Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

Amide-Derived Lysine Analogues as Substrates and Inhibitors of Histone Lysine Methyltransferases and Acetyltransferases

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1. Experimental section

All commercially available reagents were purchased and used without further purification. Reactions 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 fluorescence (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 500 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. 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.

Synthetic Procedures

Methyl (*S*)-3-amino-2-(((benzyloxy)carbonyl)amino)propanoate (1)

AcCl (14.0 mL; 199.0 mmol) was slowly added to cooled MeOH (185 mL). The solution was stirred for 5 minutes at 0 °C. Compound Cbz-L-2,3-diaminopropionic acid (9.5 g; 39.9 mmol) was added after which the solution was stirred overnight while it was allowed to warm up to room temperature. The solvent was removed and the resulting residue was dried *in vacuo* to obtain compound **5** as pure white solid in a 99% (9.4 g, 197.0 mmol) yield. R_f = 0.6 (1:4 water : MeCN), ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.33 (s, 3H), 7.94 (d, *J* = 8.3 Hz, 1H), 7.42 – 7.29 (m, 5H), 5.07 (s, 2H), 4.46 (td, *J* = 8.8, 4.7 Hz, 1H), 3.68 (s, 3H), 3.22 (dd, *J* = 13.5, 4.6 Hz, 1H), 3.08 (t, *J* = 11.2 Hz, 1H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 170.3, 155.4, 137.5, 128.9, 128.4, 128.3, 66.9, 53.6, 52.2, 39.5; ESI-MS calcd. for $C_{12}H_{16}N_2O_4$ $[M+H]^+$: 253.12, found: 253.01.

***N*-Cbz-Dap(*N*-Boc-Gly)-OMe (2)**

Prepared using general procedure A using Boc-Gly as amino acid. The product was obtained as a white solid in 88% yield (1.42 g, 3.48 mmol). R_f = 0.4 (1:4 water : MeCN); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.88 (t, *J* = 5.9 Hz, 1H), 7.64 (d, *J* = 7.6 Hz, 1H), 7.42 – 7.26 (m, 5H), 6.95 (t, *J* = 6.1 Hz, 1H), 5.03 (s, 2H), 4.15 (td, *J* = 7.3, 5.5 Hz, 1H), 3.62 (s, 3H), 3.52 – 3.46 (m, 2H), 1.38 (s, 9H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 171.5, 170.4, 156.4, 156.2, 137.2, 128.8, 128.4, 128.3, 78.6, 66.6, 54.3, 52.5, 43.7, 40.0, 28.6; HRMS calcd. for $C_{19}H_{27}N_3NaO_7$ $[M+Na]^+$: 432.175, found: 432.175.

***N*-Cbz-Dap(*N*-Boc-L-Ala)-OMe (3)**

Prepared using general procedure A using Boc-L-Ala as amino acid. The product was obtained as a white solid in 85% yield (1.43 g, 3.37 mmol). R_f = 0.4 (1:9 MeOH:DCM); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.96-7.82 (m, 1H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.42 – 7.24 (m, 5H), 6.89 (d, *J* = 7.4 Hz,

1H), 5.03 (s, 2H), 4.16 (q, J = 6.8 Hz, 1H), 3.94-3.80 (m, 1H), 3.61 (s, 3H), 3.58 – 3.45 (m, 2H), 1.36 (s, 9H), 1.12 (d, J = 7.2 Hz, 3H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 173.8, 171.5, 156.4, 155.5, 137.2, 128.8, 128.4, 128.3, 78.5, 66.1, 54.2, 52.5, 50.2, 39.6, 28.6, 18.6; HRMS calcd. for $C_{20}H_{29}N_3NaO_7$ $[M+Na]^+$: 446.190, found: 446.192.

***N*-Cbz-Dap(*N*-Boc-D-Ala)-OMe (4)**

Prepared using general procedure A using Boc-D-Ala as amino acid. The product was obtained as a white solid in 78% yield (1.96 g, 4.63 mmol). R_f = 0.6 (1:9 MeOH:DMC); ^{1}H-NMR (500 MHz, DMSO-d6) δ 7.89 (t, J = 6.0 Hz, 1H), 7.59 (d, J = 7.7 Hz, 1H), 7.42 – 7.20 (m, 5H), 6.89 (d, J = 7.5 Hz, 1H), 5.03 (s, 2H), 4.20 – 4.13 (m, 1H), 3.98-3.83 (m, 1H), 3.61 (s, 3H,), 3.56 – 3.41 (m, 1H), 3.30 – 3.21 (m, 1H) 1.36 (s, 9H), 1.13 (d, J = 7.1 Hz, 3H); ^{13}C-NMR (126 MHz, DMSO-d6) δ 173.7, 171.6, 156.4, 155.5, 137.2, 128.8), 128.4, 128.3, 78.5, 66.2, 54.2, 52.5, 50.2, 40.0, 28.6, 18.6; HRMS calcd. for $C_{20}H_{29}N_3NaO_7$ $[M+Na]^+$: 446.190, found: 446.192.

***N*-Cbz-Dap(*N*-Boc-L-Abu)-OMe (5)**

Prepared using general procedure A using Boc-L-Abu as amino acid. The product was obtained as a white solid in 84% yield (1.46 g, 3.34 mmol). R_f = 0.5 (1:9 MeOH : DCM); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.96 (t, J = 6.0 Hz, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.41-7.25 (m, 5H), 6.78 (d, J = 7.8 Hz, 1H), 5.03 (s, 2H), 4.27-4.10 (m, 1H), 3.78 (td, J = 8.0, 5.1 Hz, 1H), 3.62 (s, 3H), 3.57 – 3.48 (m, H), 3.29 (m, 2H), 1.64-1.53 (m, 1H), 1.50-1.41 (m, 1H), 1.37 (s, 9H), 0.81 (t, J = 7.3 Hz, 3H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 173.8, 171.5, 156.4, 155.8, 137.9, 128.8, 128.3, 128.2, 78.5, 66.1, 56.2, 54.3, 52.5, 40.1, 28.6, 25.6, 10.8; HRMS calcd for $C_{21}H_{31}N_3NaO_7$ $[M+Na]^+$: 460.206, found 460.207.

***N*-Cbz-Dap(*N*-Boc-D-Abu)-OMe (6)**

Prepared using general procedure A using Boc-D-Abu as amino acid. The product was obtained as a white solid in 84% yield (1.47 g, 3.36 mmol). R_f = 0.7 (1:9 MeOH : DCM) ^{1}H-NMR (400 MHz,

DMSO-d_6) δ 7.92 (t, *J* = 6.0 Hz, 1H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.46 – 7.22 (m, 5H), 6.79 (d, *J* = 7.9 Hz, 1H), 5.03 (s, 2H), 4.25 – 4.08 (m, 1H), 3.81-3.73 (m, 1H), 3.61 (s, 3H), 3.56-3.46 (m, 1H), 3.32-3.24 (m, 1H), 1.62 – 1.51 (m, 1H), 1.50 – 1.41 (m, 1H), 1.37 (s, 9H), 0.81 (t, *J* = 7.3 Hz, 3H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 173.0, 171.5, 156.4, 155.8, 137.2, 128.8, 128.4, 128.3, 78.5, 66.2, 56.2, 54.2, 52.5, 40.1, 28.6, 25.6, 10.7; HRMS calcd for $C_{21}H_{31}N_3NaO_7$ $[M+Na]^+$: 460.206, found 460.207.

***N*-Cbz-Dap(*N*-Boc-L-Phe)-OMe (7)**

Prepared using general procedure A using Boc-L-Phe as amino acid. The product was obtained as a white solid in 73% yield (1.45 g, 2.74 mmol). R_f = 0.4 (1:4 water : MeCN); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.11 (t, *J* = 6.0 Hz, 1H), 7.60 (d, *J* = 7.9 Hz, 1H), 7.40 – 7.13 (m, 10H), 6.90 (d, *J* = 8.5 Hz, 1H), 5.42 – 4.91 (m, 2H), 4.23 – 4.16 (m, 1H), 4.14 – 4.08(m, 1H), 3.63 (s, 3H), 3.60 – 3.49 (m, 1H), 3.30 – 3.22 (m, 1H), 2.92 (dd, *J* = 13.9, 4.2 Hz, 1H), 2.75 – 2.62 (m, 1H), 1.28 (s, 9H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 173.3, 171.5, 156.8, 155.7, 138.7, 137.2, 129.6, 128.8, 128.5, 128.4, 128.3, 126.6, 78.5, 66.2, 56.2, 54.2, 52.5, 40.0, 37.9, 28.6; HRMS calcd for $C_{26}H_{33}N_3NaO_7$ $[M+Na]^+$: 552.222, found: 522.222.

***N*-Cbz-Dap(*N*-Boc-D-Phe)-OMe (8)**

Prepared using general procedure A using Boc-D-Phe as amino acid. The product was obtained as a white solid in 73% yield (1.49 g, 2.82 mmol). R_f = 0.4 (1:4 water : MeCN ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.06 (t, *J* = 6.0 Hz, 1H), 7.61 (d, *J* = 7.8 Hz, 1H), 7.41 – 7.12 (m, 10H), 6.92 (d, *J* = 8.5 Hz, 1H), 5.04 (s, 2H), 4.17 (q, *J* = 6.6 Hz, 1H), 4.13 – 4.05 (m, 1H), 3.62 (s, 3H), 3.42 (t, *J* = 6.2 Hz, 2H), 2.92 (dd, *J* = 13.8, 4.3 Hz, 1H), 2.71 (dd, *J* = 13.8, 10.4 Hz, 1H), 1.28 (s, 9H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 172.7, 171.6, 156.4, 155.7, 138.7, 137.2, 129.6, 128.8, 128.5, 128.4, 128.3, 126.6, 78.5, 66.2, 56.2, 54.3, 52.5, 40.0, 37.9, 28.6; HRMS calcd for $C_{26}H_{33}N_3NaO_7$ $[M+Na]^+$: 552.222, found: 522.223.

***N*-Fmoc-Dap(*N*-Boc-Gly)-OH (9)**

Prepared using general procedure B starting from **2** (1.40 g, 3.42 mmol). The product was obtained as a white solid in 14% yield over three steps (210 mg, 0.36 mmol). R_f = 0.6 (1:4 water : MeCN); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.89 (dt, *J* = 7.5, 1.0 Hz, 2H), 7.69 (d, *J* = 7.5 Hz, 2H), 7.42 (td, *J* = 7.4, 1.2 Hz, 2H), 7.33 (td, *J* = 7.4, 1.2 Hz, 2H), 6.98 (s, 2H, H6), 4.24 (m, 3H), 3.52 – 3.40 (m, 2H), 3.27 (m, 3H), 3.76 (s, 1H), 3.48 (d, *J* = 6.0 Hz), 1.37 (s, 9H); ^{13}C-NMR (126 MHz, DMSO-d_6) δ 173.7, 172.2, 156.6, 155.4, 144.1, 141.1, 128.2, 127.7, 125.7, 120.6, 73.9, 66.3, 51.6, 50.3, 47.0, 41.0, 28.5; HRMS calcd. for $C_{25}H_{29}N_3NaO_7$ $[M+Na]^+$: 506.190, found: 506.191.

***N*-Fmoc-Dap(*N*-Boc-L-Ala)-OH (10)**

Prepared using general procedure B starting from **3** (1.40 g, 3.30 mmol). The product was obtained as a white solid in 20% yield over three steps (340 mg, 0.68 mmol). R_f = 0.3 (1:4 water: MeCN); ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.85 (d, *J* = 7.5 Hz, 2H), 7.78-7.68 (m, 1H), 7.68-7.60 (m, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 2H), 6.82 (d, *J* = 7.4 Hz, 1H), 6.70 (d, *J* = 7.1 Hz, 1H), 4.20 (m, 3H), 3.49-3.42 (m, 1H), 3.39 – 3.27 (m, 1H), 3.17 (m, 1H), 1.33 (s, 9H), 1.20 – 1.07 (m, 3H); ^{13}C-NMR (126 MHz, DMSO-d_6) δ 173.8, 172.8, 156.4, 155.7, 144.2, 141.1, 128.2, 127.7, 125.7, 120.5, 79.0, 66.3, 55.4, 50.4, 47.0, 41.6, 28.5, 18.5; HRMS calcd. for $C_{26}H_{31}N_3NaO_7$ $[M+Na]^+$: 520.206, found: 520.206.

***N*-Fmoc-Dap(*N*-Boc-D-Ala)-OH (11)**

Prepared using general procedure B starting from **4** (1.90 g, 4.49 mmol). The product was obtained as a white solid in 12% yield over three steps (260 mg, 0.52 mmol). R_f = 0.6 (1:9 water:MeCN^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.90 (d, *J* = 7.6 Hz, 2H), 7.73-7.70 (m, 1H), 7.69-7.65 (m, 2H) 7.42 (td, *J* = 7.4, 1.1 Hz, 2H), 7.3 (m, 2H), 6.92 (d, *J* = 7.3 Hz, 1H), 6.48 (s, 1H), 4.40 (m, 3H), 3.80 (s, 1H), 3.47-3.40 (m, 1H), 1.37 (m, 9H), 1.17 (m, 3H); ^{13}C-NMR (126 MHz, DMSO-d_6) δ 173.4, 172.1,

144.3, 141.4, 128.1, 127.6, 125.7, 120.6, 80.5, 55.2, 50.4, 47.1, 41.3, 28.0, 18.6; HRMS calcd for $C_{26}H_{31}N_3NaO_7$ $[M+Na]^+$: 520.206, found: 520.206.

***N*-Fmoc-Dap(*N*-Boc-L-Abu)-OH (12)**

Prepared using general procedure B starting from **5** (1.40 g, 3.00 mmol). The product was obtained as a white solid in 7% yield over 3 steps (110 mg, 0.22 mmol). R_f = 0.6 (1:4 water : MeCN); ^{1}H NMR (400 MHz, DMSO) δ 7.89 (d, *J* = 7.5 Hz, 2H), 7.69 (d, *J* = 7.5 Hz, 2H), 7.42 (td, *J* = 7.5, 1.2 Hz, 2H), 7.33 (td, *J* = 7.4, 1.2 Hz, 2H), 7.02 (s, 1H), 6.86 – 6.76 (m, 1H), 4.30 - 416 (m, 3H), 3.85 – 3.71 (m, 1H), 1.71 – 1.58 (m, 1H), 1.57-1.43 (m, 1H), 1.36 (s, 9H), 0.96 – 0.70 (m, 3H); ^{13}C NMR (126 MHz, DMSO) δ 144.11, 141.06, 127.94 (d, *J* = 69.2 Hz), 125.65, 120.53, 46.98, 28.53, 10.63; HRMS cacld. for $C_{27}H_{33}N_3NaO_7$ $[M+Na]^+$: 534.222, found: 534.223.

***N*-Fmoc-Dap(*N*-Boc-D-Abu)-OH (13)**

Prepared using general procedure B starting from **6** (2.0 g, 4.3 mmol). The product was obtained as a white solid in 21% yield over 3 steps (456 mg, 0.88 mmol).R_f = 0.6 (1:4 water : MeCN); ^{1}H NMR (500 MHz, MeOD) δ 7.89 – 7.80 (m, 1H), 7.69 (t, *J* = 7.3 Hz, 1H), 7.46 – 7.38 (m, 1H), 7.37 – 7.28 (m, 1H), 4.46 – 4.27 (m, 1H), 4.24 (t, *J* = 7.0 Hz, 0H), 3.93 (dd, *J* = 8.4, 5.3 Hz, 0H), 3.76 (dd, *J* = 13.9, 4.7 Hz, 0H), 3.51 (dd, *J* = 13.8, 7.8 Hz, 0H), 1.76 (dq, *J* = 13.6, 7.0 Hz, 0H), 1.66 – 1.53 (m, 0H), 1.45 (s, 3H), 0.94 (t, *J* = 7.4 Hz, 1H); ^{13}C NMR (126 MHz, MeOD) δ 172.1, 157.1, 143.8, 141.2, 127.4, 126.9, 24.9, 124.9, 119.5, 66.8, 56.2, 40.0, 27.3, 25.2, 9.2; HRMS cacld. for $C_{27}H_{33}N_3NaO_7$ $[M+Na]^+$: 534.222, found: 534.223.

***N*-Fmoc-Dap(*N*-Boc-L-Phe)-OH (14)**

Prepared using general procedure B starting from **7** (1.40 g, 2.80 mmol). The product was obtained as a white solid in 8% yield over 3 steps (110 mg, 0.21 mmol). R_f = 0.5 (1:4 water : MeCN); ^{1}H NMR (500 MHz, DMSO) δ 8.10 – 7.98 (m, 1H), 7.88 – 7.80 (m, 2H), 7.71 – 7.61 (m, 2H), 7.43 – 7.35 (m, 2H), 7.33 – 7.25 (m, 2H), 7.24 – 7.11 (m, 5H), 6.76 (d, *J* = 8.5 Hz, 1H), 4.30 – 4.23 (m, 2H), 4.22 –

4.16 (m, 1H), 4.13 – 4.02 (m, 1H), 3.59 – 3.51 (m, 1H), 3.28 – 3.18 (m, 1H), 2.93 (dd, *J* = 13.9, 4.3 Hz, 1H), 2.70 – 2.61 (m, 1H), 1.23 (s, 9H); ^{13}C NMR (126 MHz, DMSO) δ 172.98, 172.60, 156.62, 155.79, 144.08, 144.03, 141.08, 138.29, 129.49, 128.54, 128.21, 127.65, 126.76, 125.58, 120.54, 78.94, 66.29, 55.33, 46.96, 28.44; HRMS cacld. for $C_{32}H_{35}N_3NaO_7$[M+Na]$^+$: 596.237, found: 596.236.

***N*-Fmoc-Dap(*N*-Boc-D-Phe)-OH (15)**

Prepared using general procedure B starting from **8** (1.40 g, 2.80 mmol). The product was obtained as a white solid in 15% yield over 3 steps (210 mg, 0.41 mmol). R_f = 0.6 (1:4 water : MeCN); ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.89-784 (m, 1H), 7.83 (d, J = 5.7 Hz, 1H), 7.65 – 7.60 (m, 2H), 7.37 (t, J = 7.5 Hz, 2H), 7.29 (t, J = 7.5 Hz, 2H), 7.10 (m, 5H), 6.87 (d, *J* =7.4 hz, 1H), 6.74 (d, *J* =7.4 hz, 1H), 4.23 – 4.15 (m, 3H), 3.52 – 3.40 (m, 2H), 3.21 – 3.13 (m, 1H), 1.34 (s, 9H); **^{13}C-NMR** (126 MHz, DMSO-d_6) δ 174.5, 157.2, 156.2, 144.7, 141.72, 141.70, 139.0, 130.2, 129.2, 128.9, 128.3, 127.1, 126.3, 121.2, 79.7, 67.0, 57.0, 47.6, 29.1; HRMS calcd. for $C_{25}H_{29}N_3NaO_7$ [M+Na]$^+$: 506.190. Found: 506.191.

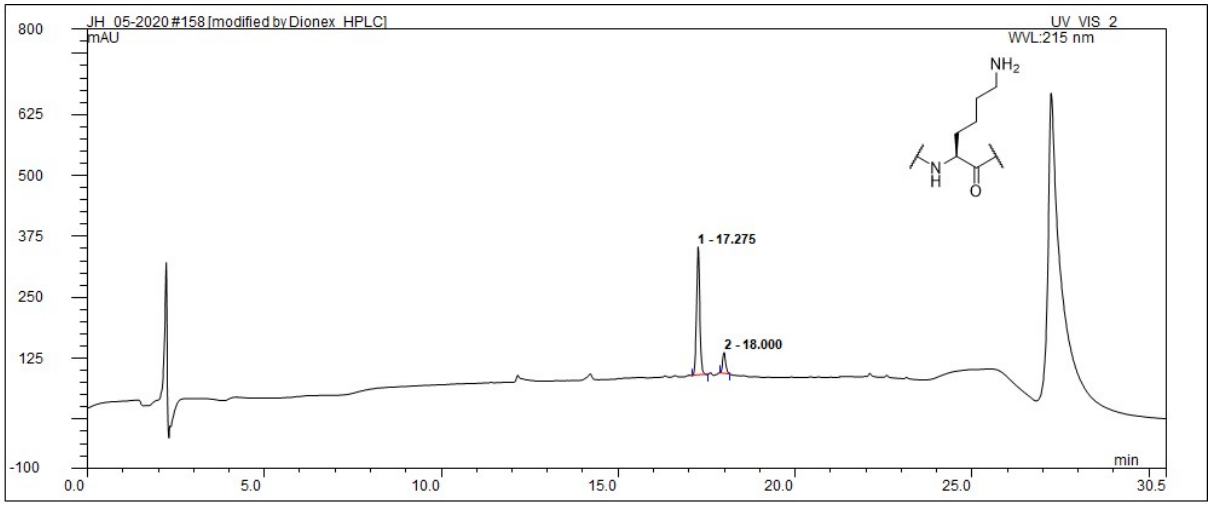
Peptide Purification and MALDI-TOF Instrumentation

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. Characterization of histone peptides

Entry	Peptide	Sequence	Formula	m/z Calculated	m/z Found
1	H3K9	ARTKQTAR**K**STGG	$C_{54}H_{101}N_{23}O_{18}$	1360.8	1361.4
2	H3K^{Gly}9	ARTKQTAR**K^{Gly}**STGG	$C_{53}H_{98}N_{24}O_{19}$	1375.7	1376.2
3	H3K$^{L\text{-}Ala}$9	ARTKQTAR**K$^{L\text{-}Ala}$**STGG	$C_{54}H_{100}N_{24}O_{19}$	1389.8	1390.0
4	H3K$^{D\text{-}Ala}$9	ARTKQTAR**K$^{D\text{-}Ala}$**STGG	$C_{54}H_{100}N_{24}O_{19}$	1389.8	1390.0
5	H3K$^{L\text{-}Abu}$9	ARTKQTAR**K$^{L\text{-}Abu}$**STGG	$C_{55}H_{102}N_{24}O_{19}$	1403.8	1403.9
6	H3K$^{D\text{-}Abu}$9	ARTKQTAR**K$^{D\text{-}Abu}$**STGG	$C_{55}H_{102}N_{24}O_{19}$	1403.8	1404.0
7	H3K$^{L\text{-}Phe}$9	ARTKQTAR**K$^{L\text{-}Phe}$**STGG	$C_{60}H_{104}N_{24}O_{19}$	1465.8	1466.2
8	H3K$^{D\text{-}Phe}$9	ARTKQTAR**K$^{D\text{-}Phe}$**STGG	$C_{60}H_{104}N_{24}O_{19}$	1465.8	1466.0

A)



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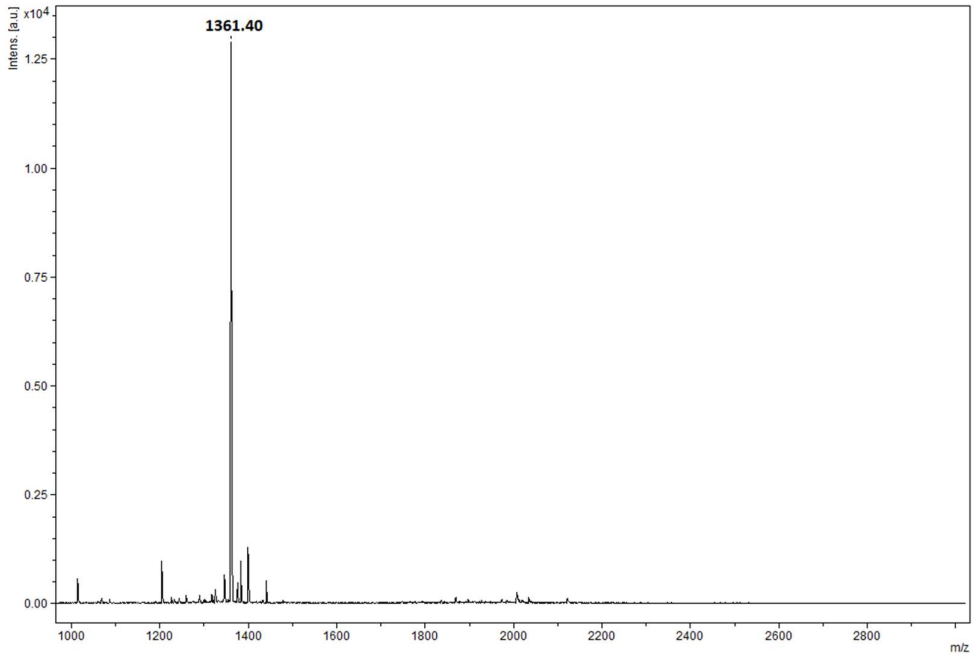
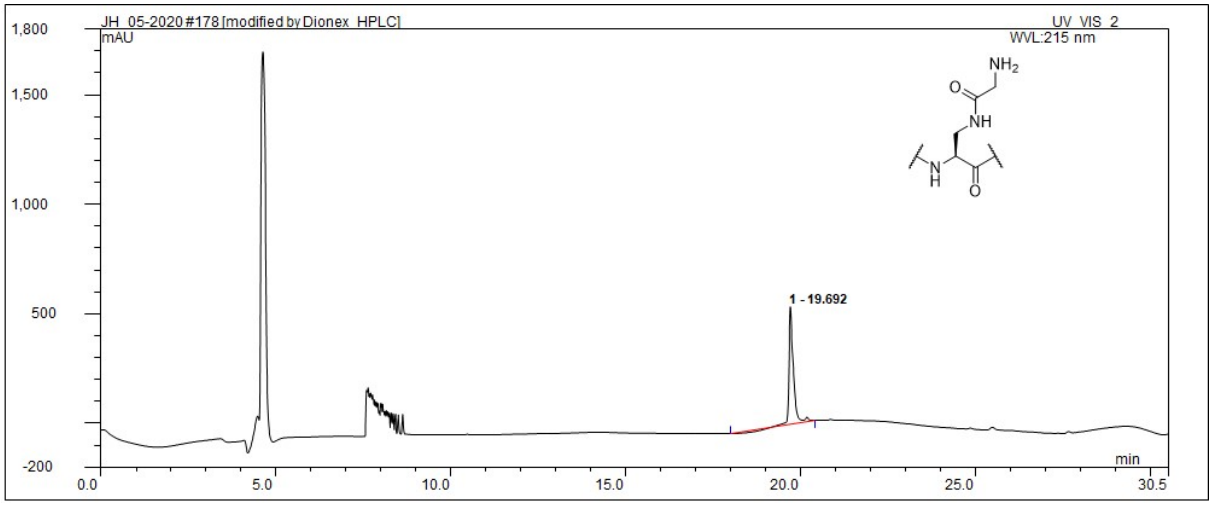


Figure S1. A) Analytical HPLC of the 13-mer H3K9 peptide after RP-HPLC purification. B) MALDI-TOF MS spectra of the purified 13-mer H3K9 peptide.

A)



B)

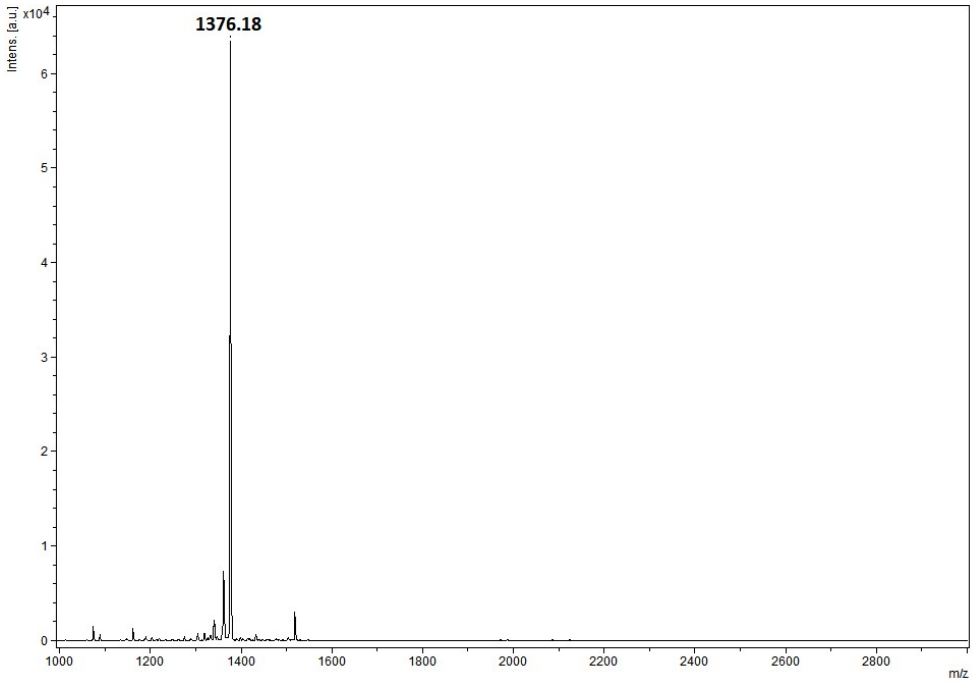
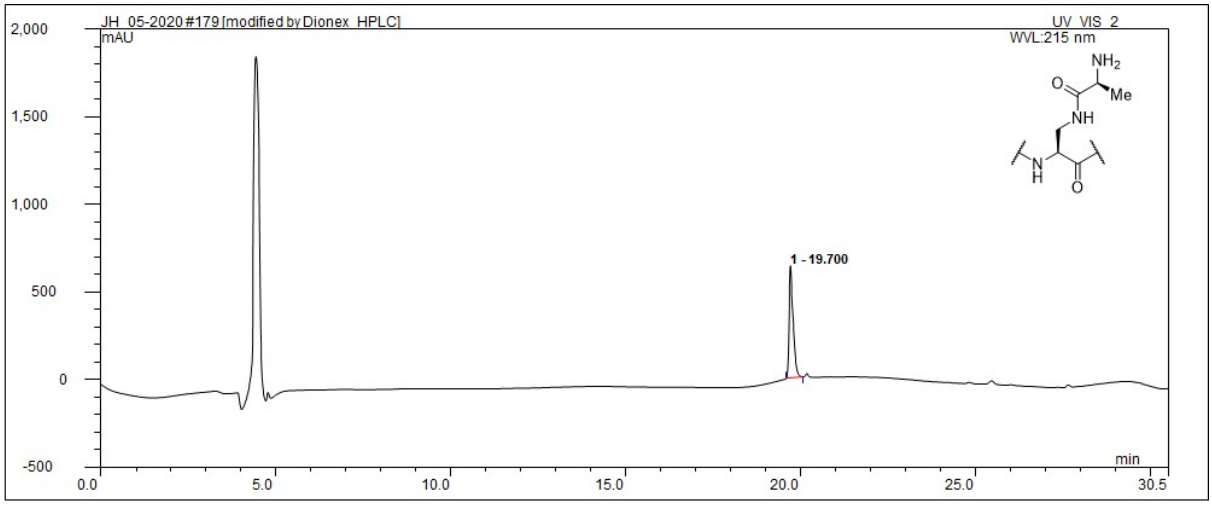


Figure S2. A) Analytical HPLC of the 13-mer H3K^{Gly}9 peptide after RP-HPLC purification. B) MALDI-TOF MS spectra of the purified 13-mer H3K^{Gly}9 peptide.

A)



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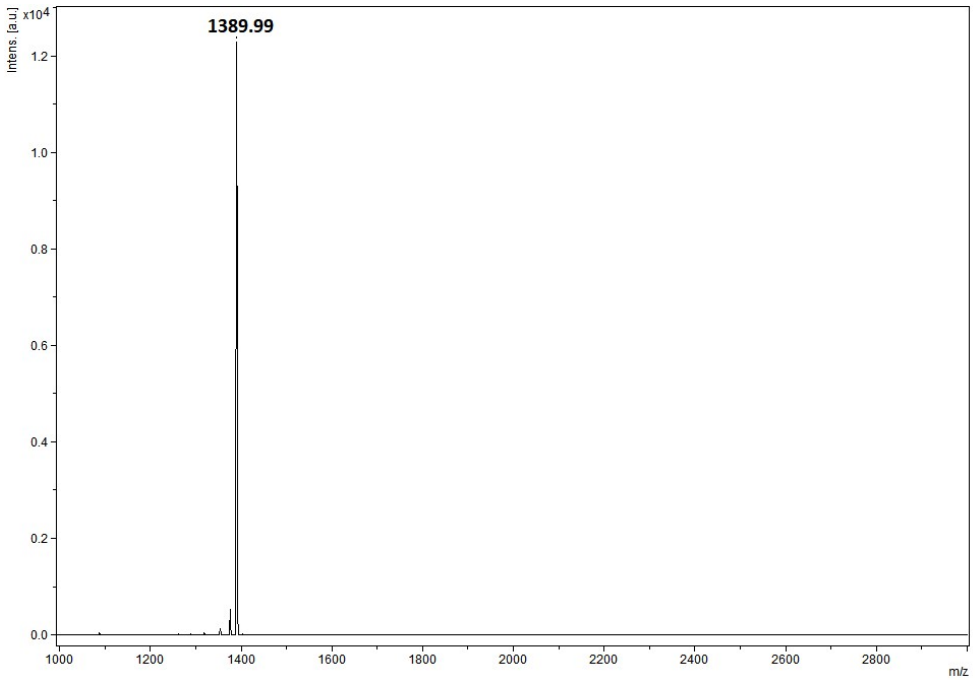
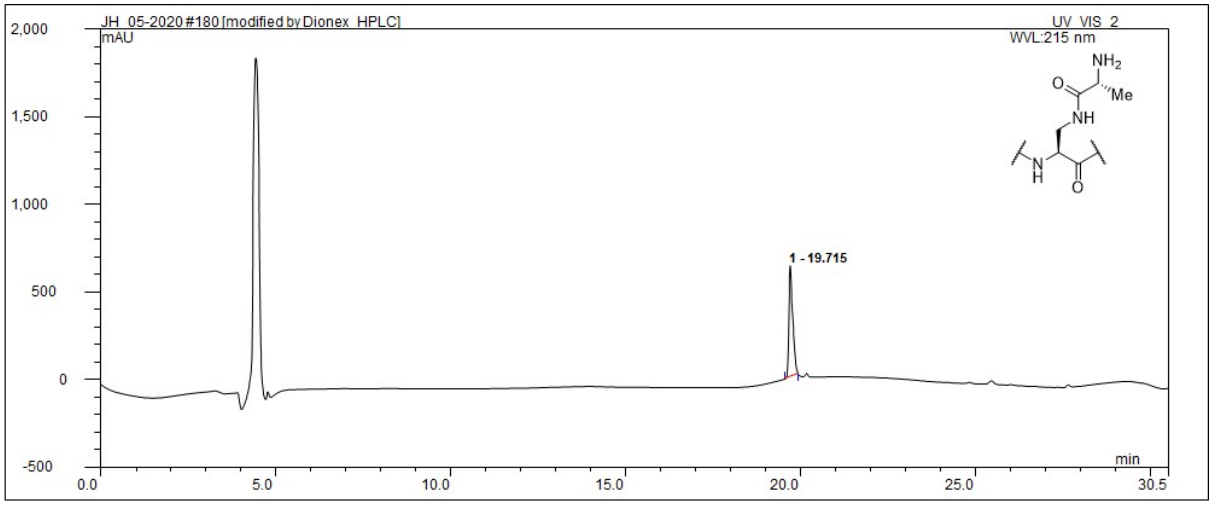


Figure S3. A) Analytical HPLC of the 13-mer H3K$^{L\text{-}Ala}$9 peptide after RP-HPLC purification. B) MALDI-TOF MS spectra of the purified 13-mer H3K$^{L\text{-}Ala}$9 peptide.

A)



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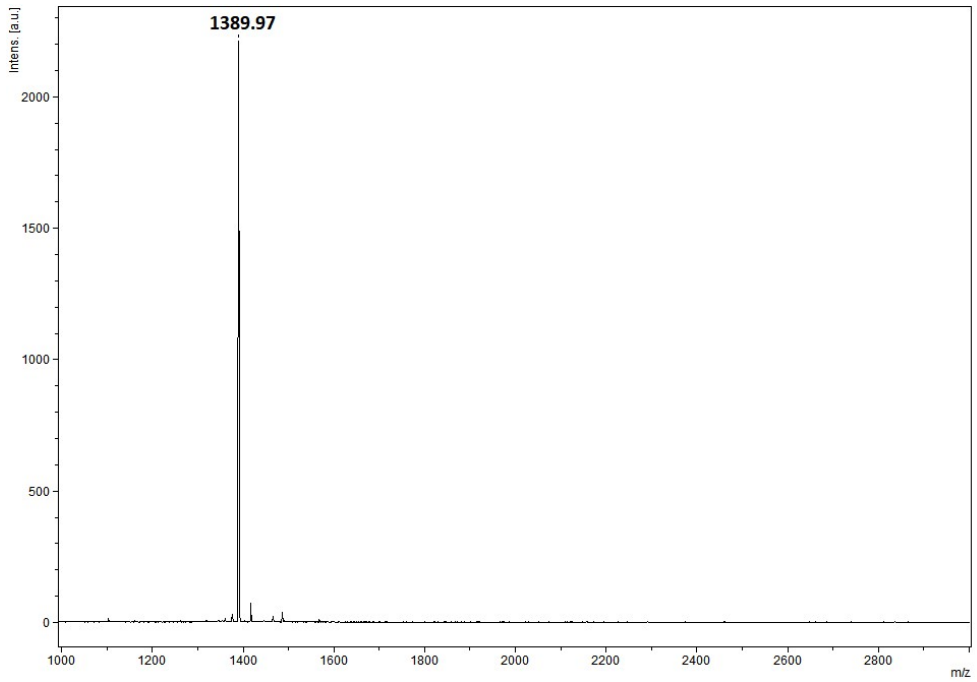
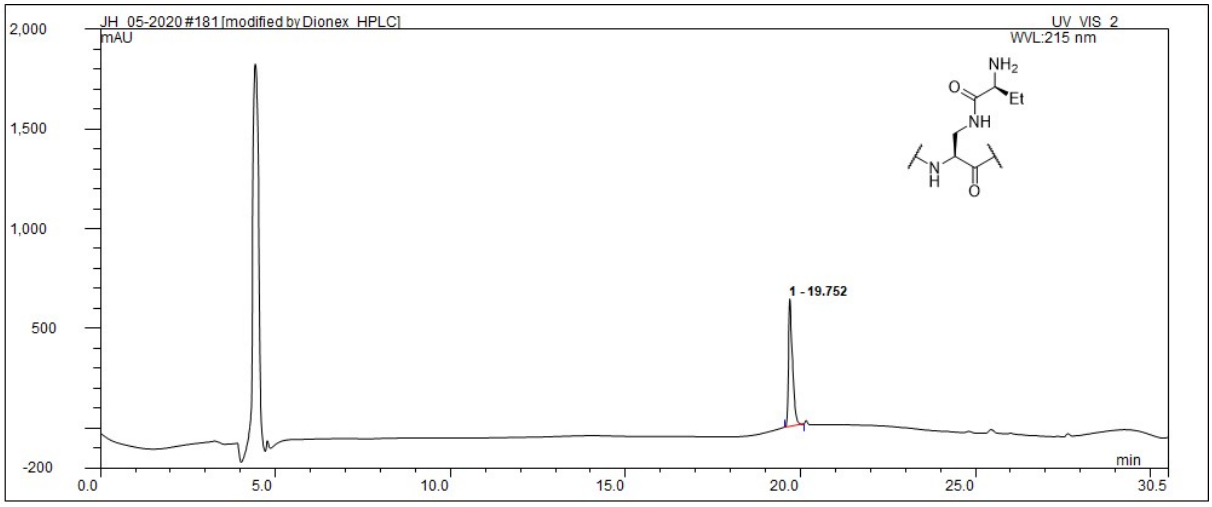


Figure S4. A) Analytical HPLC of the 13-mer H3K$^{D\text{-}Ala}$9 peptide after RP-HPLC purification. B) MALDI-TOF MS spectra of the purified 13-mer H3K$^{D\text{-}Ala}$9 peptide.

A)



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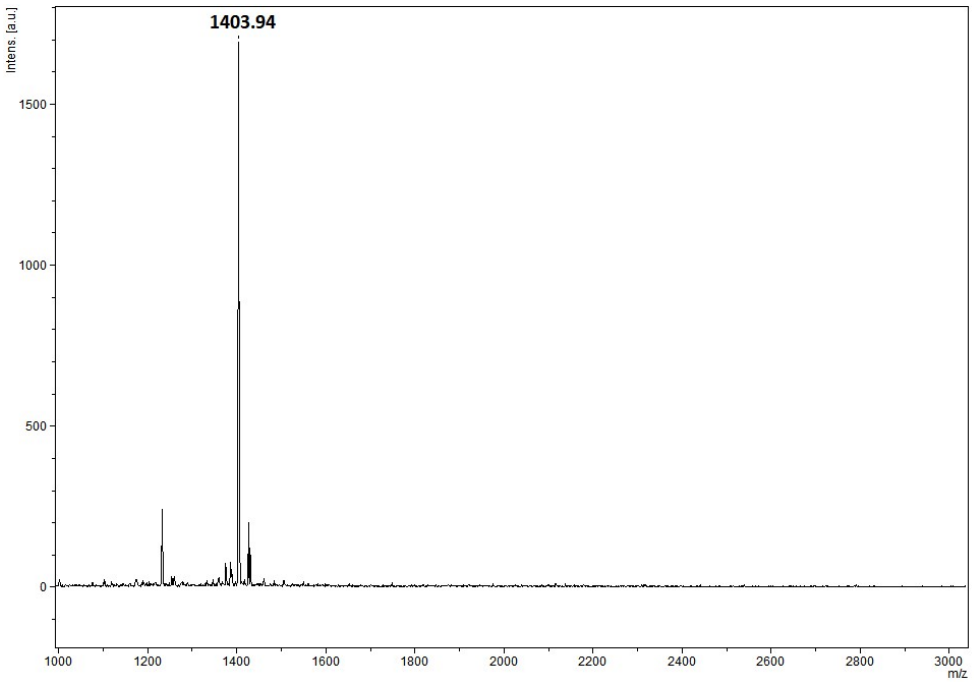
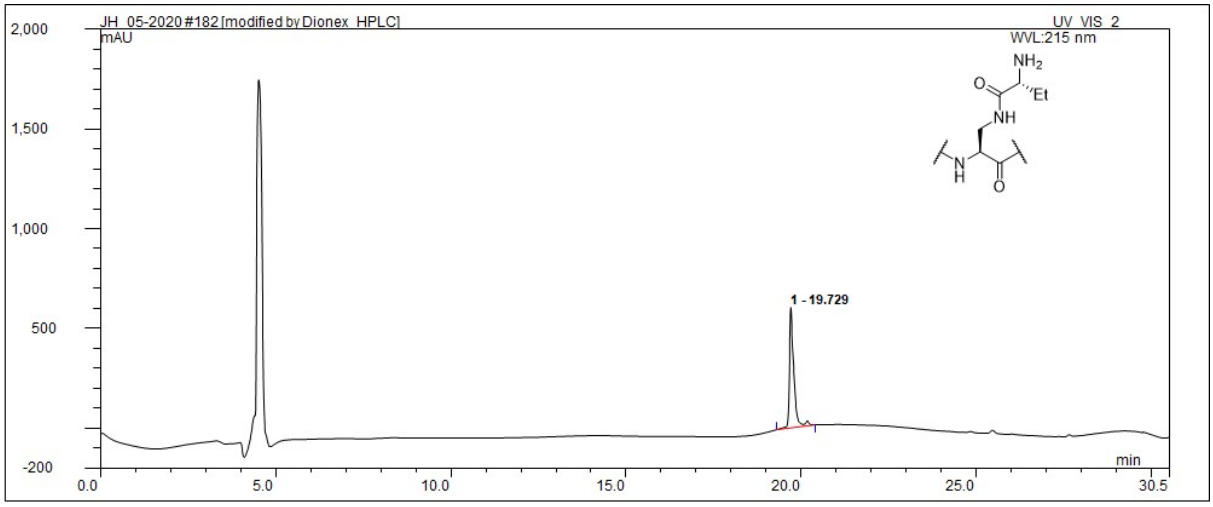


Figure S5. A) Analytical HPLC of the 13-mer H3K$^{L\text{-}Abu}$9 peptide after RP-HPLC purification. B) MALDI-TOF MS spectra of the purified 13-mer H3K$^{L\text{-}Abu}$9 peptide.

A)



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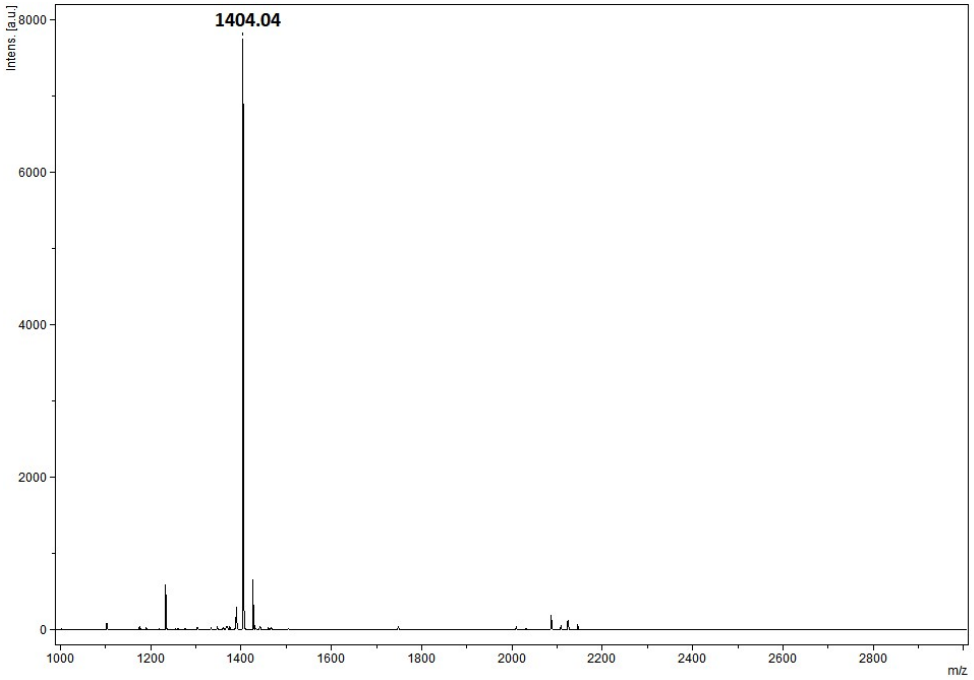
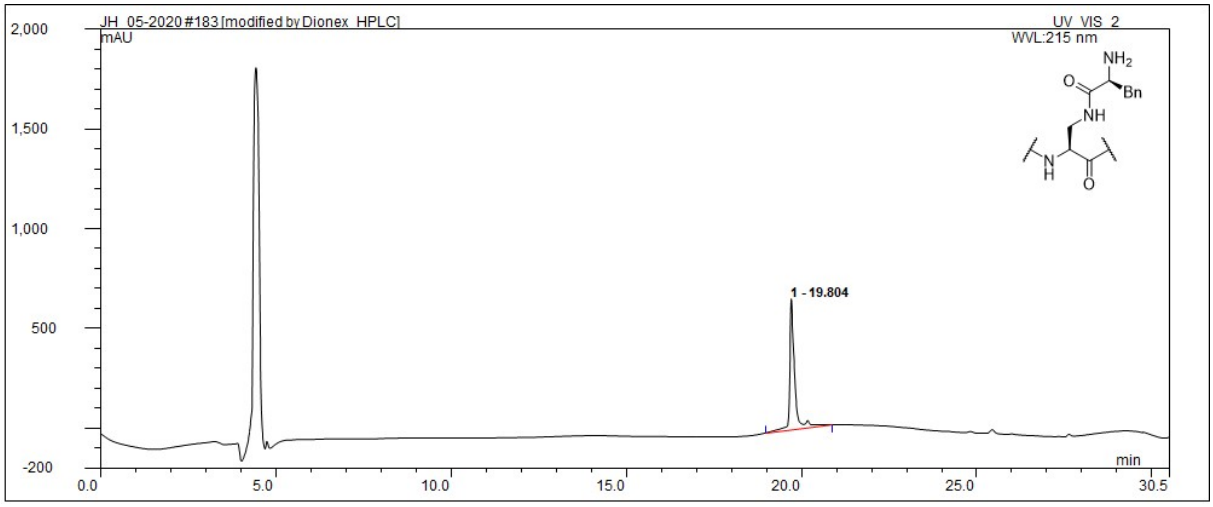


Figure S6. A) Analytical HPLC of the 13-mer H3K$^{D\text{-}Abu}$9 peptide after RP-HPLC purification. B) MALDI-TOF MS spectra of the purified 13-mer H3K$^{D\text{-}Abu}$9 peptide.

A)



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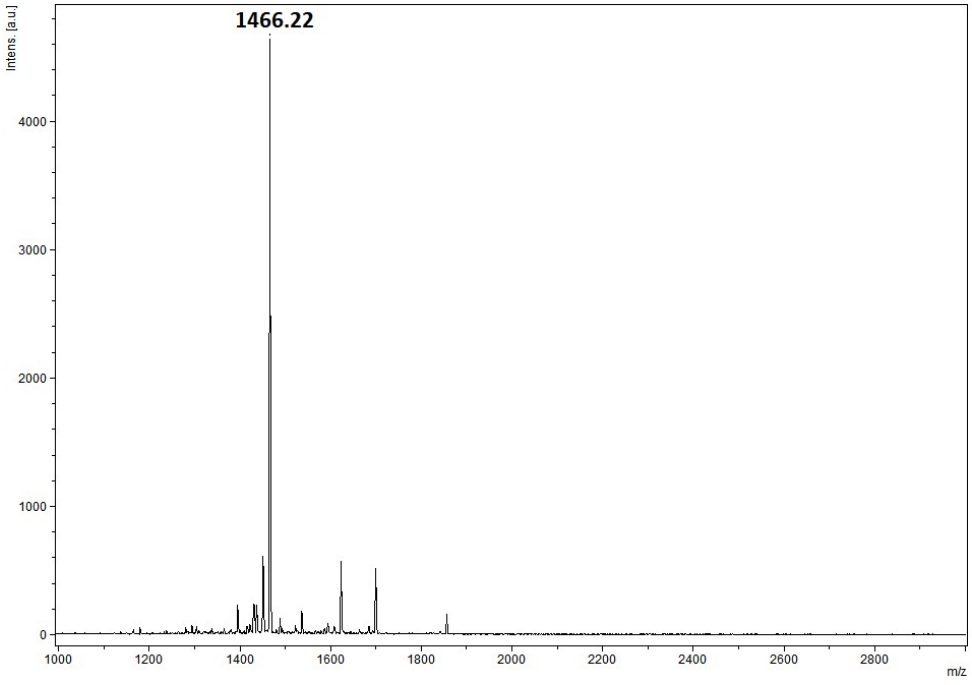
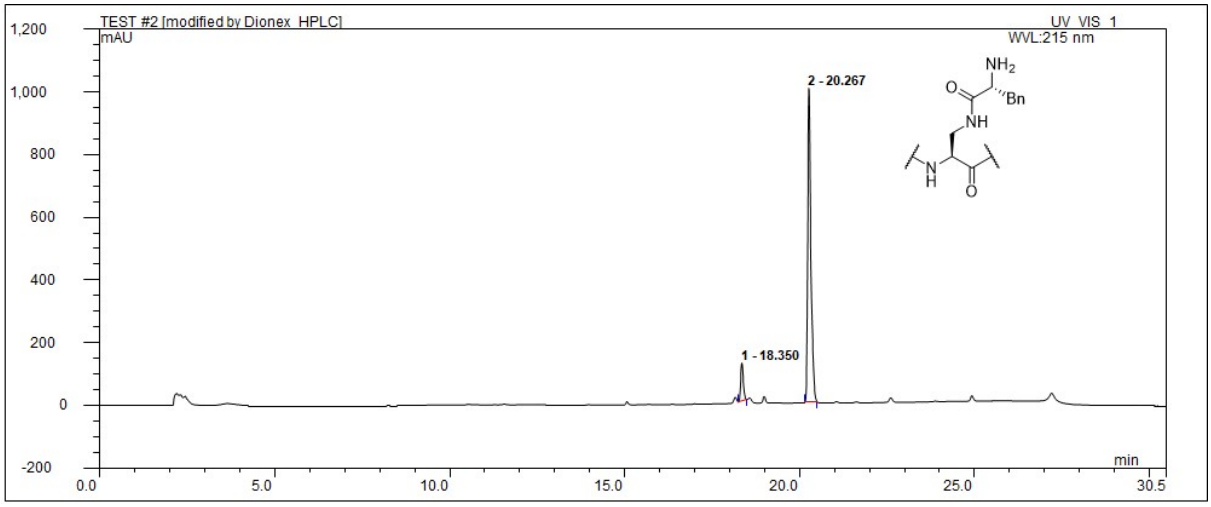


Figure S7. A) Analytical HPLC of the 13-mer H3K$^{L\text{-}Phe}$9 peptide after RP-HPLC purification. B) MALDI-TOF MS spectra of the purified 13-mer H3K$^{L\text{-}Phe}$9 peptide.

A)



B)

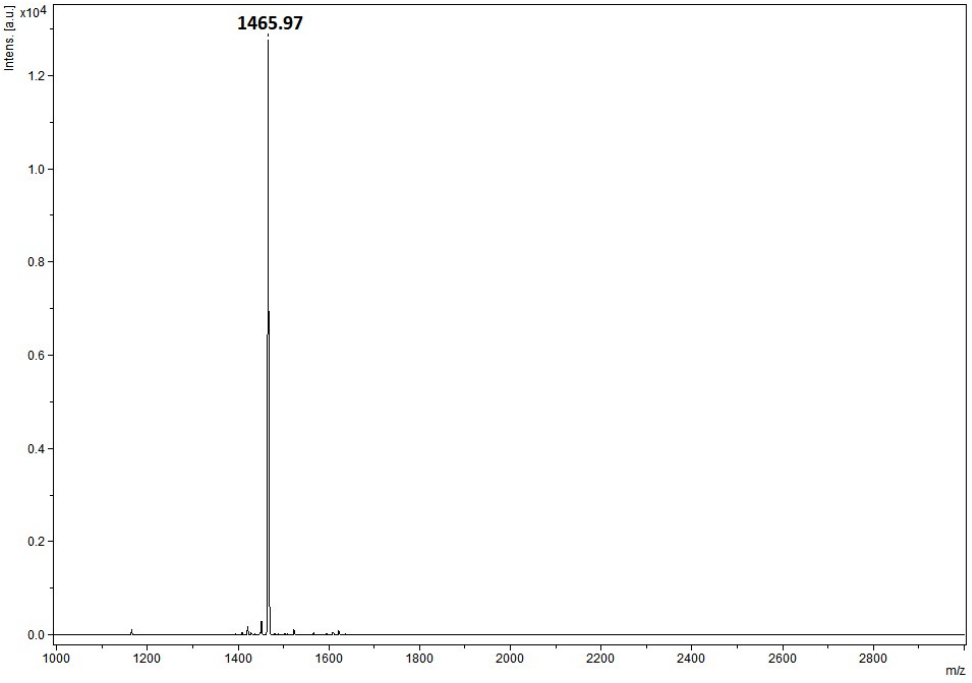


Figure S8. A) Analytical HPLC of the 13-mer H3K$^{D\text{-}Phe}$9 peptide after RP-HPLC purification. B) MALDI-TOF MS spectra of the purified 13-mer H3K$^{D\text{-}Phe}$9 peptide.

3. MALDI-TOF MS supporting figures

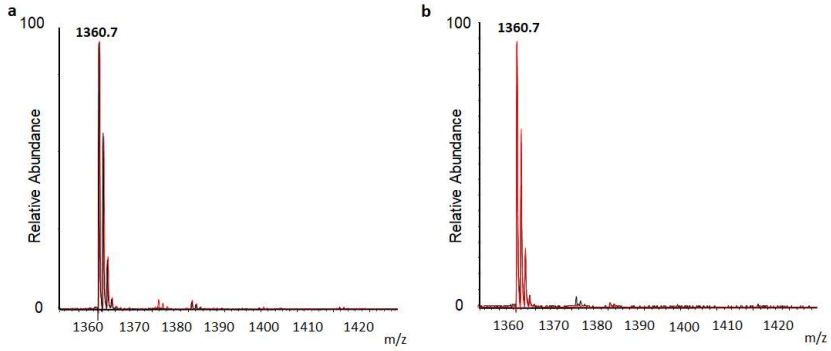


Figure S9 MALDI-TOF MS data showing control reaction of a) H3K9 in the presence of SAM and b) H3K9 in the presence of AcCoA after 3 hours.

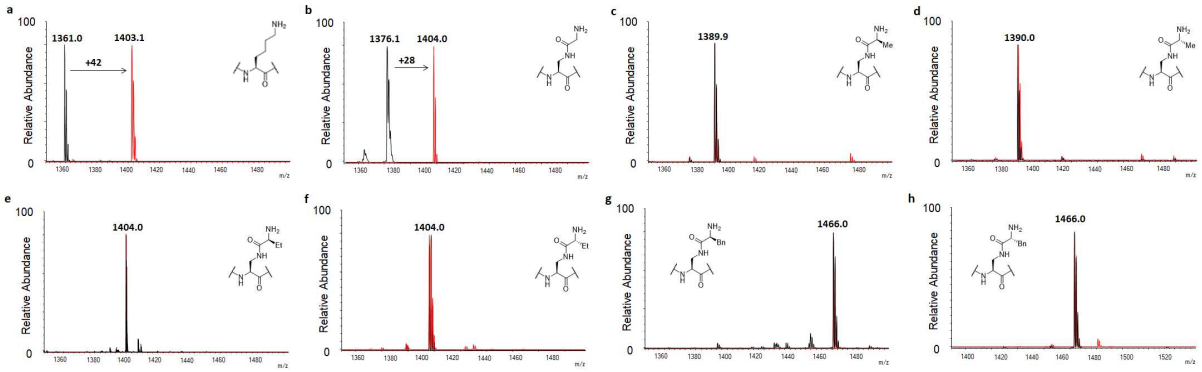


Figure S10. MALDI-TOF MS data showing methylation of H3 peptides (100 µM) in presence of G9a (2 µM) and SAM (500 µM) after 3 hours at pH 8.0. Control reactions without GLP present are shown in black, GLP-catalysed reactions are shown in red. a) H3K9, b) H3K^{Gly}9, c) H3K$^{L\text{-}Ala}$9, d) H3K$^{D\text{-}Ala}$9, e) H3K$^{L\text{-}Abu}$9, f) H3K$^{D\text{-}Abu}$9, g) H3K$^{L\text{-}Phe}$9, h) H3K$^{D\text{-}Phe}$9.

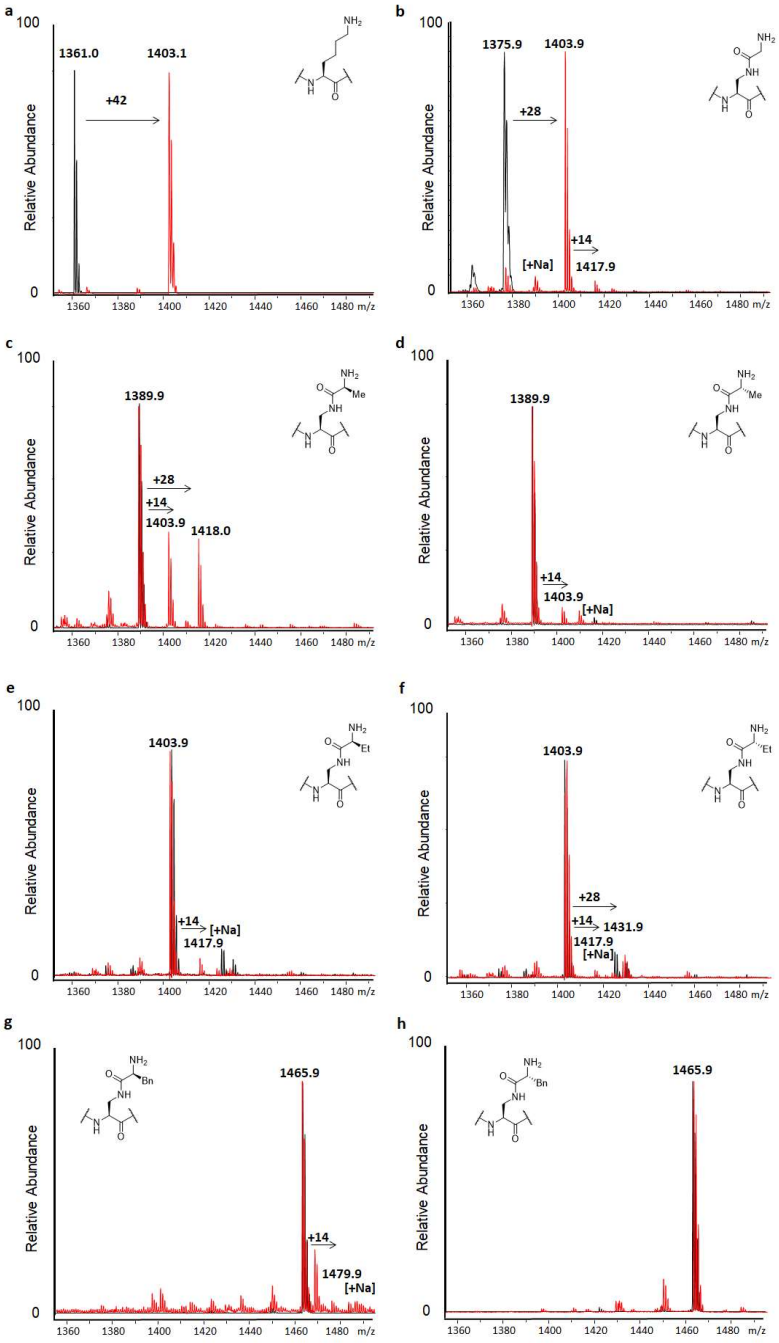


Figure S11. MALDI-TOF MS data showing methylation of H3 peptides (20 μM) in presence of GLP (2 μM) and SAM (500 μM) after 3 hours at pH 8.0. Control reactions without G9a present are shown in black, G9a-catalysed reactions are shown in red. a) H3K^{Gly}9, b) H3K$^{L\text{-}Ala}$9, c) H3K$^{D\text{-}Ala}$9, d) H3K$^{L\text{-}Abu}$9, e) H3K$^{D\text{-}Abu}$9, f) H3K$^{L\text{-}Phe}$9, g) H3K$^{D\text{-}Phe}$9.

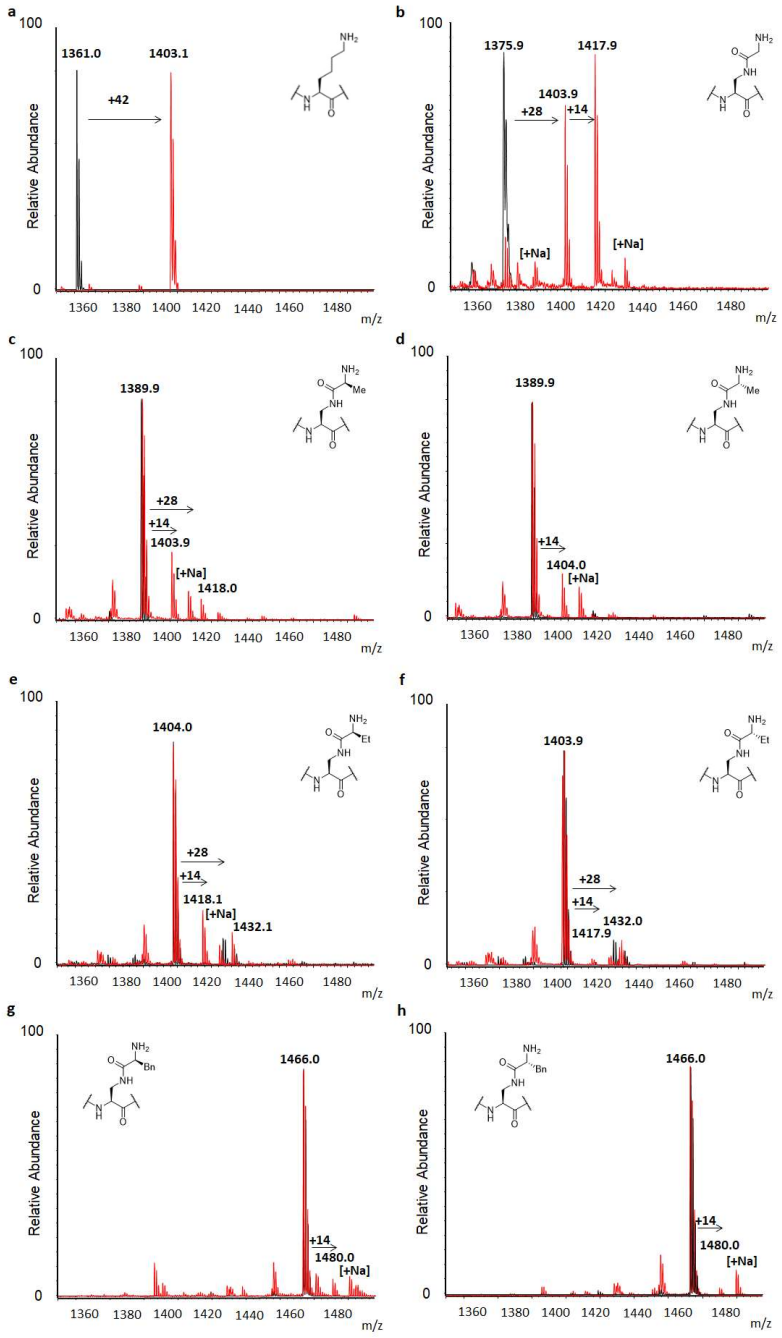


Figure S12. MALDI-TOF MS data showing methylation of H3 peptides (100 μM) in presence of GLP (10 μM) and SAM (1 mM) after 3 hours at pH 8.0. Control reactions without G9a present are shown in black, G9a-catalysed reactions are shown in red. a) H3K^{Gly}9, b) H3K$^{L\text{-}Ala}$9, c) H3K$^{D\text{-}Ala}$9, d) H3K$^{L\text{-}Abu}$9, e) H3K$^{D\text{-}Abu}$9, f) H3K$^{L\text{-}Phe}$9, g) H3K$^{D\text{-}Phe}$9.

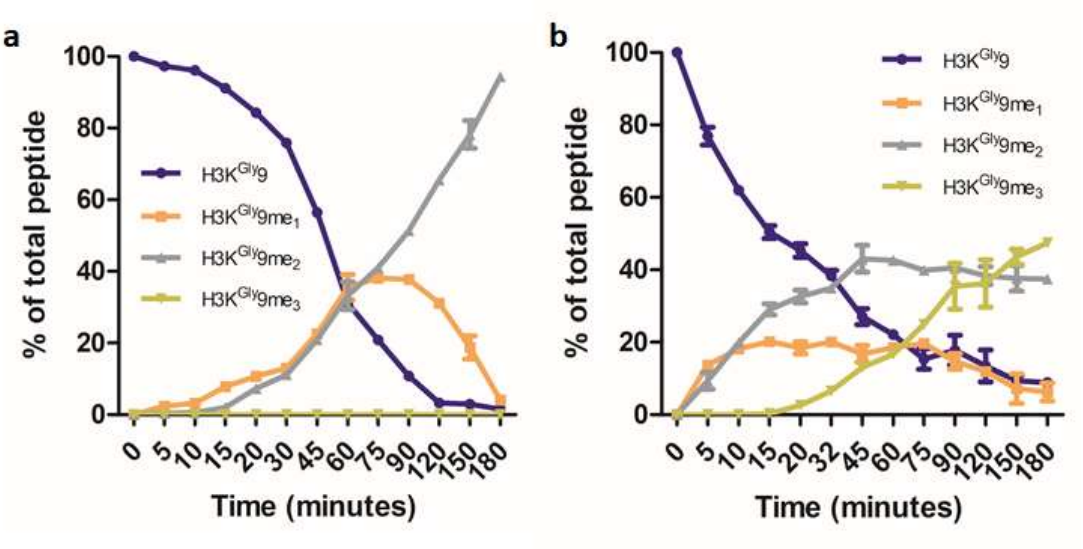


Figure S13. Consumption curve of the GLP-catalysed methylation of H3K^{Gly}9 (100 μM) at pH at **a)** 2 μM GLP and **b)** 10 μM of GLP.

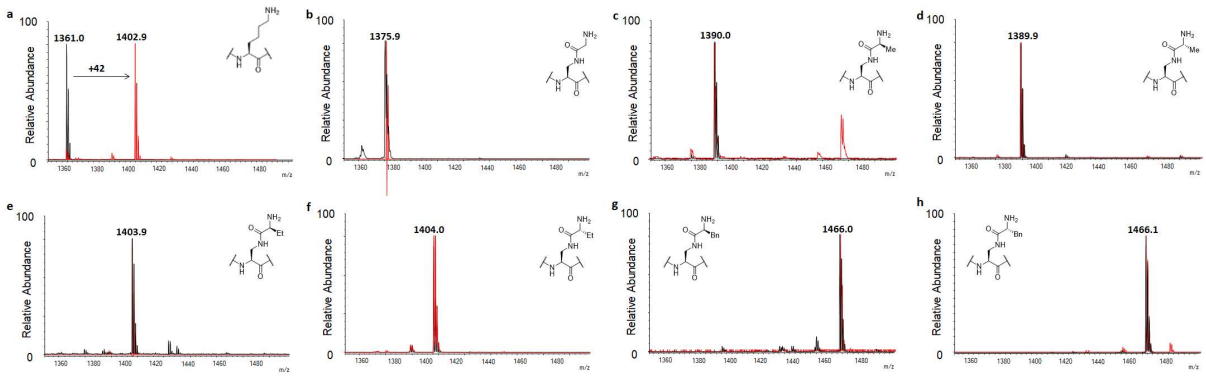


Figure S14. MALDI-TOF MS data showing acetylation of H3 peptides (100 μM) in presence of PCAF (2 μM) and AcCoA (300 μM) after 3 hours at pH 8.0. Control reactions without PCAF present are shown in black, PCAF-catalysed reactions are shown in red. a) H3K9, b) H3K^{Gly}9, c) H3K$^{L\text{-}Ala}$9, d) H3K$^{D\text{-}Ala}$9, e) H3K$^{L\text{-}Abu}$9, f) H3K$^{D\text{-}Abu}$9, g) H3K$^{L\text{-}Phe}$9, h) H3K$^{D\text{-}Phe}$9.

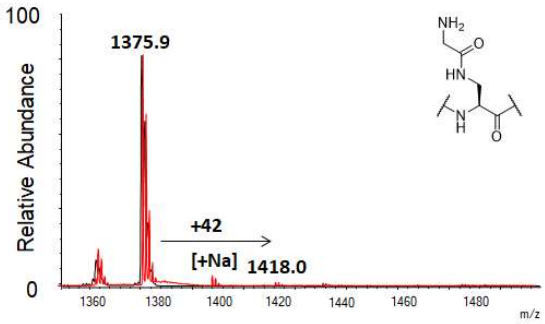


Figure S15. MALDI-TOF MS data showing acetylation of H3K^{Gly}9 (100 µM) in presence of GCN5 (10 µM) and AcCoA (500 µM) after 3 hours at pH 8.0. Control reaction without GCN5 is shown in black, GCN5-catalysed reaction is shown in red.

4. Inhibition supporting figures

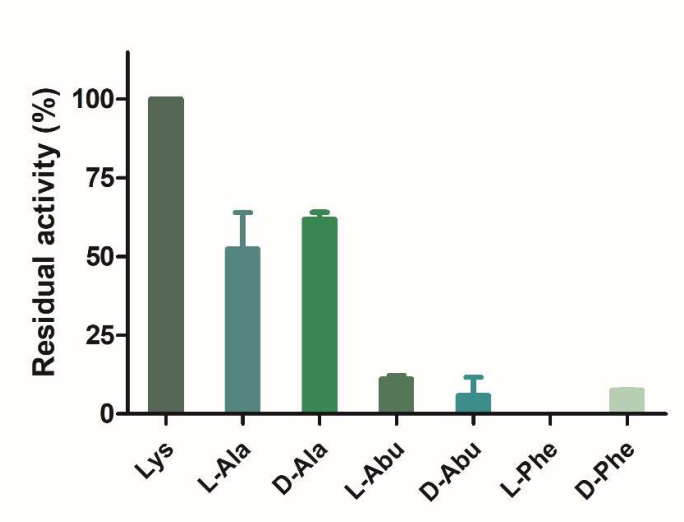


Figure S16. Single point inhibition of GLP (200 nM) by H3K9 peptides (100 µM). Error bars reported as standard error (SE), experiments carried out in duplicate.

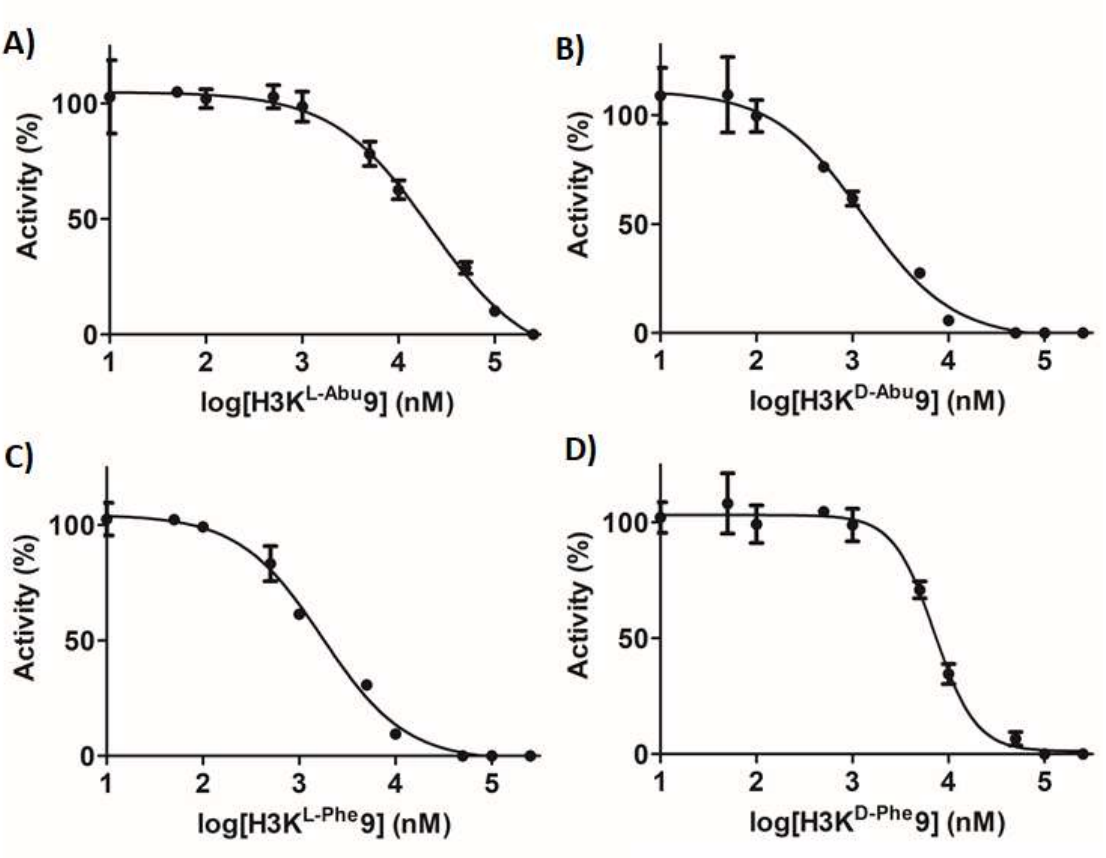


Figure S17. Dose-response curves showing inhibition of GLP-catalysed H3K9 methylation activity in the presence of different H3 peptides: a) H3K$^{L\text{-}Abu}$9, b) H3K$^{D\text{-}Abu}$9, c) H3K$^{L\text{-}Phe}$9, d) H3K$^{D\text{-}Phe}$9.

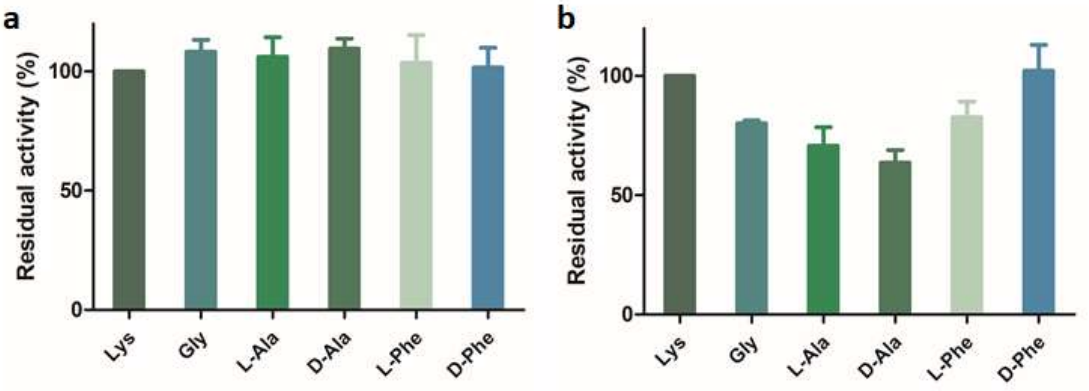


Figure S18. Single point inhibition of GCN5 (500 nM) by H3K9 peptides, a) 10 μM, b) 100 μM. Error bars reported as standard error (SE), experiments carried out in duplicate.

6. Computational docking analysis

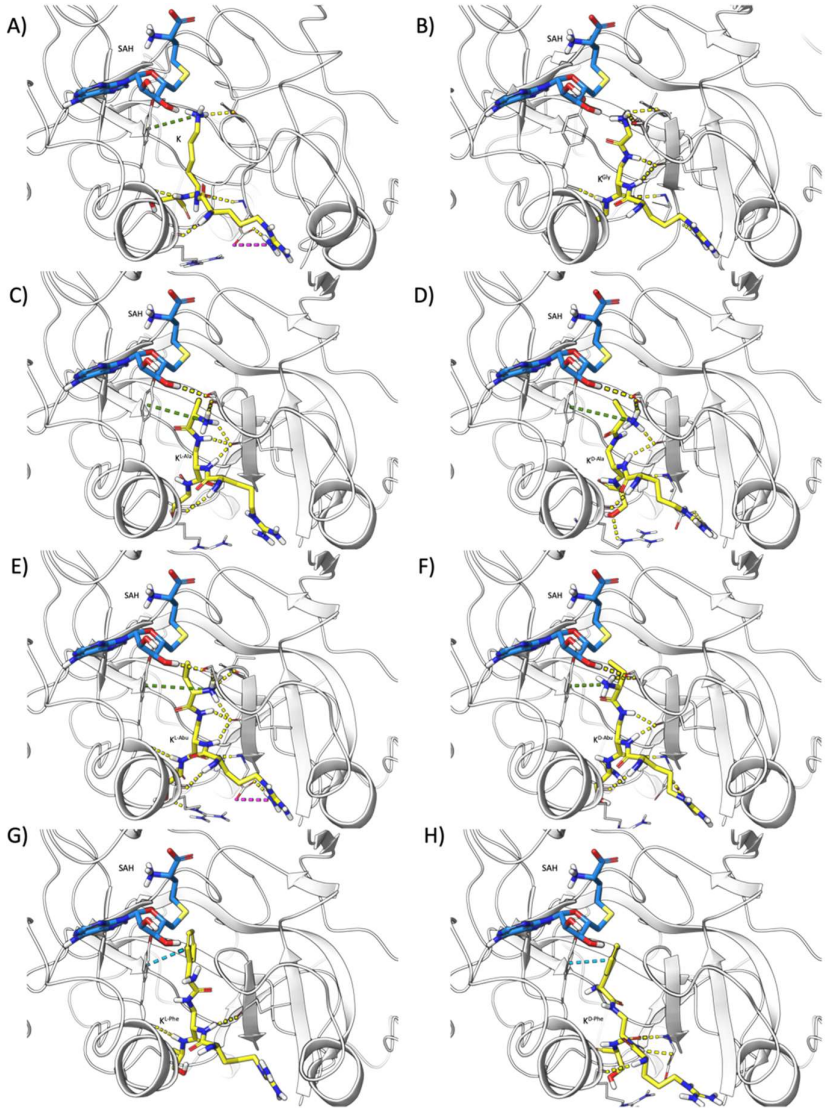


Figure S19. Detailed induced fit docking poses of lysine analog containing H3 peptides (residues 6-11) in GLP (PDB-ID: 2RFI). GLP is displayed in grey, SAH is shown in blue, and peptides are shown in yellow. Hydrogen bonding interactions are displayed as dashed yellow lines, π–π stacking is displayed as blue dashed lines. Cation-π interactions are displayed as green dashed lines. Key residues interacting with lysine analogs are displayed; a) H3K9, b) H3K^{Gly}9, c) H3K$^{L\text{-}Ala}$9, d) H3K$^{D\text{-}Ala}$9, e) H3K$^{L\text{-}Abu}$9, f) H3K$^{D\text{-}Abu}$9, g) H3K$^{L\text{-}Phe}$9, h) H3K$^{D\text{-}Phe}$9.

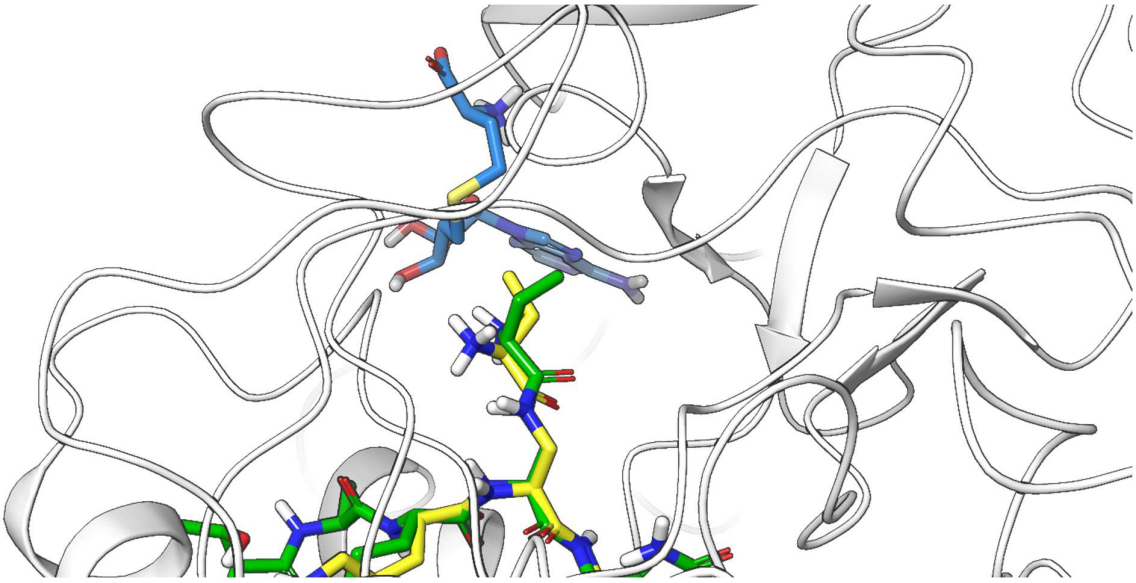
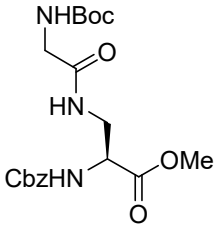


Figure S20. Overlay of H3 peptides possessing lysine analogues (residues 6-11) in GLP (PDB-ID: 2FI), overlay of H3K9$^{L\text{-}Abu}$9 (yellow) and H3K9$^{D\text{-}Abu}$9 (green). SAH is shown in blue.

7. NMR spectra of synthesised compounds



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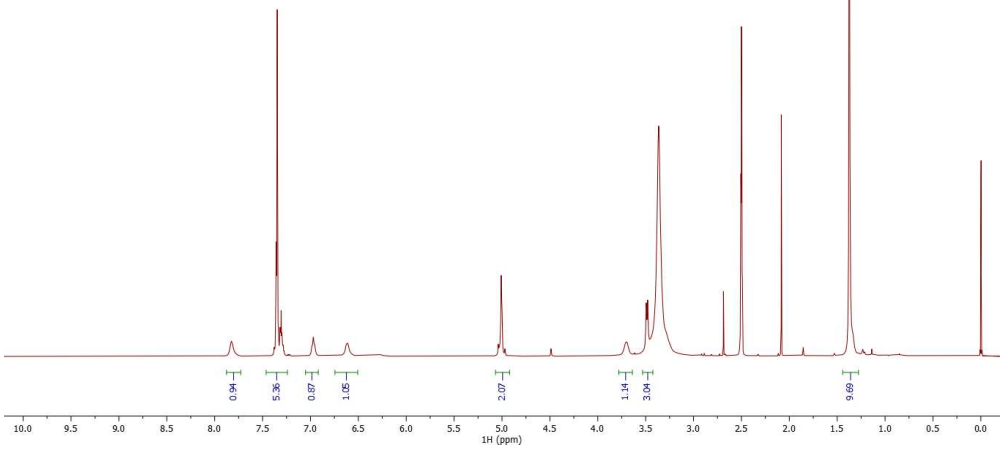

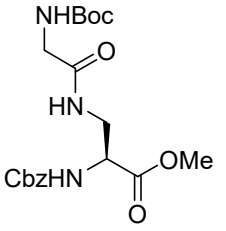

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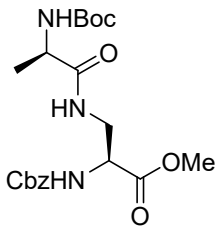

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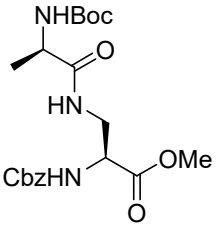

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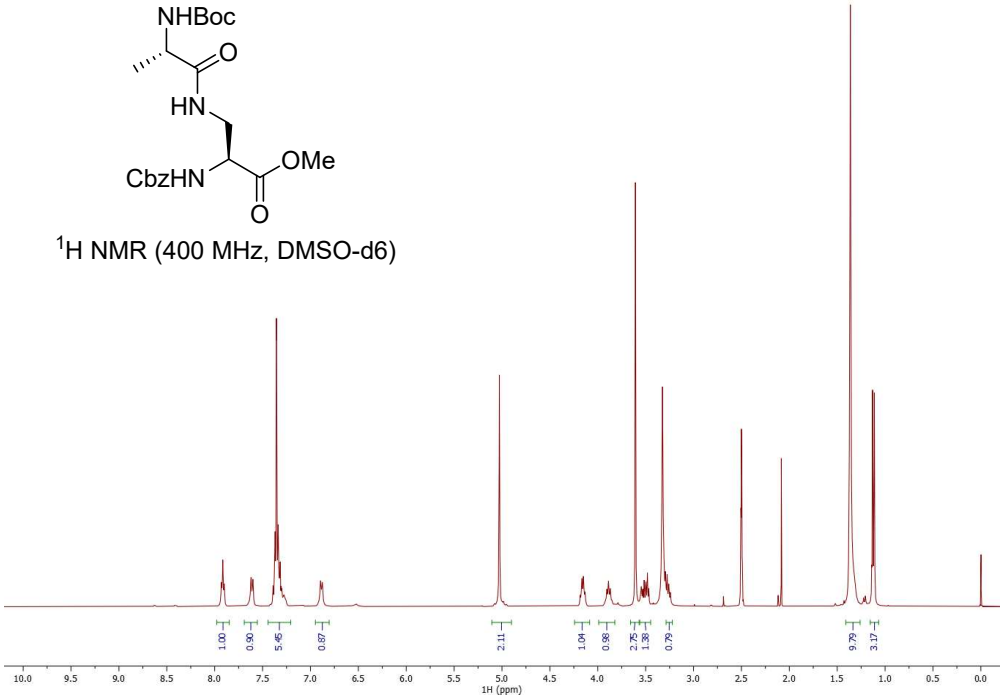

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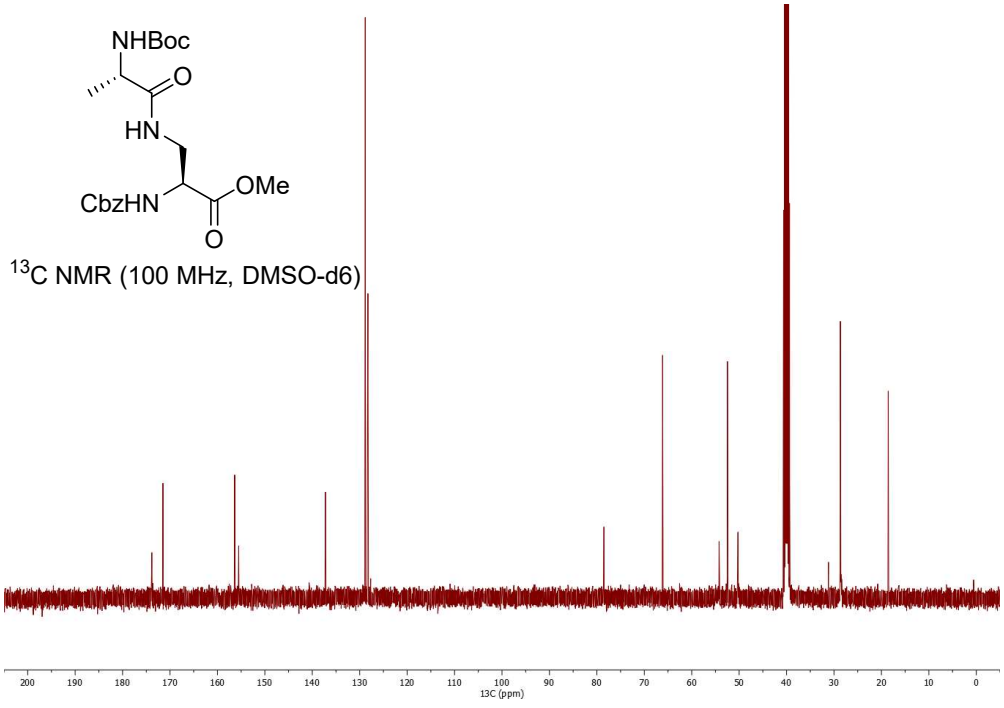

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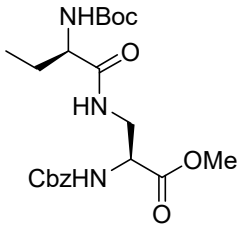

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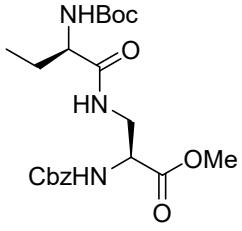

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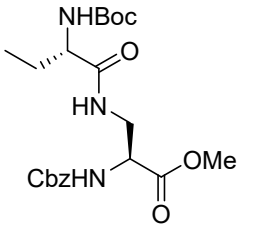

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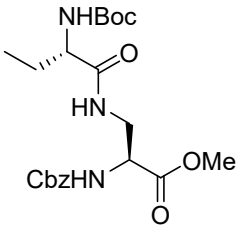

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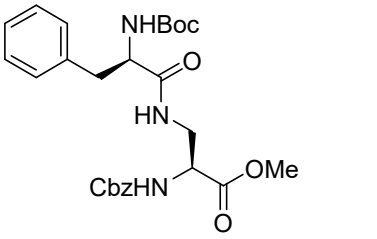

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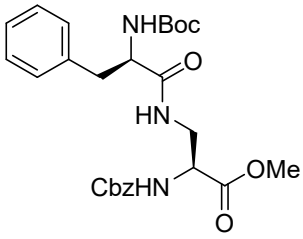

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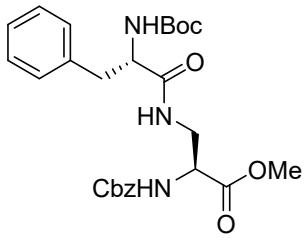

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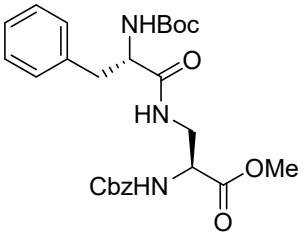

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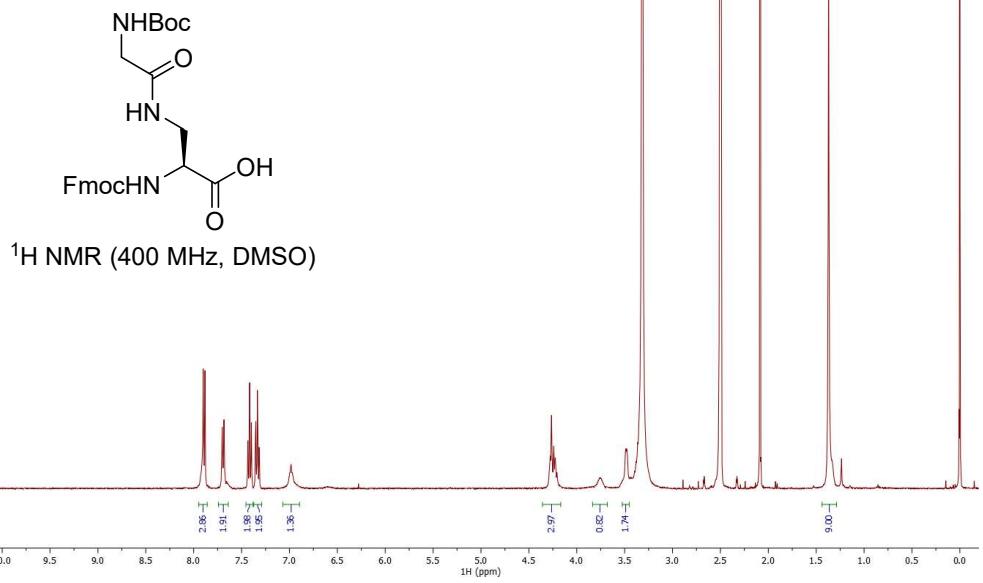
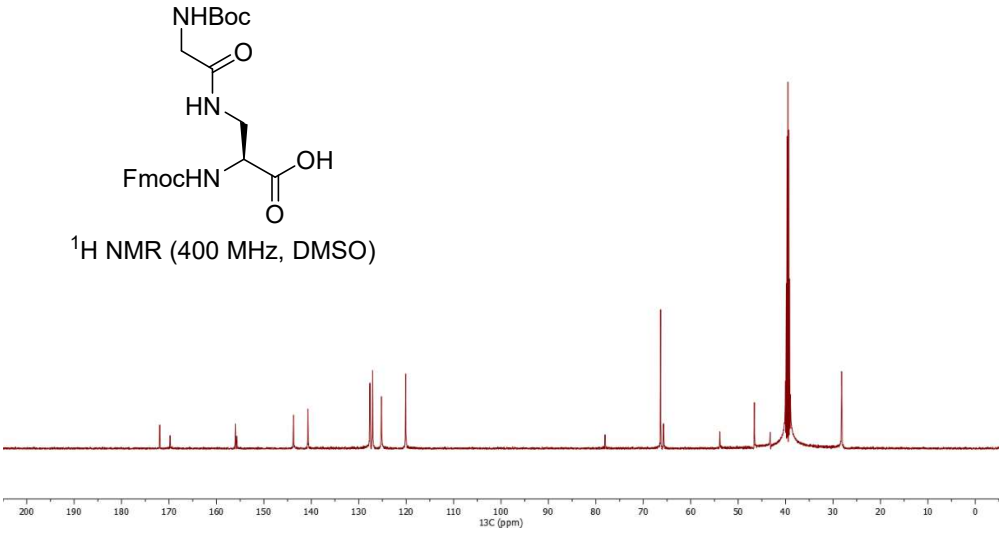
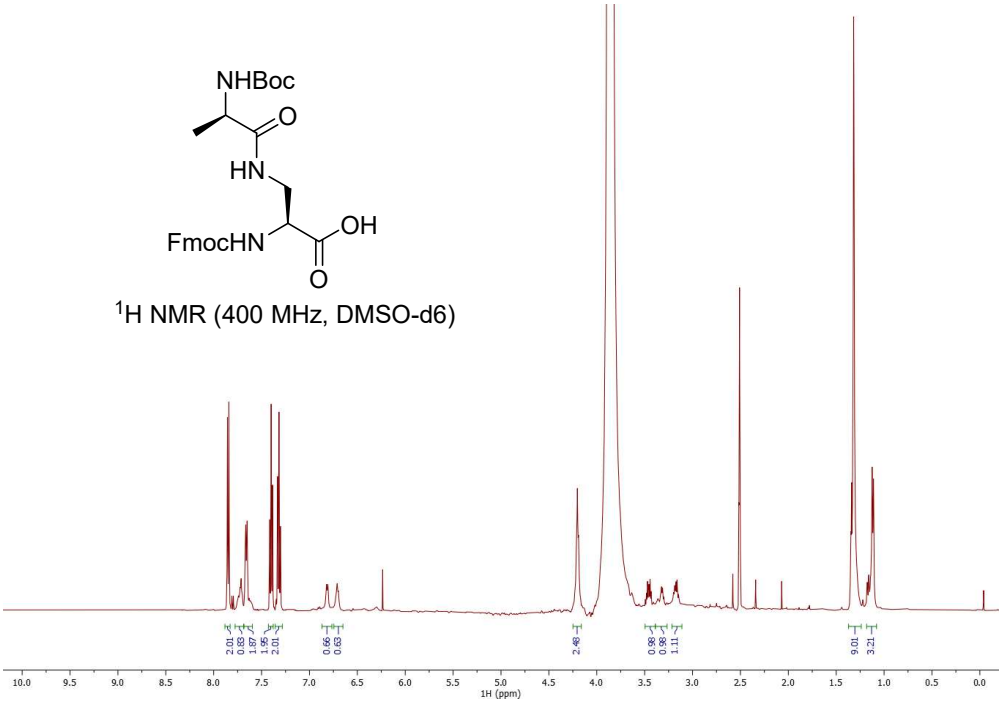


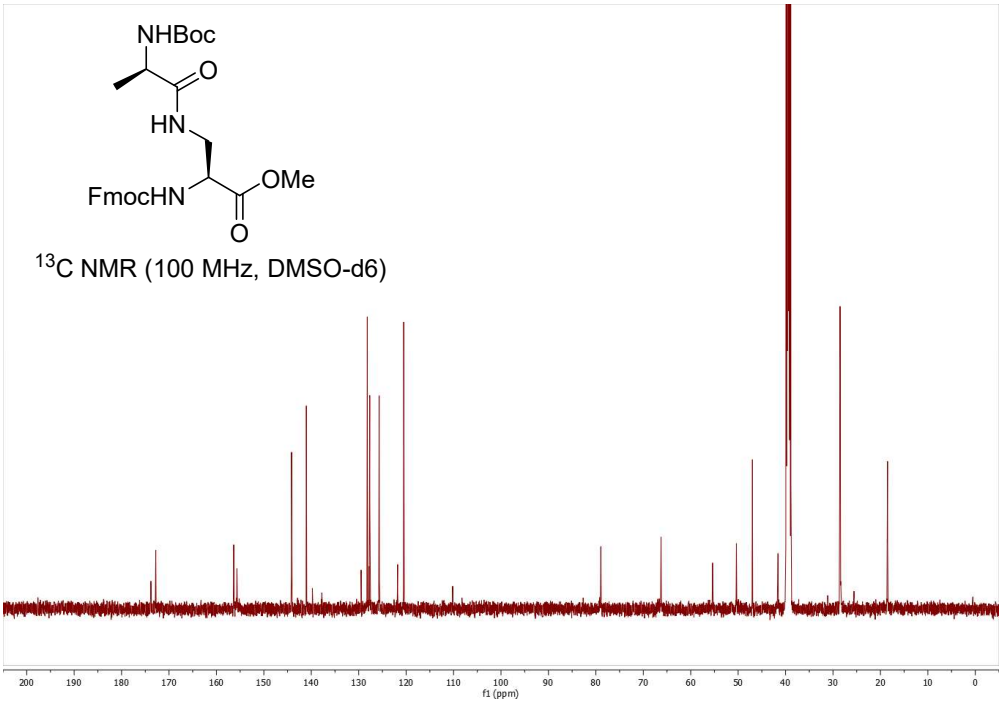
^{1}H NMR (400 MHz, DMSO)



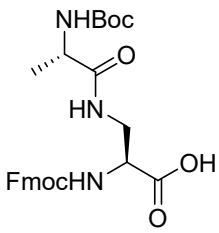
^{1}H NMR (400 MHz, DMSO)



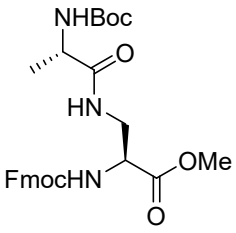
^{1}H NMR (400 MHz, DMSO-d6)



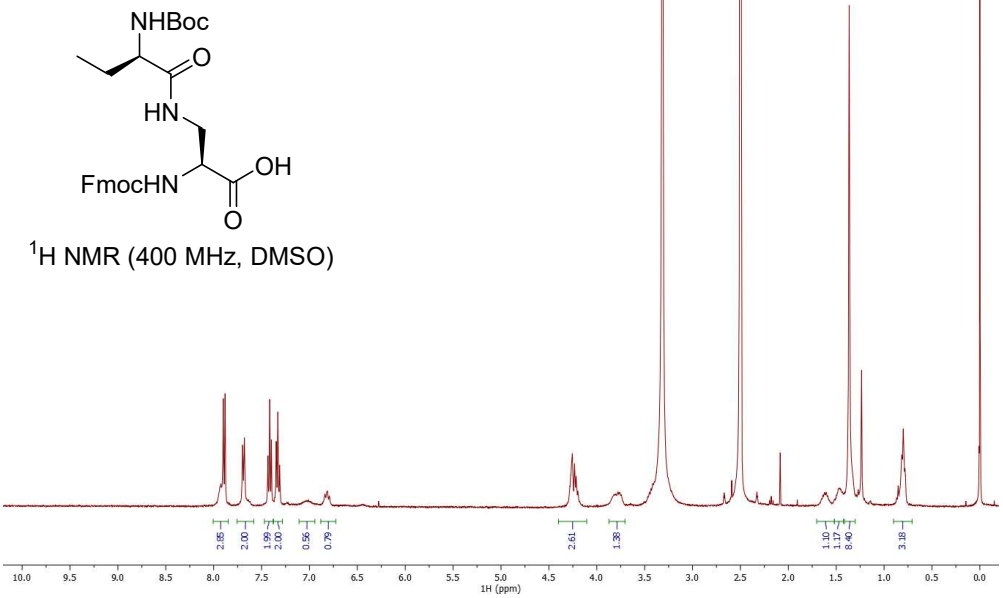
^{13}C NMR (100 MHz, DMSO-d6)



^{1}H NMR (400 MHz, DMSO-d6)

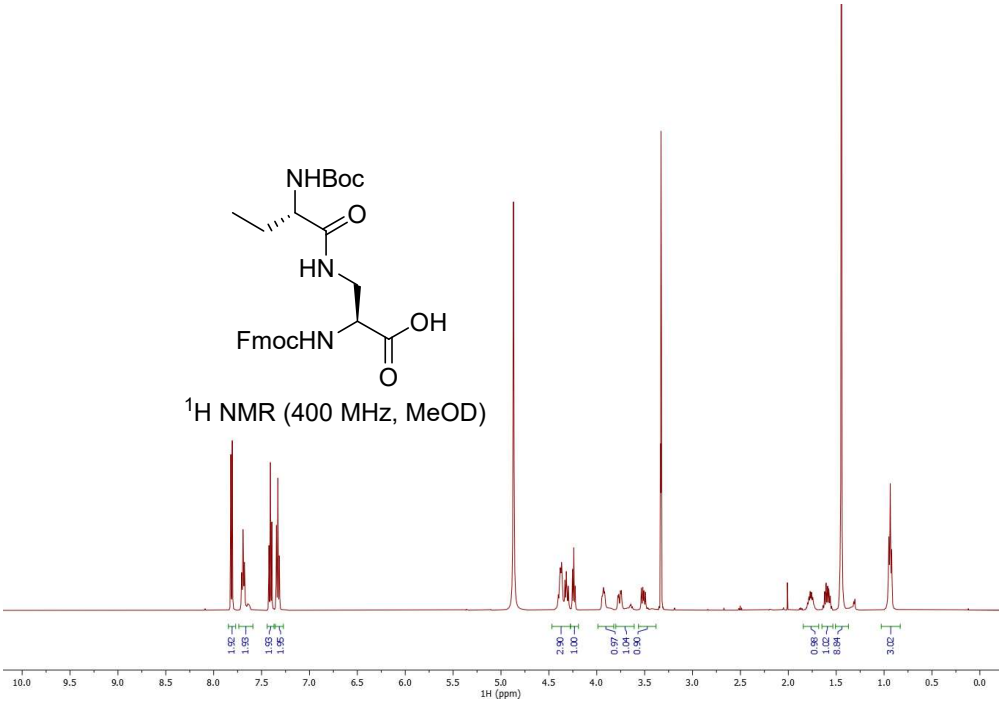


^{13}C NMR (100 MHz, DMSO-d6)

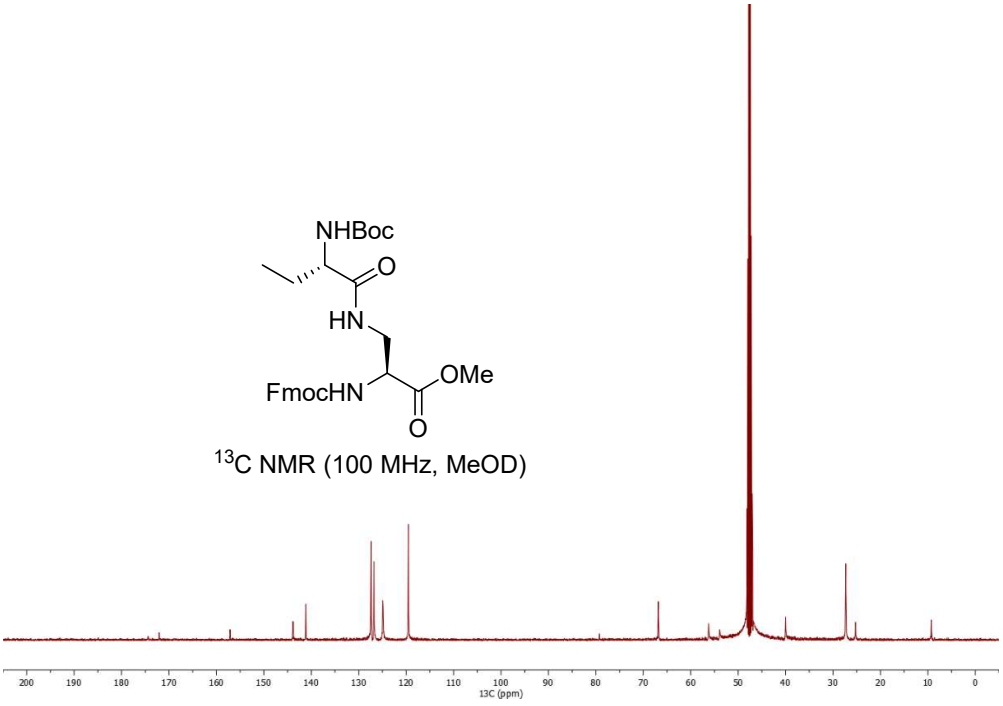


^{1}H NMR (400 MHz, DMSO)

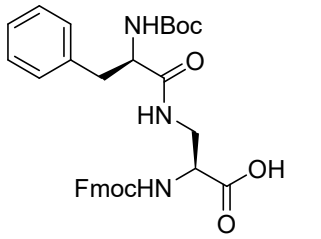
^{1}H NMR (400 MHz, DMSO)



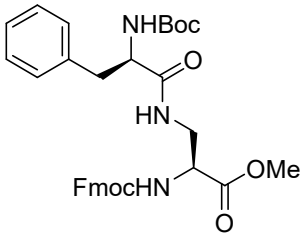
^{1}H NMR (400 MHz, MeOD)



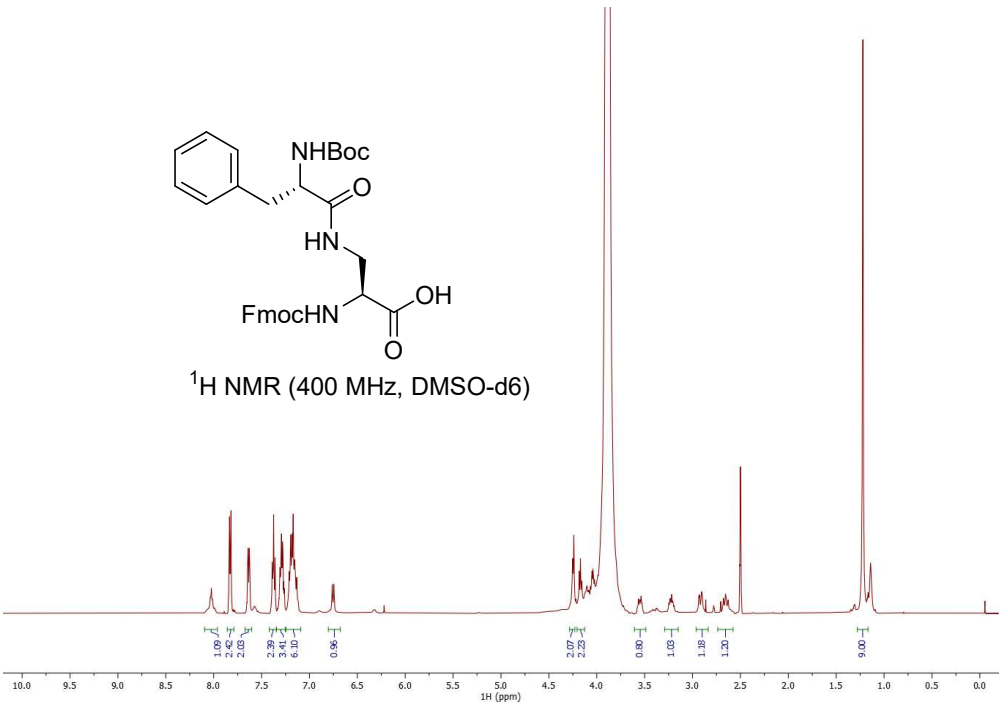
^{13}C NMR (100 MHz, MeOD)



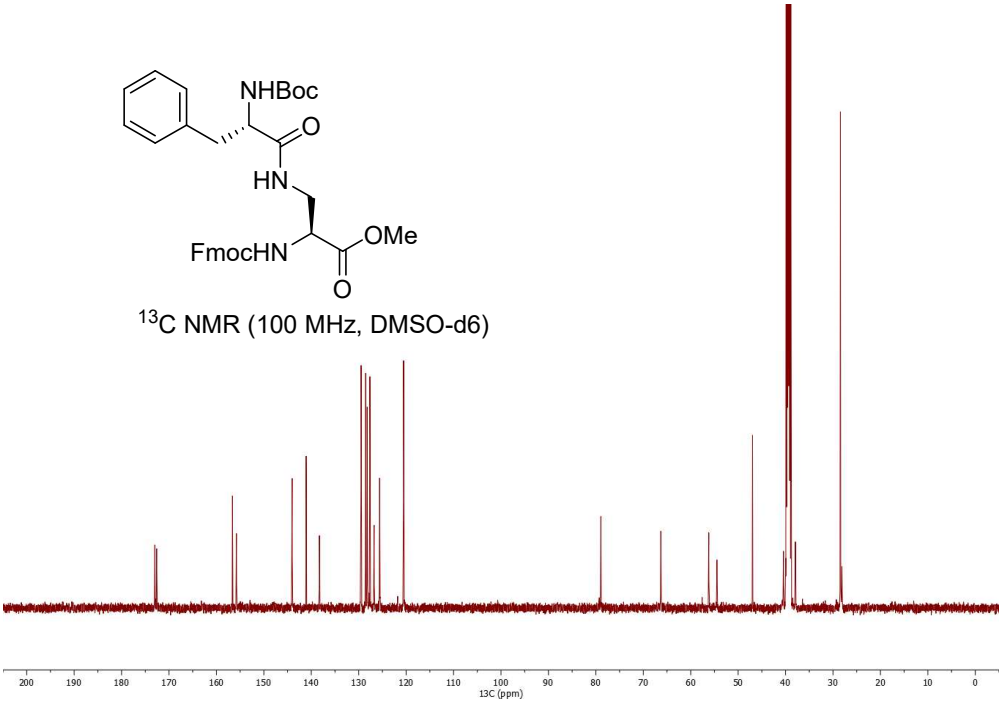
^{1}H NMR (400 MHz, DMSO-d6)



^{13}C NMR (100 MHz, DMSO-d6)



^{1}H NMR (400 MHz, DMSO-d6)



^{13}C NMR (100 MHz, DMSO-d6)