Supporting Information

Iminoboronate-based peptide cyclization that responds to pH, oxidation, and small molecule modulators

Anupam Bandyopadhyay and Jianmin Gao*

Department of Chemistry, Boston College, 2609 Beacon Street, Chestnut Hill, MA 02467

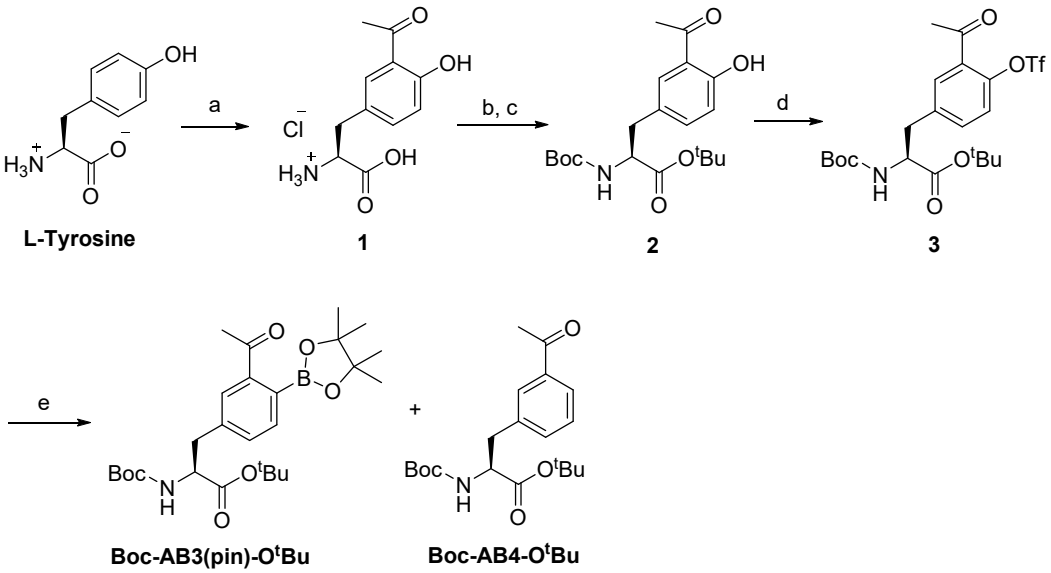
E-mail: jianmin.gao@bc.edu

Table of Contents **Pages**

I. General Remarks

Triflic anhydride, tert-Butyl 2,2,2-trichloroacetimidate, 1,1′-Bis(diphenylphosphino)ferrocene (dppf), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), sodium chloride (NaCl), phenylhydrazine, tyrosine, lysine, glutathione, fructose and lysozyme were purchased from Sigma Aldrich. Rink Amide MBHA resin and HBTU were purchased from Novabiochem (San Diego, CA). Trifluoroacetic acid, dry DCM, dry dioxane, sodium cyanoborohydraide ($NaCNBH_3$), PBS buffer, RPMI media, 0.25% Trypsin-EDTA, BSA, human serum and fetal bovine serum were purchased from Fischer Scientific. $Pd(dppf)Cl_2$ was purchased from Stream Chemicals. B_2pin_2 was purchased from Frontier Scientific. Peroxynitrite was purchased from Cayman Chemicals (Michigan, USA). Alexa Fluor 488 succinimide ester and Alexa Fluor 488 C_5-maleimide were purchased from Life Technologies (Grand Island, NY). Fmoc-protected amino acids were purchased from Chem-Impex Int'l Inc (Wood Dale, IL). AB1 was synthesized according to the published procedure.[1] UV spectra were collected on a Nanodrop UV-vis spectrometer. NMR data were collected on a VNMRS 500 and 600 MHz NMR spectrometers for small molecules and peptides respectively. Mass-spec data were generated using an Agilent 6230 LC-TOF mass spectrometer. Peptide synthesis was carried out on a Tribute peptide synthesizer from Protein Technologies. The fluorescence anisotropy experiments were performed by using a SpectraMax M5 plate reader. Confocal images were taken on a Leica SP5 confocal fluorescence microscope housed in the Biology Department of Boston College. SKOV3 (ATCC HTB-77) was a gift from Professor Weerapana in the Chemistry Department of Boston College.

II. Synthesis of AB3 and Fmoc-AB3(pin)-OH



a) $AlCl_3$, CH_3COCl, Nitrobenzene, 100 °C, 6hrs, 78%; b) $(Boc)_2O$, THF/H_2O, Na_2CO_3; c) t-Butyl 2,2,2-trichloroacetimidate, DCM, 80 °C, 12hrs, Overall 2-steps 67%, d) $(CF_3SO_2)O$, DCM, Et_3N, 97%; e) 5 mol% $Pd(dppf)Cl_2$/dppf, KOAc, B_2Pin_2, Dioxane, 90 °C, 65%; f) diethaolamine, THF, 1N HCl, 92%; g) 80% TFA in DCM.

Figure S1. Synthetic scheme of Boc-AB3(pin)-O^{t}Bu and Boc-AB4-O^{t}Bu.

IIa. Synthesis of (*S*)-2-(3-acetyl-4-hydroxyphenyl)-1-carboxyethanaminium chloride (1)[2]

Anhydrous aluminium chloride (10.8 g, 80 mmol) was added slowly to a solution of L-tyrosine (3.62 g, 20 mmol) in dry nitrobenzene (80 mL) at RT. This slightly exothermic reaction was stirred at RT for 10 minutes. Then acetyl chloride (1.88 g, 24 mmol, 1.2 equiv) was added drop wise over 1 minute, during which a color change was observed from red to yellow. The reaction mixture was heated at 100 °C with stirring for 6 hours and turned to be dark green thick gel after cooling down to room temperature. The thick gel was dissolved in cold 2N HCl (250 mL). The nitrobenzene layer was separated and the aqueous phase was washed with ethyl acetate (2 × 100 mL). The aqueous layer was concentrated to ca. 50 mL in volume and then kept at 4 °C for 14 hours. The desired product (**1**) precipitated as a white solid and was collected via filtration (4.2 g, 83%).

^{1}H NMR (500 MHz, D_2O) δ: 7.84 (s, 1H, 10), 7.51 – 7.49 (d, *J* = 8.56 Hz, 1H), 7.01-7.00 (d, *J* = 8.4 Hz, 1H), 4.27-4.26 (t, *J* = 5.4 Hz, 1H), 3.34-3.21 (m, 2H), 2.68 (s, 3H).

^{13}C NMR (125 MHz, D_2O) δ: 206.89, 171.40, 159.73, 137.84, 132.22, 126.16, 119.67, 118.27, 54.14 (d), 34.84, 16.15 (d).

MS-ESI$^+$: *m/z* calculated for $C_{11}H_{11}NO_4$ $[M+H]^+$ 224.0951, found 224.0917.

IIb. Synthesis of (*S*)-tert-butyl 3-(3-acetyl-4-hydroxyphenyl)-2-((tert-butoxycarbonyl)amino)propanoate (2)

1 (1.25 g, 5 mmol) was dissolved in a mixture of 10% Na_2CO_3 solution (20 mL) and THF (10 mL). The solution was cooled on ice bath for 5 min before Boc-anhydride (1.14 g, 5.25 mmol in 10 mL THF) was added. The reaction mixture was warmed up to room temperature and kept for 5 hours till completion. Then the reaction mixture was acidified to pH ~ 2-3 using 1N HCl and the product was extracted using ethyl acetate (3× 100 mL). The combined organic layer was washed with brine (150 mL) and dried over Na_2SO_4. Solvent removal afforded the Boc protected amino acid in quantitative yield. The product was directly dissolved in dry ethyl acetate (60 mL), to which was added tert-butyl 2,2,2-trichloroacetimidate (1.6g, 7.5 mmol). The reaction mixture was refluxed for 6 hours, at which point half equivalent more of 2,2,2-trichloroacetimidate (0.54 g, 2.5 mmol) was added and then the reaction mixture was refluxed for another 6 hours. After solvent removal, the residue was purified through silica gel using EtOAc/Hexane (1:5.6) to give the desired product **2** as a white solid (1.27 g, overall two steps 67%).

^{1}H NMR ($CDCl_3$) δ: 12.15 (s, 1H), 7.51 (s, 1H), 7.29 – 7.27 (d, *J* = 7.4 Hz, 1H), 6.91-6.89 (d, *J* = 7.6 Hz, 1H), 5.04-5.02 (d, *J* = 8.2 Hz, 1H), 4.45-4.41 (q, *J* = 4.6 Hz, 1H), 3.08-2.98 (m, 2H), 2.61 (s, 3H), 1.42 (s, 18H)

^{13}C NMR ($CDCl_3$) δ: 204.31, 170.78, 161.34, 155.0, 137.81, 131.35, 126.82, 119.42, 118.33, 82.31, 79.83, 54.71, 77.82, 28.33, 26.63

MS-ESI$^+$: *m/z* calculated for $C_{20}H_{29}NO_6$ $[M+Na]^+$ 402.1893, found 402.1963

IIc. Synthesis of (*S*)-tert-butyl 3-(3-acetyl-4-(((trifluoromethyl)sulfonyl)oxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate (3)

2 (1.20 g, 3.3 mmol), was dissolved in anhydrous dichloromethane (10 mL) and triethylamine (1.33 g, 13.2 mmol) was added while stirring. The reaction mixture was cooled to -78 ^{o}C and trifluoromethane sulfonic anhydride (1.4 g, 4.95 mmol) was added slowly during 5 min. The reaction mixture was allowed to stir at room temperature for 0.5 hr under an argon environment. After that, the reaction was quenched with saturated sodium bicarbonate (20 mL) and the mixture was allowed to stir for 5 min. The product

was extracted with dichloromethane (3 × 50 mL). The combined organic layer was washed with brine (80 mL) and dried over Na_2SO_4. After removing the solvent, the crude product was purified on silica gel column using ethyl acetate:hexane (1:5) as eluent to yield a colorless gummy product (1.64 g, 97%).

^{1}H NMR ($CDCl_3$) δ: 7.62 (s, 1H), 7.44 – 7.41 (d, *J* = 8.2 Hz, 1H), 7.26-7.25 (d, *J* = 7.2 Hz, 1H), 5.12-5.10 (d, *J* = 8.3 Hz, 1H), 4.50-4.46 (q, *J* = 5.4 Hz, 1H), 3.20-3.07 (m, 2H), 2.62 (s, 3H), 1.42 (s, 9H), 1.40 (s, 9H)

^{13}C NMR ($CDCl_3$) δ: 196.34, 170.24, 150.94, 145.63, 137.78, 134.31, 131.78, 122.11, 119.84, 117.29, 82.82, 80.07, 54.84, 37.91, 28.47, 27.96, 27.81

MS-ESI$^+$: *m/z* calculated for $C_{12}H_{12}F_3NO_6S$ $[M-(Boc+^tBu)+H]^+$ 356.0416, found 356.0391

IId. Synthesis of Boc-AB3(pin)-O^{t}Bu

3 (1.5 g, 2.935 mmol), B_2Pin_2 (1.88 g, 7.33 mmol), $Pd(dppf)Cl_2$ (0.17 g, 0. 235 mmol, 5 mol %), 1,1′-Bis(diphenylphosphino)ferrocene (dppf) (0.13 g, 0.235 mmol) and potassium acetate (0.86 g, 8.8 mmol) were was dissolved in anhydrous dioxane (20 mL). The reaction mixture was bubbled with argon for 20 minutes and it was allowed to stir at 87 ºC for 70 minutes. Completion of the reaction was monitored by LC-MS. Then water (80 mL) was added to the reaction and the product was extracted with ethyl acetate (3 × 80 mL). The combined organic layer was washed with brine (100 mL) and dried over Na_2SO_4. The solvent was removed under vacuum and the crude product was purified on silica gel using hexane:diethylether (3:2) as eluent. The desired product **Boc-AB3(pin)-O^{t}Bu** was obtained as a colorless viscous liquid (960 mg, 67%) and the protodeboronated product **Boc-AB4-O^{t}Bu** (320 mg, 30%, colorless liquid).

<u>Boc-AB3(pin)-O^{t}Bu</u>

^{1}H NMR ($CDCl_3$) δ: 7.58 (s, 1H), 7.43 – 7.42 (d, *J* = 7.6 Hz, 1H), 7.33-7.31 (d, *J* = 7.3 Hz, 1H), 4.90-4.89 (d, *J* = 8.1 Hz, 1H), 4.48-4.44 (q, *J* = 5.3 Hz, 1H), 3.19-3.08 (m, 2H), 2.57 (s, 3H), 1.41 (s, 30H)

^{13}C NMR ($CDCl_3$) δ: 199.81, 170.51, 154.99, 140.96, 137.31, 133.55, 132.28, 129.57, 83.67, 82.27, 79.77, 54.40, 37.87, 28.30, 28.0, 24.83

^{11}B NMR ($CDCl_3$) δ: 31.10

MS-ESI$^+$: *m/z* calculated for $C_{26}H_{40}BNO_7$ $[M+H]^+$ 490.2976, found 490.2996.

Boc-AB4-O^{t}Bu

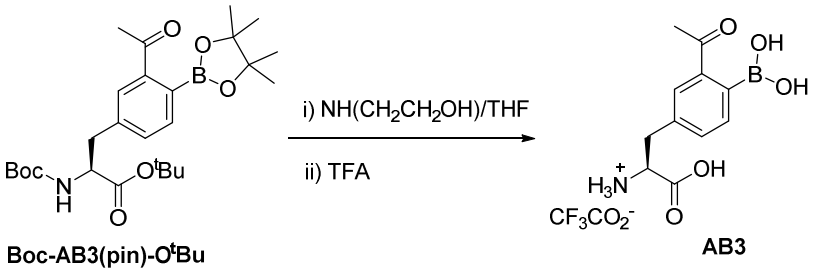
^{1}H NMR ($CDCl_3$) δ: 7.84-7.82 (m, 1H), 7.78 (s, 1H), 7.41–7.39 (m, 2H), 5.11-5.10 (d, *J* = 7.5 Hz, 1H), 4.50-4.48 (q, *J* = 4.5 Hz, 1H), 3.19-3.08 (m, 2H), 2.59 (s, 3H), 1.42 (s, 18H)

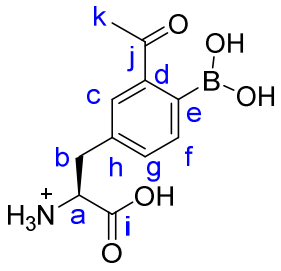
^{13}C NMR ($CDCl_3$) δ: 197.81, 170.57, 154.95, 137.09, 134.24, 129.34, 126.50, 126.89, 82.27, 79.67, 77.28, 54.67, 38.27, 28.25, 27.09, 26.57

MS-ESI$^+$: *m/z* calculated for $C_{20}H_{29}NO_5$ $[M+H]^+$ 364.2124, found 364.2158

IIe. Synthesis of AB3

Boc-AB3(pin)-O^{t}Bu (62 mg, 0.125 mmol) was dissolved in anhydrous THF (500 µL) and diethanolamine (150 mg, 1.25 mmol) was added into the solution at room temperature. The reaction was allowed to stir overnight and then quenched with 1N HCl (30 mL). The pinacol-deprotected product was extracted using EtOAc (3 × 30 mL). The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was evaporated under vacuum to yield a viscous colorless product. Without further purification the product was dissolved in 0.2 mL DCM, to which was added trifluoroacetic acid (TFA) (0.8 mL). The reaction was allowed to stir at room temperature for 30 min. LC-MS analysis indicated that the ^{t}Bu deprotection was ~80% complete. To remove the tBu group completely, TFA and dichloromethane were evaporated and the residue was again subjected to neat TFA (1 mL) for ~1 hr. Then TFA was evaporated and the residue was dissolved in water, lyophilization of which gave the crude product as a white powder. Reverse phase HPLC using purification with acetonitrile/water (0.1% TFA) mixture yielded the pure product as a white powder after lyophilization (92%, 42 mg).





AB3

^{1}H NMR (D_2O) δ: 7.97 (s, 1H, c), 7.60 – 7.58 (d, *J* = 7.7 Hz, 1H, f), 7.54-7.53 (d, *J* = 7.6 Hz, 1H, g), 4.41-4.39 (t, *J* = 5.2 Hz, 1H, a), 3.48-3.32 (m, 2H, b), 2.68 (s, 3H, k);

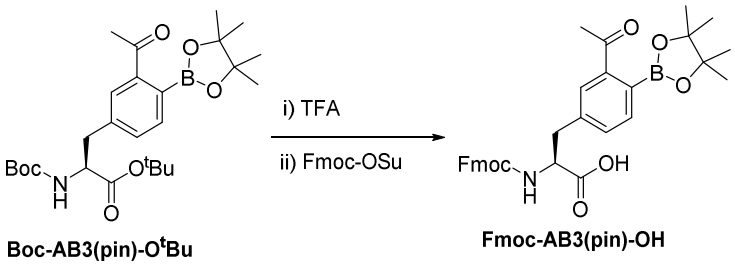
^{13}C NMR (D_2O) δ: 203.97 (j), 171.21(i), 139.36 (h), 134.62 (d), 131.28 (e), 130.34(f), 117.17(c), 115.24(g), 53.90(a), 35.28(b), 24.65(k);

^{11}B NMR (D_2O) δ: 30.30;

MS-ESI$^+$: *m/z* calculated for $C_{11}H_{15}BNO_5$ $[M-H_2O]^+$ 234.0938, found 234.0940.

IIf. Synthesis of Fmoc-AB3(pin)-OH

Boc-AB3(pin)-O^{t}Bu (0.6 g, 1.23 mmol) was dissolved in DCM (4 mL) and placed on ice bath. TFA (6 mL) was added to the solution. The reaction mixture was allowed to stir at room temperature for 1 hr. Then DCM and TFA were evaporated and the gummy residue was treated with neat TFA for another hour. Further TFA was evaporated and the residue was re-dissolved in DCM and evaporated (3 times) to remove the excess TFA. The resulting residue was subsequently dissolved in DCM (15 mL). Fmoc-OSu (430 mg, 1.23 mmol) was added to the reaction flask. The reaction was allowed to stir at room temperature for 1 hr. The resulting mixture was acidified with 2 N HCl and the product was extracted with ethyl acetate (3 × 50 mL). Combined organic layer was washed with brine (100 mL) and dried over Na_2SO_4. Solvent removal gave an oily yellowish residue, which was triturated in hexane/ethyl acetate overnight to yield an off-white solid (0.59 g, 87%). The product was directly used for peptide synthesis without further purification.



Boc-AB3(pin)-O^{t}Bu **Fmoc-AB3(pin)-OH**

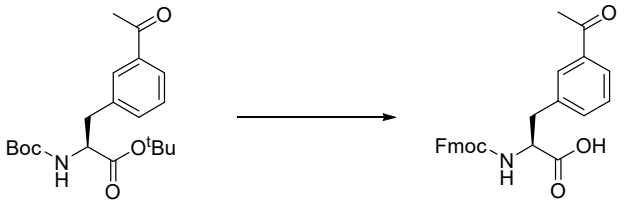
^{1}H NMR ($CDCl_3$) δ: 7.75-7.74 (d, *J* =7.4, 2H), 7.58 (s, 1H), 7.54 –7.53 (d, *J* = 5.4 Hz, 2H), 7.54-7.53 (d, *J* = 5.1 Hz, 1H), 7.39-7.37 (d, *J* = 4.8 Hz, 2H), 7.30-7.27 (m, 3H), 5.37-5.34 (t, *J* = 6.3 Hz, 1H), 4.71-4.67 (q, *J* = 5.0 Hz, 1H), 4.39-4.37 (d, *J* = 7.1 Hz, 2H), 4.18-4.16 (t, *J* = 6.3 Hz, 1H), 3.24-3.14 (m, 2H), 2.50 (s, 3H), 1.44 (s, 12H)

^{13}C NMR ($CDCl_3$) δ: 200.33, 173.65, 155.84, 143.68, 141.28, 136.77, 133.52, 132.38, 129.37, 127.75, 127.10, 125.02, 119.97, 83.97, 67.24, 54.22, 47.04, 37.47, 31.57, 24.80, 22.64, 14.11

MS-ESI$^+$: *m/z* calculated for $C_{32}H_{35}BNO_7[M+H]^+$ 556.2507, found 556.2606.

IIg. Synthesis of Fmoc-AB4-OH

The compound was synthesized from Boc-AB4-O^{t}Bu following the protocol described in IIf**.** Final trituration in ethyl acetate/hexane (1:9) solvent system gave an off-white solid with 79% yield. The product was directly used for peptide synthesis without further purification.



Boc-AB4-O^{t}Bu **Fmoc-AB4-OH**

^{1}H NMR ($CDCl_3$) δ: 7.83-7.81 (m, 2H), 7.76-7.74 (d, *J* =7.2, 2H), 7.55-7.51 (m, 2H), 7.40-7.34 (m, 4H), 7.30-7.27 (m, 2H), 5.38-5.37 (t, *J* = 5.8 Hz, 1H), 4.75-4.72 (q, *J* = 4.8 Hz, 1H), 4.44-4.34 (m, 2H), 4.18-4.16 (t, *J* = 6.3 Hz, 1H), 3.31-3.15 (m, 2H), 2.54 (s, 3H)

^{13}C NMR ($CDCl_3$) δ: 198.52,174.70, 155.80, 143.66, 143.59,141.28,137.27, 136.43, 134.26, 129.06, 128.88, 127.74, 127.45, 127.06, 125.0, 124.98, 119.99, 67.16, 54.49, 47.07, 37.66, 26.61

MS-ESI$^+$: *m/z* calculated for $C_{26}H_{23}NO_5[M+H]^+$ 430.1654, found 430.1672.

III. Lysine conjugation of AB3 and AB1

AB3 was assessed for lysine conjugation via a titration experiment and compared to AB1, an analogous amino acid reported by our group.[2] The lysine titration experiments were performed in a quartz cuvette (10 mm path length; total volume ~ 1 mL). For a typical experiment, 1 mL PBS buffer (pH 7.4) was pipetted into the cuvette, then the AB3 or AB1 stock in PBS buffer was added to give the final concentration of 50 µM and 25 µM for AB3 and AB1 respectively. The solution was mixed rapidly and then the initial absorption spectrum was recorded. The titrations were performed by titrating in a lysine stock solution (2 M in PBS buffer). The pH of all stock solutions was adjusted to 7.4. All spectra collected during titration were background corrected by subtracting that of the lysine alone at corresponding concentrations. AB3 shows an absorption peak at ~ 254 nm with a small hump at ~ 275-280 nm, while AB1 shows an absorption maximum at ~ 282 nm. Titrating lysine into AB3 elicited a red shift from ~254 nm to ~ 275 nm as a result of iminoboronate formation. The absorption at 275 nm was plotted against lysine concentration to give the titration curve. In the case of AB1, the absorption at 295 nm was plotted against lysine concentration to determine the K_d value. Each combination was repeated three times, which gave essentially overlapping curves, fitting of which gave the K_d values. The results indicate that AB3 has slightly better propensity to conjugate with lysine than earlier reported AB1.

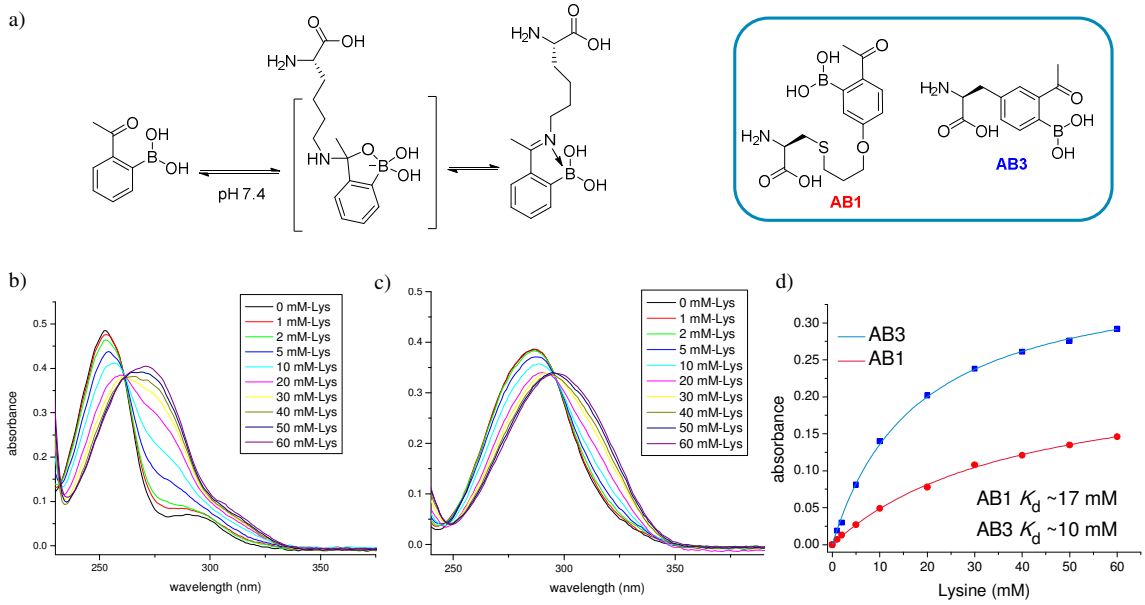


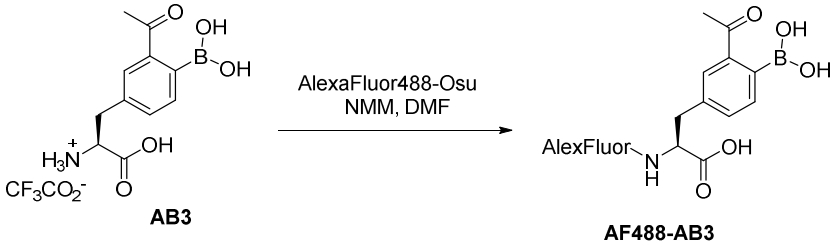
Figure S2. a) Reaction mechanism of iminoboronate formation between 2-APBA and lysine. 2-APBA moiety containing amino acid AB1 and AB3 presented in green line box. UV-vis spectral changes with titration of lysine into b) AB3 and c) AB1. d) Titration curves showcasing the superior reactivity of AB3 towards iminoboronate formation.

IV: Peptide synthesis, fluorophore labeling and characterization

All peptides were synthesized via Fmoc/^{t}Bu chemistry with the Rink Amide resin as the solid support. The syntheses were carried out on 0.05 mmole scale. Five equivalents of the commercially available amino acids were used for the coupling reaction. The incorporation of the unnatural amino acids was accomplished by using three equivalents of the Fmoc-protected amino acids and an extended coupling time (1 hr). The peptides were cleaved off resin and globally deprotected with reagent K (80% TFA, 5% H2O, 2.5% EDT, 5% Thioanisole and 7.5% phenol). Ether precipitation gave the crude peptides, which were purified by RP-HPLC (Waters Prep LC, Jupiter C18 Column). The purity of all peptides was determined by using either LC-MS (Agilent) or HPLC (Waters) to be >92%. The characterization data are presented in Table S1.

Fluorophore labeling of AB3

AB3 was directly conjugated to Alexa Fluor 488 (AF488)-OSu by following the vendor protocol from Life Technologies (http://tools.lifetechnologies.com/content/sfs/manuals/mp10168.pdf). The labeled product was purified on RP-HPLC using acetonitrile/water (1% TFA) mixture as eluent and confirmed with LC-MS for integrity and purity. The lyophilized product was dissolved in a phosphate buffer (pH = 7.4) and the stock concentration was determined by using a NanoDrop UV-VIS spectrometer with ε (495 nm) = 71,000 M^{-1} •cm^{-1}. The mass-spec result of AF488-AB3 is listed in Table S2.



AF488-C5-maleimide labeling of peptides

For a typical labeling experiment, 1.3 mM solution of AF488-C5-maleimide in DMF (100 µL) and 3 mM peptide solution in DMF (50 µL) were mixed together in a small vial. 30 mM NMM solution in DMF (6 µL) and 15 mM TCEP solution in DMF (5 µL) were added into the reaction mixture sequentially. The reaction was stirred at room temperature for 1 hr to complete. The crude products were purified by RP-HPLC (Waters Prep LC, Jupiter C18 Column). All the AF488 labeled peptides were characterized with LC-MS to confirm their identities and purities (> 95%). The mass-spec data of the labeled peptides are listed in Table S2.

Methods for LC-MS and HPLC analysis to assess the purity of peptides

Method for LC-MS (Agilent 6230 LC TOF): Agilent Extend C18 (1.8 µm, 2.1× 50 mm) analytical column using mobile phase water-acetonitrile-(0.1% formic acid) with a flow rate 0.2 mL/min. Gradient used: isocratic 5% CH_3CN for 3 min, 5% to 85% CH_3CN in 15 min, 85% to 95% CH_3CN in 4 min, then isocratic 95% CH_3CN for 2 min.

Method for analytical HPLC (Waters 2695): Phenomenex C18 (5 µm, 2.0 × 150 mm) analytical column using mobile phase water-acetonitrile-(0.1% formic acid) with a flow rate 0.2 mL/min. Gradient used, isocratic 5% CH_3CN for 5 min, then 5% to 85% CH_3CN in 14 min, then 85% to 95% CH_3CN in 3 min, then isocratic 95% CH_3CN for 2 min.

Methods for determining % peptide cyclization

Peptide cyclization was evaluated by using UV-vis, ESI-MS and NMR spectroscopy. Quantification of peptide cyclization was done by using ^{1}H-NMR, in which a chemical shift of acetyl $-CH_3$ group from ~2.56 (keto) to ~2.42 (imine) was observed upon peptide cyclization. Integration of these two peaks gives the ratio between acyclic and cyclic forms of a peptide. % peptide cyclization is listed in Figure 1c in main text. Specifically for the ^{1}H-NMR experiment, a PBS (1×, 7.4, 10% D_2O) solution of 0.5 mM peptide was analyzed immediately after the solution was made. The peptide cyclization was observed to be instantaneous and there is no incubation time required.

UV-vis experiments

UV-vis absorption was measured in quartz cuvettes (10 mm path length; total volume ~ 1 mL) with ~30 µM solutions of P1-P11 and ~20 µM solutions of the bicyclic peptide P12-P13 in a phosphate buffer (pH = 7.4) at room temperature. Then the peptide solution was adjusted to the pH 4 using 1N HCl and UV-vis absorption spectrum was recorded. All spectra collected were background corrected by subtracting the buffer absorbance at corresponding pHs.

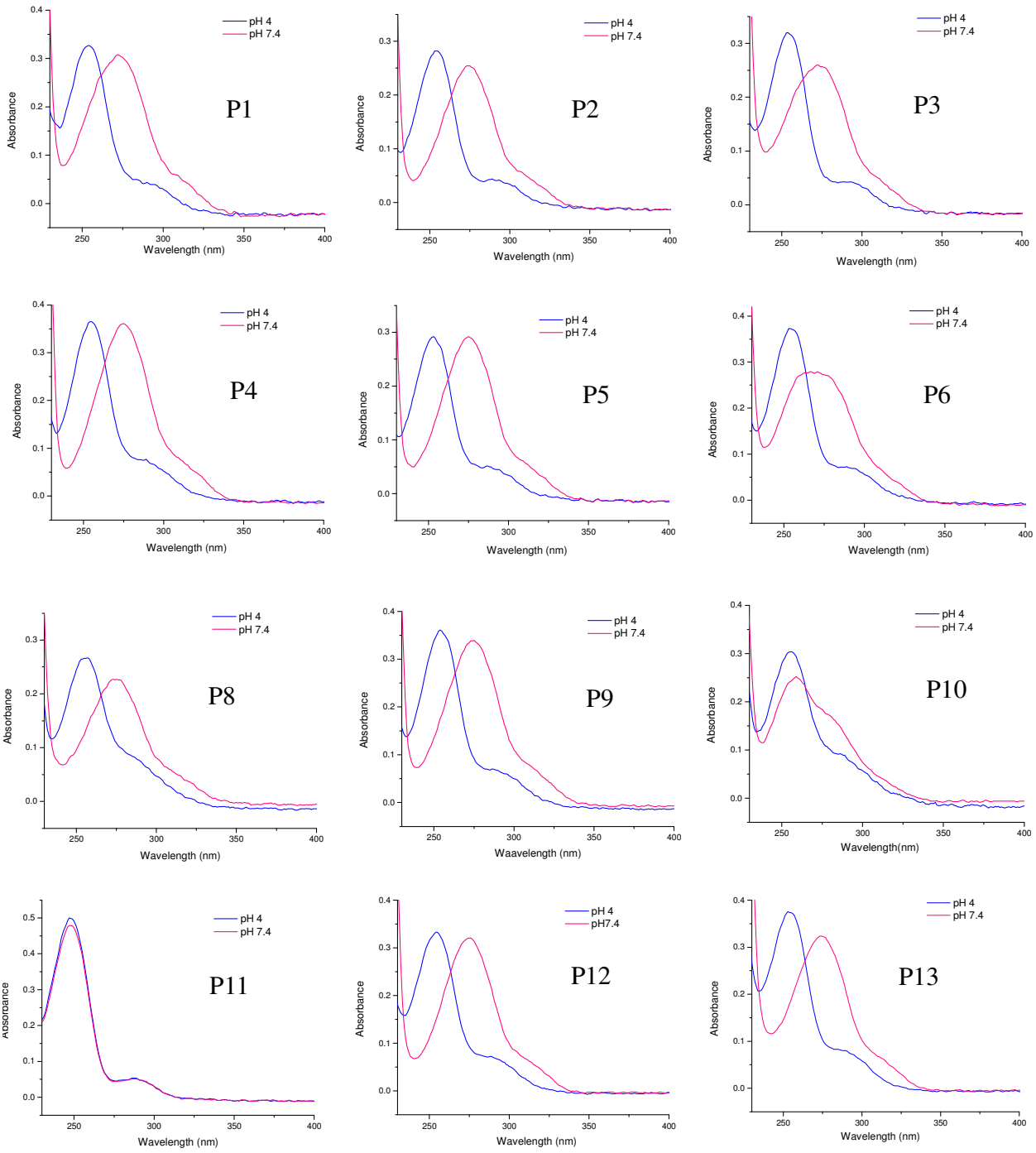


Figure S3. UV-vis spectra of various peptides at pH 7.4 and 4 respectively.

ESI-Mass experiments

5 µL peptide solution (~ 100 µM in PBS) was used for ESI-mass analysis using an Agilent 6230 LC/MS TOF mass spectrometer. The TOF mass spectrometer was operated in the positive mode with the set up of Fragmenter 200 V, Skimmer 65V and OCT 1 RF Vpp 750 V. A neutral buffer (ammonium acetate) and acetonitrile mixture (1:1 in volume) was used as the mobile phase (0.2 mL/min flow rate) to acquire the mass of the cyclic peptides. The mass of the linear peptides was acquired by using 0.05% formic acid in the water/acetonitrile mobile phase. The calculated and observed m/z values of the peptides (P1-P13) are presented in Table S1.

Table S1. Peptide sequences and the mass spec data for their linear (pH 4) and cyclic (pH 7.4) forms

Peptide	Dubbed	Mass (m/z) at pH = 4	Mass (m/z) at pH = 7.4
KAG(AB3)	P1	$[M+H]^+$ cal. 507.2739; obs. 507.2704	$[M+H-H_2O]^+$ cal. 471.2527; obs. 471.2589
KAAG(AB3)	P2	$[M+H]^+$ cal. 578.3110; obs. 578.3092	$[M+H-H_2O]^+$ cal. 542.2898; obs. 542.2954
KAAAG(AB3)	P3	$[M+H]^+$ cal. 649.3481; obs.649.3415	$[M+H-H_2O]^+$ cal. 613.3270; obs. 613.3357
KAAAAG(AB3)	P4	$[M+H]^+$ cal. 720.3852; obs.720.3731	$[M+H-H_2O]^+$ cal. 684.3641; obs. 684.3733
(AB3)AAAAGK	P5	$[M+H]^+$ cal. 720.3852; obs.720.4213	$[M+H-H_2O]^+$ cal. 684.3641; obs. 684.3933
KAAADAAADG(AB3)	P6	$[M+H-H_2O]^{2+}$ cal. 537.7531; obs. 537.7547	$[M+H-H_2O]^{2+}$ cal. 519.7379; obs. 519.7671
KTNHS(AB3)	P7	$[M+H]^+$ cal. 818.3968; obs. 818.4108	$[M+H-H_2O]^+$ cal. 782.3756; obs. 782.3703
CKRGD(AB3)	P8	$[M+H]^+$ cal. 810.3788; obs. 810.3744	$[M+H]^+$ cal. 792.3636; obs. 792.3973
C(AB3)RGDfK	P9	$[M+H]^+$ cal. 957.4472; obs. 957.4807	$[M+H-H_2O]^+$ cal. 939.4320; obs. 939.4723
KG(AB3)AAA	P10	$[M+H]^+$ cal. 649.3481; obs. 649.3674	$[M+H]^+$ cal. 649.3481; obs. 649.3507#
KAAAAG(AB4)	P11	$[M+H]^+$ cal. 676.3784; obs. 676.3975	$[M+Na]^+$ cal. 698.3601; obs. 698.3912#
(AB3)LDA(AB3)GKAAAGK	P12	$[M+H]^{2+}$ cal. 683.8533; obs. 683.8605	$[M+H]^{2+}$ cal. 665.8428; obs. 665.8378
(AB3)NGR(AB3)GRGDfK	P13	$[M+H]^{2+}$ cal. 800.4046; obs. 800.4024	$[M+H]^{2+}$ cal.782.3940; obs. 782.3866

According to the ^{1}H-NMR spectrum, the peptide should be linear regardless the varying pH.

Table S2. Alexa Fluor 488 (AF488) labeled amino acid/peptides and their mass spec data

Amino acid or peptide	Dubbed	Mass (m/z)
Alexa Fluor488-AB3	AF488-AB3	$[M-H]^+$ cal. 768.0975; obs. 768.0758
Alexa Fluor488-CKRGD(AB3)	AF488-P8	$[M+H]^{2+}$ cal. 754.7466; obs. 754.7382
Alexa Fluor488-C(AB3)RGDfK	AF488-P9	$[M+H]^{2+}$ cal. 828.2862; obs. 828.2630
Alexafluor488-C(AB3)NGR(AB3)GRGDfK	AF488-P13	$[M+H-H_2O]^{3+}$ cal. 795.3528; obs. 795.3080
Alexa Fluor488-cyclo(RGDfC)	AF488-P14	$[M+H]^+$ cal. 1277.3274; obs. 1277.3274

NMR experiments

Solutions of AB3 and all peptides (~ 0.5 mM) were prepared using lyophilized amino acid/ peptides in PBS buffer (1x, with 10% D_2O). The desired pH for the experiment was tuned using 0.5N NaOH and/or 0.5N HCl, and the final pH was confirmed by a micro pH meter. A mixture of AB3 (0.5 mM) and lysine (120 mM) was also prepared, the spectrum of which was used as a reference for iminoboronates. The NMR spectra were recorded on a 600 MHz Avance NMR spectrometer at 25 ºC with 64 scans. Further, the data were processed and stacked using MestReNova10.0.2. For better visualization, a break between 4.5 to 6 ppm is generated using MestReNova to skip the suppressed water peak. The ^{11}B-NMR spectra of P12 (1 mM) were obtained at 25 ºC with 40,000 scans. $B(OH)_3$ (1 mM) was used as internal standard (19.7 ppm) for ^{11}B-NMR studies.

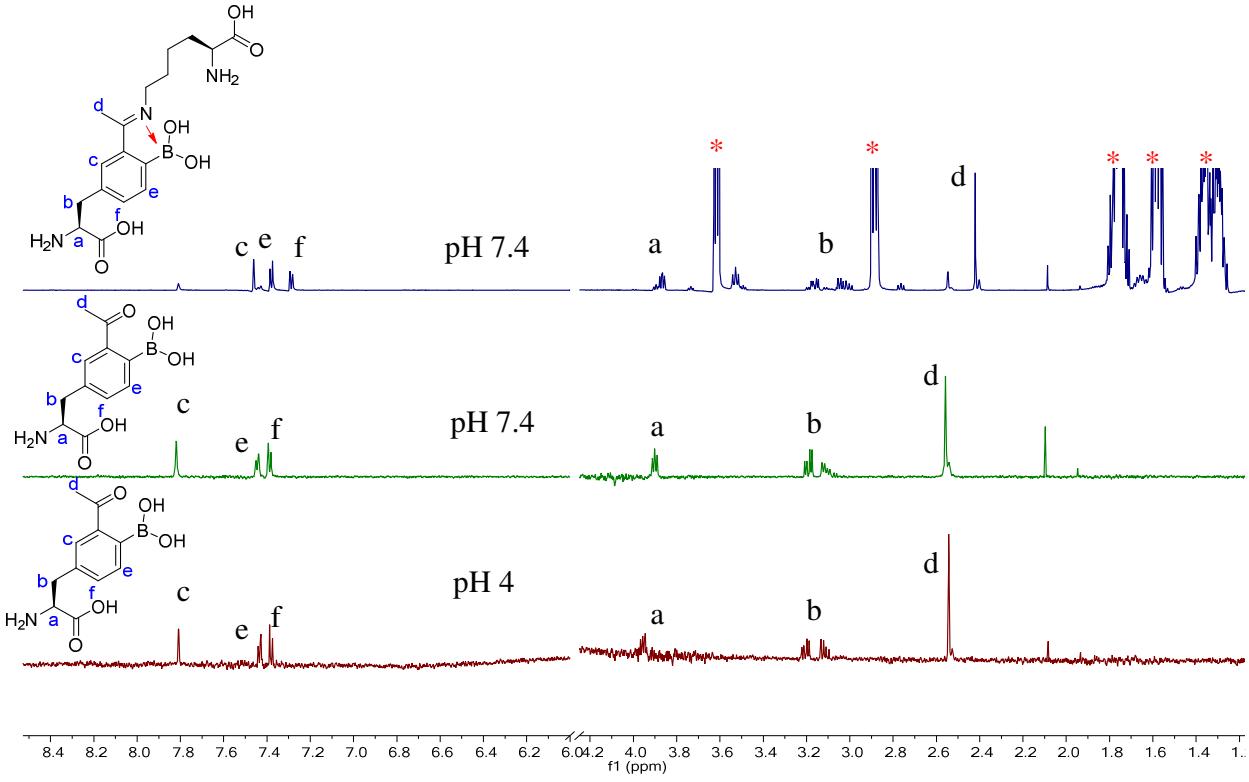


Figure S4. ^{1}H-NMR spectra of AB3 at pH 4 (bottom), pH 7.4 (middle), and in presence of excess lysine at pH 7.4 (top). The spectra are assigned with lower case letters to illustrate the chemical shift changes in AB3 after iminoboronate formation. * corresponds to the peaks of the unreacted lysine.

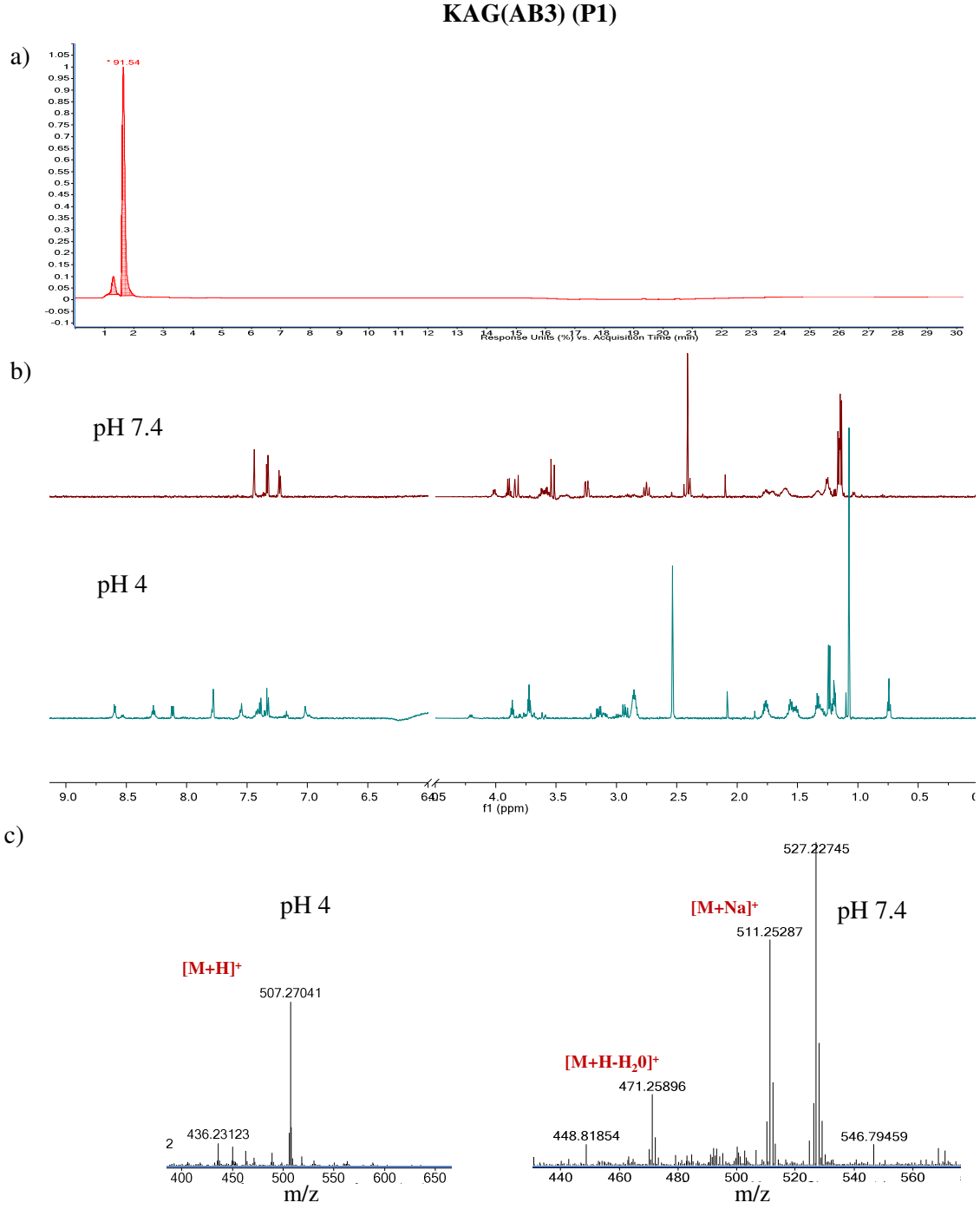


Figure S5. Characterization of P1. a) LC chromatogram; b) ^{1}H-NMR spectra at pH 4 (bottom) and pH 7.4 (top); c) ESI-MS (m/z) spectra at pH 4 (left) and pH 7.4 (right).

KAAG(AB3) (P2)

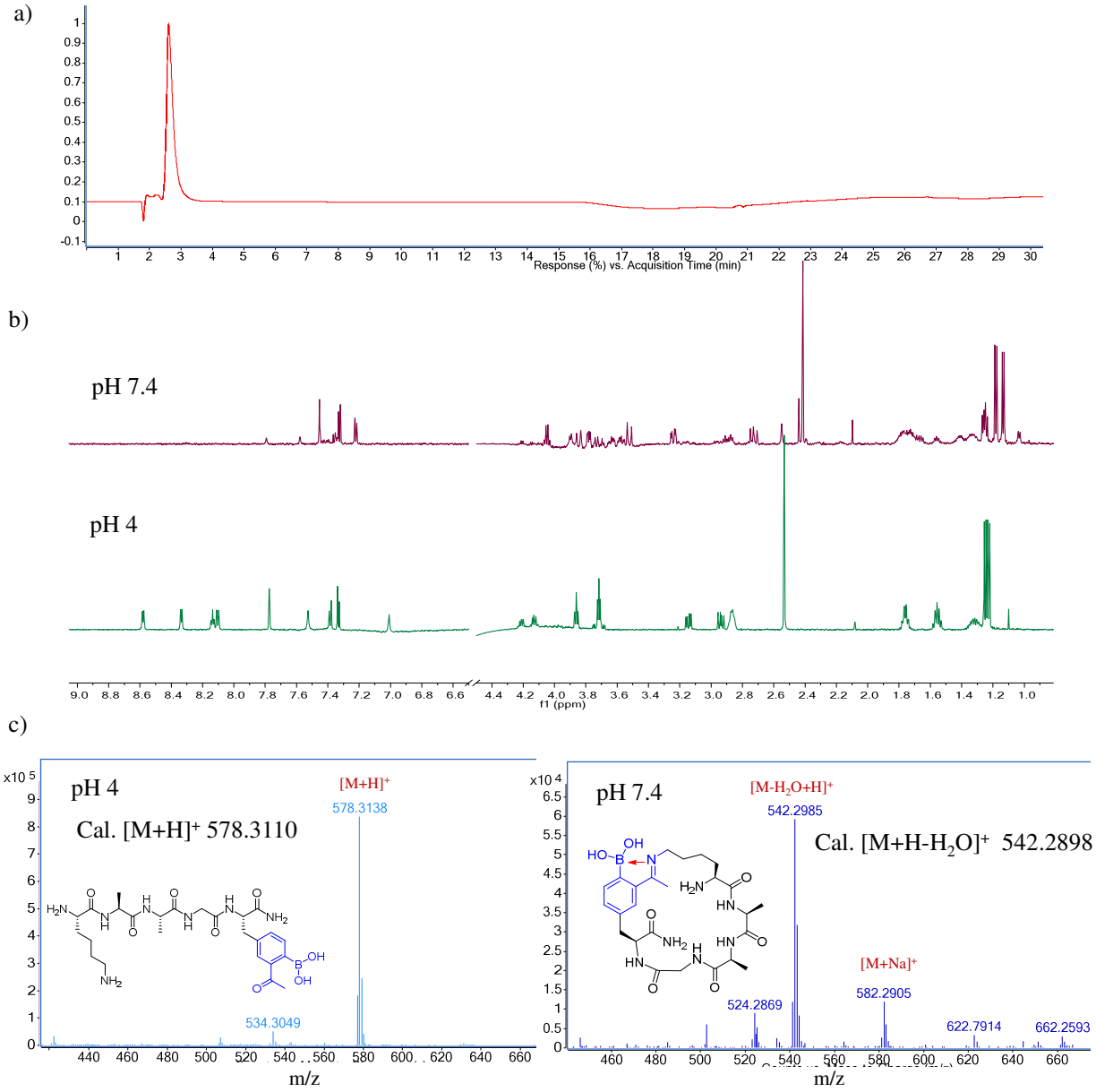


Figure S6. Characterization of P2. a) LC chromatogram; b) ^{1}H-NMR spectra at pH 4 (bottom) and pH 7.4 (top); c) ESI-MS (m/z) spectra at pH 4 (left) and pH 7.4 (right).

KAAAG(AB3) (P3)

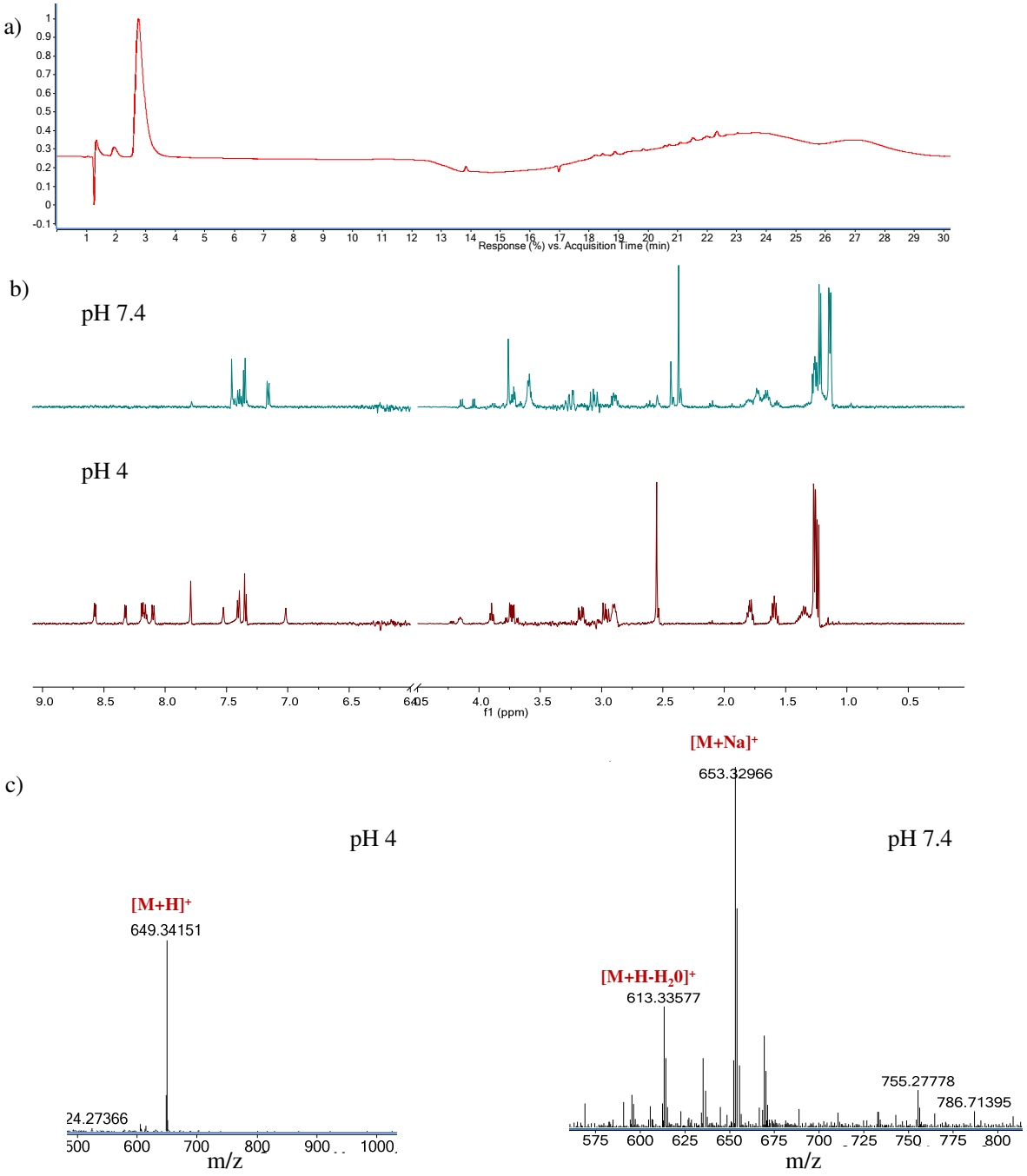


Figure S7. Characterization of P3. a) LC chromatogram; b) ^{1}H-NMR spectra at pH 4 (bottom) and pH 7.4 (top); c) ESI-MS (m/z) spectra at pH 4 (left) and pH 7.4 (right).

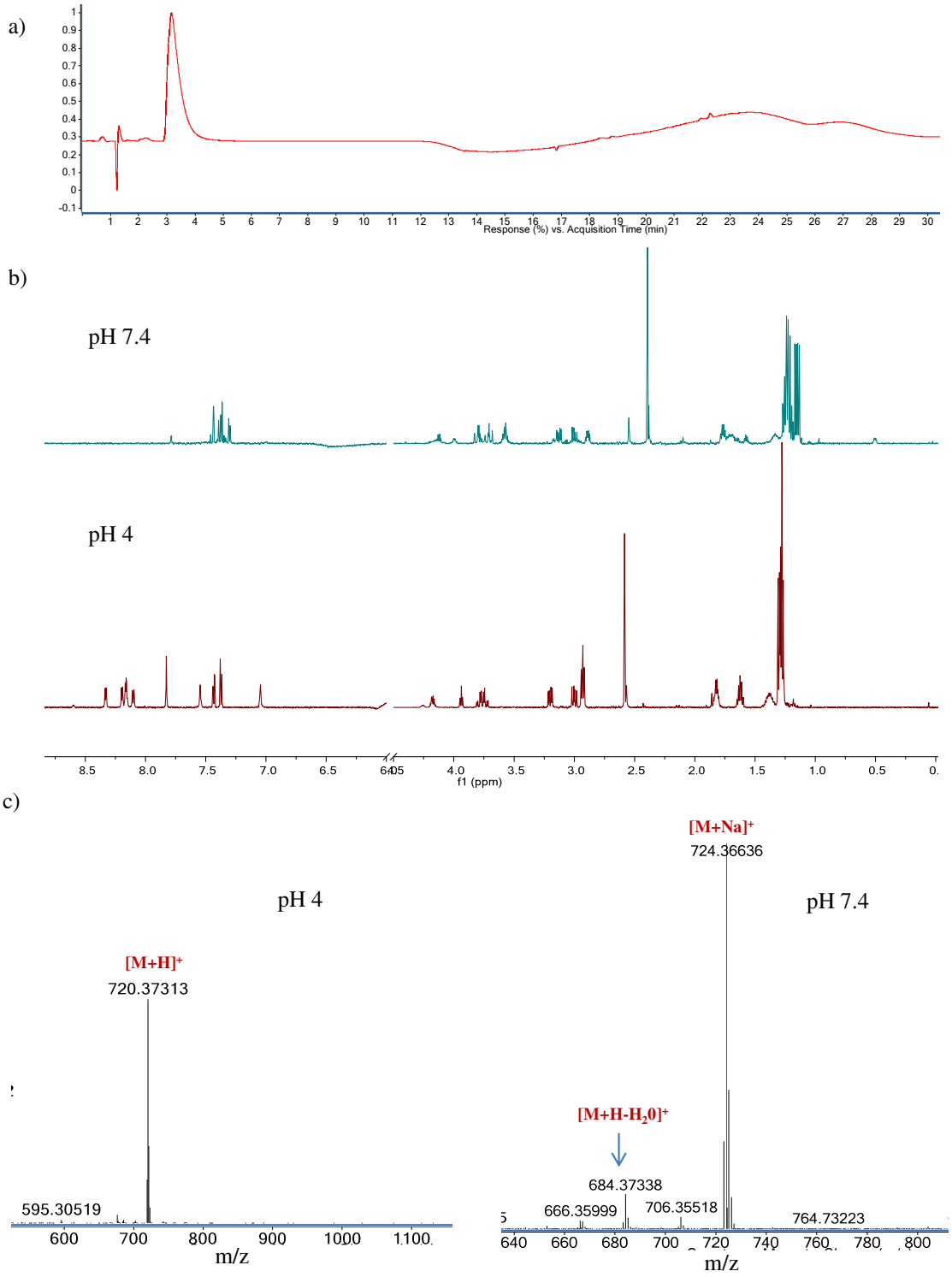


Figure S8. Characterization of P4. a) LC chromatogram; b) ^{1}H-NMR spectra at pH 4 (bottom) and pH 7.4 (top); c) ESI-MS (m/z) spectra at pH 4 (left) and pH 7.4 (right).

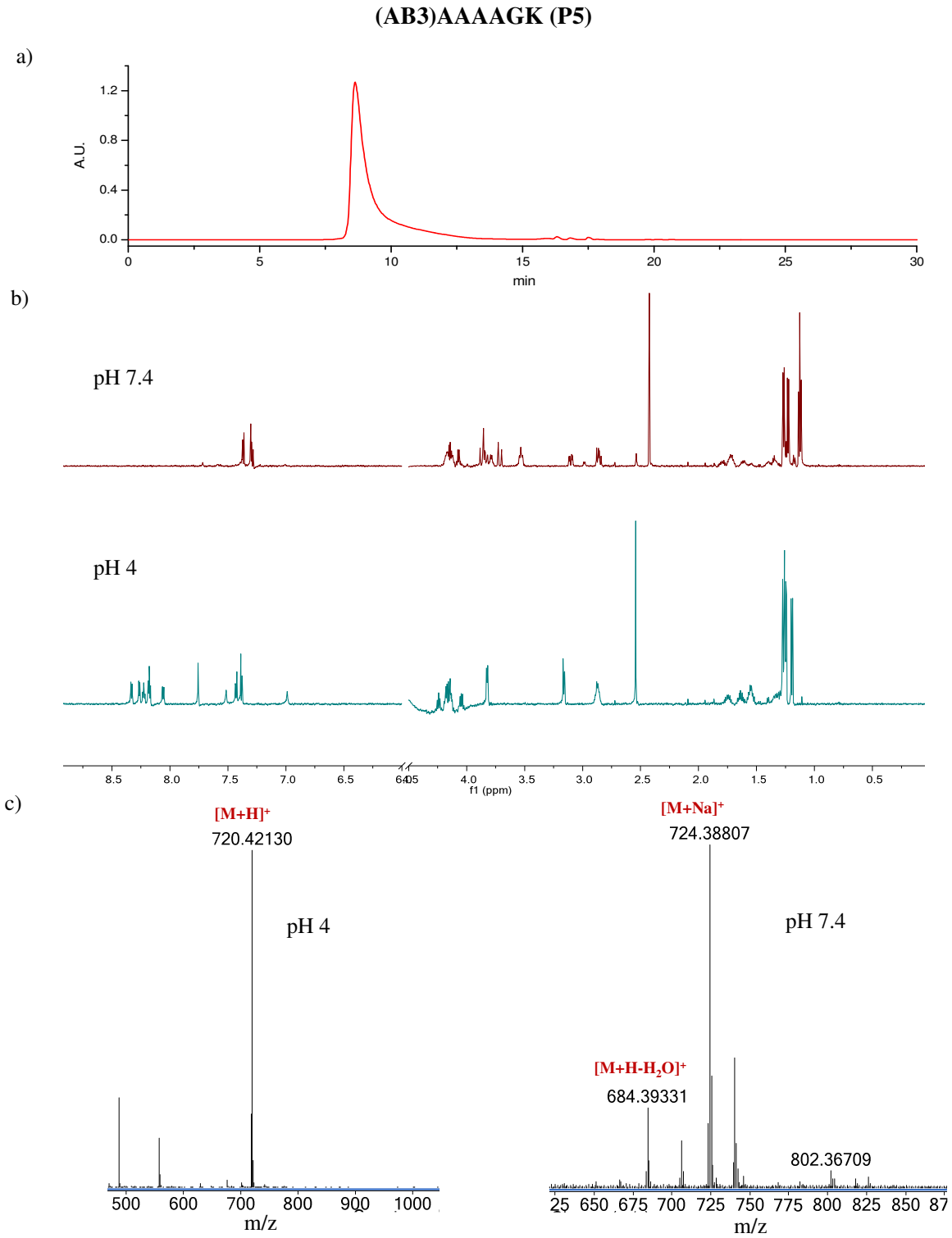


Figure S9. Characterization of P5. a) LC chromatogram; b) ^{1}H-NMR spectra at pH 4 (bottom) and pH 7.4 (top); c) ESI-MS (m/z) spectra at pH 4 (left) and pH 7.4 (right).

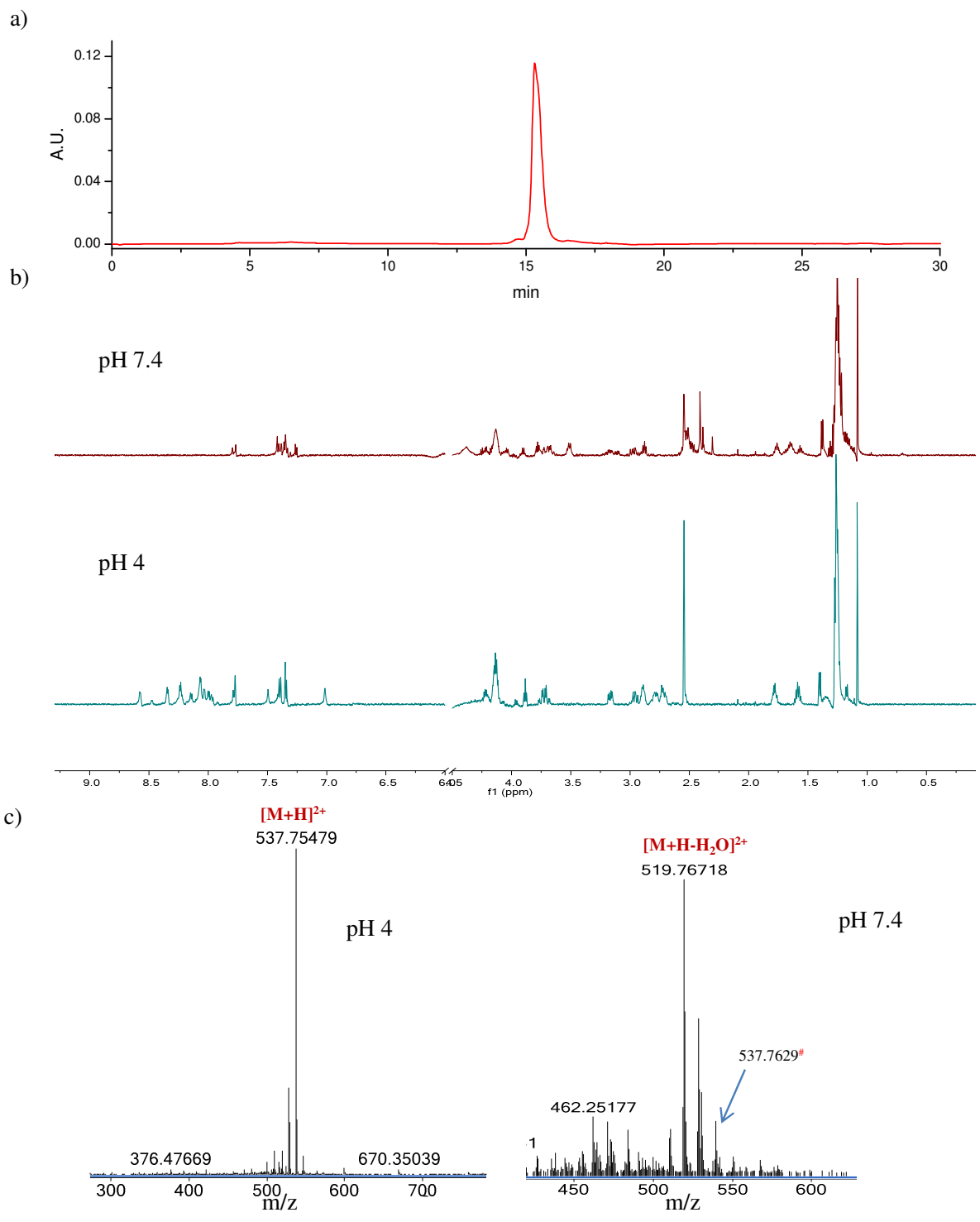


Figure S10. Characterization of P6. a) LC chromatogram; b) ^{1}H-NMR spectra at pH 4 (bottom) and pH 7.4 (top); c) ESI-MS (m/z) spectra at pH 4 (left) and pH 7.4 (right). # The mass of linear peptide is also observed in this case, which is consistent with NMR data.

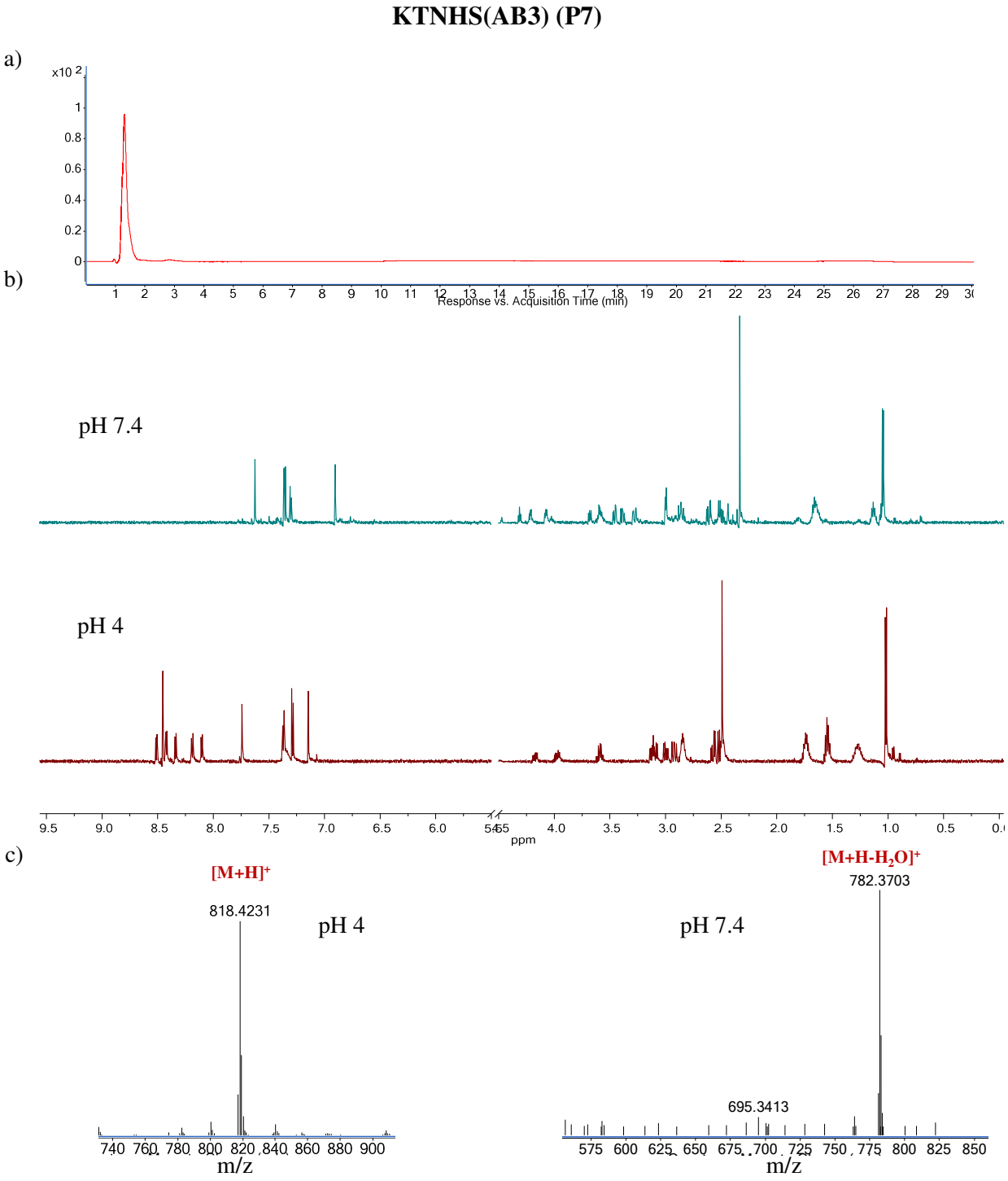


Figure S11. Characterization of P7. a) LC chromatogram; b) ^{1}H-NMR spectra at pH 4 (bottom) and pH 7.4 (top); c) ESI-MS (m/z) spectra at pH 4 (left) and pH 7.4 (right).

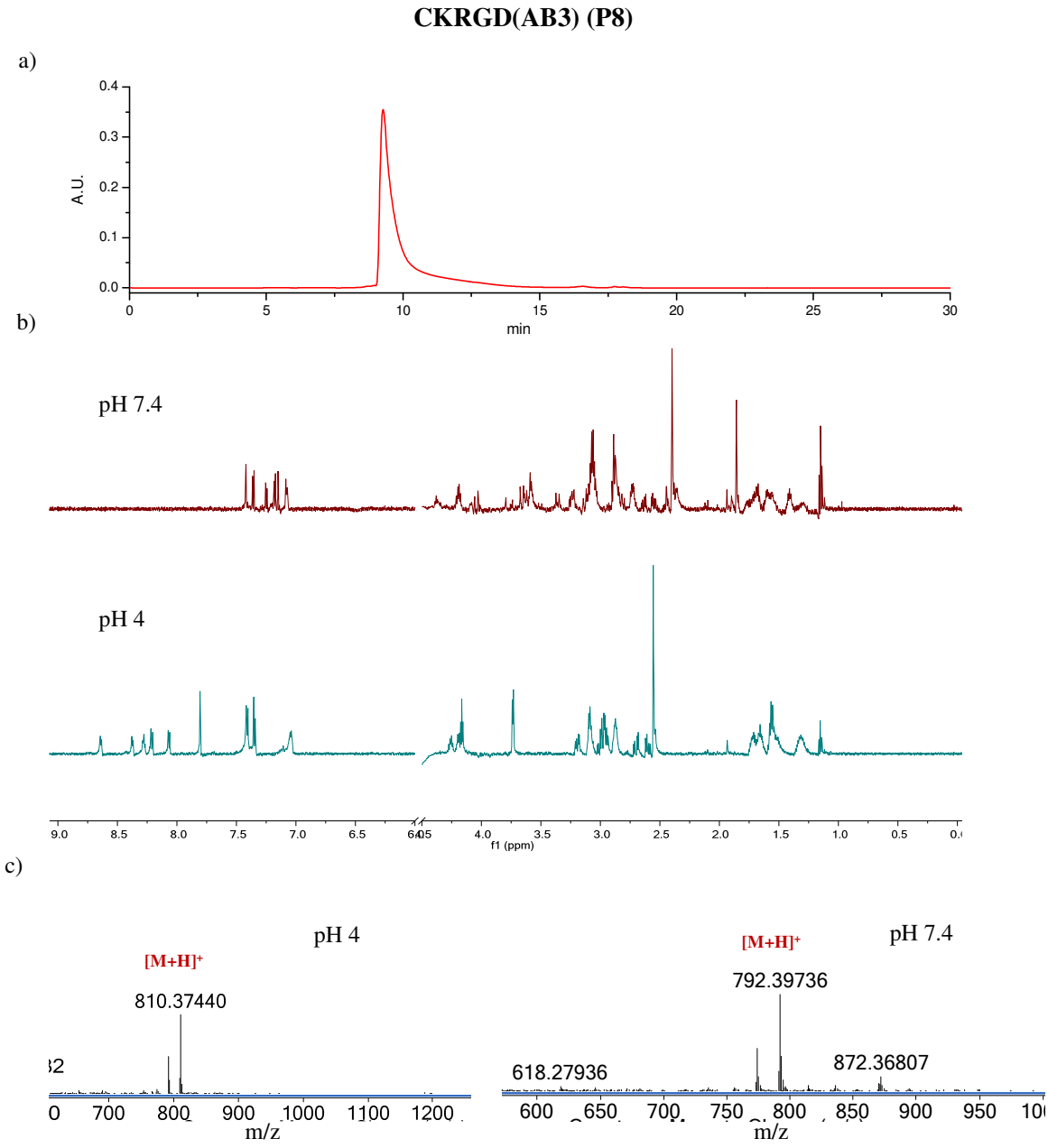


Figure S12. Characterization of P8. a) LC chromatogram; b) ^{1}H-NMR spectra at pH 4 (bottom) and pH 7.4 (top); c) ESI-MS (m/z) spectra at pH 4 (left) and pH 7.4 (right).

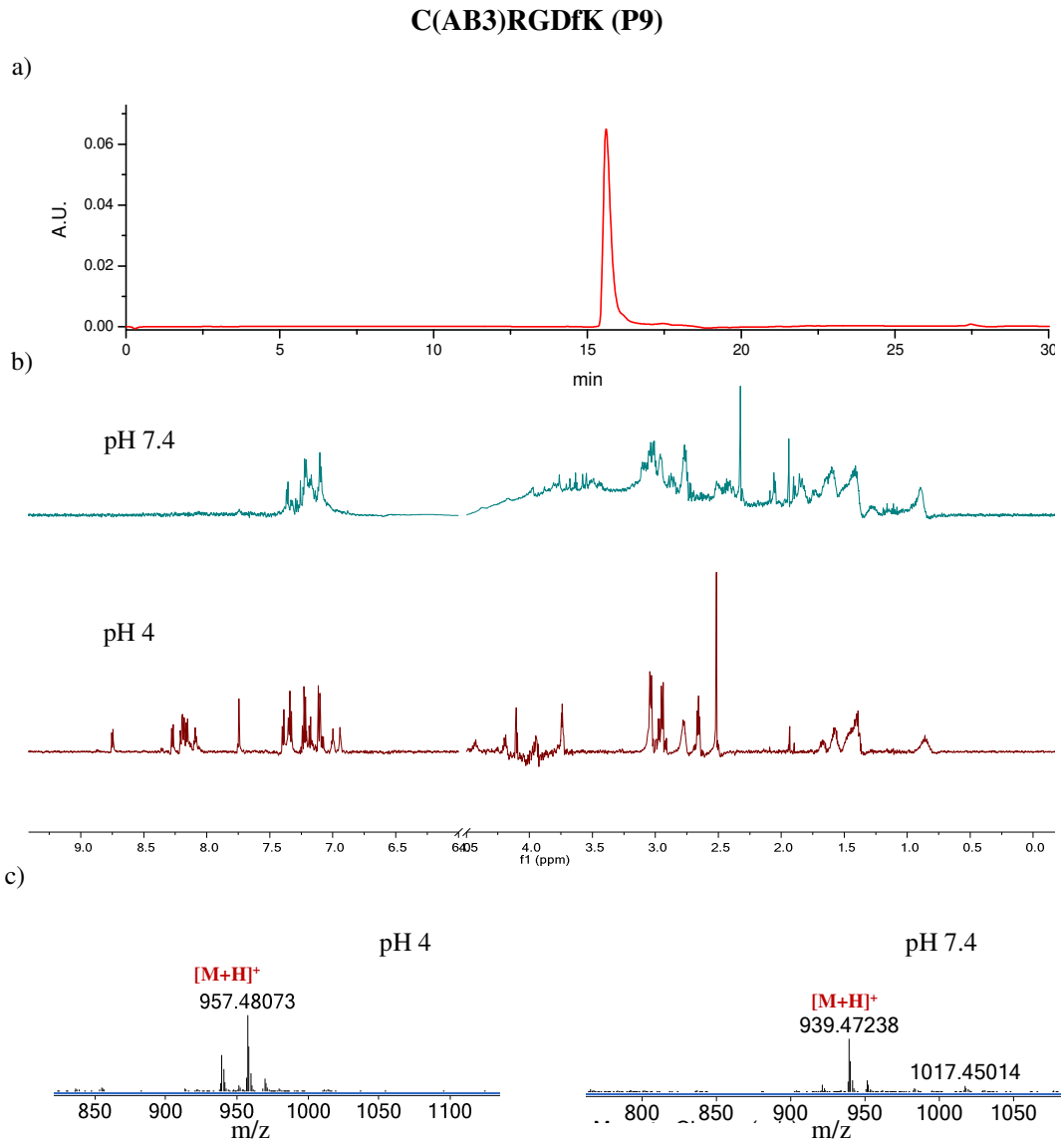


Figure S13. Characterization of P9. a) LC chromatogram; b) ^{1}H-NMR spectra at pH 4 (bottom) and pH 7.4 (top); c) ESI-MS (m/z) spectra at pH 4 (left) and pH 7.4 (right).

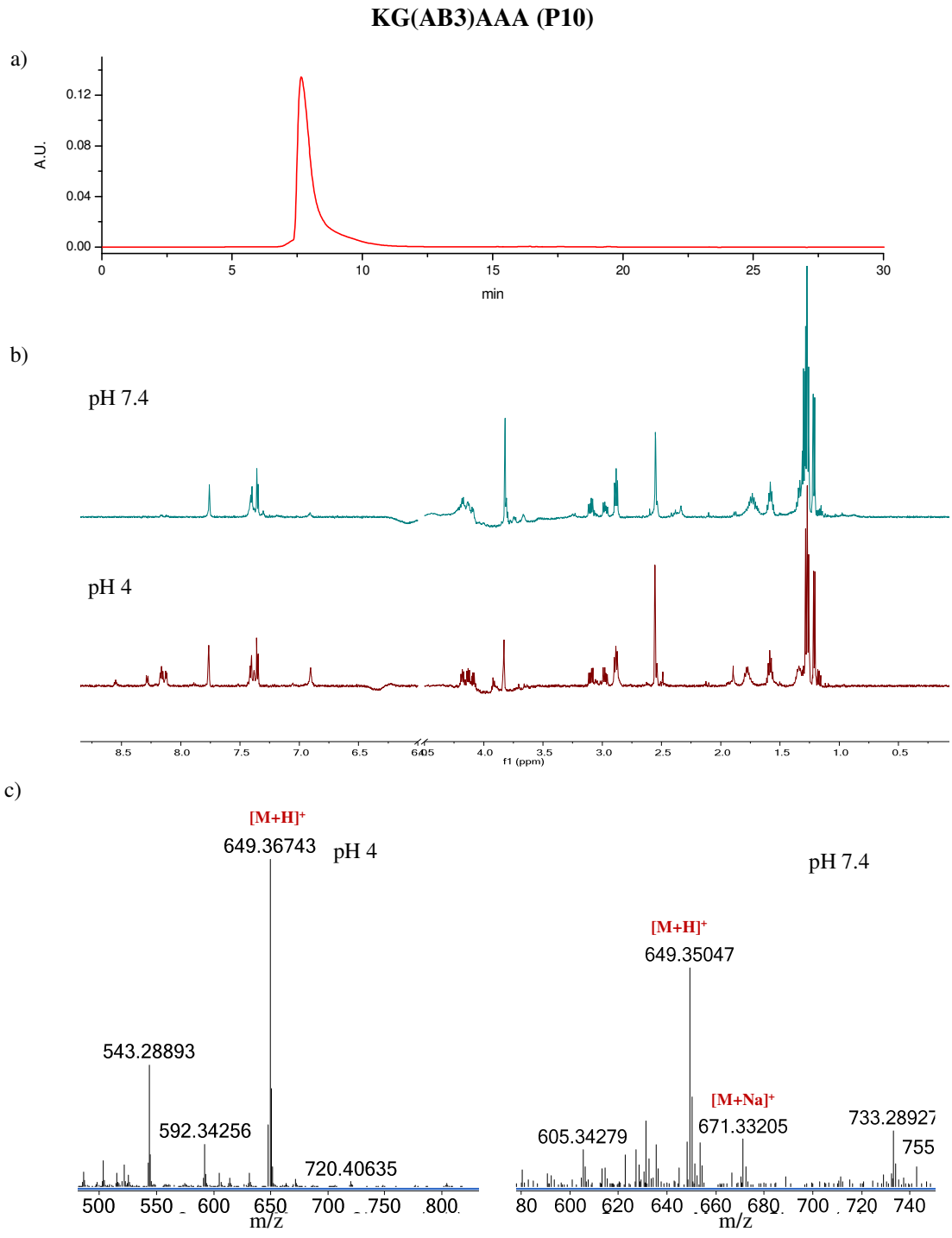


Figure S14. Characterization of P10. a) LC chromatogram; b) ^{1}H-NMR spectra at pH 4 (bottom) and pH 7.4 (top); c) ESI-MS (m/z) spectra at pH 4 (left) and pH 7.4 (right).

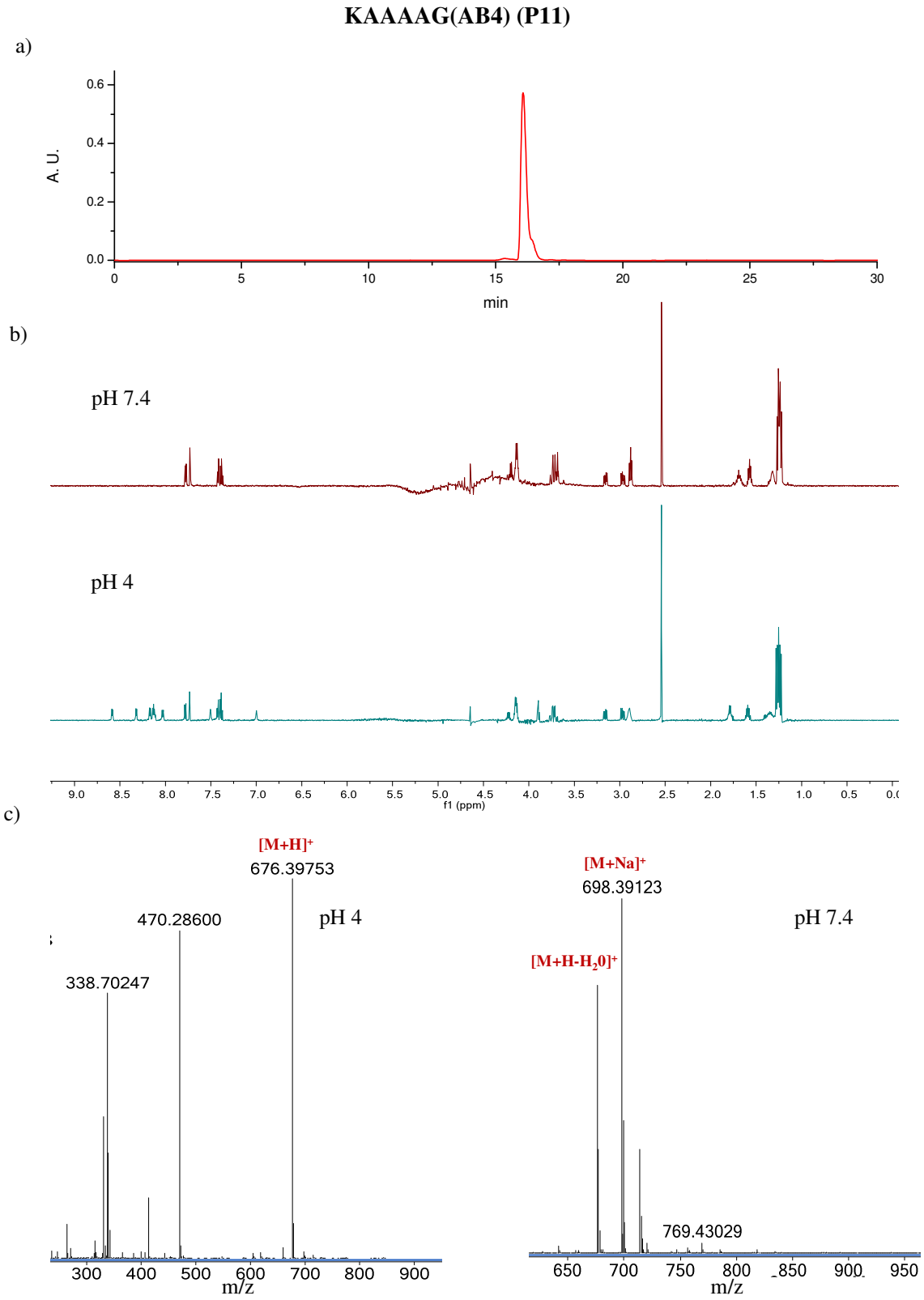


Figure S15. Characterization of P11. a) LC chromatogram; b) ^{1}H-NMR spectra at pH 4 (bottom) and pH 7.4 (top); c) ESI-MS (m/z) spectra at pH 4 (left) and pH 7.4 (right).

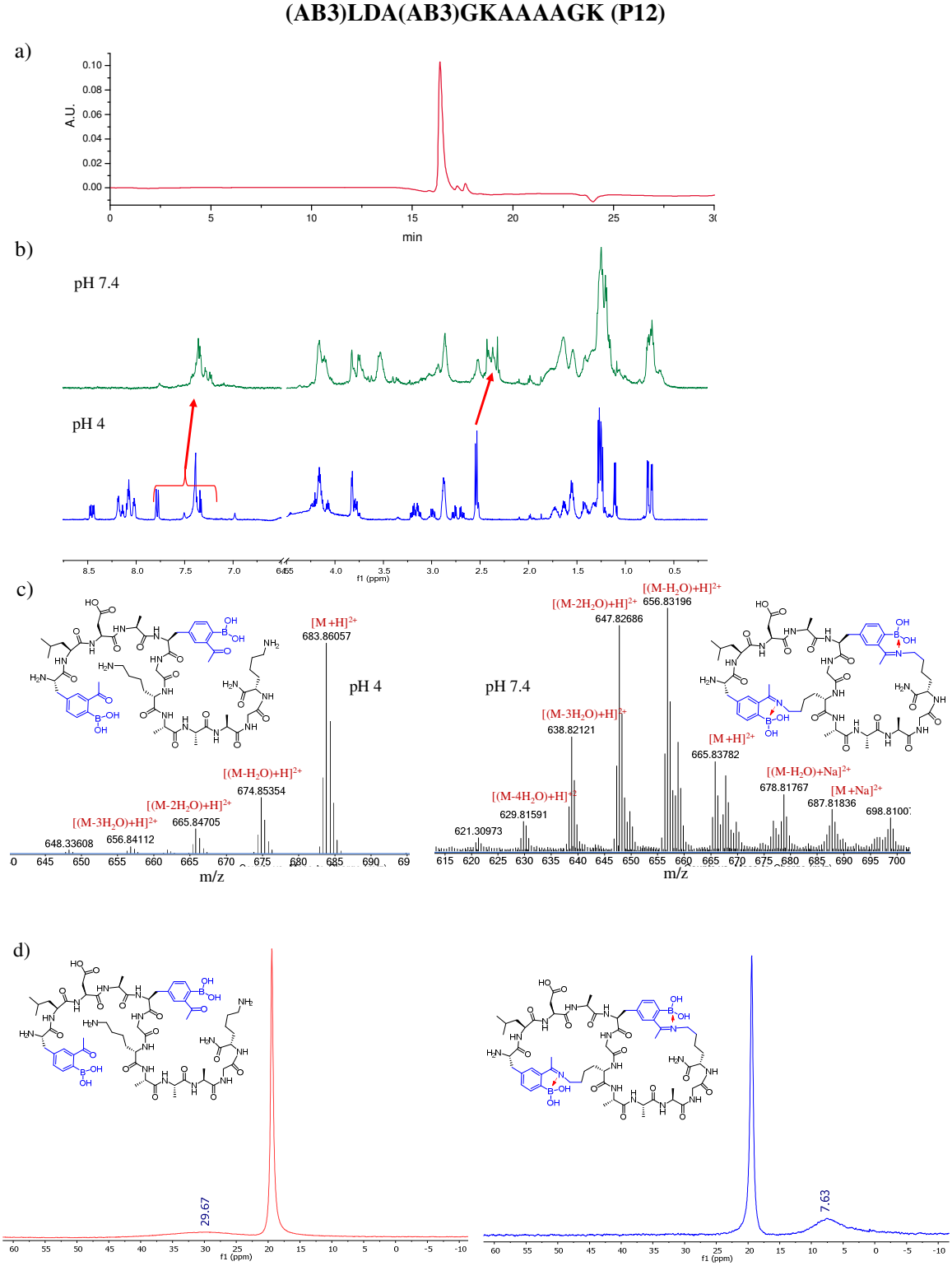


Figure S16. Characterization of P12. a) LC chromatogram; b) ^{1}H-NMR spectra at pH 4 (bottom) and pH 7.4 (top); c) ESI-MS (m/z) and d) ^{11}B-NMR data at pH 4 (left) and 7.4 (right). The 20 ppm peaks in ^{11}B-NMR correspond to boric acid used as internal reference. Zoomed-in versions of the spectra can be seen in Fig. 2 of the main text.

(AB3)NRG(AB3)GKRGDfK (P13)

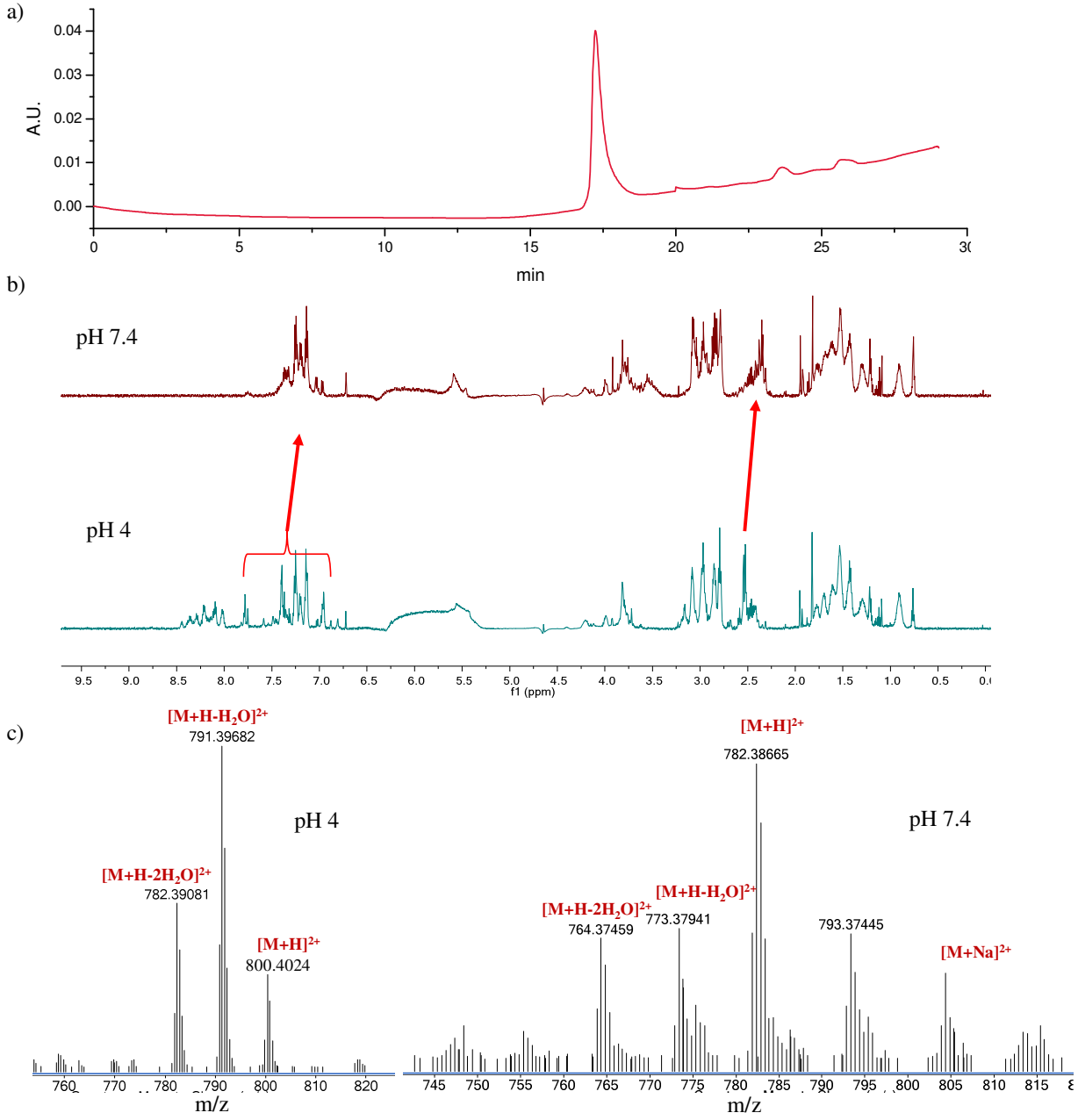


Figure S17. Characterization of P13. a) LC chromatogram; b) ^{1}H-NMR spectra at pH 4 (bottom) and pH 7.4 (top); c) ESI-MS (m/z) spectra at pH 4 (left) and pH 7.4 (right).

V. 2D-NMR structural studies of KAAAAGAB3 (P4)

TOCSY and ROESY experiments were performed with peptide P4 (1 mM in a phosphate buffer with 10% D_2O) at pH 7.8 and 4 respectively using 600 MHz Avance NMR spectrometer at 25 °C. The TOCSY was recorded with the following setting: Scans per t_1 Increment 32 and Spinlock Duration 80 ms. The ROESY was recorded using the parameters: Scans per t_1 Increment 128 and Spinlock Mixing Time 200 ms. The raw data was processed using MestReNova. The analyzed data are shown below.

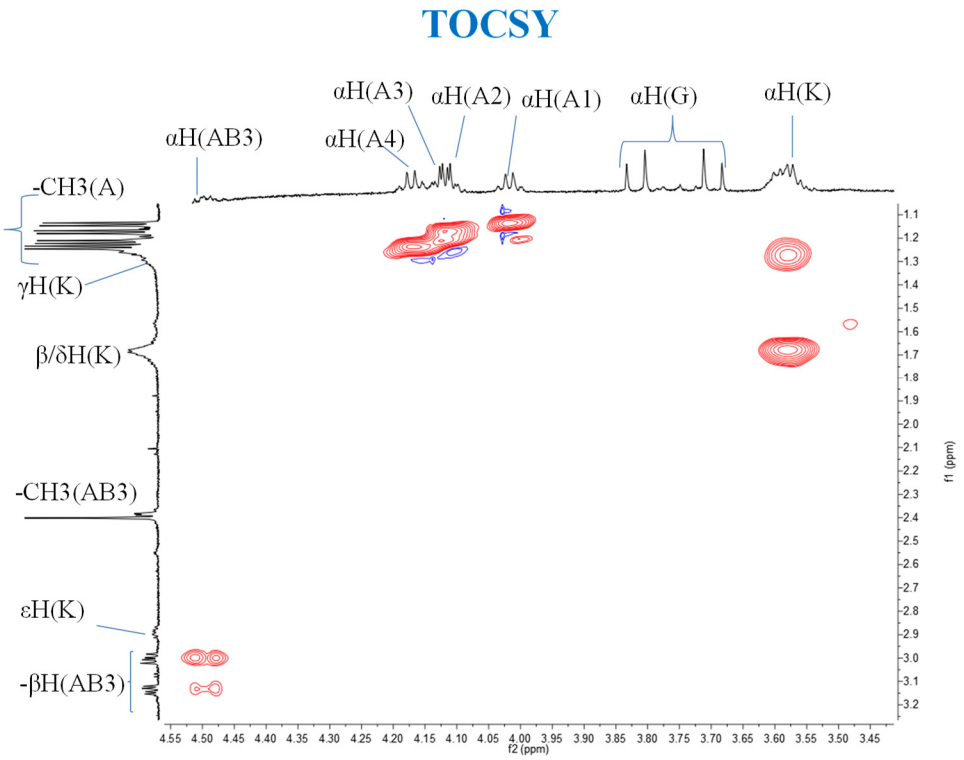


Figure S18. The assigned partial TOCSY spectrum of P4 at pH 7.8.

ROESY

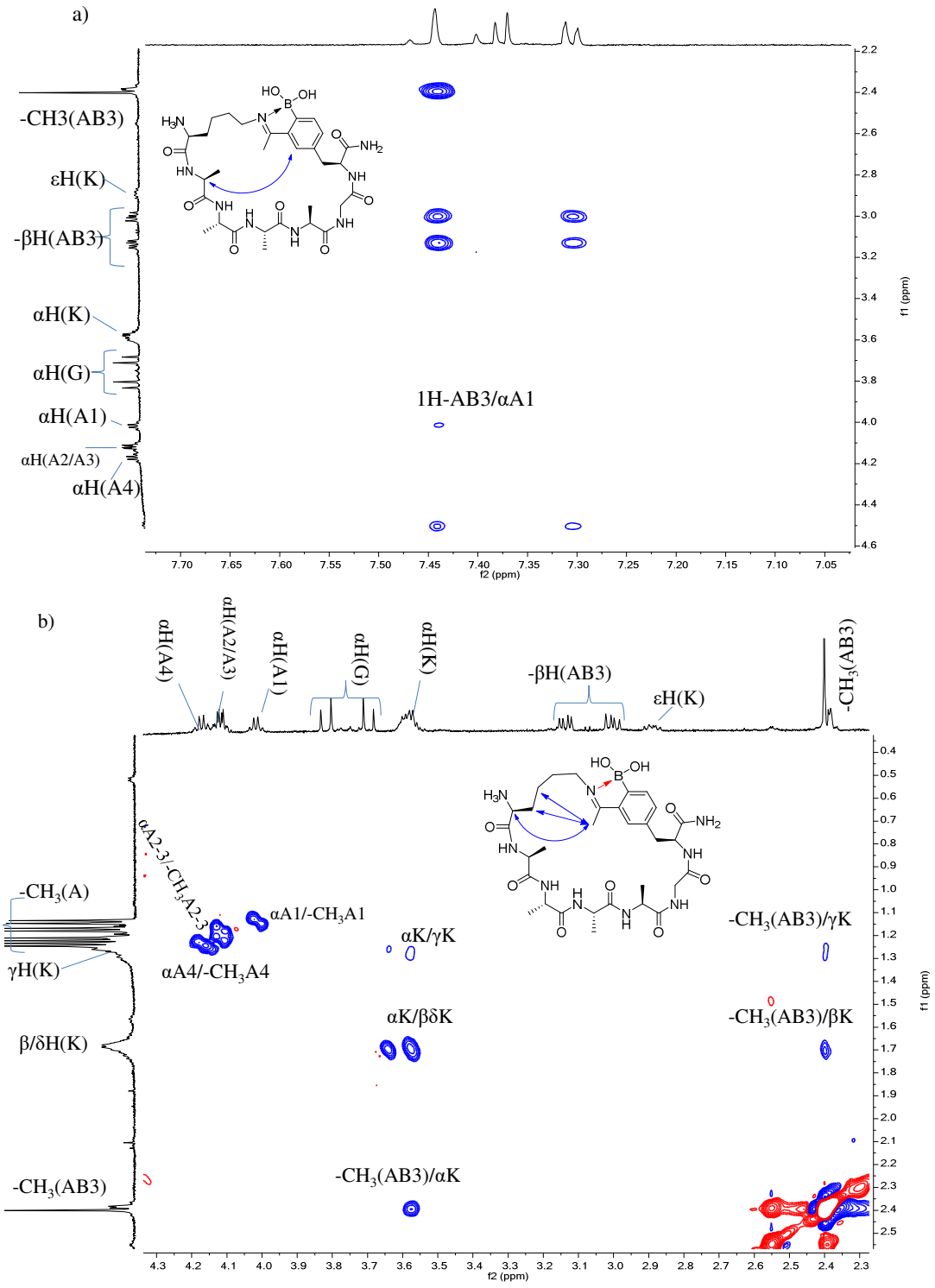


Figure S19. Partial ROESY spectra of P4 at pH 7.8. a) aromatic-H↔α,β,γ,δ and ε-H; b) acetyl -CH_3↔γ,β,α-H.

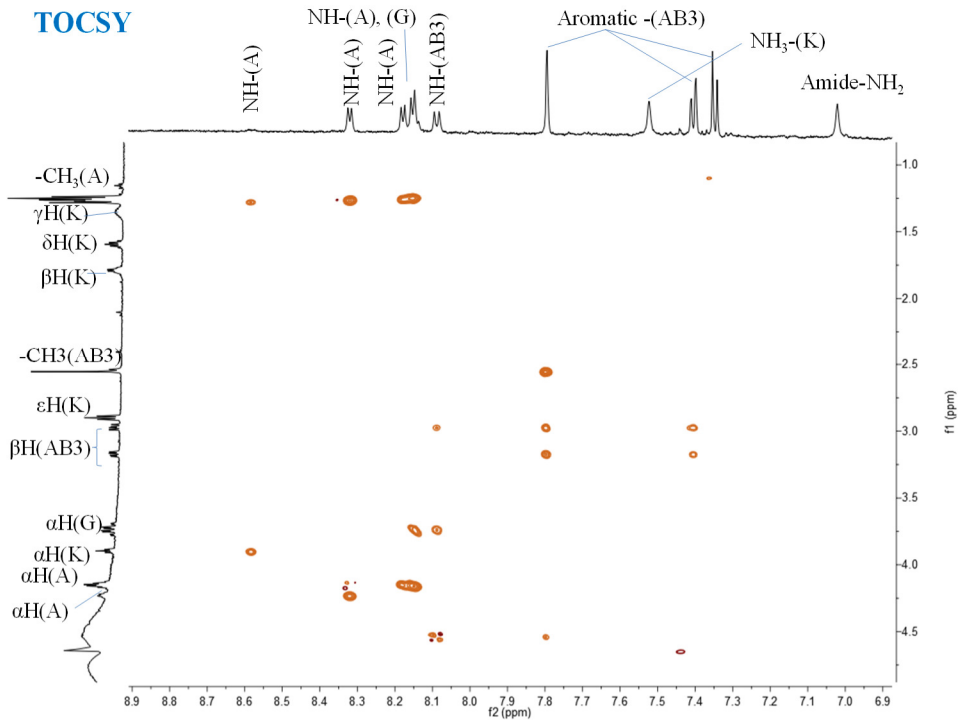


Figure S20. TOCSY spectrum of P4 at pH 4.

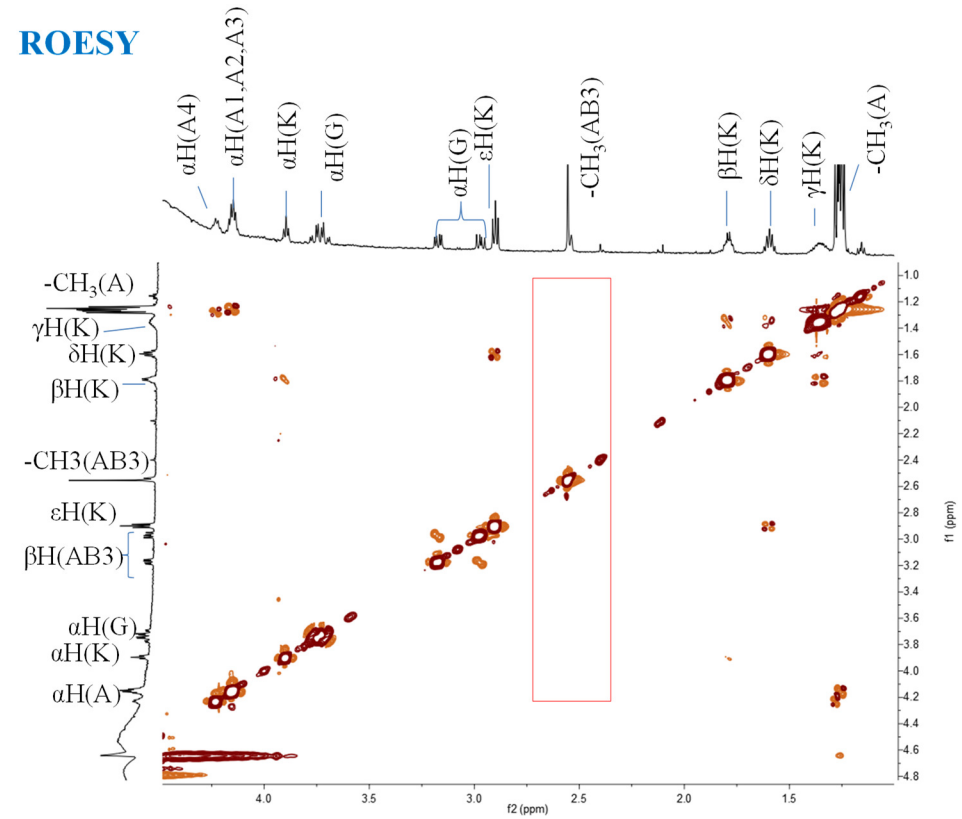


Figure S21. Partial ROESY spectrum of P4 at pH 4. The rectangular box (red) highlights the lack of NOE(s) between acetyl-CH_3 of AB3 residue and lysine residue side chain.

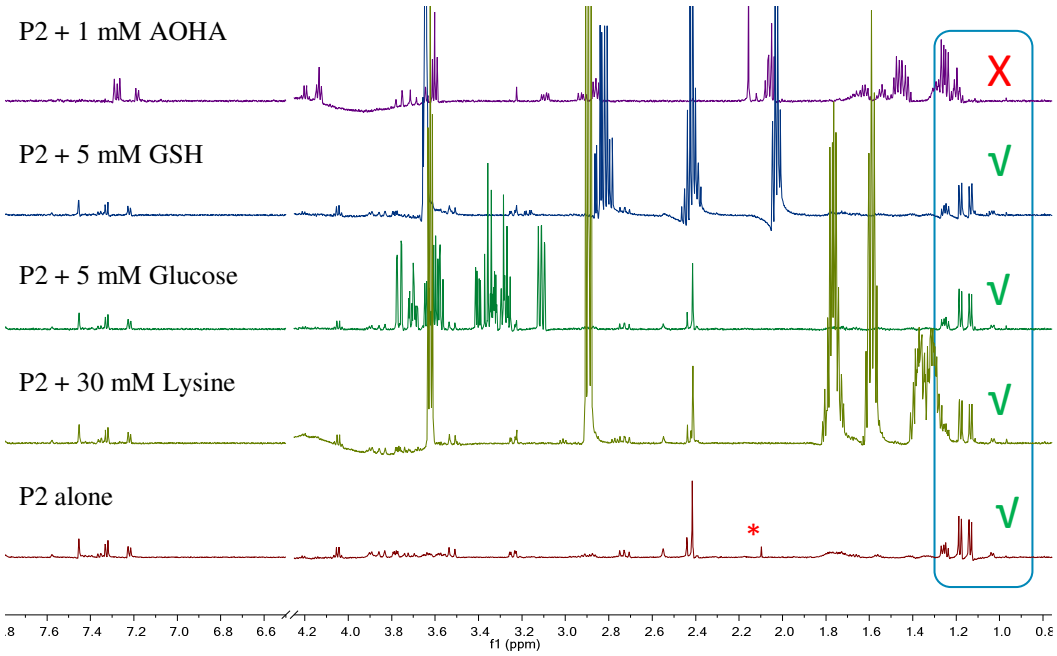
VI. Stability of the cyclic peptides in presence of biomolecules

We investigated the stability of the cyclic peptides or lack thereof in response to a variety of physiologically relevant molecules. Specifically, we examined how the cyclic peptides respond to small molecules like lysine, glucose and glutathione (GSH), proteins like lysozyme and BSA, and blood serum. The potential inhibition effect was not analyzed by monitoring the acetyl -CH_3 group due to overlapping peaks from GSH and AOHA. Instead, we found ^{1}H-NMR spectrum of P2, due to cyclization, exhibits two doublets around 1.15 ppm that correspond to the side chain methyl groups of the two Ala residues. By monitoring these characteristic peaks, the extent of P2 cyclization was assessed in presence of molecules of interest. The results (Figure S22) showed little inhibition of P2 cyclization by lysine even at 30 mM concentrations, highlighting the favorability of intramolecular reactions. Similarly, glucose (5 mM) and glutathione (5 mM) had little effect on the cyclic P2 (Fig. S22). However, when P2 (0.5 mM) was mixed with aminoxy hexanoic acid (AOHA, 1mM), the ^{1}H-NMR spectrum showed an immediate down-field shift of the Ala –CH_3 peaks, suggesting disruption of the cyclic peptide (top spectrum of Fig. S22). It turned out to be challenging to analyze peptide cyclization by ^{1}H-NMR in the presence of lysozyme or BSA, for which a fluorescence polarization assay was enlisted (Fig. S23). Towards this end, we labeled the RGD peptide P8 with Alexa Fluoro 488 (AF488). Upon mixing with BSA, AF488-P8 showed little anisotropy increase, indicating no BSA binding for this peptide. In contrast, the AF488-labeled amino acid AB3 elicited a pronounced anisotropy increase as AB3 is expected to label BSA via iminoboronate formation with surface lysines. Essentially the same results were obtained when lysozyme or fetal bovine serum (FBS, 10%) was used in this fluorescence polarization assay (Fig. S23b-c). Further, mass spectrometry analysis showed no modification of lysozyme when it was mixed with the peptide P2, while incubation with the amino acid AB3 caused complete disappearance of the mass-spec peaks of the protein (Fig. S24).

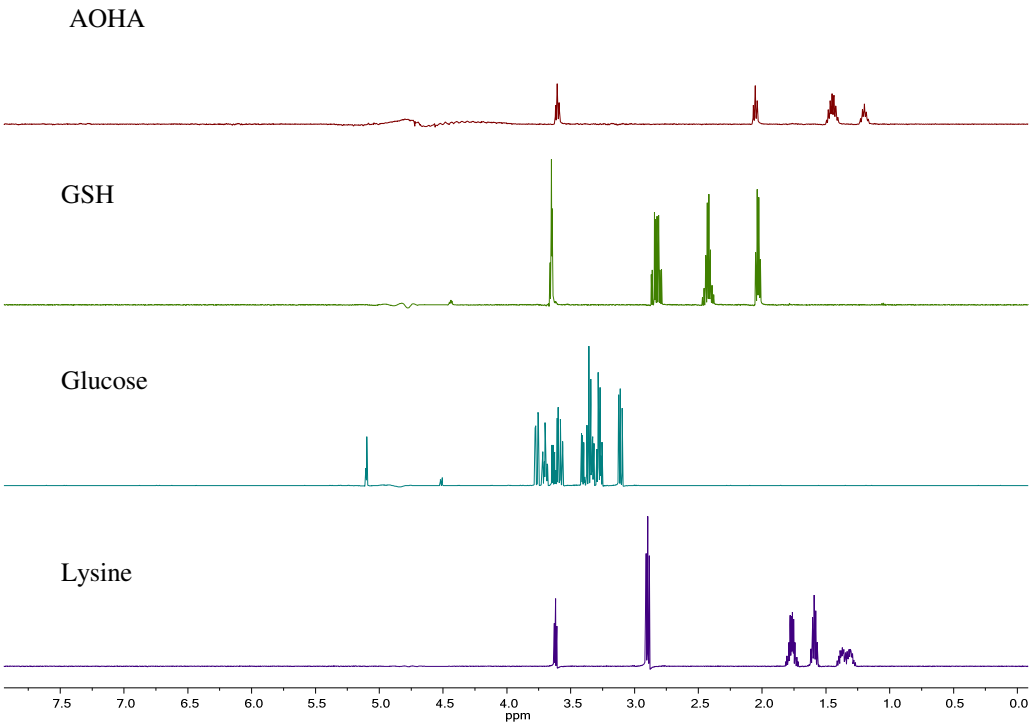
VIa. Small molecule inhibition experiments by NMR

100 µL stock solution of P2 (3 mM in PBS, pH 7.4) was diluted to 400 µL PBS buffer (1x, 10% D_2O). The solution was mixed with 100 µL stock solution of lysine (180 mM), glucose (30 mM) or glutathione (30 mM). The pH of final mixtures (volume 600 µL) was tuned to 7.4 using 0.5N NaOH and/or 0.5N HCl. The spectra were obtained using a 600 MHz Avance NMR spectrometer at 25 ^{o}C with 64 scans. The data were processed and stacked using MestReNova.

a)


P2 + 1 mM AOHA
X
P2 + 5 mM GSH
√
P2 + 5 mM Glucose
√
P2 + 30 mM Lysine
√
P2 alone
*
√
f1 (ppm)

b)


AOHA
GSH
Glucose
Lysine
ppm

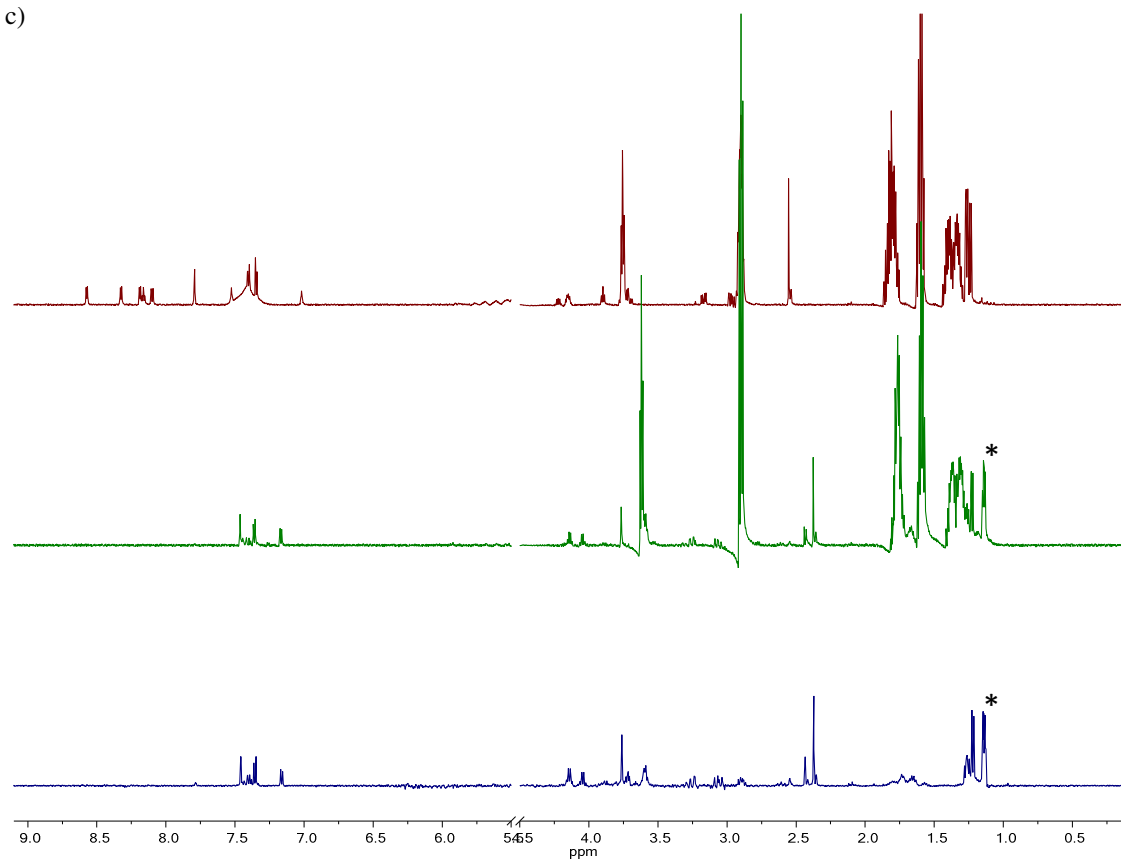


Figure S22. a) Response of an iminoboronate-cyclized peptide P2 to biological and exogenous small molecules. *The small singlet peak at 2.1 ppm of P2 alone originated from residue acetone in the NMR tube and is not related to the peptide. b) Stacked ^{1}H-NMR spectra of biological and exogenous small molecules used for inhibition experiments in a). c) NMR Spectra of a P3-lysine mixture at pH 4 (top) and neutralized to pH 7.4 (middle). The spectrum of P3 alone (bottom) is included for comparison. The fact that the middle spectrum shows signature of cyclic P3 (denoted by *) indciates the the peptide cyclization is favored thermodynamically over formation of intermolecular iminoboronates.

VIb. BSA, Lysozyme, serum inhibition experiments

Fluorescence polarization assay

BSA (0.1, 0.5, 1, 5 and 10 mg/mL), lysozyme (0.05, 0.25, 0.5, 1 and 5 mg/mL) and FBS (1, 2, 5 and 10%) were incubated with 0.5 µM of AF488-P8 for 30 min in PBS buffer (1×, pH = 7.4) at room temperature. Similarly, 0.5 µM of AF488-AB3 was incubated with BSA, lysozyme and FBS as a control. Then the fluorescence anisotropy values of each sample were recorded. All samples were measured as triplicates. The averaged data were subtracted with the anisotropy value of AF488-P8 (or AF488-AB3) alone and plotted against protein concentration. The binding curves were fitted to a hyperbola equation using Origin 8. To assess the peptide's stability in human serum, the peptide P9 (100 µM) was incubated

in 10% human serum for 1 hr. Then the sample was analyzed by HPLC and compared to the peptide without serum incubation. The HPLC analysis was performed by monitoring the UV-Vis absorption at 254 nm, where the AB3 residue absorbs.

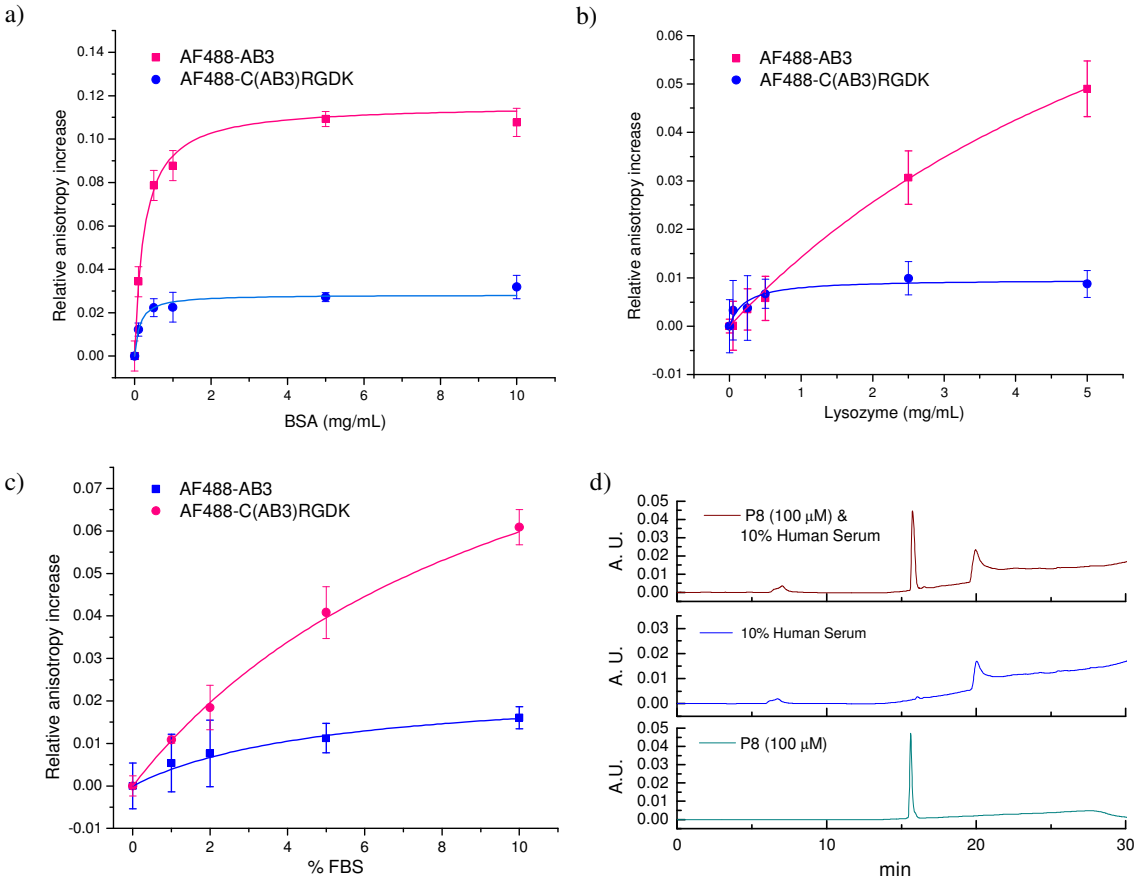
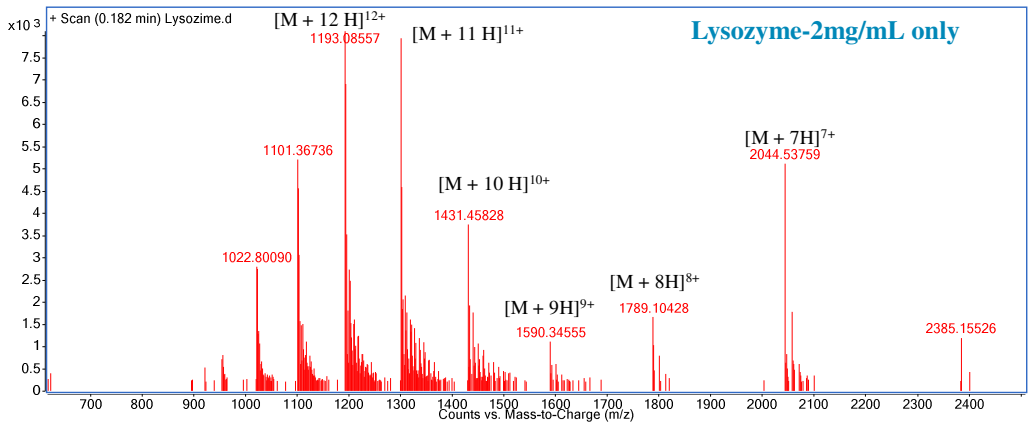


Figure S23. Fluorescence polarization experiments showing that AF488-P8 does not bind to a) BSA, b) lysozyme and c) 10% fetal bovine serum (FBS). d) HPLC analysis demonstrating that the peptide P9 stays intact after 1 hr incubation with human serum.

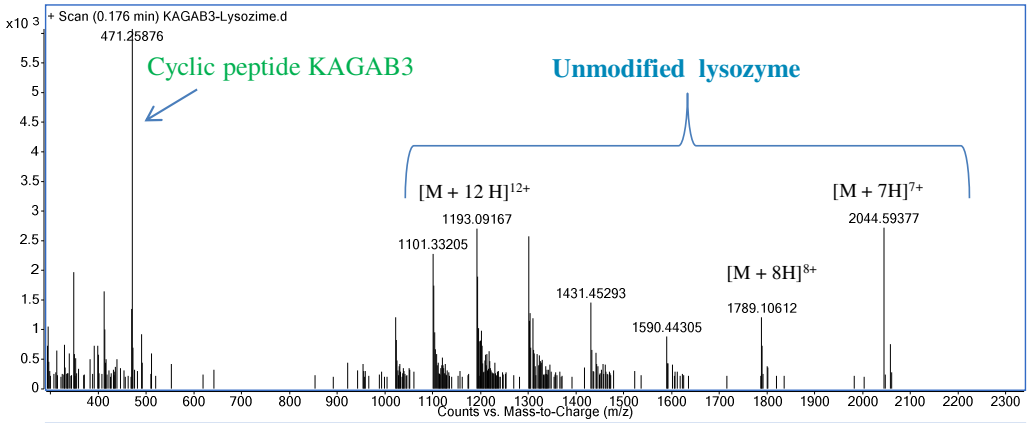
ESI-MS analysis

Peptides P1-P3 (~100 μM) were incubated with lysozyme (2 mg/mL) in PBS (pH 7.4) for 30 min at room temperature. Then 5 μL of the peptide solution was subjected to mass-spec analysis using Agilent 6230 LC/MS TOF mass spectrometer. The TOF mass spectrometer was operated in the positive mode with the set up of Fragmenter 200 V, Skimmer 65V and OCT 1 RF Vpp 750 V. A 1:1 mixture of acetonitrile and ammonium acetate buffer (pH 7) was used as the mobile phase at 0.2 mL/min. The samples were loop injected without using a column. For comparison, the same analysis was done for the mixture of AB3 (100 μM) and lysozyme (2mg/mL).

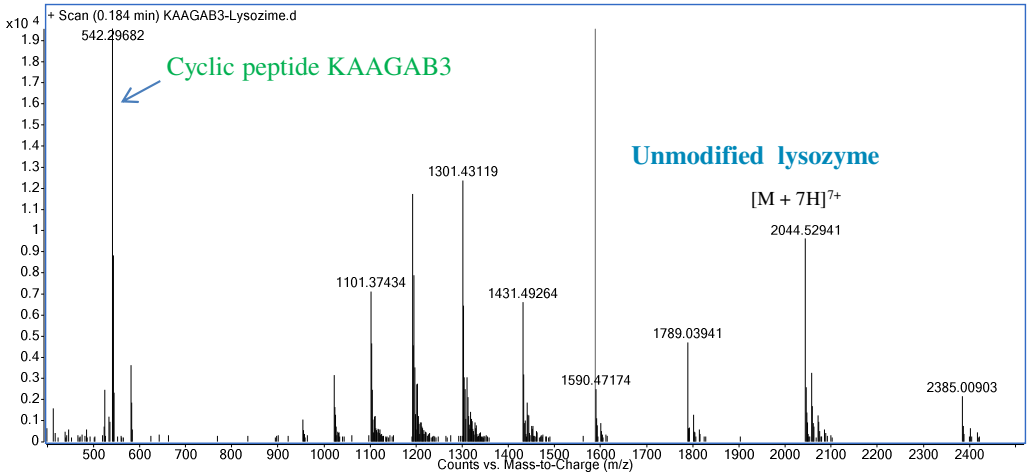
a)


+ Scan (0.182 min) Lysozime.d
x10 3
[M + 12 H]12+
1193.08557
[M + 11 H]11+
Lysozyme-2mg/mL only
1101.36736
[M + 7H]7+
2044.53759
[M + 10 H]10+
1431.45828
1022.80090
[M + 8H]8+
[M + 9H]9+
1789.10428
1590.34555
2385.15526
Counts vs. Mass-to-Charge (m/z)

b)


+ Scan (0.176 min) KAGAB3-Lysozime.d
x10 3
471.25876
Cyclic peptide KAGAB3
Unmodified lysozyme
[M + 12 H]12+
[M + 7H]7+
1193.09167
2044.59377
1101.33205
[M + 8H]8+
1431.45293
1789.10612
1590.44305
Counts vs. Mass-to-Charge (m/z)

c)


+ Scan (0.184 min) KAAGAB3-Lysozime.d
x10 4
542.29682
Cyclic peptide KAAGAB3
Unmodified lysozyme
1301.43119
[M + 7H]7+
2044.52941
1101.37434
1431.49264
1789.03941
1590.47174
2385.00903
Counts vs. Mass-to-Charge (m/z)

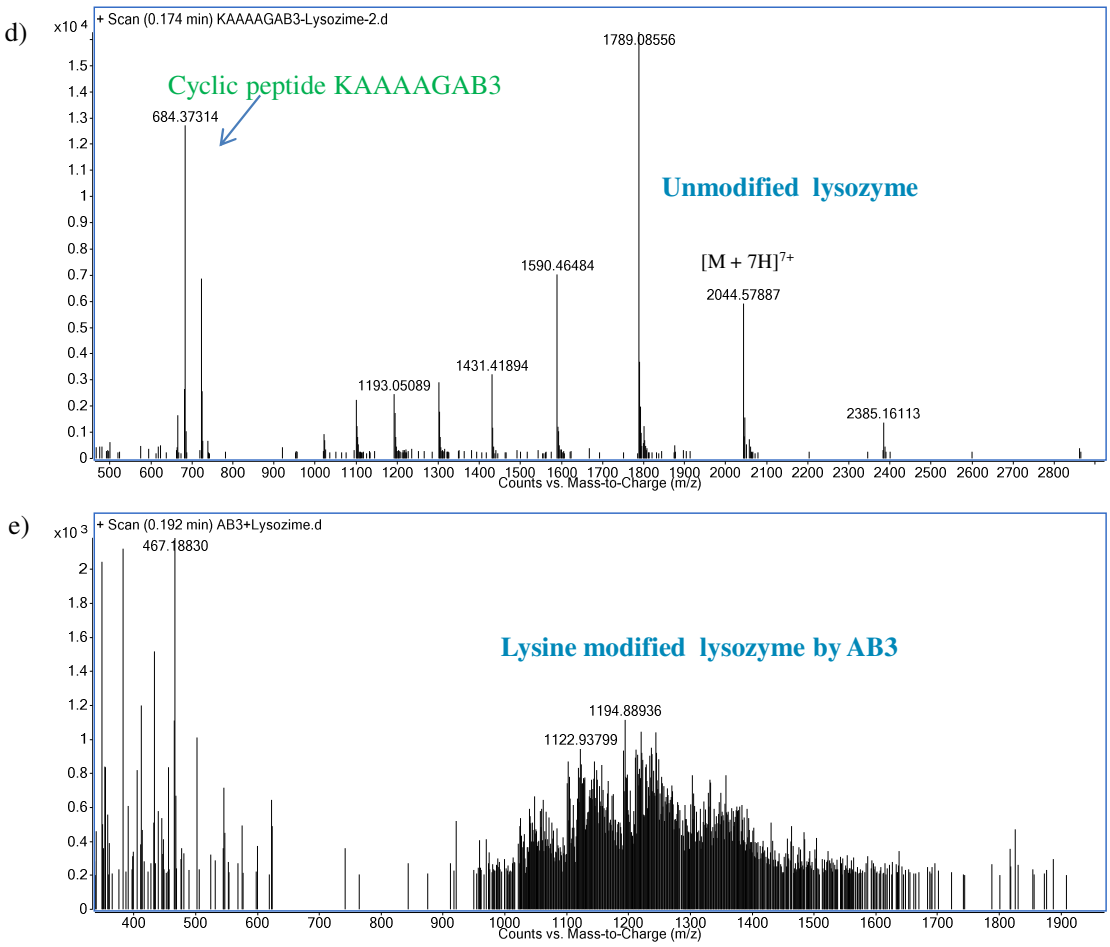


Figure S24. Mass-spec analysis of lysozyme incubated with cyclic peptides P1-P3 or with the amino acid AB3. a) lysozyme alone; b) with P1; c) with P2; d) with P3; e) with the amino acid AB3. The lysozyme peaks remained unchanged in presence of the peptides, but disappeared in presence of AB3. This is presumably because AB3 modifies the surface lysines of lysozyme.

VII. Assessing the response of peptide cyclization to various stimuli

pH titration experiment by NMR: The pH titration experiments were performed with P4 (0.5 mM) in a phosphate buffer with 10% D_2O. The desired pH was tuned using 0.5N NaOH and/or 0.5N HCl and final pH was confirmed by a micro pH meter. The ^{1}H-NMR spectra were recorded on a 600 MHz Avance NMR spectrometer at 25 ^oC with 64 scans. The data were processed and stacked using MestReNova10.0.2. For better visualization, a break between 4.5 to 6 ppm is generated using MestReNova10.0.2 to skip the suppressed water peak. The percentage of cyclic product was determined by integrating the chemical shift of –CH3 of acetyl group present in AB3 residue. No data were suppressed in that region.

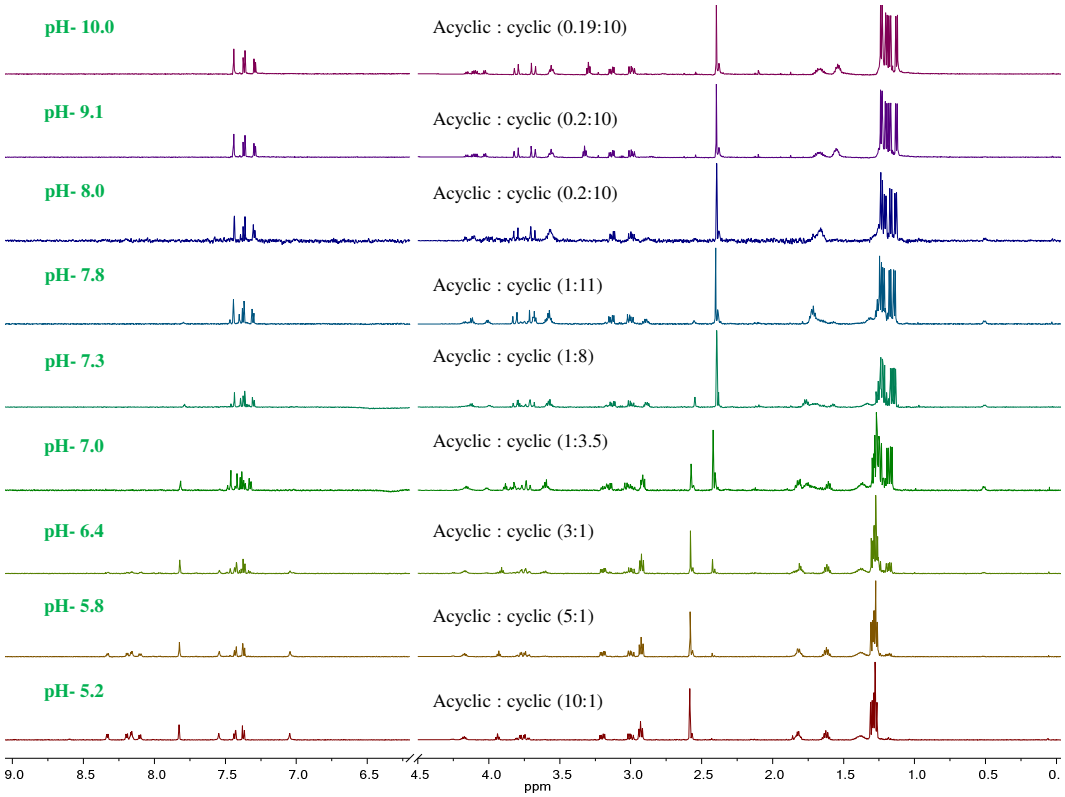


Figure S25. Stacked ^{1}H-NMR spectra of P4 at varied pH values highlighting the sensitivity of the cyclic peptide towards acidification.

Hydrogen peroxide oxidation: A solution of AF488-P8 (50 µL, 20 µM in 1x PBS, pH 7.4) was mixed with H_2O_2 (400 µM, 50 µL) and incubated for 24 hrs. The sample was then analyzed by using HPLC and ESI-MS. There was no significant change observed in the LC-traces or in the mass spec data. The data are given below.

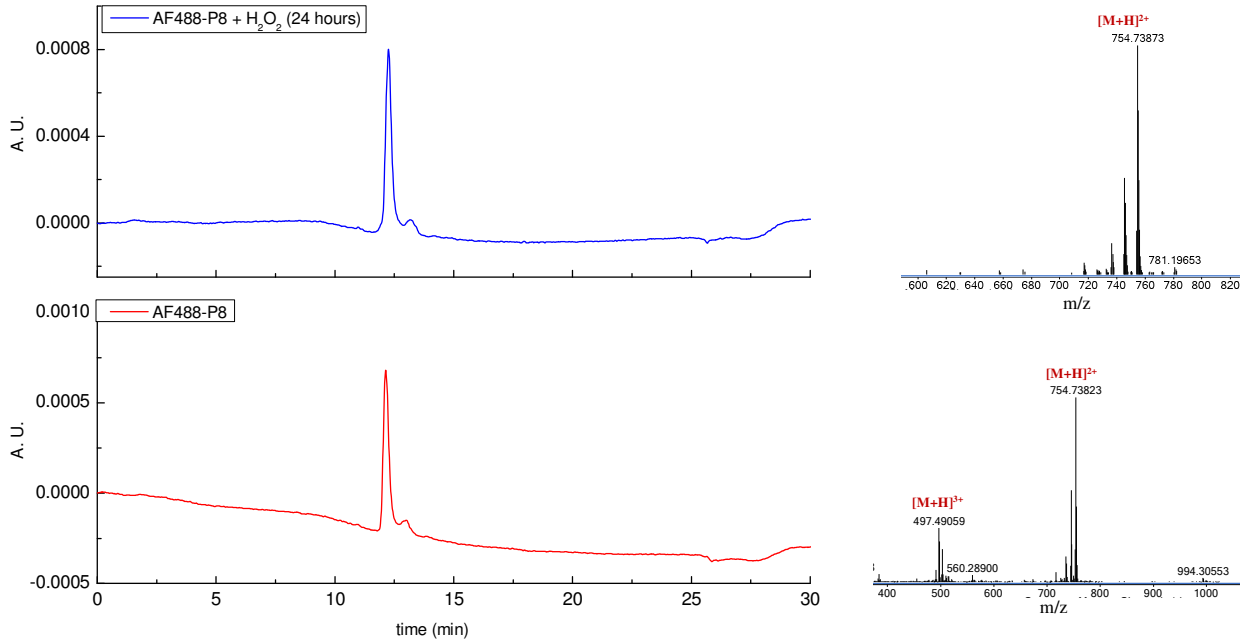
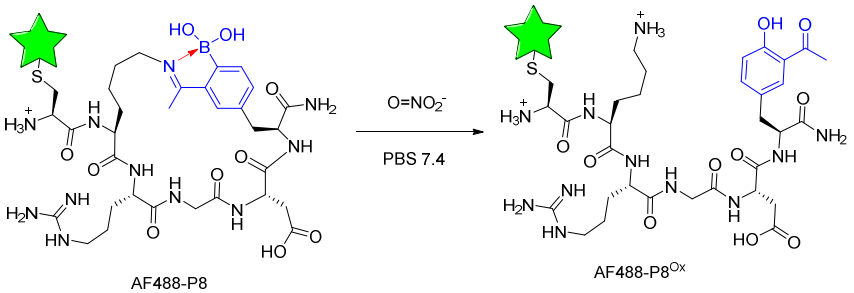


Figure S26. LC traces and ESI-MS data of AF488-P8, before (bottom) and after (top) treatment with H_2O_2 (200 µM).

Peroxynitrite oxidation: A solution of AF488-P8 (50 µL, 20 µM in 1x PBS, pH 7.4) was mixed with that of peroxynitrite ($O=N-O-O^-$) (20 µM, 50 µL) and incubated for 10 min. The sample was then analyzed by using LC-MS. A clean conversion was observed without any side product. The LC-chromatograms of the reaction are given in main text Figure 4c. The corresponding mass data of the product are given below.



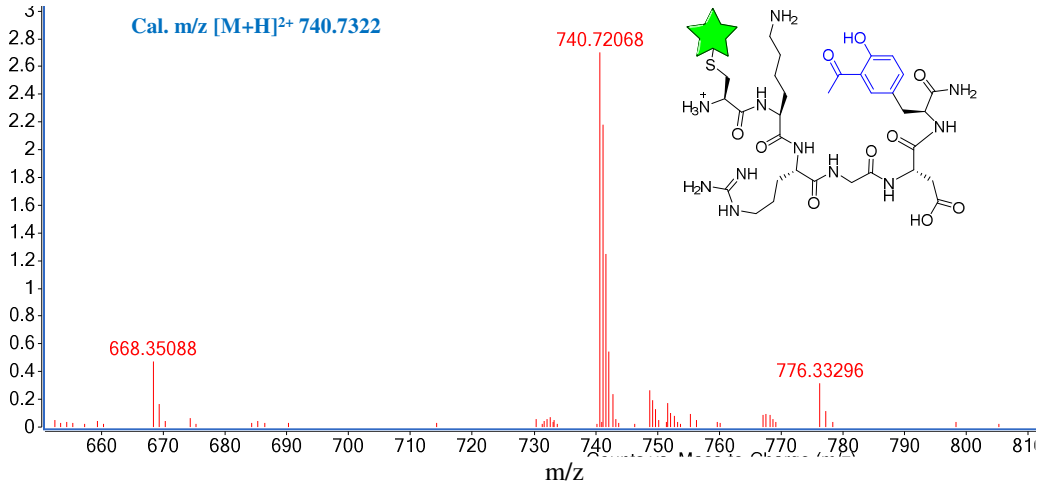


Figure S27. The mass spectrum of peroxynitrite-treated AF488-P8.The observed m/z peaks correspond to the linear oxidized product.

Phenylhydrazine cleavage: A solution of AF488-P8 (50 µL, 10 µM in 1× PBS, pH 7.4) was mixed with that of phenylhydrazine (Phzn, 11 µM, 50 µL) and incubated for ~10 minutes.[3] Then the sample was subjected to LC-MS analysis. A complete conversion was observed without any side product.

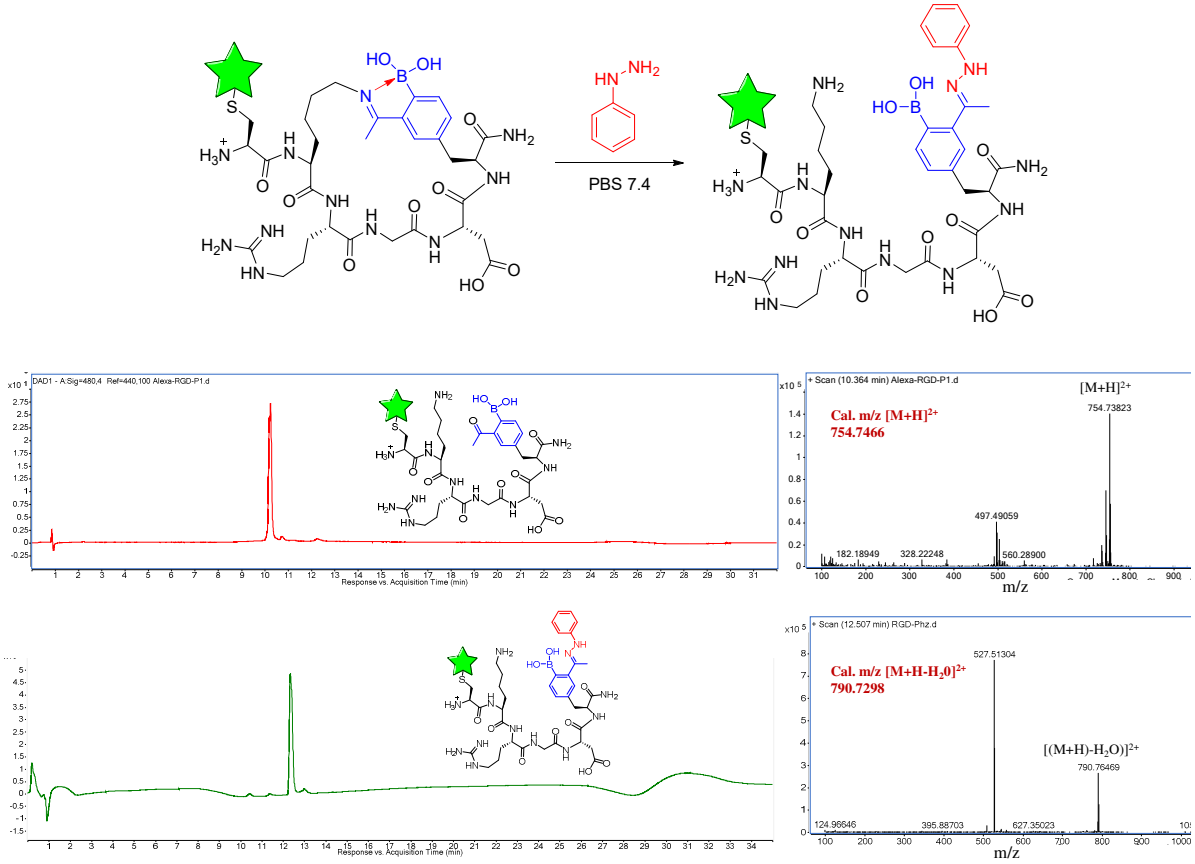


Figure S28. LC-MS data of AF488-P8 before (top) and after (bottom) cleavage by phenylhydrazine.

VIII. SKOV3 cell culture and staining experiment[4]

SKOV3 cells were grown and maintained in RPMI 1640 media with 10% FBS and 1% penicillin/streptomycin at 37 ºC, 5% CO_2 and passed for less than 50 generations. Before staining experiment with peptides, the cells (1.0–2.0 $\times 10^6$, 20 µL) were seeded on the surface of MatTek glass bottom microwell dishes (35 mm Petri dish, 14 mm microwell, No. 1.5 coverglass with thickness 0.16-0.19 mm) using 2 mL media. After 4 days, the cells were washed twice with warm binding buffer (20 mM Tris, pH = 7.4, 150 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$, and 0.1% bovine serum albumin) and then incubated at 37°C with suitable 10 µM of peptide (AF488-P9, AF488-P9L, AF488-P14) in binding buffer for 90 minutes. For binding study at acidic pH at 6.0, the binding buffer was tuned to pH 6 using 0.1 N HCl and the buffer was used for the integrin binding study with AF488-P8 and AF488-P13. After incubation for 90 minutes, the cells were washed three times with ice-cold binding buffer. Images were taken using a Leica SP5 confocal fluorescence microscope with filters that allowed detection of AF488 (488 nm excitation, 496–564 nm emission). A × 20 air objective was used with an Argon laser at 10% laser power. The gain was adjusted to 1000 HV with an offset of -0.5%. The images were captured with the software LAS 2.6 and then the brightness and contrast were adjusted with Fiji ImageJ[5] by using the same set of parameter for all the images.

IX. General procedure for reduction of iminoboronate linkage

A peptide solution (10 µM in PBS, pH 7.4) was incubated with 5 mM of $NaCNBH_3$ for 10 min at room temperature. Then the reaction mixture was quenched with 2N HCl and directly characterized using LC-MS without further purification. For NMR analysis of the reduced product, the peptide P8 (1 mM in PBS with 10% D_2O) was subjected to 10 mM $NaCNBH_3$ for 10 min at room temperature in an NMR tube. The reaction mixture was quenched with 2N HCl (30 µL) before characterization by NMR (Fig. S28). The quencehed sample was found to give a pH around 4.

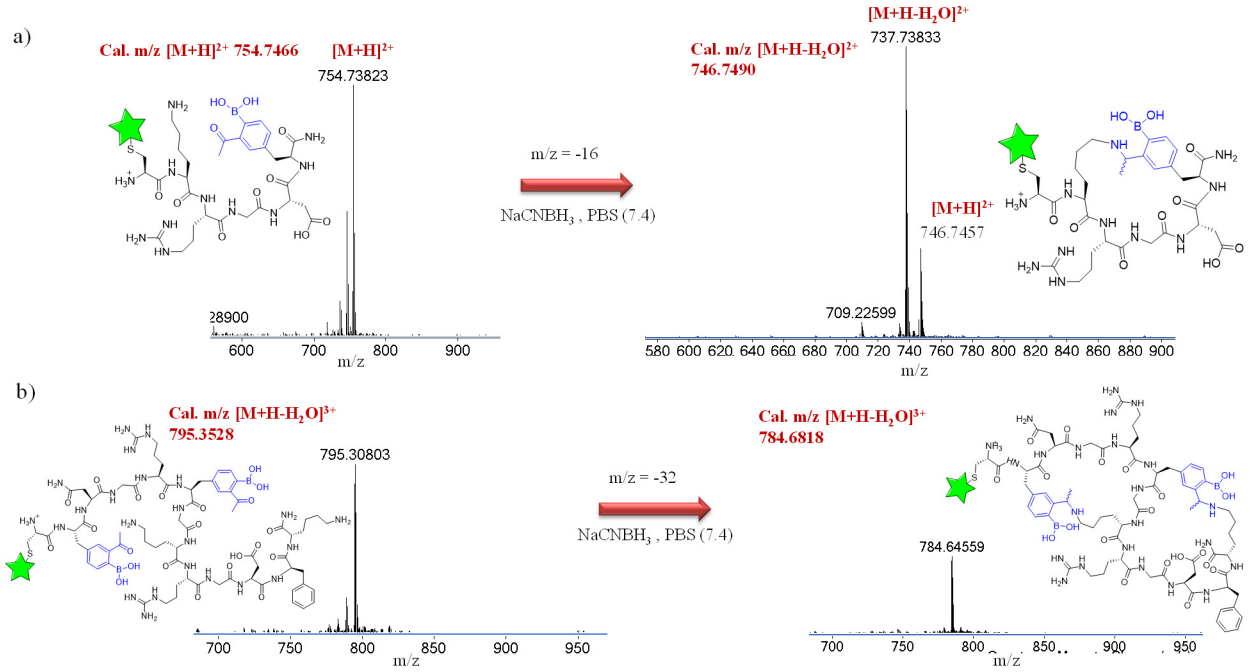


Figure S29. Mass-spec analysis of AF488-P8 (a) and AF488-P13 (b) before (left) and after (right) reduction of the iminoboronate linkage.

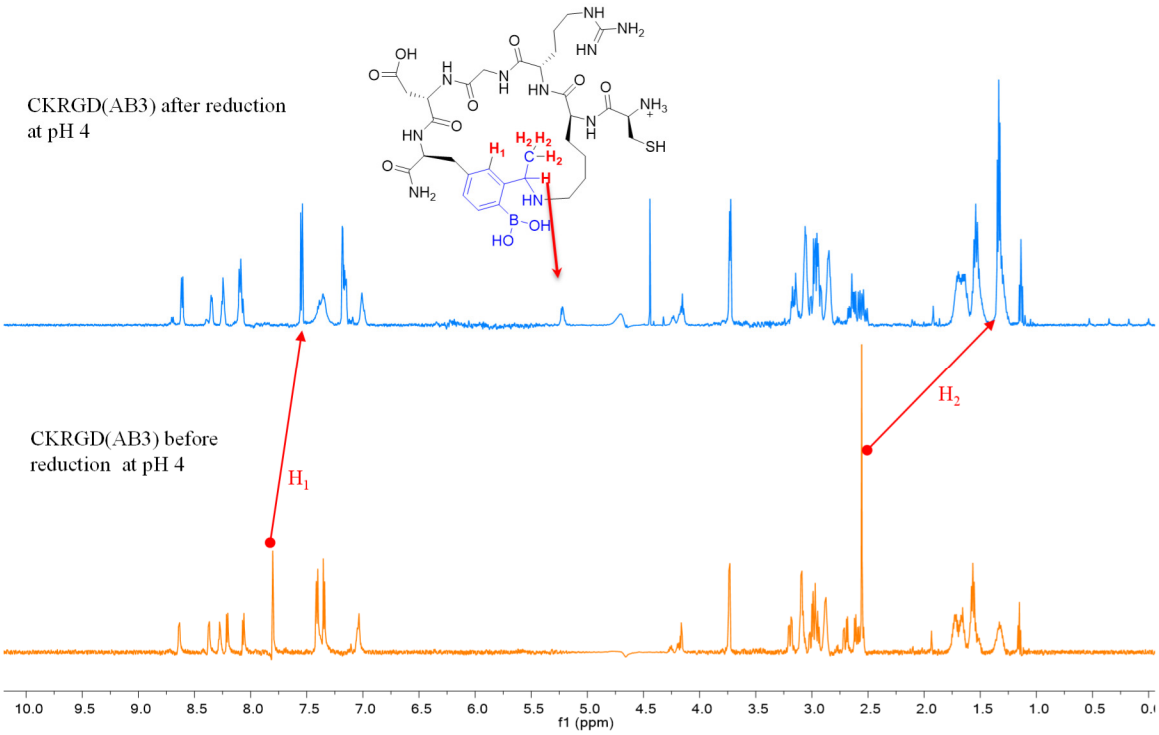
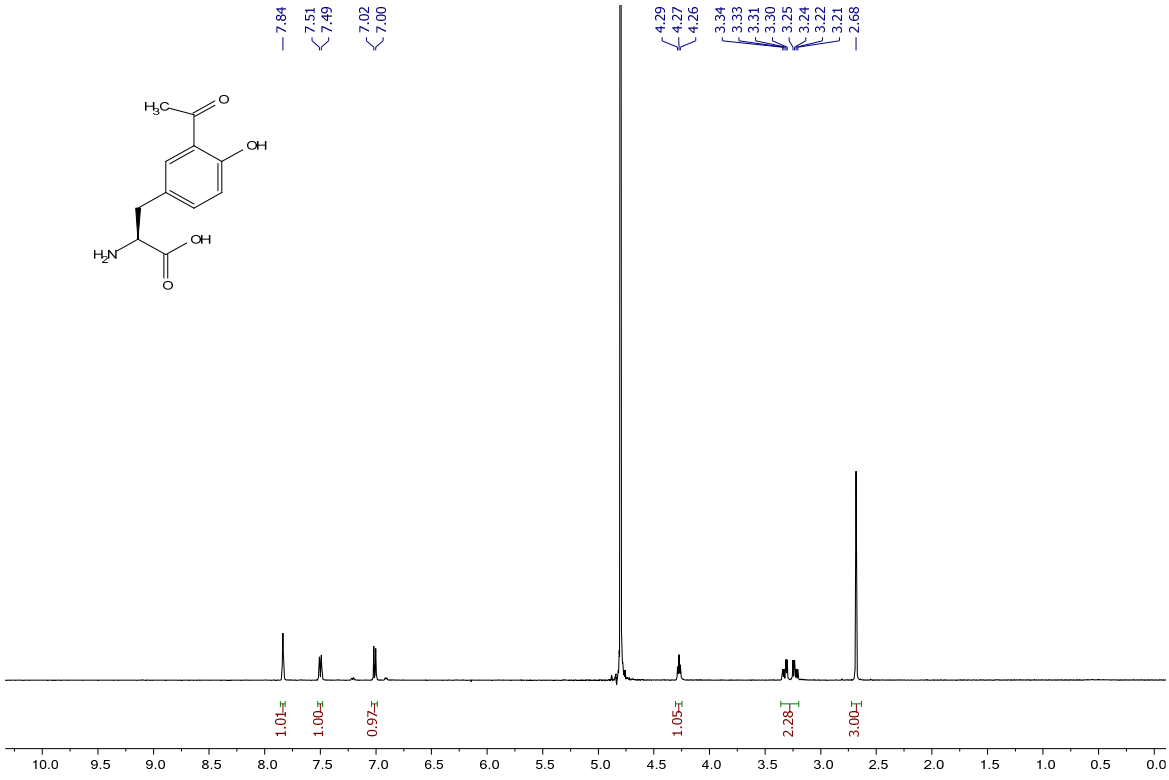


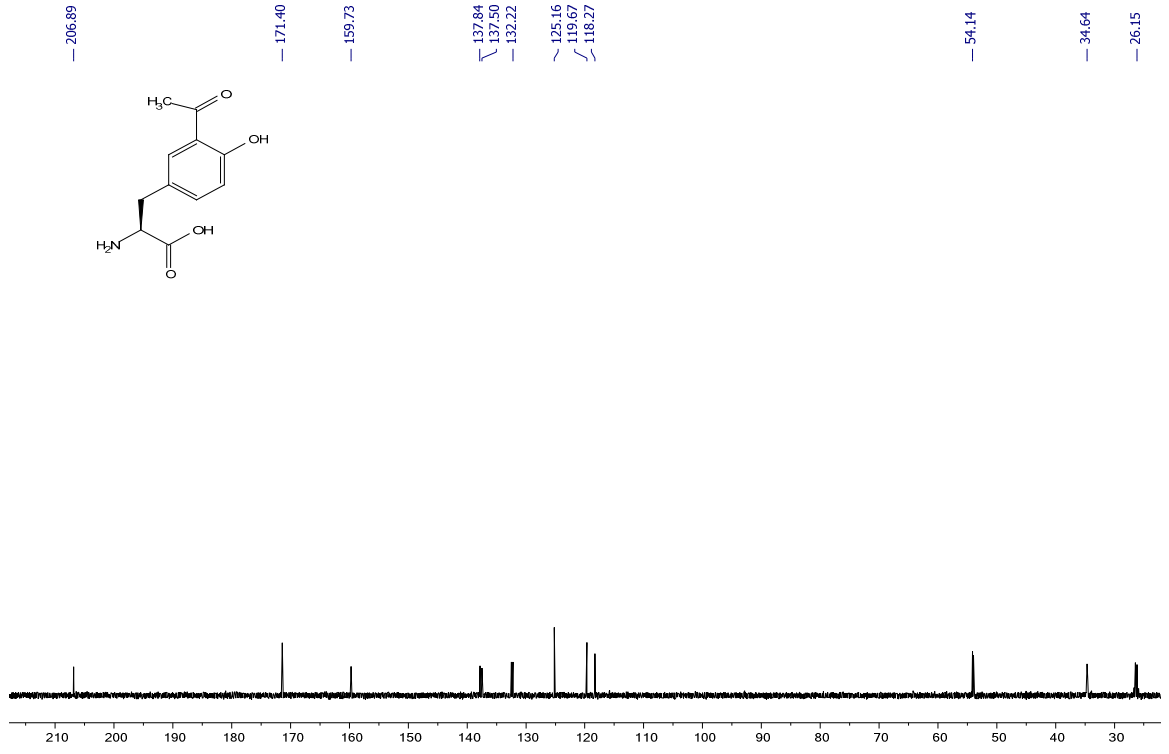
Figure S30. NMR analysis of P8 before reduction (bottom) at pH 4 and after reduction (top) by treatment of $NaCNBH_3$.

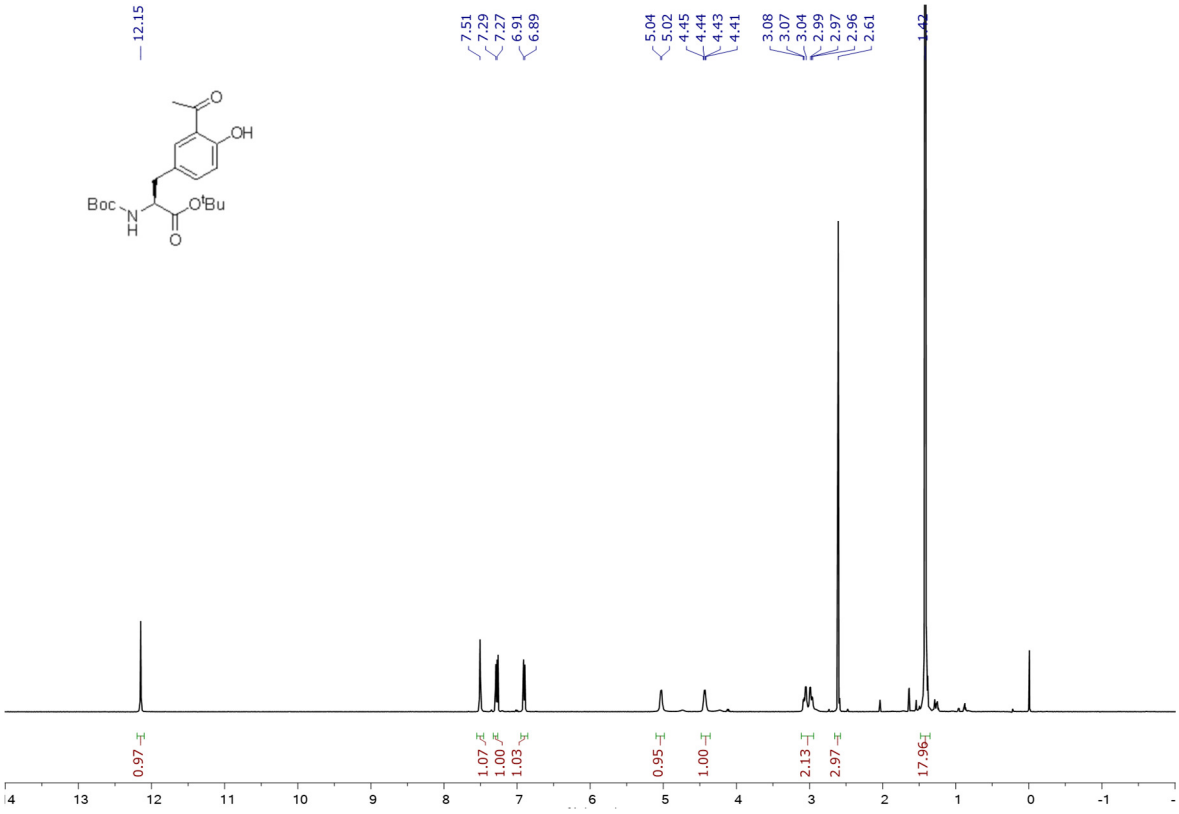
X. References

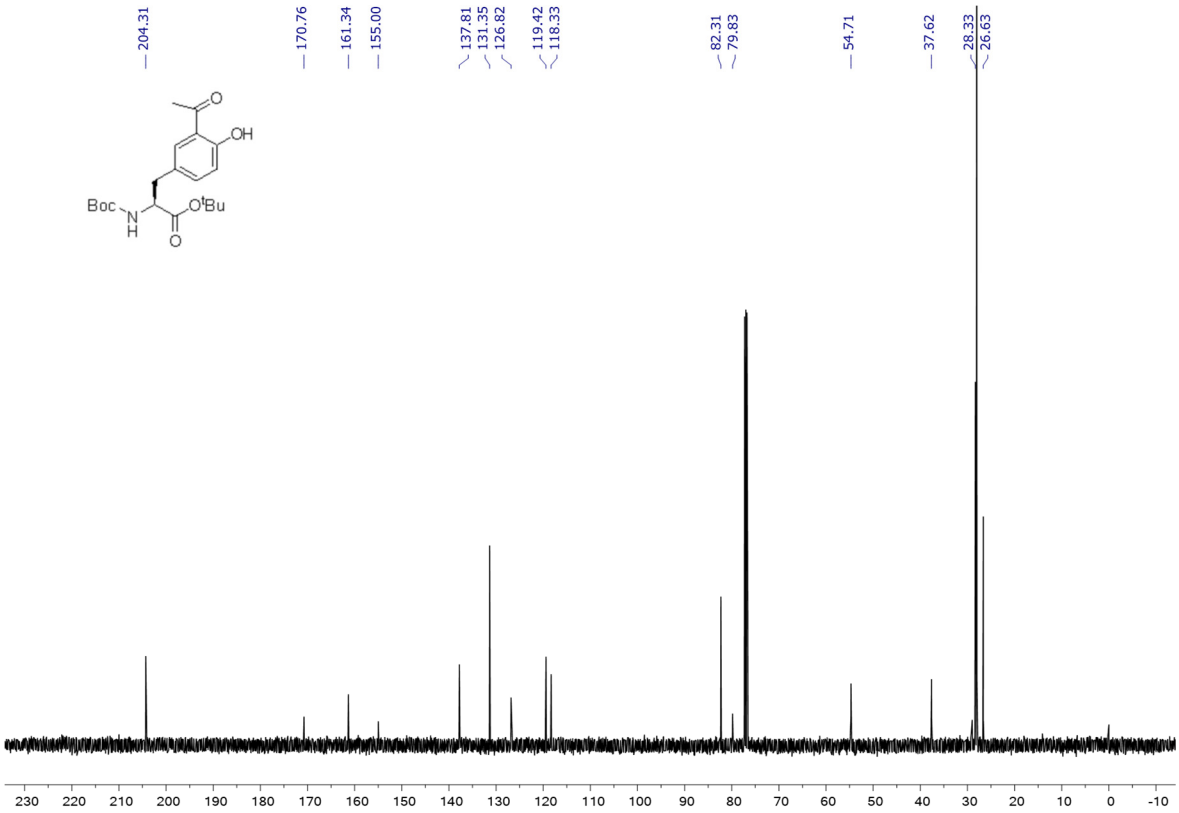
1) C. Chen, Y-F. Zhu and K. Wilcoxen, *J. Org. Chem.* **2000**, *65*, 2574-2576.
2) A. Bandyopadhyay, K. MaCarthy, M. Kelly and J. Gao, *Nat. Commun.* **2015**, *6*, 6561.
3) A. Bandyopadhyay, and J. Gao, *Chem. -Euro. J.*, **2015**, *21*, 14748-14752.
4) Y. Wu, W. Cai, and X. Chen*, Mol. Imaging Biol.* **2006**, *8*, 226–236.
5) J. Schindelin, et al. Fiji: an open-source platform for biological-image analysis. *Nat. Methods*, 2012, 9, 676-682.

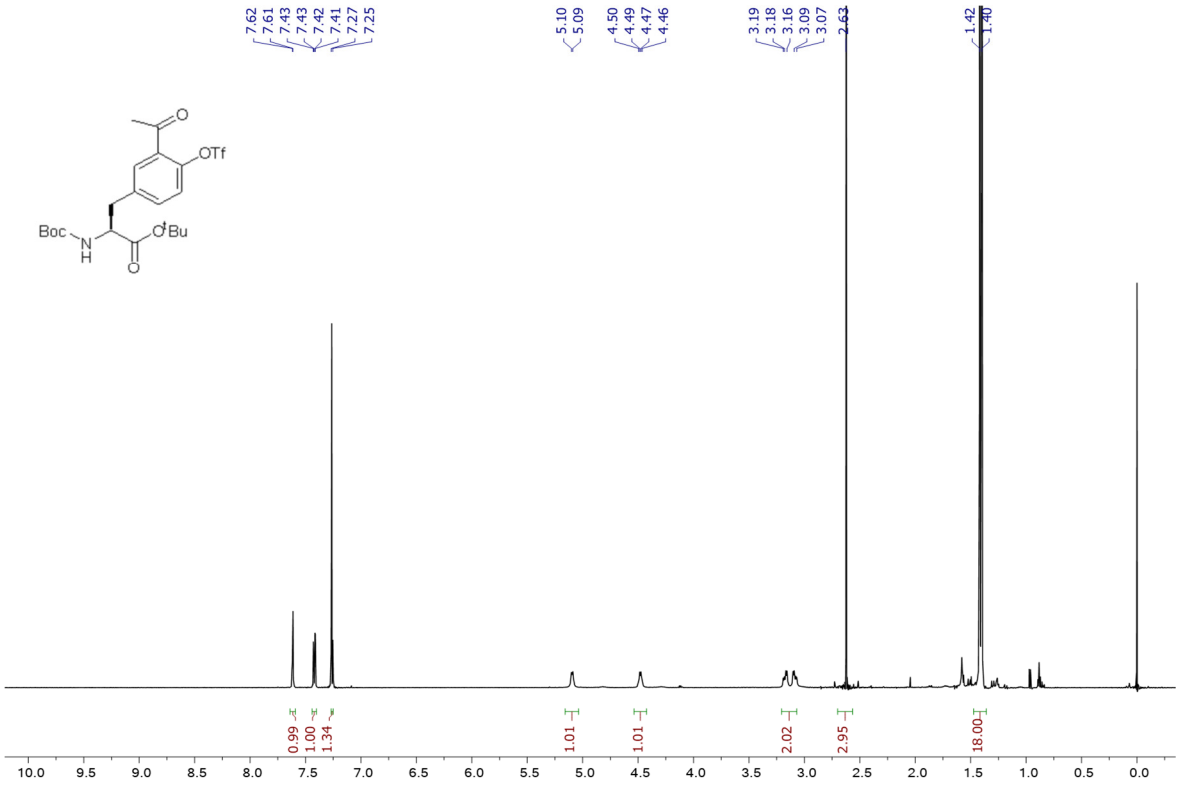
NMR Data

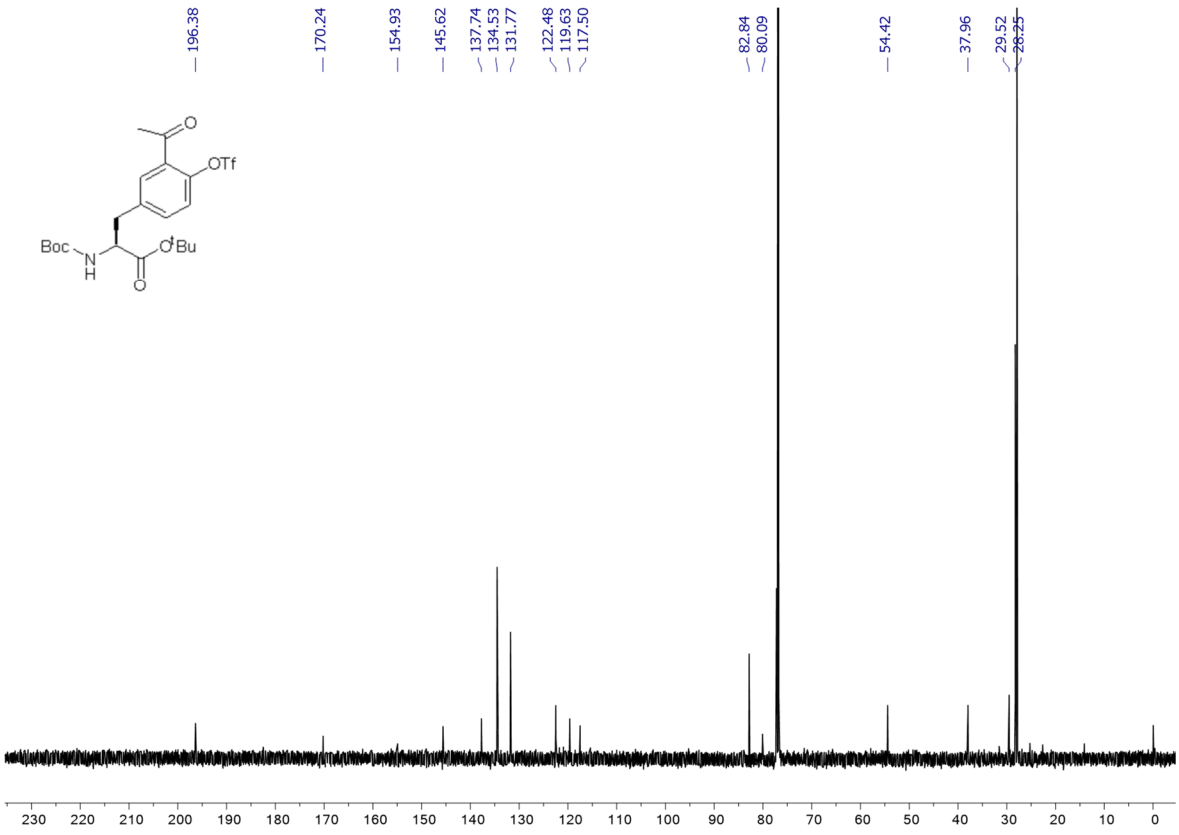

7.84
7.51
7.49
7.02
7.00
4.29
4.27
4.26
3.34
3.33
3.31
3.30
3.25
3.24
3.22
3.21
2.68
1.01
1.00
0.97
1.05
2.28
3.00
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

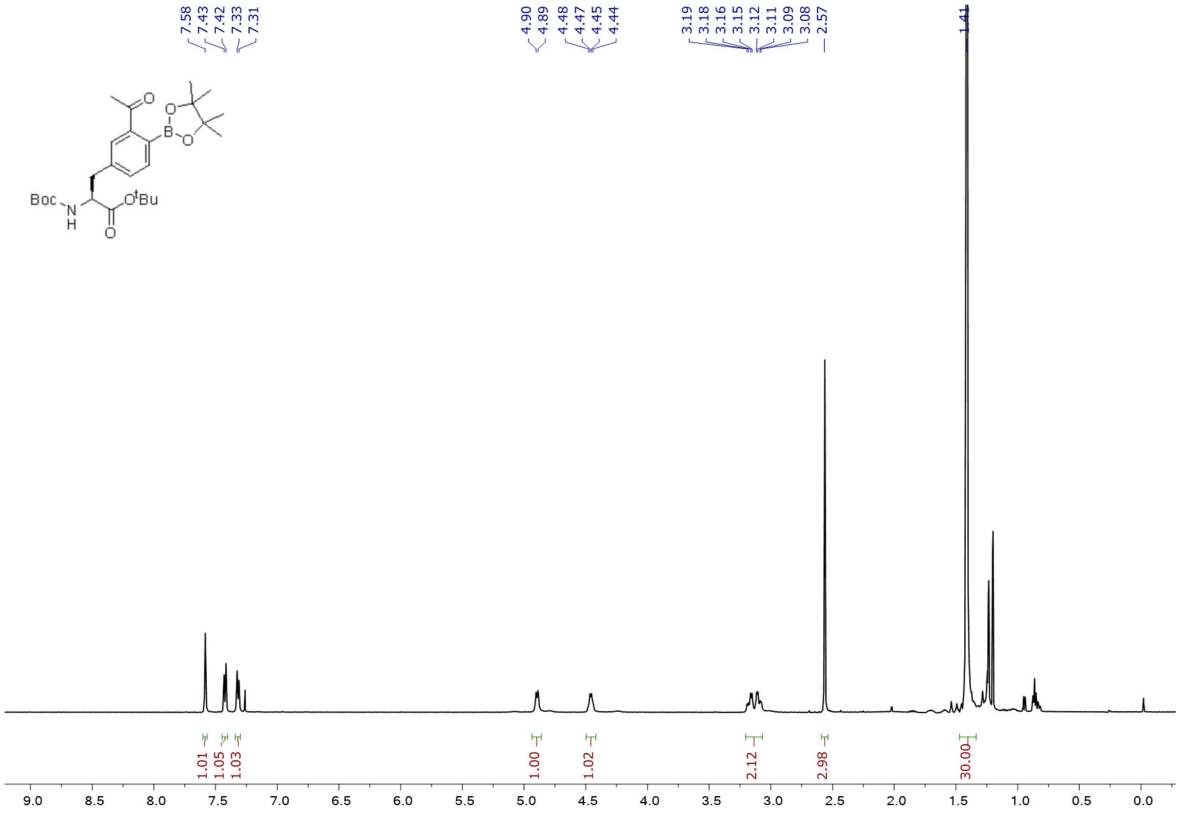

206.89
171.40
159.73
137.84
137.50
132.22
125.16
119.67
118.27
54.14
34.64
26.15
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30

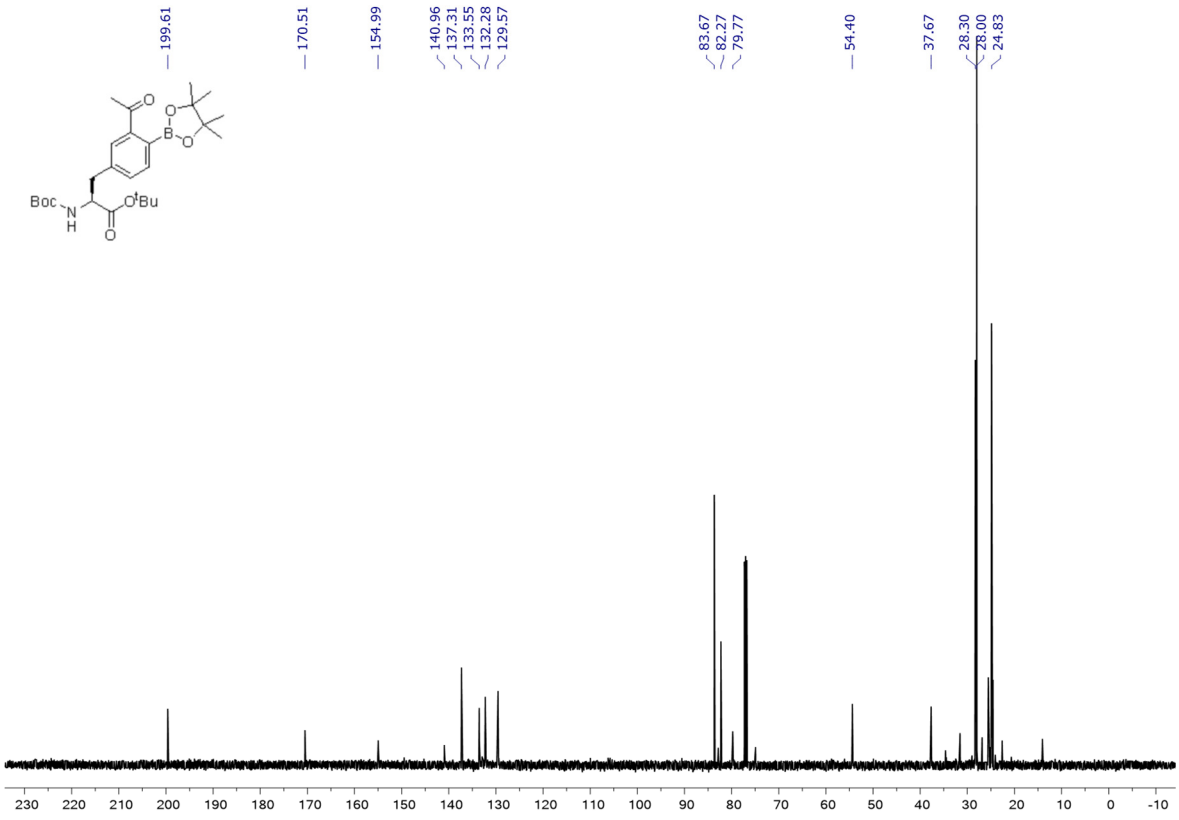

12.15
7.51
7.29
7.27
6.91
6.89
5.04
5.02
4.45
4.44
4.43
4.41
3.08
3.07
3.04
2.99
2.97
2.96
2.61
1.42
OH
Boc
N
H
O
O
O
tBu
0.97
1.07
1.00
1.03
0.95
1.00
2.13
2.97
17.96

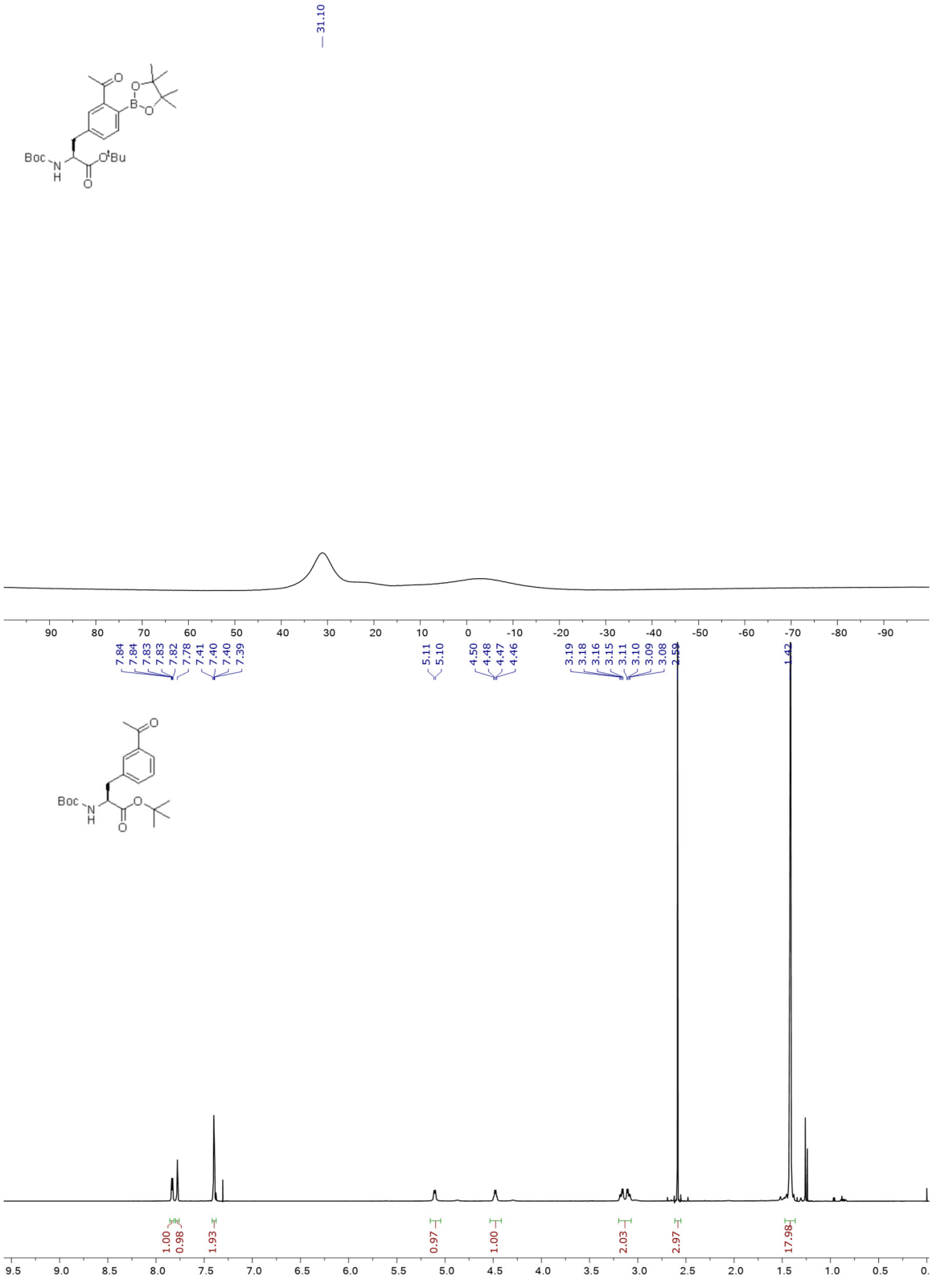

204.31
170.76
161.34
155.00
137.81
131.35
126.82
119.42
118.33
82.31
79.83
54.71
37.62
28.33
26.63
OH
Boc
N
H
O
O
O
tBu

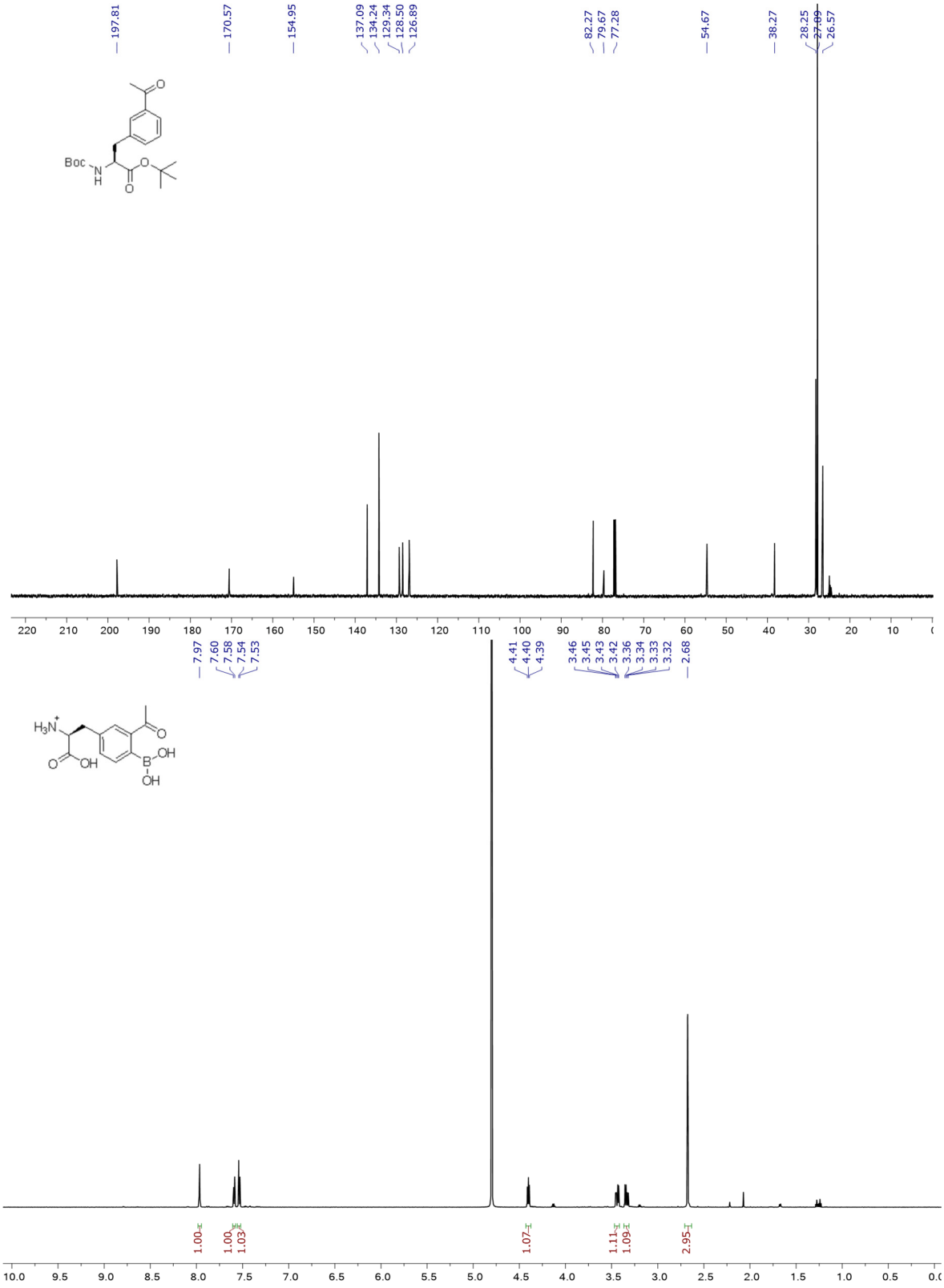

7.62
7.61
7.43
7.43
7.42
7.41
7.27
7.25
5.10
5.09
4.50
4.49
4.47
4.46
3.19
3.18
3.16
3.09
3.07
2.63
1.42
1.40
OTf
Boc
N
H
O
O
tBu
0.99
1.00
1.34
1.01
1.01
2.02
2.95
18.00
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

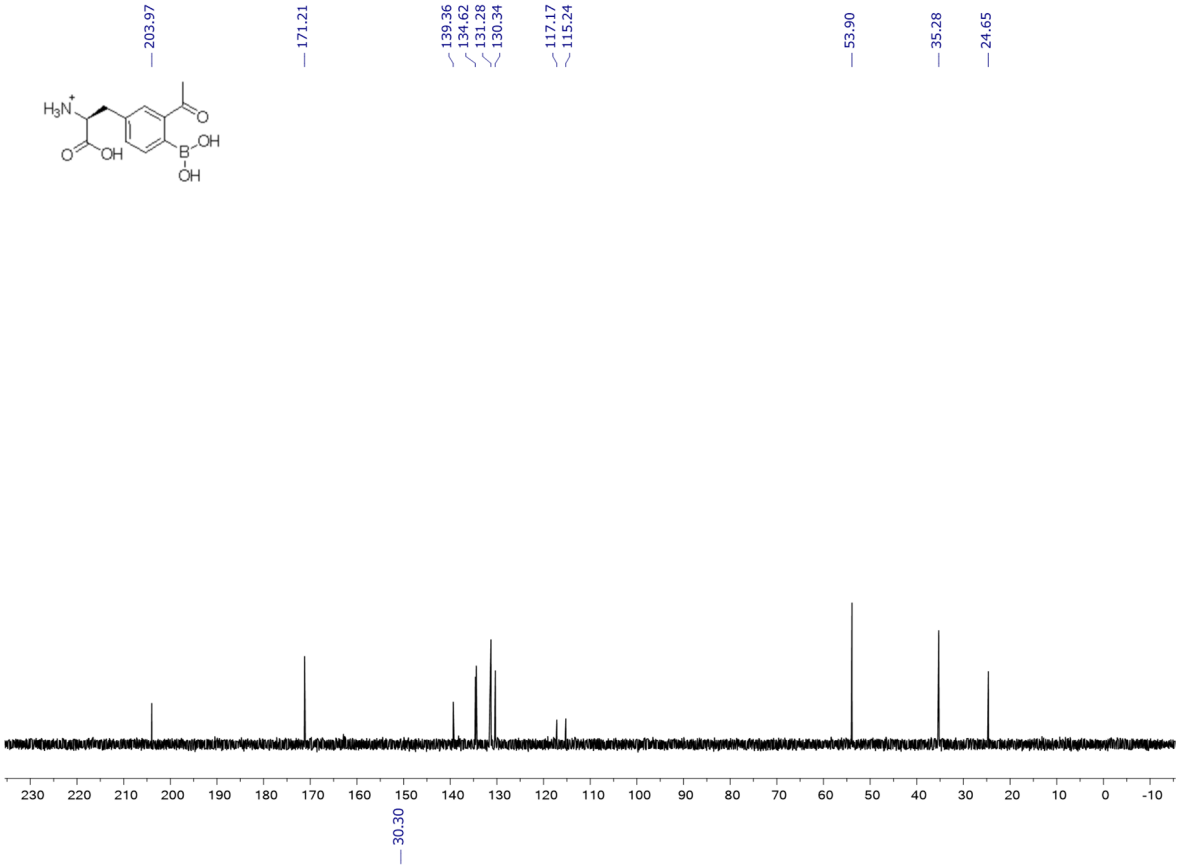

196.38
170.24
154.93
145.62
137.74
134.53
131.77
122.48
119.63
117.50
82.84
80.09
54.42
37.96
29.52
28.25
OTf
Boc
N
H
O
O
tBu
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

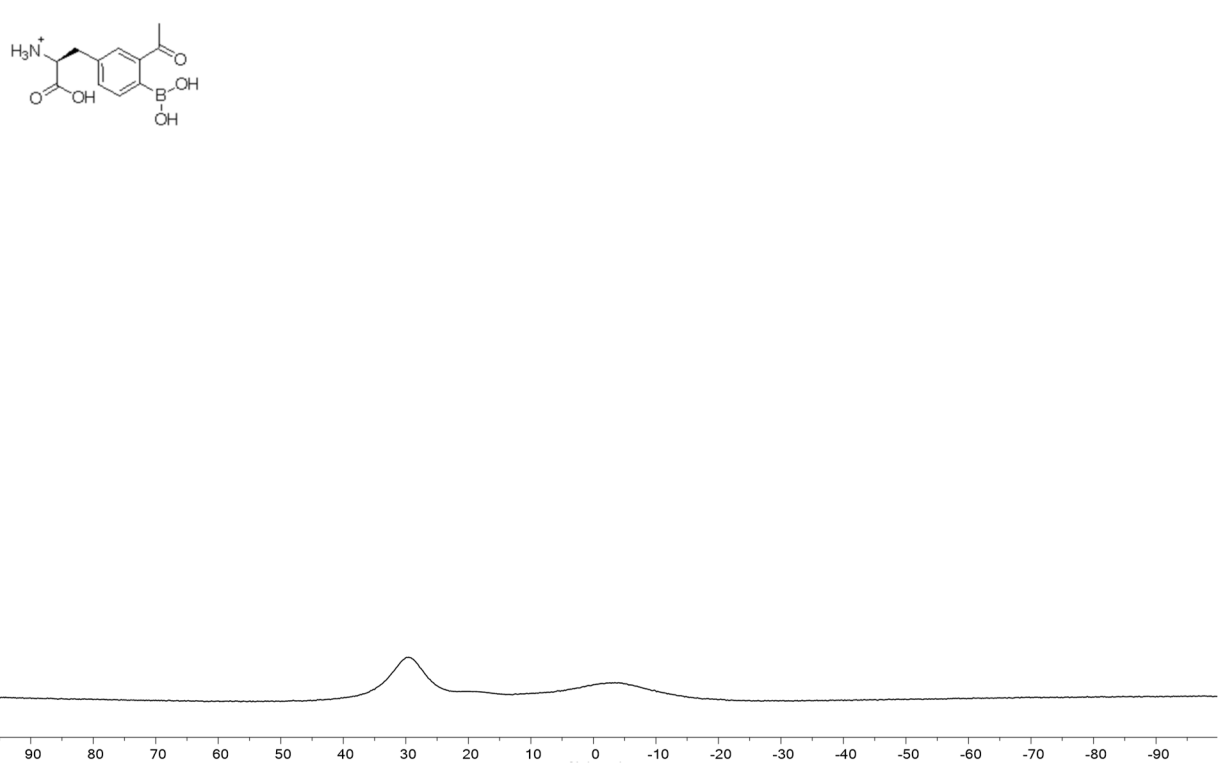

7.58
7.43
7.42
7.33
7.31
4.90
4.89
4.48
4.47
4.45
4.44
3.19
3.18
3.16
3.15
3.12
3.11
3.09
3.08
2.57
1.41
Boc
N
H
O
O
O
tBu
1.01
1.05
1.03
1.00
1.02
2.12
2.98
30.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

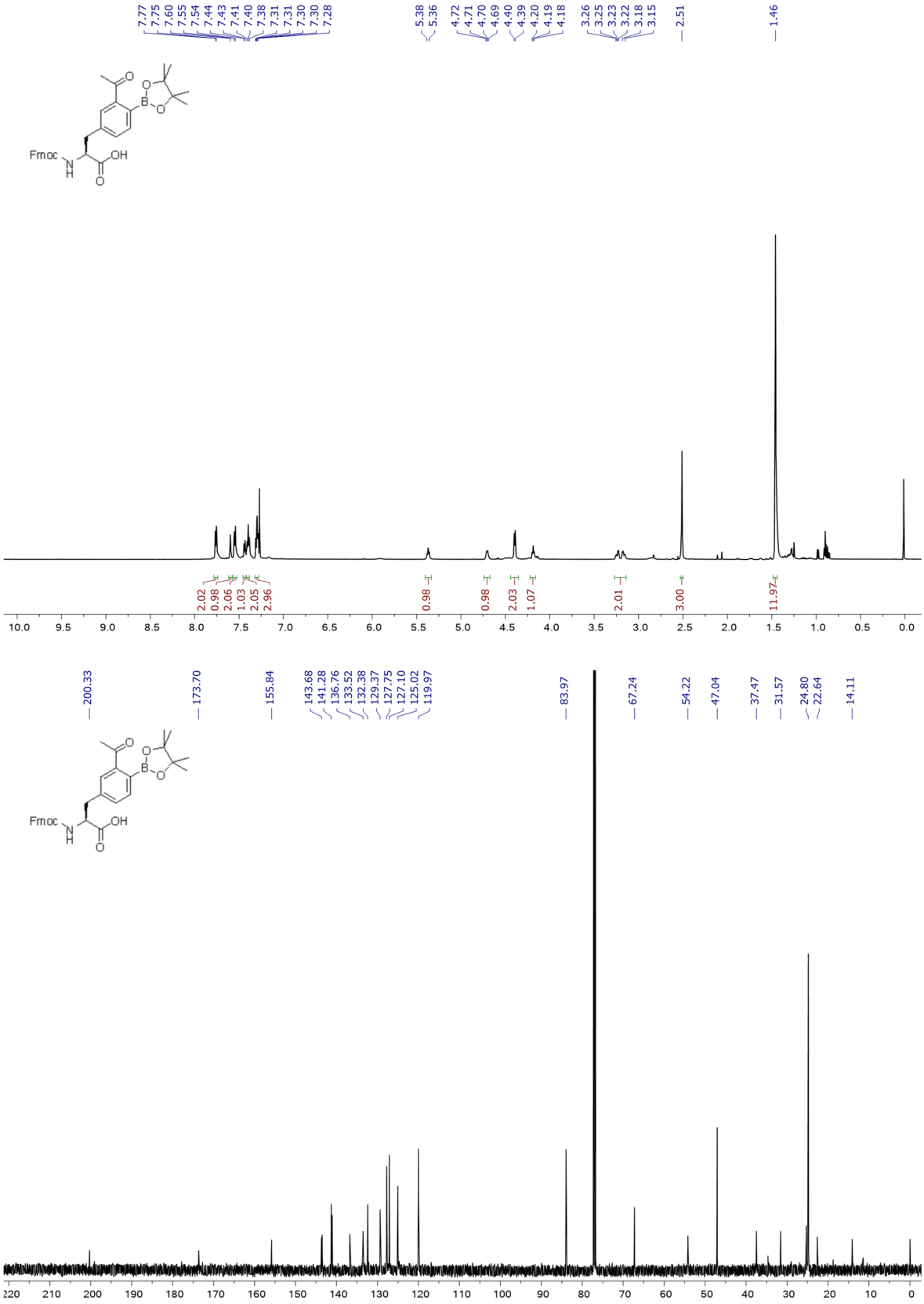

199.61
170.51
154.99
140.96
137.31
133.55
132.28
129.57
83.67
82.27
79.77
54.40
37.67
28.30
28.00
24.83
Boc
N
H
O
O
O
tBu
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

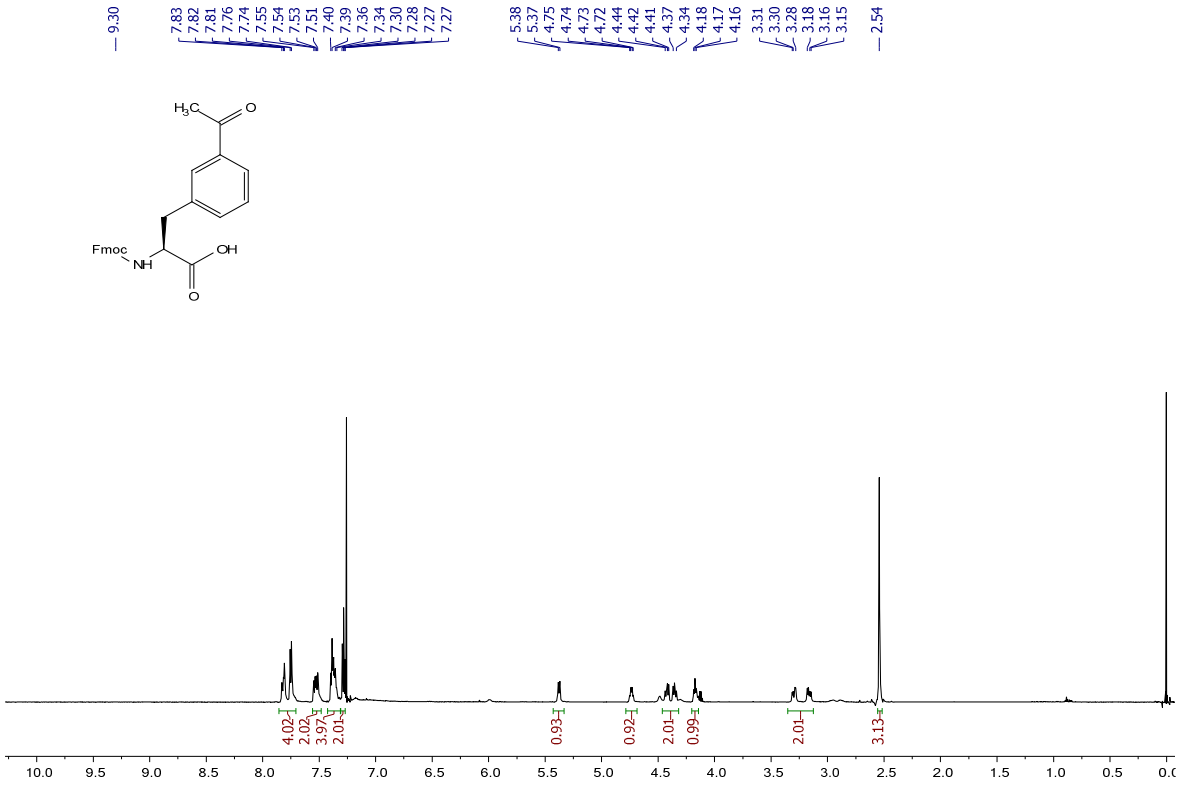

31.10
Boc
N
H
O
O
O
B
O
O
tBu
7.84
7.84
7.83
7.83
7.82
7.78
7.41
7.40
7.40
7.39
5.11
5.10
4.50
4.48
4.47
4.46
3.19
3.18
3.16
3.15
3.11
3.10
3.09
3.08
2.59
1.42
1.00
0.98
1.93
0.97
1.00
2.03
2.97
17.98

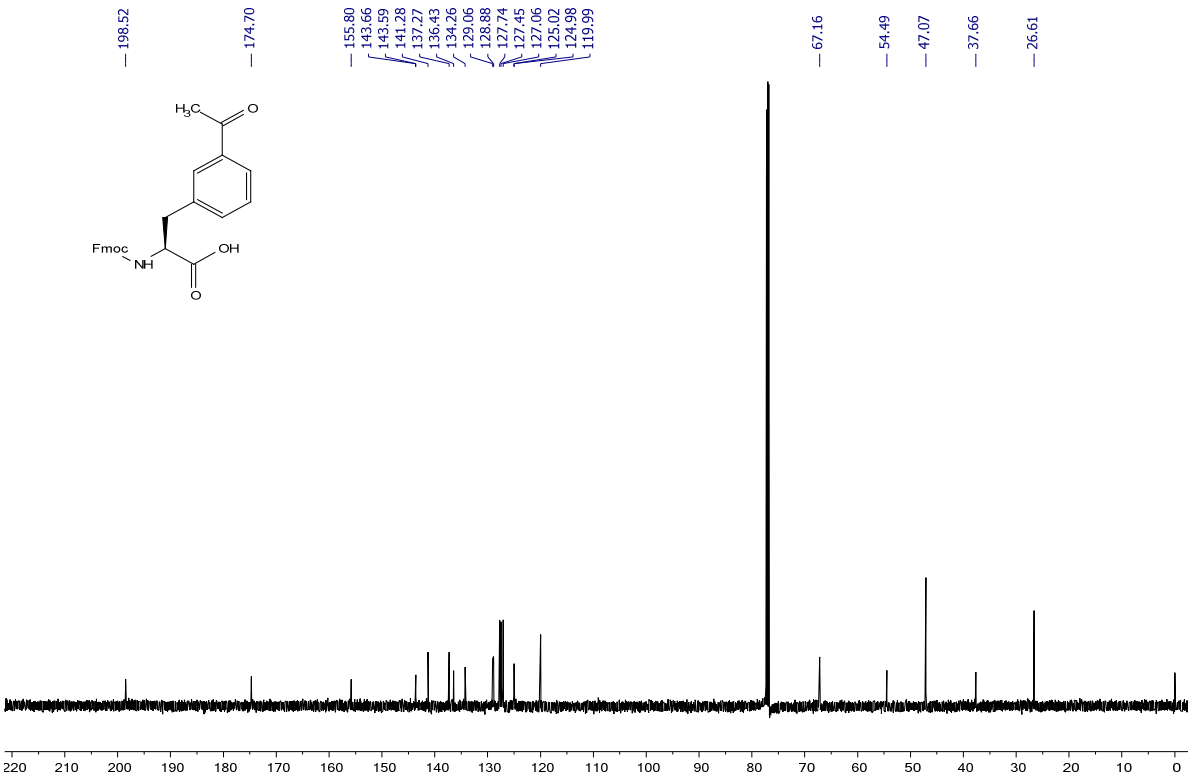

197.81
170.57
154.95
137.09
134.24
129.34
128.50
126.89
82.27
79.67
77.28
54.67
38.27
28.25
27.69
26.57
Boc
N
H
O
O
O
O
7.97
7.60
7.58
7.54
7.53
4.41
4.40
4.39
3.46
3.45
3.43
3.42
3.36
3.34
3.33
3.32
2.68
H3N+
OH
B
OH
OH
1.00
1.00
1.03
1.07
1.11
1.09
2.95


203.97
171.21
139.36
134.62
131.28
130.34
117.17
115.24
53.90
35.28
24.65
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10


30.30
90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90


7.77
7.75
7.60
7.55
7.54
7.44
7.43
7.41
7.40
7.38
7.31
7.31
7.30
7.30
7.28
5.38
5.36
4.72
4.71
4.70
4.69
4.40
4.39
4.20
4.19
4.18
3.26
3.25
3.23
3.22
3.18
3.15
2.51
1.46
Fmoc
N
H
OH
O
B
O
O
2.02
0.98
2.06
1.03
2.05
2.96
0.98
0.98
2.03
1.07
2.01
3.00
11.97
200.33
173.70
155.84
143.68
141.28
136.76
133.52
132.38
129.37
127.75
127.10
125.02
119.97
83.97
67.24
54.22
47.04
37.47
31.57
24.80
22.64
14.11


9.30
7.83
7.82
7.81
7.76
7.74
7.55
7.54
7.53
7.51
7.40
7.39
7.36
7.34
7.30
7.28
7.27
7.27
5.38
5.37
4.75
4.74
4.73
4.72
4.44
4.42
4.41
4.37
4.34
4.18
4.17
4.16
3.31
3.30
3.28
3.18
3.16
3.15
2.54
H3C
O
Fmoc
NH
OH
O
4.02
2.02
3.97
2.01
0.93
0.92
2.01
0.99
2.01
3.13
10.0
9.5
9.0
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


198.52
174.70
155.80
143.66
143.59
141.28
137.27
136.43
134.26
129.06
128.88
127.74
127.45
127.06
125.02
124.98
119.99
67.16
54.49
47.07
37.66
26.61
H3C
O
Fmoc
NH
OH
O
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0