An *Umpolung* Approach for the Chemoselective Arylation of Selenocysteine in Unprotected Peptides

Daniel T. Cohen,[†] Chi Zhang,[†] Bradley L. Pentelute* and Stephen L. Buchwald*

Department of Chemistry, Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139, United States

Supporting Information

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Materials

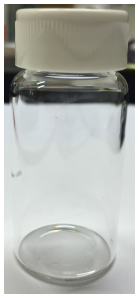
1. Chemicals

Tris(2-carboxyethyl)phosphine hydrochloride (TCEP•HCl) was purchased from Hampton Research (Aliso Viejo, CA). 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), Fmoc-L-Gly-OH, Fmoc-L-Leu-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-L-Ala-OH, Fmoc-L-Cys(Trt)-OH, Fmoc-L-Asn(Trt)-OH, Fmoc-L-Asp(tBu)-OH, Fmoc-L-Arg(Pbf)-OH, Fmoc-L-Phe-OH, Fmoc-L-Ser(tBu)-OH, Fmoc-L-Tyr(tBu)-OH, Fmoc-Ser-OH, and Fmoc-L-His(Trt)-OH were purchased from Chem-Impex International (Wood Dale, IL). Fmoc-L-4-methoxybenzyl-selenocysteine (Fmoc-L-Sec(Pmb)-OH) was prepared from Fmoc-Ser-OH using standard literature procedure.[1] Peptide synthesis-grade *N, N*-dimethylformamide (DMF), dichloromethane (DCM), diethyl ether, HPLC-grade acetonitrile, and guanidine hydrochloride were obtained from VWR International (Philadelphia, PA). All reactions were set up on the bench top open to air. Water was deionized and used as is. Ethanol, copper, ligands were purchased from commercial sources and used as received. Boronic acids were purchase from commercial sources or prepared according to standard literature procedure.[2]

2. Reaction Vessels

a) 0.6 mL Axygen Tubes (For 100 μM reaction):
Axygen Cat. No. MCT-060-L-C

b) Scintillation Vials (For 1 mM scale-up reactions):
VWR Cat. No. VW74510-20



Methods for LC-MS Analysis

LC-MS chromatograms and associated mass spectra were acquired using Agilent 6520 ESI-Q-TOF mass spectrometer unless noted. Mobile phases are: 0.1% formic acid in water (***solvent C***) and 0.1% formic acid in acetonitrile (***solvent D***) Following LC-MS methods were used:

Method A LC conditions: Zorbax SB C3 column: 2.1 x 150 mm, 5 μm, column temperature: 40 °C, gradient: 0-2 minutes 1% D, 2-11 minutes 1-61% D, 11-12 minutes 61% D, flow rate: 0.8 mL/min. MS conditions: positive electrospray ionization (ESI) extended dynamic mode in mass range 300 – 3000 m/z, temperature of drying gas = 350 °C, flow rate of drying gas = 11 L/min,

pressure of nebulizer gas = 60 psi, the capillary, fragmentor, and octupole rf voltages were set at 4000, 175, and 750, respectively.

Method B LC conditions: Zorbax SB C3 column: 2.1 x 150 mm, 5 μm, column temperature: 40 °C, gradient: 0-1 minutes 5-25% D, 1-5 minutes 25-75% D, flow rate: 0.8 mL/min. 5-6 minutes 75-95% D, flow rate: 1.5 mL/min. MS conditions are same as *Method A*.

Method C LC conditions: Zorbax SB C3 column: 2.1 x 150 mm, 5 μm, column temperature: 40 °C, gradient: 0-2 minutes 1% D, 2-23 minutes 1-61% D, 23-24 minutes 61% D, flow rate: 0.8 mL/min. MS conditions are same as *Method A*.

Method D LC conditions: Zorbax SB C18 column: 2.1 x 150 mm, 5 μm, column temperature: 40 °C, gradient: 0-2 minutes 1% D, 2-11 minutes 1-61% D, 11-12 minutes 61% D, flow rate: 0.8 mL/min. MS conditions are same as *Method A*.

All reactions for peptide stability studies were analyzed by Agilent 6550 ESI-Q-TOF mass spectrometer. Mobile phases are: 0.1% formic acid in water (***solvent C***) and 0.1% formic acid in acetonitrile (***solvent D***). Following LC-MS method was used:

Method E LC conditions: EclipsePlus C18 column: 2.1 x 50 mm, RRHD 1.8 μm, column temperature: 40 ^{o}C, gradient: 0-1 minutes 5% D, 1-6 minutes 5-50% D, 6-8 minutes 50-95% D, 8-10 minutes 95% D, flow rate: 0.5 mL/min. MS conditions: positive electrospray ionization (ESI) extended dynamic mode in mass range 300 – 3000 m/z, temperature of drying gas = 200 ^{o}C, flow rate of drying gas = 17 L/min, pressure of nebulizer gas = 35 psi, the capillary, fragmentor, and nozzle voltages were set at 3500, 380, and 500, respectively.

All data were processed using Agilent MassHunter software package. Y-axis in all chromatograms shown represents total ion current (TIC) unless noted; mass spectrum corresponds to the integration of the TIC peak unless noted.

All yields reported were determined by integrating TIC spectra. First, using Agilent MassHunter software package, the peak areas for all relevant peptidic species on the chromatogram were integrated. Then the yield was calculated as following: %yield = S_p/S_{all} where S_p is the peak area of the desired product, and S_{all} is sum of the peak areas of all peptidic species.

General Method for Preparation of Peptides

1) Fast-flow peptide synthesis

All peptide sequences *C*-terminal to selenocysteine were synthesized on a 0.2-mmol scale using manual Fmoc-SPPS (Solid phase peptide synthesis) chemistry under flow using a 3-minute cycle for each amino acid.[3] Specifically, all reagents and solvents are delivered to a stainless steel reactor containing resins at a constant flow rate using an HPLC pump; temperature of the reactor was maintained at 60 °C during the synthesis using a water bath. The procedure for each amino acid coupling cycle included a 30 second coupling with 1 mmol Fmoc-protected amino acid, 1 mmol HATU, and 500 μL of diisopropyl ethyl amine (DIEA) in 2.5 mL of DMF at a flow rate of 6 mL/min (note that for the coupling of cysteine and histidine, 190 μL of DIEA was used to prevent racemization); 1 min wash with DMF at a flow rate of 20 mL/min; 20 second deprotection with 20% (v/v) piperidine in DMF at a flow rate of 20 mL/min; and 1 minute wash with DMF at a flow rate of 20 mL/min. After completion of the fast-flow synthesis, the resins are washed with DCM (3X) and dried under vacuum. The dried resins are used in batch synthesis for coupling of selenocysteine and the rest of the peptide sequence.

Peptide sequences synthesized using fast-flow peptide synthesis are highlighted in yellow in Table S1.

2) Solid-phase peptide synthesis (SPPS) in batch

Selenocysteine and amino acids *N*-terminal to selenocysteine were coupled to the resin under batch SPPS conditions on a 0.2-mmol scale. Each amino acid was incorporated into the peptide sequence through a cycle of coupling, washing, deprotection, and washing steps. Procedure for the coupling of selenocysteine included a 20 min coupling with 0.4 mmol Fmoc-L-Sec(Pmb)-OH, 0.4 mmol HATU, and 38 μL of DIEA in 2 mL of DMF. For other amino acids, coupling was performed for 10 min with 1 mmol Fmoc-protected amino acids, 1 mmol HATU, 500 μL of DIEA in 2.5 mL of DMF. After coupling, the resin was washed with DMF (3X). Piperidine (20% (v/v) in DMF) was added to the resin for 2X 5 min each. The resin was washed with DMF (3X) and then subjected to coupling of the next amino acid.

Peptide sequences synthesized using batch SPPS are highlighted in green in Table S1.

3) Peptide cleavage and deprotection

Peptides ***containing*** selenocysteine were cleaved from the resin and the side-chain was simultaneously deprotected by treatment with 5% (v/v) water, 95% (v/v) trifluoroacetic acid (TFA), 0.4 M 2,2'-dithiobis(5-nitropyridine) (DTNP) for 7 min at 60 °C. 5 mL of cleavage cocktail was used for 0.2 mmol of peptide. The resulting solution was triturated and washed with cold diethyl ether (pre-chilled in –80 °C freezer) this was repeated a total of three times. The obtained solids were dissolved in 50% H_2O: 50% acetonitrile containing 0.1% TFA and lyophilized. ***These same solvent compositions were used in the majority of experiments and will be referred to as A: 0.1% TFA in H_2O and B: 0.1% TFA in acetonitrile.***

Peptides ***without*** selenocysteine were cleaved from the resin and the side-chain was simultaneously deprotected by treatment with 2.5% (v/v) water, 2.5% (v/v) 1,2-ethanedithiol (EDT), 1% (v/v) triisopropylsilane (TIPS) in neat TFA for 7 min at 60 °C, 5 ml of cleavage cocktail was used for 0.2 mmol of peptide. The resulting solution was triturated and washed with cold ether (pre-chilled in –80 °C freezer). The trituration was repeated a total of three times. The obtained solids were dissolved in 50% A and 50% B and lyophilized.

Peptide ***containing*** methionine was cleaved from the resin and the side-chain was simultaneously deprotected by treatment with 1% (v/v) triisopropylsilane (TIPS), 2.5% (v/v) water, 2.5% (v/v) 1,2-ethanedithiol (EDT), 2% (v/v) methyl disulfide, 92% (v/v) trifluoroacetic acid (TFA), and saturated ammonium iodide for 8 min at 60 °C. 5 mL of this cleavage cocktail was used for 0.2 mmol of peptide. The resulting solution was triturated and washed with cold diethyl ether (pre-chilled in –80 °C freezer) this was repeated a total of three times. The obtained solids were dissolved in 50% H_2O: 50% acetonitrile containing 0.1% TFA and lyophilized.

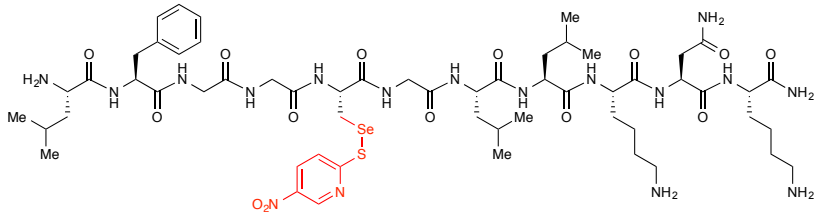
4) RP-HPLC purification of peptides

The crude peptide was dissolved in 95% A: 5% B with 6 M guanidinium hydrochloride and purified by semi-preparative RP-HPLC (Agilent Zorbax SB C18 column: 21.2 x 250 mm, 7 μm, linear gradient: 5-50% B over 90 min, flow rate: 5 mL/min). 1 μL of each HPLC fraction was mixed with 1 μL of α-cyano-4-hydroxycinnamic acid (CHCA) matrix in 75% A: 25% B, spotted with MALDI, and checked for fractions with desired molecular mass. The purity of fractions was confirmed by analytical RP-HPLC (Agilent Zorbax SB C3 column: 2.1 x 150 mm, 5 μm, gradient: 0-2 minutes 5% B, 2-11 minutes 5-65% B, 11-12 minutes 65% B, flow rate: 0.8 mL/min). HPLC fractions containing only product materials were confirmed by LC-MS analysis, combined, and then lyophilized. Peptides purified by RP-HPLC are listed in Table S1.

Table S1. Peptide Sequences. Amino acids highlighted in yellow were incorporated through fast-flow SPPS; amino acids highlighted in green were synthesized through batch SPPS.

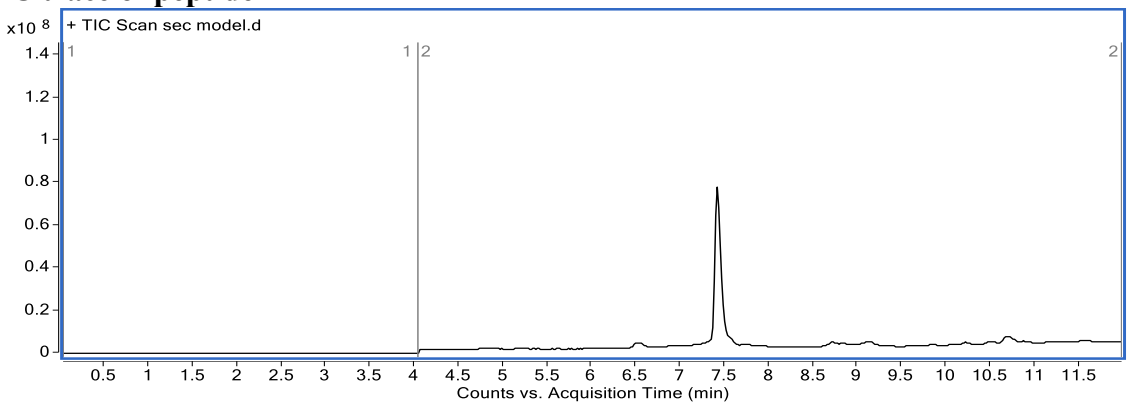
Peptide	Sequence
1	NH_2-Leu-Phe-Gly-Gly-**Sec(TNP)**-Gly-Leu-Leu-Lys-Asn-Lys-$CONH_2$
1-Ser5	NH_2-Leu-Phe-Gly-Gly-**Ser**-Gly-Leu-Leu-Lys-Asn-Lys-$CONH_2$
1-Cys5	NH_2-Leu-Phe-Gly-Gly-**Cys**-Gly-Leu-Leu-Lys-Asn-Lys-$CONH_2$
1-Cys5-TNP	NH_2-Leu-Phe-Gly-Gly-**Cys(TNP)**-Gly-Leu-Leu-Lys-Asn-Lys-$CONH_2$
1-Met5	NH_2-Leu-Phe-Gly-Gly-**Met**-Gly-Leu-Leu-Lys-Asn-Lys-$CONH_2$
7	NH_2-Gly-**Sec(TNP)**-Ala-Asn-Ser-Leu-Arg-Phe-Tyr-His-Asp-Lys-$CONH_2$
9	NH_2-Gly-**Ser**-Ala-Asn-Ser-Leu-Arg-Phe-Tyr-His-Asp-Lys-$CONH_2$

LC-MS analytical data for purified peptides

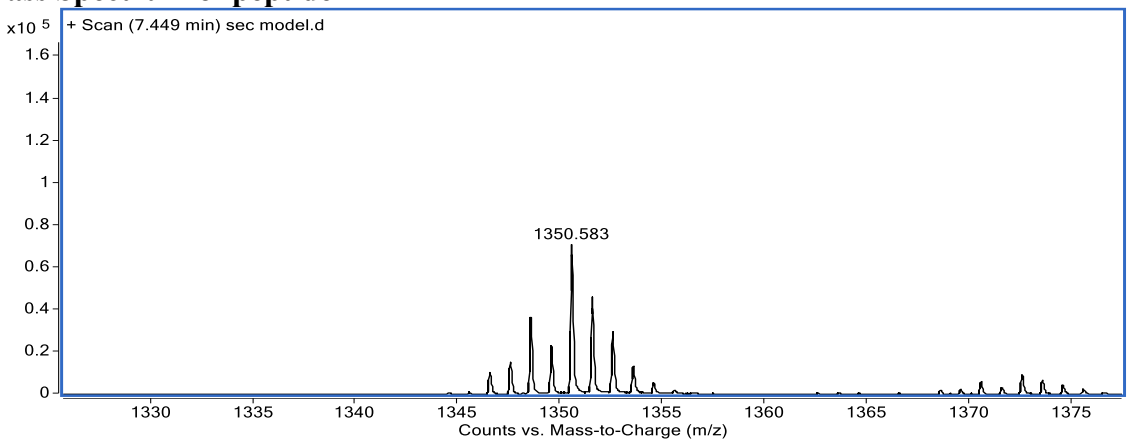


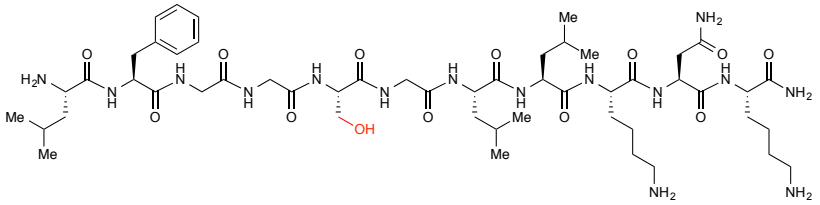
Peptide 1: LCMS Analysis *Method A*. HRMS (ESI) Mass. calcd. for $C_{57}H_{92}N_{17}O_{14}SSe$ $[M+H]^+$, 1350.58. Found $[M+H]^+$, 1350.58.

TIC trace of peptide 1



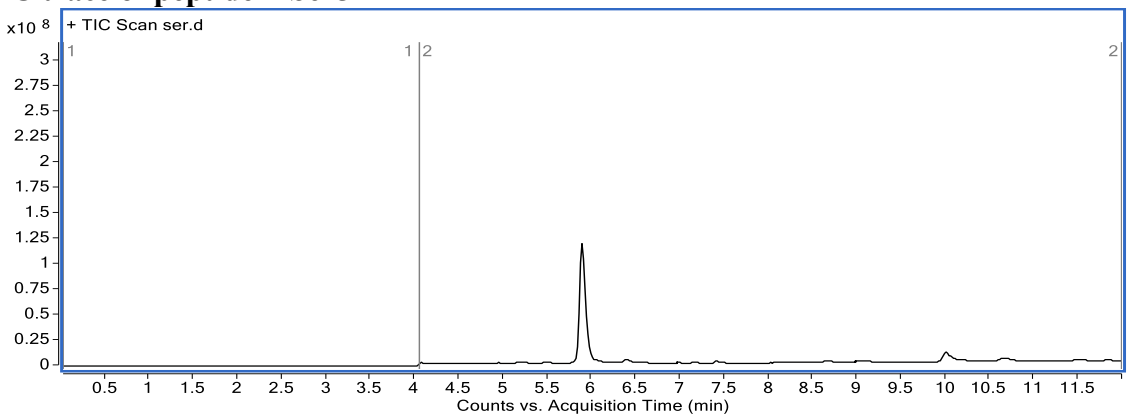
Mass Spectrum of peptide 1



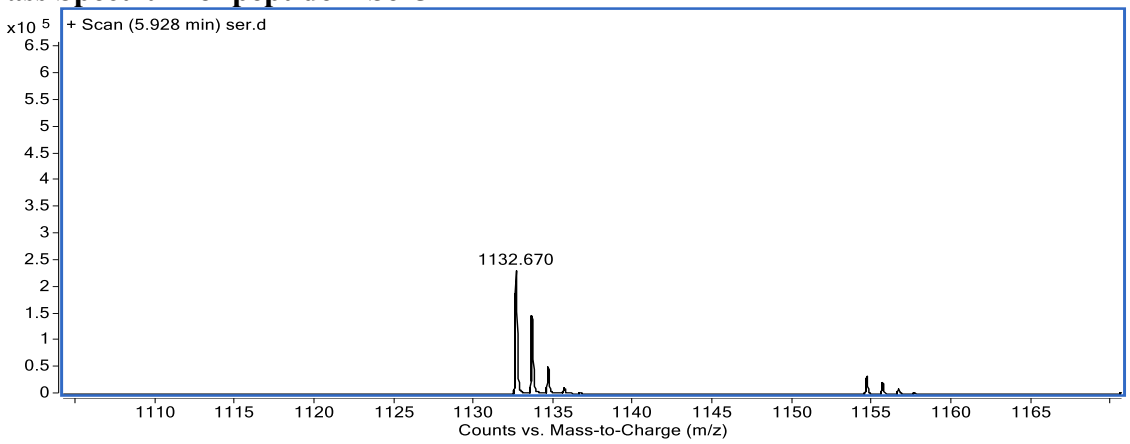


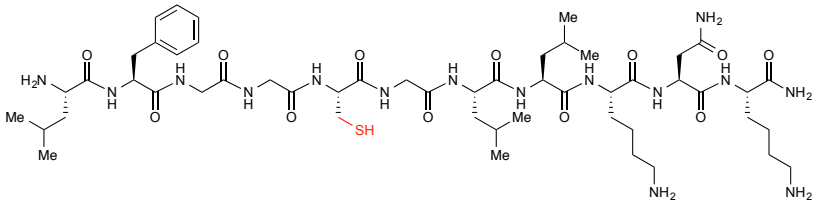
Peptide 1-Ser5: LCMS Analysis *Method A*. HRMS (ESI) Mass. calcd. for $C_{52}H_{90}N_{15}O_{13}$ $[M+H]^+$, 1132.68. Found $[M+H]^+$, 1132.67.

TIC trace of peptide 1-Ser5



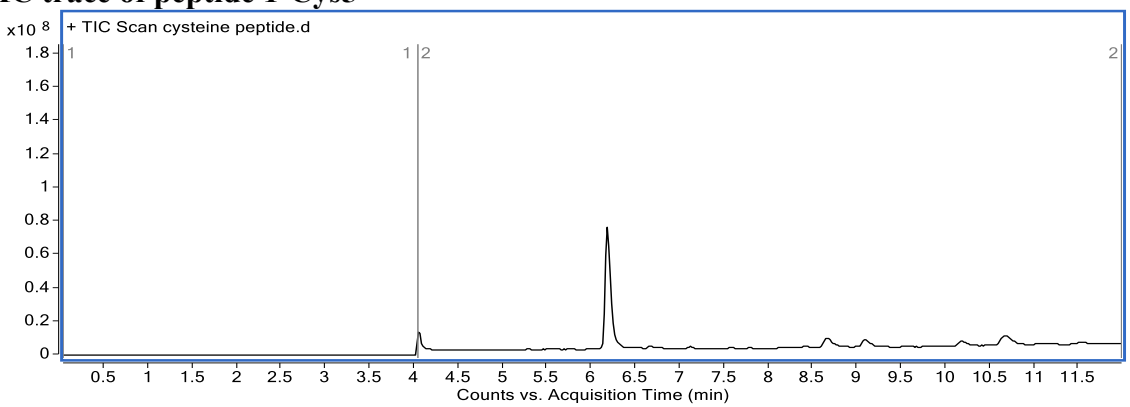
Mass Spectrum of peptide 1-Ser5



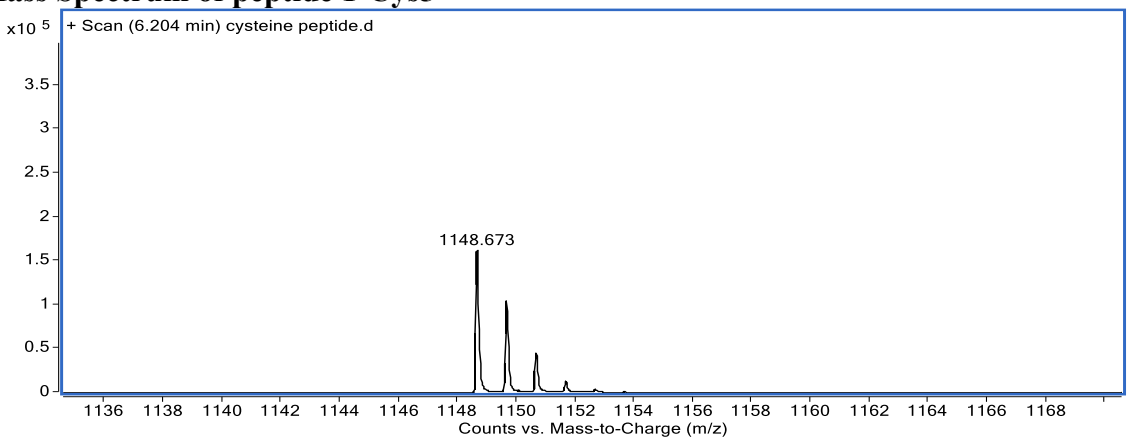


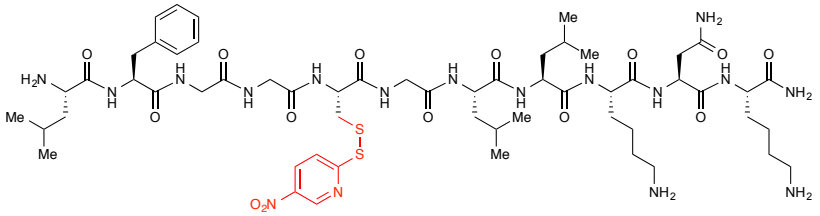
Peptide 1-Cys5: LCMS Analysis *Method A*. HRMS (ESI) Mass. calcd. for $C_{52}H_{90}N_{15}O_{12}S$ $[M+H]^+$, 1148.66. Found $[M+H]^+$, 1148.67.

TIC trace of peptide 1-Cys5



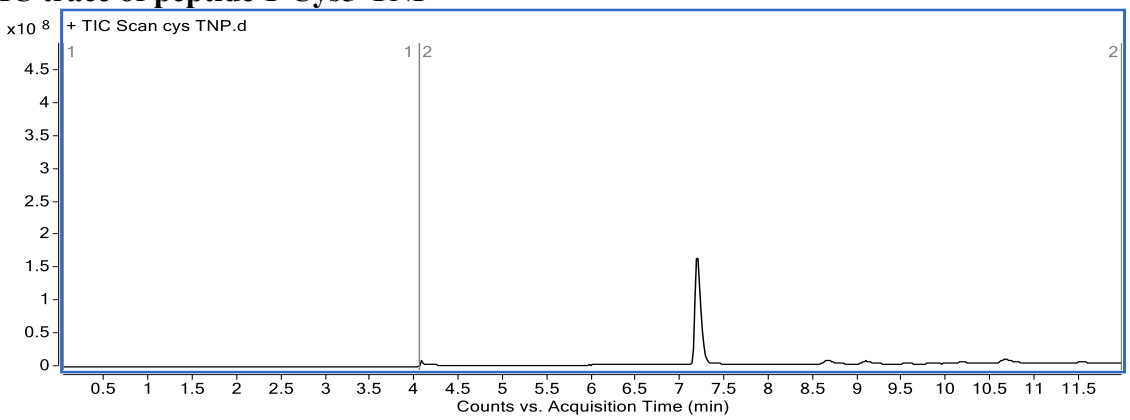
Mass Spectrum of peptide 1-Cys5



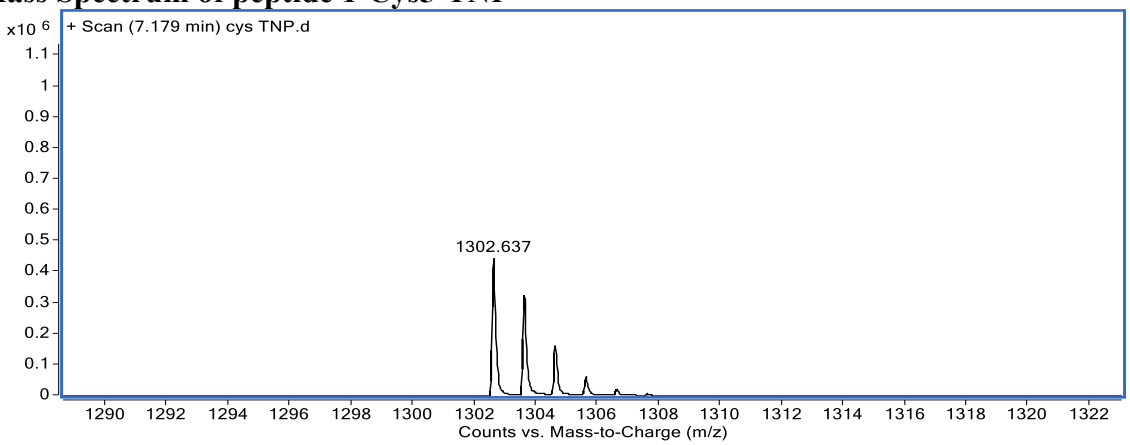


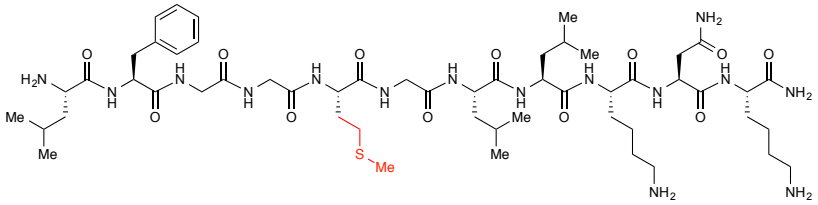
Peptide 1-Cys5-TNP: Peptide was synthesized using fast-flow peptide synthesis procedure. The TNP protecting group was installed using cleavage cocktail: 5% (v/v) H_2O, 95% (v/v) TFA, 0.4 M DTNP, at 60 °C for 5 minutes. LCMS Analysis *Method A*. HRMS (ESI) Mass. calcd. for $C_{57}H_{91}N_{17}O_{14}S_2$ $[M+H]^+$, 1302.64. Found $[M+H]^+$, 1302.64.

TIC trace of peptide 1-Cys5-TNP



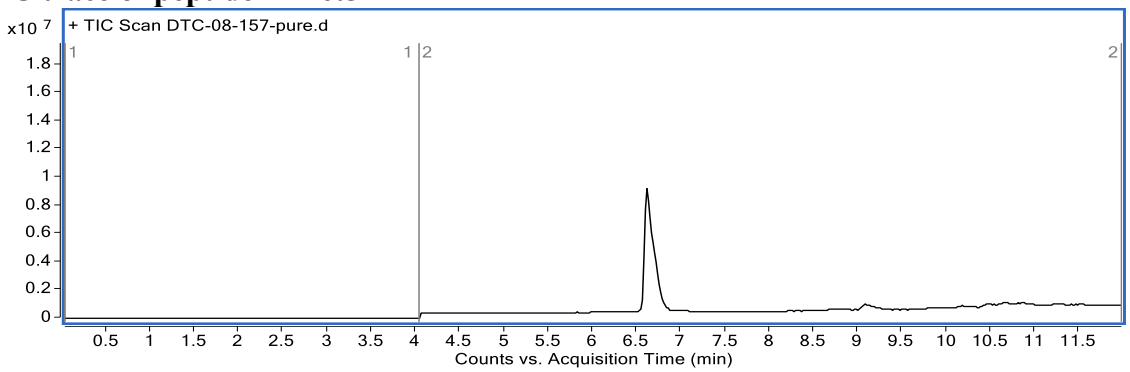
Mass Spectrum of peptide 1-Cys5-TNP



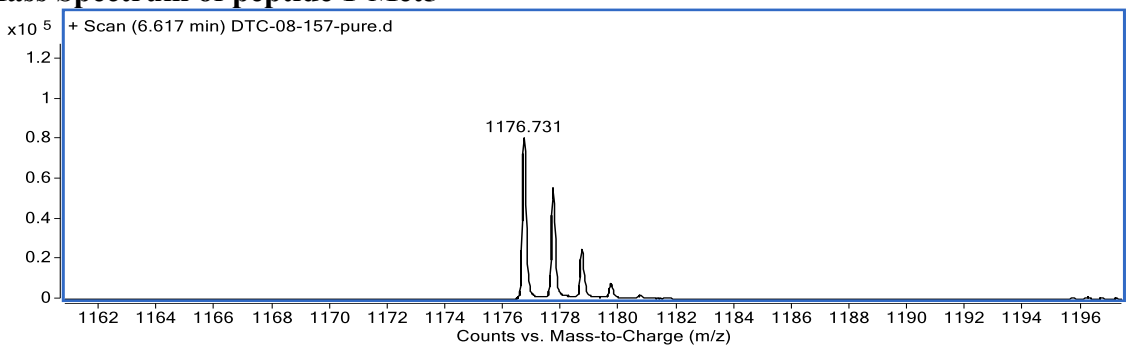


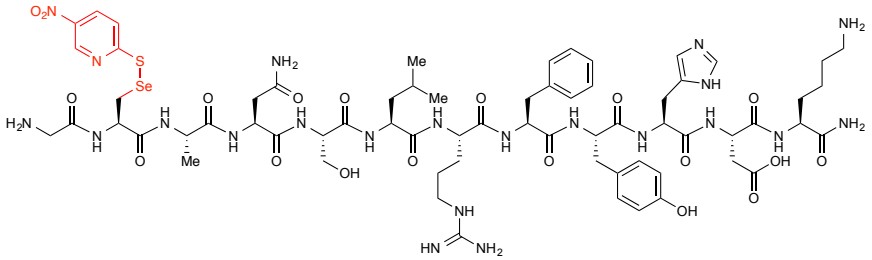
Peptide 1-Met5: LCMS Analysis *Method A*. HRMS (ESI) Mass. calcd. for $C_{54}H_{94}N_{15}O_{12}S$ $[M+H]^+$, 1176.69. Found $[M+H]^+$, 1176.73.

TIC trace of peptide 1-Met5



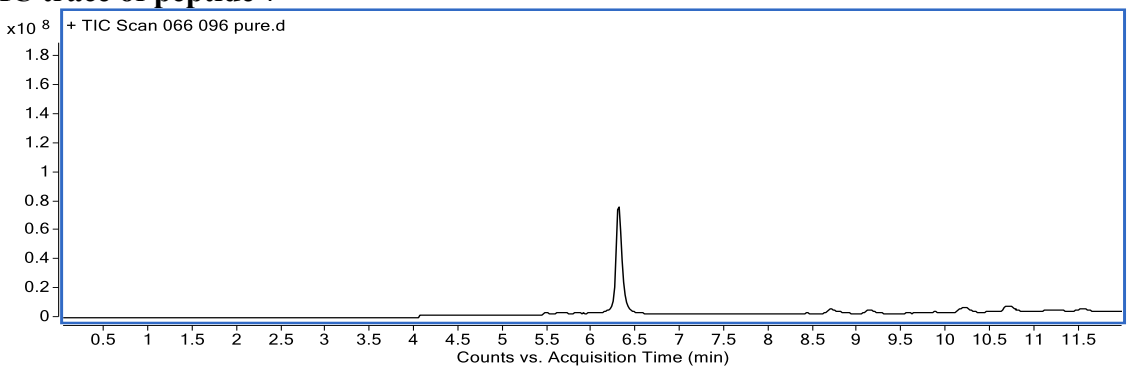
Mass Spectrum of peptide 1-Met5



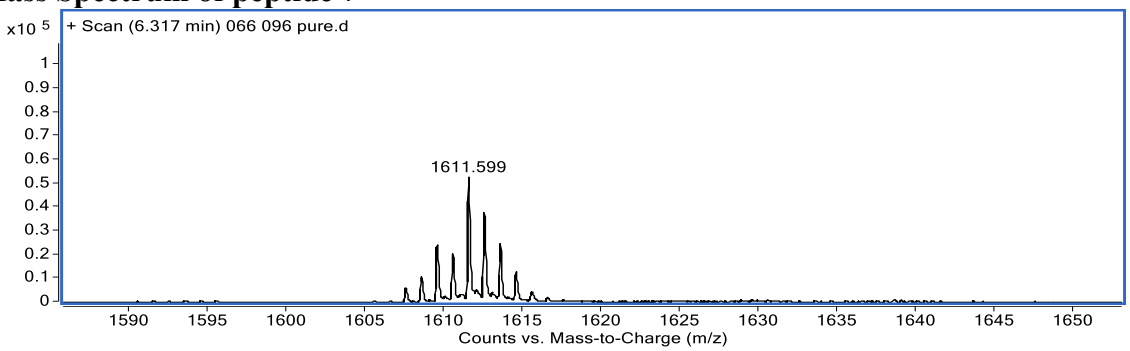


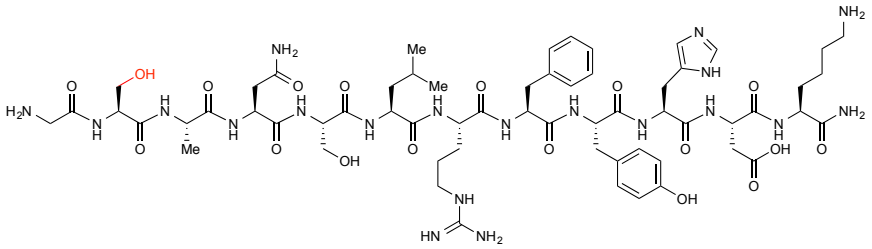
Peptide 7: LCMS Analysis *Method A*. HRMS (ESI) Mass. calcd. for $C_{66}H_{95}N_{22}O_{19}SSe$ $[M+H]^+$, 1611.60. Found $[M+H]^+$, 1611.60.

TIC trace of peptide 7



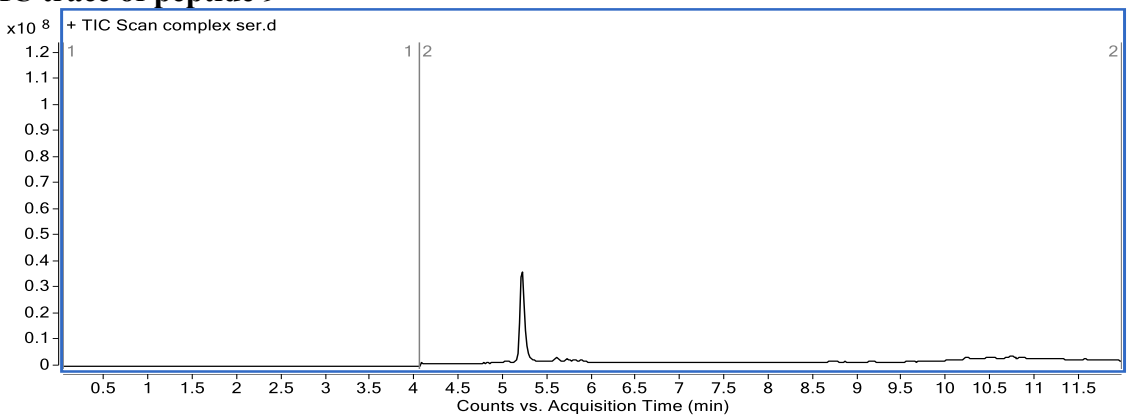
Mass Spectrum of peptide 7



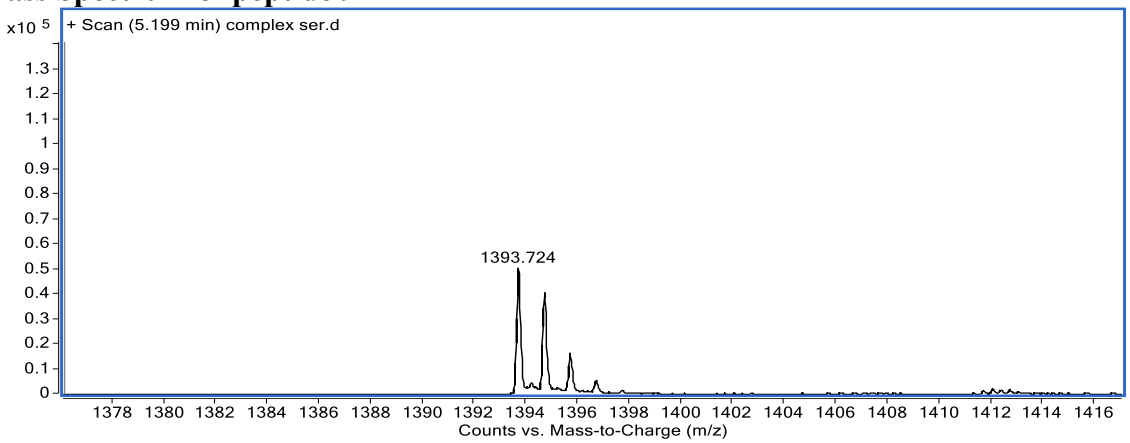


Peptide 9: LCMS Analysis *Method A*. HRMS (ESI) Mass. calcd. for $C_{61}H_{93}N_{20}O_{18}$ $[M+H]^+$, 1393.69. Found $[M+H]^+$, 1393.72.

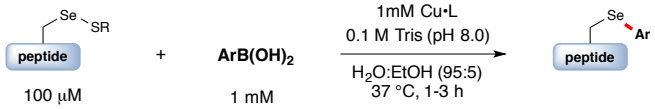
TIC trace of peptide 9



Mass Spectrum of peptide 9



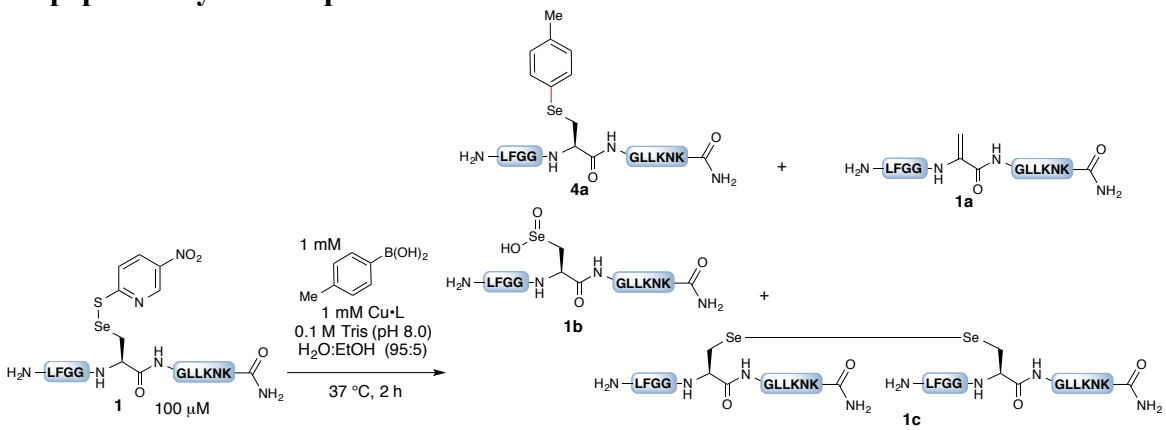
General Procedure (A) for the Synthesis of Arylated Selenocysteine



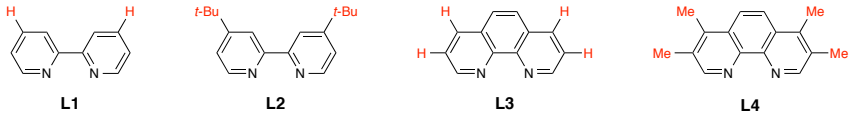
A 0.6 mL Eppendorf tube was charged with 75 μL of deionized H_2O, 10 μL of 1.0 M Tris Buffer (pH = 8.0), 10 μL of peptide (1 mM stock solution in H_2O). A separate 1.7 mL Eppendorf tube was charged with copper (20 μmol), ligand (20 μmol), arylboronic acid (20 μmol), and 1 mL or 0.5 mL of 200 proof EtOH (making a 20 or 40 mM stock solution, respectively). The heterogeneous solution was subjected to sonication for 1 min, vortexed for 30 sec, and 5 μL of the resulting solution was added to the peptide solution in the 0.6 mL Eppendorf tube. The resulting reaction mixture was capped, vortexed for 30 seconds, and placed in a 37 °C water bath for the indicated time (1-3 h). The reaction mixture was quenched with 5 μL of EDTA (200 mM in H_2O) and 100 μL of 50% A: 50% B. The quenched reaction mixture was subjected to LC-MS analysis.

Optimization Table

Selenopeptide Arylation Optimization



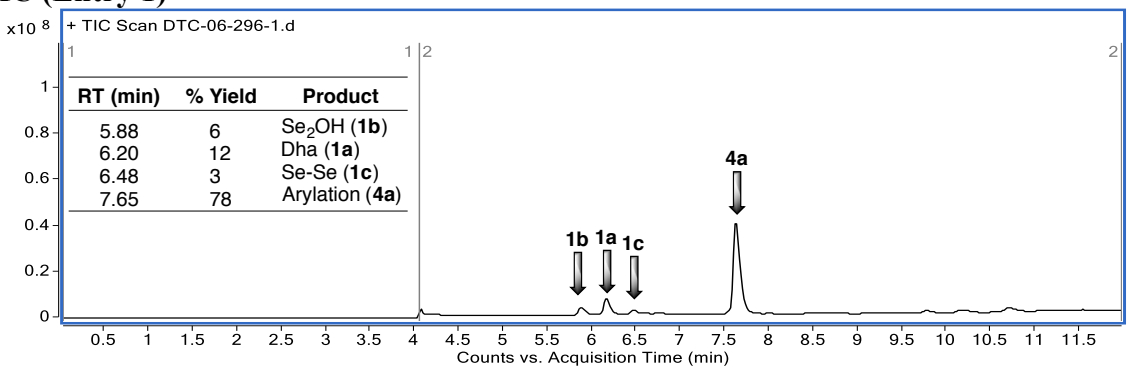
entry	copper (Cu)	ligand (L)	% conversion[b]	% yield of **4a**[a]	% yield of **1a**[a]	% yield of **1b**[a]	% yield of **1c**[a]
1	$CuSO_4$	**L1**	100	78	12	6	3
2	$CuSO_4$	**L2**	100	82	10	6	3
3	$CuSO_4$	**L3**	100	66	19	12	4
4	$CuSO_4$	**L4**	91	17	30	27	17
5[b]	$CuSO_4$	–	30	5	9	8	9
6	$CuCl_2 \cdot 2H_2O$	**L2**	100	78	13	6	2
7	$CuBr_2$	**L2**	100	79	13	6	2
8	$Cu(OAc)_2$	**L2**	100	78	14	6	2
9[c]	–	**L2**	22	0	5	0	16
10[d]	$CuSO_4$	**L2**	73	44	13	8	8
11[e]	$CuSO_4$	**L2**	28	7	8	3	10



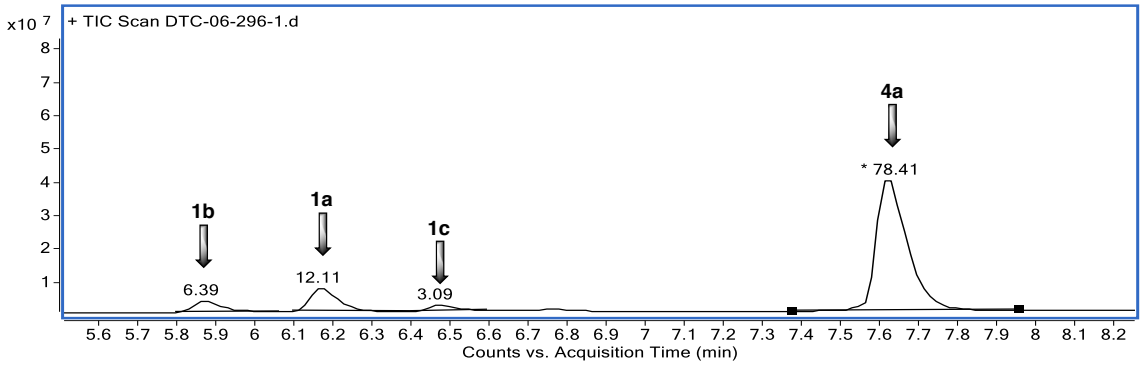
[a]Conversion and yields were determined by measuring the total ion currents (TIC) of LC-MS using *Method A*. [b]Reaction run without any ligand. [c]Reaction run without any copper. [d]0.5 mM $CuSO_4$, 0.5 mM **L2**, and 0.5 mM boronic acid were used. [e]0.25 mM $CuSO_4$, 0.25 mM **L2**, and 0.25 mM boronic acid were used.

Entry 1

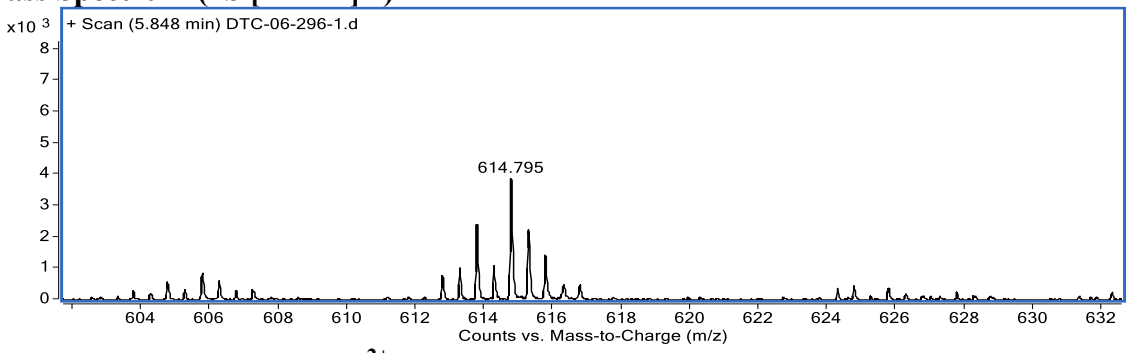
TIC (Entry 1)



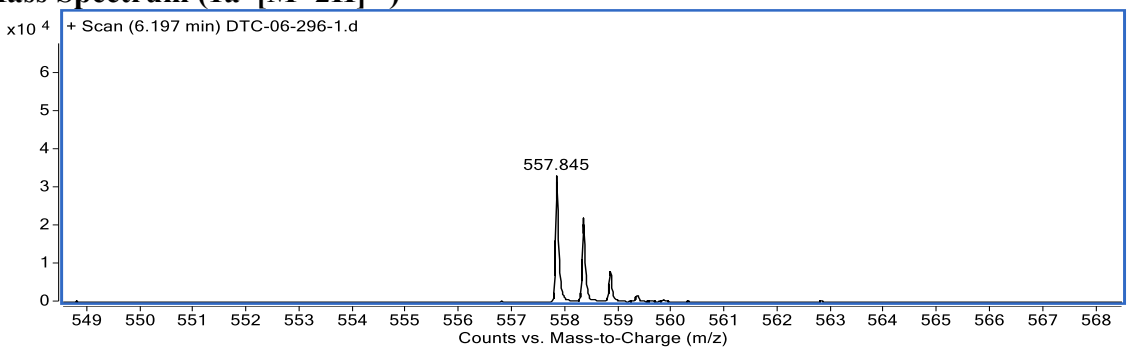
Expanded TIC with Integration (Entry 1)



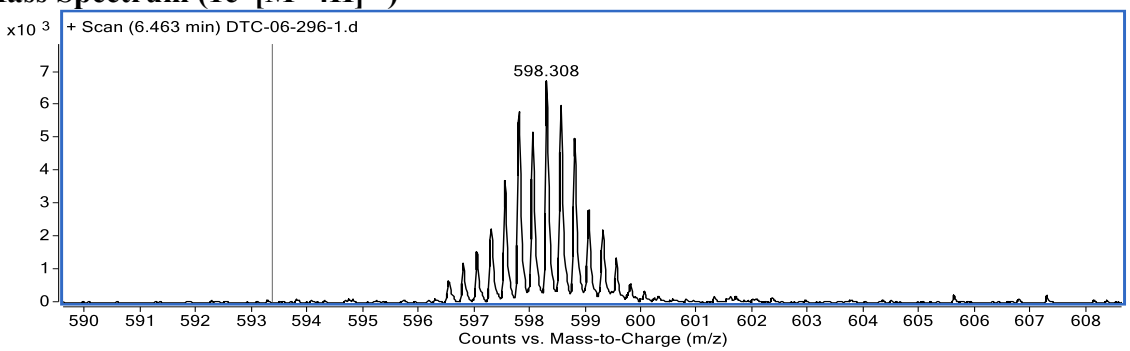
Mass Spectrum (1b $[M+2H]^{2+}$)



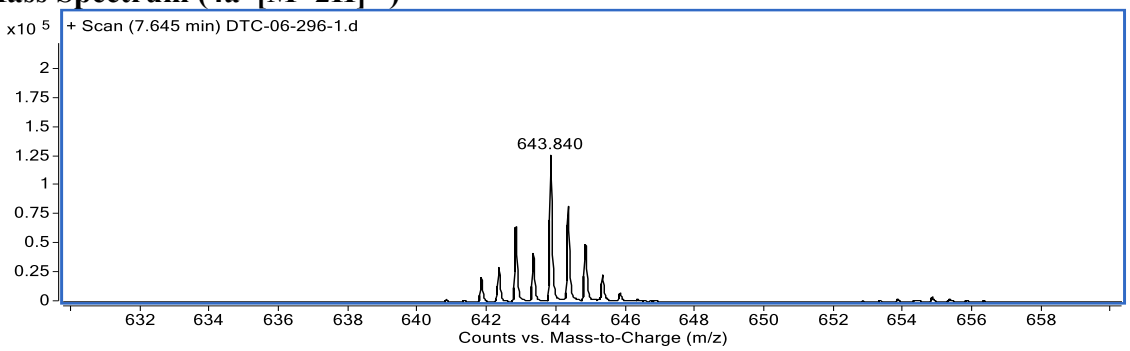
Mass Spectrum (1a $[M+2H]^{2+}$)



Mass Spectrum (1c [M+4H]$^{4+}$)

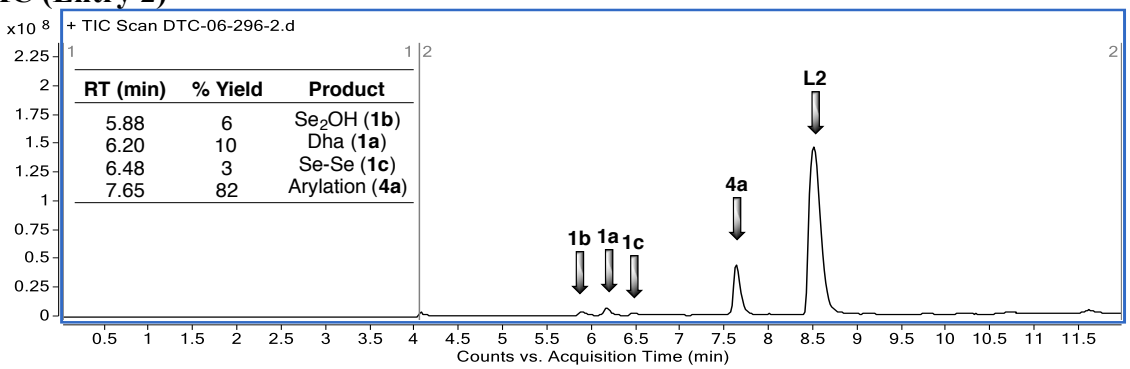


Mass Spectrum (4a [M+2H]$^{2+}$)

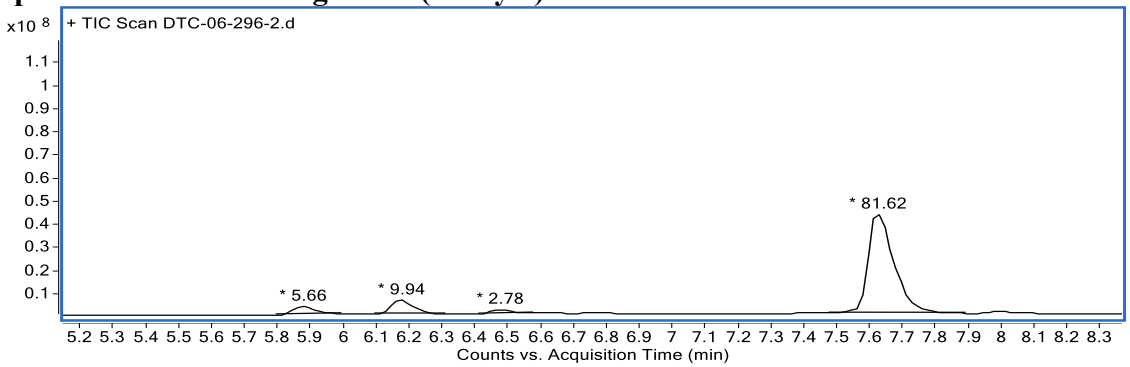


Entry 2

TIC (Entry 2)

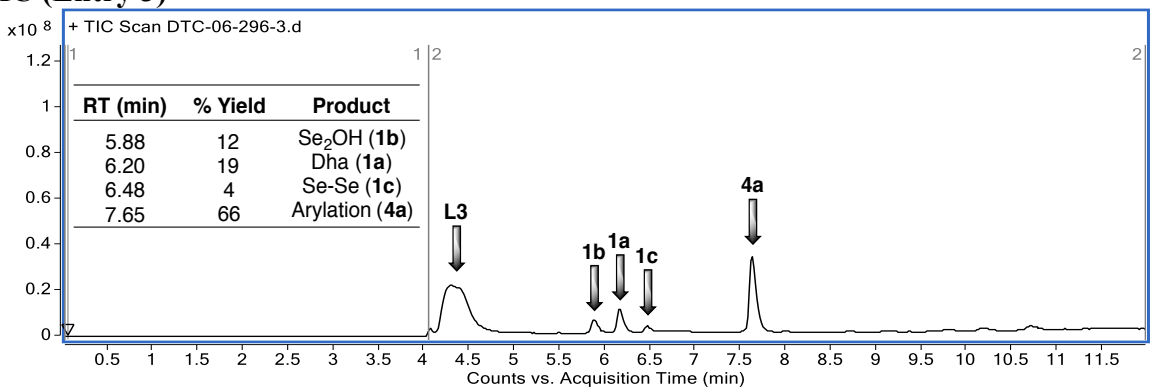


Expanded TIC with Integration (Entry 2)

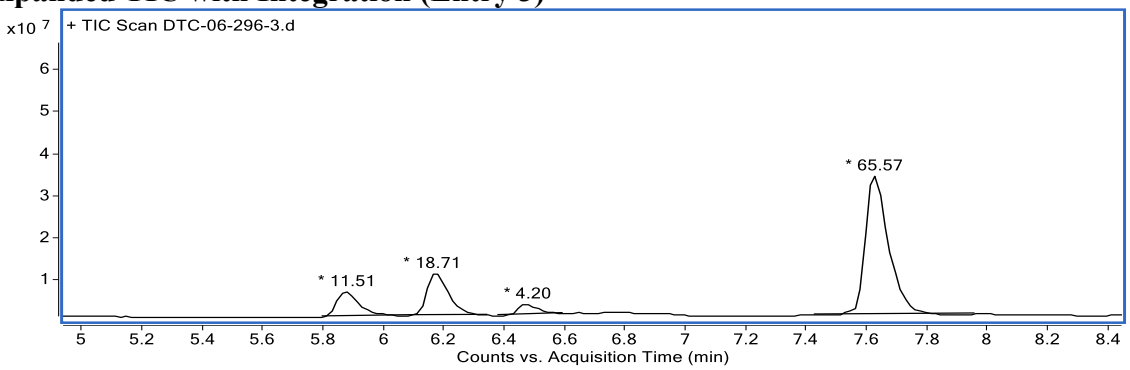


Entry 3

TIC (Entry 3)

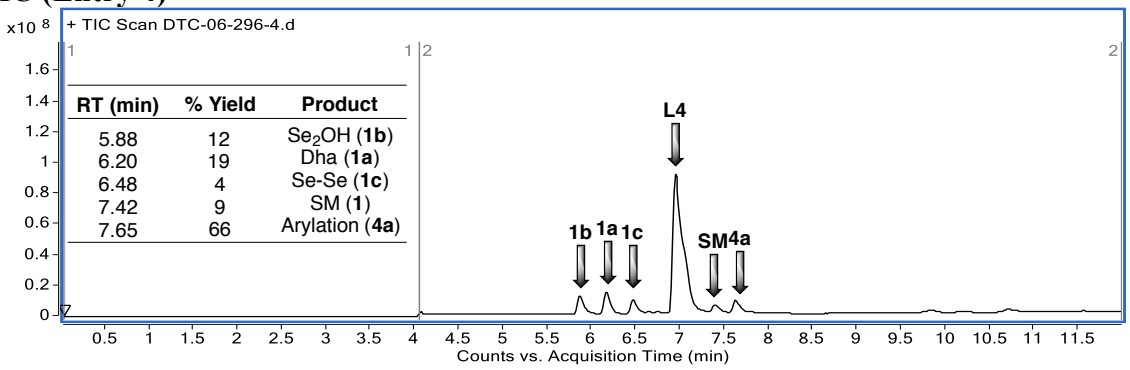


Expanded TIC with Integration (Entry 3)

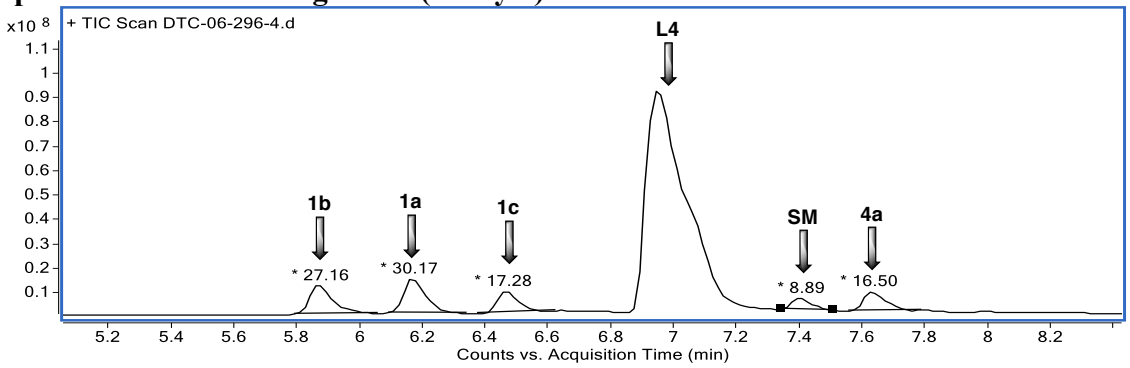


Entry 4

TIC (Entry 4)

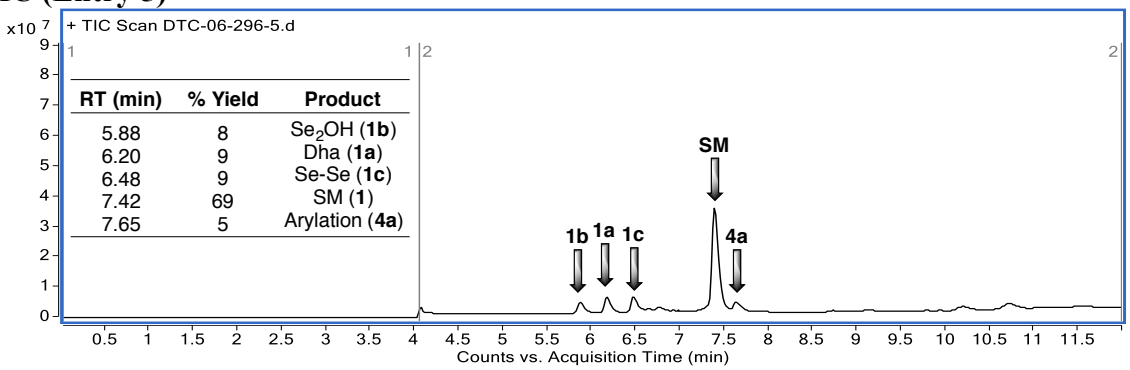


Expanded TIC with Integration (Entry 4)



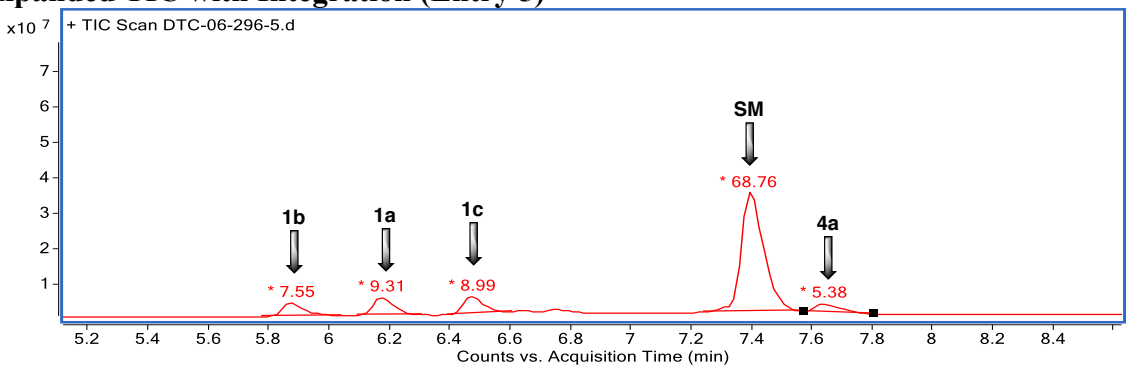
Entry 5

TIC (Entry 5)



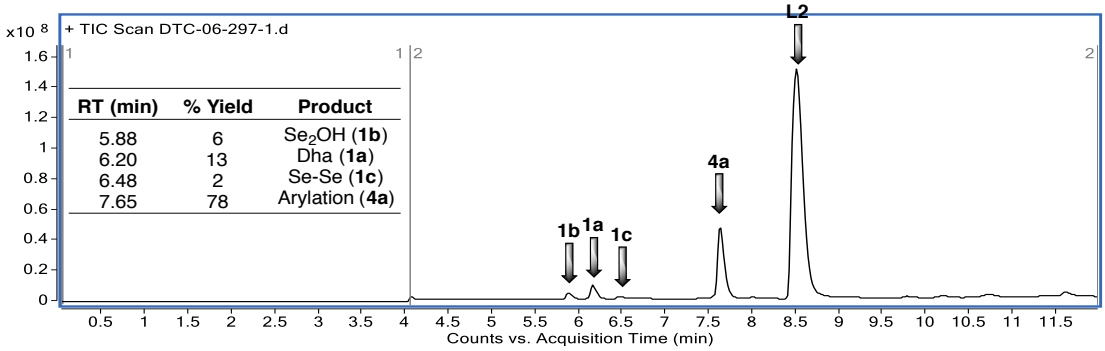
RT (min)	% Yield	Product
5.88	8	Se_2OH (**1b**)
6.20	9	Dha (**1a**)
6.48	9	Se-Se (**1c**)
7.42	69	SM (**1**)
7.65	5	Arylation (**4a**)

Expanded TIC with Integration (Entry 5)



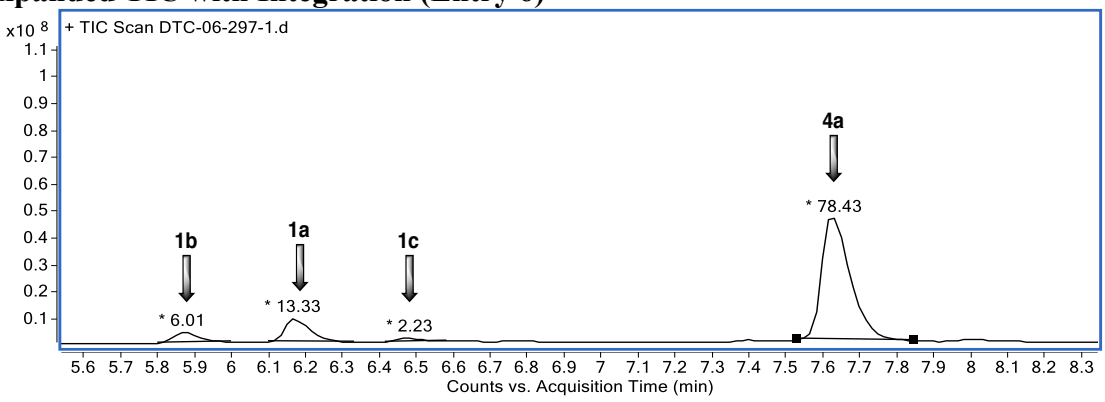
Entry 6

TIC (Entry 6)



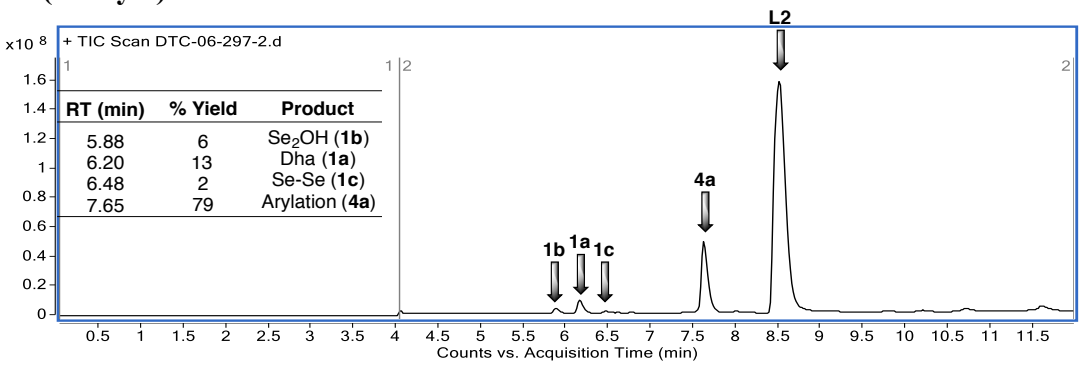
RT (min)	% Yield	Product
5.88	6	Se_2OH (**1b**)
6.20	13	Dha (**1a**)
6.48	2	Se-Se (**1c**)
7.65	78	Arylation (**4a**)

Expanded TIC with Integration (Entry 6)

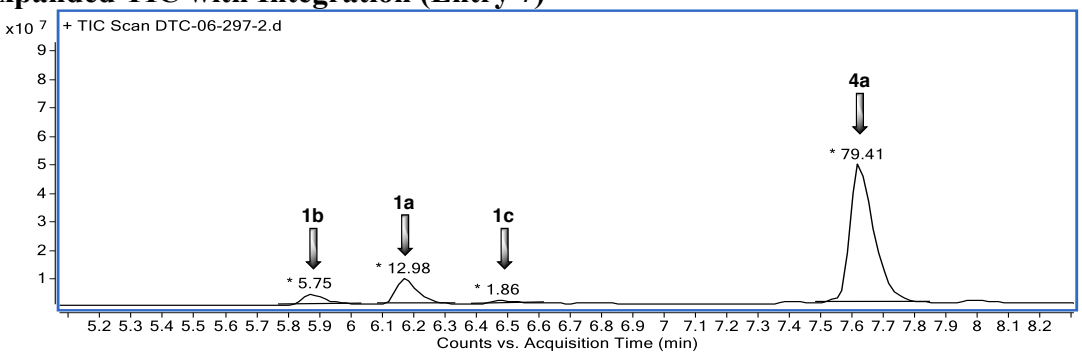


Entry 7

TIC (Entry 7)

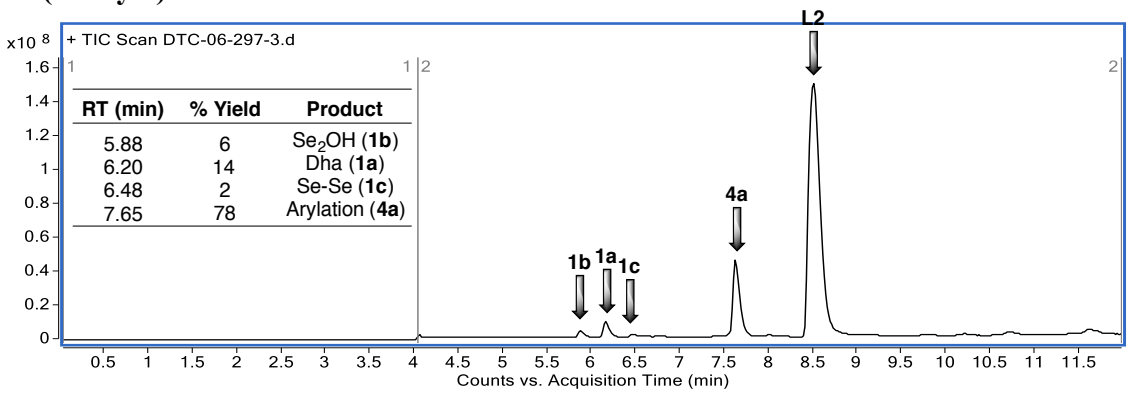


Expanded TIC with Integration (Entry 7)

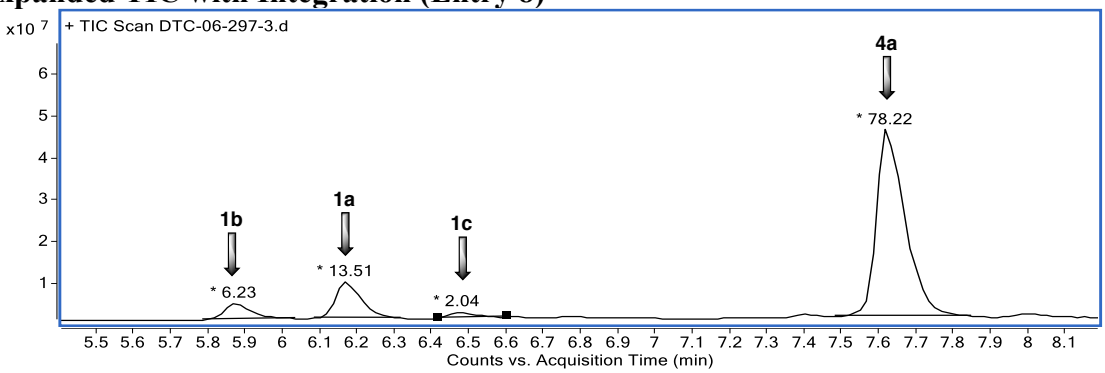


Entry 8

TIC (Entry 8)

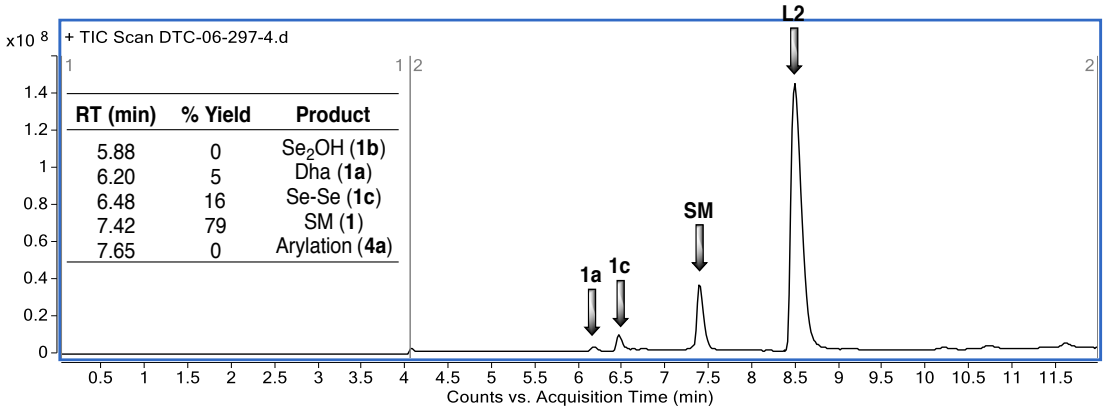


Expanded TIC with Integration (Entry 8)



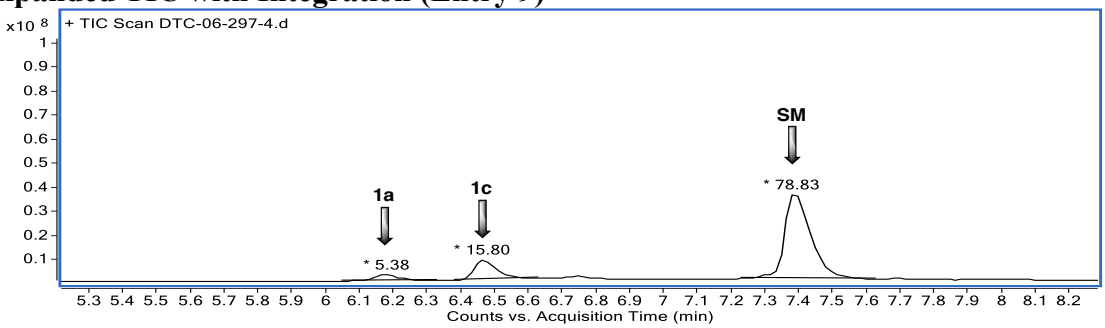
Entry 9

TIC (Entry 9)



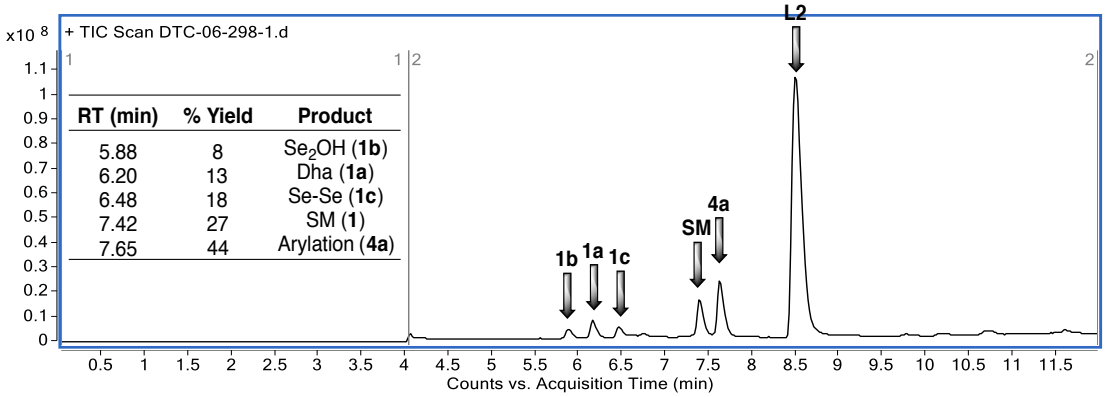
RT (min)	% Yield	Product
5.88	0	Se_2OH (**1b**)
6.20	5	Dha (**1a**)
6.48	16	Se-Se (**1c**)
7.42	79	SM (**1**)
7.65	0	Arylation (**4a**)

Expanded TIC with Integration (Entry 9)



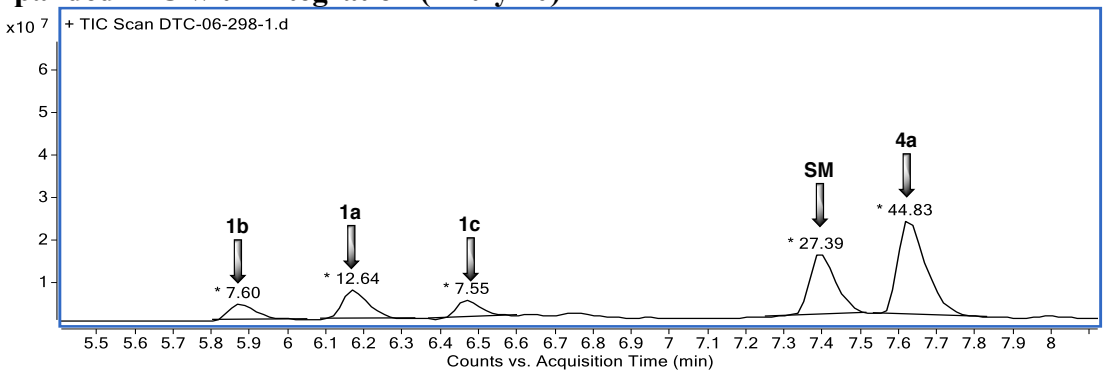
Entry 10

TIC (Entry 10)



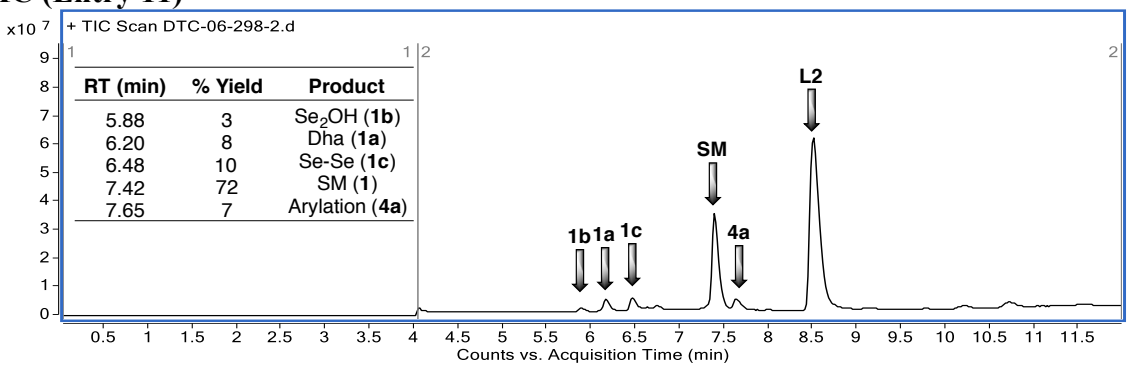
RT (min)	% Yield	Product
5.88	8	Se_2OH (**1b**)
6.20	13	Dha (**1a**)
6.48	18	Se-Se (**1c**)
7.42	27	SM (**1**)
7.65	44	Arylation (**4a**)

Expanded TIC with Integration (Entry 10)

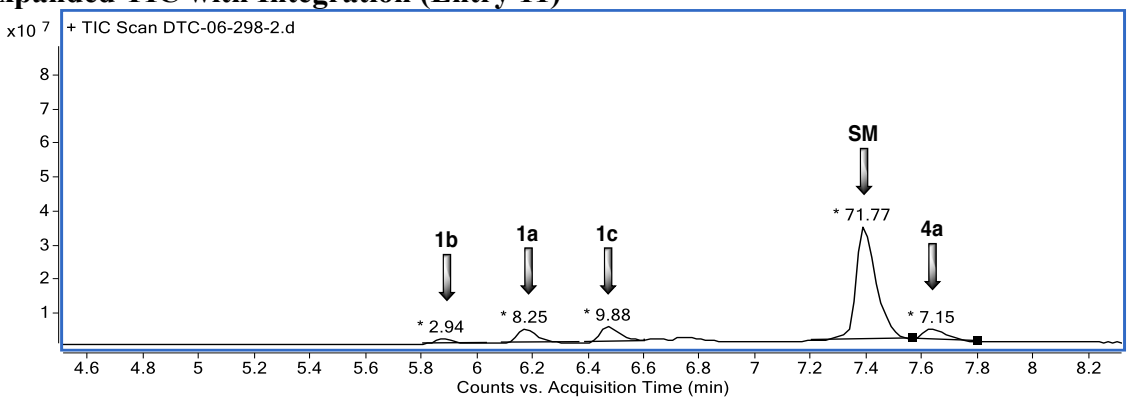


Entry 11

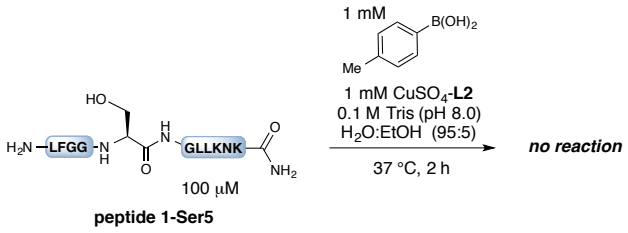
TIC (Entry 11)



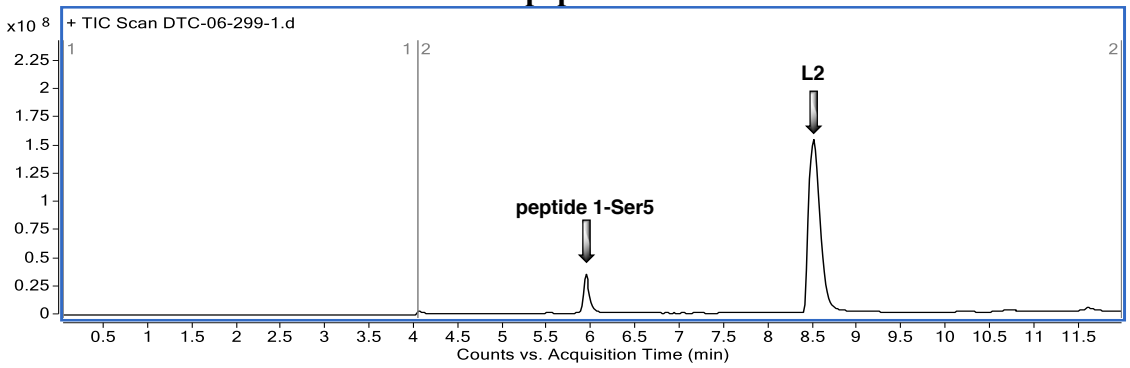
Expanded TIC with Integration (Entry 11)



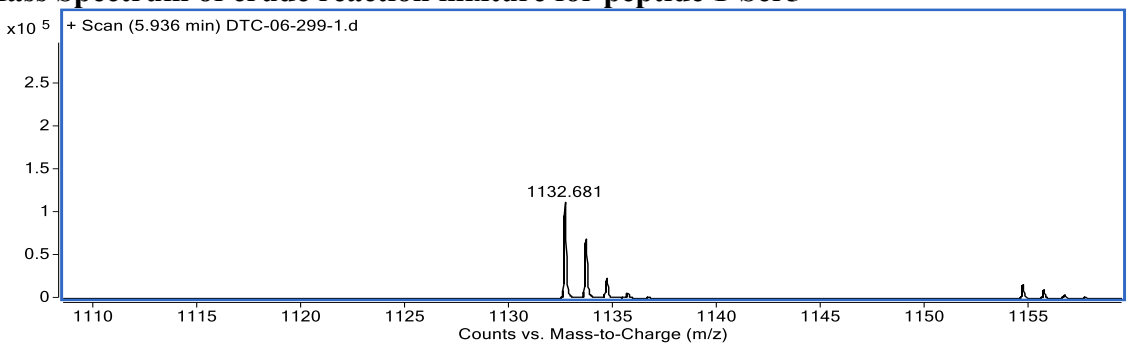
Serine Control Reaction



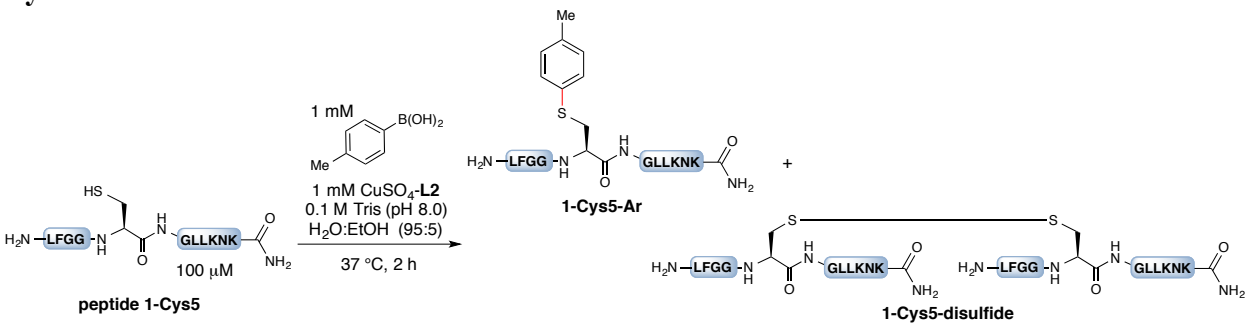
TIC trace of crude reaction mixture for peptide 1-Ser5



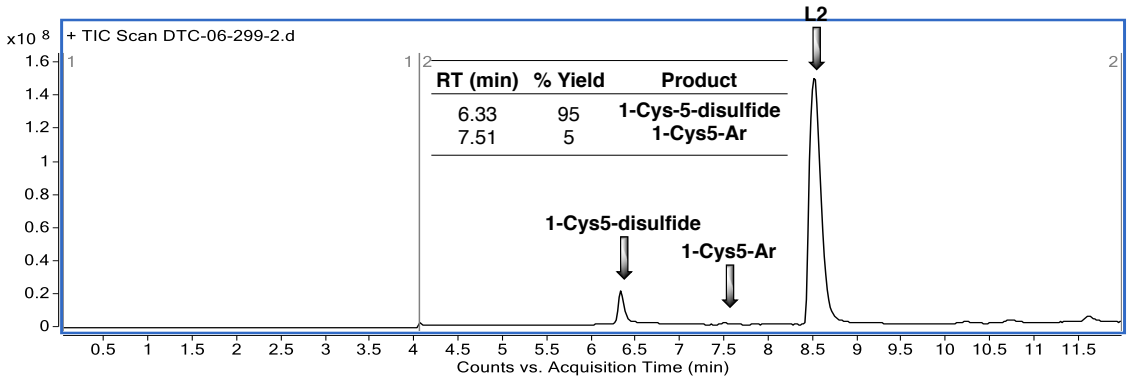
Mass Spectrum of crude reaction mixture for peptide 1-Ser5



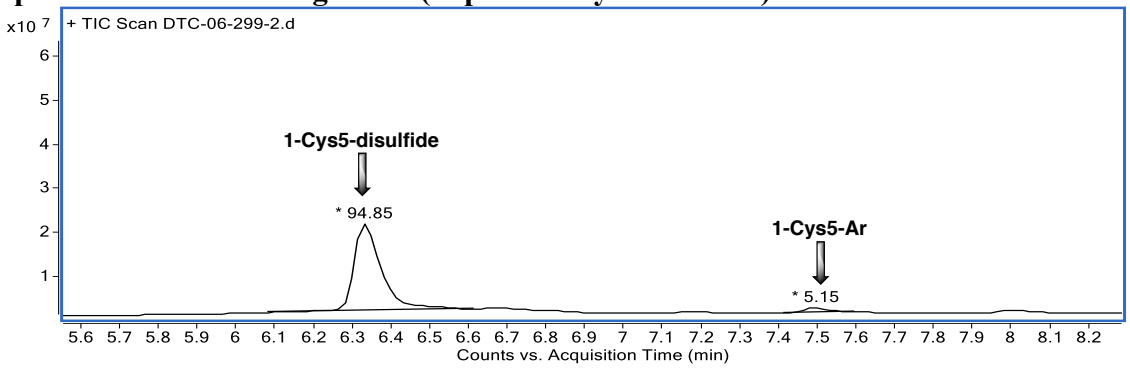
Cysteine Control Reaction



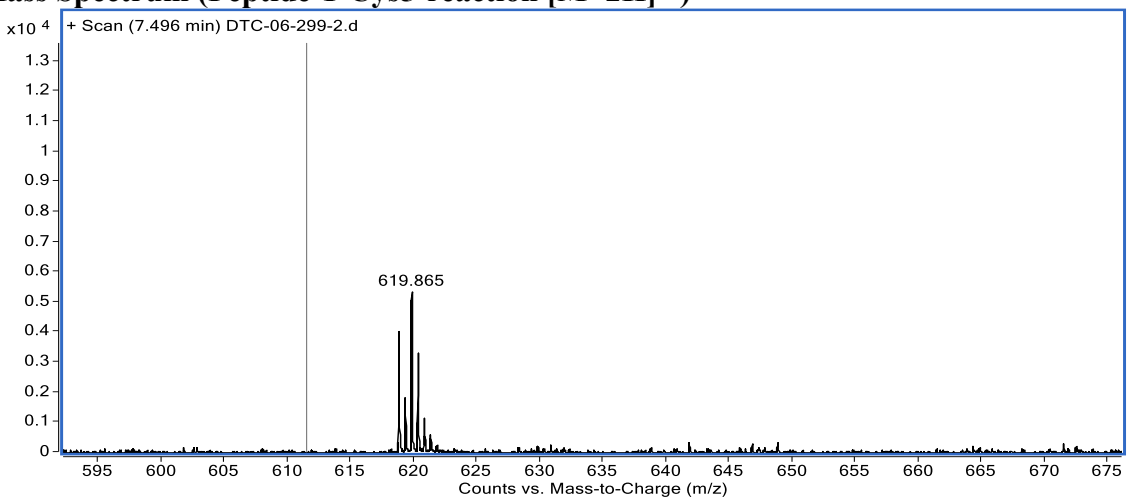
TIC (Peptide-1-Cys5-reaction)



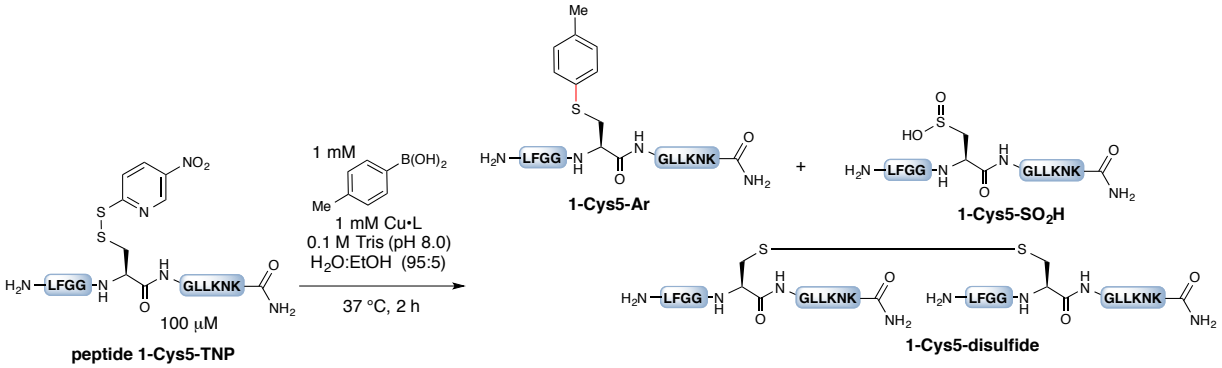
Expanded TIC with Integration (Peptide-1-Cys5-reaction)



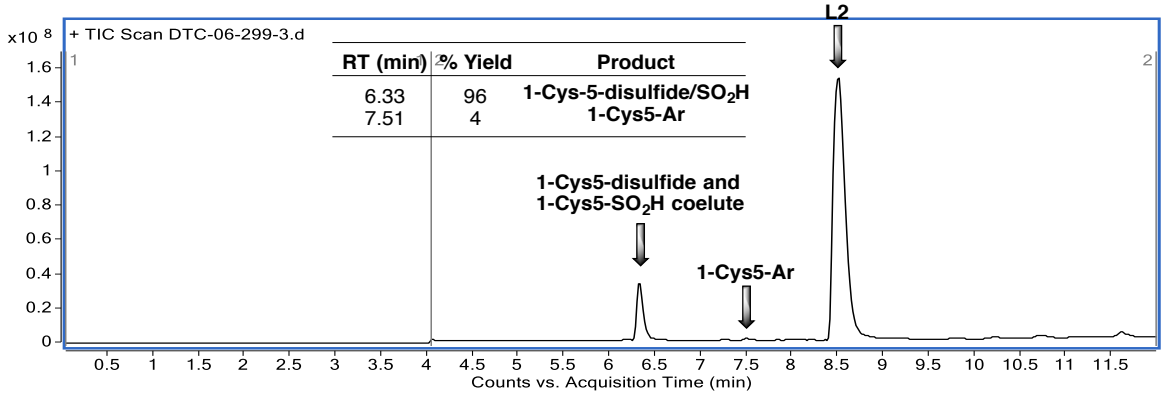
Mass Spectrum (Peptide-1-Cys5-reaction $[M+2H]^{2+}$)



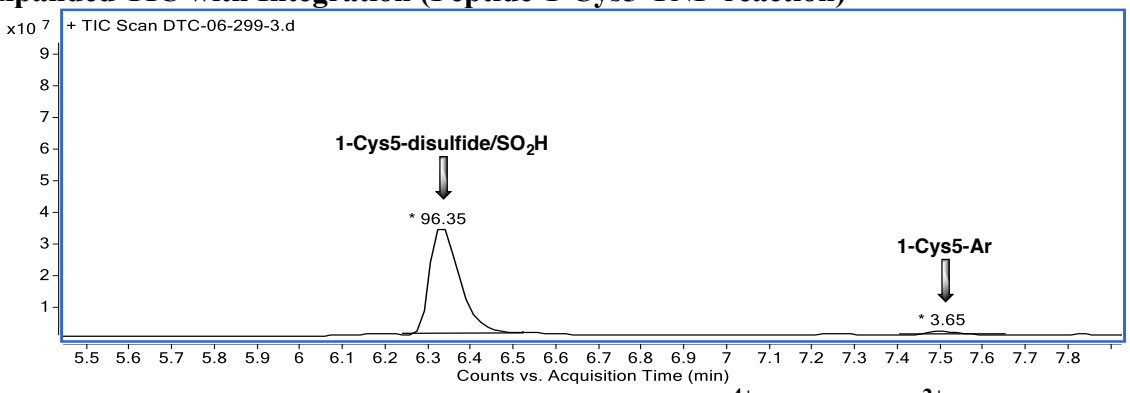
Cysteine-TNP Control Reaction



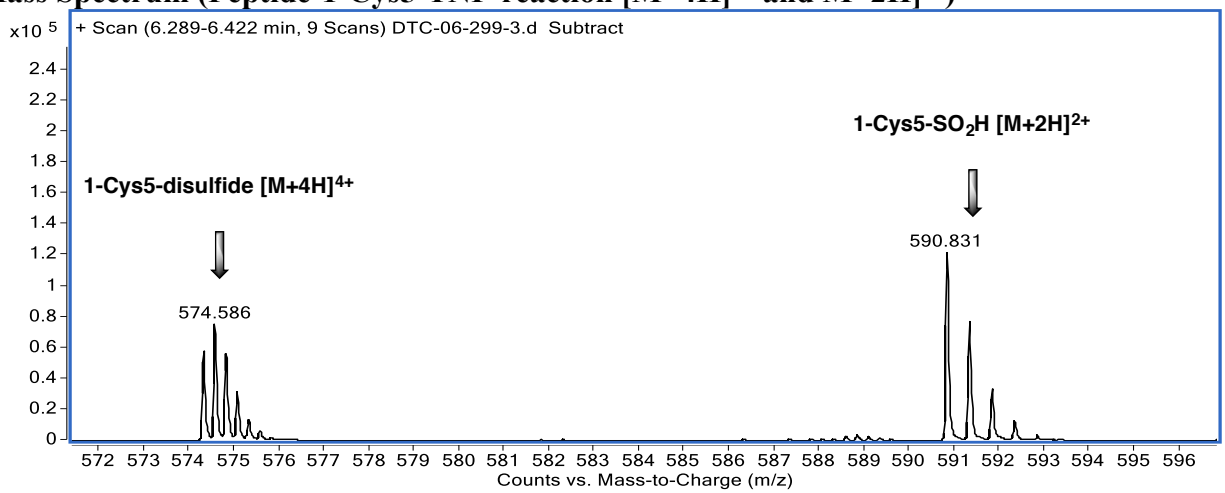
TIC (Peptide-1-Cys5-TNP-reaction)



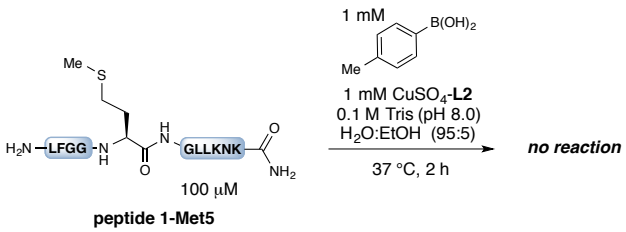
Expanded TIC with Integration (Peptide-1-Cys5-TNP-reaction)



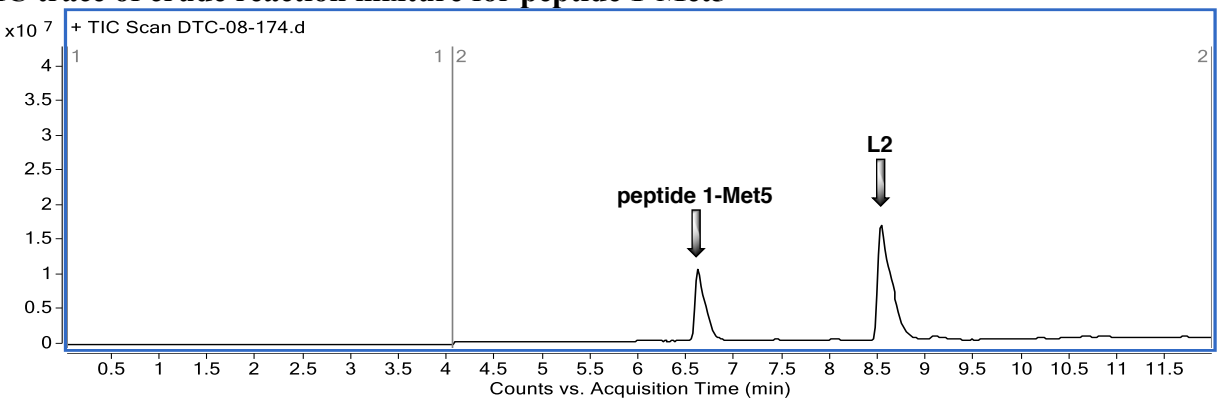
Mass Spectrum (Peptide-1-Cys5-TNP-reaction $[M+4H]^{4+}$ and $M+2H]^{2+}$)



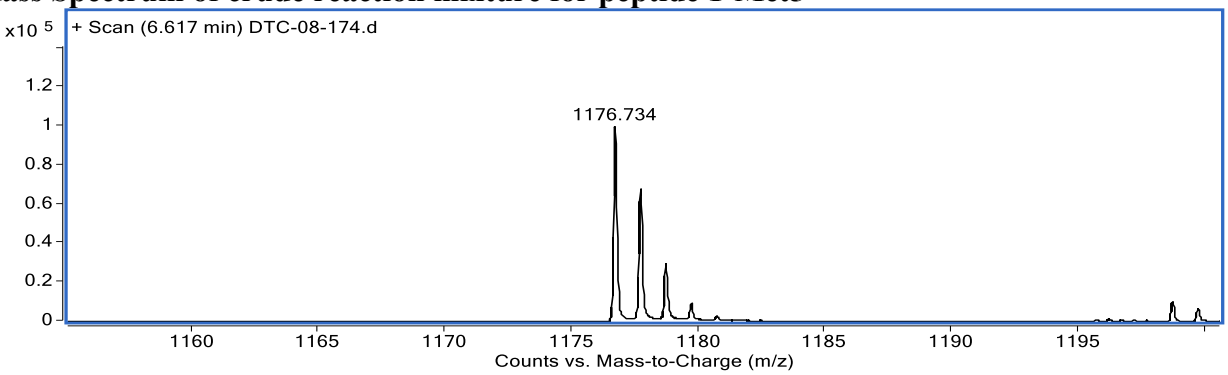
Methionine Control Reaction



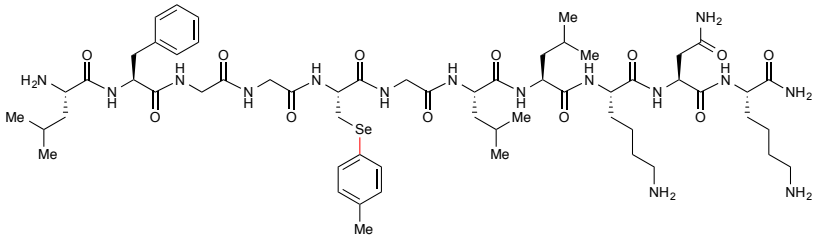
TIC trace of crude reaction mixture for peptide 1-Met5



Mass Spectrum of crude reaction mixture for peptide 1-Met5

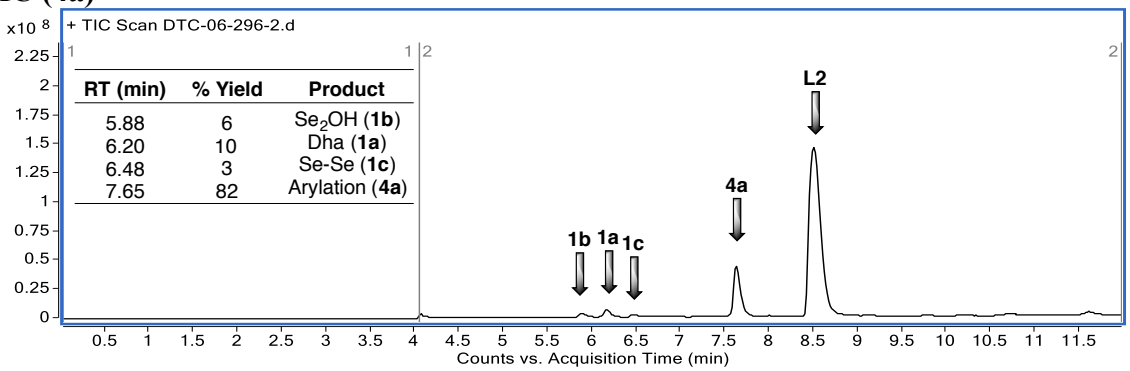


LC-MS Analysis of Arylation Reactions for Substrate Scope

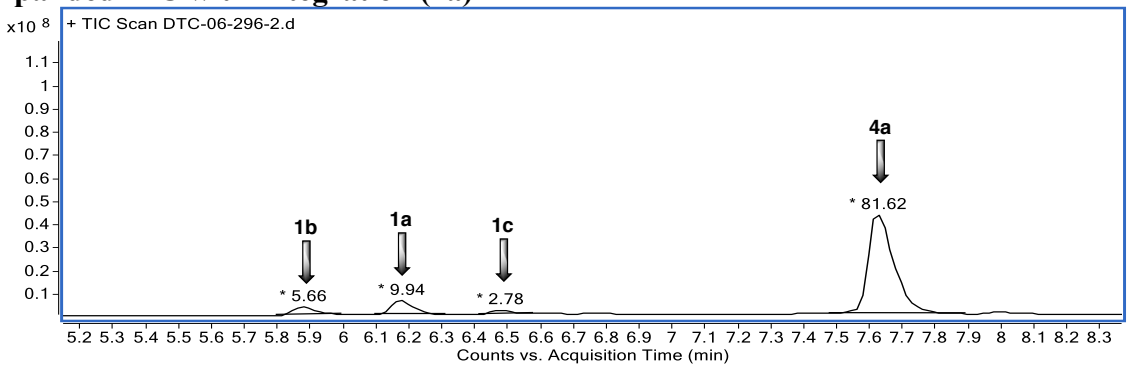


(4a): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and *p*-tolylboronic acid stock solution (1 mM) at 37 °C for 2 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **4a**: HRMS (ESI) Mass. calcd. for $C_{59}H_{97}N_{15}O_{12}Se$ $[M+2H]^{2+}$, 643.83. Found $[M+2H]^{2+}$, 643.84.

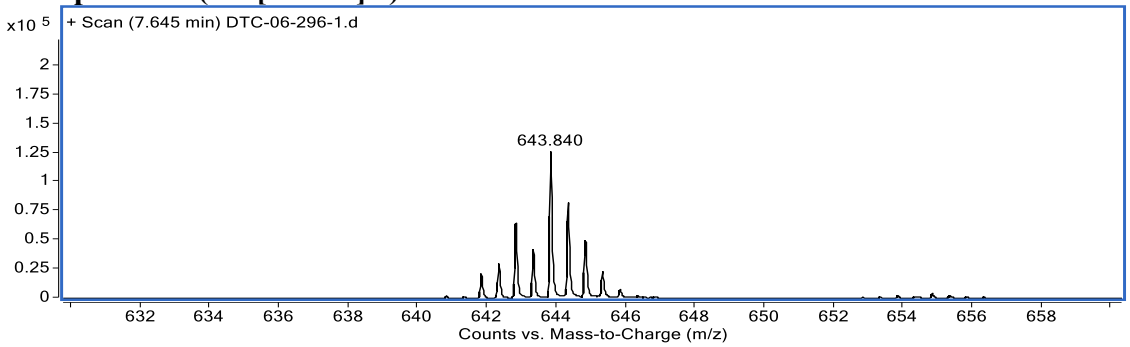
TIC (4a)

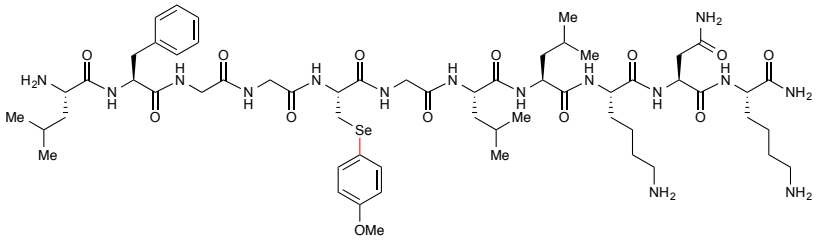


Expanded TIC with Integration (4a)



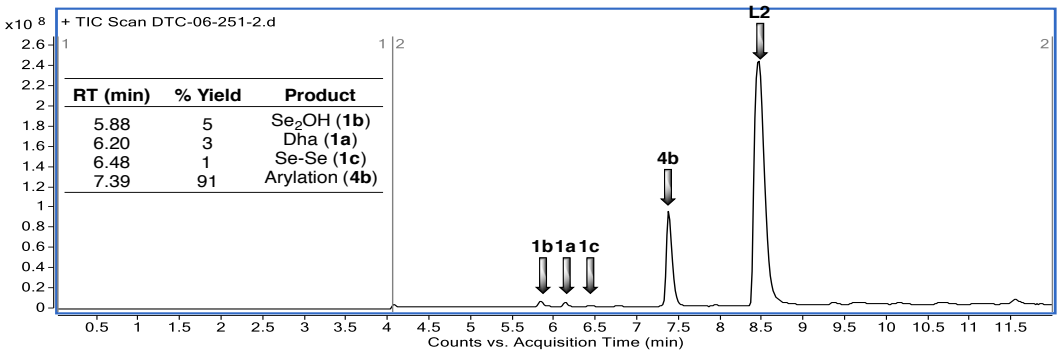
Mass Spectrum (4a $[M+2H]^{2+}$)





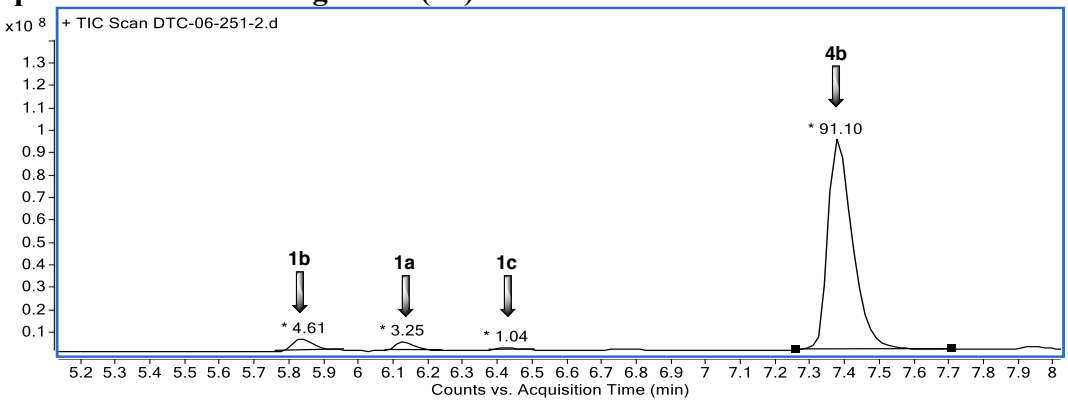
(4b): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (4-methoxyphenyl)boronic acid stock solution (1 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **4b**: HRMS (ESI) Mass. calcd. for $C_{59}H_{97}N_{15}O_{13}Se$ $[M+2H]^{2+}$, 651.83. Found $[M+2H]^{2+}$, 651.84.

TIC (4b)

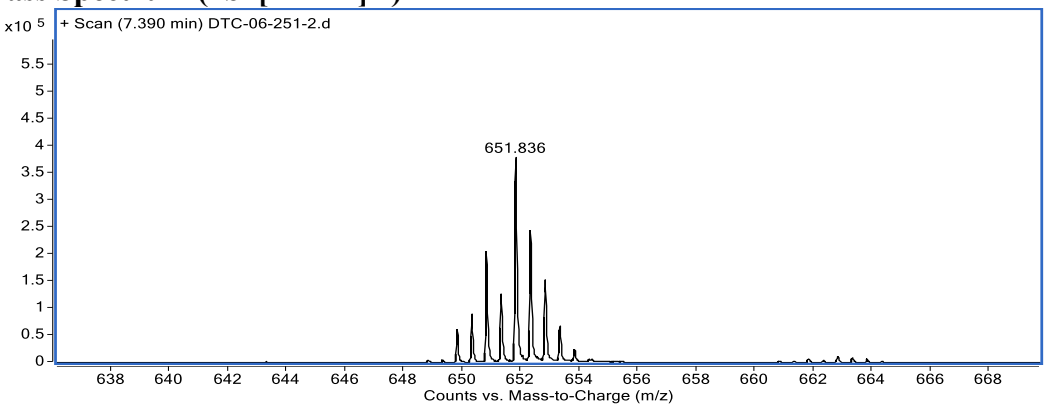


RT (min)	% Yield	Product
5.88	5	Se_2OH (**1b**)
6.20	3	Dha (**1a**)
6.48	1	Se-Se (**1c**)
7.39	91	Arylation (**4b**)

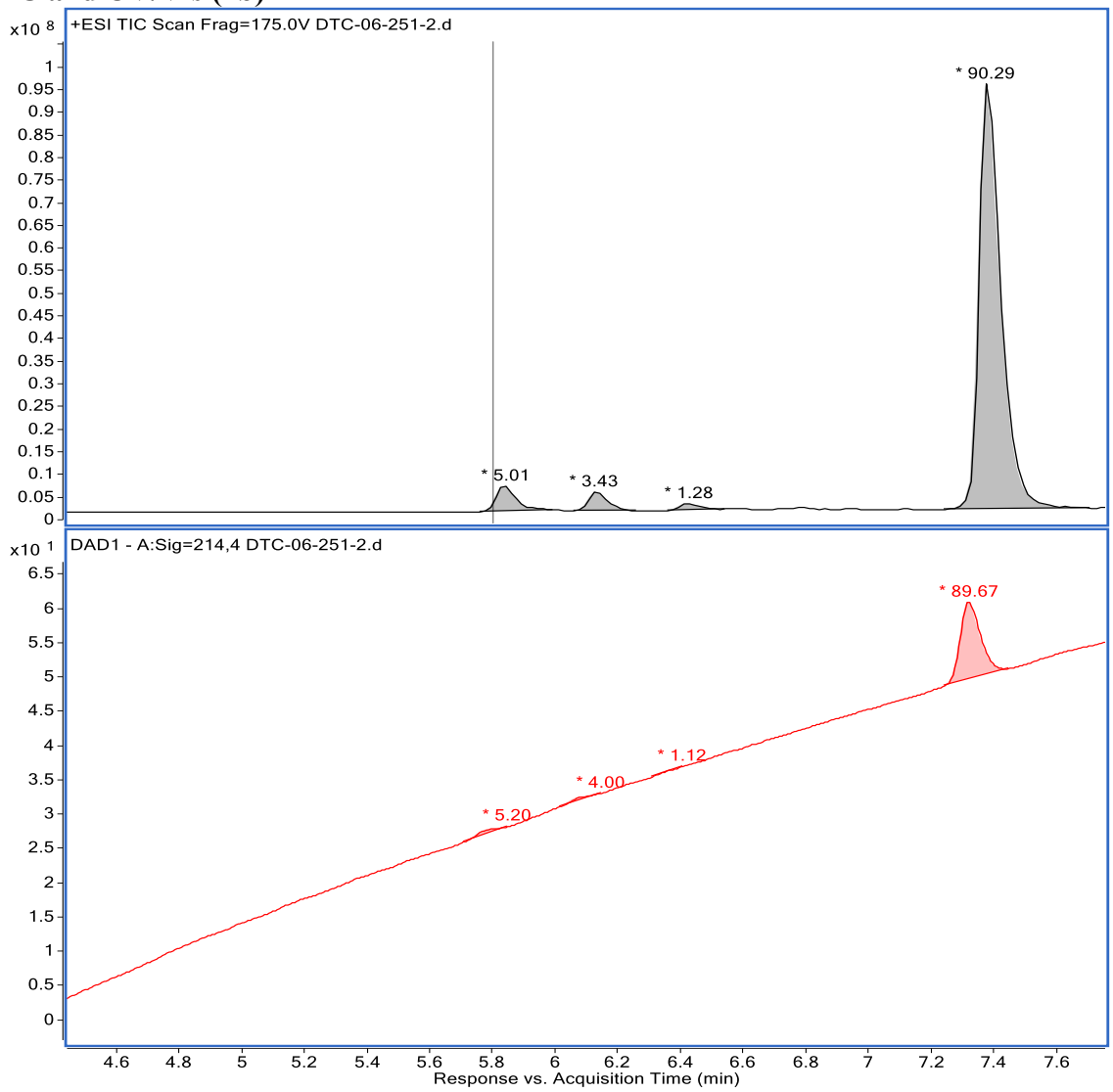
Expanded TIC with Integration (4b)

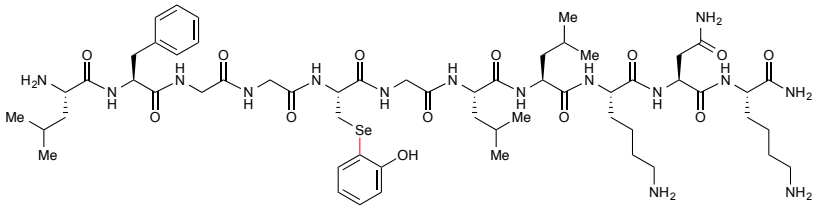


Mass Spectrum (4b $[M+2H]^{2+}$)



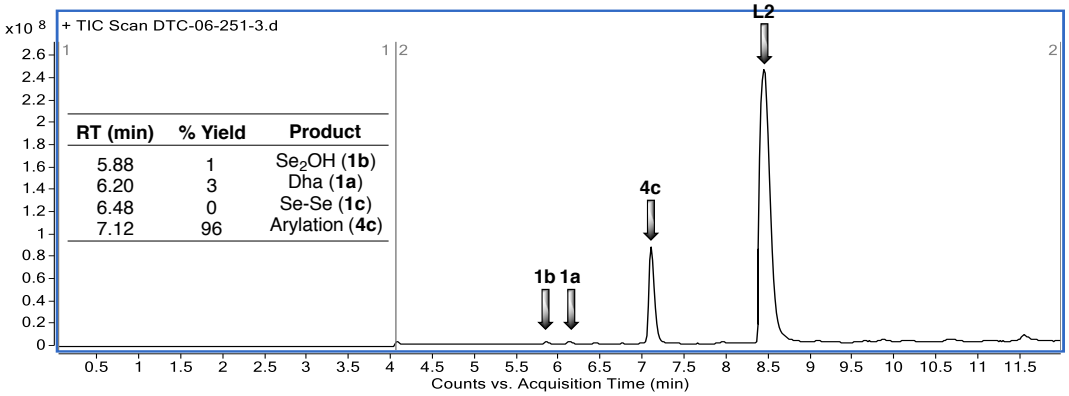
TIC and UV/Vis (4b)





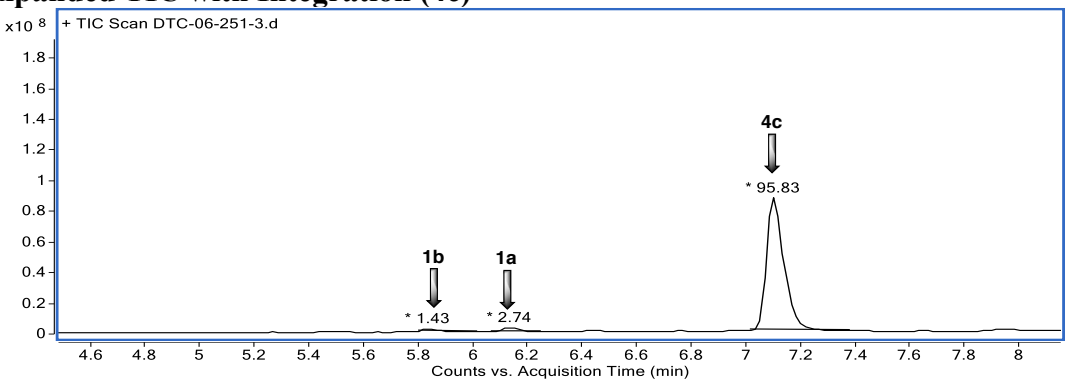
(4c): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (2-hydroxyphenyl)boronic acid stock solution (1 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **4c**: HRMS (ESI) Mass. calcd. for $C_{58}H_{94}N_{15}O_{13}Se$ $[M+H]^+$, 1288.63. Found $[M+H]^+$, 1288.61.

TIC (4c)

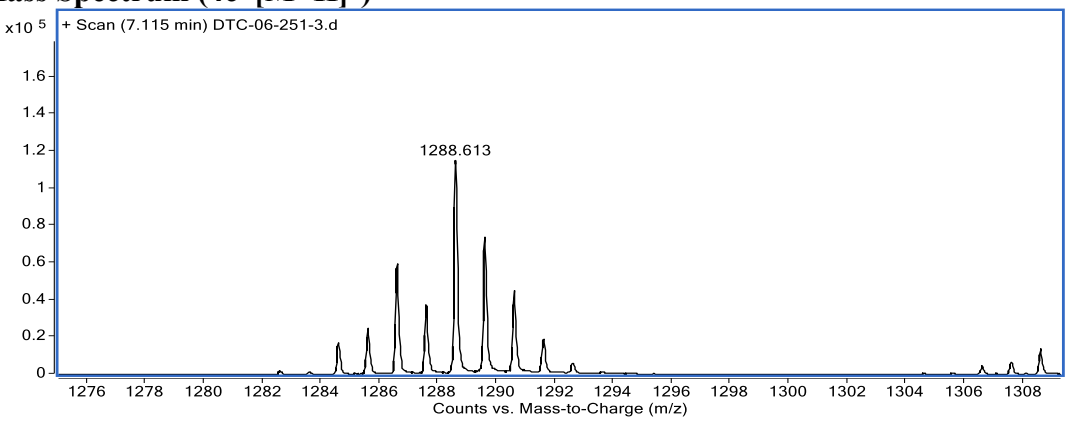


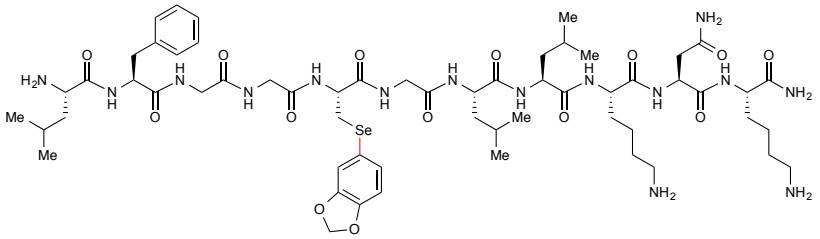
RT (min)	% Yield	Product
5.88	1	Se_2OH (**1b**)
6.20	3	Dha (**1a**)
6.48	0	Se-Se (**1c**)
7.12	96	Arylation (**4c**)

Expanded TIC with Integration (4c)



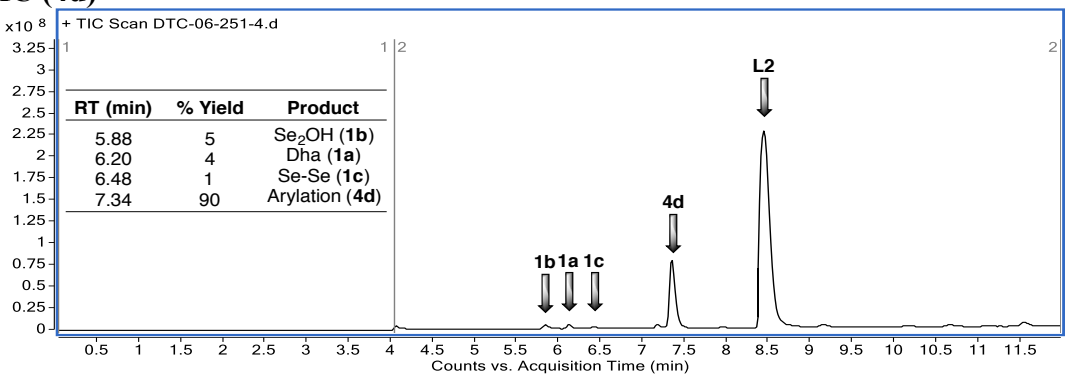
Mass Spectrum (4c $[M+H]^+$)



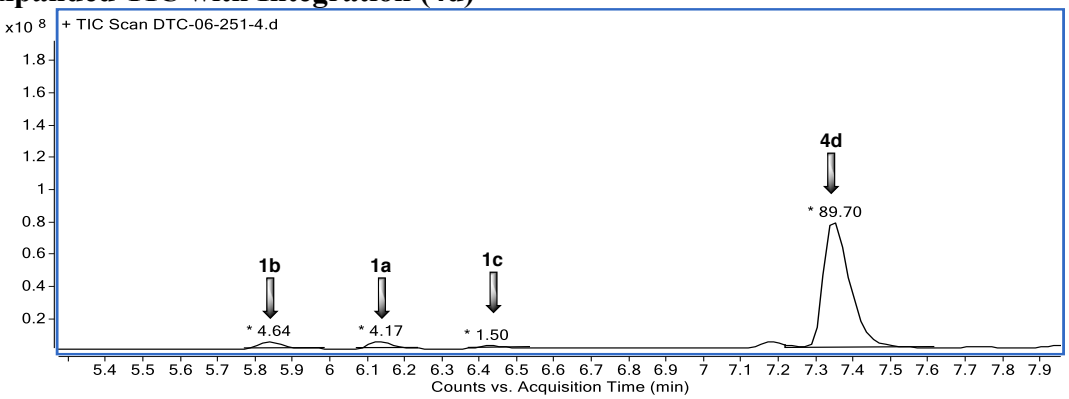


(4d): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and benzo[*d*][1,3]dioxol-5-ylboronic acid stock solution (1 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **4d**: HRMS (ESI) Mass. calcd. for $C_{59}H_{95}N_{15}O_{14}Se$ $[M+2H]^{2+}$, 658.82. Found $[M+2H]^{2+}$, 658.81.

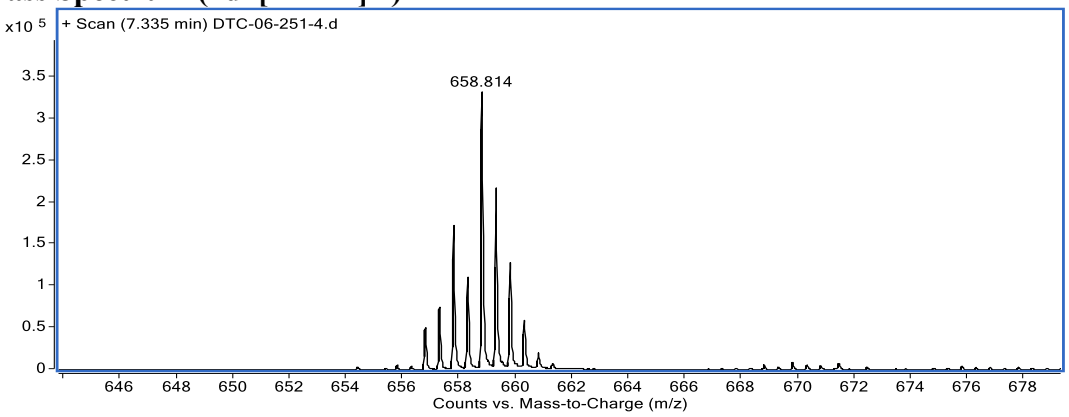
TIC (4d)

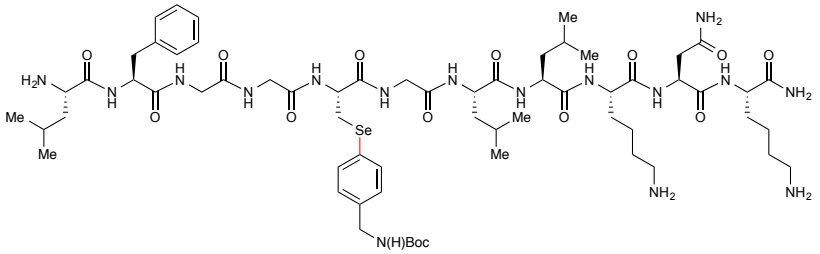


Expanded TIC with Integration (4d)



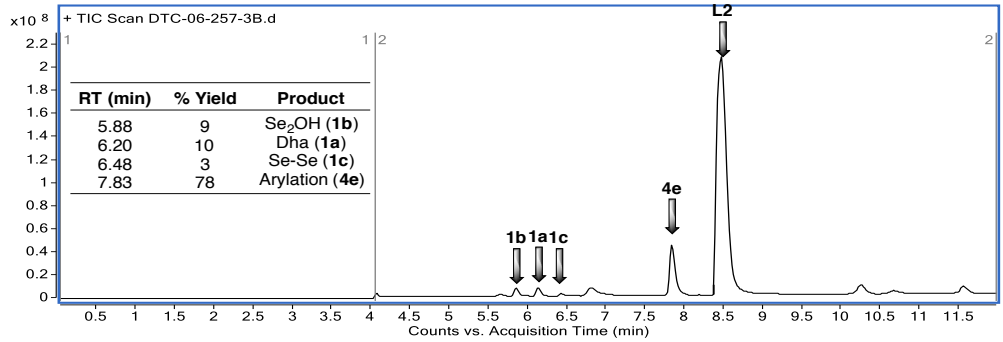
Mass Spectrum (4d $[M+2H]^{2+}$)



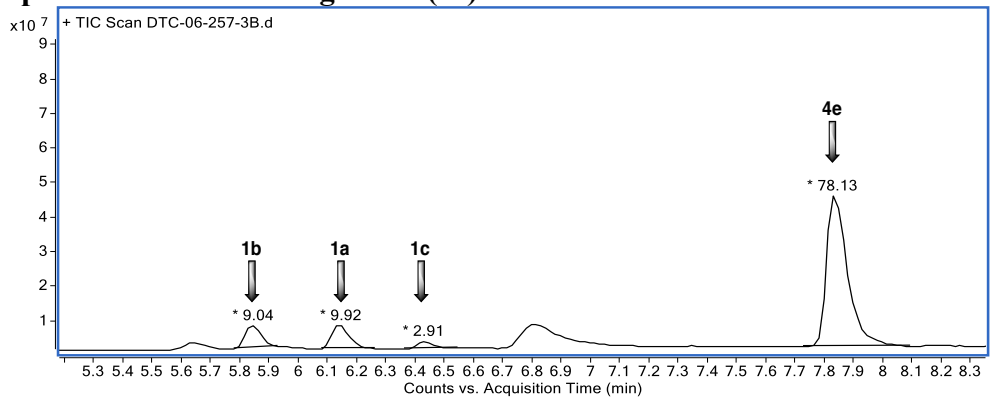


(4e): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (4-(((*tert*-butoxycarbonyl)amino)methyl)phenyl)boronic acid stock solution (1 mM) at 37 °C for 1.5 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **4e**: HRMS (ESI) Mass. calcd. for $C_{64}H_{105}N_{16}O_{14}Se$ $[M+H]^+$, 1401.72. Found $[M+H]^+$, 1401.70.

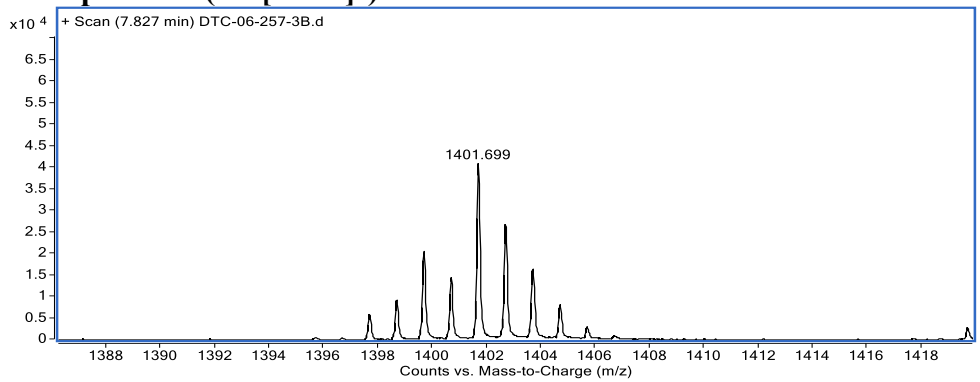
TIC (4e)

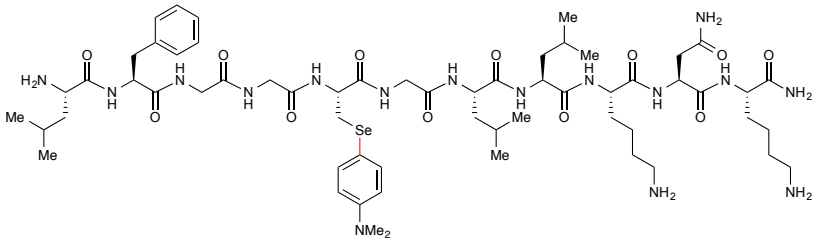


Expanded TIC with Integration (4e)



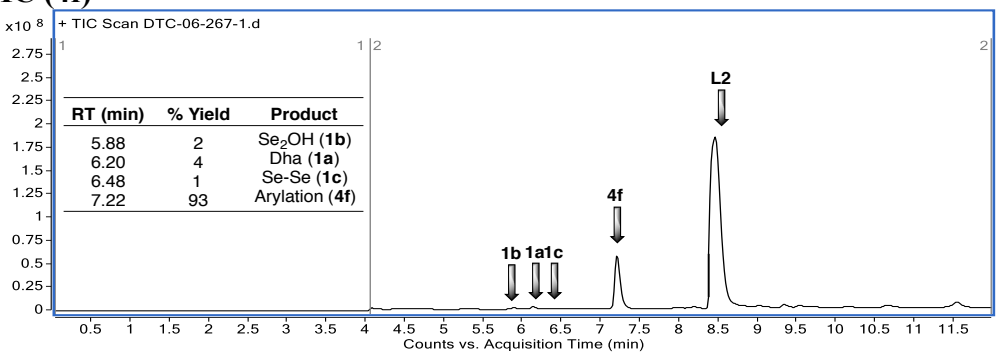
Mass Spectrum (4e $[M+H]^+$)



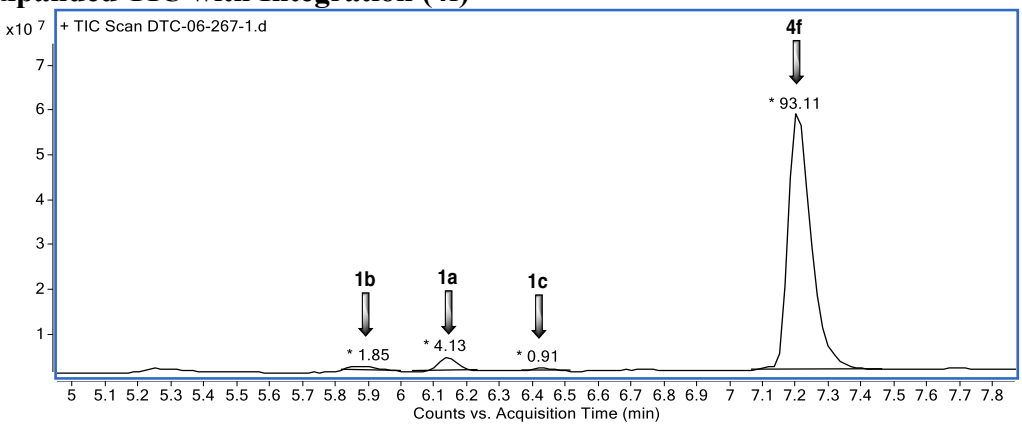


(4f): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (4-(dimethylamino)phenyl)boronic acid stock solution (1 mM) at 37 °C for 2 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **4f**: HRMS (ESI) Mass. calcd. for $C_{60}H_{99}N_{16}O_{12}Se$ $[M+H]^+$, 1315.68. Found $[M+H]^+$, 1315.69.

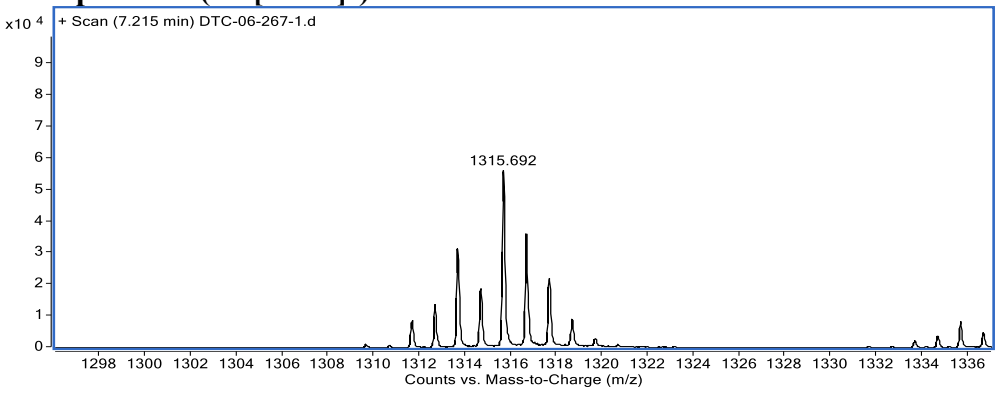
TIC (4f)

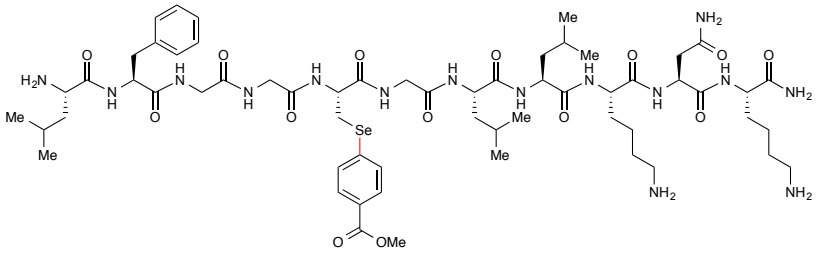


Expanded TIC with Integration (4f)



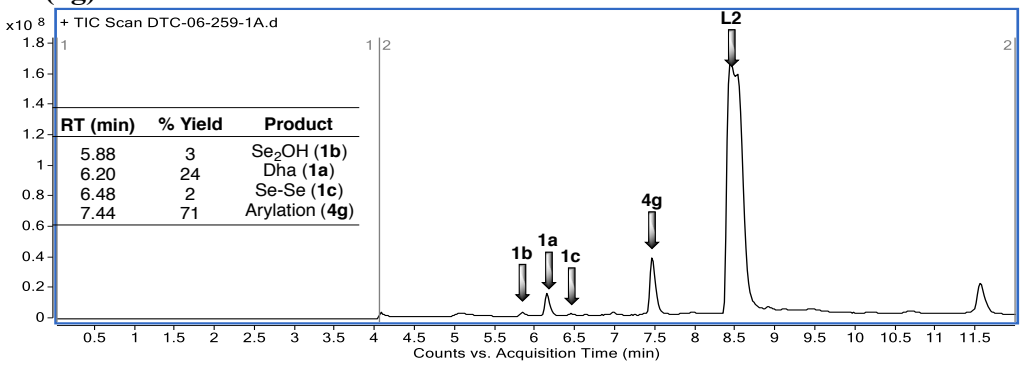
Mass Spectrum (4f $[M+H]^+$)





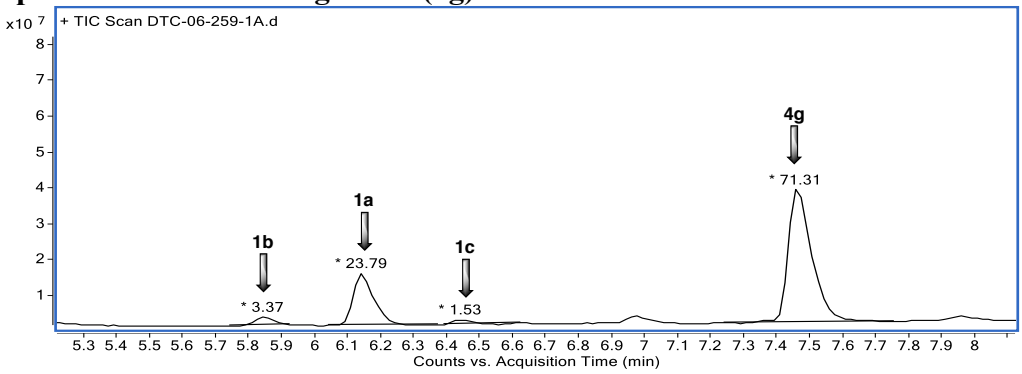
(4g): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (4-(methoxycarbonyl)phenyl)boronic acid stock solution (2 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **4g**: HRMS (ESI) Mass. calcd. for $C_{61}H_{100}N_{16}O_{13}Se$ $[M+2H]^{2+}$, 665.82. Found $[M+2H]^{2+}$, 665.82.

TIC (4g)

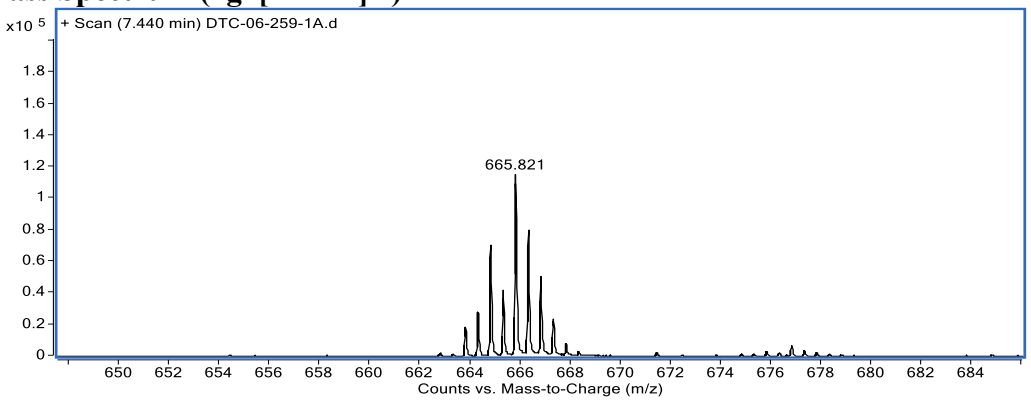


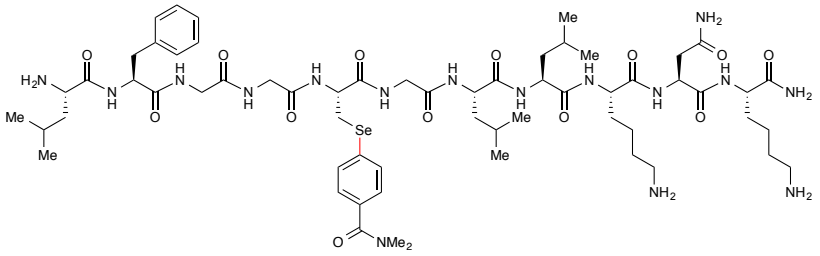
RT (min)	% Yield	Product
5.88	3	Se_2OH (**1b**)
6.20	24	Dha (**1a**)
6.48	2	Se-Se (**1c**)
7.44	71	Arylation (**4g**)

Expanded TIC with Integration (4g)



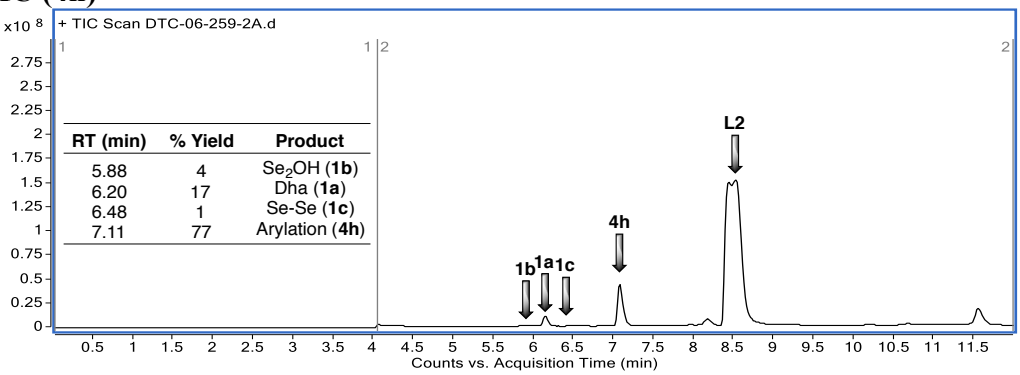
Mass Spectrum (4g $[M+2H]^{2+}$)



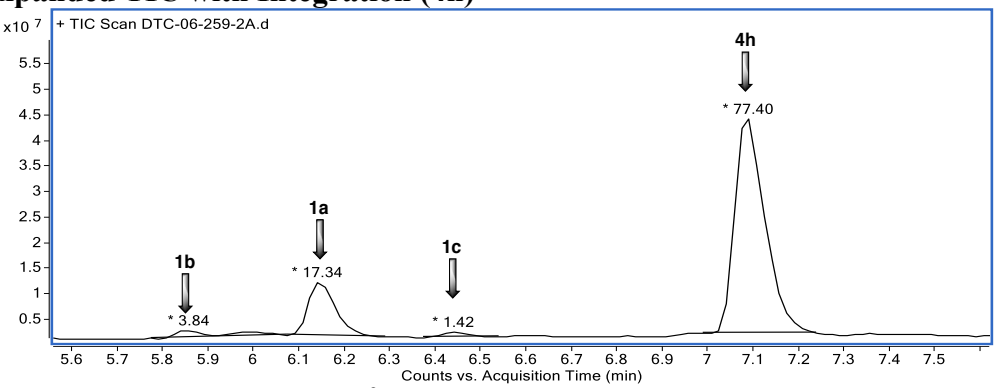


(4h): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (4-(dimethylcarbamoyl)phenyl)boronic acid stock solution (2 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **4h**: HRMS (ESI) Mass. calcd. for $C_{61}H_{100}N_{16}O_{13}Se$ $[M+2H]^{2+}$, 672.34. Found $[M+2H]^{2+}$, 672.35.

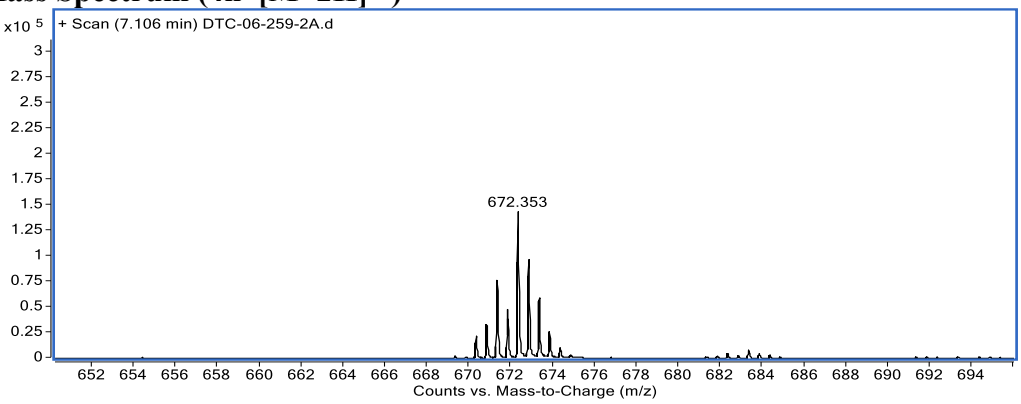
TIC (4h)

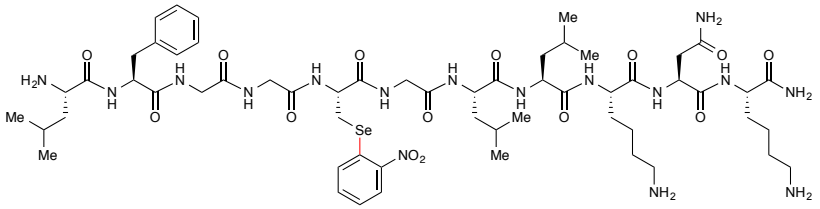


Expanded TIC with Integration (4h)



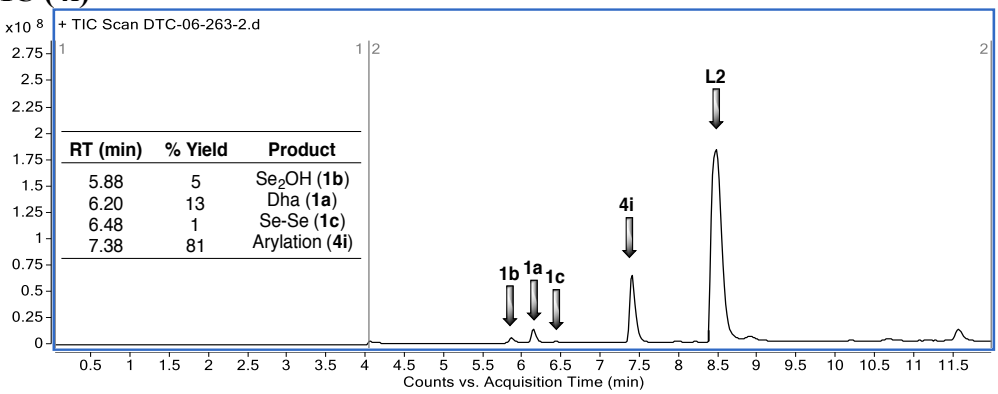
Mass Spectrum (4h $[M+2H]^{2+}$)



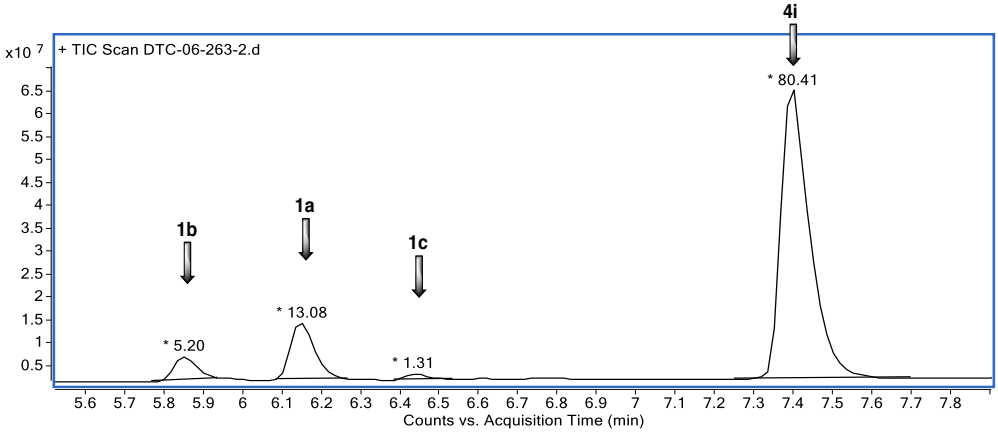


(4i): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (2-nitrophenyl)boronic acid stock solution (1 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **4i**: HRMS (ESI) Mass. calcd. for $C_{58}H_{94}N_{16}O_{14}Se$ $[M+2H]^{2+}$, 659.31. Found $[M+2H]^{2+}$, 659.32.

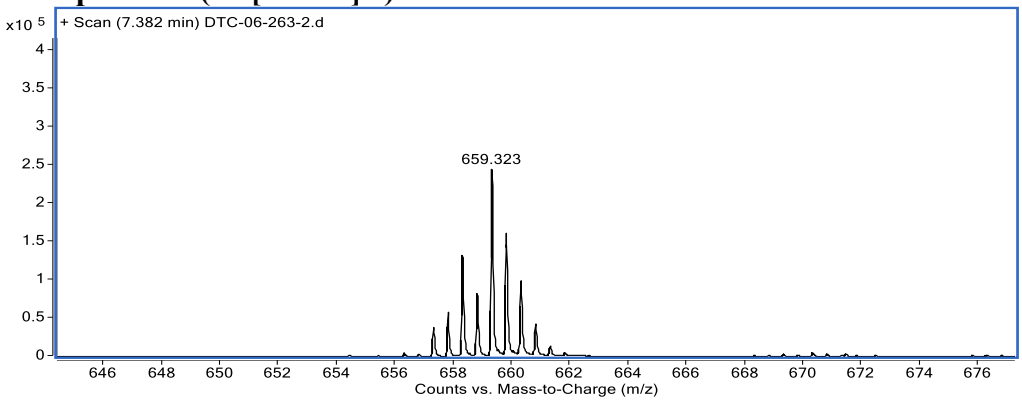
TIC (4i)



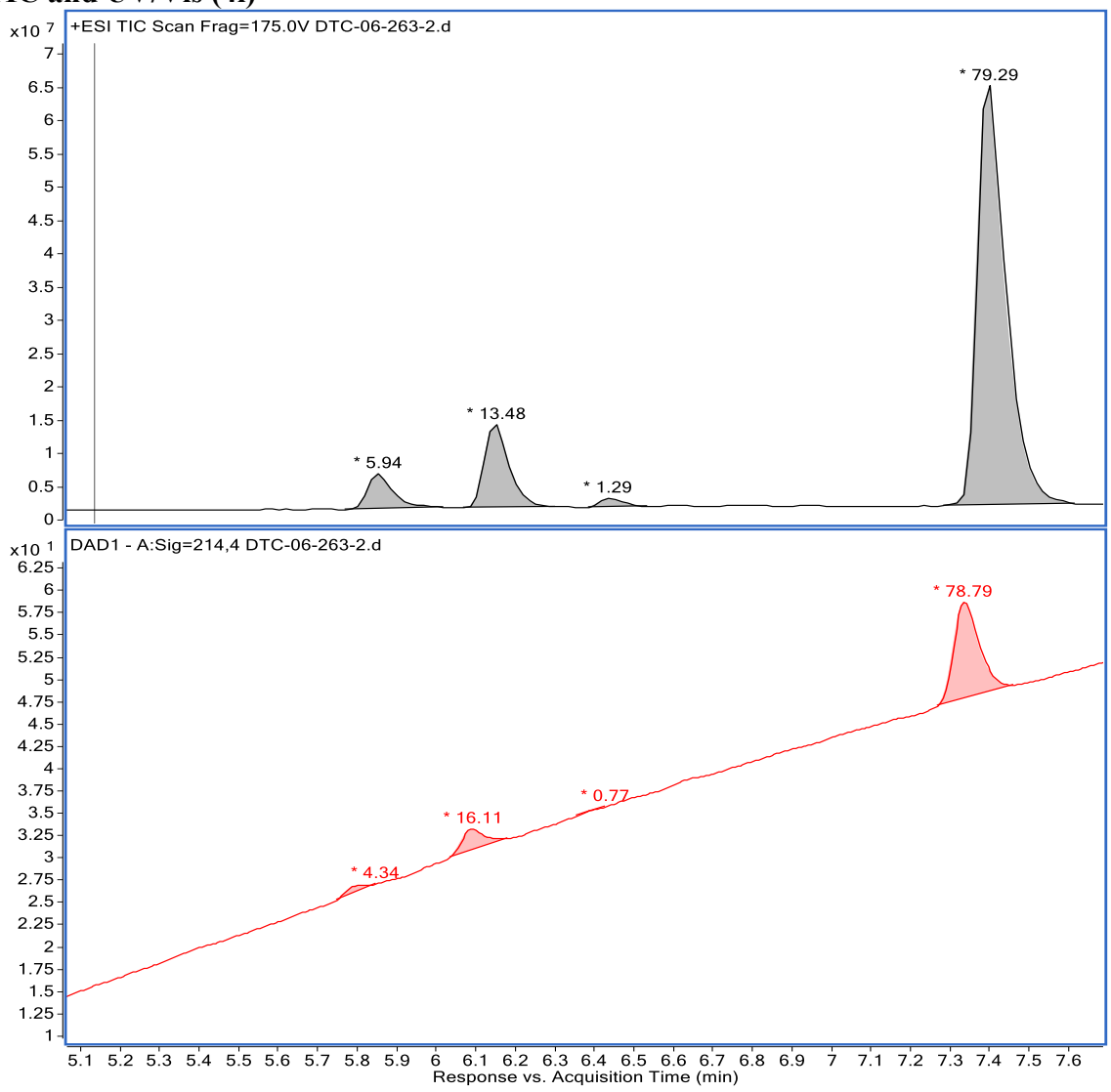
Expanded TIC with Integration (4i)

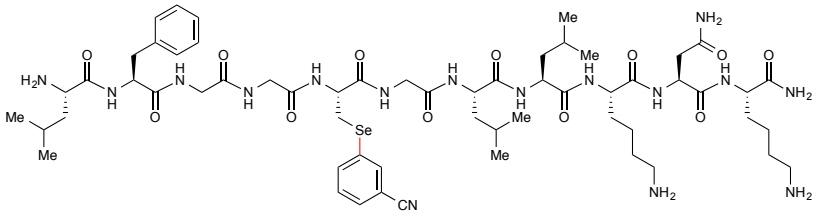


Mass Spectrum (4i $[M+2H]^{2+}$)



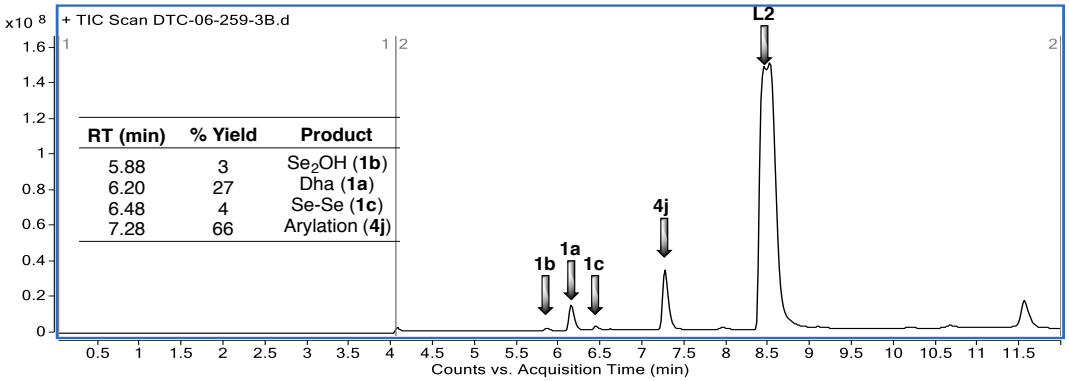
TIC and UV/Vis (4i)





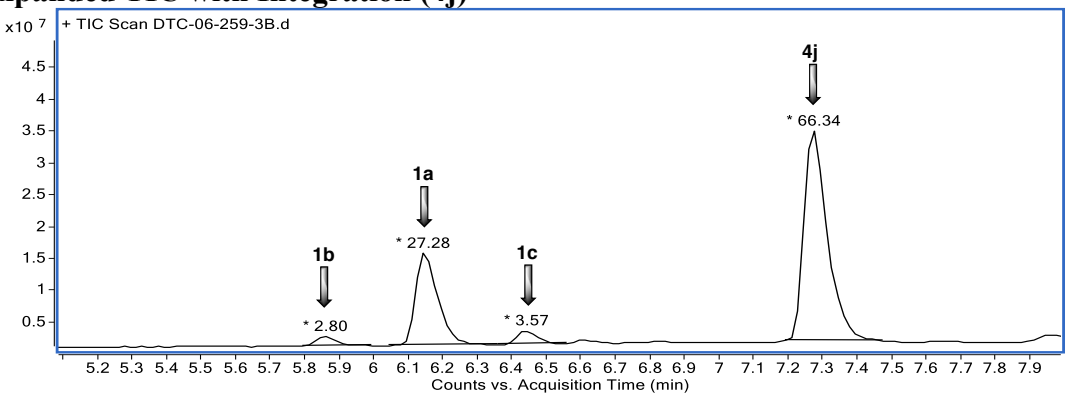
(4j): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (3-cyanophenyl)boronic acid stock solution (2 mM) at 37 °C for 2 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **4j**: HRMS (ESI) Mass. calcd. for $C_{58}H_{94}N_{16}O_{14}Se$ $[M+2H]^{2+}$, 649.32. Found $[M+2H]^{2+}$, 649.33.

TIC (4j)

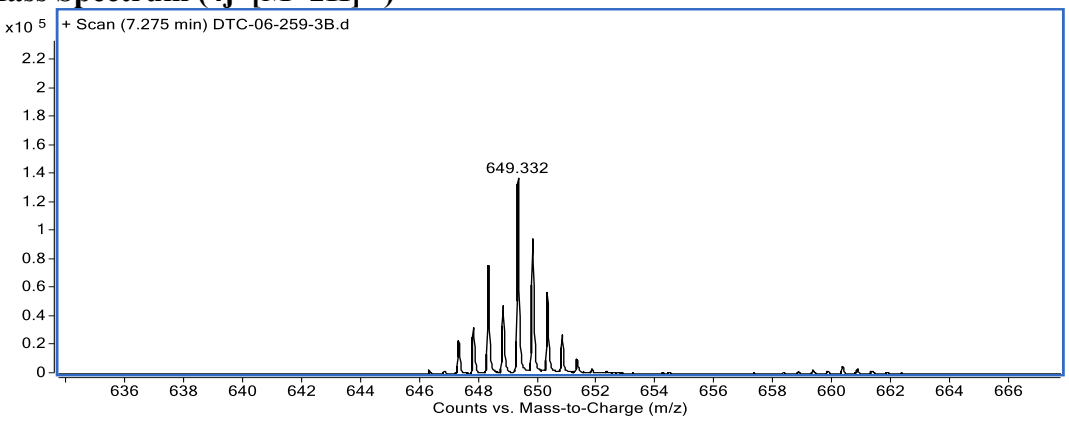


RT (min)	% Yield	Product
5.88	3	Se_2OH (**1b**)
6.20	27	Dha (**1a**)
6.48	4	Se-Se (**1c**)
7.28	66	Arylation (**4j**)

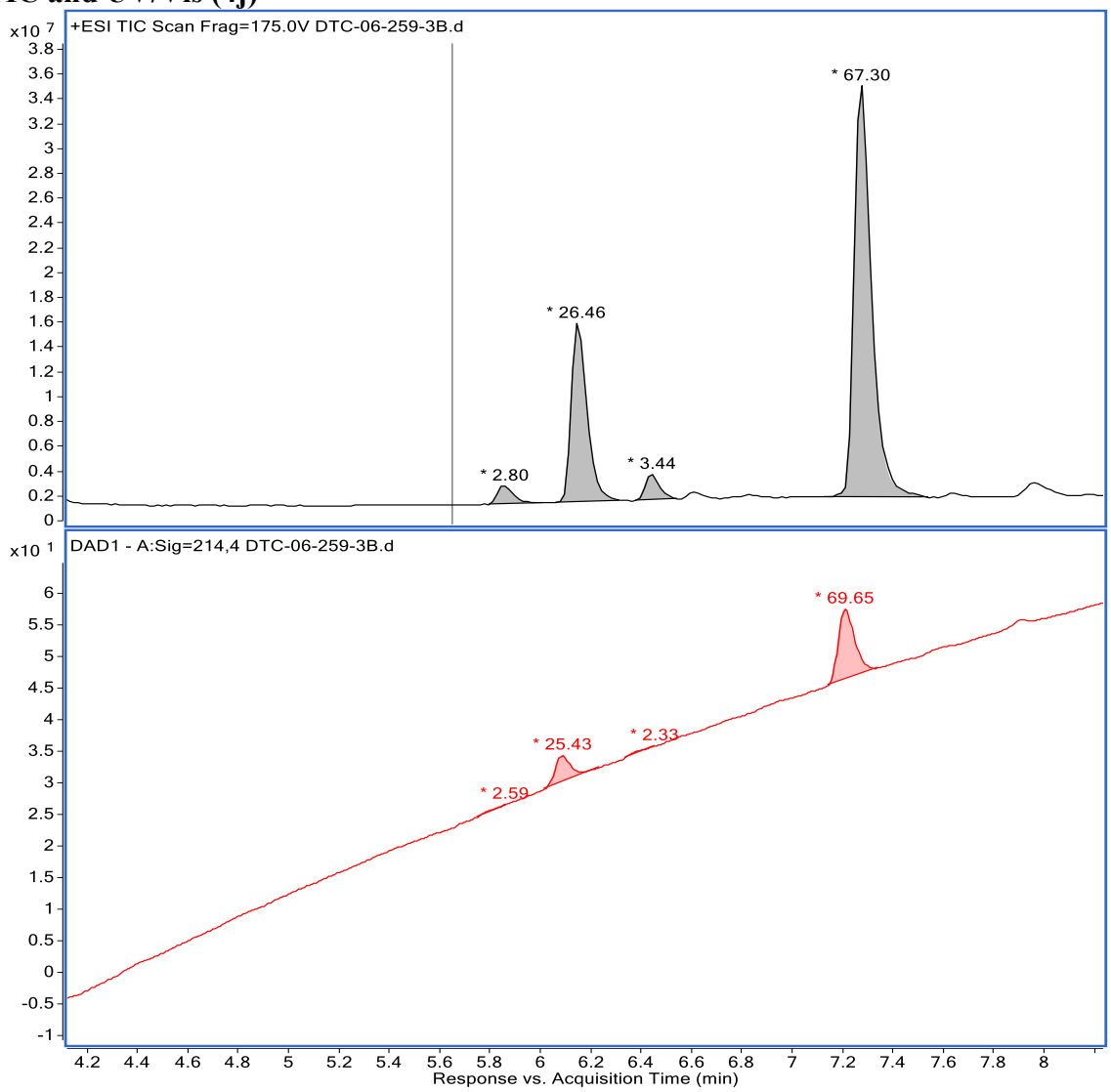
Expanded TIC with Integration (4j)

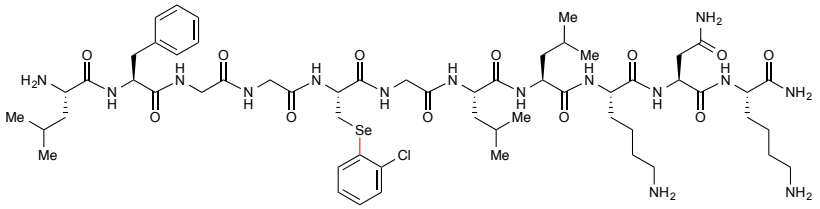


Mass Spectrum (4j $[M+2H]^{2+}$)



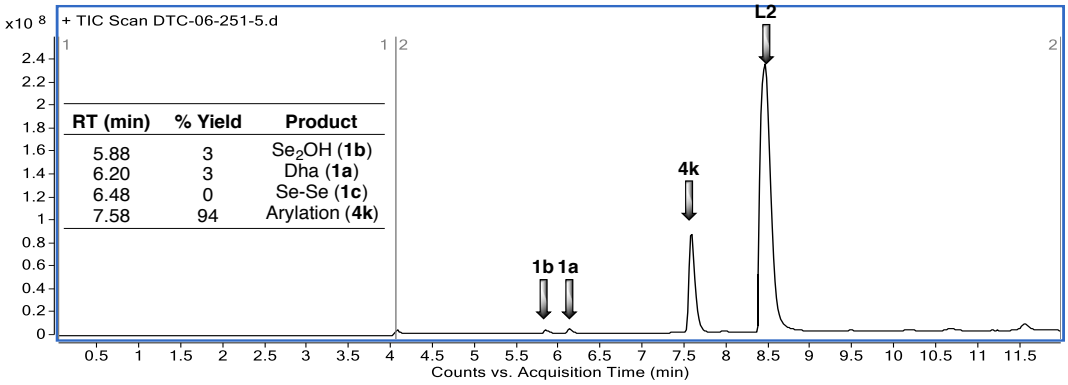
TIC and UV/Vis (4j)





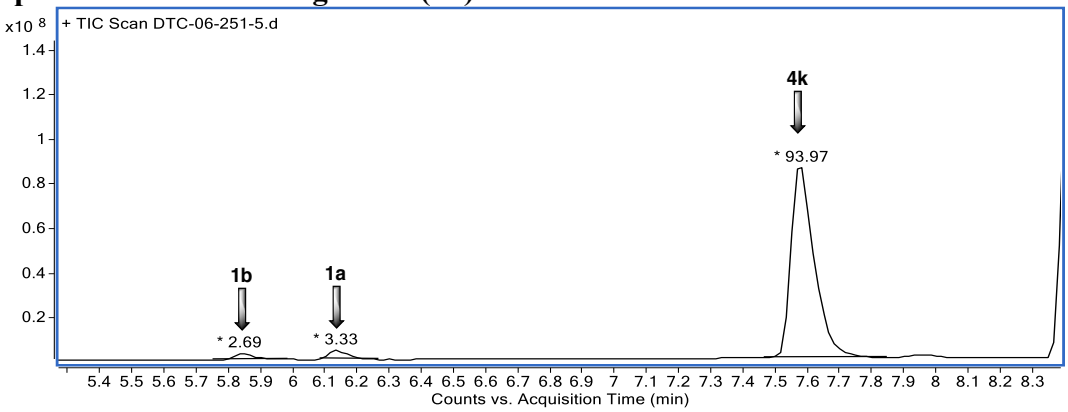
(4k): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (2-chlorophenyl)boronic acid stock solution (1 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS Method A. Analytical data for **4k**: HRMS (ESI) Mass. calcd. for $C_{58}H_{92}ClN_{15}O_{12}Se$ $[M+H]^+$, 1306.59. Found $[M+H]^+$, 1306.58.

TIC (4k)

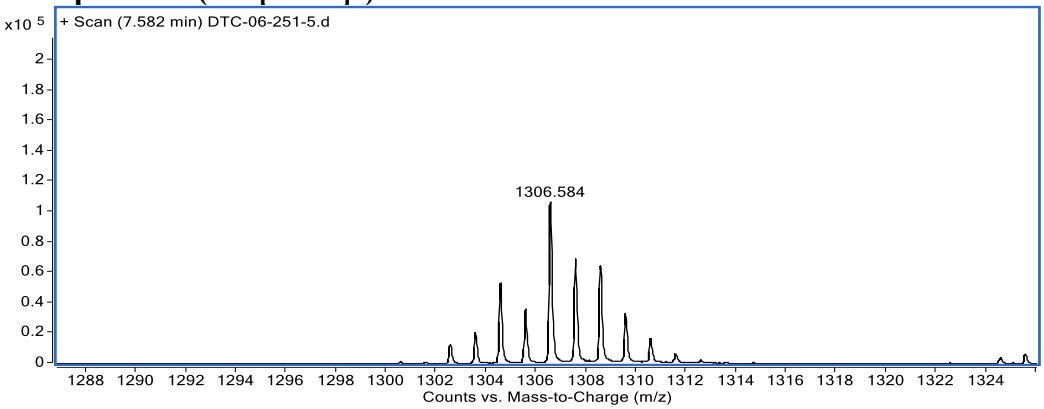


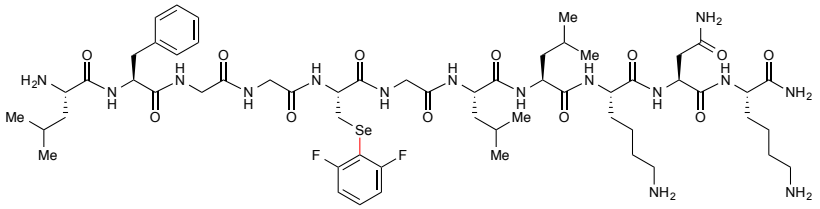
RT (min)	% Yield	Product
5.88	3	Se_2OH (**1b**)
6.20	3	Dha (**1a**)
6.48	0	Se-Se (**1c**)
7.58	94	Arylation (**4k**)

Expanded TIC with Integration (4k)



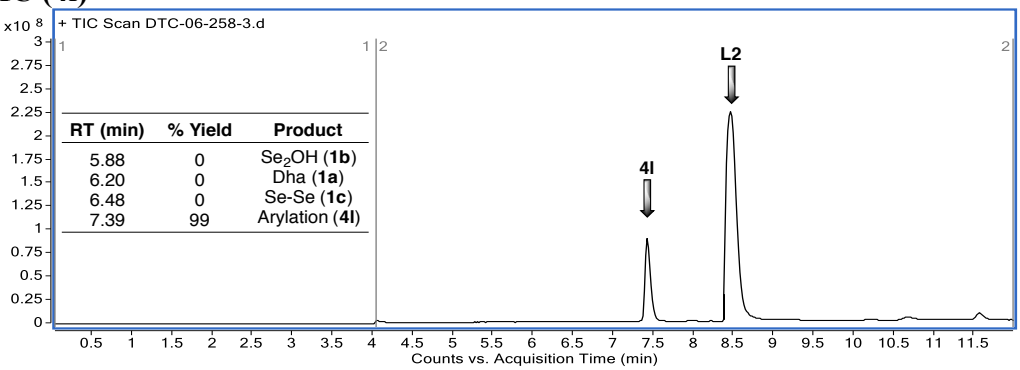
Mass Spectrum (4k $[M+H]^+$)





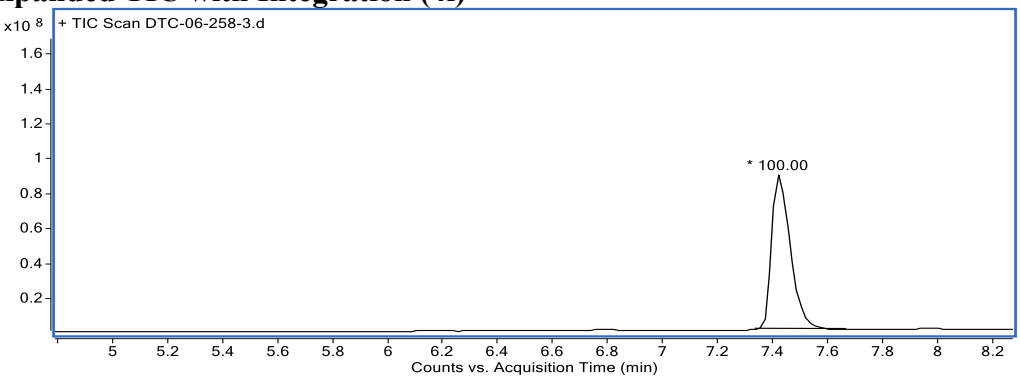
(4l): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (2,6-difluorophenyl)boronic acid stock solution (1 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **4l**: HRMS (ESI) Mass. calcd. for $C_{58}H_{91}F_2N_{15}O_{12}Se$ $[M+2H]^{2+}$, 654.81. Found $[M+2H]^{2+}$, 654.82.

TIC (4l)

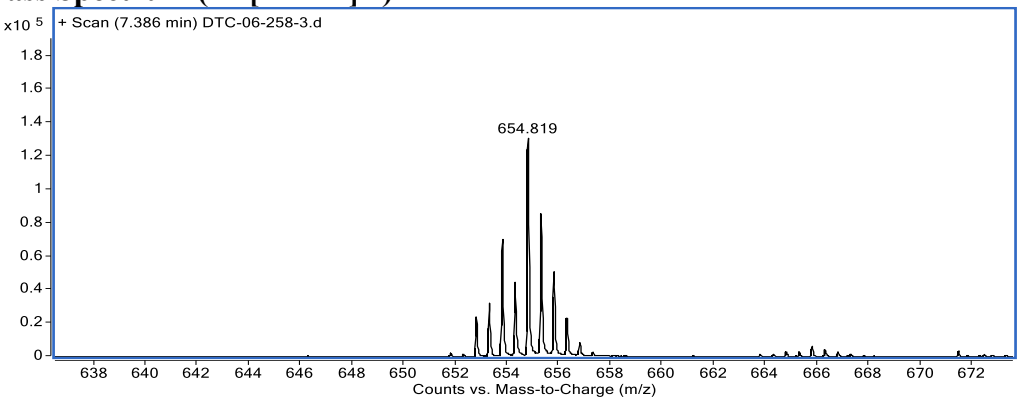


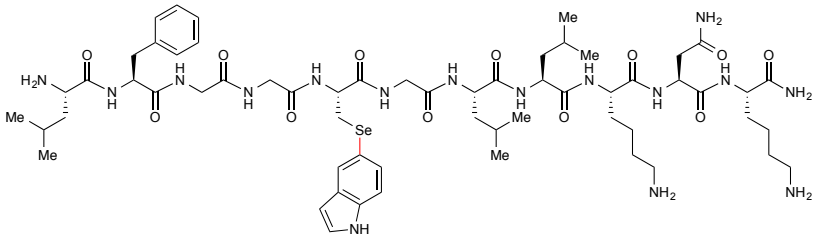
RT (min)	% Yield	Product
5.88	0	Se_2OH (**1b**)
6.20	0	Dha (**1a**)
6.48	0	Se-Se (**1c**)
7.39	99	Arylation (**4l**)

Expanded TIC with Integration (4l)



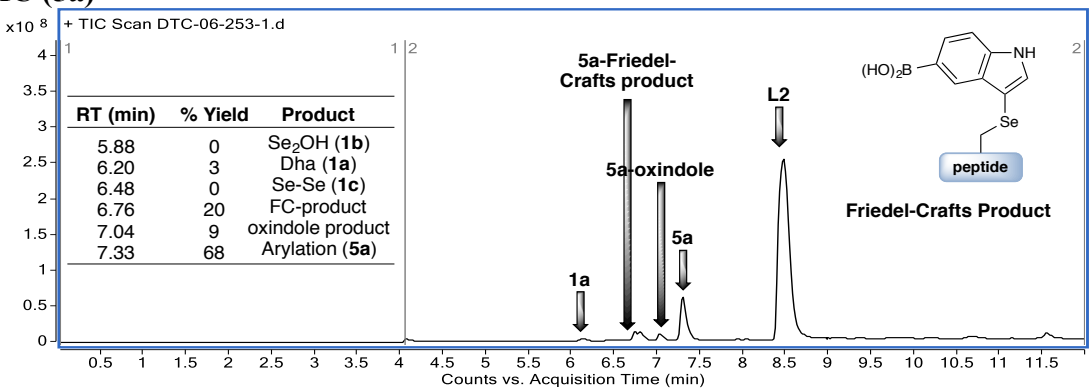
Mass Spectrum (4l $[M+2H]^{2+}$)



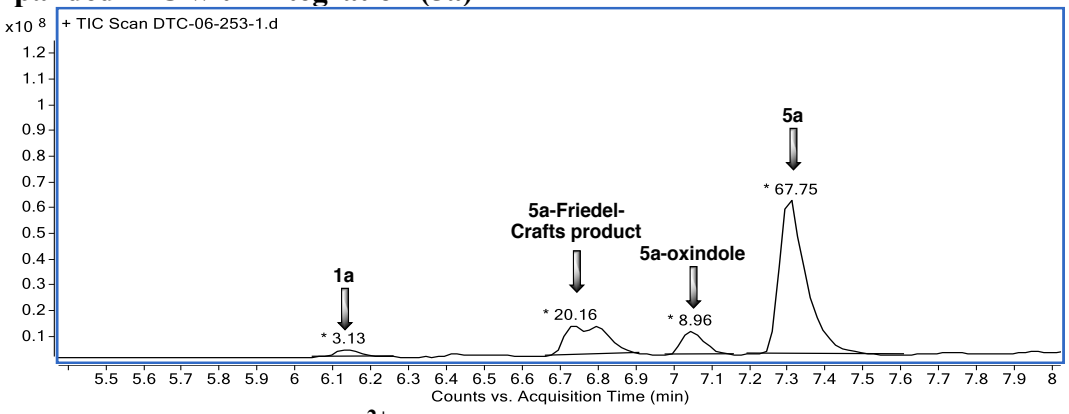


(5a): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (1*H*-indol-5-yl)boronic acid stock solution (1 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **5a**: HRMS (ESI) Mass. calcd. for $C_{60}H_{96}N_{16}O_{12}Se$ $[M+2H]^{2+}$, 656.32. Found $[M+2H]^{2+}$, 656.32.

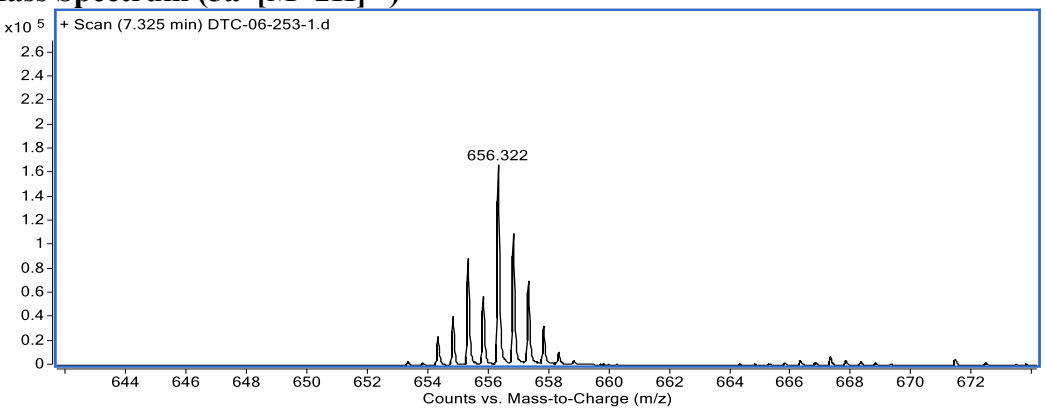
TIC (5a)

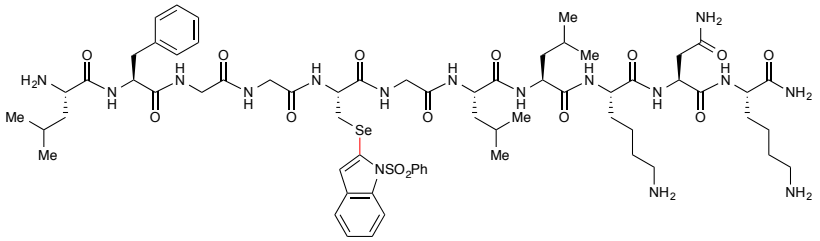


Expanded TIC with Integration (5a)



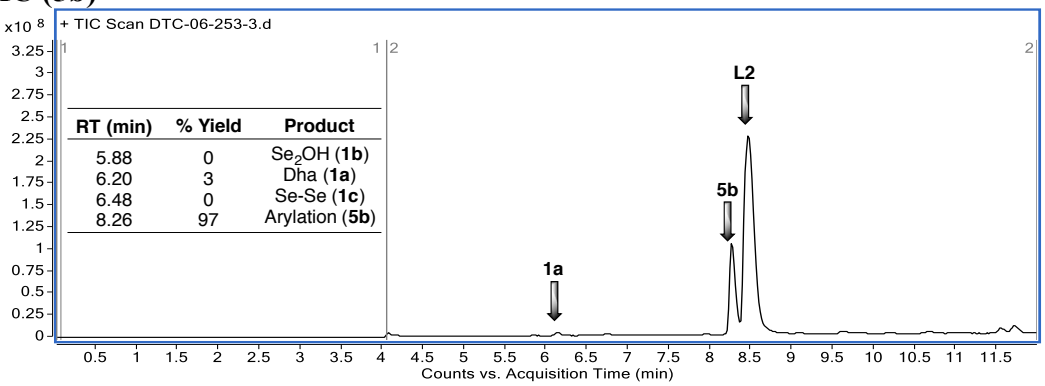
Mass Spectrum (5a $[M+2H]^{2+}$)



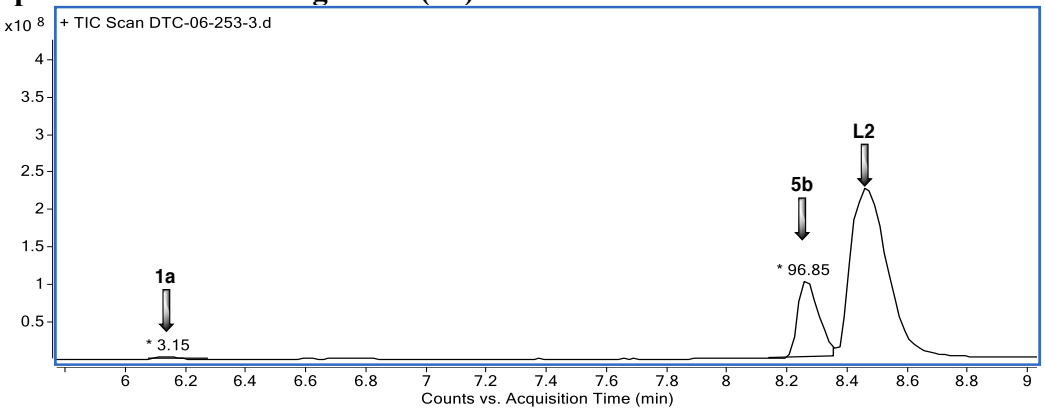


(5b): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (1-(phenylsulfonyl)-1*H*-indol-2-yl)boronic acid acid stock solution (1 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **5b**: HRMS (ESI) Mass. calcd. for $C_{66}H_{100}N_{16}O_{14}SSe$ $[M+2H]^{2+}$, 726.32. Found $[M+2H]^{2+}$, 726.33.

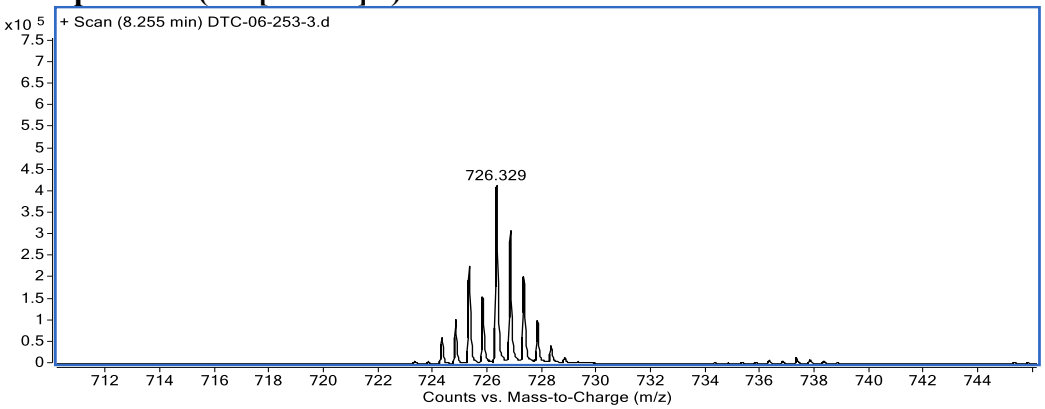
TIC (5b)

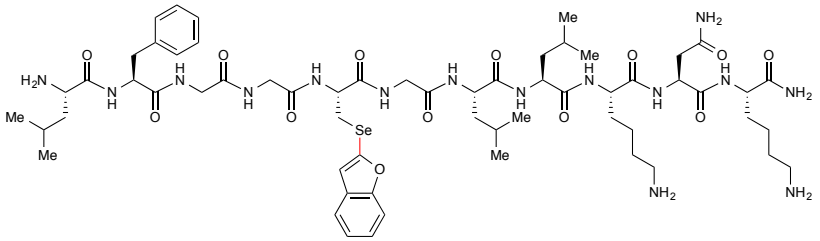


Expanded TIC with Integration (5b)



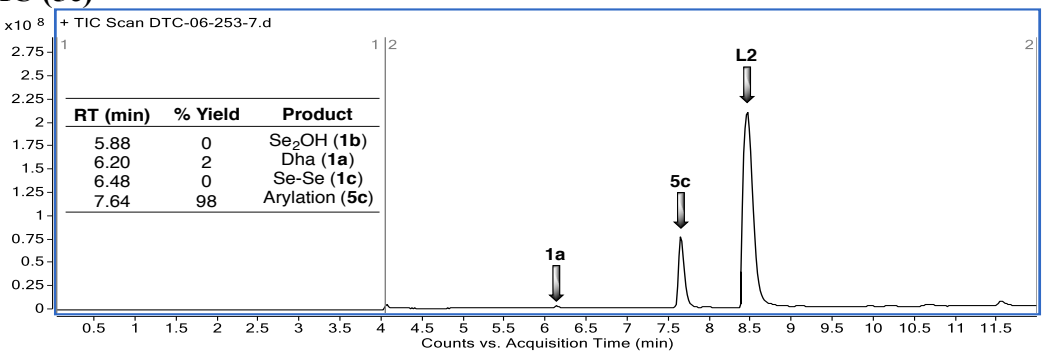
Mass Spectrum (5b $[M+2H]^{2+}$)



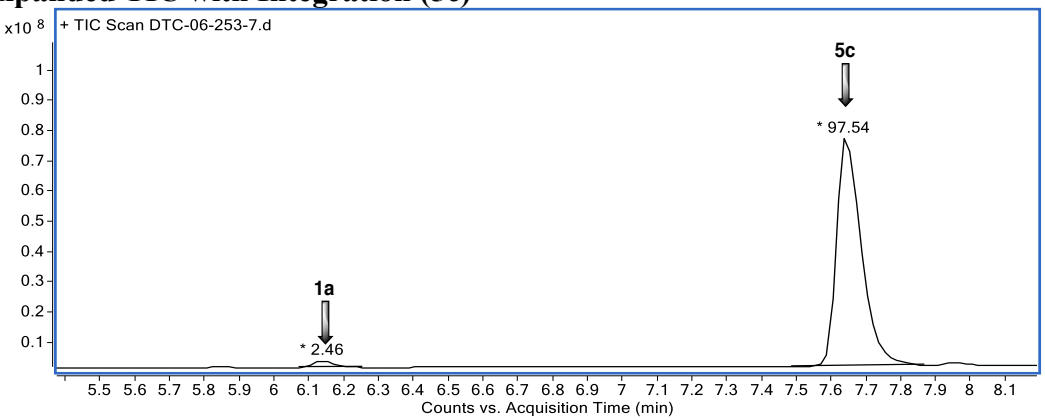


(5c): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and benzofuran-2-ylboronic acid stock solution (1 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **5c**: HRMS (ESI) Mass. calcd. for $C_{60}H_{95}N_{15}O_{13}Se$ $[M+2H]^{2+}$, 656.81. Found $[M+2H]^{2+}$, 656.82.

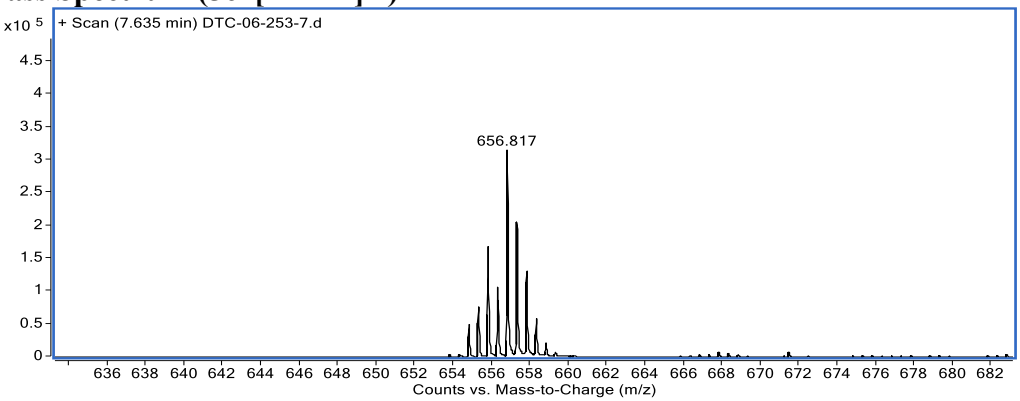
TIC (5c)

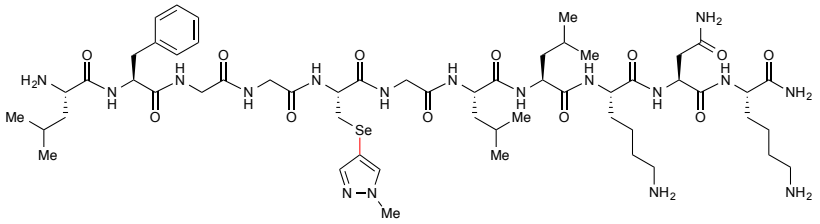


Expanded TIC with Integration (5c)



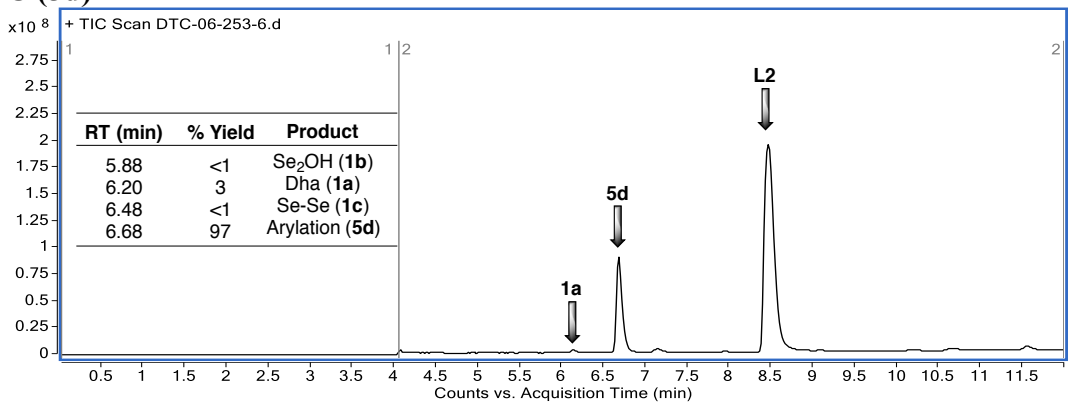
Mass Spectrum (5c $[M+2H]^{2+}$)



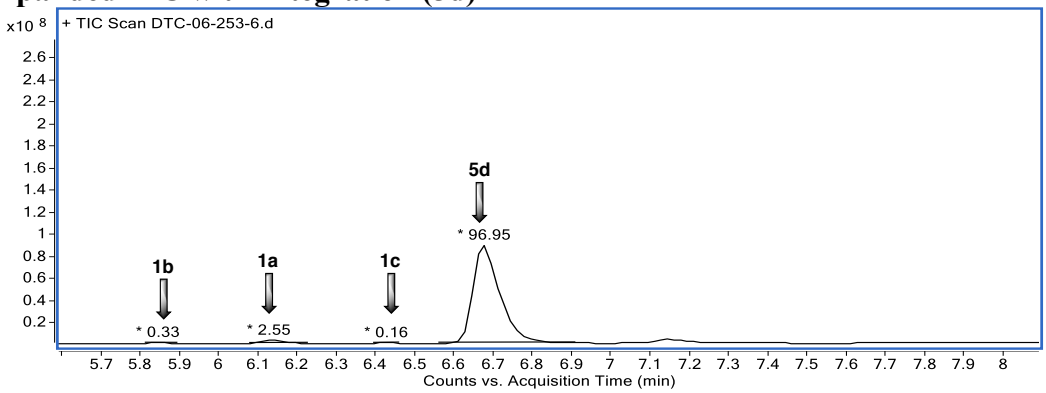


(5d): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (1-methyl-1*H*-pyrazol-4-yl)boronic acid stock solution (1 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **5d**: HRMS (ESI) Mass. calcd. for $C_{56}H_{94}N_{17}O_{12}Se$ $[M+H]^+$, 1276.64. Found $[M+H]^+$, 1276.63.

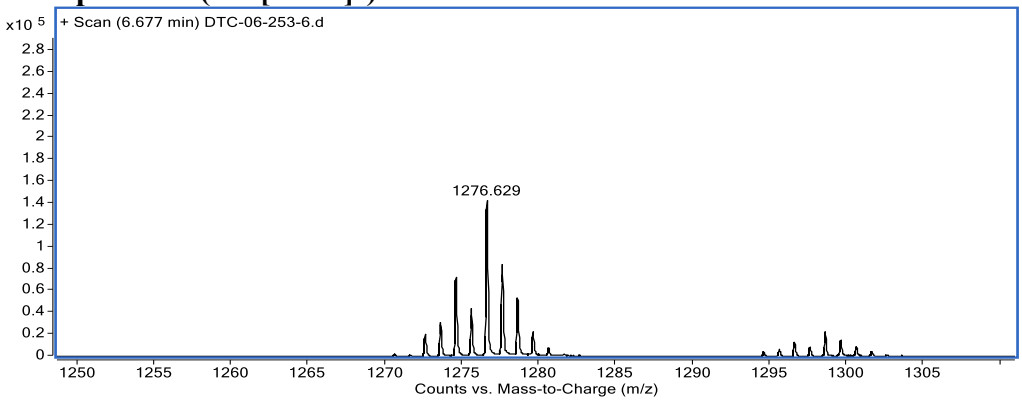
TIC (5d)

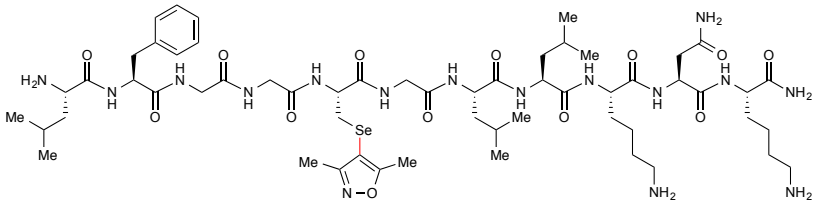


Expanded TIC with Integration (5d)



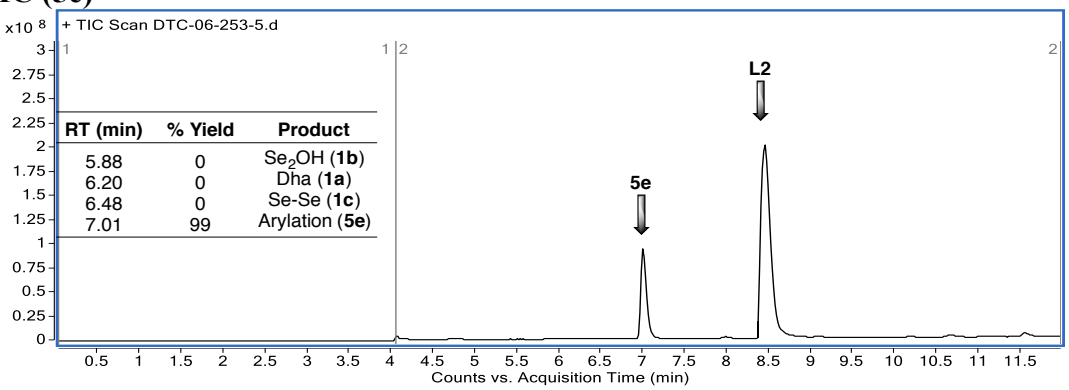
Mass Spectrum (5d $[M+H]^+$)



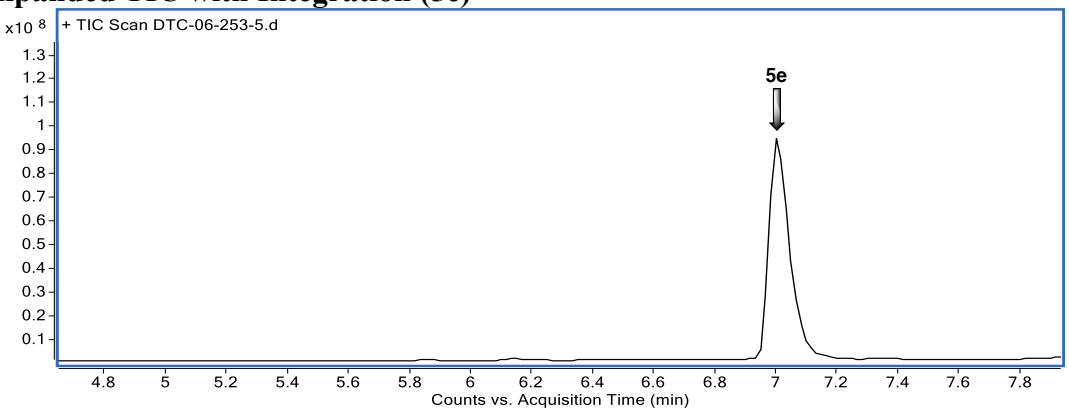


(5e): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (3,5-dimethylisoxazol-4-yl)boronic acid stock solution (1 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **5e**: HRMS (ESI) Mass. calcd. for $C_{57}H_{96}N_{16}O_{13}Se$ $[M+2H]^{2+}$, 646.32. Found $[M+2H]^{2+}$, 646.32.

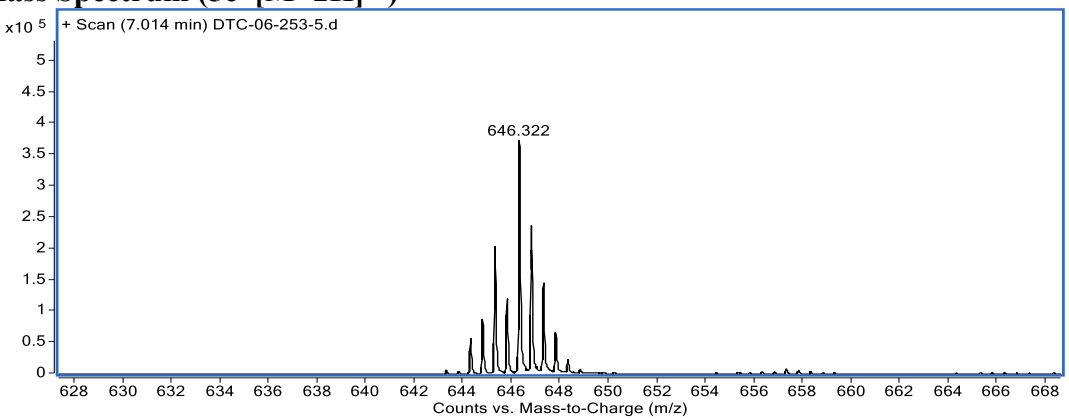
TIC (5e)

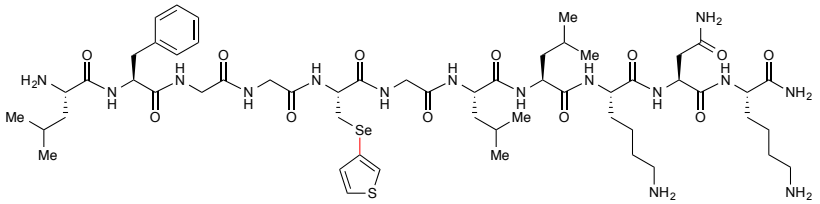


Expanded TIC with Integration (5e)



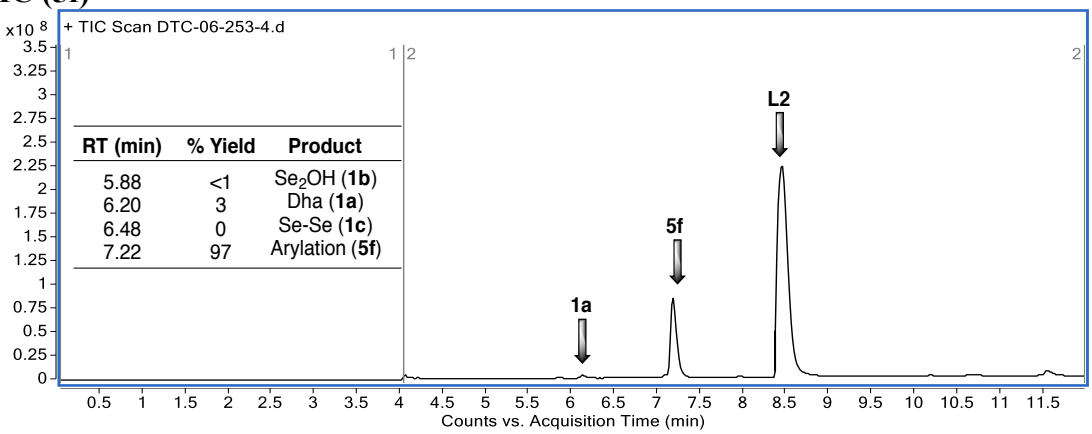
Mass Spectrum (5e $[M+2H]^{2+}$)



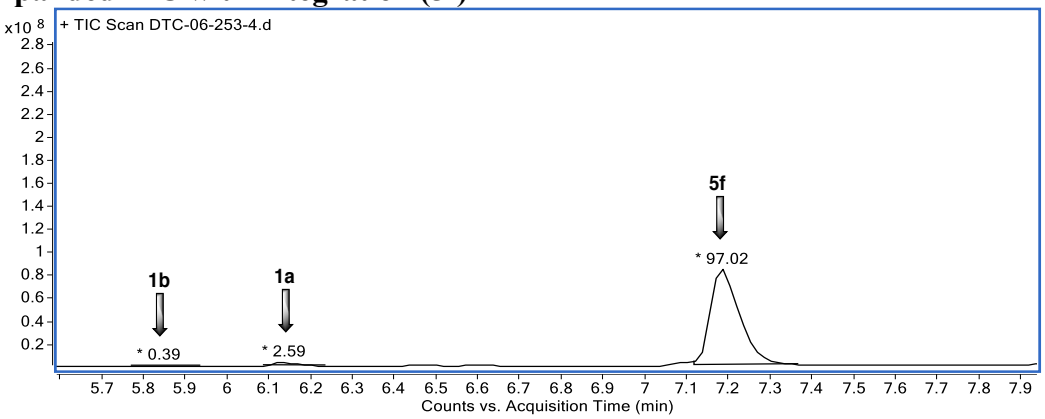


(5f): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and thiophen-3-ylboronic acid stock solution (1 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **5f**: HRMS (ESI) Mass. calcd. for $C_{56}H_{93}N_{15}O_{12}SSe$ $[M+2H]^{2+}$, 639.79. Found $[M+2H]^{2+}$, 639.79.

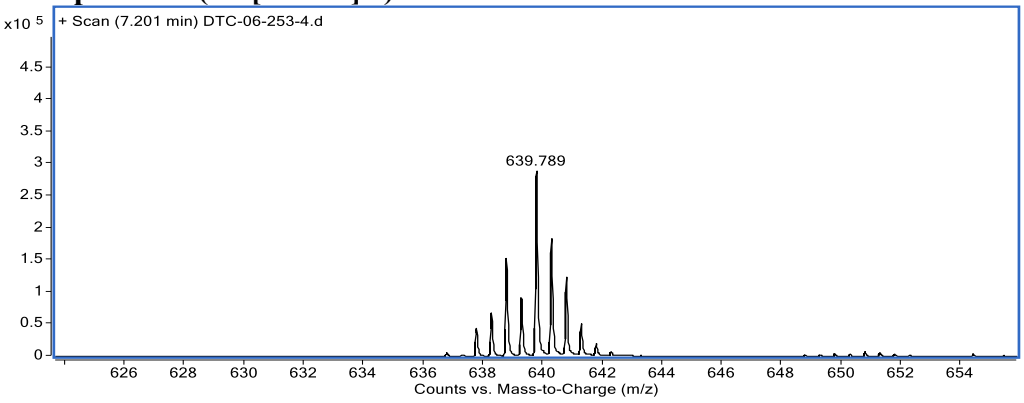
TIC (5f)

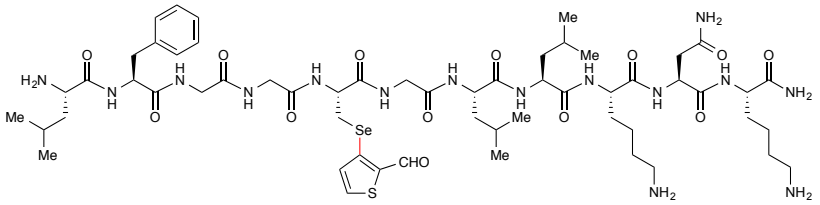


Expanded TIC with Integration (5f)



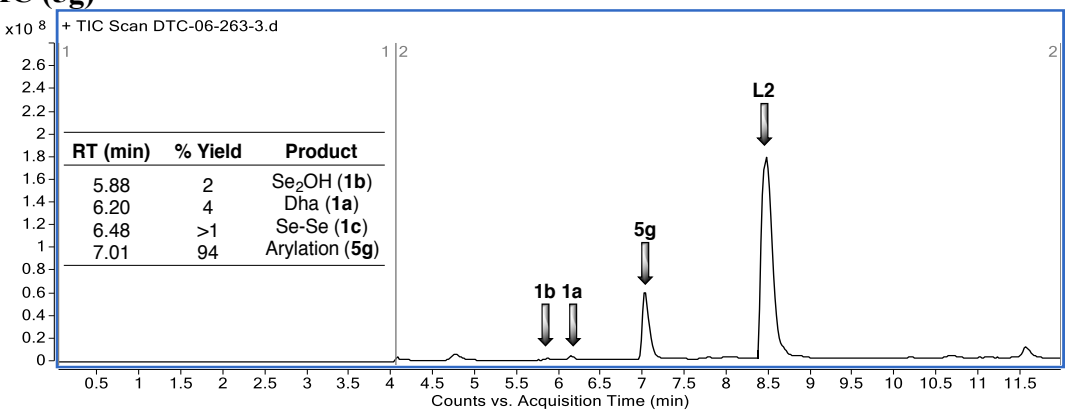
Mass Spectrum (5f $[M+2H]^{2+}$)



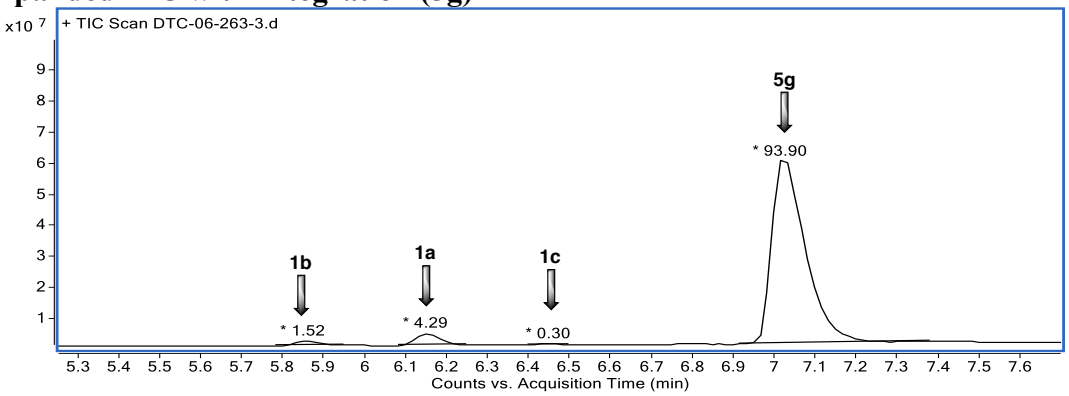


(5g): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (2-formylthiophen-3-yl)boronic acid stock solution (1 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **5g**: HRMS (ESI) Mass. calcd. for $C_{57}H_{93}N_{15}O_{13}SSe$ $[M+2H]^{2+}$, 653.80. Found $[M+2H]^{2+}$, 653.81.

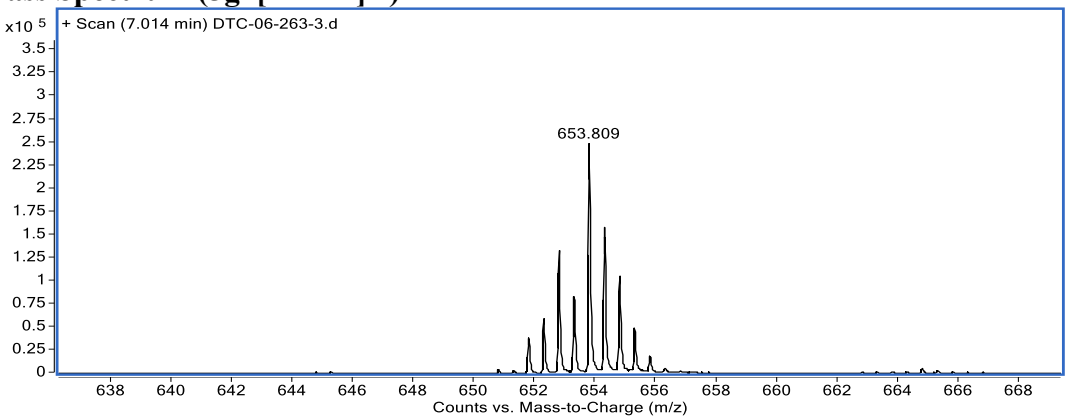
TIC (5g)

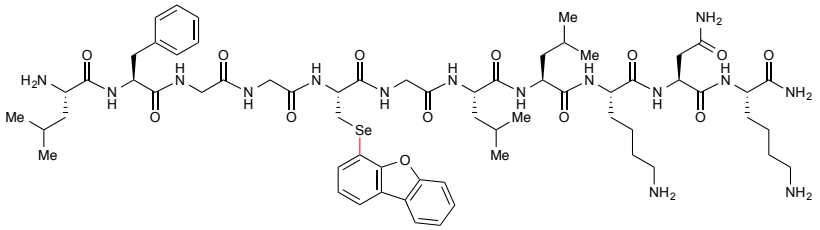


Expanded TIC with Integration (5g)



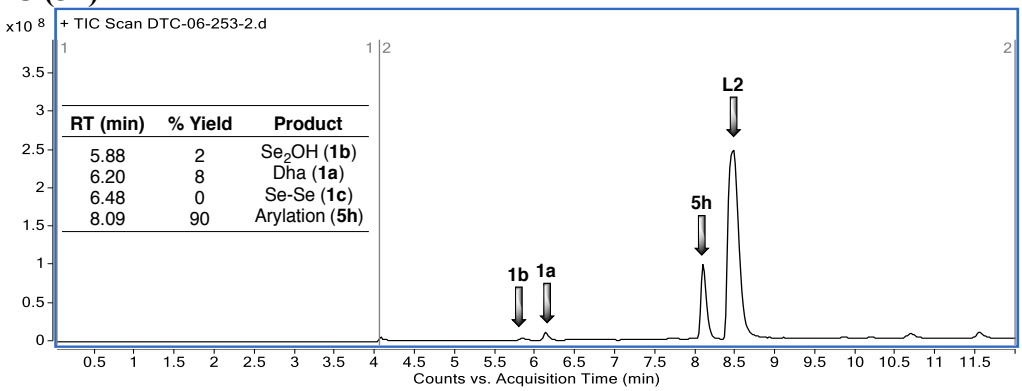
Mass Spectrum (5g $[M+2H]^{2+}$)



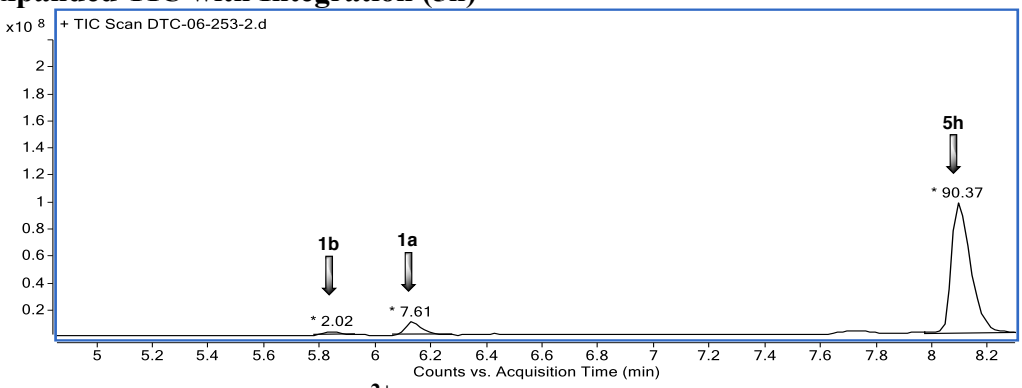


(5h): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and dibenzo[*b*,*d*]furan-4-ylboronic acid stock solution (1 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **5h**: HRMS (ESI) Mass. calcd. for $C_{64}H_{97}N_{15}O_{13}Se$ $[M+2H]^{2+}$, 681.83. Found $[M+2H]^{2+}$, 681.83.

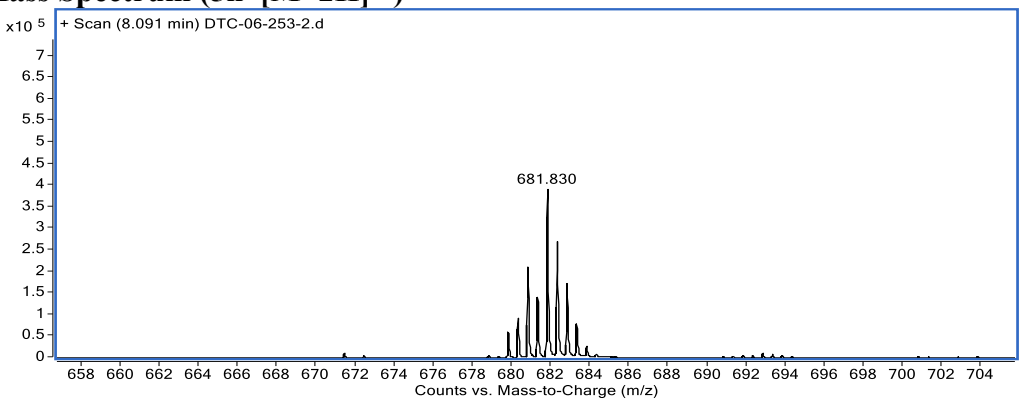
TIC (5h)

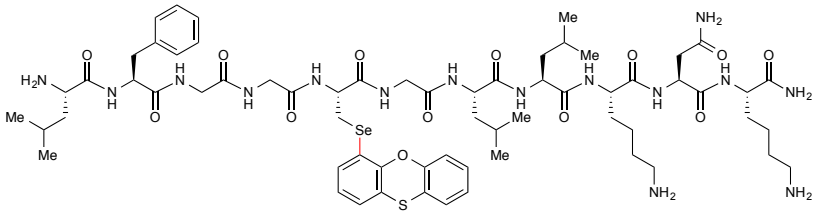


Expanded TIC with Integration (5h)



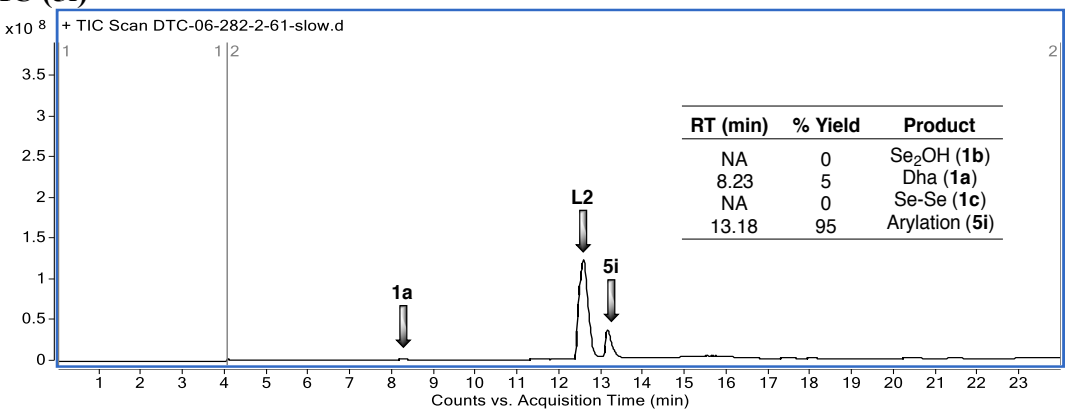
Mass Spectrum (5h $[M+2H]^{2+}$)



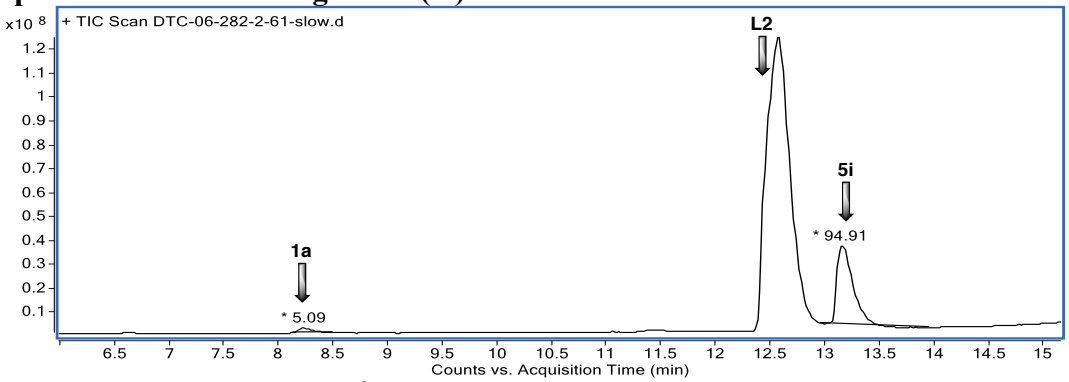


(5i): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and phenoxathiin-4-ylboronic acid stock solution (1 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method C*. Analytical data for **5i**: HRMS (ESI) Mass. calcd. for $C_{64}H_{97}N_{15}O_{13}SSe$ $[M+2H]^{2+}$, 697.81. Found $[M+2H]^{2+}$, 697.82.

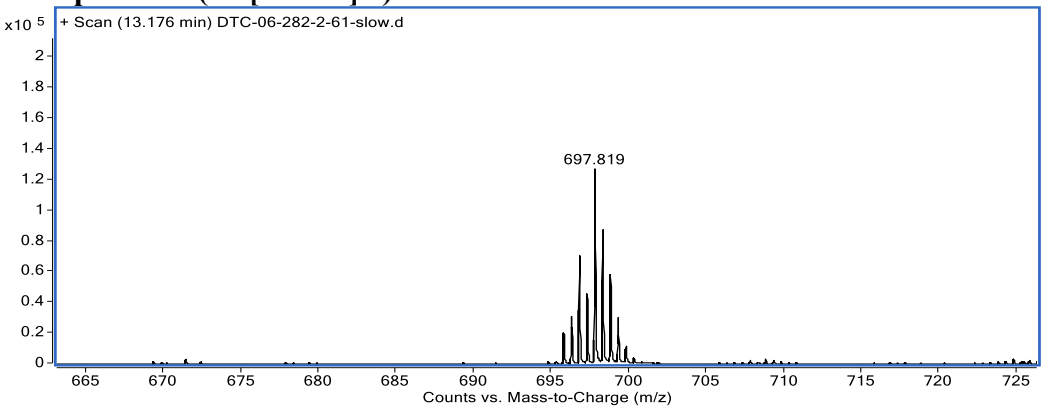
TIC (5i)

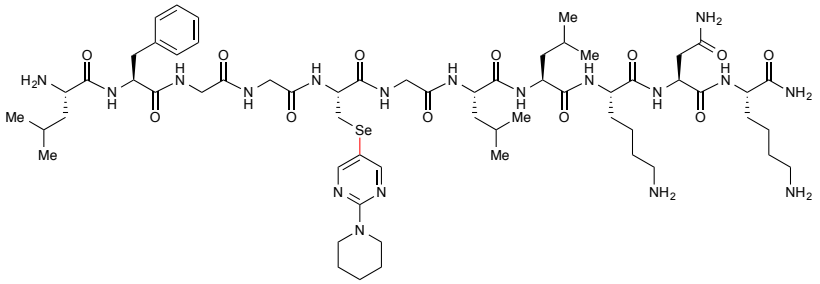


Expanded TIC with Integration (5i)



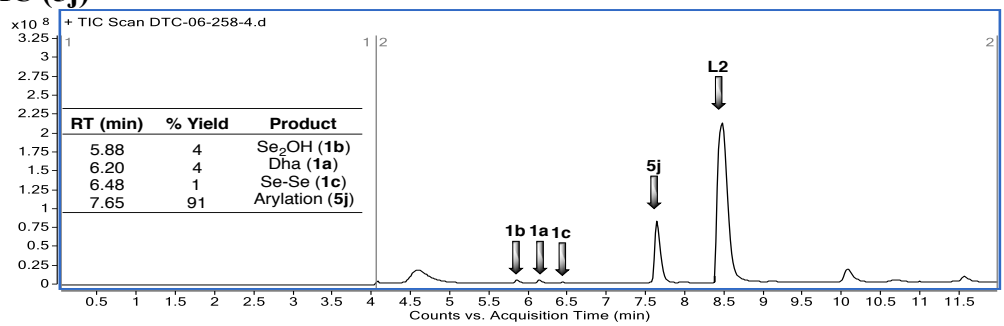
Mass Spectrum (5i $[M+2H]^{2+}$)



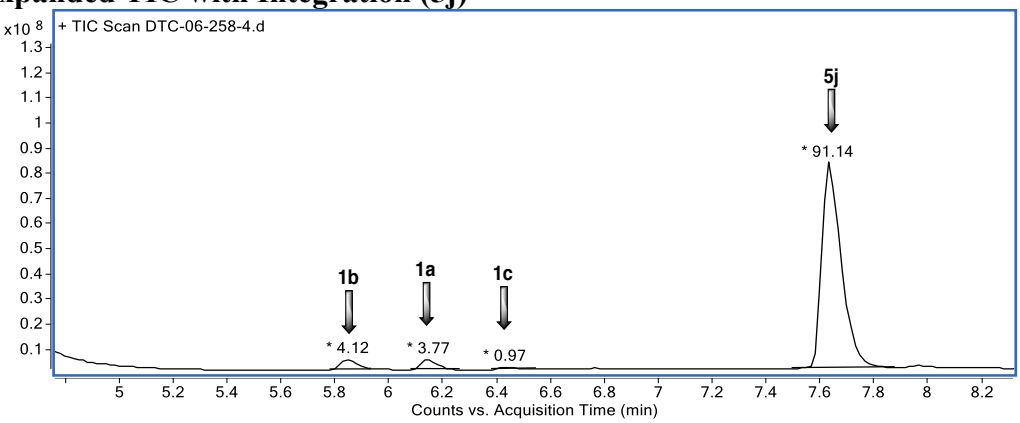


(5j): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (2-(piperidin-1-yl)pyrimidin-5-yl)boronic acid stock solution (1 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **5j**: HRMS (ESI) Mass. calcd. for $C_{61}H_{102}N_{18}O_{12}Se$ $[M+2H]^{2+}$, 679.35. Found $[M+2H]^{2+}$, 679.36.

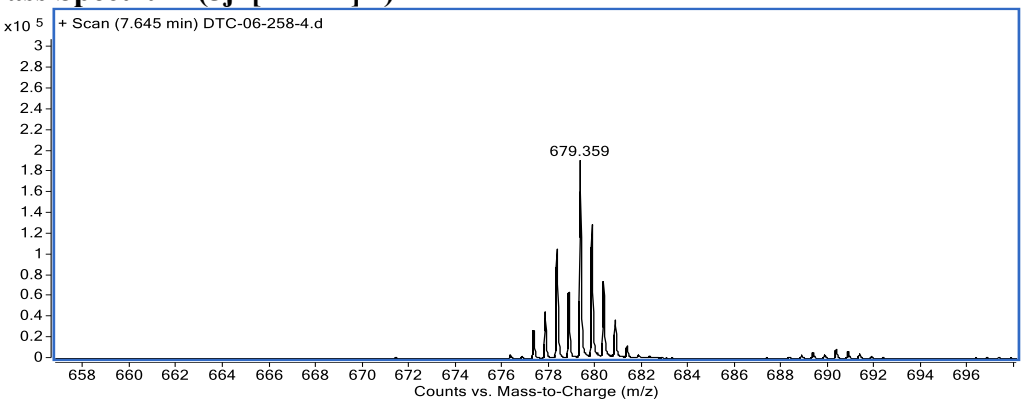
TIC (5j)

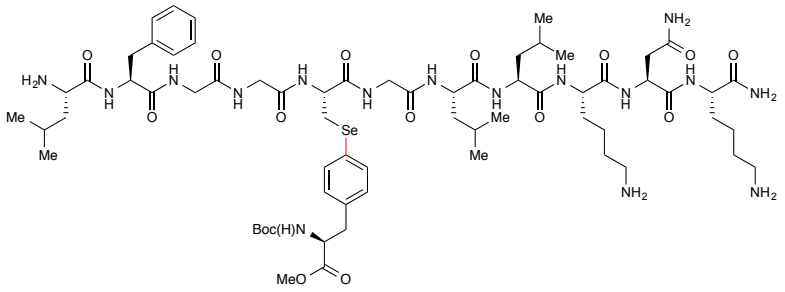


Expanded TIC with Integration (5j)



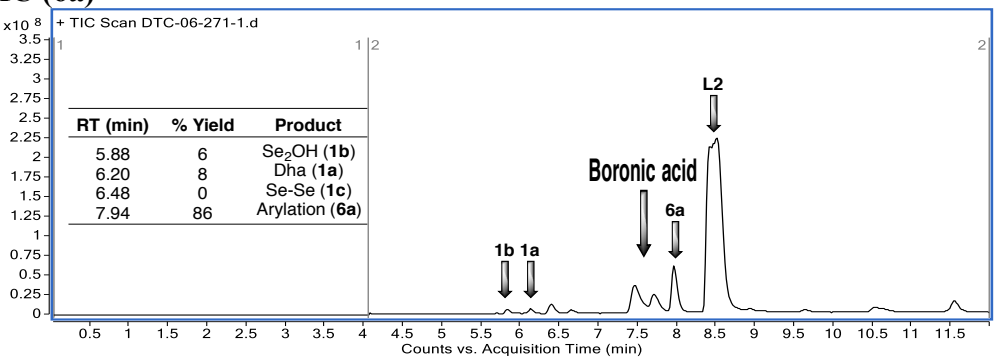
Mass Spectrum (5j $[M+2H]^{2+}$)



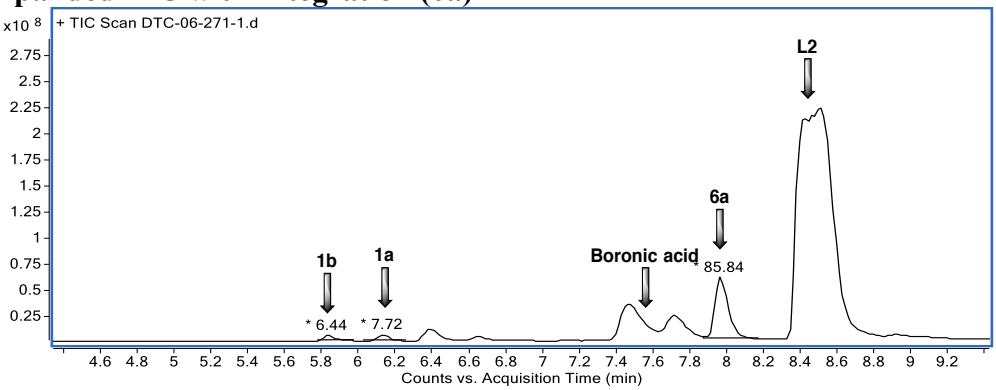


(6a): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (*S*)-(4-(2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyl)boronic acid stock solution (2 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **6a**: HRMS (ESI) Mass. calcd. for $C_{67}H_{108}N_{16}O_{16}Se$ $[M+H]^+$, 1473.73. Found $[M+H]^+$, 1473.71.

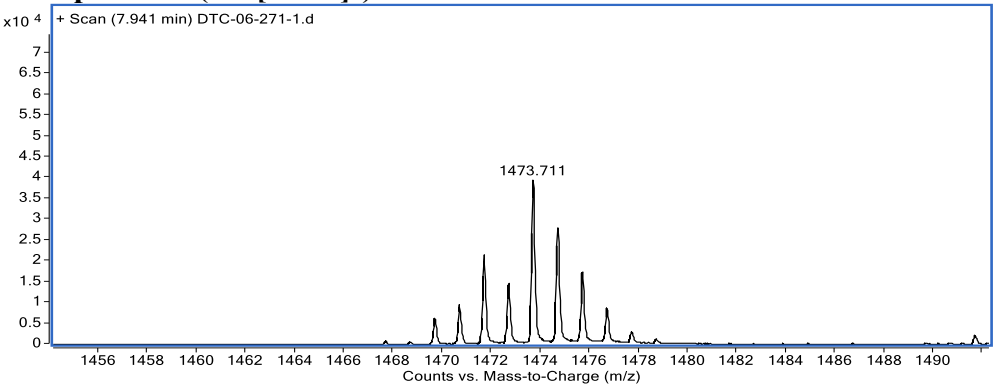
TIC (6a)

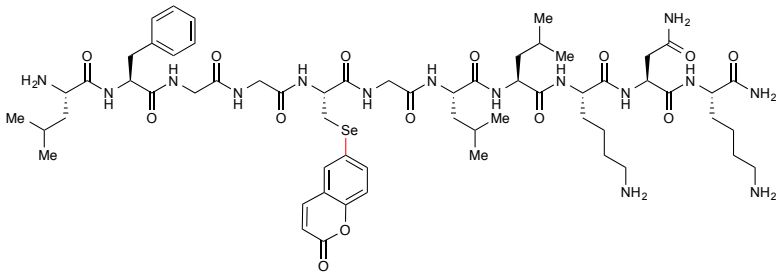


Expanded TIC with Integration (6a)



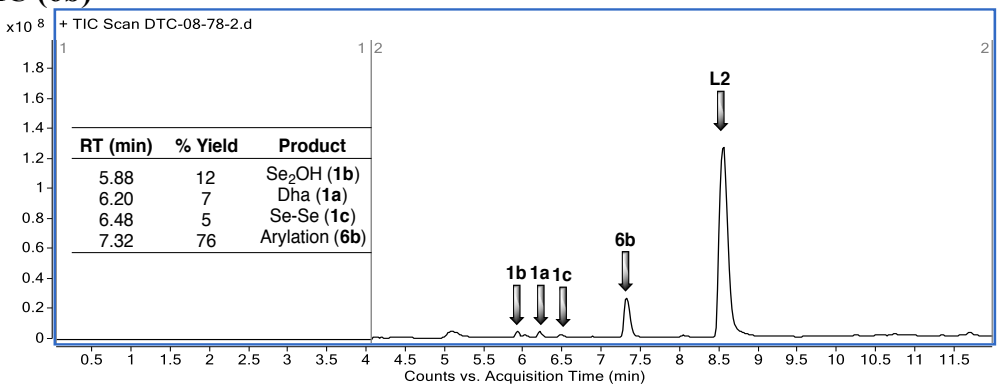
Mass Spectrum (6a $[M+H]^+$)



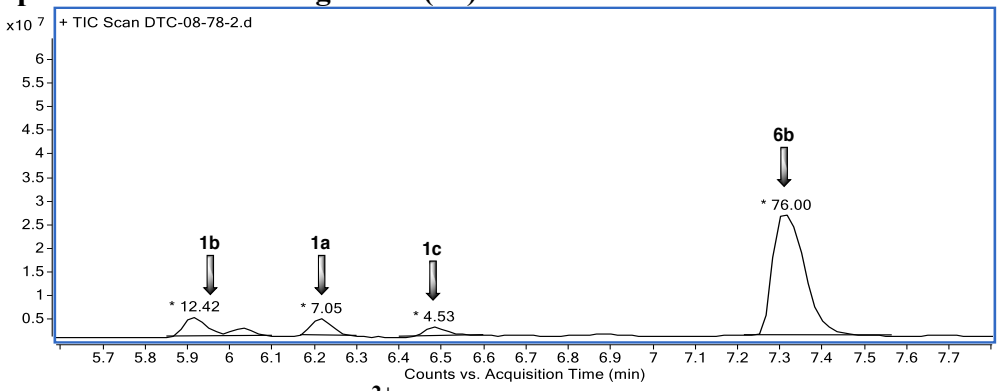


(6b): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (2-oxo-2*H*-chromen-6-yl)boronic acid stock solution (2 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **6b**: HRMS (ESI) Mass. calcd. for $C_{61}H_{95}N_{15}O_{14}Se$ $[M+2H]^{2+}$, 670.82. Found $[M+2H]^{2+}$, 670.83.

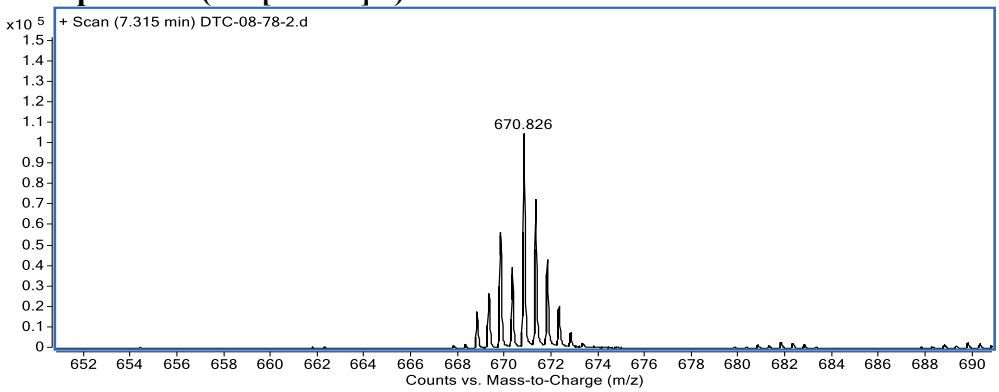
TIC (6b)

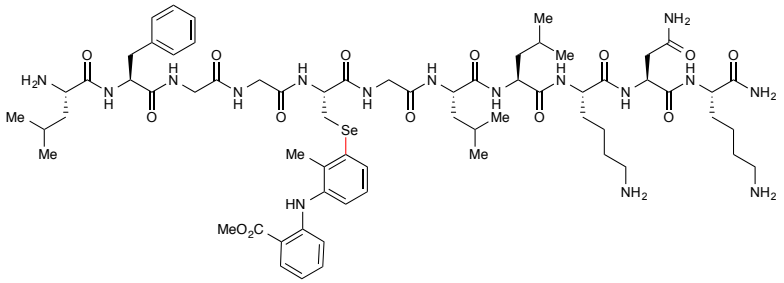


Expanded TIC with Integration (6b)



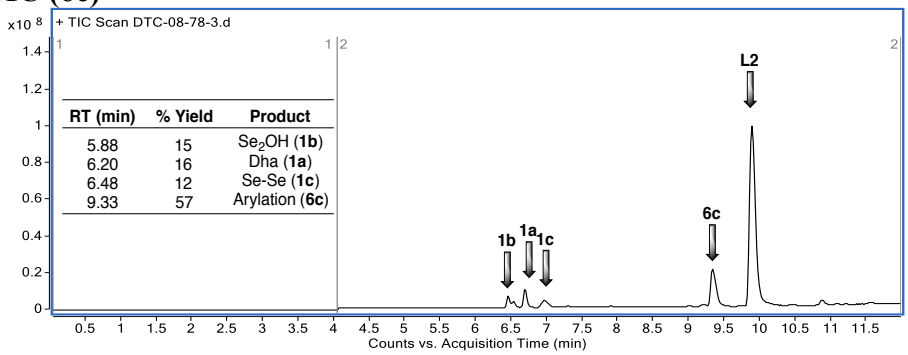
Mass Spectrum (6b $[M+2H]^{2+}$)



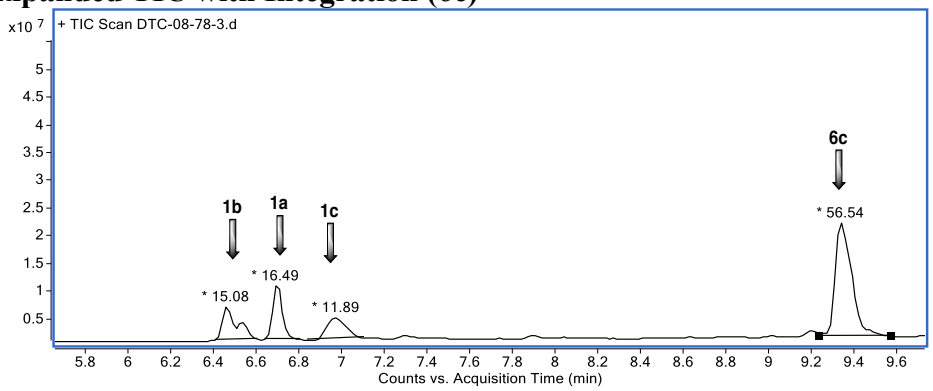


(6c): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (2-((2-(methoxycarbonyl)phenyl)amino)-6-methylphenyl)boronic acid stock solution (1 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method D*. Analytical data for **6c**: HRMS (ESI) Mass. calcd. for $C_{58}H_{92}ClN_{15}O_{12}Se$ $[M+2H]^{2+}$, 718.35. Found $[M+2H]^{2+}$, 718.36.

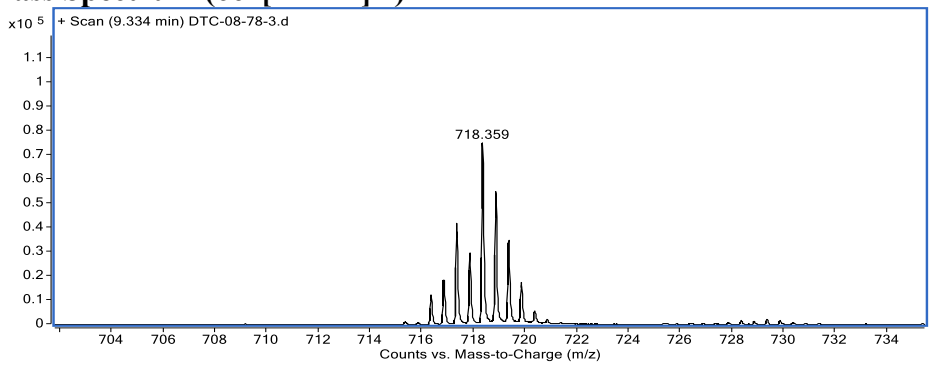
TIC (6c)

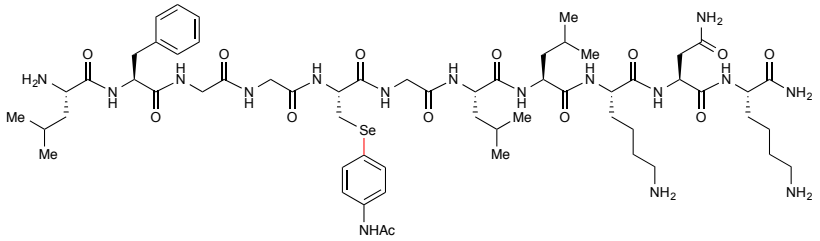


Expanded TIC with Integration (6c)



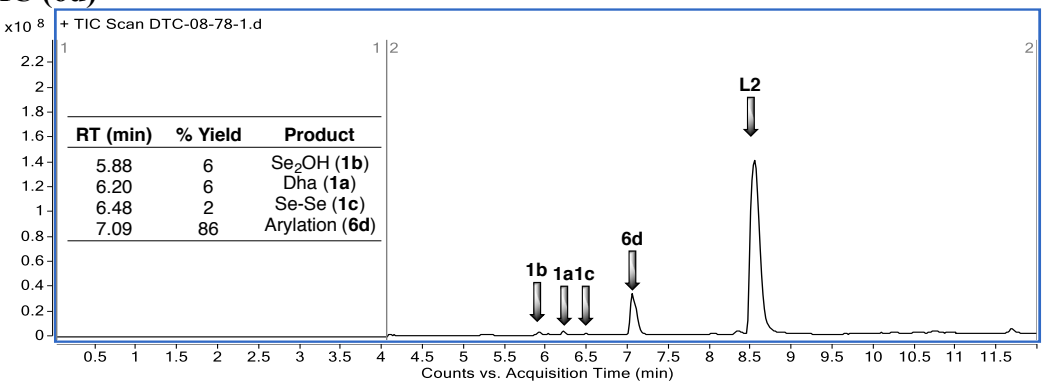
Mass Spectrum (6c $[M+2H]^{2+}$)



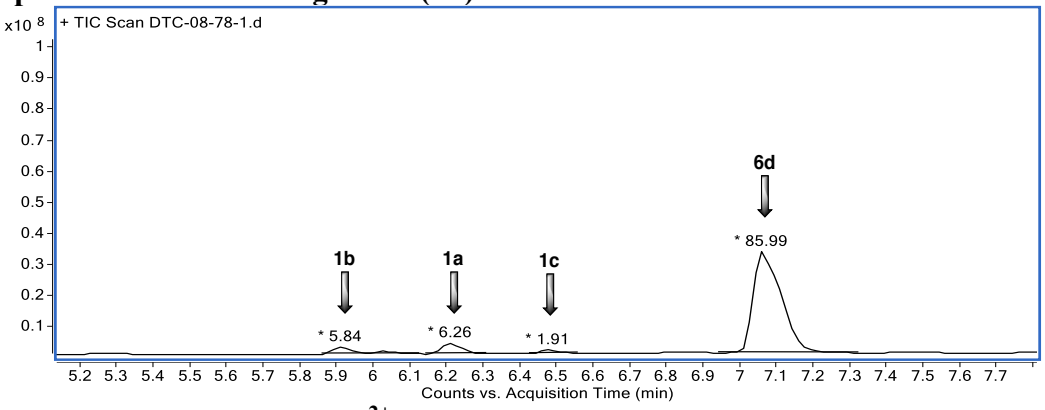


(6d): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and (4-acetamidophenyl)boronic acid stock solution (2 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **6d**: HRMS (ESI) Mass. calcd. for $C_{60}H_{98}N_{16}O_{13}Se$ $[M+2H]^{2+}$, 665.33. Found $[M+2H]^{2+}$, 665.34.

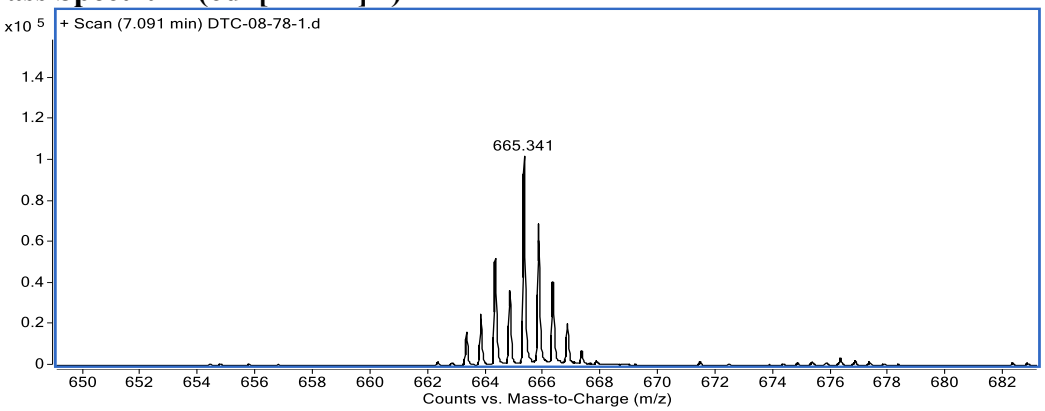
TIC (6d)

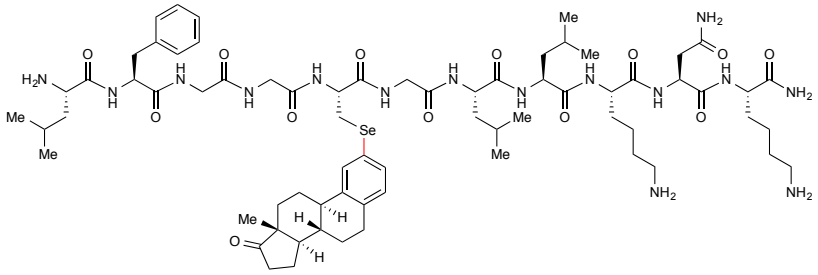


Expanded TIC with Integration (6d)



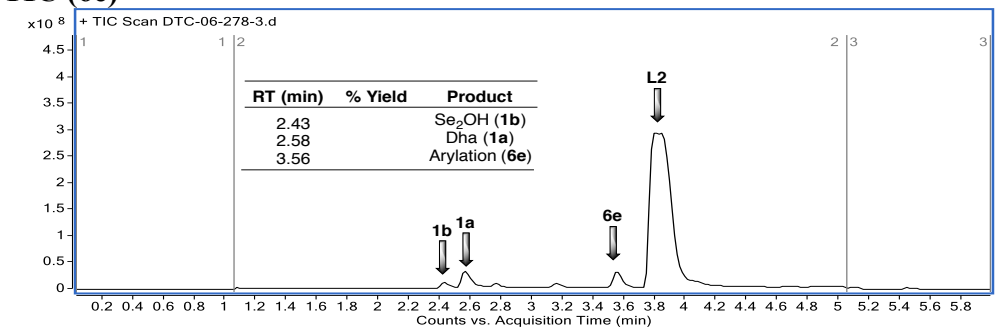
Mass Spectrum (6d $[M+2H]^{2+}$)



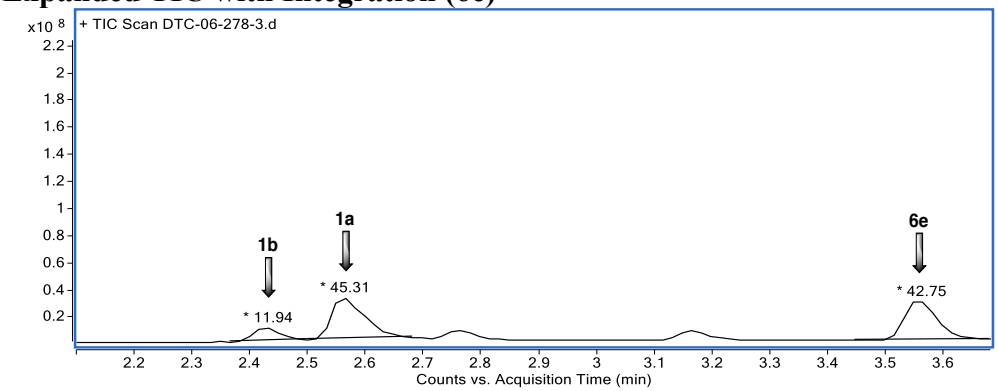


(6e): Prepared according to the general procedure (A) using peptide **1** (100 μM) and $CuSO_4$, **L2**, and ((8*R*,9*S*,13*S*,14*S*)-13-methyl-17-oxo-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[*a*]phenanthren-3-yl)boronic acid solution (2 mM) at 37 °C for 3 h. The only exception is 10% DMF was used instead of 5% EtOH. The quenched reaction mixture was analyzed using LC-MS *Method B*. Analytical data for **6e**: HRMS (ESI) Mass. calcd. for $C_{70}H_{111}N_{15}O_{13}Se$ $[M+2H]^{2+}$, 724.88. Found $[M+2H]^{2+}$, 724.89.

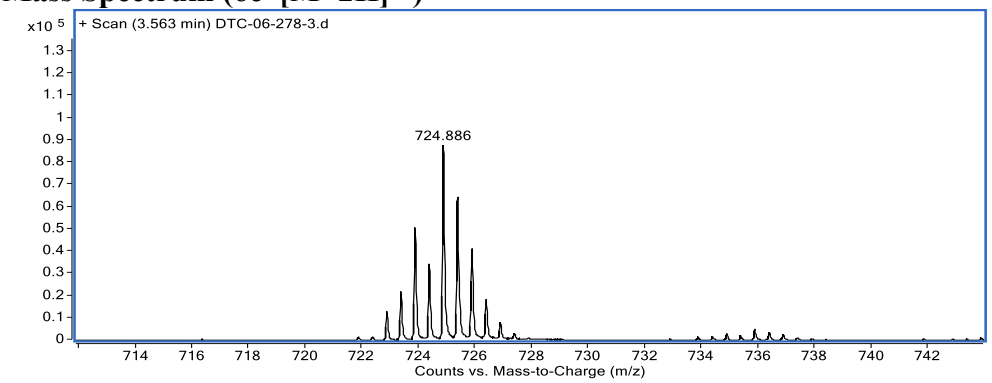
TIC (6e)

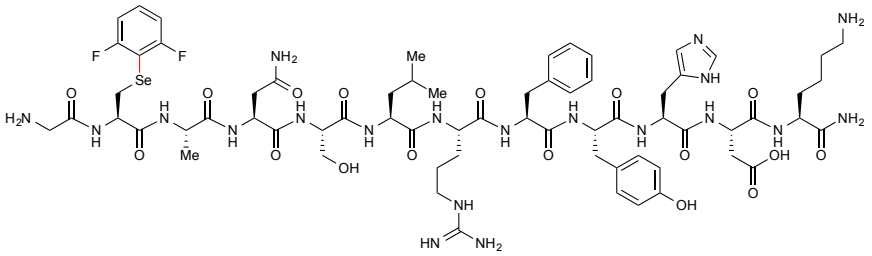


Expanded TIC with Integration (6e)



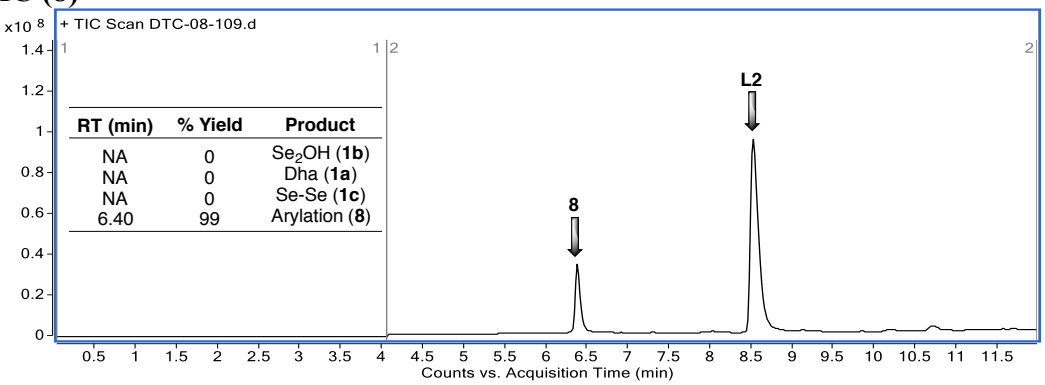
Mass Spectrum (6e $[M+2H]^{2+}$)



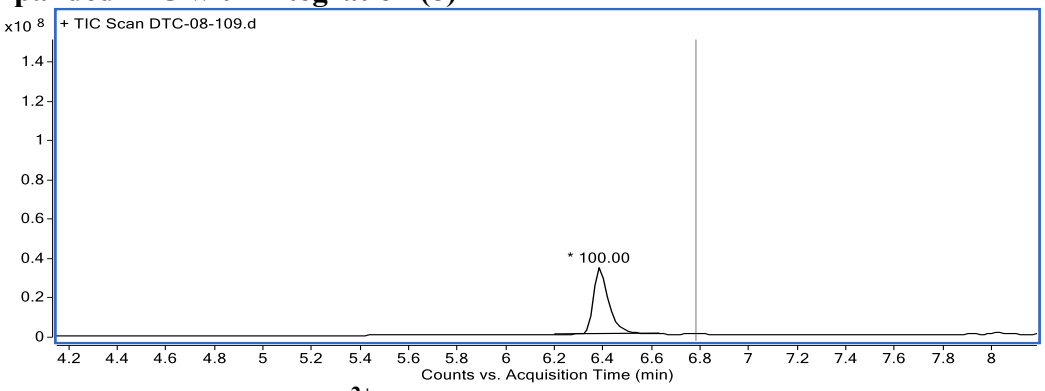


(8): Prepared according to the general procedure (A) using peptide **7** (100 μM) and $CuSO_4$, **L2**, and (2,6-difluorophenyl)boronic acid stock solution (1 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **8**: HRMS (ESI) Mass. calcd. for $C_{67}H_{96}F_2N_{20}O_{17}Se$ $[M+2H]^{2+}$, 785.32. Found $[M+2H]^{2+}$, 785.32.

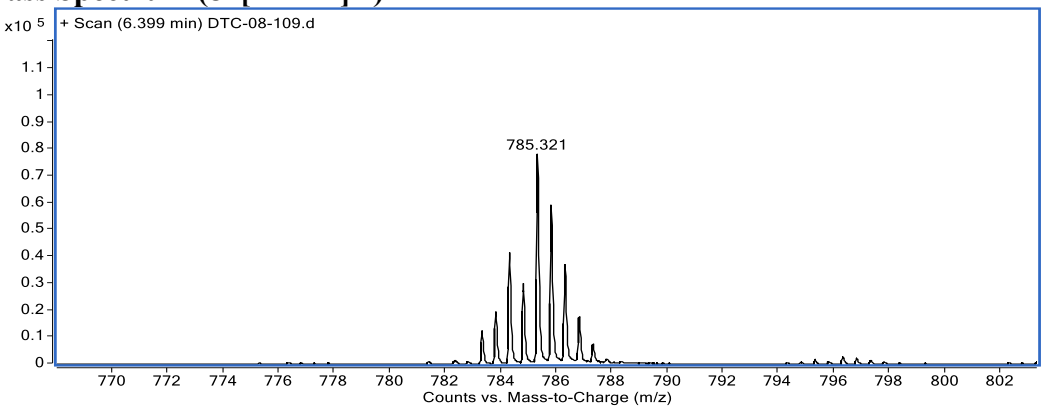
TIC (8)



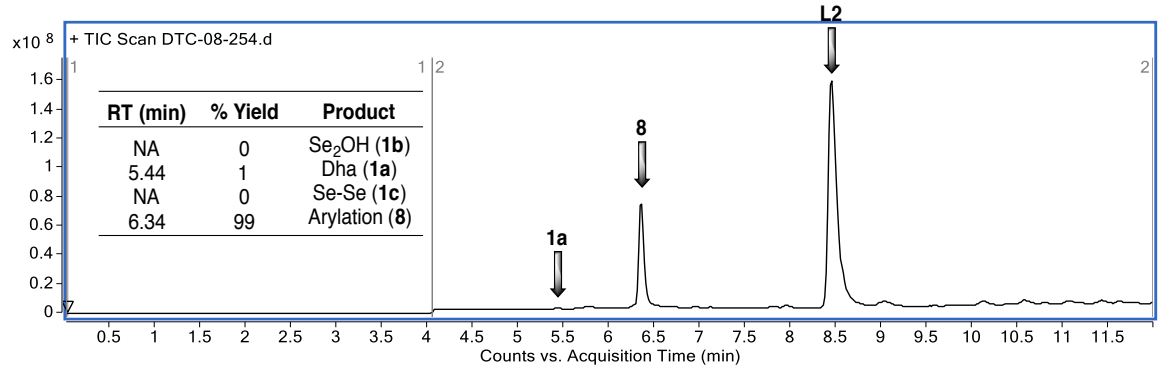
Expanded TIC with Integration (8)



Mass Spectrum (8 $[M+2H]^{2+}$)

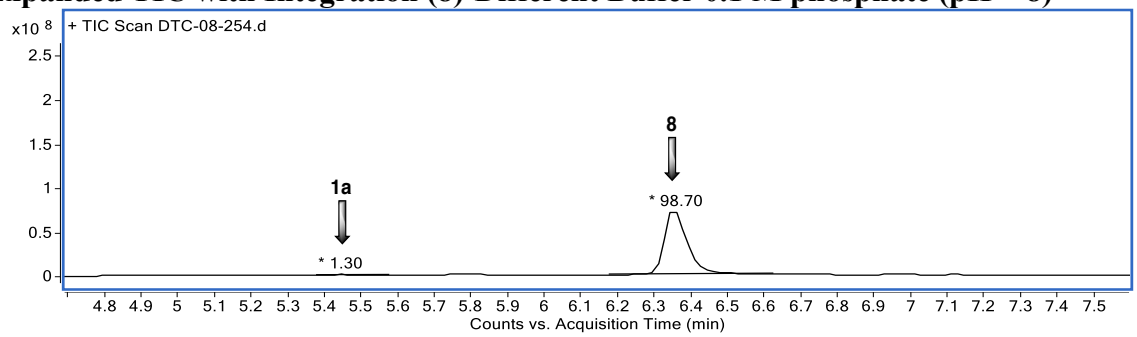


TIC (8)-Different Buffer 0.1 M phosphate (pH = 8)

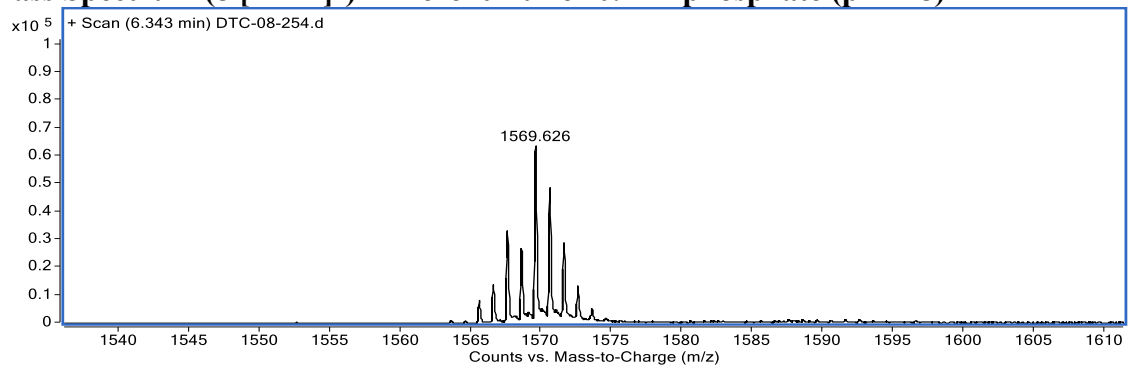


RT (min)	% Yield	Product
NA	0	Se_2OH (**1b**)
5.44	1	Dha (**1a**)
NA	0	Se-Se (**1c**)
6.34	99	Arylation (**8**)

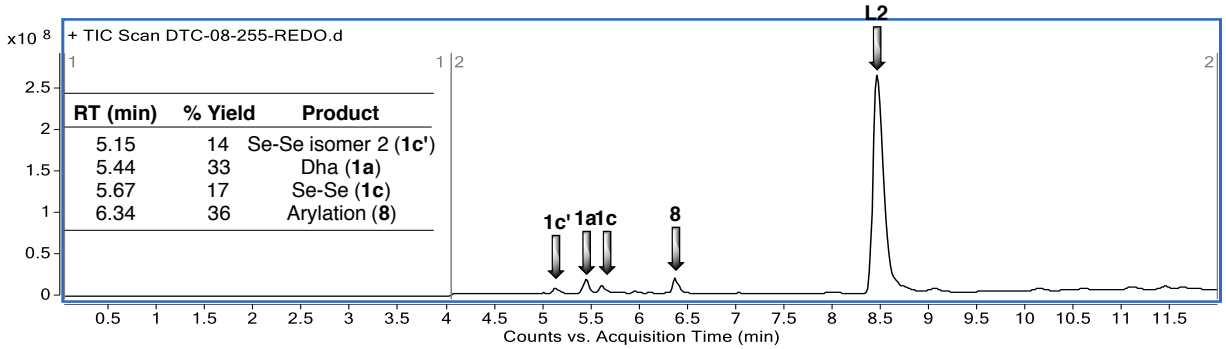
Expanded TIC with Integration (8)-Different Buffer 0.1 M phosphate (pH = 8)



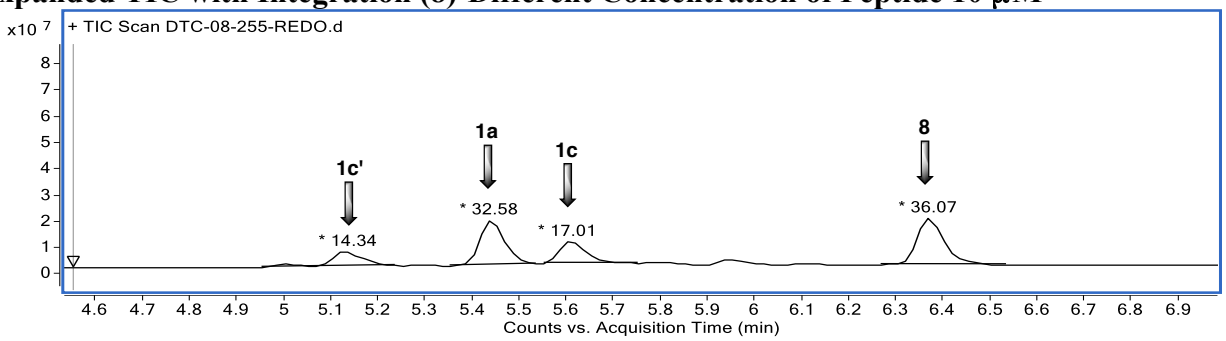
Mass Spectrum (8 $[M+H]^+$)-Different Buffer 0.1 M phosphate (pH = 8)



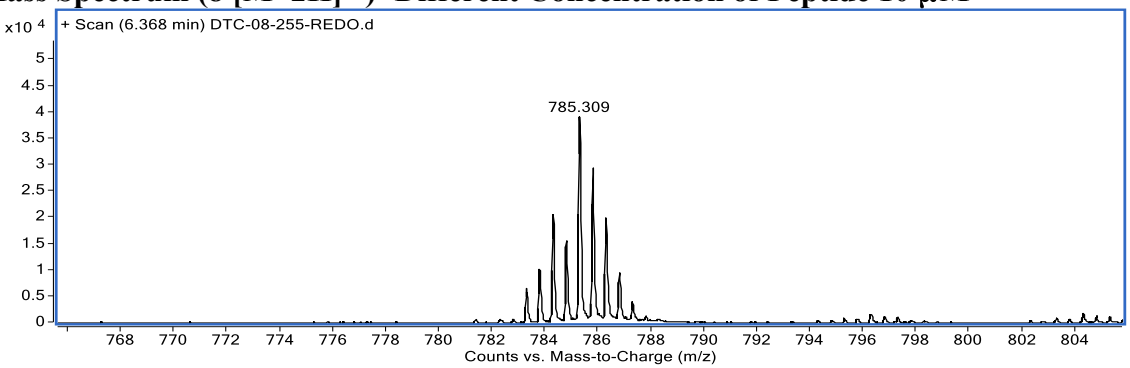
TIC (8)-Different Concentration of Peptide 10 µM



Expanded TIC with Integration (8)-Different Concentration of Peptide 10 µM

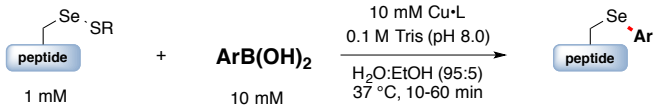


Mass Spectrum (8 $[M+2H]^{2+}$)- Different Concentration of Peptide 10 µM



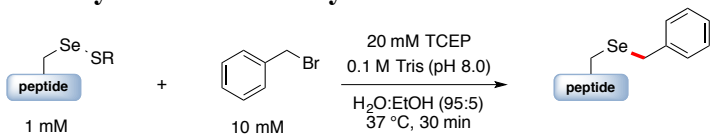
Procedure for 1mM Reactions

General Procedure (B) for arylation reactions with arylboronic acids

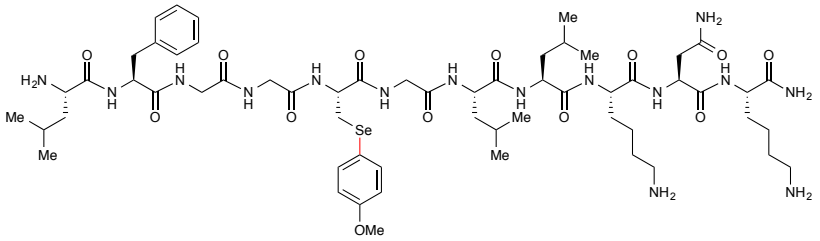


A 20 mL scintillation vial equipped with a stir bar was charged with 3 mL of deionized H_2O, 400 μL of 1.0 M Tris Buffer (pH = 8.0), 400 μL of peptide **1** (10 mM stock solution). A separate 1.7 mL Eppendorf tube was charged with copper (200 μmol), ligand (200 μmol), arylboronic acid (200 μmol), and 1 mL of 200 proof EtOH. The heterogeneous solution was subjected to sonication for 1 min, vortexed for 30 sec, and 200 μL of the resulting solution was added to the peptide solution in the 20 mL scintillation vial. The resulting reaction mixture was capped, and stirred at 800 rpm in 37 °C water bath for the indicated time (10-60 min). The reaction mixture was quenched with 4 mL of 100 mM aqueous ETDA. The resulting mixture was centrifuged at 4,000 rpm for 10 min. The supernatant was filtered through a 0.22 μm nylon filter and was subjected to purification by HPLC. ***We found that the ligands were completely removed after filtering through nylon filter.*** For copper removal efficiency using this protocol see ICP-MS analysis below.

General Procedure for alkylation of selenocysteine

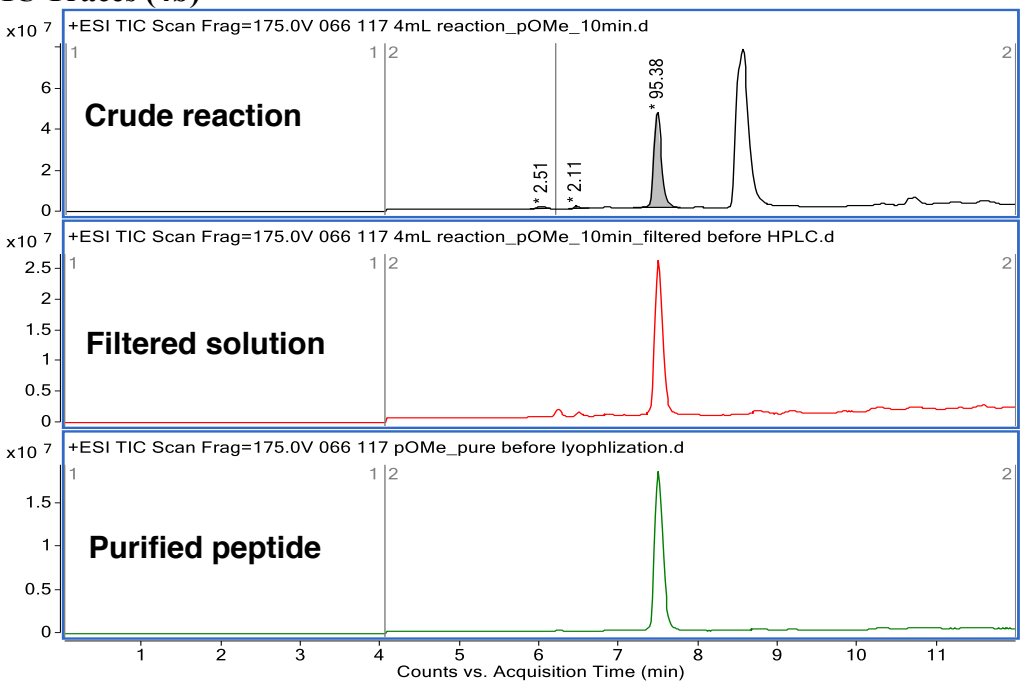


A 20 mL scintillation vial was charged with 3 mL of deionized H_2O, 400 μL of 1.0 M Tris Buffer with 200 mM TCEP (pH = 8.0), 400 μL of peptide **1** (10 mM stock solution). A separate 1.7 mL Eppendorf tube was charged with benzylbromide (200 μmol) and 1 mL of 200 proof EtOH. 200 μL of the resulting solution was added to the peptide solution in the 20 mL scintillation vial. The resulting reaction mixture was capped, and stirred at 800 rpm in 37 °C water bath for 30 min. The reaction mixture was diluted with 4 mL of deionized water. The resulting solution was filtered through a 0.22 μm nylon filter and was subjected to purification by HPLC.

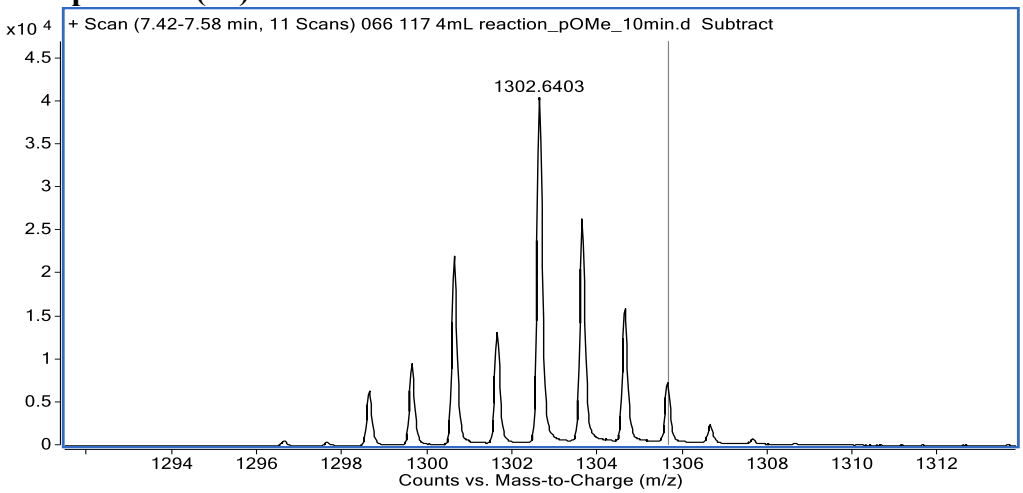


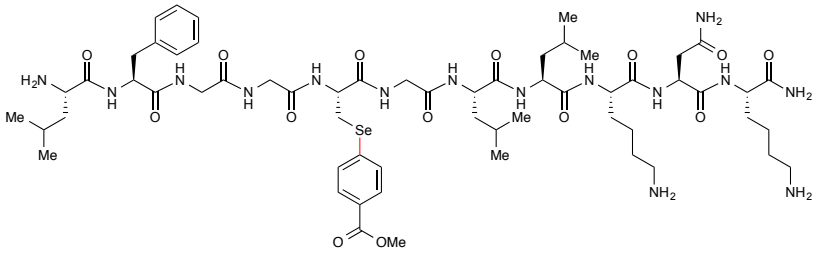
(4b): Prepared according to the general procedure (B) using peptide **1** (1 mM) and $CuSO_4$, **L2**, and (4-methoxyphenyl)boronic acid (10 mM) at 37 °C for 10 minutes. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **4b**: HRMS (ESI) Mass. calcd. for $C_{59}H_{97}N_{15}O_{13}Se$ $[M+H]^+$, 1302.64. Found $[M+H]^+$, 1302.64. **4b** was obtained as white power (3.55 mg, 68%) after HPLC purification and lyophlization.

TIC Traces (4b)



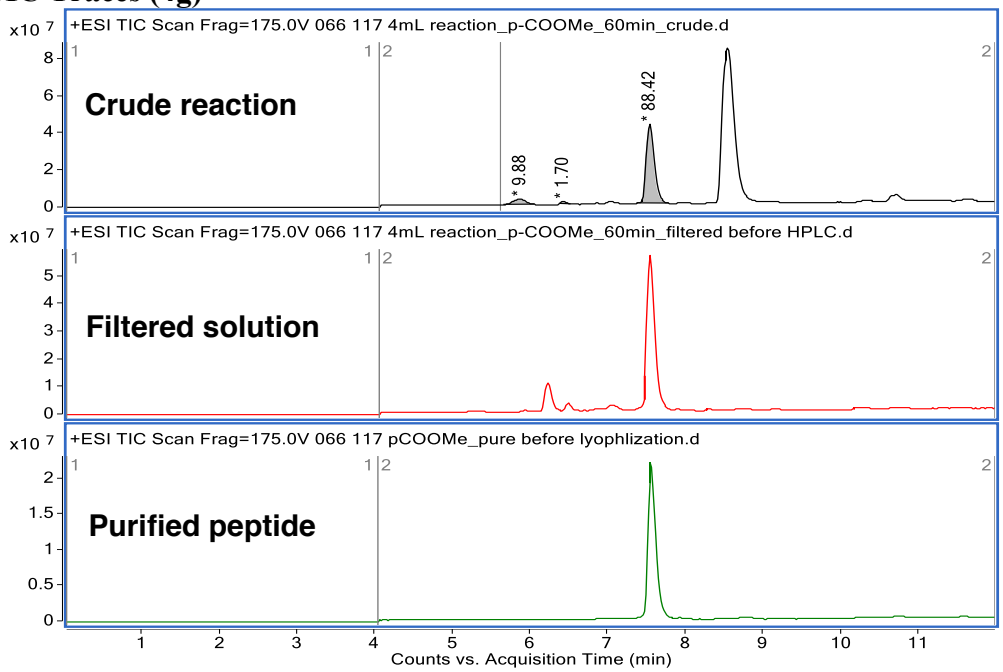
Mass spectrum (4b)



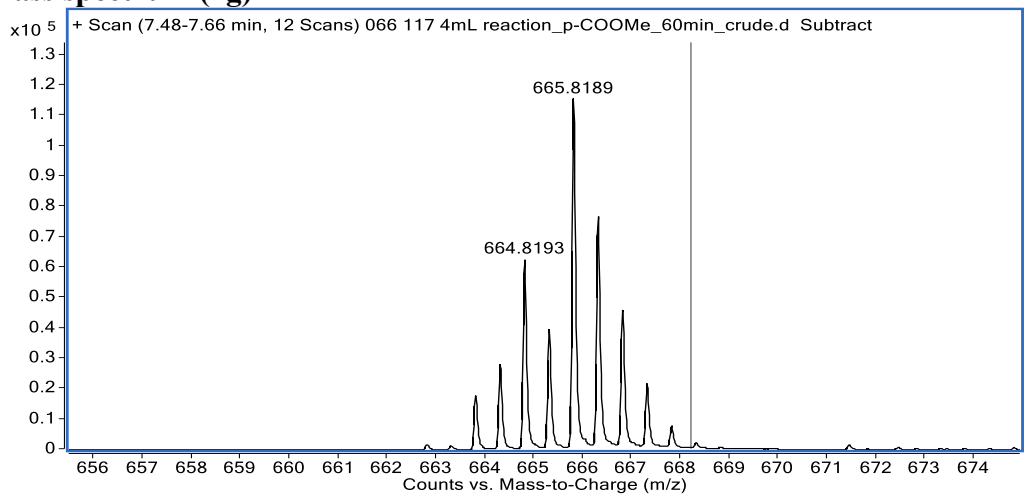


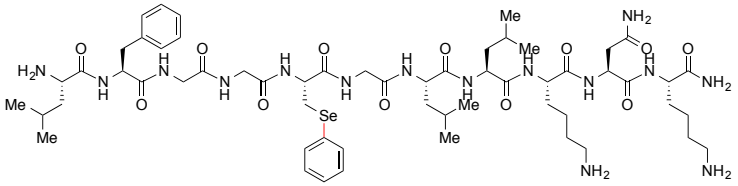
(4g): Prepared according to the general procedure (B) using peptide **1** (1 mM) and $CuSO_4$, **L2**, and (4-(methoxycarbonyl)phenyl)boronic acid (10 mM) at 37 °C for 1 h. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **4g**: HRMS (ESI) Mass. calcd. for $C_{61}H_{100}N_{16}O_{13}Se$ $[M+2H]^{2+}$, 665.82. Found $[M+2H]^{2+}$, 665.82. **4g** was obtained as white power (3.91 mg, 74%) after HPLC purification and lyophlization.

TIC Traces (4g)



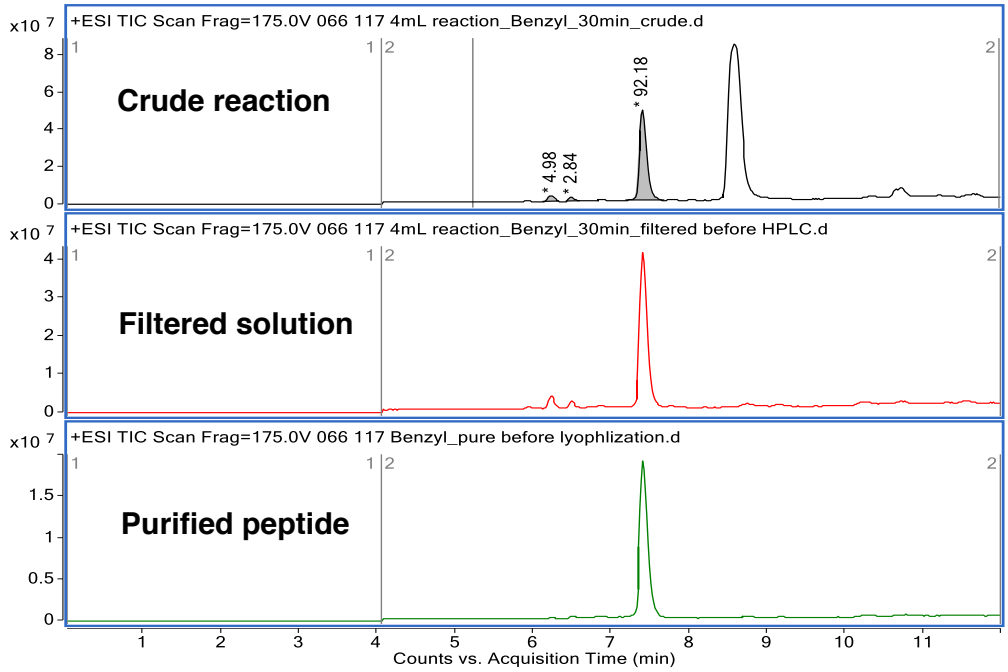
Mass spectrum (4g)



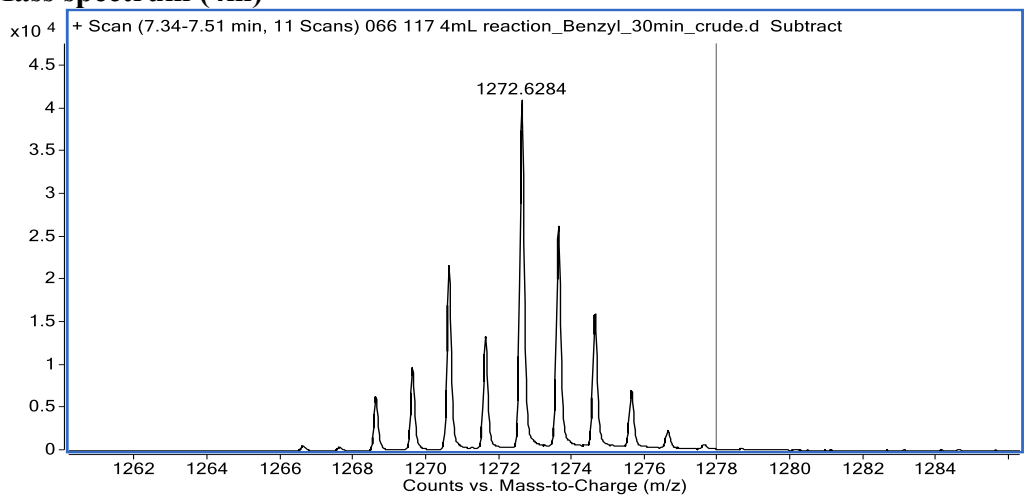


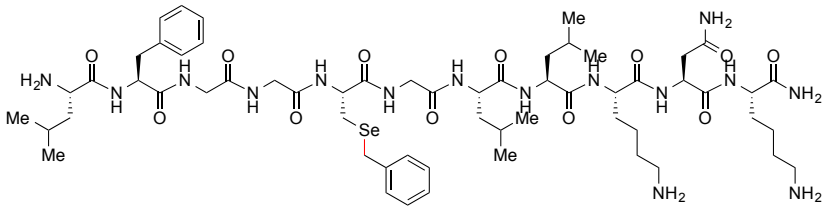
(4m): Prepared according to the general procedure (B) using peptide **1** (1 mM) and $CuSO_4$, **L2**, and phenylboronic acid (10 mM) at 37 °C for 30 minutes. The quenched reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **4m**: HRMS (ESI) Mass. calcd. for $C_{58}H_{93}N_{15}O_{12}Se$ $[M+H]^+$, 1272.63. Found $[M+H]^+$, 1272.63. **4m** was obtained as white power (3.54 mg, 70%) after HPLC purification and lyophlization.

TIC Traces (4m)



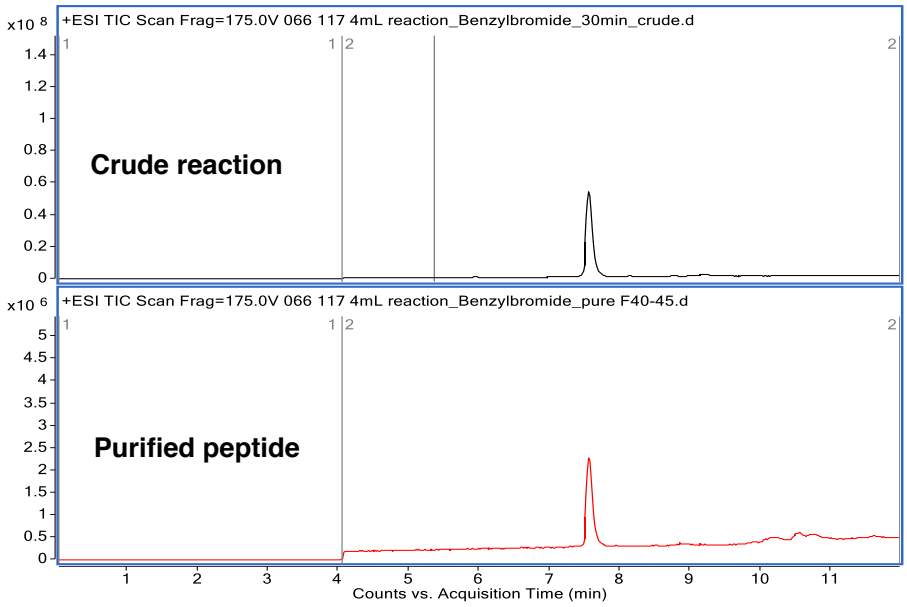
Mass spectrum (4m)



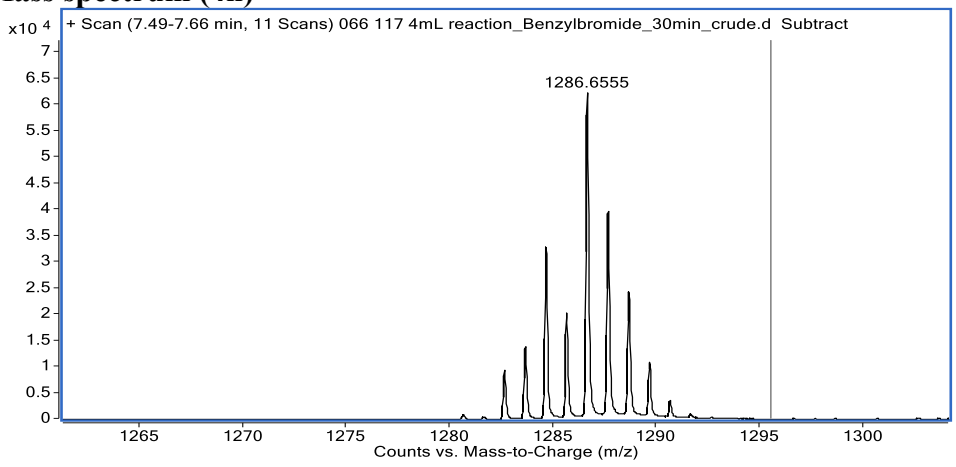


(4n): Prepared according to the alkylation procedure using peptide **1** (1 mM) and benzylbromide (10 mM) at 37 °C for 30 minutes. The reaction mixture was analyzed using LC-MS *Method A*. Analytical data for **4n**: HRMS (ESI) Mass. calcd. for $C_{59}H_{95}N_{15}O_{12}Se$ $[M+H]^+$, 1286.65. Found $[M+H]^+$, 1286.65. **4n** was obtained as white power (3.63 mg, 71%) after HPLC purification and lyophlization.

TIC Trace (4n):

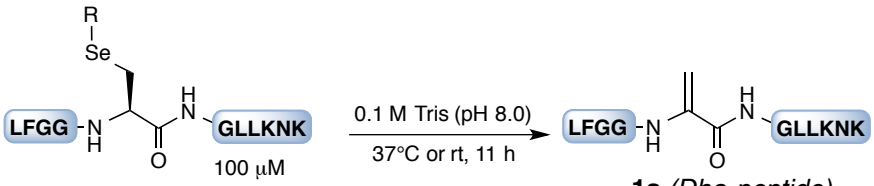


Mass spectrum (4n)



Stability Studies of Functionalized Selenocysteine Peptides

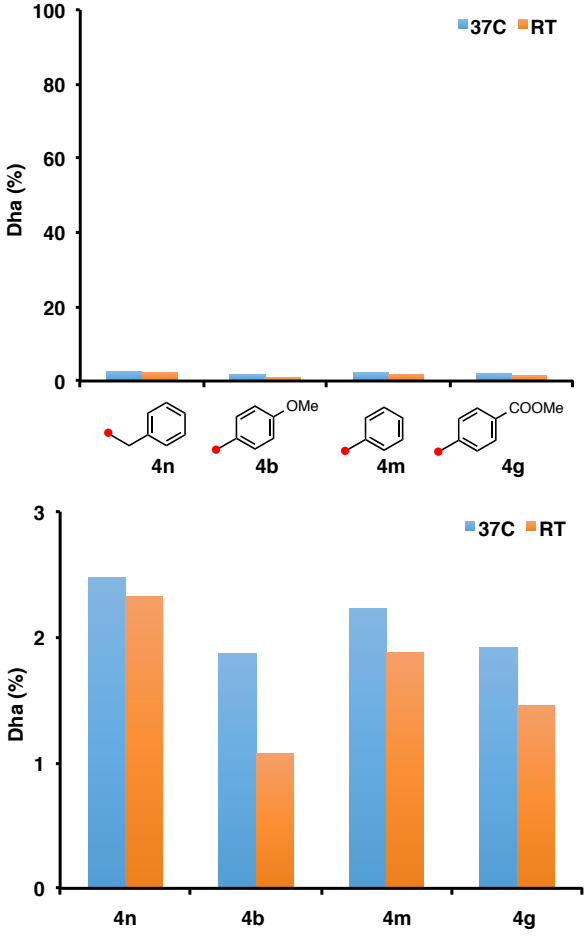
1) Stability in pH = 8.0 buffer

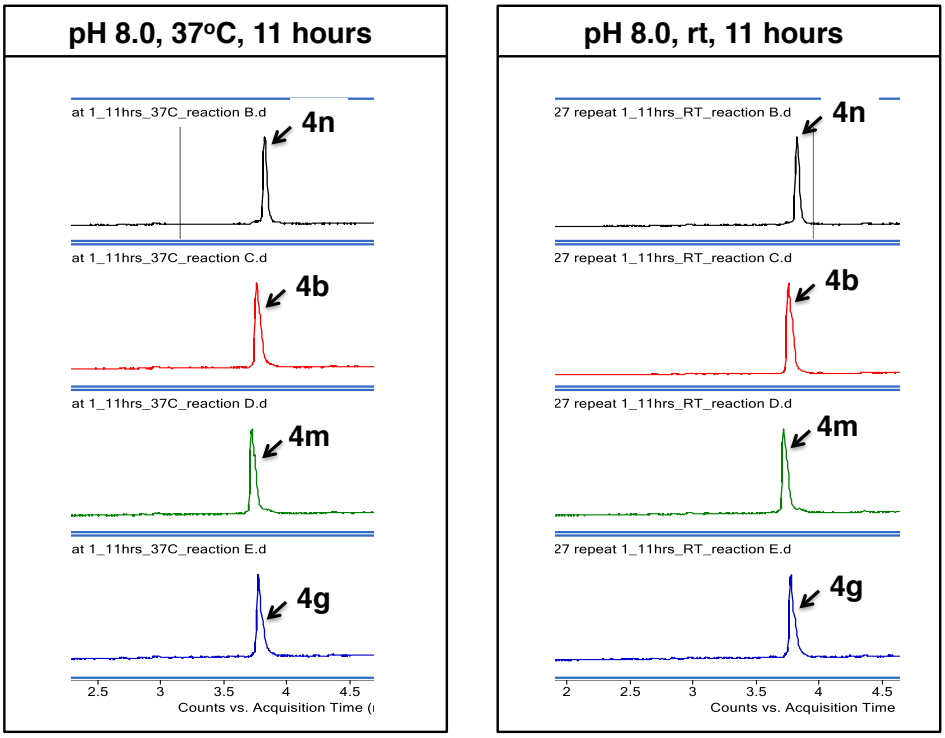


1a *(Dha peptide)*

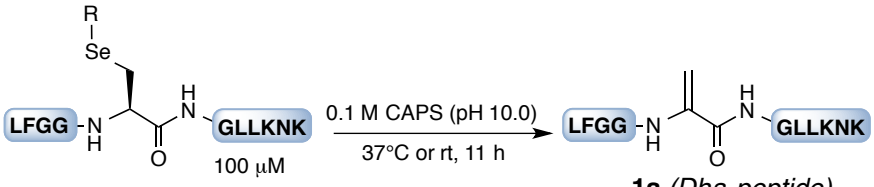
R = Bn (**4n**)
R = 4-MeOPh (**4b**)
R = Ph (**4m**)
R = 4-CO_2MePh (**4g**)

A 0.6 mL tube was charged with 178 μL of deionized H_2O, 20 μL of 1.0 M Tris Buffer (pH 8.0, 2 μL of peptide (10 mM stock solution). The resulting reaction mixture was capped and incubated in 37 °C water bath or was left at room temperature for 11 hours. 5 μL of the crude reaction mixture was quenched by addition of 200 μL of 50% A: 50% B and was subjected to LC-MS analysis *Method E*.





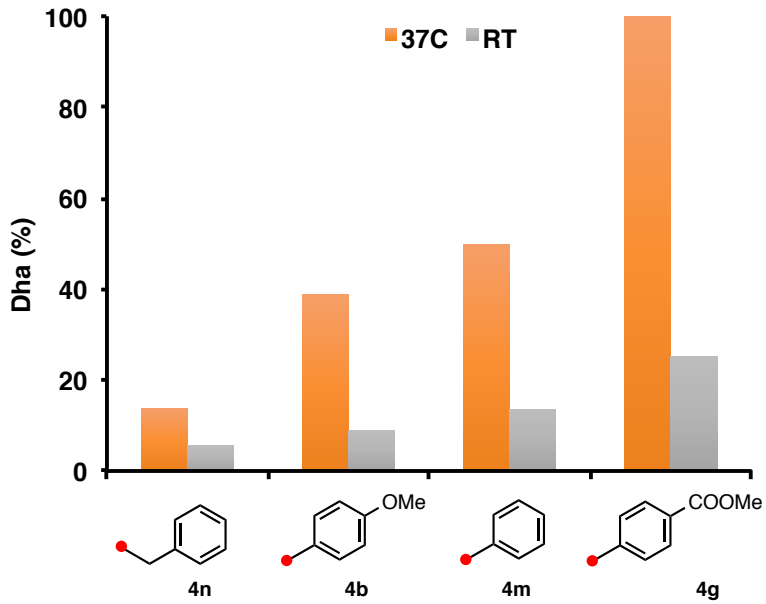
2) Stability in pH = 10.0 buffer

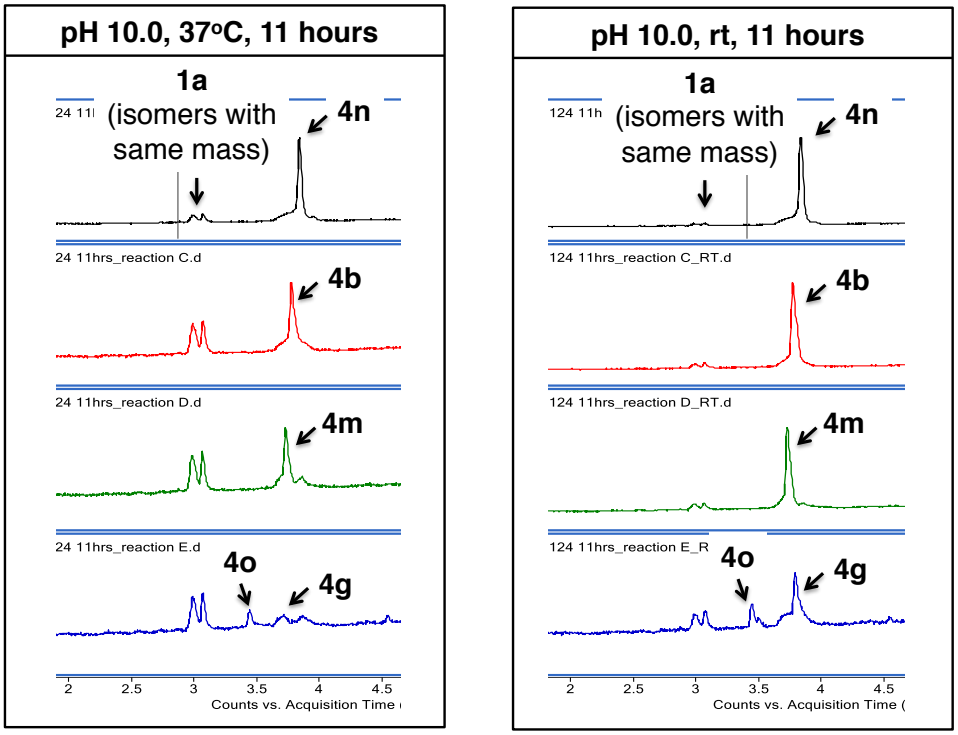


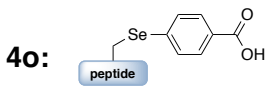
1a *(Dha peptide)*

R = Bn (**4n**)
R = 4-MeOPh (**4b**)
R = Ph (**4m**)
R = 4-CO_2MePh (**4g**)

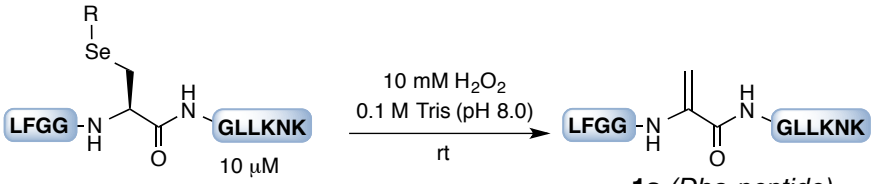
A 0.6 mL tube was charged with 178 μL of deionized H_2O, 20 μL of 1.0 M CAPS Buffer (pH 10.0), 2 μL of peptide (10 mM stock solution). The resulting reaction mixture was capped and incubated in 37 °C water bath or was left at room temperature for 11 hours. 5 μL of the crude reaction was quenched by addition of 200 μL of 50% A: 50% B and was subjected to LC-MS analysis *Method E*.


100
80
60
40
20
0
Dha (%)
37C
RT
OMe
COOMe
4n
4b
4m
4g


pH 10.0, 37°C, 11 hours
1a
(isomers with same mass)
4n
24 11hrs_reaction C.d
4b
24 11hrs_reaction D.d
4m
24 11hrs_reaction E.d
4o
4g
Counts vs. Acquisition Time
pH 10.0, rt, 11 hours
1a
(isomers with same mass)
4n
124 11hrs_reaction C_RT.d
4b
124 11hrs_reaction D_RT.d
4m
124 11hrs_reaction E_R
4o
4g
Counts vs. Acquisition Time


4o:
Se
peptide
O
OH

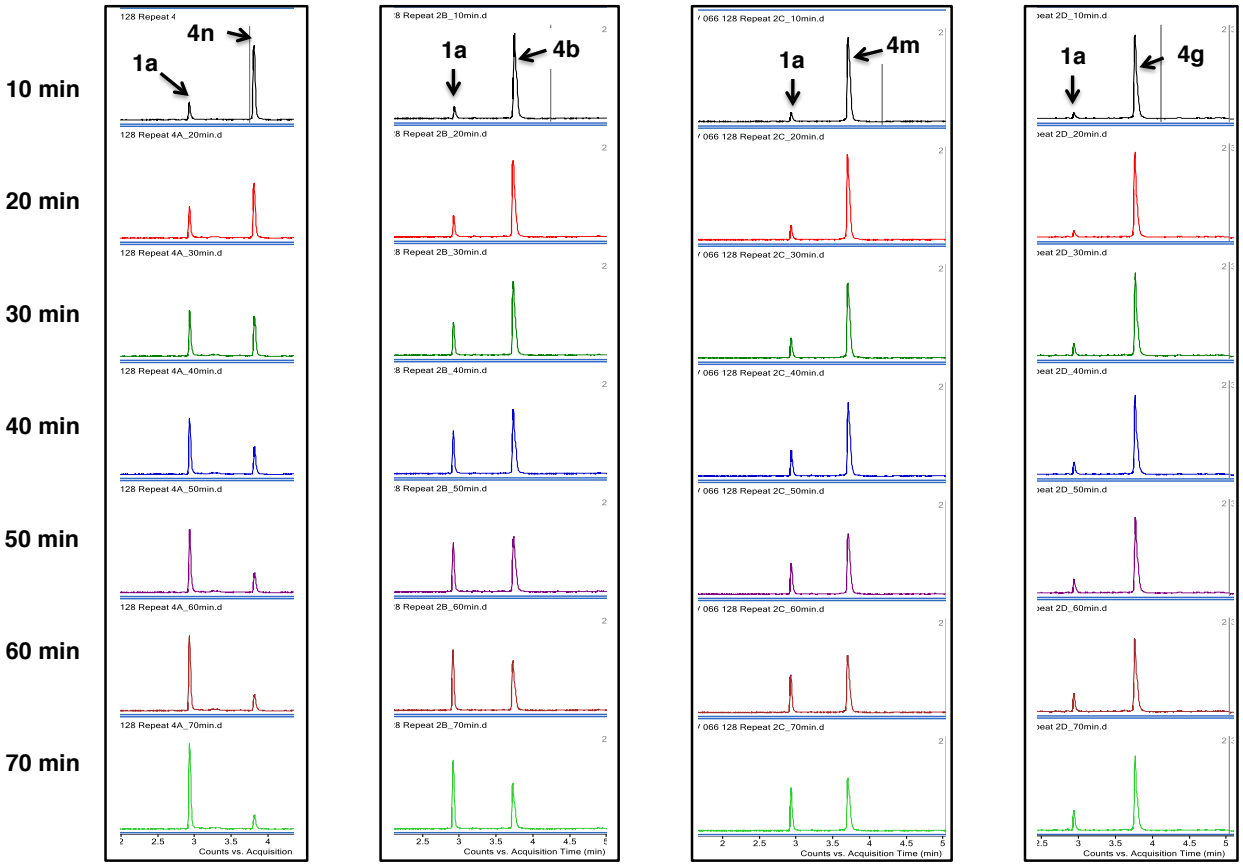
3) Stability under oxidative conditions



1a *(Dha peptide)*

R = Bn (**4n**)
R = 4-MeOPh (**4b**)
R = Ph (**4m**)
R = 4-CO_2MePh (**4g**)

A 300μL LC-MS vial was charged with 158 μL of deionized H_2O, 20 μL of 1.0 M Tris Buffer (pH = 8.0), 2 μL of peptide (1 mM stock solution), 20 μL of H_2O_2 (100 mM stock solution in water). The resulting reaction mixture was capped and was monitored by LC-MS (*Method E)*.



ICP-MS

We thank Aldo Rancier (Merck) for the copper ICP-MS analysis of purified peptides **4b**, **4m**, and **4g**. The analysis was run on an Aglient 7700 series ICP-MS. This evaluation helped quantify the copper concentration (ppm) in each of the purified Sec arylation products. For each of the purified peptides, a 20 μL of stock solution (10 mM in water) was diluted with 1 mL of phosphate buffer saline (PBS). The sample was lyophilized, re-dissolved in 80% nitric acid, and analyzed by ICP-MS. The results obtained are summarized in the following table.

Sample	Lyophilized Mass	Cu concentration
4b	10.4 mg	38 ppm
4m	10.3 mg	34 ppm
4g	10.3 mg	34 ppm

Listed below are the calculations for the copper removal efficiency.

4b

[Mass of Cu in the lyophilized sample] = (10.4 mg)*(38 ppm) = 0.40 μg

[Mass of peptide in the lyophilized sample] = (20 μL)*(10 mM)*(1301.64 g/mol) = 0.26 mg

Copper concentration in the lyophilized sample (per peptide mass) is:

[Cu concentration in the lyophilized sample] = (0.40 μg)/(0.26 mg) = 1538 ppm

Copper remaining in the total purified peptide is:

[Mass of Cu remaining] = (3.55 mg)*(1538 ppm) = 5.46 μg

Total copper added into the crude reaction is:

[Mass of total Cu] = (40 μmol)*(63.546 g/mol) = 2.54 mg

Copper removal efficiency = 1-[Mass of copper remaining]/[Mass of total Cu] = 1-(5.46 μg)/(2.54 mg) = **99.8%**

4m

[Mass of Cu in the lyophilized sample] = (10.3 mg)*(34 ppm) = 0.35 μg

[Mass of peptide in the lyophilized sample] = (20 μL)*(10 mM)*(1271.63 g/mol) = 0.26 mg

Copper concentration in the lyophilized sample (per peptide mass) is:

[Cu concentration in the lyophilized sample] = (0.35 μg)/(0.26 mg) = 1346 ppm

Copper remaining in the total purified peptide is:

[Mass of Cu remaining] = (3.54 mg)*(1346 ppm) = 4.76 μg

Total copper added into the crude reaction is:

[Mass of total Cu] = (40 μmol)*(63.546 g/mol) = 2.54 mg

Copper removal efficiency = 1-[Mass of copper remaining]/[Mass of total Cu] = 1-(4.76 μg)/(2.54 mg) = **99.8%**

4g

[Mass of Cu in the lyophilized sample] = (10.3 mg)*(34 ppm) = 0.35 μg

[Mass of peptide in the lyophilized sample] = (20 μL)*(10 mM)*(1329.64 g/mol) = 0.27 mg

Copper concentration in the lyophilized sample (per peptide mass) is:

[Cu concentration in the lyophilized sample] = (0.35 μg)/(0.27 mg) = 1296 ppm

Copper remaining in the total purified peptide is:

[Mass of Cu remaining] = (3.91 mg)*(1296 ppm) = 5.07 μg

Total copper added into the crude reaction is:

[Mass of total Cu] = (40 μmol)*(63.546 g/mol) = 2.54 mg

Copper removal efficiency = 1-[Mass of copper remaining]/[Mass of total Cu] = 1-(5.07 μg)/(2.54 mg) = **99.8%**

References

1) Schroll, A. L.; Hondal, R. J.; Flemer, S. *J. Pept. Sci.* **2012**, *18*, 155.
2) Ishiyama, T.; Murata, M.; Miyaura, N. *J. Org. Chem.*, **1995**, *60*, 7508.
3) Simon, M. D.; Heider, P. L.; Adamo, A.; Vinogradov, A. A.; Mong, S. K.; Li, X.; Berger, T.; Policarpo, R. L.; Zhang, C.; Zou, Y.; Lio, X.; Spokony, A. M.; Jensen, K. F.; Pentelute, B.L. *ChemBioChem* **2014,** *15*, 713.