Supporting Information

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General information

All of chemicals were purchased from commercial suppliers and used without further purification. IL-8 inhibitor (**1**) (AS-62401) was purchased from AnaSpec Inc. Thrombin receptor antagonist (**5**) (H-2514), Cys-Gly-His-Gly-Asn-Lys-Ser-Amyloid β-Protein (33-40) (**6**) (H-6364), oxytocin (H-2510) (**7**), somatostatin (H-1490) (**8**), and 10-mer peptide (H-PHPFHFFVYK-OH) (4010534) were purchased form BACHEM. Angiotensin I (A9650) (**9**), PAR1-AP (S1820) (**10**), HIV Tat (47-57) (H0292) (**11**), and leuprolide (pEHWSYlLRP-NH_2) (L0399) were purchased from Sigma-Aldrich. Neuromedin B (J66286MCR) (**12**) was purchased from Alfa Aesar. Bradykinin (H-RPPGFSPFR-OH) (07913) was purchased from Chem-Impex Int'l. Inc. Peptide **4** was synthesized using standard solid-phase Fmoc protocols,[1] and crude peptide was purified by reverse-phase HPLC. *N*-methylmorpholine (NMM) buffer (pH 7.0–8.0) was prepared by addition of NMM (TCI, M0370) to MilliQ water (due to the unpleasant smell of NMM, this process was conducted in a fume hood). The pH of the NMM solution was adjusted to desired pH by addition of aq HCl solution. 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (pH 5.5–6.5) was prepared by dissolution of corresponding compound in MilliQ water, and the pH was adjusted by addition of aq NaOH solution. Size exclusion columns were purchased from GE Healthcare (#17085101).

Instrumentation

Reverse-phase HPLC (RP-HPLC) was performed on a Shimadzu CBM-20a instrument with Phenomenex Jupiter 4μ Proteo 90A (250 mm×4.6 mm for analytical scale) and Phenomenex Jupiter 4μ Proteo 90A (250×15 mm for preparative scale) column. The flow rate was 1 mL/min for analytical scale and 8 mL/min for preparative scale. A gradient of acetonitrile/water with 0.1% trifluoroacetic acid (TFA) was employed. Compounds were detected by UV detector at 280 nm.

ESI-MS was conducted on a Bruker Daltonics MicroTOF spectrometer.

MALDI-TOF MS was conducted on Bruker Daltonics Autoflex Speed-MALDI-TOF/TOF spectrometer for protein experiments. A sample was mixed with sinapic acid (20 mg/mL solution in 50:50:0.1 H_2O/MeCN/TFA) or α-cyano-4-hydroxycinnamic acid (10 mg/mL solution in 50:50:0.1 H_2O/MeCN/TFA) on a MALDI plate.

Gel and blot imaging was conducted on LAS-4000 imager (Fujifilm). Coomassie-stained gels were imaged with a DIA light source. Chemical blotting with a fluorogenic azide-coumarin was conducted using a 365-nm EPI light source with a long-pass filter at 410-nm.

^{1}H and ^{13}C NMR spectra were obtained on Bruker AVANCE 500 or AVANCE 600 spectrometer.

LC-MS was conducted on a Bruker Daltonics MicroTOF spectrometer with an Agilent Pursuit 200Å Diphenyl (2.0 mm×150 mm), 5 μm column.

Additional experimental data for nickel(II)-catalyzed modification of IL-8 inhibitor 1

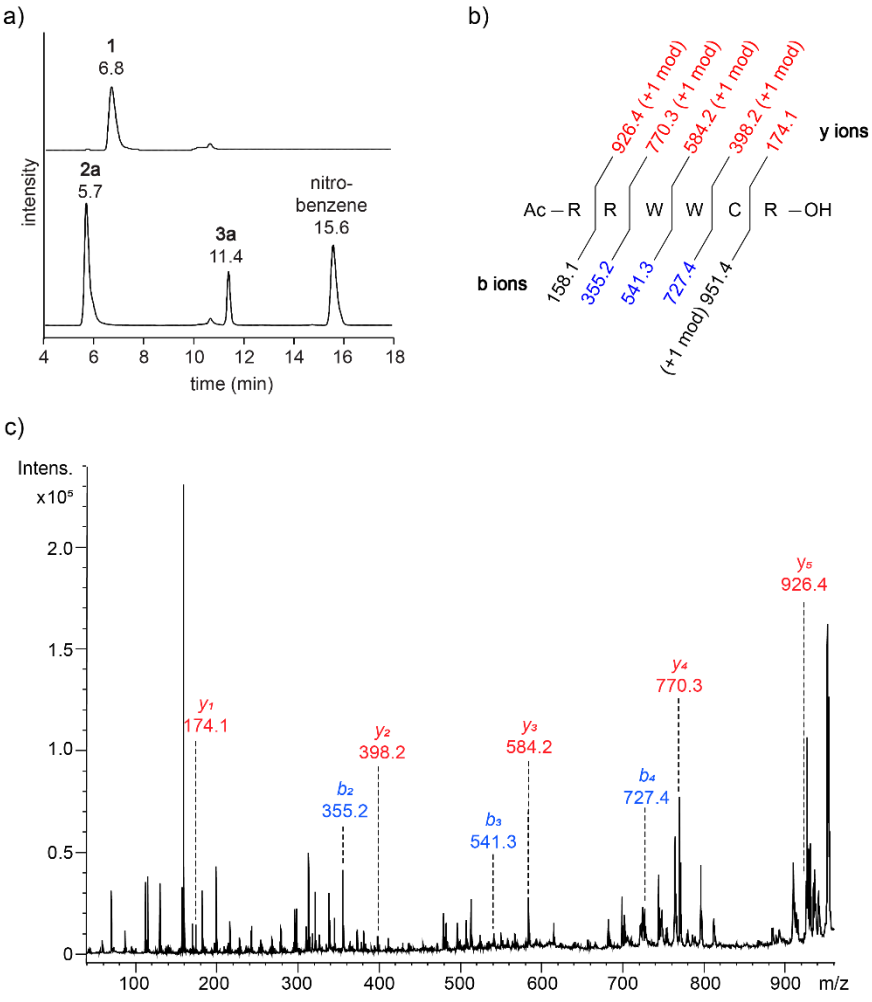


Figure S1. Nickel(II)-catalyzed cysteine modification of IL-8 inhibitor **1** with 2-nitrophenylboronic acid (**2a**). a) RP-HPLC analysis data of **1** (top) and the crude reaction mixture (bottom). Numbers on each peak represent retention times (min). b,c) MALDI-TOF MS/MS spectrum of **3a**.

^{1}H NMR characterization of the arylated product, compared with a product standard

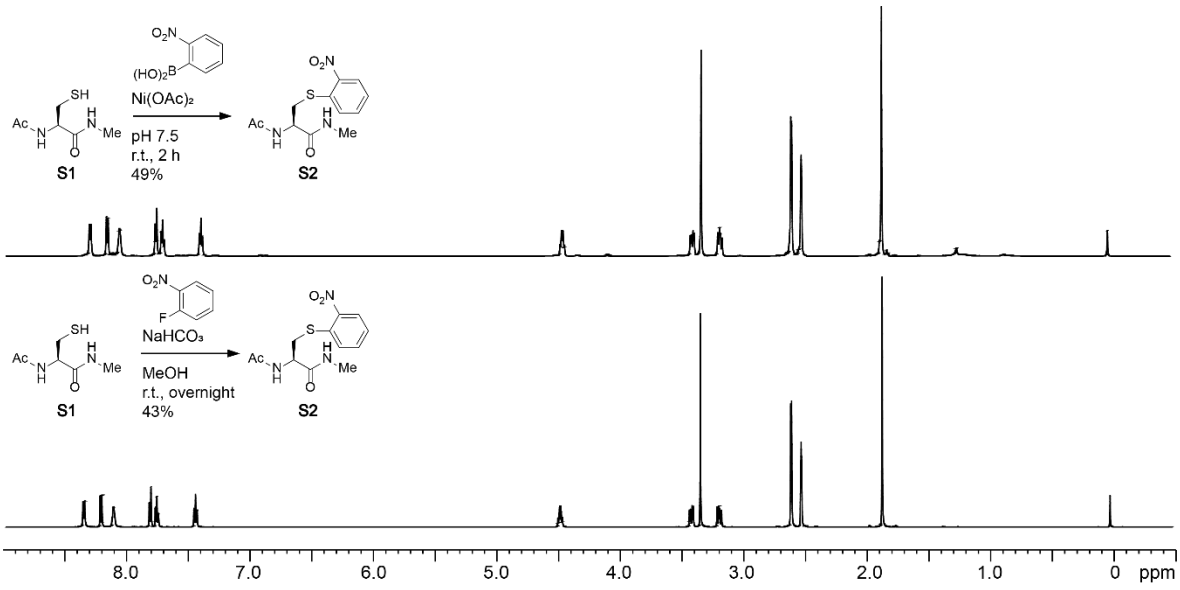


Figure S2. ^{1}H NMR study for structure identification of the *S*-arylated product **S2**. Reaction conditions were described in the organic synthesis section (vide infra). ^{1}H NMR spectra of products in nickel(II)-catalyzed *S*-arylation (top) and S_NAr reaction (bottom).

Additional experimental data for kinetics of nickel(II)-catalyzed modification of IL-8 inhibitor 1

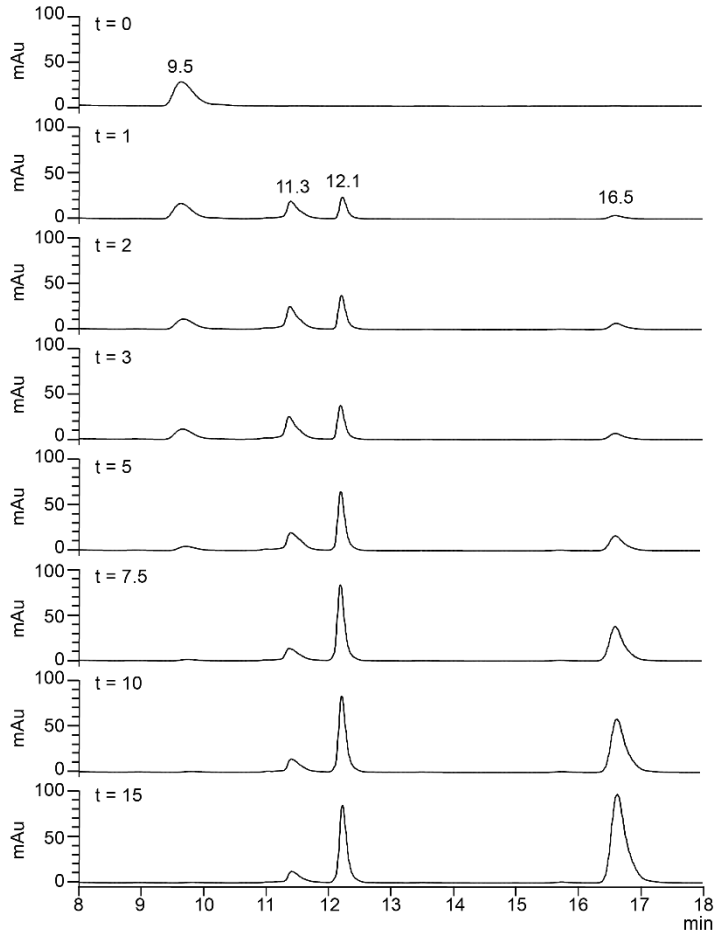


Figure S3. RP-HPLC analysis data of crude reaction mixture of nickel(II)-catalyzed cysteine modification of IL-8 inhibitor **1** with 2-nitrophenylboronic acid (**2a**) after incubation for a given time period (t min). Numbers on each peak represent retention times (min). The fractions at 9.5, 11.3, 12.1, and 16.5 min correspond to peptide **1**, dimer of **1**, modified peptide **3a**, and nitrobenzene, respectively.

pH dependency of reaction rate of nickel(II)-catalyzed cysteine *S*-arylation

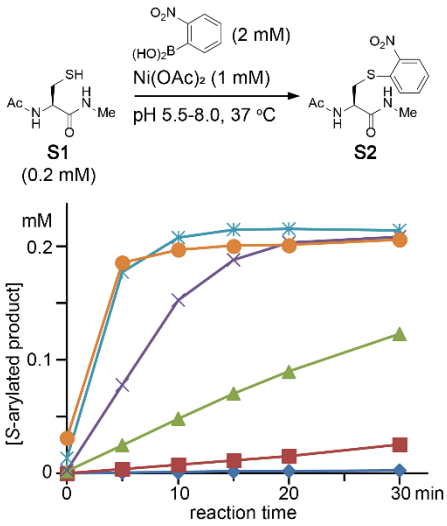


Figure S4. Kinetic data of the nickel(II)-catalyzed *S*-arylation of cysteine with 2-nitrophenylboronic acid (**2a**) in MES buffer (10 mM, pH 5.5, 6.0, and 6.5) or NMM buffer (10 mM, pH 7.0, 7.5, and 8.0) with analysis by RP-HPLC; pH 5.5 (blue rhombus), pH 6.0 (red square), pH 6.5 (green triangle), pH 7.0 (purple cross), pH 7.5 (blue asterisk), and pH 8.0 (orange circle).

Kinetic data of cysteine *S*-arylation in the presence of catalytic amount of nickel(II)

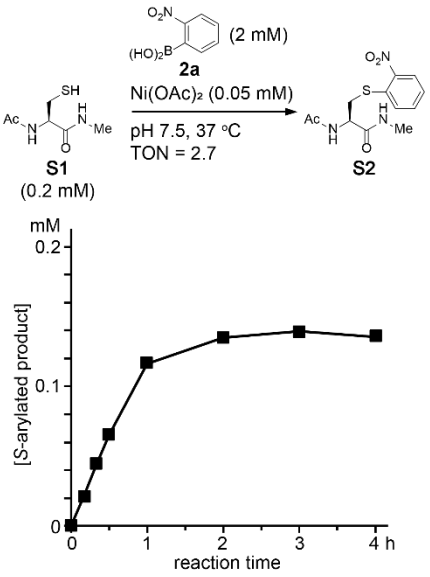


Figure S5. Kinetic data of nickel(II)-catalyzed *S*-arylation of cysteine with 2-nitrophenylboronic acid (**2a**) with analysis by RP-HPLC. Catalytic turnover number (TON) of nickel(II) was 2.7.

Additional experimental data for boronic acid screening: MALDI-TOF MS analysis data

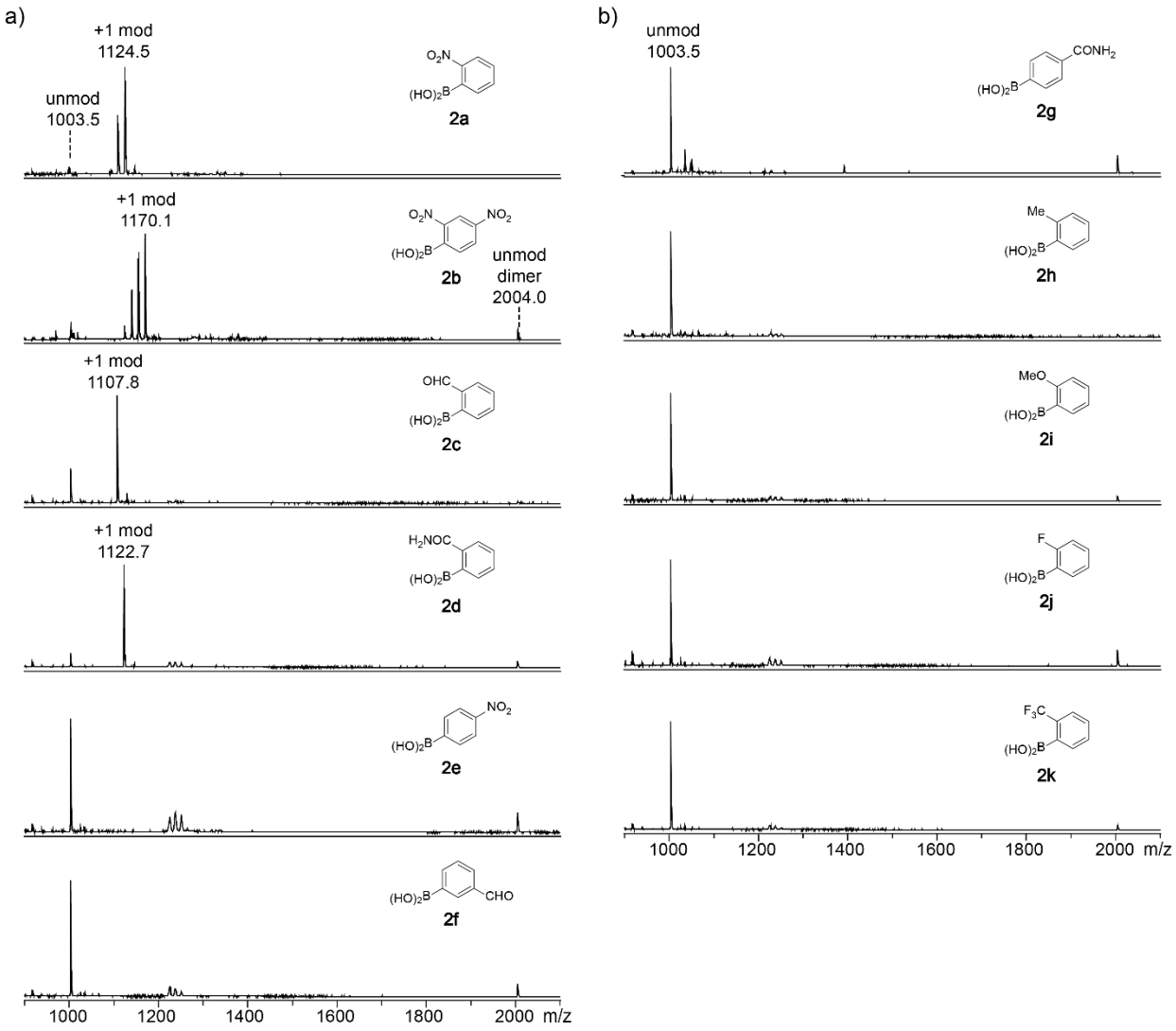


Figure S6. Nickel(II)-catalyzed cysteine modification of IL-8 inhibitor **1** with phenylboronic acid derivatives a) **2a**–**f** and b) **2g**–**k**. Mono-modified peptides were shown as "+1 mod". Peaks at 1003.5 and 2004.0 correspond to unmodified peptide **1** (unmod) ($[M+H]^+$) and its disulfide dimer (unmod dimer) ($[M+H]^+$), respectively.

Additional experimental data for peptide screening: MALDI-TOF MS analysis data for cysteine-containing peptides (Figure S7–S10)

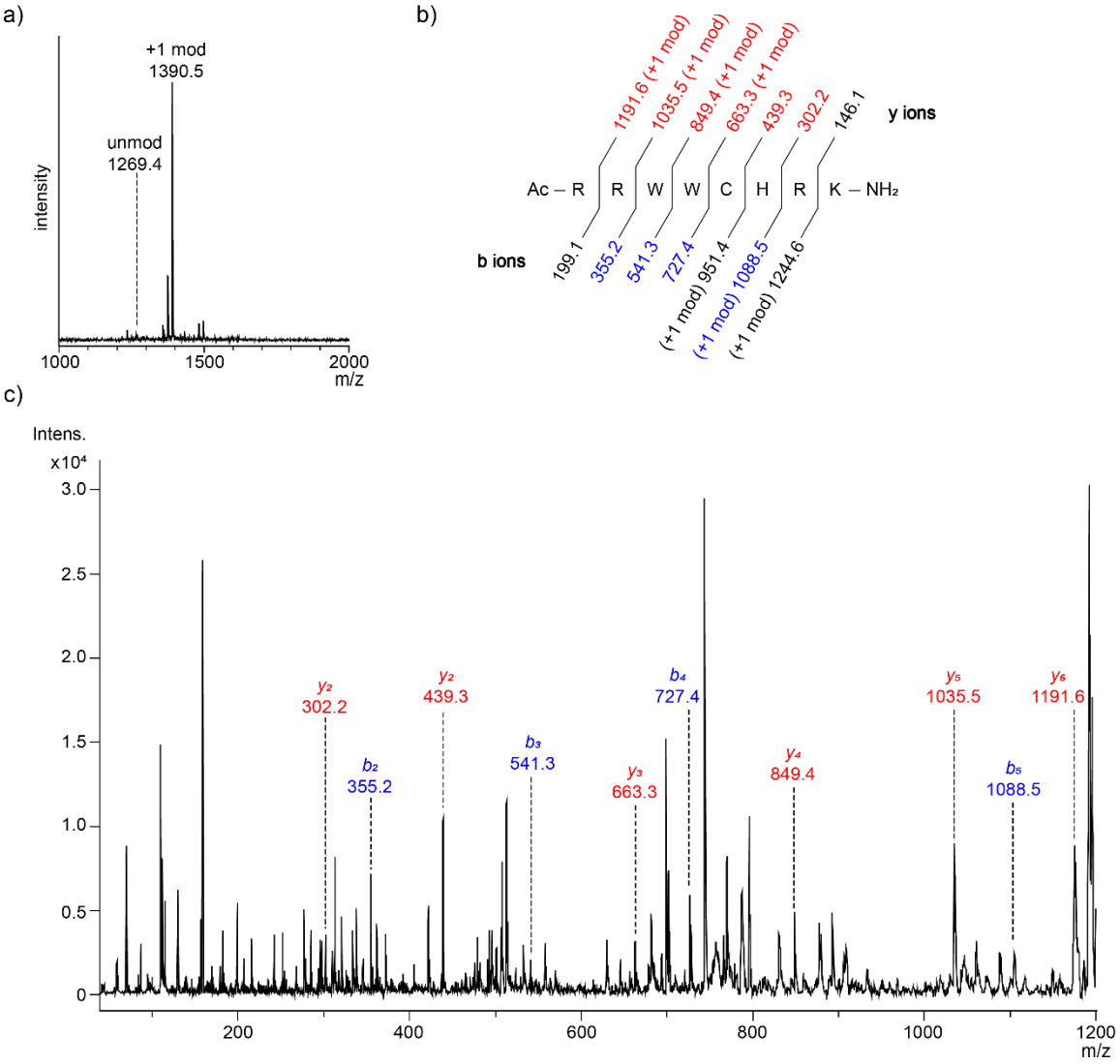


Figure S7. Nickel(II)-catalyzed cysteine modification of **4** with 2-nitrophenylboronic acid (**2a**). a) MALDI-TOF MS spectrum of the crude reaction mixture. An additional [M(+1mod)−16] peak derived from oxygen atom loss from the nitro group was observed. b,c) MALDI-TOF MS/MS spectrum of **4** modified with **2a** (+1 mod).

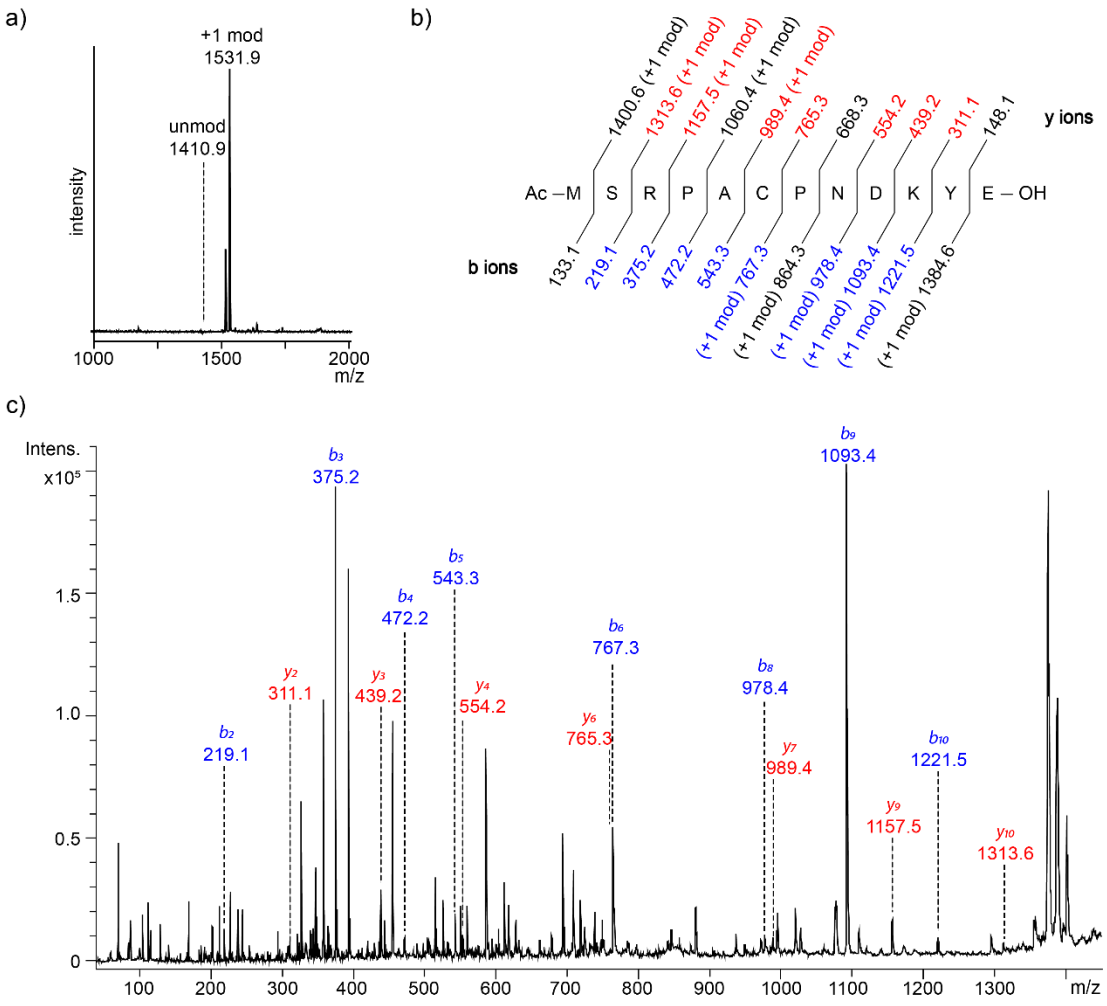


Figure S8. Nickel(II)-catalyzed cysteine modification of **5** with 2-nitrophenylboronic acid (**2a**). a) MALDI-TOF MS spectrum of the crude reaction mixture. An additional [M(+1 mod)−16] peak derived from oxygen atom loss from the nitro group was observed. b,c) MALDI-TOF MS/MS spectrum of **5** modified with **2a** (+1 mod).

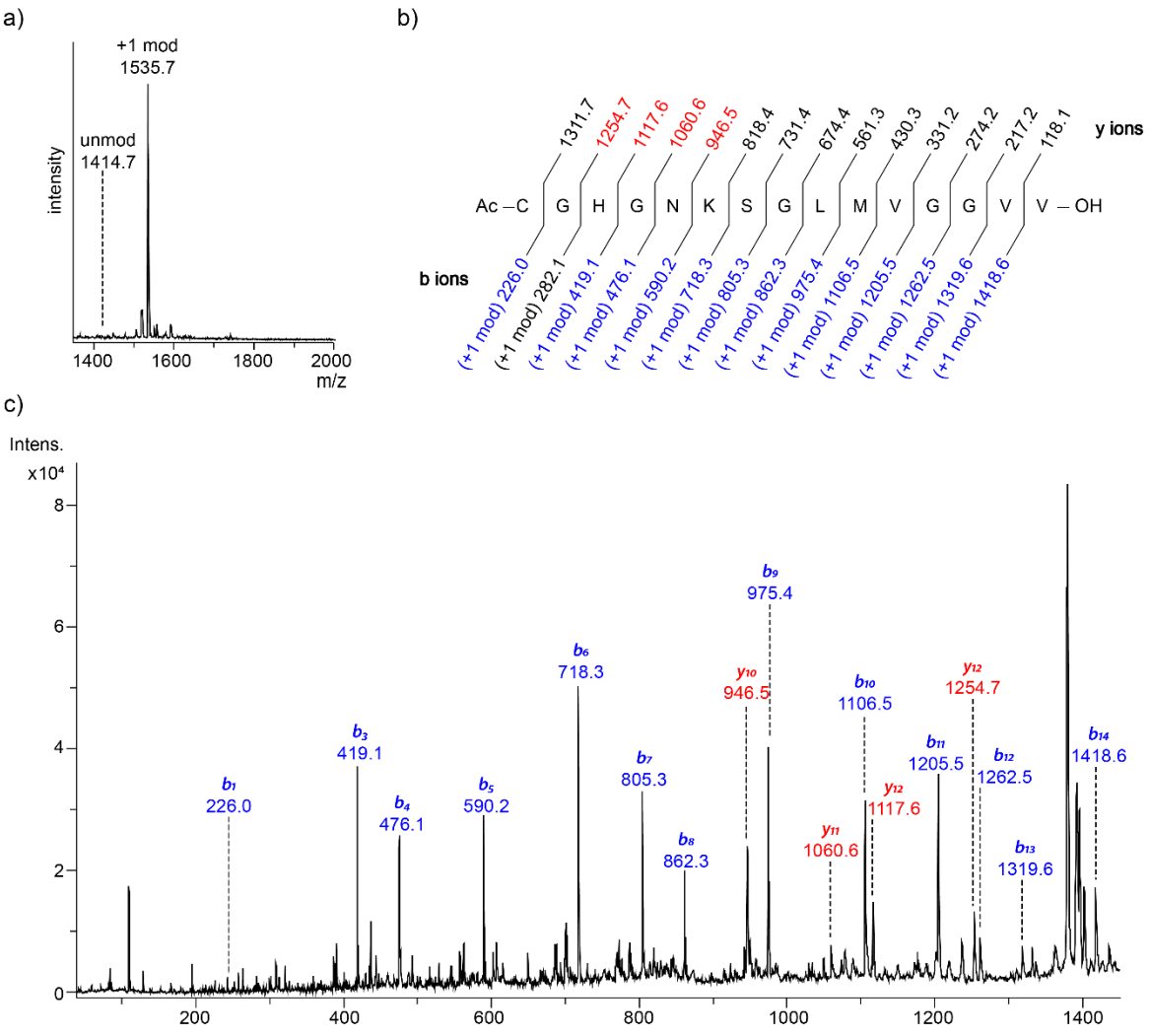


Figure S9. Nickel(II)-catalyzed cysteine modification of **6** with 2-nitrophenylboronic acid (**2a**). a) MALDI-TOF MS spectrum of the crude reaction mixture. An additional [M(+1 mod)−16] peak derived from oxygen atom loss from the nitro group was observed. b,c) MALDI-TOF MS/MS spectrum of **6** modified with **2a** (+1 mod).

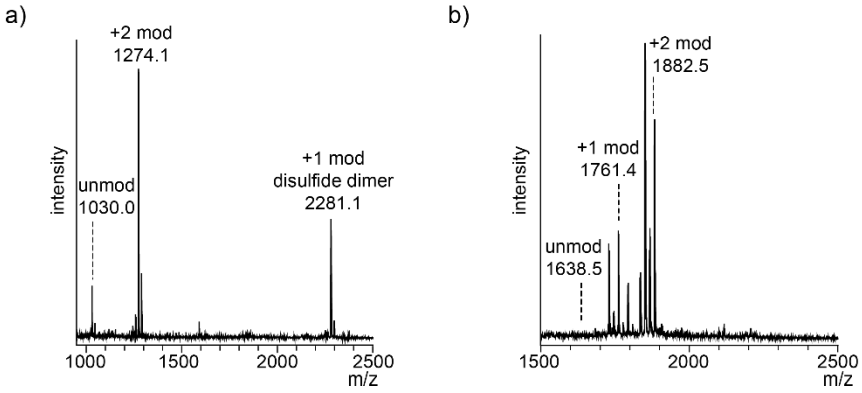


Figure S10. Nickel(II)-catalyzed cysteine modification of oxytocin (**7**, H-CYIQNCPLG-NH_2) and somatostatin (**8**, H-AGCKNFFWKTFTSC-OH) with 2-nitrophenylboronic acid (**2a**). a) MALDI-MS spectrum of the crude reaction mixture of **7**. Product ratio (+1 mod/+2 mod) was calculated from peak areas to be 1:1.6. b) MALDI-MS spectrum of the crude reaction mixture of **8**. Additional [M−16] peaks derived from oxygen atom loss from the nitro group were observed.

Additional experimental details for peptide stapling

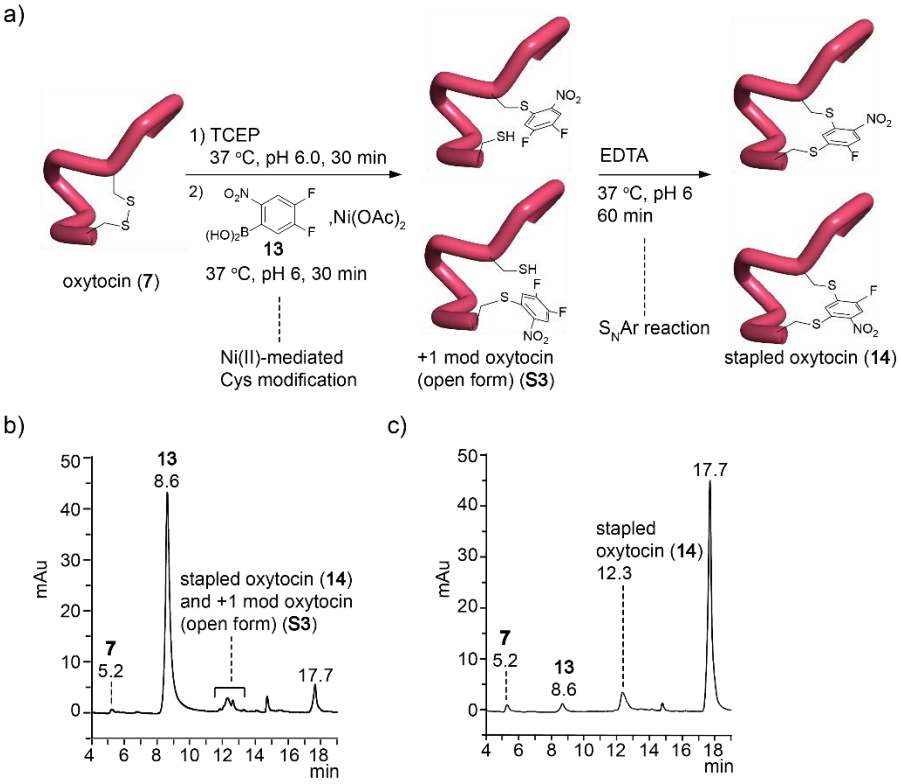


Figure S11. a) The two-step reaction scheme for stapling of oxytocin (**7**) with 4,5-difluoro-2-nitrophenylboronic acid (**13**): nickel(II)-catalyzed cysteine modification and intramolecular S_NAr reaction. The peak at 17.7 minutes corresponds to 3,4-difluoronitrobenzene. b) RP-HPLC chromatogram of the crude reaction mixture after nickel(II)-catalyzed cysteine modification (**7→S3**) with UV detection at 280 nm. c) RP-HPLC chromatogram of the crude reaction mixture after intramolecular S_NAr reaction (**S3→14**) with UV detection at 280 nm.

Stability test of stapled oxytocin 14 to chemical cleavage

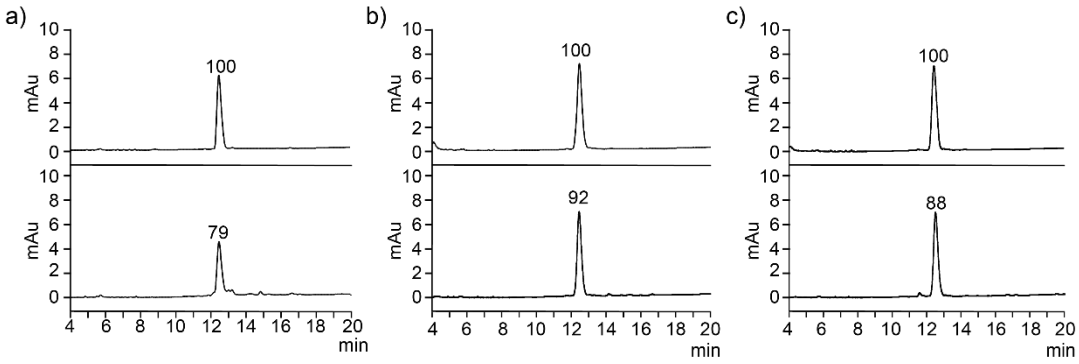


Figure S12. RP-HPLC analysis data of stapled oxytocin **14** stability test. Numbers on peak at 12.3 min represent the relative ratios of the remained intact stapled oxytocin. a) The reaction mixture after incubation in aq K_2CO_3 solution (10 mM, pH 11.1) for 0 h (top) and 24 h (bottom). b) The reaction mixture after incubation with GSH (10 mM) in NMM buffer (10 mM, pH 7.5) for 0 h (top) and 24 h (bottom). c) The reaction mixture after incubation with TCEP (1 mM) in NMM buffer (10 mM, pH 7.5) for 0 h (top) and 24 h (bottom).

Stability test of stapled oxytocin 14 to enzymatic cleavage with α-chymotrypsin

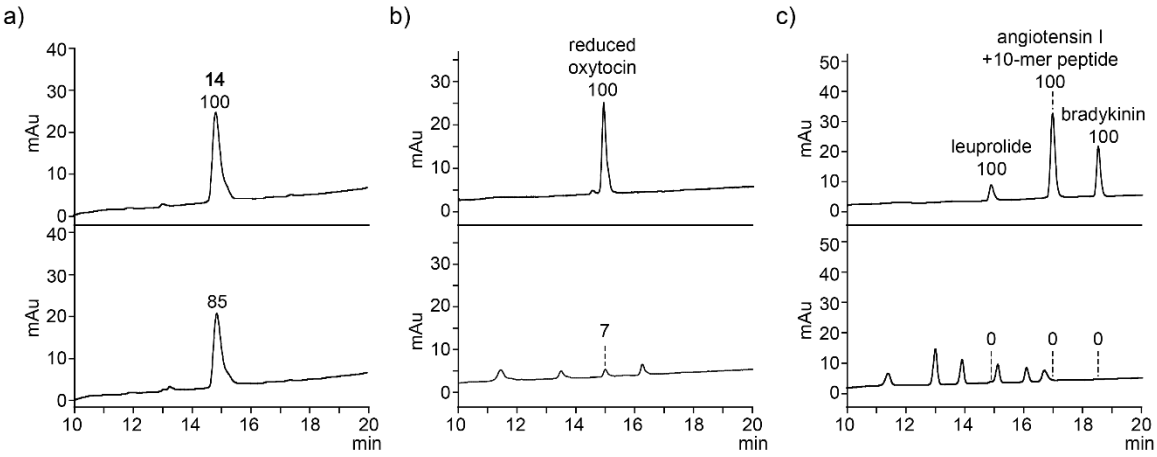


Figure S13. RP-HPLC analysis data of stability test of a) stapled oxytocin **14**, b) reduced oxytocin, and c) a peptide mixture of leuprolide, angiotensin I, 10-mer peptide, and bradykinin, to enzymatic cleavage with α-chymotrypsin. The reaction mixture after incubation in Tris buffer (100 mM Tris, 10 mM $CaCl_2$, pH 7.8) at r.t. for 0 h (top) and 2 h (bottom). Numbers on peak represent the relative ratios of the remained intact peptides.

Stability test of stapled oxytocin 14 in fetal bovine serum

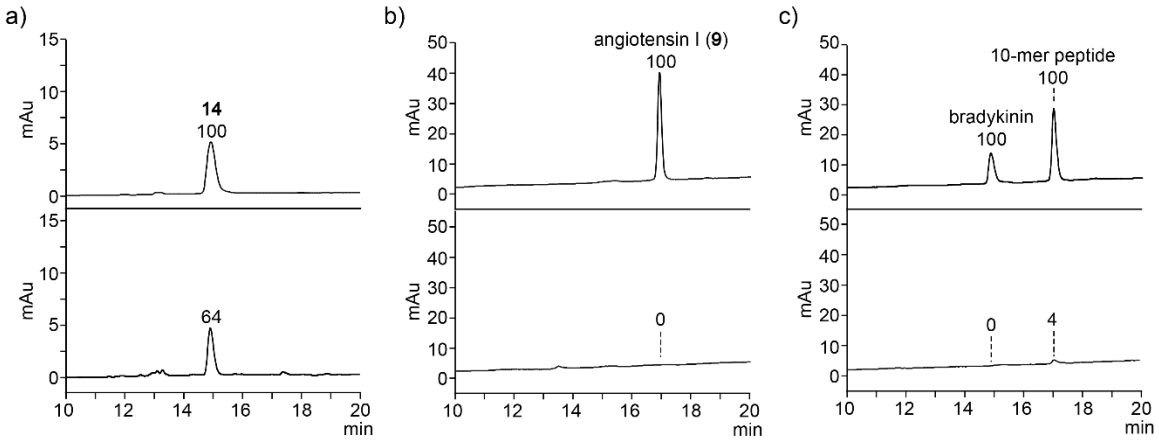


Figure S14. RP-HPLC analysis data of stability test of a) stapled oxytocin **14**, b) angiotensin I, and c) a peptide mixture of bradykinin and 10-mer peptide in fetal bovine serum (FBS). The reaction mixture after incubation in FBS at r.t. for 0 h (top) and 18 h (bottom). Numbers on peak represent the relative ratios of the remained intact peptides. "10-mer peptide" sequence: PHPFHLFVYK.

Reversible modification of cysteine in a peptide 4 by using 2,4-dinitrophenylboronic acid (2b)

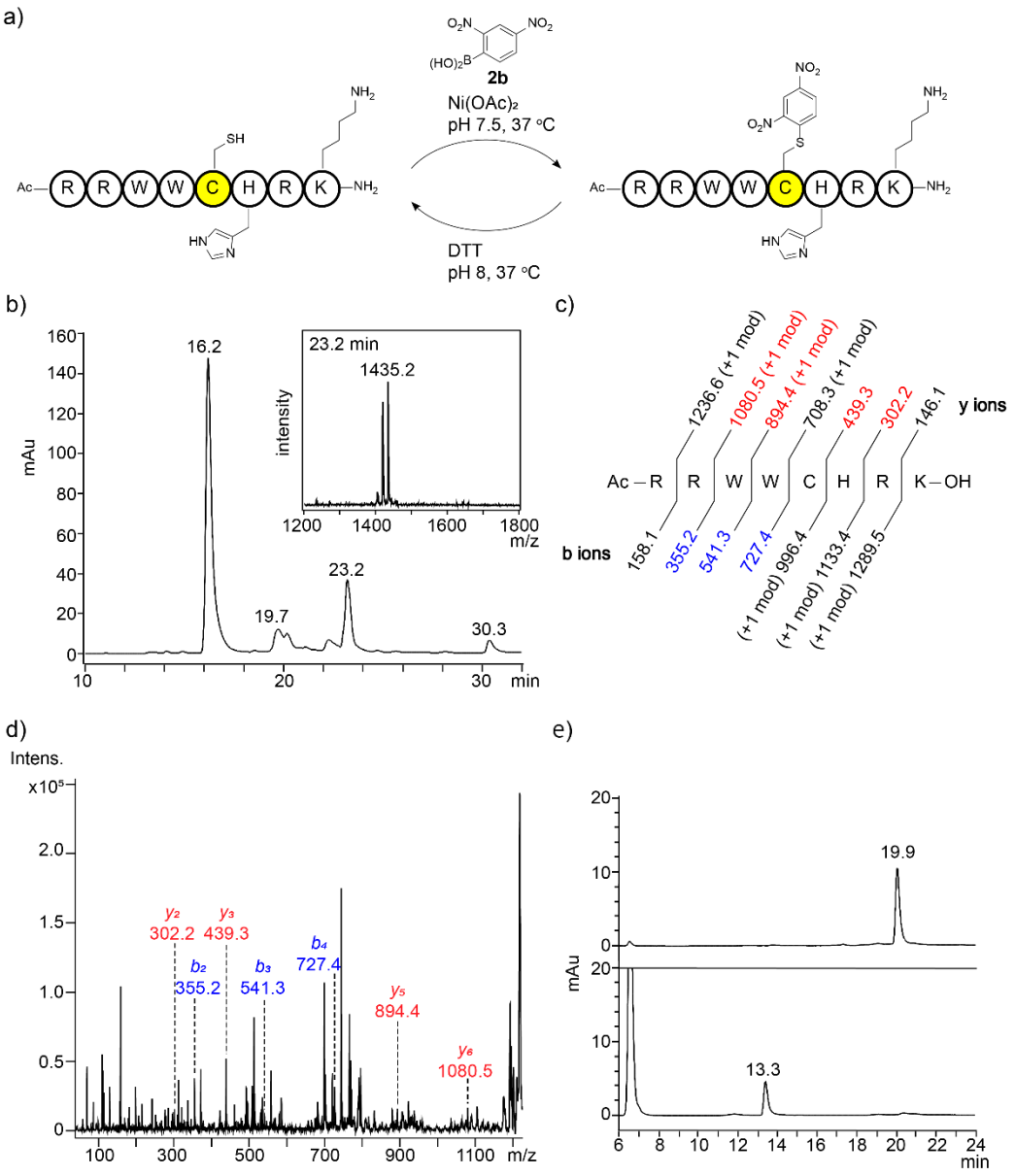


Figure S15. a) Overview of reversible cysteine modification of peptide **4**. b) RP-HPLC analysis data of the crude reaction mixture. Numbers on each peak represent retention times (min). The fractions at 16.2, 19.7, 23.2, and 30.3 min correspond to **2b**, disulfide dimer of **4**, monoarylated peptide, and diarylated peptide, respectively. Inset: MALDI-TOF MS spectrum of the fraction at 23.2 min, corresponding to *S*-arylated product. An additional [M−16] peak derived from oxygen atom loss from the nitro group was observed. c,d) MALDI-TOF MS/MS analysis of monoarylated peptide. e) RP-HPLC analysis data for the reverse reaction after incubation with DTT for 0 h (top) and 1 h (bottom). Numbers on each peak represent retention time (min). The fractions at 13.3 and 19.9 min correspond to peptide **4** and monoarylated peptide, respectively.

Additional experimental details for protein modification (Figure S16–S19)

Blot images for the ligand screening

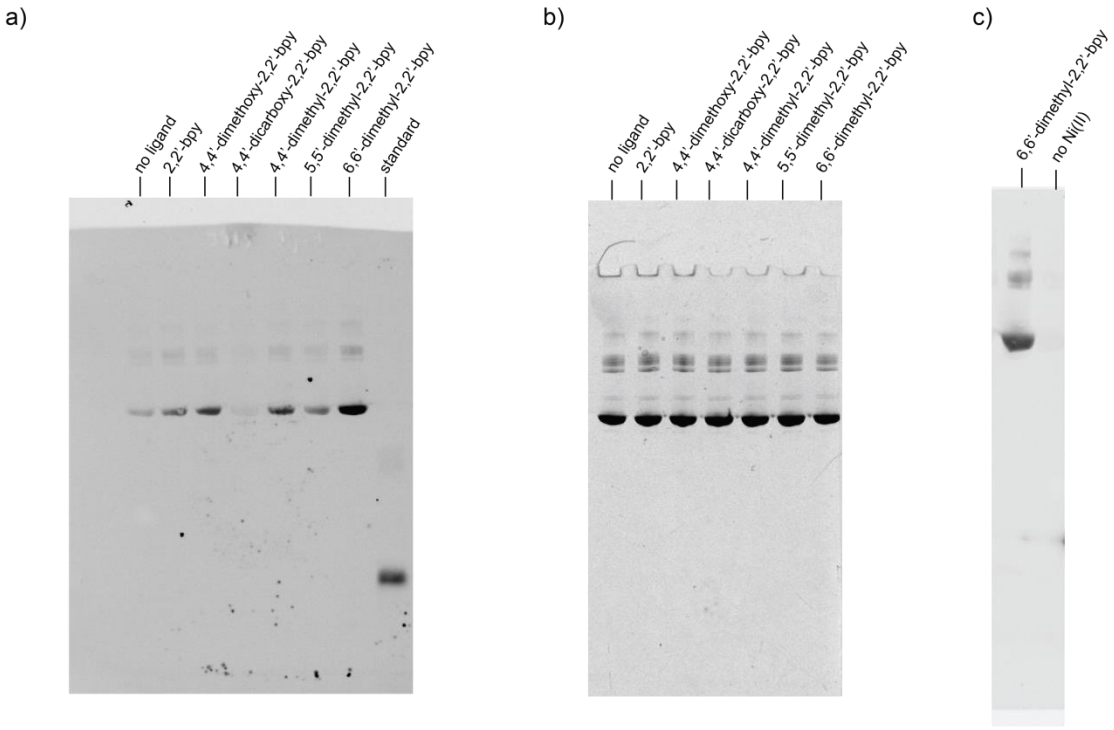


Figure S16. Full-width blot and gel images of Fig. 3a about the modification reaction of BSA with various bipyridine-based ligands. a) Chemical blotting analysis of the modification reaction. b) Coomassie total staining of the modification reaction. Conversion of each sample was calculated from the relative fluorescence intensity of bands with a singly alkyne-tagged soybean trypsin inhibitor with compound **S4** as a standard (intensity = 1.0). c) Negative control data for chemical blotting analysis of the modification reaction.

Blot images for kinetics study

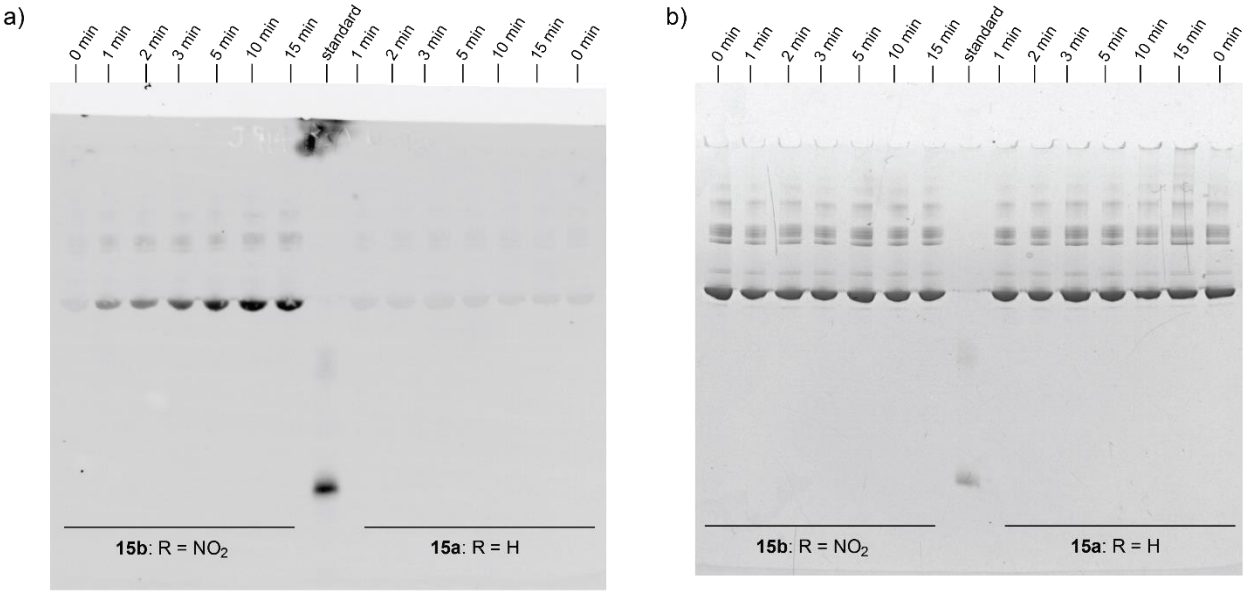


Figure S17. Full-width blot and gel images of Fig. 3b about the modification reaction of BSA with two alkyne-functionalized boronic acid at certain time point. a) Chemical blotting analysis of the modification reaction. b) Coomassie total staining of the modification reaction. Conversion of each sample was calculated from the relative fluorescence intensity of bands with a singly alkyne-tagged soybean trypsin inhibitor with compound **S4** as a standard (intensity = 1.0).

Assessment of stability of modification linkage by chemical blotting

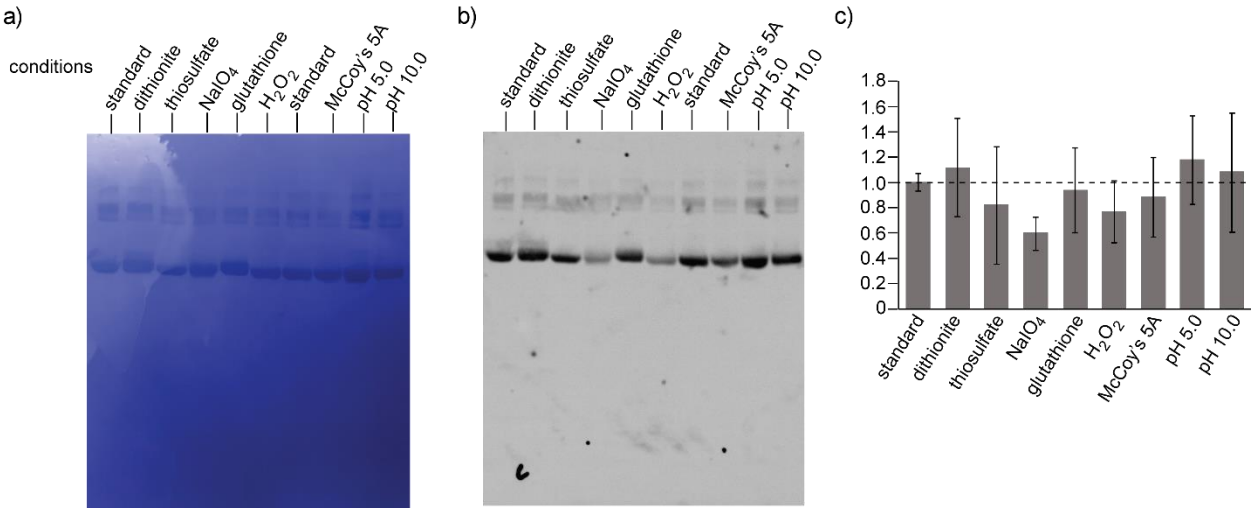
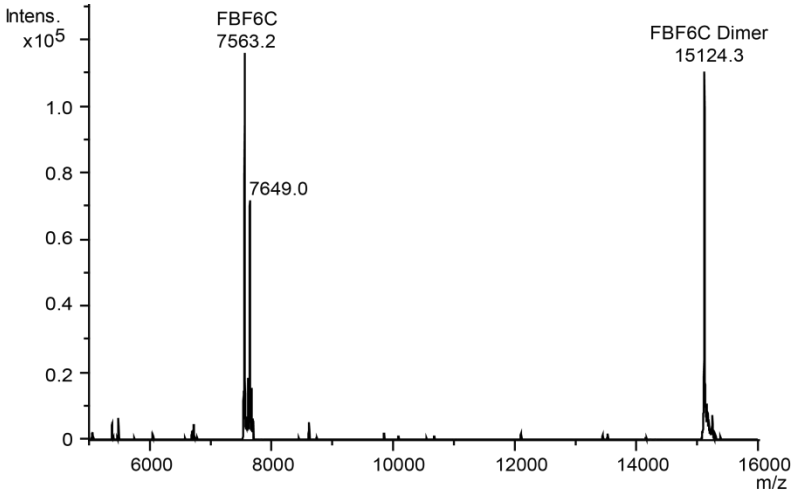


Figure S18. Chemical blot analysis data of stability tests of the alkyne-tagged BSA. a) A CBB gel image. b) A fluorescence blot membrane image by chemical blotting.[2] c) Bar graph representing stability of the alkyne-tagged BSA. Relative band intensity ± S.D. was calculated from three independent experiments. Fluorescence bands were quantified by Multi Gauge software (Fujifilm). Alkyne-tagged BSA (2 μM) was incubated in NMM buffer (50 mM) under the given conditions at rt overnight. Except for cell medium (McCoy's 5A), pH 5.0 (50 mM NaOAc buffer), and pH 10.0 (50 mM borate buffer) experiments, NMM buffer containing 1 mM of each reagent and 0.1% SDS. The rxn mixture was analyzed by chemical blotting. McCoy's 5A medium: cell culture medium containing phenol red. Standard: the alkyne-tagged BSA without incubation of reagents.

LC-MS Spectra of Modification of F6C mutant of the recombinant B-domain of *Staphylococcus aureus* protein A (FBF6C) with nickel(II) and 2-nitrophenylboronic acid (2a)

FBF6C

sequence:
MVDNKCNKEQQNAFYEILHLPNLNEEQRNAFIQSLKDDPSQSANLLAEAKKLNDAQAPKGSHH
HHHH



FBF6C after modification with nickel(II) and 2-nitrophenylboronic acid (2a)

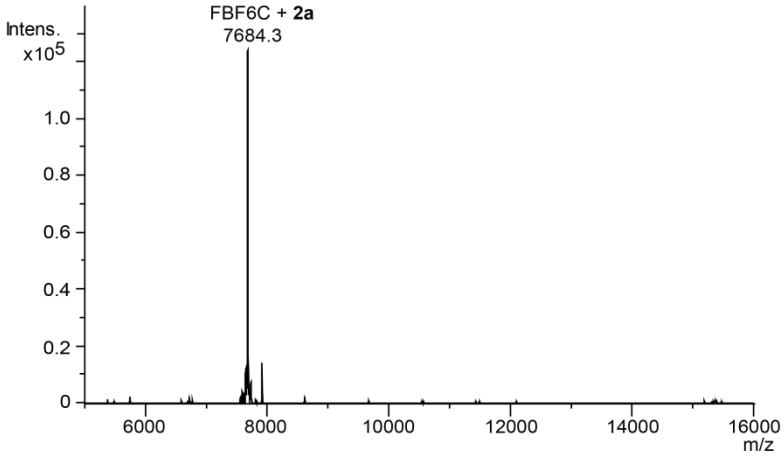


Figure S19. Deconvoluted ESI (LC-MS) spectra of FBF6C before and after modification with 2-nitrophenylboronic acid (**2a**).

Experimental procedures

Metal salt screening for cysteine modification of IL-8 inhibitor (1) with 2-nitrophenylboronic acid (2a)

IL-8 inihibitor **1** (1 μL, 2.5 mM in H_2O) was incubated with 2-nitrophenylboronic acid **2a** (0.5 μL, 50 mM in DMSO) and metal salt (0.5 μL, 25 mM in H_2O) in NMM buffer (10.5 μL, 10 mM, pH 7.5) at 37 °C for 30 min or 24 h (except for Ni(II), Cu(II), and Co(II)). Metal salts: $Ni(OAc)_2$, $Cu(OAc)_2$, $Co(OAc)_2$, $Mg(OAc)_2$, $CaCl_2$, $Mn(OAc)_2$, $Fe(NO_3)_3$, $Zn(OAc)_2$, AgOAc, and $RhCl_3$. The reaction was quenched with EDTA (1 μL, 100 mM in H_2O) and analyzed by MALDI-MS analysis without purification.

Kinetic study of nickel(II)-catalyzed cysteine modification of IL-8 inhibitor (1) with 2-nitrophenylboronic acid (2a)

IL-8 inihibitor **1** (5 μL, 2.5 mM in H_2O) was incubated with 2-nitrophenylboronic acid (**2a**) (2.5 μL, 50 mM in DMSO) and $Ni(OAc)_2$ (2.5 μL, 25 mM in H_2O) in NMM buffers (52.5 μL, 10 mM, pH 7.5) at 37 °C for a given time period. The reaction was quenched with EDTA (5 μL, 100 mM in H_2O) and 50 μL of the resulting solution was analyzed by RP-HPLC for analytical scale (22.5–70% MeCN over 15 min). Concentration of **1** and **3a** in the crude reaction mixture was calculated by using calibration curve of HPLC peak area versus concentration. The sum of the molar extinction coefficient of **1** (ε_{280} = 11000 estimated by ExPASy) and *N*-acetyl-*S*-(2-nitrophenyl)cysteine-*N*′-methylamide (ε_{280} = 3200) was used as the molar extinction coefficient of **3a** at 280 nm (ε_{280} = 14200).

Boronic acid screening for nickel(II)-catalyzed cysteine modification of IL-8 inhibitor (1)

IL-8 inhibitor **1** (1 μL, 2.5 mM in H_2O) was incubated with phenylboronic acid derivatives (**2a**, **2c**–**k**) (0.5 μL, 50 mM in DMSO), and $Ni(OAc)_2$ (1.25 μL, 10 mM in H_2O) in NMM buffer (9.75 μL, 10 mM, pH 7.5) at 37 °C for 1 h. For 2,4-dinitrophenylboronic acid (**2b**), the reaction was performed in MES buffer (10 mM, pH 6.5) at 37 °C for 30 min. The reaction was quenched with EDTA (1 μL, 100 mM in H_2O) and analyzed by MALDI-TOF MS without purification.

Peptide screening

Peptide **1**, **4**–**12** (1 μL, 2.5 mM in H_2O) was incubated with 2-nitrophenylboronic acid (**2a**) (0.5 μL, 50 mM in DMSO) and $Ni(OAc)_2$ (1.25 μL, 10 mM in H_2O) in NMM buffer (9.75 μL, 10 mM, pH 7.5) at 37 °C for 30 min. For **7** and **8**, after preincubation of peptides (1 μL, 2.5 mM in H_2O) with TCEP (1μL, 5 mM in NMM buffer (10 mM, pH 7.5)) in NMM buffer (9.5 μL, 10 mM, pH 7.5) at 37 °C for 30 min, 2-nitrophenylboronic acid **2a** (0.5 μL, 50 mM in DMSO) and nickel(II) acetate (0.5 μL, 25 mM in DMSO) was added to the reaction mixture and the resulting mixture was incubated at 37 °C for 30 min. The reaction was quenched with EDTA (1 μL, 100 mM in H_2O) and analyzed by MALDI-TOF MS without purification.

Typical preparation method of stapled oxytocin 14

After pretreatment of oxytocin (**7**) (100 μL, 2.5 mM in H_2O) with TCEP (5 μL, 100 mM in H_2O) in MES buffer (1145 μL, 10 mM, pH 6.0) at 37 °C for 30 min, 4,5-difluoro-2-nitrophenylboronic acid (**13**) (50 μL, 25 mM in DMSO) and $Ni(OAc)_2$ (50 μL, 25 mM in H_2O) were added. The reaction mixture was incubated at 37 °C for 30 min. The reaction was quenched with EDTA (100 μL, 100 mM in H_2O) and incubated at 37 °C for further 60 min. A part of the reaction mixture (25 μL) was analyzed by RP-HPLC for analytical scale (25–90% MeCN over 20 min) and the rest of the reaction mixture was purified by RP-HPLC for preparative scale (30–50% MeCN over 15 min). The collected fraction was lyophilized and the resulting powder was dissolved into H_2O (50 μL) to obtain a peptide stock solution for the stability tests.

Stability test of stapled oxytocin 15 to chemical cleavage

Stapled oxytocin **14** (3 μL of a stock solution) was incubated in following solution (34.5 μL) at 37 °C for 24 h: aq K_2CO_3 solution (10 mM, pH 11.1), glutathione (reduced form) (10 mM) in NMM buffer (10 mM, pH 7.5), and TCEP (1 mM) in NMM buffer (10 mM, pH 7.5). The reaction mixture (25 μL) was analyzed by RP-HPLC for analytical scale (25–90% MeCN over 20 min). The relative ratio of remained **14** was calculated by comparison of peak areas before and after the reaction.

Stability test of peptides to α-chymotrypsin

1) Stapled oxytocin 14

A stock solution of stapled oxytocin **14** (20 μL) was diluted with Tris buffer (103 μL, 100 mM Tris, 10 mM $CaCl_2$, pH 7.8). Prior to adding enzyme, an aliquot (15 μL) was taken as the 0 h time point. To the remaining solution was added α-chymotrypsin (2 μL, 0.16 mg/mL in Tris buffer (100 mM Tris, 10mM $CaCl_2$, pH 7.8). The resulting mixture was incubated at r.t. for 2 h and an aliquot (15 μL) was taken as the 2 h time point. Aliquots were acidified with 30% TFA in MeCN (15 μL) to deactivate the enzyme. The mixture (15 μL) was analyzed by RP-HPLC for analytical scale (15–55% MeCN over 20 min) with UV detection at 220 nm. The relative ratio of remaining **14** was calculated by comparing the peak area after 2 h to that of the peak area at 0 h.

2) Reduced oxytocin

After pretreatment of oxytocin (**7**) (17 μL, 2.5 mM in H_2O) with TCEP (4 μL, 16 mM in H_2O) at 37 °C for 40 min, a part of the reaction mixture was diluted with H_2O to prepare a solution of reduced oxytocin (100 μL, 200 μM in H_2O). Reduction of oxytocin was confirmed by RP-HPLC for analytical scale. Prior to adding enzyme, an aliquot (15 μL) was taken as the 0 h time point. To the remaining solution was added α-chymotrypsin (5.4 μL, 0.16 mg/mL in Tris buffer (100 mM Tris, 10 mM $CaCl_2$, pH 7.8)). The resulting mixture was incubated at r.t. for 2 h and an aliquot (15 μL) was taken as the 2 h time point. Aliquots were acidified with 30% TFA in MeCN (15 μL) to deactivate the enzyme. The mixture (15 μL) was analyzed by RP-HPLC for analytical scale (15–55% MeCN over 20 min) with UV detection at 220 nm. The relative ratio of remaining reduced oxytocin was calculated by comparing the peak area after 2 h to that of the peak area at 0 h.

3) Peptide mixture of angiotensin I (9), leuprolide, 10-mer peptide, and bradykinin

A mixed solution of angiotensin I (**9**), leuprolide (pEHWSYlLRP-NH_2), 10-mer peptide (H-PHPFHFFVYK-OH), and bradykinin (H-RPPGFSPFR-OH) (103 μL in total, 100 μM of each peptide) in Tris buffer (100 mM Tris, 10 mM $CaCl_2$, pH 7.8) was prepared. Prior to adding enzyme, an aliquot (15 μL) was taken as the 0 h time point. To the remaining solution was added α-chymotrypsin (2 μL, 0.16 mg/mL in Tris buffer (100 mM Tris, 10 mM $CaCl_2$, pH 7.8)). The resulting mixture was incubated at r.t. for 2 h and an aliquot (15 μL) was taken as the 2 h time point. Aliquots were acidified with 30% TFA in MeCN (15 μL) to deactivate the enzyme. The mixture (15 μL) was analyzed by RP-HPLC for analytical scale (15–55% MeCN over 20 min) with UV detection at 220 nm. The relative ratio of remaining native oxytocin was calculated by comparing the peak area after 2 h to that of the peak area at 0 h.

Reversible modification with 2,4-dinitrophenylboronic acid (2b)

Peptide **4** (50 μL, 2.5 mM in H_2O) was incubated with 2,4-dinitrophenylboronic acid (**2b**) (25 μL, 25 mM in DMSO), and $Ni(OAc)_2$ (25 μL, 25 mM in H_2O) in NMM buffer (525μL, 10 mM, pH 7.5) at 37 ^oC for 5 min. The reaction was quenched with EDTA (50 μL, 100 mM in H_2O) and purified by RP-HPLC for preparative scale (17.5–35% MeCN over 30 min). The collected fraction (R_T: 23.2 min) was lyophilized and the resulting powder was dissolved into DMSO to give stock solution of the monoarylated peptide (25 μL, 0.87 mM). The concentration of the stock peptide solution was determined by using molar extinction coefficient of *N*-acetyl-*S*-(2,4-dinitrophenyl)cysteine-*N′*-methylamide (ε_{350} = 9600). For removal of 2,4-dinitrophenyl group, modified peptide (3 μL, 0.87 mM in DMSO) was incubated with DTT (7.5 μL, 200 mM in NMM buffer (10 mM, pH8.0)) in NMM buffer (27 μL, 10 mM, pH 8.0) at 37 °C for 1 h. The reaction was analyzed by RP-HPLC for analytical scale (17.5–35% MeCN over 20 min). Retention time of peptide **4** and monoarylated peptide were 13.3 and 19.9 min, respectively.

Analysis of the protein modifications by chemical blotting[2]

The basic procedure for the detection of an alkyne handle in a modified protein is based on the previously described procedure.[2] After transfer of a protein from polyacrylamide gel to poly(vinylydene)fluoride membrane, the membrane was soaked in a methanol (2 × 2 min), rinsed with water, and placed in 1:1 mixture of H_2O/DMSO. The membrane in the solution was incubated with sodium ascorbate (1.5 mM), THPTA (100 μM), 3-azido-7-hydroxycoumarin (10 μM), and copper(II) sulfate (75 μM) at rt for 30 min to 18 h. The solution was taken out, and the membrane was rinsed with MeOH followed by a 1:1 mixture of MeOH/DMSO-d_6. The process of rinsing with MeOH and MeOH/DMSO-d_6 was repeated total 4 times. Finally, the membrane was rinsed with MeOH and 70%EtOH aq and directly used for fluorescence imaging. Quantification of the fluorescence bands was done using Image J software, using singly modified trypsin inhibitor with **S4** as a protein standard.

Preparation of singly alkyne-tagged trypsin inhibitor as a standard for chemical blotting analysis

Soybean trypsin inhibitor I (0.1 mM) was incubated with 3-(*N*-desthiobiotinyl-propargylamido-methyl)phenyl-BF_3K (**S4**) (1.5 mM) and $Cu(OAc)_2$ (1.5 mM) in HEPES buffer (0.1 M, pH 7.1, total reaction volume: 1 mL) at rt overnight. The reaction mixture was quenched with EDTA (7 mM) and passed through size exclusion column (5 mM, pH 7.4 NMM buffer as an eluent). The obtained solution was directly applied

to streptavidin beads pre-equilibrated with the NMM buffer. After the suspension was shaken for 3 h, the supernatant was removed, and the beads were washed with the NMM buffer (2 × 6 mL). The desthiobiotinylated protein on the beads was eluted with NMM buffer (50 mM, pH 7.4, 1 mL) containing 5 mM biotin at rt overnight. The eluted solution was passed through the size exclusion column (5 mM, pH 7.4 NMM buffer as an eluent) and concentrated to ~0.2 mL by centrifugal filter (MWCO: 3 kDa). The concentration of the solution was determined by UV absorbance of the protein at 280 nm. The final solution was split into aliquots, frozen with liquid nitrogen, and stored in –20 °C.

Ligand screening for protein modification

BSA (1.4 μL, 884 μM in NMM buffer (50 mM, pH 7.4)) was incubated with alkyne-tagged boronic acid **15b** (0.25 μL, 50 mM in DMSO), bipyridine derivatives (0.5 μL, 50 mM in DMSO), and $Ni(OAc)_2$ (0.5 μL, 50 mM in H_2O) in NMM buffer (22.35 μL, 50 mM, pH 7.4) at 37 ^oC for 30 min. The reaction was quenched with EDTA (0.2 μL, 1 M in H_2O) and the sample (5 μL) was analyzed by chemical blotting.

BSA modification kinetics

BSA (1.4 μL, 884 μM in NMM buffer (50 mM, pH 7.4)) was incubated with alkyne-tagged boronic acid **15a** or **15b** (0.25 μL, 50 mM in DMSO), 6,6-dimethylbipyriridne (0.5 μL, 50 mM in DMSO), and $Ni(OAc)_2$ (0.5 μL, 50 mM in H_2O) in NMM buffer (22.35 μL, 50 mM, pH 7.4) at 37 ^oC for a given time period. The reaction was quenched with EDTA (0.2 μL, 1 M in H_2O) and analyzed by chemical blotting.

Stability experiment of alkyne-tagged BSA

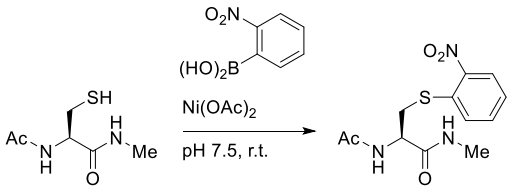
BSA (50 μM) was incubated with 4,4'-dimethoxy-2,2'-bipyridine (1 mM), alkyne-tagged nitroboronic acid **15b** (1 mM), and $Ni(OAc)_2$ (1 mM) in NMM buffer (50 mM, pH 7.4) at 37 °C for 30 min. The reaction mixture was quenched by addition of EDTA solution (5 mM) and passed through size exclusion column. The obtained solution was concentrated by centrifugal filter (MWCO: 10 kDa), split into aliquots, frozen with liq. N_2, and stored in –20 °C. Alkyne-tagged BSA (2 μM) was incubated in NMM buffer (50 mM) under given conditions at rt overnight. Except for cell medium (McCoy's 5A), pH 5.0 (50 mM NaOAc buffer), and pH 10.0 (50 mM borate buffer) experiments, NMM buffer containing 1 mM reagents was used. The reaction mixture was analyzed by chemical blotting. Heat denaturation before the SDS-PAGE process was omitted to prevent overreaction by reagents.

Modification of the F6C mutant of the recombinant B-domain of *Staphylococcus Aureus* protein A with nickel(II) and 2-nitrophenylboronic acid

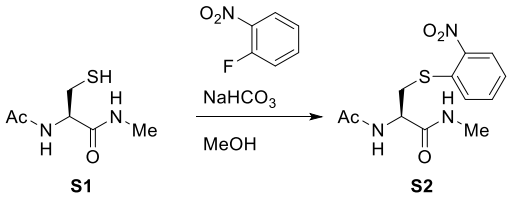
After treatment of FBF6C (1.6 μL, 763 μM in NMM buffer (50 mM, pH 7.5)) with TCEP (0.5 μL, 12.5 mM in NMM buffer (50 mM, pH 7.5)) in NMM buffer (21.4 μL, 50 mM, pH 7.5) at 37 °C for 30 min, 2-nitrophenylboronic acid (**2a**) (0.5 μL, 25 mM in DMSO), 6,6-dimethylbipyridne (0.5 μL, 50 mM in DMSO), and $Ni(OAc)_2$ (0.5 μL, 50 mM in H_2O) were added. The reaction mixture was incubated at 37 ^oC for 30 min. The reaction was quenched with EDTA (0.5 μL, 1 M in H_2O) and analyzed by LCMS.

Organic synthesis

N-Acetyl-*S*-(2-nitrophenyl)cysteine-*N′*-methylamide (S2)



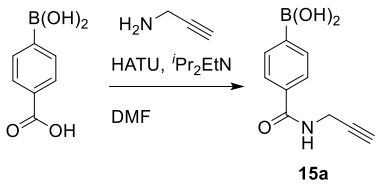
Nickel(II)-catalyzed *S*-arylation: To a solution of *N*-acetylcysteine-*N′*-methylamide (**S1**) (36 mg, 0.21 mmol) in a mixture of *N*-methylmorpholine buffer (10 mM, pH 7.5, 960 mL) and DMSO (40 mL) were added 2-nitrophenylboronic acid (338 mg, 2.02 mmol) and $Ni(OAc)_2$ (249 mg, 1.00 mmol). The solution was stirred at rt until the starting material was completely consumed, confirmed by TLC monitoring (~1 h). The reaction was quenched with EDTA solution (100 mM, 100 mL) and the resulting solution was salted-out by adding NaCl. The organic materials were extracted with EtOAc (100 mL and 2 × 60 mL). The combined organic layer was washed with aq HCl solution (2 M, 100 mL) and brine (100 mL), dried over Na_2SO_4, filtered, and evaporated in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 50:1) to afford **S2** as a yellow solid (30 mg, 49%).



Conventional S_NAr reaction: To a solution of *N*-acetylcyteine-*N′*-methylamide (**S1**) (102 mg, 0.579 mmol) in MeOH (2 mL) were added $NaHCO_3$ (145 mg, 1.73mmol) and 2-fluoronitrobenzene (91.4 μL, 0.869 mmol). The resulting suspension was stirred at rt overnight. After addition of H_2O (3 mL), the precipitates were collected by filtration and washed with MeOH to afford **S2** as a light yellow solid (75 mg, 43%).

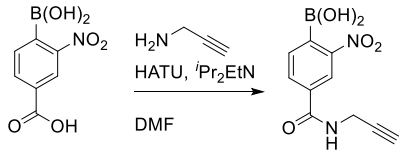
^{1}H NMR (600 MHz, DMSO-d_6): δ 8.31 (d, J = 8.1 Hz, 1H), 8.18 (dd, J = 1.0, 8.3 Hz, 1H), 8.07 (br, 1H), 7.78 (d, J = 8.1 Hz, 1H), 7.72 (ddd, J = 1.0, 7.2, 8.1 Hz, 1H), 7.41 (dd, J = 7.2, 8.3 Hz, 1H), 4.44–4.47 (m, 1H), 3.39 (dd, J = 5.7, 13.3 Hz, 1H), 3.17 (dd, J = 8.3 Hz, 13.3 Hz, 1H), 2.58 (d, J = 4.6 Hz, 3H), 1.85 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 169.2, 168.9, 145.4, 135.1, 133.7, 126.9, 125.3, 124.8, 50.8, 33.3, 25.1, 21.9. ESI-MS: calcd for $C_{12}H_{16}N_3O_4S$ $[M+H]^+$: 298.1, found 298.1.

4-(Propargylaminocarbonyl)phenylboronic acid (15a)



4-carboxyphenylboronic acid (16 mg, 0.098 mmol) and HATU (36 mg, 0.094 mmol) were dissolved in DMF (0.2 mL). Diisopropylethylamine (23 µL, 0.14 mmol) was added at 0 °C, and the solution was stirred at rt for 10 min. Propargylamine (14 µL, 0.23 mmol) was added at 0 °C, and the solution was stirred at rt for 7 h. All of volatiles were removed by gentle flow of nitrogen gas. The resulting oil was purified by reverse-phase HPLC (10–43.3% MeCN over 15 min). Lyophilization of the collected fractions afforded **15a** as a colorless powder (15 mg, 81%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.91 (t, J = 5.5 Hz, 1H), 8.18 (br, 2H), 7.85 (d, J = 8.1 Hz, 2H), 7.80 (d, J = 8.1 Hz, 2H), 4.05 (dd, J = 5.5, 2.4 Hz, 2H), 3.11 (t, J = 2.4 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 166.0, 135.0, 134.0, 126.1, 81.3, 72.8, 28.5. ESI-MS: calcd for $C_{10}H_{11}BNO_3$ $[M+H]^+$ 204.1, found 204.1.

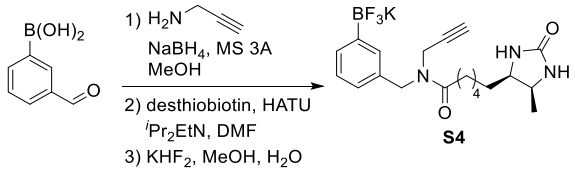
4-(Propargylaminocarbonyl)-2-nitrophenylboronic acid (15b)



15b

4-Carboxy-2-nitrophenylboronic acid (23 mg, 0.11 mmol) and HATU (42 mg, 0.11 mmol) were dissolved in DMF (0.2 mL). Diisopropylethylamine (25 µL, 0.14 mmol) was added at 0 °C, and the solution was stirred at rt for 10 min. Propargylamine (15 µL, 0.24 mmol) was added at 0 °C, and the solution was stirred at rt for 7 h. All of volatiles were removed by gentle flow of nitrogen gas. The resulting oil was purified by reverse-phase HPLC (5–20% MeCN over 22 min). Lyophilization of the collected fractions afforded the titled compound **15b** as a colorless powder (18 mg, 67%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.30 (t, J = 5.4 Hz, 1H), 8.60 (d, J = 1.3 Hz, 1H), 8.31 (br, 2H), 8.21 (dd, J = 7.6, 1.3 Hz, 1H), 7.67 (d, J = 7.6 Hz, 1H), 4.10 (dd, J = 5.4, 2.4 Hz, 2H), 3.17 (t, J = 2.4 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 164.0, 150.0, 134.5, 132.7, 132.4, 121.3, 80.9, 73.2, 28.7. ESI-MS: calcd for $C_{10}H_9BN_2O_5 \cdot CF_3COO$ $[M+CF_3COO]^-$ 361.1, found 361.0.

Potassium [3-(*N*-desthiobiotinyl-*N*-propargylaminomethyl)phenyl]trifluoroborate (S4)



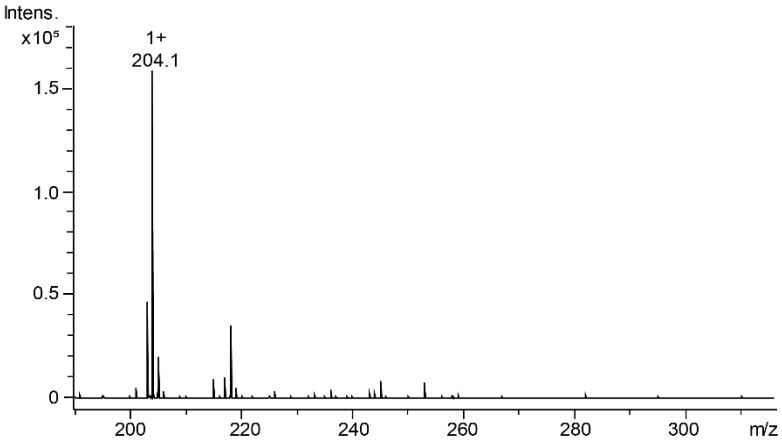
S4

3-Formylphenylboronic acid (103 mg, 0.686 mmol) was dissolved in MeOH (1.5 mL). To the solution, propargylamine (0.20 mL, 3.1 mmol) and microwave-preactivated 3A molecular sieves were added, and the suspension was stirred at rt for 2 h. $NaBH_4$ (33 mg, 0.87 mmol) was added at 0 °C in two portions, and the suspension was stirred at the same temperature for 45 min. After removal of the molecular sieves by filtration, the solution was acidified by HCl aq and stirred at rt for 1 h. All of the volatiles were removed by gentle flow of nitrogen gas, and the crude material was purified by reverse-phase HPLC (10–33.8% MeCN) to afford the secondary amine intermediate as a trifluoroacetate salt (94 mg, 45%). Desthiobiotin (17 mg, 0.081 mmol) and HATU (30 mg, 0.080 mmol) were dissolved in DMF (0.5 mL). Diisopropylethylamine (34 µL, 0.20 mmol) was added at 0 °C, and the solution was stirred at rt for 10 min.

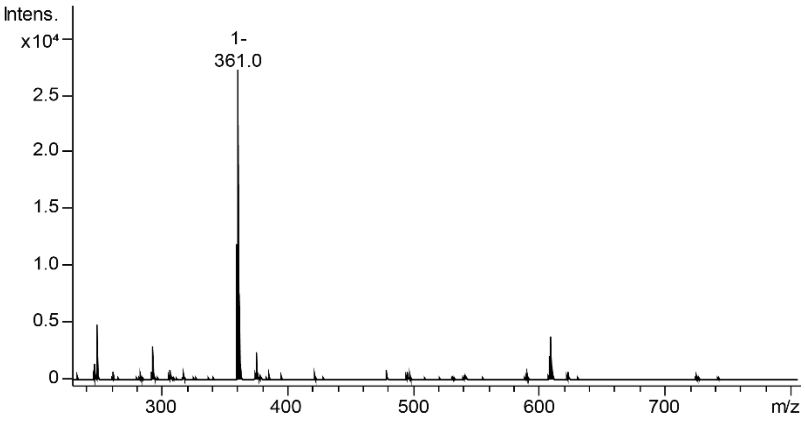
The solution was cooled to 0 °C, added to the ammonium trifluoroacetate salt (20 mg, 0.065 mmol), and stirred at rt for 4 h. Pinacol (44 mg, 0.37 mmol) was added, and the solution was stirred at rt overnight. All of volatiles were removed by gentle flow of nitrogen gas. The remaining oil was diluted with CH_2Cl_2 (10 mL). The solution was washed with sat aq $KHSO_4$ solution (3 × 5 mL), dried over Na_2SO_4, filtered, and dried under vacuum to give a boronic acid pinacol ester intermediate. The intermediate was dissolved in MeOH (0.3 mL), and solution of KHF_2 (26 mg, 0.33 mmol) in H_2O (0.2 mL) was added. After the solution was shaken for 3 h, all of volatiles were removed by gentle flow of nitrogen gas. Obtained solid was suspended in MeCN (10 mL), and insoluble materials were separated by filtration. The solution was concentrated to ~0.5 mL under vacuum, and the addition of ether (6 mL) gave precipitation. The suspension was placed in –20 °C for 3 h, and the supernatant was removed with a Pasteur pipette, followed by trituration with ether (3 × 3 mL). Trace amount of ether was removed from the solid in vacuo, and the solid was dissolved in water. Lyophilization of the solution afforded **S4** as a mixture of two amide rotamers (20 mg, 69% from the secondary amine intermediate). ^{1}H NMR (600 MHz, DMSO-d_6, mixture of rotamers): δ 7.22 (m, 1H), 7.17 (s, 1H), 7.06 (m, 1H), 6.86 (d, J = 7.2 Hz, 1H), 6.27 (m, 1H), 6.08 (m, 1H), 4.56 (s, 1.2H), 4.46 (s, 0.8H), 4.03 (s, 1.2H), 3.97 (s, 0.8H), 3.59 (m, 1H), 3.46 (m, 1H), 2.42 (m, 0.8H), 2.34 (m, 1.2H), 1.54 (m, 2H), 1.25 (m, 6H), 1.16 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6, mixture of rotamers): δ 172.1, 172.0, 162.8, 134.3, 133.9, 131.1, 130.6, 130.4, 129.8, 126.6, 126.4, 124.5, 123.3, 79.9, 79.6, 74.7, 73.9, 55.01, 54.97, 50.2, 50.1, 47.8, 40.1, 35.8, 33.5, 32.3, 32.2, 29.5, 28.8, 28.7, 25.7, 25.0, 24.7, 24.6, 15.5. ESI-MS: calcd for $C_{20}H_{26}BF_3N_3O_2$ $[M-K]^-$ 408.3, found 408.4.

ESI-MS spectra of synthesized compounds

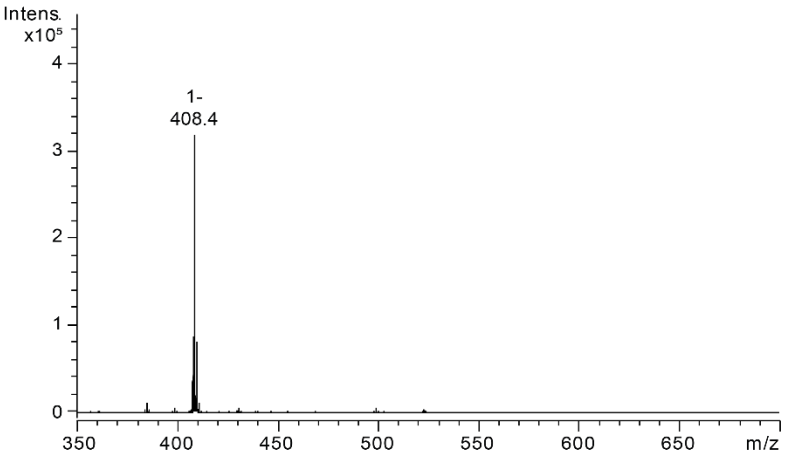
4-(Propargylaminocarbonyl)phenylboronic acid (15a)



4-(Propargylaminocarbonyl)-2-nitrophenylboronic acid (15b)

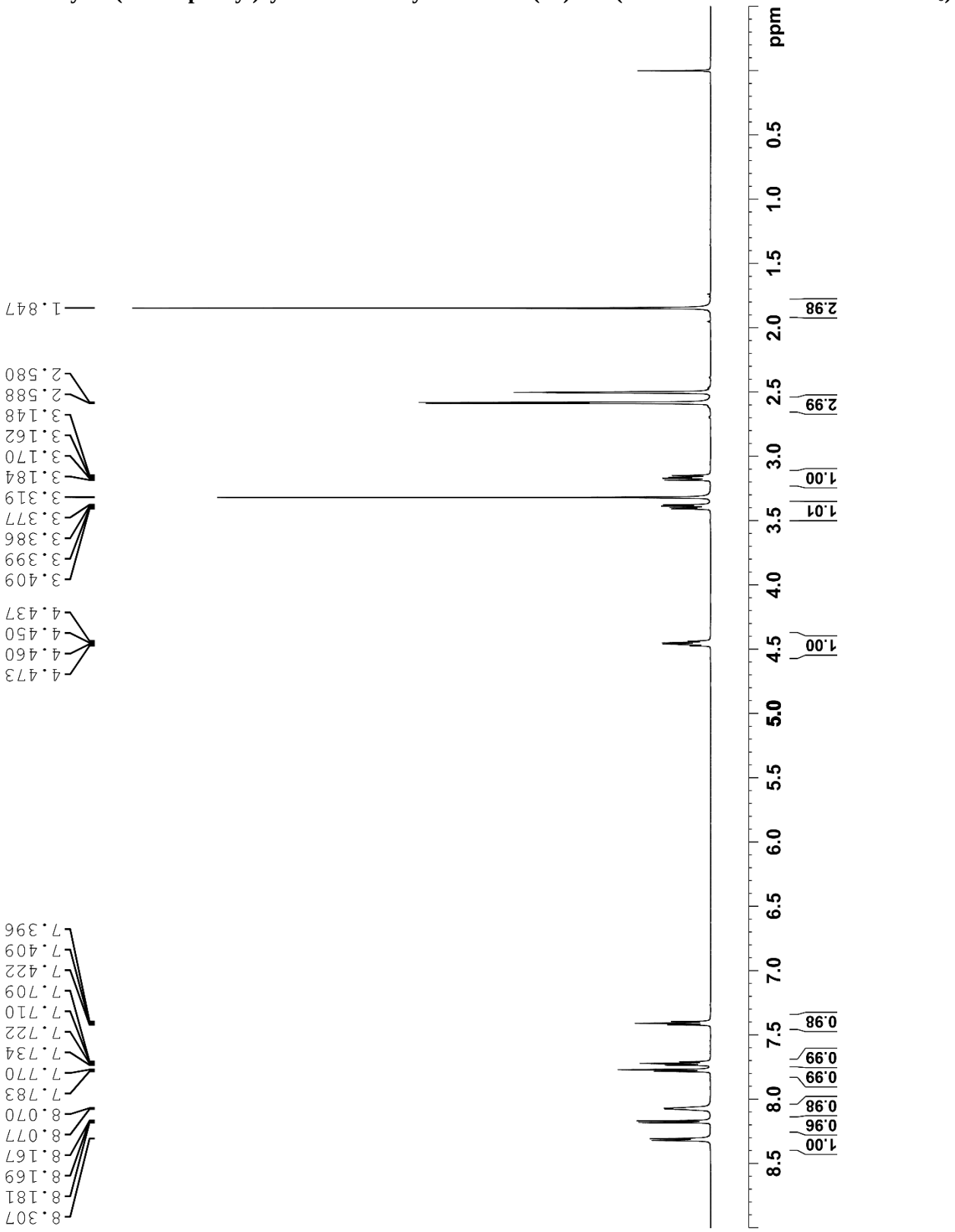


Potassium [3-(*N*-desthiobiotinyl-*N*-propargylaminomethyl)phenyl]trifluoroborate (S4)

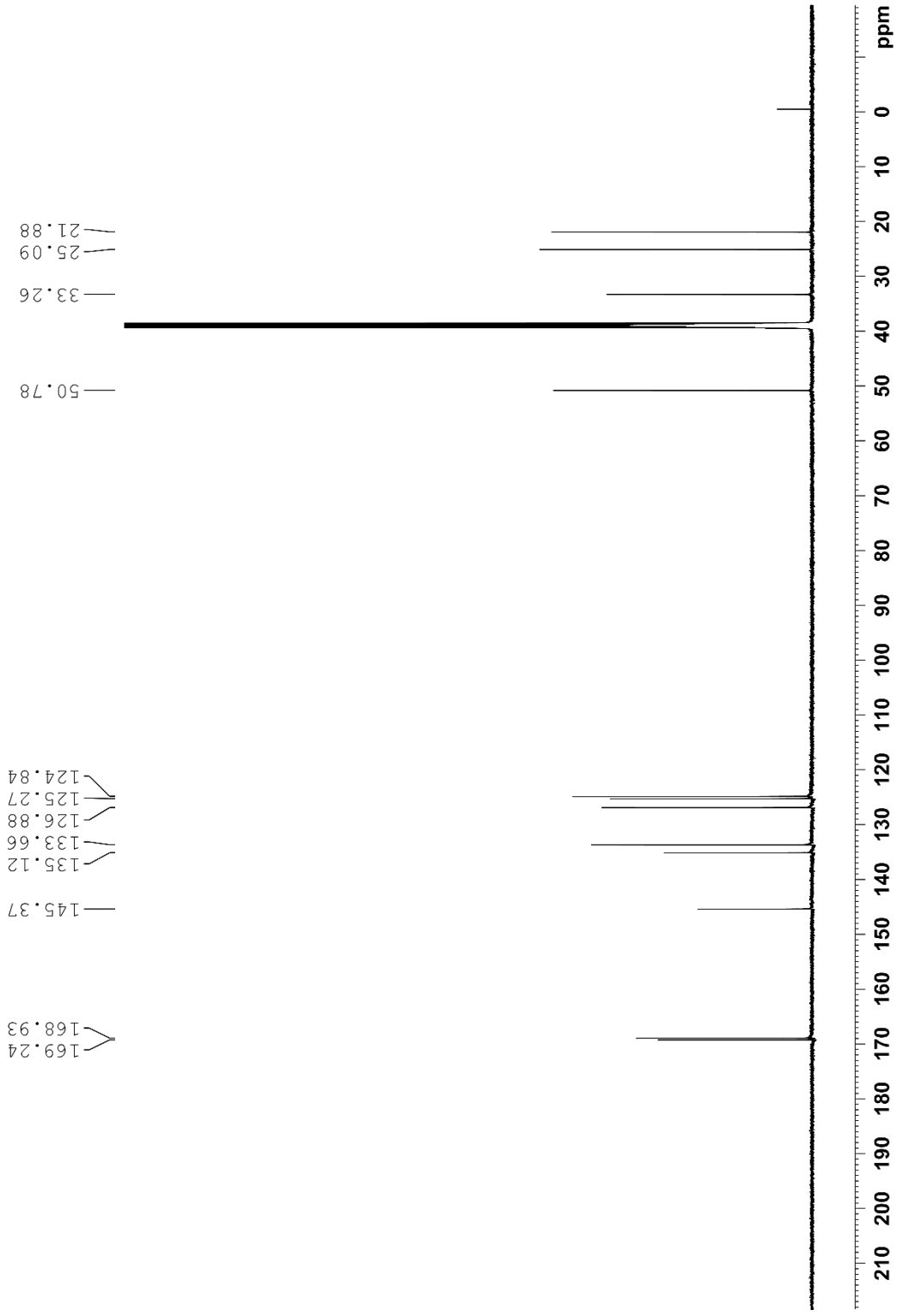


^{1}H and ^{13}CNMR spectra of synthesized compounds

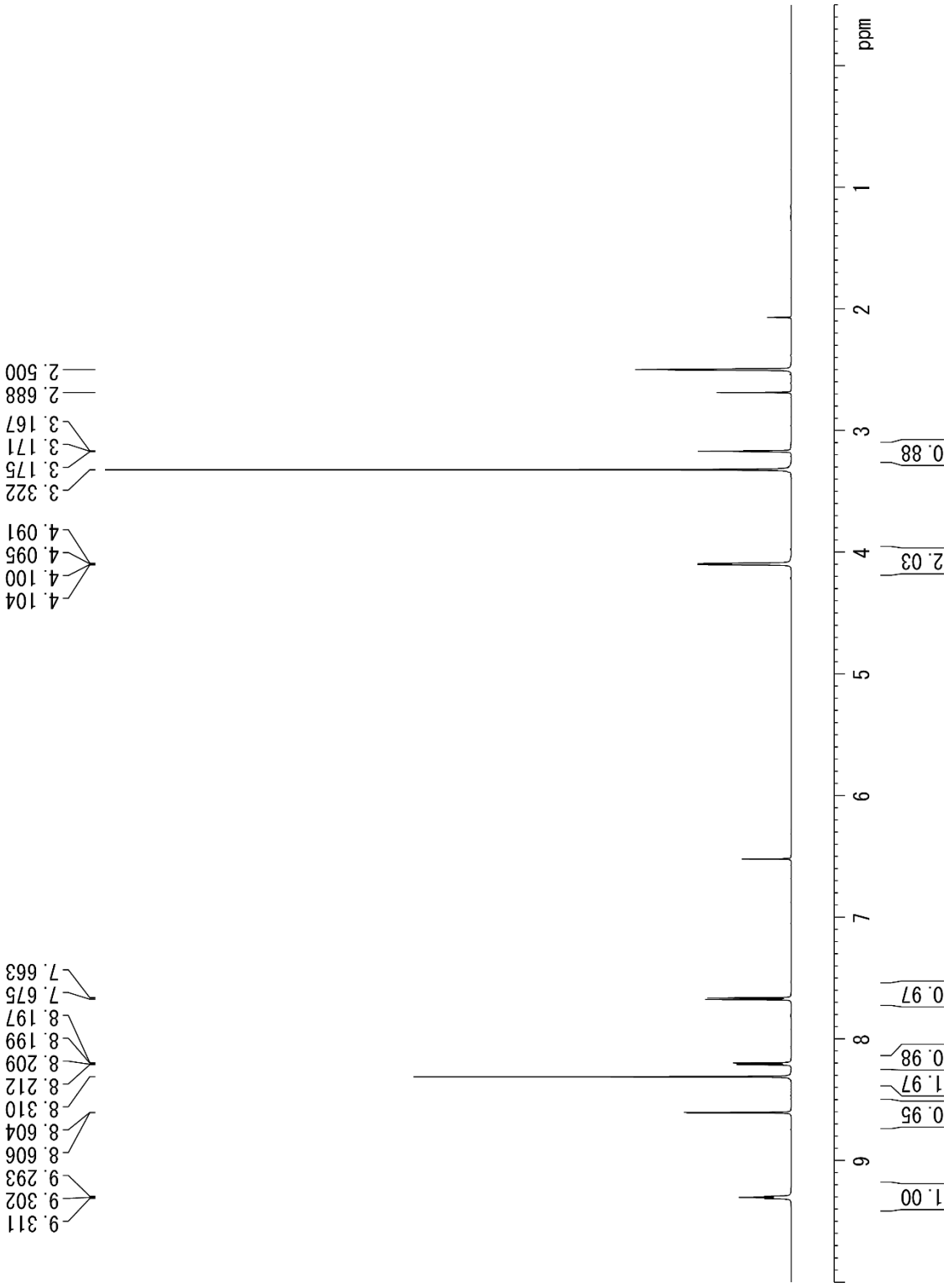
***N*-Acetyl-*S*-(2-nitrophenyl)cyteine-*N′*-methylamide (S2) (^{1}H NMR in DMSO-d_6)**



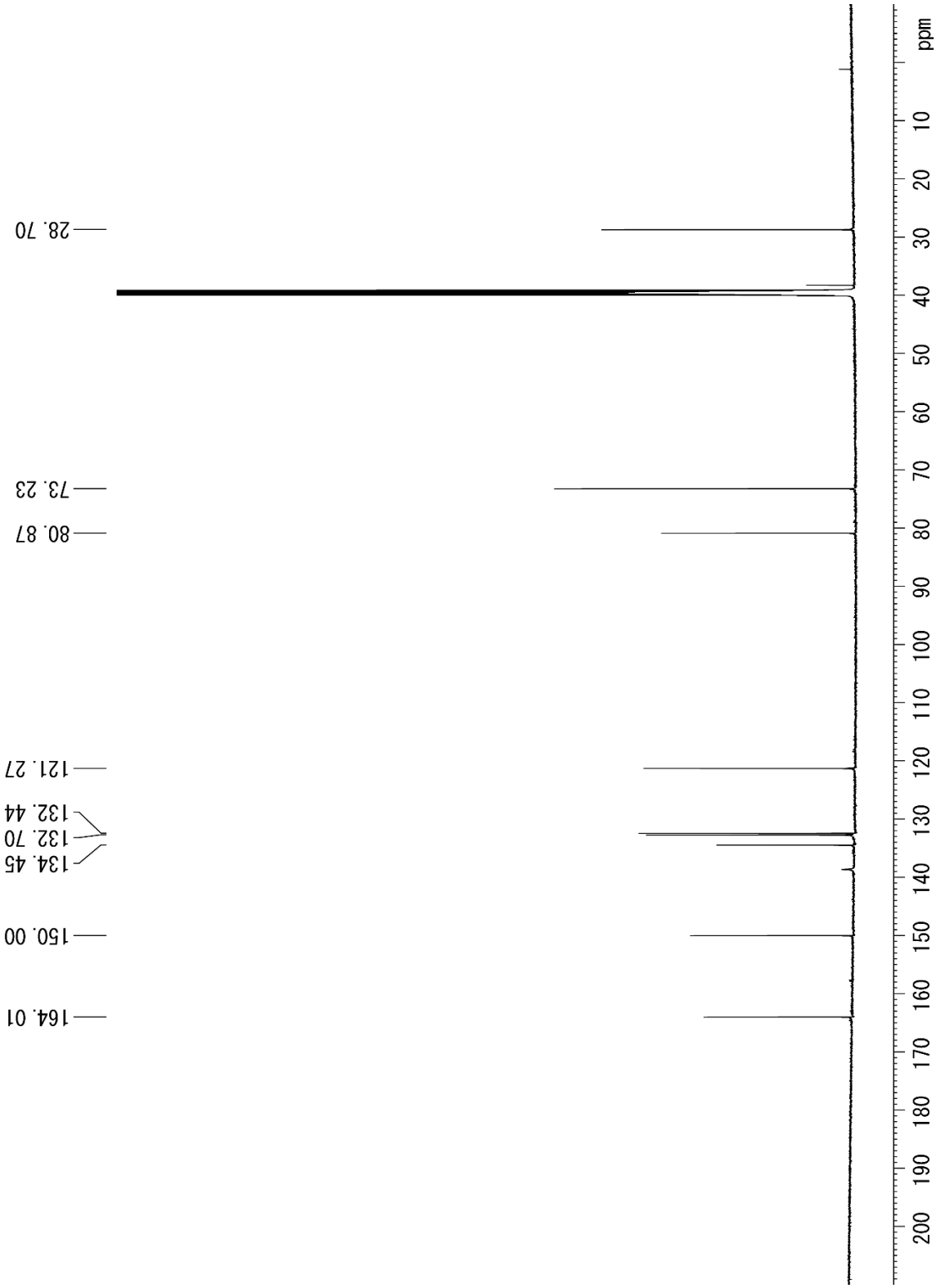
***N*-Acetyl-*S*-(2-nitrophenyl)cyteine-*N′*-methylamide (S2) (^{13}C NMR in DMSO-d_6)**



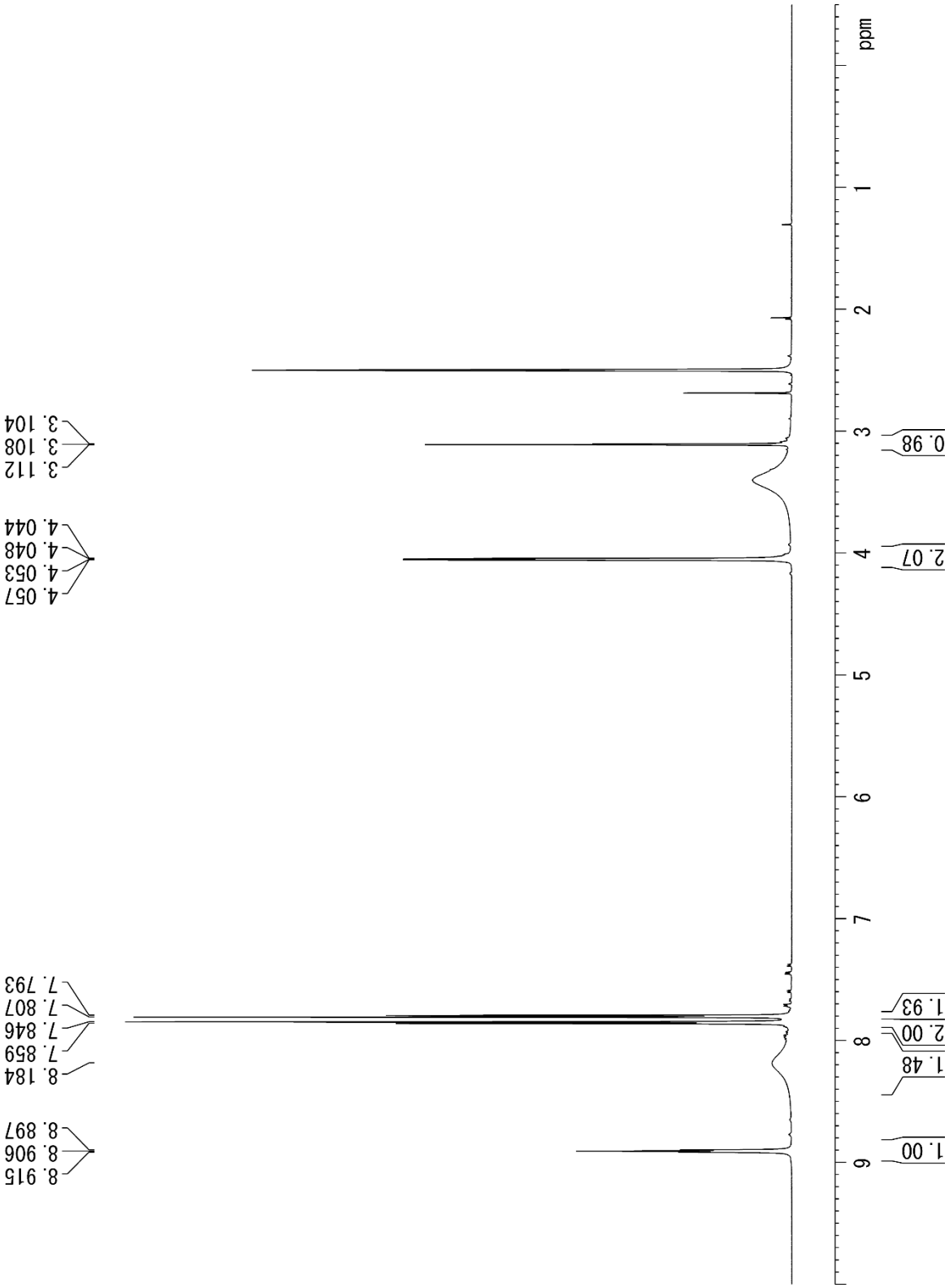
4-(Propargylaminocarbonyl)-2-nitrophenylboronic acid (15a) (^{1}H NMR in DMSO-d_6)



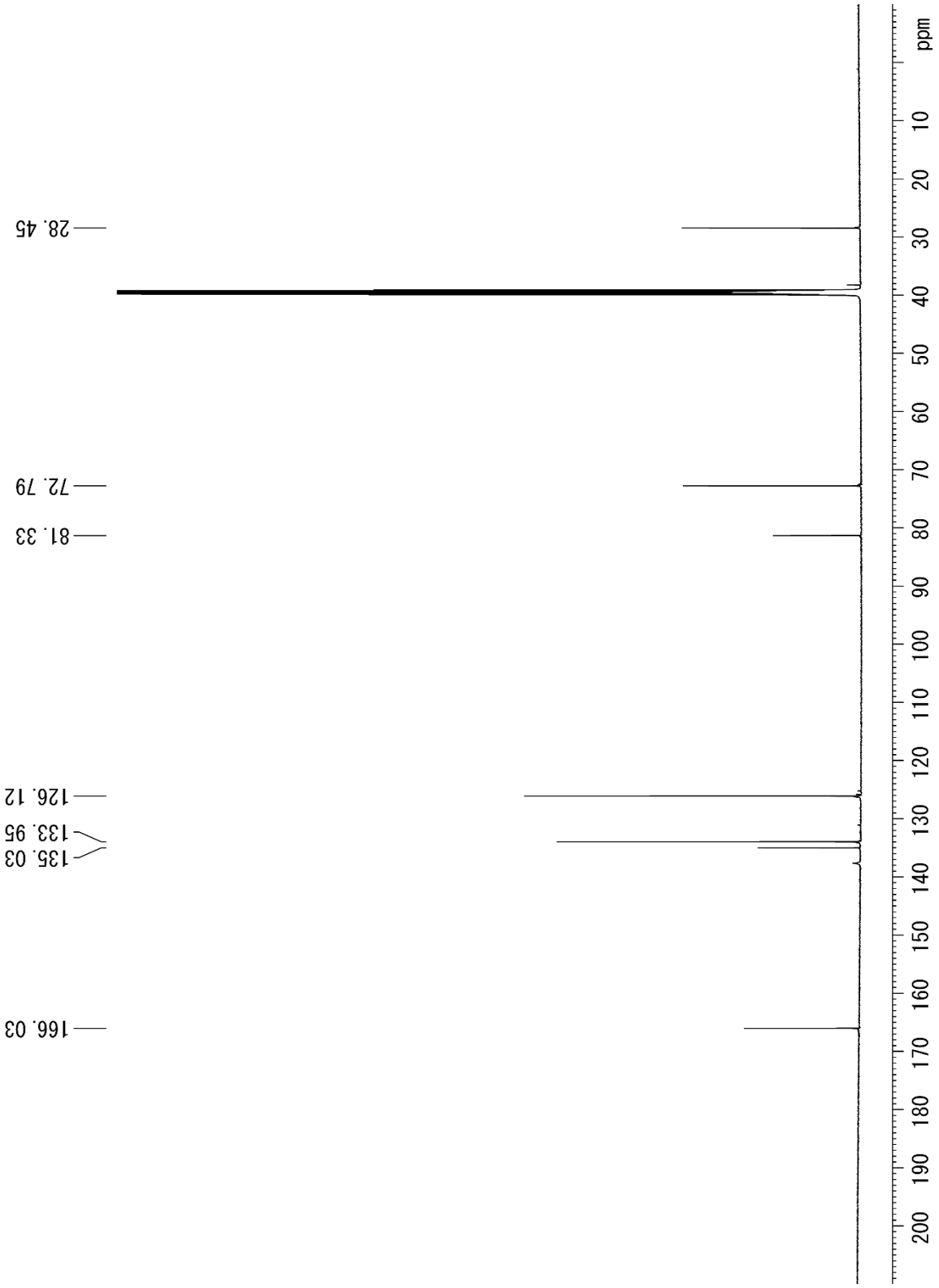
4-(Propargylaminocarbonyl)-2-nitrophenylboronic acid (15a) (^{13}C NMR in DMSO-d_6)



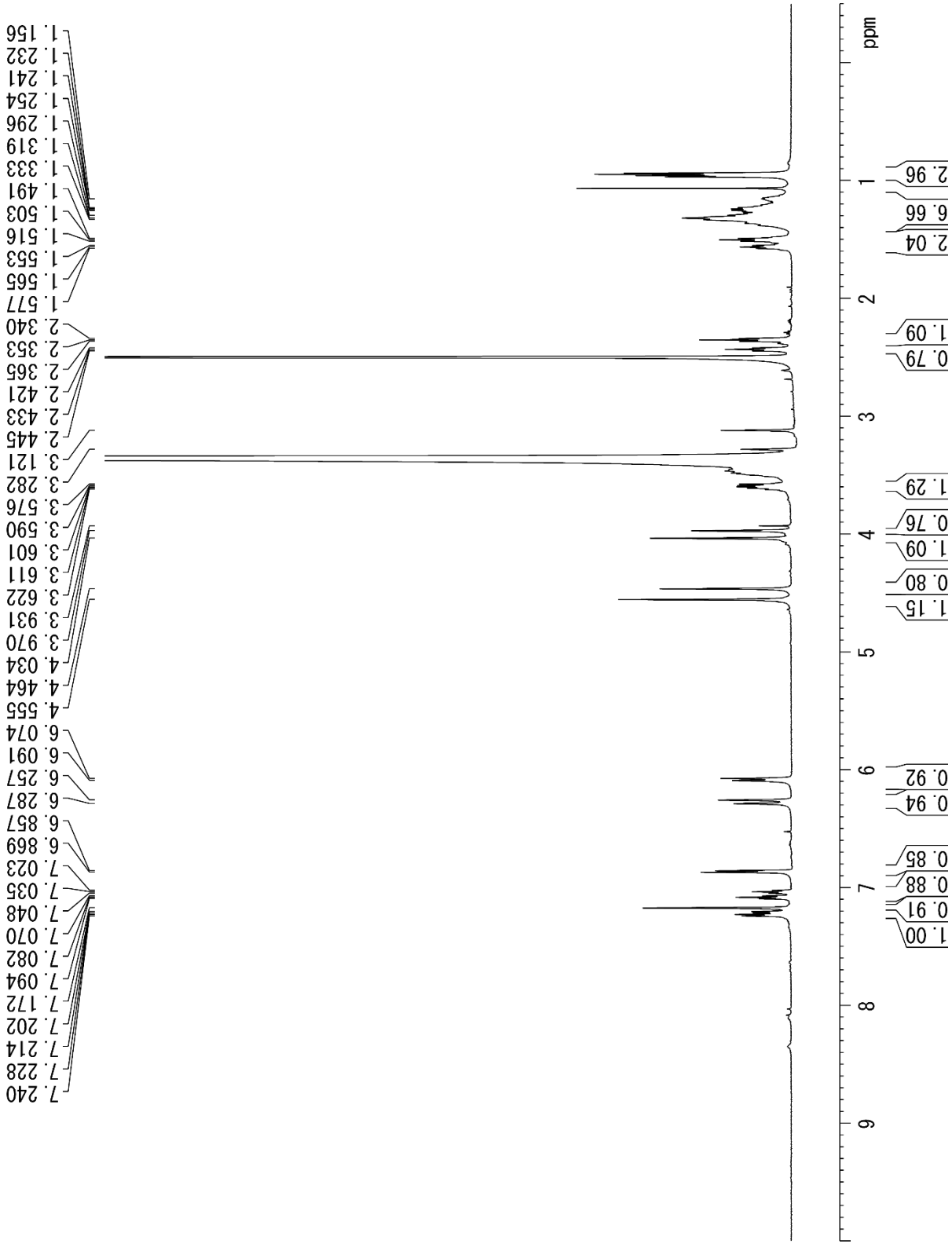
4-(Propargylaminocarbonyl)phenylboronic acid (15b) (^{1}H NMR in DMSO-d_6)



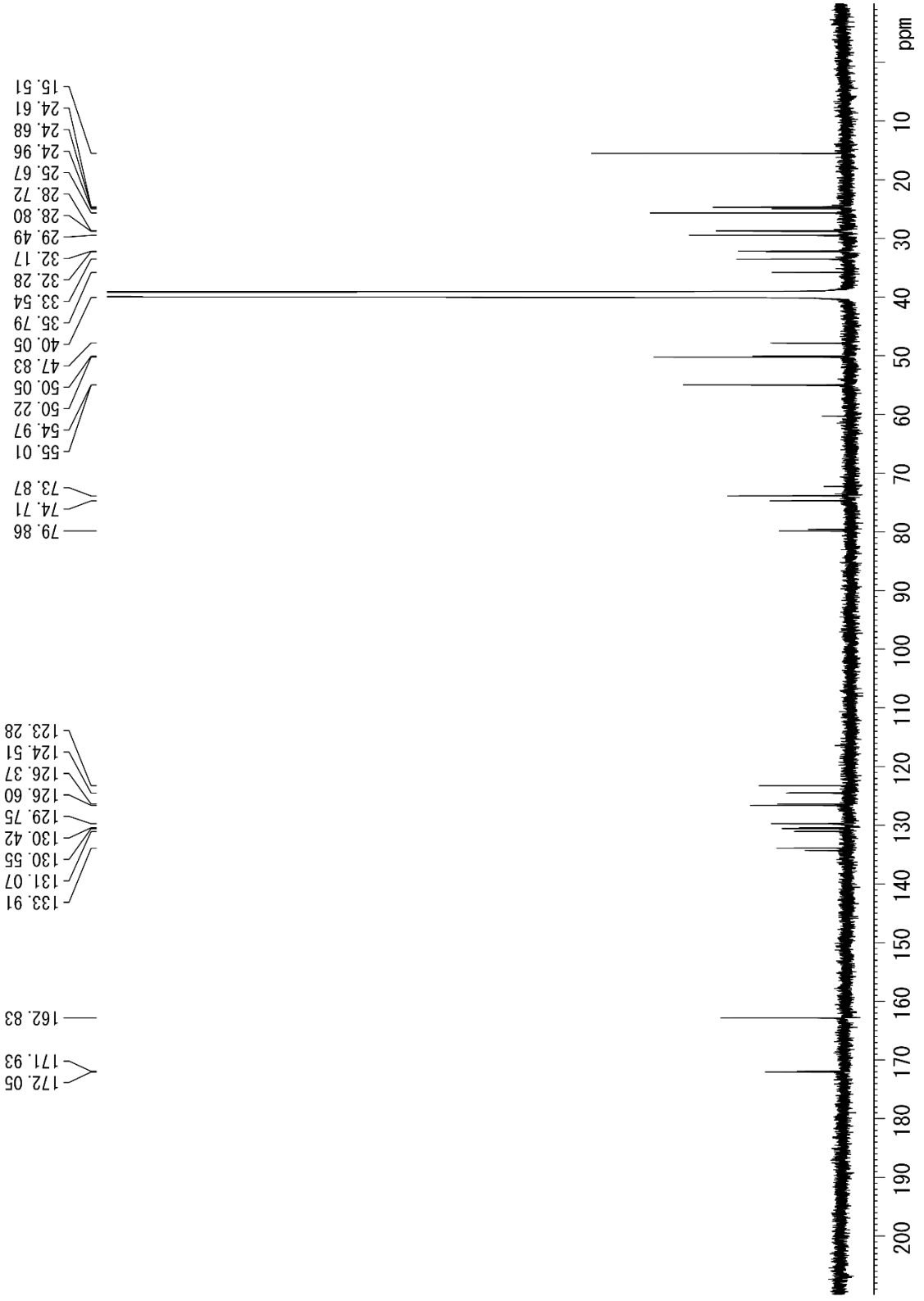
4-(Propargylaminocarbonyl)phenylboronic acid (15b) (^{13}C NMR in DMSO-d_6)



Potassium [3-(*N*-desthiobiotinyl-*N*-propargylaminomethyl)phenyl]trifluoroborate (S4) (^{1}H NMR in DMSO-d_6)



Potassium [3-(*N*-desthiobiotinyl-*N*-propargylaminomethyl)phenyl]trifluoroborate (S4) (^{13}C NMR in DMSO-d_6)



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2 J. Ohata, F. Vohidov and Z. T. Ball, *Mol. Biosyst.*, 2015, **11**, 2846–2849.