

Supporting Information

Nickel(II)-promoted amide N-H arylation of pyroglutamate-histidine with arylboronic acid reagents

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General information

All of chemicals were purchased from commercial suppliers and used without further purification. LHRH (**1**) (L7134), leuprolide (**4**) (L0399), MF- α (1–6) (**7**) (T2903), and angiotensin I (**9**) (A9650) were purchased from Sigma-Aldrich. Bombesin (**5**) (J63514, Alfa) was purchased from Alfa Aesar. H-PHPFHHFFVYK-OH (4010534) (**6**) and somatostatin (H-1490) (**12**) was purchased from BACHEM. Ac-ACTH (1–17) (**8**) (5-00544) was purchased from CHI Scientific Inc.). Bradykinin (**10**) (07913) was purchased from Chem-Impex Int'l. Inc. IL-8 inhibitor (**11**) (AS-62401) was purchased from AnaSpec Inc. *N*-Methylmorpholine (NMM) buffer (pH 8.0–8.5) and *N,N'*-diethylpiperazide (NEP) buffer (pH 9.0–9.5) were prepared by addition of NMM (TCI, M0370) or NEP (Alfa Aesar, B24743) to MilliQ water. The pH of the NMM solution and the NEP solution was adjusted to desired pH by addition of aq HCl soln. 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 4 μ Proteo 90A (250 mm \times 4.6 mm for analytical scale) and Phenomenex Jupiter 4 μ Proteo 90A (250 \times 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 absorption 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 peptide and protein experiments. A sample was mixed with sinapic acid (20 mg/mL soln in 50:50:0.1 H₂O/MeCN/TFA) or α -cyano-4-hydroxycinnamic acid (10 mg/mL soln in 50:50:0.1 H₂O/MeCN/TFA) on a MALDI plate.

Gel and blot imaging were 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.

¹H and ¹³C NMR spectra were obtained on Bruker AVANCE 600 spectrometer.

Additional experimental data for kinetics of nickel(II)-catalyzed modification of LHRH (1): RP-HPLC chromatograms

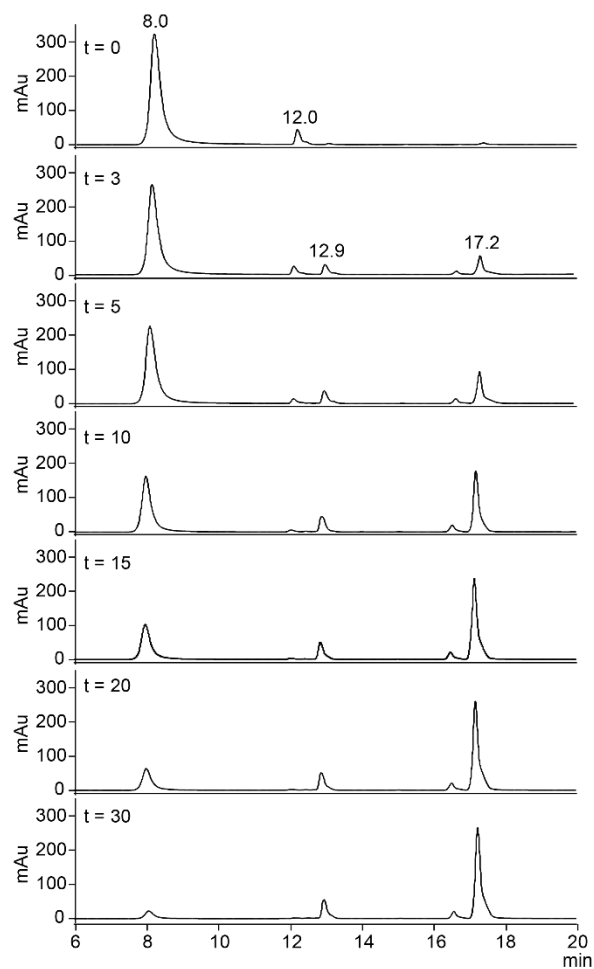


Figure S1. RP-HPLC analysis data of crude reaction mixture of nickel(II)-catalyzed Glp-His modification of LHRH (1) with 2-nitrophenylboronic acid (2a) after incubation for a given time period (t min). Numbers on each peak represent retention times (min). The fractions at 8.0, 12.0, 12.9, and 17.2 min correspond to 2-nitrophenylboronic acid (2a), peptide 1, modified peptide 3a, and nitrobenzene, respectively.

Additional experimental data for nickel(II)-catalyzed amide N-H arylation of LHRH (1)

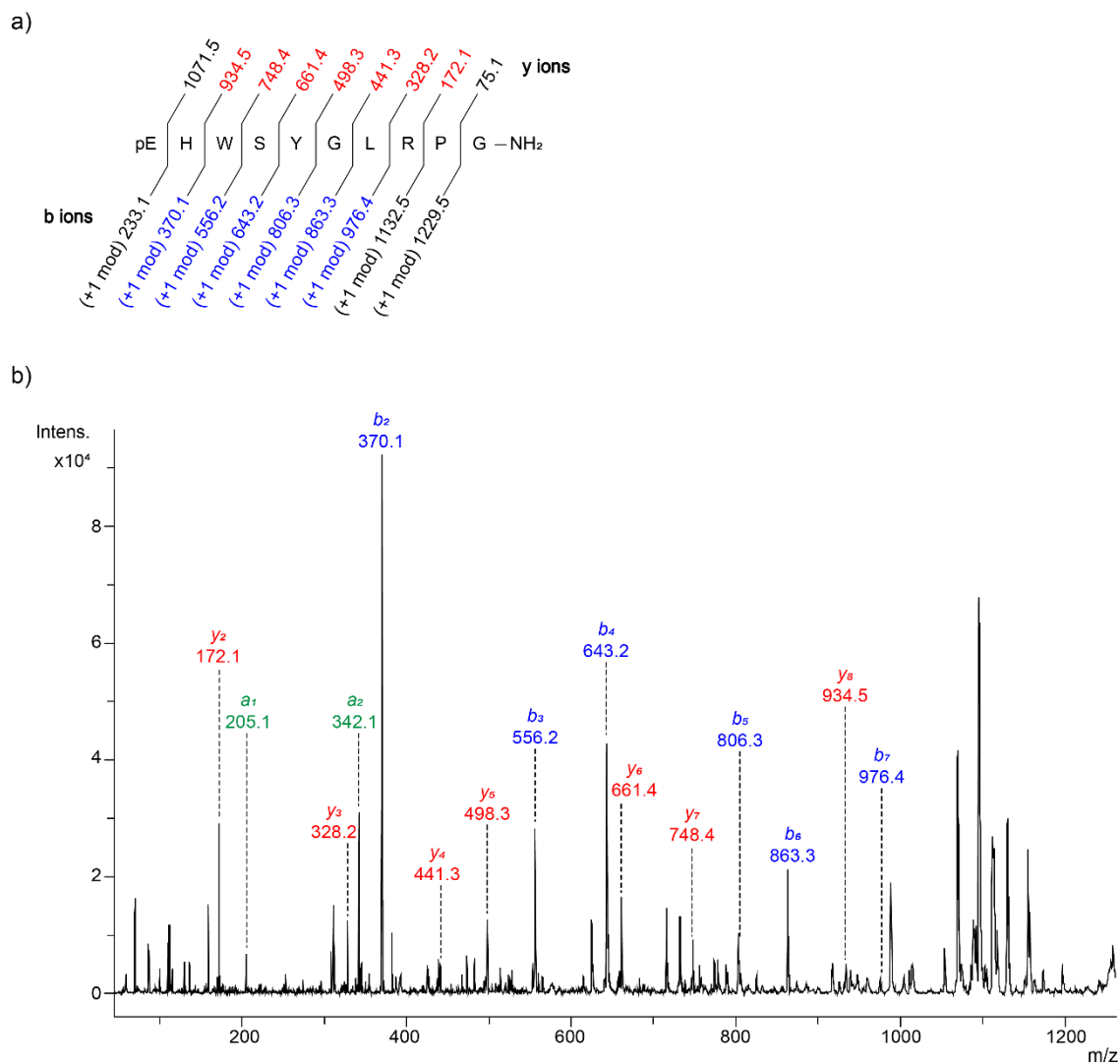


Figure S2. Nickel(II)-catalyzed amide N-H arylation of LHRH (1) with 2-nitrophenylboronic acid (2a). a) Fragmentation ladder for 1 modified with 2a at pyroglutamate (pE). b) MALDI-TOF MS/MS spectrum of 1 modified with 2a.

^1H NMR characterization of the amide N-H arylated product of tripeptide Glp-His-Pro-NH₂ (thyrotropin-releasing hormone, TRH), a model compound

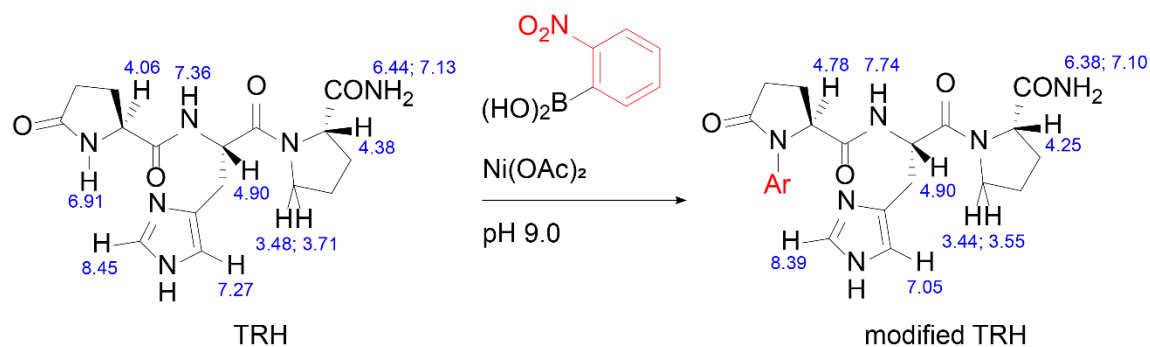


Figure S3. ^1H NMR study for structure identification of the amide N-H arylated product of thyrotropin-releasing hormone (TRH). Blue numbers represent ^1H NMR chemical shifts. Reaction condition: TRH (2 mM), boronic acid **2a** (20 mM), and Ni(OAc)₂ (2 mM) in *N,N'*-diethylpiperazine (NEP) buffer (50 mM, pH 9.0) at 37 °C for 5 h. Experimental detail and NMR spectra were shown in the organic synthesis section and NMR section, respectively (vide infra).

Effect of nickel(II) concentration on reaction rate of amide N-H arylation of LHRH (1)

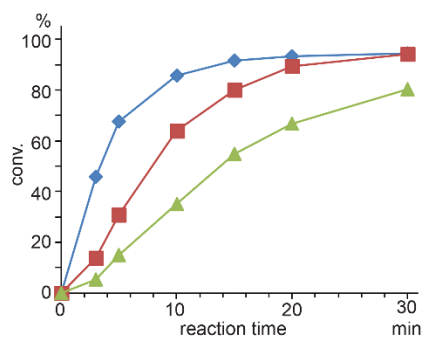
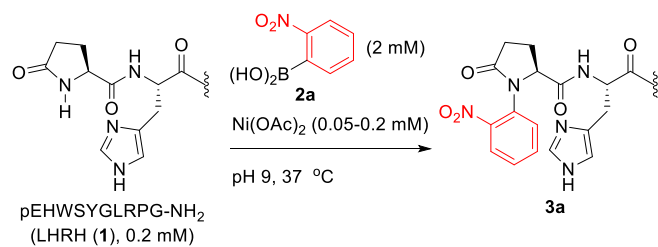


Figure S4. Time course of amide N-H arylation of LHRH (1) with 2-nitrophenylboronic acid (2a) in the presence of varying concentration of nickel(II): $[\text{Ni(OAc)}_2] = 0.05 \text{ mM}$ (green triangle), 0.1 mM (red square), and 0.2 mM (blue diamond).

Metal screen for amide N-H arylation of pyroglutamate-histidine (Glp-His) in LHRH (1)

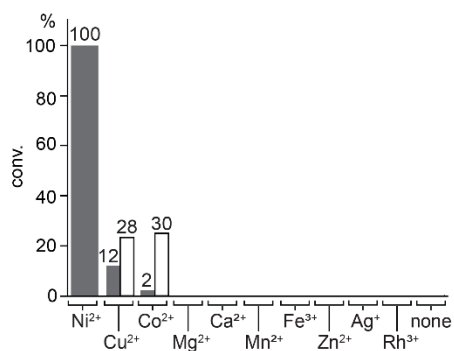
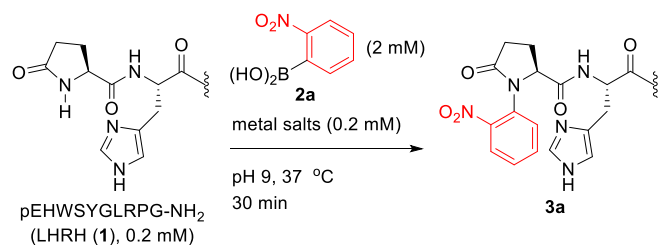


Figure S5. A bar graph representing reaction efficiency of the modification reaction in the presence of various metal salts after incubation for 30 min (solid bar): Ni(OAc)₂, Cu(OAc)₂, Co(OAc)₂, Mg(OAc)₂, CaCl₂, Mn(OAc)₂, Fe(NO₃)₃, Zn(OAc)₂, AgOAc, and RhCl₃. For Cu(II), Co(II), Mg(II), Ca(II), Mn(II), Fe(III), Zn(II), Ag(I), and Rh(III), reaction time was elongated to 24 h (plain bar). Numbers on each bar correspond to relative conversion calculated by MALDI-TOF MS of crude reaction mixture.

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

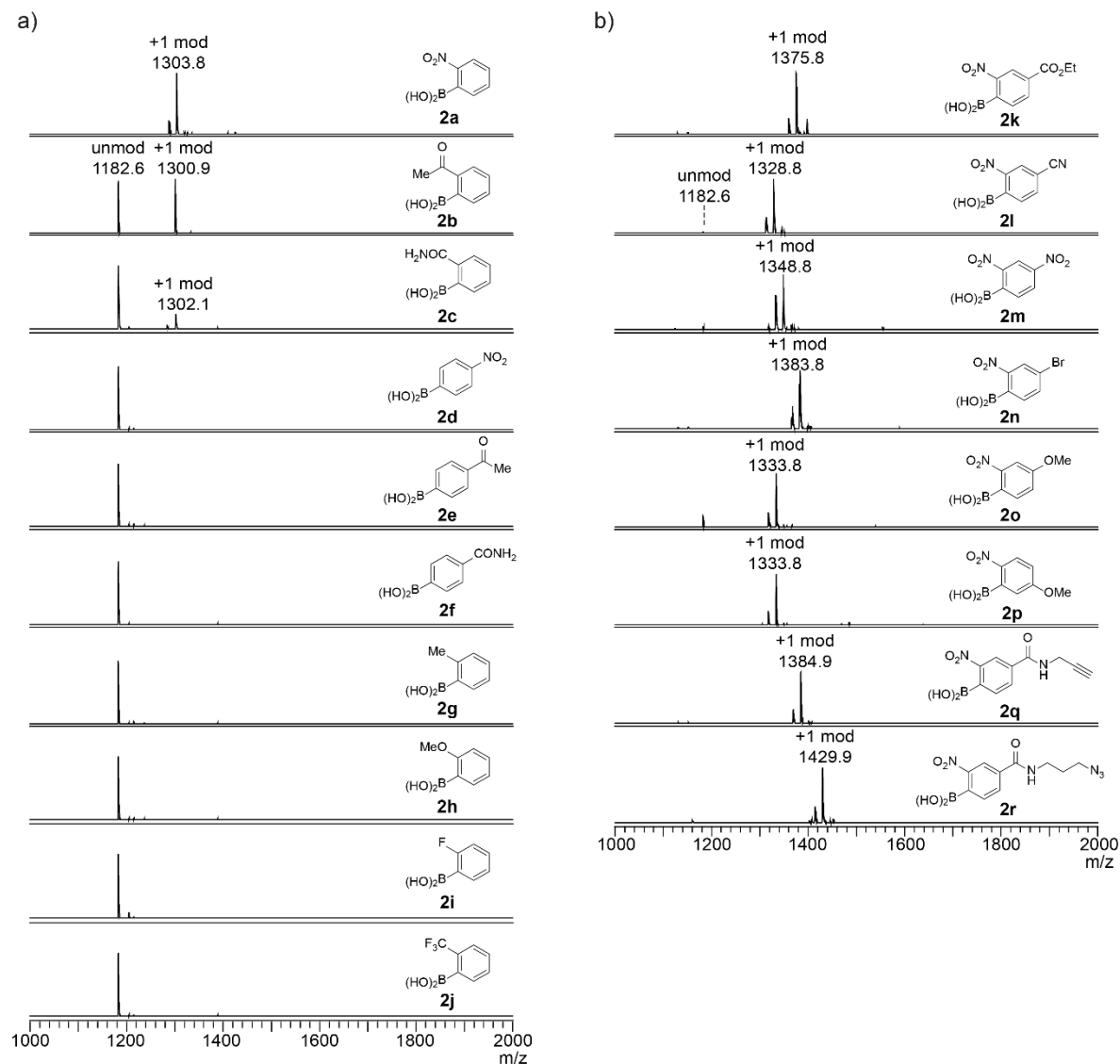


Figure S6. Nickel(II)-catalyzed cysteine modification of LHRH (**1**) with a) phenylboronic acid derivatives **2a–j** and b) 2-nitrophenylboronic acid derivatives **2k–r**. Singly modified peptides are shown as “+1 mod”. Peaks at 1182 m/z corresponds to unmodified peptide **1** (“unmod”) ([M+H]⁺).

Additional experimental data for boronic acid screening: RP-HPLC analysis data

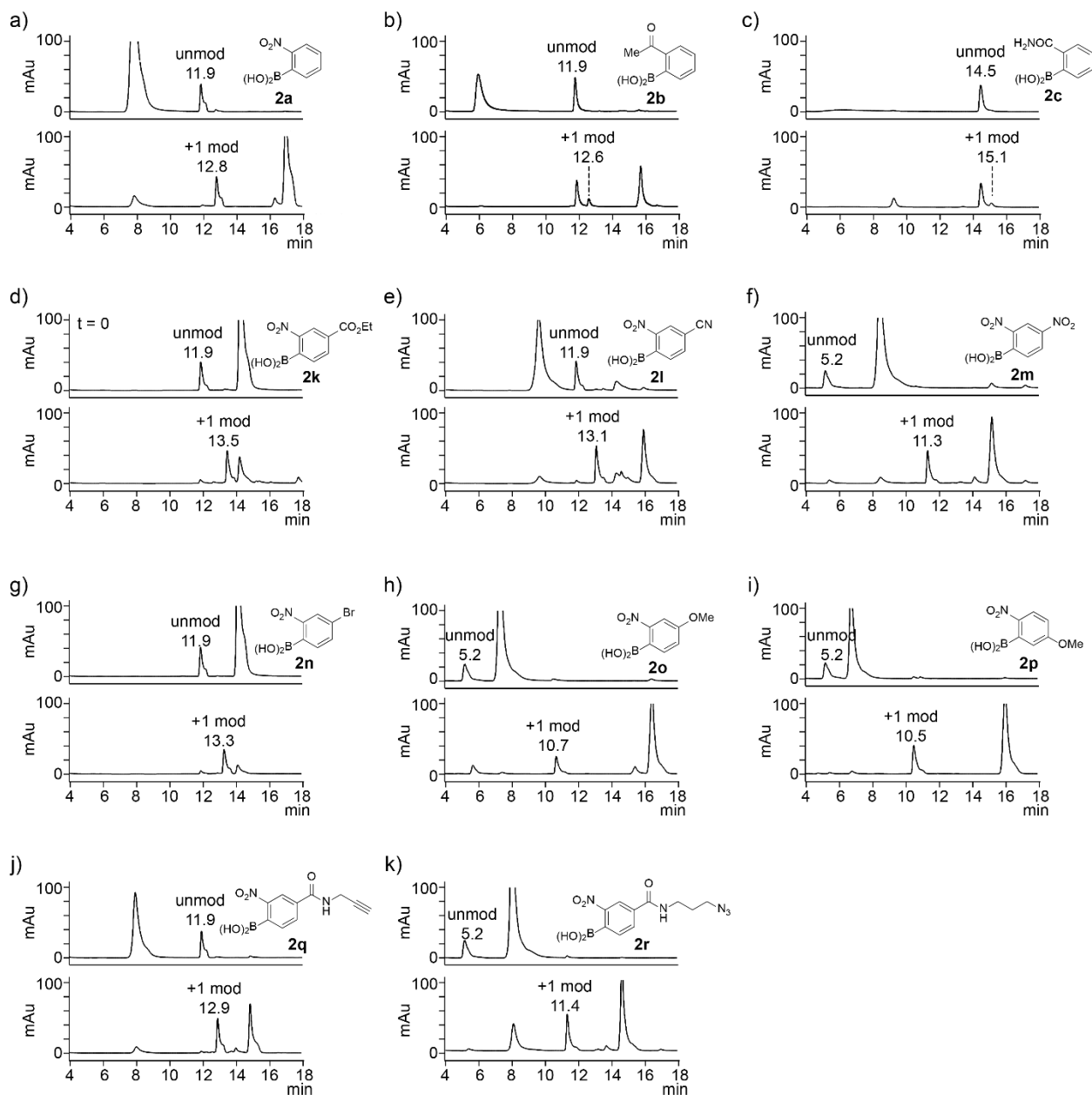


Figure S7. RP-HPLC analysis data of crude reaction mixture of nickel(II)-catalyzed Glp-His modification of LHRH (**1**) with phenylboronic acid derivatives (**2a–c** and **2k–r**) after incubation for 0 (top chromatogram) and 30 min (bottom chromatogram). Numbers on each peak represent retention times (min). Unmodified and singly modified peptides are shown as “unmod” and “+1 mod”, respectively.

Additional experimental data for peptide screening: MALDI-TOF MS analysis data for leuprolide (4)

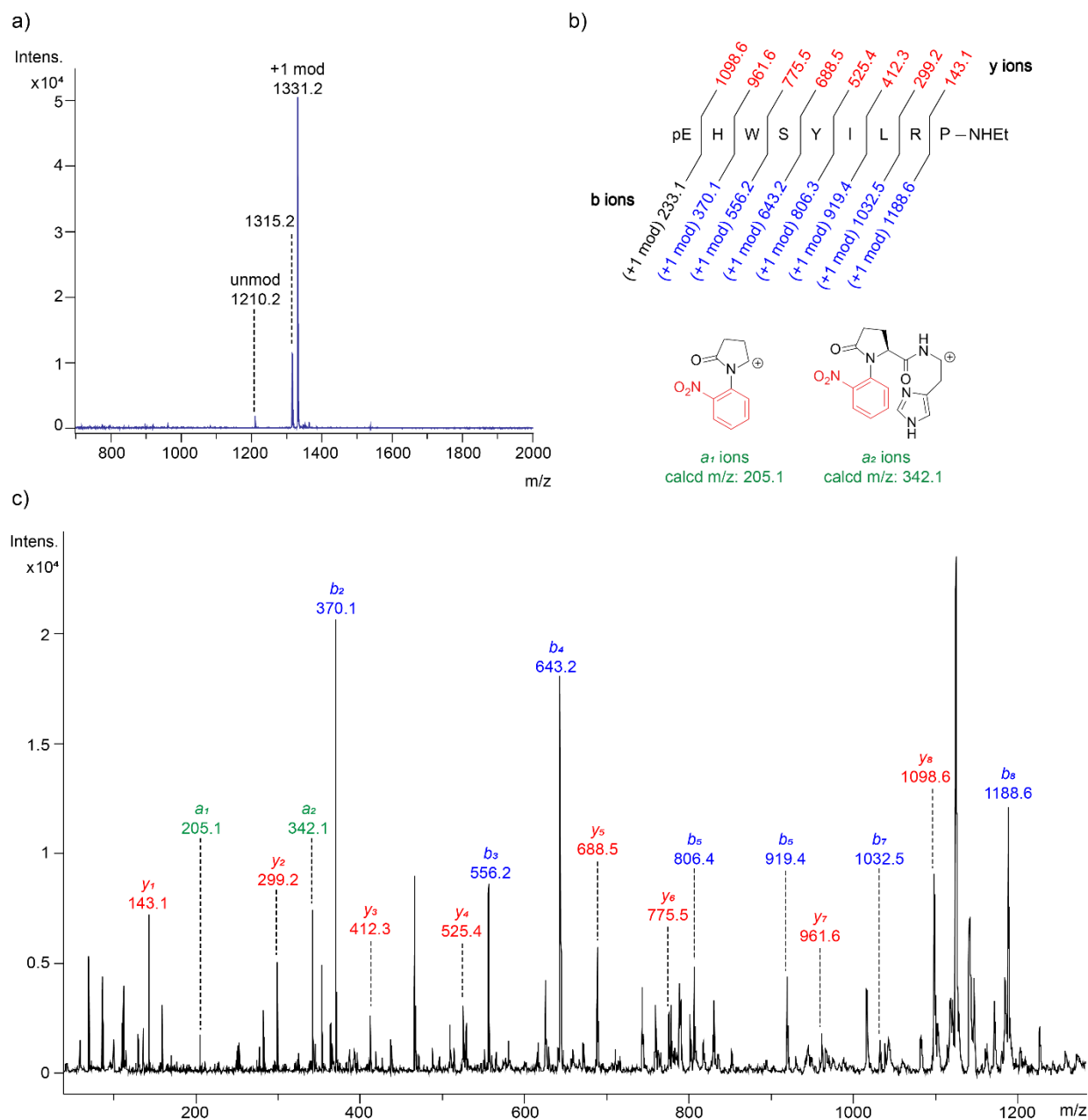


Figure S8. Nickel(II)-catalyzed amide N-H arylation of leuprolide (**4**) with 2-nitrophenylboronic acid (**2a**). a) MALDI-TOF MS spectrum of the crude reaction mixture. An additional $[M(+1\text{mod})-16]$ peak derived from oxygen atom loss from the nitro group was observed. b,c) MALDI-TOF MS/MS spectrum of **5** modified with **2a** (+1 mod).

Modification of chemically and enzymatically-incorporated Glp-His proteins

Preparation of chemically-tagged Glp-His Chymotrypsinogen A

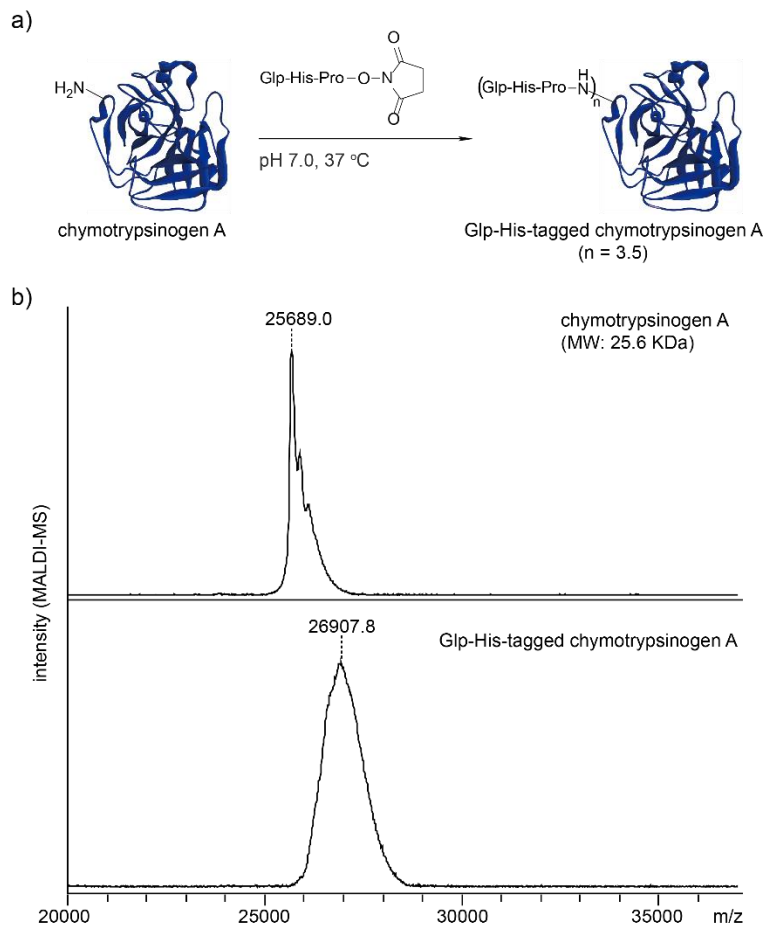


Figure S9. a) Introduction of Glp-His tag into chymotrypsinogen A via NHS ester. b) MALDI-TOF MS analyses (linear mode) of native and chemically Glp-His-tagged chymotrypsinogen A. The average number “n” of Glp-His-Pro moiety (MW: 345) was estimated to be $n = (26907.8 - 25689.0) / 345 = 3.5$.

Gel and Blot images for modification of native and Glp-His-tagged chymotrypsinogen A

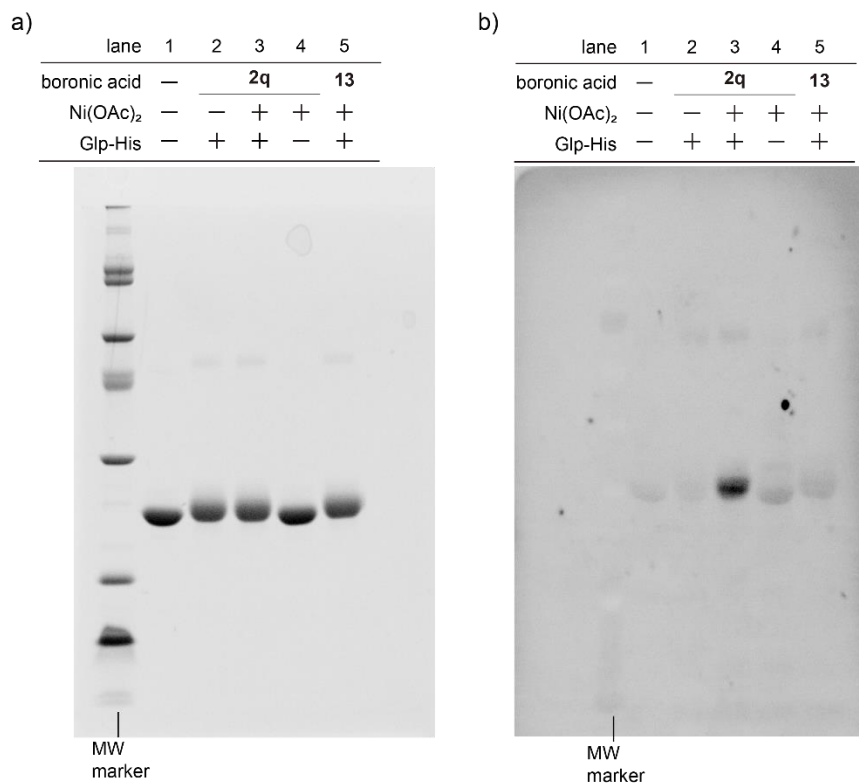


Figure S10. Full-width gel and blot images for Fig. 3b. Analysis of modification of native and Glp-His-tagged chymotrypsinogen A (20 μ M) with alkyne-functionalized boronic acids **2q** and **13** (2 mM) in the presence and absence of Ni(OAc)₂ (0.4 mM). a) Coomassie total protein staining. b) Chemical blotting analysis¹ of the modification reaction.

Full-width gel and blot images for time course of protein modification

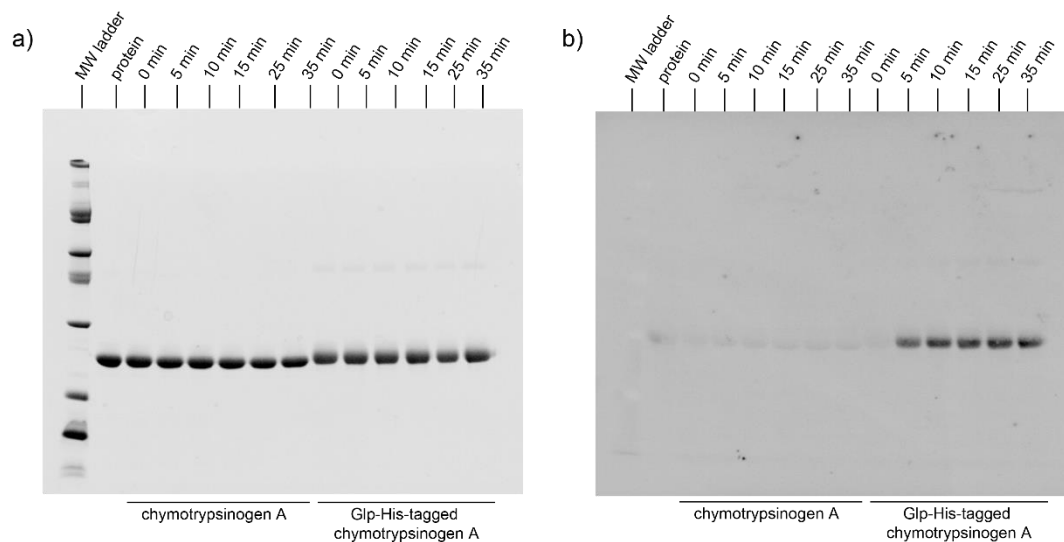


Figure S11. Full-width gel and blot images for Fig. 3c. Time course of the modification of native and Glp-His-tagged chymotrypsinogen A (20 μ M) with **2q** (2 mM) in the presence of Ni(OAc)₂ (0.4 mM) at pH 9.0. a) Coomassie total protein staining. b) Chemical blotting analysis of the modification reaction. Fluorescence bands were quantified by Image J software.

Full-width gel of the reaction of Glp-His-GFP-GST and iodoacetamide-capped Glp-His-GST with *o*-nitro PEG5k

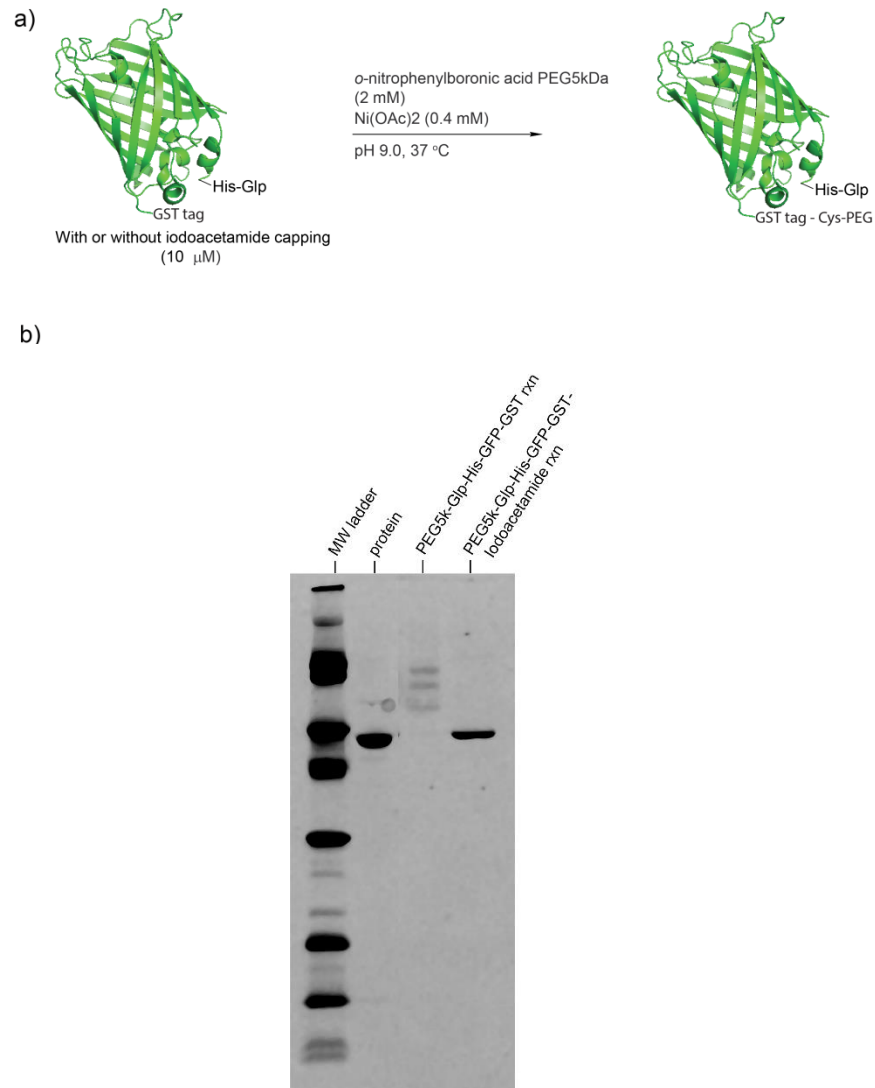


Figure S12. Gel image (CBB stain) of the reaction of Glp-His-GFP-GST before and after capping with iodoacetamide (10 μM) with a cysteine-reactive 5kDa PEGylation reagent (boronic acid, 2 mM) in the presence of Ni(OAc)₂ (0.4 mM) at pH 9.0.

Full-width gel and blot images for the modification of Glp-His-GFP-GST

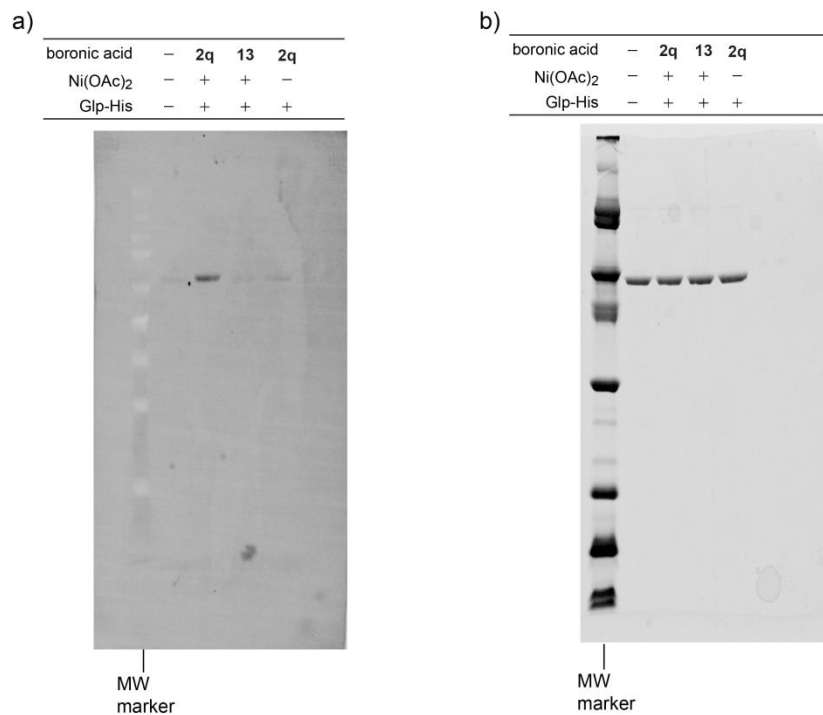


Figure S13. Full-width gel and blot images for Fig. 3e. Modification of expressed Glp-His-GFP-GST after capping free cys residues with iodoacetamide (10 μ M) with **2q** or **13** (2 mM) in the presence of Ni(OAc)₂ (0.4 mM) at pH 9.0. a) Chemical blotting analysis of the modification reaction. b) Coomassie total protein staining.

Experimental procedures

Kinetic study of nickel(II)-catalyzed Glp-His modification of LHRH (1) with 2-nitrophenylboronic acid (2a) at various pH

LHRH **1** (40 μL , 2.5 mM in H_2O) was incubated with 2-nitrophenylboronic acid (**2a**) (20 μL , 50 mM in DMSO) and $\text{Ni}(\text{OAc})_2$ (20 μL , 5 mM in H_2O) in *N*-methylmorpholine (NMM) buffer (420 μL , 10 mM, pH 8.0 and 8.5) or *N,N'*-dimethylpiperazine (NEP) buffer (420 μL , 10 mM, pH 9.0 and 9.5) at 37 $^\circ\text{C}$. The aliquot of the reaction mixture (62.5 μL) was taken after a given time period and was quenched with EDTA (5 μL , 100 mM in H_2O). The resulting solution (50 μL) was analyzed by RP-HPLC for analytical scale (15–90% MeCN over 15 min). The conversion was calculated by using calibration curve for **1** of HPLC peak area versus concentration.

Effect of nickel(II) concentration on reaction rate of amide N-H arylation of LHRH (1)

LHRH **1** (40 μL , 2.5 mM in H_2O) was incubated with 2-nitrophenylboronic acid (**2a**) (20 μL , 50 mM in DMSO) and $\text{Ni}(\text{OAc})_2$ (20 μL , 1.25, 2.5, and 5 mM in H_2O) in NEP buffer (420 μL , 10 mM, pH 9.0) at 37 $^\circ\text{C}$. The aliquot of the reaction mixture (62.5 μL) was taken after a given time period and was quenched with EDTA (5 μL , 100 mM in H_2O). The resulting solution (50 μL) was analyzed by RP-HPLC for analytical scale (15–90% MeCN over 15 min). The conversion was calculated by using calibration curve for **1** of HPLC peak area versus concentration.

Metal salt screening for Glp-His modification of LHRH (1) with 2-nitrophenylboronic acid (2a)

LHRH **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 , 5 mM in H_2O) in NEP buffer (10.5 μL , 10 mM, pH 9.0) at 37 $^\circ\text{C}$ for 30 min or 24 h (except for Ni(II)). Metal salts: $\text{Ni}(\text{OAc})_2$, $\text{Cu}(\text{OAc})_2$, $\text{Co}(\text{OAc})_2$, $\text{Mg}(\text{OAc})_2$, CaCl_2 , $\text{Mn}(\text{OAc})_2$, $\text{Fe}(\text{NO}_3)_3$, $\text{Zn}(\text{OAc})_2$, 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.

Boronic acid screening for nickel(II)-catalyzed cysteine modification of IL-8 inhibitor (1) analyzed by MALDI-TOF MS

LHRH **1** (1 μL , 2.5 mM in H_2O) was incubated with phenylboronic acid derivatives (**2a–r**) (0.5 μL , 50 mM in DMSO), and $\text{Ni}(\text{OAc})_2$ (0.5 μL , 5 mM in H_2O) in NEP buffer (10.5 μL , 10 mM, pH 9.0) at 37 $^\circ\text{C}$ for 30 min. The reaction was quenched with EDTA (1 μL , 100 mM in H_2O) and analyzed by MALDI-TOF MS without purification.

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

LHRH **1** (15 μL , 2.5 mM in H_2O) was incubated with phenylboronic acid derivatives (**2a–c** and **2k–r**) (7.5 μL , 50 mM in DMSO), and $\text{Ni}(\text{OAc})_2$ (7.5 μL , 5 mM in H_2O) in NEP buffer (157.5 μL , 10 mM, pH 9.0) at 37 $^\circ\text{C}$ for 30 min. The aliquot of the reaction mixture (62.5 μL) was taken after 0 and 30 min and was

quenched with EDTA (5 μL , 100 mM in H_2O). The resulting solution (50 μL) was analyzed by RP-HPLC for analytical scale (15–90% MeCN over 15 min for **2a**, **2b**, **2k**, **2l**, **2n** and **2q**, 15–45% MeCN over 15 min for **2c**, 25–90% MeCN over 15 min for **2m**, **2o**, **2p**, and **2r**). The conversion was calculated by using calibration curve for **1** of HPLC peak area versus concentration.

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 $\text{Ni}(\text{OAc})_2$ (0.5 μL , 5 mM in H_2O) in NEP buffer (10.5 μL , 10 mM, pH 9.0) at 37 $^\circ\text{C}$ for 30 min. The reaction was quenched with EDTA (1 μL , 100 mM in H_2O) and analyzed by MALDI-TOF MS without purification.

Preparation of Glp-His-tagged chymotrypsinogen A by conjugation with thyrotropin-releasing hormone (TRH)

The procedure for the conjugation of TRH free acid (Glp-His-Pro-OH) to chymotrypsinogen A was conducted according to literature procedure.² The solutions of TRH free acid (30 μL , 0.6 M in DMF), DCC (30 μL , 0.6 M in DMF), and *N*-hydroxysuccinimide (NHS, 30 μL , 0.6 M in DMF) were preincubated for 1 h with shaking at rt. The entire suspension was added to a solution of protein (2160 μL , 20 μM in HEPES buffer (0.1 M, pH 7.1)). The suspension was incubated at rt for 18 h. Precipitate was pelleted by centrifugation and the modified protein in the supernatant was purified by size-exclusion column with *N*-methylmorpholine (NMM) buffer (5 mM, pH 7.4) as the eluting buffer. The collected fractions were concentrated by centrifugal filtration to a final concentration of 143 μM , split into aliquots, and frozen at -20 $^\circ\text{C}$ until use.

Nickel(II)-catalyzed modification of native and Glp-His-tagged chymotrypsinogen A

Chymotrypsinogen A (2 μL , 250 μM in NMM (5 mM, pH 7.4)) or Glp-His-tagged chymotrypsinogen A (3.4 μL , 143 μM in NMM, 5 mM, pH 7.4) was incubated with **2q** or **13** (1 μL , 50 mM in DMSO) and $\text{Ni}(\text{OAc})_2$ (1 μL , 10 mM in H_2O) in NEP buffer (21 μL for chymotrypsinogen A, 19.6 μL for Glp-His-tagged chymotrypsinogen A, 10 mM, pH 9.0) at 37 $^\circ\text{C}$ for 30 min. The reaction mixtures were quenched with EDTA (1 μL , 100 mM in H_2O) and analyzed by SDS-PAGE and chemical blotting.

Time course of nickel(II)-catalyzed protein modification

Chymotrypsinogen A (2 μL , 250 μM in NMM (5 mM, pH 7.4)) or Glp-His-tagged chymotrypsinogen A (3.4 μL , 143 μM in NMM (5 mM, pH 7.4)) was incubated with **2q** (1 μL , 50 mM in DMSO) and $\text{Ni}(\text{OAc})_2$ (1 μL , 10 mM in H_2O) in NEP buffer (21 μL for chymotrypsinogen A, 19.6 μL for Glp-His-tagged chymotrypsinogen A, 10 mM, pH 9.0) at 37 $^\circ\text{C}$ for a given time period. The reaction mixtures were quenched with EDTA (1 μL , 100 mM in H_2O) and analyzed by SDS-PAGE and chemical blotting.

Reaction of free cysteine residues on Glp-His-GFP-GST with iodoacetamide

Glp-His-GFP-GST (55.2 μ L, 181 μ M in NMM (50 mM, pH 7.4) was incubated with TCEP (5 μ L, 100 mM in NH_4HCO_3 (100 mM, pH 8.4)) in NH_4HCO_3 (439.8 μ L, 100 mM, pH 8.4) at 37 °C for 30 min. To this was then added iodoacetamide (5 μ L, 100 mM in NH_4HCO_3 (100 mM, pH 8.4) and the mixture was then incubated in the dark for 2 h at rt. The reaction mixture was then purified by size-exclusion column in HEPES buffer (100 mM, pH 7.4) and concentrated by centrifugal filtration (final concentration 34 μ M), frozen with liq. N_2 , and stored at -20 °C until use. Successful making of cysteine reactivity was confirmed by the reaction of the Glp-His-GFP-GST with a PEGylated *o*-nitro boronic acid with monitoring by Coomassie brilliant blue staining.

Nickel(II)-catalyzed modification of expressed Glp-His-GFP-GST

Glp-His-GFP-GST with cys residues blocked by iodoacetamide (3.4 μ L, 34 μ M in HEPES (100 mM, pH 7.4)) was incubated with **2q** or **13** (0.5 μ L, 50 mM in DMSO) and $\text{Ni}(\text{OAc})_2$ (0.5 μ L, 10 mM in H_2O) in NEP buffer (7.8 μ L, 50 mM, pH 9.0) at 37 °C for 1 h. The reaction mixtures were quenched with EDTA (1 μ L, 200 mM in H_2O) and analyzed by SDS-PAGE and chemical blotting.

Analysis of the protein modifications by chemical blotting¹

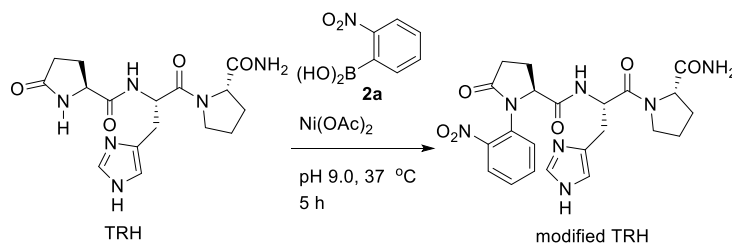
The basic procedure for the detection of an alkyne handle in a modified protein is based on the previously described procedure.² After transfer of a protein from polyacrylamide gel to poly(vinylidene)fluoride membrane, the membrane was soaked in a methanol (2 \times 2 min), rinsed with water, and placed in 1:1 mixture of H_2O /DMSO. The membrane in the soln was incubated with sodium ascorbate (1.5 mM), THPTA (100 μ M), 3-azido-7-hydroxycoumarin (10 μ M), and copper(II) sulfate (75 μ M) at rt for 30 min to 18 h. The soln was taken out, and the membrane was rinsed with MeOH followed by a 1:1 mixture of MeOH/DMSO. The process of rinsing with MeOH and MeOH/DMSO 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.

Expression of Glp-His-GFP-GST²

The expression of the Glp-His-GFP-GST protein was performed as described in a previous report.

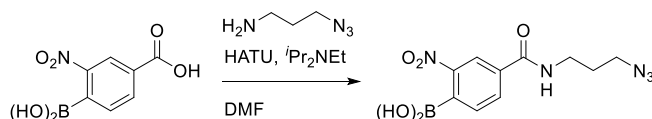
Organic synthesis

L-(N-(2-nitrophenyl)pyroglutamyl)-L-histidyl-L-prolinamide (modified TRH)



To a solution of thyrotropin-releasing hormone (TRH) (250 μ L, 25 mM in H₂O) in *N,N'*-diethylpiperazine buffer (2625 μ L, 50 mM, pH 9.0) were added 2-nitrophenylboronic acid (125 μ L, 50 mM in DMSO) and Ni(OAc)₂ (125 μ L, 50 mM in H₂O). The solution was shaken at 37 °C for 5 h. The reaction was quenched with EDTA solution (250 μ L, 100 mM in H₂O). After addition of a solution of TFA in CH₃CN (0.1%, 1125 μ L), the resulting mixture was filtered by a syringe filter. The filtrate was purified by RP-HPLC for preparative scale (5–40% acetonitrile/H₂O over 21 min) and lyophilization of the collected fractions (RT: 12.9 min) afforded the title compound as the trifluoroacetate salt (pale yellow solid, 2.5 mg). ¹H NMR (600 MHz, CD₃CN/DMSO-*d*₆, 95:5): δ 8.39 (s, 1H), 7.98 (dd, *J* = 1.2, 8.1 Hz, 1H), 7.74 (br, 1H), 7.71 (ddd, *J* = 1.2, 7.6, 8.0 Hz, 1H), 7.50 (ddd, *J* = 0.8, 7.6, 8.1 Hz, 1H), 7.37 (dd, *J* = 0.8, 8.0 Hz, 1H), 7.10 (br, 1H), 7.05 (s, 1H), 6.38 (br, 1H), 4.92–4.89 (m, 1H), 4.90–4.77 (m, 1H), 4.26–4.24 (m, 1H), 3.57–3.53 (m, 1H), 3.46–3.42 (m, 1H), 3.12 (dd, *J* = 6.0, 15.1 Hz, 1H), 3.06 (dd, *J* = 5.5, 15.1 Hz, 1H), 2.57 (m, 1H), 2.44–2.38 (m, 2H), 2.20–2.13 (m, 2H), 2.13–2.05 (m, 1H), 1.88–1.83 (m, 2H). ¹³C NMR (151 MHz, CD₃CN/DMSO-*d*₆, 95:5): δ 175.8, 175.4, 170.9, 168.9, 146.0, 134.5, 134.2, 131.4, 128.9, 128.3, 126.9, 125.8, 118.5, 63.0, 60.4, 50.6, 47.9, 30.1, 30.0, 27.1, 25.2, 24.6. ESI-MS: calcd for C₂₂H₂₆N₇O₆ [M+H]⁺ 484.1939, found 484.1929.

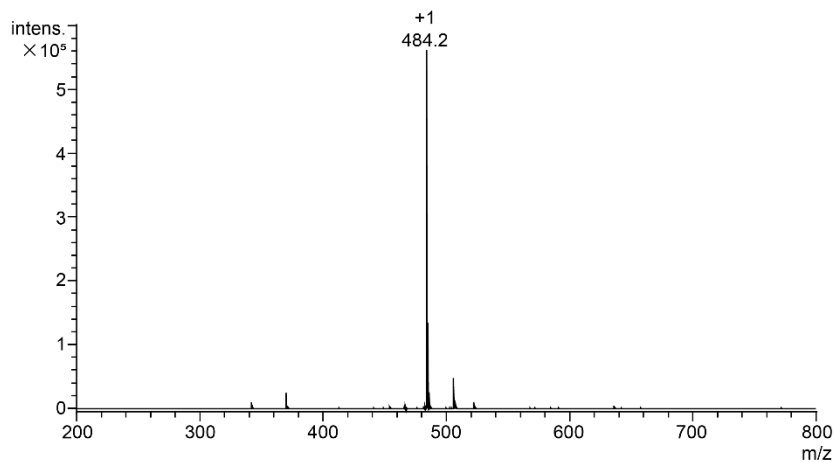
4-(3-Azidopropylaminocarbonyl)-2-nitrophenylboronic acid (2r)



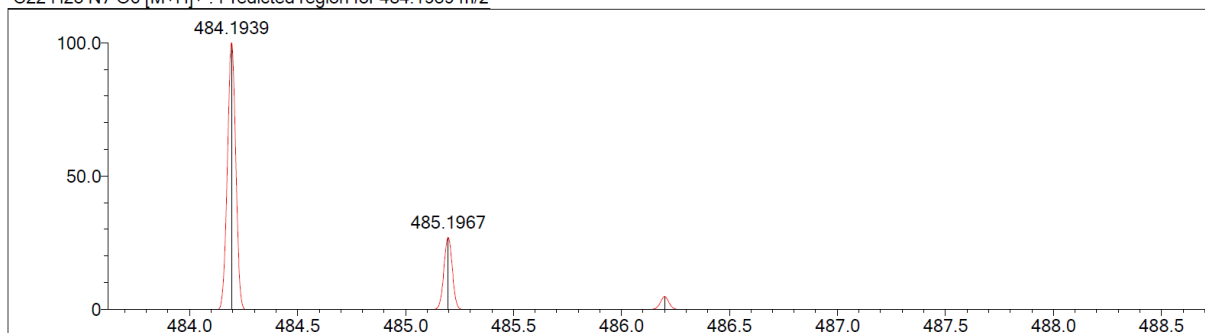
4-Carboxy-2-nitrophenylboronic acid (15 mg, 0.071 mmol) and HATU (27 mg, 0.071 mmol) were dissolved in DMF (0.2 mL). Diisopropylethylamine (19 μ L, 0.11 mmol) was added at 0 °C, and the solution was stirred at rt for 10 min. 3-Azidopropylamine (14 mg, 0.14 mmol) was added at 0 °C, and the solution was stirred at rt for 7 h. All of volatiles were removed by gentle flow of nitrogen gas. The resulting oil was purified by RP-HPLC (5–20% MeCN over 22 min). Lyophilization of the collected fractions afforded the titled compound as a colorless powder (14 mg, 68%). ¹H NMR (600 MHz, CD₃CN): δ 8.52 (s, 1H), 8.09 (d, *J* = 7.6 Hz, 1H), 7.66 (d, *J* = 7.6 Hz, 2H), 7.43 (br, 1H), 3.48–3.43 (m, 4H), 1.88 (m, 2H). ¹³C NMR (151 MHz, CD₃CN): δ 165.7, 151.0, 136.4, 133.1, 132.7, 121.9, 49.5, 37.7, 28.9. ESI-MS: calcd for C₁₀H₁₃BN₅O₅ [M+H]⁺ 294.1006, found 294.0997.

ESI-MS spectra of synthesized compounds

L-(N-(2-Nitrophenyl)pyroglutamyl)-L-histidyl-L-prolinamide (modified TRH)

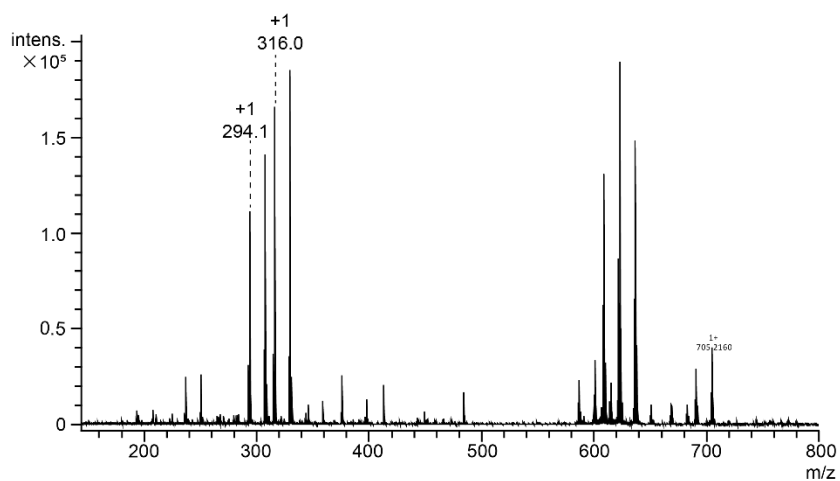


C22 H25 N7 O6 [M+H]⁺ : Predicted region for 484.1939 m/z

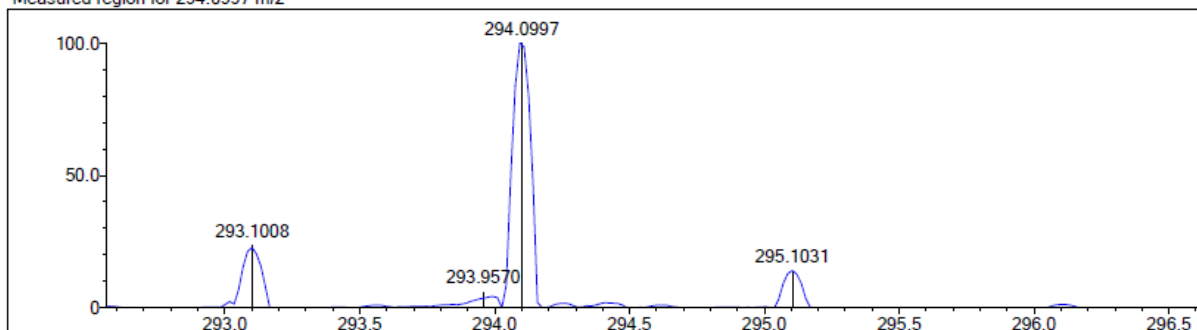


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Meas. (M)	Pred. (M)	Df. (ppm)	Iso	DBE
1	97.23	C19 H17 N17	[M+H] ⁺	484.1929	484.1926	483.1856	483.1853	0.62	97.23	20.0
2	95.48	C21 H29 N3 O10	[M+H] ⁺	484.1929	484.1926	483.1856	483.1853	0.62	95.48	9.0
> 3	91.24	C22 H25 N7 O6	[M+H] ⁺	484.1929	484.1939	483.1856	483.1866	-2.07	93.75	14.0
4	90.35	C18 H21 N13 O4	[M+H] ⁺	484.1929	484.1912	483.1856	483.1839	3.51	96.39	15.0

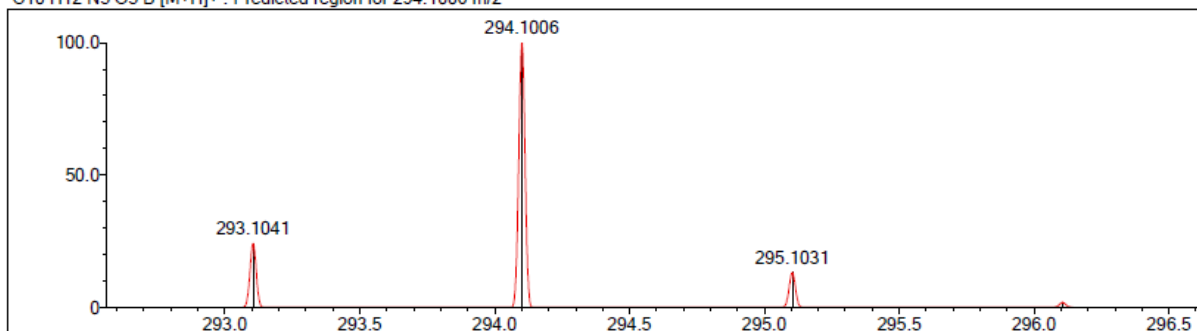
4-(3-Azidopropylaminocarbonyl)-2-nitrophenylboronic acid (2r)



Measured region for 294.0997 m/z



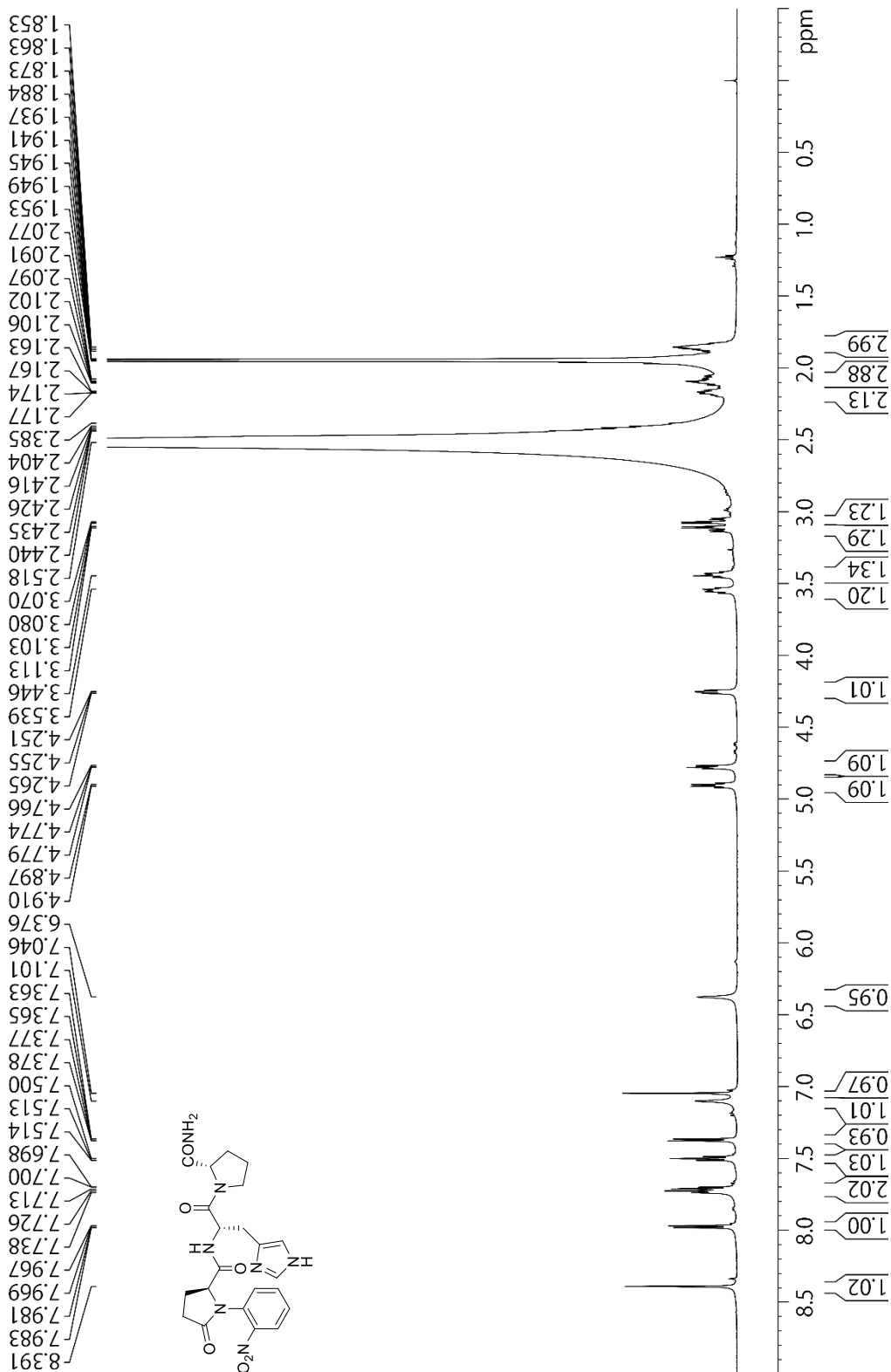
C₁₀H₁₂N₅O₅B [M+H]⁺: Predicted region for 294.1006 m/z



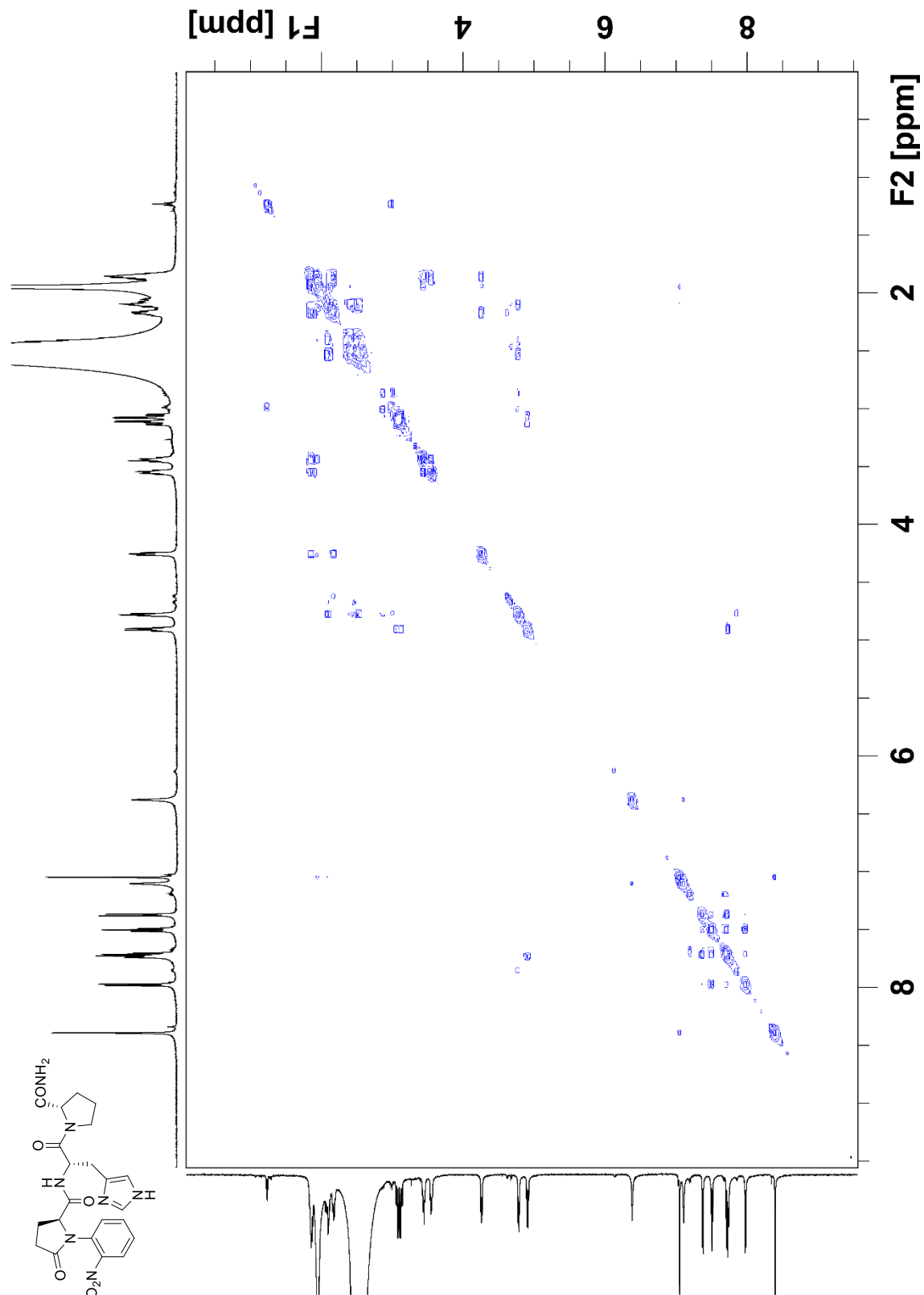
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Meas. (M)	Pred. (M)	Df. (ppm)	Iso	DBE
1	80.83	C ₉ H ₁₆ N O ₉ B	[M+H] ⁺	294.0997	294.0993	293.0924	293.0920	1.36	81.56	3.0
> 2	76.85	C ₁₀ H ₁₂ N ₅ O ₅ B	[M+H] ⁺	294.0997	294.1006	293.0924	293.0933	-3.06	81.03	8.0
3	66.09	C ₆ H ₈ N ₁₁ O ₃ B	[M+H] ⁺	294.0997	294.0979	293.0924	293.0906	6.12	83.87	9.0
4	49.28	C ₁₁ H ₈ N ₉ O B	[M+H] ⁺	294.0997	294.1020	293.0924	293.0947	-7.82	79.73	13.0

¹H and ¹³CNMR spectra of synthesized compounds

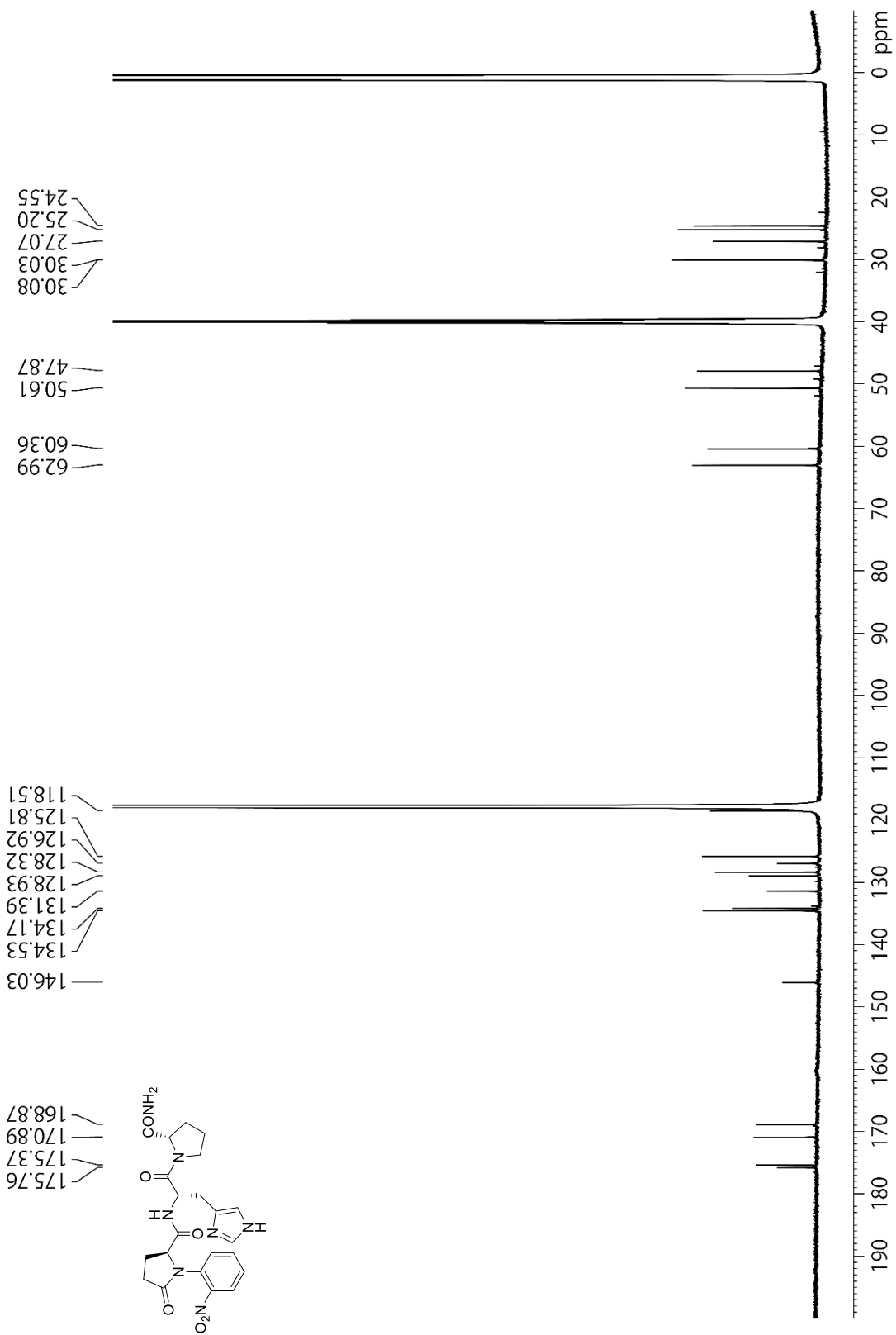
L-(N-(2-Nitrophenyl)pyroglutamyl)-L-histidyl-L-prolinamide (modified TRH) (¹H NMR in CD₃CN/DMSO-*d*₆, 95:5)



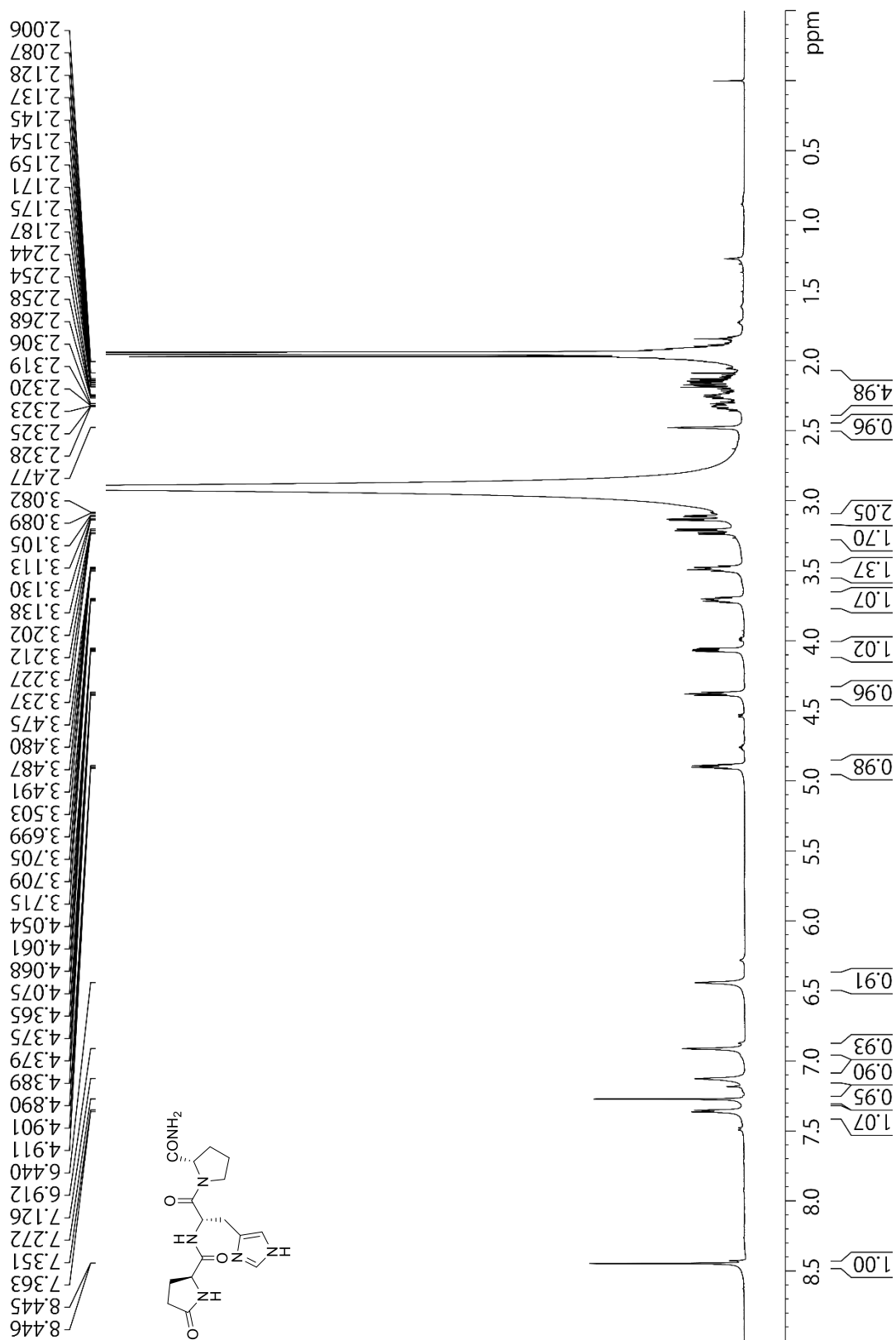
L-(N-(2-Nitrophenyl)pyroglutamyl)-L-histidyl-L-prolinamide (modified TRH) (^1H - ^1H COSY NMR in $\text{CD}_3\text{CN}/\text{DMSO-}d_6$, 95:5)



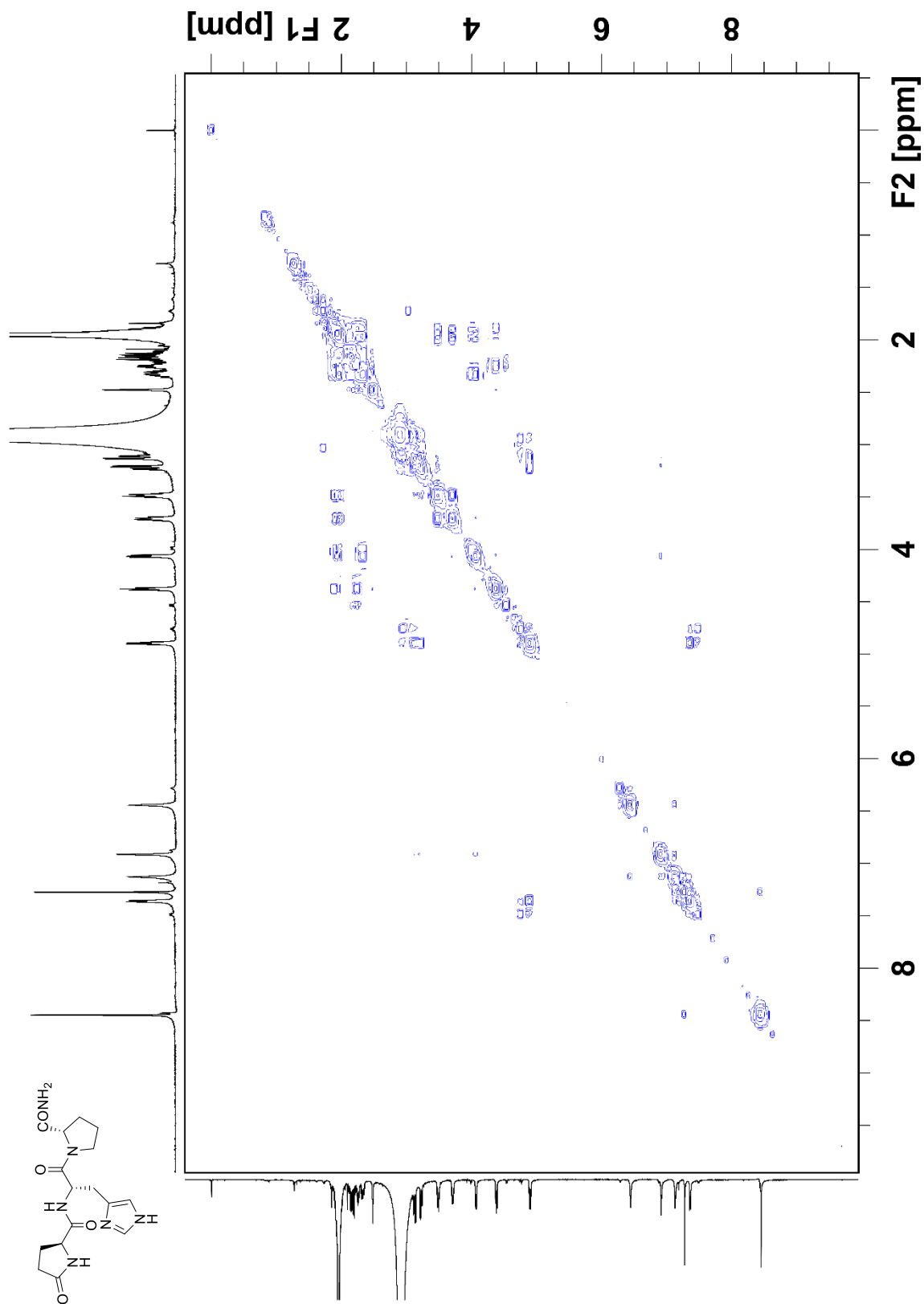
L-(N-(2-Nitrophenyl)pyroglutamyl)-L-histidyl-L-prolinamide (modified TRH) (¹³C NMR in CD₃CN/DMSO-*d*₆, 95:5)



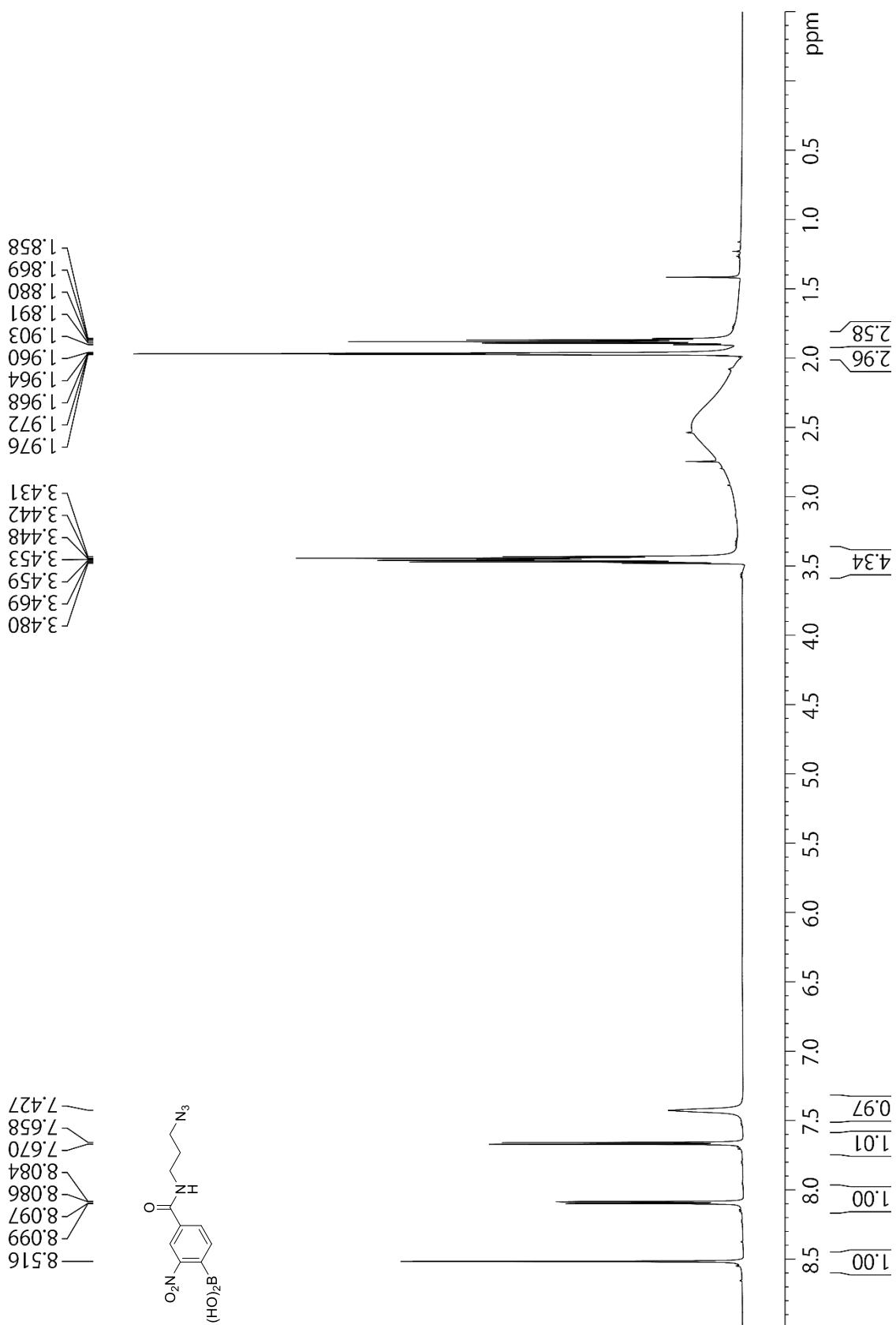
L-Pyroglutamyl-L-histidyl-L-prolinamide (TRH) (¹H NMR in CD₃CN/DMSO-d₆, 95:5)



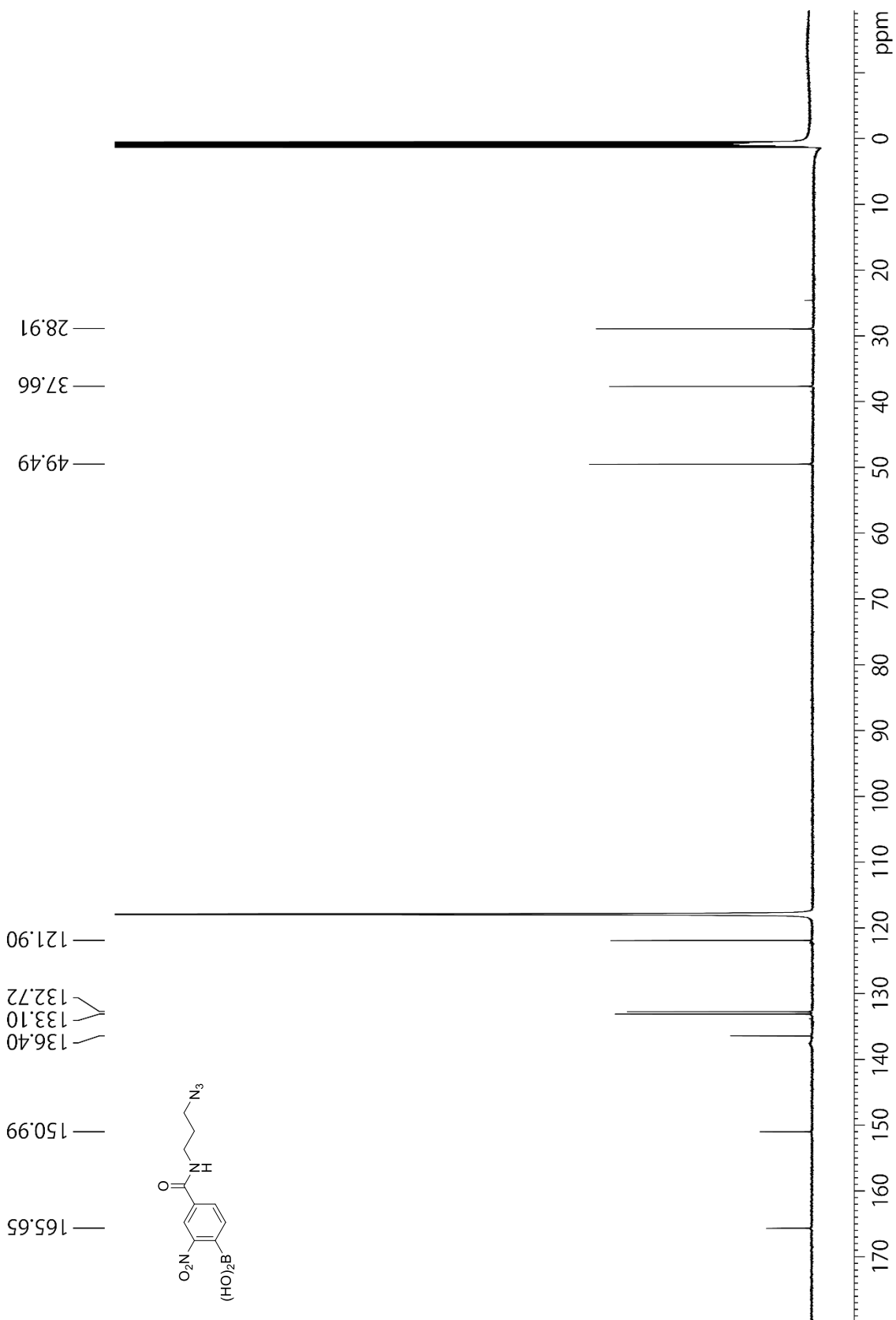
L-Pyroglutamyl-L-histidyl-L-prolinamide (TRH) (^1H - ^1H COSY NMR in $\text{CD}_3\text{CN}/\text{DMSO-}d_6$, 95:5)



4-(3-Azidopropylaminocarbonyl)-2-nitrophenylboronic acid (¹H NMR in CD₃CN)



4-(3-Azidopropylaminocarbonyl)-2-nitrophenylboronic acid (^{13}C NMR in CD_3CN)



References

- (1) Ohata, J.; Vohidov, F.; Ball, Z. T. *Mol. Biosyst.* **2015**, *11*, 2846–2849.
- (2) Ohata, J.; Zeng, Y.; Segatori, L.; Ball, Z. T. *Angew. Chem. Int. Ed.* **2018**, *57*, 4015–4019.