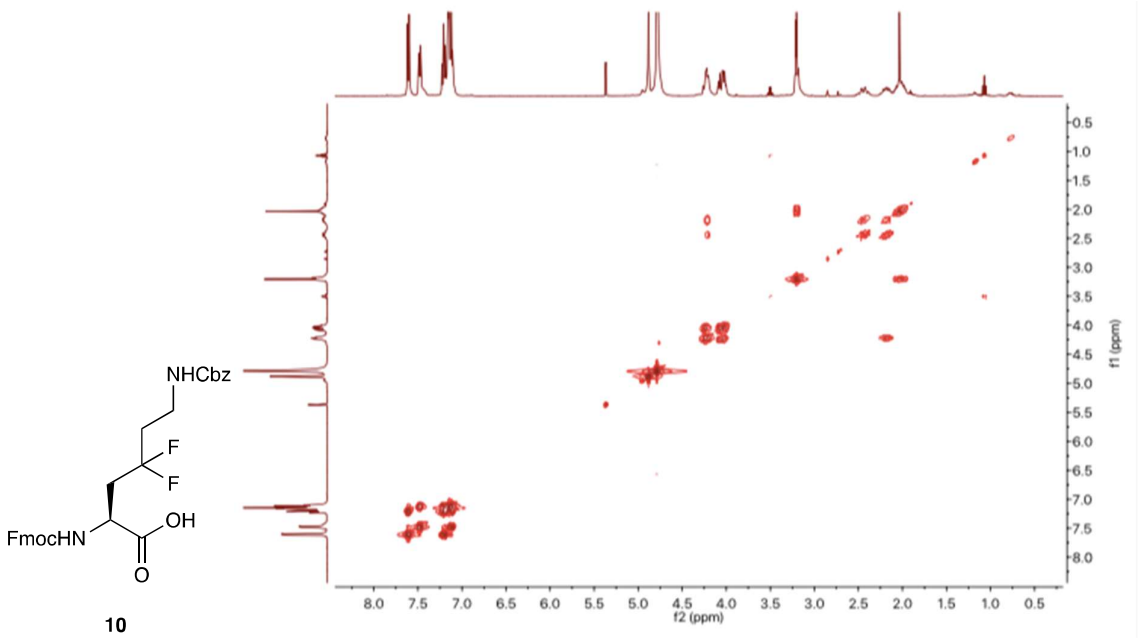
10


NHCbz
F
F
OH
FmocHN
O
-96.25
-96.48
f1 (ppm)

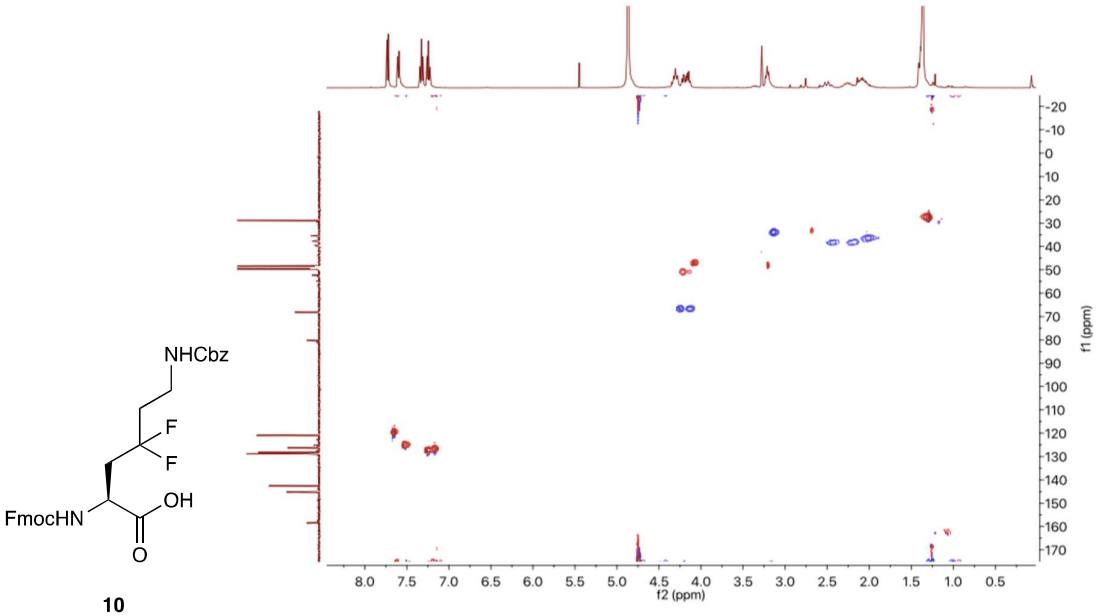
10


NHCbz
F
F
OH
FmocHN
O
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

10


NHCbz
F
F
OH
FmocHN
O
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f2 (ppm)
0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0
f1 (ppm)

10



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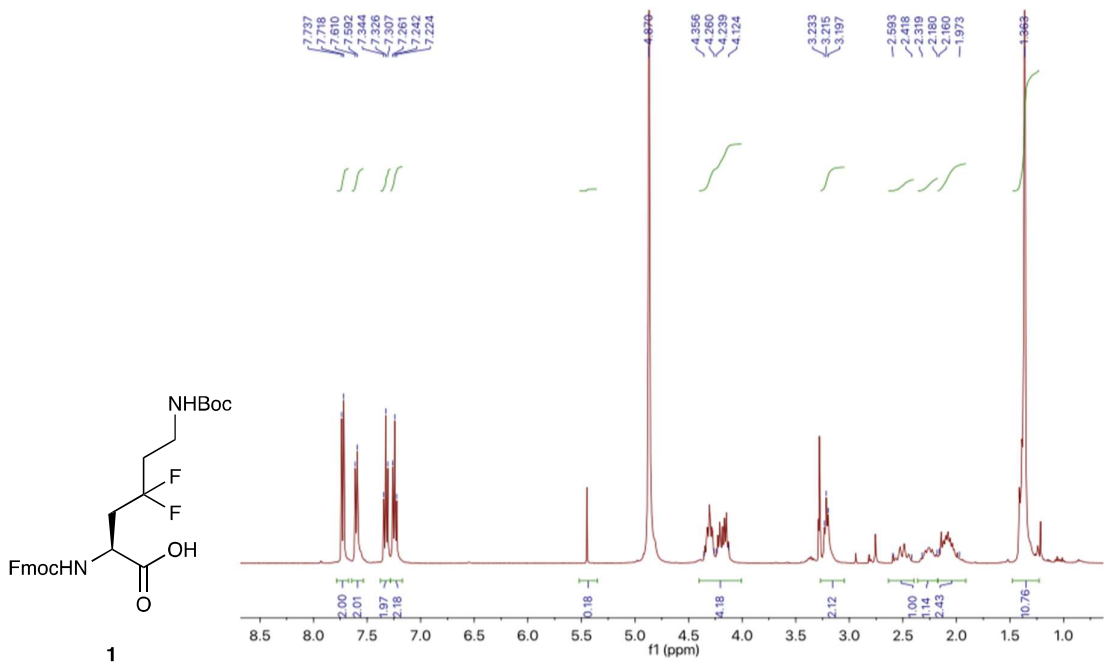

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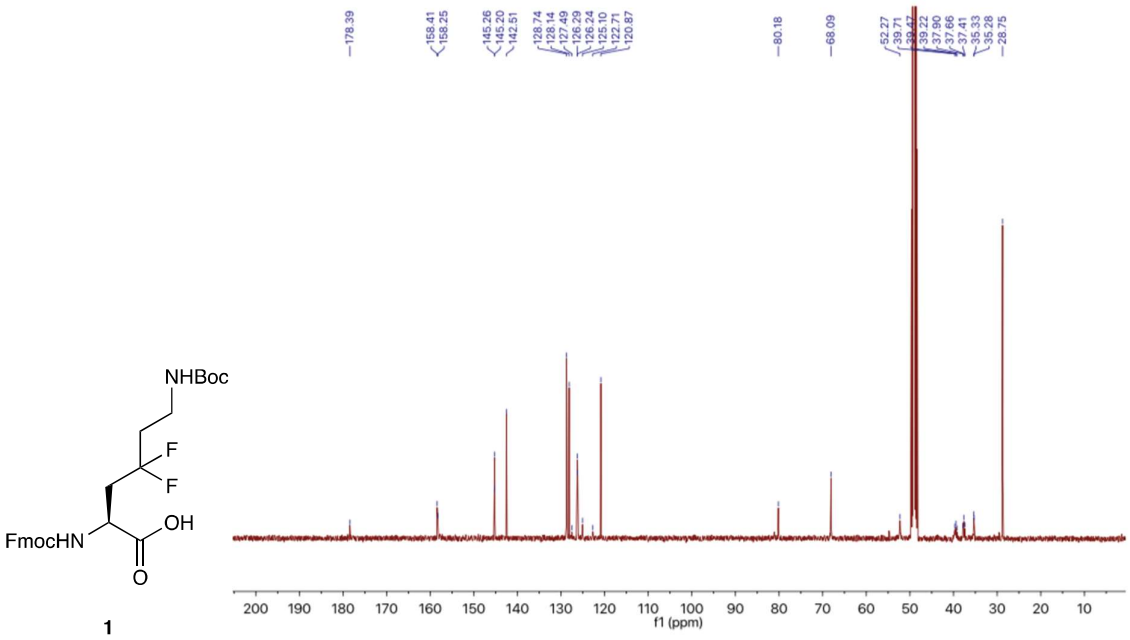
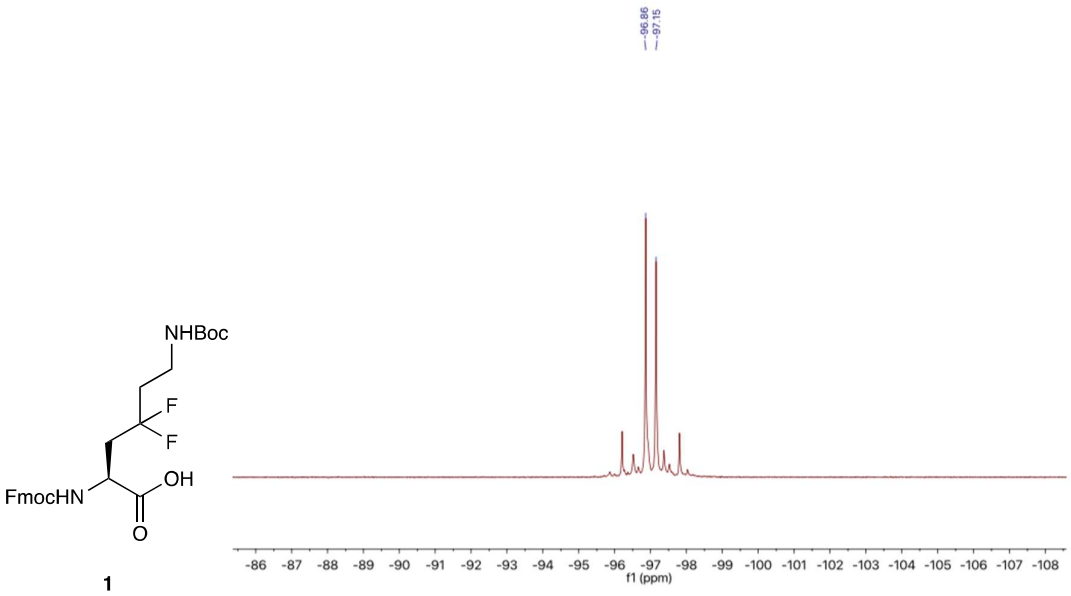
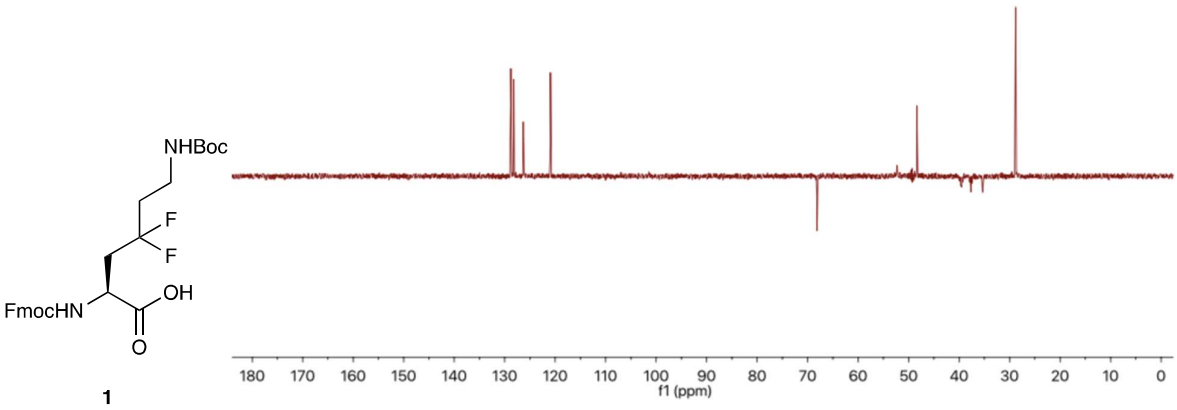

NHBoc
F
F
OH
FmocHN
O
178.39
158.41
158.25
145.26
145.20
142.51
128.74
128.14
127.49
126.89
126.24
125.10
122.71
120.87
80.18
68.09
52.27
39.71
39.47
39.22
37.90
37.66
37.41
35.33
35.28
28.75
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

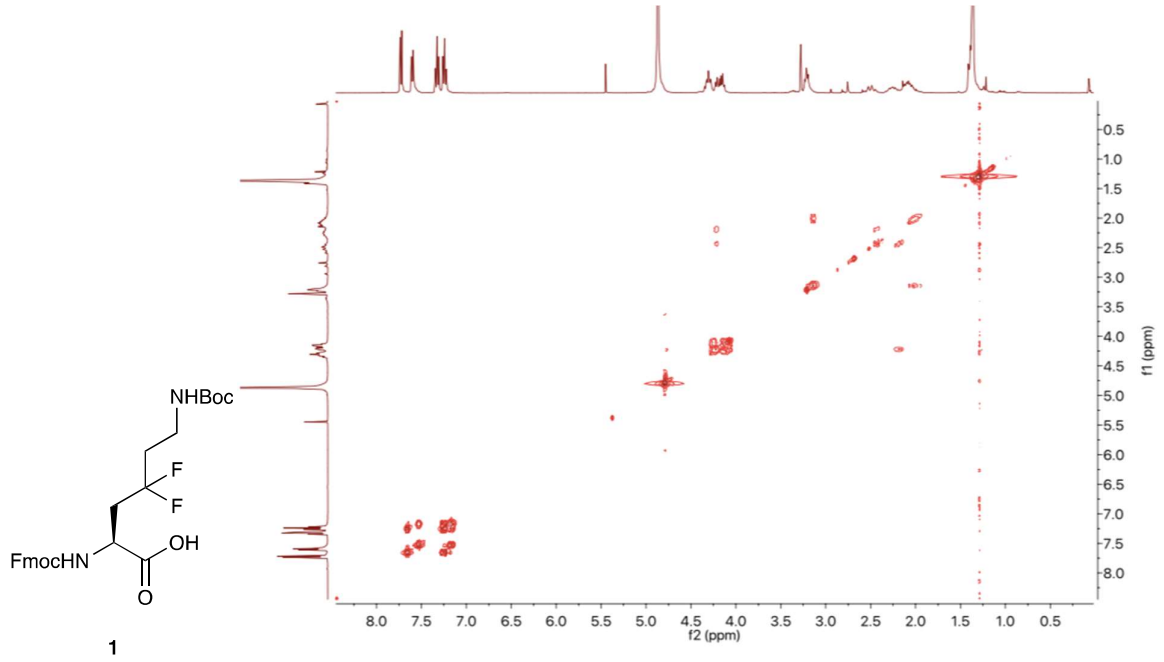
1


NHBoc
F
F
OH
FmocHN
O
96.86
97.15
-86 -87 -88 -89 -90 -91 -92 -93 -94 -95 -96 -97 -98 -99 -100 -101 -102 -103 -104 -105 -106 -107 -108
f1 (ppm)

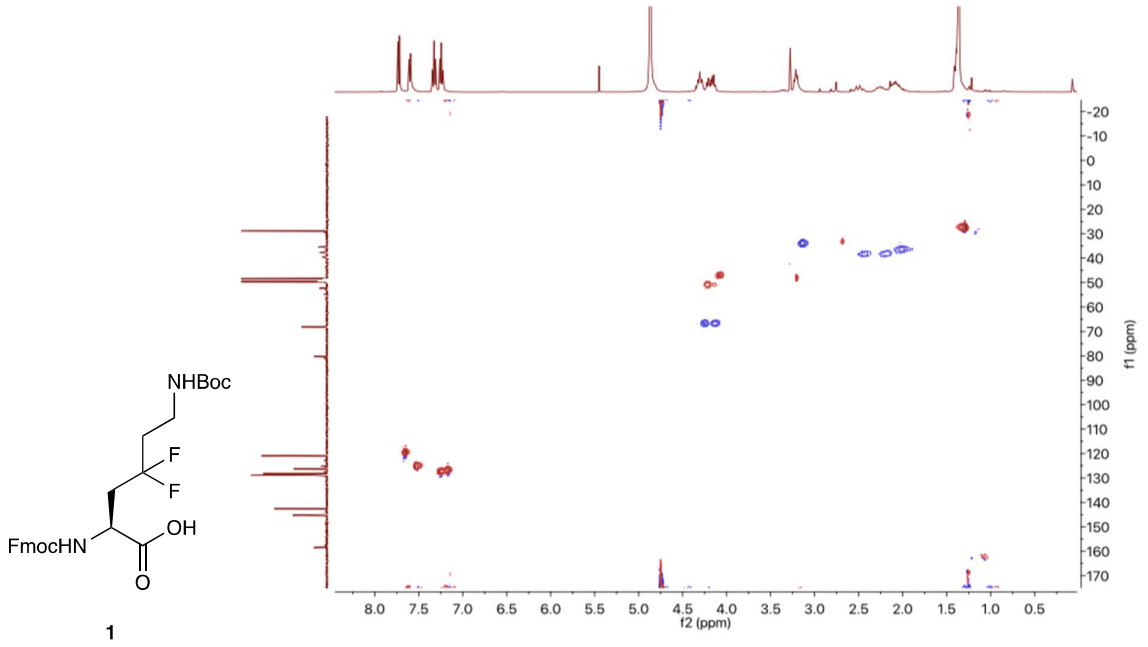
1


NHBoc
F
F
OH
FmocHN
O
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

1


NHBoc
F
F
OH
FmocHN
O
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f2 (ppm)
0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0
f1 (ppm)

1



1

13. References

1. Shinkai, Y.; Tachibana, M., H3K9 methyltransferase G9a and the related molecule GLP. *Genes Develop.* **2011,** *25*, 781-788.
2. Schuetz, A.; Bernstein, G.; Dong, A.; Antoshenko, T.; Wu, H.; Loppnau, P.; Bochkarev, A.; Plotnikov, A. N., Crystal structure of a binary complex between human GCN5 histone acetyltransferase domain and acetyl coenzyme A. *Proteins: Structure, Function, and Bioinformatics* **2007,** *68* , 403-407.
3. Al Temimi, A. H. K.; Reddy, Y. V.; White, P. B.; Guo, H.; Qian, P.; Mecinović, J., Lysine Possesses the Optimal Chain Length for Histone Lysine Methyltransferase Catalysis. *Sci. Rep.* **2017,** *7* 16148.