

Supporting Information:

**Facile Synthesis and Altered Ionization Efficiency of Diverse N^ε-Alkyllysine
Containing Peptides**

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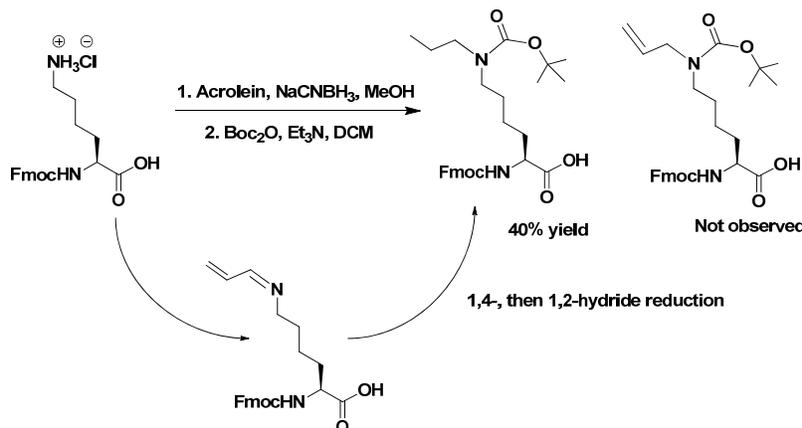
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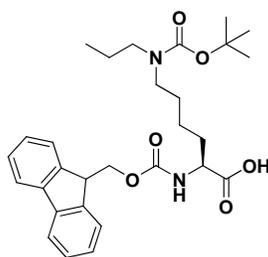
1. General Materials and Methods.

Unless stated otherwise, the following general protocols were followed. Reagents for chemical synthesis were obtained from Sigma-Aldrich or Acros Organics and used without further purification. Optima grade solvents were obtained from Fisher Scientific, degassed with argon, and purified on a solvent drying system. Optima grade acetonitrile was degassed under vacuum and used for HPLC purification. All solution-phase reactions were carried out in round-bottom flasks and stirred using Teflon-coated magnetic stirring bars. To prepare lysine derivatives, solution-phase reactions were carried out with dried solvents and under a slightly positive, static pressure of argon. Glassware was dried in an oven at 120 °C for at least 12 h prior to use and then either cooled in a desiccator cabinet over Drierite or assembled quickly while hot, sealed with rubber septa, and allowed to cool under a stream of argon. Hamilton microsyringes were dried at 60 °C for at least 24 h prior to use and cooled in the same manner as described above. Moisture- and air-sensitive liquids or solutions were handled via commercially-available, nitrogen-flushed BD Luer-Lock disposable syringes. Analytical thin layer chromatography (TLC) was performed using Whatman 250 micron aluminum backed UV F254 pre-coated silica gel flexible plates and visualized under UV light (254 nm) or by staining with potassium permanganate (KMnO₄), ceric ammonium molybdenate (CAM), or iodine (I₂). Flash column chromatography was performed on Merck 230–400 mesh silica gel 60. Reaction solvents were removed by rotary evaporation using a Büchi rotary evaporator, equipped with a dry ice-ethanol condenser. Analytical HPLC was carried out on a Waters 600 Controller HPLC/2998 diode array detector using XBridge™ Prep C18 5µm 4.6×150mm reverse phase column with UV detection at 260 nm. Preparative HPLC purification was carried out on a same HPLC using XBridge™ Prep C18 5µm OBD™ 19×150mm reverse phase column with UV detection at 260 nm. All the peptides were eluted with 0–40% gradient from H₂O (0.1%TFA) to acetonitrile (0.1% TFA) over 30 min. For preparative HPLC, the peptides were eluted at 19–25 min. Concentration and lyophilization of aqueous samples were performed using Savant Sc210A SpeedVac Concentrator (Thermo), followed by Flexi-Dry™ µP Freeze-Dryer (FTS™ System). Proton nuclear magnetic resonance spectra (¹H NMR) were recorded on Bruker Avance III 500 MHz instrument at 24 °C. Chemical shifts for ¹H NMR spectra are reported as δ in units of parts per million (ppm) relative to tetramethylsilane (δ=0.0) or using residual solvent signals: chloroform-*d* (δ=7.26, singlet) and methanol-*d*₄ (δ= 3.30, quintet). Coupling constants are expressed in Hz. High Resolution Mass spectra (HRMS) of small molecules were collected at the MSKCC Analytical Core Facility on a PE SCIEX API 100 or Waters Acuity SQD LC-MS with electrospray ionization probe (see **Section 2** below).

2. Synthesis and Characterization of Lysine Analogues and Peptides.

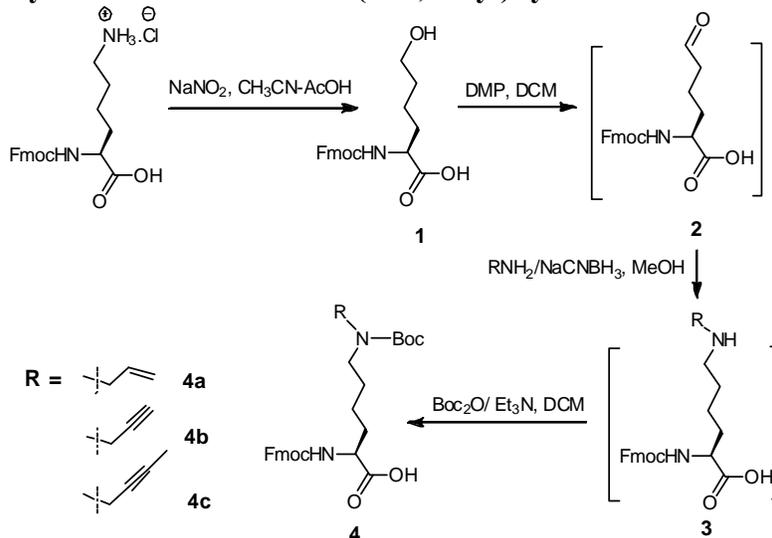


a. N^α-Fmoc-N^ε-(Boc-propyl)-lysine. Acrolein (22.4 mg, 0.4 mmol) was added to a stirred solution of N^α-Fmoc-lysine hydrochloride (100 mg, 0.25 mmol) in 5 mL of anhydrous ethanol in the presence of 3 Å molecular sieves at room temperature (22 °C). After stirring for 1 h, sodium cyanoborohydride (25.3 mg, 0.4 mmol) was added. The reaction was allowed to proceed overnight. The reaction mixture was then acidified by dropwise addition of 0.2 M hydrochloric acid, which led to a fine white precipitate. The reaction mixture was filtered and the filtrate was concentrated to afford crude mixture. Without purification, the crude mixture was subject to Boc-carbamate protection as the following. Into a stirred solution of the crude mixture, 5 mL of anhydrous dichloromethane, TEA (34.7 μL, 0.25 mmol), and Boc anhydride (54.5 mg, 0.25 mmol) were added. Upon completing the reaction, which was monitored by TLC, the reaction mixture was diluted with 10 mL dichloromethane and washed sequentially with 0.2 M HCl (5 mL × 3), saturated sodium bicarbonate (5 mL × 3), and brine (5 mL), dried over anhydrous magnesium sulfate and concentrated to give crude product. The crude product was purified by silica gel column chromatography with dichloromethane and methanol (v/v of 95:5) as eluent to afford pure N^α-Fmoc-N^ε-(Boc, propyl)-lysine with 40% yield (51 mg, 0.1 mmol) after two steps.

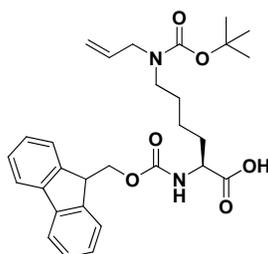


N^α-Fmoc-N^ε-(Boc, propyl)-lysine. ¹H-NMR (500 MHz, methanol-d₄): δ 7.79 (d, 2H, J=7.5 Hz), 7.67 (t, 2H, J=8.2 Hz), 7.38 (t, 2H, J=7.5 Hz), 7.3 (t, 2H, J=6.6 Hz), 4.35-4.33 (m, 2H), 4.22 (t, 1H, J=6.75), 4.14-4.13 (m, 1H), 3.19-3.16 (m, 2H), 3.13 (t, 2H, J=7.5 Hz), 1.9-1.86 (m, 1H), 1.74-1.7 (m, 1H), 1.55-1.5 (m, 4H), 1.43 (s, 9H), 1.4-1.37 (m, 2H), 0.86 (t, 3H, J=6.95); ¹³C NMR (125 MHz, methanol-d₄): δ 176.4, 158.7, 157.5, 145.4, 145.2, 142.6, 128.8, 128.2, 126.3, 120.9, 80.7, 67.9, 55.5, 48.4, 33.6, 28.8, 24.3, 11.6; HRMS *m/z* [M + H]⁺ calcd for C₂₉H₃₈N₂O₆: 511.2808, found 511.2807.

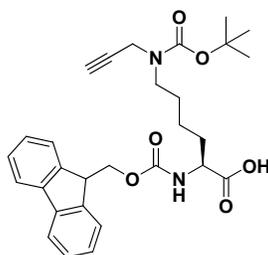
b. Solution-phase synthesis of N^α-Fmoc-N^ε-(Boc, alkyl)-lysine 4a-c.



General synthesis. The series of reactions were monitored by LC-MS as described above. Briefly, Dess-Martin periodinane (84 mg, 0.21 mmol) was added to a stirred solution of Fmoc-hydroxyornithine^{SI} (50 mg, 0.14 mmol) in dichloromethane (4 mL) at room temperature (22 °C). Reaction was allowed to continue until complete disappearance of the starting material (~1.5 h). The reaction mixture was concentrated rapidly under reduced pressure and re-dissolved in anhydrous methanol (5 mL) in the presence of 3 Å molecular sieves. The corresponding primary amine (0.27 mmol) was then added and allowed to stir for 1 h at room temperature (22 °C). The newly generated imine was subsequently reduced with sodium cyanoborohydride (17 mg, 0.27 mmol). After 8 h, the reaction mixture was quenched with a minimum amount of water and filtered through a bed of celite. The filtrate was then concentrated under reduced pressure. The crude material was re-dissolved in anhydrous dichloromethane (4 mL) and subjected to Boc protection using Et₃N (42 µl, 0.3 mmol) and Boc anhydride (36 mg, 0.17 mmol) for 5 h at room temperature (22 °C). Afterwards, the reaction mixture was diluted with 20 mL dichloromethane and washed sequentially with 0.1 N HCl, water and saturated NaCl solution, and dried with Na₂SO₄. The crude material was then subjected to silica gel flash chromatography. Elution with 5:95 methanol-dichloromethane afforded desired lysine analogues **4a-c** in 15-20% yield.

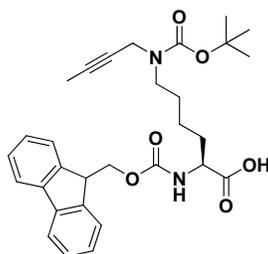


N^α-Fmoc-N^ε-(Boc, allyl)-lysine (4a). ¹H-NMR (500 MHz, methanol-d₄): δ 7.74 (d, 2H, J=7.5 Hz), 7.61 (t, 2H, J=7.5 Hz), 7.33 (t, 2H, J=7.35 Hz), 7.25 (t, 2H, J=7.4 Hz), 5.9-5.84 (m, 1H), 5.27 (d, 1H, J=17.3), 5.15 (d, 1H, J=10.35), 4.55 (m, 2H), 4.34-4.25 (m, 2H), 4.17-4.12 (m, 2H), 2.97 (t, 2H, J=5.8 Hz), 1.78-1.75 (m, 1H), 1.66-1.62 (m, 1H), 1.36 (s, 15H); ¹³C NMR (125 MHz, methanol-d₄): δ 173.8, 158.7, 158.6, 145.3, 145.2, 142.6, 133.4, 129.5, 128.8, 128.2, 126.3, 120.9, 118.6, 79.9, 67.9, 66.7, 55.5, 48.4, 41.0, 32.2, 31.2, 30.5, 28.8, 24.2; HRMS *m/z* [M + H]⁺ calcd for C₃₃H₃₈N₂O₆: 531.2471, found 531.2470. Yield: 15% (11 mg, 0.021 mmol).



N^α-Fmoc-N^ε-(Boc, propargyl)-lysine (4b). ¹H-NMR (500 MHz, methanol-d₄): δ 7.67 (d, 2H, J=7.45 Hz), 7.54 (t, 2H, J=8.55 Hz), 7.27 (t, 2H, J=7.35 Hz), 7.19 (t, 2H, J=7.4 Hz), 4.65 (q, 2H, J=15 Hz), 4.28-4.18 (m, 2H), 4.09 (t, 2H, J=6.85), 2.92 (t, 2H, J=6.55), 2.82 (s, 1H), 1.73-1.69 (m, 1H), 1.61-1.57 (m, 1H), 1.31 (s, 15H); ¹³C NMR (125 MHz, methanol-d₄): δ 173.3, 158.7, 158.6, 145.3, 145.2, 142.6, 134.0, 130.7, 129.5, 128.8, 128.2, 126.34, 126.27, 121, 79.9, 78.5,

76.7, 68.0, 55.4, 53.4, 48.4, 41.0, 32.2, 31.2, 30.5, 28.8, 24.1; **HRMS** m/z $[M + H]^+$ calcd for $C_{29}H_{34}N_2O_6$: 507.2495, found 507.2477. Yield: 16% (11 mg, 0.022 mmol).



N^α -Fmoc- N^ϵ -(Boc, 2-butynyl)-lysine (4c). 1H -NMR (500 MHz, methanol- d_4): δ 7.8 (d, 2H, $J=7.5$), 7.68 (2d, 2H, $J=7.5$), 7.39 (t, 2H, $J=7.4$), 7.31 (t, 2H, $J=7.4$), 4.69 (q, 2H, $J=15$), 4.41-4.38 (m, 1H), 4.33-4.29 (m, 1H), 4.24-4.19 (m, 2H), 3.05 (t, 2H, $J=5.45$ Hz), 1.86-1.82 (m, 1H), 1.78 (s, 3H), 1.74-1.68 (m, 1H), 1.44 (s, 15H); ^{13}C NMR (150 MHz, methanol- d_4): δ 173.5, 158.7, 158.6, 145.4, 145.1, 142.62, 142.6, 128.84, 128.8, 128.23, 128.2, 126.4, 126.3, 121, 84.1, 79.9, 74, 68, 55.4, 54.2, 48.4, 41.1, 32.1, 30.5, 28.8, 24.2, 3.2; **HR-MS** m/z $[M + H]^+$ calcd for $C_{30}H_{36}N_2O_6$: 543.2465, found 543.2471. Yield: 20% (15 mg, 0.028 mmol).

c. Solid-phase synthesis of K9- N^ϵ -alkyllsine-containing N-terminal H3 3-13 aa peptides.

Solid-phase synthesis of native and hydroxynorleucine-containing H3 N-terminal peptides.

Standard procedure for Fmoc solid phase peptide synthesis (SPPS) was utilized to assemble the N-terminal histone H3 peptide (amino acids 3-13) with H3K9 replaced by hydroxynorleucine. Here SPPS was carried out with Tribute Peptide Synthesizer (Protein Technologies Inc) according to manufacture's protocols. Briefly, SPPS was initiated from the C-terminus of the peptide with Fmoc-Gly-Wang resin (0.1 mmol). Each amino acid residue was added in the form of the Fmoc amino acid (0.5 mmol) with HBTU (0.5 mmol) as an activating agent. Here N^α -Fmoc-hydroxynorleucine-OH was installed at the position of H3K9 without protecting its free ϵ -hydroxyl group. Subsequent incorporation of amino acids were also performed in the presence of the free ϵ -hydroxyl group. The last amino acid (Thr3) was introduced as Boc-Thr(tBut)-OH. Upon completing these reactions, the resin-immobilized peptide was washed thoroughly with dichloromethane and dried under vacuum.

Resin-immobilized, fully-protected peptide (0.01 mmol) was treated with 200 μ L of acidic cocktail (45:3:1:1 of TFA, water, triisopropylsilane and phenol) for 4 h at room temperature (22 $^\circ$ C). Crude peptide was precipitated with cold diethyl ether (5 mL) and purified by HPLC (Waters) as described above. MALDI-TOF Analysis (Voyager DE-STR MALDI-TOF mass spectrometer, Applied Biosystems) of the HPLC-purified peptide confirmed its integrity (expected/observed m/z = 1136.15, Supporting Fig. S1). Unmodified histone H3 peptide TKQTARKSTGG (amino acids 3-13) was synthesized and purified in a similar manner (Supporting Fig. S1).

Solid-phase Dess-Martin oxidation/reductive amination and global deprotection of hydroxynorleucine-containing N-terminal H3 peptide. Resin-immobilized, fully-protected hydroxynorleucine-containing H3 N-terminal 3-13 aa peptide (0.01 mmol) was placed in a SPPS reactor and 1 mL of anhydrous DMF was added. The oxidation of the free ϵ -hydroxyl group was initialized by adding Dess-Martin periodinane (0.05 mmol). The SPPS reactor was gently rotated at room temperature (22 $^\circ$ C) for 5 h. DMF was removed by filtration. The resin was subject to

sequential washing with water, methanol and dichloromethane, and then dried under vacuum. The resultant resin-immobilized, ϵ -aldehydenorleucine-containing H3 N-terminal 3-13 aa peptide was resuspended in anhydrous methanol (1 mL) followed by the addition of respective primary amines (0.05 mmol). The reaction mixture was gently rotated for 3 h at room temperature (22 °C). NaCNBH₄ (0.1 mmol) was added into the reaction and gently rotated for another 10 h at room temperature (22 °C). The resultant beads were washed thoroughly with water, methanol and dichloromethane, and then dried under vacuum. Global deprotection and cleavage of the peptide from the resin were accomplished with 200 μ L of acidic cocktail (45:3:1:1 of TFA, water, triisopropylsilane and phenol) for 4 h at room temperature (22 °C). The crude products of **8a-h** were purified by HPLC as described above. The overall yield of the reaction varied from 30~50% (30% for **8a,b,e,g**; 40% for **8c**; 45% for **8h** and 50% for **8d,f**) from the first step of SPPS (Fmoc-Gly-Wang resin). The integrity and purity of **8a-h** were confirmed by MALDI-MS (Supporting Fig. S1).

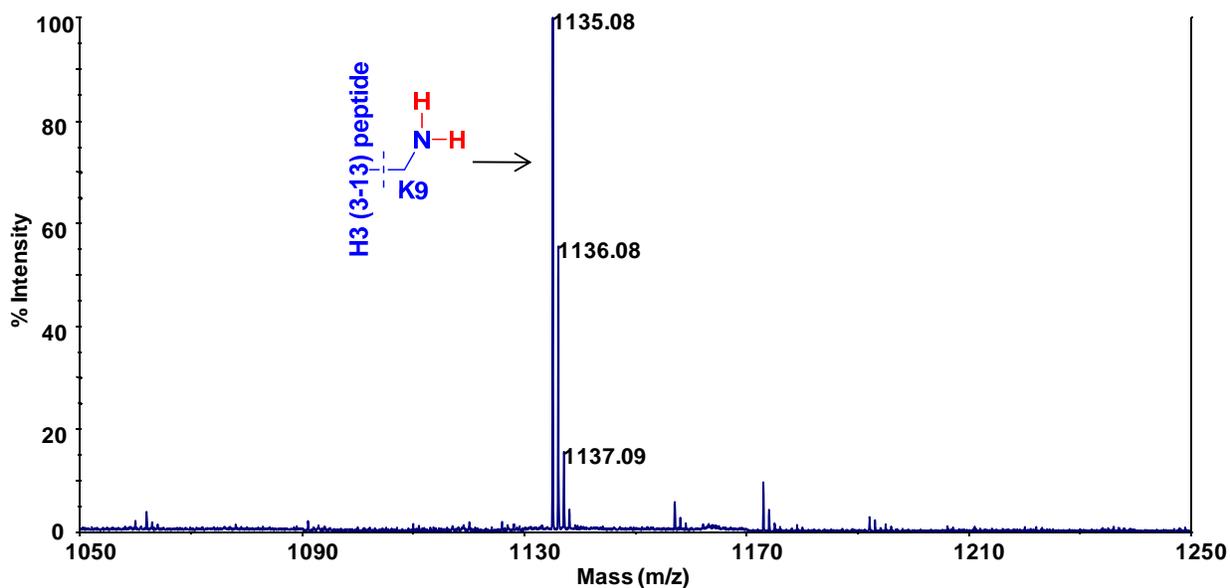
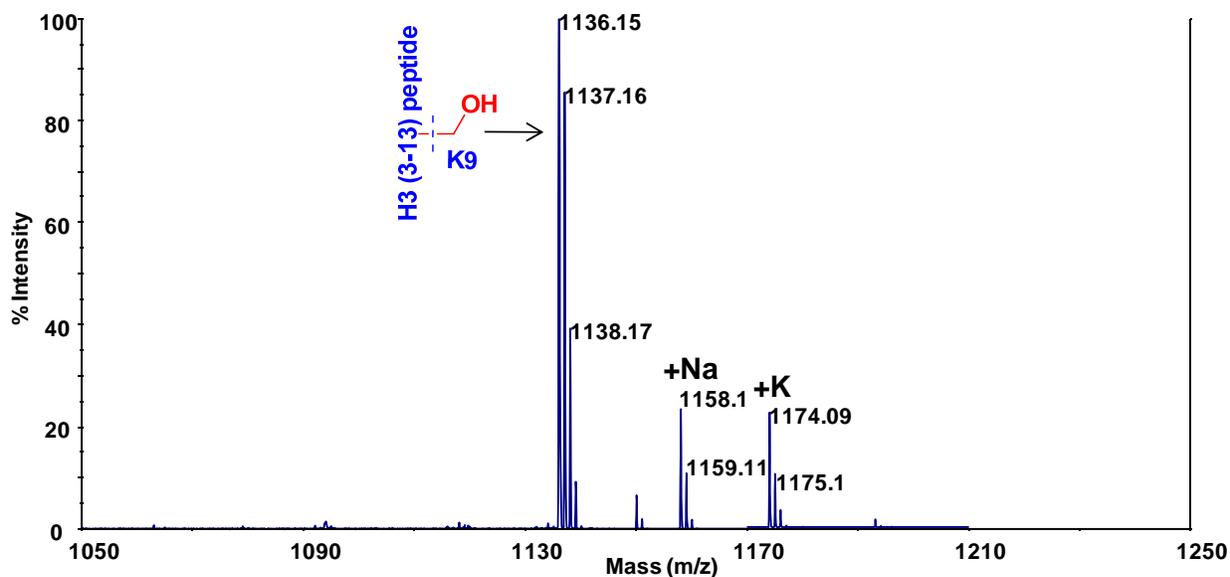
Peptide quantification and storage. After HPLC purification, all the peptides were concentrated in Speedvac, followed by overnight lyophilization to give white solids. The peptides were then dissolved in 0.1% TFA/H₂O. Concentration of these peptide stocks were quantified by UV absorbance at 205 nm for amide bonds (OD₂₀₅ of 1 mg/ml peptide = 30). The stocks were stored at -80 °C before use.

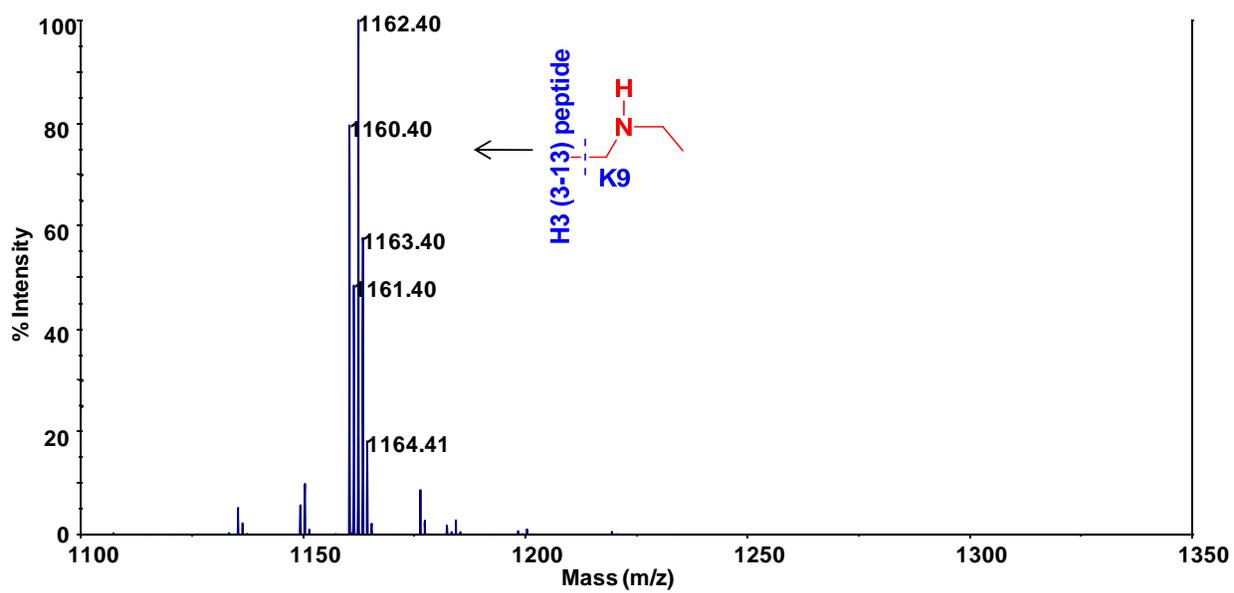
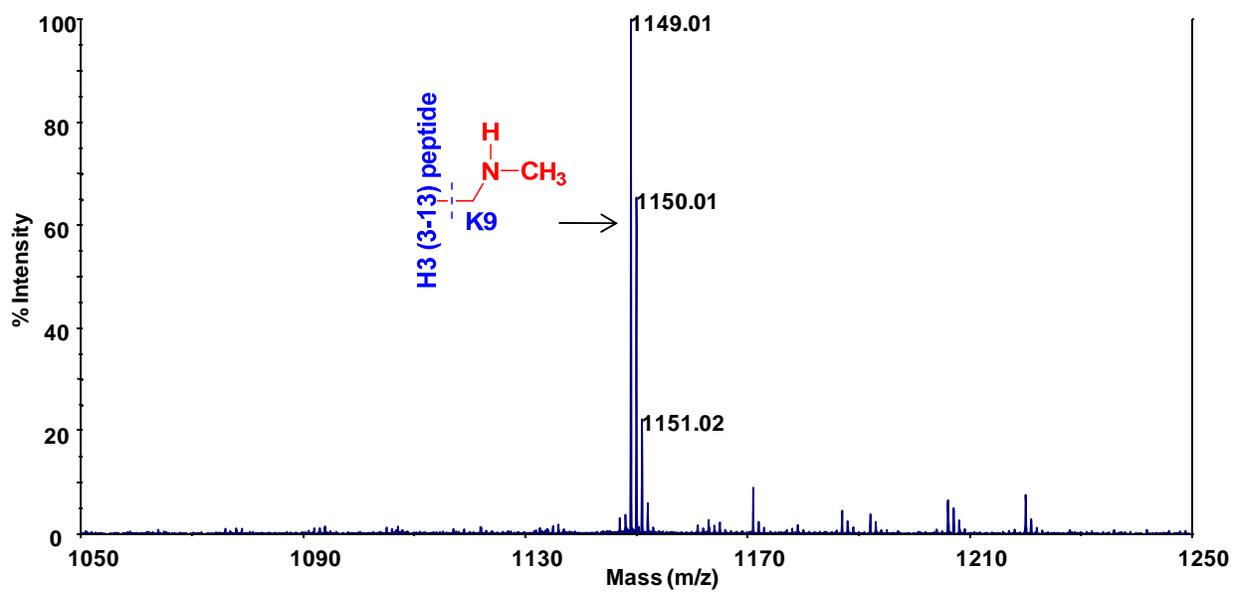
3. 3. Mass Spectroscopic Analysis of Lysine Peptides

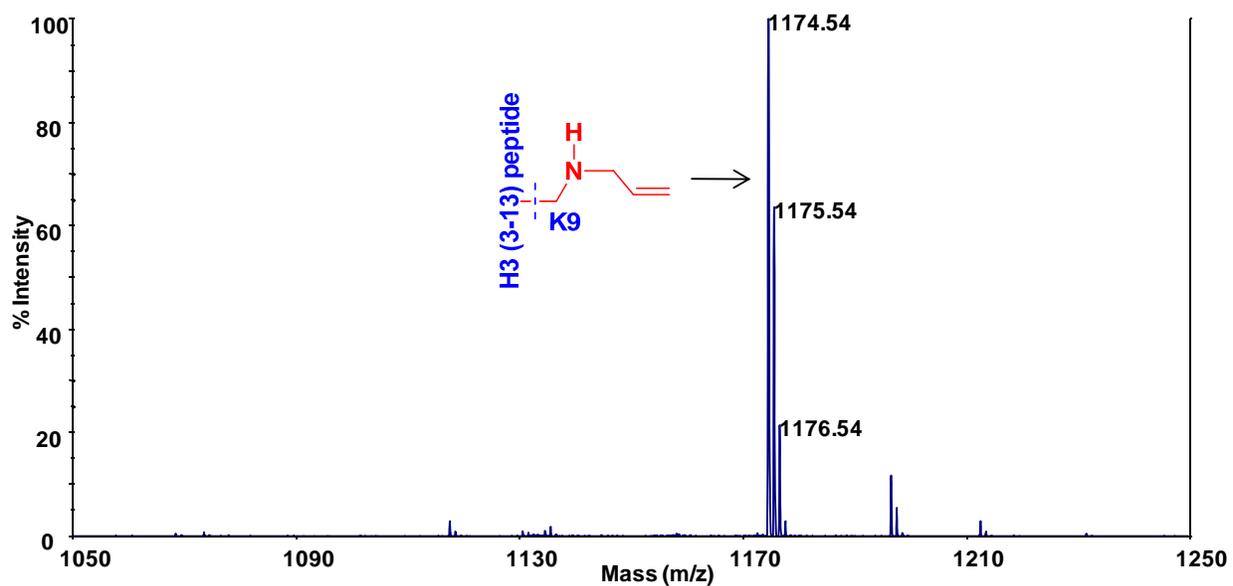
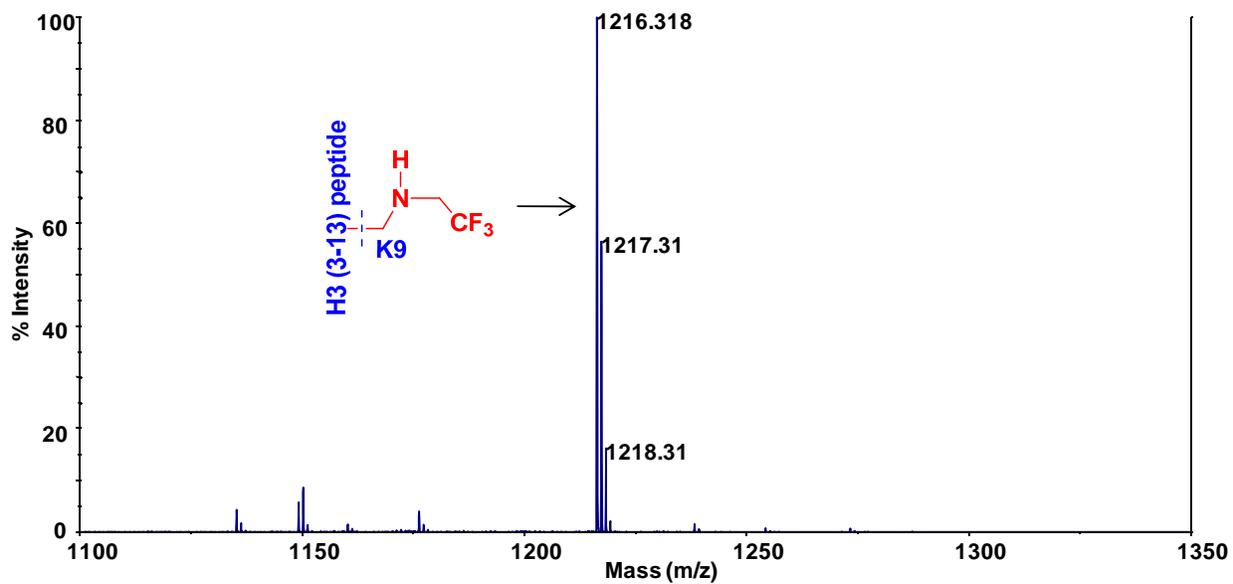
MALDI-MS (Matrix Assisted Laser Desorption/Ionization-Mass Spectrometry) analysis for the peptide samples. To analyze the peptide samples, 1 μ L of pure peptides (20 μ M solution in 0.1% TFA) was placed on a MALDI target plate. 1 μ L of the saturated solution (3:7 v/v of CH₃CN:water) of α -cyano-hydroxy-cinnamic acid (Protea Biosciences) was added and the resultant mixture was rapidly dried in air. MALDI-TOF MS analyses of these samples were performed with delayed extraction in a positive ion mode on a TOF mass spectrometer (Voyager-DE STR, Applied Biosystems, Framingham, MA, USA) with a 2.0-m flight tube. Desorption/Ionization was obtained by using a 337-nm nitrogen laser with a 3-ns pulse width. Laser power was adjusted to slightly above threshold to obtain good resolution and signal/noise ratios. External standard Calibration Mix 2 (Applied Biosystems) was used for mass calibration each time. Each measurement was obtained by accumulating three spectra collected at different positions on the plate with 500 shots per position. To acquire MALDI-MS of the mixture of two peptides (Supporting Fig. S2), 20 μ L stocks of each peptide (0.1% TFA) were mixed thoroughly. From this mixture, 2 μ L sample was spotted on placed on a MALDI target plate and processed as described above.

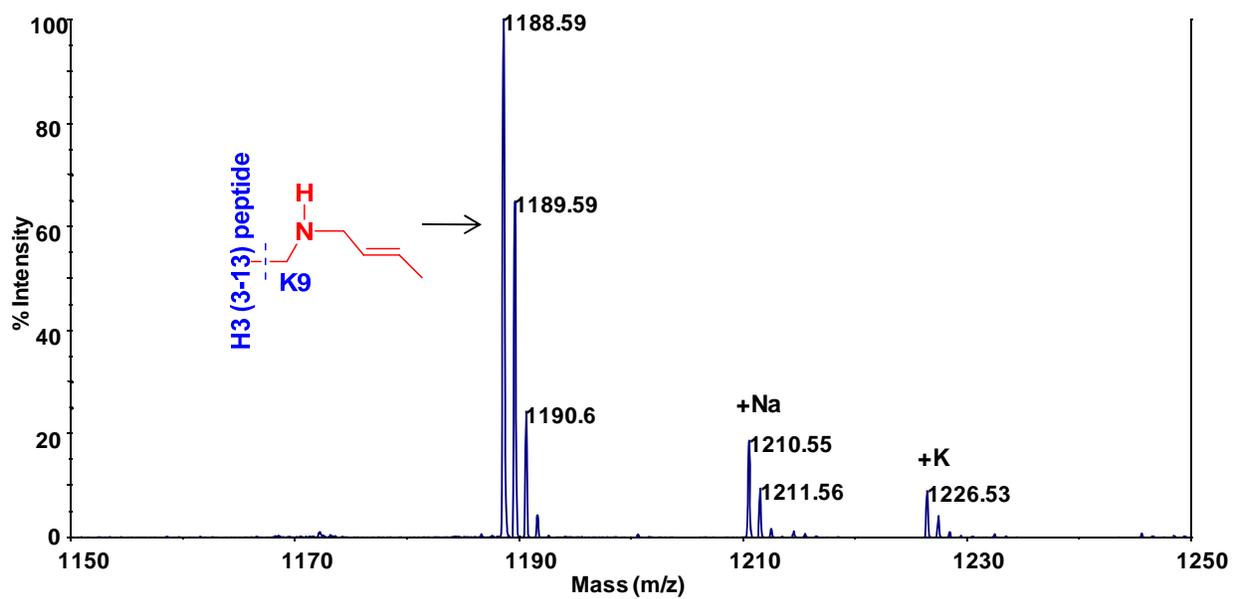
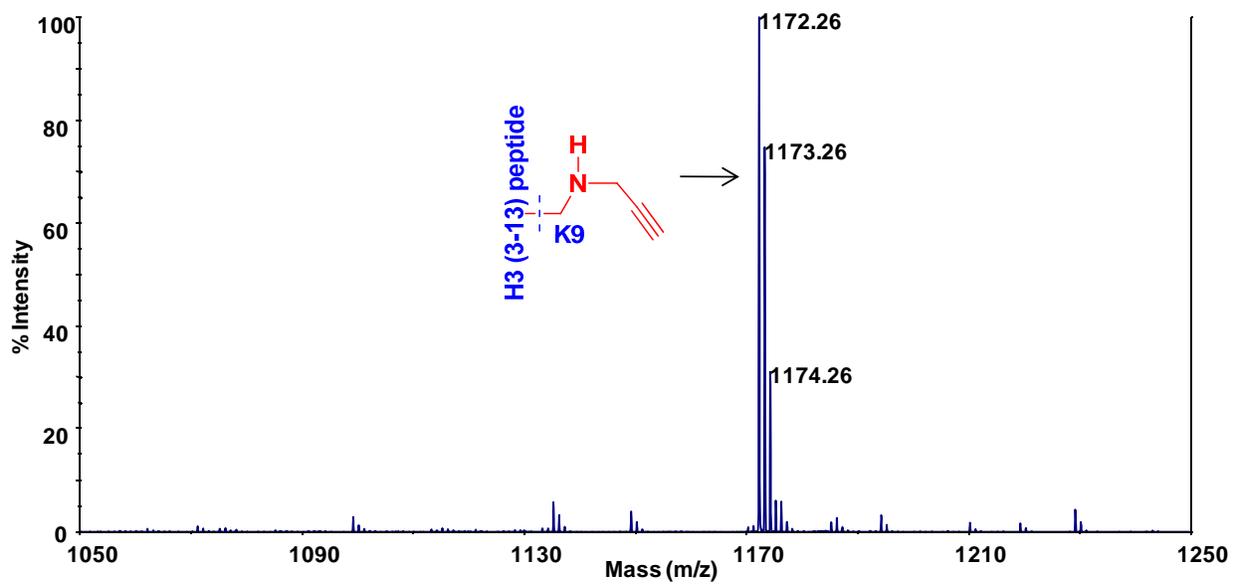
4. Supplementary Figures.

Figure S1. MALDI mass spectra of HPLC-purified peptides (hydroxynorleucine-containing/native N-terminal H3 peptides and alkyllysine-containing peptides **8a-h**). K9 denotes the lysine at the position 9 of histone H3, which was replaced by hydroxynorleucine or various N^ε-alkyllysine analogues.









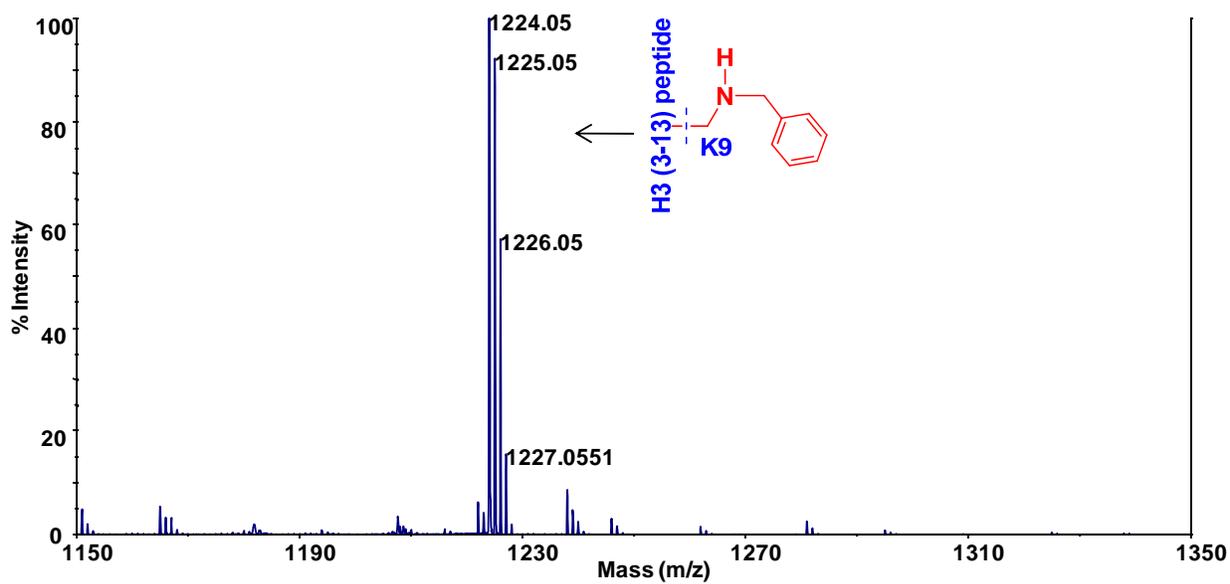
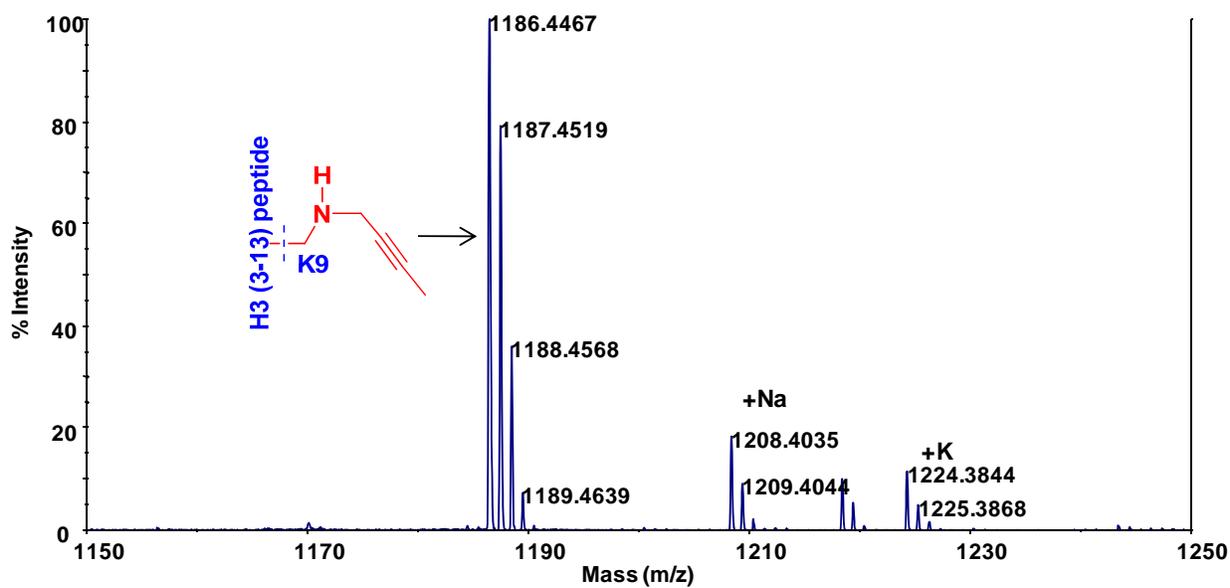
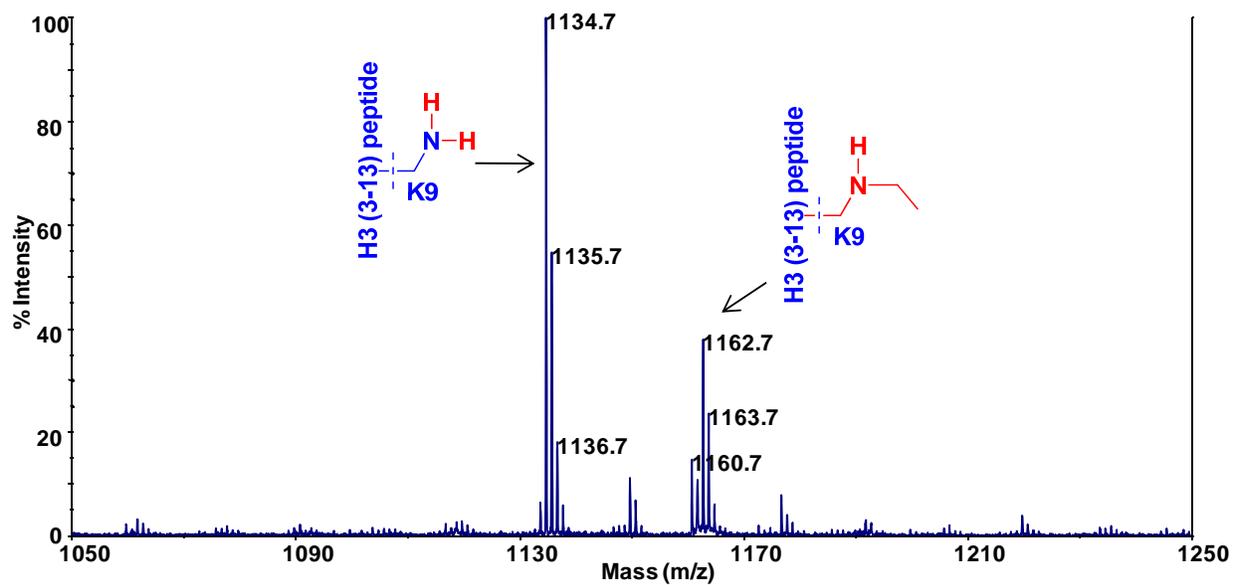
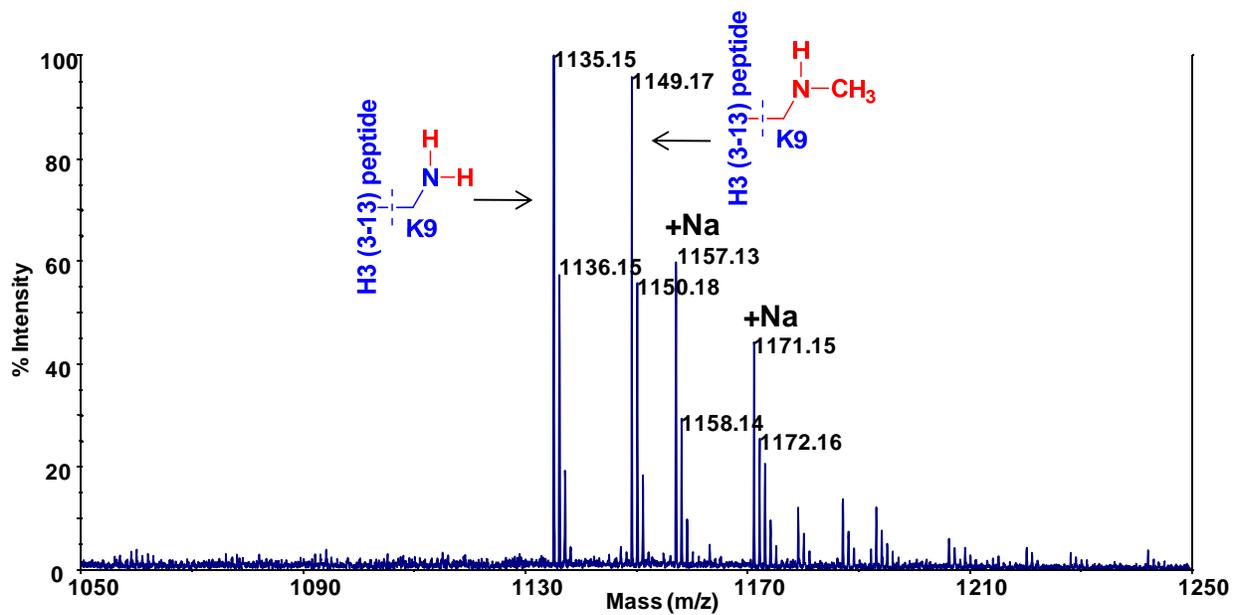
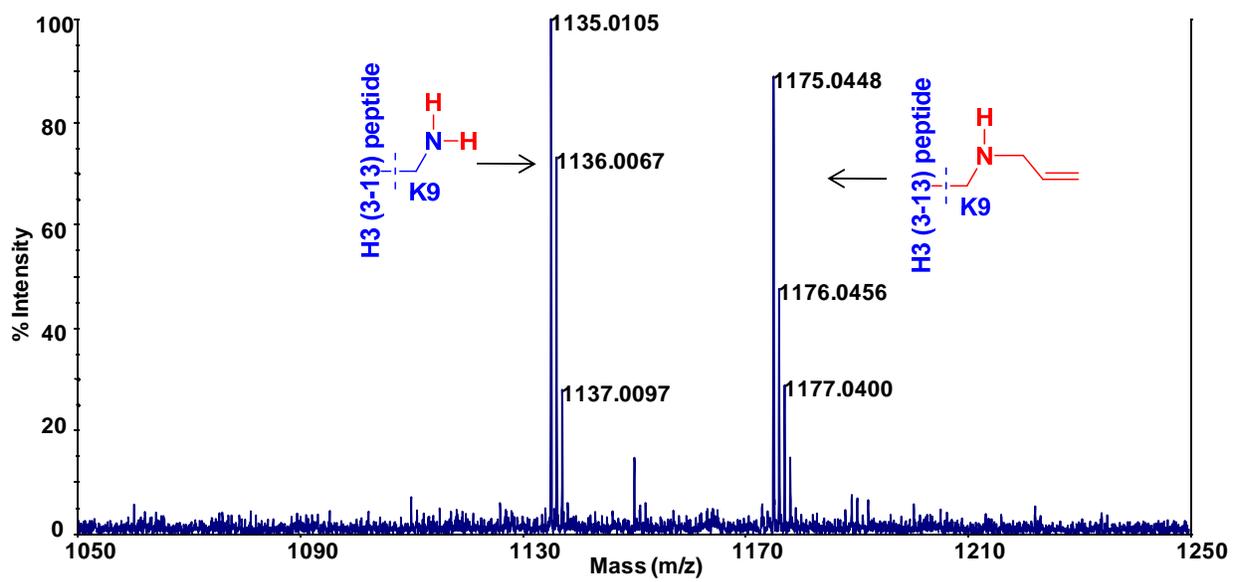
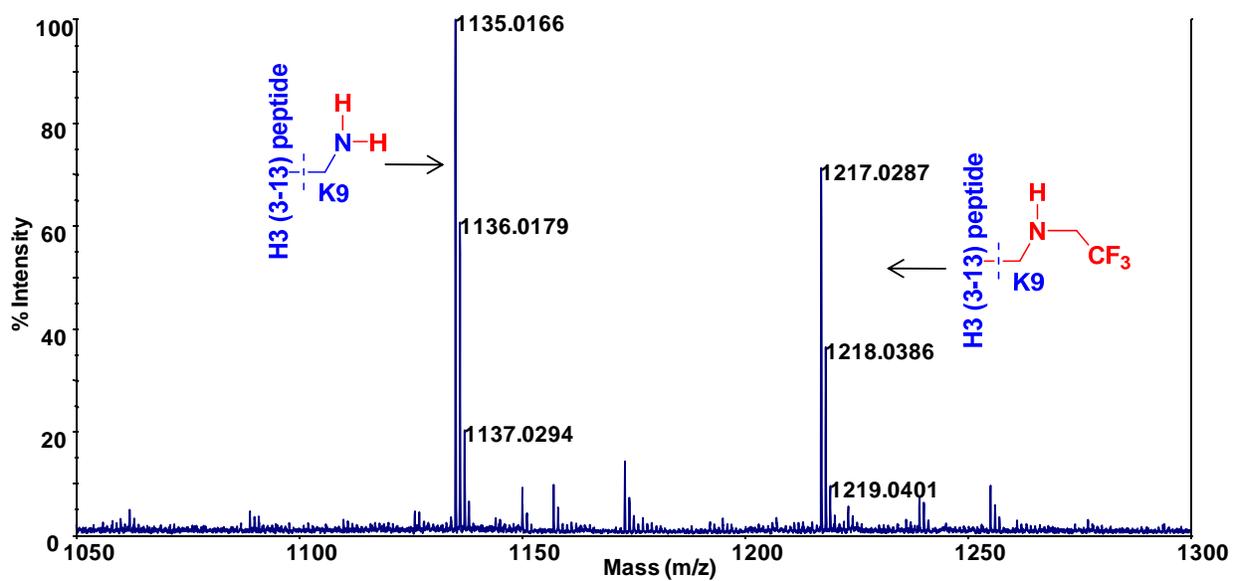
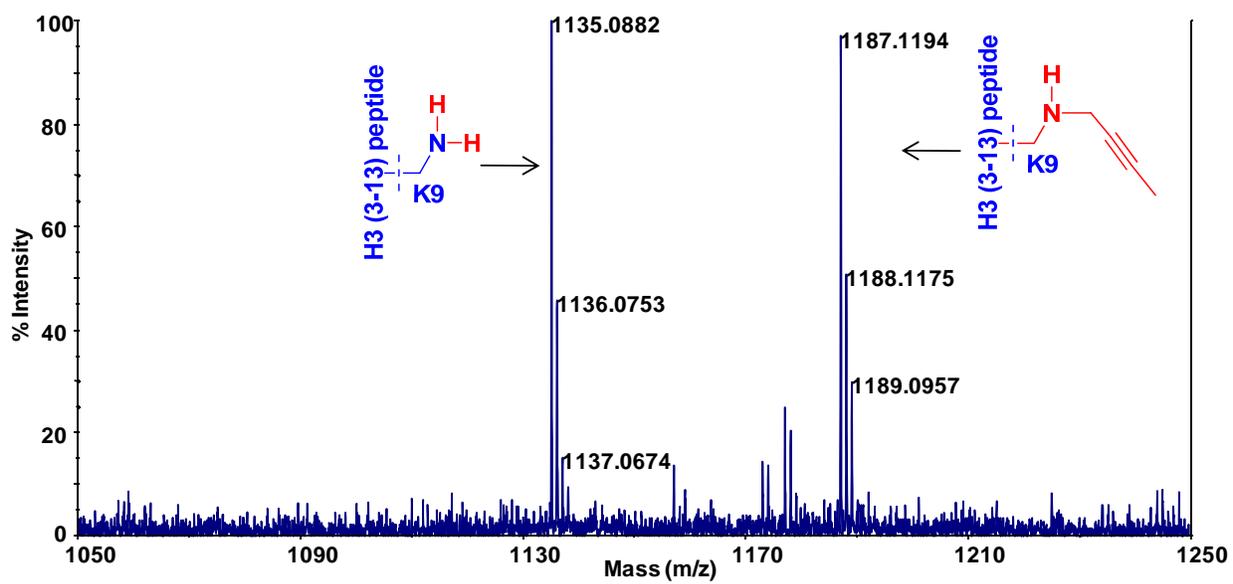
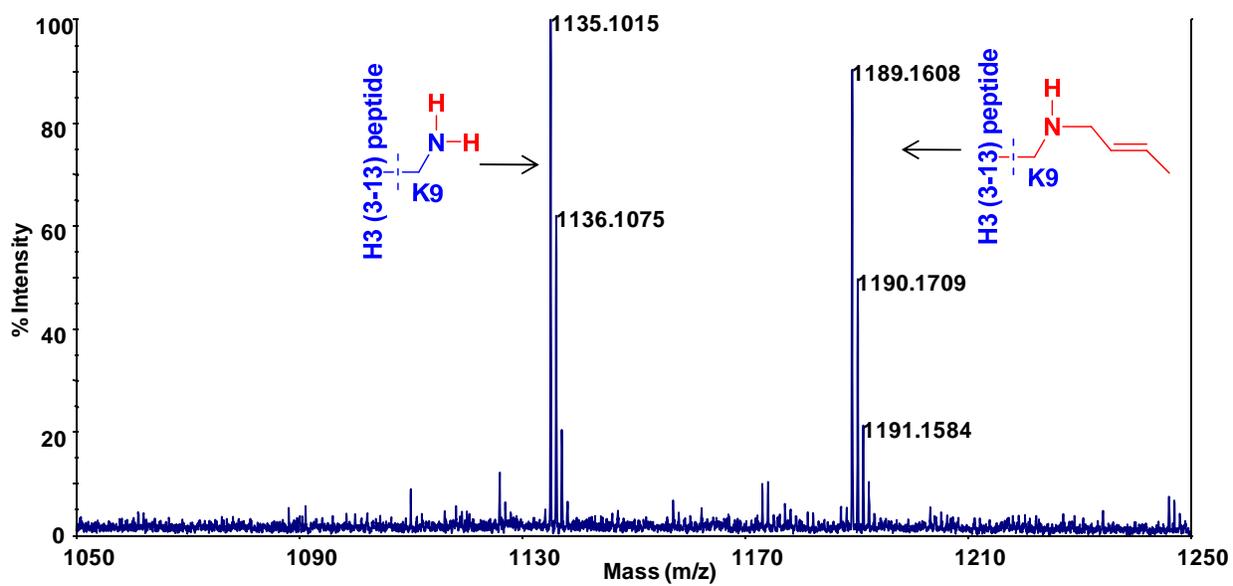
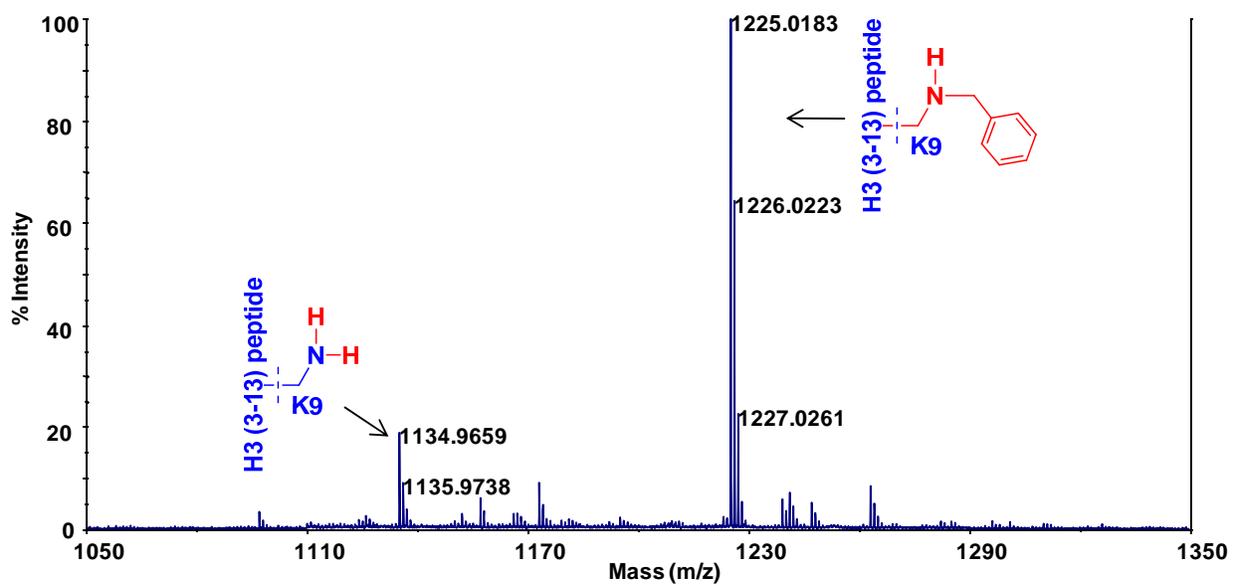


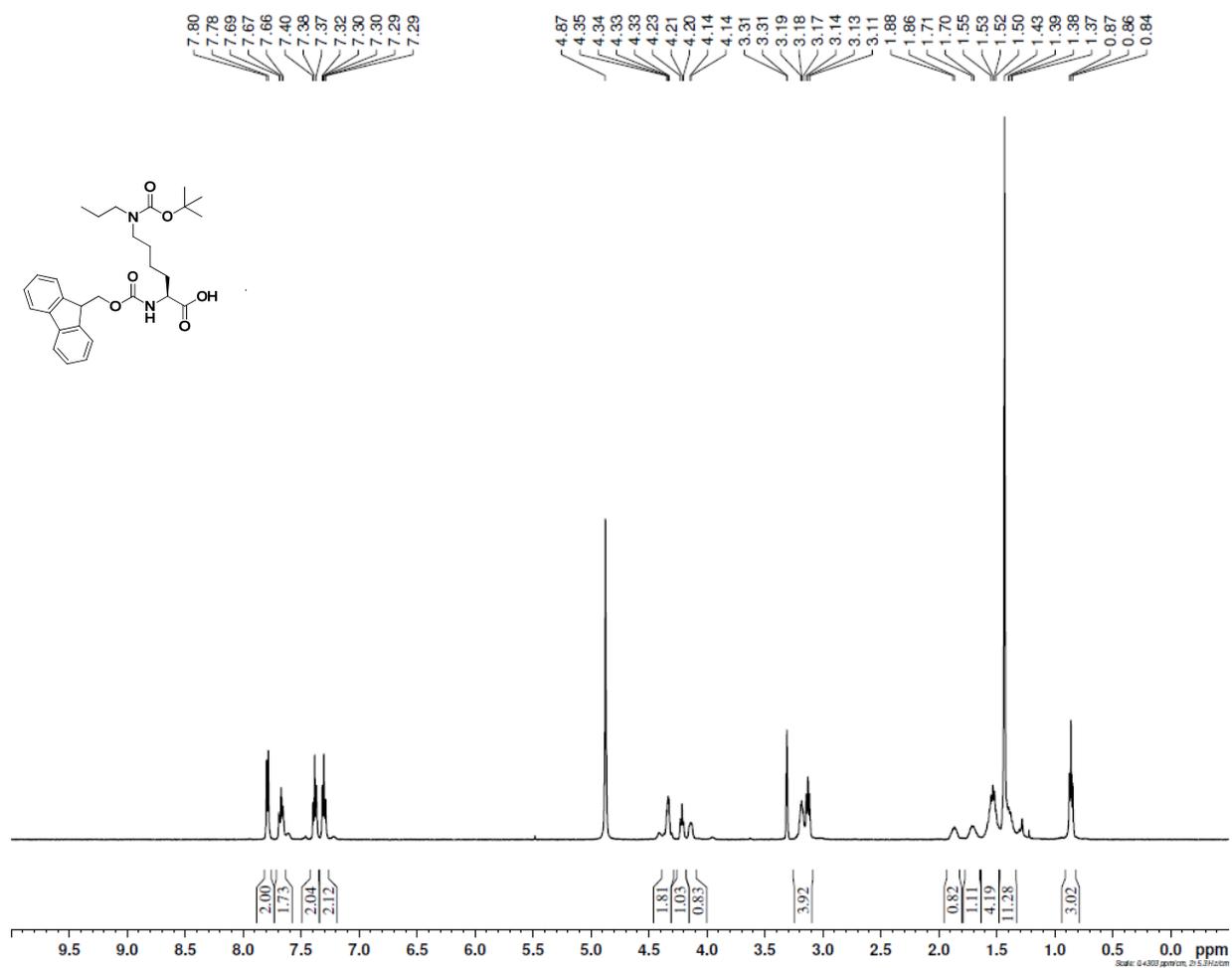
Figure S2. MALDI mass spectra of the mixture of native/alkyllysine-containing peptides.

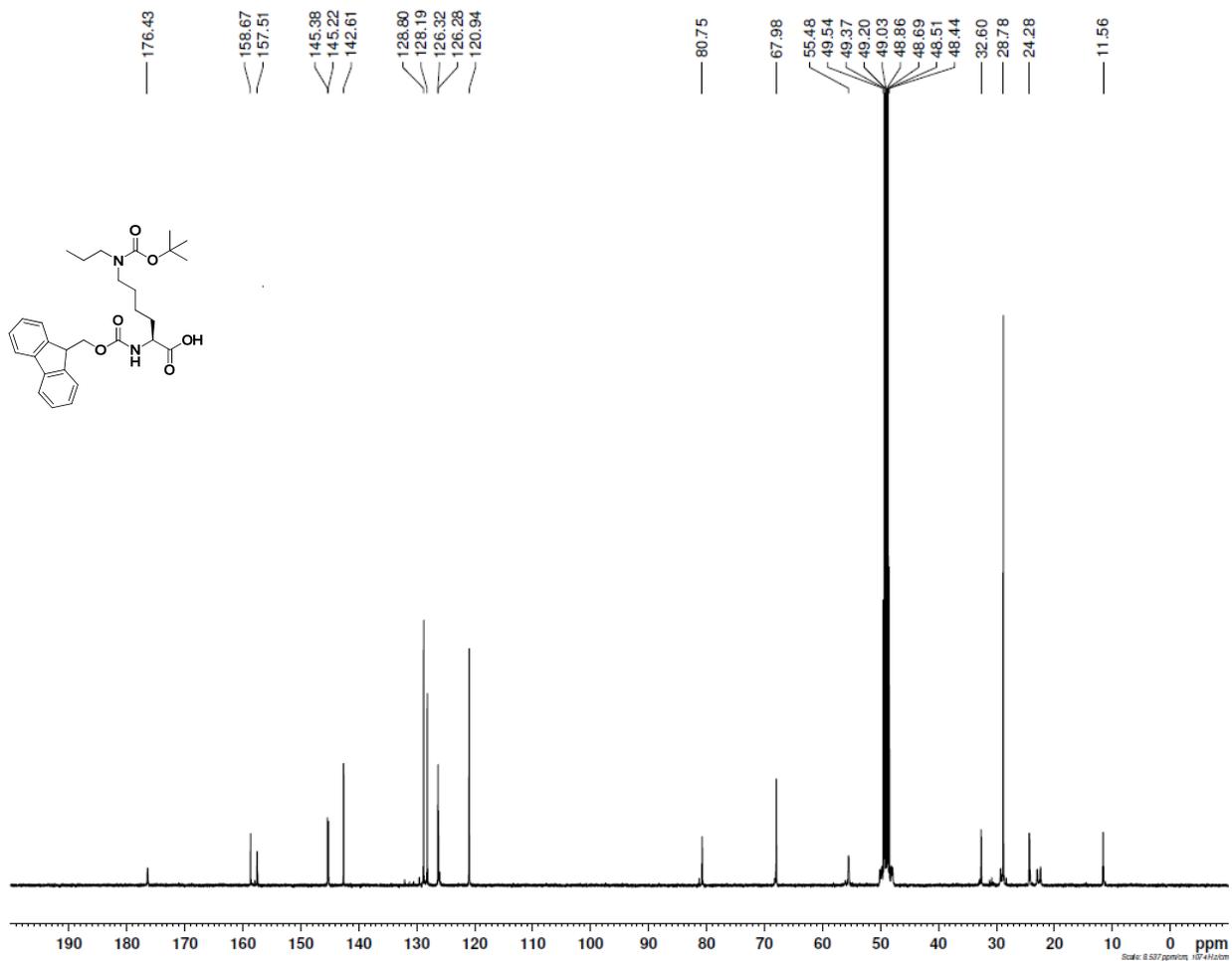


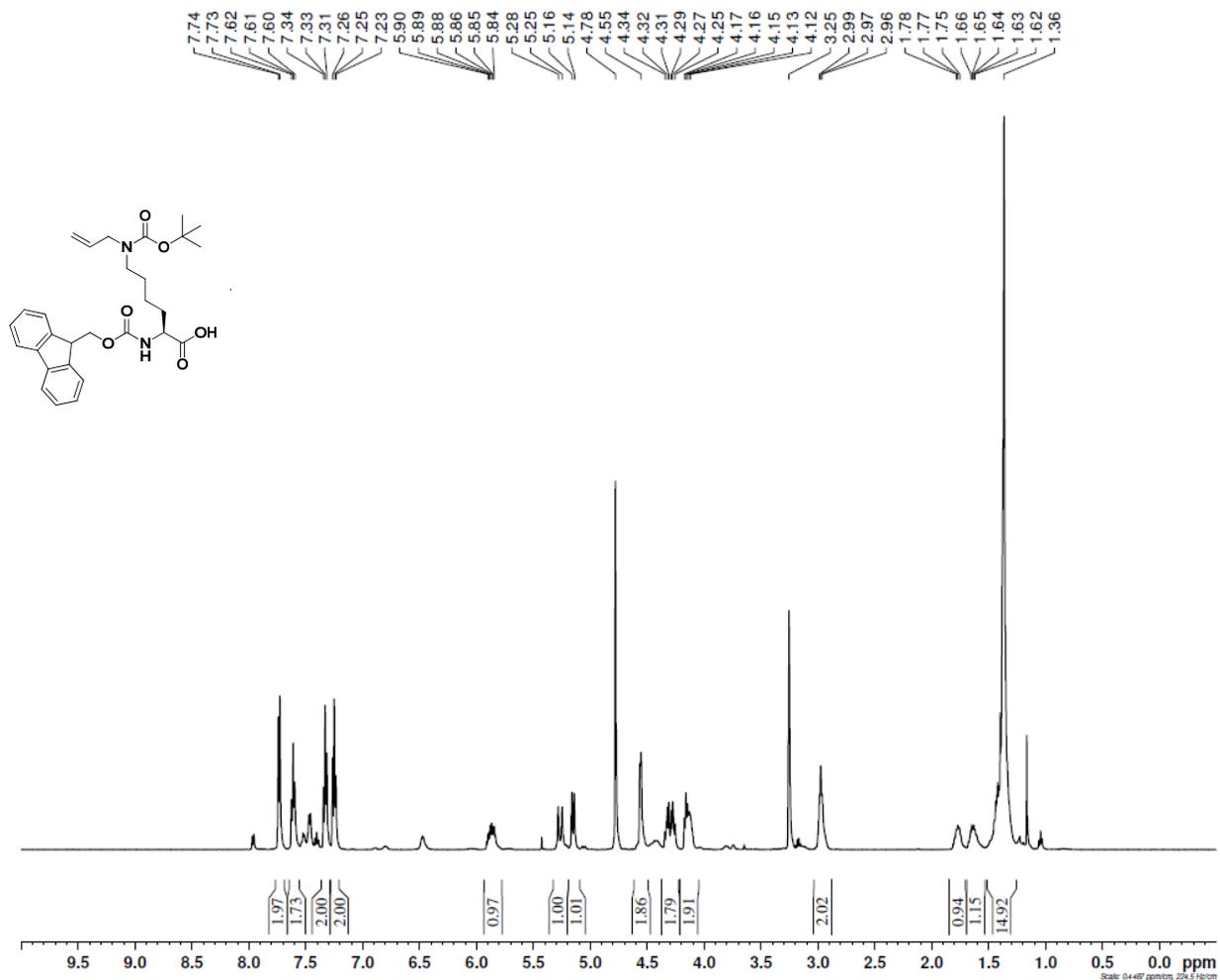


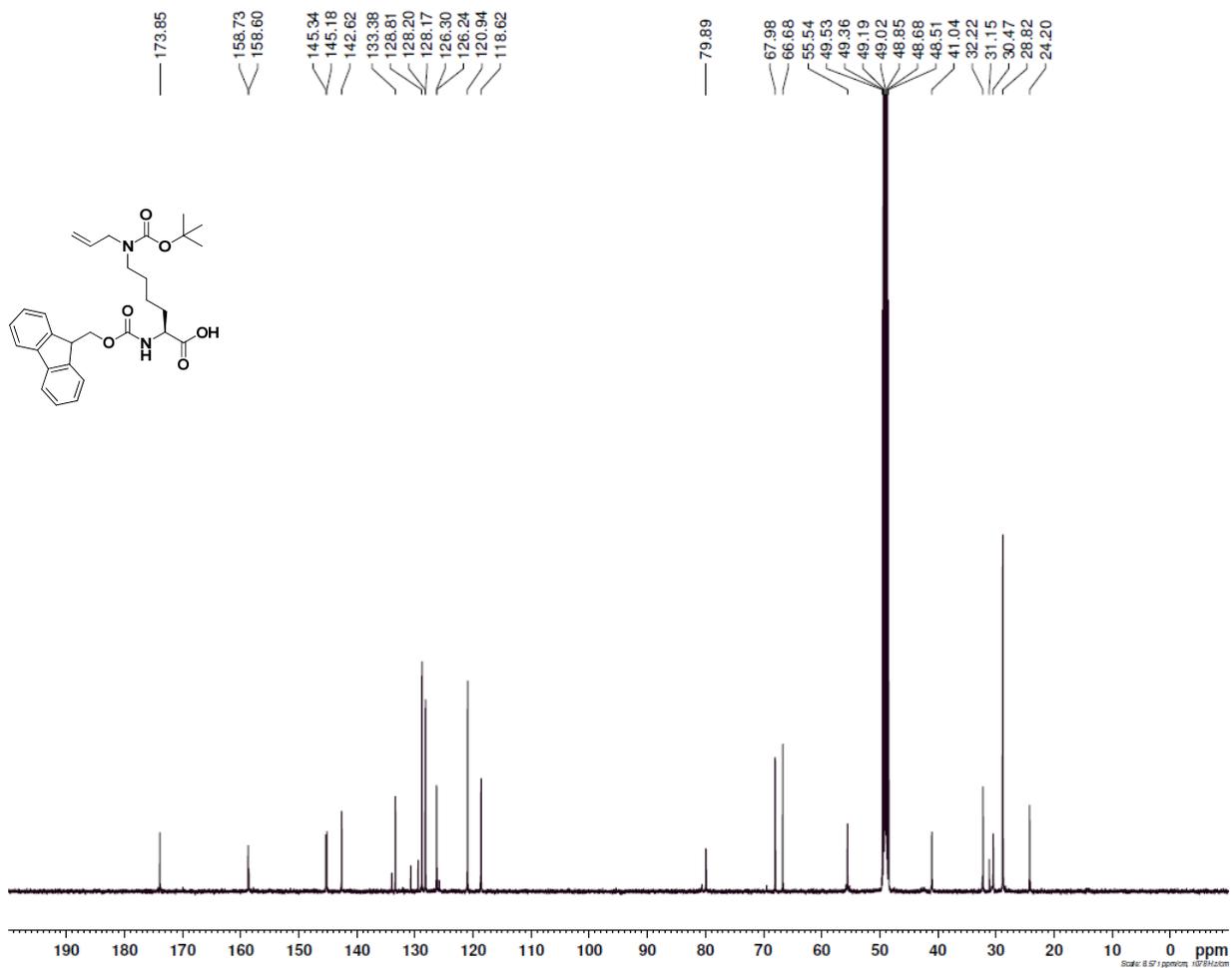


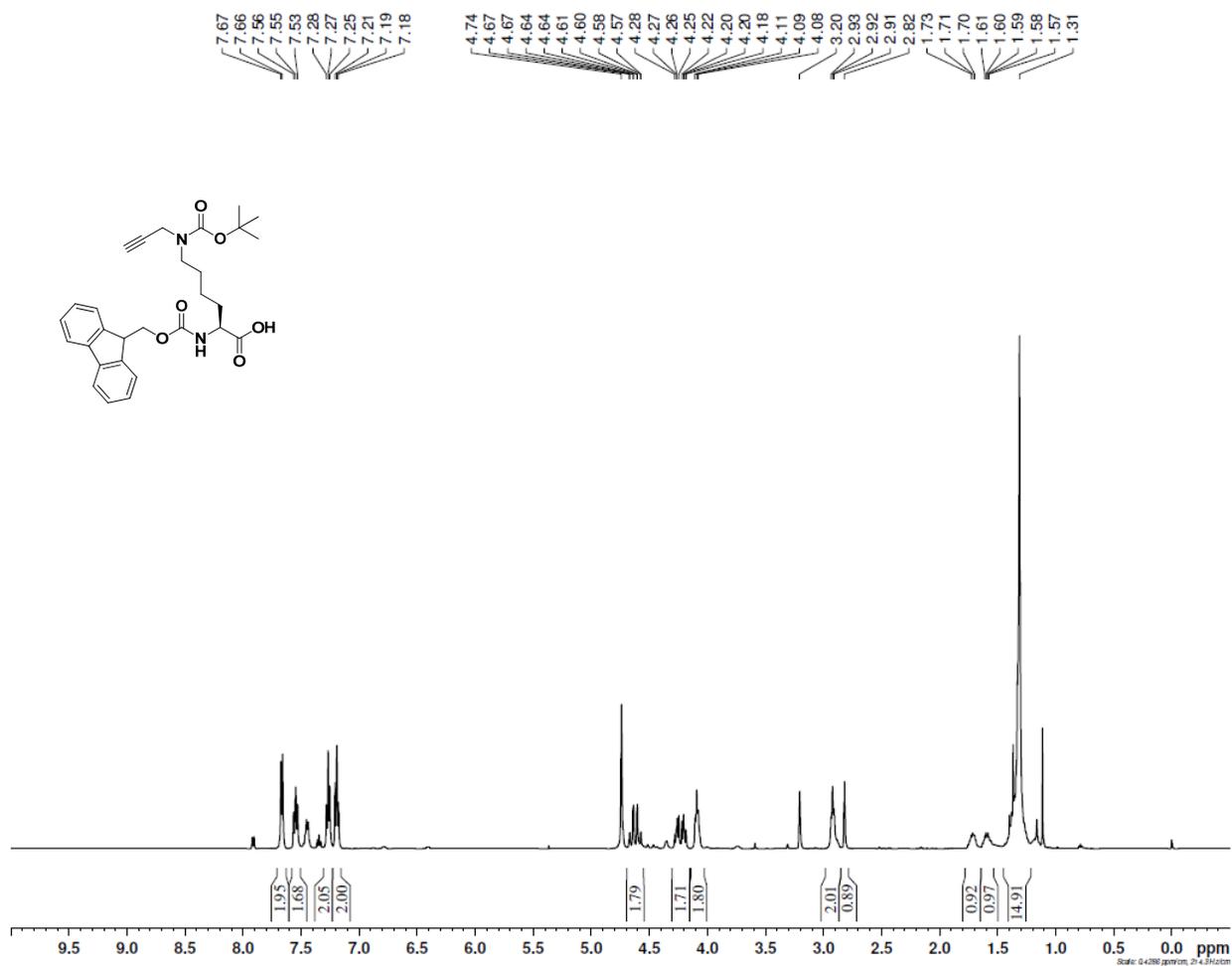


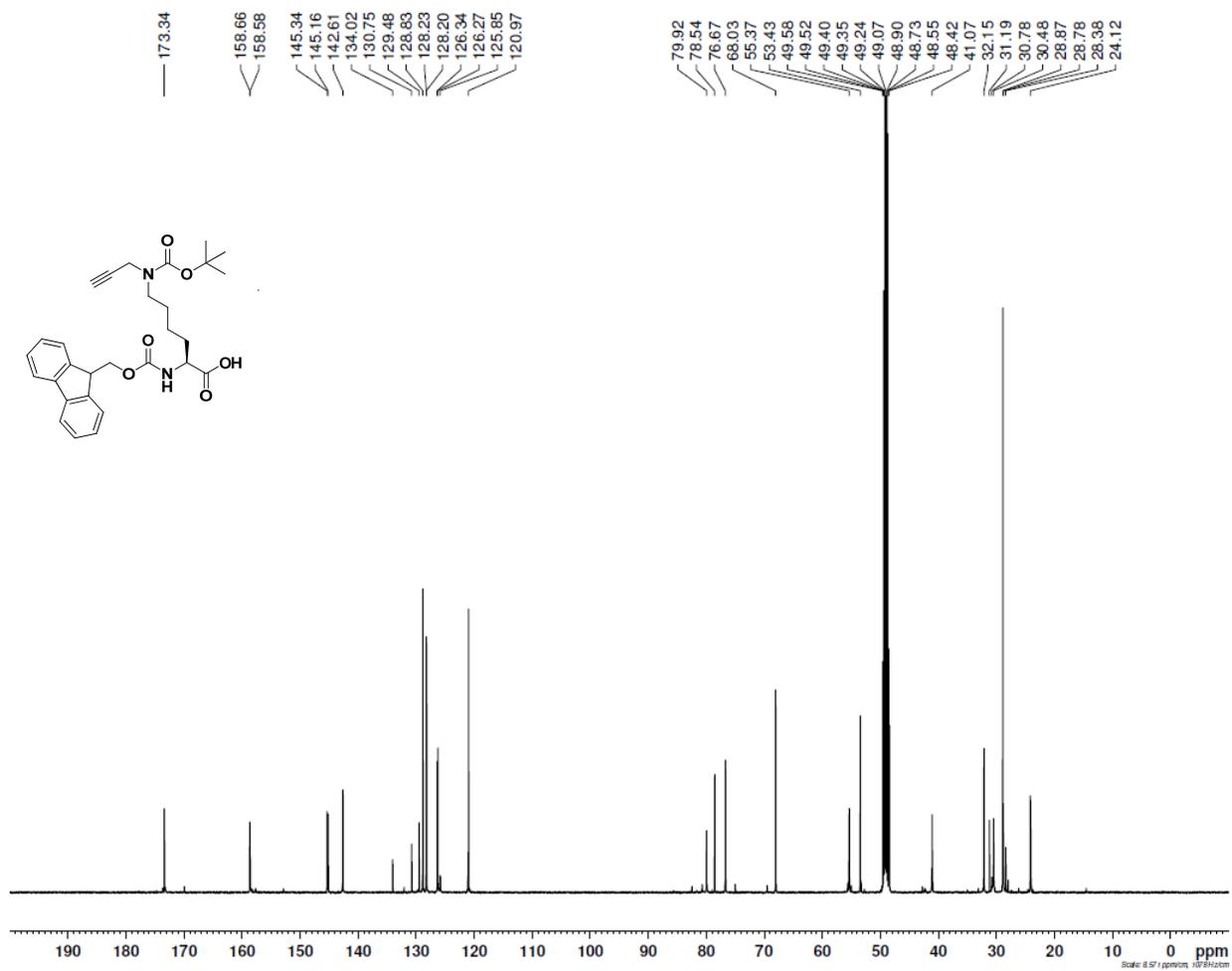


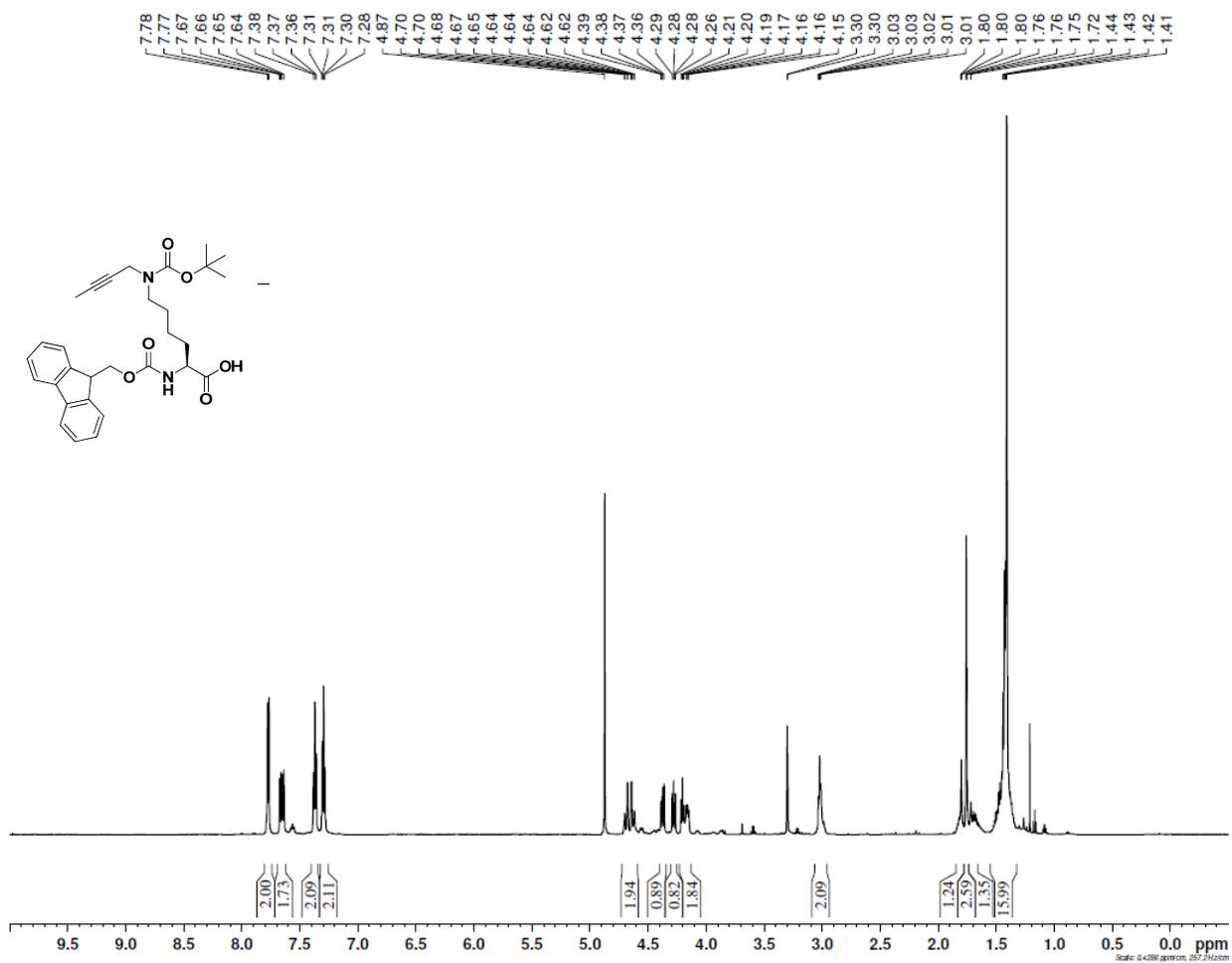


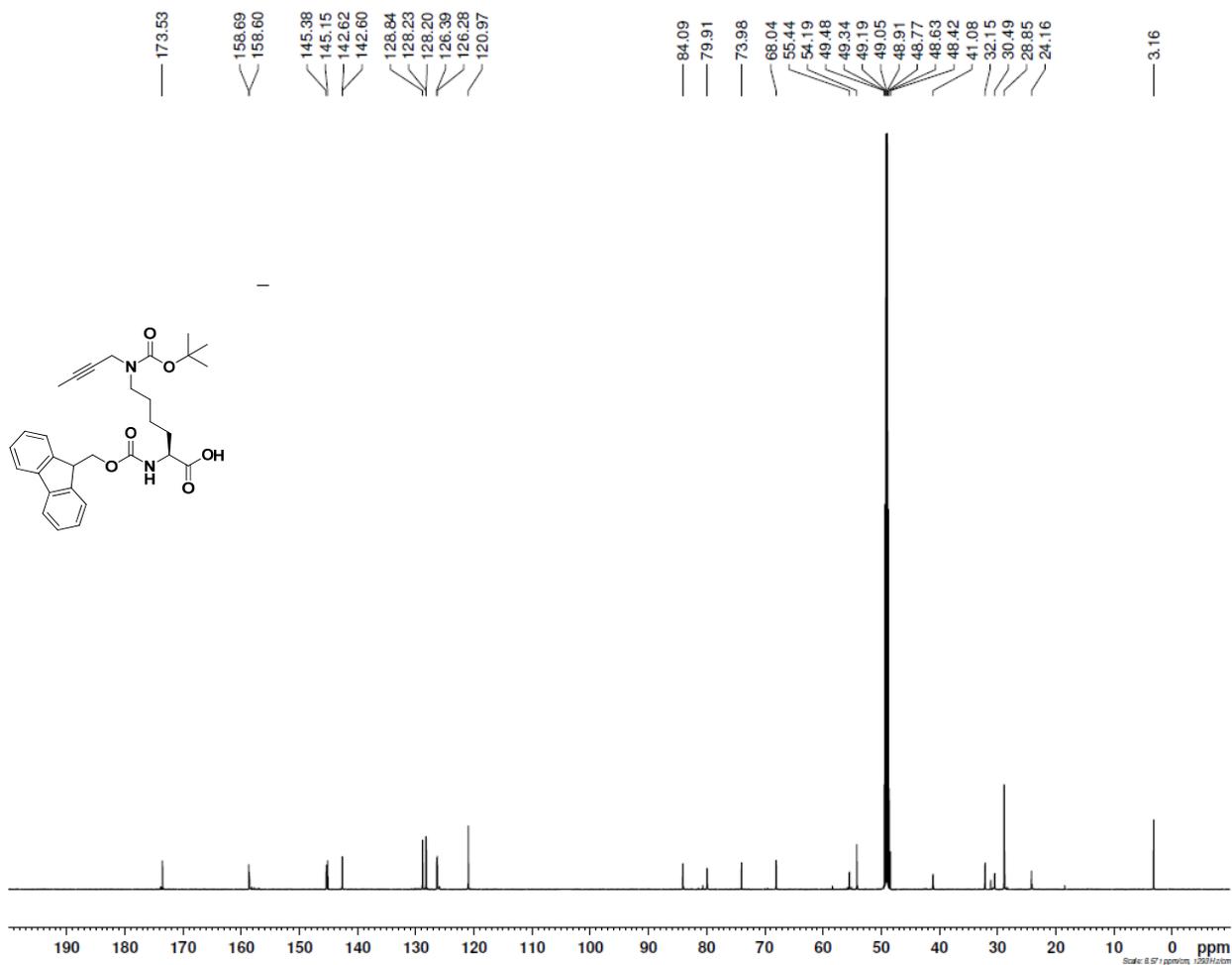












References

- (S1) J. C. Culhane, L. M. Szewczuk, X. Liu, G. P. Da, R. Marmorstein and P. A. Cole, *J. Am. Chem. Soc.*, 2006, **128**, 4536-4537; J. C. Culhane, D. Q. Wang, P. M. Yen and P. A. Cole, *J. Am. Chem. Soc.*, 2010, **132**, 3164-3176.