Supporting Information

An Organometallic Strategy for Cysteine Borylation

Authors: Mary A. Waddington,[1] Xin Zheng,[2] Julia M. Stauber,[1] Elamar Hakim Moully,[1] Hayden R. Montgomery,[1] Liban M. A. Saleh,[1] Petr Král,[2,3,4,*] Alexander M. Spokoyny[1,5,*]

Correspondence: spokoyny@chem.ucla.edu, pkral@uic.edu

Table of Contents

I. Materials and Methods

Materials:

All reagents were purchased from Sigma Aldrich, Strem Chemicals, ChemImpex, Oakwood Chemicals, TCI, Fisher Scientific, Carbosynth, Combi-Blocks, or Alfa Aesar, and used as received unless otherwise noted. Solvents (acetone, acetonitrile (MeCN), diethyl ether (Et_2O), N,N-dimethylformamide (DMF)) were used as received without further purification unless otherwise specified. Milli-Q water was used for all experiments. Fisher Water Optima™ LC-MS Grade and Fisher Acetonitrile Optima™ LC-MS Grade were used exclusively for LC-MS mobile phase solvents. MilliQ water and Fisher Acetonitrile, gradient grade, ≥99.9% were used as mobile phases for HPLC. Deuterated solvents ($CDCl_3$, and D_2O) were obtained from Cambridge Isotope Laboratories and used as received. Trifluoroacetic acid (TFA, 99%) was purchased from EMD Millipore Corporation. Angiotensin converting enzyme derived from rabbit lung (0.25 units/mL) and N-[3-(2-Furyl)acryloyl]-L-phenylalanyl-glycyl-glycine (FA-PGG) were purchased from Sigma Aldrich.

Methods:

Peptide Synthesis:
Peptide synthesis vessels (CG-1860) containing a coarse porosity fritted glass resin support and polytetrafluoroethylene (PTFE) cap were purchased from Chemglass. All peptide vessel shaking (to ensure mixing during solid phase peptide synthesis) was performed using a Burrell Wrist Action Shaker (Model 75). Specific synthetic details are summarized in Section II. All peptides were stored at -20 °C prior to use.

Peptide Purification and Characterization:
All peptide purification was carried out on an Agilent Technologies 1260 Infinity II HPLC system equipped with an Agilent ZORBAX 300SB-C18 column (5 µm, 9.4 × 250 mm) using 0.1% TFA in water and 0.1% TFA in acetonitrile as the eluent. Specific methods are described in Section III. Samples were prepared by dissolution of 20-25 mg crude peptide in water (1 mL spiked with 0.1% TFA). The borylated peptide was separated from reaction byproducts by dissolution of the crude product (0.5-5 mg) in water (1 mL spiked with 0.1% TFA), filtered using a 0.2 µm PTFE Pall syringe filter followed by purification by HPLC as described in Section III.

LC-MS analysis was utilized to assess efficiency of all bioconjugation reactions using an Agilent 6530 ESI-Q-TOF with an Agilent ZORBAX 300SB C18 column (5 µm, 2.1 × 150 mm) heated to 40 ºC and an injection volume of 0.3 µL. All mobile phase solvents were acidified with formic acid (0.1%).

LC-MS Method for Analysis of Peptide-Containing Samples:

Time (min)	Water %	Acetonitrile %	Flow (mL/min)	Max. Pressure (bar)
0.00	99.0	1.0	0.800	300.0
2.00	99.0	1.0	0.800	300.0
11.00	9.0	91.0	0.800	300.0

12.00	5.0	95.0	0.800	300.0

Table S1: LC-MS method used to characterize all peptides and products.

For all peptide-containing samples, the detector was switched on at 2 min, with the exception of **7a**/**7b**, **3a**/**3b** and **2b** when used in Proteinase K experiments (see Section IX). For these samples, the detector was switched on immediately when the run started. All samples were prepared by dissolution of the sample (0.1 mg/mL) in either water or acetonitrile spiked with 0.1% TFA. All data were analyzed using the program mMass version 5.4.1.0. Masses were collected in positive mode and are reported as protonated species. All LC traces are plotted time against the total ion count (TIC); however, the y-axis is omitted for simplicity.

Protein Purification and Characterization

Protein was purified using Pall™ microsep advance centrifugal devices with omega membrane 3K. The sample was dissolved in water (5 mL) and centrifuged at 18,000 x g for 1 hr. After, the volume beneath the membrane was discarded and the reservoir above the membrane was refilled with a second aliquot of water (5 mL) for 1 hr. The process was repeated a third time until purified protein was isolated.

LC-MS analysis was utilized to assess efficiency of all bioconjugation reactions using an Agilent 6530 ESI-Q-TOF with an Agilent ZORBAX 300SB C3 column (3.5 µm, 3 × 150 mm) heated to 40 ºC and an injection volume of 0.3 µL. All mobile phase solvents were acidified with formic acid (0.1%). All samples were prepared in water spiked with 0.1% TFA. Masses were collected in positive mode and deconvolution was conducted using Agilent Mass Hunter Qualitative Analysis software.

LC-MS Method for Analysis of Protein-Containing Samples:

Time (min)	Water %	Acetonitrile %	Flow (mL/min)	Max. Pressure (bar)
0.00	90.0	10.0	0.500	300.0
2.00	90.0	10.0	0.500	300.0
11.00	9.0	91.0	0.500	300.0
12.00	5.0	95.0	0.500	300.0

Table S2: LC-MS method used to characterize all peptides and products.

Tandem MS/MS

MS/MS was performed on purified peptide samples, both before and after borylation. Fragments before and after borylation were compared. MS/MS analysis was performed using a 30 eV collision energy and the method and fragment analysis were carried out using ProSight Lite software.

Infrared Radiation (IR)

All IR spectra were collected on a PerkinElmer UATR Two FT-IR spectrometer. PerkinElmer Spectrum Software version 10.4.4 was used to process all data. All spectra were collected on solid powders with background subtraction between samples.

NMR

All NMR spectra were obtained on a Bruker Avance 400 or a Bruker DRX 500 MHz broad band FT NMR spectrometer. ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were referenced to residual protio-solvent signals, and both ^{11}B and $^{11}B\{^{1}H\}$ NMR chemical shifts were referenced to $BF_3 \bullet Et_2O$ (15% in $CDCl_3$, δ 0.0 ppm).

Isothermal Titration Calorimetry
All titrations were performed on a GE MicroCal iTC 200 and analyzed on MicroCal Data Analysis software.

Internal Standard
For all reactions, the internal standard was prepared by alkylation of H_2N-VKGALGVCG-$CONH_2$ using iodoacetamide as described by Pentelute and Buchwald *et al*.[1] Internal standard (H_2N-VKGALGV(H_2NCOCH_2-)CG-$CONH_2$) was added to the indicated reactions (See Section III and IV) after quenching.

Inductively Coupled Plasma Atomic Emission (ICP-AE)
ICP-AES data were obtained using a Shimadzu ICPE-9000 spectrometer. Prior to measurements, samples were diluted in 4% nitric acid (Trace Metal Grade, Fisher) and a calibration curve was obtained using a platinum standard solution (1 mg/mL, Sigma-Aldrich), and diluted to 50, 75, 100, and 200 ppb Pt with 4% nitric acid.

Organometallic Reagent Synthesis
$[((Me\text{-}DalPhos)AuCl)_2C_6H_4][SbF_6]_2$ was prepared following a previously reported procedure.[2] $[PtCl(PPh_3)_2(9\text{-}B\text{-}m\text{-}C_2B_{10}H_{11})]$ was prepared according to procedures outlined by Spokoyny *et al*.[3]

Fluorescein Isothiocyanate (FITC) Labeling of Peptides
Peptide H_2N-FITC-β-ACGGA-$CONH_2$ was labeled with FITC following a procedure previously described.[4]

Buffers
Buffer for proteinase K experiments (Section IX) were adjusted with hydrochloric acid (50 mM) or sodium hydroxide (50 mM). All other aqueous buffers were adjusted with either potassium acetate (50 mM) or glacial acetic acid (50 mM) in MilliQ water.

Note on Calculation of Peptide Molecular Weight
For all peptides synthesized by solid phase synthesis, additional mass (114.02 g/mol) was added for each basic residue side chain as trifluoroacetate counterion balances the protonated basic residue.

Centrifugation
A Thermo (Sorvall) LEGEND RT+ centrifuge was utilized for all protein centrifugations. A Fisher Scientific accuSpin Micro 17 centrifuge was utilized for all peptide and CHO cell centrifugations.

Molecular Dynamics Simulations
Molecular dynamics (MD) simulations of **2a**, **2b** and **2c** solvated in 5 mM $CaCl_2$ aqueous solution was performed using NAMD[5] where a TIP3P model[6] was used to describe water and the CHARMM force field[7,8] was used to parameterize the nonapeptide and the proteinase K (PDB ID: 3PRK[9]). Ab initio calculations were done with *Gaussian09*[10] to determine unknown parameters for the carborane cluster. The carborane was optimized at the MP2/6-31g(d) level of theory, with

partial charges derived with a CHELPG algorithm[11]. Bonds, angles, and dihedrals force constants containing boron atoms were determined at the MP2/6-31g(d)//HF/6-31g level of theory with the VMD Force Field Toolkit plugin[12,13]. The alpha-carbons of residues that are 10 residues away from the substrate recognition site, which constitutes of two segments, Gly100-Tyr104 and Ser132-Gly136, in proteinase K was constrained in a harmonic potential with force constant 0.8 kcal mol^{-1} $Å^{-2}$. Nonbonded van der Waals interactions were modeled using Lennard-Jones potentials and electrostatic interactions were described by a Coulombic coupling, where the cutoff distance was 10 Å in both interactions. The Particle Mesh Ewald algorithm[14] with a grid spacing of 1 Å was used to describe long-range electrostatic interactions. A Langevin dynamics with a damping coefficient of 0.1 ps^{-1} was applied in the simulations, where the time step was 1 fs in all simulations. Each system is first minimized for 50,000 steps. Afterwards, it is heated to 300 K, with 1 K increments per 5 steps until the system reaches a temperature of 300 K. A canonical ensemble with a constant temperature and pressure (NPT, P = 1 bar, T = 300 K) was used with periodic boundary conditions applied in all directions.

II. Peptide Synthesis and Purification

The following general protocol was followed for all solid-phase peptide syntheses.

Preparation of Resin:
Rink amide resin (1.0 g, 0.44 mmol/g) was added to a 250 mL peptide synthesis vessel fitted with a coarse-porosity fritted glass resin support. Dimethylformamide (10 mL) was added to the vessel, and the mixture was shaken for a minimum of 1 h to allow the resin to swell. The resin was subsequently washed with DMF (5 × 10 mL).

First Deprotection:
A 20% solution of 4-methylpiperidine in DMF (10-15 mL/g of resin) was added to the vessel, and the mixture was shaken for 20 min. After shaking, the 4-methylpiperidine solution was removed and the resin was washed with DMF (1 x 10 mL). A fresh solution of 4-methylpiperidine in DMF (10-15 mL/g of resin) was added to the vessel, and the and the vessel was shaken for an additional 5 min. The resin was then washed with DMF (5 × 10 mL) to ensure complete removal of 4-methylpiperidine.

Amino Acid Coupling Conditions:
Amino acid (3 equiv to resin) and hexafluorophosphate benzotriazole tetramethyl uronium (HBTU, 2.9 equiv to resin) were dissolved in DMF (10 mL). N,N- Diisopropylethylamine (DIPEA, 6 equiv to resin) was then added to the mixture, and the solution was stirred for 1 min. This mixture was then added to the peptide synthesis vessel containing the resin, and the vessel was shaken for 45 min. After shaking, the solution was removed, and the resin was washed with DMF (5 × 10 mL) to ensure complete removal of excess amino acid, HBTU, and DIPEA.

Cysteine Coupling Conditions:
Cysteine coupling was performed following a procedure previously reported.[15]

Fmoc-Cys(Trt)-OH (3 equiv to resin), hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU, 4 equiv to resin), and 1-hydroxy-7-azabenzotriazole (HOAt, 0.6 M in DMF, 4 equiv to resin) were combined in DMF (6 mL) and CH_2Cl_2 (6 mL). Once dissolved, 2,4,6-trimethylpyridine (4 equiv to resin) was added and the mixture stirred quickly (1-2 sec) and added to the resin. The mixture was shaken for 1 h. After shaking, the resin was washed with DMF (5 × 10 mL) to ensure the removal of excess Fmoc-Cys(Trt)-OH, HATU, and HOAt. After cysteine coupling, the normal protocol was followed.

Amino Acid Deprotection Conditions:
A 20% solution of 4-methylpiperidine in DMF (10-15 mL/g of resin) was added to the vessel and the mixture was shaken for 15 min. After shaking, the 4-methylpiperidine solution was removed and the resin was washed once with DMF (10 mL). A fresh solution of 4-methylpiperidine in DMF (10-15 mL/g of resin) was added to the vessel, and the mixture was shaken for an additional 5 min. The solution was removed, and the resin was washed with DMF (5 x 10 mL) to ensure the compete removal of 4-methylpiperidine.

Cleavage from Resin:
After the final deprotection, the resin was washed with EtOH (2 x 10 mL), followed by DCM (3 × 10 mL). The washed resin was transferred to a 50 mL round bottom flask equipped and a magnetic stir bar was added. Nitrogen gas was flowed over the open vessel for 5 min. A cleavage cocktail (20 mL) consisting of a 95:2.5:2.5 mixture of TFA:H_2O:TIPS (TIPS = triisopropylsilane)

was added to the resin. The slurry was stirred for 3-4 hours under an atmosphere of nitrogen. Cleavage time was dependent on the amino acid composition of the peptide. Aliquots of the slurry were analyzed *via* LC-MS after filtration through a small pipette filter and dilution with water to determine full removal of peptide protecting groups. After 3-4 h, the cleavage cocktail was filtered and the filtrate was concentrated under a stream of nitrogen until 1 mL remained. To this solution was added cold (-20 °C) diethyl ether, resulting in the precipitation of the crude peptide. The supernatant was removed and the crude peptide was collected and then dried under reduced pressure.

*It is important to use fresh TIPS. TIPS stored longer than two months is less effective.

All crude peptides were purified by reversed-phase HPLC (see HPLC methods). The pure fractions were combined and lyophilized. All crude and purified peptides were stored in sealed containers at -20 °C.

Cleavage from Resin for Peptide Containing Tryptophan (9a):
After the final deprotection, the resin was washed with EtOH (2 x 10 mL), followed by DCM (3 × 10 mL). The washed resin was transferred to a 25 mL round bottom flask equipped and a magnetic stir bar was added. Nitrogen gas was flowed over the open vessel for 5 min. A cleavage cocktail (20 mL) consisting of a 95:2.5:2.5 mixture of TFA:H_2O:EDT (EDT = 1,2-ethanedithiol) was added to the resin. The slurry was stirred for 3 hours under an atmosphere of nitrogen. Aliquots of the slurry were analyzed *via* LC-MS after filtration through a small pipette filter and dilution with water to determine full removal of peptide protecting groups. After 3 h, the cleavage cocktail was filtered and the filtrate was concentrated under a stream of nitrogen until 1 mL remained. To this solution was added cold (-20 °C) diethyl ether, resulting in the precipitation of the crude peptide. The supernatant was removed and the crude peptide was collected and then dried under reduced pressure.

HPLC Methods:
For instrumentation information see Section I. The following methods were used to purify both the unmodified and borylated peptides:

A. Isocratic flush water (99%), acetonitrile (1%) for 5 min. Gradient from water (99%), acetonitrile (1%) to water (60%), acetonitrile (40%) from 5 to 45 min. Flush with 100% acetonitrile from min 45 to 48.

B. Isocratic flush water (100%) for 5 min Gradient from water (100%), acetonitrile (0%) to water (90%), acetonitrile (10%) over 85 min. Flush with 100% acetonitrile from min 85 to 88.

C. Isocratic flush water (99%), acetonitrile (1%) for 5 min. Gradient from water (99%), acetonitrile (1%) to water (40%), acetonitrile (60%) from 5 min to 80 min. Flush with 100% acetonitrile from min 80 to 83.

D. Gradient from water (99%), acetonitrile (1%) to water (65%), acetonitrile (35%) over 5 min followed by gradient from water (65%), acetonitrile (35%) to water (40%), acetonitrile (60%) from 5 min to 85 min. Flush with 100% acetonitrile from min 85 to 91 min.

E. Isocratic flush water (99%), acetonitrile (1%) for 5 min. Gradient from water (99%), acetonitrile (1%) to water (80%), acetonitrile (20%) from 5 to 85 min. Flush with 100% acetonitrile from min 85 to 88.

Sequence	Purification Method (unmodified)	Purification Method (borylated)
H_2N-γ-ECG-$CONH_2$	-	A
H_2N-DRKCAT-$CONH_2$	A	A
H_2N-FITC-β-ACGGA-$CONH_2$	C	C
H_2N-VKGALGVCG-$CONH_2$	A	A
H_2N-GCGEVKEGCG-$CONH_2$	B	A
H_2N-GSGEVKEGCG-$CONH_2$	B	A
H_2N-SCQPQPLIYP-$CONH_2$	D	D
H_2N-GCAWNHA-$CONH_2$	E	C

Table S3: Summary of purified peptidess and HPLC purification methods.

Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)

In order to assess the removal of Pt-based byproducts, ICP-AES of HPLC-purified peptide **(7b)** was conducted (see section IV for borylation of glutathione). All standards and samples were measured with a 30 sec exposure time and conducted in triplicate. An R^2 value of 1.000 was obtained and less than 50 ppb Pt was detected representing >99.9% removal of Pt by HPLC purification.

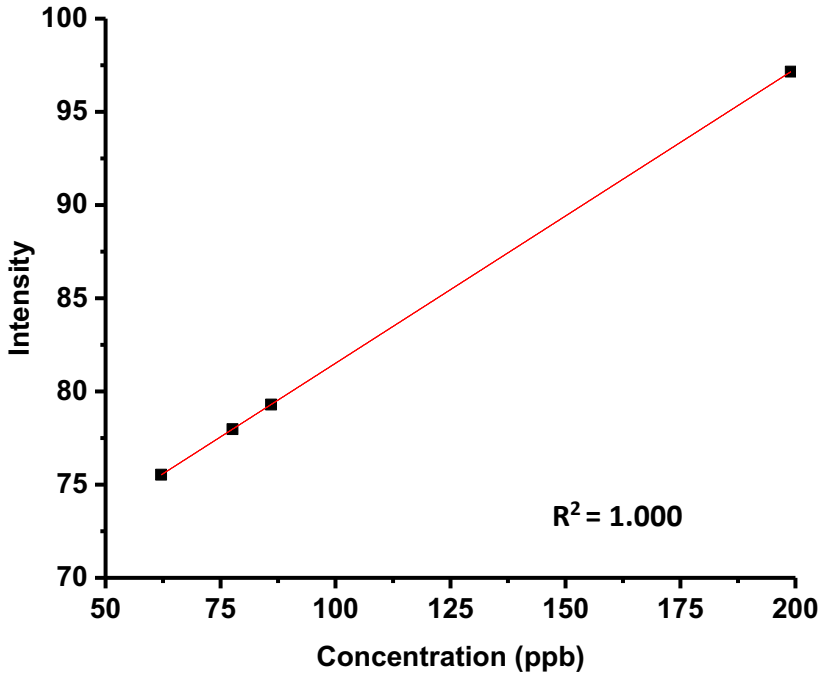

Figure S1: ICP-AES calibration curve used to measure Pt content remaining after HPLC purification of borylated peptide products.

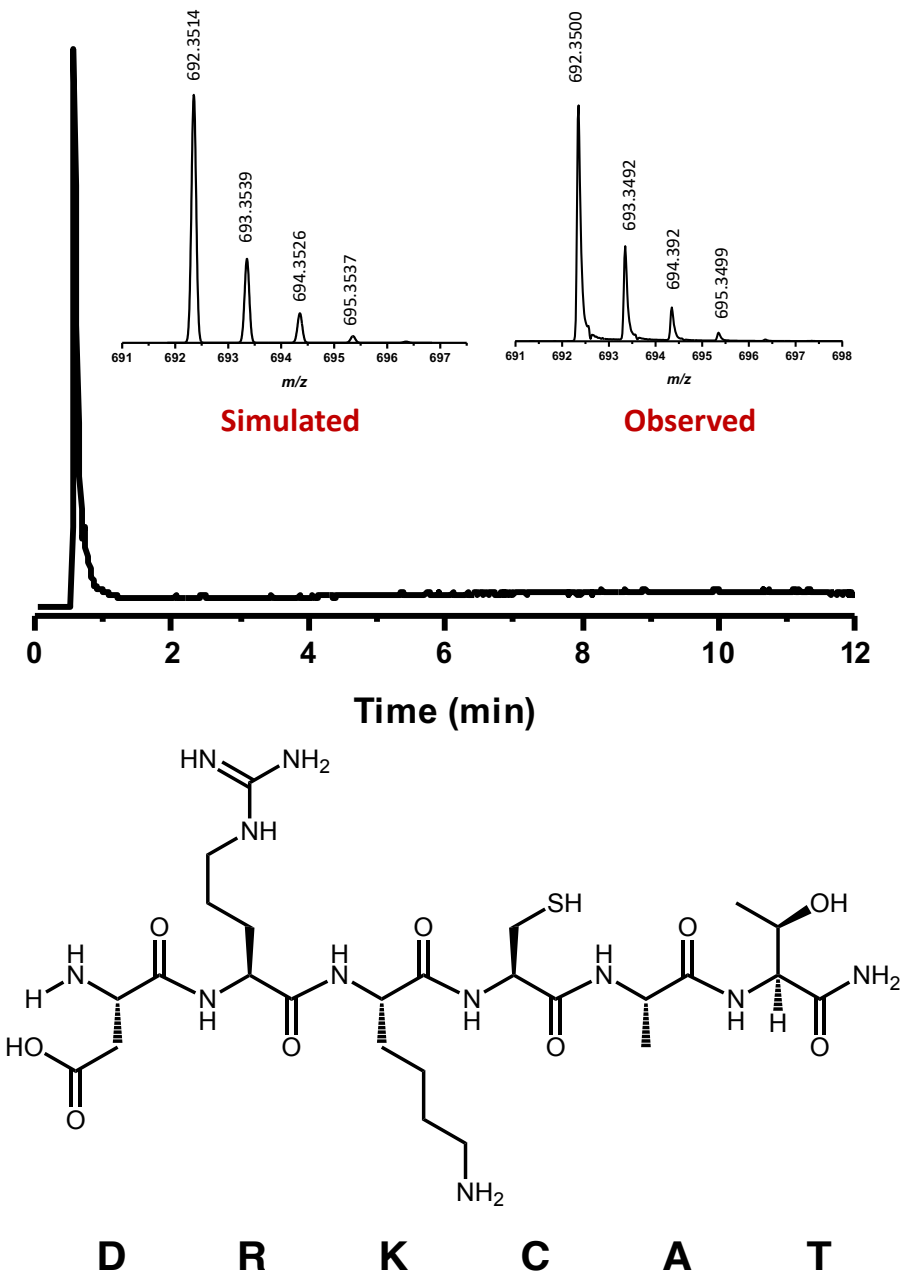

Figure S2: LC-MS data of purified peptide, H_2N-DRKCAT-$CONH_2$, **3a**, with simulated and observed masses.

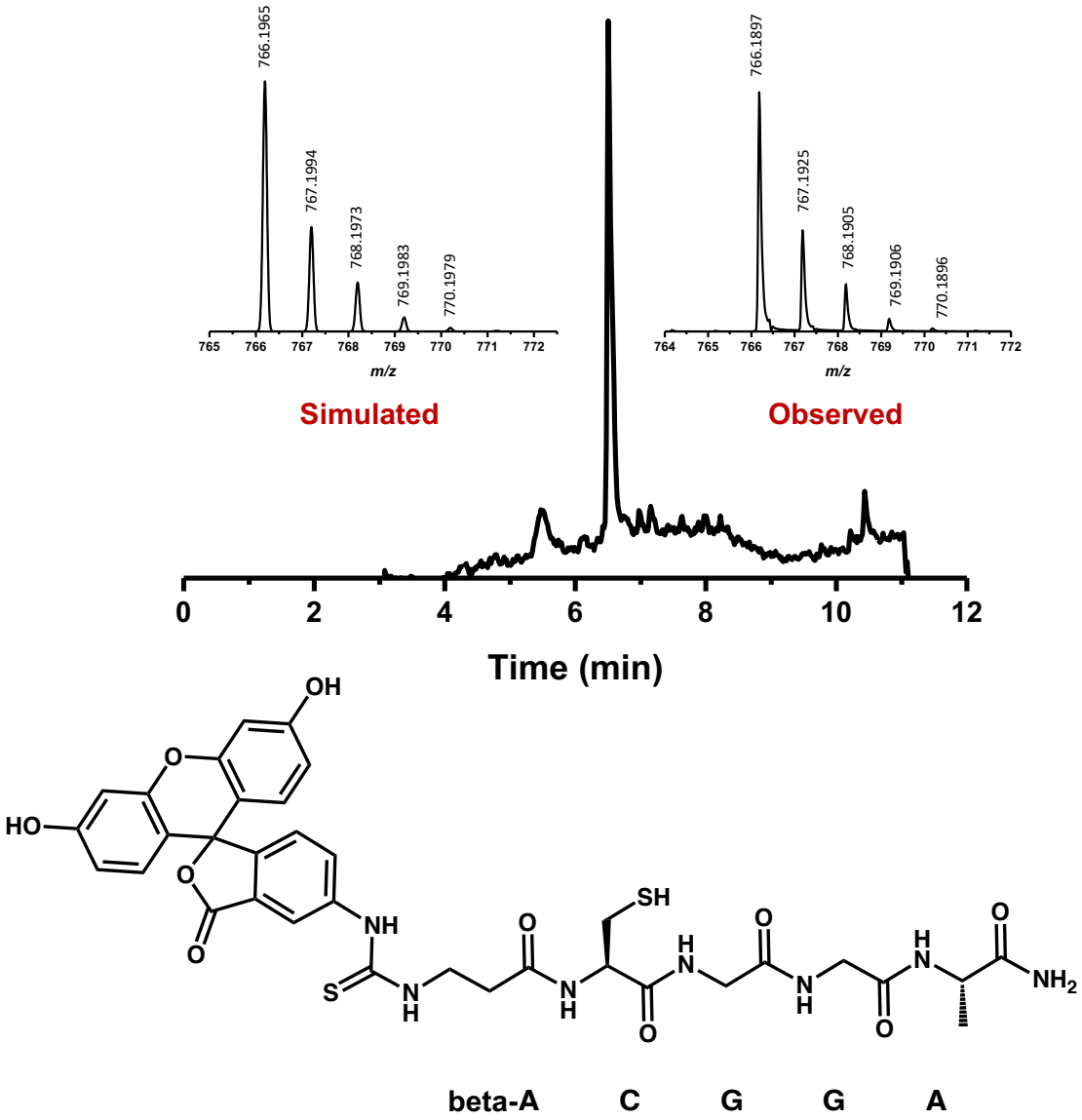

Figure S3: LC-MS data of purified peptide, FITC-β-ACGGA-CONH$_2$, **4a**, with simulated and observed masses.

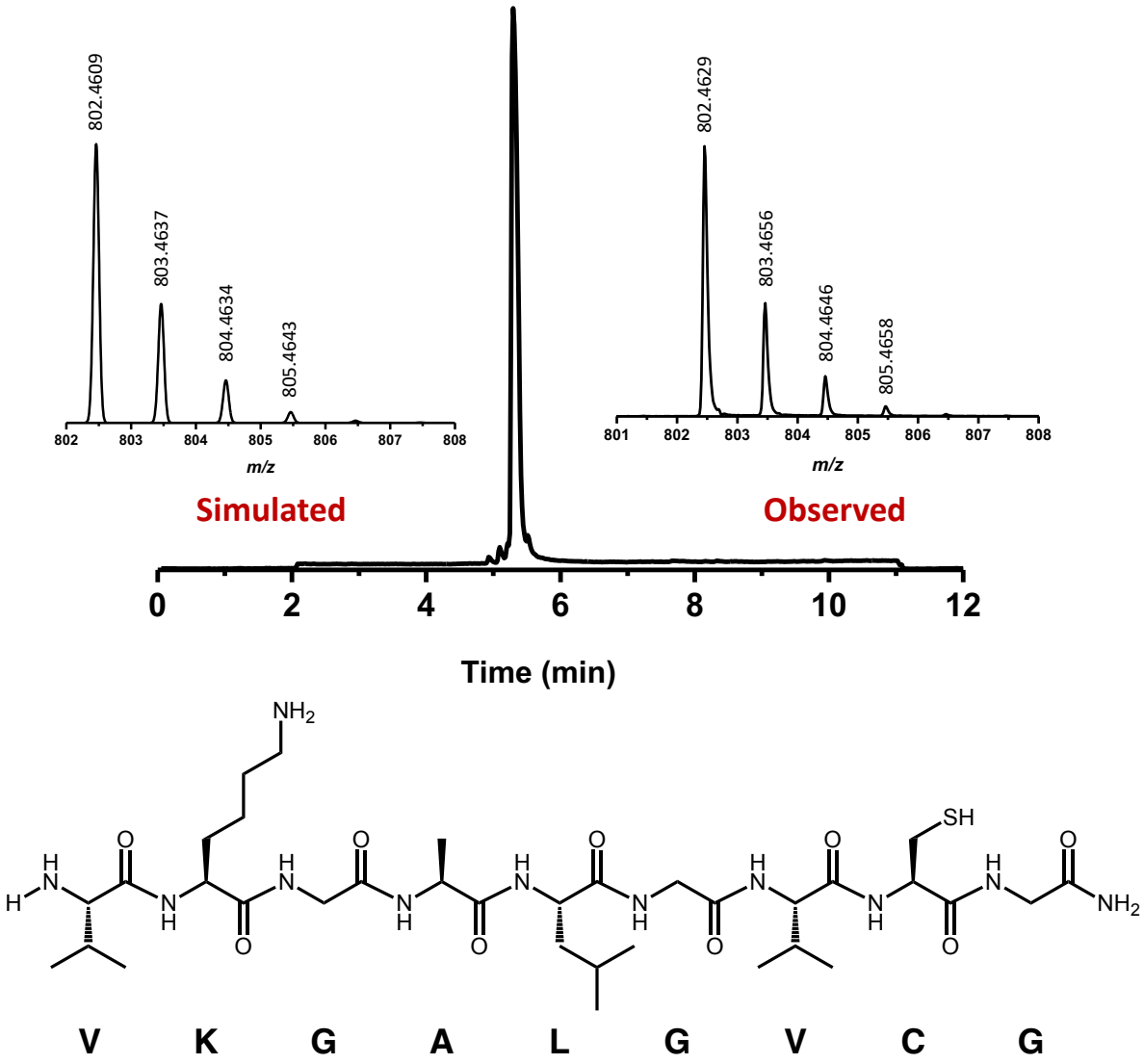

Figure S4: LC-MS data of purified peptide, H_2N-VKGALGVCG-$CONH_2$, **2a**, with simulated and observed masses.

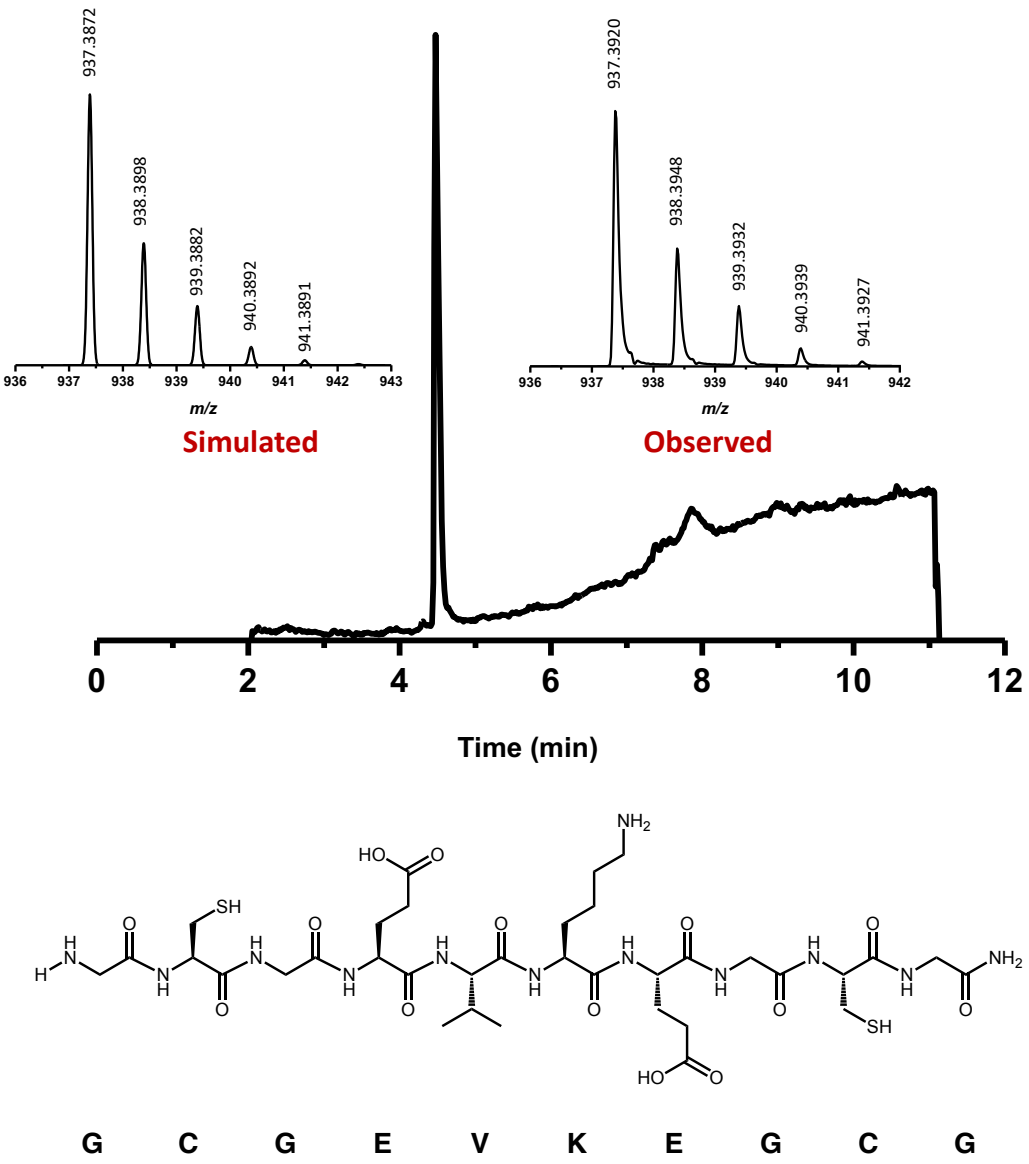

Figure S5: LC-MS data of purified peptide, H_2N-GCGEVKEGCG-$CONH_2$, **5a**, with simulated and observed masses.

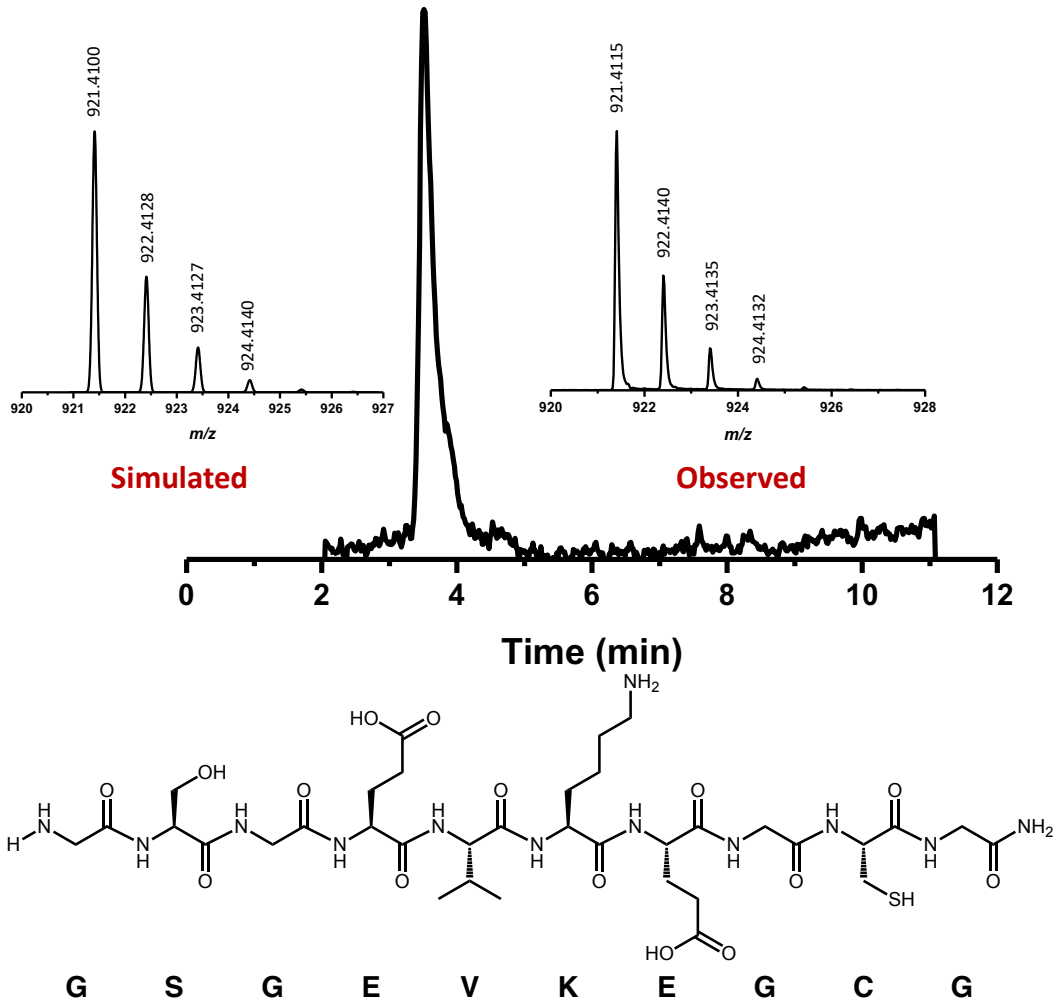

Figure S6: LC-MS data of purified peptide, H_2N-GSGEVKEGCG-$CONH_2$, **6a**, with simulated and observed masses.

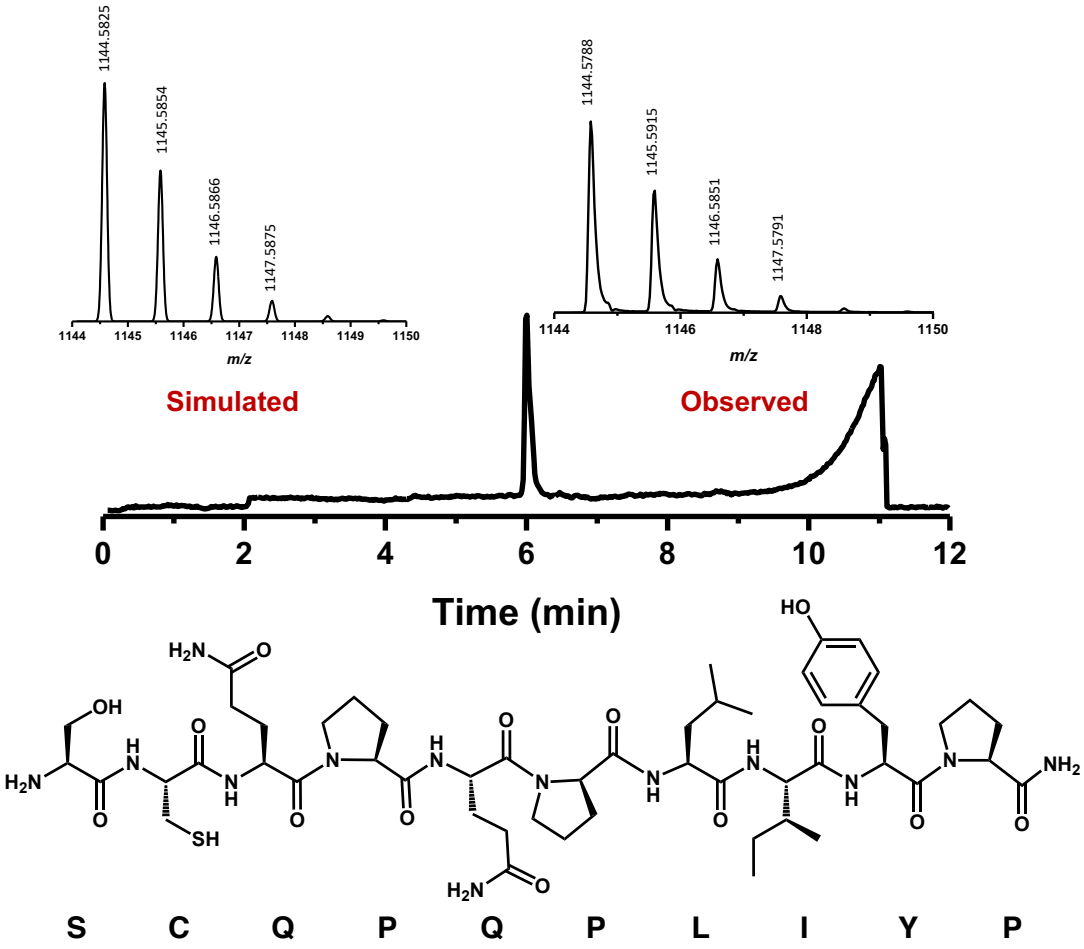

Figure S7: LC-MS data of purified peptide, H_2N-SCQPQPLIYP-$CONH_2$, **8a**, with simulated and observed masses.

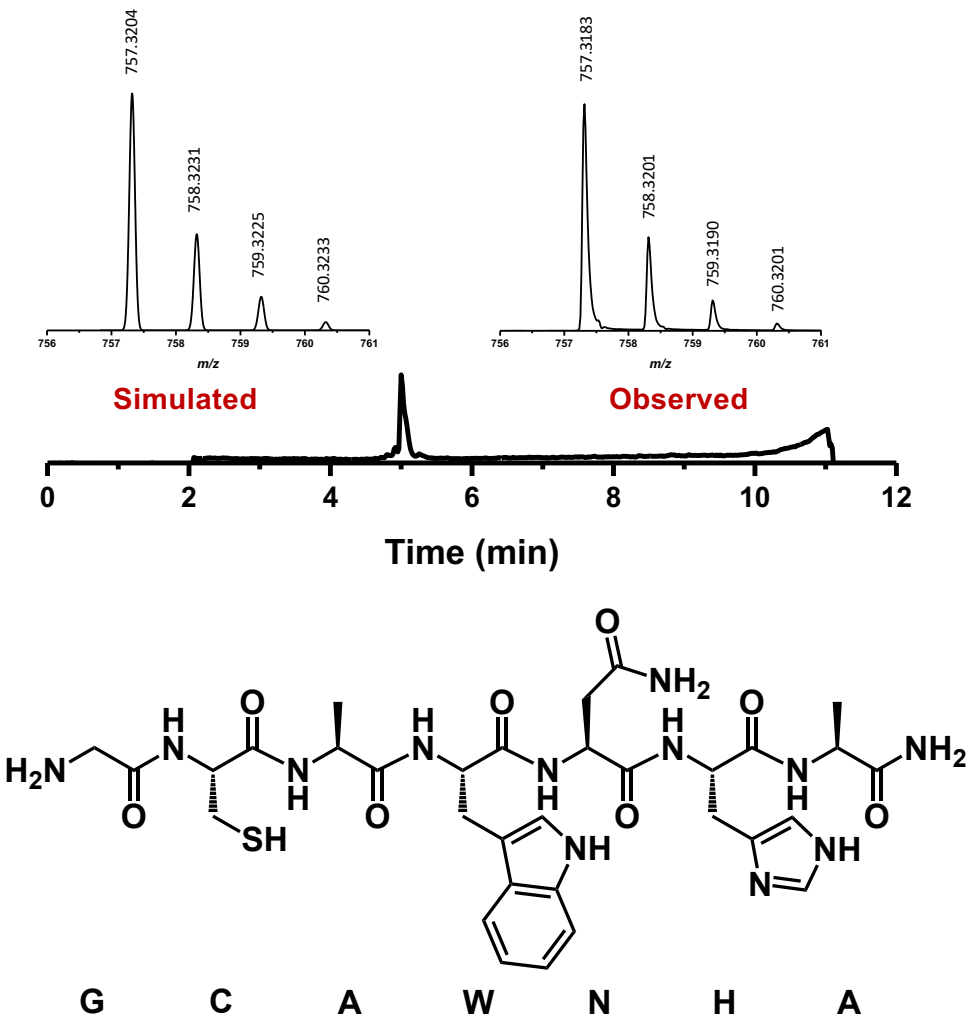

Figure S8: LC-MS data of purified peptide, H_2N-GCAWNHA-$CONH_2$, **9a**, with simulated and observed masses.

III. Peptide Borylation Reactions

General Borylation Procedure:
Each peptide (5 mM) was dissolved in Tris•HCl DMF buffer (30 mM). To this solution was added a dry stir bar and $PtCl(PPh_3)_2$-(9-B-*m*-$C_2B_{10}H_{11}$) (1.2 equiv per cysteine). The reaction was sonicated for 5 min to ensure complete solubilization of reagents, and was then allowed to stir for 1.5 h. An aliquot of the reaction mixture was removed (5 μL) and quenched with a 2:1 water:acetonitrile (v/v, spiked with 0.1% TFA) solution (175 μL). Finally, internal standard (20 μL, 1.25 mM, see Section I) was added to yield a final volume sample of 200 μL. This sample was then subjected to LCMS analysis. After ensuring conversion, the remainder of the reaction mixture was quenched through addition of a 2:1 water:acetonitrile (spiked with 0.1% TFA) mixture equaling 5 x the total reaction volume. Solvent was removed *in vacuo*, and the crude product was suspended in water (1 mL). The suspension was sonicated and then filtered with a Pall syringe filter (0.2 μm, PTFE). The filtrate was dried under reduced pressure, and purification of borylated peptides away from Pt-based byproducts was performed through reversed phase HPLC (Section II).

Depending on quantities, stock solutions of peptide (10-50 mM) and $PtCl(PPh_3)_2$-(9-B-*m*-$C_2B_{10}H_{11}$) (10-100 mM), were prepared, although storage (>24 h) of peptides in solution often resulted in disulfide bond formation between cysteine residues. Disulfide bond formation was also observed when peptide stock solutions were prepared in buffered solution and stored for >0.5 hr so concentrated stock solutions were always prepared in DMF and used immediately. Fresh $PtCl(PPh_3)_2$-(9-B-*m*-$C_2B_{10}H_{11}$) solutions were always prepared prior to use.

Borylation of Glutathione:
Glutathione (1.5 mg, 0.005 mmol, 1 equiv) was dissolved in a Tris • HCl DMF buffer (20 mL, 30 mM). To this mixture, a dry stir bar and $PtCl(PPh_3)_2$-(9-B-*m*-$C_2B_{10}H_{11}$) (5.0 mg, 0.006 mmol, 1.2 equiv) were added. The reaction was sonicated for 5 minutes to ensure solubilization and allowed to proceed for 1.5 h under constant stirring. To terminate the reaction, 5 x the reaction volume of 2:1 water:acetonitrile (0.1% TFA) was added. Quenched reactions were then sonicated for an additional 5 minutes before verifying borylation by LC-MS. After removing solvent *in vacuo*, borylated peptides were suspended in water under sonication and solutions were filtered through a Pall syringe filter (0.2 μm, polytetrafluoroethylene). Purification away from Pt byproducts was performed through reversed phase HPLC (Method A, Section III) to afford the product (0.5 mg, 30% yield).

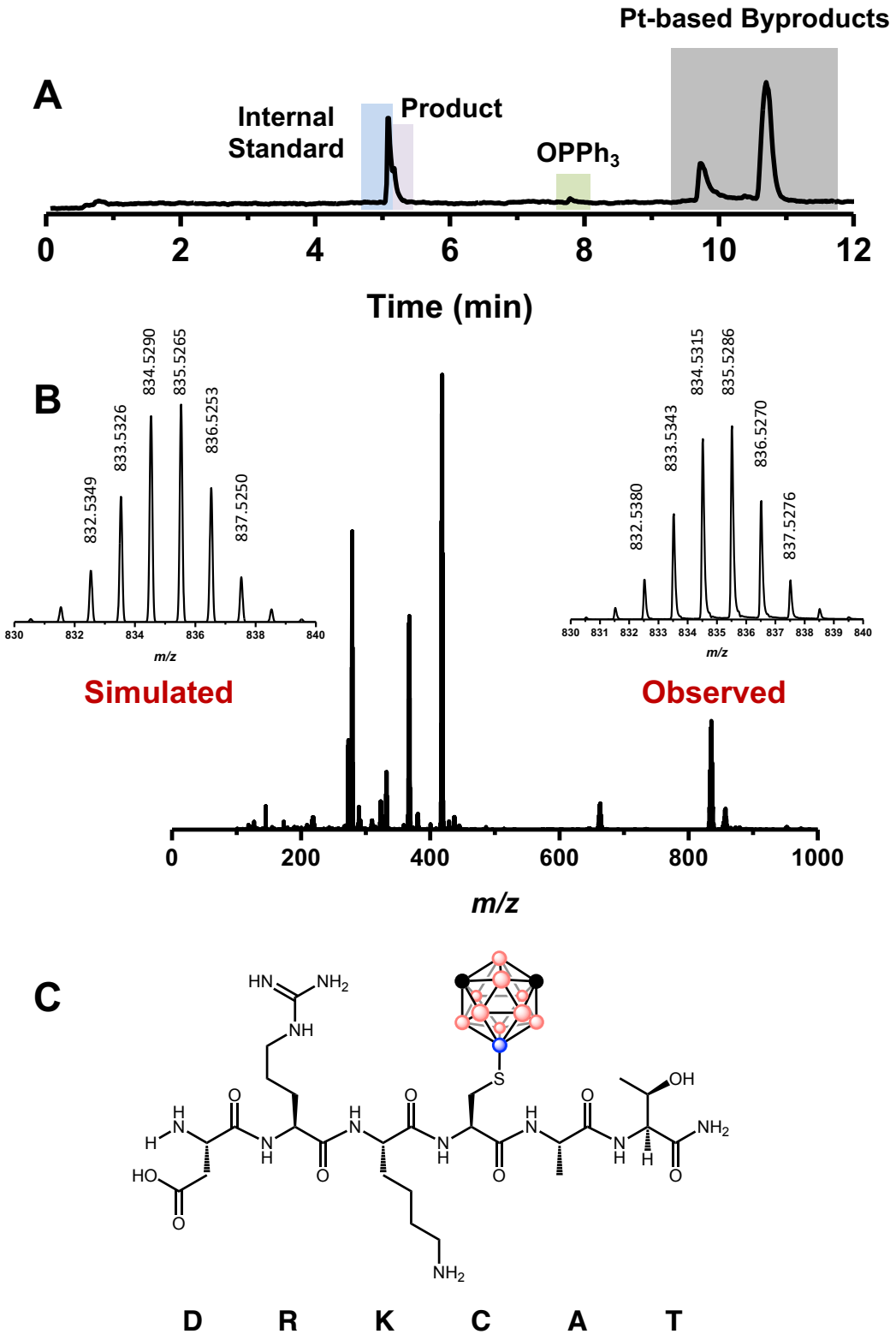

Figure S9 A: LC trace of the crude H_2N-DRKCAT-$CONH_2$ borylation reaction mixture. **B:** Simulated and observed masses for the borylated product **C:** Line drawing of the borylated product, **3b**.

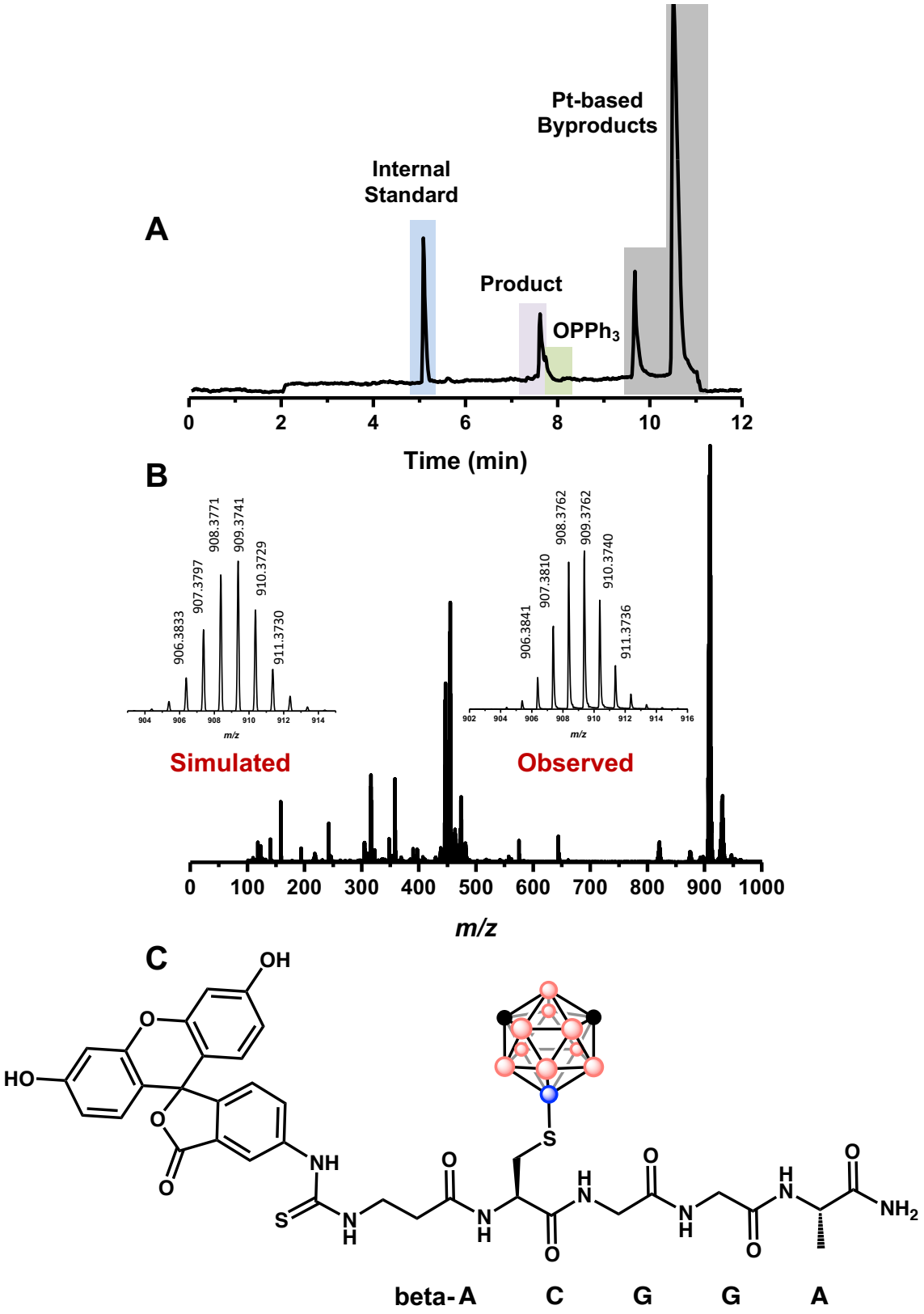

Figure S10 A: LC trace of the crude FITC-ACGGA-$CONH_2$ borylation reaction mixture. **B:** Simulated and observed masses for the borylated product **C:** Line drawing of the borylated product, **4b**.

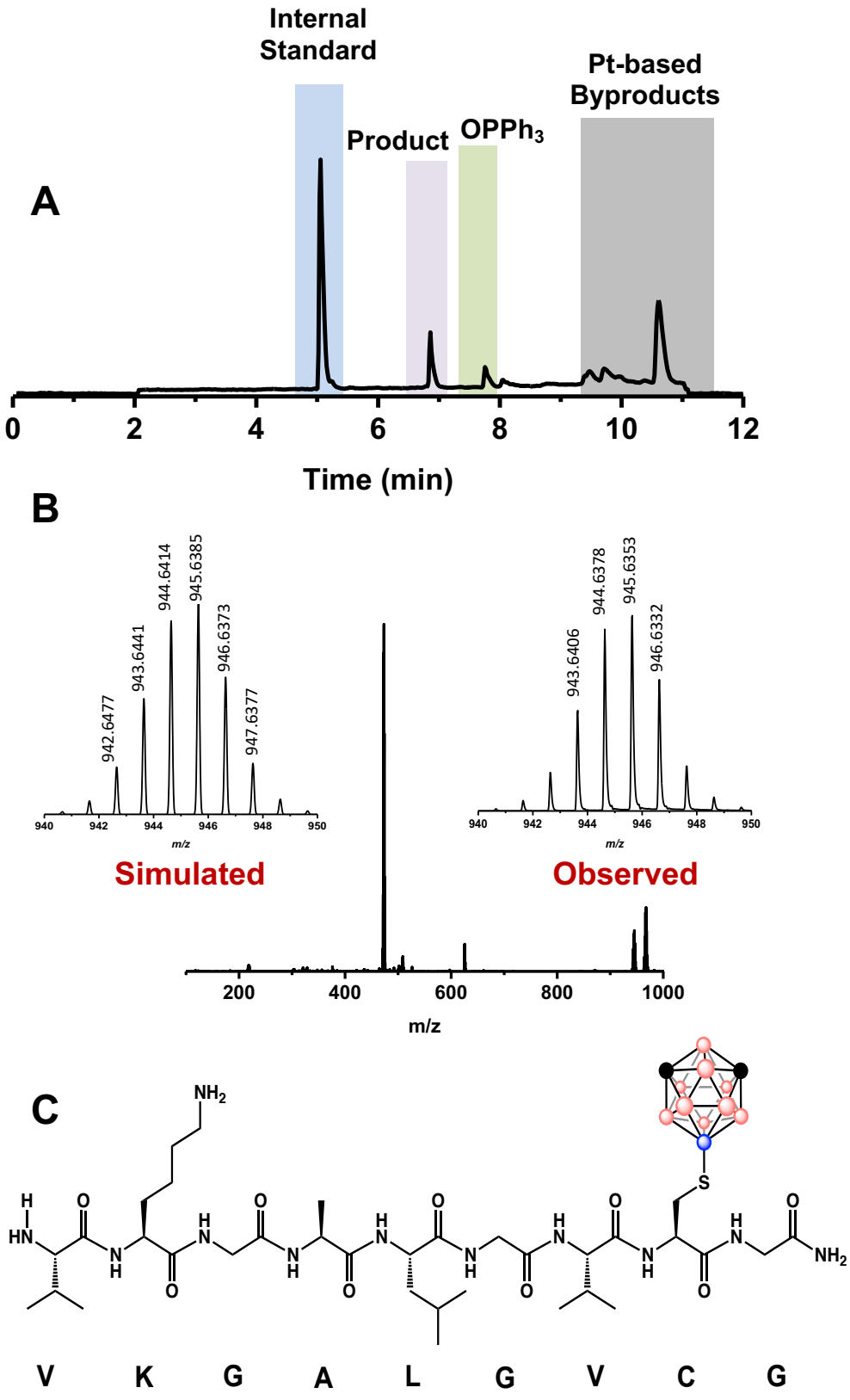

Figure S11 A: LC trace of the crude H_2N-VKGALGVCG-$CONH_2$ borylation reaction mixture. **B:** Simulated and observed masses for the borylated product **C:** Line drawing of the borylated product, **2b**.

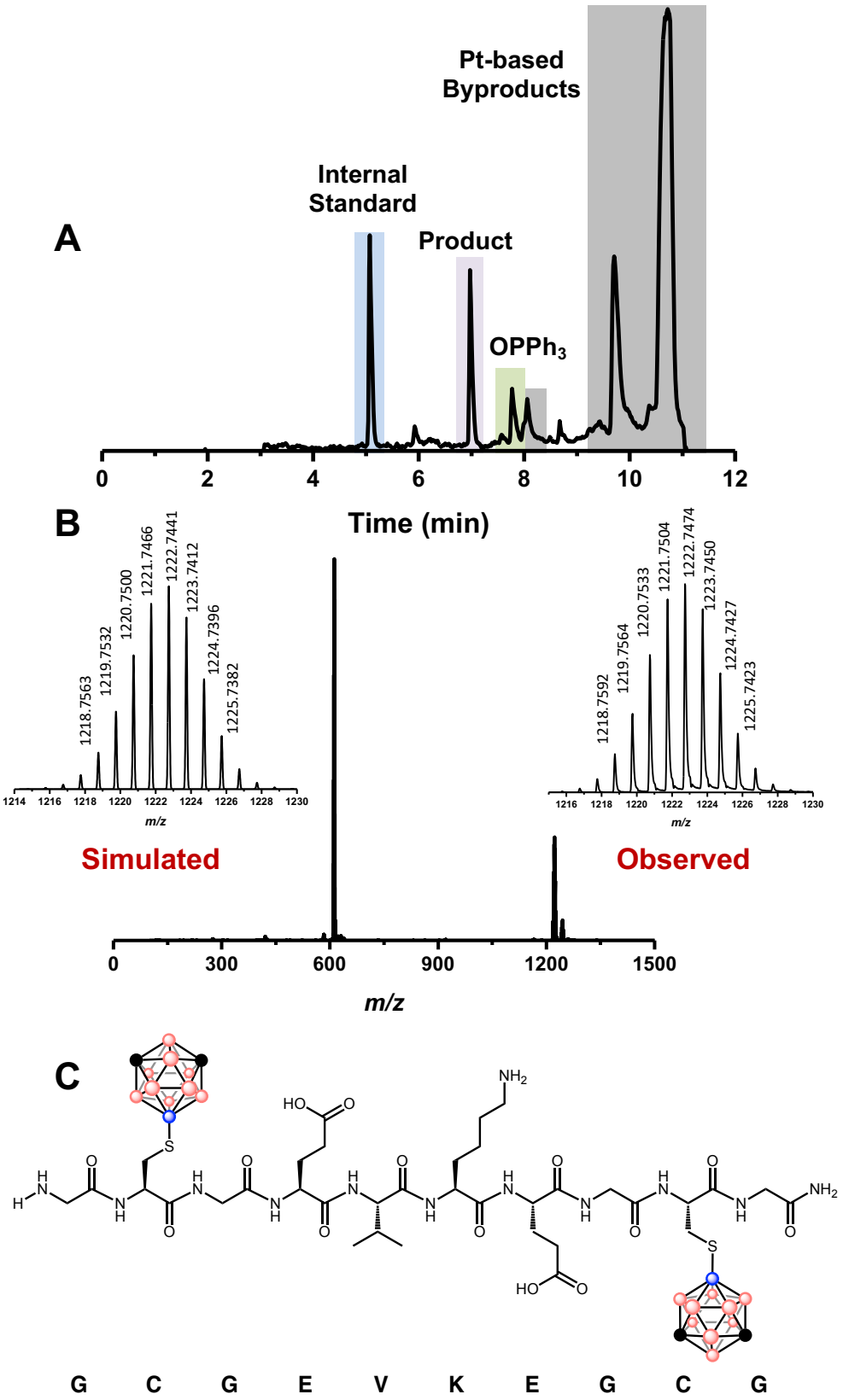

Figure S12 A: LC trace of the crude H_2N-GCGEVKEGCG-$CONH_2$ borylation reaction mixture. **B:** Simulated and observed masses for the borylated product **C:** Line drawing of the borylated product, **5b**.

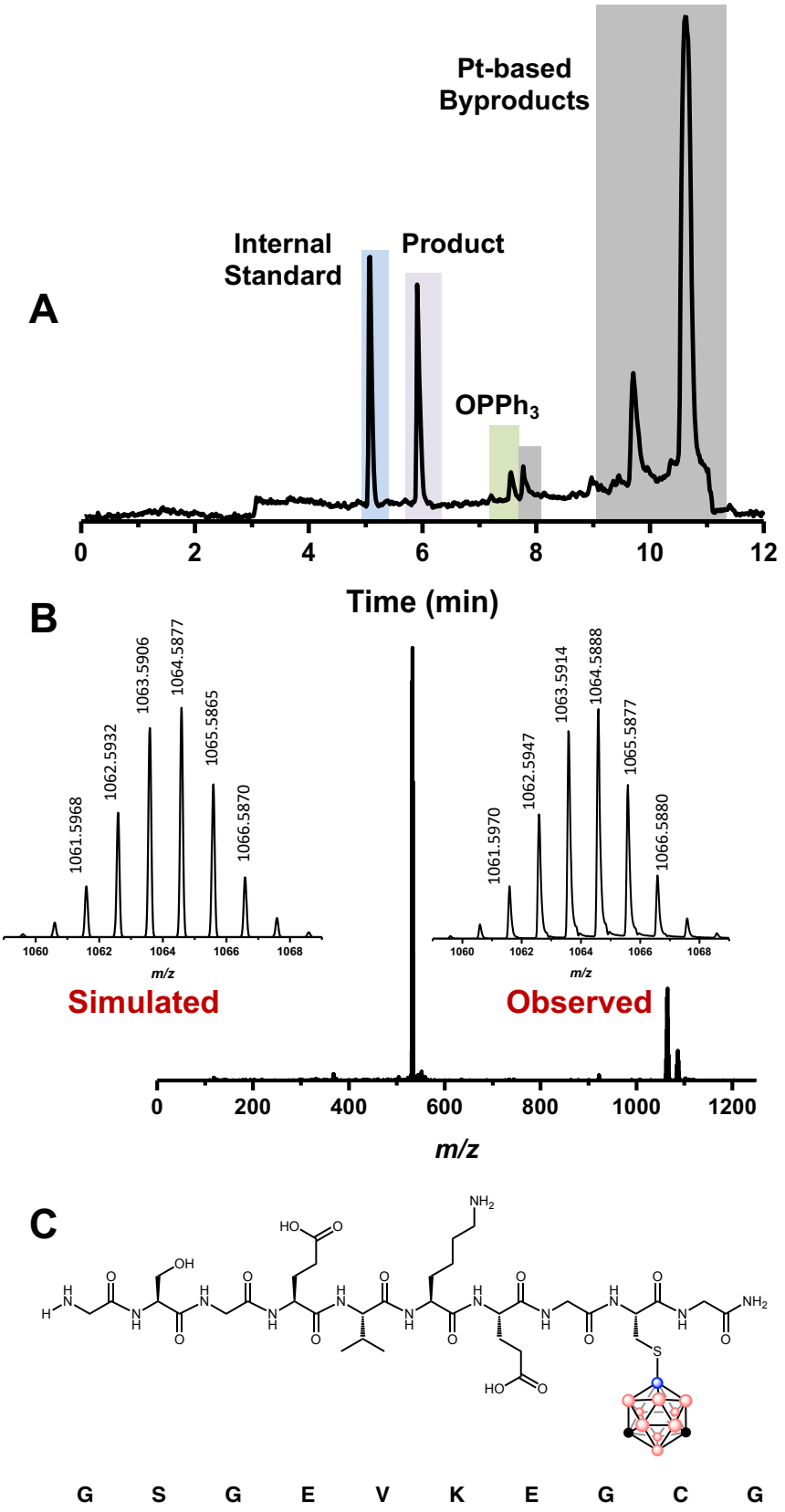

Figure S13 A: LC trace of the crude H_2N-GSGEVKEGCG-$CONH_2$ borylation reaction mixture. **B:** Simulated and observed masses for the borylated product **C:** Line drawing of the borylated product, **6b**.

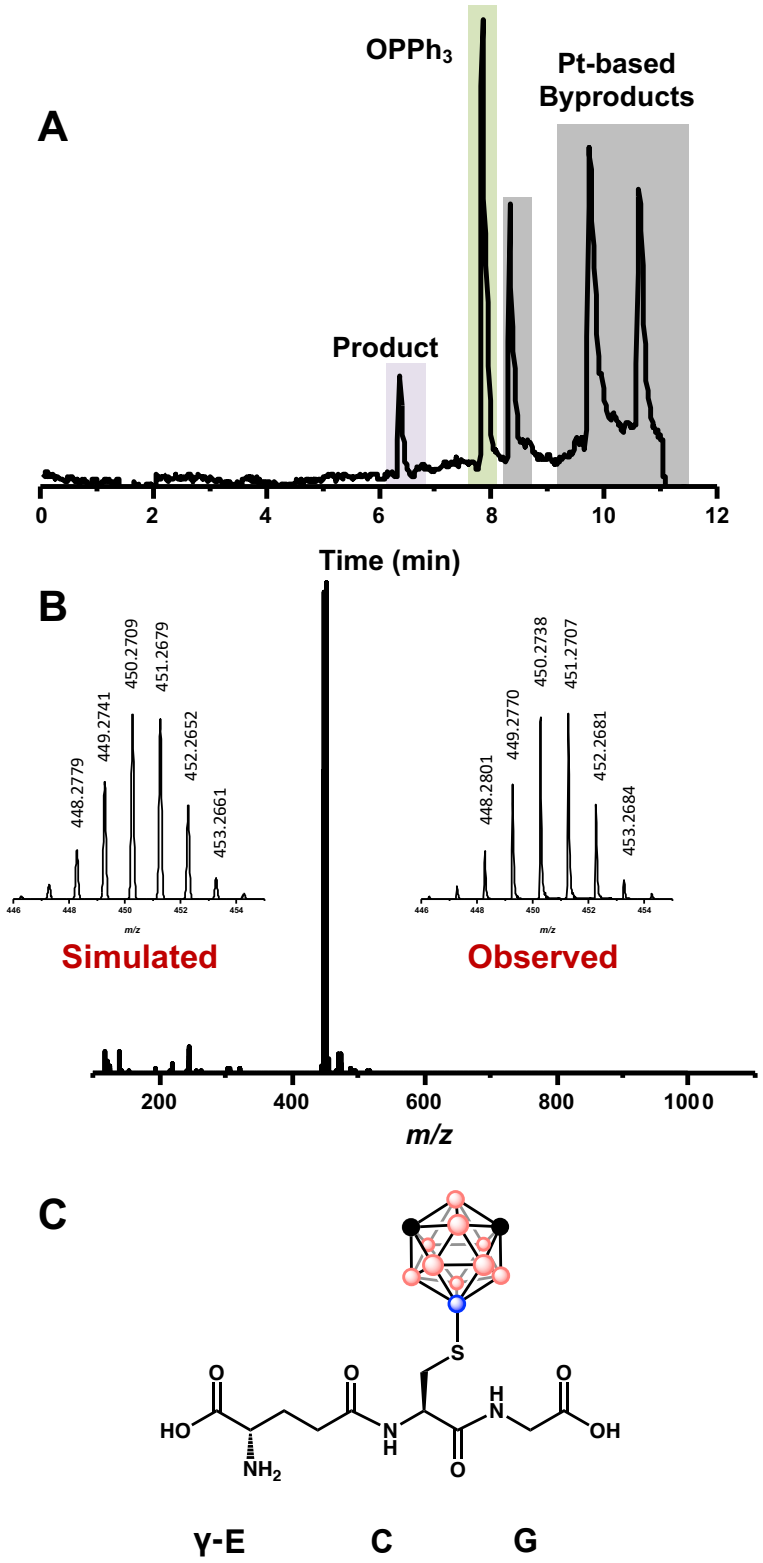

Figure S14 A: LC trace of the crude glutathione borylation reaction mixture. **B:** Simulated and observed masses for the borylated product **C:** Line drawing of the borylated product, **7b**.

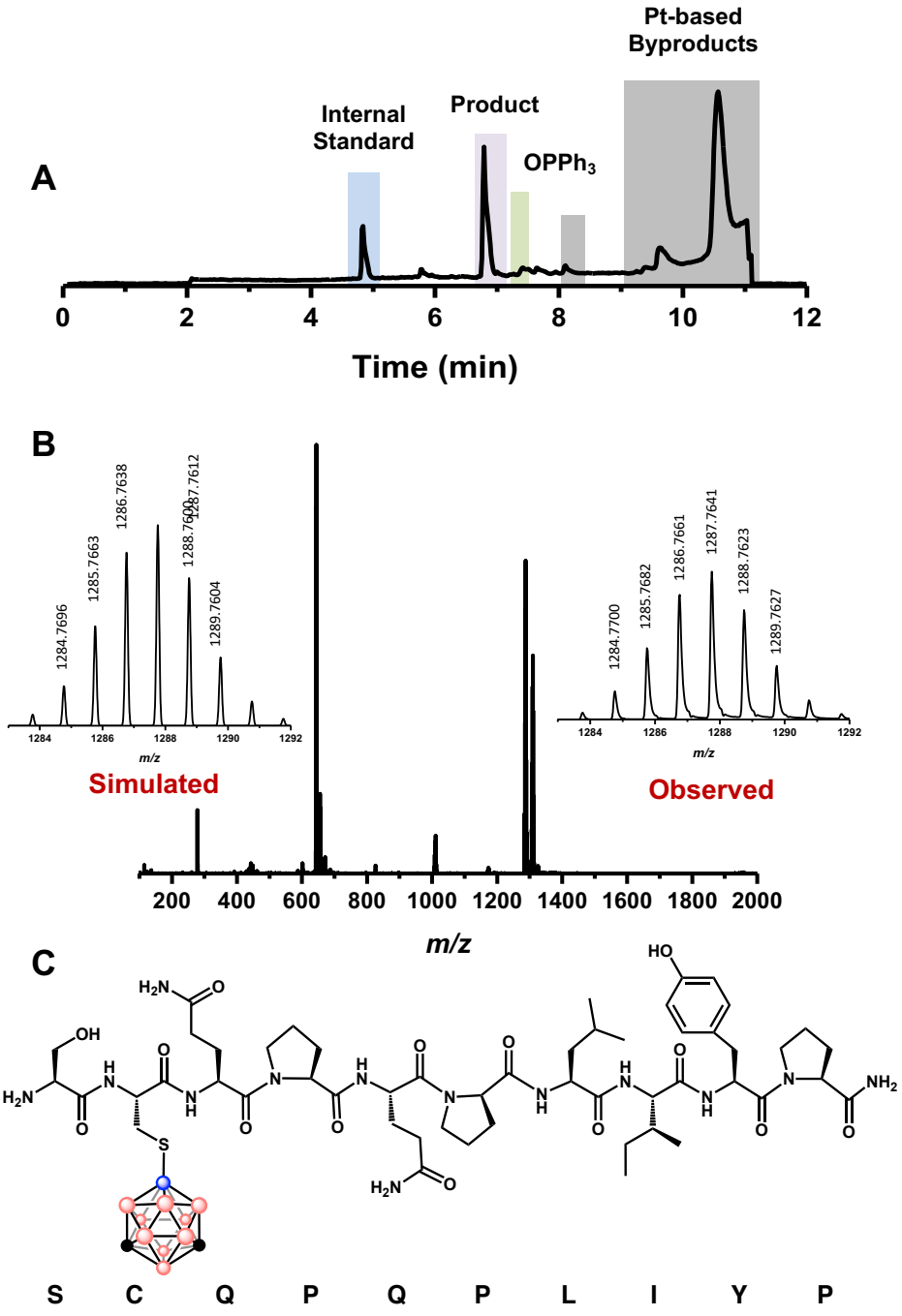

Figure S15 A: LC trace of the crude H_2N-SCQPQPLIYP-$CONH_2$ borylation reaction mixture. **B:** Simulated and observed masses for the borylated product **C:** Line drawing of the borylated product, **8b**.

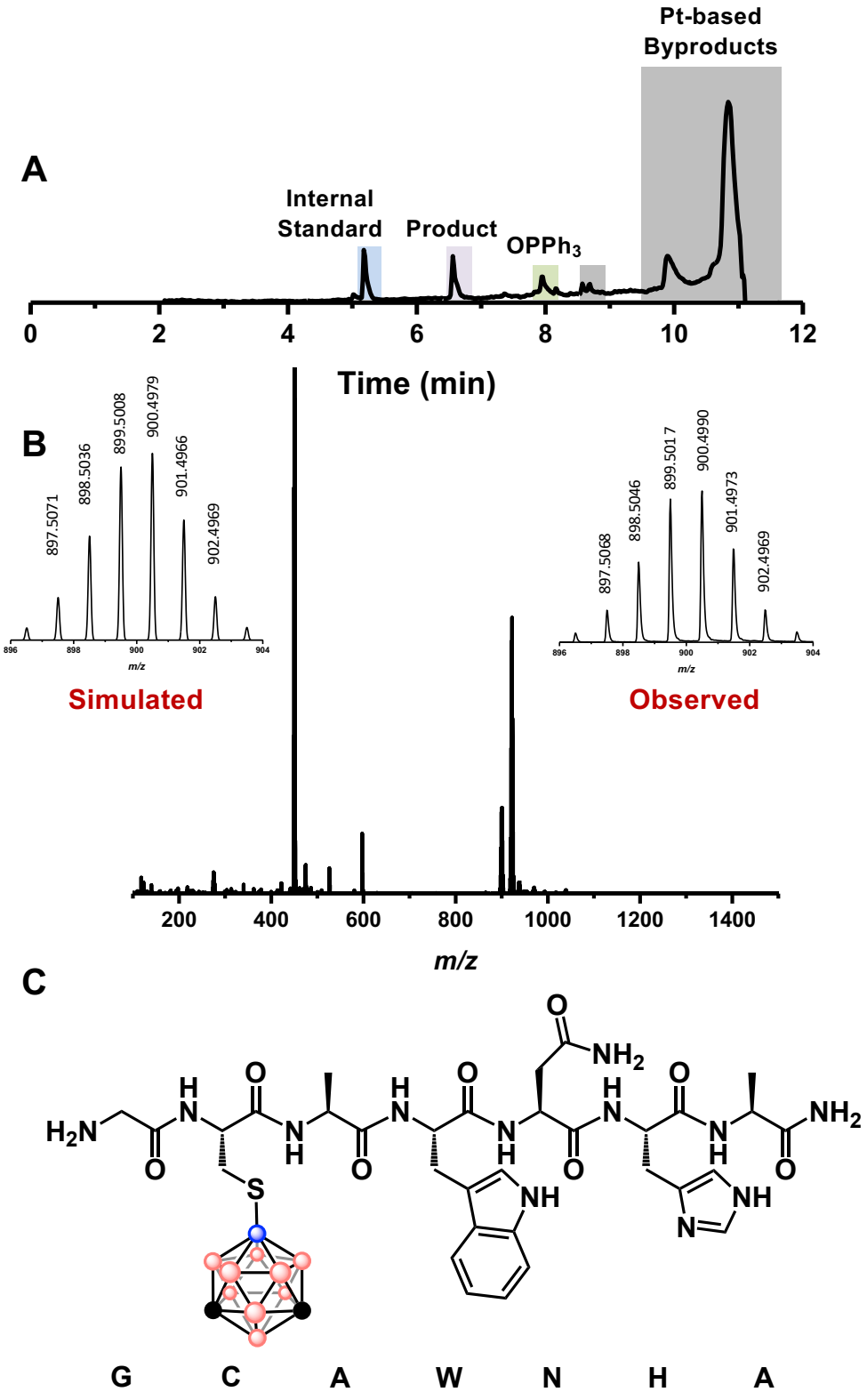

Figure S16 A: LC trace of the crude H_2N-GCAWNHA-$CONH_2$ borylation reaction mixture. **B:** Simulated and observed masses for the borylated product **C:** Line drawing of the borylated product, **9b**.

IV. Reagent Robustness Assessment

Reaction Tolerance to Water

In order to assess the borylation reaction tolerance to water, the following stock solutions were prepared in DMF: peptide (**2a**, 20 mM), Tris•HCl (200 mM) and $PtCl(PPh_3)_2$-(9-B-*m*-$C_2B_{10}H_{11}$) (50 mM). Two reaction mixtures were prepared in 1.5 mL Eppendorf tubes by the addition of dry stir bars and stock solutions and solvents as outlined in **Table S4**. An aliquot of the reaction mixture was removed (5 μL) and quenched with a 2:1 water:acetonitrile (v/v, spiked with 0.1% TFA) solution (175 μL). Finally, internal standard (20 μL, 1.25 mM, see Section I) was added to yield a final volume sample of 200 μL. This sample was then subjected to LCMS analysis as described in the General Borylation Procedure (Section III).

Total Volume (μL)	Water (μL)	DMF (μL)	Tris•HCl Buffer (μL)	Pt Stock (μL)	Peptide Stock (μL)
200	10	86	30	24	50
200	50	26	30	24	50

Table S4: Components of reaction mixtures used to assess the tolerance of peptide borylation to both 5% and 25% water.

To assess the reaction tolerance to the presence of 50% and 75% v/v water, the contents of **Table S5** were mixed, sonicated (5 min) and allowed to react for 90 min. Again, an aliquot of the reaction mixture was removed (5 μL) and quenched with a 2:1 water:acetonitrile (v/v, spiked with 0.1% TFA) solution (175 μL). Finally, internal standard (20 μL, 1.25 mM, see Section I) was added to yield a final volume sample of 200 μL. This sample was then subjected to LCMS analysis.

Total Volume (μL)	Water (μL)	DMF (μL)	Tris•HCl Buffer (μL)	Pt Stock (μL)	Peptide (mg)
194	100	40	30	24	1
233	150	33	30	24	1

Table S5: Reaction mixtures to assess the tolerance of the reaction to both 50% and 75% water.

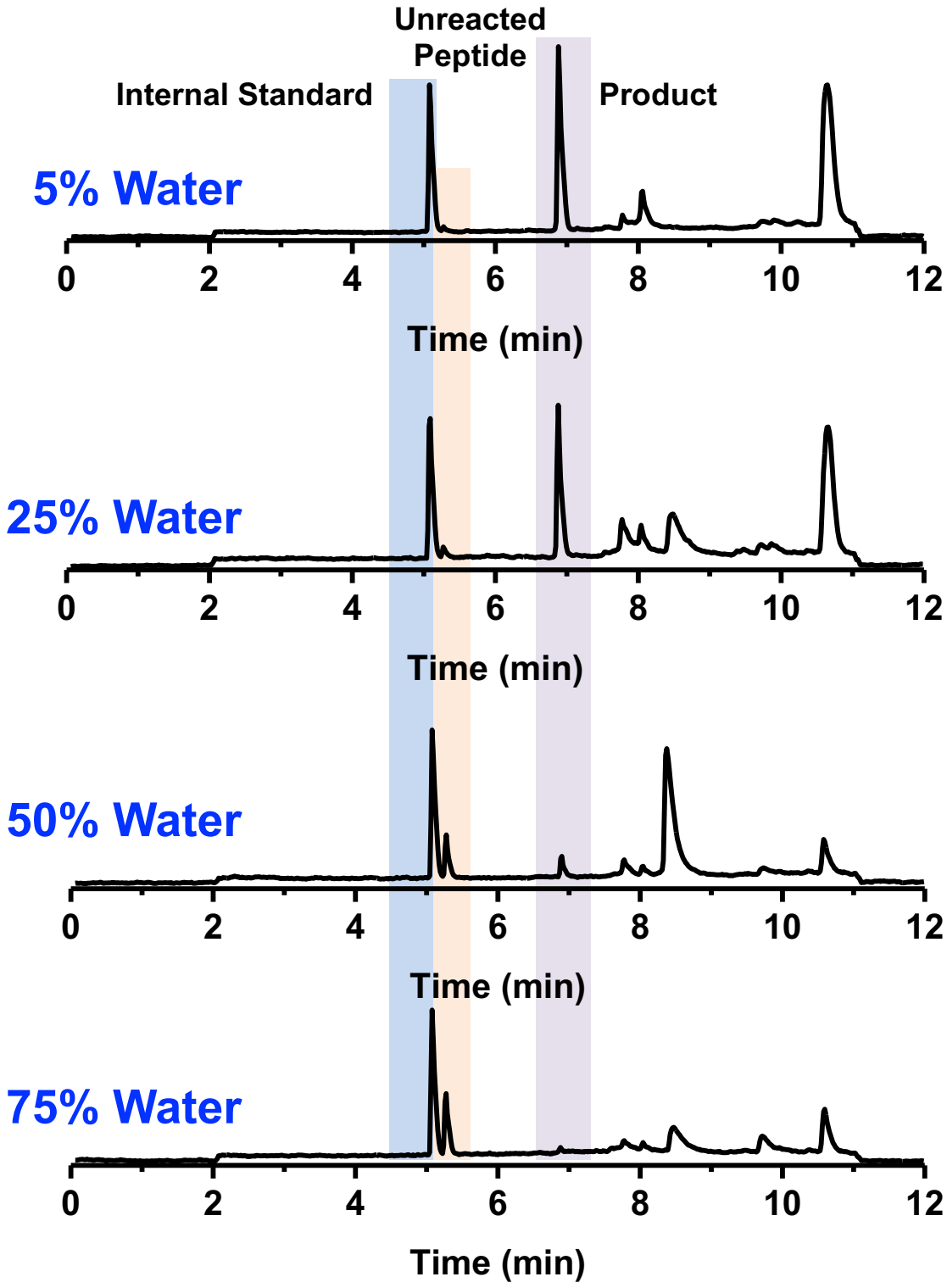

Figure S17: LC traces displaying the reaction tolerance to 5%, 25%, 50%, and 75% water.

Reaction Tolerance to Guanidine Hydrochloride (GuHCl) and Tris(2-carboxyethyl)phosphine Hydrochloride (TCEP HCl)

GuHCl:

In order to assess the reaction tolerance to GuHCl, the following stock solutions were prepared in DMF: peptide (**2a**, 20 mM), Tris•HCl (200 mM) and $PtCl(PPh_3)_2$-(9-B-*m*-$C_2B_{10}H_{11}$) (50 mM). To a 1.5 mL volume Eppendorf tube, Tris•HCl buffer (30 μL), $PtCl(PPh_3)_2$-(9-B-*m*-$C_2B_{10}H_{11}$) (24 μL), and peptide (50 μL) solutions were added. GuHCl (57 mg, 3M) and DMF (96 μL) were also added to yield a total reaction volume of 200 μL. A dry stir bar was added to the Eppendorf tube and the reaction mixture was sonicated to ensure dissolution of all reagents. The reaction was then allowed to stir for 90 min at room temperature. An aliquot of the reaction mixture was removed (5 μL) and quenched with a 2:1 water:acetonitrile (v/v, spiked with 0.1% TFA) solution (175 μL). Finally, internal standard (20 μL, 1.25 mM, see Section I) was added to yield a final volume sample of 200 μL. This sample was then subjected to LCMS analysis.

TCEP:

In order to assess the reaction tolerance to TCEP HCl, peptide **2a** (1.5 mg) was dissolved in Tris•HCl buffer (291 μL, 30 mM) to produce a 5 mM solution of **2a**. A molar excess of TCEP HCl (4.2 mg, 10 x) was added to this mixture and sonicated for 5 min. After sonication, $PtCl(PPh_3)_2$-(9-B-*m*-$C_2B_{10}H_{11}$) (1.3 mg) and a dry stir bar were added and the mixture was sonicated again for 30 sec. The reaction was then allowed to stir for 90 min at room temperature. An aliquot of the reaction mixture was removed (5 μL) and quenched with a 2:1 water:acetonitrile (v/v, spiked with 0.1% TFA) solution (175 μL). Finally, internal standard (20 μL, 1.25 mM, see Section I) was added to yield a final volume sample of 200 μL. This sample was then subjected to LCMS analysis.

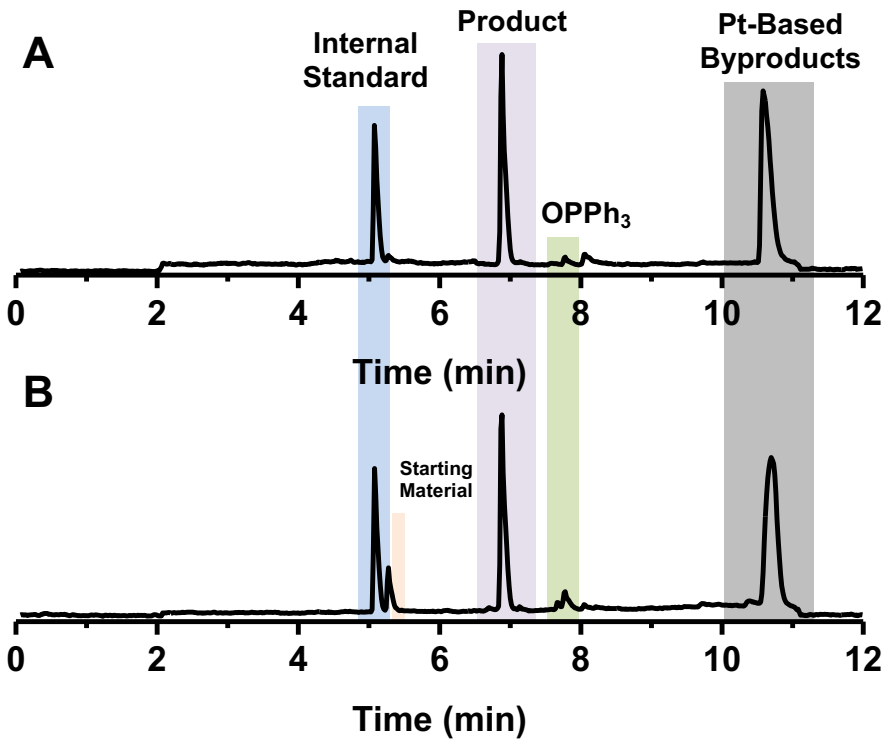

Figure S18: LC traces displaying the reaction tolerance between **1** and **2a** to **A:** 3 M guanidine HCl and **B:** TCEP HCl.

V. Data Supporting S-B Bond Formation

Tandem MS/MS

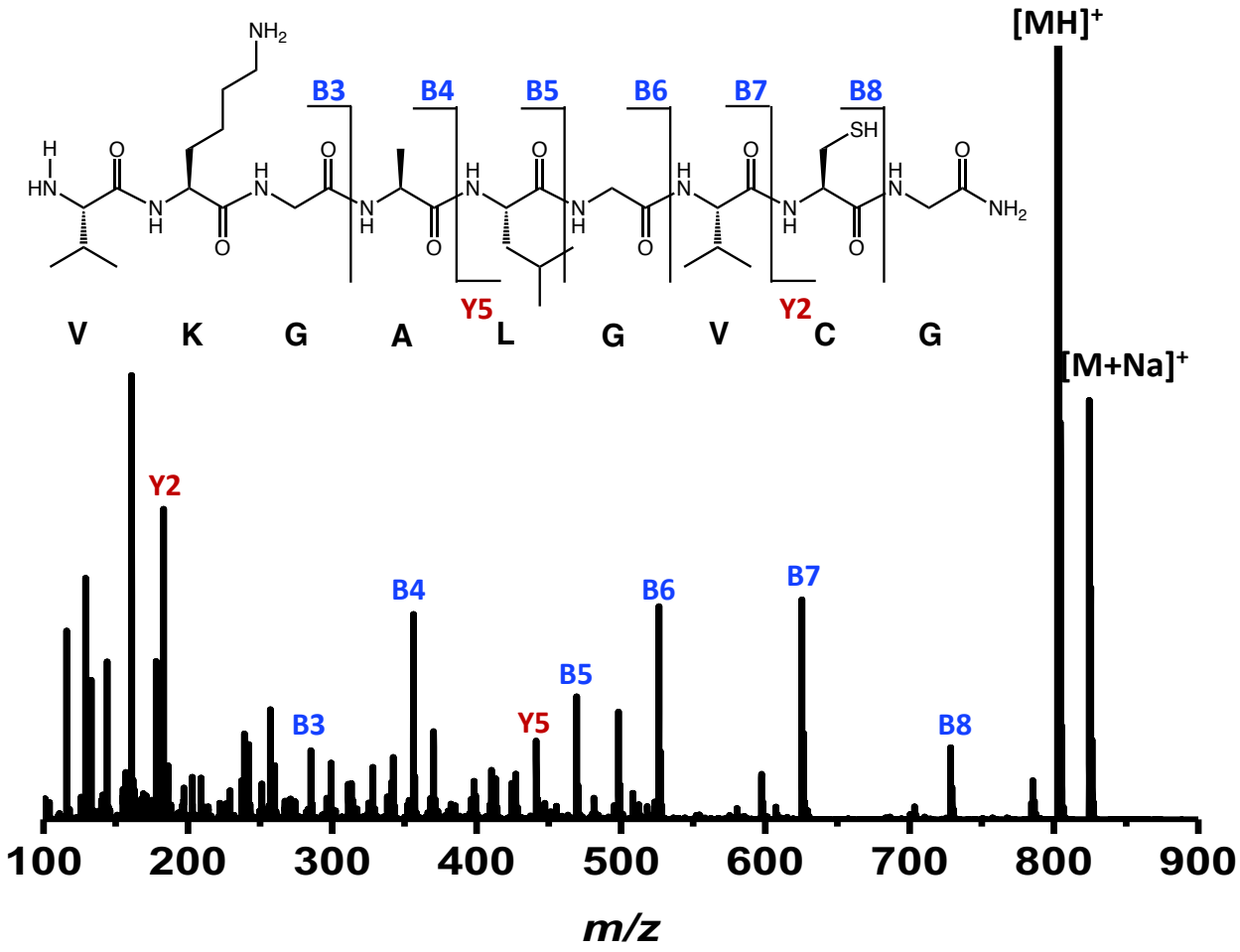

Figure S19: Mass spectrum of **2a** when 30 eV collision energy was applied.

Fragment	Type	Calculated Mass	Observed Mass
B3	B	285.193	285.191
B4	B	356.230	356.233
B5	B	469.314	469.314
B6	B	526.335	526.339
B7	B	625.404	625.408
B8	B	728.413	728.417
Y2	Y	178.065	178.067
Y5	Y	447.240	447.243

Table S6: Summary of the calculated and observed masses when **2a** was fragmented under 30 eV collision energy. These data correspond to the spectrum in **Figure S17**.

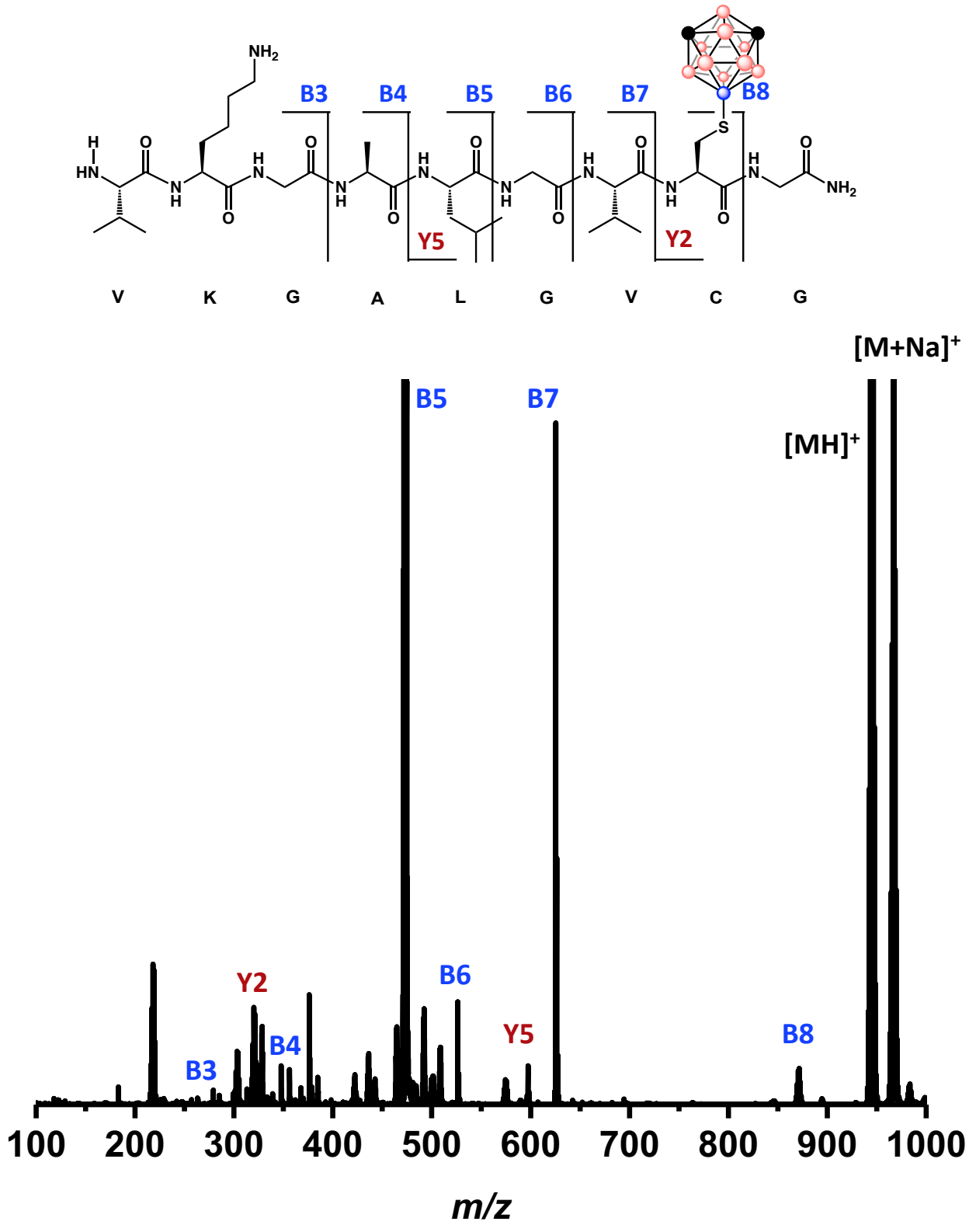

Figure S20: Mass spectrum of borylated peptide **2b** when 30 eV collision energy was applied.

Name	Type	Calculated Mass	Observed Mass
B3	B	285.193	285.191
B4	B	356.230	356.233
B5	B	469.314	469.314
B6	B	526.335	526.339
B7	B	625.404	625.408
B8	B	728.413	728.417
Y2	Y	178.065	178.067
Y5	Y	447.240	447.243

Table S7: Summary of the calculated and observed masses when **2b** was fragmented under 30 eV collision energy. These data correspond to the spectrum in **Figure S18**.

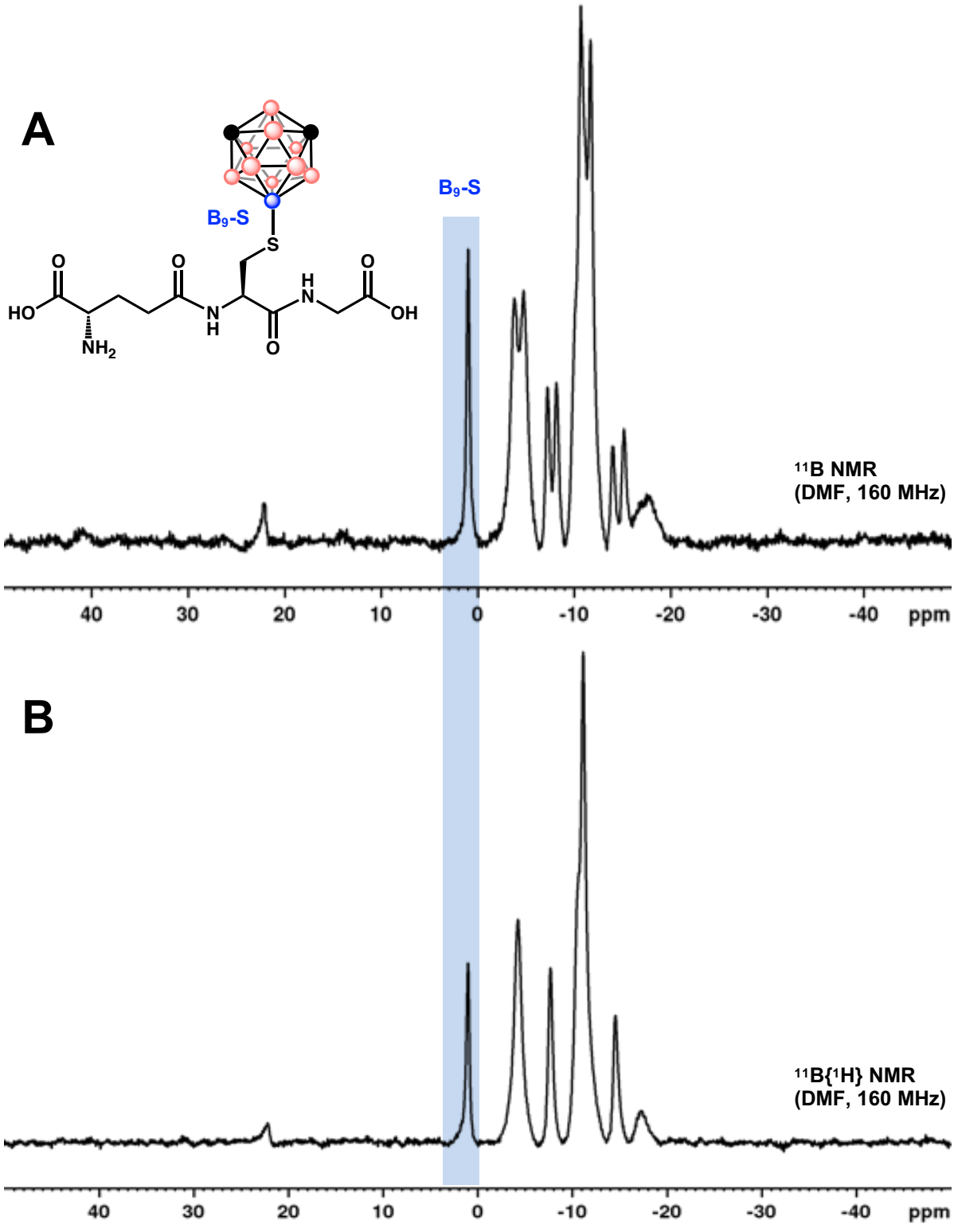

Figure S21 A: ^{11}B NMR spectrum of **7b** (DMF, 160 MHz). **B:** ^{11}B{^{1}H} NMR spectrum of **7b** (DMF, 160 MHz).

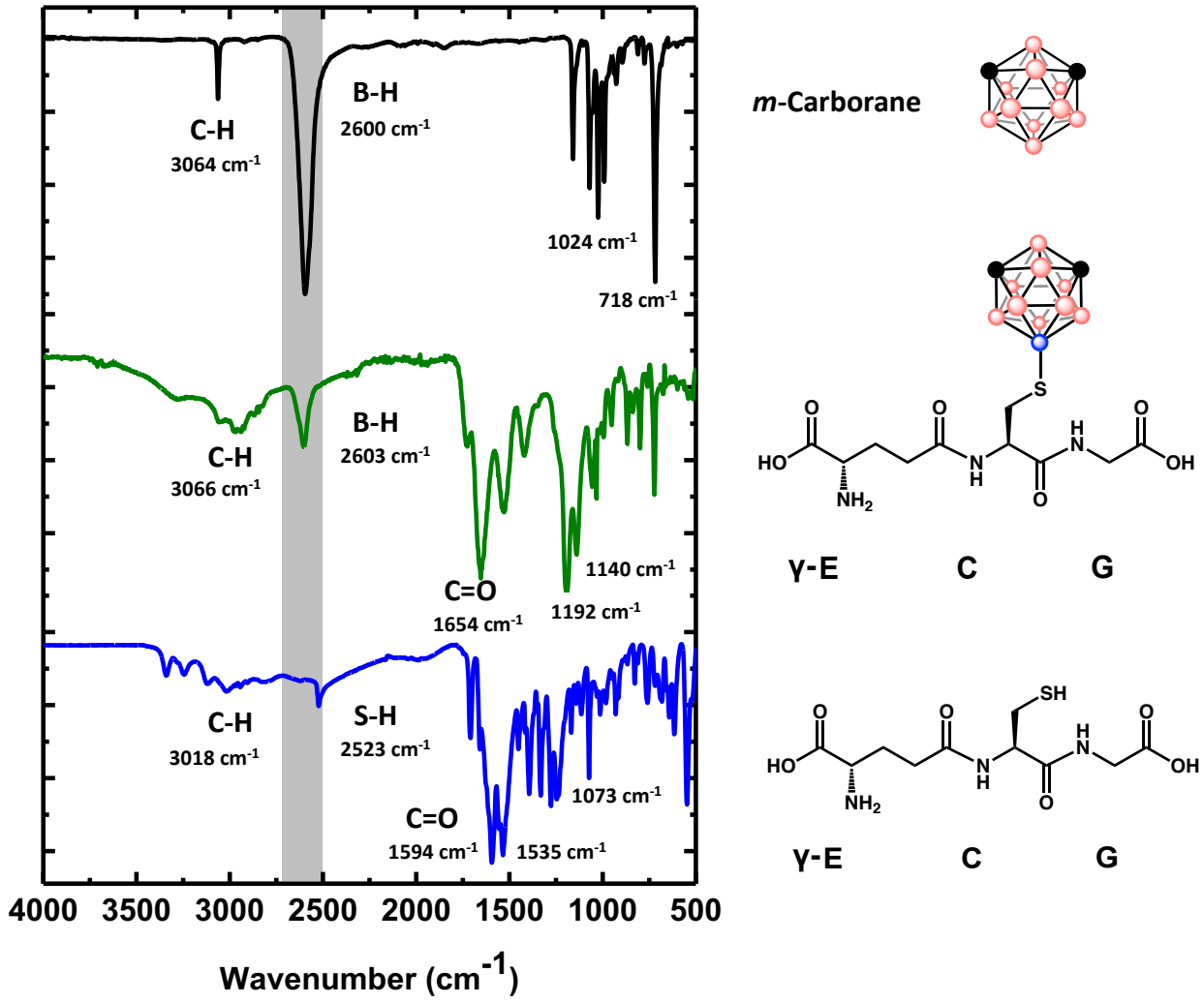

Figure S22: A comparison of the infrared spectra of peptide **7a** (blue), peptide **7b** (green) and *m*-carborane (black). The S-H stretch observed in unmodified glutathione at 2523 cm^{-1} is absent in the spectrum for borylated glutathione.

VI. DARPin Expression, Purification and Borylation

DARPin Expression and Purification

DARPin-Cys protein expression and purification was adapted from literature procedures.[1] DARPin-Cys Sequence (Calculated Mass: 15996.84 Da):

MGSDKIHHHHHHENLYFQGG**C**GGSDLGKKLLEAARAGQDDEVRILMANGADVNAYDDNGVT
PLHLAAFLGHLEIVEVLLKYGADVNAADSWGTTPLHLAATWGHLEIVEVLLKHGADVNAQDKFG
KTAFDISIDNGNEDLAEILQKLN

The plasmid was designed to have an N-terminal His_6 tag followed by a TEV protease which was left on for our purposes (model bioconjugation protein). The plasmid purchased from Twist Bioscience is a pET29b(+) vector with kanamycin resistance and the DARPin-Cys gene was cloned in via NdeI and XhoI restriction sites.

Prior to expression, the plasmid was transformed into BL21-Gold cells (Agilent) using the standard manufacturer's procedure. Overnight cultures were grown and from these, two glycerol stocks were made and stored in the -80°C freezer. To two flasks (2 L) containing 750 ml of previously autoclaved LB Broth (Miller) with kanamycin (50 ug/ml), a saturated overnight culture inoculated (5 mL) was added from one of the aforementioned glycerol stocks. The culture was grown at 37°C with 250 rpm shaking for about 4 hours before the OD600 reached ~0.4 and the culture was induced with 1 mM IPTG. The temperature was lowered and the culture was continued to shake at 30°C and 250 rpm for approximately 17 hours overnight. The final OD600 was observed to be 1.39. Uninduced and induced cell pellets from ~3 ml of culture each were normalized to 1 OD600 unit/ml and analyzed by SDS-PAGE and Coomassie Blue staining. The cultures were harvested by centrifugation at 6000 rpm for 30 min to yield a cell pellet.

The pellet was resuspended in lysis buffer containing Tris•HCl (20 mM), NaCl (150 mM), lysozyme (15 mg) and of protease inhibitor cocktail (0.5 tablet) at pH 7.5. The resulting suspension was homogenized (Avestin Emulsiflex C-3) and centrifuged at 17,000 rpm for 30 min to remove cell debris. The supernatant was loaded onto a 5 mL gravity Ni-NTA column (Qiagen) and washed with the following solutions which were prepared in buffer (Tris•HCl (20 mM) and NaCl (150 mM)): 30ml (10ml X 3) imidazole (5mM) followed by 20ml (10ml X 2) imidazole (20mM). Protein was eluted with 25ml (5ml x 5) imidazole (200mM). SDS-PAGE was run on all fractions and under reducing conditions and stained with Coomassie Blue. Pure fractions were combined and solvent exchanged into storage buffer (20mM Tris, 150mM NaCl, pH 7.5) and concentrated to ~15 ml using a using Amicon 3K Ultra-15 Centrifugal Filter (Millipore).

The purified protein was analyzed by LC-MS* and SDS-PAGE confirming sample purity and molecular weight. Concentration was determined by A280 (extinction coefficient = 15470 M^{-1} cm^{-1}) and confirmed by Ellman's (5,5'-Dithio-bis-(2-nitrobenzoic acid) Assay after treatment with and subsequent removal of (Amicon 3K Ultra-0.5 mL Centrifugal Filters) two equivalents TCEP HCl (Tris (2-carboxyethyl) phosphine hydrochloride). The protein sample was diluted with storage buffer to 300 µM and aliquots were flash frozen and stored in –80 °C freezer.

* The observed mass by LC-MS of purified protein is 15,866 Da corresponding to the calculated mass of the sequence without the initial methionine which was likely cleaved during expression.[25]

DARPin Borylation

A sample of DARPin (50 µL, 300 µM) prepared in Tris•HCl (20 mM) and NaCl (150 mM) at pH 7.5 was treated with TCEP HCl (5 µL, 17 mM) for 30 minutes. After, an aliquot of this sample (30 µL) was added to an Eppendorf tube (1 mL) containing a dry stir bar. Compound **1** dissolved in DMF (56 equiv, 100 µL, 5 mM) was added. Additionally, Tris•HCl buffer in DMF was added to bring the final reaction conditions to 15% water v/v, 85% DMF v/v under buffered conditions of Tris•HCl (33 mM) and NaCl (23 mM). The Eppendorf tube for vortexed for 5 min and the reaction was allowed to proceed for 6.5 hours. The progress of the reaction was monitored by LCMS as described in Section I.

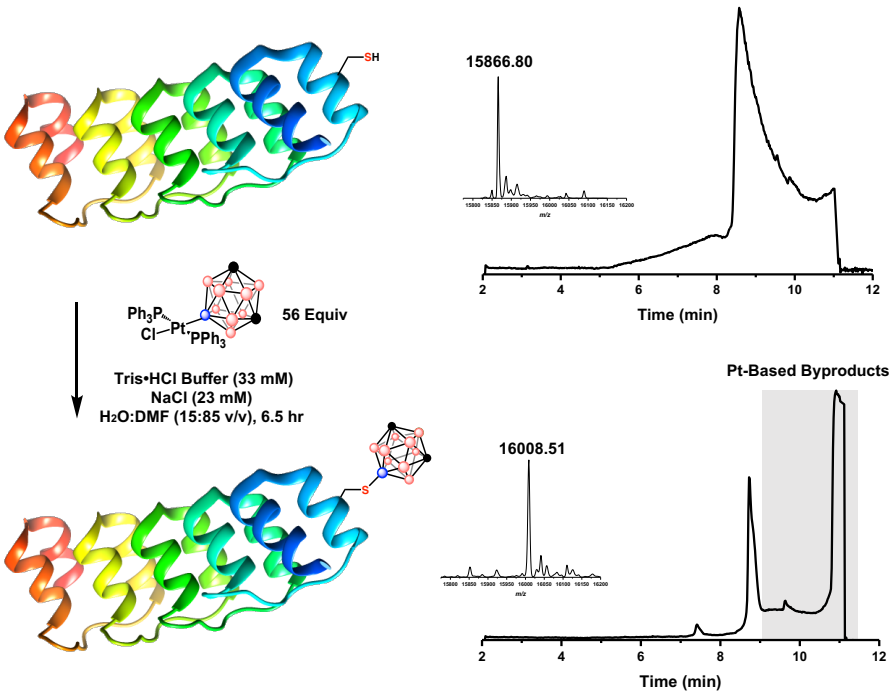

Figure S23: Borylation of DARPin with the corresponding LC traces and deconvoluted masses.

VII. Peptide Stability Assessment

Aqueous solutions of **7b** (5 mM), potassium carbonate (50 mM), hydrochloric acid (50 mM) and **7a** (50 mM) were prepared. To three 1.5 mL volume Eppendorf tubes, 20 μL of the **7b** solution was added followed by addition of the potassium carbonate solution (21 μL) to one of the three Eppendorf tubes. To the second and third tubes, the hydrochloric acid (21 μL) and **7a** (21 μL) solutions were added, respectively. All three mixtures were vortexed and allowed to sit for 24 h at r.t., after which 20 μL aliquots were removed from each tube and subjected to LC-MS analysis (see Section I). Each Eppendorf tube was then submerged in a sand bath at 37 °C for an additional 48 h. After each 24 h time period, a 20 μL aliquot was removed from each sample and subjected to LC-MS analysis.

VIII. Borylation of Peptide on Solid-Phase Resin

Peptide H_2N-QNACG-$CONH_2$ was synthesized as described in Section II. However, prior to cleavage from the resin, beads (50 mg) were removed and subjected to TIPS (1 mL), Hydroxybenzotriazole (1 mL, 0.5 M in DMF) and 2,2,2-trifluoroethanol (3 mL) to remove trityl protecting groups. After reaction for 30 minutes, the beads were isolated by vacuum filtration and washed with DMF (5 mL, 3x). A sample of beads (3 mg) were added to an Eppendorf tube (1 mL) and subjected to excess **1** (3.5 mM) in DMF Tris•HCl buffer (30 mM). After 1.5 hr, the beads were subjected to centrifugation at 18,000 x g for 3 min and the supernatant was removed. To the Eppendorf, trifluoroacetic acid (200 µL), TIPS (10 µL) and water (10 µL) were added. After 30 min, an aliquot (20 µL) was removed and diluted with 180 µL of a 2:1 water:acetonitrile mixture (spiked with 0.1% TFA). The sample was then analyzed by LCMS using the peptide characterization method described in Section I.

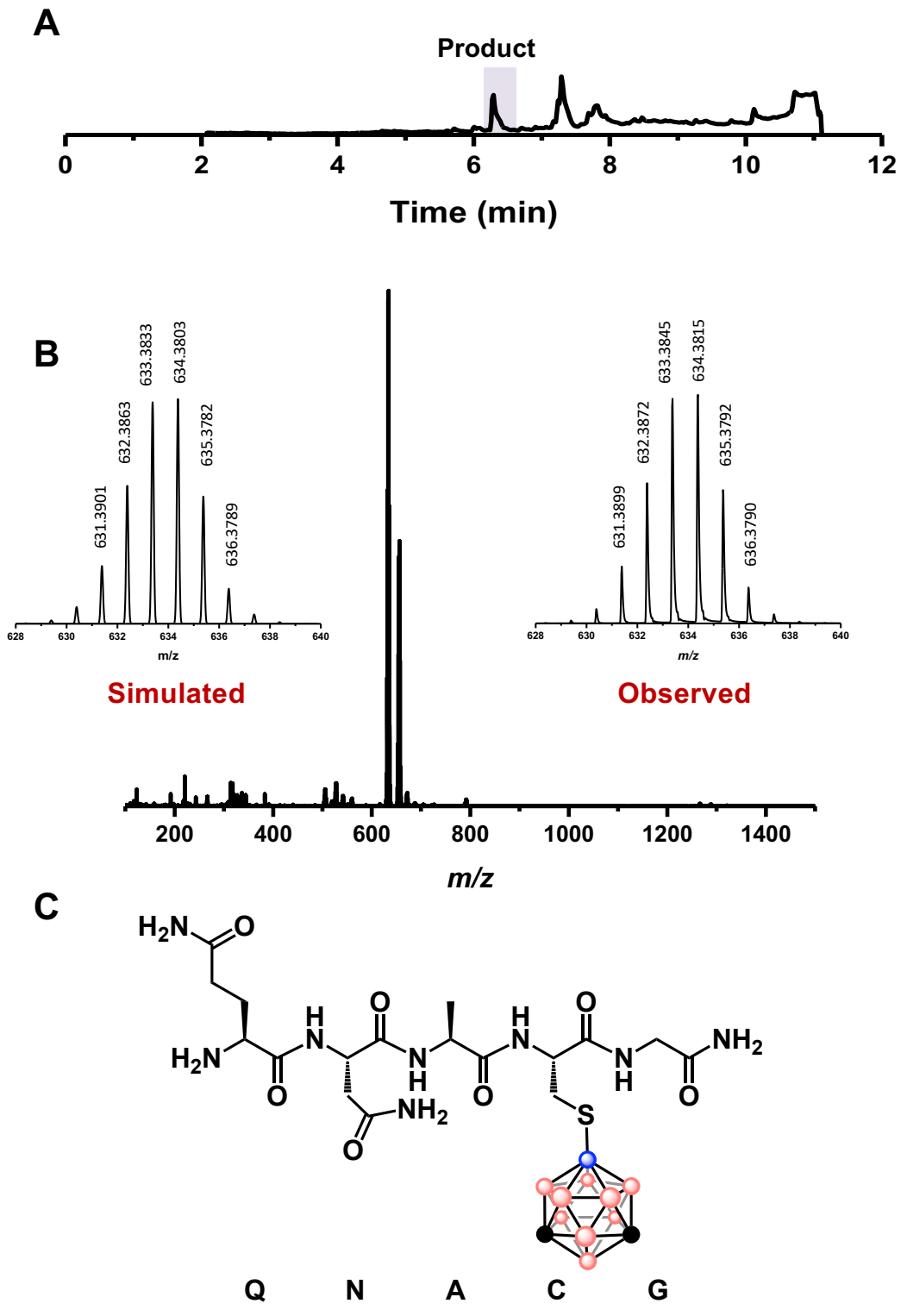

Figure S24 A: LC trace corresponding to peptide H_2N-QNAC(boryl)G-$CONH_2$. Masses corresponding to Trt-H, H_2N-QNAC(Trt)G-$CONH_2$ and H_2N-NAC(Trt)G-$CONH_2$ were observed at ~7.3 and 7.8 min. **B:** Simulated and observed masses for the borylated product **C:** Line drawing of the resulting borylated peptide.

IX. Cellular Toxicity Studies

Two stock solutions were prepared in phosphate buffered saline (1x concentration): borylated glutathione, **7b**, (1.0 mg, 356 μM) and acetamide glutathione, **7c**, (0.5 mg, 549 μM). **7c** was prepared using a previously reported procedure (see Internal Standard Procedure Section I)[1]. Both stock solutions were then filtered into autoclaved Eppendorf tubes (1.5 mL) using a 0.2 μm PTFE Pall syringe filter. To an unopened, sterile 96-well plate, Chinese hamster ovarian cells were plated to a density of 60k per well. Wells A1-C1 were allotted for the live control, A2-C2 the dead control, A3-C3 for 5 μM **7b**, D3-F3 for 5 μM **7c**, A4-C4 for 15 μM **7b**, D4-F4 for 15 μM **7c**, A5-C5 for 50 μM **7b** and D5-F5 for 50 μM **7c**. To each well, the stock solution to generate the indicated concentrations was added and the final volume was brought to 200 μL using cell media. The well plate was then incubated at 37 °C under a 5% CO_2 atmosphere for 4 h. The well plate was then removed and ~150 μL of the contents in each well was removed. Trypsin (100 μL) was added to each well and the solutions were allowed to incubate at 37 °C under a 5% CO_2 atmosphere for 5 min. After incubation, cell media (100 μL) was added to each well and the entire contents of each well was removed and transferred to 1.5 mL volume Eppendorf tubes, and centrifuged at 17,000 x g for 2 min at 4 °C. The supernatant was discarded, and the cell pellet was resuspended in phosphate buffered saline (1x concentration, 200 μL). After a second centrifugation at 17,000 x g for 2 min, the supernatant was again removed and phosphate buffered saline (1x concentration, 100 μL) and trypan blue (0.4%,100 μL) were added to each Eppendorf tube except the tubes from cells A2-C2. To these tubes, Triton X (45%, 40 μL) was added followed by phosphate buffered saline (1x concentration, 60 μL) and trypan blue (0.4%, 100 μL). After, 10 μL of each solution was pipetted onto two sides of a hemocytometer. The number of live and dead cells between both the top and bottom sides of the hemocytometer were recorded. The average measurement between triplicate solutions (e.g.: A1-C1) was then used to calculate cell viability.

Calculation of % Viability

		1		2		3		4		5	
		L	D	L	D	L	D	L	D	L	D
A	T	241	8	0	4	147	3	116	2	99	2
	B	276	1	0	2	109	1	103	0	86	4
B	T	324	5	1	2	108	4	90	3	58	9
	B	226	10	0	4	98	4	82	3	48	9
C	T	316	2	2	3	138	4	115	1	81	3
	B	192	2	0	5	130	1	115	4	65	3
D	T					117	3	56	1	96	0
	B					103	6	50	1	80	1
E	T					72	2	101	2	75	1
	B					88	4	90	6	84	0
F	T					125	3	123	3	91	0
	B					93	3	76	2	57	3

Table S8: Live (L) and dead (D) cell counts observed on hemocytometer where T is the top plate of the hemocyctometer and B is the bottom plate of the hemocytometer.

	A1	B1	C1	A2	B2	C2	A3	B3	C3	D3	E3	F3
Sum T	249	277	318	4	3	5	150	112	142	120	74	128
Sum B	277	329	194	2	4	5	110	102	131	109	92	96
Avg Total	263	303	256	3	3.5	5	130	107	136.5	114.5	83	112
Avg Live	258.5	275	254	0	0.5	1	128	103	134	110	80	109
% Viability	98.3	90.8	99.2	0	14.3	20	98.5	96.3	98.2	96.1	96.4	97.3
Average	96			10			98			96.6		
STDEV	5			10			1			0.7		

Table S9: The % viability calculated for columns 1-3 with the average and standard deviation reported.

	A4	B4	C4	D4	E4	F4	A5	B5	C5	D5	E5	F5
Sum T	118	93	116	57	103	126	101	67	84	96	76	91
Sum B	110	85	119	51	96	78	90	57	68	81	84	60
Avg Total	114	89	117.5	54	99.5	102	95.5	62	76	88.5	80	75.5
Avg Live	109.5	86	115	53	95.5	99.5	92.5	53	73	88	79.5	74
% Viability	96.0	96.6	97.9	98.1	95.98	97.5	96.9	85.5	96.1	99.4	99.4	98.0
Average	96.8			97			93			98.9		
STDEV	0.9			1			6			0.8		

Table S10: The % viability calculated for columns 4-5 with the average and standard deviation reported.

X. ITC and $^{11}B\{^1H\}$ NMR Binding Studies

ITC Binding of 7a, 7b and 7c to β-Cyclodextrin

The following solutions were prepared in acetic acid (10 mM, pH = 3.4): **7a** (1.0 mg, 10 mM), **7b** (1.8 mg, 10 mM) and **7c** (2.0, 10 mM). A solution of β-cyclodextrin (2.8 mg, 0.5 mM) was also prepared in acetic acid (10 mM, pH = 3.4). This pH represents the isoelectric point of glutathione. All solutions were sonicated (5 min) and vortexed to ensure complete solubilization. The following parameters were set for the titration of all peptide solutions into β-cyclodextrin solutions: 16 total injections at 25 °C with a reference power set to 10 μcal/sec at a stirring rate of 750 rpm. After an initial delay of 120 sec, 0.2 μL of peptide solution was injected into the cell for a duration of 0.4 sec (with a filter period of 5 sec). After this first injection, 180 sec were allowed to pass before an additional 2.4 μL was injected for 4.8 sec (with a filter period of 5 sec). This process was repeated 14 times to reach saturation. The enthalpic output (kcal mol^{-1} of injectant) of unmodified peptide was subtracted from the borylated peptide titration enthalpic output to ensure all observed binding events were due to the boryl fragment rather than side chains. The subtracted data (**Figures 4B and 4C**) are plotted alongside the data of the unmodified peptide control (**Figure 4D**).

Binding of 2a and 2b to β-Cyclodextrin

Binding of **2b** towards β-cyclodextrin was verified through ITC to yield a binding constant, K_a = 1.1 x 10^3 ± 700 M^{-1} (**SI Figure S25**). After, inclusion was also probed through $^{11}B\{^1H\}$ NMR spectroscopy by titration of a solution containing β-cyclodextrin to a solution containing **2b**. The singlet assigned to the $^{11}B(9)$-S nucleus on the intact *m*-carborane cluster attached to the peptide was shifted downfield as β-cyclodextrin was titrated to the solution. Scatchard treatment[16] of the data resulted in a binding constant, *Ka* = 1.49 x 10^3 M^{-1} (**SI Figure S27**).

ITC Parameters

Solutions of **2b** (0.5 mg, 5 mM) and **2a** (0.3 mg, 5 mM) were prepared in Tris•HCl/Trizma buffer (10 mM, pH = 8.2) containing guanidine hydrochloride (3M). A solution of β-cyclodextrin (7.1 mg, 0.25 mM) was also prepared in Tris•HCl/Trizma buffer (10 mM, pH = 8.2) containing guanidine hydrochloride (3M). This pH represents the isoelectric point of **2a**. All solutions were subjected to sonication (5 min) and mixing under vortex to ensure solubility. Guanidine hydrochloride was incorporated as a denaturing agent. The following parameters were set for the titration of all peptide solutions into β-cyclodextrin solutions: 16 total injections at 25 °C with a reference power set to 10 μcal/sec at a stirring rate of 750 rpm. After an initial delay of 120 sec, 0.2 μL of peptide solution was injected into the cell for a duration of 0.4 sec (with a filter period of 5 sec). After this first injection, 180 sec were allowed to pass before an additional 2.4 μL was injected for 4.8 sec (with a filter period of 5 sec). This process was repeated 14 times to reach saturation. The enthalpic output (kcal mol^{-1} of injectant) of unmodified peptide was subtracted from the borylated peptide titration enthalpic output to ensure all observed binding events were due to the boryl fragment rather than side chains.

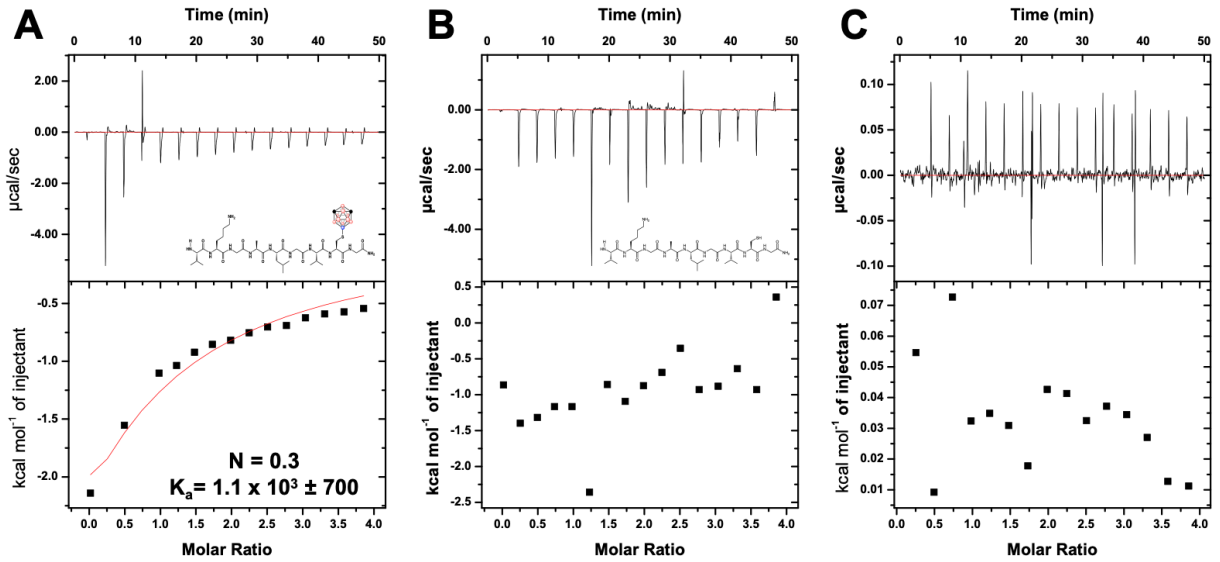

Figure S25 A: ITC plot of the titration of **2b** toβ-cyclodextrin **B:** ITC plot of the titration of **2a** to β-cyclodextrin **C:** ITC plot of the titration of buffer to β-cyclodextrin.

$^{11}B\{^{1}H\}$ NMR Binding Studies

Solutions of peptide **2b** (0.5 mg, 1.6 mM) and β-cyclodextrin (5.7 mg, 5.0 mM) were prepared in D_2O. The solution containing peptide **2b** was added to an NMR tube and a $^{11}B\{^{1}H\}$ NMR spectrum was collected as summarized by Entry A of **Table S11**. After, an aliquot of β-cyclodextrin solution (25 μL) was added to the NMR tube and mixed by tilting the NMR up and down 5 times to yield Entry B. After, a $^{11}B\{^{1}H\}$ NMR spectrum was collected. This process was repeated 5 times as described by Entries C-G of **Table S11**. Downfield shift in the singlet ascribed to the $^{11}B(9)$ nucleus was used to assess the **2b**⊂β-cyclodextrin inclusion complex as described by **Figure S26**. Scatchard treatment[16] of the data yielded an association constant K_a = 1.49 x 10^3 M^{-1} with an R^2 = 0.862 as summarized by **Figure S27**.

Entry	Chemical Shift (δ) Ascribed to $^{11}B(9)$ Nucleus	Δ Chemical Shift (δ)	Concentration 2b (M)	Concentration β-Cyclodextrin (M)
A	-0.70	-	0.0016	-
B	-0.48	0.94	0.0015	0.00037
C	-0.30	0.158	0.0014	0.00069
D	-0.11	0.2	0.0013	0.00096
E	-0.02	0.104	0.0012	0.0012
F	0.1	0.102	0.0011	0.0014
G	0.15	0.014	0.0011	0.0016

Table S11: The reaction mixtures and corresponding chemical shifts associated with the titration of β-cyclodextrin to **2b**.

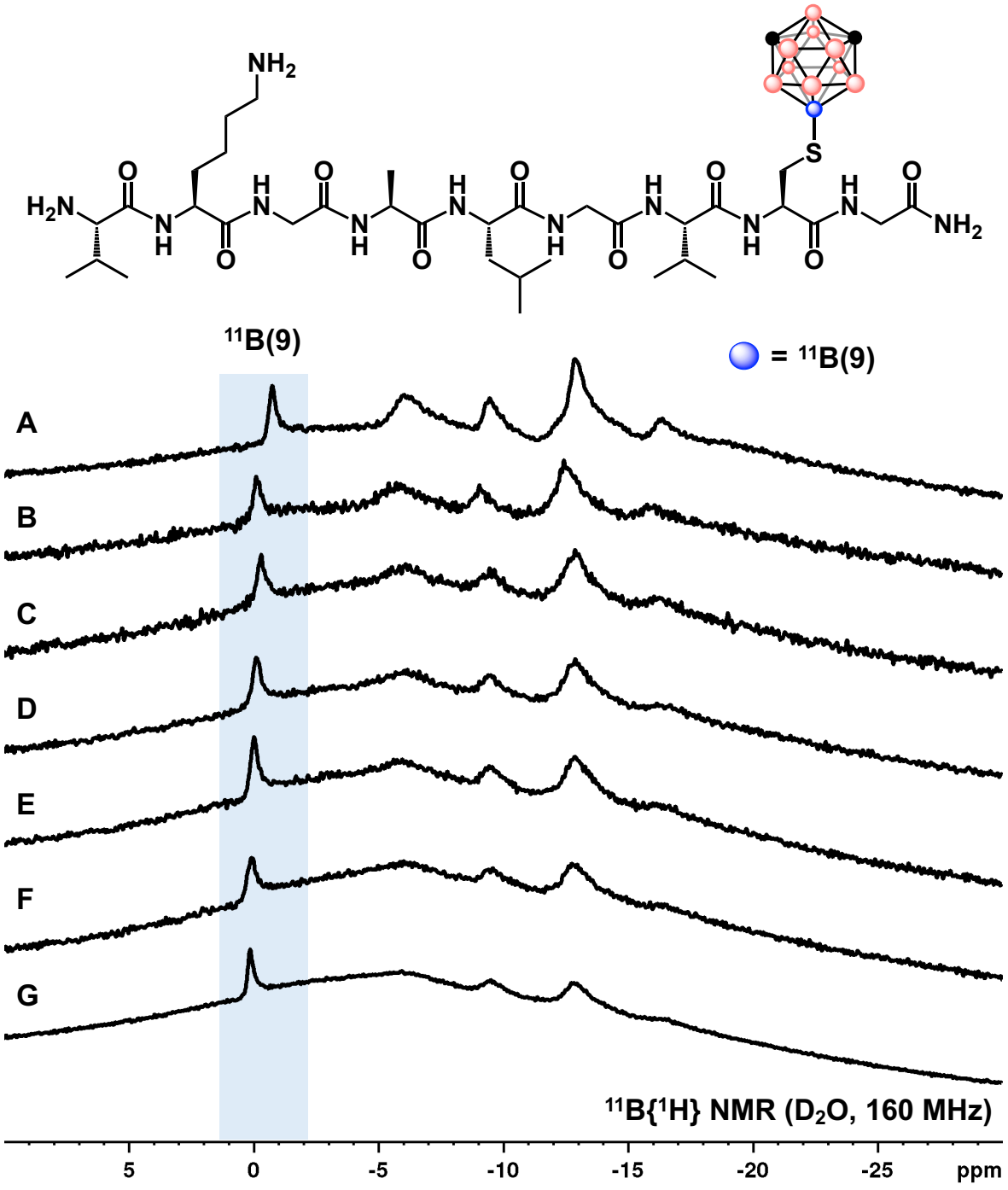

Figure S26: $^{11}B\{^{1}H\}$ NMR (D_2O, 160 MHz) spectra of the titration of β-cyclodextrin to **2b** as described by Entries A-G in **Table S7**.

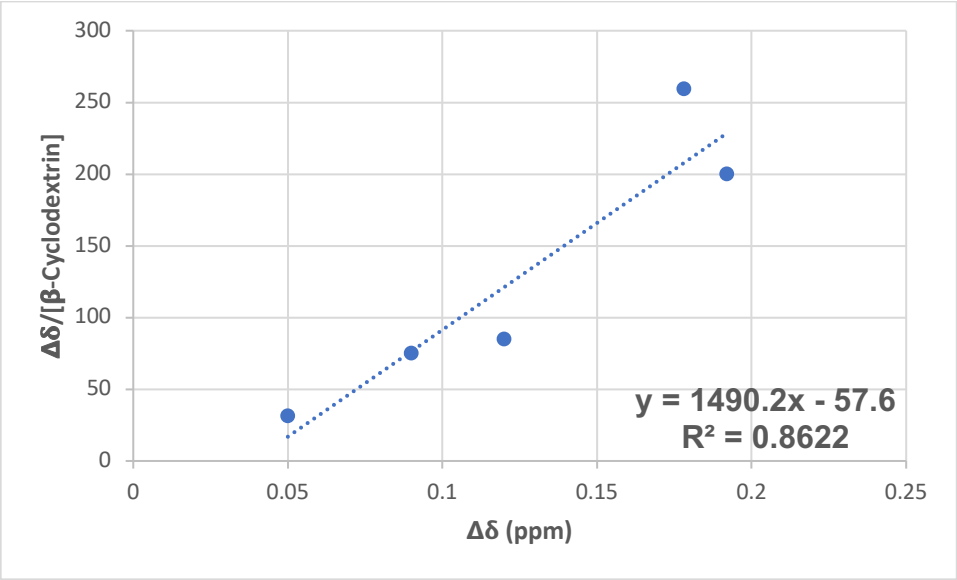

Figure S27: Scatchard plot of the titration of β-cyclodextrin to **2b** as described by Entries A-G in **Table S11** to yield an association constant K_a = 1.49 x 10^3 M^{-1} with an R^2 = 0.862.

XI. Proteinase K Degradation Studies

Stock solutions (10 μg/μL) of peptides **2a**, **2b and 2c** were prepared in water. Additionally, Proteinase K (0.8 mg) was dissolved in 1.0 mL deionized water to produce a 0.8 μg/μL solution in an Eppendorf tube (1.5 mL). After, the following stock solutions were prepared in Falcon tubes (10.0 mL): calcium chloride dihydrate (37.0 mg) in 5.0 mL deionized water to produce a 50 mM solution and β-cyclodextrin (57.0 mg) in 10.0 mL deionized water to produce a 5 mM solution. Finally, a Tris•HCl/Trizma buffered solution (50.0 mL, 100 mM) was prepared at pH 8.0. Aliquots of these stock solutions were added to an Eppendorf tube (1.5 mL) in volumes described by Table X. All entry components except Proteinase K were combined and vortexed for ~30 sec prior to the addition of Proteinase K. Finally, the Proteinase K was added and the Eppendorf tube was vortexed for ~10 sec and placed into a sand bath at 55 °C for 5 min. After 5 min, an aliquot of each reaction mixture (50 μL) was removed and placed into an Eppendorf containing protease inhibitor phenylmethyl sulfonyl fluoride (1.0 mM, 100 μL), water acidified with 0.1% TFA (25 μL) and 3-bromomethyl pyridine HBr (23.7 mM, 25 μL) as an internal standard. This entire process was repeated a second time with fresh stock solutions for error analysis. The reaction mixtures were then analyzed by LCMS (**Figure S24**) as described in Section I except the injection volume was increased from 0.3 μL to 5 μL.

Entry	Water (μL)	Buffer (μL)	Peptide 2a (μL)	Peptide 2b (μL)	Peptide 2c (μL)	$CaCl_2$ (μL)	β-Cyclodextrin (μL)	Proteinase K (μL)
A	65.0	100.0	10.0	-	-	20.0	-	5.0
B	45.0	100.0	10.0	-	-	20.0	20.0	5.0
C	70.0	100.0	-	-	10.0	10.0	-	-
D	45.0	100.0	-	-	10.0	20.0	-	5.0
E	65.0	100.0	-	-	10.0	20.0	20.0	5.0
F	70.0	100.0	-	10.0	-	20.0	-	-
G	45.0	100.0	-	10.0	-	20.0	-	5.0
H	65.0	100.0	-	10.0	-	20.0	20.0	5.0

Table S12: The reaction mixtures corresponding to entries A-H.

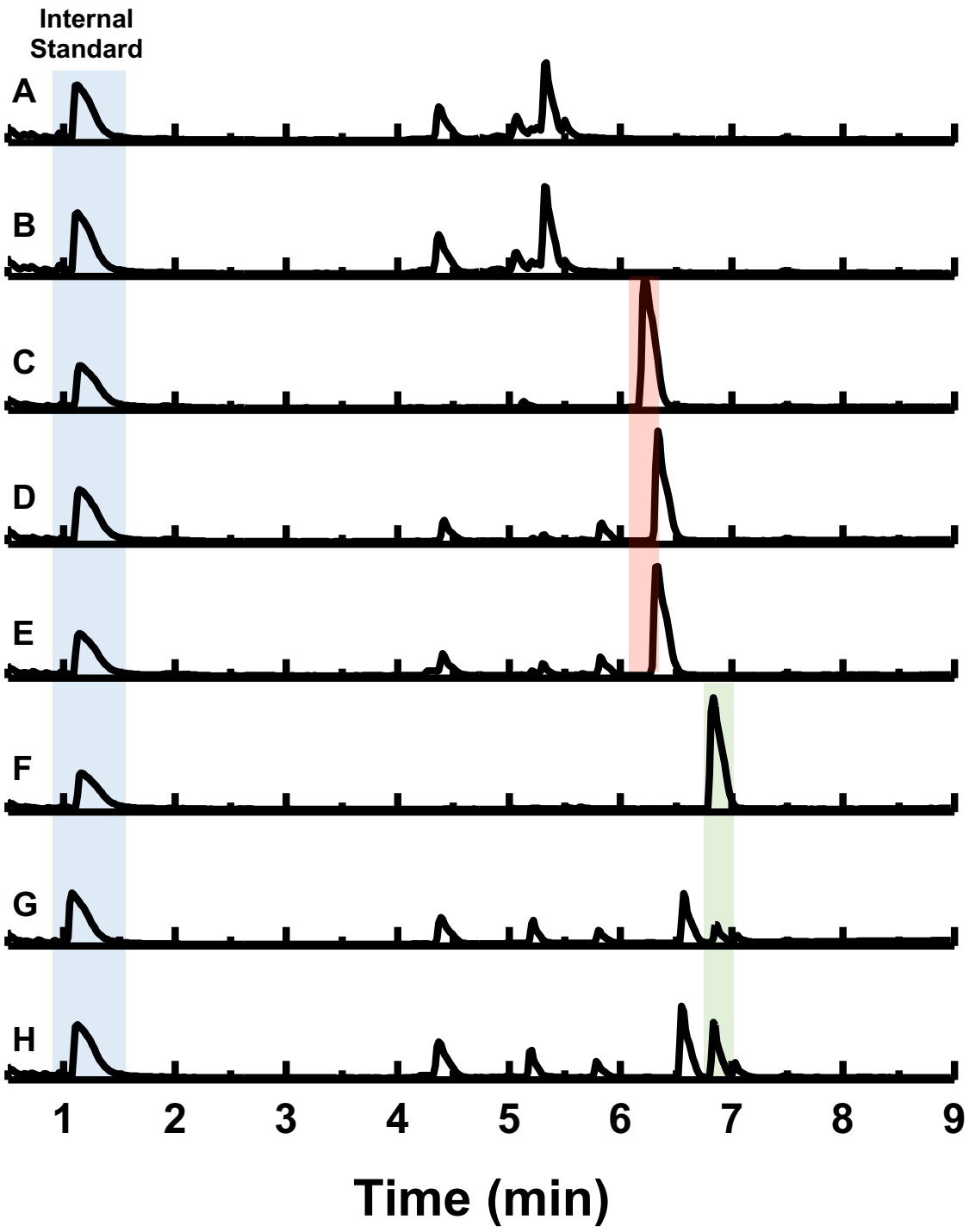

Figure S28: Representative LC traces corresponding to entries A-H described in **Table S8**. The retention time of intact **2c** is highlighted in red while intact **2b** is highlighted in green.

Calculation of % Degraded

All data are reported as an average of two trials as described by **Table S13**. As no peptide remained intact for entries A-E, a percentage of peptide remaining could not be calculated. A corrective response factor, F, was calculated using equation (1):

$$(1)\ F = \frac{Area_{Intact\ Peptide\ in\ Entry\ F} \Big/ Concentration_{Intact\ Peptide\ in\ Entry\ F}}{Area_{Internal\ Standard\ in\ Entry\ F} \Big/ Concentration_{Internal\ Standard\ in\ Entry\ F}}$$

The concentration of peptide in each entry was then calculated using this corrective factor as described by equation (2):

$$Peptide\ Remaining = \frac{F \times Concentration_{Internal\ Standard} \times Area_{Peptide}}{Area_{Internal\ Standard}}$$

The percentage of peptide remaining represents the ratio of the concentration of peptide calculated to the concentration of peptide theoretically in solution given the reactants described by **Table S13**. All data were normalized to control sample Entry F where no Proteinase K was incorporated and no degradation was observed.

Entry	Trial	Internal Standard Integration by LCMS	Intact Peptide Integration by LCMS	Response Factor, F	Concentration of Peptide in Trace (M)	Peptide Remaining (%)	Normalization	Average	Standard Deviation
F	-	1802918467	3170687750		1.83007E-05	87.98118269	100.000003	-	-
G	1	2088289113	237290391		1.18244E-06	32.11028636	44.1291064	45	1
H	2	3983531140	483082504	2.70560	1.26195E-06	51.62640758	63.6452276		
G	1	1962549068	414868096		2.19978E-06	63.54564758	75.5644676	70	10
H	2	3689486520	1462184537		4.12408E-06	65.21454314	77.2333631		

Table S13: Calculation of peptide remaining across multiple trials of entries G and H.

XII. Angiotensin-Converting Enzyme (ACE) Studies

ACE inhibition studies were derived from Otte, J. *et al*.[17] ACE from rabbit lung was prepared as a 0.25 unit/mL solution. Stock solutions of peptide **8a** (0.5 mg, 100 μM) and peptide **8b** (0.3 mg, 100 μM) were prepared in deionized water. A solution of FA-PGG (10.5 mg, 0.88 mM) was prepared in 30 mL Tris•HCl/Trizma buffer (50 mM, pH= 8.2) that contained NaCl (0.53 g, 0.3 M). These stock solutions were used to prepare the reaction mixtures outlined in Table S8. Upon mixing of the components, the reaction mixtures were transferred to a Fisher quartz cuvette (1.0 mL) and the cuvette was lowered into a sand bath set to 37 °C. After heating for 5 min, the cuvette was removed and the UV-Vis spectrum was collected from 200-800 nm. The cuvette was then returned to the sand bath until 2 minutes had elapsed. At this time, the cuvette was removed and another UV-Vis spectrum from 200-800 nm was collected. This process was repeated until a total reaction time of 21 min was reached. The absorbance at 340 nm was recorded and plotted as a function of time (**Figures S29-S38**). The % inhibition was determined by the ratio of the slope when inhibitor is present by the slope when no inhibitor is present as summarized by equation (3).

$$(3) \quad \%\ ACE\ Inhibition = \left[1 - \left(\frac{\rho A_{Inhibitor}}{\rho A_{Control}}\right)\right] X\ 100$$

All entries and the corresponding % inhibition are summarized in **Tables S14 and S15**. All reactions were conducted in duplicate.

Entry	ACE Stock (μL)	Water (μL)	FA-PGG Stock (μL)	Peptide 2a (μL)	Peptide 2b (μL)
A	40.0	560.0	200.0	-	-
B	40.0	560.0	200.0	-	-
C	40.0	540.0	200.0	-	40.0
D	40.0	540.0	200.0	-	40.0
E	40.0	540.0	200.0	40.0	-
F	40.0	540.0	200.0	40.0	
G	40.0	160.0	200.0	-	400.0
H	40.0	160.0	200.0	-	400.0
I	40.0	160.0	200.0	400.0	-
J	40.0	160.0	200.0	400.0	-

Table S14: Reaction mixtures used to assess the inhibition of peptides **8a** and **8b**.

Entry	Slope	% Inhibition	Average % Inhibition	Error
A	0.0141	0	0	-
B	0.012	0		
C	0.0112	14.2	17	4
D	0.0104	20.3		
E	0.0104	20.3	24	5
F	0.0094	28.0		
G	0.0061	53.3	52	1
H	0.0064	51.0		
I	0.0067	48.7	54	7
J	0.0054	58.6		

Table S15: The slope and % inhibition corresponding to each entry described by **Table S10.**

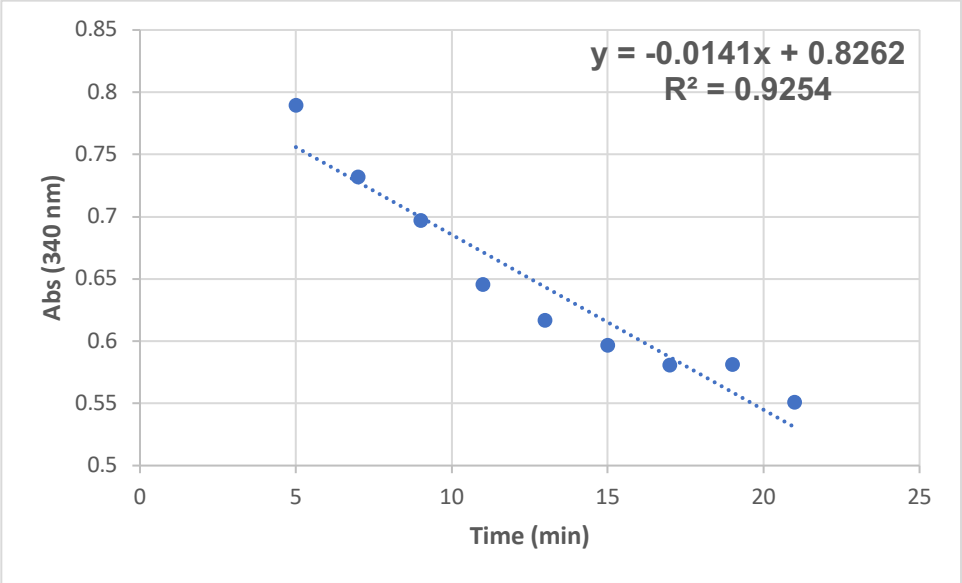

Figure S29: The absorbance of entry A detected across minutes 5-20.

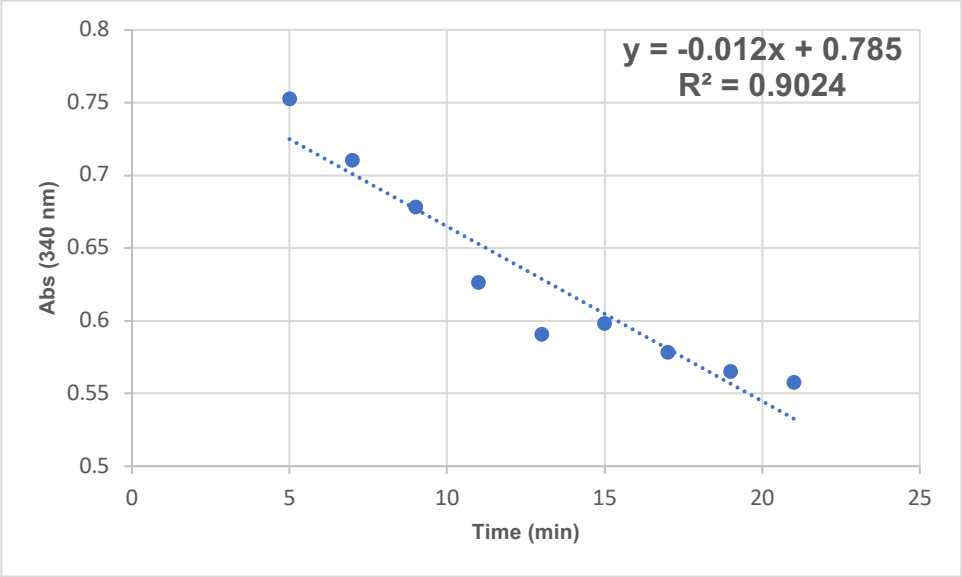

Figure S30: The absorbance of entry B detected across minutes 5-20.

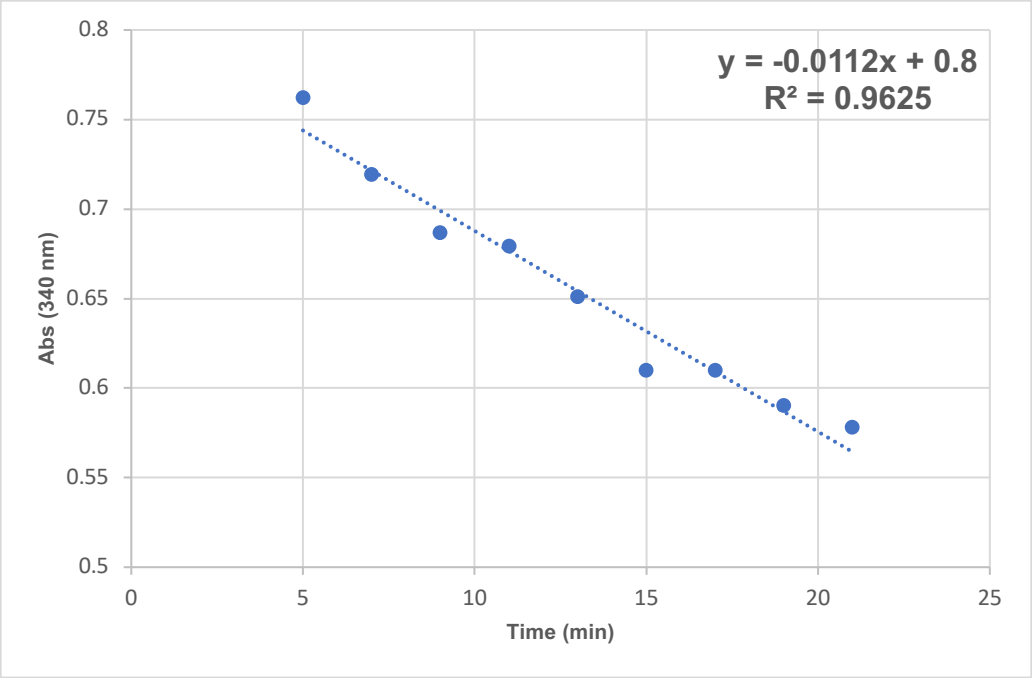

Figure S31: The absorbance of entry C detected across minutes 5-20.

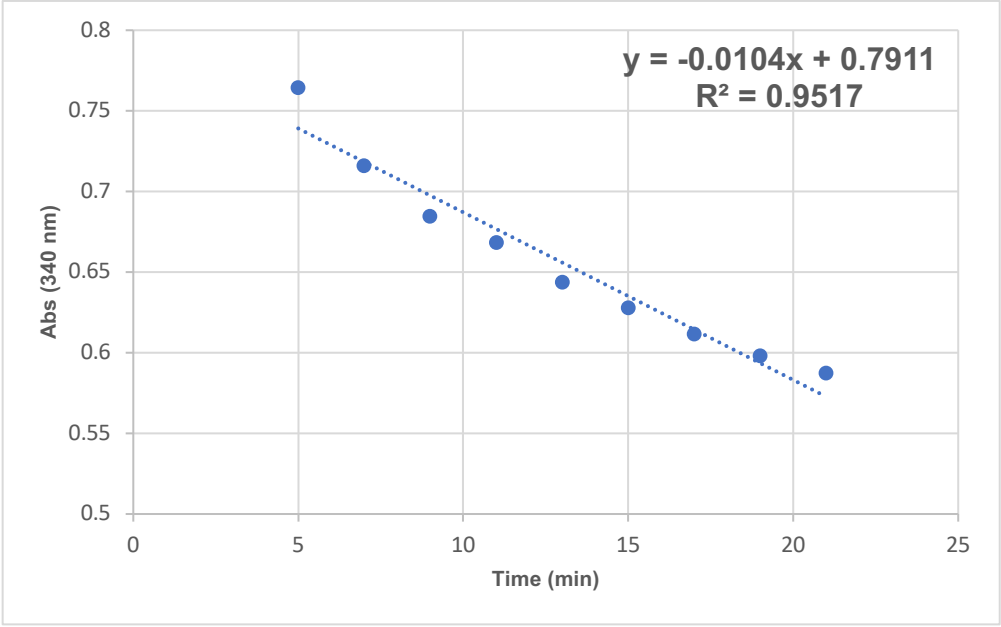

Figure S32: The absorbance of entry D detected across minutes 5-20.

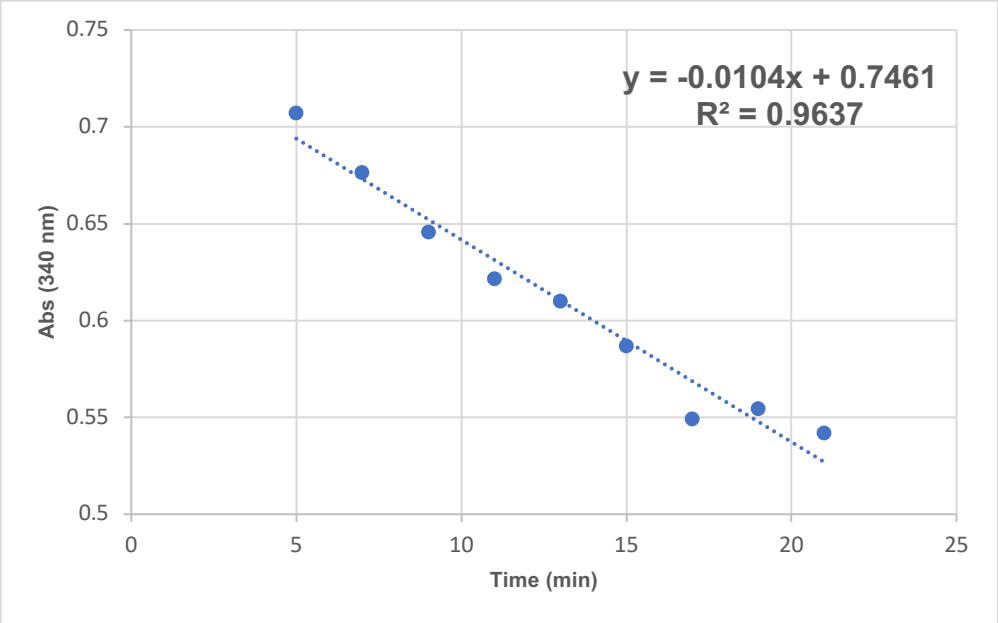

Figure S33: The absorbance of entry E detected across minutes 5-20.

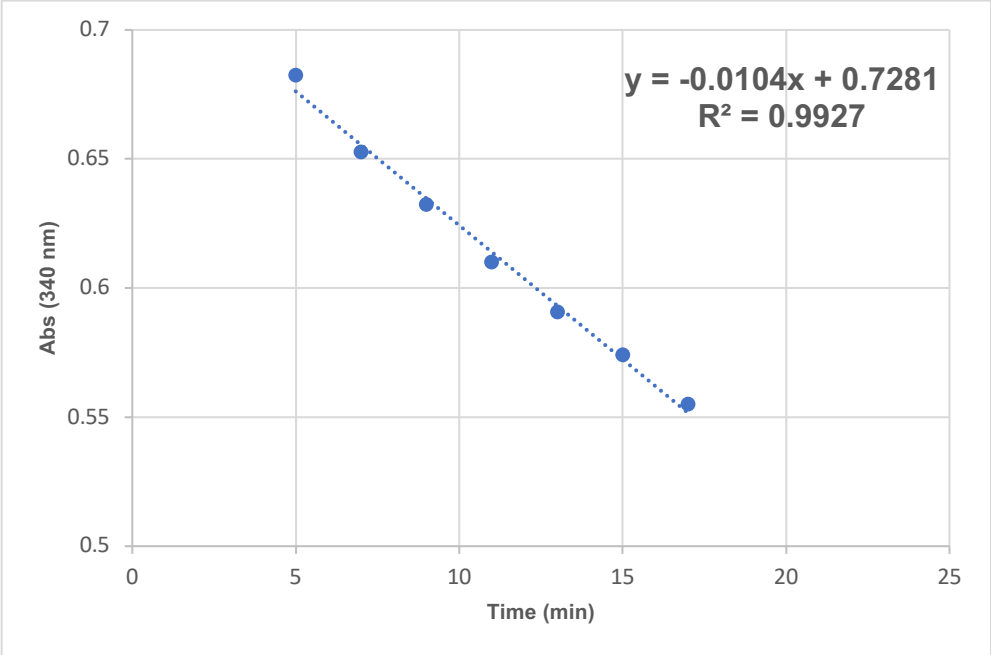

Figure S34: The absorbance of entry F detected across minutes 5-20.

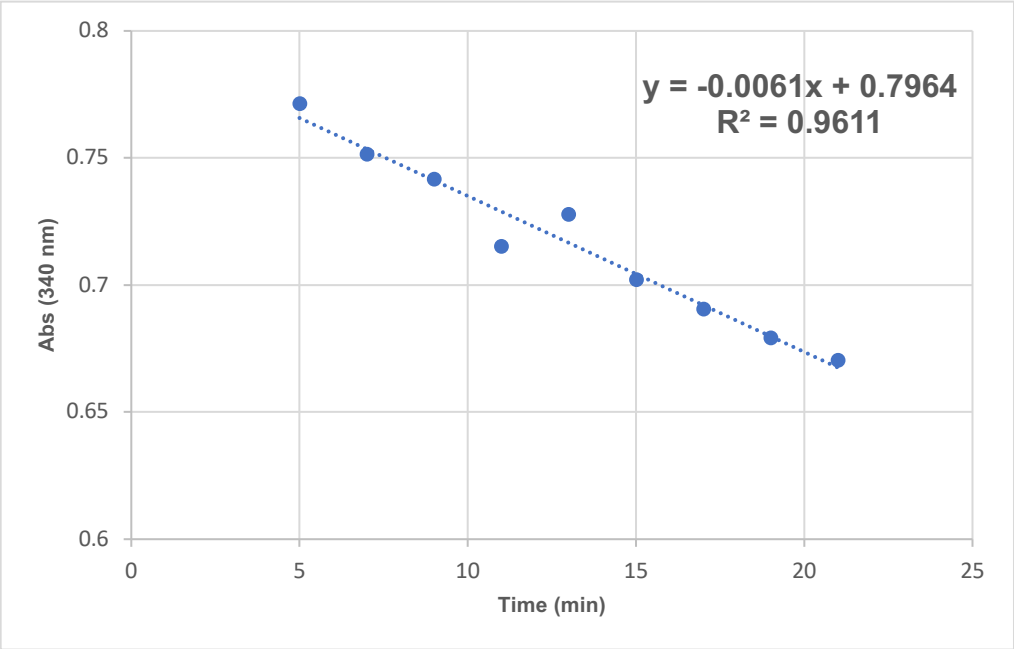

Figure S35: The absorbance of entry G detected across minutes 5-20.

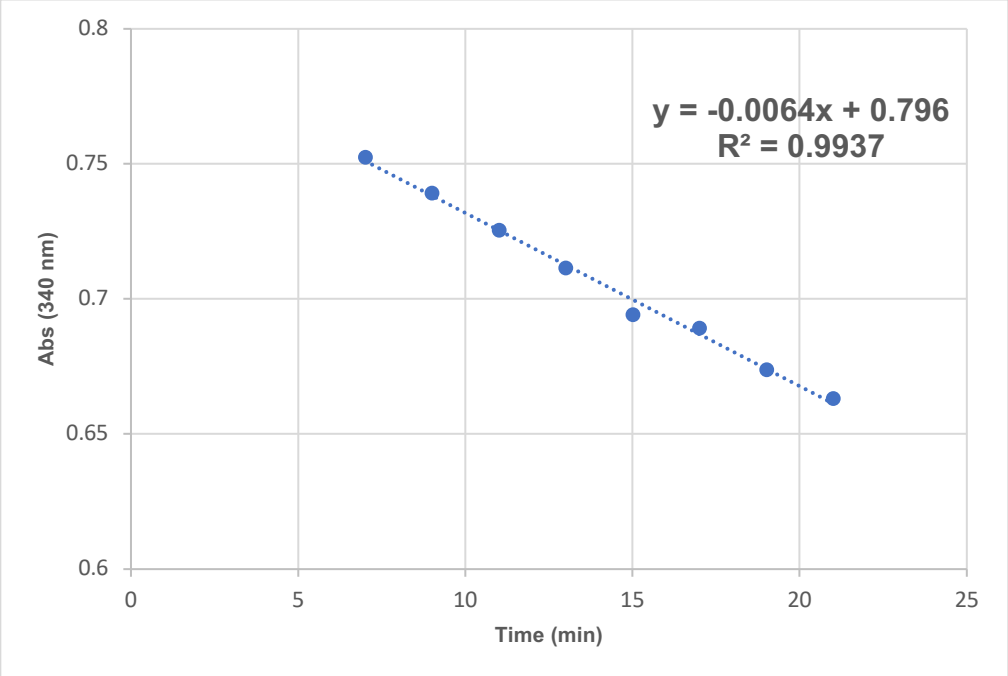

Figure S36: The absorbance of entry H detected across minutes 5-20.

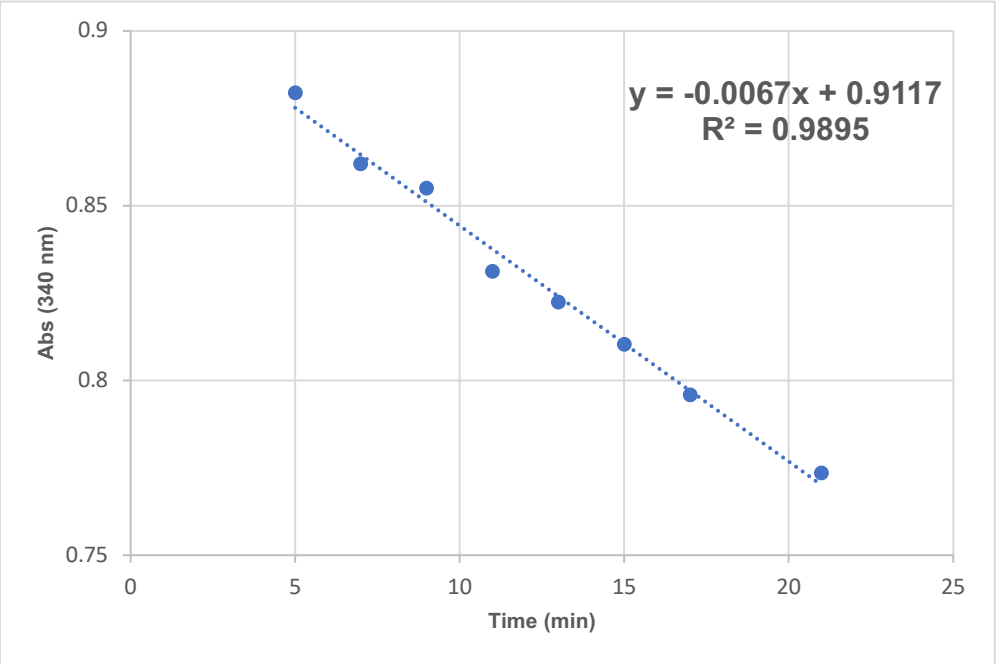

Figure S37: The absorbance of entry I detected across minutes 5-20.

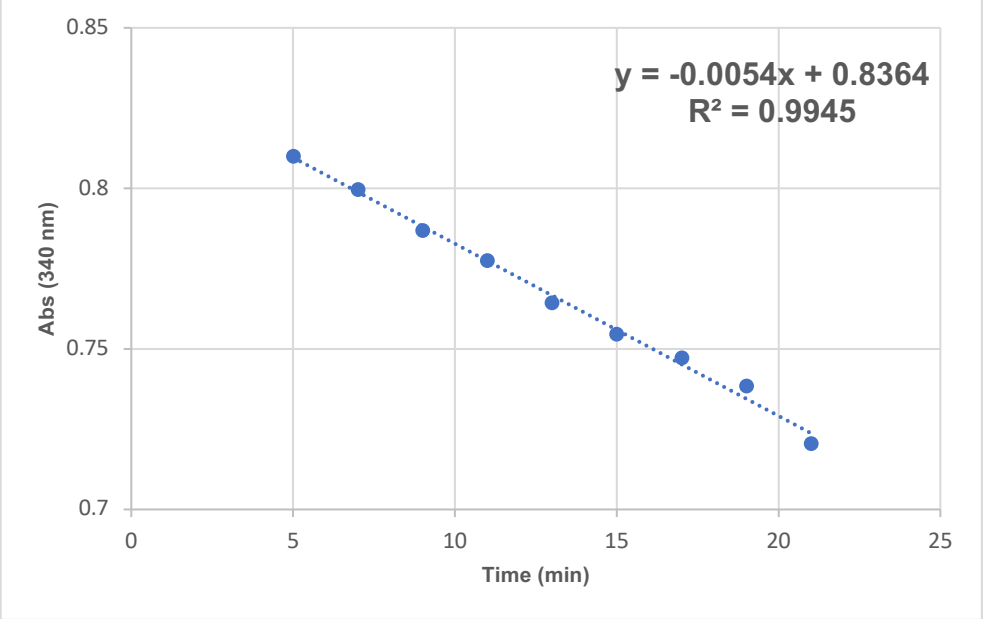

Figure S38: The absorbance of entry J detected across minutes 5-20.

XIII. Molecular Dynamics Simulations

Peptides **2a**, **2b** and **2c** were initially positioned in a similar way as transition state analog peptide inhibitors of proteinase K.[9,19] According to previous studies, the substrate recognition site of proteinase K is formed by two segments, to Gly100 to Tyr104 and Ser132 to Gly136.[9] The peptide substrate binds between these two segments as the center strand of a three-stranded antiparallel β-pleated sheet with the C-terminus of the peptide near the catalytic triad, Asp39-His69-Ser224.[9] In each binding simulation, peptides **2a**, **2b** and **2c** are prepared in β-sheet geometry with Avogadro[20] and positioned between the two-segment substrate recognition site with the C-terminus facing the catalytic triad. The initial geometries and positions of the peptides relative to proteinase K for the three systems were aligned using the "measure fit" function in VMD.[18] The three systems have all been equilibrated for 120 ns (**Figures 5, S39 Movies 1-3**). In the simulation of **2b** interacting with Proteinase K, the carborane cluster binds to a binding pocket within 5 ns of equilibration and binds to that pocket stably afterwards throughout the entire 120 ns of equilibration (**Figures S40-S41, Movie 4**).

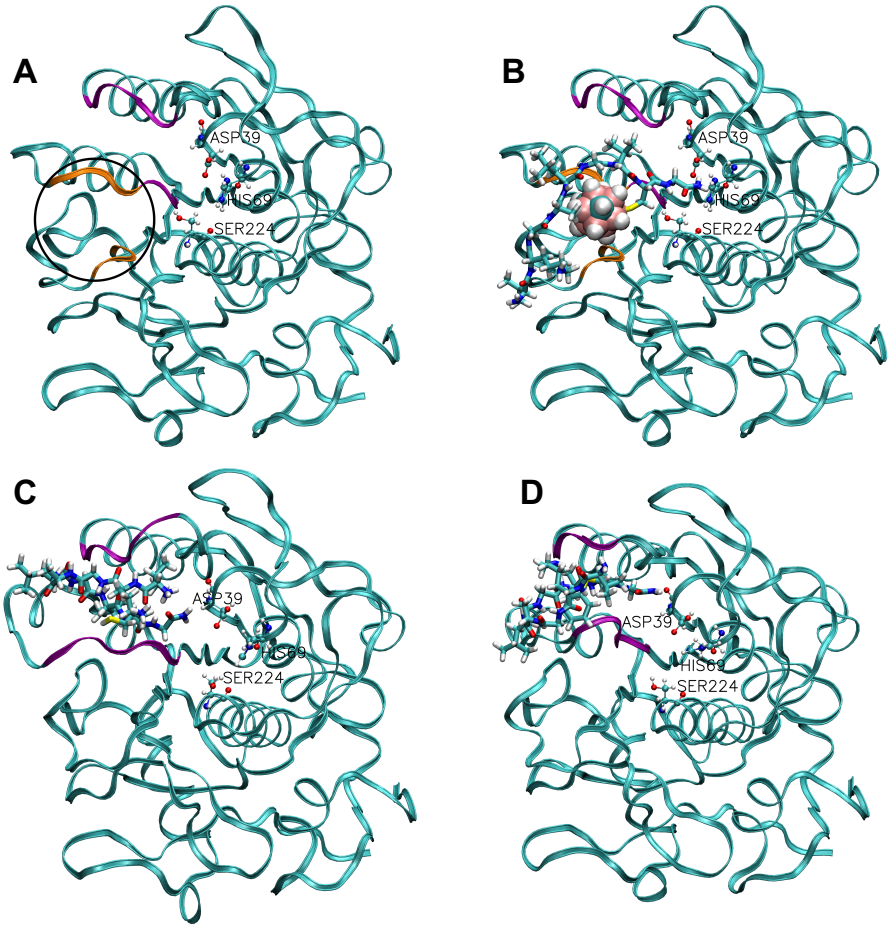

Figure S39: Molecular dynamics simulations rendered as stick and ribbon of **A:** the secondary binding pocket identified as an important docking site, **B: 2b** binding with Proteinase K, **C: 2a** binding with Proteinase K and **D: 2c** binding with Proteinase K at 120 ns of equilibration. Proteinase K is represented using QuickSurf representation in VMD.

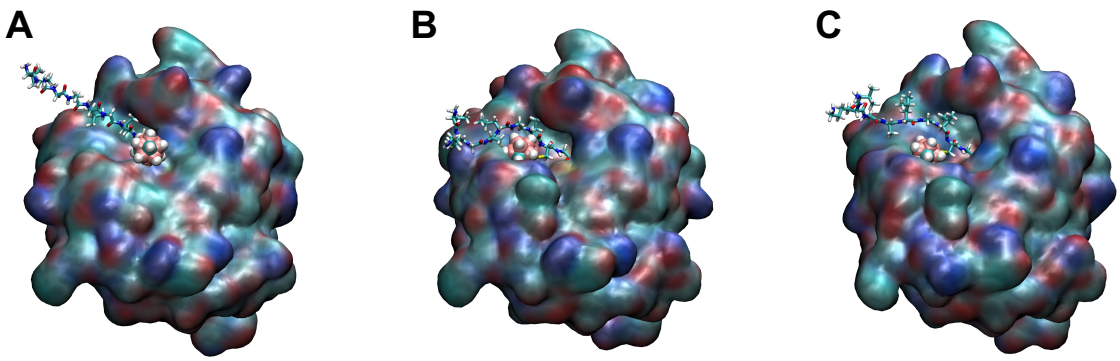

Figure S40: The binding of **2b** with proteinase K, at **A:** 0 ns, **B:** 5 ns and **C:** 120 ns of equilibration.

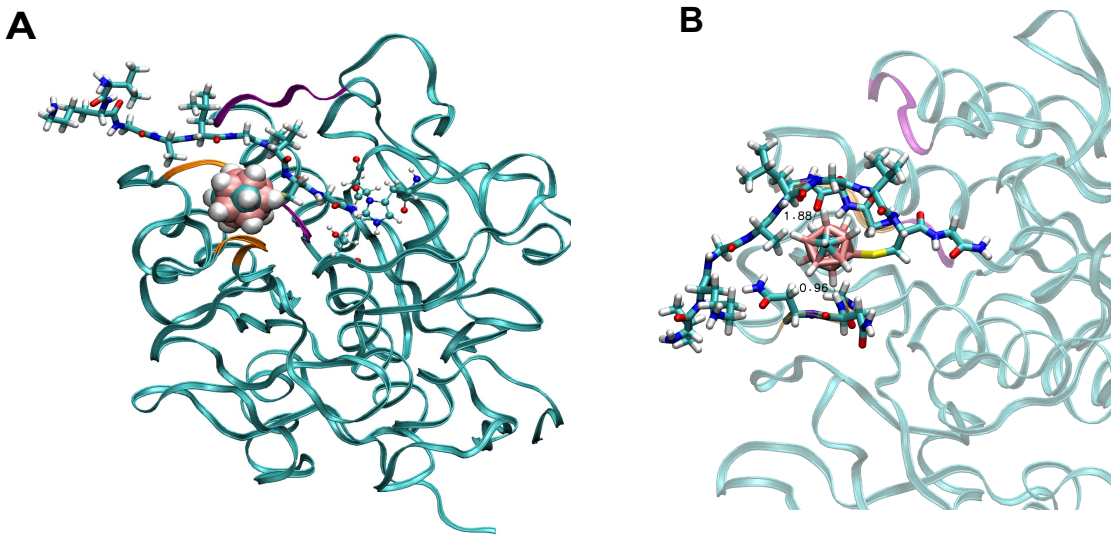

Figure S41 A: The binding of **2b** with proteinase K at 120 ns of equilibration; the proteinase K is shown in Ribbons representation with the substrate binding site in purple and carborane binding pocket in orange; the catalytic triad residues are shown in ball-and-stick model **B:** Detailed view of the carborane binding pocket with representative B-H···H-N dihydrogen bonding (H to H distance of 0.96 Å) and C-H···O hydrogen bonding (H to O distance of 1.88 Å)

The binding pocket of the carborane consists of two segments, one of which, Gly134-Gly135-Gly-136, is also part of the substrate recognition site and the other is Gly160-Asn161-Asn162. The residues that line the binding pocket contain only amide functional groups; therefore, binding through B-H···H-N dihydrogen bonds is implicated (**Figure S41B**).[21,22] The acidic C-H vertices of carborane may also form C-H···O (**Figure S41B**) or C-H···N hydrogen bonds.[22,23] Comparatively, simulations of **2a** and **2c** binding to Proteinase K indicate only 3-4 residues from the C-terminus stay bound between the two segments of the substrate recognition site without apparent interactions with other parts of the protein (**Figure S42, Movies 5-6**).

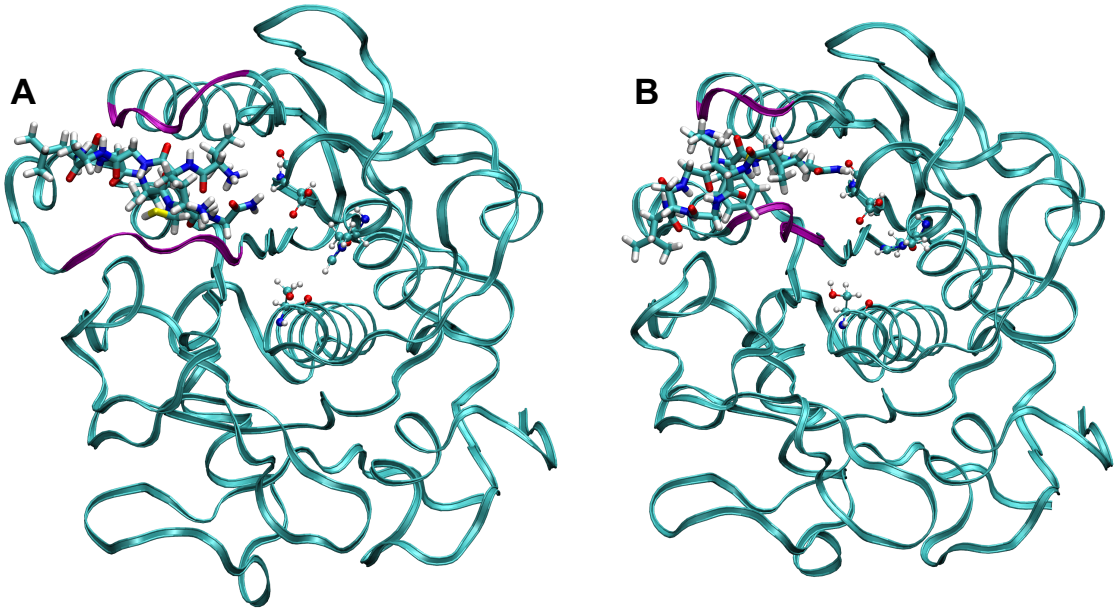

Figure S42 A: The unmodified and **B:** Cys-arylated nonapeptide binding with proteinase K at 120 ns of equilibration; the proteinase K is shown in Ribbons representation with the substrate binding site in purple.

The unique binding pocket for **2b** may pull the C-terminus of the peptide away from the catalytic triad, preventing nucleophilic attack by the hydroxyl oxygen atom of Ser224 on the carbonyl carbon atom of the peptide bond,[24] thereby protecting **2b** from hydrolysis. Over the 120-ns trajectory (**Figure S43**), the average center-to-center distance between **2a** and the catalytic triad is 13.49 Å while that between **2b** and the catalytic triad is 15.60 Å. The binding of the carborane to the binding pocket and the consequent steering of the peptide away from peptidolytic residues may be another explanation for the protection afforded by the borylation beyond the steric hindrance that obviates the approach of bulkier **2b** to the substrate binding site and the nearby catalytic triad.

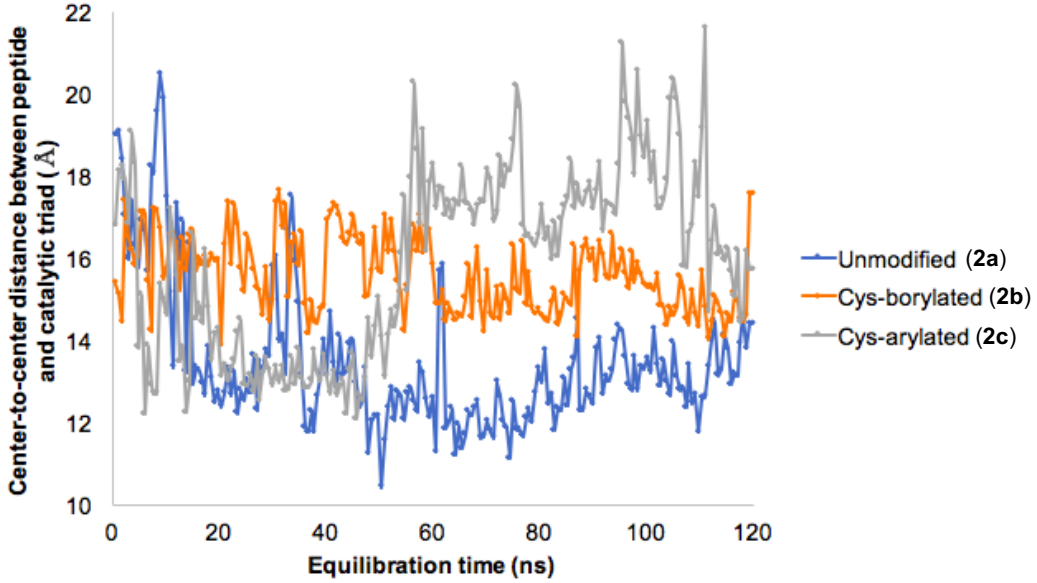

Figure S43: Center-to-center distance between the nonapeptide (unmodified, Cys-borylated or Cys-arylated) and the catalytic triad over 120 ns of equilibration.

XIV. References

1. Vinogradova, E. V., Zhang, C., Spokoyny, A. M., Pentelute, B. L. & Buchwald, S. L. Organometallic Palladium Reagents for Cysteine Bioconjugation. *Nature* **2015**, *526*, 687-691.

2. Messina, M. S., Stauber, J. M., Waddington, M. A., Rheingold, A. L., Maynard, H. D. & Spokoyny, A. M. Organometallic Gold(III) Reagents for Cysteine Arylation. *J. Am. Chem. Soc.* **2018**, *140*, 7065-7069.

3. Saleh, L. M. A., Dziedzic, R. M., Khan, S. I. & Spokoyny, A. M. Forging Unsupported Metal-Boryl Bonds with Icosahedral Carboranes. *Chem. Eur. J.* **2016**, *22*, 8466-8470.

4. Jullian, M., Hernandez, A., Maurras, A., Puget, K., Amblard, M., Martinez, J. & Subra, G. N-Terminus FITC labeling of peptides on solid support: the truth behind the spacer. *Tet. Lett.* **2009***, 50*, 260-263.

5. Phillips, J. C., Braun, R., Wang, W., Gumbart, J., Tajkhorshid, E., Villa, E., Chipot, C., Skeel, R. D., Kale L. & Schulten, K. Scalable molecular dynamics with NAMD. *J. Comput. Chem.* **2005**, *26*, 1781-1802.

6. Jorgensen, W. L., Chandrasekhar, J., Madura, J. D., Impey, R. W. & Klein, M. L. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* **1983**, *79*, 926-935.

7. Vanommeslaeghe, K., Hatcher, E., Acharya, C., Kundu, S., Zhong, S., Shim, J., Darian, E. et al. CHARMM general force field: A force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields. *J. Comput. Chem.* **2010**, *31*, 671-690.

8. MacKerell Jr, A. D., Bashford, D., Bellott, M. L. D. R., Dunbrack Jr, R. L., Evanseck, J. D., Field, M. J., Fischer, S., Gao, J., Guo, H., Ha, S., Joseph-McCarthy, D., Kuchnir, L., Kuczera, K., Lau, F. T. K., Mattos, C., Michnick, S., Ngo, T., Nguyen, D., Prodhom, B., Reiher, W., Roux, B., Schlenkrich, M., Smith, J., Stote, R., Straub, J., Watanabe, M., Yin D. & Wiorkiewicz-Kuczera, J. All-atom empirical potential for molecular modeling and dynamics studies of proteins. *J. Phys. Chem. B* **1998**, *102*, 3586-3616.

9. Wolf, W. M., Bajorath, J., Muller, A., Raghunathan, S., Singh, T. P., Hinrichs, W. & Saenger, W. Inhibition of Proteinase K by methoxysuccinyl-Ala-Ala-Pro-Ala-chloromethyl ketone. An x-ray study at 2.2-A resolution. *J. Biol. Chem.* **1991**, *266*, 17695-17699.

10. Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., Scalmani, G. et al. Gaussian 09, Revision A.02, Gaussian, Inc., Wallingford, CT, 2016.

11. Breneman, C. M. & Wiberg, K. B. Determining atom-centered monopoles from molecular electrostatic potentials. The need for high sampling density in formamide conformational analysis. *J. Comput. Chem.* **1990**, *11*, 361-373.

12. Mayne, C. G., Saam, J., Schulten, K., Tajkhorshid, E. & Gumbart, J. C. Rapid parameterization of small molecules using the force field toolkit. *J. Comput. Chem.* **2013**, *34*, 2757–2770.

13. Qian, E. A., Wixtrom, A. I., Axtell, J. C., Saebi, A., Jung, D., Rehak, P., Han, Y. Moully, E. H., Mosallaei, D., Chow, S., Messina, M., Wang, J.-Y., Royappa, A. T., Rheingold, A. L., Maynard, H. D., Kral, P. & Spokoyny, A. M.* "Atomically Precise Organomimetic Cluster Nanomolecules Assembled via Perfluoroaryl-Thiol SNAr Chemistry", *Nature Chem.* **2017,** *9*, 333-340.

14. Darden, T., York, D. & Pedersen, L. Particle mesh Ewald: An N log (N) method for Ewald sums in large systems. *J. Chem. Phys.* **1993**, *98*, 10089-10092.

15. Han, Y., Albericio, F. & Barany, G. Occurrence and Minimization of Cysteine Racemization during Stepwise Solid-Phase Peptide Synthesis[1,2]. *J. Org. Chem.* **1997**, *62*, 4307-4312.

16. Fielding, Lee. NMR Methods for the Determination of Protein-Ligand Dissociation Constants. *Curr. Top. Med. Chem.* **2003**, *3*, 39-53.

17. Shalaby, S. M., Zakora, M. & Otte, J. Performance of two commonly used angiotensin-converting enzyme inhibition assays using FA-PGG and HHL as substrates. *J. Dairy Res.* **2006**, *73*,178-186.

18. Humphrey, W., Dalke, A. & Schulten, K. VMD: visual molecular Dynamics. *J. Mol. Graph.* **1996**, *14*, 33-38.

19. Liu, S.-Q., Tao, Y., Meng, Z.-H., Fu, Y.-X. & Zhang, K.-Q. The effect of calciums on molecular motions of Proteinase K. *J*. *Mol*. *Model*. **2011**, *17*, 289-300.

20. Hanwell, M. D., Curtis, D. E., Lonie, D. C., Vandermeersch, T., Zurek, E. & Hutchison, G. R. Avogadro: An advanced semantic chemical editor, visualization, and analysis platform. *J. Cheminformatics* **2012**, *4*, 1-17.

21. Fanfrlík, J., Lepšík, M., Horinek, D., Havlas, Z. & Hobza, P. Interaction of carboranes with biomolecules: formation of dihydrogen bonds. *ChemPhysChem* **2006**, *7*, 1100-1105.

22. Sarosi, M-B., Neumann, W., Lybrand, T. P. & Hey-Hawkins, E. Molecular modeling of the interactions between carborane-containing analogs of indomethacin and cyclooxygenase-2. *J. Chem. Inf. Model.* **2017**, *57*, 2056-2067.

23. Scholz, M. & Hey-Hawkins, E. Carbaboranes as pharmacophores: properties, synthesis, and application strategies. *Chem. Rev.* **2011**, *111*, 7035-7062.

24. Liu, S.-Q., Liang, L.-M., Tao, Y., Yang, L.-Q., Ji, X.-L., Yang, J.-K., Fu, Y.-X. & Zhang, K.-Q. Structural and dynamic basis of serine proteases from nematophagous fungi for cuticle degradation. in *Pesticides in the Modern World—Pests Control and Pesticides Exposure and Toxicity Assessment* ed. Stoytcheva, M.; Intech, 2011.

25. Wingfield, P. T. N-terminal methionine processing. *Curr. Protoc. Protein Sci.* **2017**, *88*, 6.14.1–6.14.3.